

Detection and Characterization of PFAS Molecules Using Inclusion and Exclusion Features of Data Directed Analysis (DDA)

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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, however, there are challenges to their identification due to being present at very low levels.

In Data Directed Acquisition (DDA), ions satisfying certain user defined criteria are isolated by a quadrupole and subjected to elevated collision energy in order to create product ion spectra. PFAS ions exhibit a characteristic Mass Defect due to their fluorine content and this feature has been utilized to enhance their detection in mass spectrometry analyses by the use of an inclusion list. In addition, a list of background matrix *m/z* values created from prior analyses can be utilized as an exclusion list when enabled in the DDA method, further simplifying the number of ions identified by the acquisition software for quadrupole isolation and product ion formation.

Preliminary experiments utilizing inclusion and exclusion criteria were performed using a sample containing a mixture of small molecules before progressing onto PFAS samples where include list containing several hundred entries and an exclude list of several thousand, were employed.

METHODS

Liquid Chromatography^{1,2}

| | |
|--------------------|--|
| LC System | ACQUITY™ Premier System modified with PFAS Kit and Atlantis™ Premier BEH™ C18 AX Isolator Column, 2.1 x 50mm, 5 μ m (p/n 186010926). |
| Column | ACQUITY UPLC™ HSS T3 C ₁₈ (100 mm x 2.1 mm, 1.8 μ m) |
| Column Temperature | 35 °C |
| Sample Temperature | 6 °C |
| Injection Volume | 5 μ L |
| Flow Rate | 0.3 mL/min |
| Mobile Phase A | 95 H ₂ O (2 mM ammonium acetate):5 MeOH |
| Mobile Phase B | MeOH (2 mM ammonium acetate) |

Mass Spectrometry Data were acquired using a SELECT SERIES™ Cyclic™ Ion Mobility Mass Spectrometer (Figure 1) operating in ToF ESI Negative DDA (Figure 2). Survey and MSMS scan time = 100ms. Different collision energy ramps were used to produce MSMS spectra for different PFAS molecule classes

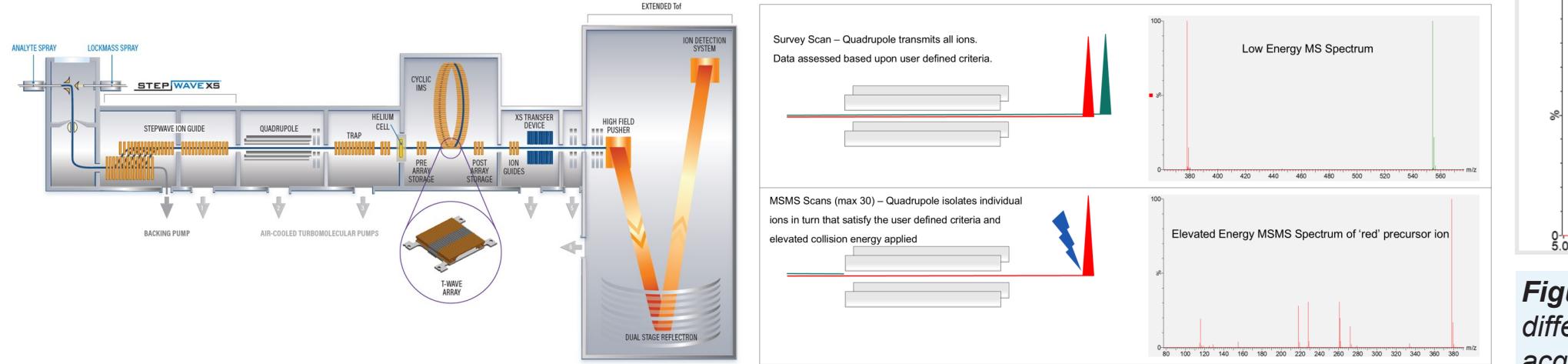


Figure 1. SELECT SERIES Cyclic IMS System.

Figure 2. DDA Acquisition method.

Samples^{1,2,3}

Waters LCMS QC Mixture (186006963),
Native PFAS Solution/mixture: PFAC30PAR (Wellington Laboratories)
Human Serum (S1-M, Sigma Aldrich)

Data Acquisition MassLynx™ v4.2 Software

Data Processing waters_connect™ Software
Searched with Waters PFAS library (130 entries)

Data Visualization Tibco Spotfire®

RESULTS



Figure 3. In small molecules analysis, ions generated by the ESI source tend to be predominantly singly charged which does not allow charge state to be a distinguishing feature from any background matrix elution peaks as it can in the analysis of larger molecules such as peptides. The use of Inclusion and Exclusion lists therefore becomes very important to minimize redundant switches on background ions. Parameters that can be specified are *m/z* and retention time. In the example shown, the LCMS QC mixture was injected and three different experiments performed; exclusion of Leucine Enkephalin throughout the run, inclusion list of all components but with an incorrect retention time for Sulfadimethoxine and switch on everything in the mixture. Green cross represents an identification and red cross represents non-identification showing the system was working correctly.

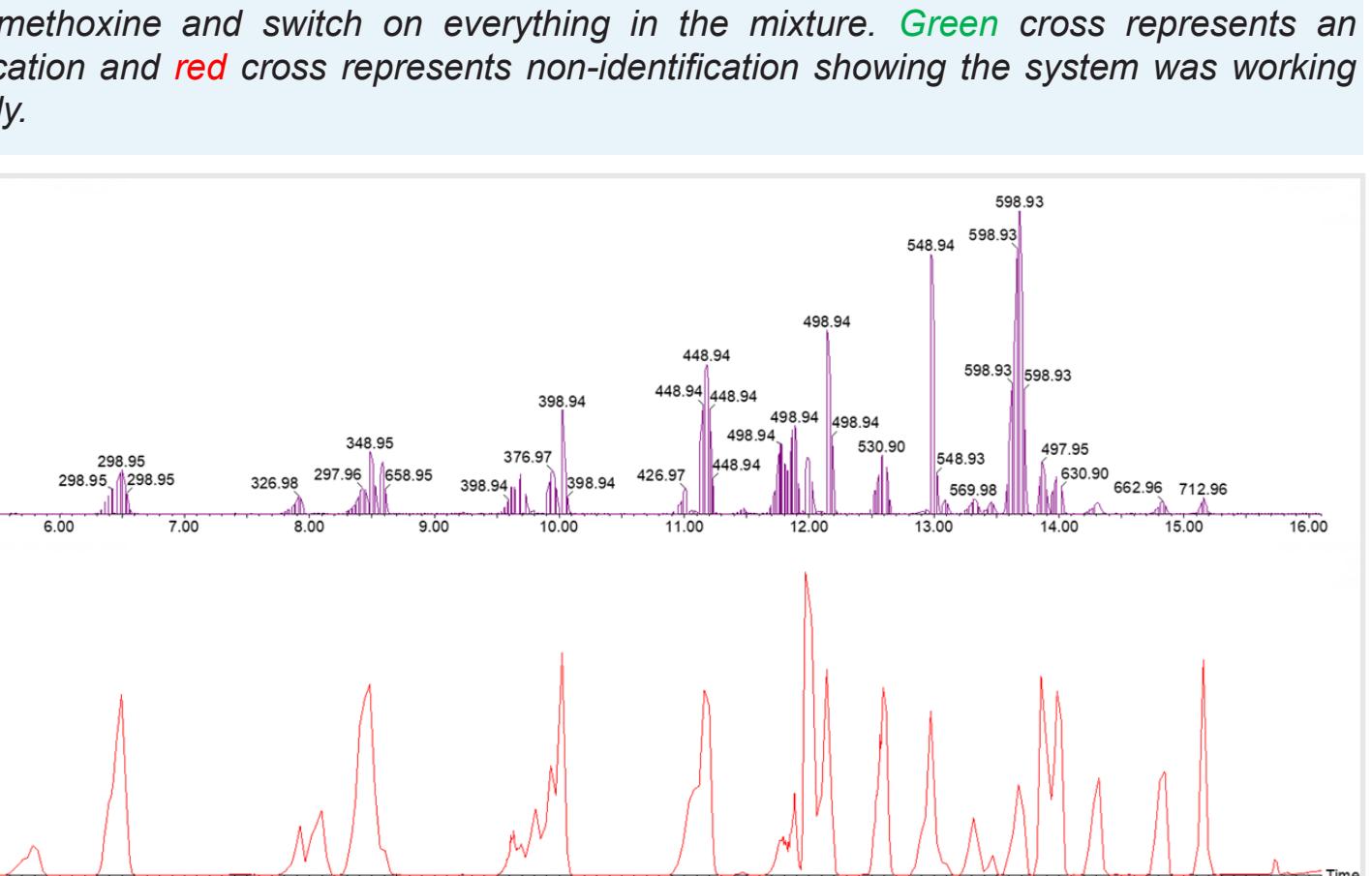


Figure 4. Kendrick mass defect is increasingly used in environmental sciences to differentiate natural organic matter from groups of environmental contaminants.⁴ In DDA acquisition methods, the characteristic mass defect of PFAS molecules can be incorporated into an inclusion list and, coupled with narrow mass windows, can aid the acquisition system in ensuring the majority of ions selected for MSMS are PFAS. The chromatogram traces above show survey and MSMS where an inclusion list comprising 600 entries has been used. The inclusion list ranged from 200.94 to 800.94 with a mass tolerance window of 50mDa. The chromatographic peak annotations in the top MSMS trace represent the masses to 2*d*₀ of the precursor ion subjected to elevated energy fragmentation.

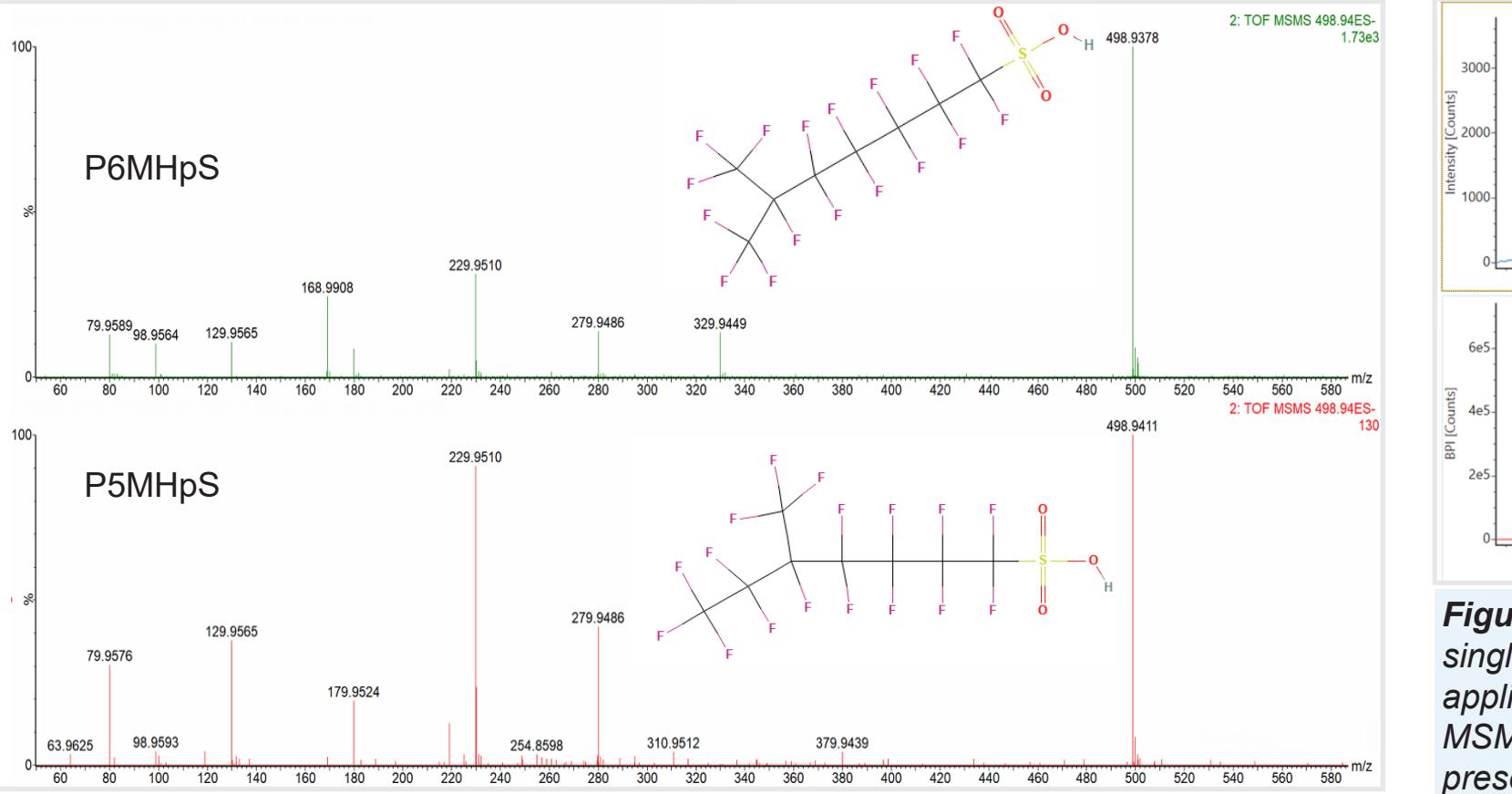


Figure 5. The use of inclusion lists allows the use of lower 'switch in to MSMS' thresholds and this aids the mass spectrometer in the production of MSMS spectra for very low abundant species. In the example shown, two fragmentation spectra for PFOS isomers³ perfluoro-5-methylheptanesulphonic acid (P5MHPs) perfluoro-6-methylheptanesulphonic acid (P6MHPs) are shown. Differences in the spectra, specifically the presence of the peak at *m/z* 329.9 in the top trace, reveal the fragmentation pathways for the two species, allowing clear identification to be achieved.

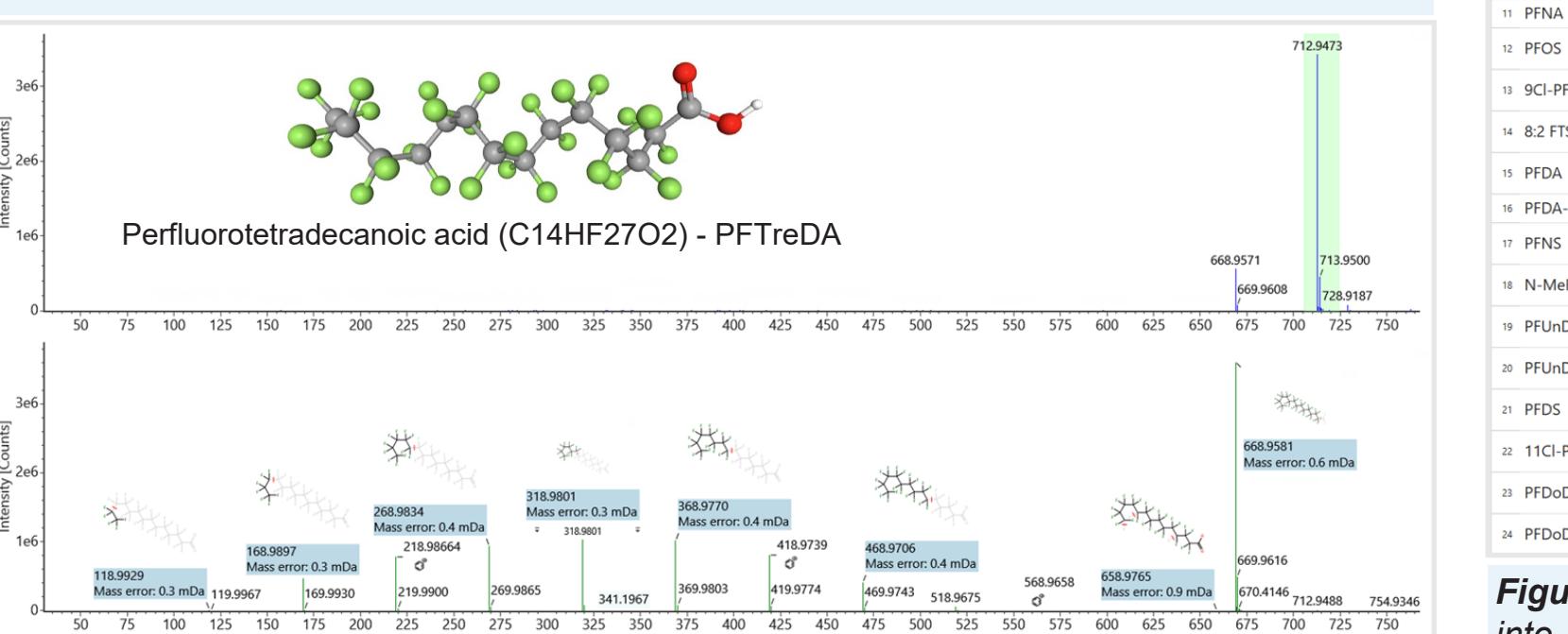


Figure 6. In the PFAS class of molecule perfluorocarboxylic acids (PFCAs), the low energy mass spectrum usually contains the CO₂ neutral loss as the base peak ion. However, an example of an identification is shown, specifically perfluorotetradecanoic acid, where the precursor ion (*m/z* approx. 712.94) rather than the neutral loss (*m/z* approx. 668.95) is observed as the dominant ion in the survey spectrum (top). The MSMS spectrum after selection for quadrupole isolation leads to clear identification of the molecule.

References

- McCullagh M, Lioupi A, Theodoridis G, Plumb R, Wilson I and Adams S. Enhanced Identification Confidence and Specificity for PFAS Analysis Using Cyclic Ion Mobility Mass Spectrometry Collision Cross Sections. 2025. Waters Application Note 720008536.
- McCullagh M, Adams S, Tudor A, Goshawk J, Mortishire-Smith R, Megson D, Ansong Asante K and Bruce-Vanderpuije P. Combining Pattern Analysis and Cyclic Ion Mobility Mass Spectrometry to Research Per- and Poly Fluoroalkyl substances (PFAS) Exposure in E-waste Handlers. 2025. Waters Application Note 720008784.
- McCullagh M, Marsden Edwards E and Adams S. Illustrating the Use of Cyclic Ion Mobility to Enhance Specificity for brominated-PFAS Isomer Analysis. 2025. Waters Application Note 720007823.
- Slemon L. The use of mass defect in modern mass spectrometry. *J Mass Spectrom.* 2012 Feb;47(2):226-36. doi: 10.1002/jms.2953. PMID: 22359333.

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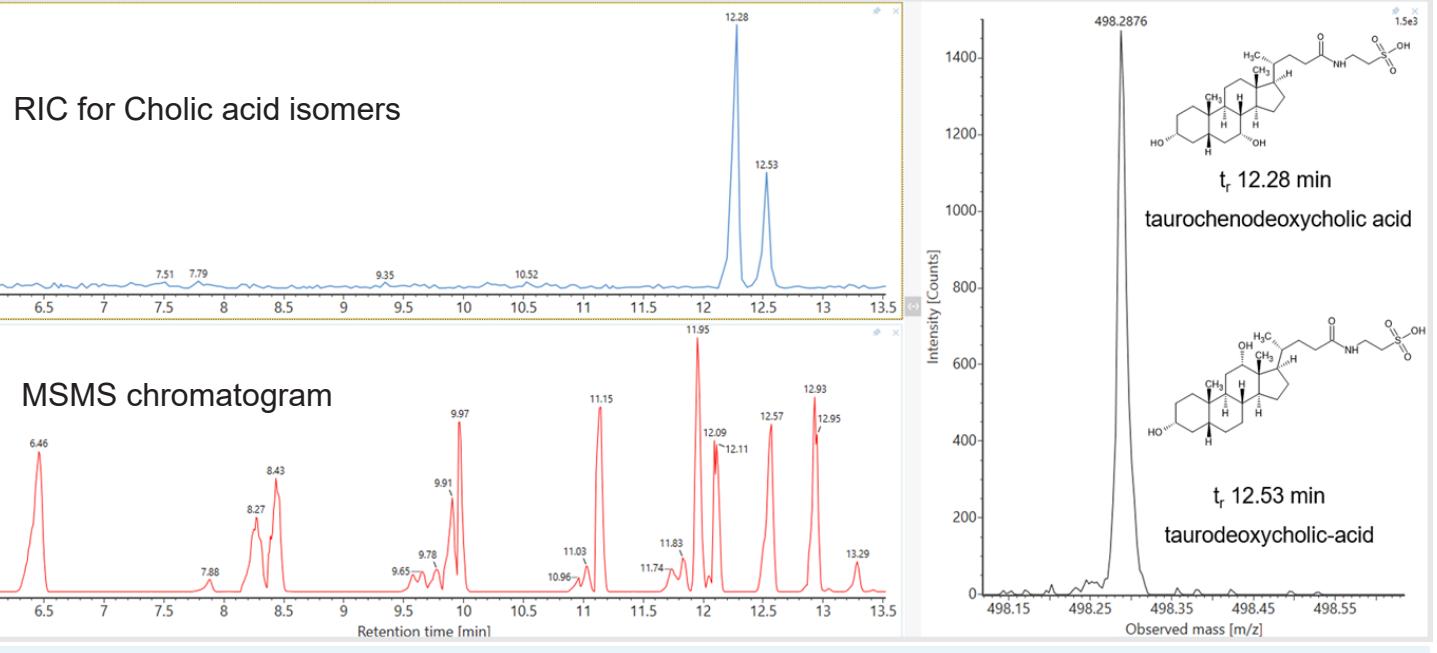


Figure 7. For small molecule analysis such as PFAS, the vast majority of ions exhibit a single charge. Therefore, the parameter charge state selection in the DDA method is not applicable. Injection of human serum and analysis with DDA and no inclusion list leads to MSMS switches on background ions and the automatic creation of a list of all the ions present, totalling >9000 entries. This can then be incorporated into subsequent analyses as a list of masses to exclude from MSMS interrogation. Present in the human serum are isomers of cholic acid and these can cause interferences in MRM analysis with lower mass resolution mass spectrometers. Using a combination of both exclusion and inclusion lists prevents the system switching upon these ions.

| Component Summary | Component name | Identification status | Observed neutral mass (Da) | Observed <i>m/z</i> | Mass error (mDa) | Mass error (ppm) | Observed RT (min) | Adducts |
|-------------------|----------------|-----------------------|----------------------------|---------------------|------------------|------------------|-------------------|---------|
| 10 | PFNA-HCO2 | Identified | 463.9706 | 418.9735 | 0.1 | 0.1 | 12.08 -HCO2 | |
| 11 | PFNA | Identified | 463.9706 | 462.9633 | 0.1 | 0.2 | 12.16 -H | |
| 12 | PFOS | Identified | 499.9375 | 498.9303 | 0.0 | 0.1 | 12.27 -H | |
| 13 | 9CI-PF3ONS | Identified | 531.9030 | 530.8957 | 0.1 | 0.3 | 12.61 -H | |
| 14 | 8.2-FTS | Identified | 527.9685 | 526.9613 | -0.3 | -0.5 | 12.88 -H | |
| 15 | PFDA | Identified | 513.9673 | 512.9600 | 0.0 | 0.0 | 12.90 -HCO2 | |
| 16 | PFDA-HCO2 | Identified | 513.9672 | 468.9701 | -0.1 | -0.2 | 12.90 -HCO2 | |
| 17 | PFNS | Identified | 549.9344 | 548.9272 | 0.1 | 0.2 | 13.01 -H | |
| 18 | N-MeFOSAA | Identified | 570.9747 | 569.9674 | 0.0 | 0.1 | 13.50 -H | |
| 19 | PFUnDA-HCO2 | Identified | 563.9641 | 518.9670 | -0.1 | -0.1 | 13.63 -HCO2 | |
| 20 | PFUnDA | Identified | 563.9643 | 562.9570 | 0.2 | 0.3 | 13.67 -H | |
| 21 | PFDS | Identified | 599.9313 | 598.9241 | 0.2 | 0.4 | 13.69 -H | |
| 22 | 11CI-PF3OUDs | Identified | 631.8963 | 630.8890 | -0.2 | -0.2 | 14.02 -H | |
| 23 | PFDoDA | Identified | 613.9614 | 612.9542 | 0.5 | 0.8 | 14.29 -H | |
| 24 | PFDoDA-HCO2 | Identified | 613.9613 | 568.9642 | 0.4 | 0.6 | 14.31 -HCO2 | |

Figure 8. A selection of the list of identifications from the injection of PFAS standard spiked into human serum background, data processed in waters_connect. In the analysis, a combination of inclusion list and 9000 entry background exclusion list was employed. Mass accuracy for the identifications was 0.9ppm RMS.

CONCLUSION

- The use of ToF Data Directed Analysis as an acquisition mode on Cyclic IMS Mass spectrometer has been demonstrated in the analysis of different classes of PFAS molecules
- Inclusion list corresponding to PFAS mass defect aids the real time detection algorithm and enables intelligent switching
- Further, an exclusion list of 'mapped' background ions produced from the injection of 'blank' matrix sample and containing 9000 entries enabled enhanced detection