Simultaneous Quantification and Screening of Per- and Polyfluoroalkyl Substances using High Resolution Mass Spectrometry

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Introduction

Per and polyfluoroalkyl substances (PFAS) are chemicals widely used in consumer products and aqueous film-forming foams (AFFF) due to their unique and desirable chemical properties. Widespread usage and environmental persistence have made legacy PFAS ubiquitous in the environment and emerging fluoro-chemicals are being identified frequently. Traditional techniques utilizing LC-MS/MS provide sensitive quantification for targeted analyte lists but do not allow for screening of emerging PFAS compounds. We will present a workflow utilizing high-resolution mass spectrometry (HR-MS) that provides high-quality data suitable for quantification and screening through a single injection. Acquired data could also be screened retrospectively for new and emerging compounds.

Databases were built with and without spectra. The larger screening database was based on a wider list of PFAS compounds, while the smaller targeted database included MSMS spectra. Sample matrix was two diluted aqueous film-forming foam formulations (AFFF) to simulate impacted groundwater. Thirty –four compounds were targeted for quantification, while screening encompassed a larger list (54 compounds), but less confident identifications were made.

Both formulations were separately analyzed with Fluoromatch Flow (v2.431)[1]. Fluoromatch is a free software that uses accurate mass, MSMS fragmentation data, presence of homologous series, etc. to provide tentative PFAS identifications with a confidence score.

Tentative screening identifications were compared with Fluoromatch data to increase the level of confidence.

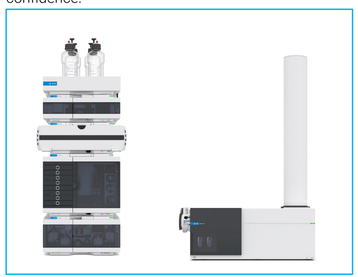

Figure 1: 1290 Infinity II UHPLC– 6546 LC/Q-TOF

Experimental

Samples

Aqueous film forming foam (AFFF) is an effective fire suppressant for petroleum-based fires. Foams are primarily composed of complex mixtures of per- and polyfluorinated substances (PFAS), but the exact composition is protected business information.

Two types of AFFF were tested. Formulation 1 (F1) is a legacy product, formulation 2 (F2) is more recent. F1 and F2 were diluted 20,000 x in 70:30 water : methanol and a labeled internal standard containing 23 labeled compounds was added at 10 ppb. Eight calibration standards were prepared in 70:30 water : methanol in the range of 0.05 ppb – 25 ppb.

LC/Q-TOF Method

Ten microliter injections were separated on a Poroshell EC –C18 column, 2.1 x 100 mm, 2.7 um with an acetonitrile and 2 mM Ammonium acetate gradient. Data was acquired in "all ions" acquisition mode meaning that 3 collision energies were collected sequentially. All ions is a data independent acquisition strategy that allows more confident identification by matching fragments with molecular ions through software. Acquisition Parameters are shown in Table 1.

Table 1. Q-TOF Acquisition Parameters

Parameter	Value
Ionization Mode	Negative
Mass range	40 -1000 m/z
Acquisition Rate	5 spectra/sec
Collision Energies	0, 10, 40 eV
Reference Masses	119.0363, 980.0164

Results and Discussion

Workflow

Data was analyzed in MassHunter Quantification using the Compound Screener Feature. The workflow is summarized in Figure 2. Two separate personal compound database libraries (PCDLs) were created. The quantification database contained retention times (RT), MSMS spectra and internal standards. The screening database contained compound name and accurate mass.

Compound Screener allows you to setup separate criteria for a compound match based on whether MSMS spectra are available, and RT is known. When matching fragments with molecular ion a coelution score is set to ensure DIA fragments are matched correctly. When importing from the PCDL fragments can be imported and designated as qualifiers. In the targeted portion of this study, 2 ions were required for a match: the molecular ion and one fragment with a coelution score of 80/100. RT was required to match database by 0.1 minutes and mass accuracy of 5 ppm was required.

For screening, RT was evaluated for the entire run and set to "greatest response". Only the molecular ion was required as spectra were not available. Mass accuracy was narrowed to 3 ppm. F or a select screening compounds that have predictable fragmentation such as carboxylic acids or sulfonic acids qualifying fragments were added.

The Compound Screener Interface is shown in Figure 3. On the left compounds are marked with red, green or orange depending on the confidence of the match based on set criteria. Chromatographic peaks and spectra are presented for easy review.

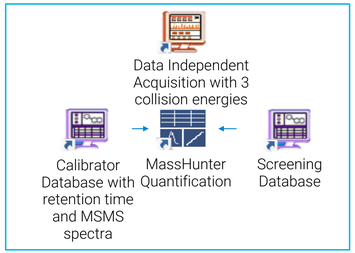

Figure 2. Workflow Diagram

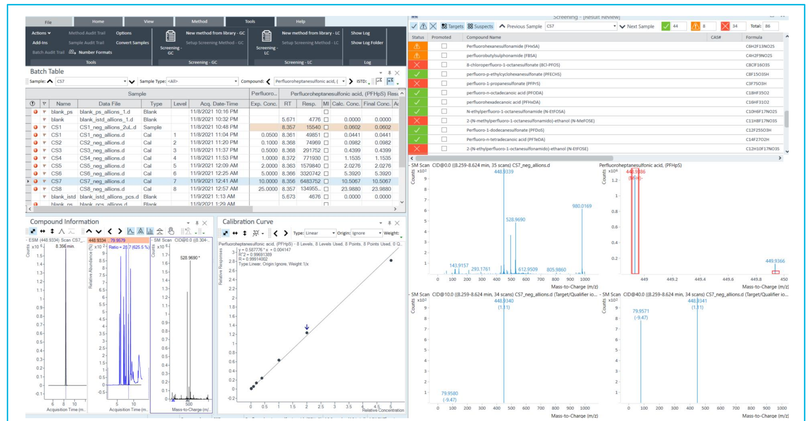

Figure 3. Compound Screener Interface

Results and Discussion

Quantification Results

Quantification for both formulations is shown in Table 2. F1 is the legacy formulations while F2 is the more recent. All concentrations have been corrected for dilution and are shown in ug/mL. Mass accuracy is also in ppm.

Table 2. Quantification Results.

Compound Name	Formula	F1	Mass Accuracy	F2	Mass Accuracy
PFBA	C4HF7O2	16.46	1.56		
PFPeA	C5HF9O2	14.91	2.22	< 1 ug/mL	1.14
4:2 FTS	C6H5F9O3S	< 1 ug/mL	-2.80		
PFHxA	C6HF11O2	45.49	2.31	46.12	0.65
PFBS	C4HF9O3S	90.65	2.30	< 1 ug/mL	2.95
PFHpA	C7HF13O2	14.55	1.43	< 1 ug/mL	-0.08
PFPeS	C5F11O3SH	85.44	2.07		
6:2 FTS	C8H5F13O3S	21.56	1.40	27.92	2.13
PFOA	C8F15O2H	34.39	1.25	24.99	1.55
PFHxS	C6HF13O3S	> 500 ug/mL	3.73	< 1 ug/mL	2.63
PFHpS	C7HF15O3S	108.25	1.78		
8:2 FTS	C10H5F17O3S	7.71	0.27	5.31	-1.71
PFDA	C10HF19O2	< 1 ug/mL	0.96	< 1 ug/mL	2.38
PFOS	C8F17O3SH	> 500 ug/mL	2.95		
PFOSA	C8H2F17NO2S	< 1 ug/mL	-2.68		

High concentrations in the legacy formulation exceed the calibration range and are indicated by > 500 ug/mL. Compounds listed with < 1 ug/mL appear to be present compared to blanks but are below the calibration range. Results are consistent with what is known about the legacy AFFF formulations with high concentrations of PFOS and PFHxS.

Screening Results

After review 6 high quality suspects were identified for F1 and are listed in Table 3. All had excellent mass accuracy and reasonable retention times. Known fragments were added for confirmation where applicable. For example, PFPrS as a sulfonic acid it is reasonable to assume that it might have the characteristic SO3- fragment (79.9574 m/z). Extraction of molecular ion and qualifier for PFPrS are shown in Figure 4. Without an analytical standard the RT and ratio between fragment and molecular ion are unknown, however its presence increases confidence that a sulfonic acid is present in the molecular structure.

Table 3. Screening Results from F1.

Name	Formula	Mass Accuracy
N-(methyl)nonafluorobutanesulfonamide (N-MeFBSA)	C5H4F9NO2S	-1.18
Perfluoro-1-decanesulfonamide (FDSA)	C10H2F21NO2S	-0.75
Perfluorohexanesulfonamide (FHxSA)	C6H2F13NO2S	0.55
perfluorobutylsulphonamide (FBSA)	C4H2F9NO2S	0.39
perfluoro-p-ethylcyclohexanesulfonate (PFECHS)	C8F15O3SH	0.92
perfluoro-1-propanesulfonate (PFPrS)	C3F7O3H	1.98

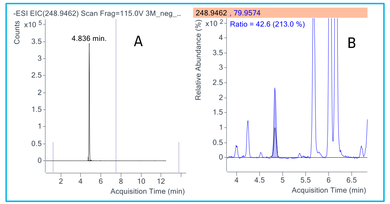

Figure 4. PFPrS Extraction Ion Chromatogram (EIC) for molecular ion (A) and overlay with EIC of SO3- fragment (B).

Comparison with Fluoromatch

Fluoromatch showed high confidence annotations in the 3 bolded formulas in Table 3 making the presence of these formulas highly likely. However, more work would be required to associate a molecular structure and name with high confidence.

Conclusions

- One legacy (F1) and one new formulation (F2) of diluted AFFF were analyzed with DIA on the 6546 QTOF.
- Two custom PFAS database were utilized. One contain spectra and RT, while the other was a broader screening list with no spectra. Data was simultaneously quantified and screened using the compound screener feature in MH Quantification software.
- Quantification results of F1 consistent with what is known about the legacy AFFF formulations with high concentrations of PFOS and PFHxS
- Six high quality suspects were identified and presumptive qualifiers added. Suspect formulas were compared to results from Fluoromatch Flow. Three formulas had highly confident annotations in Fluoromatch.
- More work would be required to associate a molecular structure and name with high confidence.

References

[1]Fluoromatch [computer software]. (2021). Retrieved from http://innovativeomics.com/software/fluoromatch-flow-covers-entire-pfas-workflow/

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