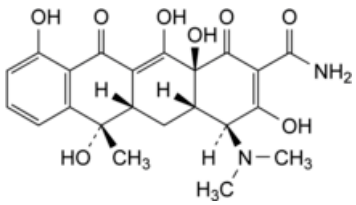
Edition: BP 2025 (Ph. Eur. 11.6 update)

Tetracycline

General Notices

(Ph. Eur. monograph 0211)



$C_{22}H_{24}N_2O_8$ 444.4 *60-54-8*

Action and use

Tetracycline antibacterial.

Ph Eur

DEFINITION

(4*S*,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.

Substance produced by certain strains of *Streptomyces aerofaciens* or obtained by any other means.

Content

88.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

Yellow, crystalline powder.

Solubility

Very slightly soluble in water, soluble in ethanol (96 per cent) and in methanol, sparingly soluble in acetone. It dissolves in dilute acid and alkaline solutions.

IDENTIFICATION

A. Thin-layer chromatography (2.2.27).

Test solution Dissolve 5 mg of the substance to be examined in *methanol R* and dilute to 10 mL with the same solvent.

Reference solution (a) Dissolve 5 mg of *tetracycline hydrochloride CRS* in *methanol R* and dilute to 10 mL with the same solvent.

Reference solution (b) Dissolve 5 mg of *tetracycline hydrochloride CRS*, 5 mg of *demeclocycline hydrochloride R* and 5 mg of *oxytetracycline hydrochloride R* in *methanol R* and dilute to 10 mL with the same solvent.

Plate *TLC octadecylsilyl silica gel F_{254} plate R*.

Mobile phase Mix 20 volumes of *acetonitrile R*, 20 volumes of *methanol R* and 60 volumes of a 63 g/L solution of *oxalic acid R* previously adjusted to pH 2 with *concentrated ammonia R*.

Application 1 µL.

Development Over 3/4 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

System suitability The chromatogram obtained with reference solution (b) shows 3 clearly separated spots.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

B. To about 2 mg add 5 mL of *sulfuric acid R*. A violet-red colour develops. Add the solution to 2.5 mL of *water R*. The colour becomes yellow.
C. Dissolve about 10 mg in a mixture of 1 mL of *dilute nitric acid R* and 5 mL of *water R*. Shake and add 1 mL of *silver nitrate solution R2*. Any opalescence in the solution is not more intense than that in a mixture of 1 mL of *dilute nitric acid R*, 5 mL of *water R* and 1 mL of *silver nitrate solution R2*.

TESTS

pH (2.2.3)

3.5 to 6.0.

Suspend 0.1 g in 10 mL of *carbon dioxide-free water R*.

Specific optical rotation (2.2.7)

-260 to -280 (dried substance).

Dissolve 0.250 g in *0.1 M hydrochloric acid* and dilute to 50.0 mL with the same acid.

Related substances

Liquid chromatography (2.2.29). *Prepare the solutions immediately before use.*

Test solution Dissolve 25.0 mg of the substance to be examined in *0.01 M hydrochloric acid* and dilute to 25.0 mL with the same acid.

Reference solution (a) Dissolve 25.0 mg of *tetracycline hydrochloride CRS* in *0.01 M hydrochloric acid* and dilute to 25.0 mL with the same acid.

Reference solution (b) Dissolve 12.5 mg of *4-epitetracycline hydrochloride CRS* in *0.01 M hydrochloric acid* and dilute to 50.0 mL with the same acid.

Reference solution (c) Dissolve 10.0 mg of *anhydrotetracycline hydrochloride CRS* in *0.01 M hydrochloric acid* and dilute to 100.0 mL with the same acid.

Reference solution (d) Dissolve 10.0 mg of *4-epianhydrotetracycline hydrochloride CRS* in *0.01 M hydrochloric acid* and dilute to 50.0 mL with the same acid.

Reference solution (e) Mix 1.0 mL of reference solution (a), 2.0 mL of reference solution (b) and 5.0 mL of reference solution (d) and dilute to 25.0 mL with *0.01 M hydrochloric acid*.

Reference solution (f) Mix 40.0 mL of reference solution (b), 20.0 mL of reference solution (c) and 5.0 mL of reference solution (d) and dilute to 200.0 mL with *0.01 M hydrochloric acid*.

Reference solution (g) Dilute 1.0 mL of reference solution (c) to 50.0 mL with *0.01 M hydrochloric acid*.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *styrene-divinylbenzene copolymer R* (8 µm);

— *temperature*: 60 °C.

Mobile phase Weigh 80.0 g of *2-methyl-2-propanol R* and transfer to a 1000 mL volumetric flask with the aid of 200 mL of *water R*; add 100 mL of a 35 g/L solution of *dipotassium hydrogen phosphate R* adjusted to pH 9.0 with *dilute phosphoric acid R*, 200 mL of a 10 g/L solution of *tetrabutylammonium hydrogen sulfate R* adjusted to pH 9.0 with *dilute sodium hydroxide solution R* and 10 mL of a 40 g/L solution of *sodium edetate R* adjusted to pH 9.0 with *dilute sodium hydroxide solution R*; dilute to 1000.0 mL with *water R*.

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 254 nm.

Injection 20 µL; inject the test solution and reference solutions (e), (f) and (g).

System suitability:

— *resolution*: minimum 2.5 between the peaks due to impurity A (1st peak) and tetracycline (2nd peak) and minimum 8.0 between the peaks due to tetracycline and impurity D (3rd peak) in the chromatogram obtained with reference solution (e); if necessary, adjust the concentration of 2-methyl-2-propanol in the mobile phase;

— *signal-to-noise ratio*: minimum 3 for the principal peak in the chromatogram obtained with reference solution (g);

— *symmetry factor*: maximum 1.25 for the peak due to tetracycline in the chromatogram obtained with reference solution (e).

Limits:

— *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (f) (5.0 per cent);

— *impurity B* (eluting on the tail of the principal peak): not more than 0.4 times the area of the peak due to impurity A in the chromatogram obtained with reference solution (f) (2.0 per cent);

— *impurity C*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (f) (1.0 per cent);

— *impurity D*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (f) (0.5 per cent).

Loss on drying (*2.2.32*)

Maximum 13.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)

Maximum 0.5 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

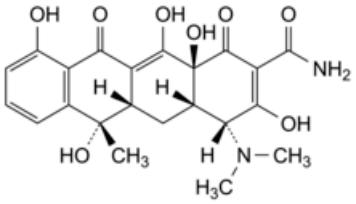
Injection Test solution and reference solution (a).

Calculate the percentage content of $C_{22}H_{24}N_2O_8$.

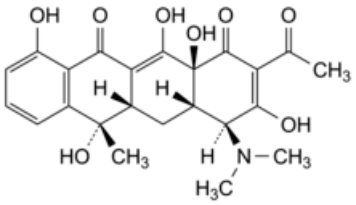
STORAGE

Protected from light.

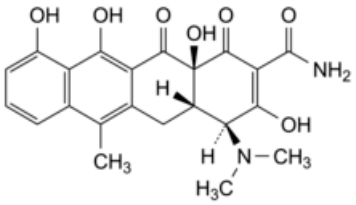
IMPURITIES



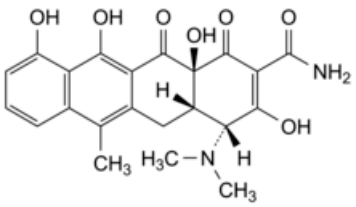
A. (4*R*,4a*S*,5a*S*,6*S*,12a*S*)-4-(dimethylamino)-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (4-epitetracycline),



B. (4*S*,4a*S*,5a*S*,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 (2-acetyl-2-decarbamoyltetracycline),



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).