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 (25 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.

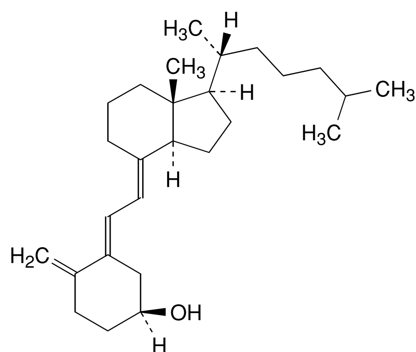
STORAGE

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.

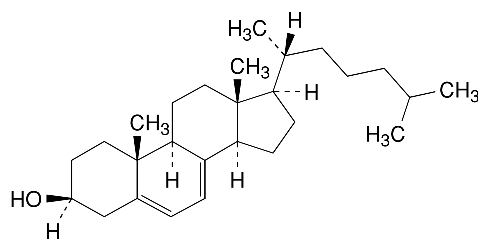
LABELLING

The label states the number of International Units per gram.

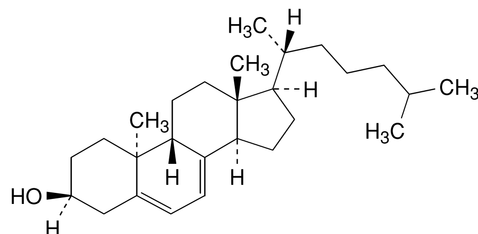
IMPURITIES



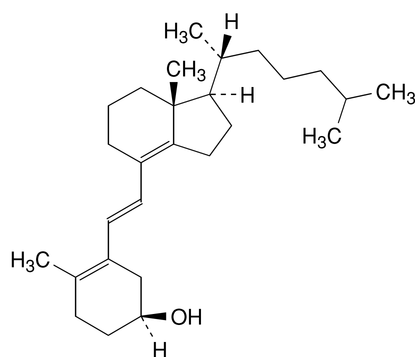
A. (5*E*,7*E*)-9,10-secocholesta-5,7,10(19)-trien-3β-ol (*trans*-cholecalciferol, *trans*-vitamin D_3),



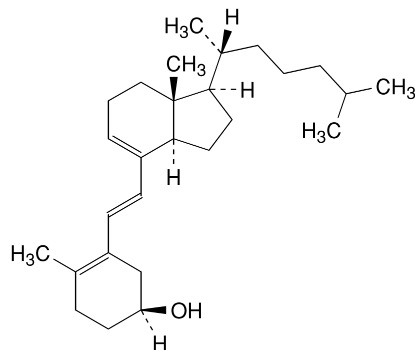
B. cholesta-5,7-dien-3β-ol (7,8-di dehydrocholesterol, provitamin D_3),



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$_3$).

01/2008:0598
corrected 6.0

CHOLECALCIFEROL CONCENTRATE (WATER-DISPERSIBLE FORM)

Cholecalciferolum in aqua dispergibile

DEFINITION

Solution of *Cholecalciferol (0072)* in a suitable vegetable fatty oil, authorised by the competent authority, to which suitable solubilisers have been added.

Content: 90.0 per cent to 115.0 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: slightly yellowish liquid of variable opalescence and viscosity.

Highly concentrated solutions may become cloudy at low temperatures or form a gel at room temperature.

IDENTIFICATION

First identification: A, C, D.

Second identification: A, B, D.

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 *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 principal 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).

D. Mix about 1 g with 10 ml of *water R* previously warmed to 50 °C, and cool to 20 °C. Immediately after cooling, a uniform, slightly opalescent and slightly yellow dispersion is obtained.

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 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, each of 10 ml, of *pentane R* and adding the washings to the 1st separating funnel. Shake the aqueous-alcoholic layer with 50 ml of *pentane R* and add the pentane layer to the 1st funnel. Wash the pentane layer with 2 quantities, each of 50 ml, of a freshly prepared 30 g/l solution of *potassium hydroxide R* in *ethanol (10 per cent V/V) R*, shaking vigorously, and then wash with successive quantities, each of 50 ml, of *water R* until the washings are neutral to phenolphthalein. Transfer the washed pentane extract to a ground-glass-stoppered flask. Evaporate the contents of the flask 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 5.0 ml of *toluene R* and add 20.0 ml of the mobile phase to obtain a solution containing about 4000 IU/ml.

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 the 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 (25 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, at the temperature stated on the label.

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 inert gas.

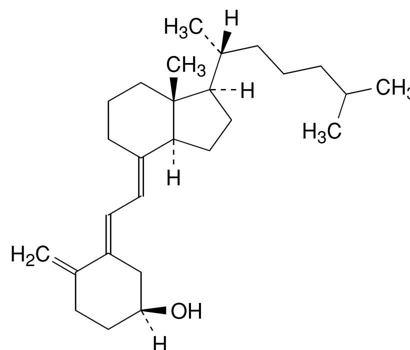
LABELLING

The label states:

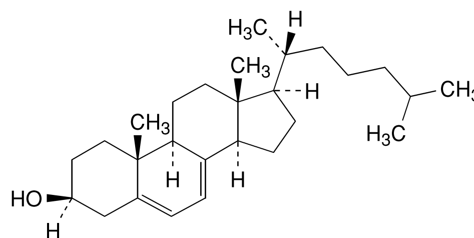
– the number of International Units per gram;

– the storage temperature.

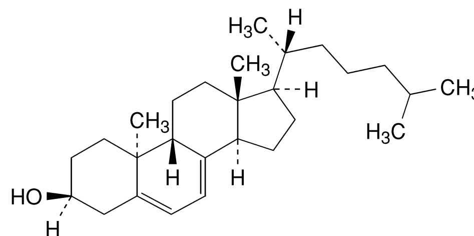
IMPURITIES



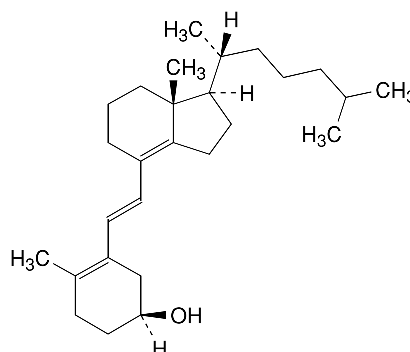
A. (5*E*,7*E*)-9,10-secocholesta-5,7,10(19)-trien-3β-ol (*trans*-cholecalciferol, *trans*-vitamin D_3),



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

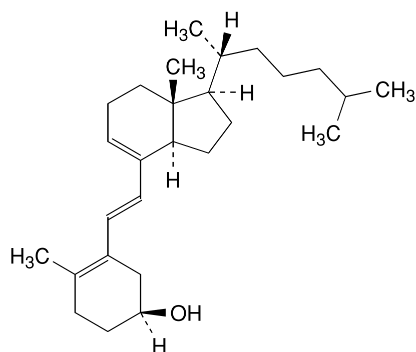


C. (9β,10α)-cholesta-5,7-dien-3β-ol (lumisterol$_3$),



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

Monographs C

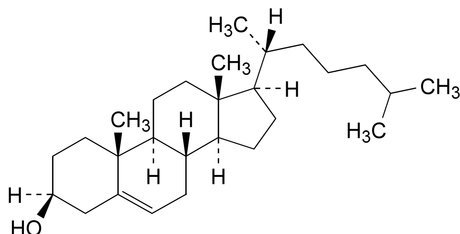


E. (6*E*)-9,10-secocholesta-5(10),6,8-trien-3β-ol (tachysterol$_3$).

01/2008:0993

CHOLESTEROL

Cholesterolum



$C_{27}H_{46}O$ M_r 386.7
[57-88-5]

DEFINITION

Cholest-5-en-3β-ol.

Content:

– *cholesterol*: minimum 95.0 per cent (dried substance);
– *total sterols*: 97.0 per cent to 103.0 per cent (dried substance).

CHARACTERS

Appearance: white or almost white, crystalline powder.

Solubility: practically insoluble in water, sparingly soluble in acetone and in ethanol (96 per cent).

It is sensitive to light.

IDENTIFICATION

A. Melting point (*2.2.14*): 147 °C to 150 °C.

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

Test solution. Dissolve 10 mg of the substance to be examined in *ethylene chloride R* and dilute to 5 ml with the same solvent.

Reference solution. Dissolve 10 mg of *cholesterol CRS* in *ethylene chloride R* and dilute to 5 ml with the same solvent.

Plate: *TLC silica gel G plate R.*

Mobile phase: *ethyl acetate R, toluene R* (33:66 *V/V*).

Application: 20 μl.

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

Drying: in air.

Detection: spray 3 times with *antimony trichloride solution R*; examine within 3-4 min.

Results: the principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

C. Dissolve about 5 mg in 2 ml of *methylene chloride R*. Add 1 ml of *acetic anhydride R*, 0.01 ml of *sulphuric acid R* and shake. A pink colour is produced which rapidly changes to red, then to blue and finally to brilliant green.

TESTS

Solubility in ethanol (96 per cent). In a stoppered flask, dissolve 0.5 g in 50 ml of *ethanol (96 per cent) R* at 50 °C. Allow to stand for 2 h. No deposit or turbidity is formed.

Acidity. Dissolve 1.0 g in 10 ml of *ether R*, add 10.0 ml of *0.1 M sodium hydroxide* and shake for about 1 min. Heat gently to eliminate ether and then boil for 5 min. Cool, add 10 ml of *water R* and 0.1 ml of *phenolphthalein solution R* as indicator and titrate with *0.1 M hydrochloric acid* until the pink colour just disappears, stirring the solution vigorously throughout the titration. Carry out a blank titration. The difference between the volumes of *0.1 M hydrochloric acid* required to change the colour of the indicator in the blank and in the test is not more than 0.3 ml.

Loss on drying (*2.2.32*): maximum 0.3 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C for 4 h.

Sulphated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Gas chromatography (*2.2.28*).

Internal standard solution. Dissolve 0.100 g of *pregnenolone isobutyrate CRS* in *heptane R* and dilute to 100.0 ml with the same solvent.

Test solution. Dissolve 25.0 mg of the substance to be examined in the internal standard solution and dilute to 25.0 ml with the same solution.

Reference solution. Dissolve 25.0 mg of *cholesterol CRS* in the internal standard solution and dilute to 25.0 ml with the same solution.

Column:

– *material*: fused silica;
– *size*: *l* = 30 m, Ø = 0.25 mm;
– *stationary phase*: *poly(dimethyl)siloxane R* (film thickness 0.25 μm).

Carrier gas: *helium for chromatography R.*

Flow rate: 2 ml/min.

Split ratio: 1:25.

Temperature:

– *column*: 275 °C;
– *injection port*: 285 °C;
– *detector*: 300 °C.

Detection: flame ionisation.

Injection: 1.0 μl.

System suitability: reference solution:

– *resolution*: minimum 10.0 between the peaks due to pregnenolone isobutyrate and cholesterol.

Calculate the percentage content of cholesterol from the declared content in *cholesterol CRS*. Calculate the percentage content of total sterols by adding together the contents of cholesterol and other substances with a