Electronic Supplementary Material (ESI) for Analyst

Supporting Information

Simple enrichment and analysis of plasma lysophosphatidic acids

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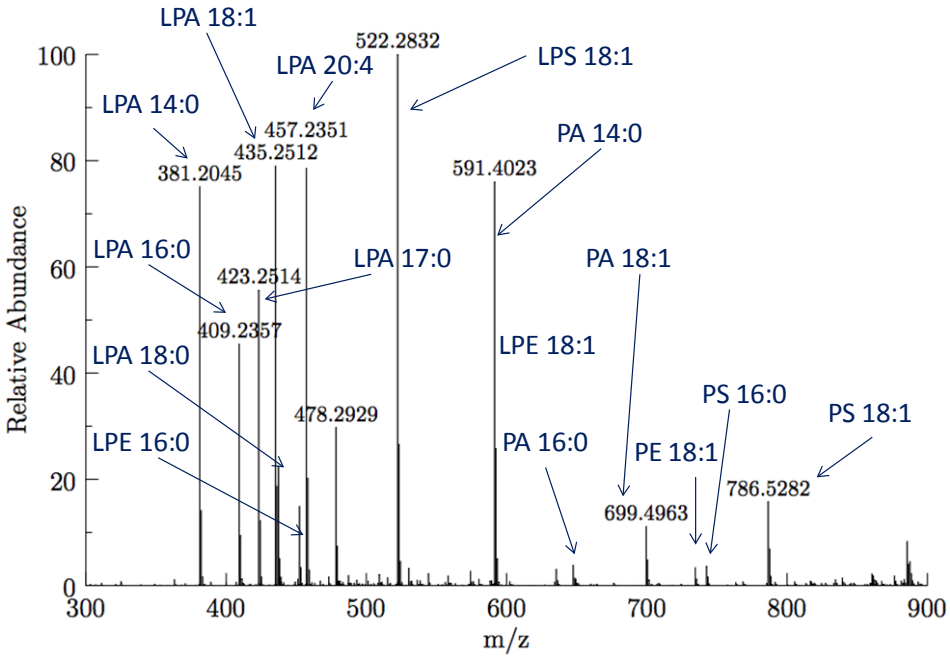

Fig. S1 Mass spectra of phospholipids mixture in negative mode.

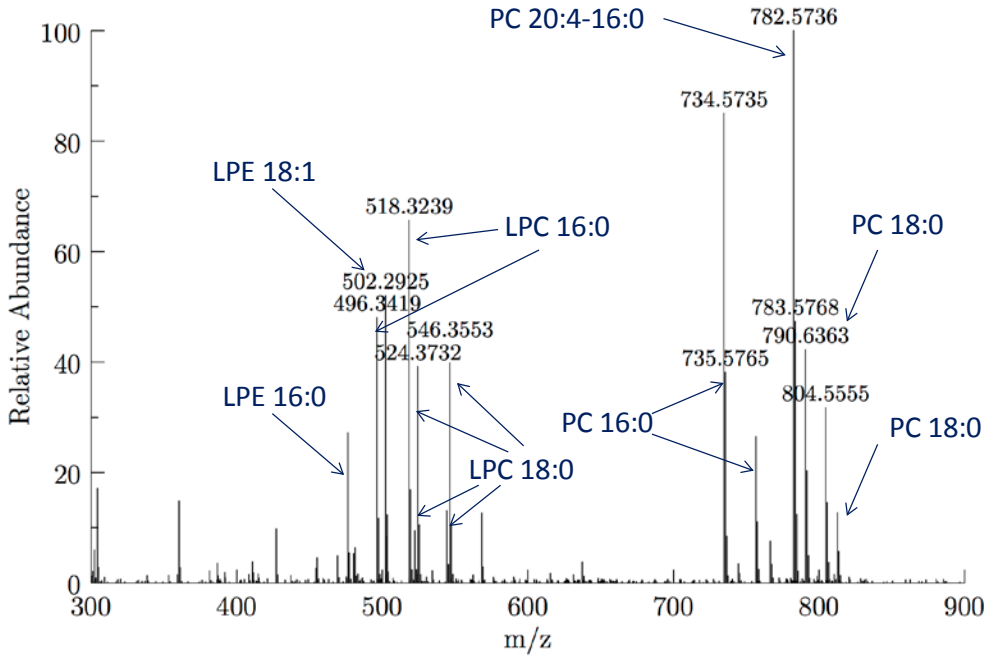

Fig. S2 Mass spectra of phospholipids mixture in positive mode.

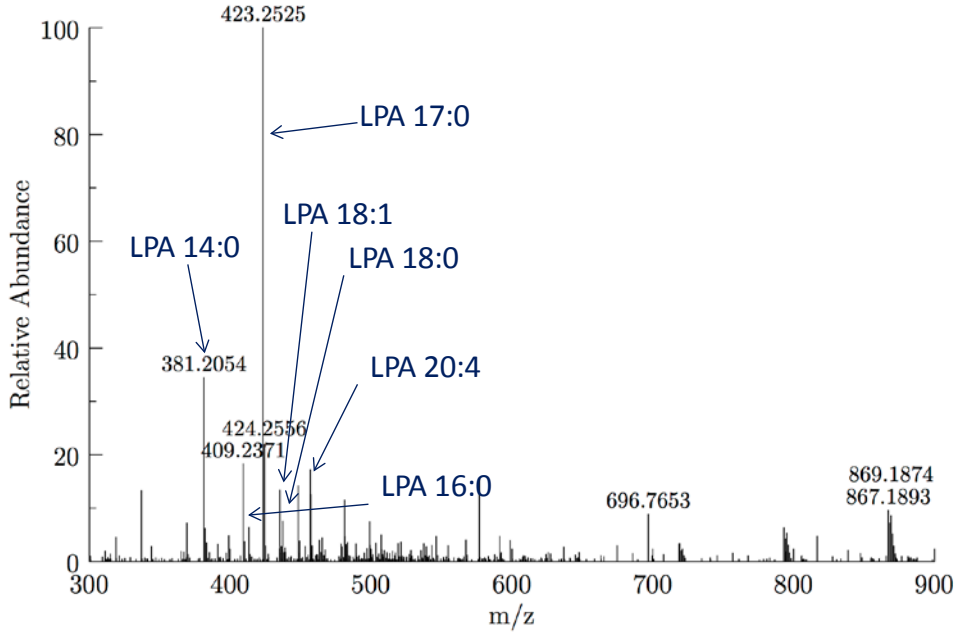

Fig. S3 Mass spectra of plasma extract in negative mode.

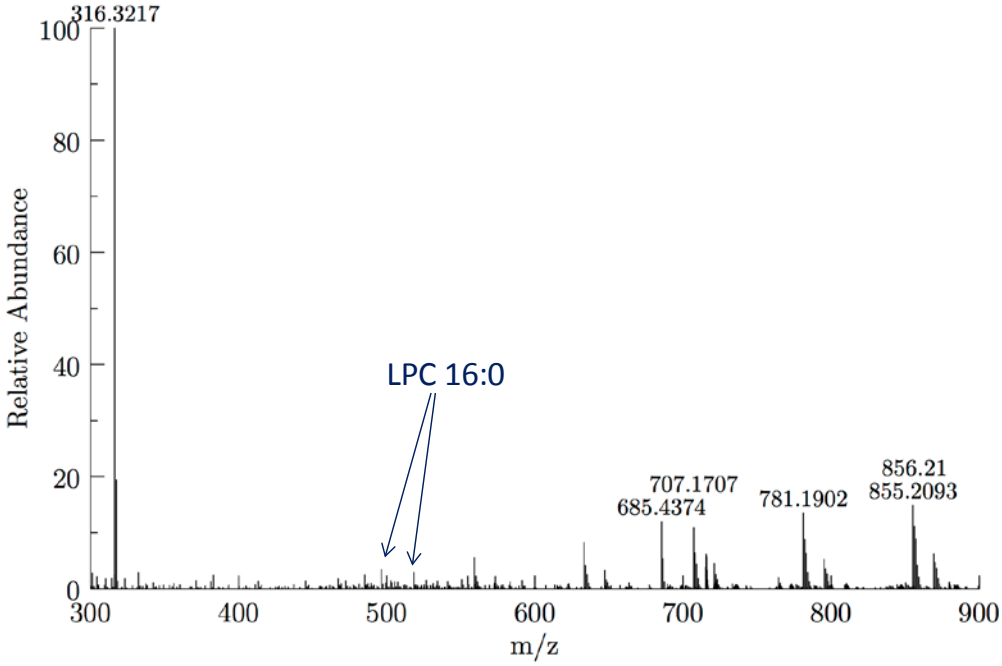

Fig. S4 Mass spectra of plasma extract in positive mode.

Table S1. Effect of column length on the resolution and theoretical plates for the separation of LPAs.[a]

	LPA 14:0		LPA 20:4		LPA 16:0		LPA 18:1		LPA 17:0		LPA 18:0
column length (mm)	*N* (*RT*, min)	R_s	*N* (*RT*, min)	R_s	*N* (*RT*, min)	R_s	*N* (*RT*, min)	R_s	*N* (*RT*, min)	R_s	*N* (*RT*, min)
100[b]	312 (4.44)	2.31	192 (5.56)	1.35	1715 (6.53)	NA	NA (7.89)	NA	2712 (8.71)	7.74	3001 (11.87)
50[c]	1280 (3.83)	4.40	1486 (4.72)	3.52	1917 (5.44)	4.30	1748 (6.61)	2.57	3416 (7.32)	10.04	4361 (10.50)

[a] *Rs* = resolution, *N* = theoretical plates, *RT* = retention time, NA = not applicable; LPA concentration: 80 μM; mobile phase: 4/1 MeOH/50 mM pH 3.0 phosphate buffer; injection volume: 5 μL.
[b]Discovery Bio wide pore (Supelco), C-8, 3 μm, 2.1 mm diameter. Flow rate: 0.20 mL/min.
[c]Luna (Phenomenex), C-8, 3 μm, 2.0 mm diameter. Flow rate: 0.20 mL/min.

Table S2. Effect of pH on the resolution and theoretical plates for the separation of LPAs.[a]

	LPA 14:0		LPA 20:4		LPA 16:0		LPA 18:1		LPA 17:0		LPA 18:0
pH	*N* (*RT*, min)	R_s	*N* (*RT*, min)	R_s	*N* (*RT*, min)	R_s	*N* (*RT*, min)	R_s	*N* (*RT*, min)	R_s	*N* (*RT*, min)
3.0	996 (3.01)	7.93	1302 (4.69)	3.14	1040 (5.59)	4.22	1860 (6.92)	1.65	397 (7.78)	5.66	2176 (11.12)
2.5	1331 (3.16)	9.71	2000 (4.85)	3.41	1862 (5.61)	4.82	1625 (6.99)	2.56	1630 (7.88)	7.57	1828 (11.21)

[a]*Rs* = resolution, *N* = theoretical plates, *RT* = retention time; LPA concentration is 5 μM; column: Luna C-8 50 × 2.0 mm; mobile phase: 7/2 MeOH/50 mM phosphate buffer; flow rate: 0.27 mL/min. Injection volume: 20 μL.

Table S3. Resolution and theoretical plates for the final optimal conditions.[a]

LPA 14:0		LPA 20:4		LPA 16:0		LPA 18:1		LPA 17:0		LPA 18:0
N (*RT*, min)	R_s	*N* (*RT*, min)	R_s	*N* (*RT*, min)	R_s	*N* (*RT*, min)	R_s	*N* (*RT*, min)	R_s	*N* (*RT*, min)
848 (3.96)	6.25	1149 (5.76)	3.75	2531 (6.84)	5.71	2105 (8.58)	2.65	1278 (9.72)	8.28	2635 (13.96)

[a] Column: Luna C-8 50 × 2.0 mm; LPA concentration is 10 μM; mobile phase: 16/5 MeOH/50 mM phosphate buffer; flow rate: 0.32 mL/min. Injection volume: 20 μL.

Table S4 Results for LPA analysis in human plasma (donor B) using the HPLC post-column fluorescence and LC/ESI/MS/MS methods (n = 3).

	non-spiked μM (average, σ)		spiked with 0.5 μM LPA μM (average, σ)		Recovery (%)	
	HPLC Post-Column	LC/ESI MS/MS	HPLC Post-Column	LC/ESI MS/MS	HPLC Post-Column	LC/ESI MS/MS
LPA 14:0	0.97(0.03)	1.03(0.01)	1.43(0.04)	1.45(0.03)	94	82
LPA 20:4	0.98(0.01)	0.94(0.01)	1.41(0.02)	1.43(0.01)	86	100
LPA 16:0	0.96(0.02)	1.04(0.02)	1.45(0.03)	1.60(0.03)	98	112
LPA 18:1	1.05(0.00)	1.03(0.02)	1.47(0.02)	1.55(0.02)	84	102
LPA 18:0	0.99(0.01)	0.93(0.01)	1.56(0.04)	1.47(0.01)	114	110
Total LPA	4.96(0.04)	4.97(0.04)	7.33(0.01)	7.50(0.08)	95	101

Table S5 Results for LPA analysis in human plasma (donor C) using the HPLC post-column fluorescence and LC/ESI/MS/MS methods (n = 3).

	non-spiked μM (average, σ)		spiked with 0.5 μM LPA μM (average, σ)		Recovery (%)	
	HPLC Post-Column	LC/ESI MS/MS	HPLC Post-Column	LC/ESI MS/MS	HPLC Post-Column	LC/ESI MS/MS
LPA 14:0	0.76(0.01)	0.68(0.02)	1.25(0.02)	1.21(0.04)	98	106
LPA 20:4	0.21(0.02)	0.27(0.02)	0.64(0.01)	0.67(0.05)	84	80
LPA 16:0	0.55(0.01)	0.42(0.04)	1.05(0.05)	0.97(0.04)	100	112
LPA 18:1	0.37(0.01)	0.32(0.01)	0.96(0.05)	0.79(0.06)	120	96
LPA 18:0	0.29(0.03)	0.23(0.01)	0.79(0.01)	0.79(0.02)	102	112
Total LPA	2.18(0.02)	1.91(0.09)	4.69(0.08)	4.44(0.12)	100	101

Table S6 Results for LPA analysis in human plasma (donor D) using the HPLC post-column fluorescence and LC/ESI/MS/MS methods (n = 3).

	non-spiked μM (average, σ)		spiked with 0.5 μM LPA μM (average, σ)		Recovery (%)	
	HPLC Post-Column	LC/ESI MS/MS	HPLC Post-Column	LC/ESI MS/MS	HPLC Post-Column	LC/ESI MS/MS
LPA 14:0	0.24(0.00)	0.23(0.01)	0.65(0.02)	0.68(0.02)	82	92
LPA 20:4	0.26(0.01)	0.28(0.01)	0.67(0.01)	0.65(0.04)	82	74
LPA 16:0	0.45(0.03)	0.43(0.01)	0.88(0.02)	0.83(0.04)	88	80
LPA 18:1	0.30(0.02)	0.38(0.01)	0.87(0.02)	0.85(0.03)	114	94
LPA 18:0	0.33(0.02)	0.31(0.00)	0.85(0.02)	0.82(0.01)	104	102
Total LPA	1.57(0.03)	1.63(0.03)	3.91(0.03)	3.83(0.12)	94	88

Table S7 Results for LPA analysis in human plasma (donor E) using the HPLC post-column fluorescence and LC/ESI/MS/MS methods (n = 3).

	non-spiked μM (average, σ)		spiked with 0.5 μM LPA μM (average, σ)		Recovery (%)	
	HPLC Post-Column	LC/ESI MS/MS	HPLC Post-Column	LC/ESI MS/MS	HPLC Post-Column	LC/ESI MS/MS
LPA 14:0	0.17(0.00)	0.18(0.01)	0.61(0.02)	0.60(0.00)	86	84
LPA 20:4	0.20(0.02)	0.23(0.01)	0.75(0.01)	0.77(0.01)	110	110
LPA 16:0	0.29(0.00)	0.28(0.02)	0.71(0.00)	0.76(0.01)	84	96
LPA 18:1	0.53(0.01)	0.47(0.02)	0.97(0.02)	1.03(0.00)	90	112
LPA 18:0	0.33(0.00)	0.30(0.01)	0.84(0.01)	0.89(0.00)	102	118
Total LPA	1.52(0.02)	1.45(0.05)	3.88(0.04)	4.06(0.01)	94	104