Precursor and Product Ion Mobility and Collision Cross Section Determination by Travelling Wave Cyclic Ion Mobility – Mass Spectrometry

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Introduction

The identification and elucidation of (bio)chemical compound structures are critically important across numerous applications, such as pharmaceutical sciences, toxicology, biotechnology, and food safety research. The potential of Travelling-Wave Cyclic Ion Mobility – Mass Spectrometry was evaluated for the identification of compounds where the position of a functional group could not be resolved using conventional tandem mass spectrometry. In addition to ion mobility measurements of intact precursor ions, ion mobility measurements of product ions were obtained and the development of workflows for the processing of multi-dimensional data sets, including precursor/product ion *m/z*, retention time, CCS, and intensity investigated.

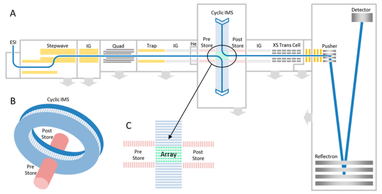


***Figure 1**. SELECT SERIES™ Cyclic™ IMS configuration/ion optics [1].*

Samples/applications

- *Flavonoids (natural products)*: isoorientin, isovitexin, orientin, vitexin
- *System Suitability Test Mixture:* acetaminophen, caffeine, sulfaguanidine, sulfadimethoxine, Val-Tyr-Val, verapamil, reserpine, terfenadine, Leu-enkephalin
- *Drug(s) (pharmaceutical):* betaxolol, ciprofloxacin
- *PFAS (environmental)*: PFAC30PAR (Wellington Laboratories)

Experimental

Chromatography

- ACQUITY™ UPLC™ BEH™ C18 Column (100 mm x 2.1 mm, 1.8 µm) operated @ 35 ºC and 0.3 mL/min
- Mobile phase(s) A 95 H_2O (2 mM ammonium acetate) : 5 MeOH, and B MeOH (2 mM ammonium acetate)
- 22 min (PFOS isomers) and 5 min (pharmaceutical/natural product standards) reversed phase separation gradients
- Injection volume: 10 µL

Mass Spectrometry

- Polarity: positive (ESI+)/negative (ESI-)
- Capillary voltage: 0.5/0.3 kV
- Source temp: 100 ºC
- Desolvation temp/gas flow: 550 ºC and 1000 L/Hr
- Acquisition methods: HDMSE (IM enabled DIA), HDMS (IM-MS) with/without quadrupole isolation

Data processing

- MassLynx™/DriftScope™ (data acquisition and review)
- ApexRT 1.17 (peak detection),
- CompareCSV 1.22 (multicolumn matching with tolerances)
- CFM-ID v2.4 (*in-silico* fragmentation)
- Streamlit, plotly, pandas, numpy, matplotlib Python libraries (user interface(s), visualization and CCS calculation)

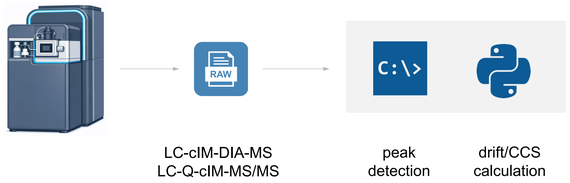


***Figure 2**. Data acquisition and processing workflow [2], illustrating multidimensional peak detection/ centroiding and CCS calculation, using development software and tools.*

Flavonoids
(natural products)

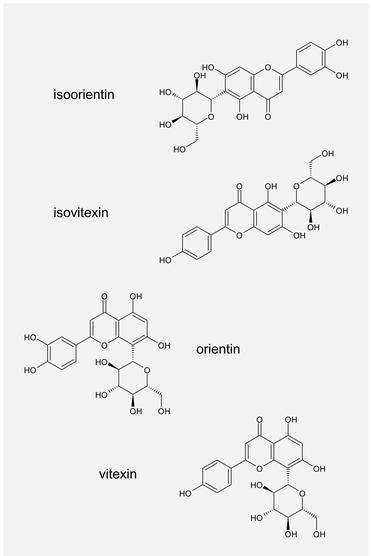


***Figure 3**. Example natural product isomeric compound structures with orientin/isoorientin and vitexin/isovitexin representing isomeric molecule pairs.*

Results

Shown, sequentially, are the intermediate and final results of *i)* peak detection and CCS calibration, including *ii)* raw data examples, followed by *iii)* matching detections against *in silico* predictions [3] and annotation.

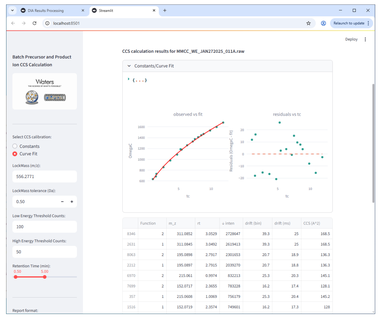
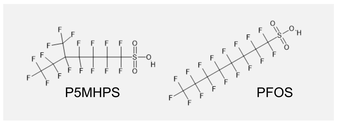


***Figure 4**. Peak detection and CCS calculation, including calibration.*



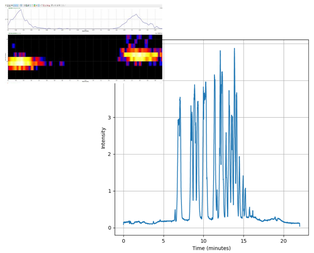


***Figure 5**. TIC and isomeric product ion drift time separation of m/z 379 product ions from linear PFOS and branched isomer P5MPHpS.*

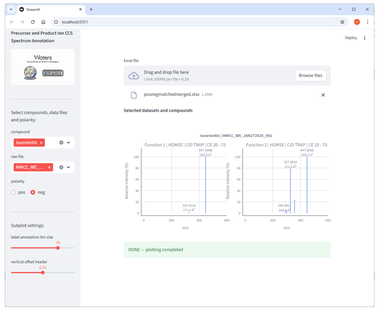


***Figure 6**. Precursor and product ion m/z and CCS annotation following in silico fragmentation prediction detection matching.*

Conclusions

- Ion mobility–derived CCS values of diagnostic precursor and product ions enabled discrimination between linear and branched PFOS isomers
- Drift time and CCS measurements allowed differentiation of isomeric flavonoid precursor and product ions.
- Highly specific CCS fingerprints of small-molecule product ions were generated for pharmaceutical-relevant compounds in combination with *in-silico* product-ion prediction.
- Fast data processing and review were facilitated through lightweight application development

Compound	Method	Polarity	Units	Precursor	Product					
Linear-PFOS	Q-cIM-MS/MS	ES-	m/z	499	-	380	-	280	-	
			CCS (Å²)	167.8	-	148.0	-	128.7		
Branched-PFOS 5MHPS	Q-cIM-MS/MS	ES-	m/z	499	430	380	330	280	-	
			CCS (Å²)	163.5	153.9	145.2	135.7	128.6		
Isoorientin	Q-cIM-MS/MS	ES-	m/z	447	357	327	299	-	-	
			CCS (Å²)	202.4	184.2	174.1	168.5	-	-	
Orientin	Q-cIM-MS/MS	ES-	m/z	447	357	327	299	-	-	
			CCS (Å²)	192.6	178.4	170.8	168.1	-	-	
Isovitexin	Q-cIM-MS/MS	ES-	m/z	431	341	311	283	-	-	
			CCS (Å²)	201.1	180.1	171.9	164.9	-	-	
Vitexin	Q-cIM-MS/MS	ES-	m/z	431	341	311	283	-	-	
			CCS (Å²)	193.7	176.2	168.8	164.4	-	-	
Verapamil	Q-cIM-MS/MS	ES+	m/z	455	303	260	165	-	-	
			CCS (Å²)	206.8	173.6	164.4	128.3	-	-	
Leucine enkephalin	Q-cIM-MS/MS	ES+	m/z	556	397	336	279	136	120	
			CCS (Å²)	227.6	193.8	180.5	171.5	128.9	124.4	
Reserpine	Q-cIM-MS/MS	ES+	m/z	609	397	365	195	174	-	
			CCS (Å²)	248.4	196.1	183.3	134.4	131.5		
Val-Tyr-Val	Q-cIM-MS/MS	ES+	m/z	380	235	136	380	-	-	
			CCS (Å²)	189.7	154.0	128.8	189.7	-	-	
Betaxolol	Q-cIM-MS/MS	ES+	m/z	308	290	266	231	177	-	
			CCS (Å²)	185.0	179.0	168.2	154.0	133.4	-	

***Table 1**. Precursor and product ion m/z and CCS values for environmental, natural products, and pharmaceutical relevant compounds. CCS differentiation is highlighted in orange, green and blue.*

References

1. *Giles et al. A Cyclic Ion Mobility-Mass Spectrometry System. Anal Chem.* ***2019*** *Jul 2;91(13):8564-8573.*
2. *Techniques for sample analysis using product ion collision-cross section information, US12332210B2*
3. *Allen et al. CFM-ID: a web server for annotation, spectrum prediction and metabolite identification from tandem mass spectra. Nucleic Acids Res.* ***2014*** *Jul;42(Web Server issue):W94-9*

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