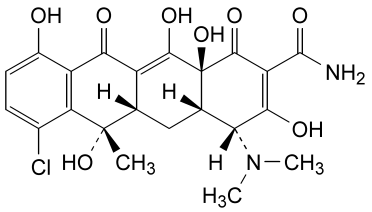
Calculate the percentage content of $C_{22}H_{24}Cl_2N_2O_8$ using the chromatogram obtained with reference solution (a). Calculate the percentage content of $C_{22}H_{25}ClN_2O_8$ using the chromatogram obtained with reference solution (e).

STORAGE

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

IMPURITIES



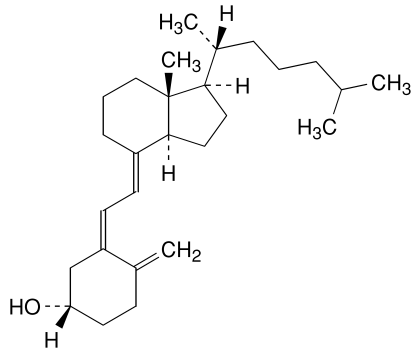
A. (4*R*,4a*S*,5a*S*,6*S*,12a*S*)-7-chloro-4-(dimethylamino)-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (4-epichlortetracycline),

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*.

STORAGE

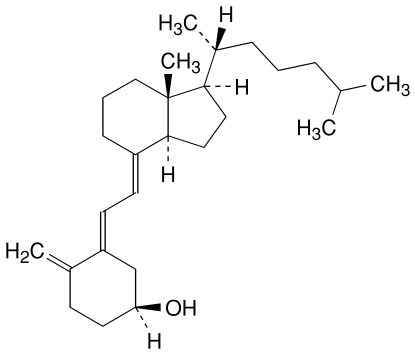
In an airtight container, under nitrogen, protected from light, at a temperature of 2 °C to 8 °C.

The contents of an opened container are to be used immediately.

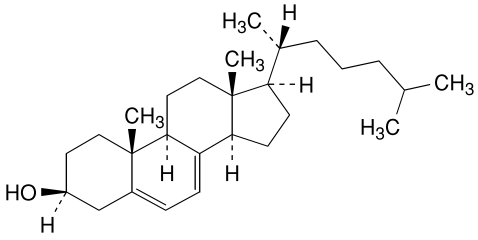
IMPURITIES

Specified impurities: *A*.

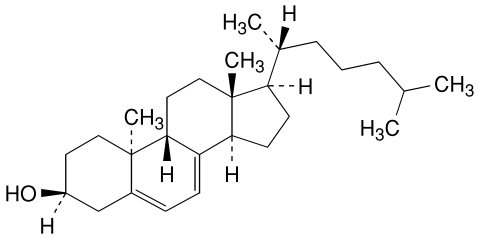
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical 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*): *B, C, D, E*.



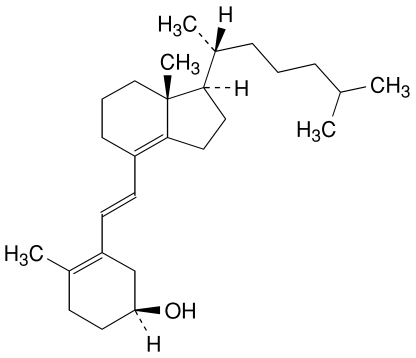
A. (5*E*,7*E*)-9,10-secocholesta-5,7,10(19)-trien-3β-ol (*trans*-cholecalciferol, *trans*-vitamin D_3),



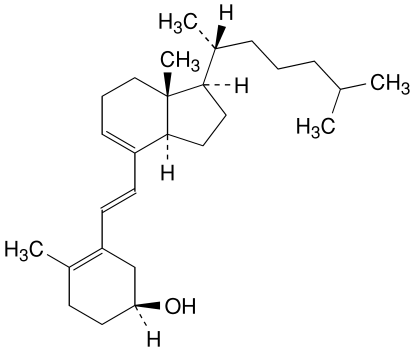
B. cholesta-5,7-dien-3β-ol (7,8-didehydrocholesterol, provitamin D_3),



C. 9β,10α-cholesta-5,7-dien-3β-ol (lumisterol$_3$),



D. (6*E*)-9,10-secocholesta-5(10),6,8(14)-trien-3β-ol (iso-tachysterol$_3$),



E. (6*E*)-9,10-secocholesta-5(10),6,8-trien-3β-ol (tachysterol$_3$).

01/2008:0575
corrected 6.0

CHOLECALCIFEROL CONCENTRATE (OILY FORM)

Cholecalciferolum densatum oleosum

DEFINITION

Solution of *Cholecalciferol (0072)* in a suitable vegetable fatty oil, authorised by the competent authority.

Content: 90.0 per cent to 110.0 per cent of the cholecalciferol content stated on the label, which is not less than 500 000 IU/g.

It may contain suitable stabilisers such as antioxidants.

CHARACTERS

Appearance: clear, yellow liquid.

Solubility: practically insoluble in water, slightly soluble in anhydrous ethanol, miscible with solvents of fats.

Partial solidification may occur, depending on the temperature.

IDENTIFICATION

First identification: A, C.

Second identification: A, B.

A. Thin-layer chromatography (*2.2.27*). *Prepare the solutions immediately before use.*

Test solution. Dissolve an amount of the preparation to be examined corresponding to 400 000 IU in *ethylene chloride R* containing 10 g/l of *squalane R* and 0.1 g/l of *butylhydroxytoluene R* and dilute to 4 ml with the same solution.