SUPPORTING INFORMATION

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

* Corresponding author: Michal Holčapek, Tel.: +420-466037087; Fax: +420-466037068; E-mail: Michal.Holcapek@upce.cz

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Table S1. Identified lipids in porcine brain and kidney extracts using the final LC/MS method, molecular weights (MW), retention times and observed ions in both positive- and negative-ion ESI-MS modes.

Identified lipid species	Observed ions		MW	Retention time [min]	Brain	Kidney
	ESI +	ESI -				
PC P-32:0; PC O-32:1	$[M+H]^+$ $[M+Na]^+$	$[M-CH_3]^-$	717.6	39.8 - 43.5	+	+
PC 32:1			731.6		+	+
PC 32:0			733.6		+	+
PC P-34:1; PC O-34:2			743.6		+	+
PC P-34:0; PC O-34:1			745.6		+	+
PC 34:2			757.6		+	-
PC 34:1			759.6		+	+
PC 34:0			761.6		+	+
PC P-36:2; PC O-36:3			769.6		+	-
PC 35:1			773.6		+	+
PC 36:4			781.6		+	+
PC 36:3			783.6		+	+
PC 36:2			785.6		+	+
PC 36:1			787.6		+	+
PC P-38:4; PC O-38:5			793.6		+	+
PC 38:6			805.6		+	+
PC 38:5			807.6		+	-
PC 38:4			809.6		+	-
PC 38:3			811.6		+	-
PC 40:6			833.6		+	-
PC 40:5			835.6		+	-
PC 40:0			845.7		-	+
SM 34:1 [*1]	$[M+H]^+$ $[M+Na]^+$	$[M-CH_3]^-$	702.6	43.5 - 46.5	+	+
SM 36:1 [*1]			730.6		+	+
SM 38:1 [*1]			758.6		+	+
SM 40:1 [*1]			786.7		+	+
SM 40:0 [*1]			788.7		+	-
SM 42:2 [*1]			812.7		+	+
SM 42:1 [*1]			814.7		+	+
PS 34:1	$[M+H]^+$ $[M+Na]^+$	$[M-H]^-$	761.5	31.7 - 33.8	+	+
PS 36:2			787.5		+	+
PS 36:1			789.5		+	+
PS 38:4			811.5		+	+
PS 38:3			813.5		+	+
PS 38:2			815.6		+	+
PS 38:1			817.6		+	+
PS 40:6			835.6		+	+
PS 40:5			837.6		+	+
PS 40:4			839.6		+	+
PS 40:3			841.6		+	-
PS 42:9			857.6		+	+
PE P-34:2; PE O-34:3	$[M+H]^+$ $[M+Na]^+$	$[M-H]^-$	699.5	29.0 - 31.6	+	+
PE P-34:1; PE O-34:2			701.5		+	+
PE 34:1			717.5		+	+
PE 34:0			719.5		+	+
PE P-36:3; PE O-36:4			725.5		+	+
PE P-36:2; PE O-36:3			727.5		+	+
PE P-36:1; PE O-36:2			729.5		+	+
PE 35:1			731.5		+	+
PE 36:4			739.5		+	+
PE 36:3			741.5		+	+
PE 36:2			743.5		+	+
PE 36:1			745.5		+	+
PE P-38:6; PE O-38:7			747.5		+	+
PE P-38:5; PE O-38:6			749.5		+	+
PE P-38:4; PE O-38:5			751.5		+	+

PE P-38:3; PE O-38:4			753.6		+	+
PE P-38:2; PE O-38:3			755.6		+	+
PE P-38:1; PE O-38:2			757.6		+	+
PE 38:6			763.5		+	+
PE 38:5			765.5		+	+
PE 38:4			767.6		+	+
PE 38:3			769.6		+	+
PE P-40:7; PE O-40:8			773.5		+	+
PE P-40:6; PE O-40:7			775.5		+	+
PE P-40:5; PE O-40:6			777.6		+	+
PE P-40:4; PE O-40:5			779.6		+	+
PE P-40:3; PE O-40:4			781.6		+	+
PE P-40:2; PE O-40:3			783.6		+	+
PE P-40:1; PE O-40:2			780.6		+	+
PE 40:7			789.5		+	+
PE 40:6			791.6		+	+
PE 40:5			793.6		+	+
PE 40:4			795.6		+	+
PI 34:1	$[M+H]^+$ $[M+Na]^+$	$[M-H]^-$	836.6	23.7 - 24.8	-	+
PI 36:1			864.6		-	+
PI 36:4			858.6		+	+
PI 38:5			884.6		+	-
PI 38:4			886.6		+	+
PI 40:7			908.6		+	-
PI 40:6			910.6		+	+
Sulfatide 36:1 *1	$[M+H-H_2SO_4]^+$	$[M-H]^-$	807.6	3.0 - 7.5	+	+
Sulfatide 36:0 *1			809.6		+	+
Sulfatide 38:1 *1			833.6		+	-
Sulfatide 38:0 *1			835.6		+	-
Sulfatide 38:1 (OH) *1			851.6		+	+
Sulfatide 40:2 *1			861.6		+	+
Sulfatide 40:1 *1			863.6		+	+
Sulfatide 41:2 *1			875.6		+	+
Sulfatide 41:1 *1			877.6		+	+
Sulfatide 40:1 (OH) *1			879.6		+	+
Sulfatide 40:0 (OH) *1			881.6		+	+
Sulfatide 42:2 *1			889.6		+	+
Sulfatide 42:1 *1			891.6		+	+
Sulfatide 41:1 (OH) *1			893.6		+	+
Sulfatide 41:0 (OH) *1			895.6		+	-
Sulfatide 43:2 *1			903.6		+	+
Sulfatide 42:2 (OH) *1			905.6		+	+
Sulfatide 42:1 (OH) *1			907.6		+	+
Sulfatide 42:0 (OH) *1			909.7		+	+
Sulfatide 44:2 *1			917.7		+	+
Sulfatide 43:2 (OH) *1			919.7		+	+
Sulfatide 43:1 (OH) *1			921.7		+	+
Sulfatide 44:2 (OH) *1			933.7		+	+
Sulfatide 44:1 (OH) *1			935.7		+	+
Sulfatide 44:0 (OH) *1			937.7		+	-
HexCer 36:1 *1	$[M+H-H_2O]^+$ $[M+Na]^+$	$[M-H]^-$	727.6	9.0 - 13.5	+	+
HexCer 41:0 *1			799.7		+	+
HexCer 42:2 *1			809.7		+	-
HexCer 42:1 *1			811.7		+	+
HexCer 42:0 *1			813.7		+	+
HexCer 43:1 *1			825.7		+	+
HexCer 43:0 *1			827.7		+	+
HexCer 44:2 *1			837.7		+	+
HexCer 44:1 *1			839.7		+	+
HexCer 44:0 *1			841.7		+	+
HexCer 36:1 (OH) *1			743.6		+	-
HexCer 38:1 (OH) *1			771.6		+	+

HexCer 40:1 (OH) [*1]			799.6		+	+
HexCer 41:1 (OH) [*1]			813.7		+	+
HexCer 42:2 (OH) [*1]			825.7		+	+
HexCer 42:1 (OH) [*1]			827.7		+	+
HexCer 43:2 (OH) [*1]			839.7		+	+
HexCer 43:1(OH) [*1]			841.7		+	+
HexCer 44:2 (OH) [*1]			853.7		+	+
Hex2Cer 36:1 [*1]	$[M+H-H_2O]^+$ $[M+Na]^+$	$[M-H]^-$	889.6	16.0 - 17.0	+	+
Hex2Cer 38:1 [*1]			917.7		+	+
Hex2Cer 40:1 [*1]			945.7		+	+
PG 34:1	$[M+H]^+$ $[M+Na]^+$	$[M-H]^-$	748.5	21.0 - 22.0	-	+
PG 36:2			774.5		-	+
PG 36:1			776.6		-	+
Cer 36:1 [*1]	$[M+H-H_2O]^+$ $[M+Na]^+$	$[M-H]^-$	565.5	5.1 - 5.7	+	+
LPC 16:0	$[M+H]^+$ $[M+Na]^+$	$[M-CH_3]^-$	495.3	47.5 - 50.0	+	+
LPC 18:1			521.4		+	+
LPC 18:0			523.4		+	+
PA 34:1	$[M+H]^+$ $[M+Na]^+$	$[M-H]^-$	674.5	25.1 - 26.0	+	-
PA 36:1			702.5		+	-
LPE 16:0	$[M+H]^+$ $[M+Na]^+$	$[M-H]^-$	453.3	36.8 - 37.6	+	-
LPE 18:0			481.3		+	-
LPE 20:3			503.3		+	-

[*1] The annotation is based on the assumption that the sphingoid base contains two hydroxyl groups.

Table S2. Relative standard deviations of retention times using six consecutive injections for the final method.

Lipid class	RSD [%]
Cer	1.6
HexCer	0.5
Hex2Cer	1.0
PG	1.4
PA	2.1
LPG	1.5
PE	1.0
PS	1.9
LPA	2.2
LPE	1.3
LPS	1.9
PC	0.9
SM	1.3
LPC	1.3

Fig. S1. Overlay of reconstructed ion current chromatograms in positive-ion HILIC/ESI-MS of PA, LPA, PS and LPS standards using Acquity UPLC BEH HILIC column (150 x 2.1 mm, 1.7 µm). Conditions are identical as for Fig. 1.

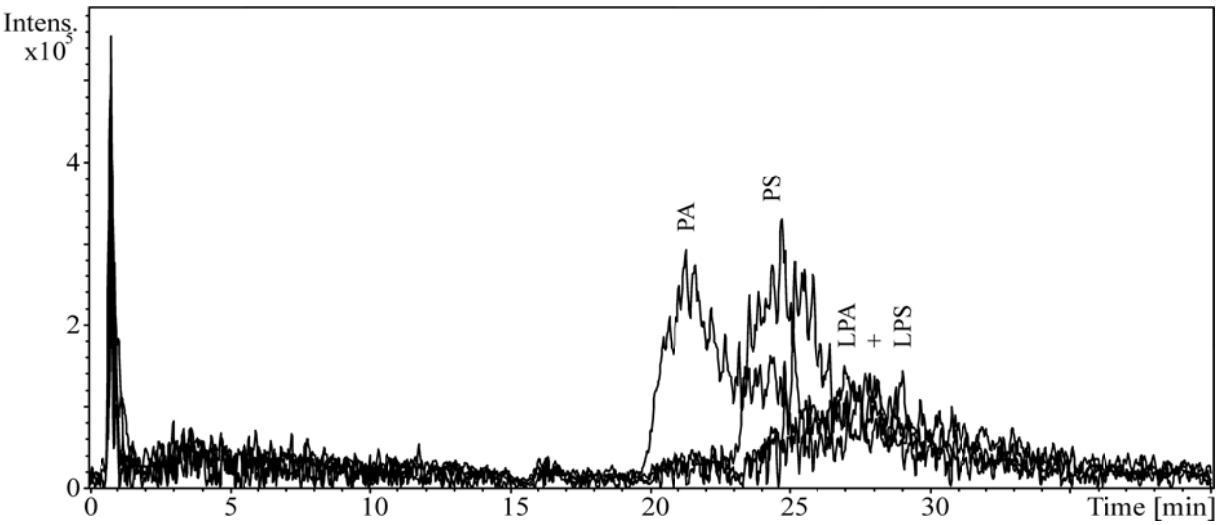

Fig. S2. Effect of pH adjusted by formic acid on HILIC/ESI-MS of lipid standard mixture: (A) pH 5, (B) pH 4, and (C) pH 3. Conditions: Ascentis column (150 x 2.1 mm, 3 μm), flow rate 0.5 mL/min, gradient 0 min – 99.5% A + 0.5% B, 40 min – 80.2% A + 19.8% B, where A is acetonitrile, and B is 20 mmol/L of aqueous ammonium formate, total ion current chromatograms in the positive-ion mode.

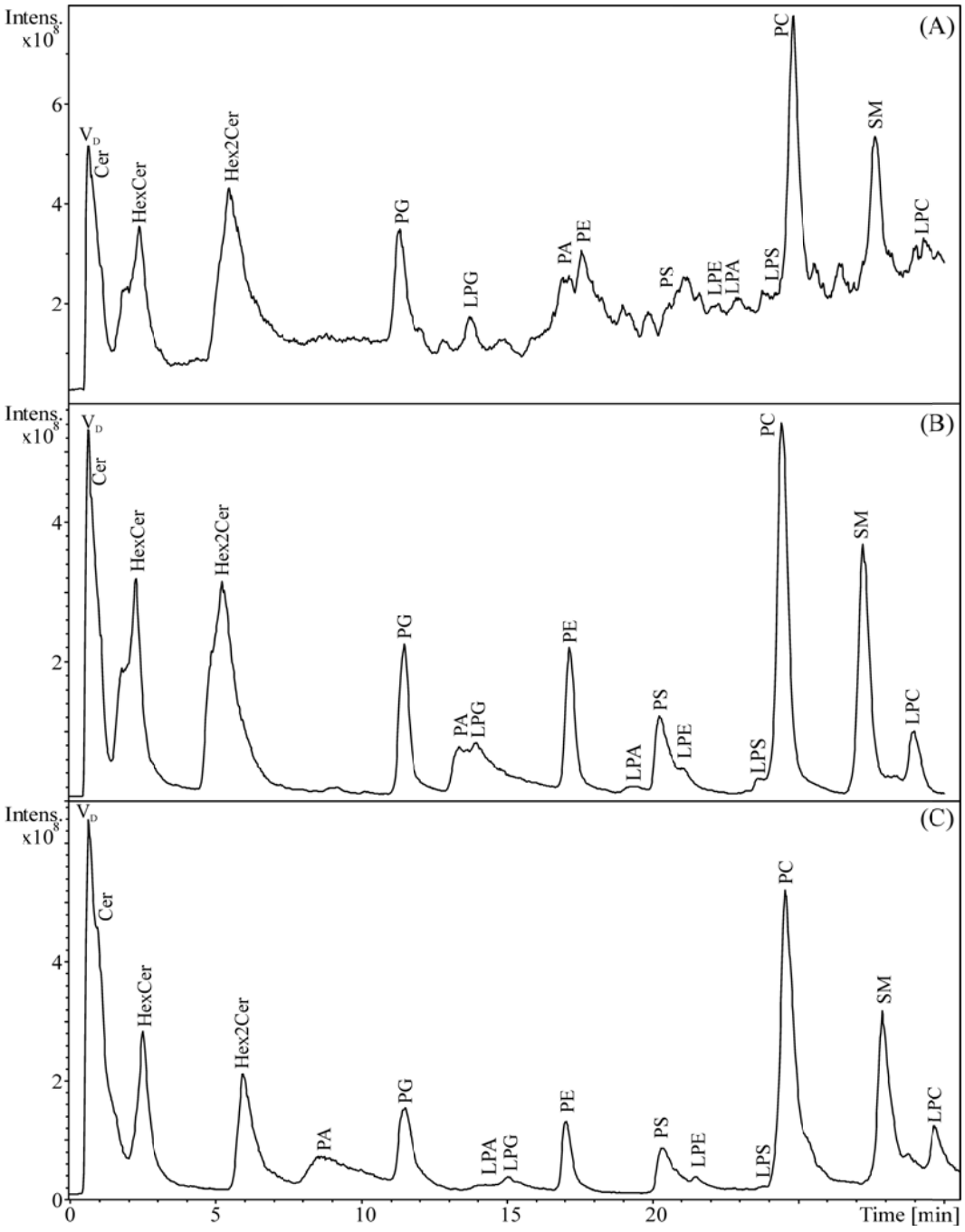

Fig. S3. Effect of pH adjusted by formic acid on HILIC/ESI-MS of lipid standard mixture: (A) pH 3.8, (B) pH 3.6, (C) pH 3.4, and (D) pH 3.2. Conditions: Ascentis column (150 x 2.1 mm, 3 μm), flow rate 0.5 mL/min, gradient 0 min – 99.5% A + 0.5% B, 40 min – 80.2% A + 19.8% B, where A is acetonitrile, and B is 20 mmol/L of aqueous ammonium formate, total ion current chromatograms in the positive-ion mode.

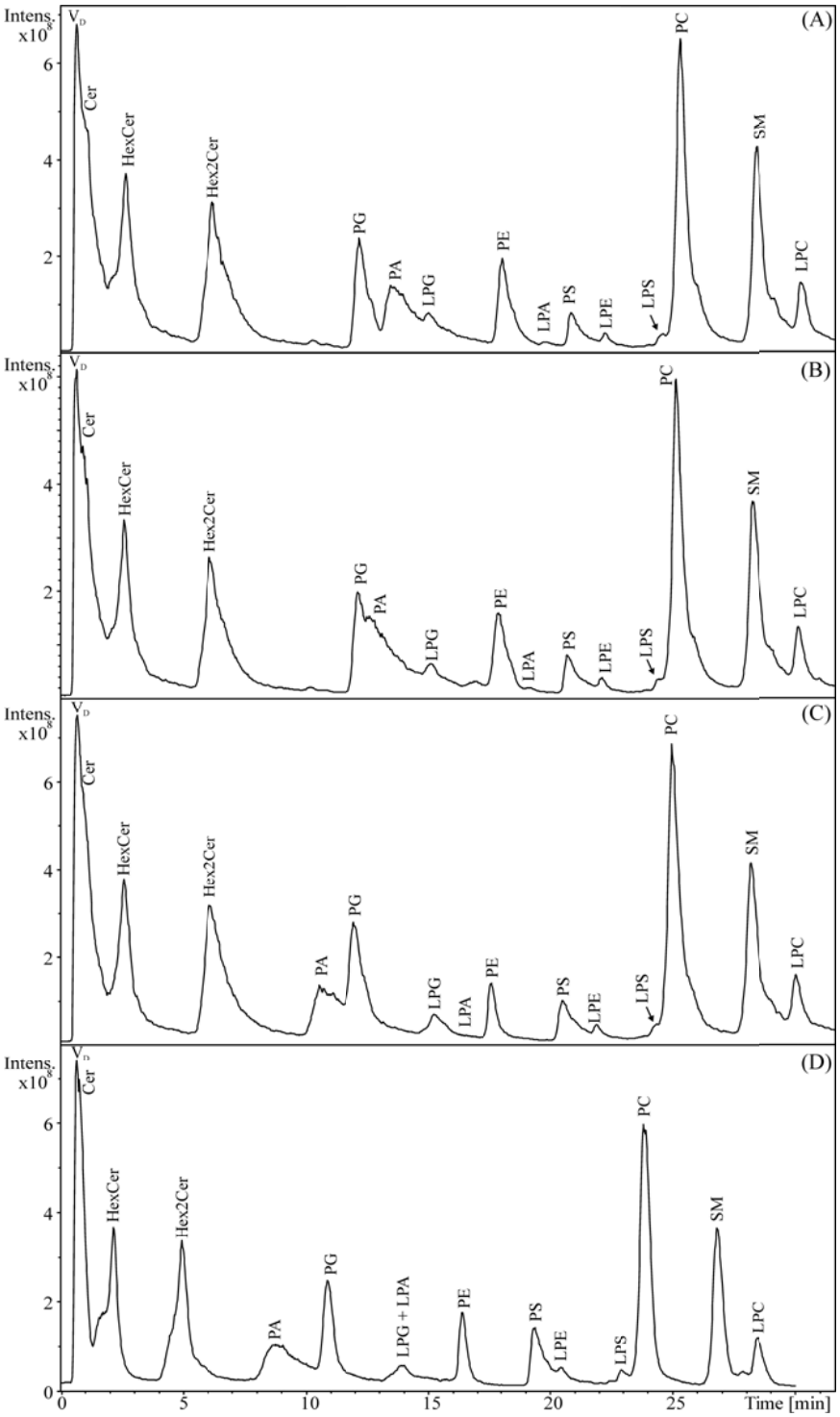

Fig. S4. Effect of aqueous ammonium formate concentration on HILIC/ESI-MS of PA, LPA, PS and LPS standards: (A) 20 mmol/L, and (B) 60 mmol/L. Conditions: Ascentis column (150 x 2.1 mm, 3 μm), flow rate 0.5 mL/min, gradient 0 min – 99.5% A + 0.5% B, 40 min – 80.2% A + 19.8% B, where A is acetonitrile, and B is aqueous ammonium formate, pH 3 adjusted by formic acid, overlay of reconstructed ion current chromatograms in the negative-ion mode.

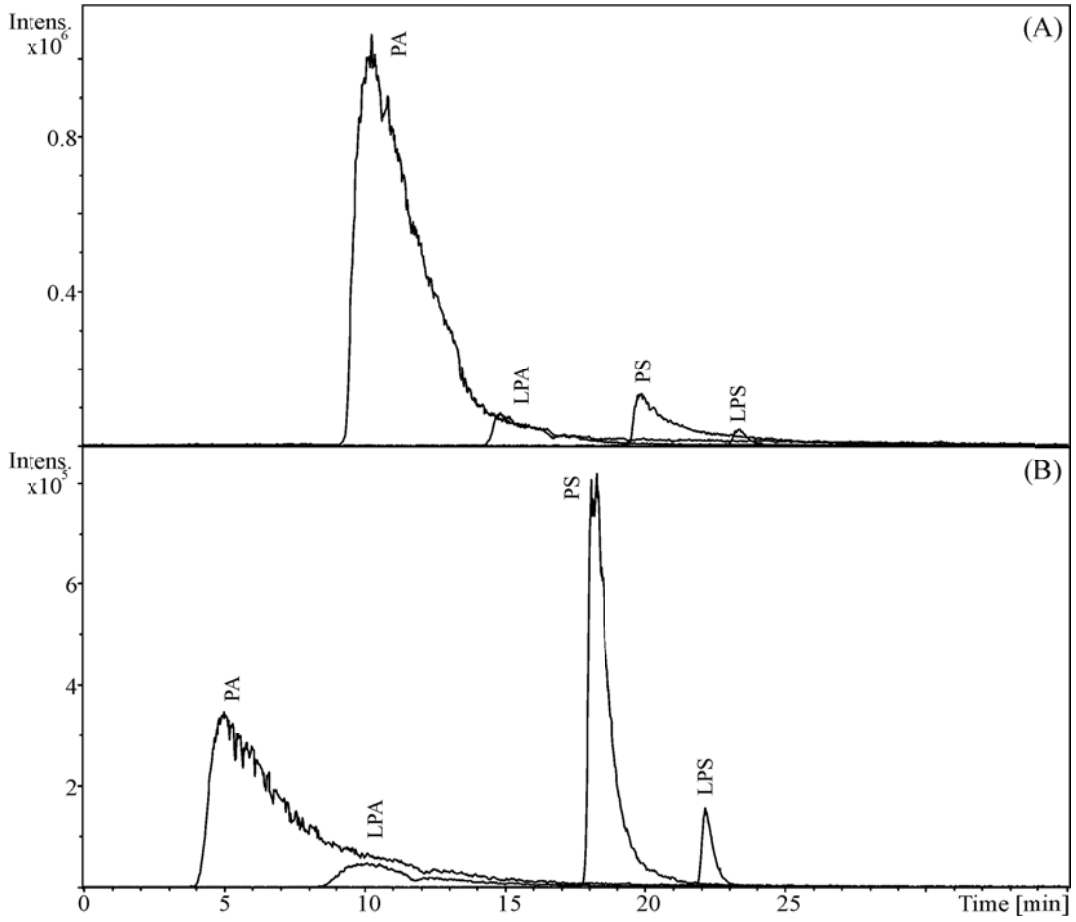

Fig. S5. Effect of column type on HILIC/ESI-MS of PA, LPA, PS and LPS standards: (A) Acquity UPC^2 Torus Diol (100 x 3 mm, 1.7 µm), (B) Acquity UPC^2 Torus 2-PIC (100 x 3 mm, 1.7 µm), (C) Acquity UPC^2 Torus DEA (100 x 3 mm, 1.7 µm), and (D) Acquity UPC^2 Torus 1-AA (100 x 3 mm, 1.7 µm). Conditions: flow rate 0.5 mL/min, gradient 0 min – 99.7% A + 0.3% B, 30 min – 75% A + 25% B, where A is acetonitrile, and B is 20 mmol/L of aqueous ammonium formate, pH 4 adjusted by formic acid, overlay of reconstructed ion current chromatograms in the negative-ion mode.

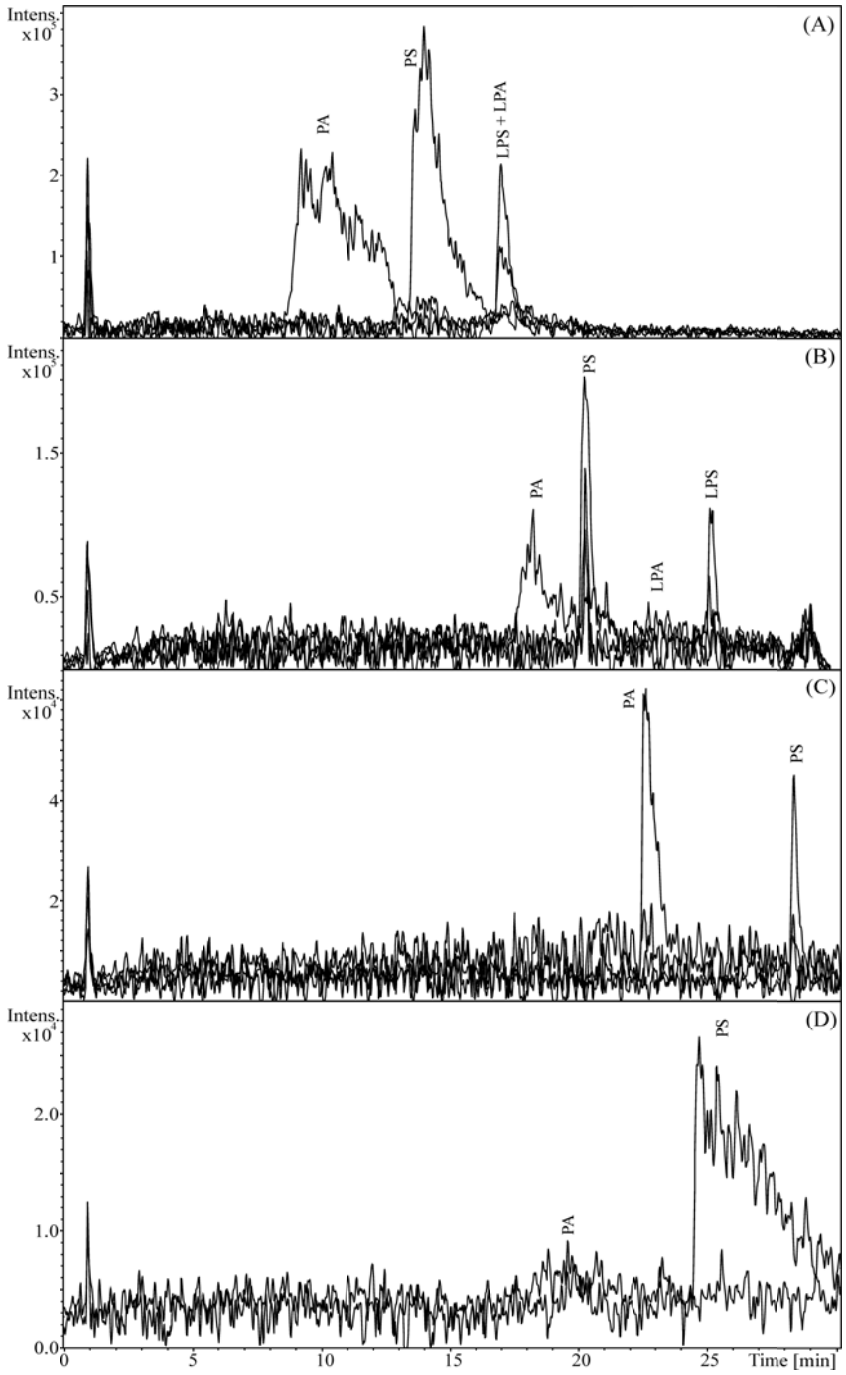

Fig. S6. Effect of pH on HILIC/ESI-MS of lipid standard mixture using hydride column, pH is adjusted by formic acid to: (A) pH 6, (B) pH 5, (C) pH 4, and (D) pH 3.5. Conditions: Cogent Diamond Hydride column (250 x 4.6 mm, 4 µm), flow rate 1 mL/min, gradient 0 min – 99.7% A + 0.3% B, 60 min – 75% A + 25% B, where A is acetonitrile, and B is 20 mmol/L of aqueous ammonium formate, total ion current chromatograms in the positive-ion mode.

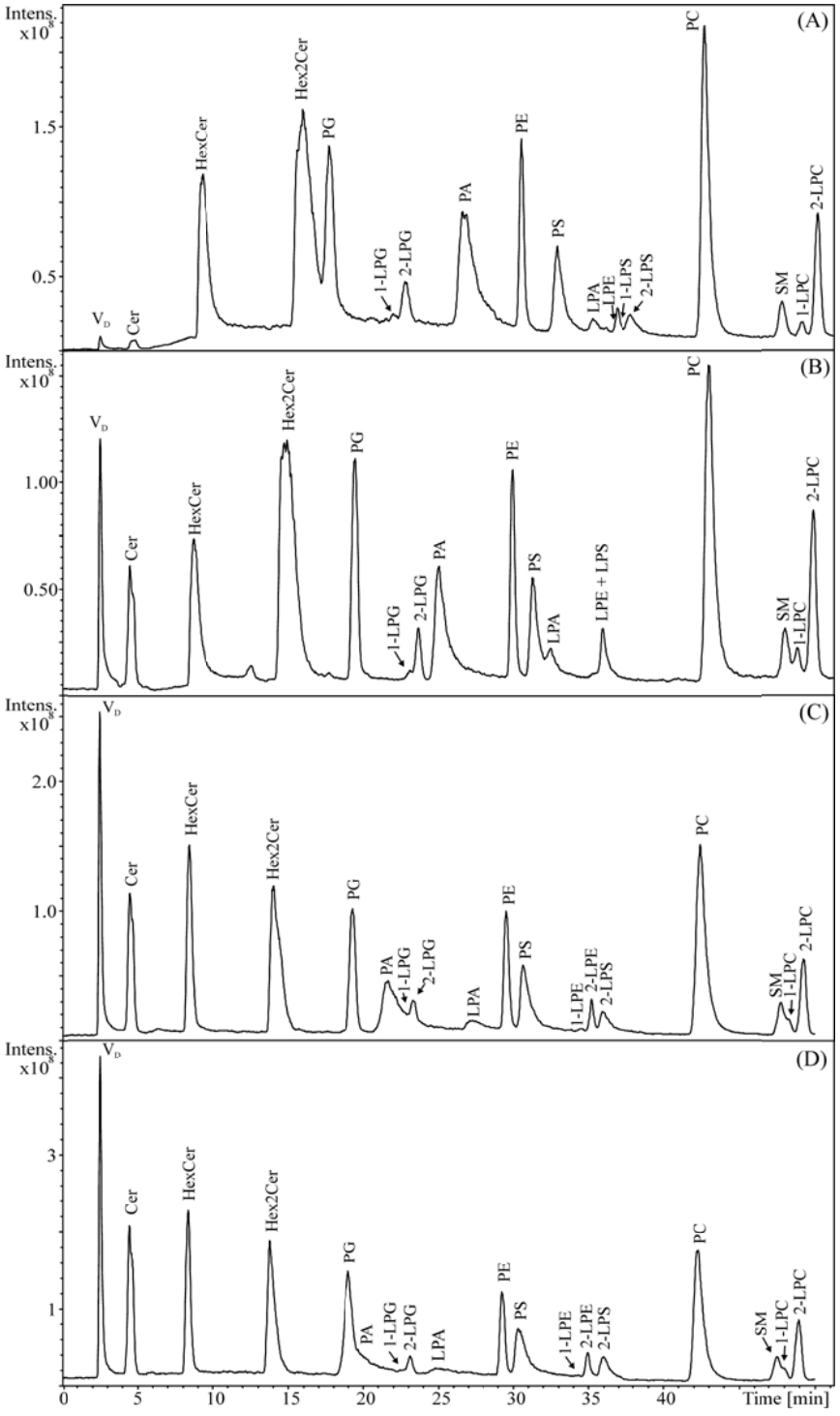

Fig. S7. Effect of buffer type on HILIC/ESI-MS of lipid standard mixture for constant pH 4 adjusted by: (A) acetic acid, and (B) formic acid. Conditions: Cogent Diamond Hydride column (250 x 4.6 mm, 4 μm), flow rate 1 mL/min, gradient 0 min – 99.7% A + 0.3% B, 60 min – 75% A + 25% B, where A is acetonitrile, and B is 20 mmol/L of aqueous ammonium acetate (A) or formate (B), total ion current chromatograms in the positive-ion mode.

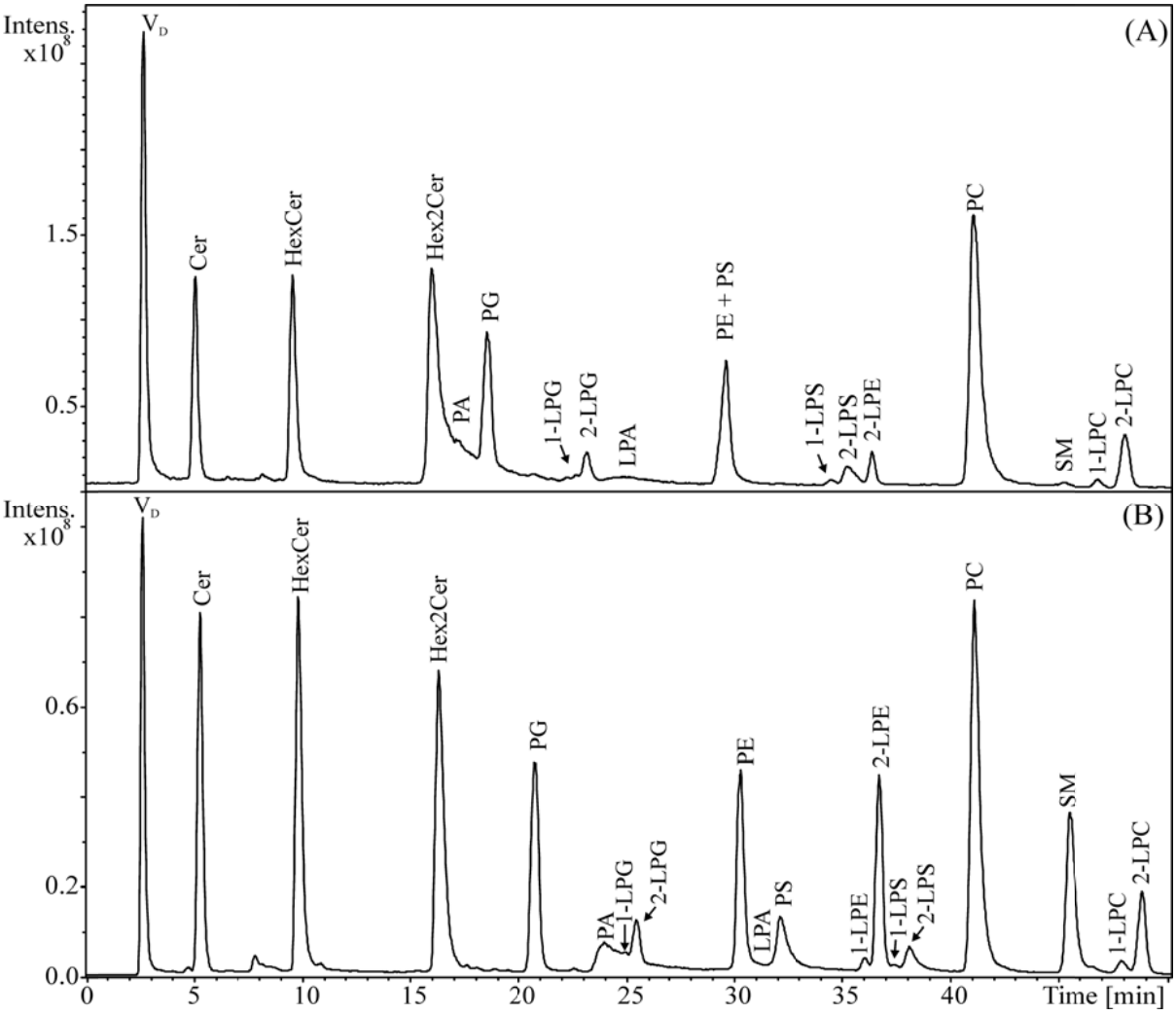

Fig. S8. Effect of ammonium formate concentration on HILIC/ESI-MS of lipid standard mixture:

(A) 20 mmol/L, (B) 30 mmol/L, (C) 40 mmol/**L**, and (D) 50 mmol/L. Conditions: Cogent Diamond Hydride column (250 x 4.6 mm, 4 μm), flow rate 1 mL/min, gradient 0 min – 99.7% A + 0.3% B, 60 min – 75% A + 25% B, where A is acetonitrile, and B is aqueous ammonium formate, pH 4 adjusted by formic acid, total ion current chromatograms in the positive-ion mode.

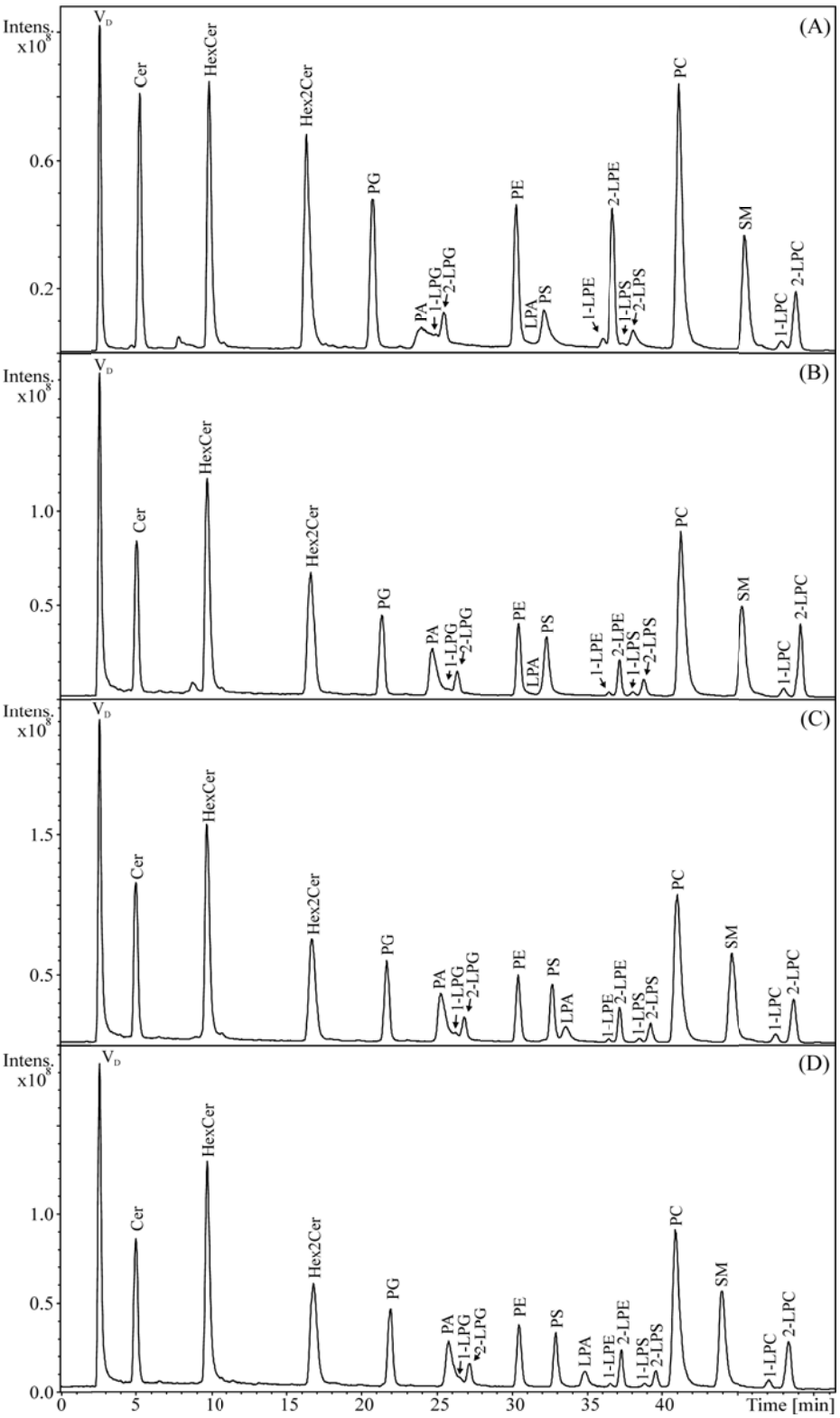

Fig. S9. Comparison of polarity modes in HILIC/ESI-MS of total lipid extract of porcine kidney: (A) positive-ion, and (B) negative-ion modes. Conditions are identical as for Fig. 5.