tents of the crucible to a 100-mL volumetric flask. Rinse the crucible with small portions of 6 N hydrochloric acid, and add the rinsings to the flask. Dilute with water to volume, and filter, discarding the first 5 mL of the filtrate. Dilute this solution quantitatively, with 0.125 N hydrochloric acid to obtain a concentration of 2 µg/mL of calcium, adding 1 mL of *Lanthanum chloride solution* per 100 mL of the final volume.

Instrumental conditions
(See *Atomic Absorption Spectroscopy* ⟨852⟩.)
Mode: Atomic absorption spectrophotometry
Analytical wavelength: Calcium emission line at 422.7 nm
Lamp: Calcium hollow-cathode
Flame: Nitrous oxide–acetylene
Blank: 0.125 N hydrochloric acid containing 1 mL of *Lanthanum chloride solution* per 100 mL

Analysis
Samples: *Standard solutions* and the *Sample solution*
Determine the absorbances of the solutions, using the *Blank*. From a linear regression equation, calculated using the absorbance of the *Standard solutions* versus concentrations, 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 = determined concentration of calcium in the *Sample solution*
C_U = nominal concentration of calcium in the *Sample solution*

Acceptance criteria: 90.0%–110.0%

CONTAMINANTS
- **MICROBIAL ENUMERATION TESTS ⟨2021⟩:** The total aerobic microbial count does not exceed 1000 cfu/g. The total combined yeasts and molds count does not exceed 100 cfu/g.
- **ABSENCE OF SPECIFIED MICROORGANISMS ⟨2022⟩:** Meet the requirements of the test for absence of *Escherichia coli*

PERFORMANCE TESTS
- **DISINTEGRATION AND DISSOLUTION OF DIETARY SUPPLEMENTS ⟨2040⟩:** Meet the requirements for *Disintegration*, 15 min
- **WEIGHT VARIATION OF DIETARY SUPPLEMENTS ⟨2091⟩:** Meet the requirements

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **LABELING:** The label states the quantity of calcium in terms of mg/Tablet.

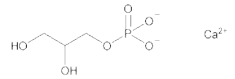
Calcium Glubionate Syrup—*see Calcium Glubionate Syrup General Monographs*

Calcium Gluceptate—*see Calcium Gluceptate General Monographs*

Calcium Gluconate—*see Calcium Gluconate General Monographs*

Calcium Gluconate Tablets—*see Calcium Gluconate Tablets General Monographs*

Calcium Glycerophosphate



$C_3H_7CaO_6P$ 210.14
1,2,3-Propanetriol, mono(dihydrogen phosphate) calcium salt (1:1);
Calcium glycerophosphate [27214-00-2].

DEFINITION
Calcium Glycerophosphate is a mixture, in variable proportions, of calcium (*RS*)-2,3-dihydroxypropyl phosphate and calcium 2-hydroxy-1-(hydroxymethyl)ethyl phosphate, which may be hydrated. Calcium Glycerophosphate contains NLT 18.6% and NMT 19.4% of calcium (Ca), calculated on the dried basis.

IDENTIFICATION
- **A.**
Analysis: Ignite 0.1 g in a crucible. Take up the residue with 5 mL of nitric acid, heat on a water bath for 1 min, and filter. Mix 1 mL of the filtrate with 2 mL of ammonium molybdate TS.
Acceptance criteria: A yellow color develops.
- **B.**
Analysis: Dissolve 20 mg of the substance being examined in 5 mL of 5 M acetic acid, and add 0.5 mL of potassium ferrocyanide solution (53 mg/mL). The resulting solution remains clear. To the clear solution, add 50 mg of ammonium chloride.
Acceptance criteria: A white crystalline precipitate is produced.

ASSAY
- **PROCEDURE**
Sample: 200 mg
Titrimetric system
(See *Titrimetry* ⟨541⟩.)
Mode: Direct titration
Titrant: 0.1 M edetate disodium VS
Endpoint detection: Colorimetric
Blank: 300 mL of water. Add 6 mL of 10 M sodium hydroxide and 15 mg of calconcarboxylic acid triturate.
Analysis: Dissolve the *Sample* in 300 mL of water, add 6 mL of 10 M sodium hydroxide and 15 mg of calconcarboxylic acid triturate. Titrate with *Titrant* until the solution is a distinct blue color.

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Calculate the percentage of calcium (Ca) in the portion of Calcium Glycerophosphate taken:

$$\text{Result} = [(V - B) \times M \times F \times 100]/W$$

V = *Sample* titrant volume (mL)
B = *Blank* titrant volume (mL)
M = titrant molarity (mM/mL)
F = equivalency factor, 40.08 mg/mM
W = weight of the *Sample* (mg)

Acceptance criteria: 18.6%–19.4% on the dried basis

IMPURITIES

- **LEAD ⟨251⟩:** NMT 4 ppm
- **IRON ⟨241⟩**

Standard solution: Dilute 1 volume of *Standard Iron Solution*, prepared as directed in the chapter, with water to 10 volumes (1 µg/mL).

Analysis: Dissolve 0.20 g in 10 mL of water. Add 2 mL of a 20% (w/v) solution of citric acid, 0.1 mL of thioglycolic acid, and mix. Make alkaline with 10 M ammonia, dilute with water to 20 mL, and allow to stand for 5 min. Any pink color produced is not more intense than that obtained by treating 4 mL of the *Standard solution* in the same manner.

Acceptance criteria: NMT 20 ppm

Delete the following:

- **HEAVY METALS,** *Method I* ⟨231⟩**:** NMT 20 ppm (Official 1-Jan-2018)
- **LIMIT OF CHLORIDE**

Standard solution: 8.24 µg/mL of sodium chloride in water

Sample solution: Dissolve 125 mg in a 10-mL mixture of 5 M acetic acid and water (2:8), and dilute with water to 15 mL.

Analysis: To the *Sample solution* add 1 mL of 2 M nitric acid, then add 1 mL of a silver nitrate solution (17 mg/mL), and allow to stand for 5 min protected from light. To 10 mL of the *Standard solution* add 5 mL of water, 1 mL of 2 M nitric acid, and 1 mL of silver nitrate solution (17 mg/mL), and allow to stand for 5 min protected from light. When viewed against a dark background, the *Sample solution* is not more turbid than the *Standard solution*.

Acceptance criteria: NMT 0.04%

- **LIMIT OF SULFATE**

Standard solution: 36.2 µg/mL of potassium sulfate in water

Sample solution: Use the *Sample solution* prepared as directed in the test for *Appearance of Solution*.

Analysis: To 15 mL of the *Standard solution* add 0.5 mL of 5 M acetic acid and 1 mL of barium chloride solution (250 mg/mL). Repeat, using 15 mL of the *Sample solution*. Allow the solutions to stand for 5 min protected from light. When viewed against a dark background, the *Sample solution* is not more turbid than the *Standard solution*.

Acceptance criteria: NMT 0.2%

- **LIMIT OF ARSENIC**

Standard stock solution: In a 250-mL volumetric flask dissolve 330 mg of arsenic trioxide in 5 mL of 2 N sodium hydroxide, and dilute with water to volume. Transfer 1 mL of this solution to a 100-mL volumetric flask, and dilute with water to volume.

Standard solution: Transfer 1 mL of *Standard stock solution* into a 10-mL volumetric flask, and dilute with water volume.

Tin(II) chloride solution: Heat 20 g of tin with 85 mL of hydrochloric acid until no more hydrogen is released. Allow to cool. Dilute 1 volume of this solution with 10 volumes of dilute hydrochloric acid (200 mg/mL of hydrochloric acid in water).

Sample solution: Transfer 330 mg of Calcium Glycerophosphate to a 25.0-mL volumetric flask, and dilute with water to volume.

Apparatus: Prepare a 100-mL side-arm conical flask containing a magnetic stirring bar. Attach to the conical flask a ground-glass stopper through which passes a glass tube 20-cm long, with an internal diameter of 5 mm. The lower end of the tube is inside the conical flask and has been drawn to a tip with an internal diameter of 1 mm. An orifice 3 mm in diameter is 15 mm from the tip and at least 3 mm below the lower surface of the stopper. The upper end of the tube has a flat ground surface at a right angle to the axis of the tube. A second glass tube of the same internal diameter and 30 mm long, with a similar flat ground surface, is placed in contact with the ground surface of the first tube and is held in position by a clamp and springs. Into the lower tube, insert 55 mg of loosely packed lead acetate cotton. Place a disk of mercuric bromide paper between the flat surfaces of the tubes.

Analysis: Before placing the tube assembly into the flask, transfer the *Sample solution* to the flask, and add 15.0 mL of hydrochloric acid, 0.1 mL of *Tin(II) chloride solution*, and 5 mL of potassium iodide TS. Allow to stand for 15 min, and add 5 g of activated zinc. Assemble the apparatus immediately, and immerse the flask in a water bath at a temperature such that a uniform evolution of gas is maintained. After not less than 2 h, examine the stain produced on the mercuric bromide paper. Perform the same procedure using 1.0 mL of the *Standard solution*. The stain produced on the mercuric bromide paper by the *Sample solution* is not more intense than that produced by the *Standard solution*.

Acceptance criteria: NMT 3 ppm

- **LIMIT OF PHOSPHATES**

Sulfomolybdic solution: Dissolve with heating 2.5 g of ammonium molybdate in 20 mL of water. Dilute 28 mL of sulfuric acid with 50 mL of water, and cool. Mix the two solutions, and dilute with water to 100 mL.

Tin(II) chloride solution: Prepare as directed in the test for *Limit of Arsenic*.

Standard stock solution: 14.3 µg/mL of monobasic potassium phosphate in water.

Standard solution: Transfer 1.0 mL of *Standard stock solution* to a 100-mL volumetric flask, and dilute with water to volume.

Sample solution: Transfer 2.5 mL of the *Sample solution* from the test for *Appearance of Solution* to a 100-mL volumetric flask, and dilute with water to volume.

Analysis: To 100 mL of the *Sample solution* add 4 mL of *Sulfomolybdic solution*, mix, and add 0.1 mL of *Tin(II) chloride solution*. To 100 mL of the *Standard solution*, add 4 mL of *Sulfomolybdic solution*, mix, and add 0.1 mL of *Tin(II) chloride solution*. Allow the preparations to stand for 10 min, then examine 20 mL of each preparation. Any color produced by the *Sample solution* is not more intense than that produced by the *Standard solution*.

Acceptance criteria: NMT 0.04%

- **CITRIC ACID**

Mercury(II) sulfate solution: 1 g of mercuric oxide in 20 mL of water and 4 mL of sulfuric acid

Analysis: Mix 5 g of Calcium Glycerophosphate with 20 mL of carbon dioxide-free water, and filter. To the filtrate add 0.15 mL of sulfuric acid, and filter. To the filtrate add 5 mL of *Mercury(II) sulfate solution*, and heat to boiling. Add 0.5 mL of 0.2 M potassium permanganate, and heat to boiling.

Acceptance criteria: No precipitate is formed.

- **GLYCEROL AND ALCOHOL-SOLUBLE SUBSTANCES**

Analysis: Mix 1 g with 25 mL of alcohol, and shake for 1 min. Filter, evaporate the filtrate to dryness on a water bath, and dry the residue at 70° for 1 h.

Acceptance criteria: The residue weighs NMT 5 mg (0.5%).

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SPECIFIC TESTS

- **APPEARANCE OF SOLUTION**

Opalescent suspension: Dissolve 1g of hydrazine sulfate in 100 mL of water and allow to stand for 4–6 h. Add 25 mL of this solution to 25 mL of a solution containing 100mg/mL of methenamine in water, and allow to stand for 24 h.

Primary reference suspension: Dilute 15.0 mL of the *Opalescent suspension* with water to 1000 mL. [NOTE—This suspension is freshly prepared and may be stored for NMT 24 h.]

Reference suspension: *Primary reference suspension* and water (30:70). [NOTE—Shake before using.]

Sample solution: Dissolve 1.5 g at room temperature in 150 mL of carbon dioxide-free water.

Analysis: Compare the opalescence of equal volumes of the *Sample solution* and the *Reference suspension*.

Acceptance criteria: The *Sample solution* is not more opalescent than the *Reference suspension*.

- **ACIDITY OR ALKALINITY**

Analysis: Dissolve 1 g of Calcium Glycerophosphate in 100 mL of water. Add 0.1 mL of 1.0% (w/v) phenolphthalein solution.

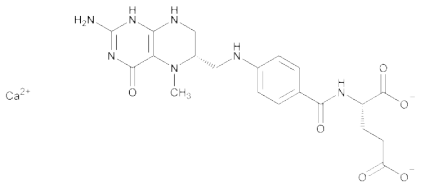
Acceptance criteria: NMT 0.5 mL of 0.1 M sodium hydroxide or 0.1 M hydrochloric acid is required to change the color of the indicator.

- **LOSS ON DRYING ⟨731⟩:** Dry a sample at 150° for 4 h: it loses NMT 12.0% of its weight.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.

Calcium L-5-Methyltetrahydrofolate



$C_{20}H_{23}CaN_7O_6 \cdot xH_2O$

$C_{20}H_{23}CaN_7O_6$ (anhydrous) 497.52

N-[4-[[(2-Amino-1,4,5,6,7,8-hexahydro-5-methyl-4-oxo-(6*S*)-pteridinyl)methyl]amino]benzoyl]-L-glutamic acid, calcium salt (1:1);
N-{4-[[((6*S*)-2-Amino-1,4,5,6,7,8-hexahydro-5-methyl-4-oxo-6-pteridinyl)methyl]amino]-benzoyl}-L-glutamic acid, calcium salt (1:1) [151533-22-1].

DEFINITION

Calcium L-5-Methyltetrahydrofolate contains NLT 95.0% and NMT 102.0% of calcium 5-methyltetrahydrofolate ($C_{20}H_{23}CaN_7O_6$), the sum of the L- and D-diastereoisomers, calculated on the anhydrous and solvent-free basis, of which NMT 1.0% corresponds to calcium D-5-methyltetrahydrofolate.

IDENTIFICATION

- **A. INFRARED ABSORPTION ⟨197K⟩**

[NOTE—If the spectra obtained show differences, dissolve the substance to be examined and the USP Calcium DL-5-Methyltetrahydrofolate RS separately in the minimum quantity of water, and add dropwise sufficient acetone to produce a precipitate. Allow to stand for 15 min, centrifuge to collect the precipitate, wash the precipitate twice with a minimum quantity of acetone, and dry. Record new spectra using the residues.]

- **B. IDENTIFICATION TESTS—GENERAL,** *Calcium* ⟨**191**⟩**:** A 5-mg/mL solution meets the requirements.
- **C. HPLC:** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. It complies with the acceptance criteria of the test for *Enantiomeric Purity*.

ASSAY

- **PROCEDURE**

Buffer: 7.8 g/L of sodium dihydrogen phosphate dihydrate in water

Solution A: Adjust the *Buffer* with 32% (w/v) sodium hydroxide solution to a pH of 6.5.

Solution B: Methanol and *Buffer* (35:65). Adjust with 32% (w/v) sodium hydroxide solution to a pH of 8.0.

Mobile phase: Gradient elution. See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
14	45	55
17	0	100
24	0	100
24.01	100	0
33	100	0

[NOTE—After analysis the column should be flushed and stored in a mixture of methanol and water (85:15).]

System suitability solution: Transfer 25 mg of USP Folic Acid RS and 25 mg of USP 4-Aminobenzoylglutamic Acid RS to a 100-mL volumetric flask. Add about 15 mg each of sodium hydrogen carbonate and sodium carbonate to the flask, add sufficient water, sonicate to dissolve, and dilute with water to volume. Transfer 1.0 mL of this solution to a second 100-mL volumetric flask containing 50 mg of USP Calcium DL-5-Methyltetrahydrofolate RS, dissolve, and dilute with water to volume.

[NOTE—The following Standard and Sample solutions must be injected immediately after preparation and injected only once.]

Standard solution: 0.5 mg/mL of USP Calcium DL-5-Methyltetrahydrofolate RS in water

Sample solution: 0.5 mg/mL of Calcium L-5-Methyltetrahydrofolate in water

Chromatographic system

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

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 32°

Flow rate: 1.1 mL/min

Injection volume: 10 µL

System suitability

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

[NOTE—For the *System suitability solution* the relative retention times of the component peaks are listed in *Table 2*. The L- and D-isomers of 5-methyltetrahydrofolate co-elute as a single peak. The 4a-hydroxy-5-methyltetrahydrofolic acid, 5-methyltetrahydropteroic acid, and dimethyltetrahydrofolic acid are included as minor components in USP Calcium DL-5-Methyltetrahydrofolate RS.]

Suitability requirements

Resolution: *System suitability solution*

NLT 6 between 4-aminobenzoylglutamic acid and 4a-hydroxy-5-methyltetrahydrofolic acid

NLT 8 between folic acid and 5-methyltetrahydrofolic acid

NLT 15 between 5-methyltetrahydrofolic acid and dimethyltetrahydrofolic acid

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