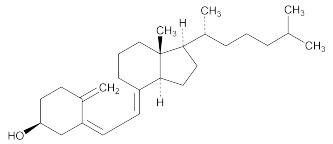
mg, of $C_7H_4ClNO_2$ in the portion of T ablets taken by the formula:

$$1000C(R_U / R_S)$$

in which C is the concentration, in mg per mL, of USP Chlorzoxazone RS in the *Standard preparation*; and R_U and R_S are the peak response ratios of the chlorzoxazone peak to the phenacetin peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Cholecalciferol



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

9,10-Secocholesta-5,7,10(19)-trien-3-ol, (3*β*,5*Z*,7*E*)-; Cholecalciferol [67-97-0].

DEFINITION

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

IDENTIFICATION

- **A. Infrared Absorption** ⟨197K⟩
  Wavelength range: 2–12 µm
- **B. Ultraviolet Absorption** ⟨197U⟩
  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%.
- **C.**
  Sample solution: 0.5 mg in 5 mL of chloroform
  Analysis: Add 0.3 mL of acetic anhydride and 0.1 mL of sulfuric acid to the *Sample solution*, and shake vigorously.
  Acceptance criteria: A bright red color is produced, and it rapidly changes through violet and blue to green.
- **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.

ASSAY

- **Procedure**
  Dehydrated hexane: Prepare a chromatographic column by packing a chromatographic tube, 8 × 60 cm, with 500 g of 50- to 250-µm chromatographic siliceous earth, activated by drying at 150° for 4 h. (See *Chromatography* ⟨621⟩, *Column Chromatography*.) Pass 500 mL of hexane through the column, and collect the eluate in a glass-stoppered flask.
  Mobile phase: *n*-Amyl alcohol in *Dehydrated hexane* (3 in 1000)
  System suitability solution: 250 mg of USP V itamin 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
  Injection size: 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,** *Specific Rotation* ⟨781S⟩
  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

Cholecalciferol Solution

DEFINITION
Cholecalciferol Solution is a solution of Cholecalciferol in an edible vegetable oil, in Polysorbate 80, or in Propylene Glycol. It contains NLT 90.0% and NMT 120.0% of the labeled amount of vitamin D as cholecalciferol ($C_{27}H_{44}O$).

ASSAY
• **PROCEDURE**
Mobile phase: Hexane and pentanol (997:3)
Standard stock solution: Dissolve USP Cholecalciferol RS in toluene, and dilute with *Mobile phase* to 50 µg/mL. [NOTE—Prepare this solution fresh daily.]
Standard solution A: 5 µg/mL from *Standard stock solution* in *Mobile phase*. [NOTE—Store at a temperature not above 0°.]
Standard solution B: Transfer 5.0 mL of *Standard stock solution* to a round-bottom flask fitted with a reflux condenser. Displace the air with nitrogen, and reflux for 1 h in a water bath under a nitrogen atmosphere to obtain a solution containing cholecalciferol and precholecalciferol. Cool, transfer the solution with the aid of several portions of *Mobile phase* to a 50-mL volumetric flask, and dilute with *Mobile phase* to volume.
Sample solution: Equivalent to 5 µg/mL of cholecalciferol in *Mobile phase* from an accurately measured volume of Cholecalciferol Solution
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L3
Flow rate: 2 mL/min
Injection size: 10 µL
System suitability
Sample: *Standard solution B*
[NOTE—The relative retention times for precholecalciferol and cholecalciferol are about 0.4 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.0 between the precholecalciferol peak and the cholecalciferol peak
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*
Cholecalciferol response factor
Calculate the cholecalciferol response factor, F_C:

$$F_C = C_S/r_S$$

C_S = concentration of USP Cholecalciferol RS in *Standard solution A* (µg/mL)
r_S = peak area of cholecalciferol from *Standard solution A*

Pre-cholecalciferol response factor
Calculate the concentration, C'_S, in µg/mL, of cholecalciferol in *Standard solution B*:

$$C'_S = F_C \times r'_S$$

F_C = response factor for cholecalciferol
r'_S = peak area of cholecalciferol from *Standard solution B*

Calculate the concentration, C'_{pre}, in µg/mL, of precholecalciferol:

$$C'_{pre} = C_S - C'_S$$

C_S = concentration of USP Cholecalciferol RS in *Standard solution A* (µg/mL)
C'_S = concentration of cholecalciferol in *Standard solution B* (µg/mL)

Calculate the response factor, F_{pre}, for pre-cholecalciferol:

$$F_{pre} = C'_{pre}/r_p$$

C'_{pre} = concentration of pre-cholecalciferol (µg/mL)
r_p = peak response of pre-cholecalciferol from *Standard solution B*

Content of vitamin D
Calculate the percentage of the labeled amount of vitamin D as cholecalciferol ($C_{27}H_{44}O$) in the portion of the Cholecalciferol Solution taken:

$$\text{Result} = \{[(F_C \times r_C) + (F_{pre} \times r_{pre})]/C_U\} \times 100$$

F_C = response factor for cholecalciferol
r_C = peak area of cholecalciferol from the *Sample solution*
F_{pre} = response factor for pre-cholecalciferol
r_{pre} = peak area of pre-cholecalciferol from the *Sample solution*
C_U = nominal concentration of cholecalciferol in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–120.0%

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
• **LABELING:** Label it to indicate the concentration, in mg/mL, of cholecalciferol. Label it also to state that it is to be used for manufacturing only.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Cholecalciferol RS

Cholestyramine Resin

Cholestyramine.
Cholestyramine [11041-12-6].

» Cholestyramine Resin is a strongly basic anion-exchange resin in the chloride form, consisting of styrene-divinylbenzene copolymer with quaternary ammonium functional groups. Each g exchanges not less than 1.8 g and not more than 2.2 g of sodium glycocholate, calculated on the dried basis.

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩**—**
USP Cholestyramine Resin RS

Identification—*Infrared Absorption* ⟨197K⟩.

pH ⟨791⟩**:** between 4.0 and 6.0, in a slurry (1 in 100).

Loss on drying ⟨731⟩**—**Dry over phosphorus pentoxide at a pressure not exceeding 50 mm of mercury at 70° for 16 hours: it loses not more than 12.0% of its weight.

Residue on ignition ⟨281⟩**:** not more than 0.1%.

Heavy metals, *Method II* ⟨231⟩**:** 0.002%.

Dialyzable quaternary amines—

pH 9.2 Buffer—Transfer 3.80 g of sodium borate decahydrate to a 1000-mL volumetric flask, dissolve in and dilute with water to volume, and mix.

Bromothymol blue solution—Transfer 150 mg of bromothymol blue and 405 mg of sodium carbonate to a 100-mL volumetric flask, dilute with water to volume, and mix.

Standard solution—Take 1 mL of 60% benzyltrimethylammonium chloride solution, accurately pipeted, and dilute quan-