Supporting Information

A Collision Cross Section Calibration Strategy for Lipid Measurements in SLIM-based High Resolution Ion Mobility

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SI Contents:

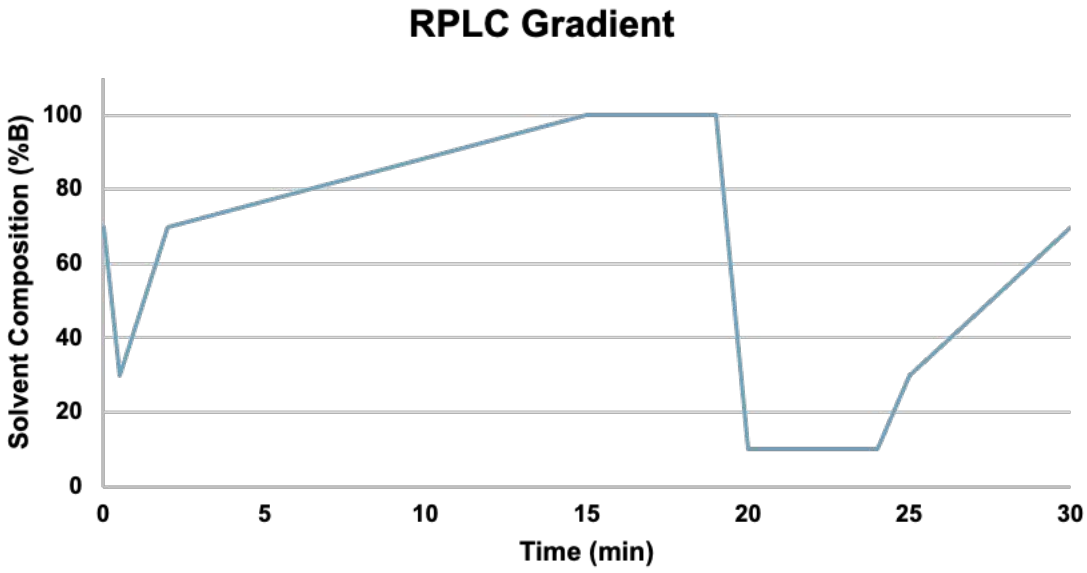

Figure S1. The standard lipid mix was analyzed using a 1290 Infinity LC system (Agilent). Reversed phase LC was performed at a flow rate of 250 µL/min using a C18 column (HypersilGold 1.9 µm, 2.1 mm x 100 mm column, Thermo Fisher) held at 40 ˚C with mobile phases consisting of 10 mM ammonium formate and 0.1% formic acid in H_2O (A) and 60:36:4 IPA:ACN:H_2O (B). The 30 minute gradient is illustrated above.

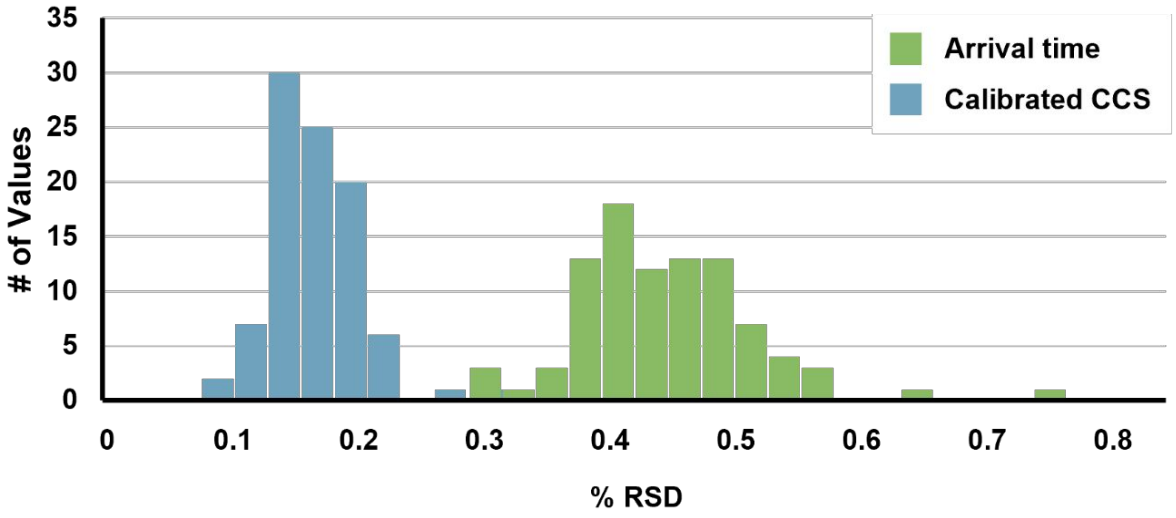

Figure S2. Inter-day reproducibility of experimental arrival times (green) and calibrated CCS values from all lipid features (n = 92).

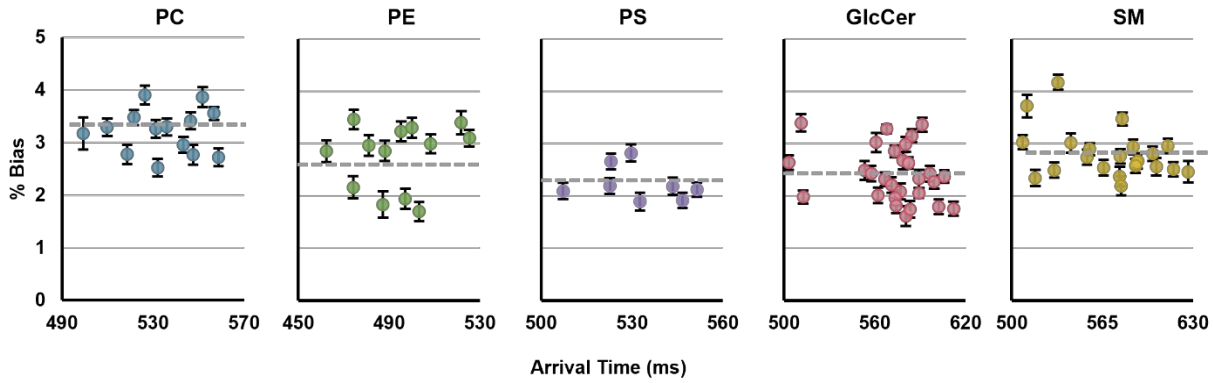

Figure S3. CCS calibration bias for all values calibrated from triplicate measurements using HFAPs with a 3rd order polynomial as compared to established ^{DT}CCS values. Dashed lines represent average biases for each subclass, and error bars represent the measurement standard deviation.

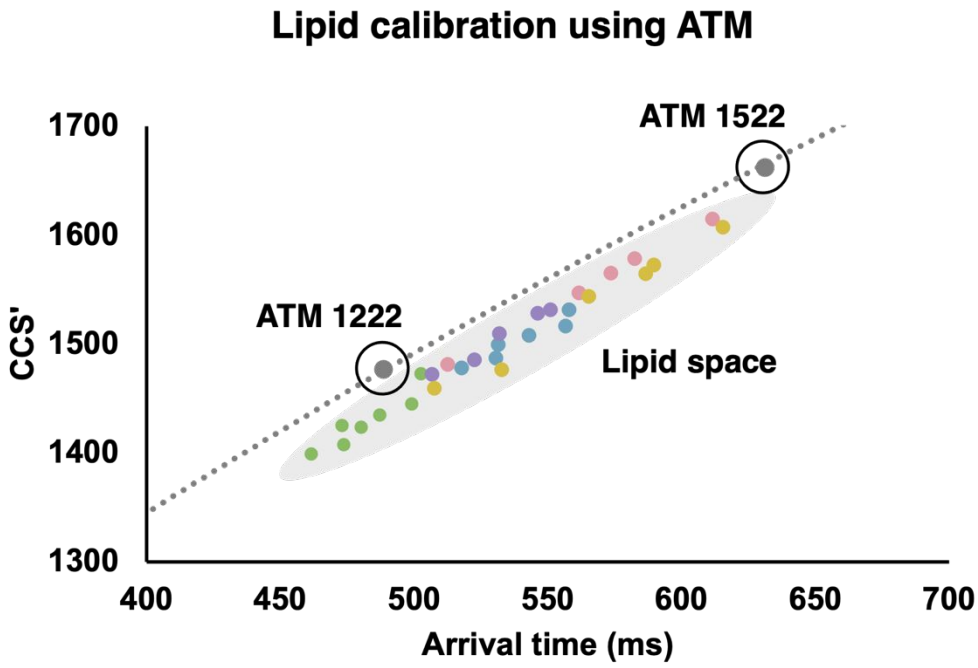

Figure S4: CCS calibration plot of the lipid analytes as compared to the Tune Mix ions *m/z* 1222 and *m/z* 1522.

Table S1. Subclass specific semi-empirically derived correction factors used for correction of HFAP-calibrated CCS values.

Class	Subclass	Correction factor
Glycerophospholipids	PC	0.0321
	PE	0.0275
	PS	0.0223
Sphingolipids	GlcCer	0.0241
	SM	0.0281
SPLASH Mix		0.0150

Table S2. Table of calibrated and corrected CCS values for all lipids using correction factors in Table S1. ^{DT}CCS values in the left column are sourced from the Unified CCS Compendium. Lipid features from each subclass that were not identified are annotated as their subclass and measured *m/z*. When arrival time features were observed for one *m/z*, peak numbers are annotated in ascending order of arrival time. Species used as calibrants in the lipid calibrant study (Figure 2) are denoted with a (*).

Tentative ID				Feature description			
Name	**Adduct**	**^{DT}CCS (Å²)**	**Bias (%)**	***m/z* (measured)**	**Peak #**	**$^{TW-SLIM}$CCS (Å²)**	**RSD (%)**
Phosphatidylcholines							
PC 34:01	[M+H]	283.1	0.21	760.5899	1	283.7	0.14
PC 34:01	[M+H]	283.1	0.64	760.5902	2	284.9	0.18
PC 34:01*	[M+Na]	286.0	0.00	782.5720	1	286.0	0.16
PC 34:02	[M+H]	280.6	0.03	758.5742	1	280.7	0.17
PC 34:02*	[M+Na]	284.2	-0.49	780.5566	1	282.8	0.18
PC 34:03	[M+H]	278.2	-0.09	756.5605	1	277.9	0.31
PC 36:01	[M+H]	289.4	0.15	788.6210	1	289.8	0.17
PC 36:01	[M+H]	289.4	0.60	788.6215	2	291.1	0.19
PC 36:01	[M+Na]	291.4	-0.50	810.6054	1	290.0	0.18
PC 36:01	[M+Na]	291.4	0.29	810.6051	2	292.2	0.13
PC 36:02	[M+H]	287.1	0.03	786.6056	1	287.2	0.17
PC 36:02*	[M+Na]	289.8	-0.31	808.5896	1	288.9	0.15
PC 36:03*	[M+Na]	288.2	-0.74	806.5746	1	286.1	0.17
PC 38:03*	[M+Na]	294.2	-0.55	834.6071	1	292.6	0.17
PC (*m/z* 782.57)	--	--	--	782.574233	1	293.3	0.19
PC (*m/z* 804.56)	--	--	--	804.558767	1	296.4	0.14
PC (*m/z* 832.59)	--	--	--	832.590633	1	302.9	0.17
PC (*m/z* 836.63)	--	--	--	836.624333	1	305.0	0.17
	Average absolute % bias		**0.33**		**Average % RSD**		**0.17**
Phosphatidylethanolamines							
PE 34:01*	[M+H]	271.1	0.67	718.5438	1	272.9	0.18
PE 34:01	[M+Na]	277.1	0.44	740.5270	1	278.3	0.19
PE 34:02*	[M+H]	269.5	0.06	716.5275	1	269.7	0.21
PE 34:02*	[M+Na]	274.1	0.17	738.5098	1	274.6	0.20
PE 36:01*	[M+H]	278.1	0.51	746.5752	1	279.5	0.19
PE 36:01*	[M+Na]	283.3	-1.09	768.5587	1	280.2	0.18
PE 36:01	[M+2Na-H]	284.9	0.31	790.5421	1	285.8	0.16
PE 36:02*	[M+H]	276.2	0.07	744.5589	1	276.4	0.19
PE 36:02	[M+Na]	281.0	0.20	766.5410	1	281.6	0.18
PE 36:02	[M+Na]	281.0	-0.85	766.5448	2	278.6	0.19
PE 36:03	[M+H]	274.4	-0.63	742.5467	1	272.7	0.22
PE 36:03	[M+Na]	278.7	-0.96	764.5288	1	276.0	0.25
PE (*m/z* 740.53)	--	--	--	740.5295	1	273.5	0.20
PE (*m/z* 768.56)	--	--	--	768.5599	1	285.0	0.22
	Average absolute % bias		**0.51**		**Average % RSD**		**0.20**

Phosphatidylserines							
PS 36:01*	[M+H]	285.6	0.40	790.5650	1	286.7	0.15
PS 36:02*	[M+H]	283.1	-0.16	788.5505	1	282.6	0.16
PS 38:04	[M+H]	286.7	0.56	812.5484	1	286.5	0.15
PS 38:04	[M+H]	286.7	-0.07	812.5490	2	288.3	0.16
PS 40:04*	[M+H]	293.5	-0.34	840.5801	1	292.5	0.15
PS 40:05	[M+H]	291.9	-0.08	838.5661	1	291.7	0.17
PS 40:06*	[M+H]	290.0	-0.37	836.5494	1	288.9	0.17
PS 40:06*	[M+Na]	294.0	-0.14	858.5327	1	293.6	0.14
	Average absolute % bias		**0.27**		**Average % RSD**		**0.16**
Cerebrosides							
GlcCer 36:01	[M+H-H2O]	281.0	0.20	710.6001	1	281.5	0.14
GlcCer 36:01	[M+H-H2O]	281.0	0.95	710.5992	2	283.7	0.16
GlcCer 36:01*	[M+Na]	285.1	-0.47	750.5925	1	283.8	0.12
GlcCer 40:00	[M+Na]	298.9	-0.23	808.6726	1	298.2	0.15
GlcCer 40:00	[M+Na]	298.9	0.54	808.6729	2	300.5	0.14
GlcCer 40:01	[M+H-H2O]	293.9	0.05	766.6641	1	294.0	0.18
GlcCer 40:01	[M+H-H2O]	293.9	0.58	766.6653	2	295.6	0.19
GlcCer 40:01*	[M+Na]	297.3	-0.44	806.6560	1	296.0	0.15
GlcCer 40:02	[M+H]	295.1	-0.02	782.6565	1	295.1	0.15
GlcCer 40:02	[M+H]	295.1	0.84	782.6593	2	297.6	0.08
GlcCer 42:00	[M+Na]	304.3	-0.02	836.7027	1	304.2	0.16
GlcCer 42:00 OH	[M+Na]	306.7	-0.08	852.6966	1	306.5	0.12
GlcCer 42:01	[M+H-H2O]	299.5	0.23	794.6925	1	300.2	0.17
GlcCer 42:01	[M+H-H2O]	299.5	0.70	794.6928	2	301.6	0.13
GlcCer 42:01	[M+Na]	302.9	-0.83	834.6867	1	300.4	0.19
GlcCer 42:01	[M+Na]	302.9	-0.12	834.6864	2	302.5	0.18
GlcCer 42:01 OH	[M+Na]	305.4	-0.18	850.6816	1	304.9	0.13
GlcCer 42:02	[M+H]	300.4	-0.36	810.6905	1	299.3	0.15
GlcCer 42:02	[M+H]	300.4	0.18	810.6890	2	300.9	0.11
GlcCer 42:02	[M+H]	300.4	0.92	810.6884	3	303.2	0.14
GlcCer 42:02	[M+H-H2O]	297.6	-0.13	792.6776	1	297.2	0.13
GlcCer 42:02	[M+H-H2O]	297.6	0.41	792.6778	2	298.8	0.12
GlcCer 42:02*	[M+Na]	300.7	-0.63	832.6701	1	298.8	0.15
GlcCer 42:02 OH*	[M+Na]	303.1	-0.70	848.6660	1	301.0	0.16
GlcCer 42:04 OH	[M+H]	300.2	-0.48	822.6523	1	298.8	0.14
GlcCer 43:02	[M+Na]	303.6	-0.39	846.6836	1	302.4	0.09
GlcCer 44:02	[M+Na]	307.5	-0.66	860.7005	1	305.5	0.14
GlcCer 46:05 OH*	[M+H]	309.9	-0.69	876.6957	1	307.8	0.14
	Average absolute % bias		**0.43**		**Average % RSD**		**0.14**
Sphingomyelins							
SM 34:01*	[M+H]	281.2	0.18	703.5808	1	281.7	0.13
SM 34:01	[M+Na]	279.9	0.87	725.5647	1	282.3	0.22
SM 36:01	[M+H]	288.4	-0.36	731.6116	1	287.4	0.15
SM 36:01*	[M+Na]	284.1	1.32	753.5268	1	287.9	0.15
SM 36:02	[M+H]	285.3	-0.51	729.5966	1	283.9	0.15
SM 38:00	[M+H]	289.8	0.16	745.6293	1	290.3	0.18

SM 38:01	[M+H]	293.4	-0.11	759.6422	1	293.1	0.15
SM 38:01	[M+Na]	293.3	0.04	781.6248	1	293.4	0.12
SM 40:00*	[M+H]	300.8	0.08	789.6903	1	301.0	0.14
SM 40:01	[M+H]	299.1	-0.10	787.6743	1	298.8	0.14
SM 40:01	[M+Na]	297.2	0.62	809.6581	1	299.0	0.13
SM 40:02*	[M+H]	296.8	-0.31	785.6599	1	295.9	0.15
SM 41:01*	[M+H]	302.3	-0.18	801.6911	1	301.7	0.15
SM 41:02	[M+H]	300.1	-0.49	799.6761	1	298.6	0.19
SM 42:00	[M+H]	306.5	0.11	817.7225	1	306.8	0.14
SM 42:01	[M+H]	304.4	-0.05	815.7058	1	304.3	0.14
SM 42:02	[M+H]	302.2	-0.19	813.6896	1	301.6	0.15
SM 42:02	[M+Na]	302.0	-0.28	835.6737	1	301.2	0.14
SM 42:03	[M+H]	300.8	-0.66	811.6745	1	298.8	0.19
SM 44:01	[M+H]	305.7	-0.29	827.7070	1	304.8	0.17
SM 44:02	[M+H]	311.0	-0.39	843.7410	1	309.8	0.21
SM 44:03*	[M+H]	308.7	-0.34	841.7226	1	307.6	0.14
SM (*m/z* 733.63)	--	--	--	733.6272	1	290.1	0.13
SM (*m/z* 761.66)	--	--	--	761.6590	1	295.6	0.14
	Average absolute % bias		**0.35**		**Average % RSD**		**0.15**
SPLASH Mix							
18:1(d7) LPC	[M+H]	232.9	-0.53	529.4026	1	231.7	0.09
18:1(d7) LPC	[M+Na]	236.0	-1.14	551.3809	1	233.4	0.10
15:0-18:1(d7) DG	[M+H-H2O]	255.6	0.75	570.5576	1	256.7	0.16
15:0-18:1(d7) DG	[M+Na]	257.1	-0.18	610.5485	1	257.6	0.12
15:0-18:1(d7) PE	[M+H]	271.7	0.31	711.5758	1	272.6	0.10
15:0-18:1(d7) PE	[M+Na]	277.8	0.03	733.5595	1	277.9	0.11
15:0-18:1(d7) PS	[M+H]	278.6	0.23	755.5674	1	279.2	0.10
18:1(d9) SM	[M+H]	286.3	0.71	738.6596	1	287.5	0.10
18:1(d9) SM	[M+Na]	286.9	0.22	760.6386	1	288.4	0.11
15:0-18:1(d7)-15:0 TG	[M+NH4]	312.1	-0.11	829.8109	1	309.3	0.10
15:0-18:1(d7)-15:0 TG	[M+Na]	310.6	-0.41	834.7644	1	311.7	0.10
	Average absolute % bias		**0.42**		**Average % RSD**		**0.11**

Calibration Simulation for Evaluation of Calibrant Error Contribution

A calibration simulation was performed to assess the contribution of uncertainty associated with the reference HFAP ^{DT}CCS values. For each HFAP ion, 10 CCS values were selected from a uniform distribution centered around the accepted value with the width of the distribution corresponding to the uncertainty of the measurement, reported as %RSD (**Table S3**). The resulting values are tabulated below (**Table S4**).

Table S3. HFAP calibrant CCS values.

HFAP ion	Arrival time (ms)	^{DT}CCS	RSD
m/z 622	223.66	202.8	0.12%
m/z 922	349.34	243.5	0.11%
m/z 1222	487.28	281.8	0.10%
m/z 1522	629.68	316.5	0.10%
m/z 1822	782.37	351.2	0.18%
m/z 2122	941.26	383.0	0.17%
m/z 2422	1103.69	412.9	0.14%
m/z 2722	1270.02	441.2	0.13%

Table S4. HFAP simulated CCS values.

HFAP ion	CCS (1)	CCS (2)	CCS (3)	CCS (4)	CCS (5)	CCS (6)	CCS (7)	CCS (8)	CCS (9)	CCS (10)
m/z 622	202.63	202.70	202.81	203.00	202.65	203.01	202.60	203.02	202.68	202.85
m/z 922	243.76	243.40	243.41	243.58	243.65	243.40	243.38	243.62	243.37	243.27
m/z 1222	281.77	281.68	281.99	282.06	281.78	281.73	282.07	281.64	281.65	281.78
m/z 1522	316.68	316.40	316.48	316.59	316.36	316.35	316.79	316.25	316.24	316.33
m/z 1822	351.64	351.48	351.22	351.26	351.19	351.56	350.67	351.48	351.79	350.83
m/z 2122	383.10	382.95	382.66	383.28	382.87	383.32	382.59	382.69	382.96	383.25
m/z 2422	413.26	412.84	413.08	413.21	413.11	413.38	412.39	413.31	412.34	412.38
m/z 2722	440.90	441.35	441.49	441.08	441.72	441.30	440.94	440.95	440.87	441.10

For each of the ten sets of CCS values, a calibration was performed using a trinomial equation. This resulted in the coefficients listed in **Table S5**.

Table S5. Calibration coefficients for simulated calibrations.

Coeff.	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
A	2.38E-07	2.52E-07	2.67E-07	2.36E-07	2.66E-07	2.18E-07	2.86E-07	2.27E-07	2.44E-07	2.47E-07
B	-9.67E-04	-9.89E-04	-1.02E-03	-9.55E-04	-1.02E-03	-9.14E-04	-1.07E-03	-9.31E-04	-9.79E-04	-9.78E-04
C	2.20E+00	2.20E+00	2.22E+00	2.18E+00	2.22E+00	2.16E+00	2.25E+00	2.16E+00	2.20E+00	2.19E+00
D	6.06E+02	6.05E+02	6.03E+02	6.10E+02	6.04E+02	6.14E+02	5.97E+02	6.14E+02	6.05E+02	6.08E+02

Applying these calibration equations to the PC lipid extract data resulted in an average uncertainty in the calibrated CCS values of 0.04% (**Table S6**).

Table S6. Summary of calibrated CCS values of PC lipids using the simulated calibrant values.

Compound	Arrival time (ms)	Avg. calibrated CCS	Standard deviation	RSD
PC 34:03	499.31	286.8	0.1159	0.040%
PC 34:02	509.89	289.6	0.1158	0.040%
PC 34:01	521.66	292.8	0.1155	0.039%
PC 34:01	526.44	294.0	0.1153	0.039%
PC 34:02	518.68	291.8	0.1156	0.040%
PC 34:01	531.21	295.1	0.1150	0.039%
PC 36:02	536.05	296.4	0.1147	0.039%
PC 36:03	532.08	295.2	0.1149	0.039%
PC 36:01	551.85	300.5	0.1138	0.038%
PC 36:01	546.60	299.1	0.1141	0.038%
PC 36:02	543.39	298.2	0.1142	0.038%
PC 36:01	556.83	301.6	0.1134	0.038%
PC 36:01	547.63	299.2	0.1140	0.038%
PC 38:03	558.81	302.0	0.1132	0.038%