Application Note

Food

Determination of 30 PFAS in Fish Oil, Coffee Powder, and Protein Powder

Using the Agilent Captiva EMR PFAS Food II passthrough cleanup and LC/MS/MS detection

Authors

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Abstract

This application note presents the development and validation of a multiresidue method for the analysis of 30 per- and polyfluoroalkyl substances (PFAS) residues in fish oil, coffee powder, and protein powder. The method employs QuEChERS extraction followed by enhanced matrix removal (EMR) mixed-mode passthrough cleanup using the Agilent Captiva EMR PFAS Food II cartridges, and subsequent liquid chromatography/triple quadrupole mass spectrometry (LC/TQ) detection. Key features include streamlined and efficient sample preparation, direct injection using Agilent 1260 Infinity II hybrid multisampler, sensitive Agilent 6495D LC/TQ detection, and reliable quantitation using neat standard calibration curves. The method was validated according to AOAC Standard Method Performance Requirements (SMPR) 2023.003 guideline, assessing suitability, sensitivity, accuracy, and precision. It successfully met the required limits of quantitation (LOQs), recovery, and repeatability (RSD) for all 30 PFAS targets in all three food matrices.

Introduction

Determination of PFAS residues in food has become an increasingly important concern in recent years. In April 2023, the European Commission implemented regulations for four PFAS compounds; PFOS, PFOA, PFNA, and PFHxS. These regulations are for various food categories including eggs, fish, seafood, meat, and offal.[1] In November 2023, AOAC released SMPR 2023.003, establishing performance requirements for the analysis of 30 PFAS compounds across 11 food categories, including produce, coffee, milk, dairy powder and plant-based protein powders, eggs, seafood, fish meat and meat of terrestrial animals, edible offal of terrestrial animals, fish oil, foods for infants and young children, and pet food and animal feed.[2]

QuEChERS extraction followed by EMR mixed-mode passthrough cleanup using Captiva EMR PFAS Food I and II cartridges has demonstrated a streamlined, efficient, and reliable sample preparation protocol. This protocol delivers excellent quantitation performance across eight food matrix categories in compliance with EU and AOAC SMPR requirements.[3-8] This study extends the method applicability to the remaining three food categories: fish oil, coffee, and plant-based protein powder, thereby completing the demonstration of the workflow for PFAS analysis across all food categories specified by AOAC SMPR 2023.003.

Additionally, the instrument method was improved by incorporating the Agilent 1260 Infinity II hybrid multisampler in feed injection mode, enabling direct injection of the sample eluent after EMR passthrough cleanup. This advancement eliminated the need for a drying and reconstitution step in the analysis of the three food matrices in this study. The modified protocol not only simplifies the workflow but also reduces sample preparation time up to 50%. This approach can be extended to other food categories with moderate to high limits of quantitation (LOQ) requirements, such as meat, fish, eggs, animal feed, and edible offal.

Experimental

Chemicals and reagents

Native PFAS and isotopically labeled internal standard (ISTD) solutions were purchased from Wellington Laboratories (Ontario, CA, U.S.). Methanol (MeOH), acetonitrile (ACN), and isopropyl alcohol (IPA) were from VWR (Randor, PA, U.S.). Acetic acid (AA) and ammonium acetate were procured from MilliporeSigma (Burlington, MA, U.S.).

Solutions and standards

Native PFAS and ISTD spiking solutions were prepared by diluting their respective stock solutions with MeOH. The native PFAS spiking solution was formulated at concentrations of 100 ng/mL for PFBA, 9Cl-PF3ONS, 11Cl-PF3OUdS, HFPO-DA, DONA, 4:2 FTS, 6:2 FTS, and 8:2 FTS, 50 ng/mL for PFPeA, and 25 ng/mL for the remaining 21 compounds. The ISTD spiking solution was prepared at a uniform concentration of 100 ng/mL for all 18 isotopically labeled ISTD compounds.

These spiking solutions were used to prepare calibration curve neat standards in ACN with 1% acetic acid, with the concentration details listed in Table 1.

After preparation, all calibration standards were diluted with 10% water, thoroughly mixed, and employed for LC/TQ injection. This dilution step was implemented to match the additional 10% dilution introduced during sample cleanup using EMR cartridges, ensuring consistency between calibration standards and sample extracts.

All standards were stored at 4 °C and used within two weeks. For routine calibration standards testing, aliquots of the calibration solutions were transferred to a separate set of vials equipped with polypropylene (PP) inserts and used for instrument injections. The standards need to be warmed up to room temperature thoroughly before use. Sonication can be used to expedite the warming up process. This step is critical to prevent the loss of long chain PFAS analytes in the vial. It is essential to vortex the sample in the insert to eliminate any air bubbles that could otherwise lead to injection errors during LC/TQ analysis.

Table 1. Calibration curve standards.

Native PFAS or ISTD	Calibration Standards (ng/mL)									
	0	1	2	3	4	5	6	7	8	9
PFBA, 9Cl-PF3ONS, 11Cl-PF3OUdS, HFPO-DA, DONA, 4:2 FTS, 6:2 FTS, 8:2 FTS	NA	0.008	0.02	0.04	0.2	0.4	2.0	4.0	8.0	20.0
PFPeA	NA	0.004	0.01	0.02	0.1	0.2	1.0	2.0	4.0	10.0
PFHxA, PFBS, PFHpA, PFPeS, PFHxS, PFOA, PFNA, PFHpS, PFDA, PFUnDA, PFDoDA, PFTrDA, PFTDA, PFOS, PFNS, PFDS, PFUnDS, PFDoS, PFTrDS, PFOSA, 10:2 FTS	NA	0.002	0.005	0.01	0.05	0.1	0.5	1.0	2.0	5.0
ISTD	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2

The ACN with 1% acetic acid extraction solvent was prepared by adding 10 mL of glacial acetic acid into 990 mL of ACN and stored at room temperature. LC mobile phase A was 5 mM NH_4OAc in water, and mobile phase B was 95:5 ACN/water. Needle wash solvents included IPA, water, and ACN.

Equipment and material

The study was performed using an Agilent 1290 Infinity II LC system consisting of an Agilent 1290 Infinity II binary pump (G7120A), an Agilent 1260 Infinity II hybrid multisampler (G7167C), and an Agilent 1290 Infinity II thermostatted column compartment (G7116B). The LC system was coupled to an Agilent triple quadrupole LC/MS system (G6495D) equipped with an Agilent Jet Stream iFunnel electrospray ion source. Agilent MassHunter workstation software was used for data acquisition and analysis.

Other equipment used for sample preparation included:

- Centra CL3R centrifuge (Thermo IEC, MA, U.S.)
- Geno/Grinder (Metuchen, NJ, U.S.)
- Multi Reax test tube shaker (Heidolph, Schwabach, Germany)
- Pipettes and Repeaters (Eppendorf, NY, U.S.)
- Agilent positive pressure manifold 48 processor (PPM-48; part number 5191-4101)
- Ultrasonic cleaning bath (VWR, PA, U.S.)

The 1290 Infinity II LC system was modified using an Agilent InfinityLab PFC-free HPLC conversion kit (part number 5004-0006), including an InfinityLab PFC delay column, 4.6 × 30 mm (part number 5062-8100). Chromatographic separation was performed using an Agilent ZORBAX RRHD Eclipse Plus C18, 95 Å, 2.1 × 100 mm, 1.8 µm (part number 959758-902), an Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 mm, 1.8 µm, 1200-bar pressure limit, and an Agilent UHPLC guard column (part number 821725-901).

The sample preparation and other consumables used included:

- Agilent Bond Elut QuEChERS EN extraction kit, EN 15662 method, buffered salts, ceramic homogenizers (part number 5982-5650CH)
- Agilent Captiva EMR PFAS Food II cartridges, 6 mL cartridges, 750 mg (part number 5610-2232)
- PP snap caps and vials, 1 mL (part numbers 5182-0567 and 5182-0542)
- PP screw cap style vials and caps, 2 mL (part numbers 5191-8121 and 5191-8151)
- Tubes and caps, 50 mL, 50/pk (part number 5610-2049)
- Tubes and caps, 15 mL, 100/pk (part number 5610-2039)

All the consumables used in the study were tested and verified with acceptable PFAS cleanliness.

LC/TQ instrument conditions

Table 2 lists the LC pump conditions.

Table 2. LC pump conditions for LC/TQ.

Parameter	Setting
Mobile Phase A	5 mM NH_4OAc in water
Mobile Phase B	95:5 ACN:water
Gradient	Time (min) A% B% Flow (mL/min) 0.00 90 10 0.400 0.50 90 10 0.400 2.00 70 30 0.400 8.50 55 45 0.400 11.50 25 75 0.400 13.25 0 100 0.460
Stop Time	15.50 min
Post Time	2.5 min

Table 3 lists the LC multisampler conditions.

Table 3. LC multisampler program for LC/TQ.

<table>
<tr><th>Parameter</th><th colspan="4">Setting</th></tr>
<tr><td rowspan="6">Feed Injection</td><td colspan="4">Mode: Feed injection</td></tr>
<tr><td colspan="4">Draw sample: 10.00 µL</td></tr>
<tr><td colspan="4">Feed speed: 10% of pump flow</td></tr>
<tr><td colspan="4">Flush out mode: automatic</td></tr>
<tr><td colspan="4">Mix 5 times with 10.00 µL air</td></tr>
<tr><td colspan="4">Inject</td></tr>
<tr><td rowspan="10">Injection Path Cleaning</td><th>Step</th><th>Task</th><th>Solvent</th><th>Duration/ Volume</th></tr>
<tr><td></td><td>Draw sample</td><td></td><td></td></tr>
<tr><td>1</td><td>Outer wash</td><td>1:1 IPA:ACN</td><td>10 sec</td></tr>
<tr><td>2</td><td>Outer wash</td><td>ACN</td><td>10 sec</td></tr>
<tr><td></td><td>Injection</td><td></td><td></td></tr>
<tr><td>1</td><td>Inner wash</td><td>2 mM Ammonium acetate</td><td>150 µL</td></tr>
<tr><td>2</td><td>Inner wash</td><td>2 mM Ammonium acetate</td><td>150 µL</td></tr>
<tr><td>3</td><td>Seat wash</td><td>1:1 IPA:ACN</td><td>150 µL</td></tr>
<tr><td>4</td><td>Seat wash</td><td>ACN</td><td>150 µL</td></tr>
<tr><td>5</td><td>Reconditioning</td><td>2 mM Ammonium acetate</td><td></td></tr>
</table>

LC column compartment: isothermal temperature 40 ± 0.8 °C.

Table 4 lists the MS electrospray ion (ESI) source settings.

Table 4. Mass spectrometer ESI source settings.

Parameter	Setting
Drying Gas	150 °C; 18 L/min
Sheath Gas	390 °C; 12 L/min
Nebulizer Gas	15 psi
Capillary Voltage	2,500 V (NEG)
Nozzle Voltage	0 V (NEG)
Ion Mode	Negative ion mode with constant fragmentor setting at 166 V
iFunnel Mode	Standard mode for all compounds except HFPO-DA

The MS acquisition conditions for PFAS targets and ISTDs were from the PFAS MRM Database (G1736AA).

Sample preparation procedure

Fish oil, coffee powder, and protein powder samples were purchased from local grocery stores and used directly for extraction. One gram of protein powder or coffee powder, or two grams of fish oil, was weighed into a 50 mL PP tube. PFAS standards and ISTDs were appropriately spiked into all prespiked quality control (QC) samples, while only ISTDs were added to matrix blanks (MBs). For procedure blanks (PBs), either 1 mL or 2 mL of water spiked with ISTDs were used.

Table 5 summarizes the spiking details for prespiked QC samples. In accordance with the sample preparation protocol, protein powder and coffee powder underwent a 10-fold dilution , while fish oil experienced a 5-fold dilution. Therefore, the spiking concentrations in QC samples were calculated by accounting for both the matrix-specific dilution factors and the required LOQs. To ensure accurate quantitation using the previously prepared calibration curve, it was critical to maintain the theoretical ISTD concentration at 0.2 ng/mL in the final ACN extract–matching the ISTD concentration in the calibration standards. The 10% water dilution applied to the sample ACN extract prior to EMR cleanup was mirrored in the calibration standards (see the "solutions and standards" section) and thus did not affect the final sample concentration calculations.

Table 5. Matrix-matched QC and matrix-zero samples prepared for coffee, protein powder, and fish oil validation batches.

	Protein Powder				Coffee Powder				Fish Oil			
Sample (g)	1				1				2			
Dilution Factor	10				10				5			
Matrix Spiked Samples	Spiking Concentration in Sample Matrix (µg/kg)											
	21 PFAS	8 PFAS	PFPeA	ISTD	21 PFAS	8 PFAS	PFPeA	ISTD	21 PFAS	8 PFAS	PFPeA	ISTD
Zero	--	--	--	2.0	--	--	--	2.0	--	--	--	1.0
QC 1	0.05	0.2	0.1	2.0	0.02	0.08	0.04	2.0	0.025	0.1	0.05	1.0
QC 2	0.08	0.4	0.2	2.0	0.05	0.2	0.1	2.0	0.05	0.2	0.1	1.0
QC 3	0.5	2.0	1.0	2.0	0.5	2.0	1.0	2.0	0.25	1.0	0.5	1.0
QC 4	2.0	8.0	4.0	2.0	2.0	8.0	4.0	2.0	1.0	4.0	2.0	1.0
QC 5	10.0	40.0	20.0	2.0	10.0	40.0	20.0	2.0	2.0	8.0	4.0	1.0

8 PFAS compounds include PFBA, HFPO-DA, DONA, 4:2 FTS, 6:2 FTS, 8:2 FTS, 9Cl-PF3ONS, and 11Cl-PF3OUdS.

After spiking, all samples were vortexed for 2 to 3 minutes to ensure equilibrium. Samples were then ready for extraction using the developed procedure, as illustrated in Figure 1.

Weigh 1 g of sample powder or 2 g of oil sample into a 50 mL tube.

Spike PFAS standard and EIS appropriately.
Vortex the sample for 2 to 3 minutes.

Add 10 mL of water. Vortex for 10 to 15 minutes.

Add 10 mL of ACN with 1% acetic acid. Vortex for 20 seconds.

Add QuEChERS EN extraction salt and two ceramic homogenizers.

Cap and shake the sample on a
Geno Grinder at 1,500 rpm for 5 minutes.

Centrifuge the tubes at 5,000 rpm for 5 minutes.

Transfer 4.5 mL of supernatant to another
15 mL tube and mix with 0.5 mL water.

Prewash the EMR PFAS Food II cartridges
with 5 mL of 1:1 ACN:MeOH with 1% AA.

Equilibrate the cartridges with 0.8 mL of corresponding sample.

Discard all the eluent and place the prelabeled
15 mL PP tubes for sample collection.

Transfer 3.5 mL of supernatant mixture
into Captiva EMR PFAS Food II cartridges.

Elute by gravity until dripping stops.

Apply 10 psi for 1 minute at the end to completely dry the sorbent bed.

Vortex gently and take an aliquot of sample eluent for analysis.

Figure 1. Sample preparation procedure using QuEChERS extraction followed by EMR mixed-mode passthrough cleanup with the Agilent Captiva EMR PFAS Food II cartridge.

Results and discussion

LC/TQ instrument method

The MS detection method used here was adopted directly from previous studies[3,5] but with modifications on the retention time window. More modifications were applied on the LC method side. The LC method still used the same LC column as the previous studies, but with different mobile phase B, gradient, and injection programs. The modified LC method provides better chromatographic distributions on native targets and ISTD compounds within the acquisition window. It also improved the chromatographic separation for some targets with their isomers and provided baseline separation for PFOS and cholic acid interferences. Figure 2 shows the chromatogram of all the targets and ISTD peaks with partial identification (A), and PFOS isomer and cholic acid interferences (B), demonstrating improved peak distribution over the retention time window and baseline separation for critical targets and possible matrix interferences. It is noteworthy that the 10 µL injection of the sample in a high percent of ACN using feed injection mode delivered excellent peak shapes for all analytes, with the exception of PFBA, which exhibited a slightly wider peak. However, the peak shape and responses remained consistent, enabling reliable integration.

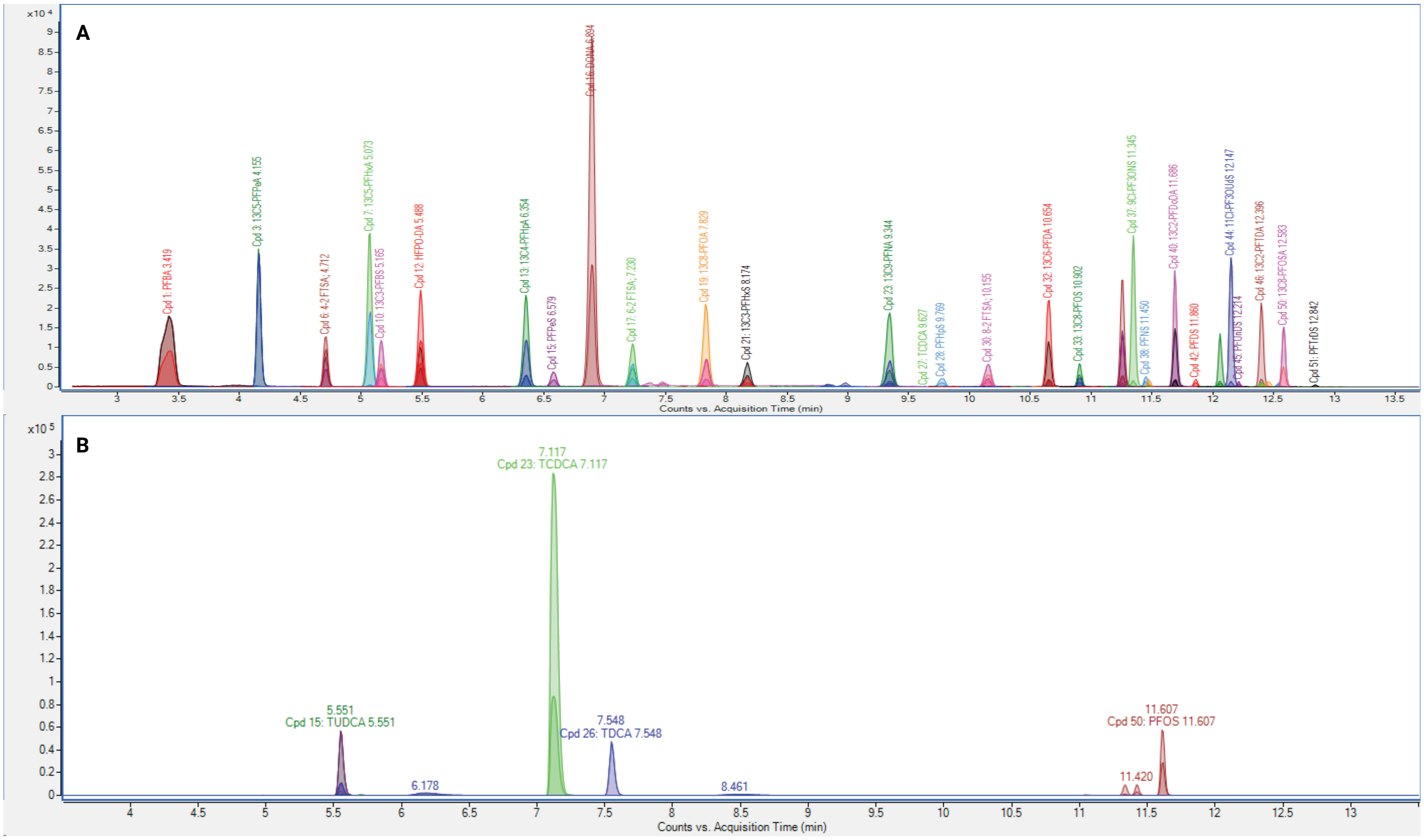

Figure 2. MRM chromatograms showing (A) all PFAS analytes and ISTD compounds and (B) PFOS alongside cholic acids–TUDCA, TCDCA, and TDCA.

Sample preparation procedure

Sample preparation generally followed the previously established protocol, with several modifications. First, the sample size was reduced to 1 g for protein powder and coffee powder, and 2 g for fish oil. Although protein powder is not highly complex, it is viscous and highly absorptive of ACN. Using a larger sample size (>1 g) led to significant loss of ACN layer after salt partitioning. Coffee powder, being dry, rich in pigments, and lipids/oils, presented a more complex and concentrated matrix. Fish oil, considered one of the most challenging oil-based matrices, was surprisingly well cleaned using EMR passthrough cleanup. Therefore, a slightly large sample size (2 g) was used for fish oil.

Second, the crude ACN extract obtained after QuEChERS extraction was diluted with 10% water and subjected to passthrough cleanup using Captiva EMR PFAS Food II cartridges. Unlike the original protocol, which used a 5 mL loading volume, the volume was reduced to 3.5 mL. The adjustment improved cleanup efficiency by preventing potential late-stage saturation of the EMR cartridge, without compromising analytes recovery. The reduced loading volume was also made feasible by the direct injection of the eluate, which does not require a large volume.

Third, following EMR cleanup, the eluate was directly injected for LC/TQ analysis. No drying, reconstitution or additional dilution with water was required. Eliminating post-treatment steps, particularly the drying step, further streamlined the workflow and reduced overall sample preparation time up to 50%.

Method validation

Method selectivity for PFAS background: Procedure blanks (PBs) were prepared using aliquots of MilliQ water and processed through the entire sample preparation protocol. One or two procedure blanks were included in each matrix validation batch. According to AOAC SMPR 2023.003, the required lowest LOQs are 0.01 µg/kg for the four core PFAS targets, 1 µg/kg for PFBA and PFPeA, and 0.1 µg/kg for other PFAS. In comparison, EU reference guidance specifies even lower LOQs for the four core targets, ranging from 0.001 to 0.004 µg/kg LOQs in the same matrices.

First, the detected four core targets, PFHxS, PFOA, PFOS, and PFNA were all below 0.001 µg/kg level in all PBs. Second, for all other PFAS targets, the background levels observed in PBs were sufficiently low to support the required method LOQs. Third, PFBA was detected below 0.005 µg/kg, which is well below the acceptance threshold of 0.3 µg/kg, based on the lowest required LOQ of 1 µg/kg.

Method selectivity for PFAS background: One of the major challenges was the suitability of food matrix blanks, as it is common to detect PFAS residues in matrix blank samples. Among the three food matrices tested, none were completely free of PFAS background contamination. This is largely attributed to the ultra-low detection limits of the analytical method and the widespread presence of PFAS in food matrices. Common PFAS contaminants observed in matrix blanks included 4:2 FTS, 6:2 FTS, PFOA, PFNA, PFOS across various foods. Protein powder exhibited the highest frequency of PFAS positive occurrence, followed by fish oil. Coffee powder showed the lowest background contamination. Notably, significantly high levels of 6:2 FTS were detected in protein powder and fish oil, which impacted the determination of LOQs and quantitation results for experimental LOQ samples.

Method LOQs: The method LOQ was defined as the lowest experimental QC spiking level that met all acceptance criteria, including target identification parameters such as retention time, signal-to-noise (S/N) ratio of the qualifier, and the ratio of quantifier to qualifier, as well as target recovery and repeatability. As previously mentioned, the determination of method LOQ was closely related to the suitability of the MB, particularly given that PFAS compounds were commonly detected across all tested food matrices. For matrices that demonstrated acceptable suitability, defined as any analyte detected in the MB being below 30% of the experimental LOQ, the experimental LOQ was reported as the method LOQ. In cases where positive detections in MBs exceeded acceptable thresholds, the experimental LOQs were deemed invalid due to unsatisfactory quantitation performance. Consequently, the method LOQs for these matrixes were calculated using Equation 1[2], based on seven MBs tested.

Equation 1.

$$\mathrm{LOQ}_{\mathrm{cal}} = \mathrm{SD}_{\mathrm{MBs}} \times 10$$

Where $\mathrm{LOQ}_{\mathrm{cal}}$ is the calculated LOQ based on PFAS detections in matrix blanks. $\mathrm{SD}_{\mathrm{MBs}}$ is the standard deviation (SD) of the detected PFAS concentrations from seven replicates of MB samples.

All matrix blank testing results and the reported LOQs for PFAS targets, whether determined experimentally or calculated and the LOQs required by AOAC SMPR, are summarized in Table 6. The PFAS detection observed in matrix blanks were confirmed based on retention time and qualifier ratio criteria.

As previously mentioned, the coffee powder matrix blank exhibited the lowest PFAS background. Only PFOA and 6:2 FTS were detected, each at approximately 0.05 µg/kg. For 6:2 FTS, the reported LOQ was based on the higher experimental spiking QC level. For PFOA, the calculated LOQ below the required threshold was used. In contrast, the fish oil matrix blank showed slightly higher PFAS background levels.

Table 6. Matrix blanks detection and the reported LOQs and required LOQs for 30 PFAS analytes in coffee powder, protein powder, and fish oil.

Target	Matrix Blank Detection and Method LOQs in Food Matrix (µg/kg)								
	Coffee Powder			Protein Powder			Fish Oil		
	MB	Reported LOQ	Required LOQ	MB	Reported LOQ	Required LOQ	MB	Reported LOQ	Required LOQ
PFBA	ND	2.0	≤ 3	0.068	0.32	≤ 1	0.032	0.2	≤ 5
PFPeA	ND	1.0	≤ 3	0.011	0.16	≤ 1	0.005	0.005	≤ 5
PFBS	ND	0.05	≤ 3	ND	0.08	≤ 0.8	ND	0.025	≤ 5
4:2 FTS	0.002	2.0	≤ 3	0.014	2	≤ 0.8	0.006	0.1	≤ 5
PFHxA	0.001	0.5	≤ 3	ND	0.05	≤ 0.8	ND	0.025	≤ 5
PFPeS	ND	0.02	≤ 3	0.037	0.5	≤ 0.8	0.014	0.25	≤ 5
HFPO-DA	ND	0.2	≤ 3	ND	0.2	≤ 0.8	ND	0.1	≤ 5
PFHpA	ND	0.5	≤ 3	0.011	0.5	≤ 0.8	0.004	0.025	≤ 5
PFHxS	ND	0.02	≤ 0.3	0.021	0.05*	≤ 0.08	0.002	0.025	≤ 0.5
DONA	0.002	0.08	≤ 3	0.010	0.2	≤ 0.8	0.005	0.1	≤ 5
6:2 FTS	0.053	2.0	≤ 3	0.808	1.87*	≤ 0.8	0.563	0.13*	≤ 5
PFOA	0.048	0.07*	≤ 0.3	0.028	0.05*	≤ 0.08	0.012	0.01*	≤ 0.5
PFHpS	ND	0.05	≤ 3	ND	0.05	≤ 0.8	ND	0.025	≤ 5
PFNA	ND	0.05	≤ 0.3	0.020	0.02*	≤ 0.08	ND	0.025	≤ 0.5
PFOS	ND	0.02	≤ 0.3	0.065	0.04*	≤ 0.08	ND	0.025	≤ 0.5
9Cl-PF3ONS	ND	0.08	≤ 3	ND	0.2	≤ 0.8	ND	0.1	≤ 5
8:2 FTS	ND	0.08	≤ 3	ND	0.2	≤ 0.8	ND	0.1	≤ 5
PFDA	ND	0.02	≤ 3	ND	0.05	≤ 0.8	ND	0.025	≤ 5
PFNS	ND	0.02	≤ 3	ND	0.05	≤ 0.8	ND	0.025	≤ 5
PFDS	ND	0.05	≤ 3	ND	0.05	≤ 0.8	ND	0.025	≤ 5
PFUnDA	ND	0.02	≤ 3	ND	0.05	≤ 0.8	ND	0.025	≤ 5
PFOSA	ND	0.05	≤ 3	ND	0.08	≤ 0.8	ND	0.025	≤ 5
11Cl-PF3OUdS	ND	0.08	≤ 3	ND	0.2	≤ 0.8	ND	0.1	≤ 5
PFUnDS	ND	0.02	≤ 3	ND	0.05	≤ 0.8	ND	0.05	≤ 5
PFDoDA	0.001	0.05	≤ 3	ND	0.05	≤ 0.8	ND	0.025	≤ 5
10:2 FTS	ND	0.05	≤ 3	ND	0.08	≤ 0.8	ND	0.025	≤ 5
PFDoS	ND	0.05	≤ 3	ND	0.05	≤ 0.8	ND	0.025	≤ 5
PFTrDA	0.001	0.05	≤ 3	ND	0.08	≤ 0.8	ND	0.05	≤ 5
PFTrDS	ND	0.05	≤ 3	ND	0.08	≤ 0.8	ND	0.05	≤ 5
PFTeDA	ND	0.5	≤ 3	ND	0.08	≤ 0.8	0.009	0.05	≤ 5

Red indicates the results failed for acceptance criteria
ND = Not detectable
* = Calculated LOQ
Bold = Core PFAS targets

Most detections were below 0.02 µg/kg, except for PFBA and 6:2 FTS, with the latter showing a notably high concentration. Calculated LOQs were reported for both 6:2 FTS and PFOA. All LOQs, whether experimental or calculated, remained below the required limits due to the higher LOQ thresholds for coffee and fish oil. The protein powder matrix exhibited the highest PFAS backgrounds, with more frequent and elevated detections. This led to a greater number of calculated LOQs being reported, and 6:2 FTS not meeting the required LOQ.

Method recovery and repeatability: Method recovery and repeatability were validated across all three matrices using five prespiking QC levels with six replicates per level. Final validation results were reported at three QC levels for each matrix: LOQ, mid, and high. When a calculated LOQ was used, the spiking QC level closest to the calculated LOQ was selected for reporting recovery and RSD at the LOQ level. Mid-level QC results correspond to concentrations 5 to 20 times the LOQ, while high-level QC results reflect concentrations 50 to 100 times the LOQ.

The validated method results are summarized in Figure 3 (coffee powder), Figure 4 (protein powder), and Figure 5 (fish oil). In each figure, the green shaded area represents the recovery acceptance window, while the blue shaded area indicates the RSD acceptance window. Solid lines depict recovery results, and dotted lines represent RSD results across the three QC levels. These QC levels are color coded: green for LOQ, purple for mid-level, and blue for high-level QCs. When positive detection of analytes in matrix blanks, the blank background was corrected for analyte recovery calculation.

All PFAS target analytes were reported across three spiking levels with acceptable recovery and RSD results. At mid- and high-level QCs, all analytes in the three matrices demonstrated excellent recovery (80 to 120%) and low RSD (< 10%). At the LOQ level, occasional deviations were observed, with recoveries falling below 80% or exceeding 120%, and RSDs exceeding 10%. Overall, the results confirm that the method reliably delivers accurate and precise quantitative measurements for 30 PFAS compounds in coffee powder, protein powder, and fish oil that meet the AOAC SMPR requirements.

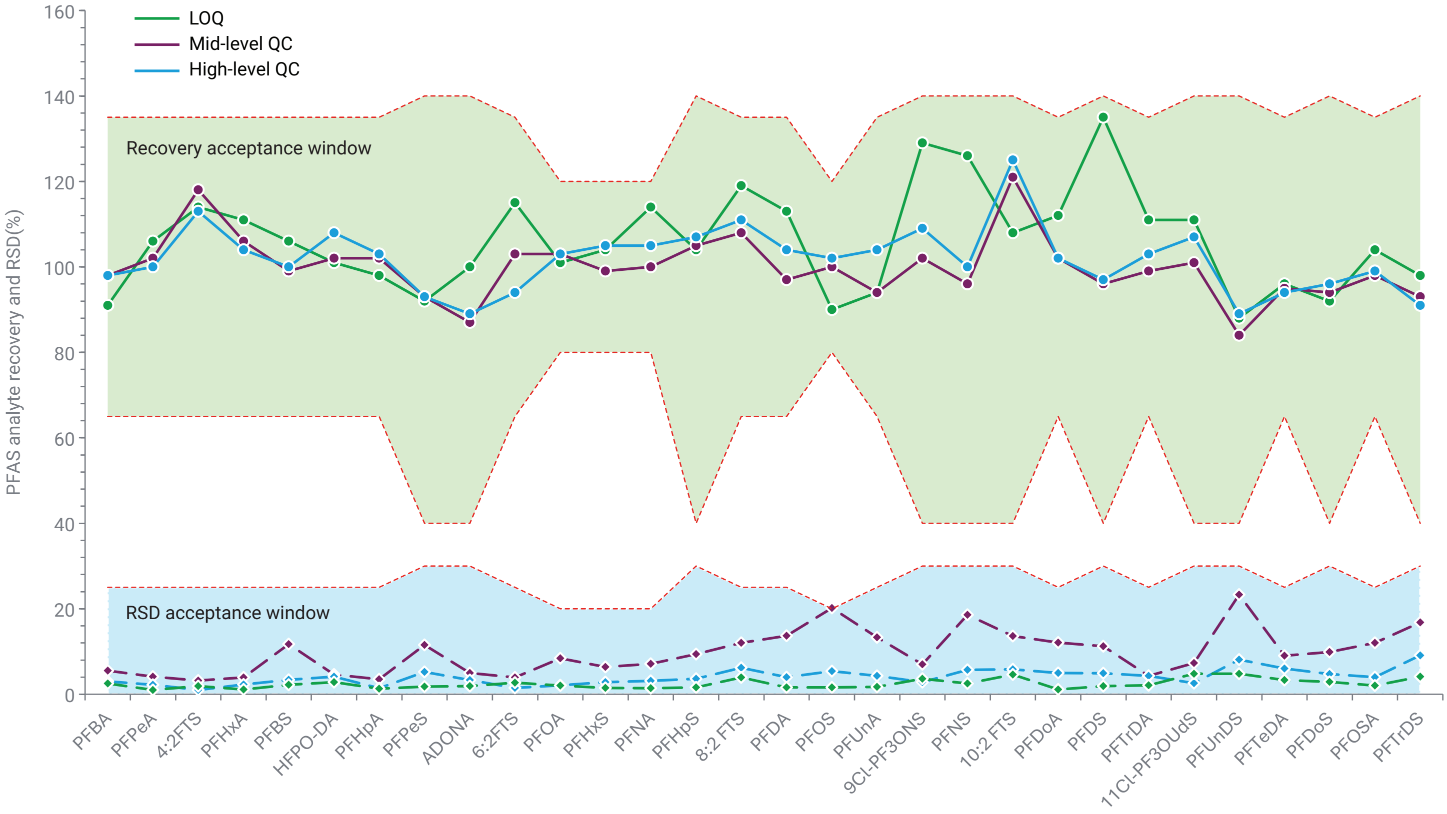

Figure 3. Validation results summary for 30 PFAS in coffee powder. Acceptance criteria based on AOAC SMPR 2023.003.

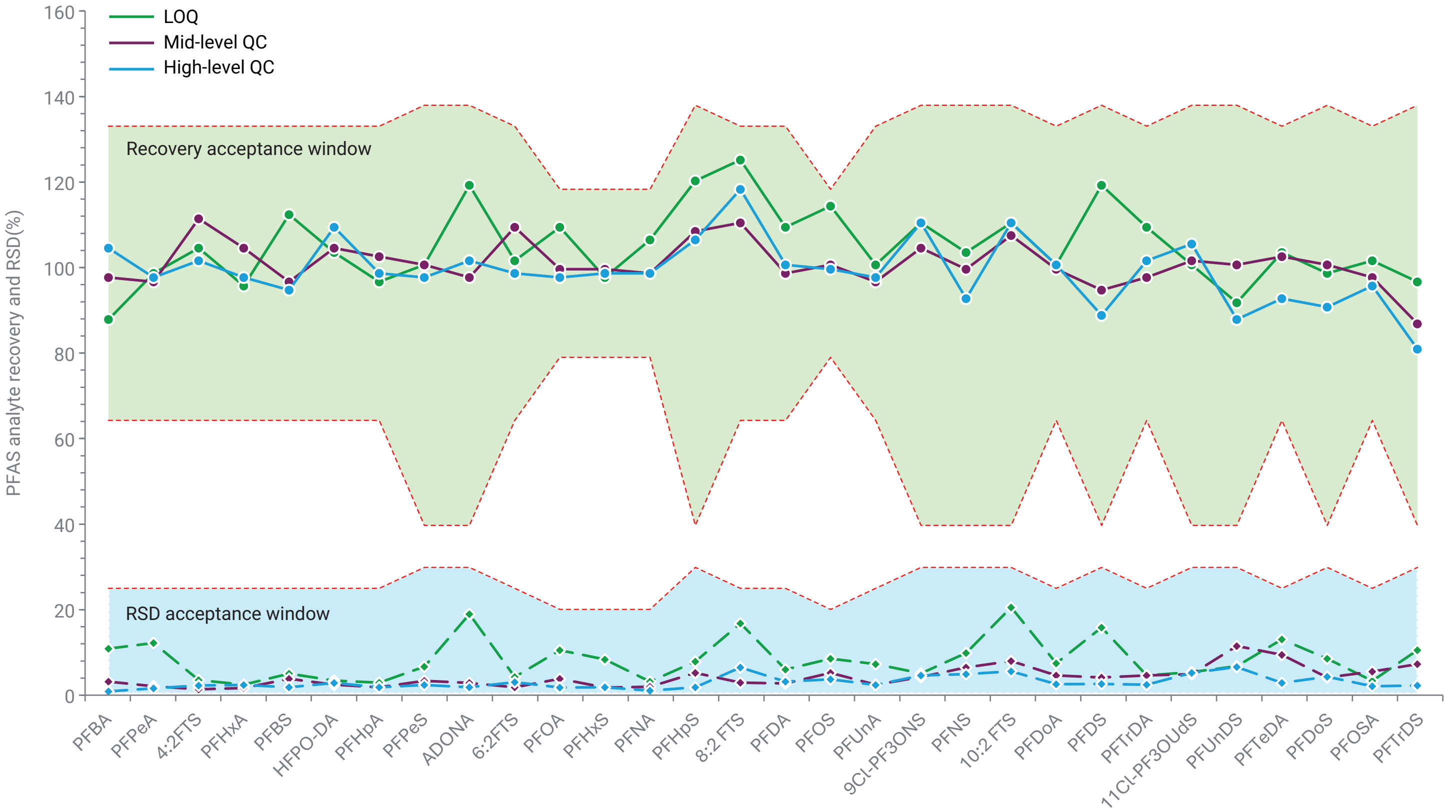

Figure 4. Validation results summary for 30 PFAS in protein powder. Acceptance criteria based on AOAC SMPR 2023.003.

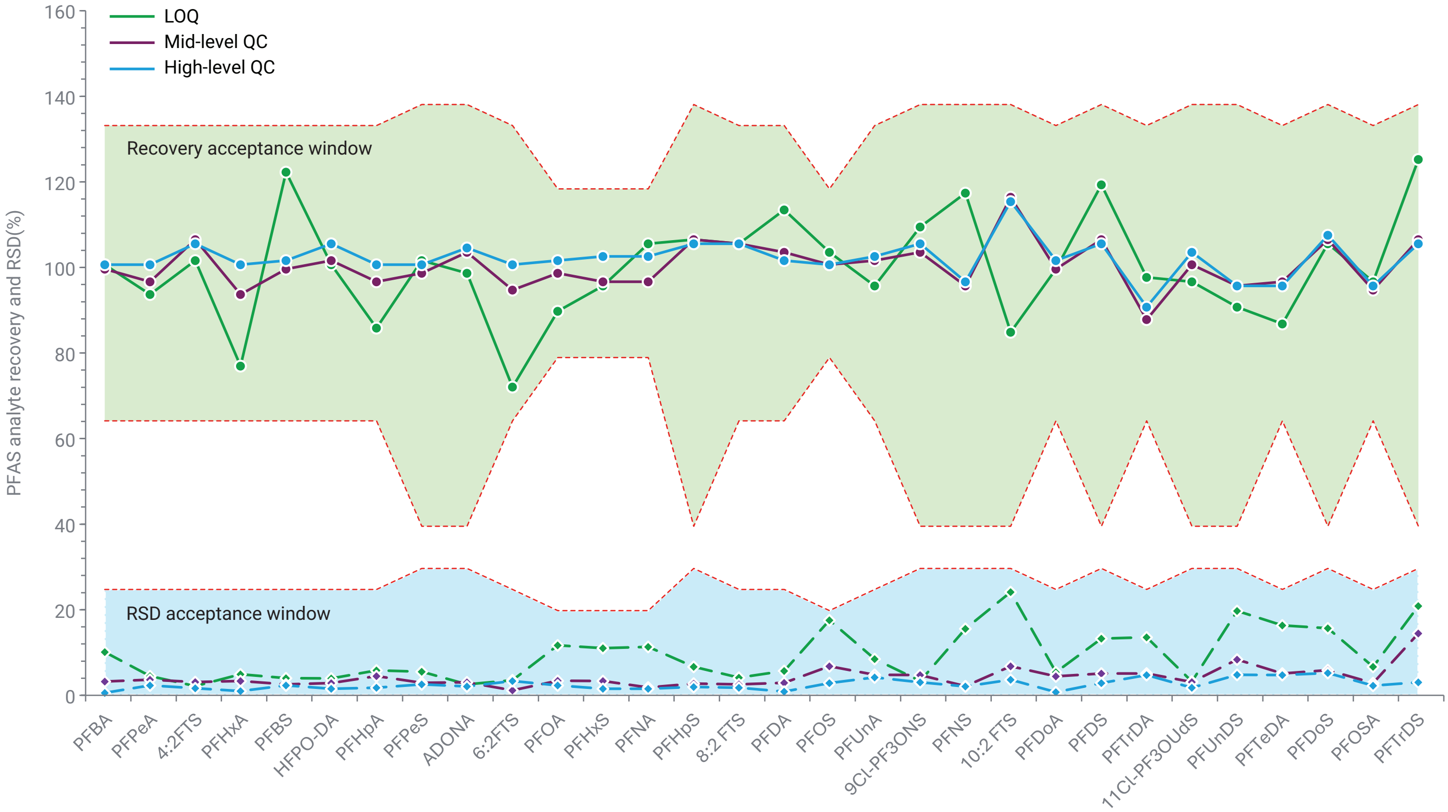

Figure 5. Validation results summary for 30 PFAS in fish oil. Acceptance criteria based on AOAC SMPR 2023.003.

Conclusion

A simplified, rapid, and reliable method was developed and validated for 30 PFAS targets in coffee powder, protein powder, and fish oil. This new method uses QuEChERS extraction followed by Captiva EMR PFAS Food II passthrough cleanup, and LC/TQ detection. The sample preparation approach is characterized by its simplicity, robustness, and cost effectiveness, offering significant time and resource saving. The method was validated to meet the acceptance criteria outlined in the AOAC SMPR 2023.003 guidelines. This work completes the demonstration of EMR-LC/MS/MS method validation across all 11 food categories required by AOAC SMPR 2023.003.

References

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