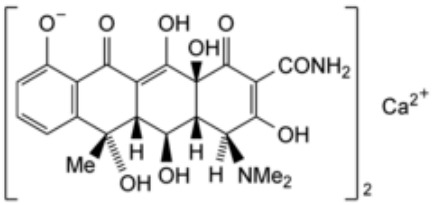
Edition: BP 2025 (Ph. Eur. 11.6 update)

Oxytetracycline Calcium

General Notices



$(C_{22}H_{23}N_2O_9)_2Ca$ 958.9 *15251-48-6*

Action and use

Tetracycline antibacterial.

DEFINITION

Oxytetracycline Calcium is the calcium salt of (4*S*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-4-dimethylamino-1,4,4a,5,5a,6,11,12a-octahydro-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxonaphthacene-2-carboxamide, a substance produced by the growth of certain strains of *Streptomyces rimosus* or obtained by any other means. It contains not less than 94.5% and not more than 102.0% of $(C_{22}H_{23}N_2O_9)_2Ca$, calculated with reference to the anhydrous substance.

CHARACTERISTICS

A pale yellow to greenish fawn, crystalline powder.

Practically insoluble in *water*; soluble in dilute acids. It dissolves slowly in 5M *ammonia*.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in 0.01 M *methanolic hydrochloric acid*.
(1) 0.05% w/v of the substance being examined.
(2) 0.05% w/v of *oxytetracycline BPCRS*.
(3) 0.05% w/v of each of *oxytetracycline BPCRS* and *demeclocycline hydrochloride BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a *silica gel* precoated plate (Merck silica gel 60 plates are suitable). Adjust the pH of a 10% w/v solution of *disodium edetate* to 7.0 with 10M *sodium hydroxide* and spray the solution evenly onto the plate (about 10 mL for a plate 100 mm × 200 mm). Allow the plate to dry in a horizontal position for at least 1 hour. Before use, dry the plate at 110° for 1 hour.
(b) Use the mobile phase as described below.
(c) Apply 1 µL of each solution.
(d) Develop the plate to 15 cm.
(e) After removal of the plate, dry it in a current of air and examine under *ultraviolet light (365 nm)*.

MOBILE PHASE

6 volumes of *water*, 35 volumes of *methanol* and 59 volumes of *dichloromethane*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position, colour and size to that in the chromatogram obtained with solution (2).

B. To 2 mg add 5 mL of *sulfuric acid*; a deep red colour is produced. Add the solution to 2.5 mL of *water*; the colour changes to yellow.
C. Yields reaction B characteristic of *calcium salts*, Appendix VI.

TESTS

Acidity or alkalinity

pH of a 2.5% w/v suspension, 6.0 to 7.5, Appendix V L.

Light absorption

Absorbance of a 0.002% w/v solution in *0.1M chloride buffer pH 2.0* at the maximum at 353 nm, 0.56 to 0.61, calculated with reference to the anhydrous substance, Appendix II B.

Specific optical rotation

In a 1% w/v solution in *0.1M hydrochloric acid*, -194 to -210, calculated with reference to the anhydrous substance, Appendix V F. Allow the solution to stand protected from light for 30 minutes before measurement.

Light-absorbing impurities

A. Dissolve 0.2 g in 6 mL of 1M *hydrochloric acid* and add sufficient *methanol* to produce 100 mL. The *absorbance* at 430 nm, when measured within 1 hour of preparing the solution, is not more than 0.30, calculated with reference to the anhydrous substance, Appendix II B.

B. Dissolve 1 g in 6 mL of 1M *hydrochloric acid* and add sufficient *methanol* to produce 100 mL. The *absorbance* at 490 nm, when measured within 1 hour of preparing the solution, is not more than 0.20, calculated with reference to the anhydrous substance, Appendix II B.

Calcium

3.90 to 4.30%, calculated with reference to the anhydrous substance, when determined by the following method. Transfer about 1 g, accurately weighed, to a Kjeldahl flask, cautiously add 10 mL of *nitric acid* and mix. Allow to stand for 5 minutes, add a glass bead and heat on a water bath for 5 minutes. Remove from the water bath, cautiously add 5 mL of 9M *perchloric acid* and heat, adding further 5 mL quantities of the perchloric acid at intervals until the liquid is almost colourless. Add 0.1 mL of *nitric acid* and allow any further reaction to subside. Do not allow the volume of the liquid in the flask to be reduced below 3 mL at any stage in the oxidation. Wash the walls of the flask with 40 mL of *water*, collecting the washings in the flask, and boil for 3 to 4 minutes to expel chlorine. Cool, transfer the contents of the flask to a conical flask with the aid of *water* and dilute to about 200 mL with *water*. Adjust to pH 9 with 5M *sodium hydroxide* and then add 100 mL of *water* followed by 12 mL of 10M *sodium hydroxide* and mix. Add about 15 mg of *calconcarboxylic acid triturate* and titrate with 0.05M *disodium edetate VS* until the colour changes from violet to full blue. Each mL of 0.05M *disodium edetate VS* is equivalent to 2.004 mg of Ca.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a mixture of 20 volumes of *acetonitrile* and 80 volumes of 0.01M oxalic acid (solvent A). *Prepare the solutions immediately before use.*

(1) 0.008% w/v of the substance being examined.
(2) Dilute 1 volume of solution (1) to 100 volumes.
(3) 0.08% w/v of *oxytetracycline for system suitability A EPCRS*.
(4) Dilute 1 volume of solution (2) to 10 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octylsilyl silica gel for chromatography* (5 µm) (Intertsil C8 is suitable).
(b) Use gradient elution and the mobile phase described below.
(c) Use a flow rate of 1.3 mL per minute.
(d) Use a column temperature of 50°.
(e) Use a detection wavelength of 254 nm.
(f) Inject 10 µL of each solution.

MOBILE PHASE

Mobile phase A 0.05% v/v *trifluoroacetic acid*.

Mobile phase B 5 volumes of *tetrahydrofuran*, 15 volumes of *methanol* and 80 volumes of *acetonitrile*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-5	90	10	isocratic
5-20	90→65	10→35	linear gradient
20-21	65→90	35→10	linear gradient
21-27	90	10	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to oxytetracycline (retention time about 7 minutes) are: impurity A, about 0.9; impurity B, about 1.2; impurity C, about 1.3; impurity D, about 1.4; impurity E, about 2.2; impurity F, about 2.3.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *peak-to-valley ratio* is at least 3.0, where *Hp* is the height above the baseline of the peak due to impurity A and *Hv* is the height above the baseline of the lowest point of the curve separating this peak from the peak due to oxytetracycline.

The test is not valid unless, in the chromatogram obtained with solution (3), the *peak-to-valley ratio* is at least 3.0, where *Hp* is the height above the baseline of the peak due to impurity B and *Hv* is the height above the baseline of the lowest point of the curve separating this peak from the peak due to oxytetracycline.

LIMITS

Identify any peak corresponding to impurities A, B, C, D, E and F in the chromatogram obtained with solution (1), using the chromatogram obtained with solution (3). Multiply the areas of the peaks due to Impurity D and E by a correction factor of 0.4.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than half the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any peak corresponding to impurity B is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%);

the area of any peak corresponding to impurity C is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2.0%);

the area of any other *secondary peak* is not greater than twice the area of the principal peak in the chromatogram obtained with solution (4) (0.2%);

the sum of the areas of all the*secondary peaks* is not greater than 3.5 times the area of the principal peak in the chromatogram obtained with solution (2) (3.5%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.10%).

Water

Not more than 15.0% w/w, Appendix IX C. Use 0.15 g.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a mixture of 20 volumes of *acetonitrile* and 80 volumes of 0.01M *oxalic acid* (solvent A). Prepare the solutions immediately before use.

(1) 0.008% w/v of the substance being examined.
(2) 0.008% w/v of *oxytetracycline BPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions stated under Related substances may be used.

DETERMINATION OF CONTENT

Calculate the content of $(C_{22}H_{23}N_2O_9)_2Ca$ using the declared content of $C_{22}H_{24}N_2O_9$ in *oxytetracycline BPCRS*. Each mg of $C_{22}H_{24}N_2O_9$ is equivalent to 1.041 mg of $(C_{22}H_{23}N_2O_9)_2Ca$.

STORAGE

Oxytetracycline Calcium should be protected from light and stored at a temperature of 2° to 8°.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Oxytetracycline Dihydrate.