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Calcium with Vitamin D Tablets

DEFINITION

Calcium with Vitamin D Tablets contain NLT 90.0% and NMT 125.0% of the labeled amount of calcium (Ca), derived from substances generally recognized as safe, and NLT 90.0% and NMT 165.0% of the labeled amount of vitamin D as cholecalciferol ($C_{27}H_{44}O$) or ergocalciferol ($C_{28}H_{44}O$). They contain no other vitamins or minerals for which nutritional value is claimed. They may contain other labeled added substances or additional ingredients in amounts that are unobjectionable.

IDENTIFICATION

Add the following:

- ▲ **A.** Characteristic absorption of calcium as obtained in the tests in *Strength* ▲ (USP 1-May-2020)

Add the following:

- ▲ **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the test for Vitamin D (Cholecalciferol or Ergocalciferol), *Method 2*. ▲ (USP 1-May-2020)

STRENGTH

Add the following:

▲ [NOTE—In the following assays, where more than one assay method is given for an individual ingredient, the requirements may be met by following any one of the specified methods, with the method used being stated in the labeling only if *Method 1* is not used.] ▲ (USP 1-MAY-2020)

• CALCIUM

[NOTE—A commercially available atomic absorption standard solution for calcium may be used where preparation of a *Calcium standard stock solution* is described in the following assay. Concentrations of the *Standard solutions* and the *Sample stock solution* may be modified to fit the linear or working range of the instrument.]

Lanthanum chloride solution: Dissolve 26.7 g of [lanthanum chloride](#) in 0.125 N [hydrochloric acid](#) to make 100 mL.

Calcium standard stock solution: Weigh 1.001 g of [calcium carbonate](#), [chelometric standard](#), previously dried at 300° for 3 h and cooled in a desiccator for 2 h. Dissolve in 25 mL of 1 N [hydrochloric acid](#). Boil to expel carbon dioxide, and dilute with water to 1000 mL to obtain a solution with a concentration of 400 µg/mL of calcium.

Standard stock solution: 100 µg/mL of calcium from a volume of *Calcium standard stock solution* in 0.125 N [hydrochloric acid](#)

Standard solutions: Into separate 100-mL volumetric flasks separately pipet 1.0, 1.5, 2.0, 2.5, and 3.0 mL of the *Standard stock solution*. To each flask add 1.0 mL of *Lanthanum chloride solution*, dilute with water to volume, and obtain *Standard solutions* with concentrations of 1.0, 1.5, 2.0, 2.5, and 3.0 µg/mL, of calcium.

Sample stock solution: Weigh and finely powder NLT 20 Tablets. Transfer the equivalent to 500 mg of calcium, in 25 mL of [hydrochloric acid](#) and heat for 30 min on a steam bath. Cool, dilute with water to 1000 mL, and filter.

Sample solution: Quantitatively dilute a volume of *Sample stock solution* with 0.125 N [hydrochloric acid](#) to obtain a concentration of 100 µg/mL of calcium. Transfer 2.0 mL of this solution to a 100-mL volumetric flask, add 1.0 mL of *Lanthanum chloride solution*, and dilute with [water](#) to volume.

Instrumental conditions

(See [Atomic Absorption Spectroscopy \(852\)](#).)

Mode: Atomic absorption spectrophotometer

Lamp: Calcium hollow-cathode

Flame: Nitrous oxide–acetylene

Analytical wavelength: Calcium emission line, 422.7 nm

Blank: 1 mL of *Lanthanum chloride solution* per 100 mL of 0.125 N [hydrochloric acid](#)

Analysis

Samples: *Standard solutions* and *Sample solution*

Determine the absorbances of the solutions, against the *Blank*. Plot the absorbances of the *Standard solutions* versus the concentration, in µg/mL, of calcium, and draw the straight line best fitting the 5 plotted points. From the graph, determine the concentration (C), in µg/mL, of calcium in the *Sample solution*.

Calculate the percentage of the labeled amount of calcium (Ca) in the portion of Tablets taken:

$$\text{Result} = (C/C_p) \times 100$$

C = measured concentration of calcium in the *Sample solution* ($\mu\text{g/mL}$)

C_U = nominal concentration of calcium in the *Sample solution* ($\mu\text{g/mL}$)

Acceptance criteria: 90.0%–125.0% of the labeled amount of calcium (Ca)

Change to read:

• **VITAMIN D (CHOLECALCIFEROL OR ERGOCALCIFEROL), ▲Method 1**

[NOTE—Where the use of vitamin D (cholecalciferol or ergocalciferol) is specified in the following procedure, use the chemical form present in the formulation and the relevant Reference Standard. ▲ (USP 1-May-2020) Use low-actinic glassware throughout this procedure.]

Mobile phase: [n-Hexane](#) and [isopropyl alcohol](#) (99:1)

Standard solution: 2 $\mu\text{g/mL}$ of [USP Ergocalciferol RS](#) or [USP Cholecalciferol RS](#) in [n-hexane](#)

System suitability solution: Heat a volume of *Standard solution* at 60° for 1 h to partially isomerize vitamin D (ergocalciferol or cholecalciferol) to its corresponding precursor.

Sample solution: Weigh and grind NLT 20 Tablets. Transfer the equivalent to 20 μg of cholecalciferol or ergocalciferol to a container having a polytetrafluoroethylene-lined screw-cap. Add 8 mL of dimethyl sulfoxide and 12 mL of [n-hexane](#), and shake for 45 min on a wrist-action shaker with tubes in a water bath maintained at 60°. Centrifuge for 10 min, withdraw the hexane layer by means of a pipet, and transfer to an evaporation flask. Add 12 mL of [n-hexane](#) to the dimethyl sulfoxide layer, mix on a vortex mixer for 5 min, and again withdraw the hexane layer by means of a pipet and add to the evaporation flask. Repeat this extraction with 3 additional 12-mL portions of [n-hexane](#), adding the hexane extracts to the evaporation flask. Evaporate the combined hexane extracts under vacuum at room temperature to dryness. Dissolve in and dilute the residue in a volume of [n-hexane](#) to obtain a concentration of 2 $\mu\text{g/mL}$.

Chromatographic system

(See [Chromatography \(621\), System Suitability](#).)

Mode: LC

Column: 4.6-mm $\times$ 15-cm; 3- μm packing [L8](#)

Detector: UV 265 nm

Flow rate: 1 mL/min

Injection volume: 100 μL

System suitability

Samples: *Standard solution* and *System suitability solution*

Suitability requirements

Resolution: NLT 10 between the vitamin D form present and its corresponding precursor, *System suitability solution*

Relative standard deviation: NMT 3.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Measure the peak areas for vitamin D.

Calculate the percentage of the labeled amount of cholecalciferol ($\text{C}_{27}\text{H}_{44}\text{O}$) or ergocalciferol ($\text{C}_{28}\text{H}_{44}\text{O}$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times F \times 100$$

r_U = peak area of cholecalciferol or ergocalciferol from the *Sample solution*

r_S = peak area of cholecalciferol or ergocalciferol from the *Standard solution*

C_S = concentration of [USP Cholecalciferol RS](#) or [USP Ergocalciferol RS](#) in the *Standard solution* ($\mu\text{g/mL}$)

C_U = nominal concentration of cholecalciferol or ergocalciferol in the *Sample solution* ($\mu\text{g/mL}$)

F = correction factor to account for the average amount of previtamin D present in the *Sample solution*, 1.09

Acceptance criteria: 90.0%–165.0% of the labeled amount of vitamin D as cholecalciferol ($\text{C}_{27}\text{H}_{44}\text{O}$) or ergocalciferol ($\text{C}_{28}\text{H}_{44}\text{O}$)

Add the following:

▲ • **VITAMIN D (CHOLECALCIFEROL OR ERGOCALCIFEROL), Method 2**

[NOTE—Where the use of a vitamin D (cholecalciferol or ergocalciferol) is specified in the following procedure, use the chemical form present in the formulation and the relevant Reference Standard. Use low-actinic glassware throughout this procedure.]

Diluent: [Water](#), [dimethylformamide](#), and [isopropyl alcohol](#) (5:10:35, v/v)

Mobile phase: [Acetonitrile](#) and [water](#) (70:30)

Cholecalciferol standard stock solution: 0.5 mg/mL of [USP Cholecalciferol RS](#) in [isopropyl alcohol](#)

Ergocalciferol standard stock solution: 0.5 mg/mL of [USP Ergocalciferol RS](#) in [isopropyl alcohol](#)

System suitability solution: Combine equal volumes of *Cholecalciferol standard stock solution* and *Ergocalciferol standard stock solution*. Dilute with *Diluent* to obtain a concentration of about 1 $\mu\text{g/mL}$ each of cholecalciferol and ergocalciferol.

Standard solution: 1 $\mu\text{g/mL}$ of [USP Cholecalciferol RS](#) or [USP Ergocalciferol RS](#) in *Diluent* from the *Cholecalciferol standard stock solution* or the *Ergocalciferol standard stock solution*, prepared accurately