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Short communication

Isolation and quantitation of phosphatidylcholine by reversed-phase liquid–liquid extraction

Jan Hradec*, Petr Dufek

Department of Cellular Biochemistry, Institute of Hematology and Blood Transfusion, U nemocnice 1, 12820, Prague 2, Czech Republic

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Abstract

A simple and rapid method is described for the determination of total phosphatidylcholines in serum. Extracts of lipids are applied to octadecyl silica cartridges and phosphatidylcholines are eluted by methanol–acetonitrile mixtures containing choline. Quantitation of these compounds is performed by colorimetry of their complexes with erythrosin B. The method is sensitive down to approximately 10 μg, exhibits good reproducibility and may be used as a preliminary step for the separation of individual molecular species of phosphatidylcholines by high-performance liquid chromatography. © 1997 Elsevier Science B.V.

Keywords: Phosphatidylcholines

1. Introduction

No specific reactions for phosphatidylcholines exist that would allow for their determination in the presence of other phospholipids. Moreover, the retention times of some of their molecular species, in particular phosphatidylethanolamines and phosphatidylglycerol, in reversed-phase high-performance liquid chromatography (HPLC) are very similar to those of phosphatidylcholines [1] and would, thus, interfere with their determination. For detailed separations of molecular species of phosphatidylcholine, their preliminary separation from the other phospholipids is necessary.

Satisfactory separations of phospholipid classes were reported by both solid–liquid [2–4] and reversed-phase HPLC [5–8]. However, all of these methods are relatively time-consuming and thus, are barely suitable for the rapid quantitative isolation of phosphatidylcholine.

For our studies on the substrate specificity of cholesterol-esterifying enzymes from rat liver cytosol [9], a simple method for the separation of phosphatidylcholine, such as that described in this paper, was required. This procedure also proved useful for the detection of alterations of phosphatidylcholine metabolism related to malignant growth, as indicated by our recent results [10].

2. Experimental

2.1. Chemicals

Phosphatidylcholine and phosphatidylethanolamine from egg yolk, phosphatidylglycerol and all

*Corresponding author.

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other chemicals were purchased from Sigma–Aldrich (Prague, Czech Republic). All solvents were of HPLC grade. Chromabond octadecyl silica cartridges (100 mg) were from Macherey–Nagel (Düren, Germany).

2.2. Extraction of total lipids

Total lipids from 0.1 ml of serum were extracted with twenty volumes of chloroform–methanol (2:1, v/v) as described by Folch et al. [10]. Extracts were evaporated to dryness at 50°C under a stream of N_2 and residues were redissolved in 200 μl of chloroform–methanol.

2.3. Liquid–liquid extraction of phosphatidylcholine

Lipid extracts were applied to Chromabond cartridges that had been equilibrated with 10 ml of 40 m*M* choline in a methanol–acetonitrile–acetic acid (20:80:5, v/v) and phosphatidylcholine was eluted using an additional 5 ml of the same solvent mixture in a Dorcus Vacuum Manifold (Tessek, Prague, Czech Republic). Eluates were collected in conical glass vials and evaporated to dryness at 50°C under a stream of N_2 in a Thermovap evaporator (Ecom, Prague, Czech Republic). Residues were dissolved in 0.5 ml of ethanol.

2.4. Quantitation of phosphatidylcholine

The colorimetric assay described by Andree and Soedjak [11] was used. Samples to be analyzed (50 μl) were mixed with 50 μl of a freshly prepared solution of 30 μg of erythrosin B in a 25 m*M* sodium citrate buffer, pH 4.25, and the absorbance at 549 nm was measured against the blank sample containing pure ethanol only. All assays were performed in triplicate.

3. Results and discussion

Phospholipids including phosphatidylcholine are firmly bound to octadecyl silica and cannot be eluted using the usual solvent mixtures. This difficulty may be overcome by the addition of choline chloride [1] or methylphosphonic acid [5] to the elution mixtures. The desorption of phospholipids is also enhanced by increasing the temperature (50°C) at which the HPLC procedure is usually performed [5,6]. This latter approach can hardly be used for liquid–liquid extraction since vaccuum manifolds do not contain heating facilities. In our hands, neither the addition of choline chloride nor methylphosphonic acid was efficient and phosphatidylcholine was eluted as a broad peak in a large volume so that its quantitative recovery could hardly be secured. However, the addition of acid to the elution mixture (Fig. 1) resulted in a quantitative elution of phosphatidylcholine (Table 1) as a sharp peak in an acceptable volume (Fig. 2). Acetic acid was used because it is volatile and can therefore easily be removed by evaporation.

Only phosphatidylcholine is eluted under the conditions described. If phosphatidylethanolamine or phosphatidylglycerol were subjected to liquid–liquid extraction, the eluate did not react with erythrosin B and no peaks could be detected if the eluate was injected into the HPLC system and chromatographed as described by Bernhard et al. [5].

The method described here yields a satisfactory recovery of added phosphatidylcholines (Table 1)

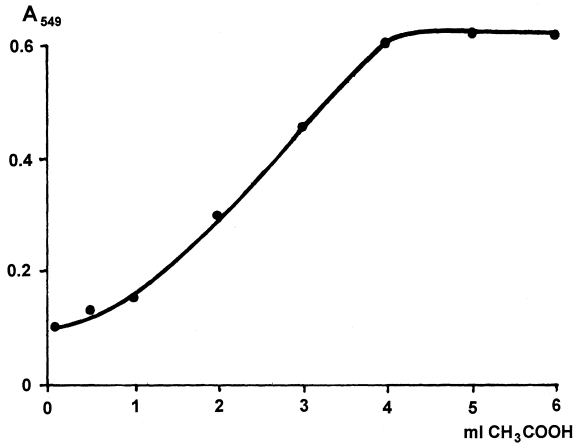

Fig. 1. Effect of acetic acid on the elution of phosphatidylcholine from octadecyl silica. Additions are indicated as ml of acid added per 100 ml of a methanol–acetonitrile (20:80, v/v) mixture. Individual points are mean values from three experiments (see Table 1 for reproducibility data).

Table 1
Recovery of added phosphatidylcholine

	Phosphatidylcholine			
	Added	Recovered		
	(mg)	Total±S.D.	Net (mg)	%
1	None	1.280±55.02	–	–
2	0.50	1.769±46.69	0.489	97.8
3	1.00	2.326±50.36	1.036	103.6
4	1.50	2.810±48.59	1.530	102.0
5	2.00	3.346±60.21	2.066	103.3
6	2.50	3.790±68.54	2.510	100.4

Quantities of phosphatidylcholine as indicated were added to the chloroform–methanol mixture used for the extraction of serum and all additions were made to each of six samples. The same batch of normal human serum was used in all samples. Extracts were applied to octadecyl silica cartridges, phosphatidylcholine was eluted and its concentration in eluates was determined as described in Section 2.

and makes it possible to quantitatively isolate these compounds in a micropreparative procedure. Quantities of up to 5 mg of phosphatidylcholine are isolated in a single run in our laboratory.

Phosphate analysis is undoubtedly the most sensitive method (down to approximately 1 nmol [11]) but it requires considerable preparation and is thus time-consuming. The colorimetric method based on complexation with erythrosin B [11] is less sensitive. Moreover, it is not specific for phosphatidylcholines. However, it is useful for the quantitation of materials containing only a single group of compounds, as in our case, where only phosphatidylcholines are present (as indicated by our control experiments with other phospholipids). It gives a linear response up to approximately 100 μg (Fig. 3) and its sensitivity is satisfactory for the determination of phosphatidylcholine in 0.05–0.1 ml of serum.

The method described in this paper was used for the determination of phosphatidylcholine in the serum of 54 blood donors and an average value of 1.865±0.360 mg/ml (ranges 1.257–2.500 mg/ml) was found. It is now also being used to analyse the serum from patients with mammary cancer, which shows very significantly increased levels ($P<0.02$) and for the quantitation of phospholipids in leukemic leukocytes (J. Hradec, to be published). In all of these applications, the procedure proved to be very useful.

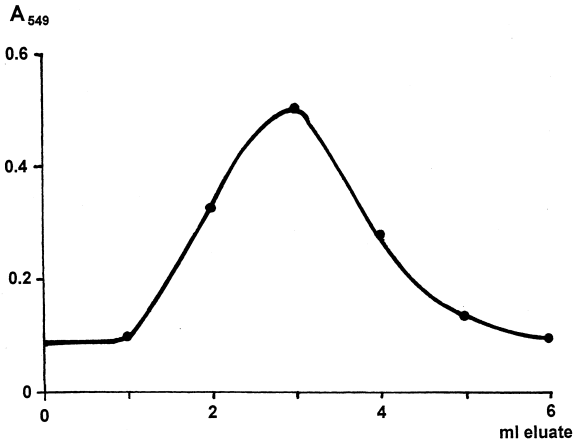

Fig. 2. Elution of phosphatidylcholine from octadecyl silica. Each ml of eluate was collected separately and the concentration of phosphatidylcholine was determined as described in Section 2. Individual points are the mean values from three experiments (see Table 1 for reproducibility data).

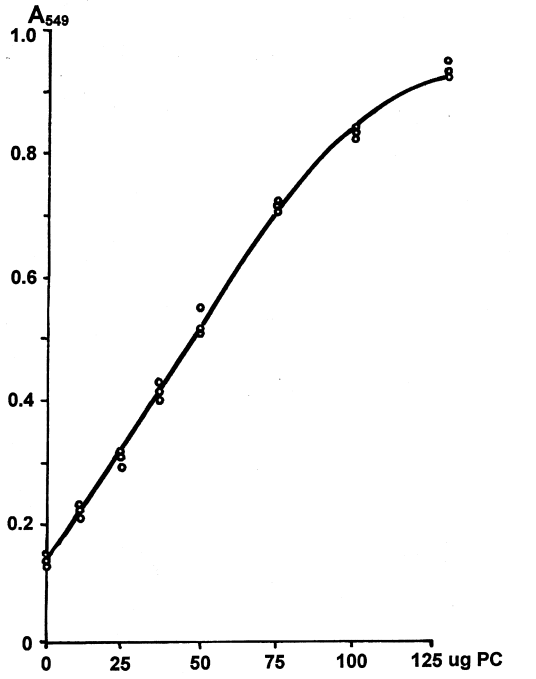

Fig. 3. Calibration curve for the determination of phosphatidylcholine (PC) with erythrosin B. Individual points were obtained in three calibrations.

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