World Health Organization

# TETRACYCLINE HYDROCHLORIDE
## *(TETRACLCLINI HYDROCHLORIDUM)*

## Draft revision for *The International Pharmacopoeia*

## (February 2019)

### *DRAFT FOR COMMENT*

Please send any comments you may have on this draft to **Dr Herbert Schmidt** (schmidth@who.int), Medicines Quality Assurance Programme, Technologies Standards and Norms, Department of Essential Medicines and Health Products, World Health Organization, 1211 Geneva 27, Switzerland, by **15 April 2019**.

In order to speed up the process for receiving draft monographs and for sending comments, please send your email address to jonessi@who.int and we will add it to our electronic mailing list. Please specify if you wish to receive monographs.

SCHEDULE FOR THE ADOPTION PROCESS OF DOCUMENT QAS/17.740:
Draft revision for *The International Pharmacopoeia*

TETRACYCLINE HYDROCHLORIDE
(TETRACYCLINI HYDROCHLORIDUM)

| **Description** | **Date** |
|---|---|
| First draft received from collaborating laboratory | September 2017 |
| Presentation to WHO Expert Committee on Specifications for Pharmaceutical Preparations | October 2017 |
| Presentation at the consultation on screening technology, sampling and specifications for medicines | 2-4 May 2018 |
| Presentation to WHO Expert Committee on Specifications for Pharmaceutical Preparations | October 2018 |
| Draft revision sent out for public consultation | February – April 2019 |
| Discussion at the informal consultation on screening technologies, laboratory tools and pharmacopoeial specifications for medicines | 02 – 03 May 2019 |
| Further follow-up action as required | |

*[**Note from the Secretariat.** It is proposed to revise the monograph on Tetracycline hydrochloride as follows:*

- *to use an LC method to the test for related substances (instead of the described TLC method),*
- *to use an LC method for assay (instead of the described microbiological method),*
- *to update the style of the monograph.*

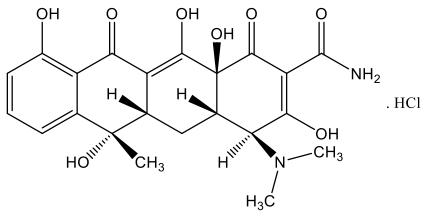
*Changes from the current monograph are indicated in the text by <u>insert</u> or ~~delete~~.]*

# TETRACYCLINE HYDROCHLORIDE
# (TETRACYCLINI HYDROCHLORIDUM)

**Molecular formula.** $C_{22}H_{24}N_2O_8,HCl$

**Relative molecular mass.** 480.9

**Graphic formula.**




**Chemical name**. (4*S*,4a*S*,5a*S*,6*S*,12α*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; [4S-(4α,4aα,5aα,6β,12aα)]-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 Reg. No. 64-75-5.

**Description.** A yellow, crystalline powder.

**Solubility.** Soluble in water R, slightly soluble in ethanol (~750 g/l) TS; practically insoluble in acetone R. It dissolves in solutions of alkali hydroxides and carbonates. Solutions in water R become turbid on standing, owing to the precipitation of tetracycline.

**Category.** Antibiotic.

**Storage**. Tetracycline hydrochloride should be kept in a tightly closed container, protected from light.

**Additional information.** Tetracycline hydrochloride decomposes rapidly in solutions below pH 2, and less rapidly in solutions above pH 7. Even in the absence of light, Tetracycline hydrochloride is gradually degraded on exposure to a humid atmosphere, the

decomposition being faster at higher temperatures. Tetracycline hydrochloride is a semi-synthetic product derived from a fermentation product.

## Requirements

**Definition.** Tetracycline hydrochloride contains not less than 95.0% and not more than 102.0% of $C_{22}H_{24}N_2O_8$,HCl, calculated with reference to the dried substance.

### Identity tests

- Either tests A, E or tests B, D and E or tests C, D and E may be applied.

A. Carry out the examination as described under *1.7 Spectrophotometry in the infrared region*. The infrared absorption spectrum is concordant with the spectrum obtained from tetracycline hydrochloride RS or with the reference spectrum of tetracycline hydrochloride RS.

B. Carry out the test as described under *1.14.1 Thin-layer chromatography* using silica gel R6 as the coating substance and a mixture of acetonitrile R, methanol R and a 63 g/L solution of oxalic acid R previously adjusted to pH 2 with ammonia (~260 g/L) TS (20:20:60 V/V/V) as the mobile phase. Apply separately to the plate 1 µL of each of the following 3 solutions in methanol R containing (A) 0.5 mg of the test substance per mL, (B) 0.5 mg of tetracycline hydrochloride RS per mL and (C) 0.5 mg of tetracycline hydrochloride RS, 0.5 mg of demeclocycline hydrochloride R and 0.5 mg oxytetracycline hydrochloride R per mL. Develop the plate for a distance of 15 cm. After removing the plate from the chromatographic chamber allow it to dry in air or in a current of air. Examine the chromatogram under ultraviolet light (254 nm). The test is not valid unless the chromatogram obtained with solution (C) shows three clearly separated spots. The principal spot in the chromatogram obtained with solution (A) corresponds in position, appearance and intensity with the spot due to tetracycline in the chromatogram obtained with solution (B).

C. Carry out the test as described under *1.14.4 High-performance liquid chromatography* using the conditions given under "Assay". The retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to the retention time of the tetracycline peak in the chromatogram obtained with solution (2).

D. To about 1 mg of the test substance add 2 mL of sulfuric acid (~1760 g/l) TS; a red-violet colour is produced which on the addition of 0.1 mL of water R changes to yellow.

E: A 0.05 g/mL solution yields reaction B described under *2.1 General identification* tests as characteristic of chlorides.

**Specific optical rotation (*1.4*).** Use a 10 mg/mL solution in hydrochloric acid (0.01 mol/L) VS; $[\alpha]_D^{20}$ = -240 to -255 with reference to the dried substance.

**Loss on drying.** Dry at 60 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury) over phosphorus pentoxide R for 3 hours; it loses not more than 20 mg/g.

**pH value (*1.13*).** pH of a 10 mg/mL solution, 1.8-2.8.

**Sulfated ash (*2.3*)**. Not more than 5.0 mg/g.

**Related substances.** Carry out the test as described under *1.14.4 High-performance liquid chromatography,* using the conditions given below under "Assay".

Use solution (1) as described under "Assay". Prepare the following additional solutions using mobile phase A as diluent. For solution (3) dissolve 12.5 mg of anhydrotetracycline hydrochloride RS and dilute to 50.0 mL. For solution (4) dissolve 12.5 mg of 4-epitetracycline hydrochloride RS and dilute to 50.0 ml. For solution (5) dissolve 12.5 mg of 4-epianhydrotetracycline hydrochloride RS and dilute to 50.0 ml. For solution (6) transfer 10.0 mL of solution (1) and 5.0 mL each of solution (3), (4) and (5) to a 50 mL volumetric flask, mix and dilute to volume. For solution (7) dilute 1 volume of solution (3)

to 500 volumes. For solution (8) dilute 1 volume of solution (4) to 100 volumes. For solution (9) dilute 1 volume of solution (5) to 500 volumes.

Inject alternately 10 µL each of solution (1), (6), (7), (8) and (9).

The following peaks are eluted at the following relative retention with reference to tetracycline (retention time about 5 minutes): impurity A (4-epitetracycline) about 0.9; impurity B (2-acetyl-2-decarbamolyltetracycline) about 1.1; impurity D (4-epianhydrotetracycline) about 1.5; and impurity C (anhydrotetracycline) about 1.7.

The assay is not valid unless in the chromatogram obtained with solution (6) the resolution between 4-epitetracycline and tetracycline is at least 2.5 and the resolution between 4-epianhydrotetracycline and anhydrotetracycline is at least 2.5.

In the chromatogram obtained with solution (1):

- the area of any peak corresponding to impurity A is not greater than 1.5 times the area of the peak due to impurity A (4-epitetracycline) in the chromatogram obtained with solution (8) (3.0%);
- the area of any peak corresponding to impurity B (2-acetyl-2-decarbamolyltetracycline) is not greater than 0.75 times the area of the peak due to impurity A (4-epitetracycline) in the chromatogram obtained with solution (8) (1.5%).
- the area of any peak corresponding to impurity C is not greater than 1.25 times the area of the peak due to impurity C (anhydrotetracycline) in the chromatogram obtained with solution (7) (0.5%);
- the area of any peak corresponding to impurity D is not greater than 1.25 times the area of the peak due to impurity D (4-epianhydrotetracycline) in the chromatogram obtained with solution (9) (0.5%).

**Assay.** Carry out the test as described under *1.14.4 High-performance liquid chromatography*, using a stainless steel column (15 cm x 4.6 mm) packed with particles of

base-deactivated silica gel, the surface of which has been modified with chemically-bonded octadecylsilyl groups (3 µm)[1].

Use the following conditions for gradient elution:

Mobile phase A: 1 volume of phosphoric acid (~1440g/L) TS in 1000 volumes of water R;

Mobile phase B: acetonitrile R.

| **Time (min)** | **Mobile phase A (% v/v)** | **Mobile phase B (% v/v)** | **Comment** |
|---|---|---|---|
| 0-7.5 | 85 to 60 | 15 to 40 | Linear gradient |
| 7.5-7.6 | 60 to 85 | 40 to 15 | Return to initial conditions |
| 7.6-10 | 85 | 15 | re-equilibration |

Operate with a flow rate of 1.0 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 280 nm. Maintain the column temperature at 50 ºC and the autosampler temperature at 10 °C.

Prepare the following solutions using mobile phase A as diluent. For solution (1) dissolve 25.0 mg of the test substance and dilute to 200.0 mL. For solution (2) dissolve 25.0 mg of tetracycline hydrochloride RS and dilute to 200.0 mL.

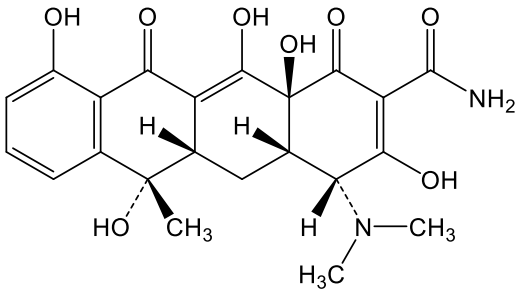
Inject alternately 10 µL each of solutions (1) and (2).

Measure the areas of the peak responses obtained in the chromatograms from solutions (1) and (2), and calculate the percentage content of $C_{22}H_{24}N_2O_8$,HCl using the declared content of $C_{22}H_{24}N_2O_8$,HCl in tetracycline hydrochloride RS.

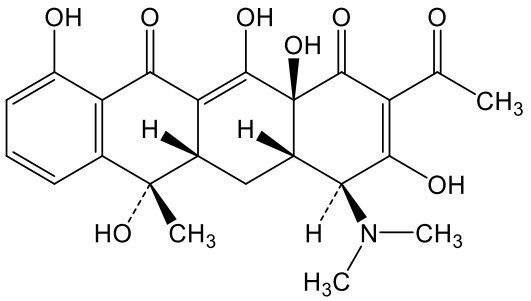
[1] A Prodigy ODS-3 column has been found suitable.

**Bacterial endotoxins.** If intended for use in the manufacture of a parenteral dosage form without a further appropriate procedure for the removal of bacterial endotoxins, carry out the test as described under *3.4 Test for bacterial endotoxins*; contains not more than 0.5 IU of endotoxin RS per mg of tetracycline hydrochloride.

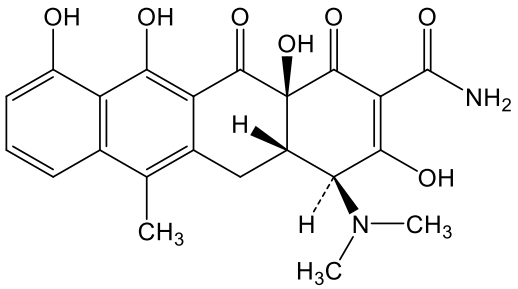
**Impurities**



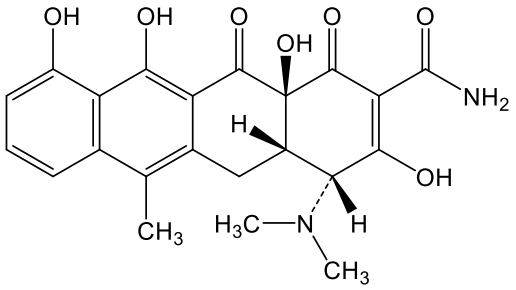

A. (4*R*,4a*S*,5a*S*,6*S*,12a*S*)-4-(dimethylamino)-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (4-epitetracycline)



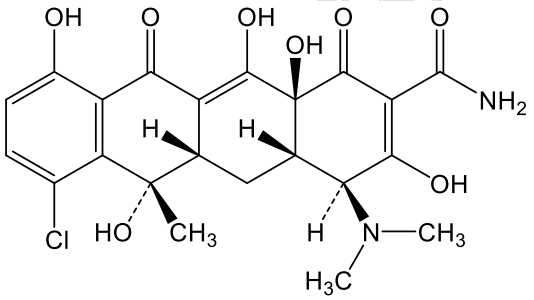

B. (4*S*,4a*S*,5a*S*, *6S*, *12aS*)-2-acetyl-4-(dimethylamino)-3,6, 0,12,12a-pentahydroxy-6-methyl-4a,5a,6,12a-tetrahydrotetracene-1,11(4*H*,5*H*)-dione (2-acetyl-2-decarbamolyltetracycline).



C. (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 (anhydrotetracycline)



D. (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 (4-epianhydrotetracycline).



E. (4*S*,4a*S*,5a*S, 6S, 12aR*)-7-Chloro-4-(dimethylamino)-1,6,10,11,12a-pentahydroxy-6-methyl-3,12-dioxo-4,4a,5,5a-tetrahydrotetracene-2-carboxamide (chlortetracycline).

***[Already established reference substances***

*Tetracycline hydrochloride ICRS*

*Anhydrotetracycline hydrochloride ICRS*

*4-Epitetracycline hydrochloride ICRS*

*4-Epianhydrotetracycline hydrochloride ICRS*

***Reagents to be established***

***Demeclocycline hydrochloride R***

*Demeclocycline hydrochloride of a suitable quality should be used.*

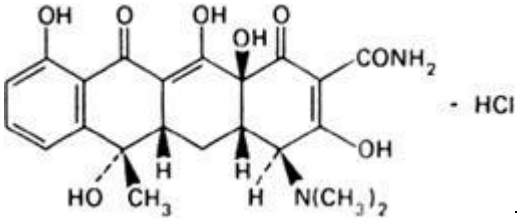
***Oxytetracycline hydrochloride R***

*Oxytetracycline hydrochloride of a suitable quality should be used.]*

**~~Molecular formula.~~** ~~$C_{22}H_{24}N_2O_8,HCl$~~

**~~Relative molecular mass.~~** ~~480.9~~

**~~Graphic formula.~~**




**~~Chemical name.~~** ~~(4*S*,4a*S*,5a*S*,6*S*,12*αS*)-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; [4*S*-(4*α*,4a*α*,5a*α*,6*β*, 12a*α*)]-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 Reg. No. 64-75-5.

**Description.** A yellow, crystalline powder; odourless.

**Solubility.** Soluble in 10 parts of water and in 100 parts of ethanol (~750 g/l) TS; practically insoluble in acetone R and ether R.

**Category.** Antibiotic.

**Storage.** Tetracycline hydrochloride should be kept in a tightly closed container, protected from light.

**Labelling.** The designation sterile Tetracycline hydrochloride indicates that the substance complies with the additional requirements for sterile Tetracycline hydrochloride and may be used for parenteral administration or for other sterile preparations.

**Additional information.** Tetracycline hydrochloride decomposes rapidly in solutions below pH 2, and less rapidly in solutions above pH 7. Even in the absence of light, Tetracycline hydrochloride is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

**Requirements**

**Definition.** Tetracycline hydrochloride contains when tested according to assay A not less than 96.0% and not more than 102.0% of $C_{22}H_{24}N_2O_8$,HCl, and when tested according to assay B not less than 950 International Units per mg, both calculated with reference to the dried substance.

**Identity tests**

A. Carry out the test as described under 1.14.1 Thin-layer chromatography, but using an unlined chamber and a cellulose coating prepared as follows: To 0.275 g of carbomer R add 120 mL of water, let the mixture stand for 1 hour while shaking it from time to time;

~~then add gradually while stirring a sufficient volume of sodium hydroxide (~80 g/l) TS to adjust to pH 7.0. To this mixture add 30 g of cellulose R1 and a sufficient quantity of water (usually 60-80 mL) to obtain a coating substance of suitable consistency. Coat the plates with a layer 0.4 mm thick, and allow them to dry at room temperature. The plates thus coated are used after a suitable treatment both for the identity test and the test of "related substances". For the identity test spray the plate with phosphate/citrate buffer pH 4.5, TS, until traces of moisture appear. Dry the plate at 50°C for 30 minutes.~~

~~Prepare the following solutions immediately before use while protected from bright light: Dissolve 5.0 mg of the test substance, 5.0 mg of chlortetracycline hydrochloride RS, 5.0 mg of oxytetracycline hydrochloride RS, and 5.0 mg of tetracycline hydrochloride RS in sufficient methanol R to produce 10 mL; this constitutes solution A. Dissolve 5.0 mg of chlortetracycline hydrochloride RS and 5.0 mg of oxytetracycline hydrochloride RS in sufficient methanol R to produce 10 mL; this constitutes solution B. Dissolve 5.0 mg of chlortetracycline hydrochloride RS, 5.0 mg of oxytetracycline hydrochloride RS, and 5.0 mg of tetracycline hydrochloride RS in sufficient methanol R to produce 10 mL; this constitutes solution C.~~

~~Apply separately to the plate 1 $\mu$l of each of solutions A, B and C, and spray it very finely and uniformly with trimethylpyridine (50 g/l) TS until traces of humidity appear (about 8 mL).~~

~~Pour the mobile phase consisting of a mixture of 60 volumes of ethyl acetate R, 30 volumes of acetone R, and 6 volumes of water in the unlined chromatographic chamber. Place the plate in the chamber in such a manner that it is not in contact with the mobile phase. Allow the plate to become impregnated with the vapours for 1 hour. Then dip the plate into the mobile phase and allow the chromatogram to develop to a distance of 15 cm. After removing the plate from the chromatographic chamber, allow it to dry in air, expose it to the vapour of ammonia (~260 g/l) TS, and examine the chromatogram immediately in ultraviolet light (365 nm). Three principal clearly separated spots are obtained with solution A corresponding in position, appearance, and intensity with those~~

obtained with solution C, two of which correspond with the spots obtained with solution B.

B. To about 1 mg add 2 mL of sulfuric acid (~1760 g/l) TS; a red-violet colour is produced which on the addition of 0.1 mL of water changes to yellow.

C. A 0.05 g/mL solution yields reaction B described under 2.1 General identification tests as characteristic of chlorides.

**Specific optical rotation.** Use a 10 mg/mL solution in hydrochloric acid (0.01 mol/l) VS and calculate with reference to the dried substance; $[\alpha]_D^{20\,°C}$ = -239° to -258°.

**Loss on drying.** Dry at 60°C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury) for 3 hours; it loses not more than 20 mg/g.

**pH value.** pH of a 10 mg/mL solution, 1.8-2.8.

**Related substances.** Carry out the test as described under 1.14.1 Thin-layer chromatography, using a plate as prepared under the identity test A. To a sufficient volume of disodium edetate (0.1 mol/l) VS add sodium hydroxide (~80 g/l) TS to adjust to pH 7.0, and use this solution to spray the plate uniformly until traces of moisture appear. Dry the plate at 50°C for 30 minutes.

Prepare the following solutions immediately before use, protecting them from bright light: Dissolve 0.10 g of the test substance in sufficient methanol R to produce 10 mL; this constitutes solution A. Dilute 2.5 mL of solution A to 10.0 mL with methanol R; this constitutes solution B. Dissolve 5.0 mg of 4-epianhydrotetracycline hydrochloride RS insufficient methanol R to produce 20 mL; this constitutes solution K. Dilute 2 mL of solution K to 10 mL with methanol R; this constitutes solution C. Dissolve 5.0 mg of 4-epitetracycline hydrochloride RS in sufficient methanol R to produce 8 mL; this constitutes solution L. Dilute 2 mL of solution L to 10 mL with methanol R; this constitutes solution D. Dissolve 5.0 mg of anhydrotetracycline hydrochloride RS in sufficient methanol R to produce 20 mL; this constitutes solution M. Dilute 2 mL of

~~solution M to 10 mL with methanol R; this constitutes solution E. Dissolve 20 mg of chlortetracycline hydrochloride RS in sufficient methanol R to produce 20 mL; this constitutes solution N. Dilute 2 mL of solution N to 10 mL with methanol R; this constitutes solution F. Dissolve 10 mg of tetracycline hydrochloride RS in sufficient methanol R to produce 20 mL; this constitutes solution P. Mix together 0.5 mL of each of the following solutions K, L, M, N and P; this constitutes solution G.~~

~~Apply separately to the plate 1 $\mu$l of each of solutions A, B, C, D, E, F, and G, and spray it very finely and uniformly with trimethylpyridine (50 g/l) TS until traces of humidity appear (about 8 mL).~~

~~As the mobile phase, use a mixture of 60 volumes of ethyl acetate R, 30 volumes of acetone R, and 6 volumes of water. Allow the chromatogram to develop to a distance of 15 cm. After removing the plate from the chromatographic chamber, allow it to dry in air, expose it to the vapour of ammonia (~260 g/l) TS, and examine the chromatogram immediately in ultraviolet light (365 nm). The spot corresponding to 4-epitetracycline hydrochloride obtained with solution B is not more intense than that obtained with solution D (5% of 4-epitetracycline hydrochloride). The spots corresponding to 4-epianhydrotetracycline hydrochloride, anhydrotetracycline hydrochloride, and chlortetracycline hydrochloride obtained with solution A are not more intense than those obtained with solution C (0.5% of 4-epianhydrotetracycline hydrochloride), solution E (0.5% of anhydrotetracycline hydrochloride), and solution F (2% of chlortetracycline hydrochloride). The test is not valid unless the chromatogram obtained with solution G shows 5 clearly separated spots.~~

~~**Anhydroderivatives.** Dissolve about 0.2 g, accurately weighed, in sufficient hydrochloric acid (0.02 mol/l) VS to produce 50 mL. Place 10.0 mL in a separator, add 10 mL of chloroform R and 10 mL of citrate buffer, pH 5.4 TS, and shake for 2 minutes. Separate the chloroform layer and measure the absorbance at 437 nm against a solvent cell containing chloroform R; not more than 0.18 (preferably use 2-cm cells for the measurement and calculate the absorbance of a 1-cm layer).~~

**Assay**

A. Dissolve about 0.25 g, accurately weighed and previously dried at 60°C under reduced pressure, in 5 mL of formic acid (~1080 g/l) TS and 10 mL of glacial acetic acid R1, add 10 mL of dioxan R, 5 mL of mercuric acetate/acetic acid TS, and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration. Method A. Each mL of perchloric acid (0.1 mol/l) VS is equivalent to 48.09 mg of $C_{22}H_{24}N_2O_8$,HCl.

B. Carry out the assay as described under 3.1 Microbiological assay of antibiotics, using either (*a*) *Bacillus pumilus* (NCTC 8241 or ATCC 14884) as the test organism, culture medium Cm1 with a final pH of 6.5–6.6, sterile phosphate buffer, pH 4.5 TS, an appropriate concentration of tetracycline (usually between 2 and 20 IU), and an incubation temperature of 37–39°C, or (*b*) *Bacillus cereus* (ATCC 11778) as the test organism, culture medium Cm1 with a final pH of 5.9–6.0, sterile phosphate buffer, pH 4.5 TS, an appropriate concentration of tetracycline (usually between 0.5 and 2 IU), and an incubation temperature of 30–33°C. The precision of the assay is such that the fiducial limits of error of the estimated potency ($P = 0.95$) are not less than 95% and not more than 105% of the estimated potency. The upper fiducial limit of error of the estimated potency ($P = 0.95$) is not less than 950 IU per mg, calculated with reference to the dried substance.

**Additional Requirements for Tetracycline Hydrochloride for sterile use**

**Bacterial endotoxins.** Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 0.5 IU of endotoxin RS per mg.

**Sterility.** Complies with 3.2 Test for sterility.

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