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 sulphate 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).

Heavy metals (*2.4.8*): maximum 50 ppm.

0.5 g complies with test C. Prepare the reference solution using 2.5 ml of *lead standard solution (10 ppm Pb) R.*

Loss on drying (*2.2.32*): maximum 13.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated 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.

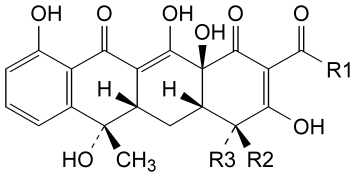
Injection: test solution and reference solution (a).

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

STORAGE

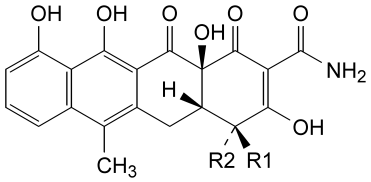
Protected from light.

IMPURITIES



A. R1 = NH_2, R2 = H, R3 = $N(CH_3)_2$: (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. R1 = CH_3, R2 = $N(CH_3)_2$, R3 = H: (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),



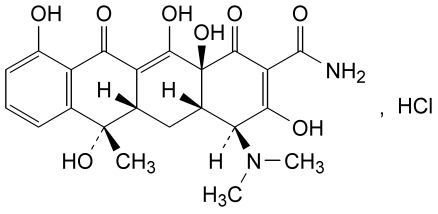
C. R1 = $N(CH_3)_2$, R2 = H: (4*S*,4a*S*,12a*S*)-4-(dimethylamino)-3,10,11,12a-tetrahydroxy-6-methyl-1,12-dioxo-1,4,4a,5,12,12a-hexahydrotetracene-2-carboxamide (anhydrotetracycline),

D. R1 = H, R2 = $N(CH_3)_2$: (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).

01/2008:0210
corrected 6.0

TETRACYCLINE HYDROCHLORIDE

Tetracyclini hydrochloridum



$C_{22}H_{25}ClN_2O_8$ M_r 480.9

[64-75-5]

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 hydrochloride.

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

Content: 95.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance: yellow, crystalline powder.

Solubility: soluble in water, slightly soluble in ethanol (96 per cent), practically insoluble in acetone. It dissolves in solutions of alkali hydroxides and carbonates. Solutions in water become turbid on standing, owing to the precipitation of tetracycline.

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 *sulphuric acid R*. A violet-red colour develops. Add the solution to 2.5 ml of *water R*. The colour becomes yellow.

C. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

pH (*2.2.3*): 1.8 to 2.8.

Dissolve 0.1 g in 10 ml of *carbon dioxide-free water R*.

Specific optical rotation (*2.2.7*): – 240 to – 255 (dried substance).

Dissolve 0.250 g in *0.1 M hydrochloric acid* and dilute to 25.0 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 15.0 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 20.0 ml of reference solution (b), 10.0 ml of reference solution (c) and 5.0 ml of reference solution (d) and dilute to 200.0 ml using *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 sulphate 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) (3.0 per cent);

– *impurity B* (eluting on the tail of the principal peak): not more than 0.5 times the area of the peak due to impurity A in the chromatogram obtained with reference solution (f) (1.5 per cent);

– *impurity C*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (f) (0.5 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).

Heavy metals (*2.4.8*): maximum 50 ppm.

0.5 g complies with test C. Prepare the reference solution using 2.5 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying (*2.2.32*): maximum 2.0 per cent, determined on 1.000 g by drying at 60 °C over *diphosphorus pentoxide R* at a pressure not exceeding 670 Pa for 3 h.

Sulphated ash (*2.4.14*): maximum 0.5 per cent, determined on 1.0 g.

Bacterial endotoxins (*2.6.14*): less than 0.5 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

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

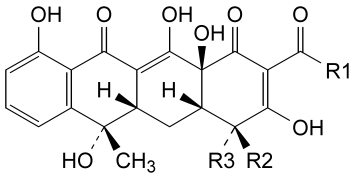
Injection: test solution and reference solution (a).

Calculate the percentage content of $C_{22}H_{25}ClN_2O_8$.

STORAGE

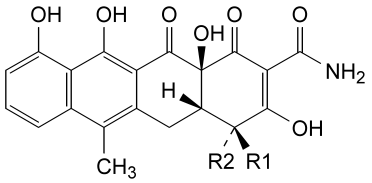
Protected from light. If the substance is sterile, store in a sterile, tamper-proof container.

IMPURITIES



A. R1 = NH_2, R2 = H, R3 = $N(CH_3)_2$: (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. R1 = CH_3, R2 = $N(CH_3)_2$, R3 = H: (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),



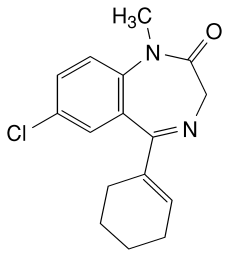
C. R1 = $N(CH_3)_2$, R2 = H: (4*S*,4a*S*,12a*S*)-4-(dimethylamino)-3,10,11,12a-tetrahydroxy-6-methyl-1,12-dioxo-1,4,4a,5,12,12a-hexahydrotetracene-2-carboxamide (anhydrotetracycline),

D. R1 = H, R2 = $N(CH_3)_2$: (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).

01/2008:1738
corrected 6.0

TETRAZEPAM

Tetrazepamum



$C_{16}H_{17}ClN_2O$ M_r 288.8
[10379-14-3]

DEFINITION

7-Chloro-5-(cyclohex-1-enyl)-1-methyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one.

Content: 99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance: light yellow or yellow crystalline powder.

Solubility: practically insoluble in water, freely soluble in methylene chloride, soluble in acetonitrile.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *Ph. Eur. reference spectrum of tetrazepam*.

TESTS

Related substances. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 25.0 mg of the substance to be examined in *acetonitrile R* and dilute to 25.0 ml with the same solvent.

Reference solution (a). Dissolve 5.0 mg of the substance to be examined and 5.0 mg of *tetrazepam impurity C CRS* in *acetonitrile R* and dilute to 10.0 ml with the same solvent. Dilute 1.0 ml of the solution to 10.0 ml with *acetonitrile R*.

Reference solution (b). Dilute 1.0 ml of the test solution to 50.0 ml with *acetonitrile R*. Dilute 1.0 ml of this solution to 10.0 ml with *acetonitrile R*.

Column:

– *size*: *l* = 0.25 m, Ø = 4.6 mm,
– *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

– *mobile phase A*: mix 40 volumes of *acetonitrile R* and 60 volumes of a 3.4 g/l solution of *potassium dihydrogen phosphate R*,
– *mobile phase B*: *acetonitrile R*,

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 35	100	0
35 - 40	100 → 55	0 → 45
40 - 50	55	45
50 - 60	55 → 100	45 → 0

Flow rate: 1.5 ml/min.

Detection: a spectrophotometer at 229 nm.

Injection: 20 µl.