Differentiating PFAS Isomers: A Multi-Pass Cyclic Ion Mobility Mass Spectrometry Approach for Detection, Identification, and Relative Quantitation

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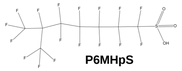
Introduction

Per– and polyfluoroalkyl substances (PFAS) are man-made, industrial compounds that are of concern due to their potential negative health effects and widespread presence in the environment. Due to their chemical manufacturing process, many PFAS exist as mixtures of linear and branched isomers. The branched isomers are not well characterized due to low abundance and lack of authentic standards. Regulatory PFAS testing methods sums branched isomers for reporting. However, there is interest in the distribution of individual isomers for fingerprinting the origin of PFAS contamination. In this study, PFAS isomers in commercial mixes and drinking water samples were characterized by combining liquid chromatography with ion mobility separation (IMS) and high-resolution mass spectrometry.

Linear PFOS

Branched PFOS Isomer



P6MHpS

Samples

The samples analyzed were technical mixes of perfluorooctanesulfonic acid (T-PFOS) and perfluorooctanoic acid (T-PFOA) from Wellington Laboratories. These samples included documentation of the relative amounts of branched isomers, as measured by ^{19}F NMR.[1] Additionally, drinking water samples that were prepared following EPA Method 533 were analyzed to measure the PFAS isomer distribution.

Experimental Parameters

LC: ACQUITY™ I-Class PLUS with a PFAS Kit

Column: CORTECS™ Premier C18, 2.7 µm; 2.1mm x 100mm @ 35C

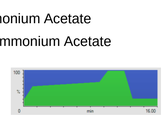
MP A: Water + 2 mM Ammonium Acetate

MP B: Methanol + 2 mM Ammonium Acetate

Flow rate: 0.300 mL/min

Gradient: 16 min

Injection Volume: 5-10 µL



MS: SELECT SERIES™ Cyclic™ IMS

Ionization: ESI-

Capillary Voltage: -0.5 kV

Mass Range: *m/z* 50-1200

MS Analyzer Mode: V mode (Resolution ~60,000 FWHM)

Acquisition: MS/MS

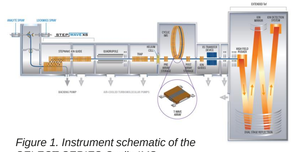


Figure 1. Instrument schematic of the SELECT SERIES Cyclic IMS

Technical PFOS:

- Of the 89 theoretical geometric PFOS isomers,[2] 11 isomers were reported in the technical PFOS standard.[1]
- High resolution IMS is needed to distinguish the PFOS isomers. With the SELECT SERIES Cyclic IMS, resolution is increased with multiple passes in the circular ion mobility path (Figure 2).

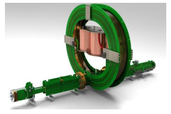


Figure 2. The cyclic Ion mobility optics in the SELECT SERIES Cyclic IMS.

- Figure 3 shows the power of combining LC separation with 6 pass IMS separation for the detection of PFOS isomers in T-PFOS.
- The isomers with single branching at carbon # 3, 4, or 5 were not separated with 6 passes. An additional IMS experiment with 'top and tail' acquisition was performed for the relative quantitation of these isomers (Figure 4).
- In total, 18 structural isomers (7 more than previously reported) were detected in T-PFOS with LC and multi-pass IMS separation. The identifications and relative amounts are reported in Table 1.
- Relative quantitation was calculated with the peak area for drift-specific extracted ion chromatograms (Figure 5) that were generated in the UNIFI™ app as part of the waters_connect™ software.

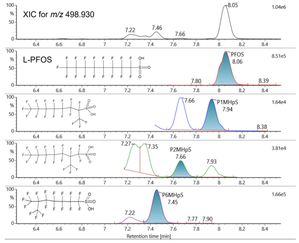


Figure 5. Top: Extracted ion chromatogram (XIC) for m/z 498.930 in the T-PFOS sample with 6 passes in the Cyclic IMS cell. Below: XICs that were created for individual PFOS isomers at m/z 498.930 and the observed drift time ± 15%. The blue color indicates the integrated peak.

Perfluorooctanesulfonic Acid (PFOS) Isomer Separation

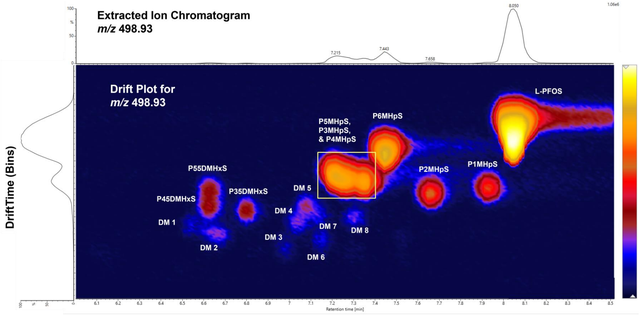


Figure 3. Detection of the PFOS Isomers in T-PFOS at approximately 50 ng/mL with chromatographic separation and six passes in the IMS cell (Resolution ~ 159 Ω/ΔΩ). The acronyms for the isomers are fully detailed in Table 1.

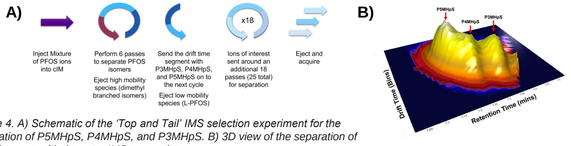


Figure 4. A) Schematic of the 'Top and Tail' IMS selection experiment for the separation of P5MHpS, P4MHpS, and P3MHpS. B) 3D view of the separation of the 3 isomers with the extra IMS separation.

Table 1. Peaks detected in the muti-pass IMS acquisition of T-PFOS standard with retention time, calculated CCS, and proposed identifications. Calculated CCS based on the procedure in McCullagh et. al.[3] Identifications were based on RT, fragmentation and CCS from authentic standards (Wellington Laboratories) measured in house and reported in literature.[4,5]

Name	Structure	Retention Time (min)	6 Pass Calculated CCS (Å²)	Percent Composition by ^{19}F-NMR	Percent Composition by LC-IMS-MS (n=3)	Difference in Percent Composition
Unknown Perfluorodimethylhexane-sulfonate (DM 1)		6.54	161.3		0.03 ± 0.01	
Perfluoro-4,4-dimethylhexanesulfonate (P44DMHxS) (DM2)		6.67	161.0		0.14 ± 0.01	
Perfluoro-4,5-dimethylhexanesulfonate (P45DMHxS)		6.60	162.8	Summed to 1.3	0.31 ± 0.03	-0.59
Perfluoro-3,5-dimethylhexanesulfonate (P35DMHxS)		6.78	162.5		0.26 ± 0.03	
Perfluoro-5,5-dimethylhexanesulfonate (P55DMHxS)		6.60	163.8	0.3	0.37 ± 0.05	+0.07
Unknown Perfluorodimethylhexane-sulfonate (DM 3)		6.99	160.0		0.03 ± 0.01	
Unknown Perfluorodimethylhexane-sulfonate (DM 4)		7.05	161.7		0.16 ± 0.01	
Unknown Perfluorodimethylhexane-sulfonate (DM 5)		7.09	162.6		0.20 ± 0.00	
Unknown Perfluorodimethylhexane-sulfonate (DM 6)		7.15	160.6		0.04 ± 0.00	
Unknown Perfluorodimethylhexane-sulfonate (DM 7)		7.14	162.2		0.05 ± 0.01	
Unknown Perfluorodimethylhexane-sulfonate (DM 8)		7.30	162.0		0.09 ± 0.00	
Perfluoro-1-methylheptanesulfonate (P1MHpS)		7.94	164.1	1.2	1.10 ± 0.01	-0.10
Perfluoro-2-methylheptanesulfonate (P2MHpS)		7.66	163.6	1.6	1.29 ± 0.05	-0.31
Perfluoro-3-methylheptanesulfonate (P3MHpS)		7.33	164.5	4.9	4.65 ± 0.34	-0.25
Perfluoro-4-methylheptanesulfonate (P4MHpS)		7.26	164.2	4.5	4.42 ± 0.24	-0.08
Perfluoro-5-methylheptanesulfonate (P5MHpS)		7.19	164.7	7.0	5.94 ± 0.55	-1.06
Perfluoro-6-methylheptanesulfonate (P6MHpS)		7.42	166.2	10.9	11.6 ± 0.5	+0.68
Linear PFOS		8.05	167.8	68.3	69.0 ± 1.6	+0.72

Drinking Water Samples:

A set of contaminated drinking water samples were prepared following EPA Method 533 and analyzed by LC-MS/MS for targeted quantitation of PFAS. Five of the samples showed a large amount of branching for PFOS. The isomer identities and relative amounts were investigated with LC-IMS-MS (Figure 6).

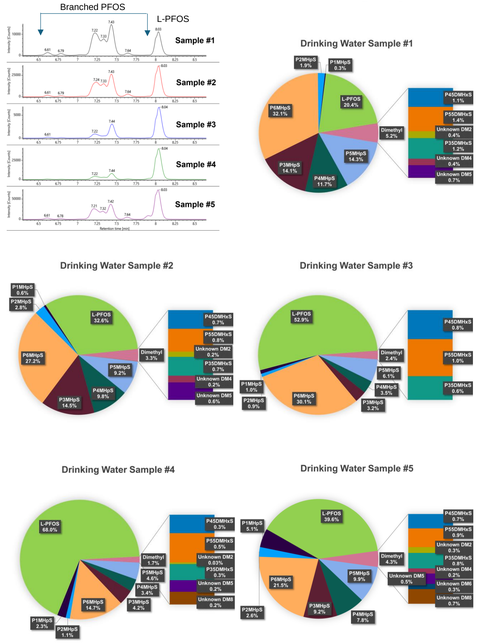


Figure 6. Top Left: XICs for PFOS (m/z 498.93) in the drinking water samples. Pie charts showing relative quantitation of PFOS Isomers in drinking water samples with chromatographic separation and six passes in the IMS cell (Resolution ~ 159 Ω/ΔΩ) and an additional 'top and tail' experiment for similar isomers. The abbreviations for the isomers are fully detailed in Table 1.

The relative abundances of PFOS isomers were unique for each of the drinking water samples. This information could be utilized for fingerprinting the source of PFAS contamination.

Conflict of Interest Disclosure: Sarah Dowd, Lindsay Hatch and Michael McCullagh are employees of Waters Corporation and presenting the poster on behalf of the company.

Perfluorooctanoic Acid (PFOA) Isomer Separation

Technical PFOA:

As PFOA readily decarboxylates in IMS, the quadrupole selected for *m/z* 412.9 and fragmented in the trap region prior to IMS (Figure 7). Three passes in the IMS cell were used to separate the isomers of the *m/z* 368.97 fragment ion (Figure 8).

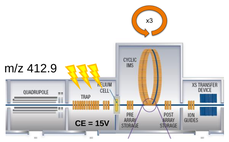


Figure 7. Schematic of the PFOA Isomer Separation. Fragmentation was done in the trap region prior to IMS separation.

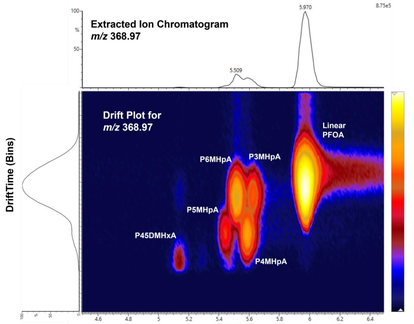


Figure 8. Detection of the PFOA Isomers in T-PFOA with chromatographic separation and three passes in the IMS cell (Resolution ~ 112 Ω/ΔΩ). The acronyms for the isomers are similar to PFOS isomers.

Conclusions

- **Combining LC separation with multi-pass IMS acquisition on the SELECT SERIES Cyclic IMS QTOF is a powerful tool for investigating the branched isomers of perfluorocarboxylic acids and perfluorosulfonic acids.**
- **Previously unreported PFOS (C8 sulfonate) isomers were detected in the T-PFOS standard and in drinking water samples.**
- **Relative quantitation of the individual isomers matched well between LC-IMS-MS and ^{19}F NMR for T-PFOS and T-PFOA.**
- **Drinking water samples showed unique distributions of PFOS isomers, which could be indicative of different sources of PFAS contamination.**

References

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