Supporting Information

Simultaneous Quantification of Cardiolipin, Bis(monoacylglycero)phosphate and their Precursors by Hydrophilic Interaction LC-MS/MS including Correction of Isotopic Overlap

Max Scherer, Gerd Schmitz, Gerhard Liebisch

Institute of Clinical Chemistry and Laboratory Medicine, University of Regensburg, Germany.

Lipid class	MRM	IS (MRM)	CE [V]	RT [min]	LOD [fmol]
PA	$[M+NH_4]^+ \rightarrow$DAG NL 115	PA 14:0/14:0 (610.5→495.5)	25	1.79	1.5 ± 0.3
PG	$[M+NH_4]^+ \rightarrow$DAG NL 189	PG 14:0/14:0 (684.5→495.5)	27	1.51	91 ± 14
BMP	$[M+H]^+ \rightarrow$MAG	BMP 14:0/14:0 (667.5→285.3)	37	1.41	119 ± 12
CL	$[M+NH_4]^+ \rightarrow$DAG	CL 14:0/14:0/14:0/14:0 (1259.0→495.5)	43	1.75	36 ± 2

Table S-1: MS parameter (MRM = multiple reaction monitoring, IS = internal standard, CE = collision energy, RT = retention time) and limit of detection (LOD) of glycerophospholipids studied. LODs (injected amount) were calculated as a signal to noise ratio of 3 determined from a triplicate measurement.

Analyte	Calibration range [pmol]	Slope (mean ± S.D.)	Correlation coefficient (mean ± S.D.)
PA 16:0/16:0	4.0 – 40	0.0670 ± 0.005	0.992 ± 0.003
PA 18:1/18:1	4.9 – 49	0.0678 ± 0.006	0.995 ± 0.002
PA 18:0/20:4	3.1 – 31	0.036 ± 0.003	0.994 ± 0.002
BMP 16:0/16:0	6.7 – 67	0.033 ± 0.003	0.996 ± 0.002
BMP 18:1/18:1	9.4 - 94	0.025 ± 0.002	0.997 ± 0.004
PG 16:0/16:0	4.3 – 43	0.171 ± 0.015	0.994 ± 0.002
PG 18:1/18:1	3.6 – 36	0.196 ± 0.013	0.998 ± 0.002
PG 18:0/20:4	1.5 – 15	0.119 ± 0.007	0.997 ± 0.004
CL 15:0/15:0/15:0/16:1	7.6 – 76	0.034 ± 0.002	0.995 ± 0.002
CL 18:1/18:1/18:1/18:1	5.9 - 59	0.047 ± 0.003	0.994 ± 0.002

Table S-2: Calibration data

Calibration was performed by standard addition to mouse heart tissue homogenates. Each value represents the average of three determinations. The indicated range was spiked to 2.5mg wet weight of heart tissue.

Analyte	Conc. [pmol/mg tissue]	Intraday-precision (%) (n=5)	Interday-precision (%) (n=5)
BMP 18:1/18:1	1.29 ± 0.10	7.5	8.7
PG 32:0	0.58 ± 0.04	7.2	12.0
PG 34:1	48.9 ± 2.1	4.2	6.8
PG 36:3	5.42 ± 0.42	7.8	7.7
PG 36:2	10.95 ± 0.66	6.0	9.73
PG 36:1	2.18 ± 0.14	6.4	6.6
PG 38:4	15.19 ± 0.58	3.8	8.7
CL 34:3-36:4	4.54 ± 0.28	6.2	8.9
CL 34:3-36:3	2.86 ± 0.23	8.0	7.3
CL 34:2-36:3	4.94 ± 0.38	7.7	4.9
CL 36:5-36:4	19.90 ± 1.15	5.8	11.1
CL 36:4-36:4	92.74 ± 3.63	3.9	5.0
CL 36:4-36:3	81.40 ± 2.4	3.0	8.8
CL 36:3-36:3	31.73 ± 2.64	8.3	10.4
CL 36:4-36:1	4.63 ± 0.30	6.5	8.6
CL 36:3-36:2	10.08 ± 1.04	10.35	11.6
CL 36:2-36:2	3.07 ± 0.32	10.3	11.8
CL 36:4-38:6	10.42 ± 0.98	9.4	9.2
CL 36:4-38:5	22.42 ± 1.29	5.8	9.0
CL 36:3-38:6	3.27 ± 0.28	8.5	7.5
CL 36:4-38:4	13.73 ± 1.16	8.5	11.3
CL 36:3-38:5	4.48 ± 0.29	6.5	8.3
CL 36:2-38:6	0.54 ± 0.06	10.9	6.6
CL 36:3-38:4	10.90 ± 0.8	6.9	12.1
CL 36:3-38:3	14.3 ± 1.3	9.0	6.8
CL 36:4-40:8	104.5 ± 6.0	5.8	7.1
CL 36:5-40:7	1.04 ± 0.13	13.0	12.6
CL 36:3-40:9	1.82 ± 0.11	6.0	7.1
CL 36:4-40:7	25.47 ± 1.25	4.9	10.5
CL 36:3-40:8	19.18 ± 1.84	9.6	12.4
CL 36:4-40:6	4.01 ± 0.41	10.0	11.8
CL 36:3-40:7	6.35 ± 0.38	5.9	8.5
CL 36:3-40:6	1.38 ± 0.14	10.3	13.7

Table S-3: Intra- and Inter-day precisions in mouse heart tissues

The displayed values are mean concentrations of mouse heart tissue and the coefficient of variation (CV) of 5 samples analyzed in series for intraday-precision and on 5 different days for interday-precision. Tissue homogenate corresponding to 2.5mg wet weight were used for quantification. Quantification was performed by standard addition using the species and range shown in table S-2. The following CL species would have been identified erroneously without isotope correction: CL 34:2-36:4, CL 34:1-36:3, CL 36:5-36:3, CL 36:4-36:2, CL 36:2-36:1, CL 36:4-38:3, CL 36:2-38:4, CL 36:2-40:8, CL 36:3-38:2, CL 36:2-38:3, CL 36:2-40:7.

Analyte	Conc. [pmol/mg cellular protein]	Intraday-precision (%) (n=5)	Interday-precision (%) (n=5)
PA 32:0	17.77 ± 1.24	7.0	9.5
PA 34:1	41.88 ± 3.56	8.5	8.1
PA 36:2	13.53 ± 0.74	5.5	6.7
PA 36:1	13.20 ± 1.37	10.3	12.3
PA 38:4	15.64 ± 1.93	12.4	11.4
BMP 16:1-18:1	43.02 ± 3.42	8.0	8.8
BMP 16:0-18:1	36.43 ± 2.28	6.3	7.9
BMP 18:2/18:2	13.89 ± 2.28	8.3	9.7
BMP 18:2-18:1	165.90 ± 15.99	9.6	10.6
BMP 18:1/18:1	388.77 ± 22.25	5.7	7.9
BMP 18:1-18:0	84.66 ± 5.35	7.5	8.1
BMP 18:1-20:4	168.12 ± 9.81	5.8	10.7
BMP 18:1-20:3	146.39 ± 14.56	9.9	8.4
BMP 18:1-20:2	70.49 ± 6.20	8.8	8.6
BMP 18:0-22:6	64.35 ± 8.22	12.8	11.3
BMP 18:1-22:5	294.03 ± 11.66	7.2	8.6
PG 34:1	18.18 ± 1.42	7.8	6.1
PG 36:3	2.54 ± 0.19	7.5	11.2
PG 36:2	4.77 ± 0.44	9.3	8.5
PG 36:1	16.53 ± 1.77	10.7	12.7
CL 34:3-34:3	40.27 ± 3.73	9.3	9.4
CL 34:2-34:2	53.63 ±4.37	8.1	6.3
CL 34:3-36:4	46.75 ± 2.32	5.0	8.0
CL 34:2-36:3	120.72 ± 9.60	8.0	11.8
CL 36:4-36:4	33.81 ± 2.58	7.6	8.9
CL 36:4-36:3	138.80 ± 6.09	4.4	3.3
CL 36:3-36:3	168.62 ± 19.42	11.5	13.1
CL 36:4-36:2	28.81 ± 2.39	8.3	11.9
CL 36:4-36:1	12.9 ± 0.83	6.4	9.8
CL 36:3-36:2	117.21 ± 8.77	7.5	4.3
CL 36:2-36:2	88.72 ± 6.53	7.4	8.6
CL 36:4-38:4	17.45 ± 1.98	11.4	7.9
CL 36:3-38:5	37.78 ± 2.98	7.9	8.7
CL 36:3-38:4	14.6 ± 1.97	13.5	12.9
CL 36:3-38:3	14.47 ± 1.58	10.9	6.5

Table S-4: Intra- and Inter-day precisions in primary human skin fibroblasts

The displayed values are mean concentrations of primary human skin fibroblasts and the coefficient of variation (CV) of 5 samples analyzed in series for intraday-precision and on 5 different days for interday-precision. Cell homogenate corresponding to 50µg protein were used for quantification. Quantification was performed by standard addition using the species and a range similar to that shown in table S-2. The following CL species would have been identified erroneously without isotope correction: CL 34:3-34:2, CL 34:2-34:1, CL 34:1-36:3, CL 34:2-36:2, CL 36:3-36:1, CL 36:2-36:1, CL 36:2-38:5.

Analyte	Spiked amount [pmol]	Recovery (S.D.) (n=4)	Matrix effect (n=4)
PA 16:0/16:0	3.1	74 ± 8	133 ± 9
PA 18:1/18:1	2.9	79 ± 9	142 ± 5
PA 18:0/20:4	2.8	78 ± 8	140 ± 10
BMP 16:0/16:0	6.7	62 ± 6	108 ± 9
BMP 18:1/18:1	6.5	71 ± 7	109 ± 4
PG 16:0/16:0	1.4	61 ± 2	128 ± 7
PG 18:1/18:1	1.3	70 ± 6	134 ± 4
PG 18:0/20:4	1.3	67 ± 8	141 ± 6
CL 15:0/15:0/15:0/16:1	7.6	64 ± 3	88 ± 6
CL 18:1/18:1/18:1/18:1	6.9	65 ± 8	91± 4

Table S-5. Recovery and matrix effects

Recovery and matrix effects were determined in mouse heart tissue homogenates corresponding to 2.5mg of wet weight. The extraction efficiency was determined by adding a glycerophospholipid standard mixture after mouse heart tissue homogenization before and after extraction. Recovery was calculated as percent of standards spiked before and after extraction. Matrix effects are calculated as the percentage of signal spiked after extraction (corrected by endogenous sphingolipid concentrations) related to a pure standard mixture. Each value represents the average of three determinations ± standard deviation.

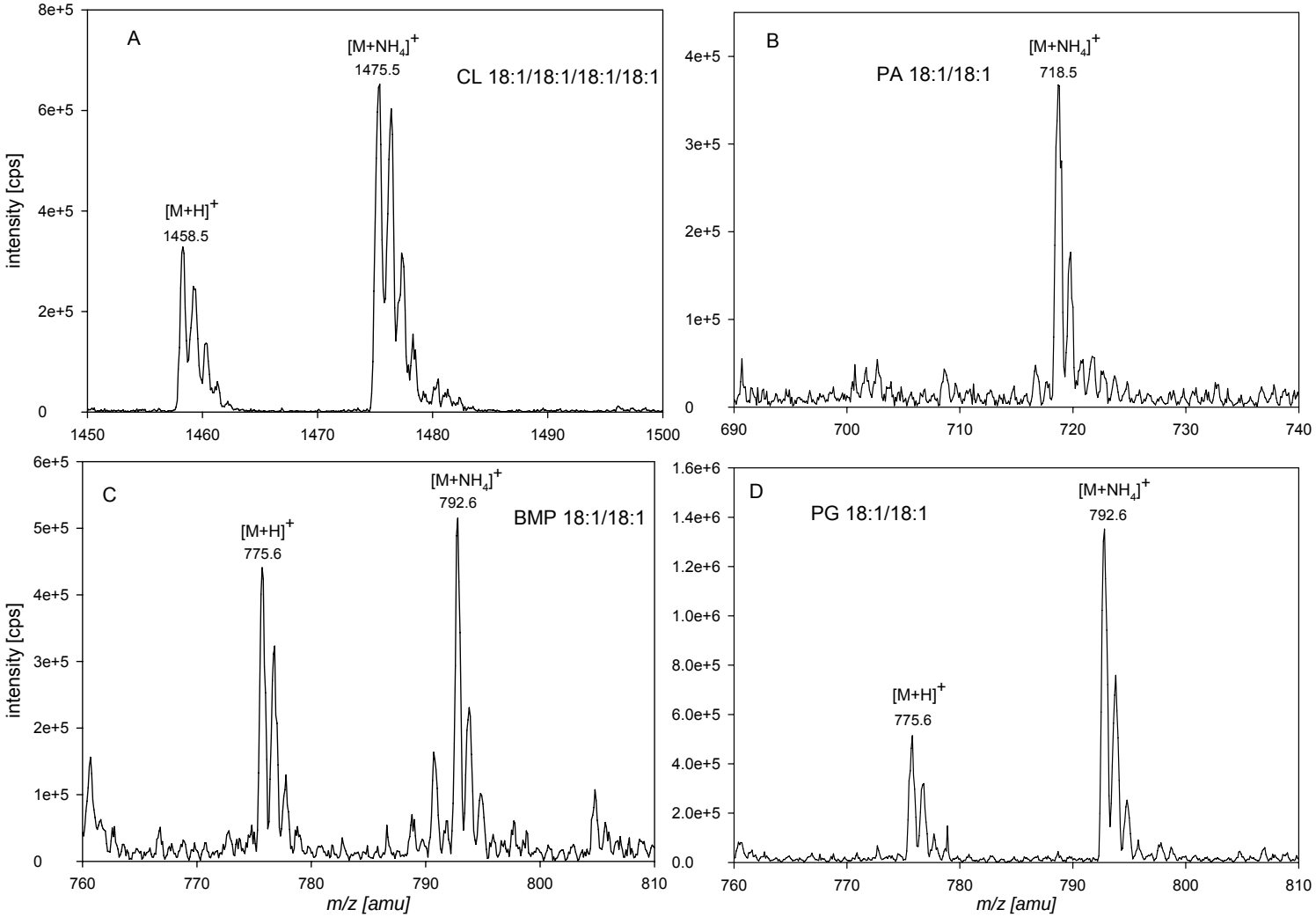

Figure S-1: MS-scan of a standard mixture containing CL 18:1/18:1/18:1/18:1 (Panel A), PA 18:1/18:1 (Panel B), BMP 18:1/18:1 (Panel C) and PG 18:1/18:1 (Panel D). Q1 scans (*m/z* 600 to 1600) were acquired during a LC run. Declustering potential was ramped from 60 to 120V. The displayed spectra were combined from the respective chromatographic peaks.

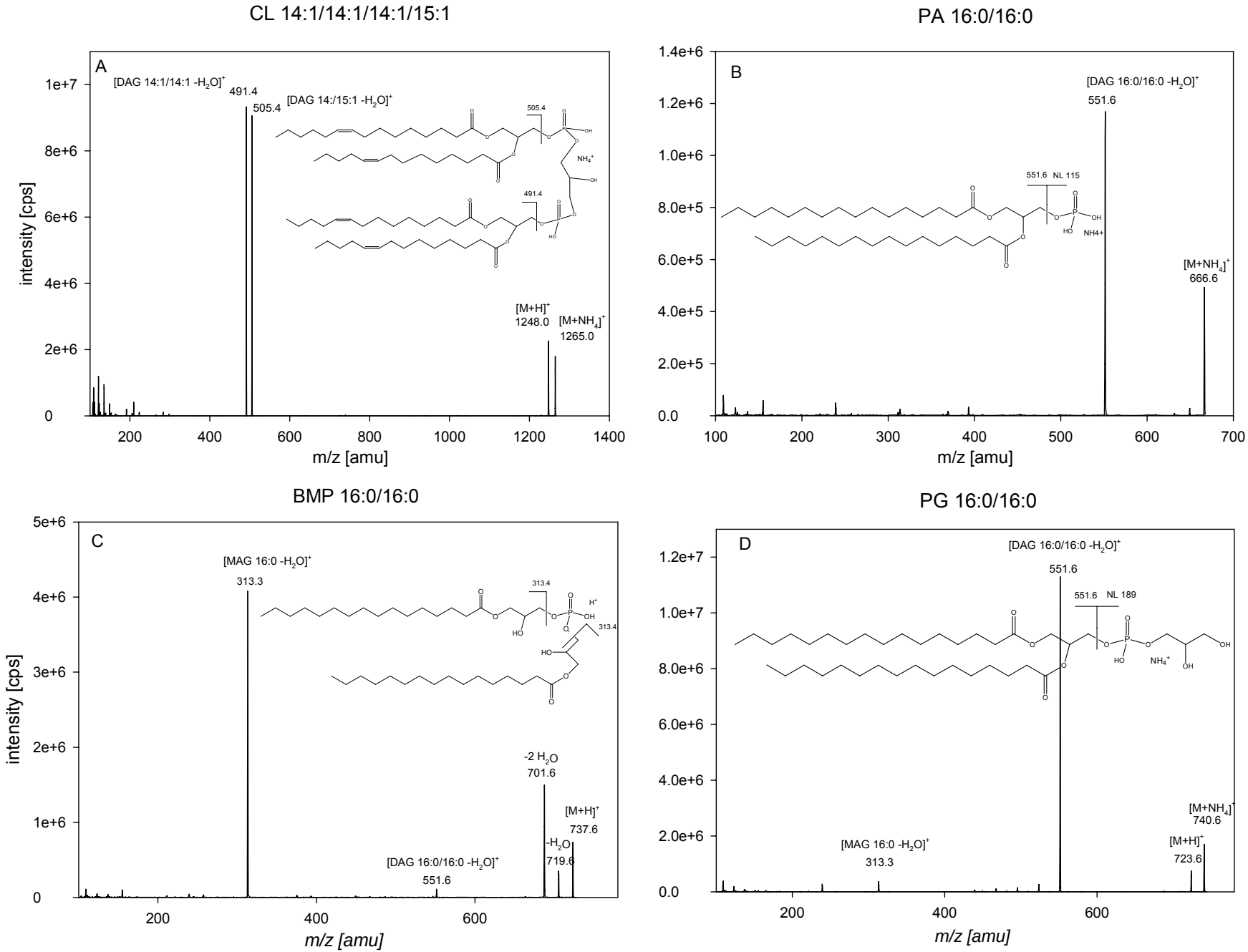

Figure S-2: Product ion spectrum and proposed fragmentation of CL 14:1/14:1/14:1/15:1 (Panel A), PA 16:0/16:0 (Panel B), BMP 16:0/16:0 (Panel C) and PG 16:0/16:0 (Panel D) in positive ionization mode. Collision energy was ramped from 5 to 130V.