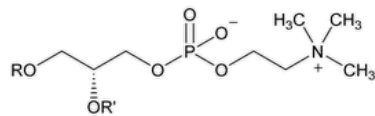


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Add the following:

^Soybean Phosphatidylcholine



R, R' = Palmitoyl or Stearoyl or Oleoyl or Linoleoyl or α-Linolenoyl

Structures of some predominant species;
Soy phosphatidylcholine;
Soya phosphatidylcholine
CAS RN®: 97281-47-5.

DEFINITION

Soybean Phosphatidylcholine contains NLT 90.0% and NMT 102.0% of phosphatidylcholine, calculated on the anhydrous basis, obtained from the soybean source. It is in an L-isomer form. It may contain a suitable stabilizer.

IDENTIFICATION

• A. IDENTIFICATION OF PHOSPHOLIPIDS BY THIN-LAYER CHROMATOGRAPHY

Developing solvent system: Chloroform, methanol, and water (65:25:4, v/v/v)
Standard solution: 10 mg/mL of USP Phosphatidylcholine (Soy) RS in the Developing solvent system
Sample solution: 10 mg/mL of Soybean Phosphatidylcholine in the Developing solvent system

Chromatographic system

(See Chromatography (621), General Procedures, Thin-Layer Chromatography.)
Mode: TLC

Plate: 20-cm × 20-cm, silica gel 60 on aluminum foil, 0.2-mm layer
Application volume: 20 µL

Spray reagent: Transfer 600 mL of water and then 80 mL of phosphoric acid to a 1-L volumetric flask. While stirring, add 100 g of anhydrous cupric sulfate. After stirring for 10 min, most of the cupric sulfate is dissolved. Add water to volume and continue stirring until the solid completely dissolves.

Analysis

Samples: Standard solution and Sample solution

Fill the chromatographic chamber with the Developing solvent system to a height of about 0.5 cm. Place a fat-free, U-shaped filter paper in the glass trough and press it against the wall. Sufficient saturation is reached once the Developing solvent system has permeated to the upper rim of the filter paper. Apply the Samples to the previously marked starting point on a TLC plate. Place the TLC plate in the saturated chromatographic chamber. When the Developing solvent system front has reached the mark (12 cm above the starting point), remove the TLC plate and dry it using a dryer. Spray or immerse the TLC plate in the Spray reagent, and dry it again with a dryer (a current of hot air). Heat the plate to 170° for 10 min. Develop all lipids by charring as dark brown spots.

Acceptance criteria: The R_F value of the major spot of the Sample solution corresponds to that of the Standard solution.

• B. IDENTITY BY FATTY ACID COMPOSITION: Soybean Phosphatidylcholine exhibits the following composition profile of fatty acids, as determined in Fats and Fixed Oils (401), Procedures, Fatty Acid Composition. See Table 1.

Table 1

Carbon Chain Length	Number of Double Bonds	Percentage (%)
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ASSAY	16	0	6–25
CONTENT OF PHOSPHATIDYLCHOLINE			
Solution A: A mixture of 1342 g (2.0 L) of n-hexane , 334.1 g (425 mL) of isopropyl alcohol , 39.4 g (38 mL) of acetic acid, glacial , and 1.45 g (2.0 mL) of triethylamine			
Solution B: A mixture of 663.5 g (850 mL) of isopropyl alcohol , 15.8 g (15 mL) of acetic acid, glacial , 140.0 g (140 mL) of water, and 0.58 g (0.8 mL) of triethylamine			
Diluent: n-Hexane , isopropyl alcohol , and water (46:46:8, v/v/v). [NOTE—To avoid the formation of two phases, mix the isopropyl alcohol and water first, and then add the n-hexane .]			
Mobile phase: See Table 2 .		3	4–9

Table 2

Program Step	Time (min)	Flow Rate (mL/min)	Solution A (%)	Solution B (%)
1	0	1.0	95	5
2	5.0	1.0	80	20
3	8.5	1.0	60	40
4	14.0	1.0	55	45
5	15.0	1.0	0	100
6	17.5	1.0	0	100
7	17.6	1.0	95	5
8	21.0	1.0	95	5
9	22.0	2.0	95	5
10	27.0	2.0	95	5
11	29.0	1.0	95	5

System suitability solution: 0.25 mg/mL of *N*-acylphosphatidylethanolamine prepared from [USP N-Acylphosphatidylethanolamine \(Soy\) Ammonium RS](#), 1.0 mg/mL of [USP Phosphatidylcholine \(Soy\) RS](#), 0.5 mg/mL of phosphatidylinositol prepared from [USP Phosphatidylinositol \(Soy\) Sodium RS](#), 0.25 mg/mL of phosphatidic acid prepared from [USP Phosphatidic Acid \(Soy\) Monosodium RS](#), 0.5 mg/mL of [USP Phosphatidylethanolamine \(Soy\) RS](#), and 0.25 mg/mL of [USP Lysophosphatidylcholine \(Soy\) RS](#) in *Diluent*. [NOTE—Due to the highly hygroscopic nature of phospholipids, take special precaution in the solution preparation. The *System suitability solution* is stable for at least 7 days after preparation.]

Phosphatidylcholine standard stock solution (2X): 2.0 mg/mL of [USP Phosphatidylcholine \(Soy\) RS](#) in *Diluent*. [NOTE—Due to the highly hygroscopic nature of phospholipids, take special precaution in the standard stock solution preparation.]

Phosphatidylcholine standard solutions: Prepare as directed in [Table 3](#).

Table 3

Concentration	Phosphatidylcholine Standard Stock Solution (2X)/Diluent (v/v)
0.6X	3:7
0.8X	4:6
1.0X	5:5

Concentration	Phosphatidylcholine Standard Stock Solution (2X)/Diluent (v/v)
1.2X	6:4
1.4X	7:3

Sample solution: 1.0 mg/mL of Soybean Phosphatidylcholine in *Diluent*

Chromatographic system

(See [Chromatography \(621\)](#), [System Suitability](#).)

Mode: LC

Detector: Evaporative light-scattering

Column: 4-mm × 12.5-cm; 5-µm packing [L20](#)

Temperatures

Column: 55°

Detector: 50°

Flow rate: 1.0 mL/min with step gradient at 2.0 mL/min. See [Table 2](#).

Injection volume: 20 µL

[NOTE—The *Detector temperature* and *Flow rate* can be adjusted as long as system suitability requirements are met.]

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for all of the specified impurities are listed in [Table 5](#).]

Suitability requirements

Resolution: NLT 2.0 between any adjacent peak pairs of the specified components

Tailing factor: NMT 2.0 for the phosphatidylcholine peak

Relative standard deviation: NMT 5.0% for the phosphatidylcholine peak

Analysis

Samples: *Phosphatidylcholine standard solutions* and *Sample solution*

Measure the areas of the phosphatidylcholine peak obtained from each of the *Phosphatidylcholine standard solutions*. Plot the logarithms of the relevant responses versus the logarithms of the concentrations, in mg/mL, of phosphatidylcholine obtained from the *Phosphatidylcholine standard solutions*, and determine the linear regression line using a least-squares analysis. The correlation coefficient for the linear regression line is NLT 0.995. From the graph so obtained, determine the concentration, *C*, in mg/mL, of phosphatidylcholine in the *Sample solution*.

Calculate the percentage of phosphatidylcholine in the portion of Soybean Phosphatidylcholine taken:

Result = $(C_U/C_S) \times 100$

C_U = concentration of phosphatidylcholine in the *Sample solution* (mg/mL), determined from the calibration curve

C_S = concentration of Soybean Phosphatidylcholine (mg/mL)

Acceptance criteria: 90.0%–102.0% on the anhydrous basis

IMPURITIES

• **PHOSPHOLIPID IMPURITIES**

Solution A, Solution B, Diluent, Mobile phase, System suitability solution, and Chromatographic system: Proceed as directed in the Assay.

Impurity standard solutions

Impurity standard solution (1X): 160 µg/mL of *N*-acylphosphatidylethanolamine prepared from [USP N-Acylphosphatidylethanolamine \(Soy\) Ammonium RS](#), 160 µg/mL of phosphatidylinositol prepared from [USP Phosphatidylinositol \(Soy\) Sodium RS](#), 160 µg/mL of phosphatidic acid prepared from [USP Phosphatidic Acid \(Soy\) Monosodium RS](#), 160 µg/mL of [USP Phosphatidylethanolamine \(Soy\) RS](#), and 400 µg/mL of [USP Lysophosphatidylcholine \(Soy\) RS](#) in *Diluent*. [NOTE—Due to the highly hygroscopic nature of phospholipids, take special precaution in the standard solution preparation.]

Prepare other levels of the standard solutions as directed in [Table 4](#). [NOTE—The solutions are prepared from the highest concentration to the lowest. It is recommended to inject the solutions from the lowest concentration to the highest.]

Table 4

Impurity Standard Solution	 Documents Document Sharing Hub
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