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Ergocalciferol Capsules

DEFINITION

Ergocalciferol Capsules usually consist of an edible vegetable oil solution of Ergocalciferol, encapsulated with Gelatin. Ergocalciferol Capsules contain NLT 100.0% and NMT 120.0% of the labeled amount of vitamin D as ergocalciferol ($C_{28}H_{44}O$).

ASSAY

Change to read:

• **Procedure**

[Note—Throughout this *Assay*, protect solutions containing, and derived from, the test specimen and the Reference Standard from the atmosphere and light, preferably by the use of a blanket of inert gas and low-actinic glassware.]

▲▲ (USP 1-Aug-2019)

Butylated hydroxytoluene solution: 10 mg/mL of butylated hydroxytoluene in *n*-hexane

Aqueous potassium hydroxide solution: 1 g/mL of potassium hydroxide in freshly boiled water. [Note—Prepare solution fresh daily.]

Alcoholic potassium hydroxide solution: 3 g of potassium hydroxide in 50 mL of freshly boiled water. Add 10 mL of alcohol, and dilute with freshly boiled water to 100 mL. [Note—Prepare solution fresh daily.]

Sodium ascorbate solution: 175 mg/mL of ascorbic acid in 1 N sodium hydroxide. [Note—Prepare solution fresh daily.]

Sodium sulfide solution: 12 g of sodium sulfide in 20 mL of water. Dilute with glycerin to 100 mL.

Mobile phase: ▲*n*-Hexane▲ (USP 1-Aug-2019) and *n*-amyl alcohol (997:3). The ratio of components and the flow rate may be varied to meet the *System suitability* requirements.

System suitability solution: Transfer 100 mg of USP Vitamin D Assay System Suitability RS to a 10-mL volumetric flask. Add a mixture of toluene and *Mobile phase* (1 in 5) to volume, and mix. Heat a portion of this solution under reflux, at 90° for 45 min, and cool.

Standard stock solution: 0.5 mg/mL of USP Ergocalciferol RS in toluene. [Note—Prepare solution fresh daily.]

Standard solution A: 20 µg/mL from the *Standard stock solution* in *Mobile phase*. [Note—Store this solution at a temperature NMT 0°.]

Standard solution B: Pipet 4 mL of the *Standard stock solution* into a round-bottomed flask fitted with a reflux condenser, and then add 2 or 3 crystals of butylated hydroxytoluene. Displace the air with nitrogen, and heat in a water bath maintained at a temperature of 90° in subdued light under a nitrogen atmosphere for 45 min to obtain a solution containing vitamin D and pre-vitamin D. Cool, transfer with the aid of several portions of *Mobile phase* to a 100-mL volumetric flask, and dilute with *Mobile phase* to volume.

Sample solution: Reflux NLT 10 Capsules with a mixture of 10 mL of *Sodium ascorbate solution* and 2 drops of *Sodium sulfide solution* on a steam bath for 10 min, crush any remaining solids with a blunt glass rod, and continue heating for 5 min. Cool, and add 25 mL of alcohol and 3 mL of *Aqueous potassium hydroxide solution*. Reflux the mixture on a steam bath for 30 min. Cool rapidly under running water, and transfer the saponified mixture to a conical separator, rinsing the saponification flask with two 15-mL portions of water, 10 mL of alcohol, and two 50-mL portions of ether. [Note—Use ether within 24 h after opening the container.] Shake the combined saponified mixture and rinsings vigorously for 30 s, and allow to stand until both layers are clear. Transfer the aqueous phase to a second conical separator, add a mixture of 10 mL of alcohol and 50 mL of solvent hexane, and shake vigorously. Allow to separate, transfer the aqueous phase to a third conical separator, and transfer the solvent hexane phase to the first separator, rinsing the second separator with two 10-mL portions of solvent hexane and adding the rinsings to the first separator. Shake the aqueous phase in the third separator with 50 mL of solvent hexane, and add the solvent hexane phase to the first separator. Wash the combined ether–solvent hexane extracts by shaking vigorously with three 50-mL portions of *Alcoholic potassium hydroxide solution*, and wash with 50-mL portions of water vigorously until the last washing is neutral to phenolphthalein. Drain any remaining drops of water from the combined ether–solvent hexane extracts, add 2 sheets of 9-cm filter paper, in strips, to the separator, and shake. Transfer the washed ether–solvent hexane extracts to a round-bottomed flask, rinsing the separator and paper with solvent hexane. Combine the solvent hexane rinsings with the ether–solvent hexane extracts, add 100 µL of *Butylated hydroxytoluene solution*, and mix. Evaporate under vacuum to dryness by swirling in a water bath maintained at a temperature NMT 40°. Cool under running water, and introduce nitrogen sufficient to restore atmospheric pressure. Without delay, dissolve and dilute the residue in an accurately measured volume of a mixture of toluene and *Mobile phase* (1 in 5), until the nominal concentration of vitamin D is about 25 µg/mL.

Chromatographic system

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

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; packing L3

Injection volume: 20 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 cholecalciferol

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Ergocalciferol response factor

Calculate the *Ergocalciferol response factor* (F_D):

$$F_D = C_S/r_S$$

C_S = concentration of USP Ergocalciferol RS in *Standard solution A* (µg/mL)

r_S = peak response of ergocalciferol from *Standard solution A*

Pre-ergocalciferol response factor

Calculate the concentration of ergocalciferol (C'_S), in µg/mL, in *Standard solution B*:

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

F_D = *Ergocalciferol response factor*, as previously determined

r'_S = peak area of ergocalciferol from *Standard solution B*

Calculate the concentration of pre-ergocalciferol (C'_{pre}), in µg/mL, in *Standard solution B*:

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

C_S = concentration of USP Ergocalciferol RS in *Standard solution A* (µg/mL)

C'_S = concentration of ergocalciferol in *Standard solution B* (µg/mL)

Calculate the *Pre-ergocalciferol response factor* (F_{pre}):

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

C'_{pre} = concentration of pre-ergocalciferol in *Standard solution B* (µg/mL)

r'_{pre} = peak response of pre-ergocalciferol from *Standard solution B*

[NOTE—The value of F_{pre} determined in duplicate, on different days, can be used during the entire procedure.]

Vitamin D content

Calculate the percentage of the labeled amount of vitamin D as ergocalciferol ($C_{28}H_{44}O$) in the portion of Capsules taken:

$$\text{Result} = \{[(F_D \times r_C) + (F_{pre} \times r'_{pre})]/C_U\} \times 100$$

F_D = *Ergocalciferol response factor*, as previously determined

r_C = peak area of ergocalciferol from the *Sample solution*

F_{pre} = *Pre-ergocalciferol response factor*, as previously determined

r'_{pre} = peak area of pre-ergocalciferol from the *Sample solution*

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

Acceptance criteria: 100.0%–120.0%

PERFORMANCE TESTS

• **DISINTEGRATION ⟨701⟩**

Buffer solution: 0.05 M acetate buffer, prepared by mixing 2.99 g of sodium acetate and 1.66 mL of glacial acetic acid with water to obtain a 1000-mL solution having a pH of 4.5 ± 0.05

Immersion fluid: *Buffer solution*

Time: 45 min

Acceptance criteria: Meet the requirements

• **Uniformity of Dosage Units ⟨905⟩:** Meet the requirements

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight, light-resistant containers.

Change to read:

• **Labeling:** Label the Capsules to indicate the content of ergocalciferol in ▲micrograms. Expression of the amount of ergocalciferol in terms of units may be added in parentheses after the mass units.[1]▲ (USP 1-Aug-2019)

• **USP Reference Standards ⟨11⟩**

USP Ergocalciferol RS

USP Vitamin D Assay System Suitability RS

[1] Where articles are labeled in terms of units in addition to the required labeling, the relationship of the USP Units or International Units (IU) to mass is as follows: 1 μg of cholecalciferol or ergocalciferol = 40 USP Units or IU.

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

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

Chromatographic Database Information: Chromatographic Database

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