THE UTILITY OF CYCLIC ION MOBILITY TO IMPROVE SELECTIVITY AND ANALYSIS EFFICIENCY OF ENVIRONMENTAL PFAS CONTAMINATION AND EXPOSURE

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INTRODUCTION

Polyfluorinated alkyl substances (PFAS) exposure is a potential contributor to increased cancer occurrence in the human population. The bio-accumulative nature of PFAS enables monitoring of levels in human biofluids to help gain understanding into exposure levels and pathways. However PFAS isomeric compounds, can be challenging to efficiently separate using liquid chromatography.

Cyclic ion mobility (cIM) provides an added dimension of separation and collision cross section (CCS) values can provide a a complimentary identification descriptor. CCS values provide an additional identification point across multiple application areas. At low concentrations where fragment information may be absent, CCS and arrival time distribution (ATD) provide an additional identification point alongside retention time (t_R).

To highlight confidence of the utility of CCS values, independent of ion mobility (IM) technology, when performing PFAS non-targeted screening assays, we have used an internally developed PFAS library to cross correlate PFAS CCS reproducibility. The SELECT SERIES™ Cyclic™ IMS mass spectrometer (see Figure 1) has been used to perform an inter-site/intra-site comparison and compared to published drift tube (DT) ion mobility PFAS CCS values.[1]

Using LC-cIM-MS, branched and linear PFAS isomers have been fully resolved. Detection of individual PFAS isomers provides opportunity to correlate cancer risk with exposure to specific PFAS isomers. Human serum samples were analysed, and data generated for PFAS isomers and isobaric biomarker isomer composition.

LC-cIM-MS (cIM resolution (R)~65-145) analyses were performed using a SELECT SERIES Cyclic IMS mass spectrometer. PFAS standards and human serum samples were analysed, using a 22 min reversed phase separation gradient. Using the combined peak capacity of LC-cIM, enabled resolution of linear and branched isomers, whilst simultaneously achieving an increase in analysis efficiency of 75 %. The enhanced resolution has the potential to facilitate a time efficient correlation between PFAS isomeric structure, concentration, and exposure. Linear and branched PFOS isomers were IM resolved in anonymized human serum extracts, using a 5.5-minute LC gradient. Importantly chromatographic separation was achieved for PFOS and bile acid biomarkers. Using ion mobility resolution (~145), we report IM enhanced specificity and resolution for biomarker isomers Taurodeoxycholic, Taurochenodeoxycholic and Tauroursodeoxycholic acid.

A focus on LC-cIM-MS as a potential strategy to generate time efficient, additional toxicological correlation specificity with individual PFAS isomeric species and resultant elevation of isobaric biomarker isomers is presented. Identification of perfluoroalkyl carboxylic acids (PFCAs), cIM conformeric profiles and corresponding CCS identification finger prints provides newfound specificity.[2]

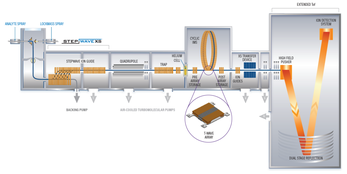


Figure 1. Schematic of SELECT SERIES Cyclic IMS mass spectrometer.

METHODS

Sample description.

Native PFAS mixture solution: PFAC30PAR and single PFAS standards: (Wellington Laboratories).

Anonymised human serum sample extracts

Library: Waters PFAS library- (130 entries)

Extraction conditions: Human serum samples were extracted using SPE 96-well µElution plates, containing a polymeric reversed-phase, weak anion exchange mixed-mode sorbent.[3]

LC Conditions:

Waters™ ACQUITY™ UPLC™ I-Class Premier chromatography system modified with PFAS Kit and ACQUITY UPLC Atlantis™ Premier BEH™ C18 AX Isolator Column, 2.1 x 50mm, 5 µm.[4]

ACQUITY UPLC HSS T3 2.1 mm x 100 mm, 1.8 µm analytical column.

Mobile phase A: 95 H_2O (2mM ammonium acetate): 5 MeOH. Mobile phase B: MeOH (2mM ammonium acetate)

Injection volume 5µL Column temperature 35 °C.

MS Conditions

Acquisition:	ES-	Data analysis and visualization: MassLynx™ v4.2 SCN1026, Driftscope™ 3.0 and Waters_connect™ software 3.8.0.23 Tibco Spotfire® 6.0.0 Software (Palo Alto, CA). Pubchem
Capillary Voltage:	0.5 kV	
Desolvation Temperature:	250 °C	
Source Temperature:	100 °C	
Cone Voltage:	10 V	
Collision Energy Ramp:	20-70 eV	
Mass Range:	*m/z* 50–1200	
MS^E Acquisition Rate:	0.156 seconds	

Scaled

TIME (minutes)	FLOW (ml/min)	%A	%B
Initial	0.30	95	5
1.0	0.30	75	25
6.0	0.30	50	50
13.0	0.30	15	85
14.0	0.30	5	95
17.0	0.30	5	95
18.0	0.30	95	5
22.0	0.30	95	5

Shortened

TIME (minutes)	FLOW (ml/min)	%A	%B
Initial	0.30	95	5
1.0	0.30	75	25
3.0	0.30	50	50
4.5	0.30	15	85
7.0	0.30	5	95
8.5	0.30	5	95
9.0	0.30	95	5
11.0	0.30	95	5

Shortened

TIME (minutes)	FLOW (ml/min)	%A	%B
Initial	0.30	95	5
0.5	0.30	75	25
1.5	0.30	50	50
3.25	0.30	15	85
3.5	0.30	5	95
4.25	0.30	5	95
4.5	0.30	95	5
5.5	0.30	95	5

RESULTS

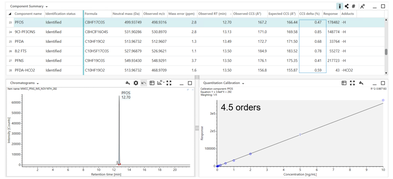


Figure 2. PFAS data review using Waters_connect, illustrating the linear response obtained for PFOS (5pg/mL to 10,000 pg/mL). $HDMS^E$ library comparison CCS values determined for "PFAC30PAR", Δ CCS < 1% illustrated.

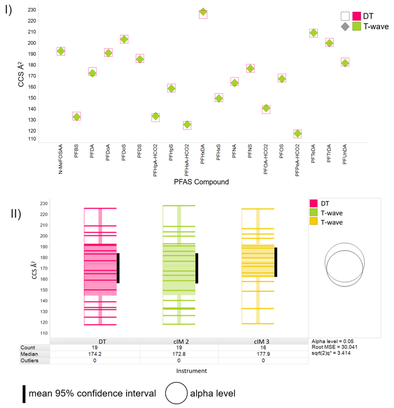


Figure 3. (I) Scatterplot comparison of PFAS cIM and DT CCS values. (II) Box plot statistical analysis ((a) intra-site and (b) inter-site) for the distribution of PFAS T-wave and DT ion mobility CCS values determined using analytical standards.

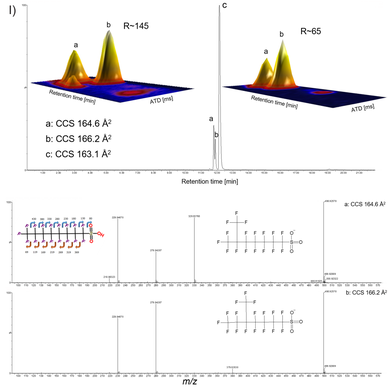


Figure 4 I) Analysis of perfluoro-1-methylheptanesulfonate and comparison of chromatographically coeluting isomeric PFOS impurity separation with increased cIM resolution. II) cIM separated single component magnified product ion spectra of isomeric PFOS impurities.

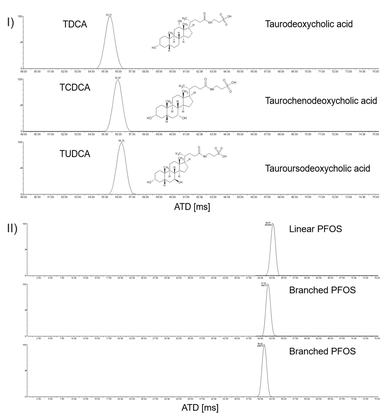
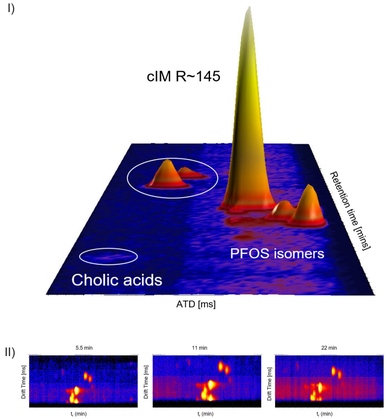


Figure 5 I) cIM differentiation of isomeric cholic acid biomarkers (R~145). II) cIM differentiation of isomeric PFOS linear and branched isomers (R~145).



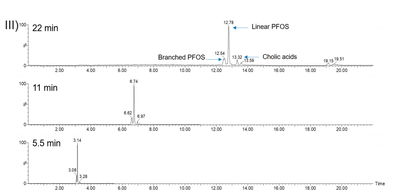
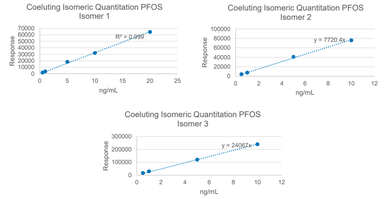
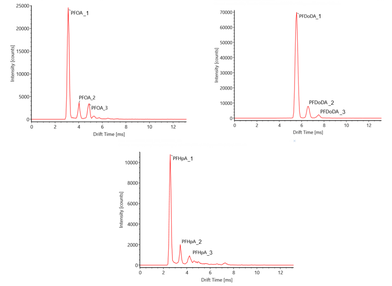


Figure 6 I) Waters_connect ion mobility data viewer 3D visualisation of ion mobility PFOS isomers separation and cholic acid biomarker isomers separation (R~ 145). II) UPLC-cIM-MS resolution (R~ 145), illustrating separation/differentiation of coeluting PFOS linear and branched isomers and separation/ differentiation of coeluting cholic acid biomarkers. III) 75 % reduction in analysis time using scaled and shortened UPLC separation for analysis of human serum samples.



Sample Name	PFOS Isomer 1 ng/mL	PFOS Isomer 2 ng/mL	PFOS Isomer 3 ng/mL
AD_48_1834	3.5	0.7	1.35
AD_56_2550	0.6	0.35	0.1
AD_46_14554	0.37	1.6	0.1

Figure 7 I) Ion mobility response curves for (0.5 ng/mL to 500 ng/mL) perfluoro-1-methylheptanesulfonate and response for cIM resolved coeluting isomeric PFOS impurities. II) Single PFAS isomer calculated concentrations determined for anonymised human serum samples.



PFOA Conformer	CCS Å²
a	154.9
b	169.7
c	182.3
d	190.1
e	205.9
f	216.8

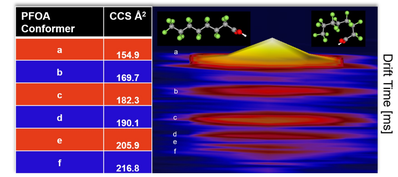


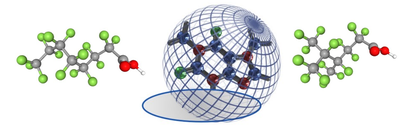
Figure 8. I) Illustration of perfluoroalkyl carboxylic acids (PFCAs) cIM conformeric profiles. II) cIM 3D PFOA m/z 412.9664 conformeric profile and corresponding CCS identification finger print.

CONCLUSION

- **Identification of perfluoroalkyl carboxylic acids cIM conformeric profiles and corresponding CCS identification finger prints provide new found identification specificity.**
- **Combined with retention time and *m/z*, a CCS value is a reproducible metric that can be used to improve cumulative specificity in non-targeted screening assays.**
- **PFAS CCS values have been shown to be reproducible for a cIM intra-site/inter-site comparison and published DT CCS values. Compared to the expected PFAS reference library value a Δ CCS < 1% can be routinely obtained.**
- **A 75 % reduction in analysis time has been shown using LC-cIM -MS. Resolution and differentiation of coeluting PFOS linear and branched isomers has been achieved. Separation and differentiation of coeluting cholic acid biomarkers has been shown.**
- **Coeluting PFAS branched and linear PFAS isomers identified in anonymised human serum samples have been fully resolved using cIM and single component calculated concentrations determined. We illustrate the potential to correlate individual PFAS isomer structure, toxicity and environmental exposure.**
- **LC-cIM-MS peak capacity facilitates structural elucidation of coeluting PFOS isomeric species.**
- **The enhanced specificity of $HDMS^E$ may be used to meet the analytical challenge of identifying PFAS "knowns" and characterise "unknown" PFAS compounds, as well as their corresponding biotransformation products.**

References

1. Dodds, JN, Hopkins ZR, Knappe DRU, and Baker ES. Rapid Characterization of Per- and Polyfluoroalkyl Substances (PFAS) by Ion Mobility Spectrometry–Mass Spectrometry (IMS-MS). *Analytical Chemistry* 2020 92 (6), 4427-4435. DOI: 10.1021/acs.analchem.9b05364
2. Schilberg RN, Wei S, Twagiravyezu S, Neill JL, Conformational dynamics of perfluorooctanoic acid (PFOA) studied by molecular rotational resonance (MRR) spectroscopy, Chemical Physics Letters, Volume 778, 2021,138789,ISSN 0009-2614.
3. Kari L. Organtini, Kenneth J. Rosnack, Mary E. Lame, Lisa J. Calton. Extracting and Analyzing PFAS from Human Serum. Waters Application Note 720007114, Revised July 2021
4. PFAS Analysis Kit for ACQUITY UPLC Systems User Guide 720006689 < https://www.waters.com/webassets/cms/support/docs/720006689en.pdf.



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