Supplementary Information

Mobilising Ion Mobility Mass Spectrometry for Metabolomics

Eleanor Sinclair[1,2], Katherine A. Hollywood[2], Cunyu Yan[2], Richard Blankley[3], Rainer Breitling[2], Perdita Barran[1,2]

[1]Michael Barber Centre for Collaborative Mass Spectrometry, Manchester Institute of Biotechnology, School of Chemistry, The University of Manchester, Princess Street, Manchester, M1 7DN, UK

[2]BBSRC/EPSRC Synthetic Biology Research Centre for Fine and Speciality Chemicals (SYNBIOCHEM), Manchester Institute of Biotechnology, The University of Manchester, Manchester, U.K

[3]Agilent Technologies, Life Sciences & Chemical Analysis Group, Cheadle Royal Business Park, Cheshire, UK, SK8 3GR

Stepped field CCS determination using DT-IM-MS:

An ion's velocity (v_d) through a mobility cell is proportional to its mobility (K) and the applied electric field (E) at a constant temperature and pressure. The electric field itself is dependent on the drift voltage (V) and length (L) of the cell (Eqn 1). (1)

$$v_d = KE = K\frac{V}{L} \qquad [1]$$

A typical ion mobility experiment will yield a measured drift time (t_D) for each ion, which corresponds to a transit time from release into the drift tube to mass detection. For a collision cross section value to be extracted the precise time an ion takes to traverse the mobility cell solely needs to be isolated, and so a corrected drift time (t_d) equation is applied (Eqn 2).

$$t_d = t_D - t_0 \qquad [2]$$

The so called 'dead time' (t_0), between the exit of the mobility cell and the mass analyser, is determined using linear regression analysis by which one experimental parameter is varied, in this case, and most commonly, the drift voltage. By exploiting the relationship between measured drift time (t_D) and the reciprocal of the drift voltage (V), the intercept of the linear regression plot returns a value for an ions dead time.(2–4)

$$t_D = \frac{L^2}{K_o}\left(\frac{273.15}{760T}\right)\left(\frac{P}{V}\right) + t_0 \qquad [3]$$

The corrected drift time (t_d) value is then substituted along with other accurately monitored experimental parameters into the Mason-Schamp Equation (Eqn 4).(5) This collision cross section (Ω) output has therefore been determined directly from the experimentally measured drift time with no requirement for calibration standardisation.

$$\Omega = \frac{(18\pi)^{1/2}}{16}\frac{ze}{(k_b T)^{1/2}}\left[\frac{1}{m_I} + \frac{1}{m_B}\right]^{1/2}\frac{t_d E}{L}\frac{760}{P}\frac{T}{273.15}\frac{1}{N} \qquad [4]$$

Where Ω is collision cross section, z is the ion charge state, k_b is the Boltzmann constant, T is the drift gas temperature, m_I is the mass of analyte ion, m_B is the mass of buffer gas molecules, t_d is the corrected drift time, E is the electric field, L is the length of the drift cell, P is the pressure in the drift cell, and N is the number density in the drift cell.

Table S1: Collision cross section values for all metabolites measured in positive ionisation mode for each replicate. CCS values have been averaged over replicates and the corresponding standard deviation (Å²) and relative standard deviation (%) is reported. The table has been ordered from low to high SD.

		CCS (Å²)			CCS (Å²)		
Metabolite	**m/z**	**Rep 1**	**Rep 2**	**Rep 3**	**Average**	**SD**	**RSD (%)**
Apigenin	271.0602	159.77	159.76	159.76	159.76	0.004	0.003
Phenylalanine	166.0863	139.62	139.61	139.63	139.62	0.008	0.006
L-Arginine	175.1190	135.61	135.59	135.61	135.60	0.009	0.007
3-Methyl-L-histidine	170.0924	133.08	133.06	133.08	133.07	0.011	0.008
Succinic acid	119.0339	136.10	136.07	136.09	136.09	0.012	0.009
Carnosine	227.1139	150.42	150.44	150.45	150.44	0.016	0.011
Malic acid	135.0288	129.64	129.61	129.62	129.62	0.017	0.013
Homocarnosine	241.1297	153.67	153.70	153.71	153.69	0.019	0.013
Hypoxanthine	137.0458	126.71	126.67	126.68	126.69	0.019	0.015
Homocystine	269.0625	157.31	157.34	157.36	157.34	0.024	0.015
Niacinamide	123.0553	124.97	124.92	124.94	124.94	0.022	0.018
L-Leucine	132.1019	124.25	124.21	124.22	124.23	0.022	0.018
Guanosine	284.0990	162.74	162.78	162.79	162.77	0.03	0.018
Uridine monophosphate	325.0432	165.65	165.70	165.71	165.69	0.034	0.02
Creatinine	114.0662	123.06	123.01	123.03	123.03	0.026	0.021
Cytidine 5'monophosphate	324.0592	166.57	166.62	166.64	166.61	0.035	0.021
Pinocembrin	257.0809	157.66	157.65	157.73	157.68	0.041	0.026
Luteolin	287.0550	164.50	164.34	164.43	164.42	0.081	0.05
Norcoclaurine	272.1281	168.24	168.23	168.41	168.29	0.098	0.058
L-Dopa	198.0711	-	149.21	149.33	149.27	0.091	0.061
Adenine	136.0618	124.71	124.62	124.78	124.70	0.084	0.068
Naringenin	273.0758	163.66	163.88	163.89	163.81	0.129	0.079
Quercetin	303.0500	167.52	167.73	-	167.62	0.151	0.09
S-Reticuline	330.1700	181.70	181.90	182.04	181.88	0.171	0.094
(-)-Riboflavin	377.1456	188.80	188.68	189.03	188.84	0.179	0.095
Tyrosine	182.0812	145.59	145.36	145.63	145.53	0.148	0.102
Folic acid	442.1470	196.18	195.85	196.27	196.10	0.223	0.114
Hesperetin	303.0864	171.67	171.96	172.07	171.90	0.205	0.119
Liquiritigenin	257.0809	162.30	161.91	162.07	162.10	0.196	0.121
Chrysin	255.0652	154.83	154.36	154.66	154.61	0.235	0.152
L-Citrulline	176.1030	137.80	137.28	137.36	137.48	0.283	0.206
L-Valine	118.0863	121.05	120.59	120.61	120.75	0.26	0.216
L-Proline	116.0706	125.15	124.66	124.67	124.83	0.279	0.223
Nicotinamide	123.0553	125.43	124.93	124.94	125.10	0.282	0.225
Cytosine	112.0505	116.70	117.17	117.35	117.07	0.333	0.285

Table S2: Collision cross section values for all metabolites measured in negative ionisation mode for each replicate. CCS values have been averaged over replicates and the corresponding standard deviation (Å²) and relative standard deviation (%) is reported. The table has been ordered from low to high SD.

		CCS (Å²)			CCS (Å²)		
Metabolite	**m/z**	**Rep 1**	**Rep 2**	**Rep 3**	**Average**	**SD**	**RSD (%)**
3-Methyl-L-histidine	168.0778	136.46	136.46	136.46	136.46	0.000	0.000
Guanosine	282.0844	162.64	162.64	162.60	162.63	0.020	0.013
Uridine triphosphate	482.9612	180.73	180.81	180.73	180.76	0.043	0.024
Chrysin	253.0506	157.56	157.60	157.49	157.55	0.053	0.034
Pinocembrin	255.0663	161.42	161.43	161.24	159.32	0.055	0.035
L-Hydroxylysine	161.0931	134.12	-	134.05	134.09	0.056	0.041
Norcoclaurine	270.1135	167.26	167.38	167.26	167.3	0.068	0.041
Quercetin	301.0354	164.92	164.83	164.79	164.85	0.069	0.042
L-Histidine	154.0622	128.66	-	128.74	128.7	0.056	0.043
L-2-Aminoadipic acid	160.0615	130.10	-	130.17	130.14	0.056	0.043
Adenosine 5'-monophosphate	346.0558	173.62	173.77	173.77	173.72	0.087	0.050
Inosine 5'-monophosphate	347.0398	171.64	171.79	171.79	171.74	0.087	0.051
Cyclic guanosine monophosphate	344.0401	168.15	168.30	168.30	168.25	0.087	0.052
L-Cysteine	120.0125	149.09	149.25	149.16	149.16	0.080	0.054
(-)-Riboflavin	375.1310	187.37	187.18	187.19	187.25	0.104	0.056
Guanosine triphosphate	521.9834	193.48	193.63	193.70	193.6	0.114	0.059
Folic Acid	440.1324	192.95	192.79	192.73	192.82	0.115	0.060
Tyrosine	180.0666	144.39	144.23	144.37	144.33	0.088	0.061
L-Tryptophan	203.0826	151.77	151.94	151.92	151.88	0.093	0.061
Liquiritigenin	255.0663	159.34	159.37	159.26	161.36	0.104	0.064
Uridine monophosphate	323.0286	161.60	161.75	-	161.67	0.107	0.066
Naringenin	271.0612	164.32	164.31	164.12	164.25	0.112	0.068
Hesperetin	301.0718	174.61	174.60	174.40	174.54	0.119	0.068
Citric Acid	191.0197	127.74	127.58	127.74	127.69	0.090	0.070
Cytidine triphosphate	427.9772	181.74	181.74	181.97	181.82	0.130	0.071
Apigenin	269.0455	159.31	159.26	159.08	159.22	0.123	0.078
Adenosine diphosphate	426.0221	183.72	184.02	183.87	183.87	0.150	0.082
Luteolin	285.0404	161.80	161.88	161.62	161.76	0.134	0.083
D-Ribose 5-phosphate	229.0119	139.22	139.15	138.99	139.12	0.117	0.084
L-Dopa	196.0565	140.94	140.96	141.18	141.03	0.134	0.095
5'-Guanylate diphosphate	442.0170	182.54	182.84	182.84	182.74	0.173	0.095
Homocarnosine	239.1149	154.86	155.02	155.17	155.02	0.153	0.099
Carnosine	225.0993	152.29	152.60	152.44	152.44	0.154	0.101
Inosine	267.0735	158.59	158.82	-	158.7	0.162	0.102
S-Reticuline	328.1554	177.50	177.54	177.18	177.41	0.194	0.109
Phenylalanine	164.0717	139.76	140.07	139.84	139.89	0.164	0.117
Guanosine 3'-phosphate	362.0507	169.34	169.72	169.64	169.57	0.199	0.118
Adenosine	266.0895	157.85	158.08	158.24	158.06	0.192	0.122
Cytidine diphosphate	402.0109	173.54	173.99	173.84	173.79	0.229	0.132
Adenine	134.0472	119.92	119.71	119.57	119.73	0.175	0.146

Inosine diphosphate	427.0061	178.98	179.51	179.28	179.26	0.263	0.147
L-Arginine	173.1044	137.46	137.20	137.04	137.23	0.214	0.156
Adeonsine triphosphate	505.9885	188.34	188.93	188.56	188.61	0.301	0.160
Uridine diphosphate	402.9949	172.48	173.00	173.00	172.83	0.303	0.176
Cytidine 5'-monophosphate	322.0446	164.42	164.64	164.04	164.37	0.306	0.186
Uridine	243.0623	151.25	151.11	151.70	151.35	0.308	0.203
Myo-inositol	179.0561	131.57	131.35	131.04	131.32	0.268	0.204
D-(+)-Glucuronic acid	193.0354	131.86	131.48	132.02	131.79	0.280	0.212
Homocystine	267.0479	151.49	151.95	-	151.72	0.324	0.213
L-Methionine	148.0438	132.39	131.61	131.85	131.95	0.396	0.300

Table S3: Instrumental parameters that deviate from Agilent MassHunter Acquisition Auto Tune 50-1700 *m/z* settings

Parameter	Value
Sheath Gas Temperature	300 °C
Sheath Gas Flow	12 L/min
Drying Gas Temperature	300 °C
Drying Gas Flow	12 L/min
Nebulizer	35 psig
Capillary Voltage	3500 V
Fragmentor	385 V
High Pressure Funnel RF	100 V
Trap Funnel RF	100 V
Rear Funnel RF	150 V
Trap Fill Time	20 ms
Trap Release Time	150 µs

References

1. Revercomb HE, Mason EA. Theory of Plasma Chromatography/Gaseous Electrophoresis. A Review. Anal Chem. 1975;47(7):970–83.

2. May JC, Goodwin CR, Lareau NM, Leaptrot KL, Morris CB, Kurulugama RT, et al. Conformational ordering of biomolecules in the gas phase: Nitrogen collision cross sections measured on a prototype high resolution drift tube ion mobility-mass spectrometer. Anal Chem. 2014;86(4):2107–16.

3. Kurulugama RT, Darland E, Kuhlmann F, Stafford G, Fjeldsted J. Evaluation of drift gas selection in complex sample analyses using a high performance drift tube ion mobility-QTOF mass spectrometer. Analyst. 2015;140(20):6834–44.

4. Creaser CS, Griffiths JR, Bramwell CJ, Noreen S, Hill C a., Thomas CLP, et al. Ion

mobility spectrometry: a review. Part 1. Structural analysis by mobility measurement. Analyst. 2004;129(11):984.

5. Mason EA, Schamp HW. Mobility of Gaseous Ions in Weak Electric Fields. Ann Phys. 1958;4:233–70.