ELECTRONIC SUPPLEMENTARY MATERIAL

Comprehensive two-dimensional liquid chromatography - electrospray ionization mass spectrometry of complex lipidomic samples

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Fig. S1 Analysis of the lipid extract of porcine brain: **A**/ RP-UHPLC/ESI-MS chromatogram, **B**/ HILIC-LC/ESI-MS chromatogram. Conditions are identical as for **Figs. 1** and **2**, respectively.

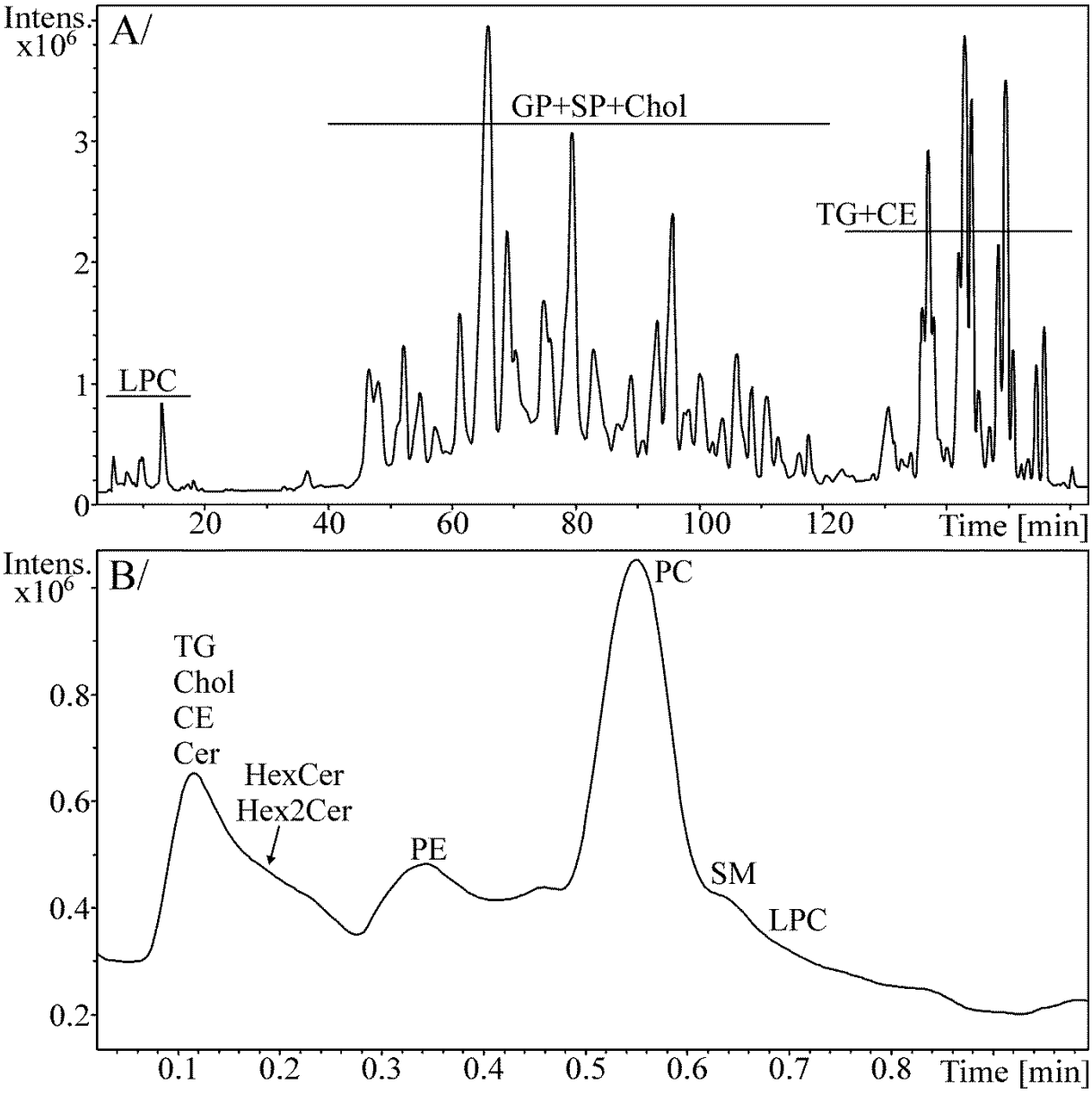

Fig. S2 Overlay of RIC HILIC-UHPLC/ESI-MS chromatograms of the mixture of lipid standards. HILIC-UHPLC conditions: Acquity UPLC BEH HILIC column (50 x 3 mm, 1.7 µm, Waters), flow rate 4 mL/min, separation temperature 40°C, gradient 0 min – 92 % B, 0.7 min – 80 % B, where A is 5 mM aqueous ammonium acetate and B is acetonitrile.

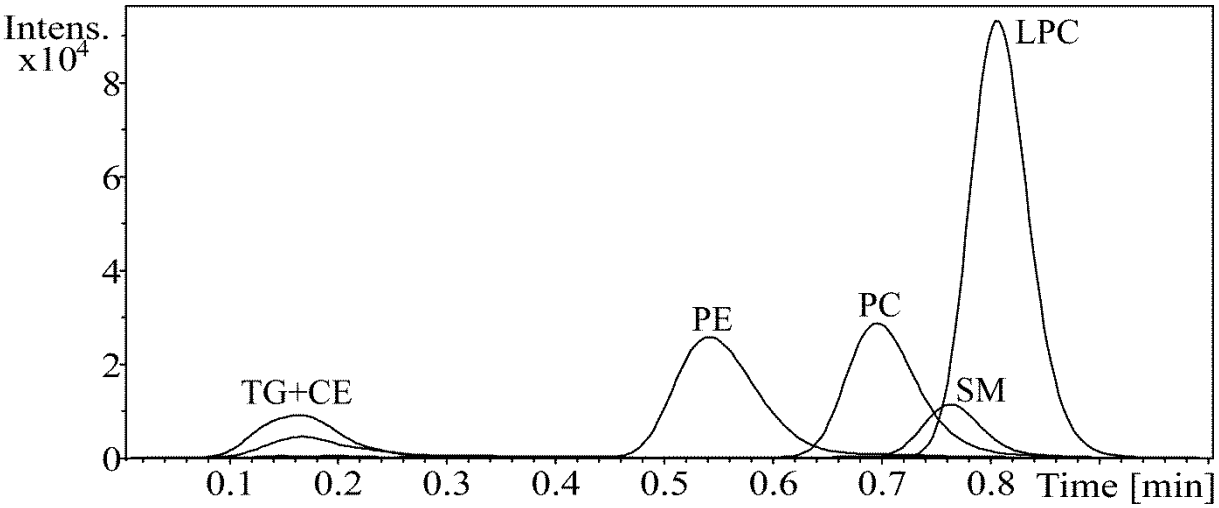

Fig. S3 RIC chromatograms for 2D-LC/ESI-MS of the porcine brain extract: **A/** *m/z* 184 characteristic for PC, SM and LPC, **B/** *m/z* 369 characteristic for cholesterol and CE.

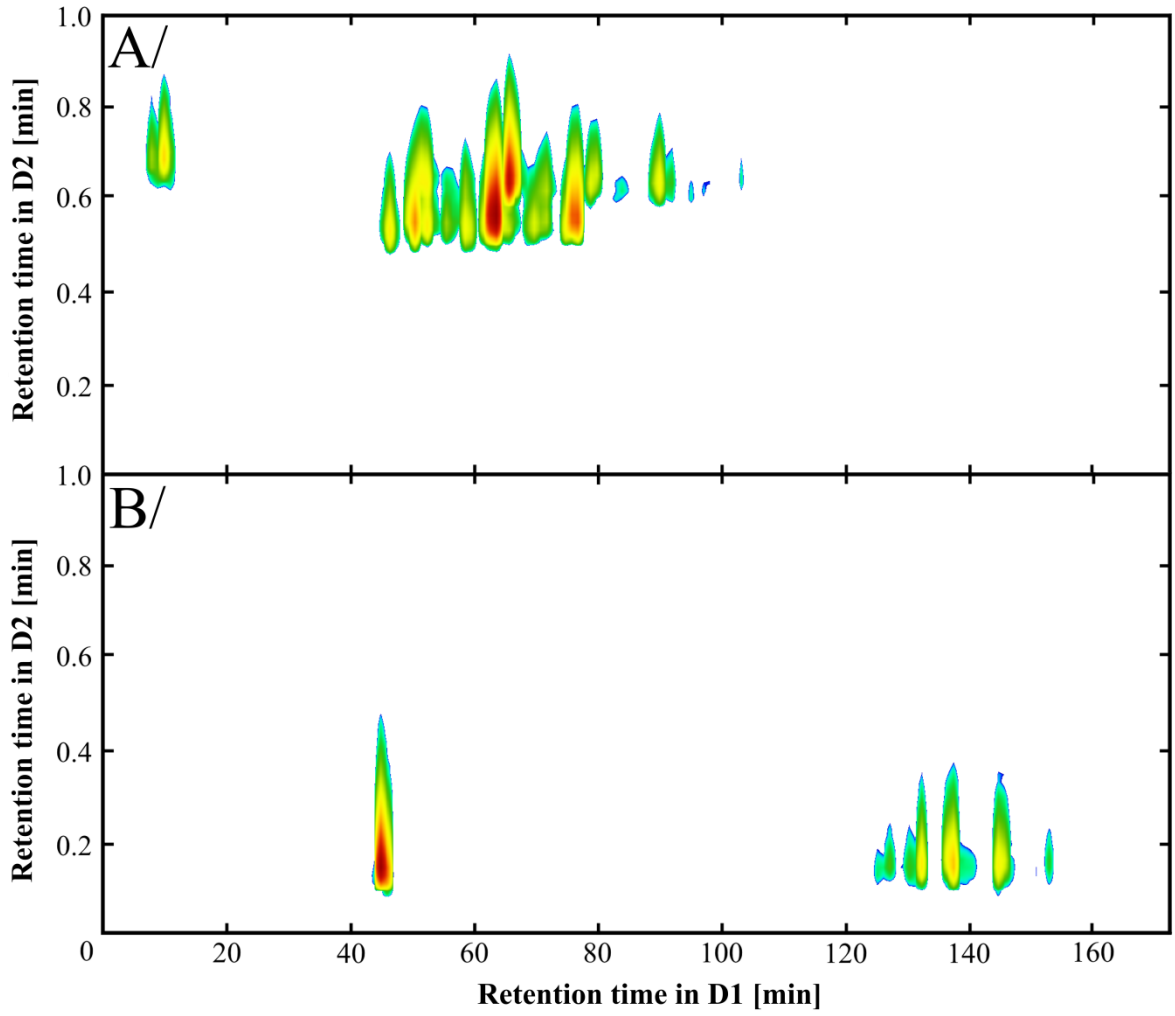

Table S1 Lipid species identified in total lipid extracts of human plasma and porcine brain

No.	Retention times [min]		Lipid species level [a]	Fatty acyl/alkyl level [a]	ECN	*m/z* experimental	*m/z* theoretical	Mass accuracy [ppm]	Plasma	Porcine brain
	1D	2D								
LYSOPHOSPHATIDYLCHOLINES (LPC)										
1	7.4	0.68	LPC 22:6	PC 22:6_0:0	10	568.3360 [b]	568.3397 [b]	-6.5		*
2	7.7	0.68	LPC 20:4	PC 20:4_0:0	12	544.3374 [b]	544.3397 [b]	-4.2		*
3	8.7	0.71	LPC 18:2	PC 18:2_0:0	14	520.3375 [b]	520.3397 [b]	-4.2	*	
4	9.7	0.70	LPC 18:1	PC 18:1_0:0	16	522.3542 [b]	522.3554 [b]	-2.3	*	*
5	9.7	0.72	LPC 16:0	PC 16:0_0:0	16	496.3391 [b]	496.3397 [b]	-1.2	*	*
6	13.7	0.71	LPC 18:0	PC 18:0_0:0	18	524.3697 [b]	524.3710 [b]	-2.4	*	
SPHINGOMYELINS (SM)										
7	38.7	0.66	SM 32:1		30	675.5468 [b]	675.5435 [b]	4.8	*	
8	45.2	0.65	SM 34:2		30	701.5574 [b]	701.5592 [b]	-2.5	*	
9	51.7	0.65	SM 34:1		32	703.5727 [b]	703.5748 [b]	-2.9	*	*
10	52.3	0.65	SM 36:2		32	729.5884 [b]	729.5905 [b]	-2.8		*
11	64.6	0.64	SM 36:1		34	731.6032 [b]	731.6061 [b]	-3.9	*	*
12	76.6	0.62	SM 42:3		36	811.6701 [b]	811.6687 [b]	1.7	*	*
13	77.6	0.63	SM 38:1		36	759.6395 [b]	759.6374 [b]	2.7	*	*
14	78.6	0.62	SM 40:2		36	785.6565 [b]	785.6531 [b]	4.3	*	*
15	83.6	0.62	SM 41:2		37	799.6708 [b]	799.6687 [b]	2.6	*	*
16	84.6	0.62	SM 39:1		37	773.6546 [b]	773.6531 [b]	1.9	*	
17	89.6	0.61	SM 42:2		38	813.6855 [b]	813.6844 [b]	1.3	*	*
18	90.6	0.62	SM 40:1		38	787.6703 [b]	787.6687 [b]	2.0	*	*
19	92.1	0.62	SM 43:2		39	827.7022 [b]	827.7000 [b]	2.6		*
20	95.6	0.62	SM 40:0		40	789.6829 [b]	789.6844 [b]	-1.8		*
21	97.6	0.62	SM 41:1		39	801.6857 [b]	801.6844 [b]	1.6	*	
22	103.3	0.62	SM 42:1		40	815.7026 [b]	815.7000 [b]	3.1	*	*
CERAMIDES (Cer), HEXOSYL CERAMIDES (HexCer) and DIHEXOSYL CERAMIDES (Hex2Cer)										
23	63.8	0.15	Cer 36:1 (OH)		34	582.5487 [b]	582.5455 [b]	5.4		*
24	65.3	0.18	Hex2Cer 36:1	Hex2Cer d18:1/18:0	34	890.6547 [b]	890.6563 [b]	-1.7		*
25	67.2	0.14	Cer 36:1		34	566.5537 [b]	566.5506 [b]	5.4		*
26	67.2	0.16	HexCer 36:1	HexCer d18:1/18:0	34	728.6049 [b]	728.6034 [b]	2.0		*
27	74.7	0.14	Cer 36:1	Cer d18:1/18:0	34	566.5524 [b]	566.5506 [b]	3.1		*
28	77.9	0.12	Cer 38:1 (OH)		36	610.5797 [b]	610.5768 [b]	4.7		*
29	81.7	0.12	Cer 38:1	Cer d18:1/20:0	36	594.5800 [b]	594.5819 [b]	-3.2		*

30	90.1	0.12	Cer 40:1 (OH)		38	638.6065 [b]	638.6081 [b]	-2.5		*
31	90.7	0.16	HexCer 42:2	HexCer d18:1/24:1	38	810.6833 [b]	810.6822 [b]	1.3		*
32	90.8	0.16	Cer 42:2	Cer d18:1/24:1	38	648.6300 [b]	648.6289 [b]	1.7		*
33	92.7	0.18	HexCer 40:1	HexCer d18:1/22:0	38	784.6679 [b]	784.6660 [b]	2.4		*
34	98.4	0.16	HexCer 41:1	HexCer d18:1/23:0	39	798.6790 [b]	798.6817 [b]	-3.3		*
35	101.5	0.17	HexCer 44:2	HexCer d18:1/26:1	40	838.7158 [b]	838.7130 [b]	3.3		*
36	104.0	0.17	HexCer 42:1	HexCer d18:1/24:0	40	812.6997 [b]	812.6973 [b]	2.9		*
37	104.8	0.18	HexCer 42:0	HexCer d18:0/24:0	42	814.7144 [b]	814.7130 [b]	1.7		*
38	106.5	0.18	HexCer 44:0	HexCer d18:0/26:0	44	842.7462 [b]	842.7443 [b]	2.2		*
PHOSPHATIDYLCHOLINES (PC)										
39	40.6	0.54	PC 34:3	PC 16:1_18:2	28	756.5531 [b]	756.5537 [b]	-0.8	*	
40	41.5	0.53	PC 36:5	PC 16:0_20:5	26	780.5517 [b]	780.5537 [b]	-2.5	*	
41	46.2	0.52	PC 38:6	PC 16:0_22:6	26	806.5674 [b]	806.5694 [b]	-2.4	*	*
42	49.5	0.52	PC 38:5	PC 18:1_20:4	28	808.5815 [b]	808.5850 [b]	-4.3	*	*
43	49.5	0.53	PC 36:4	PC 16:0_20:4	28	782.5677 [b]	782.5694 [b]	-2.1	*	*
44	51.6	0.52	PC 36:3	PC 18:1_18:2	30	784.5825 [b]	784.5850 [b]	-3.1	*	*
45	51.6	0.54	PC 34:2	PC 16:0_18:2	30	758.5706 [b]	758.5694 [b]	1.5	*	*
46	52.6	0.56	PC 32:1	PC 16:0_16:1	30	732.5553 [b]	732.5537 [b]	2.1	*	*
47	53.5	0.52	PC 38:5	PC 18:0_20:5	28	808.5808 [b]	808.5850 [b]	-5.1	*	*
48	54.6	0.52	PC 36:3	PC 16:0_20:3	30	784.5878 [b]	784.5850 [b]	3.5	*	
49	58.5	0.53	PC 40:6	PC 18:0_22:6	28	834.5988 [b]	834.6007 [b]	-2.2	*	*
50	62.5	0.52	PC 38:4	PC 18:0_20:4	30	810.5976 [b]	810.6007 [b]	-3.8	*	*
51	63.5	0.54	PC 34:1	PC 16:0_18:1	32	760.5860 [b]	760.5850 [b]	1.3	*	*
52	63.3	0.56	PC 32:0	PC 16:0_16:0	32	734.5665 [b]	734.5694 [b]	-3.9	*	*
53	65.5	0.53	PC 36:2	PC 18:0_18:2	32	786.6036 [b]	786.6007 [b]	3.6	*	*
54	68.5	0.52	PC 38:3	PC 18:0_20:3	32	812.6137 [b]	812.6163 [b]	-3.2	*	*
55	75.6	0.53	PC 36:1	PC 18:0_18:1	34	788.6199 [b]	788.6163 [b]	4.5	*	*
56	76.6	0.56	PC 34:0	PC 16:0_18:0	34	762.6026 [b]	762.6007 [b]	2.5		*
PHOSPHATIDYLETHANOLAMINES (PE)										
57	49.3	0.33	PE 38:6	PE 16:0/22:6	26	764.5204 [b]	764.5224 [b]	-2.6	*	*
58	53.3	0.33	PE 38:5	PE 18:1_20:4	28	766.5360 [b]	766.5381 [b]	-2.7	*	*
59	53.3	0.34	PE 36:4	PE 16:0/20:4	28	740.5237 [b]	740.5224 [b]	1.7	*	*
60	55.1	0.31	PE P-38:6	PE P-16:0/22:6		748.5263 [b]	748.5275 [b]	-1.6	*	*
61	55.4	0.35	PE 34:2	PE 16:1_18:1	30	716.5247 [b]	716.5224 [b]	3.2	*	*
62	58.3	0.32	PE P-38:5/PE O-38:6			750.5440 [b]	750.5432 [b]	1.1	*	*

63	58.3	0.33	PE P-36:4	PE P-16:0/20:4		724.5288 [b]	724.5275 [b]	1.8	*	*
64	61.3	0.33	PE 40:6	PE 18:0/22:6	28	792.5560 [b]	792.5537 [b]	2.9	*	*
65	63.8	0.34	PE 38:4	PE 18:0/20:4	30	768.5555 [b]	768.5537 [b]	2.3	*	*
66	64.8	0.36	PE 34:1	PE 16:0/18:1	32	718.5398 [b]	718.5381 [b]	2.3		*
67	67.3	0.35	PE 36:2	PE 18:1/18:1	32	744.5551 [b]	744.5537 [b]	1.9	*	*
68	69.3	0.33	PE 40:5	PE 18:0/22:5	30	794.5731 [b]	794.5694 [b]	4.6		*
69	69.3	0.33	PE P-38:3/PE O-38:4			754.5715 [b]	754.5745 [b]	-3.9		*
70	70.3	0.33	PE 38:3	PE 18:0_20:3	32	770.5712 [b]	770.5694 [b]	2.3	*	
71	71.3	0.32	PE P-38:4	PE P-18:0/20:4		752.5603 [b]	752.5588 [b]	1.9		*
72	74.3	0.35	PE P-36:2	PE P-18:1/18:1		728.5578 [b]	728.5588 [b]	-1.3	*	*
73	75.5	0.34	PE 40:4	PE 18:0/22:4	32	796.5868 [b]	796.5850 [b]	2.2		*
74	79.3	0.35	PE 36:1	PE 18:0/18:1	34	746.5715 [b]	746.5694 [b]	2.8		*
75	79.3	0.33	PE P-40:4	PE P-18:0/22:4		780.5924 [b]	780.5901 [b]	2.9		*
76	82.3	0.35	PE P-38:2	PE P-18:1/20:1		756.5918 [b]	756.5901 [b]	2.2		*
77	84.4	0.36	PE P-36:1/PE O-36:2			730.5763 [b]	730.5745 [b]	2.4		*
78	84.5	0.31	PE 40:2		36	800.6146 [b]	800.6163 [b]	-2.1		*
TRIACYLGLYCEROLS (TG)										
79	119.5	0.11	TG 48:3	TG 12:0_18:1_18:2	42	818.7208 [c]	818.7232 [c]	-2.9	*	
80	120.0	0.11	TG 50:4	TG 16:1_16:1_18:2	42	844.7356 [c]	844.7386 [c]	-3.5	*	
81	120.2	0.11	TG 52:5	TG 16:1_18:2_18:2	42	870.7496 [c]	870.7545 [c]	-5.6	*	
82	120.4	0.11	TG 46:2	TG 12:0_16:1_18:1	42	792.7049 [c]	792.7075 [c]	-3.2	*	
83	120.7	0.11	TG 54:6	TG 18:2_18:2_18:2	42	896.7657 [c]	896.7701 [c]	-4.9	*	
84	120.9	0.11	TG 44:1	TG 12:0_16:0_16:1	42	766.6902 [c]	766.6919 [c]	-2.2	*	
85	121.0	0.11	TG 50:4	TG 16:0_16:1_18:3	42	844.7355 [c]	844.7388 [c]	-3.9	*	
86	121.4	0.11	TG 46:2	TG 12:0_16:0_18:2	42	792.7047 [c]	792.7075 [c]	-3.5	*	
87	122.0	0.11	TG 48:3	TG 14:0_16:0_18:3	42	818.7205 [c]	818.7232 [c]	-3.3	*	
88	122.5	0.11	TG 52:5	TG 16:0_18:2_18:3	42	870.7494 [c]	870.7545 [c]	-5.8	*	
89	123.2	0.11	TG 54:6	TG 18:1_18:2_18:3	42	896.7654 [c]	896.7701 [c]	-5.2	*	
90	124.0	0.11	TG 52:5	TG 16:0_16:1_20:4	42	870.7489 [c]	870.7545 [c]	-6.4	*	
91	124.5	0.11	TG 54:6	TG 16:0_18:2_20:4	42	896.7641 [c]	896.7701 [c]	-6.7	*	
92	124.9	0.11	TG 50:4	TG 14:0_16:0_20:4	42	844.7346 [c]	844.7388 [c]	-4.9	*	
93	125.7	0.11	TG 54:6	TG 16:0_18:2_20:4	42	896.7651 [c]	896.7701 [c]	-5.5	*	
94	126.2	0.11	TG 52:4	TG 16:1_18:1_18:2	44	872.7655 [c]	872.7701 [c]	-5.2	*	
95	126.6	0.12	TG 54:5	TG 18:1_18:2_18:2	44	898.7838 [c]	898.7858 [c]	-2.2	*	*
96	126.7	0.11	TG 48:2	TG 14:0_16:1_18:1	44	820.7351 [c]	820.7388 [c]	-4.5	*	*

97	127.0	0.12	TG 50:3	TG 16:0_16:1_18:2	44	846.7564 [c]	846.7545 [c]	2.2	*	*
98	127.3	0.11	TG 52:4	TG 16:0_18:2_18:2	44	872.7710 [c]	872.7701 [c]	1.0	*	*
99	128.0	0.11	TG 48:2	TG 14:0_16:0_18:2	44	820.7364 [c]	820.7388 [c]	-2.9	*	*
100	128.8	0.11	TG 46:1	TG 12:0_16:0_18:1	44	794.7211 [c]	794.7232 [c]	-2.6		*
101	128.9	0.11	TG 52:4	TG 16:0_18:1_18:3	44	872.7667 [c]	872.7701 [c]	-3.9	*	*
102	129.2	0.12	TG 50:3	TG 16:0_16:0_18:3	44	846.7511 [c]	846.7545 [c]	-4.0	*	*
103	130.0	0.12	TG 56:6	TG 18:1_18:1_20:4	44	924.7955 [c]	924.8014 [c]	-6.3	*	*
104	130.9	0.12	TG 54:5	TG 16:0_18:2_20:3	44	898.7815 [c]	898.7858 [c]	-4.7	*	*
105	131.2	0.12	TG 56:6	TG 18:0_18:2_20:4	44	924.7953 [c]	924.8014 [c]	-6.5	*	*
106	131.9	0.11	TG 52:4	TG 16:0_16:0_20:4	44	872.7668 [c]	872.7701 [c]	-3.7	*	*
107	132.5	0.12	TG 56:6	TG 16:0_18:1_22:5	44	924.7969 [c]	924.8014 [c]	-4.8	*	
108	132.8	0.12	TG 54:4	TG 18:1_18:1_18:2	46	900.8004 [c]	900.8014 [c]	-1.1	*	*
109	133.4	0.11	TG 50:2	TG 16:0_16:1_18:1	46	848.7691 [c]	848.7701 [c]	-1.1	*	*
110	133.7	0.12	TG 52:3	TG 16:1_18:1_18:1	46	874.7832 [c]	874.7858 [c]	-2.9	*	*
111	133.9	0.12	TG 54:4	TG 16:0_18:1_20:3	46	900.7974 [c]	900.8014 [c]	-4.4	*	*
112	134.6	0.12	TG 48:1	TG 14:0_16:0_18:1	46	822.7530 [c]	822.7545 [c]	-1.8	*	*
113	134.8	0.11	TG 50:2	TG 16:0_16:0_18:2	46	848.7723 [c]	848.7701 [c]	2.6	*	*
114	134.9	0.12	TG 52:3	TG 16:0_18:1_18:2	46	874.7879 [c]	874.7858 [c]	2.4		*
115	136.3	0.12	TG 56:5	TG 16:0_18:1_22:4	46	926.8122 [c]	926.8171 [c]	-5.2		*
116	136.7	0.12	TG 46:0	TG 14:0_16:0_16:0	46	796.7351 [c]	796.7388 [c]	-4.6		*
117	136.9	0.12	TG 52:3	TG 16:0_18:0_18:3	46	874.7846 [c]	874.7858 [c]	-1.3		*
118	138.3	0.12	TG 56:5	TG 18:0_18:1_20:4	46	926.8137 [c]	926.8171 [c]	-3.6		*
119	138.6	0.11	TG 54:3	TG 18:1_18:1_18:1	48	902.8197 [c]	902.8171 [c]	2.8	*	*
120	139.5	0.12	TG 52:2	TG 16:0_18:1_18:1	48	876.8035 [c]	876.8014 [c]	2.3	*	*
121	139.7	0.11	TG 54:3	TG 18:0_18:1_18:2	48	902.8155 [c]	902.8171 [c]	-1.6	*	*
122	140.6	0.12	TG 50:1	TG 16:0_16:0_18:1	48	850.7832 [c]	850.7858 [c]	-3.0	*	
123	142.0	0.12	TG 52:2	TG 16:0_18:0_18:2	48	876.7986 [c]	876.8014 [c]	-2.8	*	*
124	142.9	0.13	TG 48:0	TG 14:0_16:0_18:0	48	824.7712 [c]	824.7701 [c]	1.3		*
125	143.0	0.12	TG 56:4	TG 16:0_18:0_22:4	48	928.8308 [c]	928.8327 [c]	-2.0		*
126	144.9	0.12	TG 56:3	TG 18:1_18:1_20:1	50	930.8497 [c]	930.8484 [c]	1.4		*
127	145.0	0.12	TG 54:2	TG 18:0_18:1_18:1	50	904.8287 [c]	904.8327 [c]	-4.4	*	*
128	145.7	0.12	TG 56:3	TG 18:0_18:1_20:2	50	930.8471 [c]	930.8484 [c]	-1.4		*
129	146.1	0.12	TG 52:1	TG 16:0_18:0_18:1	50	878.8150 [c]	878.8171 [c]	-2.4	*	*
130	147.3	0.12	TG 50:0	TG 16:0_16:0_18:0	50	852.7964 [c]	852.8014 [c]	-5.8	*	*
131	148.5	0.13	TG 56:2	TG 18:0_18:1_20:1	52	932.8661 [c]	932.8640 [c]	2.2		*

132	149.8	0.12	TG 54:1	TG 18:0_18:0_18:1	52	906.8512 [c]	906.8484 [c]	3.0		*
133	151.2	0.13	TG 52:0	TG 16:0_18:0_18:0	52	880.8358 [c]	880.8327 [c]	3.5		*
134	155.7	0.12	TG 54:0	TG 18:0_18:0_18:0	54	908.8616 [c]	908.8640 [c]	-2.6		*
STEROLS (ST) and STERYL ESTERS (SE)										
135	45.2	0.12	ST 27:1_OH	Chol		369.3531 [d]	369.3521 [d]	2.7	*	*
136	122.2	0.11	SE 27:1/20:5	CE 20:5	10	688.6003 [c]	688.6027 [c]	-3.4	*	*
137	125.1	0.12	SE 27:1/22:6	CE 22:6	10	714.6203 [c]	714.6183 [c]	2.8	*	*
138	127.2	0.12	SE 27:1/18:3	CE 18:3	12	664.6010 [c]	664.6027 [c]	-2.5	*	*
139	129.2	0.12	SE 27:1/20:4	CE 20:4	12	690.6170 [c]	690.6183 [c]	-1.8	*	*
140	134.2	0.12	SE 27:1/18:2	CE 18:2	14	666.6174 [c]	666.6183 [c]	-1.3	*	*
141	142.2	0.12	SE 27:1/18:1	CE 18:1	16	668.6354 [c]	668.6340 [c]	2.1	*	*
142	143.1	0.13	SE 27:1/16:0	CE 16:0	16	642.6190 [c]	642.6183 [c]	1.1	*	*
143	150.2	0.12	SE 27:1/18:0	CE 18:0	18	670.6483 [c]	670.6496 [c]	-1.9	*	*

[a] The lipid notation follows the shorthand nomenclature recommended by Liebisch et al. [37], where the slash separator / denotes proven *sn*-position, while the underscore separator _ is used when *sn*-position of fatty acyls is not known.

[b] $[M+H]^+$.

[c] $[M+NH_4]^+$.

[d] $[M+H-H_2O]^+$.