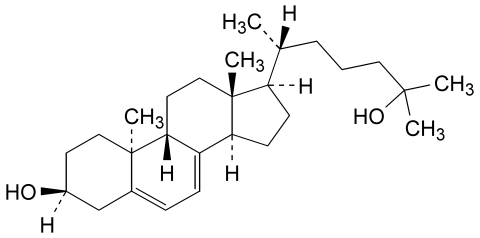
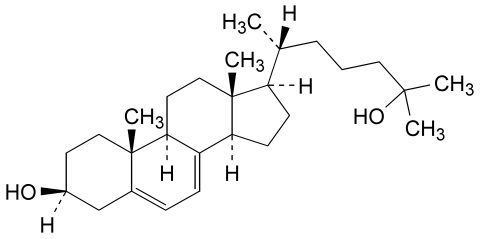
IMPURITIES

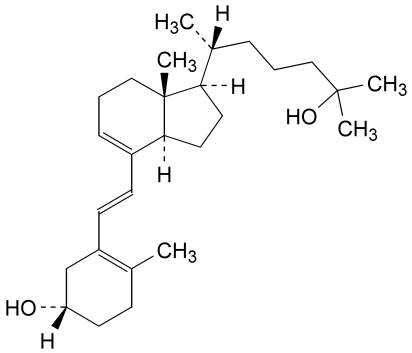
Specified impurities: *A, B, C, D.*



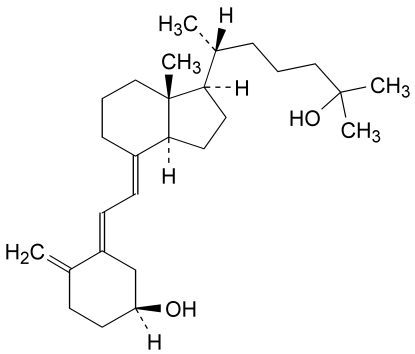
A. 9β,10α-cholesta-5,7-diene-3β,25-diol,



B. cholesta-5,7-diene-3β,25-diol,



C. (6*E*)-9,10-secocholesta-5(10),6,8-triene-3β,25-diol,

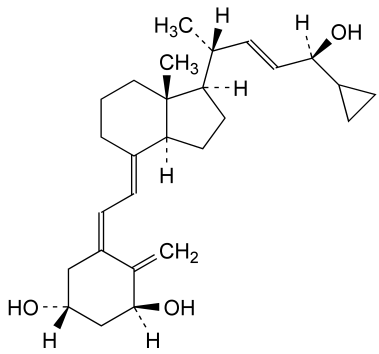


D. (5*E*,7*E*)-9,10-secocholesta-5,7,10(19)-triene-3β,25-diol.

01/2008:2011

CALCIPOTRIOL, ANHYDROUS

Calcipotriolum anhydricum



$C_{27}H_{40}O_3$ M_r 412.6

DEFINITION

(5*Z*,7*E*,22*E*,24*S*)-24-Cyclopropyl-9,10-secochola-5,7,10(19),22-tetraene-1α,3β,24-triol.

Content: 95.5 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance: white or almost white, crystalline powder.

Solubility: practically insoluble in water, freely soluble in ethanol (96 per cent), slightly soluble in methylene chloride.

It is sensitive to heat and light.

A reversible isomerisation to pre-calcipotriol 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 anhydrous calcipotriol.*

B. Loss on drying (see Tests).

Carry out the tests for related substances and the assay as rapidly as possible and protected from actinic light and air.

TESTS

Related substances

A. Thin-layer chromatography (*2.2.27*).

Solution A. To 1 ml of *triethylamine R* add 9 ml of *chloroform R*.

Test solution. Dissolve 1 mg of the substance to be examined in 100 µl of solution A.

Reference solution (a). To 10 µl of the test solution add 990 µl of solution A.

Reference solution (b). To 250 µl of reference solution (a) add 750 µl of solution A.

Reference solution (c). To 100 µl of reference solution (a) add 900 µl of solution A.

Reference solution (d). Place 2 mg of the substance to be examined in a vial and dissolve in 200 µl of solution A. Close the vial and keep it in a water bath at 60 °C for 2 h.

Plate: *TLC silica gel F_{254} plate R.*

Mobile phase: *2-methylpropanol R, methylene chloride R* (20:80 *V/V*).

Application: 10 µl of the test solution and reference solutions (b), (c) and (d).

Development: over 2/3 of the plate.

Drying: in air, then at 140 °C for 10 min.

Detection: spray the hot plate with an *alcoholic solution of sulphuric acid R*, dry at 140 °C for not more than 1 min and examine in ultraviolet light at 366 nm.

Relative retention with reference to calcipotriol (R_F = about 0.4): impurity G = about 0.4; impurity H = about 0.4; pre-calcipotriol = about 0.9; impurity A = about 1.2.

System suitability: reference solution (d):

– the chromatogram shows a secondary spot due to pre-calcipotriol.

Limits:

– *impurity A*: any spot due to impurity A is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.25 per cent),

– *impurities G, H*: any spot due to impurity G or H is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.25 per cent for the sum),

– *any other impurity*: any other spot is not more intense than the spot in the chromatogram obtained with reference solution (c) (0.1 per cent).

B. Liquid chromatography (*2.2.29*).

Solution A. Dissolve 1.32 g of *ammonium phosphate R* in *water R* and dilute to 10.0 ml with the same solvent.

Solvent mixture: solution A, *water R, methanol R* (3:297:700 *V/V/V*).

Test solution (a). Dissolve 2.00 mg of the substance to be examined in the solvent mixture and dilute to 5.0 ml with the solvent mixture.

Test solution (b). Dissolve 2.00 mg of the substance to be examined in the solvent mixture and dilute to 20.0 ml with the same solvent mixture.

Reference solution (a). Dilute 1.0 ml of test solution (a) to 100.0 ml with the solvent mixture.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 10.0 ml with the solvent mixture.

Reference solution (c). Dissolve 1.0 mg of *calcipotriol monohydrate CRS* (containing impurities B, C and D) in the solvent mixture and dilute to 2.5 ml with the solvent mixture.

Reference solution (d). Dissolve 2.00 mg of *calcipotriol monohydrate CRS* in the solvent mixture and dilute to 20.0 ml with the solvent mixture.

Column:

– *size*: *l* = 0.10 m, Ø = 4.0 mm,

– *stationary phase*: *octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase: *water R, methanol R* (30:70 *V/V*).

Flow rate: 1.0 ml/min.

Detection: spectrophotometer at 264 nm.

Injection: 20 µl of test solution (a) and reference solutions (a), (b) and (c).

Run time: twice the retention time of calcipotriol.

Relative retention with reference to calcipotriol (retention time = about 13.5 min): impurity B = about 0.86; impurity C = about 0.92; impurity D = about 1.3.

System suitability: reference solution (c):

– *peak-to-valley ratio*: minimum 1.5, where H_p = height above the baseline of the peak due to impurity C and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to calcipotriol,

– the chromatogram obtained is similar to the chromatogram supplied with *calcipotriol monohydrate CRS*.

Limits:

– *impurity B*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent),

– *impurities C, D*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent),

– *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent),

– *total*: not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (2.5 per cent),

– *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying: maximum 1.0 per cent, determined on 5 mg by thermogravimetry (*2.2.34*). Heat to 105 °C at a rate of 10 °C/min and maintain at 105 °C for 60 min.

ASSAY

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

Injection: test solution (b) and reference solution (d).

Calculate the percentage content of $C_{27}H_{40}O_3$ from the areas of the peaks and the declared content of *calcipotriol monohydrate CRS*.

STORAGE

In an airtight container, protected from light at – 20 °C or below.

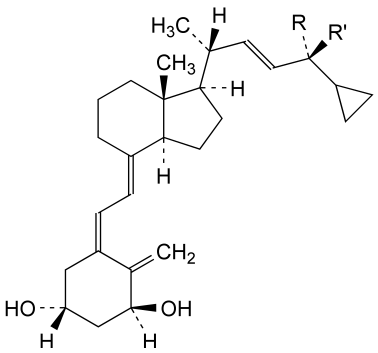
IMPURITIES

Specified impurities: A, B, C, D, G, H.

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*): *E, F, I.*

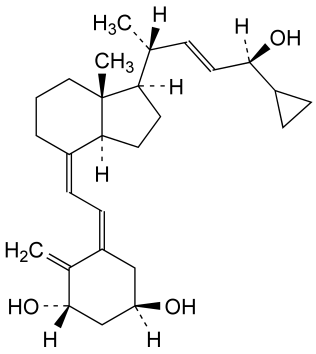
By thin-layer chromatography: A, G, H, I.

By liquid chromatography: B, C, D, E, F.

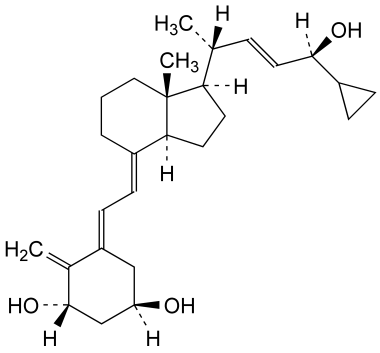


A. R + R′ = O: (5*Z*,7*E*,22*E*)-24-cyclopropyl-1α,3β-dihydroxy-9,10-secochola-5,7,10(19),22-tetraen-24-one,

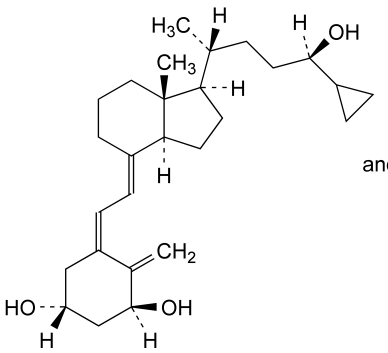
D. R = OH, R′ = H: (5*Z*,7*E*,22*E*,24*R*)-24-cyclopropyl-9,10-secochola-5,7,10(19),22-tetraene-1α,3β,24-triol (24-*epi*-calcipotriol),



B. (5*Z*,7*Z*,22*E*,24*S*)-24-cyclopropyl-9,10-secochola-5,7,10(19),22-tetraene-1α,3β,24-triol ((7*Z*)-calcipotriol),

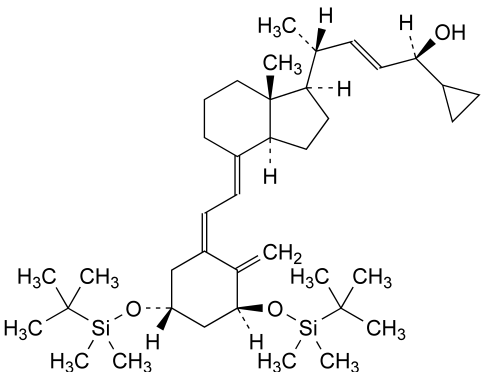


C. (5*E*,7*E*,22*E*,24*S*)-24-cyclopropyl-9,10-secochola-5,7,10(19),22-tetraene-1α,3β,24-triol ((5*E*)-calcipotriol),

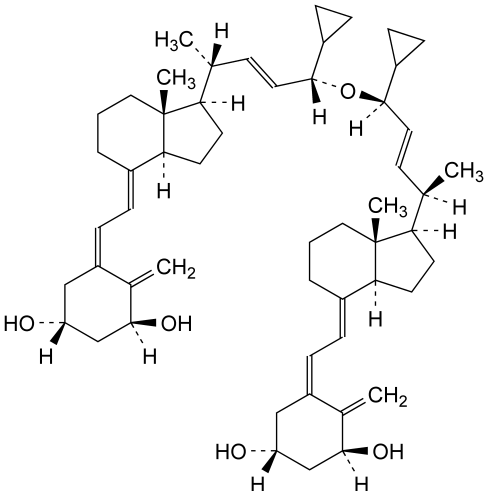


and enantiomer

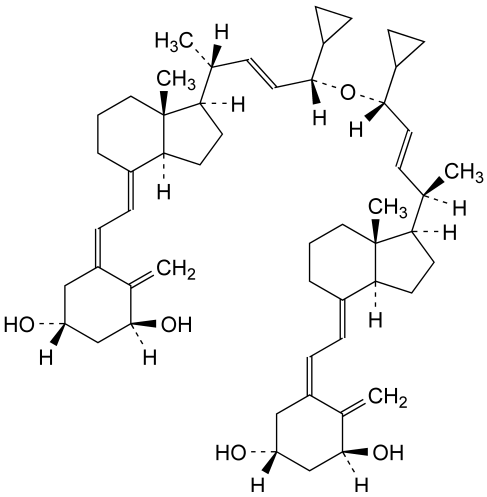
E. *rac*-(5*Z*,7*E*,22*E*,24*S*)-24-cyclopropyl-9,10-secochola-5,7,10(19)-triene-1α,3β,24-triol,



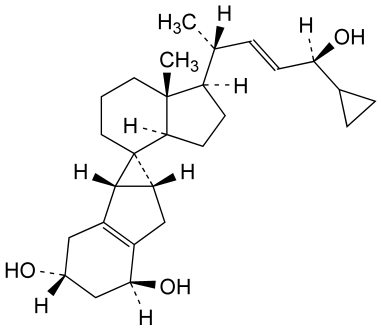
F. (5*Z*,7*E*,22*E*,24*S*)-24-cyclopropyl-1α,3β-bis[[(1,1-dimethylethyl)dimethylsilyl]oxy]-9,10-secochola-5,7,10(19),22-tetraen-24-ol,



G. 24,24′-oxybis[(5*Z*,7*E*,22*E*,24*S*)-24-cyclopropyl-9,10-secochola-5,7,10(19),22-tetraene-1α,3β-diol],



H. (5*Z*,7*E*,22*E*,24*R*)-24-cyclopropyl-24-[[(5*Z*,7*E*,22*E*,24*S*)-24-cyclopropyl-1α,3β-dihydroxy-9,10-secochola-5,7,10(19),22-tetraen-24-yl]oxy]-9,10-secochola-5,7,10(19),22-tetraene-1α,3β-diol,

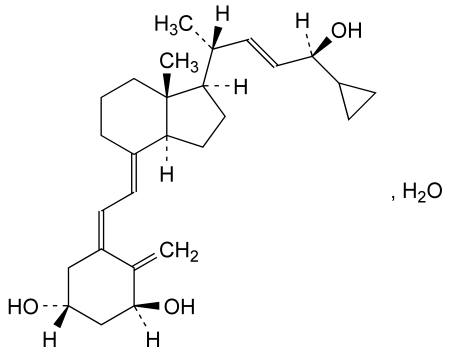


I. (6*S*,7*R*,8*R*,22*E*,24*S*)-24-cyclopropyl-6,8:7,19-dicyclo-9,10-secochola-5(10),22-diene-1α,3β,24-triol (suprasterol of calcipotriol).

01/2008:2284

CALCIPOTRIOL MONOHYDRATE

Calcipotriolum monohydricum



$C_{27}H_{40}O_3,H_2O$ M_r 430.6
[147657-22-5]

DEFINITION

(5*Z*,7*E*,22*E*,24*S*)-24-Cyclopropyl-9,10-secochola-5,7,10(19),22-tetraene-1α,3β,24-triol monohydrate.

Content: 95.5 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance: white or almost white, crystalline powder.

Solubility: practically insoluble in water, freely soluble in ethanol (96 per cent), slightly soluble in methylene chloride.

It is sensitive to light.

A reversible isomerisation to pre-calcipotriol 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 calcipotriol monohydrate.*

B. Water (see Tests).

Carry out the tests for related substances and the assay as rapidly as possible and protected from actinic light and air.

TESTS

Related substances

A. Thin-layer chromatography (*2.2.27*).

Solution A. To 1 ml of *triethylamine R* add 9 ml of *chloroform R.*

Test solution. Dissolve 1 mg of the substance to be examined in 100 µl of solution A.

Reference solution (a). To 10 µl of the test solution add 990 µl of solution A.

Reference solution (b). To 250 µl of reference solution (a) add 750 µl of solution A.

Reference solution (c). To 100 µl of reference solution (a) add 900 µl of solution A.

Reference solution (d). Place 2 mg of the substance to be examined in a vial and dissolve in 200 µl of solution A. Close the vial and keep it in a water bath at 60 °C for 2 h.

Plate: *TLC silica gel F_{254} plate R.*

Mobile phase: *2-methylpropanol R, methylene chloride R* (20:80 *V/V*).

Application: 10 µl of the test solution and reference solutions (b), (c) and (d).

Development: over 2/3 of the plate.

Drying: in air, then at 140 °C for 10 min.

Detection: spray the hot plate with an *alcoholic solution of sulphuric acid R*, dry at 140 °C for not more than 1 min and examine in ultraviolet light at 366 nm.

Relative retention with reference to calcipotriol (R_F = about 0.4): impurity G = about 0.4; impurity H = about 0.4; pre-calcipotriol = about 0.9; impurity A = about 1.2.

System suitability: reference solution (d):

– the chromatogram shows a secondary spot due to pre-calcipotriol.

Limits:

– *impurity A*: any spot due to impurity A is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.25 per cent),

– *impurities G, H*: any spot due to impurity G or H is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.25 per cent for the sum),

– *any other impurity*: any other spot is not more intense than the spot in the chromatogram obtained with reference solution (c) (0.1 per cent).

B. Liquid chromatography (*2.2.29*).

Solution A. Dissolve 1.32 g of *ammonium phosphate R* in *water R* and dilute to 10.0 ml with the same solvent.

Solvent mixture: solution A, *water R, methanol R* (3:297:700 *V/V/V*).

Test solution (a). Dissolve 2.00 mg of the substance to be examined in the solvent mixture and dilute to 5.0 ml with the solvent mixture.

Test solution (b). Dissolve 2.00 mg of the substance to be examined in the solvent mixture and dilute to 20.0 ml with the same solvent mixture.

Reference solution (a). Dilute 1.0 ml of test solution (a) to 100.0 ml with the solvent mixture.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 10.0 ml with the solvent mixture.

Reference solution (c). Dissolve 1.0 mg of *calcipotriol monohydrate CRS* (containing impurities B, C and D) in the solvent mixture and dilute to 2.5 ml with the solvent mixture.

Reference solution (d). Dissolve 2.00 mg of *calcipotriol monohydrate CRS* in the solvent mixture and dilute to 20.0 ml with the solvent mixture.