Edition: BP 2025 (Ph. Eur. 11.6 update)

Egg Phospholipids for Injection

General Notices

(Ph. Eur. monograph 2315)

Egg lecithins *93685-90-6*

Egg phosphatidylcholines *97281-44-2*

Ph Eur

DEFINITION

Mixture of natural phospholipids of egg yolk, and fractions thereof, for injection. A suitable stabiliser such as α-tocopherol may be added.

Content

— *phosphatidylcholine*: minimum 60.0 per cent (anhydrous substance);

— *phosphatidylethanolamine*: maximum 20.0 per cent (anhydrous substance);

— *lysophosphatidylcholine*: maximum 3.0 per cent (anhydrous substance).

PRODUCTION

Viral safety

The requirements of general chapter *5.1.7* apply.

CHARACTERS

Appearance

Almost white or yellow mass or powder.

Solubility

Practically insoluble in water, very soluble in ethanol (96 per cent) and freely soluble in heptane.

IDENTIFICATION

Carry out either test A or test B. If sphingomyelin is not detected with test A or B, carry out test C.

A. Thin-layer chromatography (2.2.27).

Test solution Dissolve 25 mg of the substance to be examined in methanol R and dilute to 50.0 mL with the same solvent.

Reference solution (a) Dissolve 10 mg of phosphatidylethanolamine from egg yolk CRS in methanol R and dilute to 10.0 mL with the same solvent.

Reference solution (b) Dissolve 15 mg of lysophosphatidylcholine from egg yolk CRS and 15 mg of sphingomyelin from egg yolk R in methanol R and dilute to 20.0 mL with the same solvent.

Reference solution (c) Dissolve 15 mg of phosphatidylcholine from egg yolk CRS in methanol R, add 5 mL of reference solution (a) and 1 mL of reference solution (b) and dilute to 50.0 mL with methanol R.

Plate TLC silica gel plate R (2-10 µm).

Mobile phase water R, methanol R, chloroform R (4:25:65 *V/V/V*).

Application 4 µL of the test solution and reference solution (c), as slim bands.

Development Over 2/3 of the plate.

Detection Spray with copper sulfate solution R1 and heat at 170 °C for 5-15 min; the phospholipids appear as brownish-grey bands.

Retardation factors Lysophosphatidylcholine = about 0.15; sphingomyelin = about 0.2; phosphatidylcholine = about 0.3; phosphatidylethanolamine = about 0.6.

System suitability Reference solution (c):

— the chromatogram shows 4 clearly separated bands.

Results:

— the principal band in the chromatogram obtained with the test solution is similar in position and colour to the band due to phosphatidylcholine in the chromatogram obtained with reference solution (c);

— the band due to sphingomyelin is visible in the chromatogram obtained with the test solution.

B. Liquid chromatography (2.2.29) as described in the assay with the following modifications.

Injection Test solution and reference solution (h).

Identification of peaks Use the chromatogram obtained with reference solution (h) to identify the peak due to sphingomyelin.

Relative retention With reference to phosphatidylcholine (retention time = about 8 min): sphingomyelin = about 1.1 (may be eluted as 1 or 2 peaks).

Results:

— the principal peak in the chromatogram obtained with the test solution is due to phosphatidylcholine;

— the peak due to sphingomyelin in the chromatogram obtained with the test solution has a signal-to-noise ratio not less than 3.

C. Composition of fatty acids (2.4.22, Method B). Use the mixture of calibrating substances in Table 2.4.22.-3.

Composition of the fatty-acid fraction of the substance:

— the oleic acid (C18:1) content is greater than that of linoleic acid (C18:2);

— arachidonic acid (C20:4) and docosahexaenoic acid (C22:6) are present.

TESTS

Acid value (2.5.1)

Maximum 20.

Dissolve 2.00 g in 50 mL of a mixture of equal volumes of *ethanol (96 per cent) R* and *ether R*, previously neutralised with *0.1 M potassium hydroxide* using 0.5 mL of *phenolphthalein solution R1* as indicator.

Peroxide value (*2.5.5, Method A*)

Maximum 3.

Titrate with *0.01 M sodium thiosulfate*, determining the end-point potentiometrically (*2.2.20*).

Nonphosphatidyl lipids

Maximum 7.0 per cent.

Test solution Dissolve 0.500 g of the substance to be examined in *ether R* and dilute to 15.0 mL with the same solvent.

Chromatography column *l* = 0.35-0.4 m, Ø = 10-20 mm.

Preparation of the column Transfer 1 kg of silica gel with a particle size of 0.05-0.2 mm into a container fitted with a screw cap. Add 150 g of *water R*, shake thoroughly, and allow to stand for 24 h. Suspend 15 g of the prepared adsorbent in 50 mL of *ether R* and introduce into the column. Allow the *ether R* to pass through the column to a level of about 1 cm above the silica gel bed.

Transfer the test solution to the column. Rinse with 2 quantities, each of 15 mL, of *ether R*, allowing each to pass through the column before adding the next. After rinsing, elute with 105 mL of *ether R*. Combine the rinsings and eluate and evaporate to dryness. Remove any volatile components with a stream of nitrogen and dry the residue at 105 °C for 20 min.

Calculate the percentage content of nonphosphatidyl lipids using the following expression:

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m_1 = mass of the residue, in milligrams;

m_2 = mass of the substance to be examined in the test solution, in milligrams.

Water (*2.5.12*)

Maximum 4.0 per cent, determined on 0.500-1.00 g.

Microbial contamination

TAMC: acceptance criterion 10^2 CFU/g (*2.6.12*).

Absence of *Escherichia coli* (*2.6.13*).

Absence of *Salmonella* (*2.6.13*).

Bacterial endotoxins (*2.6.14, Method A*)

Less than 6 IU/g. The suitability of the method must be verified and a risk assessment must be carried out according to general chapter *5.1.10*. *Guidelines for using the test for bacterial endotoxins* since the phospholipids can interfere with the test.

ASSAY

Liquid chromatography (*2.2.29*).

Solvent mixture Mix 8 volumes of *water R* and 46 volumes of *2-propanol R*, then add 46 volumes of *hexane R*.

Test solution Dissolve 0.100 g of the substance to be examined in the solvent mixture and dilute to 25.0 mL with the solvent mixture.

NOTE: if saturation of the peak due to phosphatidylcholine is observed, the test solution may be diluted with the solvent mixture to quantify this compound.

Reference solution (a_1) Dissolve 60 mg of *phosphatidylcholine from egg yolk CRS* in the solvent mixture and dilute to 10.0 mL with the solvent mixture.

Reference solutions (b_1), (c_1), (d_1), (e_1), (f_1) Dilute reference solution (a_1) with the solvent mixture to obtain 5 reference solutions, the concentrations of which span 60 per cent to 140 per cent of the expected content of phosphatidylcholine in the test solution.

Reference solution (a_2) Dissolve 40 mg of *phosphatidylethanolamine from egg yolk CRS* in the solvent mixture and dilute to 10.0 mL with the solvent mixture.

Reference solutions (b_2), (c_2), (d_2), (e_2), (f_2) Dilute reference solution (a_2) with the solvent mixture to obtain 5 reference solutions, the concentrations of which span 60 per cent to 140 per cent of the expected content of phosphatidylethanolamine in the test solution.

Reference solution (a_3) Dissolve 40 mg of *lysophosphatidylcholine from egg yolk CRS* in the solvent mixture and dilute to 10.0 mL with the solvent mixture.

Reference solutions (b_3), (c_3), (d_3), (e_3), (f_3) Dilute reference solution (a_3) with the solvent mixture to obtain 5 reference solutions, the concentrations of which span 60 per cent to 140 per cent of the expected content of lysophosphatidylcholine in the test solution.

Reference solution (g) Mix 5 mL of reference solution (a_1) and 5 mL of reference solution (a_2).

Reference solution (h) Dissolve 5 mg of *sphingomyelin from egg yolk R* in the solvent mixture and dilute to 50.0 mL with the solvent mixture.

Column:

— *size*: *l* = 0.125 m, Ø = 4.0 mm;

— *stationary phase*: *diol silica gel for chromatography R* (5 µm);

— *temperature*: 55 °C.

Mobile phase:

— *mobile phase A*: mix 0.08 volumes of *triethylamine R*, 1.5 volumes of *glacial acetic acid R*, 17 volumes of *2-propanol R* and 81.42 volumes of *hexane R*;

— *mobile phase B*: mix 0.08 volumes of *triethylamine R*, 1.5 volumes of *glacial acetic acid R*, 14 volumes of *water for chromatography R* and 84.42 volumes of *2-propanol R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 2.0	95	5
2.0 - 7.0	95 → 80	5 → 20
7.0 - 10.5	80 → 60	20 → 40
10.5 - 17.0	60 → 0	40 → 100
17.0 - 19.5	0	100

Flow rate 1.0 mL/min.

Detection Evaporative light-scattering detector; the following settings have been found to be suitable; if the detector has different setting parameters, adjust the detector settings so as to comply with the system suitability criteria:

— *carrier gas*: *nitrogen R*;

— *flow rate*: 2.5 mL/min;

— *evaporator temperature*: 50 °C.

Injection 20 µL of the test solution and reference solutions ($b_{1,2,3}$), ($c_{1,2,3}$), ($d_{1,2,3}$), ($e_{1,2,3}$), ($f_{1,2,3}$) and (g).

Identification of peaks Use the chromatograms obtained with reference solutions (f_1), (f_2) and (f_3) to identify the peaks due to phosphatidylcholine, phosphatidylethanolamine and lysophosphatidylcholine.

Relative retention With reference to phosphatidylcholine (retention time = about 8 min): phosphatidylethanolamine = about 0.8; lysophosphatidylcholine = about 1.2 (may be eluted as 1 or 2 peaks).

Establish calibration curves with the logarithm of the concentration of phosphatidylcholine, phosphatidylethanolamine and lysophosphatidylcholine in reference solutions ($b_{1,2,3}$), ($c_{1,2,3}$), ($d_{1,2,3}$), ($e_{1,2,3}$) and ($f_{1,2,3}$) as the abscissa and the logarithm of the corresponding peak areas as the ordinate.

System suitability:

— *resolution*: minimum 2.0 between the peaks due to phosphatidylethanolamine and phosphatidylcholine in the chromatogram obtained with reference solution (g);

— *phosphatidylcholine*:

— *repeatability*: maximum relative standard deviation of 5.0 per cent determined on 6 injections of reference solution (f_1);

— *correlation coefficient*: minimum 0.995;

— *phosphatidylethanolamine*:

— *repeatability*: maximum relative standard deviation of 5.0 per cent determined on 6 injections of reference solution (f_2);

— *correlation coefficient*: minimum 0.995;

— *lysophosphatidylcholine*:

— *repeatability*: maximum relative standard deviation of 5.0 per cent determined on 6 injections of reference solution (f_3);

— *correlation coefficient*: minimum 0.99.

Calculate the percentage contents of phosphatidylcholine, phosphatidylethanolamine and lysophosphatidylcholine using the calibration curves.

STORAGE

Under nitrogen, protected from light, at -15 °C or below.

LABELLING

The label states:

— the specified content range of phosphatidylcholine;

— the specified content range of phosphatidylethanolamine.