LC GC

Advances in Environmental Analysis of PFAS and SVOCs

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Extraction of PFAS from Mixed Matrices Using a Rapid, Simple, and Automated Extraction System

Alicia Douglas Stell

The rapid and reliable extraction of poly- and perfluoroalkyl substances is a much-needed capability, provided by the EDGE extraction system.

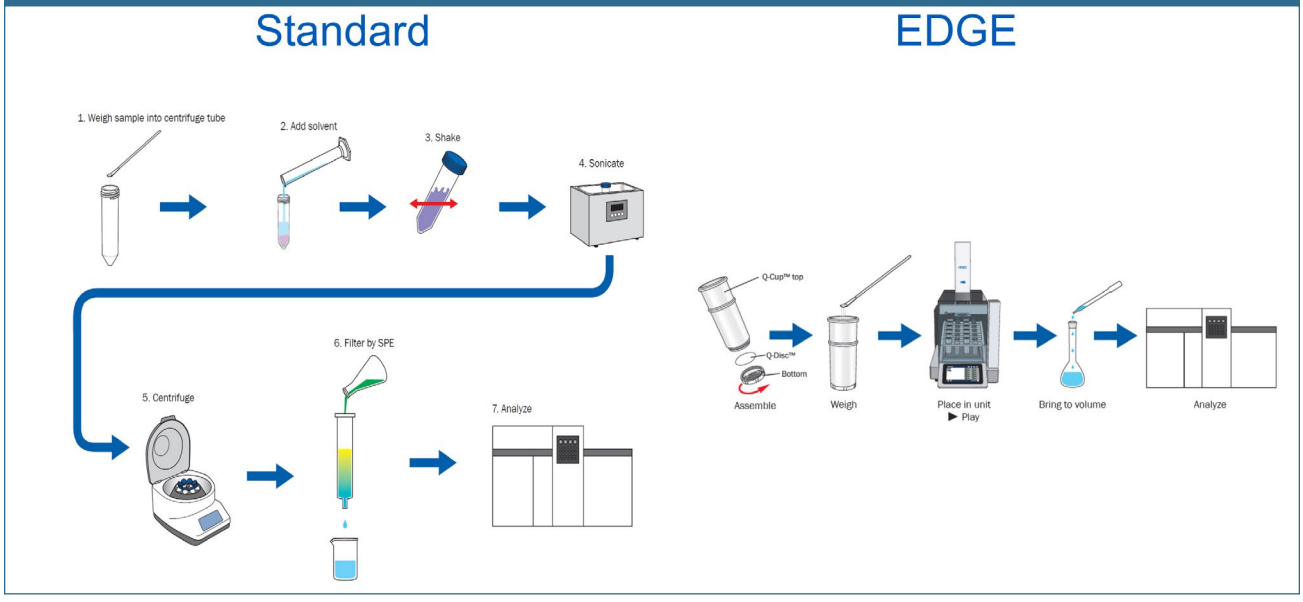
Overview

Perfluoroalkyl and polyfluoroalkyl substances (PFAS) are used widely in consumer products such as cookware, cleaning products, and food packaging because of their thermal, chemical, and fire resistance. Unfortunately, they can escape into food and the environment and are of increasing concern as environmental pollutants. Their stability becomes a downside here, making them highly persistent. As regulations continue to develop, the demand for reliable methods to extract, detect, and identify these compounds will increase.

Presently, PFAS are relatively unregulated. In the United States, there is currently no maximum contaminate level set, although the US Environmental Protection Agency (EPA) has a health advisory for perfluorooctanoic acid (PFOA) and perfluorooctane sulfuric acid (PFOS) at 70 ppt in drinking water. That value is expected to decrease as more health data comes in. There are currently two EPA methods covering 29 PFAS in drinking water. Compendial methods for other sample matrices are clearly needed. As a result, the EPA has developed an action plan that will require methods for measuring PFAS at parts per trillion levels in a variety of matrices.

The regulatory environment is also changing outside of the US. Denmark has already banned PFAS and the rest of the European Union is tightening regulations. Australia is currently in the process of phasing them out.

Figure 1: Standard versus EDGE Extraction.



Enforcement and regulatory compliance will soon require laboratories to quickly and reliably detect and quantify PFAS.

PFAS Sample Processing and the EDGE Extraction System

There is growing interest in the measurement of PFAS in solid samples, including soils and foodstuffs. The standard sample processing method can be seen on the left side of **Figure 1**. Typically, about 1 g of sample is weighed out into a centrifuge tube and 8 mL of methanol is added. The sample is then shaken for 30 minutes, sonicated for an additional 30 minutes, and then centrifuged. The liquid is then decanted and cleaned with a graphitized carbon solid-phase extraction cartridge. A 2-mL wash brings the final volume up to 10 mL. Internal standards are also added at this time.

This is a manual multi-step process that takes over an hour and does not scale well. Clearly, as the demand for PFAS analysis increases, a higher throughput and more automated system will be required. One solution is provided by the EDGE Extraction system. The typical EDGE process is seen on the right side of the figure. In the streamlined process, a 5-g sample is weighed into the Q-Cup, and then the processing method is run. Typical run time is 10 minutes or less. The resulting extract can then be brought to a consistent volume and analyzed with no further processing.

The conditions that take place during extraction increase the extraction

"To evaluate the effectiveness of the EDGE system in extracting PFAS from soil samples, a set of spike recovery experiments was performed."

efficiency in less time than traditional methods. The Q-Cups are made from an aluminum tube with a filter disk forming the bottom. During processing, the Q-Cup is moved to a sealed chamber, and solvent can be added from the top and bottom of the chamber. Heat is then applied, creating a pressure differential between the outside and inside of the Q-Cup, forcing heated solvent up through the filter, aiding in dispersal and mass transfer during the extraction. Once the desired temperature or hold time has been reached, the sample is drained through a cooling coil and collected. If necessary, multiple extraction cycles can be run on each sample. A between-sample wash of all the sample-contacted tubing eliminates the risk of carryover.

One of the challenges associated with PFAS analysis comes from their ubiquity within the laboratory. Teflon and other fluoropolymers have become a default material for a wide variety of tubing and containers. Leaching and contamination from these materials is a significant risk. The number of consumables that a sample potentially comes in contact with can be daunting, including the components of the Q-Cup (filter disc, disc packaging, the cup walls), centrifuge tubes, extraction solvents, cleaning matrixes, pipet tips, tubing, homogenizers, weigh boats, mortars, pestles, etc. Everything that comes in contact with the sample from the time it enters the laboratory until it is analyzed should be evaluated. As part of the development of the methods described here, all sample-contacting components were submitted to soak tests to establish that they would not leach PFAS into the sample.

In instrumentation, polyether ether ketone (PEEK), polypropylene, and polyethylene must be substituted for all Teflon tubing and fittings. EDGE systems are available for general use and also in a fluoropolymer-free configuration for

Figure 2: HPLC MS/MS method.

- SCIEX 4500 with Agilent pump systems
  - stainless steel lines going into the MS
  - PEEK solvent lines
  - polyethylene tubing anywhere else
  - sample chiller at 4 °C for the autosampler
- Phenomenex Gemini, 3 µm, C18, 110 Å, 50 x 3 mm column
  - Guard filter and delay column
- 10 µL injection volume
- A: 20 mM ammonium acetate in water, B: Methanol
- Flow Rate: 1200 µl/min
- Gradient

Time (min)	%A	%B
Initial	95	5
0.1	45	55
4.5	1	99
6	1	99
6.1	95	5
8.1	95	5

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dedicated PFAS analysis. Pressure during extraction is provided by an integrated pump and typically uses laboratory air. However, if easily oxidized species are being extracted, a nitrogen source can be connected.

Spike Recovery from Soil

To evaluate the effectiveness of the EDGE system in extracting PFAS from soil samples, a set of spike recovery experiments was performed. A variety of filters are available to be used as the bottom of the Q-Cup. For the experiments presented here, the Q-Disc used was a glass fiber disk that filters down to 0.3 µm. Because glass fiber disks are not particularly rugged, the fiber disc was sandwiched between two cellulose filters. This disc arrangement was sufficient for both the soil samples and the food samples described in the next section.

For each sample type, 5 g was added to the Q-Cup. Standards of 24 PFAS compounds were also spiked into the cups. The same method was used on all samples. The extraction solvent was 80:20 methanol:water with 0.3% ammonium hydroxide. The method consisted of two cycles. In each cycle, 10 mL of solvent was added to the top of the sample, and the temperature increased to 65 °C, then held for 3 minutes in the first cycle, then 4 minutes in the second. The entire process took less than 10 minutes. To avoid carryover, two washes were performed after each sample using 10 mL of methanol, followed by 10 mL of the extraction solvent. Prior to analysis, the extract was diluted to a known volume and neutralized with 20 µL of formic acid.

The samples were analyzed using a SCIEX 4500 system in which all fluoropolymer components had been replaced. The method is summarized in **Figure 2**. Separation was

Figure 3: Snack cake sample.

PFAS Detected
N-ethylperfluoro-1-octanesulfonamidoacetic acid (EtFOSAA)
N-methylperfluoro-1-octanesulfonamidoacetic acid (MeFOSAA)
perfluoro-1-pentanesulfonic acid (PFPeS)
perfluorohexanesulfonic acid (PFHxS)
perfluoro-n-butanoic acid (PFBA)
perfluoro-n-heptanoic acid (PFHpA)
perfluoro-n-hexanoic acid (PFHxA)
perfluoro-n-nonanoic acid (PFNA)
perfluoro-n-octanoic acid (PFOA)
perfluoro-n-pentanoic acid (PFPeA)

on a 50 x 3-mm Phenomenex Gemini C18 column with 3-µm particle size. Injection size was 10 µL. The aqueous phase contained 20-mM ammonium acetate. The elution was a gradient running up to 99% methanol in 4.5 minutes and then requiring another 4 minutes to re-equilibrate back to the initial conditions of 5% methanol.

In all, the total sample processing time was approximately 20 minutes. Samples were spiked at 0.1 ppb, 10 ppb, and 20 ppb of the 24 PFAS. The limit of detection for the method was approximately 0.025 ppb. Recoveries for the lowest spike were in the range of 74–101% with RSD of 10% or below. Both recovery and RSD values improved with the higher spike levels. With high spike values, particular care must be taken to prevent carry-over.

It should be noted that higher recoveries would be expected under spiked conditions compared to many real-world samples where the PFAS have become more entrained within the matrix. Subsequent studies will use certified reference materials to establish if more rigorous extraction protocols are needed.

Extraction of PFAS from Food Samples

The presence of PFAS in the food supply is becoming an increasing concern. To evaluate the ability of the EDGE extraction protocols to handle a variety of biological or food matrices, samples of cucumber, snack cakes, and savory turnovers were evaluated. No spiking was performed, and the samples were analyzed for any native PFAS content. Although the food samples could use the same extraction protocol and filter sets used for the soil samples, some accommodations had to be made to clean up potential interferants in the matrices. The high-water content in cucumber was dealt with by adding 2.5 g of Q-Matrix Hydra to the Q-Cup prior to extraction. For the high lipid content of the packaged food samples, 0.5 g of a C18 sorbent was sufficient to sequester much of the lipid and prevent problems

> **"Subsequent studies will use certified reference materials to establish if more rigorous extraction protocols are needed."**

Figure 4: Microwaveable turnover sample.

PFAS Detected	
N-ethylperfluoro-1-octanesulfonamidoacetic acid (EtFOSAA)	perfluoro-n-hexanoic acid (PFHxA)
N-methylperfluoro-1-octanesulfonamidoacetic acid (MeFOSAA)	perfluoro-n-nonanoic acid (PFNA)
perfluoro-1-butanesulfonic acid (PFBS)	**perfluoro-n-octanoic acid (PFOA)**
perfluoro-1-decanesulfonic acid (PFDS)	perfluoro-n-pentanoic acid (PFPeA)
perfluoro-1-heptanesulfonic acid (PFHpS)	perfluoro-n-butanoic acid (PFBA)
perfluoro-1-pentanesulfonic acid (PFPeS)	perfluoro-n-decanoic acid (PFDA)
perfluorohexanesulfonic acid (PFHxS)	perfluoro-n-dodecanoic acid (PFDoA)
perfluoro-n-butanoic acid (PFBA)	perfluoro-n-heptanoic acid (PFHpA)
perfluoro-n-decanoic acid (PFDA)	perfluoro-n-tridecanoic acid (PFTrDA)
perfluoro-n-dodecanoic acid (PFDoA)	perfluoro-n-u0ecanoic acid (PFUdA)
perfluoro-n-heptanoic acid (PFHpA)	**perfluorooctanesulfonic acid (PFOS)**

with the analysis. The cucumber was chopped and then homogenized prior to extraction. Five separate PFAS were identified in the cucumber, including PFOA.

The snack cakes, seen in **Figure 3** are packaged in molded plastic trays that may be a leaching source for PFAS. For the analysis, the top icing was removed. The cake was chopped, dried for 90 minutes at 100 °C, and ground in a mortar. The ten PFAS compounds that could be identified are listed in **Figure 3**.

A microwavable turnover was the second processed food item tested. This is a stuffed pastry that is packaged and microwaved in an aluminized sleeve, which is typically surface treated with fluorinated compounds. The turnovers were first microwaved per instructions.

> **"Using appropriate instrumentation, it is possible to achieve quantitative extraction of samples without contamination of PFAS in a variety of matrices."**

The outer breaded coating was then removed for testing. Like the cakes, the sample was dried for 90 minutes at 100 °C, then ground. A total of 22 PFAS compounds, listed in **Figure 4**, could be identified in the sample, including both PFOA and PFOS.

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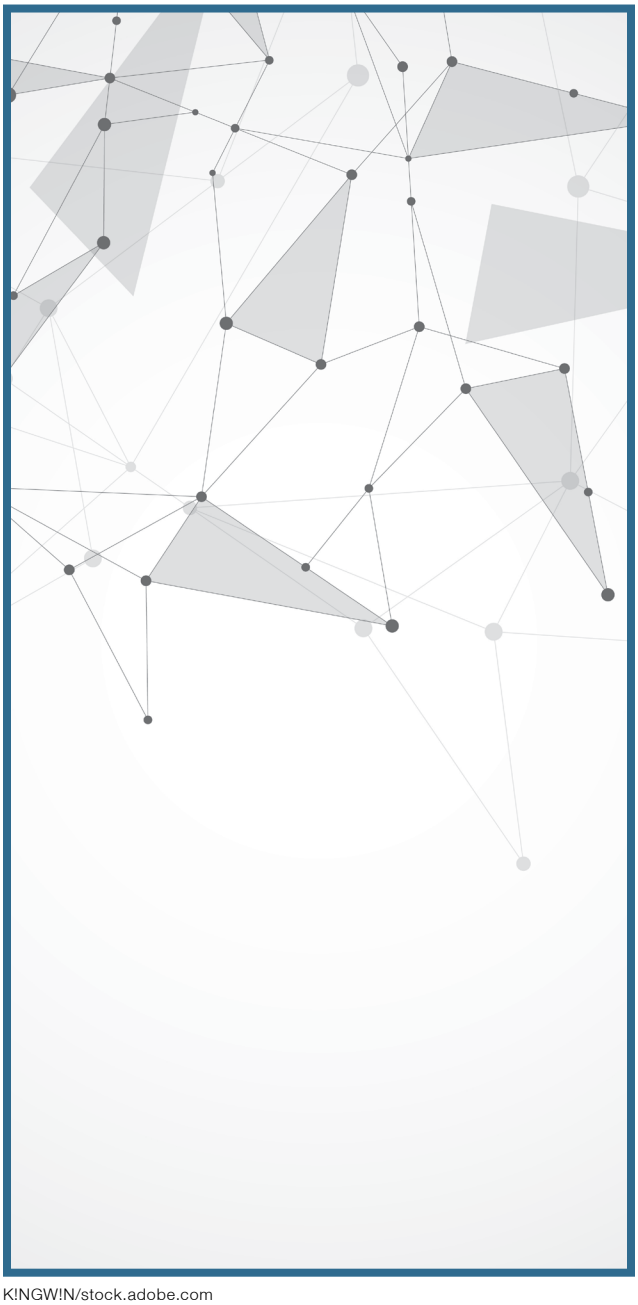
Conclusion

Using appropriate instrumentation, it is possible to achieve quantitative extraction of samples without contamination of PFAS in a variety of matrices. Instruments such as the EDGE extraction system can achieve good recoveries at low concentration levels from a variety of samples ranging from soil, to biological samples, to food items. Although not presented here, it is also possible to perform extractions from liquid samples by using a supporting material such as Q-Matrix Hydra. Extraction of packaging materials is also possible if increased temperature and hold times are used.

Analysis of PFAS requires careful consideration of laboratory materials and instrumentation must be configured to exclude possible sources of contamination. With the appropriate instrumentation, the protocol is rapid, simple, largely automated, and applicable to a broad variety of mixed matrices. This kind of analysis is likely to become increasingly in demand as the regulatory environment changes.



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From soil to plants to finished foods, PFAS contamination is everywhere.

PFAS can be a tricky substance to analyze. Known as the “forever chemicals”, they are abundant in most products we use and are in a process of rapid bioaccumulation in both plants and animals. Testing for these known carcinogens has become increasingly important, as is making sure the testing equipment is clear of all PFAS residue prior to analysis.

The EDGE is a PFAS free extraction solution that performs extractions in less than 10 minutes. Keep your lab producing accurate results in a fraction of the time of other extraction techniques.

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Improving Sample Preparation for Environmental Analysis of SVOCs and PFAS

Interview with Alicia Douglas Stell

Environmental monitoring has been a requirement since the early 1970s. Substances such as semi-volatile organic compounds (SVOCs) and heavy metals have been regulated by the U.S. Environmental Protection Agency (EPA) and other governing bodies for many years. But per- and polyfluoroalkyl substances (PFAS) are emerging contaminants that are making headlines worldwide and will require similar testing and regulation in the near future.

PFAS are man-made chemicals found in everything from food packaging and household items to outdoor gear and personal care products. They have been used in the United States since the 1940s and have been linked to adverse effects in humans. SVOCs are often found outside in soil and earth and inside in household cleaning products, dust, and air and can also pose a threat to human health. Streamlining testing is of the utmost importance to the laboratories that see hundreds of samples per day and rely on automation for consistent, repeatable, and reliable results.

Extracting PFAS can be difficult because there are so many different sources of contamination; SVOCs present challenges as well. The EDGE, an automated extraction system from CEM Corporation, is faster, simpler, and more automated than other extraction methods used to prepare samples of environmental compounds. *LCGC* recently spoke with Alicia D. Stell, Ph.D., lead R&D scientist at CEM Corporation, about preparing PFAS and SVOCs samples using the EDGE.

"Because of the low detection level used in analysis, it is imperative that PFAS are not present in the background noise, as even very low levels of contaminant can give a false positive. Therefore, it's important to remove or correct for all PFAS background."

LCGC: Why are per- and polyfluoroalkyl substances (PFAS) compounds so problematic?

Stell: There are a couple reasons why PFAS compounds are so problematic—namely persistence and abundance. PFAS compounds are often called forever compounds because they are so stable that they persist for decades after production or use. They are nearly impossible to destroy and don't naturally decompose. Because of this persistence, they bioaccumulate in animals, fish, and humans. Furthermore, it's difficult to remediate these compounds so clean-up efforts are very ineffective.

The second reason PFAS compounds are so problematic is that they are everywhere. PFAS compounds are commonly used in industrial manufacturing and can be found in trace levels in a wide variety of components, including tubing and valves. This poses a challenge for analyses of these compounds because great care must be taken to ensure that a sample is not contaminated during sampling, sample preparation, or analysis.

LCGC: Why is background such an issue with PFAS extractions in general?

Stell: It relates to the last question and the fact PFAS are abundant. Because PFAS are everywhere, there are many different sources of contamination so detecting them becomes a big problem. Researchers are working to analyze PFAS compounds at extremely low levels to understand the impact they have on human health. Because of the low detection level used in analysis, it is imperative that PFAS are not present in the background noise, as even very low levels of contaminant can give a false positive. Therefore, it's important to remove or correct for all PFAS

background when analyzing, this way you know you're getting accurate results.

LCGC: What precautions are taken to eliminate PFAS background on the EDGE?

Stell: We looked at the process from start to finish; we wanted to make sure we weren't implementing PFAS at any point during the sample preparation, i.e., anything used to prepare the sample—sample cup (CEM's Q-Cup®), filter (CEM's Q-Disc®), or any other material used within the sample such as CEM's Q-Matrix Hydra™. We also evaluated system components for PFAS contamination and created a PFAS-free EDGE by using materials such as PEEK tubing and polypropylene tubing instead of commonly used polytetrafluoroethylene (PTFE) that can lead to PFAS contamination.

To ensure that our system is PFAS-free, we did a lot of testing. We tested the EDGE system alone, and we performed soak tests on the system components and accessories to ensure that it's reliable for this unique type of application. We collected a lot of extracts, blanks, replicates, etc., to verify that at no point during the sample preparation or running the extraction on the EDGE were PFAS implemented.

LCGC: What matrices are normally tested for semi-volatile organic compounds (SVOCs)?

Stell: Historically, SVOCs are found in soils, clays, sediments, sludges, and waste solids. Soil is the most common place to look for these compounds, as they are a product of pesticides and industrial waste. However, they can be present in much more than soil. We find SVOCs in the air, which means materials such as polyurethane foam and air-monitoring filters can be extracted for these particular types of compounds.

Furthermore, foodstuffs have been tested for SVOCs. I've seen a lot of people test for SVOCs in fishmeal or other types of food capacities. So, it is a very broad sector as to where you can look for SVOCs. But traditionally, a lot of testing starts at the soil level. I think we will see the same evolution of testing with PFAS compounds, where we begin with water and soil testing and branch out into air, foodstuffs, and other items we contact that contain these compounds.

LCGC: Does EDGE have any carryover for SVOCs?

Stell: That's a great question and something we need to address because the EDGE runs extraction in series, one right after the other, and SVOCs are notorious for sticking around, and we don't want that to happen. We don't want any carryover from one sample to the next.

As a result, we've taken great care and measure to ensure that no carryover occurs. We do that by implementing a washing program that is very customizable. The customer can do up to five system washes and can run up to six different solvents, and they can control the temperature and hold time of each wash. It is even possible to collect blank extractions should post-cleaning analysis be required to confirm elimination of carryover.

The wash cycles flush fluids through the entire system's fluidics path to ensure that any surface that was in contact with the sample or extract has been thoroughly cleaned before moving on to the next sample. The wash cycles take place during the sample run of less than 10 minutes, making the wash not only efficient but very fast, too.

System washing and elimination of carryover is also important for PFAS extractions. The same variables are available for PFAS methods, including availability of up to six solvents and fully customizable wash-volume, hold-time, and wash-temperature parameters.

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Quantitative Analysis of PFAS in Drinking Water Using Liquid Chromatography Tandem Mass Spectrometry

Emily Parry and Tarun Anumol

Per- and polyfluoroalkyl substances (PFAS) are chemicals found in fire-fighting foams and consumer products requiring water-resistant and stain-repellent properties. As a result of their unique chemical properties and long-term widespread usage, these chemicals are an emerging human health concern. US Environmental Protection Agency (EPA) released analytical methods for PFAS measurement in 2009 and most recently in November of 2018. In this article, data generated using these methods with allowed analytical modifications is presented and demonstrates robustness and reproducibility while achieving low level detection limits in drinking water.

Per- and polyfluoroalkyl substances (PFAS) are a class of man-made compounds widely used in industry and manufacturing because of their uniquely desirable chemical properties. These compounds are used in non-stick cookware, food contact materials, fire-fighting foams, surfactants, and many other applications. Their chemistry makes these compounds extremely persistent, bioaccumulative, and potentially toxic to animals and humans (1). As a result of their widespread usage over the last few decades, they are now ubiquitous in the environment.

There are more than 4500 PFAS commercially manufactured, but only very few have been monitored in the environment. The most commonly measured PFAS classes in the environment are the perfluorocarboxylic acids (PFCAs), such as perfluorooctanoic acid (PFOA), and

perfluorosulfonic acids (PFSAs), such as perfluorooctanesulfonate (PFOS). Some of these PFAS compounds are currently the subject of regulation and much public and research attention (2).

US Environmental Protection Agency (EPA) indicates a drinking water health guidance for PFOA and PFOS at a combined 70 ng/L, while several US states have guidelines for PFOA, PFOS, and other PFAS (PFNA, GenX) at low ng/L levels. In Europe, the drinking water directive recommends levels of lower than 0.1 µg/L for individual PFAS, and 0.5 µg/L for total PFAS, while several member countries have guidelines for PFAS in the ng/L range in drinking water. PFOS and its salts have been listed as priority pollutants to be phased out from use under the Stockholm Convention. With PFOA and PFOS banned or in the process of being phased out by manufacturers globally, alternative compounds are being used resulting in "emerging" classes of PFAS now being detected in the environment.

The measurement of these compounds at ng/L levels is quite challenging. Therefore, the need for standard methods to measure them in the environment is critical for establishing baselines and future regulatory decisions. In 2009, the US EPA established EPA Method 537 for the quantification of 14 PFAS in drinking water, using solid-phase extraction (SPE) and liquid chromatography (LC) coupled with tandem mass spectrometry (MS/MS) (3). In late 2018, the US EPA revised this method (EPA 537.1) to include four emerging PFAS including hexafluoropropylene oxide dimer acid (HFPO-DA aka GenX), ADONA, 9Cl-PF3ONS, and 11Cl-PF3ONS (components of F-53B; replacement for PFOS) (4).

This article aims to provide a simple SPE procedure for the extraction of PFAS in drinking water analyzed in EPA Method 537, along with an LC–MS/MS method for the analysis of PFAS listed in EPA Method 537.1 to achieve the required low ng/L levels in drinking water.

Experimental

Chemicals: Standards were purchased from Wellington Laboratories, Inc. and calibration standards diluted to a desired concentration in

Table I: PFAS compound optimized transitions and estimated limit of detection on the LC-TQ system

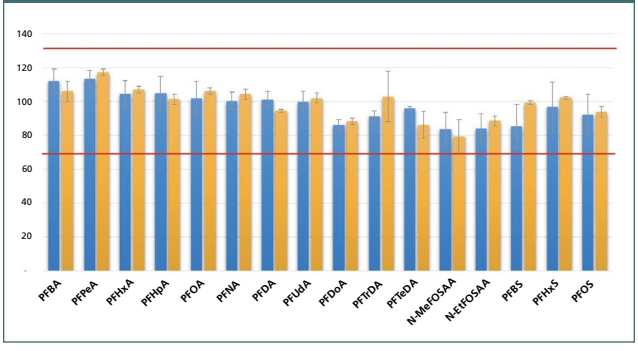
Analyte	RT	Transition	Fragmentor	Collision Energy	Estimated Instrument Level of Detection (pg on column)
PFBA[1]	5.4	213 > 168.9	60	6	0.1
PFPeA[1]	7.3	263 > 218.9	60	6	0.06
PFBS	7.6	298.9 > 80.1 298.9 > 99.1	100	34 22	0.11
PFHxA	8.5	312.9 > 119.1 312.9 > 269	70	14 6	0.7
HFPO-DA	8.9	285.1 > 169	100	0	0.44
PFHpA	9.5	362.9 > 169 362.9 > 319	72	9 0	0.18
PFHxS[2]	9.5	398.9 > 80.1 398.9 > 99.1	100 70	37 34	0.31
ADONA	9.65	377.1 > 251.1 377.1 > 84.9	95	0 30	0.04
PFOA	10.2	412.9 > 169 412.9 > 369	69	13 3	0.08
PFOS[2]	10.8	498.9 > 80.1 498.9 > 99.1	100	38 38	1.30
PFNA	10.9	462.9 > 169 462.9 > 418.9	66	13 3	0.26
9Cl- PF3ONS	11.2	531 > 351.1	90	20	1.35
PFDA	11.4	512.9 > 219 512.9 > 468.9	100 81	3 12	1.51
NMeFOSAA	11.7	570 > 482.9 570 > 418.9	115	15 12	0.47
PFUnA	11.6	562.9 > 219 562.9 > 519	100 73	15 4	1.17
NEtFOSAA	11.9	584 > 525.9 584 > 418.9	115	15 15	1.01
11Cl-P3OUdS	12.0	631 > 451	70	30	1.32
PFDoA	12.2	612.9 > 269 612.9 > 568.9	100 79	15 4	0.50
PFTrDA	12.6	662.9 > 169 662.9 > 618.9	100 91	23 7	0.18
PFTA	12.8	712.9 > 669 712.9 > 169	100	7 23	0.11

[1]Not included in EPA Method 537 or EPA Method 537.1
[2]EPA Method 537.1 requires that the 80 *m/z* product ion must be used to reduce bias between linear and branched isomers

96:4 methanol–water.

Instrumental: Five µL of the standard–sample were introduced for analysis into the LC–MS/MS system. Instrument sensitivity allowed for the reduction of 10 µL cited in the EPA 537 method. LC separation was performed on an Agilent 1260 Infinity II Prime LC system with a 3.0 × 50 mm, 1.8-µm Zorbax Eclipse Plus C18 column (Agilent). A 4.6 × 50 mm, 3.5-µm Zorbax Eclipse Plus C18 delay column (Agilent) was used after the binary pump to separate background PFAS introduced from the solvent, tubing, and the degasser from the desired analytes.

Figure 1: The average spike recoveries of PFAS in ultrapure and finished drinking water using SPE.



The Agilent Jet Stream Technology Ion Source (AJS) was used for maximum ionization. Source parameters were the same as can be seen in reference 5, with the exception of the increase of drying gas flow to 7 L/min. The Agilent Ultivo Triple Quadrupole LC/MS (LC-TQ) was operated in dynamic multiple reaction monitoring (MRM) mode to optimize sensitivity through maximizing dwell time. For most analytes, two transitions were acquired to provide quantitation and qualification ratios. MRM parameters are noted in **Table I**. EPA Method 537.1 now requires the use of 80 mass-to-charge ratio (*m/z*) for PFHxS and PFOS to reduce bias between linear and branched isomers and this was implemented.

Solid-Phase Extraction: Six replicates of 250-mL ultrapure water and finished drinking water samples were spiked at 4 ng/L for each PFAS. The samples were then extracted using a weak anion

Figure 2: Chromatogram of EPA 537.1 analytes with the addition of PFBA and PFPeA.

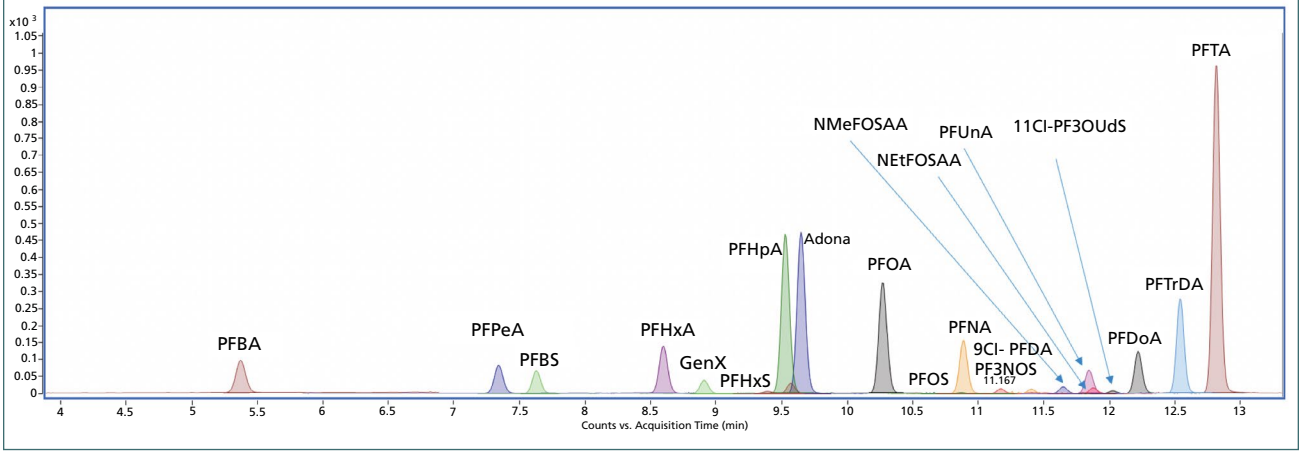
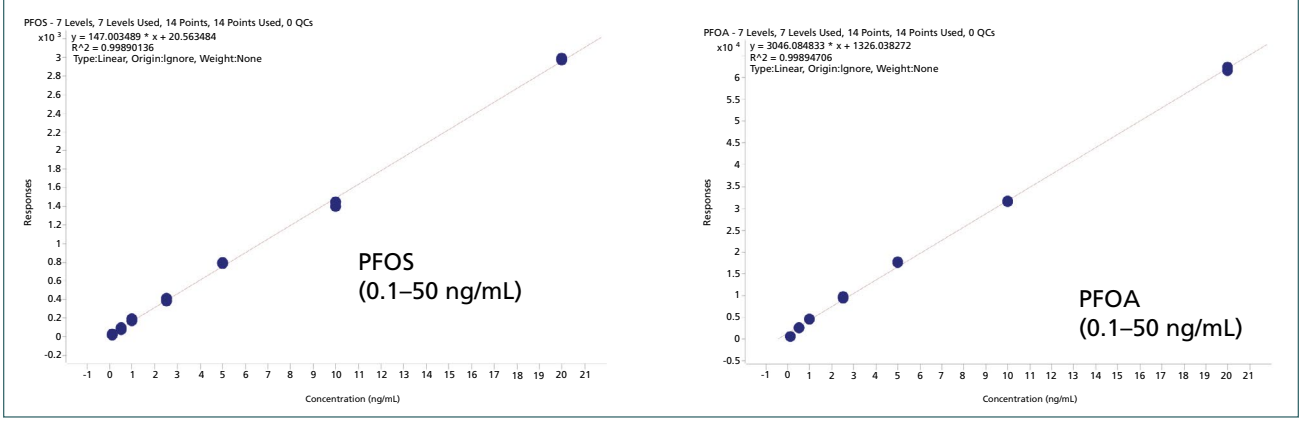


Figure 3: Linear calibration curves for PFOS and PFOS; 7-point calibration curve in duplicate (14 points) from 0.1-50 ppb in the extract.



exchange (WAX, 150 mg, 6 cc) SPE cartridge (Agilent) as in the procedure described in EPA Method 537. Details for the specific SPE procedure can be found in reference 6. The eluate was evaporated to a final volume of 1 mL constituting ~96:4 methanol–water. **Figure 1** shows that the extraction recoveries of all PFAS compounds were 70–130% and ranging from 79–112% in both ultrapure and drinking water. The relative standard deviations (RSDs) for all compounds was <15% too-within acceptable parameters for the EPA method-demonstrating that the cartridge is effective at extracting low-level PFAS from drinking water samples with high efficiency.

Results and Discussion

Background Contamination Elimination: In this study, a delay column was installed in between the pump mixer and the injection port to time resolve any background PFAS coming from the solvents or the tubing of the LC system itself.

Chromatographic Separation and Method Performance: The analysis and separation of the 18 PFAS in EPA Method 537.1 were performed with all analytes achieving good peak shapes and peak widths between 6–10 s.

Figure 2 shows a representative chromatogram of the 14 analytes in EPA Method 537, four of the emerging PFAS (GenX, ADONA, 9Cl-PF3OUdS, and 11Cl-PF3OUdS) added to EPA Method 537.1, and the addition of PFBA and PFPeA. PFBA and PFPeA were added to show the good chromatographic separation and peak shapes of the early PFAS eluters, even though these are not present in the EPA method. The mobile phase was 5-mM ammonium acetate in water and 5-mM ammonium acetate in 95:5 methanol–water, instead of the 20 mM used in the EPA methods. The EPA's method flexibility allows changes in the LC separation. However, the EPA notes that reduced RT stability was observed over time with lower concentrations. Reduced stability at the lower concentration has not been observed so far. The gradient run time was reduced from 37 min in EPA Method 537 to 19.5 min (14-min gradient and a 5.50-min post time).

Figure 4: Raw response deviation for six PFAS in the continuous calibration standards run across a 26-h batch; the number in brackets is the RSD%.

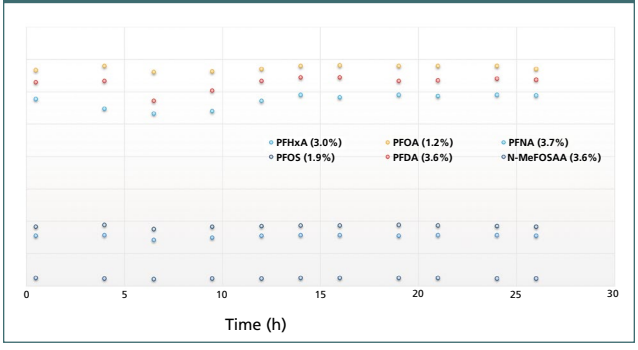


Figure 3 shows representative calibration curves for PFOA and PFOS from 0.1–50 parts per billion (ppb) in the final extract. Calibration curves were linear with R2 > 0.99. Complete details of the analytical method including method optimized parameters and method verification along with linearity, robustness, and analysis of real-world drinking water samples can be found in reference 5.

Robustness and Reproducibility: US EPA Method 537 requires sensitive analysis of PFAS and robustness of the data across samples and batches. For example, the method calls for the injection and analysis of a continuing calibration standard in a batch every 10 samples to monitor system performance and variability. In this study, this method

was evaluated by following the raw response of the PFAS standards run as continuous calibration standards every 10 samples across a batch of samples over a 26-h worklist. The standards were prepared in drinking water extracts at 1 ppb in the vial (~2.5 ng/L in sample equivalent). All PFAS analytes had response variation less than 5%RSD except N-EtFOSAA (5.6%). **Figure 4** illustrates the response stability of the calibration standards across the 26-h batch and shows that the relative response, uncorrected by internal standards (ISs), was stable across the 11 CCV samples analyzed over 26 h.

Conclusions

The analysis of PFAS at extremely low levels in drinking water is required for adequate baseline monitoring and regulatory determination. This article provides a sample extraction protocol for PFAS in the US EPA method that achieves high recoveries in the target matrix, and a robust LC–MS/MS method for excellent separation, low level detection, and reliable and robust quantification of PFAS.

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The Determination of Trace Per- and Polyfluoroalkyl Substances and Their Precursors Migrated into Food Simulants from Food Contact Materials by LC–MS/MS and GC–MS/MS

Dan Li, Zi-hao Zhang, Huai-ning Zhong, Lei Zhu, Jing-jing Pan, Jian-guo Zheng, Qin-bao Lin and Hui Liu

The determination of multiple per- and polyfluoroalkyl substances (PFAS) migrating from food contact material gained in importance as an increasing range of PFAS has been found migrating from food contact material into food. In this study, an integrated analytical approach that combines high performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) and gas chromatography–tandem mass spectrometry (GC–MS/MS) was established for detecting 36 PFAS migrating from food contact materials into four food simulants (3% acetic acid, 10% alcohol, 50% alcohol, and olive oil). The response surface methodology was applied to optimize the selection of solvents in sample treatment. This target analytical method was appropriate for the determination of multiple PFAS, with recoveries ranging from 81.8 to 118.7%. The relative standard deviations (RSDs) ranged from 2.4 to 7.8%, and detection limits were in the range of 0.3 to 10 µg/kg in relevant food simulants.

Per- and polyfluoroalkyl substances (PFAS) are a family of synthetic substances that do not occur naturally in the environment. They are a concern due to their stable physical and chemical properties with strong C-F bonds, including their chemical inertness, thermal stability, high surface activity, and hydrophobic-oleophobic properties (1–3). PFAS are widely used in consumer goods, household products and food contact materials (4–5). In food contact material (FCM), PFAS are mainly used in nonstick cookware, as well as the coatings of paper and other resistant materials, due to their oil- and

water-repellent properties (6–7). Studies have indicated that the migration of PFAS from FCM into food is likely to be the main route of consumer exposure to these substances (8–11).

PFAS have been found to be highly resistant, and could persist in the environment for long periods of time, as well as in human serum, milk, and tissues (12–17). Certain type of PFAS, such as perfluorocarboxylic acids (PFCA) and perfluorosulfonic acids (PFSA), are likely to be toxic and bioaccumulate, posing adverse effects on human health (18–21). Perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) are two of the most studied PFCA and PFSA. There is suggestive evidence from human epidemiology studies that PFOS and PFOA may cause abnormal liver enzymes, antibody response, and cancer (21–23). To reduce the occurrence of PFOS and PFOA, a number of PFAS, such as perfluoro-alcohol and its derivatives, have been used as substitutes to replace PFCA and PFSA in FCM. However, studies indicated that these substitutes could also pose health risks to humans, as PFSA and PFCA precursors are more toxic than the PFSA or PFCA themselves (24). In addition, these precursors could be absorbed into the human body, and degrade into PFOA and PFOS by an oxidation mechanism (25). For example, 8:2FTOH (flurotelomer alcohol) and 10:2FTOH could be converted into PFOA, and PFDA, and N-methyl perfluorooctane sulfonamido ethanol (N-MeFOSE) and N-ethyl perfluorooctane sulfonamido ethanol (N-EtFOSE) could be capable of conversion into PFOS. Therefore, besides known harmful PFAS, the migration of PFAS precursors from food contact material must also be taken into account to ensure its safety.

To protect consumers from exposure to PFAS migrated from FCM, stringent regulatory approaches have been adopted to control their production, application, and migration. The Danish Food and Veterinary Administration set a recommended limit for the total organic fluorine content in paper and cardboard at 0.35 µg per square decimeter of paper. The U.S. Food and Drug Administration (FDA) finalized an amendment to regulations that certain type of PFAS were not permitted as additives used in the manufacture of FCM (26). In China, the National Food Safety

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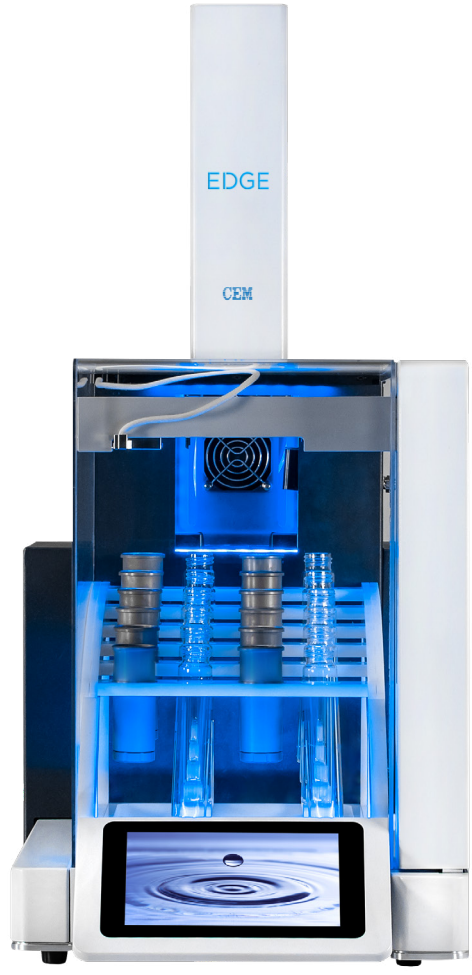

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Standard GB 9685, which is the regulation for the use of additives in FCM, added an amendment in 2016 that no longer authorized PFAS as additives in the manufacture of FCM (27). In 2017, The European Chemicals Agency (ECHA) added seven types of PFCAs and PFSAs to the Substances of Very High Concern (SVHC) list (28), which attracted much attention concerning the occurrence of PFAS, and their effects on the environment and human health.

Comparable results for measurement of PFAS migration from FCM is crucial for official control purposes. To achieve this objective, the guidance for choice of food simulants and migration test conditions for plastics have been provided in relevant regulations (29), which define the food simulants that should be used to mimic a specific foodstuff, and what standardized conditions of time, temperature, and contact should be performed. Various analytical approaches have been investigated for measuring the migration of PFAS from FCM (30–35). Gas chromatography–mass spectrometry (GC–MS) is usually used for detecting volatile fluorine-containing compounds, such as perfluoroalcohols and perfluoroalcohol acrylates. Liquid chromatography–tandem mass spectrometry (LC–MS/MS), a highly sensitive and selective tool, was applied to detect more than 10 PFAS that mainly refer to straight-chain perfluorinated carboxylic acids or perfluorinated sulfonic acids. However, an analytical approach that is suitable for determination of multiple PFAS to detect migration from food contact materials has not been well established, especially for the increasing range of precursors, including PFCAs and PFSAs. In addition, the previous studies mainly focused on measuring the residue of PFAS in different matrices of FCM, such as extracts of paper. Few studies have been carried out on detecting migration of PFAS by using conventional simulants that represent the specific foodstuff.

To meet official control purposes, this present study aims to establish an effective method for simultaneously measuring multiple PFAS migrated from FCM, and possibly containing both PFAS and their precursors, including fluorinated carboxylic acid, hydrogen-substituted fluorinated carboxylic acid, and hydrogen-substituted fluorinated alkyl acid amides. To achieve this objective, the optimization of sample treatment was carried out by using a response surface methodology. An integrated analytical approach of combining high performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) and gas chromatography–tandem mass spectrometry (GC–MS/MS) was established in four conventional food simulants (3% acetic acid, 10%

alcohol, 50% alcohol, and olive oil) that are considered to represent specific foodstuffs.

Experimental

Reagents and Materials

Ultrapure water was purified using a Milli-Q system (Millipore, Milford, Massachusetts). Alcohol and methanol (HPLC-grade) were purchased from TEDIA Company. Ammonium acetate and acetic acid were purchased from Guangzhou Chemical Reagent Factory (Guangzhou, China), and olive oil was purchased from Sinopharm (Shanghai, China).

A total of 36 PFAS standards (**Table I**) were purchased from Wellington Laboratories (Guelph, Ontario, Canada), Dr. Ehrenstorfer Company (Augsburg, Germany), Chiron Company (Trondheim, Norway), and TRC Company (North York, Ontario, Canada). An intermediate standard solution containing 27 PFAS (0.1 µg/mL, Group 1) was prepared by dissolving the commercial standards in methanol. An intermediate standard solution containing nine PFAS standards (1.0 µg/mL, Group 2) was prepared by dissolving the commercial standards in dichloromethane.

The standard working solutions used for LC–MS/MS analysis were prepared by transferring the intermediate standard (1.0 µg/mL), containing 27 PFAS (Group 1), into a 10 mL volumetric flask, spiked with 50 µL (1.0 µg/mL) 1,2,3,4-$^{13}C_4$ perfluorooctanoic acid (MPFOA) of internal standard, and then made up to 10 mL with the food simulants (10% [v/v] ethanol, 3% [w/v] acetic acid, and 50% [v/v] ethanol), respectively. The standard working solutions used for GC–MS/MS analysis were prepared by transferring the intermediate standard (1.0 µg/mL) containing nine PFAS (Group 2) into a 10 mL volumetric flask, spiked with 50 µL (0.2 µg/mL) methyl margarate-d33 of internal standard, and then made up to 10 mL with the food simulants (10% [v/v] ethanol, 3% [w/v] acetic acid, and 50% [v/v] ethanol), respectively. Then, 2 mL dichloromethane was added, vortexed for 5 min, allowed to separate, and the lower solvent layer was collected for further analysis.

The standard working solutions for the food simulant (olive oil) were prepared by transferring the intermediate standard of Group 1 (0.1 µg/mL) and Group 2 (1.0 µg/mL) into 10 g olive oil, spiked with internal standard of 10 µL MPFOA (1.0 µg/mL) and 50 µL (0.2 µg/mL) methyl margarate-d33. Then, 2 mL acetonitrile was added, vortexed for 5 min to allow stratification, and the upper solvent layer was taken for further analysis.

Equipment

An LC–triple quadrupole mass spectrometer and a GC–triple quadrupole mass spectrometer (6410

Table I: Optimized parameters of GC–EI-MS/MS and LC–ESI-MS/MS for 36 PFAS standards

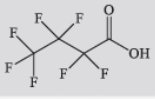
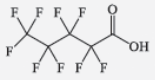
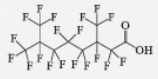
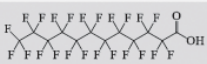
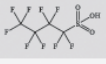
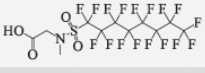
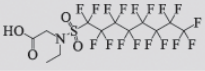
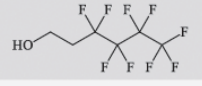
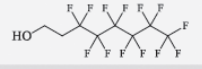
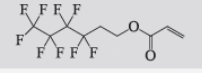
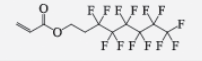
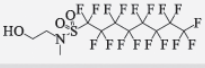
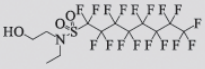
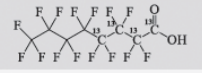
No	Compound (Abbreviation)	CAS Number	Structure	Retention Time (min)	Precursor Ion (m/z)	Product Ion (m/z)	Fragmentor (V)	Collision Energy (eV)
Group 1								
1	Perfluorobutyric acid (PFBA)	375-22-4		12.36	213.0	168.9	60	1
2	Perfluoropentanoic acid (PFPeA)	2706-90-3		13.18	262.9	218.9, 69.1	61	1, 47
3	Perfluorohexanoic acid (PFHxA)	307-24-4		13.94	313.0	269.0, 118.9	67, 68	5, 20
4	Perfluoroheptanoic acid (PFHpA)	375-85-9		14.76	362.8	319.0, 168.9	75, 75	5, 15
5	7H-Dodecafluorohep-tanoic acid(HPFHpA)	1546-95-8		14.97	344.9	280.9, 131.1	70, 70	5,25
6	Perfluorooctanoic acid (PFOA)	335-67-1		15.76	413.0	369.0, 168.9	90, 90	5, 15
7	Perfluorononanoic acid (PFNA)	375-95-1		16.71	462.8	419.0, 218.9	106, 106	5, 10
8	Perfluorodecanoic acid (PFDA)	335-76-2		17.69	512.8	468.9, 218.9	90, 90	10, 15
9	2H,2H-Perfluorodeca-noic acid (H2PFDA)	27854-31-5		17.59	476.9	393.0, 243.0	90, 90,	20, 25
10	Perfluoro-3-7-dimethyl octane carboxylate (PF-3,7-DMOA)	172155-07-6		17.69	512.8	218.9, 168.9	88, 88	20, 33
11	Perfluoroundeca-noic acid (PFUnA)	2058-94-8		18.74	562.9	518.9, 268.9	90, 90	5, 15
12	2H,2H,3H,3H-Per-fluoroundecanoic acid (H4PFUnA)	34598-33-9		20.76	490.7	366.9, 386.9	96, 96	23, 11
13	Perfluorododeca-noic acid (PFDoA)	307-55-1		19.74	613.0	569.1, 168.9	96, 96	4, 24
14	Perfluorotridecanoic acid (PFTRIDA)	72629-94-8		20.69	663.1	619.0, 168.9	101, 101	8, 28
15	Perfluorotetradeca-noic acid (PFTEDA)	376-06-7		21.60	713.0	669.1, 168.9	114, 114	8, 24
16	Perfluorohexadeca-noic acid (PFHeDA)	67905-19-5		23.27	813.0	768.9, 168.9	100, 100	5, 26
17	Perfluorobutanesul-fonic acid (PFBS)	375-73-5		13.28	299.1	80.0, 99.0	120, 120	35, 35
18	Perfluorohexanesul-fonic acid (PFHxS)	355-46-4		14.77	398.8	80.0, 99.0	161, 161	48, 36
19	Perfluoroheptanesul-fonic acid (PFHpS)	375-92-8		15.74	448.9	80.0, 99.0	114, 114	61, 56

Table I (cont'd): Optimized parameters of GC–EI-MS/MS and LC–ESI-MS/MS for 36 PFAS standards

No	Compound (Abbreviation)	CAS Number	Structure	Retention Time (min)	Precursor Ion (m/z)	Product Ion (m/z)	Fragmentor (V)	Collision Energy (eV)
20	Perfluorooctanesulfonic acid (PFOS)	1763-23-1		16.65	498.8	80.0, 99.0	120, 120	60, 60
21	1H,1H,2H,2H-Perfluorooctanesulphonic acid (H4PFOS6:2)	27619-97-2		15.71	426.8	407.0, 81.1	124, 124	43, 5
22	Perfluorodecanesulfonic acid (PFDS)	335-77-3		18.56	598.8	80.0, 99.0	195, 195	80, 60
23	Perfluorooctanesulfonamide (PFOSA)	754-91-6		20.14	497.9	77.9, 147.9	161, 161	49, 29
24	N-Methyl-Perfluorooctanesulfonamide (N-MeFOSA-M)	31506-32-8		21.67	511.8	168.9, 218.9	116, 116	31, 29
25	N-Ethyl-Perfluorooctanesulfonamide (N-EtFOSA-M)	4151-50-2		22.32	526.1	168.9, 219.1	124, 124	31, 28
26	N-Methyl-Perfluorooctanesulfonamido acetic acid (N-MeFOSAA)	n.a.		18.75	570.2	511.9, 418.9	135, 135	24, 28
27	N-Ethyl-Perfluorooctanesulfonamidoacetic acid (N-MeFOSAA)	n.a.		19.42	584.2	525.9, 418.9	135, 135	23, 25
Group 2								
28	1H,1H,2H,2H-Perfluoro-1-hexanol (4:2FTOH)	2043-47-2		1.78	196.0, 94.8	50.9, 69.0	/	25, 25
29	1H,1H,2H,2H-Perfluoro-1-octanol (6:2FTOH)	647-42-7		2.46	94.9, 131.0	69.0, 69.0	/	23, 27
30	1H,1H,2H,2H-Perfluoro-1-decanol (8:2FTOH)	678-39-7		3.69	94.9, 131.0	69.0, 69.0	/	25, 25
31	1H,1H,2H,2H-Perfluoro-1-dodecanol (10:2FTOH)	865-86-1		4.78	94.9, 131.0	69.0, 69.0	/	26, 25
32	1H,1H,2H,2H-Perfluorooctylacrylate (6:2FTA)	27619-97-2		4.40	55.1, 131.0	27.2, 69.0	/	15, 30
33	1H,1H,2H,2H-Perfluorodecylacrylate (8:2FTA)	17527-29-6		5.34	55.1, 131.0	27.2, 69.0	/	5, 20
34	1H,1H,2H,2H-Perfluorodecylacrylate (10:2 FTA)	27905-45-9		6.05	55.1, 131.0	27.2, 69.0	/	60, 45
35	N-Methyl-Perfluorooctanesulfonamidoethanol (N-MeFOSE)	24448-09-7		7.29	130.9, 526.0	69.0, 169.0	/	25
36	N-Ethyl-Perfluorooctanesulfonamidoethanol (N-EtFOSE)	1691-99-2		7.53	130.9,540.0	69.0, 169.0	/	25
Internal Standard								
37	1,2,3,4-13C4- Perfluorooctanoic acid(MPFOA)	n.a.		15.76	417.0	371.9, 168.9	95	5, 15
38	Methyl Margarate-d33	1219804-81-5		11.28	155.0	107.0, 78.9, 62.0	/	10, 15, 20

Triple Quad LC–MS, 7000c Triple Quad GC–MS, Agilent Technologies, Palo Alto, California) equipped with automatic injectors, were employed for the identification and quantification of PFAS.

The LC column employed was a 15 cm Poroshell120 EC-C18 column (2.7-µm particle diameter and 2.1-mm i.d., Agilent Technologies), while the GC column employed was 30 m DB-5 column (0.25-µm particle diameter and 0.25-mm i.d., Agilent Technologies). The LC mobile phase consisted of water (solvent A) and HPLC grade methanol (solvent B). The gradient elution procedure was as follows: 0–3 min, 10% B; 3–4 min, 20% B; 4–5 min, 45% B; 5–11 min, 70% B; 11–18 min, 85% B; 18–19 min, 100% B; 19–20 min, 75% B; 20–21 min, 50% B; 21–24 min, 20% B; 24 min, 10% B. The injection volume was 5 µL, and the flow rate was 0.2 mL/min. ESI was used in negative mode with the following conditions: spray ion voltage, 4000 V; nebulizer, 20 psi; gas flow, 8 L/min; and capillary temperature, 350 °C. The GC temperature program was as follows: 75 °C for 3 min, 30 °C/min to 250 °C for 0 min, 50 °C/min to 300 °C for 6 min. The injection volume was 2 µL and pulsed splitless. The gas flow was 1.5 mL/min, with an electron ionization (EI) source and multiple reaction monitoring (MRM) mode. All of the ionization parameters and collision cell parameters were optimized for each analyte (**Table I**).

Migration Experimental

Migration experiments were carried out in accordance with European Union (EU) regulation 10/2011 for plastic materials and articles intended to be in contact with food (29).

Each sample was exposed to the food simulants with a ratio of contact area-to-volume at 1000 mL:6 dm^2, and then placed in an incubator at 70 °C. The food simulants were collected from each sample at 2 h, followed by cooling at room temperature, and then further analysis.

Instrumental Analysis

The majority of PFAS being investigated are fatty acid compounds that can ionize protons, and negatively charged parent ions in aqueous solution. For this reason, LC–MS/MS was used to detect 27 of this type of PFAS (Group 1). For the rest of the nine PFAS being investigated (Group 2),

these are generally considered volatile compounds in which perfluoroalcohols and perfluoroalcohol acrylates would not easily be ionized in aqueous solutions; these compounds are more susceptible to ionization in an EI source than in an ESI source, therefore GC–MS/MS was preferred for their analysis.

The PFAS analytes were extracted from the relevant food simulant collected following an exposure step. For the determination of 27 PFAS (Group 1), the food simulants (10% ethanol, 3% acetic acid, and 50% ethanol) were transferred into a 10 mL volumetric flask, and filtered by a 0.22 µm syringe filter, followed by direct LC–MS/MS analysis.

The aqueous simulants containing nine PFAS (Group 2) were extracted with dichloromethane, and a volume of 10 mL of aqueous food simulants was transferred into the headspace bottle, with 50 µL (0.2 µg/mL) methyl margarate-d33 added as the internal standard. Dichloromethane was then added, and vortexed for stratification. The bottom layer was filtered, and used for GC–MS/MS analysis.

For treatment of the olive oil, 10 g of olive oil was transferred into the headspace bottle, and 10 µL (1.0 µg/mL) MPFOA and 50 µL (0.2 µg/mL) methyl margarate-d33 were added as the internal standards. After acetonitrile was added and vortexed for stratification, the supernatant liquor was filtered and analyzed by LC–MS/MS and GC–MS/MS.

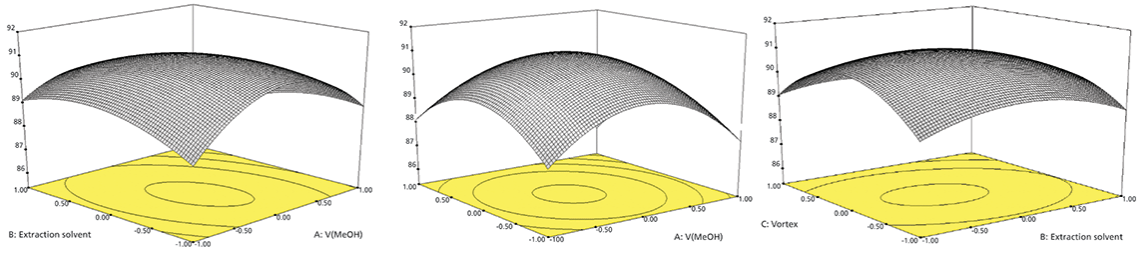
Results and Discussion

The Choice of Mobile Phase

A mixture of 27 PFAS comprising a standard at a concentration of 100 ng/mL was used to check the mass spectrometric response and separation power of four different mobile phases, including methanol–water solution, methanol–10 mmol/L ammonium acetate solution (pH = 7), methanol–(containing 0.1% ammonia) water solution, and acetonitrile–10 mmol/L ammonium acetate solution.

The results indicated that the weakest MS signal for target analytes was observed for acetonitrile–10 mmol/L ammonium acetate solution, and the strongest MS signal was observed for methanol–aqueous solution (containing 0.1% ammonia). This is likely due to the MS signal of PFAS analytes being related to the formation of the negative parent ion of PFAS, and the degree of ionization of the proton as well. Methanol allows analytes to generate target ions more easily, and be atomized on the electrospray ionization process, while acetonitrile may decrease the ionization of analytes. The poor peak shapes and baseline disturbances were observed for certain analytes, such as PFBA and PFPeA when methanol–water

Figure 1: The recovery for PFNA shown in 3D plots of response surface and contour map, where the z-axis is the recovery rate for all figures: (a) acetonitrile concentration (A, %) versus volume of extraction solvent (B, mL); (b) acetonitrile concentration (A, %) versus vortex time (C, min); and (c) extraction solvent (B, mL) versus the vortex time (C, min).



(a) (b) (c)

(containing 0.1% ammonia) was applied. Therefore, methanol–water (containing 0.1% ammonia) was not chosen as the mobile phase for further experiments.

Either methanol–water solution or methanol–10 mmol/L ammonium acetate solution was considered satisfactory as a suitable mobile phase, given that a similar ion peak response was obtained, and good chromatographic peak shapes were observed. It can be explained that the ionization of a proton occurs more often in a neutral or weak alkaline mobile phase, leading to an increase of formation of parent ions. Considering the methanol–water solution has the advantage of making maintenance of instrument pipelines simpler and more effective, the methanol–water solution was preferred for further experiments.

The Choice of Chromatography Column

Three different LC columns, including Agilent Zorbax SB-C18 (150 mm × 4.6 mm, 5-µm), Poroshell 120 EC-C18 (150 mm × 3.0 mm, 2.7-µm), and Eclipse XDB C-18 (150 mm × 4.6 mm, 3.5-µm), were compared in the separation of a mixture of standards (27 PFAS), at a concentration solution of 100 ng/mL.

The results indicated that all three columns could effectively separate the target analytes, particularly n-alkane PFAS. However, due to the occurrence of a high number of target PFAS analytes, the chromatographic peaks of various analytes may overlap each other, causing difficulty in further analysis. The Poroshell 120 EC-C18 was shown to be able to completely separate 27 PFAS analytes in 25 min with the same gradient elution program. Therefore, the Poroshell 120 EC-C18 was chosen as the stationary phase for further experiments.

For the selection of a GC column,

baseline separations could be achieved for nine target PFAS using a low or medium polarity column. Therefore, the DB-5, DB-17, or DB-35 column could be chosen to perform the analysis.

The MRM chromatogram of 36 PFAS compounds is shown in **Figures 3 and 4**.

The Choice of Extraction Solvent for Olive Oil

Olive oil, as a fatty simulant, cannot be directly analyzed due to interference from compounds it contains. Therefore, it must be converted into a suitable solution before analysis. To achieve the objective, a recovery experiment was conducted in an olive oil blank spiked with 0.1 mL PFAS standards (10 µg/mL), aimed at selecting a suitable solvent to extract the PFAS from the olive oil. Four different solvents-methanol, acetonitrile:water (1:1), acetonitrile:water (3:1), and acetonitrile-with serial extraction volumes (1 mL, 1.5 mL, 2 mL, 2.5 mL, 3 mL), were investigated. The recovery rate for each solvent is indicated in **Figure 2a**.

The results indicated that acetonitrile:water (3:1) and acetonitrile had the highest extraction efficiency, and a slightly higher recovery rate was obtained for acetonitrile compared with that of acetonitrile:water (3:1). One of the reasons might be that the PFAS being investigated are long-chain aliphatic compounds, and the pure

Table II: F-values of PFNA by Box-Behnken central composite design

Source	Model	A	B	C	AB	AC	BC	A^2	B^2	C^2
F-value	4.21	22.78	0.82	10.29	0.34	0.05	0.58	2.71	0.07	0.10

organic phase is more favorable for the extraction of the target analytes. For methanol, the separation of the methanol and olive oil layers was not clearly observed after mixing because of the emulsification effect, although the fluorinated fatty alcohol and some perfluorinated carboxylic acids had the highest recovery rates. In the case of acetonitrile:water (1:1) solvent, delamination was not clear, and the recovery rate was low, therefore acetonitrile:water (1:1) was not suitable for GC–MS analysis. Given the test results indicted, acetonitrile was chosen as the solvent to extract the PFAS from the olive oil.

The extraction conditions of PFAS in olive oil were optimized by applying a single-factor experiment derived from the response surface methodology. PFNA was taken as the sample to be investigated, and the acetonitrile concentration (A, %), volume of extraction solvent (B, mL), and the vortex time (C, min) were assigned as independent variables. The recovery rate of PFAS were taken as the response value based on the principle of the Box-Behnken central composite design. A secondary polynomial regression model was obtained

from 15 experiments with three factors and three levels: recovery = 72.46 + 7.77 × A + 1.47 × B + 5.23 × C - 1.35 × A × B + 0.50 × A × C + 1.75 × B × C + 3.70 × A^2 + 0.60 × B^2 + 0.69 × C^2. The results of the recovery of PFNA obtained by the response surface methodology are shown in **Table II** and **Figure 1**.

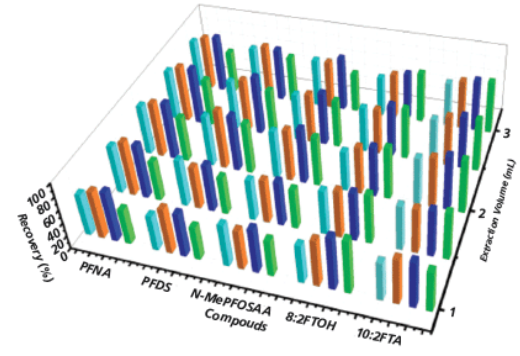
It can be found from the F-value (**Table II**) that the sequence of influence of a single factor on the recovery rate was A > C > B, suggesting that the influence of A^2 was significant in the quadratic term, while B^2 and C^2 were not. Thus, 100% acetonitrile was considered an appropriate extraction solution. The highest extraction efficiency was observed when the extraction time was 5 min, and the extraction volume was 2 mL.

The Choice of Extraction Solvent for Aqueous Simulants

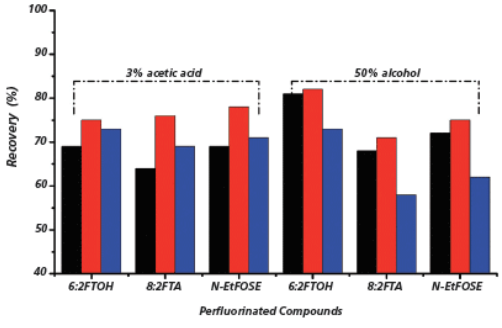
In this study, GC–MS was employed for detecting fluorinated fatty alcohols, fluorinated fatty alcohol acrylates, and fluoro-sulfonamide fatty alcohols in the extraction of aqueous simulants. A comparison experiment was conducted on two aqueous simulants (3% acetic acid and 50% ethanol) to check the extraction efficiency of the solvents n-hexane, dichloromethane, and ethyl ether:n-heptane (1:1).

The results showed that all selected solvents were well able to extract the analytes of the aqueous simulants.

Figure 2: Extraction efficiency by recovery rate of solvents: (a) recovery rate (%) of olive oil, and (b) recovery rate (%) of aqueous simulants.



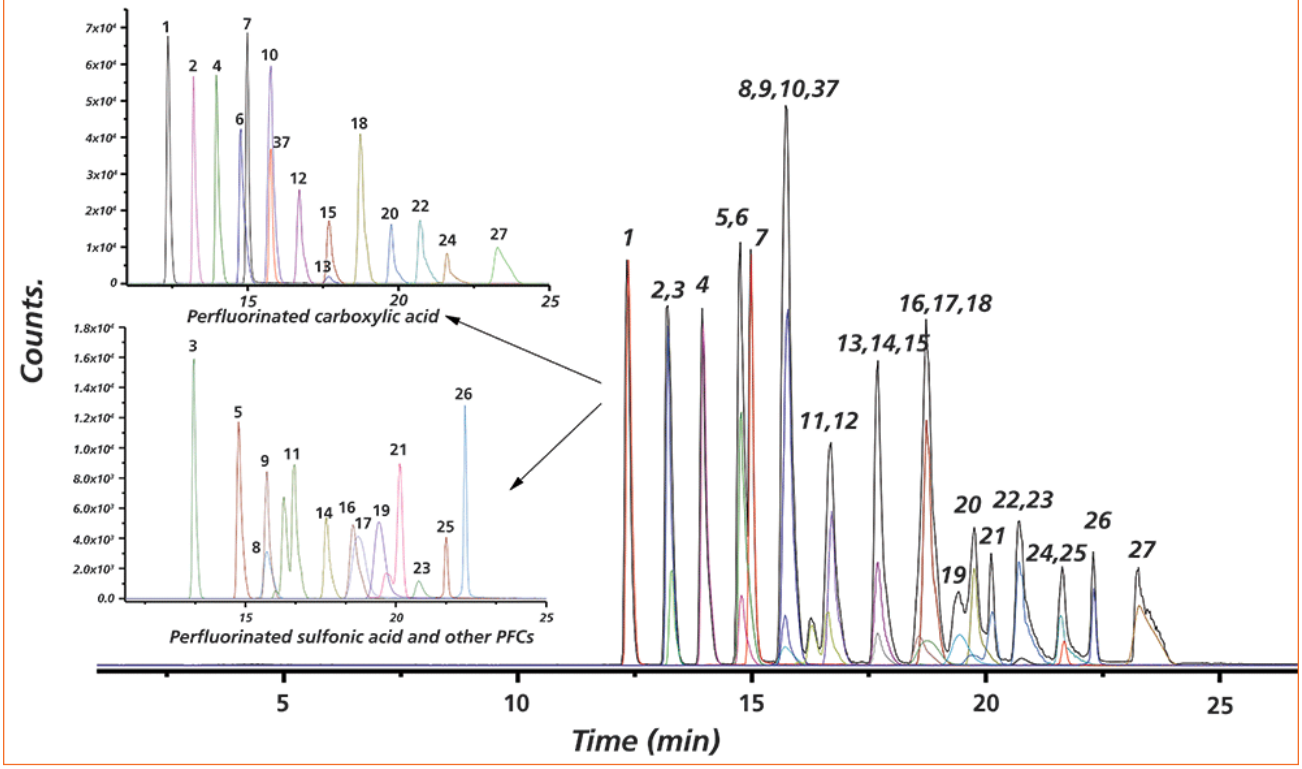
(a) Recovery rate of olive oil



(b) Recovery rate of aqueous simulants

However, n-hexane was not a suitable solvent to extract fluorinated fatty alcohols, due to its weak polarity. A mix of solvents consisting of ethyl ether and n-heptane did not demonstrate sufficient extraction efficiency. In addition, ethyl ether may cause a hazard to the analyst during vortexing at high pressures. The data indicated that the highest recovery rate was obtained when dichloromethane was applied for measuring fluorinated fatty alcohols and fluorinated fatty alcohol acrylates, which may be due to its medium polarity and

Figure 3: The MRM chromatogram of 27 PFAS compounds by HPLC–MS/MS (0.02 mg/kg)(1: PFBA, 2: PFPeA, 3: PFBS, 4: PFHxA, 5: PFHxS, 6: PFHePA, 7: HPFhpA, 8: H4PFOS 6:2, 9: PFHpS, 10: PFOA, 11: PFOS, 12: PFNA, 13: H2PFDA, 14: PF-3,7-DMOA, 15: PFDA, 16: PFDS, 17: N-MeFOSAA, 18: PFUnA, 19: N-EtFOSAA, 20: PFDoA, 21: PFOSA, 22: PFTRIDA, 23: H4PFUnA, 24: PFTEDA, 25: N-MeFOSA-M, 26: N-EtFOSA-M, 27:P FHeDA, 37: MPFOA).

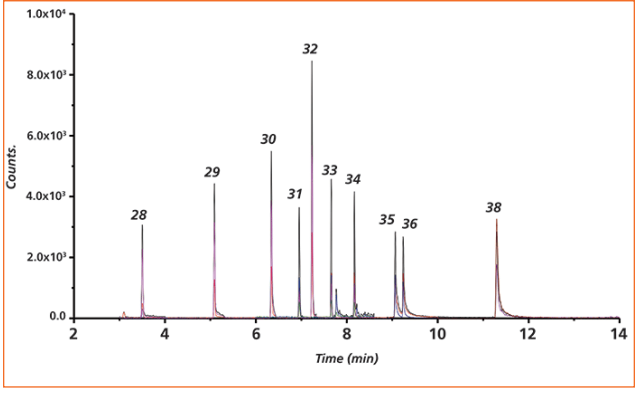


strong density that is lower than water-based solutions. Thus, dichloromethane was chosen as the extraction solvent. **Figure 2b** shows the extraction efficiency for three different solvents by measured recovery rate. The highest extraction efficiency was achieved with a 5 min extraction time with a 2 mL extraction volume.

Optimization of Mass Spectrometry

The mass spectrometry methods for 27 PFAS in Group 1 and 9 PFAS in Group 2 were optimized to directly detect each PFC (1 mg/L) in methanol. Standard solutions were scanned by performing a first-stage mass spectrometry full scan in negative ion ESI mode with corresponding transmission voltages. The results showed that the strongest peaks of the perfluorocarboxylic acid, hydrogen substituted fluorocarboxylic acid, or perfluorosulfonamide compounds were the quasi-molecular

Figure 4: The MRM chromatogram of nine perfluorinated alcohols or esters by GC–MS/MS (0.01 mg/kg) (28: 4:2FTOH, 29: 6:2FTOH, 30: 8:2FTOH, 31: 6:2FTA, 32: 10:2FTOH, 33: 8:2FTA, 34: 10:2FTA, 35: N-MeFOSE, 36: N-EtFOSE, 38: methyl margarate-d33).



ion peaks $[M-1]^-$ or the molecular ion peaks following the loss of the carboxyl group $[M-45]^-$. The strongest signal of the perfluorosulfonic acid or hydrogen substituted perfluorosulfonic acid compounds observed were quasi-molecular ion peaks $[M-1]^-$ and sulfonic acid group molecular ion peaks $[SO_3]^-$. Nine types of PFAS from Group 2 were scanned by conducting a first-stage mass spectrometry full scan under the positive EI mode. The results indicated that the fluorinated fatty alcohols and fluorinated fatty alcohol acrylates formed smaller fragmentary ion peaks $[C_3H_5F_2O]^+$ (M = 95), $(C_3H_3O)^+$ (M = 55) or $[C_3H_3F_4O]^+$ (M = 131), which are shown in **Figures 3 and 4**.

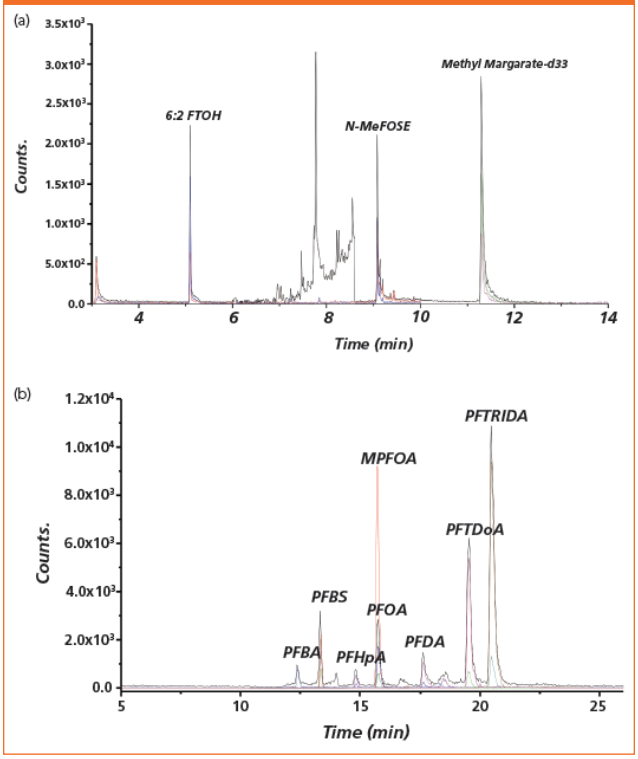
Ion beam scanning was performed with a collision energy of 0–60 eV targeting each quasi-molecular ion to obtain the daughter ion information and the collision energy values that were suitable for the daughter ion response sizes. Daughter ions of perfluorocarboxylic acid compounds are a series of molecular ions following the loss of the carboxyl group, and there are several CF_2 fragments between each molecule, including the ionic fragment of $[C_3F_7]^-$, $[C_4F_9]^-$, $[C_5F_{11}]^-$, having a general structural formula of $[C_nF_{2n+1}]^-$. The ions of the perfluorosulfonic acid compounds were those molecular ions that lost several $[C_nF_{2n+1}]^-$ saturated carbon chain groups and had sulfonic acid functional groups, such as $[C_nF_{2n}SO_3]^-$, $[SO_3]^-$, and $[FSO_3]$– ions. Both the fluorinated fatty alcohols and fluorinated fatty alcohol acrylates contained the ion-pair $[C_3H_3F_4O]^+$ (M = 131) to $[CF_3]^+$ (M = 69). Therefore, the fragment ions with the largest response and least interference were chosen as the quantitative and qualitative ions. To obtain better sensitivities, the parameters including the selection of daughter ions, fragmentation voltage, and collision energy were optimized (see **Table I**).

Quantification and Validation

Linear Range and Quantitative Limit

Using the method described above, the 36 PFAS compounds were analyzed in

Figure 5: The chromatograph profiles of a coated cardboard using (a) GC–MS/MS and (b) LC–MS/MS.



the multiple reaction monitoring mode (MRM). The concentration and peak area of each compound was taken as the abscissa and ordinate, and a standard curve was drawn. The linear range of the recovered curve was between 1 and 1000 ng/mL, and the limits of quantification (10 S/N) were 0.35–9.72 µg/kg in 3% acetic acid, 0.29–8.43 µg/kg in 10% alcohol, 0.28–8.73 µg/kg in 50% alcohol, and 0.43–9.86 µg/kg in olive oil.

Recovery and Precision

An experiment was carried out where samples were spiked with 10 and 100 ng standards in 3% acetic acid, 10% alcohol, 50% alcohol, and olive oil, respectively. The recovery rates for the four different food simulants ranged from 81.8 to 118.7%, and the relative standard deviations (RSDs) ranged from 2.4 to 7.8%.

Analysis of Real Samples

A total of 70 food contact materials were collected from local supermarkets, which include coated paper board, multilayer paper packaging, multilayer plastic food packaging, heat-resistant rubber articles, and coated heat-resistant metallic containers. The novel analytical approach was applied to perform the analysis. Some specific PFAS compounds were found in paper samples with concentrations of 0.01 mg/kg to 0.05 mg/kg of food simulants, which accounted for 5% of the total number of samples analyzed. Multilayer cardboard and coated paper board were the main types of packaging in which PFAS compounds were found with average concentrations of 0.02 mg/kg. The highest migration of PFAS was 0.08 mg/kg. The type of PFAS compounds found were mainly straight-chain perfluorinated acids and perfluorinated alcohol compounds. The chromatogram profiles of the PFAS in typical samples are shown in **Figure 5**.

Conclusion

A target approach was established for the determination of 36 PFAS migrating from food contact material into food

simulants of 3% acetic acid, 10% alcohol, 50% alcohol, and olive oil. A total of 36 PFAS were divided into two groups, and measured by LC–MS/MS and GC–MS/MS. LC–MS/MS was used to detect 27 fatty acid PFAS, while 9 PFAS with volatile properties, including fluorinated fatty alcohols and fluorinated fatty alcohol acrylates were measured by GC–MS/MS. The response surface methodology was a useful tool to simplify the selection of solvents with optimized conditions. This integrated analytical approach was appropriate for the determination of multiple PFAS with recovery rates that ranged from 81.8 to 118.7%, the relative standard deviation (RSD) ranged from 2.4 to 7.8%, and the detection limits ranged from 0.35 to 9.72 µg/kg in 3% acetic acid, 0.29 to 8.43 µg/kg in 10% alcohol, 0.28 to 8.73 µg/kg in 50% alcohol, and 0.43 to 9.86 µg/kg in olive oil. This target approach had the advantage of simultaneously measuring the migration of multiple PFAS from food contact materials with satisfactory sensitivity, accuracy, and reliability. The test results showed that PFAS were found in coated paper and board at mg/kg levels, suggesting that further investigation is needed for the migration of PFAS from coated paper and board.

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