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NATIONAL STANDARD OF THE

PEOPLE'S REPUBLIC OF CHINA

GB 5009.272-2016

National Food Safety Standard - Determination of Phosphatidylcholine, Phosphatidylethanolamine and Phosphatidylinositol in Foods

食品安全国家标准

食物中磷脂酰胆碱、磷脂酰乙醇胺、磷脂酰肌醇的测定

Issued on: December 23, 2016 **Implemented on: June 23, 2017**

Issued by: National Health and Family Planning Commission of the PRC;
State Food and Drug Administration

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National Food Safety Standard - Determination of Phosphatidylcholine, Phosphatidylethanolamine and Phosphatidylinositol

1 Scope

This Standard specifies the method for determination of the content of phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol in soybean lecithin, soybean oil, rapeseed oil, peanut oil and sunflower oil by high performance liquid chromatography.

This Standard applies to the determination of phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidylinositol (PI) in oily soybean lecithin, de-oiled soybean lecithin, soybean oil, rapeseed oil, peanut oil and sunflower oil. This Standard does not apply to the determination of soy lysophosphatidylcholine and soybean lysophosphatidylethanolamine.

2 Principle

The sample is directly dissolved or extracted by trichloromethane, and purified by amino solid phase extraction column, separated by high performance liquid chromatography, detected by ultraviolet detector, and quantified by external standard method.

3 Reagents and materials

Unless otherwise specified, all the reagents in this method are analytical reagents, the water is grade-1 water specified by GB/T 6682.

3.1 Reagents

3.1.1 n-hexane [$CH_3(CH_2)_4CH_3$]: chromatographic pure.

3.1.2 Isopropanol [$(CH_3)_2CHOH$]: chromatographic pure.

3.1.3 Acetic acid (CH_3COOH): chromatographic pure.

3.1.4 Trichloromethane (CHC_{13}).

3.1.5 Diethyl ether ($CH_3CH_2OCH_2CH_3$).

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4.2 Analytical balance: sensitivity of 1 mg and 0.1 mg.

4.3 stoppered test tube: 100 mL.

4.4 Vortex oscillator.

4.5 Rotary evaporator: rotating speed of 10 r/min ~ 120 r/min.

4.6 Centrifuge: with the speed larger than 8 000 r/min.

4.7 Nitrogen concentrator.

5 Analysis steps

5.1 Sample preparation

5.1.1 Preparation and pretreatment of soybean lecithin samples

The sample shall be protected from light in a closed and moisture-proof container. The sample shall be thoroughly mixed before use.

According to the content of phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol in the sample, weigh 15 mg ~ 50 mg (accurate to 0.1mg) of sample; use the mixed solution of n-hexane-isopropanol-acetic acid aqueous solution to dissolve and fix-volume to 5 mL. Filter through a 0.45 μm microporous membrane. Store at a temperature below -16°C after sealing.

5.1.2 Preparation and pretreatment of oil sample

For the sample, separate the sample according to GB/T 5524; take 50 g and place in a sample bottle for use.

Accurately weigh 4 g of the oil sample (accurate to 1 mg); place it in a 100 mL stoppered test tube; add 50.0 mL of trichloromethane; rotate and mix. Firstly, use 1.0 mL of trichloromethane to activate the amino solid phase extraction column; transfer 10.0 mL of the oil trichloromethane solution to the amino silica solid phase extraction column; then, sequentially use 2.0 mL of mixed solution of trichloromethane-isopropyl alcohol and 3.0 mL of mixed solution of acetic acid-diethyl ether to rinse the column; then, use 3.0 mL of methanol to elute the phospholipid; repeat it for 4 times more; collect the eluate. Use the rotary evaporator to evaporate the eluate to near dryness at 45°C; then transfer to nitrogen blowing; after drying, add 10.0 mL of mixed solution of n-hexane-isopropanol-acetic acid aqueous solution; centrifuge at 4 000 r/min for 5 min; take the supernatant for liquid chromatography analysis.

5.2 Apparatus reference conditions

5.2.1 Chromatographic column: silica gel column with a column length of 250 mm, an

inner diameter of 4.6 mm, a particle size of 5 μm, or equivalent columns;

5.2.2 Mobile phase: mixed solution of n-hexane-isopropanol-acetic acid aqueous solution (8+8+1).

5.2.3 Detection wavelength: 205 nm.

5.2.4 Flow velocity: 1 mL/min.

5.2.5 Column temperature: 30°C.

5.2.6 Injection volume: 10 μL;

5.3 Preparation of the standard curve

Inject standard series working solution into liquid chromatograph separately to test the corresponding peak areas; use the concentration of standard working solution as the abscissa and the peak area as the ordinate to draw the standard curve. For the liquid chromatogram of phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol, see Figure A.1.

5.4 Determination of sample solution

Inject the sample solution into the liquid chromatography to get the peak areas; get the concentration of phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol in the to-be-test solution according to the standard curve.

6 Description of the analysis result

6.1 Calculation of ciguatoxin in the sample

Calculate the content of phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol in the sample according to Formula (1):

$$w = \frac{\rho \times V}{m} \times K \quad \cdots\cdots (1)$$

Where:

w -- the content of phosphatidylcholine (phosphatidylethanolamine, phosphatidylinositol) in the sample, in milligrams per gram (mg/g);

ρ -- the concentration of the to-be-test component (phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol) that is obtained from the standard working curve, in milligrams per milliliter (mg/mL);

V – the volume of the sample solution, in milliliter (mL);

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