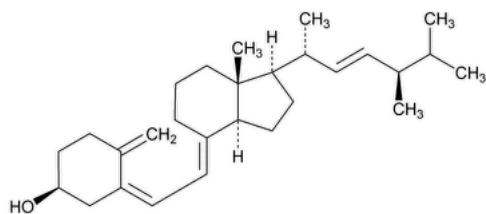


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## Ergocalciferol



$C_{28}H_{44}O$  396.65  
9,10-Secoergosta-5,7,10 (19),22-tetraen-3-ol, (3 $\beta$ ,5Z,7E,22E)-;  
Ergocalciferol CAS RN®: 50-14-6.

### DEFINITION

Ergocalciferol contains NLT 97.0% and NMT 103.0% of ergocalciferol ( $C_{28}H_{44}O$ ).

### 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 solutions* 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 A:** 50 mg/mL of [USP Ergocalciferol RS](#) in *Diluent*

**Standard solution B:** 100  $\mu$ g/mL of [USP Ergosterol RS](#) in *Diluent*

**Sample solution:** 50 mg/mL of Ergocalciferol 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 ether (1:1)

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

#### **Analysis**

**Samples:** *Standard solution A*, *Standard solution B*, 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 (ergocalciferol) having the same  $R_f$  value as the area of *Standard solution A* and may show a violet area below the ergocalciferol area. The color of the violet area is not more intense than that of the violet area from *Standard solution B*. ▲ (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 Ergocalciferol RS](#) in [toluene](#)

**Standard solution:** 120 µg/mL of [USP Ergocalciferol RS](#) in *Mobile phase*, prepared from *Standard stock solution*

**Sample stock solution:** 0.6 mg/mL of Ergocalciferol in [toluene](#)

**Sample solution:** 120 µg/mL of Ergocalciferol 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 ergocalciferol ( $C_{28}H_{44}O$ ) in the portion of Ergocalciferol 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 Ergocalciferol RS](#) in the *Standard solution* (µg/mL)

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

**Acceptance criteria:** 97.0%–103.0%

## IMPURITIES

### • REDUCING SUBSTANCES

**Standard solution:** 0.2 µg/mL of [hydroquinone](#) in [dehydrated alcohol](#)

**Sample solution:** 10 mg/mL of Ergocalciferol in [dehydrated alcohol](#)

**Blank:** [Dehydrated alcohol](#)

### Analysis

**Samples:** *Standard solution*, *Sample solution*, and *Blank*

To 10 mL each of *Standard solution*, *Sample solution*, and *Blank*, add 0.5 mL of 5 mg/mL [blue tetrazolium](#) in [methanol](#). Then add 0.5 mL of [tetramethylammonium hydroxide TS](#) in [dehydrated alcohol](#) (1 in 10). Allow the mixture to stand for 5 min, accurately timed, then add 1 mL of [glacial acetic acid](#). Determine the absorbance of the solution at 525 nm, with a suitable spectrometer, against the *Blank*.

**Acceptance criteria:** The absorbance of the *Sample solution* is NMT that of the *Standard solution*.

## SPECIFIC TESTS

- [MELTING RANGE OR TEMPERATURE \(741\)](#), [Procedures, Procedure for Class Ib, Apparatus I](#) and [Procedure for Class Ib, Apparatus II](#): 115°–119°
- [OPTICAL ROTATION \(781S\)](#), [Procedures, Specific Rotation](#)

**Sample solution:** 15 mg/mL in [alcohol](#). [NOTE—Prepare and use the solution without delay. Use Ergocalciferol from a container opened not longer than 30 min.]

**Acceptance criteria:** +103° to +106°

#### ADDITIONAL REQUIREMENTS

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

**Change to read:**

- **USP REFERENCE STANDARDS (11).**  
[USP Ergocalciferol RS](#)

▲ (USP 1-May-2020)  
[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
ERGOCALCIFEROL	<a href="#">Natalia Davydova</a> Scientific Liaison	NBDS2020 Non-botanical Dietary Supplements

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

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