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Comparison of High-Resolution Data Dependent MSMS Strategies for Best Precursor Coverage of Aqueous Film Forming Foam Formulations

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Introduction

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.

High–resolution mass spectrometry (HR-MS) is an important tool in the characterization of AFFF. However, identification of PFAS using only the accurate mass and isotope pattern of the molecular ion is not robust as formulas do not provide structural information. Fragment ions from MSMS spectra can greatly improve identification confidence with software tools such as Fluoromatch or SIRIUS. Data dependent acquisition (DDA) is a common tool used to acquire MSMS spectra when the composition of the analyte is not well understood such as AFFF. In this study, three approaches based on DDA acquisition for the MSMS fragmentation of fluorinated compounds were optimized and compared.

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

Experimental

Study Design.

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. Ten microliter injections were separated on a Poroshell EC –C18 column, 2.1 x 100 mm with a methanol and 5 mM Ammonium formate gradient. Data was collected on the 6546 QTOF.

First, the DDA parameters for iterative MSMS with automatic exclusion were optimized. This technique generates precursor ion exclusion lists based on MSMS experiments already completed. The complete iterative MSMS experiment will inject a sample up to 5 times. Each one acquiring MSMS spectra from precursors of decreasing abundance. Second, a smart preferred list generator (contact authors for free application) with inputs of mass range and mass defect was applied to generate suspect molecular ions targeted for MSMS acquisition. Parameters were tailored for fluorinated compounds based upon the U.S. EPAs CompTox Chemistry Dashboard PFAS list[1]. Finally, the preferred list was combined with iterative exclusion to verify whether multiple injections were required to acquire all suspect ions. Results from each approach were evaluated and compared to the CompTox PFAS database and processed with Fluoromatch[2].

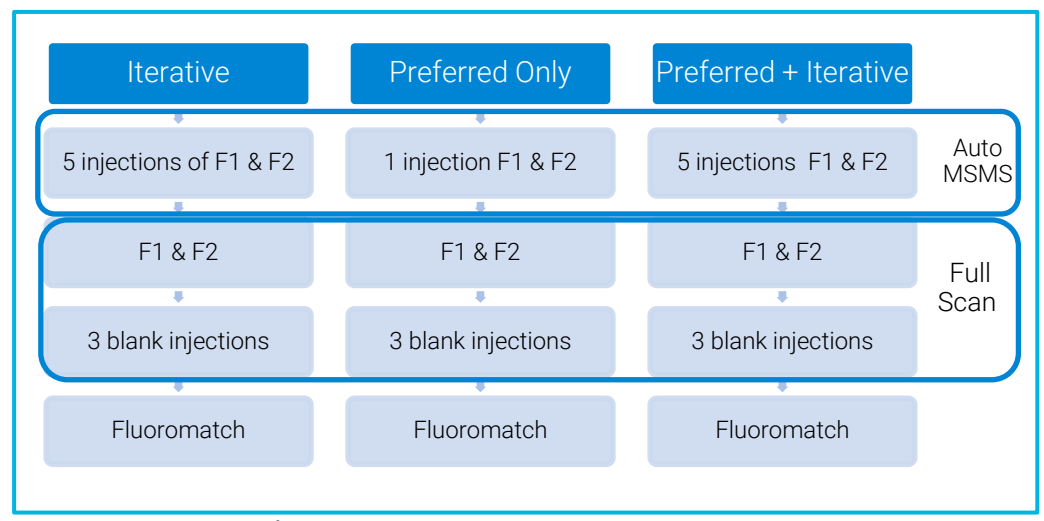

Figure 2: Study Design

Parameters were tailored for fluorinated compounds based upon the U.S. EPAs CompTox Chemistry Dashboard PFAS list[1]. Finally, the preferred list was combined with iterative exclusion to verify whether multiple injections were required to acquire all suspect ions. Results from each approach were evaluated and compared to the CompTox PFAS database[1] and processed with Fluoromatch[2].

Iterative Exclusion

Five replicate injections were collect using an iterative exclusion list. Parameters were optimized based on chromatographic peaks share and abundances.

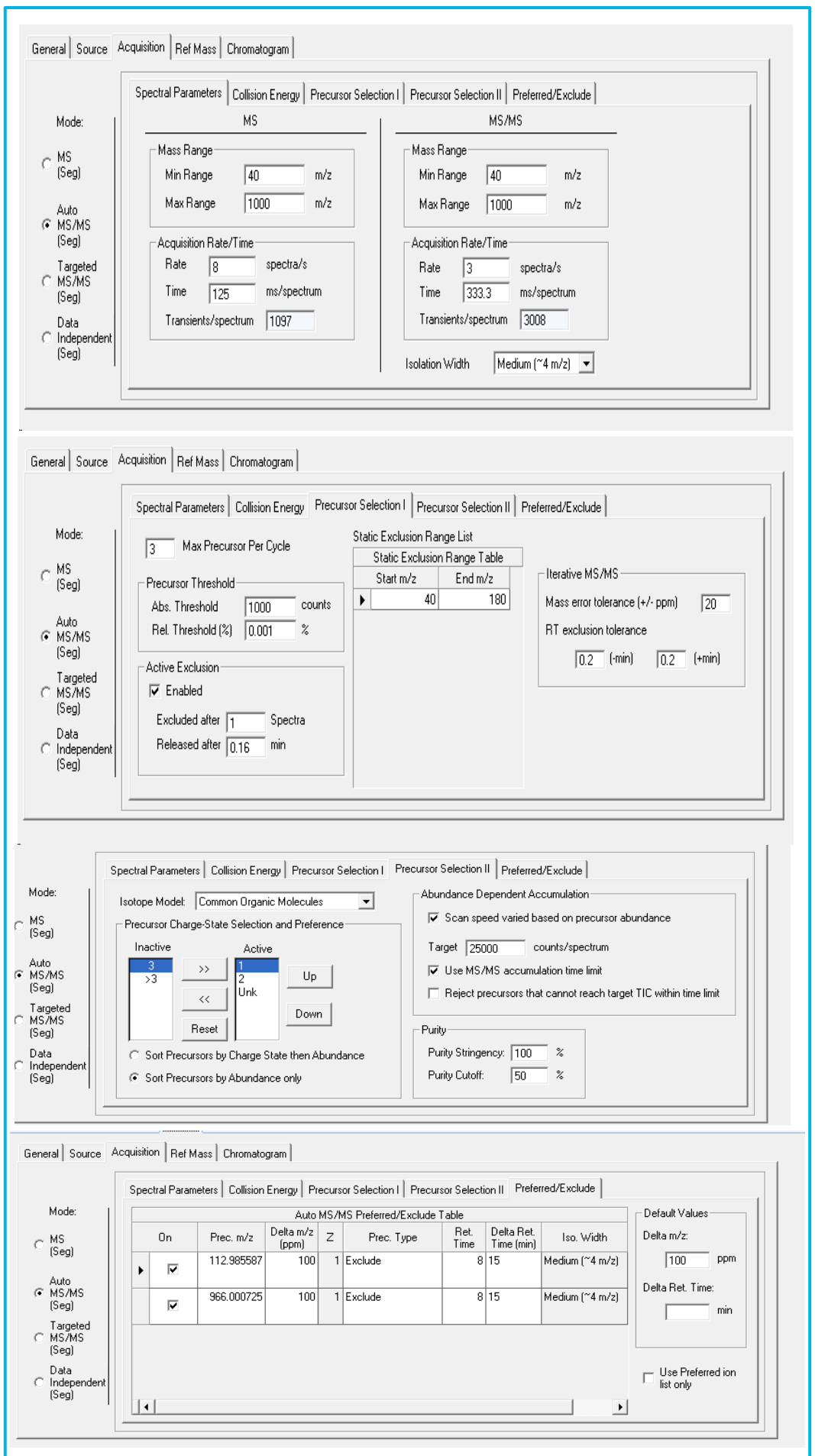

Figure 3: Auto MSMS Parameters

Preferred Only list

The Smart Preferred list was generated based on analysis of the mass defect of the CompTOX EPA Dashboard List PFASMaster[1]. The was filtered to remove entries with missing data and salts were removed.

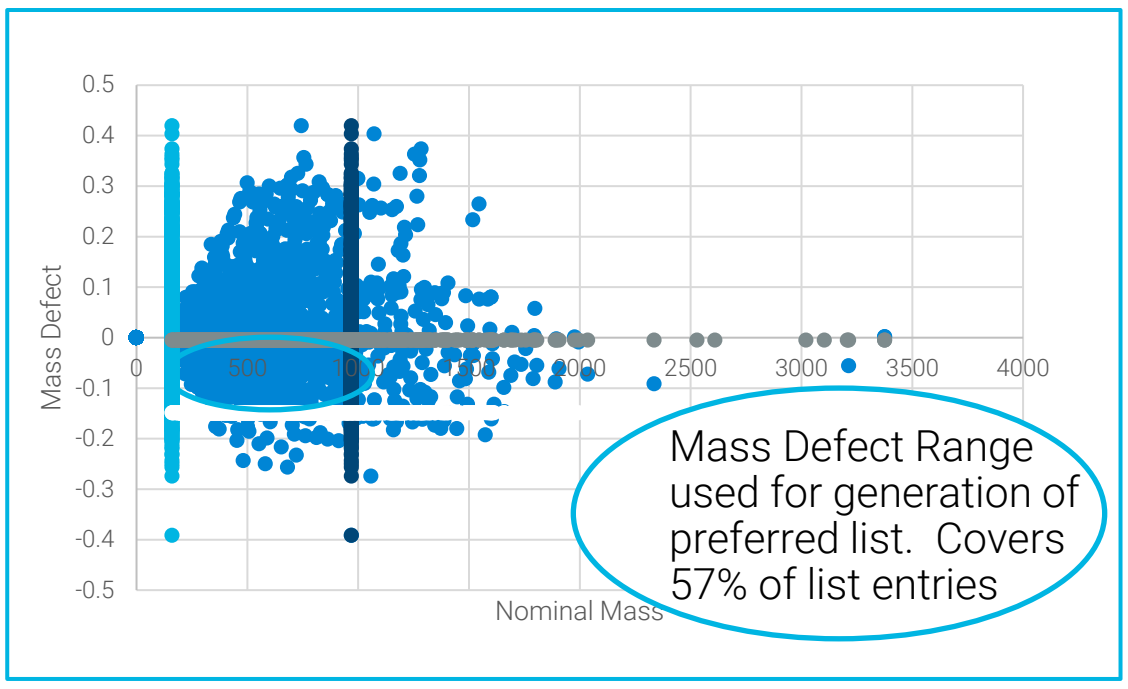

Figure 4: Plot of PFAS Master Entries nominal mass versus mass defect. Straight lines and the blue circle enclose the mass defect and nominal mass range inputed into the Smart Preferred List generator

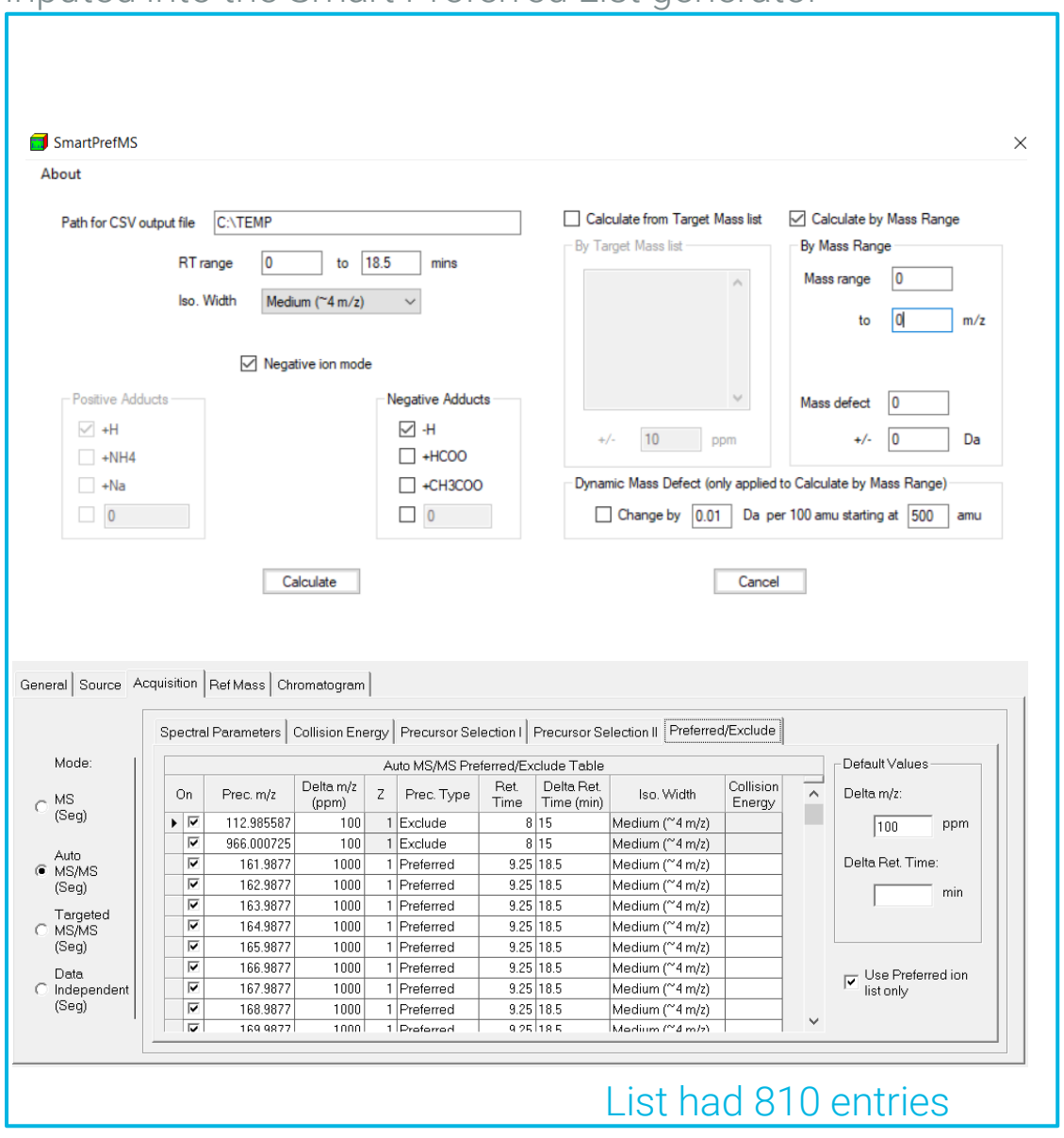

Figure 5: Smart Preferred List Generator Interface and ACQ software “preferred only” setup

Results and Discussion

Fluoromatch flow results

Each data set was processed separately with Fluoromatch Flow (v2.431)[2]. Inputs differed by the number of MSMS iterations entered. For example, F1-I1 refers to an analysis of Formulation 1 with 1 only one file of iterative MSMS, while F1-I5 used all 5 iterations.

Fluoromatch provides categories of annotation confidence. Full scoring details are provided in the user manuals available with the download[2]. In short, A is a confident ID, B tentative ID, and C possible ID. Categories below B do not have/utilize MSMS fragmentation. But are scored based on the identification of other PFAS within the same homologous series, absence of that, the score is decreased to no D, no ID, possibly PFAS, but no structural information.

Table 1: Fluoromatch Flow results for F1

	F1_I1	F1_I5	F1_P1	F1_P5
Score	# of IDs	# of IDs	# of IDs	# of IDs
A	27	27	30	30
B+	10	11	9	10
B	112	138	89	103
B-	0	0	0	0
C+	3	2	3	1
C	7	4	7	7
C-	14	11	13	11
D+	81	61	101	90
D	0	0	2	2
Total > C	59%	69%	50%	56%
Total < C	41%	31%	50%	44%

Table 2: Fluoromatch Flow results for F2

	F2_I1	F2_I5	F2_P1	F2_P2
Score	# of IDs	# of IDs	# of IDs	# of IDs
A	3	3	2	2
B+	1	2	3	3
B	23	27	17	18
B-	0	0	0	0
C+	37	32	42	41
C	0	0	0	0
C-	0	0	0	0
D+	0	0	0	0
total > C	42%	50%	34%	36%
total < C	58%	50%	66%	64%

Discussion

As noted in Koelemel et al. [3] annotation confidence improved with iterative exclusion injections. Both formulations and acquisition types showed a greater % of IDs >C when 5 injections input into the model. Annotations with a confidence of B- or higher include fragmentation, so iterative exclusion injections allow lower abundance peaks to be fragmented. In the case of the preferred only list, giving coeluting *m/z* an opportunity to be acquired through repeat injections.

Iterative exclusion outperformed the preferred only list in both formulations, likely due to the limited mass defect range input into the list generator. Widening the mass defect range input could perhaps make performance more comparable.

Optimization of the MSMS parameters for iterative exclusion (Figure 3) was demonstrated to be important though these experiments.

Conclusions

- Optimization of MSMS parameters for iterative exclusion is important for successful identifications in Fluoromatch
- Both Iterative exclusion and the preferred only list benefitted from repeat injections
- Iterative exclusion outperformed the preferred list likely due to the limited mass defect range input into the list generator

References

[1]U.S. EPA Chemistry Dashboard, PFASMASTER Chemicals, https://comptox.epa.gov/dashboard/chemical_lists (accessed 07/2021).

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

[3]Koelmel, J.P.; Paige, M.K.; Aristizabal-Henao, J.J.; Robey, N.M.; Nason, S. L.; Stelben, P.J.; Li, Y.; Kroeger, N.M.; Napolitano, M.P.; Savvaides, T.; Vasiliou, P. R.; Garrett, T.J.; Lin, E.; Deigl, C.; Jobst, K.; Townsend, T.G.; Pollitt, K.J.G.; Bowden, J. A. *Anal. Chem*. 2020, 92, 11186-11194.

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