STORAGE

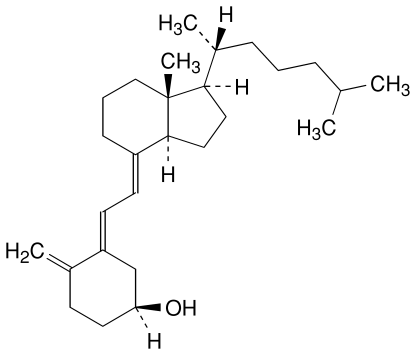
In an airtight container, under nitrogen, protected from light, at a temperature of 2 °C to 8 °C.

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

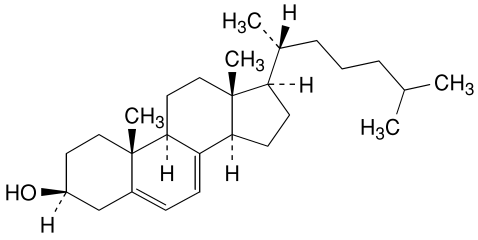
IMPURITIES

Specified impurities: *A*.

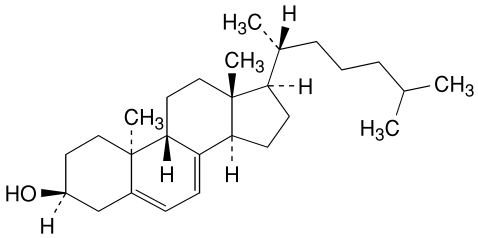
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical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



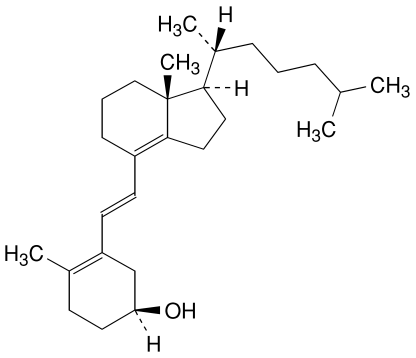
A. (5*E*,7*E*)-9,10-secocholesta-5,7,10(19)-trien-3β-ol (*trans*-cholecalciferol, *trans*-vitamin D_3),



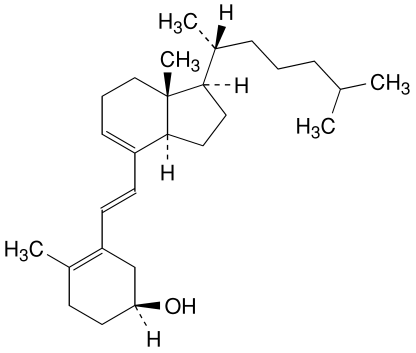
B. cholesta-5,7-dien-3β-ol (7,8-didehydrocholesterol, provitamin D_3),



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.

Reference solution (a). Dissolve 10 mg of *cholecalciferol CRS* 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.

Reference solution (b). Dissolve 10 mg of *ergocalciferol CRS* 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.

Plate: *TLC silica gel G plate R.*

Mobile phase: a 0.1 g/l solution of *butylhydroxytoluene R* in a mixture of equal volumes of *cyclohexane R* and *peroxide-free ether R.*

Application: 20 µl.

Development: immediately, protected from light, over a path of 15 cm.

Drying: in air.

Detection: spray with *sulphuric acid R.*

Results: the chromatogram obtained with the test solution shows immediately a bright yellow principal spot which rapidly becomes orange-brown, then gradually greenish-grey, remaining so for 10 min. This spot is similar in position, colour and size to the spot in the chromatogram obtained with reference solution (a). The chromatogram obtained with reference solution (b) shows immediately at the same level an orange principal spot which gradually becomes reddish-brown and remains so for 10 min.

B. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution. Prepare a solution in *cyclohexane R* containing the equivalent of about 400 IU/ml.

Spectral range: 250-300 nm.

Absorption maximum: at 267 nm.

C. 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 reference solution (a).

TESTS

Acid value (*2.5.1*): maximum 2.0.

Dissolve 5.0 g in 25 ml of the prescribed mixture of solvents.

Peroxide value (*2.5.5, Method A*): maximum 20.

ASSAY

Carry out the assay as rapidly as possible, avoiding exposure to actinic light and air.

Liquid chromatography (*2.2.29*).

Test solution. Dissolve a quantity of the preparation to be examined, weighed with an accuracy of 0.1 per cent, equivalent to about 400 000 IU, in 10.0 ml of *toluene R* and dilute to 100.0 ml with the mobile phase.

Reference solution (a). Dissolve 10.0 mg of *cholecalciferol CRS* without heating in 10.0 ml of *toluene R* and dilute to 100.0 ml with the mobile phase.

Reference solution (b). Dilute 1.0 ml of *cholecalciferol for performance test CRS* 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.

Reference solution (c). Dissolve 0.10 g of *cholecalciferol CRS* without heating in *toluene R* and dilute to 100.0 ml with the same solvent.

Reference solution (d). Dilute 5.0 ml of reference solution (c) to 50.0 ml with the mobile phase. Keep the solution in iced water.

Reference solution (e). Place 5.0 ml of reference solution (c) in a volumetric flask, add about 10 mg of *butylhydroxytoluene R* and displace air from the flask with *nitrogen R.* Heat in a water-bath at 90 °C under a reflux condenser protected from light and under *nitrogen R* for 45 min. Cool and dilute to 50.0 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 254 nm.

Injection: the chosen volume of each solution (the same volume for reference solution (a) and for the test solution); automatic injection device or sample loop recommended.

Relative retention with reference to cholecalciferol: pre-cholecalciferol = about 0.4; *trans*-cholecalciferol = about 0.5.

System suitability: reference solution (b):

– *resolution*: minimum 1.0 between the peaks due to pre-cholecalciferol and *trans*-cholecalciferol; if necessary adjust the proportions of the constituents and the flow rate of the mobile phase to obtain this resolution;
– *repeatability*: maximum relative standard deviation of 1.0 per cent for the peak due to cholecalciferol after 6 injections.

Calculate the conversion factor (*f*) using the following expression:

$$\frac{K - L}{M}$$

K = area (or height) of the peak due to cholecalciferol in the chromatogram obtained with reference solution (d);

L = area (or height) of the peak due to cholecalciferol in the chromatogram obtained with reference solution (e);

M = area (or height) of the peak due to pre-cholecalciferol in the chromatogram obtained with reference solution (e).

The value of *f* determined in duplicate on different days may be used during the entire procedure.

Calculate the content of cholecalciferol in International Units per gram using the following expression:

$$\frac{m'}{V'} \times \frac{V}{m} \times \frac{S_D + (f \times S_p)}{S'_D} \times 40\,000 \times 1000$$

m = mass of the preparation to be examined in the test solution, in milligrams;

m' = mass of *cholecalciferol CRS* in reference solution (a), in milligrams;

V = volume of the test solution (100 ml);

V' = volume of reference solution (a) (100 ml);

S_D = area (or height) of the peak due to cholecalciferol in the chromatogram obtained with the test solution;

S'_D = area (or height) of the peak due to cholecalciferol in the chromatogram obtained with reference solution (a);

S_p = area (or height) of the peak due to pre-cholecalciferol in the chromatogram obtained with the test solution;

f = conversion factor.

STORAGE

In an airtight, well-filled container, protected from light. The contents of an opened container are to be used as soon as possible; any unused part is to be protected by an atmosphere of nitrogen.

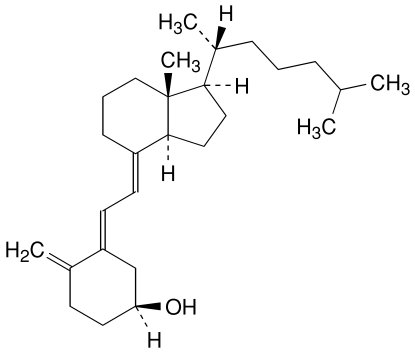
LABELLING

The label states:

– the number of International Units per gram;

– the method of restoring the solution if partial solidification occurs.

IMPURITIES



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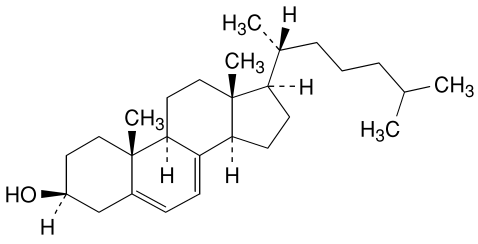
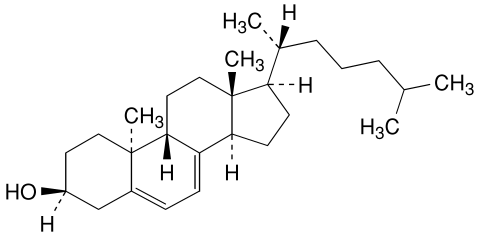
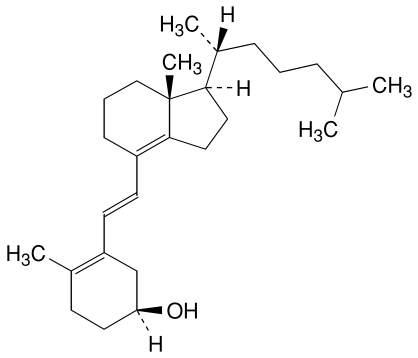


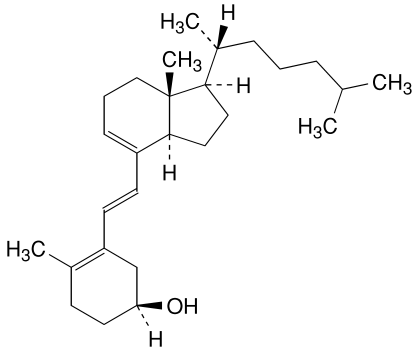
B. cholesta-5,7-dien-3β-ol (7,8-di dehydrocholesterol, provitamin D_3),



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$_3$).

01/2008:0574
corrected 6.0

CHOLECALCIFEROL CONCENTRATE (POWDER FORM)

Cholecalciferoli pulvis

DEFINITION

Powder concentrate obtained by dispersing an oily solution of *Cholecalciferol (0072)* in an appropriate matrix, which is usually based on a combination of gelatin and carbohydrates of suitable quality, 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 100 000 IU/g.

It may contain suitable stabilisers such as antioxidants.

CHARACTERS

Appearance: white or yellowish-white, small particles.

Solubility: practically insoluble, swells, or forms a dispersion in water, depending on the formulation.

IDENTIFICATION

First identification: A, C.

Second identification: A, B.

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

Test solution. Place 10.0 ml of the test solution prepared for the assay in a suitable flask and evaporate to dryness under reduced pressure by swirling in a water-bath at 40 °C. Cool under running water and restore atmospheric pressure with *nitrogen R*.