QUICK NOTE

Shining a High-Resolution Ion Mobility Light on PFAS Dark Matter for Forensic Fingerprinting

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The Objective

To demonstrate the ability of high-resolution ion mobility (HRIM) to enhance existing chromatographic methods and provide richer insights into samples.

The Challenge

Per- and polyfluoroalkyl substances (PFAS), a group of synthetic organic compounds renowned for their superior chemical stability and resistance, have emerged as an insidious and pervasive global contaminant. With their broad industrial and commercial applications spanning over half a century, these "forever chemicals" have found their way into various environmental matrices, creating significant public health concerns. The inscrutable nature of PFAS, coupled with a lack of comprehensive analytical techniques, has given rise to the concept of PFAS "dark matter."[1] Referring to the subset of PFAS compounds that currently elude detection by conventional, targeted analytical methods, these compounds have effectively remained in "darkness," and hence, their effects and prevalence are not fully understood.

In this landscape, forensic fingerprint analysis emerges as a promising tool, offering a new way to get more out of existing analytical processes. This technique, traditionally used in pollutant source identification, could provide a nuanced understanding of PFAS sources, pathways, and fates in the environment.[2] Herein we demonstrate a more comprehensive analytical technique using HRIM coupled to high-resolution mass spectrometry (HRMS) to illuminate our understanding of PFAS distribution, impact, and mitigation. Integrated with existing liquid chromatography methods, HRIM is capable of shedding light on the unseen realm of PFAS and is ultimately positioned to guide us towards a more holistic understanding of these persistent compounds.

The Key Takeaway

MOBIE® provides an additional dimension of separation that reveals size and mass relationships to provide detailed PFAS fingerprints.

The Answer

The application of HRIM to the analysis of PFAS samples provides several advantages, including the separation of branched isomers and the addition of collision cross section as a complementary identifier to mass spectra. A 26-minute liquid chromatography (LC) method was used to analyze a technical perfluorooctanoic acid standard from Wellington Labs (T-PFOA, 3825-26-1) using an Agilent 1290 LC, an integrated MOBILion Systems MOBIE instrument, and an Agilent 6546 Q-TOF in negative ion mode with electrospray ionization. Using the HRIM supplied arrival time measure, 11 isomers were identified and fully resolved due to each isomer having a unique position in the resultant two-dimensional plot (Figure 1). Of note, three observed regions, highlighted with red dashed boxes, indicate portions of the chromatogram where unresolvable compounds would reside in the absence of HRIM. The red arrow indicates a branched isomer hiding under the linear form even in this 26-minute LC separation.

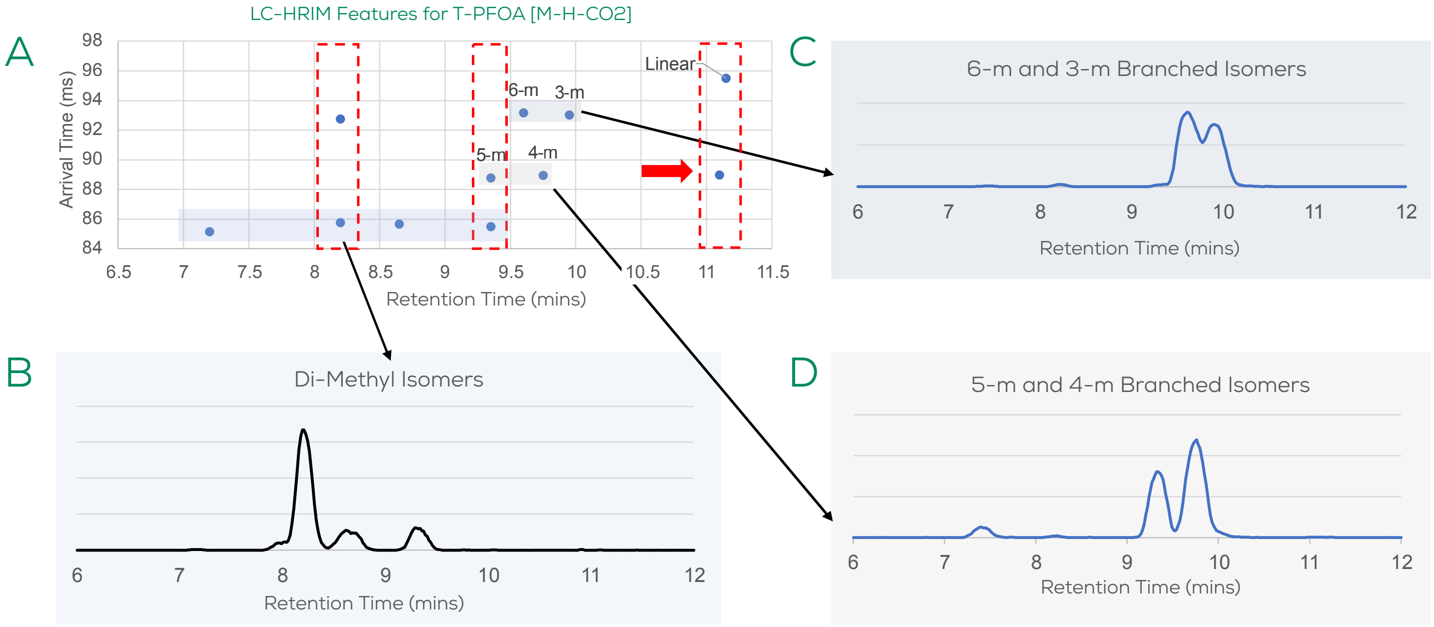

Figure 1. (A) Two-dimensional plot of chromatographic retention time versus the HRIM arrival time measure demonstrating complete separation of all 11 isomers in the T-PFOA standard. Red dashed boxes indicate three observed regions of the chromatogram where unresolvable compounds would reside in the absence of HRIM, with the red arrow highlighting a branched isomer in line with the linear form. (B-D) Mobility filtered extracted ion chromatograms (EICs) from the shaded regions of the 2D plot.

An additional advantage to using HRIM is the ability to convert arrival times to the unique molecular identifier, collision cross section (CCS). CCS is a highly reproducible measure that can be leveraged across various types of ion mobility technologies with limitations. Using a flow injection method, we established CCS values with nitrogen buffer gas as reported in Table 1, with all established CCS values within 0.3% of published drift tube CCS values.[3] The ion mobiligram for the four separate peaks is shown in Figure 2.

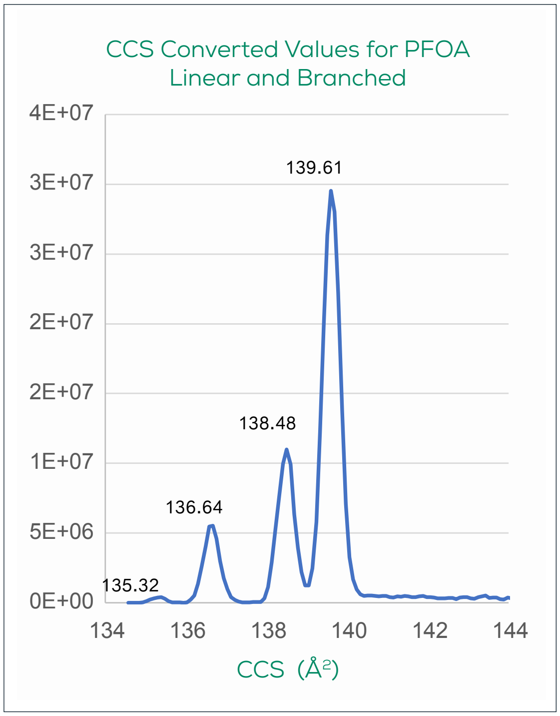

Figure 2. Ion mobiligram of a flow injection analysis with CCS calibration using Agilent reference mass solution.

PFOA Isomer	SLIM CCS (Å²)	DT CCS (Å²)	% Difference
L-PFOA	139.61	139.5 (+/- 0.1%)	0.08%
P(6M/3M)HpA	138.48	138.6 (+/- 0.4%)	0.09%
P(4M/5M)HpA	136.64	136.8 (+/- 0.3%)	0.12%
P(DiMethyl)HxA	135.32	135.7 (+/- 0.4%)	0.28%

Table 1. Measured CCS values using the structures for lossless ion manipulation-based MOBIE system. Drift tube values were taken from Dodds et al. 2020.[3] Calculated percent differences were less than 0.3% across all compounds.

The added dimension of HRIM affords the ability to generate a useful two-dimensional plot using CCS and *m/z*. This plot, referred to as a "conformational space map," provides a visual depiction of the relative size and mass of each included compound. As illustrated in Figure 3, the resultant plot can be used as a visual two-dimensional library beneficial for compound prioritization when unknown compounds are detected. The colored, dashed lines are trendlines drawn through related compounds differing by the length of the carbon chain demonstrating the highly characteristic relationship between CCS and *m/z* for each PFAS subclass. Compounds denoted with a filled circle were from the EPA-533PAR standard (Wellington Labs), while compounds denoted with an "x" were from an AFFF sample. The dotted circle in the bottom left was identified as C5F11 based on literature review as well as a CCS vs. *m/z* position falling on the extended trendline of structurally similar PFAS. The dashed square denotes an unsaturated PFOS compound that falls slightly off the perfluorooctane sulfonate (PFOS) trendline in blue illustrating a deviation from the characteristic relationship due to the loss of two fluorine atoms. A possible structure of the unsaturated PFOS compound is provided for illustrative purposes and was not confirmed. Interestingly, this subtle change provides enough of a deviation from the characteristic trendline such that the relative size and mass of the compound is no longer as consistent with the rest of the PFOS subgroup despite its substantial structural similarity. In this way, an unknown compound can be prioritized, grossly categorized, and further investigated with a head start in determining identity.

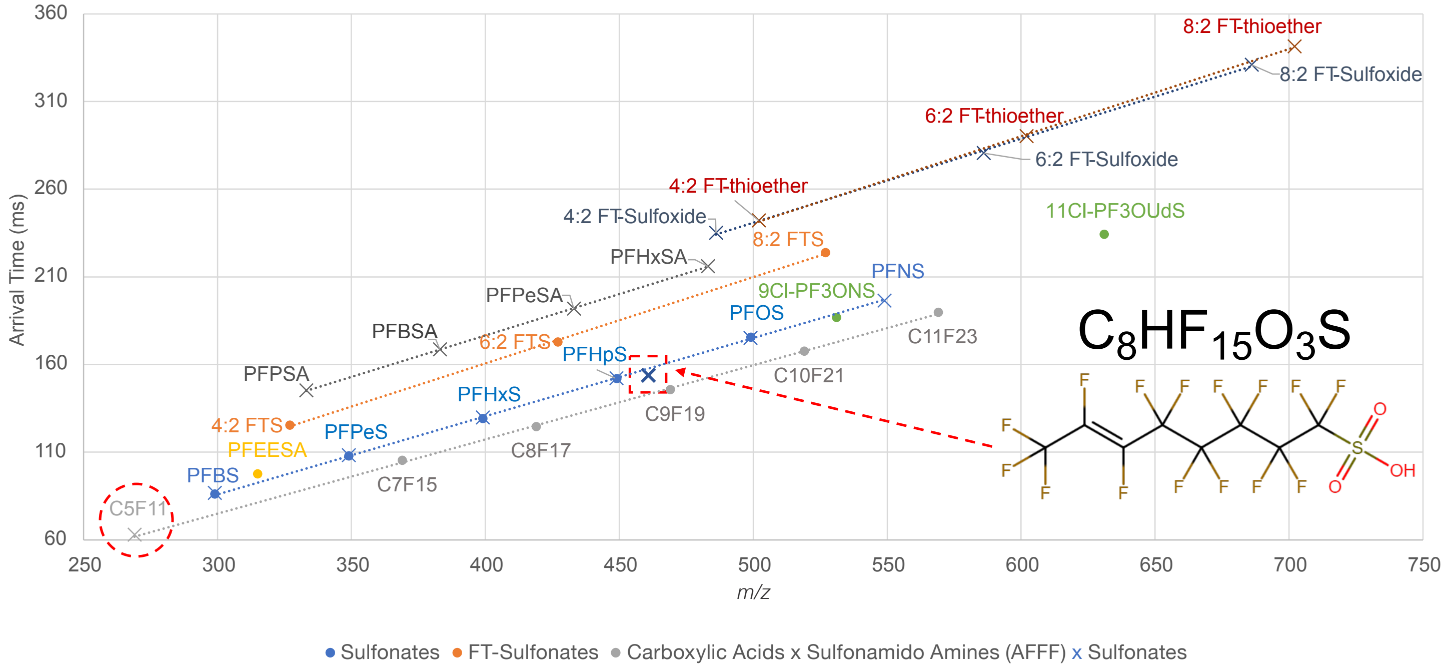

Figure 3. The conformation space map generated by plotting CCS vs. *m/z* provides a visual representation of the relative size and mass of each compound. The colored, dashed lines are trendlines drawn through related compounds differing by the length of the carbon chain demonstrating the characteristic relationship between CCS and *m/z* for each PFAS subclass. Compounds denoted as a colored, filled circle were standards, while compounds denoted with an "x" were from an AFFF sample. The dotted circle in the bottom left was identified as C5F11 based on literature review as well as a CCS vs. *m/z* position falling on the extended trendline of structurally similar PFAS. The dashed square represents an unsaturated PFOS compound that falls slightly off the PFOS trendline in blue illustrating a deviation from the characteristic relationship due to the loss of two fluorine atoms. A possible structure of the unsaturated PFOS compound is provided for illustrative purposes and was not confirmed.

Summary

The PFAS compounds that are currently regulated and routinely detected with targeted methods represent just the tip of the proverbial iceberg. The bulk of PFAS, analogous to dark matter in the universe, remains largely unobserved and unquantified. These undetected, unreported, and unregulated PFAS have the potential to silently impact environmental and human health, necessitating urgent research and the application of innovative analytical techniques. The combination of LC, HRIM, and high-resolution mass spectrometry is a powerful arrangement that can provide highly informative fingerprints of the various PFAS signatures in the environment.

Acknowledgments

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References

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