PFAS Analysis in Aqueous Film-Forming Foams (AFFF) Using a High-Resolution Mass Spectrometer and Newly Integrated Software Suite

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1. Introduction

Aqueous Film-Forming Foams (AFFF) are specialized firefighting agents that are engineered to rapidly extinguish flammable liquid fires by creating a barrier that cools the fire and suppresses vapor release. They are widely used in high-risk environments such as airports, military bases, oil refineries, and chemical processing facilities, where quick and effective fire suppression is critical. However, the widespread use of AFFF has raised environmental and health concerns due to the presence of per- and polyfluoroalkyl substances (PFAS), a group of chemicals known for their persistence and potential health risks.

Early formulations of AFFF, developed in the 1960s and 1970s, are often referred to as “Legacy PFOS-based AFFF.” These foams primarily utilized long-chain PFAS such as perfluorooctanesulfonic acid (PFOS) and perfluorohexanesulfonic acid (PFHxS) in various ratios, valued for their exceptional film-forming and firefighting properties. By the 1970s, new formulations emerged under the classification of “Legacy fluorotelomer-based AFFF,” which incorporated a mixture of long-chain fluorotelomers alongside compounds including 6:2 fluorotelomer sulfonate (6:2 FTS), 8:2 fluorotelomer sulfonate (8:2 FTS), and 6:2 fluorotelomer sulfonamide betaine (6:2 FTAB). These blends further optimized foam performance but continued to rely on environmentally persistent chemicals. By the 2010s, responding to growing health and environmental concerns, manufacturers transitioned to "modern fluorotelomer AFFF" formulations, which employed shorter-chain PFAS such as perfluorobutane sulfonate (PFBS), 6:2 FTS, and 4:2 FTS. These shorter-chain compounds are less prone to bioaccumulation in living organisms but remain highly persistent in the environment, continuing to raise concerns about long-term ecological impacts and regulatory challenges.[1]

This application note demonstrates the use of the Shimadzu LCMS-9030 Quadrupole Time-of-Flight (QTOF) mass spectrometer for untargeted analysis of AFFF (**Fig. 1**). This high-resolution analytical technique provides a detailed chemical profile, enabling the comprehensive characterization of PFAS compounds found in AFFF across two generations. Integration of FluoroMatch software into the workflow allows for rapid identification of the generation of AFFF based on its distinct chemical composition, including the specific types of PFAS present.

Figure 1. Shimadzu LCMS-9030

2. Methods

First- and second-generation AFFF samples were prepared as 3% (v/v) aqueous solutions, consistent with concentrations used in firefighting applications. Each foam sample was then diluted by a factor of 5x, 25x, 100x, 250x, and 500x into a solution of 50:50 water/methanol containing 0.1% acetic acid (diluent). The sample processing was performed in a separate space using laboratories supplies that were not used for any routine PFAS sample processing to ensure there was not laboratory contamination from use of the AFFF gel concentrate, because it contains extremely high concentrations of PFAS compounds.

Diluent was run in triplicate first with the method conditions outlined in **Table 1**. The MS data was then processed with LabSolutions Insight Explore and the top intensity hits were selected to be included within the exclusion list for all the AFFF sample runs. A prior ion list was also constructed from previous work with 56 known PFAS and their matched retention time. LabSolutions LCMS was configured to automatically export each data file as an .mzML which is required for FluoroMatch processing.

Table 1. Analytical method conditions for AFFF Untargeted PFAS assay

[LC] Nexera	
Mobile Phase (LCMS Grade)	A: 2 mmol/L Ammonium Acetate in H_2O/ Acetonitrile = 95/5 B: Acetonitrile
Delay Column	Shimadzu Nexcol PFAS Delay 50 mm x 3.0 mm, 5 µm (P/N: 220-91394-09)
Analytical Column	Shim-pack Scepter C18-120 2.1 mm x 100 mm, 3 µm (P/N: 227-31014-05)
Gradient (%B)	10% (0.5 min) ⇒95% (25 min) ⇒95% (30 min) ⇒ 10% (30.1-35 min)
Column Oven Temp.	45 ℃
Flow rate	0.45 mL/min
Injection Volume	40 µL
Multiple draw injection program	Co-injection 20 µL Sample → 25 µL 0.1% Acetic acid in H_2O → Co-injection 20 µL Sample → 25 µL 0.1% Acetic acid in H_2O
Autosampler Rinsing	60/40 Acetonitrile/2-propanol, Before/After Aspiration 4 seconds
[MS] LCMS-9030	
Interface Temp.	170℃
Probe position	+1 mm
Nebulizer gas flow	3 L/min
Heating gas flow	15 L/min
Interface Voltage	-3 kV
DL Temp.	200 ℃
Heatblock Temp.	300 ℃
Drying gas flow	8 L/min
Ionization Mode	ESI (-) and ESI (+)
MS Acquisition Type	DDA, MS (110-1300m/z), MS/MS (40-1300m/z) 6 Dependent Events with Exclusion and Prior Ions Lists
Collision Energy	35 +/- 22

All required sample types were loaded into the FluoroMatch software (Flow version 5.4 was at the time of this analysis) and processed using the parameters summarized in **Table 2.**

Table 2. Processing parameters for FluoroMatch Flow

FluoroMatch Parameters	
Peak Picking Algorithm	Mzmine 2
MS/MS intensity threshold	25
Full-scan intensity threshold	200
Full-scan Peak Height Minimum	1500
Noise Level (MS1)	100
Full-Scan Mass Accuracy Tolerance	15 ppm/0.007 Da
MS/MS intensity threshold	25
m/z Search Window MS/MS	30 ppm

3. Results

Once FluoroMatch has processed the .mzML files the software outputs the results into a format that can be loaded into a customized PowerBI Visualizer to help review the complex set of identified features. An example of the comparison found at a feature level is shown in **Figure 2**. This figures shows the Kendrick mass defect plot of all the features found (showing only features that scored between A-C+). As you can tell immediately each generation of AFFF can be shown to contain over 3,000 features.

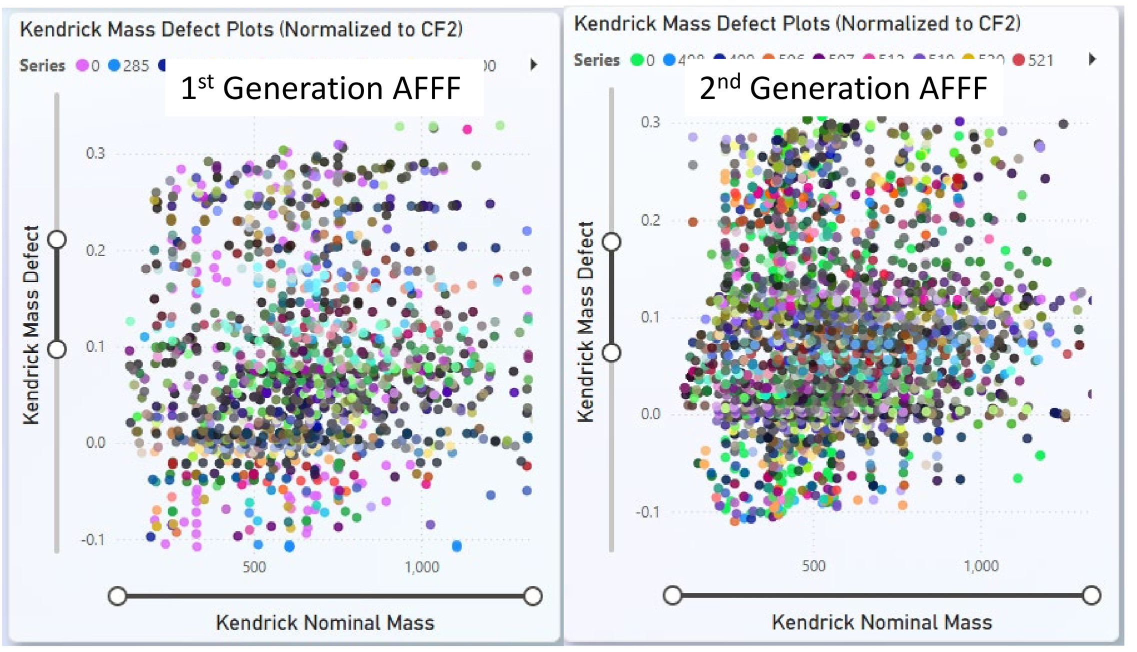

Figure 2. Kendrick mass plots of feature landscape for 1st and 2nd generation AFFF (plotting scores A-C+)

Deeper investigation into the feature landscape you will begin to see generalized trends of similar series of compounds appearing across similar mass defect and nominal mass range. Additionally, these results should also correlate in the retention time plot of their m/z ratio, shown in **Figure 3**. As can be seen in the figure C5-C16 fluorinated acids can be found in the 2nd generation AFFF whereas in the 1st generation only C8, C9, and C13 are found. It is important to note that the series results show follow a linear relationship as shown below, if there are outliers that is usually an indication that the found feature may be erroneous. Similar results can be seen for the fluorotelomer analytes in **Figure 4**.

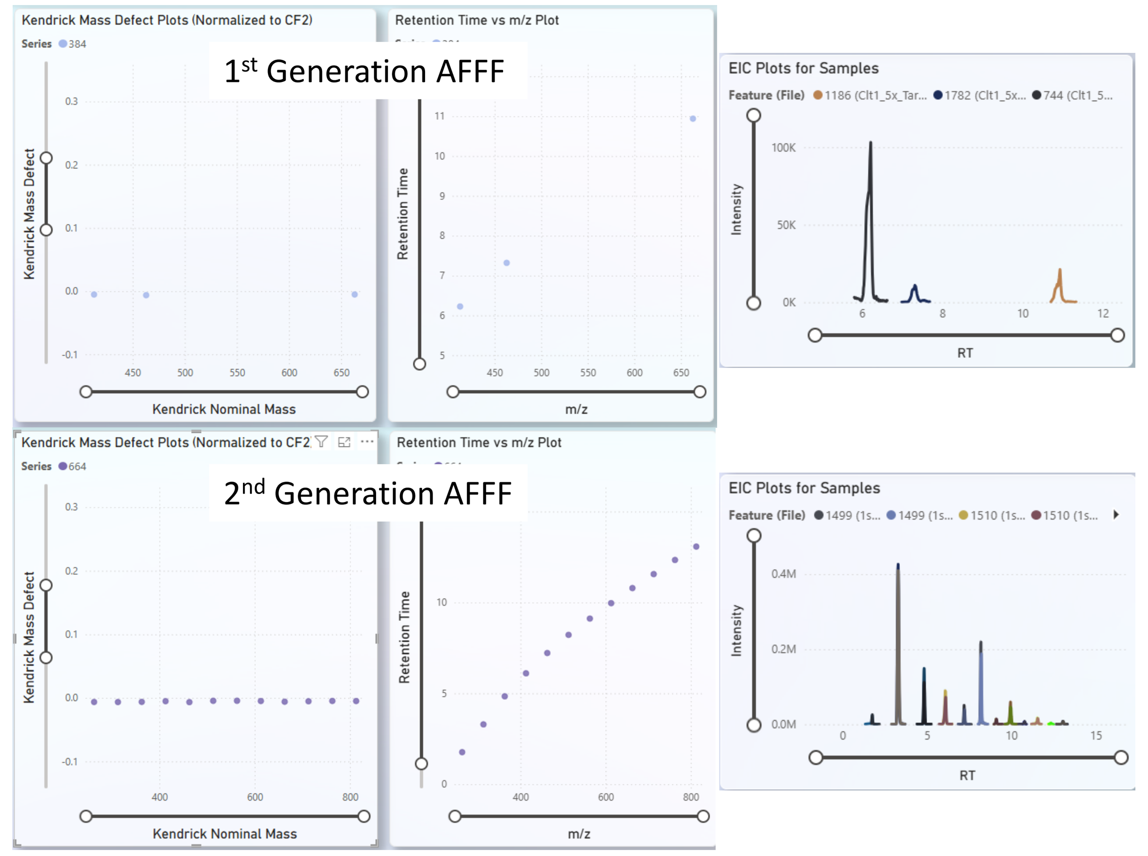

Figure 3. Comparison of 1st and 2nd generation AFFF acid series and the corresponding EIC sample plots.

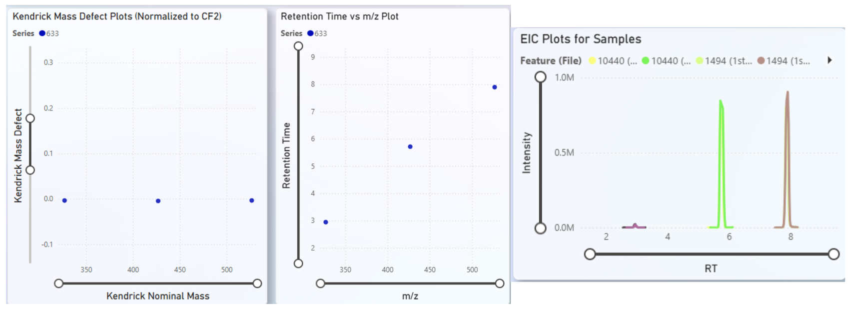

Figure 4. 2nd generation AFFF 4:2/6:2/8:2 FTS compounds found (characteristics of 2nd generation foams)

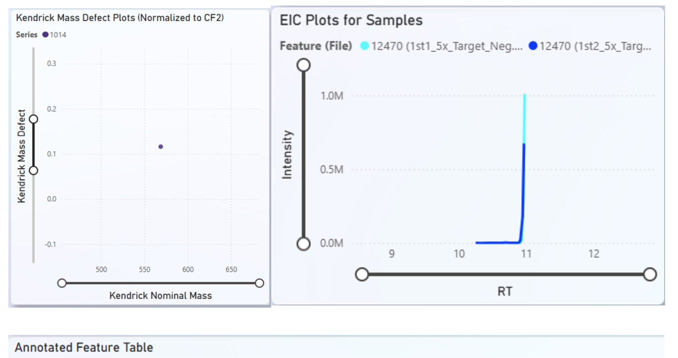

Figure 5. 2nd generation AFFF 6:2 FTAB (saturated chromatogram)

The FTS compounds were not found in the 1st generation AFFF at all. A unique compound found primarily in AFFF is 6:2 FTAB, a unique compound only found in the later generation material. 6:2 FTAB is unique compound because it is one of the zwitterionic PFAS compounds, we confirmed the compound was in the sample in both -/+ ESI. **Figure 5** highlights the 6:2 FTAB results found in the 2nd generation AFFF. The results from FluoroMatch were also confirmed through LabSolutions Insight Explore using the Assign function to identify and score the MS/MS fragments. A high Assign score of 88% indicated with high confidence that it was the compound found through FluoroMatch. This also highlighted the importance of sample dilution because an incomplete chromatogram was obtained in FluoroMatch for 6:2 FTAB because of the high concentration (saturation). **Figure 6** shows the structure and Assigned fragments found through LabSolutions Insight Explore software.

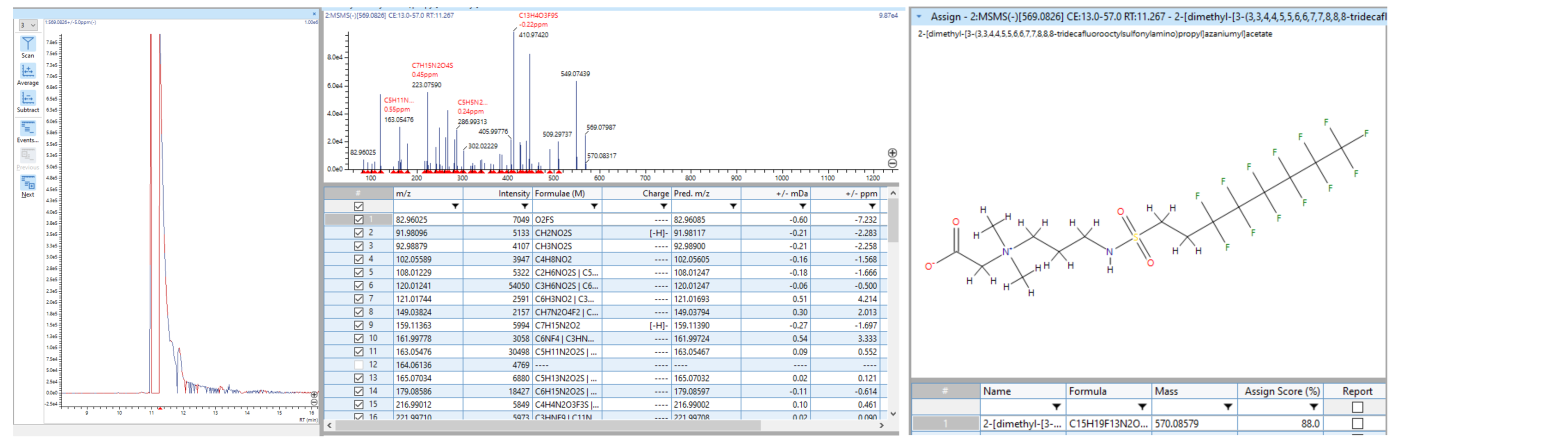

Figure 6. LabSolutions Insight Explore 6:2 FTAB chromatogram, Assign MS/MS results, and compound match from PubChem (Assign score 88%)

4. Conclusion

With the incorporation of Shimadzu .mzML file support into FluoroMatch untargeted QTOF PFAS analysis has gained an additional tool to help characterize typically very complex sample sets. This tool in addition to LabSolutions Insight Explore provide a means to fully characterize suspected PFAS containing samples. These tools allowed for the delineation of the AFFF samples based on the class of PFAS found in each sample set including carboxylic acids, FTS’s, and 6:2 FTAB. There are many other examples within the data sets that also highlight the same behavior amongst AFFF manufacturing.

Reference

1) pfas-1.itrcweb.org/3-firefighting-foams

2) Disclaimer: For Research Use Only. Not for use in diagnostic procedures.

Conflict of Interest Statement:

(2) authors are affiliated/funded by Shimadzu Corporation, (1) authors are affiliated/funded by RJ Lee Group