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Calcium and Vitamin D with Minerals Tablets

DEFINITION

Calcium and Vitamin D with Minerals Tablets contain vitamin D as Ergocalciferol (vitamin D_2) or Cholecalciferol (vitamin D_3), Calcium, and one or more minerals derived from substances generally recognized as safe, furnishing one or more of the following elements in ionizable form: copper, magnesium, manganese, and zinc. Tablets contain NLT 90.0% and NMT 165.0% of the labeled amount of vitamin D as cholecalciferol ($C_{27}H_{44}O$) or ergocalciferol ($C_{28}H_{44}O$), and NLT 90.0% and NMT 125.0% of the labeled amount of calcium (Ca), copper (Cu), magnesium (Mg), manganese (Mn), and zinc (Zn). Calcium and Vitamin D with Minerals Tablets may contain other labeled added substances that are generally recognized as safe, in amounts that are unobjectionable.

IDENTIFICATION

Add the following:

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

Add the following:

▲• **B.** The retention time of the vitamin D 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

Change to read:

▲[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)

Change to read:

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

[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.▲ (USP 1-May-2020) Use low-actinic glassware throughout this procedure.]

Mobile phase: *n-Hexane* and isopropyl alcohol (99:1)

Standard solution: 2 µg/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 polytef-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 µg/mL.

Chromatographic system

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

Mode: LC

Detector: UV 265 nm

Column: 4.6-mm × 15-cm; 5-µm packing L8

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*

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Measure the responses for the vitamin D peaks.

Calculate the percentage of the labeled amount of cholecalciferol ($C_{27}H_{44}O$) or ergocalciferol ($C_{28}H_{44}O$) in the portion of Tablets taken:

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

r_U = peak height for cholecalciferol or ergocalciferol from the *Sample solution*

r_S = peak height for cholecalciferol or ergocalciferol from the *Standard solution*

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

C_U = nominal concentration of ergocalciferol or cholecalciferol in the *Sample solution* (µg/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 cholecalciferol ($C_{27}H_{44}O$) or ergocalciferol ($C_{28}H_{44}O$)

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 µg/mL each of cholecalciferol and ergocalciferol.

Standard solution: 1 µg/mL of USP Cholecalciferol RS or USP Ergocalciferol RS in *Diluent* from the *Cholecalciferol standard stock solution* or *Ergocalciferol standard stock solution*, prepared accurately

Sample solution: Finely powder NLT 20 Tablets. Transfer a portion of the powder, nominally equivalent to 50 µg of cholecalciferol (or ergocalciferol), into a 50-mL volumetric flask, add 5 mL of water, and sonicate for 10 min with intermittent shaking. Add 10 mL of dimethylformamide, sonicate for 15 min with intermittent shaking, cool to room temperature, and dilute with isopropyl alcohol to volume. Mix well and pass through a nylon filter of 0.45-µm pore size.

Chromatographic system

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

Mode: LC

Detector: UV 265 nm

Column: 4.6-mm × 15-cm; 2.7-µm packing L96

Column temperature: 40°

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

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

[Note—The relative retention times for cholecalciferol and ergocalciferol are 1.0 and 1.1, respectively.]

Suitability requirements

Resolution: NLT 1.5 between the cholecalciferol and ergocalciferol peaks, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of cholecalciferol ($C_{27}H_{44}O$) or ergocalciferol ($C_{28}H_{44}O$) in the portion of Tablets taken:

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

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* (µg/mL)

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

Acceptance criteria: 90.0%–165.0%▲ (USP 1-May-2020)

• **Calcium,** *Method 1*

[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

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linear or working range of the instrument.]

Lanthanum chloride solution: 267 mg/mL of lanthanum chloride heptahydrate in 0.125 N hydrochloric acid

Calcium standard stock solution: 400 µg/mL of calcium. Dissolve 1.001 g of calcium carbonate, 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.

Standard stock solution: 100 µg/mL of calcium from *Calcium standard stock solution* diluted with 0.125 N hydrochloric acid

Standard solutions: Into separate 100-mL volumetric flasks 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*, and dilute with water to volume to 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. Mix a portion of the powder equivalent to a nominal amount of 500 mg of calcium with 25 mL of concentrated 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 the *Sample stock solution* with 0.125 N hydrochloric acid to obtain a nominal 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 spectrophotometry

Lamp: Calcium hollow-cathode

Flame: Nitrous oxide–acetylene

Analytical wavelength: Calcium emission line, 422.7 nm

Blank: 0.125 N hydrochloric acid containing 1 mL of *Lanthanum chloride solution* per 100 mL

Analysis

Samples: *Standard solutions* and *Sample solution*

Determine the absorbances of the solutions against the *Blank*. Plot the absorbances of the *Standard solutions* versus 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:

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

C = measured concentration of calcium in the *Sample solution* (µg/mL)

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

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

• **Copper,** *Method 1*

Copper standard solution: Dissolve 1.00 g of copper foil in a minimum volume of a 50% (v/v) solution of nitric acid solution, and dilute with a 1% (v/v) solution of nitric acid to 1000 mL. This solution contains 1000 µg/mL of copper.

Standard stock solution: 100 µg/mL of copper from *Copper standard solution* diluted with 0.125 N hydrochloric acid

Standard solutions: To separate 200-mL volumetric flasks transfer 1.0, 2.0, 4.0, 6.0, and 8.0 mL of the *Standard stock solution*. Dilute with water to volume to obtain concentrations of 0.5, 1.0, 2.0, 3.0, and 4.0 µg/mL of copper.

Sample solution: Weigh and finely powder NLT 20 Tablets. Transfer the equivalent of 5 mg of copper from powdered Tablets to a porcelain crucible. Heat for 6–12 h in a muffle furnace maintained at 550°, and cool. Add 15 mL of hydrochloric acid, and boil gently on a hot plate or a steam bath for 30 min, intermittently rinsing the inner surface of the crucible with 6 N hydrochloric acid. Cool, and quantitatively transfer the contents of the crucible to a 100-mL volumetric flask, rinsing the crucible with portions of 6 N hydrochloric acid. Dilute the contents of the flask with water to volume, and filter, discarding the first 5 mL of the filtrate. Dilute the filtrate quantitatively with 0.125 N hydrochloric acid to obtain a concentration of 2 µg/mL of copper.

Instrumental conditions

(See *Atomic Absorption Spectroscopy (852)*.)

Mode: Atomic absorption spectrophotometry

Lamp: Copper hollow-cathode

Flame: Air–acetylene

Analytical wavelength: Copper emission line, 324.7 nm

Blank: 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 concentration, in µg/mL, of copper, and draw the straight line best fitting the 5 plotted points. From the graph, determine the concentration (*C*), in µg/mL, of copper in the *Sample solution*.

Calculate the percentage of the labeled amount of copper (Cu) in the portion of Tablets taken:

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

C = measured concentration of copper in the *Sample solution* (µg/mL)

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