

# 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, [4S-(4 $\alpha$ ,4a $\alpha$ ,5a $\alpha$ ,6 $\beta$ ,12a $\alpha$ )]-;  
(4S,4aS,5aS,6S,12aS)-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 <sup>a</sup>	1.3	1.5
4-Epianhydrotetracycline	1.7	2.0
Anhydrotetracycline	1.9	0.5
Any individual unspecified impurity	—	0.10

<sup>a</sup> 2-Acetyl-2-decarbamoylepianhydrotetracycline; (4S,4aS,5aS,6S,12aS)-2-Acetyl-4-(dimethylamino)-3,6,10,12,12a-pentahydroxy-6-methyl-4a,5a,6,12a-tetrahydrotetracene-1,11(4H,5H)-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.

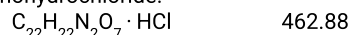
#### Change to read:

- **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](#)

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



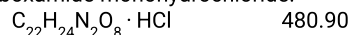
[USP 4-Epi-anhydrotetracycline Hydrochloride RS](#)

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



[USP Epi-tetracycline Hydrochloride RS](#)

(4R,4aS,5aS,6S,12aS)-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.



[USP Tetracycline Hydrochloride RS](#)

**Auxiliary Information** - Please [check for your question in the FAQs](#) before contacting USP.

Topic/Question	Contact	Expert Committee
TETRACYCLINE HYDROCHLORIDE	<a href="#">Documentary Standards Support</a>	SM12020 Small Molecules 1
REFERENCE STANDARD SUPPORT	RS Technical Services <a href="mailto:RSTECH@usp.org">RSTECH@usp.org</a>	SM12020 Small Molecules 1

**Chromatographic Database Information:** [Chromatographic Database](#)

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