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Short communication

Separation of phospholipids by nonaqueous capillary electrophoresis with electrospray ionisation mass spectrometry

Klaus Raith*, Raik Wolf, Jens Wagner, Reinhard H.H. Neubert

Martin-Luther-University, Department of Pharmacy, Institute of Pharmaceutics and Biopharmaceutics, Wolfgang-Langenbeck-Strasse 4, 06120 Halle (Saale), Germany

Abstract

A new method for separation of the main phospholipid classes by means of nonaqueous CE is described. Detection was done by electrospray ionisation MS in the negative mode. The structure of phospholipids was elucidated by MS–MS and MS^n experiments prior to CE separation. © 1998 Elsevier Science B.V.

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1. Introduction

While capillary zone electrophoresis (CZE) is well established for analyzing a wide range of substances, its applicability to lipids is still limited. An alternative to micellar electrokinetic capillary chromatography (MEKC), which is often unsuitable to MS detection, is nonaqueous CE (see [1] and literature given there). A MEKC method for phospholipid separation with UV detection was described by Ingvardsen et al. [2]. The potential of MS–MS and MS^n in structure elucidation of phospholipids was shown by Kerwin et al. [3]. However, CE–MS methods have to be compared with HPLC–MS (for phospholipids see [4]).

In this paper a quick and simple procedure using CE–electrospray ionisation(ESI) MS for the separation of different phospholipid classes according to the head groups is described.

*Corresponding author.

2. Experimental

2.1. Instrumentation

HPCE operations were carried out with a $HP^{3D}CE$ system (Hewlett-Packard, Waldbronn, Germany), which was coupled to an LCQ ion trap mass spectrometer (Finnigan MAT, San Jose, CA, USA) equipped with an ESI interface. Methyl-Sil deactivated capillaries (75 μm I.D.× 360 μm O.D., cut to a length of ~600 mm; CS, Langerwehe, Germany) were used to prevent wall interactions.

2.2. Reagents

1-Palmitoyl-2-oleyl-phosphatidylcholine (POPC), 1 - palmitoyl - 2 - oleyl - phosphatidylethanolamine (POPE), 1-palmitoyl-2-oleyl-phosphatidylglycerol (POPG) and 1-palmitoyl-2-oleyl- phosphatidylserine (POPS) were purchased from Avanti Polar Lipids (Alabaster, AL, USA). Ammonium acetate was purchased from Sigma (Deisenhofen, Germany). All

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solvents for CE were of HPLC grade from different suppliers. For MS sheath liquid methanol of gradient grade (Merck, Darmstadt, Germany) and doubly distilled water were used.

2.3. CE buffer and sample preparation

Ammonium acetate (20 m*M*) was solved in a mixture of acetonitrile–2-propanol–*n*-hexane (57:38:5, v/v) degassed by ultrasound for ~10 min and filtered through a 0.2-μm PTFE membrane filter before use. The samples were prepared as following: 50 μl of a chloroform solution (10 mg/ml) were diluted with 1 ml acetonitrile–2-propanol–*n*-hexane (57:38:5, v/v); 10 μl glacial acetic acid was added to prevent current breakdowns in case of large volume injection.

2.4. Capillary electrophoresis

The capillary was conditioned only with the CE electrolyte described above for 4 min. The samples were injected by pressure (200 mbar s). Separations were done at 25°C with +30 kV at the capillary inlet. If negative voltage is applied, the migration order is inverse. The effective separation voltage is calcu-

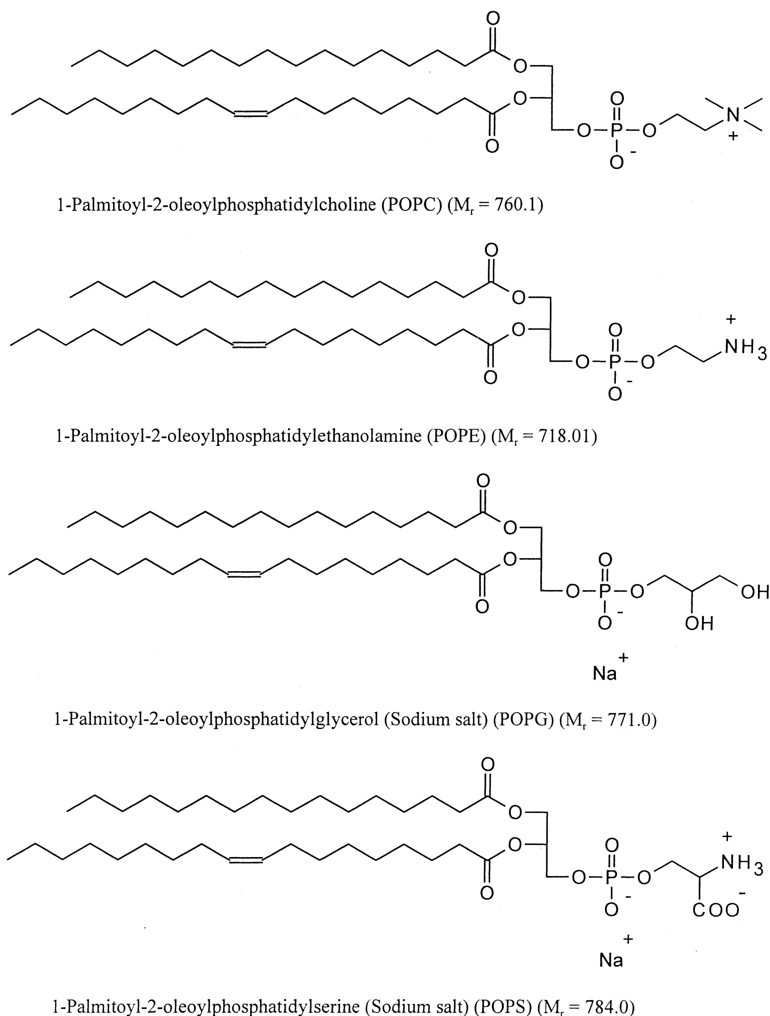

Fig. 1. Structures of the used phospholipids.

lated from the difference between nominal CE voltage applied to the capillary inlet and electrospray voltage applied to the outlet instead of the ground potential in normal CE experiments. Since no EOF exists, a small pressure difference must be applied to guarantee a sufficient liquid flow between CE capillary and ESI needle, which is necessary to prevent flashovers. However, it may be necessary to apply negative pressure from CE to compensate vacuum suction from MS.

2.5. Mass spectrometry

Detection was mainly done in the negative mode (ESI voltage: −4.5 kV). For stable electrospray conditions it was necessary to add sheath liquid (methanol–water, 80:20) via a syringe pump at rates of at least 0.5 μl/min (positive) or 5.0 μl/min (negative mode), respectively. Measurements can be performed in the full scan as well as in the selected ion monitoring (SIM) mode (for up to 10 known m/z ranges). MS–MS and MS^n was done by mass analyzer collision induced dissociation of the chosen parent ions at the ion trap.

3. Results and discussion

For understanding the electrophoretical properties it is important to visualize the structures of the investigated phospholipid classes shown in Fig. 1. To meet the solubility requirements of all types of phospholipids a solvent mixture of acetonitrile–2-propanol–*n*-hexane (57:38:5, v/v) was chosen with the volatile ammonium acetate as CE electrolyte. Glacial acetic acid (1%) was added to improve electrophoretical separation, which caused a not detrimental increase in CE current.

Fig. 2 shows the separation of the 1-palmitoyl-2-oleyl-derivatives of each phospholipid class. A separation was obtained within a short time (10 min). Although a separation of phospholipids with identical head groups which differ only in fatty acids was not successful until now, a separate quantitation is possible at different m/z. However, the limit of

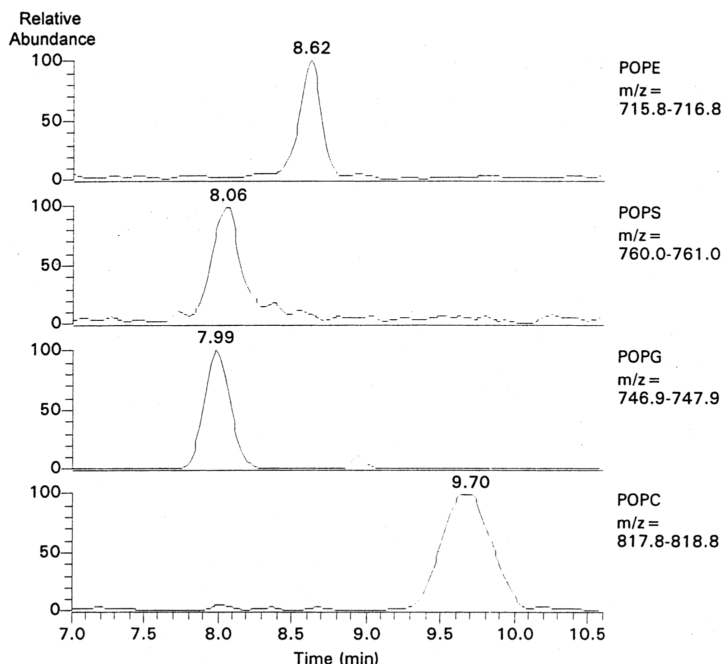

Fig. 2. Electropherogram of a phospholipid separation. The four peaks are labelled with their migration times (min). For an explanation of m/z see Table 1.

Table 1
Most important adducts and fragmentation of the phospholipids by MS–MS and MS^n

	POPC	POPE	POPG (Na salt)	POPS (Na salt)
(a) Positive ionization mode (H adducts, [a]: Na adducts)				
PL	760, 782[a]	718, 740[a]	771, 793[a]	784, 806[a]
OA	478			
PA	504			
PL HG	723[b]	697		719
OA				437
PA				463
PL HG-phosphate	577, 599[a]	577, 599[a]		
(b) Negative ionization mode ($[M-H]^-$, [c]: $[M+acetate]^-$ (acetate from CE buffer))				
PL	818[c]	716	747	760
OA[d]		452	465	
PA[e]		478	491	
PL HG			673	673
OA[d]		391	391	391
PA[e]		417	417	417
(OA+PA)[d,e]		153	153	153

PL, phospholipid, −, minus (fragmentation), HG, headgroup, OA, oleic acid, PA, palmitic acid.
All numbers are m/z ratios but can be taken as molecular masses because $z=1$.
[b] Headgroup not completely split off.
[d] $m/z=281$ resulting from OA ($[M-H]^-$).
[e] $m/z=255$ resulting from PA ($[M-H]^-$).

detection suffers from the dilution by sheath liquid. Progress in electrospray technology may solve this fundamental problem [5].

The m/z of the most important adducts of each phospholipid were determined in preliminary MS experiments. The quick and simple fragmentation of phospholipid headgroups and fatty acids is possible by MS^n, which is a major advantage of the ion trap technology (see Table 1). This is important for the identification of unknown phospholipids.

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