Status: Currently Official on 16-Feb-2025
Official Date: Official as of 01-May-2022
Document Type: USP Monographs
DocId: GUID-7274D207-A029-4E9F-BC37-DB9074731716_3_en-US
DOI: https://doi.org/10.31003/USPNF_M81820_03_01
DOI Ref: 8i8kd

Tetracycline Hydrochloride Capsules

DEFINITION

Tetracycline Hydrochloride Capsules contain NLT 90.0% and NMT 125.0% of the labeled amount of tetracycline hydrochloride ($C_{22}H_{24}N_2O_8 \cdot HCl$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum 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 4-Epianhydrotetracycline Hydrochloride RS, USP Epitetracycline Hydrochloride RS, and USP Tetracycline Hydrochloride RS in *Solution A*

Standard solution: 100 µg/mL of USP Tetracycline Hydrochloride RS in *Solution A*. [Note—If needed, dilute the *Standard solution* concentration for *Identification B*.]

Sample solution: Nominally equivalent to 100 µg/mL of tetracycline hydrochloride in *Solution A* from NLT 20 Capsules prepared as follows. Transfer a portion of Capsule contents, equivalent to 25 mg of tetracycline hydrochloride, to a 250-mL volumetric flask. Dissolve with the aid of sonication, and dilute with *Solution A* to volume. Pass through a syringe filter of 0.22-µm pore size and discard the first 2 mL of the filtrate. [Note—If needed, dilute the *Sample solution* concentration for *Identification B*.]

Chromatographic system

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

Mode: LC

Detector: UV 280 nm. For *Identification B*, use a diode array detector in the range of 220–400 nm.

Column: 4.6-mm × 15-cm; 3-µm packing L1

Temperatures

Autosampler: 4°

Column: 50°

Flow rate: 1.0 mL/min

Injection volume: 10 µL

System suitability

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

[NOTE—The relative retention times for epitetracycline, tetracycline, 4-epianhydrotetracycline, and anhydrotetracycline are 0.9, 1.0, 1.6, and 1.8, respectively.]

Suitability requirements

Tailing factor: NMT 1.5, *Standard solution*

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

Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of tetracycline hydrochloride ($C_{22}H_{24}N_2O_8 \cdot HCl$) in the portion of Capsules taken:

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

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 = nominal concentration of tetracycline hydrochloride in the *Sample solution* (µg/mL)

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

F = correction factor, 0.001 mg/µg

Acceptance criteria: 90.0%–125.0%

PERFORMANCE TESTS

Change to read:

• **DISSOLUTION** ⟨711⟩

Test 1

Medium: Water; 900 mL

Apparatus 2: 75 rpm. Maintain a distance of 45 ± 5 mm between the blade and the inside bottom of the vessel.

Times

For Capsules labeled to contain 250 mg: 60 min

For Capsules labeled to contain 500 mg: 90 min

Standard solution: A known concentration of USP Tetracycline Hydrochloride RS in *Medium*

Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute with *Medium*, if necessary.

Instrumental conditions

▲(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)▲ (IRA 1-May-2022)

Mode: UV

Analytical wavelength: 276 nm

▲**Analysis:**

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of tetracycline hydrochloride ($C_{22}H_{24}N_2O_8 \cdot HCl$) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times D \times 100$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

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

L = label claim (mg/Capsule)

V = volume of *Medium*, 900 mL

D = dilution factor for the *Sample solution*, if applicable▲ (IRA 1-May-2022)

Tolerances: NLT 80% (*Q*) of the labeled amount of tetracycline hydrochloride ($C_{22}H_{24}N_2O_8 \cdot HCl$) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.

Medium: Water; 900 mL, deaerated

Apparatus 2: 75 rpm. Maintain a distance of 45 ± 5 mm between the blade and the inside bottom of the vessel.

Times

For Capsules labeled to contain 250 mg: 30 and 60 min

For Capsules labeled to contain 500 mg: 30, 60, and 90 min

Standard solution: A known concentration of USP Tetracycline Hydrochloride RS in *Medium*

Sample solution: At the times specified, withdraw a known volume of the solution under test and pass it through a suitable filter. Dilute with *Medium*, if necessary. Replace the volume withdrawn with the same amount of *Medium*, preheated at 37.0 ± 0.5°.

Blank: *Medium*

Instrumental conditions

▲(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)▲ (IRA 1-May-2022)

Mode: UV

Analytical wavelength: 276 nm

▲**Analysis**

Samples: *Standard solution* and *Sample solution*

Calculate the concentration (C_i) of tetracycline hydrochloride ($C_{22}H_{24}N_2O_8 \cdot HCl$) in the sample withdrawn at each time point (*i*):

$$\text{Result}_i = (A_U/A_S) \times C_S \times D$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

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

D = dilution factor for the *Sample solution*, if applicable

Calculate the percentage of the labeled amount of tetracycline hydrochloride ($C_{22}H_{24}N_2O_8 \cdot HCl$) dissolved at each time point (*i*):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = [(C_2 \times V) + (C_1 \times V_S)] \times (1/L) \times 100$$

$$\text{Result}_3 = \{(C_3 \times V) + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of tetracycline hydrochloride in the portion of sample withdrawn at the specified time point (mg/mL)

V = volume of *Medium*, 900 mL

L = label claim (mg/Capsule)

V_S = volume of the *Sample solution* withdrawn at each time point and replaced with *Medium* (mL)▲ (IRA 1-May-2022)

Tolerances: See *Table 2* and *Table 3*.

Table 2

For Capsules Labeled to Contain 250 mg		
▲**Time Point (*i*)**▲ (IRA 1-May-2022)	**Time (min)**	**Amount Dissolved [% (*Q*)]**
▲1▲ (IRA 1-May-2022)	30	NLT 60
▲2▲ (IRA 1-May-2022)	60	NLT 85

Table 3

For Capsules Labeled to Contain 500 mg		
▲Time Point (*i*)▲ (IRA 1-May-2022)	Time (min)	Amount Dissolved [% (*Q*)]
▲1▲ (IRA 1-May-2022)	30	NLT 50
▲2▲ (IRA 1-May-2022)	60	NLT 70
▲3▲ (IRA 1-May-2022)	90	NLT 85

• **Uniformity of Dosage Units ⟨905⟩:** Meet the requirements

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 4-Epianhydrotetracycline Hydrochloride RS, USP Epitetracycline 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: 3 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

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 2.0%

Analysis

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

Calculate the percentage of anhydrotetracycline hydrochloride and epitetracycline hydrochloride in the portion of Capsules 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 = nominal concentration of tetracycline hydrochloride in the *Sample solution* (µg/mL)

Calculate the percentage of 4-epianhydrotetracycline hydrochloride in the portion of Capsules 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 = nominal concentration of tetracycline hydrochloride in the *Sample solution* (µg/mL)▲ (IRA 1-May-2022)

Calculate the percentage of each unspecified impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \text{ ▲▲ (IRA 1-May-2022)} \times 100$$

r_U = peak response of each 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 = nominal concentration of tetracycline hydrochloride in the *Sample solution* (µg/mL)

▲▲ (IRA 1-May-2022)

Acceptance criteria: See *Table 4*. ▲The reporting threshold is▲ (IRA 1-May-2022) 0.05%.

Table 4

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Epitetracycline▲▲ (IRA 1-May-2022)	0.9	3.0
Tetracycline	1.0	—
2-Acetyl analog[a,b]	1.3	—
4-Epianhydrotetracycline▲▲ (IRA 1-May-2022)	1.7	3.0
Anhydrotetracycline▲▲ (IRA 1-May-2022)	1.8	0.5
Any individual unspecified impurity	—	0.1

[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.

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

ADDITIONAL REQUIREMENTS

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

• **Labeling:** When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.

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

Topic/Question	Contact	Expert Committee
TETRACYCLINE HYDROCHLORIDE CAPSULES	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-7274D207-A029-4E9F-BC37-DB9074731716_3_en-US

DOI: https://doi.org/10.31003/USPNF_M81820_03_01

DOI ref: 8i8kd