thermo scientific

APPLICATION NOTE 667

A comparison between HRAM Orbitrap technology and MS/MS for the analysis of polyfluoroalkyl substances by EPA Method 537

Authors

Ali Haghani,[1] Andy Eaton,[1] Richard F. Jack,[2] Ed George,[2] Dipankar Ghosh[2]

[1]Eurofins Eaton Analytical, Inc. Monrovia, CA; [2]Thermo Fisher Scientific, San Jose, CA

Keywords

Contaminants of emerging concern, CEC, perfluorinated compound, perfluoroalkyl acid, PFOA, PFOS, perfluorinated alkyl substances, PFAS, perfluoroalkyl acids, PFAA, persistent organic pollutants, POPs, fire-fighting foam, Orbitrap

Goal

To demonstrate a liquid chromatography – high-resolution, accurate-mass (LC-HRAM) methodology using Orbitrap™ technology as a sensitive, accurate, and reliable quantitative alternative to the use of triple quadrupole mass spectrometers while simultaneously determining unknown perfluorinated compounds in the same drinking water extracts.

Introduction

The unique water-, oil-, grease-, stain- and heat-resistant properties of perfluoroalkyl substances (PFASs) have led to their widespread use in diverse industrial applications and multiple consumer products for over fifty years.

Perfluoroalkyl substances are compounds for which all hydrogens on all carbons (except for carbons associated with functional groups) have been replaced by fluorines, e.g., perfluoroalkyl acids (e.g., PFOA, PFOS). Polyfluoroalkyl substances are compounds for which all hydrogens on at least one (but not all) carbons have been replaced by fluorines, e.g., fluorotelomer-based compounds.[1] The carbon-hydrogen linkages allow for biotic and abiotic degradation in the environment. However, the C–F bond

ThermoFisher SCIENTIFIC

is considered the strongest single bond in organic chemistry with a bond enthalpy of 481 kJ/mol in CH_3F, which is substantially higher than that of other bonds. This pronounced bond strength is reflected in the notorious environmental and chemical stability of these compounds.[2] (See Figure 1.)

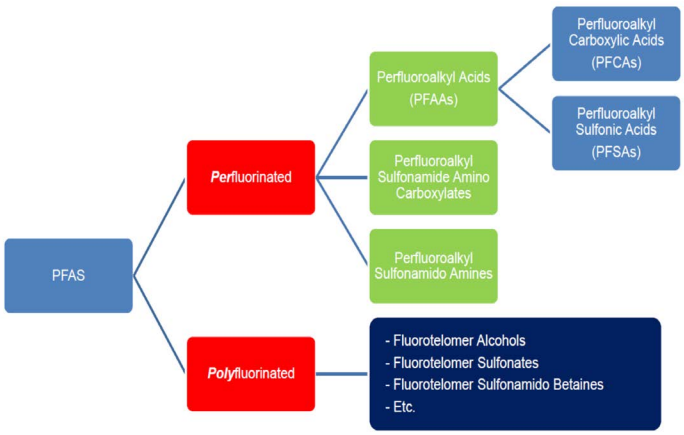


Figure 1. Perfluorinated and polyfluorinated compounds as emerging contaminants in the environment.

The National Institute of Environmental Health Sciences and the National Toxicology Program are supporting research to better understand the potential health effects in humans of exposure to PFASs. In animal studies, some PFASs disrupt normal endocrine activity; reduce immune function; cause adverse effects on multiple organs, including the liver and pancreas; and cause developmental problems in rodent offspring exposed in the womb.[3]

As a result, the United States Environmental Protection Agency (EPA) developed EPA Method 537[4] for the Unregulated Contaminant Monitoring Rule (UCMR 3) program, which collects data for contaminants suspected to be present in drinking water but that do not currently have health-based standards set under the Safe Drinking Water Act (SDWA).[5] In 2012, six PFASs were added to the UCMR 3 list to be monitored, including PFOS and PFOA using EPA Method 537. EPA Method 537 is an offline SPE method using LC-MS/MS detection for the quantitation of linear PFASs in drinking water. In October 2015, occurrence data from the study was released (Figure 2). It is important to note that this is only a small fraction of the hundreds of compounds that can potentially exist in the environment, such as the multiple branched and polyfluorinated PFASs breakdown products that have been known to be in environmental waters. However, standards do not exist for many of these compounds.

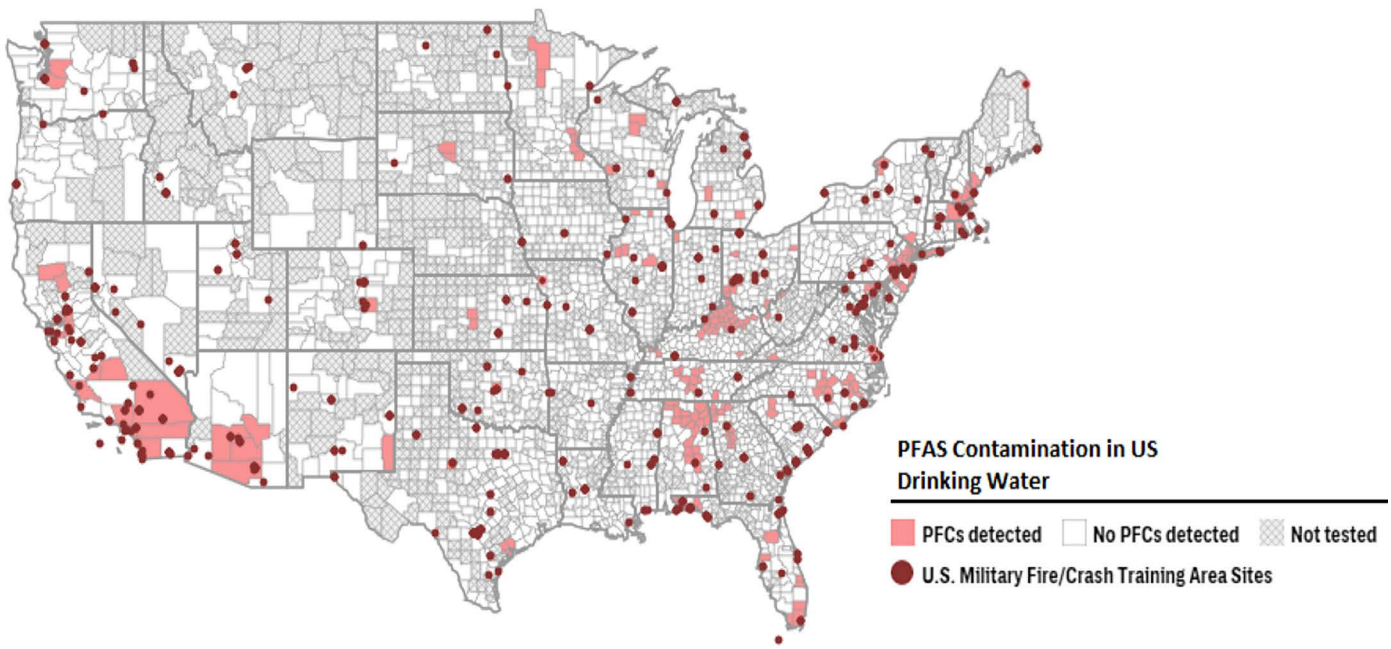


Figure 2. PFAS occurrence data released by EPA for UCMR 3, using EPA Method 537 and monitoring six PFAS compounds.
Data visualization by Moiz Syed. Sources: EPA and Departmennt of Defense. https://theintercept.com/2015/12/16/toxic-firefighting-foam-has-contaminated-u-s-drinking-water-with-pfcs/

Liquid chromatography-tandem mass spectrometry (LC/MS/MS) has been the method of choice for the analysis of PFASs in a variety of matrices. EPA Method 537 is based on this technique, as it allows monitoring of select target analytes in public water supplies. However, other screening strategies taking into account full scan with other advanced MS/MS scan modes can potentially offer a valuable alternative to SRM based methodology due to the development of selective instrumentation for the simultaneous determination of known and unknown contaminants. In addition, high-resolution, accurate-mass (HRAM) capability also provides the ruggedness and sensitivity of MS/MS-based methods without the limitations of unknown identification.

HRAM Orbitrap technology allows for excellent full scan quantitation of target PFASs with MS/MS confirmation. In addition, screening for other contaminants is possible with powerful software tools utilizing comprehensive compound databases and spectral libraries. For this application, we evaluate HRAM Orbitrap quantitation and sensitivity on the Thermo Scientific™ Q Exactive™ mass spectrometer using EPA Method 537 with some minor changes to expand the scope of compounds that can be analyzed using the method. A comparison of HRAM Orbitrap and triple quadrupole mass spectrometry will be described in terms of lowest concentration minimum reporting limit (LCMRL) for the six PFAS compounds in the current EPA Method 537. The results show that HRAM Orbitrap technology provides equal or better quantitation in full scan as compared to traditional triple quadrupole techniques, with the additional capability to screen for unknown PFASs.

Experimental

Sample preparation

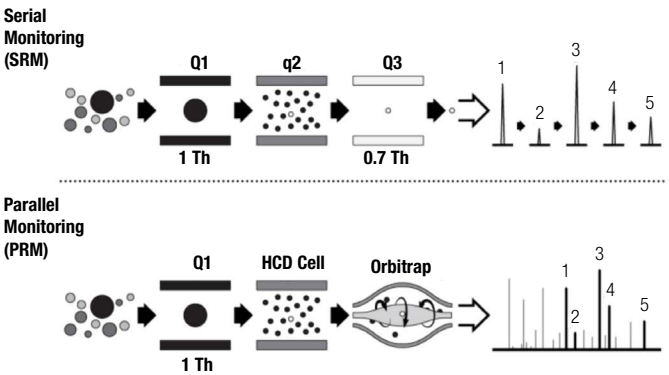
A 250-mL water sample was preserved with Trizma® buffer (MilliporeSigma), fortified with surrogate standards, and passed through a solid phase extraction (SPE) cartridge containing Thermo Scientific™ Dionex™ SolEx™ HRPHS material to extract the method analytes and surrogates. The compounds were eluted from the solid phase with a small amount of methanol. The extract was concentrated to dryness with nitrogen in a heated water bath, and then adjusted to a 1 mL volume with 96:4% (vol/vol) methanol/water after adding the internal standards. A 5 µL injection was made into an LC equipped with a C18 column that was interfaced to a Q Exactive hybrid mass spectrometer capable of producing full scan and MS/MS data. Note: The use of the modified SPE material mentioned above enabled the capture of smaller PFAS compounds that are beyond the scope of the original EPA Method 537.

Separation

LC:	Thermo Scientific™ UltiMate™ 3000 RS UHPLC system, binary pump, autosampler, and column heater set at 30 °C with 25 µL sample loop
Column:	Thermo Scientific™ Hypersil GOLD™ aQ, 2.1 × 150 mm (3 µm)
Mobile Phase:	A: 20 mM ammonium acetate in water B: Methanol
Gradient:	Start at 30% B, hold for 0.5 minutes and then use a linear gradient to 90% B at 15 minutes, hold for one minute, then drop to original 30% B and equilibrate for additional 3 minutes for a total 19 minutes run time.

Q Exactive MS scan modes and settings

The Q Exactive hybrid mass spectrometer was evaluated using two scan modes: 1) Full scan analysis from *m/z* 100–1000 at mass resolution 70,000 (FWHM) at *m/z* 200, and 2) Parallel reaction monitoring mode (PRM), described in Figure 3, at mass resolution 35,000 (FWHM) at *m/z* 200 and isolation width of 1 Da.



Parallel reaction monitoring for high resolution and high mass accuracy quantitative, targeted proteomics. *Peterson et al., MCP 2012, 0112.020131.*

Figure 3. Parallel reaction monitoring (PRM) in a Q Exactive Orbitrap MS compared to traditional triple quadrupole (serial) MRM analysis.

Full scan acquisition does not require compound optimization for target compounds with the added benefit to perform non-targeted and retrospective data analysis. Accurate quantitation depends upon low ppm mass accuracy and high resolution to discriminate the analyte from matrix components.

PRM is similar to the typical SRM experiment used in a triple quadrupole mass analyzer. The principal difference is that all fragments are collected in a full scan high resolution mass analysis. As a result, multiple MS/MS fragments can be associated with a single precursor. This technique is used for targeted quantitation; thus, retention time, selective compound formula or monoisotopic molecular weight, and collision energy are required to be used in an inclusion list for data acquisition. This experiment empirically has more specificity for the target compound than a full scan experiment since a specific precursor is isolated and fragmented. However, non-targeted analysis is not possible using PRM.

Table 1 describes some key Q Exactive MS settings for each acquisition mode.

Table 1. Q Exactive MS settings.

Full Scan Analysis	
Resolution (FWHM):	70,000
AGC Target:	1.00E+06
Maximum Ion Time:	100 ms
Mass Scan Range:	100–1100 *m/z*
Ion Polarity:	Negative
PRM Analysis	
Resolution (FWHM):	35,000
AGC Target:	2.00E+05
Maximum Ion Time:	100 ms
Isolation Width:	1 Da
Ion Polarity:	Negative

Results and discussion

The liquid chromatography parameters were optimized to ensure good peak symmetry, especially for the early eluting compounds. As the homologous CF_2 backbone increases, the compounds become less polar, exhibiting greater retention on the reversed phase column. The sulfonates are less ionic than those compounds containing carboxylic acids, hence they elute later than PFCA with equal number of carbon atoms in the backbone, e.g., PFOS elutes later than PFOA, although both are C8. Figure 4 displays the observed peak shape and separation obtained with this method.

The sensitivity and linearity for the target compounds on the Q Exactive HRAM Orbitrap mass spectrometer in both the full scan and PRM acquisitions were compared. Example result for PFOA shows comparable sensitivity, specificity, and calibration linearity in both modes (Figure 5). Confirmation of the result in full scan is obtained through isotopic pattern match, retention time confirmation, and mass accuracy (i.e. mass extraction window (MEW)), which is typically 2–3 ppm on the Q Exactive instrument. In PRM mode, a full scan product ion spectrum is obtained and can be used to search against a spectral library. In addition, ion ratio confirmation is possible for further confidence in the identification.

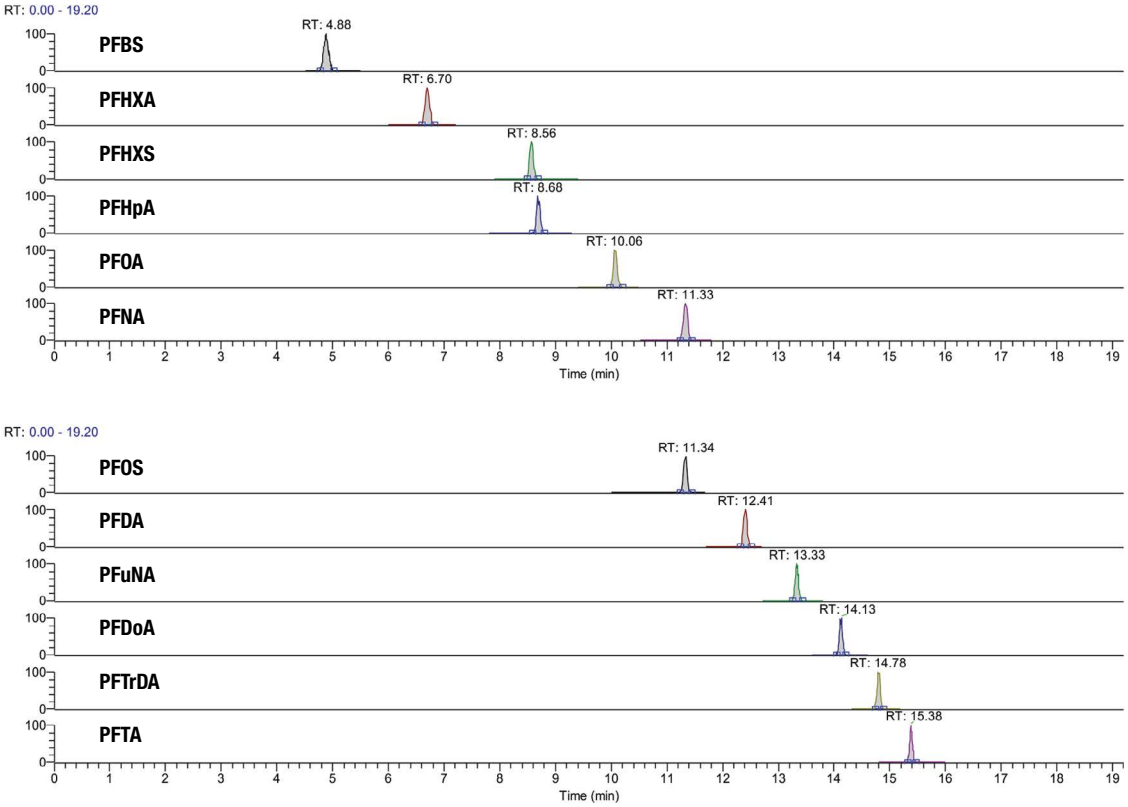


Figure 4. Full scan extracted ion chromatogram of target compounds at 70K resolution, showing good peak shapes and S/N for a 2.5 ppt standard.

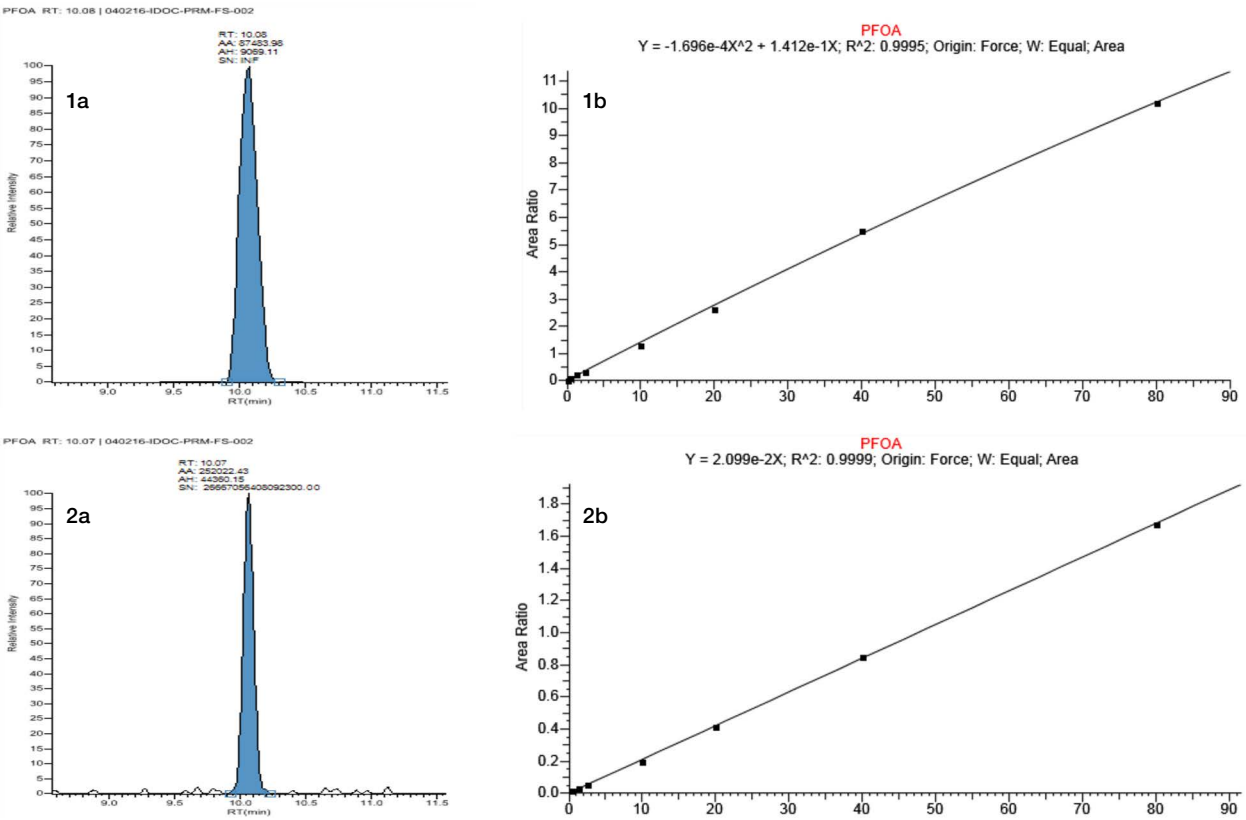


Figure 5. Comparison of full scan extracted ion and PRM scan modes for the compound PFOA at 0.5 ppt and calibration linearity from 0.5 to 80 ppt. (1a, 1b) PRM with primary MS2 transition used for quantitation; (2a, 2b) Full scan extracted ion at 70,000 FWHM used for quantitation.

In Figure 6, a comparison of triple quadrupole SRM, full scan Q Exactive, and PRM Q Exactive analyses is shown for PFOA. Excellent quantitation and sensitivity is obtained with HRAM Orbitrap technology in comparison to QQQ analysis.

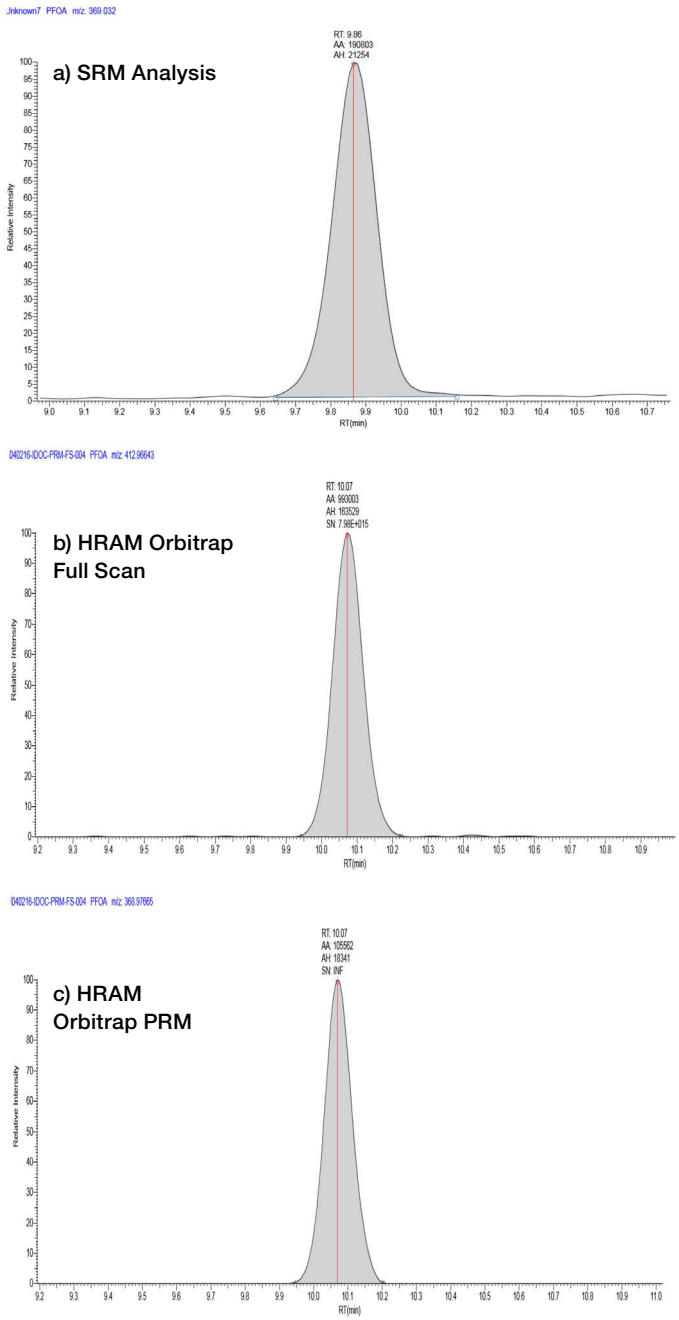


Figure 6. A 2.5 ppt standard of PFOA analyzed by both Q Exactive MS and triple quadrupole mass analyzers under similar conditions (all 5 µL injections). Excellent quantitation and sensitivity is obtained with HRAM Orbitrap in comparison to QQQ analysis.

For EPA Method 537, the lowest concentration minimum reporting limit (LCMRL) is used to evaluate method performance. The LCMRL is defined as the lowest spiking concentration at which recovery of between 50 and 150% is expected 99% of the time by a single analyst. The procedure requires, at a minimum, four replicates at each of seven fortification levels. Four laboratory reagent blanks must also be included. All samples must be processed through the entire method procedure.[6] Test data is entered into a calculator provided by the EPA.

Calculated LCMRLs are shown for both scan modes on the Q Exactive instrument in Figure 7. All results obtained were equal to or better than the published LCMRLs using triple quadrupole SRM analysis for the target analytes in EPA Method 537. Note: the less than values on the LCMRL table means a lower concentration is needed for calculation of LCMRL.

PFOS quantitation

It is important to note that the quantification of environmental samples containing PFOS can be challenging as there is no perfect practical way for accurate quantification of all branched isomers due to different ratios in existing samples and relative response factors. These ratios will differ from calibration standards and between samples from different locations. For PFOS, the 499→99 SRM transition representing a specific branched isomer is generally lower biased relative to the branch representing the SRM transition 499→80 (higher bias). Figure 8 shows a sample containing PFOS compared to a calibration standard. Note that the ratios are not the same, resulting in a biased result if quantitated using EPA Method 537 (the method uses the 499→80 SRM transition). In the Q Exactive instrument, full scan can be used to observe all the branches and appears to be more reliable for quantitation of PFOS. Full scan is closer to the average of the two MRMs and less prone to other factors effecting isomer response factors.

PRM			
EPA Method 537 Target List			
	Critical Level (ng/L)	**DL (ng/L)**	**LCMRL (ng/L)**
PFBS	0.077	0.12	<0.5
PFDA	0.18	<0.5	<0.5
PFDoA	0.14	0.29	<0.5
PFHpA		0.35	0.97
PFHxA	0.16	0.27	<0.5
PFHxS		0.52	0.77
PFNA	0.14	0.26	<0.5
PFOA		0.36	0.5
PFOS	0.14	0.21	<0.5
PFTA		0.48	0.71
PFTrDA	0.18	0.32	<0.5
PFuNA		0.31	0.72

Full Scan			
EPA Method 537 Target List			
	Critical Level (ng/L)	**DL (ng/L)**	**LCMRL (ng/L)**
PFBS	0.15	0.2	<0.5
PFDA	0.15	0.26	<0.5
PFDoA		0.47	0.73
PFHpA	0.09	0.15	<0.5
PFHxA	0.13	0.19	<0.5
PFHxS		1.7	2.4
PFNA	0.11	0.17	<0.5
PFOA		0.22	0.5
PFOS		0.26	0.5
PFTA	0.15	0.2	<0.5
PFTrDA		0.31	0.55
PFuNA		0.38	1
PFBA		0.19	0.64
PFODA		0.55	1
PFDS	0.13	0.19	<0.5
PFHxDA		0.12	0.5
PFPA	0.18	0.19	<0.5

Figure 7. LCMRL tables for both Q Exactive HRAM Orbitrap scan modes. The compounds highlighted in red are additional analytes that are not part of the original EPA Method 537 list but were found in processed drinking water from the same UCMR3 water extracts.

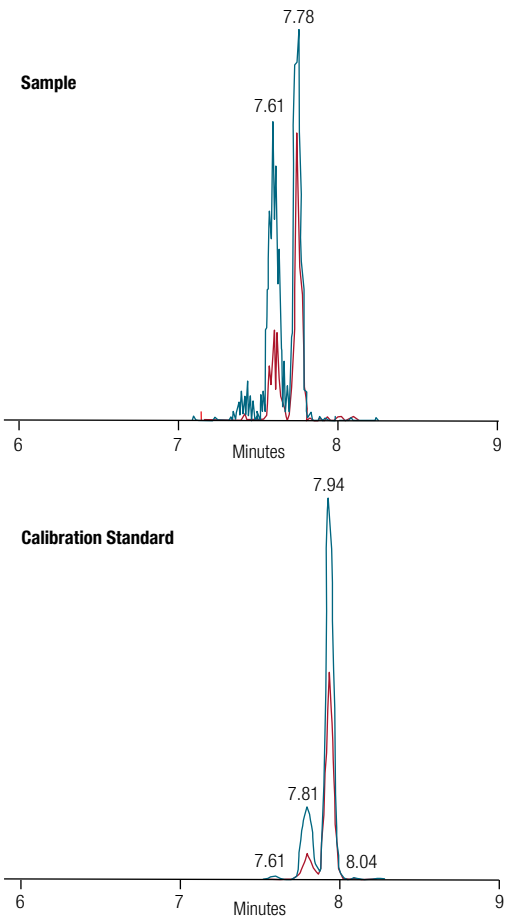


Figure 8. PFOS branch ratio comparison in a sample vs. a calibration standard. These ratios are represented by overlay of the SRM transitions 499→80 (blue trace) and 499→99 (red trace).

Outside of the US, the 499→99 transition is commonly used, whereas EPA Method 537 uses 499→80. The United Nations Environment Program (UNEP) has suggested to take the average of the two using triple quadrupole MS, which makes the results closer to full scan quantitation (Figure 9).

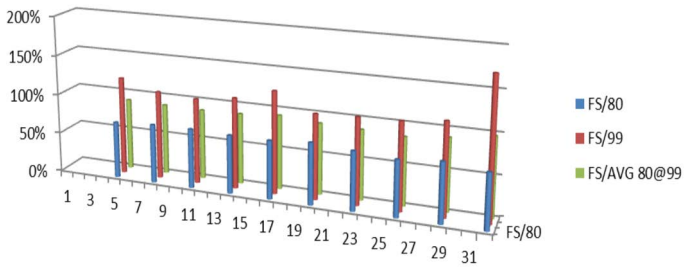


Figure 9. Quantitation comparison of full scan in a Q Exactive MS to SRMs 499→80 and 499→99 (represented as peak area ratios). Results suggest that full scan will have less bias and be close to average of using two SRMs for quantitation.

Screening for other PFASs

As mentioned earlier, an advantage of Q Exactive HRAM Orbitrap instrumentation over targeted analysis using a triple quadrupole MS is the ability to screen for related compounds and other PFASs in samples. For full scan data, retrospective analysis and identification of compounds are possible using spectral libraries, along with retention time and isotope pattern matching for confirmation. Figure 10 shows a sample taken during the UCMR with detection of a non-targeted compound, PFDS using this approach. As predicted, the branched isomer is also detected.

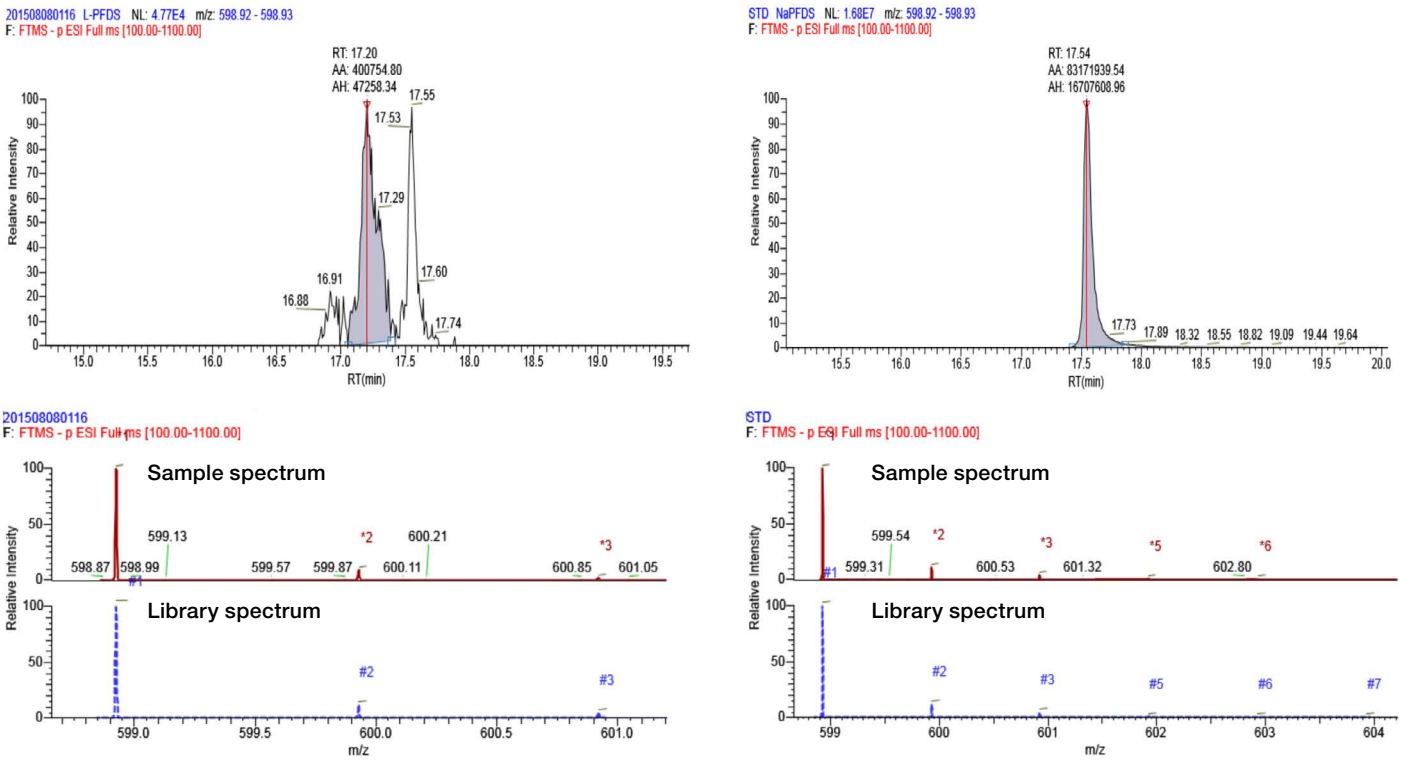


Figure 10. A UCMR3 sample shown having trace hits for a non-targeted compound (PFDS). Post-run identification was performed using an in-house spectral library with isotopic pattern recognition, accurate mass, and retention time for confirmation.

Further interrogation of samples can be performed utilizing a full scan data-dependent acquisition such that both full scan and MS/MS fragments for the top five most intense ions in the mass spectrum are recorded. Powerful data mining tools, such as Thermo Scientific™ Compound Discoverer™ software, allow easy setup of flexible, customized workflows. An example workflow is shown in Figure 11. The software has powerful statistical tools and filters to help narrow down the potential structures of selected compounds, and they can be drawn in a 'custom explanation' using Thermo Scientific™ Mass Frontier™ software to check against accurate mass, isotope pattern, MS, and MS2 data. Known characteristic patterns for suspects can be visualized and used for data filtering. For example, fluorine has a negative mass defect—it has an atomic number of 9 and a relative atomic weight of 18.9984 u. This negative mass defect leads to substantially lower monoisotopic masses of highly fluorinated compounds than the respective nominal mass. Figure 12 is an example of the 'custom explanations' view within the software.

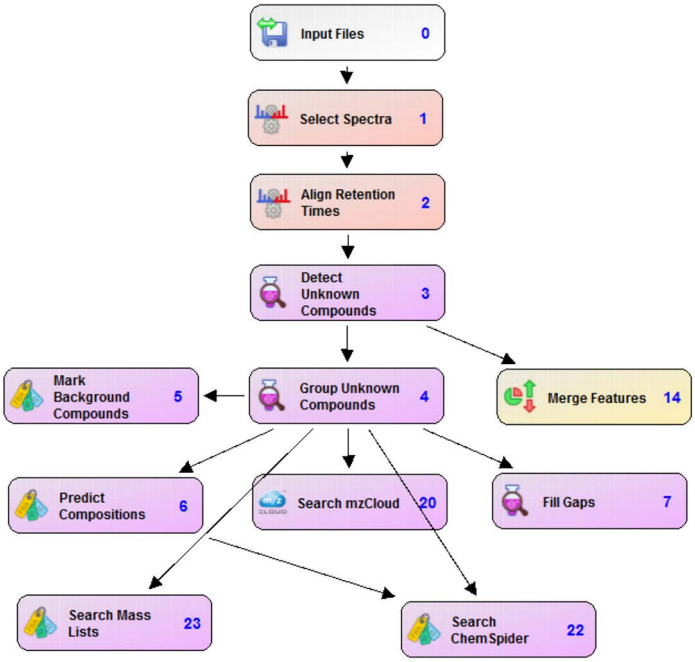


Figure 11. Workflow example in Compound Discoverer software. Flow-chart style elements can be easily dragged and dropped into place for easy customization.

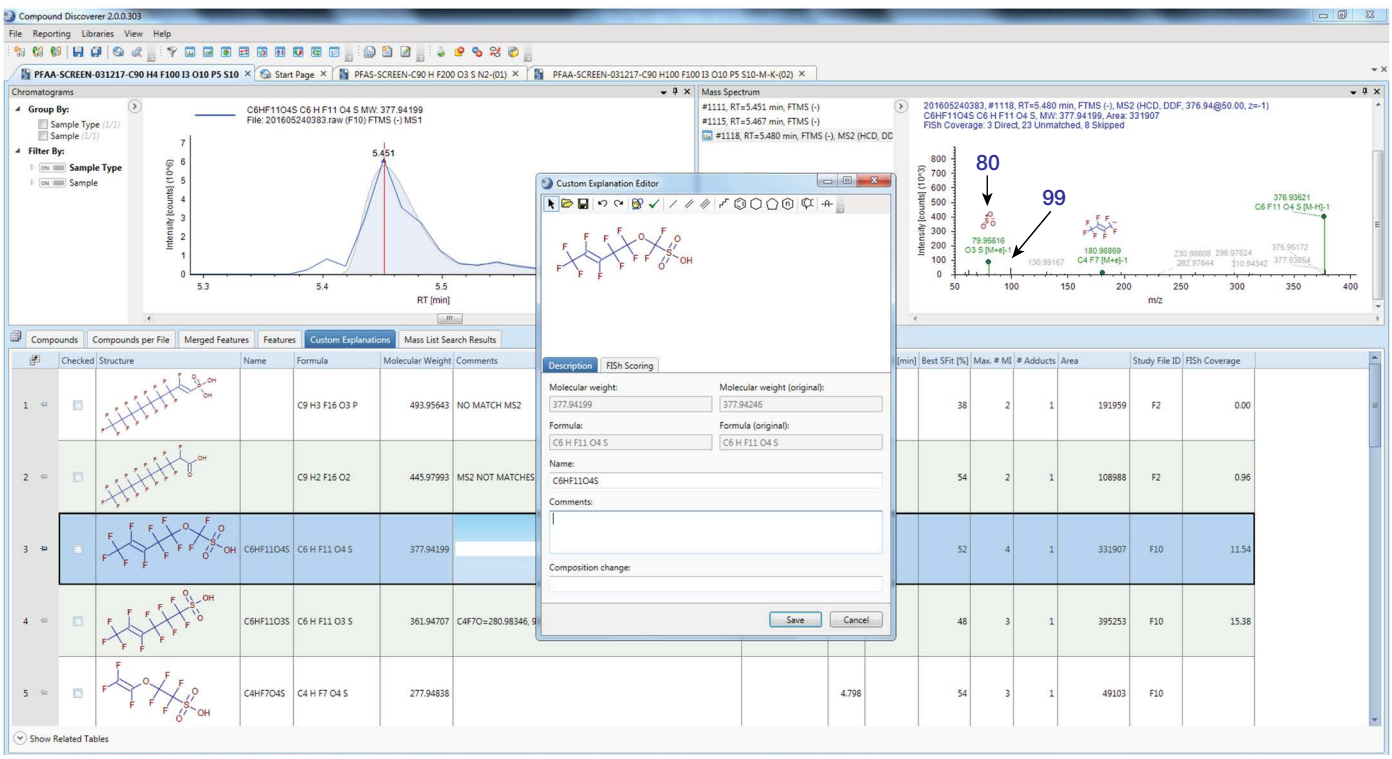


Figure 12. A proposed structure can be drawn in a 'custom explanations' window in Compound Discoverer software using Mass Frontier software for FISH coverage and to check against accurate mass, isotope pattern, with MS2 data displayed in the same workspace.

Conclusion

- Based on the EPA method flexibility rule, QA/QC requirements and guidance within EPA Method 537, HRAM Orbitrap technology should be permissible for potential compliance monitoring if PFASs become regulated compounds in US drinking waters. Q Exactive HRAM Orbitrap instrumentation in the PRM scan mode can be used for quantitation with performance like a triple quadrupole in SRM mode with added specificity, selectivity, and comparable sensitivity.
- Full scan HRAM Orbitrap technology can likely produce more accurate quantitative data for compounds that contain branched isomers such as PFOS.
- Routine quantitative workflows and non-targeted analysis can be performed in a single analysis.
- With complex samples with unknown amounts of other PFASs, utilization of Compound Discoverer software can lower the data processing time and quickly show results.
- Other techniques may be necessary for further confirmation of suspects/unknown structures such as MS^n, ^{13}C, and ^{19}F NMR, when standards are not commercially available.

References

1. EPA. Per- and Polyfluoroalkyl Substances (PFASs) in Your Environment -Includes Information on Perfluorooctanoic Acid (PFOA), Perfluorooctyl Sulfonate (PFOS), and All Other PFASs, and on PFCs, https://www.epa.gov/pfas (accessed June 4, 2017).
2. EPA. Drinking Water Health Advisories for PFOA and PFOS, https://www.epa.gov/ground-water-and-drinking-water/drinking-water-health-advisories-pfoa-and-pfos (accessed June 4, 2017).
3. National Institute of Environmental Health Sciences. Perfluorinated Chemicals (PFCs), https://www.niehs.nih.gov/health/materials/perflourinated_chemicals_508.pdf (accessed June 4, 2017).
4. EPA Method 537 'Determination of Selected Perfluorinated Alkyl Acids in Drinking Water by Solid Phase Extraction and Liquid Chromatography / Tandem Mass Spectrometry LC/MS/MS' version 1.1 (2009).
5. EPA. Unregulated Contaminant Monitoring Rule 3 (UCMR3) http://water.epa.gov/lawsregs/rulesregs/sdwa/ucmr/ucmr3/ (accessed June 4, 2017).
6. Winslow, S.D.; Pepich, B.V.; Martin, J. J.; Hallberg, G.R.; Munch D.J.; Frebis, C.P.; Hedrick, E.J.; Krop, R.A. Statistical Procedures for Determination and Verification of Minimum Reporting Levels for Drinking Water Methods. *Environ. Sci. Technol.* **2006,** *40,* 281–288.

Find out more at **thermofisher.com**

ThermoFisher SCIENTIFIC