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Doxycycline for Injection

DEFINITION

Doxycycline for Injection contains an amount of Doxycycline Hyclate equivalent to NLT 90.0% and NMT 120.0% of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$).

IDENTIFICATION

- **A.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **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, 0.5 g of edetate disodium, and 0.5 mL of triethylamine to a 1000-mL volumetric flask. Add about 850 mL of water and mix. Dilute with water to volume and adjust with 1 N sodium hydroxide to a pH of 8.5 ± 0.1. Pass through a suitable filter of 0.22-µm pore size.

Solution B: Methanol

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	90	10
2.0	90	10
4.0	60	40
6.0	90	10
9.0	90	10

Diluent: 0.01 N hydrochloric acid

Standard solution: 0.12 mg/mL of USP Doxycycline Hyclate RS in *Diluent*. Sonicate as needed to dissolve.

Sample solution: Nominally 0.1 mg/mL of doxycycline prepared as follows. Dissolve the contents of one container of Doxycycline for Injection using 1% of the final volume of *Diluent* and transfer to a suitable volumetric flask. Dilute with *Diluent* to volume and mix.

Chromatographic system

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

Mode: LC

Detector: UV 270 nm. For *Identification A*, use a diode array detector in the range of 200–400 nm.

Column: 2.1-mm × 5-cm; 1.7-µm packing L7. [Note—A 1.7-µm guard column with packing L7 was used during method validation.]

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 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) in the portion of Doxycycline for Injection taken:

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

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 = nominal concentration of doxycycline in the *Sample solution* (mg/mL)

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

F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–120.0%

IMPURITIES

• **Organic Impurities**

Protect solutions containing doxycycline from light.

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

System suitability stock solution 1: 1 mg/mL each of USP Doxycycline Related Compound A RS and USP Methacycline Hydrochloride 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, doxycycline related compound A, methacycline, and doxycycline. [Note—The solution is stable up to 14 days when stored in a refrigerator.]

Standard solution: 2.3 µg/mL of USP Doxycycline Hyclate RS in *Diluent*

Sample solution: Nominally 2.0 mg/mL of doxycycline prepared as follows. Dissolve the contents of one container of Doxycycline for Injection using 20% of the final volume of *Diluent* and transfer to a suitable volumetric flask. Dilute with *Diluent* to volume and mix.

System suitability

Samples: *System suitability 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% for the doxycycline peak, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Doxycycline 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 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 = nominal concentration of doxycycline in the *Sample solution* (mg/mL)

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

F = conversion factor, 0.001 mg/µg

Acceptance criteria: See *Table 2*. Disregard peaks less than 0.1%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Methacycline[a,b]	0.64	—
4-Epidoxycycline[c]	0.79	2.2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Doxycycline related compound A (6-epidoxycycline)[b,d]	0.88	—
Doxycycline	1.0	—
Any individual unspecified impurity	—	0.5
Total impurities	—	5.5

[a] (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.

[b] Process impurities that are controlled in the drug substance are not to be reported. They are listed here for information only.

[c] (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.

[d] (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.

SPECIFIC TESTS

• **Sterility Tests ⟨71⟩:** Meets the requirements. If the membrane filtration test is used, use *Fluid D* instead of *Fluid A*.

• **pH ⟨791⟩**

Sample solution: Constitute as directed in the labeling.

Acceptance criteria: 1.8–3.3

• **Loss on Drying ⟨731⟩**

Analysis: Dry 100 mg in a capillary-stoppered bottle under vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 h.

Acceptance criteria: NMT 2.0% for the article containing added substances; NMT 4.0% for the article containing no added substances

• **Particulate Matter in Injections ⟨788⟩:** Meets the requirements for small-volume injections

• **Bacterial Endotoxins Test ⟨85⟩:** Contains NMT 1.14 USP Endotoxin Units/mg of doxycycline

• **Constituted Solution:** At the time of use, it meets the requirements in *Injections and Implanted Drug Products ⟨1⟩, Product Quality Tests Common to Parenteral Dosage Forms, Specific Tests, Completeness and Clarity of Solutions*.

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve as described in *Packaging and Storage Requirements ⟨659⟩, Injection Packaging*, protected from light.

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-Jul-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 ▲hydrochloride▲ (ERR 1-Jul-2022) ·

$C_{22}H_{24}N_2O_8 \cdot HCl$ ▲480.90▲ (ERR 1-Jul-2022)

USP Methacycline Hydrochloride RS

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

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

Chromatographic Database Information: Chromatographic Database

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