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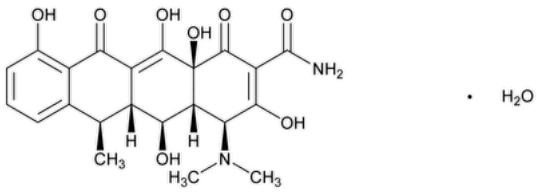
Doxycycline

Change to read:



$C_{22}H_{24}N_2O_8 \cdot H_2O$ ▲462.46▲ (CN 1-Aug-2023)
$C_{22}H_{24}N_2O_8$ 444.44
2-Naphthacenecarboxamide, 4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-, [4*S*-(4α,4aα,5α,5aα,6α,12aα)]-, monohydrate;
(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 monohydrate CAS RN®: 17086-28-1; UNII: N12000U13O.
Anhydrous CAS RN®: 564-25-0; UNII: 334895S862.

DEFINITION

Doxycycline has a potency equivalent to NLT 880 µg/mg and NMT 980 µg/mg of doxycycline ($C_{22}H_{24}N_2O_8$).

IDENTIFICATION

- **A. Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*:** 197A
- **B.** The retention time 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 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 Monohydrate RS in *Diluent*. Sonicate as needed to dissolve.

Sample solution: 0.1 mg/mL of Doxycycline 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 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 Monohydrate RS in the *Standard solution* (mg/mL)

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

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

Acceptance criteria: 880–980 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 Monohydrate RS in *Diluent*

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

Sample solution: 2 mg/mL of Doxycycline 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 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 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 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 Monohydrate RS in the *Standard solution* (mg/mL)

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

P = potency of doxycycline in USP Doxycycline Monohydrate 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]	0.60	1.0	0.5
Doxycycline related compound A (6-epidoxycycline)	0.72	0.67	2.0
Doxycycline	1.0	—	—
Doxycycline related compound F[b]	1.20	0.66	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.▲ (CN 1-Aug-2023)

SPECIFIC TESTS

- **Crystallinity ⟨695⟩:** Meets the requirements
- **pH ⟨791⟩**

  Sample solution: An aqueous suspension containing 10 mg/mL

  Acceptance criteria: 5.0–6.5
- **Water Determination ⟨921⟩, *Method I*:** 3.6%–4.6%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers.

Change to read:

- **USP Reference Standards ⟨11⟩**

  USP Doxycycline Hyclate RS

  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)]-.

$(C_{22}H_{24}N_2O_8 \cdot HCl)_2 \cdot C_2H_6O \cdot H_2O$ 1025.87

USP Doxycycline Monohydrate 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

(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, hydrochloride.▲ (CN 1-Aug-2023) $C_{22}H_{24}N_2O_8 \cdot HCl$ ▲480.90▲ (CN 1-Aug-2023)

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	Documentary Standards Support	SM12020 Small Molecules 1

Chromatographic Database Information: Chromatographic Database

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