Assessing Relative Response of Four European-Regulated PFAS in Human Serum Using Cyclic Ion Mobility MS

Pennante Bruce-Vanderpuije [4], Kabre Heck [2], Sara Beverley [1], David Megson [3], Stuart Adams [1]*, Kwadwo Ansong Asante [4] and Michael McCullagh [1].
[1] Waters Corporation, Stamford Avenue, Altrincham Road, Wilmslow, SK9 4AX. UK. [2] Waters Corporation, Milford, Massachusetts, USA.,[3]Manchester Metropolitan, University, Manchester, UK. [4]CSIR-Water Research Institute, Accra, Ghana.

Introduction

Using LC-Cyclic-IMS-MS, we evaluated the relative response of four European-regulated PFAS (PFOS, PFOA, PFNA, and PFHxS) in human serum. Stringent regulatory guidelines have been introduced due to the documented toxicity associated with environmental PFAS exposure.[1-5] PFNA, in particular, has been linked to strong developmental toxicity, neonatal mortality, delayed developmental milestones, and hepatotoxicity often equal to or greater than PFOA and PFOS.[6] Recent studies also show that some alternative PFAS exhibit toxicity comparable to previously regulated compounds.[7] The bioaccumulative nature of PFAS enables monitoring in human biofluids to better understand exposure pathways and health risk. Ion mobility (IM) using the SELECT SERIES™ Cyclic™ IMS Instrument provides an additional dimension of separation, and collision cross section (CCS) values offer enhanced identification confidence and feature-selective specificity (see Figure 1).[8]

Experimental

LC-Cyclic-IMS-MS analyses were performed using a quadrupole-Cyclic IM-time-of-flight mass spectrometer (Cyclic IMS resolution R ~65–145). Reversed-phase LC separation employed mobile phases of: A (95:5 water: methanol, with 2 mM ammonium acetate) and B (methanol with 2 mM ammonium acetate), using a 22 min gradient at 0.3 mL/min. An ACQUITY™ UPLC™ BEH™ C18 column (100 mm × 2.1 mm, 1.8 µm) at 35 ° C with a 10 µL injection volume was used. The modified ACQUITY i-Class System included a PFAS-free conversion kit and Atlantis™ Premier BEH C18 AX Isolator Column, 2.1 x 50mm, 5 µm.[9] Human serum samples were collected from Ghanaian firefighters and e-waste handlers. Sample preparation used SPE 96-well µElution plates with polymeric reversed-phase, weak anion exchange sorbent.

Results

LC-Cyclic-IMS-MS was used to analyse human serum samples to assess environmental exposure to PFAS. A subset of data focusing on four EU-regulated PFAS is presented. Ion mobility CCS values provided additional identification specificity, with good agreement to library values (Table 1). An example detection is shown in Figure 2. Calculated concentrations for PFOS, PFHxS, PFNA, and PFOA are presented in Figure 3. A combined 83% detection rate has been determined for the four EU-regulated PFAS in human serum samples and NIST reference standards. Confident identification at trace levels was achieved using isotopically labelled internal standards, retention time, accurate mass, and CCS values. Despite soft ionisation conditions, compared to PFCA [M-H]$^-$ species, dominant [M–CO_2H]$^-$ fragments form due to labile neutral loss pathways, but enable reliable ion mobility-based feature classification at trace levels (see Figure 4).[10]

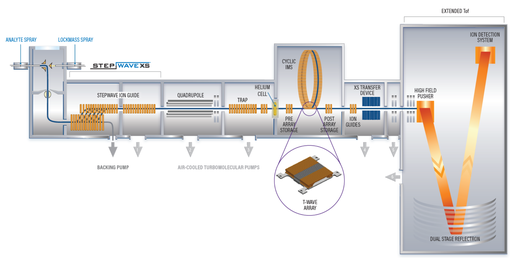

Figure 1. SELECT SERIES Cyclic IMS System.

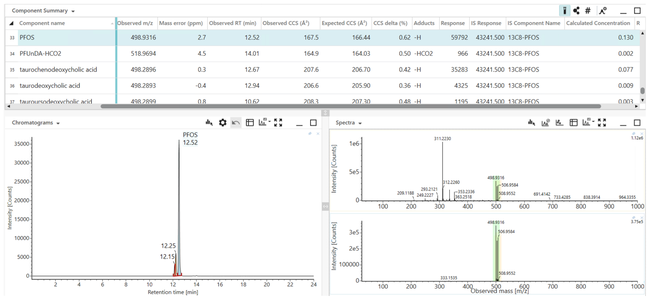

Figure 2. Component summary showing the calculated PFOS concentration (0.13 ng/mL) in firefighter sample F12 (Δ CCS = 0.62%). The mass spectra (right) include an ion mobility-resolved region (lower pane), enabling separation of PFOS ions from co-eluting matrix background signals (upper pane), and discrimination of native PFOS ($C_8HF_{17}O_3S$), the ($C4[^{13}C]_4HF_{17}O_3S$) injection internal standard, and the ($[^{13}C]_8HF_{17}O_3S$) extraction internal standard.

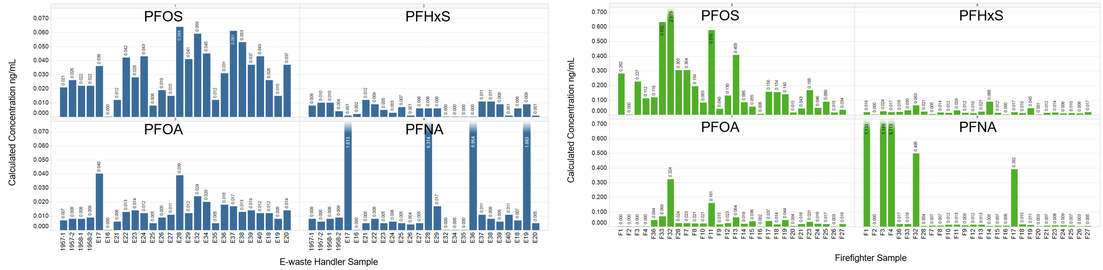

Figure 3. Summary plot of calculated concentrations for PFOS, PFHxS, PFNA, and PFOA determined to be present in firefighter and e-waste handler human serum. The calculated concentration ranges were as follows: for e-waste handlers, PFOS (0.008–0.064 ng/mL), PFHxS (0.001–0.012 ng/mL), PFOA (0.005–0.04 ng/mL), and PFNA (0.004–1.513 ng/mL). In firefighter serum samples, higher concentration ranges were observed: PFOS (0.006–2.979 ng/mL), PFHxS (0.001–0.088 ng/mL), PFOA (0.002–0.324 ng/mL), and PFNA (0.003–5.713 ng/mL).

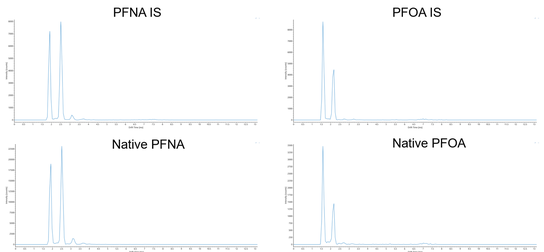

Figure 4. PFNA AND PFOA IM arrival time distributions for PFNA and PFOA native and labelled extraction internal standards, showing reproducible characteristic arrival time distribution (ATD) features, which have been determined to be characteristic of perfluoroalkyl carboxylic acids (PFCAs), providing specificity that can be utilised to facilitate PFCA identification.

Table 1. RMS Δ CCS (Å²) for EU-regulated PFAS (PFOS, PFHxS, PFOA, and PFNA) in firefighter and e-waste handler samples.

PFAS Compound	Fire Fighters RMS Δ CCS Å²	E Waste Handlers RMS Δ CCS Å²
PFOS	0.64	0.58
PFHxS	0.67	0.52
PFOA	0.13	0.09
PFNA	0.39	0.31

Conclusions

- **Initial findings indicate elevated PFAS in both E-waste handlers and firefighters relative to control samples, with firefighters exhibiting higher exposure levels to PFOS, PFHxS, PFNA and PFOA.**
- **LC-Cyclic-IMS-MS can form a critical part of analytical strategy to enhance identification specificity of PFAS and provides an opportunity to correlate genotoxicity and environmental fate.**
- **LC-Cyclic-IMS-MS is a highly specific non-targeted analysis strategy that can be used to identify known and emerging PFAS in complex samples.**

References

1. https://www.epa.gov/system/files/documents/2024-01/method-1633-final-for-web-posting.pdf,
2. https://eurl-pops.eu/working-groups#_pfas
3. https://cdn.dwj.gov.uk/wp-content/uploads/2021/10/04203217/Information-Letter-PFAS-Monitoring.pdf
4. https://www.maine.gov/dep/spills/topics/pfas/PFAS-products/
5. https://www.assemblee-nationale.fr/dyn/16/textes/l16b0276_texte-adopte-provisoire.pdf
6. U.S. Environmental Protection Agency. Body Weight Data for IRIS PFNA Assessment. Washington (DC): EPA; 2024. Available from: https://assessments.epa.gov/risk/document/&deid=363057 [epa.gov]
7. Conley JM, Lambright CS, Evans N, McCord J, Strynar MJ, Hill D, Medlock-Kakaley E, Wilson SV, Gray Jr LE. Hexafluoropropylene oxide-dimer acid (HFPO-DA or GenX) alters maternal and fetal glucose and lipid metabolism and produces neonatal mortality, low birthweight, and hepatomegaly in the Sprague-Dawley rat. Environ Int. 2021 Jan;146:106204.
8. Enhanced Identification Confidence and Specificity for PFAS Analysis Using Cyclic Ion Mobility Mass Spectrometry Collision Cross Sections. 2024. Waters Application Note 720008536. PFAS Analysis Kit for ACQUITY UPLC Systems User Guide 720006689 < https://www.waters.com/webassets/cms/support/docs/720006689en.pdf.
9. PFAS Analysis Kit for ACQUITY UPLC Systems User Guide 720006689 < https://www.waters.com/webassets/cms/support/docs/720006689en.pdf.
10. McCullagh, M., Kass, I., Lioupi, A., Theodoridis, G., Plumb, R., Dowd, S., & Adams, S. (2024, June). *The utility of Cyclic ion mobility to improve selectivity and analysis efficiency of environmental PFAS contamination and exposure* [Poster presentation]. ASMS Conference on Mass Spectrometry and Allied Topics, Anaheim, CA, United States.

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*Present address, Fera Science Ltd., Sand Hutton, York YO41 1LZ
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