Comprehensive evaluation of the analytical efficiency of combustion ion chromatography for per- and polyfluoroalkyl substances in biological and environmental matrices

John Michael N. Aguilar[1*], Mindula K. Wijayahena[1*], Logan S. Running[1], Kharittha Phatthanawiwat[2], Aree Choodum[2], Joshua S. Wallace[1,3], Diana S. Aga[1,3#]

[1]Department of Chemistry, University at Buffalo, The State University of New York, Buffalo, NY 14260, United States

[2]Intergated Science and Technology Research Center, Faculty of Technology and Environment, Prince of Songkla University, Phuket Campus, Kathu, Phuket 83120, Thailand

[3]Research and Education in Energy, Environment and Water (RENEW) Institute, University at Buffalo, The State University of New York, Buffalo, NY 14260, United States

*These authors have contributed equally

[#]Corresponding author: Email address: dianaaga@buffalo.edu

Abstract

Combustion ion chromatography (CIC) has emerged as a highly valuable tool in the analysis of per- and polyfluoroalkyl substances (PFAS) in biological and environmental samples, to determine total fluorine content. The information from CIC complements results from targeted analysis using liquid chromatography-tandem mass spectrometry (LC-MS/MS) and indicates the presence of many other organofluorine compounds that remain unaccounted for by the latter technique. However, the effect of different matrices and PFAS types on combustion efficiency has not been systematically evaluated, such as varying chain lengths and head groups, different combustion times, and different types of matrices. Comparison of PFAS with chain lengths of C_4 to C_{12} using equal fluorine equivalents of 5.27 nmol (100 ng) demonstrated no significant differences in the CIC responses, indicating uniform combustion. Further, no significant differences in signal were observed between combustion times ranging from 7.5 to 15 min. However, fluorotelomer alcohols (FTOHs) exhibited losses due to volatility, which could be mitigated using activated carbon,

improving intensities by 200-1400%. Variations in matrix exhibited no observable changes in PFAS combustion efficiency for all chain lengths in water, blood, and biosolid matrix. To validate further, CIC was applied in the analysis of real blood and biosolid samples to compare the total fluorine concentrations observed with the concentrations determined by LC-MS/MS. The results of this study highlight the strengths and limitations of CIC as an important complementary method for PFAS analysis for different matrices and provide guidance for optimizing conditions for specific applications and for proper interpretation of CIC results.

Introduction

Concerns surrounding organofluorine (OF) chemicals as environmental pollutants, especially per- and polyfluoroalkyl substances (PFAS), have increased worldwide due to the growing evidence of their ubiquity, persistence, and negative health impacts.[1-3] PFAS are a broad class of chemicals with over 12,000 unique structures with varying degrees of fluorination, ranging from partially to fully fluorinated carbon backbones, with a polar head group such as a carboxylate, sulfonate, sulfonamide, quaternary amines or alcohols.[4] PFAS have been detected in diverse environmental samples, from groundwater[5-7] to dust and atmospheric sources,[8-10] in addition to drinking water.[7] Recently, the U.S. Environmental Protection Agency (EPA) established health-based maximum contaminant levels (MCLs) for five PFAS in drinking water, including 4.0 part per trillion (ppt) limits for perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS), and 10 ppt limits for perfluorohexane sulfonic acid (PFHxS), perfluorononanoic acid (PFNA), and hexafluoropropylene oxide dimer acid (HFPO-DA) (also known as GenX Chemicals).[11] The EPA is also regulating mixtures of PFHxS, PFNA, HFPO-DA, and perfluorobutane sulfonic acid (PFBS). The potential for negative environmental and human health impacts posed by PFAS

coupled to their diverse chemistries and ubiquity necessitates a comprehensive and sensitive approach to PFAS analysis.[12]

The most utilized tool for analyzing individual PFAS is liquid chromatography with tandem mass spectrometry (LC-MS/MS), targeting a discrete set of analytes for quantification based on isotope dilution with ^{13}C-labeled PFAS analogues.[12-14] Current methods established by EPA for PFAS in drinking water are EPA methods 533[15] and 537.1,[16] which enable detection of 18 and 28 PFAS, respectively, while EPA Method 1633 is generally used to analyze for 40 PFAS in biosolids and tissue samples.[17] Despite the emphasis on the analysis of a limited number of PFAS by LC-MS/MS, the environmental burden attributable to PFAS is the result of thousands of different PFAS species, many of which are not amenable to LC-MS/MS analysis due to low recoveries during sample extraction, lack of retention in the LC column, and poor ionization efficiencies during MS detection. Consequently, LC-MS/MS analysis is likely to underestimate the quantity of PFAS in the samples of interest. Subsequently, total oxidizable precursor (TOP) assay is often used to gain more information on the presence of unknown PFAS that may be precursors to perfluoroalkyl acids; however, not all fluorinated compounds can be oxidized and not all products have been identified.[18, 19] The use of high-resolution mass spectrometry (HRMS) techniques are increasingly being used for non-target analysis and suspect screening of unknown PFAS for more robust identification; however, HRMS requires the compounds be ionizable, which may not be the case, while the lack of available reference standards limits the ability to fully quantify newly identified compounds, allowing only for semi-quantification.[20] Hence, there is a critical need for a method to complement traditional LC-MS/MS and HRMS analyses and capture the distribution of fluorinated compounds to evaluate the total organofluorine (TOF) burden in environmental and biological compartments.

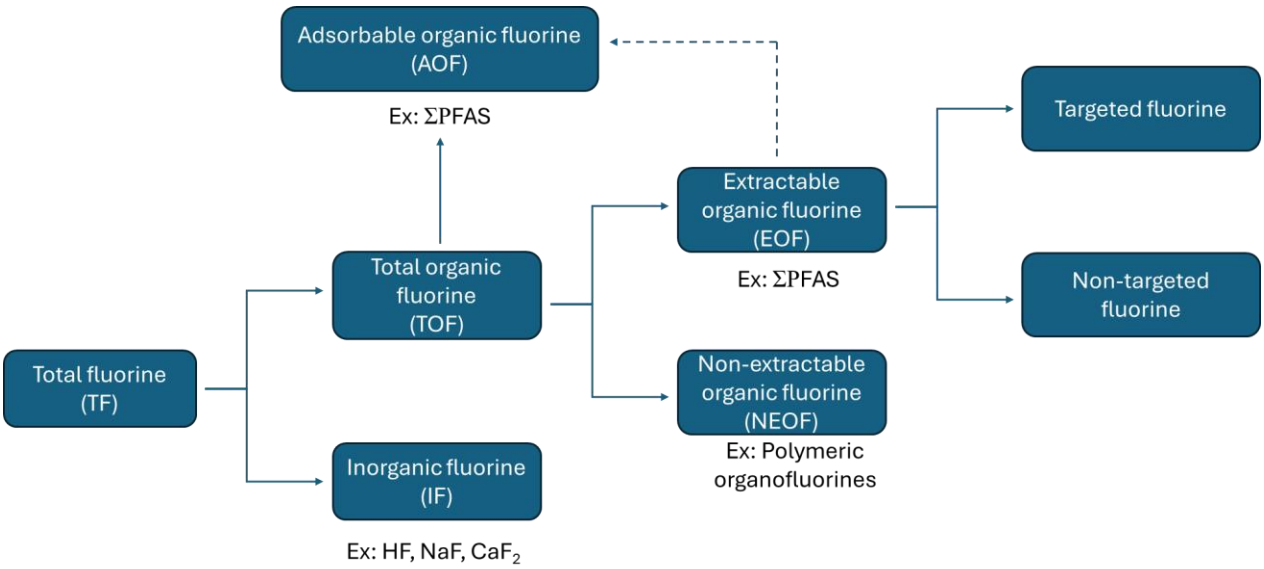

Figure 1. Breakdown of fluorinated compounds and traditional analytical approaches. Total fluorine (TF) is comprised of both inorganic fluorine (IF) and total organofluorine (TOF). The TOF is divided into three categories: (1) adsorbable organofluorine (AOF), defined as the mass of organofluorine (OF) that can be adsorbed from an aqueous sample onto an activated carbon,[21] (2) extractable organofluorine (EOF), defined as the mass of OF that can be extracted into a solvent system,[22] and (3) non-extractable organofluorine (NEOF), defined as the mass of OF not amenable to extraction by solvents (e.g. polymeric OF).[22] In solid samples, EOF Can be followed by adsorption process as indicated by the broken lines. The EOF is further divided into (i) targeted fluorine, such as known PFAS that are included in a targeted list of analytes, and (ii) non-targeted fluorine, such as emerging and previously unidentified PFAS, which account for the discrepancy between the EOF and the fluorine content of the targeted PFAS.[22]

Combustion ion chromatography (CIC) is an attractive complement for the analysis of halogen-containing compounds.[22] CIC combines traditional ion chromatography (IC) with a combustion oven to burn solid and liquid samples in the presence of oxygen and hydrated argon to reduce organohalides (such as brominated flame retardants, chlorinated pesticides, iodinated pharmaceuticals, and PFAS) to HX (X= Br, Cl, I, F). The reduced halogens are then transferred to an absorption tube containing a known volume of deionized water for subsequent analysis by IC. One study evaluated the limit of detection (LOD) for F^-, Cl^-, Br^-, and I^- in CIC and reported 4.3, 1.4, 2.2, and 16 ng/L LOD in water samples, respectively; the CIC was then applied to the detection of polybrominated biphenyls, brominated flame retardants, and PFOS and demonstrated the

applicability of the CIC method to assess flue gas and fly ash contamination by organohalogens.[23] Typically, the analysis of PFAS in water involves solid-phase extraction (SPE) to pre-concentrate and prepare the samples for LC-MS/MS analysis. However, this conventional approach results in varying recovery efficiencies for different PFAS species due to their differences in solubilities and adsorption affinities to the SPE sorbent, which are also affected differently by the types of interferences present in the samples such as natural organic matter, ions, and other compounds that affect extraction. Additionally, shorter-chain PFAS (C_2–C_4) may not be effectively captured by common SPE sorbents, resulting in a systematic underestimation of PFAS levels. Recoveries for PFAS using SPE exhibits recoveries as low as 16%, with the shorter chain (C_4-C_5) PFAS exhibiting the lowest recoveries along with sulfonyl fluorides.[24] In contrast, CIC analysis does not require extensive sample cleanup, providing a robust capacity to detect nearly all PFAS by eliminating the potential loss resulting from the sample preparation steps.

CIC analysis enables the quantification of total fluorine (TF), and differentiates between TOF, and inorganic fluorine (IF) (Figure 1) with limited sample preparation. Furthermore, the use of CIC can provide both extractable organofluorine (EOF) and adsorbable organofluorine (AOF), potentially closing the fluorine mass balance to account for PFAS contamination in the absence of commercially available standards. Hence, results from CIC analysis complement the information obtained from targeted LC-MS/MS methods[25] and reveal a wider PFAS chemical space that would otherwise remain unreported in targeted analysis[26] because CIC can account for fluorine from both known PFAS and unidentified extractable PFAS in EOF.

Analyses using CIC can have important applications in biomonitoring, including PFAS analysis in tissues, serum, and blood.[27] Recent examples include analysis of human blood from cohorts of individuals with historical drinking water contamination from firefighting foams using CIC which

reported EOF concentration ranges of <107 – 592 ng/mL F.[28] The elevated EOF levels in blood enables determination of samples with high PFAS content, which was validated by a fluorine mass balance analysis using LC-MS/MS target analysis, revealing an average ΣPFAS concentration of 346 ng/mL.[28] Another study analyzing 39 PFAS compounds in industrial wastewater, river water, and air samples reported recoveries of 46-112% and 72-99% and LODs of 0.3 – 0.5 µg/L and 0.1 – 0.2 µg/L using the AOF and EOF, respectively.[28] Significantly higher TOF were measured in the samples compared to the LC-MS/MS, demonstrating that the combination of the two techniques have the ability to comprehensively measure total PFAS, including known and unknown OF species.[29] Despite the increasing number of CIC applications in PFAS analysis, questions related to the combustion efficiency of different PFAS representing diverse chain-lengths, degrees of fluorination, and headgroup functionalities remain unanswered. A previous study suggested that combustion efficiency is dependent on the chain length, with longer chain PFAS (C_{11}) exhibiting incomplete combustion compared to the shorter chain PFAS.[22]

This study aims to systematically evaluate factors affecting the combustion efficiency and analytical performance of CIC for PFAS analysis. Several factors were investigated to determine whether: (1) the combustion efficiencies are the same between IF (e.g. potassium chloride, KF) and OF compounds (e.g. PFAS); (2) the length of combustion time impacts combustion efficiencies of PFAS analysis by CIC; (3) the PFAS structure (e.g. carbon backbone length, degree of fluorination, different head groups) affect combustion efficiency; and (4) the different types of sample matrices influence combustion efficiencies of PFAS. To comprehensively evaluate these variables, combustion efficiencies of different PFAS compounds with different head groups and varying carbon chain length (C_3–C_{13}) were investigated and compared to KF that is traditionally used as the calibration standard for fluoride ion quantification. To determine the effect of

combustion times, different carboxylated PFAS with increasing chain lengths were combusted at times ranging from 7.5 – 15 min. The effect of sample matrix on CIC signal was investigated by comparing combustion efficiencies of PFAS in biosolids and blood. Based on the results of these studies, an optimized CIC method for biosolids was developed and validated for determination of TF, AOF, and targeted PFAS in two types of biosolids with varying organic matter content. Results from this study provide critical information that will advance the quantitative applications of CIC as a complementary analytical technique for closing the gap in accounting of the fluorine content in PFAS contaminated samples.

Materials and Methods

Chemicals

The list of sources of PFAS standards are listed in Table S1. The American Chemical Society (ACS) grade >99% potassium fluoride and methyl tert-butyl ether (MTBE) were purchased from Sigma Aldrich (Saint Louis, MO). LC-MS grade methanol (MeOH) and acetonitrile (ACN) were purchased from Omnisolv® through EMD Millipore Sigma (Saint Louis, MO). Ammonium acetate (ACS grade) formic acid (88%, ACS grade) was purchased from J.T. Baker (Phillipsburg, NJ). Nanopure^{TM}, 18.2 MΩ water was generated using a Barnstead Nanopure^{TM} Diamond (Waltham, MA) system. Carbopack^{TM} graphitized carbon black (GCB) (120 – 400 mesh) was obtained from EMD Millipore Sigma (Saint Louis, MO).

Combustion Ion Chromatography (CIC) Instrumentation and Quantification

Fluoride ion concentrations were determined using ThermoScientific Dionex ICS-6000 DC ion chromatograph (IC) (West Palm Beach, FL) coupled with a Mandel AQF-2100H combustion system (Houston, TX). Inlet and outlet furnace temperatures were set at 900^{o}C and 1000^{o}C,

respectively. Argon and oxygen gases were set at flow\ rates of 200 and 400 mL/min, respectively. Oxygen was used as combustion gas ensuring the sample burns completely and converts any halogens into their corresponding volatile compounds such as hydrogen halides, while argon transfers the combustion products into the subsequent steps without reacting with them. The resulting combustion gases are passed through an absorption solvent (Nanopure^{TM} water) set at 10 mL. This solution is then injected into the IC unit where anion separation was achieved using a Dionex IonPac^{TM} AS18-Fast column (2 x 150 mm, 4 µm) (West Palm Beach, FL) at a flow rate of 0.25 mL/min with varying concentrations of potassium hydroxide (KOH) in Nanopure^{TM} water as the eluent. The ion chromatography gradient elution program started with a 10 mM of KOH held for 4 min, then increased to 40 mM KOH in 1 min, and held for 3 min before returning to 10 mM KOH in 1 min; The total run is 13 min with equilibration time of 4 min. An external standard calibration of KF ranging from 0.12 – 24.8 nmol of fluoride as KF in Nanopure^{TM} was used for quantification.

PFAS Extractions in Blood for LC-MS/MS Analysis

Whole blood samples (100 µL) were placed in a centrifuge tube with 200 µL of 0.1 M formic acid. The samples were vortex for 1 min and sonicated for 10 min. A 900-µL aliquot of cold (-20°C) 50:50 ACN:MTBE solution (extraction solution A) was added, vortexed for 1 min and centrifuged at 20,817 × *g* for 5 min. The supernatant was removed and placed in a clean 1.7-mL polypropylene (PP) centrifuge tube. A second 900-µL aliquot of cold (-20°C) extraction solution A was added to the remaining blood materials, vortexed for one min, and centrifuged at 20,817 × *g* for 5 min. The supernatants were combined, and 50 mg of GCB were added for sample cleanup and vortexed for 10 min and centrifuged for 10 min at 3234 × *g*. The supernatant was removed and placed in a clean PP centrifuge tube. A 1-mL portion of cold (-20°C) extraction solution A was added to the

remaining GCB, vortexed for 10 min, and centrifuged for 10 min at 3234 × *g* as a wash. The supernatant was removed, combined with the others, then dried under nitrogen (25°C). Extracts were fortified with internal standards (100 µg/L of $^{13}C_4$-PFOA and $^{13}C_4$-PFOS) and resuspended in 50% mobile phase A (95:5 (v/v) 5 mM ammonium acetate:ACN) and 50% of mobile phase B (50:50 (v/v) MeOH:ACN) to a final volume of 200 µL for LC-MS/MS analysis following the method reported in Camdzic et al (2023).[30]

PFAS Extractions in Biosolids for LC-MS/MS Analysis

For each sample, 250 mg of lyophilized and homogenized biosolids were placed in a 50-mL PP centrifuge tube for extraction.[13] Samples were fortified with 25 µL of 1 ppm labelled PFAS mix (MPFAC-24ES, Wellington Labs, Guelph, ON, Canada) and vortexed for 30 s to homogenize. A 7.5 mL volume of 1% acetic acid in Nanopure™ water was added, vortexed for 30 s, and sonicated at 60°C for 20 min (35 kHz, 180 W). Samples were then centrifuged at 3234 × *g*, for 40 min at ambient temperature, and the supernatants collected in a clean 50-mL PP tube. A 1.7 mL solution of 1% acetic acid in 90:10 MeOH:H_2O was then added, vortexed, sonicated at 60°C for 20 min, then centrifuged at 3234 × *g* for 40 min at ambient temperature. The extraction was performed in three cycles, combining each sample's supernatant. Pooled supernatants were then loaded onto C_{18} cartridges (3 cc, 500 mg, Waters™ Sep-Pak, Milford, MA). Cartridges were pre-conditioned with 3 mL of methanol followed by 3 mL of 1% acetic acid in water, leaving some water in the cartridge to prevent drying out. The loaded samples in the SPE cartridges were eluted with 6 mL of methanol (by gravity flow) into a clean 15 mL PP centrifuge tube. Eluents were cleaned by adding 50 mg of GCB, vortexed for 30 s, then centrifuged at 3234 × *g* for 15 min at 4°C. Supernatant was transferred to a clean 15-mL PP tube and dried at ambient temperature under nitrogen gas. Dried extracts were finally resuspended in 250 µL of 50% mobile phase A (95:5 (v/v)

5 mM ammonium acetate:ACN) and 50% of mobile phase B (50:50 (v/v) MeOH:ACN), and was analyzed by LC-MS/MS.[30]

Results and Discussions

Comparison of calibration based on IF and OF

To determine whether the CIC signals generated from equimolar concentrations of fluoride from an IF source versus an OF source are comparable, stock solutions of potassium fluoride (KF) and PFPrA, each containing 5.27 nmol or 100 ng of fluorine, were separately prepared in Nanopure^{TM} water and then analyzed using CIC. PFPrA was selected as a representative organofluorine compound to be compared with KF, as it is an ultra-short chain PFAS with limited and manageable volatility which would otherwise bias the results. No statistical difference in the signals obtained from combustion of KF and PFPrA were observed ($p > 0.05$, t-test), suggesting that both organic and inorganic forms of fluorine result in similar combustion efficiencies and responses (Figure 2A). Consequently, a calibration curve of 0.12 – 24.8 nmol of fluoride as KF in Nanopure^{TM} water was constructed demonstrating a high degree of linearity (R^2 = 0.9999) (Figure 2B). The calibration curve was then used to quantify the fluorine content of different PFAS analyzed by CIC relative to the KF standard. Experimental results were compared to the theoretical quantity of fluorine for each PFAS (2.63 nmol or 50 ng F^-), by determining the combustion response as a surrogate for recovery (Equation 1). Recoveries ranged from 85.8 – 117% (Table 1), indicating that the amount of fluorine expected from different PFAS compounds can be accurately quantified using a calibration curve generated from KF. Using a single standardized calibration curve is essential to avoid complications when dealing with PFAS mixtures that have varying degrees of fluorination and other unknown fluorinated compounds.

$$Equation\ 1: Recovery\ (\%) = \frac{calculated\ amount\ of\ fluorine}{theoretical\ amount\ of\ fluorine}\ x\ 100\%$$

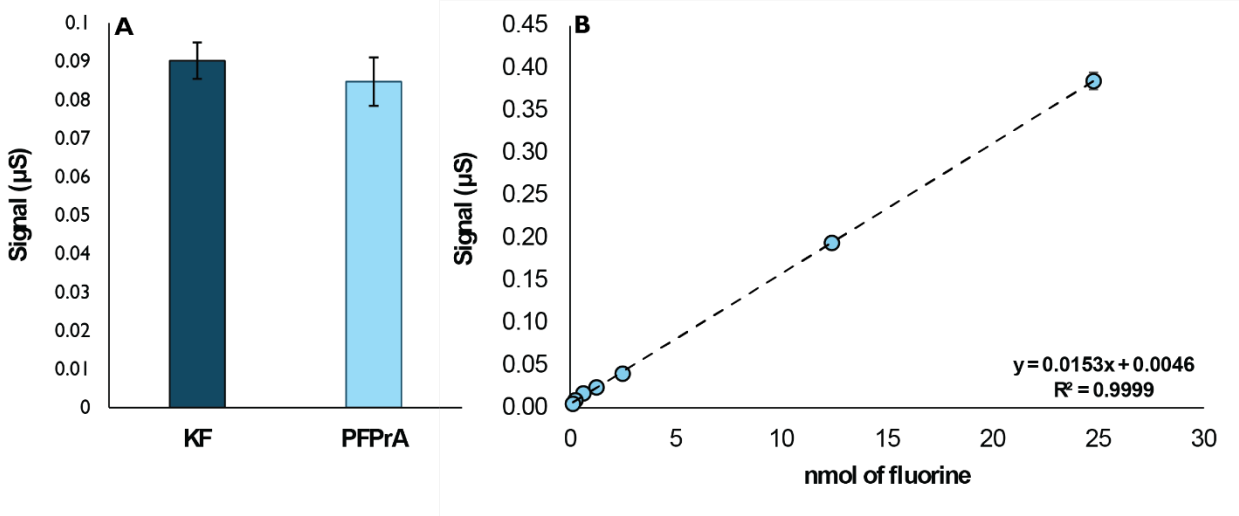

Figure 2. (A) Peak area response of 5.27 nmol (100 ng) of fluoride in potassium fluoride (KF) and perfluoropropanoic acid (PFPrA) (n=3) ($p > 0.05$, t-test). (B) Calibration curve obtained from spiking 50 µL of 0.12 – 24.8 nmol of fluoride from KF solution in Nanopure^{TM} water to the sample boats (n=3).

Table 1. Fluoride quantities (nmol) (n=3) observed following direct combustion of various PFAS species containing a total of 2.63 nmol of fluorine assuming 100% combustion.

PFAS	Measured fluoride (nmol)			Recovery (%)			
	I	II	III	I	II	III	Average ± SD
PFPrA	2.45	2.24	2.08	93.1	85.2	79.2	85.8± 7
PFPeA	2.48	2.37	2.36	94.1	90.1	90	91.3 ± 2
PFHxA	2.87	2.52	2.98	109	95.6	113	106 ± 9
PFHpA	2.68	2.65	2.51	102	101	95.3	99.3 ± 3
PFOA	2.95	3.13	3.17	112	119	121	117 ± 5
PFDA	2.81	2.5	2.31	107	94.8	87.7	97 ± 10
PFUnDA	2.83	2.66	2.82	107	101	107	105 ± 4
PFDoA	2.81	2.48	2.78	107	94.3	105	102 ± 7
PFTrDA	3.08	2.79	2.83	117	106	108	110 ± 6

Assessing the combustion efficiency for PFAS

Effect of chain length, head group and combustion time

Studies have demonstrated the utility of CIC for evaluating the total fluorine or EOF but with the assumption that all fluorinated analytes have the same combustion efficiency.[31] Combustion efficiencies for perfluoroalkyl carboxylic acids (PFCAs) with increasing chain lengths (C_4–C_{12}), dissolved in methanol, were evaluated at different combustion times, 7.5, 10, and 15 min. Short-chain PFAS (C_3-C_5) are considered volatile to semi-volatile (vapor pressures (VP) from 7.0-19.0 mmHg), while longer chains are not (VP around 1.0×10^{-03} mmHg).[32-34] Consequently, we suspected that the short-chain PFAS will require shorter combustion times, while longer chains would require longer combustion times. The resulting signals were normalized to the signal of an equimolar standard of KF (Figure 3A). The responses for the C_4 to C_{12} PFAS were not significantly different ($p > 0.05$, ANOVA), even at different combustion times. This finding is in contrast to the report by Aro et al.[22] which indicated that combustion efficiencies decreased with increasing chain lengths of PFCAs. The CIC responses for carboxylates, sulfonates, and fluorotelomer alcohols (FTOHs) of varying chain lengths (C_6, C_8, C_{10}, and C_{12}), each containing 5.27 nmol of fluorine, were combusted for 10 min to evaluate the impact of different head groups on combustion efficiencies (Figure 3B and 3C). The resulting signals were again normalized to an equimolar KF standard (Figure 3B). No significant differences were observed in the combustion responses for the carboxylates ($p > 0.05$, ANOVA) across the evaluated chain lengths, with recoveries ranging from 80-140% (Figure 3C). On the other hand, varying chain lengths of the sulfonated PFAS showed significant differences in the responses due to the low signal of the longest chain length tested (C_{12}, PFDoS, perfluorododecanesulfonic acid). Interestingly, the shorter chain FTOHs (C_4 – C_8) generated lower signals compared to the longest chain length (C_{12}) FTOHs. This can be likely attributed to the volatilization of the shorter chain FTOHs prior to injection to the CIC. FTOHs are known to be the volatile PFAS group as their vapor pressure range from 7.44 – 1.01 mmHg.[35]

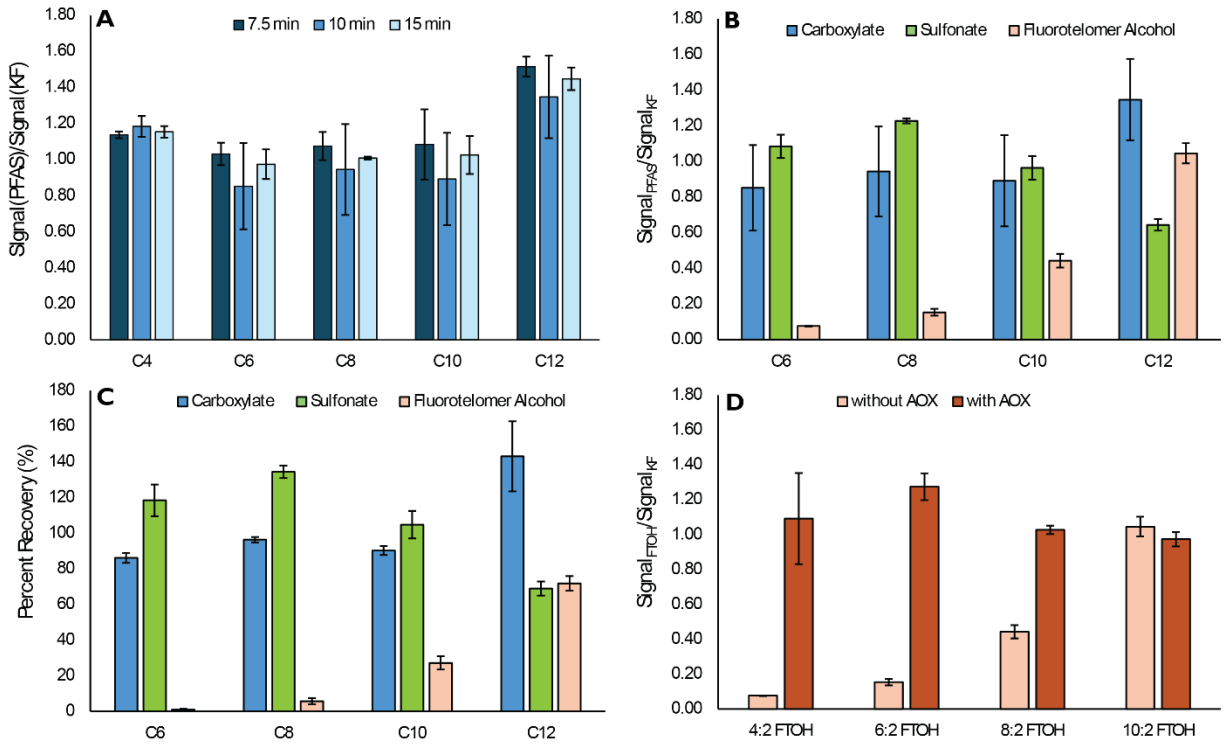

Figure 3. Relative signal and percent recoveries of all PFAS solutions prepared to contain equimolar concentrations of fluorine (5.27 nmol, 100 ng) in Nanopure™ water. (A) Normalized responses (F^- peak area) of different chain lengths of perfluorocarboxylic acids (PFCAs) at different combustion times ($p > 0.05$, ANOVA); (B) Normalized responses of different PFAS head groups at varying chain lengths combusted for 10 min; (C) Calculated recovery of different PFAS head groups with varying chain lengths compared to equimolar KF standard; (D) Normalized responses for FTOHs of increasing chain lengths with and without the use of activated carbon as adsorbable material combusted for 10 mins.

The use of adsorbent material for volatile PFAS

To address the loss of volatile PFAS, such as FTOH, in aqueous samples during combustion in CIC, the use of an adsorptive material was explored to minimize volatilization in the pre-analysis steps. Each FTOH, containing 5.27 nmol of fluorine in Nanopure™ water, were spiked onto 40 mg of activated carbon (Mandel Scientific Inc, Houston, TX), combusted for 10 min, and analyzed by ion chromatography. Activated carbon effectively adsorbs PFAS due to its high surface area, porous structure, and hydrophobic nature, which allows it to trap PFAS. It also has electrostatic properties that enhance its ability to attract and retain negatively charged PFAS headgroups,

making it suitable for removing PFAS from various environmental matrices, including water.[36] Additionally, it enables capture of volatile PFAS compounds like FTOHs that may otherwise be lost.[37] Results indicated a significant increase in normalized responses for all the FTOHs with no significant differences ($p > 0.05$, ANOVA) across different FTOH chain lengths. Utilizing adsorption methods with activated carbon material presents a promising approach for minimizing the loss of volatile PFAS in liquid samples during CIC analysis.

Effect of different sample matrices on combustion

The combustion efficiency of different chain lengths of PFCAs were also investigated in biosolids and blood samples to represent environmental and biological matrices, respectively. Different PFCAs with increasing chain lengths were spiked into Nanopure^{TM} water, blood and biosolids such that each sample received equal quantities of fluorine (5.27 nmol) from each PFCA evaluated. Because the biosolid samples utilized in this experiment contained IF, 10 different biosolid aliquots were homogenized and analyzed by CIC to determine the baseline total fluorine response per milligram of biosolid. The signals for spiked biosolid samples were then corrected by subtracting the average F^- signal of non-spiked biosolid samples. Blood samples used in this experiment had undetectable background fluorine levels. Figure 4 illustrates the PFCA responses at varying chain lengths across the tested matrices. Generally, the PFCAs showed no significant variation between matrices, nor were there significant differences observed within each matrix ($p > 0.05$, ANOVA). However, responses for longer-chain PFCAs in biosolids were statistically different ($p < 0.05$, pairwise t-test) and lower than the responses observed in water and blood. This may be attributed to the manner by which IF signals were subtracted from the TF signals as required to determine the signals originating from PFAS spiked in the biosolids. This suggests that

an extraction method to separate IF may be necessary to estimate OF content in biosolids using EOF.

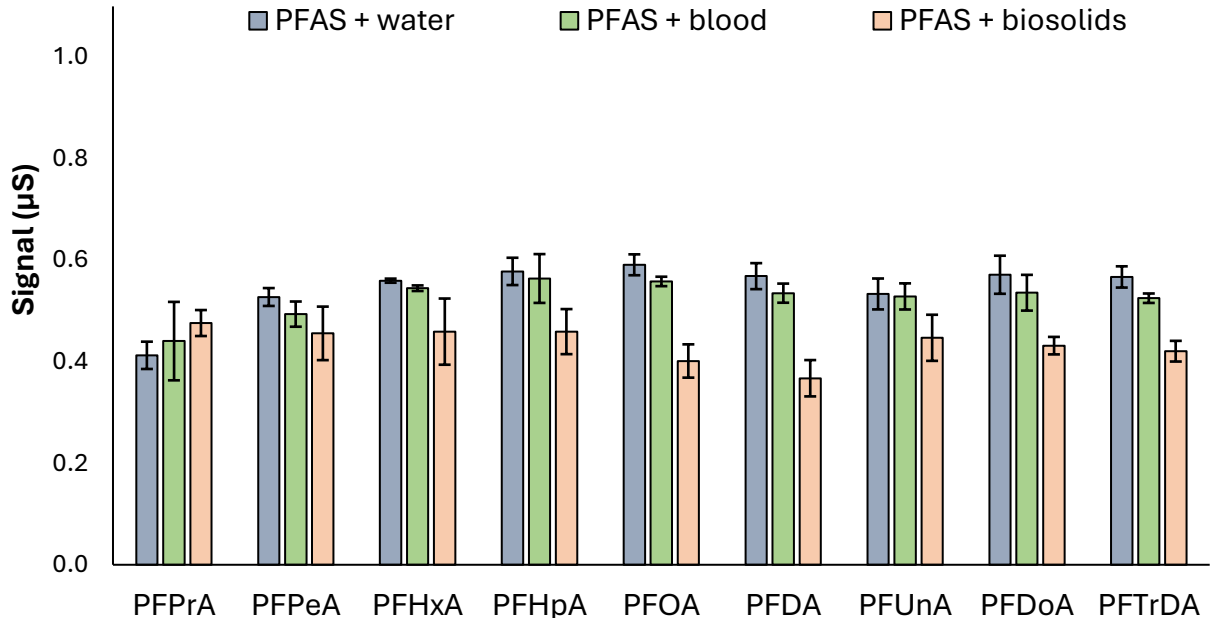

Figure 4. Responses of different carboxylic PFAS in water, blood, and biosolids, each containing the same quantity of fluorine (5.27 nmol), combusted at 10 min (n=10).

Optimizing sample loop volume in absorption unit

As previously described, CIC requires combustion of fluorinated compounds at high-temperature conditions to reduce OF to hydrogen fluoride (HF), which is transferred to an absorption tube and analyzed via ion chromatography. However, the injection volume may be critical to obtaining measurable F- signals, particularly for trace levels of OF. The loop volume determines the volume of sample injected into the ion chromatograph with larger volume loops allowing for increased sample loading and enhanced sensitivity. On the other hand, excessively large loops may lead to column or suppressor overload, adversely affecting chromatographic peak shapes and resolution. The effect of sample loop volume on the F^- response from KF was evaluated using three different injection loop volumes: 100 µL, 200 µL, and 500 µL (Figure S1). Altering the loop volume resulted in linear response curves with increasing fluoride mass injected. As expected, the

calculated slopes increased with larger volume injections with slopes of 0.0153 µS/nmol, 0.0238 µS/nmol, and 0.0446 µS/nmol observed for 100 µL, 200 µL, and 500 µL loops, respectively (Figure 5). These observed results demonstrate improved sensitivity, enabling quantitative analysis of lower fluoride concentrations at detection limits of 4.22 ng (0.22 nmol), 2.01 ng (0.11 nmol), and 1.09 ng (0.057 nmol), for the 100 µL, 200 µL, and 500 µL loops, respectively. Determining the optimal loop length for CIC analysis is often overlooked. However, it is important to emphasize that the choice of loop and injection volume should be a critical parameter in developing a CIC method to detect low concentrations of PFAS. Miyake et al. has demonstrated that by using a 1500 µL injection volume of absorbent, they were able to achieve an LOD of 1.5 ng/mL of fluorine in human blood samples.[31]

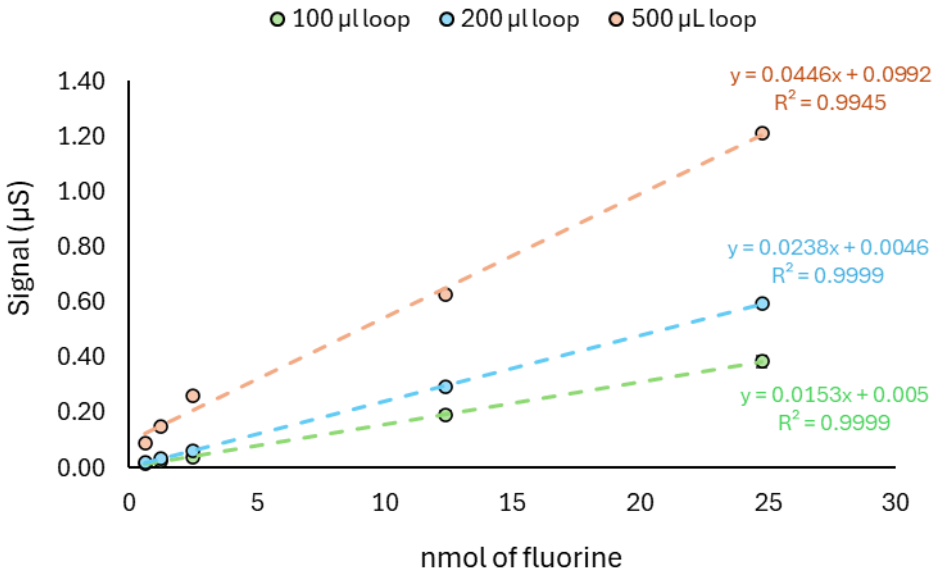

Figure 5. Fluoride response (µS)from KF curves representing of increasing amounts of fluorine using different loop lengths (n=3).

Application in biosolid and real human serum samples

The TF and TOF content in biosolids samples collected from a wastewater treatment facility (n = 3, from a blind study) and random human serum samples (n = 7, from a blind study) were determined using CIC and compared to the targeted PFAS detected via LC-MS/MS for comparison. Two types of biosolids, primary solids (PS) and waste activated sludge (WAS) were extracted and analyzed. Activated sludge is a common biological treatment approach,[38] characterized by the aggregation and sedimentation of microorganisms and organic substances within the clarification chamber, resulting in the formation of WAS.[39] The accumulated sludge is routinely collected, subjected to digestion processes, and subsequently treated with lime for stabilization to generate PS and facilitate disposal via land application,[13] presenting an important point of PFAS release to the environment.[12, 40] Of 40 PFAS targeted by LC-MS/MS, 11 were detected in PS samples: 1H, 1H, 2H, 2H-Perfluorohexane sulfonic acid (4:2 FTS), 1H, 1H, 2H, 2H-Perfluorodecane sulfonic acid (8:2 FTS), N-ethyl perfluorooctanesulfonamidoacetic acid (N-EtFOSAA), perfluorobutanesulfonic acid (PFBS), PFOS, perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), PFOA, PFNA, perfluorodecanoic acid (PFDA), and perfluorododecanoic acid (PFDoA), while 15 PFASdetected in WAS: 4:2 FTS, 8:2 FTS, FOSA, N-EtFOSAA, PFBS, PFOS, PFHxA, PFPHpA, PFOA, PFNA, PFDA, perfluoroundecanoic acid (PFUDA) and PFDoA, perfluorotridecanoic acid (PFTrDA) and perfluorotetradecanoic acid (PFTeDA) (Table 2). Concentrations of PFAS detected in PS and WAS ranged from 0.5 - 17.6 µg/L and 0.5 – 41.7 µg/L, respectively. These detections showed WAS samples contained higher concentrations of PFAS than the PS samples. CIC analysis revealed total fluoride quantities ranging from 117.5 – 144.1 ng and 417.2 – 458.7 ng for PS and WAS, respectively. The elevated PFAS levels found in WAS compared to PS indicate that dewatering and treatment processes may help lower the fluorinated compounds in biosolid samples.[13] Importantly, the fluorine mass

attributable to the PFAS detected by LC-MS/MS in the biosolids was substantially lower than indicated by CIC analysis (Figure 6). These results indicate that biosolids contain additional OF compounds beyond the 40 targeted PFAS in the LC-MS/MS method. As biosolids originate from wastewater sludges, the presence of IF from domestic waste, for example from sodium fluoride (NaF) (commonly used in drinking water fluoridation and found in most toothpaste products), adds to the overall fluorine content in biosolids and would not be captured by LC-MS/MS analysis. Further, conventional LC-MS/MS methods using C_{18} LC columns underreport PFAS content, as they fail to retain trifluoroacetic acid (TFA) and other ultra-short chain PFAS, however, this study also detected significant amounts of TFA in wastewater samples using fluorine nuclear magnetic resonance (^{19}F-NMR).[41] Moreover, targeted LC-MS/MS analyses generally only include negatively charged PFAS, systematically excluding positively charged and neutral and amphoteric PFAS.[12] Further, any excreted fluorinated pharmaceutical compounds that are discharged into domestic waste streams, such as fluoroquinolone antibiotics, antidepressants, and other fluorinated materials, will also contribute to the total fluorine content in the biosolids.[42]

Table 2. LC-MS/MS and CIC results for primary sludge (PS) and waste activated sludge (WAS) samples (n=3, each) from municipal wastewater treatment plants.

Concentration (µg/L) detected in LC-MS/MS						
Detected Compounds	**PS1**	**PS2**	**PS3**	**WAS1**	**WAS2**	**WAS3**
6:2 FTS	5.7	5.9	5.8	1.4	1.4	1.5
8:2 FTS	0.5	0.5	0.6	0.8	0.5	0.6
FOSA	<LOD	<LOD	<LOD	4.7	2.2	1.8
N-EtFOSAA	6.7	9.6	8.5	5.6	4.6	3.1
PFBS	3.4	1.0	3.1	3.7	1.7	3.5
PFOS	11.8	9.7	9.7	41.7	39.2	39.7
PFHxA	16.3	17.6	16.4	10.1	9.9	10.4
PFHpA	2.2	2.3	2.6	2.3	2.0	2.1
PFOA	7.2	6.9	8.4	6.3	5.6	5.5
PFNA	0.8	0.6	0.7	1.1	1.3	1.5
PFDA	4.3	4.6	4.9	9.4	9.3	10.0

PFUDA	<LOD	<LOD	<LOD	23.1	24.8	22.5
PFDoA	2.1	1.5	2.1	9.3	8.7	9.4
PFTrDA	<LOD	<LOD	<LOD	7.3	7.0	7.1
PFTeDA	<LOD	<LOD	<LOD	1.5	1.2	1.2
Total Fluoride in CIC (ng)	144.1	117.5	129.4	458.7	429.7	417.2

Of the 40 PFAS targeted by LC-MS/MS, five were detected in seven serum samples, including: PFHxA, PFOA, PFNA, PFHxS, and PFOS. Concentrations ranged from 0.5 µg/L to 4.6 µg/L (Table 3). PFOA was detected in all serum samples, while PFOS and PFNA were present in most samples with detection frequencies of 57% and 71%, respectively. However, CIC analysis using a 200 µL sample loop did not detect elevated TF content, indicating that blood samples' TF content were below the 2.5 ng F^- detection limit. The fluoride content in each blood sample, based on the detected PFAS via target analysis, ranged from 0.09 to 0.95 ng, which suggests that there are likely no other types of PFAS in the blood sample that are not included in the targeted analytes.

Table 3. LC-MS/MS and CIC results for serum samples (n=7).

Concentration (µg/L) detected in LC-MS/MS							
Detected Compounds	**Sample 1**	**Sample 2**	**Sample 3**	**Sample 4**	**Sample 5**	**Sample 6**	**Sample 7**
PFHxA	<LOD	0.6	1.0	1.1	0.9	1.6	<LOD
PFOA	2.0	1.4	2.8	1.2	4.6	3.7	1.3
PFNA	0.7	0.8	0.7	2.6	<LOD	0.8	<LOD
PFHxS	<LOD	<LOD	1.6	<LOD	<LOD	<LOD	<LOD
PFOS	1.6	<LOD	0.7	0.8	9.0	<LOD	<LOD
Total Fluoride in CIC (ng)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

Detection of TF, AOF and targeted PFAS in primary solids and waste activated sludge

Analysis by CIC can determine TF but it is not able to differentiate between different types of fluorine sources. While the EOF compounds are present in the extracts prepared for LC-MS/MS analysis, it is important to note that the extracts may still contain IF. Implementing an AOF preparation procedure effectively removes the IF, leaving only the OF.[29] Adsorbable OF was used prior to combustion analysis of the biosolids. The aqueous extracts from the PS and WAS were passed through an activated carbonF sorbent, which was then analyzed via CIC. At the same time, 2.5 mg of the original samples were also directly analyzed by CIC to account for the TF present in the biosolids. Interestingly, the mass of fluoride determined by LC-MS/MS analysis only accounted for 5.11 ± 0.01 % and 0.748 ± 0.001 % of all the fluorine compounds detected by direct CIC, while AOF captured 27.00 ± 0.03% and 17.82 ± 0.04% in PS and WAS samples, respectively (Figure 6). The additional fluoride detected via AOF may be attributed to the presence of other PFAS or organofluorine compounds. The remaining fluoride representing 67.89 ± 0.04% and 81.44 ± 0.04% for PS and WAS, respectively, (Figure 6) may either be in the form of IF, additional EOF that are not adsorbed on the activated carbon used in AOF, or NEOF. These results demonstrate the capacity of CIC to complement LC-MS/MS analysis for screening PFAS and other fluorinated compounds in environmental samples. Because CIC is unable to differentiate between IF and TOF, a suitable extraction procedure followed by an AOF cleanup is necessary to effectively distinguish OF, such as PFAS, from IF compounds.

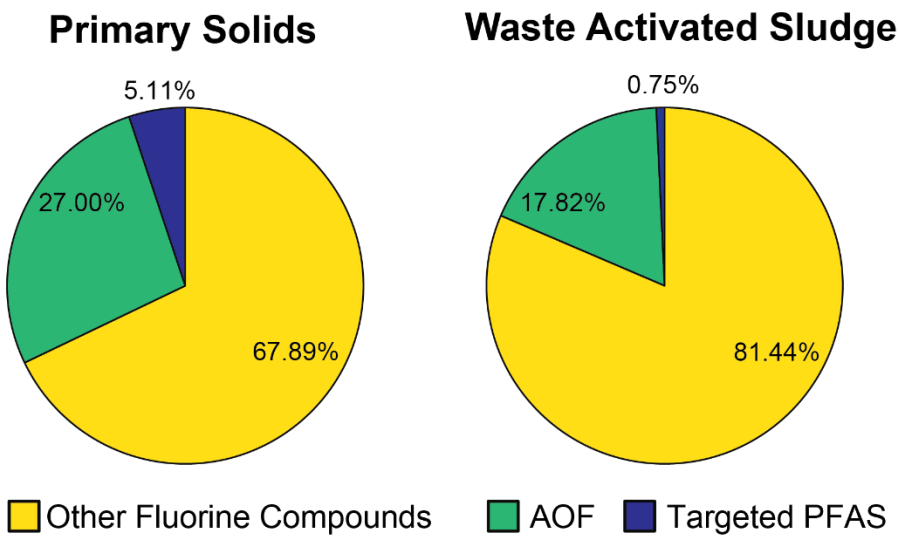

Figure 6. Relative composition of different forms of fluorine in primary solids and waste activated sludge originating from a municipal wastewater treatment plant (n = 3, each).

Conclusions

The complexity of PFAS in environmental and biological matrices pose significant challenges for accurate detection and quantification. With over 12,000 unique PFAS structures, traditional analytical methods face important limitations in their ability to capture the full spectrum of these compounds. LC-MS/MS has been instrumental in detecting PFAS in environmental and biological samples, offering robust detection capabilities. However, the targeted nature of LC-MS/MS methods underreport PFAS burden. To address this limitation, CIC emerged as a promising technique for assessing TOF content in various matrices, including complex samples such as biosolids and blood.

Efforts in this study to optimize CIC for PFAS analysis have revealed insights into various factors affecting combustion efficiency and sensitivity of the method. Assessment of combustion efficiency showed combustion times and PFAS chain lengths did not significantly influence CIC responses, with the exception of the signal detected in PFDoS. Overall, uniform combustion

capacity across different PFAS compounds were observed. It was obseved that the use of adsorbable materials such as activated carbon can minimize losses of volatile PFAS during analysis, resulting in improved recoveries. Matrix effects were also evaluated, demonstrating consistent PFAS combustion efficiency in complex environmental matrices like biosolids and blood. No significant differences were observed between the responses of IF and OF standard, hence, quantification of PFAS in various matrices can be achieved using a KF calibration curve.

The use of CIC to complement LC-MS/MS analyses offers a comprehensive approach to PFAS analysis, enabling the determination of TOF content alongside targeted PFAS species. Additionally, the application of CIC in conjunction with SPE and adsorption techniques allows for differentiation between AOF and IF, shedding light on the distribution of AOF compounds in environmental samples. Overall, the development and optimization of CIC methodologies represent a crucial step towards accurate reporting of PFAS and enhancing our understanding of PFAS contamination in the environment and elsewhere.

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