

ICS 67.050

CCS X 04



**NATIONAL STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA**

GB/T 46804-2025

Determination of phosphatidylserine in food

食品中磷脂酰丝氨酸的测定

Issued on: December 2, 2025

Implemented on: July 1, 2026

**Issued by: State Administration for Market Regulation;
Standardization Administration of the PRC**

Table of Contents

1	Scope
2	Normative References
3	Terms and Definitions
4	Principle
5	Reagents and Materials
5.1	Reagents
5.2	Reagent Preparation
5.2.8	Sodium Hydroxide Solution (1 mol/L). Weigh
5.2.9	Phosphate Buffer Solution. Weigh
5.3	Materials
5.5	Preparation of Standard Solutions
5.5.3	Phosphatidylserine Working Standard Solutions. Pipette
6	Instruments and Equipment
6.2	Analytical Balance. Accuracy of
7	Analytical Procedure
7.1	Sample Preparation
8	Calculation and Expression of Results...

1 Scope

GB/T 46804-2025 is the Chinese national standard covering measuring phosphatidylserine in food - the extraction of the phospholipid fraction, the separation from the other phospholipids that accompany it, and the quantification, which is what an infant formula or a supplement claim rests on. First edition. Issued on 2 December 2025, it has been in force since 1 July 2026.

This document describes a method for the determination of phosphatidylserine content in foods. This document is applicable to the determination of phosphatidylserine content in foods such as beverages, confectionery (compressed candies, gel candies), convenience foods, and dairy products.

2 Normative References

The following documents, through normative references herein, constitute essential provisions of this document. For dated references, only the edition cited applies. For

undated references, the latest edition of the referenced document (including any amendments) applies.

GB/T 6682-2008 Water for Analytical Laboratory Use - Specification and Test Methods

3 Terms and Definitions

This document does not contain any terms or definitions that require further clarification.

4 Principle

After extraction and purification from the test sample, phosphatidylserine is separated using normal-phase liquid chromatography, detected using an Evaporative Light Scattering Detector (ELSD), and quantified using the external standard method.

5 Reagents and Materials

Unless otherwise specified, all reagents shall be of analytical reagent grade.

5.1 Reagents

5.1.1 Methanol (CH_3OH). Chromatographic grade.

5.1.2 Acetonitrile (CH_3CN). Chromatographic grade.

5.1.3 Chloroform (CHCl_3). Chromatographic grade.

5.1.4 n-Hexane [$\text{CH}_3(\text{CH}_2)_4\text{CH}_3$]. Chromatographic grade.

5.1.5 Isopropanol [$(\text{CH}_3)_2\text{CHOH}$]. Chromatographic grade.

5.1.6 n-Propanol ($\text{C}_3\text{H}_7\text{OH}$). Chromatographic grade.

5.1.7 Triethylamine [$(\text{CH}_3\text{CH}_2)_3\text{N}$].

5.1.8 Acetic acid (CH_3COOH).

5.1.9 Hydrochloric acid (HCl).

5.1.10 Phosphoric acid (H_3PO_4).

5.1.11 Sodium chloride (NaCl).

5.1.12 Sodium dihydrogen phosphate dihydrate ($\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$).

5.1.13 Disodium hydrogen phosphate dodecahydrate ($\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$).

5.1.14 Sodium hydroxide (NaOH).

5.2 Reagent Preparation

5.2.1 Chloroform-Methanol Solution (90+10). Take 900 mL of chloroform (5.1.3) and 100 mL of methanol. Mix thoroughly and set aside for use.

5.2.2 Methanol-Hydrochloric Acid Solution (90+10). Take 90 mL of methanol (5.1.1) and 10 mL of hydrochloric acid (5.1.9). Mix thoroughly and set aside for use.

5.2.3 Acetonitrile-n-Propanol Solution (60+30). Take 60 mL of acetonitrile (5.1.2) and 30 mL of n-propanol (5.1.6). Mix thoroughly and set aside for use.

5.2.4 Isopropanol-Methanol-Hydrochloric Acid Solution (80+20). Take 80 mL of isopropanol (5.1.5) and 20 mL of the methanol-hydrochloric acid solution (5.2.2). Mix thoroughly and set aside for use.

5.2.5 n-Hexane-Isopropanol-Acetic Acid-Triethylamine Solution (820+170+10+0.8). Take 820 mL of n-hexane (5.1.4), 170 mL of isopropanol (5.1.5), 10 mL of acetic acid (5.1.8), and 0.8 mL of triethylamine (5.1.7). Mix thoroughly and set aside for use.

5.2.6 Isopropanol-Water-Acetic Acid-Triethylamine Solution (850+140+10+0.8). Take 850 mL of isopropanol (5.1.5), 140 mL of water, 10 mL of acetic acid (5.1.8), and 0.8 mL of triethylamine (5.1.7). Mix thoroughly and set aside for use.

5.2.7 Saturated Sodium Chloride Solution. Weigh 100 g of sodium chloride (5.1.11), add 200 mL of hot water, and mix thoroughly using an ultrasonic bath. Cool until a saturated solution containing both solid and liquid phases is formed.

5.2.8 Sodium Hydroxide Solution (1 mol/L). Weigh

4.0 g of sodium hydroxide (5.1.14) into a beaker and dissolve it with stirring. After cooling to room temperature, transfer the solution to a 100 mL volumetric flask, dilute to the mark with water, and mix thoroughly.

5.2.9 Phosphate Buffer Solution. Weigh

27.4 g of sodium dihydrogen phosphate dihydrate (5.1.12) and

8.80 g of disodium hydrogen phosphate dodecahydrate (5.1.13) into a 1000 mL beaker. Add 800 mL of water to dissolve the solids, then adjust the pH to

6.0 using the 1 mol/L sodium hydroxide solution (5.2.8). Transfer the solution to a 1000 mL volumetric flask, dilute to the mark with water, and mix thoroughly.

5.3 Materials

5.3.1 Amino Solid-Phase Extraction (SPE) Column. 500 mg/6 mL, or an equivalent product with comparable performance.

5.3.2 Filter Membrane. 0.22 μ m, suitable for organic phases.

5.4 Reference Standards Phosphatidylserine (C₄₂H₈₂NO₁₀P, CAS No.. 51446-62-9). Purity $\geq$ 97%, or a certified reference material/standard sample accompanied by a certificate issued by a national certifying body.

5.5 Preparation of Standard Solutions

5.5.1 Phosphatidylserine Standard Stock Solution (10 mg/mL). Accurately weigh 100.0 mg of the phosphatidylserine reference standard (weighed to the nearest 0.0001 g). Dissolve it in an appropriate amount of chloroform-methanol solution (5.2.1), transfer the solution to a 10 mL volumetric flask, dilute to the mark with chloroform-methanol solution (5.2.1), and mix thoroughly. Store frozen at -20°C. the solution is stable for 1 month.

5.5.2 Phosphatidylserine Intermediate Standard Solution (1 mg/mL). Accurately transfer 1 mL of the Phosphatidylserine Standard Stock Solution (5.5.1) into a 10 mL volumetric flask, and dilute to the mark with the chloroform-methanol solution (5.2.1). Prepare immediately before use.

5.5.3 Phosphatidylserine Working Standard Solutions. Pipette

0.2 mL,

0.5 mL, 1 mL,

1.5 mL, 2 mL, and 3 mL aliquots of the Phosphatidylserine Intermediate Standard Solution (5.5.2) into separate 10 mL volumetric flasks. Dilute each to the mark with the chloroform-methanol solution (5.2.1) and mix thoroughly. The mass concentrations of this series of phosphatidylserine standard solutions are 20 µg/mL, 50 µg/mL, 100 µg/mL, 150 µg/mL, 200 µg/mL, and 300 µg/mL, respectively. Prepare immediately before use.

6 Instruments and Equipment

6.1 High-Performance Liquid Chromatograph (HPLC). Equipped with an Evaporative Light Scattering Detector (ELSD).

6.2 Analytical Balance. Accuracy of

0.001 g and 0.0001 g.

6.3 Ultrasonic Cleaner. Temperature range of 10°C to 80°C.

6.4 Vortex Mixer. Maximum speed of not less than 500 r/min.

6.5 Centrifuge. Maximum speed of not less than 10,000 r/min.

7.1 Sample Preparation

7.1.1 Solid Samples Solid samples are primarily classified into. -- Block samples (e.g.,