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⟨91⟩ CALCIUM PANTOTHENATE ASSAY

INTRODUCTION

The following liquid chromatographic procedures are provided for the determination of calcium pantothenate as an active pharmaceutical ingredient, a dietary supplement ingredient, or a component in dietary supplements or pharmaceutical dosage forms.

While conducting these procedures, protect solutions containing and derived from the test specimen and the Reference Standards from atmosphere and light, preferably with the use of low-actinic glassware. Use the appropriate USP Reference Standards.

ASSAY

• **Chromatographic Methods, Procedure 1**

This procedure can be used to determine calcium pantothenate in:

- *Oil- and Water-Soluble Vitamins Capsules*
- *Oil- and Water-Soluble Vitamins Tablets*
- *Oil- and Water-Soluble Vitamins with Minerals Capsules*
- *Oil- and Water-Soluble Vitamins with Minerals Tablets*
- *Water-Soluble Vitamins Capsules*
- *Water-Soluble Vitamins Tablets*
- *Water-Soluble Vitamins with Minerals Capsules*
- *Water-Soluble Vitamins with Minerals Tablets*

This procedure involves the extraction of calcium pantothenate from the formulation by the *Internal standard solution*.

Unless specified in the individual monographs, the *Internal standard solution*, *Standard solution*, *Sample solution*, and reagent solutions are prepared as follows.

Mobile phase: Phosphoric acid and water (1:1000)

Internal standard solution: 80 mg of *p*-hydroxybenzoic acid in 3 mL of alcohol. Add 50 mL of water and 7.1 g of dibasic sodium phosphate, and dilute with water to 1000 mL. Adjust with phosphoric acid to a pH of 6.7.

Standard solution: 0.6 mg/mL of USP Calcium Pantothenate RS in *Internal standard solution*

Sample solution (for capsules)**:** Weigh NLT 20 capsules in a tared weighing bottle. Open the capsules, without losing shell material, and transfer the contents to a 100-mL beaker. Remove any contents adhering to the empty shells by washing, if necessary, with several portions of ether. Discard the washings, and dry the capsule shells with the aid of a current of dry air until the odor of ether is no longer perceptible. Weigh the empty capsule shells in the tared weighing bottle, and calculate the average net weight per capsule. Transfer a quantity of mixed capsule contents and a volume of *Internal standard solution* to a centrifuge tube to obtain a concentration of 0.6 mg/mL in the *Sample solution*.

Sample solution (for tablets)**:** Finely powder NLT 30 tablets. Transfer a portion of the powder, equivalent to a nominal amount of 15 mg of calcium pantothenate, to a centrifuge tube. Add 25.0 mL of the *Internal standard solution*, and shake vigorously for 10 min. Centrifuge, filter, and use the clear filtrate.

Chromatographic system

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

Mode: LC

Detector: UV 210 nm

Column: 3.9-mm × 15-cm; packing L1

Flow rate: 1.5 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

[Note—The relative retention times for calcium pantothenate and *p*-hydroxybenzoic acid are about 0.5 and 1.0, respectively.]

Suitability requirements

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of calcium pantothenate ($C_{18}H_{32}CaN_2O_{10}$) in the portion of the sample taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of calcium pantothenate to *p*-hydroxybenzoic acid from the *Sample solution*

R_S = peak response ratio of calcium pantothenate to *p*-hydroxybenzoic acid from the *Standard solution*

C_S = concentration of USP Calcium Pantothenate RS in the *Standard solution* (mg/mL)

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

- **Chromatographic Methods, Procedure 2**

This procedure can be used to determine calcium pantothenate in:

- *Oil- and Water-Soluble Vitamins Capsules*
- *Oil- and Water-Soluble Vitamins Tablets*
- *Oil- and Water-Soluble Vitamins with Minerals Capsules*
- *Oil- and Water-Soluble Vitamins with Minerals Tablets*
- *Water-Soluble Vitamins Capsules*
- *Water-Soluble Vitamins Tablets*
- *Water-Soluble Vitamins with Minerals Capsules*
- *Water-Soluble Vitamins with Minerals Tablets*

This procedure involves dissolving an appropriate quantity of the sample equivalent to 10 mg of calcium pantothenate in 10 mL of methanol and diluting with water to 250 mL.

Unless specified in the individual monographs, the *Standard solution*, *Sample solution*, and reagent solutions are prepared as follows.

Buffer solution: Dissolve 10.0 g of monobasic potassium phosphate in 2000 mL of water, and adjust with phosphoric acid to a pH of 3.5.

Mobile phase: Methanol and *Buffer solution* (1:9)

Standard stock solution: 0.25 mg/mL of USP Calcium Pantothenate RS in water. Prepare fresh every 4 weeks. Store in a refrigerator.

Standard solution: 40 µg/mL of USP Calcium Pantothenate RS from the *Standard stock solution* diluted with water

Sample solution (for capsules)**:** Weigh NLT 20 capsules in a tared weighing bottle. Open the capsules, without losing shell material, and transfer the contents to a 100-mL beaker. Remove any contents adhering to the empty shells by washing, if necessary, with several portions of ether. Discard the washings, and dry the capsule shells with the aid of a current of dry air until the odor of ether is no longer perceptible. Weigh the empty capsule shells in the tared weighing bottle, and calculate the average net weight per capsule. Transfer a portion of the capsule contents, equivalent to 10 mg of calcium pantothenate, to a 250-mL volumetric flask. Add 10 mL of methanol, and swirl the flask to disperse the capsule contents. Dilute with water to volume, mix, and filter.

Sample solution (for tablets)**:** Finely powder NLT 20 tablets. Transfer a portion of the powder, equivalent to 10 mg of calcium pantothenate, to a 250-mL volumetric flask. Add 10 mL of methanol, and swirl the flask to disperse. Dilute with water to volume, mix, and filter.

Chromatographic system

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

Mode: LC

Detector: UV 205 nm

Column: 3.9-mm × 30-cm; 5-µm packing L1

Column temperature: 50°

Flow rate: 2 mL/min

Injection volume: 25 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of calcium pantothenate ($C_{18}H_{32}CaN_2O_{10}$) in the portion of the sample taken:

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

r_U = peak response of calcium pantothenate from the *Sample solution*

r_S = peak response of calcium pantothenate from the *Standard solution*

C_S = concentration of USP Calcium Pantothenate RS in the *Standard solution* (mg/mL)

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

- **Chromatographic Methods, Procedure 3**

This procedure can be used to determine calcium pantothenate in:

- *Oil- and Water-Soluble Vitamins Oral Solution*
- *Oil- and Water-Soluble Vitamins with Minerals Oral Solution*
- *Water-Soluble Vitamins with Minerals Oral Solution*

This procedure involves diluting an appropriate quantity of the liquid sample with water to a final concentration of 80 µg/mL of calcium pantothenate.

Unless specified in the individual monographs, the *Standard solution*, *System suitability solution*, *Sample solution*, and reagent solutions are prepared as follows.

Mobile phase: 0.2 M monobasic sodium phosphate and methanol (97:3). Adjust with 1.7 M phosphoric acid to a pH of 3.2 ± 0.1.

Standard solution: 80 µg/mL of USP Calcium Pantothenate RS in *Mobile phase*

System suitability solution: 80 µg/mL of USP Racemic Panthenol RS in *Mobile phase*. Mix the resulting solution and *Standard solution* (1:1).

Sample solution: Equivalent to 80 µg/mL of calcium pantothenate from the oral solution in *Mobile phase*

Chromatographic system

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

Mode: LC

Detector: UV 210 nm

Column: 4.0-mm × 10-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

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

Suitability requirements

Resolution: NLT 1.5 between panthenol and calcium pantothenate, *System suitability solution*

Tailing factor: NMT 2.0 for the calcium pantothenate peak, *Standard solution*

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

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of calcium pantothenate ($C_{18}H_{32}CaN_2O_{10}$) in the portion of the sample taken:

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

r_U = peak response of calcium pantothenate from the *Sample solution*

r_S = peak response of calcium pantothenate from the *Standard solution*

C_S = concentration of USP Calcium Pantothenate RS in the *Standard solution* (mg/mL)

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

• **Chromatographic Methods, Procedure 4**

This procedure can be used to determine calcium pantothenate in:

- Active pharmaceutical ingredients
- Dietary ingredients

This procedure involves the dissolution of the sample directly into water.

Unless specified in the individual monographs, the *Standard solution*, *System suitability solution*, *Sample solution*, and reagent solutions are prepared as follows.

Buffer solution: Dissolve 3.2 g of monobasic sodium phosphate in 1 L of water and adjust with 1 N sodium hydroxide to a pH of 5.5.

Mobile phase: Acetonitrile and *Buffer solution* (2:98)

System suitability solution: 0.1 mg/mL of USP Calcium Pantothenate RS and 0.5 mg/mL each of USP Beta Alanine RS, USP Sodium D-Pantoate RS, and USP Pantolactone RS in water

Standard solution: 0.5 mg/mL of USP Calcium Pantothenate RS in water

Sample solution: 0.5 mg/mL of Calcium Pantothenate in water

Chromatographic system

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

Mode: LC

Detector: UV 200 nm

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

Column temperature: 35°

Flow rate: 2 mL/min

Injection volume: 10 µL

System suitability

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

[NOTE—The relative retention times for beta alanine, pantoic acid, pantothenic acid, and pantolactone are 0.3, 0.6, 1.0, and 1.9 min, respectively.]

Suitability requirements

Resolution: NLT 5.0 between the pantothenic acid and pantoic acid peaks, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

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

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of calcium pantothenate ($C_{18}H_{32}CaN_2O_{10}$) in the portion of the sample taken:

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

r_U = peak response of calcium pantothenate from the *Sample solution*

r_S = peak response of calcium pantothenate from the *Standard solution*

C_S = concentration of USP Calcium Pantothenate RS in the *Standard solution* (mg/mL)

C_U = concentration of Calcium Pantothenate in the *Sample solution* (mg/mL)

ADDITIONAL REQUIREMENTS

- **USP REFERENCE STANDARDS ⟨11⟩**
  - USP Beta Alanine RS
  - USP Calcium Pantothenate RS
  - USP Pantolactone RS
  - USP Racemic Panthenol RS
  - USP Sodium D-Pantoate RS

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

Topic/Question	Contact	Expert Committee
<91> CALCIUM PANTOTHENATE ASSAY	Natalia Davydova Scientific Liaison	NBDS2020 Non-botanical Dietary Supplements

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