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Building a Collision Cross Section Library of Pharmaceutical Drugs Using the Vion IMS QTof Platform: Verification of System Performance, Precision and Deviation of CCS Measurements

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APPLICATION BENEFITS

- Routine collision cross section measurement using bench top MS platform
- Ease of CCS experimental set up on a Tof platform
- High mass accuracy and CCS measurement reproducibility
- CCS library of 134 FDA approved drug standards
- QC-based protocol for routine CCS measurement

WATERS SOLUTIONS

ACQUITY UPLC® I-Class System

Vion® IMS QTof Ion Mobility Quadrupole Tome-of-flight Mass Spectrometry

ACQUITY UPLC 1.7 µm BEH C_{18} Column

UNIFI® Scientific Information System

QC Reference Standard Solution from Waters [part number 186006963]

KEYWORDS

Ion Mobility, QC Mix, collision cross section, CCS, QTof, library, UNIFI, Vion, FDA drugs

INTRODUCTION

For a typical LC-MS analytical separation and identification, compounds of interest are resolved in the LC dimension (with a defined retention time), then their *m/z* values are measured on the mass spectrometer. High resolution mass spectrometry (HRMS) provides selectivity and specificity for the specific ion/charge or mass/charge ratio of the analyte of interest and can be reproducibly measured across instrument platforms. However, in the case of complex and variable matrices, or high abundance background signals, identifications based solely on the combination of retention time and *m/z* may be insufficient due to interference and/or chromatographic variability. A physical property that can help differentiate and identify ions having similar retention time and help resolving multiple species in narrow *m/z* ranges would be useful both for separation and for confident identification.

Ion mobility is a measurable property, which can be used to derive the collision cross section (CCS) of a molecule under specific gas and temperature conditions. Waters SYNAPT® and Vion MS platforms are capable of sensitive and accurate CCS measurements. On the Vion IMS QTof platform, the ion mobility separation device is located between the StepWave™ device and the quadrupole (Figure 1). Ion mobility separates ions according to their size and shape and reports the separation as either the drift time or the collision cross section (CCS) value.

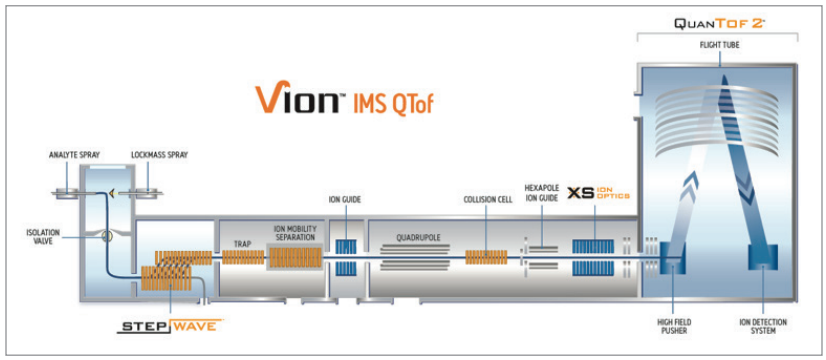

Figure 1. System diagram of Vion IMS QTof platform, showing ion mobility separation device installed between the StepWave device and a mass resolving quadrupole.

For two ions having the same *m/z*, the ion with a smaller size/shape moves faster through the gas cell and elutes first from the ion mobility cell. This ion has a shorter drift time and a correspondingly smaller CCS value (Figure 2) versus the later eluting ion. The CCS value is a physical property of the compound of interest and is independent of matrix, LC and MS (cone voltage – CV and collision energy – CE) conditions. We can use this property alongside the *m/z* and the retention time in order to improve the specificity of identifications. In order to be effective, the measurement of CCS must be accurate and precise.

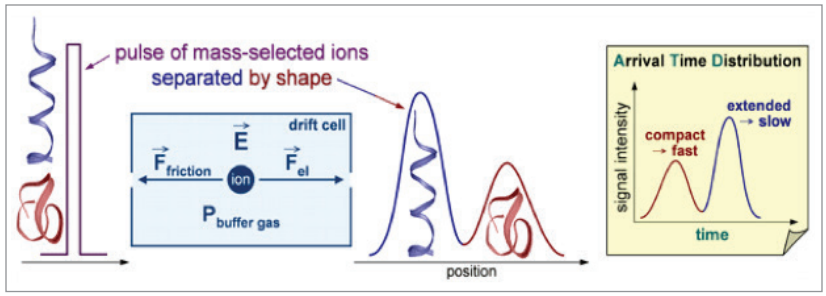

Figure 2. Schematics for the principle of ion separation in an ion mobility device. For two ions having the same m/z, the ion with smaller size and shape moves faster and will have a shorter drift time.

In this application note, the CCS values are measured for 134 small molecule FDA-approved drug standards. The deviation of the experiment was determined based on 30 measurements of eight quality control compounds for which CCS values have been reported.[1] The assay precision was determined based on triplicate measurements of the CCS values for each compound at two different concentrations.

EXPERIMENTAL

SAMPLE DESCRIPTION

The sample preparation and data acquisition followed an internal QC protocol for the CCS measurement as shown in Figure 3. A collection of 134 diverse FDA-approved drugs obtained as 10 mM solutions in DMSO was purchased from an external vendor. The solution was diluted using 30% acetonitrile/70% H_2O to two concentrations, a high concentration of 1 µM and a low concentration of 0.2 µM. A total of 6 sample sets were prepared, labeled as P1_high, P1_low, P2_high, P2_low, P3_high, and P3_low (triplicate measurements at high and low concentration). The LC-MS QC Reference Standard solution from Waters [part number 186006963] was used and analyzed before, during, and after each set of analyses.

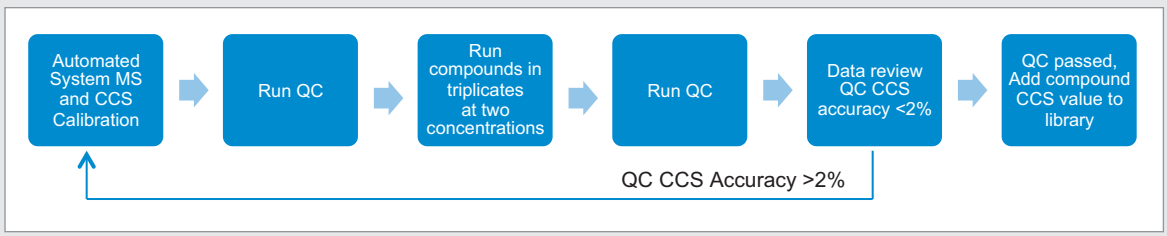

Figure 3. Protocol for CCS measurement. QC compounds are run before, during, and after a set of test compounds and reviewed for its deviation from known value. In the event of QC compounds exceeding the 2% criteria, the instrument must be recalibrated using the auto setup procedure and the measurement repeated.

Method conditions

The analytical LC-MS experiments were performed on a Waters® ACQUITY UPLC I-Class System and a Vion IMS QTof Mass Spectrometer. UNIFI Scientific Information System was used for data acquisition and data processing.

LC conditions

LC system:	ACQUITY UPLC I-Class
Column:	ACQUITY BEH C_{18} 2.1 x 50 mm, 1.7 µm (p/n 186002350)
Column temp.:	45 °C
Sample temp.:	10 °C
Injection volume:	1–3 µL
Flow rate:	0.8 mL/min
Mobile phase A:	Water with 0.1% formic acid
Mobile phase B:	Acetonitrile with 0.1% formic acid
Gradient:	Rapid generic gradient conditions with 3 min run time (Table 1)

Gradient:

	Time (min)	Flow rate (mL/min)	Composition A (%)	Composition B (%)	Curve
1	0.00	0.800	98.0	2.0	Initial
2	2.20	0.800	10.0	90.0	6
3	2.50	0.800	10.0	90.0	6
4	2.80	0.800	98.0	2.0	6
5	3.00	0.800	98.0	2.0	6

Table 1. Gradient table.

MS conditions

MS system:	Vion IMS QTof
Ionization mode:	ESI+, resolution mode (>40,000 FWHM)
Acquisition range:	50–1000 *m/z*
Capillary voltage:	0.5 kV
Cone voltage:	40 V
Cone gas flow:	20 L/h
Source temp.:	120 °C
Desolvation gas temp.:	550 °C
Desolvation gas flow:	800 L/h Scan time = 0.1 s
Experiment:	$HDMS^E$: Collision Energy (CE) settings: low CE, 6.0 eV; high CE, ramp 20-55 eV

RESULTS AND DISCUSSION

HDMS^E DATA ACQUISITION

All data were collected using HDMS^E (settings shown in Figure 4). HDMS^E enables collection of *m/z*, CCS, and detailed fragment ion spectra in a non-targeted manner. The selectivity afforded by HDMS^E improves both the precursor as well as the fragment ion information. A representative dataset including extracted ion chromatogram (XIC), ion mobilogram, spectra with structure annotation, and the result table is shown in Figure 5. The mass error in ppm and observed CCS value is automatically determined for each compound as shown in the component summary table in Figure 5a.

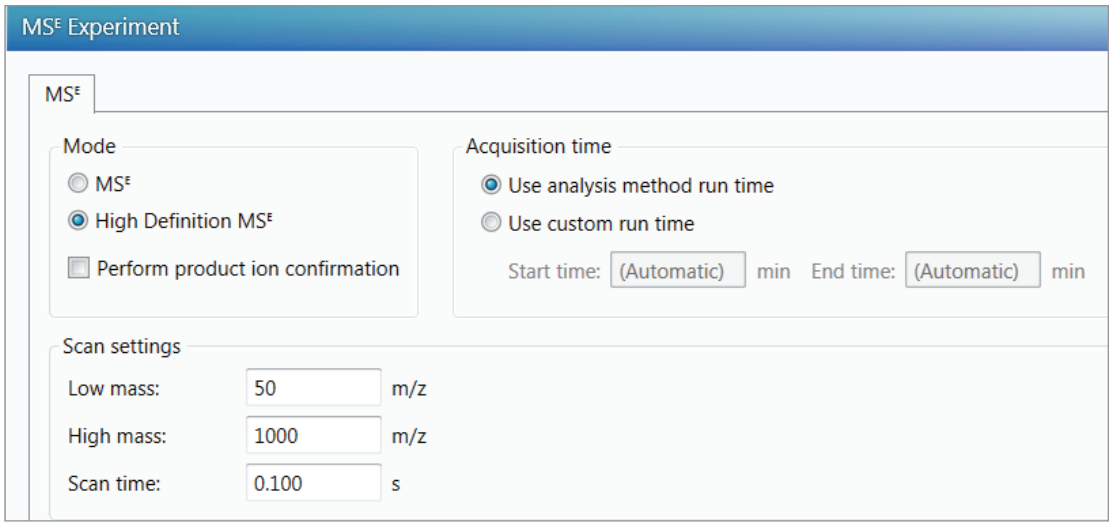

Figure 4. Setting HDMS^E mode of acquisition for collecting CCS Library information.

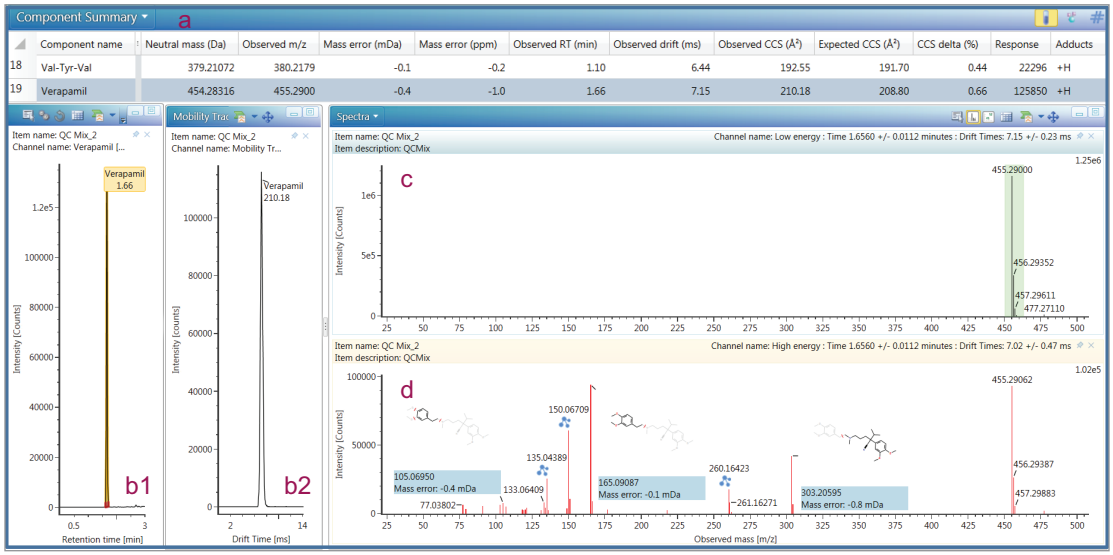

Figure 5. Verapamil dataset from the HDMS^E measurement, showing a) the result table, b1) XIC chromatogram, b2) ion mobilogram, and c) low and d) high energy spectra with structure annotation.

DEVIATION OF CCS MEASUREMENTS FOR QC COMPOUNDS

The QC reference solution from Waters contains a mixture of 9 compounds with reported CCS values.[1] These compounds are included in MajorMix set up solution (p/n 186008113) used in automated instrument setup. For the QC calibration to pass, the acceptance criteria for both intra and inter instrument measurement require that the observed CCS value is within 2% of the expected value (as shown in Figure 3). In the present study, the CCS value of QC compounds was collected before, during and after each compound set. A total of 30 CCS measurements were recorded for each compound in 6 sets of samples over two weeks. Table 4 summarizes the expected CCS, averaged observed CCS, averaged deviation of the measurement in terms of % difference from reported value (% Delta), and number of measurements. Figure 6 is a plot of % Delta of each compound within each injection of the 6 sets of measurements. Results show excellent deviation, with all individual measurement well within the acceptance criterion of 2%.

Table 2. Summary of averaged measured, expected CCS values, and deviation of QC compounds.

Component name#	*m/z*	Expected CCS (Å²)*	Observed CCS(Å²)	Deviation (%Delta)	Number of measurements
Caffeine	195.0876	138.2	137.7 ± 0.5	-0.36	30
Leu_Enkephalin	556.2766	229.8	228.5 ± 0.8	-0.57	30
Reserpine	609.2806	252.3	251.0 ± 1.0	-0.52	30
Sulfadimethoxine	311.0808	168.4	168.3 ± 0.6	-0.06	30
Sulfaguanidine	215.0597	146.8	146.0 ± 0.5	-0.55	30
Terfenadine	472.3210	228.7	230.1 ± 0.6	0.61	30
Val-Tyr-Val	380.2179	191.7	193.1 ± 0.8	0.73	30
Verapamil	455.2904	208.8	209.8 ± 0.9	0.48	30

** The expected CCS values are listed in "referencecompound.xml" file (dated 7/18/2016) in UNIFI folder.*
 # Acetaminophen elutes at solvent front and is not included in the summary.

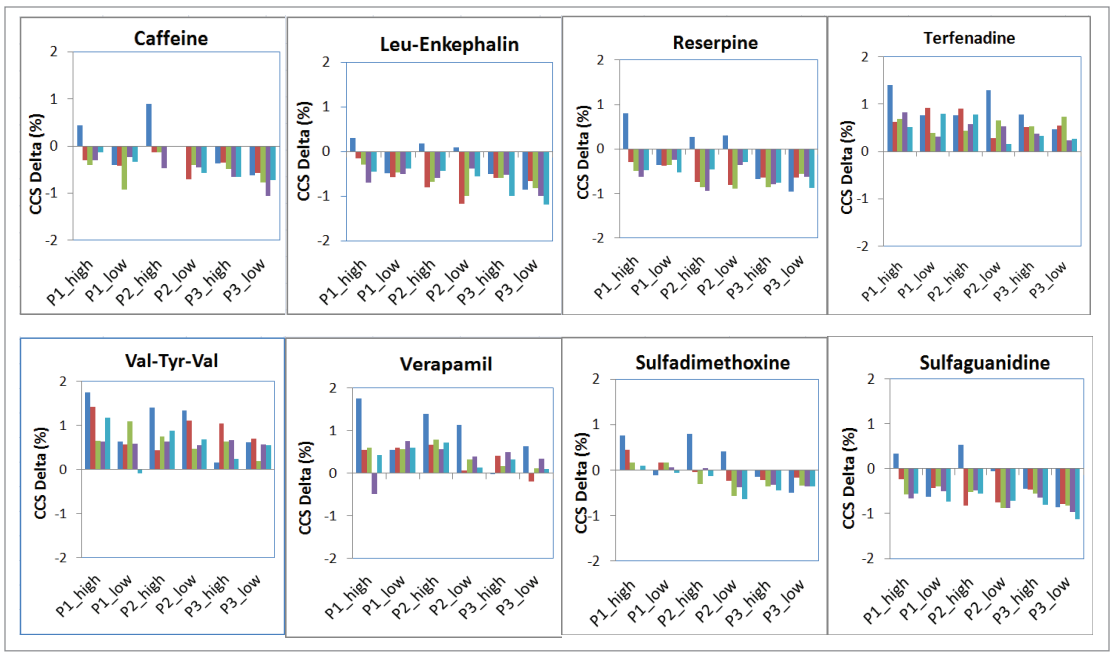

Figure 6. Plot of % Delta vs. sample sets. Within each sample set, there are 5 independent CCS measurements, and there are 6 sets of samples for a total of 30 independent CCS measurements for each compound. The data show the CCS measurement of all sample sets and all outcomes are well within the Vion specification.

PRECISION OF CCS MEASUREMENT

CCS values were measured for 134 diverse compounds with molecular weights ranging from 200 to 900 daltons. The data for representative compounds are summarized in Table 3. For each compound, the reported value is the average of triplicate measurements at two concentrations, for a total of 6 measurements. When data quality is poor at the low sample concentration, the reported value is the average of 3 independent measurements at the higher concentration. Table 3 is a summary of all data obtained, including the number of measurements, averaged CCS value and %RSD of CCS replicates. Figure 7 is a histogram plot showing distribution for the %RSD of CCS reproducibility. The data shows an excellent reproducibility of CCS measurement, for more than 99.3% compounds, the %RSD is ≤0.5%.

Table 3. Summary of measured CCS value.

entry	Component name	Neutral mass (Da)	Average observed *m/z*	Average mass error (ppm)	Average CCS	%RSD CCS	Number of measurement	Adduct
1	(S)-Timolol	316.1569	317.1644	0.58	175.79	0.17	6	+H
2	Altretamine	210.1593	211.1664	-0.67	145.99	0.17	6	+H
3	Amoxapine	313.0982	314.1055	-0.10	171.75	0.28	6	+H
4	Anastrozole	293.1641	294.1712	-0.52	183.63	0.19	6	+H
5	Aripiprazole	447.1480	448.1555	0.37	204.45	0.17	6	+H
6	Atazanavir	704.3898	705.3977	0.97	263.63	0.26	6	+H
7	Azithromycin	748.5085	749.5164	0.75	265.12	0.26	6	+H
8	Betaxolol	307.2147	308.2219	-0.23	187.29	0.30	6	+H
9	Bisacodyl	361.1314	362.1388	0.32	193.91	0.25	6	+H
10	Bumetanide	364.1093	365.1163	-0.73	186.17	0.23	6	+H
11	Buspirone	385.2478	386.2552	0.33	197.85	0.05	6	+H
12	Butenafine	317.2144	318.2216	-0.17	185.34	0.33	6	+H
13	Cabergoline	451.2947	452.3023	0.68	221.73	0.25	6	+H
14	Carvedilol	406.1893	407.1968	0.53	188.94	0.12	6	+H
15	Celecoxib	381.0759	382.0831	-0.18	186.52	0.23	6	+H
16	Chlorpromazine	318.0958	319.1032	0.38	170.58	0.18	6	+H
17	Citalopram	324.1638	325.1711	0.03	179.06	0.18	6	+H
18	Clindamycin	424.1799	425.1872	0.00	202.19	0.50	12	+H
19	Clofarabine	303.0535	304.0605	-0.63	158.41	0.32	6	+H
20	Clozapine	326.1298	327.1371	0.07	178.40	0.16	6	+H
21	Diltiazem	414.1613	415.1689	0.62	196.07	0.16	6	+H
22	Dipyridamole	504.3173	505.3248	0.60	225.27	0.21	6	+H
23	Dobutamine	301.1678	302.1750	-0.27	168.93	0.31	6	+H
24	Docetaxel	807.3466	830.3359	0.03	286.24	0.24	6	+Na
25	Dolasetron	324.1474	325.1547	0.20	178.45	0.23	6	+H
26	Donepezil	379.2147	380.2223	0.70	198.10	0.40	6	+H
27	Dorzolamide	324.0272	325.0347	0.77	170.11	0.16	6	+H
28	Eprosartan	424.1457	425.1531	0.38	204.57	0.14	6	+H
29	Erlotinib	393.1689	394.1762	0.13	200.61	0.12	6	+H
30	Escitalopram	324.1638	325.1712	0.30	179.66	0.24	6	+H
31	Fluphenazine	437.1749	438.1825	0.77	197.24	0.22	6	+H
32	Gefitinib	446.1521	447.1597	0.83	204.93	0.13	6	+H
33	Haloperidol	375.1401	376.1475	0.12	193.50	0.31	6	+H
34	Imipramine	280.1940	281.2011	-0.62	165.45	0.24	6	+H
35	Ivermectin	874.5079	897.4966	-0.53	299.90	0.17	3	+Na
36	Ketoconazole	530.1488	531.1563	0.50	214.64	0.14	6	+H

entry	Component name	Neutral mass (Da)	Average observed *m/z*	Average mass error (ppm)	Average CCS	%RSD CCS	Number of measurement	Adduct
37	Lamotrigine	255.0079	256.0154	0.98	151.08	0.38	6	+H
38	Lapatinib	580.1347	581.1421	0.20	236.48	0.20	6	+H
39	Lidocaine	234.1732	235.1804	-0.47	156.76	0.17	6	+H
40	Lincomycin	406.2138	407.2213	0.63	200.56	0.20	6	+H
41	Linezolid	337.1438	338.1509	-0.55	180.43	0.36	6	+H
42	Loperamide	476.2231	477.2306	0.60	221.90	0.12	6	+H
43	Meloxicam	351.0348	352.0419	-0.28	175.45	0.16	6	+H
44	Moxifloxacin	401.1751	402.1826	0.50	196.84	0.12	6	+H
45	Mycophenolate	433.2101	434.2178	1.05	196.59	0.24	6	+H
46	Naltrexone	341.1627	342.1702	0.70	176.69	0.15	6	+H
47	Olanzapine	312.1409	313.1483	0.37	176.04	0.47	6	+H
48	Olopatadine	337.1678	338.1753	0.63	178.32	0.08	6	+H
49	Pilocarpine	208.1212	209.1284	-0.40	146.69	0.19	6	+H
50	Pindolol	248.1525	249.1598	0.03	159.15	0.17	6	+H
51	Pioglitazone	356.1195	357.1270	0.57	177.85	0.21	6	+H
52	Piroxicam	331.0627	332.0700	0.03	171.61	0.14	6	+H
53	Prazosin	383.1594	384.1670	0.97	193.62	0.20	6	+H
54	Promethazine	284.1347	285.1420	-0.08	163.61	0.21	6	+H
55	Propafenone	341.1991	342.2065	0.45	178.14	0.53	6	+H
56	Quetiapine	383.1668	384.1744	0.95	191.83	0.14	6	+H
57	Rifampicin	822.4051	823.4122	-0.28	286.36	0.08	6	+H
58	Riluzole	234.0075	235.0150	0.93	143.41	0.19	6	+H
59	Risperidone	410.2118	411.2196	1.30	205.04	0.27	6	+H
60	Sertaconazole	435.9971	437.0046	0.47	189.13	0.32	6	+H
61	Sirolimus	913.5551	936.5428	0.10	323.06	0.21	3	+Na
62	Sumatriptan	295.1355	296.1427	-0.08	162.29	0.14	6	+H
63	Tacrine	198.1157	199.1230	-0.03	141.20	0.13	6	+H
64	Terbinafine	291.1987	292.2058	-0.73	187.64	0.07	6	+H
65	Tizanidine	253.0189	254.0264	1.00	148.99	0.21	6	+H
66	Vardenafil	488.2206	489.2279	0.05	227.22	0.15	6	+H
67	Vinblastine	810.4204	811.4278	0.23	284.23	0.32	6	+H
68	Vinorelbine	778.3942	779.4019	0.62	279.25	0.15	6	+H

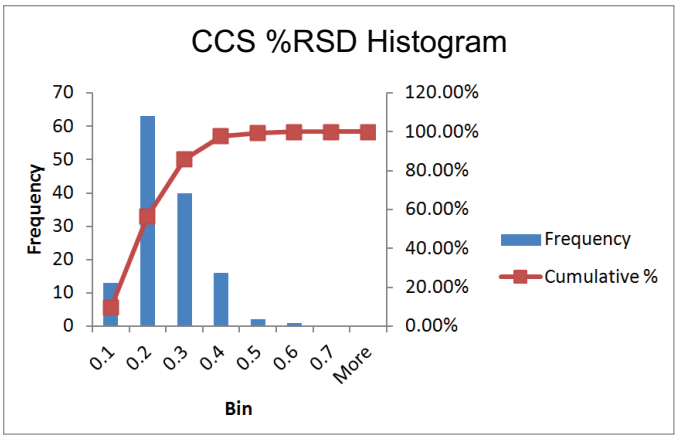

Figure 7. Histogram plot of CCS %RSD value. The data show >99% data has %RSD <0.5%.

RELATIONSHIP BETWEEN CCS VALUE AND *m/z*

The CCS value of a given class of compounds is broadly correlated with *m/z*.[2,3] In this study, when the CCS value is plotted against *m/z*, there is a linear relationship having slope = 0.2312, intercept = 101, and a coefficient of determination R^2 = 0.96 (Figure 8). At a given *m/z*, the spread of CCS value extends to ~30 Å^2. For example, around *m/z* of 290, CCS value ranges from 160 to 190 Å^2. This relationship suggests that CCS prediction based on mass alone is insufficient, additional molecular descriptors such as 3D conformation and partical charge distribution have been shown to be important in CCS prediction.[3] The expanded graph in Figure 8 includes the standard deviation of CCS for each compound as the error bar, where the reproducibility is less than 1 Å^2. The data suggests that for compounds with the same *m/z*, high precision of the CCS measurement will help in unambigously identifying the peaks of interest, making CCS highly valuable in discriminating compounds compared to using *m/z* alone.

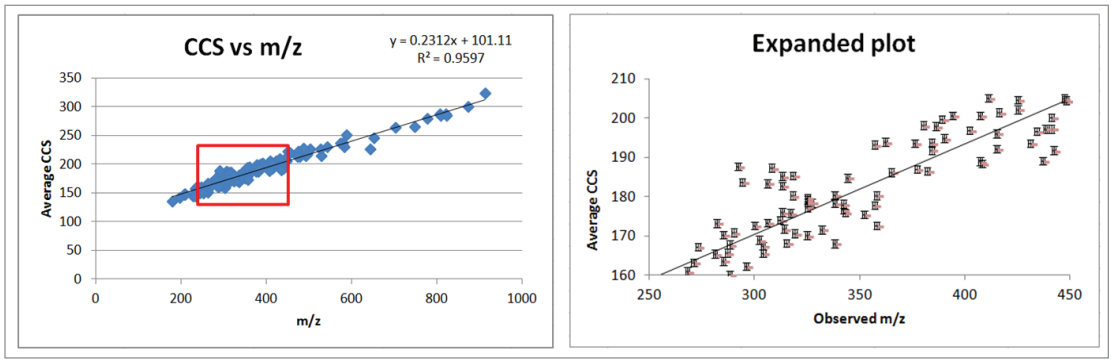

Figure 8. (left) Plot of CCS value vs m/z. The line shown is a linear regression with the equation y = 0.2312 x + 101.11 and R^2 = 0.9597. The inset (red region) of m/z from 250 to 450 is shown on the right.

MASS ACCURACY OF THE VION IMS QTof

For accurate library building or measurements, it is equally important to have high mass accuracy. Vion is the first generation of Waters Tof products to use the new QuanTof 2™ Detector (see Figure 1). Compared to the previous detector, QuanTof 2 has increased performance which prevents signal saturation and improves sensitivity and linearity when ion mobility is enabled. These enhancements significantly improve the suitability of the detector for its use in data acquisition across a variety of concentrations and for routine use. The reported mass error for representative compounds is included in Table 3. For the 134 compounds determined in the present study, the RMS is 0.86 ± 0.36 ppm.

CONCLUSIONS

The deviation and precision of a set of CCS measurements were determined on the Vion IMS QTof platform. CCS was measured for QC and 134 additional small molecule compounds. Deviation of the measurement was determined based on 30 measurements for each of the eight quality control compounds and found to be well within 2%. Precision was determined based on 6x repeated measurements at two concentrations for each of the 134 commercially available FDA approved drugs. Results showed excellent CCS precision with %RSD less than 0.6% for all compounds. The mass accuracy of the instrument is excellent with RMS less than 1 ppm for the set of compounds measured. In conclusion, the present study suggests that Vion is a robust platform for routine qualitative and quantitative analysis. The high accuracy in CCS and *m/z* measurement enables its utility for ion mobility and *m/z*-based compound identification and measurements.

Companion document: UNIFI library of QC and CCS compounds measured in this study.

References

1. The expected CCS values are listed in "referencecompound.xml" file (dated 7/18/2016) in UNIFI program file folder.
2. J. Regueiro, et. al. *Anal. Chem.* 2016, *in press*, DOI: 10,1021.
3. G.B. Gonzales, et. al. *J. Anal. Chim. Acta* 2016, 924, 68.

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