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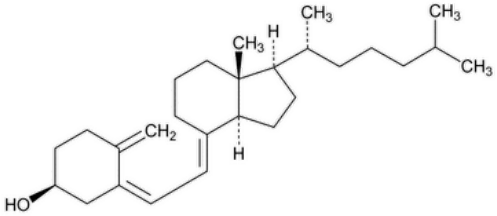
Cholecalciferol



$C_{27}H_{44}O$ 384.64

9,10-Secocholesta-5,7,10(19)-trien-3-ol, (3β,5*Z*,7*E*)-;

Cholecalciferol CAS RN®: 67-97-0; UNII: 1C6V77QF41.

DEFINITION

Cholecalciferol contains NLT 97.0% and NMT 103.0% of cholecalciferol ($C_{27}H_{44}O$).

IDENTIFICATION

Change to read:

• **A.** ▲**Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*: 197K**▲ (USP 1-May-2020)

Wavelength range: 2–12 µm

Acceptance criteria: Meets the requirements in the chapter

Change to read:

• **B.** ▲**Spectroscopic Identification Tests ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: 197U**▲ (USP 1-May-2020)

Analytical wavelength: 265 nm

Sample solution: 10 µg/mL in alcohol

Acceptance criteria: Meets the requirements in the chapter. Absorptivities do not differ by more than 3.0%.

Change to read:

• **C.** ▲The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-May-2020)

Delete the following:

▲• **D. Thin-Layer Chromatography**

[Note—For the *Standard solution* and the *Sample solution*, follow these procedures: use low-actinic glassware, dissolve the samples without heating, and use the solutions immediately.]

Diluent: 10 mg/mL of squalane in chloroform

Standard solution: 50 mg/mL of USP Cholecalciferol RS in *Diluent*

Sample solution: 50 mg/mL of Cholecalciferol in *Diluent*

Chromatographic system

(See *Chromatography ⟨621⟩, Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 10 µL

Developing solvent system: Cyclohexane and diethyl ether (1:1)

Spray reagent: 20 mg/mL of acetyl chloride in antimony trichloride TS

Analysis

Samples: *Standard solution* and *Sample solution*

[NOTE—Perform the development and subsequent operations in the dark.]

Place the plate in a chamber containing and equilibrated with *Developing solvent system*. Develop until the solvent front has moved about 15 cm above the line of application. Remove the plate, allow the solvent to evaporate, and spray with *Spray reagent*.

Acceptance criteria: The *Sample solution* shows a yellowish-orange area (cholecalciferol) having the same R_F value as the area of the *Standard solution* and may show below the cholecalciferol area a violet area, attributed to 7-dehydrocholesterol.▲ (USP 1-May-2020)

ASSAY

Change to read:

• **PROCEDURE**

▲▲ (USP 1-May-2020)

Mobile phase: *n*-Amyl alcohol in ▲hexane, solvent, chromatographic▲ (USP 1-May-2020) (3 in 1000)

System suitability solution: 250 mg of USP Vitamin D Assay System Suitability RS in 10 mL of a mixture of toluene and *Mobile phase* (1:1). Heat this solution, under reflux, at 90° for 45 min, and cool. [NOTE—This solution contains cholecalciferol, precholecalciferol, and *trans*-cholecalciferol.]

[NOTE—For the stock solutions, follow these procedures: use low-actinic glassware, dissolve the samples without heating, and prepare the solutions fresh daily.]

Standard stock solution: 0.6 mg/mL of USP Cholecalciferol RS in toluene

Standard solution: 120 µg/mL of USP Cholecalciferol RS in *Mobile phase*, prepared from *Standard stock solution*

Sample stock solution: 0.6 mg/mL of Cholecalciferol in toluene

Sample solution: 120 µg/mL of Cholecalciferol in *Mobile phase*, prepared from *Sample stock solution*

Chromatographic system

(See *Chromatography ⟨621⟩, System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; packing L3

▲**Flow rate:** 1.5 mL/min▲ (USP 1-May-2020)

Injection volume: 5–10 µL

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for precholecalciferol, *trans*-cholecalciferol, and cholecalciferol are 0.4, 0.5, and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.0 between *trans*-cholecalciferol and precholecalciferol

Relative standard deviation: NMT 2.0% for the peak response of cholecalciferol

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of cholecalciferol ($C_{27}H_{44}O$) in the portion of Cholecalciferol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Cholecalciferol RS in the *Standard solution* (µg/mL)

C_U = concentration of Cholecalciferol in the *Sample solution* (µg/mL)

Acceptance criteria: 97.0%–103.0%

SPECIFIC TESTS

• **OPTICAL ROTATION** ⟨781S⟩, *Procedures, Specific Rotation*

Sample solution: 5 mg/mL in alcohol. [NOTE—Prepare and use the solution without delay. Use Cholecalciferol from a container opened not longer than 30 min.]

Acceptance criteria: +105° to +112°

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in hermetically sealed containers under nitrogen, and store in a cool place protected from light.

- **USP Reference Standards ⟨11⟩**

USP Cholecalciferol RS
USP Vitamin D Assay System Suitability RS

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
CHOLECALCIFEROL	Natalia Davydova Scientific Liaison	NBDS2020 Non-botanical Dietary Supplements

Chromatographic Database Information: Chromatographic Database

Most Recently Appeared In:
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