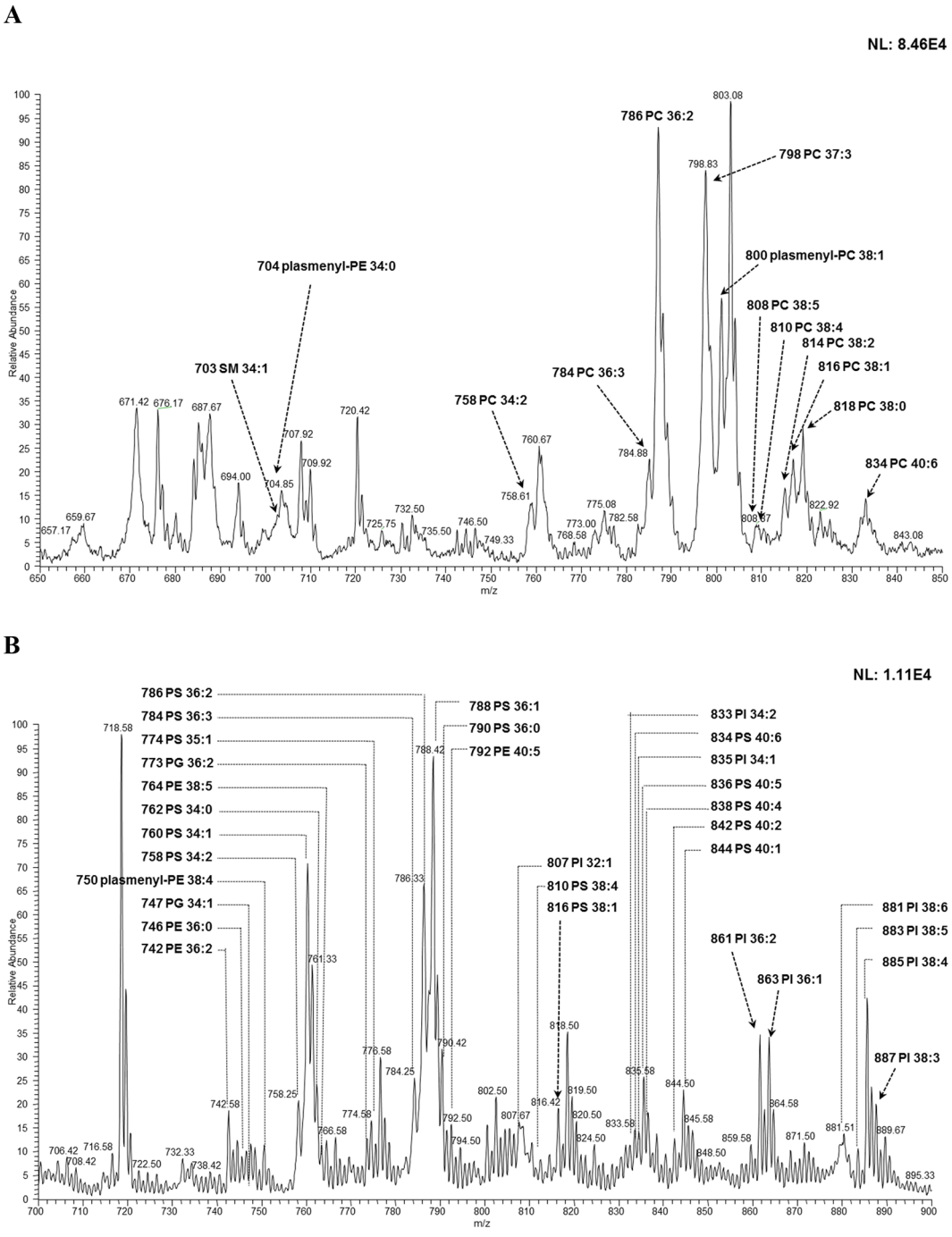
Comparative metabolic and lipidomic profiling of human breast cancer cells with different metastatic potentials

SUPPLEMENTARY FIGURES AND TABLES



Supplementary Figure S1: Representative spectra of lipid profiles from mammary epithelial and breast cancer cells, derived from nano-electrospray mass spectrometry in A. positive ion mode and B. negative ion mode.

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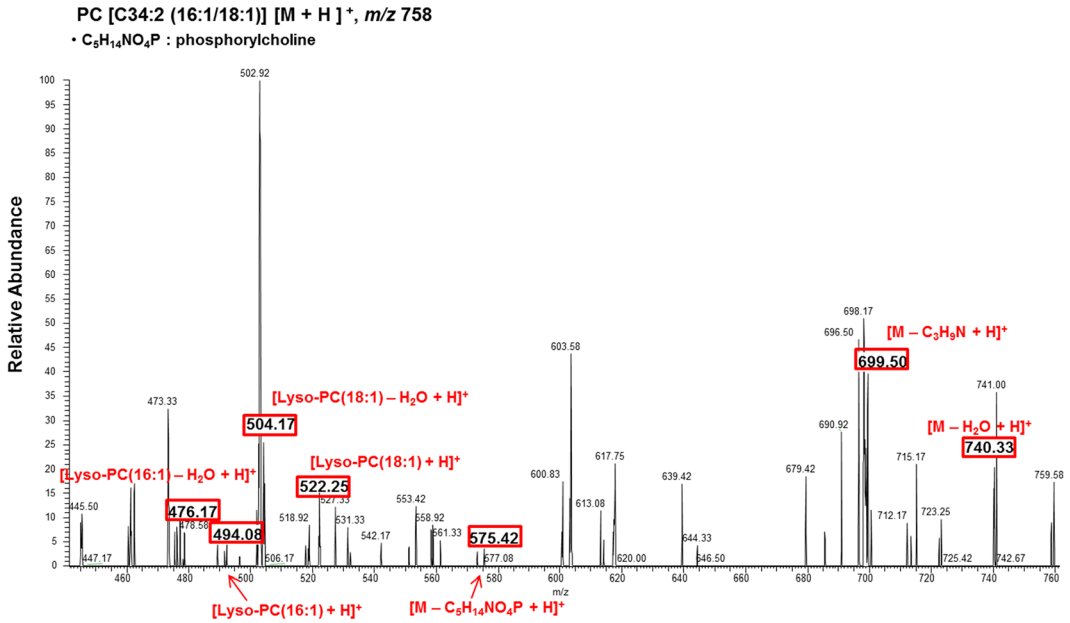

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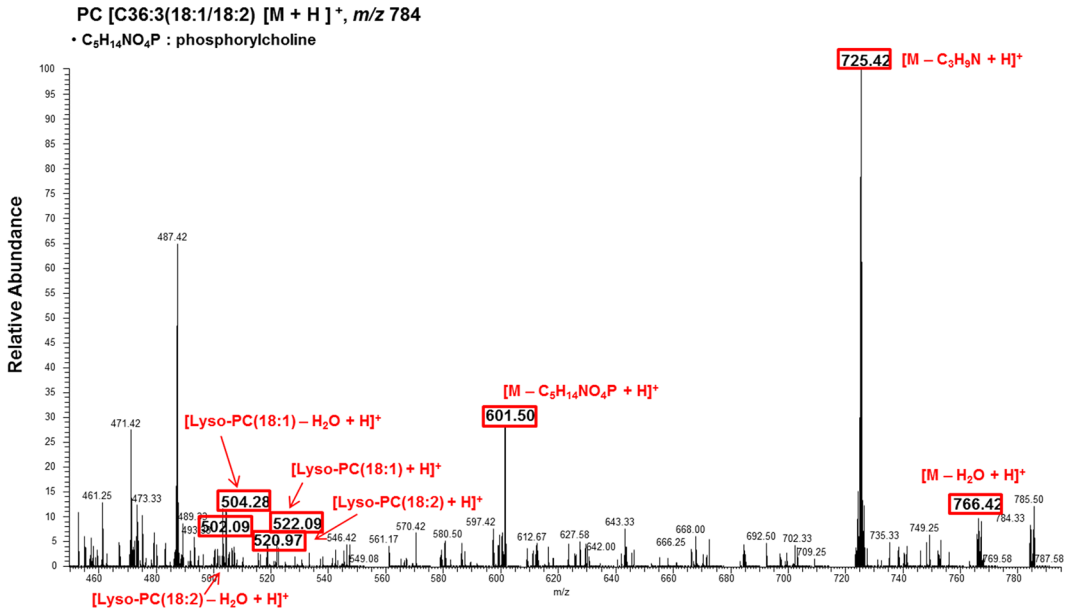

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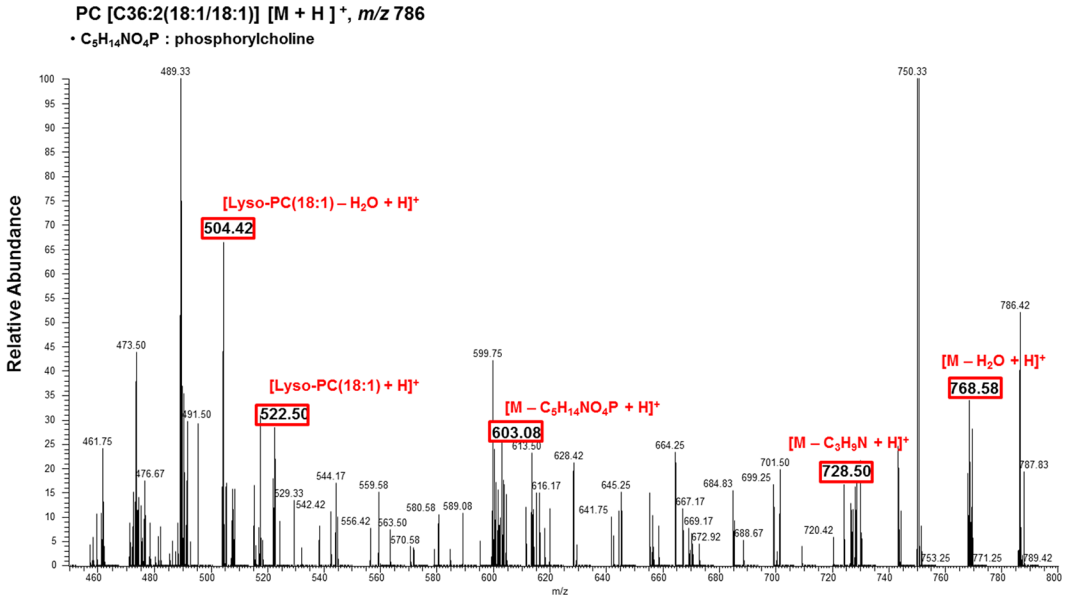

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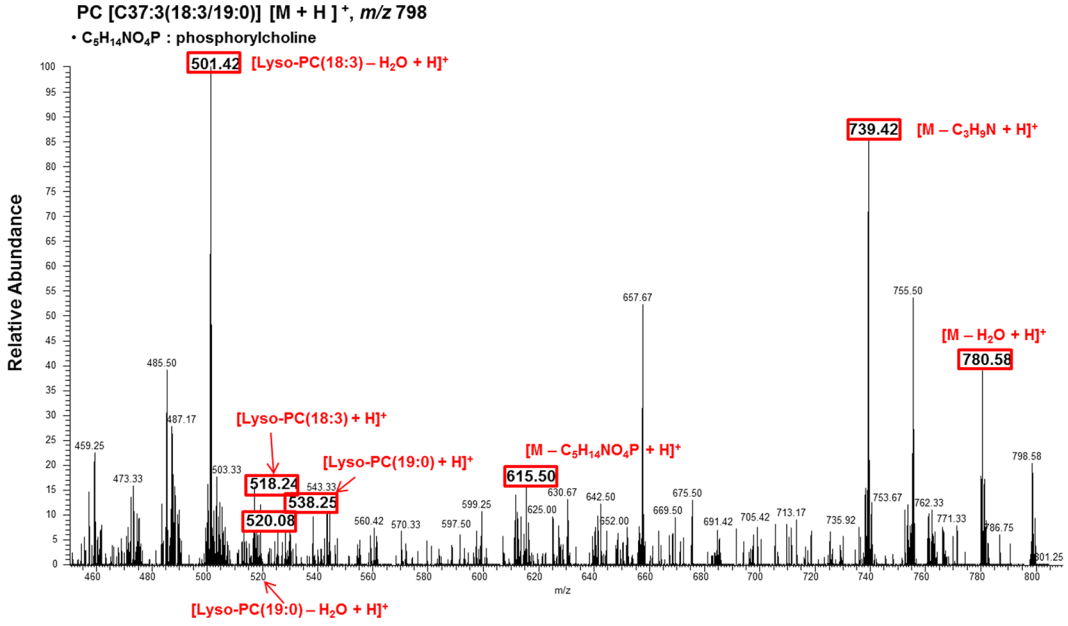

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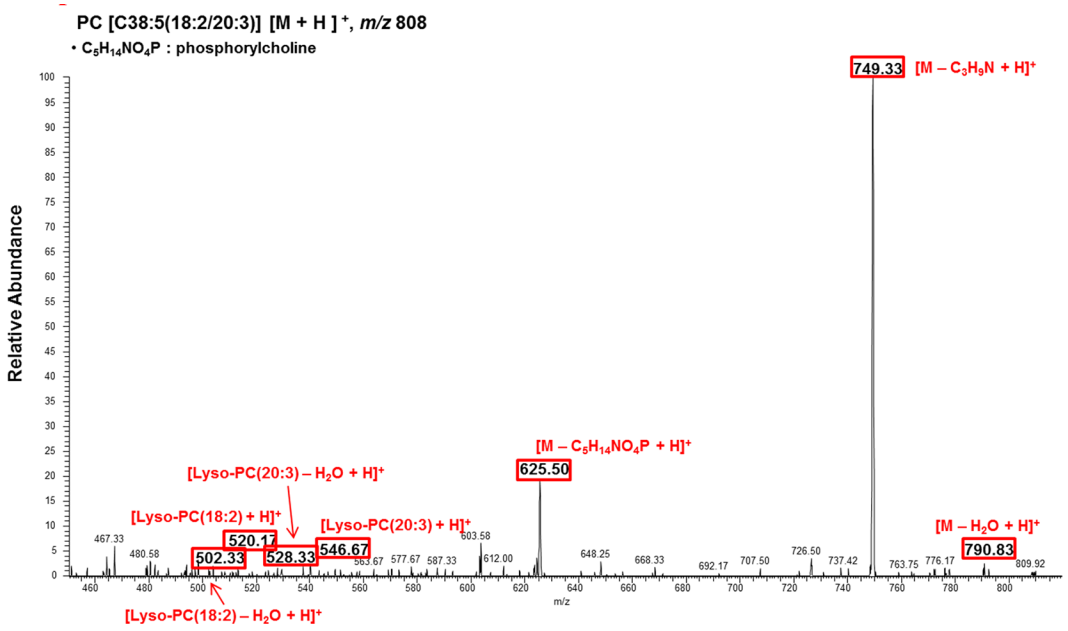

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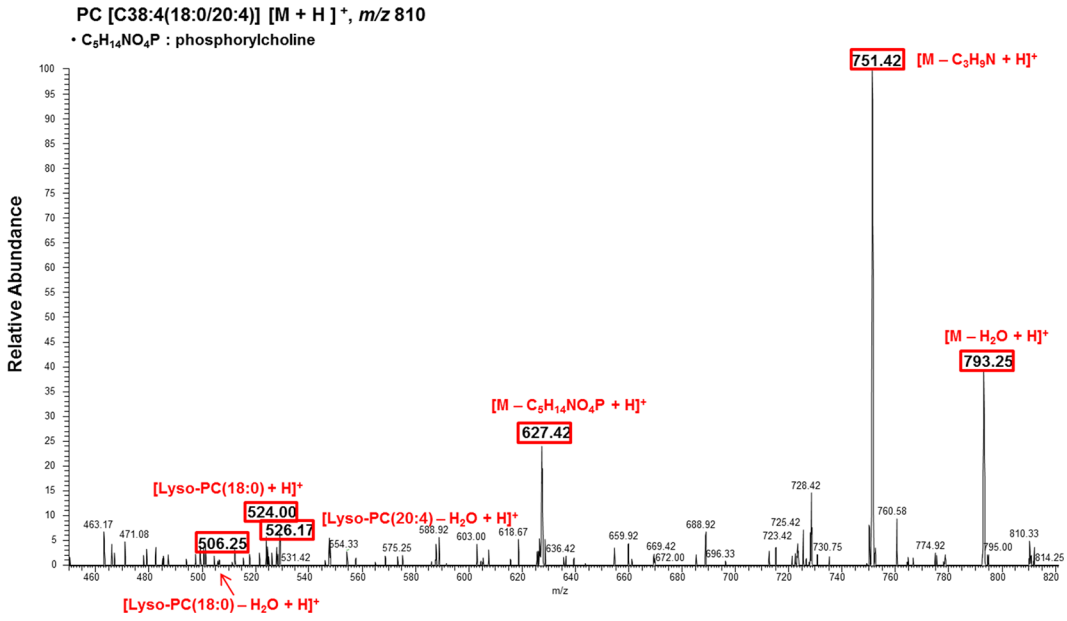

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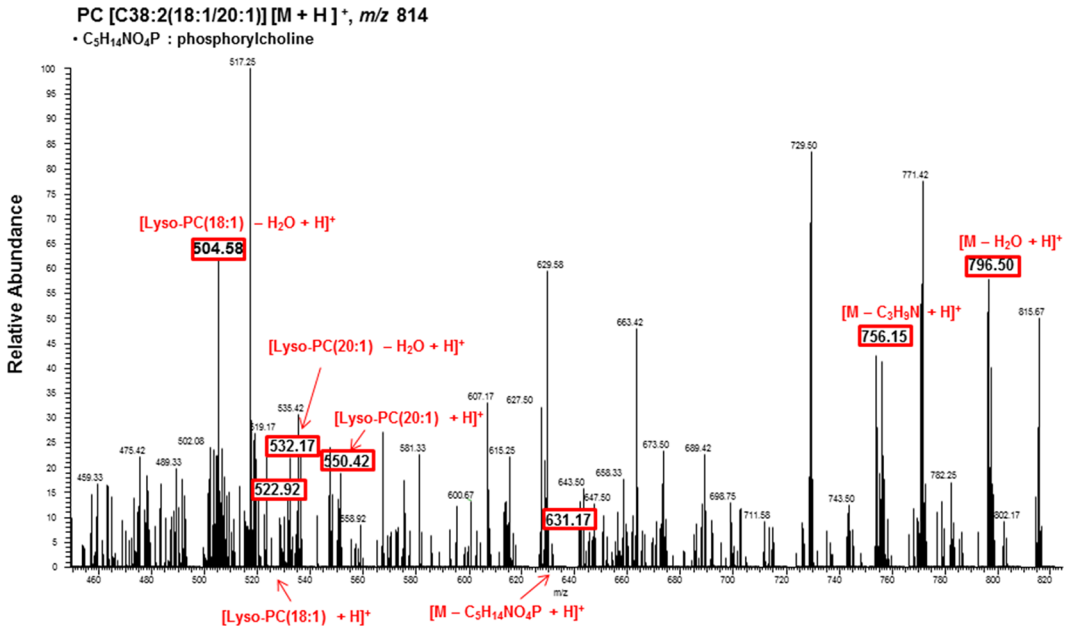

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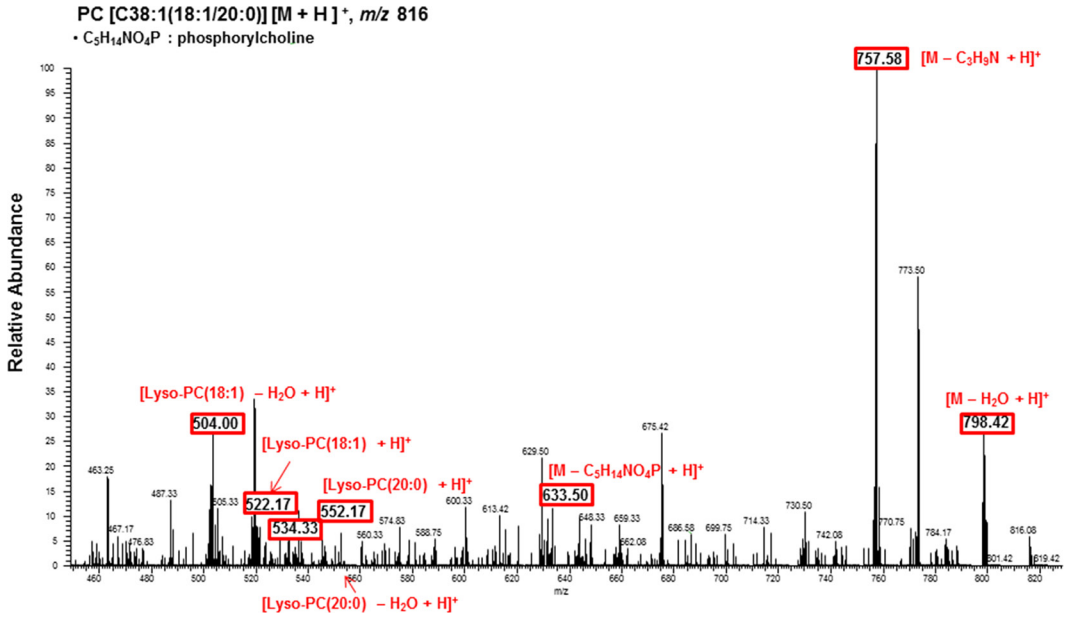

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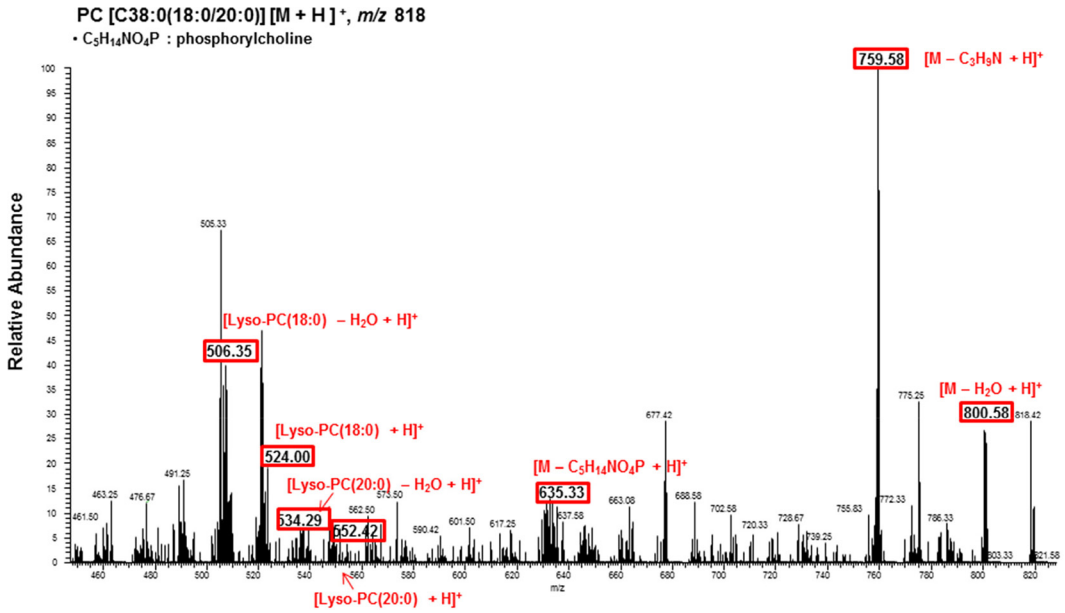

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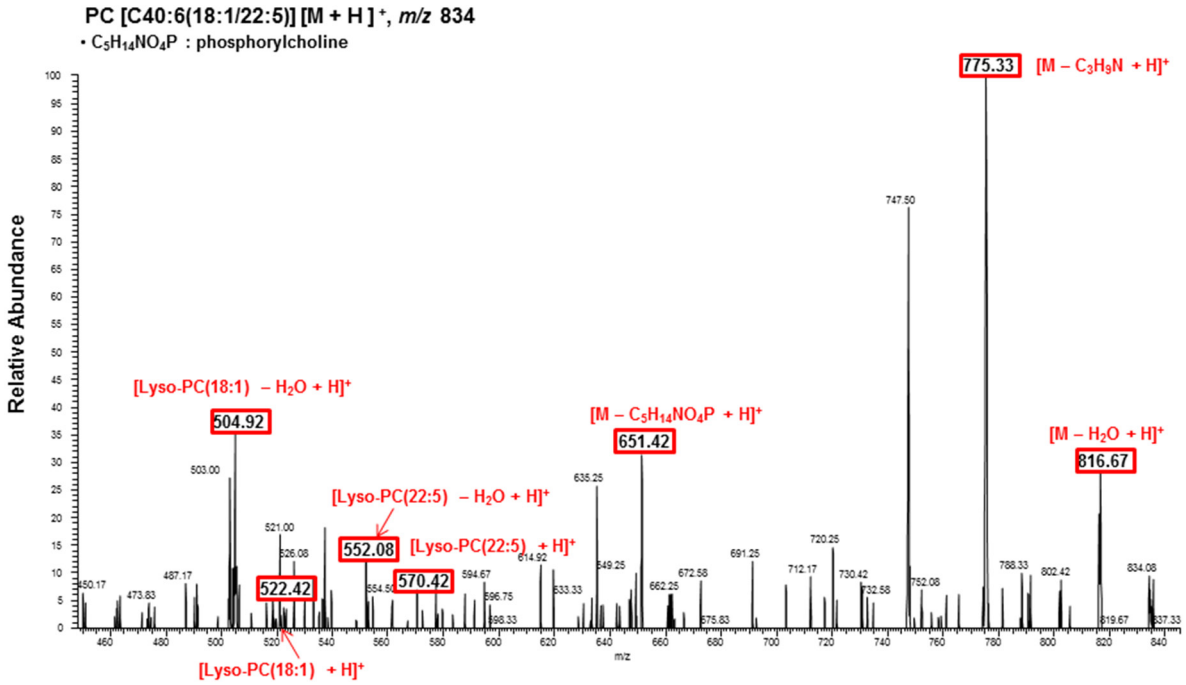

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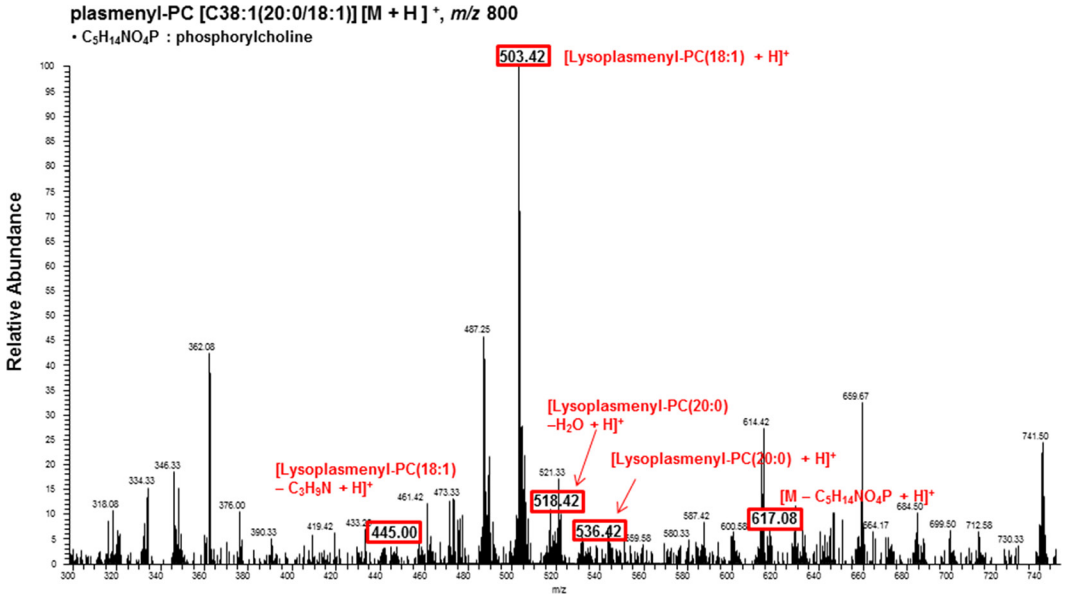

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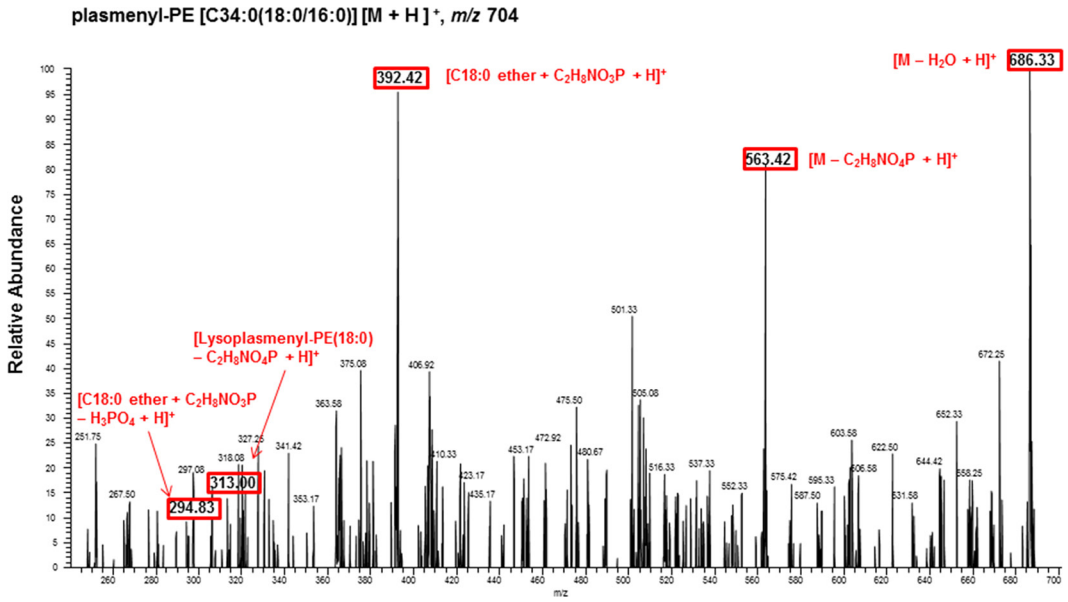

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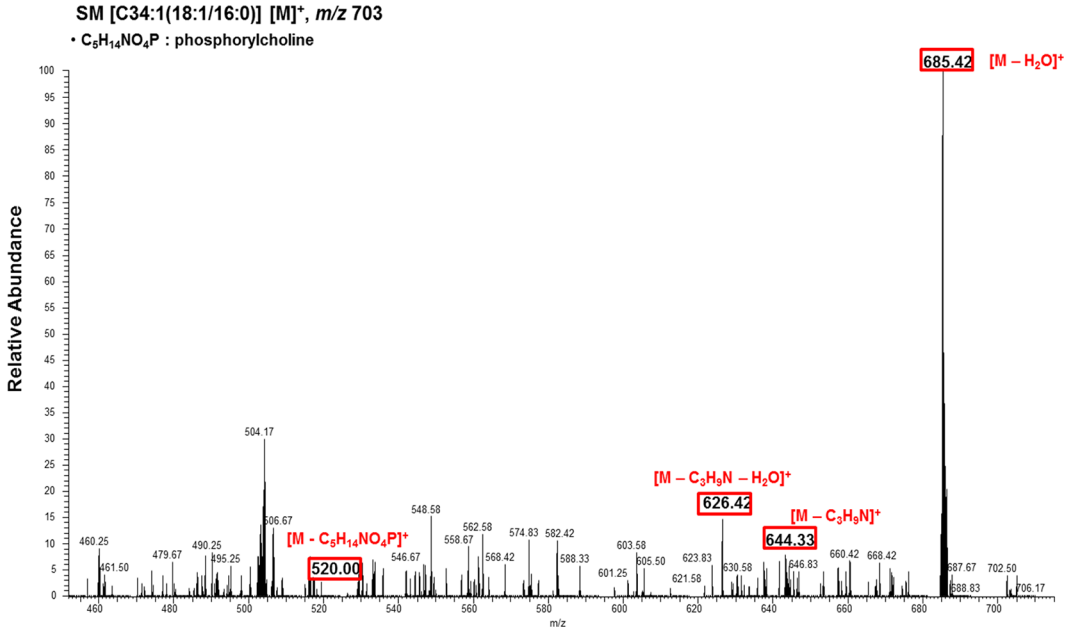

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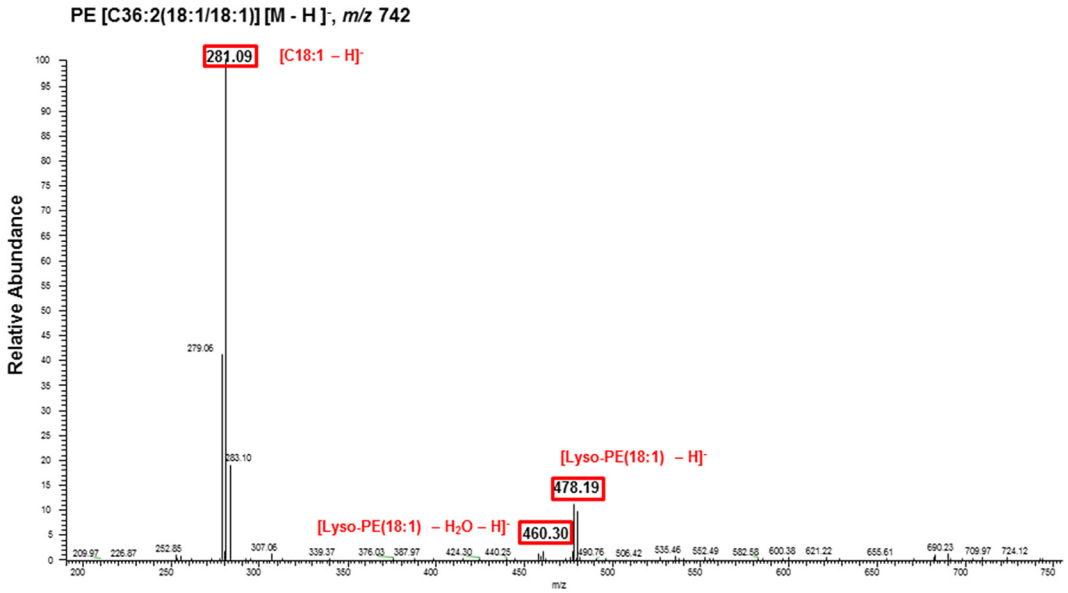

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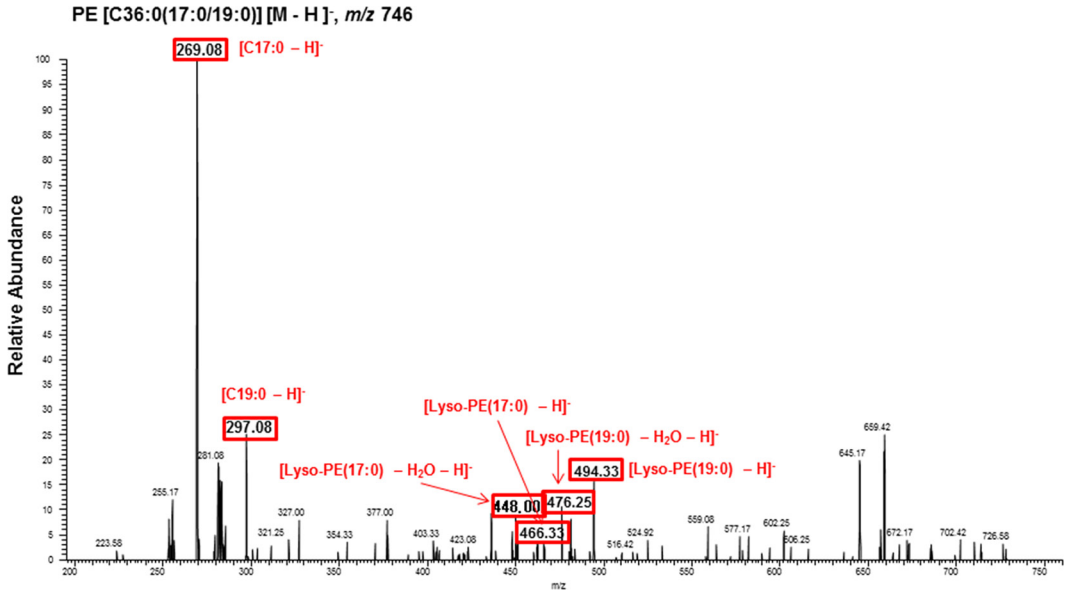

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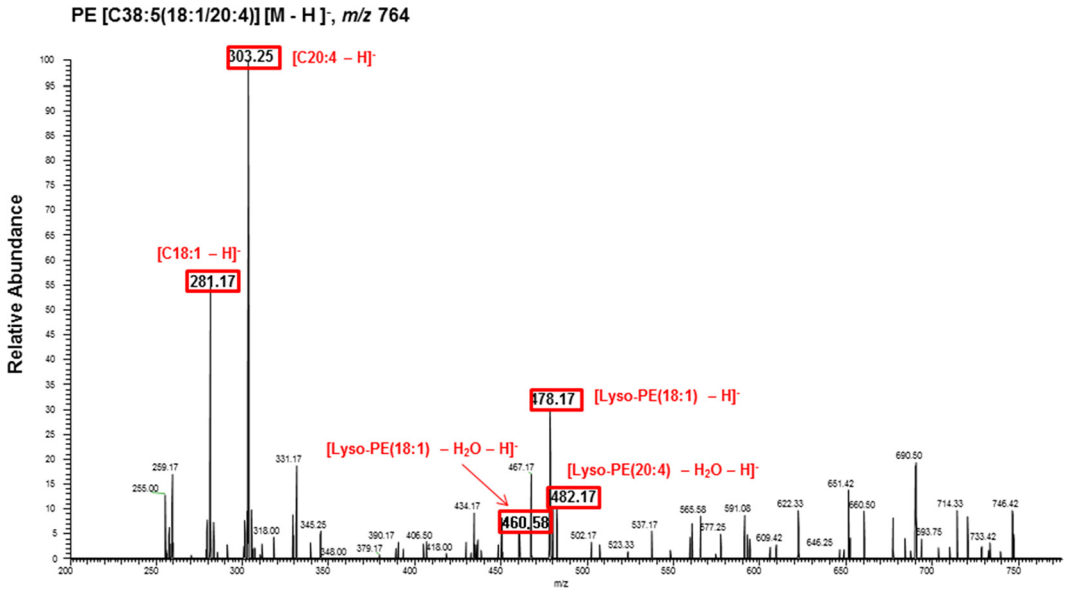

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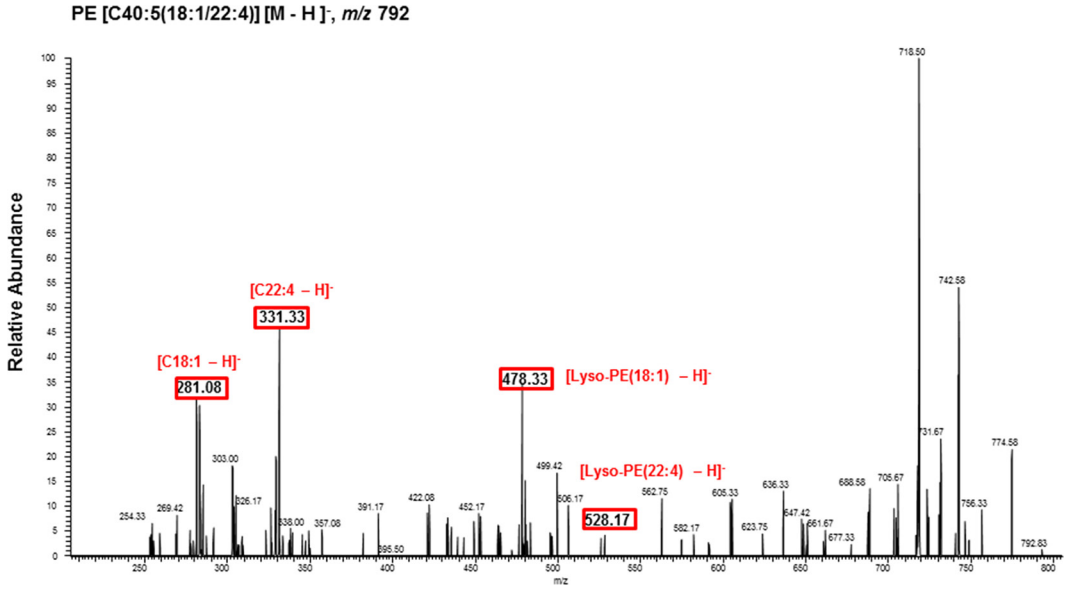

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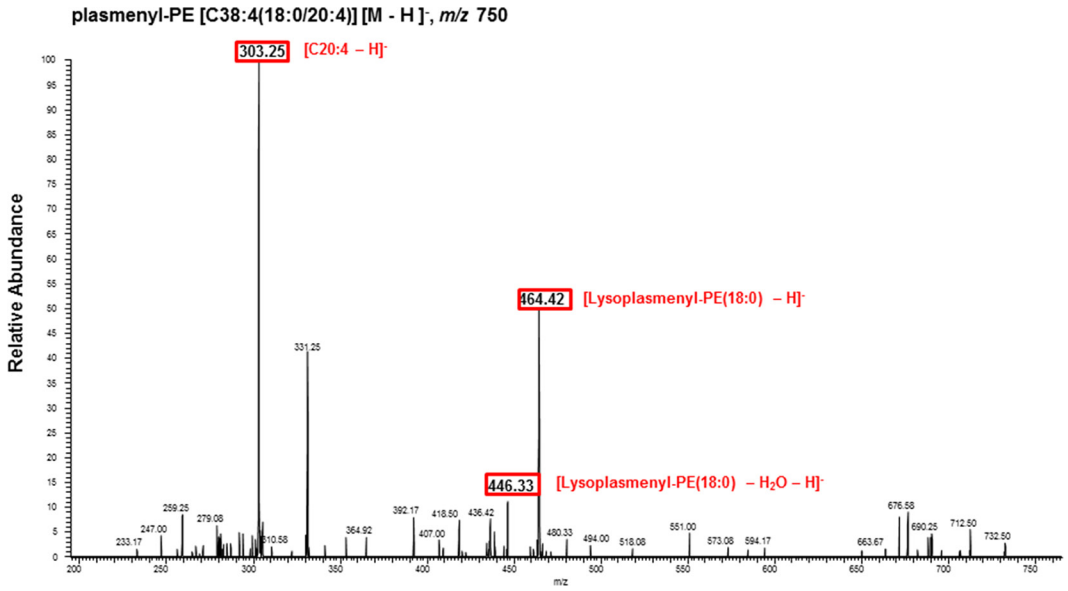

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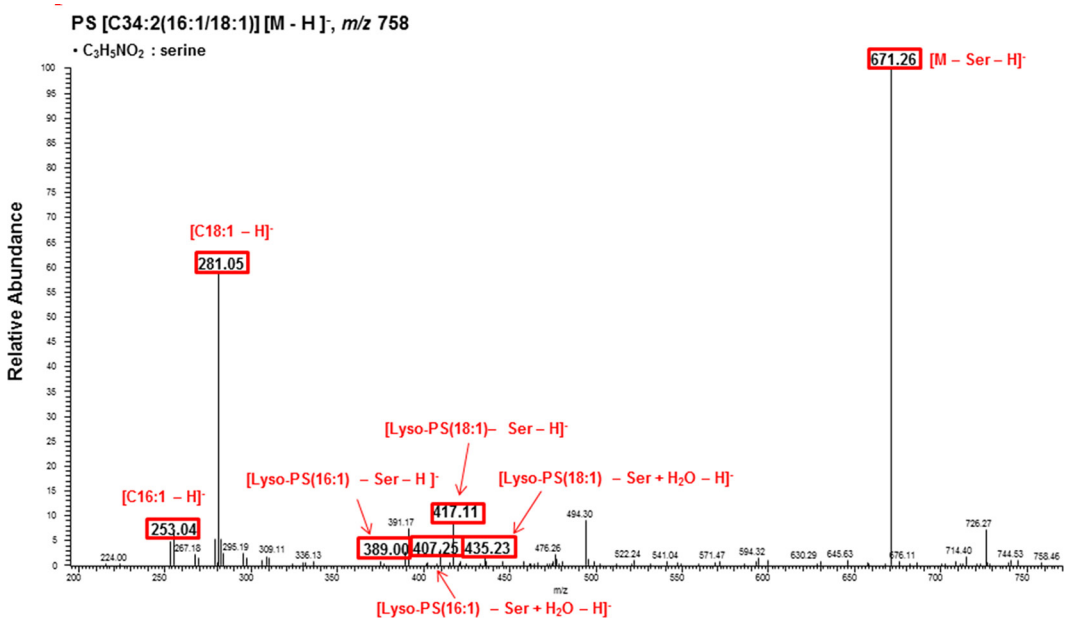

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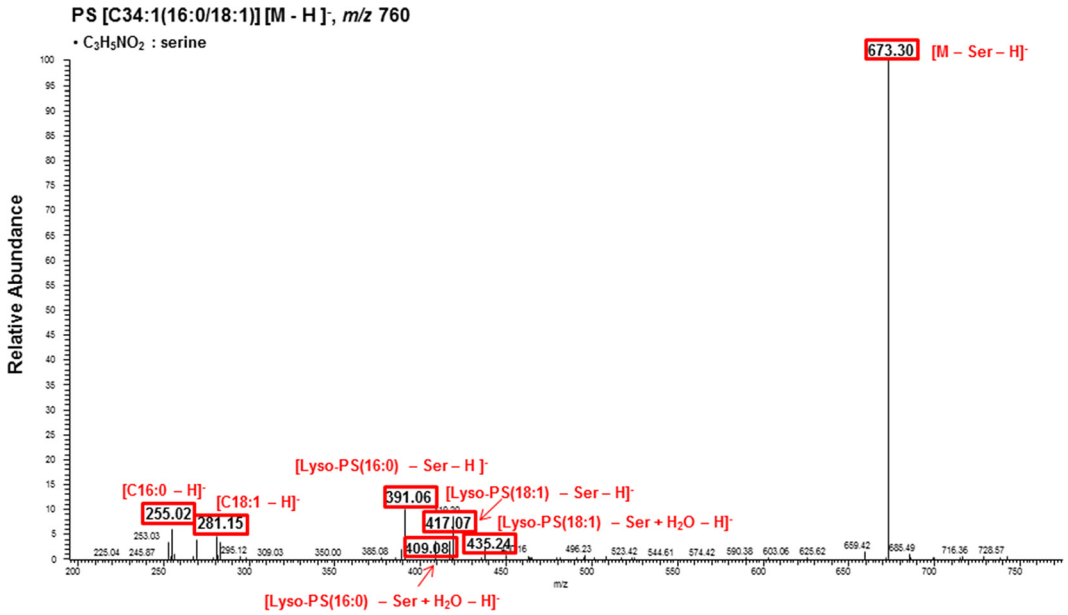

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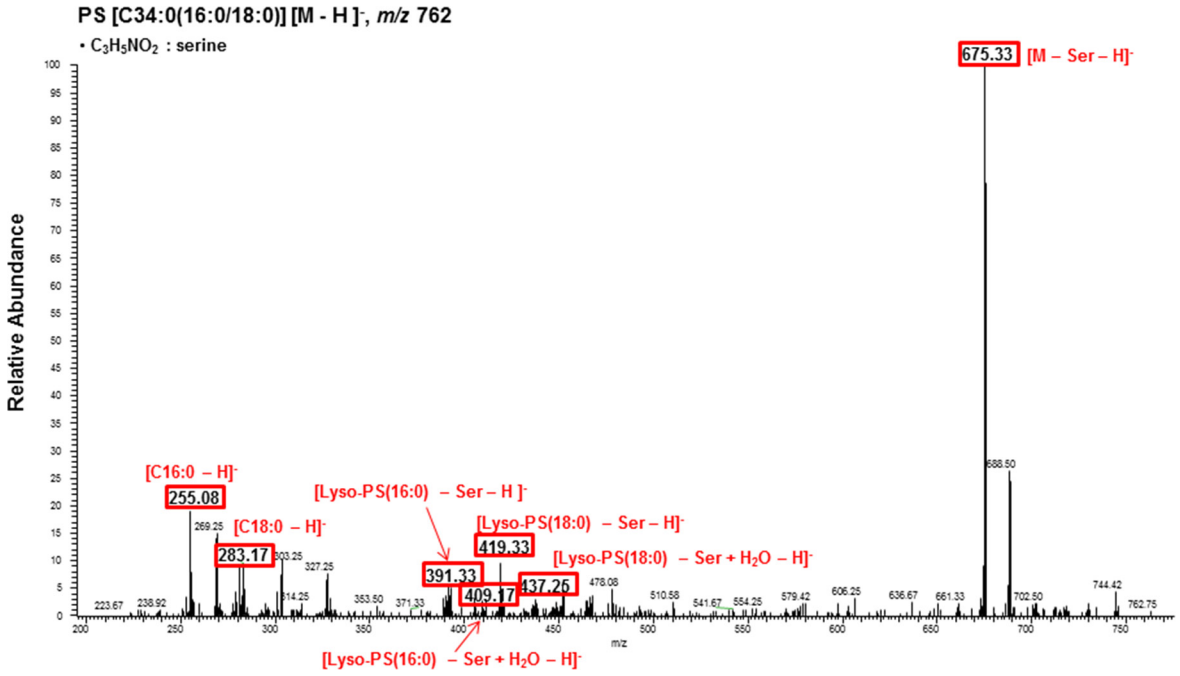

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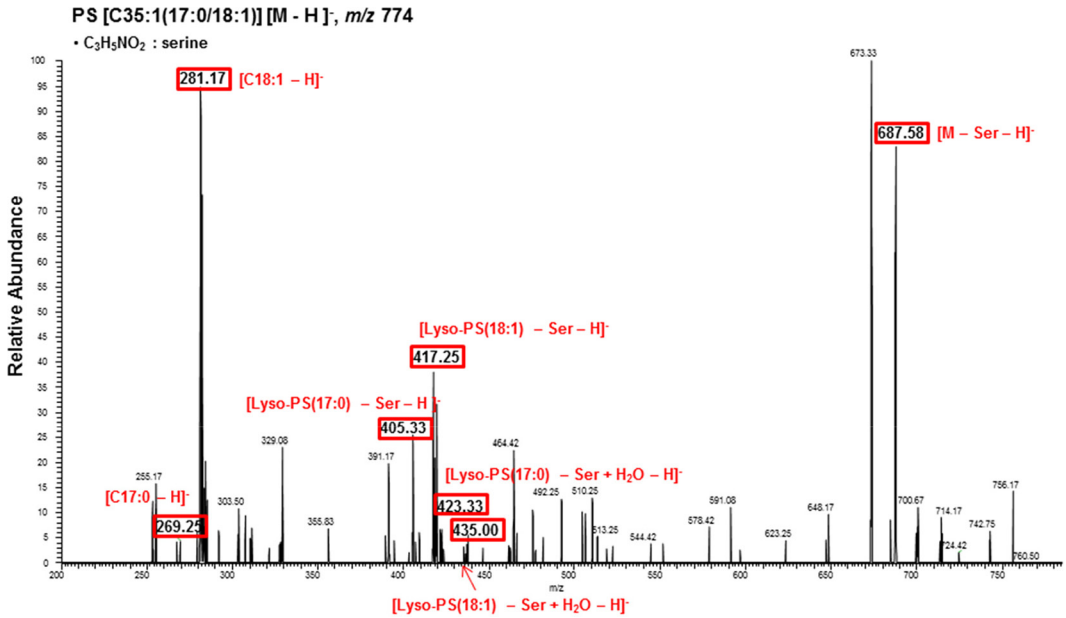

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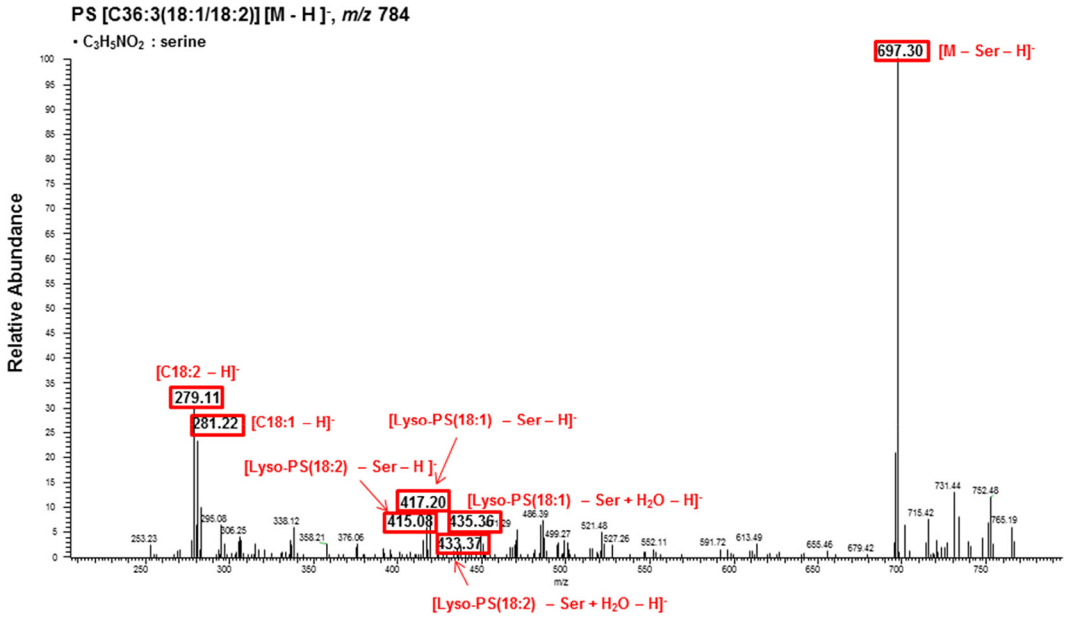

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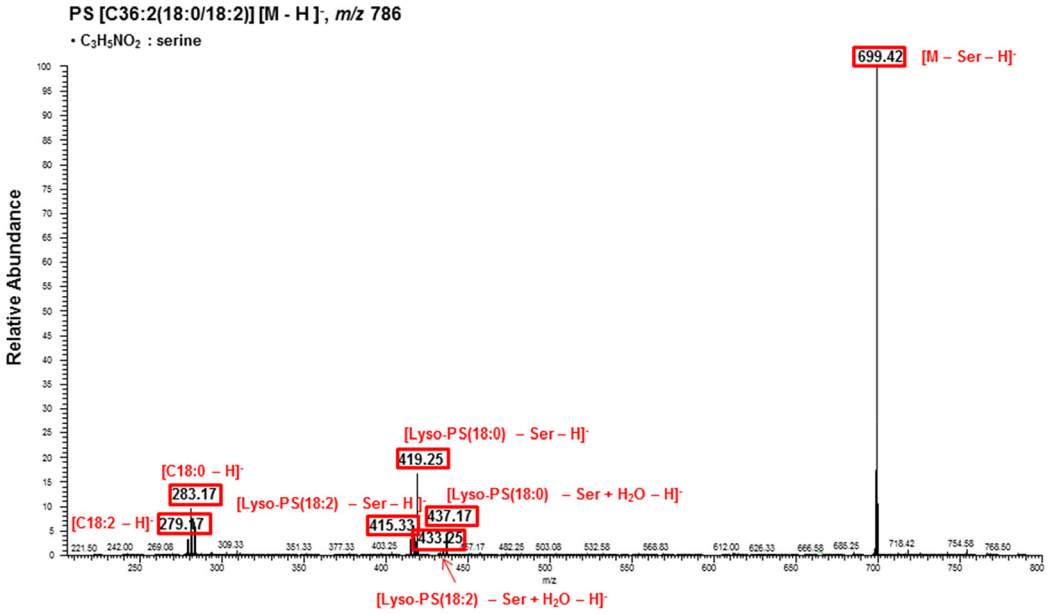

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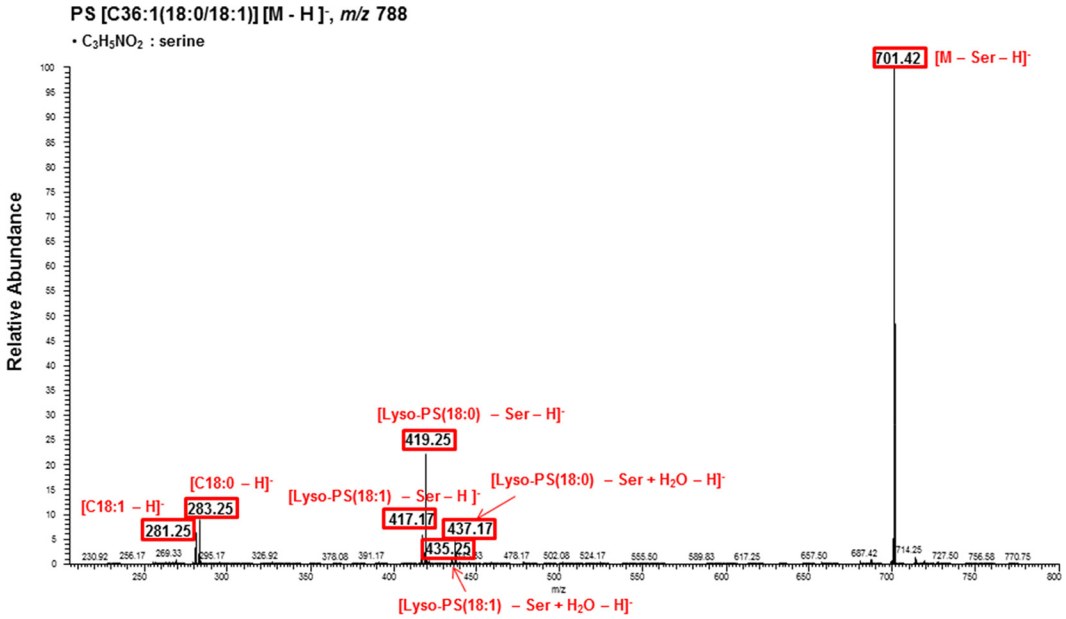

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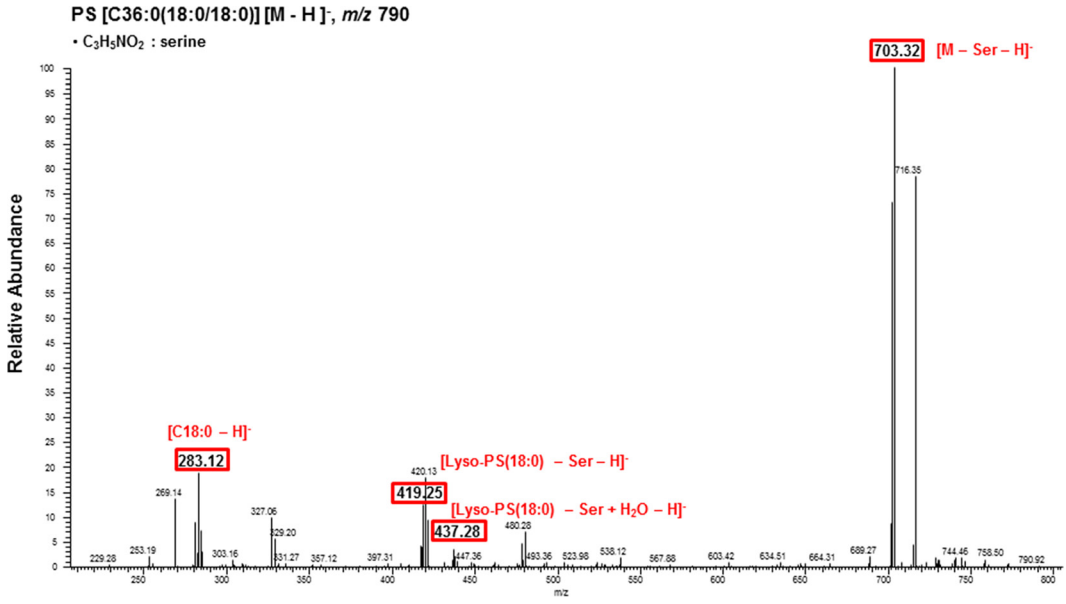

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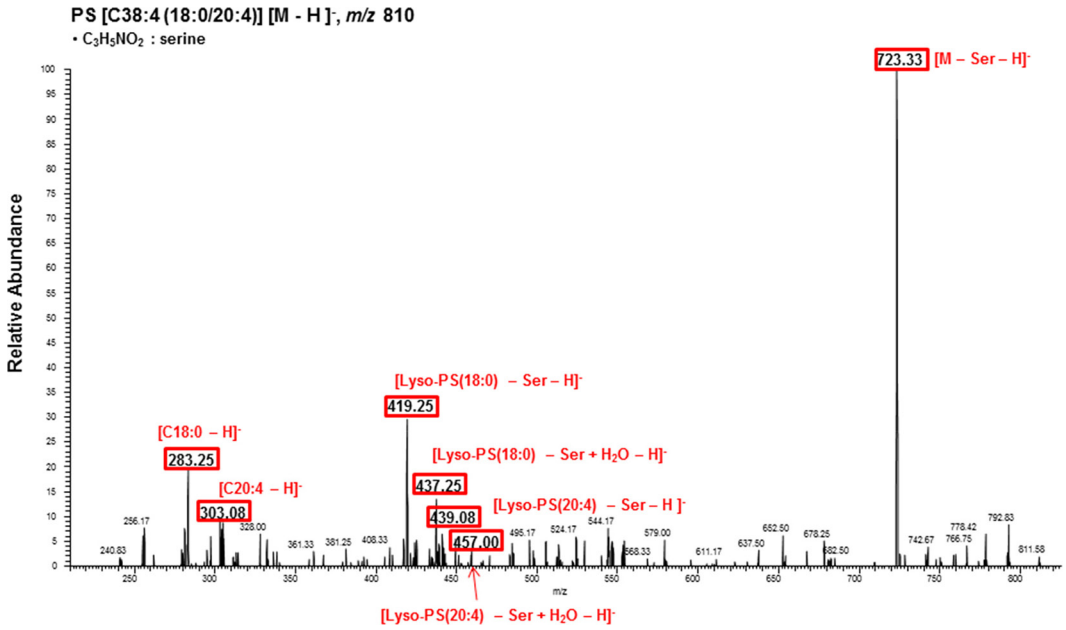

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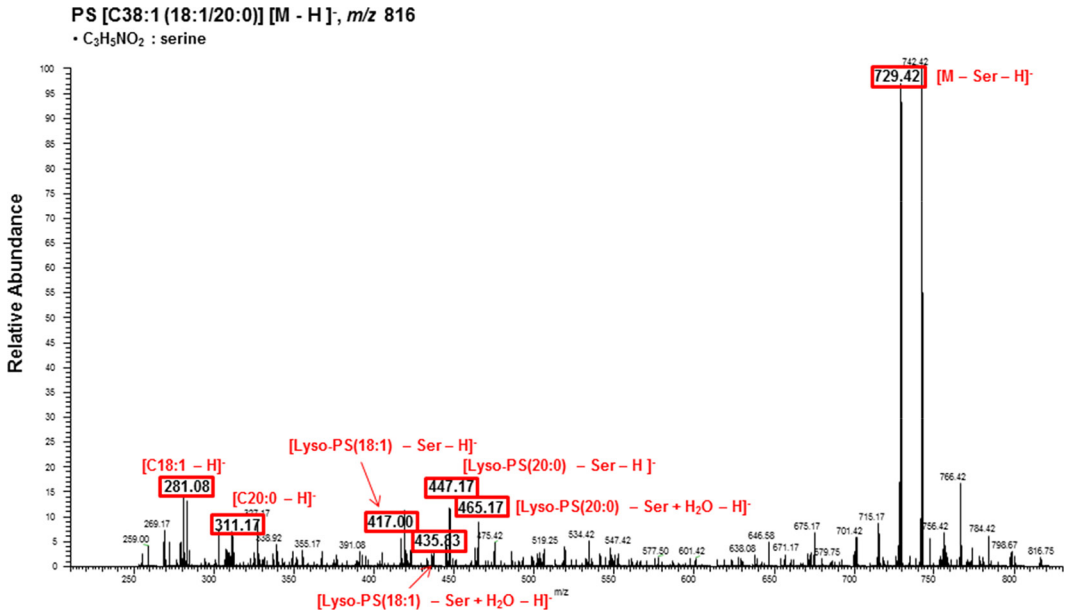

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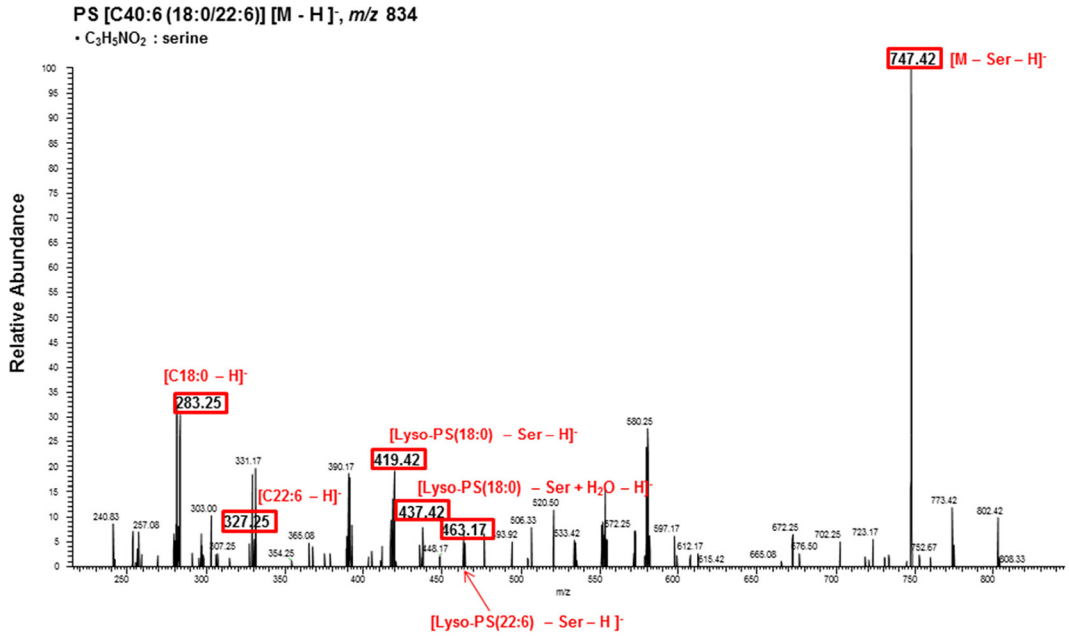

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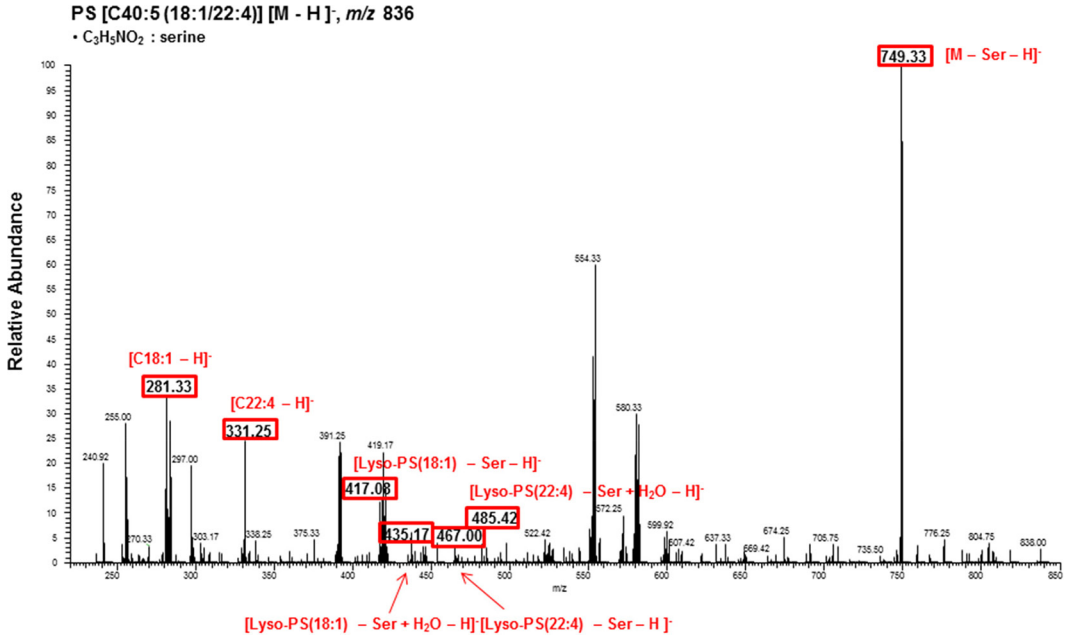

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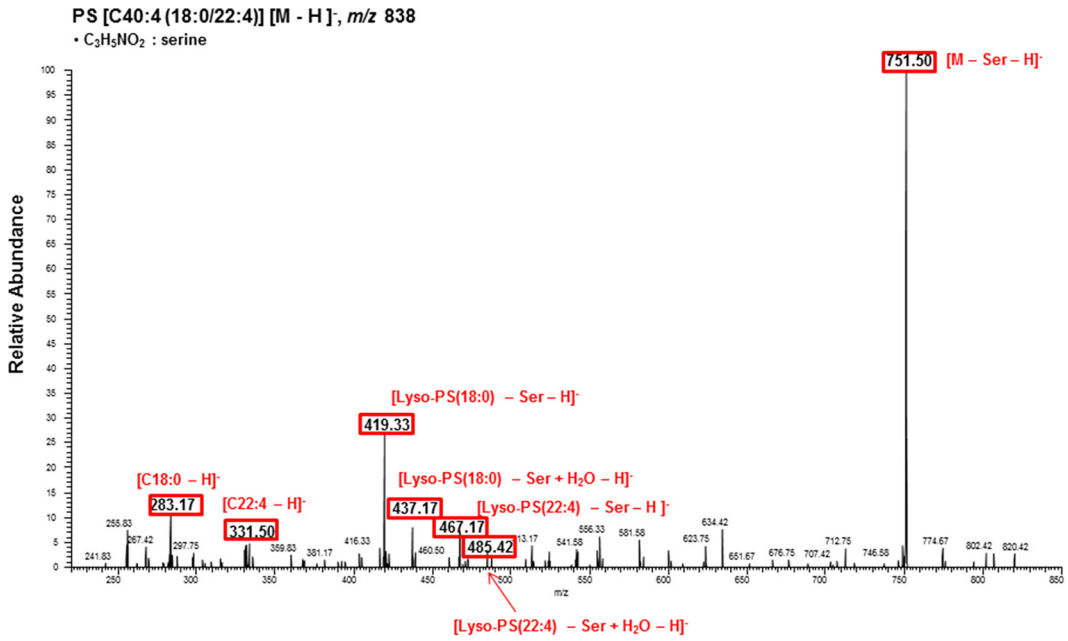

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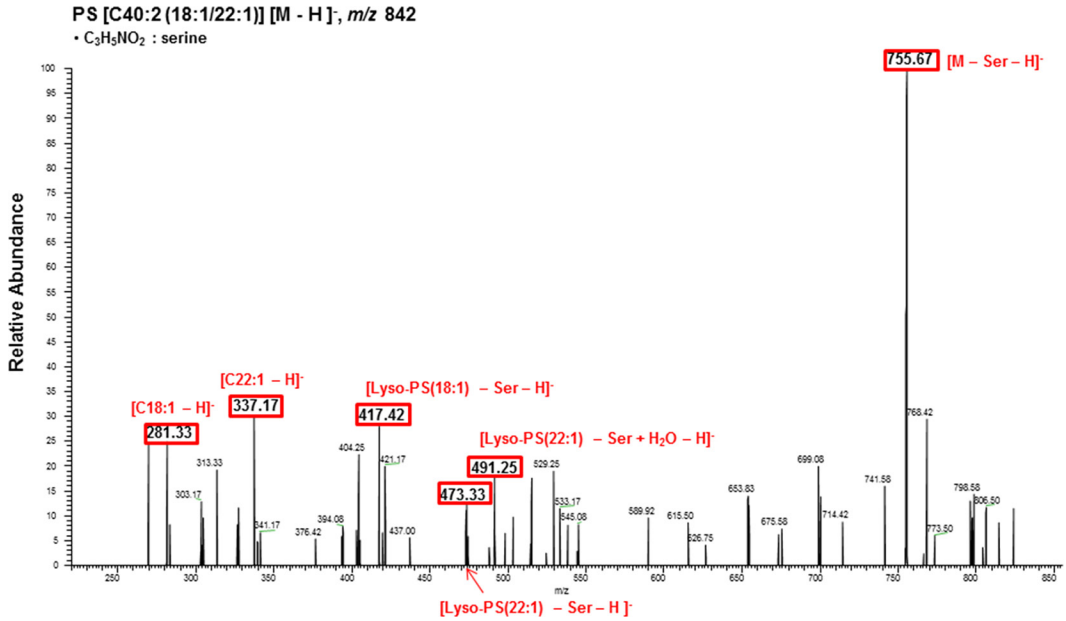

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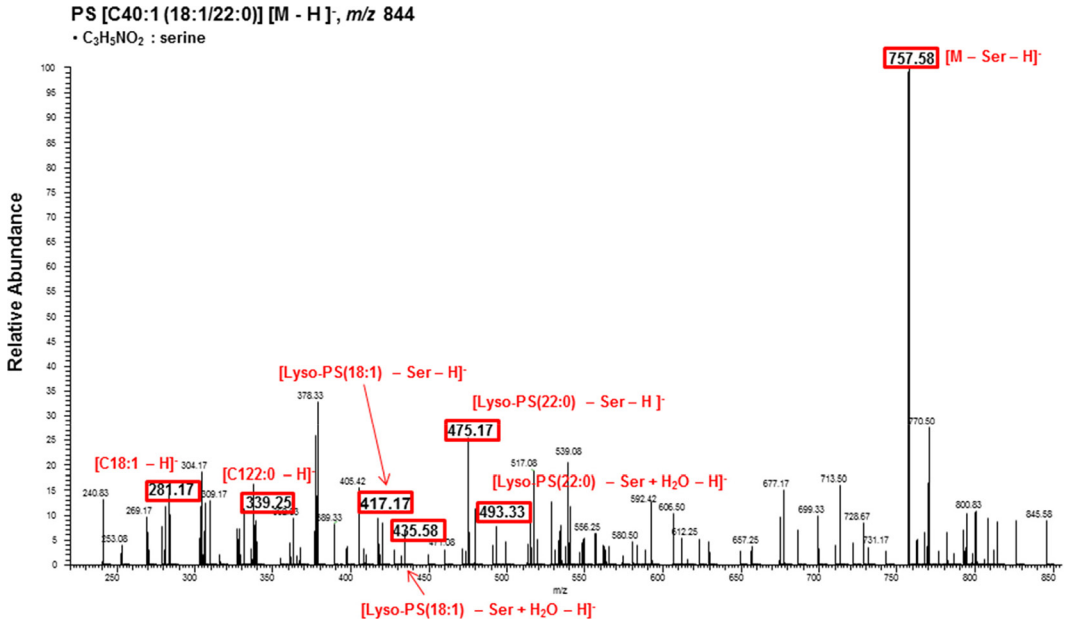

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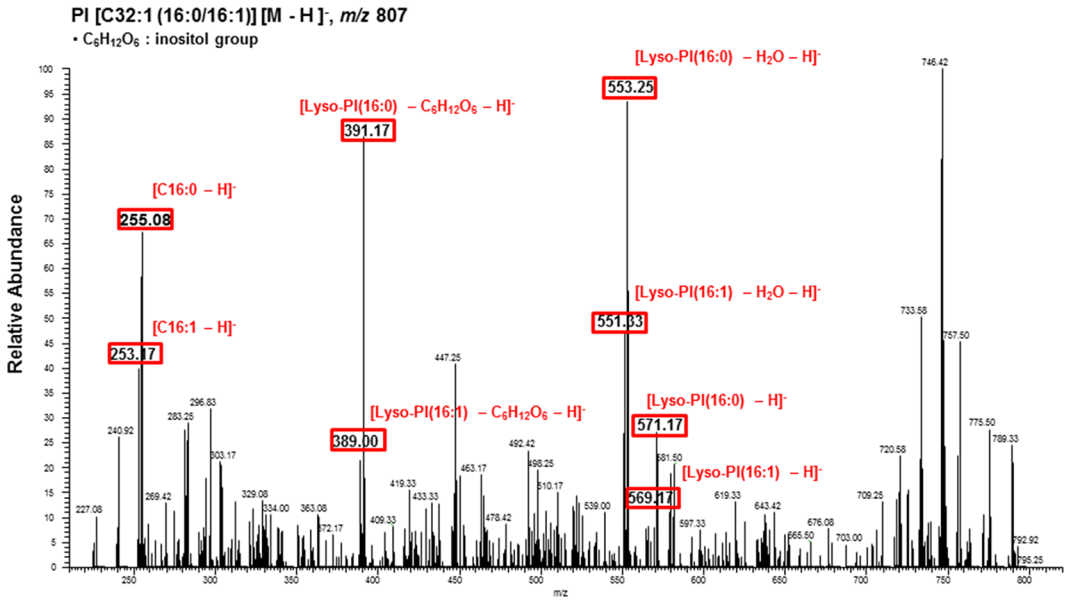

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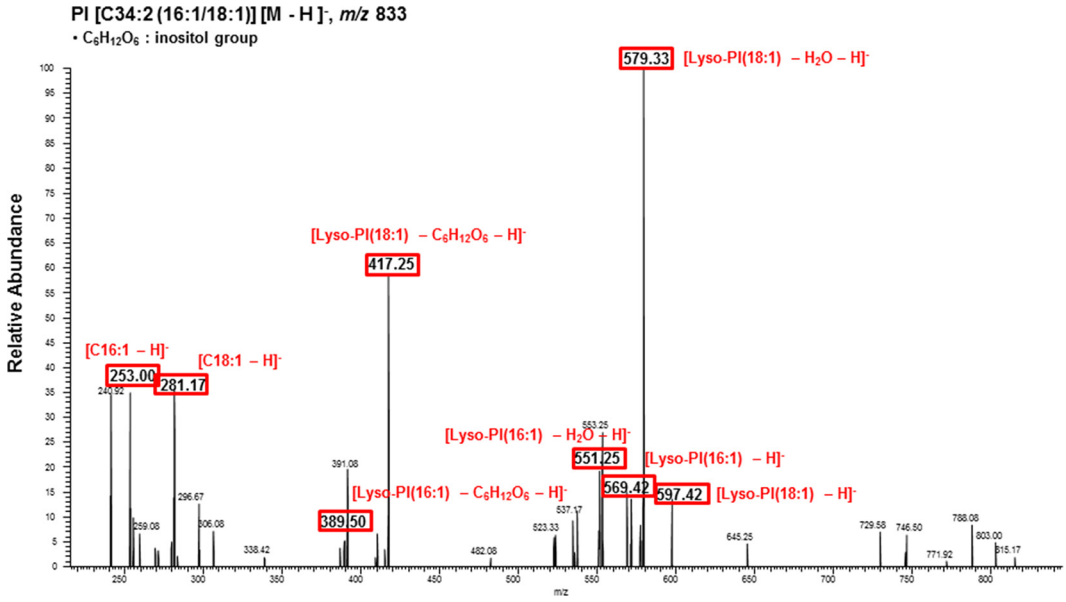

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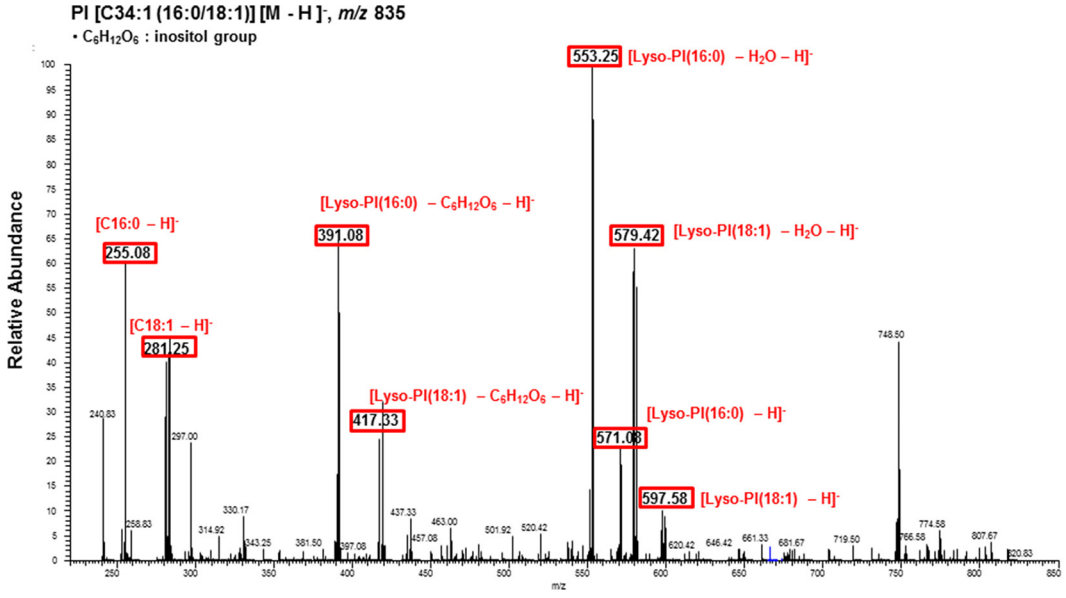

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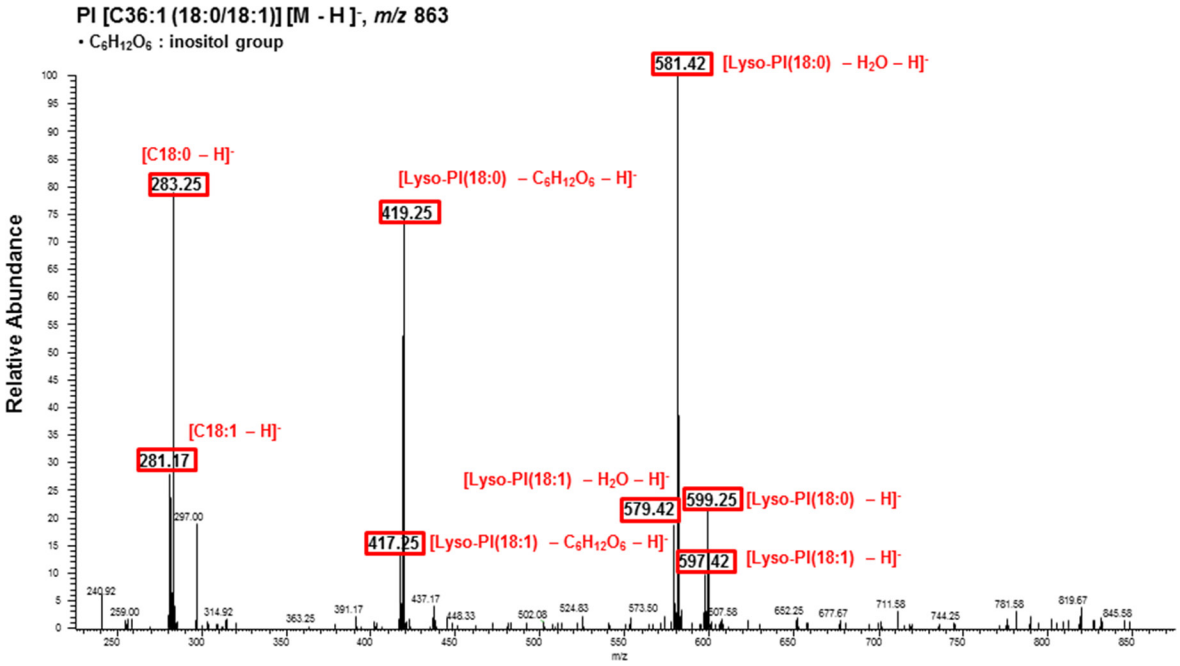

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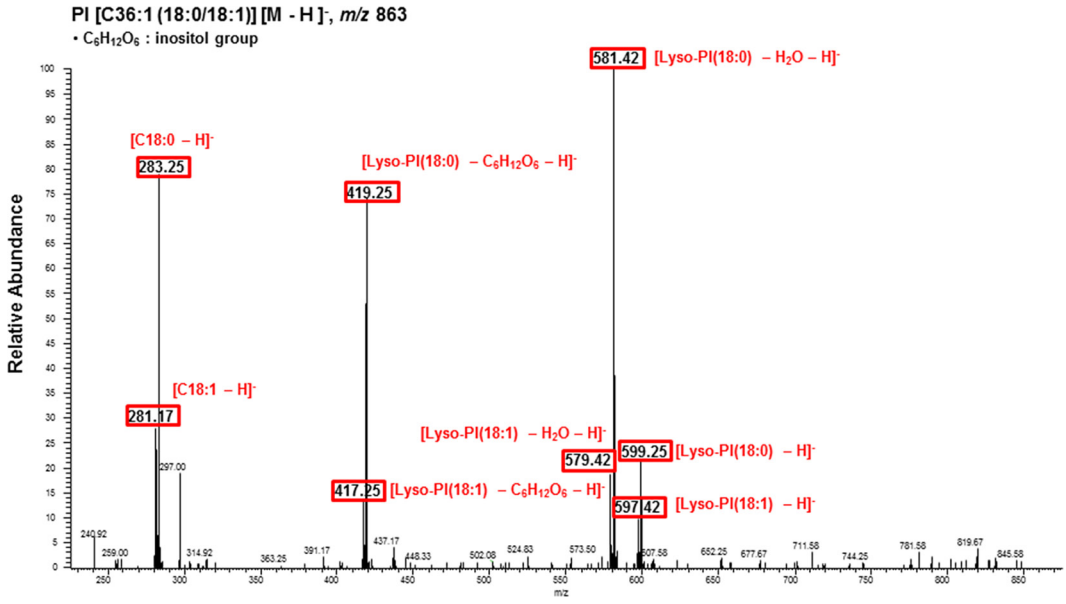

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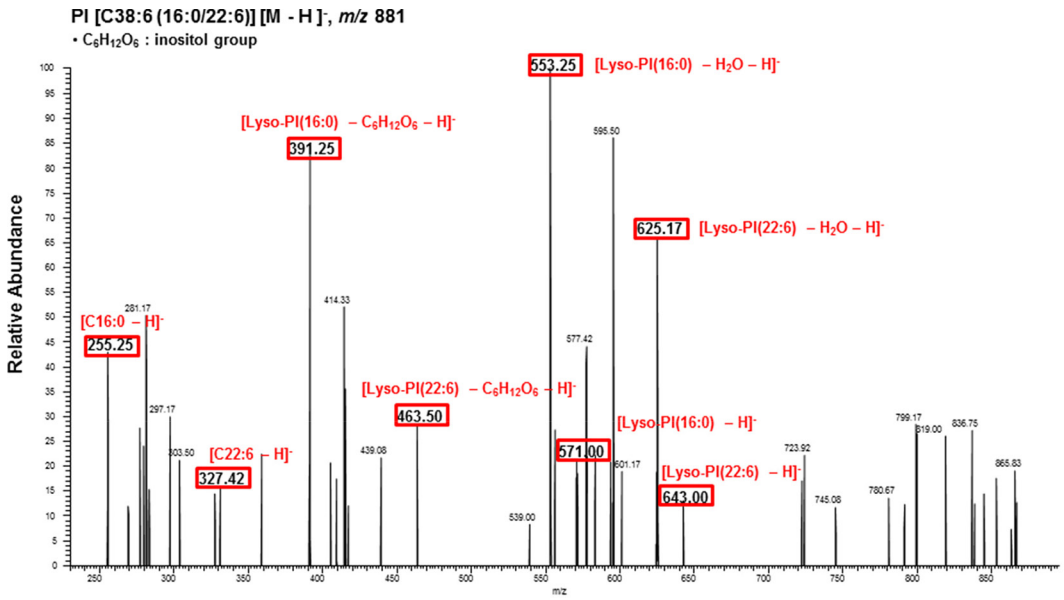

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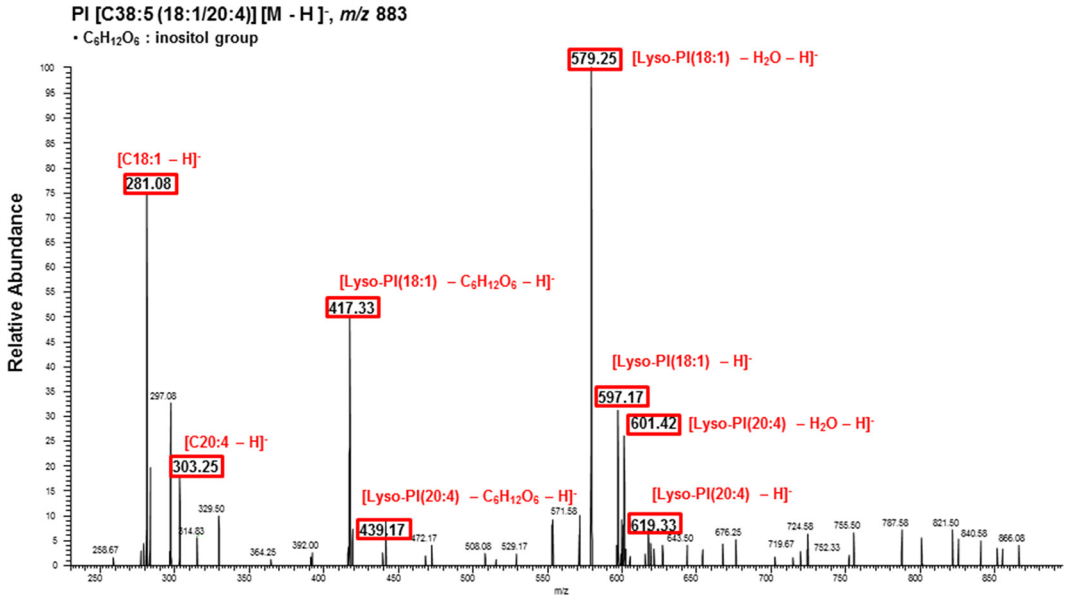

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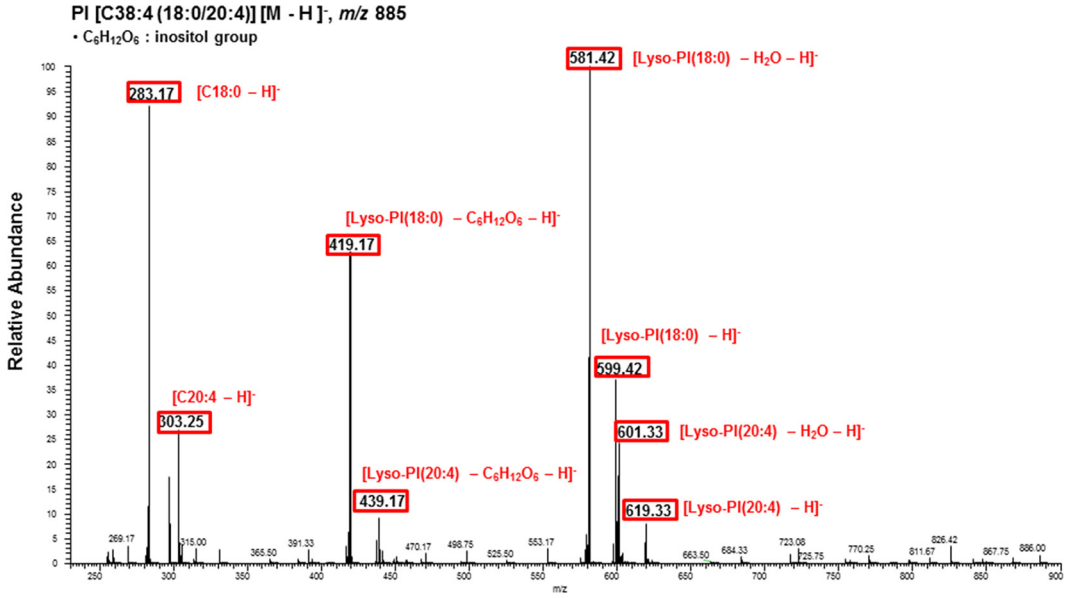

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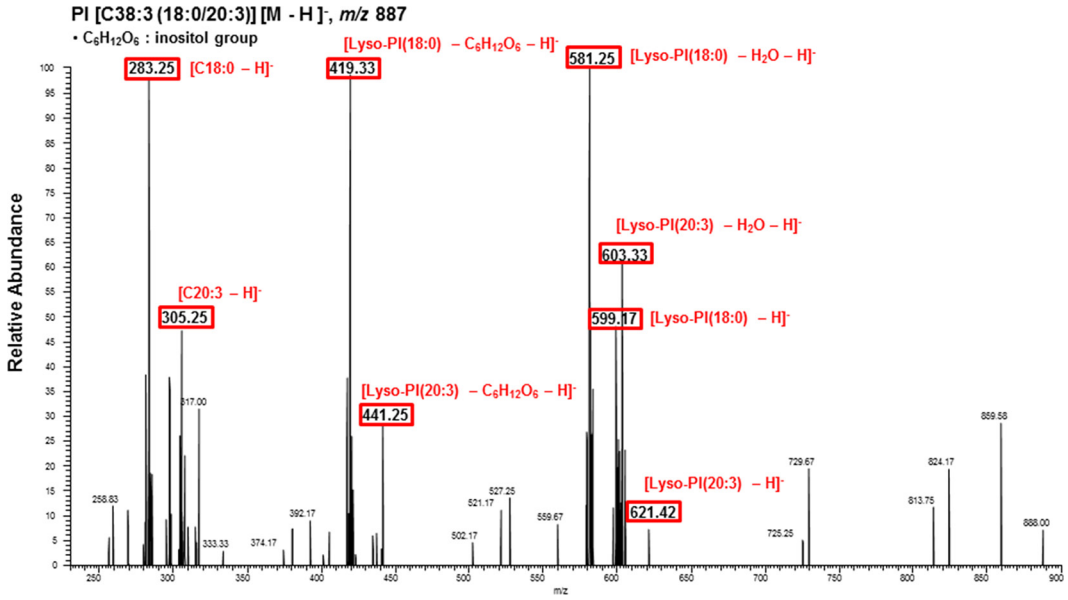

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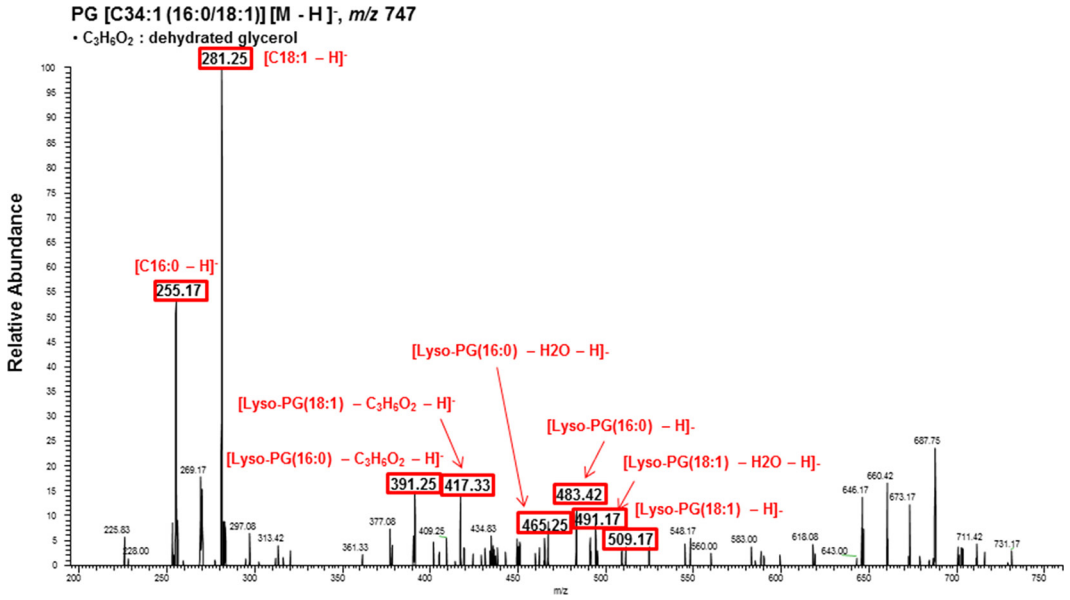

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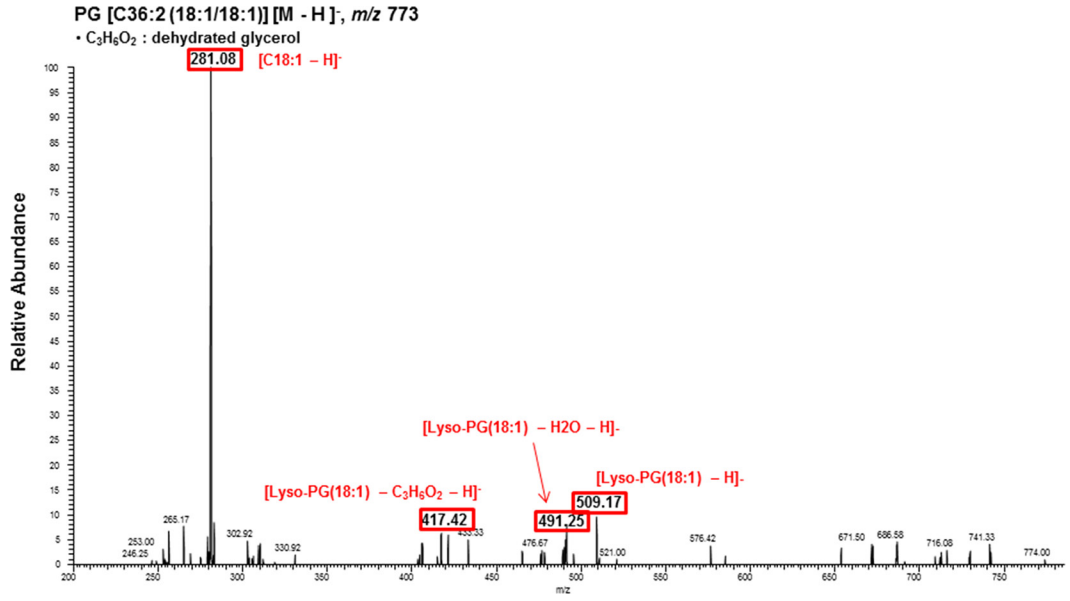

Supplementary Figure S2: MS/MS spectra of the assigned 44 intact lipids in mammary epithelial and breast cancer cells using LTQ-XL mass spectrometry. All lipids were identified by matching against the in-house MS/MS library, databases of Lipidmaps and LipidBlast, based on their major structural features, and the major precursor and fragment ions were indicated as red characters. Each compound corresponding to compound # was listed in Supplementary Table S2.

Supplementary Table S1: A GC-MS-based metabolic profile of breast cancer cells in different progression stages

No.	Compound	RT	MCF-10A	MCF-7	MDA-MB-231
	Alcohol				
1	Erythritol	19.03	3.94 ± 0.75[a]	14.55 ± 7.03[b]	1.50 ± 0.27[a]
		19.05			
2	Myo-inositol	28.95	130.11 ± 41.21[a]	62.12 ± 28.52[a]	242.86 ± 87.10[b]
	Amino acid				
3	Alanine	7.00	110.92 ± 49.66[ab]	169.45 ± 56.46[a]	74.36 ± 16.61[b]
4	Asparagine	20.85	9.08 ± 2.84[a]	15.92 ± 5.47[b]	6.15 ± 1.27[a]
5	Aspartic acid	15.06	119.17 ± 34.00[a]	85.77 ± 42.08[ab]	60.90 ± 13.09[b]
		17.41			
		17.43			
6	Glutamine	19.78	343.20 ± 46.94[a]	1401.87 ± 770.11[b]	355.54 ± 45.65[a]
		19.79			
		19.81			
		23.11			
7	Glycine	7.47	221.07 ± 47.07	315.14 ± 99.45	362.64 ± 138.97
		12.02			
		12.03			
8	Leucine	8.30	54.91 ± 7.33	101.75 ± 42.91	68.48 ± 24.12
		11.72			
9	Lysine	26.08	89.80 ± 18.56	150.53 ± 65.83	91.28 ± 26.41
10	Ornithine	24.00	5.09 ± 1.33[a]	6.96 ± 2.16[a]	17.80 ± 3.10[b]
11	Proline	11.81	38.33 ± 11.90[a]	112.39 ± 50.66[b]	25.09 ± 8.62[a]
12	Serine	10.81	43.61 ± 12.39[a]	191.98 ± 104.09[b]	38.99 ± 12.56[a]
		13.45			
13	Threonine	11.74	39.35 ± 9.52[a]	258.60 ± 80.39[b]	33.90 ± 9.90[a]
		11.75			
		14.08			
		14.09			
14	Tryptophan	30.92	25.12 ± 8.06[ab]	42.38 ± 15.92[a]	18.97 ± 5.16[b]
15	Tyrosine	26.37	137.08 ± 34.37[a]	389.82 ± 193.48[b]	101.24 ± 15.73[a]
16	Valine	6.71	98.82 ± 30.15[a]	323.69 ± 105.30[b]	78.92 ± 26.64[a]
		9.74			
		9.76			

(*Continued*)

No.	Compound	RT	MCF-10A	MCF-7	MDA-MB-231
	Fatty acid				
17	3-hydroxybutanoic acid	8.05	26.96 ± 6.97^{a}	22.64 ± 11.89^{a}	49.06 ± 12.80^{b}
18	Oleic acid	31.08	1.41 ± 0.71	1.68 ± 1.33	2.44 ± 0.60
19	Palmitic acid	28.36	13.73 ± 2.11^{ab}	12.54 ± 4.31^{a}	20.13 ± 7.57^{b}
20	Stearic acid	31.49	7.84 ± 3.60	8.26 ± 2.33	11.92 ± 3.58
	Organic acid				
21	Isocitric acid	24.07	2.14 ± 0.76^{a}	12.26 ± 4.53^{b}	8.64 ± 3.01^{b}
22	Lactic acid	6.04	32.16 ± 7.87^{a}	199.70 ± 85.43^{b}	59.49 ± 23.99^{a}
23	Malic acid	16.67	7.74 ± 2.46^{a}	14.68 ± 4.87^{b}	5.10 ± 1.28^{a}
24	Oxalic acid	7.90	248.51 ± 94.00^{a}	388.76 ± 128.54^{ab}	509.14 ± 194.63^{b}
25	Pyruvic acid	7.63	11.16 ± 7.74	11.97 ± 4.66	15.44 ± 3.16
	Purine				
26	Adenine	24.95	6.71 ± 1.03^{a}	8.28 ± 1.48^{a}	109.12 ± 13.81^{b}
27	Guanine	29.59	0.37 ± 0.06^{a}	116.02 ± 58.59^{b}	137.75 ± 18.65^{b}
28	Hypoxanthine	23.73	244.10 ± 92.38^{a}	167.28 ± 97.68^{ab}	84.17 ± 9.68^{b}
29	Inosine	36.11	11.30 ± 1.94^{a}	22.60 ± 11.00^{b}	2.34 ± 1.21^{a}
30	Uracil	12.86	22.51 ± 3.40^{a}	2.05 ± 0.76^{b}	29.13 ± 6.33^{a}
31	Xanthine	27.80	20.49 ± 6.32^{a}	0.23 ± 0.35^{b}	2.71 ± 0.74^{b}
	Sugar				
32	6-phosphogluconic acid	33.93	4.58 ± 1.35	3.18 ± 2.67	4.61 ± 2.15
33	Fructose-6-phosphate	32.31	34.04 ± 13.35^{a}	5.94 ± 4.14^{b}	12.92 ± 3.98^{b}
34	Glucose	27.15	11.88 ± 8.37^{ab}	20.67 ± 12.67^{a}	7.15 ± 1.70^{b}
35	Glucose-6-phosphate	32.32	187.35 ± 36.35^{a}	41.77 ± 23.83^{b}	118.50 ± 30.10^{c}
		32.49			
		32.74			
		33.03			
		33.33			
		33.50			
36	Glyceric acid	12.67	0.94 ± 0.37^{a}	2.43 ± 0.99^{b}	0.92 ± 0.62^{a}
37	Mannose-6-phosphate	35.72	59.41 ± 18.13^{a}	17.48 ± 9.79^{b}	11.95 ± 3.52^{b}
38	Sucrose	36.96	6.20 ± 2.29	5.85 ± 0.90	8.06 ± 3.77
	Other				
39	Creatinine	18.10	3.86 ± 2.02^{a}	14.50 ± 6.90^{b}	3.10 ± 1.48^{a}

Different letters in the same row indicate a significant difference. Mean ± SD value for quintuplicate measurements are shown. The values are multiplied by '10e3' due to significantly small values

Supplementary Table S2: Normalized ion intensities of identified lipids in mammary epithelial cells (MCF-10A) and metastatic breast cancer cells (MCF-7 and MDA-MB-231) using nano-electrospray mass spectrometry

No.	Lipid molecular species (total acyl carbons:total double bonds)	Proposed composition	Ion species	m/z	MCF-10A	MCF-7	MDA-MB-231
Positive ion mode							
Phosphatidylcholine (PC)							
1	PC 34:2	C16:1/C18:1	$[M + H]^+$	758	1.77 ± 0.26^a	3.52 ± 0.39^b	4.37 ± 1.07^b
2	PC 36:3	C18:1/C18:2	$[M + H]^+$	784	2.05 ± 0.42^a	4.93 ± 0.58^{ab}	8.42 ± 3.58^b
3	PC 36:2	C18:1/C18:1	$[M + H]^+$	786	9.72 ± 2.33^a	24.65 ± 2.90^{ab}	39.53 ± 15.19^b
4	PC 37:3	C18:3/C19:0	$[M + H]^+$	798	7.45 ± 5.28a	10.67 ± 3.46a	8.63 ± 0.97a
5	PC 38:5	C18:2/C20:3	$[M + H]^+$	808	0.37 ± 0.12^a	1.19 ± 0.32^b	1.68 ± 0.75^b
6	PC 38:4	C18:0/C20:4	$[M + H]^+$	810	0.39 ± 0.05^a	0.63 ± 0.12^a	1.76 ± 0.27^b
7	PC 38:2	C18:1/C20:1	$[M + H]^+$	814	1.36 ± 0.22^a	2.54 ± 0.29^b	1.39 ± 0.23^a
8	PC 38:1	C18:1/C20:0	$[M + H]^+$	816	2.88 ± 0.49^a	4.52 ± 0.60^b	5.27 ± 0.83^b
9	PC 38:0	C18:0/C20:0	$[M + H]^+$	818	3.39 ± 1.63^a	5.11 ± 2.16^{ab}	7.73 ± 2.77^b
10	PC 40:6	C18:1/C22:5	$[M + H]^+$	834	1.33 ± 0.17^a	2.10 ± 0.19^b	2.03 ± 0.21^b
Plasmenylphosphatidylcholine (plasmenyl-PC)							
11	Plasmenyl-PC 38:1	C20:0/C18:1	$[M + H]^+$	800	3.87 ± 1.43^a	7.46 ± 0.64^b	3.67 ± 2.00^a
Plasmenylphosphatidylethanolamine (plasmenyl-PE)							
12	plasmenyl-PE 34:0	C18:0/C16:0	$[M + H]^+$	704	1.33 ± 0.49^a	1.98 ± 0.64^a	2.02 ± 0.32^a
Sphingomyelin (SM)							
13	SM 34:1	D18:1/16:0	$[M]^+$	703	2.07 ± 0.94^a	2.26 ± 0.92^a	3.10 ± 0.76^a
Negative ion mode							
Phosphatidylethanolamine (PE)							
14	PE 36:2	C18:1/C18:1	$[M - H]^-$	742	10.01 ± 0.69^a	16.17 ± 0.67^b	4.68 ± 0.30^c
15	PE 36:0	C17:0/C19:0	$[M - H]^-$	746	2.79 ± 0.57^a	7.30 ± 0.37^b	4.35 ± 1.37^c
16	PE 38:5	C18:1/C20:4	$[M - H]^-$	764	3.31 ± 0.27^a	8.87 ± 0.17^b	4.09 ± 0.62^c
17	PE 40:5	C18:1/C22:4	$[M - H]^-$	792	4.86 ± 0.36^a	8.11 ± 0.47^b	7.51 ± 1.27^b
Plasmenylphosphatidylethanolamine (plasmenyl-PE)							
18	plasmenyl-PE 38:4	C18:0/C20:4	$[M - H]^-$	750	4.55 ± 0.28^a	1.65 ± 0.18^b	13.03 ± 1.02^c
Phosphatidylserine (PS)							
19	PS 34:2	C16:1/C18:1	$[M - H]^-$	758	7.56 ± 0.72^a	15.01 ± 1.36^b	25.11 ± 4.40^c
20	PS 34:1	C16:0/C18:1	$[M - H]^-$	760	33.19 ± 4.34^a	48.96 ± 3.55^b	80.84 ± 12.65^c

(*Continued*)

No.	Lipid molecular species (total acyl carbons:total double bonds)	Proposed composition	Ion species	m/z	MCF-10A	MCF-7	MDA-MB-231
21	PS 34:0	C16:0/C18:0	$[M-H]^-$	762	9.76 ± 0.96^a	11.87 ± 0.82^a	20.15 ± 2.89^b
22	PS 35:1	C17:0/C18:1	$[M-H]^-$	774	5.22 ± 0.56^a	12.82 ± 1.34^b	6.31 ± 0.69^a
23	PS 36:3	C18:1/C18:2	$[M-H]^-$	784	10.54 ± 1.10^a	6.36 ± 0.63^a	34.69 ± 7.56^b
24	PS 36:2	C18:0/C18:2	$[M-H]^-$	786	36.71 ± 2.26^a	36.22 ± 3.41^a	77.96 ± 12.92^b
25	PS 36:1	C18:0/C18:1	$[M-H]^-$	788	51.25 ± 3.70^a	44.11 ± 2.35^a	104.38 ± 12.65^b
26	PS 36:0	C18:0/C18:0	$[M-H]^-$	790	11.46 ± 0.72^a	23.03 ± 0.96^b	24.21 ± 1.86^b
27	PS 38:4	C18:0/C20:4	$[M-H]^-$	810	2.70 ± 0.50^a	2.88 ± 0.18^a	11.97 ± 1.30^b
28	PS 38:1	C18:1/C20:0	$[M-H]^-$	816	7.29 ± 0.42^a	11.43 ± 0.52^b	10.23 ± 0.88^c
29	PS 40:6	C18:0/C22:6	$[M-H]^-$	834	2.91 ± 0.33^a	5.63 ± 0.55^b	12.13 ± 1.18^c
30	PS 40:5	C18:1/C22:4	$[M-H]^-$	836	5.35 ± 0.60^a	13.14 ± 0.75^b	12.88 ± 1.32^b
31	PS 40:4	C18:0/C22:4	$[M-H]^-$	838	2.82 ± 0.32^a	3.01 ± 0.26^a	10.62 ± 1.61^b
32	PS 40:2	C18:1/C22:1	$[M-H]^-$	842	5.38 ± 0.26^a	3.46 ± 0.06^b	5.76 ± 0.52^a
33	PS 40:1	C18:1/C22:0	$[M-H]^-$	844	9.54 ± 0.58^a	15.09 ± 0.72^b	13.10 ± 1.15^c
Phosphatidylinositol (PI)							
34	PI 32:1	C16:0/C16:1	$[M-H]^-$	807	2.22 ± 0.15^a	7.17 ± 0.29^a	37.76 ± 8.27^b
35	PI 34:2	C16:1/C18:1	$[M-H]^-$	833	5.50 ± 0.44^a	6.28 ± 0.43^a	11.56 ± 1.42^b
36	PI 34:1	C16:0/C18:1	$[M-H]^-$	835	9.49 ± 0.76^a	23.30 ± 1.44^b	13.32 ± 2.38^c
37	PI 36:2	C18:1/C18:1	$[M-H]^-$	861	27.51 ± 1.95^a	18.17 ± 1.25^b	6.75 ± 0.60^c
38	PI 36:1	C18:0/C18:1	$[M-H]^-$	863	21.06 ± 1.47^a	35.18 ± 2.23^b	3.39 ± 0.41^c
39	PI 38:6	C16:0/C22:6	$[M-H]^-$	881	2.02 ± 1.11^a	3.19 ± 0.36^a	7.73 ± 3.08^b
40	PI 38:5	C18:1/C20:4	$[M-H]^-$	883	1.63 ± 0.27^a	2.04 ± 0.19^a	6.89 ± 1.28^b
41	PI 38:4	C18:0/C20:4	$[M-H]^-$	885	10.73 ± 0.86^a	7.42 ± 0.57^a	62.18 ± 7.40^b
42	PI 38:3	C18:0/C20:3	$[M-H]^-$	887	11.90 ± 0.82^a	5.74 ± 0.49^b	13.90 ± 1.61^c
Phosphatidylglycerol (PG)							
43	PG 34:1	C16:0/C18:1	$[M-H]^-$	747	4.99 ± 0.61^a	8.17 ± 0.61^b	3.61 ± 0.95^c
44	PG 36:2	C18:1/C18:1	$[M-H]^-$	773	4.40 ± 0.61^a	8.90 ± 1.34^b	0.96 ± 0.27^c

[a] Each value represents the mean ± standard deviation (SD) (n = 5).

[b] For simplicity, '×10e1' and '×10e2' were omitted from the values of identified lipids in positive mode and negative mode, respectively.