

# 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:

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