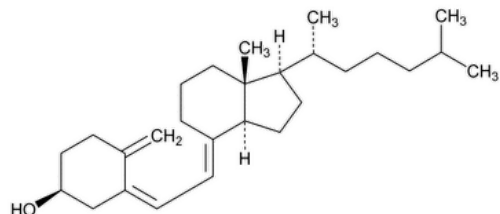


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# Cholecalciferol



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

9,10-Secocholesta-5,7,10(19)-trien-3-ol, (3 $\beta$ ,5Z,7E)-;

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  $\mu$ m

**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  $\mu$ g/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  $\mu$ L

**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 | <a href="#">Natalia Davydova</a><br>Scientific Liaison | NBDS2020 Non-botanical Dietary Supplements |

**Chromatographic Database Information:** [Chromatographic Database](#)

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