Electronic Supplementary Information for

Selective Detection of Phospholipids from Human Blood Plasma and Single Cells for Cancer Differentiation using Dispersed Solid-Phase Microextraction Combined with Extractive Electrospray Ionization Mass Spectrometry

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Table S5: Analytical preference of d-SPME-iEESI-MS/MS

References

Materials and Chemicals

Biofluid samples involved in this study include human blood plasma and cancer cells. Human blood plasma samples from ovarian carcinoma patients, pancreatic cancer patients, and health volunteers were provided by The First Hospital of Jilin University (Changchun, China), with full consent from all the volunteers. A total of 102 human blood plasma samples containing 59 plasma samples of ovarian cancer and 43 samples from health volunteers as control group, and a total of 58 human blood plasma samples containing 28 samples from pancreatic cancer patients and 23 samples from health volunteers were used in this study. Cell lines (MV4-11 and NB4) were purchased from American Type Culture Collection, and were cultured in RPMI 1640 containing 10% fetal bovine serum (FBS) (Life Technology). Note that the experiments of human blood plasma and cancer cells were adhered to the tenets of Helsinki Declaration,[1] and approved by the Ethics Committee of the Jilin University and The First Hospital of Jilin University.

Both Methanol and isopropyl alcohol are HPLC grade and purchased from Merck KGaA (Darmstadt, Germany), and ammonium hydroxide solution (w/w, 20%) was purchased from CNW Technologies GmbH (Düsseldorf, Germany). Trifluoroacetic acid (TFA) (HPLC grade) was brought from Fisher Scientific (Waltham, MA, USA). Titanium butoxide (99%), ethylene glycol (EG) (99%), ethanol (99.99%), ethylene diamine (ED) (99.99%), ferric trichloride hexahydrate ($FeCl_3 \cdot 6H_2O$) (99%), and sodium acetate (NaAc) (99%), and ammonium bicarbonate (99%) were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Phospholipids of 1-palmitoyl-2-hydroxy-sn-glycero-3-phosphocholine (LysoPC(16:0)) and 2-Oleoyl-1-palmitoyl-sn-glycero-3-phosphocholine

(PC(34:1)) were purchased from Avanti Polar Lipids (Alabaster, AL, USA). 1,2-Diacyl-sn-glycero-3-phospho-L-serine (PS(18:1/18:1)) from bovine brain, L-α-Phosphatidylethanolamine (PE(18:2/18:0)) from egg yolk, and sphingomyelin (SM(d18:1/16:0)) from chicken egg yolk were purchased form Sigma-Aldrich (St. Louis, USA). Phosphate-Buffered Saline (PBS, 10X, pH 7.4) was brought from Life Technology. The PBS solution was diluted to 1X working concentration (containing 137 mM NaCl, 2.7 mM KCl, 8 mM Na2HPO4, and 2 mM KH2PO4) before use. The stock solutions of LysoPC(16:0) and LysoPC(34:1) were prepared in isopropyl alcohol at a concentration of 0.1 mg mL^{-1}, respectively, and stored at 4 °C. A 4 mg aliquot of Fe_3O_4@TiO_2 magnetic nanocomposite material was ultrasonic dispersed in 1 mL ultrapure water and added 1% TFA into the suspension solution before use. The silver coated fused-silica electrospray emitters were propused from Thermo Scientific (San Jose, CA, USA) with a tip diameter of about 10 μm. Ultrapure water was obtained from a Millipore water purification system (Milli-Q, Millipore; Bedford, MA, USA).

Preparation of Fe_3O_4@TiO_2 magnetic nanocomposites

Firstly, Fe_3O_4 magnetic nanoparticles were synthesized by hydrothermal method. Briefly, NaAc (15.0 g) and $FeCl_3 \cdot 6H_2O$ (5.0 g) were added in 100 mL ethylene glycol, followed with ED (50 mL) added inside. After vigorously vortexing for 30 min, the homogeneous mixture was sealed in a Teflon-lined stainless-steel autoclave (200 mL) and maintained at 200 °C heating condition for 8 h, and then cooled to room temperature (25 °C). The product was cleaned several times using water/ethanol (v/v, 1/1), and then vacuum dried at 60 °C

for 6 h. Thus, Fe_3O_4 magnetic nanoparticles were obtained.

Secondly, the produced Fe_3O_4 magnetic nanoparticles were then coated with TiO_2 by sol-gel process. A 10 mL aliquot of titanium butoxide was added into 37 mL ethanol under vigorous vortexing, which treated as solution A. Solution B was obtained by adding 0.5 g Fe_3O_4 magnetic nanoparticles into the mixture of 3 mL ethanol and 5 mL water. The Fe_3O_4 magnetic nanoparticles were ultrasonic dispersed. Then, solution B was slowly added into solution A under vigorous vortexing, and a brown colloidal solution was obtained after 2 h stirring. The colloidal solution was dried at 80 °C, and the solid product further annealed at 550 °C for 2 h. The annealed treated product was cooled at room temperature and grinded into fine particles. Lastly, $Fe_3O_4@TiO_2$ magnetic nanocomposite product was obtained.

The morphological, structural and elemental properties of the synthesized $Fe_3O_4@TiO_2$ magnetic nanocomposite were characterized using scanning electron microscopes (SEM) and energy dispersive X-ray analysis (EDX) (FIB-SEM instrument, Helios Nanolab 600i from FEI Co., USA). The electron beam voltage was set to 10–20 kV. Working distance of the instrument was set to 4 mm. Transmission electron microscopy (TEM) images of the material was obtained using a high resolution transmission electron microscopy (HRTEM, FEI Tecnai G2 S-Twin F20). X-ray diffraction (XRD) data were obtained using a Rigaku D/Max 2550 diffractometer (Tokyo, Japan) with a graphite monochromator using Cu-K_α radiation (λ = 1.5418 Å) operating at 50 kV and 200 mA. The high similarity between the morphological, structural and elemental properties as well as the content of the synthesized $Fe_3O_4@TiO_2$ nanocomposites from different batches was confirmed by SEM, EDX, TEM,

and XRD analyses.

Liquid-liquid extraction combined with ESI-MS (LLE-ESI-MS) analysis of phospholipids in human blood plasma samples

The traditional liquid-liquid extraction process of the phospholipids from the human blood plasma samples was done according to earlier literature.[2, 3] Briefly, an aliquot of 100 blood plasma sample was loaded into a 1.5 mL Eppendorf vial. After that, 300 μL methanol was added inside the vial for protein precipitation and metabolite extraction. The mixture was vigorously vortexed for 20 s and stored at −20 °C for 20 min. Then, the sample was centrifuged under 12000 rpm at 4 °C for 10 min, and 100 μL of supernatant was collected into a vial for the subsequent ESI-MS analysis. The ESI-MS analysis was carried out on Orbitrap Fusion™ Tribrid™ mass spectrometer (Thermo Scientific, San Jose, CA, USA). Mass spectra were collected in the mass range of *m/z* 400–1000 in positive ion detection mode. For each test the 5 μL sample solution was injected using a Valco 6 port valve at a flow rate of 8 μL min^{-1}. The ionization voltage was +4.0 kV. The heated LTQ capillary was maintained at 320 °C. The pressure of nitrogen sheath gas was 20 instrument units. Other instrumental conditions were maintained the same as the experimental conditions in d-SPME-iEESI-MS experiments.

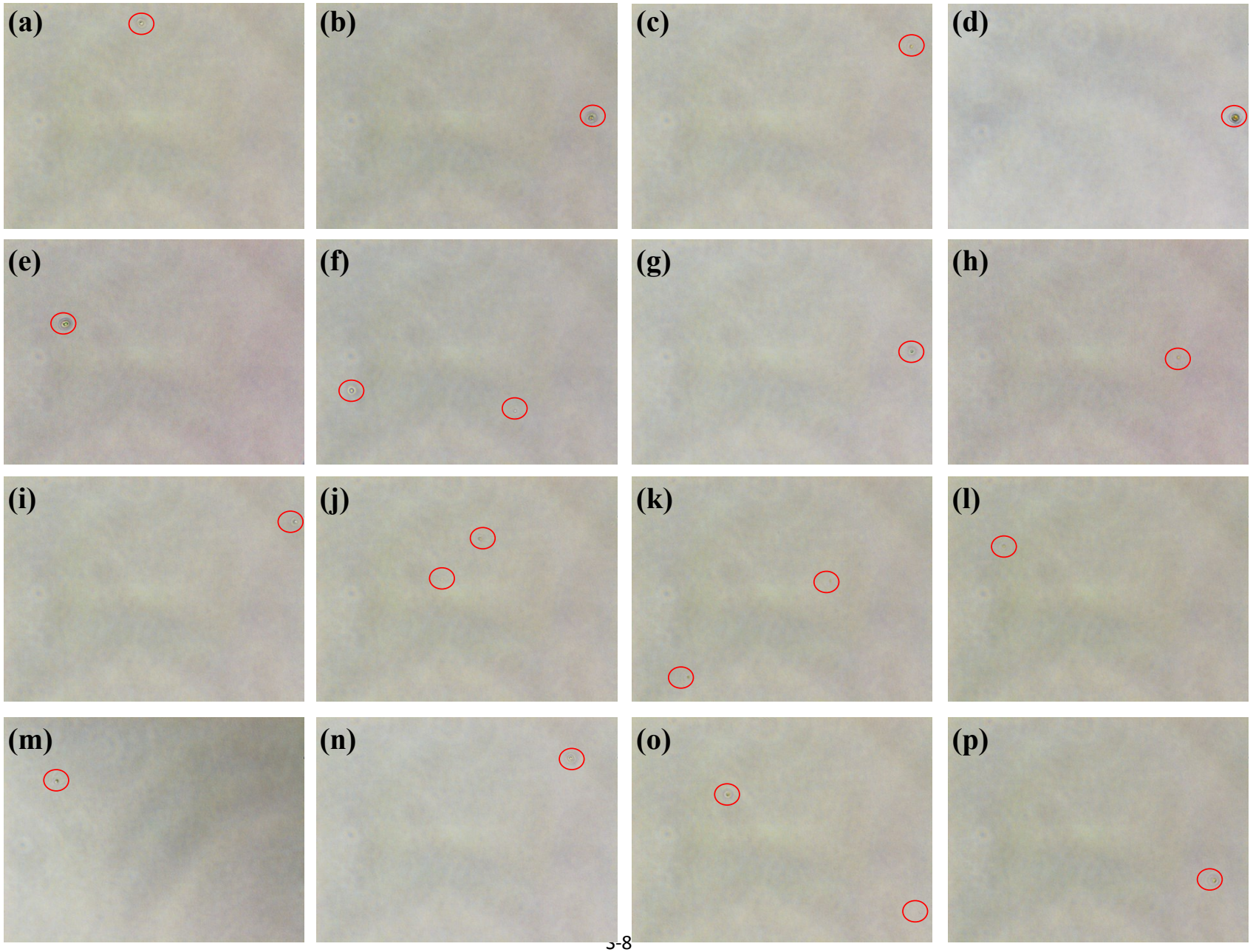
(a)
(b)
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Fig. S1 Optical images of single-cell samples in the 96-well microtiter plate (the cells are highlighted with ellipses). (a–p) representative optical images of cell samples in the 96-well microtiter plate. Only the samples with single-cell distribution were used for the d-SPME-iEESI-MS analysis.

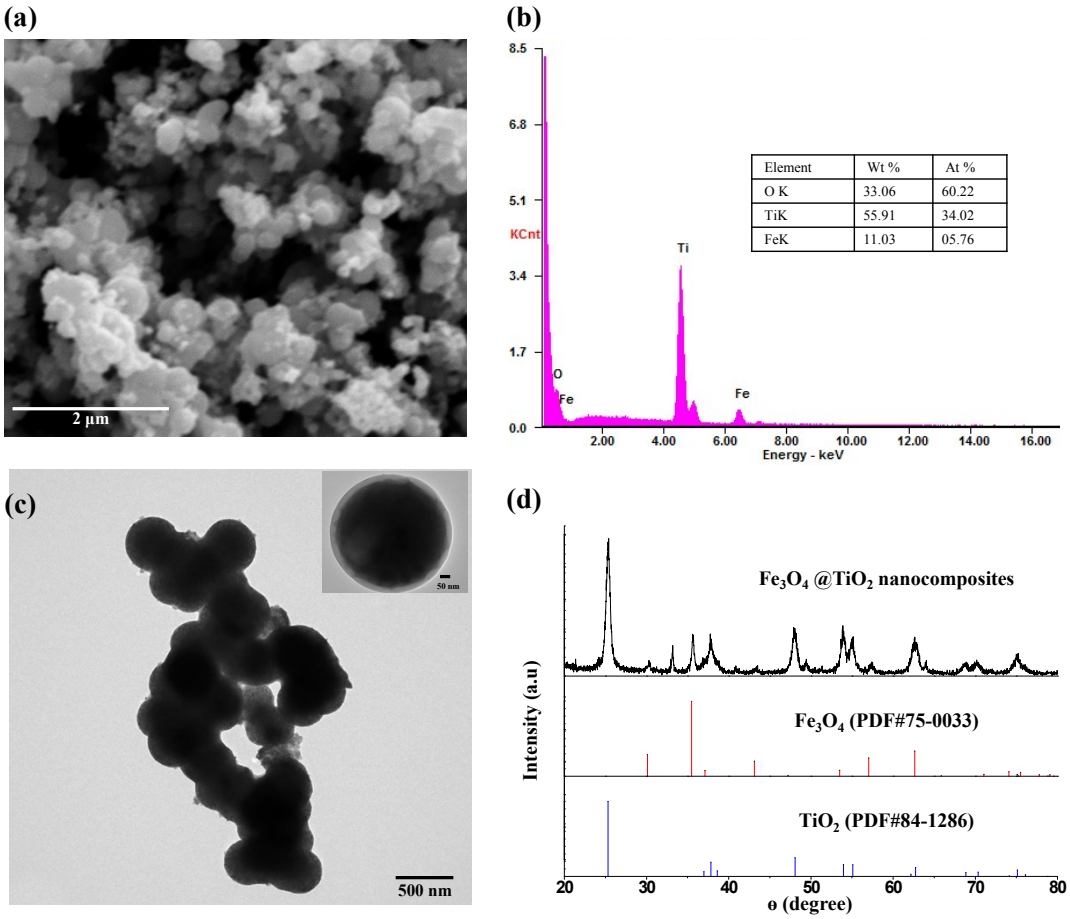

Fig. S2 Characterization of Fe_3O_4@TiO_2 nanocomposites. (a) SEM images of the Fe_3O_4@TiO_2 nanocomposites, (b) elemental analysis of the Fe_3O_4@TiO_2 nanocomposites, (c) TEM image of Fe_3O_4@TiO_2 nanocomposites (the inset is a high-resolution TEM image), (d) XRD patterns of the Fe_3O_4@TiO_2 nanocomposites.

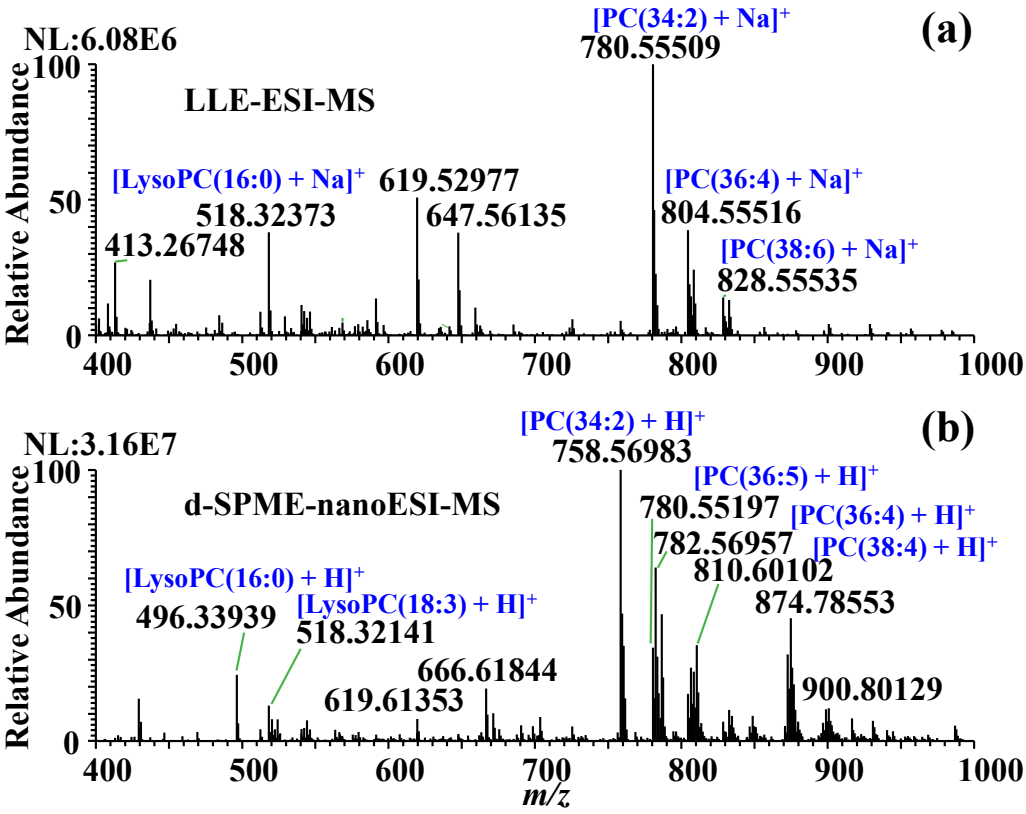

Fig. S3 Mass spectra of human blood plasma sample. (a) LLE-ESI-MS and (b) d-SPME-iEESI-MS.

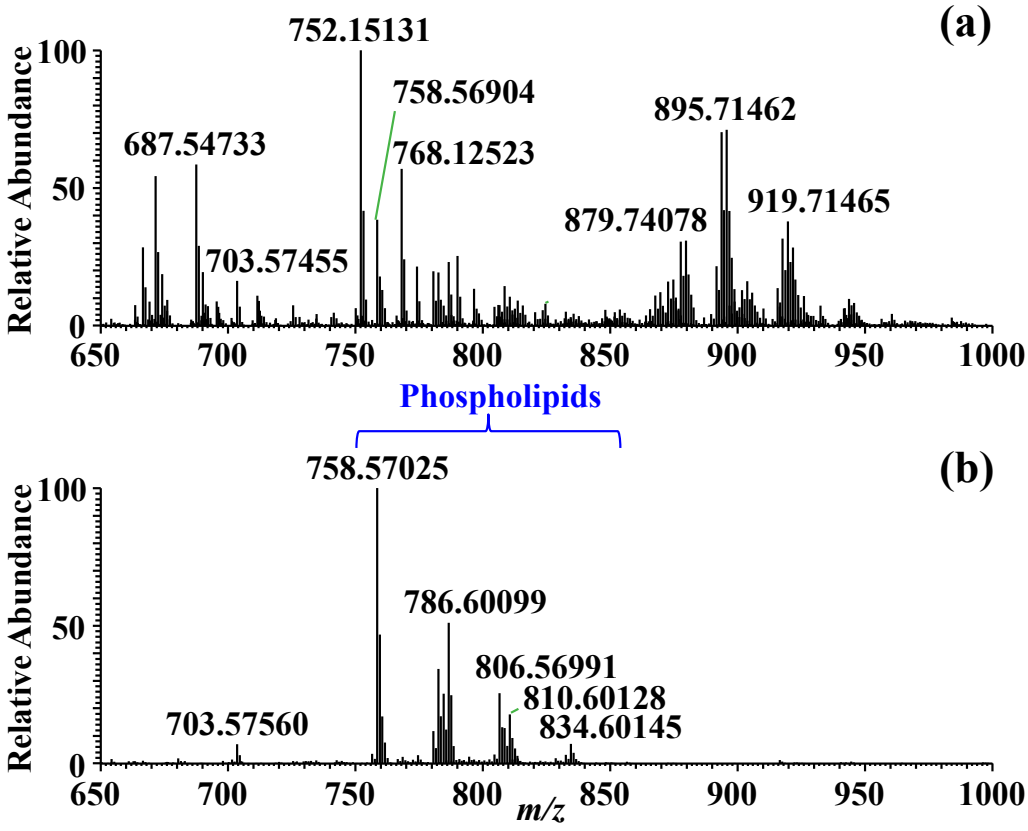

Fig. S4 Mass spectra of human blood plasma sample. (a) Direct nanoESI-MS without d-SPME step (b) d-SPME-iEESI-MS.

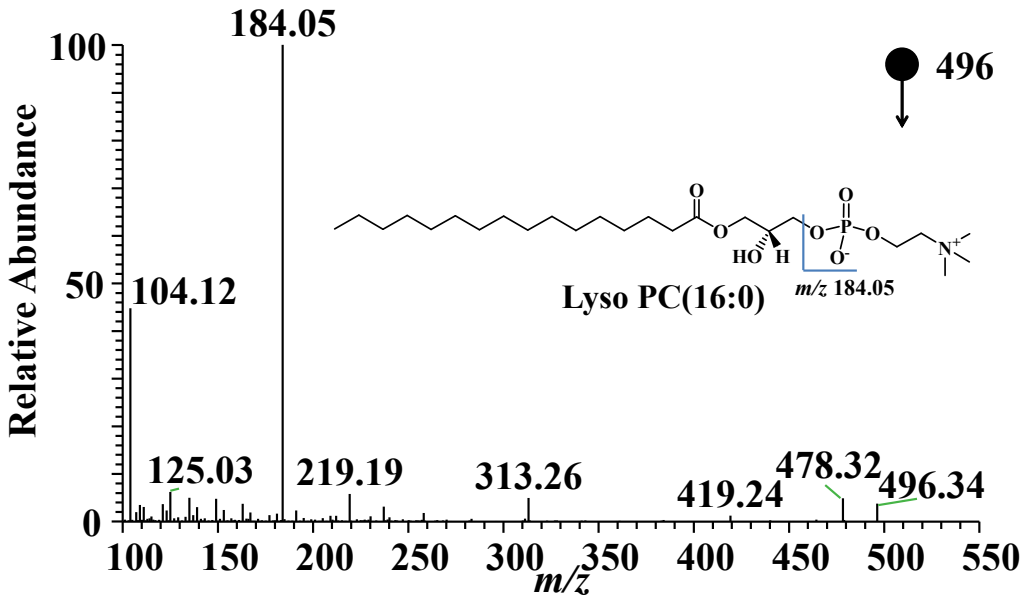

Fig. S5 d-SPME-iEESI-MS/MS analysis of LysoPC(16:0) (5 μg L^{-1}) in PBS samples.

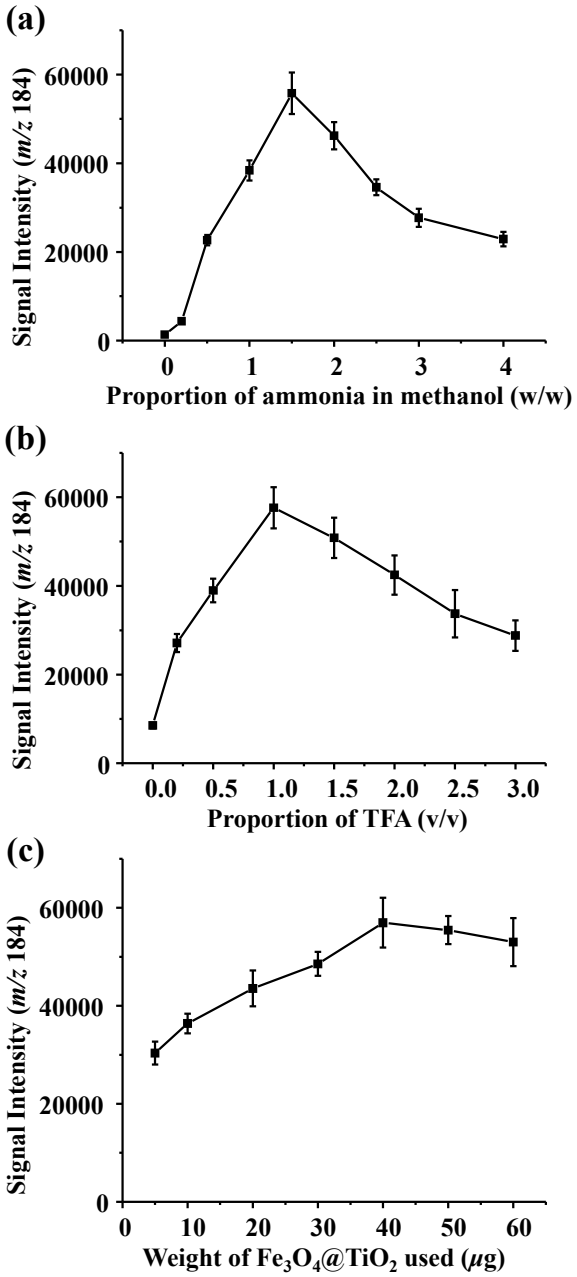

Fig. S6 The influence of the ammonia concentration in the desorption solution, the proportion of TFA, and the magnetic nanoparticle amounts for the detection of LysoPC(16:0). (a) The proportion of ammonia in methanol (%, w/w) in the desorption solution, (b) TFA concentration in the the Fe_3O_4@TiO_2 nanocomposites solution, and (c) different Fe_3O_4@TiO_2 nanocomposites amounts for the capture of LysoPC(16:0) (5 μg L^{-1}) in the PBS solution.

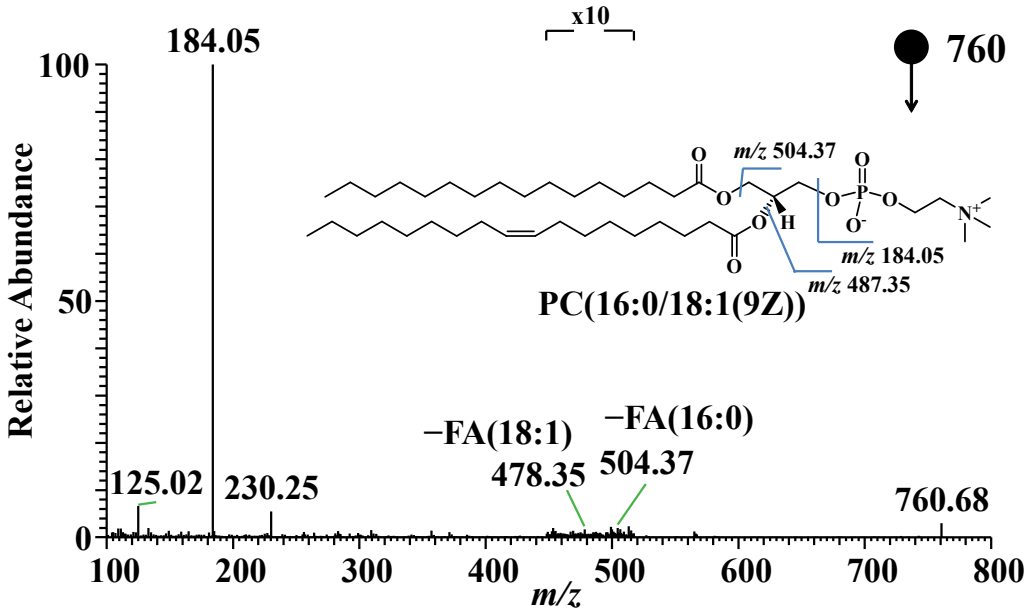

Fig. S7 d-SPME-iEESI-MS/MS analysis of PC(16:0/18:1) (5 μg L^{-1}) in PBS samples.

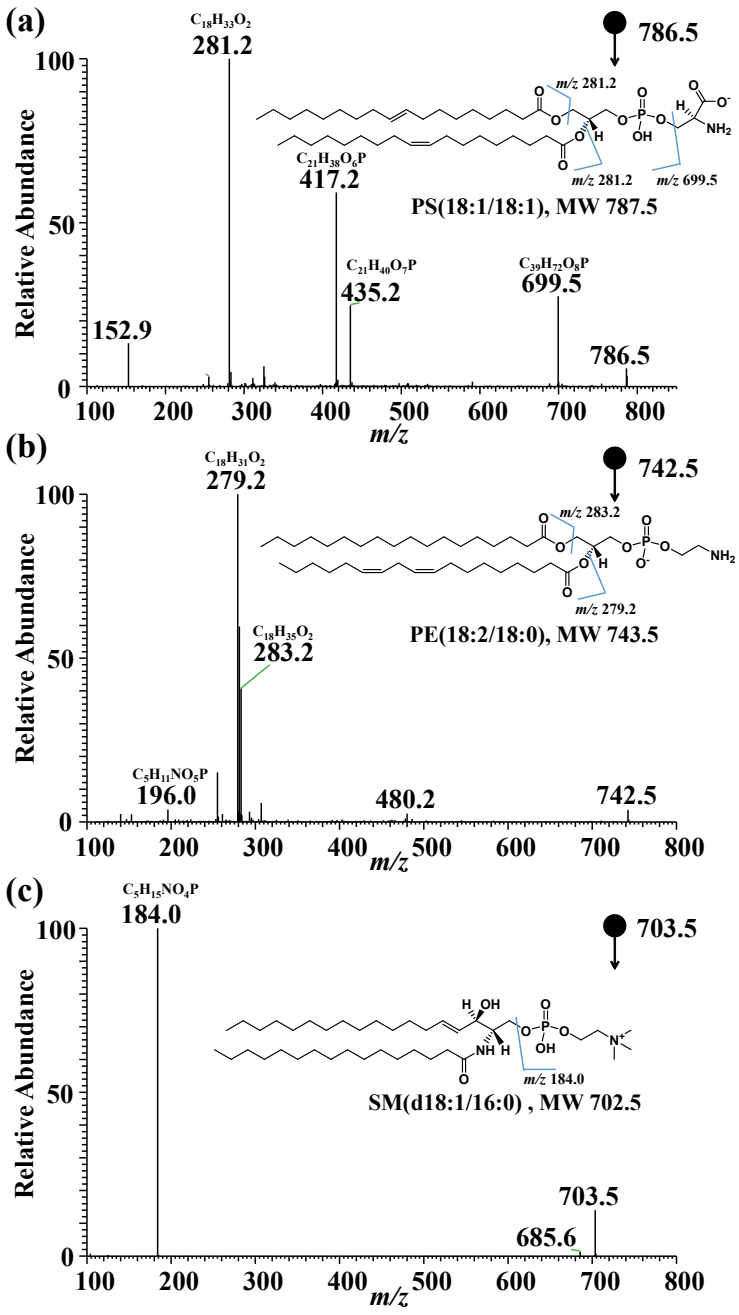

Fig. S8 d-SPME-iEESI-MS/MS analysis of phospholipid standards (10 μg L^{-1} in PBS solution): a) PS(18:1/18:1) in negative ion detection mode: b) PE(18:2/18:0) in negative ion detection mode; c) SM(d18:1/16:0) in positive ion detection mode.

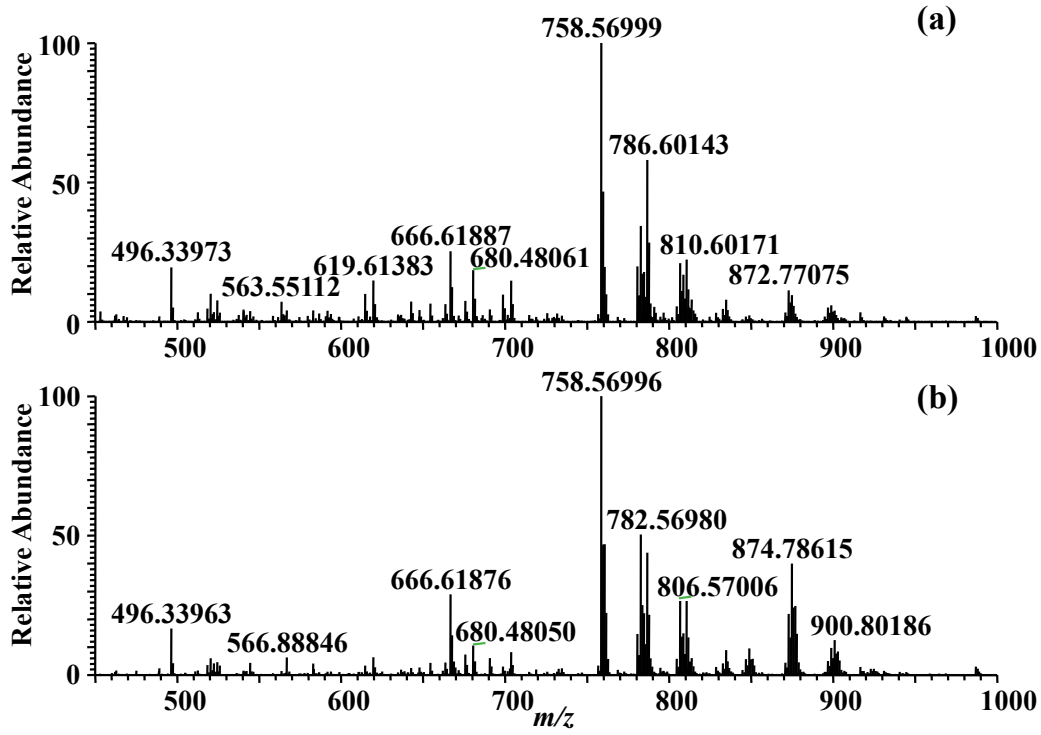

Fig. S9 d-SPME-iEESI-MS analysis of human blood plasma samples donated from healthy volunteers and pancreatic cancer patients. (a) Healthy volunteers, (b) patients with pancreatic cancer.

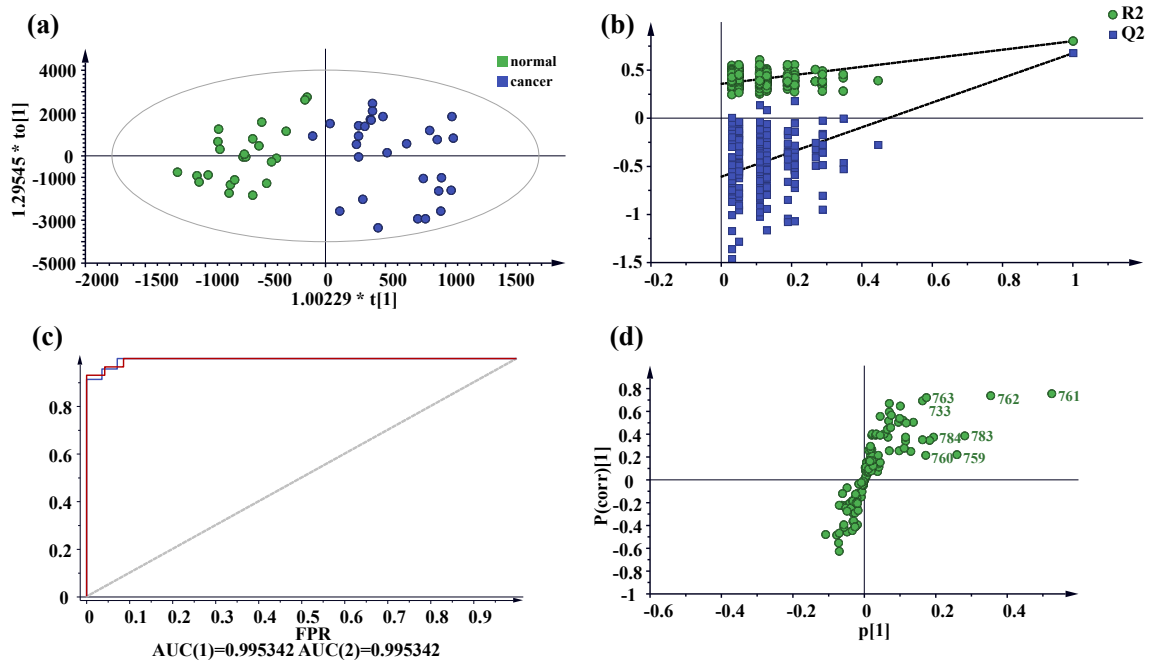

Fig. S10 OPLS-DA analysis of blood plasma samples data obtained from the pancreatic cancer patients and healthy controls. (a) OPLS-DA score plot of MS data collected from health controls (green) and pancreatic cancer patients (blue), (b) the S-plot loading plot of the MS data, (c) 200 permutations test result of the OPLS-DA model, (d) ROC plot of the OPLS-DA model.

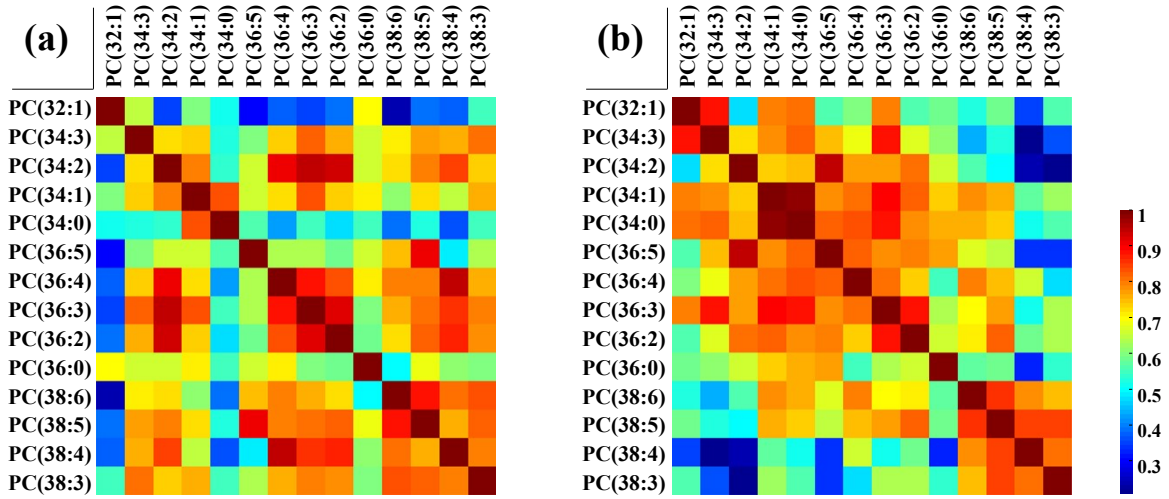

Fig. S11 Correlation among 14 kinds of phospholipids (VIP >1) in healthy controls and pancreatic cancer. (a) healthy controls and (b) pancreatic cancer.

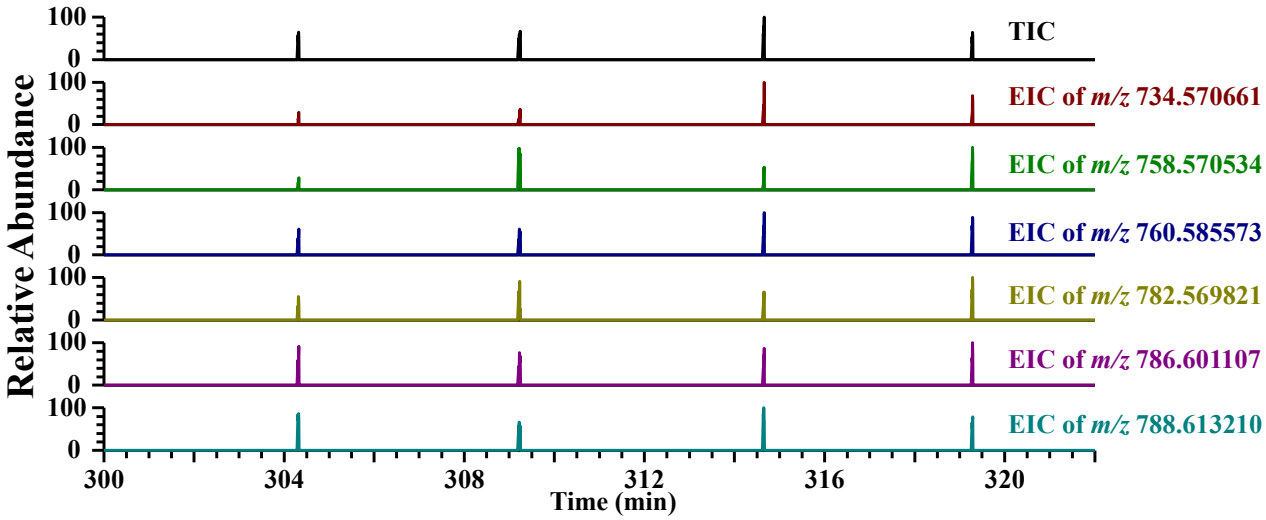

Fig. S12 Total ion chromatogram (TIC) and extracted ion chromatogram (EIC) of target phospholipids in the analysis of signle MV4-11 cells.

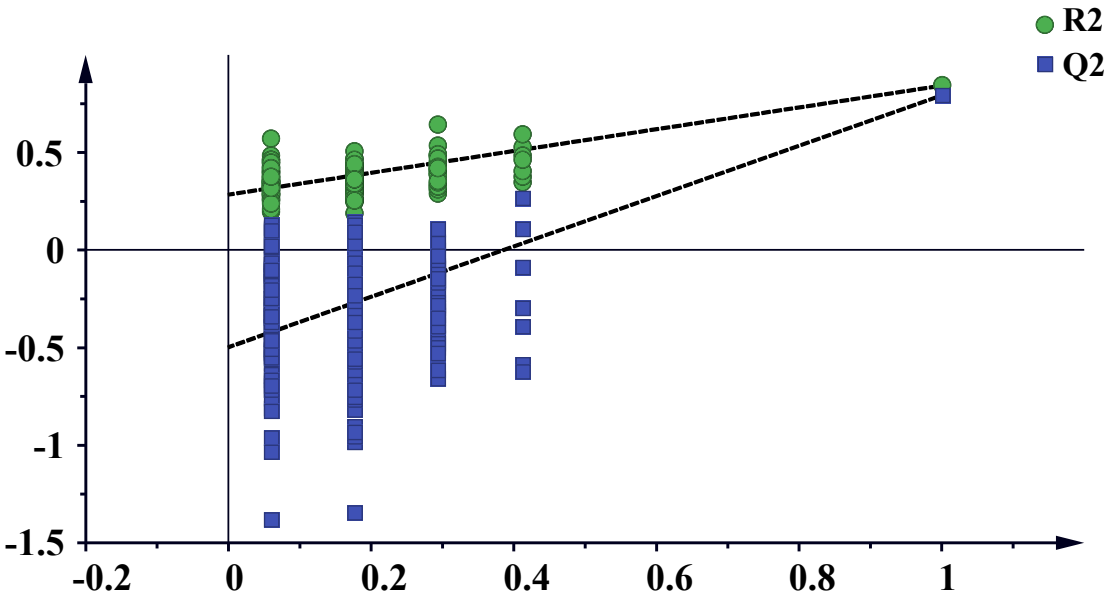

Fig. S13 200 permutations test result of the OPLS-DA model for the process of two kinds of cell types.

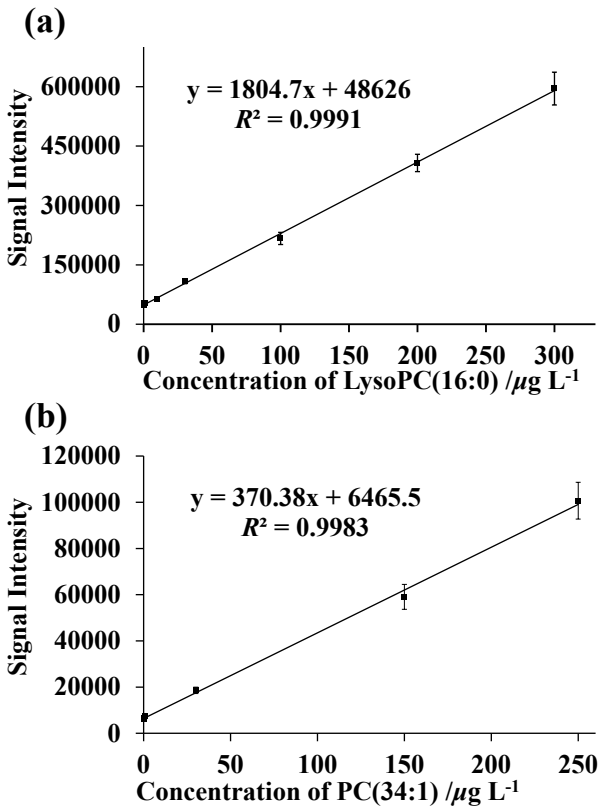

Fig. S14 Signal response curves of *m/z* 184 for the determination of phospholipids with d-SPME-iEESI-MS/MS analysis: (a) LysoPC(16:0) and (b) PC(16:0/18:1).

Table S1. Clinical information of blood plasma samples from ovarian cancer and pancreatic cancer patients

Cancerous type	Ovarian cancer	Pancreatic cancer
Number of samples	59	28
Sex (F/M)	59/0	16/13
Age (mean ± SD, range)	51.9 ± 11.9, 18–75	61.4 ± 8.4, 43–74
Stage		
S1	16	3
S2	9	11
S3	32	14
S4	2	1

Table S2. List of the identified lipid species from blood plasma sample using d-SPME-iEESI-MS

No.	Lipids [a]	Formula	Experimental *m/z*	Adduct	Monoisotopic mass	Theoretical adduct [b] *m/z*	Error [c] (ppm)
1	LysoPC(16:0)	$C_{24}H_{50}NO_7P$	496.339670	M+H	495.3325	496.3398	0
2	LysoPC(16:0)	$C_{24}H_{50}NO_7P$	518.321690	M+Na	495.3325	518.3217	0
3	LysoPC(18:2)	$C_{26}H_{50}NO_7P$	520.339580	M+H	519.3325	520.3398	0
4	LysoPC(18:0)	$C_{26}H_{54}NO_7P$	524.371050	M+H	523.3638	524.3711	0
5	LysoPC(20:4)	$C_{28}H_{50}NO_7P$	544.339670	M+H	543.3325	544.3398	0
6	LysoPC(20:3)	$C_{28}H_{52}NO_7P$	546.354190	M+H	545.3481	546.3554	2
7	PE(28:1)	$C_{33}H_{64}NO_8P$	634.444671	M+H	633.437	634.4442	1
8	SM(32:1)	$C_{37}H_{75}N_2O_6P$	675.544117	M+H	674.5363	675.5436	1
9	SM(32:1)	$C_{37}H_{75}N_2O_6P$	697.526424	M+Na	674.5363	697.5255	1
10	SM(34:1)	$C_{39}H_{80}N_2O_6P$	703.575660	M+H	703.5754	703.5754	0
11	SM(34:1)	$C_{39}H_{80}N_2O_6P$	725.557617	M+Na	703.5754	725.5573	0
12	SM(36:2)	$C_{41}H_{81}N_2O_6P$	729.591685	M+H	728.5832	729.5905	2
13	PC(32:2)	$C_{40}H_{76}NO_8P$	730.539537	M+H	729.5309	730.5381	2
14	SM(36:1)	$C_{41}H_{84}N_2O_6P$	731.607062	M+H	731.6067	731.6067	0
15	PC(32:1)	$C_{40}H_{78}NO_8P$	732.555074	M+H	731.5465	732.5538	2
16	PC(32:0)	$C_{40}H_{80}NO_8P$	734.570661	M+H	733.5622	734.5694	2
17	SM(34:1)	$C_{39}H_{80}N_2O_6P$	741.531872	M+K	703.5754	741.5313	1
18	PE(34:0)	$C_{39}H_{78}NO_8P$	742.536580	M+Na	719.5465	742.5357	1
19	PE(36:2)	$C_{41}H_{78}NO_8P$	744.555358	M+H	743.5465	744.5538	2
20	PE(36:1)	$C_{41}H_{80}NO_8P$	746.569949	M+H	745.5622	746.5694	1
21	PC(34:0)	$C_{42}H_{84}NO_7P$	746.608326	M+H	745.5985	746.6058	3
22	SM(36:2)	$C_{41}H_{81}N_2O_6P$	751.573739	M+Na	728.5832	751.5724	2
23	SM(36:1)	$C_{41}H_{84}N_2O_6P$	753.589317	M+Na	731.6067	753.5886	1
24	PC(32:1)	$C_{40}H_{78}NO_8P$	754.538498	M+Na	731.5465	754.5357	4
25	PC(34:3)	$C_{42}H_{78}NO_8P$	756.554856	M+H	755.5465	756.5538	1
26	PC(34:2)	$C_{42}H_{80}NO_8P$	758.570534	M+H	757.5622	758.5694	1
27	SM(38:1)	$C_{43}H_{87}N_2O_6P$	759.637806	M+H	758.6302	759.6375	0

28	PC(34:1)	$C_{42}H_{82}NO_8P$	760.585573	M+H	759.5778	760.5851	1
29	PC(34:0)	$C_{42}H_{84}NO_8P$	762.59229	M+H	761.5929	762.5935	1
30	PE(38:6)	$C_{43}H_{74}NO_8P$	764.523057	M+H	763.5152	764.5225	1
31	PE(38:3)	$C_{43}H_{76}NO_8P$	766.537281	M+H	765.5309	766.5381	1
32	PC(34:0)	$C_{42}H_{84}NO_7P$	768.591104	M+Na	745.5985	768.5878	4
33	SM(36:1)	$C_{41}H_{83}N_2O_6P$	769.561880	M+K	730.5989	769.562	0
34	PE(28:3)	$C_{43}H_{80}NO_8P$	770.568303	M+H	769.5622	770.5694	1
35	PC(36:2)	$C_{44}H_{84}NO_7P$	770.606981	M+H	769.5985	770.6058	2
36	PE(38:2)	$C_{43}H_{82}NO_8P$	772.586005	M+H	771.5778	772.5851	1
37	PE(38:1)	$C_{43}H_{84}NO_8P$	774.601190	M+H	773.5935	774.6007	1
38	SM(38:2)	$C_{43}H_{83}N_2O_6P$	777.584652	M+Na	754.5989	777.5881	4
39	PC(36:6)	$C_{44}H_{76}NO_8P$	778.536908	M+H	777.5309	778.5381	2
40	PC(34:2)	$C_{42}H_{80}NO_8P$	780.552162	M+Na	757.5622	780.5514	1
41	PC(36:5)	$C_{44}H_{78}NO_8P$	780.552162	M+H	779.5465	780.5538	2
42	PC(36:4)	$C_{44}H_{80}NO_8P$	782.569821	M+H	781.5622	782.5694	0
43	PC(34:1)	$C_{42}H_{82}NO_8P$	782.569821	M+Na	759.5778	782.567	4
44	PC(36:3)	$C_{44}H_{82}NO_8P$	784.585886	M+H	783.5778	784.5851	1
45	SM(40:2)	$C_{45}H_{89}N_2O_6P$	785.653071	M+H	784.6458	785.6531	0
46	PC(36:2)	$C_{44}H_{84}NO_8P$	786.601107	M+H	785.5935	786.6007	0
47	SM(40:1)	$C_{45}H_{91}N_2O_6P$	787.668780	M+H	786.6615	787.6688	0
48	PC(36:1)	$C_{44}H_{86}NO_8P$	788.613210	M+H	787.6091	788.6164	4
49	PC(36:0)	$C_{44}H_{88}NO_8P$	790.62389	M+H	789.6242	790.6248	1
50	PE(38:4)	$C_{46}H_{84}NO_7P$	794.606059	M+H	793.5985	794.6058	0
51	PC(34:2)	$C_{42}H_{80}NO_8P$	796.525731	M+K	757.5622	796.5253	1
52	PE(40:4)	$C_{45}H_{82}NO_8P$	796.583794	M+H	795.5778	796.5851	2
53	SM(38:1)	$C_{43}H_{87}N_2O_6P$	797.590290	M+K	758.6302	797.5933	4
54	PC(34:1)	$C_{42}H_{82}NO_8P$	798.541569	M+K	759.5778	798.541	1
55	PE(38:0)	$C_{43}H_{86}NO_8P$	798.599467	M+Na	775.6091	798.5983	1
56	PE(40:3)	$C_{45}H_{84}NO_8P$	798.599467	M+H	797.5935	798.6007	2
57	SM(40:1)	$C_{45}H_{89}N_2O_7P$	801.649292	M+H	800.6407	801.648	2
58	PC(38:8)	$C_{46}H_{76}NO_8P$	802.536663	M+H	801.5309	802.5381	2
59	PC(38:7)	$C_{46}H_{78}NO_8P$	804.552642	M+H	803.5465	804.5538	1
60	PC(38:6)	$C_{46}H_{80}NO_8P$	806.570470	M+H	805.5622	806.5694	1
61	SM(40:2)	$C_{45}H_{90}N_2O_6P$	807.635374	M+Na	785.6537	807.6356	0

62	PC(38:5)	$C_{46}H_{82}NO_8P$	808.584811	M+H	807.5778	808.5851	0
63	PC(40:1)	$C_{45}H_{91}N_2O_6P$	809.651754	M+Na	786.6615	809.6507	1
64	PC(38:4)	$C_{46}H_{84}NO_8P$	810.601721	M+H	809.5935	810.6007	1
65	SM(40:0)	$C_{45}H_{93}N_2O_6P$	811.669518	M+Na	788.6771	811.6663	4
66	PE(40:7)	$C_{45}H_{76}NO_8P$	812.520541	M+Na	789.5309	812.5201	1
67	PE(42:10)	$C_{47}H_{74}NO_8P$	812.520541	M+H	811.5152	812.5225	2
68	PC(38:3)	$C_{46}H_{86}NO_8P$	812.617775	M+H	811.6091	812.6164	2
69	PC(36:0)	$C_{44}H_{88}NO_8P$	812.617775	M+Na	789.6248	812.614	5
70	SM(42:2)	$C_{47}H_{93}N_2O_6P$	813.685120	M+H	812.6771	813.6844	1
71	SM(42:1)	$C_{47}H_{96}N_2O_6P$	815.701156	M+H	815.7006	815.7006	1
72	SM(40:3)	$C_{45}H_{85}N_2O_6P$	819.573775	M+K	780.6145	819.5777	5
73	PC(36:4)	$C_{44}H_{80}NO_8P$	820.528243	M+K	781.5622	820.5253	4
74	PE(42:6)	$C_{47}H_{82}NO_8P$	820.583634	M+H	819.5778	820.5851	2
75	PC(40:5)	$C_{48}H_{86}NO_7P$	820.623023	M+H	819.6142	820.6215	2
76	SM(40:2)	$C_{45}H_{87}N_2O_7P$	821.611065	M+Na	798.6251	821.6143	4
77	SM(40:1)	$C_{45}H_{89}N_2O_7P$	823.630461	M+Na	800.6407	823.63	1
78	PC(36:2)	$C_{44}H_{84}NO_8P$	824.557887	M+K	785.5935	824.5566	2
79	PC(40:9)	$C_{48}H_{78}NO_8P$	828.553228	M+H	827.5465	828.5538	1
80	SM(42:1)	$C_{47}H_{93}N_2O_7P$	829.679989	M+H	828.672	829.6793	1
81	PC(40:8)	$C_{48}H_{80}NO_8P$	830.570658	M+H	829.5622	830.5694	1
82	PC(40:7)	$C_{48}H_{82}NO_8P$	832.584889	M+H	831.5778	832.5851	0
83	PC(40:6)	$C_{48}H_{84}NO_8P$	834.602427	M+H	833.5935	834.6007	2
84	SM(42:2)	$C_{47}H_{93}N_2O_6P$	835.605623	M+Na	812.6771	835.6663	1
85	PC(40:5)	$C_{48}H_{86}NO_8P$	836.617938	M+H	835.6091	836.6164	2
86	SM(42:1)	$C_{47}H_{96}N_2O_6P$	837.682688	M+Na	815.7006	837.6825	0
87	PC(40:4)	$C_{48}H_{88}NO_8P$	838.632557	M+H	837.6248	838.632	1
88	PC(38:4)	$C_{46}H_{84}NO_8P$	848.560323	M+K	809.5935	848.5566	4
89	SM(42:1)	$C_{47}H_{93}N_2O_7P$	851.661836	M+Na	828.672	851.6613	1
90	PC(42:9)	$C_{50}H_{82}NO_8P$	856.585309	M+H	855.5778	856.5851	0
91	PC(40:6)	$C_{48}H_{84}NO_8P$	856.585309	M+Na	833.5935	856.5827	3
92	PE(42:10)	$C_{49}H_{78}NO_8P$	862.536159	M+Na	839.5465	862.5357	1
93	PC(44:12)	$C_{52}H_{80}NO_8P$	916.527037	M+K	877.5622	916.5253	2
94	PC(44:11)	$C_{52}H_{82}NO_8P$	918.542054	M+K	879.5778	918.541	1

[a] PC, phosphatidylcholine; PE, phosphatidylethanolamine; SM, sphingomyelin

[b] Theoretical adduct *m/z* refers to databases of Human Metabolome Database (http//: www.hmdb.ca) and LIPID MAPS (http//: www. lipidmaps.org)

[c] Error = (abs(experimental mass - theoretical adduct mass)/ theoretical adduct mass)*1000000

Table S3. 12 kinds of phospholipids (VIP >1) in ovarian cancer and healthy controls.

Lipids	Experimental m/z	Adduct	VIP value
PC(34:2)	758.570534	M+H	4.25
PC(36:2)	786.601107	M+H	3.33
PC(36:4)	782.569821	M+H	2.74
PC(38:6)	806.570470	M+H	2.12
PC(36:3)	784.585886	M+H	2.12
PC(36:5)	780.552162	M+H	2.09
PC(38:4)	810.601721	M+H	2.08
PC(34:1)	760.585573	M+H	2.05
PC(38:5)	808.584811	M+H	2.04
PC(36:1)	788.613210	M+H	1.22
PC(38:7)	804.552642	M+H	1.21
PC(38:3)	812.617775	M+H	1.14

Table S4. 14 kinds of phospholipids (VIP >1) in pancreatic cancer and healthy controls.

Lipids	Experimental m/z	Adduct	VIP value
PC(34:1)	760.585573	M+H	4.55
PC(34:2)	758.570534	M+H	4.06
PC(36:4)	782.569821	M+H	3.00
PC(36:3)	784.585886	M+H	2.05
PC(36:2)	786.601107	M+H	2.03
PC(38:6)	806.570470	M+H	1.76
PC(38:4)	810.601721	M+H	1.64
PC(34:0)	762.592290	M+H	1.51
PC(36:5)	780.552162	M+H	1.42
PC(32:1)	732.555074	M+H	1.41
PC(34:3)	756.554856	M+H	1.26
PC(38:3)	812.617775	M+H	1.20
PC(38:5)	808.584811	M+H	1.16
PC(36:0)	790.623890	M+H	1.08

Table S5. Analytical preference of d-SPME-iEESI-MS in the analysis of LysoPC(16:0) and (b) PC(16:0/18:1).

Analytes	Linear range (μg L^{-1})	RSDs (%)	R^2	LOD (μg L^{-1})	Recovery experiments		
					Spiked concentration	Recovery (%, n=6)	RSD (%, n=6)
LysoPC (16:0)	0.1-300	3.3-7.1	0.9991	0.015	0.2	112.9	3.3
					150	96.3	7.0
					250	96.7	7.1
PC(16:0/18:1)	0.1-250	2.7-9.1	0.9983	0.013	0.2	111.8	2.7
					100	102.2	4.9
					200	98.6	6.1

References

1. M. D. E. Goodyear, K. Krleza-Jeric and T. Lernmens, *Brit. Med. J.*, 2007, **335**, 624-625.
2. D. F. Wang, S. L. Cheng, Q. Fei, H. W. Gu, D. Raftery, B. Cao, X. Y. Sun, J. J. Yan, C. B. Zhang and J. Wang, *Psychiat. Res.*, 2019, **272**, 18-29.
3. T. Cajka and O. Fiehn, *Trac-trend. Anal. Chem.*, 2014, **61**, 192-206.