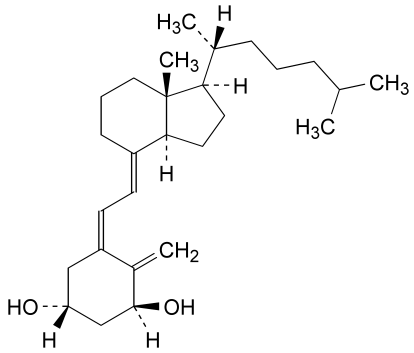
01/2008:1286

ALFACALCIDOL

Alfacalcidolum



$C_{27}H_{44}O_2$ M_r 400.6
[41294-56-8]

DEFINITION

(5*Z*,7*E*)-9,10-Secocholesta-5,7,10(19)-triene-1α,3β-diol.

Content: 97.0 per cent to 102.0 per cent.

CHARACTERS

Appearance: white or almost white crystals.

Solubility: practically insoluble in water, freely soluble in ethanol (96 per cent), soluble in fatty oils.

It is sensitive to air, heat and light.

A reversible isomerisation to pre-alfacalcidol takes place in solution, depending on temperature and time. The activity is due to both compounds.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *Ph. Eur. reference spectrum of alfacalcidol.*

B. Examine the chromatograms obtained in the test for related substances.

Results: the principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (a).

TESTS

Related substances. Liquid chromatography (*2.2.29*): use the normalisation procedure. *Carry out the test as rapidly as possible, avoiding exposure to actinic light and air.*

Test solution. Dissolve 1.0 mg of the substance to be examined without heating in 10.0 ml of the mobile phase.

Reference solution (a). Dissolve 1.0 mg of *alfacalcidol CRS* without heating in 10.0 ml of the mobile phase.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 100.0 ml with the mobile phase.

Reference solution (c). Heat 2 ml of reference solution (a) in a water-bath at 80 °C under a reflux condenser for 2 h and cool.

Column:

– *size*: *l* = 0.25 m, Ø = 4.0 mm;
– *stationary phase*: *octadecylsilyl silica gel for chromatography R2* (5 μm).

Mobile phase: *ammonia R, water R, acetonitrile R* (1:200:800 *V/V/V*).

Flow rate: 2.0 ml/min.

Detection: spectrophotometer at 265 nm.

Injection: 100 μl of the test solution and reference solutions (b) and (c).

Run time: twice the retention time of alfacalcidol.

Relative retention with reference to alfacalcidol: pre-alfacalcidol = about 1.3.

System suitability: reference solution (c):

– *resolution*: minimum 4.0 between the peaks due to pre-alfacalcidol and alfacalcidol; if necessary, adjust the proportions of the constituents of the mobile phase.

Limits:

– *impurities A, B, C*: for each impurity, maximum 0.5 per cent;
– *total*: maximum 1.0 per cent;
– *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent); disregard the peak due to pre-alfacalcidol.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection: the test solution and reference solutions (a) and (c).

System suitability: reference solution (c):

– *repeatability*: maximum relative standard deviation of 1 per cent for the peak due to alfacalcidol after 6 injections.

Calculate the percentage content of $C_{27}H_{44}O_2$ from the declared content of *alfacalcidol CRS*.

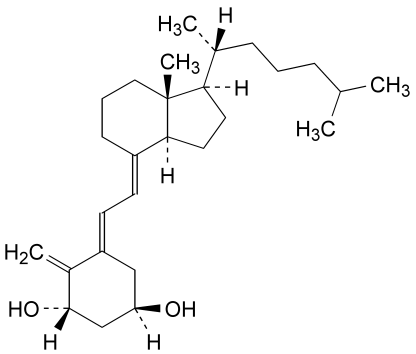
STORAGE

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

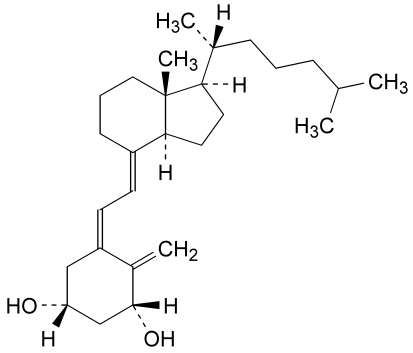
The contents of an opened container are to be used immediately.

IMPURITIES

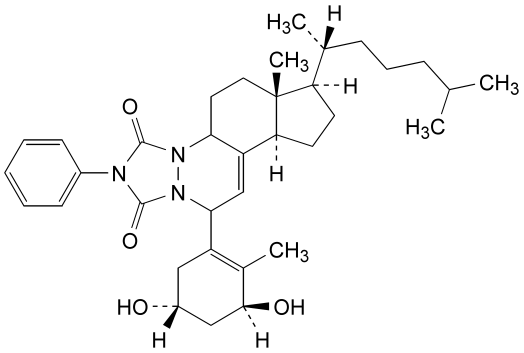
Specified impurities: *A, B, C.*



A. (5*E*,7*E*)-9,10-secocholesta-5,7,10(19)-triene-1α,3β-diol (*trans*-alfacalcidol),



B. (5*Z*,7*E*)-9,10-secocholesta-5,7,10(19)-triene-1β,3β-diol (1β-calcidol),



C. triazoline adduct of pre-alfacalcidol.

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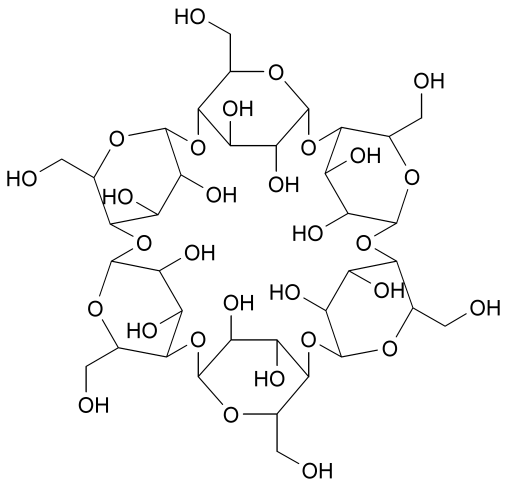
ALFADEX

Alfadexum



$[C_6H_{10}O_5]_6$ M_r 973
[10016-20-3]

DEFINITION

Cyclohexakis-(1→4)-(α-D-glucopyranosyl) (cyclomaltohexaose or α-cyclodextrin).

Content: 98.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance: white or almost white, amorphous or crystalline powder.

Solubility: freely soluble in water and in propylene glycol, practically insoluble in anhydrous ethanol and in methylene chloride.

IDENTIFICATION

A. Specific optical rotation (see Tests).

B. Examine the chromatograms obtained in the assay.

Results: the principal peak in the chromatogram obtained with test solution (b) is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (c).

C. Dissolve 0.2 g in 2 ml of *iodine solution R4* by warming on a water-bath, and allow to stand at room temperature; a yellowish-brown precipitate is formed.

TESTS

Solution S. Dissolve 1.000 g in *carbon dioxide-free water R* and dilute to 100.0 ml with the same solvent.

Appearance of solution. Solution S is clear (*2.2.1*).

pH (*2.2.3*): 5.0 to 8.0.

Mix 1 ml of a 223.6 g/l solution of *potassium chloride R* and 30 ml of solution S.

Specific optical rotation (*2.2.7*): + 147 to + 152 (dried substance), determined on solution S.

Reducing sugars: maximum 0.2 per cent.

Test solution. To 1 ml of solution S add 1 ml of *cupri-tartaric solution R4*. Heat on a water-bath for 10 min, cool to room temperature. Add 10 ml of *ammonium molybdate reagent R1* and allow to stand for 15 min.

Reference solution. Prepare a reference solution at the same time and in the same manner as the test solution, using 1 ml of a 0.02 g/l solution of *glucose R*.

Measure the absorbance (*2.2.25*) of the test solution and the reference solution at the absorption maximum at 740 nm using *water R* as the compensation liquid. The absorbance of the test solution is not greater than that of the reference solution.

Light-absorbing impurities. Examine solution S between 230 nm and 750 nm. Between 230 nm and 350 nm, the absorbance (*2.2.25*) is not greater than 0.10. Between 350 nm and 750 nm, the absorbance (*2.2.25*) is not greater than 0.05.

Related substances. Liquid chromatography (*2.2.29*).

Test solution (a). Dissolve 0.25 g of the substance to be examined in *water R* with heating, cool and dilute to 25.0 ml with the same solvent.

Test solution (b). Dilute 5.0 ml of test solution (a) to 50.0 ml with *water R*.

Reference solution (a). Dissolve 25.0 mg of *betadex CRS* (impurity A), 25.0 mg of *gammacyclodextrin CRS* (impurity B) and 50.0 mg of *alfadex CRS* in *water R*, then dilute to 50.0 ml with the same solvent.

Reference solution (b). Dilute 5.0 ml of reference solution (a) to 50.0 ml with *water R*.

Reference solution (c). Dissolve 25.0 mg of *alfadex CRS* in *water R* and dilute to 25.0 ml with the same solvent.