System suitability: reference solution (d):

- *resolution*: minimum 4.0 between the peaks due to impurity A (1st peak) and oxytetracycline (2nd peak) and minimum 5.0 between the peaks and due to oxytetracycline and impurity B (3rd peak); adjust the 2-methyl-2-propanol content in the mobile phase if necessary;
- *symmetry factor*: maximum 1.25 for the peak due to oxytetracycline.

Limits:

- *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (e) (0.5 per cent);
- *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (e) (2.0 per cent);
- *impurity C* (eluting on the tail of the principal peak): not more than 4 times the area of the peak due to impurity A in the chromatogram obtained with reference solution (e) (2.0 per cent);
- *disregard limit*: 0.02 times the area of the peak due to oxytetracycline in the chromatogram obtained with reference solution (d) (0.1 per cent).

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

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

Water (*2.5.12*): 6.0 per cent to 9.0 per cent, determined on 0.250 g.

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.

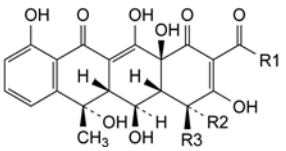
Injection: test solution and reference solution (a).

Calculate the percentage content of $C_{22}H_{24}N_2O_9$.

STORAGE

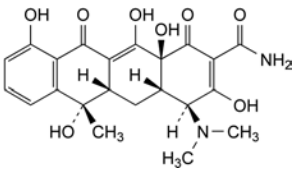
In an airtight container, protected from light.

IMPURITIES



A. R1 = NH_2, R2 = $N(CH_3)_2$, R3 = H: (4*R*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-4-(dimethylamino)-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (4-epioxytetracycline),

C. R1 = CH_3, R2 = H, R3 = $N(CH_3)_2$: (4*S*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-2-acetyl-4-(dimethylamino)-3,5,6,10,12,12a-hexahydroxy-6-methyl-4a,5a,6,12a-tetrahydrotetracene-1,11(4*H*,5*H*)-dione (2-acetyl-2-decarbamoyloxytetracycline),



B. (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 (tetracycline).

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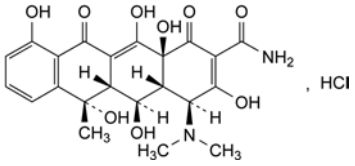
OXYTETRACYCLINE HYDROCHLORIDE

Oxytetracyclini hydrochloridum



$C_{22}H_{25}ClN_2O_9$ M_r 496.9

[2058-46-0]

DEFINITION

(4*S*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-4-(Dimethylamino)-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide hydrochloride.

Substance produced by the growth of certain strains of *Streptomyces rimosus* or obtained by any other means.

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

CHARACTERS

Appearance: yellow, crystalline powder, hygroscopic.

Solubility: freely soluble in water, sparingly soluble in ethanol (96 per cent). Solutions in water become turbid on standing, owing to the precipitation of oxytetracycline.

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 *oxytetracycline hydrochloride CRS* in *methanol R* and dilute to 10 mL with the same solvent.

Reference solution (b). Dissolve 5 mg of *oxytetracycline hydrochloride CRS*, 5 mg of *tetracycline hydrochloride R* and 5 mg of *minocycline 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 deep 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*): 2.3 to 2.9.

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

Specific optical rotation (*2.2.7*): – 188 to – 200 (anhydrous substance).

Dissolve 0.250 g in *0.1 M hydrochloric acid* and dilute to 25.0 mL with the same acid.

Specific absorbance (*2.2.25*): 270 to 290 determined at 353 nm (anhydrous substance).

Dissolve 20.0 mg in *buffer solution pH 2.0 R* and dilute to 100.0 mL with the same buffer solution. Dilute 10.0 mL of the solution to 100.0 mL with *buffer solution pH 2.0 R*.

Light-absorbing impurities. *Carry out the measurements within 1 h of preparing the solutions.*

Dissolve 20.0 mg in a mixture of 1 volume of *1 M hydrochloric acid* and 99 volumes of *methanol R* and dilute to 10.0 mL with the same mixture of solvents. The absorbance (*2.2.25*) determined at 430 nm has a maximum of 0.50 (anhydrous substance).

Dissolve 0.100 g in a mixture of 1 volume of *1 M hydrochloric acid* and 99 volumes of *methanol R* and dilute to 10.0 mL with the same mixture of solvents. The absorbance (*2.2.25*) determined at 490 nm has a maximum of 0.20 (anhydrous substance).

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

Test solution. Dissolve 20.0 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 20.0 mg of *oxytetracycline CRS* in *0.01 M hydrochloric acid* and dilute to 25.0 mL with the same acid.

Reference solution (b). Dissolve 20.0 mg of *4-epioxytetracycline CRS* in *0.01 M hydrochloric acid* and dilute to 25.0 mL with the same acid.

Reference solution (c). Dissolve 20.0 mg of *tetracycline hydrochloride CRS* in *0.01 M hydrochloric acid* and dilute to 25.0 mL with the same acid.

Reference solution (d). Dissolve 8.0 mg of *α-apo-oxytetracycline CRS* in 5 mL of *0.01 M sodium hydroxide* and dilute to 100.0 mL with *0.01 M hydrochloric acid*.

Reference solution (e). Dissolve 8.0 mg of *β-apo-oxytetracycline CRS* in 5 mL of *0.01 M sodium hydroxide* and dilute to 100.0 mL with *0.01 M hydrochloric acid*.

Reference solution (f). Mix 1.5 mL of reference solution (a), 1.0 mL of reference solution (b), 3.0 mL of reference solution (c), 3.0 mL of reference solution (d) and 3.0 mL of reference solution (e) and dilute to 25.0 mL with *0.01 M hydrochloric acid*.

Reference solution (g). Mix 1.0 mL of reference solution (b), 4.0 mL of reference solution (c) and 40.0 mL of reference solution (e) and dilute to 200.0 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 30.0 g (for mobile phase A) and 100.0 g (for mobile phase B) of *2-methyl-2-propanol R* and transfer separately to 1000 mL volumetric flasks with the aid of 200 mL of *water R*; to each flask add 60 mL of *0.33 M phosphate buffer solution pH 7.5 R*, 50 mL of a 10 g/L solution of *tetrabutylammonium hydrogen sulfate R* adjusted to pH 7.5 with *dilute sodium hydroxide solution R* and 10 mL of a 0.4 g/L solution of *sodium edetate R* adjusted to pH 7.5 with *dilute sodium hydroxide solution R*; dilute each solution to 1000 mL with *water R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 15	70	30
15 - 30	30	70
30 - 45	70	30

Flow rate: 1 mL/min.

Detection: spectrophotometer at 254 nm.

Injection: 20 µL of the test solution and reference solutions (f) and (g).

System suitability: reference solution (f):

- *resolution*: minimum 4.0 between the peaks due to impurity A (1st peak) and oxytetracycline (2nd peak), minimum 5.0 between the peaks due to oxytetracycline and impurity B (3rd peak) and minimum 3.5 between the peaks due to impurity D (4th peak) and impurity E (5th peak); if necessary, adapt the ratio mobile phase A: mobile phase B and/or adjust the time programme used to produce the 1-step gradient elution;
- *symmetry factor*: maximum 1.25 for the peak due to oxytetracycline.

Limits:

- *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (g) (0.5 per cent);
- *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (g) (2.0 per cent);
- *impurity C* (eluting on the tail of the main peak): not more than 4 times the area of the peak due to impurity A in the chromatogram obtained with reference solution (g) (2.0 per cent);
- *total of impurities D, E and F* (eluting between the latter two): not more than the area of the peak due to impurity E in the chromatogram obtained with reference solution (g) (2.0 per cent);
- *disregard limit*: 0.02 times the area of the peak due to oxytetracycline in the chromatogram obtained with reference solution (f) (0.1 per cent).

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

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

Water (*2.5.12*): maximum 2.0 per cent, determined on 0.500 g.

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

Bacterial endotoxins (*2.6.14*): less than 0.4 IU/mg, if intended for use in the manufacture of parenteral preparations 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.

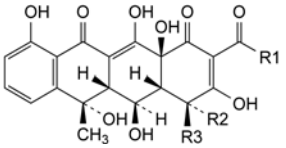
Injection: test solution and reference solution (a).

Calculate the percentage content of $C_{22}H_{25}ClN_2O_9$ taking 1 mg of oxytetracycline as equivalent to 1.079 mg of oxytetracycline hydrochloride.

STORAGE

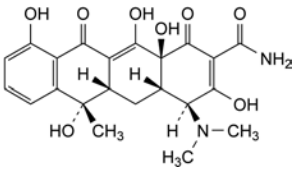
In an airtight container, protected from light. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

IMPURITIES

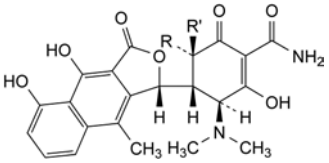


A. R1 = NH_2, R2 = $N(CH_3)_2$, R3 = H: (4*R*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-4-(dimethylamino)-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (4-epioxytetracycline),

C. R1 = CH_3, R2 = H, R3 = $N(CH_3)_2$: (4*S*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-2-acetyl-4-(dimethylamino)-3,5,6,10,12,12a-hexahydroxy-6-methyl-4a,5a,6,12a-tetrahydrotetracene-1,11(4*H*,5*H*)-dione (2-acetyl-2-decarbamoyloxytetracycline),

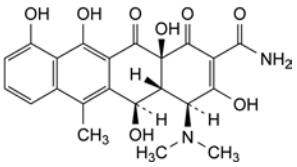


B. (4*S*,4a*S*,5a*S*,6*S*,12a*S*)-4-(dimethylamino)-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (tetracycline),



D. R = OH, R′ = H: (3*S*,4*S*,5*S*)-4-[(1*R*)-4,5-dihydroxy-9-methyl-3-oxo-1,3-dihydronaphtho[2,3-*c*]furan-1-yl]-3-(dimethylamino)-2,5-dihydroxy-6-oxocyclohex-1-enecarboxamide (α-apo-oxytetracycline),

E. R = H, R′ = OH: (3*S*,4*S*,5*R*)-4-[(1*R*)-4,5-dihydroxy-9-methyl-3-oxo-1,3-dihydronaphtho[2,3-*c*]furan-1-yl]-3-(dimethylamino)-2,5-dihydroxy-6-oxocyclohex-1-enecarboxamide (β-apo-oxytetracycline),

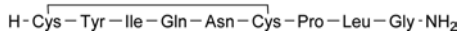


F. (4*S*,4a*R*,5*R*,12a*S*)-4-(dimethylamino)-3,5,10,11,12a-pentahydroxy-6-methyl-1,12-dioxo-1,4,4a,5,12,12a-hexahydrotetracene-2-carboxamide (anhydrooxytetracycline).

01/2008:0780
corrected 6.0

OXYTOCIN

Oxytocinum



$C_{43}H_{66}N_{12}O_{12}S_2$ M_r 1007
[50-56-6]

DEFINITION

Synthetic cyclic nonapeptide having the structure of the hormone produced by the posterior lobe of the pituitary gland that stimulates contraction of the uterus and milk ejection in receptive mammals. It is available in the freeze-dried form as an acetate.

Content: 93.0 per cent to 102.0 per cent (anhydrous and acetic acid-free substance).

By convention, for the purpose of labelling oxytocin preparations, 1 mg of oxytocin peptide ($C_{43}H_{66}N_{12}O_{12}S_2$) is equivalent to 600 IU of biological activity.

L-Cysteinyl-L-tyrosyl-L-isoleucyl-L-glutamyl-L-asparaginyl-L-cysteinyl-L-prolyl-L-leucylglycinamide cyclic (1→6)-disulfide.

CHARACTERS

Appearance: white or almost white, hygroscopic powder.

Solubility: very soluble in water. It dissolves in dilute solutions of acetic acid and of ethanol (96 per cent).

IDENTIFICATION

A. Examine the chromatograms obtained in the assay.

Results: the principal peak in the chromatogram obtained with the test solution is similar in retention time to the principal peak in the chromatogram obtained with the reference solution.

B. Amino acid analysis (*2.2.56*). For hydrolysis use Method 1 and for analysis use Method 1.

Express the content of each amino acid in moles. Calculate the relative proportions of the amino acids, taking 1/6 of the sum of the number of moles of aspartic acid, glutamic acid, proline, glycine, isoleucine and leucine as equal to 1. The values fall within the following limits: aspartic acid: 0.90 to 1.10; glutamic acid: 0.90 to 1.10; proline: 0.90 to 1.10; glycine: 0.90 to 1.10; leucine: 0.90 to 1.10; isoleucine: 0.90 to 1.10; tyrosine: 0.7 to 1.05; half-cystine: 1.4 to 2.1. Not more than traces of other amino acids are present.

TESTS

pH (*2.2.3*): 3.0 to 6.0.

Dissolve 0.200 g in *carbon dioxide-free water R* and dilute to 10.0 mL with the same solvent.

Related substances. Liquid chromatography (*2.2.29*): use the normalisation procedure.

Test solution. Prepare a 0.25 mg/mL solution of the substance to be examined in a 15.6 g/L solution of *sodium dihydrogen phosphate R*.

Resolution solution. Dissolve the contents of a vial of *oxytocin/desmopressin validation mixture CRS* in 1 mL of a 15.6 g/L solution of *sodium dihydrogen phosphate R*.

Column:

– *size*: *l* = 0.125 m, Ø = 4.6 mm;
– *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

– *mobile phase A*: 15.6 g/L solution of *sodium dihydrogen phosphate R*;
– *mobile phase B*: *acetonitrile for chromatography R*, *water R* (50:50 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 30	70 → 40	30 → 60
30 - 30.1	40 → 70	60 → 30
30.1 - 45	70	30

Flow rate: 1 mL/min.

Detection: spectrophotometer at 220 nm.

Injection: 50 µL.

Retention time: oxytocin = about 7.5 min; desmopressin = about 10 min.