Journal of Chromatography A, 1439 (2016) 65–73

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Hydrophilic interaction liquid chromatography–mass spectrometry of (lyso)phosphatidic acids, (lyso)phosphatidylserines and other lipid classes

Eva Cífková, Roman Hájek, Miroslav Lísa, Michal Holčapek*

Department of Analytical Chemistry, Faculty of Chemical Technology, University of Pardubice, Studentská 573, 53210 Pardubice, Czech Republic

ARTICLE INFO

Article history:
Received 9 November 2015
Received in revised form 15 January 2016
Accepted 25 January 2016
Available online 29 January 2016

Keywords:
Phosphatidic acid
Phosphatidylserine
Phospholipids
Lysophospholipids
HILIC
LC/MS

ABSTRACT

The goal of this work is a systematic optimization of hydrophilic interaction liquid chromatography (HILIC) separation of acidic lipid classes (namely phosphatidic acids—PA, lysophosphatidic acids—LPA, phosphatidylserines—PS and lysophosphatidylserines—LPS) and other lipid classes under mass spectrometry (MS) compatible conditions. The main parameters included in this optimization are the type of stationary phases used in HILIC, pH of the mobile phase, the type and concentration of mobile phase additives. Nine HILIC columns with different chemistries (unmodified silica, modified silica using diol, 2-picolylamine, diethylamine and 1-aminoanthracene and hydride silica) are compared with the emphasis on peak shapes of acidic lipid classes. The optimization of pH is correlated with the theoretical calculation of acidobasic equilibria of studied lipid classes. The final method using the hydride column, pH 4 adjusted by formic acid and the gradient of acetonitrile and 40 mmol/L of aqueous ammonium formate provides good peak shapes for all analyzed lipid classes including acidic lipids. This method is applied for the identification of lipids in real samples of porcine brain and kidney extracts.

1. Introduction

Phosphatidic acids (PA) are found in small amounts in biological membranes, but they are crucial biosynthetic precursors of all phospholipids (PL) and signaling molecules in biochemical and physiological processes in cells [1–3]. PA are a source of diacylglycerols (DG), which are precursors for triacylglycerols (TG), phosphatidylcholines (PC) and phosphatidylethanolamines (PE) via the Kennedy pathway [4], and precursors of phosphatidylglycerols (PG), phosphatidylserines (PS), phosphatidylinositols (PI) and cardiolipins via cytidine diphosphate DG (CDP-DG) pathway [1]. Lysophosphatidic acids (LPA) are biosynthetic precursors with regulatory functions in the mammalian reproduction system. PS are important components of cellular membranes, where they comprise 5–10% of total PL with important roles in the regulation of apoptosis, lipid synthesis and transport [1,3]. Lysophosphatidylserines (LPS) belong to signaling PL with the important role during inflammatory processes [5]. PA and PS are acidic lipids with the potential to carry two negative charges—both charges on the phosphate group for PA, while one negative charge is on the phosphate and the second one on the carboxylate group in case of PS (Fig. 1).

The comprehensive lipidomic analysis of various biological tissues is a challenging task due to the extreme complexity of individual lipid classes varying in the structure, attached functional groups, polarity, dissociation and ionization behavior, *etc.* The important part of lipidomic analysis is the quantitation, which can be performed using the MS with the direct infusion (shotgun lipidomics) [6–8] or the liquid chromatography–mass spectrometry (LC/MS) approaches [9,10]. Shotgun lipidomics enables fast and robust analysis using precursor ion and neutral loss scans characteristic for the fragmentation behavior of functional group of individual lipid classes [6–8]. LC/MS approaches can be divided into the lipid class separation according to the polarity using HILIC mode for polar lipid classes [9–11] or normal phase (NP) LC for nonpolar lipid classes [12,13]. Individual lipid species can be separated according to the fatty acyl chain length and the number of double bonds using reversed phase (RP) LC [11,14–16] or non-aqueous reversed-phase (NARP) LC [17–19]. Recently, the coupling of ultrahigh-performance supercritical fluid chromatography and mass spectrometry (UHPSFC/MS) approach [20] has

* Corresponding author. Fax: +420 466037068.
E-mail address: Michal.Holcapek@upce.cz (M. Holčapek).

http://dx.doi.org/10.1016/j.chroma.2016.01.064
0021-9673/

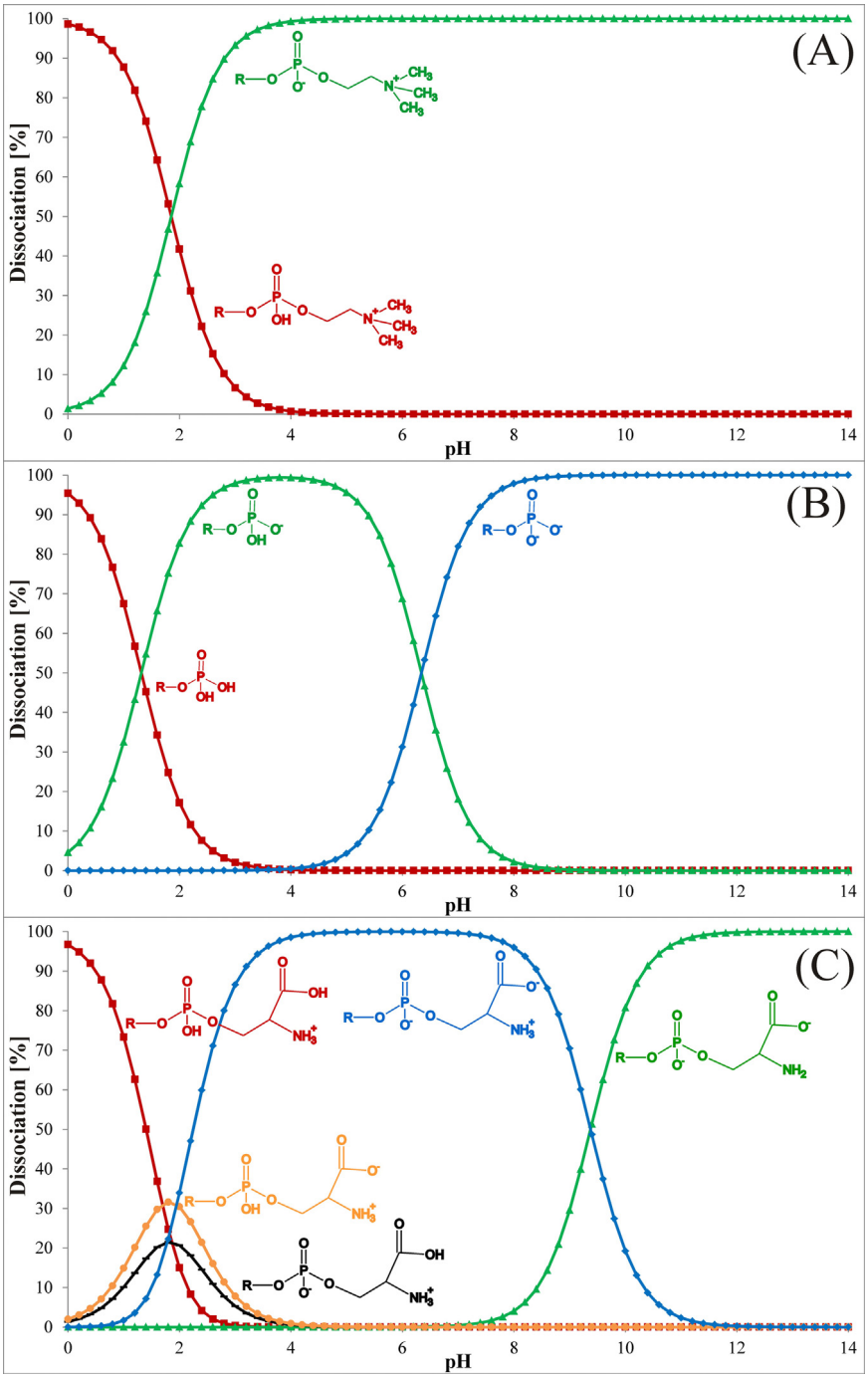

Fig. 1. Dissociation equilibria of (A) PC, (B) PA and (C) PS calculated by the Marvin demo software in the pH range 0–14, where R means $C_{17}H_{33}COOCH_2(C_{17}H_{33}COO)CHCH_2$-.

been introduced with the applicability for both polar and nonpolar lipid classes. The quantitative analysis in LC/MS approaches was described using internal standards (IS) per each lipid class [9] or the combination of single IS and response factors of individual lipid classes related to this IS [21]. The lipid class separation using HILIC or NP mode is more convenient for LC/MS quantitation, because lipid class IS are coeluting with lipid species inside particular lipid classes, therefore they are ionized at identical matrix conditions similarly as for shotgun lipidomics. This way of lipidomic quantitation should provide the most reliable data. Successful separations of PA and PS in HILIC mode have been previously reported [22–28], but they are based on the use of additives not well compatible with MS detection with possible strong ion suppression and memory effects, such as the use of alkyl ammonium salts as ion-pairing agents [29].

The main goal of this work is the optimization of HILIC/MS method for the analysis of the highest number of individual lipid classes with the emphasis on acidic lipid classes, such as PA, PS and their lysoderivatives using MS compatible conditions. We describe the separation in HILIC mode using a silica hydride column, which enables the characterization of 18 lipid classes including PA, PS and regioisomeric pairs of lysoderivatives in one analytical run. The developed method is applied for the analysis of lipid class composition of porcine brain and kidney.