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アプリケーションノート

Determination and Characterization of Perfluoroalkyl and Polyfluoroalkyl Substances (PFAS’ s) in Environmental Samples Using UPLC Ion Mobility MS

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Abstract

In this application note we explore the use of the ACQUITY UPLC I-Class System combined with ion mobility as an important tool for the unequivocal identification of PFOS isomers in environmental samples.This technique has been utilized to analyze a series of environmental extracts, including mink and fish muscle, to determine the presence of PFOS. There are some unique advantages to profiling complex matrices using this technique which employs a combination of high resolution mass spectrometry, with high efficiency ion mobility-based measurements and separations. Ion mobility spectrometry (IMS) is a rapid, orthogonal, gas phase separation technique which allows another dimension of separation to be obtained within an LC timeframe. Compounds can be differentiated based on size, shape, and charge. In addition, both precursor ion and fragment ion information can be acquired in a single injection for all components analyzed.

Benefits

- Individual PFOS isomers are resolved from chromatographically co-eluting isobaric biological matrix interferences with ion mobility.
- Efficiency of structrural elucidation is improved through mobility spectral clean up.
- Ion mobility drift times can be used as identification information in a routine screening workflow to identify PFOS isomers.
- Identification based on retention time, precursor ion accurate mass measurements, fragmentation data with accurate mass measurements, and ion mobility drift times can be generated in one analysis.

Introduction

Perfluoroalkyl and polyfluoroalkyl substances (PFAS’ s) are a class of man-made compounds that are frequently detected in biological and environmental samples. PFAS’ s are used in a multitude of commercial/industrial processes and products, ranging from fire-fighting foams, insecticide formulations, water-resistant coatings, and floor polishes, to oil-resistant coatings for paper products approved for food contact. As with many anthropogenic compounds, the incidence of cancers resulting from exposure has become a cause for concern.

Perfluorooctane sulfonate (PFOS) is frequently detected in biological and environmental samples and occurs due to abiotic or biotic environmental processes. MRM transition based LC-MS/MS analyses have been used previously to investigate PFOS in marine animals and human serum. Benskin et al. reported that

common matrix interferents (taurodeoxycholate [TDCA]) can complicate PFOS quantification because they undergo the same transition (*m/z* 499 ⇒ *m/z* 80) and tend to co-elute with PFOS, leading to a positive bias.[1,2]

In this application note we explore the use of the Waters ACQUITY UPLC I-Class System with ion mobility as an important tool for the unequivocal identification of PFOS isomers in environmental samples.[3,4,5] This technique has been utilized to analyze a series of environmental extracts, including mink and fish muscle, to determine the presence of PFOS. There are some unique advantages to profiling complex matrices using this technique which employs a combination of high resolution mass spectrometry, with high efficiency ion mobility-based measurements and separations. Ion mobility spectrometry (IMS) is a rapid, orthogonal, gas phase separation technique which allows another dimension of separation to be obtained within an LC timeframe. Compounds can be differentiated based on size, shape, and charge. In addition, both precursor ion and fragment ion information can be acquired in a single injection for all components analyzed

Experimental

UPLC conditions

UPLC system:	ACQUITY UPLC I-Class (equipped with PFC Kit)
Column:	ACQUITY UPLC BEH C_{18} 100 mm x 2.1 mm, 1.7 µm
Column temp.:	50 °C
Flow rate:	0.3 mL/min
Mobile phase:	70% 2 mM NH_4Ac in water / 30% 2 mM NH_4Ac in methanol/ACN 80/20 (A) mM NH_4Ac in methanol/ACN 80/20 (B)

Gradient:

Time(min)	%A	%B
Initial	100.0	0.0
0.50	100.0	0.0
16.00	65.0	35.0
22.00	65.0	35.0
27.00	10.0	90.0
27.10	0.0	100.0
28.00	0.0	100.0
28.10	100.0	0.0
34.00	100.0	0.0

MS conditions

MS system:	SYNAPT G2-S
Ionization mode:	ES –
Desolvation temp.:	550 °C
Acquisition mode:	Ion mobility
Mass range:	50 to 600 Da
Acquisition rate:	10 spectra/s
Capillary voltage:	2.3 kV
Cone voltage:	15V

Ion mobility gas:	CO_2 and N_2
Collision energy ramp:	35 to 75 eV
IMS wave velocity range:	400 *m/s* and 550 *m/s*
IMS wave height:	40 V
IMS duty cycle:	10.8 ms

Sample preparation

Whole mink carcasses (juvenile and adult males) were provided by licensed hunters and kept frozen (-20 °C) before being autopsied at the Swedish University of Agricultural Sciences.

The procedure used was in accordance with the method reported by Kärrman *et al.,*[7] with minor modifications. Liver samples were homogenized using Ultra-Turrax (IKA). A sub-sample of liver (1 g) was taken and 10-mL acetonitrile was added. The mixture was repeatedly vortex mixed and sonicated for 30 min. The supernatant was removed after centrifugation (10,000 x g, 30 min), and the extraction procedure was repeated upon the resulting pellet. The acetonitrile fractions were combined and reduced in volume to 10-mL after which 25-mL water was added. After mixing and centrifugation, the extract was passed through an Oasis WAX Solid Phase Extraction (SPE) Cartridge previously conditioned using 4-mL methanol followed by 4-mL water. After sample loading, the Oasis SPE Cartridge was washed with 4-mL 25 mM sodium acetate (pH 4) and 4-mL 40% methanol in water, followed by drying under vacuum. A final wash with 8-mL methanol was employed before the perfluorinated compounds were eluted using 2-mL 2% ammonium hydroxide in methanol into a tube with 50-mg ENVI-Carb (Supelclean, 120/400 mesh) and 100-µL acetic acid. The carbon solution was mixed by vortexing for 30 s, then filtered through a 0.2-µm GHP membrane, and reduced to 200 µL using N_2, after which 300-µL 2 mM ammonium acetate in water and the performance standard 7HPFHpA were added.

The study undertaken investigates the use of the Waters SYNAPT G2-S Platform to determine if UPLC in combination with ion mobility can provide a route to specific and unambiguous identification, enabling the unequivocal distinction of PFOS isomers. A schematic representation and illustration of the mechanism of ion mobility are shown in Figure 1. For additional research purposes only, the separation power of ion mobility was enhanced by substituting N_2 with CO_2 for the mobility drift gas.6 Use of CO_2 enabled characteristic drift time separation for the PFOS isomers analyzed. It is important that PFOS isomers are identified correctly because their physical, chemical, and biological properties may be affected by perfluoromethyl branching. As a result there has been increased scientific interest in relating toxicity,

environmental transport, degradation, and bioaccumulation to perfluoromethyl branching patterns.

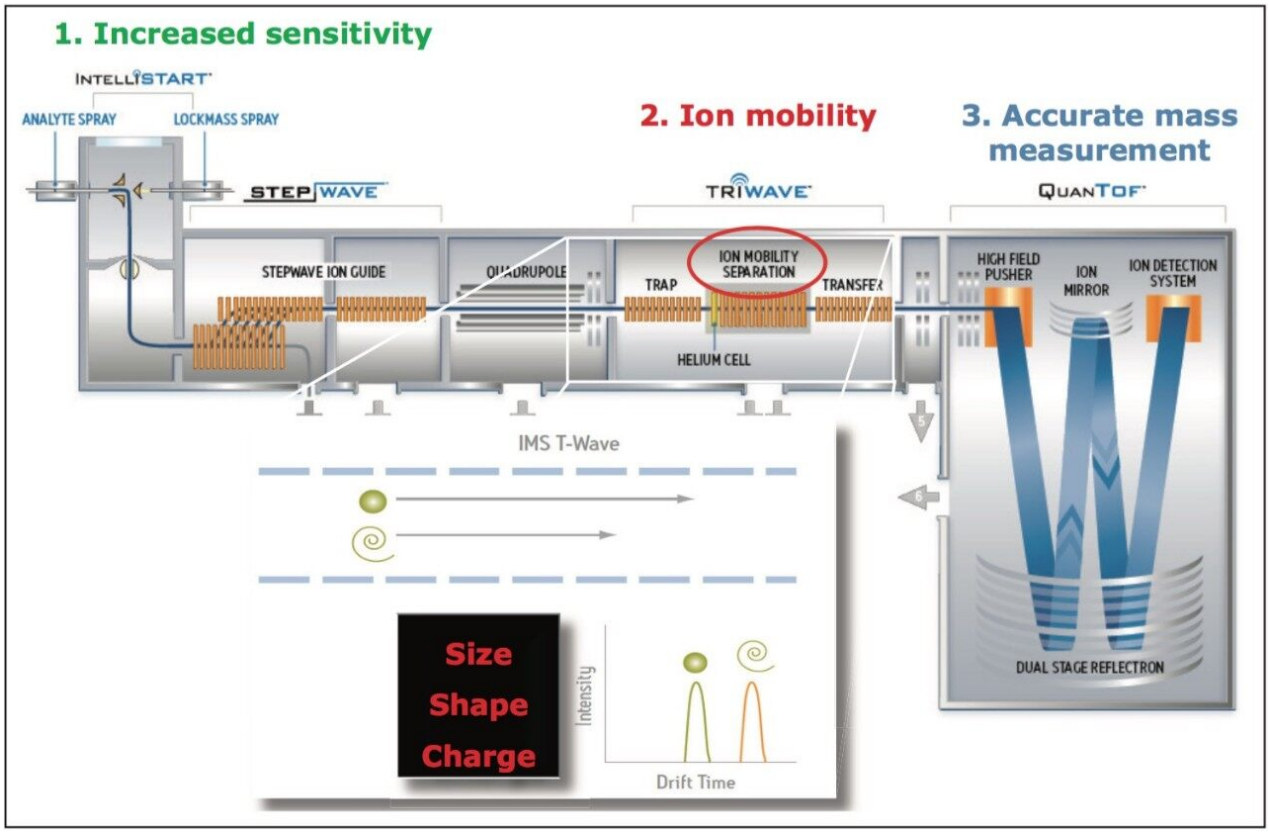

Figure 1. Schematic of the SYNAPT G2-S Mass Spectrometer and illustration of the mechanism of ion mobility.

Results and Discussion

The UNIFI Scientific Data Management System is designed to enable the day-to-day use of ion mobility in screening assays. Data were acquired using MassLynx Software, and processed using UNIFI. Using mass spectral information generated from a standard mixture of PFOS isomers, a scientific library in UNIFI was created incorporating the expected retention times and drift times of PFOS isomers. This allowed non-targeted acquisition and a targeted screen of the environmental extracts analyzed with the combination of orthogonal ion mobility separation and UPLC chromatographic separation.

The results obtained in determining the presence of PFOS in mink clearly show the benefits of using ion mobility. It is possible to separate co-eluting isobaric interferences from PFOS isomers. In Figure 2, accurate mass extracted ion chromatograms are presented. The interferences (peaks A and B) overlap with

the different major PFOS isomers (peaks C-F). Since TCDCA (taurochenodeoxycholate)/TDCA interferences and PFOS isomers also produce isobaric fragments, it is difficult to characterize and accurately quantify these components using conventional MS techniques. PFOS and TDCA, as well as other cholic acids, have similar isomeric profiles, retention times, and transitions (*m/z* 499 ⇒ *m/z* 80). Using quadrupole technology' s selectivity to target PFOS 498.9, typical mass resolution would result in a 1 Da span across the mass selected when performing MS/MS. With this information, it is easy to understand how the interferences can be mistaken for PFOS.

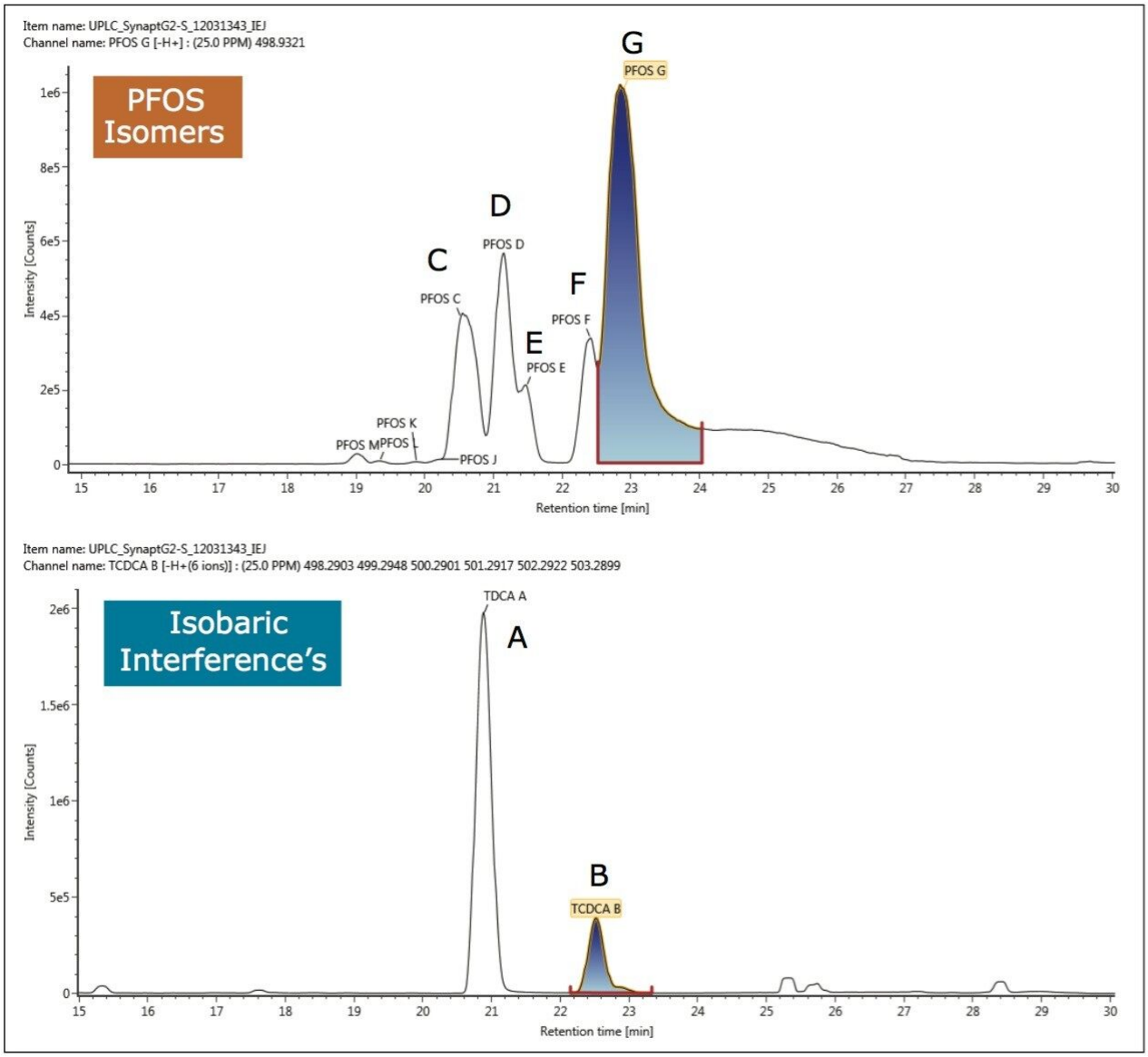

Figure 2. Accurate mass extracted ion chromatograms for the isobaric biological matrix interferences (peaks A and B) and PFOS isomers (peaks C-G).

The approach undertaken here negates the need to use complex chromatography, sample clean up and highly specific MS experimental design. Using ion mobility, it is possible to acquire mobility resolved mass spectral information from the sample, including precursor and fragmentation information, as well as drift times to enable further characteristic profiling. PFOS isomers can be resolved from interfering components as they have vastly different mobility drift times, as shown in the component drift plot in Figure 3. The orthogonal separation of TDCA and TCDCA produces a drift time differentiation of 2 ms from PFOS isomers.

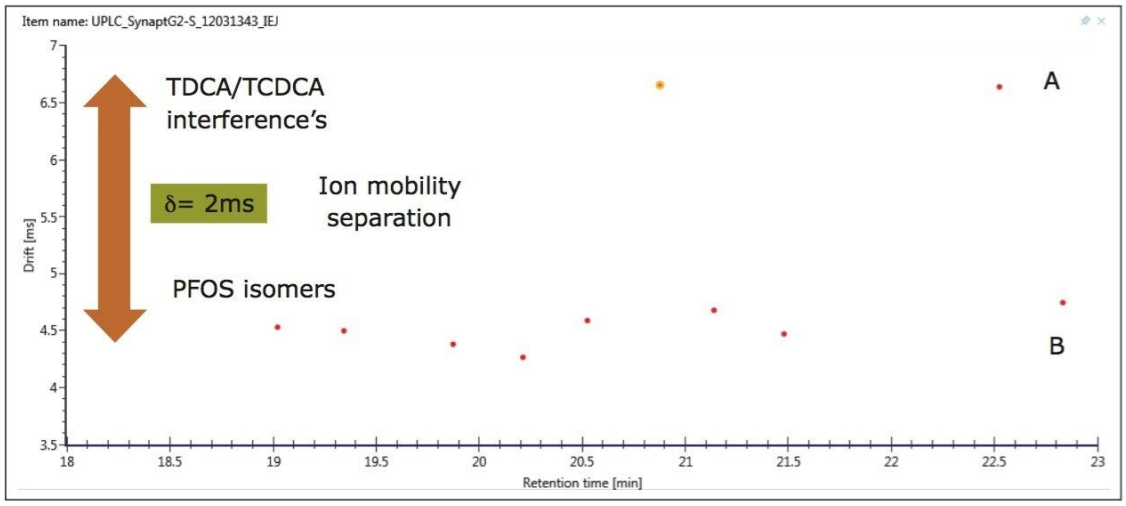

Figure 3. Component drift plot of drift time versus retention time for the nominally isobaric interferences (A) and the PFOS isomers (B).

Drift times of PFOS isomers provide more definitive information regarding their identity. Ordinarily, retention time information and subtle differences in observed product ions, as well as their intensity are used to characterize individual isomers. This can be very challenging at low concentrations. Using drift times that directly relate to collison cross section (CCS), provide an additional identification point related to the physical properties of the molecules, (over *m/z* and chromatographic retention time).

Through the combination of orthogonal ion mobility separation and UPLC chromatographic separation, peak capacity is increased, hence, single component accurate mass spectra can be obtained. It is now possible to create a characteristic assignment profile of PFOS isomers using drift time, retention time, and accurate mass measurements (Table 1). The highly specific mobility aligned precursor and fragment ions, which are resolved from matrix interferences are also used to produce elemental composition information and proposed fragmentation pathways.

	PFOS isomer identification					
PFOS isomers	**J** **3mPFOS**	**C** **5mPFOS**	**D** **IsoPFOS**	**E** **2,2 perfluoro methyl PFOS (tentative)**	**F** **1mPFOS**	**G** **nPFOS**
Drift time (ms) mass measurement error	4.27 -0.23 ppm	4.59 3.4 ppm	4.68 3.66 ppm	4.47 3.12 ppm	4.43 3.72 ppm	4.75 -14.91 (2.68 ppm HE)
Retention time (min)	20.21	20.55	21.14	21.48	22.40	22.80
TDCA interferences		**A** **TDCA**	**B** **TCDCA**			
Drift time (ms) mass measurement error		6.65 3.59 ppm	6.64 1.64 ppm			
Retention time (min)		20.88	22.52			

Table 1. A summary of drift times, retention time, and isomer assigment for major PFOS isomers and co-eluting biological matrix interferences.

Examples of individual ion mobility resolved fragmentation spectra that were obtained for isomers of co-eluting PFOS and co-eluting isobaric biological matrix interferences, are shown in Figures 4, 5, 6, and 7. In Figure 4, the fragmentation spectrum and mobility trace for 3mPFOS is presented; it is mobility separated from 5mPFOS (C) and TDCA (A), which chromatographically co-elute. The enhanced peak capacity obtained with ion mobility enabled the comparison of fragment ion spectra from the different PFOS isomers without interference from the co-eluting TDCA/TCDCA. TDCA co-elutes with PFOS isomers C, and D at retention times 20.55 and 21.14 mins (see Figure 2). The characteristic fragmentation spectrum of isobaric interference TDCA is shown in Figure 5. A commonality of fragments to those obtained for PFOS isomers can be seen in Figures 6 and 7. During the targeted screen, retention time, drift time, and precursor ion/fragmentation spectra were produced for nine PFOS isomers. Spectra associated with matrix interferences were also generated. It can be seen that PFOS isomers C and D have different fragmentation profiles as well as drift times of 4.59 and 4.68 ms respectively (Figures 6 and 7). This data demonstrates that drift time information can be used as specific identification criteria for PFOS isomers and would greatly increase confidence in isomer identification. The use of drift time information greatly reduces reliance upon chromatographic retention time for correct identification of isomers. The complexity of matrices used to study PFOS in environmental samples has been shown to cause shifts in retention times.

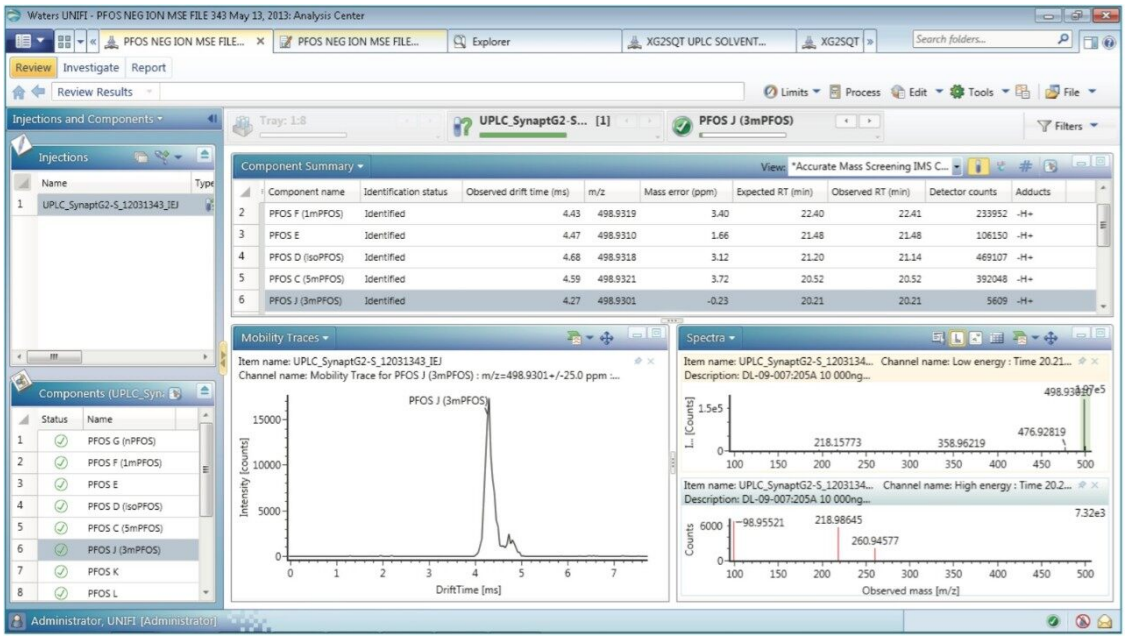

Figure 4. Minor PFOS (J) (3mPFOS) isomer fragmentation spectra, ion mobility resolved from co-eluting PFOS isomer C at retention time 20.21 mins.

Figure 5. Ion mobility resolved characteristic fragmentation spectra of isobaric interference TDCA (A) at retention time 20.88 mins were generated using the combination of orthogonal ion mobility separation and UPLC chromatographic separation.

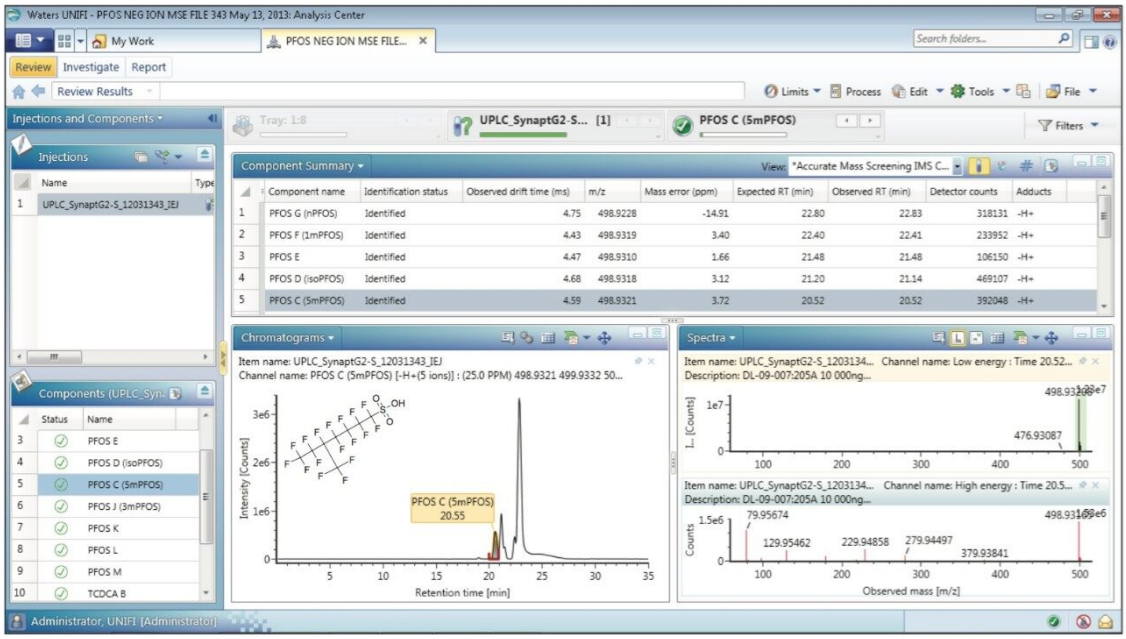

Figure 6. PFOS (C) (5mPFOS) isomer ion mobility precursor ion and characteristic fragmetation spectra, ion mobility resolved from isobaric interference TDCA (A) at retention time 20.55 mins.

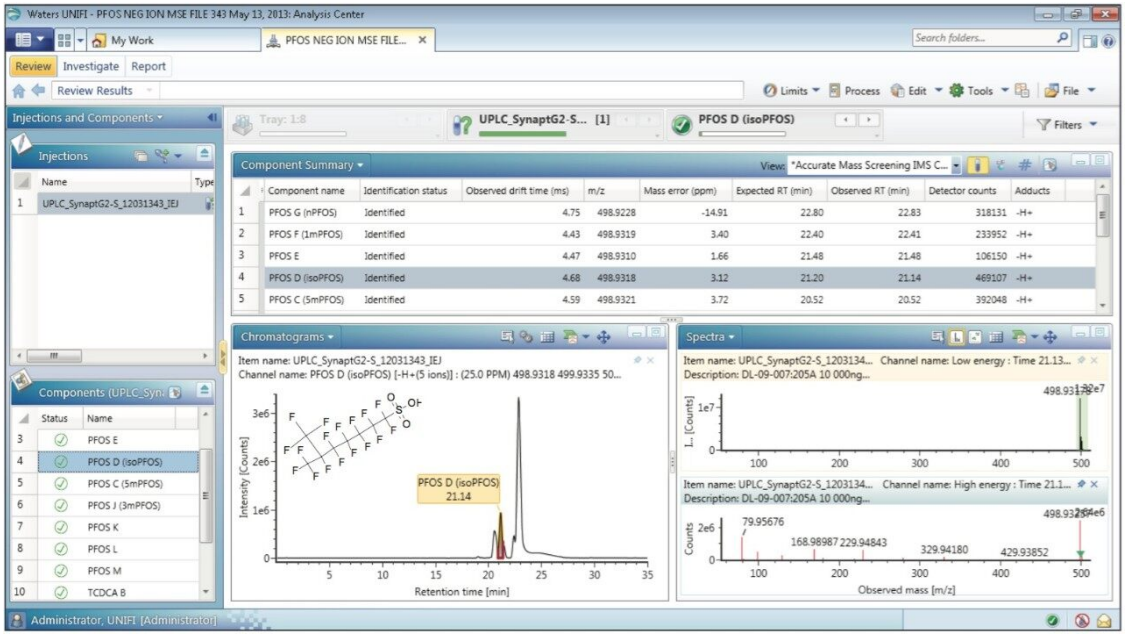

Figure 7. PFOS (D) (isoPFOS) isomer ion mobility precursor ion and characteristic fragmetation spectra, ion mobility resolved from isobaric interference TDCA (A), at retention time 21.14 mins.

Beskin *et al.* have previously shown the elution order of PFOS isomers.[1] Where performed, the PFOS isomer

assignments are presented in Table 1. Structural elucidation was performed according to the methodology introduced by Langlois and Oehme.[8] Unbranched nPFOS is known to be retained strongest at 22.80 mins under reversed phase conditions. This was confirmed with the data presented which showed a combination of the presence of “O” series and “9” series ions in the fragmentation data occurs. From the elucidation of fragments it is concluded that there is no branching in the isomer fragmented. Elucidation based on formation of product ions is indeed a challenging task where a reliance on low intensity ions is required. Optimum sensitivity and collision energies are required. Also the challenge of lower intensity product ions as chain length increases needs to be overcome. A profile of nine PFOS isomer drift times has been generated and warrants further studies to expand the applicability of ion mobility to specific PFOS isomer identification. Our initial investigation indicates that PFOS isomers with a more linear structure have longer drift times, compared to those that have branching further away from the PFOS end groups.

In total, the drift times of nine PFOS isomers have been determined and where sufficient response was obtained, identities were confirmed by examination of their fragmentation spectra. Drift times of PFOS isomers have been shown to increase with linear chain length, and this is a trend that might be used in structural elucidation exercises in future studies. The data presented shows that ion mobility has utility in isomer-specific analysis of PFOS in environmental samples and that further investigation of its application in this field is warranted. Software enhancements will enable further studies to be performed with direct determination of collision cross sections of PFOS isomers.

Conclusion

- Co-eluting isobaric biological interferences TDCA and TCDCA have been resolved from PFOS isomers using ion mobility N_2 and CO_2 as a drift gas.
- Increased confidence can be obtained from distinct drift times for the PFOS isomers and used as an additional identification point to reduce the dependence upon chromatographic retention times.
- Using UPLC IMS-MS, single component precursor ion and fragmentation spectra have been generated for PFOS isomers and TDCA/TCDCA isomers.
- UPLC IMS-MS offers an uncompromised unique approach for the determination and characterization of PFOS within the environment by enabling isomer-specific analysis.
- UPLC IMS-MS in combination with targeted screening can provide an efficient route to specific identification of PFOS isomers.

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