Status: Currently Official on 16-Feb-2025
Official Date: Official as of 01-Nov-2022
Document Type: USP Monographs
DocId: GUID-F4D422FE-8519-4343-ABB5-FB0F228F7717_6_en-US
DOI: https://doi.org/10.31003/USPNF_M81810_06_01
DOI Ref: 37caj

Tetracycline Hydrochloride

$C_{22}H_{24}N_2O_8 \cdot HCl$ 480.90

2-Naphthacenecarboxamide, 4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-, monohydrochloride, [4*S*-(4α,4aα,5aα,6β,12aα)]-;

(4*S*,4a*S*,5a*S*,6*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 monohydrochloride CAS RN®: 64-75-5; UNII: P6R62377KV.

DEFINITION

Tetracycline Hydrochloride has a potency of NLT 900 µg/mg of tetracycline hydrochloride ($C_{22}H_{24}N_2O_8 \cdot HCl$).

IDENTIFICATION

- **A. Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*:** 197A or 197K. Do not dry the specimen.
- **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***

  Sample solution: 1 mg/mL of Tetracycline Hydrochloride in methanol

  Acceptance criteria: Meets the requirements

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, USP 4-Epianhydrotetracycline Hydrochloride RS, and USP Tetracycline Hydrochloride RS in *Solution A*

Standard solution: 100 µg/mL of USP Tetracycline Hydrochloride RS in *Solution A*

Sample solution: 100 µg/mL of Tetracycline Hydrochloride 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 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the potency, in µg/mg, of tetracycline hydrochloride ($C_{22}H_{24}N_2O_8 \cdot HCl$) in the portion of Tetracycline Hydrochloride 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 Hydrochloride in the *Sample solution* (µg/mL)

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

Acceptance criteria: NLT 900 µg/mg

IMPURITIES

• **Organic Impurities**

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

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*

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 2.5 between epitetracycline and tetracycline; NLT 2.5 between anhydrotetracycline and 4-epianhydrotetracycline

Relative standard deviation: NMT 2%

Analysis

Samples: *Standard solution 1*, *Standard solution 2*, *Standard solution 3*, and *Sample solution*

Calculate the percentage of anhydrotetracycline hydrochloride and epitetracycline hydrochloride in the portion of Tetracycline Hydrochloride 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 Hydrochloride in the *Sample solution* (µg/mL)

Calculate the percentage of 4-epianhydrotetracycline hydrochloride in the portion of Tetracycline Hydrochloride 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 USP 4-Epianhydrotetracycline Hydrochloride RS in *Standard solution 2* (µg/mL)

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

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

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

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*

C_S = concentration of USP Tetracycline Hydrochloride RS in *Standard solution 3* (µg/mL)

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

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

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Epitetracycline	0.9	3.0
Tetracycline	1.0	—
2-Acetyl analog[a]	1.3	1.5
4-Epianhydrotetracycline	1.7	2.0
Anhydrotetracycline	1.9	0.5
Any individual unspecified impurity	—	0.10

[a] 2-Acetyl-2-decarbamoyltetracycline; (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 Hydrochloride in 0.1 N hydrochloric acid
  Acceptance criteria: −240° to −255° on the dried basis
- **Crystallinity** ⟨695⟩**:** Meets the requirements
- **pH** ⟨791⟩
  Sample solution: Prepare in a solution (1 in 100).
  Acceptance criteria: 1.8–2.8
- **Loss on Drying** ⟨731⟩
  Sample: 100 mg of Tetracycline Hydrochloride
  Analysis: Dry the *Sample* 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%
- **Sterility Tests** ⟨71⟩**:** Where the label states that Tetracycline Hydrochloride is sterile, it meets the requirements.

Change to read:

- **Bacterial Endotoxins Test** ⟨85⟩**:** Where the label states ▲Tetracycline Hydrochloride▲ (ERR 1-Nov-2022) must be subjected to further processing

during the preparation of injectable dosage forms, the level of bacterial endotoxins are such that the requirement under the relevant dosage form monograph(s) in which ▲Tetracycline Hydrochloride▲ (ERR 1-Nov-2022) is used can be met.

ADDITIONAL REQUIREMENTS

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

Change to read:

• **Labeling:** Where ▲Tetracycline Hydrochloride▲ (ERR 1-Nov-2022) must be sterile or subjected to further processing during the preparation of injectable dosage forms to ensure acceptable levels of bacterial endotoxins, it is so labeled.

• **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

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

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

USP Tetracycline Hydrochloride RS

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

Topic/Question	Contact	Expert Committee
TETRACYCLINE HYDROCHLORIDE	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

Most Recently Appeared In:
Pharmacopeial Forum: Volume No. 47(4)

Current DocID: GUID-F4D422FE-8519-4343-ABB5-FB0F228F7717_6_en-US

DOI: https://doi.org/10.31003/USPNF_M81810_06_01

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