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CASE STUDY 3: Quantitative and Qualitative Analysis of Perfluoroalkyl Substances (PFASs) in Wildlife Samples Using the Xevo G2-XS Q-Tof

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APPLICATION BENEFITS

Reviewing complex high resolution, non-targeted MS^E or HDMS®^{E} datasets using workflows, filters, and views within an integrated scientific information system allows:

- Screening for a theoretical unlimited number of compounds in a single injection.
- Simultaneous collection of qualitative and quantitative unbiased data for either targeted or non-targeted analysis.
- Historical data review performed using accurate mass precursor and fragment ion information.

WATERS SOLUTIONS

ACQUITY UPLC® I-Class System

ACQUITY UPLC BEH C_{18} Column

Xevo® G2-XS Q-Tof™

PFC Analysis Kit

UNIFI® Scientific Information System

KEYWORDS

PFASs, PFCs, matrix interference, non-targeted screening, environmental analysis, MS^E, High Resolution Accurate Mass, HRAM, High resolution mass spectrometry, HRMS, data independent analysis

GOAL

Demonstrate low-level detection and comprehensive data review of fluorinated environmental contaminants in wildlife using HRMS technologies and an integrated scientific information system.

INTRODUCTION

Perfluoroalkyl substances (PFASs) encompass a range of fully fluorinated alkyl compounds, typically with an anionic end group (Figure 1). These compounds have been implemented in a range of consumer goods and industrial processes due to their hydro- and lipo-phobic properties. As a result of their widespread use and subsequent leaching from materials, they have been found in various environmental and biological samples. Concern that these compounds exhibit characteristics of persistent organic pollutants (POPs) has resulted in study and legislation against their use.[1] For monitoring and research purposes, sub-ppb detection of these compounds is often required. Traditionally, this type of analysis has been performed using the selective MRM approach on a tandem quadrupole MS. However, the ability to identify other contaminants of concern post acquisition or matrix components such as co-extracted bile acids[2] supports the use of high resolution mass spectrometry (HRMS).

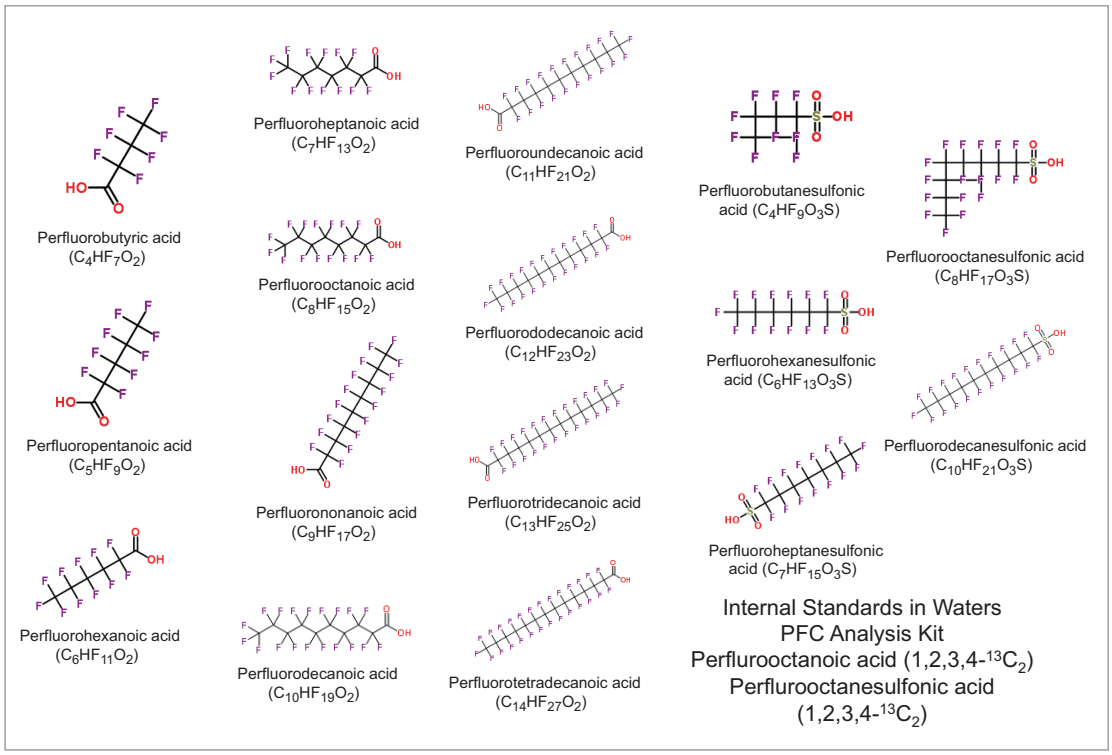

Figure 1. Various carbon chain length PFASs commonly monitored in the environment, with carboxylic or sulfonic anionic end groups. PFASs displayed in this figure are provided in Waters PFC Analysis Kit at various concentration levels. All structures were taken from www.chemspider.com.

High resolution mass spectrometry (HRMS) screening techniques can, in theory, monitor an unlimited number of targets at the same time as providing information to help discover unknown compounds or metabolites of interest. The ease of use and efficacy of a non-targeted, data independent, analysis type (MS^E),[3] coupled with a state-of-the-art scientific information system (UNIFI) for multi-analyte screening in environmental samples is demonstrated with this case study. This approach was undertaken on an authentic sample analysis of mink liver for low level detection (ppb and sub-ppb) and quantification of identified PFASs. This particular application note will focus on introducing the novel way a user in a routine environment can customize data review within the scientific information system to establish a concise, rapid, facile, and consistent approach to reviewing HRMS data.

EXPERIMENTAL

LC conditions

LC system:	ACQUITY UPLC I-Class with the PFC Analysis Kit
Column:	ACQUITY UPLC BEH C_{18}, 1.7 µm 2.1 x 50 mm
Column temp.:	55 °C
Mobile phase A:	98:2 Water: MeOH 2 mM ammonium acetate
Mobile phase B:	MeOH 2 mM ammonium acetate

Gradient:

Min.	Flow rate (mL/min.)	%A	%B
Initial	0.65	90	10
0.5	0.65	90	10
5.1	0.65	0	100
6.6	0.65	0	100
6.7	0.65	90	10
8.5	0.65	90	10

MS conditions

MS system:	Xevo G2-XS Q-Tof
Acquisition range:	50 to 1200 *m/z*
Ionization mode:	ESI-
Capillary voltage:	1.5 kV
Cone voltage:	15 V
Source temp.:	120 °C
Desolvation temp.:	550 °C
Cone gas flow:	50 L/hr
Desolvation gas flow:	1000 L/hr min.
Scan time:	0.2 s
Low collision energy:	6 V
High collision energy:	35 to 75 V
Lock mass:	Leucine enkephalin (554.2610)

Sample analysis and data processing

Solvent standards of the PFASs investigated were diluted in methanol from a mixed standard provided in Waters® PFC Analysis Kit (p/n 176001744). Mink liver samples were provided by a collaborator as extracts, per the methodology described in Kärrman et. al.[4] Mink liver samples were stored in vials prior to analysis, and were diluted 1:10 in methanol before injection onto the system.

In order to ensure that possible sources of PFASs contamination present within all LC analytical equipment would not bias the analysis, modifications to the UPLC® System were made. Using components from the PFC Analysis Kit, Teflon (a common source of PFAS contamination) solvent tubing was replaced with PEEK tubing provided in the kit. A 50-mm ACQUITY UPLC BEH C_{18} Isolator Column was also placed after the solvent mixing mechanism, and prior to the sample manager, to retain any residual PFASs that were present in the system or solvents. More information regarding the PFC Analysis Kit can be found in Waters Application Note no. 720002813en.[5] Figure 2 shows the placement of the BEH C_{18} Isolator Column.

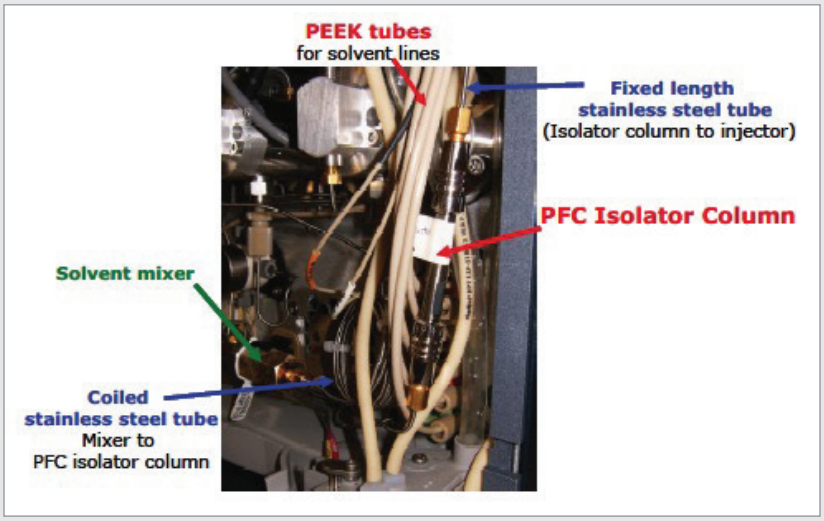

Figure 2. The PFC Analysis Kit components incorporate an isolator column to remove any PFASs introduced from the lab or system components containing PTFE. Also, PEEK tubing is included to replace Teflon tubing that contribute to PFASs background in LC-MS analyses.[5]

Standards and samples were analyzed using the ACQUITY UPLC I-Class System coupled to the Xevo G2-XS Q-Tof Mass Spectrometer. A non-targeted, data independent analysis, (MS^E)[1] was collected and processed in the UNIFI Scientific Information System.

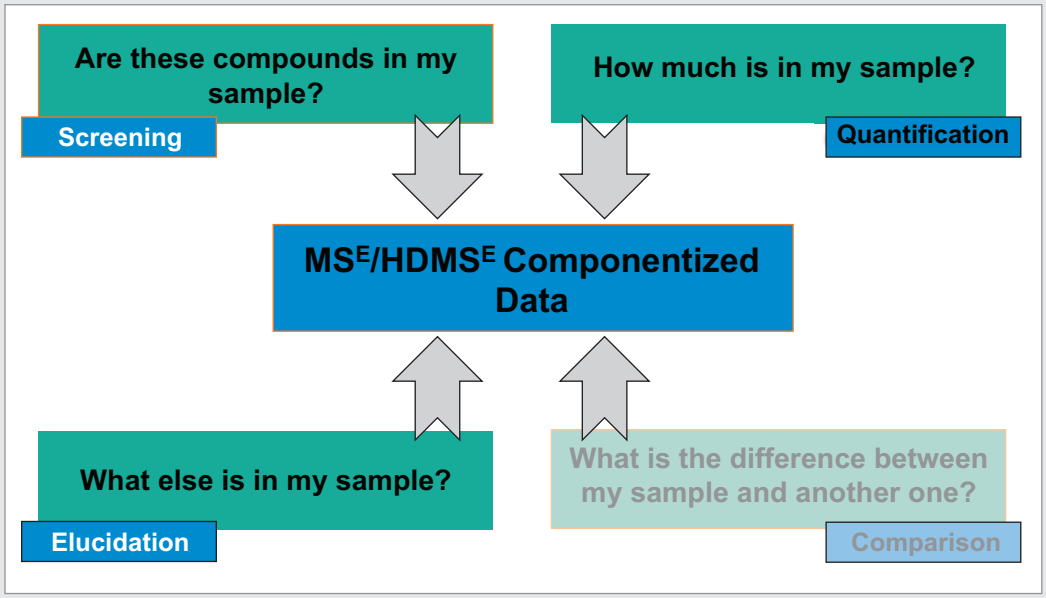

Figure 3. This analysis focuses on the qualitative and quantitative screening capabilities of Waters' Pesticide Screening Application Solution (PSAS), attempting to answer the highlighted questions for the fundamental screening questions in Figure 3.

The aim of these case studies is to show how a user can get from injection of a sample to submission of an accurate report in a quick, efficient, systematic, and reproducible way using the workflows, views filters, and software tools in UNIFI.[6]

Here, the component list provided a flexible and information rich approach to making identifications in the analyzed samples. The component list displayed in Figure 4 contains detection results such as retention time and theoretical accurate mass product ions. It can be updated at any time following data acquisition, thus affording historical data review for emerging compounds that become of interest following an original analysis. UNIFI's Scientific Library functionality makes this informative updating of screening lists possible, and is described later in this application note.

Manage Components

Create Import Paste Results Delete Edit Fragments... Edit Adducts... Add To Common Fragments Add To Neutral Losses

	Component name	Expected RT (min)	Expected neutral mass (Da)	Expected fragment (m/z)	Excluded	Formula
1	Perfluorotetradecanoic acid	4.68	713.9545	668.9574, 127.0012, 168.9894, 218.9862		C14HF27O2
2	Perfluorotridecanoic acid	4.57	663.9577	618.9606, 168.9894, 218.9855, 118.9926		C13HF25O2
3	Perfluorododecanoic acid	4.43	613.9609	568.9638, 168.9894, 118.9926		C12HF23O2
4	Perfluorodecanesulfonic acid	4.28	599.9311	79.9574, 229.9478, 279.9446, 168.9894		C10HF21O3S
5	Perfluoroundecanoic acid	4.27	563.9641	518.9670, 168.9894, 118.9926		C11HF21O2
6	Perfluorodecanoic acid	4.09	513.9673	568.9638, 168.9894, 118.9926		C10HF19O2
7	Perfluorooctanesulfonic acid	3.89	499.9375	79.9574, 168.9894, 129.9535, 197.9779		C8HF17O3S
8	C13PFOS	3.89	503.9509			12C4(13C) 4HF17O3S
9	Perfluorononanoic acid	3.86	463.9705	418.9734, 168.9894, 155.9840, 118.9926, 218.9881, 203.9652		C9HF17O2
10	Perfluoroheptanesulfonic acid	3.64	449.9407	79.9574, 368.9766, 168.9894, 118.9926, 129.9542		C7HF15O3S
11	Perfluorooctanoic acid	3.60	413.9737	368.9766, 168.9894,		C8HF15O2

Tips for defining expected components

Tips for using internal standards

Figure 4. Targeted component list imported from the UNIFI Scientific Library.

RESULTS AND DISCUSSION

For the 11 carboxylic and sulfonic acid PFASs, instrumental performance with respect to determination of limits of detection (LOD; peak-to-peak S/N 1:3), quantification (LOQ; peak-to-peak S/N 1:10), and linear dynamic range were carried out with solvent standards that are listed in Table 1. Theoretical exact mass product ion information was also obtained, where at least two ions were observed for each compound.

As a result of full spectral acquisition, the entirety of the fragmentation pathways of the analytes of interest is obtained, as is shown in Figure 5. Chromatographic separation of the PFASs standards analyzed is shown in Figure 6. Peaks were approximately 6 s in width at the base, and between 12 to 15 points across the peak. This was maintained for both low and high energy data, due to the sufficient scan speeds of the Xevo G2-XS Q-Tof instrument.

Compound	LOD (ng/mL)	LOQ (ng/mL)	Correlation Coefficient (R^2)	Range (ng/mL)	RT (min)	Formula	Fragment Ion 1	Fragment Ion 2	Fragment Ion 3	Fragment Ion 4	Fragment Ion 5	Fragment Ion 6
Perfluorobutanesulfonic acid (PFBS)	0.05	0.25	0.999	0.25–25	2.36	$C_4HF_9O_3S$	79.9574	98.9885				
Perfluorohexanesulfonic acid (PFHxS)	0.01	0.05	0.996	0.05–25	3.34	$C_6HF_{13}O_3S$	79.9574	98.9558	118.9926			
Perfluoroheptanesulfonic acid (PFHpS)	0.01	0.05	0.999	0.05–10	3.64	$C_7HF_{15}O_3S$	79.9574	98.9558	368.9766	168.9894	118.9926	129.9542
Perfluorononanoic acid (PFNA)	0.25	0.5	0.993	0.5–25	3.86	$C_9HF_{17}O_2$	418.9734	168.9894	155.984	118.9926	218.9881	203.9652
Perfluorooctanesulfonic acid (PFOS)	0.01	0.05	0.999	0.05–10	3.89	$C_8HF_{17}O_3S$	79.9574	98.9558	168.9894	129.9535	197.9779	
Perfluorodecanoic acid (PFDA)	0.1	0.5	0.998	0.5–25	4.09	$C_{10}HF_{19}O_2$	468.9702	168.9894	218.9588	268.9823		
Perfluoroundecanoic acid (PFUA)	0.1	0.25	0.995	0.25-25	4.27	$C_{11}HF_{19}O_2$	518.967	168.9894	118.9926			
Perfluorodecanesulfonic acid (PFDS)	0.01	0.05	0.998	0.05-10	4.28	$C_{10}HF_{21}O_3S$	79.9574	98.9885	229.9478	279.9446	168.9894	
Perfluorododecanoic acid (PFDoA)	0.1	0.25	0.997	0.25-25	4.43	$C_{12}HF_{23}O_2$	568.9638	168.9894	118.9926			
Perfluorotridecanoic acid (PFTriA)	0.1	0.25	0.995	0.25-25	4.57	$C_{13}HF_{25}O_2$	618.9606	168.9894	218.9588	118.9926		
Perfluorotetradecanoic acid (PFTeA)	0.1	0.25	0.997	0.25-25	4.68	$C_{14}HF_{27}O_2$	668.9574	127.0012	168.9894	218.9862		

Table 1. LODs, LOQs, linearity and linear dynamic range for 11 PFASs with sulfonic acids formulae (green) and carboxylic acids (orange). High energy product ions obtained from solvent standards are also described for each PFAS.

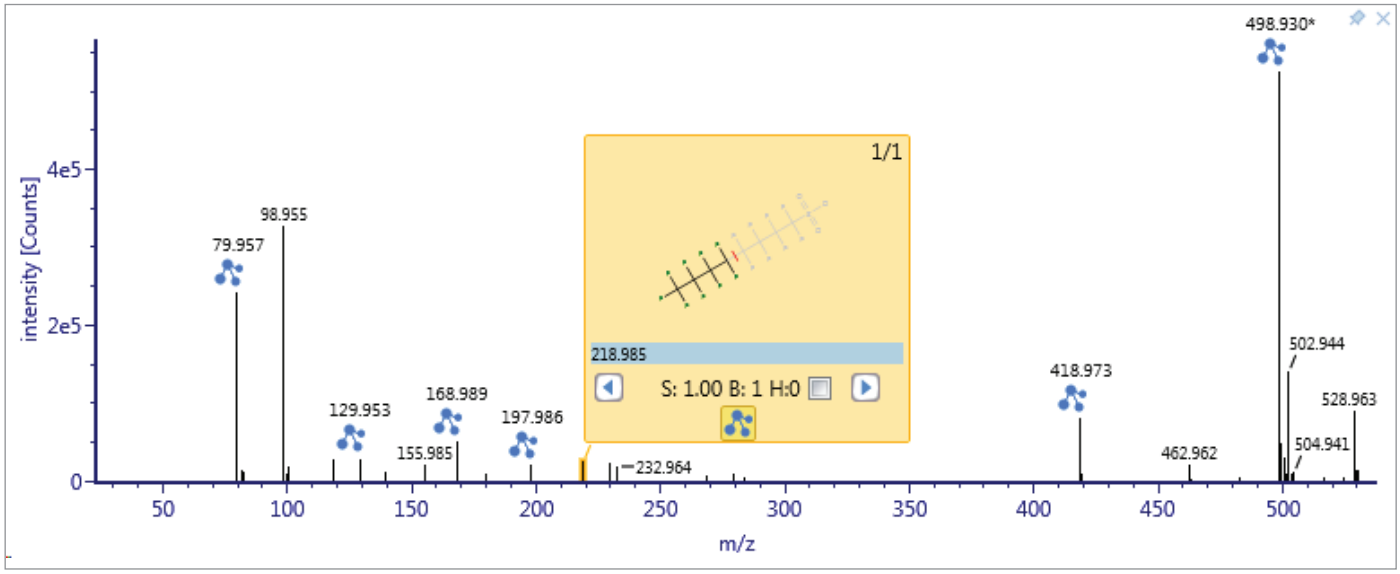

Figure 5. The determination of product ions was obtained through the use of automated Fragment Match in UNIFI which dissects the parent molecule structure provided in the processing method with chemical intelligence and matches potential elevated energy accurate mass spectral peaks. The elevated energy data for PFOS is shown. Blue molecule symbols indicate a proposed structure with the inset showing an example product ion.

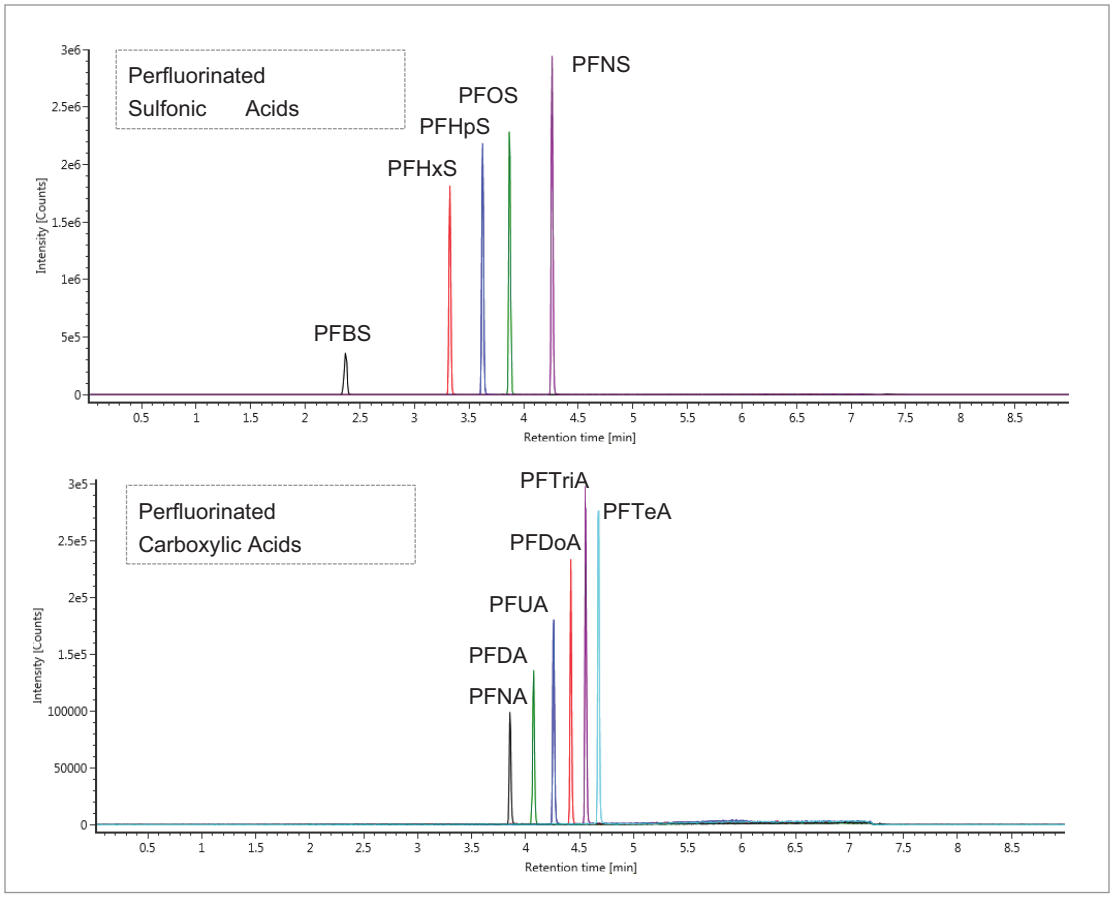

Figure 6. Extracted ion chromatograms (XICs) of sulfonic (top) and carboxylic (bottom) perfluorinated acids.

Diluted mink liver extracts contained various PFASs including PFHpS, PFHxS, PFBS, PFOS, PFDS, and PFNA that were detected at levels ranging from 0.2 to 0.8 ng/mL (without dilution and sample mass correction). In addition to isolating the PFASs of interest, the extraction method also resulted in the co-extraction of the bile acid taurodeoxcyholate (TDCA), which co-elutes chromatographically with PFOS. This can result in a high intensity peak in the analysis. However using exact mass measurements afforded by QTof MS, TDCA can be easily distinguished from PFOS (Figure 7).

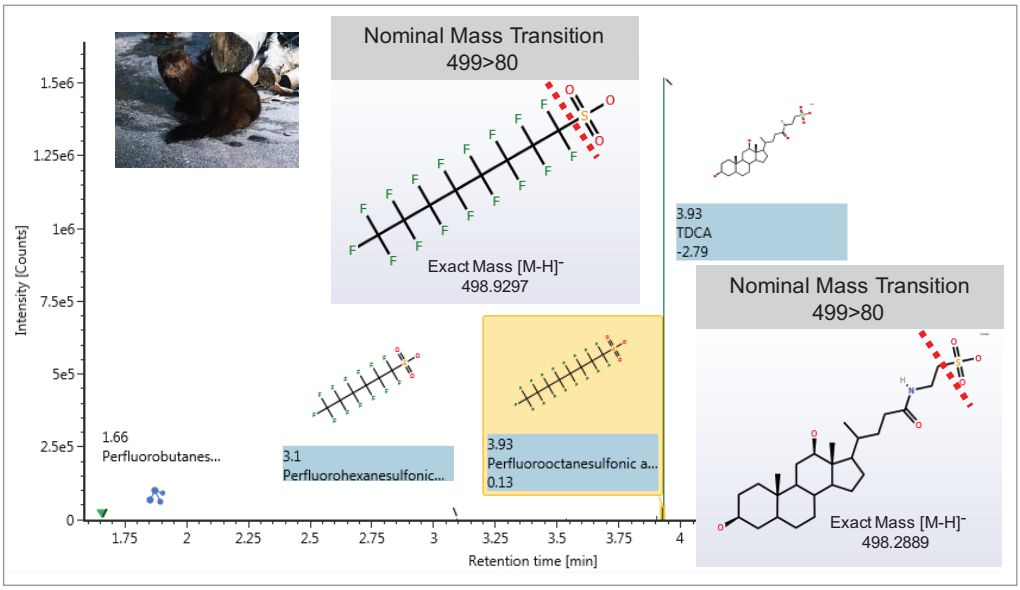

Figure 7. PFOS identification in mink liver samples chromatographically co-elutes with the bile acid, TDCA, in addition to sharing the loss of the –SO3. Although this is problematic for tandem quadrupole analyses which utilize nominal mass differentiation, the use of exact mass measurements shows a mass difference of 0.6 Da and is easily mass resolved using Tof MS.

CONCLUSIONS

The Xevo G2-XS Q-Tof is a highly sensitive high resolution mass spectrometer able to detect carboxylic and sulfonic PFASs at sub-ppb levels with exact mass measurements for both precursor and product ions.

Exact mass fragment information obtained in the same analysis for any analyte of interest can easily be compared with the parent structure to provide specific and comprehensive structural information.

Data independent full spectral acquisition allows the user to see various unexpected or unintended aspects of a sample, as well as the ability to perform historical data review.

ACKNOWLEDGEMENTS

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References

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APPENDIX: UNIFI WORKFLOW STEP SPOTLIGHT: QUANTIFICATION

Following qualification of PFASs in the mink liver samples, quantification was performed for all identified analytes present above the LOQs and within the linear dynamic range. A user created workflow step (Figure 8) was created to show relevant displays such as a calibration curve (which includes the R^2 value, slope calculation, and weighting used, as well as %RSD value), calculated concentration, identified and expected high energy product ions, and an extracted ion chromatogram for the identified analyte – all with a single mouse click. The workflow step is applied to the samples only, which is also user defined when designing the workflow step. Figure 9 shows these results for PFOS in mink liver.

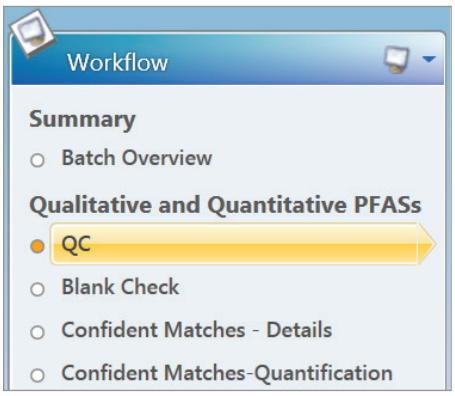


Figure 8. User-created workflow steps for this analysis. The quantification step (step 4) specifies the sample type viewed, and the information for that sample step such as linearity (R^2) of calibration curve, calculated concentration in the sample, and product ions found in the high energy spectrum for the sample. Workflow steps are a streamlined and efficient way of reviewing HRMS data.

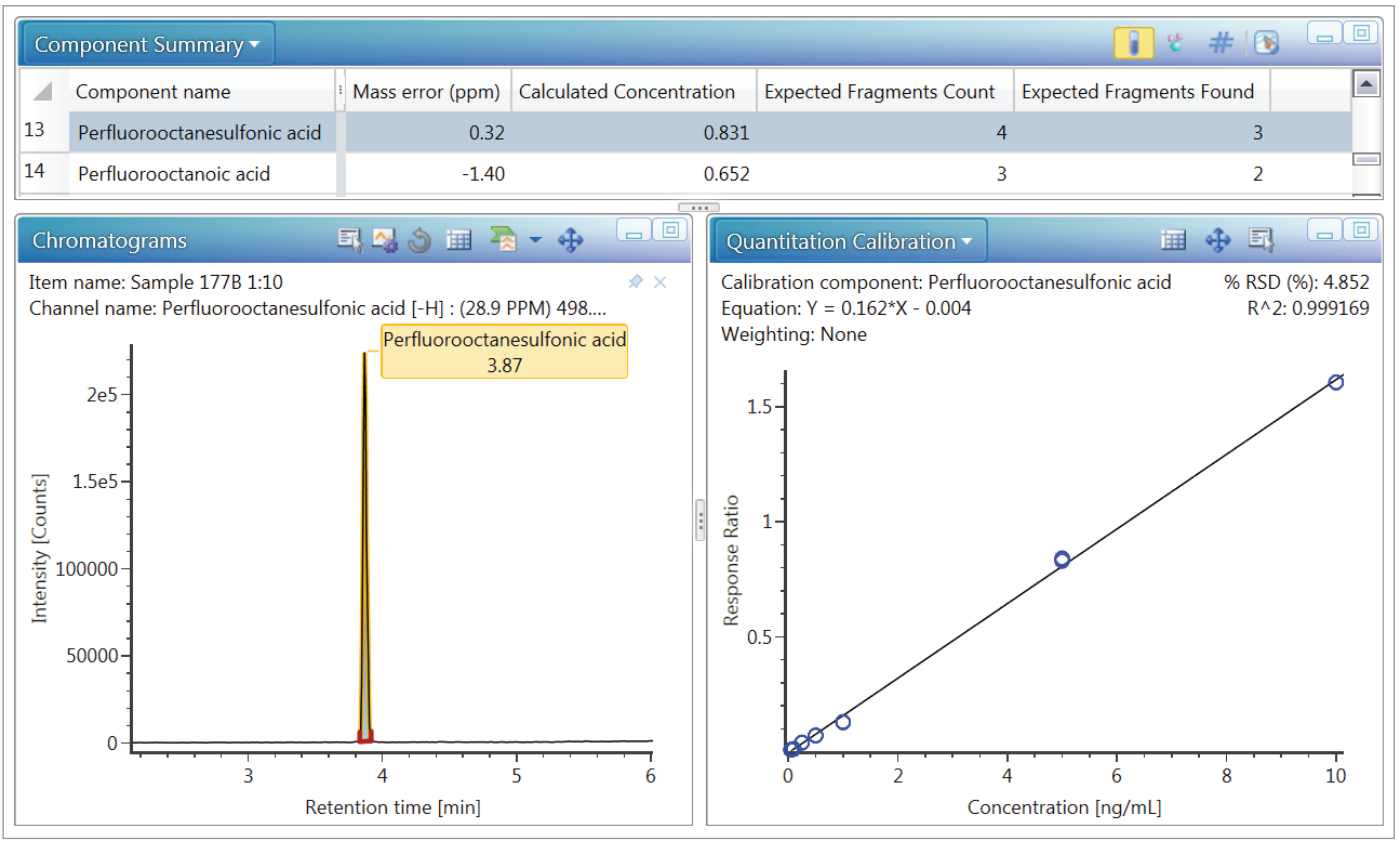


Figure 9. PFOS Identification in mink liver sample, as well as the calibration curve for the solvent standards.

Internal standards (^{13}CPFOS and ^{13}CPFOA) were used to correct for any variations in injection or standard conditions. Their application to the quantitative analysis are input in the analysis method target list, and coupled to the analytes through a drop down menu. This is shown in Figure 10. This information is input prior to injection, such that all quantitative and qualitative analyses performed occur during the live injection time, within UNIFI Software.

Component name	Expected RT (min)	Expected fragment (m/z)	Formula	Internal standard?	Use internal standard
Perfluorooctanesulfonic acid	3.89	79.9574, 168.9894, 129.9535, 197.9779	C8HF17O3S	☐	C13PFOS
C13PFOS	3.89		12C4(13C)4HF17O3S	☑	

Figure 10. Component tab, containing targeted compounds (in this example, PFOS) imported from the Scientific Library, and the application of internal standards.

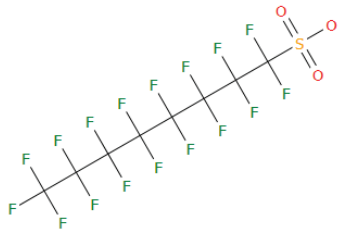
SOFTWARE SPOTLIGHT: UNIFI SCIENTIFIC LIBRARIES

Componentization of the data[7] allows users to interrogate a sample injection for target compounds and non-targeted (unknown) candidate masses of interest in the same dataset, without the need to reprocess the raw data. Information included in the target list comes from the scientific library functionality included in the UNIFI Scientific Information System. Libraries are user created and contain compound information such as structure, retention times and exact mass product ions, as well as capacity to store spectra and documentation about the compound. Figure 11 shows a library entry for PFOS, with detection results obtained from a standard injection.

Perfluorooctanesulfonic acid [PFC QC Library]

Tools

Property	Value
Item type	Compound
Item description	PFOS
IUPAC name	Perfluorooctanesulfonic acid
Formula	C8HF17O3S
Hill formula	C8HF17O3S
Average molar mass	500.1296



Detection results

Add Edit Delete

Priority	Neutral Mass (Da)	Adduct	Fragmentation type	Expected m/z	Expected RT (min)	Ionization technique	Detail type
Detection result:	Instrument model: Unknown, Instrument serial no: , Manually created, Created by administrator on Dec 17, 2015 Imported from Excel						(5 items)
1	498.9302	-H	Unknown	498.9302	3.890	ESI-	MSe
2			CID	79.9574	3.890	ESI-	MSe
3			CID	168.9894	3.890	ESI-	MSe
4			CID	129.9535	3.890	ESI-	MSe
5			CID	197.9779	3.890	ESI-	MSe

Figure 11. UNIFI Scientific Library entry for PFOS. Structure information, as well as detection results are entered here and used to make positive identifications in the analyzed sample. Detection results were obtained from running a solvent standard at various concentrations and determining product ions using Fragment Match tool within UNIFI Software.

In the case of emerging PFASs, or any other newly identified contaminants for which analytical standards may not be readily available, the use of compound structure can be used as a means to make tentative identifications. Because both low and high energy full spectral acquisition is achieved, UNIFI's MassFragment™ tool will search not only for the precursor mass but also match proposed losses from the structure to masses observed in the high energy spectrum.

The UNIFI Scientific Libraries and target lists within an analysis method can be updated anytime, and the analysis re-interrogated to continually increase the numbers of compounds. This particular functionality is most useful in cases where the compounds are not yet found in online chemical databases, and users must generate their own structural information. Additionally, the Scientific Library functionality provides useful storage tools for experimental and compound information. Figure 12 shows a stored spectrum for PFOS, which can be used in future analyses to add confidence to a compound match.

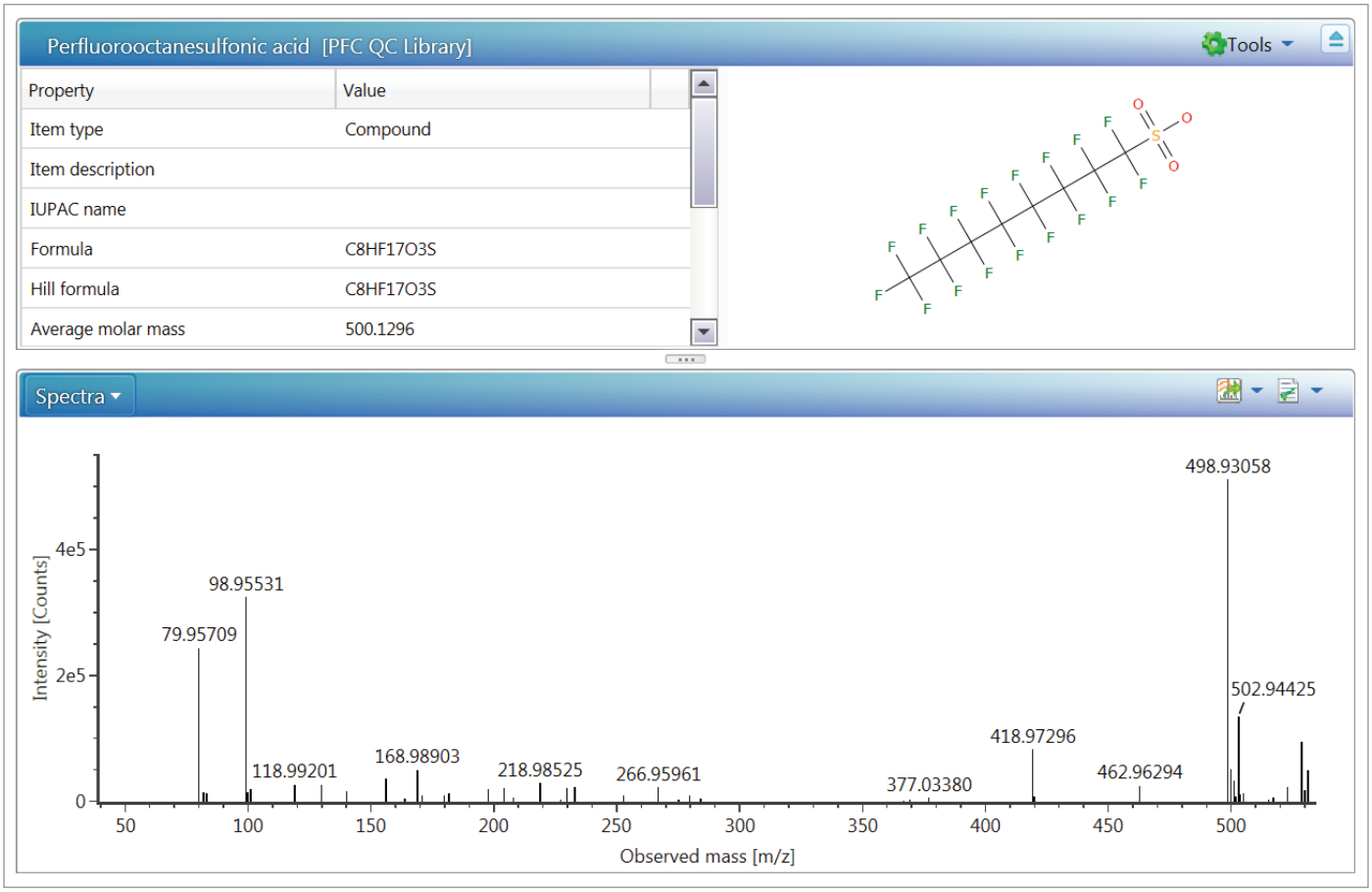


Figure 12. Spectrum from the high-energy channel and componentized data for PFOS, in a 25 ng/mL level standard. This spectrum is stored in the UNIFI Scientific Library (author named "PFC QC Library"), and contains the product ions and their abundance observed for PFOS.

Data processing using UNIFI's Scientific Library functions greatly enhances the ability to rapidly identify expected as well as emerging compounds of interest. Historical data review is a powerful approach in particular for environmental and food analysis. It is made possible by full spectral acquisition and exact mass measurements of low and high collision energy data.