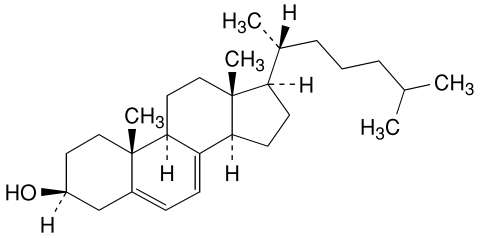
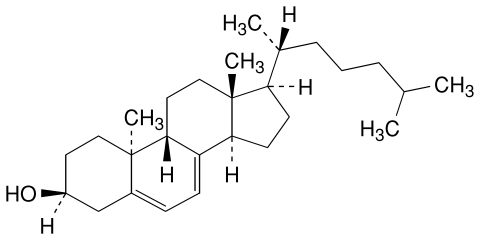
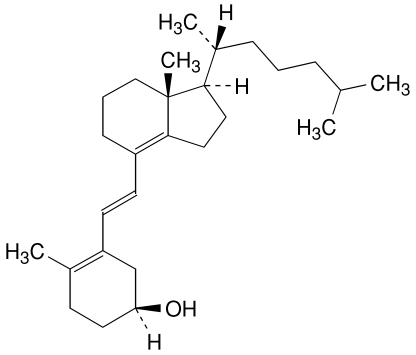


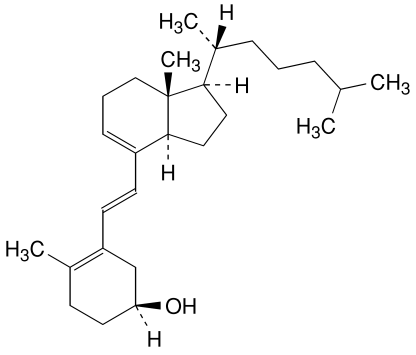
B. cholesta-5,7-dien-3β-ol (7,8-di dehydrocholesterol, provitamin D_3),



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$_3$).

01/2005:0574

CHOLECALCIFEROL CONCENTRATE (POWDER FORM)

Cholecalciferoli pulvis

DEFINITION

Cholecalciferol concentrate (powder form) is 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.

The declared content of cholecalciferol is not less than 100 000 IU/g and the concentrate contains not less than 90.0 per cent and not more than 110.0 per cent of the content stated on the label. The concentrate may contain suitable stabilisers such as antioxidants.

CHARACTERS

White or yellowish-white, small particles which, depending on their formulation, may be practically insoluble in water or may swell or form a dispersion.

IDENTIFICATION

First identification: A, C.

Second identification: A, B.

A. Examine by thin-layer chromatography (*2.2.27*), using a *TLC silica gel G plate R*.

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*. Prepare immediately before use.

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 solvent. Prepare immediately before use.

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 solvent. Prepare immediately before use.

Apply to the plate 20 µl of each solution. Develop immediately, protected from light, over a path of 15 cm using a mixture of equal volumes of *cyclohexane R* and *peroxide-free ether R*, the mixture containing 0.1 g/l of *butylhydroxytoluene R*. Allow the plate to dry in air and spray with *sulphuric acid R*. Compare the principal spot in the chromatogram obtained with the test solution with the principal spot in each of the chromatograms obtained with reference solution (a) and reference solution (b), respectively. The chromatogram obtained with the test solution shows immediately a bright-yellow principal spot which rapidly becomes orange-brown, then gradually greenish-grey and remains 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. 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*. Examined between 250 nm and 300 nm (*2.2.25*), the solution shows an absorption maximum at 265 nm.

C. Examine the chromatograms obtained in the assay. The principal peak in the chromatogram obtained with the test solution has a similar retention time to the principal peak in the chromatogram obtained with reference solution (a).

ASSAY

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

Examine by 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 *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 under a reflux condenser on a water-bath for 30 min. Cool rapidly under running water. Transfer the liquid to a separating funnel with the aid of two quantities, each of 15 ml, of *water R*, one quantity of 10 ml of *alcohol R* and two quantities, each of 50 ml, of *pentane R*. Shake vigorously for 30 s. Allow to stand until the two layers are clear. Transfer the lower aqueous-alcoholic layer to a second separating funnel and shake with a mixture of 10 ml of *alcohol R* and 50 ml of *pentane R*. After separation, transfer the aqueous-alcoholic layer to a third separating funnel and the pentane layer to the first separating funnel, washing the second separating funnel with two quantities, each of 10 ml, of *pentane R* and adding the washings to the first separating funnel. Shake the aqueous-alcoholic layer with 50 ml of *pentane R* and add the pentane layer to the first funnel. Wash the pentane layer with two quantities, each of 50 ml, of a freshly prepared 30 g/l solution of *potassium hydroxide R* in *alcohol (10 per cent V/V) R*, shaking vigorously, 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 g 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.

The chromatographic procedure may be carried out using:

- a stainless steel column 0.25 m long and 4.6 mm in internal diameter packed with a suitable silica gel (5 µm),
- as mobile phase at a flow rate of 2 ml/min, a mixture of 3 volumes of *pentanol R* and 997 volumes of *hexane R*,
- as detector a spectrophotometer set at 254 nm.

An automatic injection device or a sample loop is recommended. Inject a suitable volume of reference solution (b). Adjust the sensitivity of the system so that the height of the principal peak is at least 50 per cent of the full scale of the recorder. Inject reference solution (b) 6 times. When the chromatograms are recorded in the prescribed conditions, the approximate relative retention times with reference to cholecalciferol are 0.4 for pre-cholecalciferol and 0.5 for *trans*-cholecalciferol. The relative standard deviation of the response for cholecalciferol is not greater than 1 per cent and the resolution between the peaks due to pre-cholecalciferol and *trans*-cholecalciferol is not less than 1.0. If necessary adjust the proportions of the constituents and the flow rate of the mobile phase to obtain this resolution.

Inject a suitable volume of reference solution (d) and of reference solution (e).

Calculate the conversion factor (*f*) from the expression:

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

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.

Inject a suitable volume of reference solution (a). Adjust the sensitivity of the system so that the height of the principal peak is at least 50 per cent of the full scale of the recorder. Inject the same volume of the test solution and record the chromatogram in the same manner.

Calculate the content of cholecalciferol in International Units per gram from the 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 substance 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

Store 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.

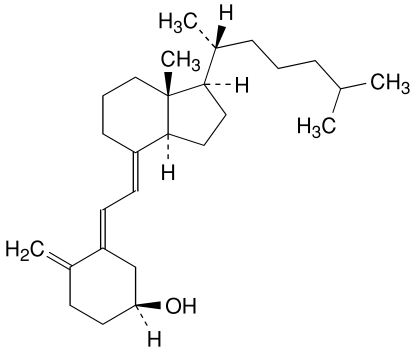
LABELLING

The label states:

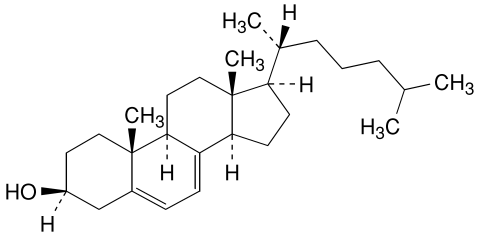
– the number of International Units per gram,

– the name of any added stabilisers.

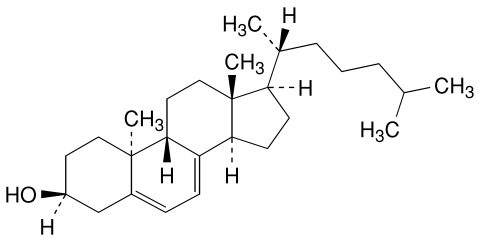
IMPURITIES



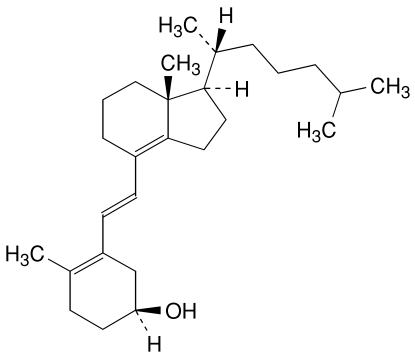
A. (5*E*,7*E*)-9,10-secocholesta-5,7,10(19)-trien-3β-ol (*trans*-cholecalciferol, *trans*-vitamin D_3),



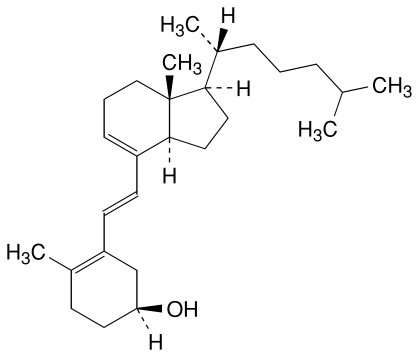
B. cholesta-5,7-dien-3β-ol (7,8-di dehydrocholesterol, provitamin D_3),



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$_3$).

01/2005:0598

CHOLECALCIFEROL CONCENTRATE (WATER-DISPERSIBLE FORM)

Cholecalciferolum in aqua dispergibile

DEFINITION

Cholecalciferol concentrate (water-dispersible form) consists of a solution of *Cholecalciferol (0072)* in a suitable vegetable fatty oil, authorised by the competent authority, to which suitable solubilisers have been added.

The declared content of cholecalciferol is not less than 100 000 IU/g and the concentrate contains not less than 90.0 per cent and not more than 115.0 per cent of the content stated on the label. The concentrate may contain suitable stabilisers such as antioxidants.

CHARACTERS

A 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. Examine by thin-layer chromatography (*2.2.27*), using a *TLC silica gel G plate R*.

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*. Prepare immediately before use.

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 solvent. Prepare immediately before use.

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 solvent. Prepare immediately before use.

Apply to the plate 20 µl of each solution. Develop immediately, protected from light, over a path of 15 cm using a mixture of equal volumes of *cyclohexane R* and *peroxide-free ether R*, the mixture containing 0.1 g/l of *butylhydroxytoluene R*. Allow the plate to dry in air and spray with *sulphuric acid R*. Compare the principal spot