

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, $\varnothing = 4.6$ mm;
- stationary phase: silica gel for chromatography R (5 μ m).

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

Flow rate: 2 mL/min.

Detection: spectrophotometer at 265 nm.

Injection: 5 μ L 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

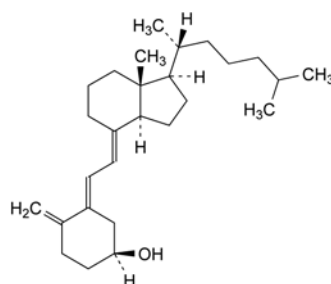
In an airtight container, under nitrogen, protected from light, at a temperature of 2 °C to 8 °C.

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

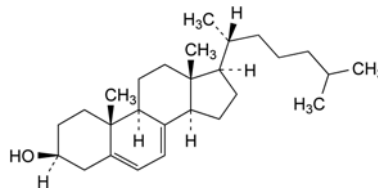
IMPURITIES

Specified impurities: A.

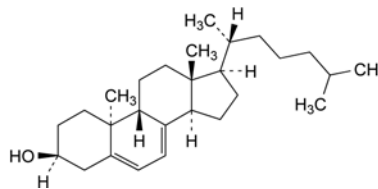
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical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



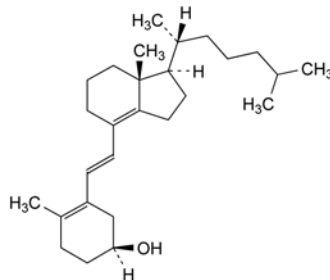
A. (5*E*,7*E*)-9,10-secocholesta-5,7,10(19)-trien-3 β -ol (*trans*-cholecalciferol, *trans*-vitamin D₃),



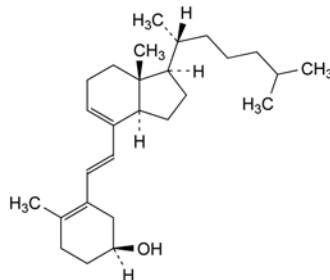
B. cholesta-5,7-dien-3 β -ol (7,8-didehydrocholesterol, provitamin D₃),



C. 9 β ,10 α -cholesta-5,7-dien-3 β -ol (lumisterol₃),



D. (6*E*)-9,10-secocholesta-5(10),6,8(14)-trien-3 β -ol (iso-tachysterol₃),



E. (6*E*)-9,10-secocholesta-5(10),6,8-trien-3 β -ol (tachysterol₃).

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corrected 6.5

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 sulfuric 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.

Related substances

The thresholds indicated under Related substances (Table 2034.-1) in the general monograph *Substances for pharmaceutical use (2034)* do not apply.

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 system suitability 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, $\varnothing = 4.6$ 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.

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corrected 6.5

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. Dissolve the residue immediately in 0.4 mL of ethylene chloride R containing 10 g/L of squalane R and 0.1 g/L of butylhydroxytoluene R.

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 sulfuric 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. Place 5.0 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. Dissolve the residue immediately in 50.0 mL of cyclohexane R.

Spectral range: 250-300 nm.

Absorption maximum: at 265 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

Related substances

The thresholds indicated under Related substances (Table 2034-1) in the general monograph *Substances for pharmaceutical use (2034)* do not apply.

ASSAY

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

Liquid chromatography (2.2.29).

Test solution. Introduce into a saponification flask a quantity of the preparation to be examined, weighed with an accuracy of 0.1 per cent, equivalent to about 100 000 IU. Add 5 mL of water R, 20 mL of anhydrous ethanol R, 1 mL of sodium ascorbate solution R and 3 mL of a freshly prepared 50 per cent m/m solution of potassium hydroxide R. Heat in a water-bath under a reflux condenser for 30 min. Cool rapidly under running water. Transfer the liquid to a separating funnel with the aid of 2 quantities, each of 15 mL, of water R, 1 quantity of 10 mL of ethanol (96 per cent) R and 2 quantities, each of 50 mL, of pentane R. Shake vigorously for 30 s. Allow to stand until the 2 layers are clear. Transfer the lower aqueous-alcoholic layer to a 2nd separating funnel and shake with a mixture of 10 mL of ethanol (96 per cent) R and 50 mL of pentane R. After separation, transfer the aqueous-alcoholic layer to a 3rd separating funnel and the pentane layer to the 1st separating funnel, washing the 2nd separating funnel with 2 quantities,