

## **Supporting Information**

### **Evaluation of Collision Cross Section Calibrants for Structural Analysis of Lipids by Traveling Wave Ion Mobility-Mass Spectrometry**

Kelly M. Hines<sup>1</sup>, Jody C. May<sup>2</sup>, John A. McLean<sup>2</sup> and Libin Xu\*,<sup>1</sup>

<sup>1</sup>Department of Medicinal Chemistry, University of Washington, Seattle, WA 98195;

<sup>2</sup>Department of Chemistry, Center for Innovative Technology, Vanderbilt Institute of Chemical Biology, Vanderbilt Institute for Integrative Biosystems Research and Education, Vanderbilt University, Nashville, TN 37235

Address correspondence to:

Libin Xu, Ph.D.

Department of Medicinal Chemistry

University of Washington

Tel: (206) 543-1080

Fax: (206) 685-3252

Email: libinxu@uw.edu

## Supplemental Experimental Section

**Experimental Parameters for the Agilent 6560 IM-MS.** The nitrogen drift gas was maintained at a constant pressure and temperature which was *ca.* 4.0 Torr and 31 °C, respectively, for all experiments. Pressure regulation was achieved using a gas flow controller (640B MKS Instruments) which responds to the readout from an absolute pressure capacitance gauge (CDG 500, Agilent) which is mounted directly to the drift tube. The drift tube is at ambient temperature with gradients minimized using a thermal blanket and is monitored using a type K thermocouple (Omega Engineering) connected to the center of the drift chamber. Mass analysis was performed in “extended dynamic range” mode (digitizer set to 2 GHz) using an *m/z* range from 50 to 3200 ( $m/\Delta m = 20\text{-}30,000$ ). Samples were directly infused into the primary nebulizer of the dual-inlet “Jet Stream” ion source at a rate of 5 µL/min using a syringe pump (Cole-Parmer). The primary nebulizer of was operated with the following conditions: gas temperature 300 °C, drying gas 8 L/min, nebulizer 10 psig, sheath gas temperature 200 °C, sheath gas flow 8 L/min, capillary voltage 3.5 kV, nozzle voltage 2.0 kV. For the HFAP series, the “Calibrant B” pressurized infusion line of the 6560 instrument was used to infuse the sample into secondary nebulizer. In this mode, the HFAP sample is introduced concurrent with the samples originating from the primary nebulizer, yielding an internal calibrant for all IM-MS spectra. The drift field was stepped ( $n = 9$ ; 7.1, 8.3, 9.6, 10.9, 12.2, 13.5, 14.8, 17.3, and 19.9 V/cm) over the course of the data acquisition. In this manner, the time corresponding to ion transit outside of the drift region could be determined using a linear regression analysis.

**Experimental Parameters for the Waters Synapt G2-Si.** Source and T-Wave parameters for positive and negative modes (displayed as “positive/negative” where settings differ) were: capillary voltage, +3.5 kV/-3.0 kV; source temperature, 100 °C/120 °C; sampling cone, 30 V;

source offset, 10 V; desolvation temperature, 200 °C; source gas flow, 0 mL/min; cone gas flow, 10 L/hr; desolvation gas flow, 800 L/hr; nebulizer gas flow, 5 bar; IMS wave delay, 1000 µs; trap gas flow, 2 mL/min; helium cell gas flow, 180 mL/min; IMS gas flow, 90 mL/min; IMS pressure, 2.87 mbar Nitrogen; trap wave velocity, 311 m/s; trap wave height, 4.0 V; transfer wave velocity, 175 m/s; transfer wave height, 4.0 V; enhanced duty cycle (EDC) delay coefficient, 1.35/1.41.

## Supplemental Figures and Tables

**Table S1.** Details for lipid standards from Avanti Polar Lipids, their respective concentrations in the calibration mixture, and drift tube CCS measurements of PEs and PCs in N<sub>2</sub>.

PE	Catalog No.	uM	[M+H] <sup>+</sup>	CCS (Å <sup>2</sup> )	RSD (%)	[M-H] <sup>-</sup>	CCS (Å <sup>2</sup> )	RSD (%)
PE 6:0/6:0	850697C	5	412.21	202.1	0.3	410.20	199.3	0.3
PE 8:0/8:0	850699C	5	468.27	217.9	0.5	466.26	210.8	0.3
PE 10:0/10:0	850700C	5	524.33	233.0	0.4	522.32	223.2	0.4
PE 12:0/12:0	850702X	5	580.40	246.7	0.4	578.38	235.7	0.4
PE 14:0/14:0	850745X	5	636.46	259.2	0.4	634.45	247.7	0.4
PE 15:0/15:0	850704X	10	664.49	265.0	0.4	662.48	253.3	0.3
PE 16:1/16:1	850706C	10	688.49	263.8	0.4	686.48	256.3	0.4
PE 16:0/16:0	850705X	10	692.52	271.5	0.3	690.51	259.4	0.4
PE 16:0/18:1	850757C	10	718.54	273.4	0.4	716.52	264.5	0.4
PE 17:0/17:0	830756X	10	720.55	277.4	0.4	718.54	265.5	0.4
PE 18:1/18:1	850725C	10	744.55	277.9	0.4	742.54	269.2	0.4
PE 18:0/18:1	850758C	10	746.57	280.2	0.4	744.56	270.7	0.4
PE 18:0/18:0	850715X	10	748.59	282.7	0.4	746.57	271.6	0.4
PE 20:4/20:4	850800C	10	788.52	280.1	0.4	786.51	274.2	0.4

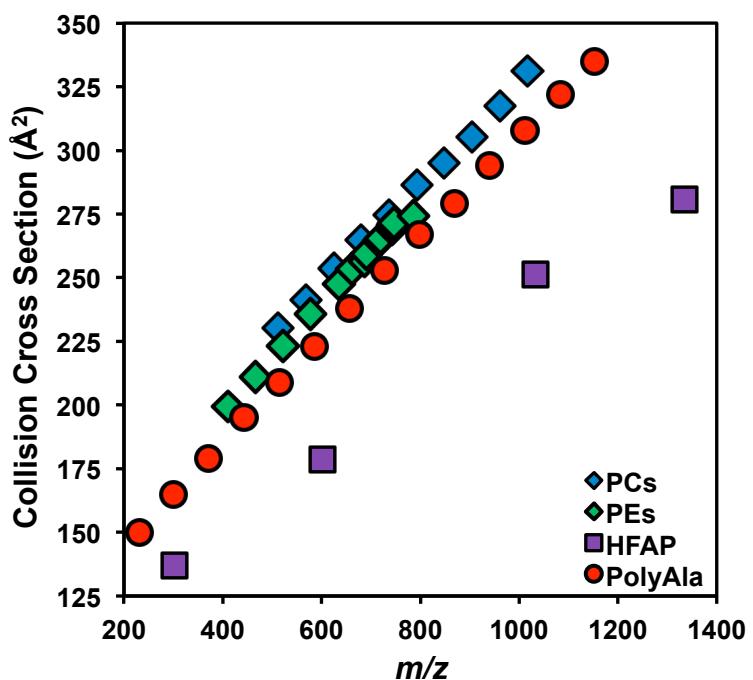
PC	Catalog No.	uM	[M+H] <sup>+</sup>	CCS (Å <sup>2</sup> )	RSD (%)	[M+CH <sub>3</sub> COO] <sup>-</sup>	CCS (Å <sup>2</sup> )	RSD (%)
PC 6:0/6:0	850305C	5	454.26	213.3	0.4	512.26	230.2	0.3
PC 8:0/8:0	850315C	5	510.32	230.6	0.4	568.33	241.4	0.4
PC 10:0/10:0	850325C	5	566.38	245.4	0.4	624.39	253.6	0.4
PC 12:0/12:0	850335C	5	622.44	258.4	0.4	680.45	264.6	0.4
PC 14:0/14:0	850345C	5	678.51	270.4	0.4	736.51	274.4	0.3
PC 16:0/16:0	850355C	10	734.57	282.5	0.4	792.58	286.2	0.4
PC 18:0/18:0	850365C	10	790.63	294.5	0.4	848.64	295.1	0.5
PC 20:0/20:0	850368C	10	846.69	306.4	0.4	904.70	305.1	0.6
PC 22:0/22:0	850371C	10	902.76	319.1	0.4	960.76	317.4	0.6
PC 24:0/24:0	850373C	10	958.82	330.9	0.7	1016.83	331.0	0.7

**Table S2.** Drift tube CCS measurements of PolyAla in N<sub>2</sub>.

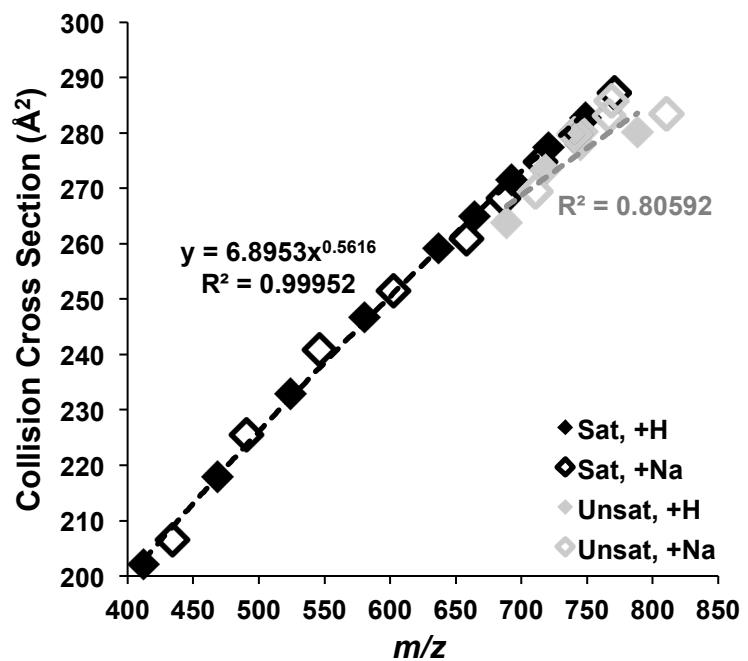
PolyAla	[M+H] <sup>+</sup>	CCS (Å <sup>2</sup> )	RSD (%)	[M-H] <sup>-</sup>	CCS (Å <sup>2</sup> )	RSD (%)
A <sub>3</sub>	161.09	132.1	0.2	159.08	139.8	0.5
A <sub>4</sub>	232.13	152.2	0.5	230.11	155.2	0.2
A <sub>5</sub>	303.17	171.5	0.4	301.15	169.0	0.3
A <sub>6</sub>	374.20	180.8	0.3	372.19	183.1	0.2
A <sub>7</sub>	445.24	193.3	0.3	443.23	198.1	0.2
A <sub>8</sub>	516.28	207.6	0.3	514.26	211.4	0.2
A <sub>9</sub>	587.32	223.2	0.3	585.30	224.6	0.1
A <sub>10</sub>	658.35	236.3	0.3	656.34	238.6	0.1
A <sub>11</sub>	729.39	248.9	0.3	727.37	251.9	0.1
A <sub>12</sub>	800.43	261.9	0.3	798.41	264.2	0.1
A <sub>13</sub>	871.46	274.9	0.2	869.45	274.5	0.2
A <sub>14</sub>	942.50	287.5	0.2	940.49	288.7	0.2
A <sub>15</sub>	1013.54	299.0	0.3	1011.52	302.2	0.1
A <sub>16</sub>	1084.58	308.1	0.3	1082.56	314.8	0.1

**Table S3.** Drift tube CCS values for HFAP in N<sub>2</sub>.

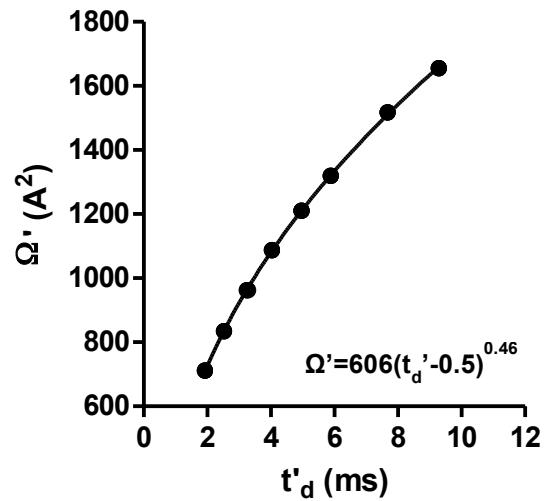
[M+H] <sup>+</sup>	CCS (Å <sup>2</sup> )	RSD (%)	[M-F] <sup>-</sup>	CCS (Å <sup>2</sup> )	RSD (%)
322.05	154.1	0.3	302.00	139.1	0.3
622.03	202.9	0.3	601.98	179.3	0.3
922.01	243.0	0.4	1033.99	252.8	0.3
1221.99	281.0	0.4	1333.97	281.3	0.4



**Figure S1.** IM-MS conformational space plot showing the trends in CCS- $m/z$  for each of the four calibrants from negative ESI DTIM-MS measurements in N<sub>2</sub>.



**Figure S2.** Comparison of the power fits for *m/z* vs. CCS for saturated (*n*=9) versus unsaturated PEs (*n*=5).



**Figure S3.** TWIM CCS calibration plots for TAAs, where  $\Omega'$  is the corrected drift tube CCS and  $t'_d$  is the mass-independent drift time. Calibration was performed in positive mode with wave settings of 40 V and 550 m/s. The calibration fit had  $R^2 \geq 0.995$ .

**Table S4.** Summary of CCS calibration precision and accuracy at wave velocity 550 m/s and wave height 40 V.

Average RSD of Calibrated CCSs (%)

Analyte	PE	PC	HFAP	PolyAla	TAA
PC (+)	0.1	0.0	0.3	0.1	0.1
PE (+)	0.1	0.1	0.3	0.1	0.1
PC (-)	0.2	0.0	0.2	0.1	-
PE (-)	0.1	0.1	0.2	0.1	-

Average Error of Calibrated CCSs (%)

Analyte	PE	PC	HFAP	PolyAla	TAA
PC (+)	0.3	0.3	0.9	1.7	4.4
PE (+)	0.4	0.5	1.0	1.6	2.2
PC (-)	0.9	0.3	0.4	2.8	-
PE (-)	0.2	0.8	0.4	2.7	-

**Table S5.** Summary of results from TWIM CCS calibration of PEs with PEs at 550 m/s and 40V.

Compound [M+H] <sup>+</sup>	Avg. Cal. CCS (Å <sup>2</sup> , n=9)	RSD (%)	DTIM CCS (Å <sup>2</sup> )	% Error (DTIM:Cal)
PE 6:0/6:0	202.0	0.0	202.1	0.0
PE 8:0/8:0	218.8	0.1	217.9	0.4
PE 10:0/10:0	233.6	0.0	233.0	0.3
PE 12:0/12:0	246.9	0.0	246.7	0.1
PE 14:0/14:0	258.8	0.0	259.2	-0.2
PE 15:0/15:0	265.6	0.1	265.0	0.2
PE 16:1/16:1	262.8	0.0	263.8	-0.4
PE 16:0/16:0	271.4	0.1	271.5	0.0
PE 16:0/18:1	273.3	0.1	273.4	0.0
PE 17:0/17:0	277.3	0.2	277.4	-0.1
PE 18:1/18:1	276.3	0.1	277.9	-0.6
PE 18:0/18:1	279.9	0.1	280.2	-0.1
PE 18:0/18:0	283.9	0.0	282.7	0.4
PE 20:4/20:4	277.2	0.1	280.1	-1.0

Compound [M-H] <sup>-</sup>	Avg. Cal. CCS (Å <sup>2</sup> , n=9)	RSD (%)	DTIM CCS (Å <sup>2</sup> )	% Error (DTIM:Cal)
PE 6:0/6:0	198.9	0.1	199.3	-0.2
PE 8:0/8:0	211.5	0.2	210.8	0.3
PE 10:0/10:0	223.3	0.1	223.2	0.0
PE 12:0/12:0	235.4	0.1	235.7	-0.1
PE 14:0/14:0	247.6	0.1	247.7	0.0
PE 15:0/15:0	253.3	0.1	253.3	0.0
PE 16:1/16:1	255.7	0.1	256.3	-0.2
PE 16:0/16:0	259.9	0.1	259.4	0.2
PE 16:0/18:1	264.4	0.1	264.5	-0.1
PE 17:0/17:0	265.8	0.1	265.5	0.1
PE 18:1/18:1	268.6	0.1	269.2	-0.2
PE 18:0/18:1	270.7	0.0	270.7	0.0
PE 18:0/18:0	272.8	0.1	271.6	0.5
PE 20:4/20:4	273.7	0.1	274.2	-0.2

**Table S6.** Summary of results from TWIM CCS calibration of PCs with PCs at 550 m/s and 40V.

<b>Compound [M+H]<sup>+</sup></b>	<b>Avg. Cal. CCS (Å<sup>2</sup>, n=9)</b>	<b>RSD (%)</b>	<b>DTIM CCS (Å<sup>2</sup>)</b>	<b>% Error (DTIM:Cal)</b>
PC 6:0/6:0	214.2	0.0	213.3	0.4
PC 8:0/8:0	230.2	0.0	230.6	-0.2
PC 10:0/10:0	244.6	0.0	245.4	-0.3
PC 12:0/12:0	258.3	0.0	258.4	0.0
PC 14:0/14:0	270.8	0.0	270.4	0.1
PC 16:0/16:0	282.3	0.1	282.5	-0.1
PC 18:0/18:0	294.0	0.0	294.5	-0.2
PC 20:0/20:0	306.0	0.1	306.4	-0.1
PC 22:0/22:0	318.1	0.1	319.1	-0.3
PC 24:0/24:0	329.8	0.0	330.9	-0.3

<b>Compound [M+CH<sub>3</sub>COO]<sup>-</sup></b>	<b>Avg. Cal. CCS (Å<sup>2</sup>, n=9)</b>	<b>RSD (%)</b>	<b>DTIM CCS (Å<sup>2</sup>)</b>	<b>% Error (DTIM:Cal)</b>
PC 6:0/6:0	230.5	0.0	230.2	0.1
PC 8:0/8:0	242.1	0.0	241.4	0.3
PC 10:0/10:0	252.6	0.0	253.6	-0.4
PC 12:0/12:0	264.0	0.0	264.6	-0.2
PC 14:0/14:0	274.1	0.0	274.4	-0.1
PC 16:0/16:0	285.2	0.0	286.2	-0.3
PC 18:0/18:0	296.1	0.0	295.1	0.4
PC 20:0/20:0	306.6	0.0	305.1	0.5
PC 22:0/22:0	318.0	0.1	317.4	0.2
PC 24:0/24:0	329.4	0.1	331.0	-0.5

**Table S7.** Summary of results from TWIM CCS calibration of PolyAla with PolyAla at 550 m/s and 40V.

Compound [M+H] <sup>+</sup>	Avg. Cal. CCS (Å <sup>2</sup> , n=9)	RSD (%)	DTIM CCS (Å <sup>2</sup> )	% Error (DTIM:Cal)
A <sub>3</sub>	154.7	0.1	152.2	1.7
A <sub>4</sub>	167.9	0.0	171.5	-2.1
A <sub>5</sub>	180.3	0.1	180.8	-0.3
A <sub>6</sub>	193.8	0.1	193.3	0.3
A <sub>7</sub>	207.6	0.0	207.6	0.0
A <sub>8</sub>	223.7	0.1	223.2	0.2
A <sub>9</sub>	237.0	0.2	236.3	0.3
A <sub>10</sub>	249.2	0.1	248.9	0.1
A <sub>11</sub>	262.1	0.1	261.9	0.1
A <sub>12</sub>	274.9	0.0	274.9	0.0
A <sub>13</sub>	287.2	0.1	287.5	-0.1
A <sub>14</sub>	298.6	0.0	299.0	-0.1

Compound [M-H] <sup>-</sup>	Avg. Cal. CCS (Å <sup>2</sup> , n=9)	RSD (%)	DTIM CCS (Å <sup>2</sup> )	% Error (DTIM:Cal)
A <sub>3</sub>	155.3	0.1	155.2	0.1
A <sub>4</sub>	169.0	0.0	169.0	0.0
A <sub>5</sub>	183.5	0.1	183.1	0.2
A <sub>6</sub>	197.4	0.1	198.1	-0.4
A <sub>7</sub>	211.6	0.1	211.4	0.1
A <sub>8</sub>	223.7	0.1	224.6	-0.4
A <sub>9</sub>	238.3	0.1	238.6	-0.1
A <sub>10</sub>	251.6	0.1	251.9	-0.1
A <sub>11</sub>	264.4	0.1	264.2	0.1
A <sub>12</sub>	276.3	0.1	274.5	0.7
A <sub>13</sub>	289.4	0.1	288.7	0.3
A <sub>14</sub>	301.9	0.1	302.2	-0.1
A <sub>15</sub>	314.1	0.1	314.8	-0.2
A <sub>16</sub>	325.8	0.1	326.1	-0.1

**Table S8.** Summary of results from TWIM CCS calibration of HFAP with HFAP at 550 m/s and 40V.

Compound [M+H] <sup>+</sup>	Avg. Cal. CCS (Å <sup>2</sup> , n=9)	RSD (%)	DTIM CCS (Å <sup>2</sup> )	% Error (DTIM:Cal)
322.06	153.2	0.0	153.4	-0.1
622.06	202.5	0.1	202.4	0.0
922.05	242.4	0.1	242.7	-0.1
1222.04	281.0	0.0	280.9	0.0

Compound [M-F] <sup>-</sup>	Avg. Cal. CCS (Å <sup>2</sup> , n=9)	RSD (%)	DTIM CCS (Å <sup>2</sup> )	% Error (DTIM:Cal)
302.01	136.7	0.0	136.9	-0.2
602.01	178.3	0.1	178.4	0.0
1034.03	251.2	0.1	251.2	0.0
1334.03	280.9	0.1	280.9	0.0

**Table S9.** Summary of results from TWIM CCS calibration of TAAs with TAAs at 550 m/s and 40V.

Compound M+	Avg. Cal. CCS ( $\text{\AA}^2$ , n=9)	RSD (%)	DTIM CCS ( $\text{\AA}^2$ )	% Error (DTIM:Cal)
TAA3	143.3	0.2	144.1	-0.5
TAA4	167.7	0.3	166.6	0.6
TAA5	191.0	0.1	190.1	0.5
TAA6	212.8	0.1	213.5	-0.3
TAA7	236.1	0.1	236.4	-0.1
TAA8	256.9	0.1	256.6	0.1
TAA10	291.9	0.1	293.5	-0.5
TAA12	320.2	0.0	319.0	0.4

**Table S10.** Summary of results from TWIM CCS calibration of PEs with HFAP at 550 m/s and 40V.

<b>Compound [M+H]<sup>+</sup></b>	<b>Avg. Cal. CCS (Å<sup>2</sup>, n=9)</b>	<b>RSD (%)</b>	<b>DTIM CCS (Å<sup>2</sup>)</b>	<b>% Error (DTIM:Cal)</b>
PE 6:0/6:0	197.5	0.1	202.1	-2.3
PE 8:0/8:0	217.4	0.1	217.9	-0.2
PE 10:0/10:0	234.1	0.2	233.0	0.5
PE 12:0/12:0	248.5	0.2	246.7	0.7
PE 14:0/14:0	261.0	0.2	259.2	0.7
PE 15:0/15:0	268.0	0.4	265.0	1.1
PE 16:1/16:1	265.2	0.2	263.8	0.5
PE 16:0/16:0	273.9	0.2	271.5	0.9
PE 16:0/18:1	275.9	0.2	273.4	0.9
PE 17:0/17:0	279.9	0.3	277.4	0.9
PE 18:1/18:1	278.9	0.2	277.9	0.4
PE 18:0/18:1	282.5	0.4	280.2	0.8
PE 18:0/18:0	286.5	0.4	282.7	1.3
PE 20:4/20:4	279.8	0.2	280.1	-0.1

<b>Compound [M-H]<sup>-</sup></b>	<b>Avg. Cal. CCS (Å<sup>2</sup>, n=9)</b>	<b>RSD (%)</b>	<b>DTIM CCS (Å<sup>2</sup>)</b>	<b>% Error (DTIM:Cal)</b>
PE 6:0/6:0	197.1	0.4	199.3	-1.1
PE 8:0/8:0	210.5	0.5	210.8	-0.1
PE 10:0/10:0	222.6	0.3	223.2	-0.3
PE 12:0/12:0	234.9	0.2	235.7	-0.3
PE 14:0/14:0	247.0	0.4	247.7	-0.3
PE 15:0/15:0	252.6	0.4	253.3	-0.3
PE 16:1/16:1	254.9	0.3	256.3	-0.5
PE 16:0/16:0	259.0	0.1	259.4	-0.2
PE 16:0/18:1	263.3	0.1	264.5	-0.4
PE 17:0/17:0	264.8	0.3	265.5	-0.3
PE 18:1/18:1	267.4	0.0	269.2	-0.7
PE 18:0/18:1	269.4	0.1	270.7	-0.5
PE 18:0/18:0	271.5	0.2	271.6	0.0
PE 20:4/20:4	272.3	0.2	274.2	-0.7

**Table S11.** Summary of results from TWIM CCS calibration of PEs with PCs at 550 m/s and 40V.

Compound [M+H] <sup>+</sup>	Avg. Cal. CCS (Å <sup>2</sup> , n=9)	RSD (%)	DTIM CCS (Å <sup>2</sup> )	% Error (DTIM:Cal)
PE 6:0/6:0	202.6	0.0	202.1	0.2
PE 8:0/8:0	219.0	0.0	217.9	0.5
PE 10:0/10:0	233.5	0.0	233.0	0.2
PE 12:0/12:0	246.5	0.0	246.7	-0.1
PE 14:0/14:0	258.2	0.0	259.2	-0.4
PE 15:0/15:0	264.9	0.1	265.0	0.0
PE 16:1/16:1	262.2	0.0	263.8	-0.6
PE 16:0/16:0	270.6	0.0	271.5	-0.3
PE 16:0/18:1	272.6	0.0	273.4	-0.3
PE 17:0/17:0	276.5	0.2	277.4	-0.3
PE 18:1/18:1	275.5	0.0	277.9	-0.9
PE 18:0/18:1	279.1	0.1	280.2	-0.4
PE 18:0/18:0	283.1	0.1	282.7	0.1
PE 20:4/20:4	276.4	0.0	280.1	-1.3

Compound [M-H] <sup>-</sup>	Avg. Cal. CCS (Å <sup>2</sup> , n=9)	RSD (%)	DTIM CCS (Å <sup>2</sup> )	% Error (DTIM:Cal)
PE 6:0/6:0	204.7	0.0	199.3	2.7
PE 8:0/8:0	215.5	0.2	210.8	2.2
PE 10:0/10:0	225.7	0.0	223.2	1.1
PE 12:0/12:0	236.5	0.0	235.7	0.3
PE 14:0/14:0	247.5	0.2	247.7	-0.1
PE 15:0/15:0	252.7	0.2	253.3	-0.2
PE 16:1/16:1	254.8	0.2	256.3	-0.6
PE 16:0/16:0	258.6	0.0	259.4	-0.3
PE 16:0/18:1	262.8	0.0	264.5	-0.6
PE 17:0/17:0	264.2	0.2	265.5	-0.5
PE 18:1/18:1	266.7	0.0	269.2	-0.9
PE 18:0/18:1	268.7	0.1	270.7	-0.8
PE 18:0/18:0	270.7	0.2	271.6	-0.3
PE 20:4/20:4	271.5	0.2	274.2	-1.0

**Table S12.** Summary of results from TWIM CCS calibration of PEs with PolyAla at 550 m/s and 40V.

Compound [M+H] <sup>+</sup>	Avg. Cal. CCS (Å <sup>2</sup> , n=9)	RSD (%)	DTIM CCS (Å <sup>2</sup> )	% Error (DTIM:Cal)
PE 6:0/6:0	200.6	0.2	202.1	-0.7
PE 8:0/8:0	220.0	0.2	217.9	1.0
PE 10:0/10:0	236.5	0.2	233.0	1.5
PE 12:0/12:0	250.8	0.1	246.7	1.7
PE 14:0/14:0	263.3	0.1	259.2	1.6
PE 15:0/15:0	270.3	0.2	265.0	2.0
PE 16:1/16:1	267.5	0.1	263.8	1.4
PE 16:0/16:0	276.3	0.1	271.5	1.8
PE 16:0/18:1	278.3	0.1	273.4	1.8
PE 17:0/17:0	282.3	0.1	277.4	1.8
PE 18:1/18:1	281.3	0.1	277.9	1.2
PE 18:0/18:1	285.0	0.2	280.2	1.7
PE 18:0/18:0	289.0	0.1	282.7	2.2
PE 20:4/20:4	282.2	0.1	280.1	0.8

Compound [M-H] <sup>-</sup>	Avg. Cal. CCS (Å <sup>2</sup> , n=9)	RSD (%)	DTIM CCS (Å <sup>2</sup> )	% Error (DTIM:Cal)
PE 6:0/6:0	204.2	0.1	199.3	2.5
PE 8:0/8:0	217.7	0.2	210.8	3.3
PE 10:0/10:0	229.9	0.1	223.2	3.0
PE 12:0/12:0	242.3	0.1	235.7	2.8
PE 14:0/14:0	254.6	0.2	247.7	2.8
PE 15:0/15:0	260.3	0.2	253.3	2.7
PE 16:1/16:1	262.6	0.1	256.3	2.5
PE 16:0/16:0	266.7	0.1	259.4	2.8
PE 16:0/18:1	271.1	0.1	264.5	2.5
PE 17:0/17:0	272.5	0.1	265.5	2.6
PE 18:1/18:1	275.2	0.1	269.2	2.2
PE 18:0/18:1	277.2	0.1	270.7	2.4
PE 18:0/18:0	279.3	0.1	271.6	2.9
PE 20:4/20:4	280.2	0.1	274.2	2.2

**Table S13.** Summary of results from TWIM CCS calibration of PEs with TAAs at 550 m/s and 40V.

Compound [M+H] <sup>+</sup>	Avg. Cal. CCS (Å <sup>2</sup> , n=9)	RSD (%)	DTIM CCS (Å <sup>2</sup> )	% Error (DTIM:Cal)
PE 6:0/6:0	200.9	0.1	202.1	-0.6
PE 8:0/8:0	221.4	0.1	217.9	1.6
PE 10:0/10:0	238.3	0.1	233.0	2.3
PE 12:0/12:0	252.7	0.1	246.7	2.5
PE 14:0/14:0	265.3	0.0	259.2	2.3
PE 15:0/15:0	272.2	0.1	265.0	2.7
PE 16:1/16:1	269.4	0.0	263.8	2.1
PE 16:0/16:0	278.1	0.1	271.5	2.4
PE 16:0/18:1	280.1	0.1	273.4	2.4
PE 17:0/17:0	284.0	0.2	277.4	2.4
PE 18:1/18:1	283.0	0.1	277.9	1.8
PE 18:0/18:1	286.6	0.1	280.2	2.3
PE 18:0/18:0	290.5	0.1	282.7	2.8
PE 20:4/20:4	283.9	0.1	280.1	1.3

**Table S14.** Summary of results from TWIM CCS calibration of PCs with HFAP at 550 m/s and 40V.

<b>Compound [M+H]<sup>+</sup></b>	<b>Avg. Cal. CCS (Å<sup>2</sup>, n=9)</b>	<b>RSD (%)</b>	<b>DTIM CCS (Å<sup>2</sup>)</b>	<b>% Error (DTIM:Cal)</b>
PC 6:0/6:0	211.8	0.1	213.3	-0.7
PC 8:0/8:0	230.4	0.2	230.6	-0.1
PC 10:0/10:0	246.4	0.2	245.4	0.4
PC 12:0/12:0	261.1	0.2	258.4	1.1
PC 14:0/14:0	274.1	0.2	270.4	1.4
PC 16:0/16:0	285.7	0.4	282.5	1.1
PC 18:0/18:0	297.3	0.3	294.5	1.0
PC 20:0/20:0	309.1	0.4	306.4	0.9
PC 22:0/22:0	320.5	0.3	319.1	0.4
PC 24:0/24:0	331.5	0.4	330.9	0.2

<b>Compound [M+CH<sub>3</sub>COO]<sup>-</sup></b>	<b>Avg. Cal. CCS (Å<sup>2</sup>, n=9)</b>	<b>RSD (%)</b>	<b>DTIM CCS (Å<sup>2</sup>)</b>	<b>% Error (DTIM:Cal)</b>
PC 6:0/6:0	228.1	0.3	230.2	-0.9
PC 8:0/8:0	241.2	0.2	241.4	-0.1
PC 10:0/10:0	252.5	0.1	253.6	-0.4
PC 12:0/12:0	264.6	0.0	264.6	0.0
PC 14:0/14:0	275.0	0.0	274.4	0.2
PC 16:0/16:0	286.1	0.1	286.2	0.0
PC 18:0/18:0	296.9	0.2	295.1	0.6
PC 20:0/20:0	306.9	0.2	305.1	0.6
PC 22:0/22:0	317.7	0.2	317.4	0.1
PC 24:0/24:0	328.3	0.4	331.0	-0.8

**Table S15.** Summary of results from TWIM CCS calibration of PCs with PEs at 550 m/s and 40V.

<b>Compound [M+H]<sup>+</sup></b>	<b>Avg. Cal. CCS (Å<sup>2</sup>, n=9)</b>	<b>RSD (%)</b>	<b>DTIM CCS (Å<sup>2</sup>)</b>	<b>% Error (DTIM:Cal)</b>
PC 6:0/6:0	214.0	0.0	213.3	0.3
PC 8:0/8:0	230.3	0.0	230.6	-0.1
PC 10:0/10:0	244.9	0.0	245.4	-0.2
PC 12:0/12:0	258.9	0.0	258.4	0.2
PC 14:0/14:0	271.5	0.1	270.4	0.4
PC 16:0/16:0	283.1	0.1	282.5	0.2
PC 18:0/18:0	294.9	0.1	294.5	0.1
PC 20:0/20:0	307.1	0.1	306.4	0.2
PC 22:0/22:0	319.2	0.3	319.1	0.0
PC 24:0/24:0	330.9	0.3	330.9	0.0

<b>Compound [M+CH<sub>3</sub>COO]<sup>-</sup></b>	<b>Avg. Cal. CCS (Å<sup>2</sup>, n=9)</b>	<b>RSD (%)</b>	<b>DTIM CCS (Å<sup>2</sup>)</b>	<b>% Error (DTIM:Cal)</b>
PC 6:0/6:0	228.6	0.1	230.2	-0.7
PC 8:0/8:0	241.7	0.1	241.4	0.1
PC 10:0/10:0	253.2	0.1	253.6	-0.2
PC 12:0/12:0	265.7	0.1	264.6	0.4
PC 14:0/14:0	276.5	0.1	274.4	0.8
PC 16:0/16:0	288.4	0.2	286.2	0.8
PC 18:0/18:0	299.9	0.2	295.1	1.6
PC 20:0/20:0	310.8	0.2	305.1	1.9
PC 22:0/22:0	322.6	0.3	317.4	1.6
PC 24:0/24:0	334.3	0.4	331.0	1.0

**Table S16.** Summary of results from TWIM CCS calibration of PCs with PolyAla at 550 m/s and 40V.

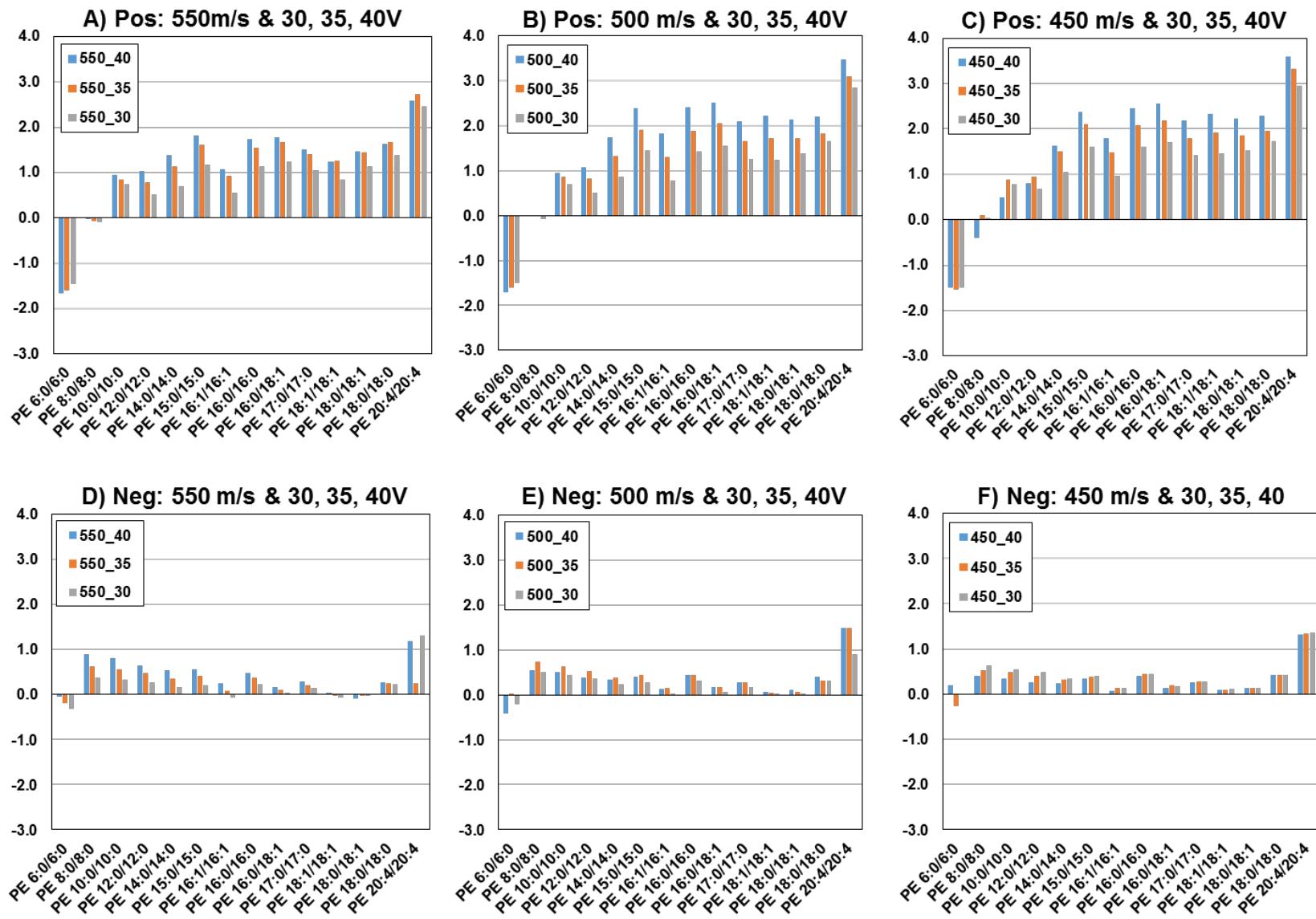
Compound [M+H] <sup>+</sup>	Avg. Cal. CCS (Å <sup>2</sup> , n=9)	RSD (%)	DTIM CCS (Å <sup>2</sup> )	% Error (DTIM:Cal)
PC 6:0/6:0	214.5	0.2	213.3	0.6
PC 8:0/8:0	232.8	0.2	230.6	1.0
PC 10:0/10:0	248.7	0.2	245.4	1.3
PC 12:0/12:0	263.4	0.1	258.4	1.9
PC 14:0/14:0	276.4	0.1	270.4	2.2
PC 16:0/16:0	288.2	0.1	282.5	2.0
PC 18:0/18:0	300.0	0.0	294.5	1.9
PC 20:0/20:0	311.9	0.1	306.4	1.8
PC 22:0/22:0	323.6	0.0	319.1	1.4
PC 24:0/24:0	334.9	0.1	330.9	1.2

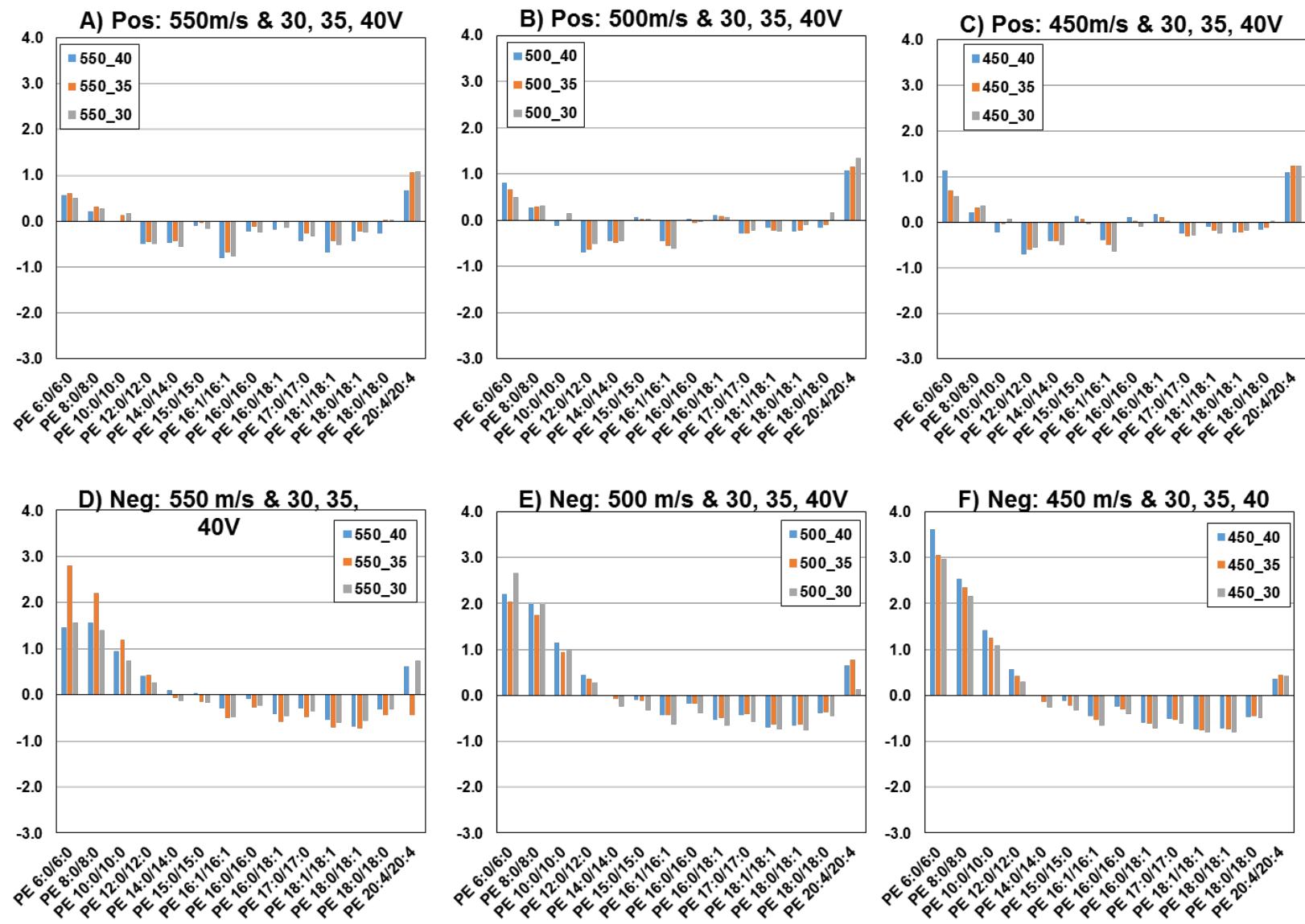
Compound [M+CH <sub>3</sub> COO] <sup>-</sup>	Avg. Cal. CCS (Å <sup>2</sup> , n=9)	RSD (%)	DTIM CCS (Å <sup>2</sup> )	% Error (DTIM:Cal)
PC 6:0/6:0	235.4	0.1	230.2	2.3
PC 8:0/8:0	248.6	0.1	241.4	3.0
PC 10:0/10:0	260.1	0.1	253.6	2.6
PC 12:0/12:0	272.4	0.1	264.6	2.9
PC 14:0/14:0	282.9	0.1	274.4	3.1
PC 16:0/16:0	294.2	0.1	286.2	2.8
PC 18:0/18:0	305.1	0.1	295.1	3.4
PC 20:0/20:0	315.4	0.1	305.1	3.4
PC 22:0/22:0	326.4	0.0	317.4	2.8
PC 24:0/24:0	337.2	0.1	331.0	1.9

**Table S17.** Summary of results from TWIM CCS calibration of PCs with TAAs at 550 m/s and 40V.

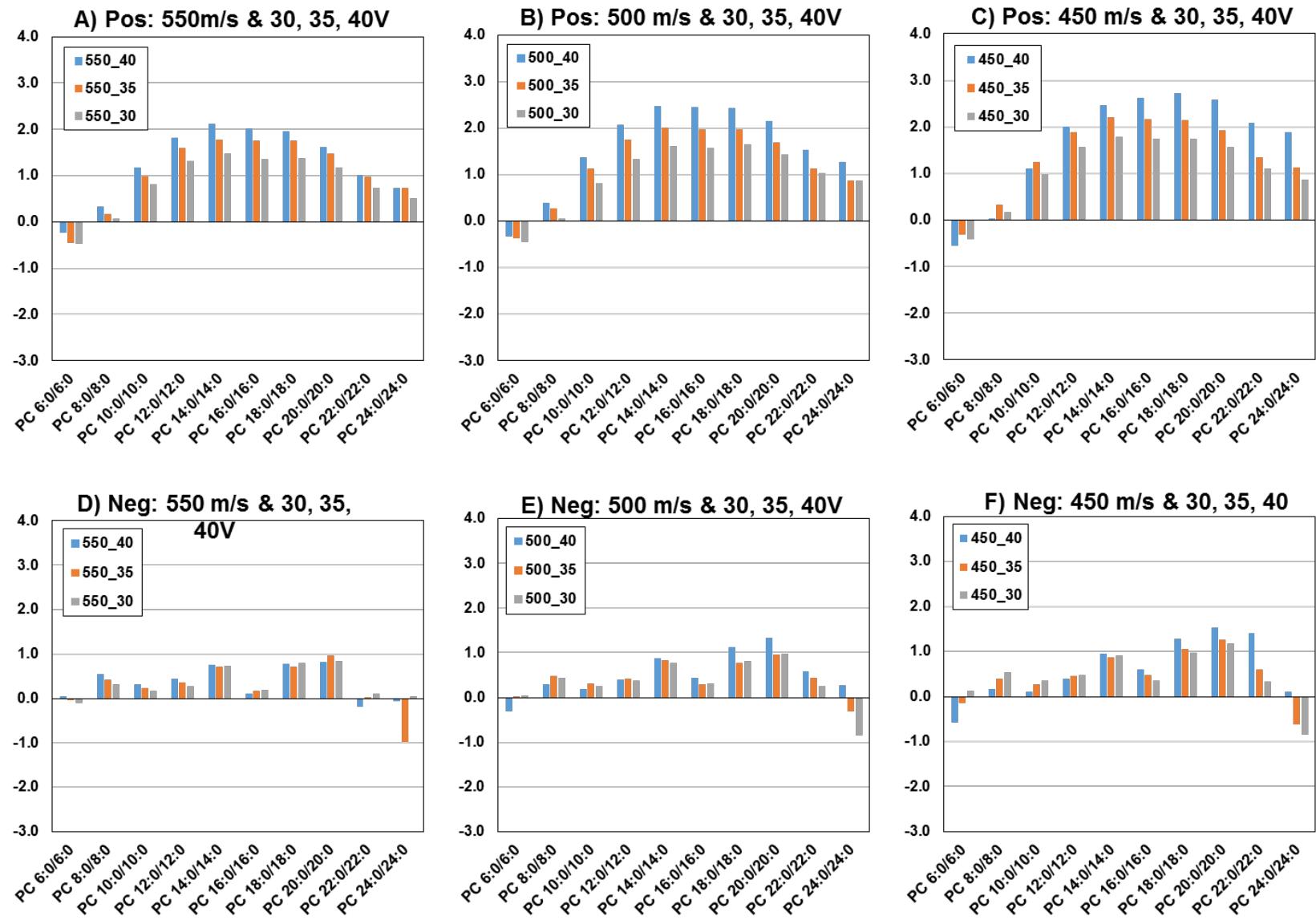
Compound [M+H] <sup>+</sup>	Avg. Cal. CCS (Å <sup>2</sup> , n=9)	RSD (%)	DTIM CCS (Å <sup>2</sup> )	% Error (DTIM:Cal)
PC 6:0/6:0	225.1	0.1	213.3	1.1
PC 8:0/8:0	242.6	0.1	230.6	1.7
PC 10:0/10:0	258.0	0.1	245.4	2.1
PC 12:0/12:0	271.8	0.0	258.4	2.7
PC 14:0/14:0	284.0	0.0	270.4	2.9
PC 16:0/16:0	295.5	0.1	282.5	2.6
PC 18:0/18:0	307.0	0.0	294.5	2.3
PC 20:0/20:0	318.3	0.1	306.4	2.1
PC 22:0/22:0	329.3	0.1	319.1	1.5
PC 24:0/24:0	334.7	0.1	330.9	1.1



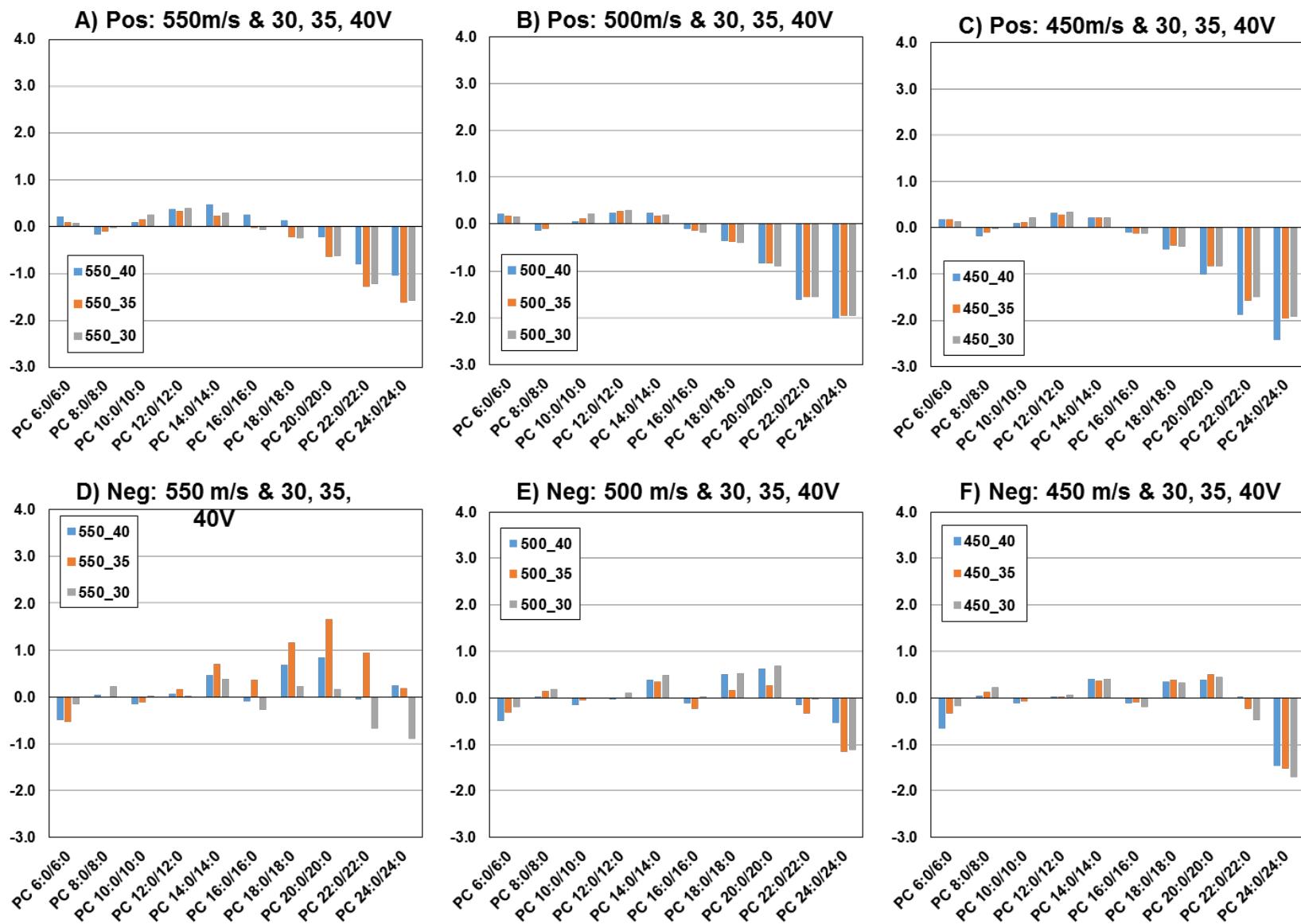
**Figure S4.** Results from the HFAP calibration of PEs at varied traveling wave heights and velocities in positive (A-C) and negative (D-F) mode.



**Figure S5.** Results from the PC calibration of PEs at varied traveling wave heights and velocities in positive (A-C) and negative (D-F) modes.



**Figure S6.** Results from the HFAP calibration of PCs at varied traveling wave heights and velocities in positive (A-C) and negative (D-F) modes.



**Figure S7.** Results from the PE calibration of PCs at varied traveling wave heights and velocities in positive (A-C) and negative (D-F) modes.

**Table S18.** Summary of results from TWIM CCS calibration of PEs with HFAP at varied wave heights and velocities (at 550, 500 and 450 m/s and 40, 35, and 30V), as shown in Figure S4.

Compound [M+H] <sup>+</sup>	Avg. Cal. CCS (Å <sup>2</sup> , n=9)	RSD (%)	DTIM CCS (Å <sup>2</sup> )	% Error (DTIM:Cal)
PE 6:0/6:0	198.9	0.1	202.1	1.6
PE 8:0/8:0	217.8	0.1	217.9	0.1
PE 10:0/10:0	234.9	0.1	233.0	0.8
PE 12:0/12:0	248.7	0.2	246.7	0.8
PE 14:0/14:0	262.4	0.3	259.2	1.3
PE 15:0/15:0	269.8	0.4	265.0	1.8
PE 16:1/16:1	266.9	0.4	263.8	1.2
PE 16:0/16:0	276.4	0.4	271.5	1.8
PE 16:0/18:1	278.6	0.4	273.4	1.9
PE 17:0/17:0	281.8	0.3	277.4	1.6
PE 18:1/18:1	282.3	0.5	277.9	1.6
PE 18:0/18:1	284.8	0.3	280.2	1.7
PE 18:0/18:0	287.8	0.3	282.7	1.8
PE 20:4/20:4	288.5	0.4	280.1	3.0

Compound [M-H] <sup>-</sup>	Avg. Cal. CCS (Å <sup>2</sup> , n=9)	RSD (%)	DTIM CCS (Å <sup>2</sup> )	% Error (DTIM:Cal)
PE 6:0/6:0	199.0	0.18	199.3	0.1
PE 8:0/8:0	212.0	0.15	210.8	0.6
PE 10:0/10:0	224.3	0.14	223.2	0.5
PE 12:0/12:0	236.7	0.11	235.7	0.4
PE 14:0/14:0	248.5	0.10	247.7	0.3
PE 15:0/15:0	254.3	0.09	253.3	0.4
PE 16:1/16:1	256.5	0.08	256.3	0.1
PE 16:0/16:0	260.4	0.07	259.4	0.4
PE 16:0/18:1	264.9	0.05	264.5	0.1
PE 17:0/17:0	266.1	0.05	265.5	0.2
PE 18:1/18:1	269.3	0.06	269.2	0.0
PE 18:0/18:1	270.8	0.08	270.7	0.1
PE 18:0/18:0	272.5	0.07	271.6	0.3
PE 20:4/20:4	277.4	0.37	274.2	1.2

**Table S19.** Summary of results from TWIM CCS calibration of PEs with PCs at varied wave heights and velocities (at 550, 500 and 450 m/s and 40, 35, and 30V), as shown in Figure S5.

Compound [M+H] <sup>+</sup>	Avg. Cal. CCS (Å <sup>2</sup> , n=9)	RSD (%)	DTIM CCS (Å <sup>2</sup> )	% Error (DTIM:Cal)
PE 6:0/6:0	203.5	0.1	202.1	0.7
PE 8:0/8:0	218.5	0.1	217.9	0.3
PE 10:0/10:0	233.0	0.1	233.0	0.0
PE 12:0/12:0	245.3	0.1	246.7	0.6
PE 14:0/14:0	258.0	0.1	259.2	0.5
PE 15:0/15:0	265.0	0.1	265.0	0.0
PE 16:1/16:1	262.2	0.1	263.8	0.6
PE 16:0/16:0	271.3	0.1	271.5	0.1
PE 16:0/18:1	273.5	0.1	273.4	0.0
PE 17:0/17:0	276.6	0.1	277.4	0.2
PE 18:1/18:1	277.0	0.2	277.9	0.3
PE 18:0/18:1	279.6	0.1	280.2	0.2
PE 18:0/18:0	282.5	0.1	282.7	0.1
PE 20:4/20:4	283.2	0.2	280.1	1.1

Compound [M-H] <sup>-</sup>	Avg. Cal. CCS (Å <sup>2</sup> , n=9)	RSD (%)	DTIM CCS (Å <sup>2</sup> )	% Error (DTIM:Cal)
PE 6:0/6:0	204.3	0.7	199.3	2.5
PE 8:0/8:0	215.0	0.3	210.8	2.0
PE 10:0/10:0	225.6	0.2	223.2	1.1
PE 12:0/12:0	236.6	0.1	235.7	0.4
PE 14:0/14:0	247.5	0.1	247.7	0.1
PE 15:0/15:0	252.9	0.1	253.3	0.2
PE 16:1/16:1	255.0	0.1	256.3	0.5
PE 16:0/16:0	258.7	0.1	259.4	0.3
PE 16:0/18:1	263.0	0.1	264.5	0.6
PE 17:0/17:0	264.3	0.1	265.5	0.5
PE 18:1/18:1	267.3	0.1	269.2	0.7
PE 18:0/18:1	268.8	0.1	270.7	0.7
PE 18:0/18:0	270.5	0.1	271.6	0.4
PE 20:4/20:4	275.3	0.4	274.2	0.4

**Table S20.** Summary of results from TWIM CCS calibration of PCs with HFAP at varied wave heights and velocities (at 550, 500 and 450 m/s and 40, 35, and 30V), as shown in Figure S6.

<b>Compound [M+H]<sup>+</sup></b>	<b>Avg. Cal. CCS (Å<sup>2</sup>, n=9)</b>	<b>RSD (%)</b>	<b>DTIM CCS (Å<sup>2</sup>)</b>	<b>% Error (DTIM:Cal)</b>
PC 6:0/6:0	212.4	0.1	213.3	0.4
PC 8:0/8:0	231.1	0.1	230.6	0.2
PC 10:0/10:0	248.0	0.2	245.4	1.1
PC 12:0/12:0	262.8	0.3	258.4	1.7
PC 14:0/14:0	275.8	0.3	270.4	2.0
PC 16:0/16:0	288.0	0.4	282.5	2.0
PC 18:0/18:0	300.3	0.4	294.5	2.0
PC 20:0/20:0	311.7	0.4	306.4	1.7
PC 22:0/22:0	323.0	0.4	319.1	1.2
PC 24:0/24:0	334.1	0.4	330.9	0.1

<b>Compound [M+CH<sub>3</sub>COO]<sup>-</sup></b>	<b>Avg. Cal. CCS (Å<sup>2</sup>, n=9)</b>	<b>RSD (%)</b>	<b>DTIM CCS (Å<sup>2</sup>)</b>	<b>% Error (DTIM:Cal)</b>
PC 6:0/6:0	230.0	0.2	230.2	0.1
PC 8:0/8:0	242.4	0.1	241.4	0.4
PC 10:0/10:0	254.2	0.1	253.6	0.2
PC 12:0/12:0	265.7	0.1	264.6	0.4
PC 14:0/14:0	276.7	0.1	274.4	0.8
PC 16:0/16:0	287.1	0.2	286.2	0.3
PC 18:0/18:0	297.8	0.2	295.1	0.9
PC 20:0/20:0	308.4	0.2	305.1	1.1
PC 22:0/22:0	318.6	0.4	317.4	0.4
PC 24:0/24:0	329.8	0.5	331.0	0.4

**Table S21.** Summary of results from TWIM CCS calibration of PCs with PEs at varied wave heights and velocities (at 550, 500 and 450 m/s and 40, 35, and 30V), as shown in Figure S7.

<b>Compound [M+H]<sup>+</sup></b>	<b>Avg. Cal. CCS (Å<sup>2</sup>, n=9)</b>	<b>RSD (%)</b>	<b>DTIM CCS (Å<sup>2</sup>)</b>	<b>% Error (DTIM:Cal)</b>
PC 6:0/6:0	213.6	0.0	213.3	0.2
PC 8:0/8:0	230.4	0.1	230.6	0.1
PC 10:0/10:0	245.8	0.1	245.4	0.2
PC 12:0/12:0	259.2	0.1	258.4	0.3
PC 14:0/14:0	271.1	0.1	270.4	0.3
PC 16:0/16:0	282.3	0.1	282.5	0.1
PC 18:0/18:0	293.6	0.2	294.5	0.3
PC 20:0/20:0	304.1	0.2	306.4	0.8
PC 22:0/22:0	314.5	0.3	319.1	1.4
PC 24:0/24:0	324.9	0.4	330.9	1.8

<b>Compound [M+CH<sub>3</sub>COO]<sup>-</sup></b>	<b>Avg. Cal. CCS (Å<sup>2</sup>, n=9)</b>	<b>RSD (%)</b>	<b>DTIM CCS (Å<sup>2</sup>)</b>	<b>% Error (DTIM:Cal)</b>
PC 6:0/6:0	229.3	0.2	230.2	0.4
PC 8:0/8:0	241.7	0.1	241.4	0.1
PC 10:0/10:0	253.4	0.1	253.6	0.1
PC 12:0/12:0	264.7	0.1	264.6	0.1
PC 14:0/14:0	275.6	0.1	274.4	0.4
PC 16:0/16:0	286.0	0.2	286.2	0.1
PC 18:0/18:0	296.5	0.3	295.1	0.5
PC 20:0/20:0	307.0	0.4	305.1	0.6
PC 22:0/22:0	317.0	0.4	317.4	0.1
PC 24:0/24:0	328.1	0.7	331.0	0.9

**Table S22.** Summary of results from TWIM CCS calibration of PEs with HFAP with a ramped wave velocity (900-300 m/s) and 40V wave height.

Compound [M+H] <sup>+</sup>	Avg. Cal. CCS (Å <sup>2</sup> , n=3)	DTIM CCS (Å <sup>2</sup> )	% Error (DTIM:Cal)
PE 6:0/6:0	190.3	202.1	5.8
PE 8:0/8:0	209.0	217.9	4.1
PE 10:0/10:0	225.9	233.0	3.0
PE 12:0/12:0	240.0	246.7	2.7
PE 14:0/14:0	251.7	259.2	2.9
PE 15:0/15:0	256.6	265.0	3.2
PE 16:1/16:1	254.4	263.8	3.6
PE 16:0/16:0	262.9	271.5	3.2
PE 16:0/18:1	264.5	273.4	3.3
PE 17:0/17:0	267.8	277.4	3.5
PE 18:1/18:1	267.1	277.9	3.9
PE 18:0/18:1	269.8	280.2	3.7
PE 18:0/18:0	273.0	282.7	3.4
PE 20:4/20:4	268.3	280.1	4.2

Compound [M-H] <sup>-</sup>	Avg. Cal. CCS (Å <sup>2</sup> , n=3)	DTIM CCS (Å <sup>2</sup> )	% Error (DTIM:Cal)
PE 6:0/6:0	190.3	199.3	4.5
PE 8:0/8:0	203.5	210.8	3.5
PE 10:0/10:0	214.8	223.2	3.8
PE 12:0/12:0	225.9	235.7	4.2
PE 14:0/14:0	237.0	247.7	4.3
PE 15:0/15:0	242.3	253.3	4.3
PE 16:1/16:1	245.0	256.3	4.4
PE 16:0/16:0	247.8	259.4	4.5
PE 16:0/18:1	251.7	264.5	4.8
PE 17:0/17:0	253.2	265.5	4.6
PE 18:1/18:1	255.9	269.2	4.9
PE 18:0/18:1	258.7	270.7	4.4
PE 18:0/18:0	259.9	271.6	4.3
PE 20:4/20:4	259.6	274.2	5.3

**Table S23.** Summary of results from TWIM CCS calibration of PEs with PCs with a ramped wave velocity (900-300 m/s) and 40V wave height.

Compound [M+H] <sup>+</sup>	Avg. Cal. CCS (Å <sup>2</sup> , n=3)	DTIM CCS (Å <sup>2</sup> )	% Error (DTIM:Cal)
PE 6:0/6:0	199.4	202.1	1.3
PE 8:0/8:0	218.3	217.9	0.2
PE 10:0/10:0	235.8	233.0	1.2
PE 12:0/12:0	250.4	246.7	1.5
PE 14:0/14:0	262.8	259.2	1.4
PE 15:0/15:0	267.9	265.0	1.1
PE 16:1/16:1	265.6	263.8	0.7
PE 16:0/16:0	274.6	271.5	1.1
PE 16:0/18:1	276.3	273.4	1.1
PE 17:0/17:0	279.8	277.4	0.9
PE 18:1/18:1	279.1	277.9	0.4
PE 18:0/18:1	282.0	280.2	0.6
PE 18:0/18:0	285.4	282.7	1.0
PE 20:4/20:4	280.3	280.1	0.1

Compound [M-H] <sup>-</sup>	Avg. Cal. CCS (Å <sup>2</sup> , n=3)	DTIM CCS (Å <sup>2</sup> )	% Error (DTIM:Cal)
PE 6:0/6:0	200.7	199.3	0.7
PE 8:0/8:0	213.7	210.8	1.4
PE 10:0/10:0	225.1	223.2	0.9
PE 12:0/12:0	236.5	235.7	0.4
PE 14:0/14:0	248.2	247.7	0.2
PE 15:0/15:0	253.8	253.3	0.2
PE 16:1/16:1	256.6	256.3	0.1
PE 16:0/16:0	259.6	259.4	0.1
PE 16:0/18:1	263.8	264.5	0.3
PE 17:0/17:0	265.5	265.5	0.0
PE 18:1/18:1	268.3	269.2	0.3
PE 18:0/18:1	271.3	270.7	0.2
PE 18:0/18:0	272.7	271.6	0.4
PE 20:4/20:4	272.4	274.2	0.7

**Table S24.** Summary of results from TWIM CCS calibration of PCs with HFAP with a ramped wave velocity (900-300 m/s) and 40V wave height.

Compound [M+H] <sup>+</sup>	Avg. Cal. CCS (Å <sup>2</sup> , n=3)	DTIM CCS (Å <sup>2</sup> )	% Error (DTIM:Cal)
PC 6:0/6:0	203.4	213.3	4.7
PC 8:0/8:0	220.4	230.6	4.4
PC 10:0/10:0	235.6	245.4	4.0
PC 12:0/12:0	248.0	258.4	4.0
PC 14:0/14:0	260.2	270.4	3.8
PC 16:0/16:0	270.8	282.5	4.2
PC 18:0/18:0	281.5	294.5	4.4
PC 20:0/20:0	292.9	306.4	4.4
PC 22:0/22:0	302.5	319.1	5.2
PC 24:0/24:0	312.8	330.9	5.5

Compound [M+CH <sub>3</sub> COO] <sup>-</sup>	Avg. Cal. CCS (Å <sup>2</sup> , n=3)	DTIM CCS (Å <sup>2</sup> )	% Error (DTIM:Cal)
PC 6:0/6:0	219.2	230.2	4.8
PC 8:0/8:0	230.5	241.4	4.5
PC 10:0/10:0	241.3	253.6	4.9
PC 12:0/12:0	252.0	264.6	4.8
PC 14:0/14:0	262.7	274.4	4.3
PC 16:0/16:0	271.8	286.2	5.0
PC 18:0/18:0	282.1	295.1	4.4
PC 20:0/20:0	291.0	305.1	4.6
PC 22:0/22:0	299.8	317.4	5.5
PC 24:0/24:0	310.0	331.0	6.4

**Table S25.** Summary of results from TWIM CCS calibration of PCs with PEs with a ramped wave velocity (900-300 m/s) and 40V wave height.

Compound [M+H] <sup>+</sup>	Avg. Cal. CCS (Å <sup>2</sup> , n=3)	DTIM CCS (Å <sup>2</sup> )	% Error (DTIM:Cal)
PC 6:0/6:0	212.4	213.3	0.4
PC 8:0/8:0	229.3	230.6	0.6
PC 10:0/10:0	244.5	245.4	0.4
PC 12:0/12:0	257.0	258.4	0.6
PC 14:0/14:0	269.4	270.4	0.4
PC 16:0/16:0	280.2	282.5	0.8
PC 18:0/18:0	291.2	294.5	1.1
PC 20:0/20:0	303.0	306.4	1.1
PC 22:0/22:0	312.9	319.1	1.9
PC 24:0/24:0	323.6	330.9	2.2

Compound [M+CH <sub>3</sub> COO] <sup>-</sup>	Avg. Cal. CCS (Å <sup>2</sup> , n=3)	DTIM CCS (Å <sup>2</sup> )	% Error (DTIM:Cal)
PC 6:0/6:0	228.3	230.2	0.8
PC 8:0/8:0	240.4	241.4	0.4
PC 10:0/10:0	252.2	253.6	0.5
PC 12:0/12:0	264.1	264.6	0.2
PC 14:0/14:0	276.2	274.4	0.7
PC 16:0/16:0	286.7	286.2	0.2
PC 18:0/18:0	298.5	295.1	1.2
PC 20:0/20:0	309.0	305.1	1.3
PC 22:0/22:0	319.5	317.4	0.7
PC 24:0/24:0	331.6	331.0	0.2

**Table S26.** Validation of lipid CCS calibration against DTIM measurements of PC and PE extracts.

Identification <sup>a,b</sup>	<i>m/z</i> Observed	TWIM CCS ( $\text{\AA}^2$ ) [Adduct]	DTIM CCS ( $\text{\AA}^2$ ) [Adduct] <sup>c</sup>	CCS Abs. Error (%)	RSD (%) TWIM CCS
PC 34:02	758.58	282.4 [H]	279.5 [H]	1.0	0.4
PC 34:01	760.59	285.9 [H]	282.0 [H]	1.4	0.3
PC 36:02	786.61	289.6 [H]	285.9 [H]	1.3	0.5
PC 36:01	788.62	294.5 [H]	288.2 [H]	2.2	0.6
PC 36:01	810.60	291.1 [Na]	290.3 [Na] <sup>d</sup>	0.3	0.1
PE 34:01	718.54	271.2 [H]	270.1 [H]	0.4	0.1
PE 36:02	744.56	273.9 [H]	275.1 [H]	-0.4	0.1
PE 36:01	746.57	276.9 [H]	277.0 [H]	-0.1	0.1
PE 36:02	766.53	277.0 [Na]	279.9 [Na]	-1.0	0.2
PE 36:01	768.55	278.4 [Na]	282.2 [Na] <sup>d</sup>	-1.4	0.1

<sup>a</sup> All identifications made within 10 ppm of the observed *m/z*; <sup>b</sup> PE P, plasmalogen ether PE; <sup>c</sup> DTIM values from Ref. 52 unless otherwise noted; <sup>d</sup> DTIM values from Ref. 26.