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Egg Phospholipids

DEFINITION

Egg Phospholipids is a mixture of naturally occurring phospholipids obtained from the yolk of hens' eggs that is suitable for use as an emulsifying agent in injectable emulsions. The content of phosphatidylcholine, phosphatidylethanolamine, lysophosphatidylcholine, and other related phospholipids is to be reported in the certificate of analysis. It may also contain a suitable stabilizer.

ASSAY

• **Content of Phospholipids**

Solution A: 1341.6 g of *n*-hexane, 334.1 g of 2-propanol, 39.4 g of glacial acetic acid, and 1.45 g of triethylamine (or 2.0 mL triethylamine)

Solution B: 663.5 g of 2-propanol, 140.0 g of water, 15.8 g of glacial acetic acid, and 0.58 g of triethylamine

Solvent: *n*-Hexane, 2-propanol, and water (23:23:4). [Note—To avoid the formation of two phases, mix the 2-propanol and water first, and then add the *n*-hexane.]

Mobile phase: See the gradient table below.

Program Step	Time (min)	Flow (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	15.0	1.0	0	100
5	17.5	1.0	0	100
6	17.6	1.0	95	5
7	21.0	1.0	95	5
8	22.0	2.0	95	5
9	27.0	2.0	95	5
10	29.0	1.0	95	5

Standard solutions: Transfer USP Phosphatidylcholine RS, USP Phosphatidylethanolamine RS, and USP Lysophosphatidylcholine RS to separate flasks, dissolve each in *Solvent*, and dilute. *Standard solutions* of five different concentrations are prepared on the basis of the expected content of phosphatidylcholine, phosphatidylethanolamine, and lysophosphatidylcholine in the sample. The *Standard solutions* should cover a range of 60% to 140%. Calculate the concentrations of the Standards:

$$\text{Result} = WP/V$$

W = weight of the Standard (mg)

P = purity of the designated Reference Standard

V = volume of each of the *Standard solutions* (mL)

Sample solution: 100 mg of Egg Phospholipids in a 25-mL volumetric flask. Dissolve in *Solvent,* and dilute. Calculate the concentration, in mg/mL: this value is used as the sample amount.

Chromatographic system

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

Mode: LC

Detector: evaporative light-scattering detector

Column: 4-mm × 125-mm; 5-µm packing L20

Column temperature: 55°

Injection size: 20 µL

System suitability

Sample: *Standard solutions*

[Note—The relative retention times for phosphatidylcholine, phosphatidylethanolamine, and lysophosphatidylcholine are 1.00, 0.85, and 1.25, respectively.]

Suitability requirements

Relative standard deviation: NMT 5.0%

Analysis

Samples: Each of the *Standard solutions* and *Sample solution*

Identify the peaks of the relevant analytes in the chromatogram of the *Sample solution* by comparison with the chromatograms obtained from the *Standard solutions.* Measure the areas of the analyte peaks. Plot the logarithms of the relevant responses versus the logarithms of the concentrations, in mg/mL, of each analyte obtained from the *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 graphs so obtained, determine the concentration, C, in mg/mL, of the relevant analyte in the *Sample solution.*

Separately calculate the percentages of phosphatidylethanolamine, phosphatidylcholine, and lysophosphatidylcholine in the portion of Egg Phospholipids taken:

$$\text{Result} = (CV/W) \times 100$$

C = concentration of the relevant analyte in the *Sample solution* (mg/mL)

V = volume of the relevant analyte in the *Sample solution* (mL)

W = weight of Egg Phospholipids in the *Sample solution* (mg)

Acceptance criteria: NMT 3.0% of lysophosphatidylcholine

IMPURITIES

Organic Impurities

• **Procedure: Limit of Nonphosphatidyl Lipids**

Solvent: Diethyl ether

Sample solution: 500 mg of Egg Phospholipids, dissolved in 15 mL of *Solvent*, in a 50-mL conical flask

Chromatographic system

(See *Chromatography ⟨621⟩, Column Chromatography*.)

Mode: Column

Chromatographic column:

Transfer 1000 g of silica gel having a particle size of 0.05–0.2 mm into a container with well-closing screw caps. Add 150 g of water, shake well, and allow to stand for 24 h. Suspend 15 g of prepared adsorbent in 50 mL of *Solvent*, and introduce into a 1- to 2-cm chromatographic column. Drain the *Solvent* through the column to a level of about 1 cm above the silica gel bed.

Analysis

Sample: *Sample solution*

Transfer the *Sample solution* to the *Chromatographic column.* Rinse the column containing the *Sample solution* with two 15-mL portions of *Solvent,* allowing each rinse to pass through the column before adding the next. After rinsing, elute with 105 mL of *Solvent*. Evaporate the eluate (150 mL) in a tared, round–bottom, 250-mL conical flask to dryness, using a suitable rotary evaporator. The volatiles are blown out with a stream of nitrogen, and the residue is dried at 105° for 20 min. The weight of the residue gives the oil fraction, determined as nonpolar lipids, in Egg Phospholipids.

Calculate the percentage of the nonphosphatidyl lipids taken:

$$\text{Result} = A/W \times 100$$

A = weight of the residue (mg)

W = weight of Egg Phospholipids taken in the *Sample solution* (mg)

Acceptance criteria: NMT 7.0%

SPECIFIC TESTS

• **Fats and Fixed Oils, *Acid Value* ⟨401⟩:** NMT 20.0

• **Fats and Fixed Oils, *Peroxide Value* ⟨401⟩:** NMT 3

• **Bacterial Endotoxins Test ⟨85⟩:** NMT 6 USP Endotoxin Units/g

• **Microbial Enumeration Tests ⟨61⟩** and **Tests for Specified Microorganisms ⟨62⟩:** The total microbial count does not exceed 100 cfu/g. It meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli*.

• **Water Determination, *Method I* ⟨921⟩**
Sample: 2 g in 50 mL of anhydrous methyl alcohol
Acceptance criteria: NMT 6.0%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve under nitrogen in a sealed container, and store at a temperature of −10° or below.

• **USP Reference Standards ⟨11⟩**
USP Phosphatidylcholine RS
USP Phosphatidylethanolamine RS
USP Lysophosphatidylcholine RS

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

Topic/Question	Contact	Expert Committee
EGG PHOSPHOLIPIDS	Documentary Standards Support	CE2020 Complex Excipients

Chromatographic Database Information: Chromatographic Database

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