Electronic Supplementary Material (ESI) for Analyst.

High resolution ion mobility-mass spectrometry for separation and identification of isomeric lipids

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Supplementary Information

Calculation of reduced mobilities and collision cross sections

Reduced mobilities (K_0) and collision cross sections (Ω) can be calculated be normalising the ion mobility drift time to drift length, potential, pressure and temperature and a modified zero field (so-called Mason-Schamp) equation. This momentum transfer scan law includes field-dependent corrections for both collisional momentum transfer and collision frequency (α and β terms, respectively). See Siems et al., Anal. Chem. 2012, 84, 9782−9791, for more detailed information.

$$K_0 = \frac{L^2}{V\ t_d}\frac{P}{1000\ mbar}\frac{273\ K}{T}$$

$$\Omega = \frac{3}{16}\left(\frac{2\pi}{\mu k T}\right)^{1/2}\frac{qzE}{v_d N}\left[1+\left(\frac{\beta_{MT}}{\alpha_{MT}}\right)^2\left(\frac{v_d}{v_T}\right)^2\right]^{-1/2}$$

K = reduced mobility [cm²/Vs]

L = drift length [cm]

V = drift potential [V]

t_d = drift time [s]

P = pressure in the drift cell [mbar]

T = temperature of the drift cell [K]

Ω = integrated collision cross section

μ = reduced mass of the analyte and the drift gas

k = Boltzmann's constant

q= elementary charge

z = charge number

E = electric field

v_d = drift velocity

N = neutral gas number density

β_{MT} = correction coefficient for momentum transfer

α_{MT} = correction coefficient for collision frequency

v_T = thermal velocity

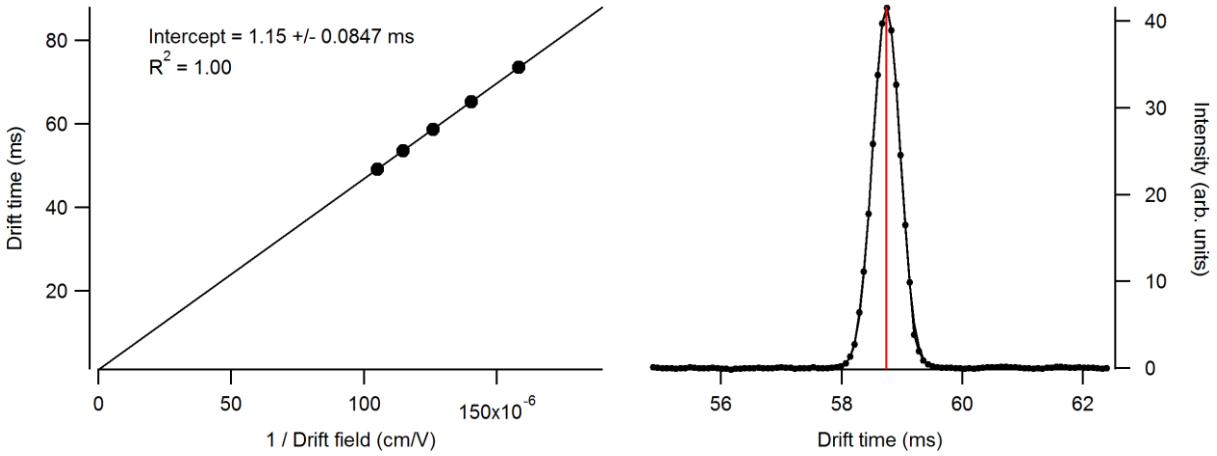

Figure S1. *Left*: Determination of corrected drift times for PI 16:0/18:1 (in yeast polar lipid extract) from a plot of the measured drift time (y-axis) versus the inverse drift field (x-axis). Drift time measurements were carried out at five different drift fields (between 8 and 12 kV over 20 cm) and the y-intercept of the plot (the "nonmobility" component of the drift time) was subtracted from the measured drift time to obtain the corrected drift time. The corrected drift time is then used to calculate reduced mobilities (K_0) and collision cross sections (Ω). *Right*: IM spectrum obtained at 10 kV shows a highly symmetric peak for PI 16:0/18:1 that allows precise extraction of the peak maximum (red line) for drift time correction.

Estimation of measurement uncertainty

For the IMS-TOF instrument configuration described in this paper, we estimate the following uncertainties for the measurement of instrumental parameters: pressure +- 1mbar (0.1%), temperature +- 4 K (1%), drift voltage +- 2V (0.03 %) and drift time extraction +- 0.01 ms (0.03%). Applying conventional propagation of error, this results in a combined uncertainty of 1.1 %.

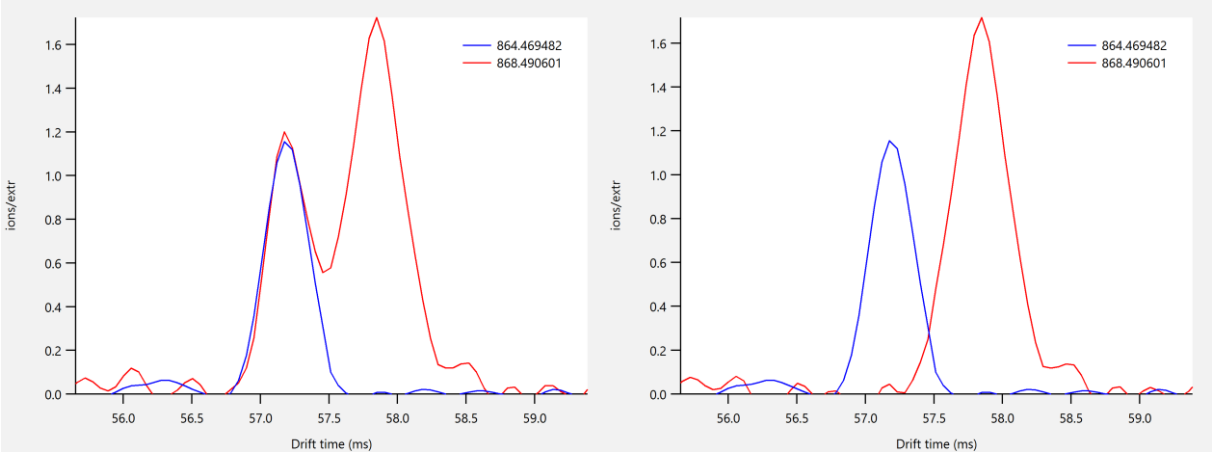

Figure S2. Demonstration of isotopic correction for determination of IMS peak areas. *Left*: Overlay of extracted ion mobilograms for [PC 34:2 + ^{107}Ag]$^+$ (blue) with [PC 34:1 + ^{109}Ag]$^+$ (red), measured in a polar lipid extract of yeast. The blue trace has been rescaled by a factor of 0.114 corresponding to the calculated isotopic abundance of $C_{42}H_{82}NO_8PAg$ (PC 34:2) at the [M+4] isotope. It can be clearly seen that the peak at 57.1 ms in the red trace corresponds to the M+4 isotope of PC 34:2 rather than an isomer of PC 34:1. *Right*: Trace for [PC 34:1 + ^{109}Ag]$^+$ (red) after isotopic correction, *i.e.* subtraction of the scaled blue trace, which corresponds to [PC 34:2 + ^{107}Ag]$^+$.

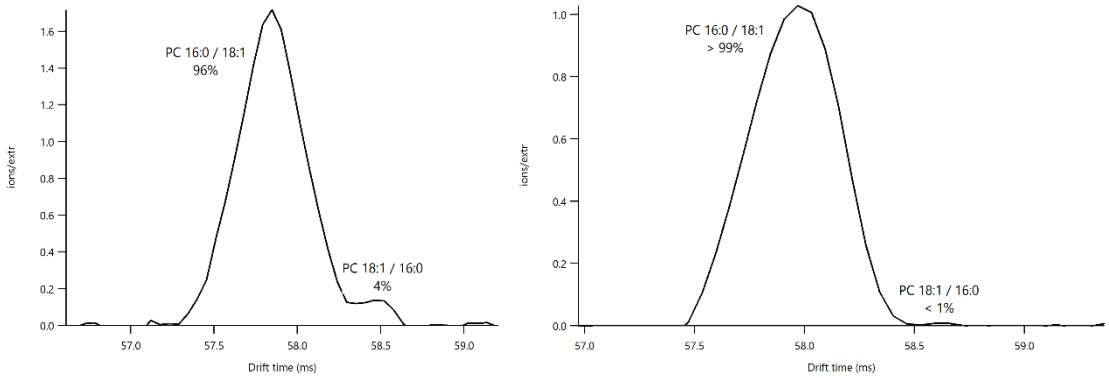

Figure S3. Extracted ion chromatograms for parallel quantification of PC 16:0/18:1 and PC 18:1/16:0 in yeast (*left*) and bovine heart (*right*) polar lipid extracts.

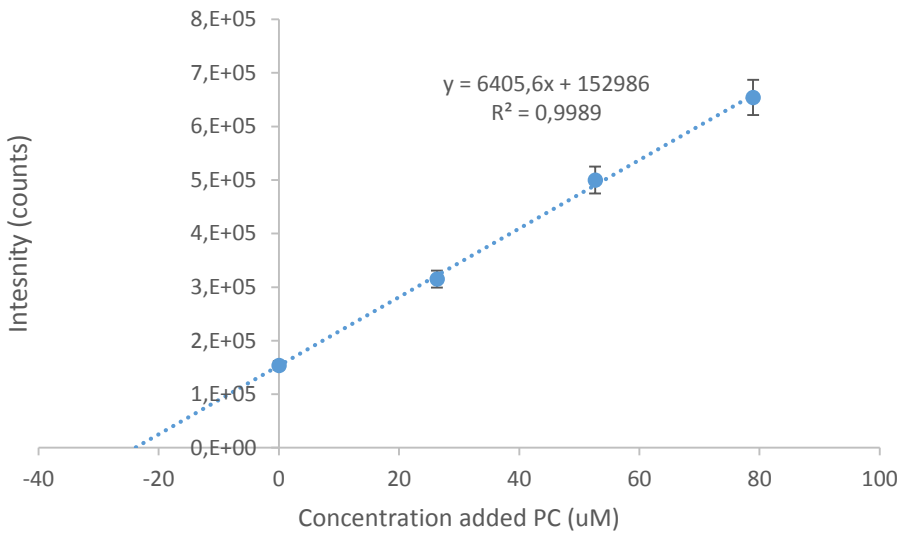

Figure S4. Standard addition experiment for the determination of PC 16:0/18:1 and PC 18:1/16:0 in a porcine brain polar lipid extract. Extrapolation to Intensity = 0 gives the absolute value of the concentration in the 1:20 diluted sample.

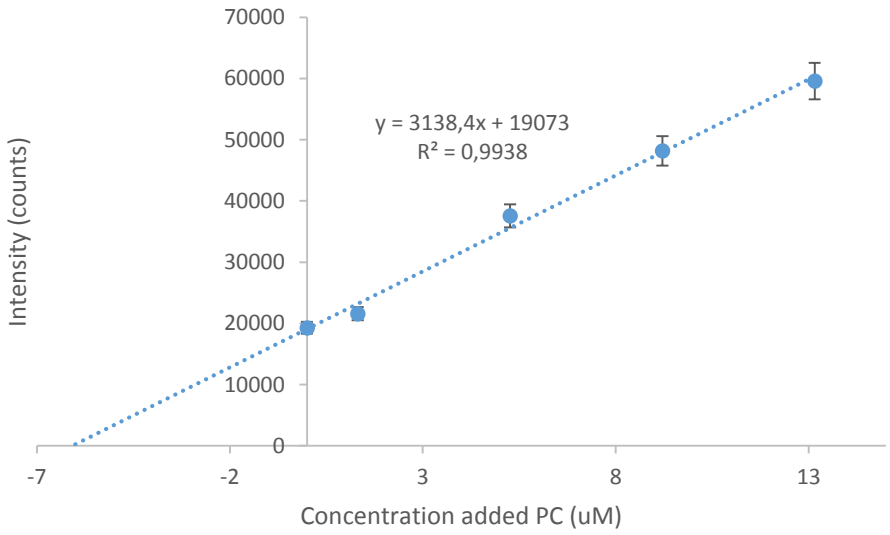

Figure S5. Standard addition experiment for the determination of PC 16:0/18:1 and PC 18:1/16:0 in a yeast polar lipid extract. Extrapolation to Intensity = 0 gives the absolute value of the concentration in the 1:20 diluted sample.

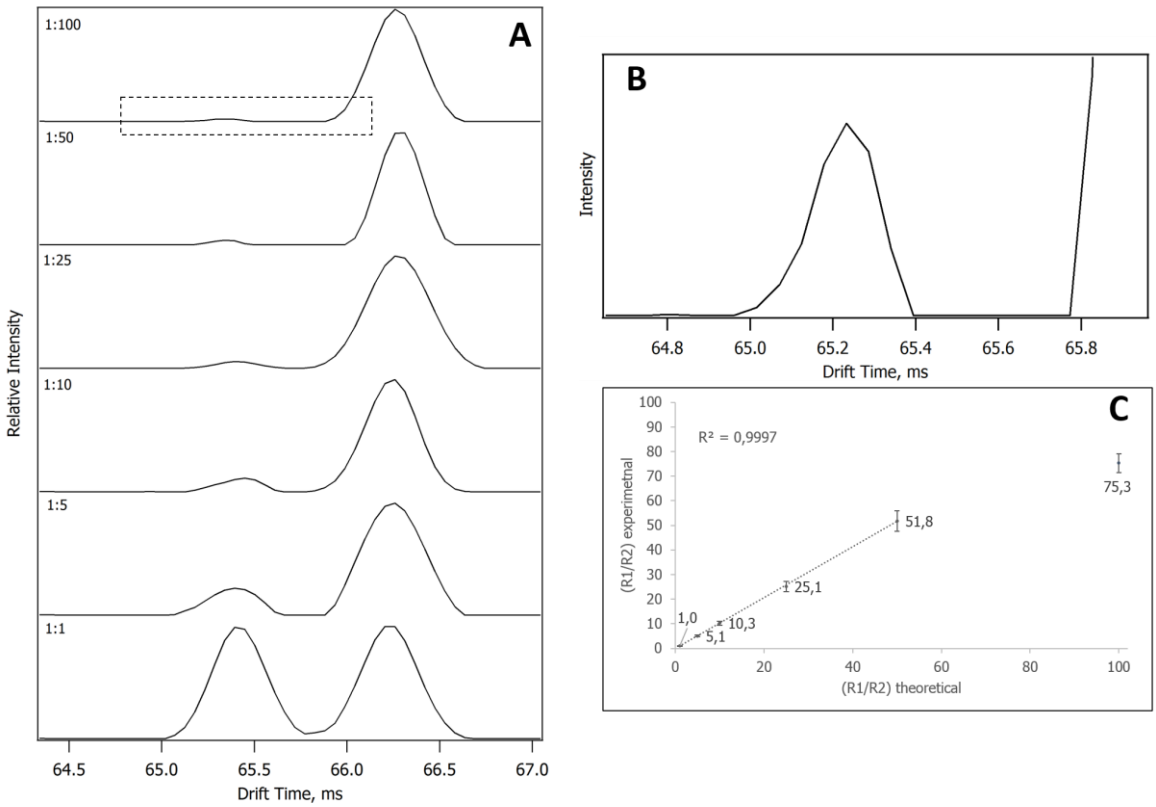

Figure S6. A IMS traces for calibration standards containing PC 16:0/18:1 and PC 18:1/16:0 in known ratios (from 1:1 to 1:100). Concentration of PC 18:1/16:0 was kept constant at 500 nM. At a ratio of 1:100, corresponding to a concentration of 5 nM for PC 16:0/18:1 which is close to the limit of detection of 2 nM (4 fmol/min), it is still possible to measure high quality IM spectra (**B** is a zoom of marked region in the upper trace of **A**). **C** In this concentration range, quantitation is linear for a ratio of up to 1:50 and limited by the LOD (R1 and R2 correspond to concentrations of PC 16:0/18:1 and PC 18:1/16:0, respectively).

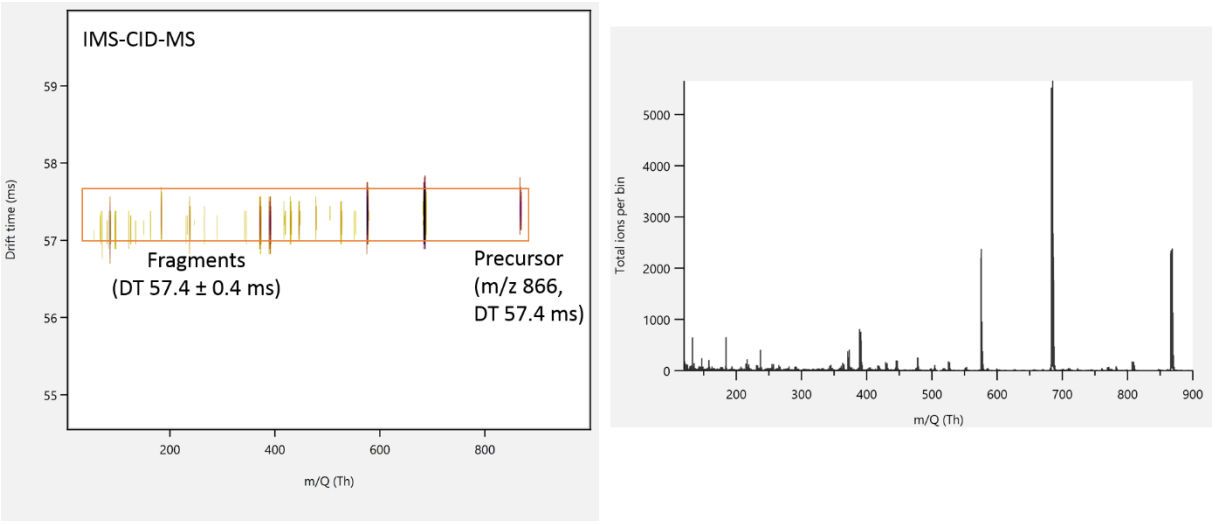

Figure S7. In IMS-CID-MS, fragments are identified based on identical IMS drift times as their precursors, here shown for fragmentation of the silver adduct of PC 16:0/18:1 (left). The drift time regions can be automatically extracted to produce clean, precursor-specific CID MS spectra.

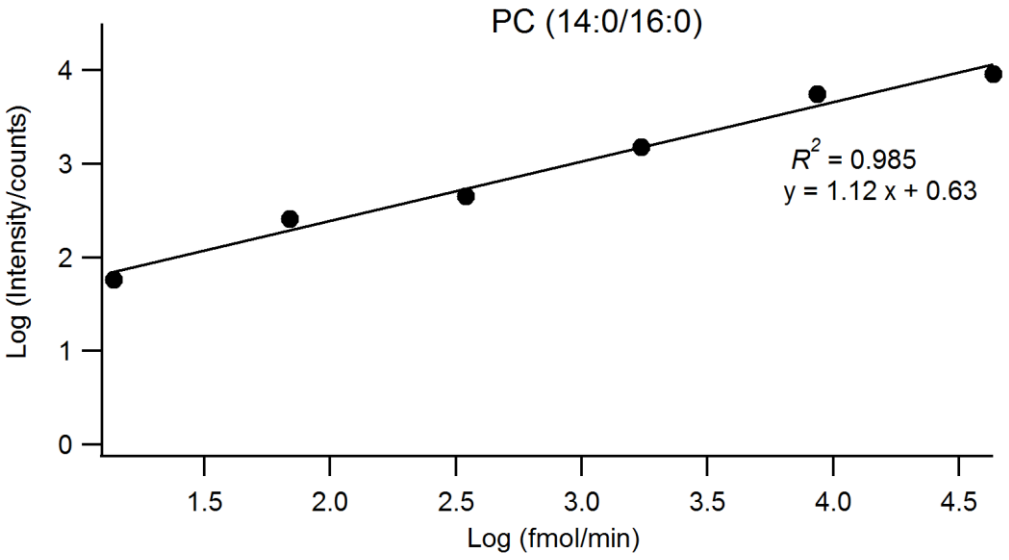

Figure S8. Calibration plot for PC 14:0/16:0 showing linear instrument response for more than 3 orders of magnitude.

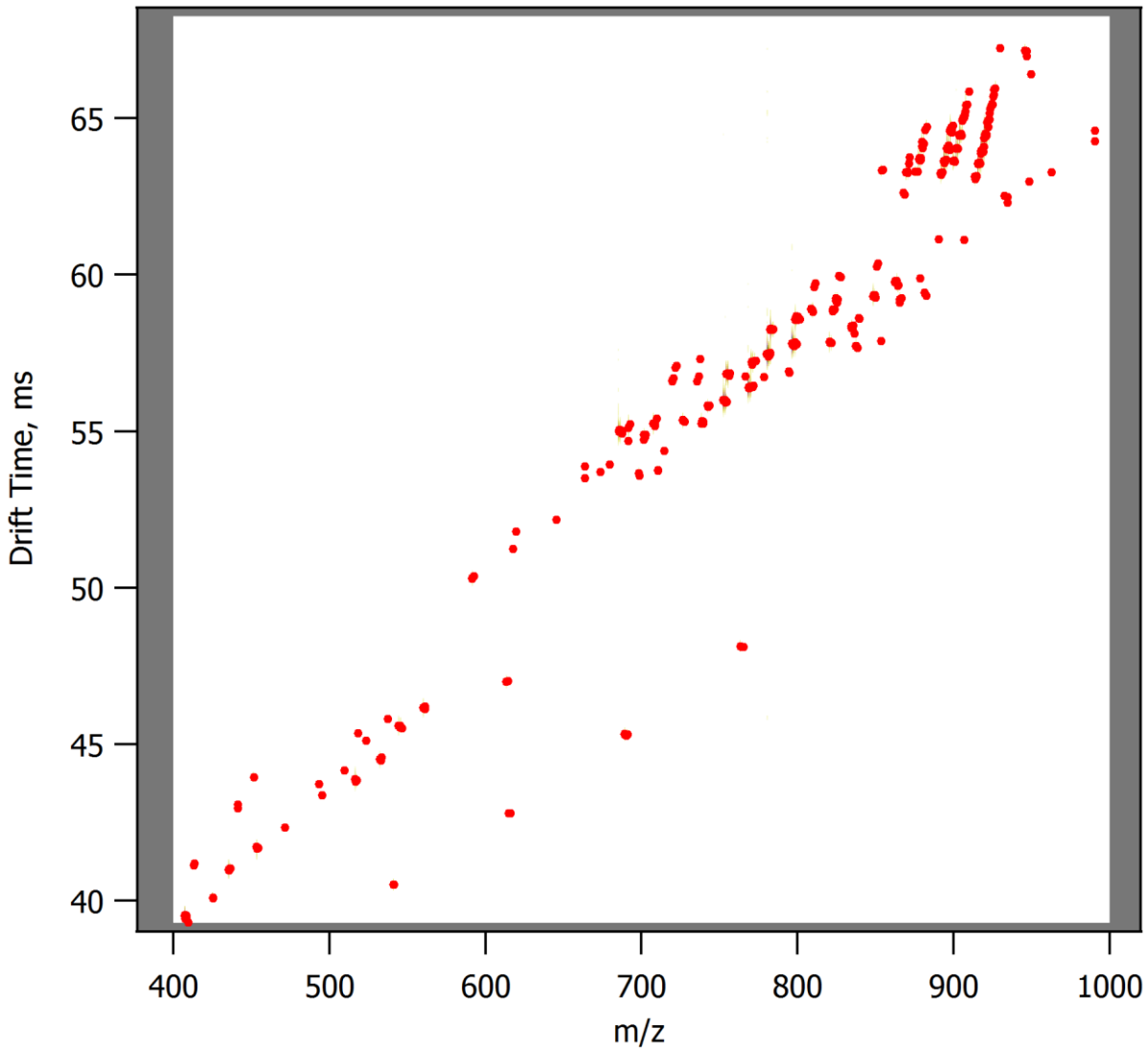

Figure S9. 2d IMS-MS plot showing detection of polar lipid features in yeast. 416 features are detected in IMS-MS mode whereas only 255 peaks were detected in MS only mode, corresponding to an increase of 63% in IMS-MS mode. The features are sharp and difficult to visualize, and are therefore marked by dots. Peaks larger than 1% relative intensity are shown.

Positive Ion Mode

Lipid	Sum Formula	K_0 (cm^2/Vs)	CCS ($Å^2$)	Origin
LPC 16:1	C24 H48 O7 N1 P1 Na1	0.810	227.7	yeast
LPC 18:1	C26 H52 O7 N1 P1 Na1	0.780	236.2	yeast
PC [34:0]	C42 H84 O8 N1 P1 Na1	0.609	301.1	egg yolk
PC [34:1]	C42 H82 O8 N1 P1 Na1	0.608	300.3	egg yolk
PC [34:2]	C42 H80 O8 N1 P1 Na1	0.604	302.6	egg yolk
PC [34:3]	C43 H82 O7 N1 P1 Na1	0.625	292.3	bovine heart
PC [36:1]	C44 H86 O8 N1 P1 Na1	0.597	306.2	egg yolk
PC [36:2]	C44 H84 O8 N1 P1 Na1	0.603	303.1	egg yolk
PC [36:3]	C44 H82 O8 N1 P1 Na1	0.618	295.8	egg yolk
PC [38:6]	C46 H80 O8 N1 P1 Na1	0.609	299.9	egg yolk
PC 14:0-16:1	C38 H76 O8 N1 P1 Na1	0.645	283.6	yeast
PC 14:1-16:1	C38 H74 O8 N1 P1 Na1	0.655	279.6	yeast
PC 16:0-18:1	C40 H78 O8 N1 P1 Na1	0.628	291.4	yeast
PC 16:0-18:1	C42 H82 O8 N1 P1 Na1	0.611	299.1	yeast
PC 16:1-16:1	C40 H76 O8 N1 P1 Na1	0.636	287.8	yeast
PC 16:1-18:1	C42 H80 O8 N1 P1 Na1	0.618	295.3	bovine heart
PC 16:1-18:1	C42 H80 O8 N1 P1 Na1	0.620	295.1	yeast
PC 18:0-18:1	C44 H86 O8 N1 P1 Na1	0.597	306.1	bovine heart
PC 18:0-18:1	C44 H86 O8 N1 P1 Na1	0.597	306.0	yeast
PC 18:1-18:1	C44 H84 O8 N1 P1 Na1	0.603	302.7	bovine heart
PC 18:1-18:1	C44 H84 O8 N1 P1 Na1	0.604	302.8	yeast
PC-O [34:2]	C41 H78 O8 N1 P1 Na1	0.615	297.2	bovine heart
PC-O [34:3]	C42 H80 O7 N1 P1 Na1	0.622	294.0	bovine heart
PC-O [36:2]	C44 H86 O7 N1 P1 Na1	0.603	303.1	bovine heart
PC-O [36:3]	C44 H84 O7 N1 P1 Na1	0.607	301.0	bovine heart
PC-O [36:4]	C43 H78 O8 N1 P1 Na1	0.615	297.2	bovine heart
PC-O [36:5]	C44 H80 O7 N1 P1 Na1	0.622	293.8	bovine heart
PE [36:2]	C41 H78 O8 N1 P1 Na1	0.624	293.0	bovine heart
PE [38:1]	C43 H84 O8 N1 P1 Na1	0.609	299.8	bovine heart
PE [38:2]	C43 H82 O8 N1 P1 Na1	0.612	298.6	bovine heart
PE [38:3]	C43 H80 O8 N1 P1 Na1	0.615	297.1	bovine heart
PE 16:0-18:1	C39 H76 O8 N1 P1 Na1	0.640	286.1	yeast
PE 16:1-16:1	C37 H70 O8 N1 P1 Na1	0.662	276.6	yeast
PE 16:1-18:1	C39 H74 O8 N1 P1 Na1	0.645	283.8	yeast
PE-O [36:3]	C41 H78 O7 N1 P1 Na1	0.631	290.1	bovine heart
PE-O [38:3]	C43 H82 O7 N1 P1 Na1	0.614	297.6	bovine heart

Negative Ion Mode

Lipid	Sum Formula	K_0 (cm^2/Vs)	CCS ($Å^2$)	Origin
CL [72:6]	H143 C81 O17 P2	0.440	412.1	bovine heart
CL [72:7]	H141 C81 O17 P2	0.441	411.5	bovine heart
LPA 16:0	H38 C19 O7 P1	0.954	194.5	yeast
LPA 16:1	H36 C19 O7 P1	0.971	191.1	yeast

LPA 18:0	H42 C21 O7 P1	0.898	206.2	yeast
LPA 18:1	H40 C21 O7 P1	0.932	198.8	yeast
LPE 16:1	H41 C21 N1 O7 P1	0.920	201.1	yeast
LPE 18:1	H45 C23 N1 O7 P1	0.879	210.1	e. coli
LPE 18:1	H45 C23 N1 O7 P1	0.879	210.1	yeast
LPG 16:0	H44 C22 O9 P1	0.862	214.3	e. coli
LPG 16:1	H42 C22 O9 P1	0.871	212.0	e. coli
LPG 17:1	H44 C23 O9 P1	0.865	213.5	e. coli
LPG 18:0	H48 C24 O9 P1	0.831	221.9	e. coli
LPG 18:1	H46 C24 O9 P1	0.846	217.9	e. coli
LPG 18:1	H46 C24 O9 P1	0.851	216.9	yeast
LPI 16:0	H48 C25 O12 P1	0.804	228.7	yeast
LPI 16:1	H46 C25 O12 P1	0.814	226.1	yeast
LPI 18:0	H52 C27 O12 P1	0.778	236.1	yeast
LPI 18:1	H50 C27 O12 P1	0.787	233.4	yeast
mLCL [54:5]	H111 C63 O16 P2	0.502	361.9	bovine heart
PA 16:0-16:1	H66 C35 O8 P1	0.728	251.8	yeast
PA 16:0-18:1	H70 C37 O8 P1	0.707	259.2	yeast
PA 16:1-16:1	H64 C35 O8 P1	0.734	250.0	yeast
PA 16:1-18:1	H68 C37 O8 P1	0.713	257.0	yeast
PA 18:0-18:1	H74 C39 O8 P1	0.684	268.0	yeast
PA 18:1-18:1	H72 C39 O8 P1	0.694	264.0	yeast
PE [33:1]	H73 C38 N1 O8 P1	0.687	266.7	e. coli
PE [36:2]	H77 C41 N1 O8 P1	0.663	276.0	bovine heart
PE [36:2]	H77 C41 N1 O8 P1	0.663	275.9	porcine brain
PE [38:4]	H77 C43 N1 O8 P1	0.657	278.4	bovine heart
PE [38:4]	H77 C43 N1 O8 P1	0.659	277.4	porcine brain
PE [40:4]	H81 C45 N1 O8 P1	0.635	287.9	bovine heart
PE [40:4]	H81 C45 N1 O8 P1	0.635	287.8	porcine brain
PE [40:6]	H77 C45 N1 O8 P1	0.632	289.4	porcine brain
PE-O [34:3]	H73 C39 N1 O7 P1	0.687	266.5	bovine heart
PE-O [36:3]	H77 C41 N1 O7 P1	0.670	273.1	bovine heart
PE-O [36:4]	H75 C41 N1 O7 P1	0.678	270.0	bovine heart
PE-O [36:5]	H73 C41 N1 O7 P1	0.677	270.4	bovine heart
PE-O [38:5]	H77 C43 N1 O7 P1	0.659	277.8	bovine heart
PG [30:0]	H70 C36 O10 P1	0.690	265.6	e. coli
PG [32:0]	H74 C38 O10 P1	0.677	270.3	e. coli
PG [32:1]	H72 C38 O10 P1	0.677	270.4	e. coli
PG [33:1]	H74 C39 O10 P1	0.668	273.7	e. coli
PG [34:0]	H78 C40 O10 P1	0.659	277.6	bovine heart
PG [35:1]	H78 C41 O10 P1	0.653	280.1	e. coli
PG [35:2]	H76 C41 O10 P1	0.656	278.7	e. coli
PG [36:1]	H80 C42 O10 P1	0.646	282.9	e. coli
PG [36:1]	H80 C42 O10 P1	0.644	284.1	bovine heart
PG [36:2]	H78 C42 O10 P1	0.648	282.3	e. coli
PG [36:2]	H78 C42 O10 P1	0.648	281.9	bovine heart
PG [36:3]	H76 C42 O10 P1	0.653	280.0	bovine heart
PG [36:4]	H74 C42 O10 P1	0.658	278.0	bovine heart
PG [37:2]	H80 C43 O10 P1	0.640	285.5	e. coli
PG [38:4]	H78 C44 O10 P1	0.640	285.6	bovine heart

PG 16:0-16:1	H72 C38 O10 P1	0.681	268.7	yeast
PG 16:0-18:1	H76 C40 O10 P1	0.661	276.7	yeast
PG 16:0-18:1	H76 C40 O10 P1	0.660	277.1	e. coli
PG 16:0-18:1	H76 C40 O10 P1	0.660	277.0	bovine heart
PG 16:1-16:1	H70 C38 O10 P1	0.685	267.1	yeast
PG 16:1-18:1	H74 C40 O10 P1	0.665	274.0	bovine heart
PG 16:1-18:1	H74 C40 O10 P1	0.666	273.8	yeast
PG 16:1-18:1	H74 C40 O10 P1	0.665	274.3	e. coli
PI [36:3]	H80 C45 O13 P1	0.616	296.3	bovine heart
PI [36:4]	H78 C45 O13 P1	0.614	297.2	porcine brain
PI [38:3]	H84 C47 O13 P1	0.605	301.7	bovine heart
PI [38:3]	H84 C47 O13 P1	0.600	303.9	porcine brain
PI [38:4]	H82 C47 O13 P1	0.601	303.6	porcine brain
PI [38:4]	H82 C47 O13 P1	0.607	300.7	bovine heart
PI [38:5]	H80 C47 O13 P1	0.604	302.0	porcine brain
PI [38:5]	H80 C47 O13 P1	0.609	299.6	bovine heart
PI 12:0-16:0	H70 C37 O13 P1	0.669	273.5	yeast
PI 14:0-16:0	H74 C39 O13 P1	0.653	280.1	yeast
PI 14:0-16:1	H72 C39 O13 P1	0.657	278.4	yeast
PI 14:1-16:1	H70 C39 O13 P1	0.645	283.6	yeast
PI 16:0-16:1	H76 C41 O13 P1	0.642	284.6	yeast
PI 16:0-18:1	H80 C43 O13 P1	0.628	290.9	yeast
PI 16:1-16:1	H74 C41 O13 P1	0.646	282.9	yeast
PI 16:1-18:1	H78 C43 O13 P1	0.631	289.2	yeast
PI 18:0-18:1	H84 C45 O13 P1	0.613	297.5	yeast
PI 18:0-18:1	H84 C45 O13 P1	0.611	297.7	bovine heart
PI 18:1-18:1	H82 C45 O13 P1	0.617	295.9	yeast
PI 18:1-18:1	H82 C45 O13 P1	0.614	296.2	bovine heart
PS [36:1]	H79 C42 N1 O10 P1	0.632	289.4	porcine brain
PS [36:2]	H77 C42 N1 O10 P1	0.641	285.0	bovine heart
PS [36:2]	H77 C42 N1 O10 P1	0.639	286.5	porcine brain
PS [38:1]	H83 C44 N1 O10 P1	0.617	296.1	porcine brain
PS [38:2]	H81 C44 N1 O10 P1	0.621	294.3	porcine brain
PS [38:3]	H79 C44 N1 O10 P1	0.625	292.4	porcine brain
PS [38:4]	H77 C44 N1 O10 P1	0.627	291.4	porcine brain
PS [40:1]	H87 C46 N1 O10 P1	0.604	302.2	porcine brain
PS [40:2]	H85 C46 N1 O10 P1	0.607	301.0	porcine brain
PS [40:3]	H83 C46 N1 O10 P1	0.611	299.0	porcine brain
PS [40:4]	H81 C46 N1 O10 P1	0.614	297.6	porcine brain
PS [40:5]	H79 C46 N1 O10 P1	0.616	296.4	porcine brain
PS [40:6]	H77 C46 N1 O10 P1	0.618	295.4	porcine brain
PS 16:0-16:1	H71 C38 N1 O10 P1	0.671	272.6	yeast
PS 16:0-18:1	H75 C40 N1 O10 P1	0.656	278.8	yeast
PS 16:1-16:1	H69 C38 N1 O10 P1	0.676	270.6	yeast
PS 16:1-18:1	H73 C40 N1 O10 P1	0.661	276.9	yeast