Application Note

Food & Beverage Testing

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# Determination of 30 Per- and Polyfluoroalkyl Substances in Beef, Tuna, and Shrimp

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


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## Abstract

This application note presents the development and validation of a multiresidue method for the analysis of per- and polyfluoroalkyl substances (PFAS) residues in beef, tuna, and shrimp. The method uses QuEChERS extraction, followed by enhanced matrix removal (EMR) mixed-mode passthrough cleanup with the Agilent Captiva EMR PFAS Food II cartridge, then LC/MS/MS detection. The method features simplified and efficient sample preparation, sensitive LC/MS/MS detection, and reliable quantitation using neat standard calibration curves. The Captiva EMR PFAS Food II cartridges were developed and optimized specifically for PFAS analysis in animal-origin foods and plant-origin seeded dry foods. The method was validated based on the AOAC Standard Method Performance Requirements (SMPR), including method suitability, sensitivity, accuracy, and precision. The method was demonstrated to meet the required limits of quantitation (LOQs), recovery, and repeatability for four core PFAS targets—perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), and perfluorohexane sulfonic acid (PFHxS)—and the remaining 26 PFAS targets in the three food matrices evaluated in this study.

## Introduction

Determination of PFAS residues in food has become a topic of rising concern, gaining more attention over the last several years. In April 2023, the European Commission enforced regulations for four PFAS compounds—PFOS, PFOA, PFNA, and PFHxS—in eggs, fish, seafood, meat, and offal.[1] In November 2023, the AOAC released the SPMR 2023.003 for the analysis of 30 PFAS in produce, beverages, dairy products, eggs, seafood, meat products, and feed.[2]

Agilent Captiva EMR PFAS Food cartridges were developed and optimized specifically for PFAS analysis in foods. Two types of cartridges (I and II) were designed to cover the large variety of food matrices. Methods developed for PFAS analysis PFAS in infant formula, milk, and eggs using Captiva EMR PFAS II cartridges[3] and PFAS analysis in baby food using Captiva EMR PFAS I cartridges[4] demonstrated excellent performance, reliability, and simplicity. The objectives of this study were to develop and validate a complete workflow for the determination of 30 PFAS in beef, tuna, and shrimp, which uses QuEChERS extraction followed by EMR mixed-mode passthrough cleanup using the Captiva EMR PFAS Food II cartridge and detection with the Agilent 6495D triple quadrupole LC/MS.

## Experimental

### Chemicals and reagents

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

### Solutions and standards

The preparation of standard solutions and other reagents are listed in a previous application note.[3]

### Equipment and material

The study was performed using an Agilent 1290 Infinity II LC system consisting of a 1290 Infinity II high-speed pump (G7120A), an Agilent 1290 Infinity II multisampler (G7167B), and an Agilent 1290 Infinity II multicolumn thermostat (G7116A). The LC system was coupled to an Agilent 6495D LC/TQ 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, USA)
- Geno/Grinder (Metuchen, NJ, USA)
- Multi Reax test tube shaker (Heidolph, Schwabach, Germany)
- Pipettes and repeater (Eppendorf, NY, USA)
- Agilent positive pressure manifold 48 processor (PPM-48; part number 5191-4101)
- CentriVap and CentriVap Cold Trap (Labconco, MO, USA)
- Ultrasonic cleaning bath (VWR, PA, USA)

The 1290 Infinity II LC system was modified using an Agilent InfinityLab PFC-free HPLC conversion kit (part number 5004-0006), including an Agilent InfinityLab PFC delay column, 4.6 × 30 mm (part number 5062-8100). Chromatographic separation was performed using an Agilent ZORBAX RRHD Eclipse Plus C18 column, 2.1 × 100 mm, 1.8 µm (part number 959758-902), and an Agilent ZORBAX RRHD Eclipse Plus C18 column, 2.1 mm, 1.8 µm, 1,200 bar pressure limit, UHPLC guard (part number 821725-901).

**Other Agilent consumables used included:**

- Agilent Bond Elut QuEChERS EN extraction kit, EN 15662 method, buffered salts, ceramic homogenizers (part number 5982-5650CH)
- Captiva EMR PFAS Food II cartridges, 6 mL cartridges, 750 mg (part number 5610-2232)
- Polypropylene (PP) snap caps and vials, 1 mL and 2 mL (part numbers 5182-0567 and 5182-0542)
- PP screw cap style vials and caps, 50 mL (part numbers 5191-8150 and 5191-8151)
- Tubes and caps, 50 mL, 50/pk (part number 5610-2049)
- Tubes and caps, 15mL, 100/pk (part number 5610-2039)

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

### LC/MS/MS instrument conditions

The LC/MS/MS method conditions are described in a previous application note.[3]

## Sample preparation

Beef, canned tuna, and shrimp samples were purchased from local grocery stores. Fresh beef and shrimp were cut into small cubic pieces and frozen at –20 °C. The frozen sample was then blended into fine powder using a mechanical blender. Canned tuna was blended directly into fine paste. All the homogenized samples were either used for extraction or stored at –20 °C for future use.

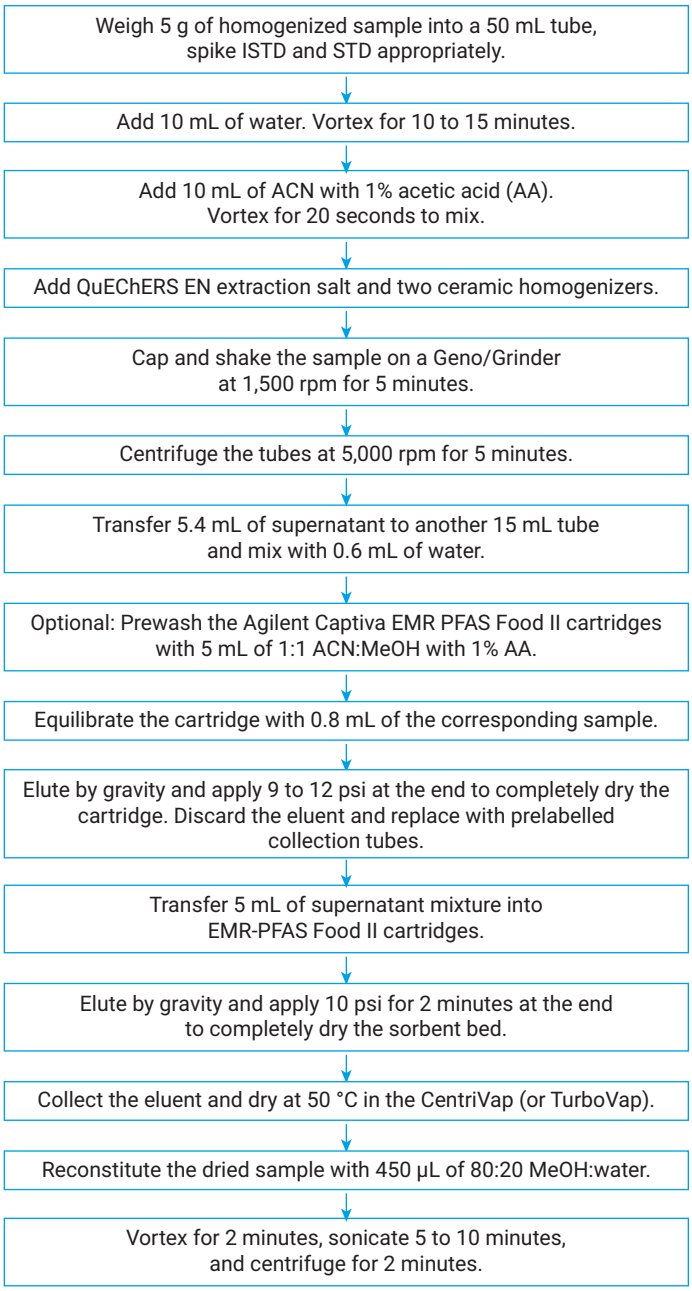


**Figure 1.** Sample preparation procedure for PFAS analysis in beef, tuna, and shrimp.

For all the homogenized samples, a 5 g sample was weighed into clean PP 50 mL tubes for extraction. The native PFAS spiking and ISTD spiking solutions were added to the QC samples appropriately, and only ISTD to matrix blanks. The samples were vortexed for 10 to 15 seconds after spiking. The samples were then ready for the procedure (Figure 1).

## Method performance evaluation

The EMR mixed-mode passthrough cleanup using Captiva EMR PFAS Food II cartridges was evaluated thoroughly in the previous study in terms of matrix removal, target recovery, and repeatability during sample cleanup with the cartridge.[3] The entire method was then validated, which included a calibration study, method LOQ determination, and recovery accuracy and precision. Due to the different requirements of the target LOQs, five prespiked QC-level samples were prepared in replicates of four to five at each level. In addition, the matrix blanks were prepared in replicates of five to seven for quantitation of the targets in the matrix control sample. This is important for accuracy evaluation, as the contribution from the matrix for some PFAS is unavoidable. Table 1 shows the matrix zero blanks and prespiked QC PFAS standard and ISTD spiking.

**Table 1.** Matrix-matched QC and matrix-zero samples in group II food matrices.

| | Beef | | Tuna | | Shrimp | |
|---|---|---|---|---|---|---|
| Sample Size (g) | 5 | | 5 | | 5 | |
| Concentration Factor | 5x | | 5x | | 5x | |
| Matrix Spiked Samples | Spiking Concentration (µg/kg) | | | | | |
| | STD* | ISTD | STD* | ISTD | STD* | ISTD |
| Zero | – | 0.2 | – | 0.2 | – | 0.2 |
| PR-QC 1 | 0.02 | 0.2 | 0.02 | 0.2 | 0.02 | 0.2 |
| PR-QC 2 | 0.04 | 0.2 | 0.04 | 0.2 | 0.04 | 0.2 |
| PR-QC 3 | 0.1 | 0.2 | 0.1 | 0.2 | 0.1 | 0.2 |
| PR-QC 4 | 0.4 | 0.2 | 0.4 | 0.2 | 0.4 | 0.2 |
| PR-QC 5 | 1.0 | 0.2 | 1.0 | 0.2 | 1.0 | 0.2 |

* Concentrations only indicated for generic concentration of 28 PFAS targets. Concentrations of PFBA and PFPeA were 10x and 2x the generic concentrations, respectively.

## Results and discussion

### EMR mixed-mode passthrough cleanup

The Captiva EMR PFAS Food cartridges provide comprehensive matrix removal after traditional QuEChERS extraction, which demonstrates a simplified yet efficient procedure to remove matrix interferences including carbohydrates, organic acids, pigments, fats and lipids, and other hydrophobic and hydrophilic matrix co-extractives. The Captiva EMR PFAS Food I cartridges contain less sorbent with a simpler formula and are recommended for fresh and processed fresh foods of plant origin, such as fruits and vegetables, baby food, and juices. The EMR PFAS Food II cartridges contain more sorbent with a more complex formulation and are recommended for fresh and processed fresh and dry foods of animal origin, such as milk, eggs, meat, fish, and infant formula; dry seed feed and food of plant origin; and oils. Comparing to traditional dispersive SPE (dSPE) cleanup used after QuEChERS extraction, the EMR mixed-mode passthrough cleanup provided significant improvement on PFAS recovery and reproducibility, as well as matrix removal in multiple food matrices.[3,4]

The matrix removal during sample cleanup was also evaluated using GC/MS full scan and LC/Q-TOF total ion chromatogram (TIC) scan. Figure 2 shows the chromatogram comparison using GC/MS full scan evaluation for beef, tuna, and shrimp sample extract with and without EMR mixed-mode passthrough cleanup. Figure 3 shows the chromatogram comparison using LC/Q-TOF TIC scan evaluation for tuna sample extract with EMR cleanup versus traditional dSPE cleanup. The results demonstrate significant improvement in matrix removal using EMR mixed-mode passthrough cleanup.

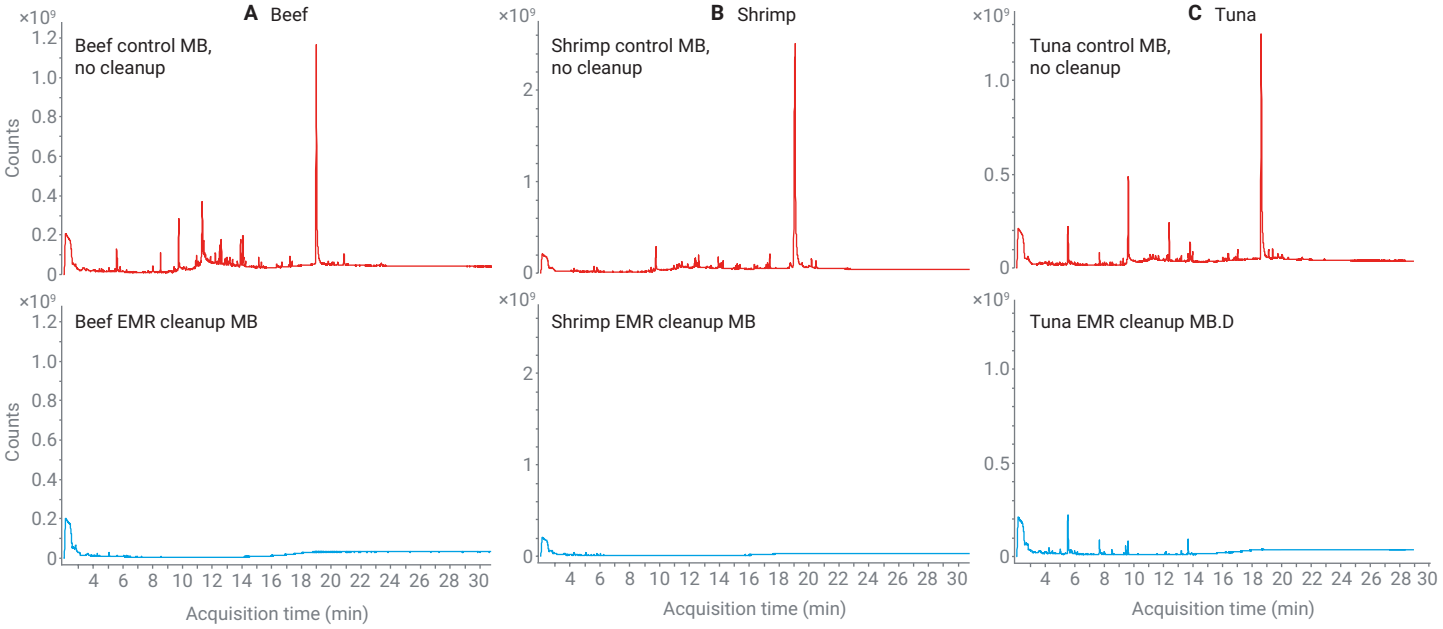


**Figure 2.** Food matrix removal by EMR mixed-mode passthrough cleanup using Agilent Captiva EMR PFAS Food II cartridges, by GC/MS full scan for beef extract (A), shrimp extract (B), and tuna extract (C).

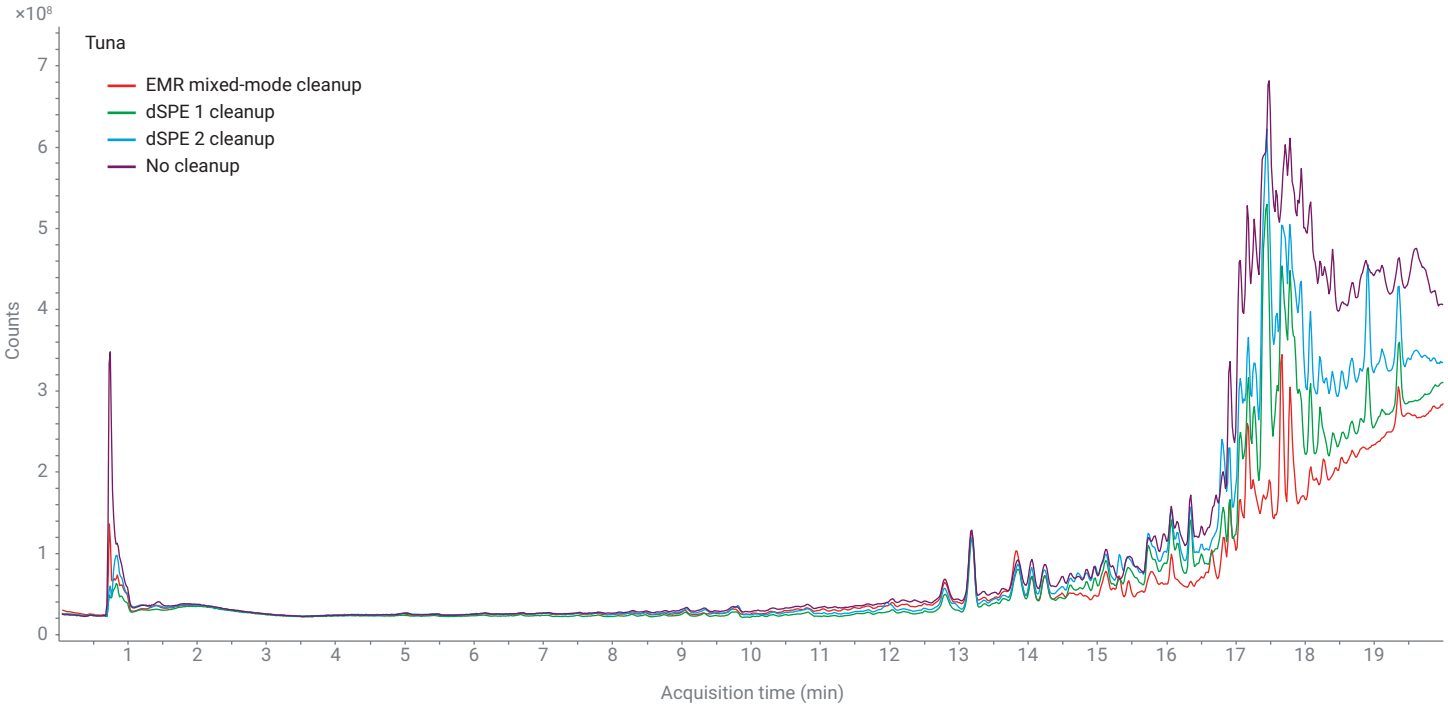


**Figure 3.** Food matrix removal comparison between EMR mixed-mode passthrough cleanup and traditional dSPE cleanups using LC/Q-TOF TIC (+) scan for tuna sample extract after QuEChERS extraction.

Besides the improvement of PFAS targets recovery and matrix removal, another important feature provided by EMR mixed-mode passthrough cleanup is the higher sample volume recovery. Sample volume recovery usually is critical for PFAS analysis in food, since the required LOQs are in the low- to mid-range ppt level, requiring the use of a postconcentration step to boost the method sensitivity. Comparing to the ~50% loss on sample volume when using traditional dSPE cleanup, the EMR mixed-mode cleanup provides > 90% volume recovery, which allows easy postconcentration and consistent sample reconstitution.

## Sample preparation procedure

The use of EMR mixed-mode passthrough cleanup simplifies the entire sample preparation procedure with fewer steps, which saves time, effort, and consumables. The newly developed method includes two major processes: QuEChERS extraction and EMR passthrough cleanup. The traditional method includes three major processes: QuEChERS extraction, dSPE cleanup, and WAX SPE extraction.[5] Figure 5, from a previous application note[3], shows a comparison of the two sample preparation method procedures. The new method using the simplified procedure with fewer steps clearly demonstrates the savings on time and effort. Given the same sample quantity for preparation, the time needed using the traditional method is double to triple that of the time needed using the new method. The new method also uses less solvent and fewer consumables than the traditional method. All of these features using the new sample preparation method led to the improved overall lab productivity for sample analysis.

## Entire method validation

The new method was validated for the determination of 30 PFAS targets in beef, tuna, and shrimp by following the AOAC SMPR guidance. The requirements for PFAS target LOQs in the tested food matrices are listed in Table 2.

**Table 2.** AOAC SMPR requirements for LOQs in beef, tuna, and shrimp.

| Food Matrix | LOQ (µg/kg) | | |
|---|---|---|---|
| | PFHxS, PFOA, PFNA, PFOS | PFBA and PFPeA | Other PFAS |
| Beef | ≤ 0.1 | ≤ 1 | ≤ 1 |
| Tuna | ≤ 0.1 | ≤ 1 | ≤ 1 |
| Shrimp | ≤ 0.3 | ≤ 3 | ≤ 3 |

## Method LOQs

The three food matrices being evaluated in the study all showed positive incurrence in matrix blanks. As a result, matrix background correction was necessary and used for method validation for target recovery. Matrix blanks were prepared in five to seven replicates, and then the lowest reportable LOQs from the method were calculated according to the following equation:

$LOQ_{cal} = 10 \times SD_{MBs}$

Where:

- $LOQ_{cal}$ is the method's lowest reportable limit of quantitation
- $SD_{MBs}$ is the standard deviation (SD) of detected incurred targets from five to seven replicates of matrix blanks (MBs)

The method LOQs were then decided based on the lowest validated QC spiking level that was equal to or above the lowest reportable LOQs. Table 3 shows the calculated lowest reportable ($LOQ_{cal}$) and validated ($LOQ_{val}$) method for each target in each matrix.

For the core PFAS targets, the validated method LOQs were demonstrated to be below or equal to the required LOQs for PFHxS, PFOA, and PFOS in all three tested matrices. The validated method LOQ for PFNA was below or equal to the required LOQs listed for tuna and shrimp, but was higher than the required LOQ in beef due to matrix-positive incurrence. For other PFAS targets, the validated method LOQs are demonstrated to be below or equal to the required LOQs in all three matrices. The cholic acid (TCDCA) showed up in the acquisition window of PFOS for the tuna sample, and TCDCA showed up in the acquisition window of PFOS for the beef sample. However, the chromatographic separation provided a baseline separation of these interferences with PFOS, and thus did not impact the PFOS target peak identification and integration. Figure 4 shows the chromatograms of matrix blanks and method-validated LOQs for the core targets in beef, tuna, and shrimp.

**Table 3.** Lowest reportable method calculated ($LOQ_{cal}$) and validated ($LOQ_{val}$) for 30 PFAS targets in three food matrices.

| Target | Beef | | Tuna | | Shrimp | |
|---|---|---|---|---|---|---|
| | $LOQ_{cal}$ | $LOQ_{val}$ | $LOQ_{cal}$ | $LOQ_{val}$ | $LOQ_{cal}$ | $LOQ_{val}$ |
| PFBA | 0.248 | 0.4 | 0.308 | 0.4 | 0.056 | 0.4 |
| PFPeA | 0.005 | 0.04 | 0.025 | 0.04 | NA | 0.04 |
| PFBS | 0.002 | 0.02 | 0.005 | 0.02 | 0.007 | 0.02 |
| 4:2 FTS | 0.003 | 0.02 | 0.006 | 0.02 | NA | 0.02 |
| PFPeS | 0.011 | 0.02 | 0.008 | 0.02 | NA | 0.02 |
| PFHxA | 0.002 | 0.02 | 0.006 | 0.02 | NA | 0.02 |
| HFPO-DA | NA | 0.02 | 0.006 | 0.02 | 0.006 | 0.02 |
| PFHpA | 0.009 | 0.02 | 0.01 | 0.02 | 0.005 | 0.02 |
| PFHxS* | 0.010 | 0.02 | 0.005 | 0.02 | NA | 0.02 |
| DONA | NA | 0.02 | NA | 0.02 | NA | 0.02 |
| 6:2 FTS | 0.004 | 0.02 | 0.005 | 0.02 | 0.007 | 0.02 |
| PFOA* | 0.008 | 0.02 | 0.01 | 0.02 | 0.025 | 0.04 |
| PFHpS | NA | 0.02 | NA | 0.02 | 0.001 | 0.02 |
| PFNA* | 0.134 | 0.4 | 0.01 | 0.02 | 0.026 | 0.1 |
| PFOS* | 0.006 | 0.02 | 0.021 | 0.04 | 0.025 | 0.1 |
| 9Cl-PF3ONS | NA | 0.02 | 0.005 | 0.02 | 0.001 | 0.02 |
| 8:2 FTS | NA | 0.02 | NA | 0.02 | 0.001 | 0.02 |
| PFNS | 0.008 | 0.02 | NA | 0.02 | 0.054 | 0.1 |
| PFDA | NA | 0.02 | NA | 0.02 | 0.001 | 0.02 |
| PFDS | NA | 0.02 | NA | 0.02 | 0.006 | 0.02 |
| PFUnDA | 0.011 | 0.02 | 0.037 | 0.02 | 0.049 | 0.1 |
| PFOSA | 0.002 | 0.02 | 0.005 | 0.02 | 0.007 | 0.02 |
| 11Cl-PF3OUdS | NA | 0.02 | NA | 0.02 | NA | 0.02 |
| PFUnDS | NA | 0.02 | NA | 0.02 | NA | 0.02 |
| PFDoDA | NA | 0.02 | 0.013 | 0.04 | 0.033 | 0.04 |
| 10:2 FTS | 0.003 | 0.02 | NA | 0.02 | 0.001 | 0.02 |
| PFDoS | 0.001 | 0.02 | NA | 0.02 | NA | 0.02 |
| PFTrDA | NA | 0.02 | 0.013 | 0.1 | 0.050 | 0.1 |
| PFTrDS | NA | 0.02 | NA | 0.02 | 0.008 | 0.02 |
| PFTeDA | NA | 0.02 | 0.006 | 0.02 | 0.021 | 0.1 |

* Core PFAS targets.

Red text indicates the $LOQ_{val}$ level is above the required LOQ level for the target in this matrix.

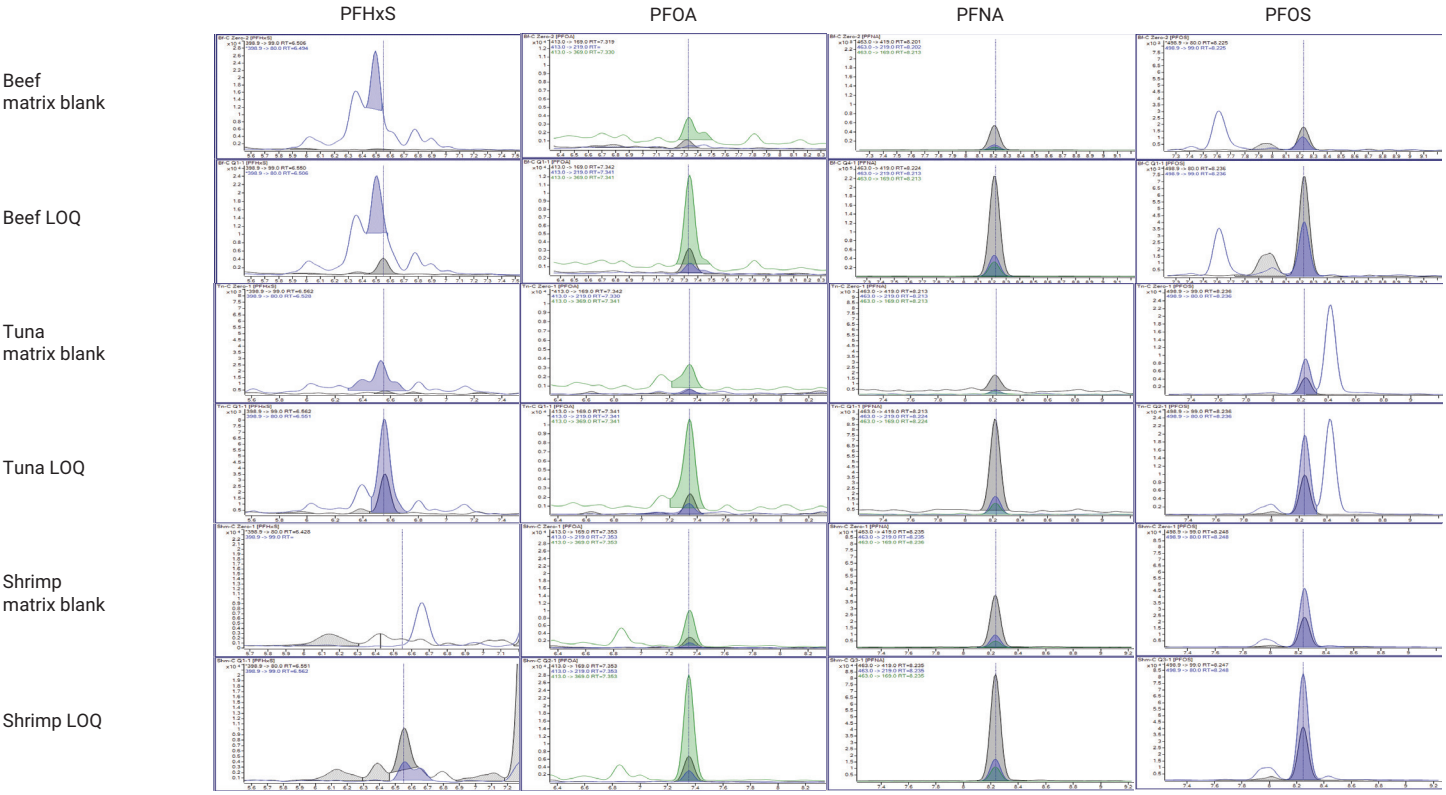


**Figure 4.** Beef, tuna, and shrimp matrix blanks and LOQ chromatograms for the core PFAS targets: PFHxS, PFOA, PFNA, and PFOS. LOQ levels in each matrix are listed in Table 3.

## Method calibration

The use of 18 PFAS isotopically labeled ISTDs allows the same standard calibration curve to be used for PFAS quantitation in different food matrix samples. Therefore, a matrix-matched calibration curve is not needed for each food matrix. This significantly increases sample testing productivity, saves time and costs, and improves sample analysis consistency.

The calibration curve range was decided based on the required LOQs in the food matrices, the concentration factor introduced through sample preparation, and the instrument method sensitivity. Due to the higher detection levels required for beef, tuna, and shrimp, a calibration set range from 20 to 10,000 ng/L was used. The results confirmed a 500x calibration curve dynamic range with correlation coefficient $R^2$ > 0.99 for all 30 PFAS targets.

## Method accuracy and precision

Method recovery and repeatability (RSD) were validated in beef, tuna, and shrimp. The acceptance criteria[2] is 80 to 120% recovery for PFOS, PFOA, PFHxS, and PFNA, and repeatability (RSD%) is ≤ 20% in all three matrices. For the other PFAS targets with corresponding isotopic ISTD, the acceptance criteria for recovery is 65 to 135% and for RSD is ≤ 25%. For other PFAS targets without corresponding isotopic ISTD, the acceptance criteria for recovery is 40 to 140%, and for RSD is ≤30%.

The final reporting validation results include three QC levels in each matrix, including LOQ-, mid-, and high-level QCs. The method-validated LOQs are listed in Table 3, the mid-level results are reported at 5 to 10x LOQ, and the high-level QCs are reported at 20 to 50x LOQ. There were two exceptions for PFNA in beef and PFTrDA in shrimp where two levels at 0.4 and 1 µg/kg were reportable due to significant high positive occurrence in the sample matrix control.

Figure 5 shows the method validation recovery RSD summary for PFAS analysis in beef, tuna, and shrimp. Overall, the method delivered favorable RSD results for all 30 targets in the tested food matrices. The core PFAS targets all generated acceptable recovery and RSD for all spiking levels in all matrices. Other PFAS targets generated acceptable recovery and RSD for all spiking levels in three matrices, except PFOSA LOQ level recovery (62%) in tuna. Targets with corresponding isotopically ISTD generated better quantitation results than targets without corresponding isotopically ISTD. Significant matrix positive incurrence also impacted the spiking recovery results.

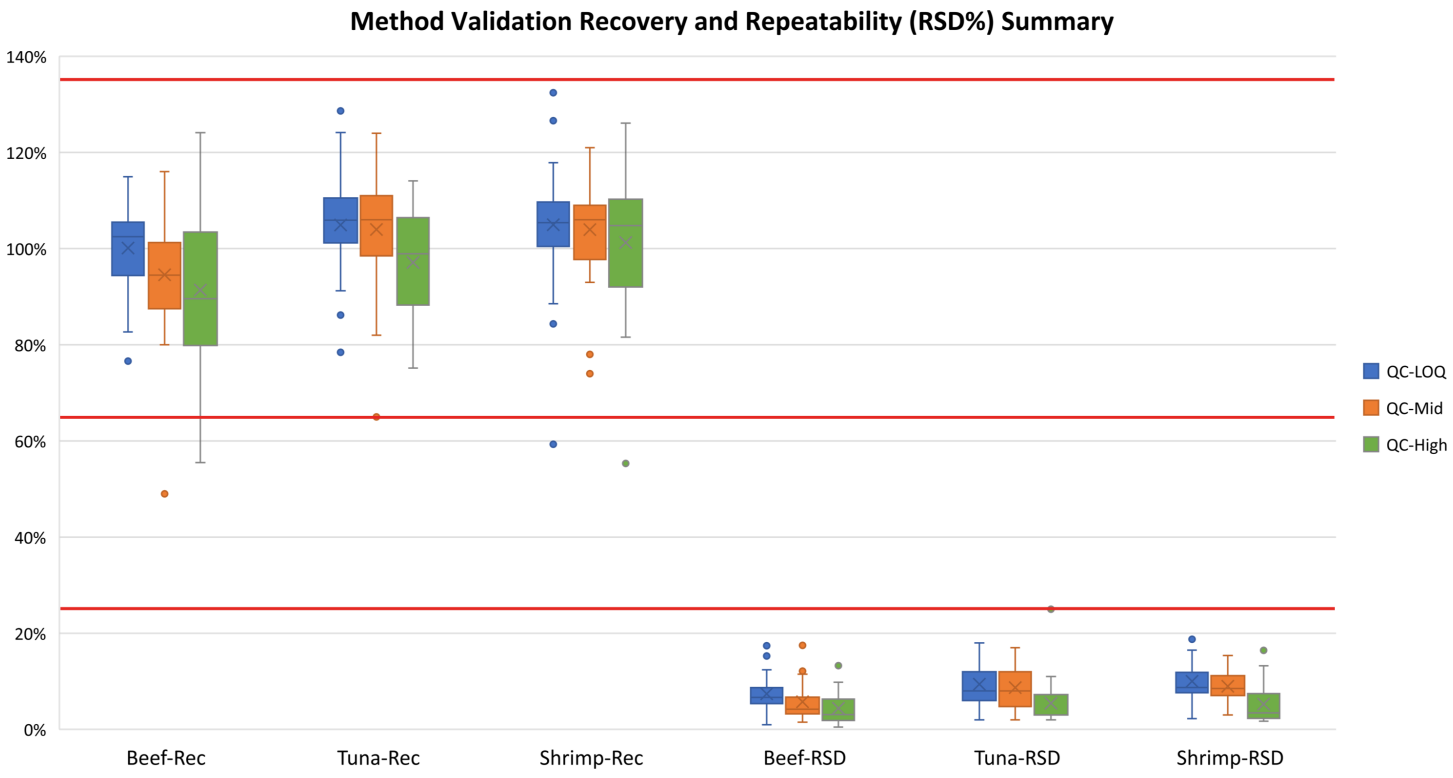


**Figure 5.** Method validation recovery and repeatability (RSD%) summary for PFAS analysis in beef, tuna, and shrimp.

## Conclusion

A simplified, rapid, and reliable method using QuEChERS extraction followed by EMR mixed-mode passthrough cleanup with the Agilent Captiva EMR PFAS Food II cartridge and LC/MS/MS detection was developed and validated for 30 PFAS targets in beef, tuna, and shrimp. The EMR mixed-mode passthrough cleanup demonstrated a significant improvement on traditional dSPE cleanup in terms of matrix removal, PFAS recovery, and sample volume recovery. The method is also simpler, saving time and effort, and thus it improves overall lab productivity. The entire method was validated with acceptance criteria, and method performance was shown to meet the requirements described in AOAC SMPR 2023.003.

## References


1. EUR-Lex (**2023**) Consolidated Text: Commission Regulation (EU) 2023/915 of 25 April 2023 on Maximum Levels for Certain Contaminants in Food and Repealing Regulation (EC) No 1881/2006.
2. AOAC (**2023**) Standard Method Performance Requirements (SMPRs) for Per- and Polyfluoroalkyl Substances (PFAS) in Produce, Beverages, Dairy Products, Eggs, Seafood, Meat Products, and Feed (AOAC SMPR 2023.003).
3. Zhao, L.; Giardina, M.; Parry, E. Determination of 30 Per- and Polyfluoroalkyl Substances in Infant Formula, Milk, and Eggs Using Captiva EMR PFAS Food II Passthrough Cleanup and LC/MS/MS Detection, *Agilent Technologies application note*, publication number 5994-7366EN, **2024**.
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5. Genualdi, S.; Young, W.; Peprah, E.; *et al.* Analyte and Matrix Method Extension of Per- And Polyfluoroalkyl Substances in Food and Feed. *Anal. and Bioanal. Chem.* **2024**, *416*, 627–633. doi: 10.1007/s00216-023-04833-1.



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