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Analytical and Informatic Benefits from Automated Conversion of Ion Mobility Arrival Times to Collision Cross Sections in Raw Data Files

Sarah M. Stow[1], Aivett Bilbao[2], Bryson C. Gibbons[2], Rachel Harris[3], Kelly W. Moser[3], J. Daniel DeBord[3], John C. Fjeldsted[1]

[1]Agilent Technologies, Santa Clara, CA

[2]Pacific Northwest National Laboratory, Richland, WA

[3]MOBILion Systems, Inc., Chadds Ford, PA

Introduction

Achieving faster separations prior to MS analysis is a common goal for analytical workflows. While LC is still the primary choice, ion mobility (IM), now available in various configurations from multiple vendors, is actively used in many labs. During IM, ions are separated based on their gas phase structure and mobility measured by ion arrival time (AT). The AT of the ions can then be converted into a collision cross section (CCS) or rotationally averaged cross-sectional area of the ion which is less susceptible to changes in method parameters[1] and measurements made on different instruments. An automated preprocessing conversion of the IM-MS raw measurements to CCS space will be discussed in this presentation and evaluated across various method parameters.

Figure 1. SLIM (structures for lossless ion manipulation)-based HRIM device coupled to a 6546 LC/Q-TOF

Mapping Arrival Time to Collision Cross Section Space

The PNNL Preprocessor[2] was used to map arrival time data to CCS space. This mapping is performed by converting every individual (x,y) coordinate of (*m/z*, arrival time) to (*m/z*, CCS).

$$\Omega/z = \left[\frac{m_g + m_i}{m_i}\right]^{\frac{1}{2}} \left(a + bt_i + ct_i^2 + dt_i^3\right)$$

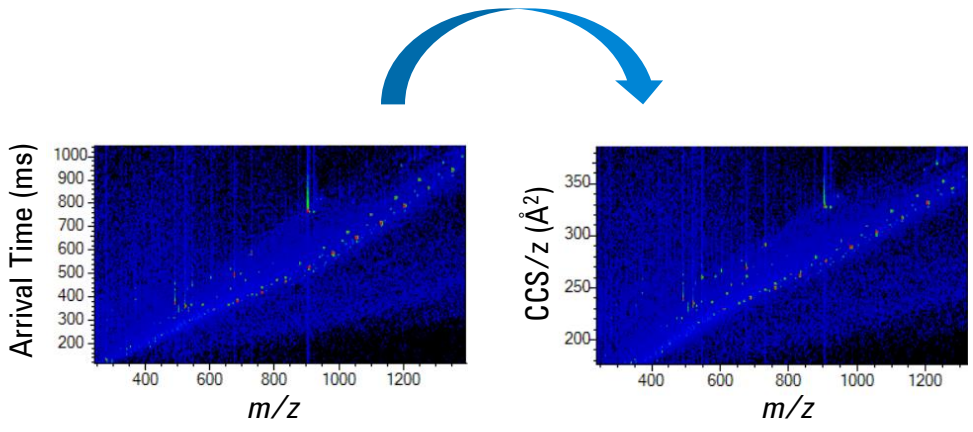

Figure 2. CCS space mapping performed by the PNNL PreProcessor

Experimental

Experimental Parameters

Experiments were performed on a commercial High Resolution Ion Mobility (HRIM) device (MOBILion Systems, Inc., Chadds Ford, PA) and a 6546 LC/QTOF (Agilent Technologies, Santa Clara, CA). A commercial LC (1290 Infinity II Series, Agilent Technologies, Santa Clara, CA) was used for sample introduction for both flow injection and a HILIC (RX-Sil, 3.0 x 100 mm, 1.8 micron) separation prior to SLIM-MS analysis. Agilent Tune Mix as well as lipid standards (Avanti Polar Lipids, Alabaster, AL) were run to evaluate performance. Two LC gradients (Table 1.) were run to evaluate RT variations across the three methods. LC gradient 1 was run at both 0.36 mL/min and 0.45 mL/min flow rates. Three separation wave settings (Table 2.) were run to evaluate arrival time and CCS variations across the three methods. Agilent Tune Mix was used for all CCS calibrations.

Table 1. Two LC gradients used in this study. LC gradient 1 was run at both 0.36 mL/min and 0.45 mL/min

LC Gradient 1			LC Gradient 2		
Min	ACN (0.1% FA)	ACN:MeOH:H_2O (50:20:30 v/v) (20 mM NH_4HCO_2)	Min	ACN (0.1% FA)	ACN:MeOH:H_2O (50:20:30 v/v) (20 mM NH_4HCO_2)
0	70%	30%	0	70%	30%
2	40%	60%	3	40%	60%
4	30%	70%	4	20%	80%
5	0%	100%	6	0%	100%
8	0%	100%	8	0%	100%
9-12	70%	30%	9-12	70%	30%

Table 2. Three separation wave settings for the SLIM experiments

Method	Wave Speed	Wave Amplitude
1	180 m/s	40 V_{pp}
2	180 m/s	30 V_{pp}
3	145 m/s	30 V_{pp}

Data Preprocessing Parameters

The PNNL PreProcessor[2] was used for data pretreatment including drift bin summing (5), drift and mass smoothing (3), thresholding (min20), and spike removal (1). These techniques improve peak shape and reduce data file size. IM data files were also compressed into a single frame and converted to 3D data files (IM dimension mapped to LC space). Arrival time space was also converted to CCS space using the PNNL PreProcessor. Agilent IM-MS Browser was used to create and apply the CCS calibration as well as perform feature finding to facilitate CCS reporting.

Comparing Reproducibility for Retention Time, Arrival Time, and Collision Cross Section Space

To see the benefit of calibrating to CCS space, the lipid standard sample was run at various experimental conditions. Extracted ion chromatograms for 5 lipids in the sample (Ceramide, PE, PC, SM, and LPC) are shown in Figure 3. Shifts in RT are shown in the A) column and shifts in AT are shown in the B) column. When AT values are calibrated to CCS space using the CCS mapping in the PNNL PreProcessor, the lipids align as shown in column C). Each experimental condition was performed in triplicate and results are shown in Table 3. for 3 lipids (Ceramide, PC, and SM). Changes in LC gradient and flow rate result in ~12-13.5% shifts in RT. Changes in separation wave settings on the SLIM result in even larger ~30% shifts. Ion mobility provides a built-in calibration to CCS space which standardizes changes in parameters to less than 0.2% shifts when experimental parameters are changed. While comparing results from different LC and SLIM parameters is not recommended, it is shown here for proof of principal. Results are also shown within each experimental method highlighting the reproducibility of IM measurements compared to LC. The relative intensity of the lipids is worth noting as a benefit to LC separations is reducing the number of ions competing for ionization at the same time.

Table 3. Reproducibility results for the Ceramide, PC, and SM lipids for RT, Arrival Time, and mapped CCS. Results are shown across methods and within each method

		RT	AT	CCS
Ceramide	All	1.23 ± 0.15 (12.01%)	620.49 ± 165.93 (26.74%)	259.05 ± 0.25 (0.10%)
	Method 1 n=3	1.32 ± 0.01 (0.44%)	451.68 ± 0.05 (0.01%)	258.99 ± 0.01 (<0.01%)
	Method 2 n=3	1.32 ± 0.00 (0.31%)	828.75 ± 1.18 (0.14%)	259.30 ± 0.32 (0.12%)
	Method 3 n=3	1.06 ± 0.01 (0.60%)	581.04 ± 0.18 (0.03%)	258.87 + 0.03 (0.01%)
PC	All	4.35 ± 0.59 (13.53%)	677.40 ± 180.53 (26.65%)	268.63 ± 0.04 (0.02%)
	Method 1 n=3	4.35 ± 0.03 (0.76%)	493.25 ± 0.07 (0.01%)	268.68 ± 0.02 (0.01%)
	Method 2 n=3	4.93 ± 0.01 (0.12%)	903.72 ± 0.06 (0.01%)	268.60 ± 0.01 (<0.01%)
	Method 3 n=3	3.75 ± 0.01 (0.20%)	635.23 ± 0.04 (0.01%)	268.60 ± 0.01 (<0.01%)
SM	All	4.62 ± 0.60 (13.05%)	805.51 ± 212.03 (26.32%)	291.17 ± 0.47 (0.16%)
	Method 1 n=3	4.64 ± 0.03 (0.71%)	588.32 ± 0.09 (0.01%)	291.66 ± 0.02 (0.01%)
	Method 2 n=3	5.21 ± 0.00 (0.08%)	1070.83 ± 0.15 (0.01%)	290.58 ± 0.02 (0.01%)
	Method 3 n=3	4.00 ± 0.01 (0.19%)	757.38 ± 0.07 (0.01%)	291.28 ± 0.01 (<0.01%)

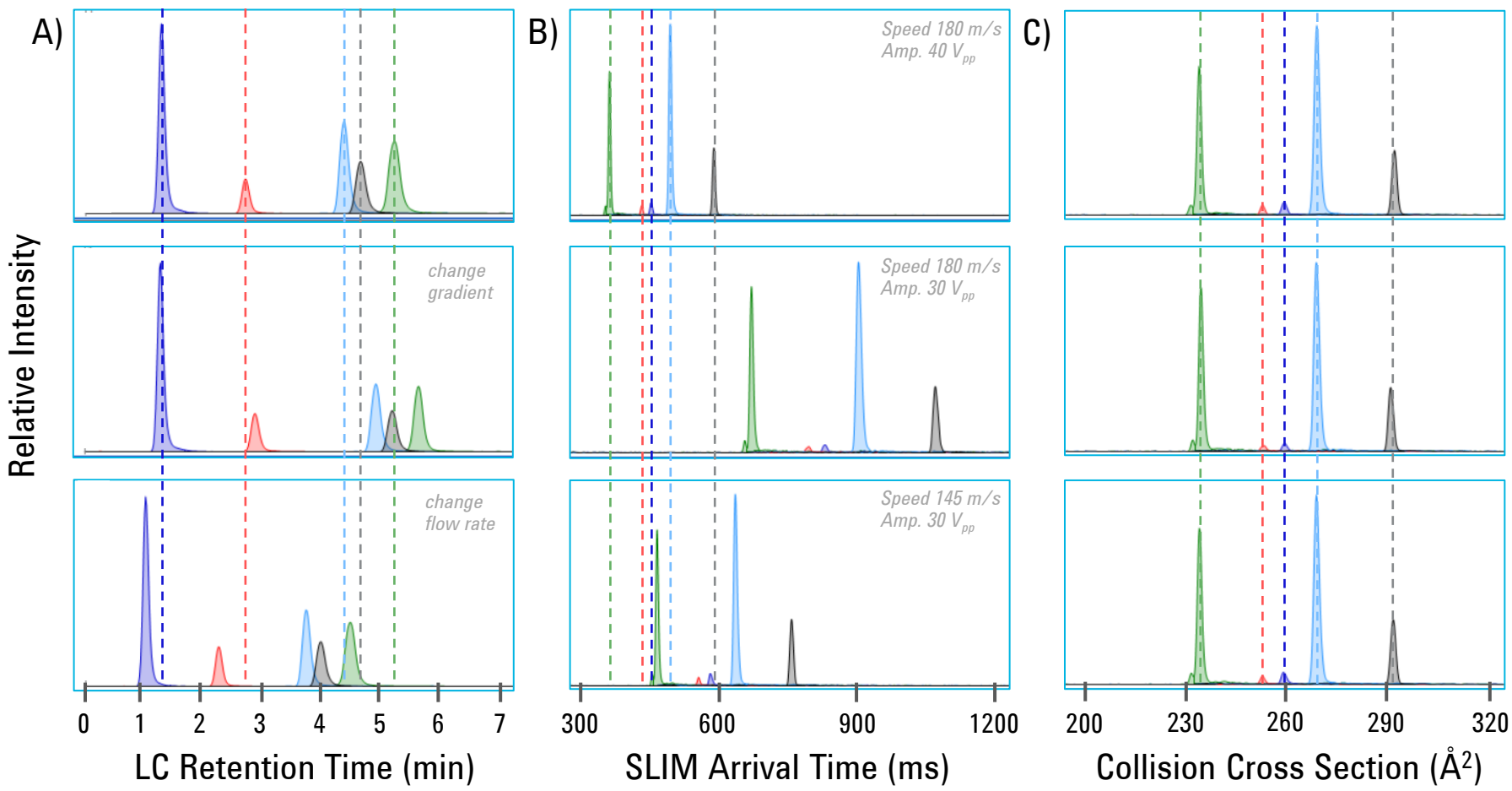

Figure 3. Extracted ion chromatograms for A) LC separation, B) SLIM separation, and C) calibrated SLIM separation to collision cross section space for 5 lipid standards (Ceramide – dark blue, PE – red, PC – light blue, SM – black, and LPC – green). Changes in LC gradient and flow rate cause LC peaks to shift. Changes in SLIM wave settings cause major changes in arrival time for the ions. Calibrating SLIM results to CCS space results in the most reproducible results across various experiment parameters.

Results and Discussion

CCS Agreement Across Separation Wave Settings and Between Feature Finding & CCS Space Mapping

CCS results are shown in Figure 4. for 8 lipids across 3 separation wave settings (green, red, and blue) and for feature finding (circle) and CCS space mapping (square). Previous to the CCS mapping on the raw data presented in this study, CCS values are determined for features which are based on IM peak detection using the arrival time centroid. Agreement between feature finding and CCS mapping is less than 0.05% across the lipids. Percent difference across the wave settings is less than 0.54% with larger differences observed for the red setting due to peak broadening at longer arrival times observed at this setting.

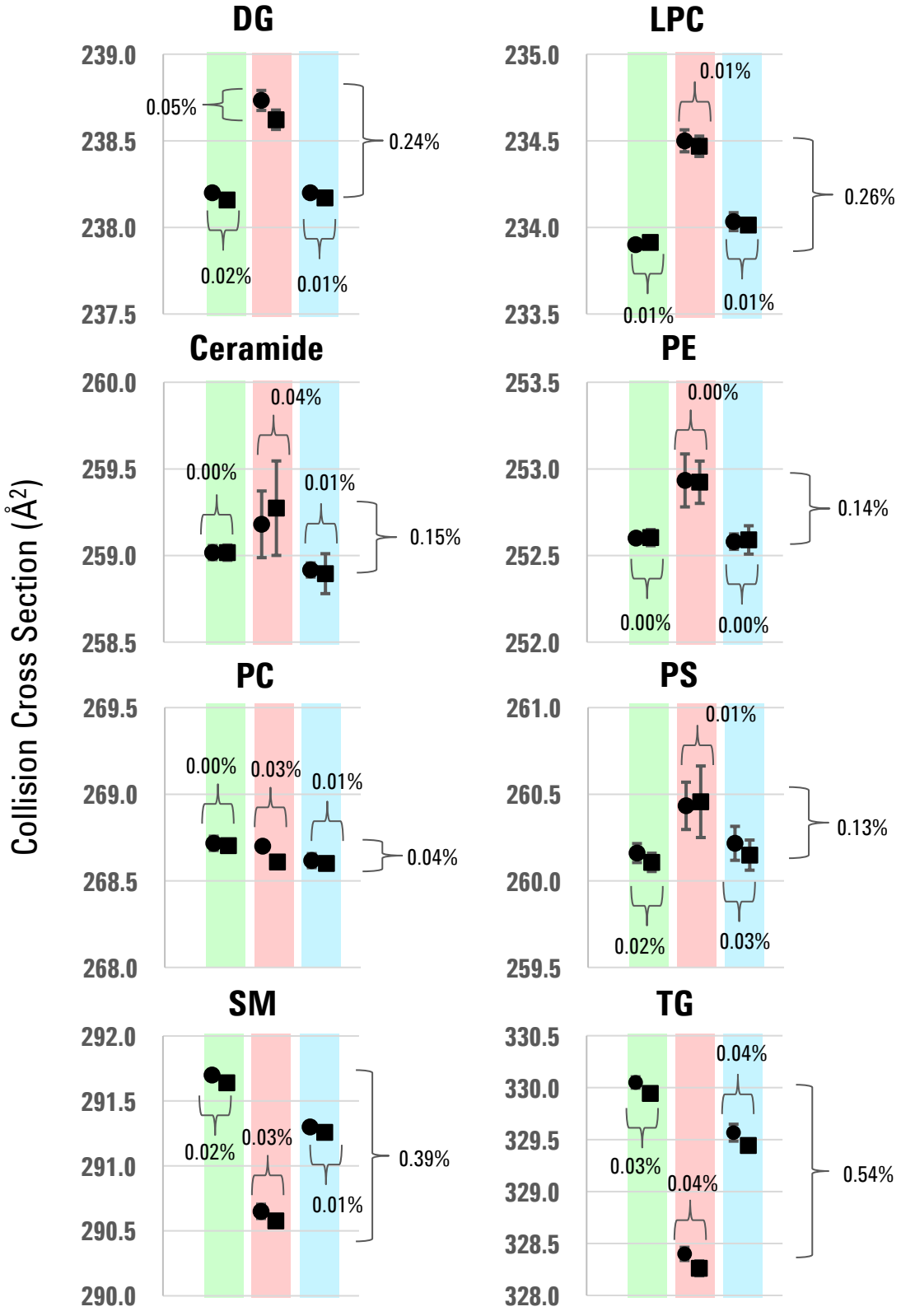

Figure 4. Scatter plots to show variations in CCS across separation wave settings (180m/s & 40V_{pp} - green, 180 m/s & 30 V_{pp} - red, 145m/s & 30 V_{pp} - blue) and between feature finding and mapped CCS values (feature finding – circle, mapping – square).

Comparison of SLIM and 6560 Drift Tube CCS Values

Calibrated CCS values from SLIM were compared with Single Field CCS values from the 6560 Drift Tube instrument for positive and negative ions. Single Field ^{SLIM}CCS have a percent difference less than 2.0% when compared to Single Field ^{DT}CCS values for the lipid standards analyzed in this study. A percent difference of 2.0% is small, but does suggest that libraries of ^{SLIM}CCS values may benefit future informatics workflows.

Table 4. Comparison of SLIM and 6560 DT CCS Values

+	Single DT	SLIM (%diff.)	-	Single DT	SLIM (%diff.)
DG	235.23	238.20 (1.3%)	LPC	241.90	243.20 (0.5%)
LPC	232.30	233.90 (0.7%)	PA	238.40	241.10 (1.1%)
Cer	255.10	259.02 (1.5%)	Cer	258.45	262.60 (1.6%)
PE	250.10	252.60 (1.0%)	PE	246.55	249.70 (1.3%)
PC	264.87	268.72 (1.4%)	PG	253.60	257.30 (1.4%)
PS	259.73	260.16 (0.2%)	PS	257.15	261.20 (1.6%)
SM	285.97	291.70 (2.0%)	PC	272.55	276.53 (1.4%)
TG	324.77	330.05 (1.6%)	PI	269.35	273.53 (1.5%)

Conclusions

In this study we evaluated the benefits of converting to CCS space for IM data.

- Results can be compared across various instrument settings when data is mapped to CCS space
- Percent difference from CCS values from different wave settings is within 0.54%
- CCS agreement between feature finding results and mapping is within 0.05%

References

[1]J. C. May et. al. Resolving Power and Collision Cross Section Measurement Accuracy of a Prototype High-Resolution Ion Mobility Platform Incorporating Structures for Lossless Ion Manipulation. Journal of the American Society for Mass Spectrometry 2021, 32, 4, 1126-1137.

[2]A. Bilbao et. al. A Preprocessing Tool for Enhanced Ion Mobility-Mass Spectrometry-Based Omics Workflows. Journal of Proteome Research 2021.

https://explore.agilent.com/asms

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