From Packaging to Plate:

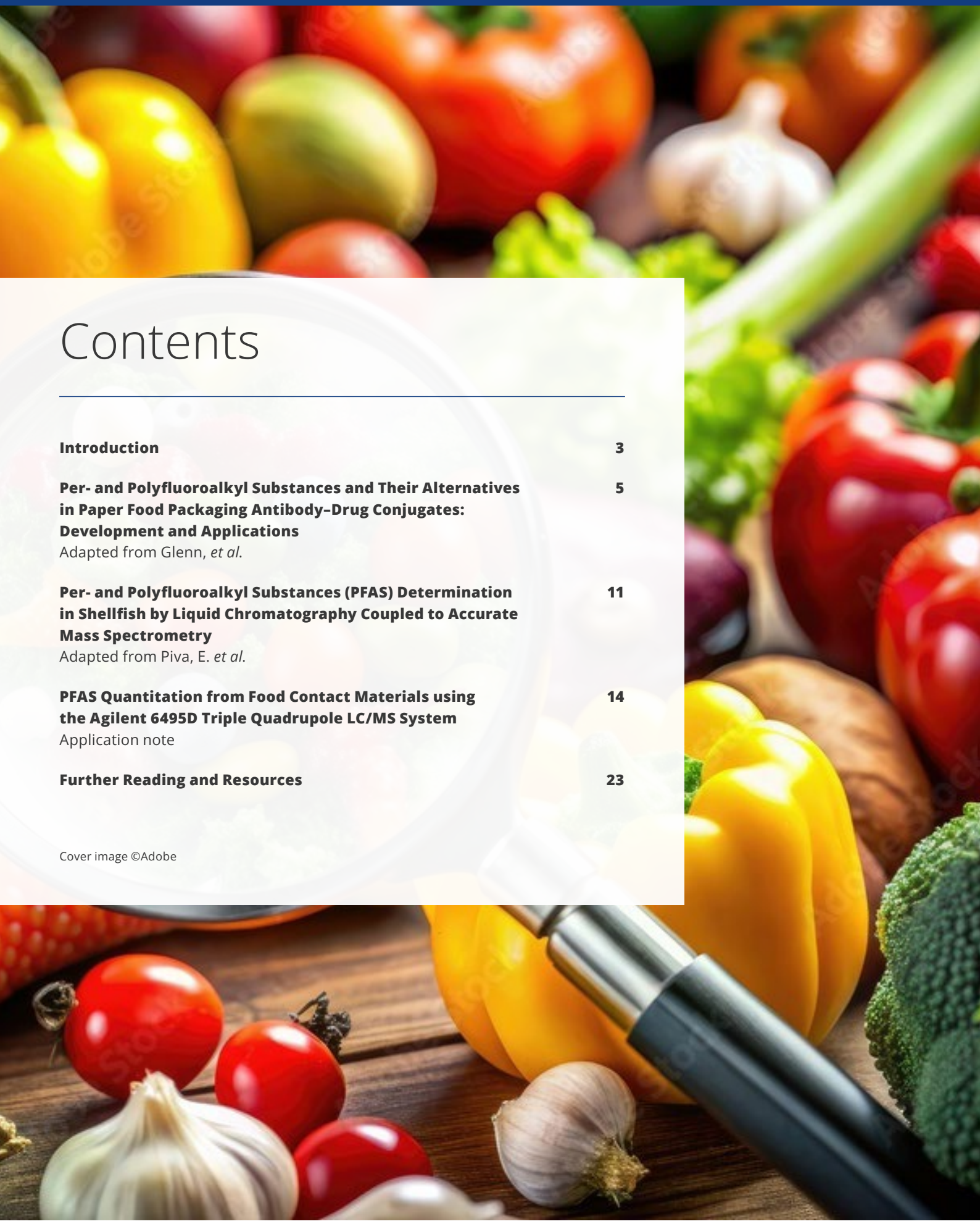
PFAS and Food Safety

Expert Insights

Sponsored by Agilent
Trusted Answers

CURRENT PROTOCOLS
A Wiley Brand

WILEY



Contents

Cover image ©Adobe

Introduction

Per- and polyfluoroalkyl substances (PFAS) are a group of synthetic chemicals widely used for their water- and grease-resistant properties. Their applications span various industries, including the food sector, where they are utilized in both food packaging and other food contact materials. However, due to their persistence in the environment and potential health risks, PFAS have become a major concern.

PFAS can enter the food supply through several routes. They may accumulate in food items such as fish, meats, eggs, and produce grown in contaminated areas. Food packaging materials treated with PFAS for moisture and grease resistance can also lead to PFAS migration into food, especially when in contact with hot or fatty foods.

PFAS are commonly used in paper-based food packaging, such as fast-food wrappers and microwave popcorn bags, to provide grease resistance. Despite their effectiveness, the potential for PFAS to leach into food has prompted regulatory scrutiny and efforts to find safer alternatives.

Mass spectrometry is a powerful analytical tool used to detect and quantify PFAS in various matrices, including food and environmental samples. Its high sensitivity and precision make it ideal for identifying even trace levels of PFAS compounds. It can detect a wide range of PFAS, including both long-chain and emerging short-chain alternatives, providing valuable data for regulatory compliance and risk assessment. Recent advancements in mass spectrometry, such as the development of high-resolution and accurate mass spectrometers, have enhanced the ability to perform untargeted analysis and identify unknown PFAS degradation products. These capabilities are crucial for understanding the environmental fate and potential health impacts of PFAS.

This Expert Insight begins with a review paper, adapted from G *et al.* [1], on the use of PFAS in food packaging, particularly paper-based materials, for moisture and oil resistance. It highlights the environmental and health concerns associated with PFAS, such as bioaccumulation and links to reproductive and immune system issues. The study explores current alternatives to PFAS, including non-biodegradable options like waxes and polymer films, and emerging biodegradable alternatives such as PLA. It emphasizes the need for cost-effective, biodegradable replacements that match PFAS performance while ensuring environmental sustainability and commercial viability.

The next study, adapted from Piva *et al.* [2], presents a method for detecting PFAS in bivalves using liquid chromatography coupled to accurate mass spectrometry (LC-QTOF). It addresses concerns about PFAS bioaccumulation and toxicity, particularly in marine ecosystems. The study highlights the detection of both linear and branched PFAS isomers in shellfish, with a focus on the Mediterranean region. It demonstrates the method's sensitivity and precision, contributing valuable data on PFAS distribution patterns in marine environments and indicating ongoing contamination despite regulatory efforts to limit PFAS use.

In summary, while PFAS offer functional benefits in food packaging, their persistence and potential health risks necessitate careful monitoring. Mass spectrometry plays a critical role in determining PFAS in food and food packaging, aiding regulatory efforts and the development of safer alternatives.

Through the methods and applications presented in this Expert Insi we hope to educate scientists on PFAS analysis in food. To gain a deeper understanding of available options for improving your research, we encourage you to visit Agilent.

Róisín Murtagh
Senior Content Strategist, Wiley

References

[1] Glenn, G. *et al.* (2021). Per- and polyfluoroalkyl substances and their alternatives in paper food packaging. *Comprehensive Reviews in Food Science and Food Safety.* https://doi.org/10.1111/1541-4337.12726.

[2] Piva, E. *et al.* (2022). Per- and polyfluoroalkyl substances (PFAS) determination in shellfish by liquid chromatography coupled to accurate mass spectrometry. *Drug Testing and Analysis*. https://doi.org/10.1002/dta.3282.

Agilent
Trusted Answers

Are You up to Speed?

Detect both regulated and non-regulated PFAS compounds in your food

Now you can test for target and non-target PFAS compounds with comprehensive profiling capabilities to keep ahead of legislation.

The Agilent Revident LC/Q-TOF gives you a faster path to rock-solid yes/no answers, with the ability to look deeper into complex samples than ever before. With simultaneous high sensitivity, high resolution, high speed and wide dynamic range all in one instrument, you no longer have to compromise.

Accelerate your capabilities with the Revident LC/Q-TOF.

www.agilent.com/chem/revident

DE-000050
© Agilent Technologies, Inc. 2024

Per- and Polyfluoroalkyl Substances and Their Alternatives in Paper Food Packaging

Adapted from Glenn, G. *et al.*[1]

Per- and polyfluoroalkyl substances (PFAS), long used in food packaging for moisture and oil resistance, have raised health concerns due to bioaccumulation and links to reproductive abnormalities, immunosuppression, and tumor formation. While second-generation PFAS exhibit shorter biological half-lives, chronic exposure risks remain concerning. Current alternatives include non-biodegradable options like waxes and polymer film laminates (polyethylene, EVOH, PET) and emerging biodegradable alternatives such as PLA, though these show suboptimal performance. Surface coatings using starches, chitosan, alginates, and nanocellulose provide adequate oil barriers but poor moisture resistance without modification. Internal sizing agents improve moisture resistance but lack oil barrier properties. The challenge remains to develop cost-effective, biodegradable alternatives that match PFAS performance while ensuring environmental sustainability and commercial viability.

Introduction

This review discusses the challenges and environmental implications of food service packaging materials, particularly in the context of the UN's 2030 goal to reduce food waste by 50%. The article examines two primary categories of food service ware: Plastic (primarily polystyrene [PS]) and paper-based materials, analyzing their respective advantages and limitations.

Plastic food service ware, while offering excellent functional properties, presents significant environmental and health concerns. These include limited end-of-life options, environmental persistence, and the migration of potentially harmful molecules, including styrene monomers, BPA, phthalates, and various plasticizers, into food. Microplastic dissemination is also an emerging concern.

Paper-based alternatives, derived from plant fibers, are generally considered more sustainable but lack PS's functional properties. To enhance paper packaging performance, various additives have been developed, notably per- and polyfluoroalkyl substances (PFAS). While PFAS effectively provide moisture and grease resistance, their environmental persistence, bioaccumulation potential, and associated health concerns have led to increasing public scrutiny. The FDA has historically approved over 90 unique PFAS for food contact paper products, though many long-chain PFAS have been phased out. This review discusses PFAS development, applications in food service items, and potential alternatives for providing moisture and oil resistance in food packaging.

PFAS History

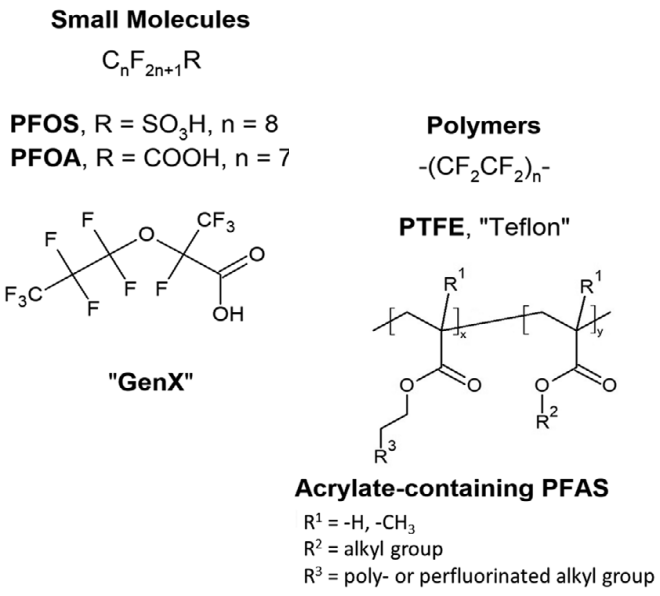
Year/Period	Event/Development
1930s	• PFAS emerged with DuPont's discovery of polytetrafluoroethylene, the key polymer in Teflon.
Pre-2001	• Long-chain (C8) PFAS compounds, particularly PFOA and PFOS, were widely used in industrial applications.
2001	• Elevated PFOA levels detected in drinking water near a DuPont facility; PFAS found in human serum and wildlife globally, including remote arctic regions.
2002	• 3M began voluntarily phasing out legacy PFAS compounds (C6+ sulfonates and C7+ carboxylates).
Post-2002	• Increased PFAS production in foreign countries. • The U.S. transitioned to short-chain PFAS alternatives, less bioaccumulative in humans. • New environmental challenges arose, including increased mobility in waterways and higher bioaccumulation in crops, with similar environmental persistence as long-chain PFAS.

PFAS Properties

Chemical and Physical

PFAS represent a diverse class of synthetic chemicals, with over 3,000 variants currently in the global market. These anthropogenic compounds are characterized by their fluorinated alkyl chains and at least one perfluoroalkyl moiety (-CnF2n) (Fig. 1).

Figure 1



Chemical structure of selected PFAS

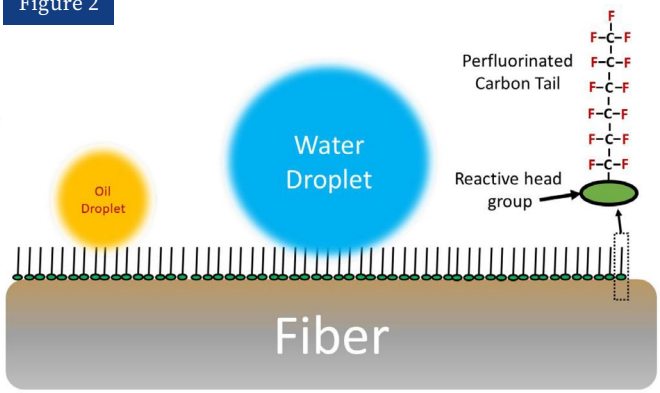
PFAS can be broadly categorized into two main groups: High-molecular-weight polymers (like those used in non-stick coatings) and non-polymeric fluorosurfactants. The non-polymeric PFAS are smaller, mobile molecules comprising a reactive head group that interacts strongly with water molecules, and a fluorinated carbon tail. They can be further classified as perfluoroalkyl (fully fluorinated carbon backbone) or polyfluoroalkyl (partially fluorinated) molecules.

The exceptional strength of the carbon-fluorine bond confers extreme environmental persistence, with resistance to thermal degradation, hydrolysis, photolysis, and biodegradation. This stability has led to their designation as "forever chemicals," as virtually all manufactured PFAS or their C-F bond-containing degradation products persist in the environment.

Commercial Uses

PFAS coatings provide moisture, oil/grease repellency, and chemical stability across diverse applications. In food packaging, the perfluorinated tail orientation creates both water and oil resistance (Fig. 2). These compounds have found extensive use across four primary sectors: (1) durable moisture/stain-resistant treatments for textiles and furnishings, (2) fire-resistant surfactants in firefighting foams, (3) chemical-resistant coatings for packaging and consumer goods, and (4) process surfactants or precursors. Their applications range from consumer products (cookware, cosmetics, cleaning products) to industrial uses (metal plating, hydraulic fluids, pesticides), potentially replacing various non-sustainable chemical alternatives.

Figure 2



Schematic of PFAS coating and orientation on the surface of fibers. PFAS chemicals tend to coat the surfaces of fibers, including fibers located internally when internal sizing containing PFAS is used such as with molded pulp paper packaging.

Bioaccumulation

Bioaccumulation occurs when chemicals accumulate in organisms faster than they can be eliminated, posing chronic toxicity risks for substances with long half-lives. While polymeric PFAS like Teflon are generally too large for bioaccumulation, some polymeric PFAS can degrade into bioaccumulative fragments. Nonpolymeric PFAS, being smaller molecules, can enter the body through multiple exposure routes including drinking water, food, dust, and inhalation.

National Health and Nutrition Examination Survey data (1999-2008) revealed PFAS presence in 95% of US blood samples, particularly long-chain variants like PFOA, PFOS, and PFHxS. While the phase-out of long-chain PFAS led to declining serum levels, PFAS with chains longer than C8 showed persistence or increases. These compounds' environmental persistence, long biological half-lives (3.3-15.5 years), and biomagnification potential continue to raise concerns about chronic toxicity risks.

Second-generation PFAS chemicals, developed as alternatives to long-chain PFAS, feature significantly shorter biological half-lives (e.g., PFBS: 4 weeks vs PFOS: 3.3 years; GenX [trade name]: a few days). However, these compounds still raise concerns due to their persistent environmental contamination of food and water sources, high environmental mobility, and unknown chronic exposure effects, despite their reduced bioaccumulation potential.

The FDA assesses PFAS levels in common foods using EPA's reference dose for PFOA and PFOS (0.02 µg/kg body weight/day) as a toxicity benchmark. These two compounds remain the primary focus due to their extensive characterization, prevalent detection in human serum and environmental samples, and continued global production despite US and European manufacturing bans. While second-generation PFAS are present in food and water, their short half-lives often result in low or undetectable blood serum levels. The FDA and EPA specifically assess PFAS accumulation in produce from contaminated areas and in retail seafood.

While FDA testing indicates PFAS levels in the general food supply pose no immediate health concerns, produce from contaminated areas can accumulate significant PFAS. Consumption limits vary notably between the EPA's reference dose and EFSA's more stringent guidelines, reflecting regulatory disagreement about safe exposure levels. EFSA's stricter standards, prompted by evidence of immune system effects at very low concentrations, are more easily exceeded through regular consumption of foods prone to PFAS accumulation, such as fish, meats, eggs, and fruits.

Chronic exposure to PFAS raises health concerns due to their bioaccumulative properties and largely unknown health effects. Primary exposure routes for the general population include food (66%), water (26%), and household dust (8.9%), though these proportions vary globally. Assessment of exposure sources is complicated by factors including proximity to contamination sites, analytical methods, demographics, and cultural dietary differences. Food packaging also contributes significantly to exposure through PFAS migration, particularly into liquid or hot foods, with higher serum levels observed in consumers of microwave popcorn and fast foods.

Unlike lipophilic persistent organic pollutants that accumulate in fatty tissue, PFAS bind to proteins (oleophobic). They accumulate differentially in human tissues, with the highest concentrations being found in lung tissue across 29 tested PFAS chemicals. Legacy compounds like PFOA predominantly accumulate in the liver and are associated with reproductive, developmental, and oncological effects.

Regulatory Interventions

Year/Period	US & International Initiatives
2006	EPA launched PFOA Stewardship Program to phase out long-chain PFAS by 2015.
2009	The Stockholm Convention listed PFOS as a persistent organic pollutant.
2013	Norway banned PFOA in consumer products.
2015	Madrid Statement signed by 230 scientists to limit PFAS production.
2015	EPA's program achieved the goal of phasing out long-chain PFAS.
2016	EPA set health advisory limits for PFOA and PFOS in drinking water.
2016	The EU decided to restrict PFOA and related chemicals.
2020 (July)	The EU restricted the use/import of PFOA and its precursors.
2020	Michigan sued manufacturers over PFAS contamination.
2022	Washington State's PFAS ban in food packaging took effect.
Ongoing	New York and other states considering similar PFAS restrictions.

PFAS uses in Food Packaging

Agilent's 6495C Triple Quadrupole LC/MS could enable precise quantification of PFAS migration from packaging into food, helping establish safety thresholds. The instrument's sensitivity down to femtogram levels would be particularly valuable for detecting short-chain PFAS.

PFAS compounds are used extensively in paper food service items for grease and moisture resistance. The FDA's historical position, based on 8 surveys of the general food supply, indicated minimal public risk from PFAS in food packaging, with only tilapia and ground turkey showing detectable levels below precautionary limits. However, in July 2020, the FDA announced a phase-out of packaging containing 6:2 fluorotelomer alcohol (6:2 FTOH) following evidence of bioaccumulation in animal studies.

A significant concern emerges regarding the environmental persistence of PFAS chemicals and their end products beyond the packaging's useful life. This issue became particularly relevant when molded pulp fiber containers emerged as alternatives to banned polystyrene foam containers. Despite being marketed as 100% compostable and meeting ASTM D6400 standards, these products often contain non-degradable PFAS additives that persist in composting streams. Compost containing food packaging waste was shown to have PFAS levels 10 times higher than packaging-free compost. While most detected PFAS were second-generation chemicals, the presence of FDA-banned legacy PFAS raises concerns about potential sources, including contaminated recycled fiber, tainted water in composting operations, and non-compliant imported food packaging.

In response to these concerns, the Biodegradable Products Institute (BPI) revised its certification standards. As of January 2020, BPI certification requires products to contain less than 100 parts per million of total fluorine, aiming to protect composting facilities and maintain compost quality.

Sources of PFAS Leakage

Agilent's 7250 GC/Q-TOF could help identify unknown PFAS degradation products in environmental samples, leveraging its high resolution and accurate mass capabilities.

Manufacturing accounts for ~15% of environmental PFAS contamination, with consumer products including food packaging contributing ~85%. PFAS enter the environment through production waste, landfill leachate, and degradation of treated products.

Alternatives to PFAS

Agilent's 6545XT AdvanceBio LC/Q-TOF could be used to analyze the molecular weight distributions and structures of novel biopolymer alternatives, supporting the development of optimal barrier properties.

Lamination

Commercial paper lamination primarily involves extrusion coating or melted plastic films, with synthetic petroleum-based polymers accounting for over 99% of applications to achieve water and oil resistance. Examples of conventional polymers include PE, EVOH, and PET. Examples of biodegradable polymers include PLA, PBAT, PBS, and PHA.

Surface sizing

Category	Key Points
General Overview	• Sizing agents enhance liquid resistance and structural integrity in papermaking. • Applied as surface coatings or incorporated into pulp. • Delivered as aqueous solutions or dispersions.
Polysaccharides	• Starches are cost-effective but swell in water; modifications improve properties. • Chemical modifications include oxidation, cationization, or esterification. • Alternative polysaccharides: Cellulose, chitosan, alginates. • Cellulose barriers: Glassine, parchment paper, MFC/NFC, cellulose nanocrystals. • Chitosan: Excellent oxygen and grease barriers; higher cost and poor water resistance. • Alginates: Effective grease resistance and air barriers; growing commercial interest. • Challenges: High production costs, need for improved water resistance.
Synthetic Polymers	• Water-dispersed polymers provide moisture and grease resistance but are not biodegradable. • Common types: Styrene-butadiene copolymers, acrylates, vinyl esters, polyvinyl alcohol. • Recent improvements include nano-fillers and latex-wax combinations.
Proteins	• Offer excellent grease and oxygen barriers but poor water vapor resistance. • Sources: Animal-derived (gelatin, casein, whey) and plant-based (soy, wheat gluten, corn zein). • Zein and kafirin provide enhanced water resistance; high costs limit use. • Zein-coated surfaces achieve superhydrophobic properties (water contact angles up to 155°).
Polyesters	• PLA and PHAs offer oil and water resistance and are biodegradable. • Dispersions created through controlled precipitation or extrusion. • Bio-based alternatives: Cross-linked systems from vegetable oils, inspired by natural leaf cuticles.
Other	• Mineral fillers enhance barrier properties and reduce costs. • Specialized coatings: Silica nanoparticles, vapor-deposited metals. • Natural alternatives: Kraft lignin, shellac, wax coatings, organo-silane coatings.
Key Limitations	• Realistic testing conditions are needed. • Cost-effectiveness challenges. • Balance between water and oil resistance. • Processing efficiency and scalability.

Internal sizing

Category	Key Points
Alkyl Ketene Dimers (AKD)	• Widely used internal sizing agent; effective at low concentrations and stable. • Synthesized from long-chain fatty acids; applied as aqueous dispersion. • Creates water-resistant surfaces; limited oil resistance. • Recent enhancements: combined with nanocrystalline cellulose, polyvinyl alcohol, and mineral additives.
Alkenyl Succinic Anhydride (ASA)	• More reactive than AKD; requires careful handling and immediate use after dispersion. • Made by combining maleic anhydride with long-chain alkenes. • Provides water resistance; minimal oil resistance. • The FDA limits ASA content to 1% by paper weight.
Rosin	• Natural resin from softwood trees, used as paper size since the 1800s. • Consists mainly of abietic acid; requires acidic conditions for sizing. • Applied as an emulsion with alum; binds to cellulose fibers for water resistance. • Use declined after AKD and ASA emerged due to incompatibility with $CaCO_3$ and potential degradation. • Oil resistance properties remain unexplored.

Conclusion

While numerous alternatives exist, a cost-effective, fully biodegradable replacement matching PFAS performance remains elusive. Key research needs include:

- Improving water resistance of bio-based coatings
- Developing more efficient production processes
- Enhancing barrier properties while maintaining compostability
- Establishing migration and safety profiles of alternatives

The article emphasizes the pressing need for safer alternatives to PFAS in food packaging. Agilent's advanced mass spectrometry capabilities could help address critical knowledge gaps in performance, safety assessment, and environmental impact of both current PFAS and emerging alternatives.

A comprehensive analytical approach using Agilent's mass spectrometry portfolio could accelerate alternative development by:

- Characterizing molecular structures of novel barrier materials
- Quantifying migration of coating components
- Identifying degradation products and environmental fate
- Validating safety and performance metrics

References

[1] Glenn, G. *et al.* (2021). Per- and polyfluoroalkyl substances and their alternatives in paper food packaging. *Comprehensive Reviews in Food Science and Food Safety.* https://doi.org/10.1111/1541-4337.12726.

Per- and Polyfluoroalkyl Substances (PFAS) Determination in Shellfish by Liquid Chromatography Coupled to Accurate Mass Spectrometry

Adapted from Piva, E. *et al.* [1]

Per- and polyfluoroalkyl substances (PFAS) are chemical compounds with a C-F backbone and either sulfonic or carboxylic acid groups, that have been manufactured for over 70 years. Recent bioaccumulation and toxicity concerns about legacy PFAS (e.g., PFOA, PFOS) led to their replacement with emerging compounds, such as GenX, ADONA, and C6O4. While newer short-chain PFAS show lower bioaccumulation, they demonstrate similar toxicological effects. Studies have shown different behaviors between branched and linear PFAS isomers, with branched forms showing varied environmental distribution and biological processing. Marine ecosystems, particularly shellfish, serve as key indicators for PFAS contamination. This study presents a method for the detection of PFAS in bivalves using liquid chromatography coupled to accurate mass spectrometry (LC-QTOF).

Materials and methods

Materials

Analytes (>98% purity) and mass-labeled standards were obtained from Wellington Laboratories. Isotope-labeled compounds were used as surrogates and injection standards. The method followed EPA-533 guidelines for cases where direct isotope-labeled analogs weren't available. Reference solutions for accurate mass measurement and WAX polymer (150 mg, 6 ml) cartridges were from Agilent Technologies.

Sample Collection

Four pooled mussel samples, four pooled clam samples, and one pooled oyster sample were provided by the National Reference Laboratory for Marine Biotoxins in Italy. Atlantic scallops and Atlantic and Pacific clams were purchased from local stores. Samples were collected from both natural and cultivation settings along the Adriatic Sea coast, with each pool containing 2 kg of material. The bivalve content was homogenized and 100 g portions were prepared for analysis.

Sample Preparation

Sample preparation involved weighing approximately 1 g of tissue into a polypropylene tube and adding internal standards and acetonitrile. Samples underwent mechanical agitation followed by ultrasonic extraction. After centrifugation to remove debris, the supernatant was collected and underwent a liquid-liquid extraction with hexane to remove lipids. The samples were then purified using weak anion exchange SPE, including conditioning, washing, and elution steps. Finally, the extracts were dried under nitrogen, reconstituted in a methanol/water mixture, and spiked with injection standards before analysis.

Instrumentation and Analysis

Analyses were carried out using an Agilent 1290 Infinity II LC coupled to a 6546 ~~LC/Q-TOF~~ mass spectrometer. Chromatographic separation employed a Poroshell EC-C18 column (Agilent Technologies) with mobile phases of 20 mM ammonium acetate in water and methanol, both containing 0.1% formic acid. The ~~LC/~~Q-TOF operated in negative mode with optimized source parameters. Data analysis was performed by the Masshunter software (version B.10.1), Agilent Technologies).

PFAS Quantification and Untargeted Analysis

Identification of compounds was done by considering accurate mass (≤5 ppm) of the $[M - H]^-$ or relative adducts, isotopic pattern distribution, and retention time compared with reference material. Quantification used an isotope dilution technique with matrix-matched calibration. For untargeted analysis, an in-house database of 150 PFAS compounds was prepared.

Method Quality Assurance

PFAS quantification used isotopic dilution with ^{13}C-labelled standards. Seven-point calibration curves (0.05-10 ng/ml) were prepared with 1/x weighting. Matrix-matched calibration wasn't needed due to isotope-labeled internal standards. Quality controls at 0.6 and 1.2 ng/ml were analyzed in triplicate over three days. LOD and LOQ were determined using signal-to-noise ratios of 3 and 10, respectively, and verified through spike recovery. Laboratory blanks were run alongside samples to monitor contamination.

Results

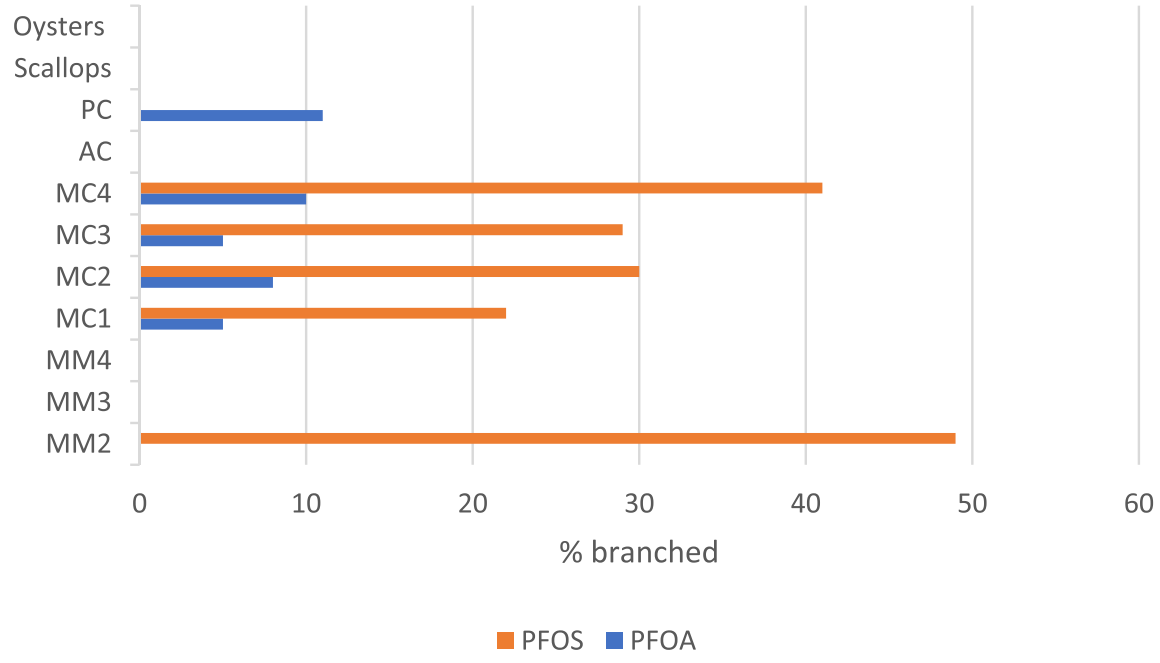
Method Optimization and Quality Assurance Parameters

The optimized extraction method used plain acetonitrile, which provided the best recovery and consistency for PFAS in clam samples compared to acetonitrile/water mixtures. A hexane cleaning step removed lipids and improved mass accuracy, particularly for ADONA. The method achieved 12-minute chromatographic separation with LODs of 0.002-0.05 ng/g and LOQs of 0.007-0.15 ng/g. Method validation showed good precision (RSD <15%), minimal matrix effects (80-110% for most compounds), and accuracy bias within 1-15%. No PFAS contamination or carryover was observed in laboratory blanks.

Sample Analysis

The analysis of 12 bivalve samples detected at least one PFAS in each sample, with 12 PFAS detected above LOD and 7 quantified. Total PFAS concentrations ranged from 0.03 to 0.57 ng/g. PFOS and PFOA showed the highest detection frequency, followed by PFBS. Species-specific patterns emerged, with clams showing higher concentrations than mussels, particularly in Mediterranean samples (0.38 ng/g vs 0.03 ng/g). The study identified both linear and branched isomers, with branched PFOA found in Mediterranean and Pacific clams (5-11%) and branched PFOS accounting for 22-49% of total PFOS (Fig. 1). Untargeted analysis revealed three PFOS precursors (N-MeFOSA, N-EtFOSA, and N-MeFOSAA) in Mediterranean samples, with N-MeFOSAA confirmed at the highest confidence level.

Figure 1



Percentage of branched PFOA and PFOS detected in samples. MM: Mediterranean mussels; MC: Mediterranean clams; PC: Pacific clams; AC: Atlantic clams

Discussion

The study highlights the evolution of PFAS analysis techniques, noting that while LC-MS/MS remains the standard method, it has limitations for analyzing emerging short-chain PFAS due to restricted MRM transitions. The authors justify their use of LC/Q-TOF as it offers both high resolution and the ability to perform untargeted analysis.

The authors compared their findings with recent literature (2016-2021) on PFAS in bivalves. Despite efforts to limit their use, PFOS and PFOA remain the predominant compounds in biomonitoring studies, likely due to their resistance to biodegradation and ongoing formation from precursor compounds.

Key comparative findings included:

- PFOS > PFCA in mussels, contrasting with a French study showing PFOS < PFCA
- Overall PFAS concentrations aligned with French studies, confirming consistent Mediterranean Sea contamination
- South African studies found higher concentrations in mussels versus oysters (5.7 vs 0.6 ng/g)
- Their findings matched a previously published pattern of contamination distribution (oysters < scallops < mussels < clams)

The study identified both linear and branched PFAS isomers, with branched forms predominantly found in Mediterranean clams and mussels. The presence of branched isomers likely relates to the proximity of Italy's Veneto region and Po River, known areas of PFAS contamination. The detection of branched isomers is particularly significant given their different pharmacokinetics and toxicity compared to linear forms, potentially presenting additional human health concerns through food consumption.

The study also detected PFAS precursors, including fluorotelomer compounds and N-MeFOSAA, particularly in Mediterranean samples, indicating ongoing contamination despite regulatory efforts.

Conclusions

The study reported the successful development and validation of an LC/Q-TOF method for PFAS analysis in shellfish. The method demonstrated good sensitivity, precision, and accuracy for both targeted and untargeted analysis. Species-specific accumulation patterns emerged, with clams showing higher PFAS levels than mussels. The method successfully detected both linear and branched isomers, contributing to understanding PFAS distribution patterns in marine environments. The presence of PFOS precursors in Mediterranean samples suggests ongoing contamination despite regulatory efforts to limit PFAS use.

The study's strength lies in its comprehensive analytical approach, combining targeted and untargeted analysis while maintaining high analytical standards through thorough method validation. The detection of branched isomers and precursor compounds provides valuable insights into PFAS environmental fate and distribution patterns.

References

[1] Piva, E. *et al*. (2022). Per- and polyfluoroalkyl substances (PFAS) determination in shellfish by liquid chromatography coupled to accurate mass spectrometry. *Drug Testing and Analysis*. https://doi.org/10.1002/dta.3282.

Application Note

Food Testing

Agilent

Trusted Answers

PFAS Quantitation from Food Contact Materials Using the Agilent 6495D Triple Quadrupole LC/MS System

Suitable for Agilent

1290 Infinity III LC

Author

Aimei Zou

Agilent Technologies, Inc.

Abstract

There has been growing concern about per- and polyfluoroalkyl substances (PFAS) in food contact materials (FCMs) and their potential migration into food. This has necessitated the development of methods for accurate and reliable PFAS characterization. In this application note, 110 PFAS, comprised of 73 native and 37 labeled compounds, were quantified from paper straws using an Agilent 6495D triple quadrupole LC/MS system. The method detection limit was within 0.2 µg/kg for all 73 target analytes. For most analytes, R^2 values were greater than 0.99, confirming linearity. Matrix-spiked quality control (QC) recovery was 65 to 120% in 90% of analytes, with precision (%RSD) ≤ 20%. These performance attributes confirm the sensitivity and reliability of the 6495D LC/TQ system for PFAS screening in paper straws.

Introduction

PFAS are a large group of manufactured chemicals used in various industries worldwide due to their unique properties.[1,2] Since the 1950s, PFAS-containing materials have been used in food packaging as coatings to prevent the paper from absorbing fats and water, and to serve as barriers to printing inks and moisture.[3,4] However, there have been increasing concerns about the potential health impacts and environmental safety of specific critical PFAS such as perfluorooctanoic acid (PFOA), perfluorooctanesulfonic acid (PFOS), perfluorohexanesulfonic acid (PFHxS), perfluorononanoic acid (PFNA), their salts, and related compounds.

Regulatory bodies such as the Stockholm Convention, US Food and Drug Administration (FDA), European Union (EU), and the Registration, Evaluation, Authorization, and Restriction of Chemicals (REACH), have introduced legislation to restrict the use of PFAS in many applications. For example, the Regulation EU No. 10/2011 limits the use of PFAS in plastic FCMs.[5] Additionally, in 2016, the FDA revoked regulations authorizing the use of long-chain PFAS, such as PFOS and PFOA, in food contact applications. In January 2023, Germany, Denmark, the Netherlands, Norway, and Sweden jointly proposed to the European Chemicals Agency (ECHA) that a broad ban be placed on PFAS in the EU market (REACH Appendix XV).[6] More regulations and voluntary actions are anticipated to monitor and mitigate PFAS contamination in food packaging and contact materials.

Therefore, it is critical to establish a sensitive and accurate quantitative analytical approach that ensures the safety of FCMs. Technologies such as liquid chromatography (LC) and gas chromatography (GC), combined with tandem mass spectrometry (MS/MS), are often used to analyze different PFAS groups based on their properties. Fluorotelomer alcohols (FTOHs), a type of fluorotelomer with an alcohol functional group, are volatile compounds suitable for GC/MS analysis. However, FTOHs may degrade into perfluorinated carboxylic acids (PFCAs) such as PFOA, PFDA, and PFNA, which are specific to LC/MS analysis.[4]

Special care must be taken during sample collection, handling, and laboratory analysis to reduce sources of contamination. However, analytical instrumentation, reagents, and consumables must also be selected carefully, as they can serve as a significant source of contamination by PFAS and lead to false positive results.

In this application note, a sensitive and reliable method using a 6495D triple quadrupole LC/MS system (LC/TQ) equipped with a PFAS-free flow path was developed for the quantitation of PFAS in a food contact material, specifically paper straws.

Experimental

A total of 110 PFAS, including 73 native and 37 labeled compounds (34 surrogates and three internal standards) were analyzed based on the solvent extraction principle, followed by a dilute-and-shoot method.

Chemicals and reagents

All chemicals and solvents used for this study were LC/MS grade and purchased from Sigma-Aldrich (St. Louis, MO, USA). Agilent InfinityLab Ultrapure LC/MS grade water (part number 5191-4498) was also used.

Consumables

Variability in consumable geometry and composition can greatly impact background levels as well as contribute to unplanned downtime and troubleshooting. Therefore, to remove uncertainty from measurements, it is important to source consumables that have strict quality control (QC) protocols in place during production and that are proven to deliver specific results. All consumables used in this work were from Agilent, and all were tested and verified for their suitability in PFAS analysis to deliver ultra-low PFAS background levels.[7] These consumables included:

- 15 mL Falcon tubes (part number 5610-2039)
- Agilent Captiva 5 mL polypropylene (PP) syringe (part number 9301-6476)
- Agilent Captiva Premium syringe filter, nylon membrane (part number 5190-5092)
- Agilent 2 mL polyfluorinated compound (PFC)-free PP vials (part number 5191-8150)
- Agilent 250 µL PP vials and caps (part numbers 5190-2242 and 5191-8151)

Standards and calibration preparation

Native and isotopically labeled PFAS standards were sourced from Wellington Laboratories Inc. (Guelph, ON, Canada) and Toronto Research Chemicals (Toronto, ON, Canada) as stock solutions, solution mixes, or powdered standards. Twelve calibration standards were prepared ranging from 1 to 50,000 ng/L (ppt) in methanol:water (80:20, v:v). Each calibration level included a constant amount of surrogate mix (used as an extracted internal standard, EIS) and isotope performance standard mix (EPA 533IS, used as a nonextracted internal standard, IPS).

Sample extraction procedure

A commonly used paper straw, purchased from a local store, was chosen as the FCM for testing in this study. Prior to weighing, the paper straw was cut into pieces smaller than 5 × 5 mm^2 using a stainless-steel cutter. The cutter was precleaned using isopropanol (IPA) to avoid contamination.

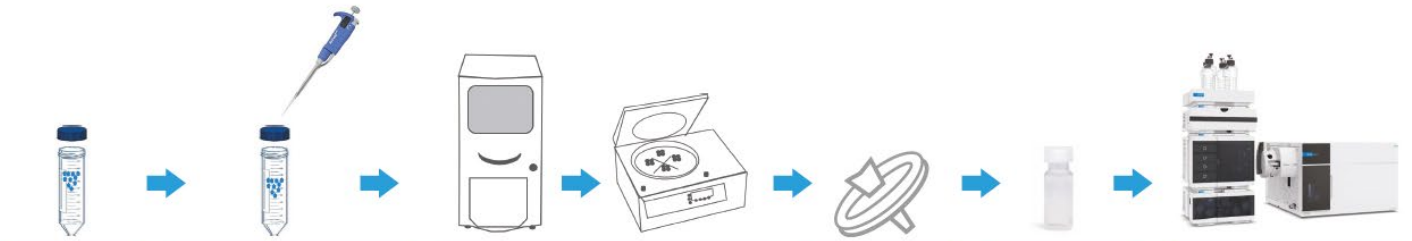
Solvent extraction is a common method used to analyze and test packaging materials, particularly for identifying compounds that can migrate from packaging materials into products or food.[5,8] In this study, a simple and fast solvent extraction method was developed for leaching PFAS from paper straw samples (Figure 1). A 1 ± 0.01 g sample was weighed into a 15 mL PP Falcon tube for extraction.

To prepare QC samples, an appropriate amount of native PFAS spike mix and surrogate spike (EIS) was added to the tube. QC samples were spiked to achieve low, middle, and high concentrations of 1.0 µg/kg (low spike quantity, LSQ), 10 µg/kg (middle spike quantity, MSQ), and 50 µg/kg (high spike quantity, HSQ), respectively. A matrix blank, which lacked the native PFAS standard mix, was also prepared.

Next, 10 mL of methanol was added into each sample tube. The samples were then mechanically shaken at 2,000 rpm for 30 minutes, followed by ultrasonic assisted extraction (UAE) at 60 °C for one hour. The mechanical shaking and UAE step was repeated twice. The samples were then centrifuged at 4,200 rpm for 15 minutes. After centrifugation, the supernatant extract was filtered into a PP vial. If not analyzed immediately, these extracts were stored at −20 °C. Prior to analysis, a final dilution was performed in which 800 µL of the filtered extract was transferred to a PP vial, with the addition of 150 µL of water and 50 µL of IPS mix (non-extracted internal standard). The solution was thoroughly vortexed and prepared for LC/TQ injection (Figure 1). Two technical preparations were performed for each QC sample concentration.

Instrumentation

An Agilent 1290 Infinity II UHPLC system was used for chromatographic separation. To minimize PFAS contamination, the standard LC system fluid path was replaced with an Agilent InfinityLab PFC-free HPLC conversion kit (part number 5004-0006), including bottle head assembly, pump head adapter assembly, inline filter, multiwash tubing kit, and a PFC delay column. An Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 100 mm, 1.8 µm column (part number 959758-902) was installed on the multicolumn thermostat. A gradient method with less than 15 minutes elution time, as outlined in the Agilent PFAS eMethod (part number G5285AA), was used. This method used 5 mM ammonium acetate in water (mobile phase A) and 100% methanol (mobile phase B) at a flow rate of 0.4 mL/min. Targeted



Sample
Finely cut the paper straw. Weigh a 1 ± 0.01 g sample into a 15 mL polypropylene (PP) Falcon tube (p/n 5610-2039).

Spiking/solvent addition
For QC sample preparation, spike an appropriate amount of surrogate mix and PFAS analyte mix. Add 10 mL of methanol.

Extraction
Place the samples on a mechanical shaker at 2,000 rpm for 30 minutes, followed by ultrasonic assisted extraction (UAE) at 60 °C for 1 hour. Repeat this step twice. Centrifuge the tube at 4,200 rpm for 15 minutes.

Filtration
Filter the supernatant extract using a PP syringe (p/n 9301-6476) and nylon membrane syringe filter (p/n 5190-5092). If the analysis is not immediate, store the extract at −20 °C.

Final dilution
Transfer 800 µL of the filtered extract into a 2 mL PFC-free PP vial (p/n 5191-8150), then add 150 µL of H_2O and 50 µL of isotope performance standard. Vortex well.

Analysis
The sample is ready for LC/TQ injection (dilution factor: 12.5-fold).

Figure 1. Workflow for PFAS extraction from a paper straw.

quantification was performed using the 6495D LC/TQ system equipped with an Agilent Jet Stream (AJS) ion source operating in negative ionization mode. Autotuning was performed in standard quadrupole mode to optimize instrument parameters. Data processing was performed using Agilent MassHunter LC/MS Acquisition software version 12.1 Update 3 and Agilent MassHunter Quantitative Analysis software version 12.1. The acquisition method was based on the Agilent PFAS multiple reaction monitoring (MRM) database for 108 compounds (part number G1736AA) and two other analytes (PFUnDS and PFTrDS). This method covers the four regulated PFAS in EU 2023/915 and forty PFAS in EPA 1633, as well as the recommended targets under EURL POPs for PFAS in food and feed, AOAC SMPR 2023.003, US FDA C-010.03, and USDA CLG-PFAS 2.04 for PFAS in food. The full list of PFAS targets and CAS numbers is shown in Table 1.

Table 1. Analytical results summary.

Target Number	Compound Name	CAS Number	Surrogate	RT (min)	MDL (µg/kg)	LOQ (Validated) (µg/kg)	% Recovery LSQ (1 µg/kg)	% Recovery MSQ (10 µg/kg)	% Recovery HSQ (50 µg/kg)
1	PFBPA	52299-24-8	Cl-PFOPA	1.2	0.07	1	67	61	59
2	PFBA	375-22-4	$^{13}C_4$-PFBA	3.1	0.08	1	98	97	90
3	PFMPA	377-73-1	$^{13}C_4$-PFBA	3.2	0.07	1	81	88	85
4	PFPeA	2706-90-3	$^{13}C_5$-PFPeA	3.5	0.06	1	112	88	82
5	3:3 FTCA	356-02-5	$^{13}C_5$-PFPeA	3.5	0.10	1	89	94	91
6	PFBS	375-73-5	$^{13}C_3$-PFBS	3.5	0.04	1	82	86	83
7	PFHxPA	40143-76-8	Cl-PFOPA	3.6	0.09	1	110	101	89
8	PFMBA	863090-89-5	$^{13}C_5$-PFPeA	3.6	0.03	1	73	82	80
9	Cl-PFHxPA	N/A	Cl-PFOPA	3.7	0.11	1	98	94	86
10	PFEESA	113507-82-7	$^{13}C_3$-PFBS	3.7	0.03	1	80	87	84
11	NFDHA	151772-58-6	$^{13}C_5$-PFHxA	3.9	0.04	1	74	81	79
12	4:2 FTSA	757124-72-4	$^{13}C_2$-4:2 FTSA	3.9	0.06	1	87	93	76
13	PFHxA	307-24-4	$^{13}C_5$-PFHxA	4.0	0.03	1	94	82	79
14	PFPeS	2706-91-4	$^{13}C_3$-PFHxS	4.0	0.07	1	74	82	80
15	HFPO-DA	13252-13-6	$^{13}C_3$-HFPO-DA	4.1	0.04	1	99	99	97
16	FBSA	30334-69-1	$^{13}C_3$-PFHxS	4.2	0.04	1	80	88	86
17	P5MeODIOXOAc	1190931-41-9	$^{13}C_3$-HFPO-DA	4.4	0.17	1	100	93	91
18	PFHpA	375-85-9	$^{13}C_4$-PFHpA	4.6	0.06	1	120	81	78
19	PFHxS	355-46-4	$^{13}C_3$-PFHxS	4.7	0.07	1	77	84	83
20	DONA	919005-14-4	$^{13}C_4$-PFHpA	4.7	0.03	1	71	75	76
21	PFOPA	40143-78-0	Cl-PFOPA	4.8	0.11	1	80	100	100
22	5:3 FTCA	914637-49-3	$^{13}C_2$-6:2 FTUCA	4.8	0.08	1	78	84	82
23	6:2 FTUCA	70887-88-6	$^{13}C_2$-6:2 FTUCA	4.8	0.07	1	86	90	89
24	6:2 FTCA	53826-12-3	$^{13}C_2$-6:2 FTCA	5.0	0.09	1	111	111	114
25	4-PFecHS	646-83-3	$^{13}C_8$-PFOS	5.3	0.10	1	82	90	89
26	6:2 FTSA	27619-97-2	$^{13}C_2$-6:2 FTSA	5.4	0.06	1	89	92	71
27	PFOA	335-67-1	$^{13}C_8$-PFOA	5.4	0.02	1	82	78	79
28	PFHpS	375-92-8	$^{13}C_8$-PFOS	5.5	0.06	1	78	84	84
29	MeFBSA	68298-12-4	$^{13}C_8$-PFOSA	5.7	0.15	1	69	69	73
30	FHxSA	41997-13-1	$^{13}C_8$-PFOS	6.0	0.04	1	83	90	89
31	PFNA	375-95-1	$^{13}C_9$-PFNA	6.3	0.03	1	81	82	81
32	PFOS	1763-23-1	$^{13}C_8$-PFOS	6.4	0.05	1	76	82	81
33	8:2 FTUCA	70887-84-2	$^{13}C_2$-8:2 FTUCA	6.6	0.05	10	52	77	79
34	PFDPA	52299-26-0	Cl-PFOPA	6.6	0.14	1	79	112	108
35	7:3 FTCA	812-70-4	$^{13}C_2$-8:2 FTUCA	6.7	0.11	1	80	85	86

Target Number	Compound Name	CAS Number	Surrogate	RT (min)	MDL (µg/kg)	LOQ (Validated) (µg/kg)	% Recovery LSQ (1 µg/kg)	% Recovery MSQ (10 µg/kg)	% Recovery HSQ (50 µg/kg)
36	HFPO-TA	13252-14-7	$^{13}C_9$-PFNA	6.7	0.07	1	75	81	80
37	8:2 FTCA	27854-31-5	$^{13}C_2$-8:2 FTCA	6.7	0.09	1	94	90	80
38	9Cl-PF3ONS	756426-58-1	$^{13}C_8$-PFOS	6.9	0.09	1	68	74	74
39	FOSAA	2806-24-8	$^{2}H_3$-N-MeFOSAA	7.1	0.09	1	83	89	86
40	8:2 FTSA	39108-34-4	$^{13}C_2$-8:2 FTSA	7.2	0.06	1	88	92	75
41	PFNS	68259-12-1	$^{13}C_8$-PFOS	7.2	0.08	1	83	91	91
42	PFDA	335-76-2	$^{13}C_6$-PFDA	7.2	0.09	1	74	79	79
43	8:3 FTCA	34598-33-9	$^{13}C_6$-PFDA	7.6	0.07	1	97	100	98
44	N-MeFOSAA	2355-31-9	$^{2}H_3$-N-MeFOSAA	7.6	0.08	1	82	89	87
45	MeFHxSA	68259-15-4	$^{13}C_8$-PFOSA	7.8	0.10	1	71	74	75
46	PFDS	335-77-3	$^{13}C_8$-PFOS	7.9	0.09	1	80	90	89
47	PFUnDA	2058-94-8	$^{13}C_7$-PFUnDA	8.0	0.11	1	67	73	75
48	N-EtFOSAA	2991-50-6	$^{2}H_5$-N-EtFOSAA	8.0	0.04	1	76	86	84
49	PFOSA	754-91-6	$^{13}C_8$-PFOSA	8.0	0.03	1	77	82	81
50	10:2 FTUCA	70887-94-4	$^{13}C_2$-10:2 FTUCA	8.2	0.07	1	77	82	82
51	11Cl-PF3OUdS	763051-92-9	$^{13}C_8$-PFOS	8.3	0.06	1	65	72	71
52	PFUnDS	749786-16-1	$^{13}C_7$-PFUnDA	8.5	0.07	10	64	72	76
53	PFDoDA	307-55-1	$^{13}C_2$-PFDoDA	8.5	0.08	1	72	73	71
54	10:2 FTSA	120226-60-0	$^{13}C_2$-8:2 FTSA	8.5	0.07	1	107	115	96
55	10:2 FTCA	53826-13-4	$^{13}C_2$-10:2 FTCA	8.5	0.08	10	N.A.	98	99
56	6:6 PFPi	40143-77-9	$^{13}C_2$-PFDoDA	8.7	0.09	1	77	82	82
57	PFDoS	79780-39-5	$^{13}C_8$-PFOS	8.9	0.06	1	83	78	78
58	PFTrDA	72629-94-8	$^{13}C_2$-PFDoDA	8.9	0.11	1	66	74	76
59	N-MeFOSA	31506-32-8	$^{2}H_3$-N-MeFOSA	9.2	0.12	1	93	97	96
60	FDSA	N/A	$^{13}C_8$-PFOSA	9.2	0.04	1	66	72	70
61	MeFOSE	24448-09-7	$^{2}H_7$-MeFOSE	9.2	0.08	1	79	82	85
62	PFTrDS	791563-89-8	$^{13}C_2$-PFTDA	9.3	0.08	10	57	75	74
63	6:2 diPAP	57677-95-9	$(^{13}C_2)_2$-6:2 diPAP	9.3	0.07	1	84	87	86
64	PFTDA	376-06-7	$^{13}C_2$-PFTDA	9.3	0.11	1	67	72	72
65	6:8 PFPi	610800-34-5	$(^{13}C_2)_2$-6:2 diPAP	9.4	0.07	N.D.*	24	26	27
66	N-EtFOSA	4151-50-2	$^{2}H_5$-N-EtFOSA	9.6	0.10	1	74	79	79
67	EtFOSE	1691-99-2	$^{2}H_5$-EtFOSE	9.6	0.08	1	93	90	92
68	6:2/8:2 diPAP	943913-15-3	$(^{13}C_2)_2$-6:2 diPAP	9.9	0.06	10	61	65	67
69	8:8 PFPi	40143-79-1	$(^{13}C_2)_2$-6:2 diPAP	10.0	0.05	N.D.*	24	25	28
70	PFHxDA	67905-19-5	$^{13}C_2$-PFHxDA	10.1	0.05	1	84	83	80
71	8:2 diPAP	678-41-1	$(^{13}C_2)_2$-8:2 diPAP	10.4	0.04	1	75	77	77
72	PFODA	16517-11-6	$^{13}C_2$-PFHxDA	10.7	0.08	1	76	85	83
73	diSAmPAP	2965-52-8	$(^{13}C_2)_2$-8:2 diPAP	11.0	0.04	N.D.*	54	58	59

* N.D.: Not determined. The LOQ for three compounds were not determined due to lower recovery.

Results and discussion

Method sensitivity and linearity

By implementing the described LC/TQ acquisition method setup and data processing steps, the 6495D LC/TQ system demonstrated excellent sensitivity for 73 native PFAS across 14 target groups (Figure 2). The abbreviations for these 14 target groups are defined in Table 2. The MRM overlay of 15 PFCA targets in the LSQ illustrates the symmetric separation and superior sensitivity of the 6495D LC/TQ system for the determination of PFAS in FCMs (Figure 3). Despite the close elution of two pairs of PFCAs (PFUnDS with PFDoA, and PFTrDS with PFTDA), the unique MRMs of these targets enable unambiguous compound quantitation and superior accuracy.

Method detection limit (MDL) and the limit of quantification (LOQ) assessments were performed to evaluate the sensitivity of the entire workflow. The MDL was calculated using MassHunter Quantitative Analysis software version 12.1, based on nine continuous injections derived from two technical replicates of LSQ samples.[9] A similar procedure is described in 40 CFR Part 136 Appendix Revision 2, US EPA.[10] The MDL values (based on sample weight) for each target are summarized in Table 1.

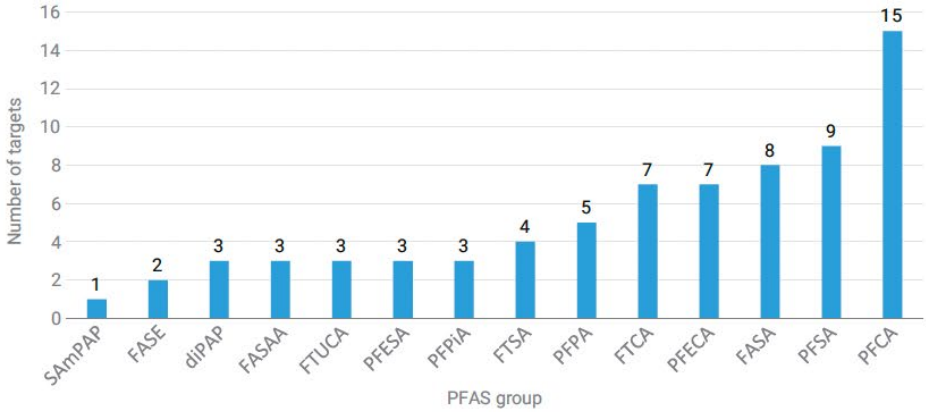


Figure 2. Distribution of 73 native PFAS across different groups.

Table 2. Abbreviations for 14 PFAS groups.

Abbreviation	Description
diPAP	Polyfluoroalkyl phosphoric diester
SAmPAP	Perfluorooctane sulfonamido-ethanol-based phosphate diester
FASA	Perfluoroalkane sulfonamides
FASAA	Perfluoroalkane sulfonamido acetic acid
FASE	Perfluoroalkane sulfonamido ethanol
FTCA	Fluorotelomer carboxylic acid
FTSA	Fluorotelomer sulfonic acid
FTUCA	Fluorotelomer unsaturated carboxylic acid
PFCA	Perfluoroalkyl carboxylic acid
PFECA	Perfluoroether carboxylic acid
PFESA	Perfluoroether sulfonic acid
PFPA	Perfluoroalkyl phosphonic acid
PFPiA	Perfluoroalkyl phosphinic acid
PFSA	Perfluoroalkyl sulfonic acid

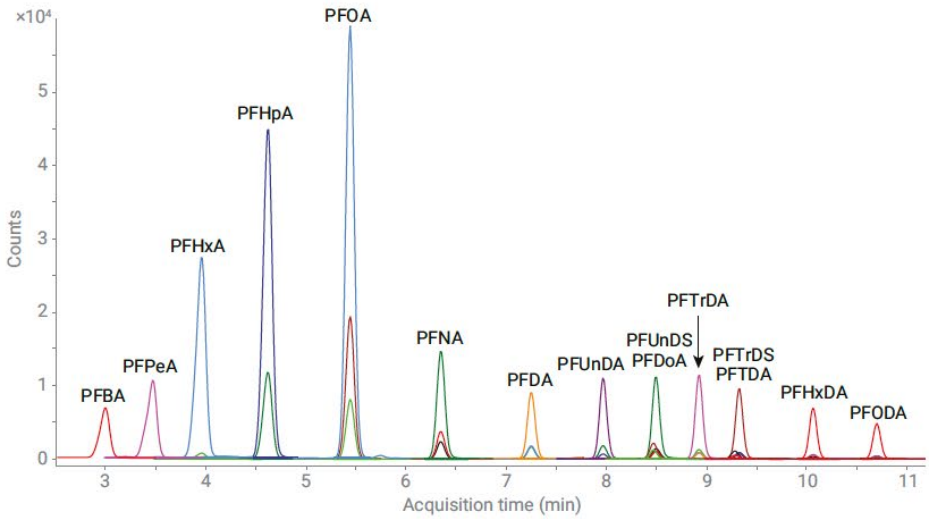


Figure 3. Overlaid MRM chromatogram of 15 PFCA targets in the paper straw LSQ at a spiking level of 1.0 µg/kg (target concentration in ready-to-inject sample vial is 80 ng/L).

Figure 4 illustrates the MDL distribution for all targets. Notably, all 73 analytes had an MDL of ≤ 0.2 µg/kg. Among these, 20 analytes exhibited an MDL of ≤ 0.05 µg/kg, while 43 targets fell within an MDL range of 0.05 to 0.1 µg/kg. Furthermore, the MDLs for PFOA (0.02 µg/kg), PFOS (0.05 µg/kg), PFDA (0.09 µg/kg), PFNA (0.03 µg/kg), and PFHxS (0.07 µg/kg) were < 0.1 µg/kg. These values are significantly lower than the typical regulatory requirements in similar food market spaces. These results underscore the exceptional sensitivity of PFAS analysis from FCMs using the 6495D LC/TQ system equipped with the dedicated PFC-free conversion kit. This provides assurance that the compounds of interest can be quantified accurately without false positives.

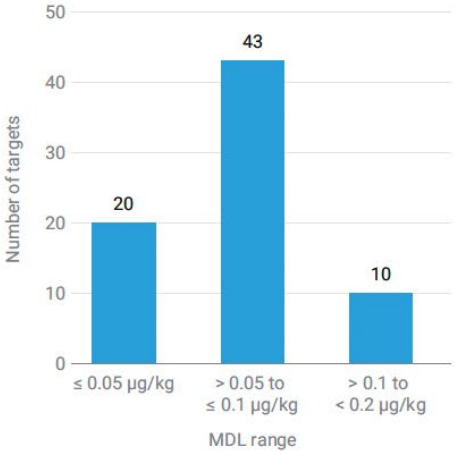


Figure 4. MDL distribution of all 73 targets.

LOQ is the lowest concentration of the analyte in the test material that has been validated with acceptable recovery and repeatability, using the entire workflow and identification criteria.[11] In this work, prespiked sample QCs (LSQ, MSQ, and HSQ) were used to establish the method LOQ following these identification criteria:

- Recovery between 65 and 135% with percent relative standard deviation (%RSD) ≤ 20%
- Intrabatch retention time (RT) tolerance 1%
- Signal-to-noise ratio (S/N) ≥ 3:1
- Ion ratio of quantifier and qualifier within ± 30%

The LOQ values for each analyte are summarized in Table 1. Notably, 65 out of 73 analytes (89%), including PFOA, PFOS, PFNA, and PFHxS, achieved an LOQ of 1 µg/kg, demonstrating the outstanding performance of this workflow for quantifying PFAS in FCM samples.

The method linearity range for each of the targets was established using linear regression with 1/x weighting for most of the 73 analytes and a minimum of five calibration levels. All target analytes exhibited excellent R^2 values, exceeding 0.99 (except for 8:2 FTCA). Additionally, the accuracy of each calibration standard fell within the commonly accepted limits of 70 to 130%.

Method recovery and precision

Matrix-spiked QC recovery was employed to evaluate the accuracy of this PFAS analysis workflow. The method was set up for the analysis of 73 native PFAS analytes and 34 labeled compounds (surrogates), which served as EIS for isotope dilution or internal standard quantification of the native PFAS. This isotope dilution method effectively corrects matrix effects and reduces target loss, significantly boosting the accuracy of analytical performance.[11-13] The EPA 533 isotope performance standard mix includes three labeled PFAS compounds ($^{13}C_3$-PFBA, $^{13}C_2$-PFOA, and $^{13}C_4$-PFOS), which are used as nonextracted internal standards (NIS) for calculating the surrogate recoveries. To correct for native PFAS levels, the measured concentration of each analyte in the spiked QC sample was adjusted by subtracting its presence in the unspiked FCM matrix blank sample.

The method recovery was calculated based on the mean percent recovery. Method precision was assessed using the %RSD of recoveries, calculated from replicate injections of duplicate technical preparations (n = 6). Table 1 lists recovery values of each analyte. For LSQ, MSQ, and HSQ samples, approximately 90% of analytes achieved recovery within a range of 65 to 120%, meeting the commonly accepted range for food matrices (Figure 5).[11] Among these,

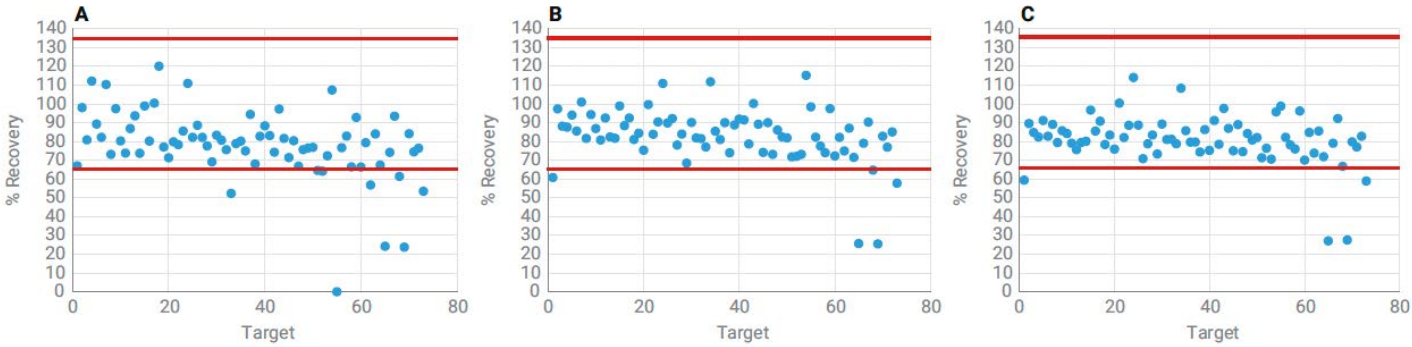


Figure 5. Mean recovery (n = 6) distribution of all 73 targets, (A) LSQ, (B) MSQ, and (C) HSQ. The recovery limit of 65 to 135% is marked using a red line.

PFNA, PFOS, PFOA, and PFHxS achieved recovery > 75% across all three QC levels.

Recovery analysis of 10:2 FTCA in the LSQ sample was impacted by matrix interference. Poor recovery was observed for two targets, 8:8 PFPi and 6:8 PFPi, in all three QC samples. The recovery repeatability for all targets was ≤ 20% RSD for all spiked QC samples. Notably, 96% of the samples were below 10% RSD, except for three outliers in LSQ and one outlier in MSQ (Figure 6). The repeatability of measures for 8:8 PFPi and 6:8 PFPi was within 10%, despite the poor recovery. However, these targets are currently not listed in any of the regulatory guidelines discussed here. These results confirm the excellent extraction efficiency of PFAS compounds from FCM and the reproducibility of measurements using these methods.

In various regulatory guidelines, PFAS compounds from the PFCA and PFSA groups, such as PFOA, PFDA, and PFOS, are consistently highlighted as critical concerns. Special evaluation of these three targets was performed. MRM overlays of duplicate LSQs demonstrate consistency between the two technical preparations (Figure 7). The response reproducibility of the targets confirms the reliability of this workflow for routine PFAS analysis in FCM samples.

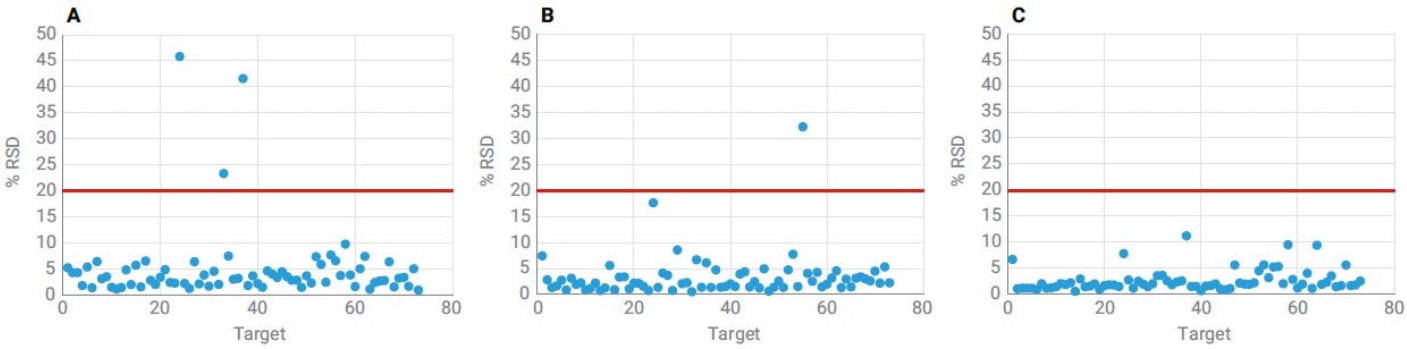


Figure 6. Recovery %RSD summary of all 73 targets calculated from replicate injections of duplicate spiked samples (n = 6), (A) LSQ, (B) MSQ, and (C) HSQ. The 20% RSD limit is marked using a red line.

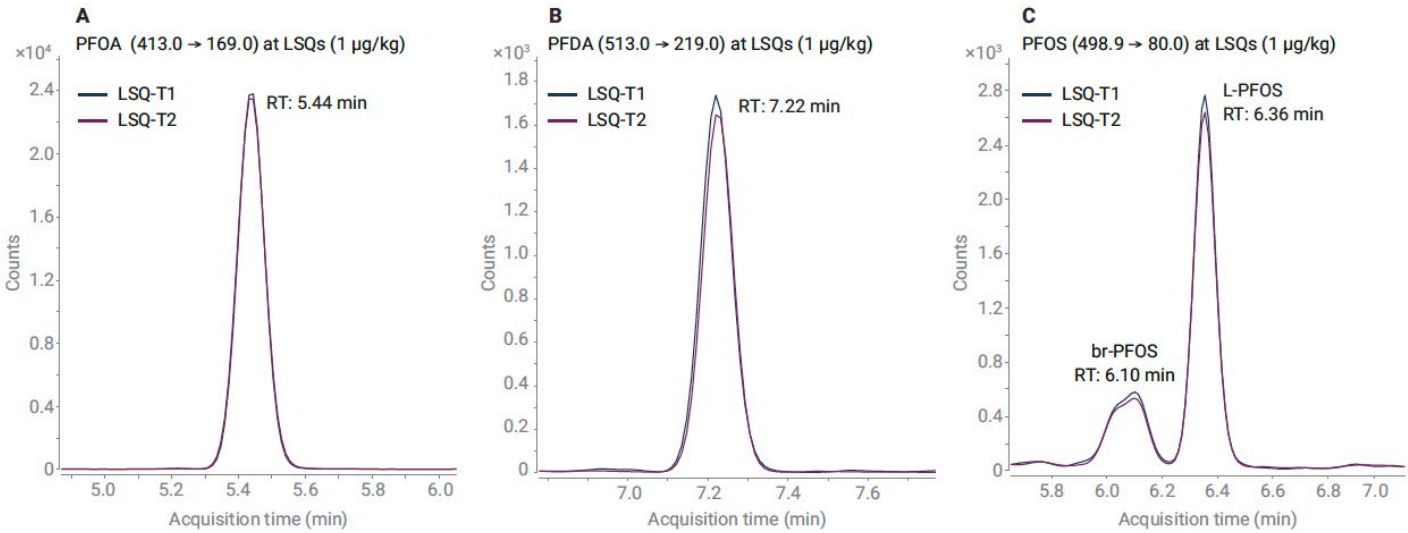


Figure 7. Overlay of MRM traces of critical targets: PFOA (A), PFDA (B), and PFOS (C) from two LSQ technical preparations (T1: techinical preparation 1, T2: techinical preparation 2). **Note:** The ready-to-injection concentration of targets at the LSQ level is 80 ng/L.

Quantitation of PFAS from blank food contact material matrix

The native concentration of PFAS in FCM samples was investigated to validate the reliability of this newly developed analytical method. Reagent blank (procedural blank without sample addition) and unspiked FCM samples (matrix blanks) were extracted in triplicate using the same preparation procedure and then analyzed by LC/TQ. The data revealed no contamination in the reagent blank, while approximately 10 native PFAS compounds at trace levels were observed in the matrix blank. Noteworthy compounds with residue concentrations greater than the MDL were PFHxA, PFHpA, PFOA, PFNA, and 10:2 FTCA.

Conclusion

This study successfully demonstrated an end-to-end workflow for the quantitative analysis of 73 native PFAS from food contact material (FCM) samples. A simple and rapid solvent extraction procedure was used to leach PFAS compounds from the matrix. This was followed by a dilute-and-shoot approach to LC/TQ without the need for drying and reconstitution. Superior chromatographic separation was achieved for all 110 PFAS compounds in the initial 12 minutes. This demonstrates the performance efficiency of the Agilent 1290 Infinity II LC for routine laboratory use and enhanced lab productivity. The Agilent 6495D triple quadrupole LC/MS equipped with the PFC-free conversion kit offered excellent background contamination removal and ppt-level sensitivity for precise PFAS quantitation from the FCM matrix.

The method verification results, including sensitivity and recovery, confirm the applicability of this workflow for PFAS measurements at lower concentrations. These high-quality analytical outcomes enable FCM manufacturers to make informed decisions during production, ensuring compliance with upcoming regulatory standards and enhancing consumer safety. Additionally, the use of the dedicated PFAS MRM database provided ease of method creation while helping to reduce the MS parameter optimization time.

References

1. U.S. Environmental Protection Agency. PFAS Explained. **https://www.epa.gov/pfas/pfas-explained** (accessed Oct 10, **2024**).
2. National Institute of Environmental Health Sciences. Perfluoroalkyl and Polyfluoroalkyl Substances (PFAS). **https://www.niehs.nih.gov/health/topics/agents/pfas/index.cfm** (accessed Oct 10, **2024**).
3. Consumer Reports. Dangerous PFAS Chemicals Are in Your Food Packaging. **https://www.consumerreports.org/health/food-contaminants/dangerous-pfas-chemicals-are-in-your-food-packaging-a3786252074/** (accessed Oct 10, **2024**).
4. OECD. PFASs and Alternatives in Food Packaging (Paper and Paperboard) Report on the Commercial Availability and Current Uses; OECD Series on Risk Management, No. 58; Environment, Health and Safety, Environment Directorate, OECD: Paris, **2020**.
5. Commission Regulation (EU) No. 10/2011.
6. European Chemicals Agency. ANNEX XV Restriction Report Proposal for a Restriction; ECHA: Helsinki, March **2023**.
7. Per- and Polyfluoroalkyl Substances (PFAS) Analysis for Environmental Samples. *Agilent Technologies ordering guide*, publication number 5994-2357EN, **2024**.
8. Method Development and Screening of Extractable Organofluorine (EOF) and Targeted PFAS Analysis in Food Packaging Materials; Spring, **2022**.
9. Wells, G.; Prest, H.; William Russ, C. Signal, Noise, and Detection Limits in Mass Spectrometry, *Agilent Technologies application note*, publication number 5990-7651EN, **2023**.
10. U.S. Environmental Protection Agency. Definition and Procedure for the Determination of the Method Detection Limit, Revision 2; EPA 821-R-16-006; U.S. EPA: Washington, DC, December **2016**.
11. AOAC International. Standard Method Performance Requirements (SMPRs) for Per- and Polyfluoroalkyl Substances (PFAS) in Produce, Beverages, Dairy Products, Eggs, Seafood, Meat Products, and Feed; AOAC SMPR 2023.003.
12. U.S. Environmental Protection Agency. EPA Method 1633 Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/MS; U.S. EPA: Washington, DC, January **2024**.
13. U.S. Food & Drug Administration. Determination of 30 Per and Polyfluoroalkyl Substances (PFAS) in Food and Feed using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS); U.S. FDA: Silver Spring, MD, April **2024**.

www.agilent.com

DE-001948

This information is subject to change without notice.

© Agilent Technologies, Inc. 2024
Printed in the USA, November 4, 2024
5994-7889EN

Agilent
Trusted Answers

Further Reading and Resources

Analysis of Per and Polyfluoroalkyl Substances in Edible Fish Tissue Using Agilent Captiva EMR–Lipid and LC/MS/MS

Application note

Quantitation of Per- and Polyfluoroalkyl Substances (PFAS) in Chicken Eggs for Human Consumption

Application note

The Forefront of Environmental Toxicology

On-demand virtual event where several experts delve into the complexities of emerging contaminants, discussing these contaminants' sources, distribution, and potential impacts on ecosystems and human health.

Wiley's content on PFAS

Access all of Wiley's content on PFAS, presented in infographics, article collections, and expert insights.

WILEY

Imprint

© Wiley-VCH GmbH, Boschstr. 12, 69469 Weinheim, Germany

Cover: © 202X Adobe

Email: info@wiley-vch.de

Editor: Dr. Róisín Murtagh

xxxxxx.xxxxxx