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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 pre-cholecalciferol:

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

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](#)

Auxiliary Information - Please [check for your question in the FAQs](#) before contacting USP.

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

Chromatographic Database Information: [Chromatographic Database](#)

Most Recently Appeared In:

Pharmacopeial Forum: Volume No. Information currently unavailable

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