Application note | 003644

Food and beverage

Comprehensive screening of per- and polyfluoroalkyl substances (PFAS) in food contact materials

Utilizing combustion ion chromatography for total organic fluorine (TOF) analysis

Authors

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Keywords

Combustion ion chromatography (CIC), total organic fluorine (TOF), extractable organic fluorine (EOF), food contact materials (FCM), non-targeted analysis (NTA)

Goal

To develop a method to measure total organic fluorine (TOF) and extractable organic fluorine (EOF) in food contact materials (FCM) using combustion ion chromatography (CIC)

Introduction

Per- and polyfluoroalkyl substances (PFAS) are used globally in many industries and comprise thousands of individual compounds. They have been intentionally added to food contact materials (FCM) for decades to confer grease and water repellency.[1] PFAS are highly persistent, bioaccumulative, and toxic. PFAS can migrate from FCM into food, with migration rates dependent on the food's temperature, acidity, storage time, and fat content.[2-4] When disposed in a landfill, PFAS-treated FCM can release PFAS into compost, contaminating surface waters and the surrounding environment.[5] Consequently, the use of PFAS in FCM presents significant concerns related to direct human exposure and environmental pollution at the end of their lifecycle.

The adverse health and environmental impacts of PFAS in FCM have prompted legislative changes. In 2020, Denmark banned PFAS compounds in paper and

paperboard FCM, setting an indicator threshold value of 20 micrograms of organic fluorine (OF) per gram of paper to help the industry identify intentionally added PFAS.[6] In 2023, five European Union member states proposed to the European Chemicals Agency (ECHA) a restriction on all nonessential uses of PFAS, including food packaging.[7] As of 2024, twelve states in the United States have or will legislate the use of PFAS in food packaging due to the absence of federal action. For instance, California banned all plant fiber-based food packaging containing PFAS that are either intentionally added or present at levels exceeding 100 parts-per-million total organic fluorine (TOF), effective January 1, 2023.[8] On February 28, 2024, the Food and Drug Administration (FDA) announced that grease-proofing substances containing PFAS materials are "no longer being sold by manufacturers for food contact use in the U.S. market."[9] However, this restriction only applies to grease-proofing substances, and many other types of PFAS can still exist in food packaging. Additionally, the restriction is a voluntary commitment from current food packaging manufacturers and does not extend to new and international manufacturers.

PFAS testing has been performed primarily using liquid chromatography coupled with triple quadrupole mass spectrometry (LC-QQQ). As a targeted analytical technique, LC-QQQ results are limited to compounds with available standards. As a result, these targeted studies do not necessarily provide a comprehensive measurement of the total PFAS that may exist in samples. Recently, laboratories have focused on developing and validating lower-cost alternatives that provide a more comprehensive measure of total PFAS content. This has led to the development of several methods for measuring total fluorine (TF) as a proxy for total PFAS contamination in FCM. These methods employ technologies such as combustion ion chromatography (CIC)[10-13] and particle-induced γ-ray emission spectroscopy (PIGE).[14-16] PIGE is a surface measurement technique. Due to the limited penetration depth of the particle beam, sample heterogeneity and surface coatings can lead to higher measurements compared to bulk volume techniques such as CIC. However, measuring only TF is not a reliable proxy for PFAS, as it includes both organic and inorganic fluorine, the latter of which is not considered PFAS. Using TF as a measure of PFAS may overestimate the amount of PFAS in samples. A method for determining TOF in packaging was developed using oxygen combustion sample preparation with a fluoride ion-selective electrode.[17] However, this method involves tedious manual steps and lacks the sensitivity of CIC. Alternatively, some studies have used extractable organic fluorine (EOF) to better indicate the total amount of PFAS in samples.[10-12] However, these studies provided a screening technique for PFAS with CIC without specifying the type of fluorine extracted (inorganic vs. organic). This can overestimate EOF if inorganic fluorine (IF) is not subtracted from the measurement, as there is the potential for co-extraction of IF using methanol and other polar organic solvent.[18] Additionally, previous studies only cut FCM into small pieces without grinding them into powder, likely resulting in lower extraction efficiency compared to samples ground to increase the surface area for extraction.

CIC offers excellent sensitivity and versatility, independence on sample thickness, and the possibility for direct ion chromatography (IC) analysis to determine IF. Consequently, CIC has been successfully used to determine adsorbable organic fluorine (AOF) and TOF in environmental sample matrices.[19-21]

In this study, we developed a method to screen for total PFAS using CIC to characterize fluorine content in FCM. This method allows for full quantification of TOF and EOF fractions of fluorine. Furthermore, we characterize the organic fluorine fractions by identifying individual PFAS compounds using high resolution mass spectrometry (HRMS).

Experimental

Equipment

- A Thermo Scientific™ Dionex™ Integrion™ HPIC™ system (P/N 22153-60306) including:
  - Eluent generator
  - Pump
  - Degasser
  - Conductivity detector
  - Column oven temperature control
  - Detector-suppressor compartment temperature control
- Nittoseiko™ Automatic Combustion Unit Model AQF–2100H system* including:
  - Automatic Sample Changer ASC-Controlasc-270LS
  - Horizontal Furnace Model HF-210
  - Gas Absorption Unit GA-211
  - External Solution Selector ES-210

*Any combustion oven with equivalent performance will work.

- Thermo Scientific™ EXTREVA™ ASE™ Accelerated Solvent Extractor (P/N 22184-60101)
- Genevac™ Rocket Synergy™ 2 Benchtop Evaporator (ATC Scientific Product, Warminster, PA)
- Thermo Scientific™ Orbitrap Exploris™ 240 mass spectrometer (P/N BRE725535)
- Thermo Scientific™ Vanquish™ Flex UHPLC system

Software

- All CIC data were acquired using the Thermo Scientific™ Chromeleon™ Data System (CDS) Version 7.3 1 with DDK driver to control the combustion system.
- All LC-HRMS data were acquired and processed using Chromeleon CDS, version 7.3.2.
- Thermo Scientific™ Compound Discoverer™ 3.3 SP3 software package

Consumables

- Thermo Scientific™ Dionex™ EGC 500 KOH Cartridge (P/N 075778)
- Thermo Scientific™ Dionex™ CR-ATC 600 Continuously Regenerated Anion Trap Column (P/N 088662)
- Thermo Scientific™ Dionex™ ADRS 600 Anion Dynamically Regenerated Suppressor, 2 mm (P/N 088667)
- Fisherbrand™ Narrow-Mouth field sample bottles, high density polyethylene (HDPE), for storage of standards and samples, 125 mL (Fisher Scientific P/N 02-895A) and 250 mL sizes (Fisher Scientific P/N 02-895B)
- Thermo Scientific™ Dionex™ ASE™ Collection Vials, 60 mL (P/N 048784)
- Thermo Scientific™ Dionex™ Extraction Cell Filters, Cellulose, 27 mm (P/N 068093)
- Stainless steel extraction cells 10 mL (P/N 060070)
- Polyethersulfone (PES) filter 0.2 µm pore size (Fisher Scientific P/N 09-740-113)
- Disposable syringe filters, 22 mm, 0.2 µm, nylon membrane (P/N CH4513-NN)
- Polypropylene autosampler vials 1.5 mL (P/N 6ESV9-1PP)
- Polypropylene caps, 9 mm, screw-thread (P/N C5000-50)
- Polypropylene centrifuge tube 15 mL (Fisher Scientific P/N 05-539-12), 50 mL (Fisher Scientific P/N 05-539-13)

Reagents and standards

- Deionized (DI) water, Type 1 reagent grade, 18 MΩ·cm resistivity or better
- Certified fluoride standard (1,000 mg/L) (Fisher Scientific P/N NC1145532)
- Certified seven-anion standard mixture (Fluoride 20 mg/L, Bromide 100 mg/L, Chloride 100 mg/L, Nitrite 100 mg/L, Nitrate 100 mg/L, Sulfate 100 mg/L, Phosphate 200 mg/L) (Fisher Scientific P/N NC1145568)
- Perfluorooctanesulfonamide (Sigma-Aldrich P/N CDS010729)
- Sodium perfluoro-1-octanesulfonate (PFOS) (Wellington Laboratories, P/N ULM-9001-1.2)
- PFAS authentic reference standards (Wellington Laboratories)
- Methanol, UHPLC-MS grade, 1 L, Thermo Scientific™ (P/N A458-1) for LC-MS analysis
- Acetonitrile, UHPLC/MS grade, 1 L, Thermo Scientific™ (P/N A956-1) for LC-MS analysis
- Acetic acid, LC-MS grade, 1 mL ampoules, Fisher Chemical™ (Fisher Scientific P/N A1131AMP)
- Ammonium acetate, LC-MS grade, 50 g, Fisher Chemical™ (Fisher Scientific P/N A114-50)
- Water, UHPLC-MS grade, 1 L, Thermo Scientific™ (P/N W81)
- Methanol, HPLC grade (Fisher Scientific P/N A454-4) for sample solvent extraction
- Acetonitrile, HPLC grade (Fisher Scientific P/N A996-4) for sample solvent extraction

Preparation of solutions and reagents

Calibration standard

An 8-point calibration curve was prepared over a concentration range of 1 to 200 mg/L by diluting the fluoride certified 1,000 mg/L standard solution with DI water.

Perfluorooctanesulfonamide

A standard solution of perfluorooctanesulfonamide with a concentration of 1 mg/mL in methanol was prepared for direct combustion and extraction recovery analysis. It was prepared by dissolving 25 mg of perfluorooctanesulfonamide in 25 mL of methanol.

Samples

FCMs were either purchased from an online store or collected from a local restaurant. Eight samples were analyzed: a compostable disposable paper bowl (Sample #1), a kraft paper bakery bag (Sample #2), a compostable disposable paper plate (Sample #3), a sandwich wrap (Sample #4), a pizza box (Sample #5), a french fry cup (Sample #6), a french fry bag (Sample #7), and a piece of baking paper (Sample #8).

Sample preparation

FCM were first cut into pieces using stainless steel scissors that had been washed with methanol. Samples intended for the determination of TOF and EOF were finely ground using a Freezer/Mill model 6770 (SPEX SamplePrep LLC, Methucen, NJ) and a 6751 small grinding vial set, which includes magnetic stainless steel end caps, impactor rods, and polycarbonate center cylinders. The process, known as cryogenic milling, involves grinding the sample while cooling it with liquid nitrogen. The extreme cold makes the material brittle, facilitating its reduction into fine particles. The sample particle size after grinding, measured by a Beckman Coulter™ Multisizer™ 3 Particle Counter, is approximately 30–50 µm. Approximately two grams of each sample were placed into a small freezer mill grinding vial and ground according to the manufacturer's instructions. After grinding, vials were removed from the instrument and allowed to sit at room temperature for 5–10 min to warm up before the removal of the magnetic stainless steel end caps.

Inorganic fluorine was then extracted from the ground samples as follows: 1 g of pulverized sample was weighed in a 50 mL centrifuge tube and mixed with 20 mL of deionized (DI) water. The tube was sonicated in a water bath for 10 min and centrifuged at 15,000 × g for 10 min. The supernatant was filtered with a polyethersulfone (PES) filter (pore size = 0.2 µm), and the filtrate was collected in a 15 mL polypropylene vial. This extract was directly injected into the IC to determine total inorganic fluorine (TIF).

One gram of ground sample was weighed in 10 mL accelerated solvent extraction (ASE) cells and then extracted with a solvent mixture of methanol (Φ = 80%) and acetonitrile (Φ = 20%) using an EXTREVA ASE system at 60 °C for 15 min with a solvent flow rate at 1 mL/min, gas-assisted flow rate at 10 mL/min, cell fill volume 50%, and purge time at 45 s. The extract was collected in a clear 60 mL collection vial and then transferred to a Genevac Rocket Synergy 2 Benchtop Evaporator. The extract was dried completely using the preprogrammed low boiling point program for one hour and then reconstituted in 1.4 mL of extracting solvent. To prevent the loss of volatile PFAS during evaporation, a low boiling point program was selected.

The extractable fluorine levels of both direct extracts and concentrated extracts from the three samples were compared, and similar results were observed. This indicates that PFAS are not lost during the evaporation process for these three samples. However, the methods may need optimization for other samples, as the volatile PFAS molecules in different samples may vary. The concentrated extract was transferred to a 1.5 mL centrifuge tube and centrifuged at 15,000 × g for 10 min. The supernatant was transferred into another 1.5 mL centrifuge tube. This extract was used to determine extractable fluorine (EF), containing both organic and inorganic extractable fluorine by CIC and non-targeted PFAS analysis by LC-HRMS.

Another 1 g aliquot of ground sample was extracted following the above procedure. The extract was redissolved in 20 mL of DI water after being completely dried. The solution was centrifuged at 15,000 × g for 10 min and filtered through a PES syringe filter (pore size = 0.2 µm). The amount of extractable inorganic fluorine (EIF) was determined by directly injecting this solution into the IC system.

Table 1. Combustion ion chromatography conditions

Method parameter	Value
Eluent source	Dionex EGC 500 KOH eluent generator cartridge, Dionex CR-ATC 600 trap column
KOH gradient	Time (min) / KOH (mM) 0–6 / 8 6–10.25 / 8–75 10.25–12 / 75 12–15 / 75–8 15–20 / 8
Injection volume	25 µL
Columns	Thermo Scientific™ Dionex™ IonPac™ AG24 guard, AS24 analytical anion-exchange columns, 2 mm
Column temp.	30 °C
Flow rate	0.30 mL/min
Detection	Suppressed conductivity, Dionex ADRS 600 suppressor, recycle mode, 56 mA
Furnace temp.	950 °C inlet, 1,000 °C outlet
Boat program	Position (mm) / Wait time (s) / Boat speed (mm/s) 90 / 60 / 10 End / 600 / 10 Cool / 60 / 40 Home / 120 / 20
Gas	Ar: 200 mL/min; O_2: 400 mL/min
Hydration	Water: pump scale 2, 125 µL/min /Ar: 100 mL/min

TOF and EOF by CIC

TOF and EOF were measured using a hybrid CIC system consisting of a Thermo Scientific Dionex IC system and Nittoseiko modules, although any combustion oven with equivalent performance will work.

The CIC method combines an automated Nittoseiko Analytech AQF-2100H combustion-absorption unit with a Dionex Integrion IC system. There are two modes to introduce a sample into the CIC system as shown in Figure 1. One mode is combustion mode. In this mode, samples (both neat material and extracts) were placed onto a ceramic boat that was introduced into a combustion oven (HF-210, Nittoseiko) heated to 1,100 °C under an atmosphere of argon (200 mL/min) and oxygen (400 mL/min). Hydration with water at pump scale 2 (125 µL/min) and argon gas at 100 mL/min was supplied to the branch tube of the pyrolysis tube with the Nittoseiko GA-211 module to prevent the formation of HF, which will react with the glass/quartz of the furnace tube.

All gaseous acidic combustion products were absorbed in 10 mL of DI water (Nittoseiko GA-211 module), and an aliquot of 25 µL was injected into the IC system. In this mode, all fluorine derived from organic and inorganic compounds from solid or liquid samples is converted to and determined as fluoride by IC.

The other mode is called direct injection mode. In this mode, aqueous samples were introduced directly to the IC sample loop. Fluoride was measured using a Dionex Integrion IC system, equipped with a 25 µL sample loop, Dionex ADRS 600 suppressor, and Dionex EGC 500 KOH cartridge. Fluoride was separated from other anions using a Dionex AS24 analytical column and AG24 guard column maintained at 30 °C. The detailed CIC conditions are listed in Table 1.

Figure 2 describes the overall workflow for analyzing total fluorine (TF) and total inorganic fluorine (TIF), extractable fluorine (EF), and extractable inorganic fluorine (EIF) analysis in samples using the CIC method.

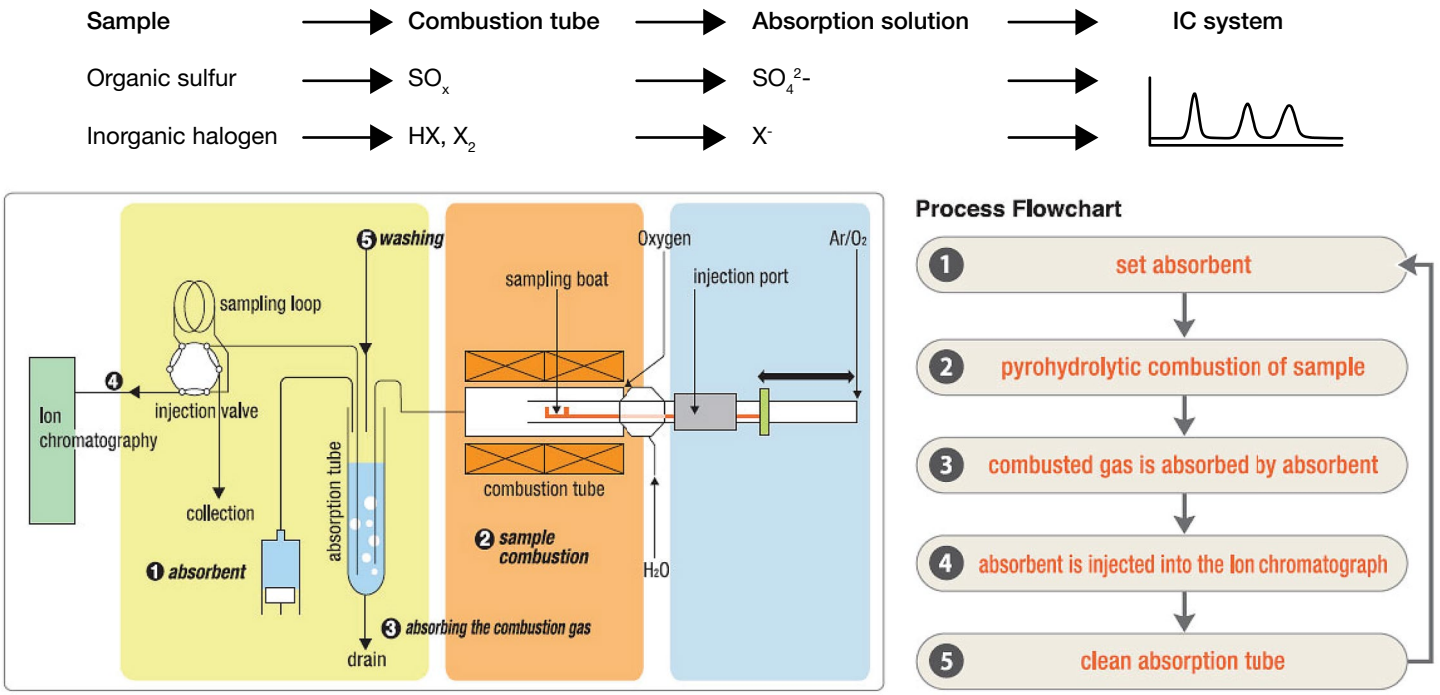

Figure 1. Diagram of a combustion ion chromatography system

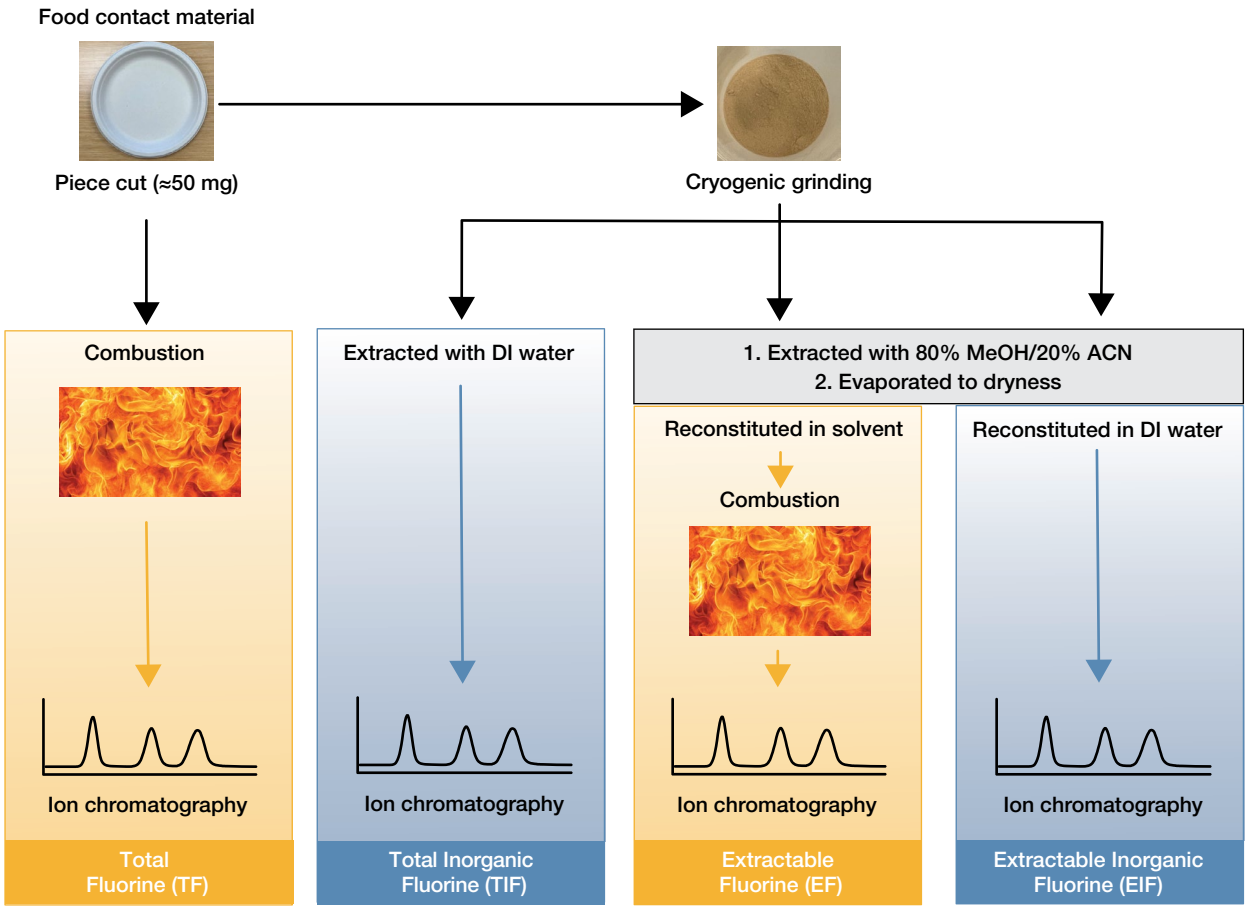

Figure 2. TOF and EOF analysis workflow

For TF analysis, the sample amount needed may vary based on the concentration of PFAS in the samples. For the analyses described here, 10–50 mg of FCM was cut and placed onto a pre-baked ceramic boat for analysis by CIC operated in combustion mode. To avoid carryover, two to three boat blanks were measured after samples with expected high fluorine content. For example, Sample #3 had the highest fluorine level measured. After sample injection, a boat blank was run, and we observed a 0.96% carryover. Following this, an additional boat blank was run, and a 0.098% carryover was observed. To determine TIF, the water extract was analyzed by CIC operated in direct injection mode. TOF was calculated by subtracting TIF from TF.

To determine EF, 200 µL of concentrated organic extract was placed on a ceramic boat and analyzed by CIC in combustion mode. To determine EIF, the organic extract was dried and reconstituted in 20 mL of water and then analyzed by CIC direct injection mode. EOF was calculated by subtracting EIF from the EF. To avoid carryover, two to three boat blanks were measured after samples with expected high fluorine content.

Identification of PFAS using HPLC-HRMS

HPLC-HRMS analysis was performed using an Orbitrap Exploris 240 mass spectrometer coupled to a Vanquish Flex UHPLC system. The liquid chromatography system was fitted with a PFAS conversion kit to remove fluoropolymers from the wetted flow path of the UHPLC system. A Hypersil GOLD (3.0 × 50 mm, 1.9 µm) delay column was installed between the HPLC pump and autosampler to separate PFAS present in the mobile phase from compounds present in samples. The system was also fitted with a strong solvent loop between the autosampler and analytical column to allow for larger injections of solutions containing a high percentage of organic solvent.

The extracts of the FCM were transferred to polypropylene autosampler vials with polypropylene caps containing a polypropylene septum and analyzed by LC-HRMS. To monitor instrument performance across the sequence, samples were spiked with a solution containing 24 isotopically labelled PFAS internal standards (MPFAC-HIF-ES, Wellington Laboratories). All analyses were performed using a 22-minute reverse phase chromatography method with an Acclaim RSLC C18 (2.1 × 100 mm, 2.2 µm) analytical column and a 5 µL injection volume. To reduce the adsorption of long chain PFAS to surfaces within the autosampler vial, the autosampler temperature was maintained at 22 °C throughout all analyses. Since the polypropylene septa of the autosampler vial caps do not reseal following each injection, all replicate injections were performed by injection of separate vials of the same solution.

All HRMS analyses were performed using heated electrospray ionization (HESI). All source conditions were optimized to provide the highest levels of signal sensitivity and stability for PFAS. The HESI spray voltage was set to -1,000 V, and the source nitrogen gas flows were set as follows: sheath gas of 55 arbitrary units (arb), aux gas of 12 arb, and sweep gas of 0.5 arb. To reduce in-source fragmentation of PFAS ether acids, an ion transfer tube temperature of 225 °C and vaporizer temperature of 250 °C were used. Data were collected using a top 4 data-dependent MS^2 method with a quadrupole isolation width of 1.5 Daltons, stepped collision energies (absolute) of 2, 10, 25, and 55 V, and a maximum ion injection time of 50 ms. A mass resolution of 240,000 FWHM (full width half maximum; defined at *m/z* 200) was used for Full Scan and 30,000 FWHM for $ddMS^2$. Mild trapping was enabled to reduce precursor ion fragmentation during the acquisition of Full Scan spectra. To consistently provide mass accuracies ≤1 ppm in all spectra collected across each HPLC-HRMS run, the Thermo Scientific™ EASY-IC™ source was set to scan-to-scan mode. Further details regarding the HPLC-HRMS method can be found in Table 2.

All HPLC-HRMS data were processed within Compound Discoverer software, which contained a workflow specifically designed for detecting and annotating unknown PFAS compounds. Briefly, a compound detection was implemented using a spectral intensity threshold of 10,000 cps, mass tolerance of 2 ppm, and minimum of 5 data points across a detected chromatographic peak. Compounds were detected across all files with the following possible ions and adducts: $[M-H]^-$, $[M-H-CO_2]^-$, $[M-H-H_2O]$-, $[M-H-SO_3]^-$, and $[2M-H]^-$. All compounds detected within individual files were then grouped into a single list of final detected compounds using a mass tolerance of 2 ppm and retention time tolerance of 0.2 min. Additionally, all detected peaks, within each file, were rated using a series of peak rating factors, producing a final rating score between 0–10. All compounds that had detected peaks with a rating factor >6 in at least one sample were retained, while all others were removed. The retained compounds were then annotated using multiple spectral libraries and chemical databases. Three spectral libraries were used: the Thermo Scientific™ mzCloud™ MS^n spectral library, 2023 NIST MSMS library, and the Getzinger *in silico* PFAS library, which contains *in silico*-generated MS^2 spectra of more than 40,000 PFAS compounds.[22] In addition, the measured monoisotopic mass of the $[M-H]^-$ ion of each compound was compared against four different PFAS-specific mass lists: a user-defined list of target PFAS compounds containing retention times measured from reference standards, NIST Suspect List, EPA PFAS Structure List, and the Getzinger *in silico* PFAS library (mass list version of the spectral library described above). Lastly, observed MS^2 fragments for each detected compound were cross-referenced against an offline version of the FluoroMatch PFAS fragment library.[23] For detected compounds that matched to more than one spectral library or mass list, an annotation priority was used that prioritized libraries or mass lists generated from authentic standards analyzed with Thermo Scientific™ instrumentation. This includes the mzCloud MS^n spectral library and the user-defined list of targeted PFAS compounds with retention times. See Table 3 for more information on annotation priority.

After the finalized list of detected and annotated PFAS compounds was obtained, an initial data reduction step was implemented to only retain compounds with a standard mass defect within -0.11–0.12 and the number of fluorine in the calculated molecular formula ≥3. A standards mass defect range of -0.11–0.12 was used as previous work has shown that 90% of all PFAS within the EPA PFAS structure list have a standard mass defect that falls within this range.[24] Following, all annotations were then categorized into confidence levels between 1–5, following criteria and guidance developed specifically for PFAS compounds by Charbonnet *et al*.[25] Table 3 shows the criteria used in this work to assign confidence levels to each annotated PFAS compound.

Table 2. HPLC conditions

Method parameter	Value		
Mobile phase A	UHPLC-MS grade water + 0.1% LC/MS grade glacial acetic acid		
Mobile phase B	78% UHPLC-MS grade acetonitrile + 20% UHPLC grade methanol + 2 mM ammonium acetate (aqueous)		
Analytical column	Thermo Scientific™ Acclaim™ RSLC C18 (2.1 × 100 mm, 2.2 µm)		
Delay column	Thermo Scientific™ Hypersil GOLD™ (3.0 × 50 mm, 1.9 µm)		
Flow rate	0.4 mL/min		
Gradient	Time (min)	%B	Curve
	0	5	5
	1	30	5
	2	45	5
	3	55	5
	14.5	100	5
	17.5	100	5
	17.7	5	5
	22	5	5

Table 3. Criteria for annotating unknown PFAS compounds at different confidence levels

Annotation criteria	Annotation confidence level				
	5	4	3	2	1
Measured mass ±2 ppm of entry in at least one PFAS Mass List[a]	✓	✓	✓	✓	✓
Standard mass defect is between -0.11–0.12	—	✓	✓	✓	✓
Isotopic pattern match[b]	—	✓	✓	✓	✓
≥1 MS^2 fragment with match to FluoroMatch database and/or >50% similarity match to *in silico* PFAS spectral libraries[c]	—	—	✓	—	—
>50% similarity match to mzCloud or 2023 NIST MS/MS spectral libraries[d]	—	—	—	✓	✓
Retention time match to Reference Standard[e]	—	—	—	—	✓

[a]Annotations based on Mass Lists only (Levels 4–5) used the following mass list priority: NIST Suspect List, EPA PFAS Structure List, Getzinger *in silico* PFAS library.

[b]Isotope pattern matching score compared the difference between the isotope distribution of the measured spectral peaks and hypothetical isotope distribution of calculated empirical formula, with the assigned formula requiring mass accuracy within 2 ppm and minimum spectral fit of 50%.

[c]For Level 3 annotation, if multiple compounds produced matching scores >50% (using the NIST search algorithm) against the Getzinger *in silico* PFAS library, then the compound with the highest score was used for the final compound annotation. For Level 3 annotations based only on a match to the FluoroMatch database, mass lists were used for annotations, taking into account similarity between the matching MS^2 fragments and the structure of compounds within the mass lists.

[d]Similarity reverse scores for the mzCloud spectral library were calculated using the Cosine identity search algorithm. The Similarity scores for the 2023 NIST MSMS library were calculated using the NIST search algorithm. For both libraries a precursor mass tolerance of ±2 ppm was used.

[e]Retention times for 91 PFAS compounds were determined using the same UHPLC-MS method used for the non-targeted analysis and were saved as a user-defined mass list, which was then used for Level 1 annotations.

Results and discussion

Separation

Establishing the appropriate eluent was essential for determining the optimal separation of fluoride from the water dip and other common anions. Carbonate and hydroxide are commonly used eluents in IC. Hydroxide eluent was chosen due to its effectiveness in separating fluoride from the water dip and its ability to yield a higher signal-to-noise ratio (S/N) after suppression compared to carbonate eluent. The use of hydroxide eluent resulted in an increased overall method sensitivity.

Figure 3 illustrates the separation of seven common anions using combustion and direct injection modes. Only fluoride, chloride, bromide, and sulfate were detected in the chromatogram obtained from the combustion mode. Notably, fluoride was well-separated from the water dip and other anions, enabling accurate determination. Measuring fluoride using combustion mode and direct injection mode yields the same result of 100% recovery. Other halogen and sulfur species do not have perfect recovery because we used only deionized water as the absorption solution. To convert the gaseous vapors of other halogen and sulfur species into their respective ions, adding hydrogen peroxide is necessary. Since this study focuses solely on determining fluorine, we chose deionized water as the absorption solution to minimize contamination from additional chemicals.

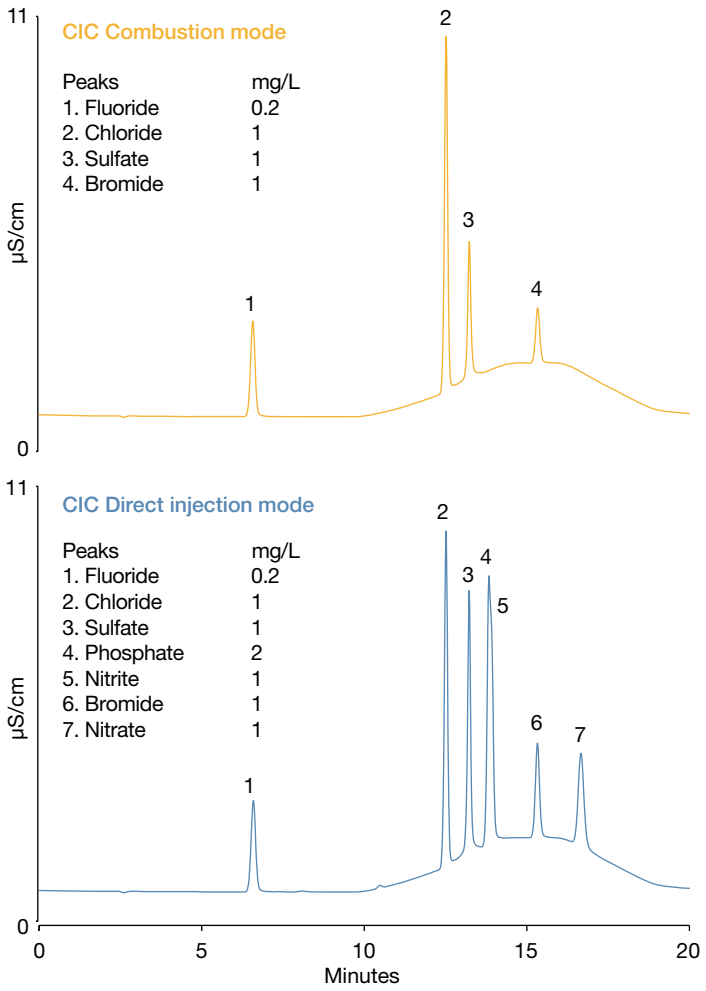

Figure 3. Separation of seven anions standard (combustion mode vs. direct injection mode)

Calibration

Both combustion mode and direct injection mode can be used to build fluoride calibration curves. In this study, we choose combustion mode as recommended by EPA Method 1621 for the Determination of AOF in Aqueous Matrices by CIC.[19] An 8-point calibration curve was prepared over a concentration range of 1–200 mg/L by diluting the fluoride certified 1,000 mg/L standard solution with DI water. Each calibration standard was analyzed by pipetting 200 µL into clean ceramic boats. The regression coefficients of the calibration curve were >0.999 with a quadratic fitting, and calculated concentrations of the calibration standards were within 97–110% of the true value for all calibration levels, meeting the EPA requirements of 80–120%. The calibration was also assessed based on the relative standard error (RSE) method as listed in EPA Method 1621, and the RSE for the calibration curve was <5%, meeting the EPA requirement of 20% or less.

Method detection limit (MDL)

The method detection limit (MDL), which depends on blank values and standard deviations of blank measurements, was calculated according to the guideline in the Definition and Procedure for the Determination of the Method Detection Limit, Revision 2 EPA document.[26] We report MDL_b if the blank for an individual analyte gives a numerical result. The MDL_b is calculated as: $MDL_b = X + (3.143 \cdot \sigma)$, where X is the average of the method blanks, 3.143 is the Student's *t*-test value for n=7, and σ is the standard deviation of the replicate method blanks. MDL_s is reported if the blank does not give a numerical value and a spiked MDL standard was prepared, calculated as: $MDL_s = 3.14 \cdot \sigma$, where 3.143 is Student's *t*-test value for n=7, and σ is the standard deviation.

For TF, seven boats without samples were analyzed, yielding an average blank value of 10.6 ng and standard deviation 4.6. This translates to an MDL_b of 0.51 µg/g, assuming a sample weight of 50 mg.

For TIF and EIF, external water injection did not yield a value. Therefore, 2 µg/L fluoride MDL standard was prepared. Seven injections of the MDL standard were run, and MDL_s was calculated as 24.2 ng/g assuming a sample weight of 1 g.

For EF, seven blank solvents extracted by the EXTREVA ASE system were analyzed, yielding an average value of 224 ng of fluorine and a standard deviation of 23 ng. This translates to an MDL_b of 2.07 µg/g assuming a sample weight of 1 g. The blank value of fluorine is primarily contributed by the extraction solvent itself, as direct combustion of the extraction solvent also yielded a similar blank value.

Method accuracy and precision

Accuracy and precision were evaluated using two approaches. First, the direct combustion of the PFAS standard was performed to verify the accuracy of the CIC system. Second, samples were spiked with PFAS standards and subjected to the complete workflow to assess accuracy. The spiked samples were analyzed in triplicate to evaluate method precision.

To monitor for background contamination, extraction blanks were processed in every batch. The obtained results were blank corrected by subtracting the corresponding average blank values.

Testing the combustion efficiency of PFAS is crucial. However, evaluating the combustion efficiency of every possible organofluorine compound detected with this technique is not feasible. PFOS specifically was tested because it is a C8 PFAS compound commonly used in grease-proofing agents in FCM, but it is less volatile compared to other C8 PFAS compounds. As a result of its lower volatility, it can be harder to combust to completion relative to other C8 PFAS compounds. A standard solution of sodium perfluoro-1-octanesulfonate (PFOS) with a concentration of 50 µg/mL in methanol was used for direct combustion analysis. For the test, 200 µL of this solution were added to the CIC sample boat, and the fluoride value was determined using a previously established calibration curve. A recovery rate of 101% was achieved, confirming that the chosen combustion conditions effectively convert the fluorine in PFAS compounds to fluoride. Additionally, a combustion efficiency test was performed using another more volatile C8 PFAS compound, perfluorooctanesulfonamide. For this test, 20 µL of perfluorooctanesulfonamide with a concentration of 1 mg/mL in methanol was prepared, achieving a recovery rate of 99.3%.

To assess extraction recovery, 154 µL of a 1 mg/mL perfluorooctanesulfonamide standard solution was added to 1 g of ground FCM sample, resulting in a spike of 100 µg/g of fluorine in the samples. The samples were incubated at room temperature overnight to ensure absorption of PFAS into the FCM matrix. The samples and spiked samples were then extracted using the previously described EXTREVA ASE method. The EF content was determined using CIC. Extraction recoveries ranging from 95% to 101% were obtained for all three samples, confirming the effectiveness of the chosen extraction conditions in extracting PFAS from the FCM matrix. The average relative standard deviation between the triplicates was less than 5%, indicating high precision.

TOF and EOF in FCM samples by CIC

The TF in a sample is defined as the sum of TIF and TOF. In this study, TOF was determined by subtraction of TIF from TF. After combusting the solid sample, TF was measured using IC. Before working on food contact materials, Kimtech Science™ Kimwipes™ Delicate Task Wipes were used as a surrogate sample control to ensure that no contamination was introduced during the sample preparation process, including cutting and transferring.

The total fluorine in the Kimwipes was found to be below the limit of quantification. High concentrations of TF, ranging from 1,083 ppm to 2,142 ppm, were measured in three of the samples (Table 4). Three replicates of sample analysis were conducted in this study. The relative standard deviation (RSD) for these replicates is less than 6% for samples with high TF (greater than 10 ppm) and less than 8% for samples with low TF (less than 10 ppm). Molded fiber-based FCM (Samples #1 and #3) required large quantities of PFAS to be mixed into the raw pulp to confer mechanical strength and prevent disintegration upon contact with liquids. Paper bags (Sample #2), used for oily foods like pastries, donuts, or hamburgers, also contained high levels of total fluorine. Many brand owners and retailers have adopted a threshold of 100 ppm of TF as a concentration that is indicative of intentionally added PFAS treatments.[27,28] The next step for the samples with high levels of TF was to analyze TOF.

TIF was measured using IC after extracting the ground FCM samples with DI water. The term total inorganic fluorine (TIF) was introduced to differentiate from extractable inorganic fluorine (EIF), as water is a more effective extraction solvent for inorganic fluorine than organic solvents. Due to the production process of paper and board, there may be high concentrations of calcium present, which can form insoluble calcium fluoride. We evaluated the total inorganic fluorine levels in our samples and found that the fluorine levels were well below the saturation concentration of calcium fluoride, regardless of the presence of calcium carbonate. However, if the TIF values are above the saturation concentration of calcium fluoride, additional experiments should be run to understand the amount of fluoride potentially bound to calcium and therefore not reflected in the TIF measurements. Therefore, the calculation we provided for total organic fluorine (TOF) = (TF - TIF) is only valid for samples with fluoride concentrations below the saturation concentration. The contribution of inorganic fluorine to TF was found to be very small, as shown in Table 4. Even after subtracting TIF, the TOF remained above 1,000 ppm. Therefore, the three samples did not meet the regulatory limit of 100 ppm TOF set by the state of California.[8]

Figure 4 shows the IC chromatogram of TF and TIF after combusting Sample #1. Fluoride is separated from other anions and detected by suppressed conductivity. It is extracted quantitatively with DI water from samples, and therefore, it can be accurately determined.

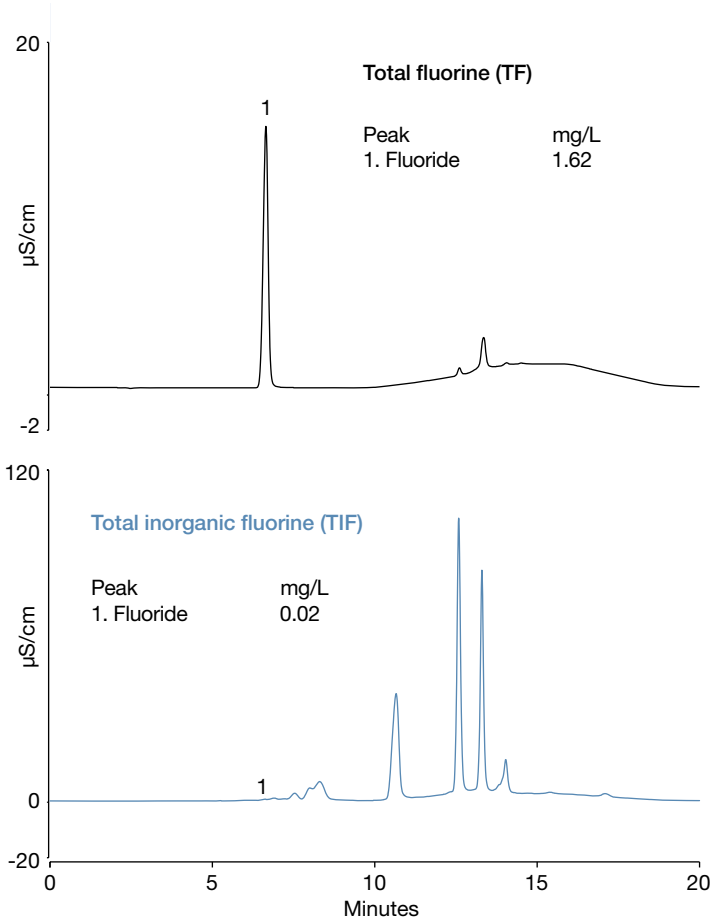

Figure 4. TF and TIF chromatogram (Sample #1)

Table 4. TOF and EOF in food contact material, ppm (µg/g) (n=3, RSD <8%)

Sample	TF	TIF	TOF (TF-TIF)	EF	EIF	EOF (EF-EIF)	EOF/TOF (%)
1	1,083	0.4	1,082.6	139	0.5	138.5	12.8
2	1,369	0.3	1,368.7	90.3	0.3	90	6.6
3	2,142	0.2	2,141.8	72.8	0.2	72.6	3.4
4	8.31						
5	20.2						
6	5.39						
7	41.7						
8	3.29						

EOF was determined for each sample using CIC to gain further insight into the PFAS content in these samples. Comparing the EF content obtained from cut pieces and ground samples of the three samples showed increased extraction yields (plus 85–171%) for ground samples. Therefore, ground samples were used for extraction in this study.

Ground solid samples were extracted using the previously described method with the EXTREVA ASE extractor. This extraction technique employs dynamic extraction, unlike traditional static extraction. In this method, the extraction solvent is continuously added to the extraction cell, and the extract is continuously collected into a collection bottle. The conditions selected were effective in completely extracting fluorine compounds from the three samples tested, as determined by analysis of a second subsequent extraction following the first. However, extraction parameters such as extraction time, solvent delivery speed, and extraction temperature can be adjusted to suit the specific sample matrix. The extract was divided, with a 200 µL portion subjected to EF analysis by CIC, while the remaining extract was used for non-targeted LC-HRMS PFAS analysis. The EF amount in the three samples ranged from 72 to 139 ppm (Table 4). The amount of EIF was minimal, similar to the TIF determined after DI water extraction. After subtracting the EIF from the EF, the range of extractable organic fluorine remained at 72–139 ppm.

Among the three samples we tested, Sample #3 had the highest TOF, while Sample #1 had the highest EOF. This suggests that EOF and TOF are not correlated in the three samples we tested. However, the sample size in this study is insufficient to draw comprehensive conclusions about the correlation between EOF and TOF universally for FCM. Figure 5 demonstrates the separation of EF and EIF in sample #1. For the EIF study, we used a solvent mixture of 80% methanol and 20% acetonitrile. This solvent mix can extract not only fluoride but also other anions such as organic acids and chloride, which may interfere with the integration of the fluoride peak. However, as shown in the chromatogram, fluoride was successfully separated from these anions using ion chromatography, allowing for accurate determination.

Figure 6 presents an overview of the different forms of fluorine in sample #1. This study did not conduct targeted PFAS analysis to calculate the fluorine mass balance. Previous research has shown that targeted PFAS analysis can identify only a small fraction of the extractable organic fluorine (EOF) due to limited availability of

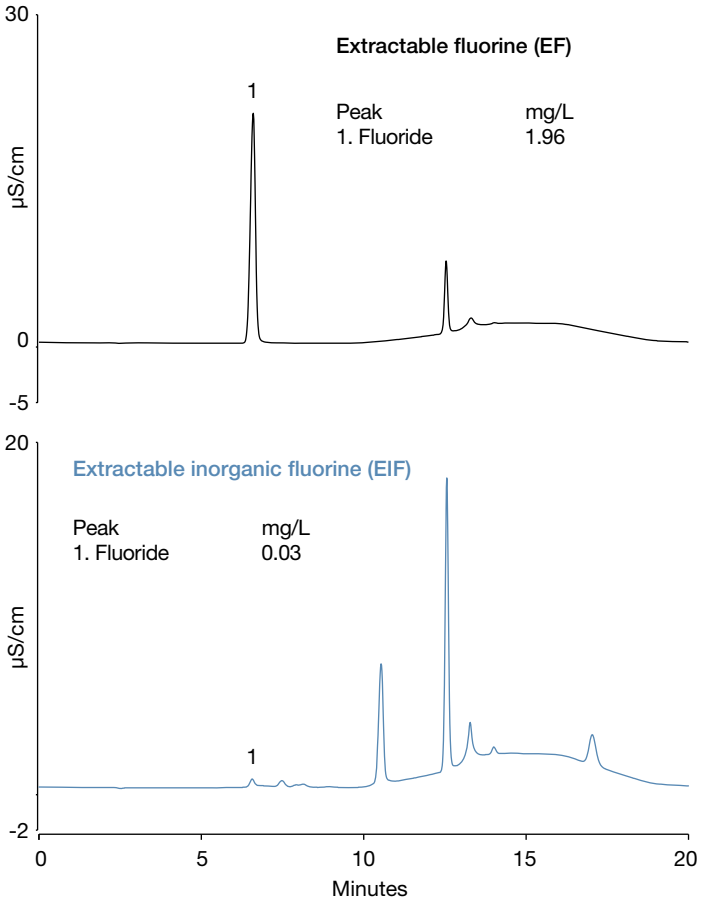

Figure 5. EF and EIF chromatogram (Sample #1)

PFAS standards and inefficient ionization of certain fluorinated alkanes/alkenes and PFAS precursors.[10-12] The effectiveness of targeted LC-MS PFAS analysis is heavily influenced by the total amount of PFAS that can be extracted from a solid sample.

Figure 6 shows that EOF accounts for less than 15% of TOF, and LC-MS-based targeted PFAS analysis will miss unidentified organic and non-extractable organic fluorine compounds. In assessing overall exposure to organic fluorinated chemicals, EOF provides a more accurate estimate than targeted LC-MS analysis. TOF better describes the total PFAS contamination in FCM, including organofluorines that are less or not extractable using organic solvents, such as polymeric PFAS or lipophilic species like fluorinated alkanes.

To ensure accuracy and precision, it is essential to utilize a combination of analytical techniques that complement each other and offer a comprehensive understanding of the content and composition profile of the samples. A general decision tree approach was adopted when determining which measure

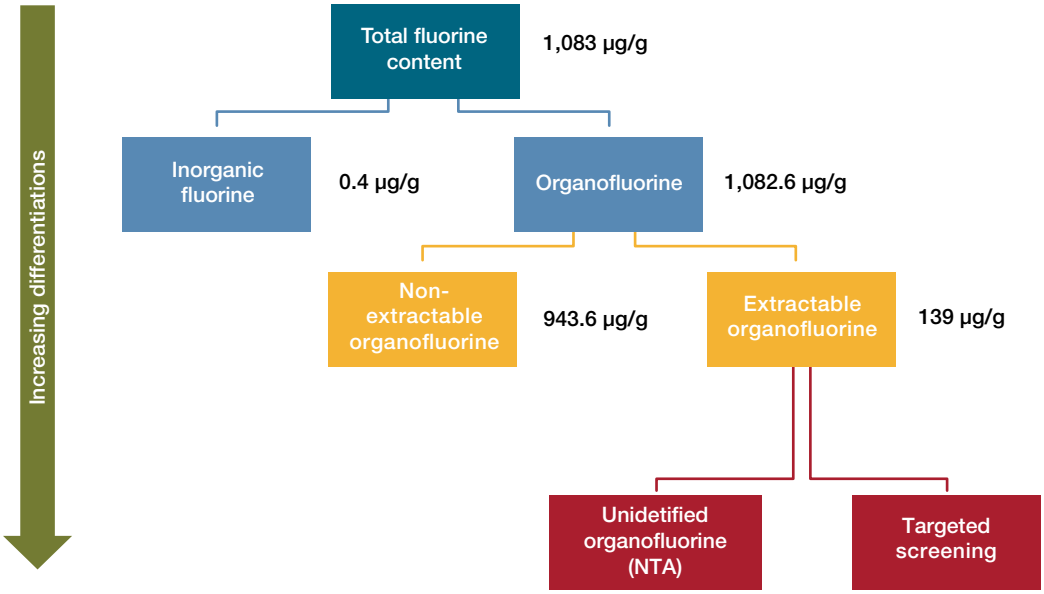

Figure 6. TOF and EOF mass balance (Sample #1)

of fluorine to use in the study. We began by measuring the TF content through the combustion of the cut sample. If the TF was below the compliance threshold of 100 ppm, no further analysis was required, as the TOF would also be under the limit. However, if the TF exceeded 100 ppm, we proceeded to the next step for additional measurements. We determined the TIF and subtracted this from the TF to calculate TOF. For regulatory compliance, these two steps were sufficient because only TOF is regulated. If a deeper understanding of the PFAS compounds in your samples is desired, perform solvent extraction to determine the EOF.

Human exposure to PFAS occurs through multiple pathways. Direct ingestion of PFAS-containing materials represents one significant risk, where extractable organic fluorine (EOF) can serve as a useful indicator if the PFAS is extracted directly from food contact materials (FCM). However, environmental contamination also plays a crucial role in PFAS exposure. Non-extractable PFAS released into the environment can degrade over time, posing substantial risks through subsequent human contact. To understand these risks better, further research is needed to quantify the exposure fraction from precursors that degrade into terminal PFAS, which are associated with adverse health effects.[29] Regulatory bodies often regulate total organic fluorine (TOF) over EOF due to the potential for PFAS in landfills to contaminate the environment and because the EOF method is more time-consuming and costly.

Identification of PFAS in FCM using HPLC-HRMS

The levels of total organic fluorine (TOF) detected in the three samples exceed the regulated limit for PFAS in food contact materials (>100 ppm). Sample #1 also surpasses the regulated limit of 100 ppm for extractable organic fluorine (EOF). To better understand the specific molecular composition of the EOF fraction collected from FCM food contact materials, a non-targeted analysis approach using LC-HRMS was implemented for extracts of all three food contact material samples. Following recommendations from Charbonnet *et al.*, specific criteria were established to classify the annotations of each detected PFAS compound into five confidence levels (Table 3). Confidence Levels 1 and 2 require strong matches to mass spectra within libraries of spectra obtained from authentic reference standards. Level 3 necessitates a combination of high matching scores to spectra within an *in silico* mass spectra library or a PFAS fragment database. Annotations in Levels 4 and 5 rely on a match between the measured monoisotopic mass (within 2 parts per million) and/or a high similarity between the measured and hypothetical isotope pattern.

Table 5 displays the final list of detected PFAS compounds, with 46 compounds identified and annotated across the three samples. The peak area intensities of these compounds varied by four orders of magnitude.

Table 5 (part 1). List of PFAS compounds detected and annotated with Levels 1–5 confidence, using the non-targeted analysis workflow. The table is sorted by maximum observed peak area across all samples, in descending order.

Measured *m/z* (amu)	Sample detected in	Annotated name or InChIKey[a]	Annotated formula[b]	Confidence level	Number of matches to library or database			
					mzCloud Library[d]	2023 NIST Library[e]	*in silico* PFAS Library[f]	Mass lists and ChemSpider[g],[h]
312.97284	All	Perfluorohexanoic acid (PFHxA)	$C_6HF_{11}O_2$	1	1	1	10	24
639.08105	All	MOBOLROZYFUWDB-UHFFFAOYSA-N	$C_{19}H_{16}F_{20}O$	4	0	0	0	1
291.98872	1	VZNMWWFYPBHICQ-UHFFFAOYSA-N	$C_5H_6F_7NO_3S$	4	0	0	0	2
162.98242	All	Perfluoropropanoic acid (PFPrA)	$C_3H_5O_2$	3	0	0	2	13
269.02448	3	FPCVLTWBCITESZ-UHFFFAOYSA-N	$C_{10}H_7F_5O_3$	5	0	0	0	8
222.99957	All	DJKYVLNTLUJRAO-UHFFFAOYSA-N	$C_7H_3F_7O_3$	5	0	0	1	6
338.98782	All	QCGLRGPTQJMLID-UHFFFAOYSA-N	$C_8H_3F_{11}O_2$	4	0	0	0	20
376.98468	All	2-Perfluorohexyl ethanoic acid (6:2 FTCA)	$C_8H_3F_{13}O_2$	4	0	0	0	19
365.01481	1,3	VFPXSKFHUQRSGX-UHFFFAOYSA-N	$C_9H_5F_{11}N_2O$	5	0	0	0	3
212.97921	All	Perfluorobutanoic acid (PFBA)	$C_4HF_7O_2$	1	1	0	2	21
362.96902	All	Perfluoroheptanoic acid (PFHpA)	$C_7HF_{13}O_2$	1	1	1	4	21
298.94301	All	Perfluorobutanesulfonic acid (PFBS)	$C_4HF_9O_3S$	1	1	1	0	17
310.10472	2	2,2,3,3,4,4,4-heptafluoro-N-heptan-2-ylbutanamide	$C_{11}H_{16}F_7NO$	5	0	0	0	4
277.05010	2	DBICBVPPQHNOJS-UHFFFAOYSA-N	$C_9H_{11}F_5O_4$	5	0	0	0	6
423.02666	All	WVGVZMFVHJSROX-UHFFFAOYSA-N	$C_{10}H_9F_{13}O_3$	4	0	0	0	2
262.97601	All	Perfluoropentanoic acid (PFPeA)	$C_5HF_9O_2$	1	1	1	3	28
213.01530	All	3:2 Fluorotelomer alcohol (3:2 FTOH)	$C_5H_5F_7O$	4	0	0	0	36
418.99607	All	Ethyl 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluoro-2-oxooctanoate	$C_{10}H_5F_{13}O_3$	3	0	0	0	24
280.98297	All	perfluoro methyl cyclopentane carboxylic acid	$C_7HF_{11}O_2$	3	0	0	9	15
526.02108	All	AWGGYVBRXBKTHH-UHFFFAOYSA-N	$C_{17}H_{13}F_8NO_7S$	5	0	0	0	1
338.98783	2,3	Ethenyl undecafluorohexanoate	$C_8H_3F_{11}O_2$	3	0	0	0	19
473.00715	1,3	AMHRIPXYMJFVNS-UHFFFAOYSA-N	$C_{13}H_7F1_3O_4$	3	0	0	0	8
493.03227	1,2	DNHMQQZUPYTBHR-UHFFFAOYSA-N	$C_{13}H_{11}F_{13}O_5$	3	0	0	0	4
219.05100	1,3	Butanoic acid, 3,3,4,4-tetrafluoro-2-hydrazino-2-hydroxy-, hydrazide	$C_4H_8F_4N_4O_2$	4	0	0	0	2
221.00678	1,3	S-Propan-2-yl pentafluoropropanethioate	$C_6H_7F_5OS$	3	0	0	0	7
242.98971	1	Perfluoromethoxypropionic acid methyl ester	$C_5H_3F_7O_3$	3	0	0	0	25
442.96239	All	6:2 fluorotelomer sulfate (6:2 FTS)	$C_8H_5F_{13}O_4S$	3	0	0	0	10
253.10576	1,2	AUNCOBDUGAGLPF-UHFFFAOYSA-N	$C_{11}H_{17}F_3O_3$	4	0	0	0	9
719.98837	All	JQMYFIYSOVPACE-UHFFFAOYSA-N	$C_{16}H_5F_{26}NO$	5	0	0	0	2
650.03535	All	N,N-dimethyl-N-3-(perfluoroalkylsulfonamidopropan-1-yl)amine N-oxide	$C_{14}H_{14}F_{19}N_2O_3S$	5	0	0	0	1

[a]PFAS compounds annotated using the Getzinger *in silico* PFAS library are annotated with the InChIKeys. For compounds annotated at Level 3 confidence without a match to the *in silico* PFAS library, annotation was determined by comparison between MS^2 fragments matching the FluoroMatch database and mass lists, using the following mass list priority: NIST Suspect List, EPA PFAS Structure List, Getzinger *in silico* PFAS library.

[b]For Levels1-4 confidence, formulas were determined through the Predict Composition node of the workflow, requiring mass accuracy within 2 ppm and minimum spectral fit of 30%. For Level 5 confidence, formulas were derived from the mass list (within 2 ppm mass error).

[c]The annotated mass is the numerical difference between the hypothetical monoisotopic *m/z* based on the empirical formula and the measured *m/z*.

[d]Number of compounds within the online mzCloud database that produced similarity reverse scores >50% and precursor mass within ±2 ppm of the measured mass. The compound with the highest similarity reverse score was used for the final compound annotation.

[e]Number of compounds within the offline 2023 NIST Tandem Mass Spectrometry library (as an mzVault library database) that products matching scores >50% (using the NIST search algorithm) and precursor mass within ±2 ppm of the measured mass, with the highest score used for the final compound annotation.

[f]Number of compounds within the Getzinger *in silico* PFAS library (as an mzVault library database) that products matching scores >50% (using the NIST search algorithm) and precursor mass within ±2 ppm of the measured mass, with the highest score used for the final compound annotation.

[g]Number of compounds with monoisotopic mass within 2 ppm of the measured mass in the Full Scan spectrum across all searched Mass Lists and ChemSpider. For ChemSpider, a direct match to the calculated empirical formula also had to be met.

[h]For Level 5 annotations, annotation priority was based on the following order of mass lists: NIST Suspect List, EPA PFAS Structure List, Getzinger *in silico* PFAS library.

Table 5 (part 2). List of PFAS compounds detected and annotated with Levels 1–5 confidence, using the non-targeted analysis workflow. The table is sorted by maximum observed peak area across all samples, in descending order.

Measured *m/z* (amu)	Sample detected in	Annotated name or InChIKey[a]	Annotated formula[b]	Confidence level	Number of matches to library or database			
					mzCloud Library[d]	2023 NIST Library[e]	*in silico* PFAS Library[f]	Mass lists and ChemSpider[g,h]
412.96596	1,3	Perfluorooctanoic acid (PFOA)	$C_8HF_{15}O_2$	1	1	1	10	159
365.10245	All	ZZRSJSRLKXMVOV-KRXBUXKQSA-N	$C_{16}H_{18}F_4O_5$	4	0	0	0	1
268.11666	1,3	BNBAMNCUDSVRLU-UHFFFAOYSA-N	$C_{11}H_{18}F_3NO_3$	4	0	0	0	4
223.09516	2	DXRFMGLMQPWABC-UHFFFAOYSA-N	$C_{10}H_{15}F_3O_2$	4	0	0	0	10
195.06399	All	JQDPYJDDNDLDHS-UHFFFAOYSA-N	$C_8H_{11}F_3O_2$	4	0	0	0	34
711.01149	All	FWEMIOJVGQCPKL-UHFFFAOYSA-N	$C_{17}H_{11}F_{22}O_5$	5	0	0	0	2
223.04003	3	NXGQSXKLTXUFDH-UHFFFAOYSA-N	$C_6H_9F_5O_3$	4	0	0	0	2
267.06223	All	2-(2,3,3,4,4,5,5-Heptafluoropentyl)oxolane	$C_9H_{11}F_7O$	4	0	0	0	19
203.03259	1,3	MZIRLHDDNVDDSK-UHFFFAOYSA-N	$C_9H_7F_3O_2$	4	0	0	0	79
250.88596	2,3	1-Chloro-2-[dichloro(fluoro)methoxy]-1,1,2,2-tetrafluoroethane	$C_3Cl_3F_5O$	5	0	0	0	5
342.99630	All	(2-Chloroethenyl)bis(2,2,3,3-tetrafluorocyclobutyl)silane	$C_{10}H_9ClF_8Si$	5	0	0	0	4
185.04324	1,3	GTNPZDVGDNDIPF-UHFFFAOYSA-N	$C_6H_9F_3O_3$	4	0	0	0	18
357.01196	All	LYHBSHCRNDNIRA-UHFFFAOYSA-N	$C_{12}H_5F_7N_2O_3$	5	0	0	0	3
201.03811	1,2	MECXAEIDGZNWJG-UHFFFAOYSA-N	$C_6H_9F_3O_4$	4	0	0	0	7
455.01028	1	HNNXJRYSGJHZSQ-UHFFFAOYSA-N	$C_9H_9F_{13}N_2O_2S$	5	0	0	0	1
417.03327	3	GXEGVBAPJYEZMC-UHFFFAOYSA-N	$C_{13}H_{18}C_{l3}F_5N_2O$	5	0	0	0	2

[a]PFAS compounds annotated using the Getzinger *in silico* PFAS library are annotated with the InChIKeys. For compounds annotated at Level 3 confidence without a match to the *in silico* PFAS library, annotation was determined by comparison between MS^2 fragments matching the FluoroMatch database and mass lists, using the following mass list priority: NIST Suspect List, EPA PFAS Structure List, Getzinger *in silico* PFAS libraryy.

[b]For Levels1-4 confidence, formulas were determined through the Predict Composition node of the workflow, requiring mass accuracy within 2 ppm and minimum spectral fit of 30%. For Level 5 confidence, formulas were derived from the mass list (within 2 ppm mass error).

[c]The annotated mass is the numerical difference between the hypothetical monoisotopic *m/z* based on the empirical formula and the measured *m/z*.

[d]Number of compounds within the online mzCloud database that produced similarity reverse scores >50% and precursor mass within ±2 ppm of the measured mass. The compound with the highest similarity reverse score was used for the final compound annotation.

[e]Number of compounds within the offline 2023 NIST Tandem Mass Spectrometry library (as an mzVault library database) that products matching scores >50% (using the NIST search algorithm) and precursor mass within ±2 ppm of the measured mass, with the highest score used for the final compound annotation.

[f]Number of compounds within the Getzinger *in silico* PFAS library (as an mzVault library database) that products matching scores >50% (using the NIST search algorithm) and precursor mass within ±2 ppm of the measured mass, with the highest score used for the final compound annotation.

[g]Number of compounds with monoisotopic mass within 2 ppm of the measured mass in the Full Scan spectrum across all searched Mass Lists and ChemSpider. For ChemSpider, a direct match to the calculated empirical formula also had to be met.

[h]For Level 5 annotations, annotation priority was based on the following order of mass lists: NIST Suspect List, EPA PFAS Structure List, Getzinger *in silico* PFAS library.

By analyzing the relative peak area intensities, it was found that a significant number of the most abundant PFAS compounds belonged to a homologous series of perfluorocarboxylic acids (PFCAs) ranging from perfluoropropionic acid (C3; PFPrA) to perfluorooctanoic acid (C8; PFOA). All of these PFCAs except one (perfluoropropionic acid) were confidently annotated at Level 1 and observed in all three samples. PFOA, however, was only detected in the paper bowl and paper plate. The cookie bag contained the highest total summed peak areas for all PFCAs, followed by the paper plate and the paper bowl. Previous studies have also reported PFCAs in various food contact materials.

In addition to the linear PFCAs, a perfluorinated cyclic isomer of perfluorocarboxylic acids, perfluoro methyl cyclopentane carboxylic acid, was detected in all three samples, with the highest peak area observed in the cookie bag. Furthermore, perfluorobutanesulfonic acid (PFBS) was detected in all three samples, with the paper bowl having the highest abundance. Both PFBS and the PFCAs were confidently annotated at Level 1.

In addition to perfluorinated compounds, several polyfluorinated compounds were detected and confidently annotated at Levels 3-5. This includes 2-perfluorohexyl ethanoic acid (6:2 FTCA), 3:2 fluorotelomer alcohol (3:2 FTOH), and 6:2 fluorotelomer sulfate (6:2 FTS), all of which were found in all three samples. Among them, 6:2 FTCA exhibited the highest peak area response in the cookie bag, while 3:2 FTOH and 6:2 FTS were most prevalent in the paper bowl. Previous research has reported the presence of 6:2 FTCA and 6:2 FTS in food contact materials. Fluorotelomer alcohols (FTOH) like 6:2 FTOH and 8:2 FTOH have also been documented in prior studies.[2,3] However, the observation of 3:2 FTOH in this study is novel to the best of our knowledge.

When considering the total peak areas of different species within major homologous groups (perfluorocarboxylic acids, PFCAs; perfluorosulfonic acids, PFSAs; fluorotelomer alcohols, FTOHs; fluorotelomer carboxylic acids, FTCAs), PFCAs were found to be the most abundant group, accounting for 27–41% of the total peak area of all detected compounds (Figure 7). Across all samples, these four groups collectively represented 50–63% of the total peak areas of all detected compounds.

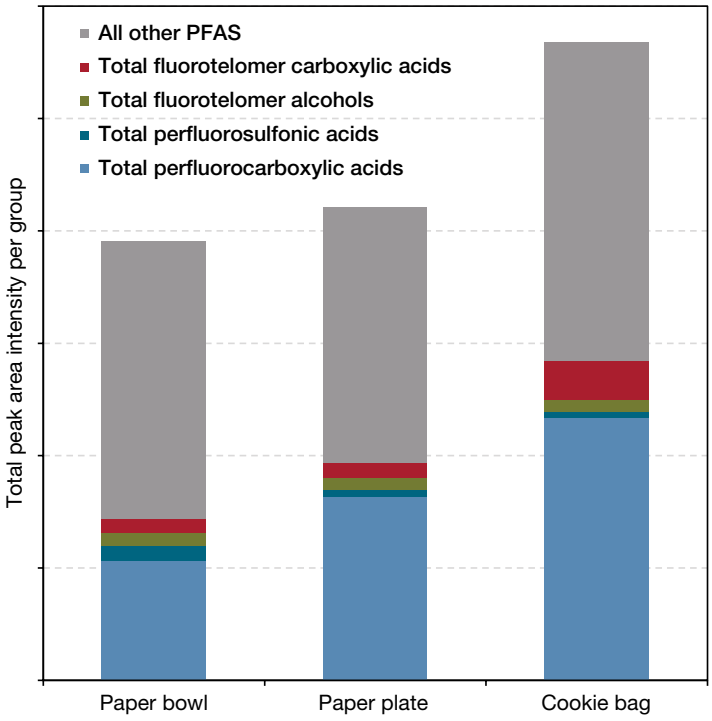

Figure 7. Comparison of total peak areas for the four major homologous groups of detected and annotated poly- and perfluoro-compounds

Conclusion

We developed a sensitive and automated method using CIC to determine both TOF and EOF in FCM. The TOF method is beneficial for manufacturers to comply with current state regulations. Interestingly, we found that the amount of extractable fluorine in FCM is limited, indicating that targeted LC-MS approaches may overlook a significant portion of the fluorinated content in the samples, including unidentified EOF compounds and non-extractable organic fluorine.

To address this limitation, we employed a non-targeted LC-HRMS approach, which allowed us to detect 46 PFAS compounds in the EOF fraction, many of which fall outside of the typical list of targeted screening or quantification methods. Among these compounds, perfluorocarboxylic acids emerged as the most abundant homologous group. These findings demonstrate that this CIC workflow provides a more comprehensive understanding of the total PFAS and fluorinated content in FCM compared to LC-MS targeted approaches, offering greater clarity about the PFAS contamination in FCM.

List of abbreviations

PFAS: Per-and polyfluoroalkyl substances

FCM: Food contact material

IC: Ion chromatography

CIC: Combustion ion chromatography

TF: Total fluorine

TOF: Total organic fluorine

TIF: Total inorganic fluorine

EF: Extractable fluorine

EOF: Extractable organic fluorine

EIF: Extractable inorganic fluorine

LC: Liquid chromatography

MS: Mass spectrometry

HRMS: High resolution mass spectrometry

MeOH: Methanol

ACN: Acetonitrile

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