**Development of a Total Organic Fluorine Method for the Analysis of
Process Wastewater Streams and Air from Fayetteville Works (NC)**

FINAL REPORT

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<u>**EXECUTIVE SUMMARY**</u>

Per- and polyfluoroalkyl substances (PFAS) are of interest because many have long persistence in the environment and widespread presence in humans and wildlife [1]. PFAS have been used in many products since the 1950s. Products containing PFAS include food packaging, nonstick cooking pans, electronics, carpets, fabrics, paints, adhesives, personal care products, and firefighting foams. More than 6000 PFAS have been found in the environment, and that number is likely to continue to grow as more are discovered, including transformation products and metabolites from the parent compounds manufactured [1]. Perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) were previously phased out of products in North America and have been restricted in Europe under the Registration, Evaluation, and Authorization of Chemicals (REACH) program. PFAS replacements are now being found in the environment, including drinking water sources. Liquid chromatography (LC)-mass spectrometry (MS) is the most popular tool for measuring PFAS in environmental samples, but this is limited to PFAS compounds that have available standards and analytical methods; thus, much of the organic fluorine present can be missed.

In order to more comprehensively capture all organic fluorine species, we developed two robust methods using combustion ion chromatography (CIC) for measuring total organic fluorine (TOF) in industrial wastewaters, air, and river water. These two methods, adsorbable organic fluorine (AOF) and extractable organic fluorine (EOF), have been optimized and compared using 39 different PFAS standards, including newer replacement PFAS compounds.

The finalized AOF method is as follows: Samples are filtered with a 0.22 μm filter to remove particulates and solids. Samples are then analyzed for background inorganic fluoride using ion chromatography. If the inorganic fluoride is found to be above 5 mg/L, samples are diluted to lower the inorganic fluorine below 5 mg/L in order to avoid false high signals since ion chromatography cannot distinguish between inorganic and organic fluorine. After analyzing for inorganic fluoride, samples are adjusted to pH < 1 with concentrated nitric acid. Samples (50 mL or 500 mL) are then passed through two activated carbon (AC) columns in series, followed by a rinsing step with 15 mL of 0.1% ammonium hydroxide solution. Both AC columns from each sample are pyrolyzed in the same ceramic boat with a quick furnace and gasses are collected with an aqueous solution containing 1 mM sodium bicarbonate and 1.0% hydrogen peroxide. This solution is then analyzed for fluoride with ion chromatography.

The finalized EOF method is as follows: Samples are filtered with a 0.22 μm filter to remove particulates and solids. Samples are then analyzed for background inorganic fluoride using ion chromatography. If the inorganic fluorine is found to be above 5 mg/L, samples are diluted to lower the inorganic fluorine below

1

5 mg/L in order to avoid false high signals. After analyzing for inorganic fluoride, samples are adjusted to pH 5 with concentrated nitric acid. Strata XAW/GCB SPE cartridges are conditioned with 10 mL of 0.3% NH$_4$OH in methanol followed by 10 mL of methanol. Cartridges are then equilibrated with 10 mL of ultrapure water at pH 5. Samples with a volume of 500 mL are then loaded on the SPE cartridge at a flow rate of 4 mL/min. To remove interfering analytes from the sample, cartridges are next washed with 10 mL of 0.1% ammonium hydroxide in ultrapure water and dried for 10 minutes under nitrogen gas. Organics are eluted from the cartridges with 0.3% ammonium hydroxide in methanol after the eluent has been allowed to soak for 5 minutes on the cartridge. Eluted organics are then concentrated under nitrogen gas to 200 µg/L. An aliquot of 100 µL is directly injected into a ceramic boat containing quartz wool. Samples are pyrolyzed and off-gasses are collected with an aqueous solution containing 1 mM sodium bicarbonate and 1.0% hydrogen peroxide. This solution is then analyzed for fluoride with ion chromatography.

For the optimized AOF method, we report a recovery of 79 and 87% for a mix of 39 PFAS standards in ultrapure water using a sample volume of 50 mL and 500 mL respectively. Our method reporting limit of detection (LOD) and quantification (LOQ) for a volume of 50 mL is 0.5 and 1.6 µg/L, respectively. For lower LOD and LOQ values of 0.2 and 0.5 µg/L, respectively, a larger sample volume of 500 mL can be used; however, a larger volume has been shown to decrease the recovery of PFAS. Comparatively, our optimized EOF method reporting LOD and LOQ values are even lower than the AOF method at 0.2 and 0.5 µg/L, respectively. Another advantage of the optimized EOF method is a higher percent recovery (91%) for the same mix of 39 PFAS standards in ultrapure water.

Both optimized methods were applied to process wastewater samples from four locations at the Chemours Fayetteville Works, North Carolina plant for method comparison and validation. We also report a total organic fluorine value for 16 extracted air samples from the plant.

Background

Combustion ion chromatography (CIC) is a popular technology for capturing total organic fluorine (TOF), as well as total organic chlorine (TOCl), total organic bromine (TOBr), and total organic iodine (TOI) [2-16]. CIC methods involve sample extraction, concentration, combustion at 1000°C in a furnace, collection of generated gases in an aqueous adsorbing solution, and analysis of the resulting anions by ion chromatography (IC). In this way, organic fluorine, chlorine, bromine, and iodine are extracted and converted into inorganic fluoride, chloride, bromide, and iodide, which are easily measured by IC.

For total organic fluorine (TOF), previously published CIC methods include the extractable organic fluorine (EOF) and the adsorbable organic fluorine (AOF) assays. EOF and AOF differ in how the samples are extracted: EOF uses solid-phase extraction (SPE) with ion pairing methods (e.g., Oasis WAX cartridges), and AOF uses a polystyrene divinylbenzene-based AC column. Both methods have been shown to effectively extract and quantify TOF in aqueous samples and in other kinds of samples [5-14]. Other TOF/total fluorine methods include the total oxidizable precursor (TOP) assay, particle-induced gamma ray emission spectroscopy (PIGE), instrumental neutron activation analysis (INAA), and ^{19}F nuclear magnetic resonance (NMR) [13, 14]. However, each of these has issues for TOF analysis. First, because the TOP assay selects only for compounds that can be oxidized into target per- and polyfluoroalkyl substances (PFASs), it will miss newer fluorinated compounds like HFPO-DA and ADONA that either do not oxidize or do not oxidize into familiar perfluoroalkyl acids [14]. Also, because the TOP assay relies on reversed-phase liquid chromatography (LC), short-chain compounds that are not retained by traditional LC columns are lost.

PIGE is a surface analysis technique that uses an accelerated beam of protons to excite ^{19}F nuclei, which emit gamma rays proportional to the number of fluorine atoms. However, PIGE does not allow inorganic fluoride to be distinguished from organic fluorine [14]. Instrumental neutron activation analysis (INAA) is another nuclear technique recently used to measure total fluorine in biological, environmental, and food packaging samples [13]. However, this technique cannot distinguish between inorganic and organic fluorine, and it suffers from interferences with aluminum. Finally, ^{19}F NMR can be used to quantify TOF by integrating multiple peaks associated with organic fluorine compounds, but it is not often applied to water samples due to high detection limits (10 µg/L for a 100 mL water sample) [14]. In a recent comparison of CIC, PIGE, and INAA for measuring total fluorine in food packaging, CIC had the lowest detection limits [13]. Earlier this year, a new CIC method reported detection limits of 0.3 µg/L for adsorbable organic fluorine, using custom-packed activated carbon columns and 300 mL volume of sample [16]. While this AOF method offered lower detection limits, there were still aspects to this method that could be improved to further optimize performance.

Project Goal: The goal of this project was to create a Combustion IC Total Organic Fluorine method that will optimize the recovery of organofluorine chemicals, including newer compounds like HFPO-DA and other perfluoroether carboxylic acids, and allow rugged and reliable measurements of TOF in process wastewater, river water, and air. A TOF analysis can be combined with results from a target analysis of known PFAS compounds in the same samples for a more complete analysis.

Materials and Methods

Reagents and Solutions. Pentafluoropropionic acid (98%), perfluoropentanoic acid (98%), perfluorohexanoic acid (97%), perfluoroheptanoic acid (97%), perfluorooctanoic acid (95%), perfluorononanoic acid (97%), perfluorodecanoic acid (96%), perfluoroundecanoic acid (97%), perfluorododecanoic acid (96%), perfluorotetradecanoic acid (97%), heptafluoroobutyric acid (98%), hexafluoropropylene oxide trimer acid (95%), nonafluoroheptanesulfonic acid (97%), and perfluorooctanesulfonic acid (97%) were purchased from Synquest Labs (Alachua, FL). All other PFAS standards were received from Chemours (Fayetteville, NC) as 1000 mg/L solutions in methanol (Table 1). Potassium phosphate monobasic (≥99.0%), sodium carbonate anhydrous (≥99.5%), sodium fluoride (99%), sodium sulfate (99%), sodium nitrate (99%), hydrogen peroxide (30%), potassium hydrogen phthalate (99%), and nitric acid (70%) were purchased from Fisher Scientific (Pittsburgh, PA). High-performance liquid chromatography (HPLC) grade methanol and acetonitrile (Honeywell B&J) were purchased from VWR (Radnor, PA). Ammonium hydroxide solution (28%) was purchased from Sigma-Aldrich (St. Louis, MO).

Table 1. Full List of PFAS Standards Used in This Study and Included in the PFAS Standard Mix.

Abbreviation	Analyte
1. PFPeA *	Perfluoropentanoic acid
2. PFHpA *	Perfluoroheptanoic acid
3. PFHxA *	Perfluorohexanoic acid
4. PFUdA *	Perfluoroundecanoic acid
5. PFDoA *	Perfluorododecanoic acid
6. PFOA *	Perfluorooctanoic acid
7. PFNA *	Perfluorononanoic acid
8. PFDA *	Perfluorodecanoic acid
9. PFTeDA *	Perfluorotetradecanoic acid
10. PFBS *	Perfluorobutanesulfonate
11. PFPrA *	Perfluoropropanoic acid
12. PFBA *	Perfluorobutanoic acid
13. HFPO-TA *	Hexafluoropropylene oxide trimer acid
14. PFOS *	Perfluorooctanesulfonate
15. HPFO-DA	Hexafluoropropylene oxide dimer acid
16. PFO3OA	Perfluoro-3,5,7-trioxaoctanoic acid
17. PFO4DA	Perfluoro-3,5,7,9-tetraoxadecanoic acid
18. PFO5DA	Perfluoro-3,5,7,9,11-pentaoxadodecanoic acid
19. PEPA	Perfluoro-2-ethoxypropanoic acid
20. PMPA	Perfluoro-2-methoxypropanoic acid
21. EVE Acid	Perfluoroethoxypropionic acid
22. Hydro-EVE Acid	Propanoic acid, 3-[1-[difluoro(1,2,2,2-tetrafluoroethoxy)methyl-
23. NVHOS	1,1,2,2-Tetrafluoro-2-(1,2,2,2-tetrafluoro-ethoxy)ethane sulfonic acid
24. R-PSDA	Pentanoic acid, 2,2,3,3,4,5,5,5-octafluoro-4-(1,1,2,2-tetrafluoro-2-sulfoethoxy)-
25. R-PSDCA	Ethanesulfonic acid, 1,1,2,2-tetrafluoro-2-[1,2,2,3,3-pentafluoro-1-(trifluoromethyl)propoxy-
26. Hydrolyzed PSDA	Acetic acid, 2-fluoro-2-[1,1,2,3,3,3-hexafluoro-2-(1,1,2,2-tetrafluoro-2-sulfoethoxy)propoxy-
27. PFMOAA	Perfluoro-2-methoxyacetic acid
28. PFECA G	Perfluoro-4-isopropoxybutanoic acid
29. PFECA B	Perfluoro-3,6-dioxaheptanoic acid
30. MTP	Perfluoro-2-methoxypropanoicacid
31. Hydrolyzed TAF n=1 (PFO2HxA)	Perfluoro(3,5-dioxahexanoic) acid
32. PS Acid	Ethanesulfonic acid, 2-[1-[difluoro[(1,2,2-trifluoroethenyl)oxy]methyl]-1,2,2,2-tetrafluoroethoxy]-1,1,2,2-tetrafluoro-
33. Hydro-PS Acid	Ethanesulfonic acid, 2-[1-[difluoro(1,2,2,2-tetrafluoroethoxy)methyl]-1,2,2,2-tetrafluoroethoxy]-1,1,2,2-tetrafluoro
34. PES	Perfluoro-2-ethoxyethanesulfonic acid
35. DFSA	Difluoro-sulfo-acetic acid
36. BP5	Byproduct 5
37. MMF	Difluoromalonic acid
38. R-EVE	4-(2-Carboxy-1,1,2,2-tetrafluoroethoxy)-2,2,3,3,4,5,5,5-octafluoropentanoic acid
39. PFHxS	Perfluorohexanesulfonate

* Standard purchased from Synquest labs. All other standards were received from Chemours.

Individual PFAS standards and a standard PFAS mix containing the 39 compounds listed in Table 1 were used to determine method recovery. Notably, these compounds include newer perfluoroether carboxylic acids (including HFPO-DA), as well as perfluoroalkyl carboxylates, perfluoroalkyl sulfonates, and perfluoroether sulfonic acids. Sodium fluoride was used to evaluate the efficiency of the aqueous absorption solution to absorb fluoride. Stock solutions were prepared by dissolving each pure standard in methanol or ultrapure water at 100.0 mg/L; these were diluted further to 1.0 mg/L for working solutions used for further experiments. For recovery experiments, a mass balance approach was used to spike samples at a total concentration of 50 µg/L F⁻ for individual standard recoveries, as well as standard mix recoveries.

Ultrapure water (≥18 MΩ/cm) was used to prepare all solutions in this study. A Thermo Electron ROSS Ultra pH electrode connected to a Thermo Orion Star 211 pH Benchtop meter (Thermo Fisher Scientific, Waltham, MA) was used to measure pH. Electrode calibration was performed with commercial pH 4, 7, and 10 standards.

Potassium hydrogen phthalate (KHP) solutions were prepared at 1000 mg/L carbon by dissolving KHP in ultrapure water. KHP was used as a calibrant for dissolved organic carbon (DOC) measurements of collected water samples.

Collection of Samples. River water samples (Broad River, Columbia, SC) were collected and spiked with PFAS stock solutions to test the performance of the AOF and EOF methods. Process wastewater samples were collected by Chemours (Fayetteville, NC), and air samples extracted in methanol were shipped to the University of South Carolina from Eurofins (Knoxville, TN) to demonstrate the final AOF and EOF methods. Samples were stored at 4°C until use.

Experimental Procedure and Instrumentation. Dissolved organic carbon was measured using a Shimazdu Total Organic Carbon analyzer. Prior to analysis by AOF or EOF, samples were first analyzed for inorganic fluoride by ion chromatography and for dissolved organic carbon. Samples used for spiked recovery experiments were also first analyzed for total organic fluorine in order to determine background levels of both inorganic and organic fluoride. For AOF analysis, water samples were processed using a Total Organic Halogen Analyzer (Mitsubishi Chemical Analytech, Chigasaki, Japan; formerly distributed by Cosa Xentaur, Yaphank, USA, now distributed by Mandel Scientific), which includes an adsorption unit, automatic solid sampler, combustion furnace, and absorption unit (Figure 1). Samples were loaded onto two pre-packed activated carbon (AC) columns in series (Mitsubishi Chemical Analytech, Chigasaki, Japan) with an adsorption module (TXA-04, Figure 1A). Both ACs were then loaded onto a ceramic combustion vessel (henceforth "ceramic boat") using a T-tool. For EOF analysis, water samples were loaded onto pre-packed SPE cartridges (Strata WAX/GVB) and processed with a SPE vacuum manifold. Cartridges were then dried under nitrogen gas to remove the aqueous wash solution, followed by elution with methanol containing 0.3% ammonium hydroxide, and then concentrated under nitrogen gas. Concentrated samples were then directly injected into ceramic boats containing quartz wool.

Ceramic boats from both AOF and EOF analysis were automatically loaded into a quick furnace (AQF-2100H) using an automatic solid sampler (ASC-240S, Figure 1B). Ceramic boats were pre-baked at 1000°C for 10 min to remove any prior contamination. Samples were pyrolyzed inside the furnace at 1000°C, and the effluent gasses were bubbled into 50 mL polypropylene centrifuge tubes containing 5 mL of 1 mM sodium bicarbonate and 0.1% hydrogen peroxide as the absorption solution, using a gas absorption unit (AU-250, Figure 1C). Samples were combusted in the furnace for 10 minutes. Argon and oxygen gas flow

rates of 400 and 200 mL/min were used, respectively, through the furnace. Ultrapure water was supplied to the furnace at 100 µL/min to help the dissolution of the hydrogen fluoride from the quartz tube. Each centrifuge tube was weighed with an analytical balance before and after each run to obtain the exact mass of each sample.
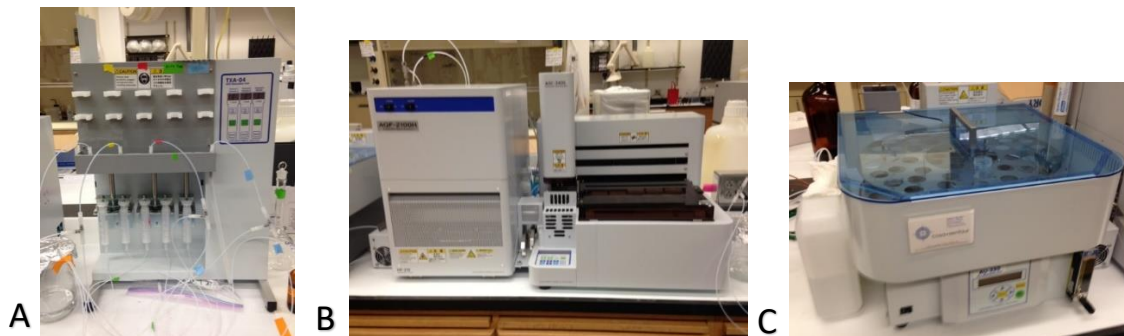

Figure 1. Water sample adsorption and combustion process using a Mitsubishi Total Organic Halogen Analyzer with adsorption module (A), automatic quick furnace and automatic solid sampler (B), and a gas absorption unit (C).

The absorption solution containing the off-gasses was analyzed for fluoride with a Thermo Dionex Integrion HPIC ion chromatograph equipped with a 500 µL sample loop, Dionex ADRS 600 suppressor, a Dionex IonPac AS20 analytical column and guard column, and an EGC II KOH cartridge. The following gradient hydroxide elution was used: 5 mM from 0-5 min, 30 mM for 5-15 min, and 20 mM for 15-30 min, at a flow rate of 1 mL/min. The fluoride limit of quantification by IC was 1.0 µg/L. The final TOF values in the original sample were calculated using:

$$TOF = \frac{[F] \times V_f - BKG}{V_i} \times 100$$

Eq. (1)

Where [F] is the fluoride concentration (µg/L) analyzed by IC, V_f is the final volume (mL) of the absorption solution, and V_i (mL) is the volume of the loaded water sample. BKG is the mass of background fluorine (µg) in the procedural blank determined by loading ultrapure water with the same analytical procedure described above.

TOF recovery was calculated using:

$$percent\ recovery = \frac{measured\ TOF}{theoretical\ TOF} \times 100$$

Eq. (2)

Where measured TOF is calculated based on Eq. 1, and theoretical TOF is calculated based on the PFAS concentrations and the fluorine mass fractions in the PFAS chemical formulas. For river water samples that contained a background TOF level but were used for spiked recovery experiments, measured TOF was corrected by measured background TOF levels and subtracting that value from the measured TOF value.

Adsorbable Organic Fluorine (AOF) Method Optimization

An adsorbable organic fluorine (AOF) approach was developed, evaluated, and optimized for recovery and method performance. Several key parameters were evaluated for maximum recovery of organic fluorine, lack of co-extraction of inorganic fluoride, linearity of calibration curves, detection limits, precision, and accuracy. The key parameters that were tested are as follows.

Activated Carbon Choice and Pretreatment. Ion chromatography cannot distinguish between combusted organic analytes and inorganic fluoride; therefore, background inorganic fluoride must be removed as efficiently as possible to minimize an artificially high signal. In order to lower background levels of fluoride in the whole system, the system was flushed a series of times with ultrapure water until background signals were non-detect. AC sources were also evaluated and compared for inorganic fluoride impurities (Table 2) before and after rinsing with 10 mL of 5000 mg/L potassium nitrate. The AC was directly combusted and analyzed for fluoride using ion chromatography. Mitsubishi AC columns were found to have the lowest background fluoride levels and were chosen for further method optimization. In addition to having the lowest fluorine background of any AC currently available, the use of these pre-packed AC columns avoids the need for analysts to pack columns themselves with loose AC, which can introduce error and require a clean room to avoid sorption of airborne organics onto the fine AC material.

Table 2. Background Inorganic Fluoride in Activated Carbon Columns

	Activated Carbon Source	*Fluoride (ng per cartridge)*
No Pre-Rinse	Cosa Xentaur*	44 (39-49)
	Mitsubishi Chemical Analytech	77 (75-80)
	EnviroScience	632 (629-634)
Pre-Rinse	Mitsubishi Chemical Analytech	4 (ND-26)
	EnviroScience	311 (230-363)

*Cannot be purchased anymore

Inorganic fluoride can be expected in environmental and process wastewater samples since fluoride is naturally occurring in environmental water matrices up to 1 mg/L and was detected in processed wastewaters from Chemours up to 5 mg/L. To assist in further lowering background levels of inorganic fluoride, a pre-rinse of the ACs was evaluated to lower the adsorption affinity of fluoride to the AC. A pre-rinse of 10 mL potassium nitrate (as 5000 mg/L nitrate) was evaluated for inorganic fluoride removal using a solution of ultrapure water containing 5 mg/L inorganic fluoride. To assess the effect of a pre-rinse on the adsorption of organics, a separate experiment was done using a solution of ultrapure water containing a standard mix of 39 PFAS (Table 1). Results show that a pre-rinse of the AC can be used to lower inorganic fluoride levels; however, results also showed that a pre-rinse lowered the percent recovery of organic fluorine by 21% (Table3) The decrease in percent recovery of TOF could potentially be due to ions from the pre-rinse adsorbing to the AC, leaving less surface area for the organics to adsorb. For this reason, a pre-rinse of the AC was omitted from this method.

Table 3. Comparison of Pre-Rinsing vs. Not Pre-Rinsing Activated Carbon for Simulated Samples

	Inorganic F⁻ Retention (µg/L) (Ultrapure water + fluoride)	Percent Fluorine Recovery (µg/L) (39 PFAS)
Pre-Rinse	17.4 ± 2.1	79 ± 4.4
No Pre-Rinse	19.3 ± 4.7	102 ± 4.2

*Results represent a mean of 3 replicates.

Sample pH Pretreatment. The pH of the sample can be adjusted in order to increase the adsorption affinity of organics to AC by deionizing the organics (making anionic compounds neutral) at a pH value that is lower than the target analyte's pK_a value. For example, if these compounds are anions or cations, they will be more polar and less likely to adsorb to the AC, thus, passing through the AC and not being retained. Although the pK_as of many PFAS compounds are unknown, the PFAS compounds evaluated in this study all contain carboxylic acid and/or sulfonic acid hydrophilic head groups, as well as electronegative fluorine atoms, so it was predicted that a pH <1 would be necessary to deionize many of the PFAS compounds evaluated for this study. To evaluate the effect of the pH adjustment of the sample, samples containing a standard mix of PFAS standards 1-14 (standards with known high purity at the time of these analyses) (Table 1) prepared as 50 µg/L fluorine in ultrapure water were adjusted to several pH values (1, 3, 5, and 11) using concentrated nitric acid and concentrated sodium hydroxide (Figure 2). Several individual PFAS standards were also evaluated at a pH of 1 and 2 in ultrapure water for percent recovery (Figure 3).
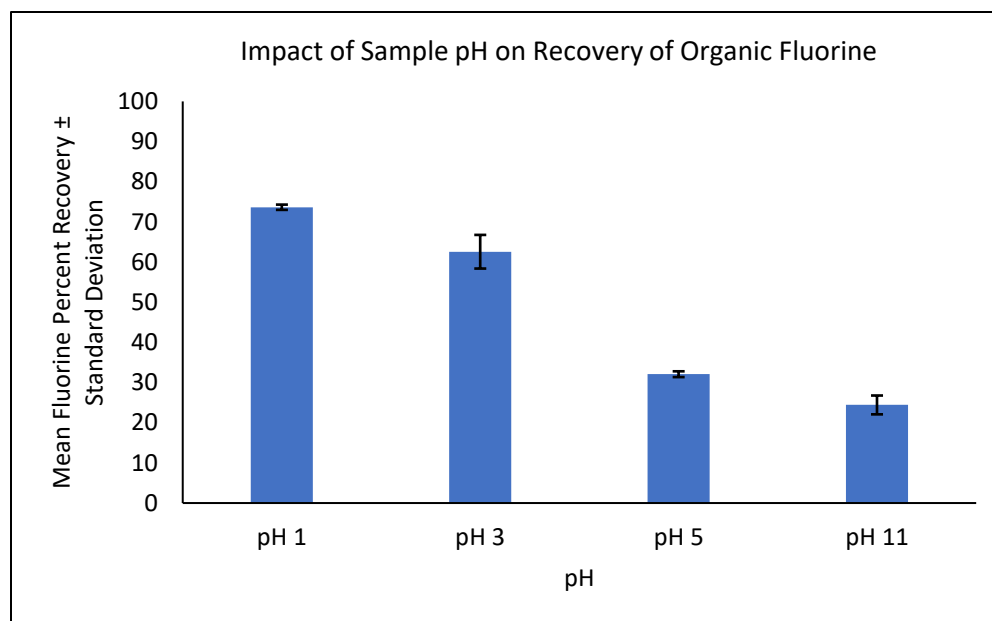
Figure 2. Recovery of fluorine from a PFAS mix of 14 compounds spiked into ultrapure water and adjusted to varying pH values with concentrated nitric acid or sodium hydroxide. Results represent a mean of 3 replicates.
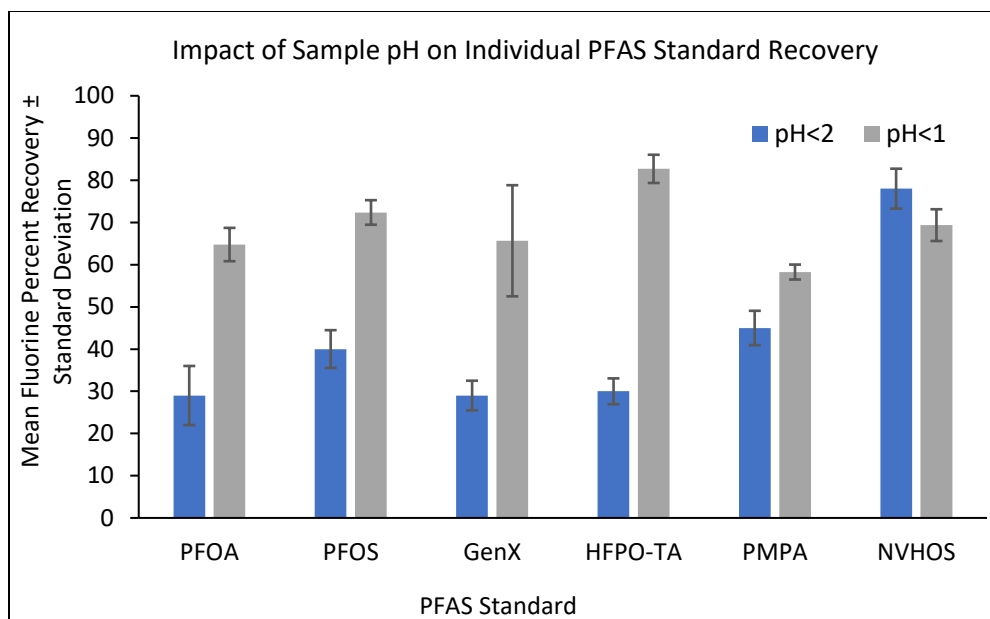
Figure 3. Recovery of fluorine from several individual PFAS standards in ultrapure water at a pH adjustment below 2 vs below 1. Results represent a mean of 3 replicates.

Results show that a pH adjustment below 1.0 improves the overall percent recovery of organic fluorine, but a caveat is that the pK_a of HF is 3.1. If the pH of the sample is adjusted to <1, inorganic fluoride (F^-) will be converted to a protonated form (HF), which could have a higher bonding affinity to AC compared to its anionic form.[14] In order to ensure that inorganic fluoride is still efficiently removed at a pH below 1, sample pH adjustment was also evaluated for inorganic fluoride retention. Samples containing two realistic concentrations of inorganic fluoride (0.5 and 1.0 mg/L) in ultrapure water and samples containing a standard mix of PFAS compounds 1-14 (Table 1) as 50 µg/L fluorine in ultrapure water were either adjusted to pH below 1 or not adjusted and evaluated for fluoride retention and percent fluorine recovery, respectively (Table 4). A pH adjustment below 1 showed the lowest level of inorganic fluoride retained, as well as a higher percent recovery of organic fluorine. Thus, a pH adjustment of the sample below 1 was chosen for this method.

Table 4. Comparison of Adjusting the pH of the Sample to Below 1 vs. No pH Adjustment

	500 µg/L F^- retained	1 mg/L F^- retained	Percent Fluorine Recovery
pH adjusted	24.6 ± 5.7	19.3 ± 4.7	75 ± 4.7
pH not adjusted	42.0 ± 1.1	31.1 ± 2.1	57 ± 4.4

*Results represent a mean of 3 replicates.

Sample Dilution Factor and Volume. The volume of the sample was optimized to lower detection limits for trace analysis. A larger sample volume can be used to increase the concentration factor and can therefore improve detection limits for trace analysis, but sample volume must also be optimized in order to *prevent breakthrough* of the analyte from the AC and to waste. Other considerations with an increased

9

volume size include required sample processing time and sample availability. To optimize the sample volume for potential breakthrough, experiments were completed with a standard mix of PFAS standards 1-14 (Table 1) as 50 µg/L fluorine in ultrapure water at varying volumes (50, 100, 150, 250, 350, and 500 mL) and were evaluated for percent recovery. Samples were loaded onto two AC columns in series, but the AC from each column was pyrolyzed separately to analyze each AC for fluorine (Figure 4). For increased sample volumes, a trend of decreased percent recovery was observed, as well as an increased breakthrough. Experiments were completed using ultrapure water that theoretically only contained organics resulting from the spiked PFAS mix, but in a real scenario, the matrix would likely contain more organics, which might increase breakthrough values even higher when larger volumes are used for analysis.
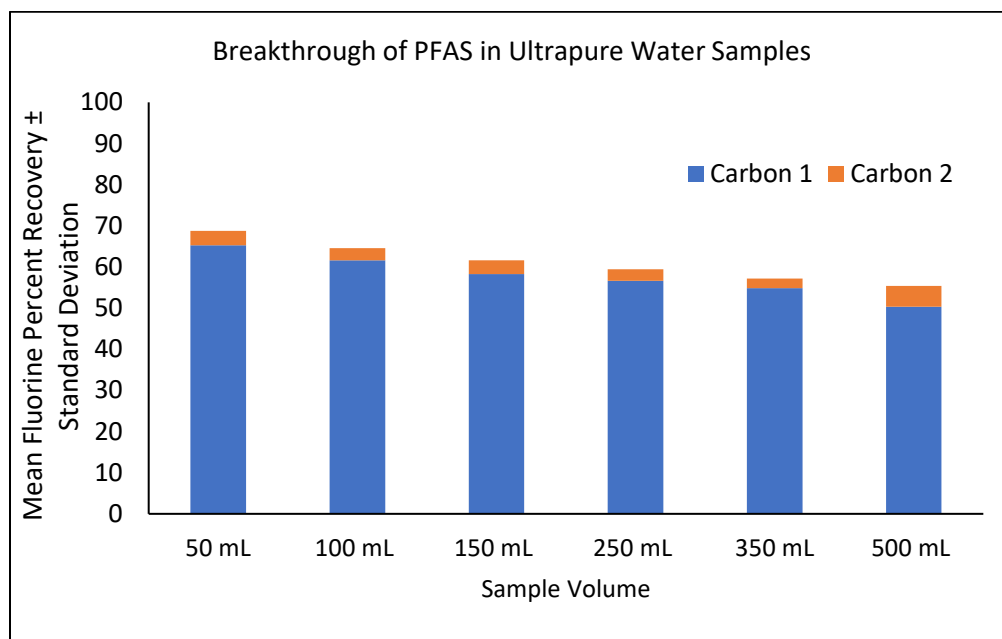
Figure 4. Breakthrough analysis of organic fluorine absorbed onto two AC columns in series from ultrapure water and measured using the AOF procedure previously described. Results represent a mean of 3 replicates.

To evaluate the effect of a more complicated environmental matrix on the adsorption of PFAS compounds, river water samples were diluted at different ratios and tested at different volumes to reduce possible matrix effects and to prevent breakthrough of organics to waste. River water used for these experiments was first analyzed for dissolved organic carbon (DOC). The resulting DOC value was 2.4 mg/L. Three dilution factors (10, 50, and 100) and four volumes (50, 100, 150, and 250 mL) were tested. For dilution factor experiments, river water samples spiked with a standard mix of PFAS compounds 1-14 (Table 1) as 50 µg/L F$^-$ were passed through two AC columns in a series, but the AC from each column was pyrolyzed individually for analysis of TOF in each column (Figure 5).

Compared to breakthrough experiments in which ultrapure water is used as the matrix (Figure 4), a higher DOC value, or more complex matrix, might result in a higher breakthrough value of the analyte from the first AC column into the second AC column. If samples contain high DOC values, a third AC column might be added in series to help capture the analytes breaking through the second AC column. Results from dilution factor experiments also show a general trend in increased breakthrough values as the volume of

the sample increases. In order to achieve a higher recovery at a dilution factor of 100 when compared to a dilution factor of 50, a larger sample volume is required. For complex samples with high DOC values, a dilution factor may be necessary, but sample volume and breakthrough should also be considered.
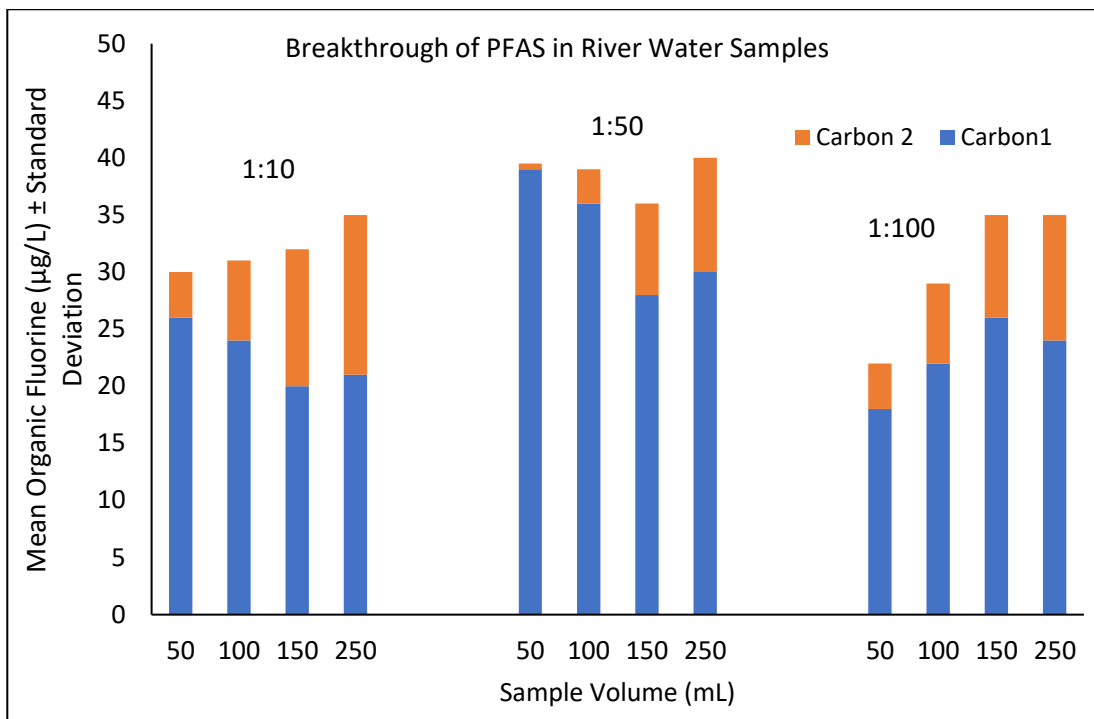

Figure 5. Breakthrough analysis of organic fluorine adsorbed onto two AC columns in series from river water and measured using the AOF procedure previously described. Results represent a mean of 3 replicates.

Inorganic Fluoride Rinse Step. As mentioned previously, removing inorganic fluoride is a key factor for this method in order to avoid an artificially high signal from inorganic fluoride. Although inorganic fluoride interference may be impossible to completely avoid, it can be lowered by utilizing an ion exchange rinse step after the sample has been loaded onto the AC cartridges. Samples containing varying concentrations of sodium fluoride in ultrapure water (0.5, 1.0, and 5.0 mg/L) were adsorbed onto the AC; then several rinsing solutions (sodium nitrate, sodium sulfate, and ammonium hydroxide) were evaluated for inorganic fluoride removal using 10 mL of each solution (Figure 6). Rinsing solutions were also evaluated for percent recovery of organic fluorine using samples of ultrapure water spiked with a standard mix of PFAS standards 1-14 (Table 1) as 50 µg/L fluorine. (Figure 7). Ammonium hydroxide showed the highest removal of inorganic fluoride while also maintaining the highest percent recovery of organic fluorine. Thus, *ammonium hydroxide was chosen as the rinsing solution* for further optimization.
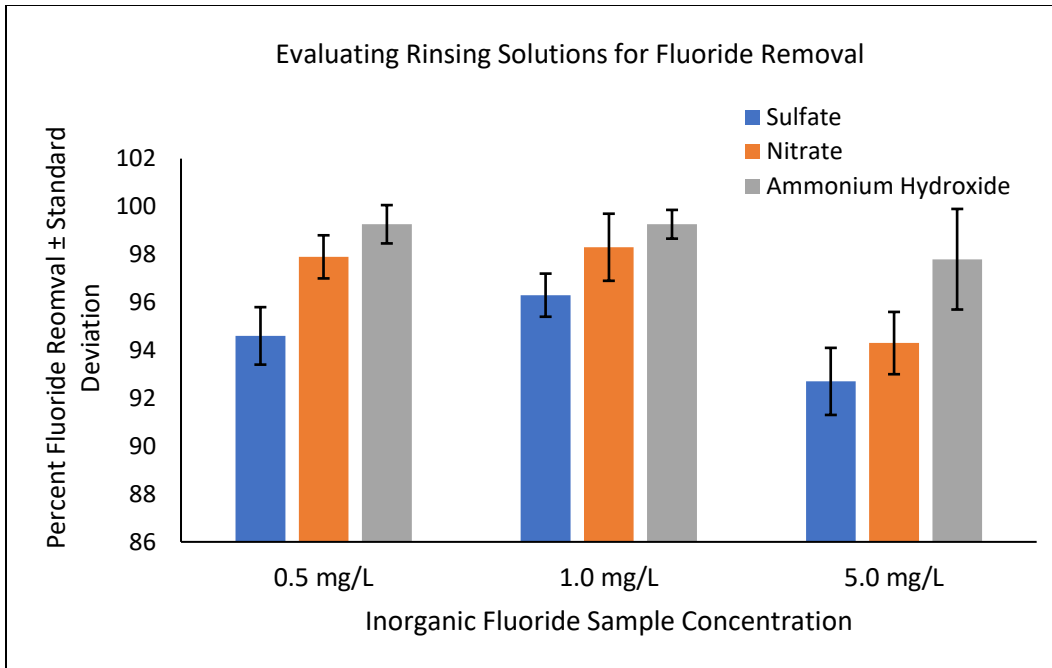
Figure 6. Inorganic fluoride removal at three concentrations of inorganic fluoride using different rinsing solutions for fluoride removal. Results shown are the percent inorganic fluoride remaining from a 5 mg/L inorganic fluoride in ultrapure water sample. Results represent a mean of 3 replicates.
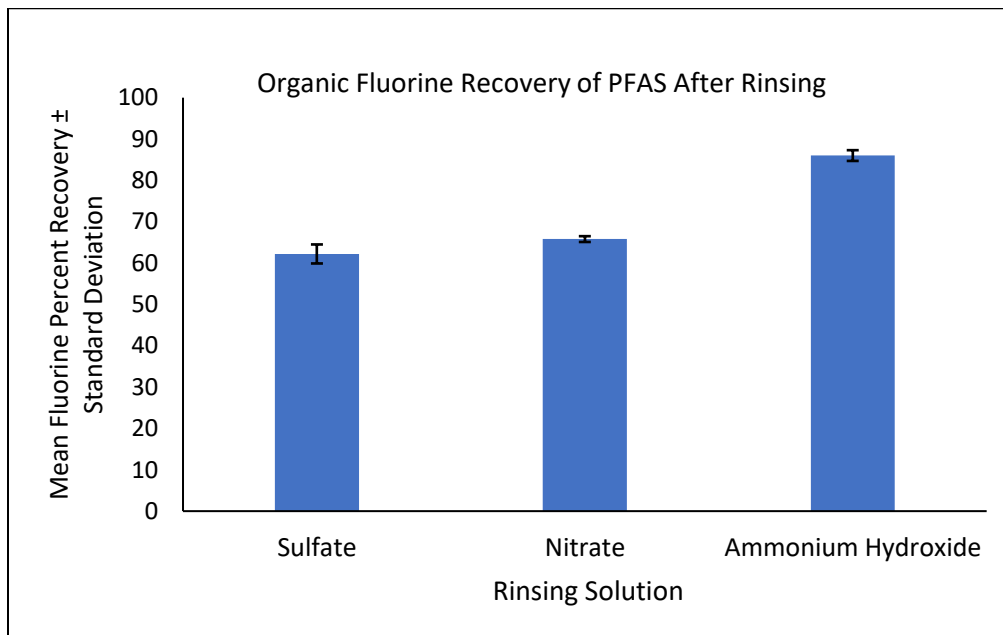

Figure 7. Recovery of organic fluorine resulting from the varying composition of the rinsing solution for inorganic fluoride removal. Results represent a mean of 3 replicates.

To further optimize the ammonium hydroxide rinsing solution, ammonium hydroxide solutions of varying concentration (0.01 and 0.1%) and varying volumes (5, 10, 15, and 20 mL) were evaluated for percent fluoride removal with samples containing 5 mg/L inorganic fluoride. (Figure 8). Percent recovery of organic

fluorine was also evaluated at the same ammonium hydroxide concentrations and volumes mentioned above using a standard mix of PFAS standards 1-14 (Table 1) as 50 µg/L fluorine spiked into ultrapure water (Figure 9). *Overall results show that using 15 mL of 0.1% ammonium hydroxide is the most optimal rinsing solution condition for inorganic fluoride removal and organic fluorine recovery*.
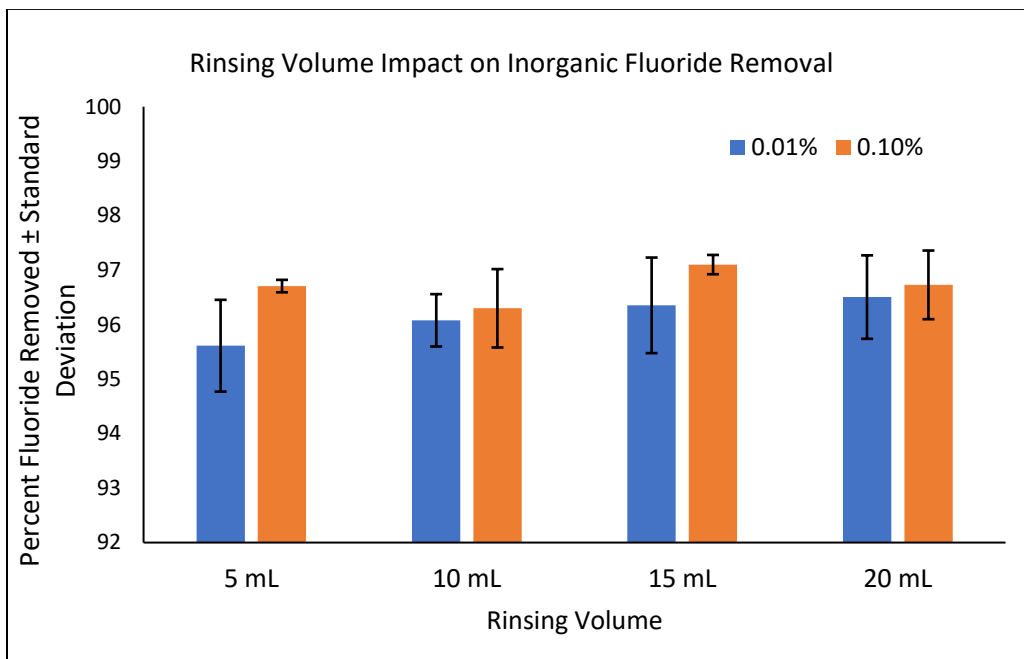
Figure 8. Inorganic fluoride removal results of two concentrations of ammonium hydroxide rinsing solution at varying volumes. Results shown are the percent inorganic fluoride remaining from a 5 mg/L inorganic fluoride in ultrapure water sample. Results represent a mean of 3 replicates.
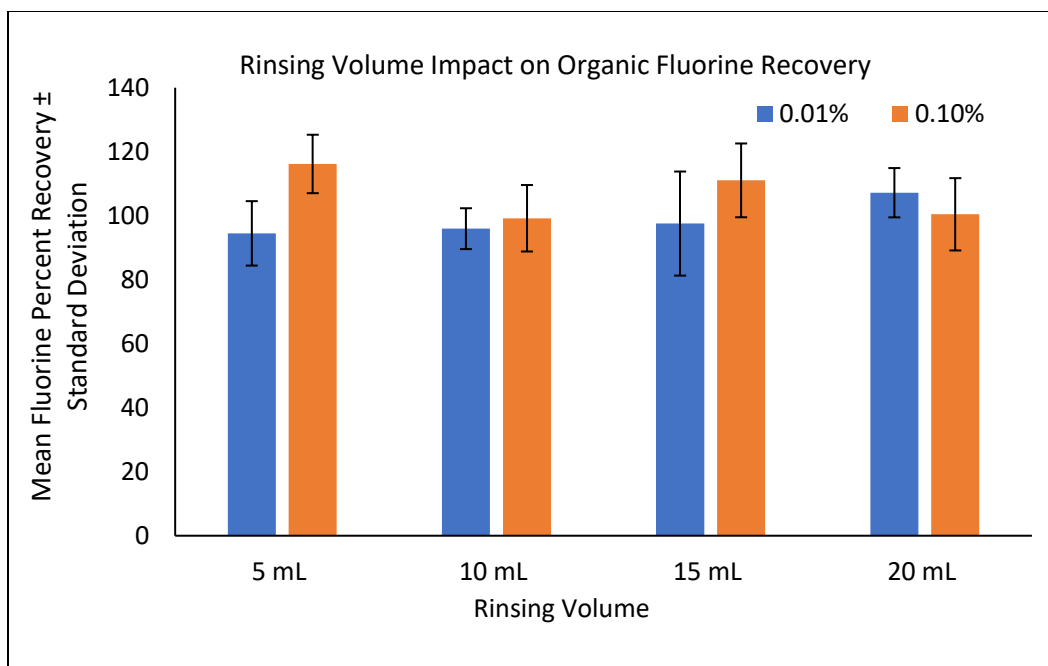
Figure 9. Rinsing volume analysis of organic fluorine adsorbed to two AC columns in series from ultrapure water. Results represent a mean of 3 replicates.

The next step in the AOF method is to combust the ACs, and because samples extracted from the EOF method must also be combusted, method optimizations for AOF and EOF combustion, absorption, and ion chromatography analysis were treated the same and are discussed after EOF method optimization in this report.

Limit of Quantification (LOQ) and Limit of Detection (LOD). The method limit of detection was calculated using three times the average signal of the procedural blank (n=7), and the method limit of quantification was calculated using nine times the average signal of the procedural blank (n=3). For comparison to previously published methods, values were calculated at sample volumes of 50 mL and 500 mL using a sample of ultrapure water containing sodium fluoride as 1 mg/L of fluoride. As mentioned previously, a larger sample volume can be used for trace analysis, however time of analysis and sample availability should be considered.

Table 5. Method LOD and LOQ Using Two Different Sample Volumes

Sample Volume	LOD (µg/L)	LOQ (µg/L)
50 mL	0.53	1.6
500 mL	0.33	1.0

Note: LOD and LOQ values in Table 5 are represented in concentrations of fluoride. For comparison to quantification methods for PFAS compounds, these values were converted to several common PFAS structures using a mass balance approach. Values are listed in Table 6.

Table 6. AOF Method LOD and LOQ Converted to Concentrations for Several PFAS Compounds

	HFPO-DA (µg/L)		PFOA (µg/L)		PFOS (µg/L)		ADONA (µg/L)	
	LOD	LOQ	LOD	LOQ	LOD	LOQ	LOD	LOQ
50 mL Sample Volume	0.8	2.7	0.7	2.5	0.8	2.5	0.9	2.8
500 mL Sample Volume	0.5	1.7	0.4	1.5	0.5	1.5	0.5	1.7

Extractable Organic Fluorine (EOF) Method Optimization

Background. A second extraction method, an extractable organic fluorine (EOF) approach was developed, evaluated, and optimized for recovery and method performance. Several key parameters were evaluated for maximum recovery of organic fluorine, lack of co-extraction of inorganic fluoride, linearity of calibration curves, detection limits, precision, and accuracy. The results of that parameterization are as follows.

Solid Phase Extraction (SPE) Cartridge Selection. Several SPE phases were compared for inorganic fluoride removal, as well as maximum percent recovery of organic fluorine. Since the PFAS compounds of interest for this method are acidic in nature, weak anion exchange phases and one universal reverse-phase sorbent were chosen for comparison. For inorganic removal comparison experiments, samples containing 1 mg/L of fluoride in ultrapure water were loaded onto the cartridges, and for percent recovery comparison experiments, ultrapure water samples were spiked with a standard mix of PFAS compounds 1-14 (Table 1) as 50 µg/L fluorine and loaded onto the cartridges. Results show that Strata WAX/GCB cartridges removed inorganic fluoride to non-detect levels, and these cartridges also had the highest percent recovery of organic fluorine when compared to other phases. Thus, *Strata WAX/GCB SPE cartridges were chosen* for further method optimization. (Table 7)

Unlike the other cartridges tested, the Strata WAX/GCB cartridges contain a layer of activated carbon beneath the solid phase polymer sorbent. The added activated carbon layer eliminates the need to place multiple different sorbent phases in series for the purpose of capturing a wider range of PFAS compounds.

Table 7. Solid Phase Extraction (SPE) Cartridge Comparison

SPE Cartridge	Percent Recovery	Inorganic Fluoride Retained from 1 mg/L F⁻ sample
Strata WAX/GCB	89	ND*
Strata X-AW	67	3.0 µg/L
Oasis WAX	62	ND*
Oasis HLB	73	ND*

* The limit of detection for fluoride by ion chromatography is 1.0 µg/L

Sample Pretreatment. For solid phase extraction, the pH of the sample should be adjusted in order to increase the binding efficiency of PFAS compounds to the sorbent in the SPE cartridge. For weak anion exchange sorbents, the optimal pH adjustment is typically two units above the target analyte's pK_a. Several sample pH values (3, 5, 6, 9, and 11) were evaluated using samples containing a mix of PFAS compounds

1-14 (Table 1) as 50 µg/L fluorine in ultrapure water (Figure 10). *Results show that the optimal sample pH adjustment is a pH of 5.*
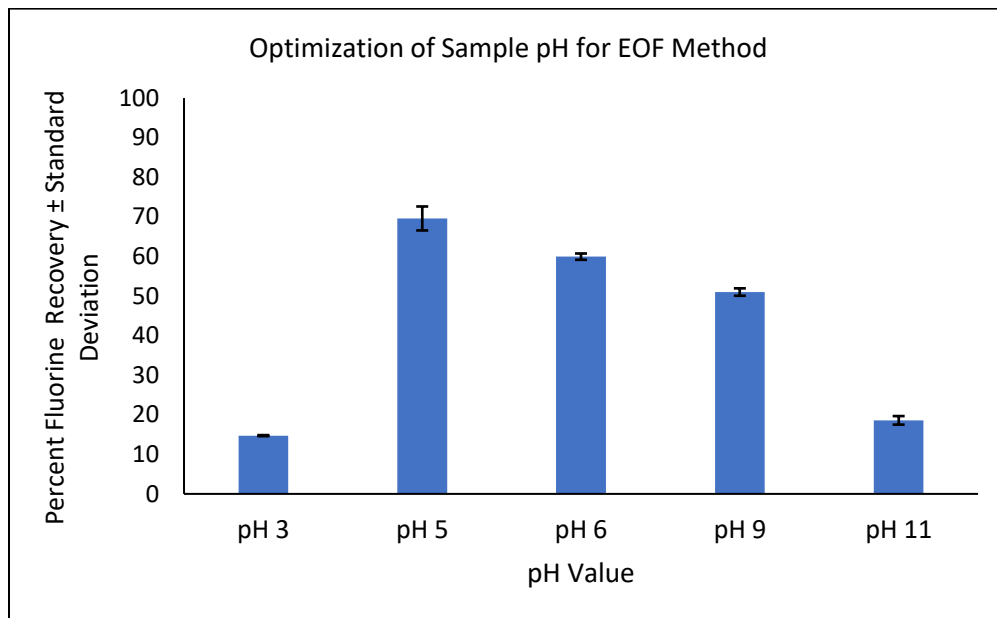

Figure 10. Sample pH adjustment analysis of organic fluorine from a PFAS mix spiked into ultrapure water. Results shown are fluoride concentrations measured from the absorption solution that represents recovered organic fluorine resulting from pyrolysis of the concentrated SPE eluent. Results represent a mean of duplicates.

Conditioning and Equilibration Steps. In addition to adjusting the pH of the sample, and before the sample is loaded onto the SPE cartridges, the cartridges must be conditioned and equilibrated for the purpose of removing background contamination and preparing the sorbent with a matrix similar to the sample matrix. A two-step conditioning is recommended by the SPE cartridge manufacturers, Phenomenx. Conditioning and equilibration steps were tested at various volumes (5, 10, 15, and 20 mL) using a standard mix of PFAS compounds 1-14 (Table 1) as 50 µg/L fluorine in ultrapure water (Figures 11-13). Solutions used for conditioning and equilibration were chosen based on results from elution composition experiments discussed later in this report. *Results show that a conditioning and equilibration volume of 10 mL is the most optimal volume* for this method.
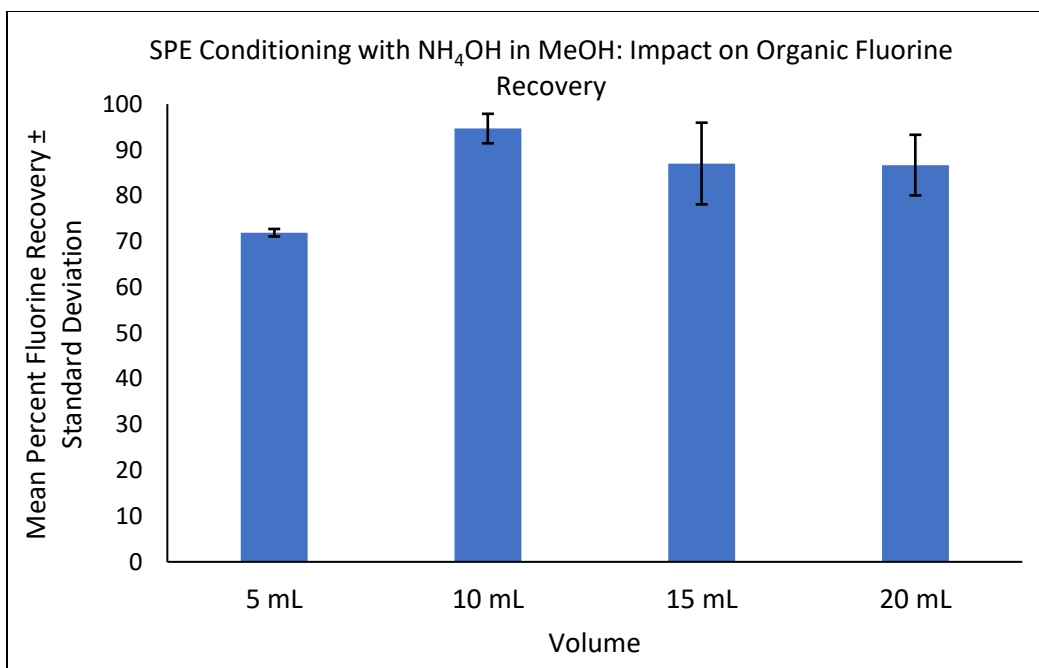
Figure 11. SPE conditioning volume analysis of organic fluorine using varying volumes of a 0.3% NH$_4$OH solution in methanol. Results shown are fluoride concentrations measured from the absorption solution that represents recovered organic fluorine resulting from pyrolysis of the concentrated SPE eluent. Results represent a mean of duplicates.
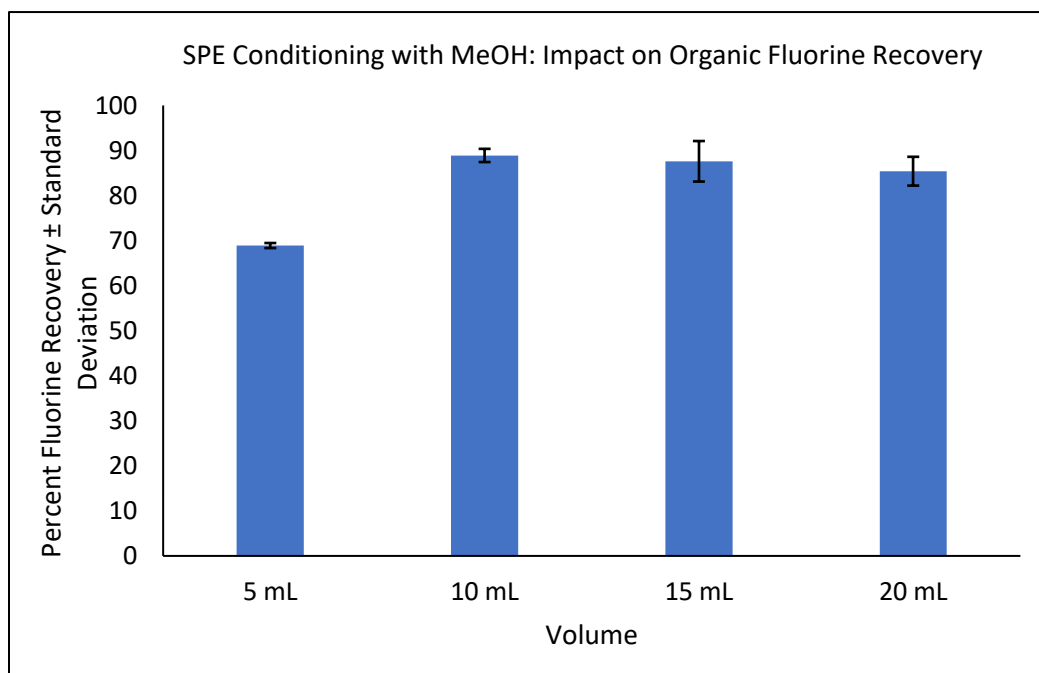
Figure 12. SPE conditioning volume analysis of organic fluorine using varying volumes of methanol. Results shown are fluoride concentrations measured from the absorption solution that represents recovered organic fluorine resulting from pyrolysis of the concentrated SPE eluent. Results represent a mean of duplicates.
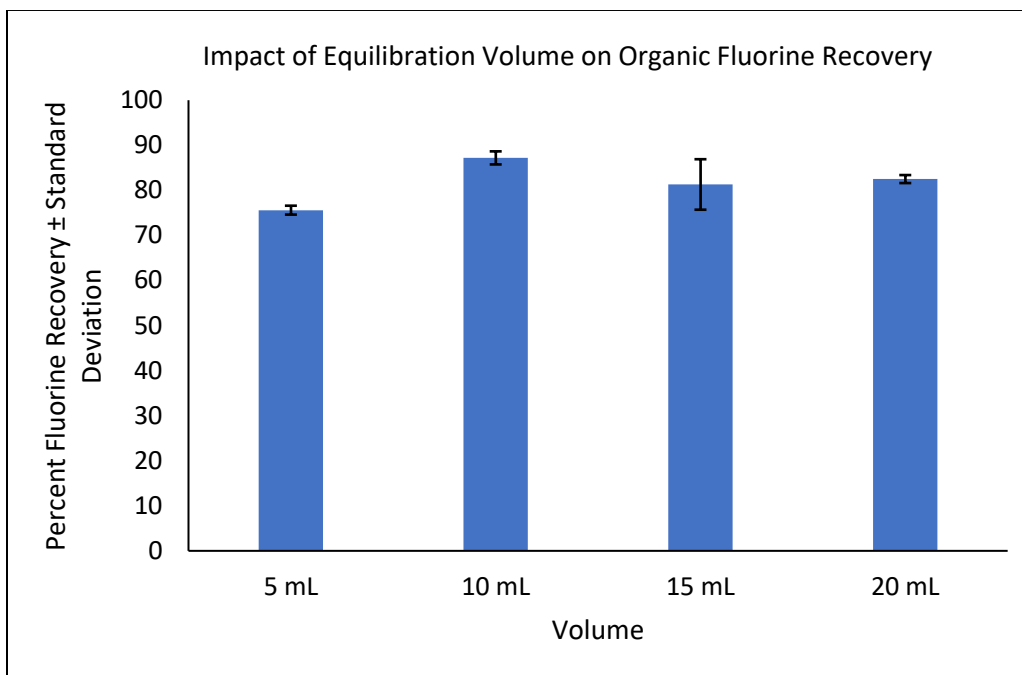
Figure 13. Equilibration volume analysis of organic fluorine using varying volumes of ultrapure water adjusted to a pH of 5. Results shown are fluoride concentrations measured from the absorption solution that represents recovered organic fluorine resulting from pyrolysis of the concentrated SPE eluent. Results represent a mean of duplicates.

Sample Volume. After the cartridges have been properly conditioned and equilibrated, the sample is then loaded onto the cartridges. Like AOF optimization, the volume of the sample was optimized in order to avoid overloading the cartridges, potentially rinsing organic fluorine to waste, while using a large enough volume for trace analysis. Samples of a standard mix of PFAS compounds 1-14 (Table 1) as 50 µg/L in ultrapure water were loaded onto cartridges at varying volumes (100, 200, 400, 600, 800, 1000 mL) (Figure 14). Volumes were chosen based on the amount of sorbent within the cartridge (200mg/50mg). At the time of these experiments, only one size Strata WAX/GCB cartridge was available for purchase; however, larger size cartridges are now available (500mg/50mg). Further experiments could be done with a larger cartridge size so that more volume of the sample can be loaded onto the cartridges to further lower the LODs and LOQs if samples are expected to contain trace levels of organic fluorine.

Experiments show a trend of decreased percent recovery organic fluorine as sample volume increases; however, an increased sample volume can also be used for low detection limits for trace analysis. Other factors to consider when choosing sample volume include sample analysis time and availability of sample. Due to the availability of process wastewater samples for this study, *a sample volume of 500 mL was chosen* for this method.
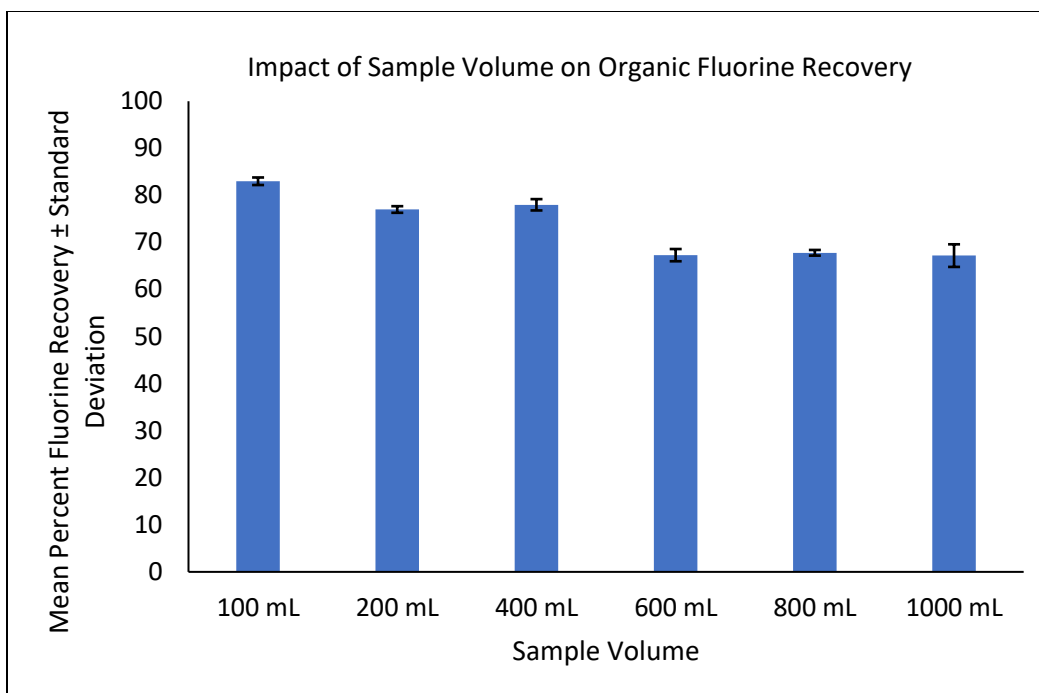
Figure 14. Sample volume analysis of organic fluorine using varying volumes of sample. Results shown are fluoride concentrations measured from the absorption solution that represents the recovered organic fluorine resulting from pyrolysis of the concentrated SPE eluent. Results represent a mean of duplicates.

Wash Step. Since inorganic fluoride is unavoidable in real samples, there is potential that inorganic fluoride from the sample may adsorb to the solid phase during sample loading. In order to remove inorganic fluoride, an aqueous wash step is employed after the sample has been loaded. Ammonium hydroxide was added to ultrapure water at various concentrations (1.0, 0.1, and 0.01%) and evaluated for inorganic fluoride removal using a 5 mg/L inorganic fluoride solution in ultrapure water (Figure 15). Based on the results from these experiments, ammonium hydroxide concentrations of 0.1 and 1.0% were evaluated at various volumes (5, 10, 15, and 20 mL) for percent recovery of organic fluorine using a standard mix of PFAS compounds 1-14 (Table 1) as 50 µg/L fluorine in ultrapure water (Figure 16). Results show that a higher percent ammonium hydroxide works more effectively for the removal of *inorganic fluoride*, but the opposite is true for percent recovery of *organic fluorine*. It was observed that using *10 mL of a wash solution containing 0.1% ammonium hydroxide yields a higher percent recovery of organic fluorine*. Thus, this was chosen as the wash step for this method.
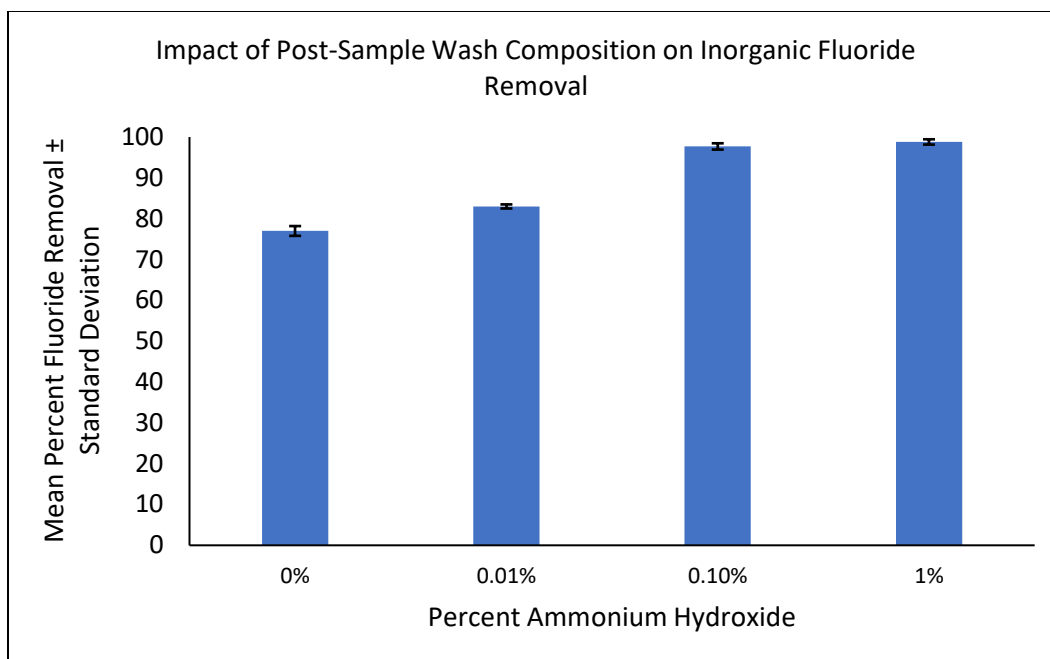
Figure 15. Inorganic fluoride removal using varying concentrations of ammonium hydroxide in ultrapure water. Results shown are fluoride concentrations measured from the absorption solution that represents the percent fluoride removed following pyrolysis of the concentrated SPE eluent. Results represent a mean of duplicates.
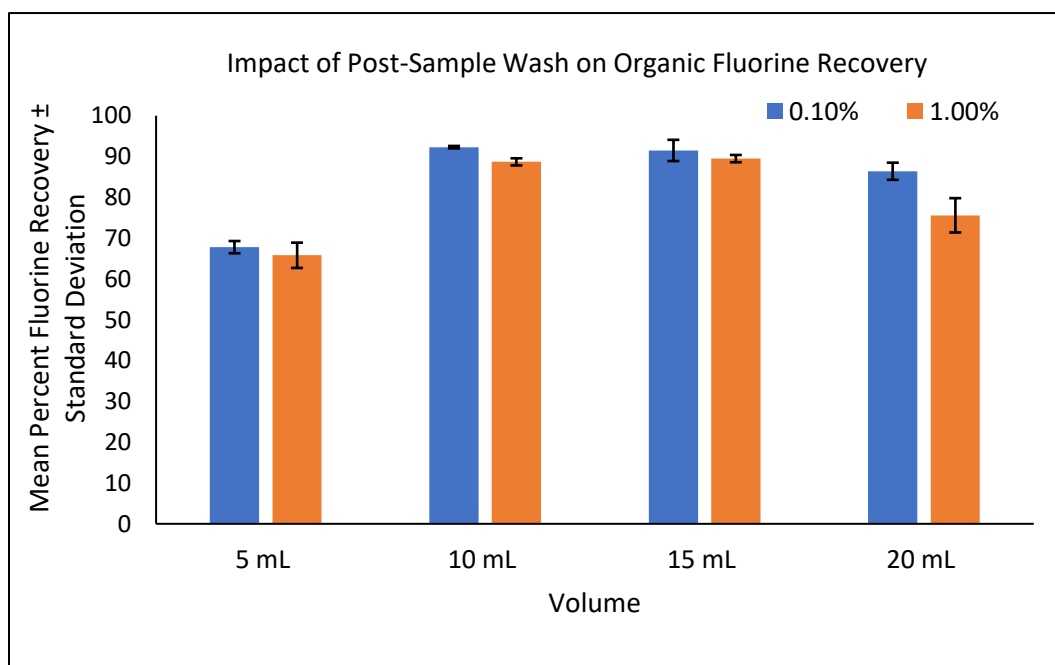

Figure 16. Organic fluorine recovery using varying wash volumes and concentration of ammonium hydroxide. Results shown are fluoride concentrations measured from the absorption solution that represents recovered organic fluorine resulting from pyrolysis of the concentrated SPE eluent. Results represent a mean of duplicates.

Drying Time. After the SPE cartridges are washed for the removal of interfering analytes, the cartridges must be dried under nitrogen gas to remove water before organics can be eluted with an organic solvent. The drying time of the cartridges was optimized by evaluating various drying times of 5, 10, 15, and 20 min using a standard mix of PFAS compounds 1-14 (Table 1) as 50 µg/L fluorine in ultrapure water (Figure 20). *A drying time of 10 minutes yielded the highest percent recovery of organic fluorine* and was therefore chosen as the drying time for this method.
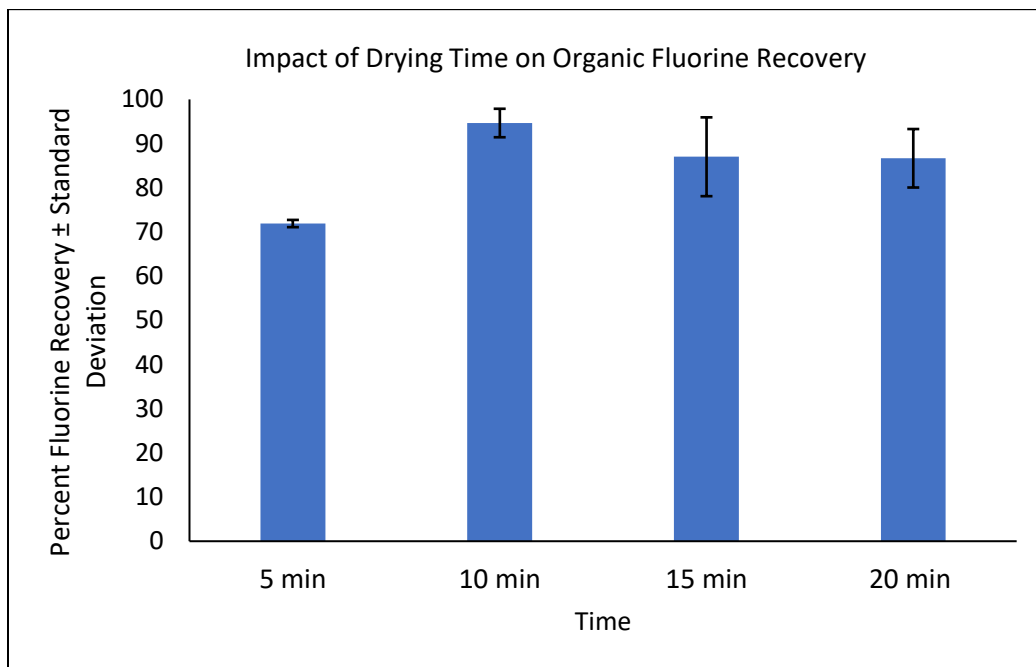
Figure 17. Recovery of organic fluorine resulting from varying the drying time of SPE cartridges under nitrogen gas after a wash solution was passed through the cartridges. Results represent a mean of duplicates.

Elution. When choosing an organic solvent for elution of organics, the polarity of the solvent should be similar to the polarity of the target analyte. Because PFAS compounds contain polar head groups, a strong polar organic solvent should be used for elution. Thus, methanol and acetonitrile were chosen to be compared as elution solutions. Various percent organic-to-aqueous solutions of both acetonitrile and methanol were evaluated using a standard mix of PFAS compounds 1-14 (Table 1) as 50 µg/L fluorine in ultrapure water for evaluating recovery of organic fluorine (Figures 18 and 19). Results show that acetonitrile is not as strong an eluent compared to methanol, and that in order *to recover the most organic fluorine as possible, 100% methanol should be used as the eluent*.
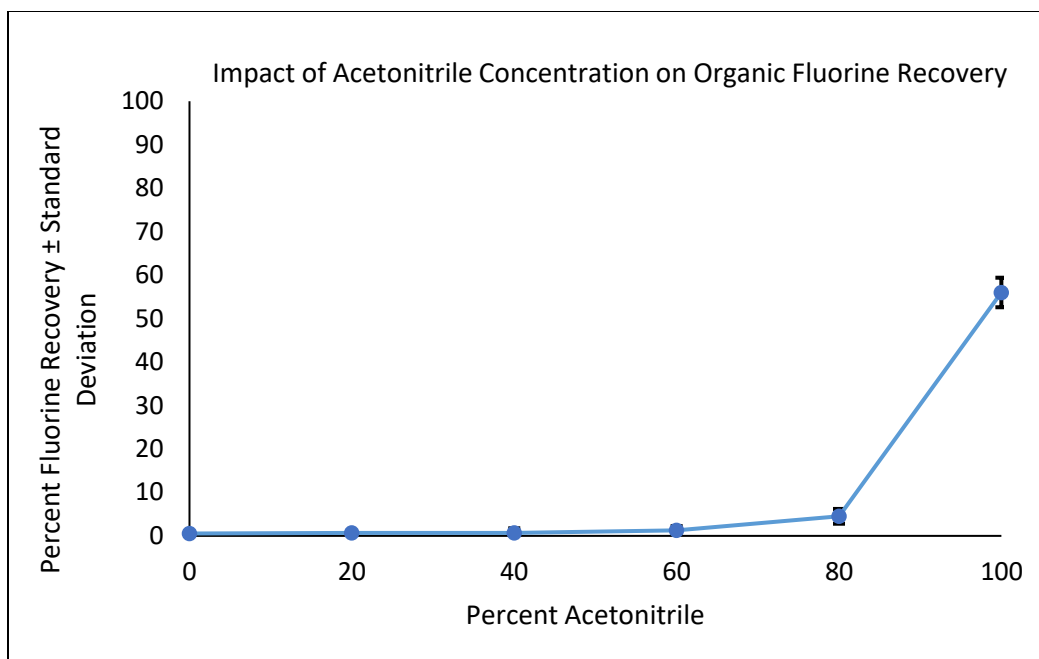
Figure 18. Recovery of organic fluorine using varying percent acetonitrile-to-ultrapure water to elute organics from the SPE cartridges. Results represent a mean of duplicates.
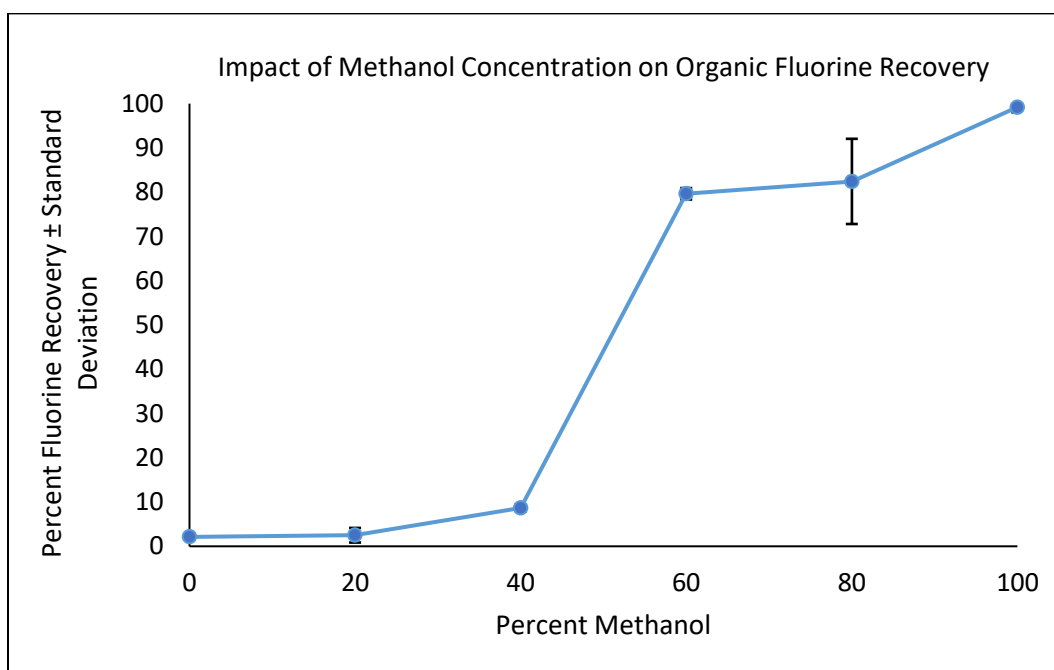

Figure 19. Recovery of organic fluorine using varying percent methanol-to-ultrapure water to elute organics from the SPE cartridges. Results represent a mean of duplicates.

Adding a more selective counter ion into the eluent can be used to displace target analytes effectively by selectively replacing the analyte on the sorbent bed. To further increase the elution of PFAS compounds, various concentrations of ammonium hydroxide (0.1, 0.3, 0.5, 1.0, and 2.0%) were added to methanol and evaluated for percent recovery of organic fluorine using a standard mix of PFAS compounds 1-14 (Table 1) as 50 μg/L fluorine in ultrapure water (Figure 20). Results showed that *adding 0.3% ammonium hydroxide to methanol increased the percent recovery of organic fluorine and was chosen as the composition of the eluent*.
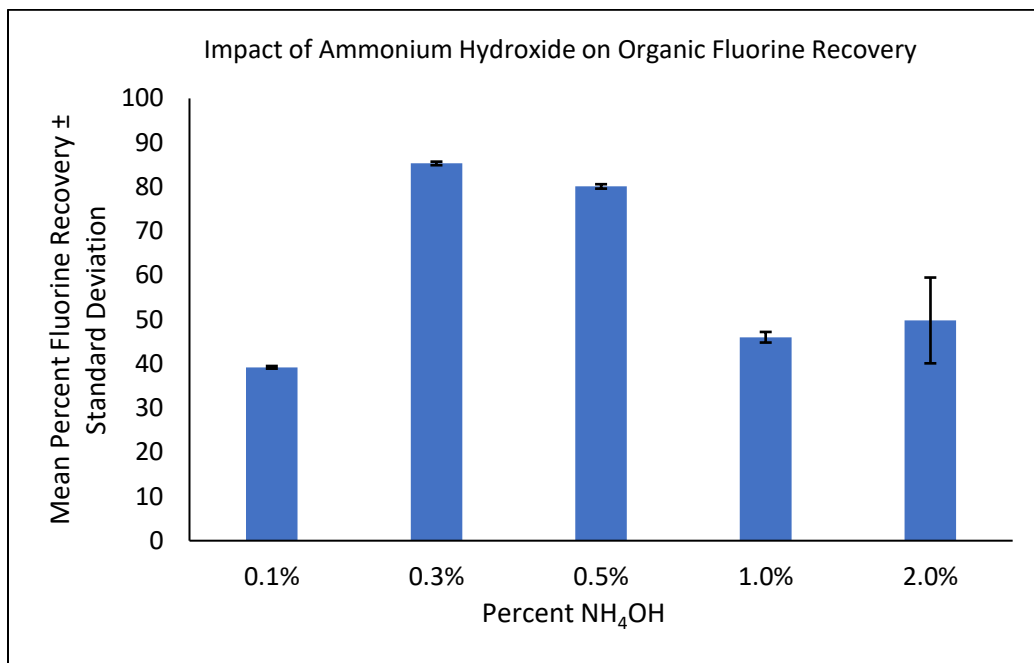

Figure 20. Recovery of organic fluorine from varying concentrations of ammonium hydroxide in methanol used to elute organics from the SPE cartridges. Results represent a mean of duplicates.

Once the eluent composition was determined, a pre-soak step, as well as eluent volume, was also evaluated for this method. The purpose of a pre-soak of the eluent on the cartridge is to allow the eluent to interact with the sorbent to give the solution more time to displace organics. Pre-soak times were varied (0, 1, 5, and 10 min) and compared for percent recovery of organic fluorine using a standard mix of PFAS compounds 1-14 (Table 1) as 50 μg/L fluorine in ultrapure water (Figure 21). The elution volume was evaluated at varying volumes (5, 10, 15, and 20 mL) and compared for percent recovery of organic fluorine using a standard PFAS mix in ultrapure water (Figure 22). *For the highest percent recovery of organic fluorine, a 10 mL elution volume of 0.3% ammonium hydroxide in methanol should be used after cartridges have been soaked with the elution solution for 5 minutes*.
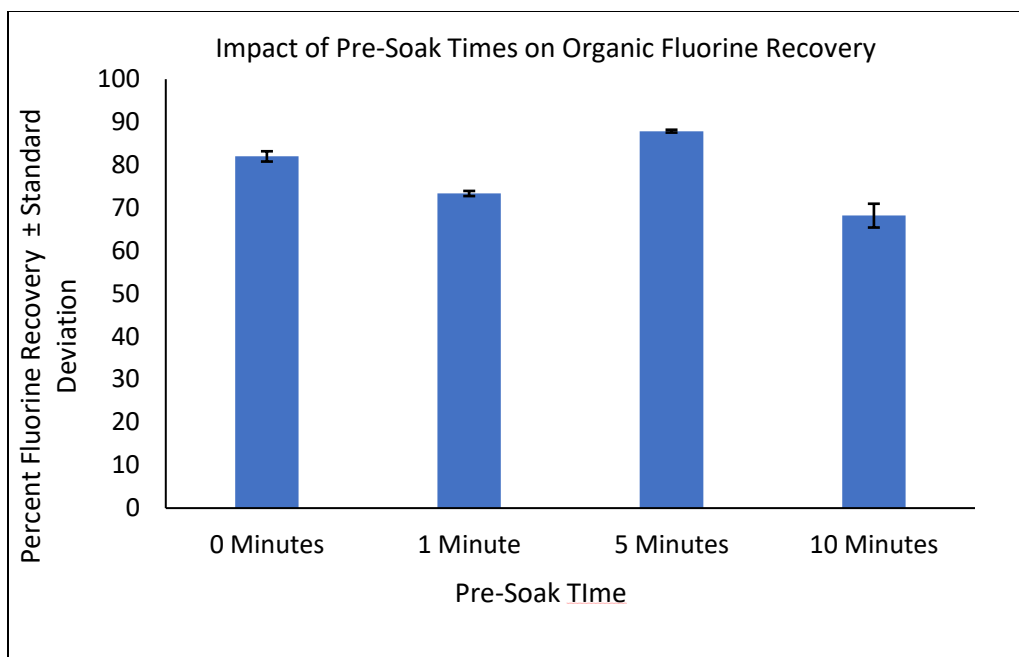
Figure 21. Recovery of organic fluorine resulting from varying pre-soak times of 0.3% ammonium hydroxide allowed to soak on the SPE cartridges until organics were eluted from the cartridges. Results represent a mean of duplicates.
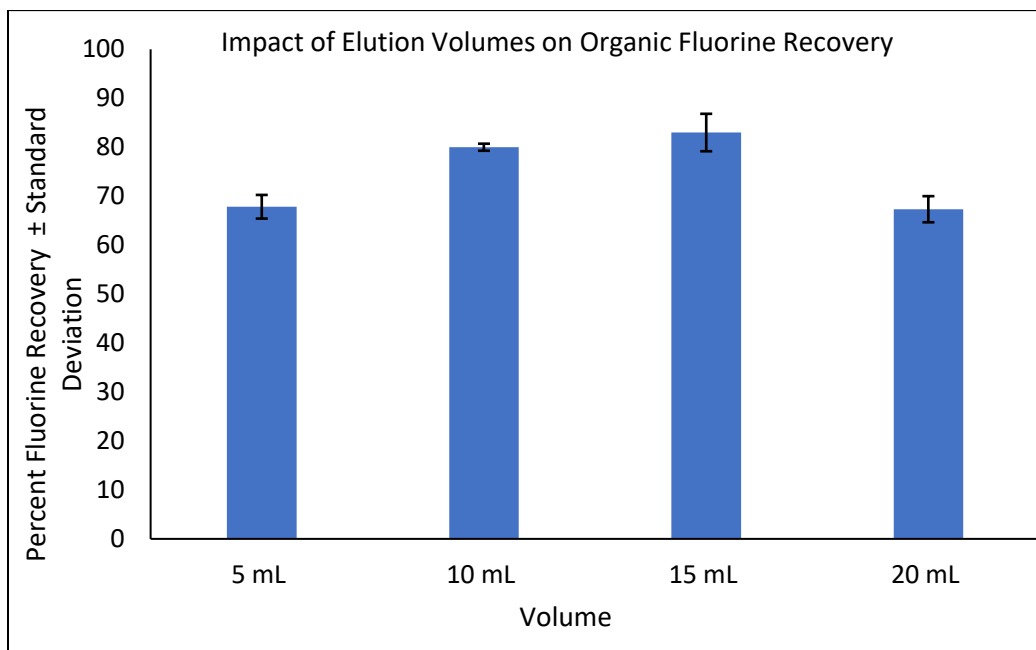

Figure 22. Recovery of organic fluorine from various elution volumes using 0.3% ammonium hydroxide in methanol. Results represent a mean of duplicates.

Limit of Detection (LOD) and Limit of Quantification (LOQ). The method limit of detection was calculated using three times the average signal of the procedural blank (n=7), and the method limit of quantification was calculated using nine times the average signal of the procedural blank (n=3). For comparison to

previously published methods, values were calculated at sample volumes of 500 mL using a sample of ultrapure water containing 1 mg/L of fluoride. As mentioned previously, a larger sample volume can be used for trace analysis, however time of analysis and sample availability should be considered.

Table 8. EOF Method LOD and LOQ Values

LOD (µg/L)	0.2
LOQ (µg/L)	0.5

LOD and LOQ values in Table 8 are represented in concentration of fluoride. For comparison to quantification methods for PFAS compounds, these values were converted to several common PFAS structures using a mass balance approach. Values are listed in Table 9.

Table 9. EOF Method LOD and LOQ Converted to Concentrations of Several PFAS Compounds

	HFPO-DA (µg/L)		PFOA (µg/L)		PFOS (µg/L)		ADONA (µg/L)	
	LOD	LOQ	LOD	LOQ	LOD	LOQ	LOD	LOQ
500 mL Sample Size	0.5	0.8	0.4	0.7	0.5	0.8	0.5	0.9

Combustion. After extraction, samples from either AOF or EOF extraction methods are then placed into a ceramic boat for combustion in order to *break the carbon-fluorine bond*. Ceramic boats are pre-baked before combustion to minimize carryover by lowering background fluoride levels. Pre-bake times were evaluated at varying times (0, 5, and 10 min) using empty ceramic boats and analyzing the absorption solution for fluoride. Results show that *a pre-bake time of 10 minutes lowered background levels to below detection* (Table 10), and was therefore used for this method.

Table 10. Pre-bake Time for Ceramic Boats

Time (minutes)	Inorganic Fluoride (µg/L)
0	7.1
5	6.9
10	ND*

***The detection limit for fluoride by ion chromatography is 1.0 µg/L.

Once the ceramic boats have been pre-baked, samples can be placed into the boats for combustion in the quick furnace. For the AOF extraction technique, the AC from both AC columns can be directly placed into boats using a T-tool to push the material out of the quartz columns. Concentrated samples (SPE eluent) from the EOF extraction technique can be directly injected into ceramic boats containing quartz wool. The combustion time of the samples was optimized to ensure complete combustion of organics by directly combusting a standard mix of PFAS compounds 1-14 (Table 1) at two different concentrations (50 and 100 µg/L as fluorine) in ultrapure water at varying total combustion times (5, 10, and 15 min) (Figure 23). Combustion times greater than 15 minutes were unable to be evaluated due to manufacturer safety settings. No significant difference was found between a combustion time of 10 and 15 minutes, so for a

shorter analysis time, *a combustion schedule of 260 sec at the end position, 200 sec at the cooling position, and 200 sec at the home position for a total time of 10.6 minutes* was chosen for this method.

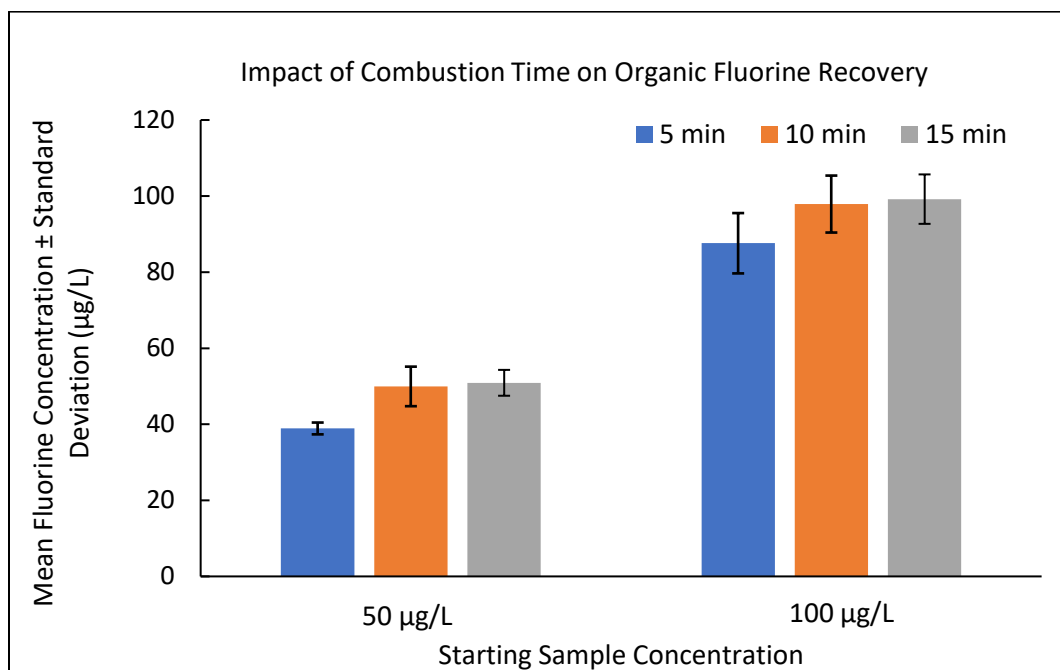**Impact of Combustion Time on Organic Fluorine Recovery**

Figure 23. Total organic fluorine concentration resulting from varying combustion times. Results represent a mean of duplicates.

Little data exists for the temperature needed for complete combustion of PFAS compounds. *To ensure the most complete combustion as possible, a combustion temperature of 1000°C, the highest possible temperature for this instrument (due to safety precautions from the manufacturer), is used for this method.*

Absorption of Effluent Gasses. After combustion, effluent gasses are bubbled into an absorption solution often referred to as a trapping solution. The absorption solution composition was optimized for highest percent recovery of fluoride using a solution of inorganic fluoride in ultrapure water. Samples were directly spiked into ceramic boats containing quartz wool and combusted so that fluoride was converted into HF gas. HF gas is then trapped within the aqueous solution in which an equilibrium exists between aqueous HF and inorganic fluoride with hydrogen (Eq. 3). To drive the equilibrium toward producing more inorganic fluoride, a buffer solution can be added to the absorption solution.

$$HF \rightleftharpoons H^+ + F^- \qquad \text{Eq. (3)}$$

Various buffer solutions were evaluated at different concentrations (0.1 and 1 mM) with and without hydrogen peroxide for fluoride recovery. Potential for coelution of buffer ions with fluoride in ion chromatography analyses was also evaluated. Hydrogen peroxide was added to the solution as a reducing agent for the purpose of trapping potentially overly oxidized fluoride (Figures 24 and 25). Separate experiments were done to optimize the concentration of hydrogen peroxide (0.1, 0.5, and 1.0 %) in 1 mM

buffer solutions (Figure 26). *For the highest recovery of fluoride, an absorption solution of 1 mM sodium bicarbonate buffer containing 1.0 % hydrogen peroxide was used for this method.*
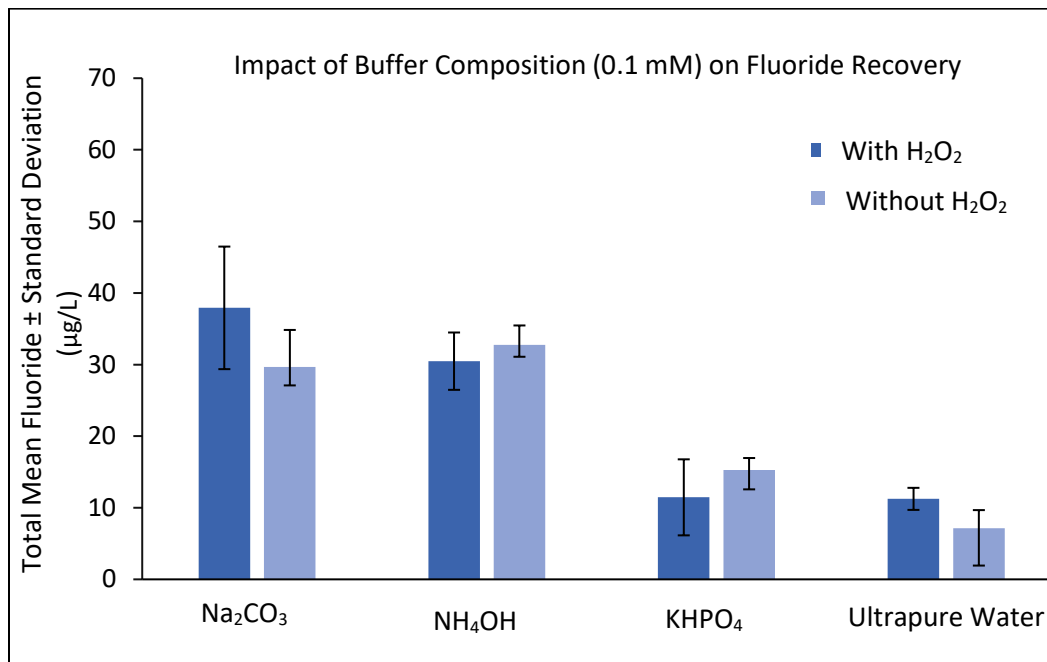

Figure 24. Total inorganic fluoride recovery from selected 0.1 mM buffer solutions with and without hydrogen peroxide. Results represent a mean of duplicates.
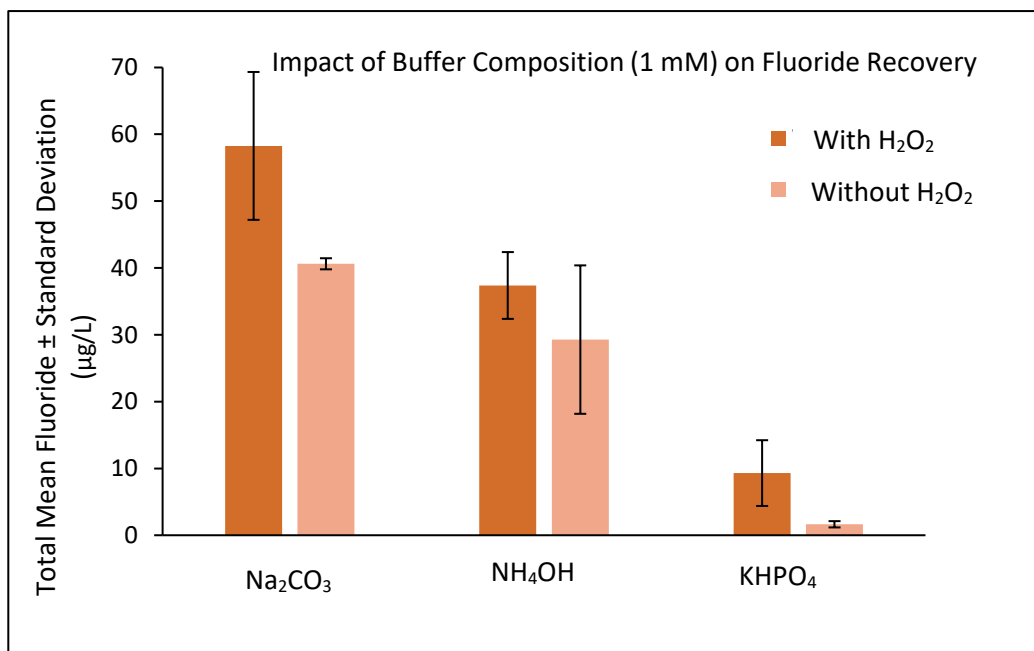

Figure 25. Total inorganic fluoride recovery from selected 1 mM buffer solutions with and without hydrogen peroxide. Results represent a mean of duplicates.
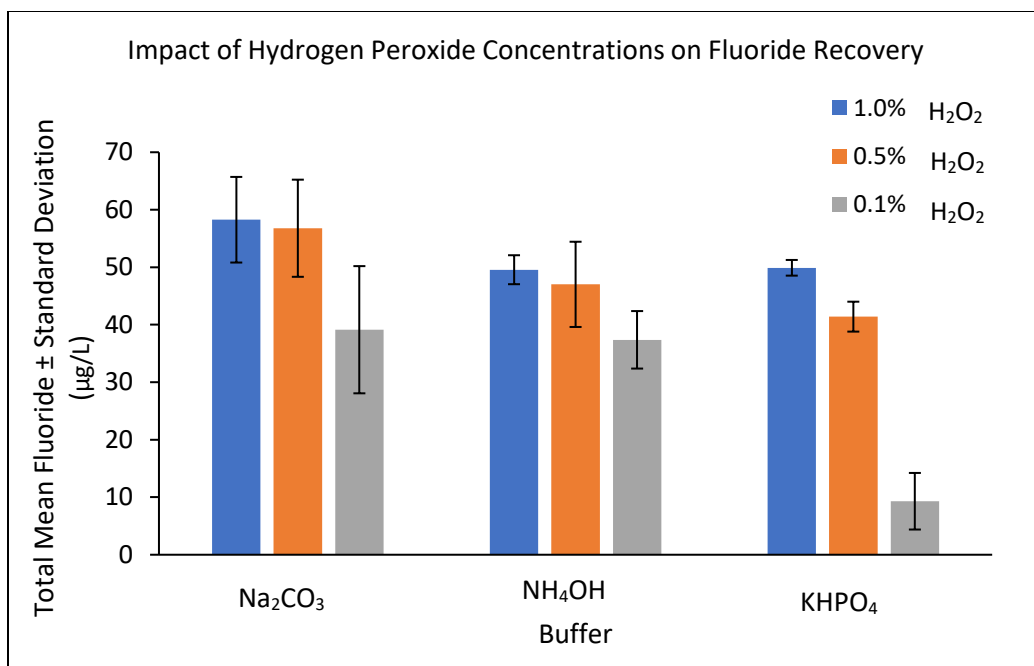
Figure 26. Total inorganic fluoride recovery from selected 1 mM buffer solutions with varying concentrations of hydrogen peroxide. Results represent a mean of duplicates.

Ion Chromatography Analysis

Once organic fluorine is converted into inorganic fluoride and trapped in the absorption solution, the sample can then be analyzed for fluoride using ion chromatography. Several key parameters were optimized.

Eluent. Ion chromatography utilizes an eluent as the "carrier" portion of the mobile phase for chromatography. Because ion chromatography uses a conductivity detector, the baseline signal of the chromatogram is converted to the conductivity of the eluent used. If carbonate is used as the eluent, the baseline signal is higher than that of water, causing a characteristic water dip (Figure 27). The water dip appears at the beginning of the chromatogram, which is problematic when quantifying fluoride. Due to fluoride's strong electronegative ionic characteristics, fluoride elutes towards the beginning of the chromatographic run. We observed that as the analytical and guard columns use and lifetime increases, the fluoride peak moves closer to the water dip, and can eventually elute within the water dip. To avoid the water dip interference with the fluoride signal, the eluent was switched to sodium hydroxide for improved chromatography (Figure 28). The water dip is not observed with a hydroxide eluent since the baseline signal is lowered to a conductivity closer to water. Another advantage to switching to hydroxide as the eluent was that an eluent generator could be used with the Thermo Dionex Integrion system to create an eluent gradient for better separation of peaks. Thus, for fluoride analysis, *a hydroxide eluent using an eluent generator is recommended*.
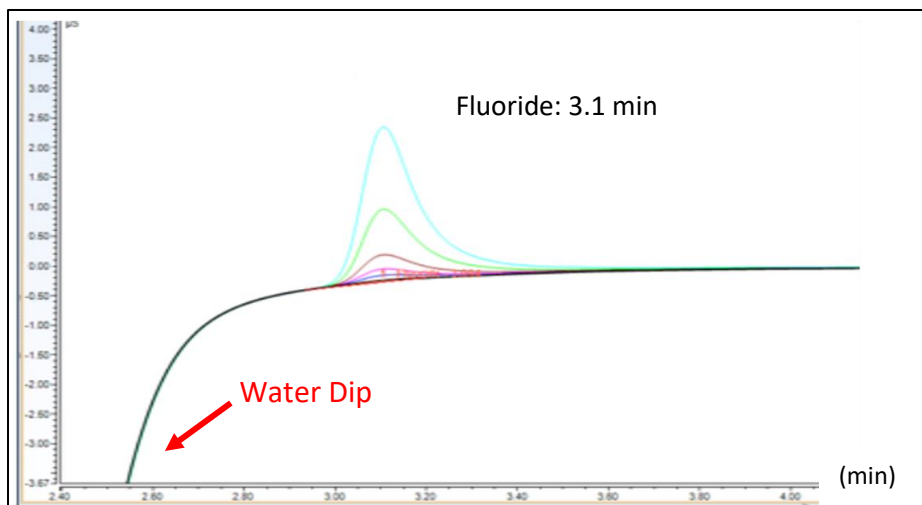
Figure 27. Ion chromatogram of the peak from fluoride in ultrapure water using carbonate as the eluent. Shown to the left of the chromatogram is the characteristic water dip.
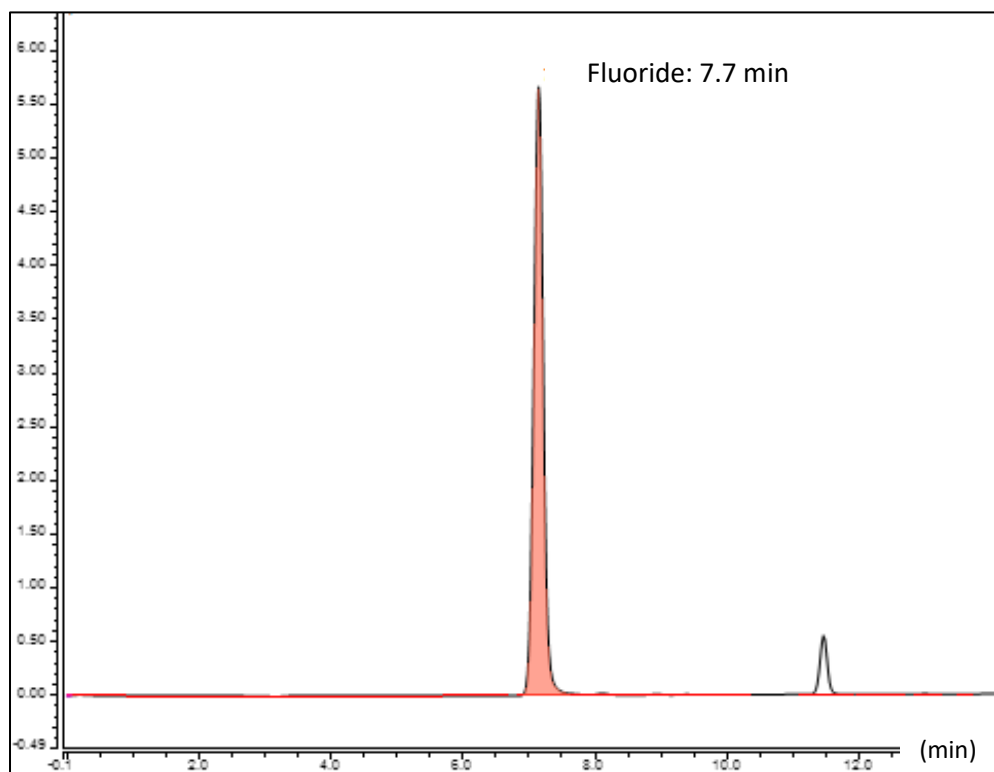

Figure 28. Ion chromatogram of the peak from fluoride in ultrapure water using hydroxide as the eluent. No water dip is observed.

Column. Several IC columns were evaluated for the best chromatographic separation of fluoride from potential interfering peaks in the sample, as well as maximum sensitivity of the signal. Columns were evaluated for coelution using a solution of sodium fluoride, sodium sulfate, and sodium nitrate in

ultrapure water. Anions chosen for coelution evaluation were based on example applications from the manufacturer. Column efficiency was evaluated throughout method optimization by running check standards and comparing signals to check standards from previous runs. For the two IC columns best for analysis of anions with a hydroxide eluent (AS16 and AS20), chloride was found to interfere with the fluoride signal using the AS16 column (Figure 29); however, the AS20 column provided excellent separation and was chosen as the analytical column (Figure 30).
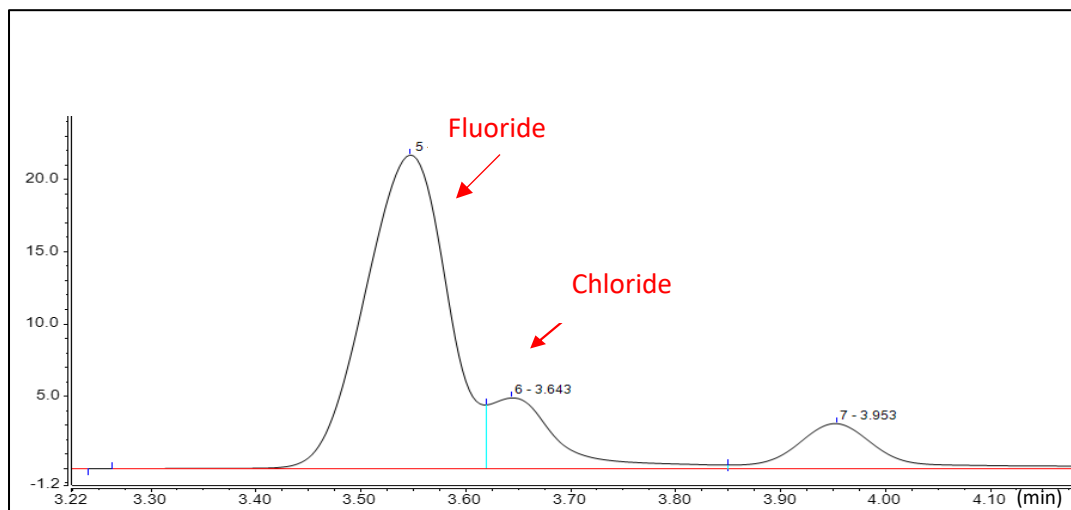

Figure 29. Ion chromatogram of the fluoride and chloride peaks in ultrapure water using the AS16 column and hydroxide eluent.
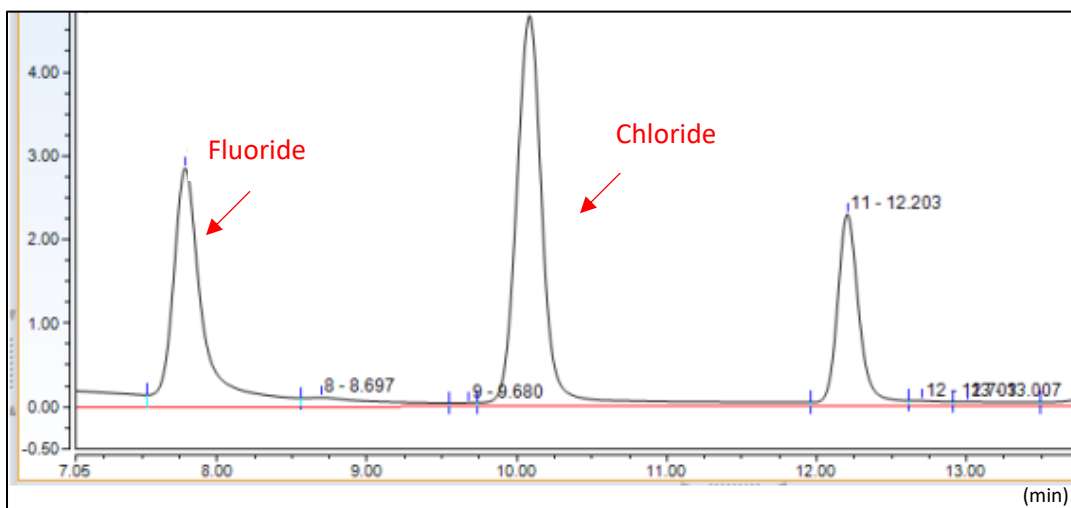

Figure 30. Ion chromatogram of the fluoride and chloride peaks in ultrapure water using the AS20 column and hydroxide eluent.

Calibration. Two stock solution mixes of 10 and 100 mg/L sodium fluoride in ultrapure water were used to prepare a calibration curve (1, 5, 10, 20, 30, 75, 100, 200, 300, 500, and 750 µg/L) with a coefficient of

determination ≥ 0.99 in the IC system. Limits of quantification for fluoride were 1.0 μg/L. Calibration curves were prepared every month and checked before each run with two freshly prepared calibration point checks (20 and 75 μg/L).

FINAL OPTIMIZED METHODS

AOF Optimized Method

The final optimized adsorbable organic fluorine (AOF) method is as follows:

1. Filter samples with a 0.22 μm filter to remove particulates and solids.

2. Analyze samples for background inorganic fluoride using ion chromatography. If samples contain more than 5 mg/L inorganic fluoride, dilute samples in order to avoid false high signal for total organic fluorine measurements.

3. Analyze samples for dissolved organic carbon (DOC) in order to determine if dilution is necessary to avoid breakthrough of the analytes.

4. Adjust sample pH to < 1 with concentrated nitric acid.

5. Pass samples (50 mL or 500 mL) through two activated carbon (AC) columns in series.

6. Pass 15 mL of rinsing solution containing 0.1% ammonium hydroxide in ultrapure water through both AC columns in series.

7. Insert both ACs into one ceramic boat that has been pre-baked for 10 minutes using a T-tool.

8. Insert the ceramic boat containing the sample into the furnace for 10 minutes at 1000°C to convert organically bonded fluorine into HF gas.

9. Collect off-gasses with an aqueous solution containing 1 mM sodium bicarbonate and 1.0% hydrogen peroxide. The aqueous solution is received in a 40 mL centrifuge tube that is weighed before and after analysis in order to obtain the total weight of the sample.

10. Analyze the absorption solution containing the sample for fluoride with ion chromatography (using AS20 column and hydroxide eluent).
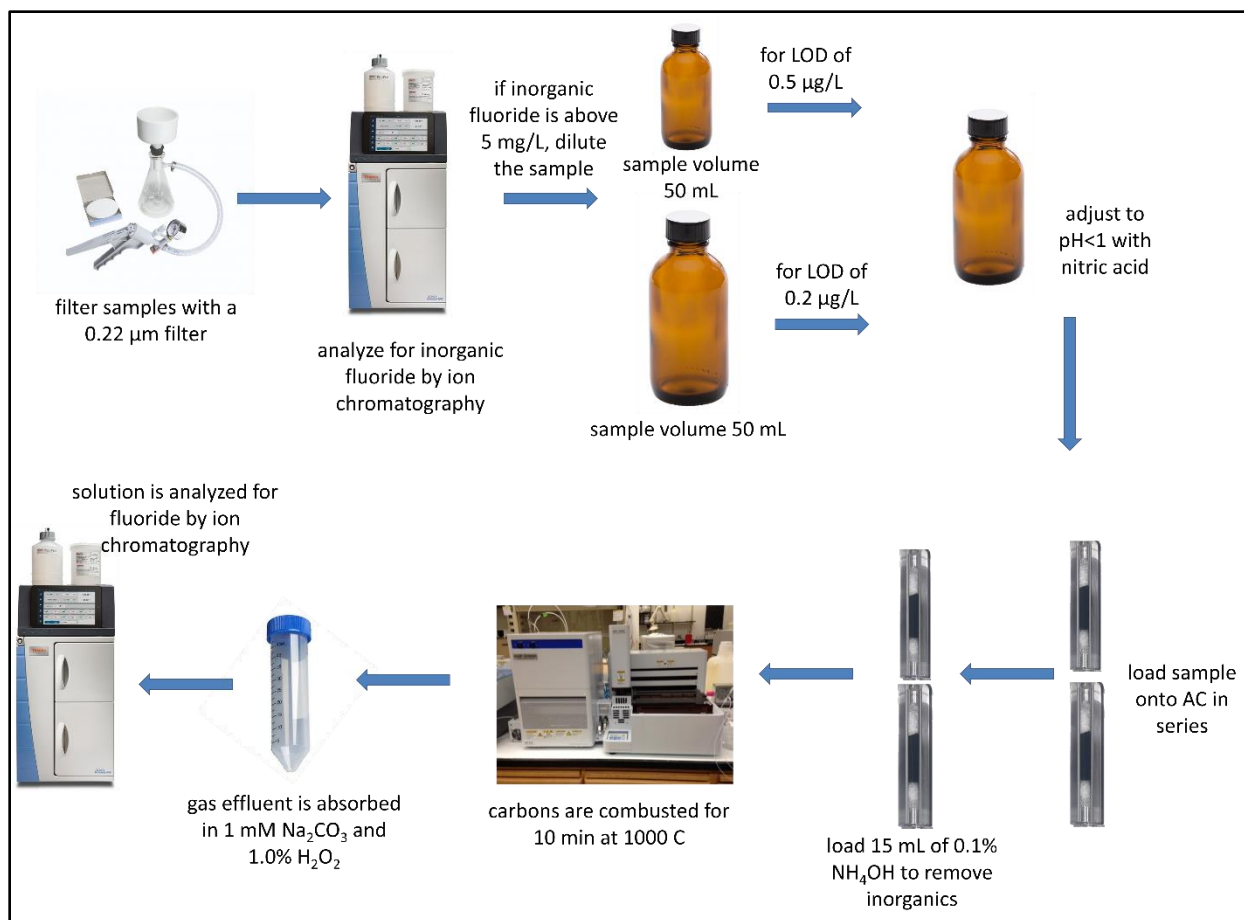
Figure 31. Final optimized AOF method.

AOF Method Evaluation

To evaluate the efficiency of the finalized AOF method, individual PFAS standards were tested for percent recovery of organic fluorine in ultrapure water, as well as river water, at a volume of 50 mL (Figure 32 and 33). River water was from the Broad River (Columbia, SC) containing dissolved organic carbon (DOC) of 2.4 mg/L. PFAS mix percent recoveries in ultrapure water and river water were evaluated at a volume of 50 mL and 500 mL (Table 11) using a PFAS mix containing all 39 standards as 50 µg/L fluorine. Percent recoveries slightly decreased in river water compared to ultrapure water potentially due to matrix effects. It can be expected that with higher DOC in a sample, the percent recovery of organic fluorine may decrease due to other organics outcompeting PFAS for sorption sites on the AC. While in general, shorter chain PFAS compounds yielded lower percent recoveries compared to longer chain PFAS compounds, no consistent trend was observed with increasing chain lengths.

Table 11. PFAS Mix Recoveries

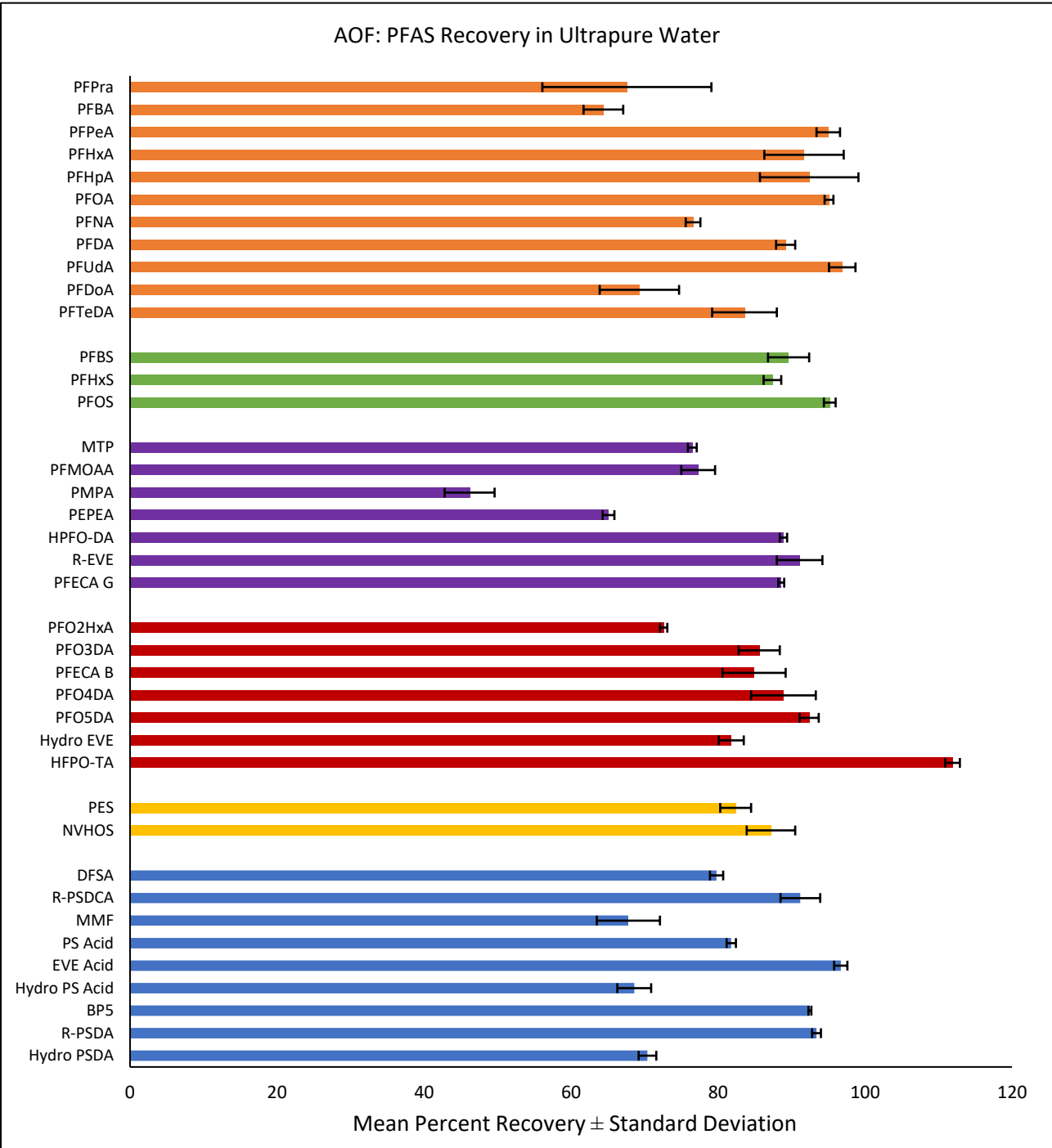|	50 mL Sample Volume	500 mL Sample Volume
Recovery in Ultrapure Water	87%	79%
Recovery in River Water	81%	72%

Figure 32. Organic fluorine recovery of 39 PFAS standards individually spiked into ultrapure water by AOF analysis. Percent recoveries range from 46-112%. PFAS standards are organized by PFAS class based on organic functional groups. Results represent a mean of 3 replicates.
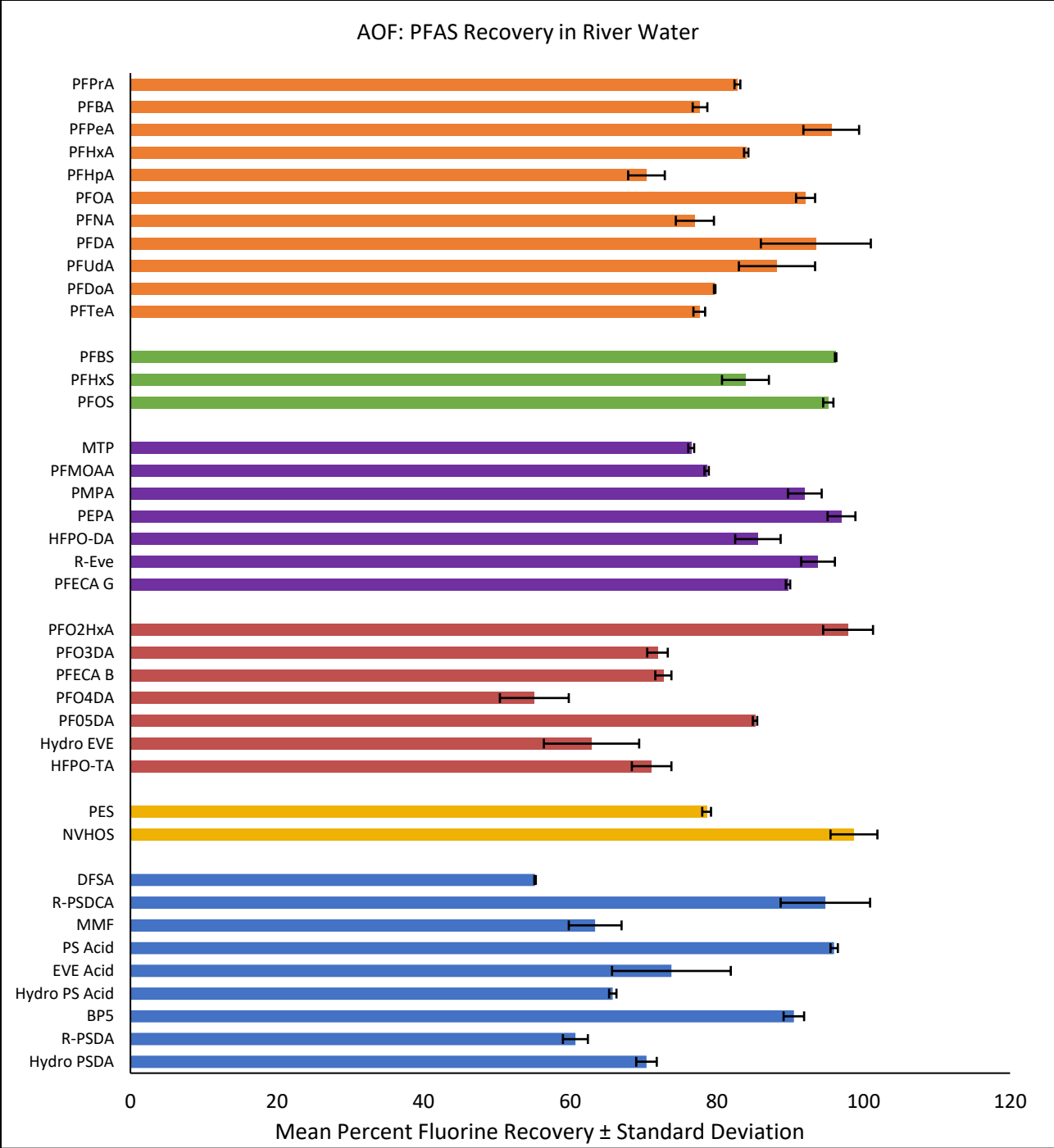
Figure 33 . Percent organic fluorine recovery of 39 PFAS standards individually spiked into river water by AOF analysis. Recoveries range from 55-98%. PFAS standards are organized by PFAS class based on organic functional groups. Results represent a mean of 3 replicates.

EOF Optimized Method

The final optimized extractable organic fluorine (EOF) method is as follows:

1. Filter samples to remove particulates and solids using a 0.22 μm filter.

2. Analyze samples for background inorganic fluoride using ion chromatography. If samples contain more than 5 mg/L inorganic fluorine, dilute samples in order to avoid false high signal for total organic fluorine measurements.

3. Adjust samples to pH 5 with concentrated nitric acid or concentrated sodium hydroxide.

4. Condition Strata WAX/GCB cartridges with 10 mL of 0.3% NH_4OH in methanol.

5. Condition cartridges with 10 mL of methanol.

6. Equilibrate cartridges with 10 mL of ultrapure water at pH 5.

7. Load 500 mL of sample onto cartridges at a flow rate of 4 mL/min.

8. Wash cartridges with 10 mL of 0.1% ammonium hydroxide in ultrapure water.

9. Allow cartridges to dry for 10 minutes under nitrogen gas.

10. Load 5 mL of 0.3% ammonium hydroxide in methanol onto cartridge and allow to soak for 5 minutes.

11. Elute organics from the cartridges with 10 mL of 0.3% ammonium hydroxide in methanol.

12. Concentrate eluted organics to 200 μL under nitrogen gas.

13. Directly inject 100 μL into a ceramic boat containing quartz wool that has been pre-baked for 10 minutes.

14. Insert the ceramic boat containing the sample into the furnace for 10 minutes at 1000°C to convert organically bonded fluorine into HF gas.

15. Collect the off-gasses with an aqueous solution containing 1 mM sodium bicarbonate and 1.0% hydrogen peroxide. The aqueous solution is received in a 40 mL centrifuge tube that should be weighed before and after analysis in order to obtain the total weight of the sample.

16. Analyze the absorption solution containing the sample for fluoride with ion chromatography (using AS20 column and hydroxide eluent).
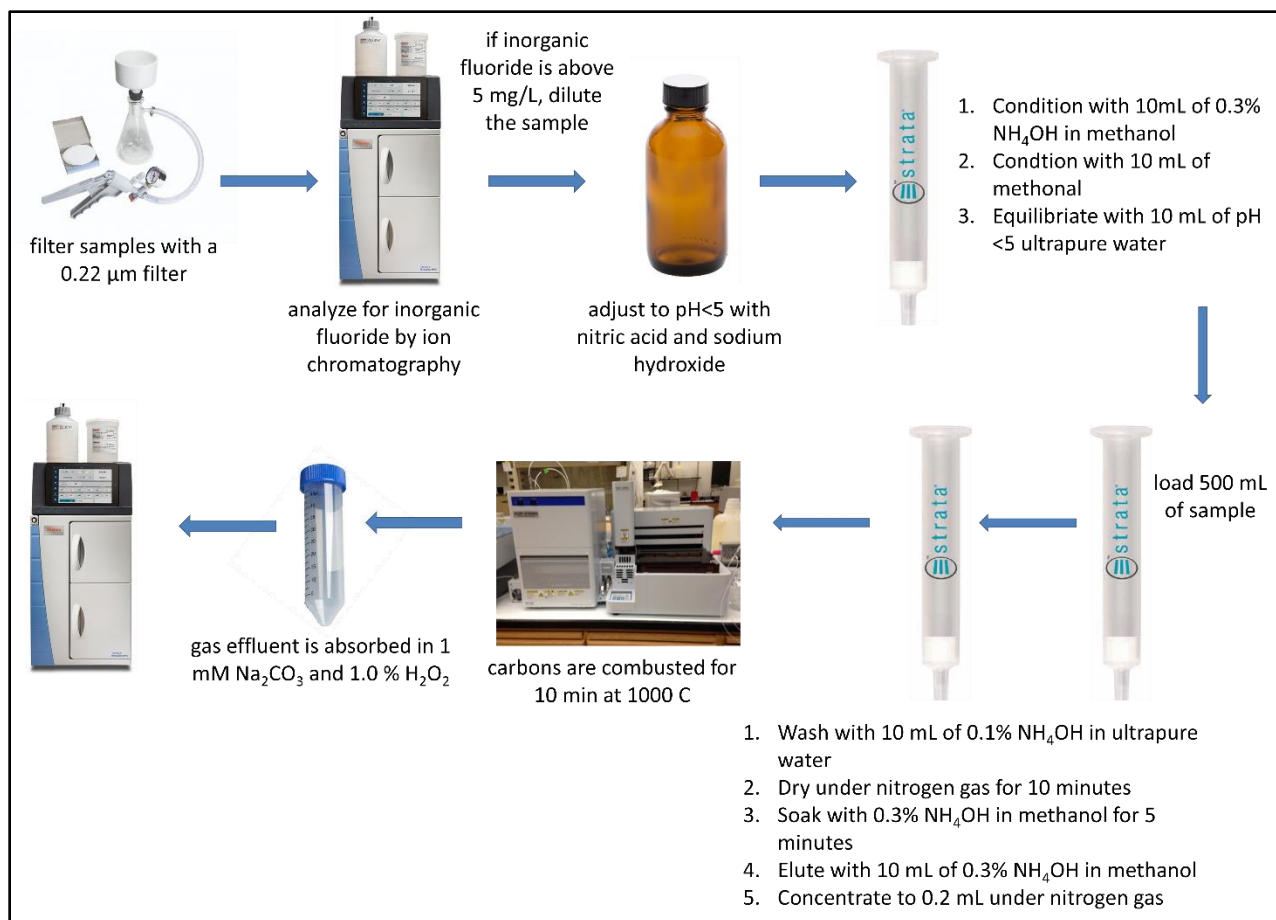
Figure 34. Final optimized EOF method.

EOF Method Evaluation

To evaluate the efficiency of the finalized EOF method, 39 individual PFAS standards were evaluated for percent recovery of organic fluorine in ultrapure water and river water (Figures 35 and 36). PFAS mix recoveries in ultrapure water and river water were evaluated at a sample volume of 500 mL (Table 12) using a mix of all 39 PFAS standards at as 50 µg/L fluorine. Percent recoveries decreased slightly in river water compared to ultrapure water potentially due to matrix effects. While in general, shorter chain PFAS compounds yielded lower percent recoveries compared to longer chain PFAS compounds, no consistent trend was observed with increasing chain lengths.

Table 12. EOF PFAS Mix Recoveries (39 PFAS at 50 ug/L fluorine each)

	Ultrapure Water	River Water
Percent Recovery	91%	87%

Figure 35. Organic fluorine recovery of 39 PFAS standards individually spiked into ultrapure water by EOF analysis. Percent recoveries range from 72-99%. PFAS standards are organized by PFAS class, based on organic functional groups. Results represent a mean of duplicates.
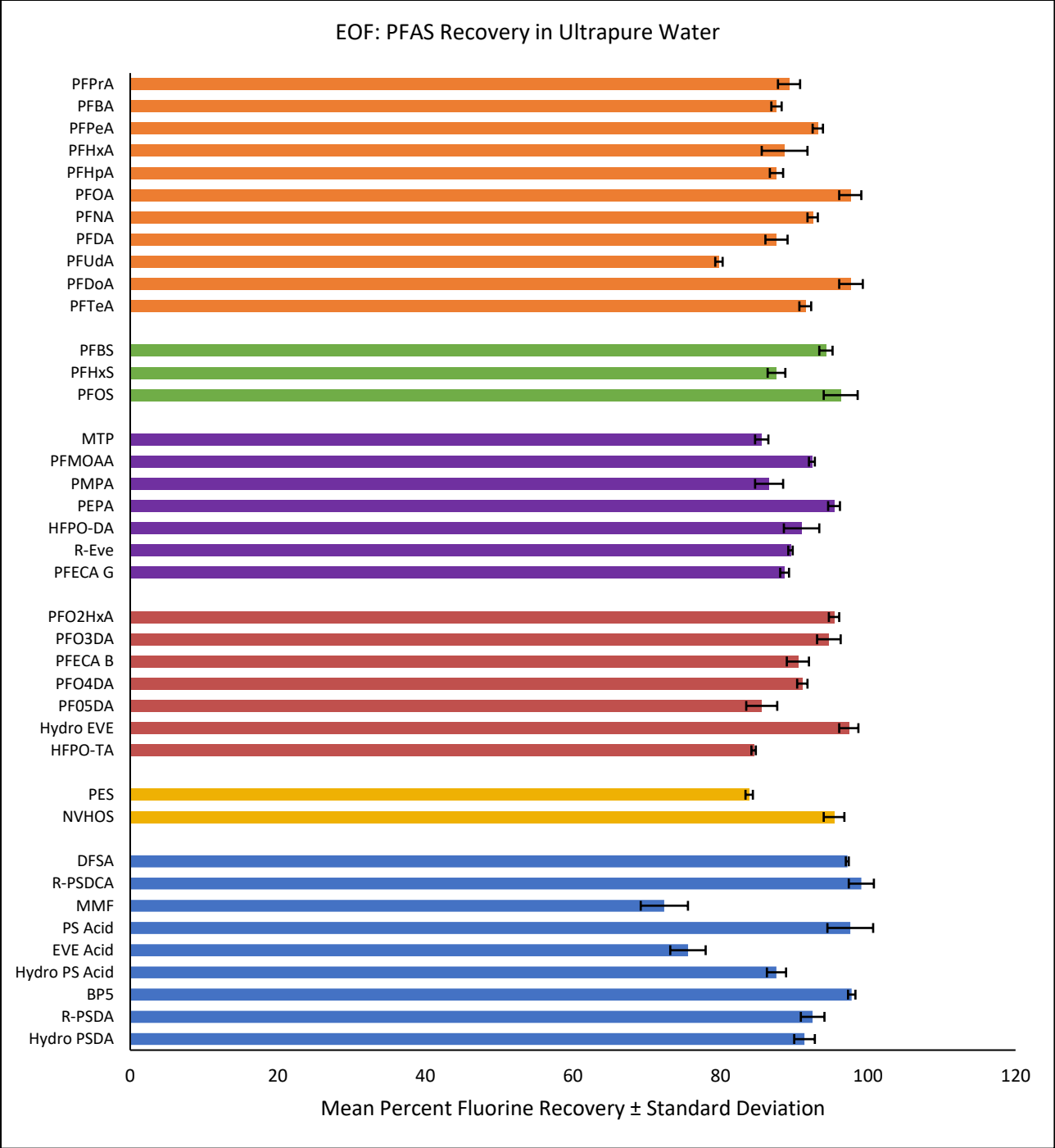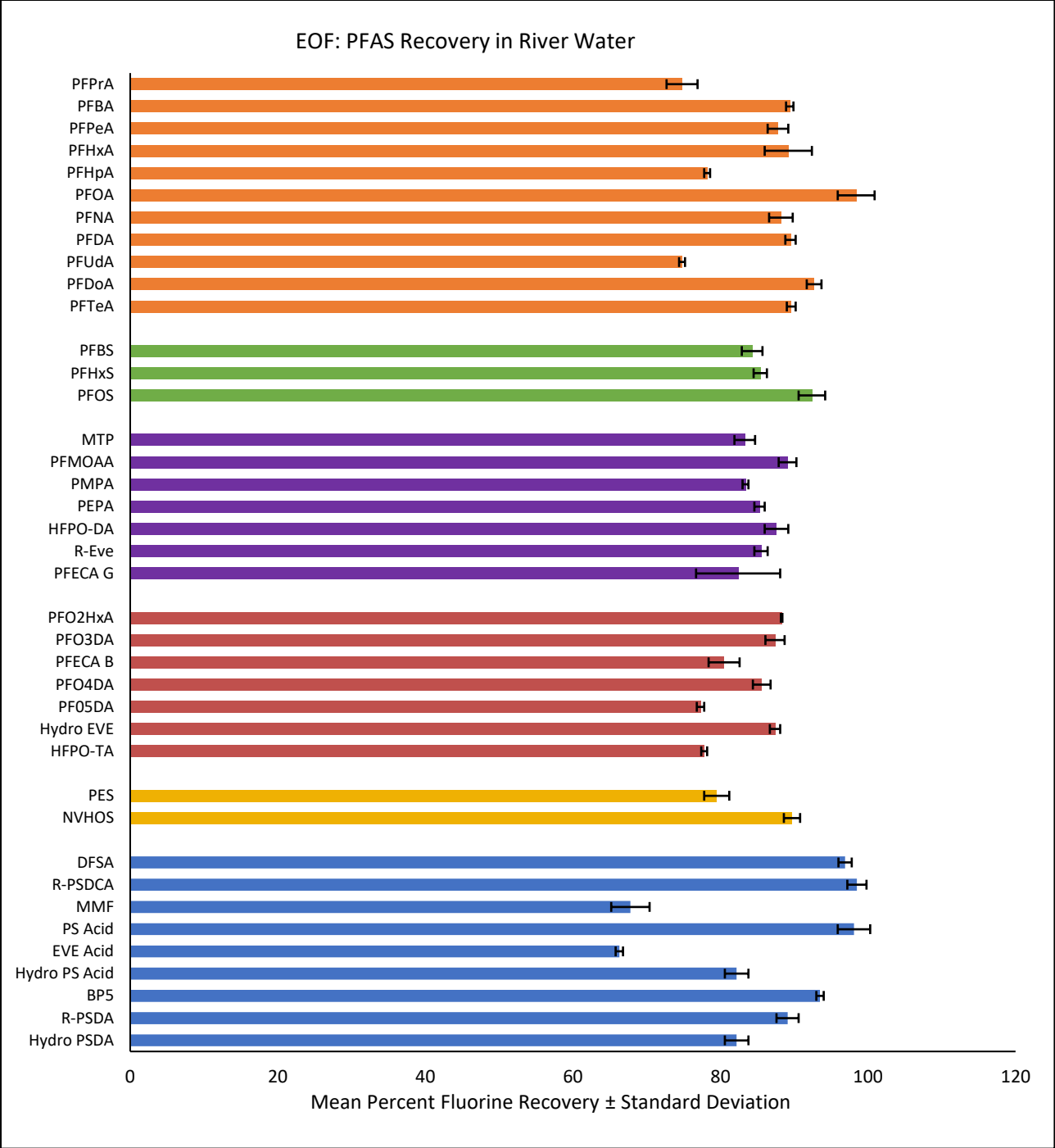
Figure 36. Organic fluorine recovery of 39 PFAS standards individually spiked into collected river water by EOF analysis. Percent recoveries range from 66-98%. PFAS standards are organized by PFAS class, based on organic functional groups. Results represent a mean of duplicates.

AOF AND EOF METHOD COMPARISONS AND CONCLUSIONS

Two new and sensitive extraction methods (AOF and EOF) that quantify total organic fluorine have been developed. Compared to previously published methods, we report higher percent recoveries for a larger mix of PFAS compounds (Table 13). The highest percent recovery for PFAS compounds using a previous AOF method reported a 64-86% recovery range for a mixture of 29 PFAS compounds in different water matrices [16]. This method also required that ACs be packed in the lab rather than purchasing pre-packed ACs. To our knowledge, there is no currently published EOF method for water matrices using the Strata-WAX/GCB SPE cartridges or utilizing a mix containing as many as 39 PFAS compounds.

Both methods can be used to quantify TOF in river water and process wastewater. Method comparisons show that, compared to AOF, EOF extractions have a higher percent recovery of the 39 PFAS standards analyzed in this study, as well as lower LOD and LOQ values (Table 13). When considering which technique is suitable for analysis, sample volume, analysis time, and sample size availability should also be considered.

Table 13. Comparison of Developed AOF and EOF Methods

	AOF (50 mL Sample Volume)	AOF (500 mL Sample Volume)	EOF (500 mL Sample Volume)
Percent Recovery in Ultrapure Water	87%	79%	91%
Percent Recovery in River Water	81%	72%	87%
Percent Inorganic Fluoride Removed	98%	98%	≥ 99%
LOD (µg/L)	0.5	0.3	0.2
LOQ (µg/L)	1.6	1.0	0.5
Full Analysis Time (one sample in triplicate)	~3 hours	~11 hours	~5 hours

METHOD APPLICATION

Process Wastewater from Chemours. Both AOF and EOF methods optimized in this study were applied to four sampling locations at the Chemours plant located in Fayetteville, North Carolina for method comparison and validation. Total organic fluorine levels were measured in all four samples (Table 14). Samples were first analyzed without dilution, but this led to an overload on the IC column, so samples were reprocessed with a dilution factor of 1:50 and a sample volume of 50 mL for AOF analysis and 500 mL for EOF analysis. To further avoid overloading the column, the absorption solution was also diluted by a factor of 1:100 before analyzing by ion chromatography. The two optimized methods were built for trace analysis; however, the process wastewater results show elevated levels of organic fluorine. Therefore, dilution may be necessary when analyzing concentrated process wastewaters.

Table 14. Total Organic Fluorine Results for Process Wastewater from Four Sampling Locations*

Location	AOF ± Standard Deviation (mg/L)	EOF ± Standard Deviation (mg/L)
16	0.52 ± 0.02	1.7 ± 0.1
17A	25.1 ± 2.2	27.1 ± 1.7
17B	14.8 ± 1.7	17.0 ± 2.8
18	0.10 ± 0.01	0.14 ± 0.03

*Samples received at the University of South Carolina on November 18, 2021; results represent a mean of triplicates.

Dissolved organic carbon (DOC) for these four process wastewater samples was ~60-1000 times larger in magnitude (Table 15) compared to river water samples (2.4 mg/L) that were used for method optimization and validation. Further experiments should be done using a more complex matrix with a higher DOC value to better understand the effect DOC plays on the extraction of PFAS compounds from process wastewaters.

Table 15. Dissolved Organic Carbon Results for Process Wastewater from Four Sampling Locations

Sample Location	Dissolved Organic Carbon (mg/L)
16	2,500
17A	306
17B	1,319
18	250

Air Samples from Chemours. Air samples (extracted in methanol using an impinger) were received from Eurofins Scientific and contained an internal standard that was used for target LC-MS/MS analysis of HFPO-DA. Samples were directly combusted in the quick furnace and analyzed for fluoride by ion chromatography (Table 16) since the organics were already extracted. Total organic fluorine concentrations were calculated for the volume of sample that was received by also subtracting the theoretical organic fluorine value resulting from the internal standard. TOF results (Table 16) show that inlet locations (sample ID's ending in 48, located before granular activated carbon (GAC) bed) have higher TOF levels than outlet locations (sample ID's ending in 49, following GAC filtration), demonstrating significant removal of PFAS from the Carbon Bed.

Target LC-MS/MS analysis results from Eurofins Scientific for HFPO-DA are also included in Table 16. The concentration of HFPO-DA was converted to a concentration of known organic fluorine using a mass balance approach so that percent unknown organic fluorine could be calculated. The known organic fluorine value was subtracted from the total organic fluorine value to calculate a percent unknown organic fluorine. These results demonstrate how *target analysis using LC-MS/MS can vastly underestimate the amount of organic fluorine in real samples*, with >99% of organic fluorine unaccounted for by target analysis. This illustrates the power of using a total organic fluorine approach, which can capture both known and unknown organic fluorine.

Table 16. Total Organic Fluorine Results Compared to Target LC-MS/MS Analysis for Air Samples*

Sample ID	Volume Received (mL)	Total Organic Fluorine (µg/L)	LC-MS/MS Results for HFPO-DA (µg/L)	Unknown Organic Fluorine (%)
140-24648-1	8	580 ± 1.7	15.4	98
140-24648-2	8	430 ± 1.6	573	15
140-24648-3	7	230 ± 0.9	46.0	87
140-24648-4	8	230 ± 2.1	1.13	> 99
140-24648-5	8	160 ± 0.6	5.39	98
140-24648-6	8	280 ± 1.3	911	- 106
140-24648-7	8	120 ± 1.2	58.3	69
140-24648-8	8	190 ± 2.4	2.86	99
140-24649-1	7	150 ± 1.3	0.667	> 99
140-24649-2	8	900 ± 1.1	0.193	> 99
140-24649-3	7	170 ± 0.7	0.0239	> 99
140-24649-4	8	110 ± 3.2	0.0009	> 99
140-24649-5	9	50 ± 1.7	1.04	> 99
140-24649-6	8	100 ± 1.7	0.158	> 99
140-24649-7	8	140 ± 2.4	0.0286	> 99
140-24649-8	8	90 ± 0.9	0.0014	> 99

*Air samples were provided as methanol extracts and were received at the University of South Carolina on December 2, 2021; results represent a mean of duplicates.

Acknowledgement

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