GB

NATIONAL STANDARD OF THE

PEOPLE'S REPUBLIC OF CHINA

ICS 67.050

CCS X 83

GB/T 22246-2025

Replacing GB/T 22246-2008

Determination of Pantothenic Acid in Health Foods

保健食品中泛酸的测定

Issued on: January 24, 2025 **Implemented on: August 1, 2025**

Issued by: State Administration for Market Regulation;

Standardization Administration of the People's Republic of China.

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Determination of Pantothenic Acid in Health Foods

1 Scope

This document describes a high-performance liquid chromatography (HPLC) for the determination of pantothenic acid in health foods.

This document is applicable to the determination of pantothenic acid in health foods in dosage forms, such as tablets, hard capsules, soft capsules, granules, powders, oral liquids and jelly candies, etc.

2 Normative References

The contents of the following documents constitute indispensable clauses of this document through the normative references in the text. In terms of references with a specified date, only versions with a specified date are applicable to this document. In terms of references without a specified date, the latest version (including all the modifications) is applicable to this document.

GB/T 6682 Water for Analytical Laboratory Use - Specification and Test Methods

3 Terms and Definitions

This document does not have terms or definitions that need to be defined.

4 Principle

Pantothenic acid in the specimen is extracted with hot water, separated by high-performance liquid chromatography, and detected by ultraviolet light. Qualitative determination is based on retention time, and quantitative determination is based on the external standard method.

5 Reagents or Materials

Unless otherwise specified, only analytically pure reagents shall be used.

5.1 Reagents

5.1.1 Water, Grade 1 water as specified in GB/T 6682.

5.1.2 Hydrochloric acid (HCl).

5.1.3 Sodium hydroxide (NaOH).

5.1.4 Phosphoric acid (H_3PO_4).

100 mL volumetric flasks, add water to dilute to the scale and mix them well to obtain pantothenic acid series standard working solutions with concentrations of 1 μg/mL, 2 μg/mL, 4 μg/mL, 8 μg/mL, 16 μg/mL and 32 μg/mL. Prepare immediately prior to use.

5.4 Materials

Filter membrane: 0.45 μm, aqueous.

6 Instruments and Equipment

6.1 High-performance liquid chromatograph: equipped with UV detector or equivalent.

6.2 Balance: with a division value of 0.001 g and 0.1 mg.

6.3 Constant-temperature oscillating water bath: with an oscillating frequency of 100 r/min ± 20 r/min.

6.4 Ultrasonic cleaner.

6.5 pH meter: with an accuracy of ±0.01.

6.6 High-speed centrifuge: speed not less than 8,000 r/min.

7 Analytical Procedures

7.1 Specimen Preparation

7.1.1 Solid specimens (hard capsules, tablets and powders, etc.): take at least 20 capsules or at least 5 g of sample (for hard capsules, remove the shells and take the contents; for tablets, crush them), finely grind (if necessary), mix well and set aside.

7.1.2 Solid specimens (jelly candies, etc.): take at least 10 capsules (for mixed specimens of different colors or flavors, evenly conduct the sampling by color or flavor) or at least 10 g of sample, cut into small pieces and set aside.

7.1.3 Semi-solid specimens (soft capsules, etc.): take at least 20 capsules or at least 5 g of sample, remove the shells, take the contents, mix well and set aside.

7.1.4 Liquid specimens (oral liquids, etc.): take 5 minimum-size packages or at least 50 mL of sample (if the sample contains carbonic acid, use ultrasound to remove carbon dioxide), mix well and set aside.

7.2 Specimen Preparation

Weigh-take 1 g ~ 3 g of specimen (accurate to 0.001 g), or transfer-take 1 mL ~ 3 mL of specimen, or a volume that obtains approximately 1 mg of pantothenic acid in the specimen. Place it in a 100 mL conical flask. Add 30 mL of warm water at 40 °C ~ 50 °C; vortex or stir it,

until the sample is thoroughly dissolved. Perform ultrasound for 20 minutes, and cool to room temperature. Use 0.1 mol/L hydrochloric acid solution (5.1.8), 1.0 mol/L hydrochloric acid solution (5.1.9), 0.01 mol/L sodium hydroxide solution (5.1.10) or 0.1 mol/L sodium hydroxide solution (5.1.11) to adjust the pH to 5.0. Add 5 mL of 0.5 mol/L zinc sulfate solution (5.1.12) and mix it thoroughly. Transfer to a 50 mL volumetric flask, use water to dilute to the scale, mix it thoroughly, and transfer to a centrifuge tube. At 8,000 r/min, centrifuge for 2 min. Pass the supernatant through a 0.45 μm filter membrane (5.4) and prepare the filtrate for determination on the machine.

NOTE: if necessary, based on the content of the components in the specimen, appropriately increase the dilution factor f to ensure that the mass concentration of pantothenic acid in the specimen is within the range of 1.0 μg/mL ~ 32.0 μg/mL.

7.3 Chromatographic Reference Conditions

The chromatographic reference conditions are as follows:

a) Chromatographic column: C_{18} (particle size 5 μm, 250 mm × 4.6 mm), or equivalent chromatographic column;

b) Mobile phase: potassium dihydrogen phosphate solution–acetonitrile (5.1.14);

c) Flow rate: 1 mL/min;

d) Column temperature: 35 °C;

e) Injection volume: 10 μL or 20 μL;

f) Detection wavelength: 200 nm.

7.4 Standard Curve Determination

Successively inject the pantothenic acid standard working solution into the high-performance liquid chromatograph and determine the peak area of each component. With the mass concentration of the corresponding standard working solution as the x-coordinate and the peak area as the y-coordinate to draw a standard curve. The high-performance liquid chromatogram of the pantothenic acid standard solution (16 μg/mL) is shown in Figure A.1 in Appendix A.

7.5 Determination of Specimen Solution

Inject the specimen solution into the high-performance liquid chromatograph to obtain the corresponding peak area. In accordance with the standard curve, use the external standard method to calculate the mass concentration of pantothenic acid in the specimen solution to be determined. The high-performance liquid chromatogram of pantothenic acid in the specimen (tablets) is shown in Figure A.2.

Perform two tests in parallel.

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