Status: Currently Official on 18-Feb-2025
Official Date: Official as of 01-Dec-2024
Document Type: NF Monographs
DocId: GUID-81DE8B6C-77FD-4031-A3AD-A7F8618A2247_5_en-US
DOI: https://doi.org/10.31003/USPNF_M44420_05_01
DOI Ref: 5nasq

Lecithin

CAS RN®: 8002-43-5.

DEFINITION

Lecithin is a complex mixture of acetone-insoluble phosphatides, which consist chiefly of phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, and phosphatidic acid, present in conjunction with various amounts of other substances such as triglycerides, fatty acids, and carbohydrates, as separated from the crude vegetable oil source. The content of each of the phospholipids (phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, and phosphatidic acid) is indicated on the certificate of analysis.

IDENTIFICATION

• **A. Identification of Phospholipids by Thin-Layer Chromatography**

Developing solvent system: Chloroform, methanol, and water (65:25:4, v/v/v)

Standard solution A: 10 mg/mL of USP Phosphatidic Acid (Soy) Monosodium RS and 10 mg/mL of USP Phosphatidylcholine (Soy) RS in the *Developing solvent system*

Standard solution B: 7 mg/mL of USP Phosphatidylethanolamine (Soy) RS and 7 mg/mL of USP Lysophosphatidylcholine (Soy) RS in the *Developing solvent system*

Sample solution: 20 mg/mL of Lecithin 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 A*, *Standard solution B*, 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* in different bands 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 retardation factor (R_F) values of the spots for phosphatidylcholine, phosphatidylethanolamine, phosphatidic acid, and lysophosphatidylcholine from the *Sample solution* correspond to those from *Standard solution A* and *Standard solution B*. [Note—Depending on the sample tested, if a phospholipid component presents in a low amount in the sample, the corresponding spot in the *Sample solution* on the TLC may not be visualized.]

ASSAY

Change to read:

• **Content of Phospholipids**

[Note—Analyze Lecithin for lysophosphatidylcholine ▲if needed.▲ (NF 1-Dec-2024)]

Solution A: Mix 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 2.0 mL of triethylamine.

Solution B: Mix 663.5 g (850 mL) of isopropyl alcohol, 15.8 g (15 mL) of acetic acid, glacial, 140 g (140 mL) of water, and 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 1*.

Table 1

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

Phospholipids standard stock solution (2X): 0.8 mg/mL of USP Phosphatidylcholine (Soy) RS, 0.4 mg/mL of USP Phosphatidylethanolamine (Soy) RS, 0.4 mg/mL of phosphatidylinositol prepared from USP Phosphatidylinositol (Soy) Sodium RS, and 0.2 mg/mL of phosphatidic acid prepared from USP Phosphatidic Acid (Soy) Monosodium RS in *Diluent*. [Note—Due to the highly hydroscopic nature of phospholipids, take special precaution in the Standard preparation.]

Phospholipids standard solutions: Prepare as directed in *Table 2*.

Table 2

Concentration	Phospholipids standard stock solution (2X) and Diluent (v/v)
0.6X	3:7
0.8X	4:6
1.0X	5:5
1.2X	6:4
1.4X	7:3

System suitability solution: *Phospholipids standard solution 1.0X*

Lysophosphatidylcholine standard stock solution (2X): 60 µg/mL of USP Lysophosphatidylcholine (Soy) RS in *Diluent*

Lysophosphatidylcholine standard solution: 30 µg/mL of USP Lysophosphatidylcholine (Soy) RS in *Diluent*

▲**Reference**▲ (NF 1-Dec-2024) **solution:** Mix equal volumes of *Phospholipids standard stock solution (2X)* and *Lysophosphatidylcholine standard stock solution (2X)*.

Sample solution: 1 mg/mL of Lecithin in *Diluent*. [Note—If necessary, adjust the concentration of the *Sample solution* to obtain the concentration of each of the phospholipids within the calibration range. ▲For analysis of Lecithin for lysophosphatidylcholine, the concentration of the *Sample solution* cannot be adjusted.▲ (NF 1-Dec-2024)]

Chromatographic system

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

Mode: LC

Detector: Evaporative light-scattering

Column: 4-mm × 12.5-cm; 5-µm packing L20

Temperatures

Detector: 50°

Column: 55°

Flow rate: 1.0 mL/min with step gradient at 2.0 mL/min (see *Table 1*)

Injection volume: 20 µL

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

System suitability

Samples: *System suitability solution* and ▲*Reference*▲ *(NF 1-Dec-2024) solution*

[NOTE—The relative retention times for phosphatidic acid, phosphatidylethanolamine, phosphatidylcholine, phosphatidylinositol, and lysophosphatidylcholine are 0.4, 0.9, 1.0, 1.2, and 1.3, respectively, for the ▲*Reference*▲ *(NF 1-Dec-2024) solution*.]

Suitability requirements

Resolution: NLT 2.0, *System suitability solution*

Relative standard deviation: NMT 5.0%, *System suitability solution*

Analysis

Samples: *Phospholipids standard solutions*, *Lysophosphatidylcholine standard solution*, and *Sample solution*

Identify the peaks of the relevant phospholipids from the *Sample solution* by comparison with those in the *Phospholipids standard solutions*. Measure the areas of the phospholipid peaks from the *Phospholipids standard solutions* and *Sample solution*. Plot the logarithms of the relevant responses versus the logarithms of the concentrations, in mg/mL, of each of the phospholipids from the *Phospholipids 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, determine the concentration (C), in mg/mL, of the relevant phospholipid in the *Sample solution*.

Calculate the percentage of each of the phospholipids (phosphatidic acid, phosphatidylethanolamine, phosphatidylcholine, and phosphatidylinositol) in the portion of Lecithin taken:

$$\text{Result} = (C_U/C_S) \times 100$$

C_U = concentration of each of the phospholipids in the *Sample solution* (mg/mL)

C_S = concentration of Lecithin in the *Sample solution* (mg/mL)

Identify the peak of lysophosphatidylcholine from the *Sample solution* by comparison with that in the *Lysophosphatidylcholine standard solution*. Compare the peak area of lysophosphatidylcholine from the *Lysophosphatidylcholine standard solution*▲, which corresponds to 3.0% of lysophosphatidylcholine in Lecithin in the *Sample solution*,▲ (NF 1-Dec-2024) with that of the *Sample solution*.

Acceptance criteria

Content of phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, and phosphatidic acid: Within the respective ranges stated on the label

▲▲ (NF 1-Dec-2024)

IMPURITIES

• **LEAD ⟨251⟩, *Procedures, Procedure 1*:** NMT 10 ppm

• **HEXANE-INSOLUBLE MATTER**

Sample: If the substance under test is plastic or semisolid, soften the Lecithin by warming it at a temperature not exceeding 60°, and then mix. Weigh 10.0 g into a 250-mL conical flask.

Analysis: Add 100 mL of hexane, solvent to the *Sample*. Shake until the *Sample* is completely dissolved or until no more residue seems to be dissolving. Pass through a coarse-porosity filtering funnel that previously has been heated at 105° for 1 h, cooled, and weighed. Wash the flask with two 25-mL portions of hexane, solvent, and pour both washings through the funnel. Dry the funnel at 105° for 1 h. [CAUTION—Hexane, solvent is flammable.] Cool to room temperature, and determine the gain in weight.

Acceptance criteria: NMT 0.3%

For Sunflower Lecithin: NMT 1.0%

SPECIFIC TESTS

Change to read:

• **CONTENT OF ACETONE-INSOLUBLE MATTER**

Sample: If the substance under test is plastic or semisolid, soften the Lecithin by warming it briefly at a temperature not exceeding 60°, and then mix. Transfer 2 g to a 40-mL centrifuge tube that previously has been tared along with a stirring rod, cool, and weigh.

Analysis: Add 15.0 mL of acetone to the *Sample*, and warm carefully in a water bath to melt the test specimen without evaporating the acetone. Stir to help dissolve completely, and place in an ice-water bath for 5 min. De-oiled lecithin and fractions are suspended in acetone by stirring. Add acetone that has been previously chilled to 0°–5° to the 40-mL mark on the tube, stirring during the addition. Cool in an ice-water bath for 15 min, stir, remove the rod, clarify by centrifuging at about 2000 rpm for 5 min, and decant. Break up the residue with the stirring rod, and refill the centrifuge tube to the 40-mL mark with chilled acetone, while stirring. Cool in an ice-water bath for 15 min, stir, remove the rod, centrifuge, and decant. Break up the residue with the stirring rod. Place the tube in a horizontal position until most of the

acetone has evaporated. Mix again, and heat the tube containing the acetone-insoluble residue and the stirring rod at 105° to constant weight. [**Caution**—Acetone is flammable.]

Determine the weight of the residue, and calculate the percentage of acetone-insoluble matter.

Acceptance criteria: NLT 50.0%

▲▲ (NF 1-Dec-2024)

• **Fats and Fixed Oils ⟨401⟩, *Procedures, Acid Value***

Sample: If the substance under test is plastic or semisolid, soften the Lecithin by warming it briefly at a temperature not exceeding 60°, and then mix. Transfer 2 g to a 250-mL conical flask.

Analysis: Dissolve the *Sample* in 50 mL of petroleum ether with 100°–120° boiling range. To this solution add 50 mL of alcohol, previously neutralized to phenolphthalein with 0.1 N sodium hydroxide VS, and mix. Add phenolphthalein TS. Titrate with 0.1 N sodium hydroxide VS to a pink endpoint that persists for 5 s.

Calculate the acid value, in mg, of potassium hydroxide required to neutralize the free acids in 1.0 g of Lecithin:

$$\text{Result} = (M_r \times N \times V)/W$$

M_r = molecular weight of potassium hydroxide, 56.11

N = normality of the sodium hydroxide VS

V = volume of the sodium hydroxide VS consumed in the titration (mL)

W = weight of Lecithin taken (g)

Acceptance criteria: NMT 36

Change to read:

• ▲**Fats and Fixed Oils ⟨401⟩, *Procedures, Peroxide Value***▲ (NF 1-Dec-2024)

Sample: 5 g of Lecithin

Analysis: Transfer the *Sample* into a 250-mL Erlenmeyer flask with a ground-glass stopper, add 35 mL of a mixture of chloroform and acetic acid, glacial (2:1), and mix. Completely dissolve the test specimen while shaking gently. The solution becomes transparent. Completely replace the air in the flask with nitrogen. While purging with nitrogen, add 1 mL of potassium iodide solution (165 mg/mL of potassium iodide), then stop the flow of the nitrogen, and immediately place a stopper in the flask. Shake for 1 min, and allow to stand in a dark place for 5 min. Add 75 mL of water, replace the stopper again, and shake vigorously. Titrate with 0.01 N sodium thiosulfate VS, adding starch TS as the endpoint is approached, and continue the titration until the blue starch color has just disappeared. Perform a blank determination ▲under the same conditions,▲ (NF 1-Dec-2024) and make any necessary correction.

Calculate the peroxide value, as mEq of peroxide per 1000 g of Lecithin:

$$\text{Result} = (S \times N/W) \times 1000$$

S = net volume of sodium thiosulfate solution required for titration (mL)

N = normality of the sodium thiosulfate solution

W = weight of Lecithin taken (g)

Acceptance criteria: NMT 10

▲▲ (NF 1-Dec-2024)

• **Microbial Enumeration Tests ⟨61⟩** and **Tests for Specified Microorganisms ⟨62⟩:** The total aerobic microbial count does not exceed 10^3 cfu/g, and the total combined molds and yeasts count does not exceed 10^2 cfu/g. It meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli*.

• **Water Determination ⟨921⟩, *Method I*:** NMT 2.0%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in well-closed, light-resistant containers. Store at the temperature indicated on the label. Protect from excess heat and moisture.

Change to read:

• **Labeling:** Label to indicate the content of phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, and phosphatidic acid. The labeling also indicates the natural source of lecithin. ▲▲ (NF 1-Dec-2024) Label it to indicate the storage conditions.

• **USP Reference Standards ⟨11⟩**

USP Lysophosphatidylcholine (Soy) RS
USP Phosphatidic Acid (Soy) Monosodium RS
USP Phosphatidylcholine (Soy) RS
USP Phosphatidylethanolamine (Soy) RS
USP Phosphatidylinositol (Soy) Sodium RS

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

Topic/Question	Contact	Expert Committee
LECITHIN	Documentary Standards Support	CE2020 Complex Excipients
REFERENCE STANDARD SUPPORT	RS Technical Services RSTECH@usp.org	CE2020 Complex Excipients

Chromatographic Database Information: Chromatographic Database

Most Recently Appeared In:
Pharmacopeial Forum: Volume No. 48(2)

Current DocID: GUID-81DE8B6C-77FD-4031-A3AD-A7F8618A2247_5_en-US

DOI: https://doi.org/10.31003/USPNF_M44420_05_01

DOI ref: 5nasq