Waters™

Application Note

Using the Combined Peak Capacity of Liquid Chromatography and Cyclic Ion Mobility Mass Spectrometry to Enhance PFAS Analysis Efficiency and Specificity

Michael McCullagh, Robert S. Plumb, Stuart Adams, Ian D. Wilson, Artemis Lioupi, Georgios Theodoridis

Waters Corporation, Laboratory of Analytical Chemistry, School of Chemistry, Aristotle University of Thessaloniki,54124 Thessaloniki, Greece, Division of Systems Medicine, Department of Metabolism, Digestion and Reproduction, Imperial College London, London W12 0NN, United Kingdom

Abstract

The World Health Organization (WHO) has designated perfluorooctanesulfonic (PFOS) acid and perfluorooctanoic acid (PFOA) as carcinogenic to humans. Stringent per and polyfluorinated alkyl substances (PFAS's) regulations are in place and are regional and dynamic. The number of PFAS screening samples is predestined to increase and therefore strategies to improve analysis efficiency and specificity, will be aspired to.

Liquid chromatography ion mobility mass spectrometry (LC-IM-MS) provides enhanced specificity to monitor PFAS environmental exposure cause, and simultaneously opportunity to improve understanding of biological effect. The SELECT SERIES™ Cyclic™ Ion Mobility Mass Spectrometer has been used to illustrate a strategy to increase sample throughput and show the potential to improve selectivity and specificity of the detection of PFOS isomers in human serum analysis. A 75% reduction in analysis time has been shown using LC-IM-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, providing opportunity to facilitate a time

efficient correlation between PFAS isomeric structure, concentration, and exposure.

Benefits

- LC-IM-MS is a routine and robust non-targeted screening approach that provides enhanced peak capacity, using a combination of chromatographic, *m/z* and ion mobility resolution. The combined resolution can be used to differentiate isobaric and isomeric analytes, including PFOS isomers
- Enhanced peak capacity facilitates a 75% improvement in analysis efficiency, while simultaneously retaining resolution of PFOS isomers and isobaric cholic acid isomers biomarkers
- Chromatographically coeluting PFOS isomers can be quantified based on ion mobility separation, the individual PFOS isomer concentrations, and structure can be cross correlated with biological effect
- Dial up ion mobility resolution enables differentiation of cholic acid biomarkers at resolution of R~250 which can be obtained in combination with ultra-high performance liquid chromatography (UHPLC) peak widths

Introduction

The properties of PFAS and their incorporation into products used within society have previously been described.[1] Exposure to PFAS has been correlated with adverse health conditions including cancer, immunodeficiencies, and elevated cholesterol levels. As a result, industrial production of long chain PFAS has been phased out over the last two decades. For the monitoring of environmental PFAS, current legislation focuses on the use of targeted LC-MS analysis strategies, EPA method 1633 targets 40 PFAS compounds, incorporating 0.003% of the EPA fluorinated compound data base.[2,3] The method is applicable to analysis of aqueous, solid, biosolids, and tissue samples. Whereas EU 2020/2184 (updated Jan 2021): employs a PFAS sum parameter for 20 sulfonic and carboxylic acids in drinking water, however, in the England the drinking water inspectorate (DWI) guidance proposes an extended list of 47 PFAS are monitored.[4,5] Global guidelines and legislation are dynamic, for example United States 2021: Maine has enacted groundbreaking law to ban the use of toxic PFAS compounds in all products by 2030, with France and Denmark heading towards a bilateral ban of PFAS to combat health risk.[6,7] Notably “Forever pollution” is also considered, where a fee is imposed upon the industries responsible for pollution. Although developed countries are implementing more stringent guidelines for PFAS, developing/underdeveloped countries continue production and often lack regulations and mechanisms to address emerging PFAS. A move towards alternative PFAS has occurred, for which the toxicity, and

environmental fate must be ascertained, although several studies indicate that new alternative PFAS have similar toxicity to banned PFAS.[8]

Given the worldwide concerns regarding known PFAS and regional legislation to ban all PFAS, the number of analyte targets will be beyond the feasibility of using a targeted LC-MS strategy. It is therefore inevitable, there will be an increase in the implementation of non-targeted screening strategies to detect, and identify, known, unknown, emerging PFAS, degradation products, and PFAS precursors. The number of PFAS being detected are increasing and there will be an inevitable increase in the number of sample analyses required. [9]

Application and utility of CCS to provide enhanced specificity for non-targeted PFAS analysis has previously been discussed.[1] Additionally, the utility of LC IM affords the opportunity to increase peak capacity (Pc). LC-IM-MS enhanced Pc is used routinely to facilitate enhanced resolution of isobaric analytes and reduce false detection rates.[9] Unlike targeted LC-MS, chromatographically coeluting isomers can also be resolved and quantified. Enhanced fragmentation pathway specificity can also be obtained, to facilitate structural elucidation efficiency.[11–13] Additionally enhanced Pc can be utilised to improve analysis efficiency.[14,15] Here in we illustrate how LC-IM-MS using a SELECT SERIES Cyclic IMS System (see Figure 1), can enhance Pc to provide a 75% improvement in analysis efficiency and quantify chromatographically coeluting PFOS isomers, as well as simultaneously resolve coeluting isobaric cholic acid biomarkers.

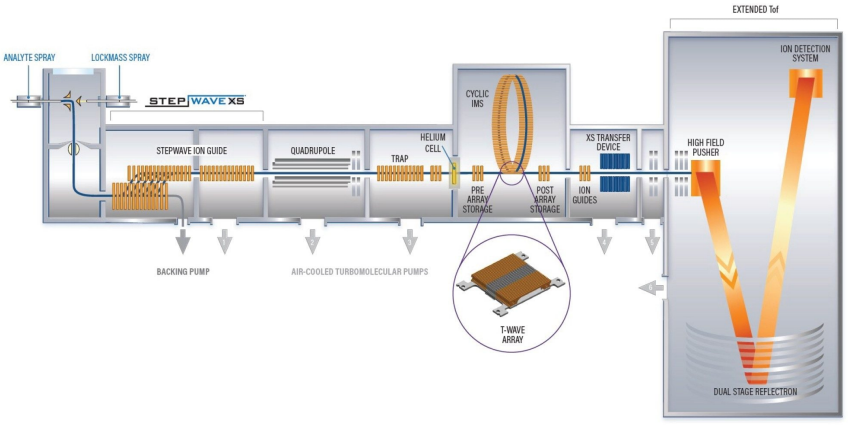


Figure 1. Schematic of SELECT SERIES Cyclic IMS System.

Experimental

Sample Description

Native PFAS Solution/mixture: PFAC30PAR (Wellington Laboratories) and Waters™ LCMS QC Reference Standard (186006963 <https://www.waters.com/nextgen/global/shop/standards--reagents/186006963-lcms-qc-reference-standard.html>), PFOS/PFOA linear and branched standards (Wellington Laboratories) Cholic acid standards: Tauroursodeoxycholic acid, taurochenodeoxycholic acid, taurodeoxycholic acid (Merck) Anonymised human serum samples.

Extraction Conditions

Anonymised human serum samples were extracted using SPE 96-well µElution plates, containing a polymeric reversed-phase, weak anion exchange mixed-mode sorbent. The method used is outlined in Figure 2 below.[16]

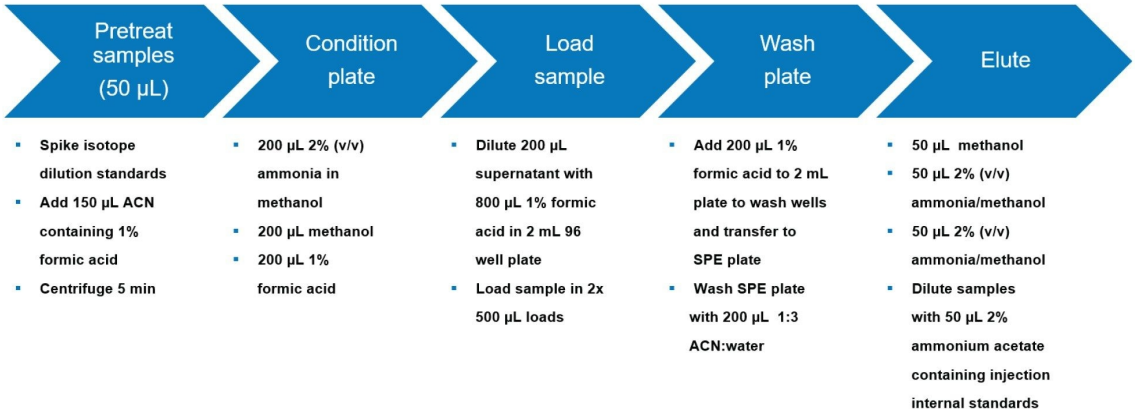


Figure 2. Extraction Method for PFAS in human serum samples.

Gradient Tables

The original method used in previously published work was scaled from an ACQUITY™ UPLC™ HSS T3 100 mm Column to a 50 mm column.[16] The method was then shortened on the 50 mm column by increasing the flowrate from 0.3 to 0.6 mL/min. The separation methods are listed out in Figure 3.

2.1 x 100 mm

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

Scaled

2.1 x 50 mm

TIME (minutes)	FLOW (ml/min)	%A	%B
Initial	0.30	95	5
1.0	0.30	75	25
3.0	0.30	50	50
6.5	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	0.60	95	5
0.5	0.60	75	25
1.5	0.60	50	50
3.25	0.60	15	85
3.5	0.60	5	95
4.25	0.60	5	95
4.5	0.60	95	5
5.5	0.60	95	5

Figure 3. UHPLC scaled and shortened reversed phase gradients.

LC Conditions

LC system:	ACQUITY Premier System modified with PFAS Kit and Atlantis™ Premier BEH™ C18 AX Isolator Column, 2.1 x 50 mm, 5 µm (p/n: 186010926).[17]
Column:	ACQUITY UPLC Premier HSS T3 C_{18} Column (100 mm x 2.1 mm, 1.8 µm & 50 mm x 2.1 mm, 1.8 µm)
Column temperature:	35 °C
Sample temperature:	6 °C
Injection volume:	5 µL
Flow rate:	0.3 and 0.6 mL/min
Mobile phase A:	95 H_2O (2 mM ammonium acetate):5 MeOH

Mobile phase B:	MeOH (2 mM ammonium acetate)

MS Conditions

Ionization mode:	ES-
Acquisition range:	m/z 50–1200 (HDMS^E) and *m/z* 499 (HDMS/MS)
Capillary voltage:	0.5 kV
Collision energy:	HDMS^E (CE 20–70 Ev), HDMS/MS (CE 6eV)
Cone voltage:	10 V
Desolvation temperature:	350 °C
Source temperature:	100 °C

Data Management

Chromatography and MS:	MassLynx™ 4.2 SCN1026 Software
Informatics:	waters_connect™ 3.1.0.243 Software
Library:	Waters PFAS library- (130 entries)

Results and Discussion

Pc can be used to represent the maximum number of resolved peaks in a separation window ***e.g.*** chromatogram or ion mobility spectrum. Using IM a factor of 3 to 10 increase in peak capacity can be achieved, depending on

IM resolution, *m/z* resolution and sample complexity.[18,19] Increasing the travelling wave ion mobility spectrometry (TWIMS) separation can enhance the resultant LC-IM-MS peak capacity, which enables more specific precursor fragment alignment, to facilitate attainment of single component ion mobility product ions, that can enable PFOS isomer differentiation. The SELECT SERIES Cyclic IMS System has a default IM resolution of 65, facilitating partial separation of PFOS isomers, using default system parameters, and a nitrogen drift gas. Additional enhanced peak capacity can be obtained using dial up IM resolution, enabling resolution of PFOS isomers. Cyclic ion mobility mass spectrometry provides ppm mass accuracy, combined with enhanced flexibility, and enhanced specificity. This is an ideal analytical combination to identify isomeric PFAS and differentiate isobaric matrix analytes from PFAS using non-targeted screening.

In Figure 4 (I) the impact upon chromatographic separation is presented, where, as the total analysis time decreases from 22 minutes to 5.5 minutes, chromatographic coelution of detected PFOS isomers and cholic acid biomarkers are observed. Note, cholic acids isomers and PFOS isomers are isobaric. Additionally using targeted analysis, the potential exists for false PFOS detections or erroneous quantified calculated concentrations, the nominal MRM transitions, precursor *m/z* 499 à *m/z* 80 would not be differentiated, unless cholic acid biomarkers, and PFOS isomers are fully resolved. In accordance with EPA Method 1633, a 1-minute retention time separation of PFOS isomers and cholic acid biomarkers is required. In the case of human serum analysis cholic acids become elevated because of environmental exposure to PFOS.[20] However, using LC-IM-MS/MS (R~145, *m/z* 499, and CE 6eV), the PFOS and cholic acids isomers remain resolved (see Figure 4 (II and III). Regardless of chromatographic conditions used, the isomeric isobaric cholic acids and isomeric PFOS are fully resolved using LC-IM-MS/MS. The enhanced peak capacity of LC-IM can be used to facilitate increased sample throughput, a 75% reduction in analysis time has been achieved. Additionally, the cholic acid biomarkers identified in the human serum extract are differentiated by their respective arrival time distribution (ATD). Using cholic acid standards, further differentiation was achieved using an IM resolution of R~250 in an Ultra-High-Performance Liquid Chromatography (UHPLC) time frame (half-height peak width ~3 seconds), see Figure 5.

LC-IM-MS also presents opportunity to determine the individual calculated concentrations of chromatographically coeluting PFOS isomers and correlate the isomer structure with observed biomarker concentrations and in turn, gain a greater understanding of PFOS isomer structure and biological effect. Performing a feasibility study, calculated concentrations were determined for three PFOS isomers detected in anonymised human serum samples (see Figure 6). Using the waters_connect ion mobility data viewer, the response of the chromatographically coeluting PFOS isomers obtained at (R~145) was determined by manually generating each respective extracted mass chromatogram. The extracted mass chromatogram intensities were used to produce linearity curves, for a dilution series of L-PFOS standard (range 0.1 ng/mL to 50 ng/mL in vial)

and subsequently calculate the PFOS isomer concentrations in human serum samples (calculated concentrations were determined using Excel™ spreadsheet). The expected working range was predetermined in a parallel comparative targeted analysis study, 4.5 orders of dynamic range have previously been illustrated.[1] The L-PFOS standard (78.8% L-PFOS, 10% P6MHPS (perfluoro-6-methylheptanesulfonic acid) and 4.5% P5MHPS (perfluoro-5-methylheptanesulfonic acid) and 7.7% (other), facilitated generation of three respective correlation curves, corresponding to the individual PFOS isomer concentrations in vial. Each human serum sample exhibits a different isomer concentration profile and summed PFOS isomer concentration, (Human1(4.71 ng/mL)), Human2(0.80 ng/mL)), Human3(1.28 ng/mL)). At the concentrations observed, sufficiently distinguishing PFOS isomer fragmentation spectra were not obtained. However, CCS values provide an additional identification descriptor at low ion intensities where the mass spectra may exhibit insufficient characteristic product ion information, may be weak or unobserved.[10] CCS values of linear PFOS and branched PFOS isomers have been characterised, enabling identification of individual PFOS isomers in human serum samples. Subsequently, it was possible to use a single linear L-PFOS standard (containing known percentages of P5MHPS and P6MHPS) to produce a dilution series to quantify chromatographically coeluting branched PFOS isomers and linear PFOS. Using relative retention time and CCS values the PFOS isomers detected in the human serum samples are identified as P5MHPS (CCS Δ0.8%), P6MHPS (CCS Δ0.4%) and linear PFOS (CCS Δ1%).

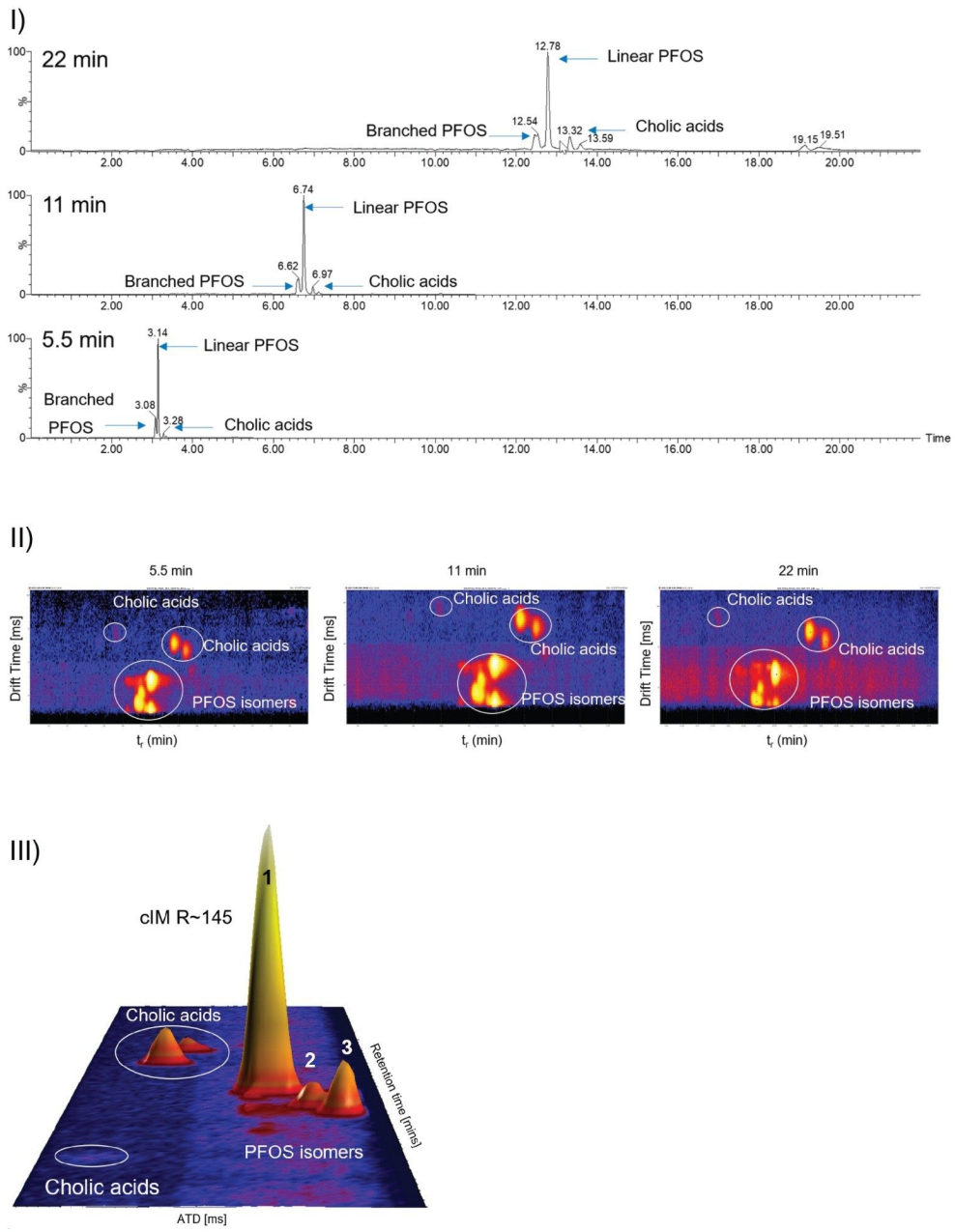


Figure 4. I) 75% reduction in analysis time using scaled and shortened reversed phase LC gradients for analysis of human serum samples. II) LC-IM-MS resolution (R~145), illustrating separation/differentiation of coeluting L-PFOS linear and PFOS branched isomers and separation/differentiation of coeluting cholic acid biomarkers. III) waters_connect ion mobility data viewer 3D visualisation of ion mobility PFOS isomers

separation and cholic acid biomarker isomers separation.

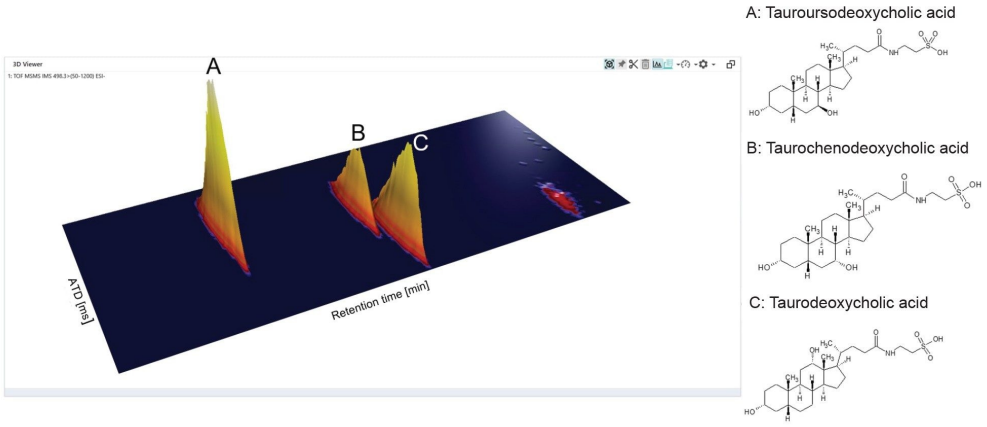
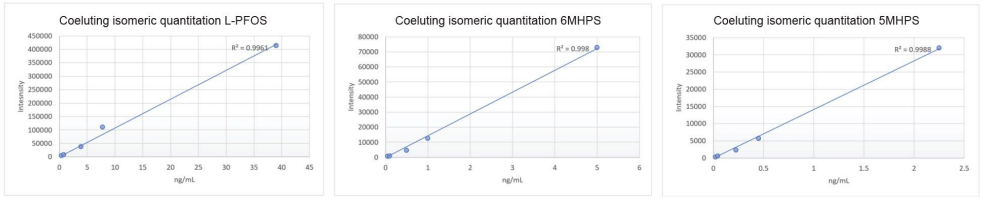


Figure 5. LC-IM of isomeric cholic acid biomarkers using resolution R~250. A:B Δ ATD~0.5 ms and B:C Δ ATD 1.5 ms.



Sample name	L-PFOS isomer 1 ng/mL	P6MHPS isomer 2 ng/mL	P5MHPS isomer 2 ng/mL
Human serum 1	3.4	0.57	0.73
Human serum 2	0.40	0.25	0.16
Human serum 3	0.71	0.34	0.24

Figure 6. PFOS isomer linearity curves and single component calculated concentrations of chromatographically coeluting PFOS isomers detected in human serum samples.

Conclusion

With worldwide concern of PFAS contamination and human exposure, it is inevitable that PFAS sample analysis will continue to increase. LC-IM-MS can be used to perform non-targeted screening analysis, to not only detect and identify unknown and known PFAS but also for complex concentration calculations regarding isomer patterns where standards are available.

LC-IM-MS provides specificity using a combination of chromatographic, *m/z* and ion mobility resolution. The combined resolution can be used to differentiate, isobaric, and isomeric analytes. Additionally, analysis times can be reduced by 75% while simultaneously retaining resolution of chromatographically coeluting PFOS isomers and maintaining resolution of isobaric cholic acid isomers. The approach illustrates a complementary strategy to EPA 1633 to differentiate isomeric PFOS and isobaric isomeric cholic acid biomarkers.

Utilizing LC-IM-MS as an analytical strategy provides flexibility and enhanced specificity simultaneously, this is highlighted by the first illustration that it is possible to quantify chromatographically coeluting PFOS isomers. Thereafter the opportunity exists to cross corelate the calculated concentrations of PFOS isomers with observed biological effect.

References

1. Michael McCullagh, Stuart Adams, Sarah Dowd, Isabel Riba, Hania Khoury-Hollins. Enhanced Identification Confidence and Specificity for PFAS Analysis Using Cyclic Ion Mobility Mass Spectrometry Collision Cross Sections. 2024. Waters Application Note. 720008536. September, 2024.
2. https://www.epa.gov/system/files/documents/2024-01/method-1633-final-for-web-posting.pdf < https://www.epa.gov/system/files/documents/2024-01/method-1633-final-for-web-posting.pdf> .
3. U.S. EPA Chemistry Dashboard, https://comptox.epa.gov/dashboard/chemical-lists/PFASSTRUCTboard (epa.gov) <https://comptox.epa.gov/dashboard/chemical-lists/pfasstruct> (accessed 2024–20–03).
4. Directive (EU) 2020/2184 of the European Parliament and of the Council of 16 December 2020 on the Quality of Water Intended for Human Consumption. https://eur-lex.europa.eu/eli/dir/2020/2184/oj <https://eur-lex.europa.eu/eli/dir/2020/2184/oj> .

5. https://cdn.dwi.gov.uk/wp-content/uploads/2021/10/04203217/Information-Letter-PFAS-Monitoring.pdf < https://cdn.dwi.gov.uk/wp-content/uploads/2021/10/04203217/Information-Letter-PFAS-Monitoring.pdf> .

6. https://www.maine.gov/dep/spills/topics/pfas/PFAS-products/ < https://www.maine.gov/dep/spills/topics/pfas/PFAS-products/> .

7. https://www.assemblee-nationale.fr/dyn/16/textes/l16t0276_texte-adopte-provisoire.pdf < https://www.assemblee-nationale.fr/dyn/16/textes/l16t0276_texte-adopte-provisoire.pdf> .

8. 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.

9. Kirkwood-Donelson KI, Dodds JN, Schnetzer A, Hall N, Baker ES. Uncovering per- and polyfluoroalkyl substances (PFAS) with nontargeted ion mobility spectrometry–mass spectrometry analyses. *Sci. Adv.* 9(43):eadj7048(2023).

10. Goscinny S, McCullagh M, Far J, De Pauw E, Eppe G. Towards the use of ion mobility mass spectrometry derived collision cross section as a screening approach for unambiguous identification of targeted pesticides in food. *Rapid Commun Mass Spectrom*. 2019 Jul:33.

11. McCullagh M, Douce D, Van Hoeck E, Goscinny S. Exploring the Complexity of Steviol Glycosides Analysis Using Ion Mobility Mass Spectrometry. *Anal Chem*. 2018 Apr 3;90(7):4585–4595.

12. McCullagh M, Pereira CAM, Yariwake JH. Use of ion mobility mass spectrometry to enhance cumulative analytical specificity and separation to profile 6-C/8-C-glycosylflavone critical isomer pairs and known-unknowns in medicinal plants. *Phytochem Anal*. 2019 Jul;30(4):424–436.

13. McCullagh M, Giles K, Richardson K, Stead S, Palmer M. Investigations into the performance of travelling wave enabled conventional and cyclic ion mobility systems to characterise protomers of fluoroquinolone antibiotic residues. *Rapid Commun Mass Spectrom*. 2019. Jul:33.

14. Rainville PD, Wilson ID, Nicholson JK, Isaac G, Mullin L, Langridge JL, Plumb RS. Ion mobility spectrometry combined with ultra performance liquid chromatography/mass spectrometry for metabolic phenotyping of urine: Effects of column length, gradient duration and ion mobility spectrometry on metabolite detection. *Analytica Chimica Acta*. Volume 982, 22 August 2017, Pages 1–8.

15. McCullagh M, Goshawk J, Mortishire-Smith R. High throughput non-targeted screening using a rapid gradient microbore UPLC method and a library of FDA-approved small molecule drugs. Waters Application Note. 720007174. March, 2021.

16. Kari L. Organtini, Kenneth J. Rosnack, Mary E. Lame, Lisa J. Calton. Extracting and Analyzing PFAS from Human Serum 2023. Waters Application Note. 720007114. July, 2021.

17. PFAS Analysis Kit for ACQUITY UPLC Systems User Guide. 720006689 < https://www.waters.com/webassets/cms/support/docs/720006689en.pdf> . January, 2024.

18. Dwivedi P, Puzon G, Tam M, Langlais D, Jackson S, Kaplan K, William F Siems, Albert J Schultz, Luying Xun, Amina Woods, Herbert H Hill Jr. Metabolic profiling of *Escherichia coli* by ion mobility-mass spectrometry with MALDI ion source. *J Mass Spectrom*., 2010,45(12), 1383–1393.

19. Grabarics M, Lettow M, Kirk AT, von Helden G, Causon TJ, Pagel K. Plate-height model of ion mobility-mass spectrometry: Part 2—Peak-to-peak resolution and peak capacity. *J Sep Sci*. 2021 Jul;44(14):2798–2813.

20. Zhang X, Zhao L, Ducatman A, Deng C, von Stackelberg KE, Danford CJ, Zhang X. Association of per- and polyfluoroalkyl substance exposure with fatty liver disease risk in US adults. *JHEP Reports*, Volume 5, Issue 5, 2023, 100694.

21. http://archives.esf.org/fileadmin/Public_documents/Publications/ESPB10.pdf < http://archives.esf.org/fileadmin/Public_documents/Publications/ESPB10.pdf> .

"For Research Use Only. Not for use in diagnostic procedures."

This study was performed in collaboration with the 3rd Department of Neurology of Aristotle University of Thessaloniki who followed full management and ethical review, in accordance with both national and EU guidelines.[21]

Acknowledgement

We thank the authors Artemis Lioupi, Georgios Theodoridis, from the Laboratory of Analytical Chemistry, School of Chemistry, Aristotle University of Thessaloniki and Ian Wilson from Division of Systems Medicine, Department

of Metabolism, Digestion and Reproduction, Imperial College London, for their valuable contributions to this application note.

Featured Products

ACQUITY Premier System <https://www.waters.com/nextgen/global/products/chromatography/chromatography-systems/acquity-premier-system.html>

SELECT SERIES Cyclic IMS <https://www.waters.com/nextgen/global/products/mass-spectrometry/mass-spectrometry-systems/select-series-cyclic-ims.html>

MassLynx Mass Spectrometry Software <https://www.waters.com/nextgen/global/products/informatics-and-software/mass-spectrometry-software/masslynx-mass-spectrometry-software.html>

waters_connect Software Solutions <https://www.waters.com/nextgen/global/products/informatics-and-software/waters_connect.html>

720008671, February 2025