Relative retention with reference to chlortalidone (retention time = about 7 min): impurity B = about 0.7; impurity J = about 0.9; impurity G = about 6.

System suitability: reference solution (b):

– *resolution*: minimum 1.5 between the peaks due to impurity J and chlortalidone.

Limits:

– *impurity B*: not more than 7 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.7 per cent);

– *impurity J*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);

– *impurity G*: not more than 2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

– *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

– *total*: not more than 12 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.2 per cent);

– *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Chlorides (*2.4.4*): maximum 350 ppm.

Triturate 0.3 g finely, add 30 ml of *water R*, shake for 5 min and filter. 15 ml of the filtrate complies with the test. Prepare the standard using 10 ml of *chloride standard solution (5 ppm Cl) R*.

Loss on drying (*2.2.32*): maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*): maximum 0.1 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.

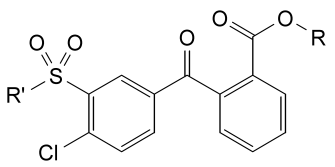
Injection: 20 µl of test solution (b) and reference solution (c).

Calculate the percentage content of $C_{14}H_{11}ClN_2O_4S$ from the declared content of *chlortalidone CRS*.

IMPURITIES

Specified impurities: B, G, J.

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *A, C, D, E, F, H, I.*

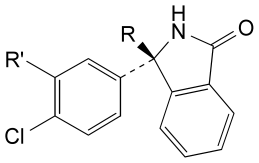


A. R = H, R′ = OH: 2-(4-chloro-3-sulphobenzoyl)benzoic acid,

B. R = H, R′ = NH_2: 2-(4-chloro-3-sulphamoylbenzoyl)benzoic acid,

C. R = C_2H_5, R′ = NH_2: ethyl 2-(4-chloro-3-sulphamoylbenzoyl)benzoate,

I. R = $CH(CH_3)_2$, R′ = NH_2: 1-methylethyl 2-(4-chloro-3-sulphamoylbenzoyl)benzoate,



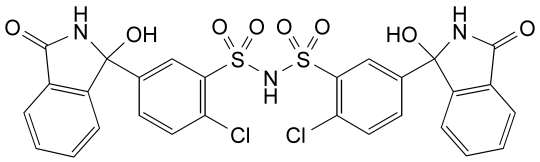
and enantiomer

D. R = OC_2H_5, R′ = SO_2-NH_2: 2-chloro-5-[(1*RS*)-1-ethoxy-3-oxo-2,3-dihydro-1*H*-isoindol-1-yl]benzenesulphonamide,

E. R = H, R′ = SO_2-NH_2: 2-chloro-5-[(1*RS*)-3-oxo-2,3-dihydro-1*H*-isoindol-1-yl]benzenesulphonamide,

G. R = OH, R′ = Cl: (3*RS*)-3-(3,4-dichlorophenyl)-3-hydroxy-2,3-dihydro-1*H*-isoindol-1-one,

H. R = $OCH(CH_3)_2$, R′ = SO_2-NH_2: 2-chloro-5-[(1*RS*)-1-(1-methylethoxy)-3-oxo-2,3-dihydro-1*H*-isoindol-1-yl]benzenesulphonamide,



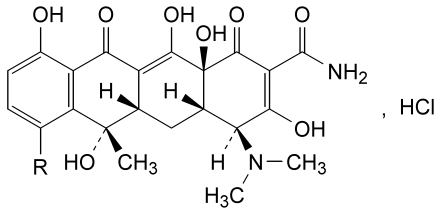
F. bis[2-chloro-5-(1-hydroxy-3-oxo-2,3-dihydro-1*H*-isoindol-1-yl)benzenesulphonyl]amine,

J. impurity of unknown structure with a relative retention of about 0.9.

01/2008:0173

CHLORTETRACYCLINE HYDROCHLORIDE

Chlortetracyclini hydrochloridum



Compound	R	Molecular formula	M_r
Chlortetracycline hydrochloride	Cl	$C_{22}H_{24}Cl_2N_2O_8$	515.3
Tetracycline hydrochloride	H	$C_{22}H_{25}ClN_2O_8$	480.9

DEFINITION

Mixture of antibiotics, the main component being the hydrochloride of (4*S*,4a*S*,5a*S*,6*S*,12a*S*)-7-chloro-4-(dimethylamino)-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (chlortetracycline hydrochloride), a substance produced by the growth of certain strains of *Streptomyces aureofaciens* or obtained by any other means.

Content:

– $C_{22}H_{24}Cl_2N_2O_8$: minimum 89.5 per cent (anhydrous substance),

- $C_{22}H_{25}ClN_2O_8$: maximum 8.0 per cent (anhydrous substance),
- 94.5 per cent to 102.0 per cent for the sum of the contents of chlortetracycline hydrochloride and tetracycline hydrochloride (anhydrous substance).

CHARACTERS

Appearance: yellow powder.

Solubility: slightly soluble in water and in alcohol. It dissolves in solutions of alkali hydroxides and carbonates.

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 *chlortetracycline hydrochloride CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (b). Dissolve 5 mg of *chlortetracycline hydrochloride CRS*, 5 mg of *doxycycline R* and 5 mg of *demeclocycline 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 deep blue colour develops which becomes bluish-green. Add the solution to 2.5 ml of *water R*. The colour becomes brownish.

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

TESTS

pH (*2.2.3*): 2.3 to 3.3.

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

Specific optical rotation (*2.2.7*): – 235 to – 250 (anhydrous substance).

Dissolve 0.125 g in *water R* and dilute to 50.0 ml with the same solvent.

Absorbance (*2.2.25*): maximum 0.40 at 460 nm.

Dissolve 0.125 g in *water R* and dilute to 25.0 ml with the same solvent.

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 *chlortetracycline hydrochloride CRS* in *0.01 M hydrochloric acid* and dilute to 25.0 ml with the same acid.

Reference solution (b). Dissolve 10.0 mg of *4-epichlortetracycline hydrochloride 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). Mix 5.0 ml of reference solution (a) and 10.0 ml of reference solution (b) and dilute to 25.0 ml with *0.01 M hydrochloric acid*.

Reference solution (e). Mix 5.0 ml of reference solution (b) and 5.0 ml of reference solution (c) and dilute to 50.0 ml with *0.01 M hydrochloric acid*.

Reference solution (f). Dilute 1.0 ml of reference solution (c) to 20.0 ml with *0.01 M hydrochloric acid*. Dilute 5.0 ml of this solution to 200.0 ml with *0.01 M hydrochloric acid*.

Column:

- *size*: *l* = 0.25 m, Ø = 4.6 mm,
- *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm),
- *temperature*: 35 °C.

Mobile phase: to 500 ml of *water R*, add 50 ml of *perchloric acid solution R*, shake and add 450 ml of *dimethyl sulphoxide R*,

Flow rate: 1 ml/min.

Detection: spectrophotometer at 280 nm.

Injection: 20 µl; inject the test solution and reference solutions (d), (e) and (f).

System suitability: reference solution (d):

- *resolution*: minimum 2.0 between the peaks due to impurity A and to chlortetracycline; if necessary, adjust the dimethyl sulphoxide content in the mobile phase,
- *symmetry factor*: maximum 1.3 for the peak due to chlortetracycline.

Limits:

- *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (e) (4.0 per cent),
- *total of other impurities eluting between the solvent peak and the peak corresponding to chlortetracycline*: not more than 0.25 times the area of the peak due to impurity A in the chromatogram obtained with reference solution (e) (1.0 per cent),
- *disregard limit*: area of the principal peak 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 limit test C. Prepare the standard using 2.5 ml of *lead standard solution (10 ppm Pb) R*.

Water (*2.5.12*): maximum 2.0 per cent, determined on 0.300 g.

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

Bacterial endotoxins (*2.6.14*): less than 1 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.

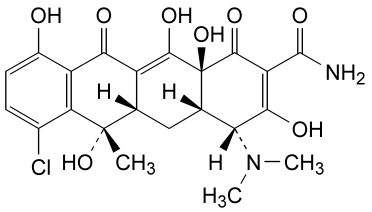
Injection: test solution and reference solutions (a) and (e).

Calculate the percentage content of $C_{22}H_{24}Cl_2N_2O_8$ using the chromatogram obtained with reference solution (a). Calculate the percentage content of $C_{22}H_{25}ClN_2O_8$ using the chromatogram obtained with reference solution (e).

STORAGE

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

IMPURITIES



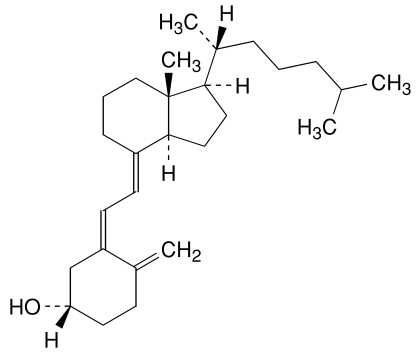
A. (4*R*,4a*S*,5a*S*,6*S*,12a*S*)-7-chloro-4-(dimethylamino)-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (4-epichlortetracycline),

B. demeclocycline.

01/2008:0072

CHOLECALCIFEROL

Cholecalciferolum



$C_{27}H_{44}O$ M_r 384.6
[67-97-0]

DEFINITION

(5*Z*,7*E*)-9,10-Secocholesta-5,7,10(19)-trien-3β-ol.

Content: 97.0 per cent to 102.0 per cent.

1 mg of cholecalciferol is equivalent to 40 000 IU of antirachitic activity (vitamin D) in rats.

CHARACTERS

Appearance: white or almost white crystals.

Solubility: practically insoluble in water, freely soluble in ethanol (96 per cent), soluble in trimethylpentane and in fatty oils.

It is sensitive to air, heat and light. Solutions in solvents without an antioxidant are unstable and are to be used immediately.

A reversible isomerisation to pre-cholecalciferol takes place in solution, depending on temperature and time. The activity is due to both compounds.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *cholecalciferol CRS*.

TESTS

Specific optical rotation (*2.2.7*): + 105 to + 112, determined within 30 min of preparing the solution.

Dissolve 0.200 g rapidly in *aldehyde-free alcohol R* without heating and dilute to 25.0 ml with the same solvent.

Related substances. Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use, avoiding exposure to actinic light and air.*

Test solution. Dissolve 10.0 mg of the substance to be examined in *trimethylpentane R* without heating and dilute to 10.0 ml with the same solvent.

Reference solution (a). Dissolve 10.0 mg of *cholecalciferol CRS* in *trimethylpentane R* without heating and dilute to 10.0 ml with the same solvent.

Reference solution (b). Dilute 1.0 ml of *cholecalciferol for system suitability CRS* (containing impurity A) to 5.0 ml with the mobile phase. Heat in a water-bath at 90 °C under a reflux condenser for 45 min and cool (formation of pre-cholecalciferol).

Reference solution (c). Dilute 10.0 ml of reference solution (a) to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 100.0 ml with the mobile phase.

Column:

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

Mobile phase: *pentanol R, hexane R* (3:997 *V/V*).

Flow rate: 2 ml/min.

Detection: spectrophotometer at 265 nm.

Injection: 5 µl of the test solution and reference solutions (b) and (c).

Run time: twice the retention time of cholecalciferol.

Relative retention with reference to cholecalciferol (retention time = about 19 min): pre-cholecalciferol = about 0.5; impurity A = about 0.6.

System suitability: reference solution (b):

– *resolution*: minimum 1.5 between the peaks due to pre-cholecalciferol and impurity A.

Limits:

– *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent);
– *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.10 per cent);
– *total*: not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent);
– *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent); disregard the peak due to pre-cholecalciferol.

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 cholecalciferol ($C_{27}H_{44}O$) from the declared content of *cholecalciferol CRS*.