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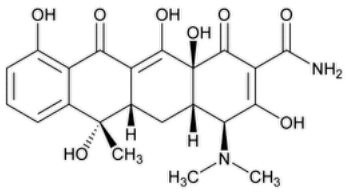
Tetracycline

Change to read:



$C_{22}H_{24}N_2O_8$ 444.44
2-Naphthacenecarboxamide, 4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-, [4*S*-(4α,4aα,5aα,6β,12aα)]-;
(4*S*,4a*S*,5a*S*,12a*S*)-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide CAS RN®: 60-54-8; ▲UNII: F8VB5M810T.▲ (IRA 1-May-2022)

Trihydrate 498.49 CAS RN®: 6416-04-2; UNII: 93V6NC52SB.

DEFINITION
Tetracycline has a potency equivalent to NLT 975 µg/mg of tetracycline hydrochloride ($C_{22}H_{24}N_2O_8 \cdot HCl$), calculated on the anhydrous basis.

IDENTIFICATION

• **A. Spectroscopic Identification Tests ⟨197⟩, *Ultraviolet-Visible Spectroscopy*:** 197U
Sample solution: 20 µg/mL in 0.25 N sodium hydroxide
Analytical wavelength: 380 nm
Analysis: Measure the absorptivity 6 min after preparation.
Acceptance criteria: Absorptivity, calculated on the anhydrous basis and taking into account the potency of the Reference Standard, is between 104.5% and 111.95% of the absorptivity of USP Tetracycline Hydrochloride RS.

• **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**
Solution A: Dilute 1 mL of phosphoric acid with water to 1 L.
Solution B: Acetonitrile
Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	85	15
7.5	60	40
7.6	85	15
10	85	15

System suitability solution: 25 µg/mL each of USP Anhydrotetracycline Hydrochloride RS, USP Epitetracycline Hydrochloride RS, and USP 4-Epianhydrotetracycline Hydrochloride RS, and 100 µg/mL of USP Tetracycline Hydrochloride RS in *Solution A*
Standard solution: 100 µg/mL of USP Tetracycline Hydrochloride RS in *Solution A*
Sample solution: 90 µg/mL of Tetracycline in *Solution A*
Chromatographic system
(See *Chromatography ⟨621⟩, System Suitability*.)
Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 15-cm; 3-µm packing L60. [NOTE—Column with L1 packing is also suitable.]

Temperatures

Autosampler: 10°

Column: 50°

Flow rate: 1.0 mL/min

Injection volume: 10 µL

System suitability

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

Suitability requirements

Resolution: NLT 2.5 between epitetracycline and tetracycline; NLT 2.5 between anhydrotetracycline and 4-epianhydrotetracycline, *System suitability solution*

Tailing factor: NMT 1.5, *Standard solution*

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

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the potency equivalent, in µg/mg, of tetracycline hydrochloride ($C_{22}H_{24}N_2O_8 \cdot HCl$) in the portion of Tetracycline taken:

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

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

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

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

C_U = concentration of Tetracycline in the *Sample solution* (µg/mL)

P = potency of USP Tetracycline Hydrochloride RS (µg/mg)

Acceptance criteria: NLT 975 µg/mg on the anhydrous basis

IMPURITIES

Change to read:

• **ORGANIC IMPURITIES**

Solution A, Solution B, Mobile phase, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

System suitability solution: 25 µg/mL each of USP Anhydrotetracycline Hydrochloride RS, USP Epitetracycline Hydrochloride RS, USP 4-Epianhydrotetracycline Hydrochloride RS, and USP Tetracycline Hydrochloride RS in *Solution A*

▲**Standard solution 1:** 0.5 µg/mL of USP Anhydrotetracycline Hydrochloride RS and 3 µg/mL of USP Epitetracycline Hydrochloride RS in *Solution A*

Standard solution 2: 2 µg/mL of USP 4-Epianhydrotetracycline Hydrochloride RS in *Solution A*

Standard solution 3: 0.1 µg/mL of USP Tetracycline Hydrochloride RS in *Solution A*▲ (IRA 1-May-2022)

System suitability

Samples: *System suitability solution*, ▲*Standard solution 1*, *Standard solution 2*, and *Standard solution 3*▲ *(IRA 1-May-2022)*

Suitability requirements

Resolution: NLT 2.5 between epitetracycline and tetracycline; NLT 2.5 between anhydrotetracycline and 4-epianhydrotetracycline, *System suitability solution*

Relative standard deviation: ▲NMT 2.8% for anhydrotetracycline and epitetracycline, *Standard solution 1*; NMT 2.8% for 4-epianhydrotetracycline, *Standard solution 2*; NMT 2.8% for tetracycline, *Standard solution 3*▲ (IRA 1-May-2022)

Analysis

Samples: ▲*Standard solution 1*, *Standard solution 2*, *Standard solution 3*,▲ (IRA 1-May-2022) and *Sample solution*

▲Calculate the percentage of anhydrotetracycline hydrochloride and epitetracycline hydrochloride in the portion of Tetracycline taken:

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

r_U = peak response of anhydrotetracycline or epitetracycline from the *Sample solution*

r_S = peak response of the corresponding USP Reference Standard from *Standard solution 1*

C_S = concentration of the corresponding USP Reference Standard in *Standard solution 1* (µg/mL)

C_U = concentration of Tetracycline in the *Sample solution* (µg/mL)

Calculate the percentage of 4-epianhydrotetracycline hydrochloride in the portion of Tetracycline taken:

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

r_U = peak response of 4-epianhydrotetracycline from the *Sample solution*

r_S = peak response of 4-epianhydrotetracycline from *Standard solution 2*

C_S = concentration of the USP 4-Epianhydrotetracycline Hydrochloride RS in *Standard solution 2* (µg/mL)

C_U = concentration of Tetracycline in the *Sample solution* (µg/mL)▲ (IRA 1-May-2022)

Calculate the percentage of 2-acetyl analog or any unspecified impurity in the portion of Tetracycline taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of 2-acetyl analog or any unspecified impurity from the *Sample solution*

r_S = peak response of tetracycline from ▲*Standard solution 3*▲ (IRA 1-May-2022)

C_S = concentration of USP Tetracycline Hydrochloride RS in ▲*Standard solution 3*▲ (IRA 1-May-2022) (µg/mL)

C_U = concentration of Tetracycline in the *Sample solution* (µg/mL)

M_{r1} = molecular weight of tetracycline, 444.44

M_{r2} = molecular weight of tetracycline hydrochloride, 480.90

Acceptance criteria: See *Table 2*. ▲The reporting threshold is▲ (IRA 1-May-2022) 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
▲Epitetracycline▲ (IRA 1-May-2022)	0.9	3.0
Tetracycline	1.0	—
2-Acetyl analog[a]	1.3	2.0
4-Epianhydrotetracycline▲▲ (IRA 1-May-2022)	1.7	2.0
Anhydrotetracycline▲▲ (IRA 1-May-2022)	1.9	0.5
Any individual unspecified impurity	—	0.10

[a] 2-Acetyl-2-decarbamoyltetracycline; also known as (4*S*,4a*S*,5a*S*,6*S*,12a*S*)-2-Acetyl-4-(dimethylamino)-3,6,10,12,12a-pentahydroxy-6-methyl-4a,5a,6,12a-tetrahydrotetracene-1,11(4*H*,5*H*)-dione.

SPECIFIC TESTS

• **Optical Rotation** ⟨781S⟩, *Procedures, Specific Rotation*
Sample solution: 5 mg/mL of tetracycline in 0.1 N hydrochloric acid
Acceptance criteria: −260° to −280° on the anhydrous basis
• **Crystallinity** ⟨695⟩**:** Meets the requirements
• **pH** ⟨791⟩
Sample solution: Prepare in an aqueous suspension (1 in 100).
Acceptance criteria: 3.0–7.0
• **Water Determination** ⟨921⟩, *Method I***:** NMT 13.0%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight, light-resistant containers.
• **Labeling:** Label it to indicate that it is to be used in the manufacture of nonparenteral drugs only.
Change to read:

• **USP Reference Standards ⟨11⟩**

USP Anhydrotetracycline Hydrochloride RS

▲(4*S*,4a*S*,12a*S*)-4-(Dimethylamino)-3,10,11,12a-tetrahydroxy-6-methyl-1,12-dioxo-1,4,4a,5,12,12a-hexahydrotetracene-2-carboxamide monohydrochloride.

$C_{22}H_{22}N_2O_7 \cdot HCl$ 462.88▲ (IRA 1-May-2022)

USP 4-Epianhydrotetracycline Hydrochloride RS

▲(4*R*,4a*S*,12a*S*)-4-(Dimethylamino)-3,10,11,12a-tetrahydroxy-6-methyl-1,12-dioxo-1,4,4a,5,12,12a-hexahydrotetracene-2-carboxamide hydrochloride.

$C_{22}H_{22}N_2O_7 \cdot HCl$ 462.88▲ (IRA 1-May-2022)

USP Epitetracycline Hydrochloride RS

▲(4*R*,4a*S*,5a*S*,6*S*,12a*S*)-4-(Dimethylamino)-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide monohydrochloride.

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

USP Tetracycline Hydrochloride RS

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

Topic/Question	Contact	Expert Committee
TETRACYCLINE	Documentary Standards Support	SM12020 Small Molecules 1
REFERENCE STANDARD SUPPORT	RS Technical Services RSTECH@usp.org	SM12020 Small Molecules 1

Chromatographic Database Information: Chromatographic Database

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