Supplementary information for

Fast broad-coverage lipidomics enabled by ion mobility-mass spectrometry

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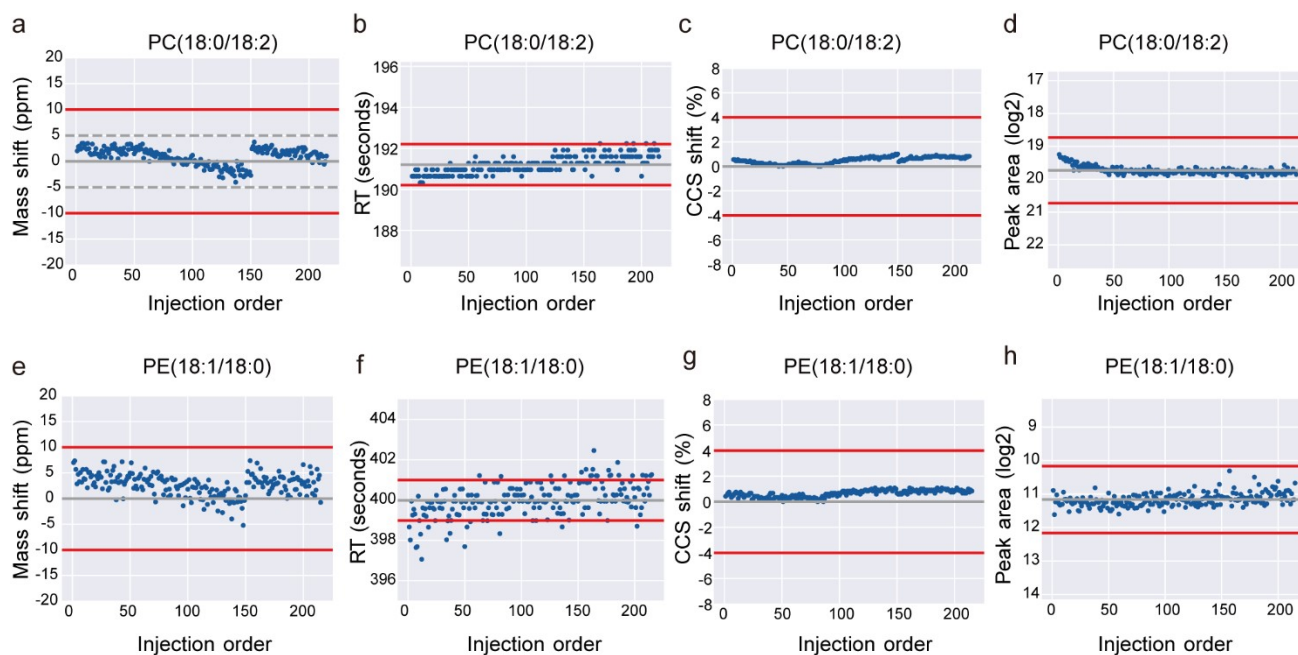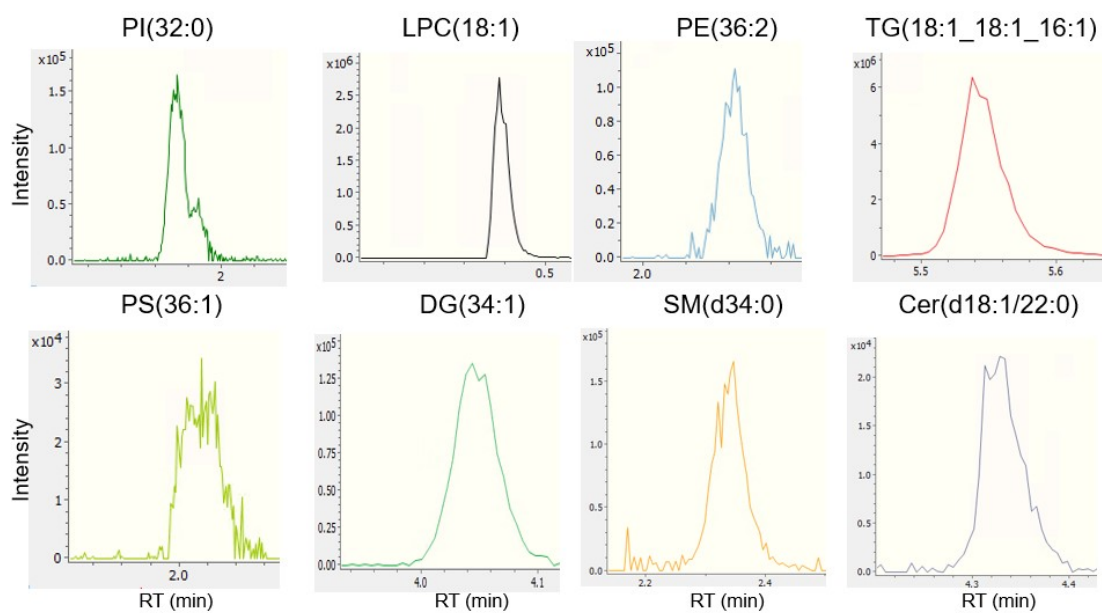
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Fig. S1. Stability of the 8-minute LC–IM–MS method regarding mass accuracy, retention time, CCS, and lipid abundance. (a) Mass shift, (b) RT shift, (c) CCS shift, and (d) intensity deviation of PC(18:0/18:2) over the course of 215 sample injections. (e) Mass shift, (f) RT shift, (g) CCS shift, and (h) intensity deviation of PE(18:1/18:0) over the course of 215 sample injections.

Supplementary Fig. S2. The extracted ion chromatograms (EICs) of different lipid species from NIST human plasma and BioMix sample.
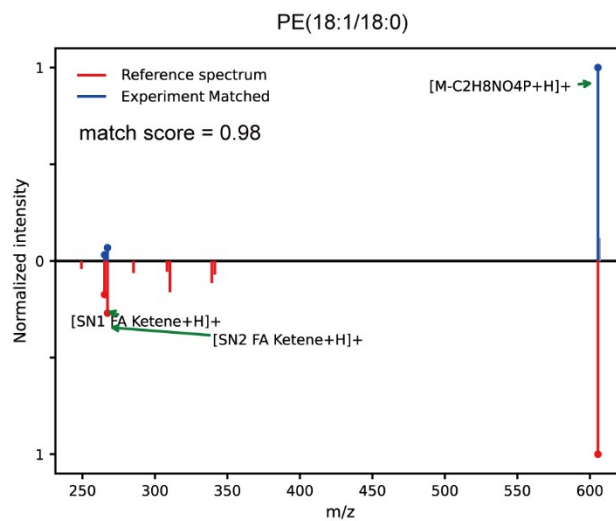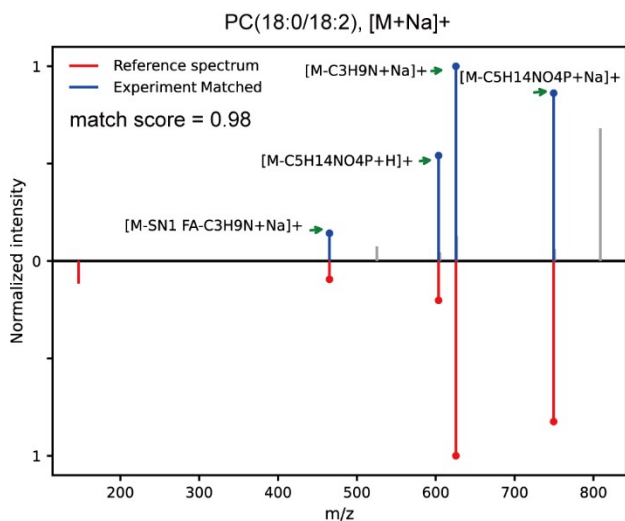
Fig. S3. Mirror plots of MS/MS match for PC(18:0/18:2) and PE(18:1/18:0).

a PC(16:0/18:0)

y = 0.0203x - 0.0081
R2= 0.998

b PC(P-18:0/18:1)

y = 0.0321x - 0.0463
R2= 0.9921

c PE(18:0/18:2)

y = 0.9057x - 0.8296
R2= 0.992

d PE(P-18:0/18:1)

y = 1.5418x - 4.6384
R2 = 0.991

e LPE(18:0)

y = 0.0011x + 0.0059
R2= 0.9929

f LPE(P-18:0)

y = 0.4656x - 0.2307
R2= 0.9978

g LPC(O-16:1)

y = 0.1076x + 0.0599
R2= 0.9938

h LPC(16:0)

y = 0.1052x + 0.0555
R2= 0.9947

i SM(d18:1/17:0)

y = 0.1811x - 0.1028
R2= 0.9933

j TG(18:1/16:0/18:1)

y = 0.0929x - 0.0486
R2= 0.9982

k DG(17:1/17:1)

y = 0.6048x - 0.5845
R2= 0.9954

l PE(P-18:0/18:1)
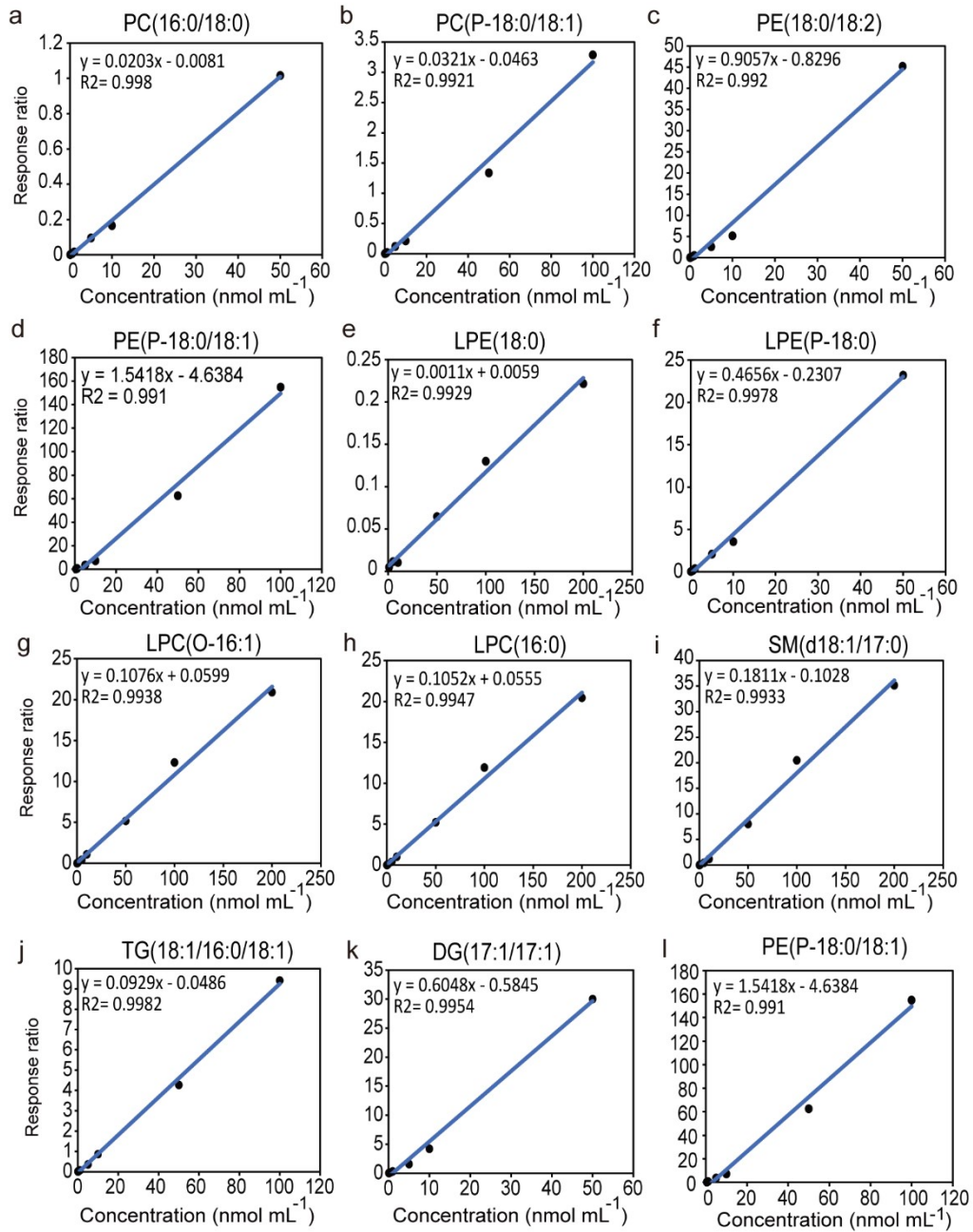
y = 1.5418x - 4.6384
R2= 0.991

Fig. S4. Calibration curves for quantification of individual lipid classes.
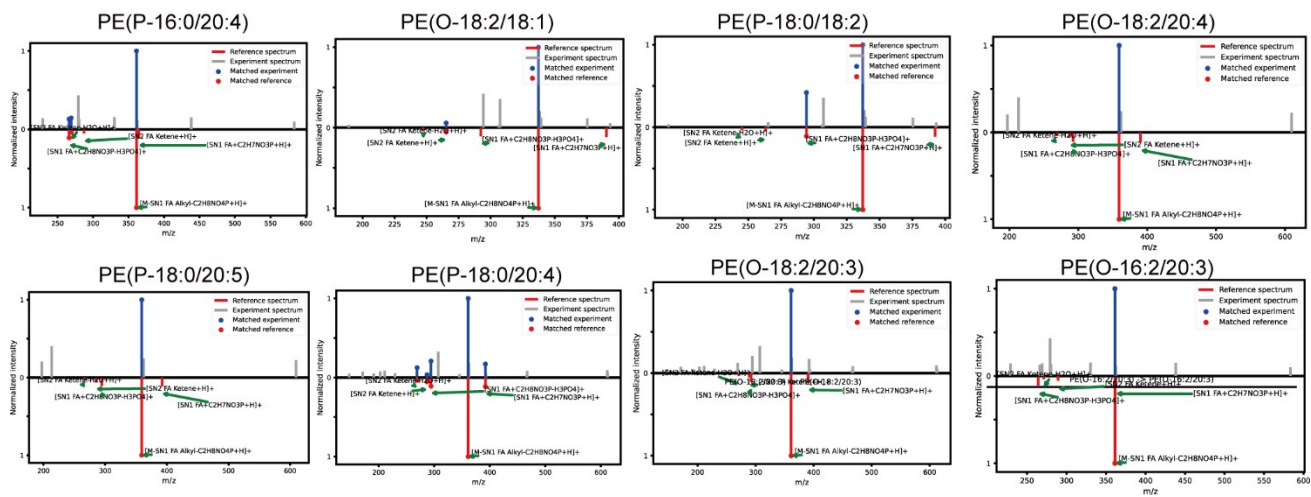
Fig. S5. Mirror plots of MS/MS match for ether phospholipid species.

Supplementary Table S1. Parameter set of Me4DX for data processing.

Parameter	Value
# MS2 spectral dereplication	
Absolute intensity cutoff of fragment ion	30 counts
Relative intensity cutoff of fragment ion	0.01
m/z tolerance for precursor ion match	20 ppm or 0.004 Da for m/z < 200 Da
Maximum/minimum RT tolerance for clustering MS2	20 s and 10 s
Maximum/minimum mobility tolerance for clustering MS2	0.030 and 0.015 V·s/cm^2
3D distance cutoff in HCA	1
# The bottom-up assembly algorithm for 4D peak detection	
m/z tolerance for MS1 data point search	20 ppm or 0.004 Da for m/z < 200 Da
Mobility range to reconstruct ion mobilogram	0.1 V·s/cm^2
Frame range to reconstruct ion chromatogram	30 frames
Peak span of EIM and EIC	13 points and 11 points
RT difference tolerance of detected EIC apex and precursor RT	10 s
Mobility difference tolerance of detected EIC apex and precursor RT	0.015 V·s/cm^2
Frame range for signal integration	5 frames
Mobility range for signal integration	0.015 V·s/cm^2
Signal-to-noise ratio cutoff	3
Normalized standard noise cutoff	0.35
# 4D peak alignment and grouping	
m/z tolerance for landmark match	20 ppm or 0.004 Da for m/z < 200 Da
RT tolerance for landmark match	30 s
CCS tolerance for landmark match	2%
Dot-product cutoff for MS2 spectral match for landmark match	0.8
m/z bin size	0.015 Da
Mobility bin size	0.015 V·s/cm^2
Bandwidth of gaussian smoothing kernel to apply to the peak density chromatogram	5 s
Minimum fraction of samples necessary in at least one of the sample groups	0.5
Frame range for gap filling	5 frames
Mobility range for gap filling	0.015 V·s/cm^2
m/z tolerance for gap filling	20 ppm or 0.004 Da for m/z < 200 Da

Supplementary Table S2. Quantitative analysis information on each lipid class for NIST SRM 1950 human plasma and plasma samples of patients with CRC.

Lipid class	Adduct form	Internal standard	Concentration of internal standard (nmol/mL)
LPC	$[M+H]^+$	d7-LPC(18:1)	9.46
LPC(P)/LPC(O)	$[M+H]^+$	d7-LPC(18:1)	9.46
LPE	$[M+H]^+$	d7-LPE(18:1)	2.05
LPE(P)/LPE(O)	$[M+H]^+$	d7-LPE(18:1)	2.05
PC	$[M+H]^+$	d7-PC(15:0/18:1)	42.49
PC(P)/PC(O)	$[M+H]^+$	d7-PC(15:0/18:1)	42.49
PE	$[M+H]^+$	d7-PE(15:0/18:1)	1.41
PE(P)/PE(O)	$[M+H]^+$	d7-PE(15:0/18:1)	1.41
SM	$[M+H]^+$	d9-SM(d18:1/18:1)	8.13
DG	$[M+NH_4]^+$	d7-DG(15:0/18:1)	3.40
TG	$[M+NH_4]^+$	d7-TG(15:0/18:1/15:0)	13.54
Cholesterol	$[M-H_2O+H]^+$	d7-Cholesterol	50.84
CE	$[M+NH_4]^+$	d7-CE(18:1)	106.44

Time (min)	Time (min)	Time (min)	Time (min)	Time (min)	A(%)	B(%)	Flow(mL/min)
0.00	0.00	0.00	0.00	0.00	70.00	30.00	0.50
1.00	1.00	0.50	1.50	1.50	70.00	30.00	0.50
1.50	3.00	5.00	9.00	14.00	30.00	70.00	0.50
1.70	3.50	6.00	10.00	15.00	30.00	70.00	0.50
1.80	3.60	6.10	10.10	15.10	10.00	90.00	0.50
2.00	3.90	6.50	11.00	16.00	10.00	90.00	0.50
2.10	4.00	6.70	11.10	16.10	70.00	30.00	0.50
3.00	5.00	8.00	13.00	18.00	70.00	30.00	0.50

Supplementary Table S3. The LC gradients of individual LC–IM–MS methods.

Supplementary Table S4. Summary of linear dynamic ranges, LOD, LOQ, and reproducibility for quantification.

Lipid	Conc. range (nmol mL^{-1})	Equation	R2	LOD* (nmol mL^{-1})	LOQ# (nmol mL^{-1})	RSD of corresponding IS
PC(16:0/18:0)	0.05-50	y = 0.0203x-0.0081	0.9987	0.01	0.05	13.7%
PC(P-18:0/18:1)	0.05-100	y = 0.0321x-0.0463	0.9921	0.01	0.05	13.7%
PE(18:0/18:2)	0.01-50	y = 0.9057x-0.8296	0.9920	0.01	0.01	5.8%
PE(P-18:0/18:1)	0.5-100	y = 1.5418-4.6384	0.9910	0.01	0.5	8.7%
LPE(18:0)	1-200	y = 0.0011x-0.0059	0.9929	1	1	10.5%
LPE(P-18:0)	0.05-50	y = 0.4656x-0.2307	0.9579	0.01	0.05	8.1%
LPC(O-16:1)	0.01-200	y = 0.1076x+0.0599	0.9938	0.005	0.01	4.8%
LPC(16:0)	0.01-200	y = 0.1052x+0.0555	0.9947	0.005	0.01	9.8%
SM(d18:1/17:0)	0.05-200	y = 0.1811x-0.1028	0.9933	0.01	0.05	4.3%
TG(18:1/16:0/18:1)	0.01-100	y = 0.0929x-0.0486	0.9982	0.005	0.01	3.4%
DG(17:1/17:1)	0.05-50	y = 0.6048x-0.5845	0.9954	0.01	0.05	3.5%
CE(17:0)	0.1-10	y = 0.0089x+0.0037	0.9824	0.01	0.1	2.8%
cholesterol	5-200	y = 0.0186x+0.0444	0.9907	0.5	5	8.1%

*LOD was estimated with signal to noisy (S/N) more than three.

#LOQ was estimated with signal to noisy (S/N) more than ten.

Supplementary Table S5. Demographics of patients with CRC recruited in this study.

Characteristics	Total (n=23)
Age (mean±s.d., year)	58.74±12.42
BMI (mean±s.d.)	23.43±2.97
Stage (II/III/IV)	
I	4
II	14
III	10
IV	2