Application Note

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Expanding Coverage for the Analysis of PFAS in Paper-Based Food Packaging Materials

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Abstract

In this study, 40 paper-based food contact materials (FCMs), including to-go boxes, microwave popcorn bags, wrappers, paper straws, and baking liners, were analyzed for the occurrence of per- and polyfluoroalkyl substances (PFAS). Chromatographic separation and detection were accomplished by liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (LC/Q-TOF). FluoroMatch software was used to perform nontargeted PFAS analysis and gain a more comprehensive profile of PFAS in FCMs. The highest concentrations of PFAS were detected in clamshell to-go boxes, with levels reaching up to 356.6 ng/g. Major contributors were PFOA (up to 187.2 ng/g) and PFDA (up to 92.2 ng/g), which exceed European Union regulatory limits (25 µg/kg).[1]

Introduction

PFAS are manufactured chemicals used in food packaging for their grease- and waterproof properties. However, PFAS are persistent in the environment and may pose health risks.[2] This study presents a workflow to screen for PFAS in FCMs using LC/Q-TOF. The FluoroMatch software suite was used to automate PFAS data annotation of knowns and unknowns as well as generate an interactive visualization dashboard, enhancing analysis accuracy and coverage.

The FluoroMatch suite is an open-source set of tools designed to streamline the suspect and nontarget screening of PFAS compounds. It automates several processes, including file conversion, chromatographic peak picking, blank feature filtering, PFAS annotation based on precursor and fragment masses, homologous series detection, compound classification (PFAS versus not PFAS), and reporting annotation confidence. The software library contains 15,643 PFAS species and associated fragmentation patterns, with the capability to add more.[3]

Various FCM samples, including takeaway containers and microwave popcorn bags, were rigorously extracted and analyzed. Suspect screening was employed for PFAS quantitation, while nontargeted workflows enabled the identification and semi-quantitation of PFAS that were lacking standards.

Experimental

Chemicals and reagents

Ammonium acetate (LC/MS grade) and HPLC-grade solvents (water, methanol, and reagent alcohol) were obtained from Fisher Scientific. Ultrapure water was sourced from a Milli-Q Reference Water Purification system. A mixture of 18 PFAS standards at 2 µg/mL in methanol, including HFPO-DA, PFBS, PFHxA, PFHpA, ADONA, PFHxS, PFOA, PFNA, PFOS, PFDA, 9Cl-PF3ONS, PFUnDA, MeFOSAA, EtFOSAA, PFDoDA, 11Cl-PF3OUdS, PFTrDA, and PFTeDA, was obtained from Agilent (part number PFS-537-APDS).

Sample collection and preparation

Forty paper-based FCMs were collected from restaurants and shops in Montreal between 2022 and 2023 (Table A1). The samples included popcorn bags, clamshell to-go boxes, takeaway boxes and trays, wrappers and snack bags, paper straws, and bakery baking liners. Each sample was cut into thin strips, and 0.2 g (± 0.01 g) was weighed and placed into 15 mL polypropylene centrifuge tubes. Four milliliters of fresh EPA 1633 diluent (methanol) was added to each tube. The samples were vortexed for 2 minutes, sonicated for 8 minutes, and extracted at room temperature for 1 hour. This extraction procedure was repeated five times. After extraction, the samples were centrifuged at 4,500 rpm for 10 minutes. The supernatant was filtered through a 0.22 µm filter into HPLC vials and stored at −20 °C until analysis.

LC/MS analysis

Extracts were analyzed using an Agilent 1290 Infinity II LC system coupled with an Agilent 6545 LC/Q-TOF. An Agilent PFC-Free HPLC Conversion kit (part number 5004-0006) and an Agilent InfinityLab PFC delay column (part number 5062-8100) were used to minimize background PFAS levels. Liquid chromatographic separation was performed on a 1.8 µm, 2.1 × 100 mm, Agilent ZORBAX RRHD StableBond SB-C18 column (part number 858700-902) with a corresponding guard column (Agilent part number 821725-902). The mobile phases were (A) water with 20 mM ammonium acetate and (B) methanol. The flow rate was 0.4 mL/min. The elution gradient was: 5% B (0 to 0.5 minutes), linear increase to 40% B (0.5 to 3 minutes), linear increase to 80% B (3 to 16 minutes), hold 80% B (16 to 18 minutes), linear increase to 100% B (18 to 22 minutes), hold 100% B (20 to 22 minutes), decrease to 5% B (22 to 22.5 minutes), followed by a 4 minutes post-run re-equilibration. The injection volume was 10 µL, and the column temperature was maintained at 50 °C. For the LC/Q-TOF, the drying gas was set to 4 L/min at 230 °C, the nebulizer to 20 psi, and the sheath gas to 12 L/min at 375 °C.

Method validation

Method detection limits (MDLs) and quantification limits (MQLs) were assessed as 3σ and 10σ of the signal using ten procedural blanks. Procedural blanks were prepared by adding 4 mL of fresh EPA 1633 diluent to 15 mL centrifuge tubes and extracting the solution. Matrix-matched calibration was conducted by spiking standard solutions at concentrations of 0.1, 0.5, 1, 2.5, 5, 7.5, and 10 ng/mL. Quality control samples spiked at 10 ng/mL were tested five times to evaluate method repeatability. Samples from each category were spiked at 5 ng/mL to assess recovery.

Data processing

While FluoroMatch Flow can be used to cover the entire nontargeted workflow, the use of Agilent Profinder for peak picking is preferred as it has been shown to perform higher quality peak picking.[4] In this study, the FluoroMatch Modular software workflow was integrated with the Agilent peak picking software. In this case, we used Profinder batch-recursive molecular feature extraction to perform peak picking, alignment, and blanks subtraction. After the files underwent recursive peak picking in Profinder, the results were exported as a CSV feature table. The neutral mass values were converted to the $[M-H]^-$ *m/z* value as a required column for FluoroMatch Modular.[4]

Note: in the more recent iteration of Agilent MassHunter Explorer, *m/z* values are directly provided.

FluoroMatch IonDecon was used to filter All Ions MS/MS data to retain only fragments correlating with precursor ions. This software can deconvolute any All Ions files and generate open-source data-dependent acquisition (DDA) formatted files for downstream nontargeted analysis workflows.[5] In complex samples, incorporating All Ions fragmentation (AIF) and IonDecon can enhance MS/MS coverage of PFAS. It generates .ms2 files, which are formatted like DDA files and are filtered to only contain fragments that correlate with precursor ions found in the feature table. These .ms2 files are used alongside the feature table from Profinder output. For a more detailed description of this process, please see reference four.[4]

Note: IonDecon is embedded in FluoroMatch Flow and Modular; the .mzML files (converted using Agilent MassHunter Qualitative Analysis software) can be directly imported in the software with All Ions files containing "All_Ions" in the file name, and they will automatically be processed.

FluoroMatch Modular was then used to perform homologous series detection, rule-based fragmentation matching, fragment screening, accurate mass matching, in silico MS/MS matching, confidence scoring, formula prediction, and Kaufmann analysis.

Results and discussion

Method validation

The method for analyzing PFAS compounds showed excellent linearity ($R^2 \geq 0.99$) within the 0.1 to 10 ng/mL range. Precision was high, with relative standard deviation (RSD) values below 5%, indicating good repeatability. Recovery rates ranged from 82 to 94%, demonstrating the method reliability for PFAS quantification. Method validation results are shown in Table 1.

Table 1. Method validation results for the analysis of per- and polyfluoroalkyl substances in food contact materials.

No.	Compound	Linearity (R^2) from 0.1 to 10 ng/mL	MDL (ng/g)	MQL (ng/g)	Precision (RSD)	Recovery
1	HFPO-DA	–	–	–	–	–
2	PFBS	0.99	0.003	0.009	0.02	91 ± 1.9
3	PFHxA	0.99	0.10	0.33	0.01	91 ± 5.3
4	PFHpA	0.99	0.07	0.22	0.03	90 ± 1.6
5	ADONA	0.99	0.07	0.23	0.01	85 ± 4.1
6	PFHxS	0.99	0.01	0.03	0.03	82 ± 3.3
7	PFOA	0.99	0.02	0.08	0.02	86 ± 3.1
8	PFNA	0.99	0.04	0.14	0.02	85 ± 1.9
9	PFOS	0.99	0.006	0.02	0.06	88 ± 6.4
10	PFDA	0.99	0.02	0.07	0.01	84 ± 3.8
11	9Cl-PF3ONS	0.99	0.009	0.03	0.03	90 ± 3.9
12	PFUnDA	0.99	0.17	0.56	0.02	90 ± 4.4
13	MeFOSAA	0.99	0.23	0.76	0.02	83 ± 1.5
14	EtFOSAA	0.99	0.06	0.19	0.04	85 ± 3.7
15	PFDoDA	0.99	0.07	0.22	0.04	92 ± 3.1
16	11Cl-PF3OUdS	0.99	0.01	0.03	0.01	94 ± 5.9
17	PFTrDA	0.99	0.09	0.28	0.03	91 ± 4.7
18	PFTeDA	0.99	0.61	2.04	0.05	87 ± 2.2

PFAS identification using FluoroMatch

Using FluoroMatch Visualizer, interactive tools for visualizing PFAS data were generated, including mass defect plots, accurate mass versus retention time plots, MS/MS fragmentation plots, annotation tables, and fragment screening. Individual homologous series were selected based on nominal mass and normalized mass defect, allowing for the observation of patterns and identification of outliers. This interactive cross-filtering simplified the evaluation of PFAS features and enhanced confidence in nontargeted results.

FluoroMatch was used to annotate a homologous series (C3 to C14) of perfluoroalkyl carboxylic acids (PFCAs), including PFHxA (perfluorohexanoic acid), PFHpA (perfluoroheptanoic acid), PFOA (perfluorooctanoic acid), PFNA (perfluorononanoic acid), PFDA (perfluorodecanoic acid), and PFDoDA (perfluorododecanoic acid). This workflow demonstrated that the suspect screening approach successfully identified common PFAS with available standards, while FluoroMatch was able to annotate additional PFAS that were lacking standards.

Additionally, FluoroMatch annotated C6 and C8 perfluoroalkyl sulfonic acids (PFSAs), which were not identified by suspect screening. This highlights the value of a hybrid workflow that combines traditional suspect screening with nontargeted tools like FluoroMatch, enhancing confidence in the nontargeted results through their complementary nature. Specifically, FluoroMatch allows for homologous series detection to help annotate incomplete or noisy spectra. FluoroMatch Visualizer evaluation helps incorporate additional lines of evidence to annotation like retention time patterns and Kendrick mass defect to aid in annotation.

Example results from FluoroMatch Visualizer are shown in Figures 1 and 2.

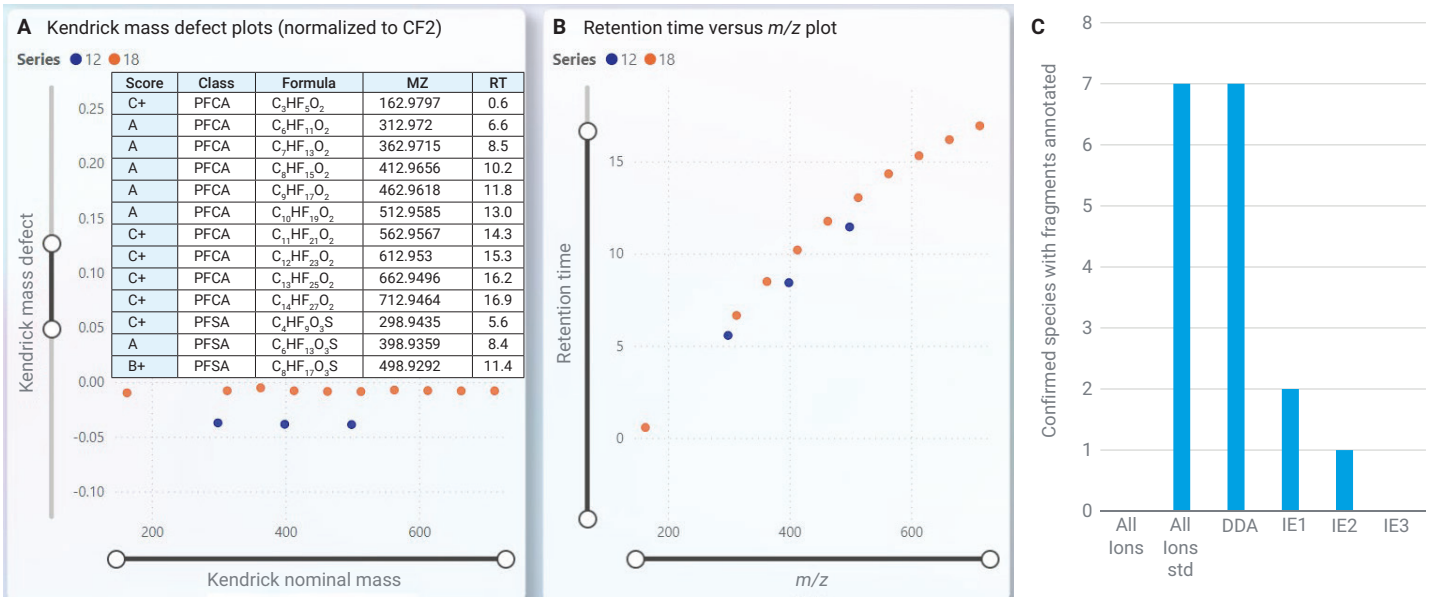

Figure 1. FluoroMatch Visualizer results. (A) The annotated species using the Schimanski scoring framework plotted by their Kendrick mass defect. (B) The same annotated compounds plotted by retention time versus *m/z*. (C) A confirmation of All Ions, DDA (Auto MS/MS), and the improvements with three rounds of iterative exclusion.

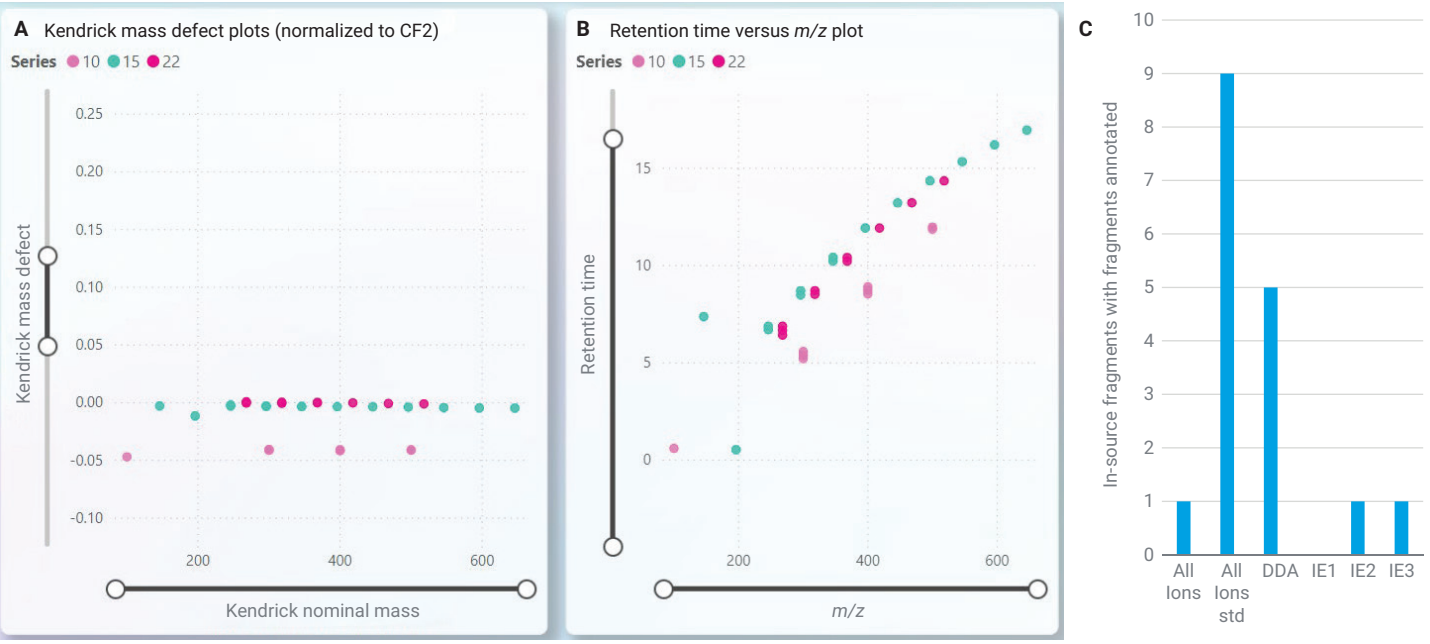

Figure 2. FluoroMatch Visualizer can identify in-source fragmentation by the features in teal, dark pink, and light pink sharing the same retention time (forming horizontal lines) in the retention time versus *m/z* plot. These in-source fragments can be used for further validation of species chemical identity (for example, PFCAs generally occur as both the $[M–H]^-$ ions and $[M–CO_2]^-$ in-source fragments).

PFAS in food contact materials

Of the 40 tested FCMs, the samples that tested positive for PFAS are show in Figure 3. The highest concentrations of PFAS were found in clamshell to-go boxes, with levels reaching up to 356.6 ng/g. Specifically, PFOA and PFDA were major contributors, with concentrations up to 187.2 ng/g and 92.2 ng/g, respectively. Snack wrappers showed PFAS concentrations up to 75.2 ng/g, with PFHxA being the most prevalent at 62 ng/g. Microwave popcorn bags had PFAS concentrations up to 9.4 ng/g. The most prevalent targeted analyte was PFHxA, which was found in all positive samples.

PFAS were detected in all clamshell to-go boxes, 50% of microwave popcorn bags, and 16.7% of snack wrappers, but were not found in takeaway trays, paper straws, or baking liners. Several perfluoroalkyl carboxylic acids (PFCAs) were identified in the materials, including PFNA, PFDoDA, PFDA, PFOA, PFHpA, and PFHxA. Notably, PFOA accounted for approximately 50% of the total PFAS concentration in clamshell to-go boxes, and PFDA for about 20%. Some clamshell to-go boxes exceeded the European Union's regulatory limit of 25 µg/kg for PFOA and PFDA.

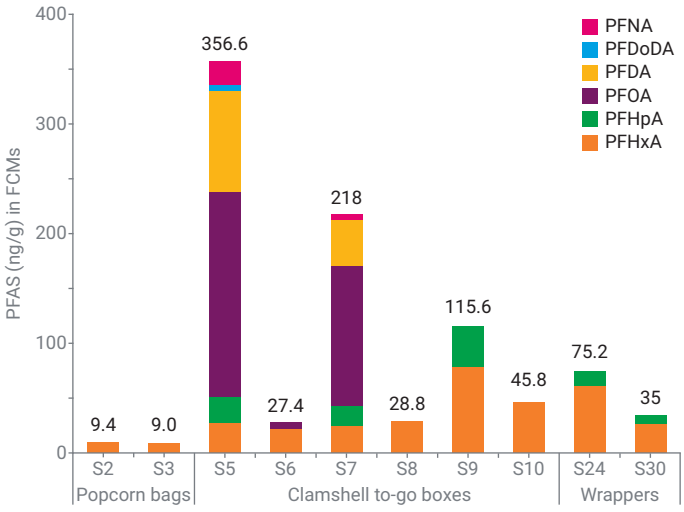

Figure 3. Food contact materials that tested positive for PFAS and their concentrations (ng/g).

Conclusion

This study successfully demonstrates the effectiveness of FluoroMatch software in automating the annotation and visualization of PFAS compounds in food packaging materials. The integration of FluoroMatch Visualizer provides a comprehensive approach to identifying both targeted and nontargeted PFAS compounds.

The results highlight the prevalence of PFAS in various paper-based FCMs, with clamshell to-go boxes showing the highest concentrations, particularly PFOA and PFDA, which exceed European Union regulatory limits. As PFAS can migrate into food, especially at higher temperatures, the pervasiveness of PFAS in FCMs raises concerns about potential exposure through hot meals and microwave heating. The widespread detection of PFAS in clamshell to-go boxes and other FCMs indicates a need for stricter regulations to reduce PFAS use in food packaging to protect consumer health.

References

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3. Koelmel, J.; *et al.* Toward Comprehensive Per- and Polyfluoroalkyl Substances Annotation Using FluoroMatch Software and Intelligent High-Resolution Tandem Mass Spectrometry Acquisition. *Analytical Chemistry* **2020**, *92(16)*, 11186–11194.
4. Koelmel, J.; *et al.* Non-Target PFAS Analysis in Dried Blood Spots Using the Agilent 6546 LC/Q-TOF with Profinder and FluoroMatch. *Agilent Technologies application note*, publication number 5994-7023EN, **2024**.
5. Koelmel, J.; *et al.* Expanding Per- and Polyfluoroalkyl Substances Coverage in Nontargeted Analysis Using Data-Independent Analysis and IonDecon. *J. Am. Soc. Mass Spectrom.* **2023**, *34*, 2525–2537.

Appendix

Table A-1. Sample information for food contact materials.

Code	Group	Paper Type	Food	Color	Store	Year
1	Popcorn bags (4)	Microwave popcorn bag	Popcorn	Brown	Supermarket	2022
2		Microwave popcorn bag	Popcorn	Purple	Supermarket	2022
3		Microwave popcorn bag	Popcorn	Yellow	Supermarket	2022
4		Microwave popcorn bag	Popcorn	Brown	Supermarket	2022
5	Clamshell to-go boxes (6)	Clamshell to-go box	Hot meal	White	Fast-food restaurant	2022
6		Clamshell to-go box	Hot meal	White	Fast-food restaurant	2022
7		Clamshell to-go box	Hot meal	White	Fast-food restaurant	2022
8		Clamshell to-go box	Hot meal	White	Fast-food restaurant	2022
9		Clamshell to-go box	Hot meal	White	Fast-food restaurant	2022
10		Clamshell to-go box	Hot meal	Wood	Fast-food restaurant	2022
11	Other takeaway boxes/trays (10)	Carboard burger box	Burger/fast food	Multicolored	Fast-food restaurant	2022
12		Carboard burger box	Burger/fast food	Multicolored	Fast-food restaurant	2022
13		Carboard burger box	Burger/fast food	Multicolored	Fast-food restaurant	2022
14		French fries holder	French fries	Multicolored	Fast-food restaurant	2022
15		Small carboard soup bowl	Soup/ porridge	White	Supermarket	2022
16		Large carboard soup bowl	Soup/ porridge	White	Supermarket	2022
17		Carboard cup	Drinks	White	Supermarket	2022
18		Kraft snack tray	Snacks	Brown	Fast-food restaurant	2022
19		Corrugated pizza box	Pizza	White	Fast-food restaurant	2022
20		Carboard fast-food box	Fast food	Red	Fast-food restaurant	2022
21	Snack wrapper/bags (12)	Sandwich wrapper	Sandwich	Multicolored	Fast-food restaurant	2022
22		Pizza wrapping sheet	Pizza	Multicolored	Fast-food restaurant	2022
23		Small glassine snack bag	Fast food	Brown	Fast-food restaurant	2022
24		Small glassine snack bag	Fast food	White	Fast-food restaurant	2023
25		Small glassine snack bag	Fast food	Brown	Fast-food restaurant	2023
26		Small glassine snack bag	Pizza	Brown	Fast-food restaurant	2023
27		Small glassine snack bag	Pizza	White	Fast-food restaurant	2023
28		Small glassine snack bag	Beverages	White	Fast-food restaurant	2023
29		Small glassine snack bag	Beverages	Brown	Fast-food restaurant	2023
30		Small glassine snack bag	Beverages	Multicolored	Fast-food restaurant	2023
31		Kraft paper bag	Fast food	Brown	Fast-food restaurant	2023
32		Kraft paper bag	Fast food	Brown	Fast-food restaurant	2023
33	Paper straws (5)	Paper straw	Beverages	White	Fast-food restaurant	2023
34		Paper straw	Beverages	White	Fast-food restaurant	2023
35		Paper straw	Beverages	White	Bubble tea shop	2023
36		Paper straw	Beverages	Dark green	Bubble tea shop	2023
37		Paper straw	Beverages	White	Coffee shop	2023
38	Baking paper liners (3)	Grease-proof bread baking liner	Bakery products	Gray	Bakery	2022
39		Grease-proof bread baking liner	Bakery products	Brown	Bakery	2022
40		Grease-proof bread baking liner	Bakery products	Yellow	Bakery	2022

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