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# Determination of 30 Per- and Polyfluoroalkyl Substances (PFAS) in Baby Food

## Using Captiva EMR PFAS Food I 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) in baby food. The method uses QuEChERS extraction, followed by Enhanced Matrix Removal (EMR) mixed-mode passthrough cleanup using the Agilent Captiva EMR PFAS Food I 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 novel Captiva EMR PFAS Food I cartridge was developed and optimized specifically for PFAS analysis in fresh and processed foods of plant origin. The method was validated based on the AOAC Standard Method Performance Requirements (SMPR) requirements, 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 baby food.

## 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, AOAC released the SMPR 2023.003 for the analysis of 30 PFAS in produce, beverages, dairy products, eggs, seafood, meat products, and feed.[2]

For food analysis, the sample preparation method plays a critical role for the entire method providing the efficient PFAS extraction and removal of matrix co-extractives. The large variety and high complexity of food matrices challenge the sample preparation method not only in terms of sample extraction and matrix cleanup efficiency but also the overall method simplicity, sample processing efficiency, and accommodation of different matrices. Weak anion exchange (WAX) sorbent-based solid phase extraction (SPE) methods have been used widely for PFAS analysis in environmental samples such as water and soil, as well as other matrices.[3, 4] However, the SPE method is challenging for sample preparation of complex solid food matrices, as food samples need to be extracted before loading into the cartridge. Also, the typical SPE procedure involving conditioning, equilibrium, loading, washing, and eluting requires a lot of time and solvent.

QuEChERS extraction followed with typical dispersive SPE (dSPE) cleanup has been reported for PFAS in food sample preparation.[5] However, dSPE cleanup does not provide efficient matrix removal for many food matrices, which cannot support the lower LOQ requirement in food. Thus, additional WAX SPE cleanup is added after dSPE cleanup.[5] This causes the method to be time consuming and labor intensive, which significantly impacts sample process productivity. This dSPE sample cleanup also results in loss of PFAS targets.

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. The objectives of this study were to develop and validate a complete workflow for the determination of 30 PFAS in baby food, which uses QuEChERS extraction followed by EMR mixed-mode passthrough cleanup using Captiva EMR PFAS Food I cartridge and detection with the Agilent 6495D LC/MS/MS.

## Experimental

### Chemicals and reagents

Native PFAS and isotopically labeled internal standard (ISTD) primary standard stock solutions were purchased from Wellington Laboratories (Ontario, CA). 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

Three native PFAS spiking solutions (I, II, and III) were prepared by diluting the native PFAS primary solutions with MeOH, at concentrations of 200, 20, and 2 ng/mL for 28 PFAS targets, respectively. The exceptions were for PFBA and PFPeA, where the concentrations were a factor of 10 and five times the concentration of the other 28 targets.

The ISTD spiking solution was prepared by diluting the ISTD primary solution with MeOH at a concentration of 100 ng/mL.

The native PFAS and ISTD spiking solutions were used for preparing neat calibration standards at 10, 20, 50, 100, 200, 500, 1,000, 2,000, and 5,000 ng/L for native PFAS targets and ISTD concentration of 1,000 ng/L in MeOH. They were also used for matrix prespiked quality control (QC) samples. All standards were stored at 4 °C and used for no more than 2 weeks.

The ACN with 1% acetic acid extraction solvent was prepared by adding 10 mL 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 MeOH.

### Equipment and materials

The study was performed using an Agilent 1290 Infinity II LC system consisting of a 1290 Infinity II high-speed pump (G7120A), a 1290 Infinity II multisampler (G7167B), and a 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 x 30 mm (part number 5062-8100). Chromatographic separation was performed using an Agilent ZORBAX RRHD Eclipse Plus C18 column, 95 Å, 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 I cartridges, 6 mL cartridges, 340 mg (part number 5610-2230)
- Polypropylene (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 5191-8150 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 for acceptable PFAS cleanliness.

## LC/MS/MS instrument conditions

The LC/MS/MS settings can be found in Agilent Technologies application note 5994-7366EN.[6]

## Sample preparation

Baby foods were purchased from local grocery stores, and contained multiple types of fruit and vegetables, such as apple, sweet potato, carrot, banana, beet, squash, blueberry, pear, blackberry, kiwi, kale, spinach, mango, chia, and peach. For consistent matrix sampling, the multiple bags of baby food were premixed thoroughly in a polypropylene bottle.

Baby food sample preparation used 10 g of sample for extraction. The native PFAS and ISTD spiking solutions were added to the QC samples appropriately, and only the ISTD spiking solution was added to the matrix blanks. The samples were vortexed for 10 to 15 seconds after spiking. The samples were then ready for the procedure, which is described in Figure 1.

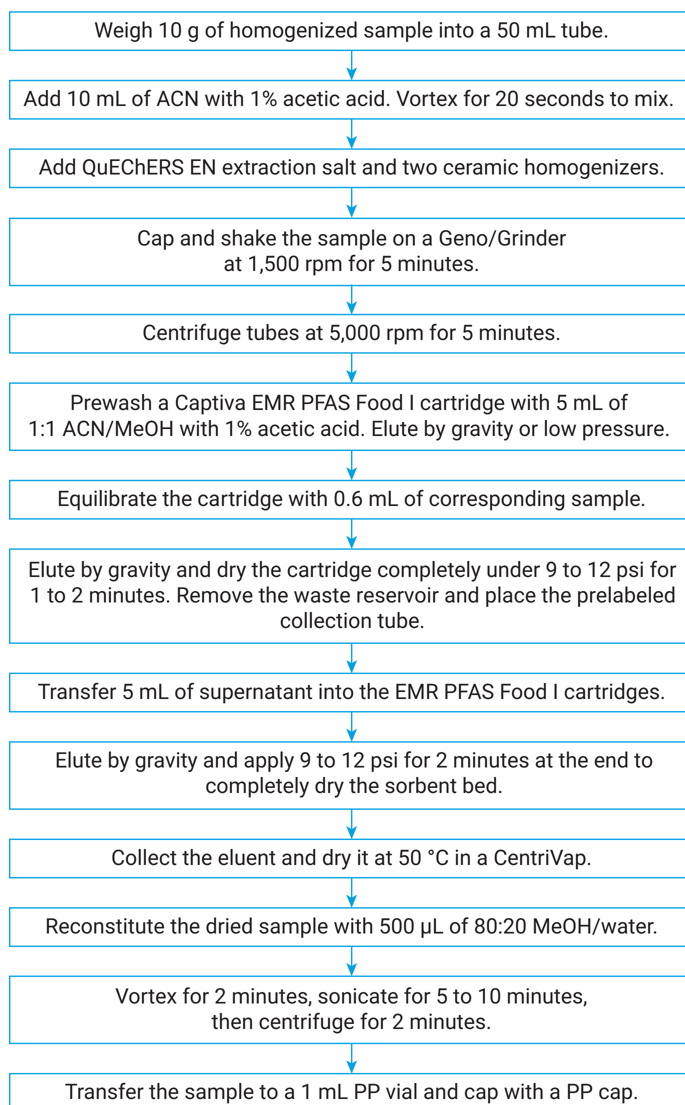


**Figure 1.** Sample preparation procedure for PFAS analysis in baby food by LC/MS/MS.

### Method performance evaluation

The EMR mixed-mode passthrough cleanup using Captiva EMR PFAS Food I cartridges was evaluated in terms of matrix removal, target recovery, and repeatability during sample cleanup with the cartridge. The entire method was then validated, which included a calibration study, method LOQ determination, and recovery and precision. Due to the different requirements of the target LOQs and the ultralow LOQ requirement for PFOA, PFOS, PFNA, and PFHxS in produce[1], seven prespiked QC-level samples were prepared in replicates of four or 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. The PFAS spiking levels for prespiked QC samples were 0.001, 0.002, 0.004, 0.01, 0.02, 0.1, and 0.2 µg/kg for 28 PFAS with 10 times the concentration for PFBA and 5 times concentration of PFPeA in baby food. The ISTD spiking level in all the prespiked QC samples and matrix blanks was 0.1 µg/kg.

## Results and discussion

### Enhanced matrix removal mixed-mode passthrough cleanup

The Captiva EMR PFAS Food cartridges provide comprehensive matrix removal after traditional QuEChERS extraction through a mixed-mode mechanism. Passthrough cleanup is 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 foods of plant origin, such as fruits and vegetables, baby food, and juices. The Captiva EMR PFAS Food II cartridges contain more sorbent with a more complex formulation, and are recommended for fresh and processed foods of animal origin, such as milk, eggs, meat, fish, infant formula, as well as some foods of plant origin like dry-seed feed and food, and oils.

Compared to traditional dSPE cleanup used after QuEChERS extraction, the EMR mixed-mode passthrough cleanup provided significant improvement on PFAS recovery and reproducibility. The PFAS recovery after using the Captiva EMR PFAS Food cartridge passthrough cleanup was evaluated in baby food crude extract after QuEChERS extraction, and was compared to typical dSPE cleanup. Figure 2 shows the comparison results based on the average recovery of each target in baby food extract, demonstrating significant improvement in recovery using EMR mixed-mode passthrough cleanup using Captiva EMR PFAS Food I cartridges compared to dSPE cleanup.

The matrix removal during sample cleanup was also evaluated using GC/MS full scan and LC/Q-TOF total ion chromatogram (TIC) scan, as shown in the chromatogram comparison in Figure 3. The results demonstrate significant improvement in matrix removal using EMR mixed-mode passthrough cleanup.

Besides the improvement on PFAS target recovery and matrix removal, another important feature provided by EMR mixed-mode passthrough cleanup is the increased sample volume recovery. Sample volume recovery is usually not a concern in other common food safety analyses, such as pesticides and vet drugs analyses; however, it can be critical for PFAS analysis in food since the required LOQs are in the low- to mid-ppt level. The ultralow LOQs require the use of a postconcentration step to boost method sensitivity. It is common to apply a 5 to 10 times postconcentration factor after sample cleanup using a dry-and-reconstitute step. As a result, the sample volume becomes important to achieve a high concentration factor and consistent reconstitution. Usually, the dSPE cleanup only provides ~ 50% of sample volume recovery, which means the cleaning of 5 mL sample extract can only generate ~ 2.5 mL cleaned sample volume. However, the EMR mixed-mode cleanup volume recovery is > 90%, which means the cleaning of 5 mL sample extract delivers ~ 4.5 mL of sample. This large volume provides easy postconcentration and consistent sample reconstitution.

EMR PFAS dSPE 1 dSPE 2

Average recovery % (n = 3)

0 20 40 60 80 100 120

PFBA PFBS PFPeA 4:2 FTA PFPeS HFPO-DA PFHxA PFHpA PFHxS DONA 6:2 FTSA PFOA PFHpS PFNA PFOS 9Cl-PF3ONS PFNS 8:2 FTS PFDA PFDS PFUnDA PFOSA 11Cl-PF3OUdS PFUnDS PFDoDA 10:2 FTS PFDoS PFTrDA PFTeDA PFTrDS

**Figure 2.** PFAS recovery in baby food after QuEChERS extraction using either EMR mixed-mode passthrough cleanup with Agilent Captiva EMR PFAS Food I cartridges or traditional dSPE cleanups.

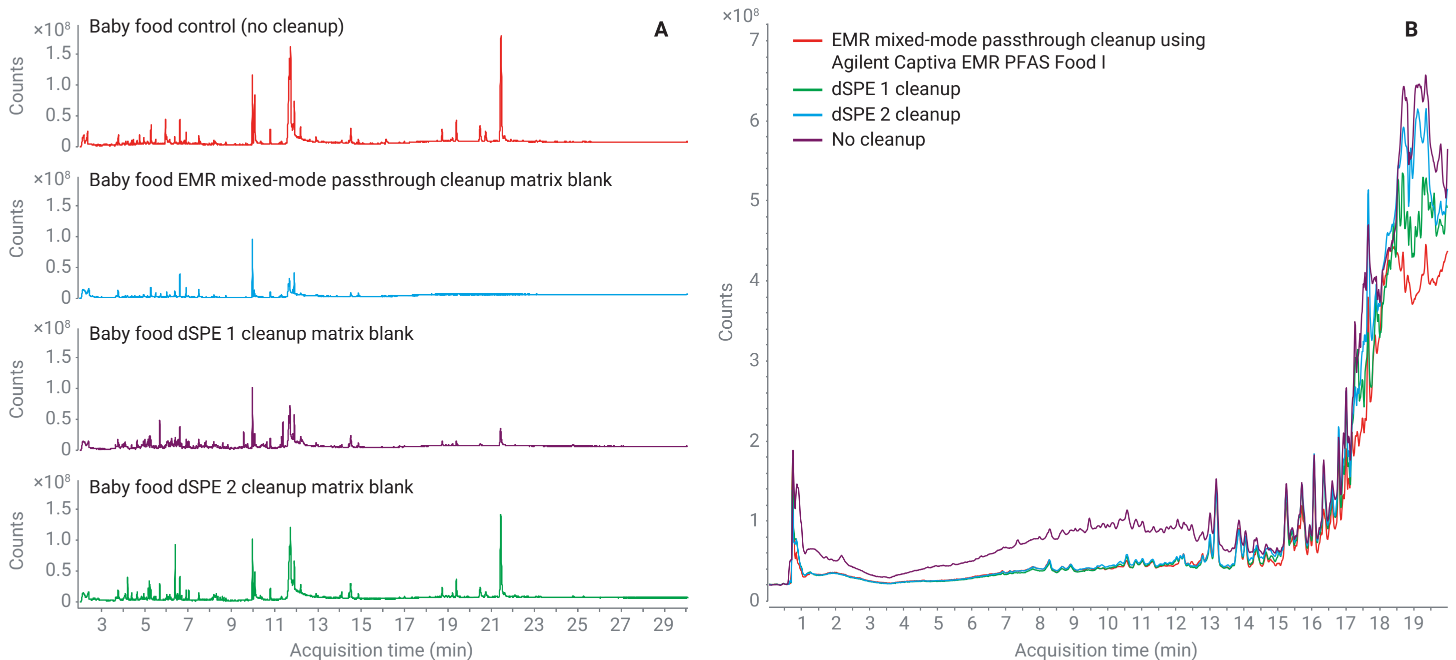


**Figure 3.** Baby food matrix removal comparison between EMR mixed-mode passthrough cleanup using Agilent Captiva EMR PFAS Food I cartridges versus traditional dSPE cleanups using GC/MS full scan (A) and LC/Q-TOF TIC + scan (B).

## 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, while the traditional method includes three major processes: QuEChERS extraction, dSPE cleanup, and WAX SPE extraction.[5]

Figure 4 shows a comparison of the two sample preparation methods. The WAX SPE step used in the traditional method was added to further clean the sample extract after dSPE cleanup.[5] However, the SPE method is challenging to implement with the previous sample extraction and dSPE cleanup steps. The crude organic (ACN) extract needs to be diluted to a solution containing 90% water before loading on the cartridge, either by drying and reconstituting in a highly aqueous solution, or by direct dilution with water, resulting in large sample loading, which is time- and effort-consuming for just the sample loading step. The typical SPE procedure involving conditioning, equilibrium, loading, washing, and eluting also requires more time and uses more solvent. Given the same sample quantity for preparation, the traditional method can take up to triple the time of the new method. Also, less solvents and fewer consumables are used in the new method compared to the traditional method. Collectively, these benefits of the new method can improve overall lab productivity.

Even for the simple, fresh food samples of plant origin (where the WAX SPE step can be skipped), the use of EMR mixed-mode passthrough cleanup still provides greater simplicity than traditional dSPE cleanup by obsoleting multiple steps like uncapping and capping, transferring sample, centrifuging, and more. These benefits also improve overall productivity.

## Entire method validation

The new method was validated for the determination of 30 PFAS targets in baby food by following the AOAC SMPR guidance. The method needed to meet the requirements for PFAS target LOQs, which were ≤ 0.01 µg/kg for the core PFAS targets; ≤ 1 µg/kg for PFBA and PFPeA; and ≤ 0.1 µg/kg for the remaining PFAS targets.

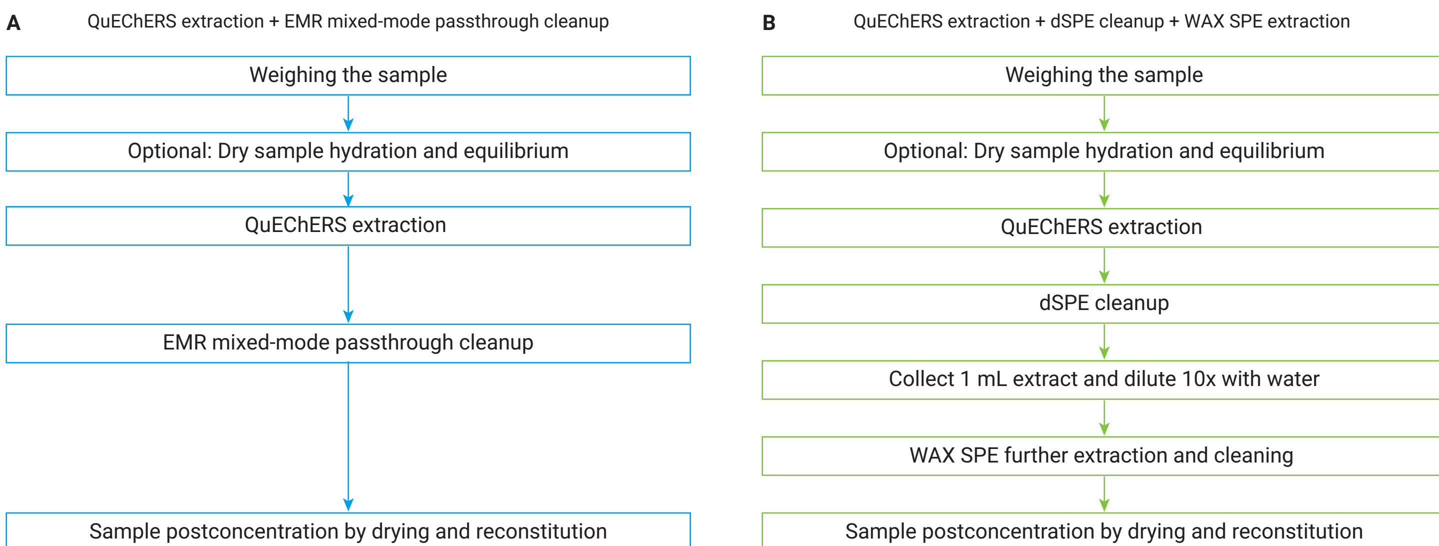


**Figure 4.** Sample preparation methods procedure comparison for PFAS in food analysis using the new method (A) versus the traditional method (B).

## Method LOQs and validation levels

The baby food matrices evaluated in this study all showed positive detection in matrix blanks. Different baby foods were screened, and those with the lowest PFAS background were used as the control for method validation. However, matrix background correction was still necessary, and was used for method validation for target recovery. Matrix blanks were prepared in five to seven replicates. The lowest method reportable LOQs were calculated based on the matrix blanks detection according to Equation 1.

**Equation 1.**

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

Where:

- $LOQ_{cal}$ is the method's lowest reportable LOQ
- $SD_{MBs}$ is the standard deviation (SD) of detected 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 1 shows the calculated lowest reportable LOQs and validated method LOQs for each target in baby food. The mid and high levels for validation are also listed in Table 1.

For the core PFAS targets, the validated method LOQs were demonstrated to be below or equal to the required LOQs in baby food. The validated LOQs for PFNA, PFOS, and PFHxS also met the EU LOQ regulations, which are 0.001 µg/kg for PFOA and PFNA, 0.002 µg/kg for PFOS, and 0.004 µg/kg for PFHxS. Both PFBA and PFBS were detected in the matrix blank, resulting in higher validated LOQs for these two targets in baby food. PFOA also showed positive matrix contribution, resulting in failure of validation at the 0.001 µg/kg level for LOQ. Figure 5 shows the chromatograms of matrix blanks and validated method LOQs for the core targets in baby food.

**Table 1.** Method lowest reportable calculated LOQ ($LOQ_{cal}$), validated LOQ ($LOQ_{val}$), and mid and high levels for validation for 30 PFAS targets in baby food.

| Target | $LOQ_{cal}$ (µg/kg) | $LOQ_{val}$ (µg/kg) | Mid $Level_{val}$ (µg/kg) | High $Level_{val}$ (µg/kg) |
|---|---|---|---|---|
| PFBA | 0.783 | 1 | 2 | – |
| PFPeA | 0.007 | 0.01 | 0.1 | 1 |
| PFBS | 0.038 | 0.1 | 0.2 | – |
| 4:2 FTS | 0.002 | 0.01 | 0.02 | 0.2 |
| PFPeS | 0.003 | 0.004 | 0.01 | 0.2 |
| PFHxA | NA | 0.002 | 0.01 | 0.2 |
| HFPO-DA | NA | 0.001 | 0.01 | 0.2 |
| PFHpA | 0.002 | 0.001 | 0.01 | 0.2 |
| PFHxS* | 0.001 | 0.002 | 0.01 | 0.2 |
| DONA | NA | 0.001 | 0.01 | 0.2 |
| 6:2 FTS | 0.001 | 0.001 | 0.01 | 0.2 |
| PFOA* | 0.002 | 0.002 | 0.01 | 0.2 |
| PFHpS | NA | 0.004 | 0.01 | 0.2 |
| PFNA* | 0.001 | 0.001 | 0.01 | 0.2 |
| PFOS* | 0.001 | 0.001 | 0.01 | 0.2 |
| 9Cl-PF3ONS | NA | 0.002 | 0.01 | 0.2 |
| 8:2 FTS | NA | 0.001 | 0.01 | 0.2 |
| PFNS | 0.001 | 0.001 | 0.01 | 0.2 |
| PFDA | 0.001 | 0.001 | 0.01 | 0.2 |
| PFDS | NA | 0.01 | 0.02 | 0.2 |
| PFUnDA | 0.001 | 0.002 | 0.01 | 0.2 |
| PFOSA | 0.001 | 0.01 | 0.02 | 0.2 |
| 11Cl-PF3OUdS | NA | 0.002 | 0.01 | 0.2 |
| PFUnDS | 0.002 | 0.01 | 0.1 | 0.2 |
| PFDoDA | 0.002 | 0.004 | 0.01 | 0.2 |
| 10:2 FTS | NA | 0.002 | 0.01 | 0.2 |
| PFDoS | NA | 0.01 | 0.02 | 0.2 |
| PFTrDA | NA | 0.002 | 0.01 | 0.2 |
| PFTrDS | NA | 0.02 | 0.1 | 0.2 |
| PFTeDA | 0.003 | 0.01 | 0.02 | 0.2 |

* Core PFAS targets

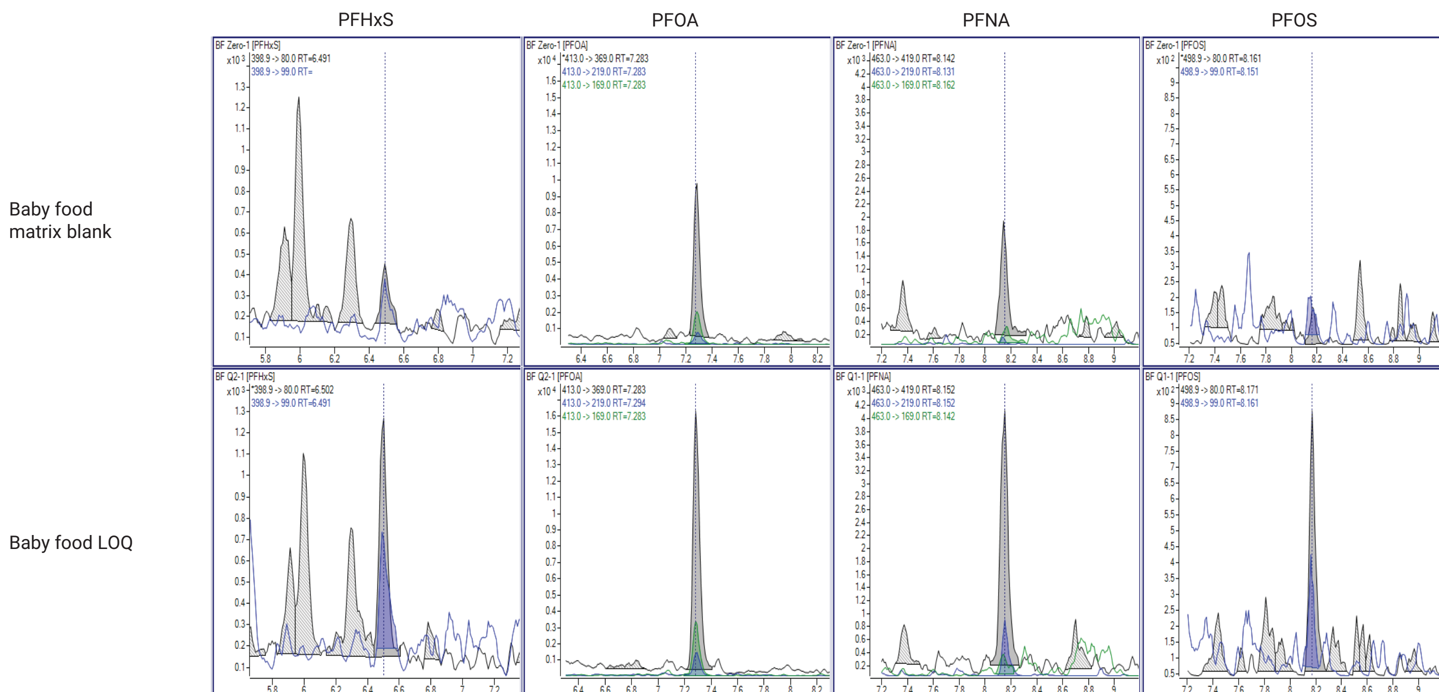


**Figure 5.** Baby food matrix blanks and LOQ chromatograms for the core PFAS targets: PFHxS (0.002 µg/kg), PFOA (0.002 µg/kg), PFNA (0.001 µg/kg), and PFOS (0.001 µg/kg).

## 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, saving time and costs, and improving 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 lower LOQ levels required in baby food, a calibration set range from 10 to 5,000 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. Figure 6 shows the calibration curves of the core PFAS targets—PFHxS, PFOA, PFNA, and PFOS—established in the dynamic range.

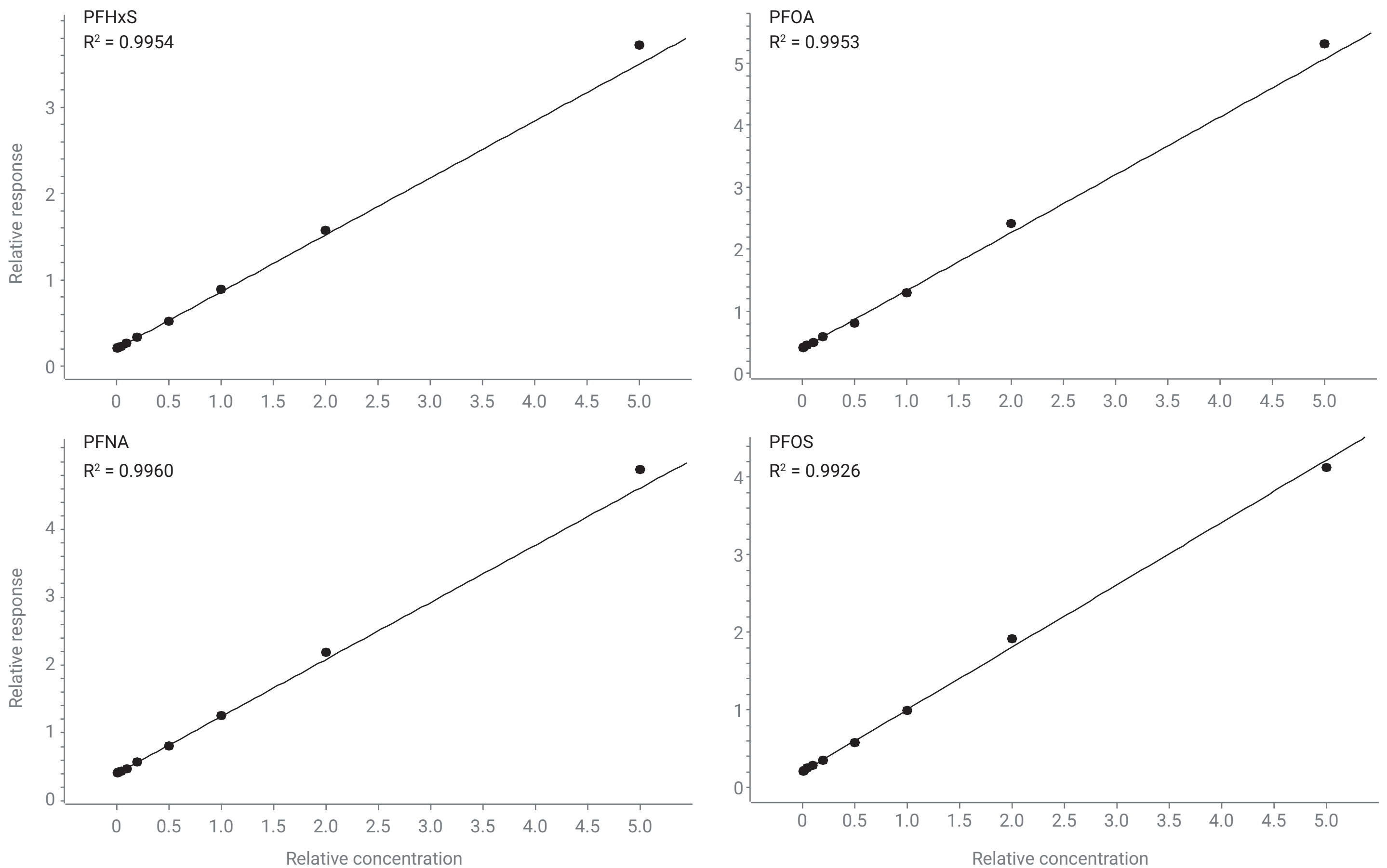


**Figure 6.** Calibration curves for core PFAS targets with a dynamic range of 10 to 5,000 ng/L in MeOH.

## Method accuracy and precision

Method recovery and repeatability were validated. Seven prespiking QC levels were designed to accommodate the different LOQ requirements on the core PFAS targets, PFBA and PFPeA, and the rest of the PFAS targets in food. For each QC level, four to five replicates were prepared for method repeatability evaluation. The acceptance criteria for baby food is 65 to 135% recovery and ≤ 25% RSD.[2] The three levels of prespiked QCs were reported for method validation, including LOQ and mid and high levels. Table 1 lists the detailed concentration for each analyte. There were two exceptions: PFBA and PFBS, where only two levels were reportable due to significantly high positive occurrence in sample matrix control.

Figure 7 shows the method validation recovery and repeatability (RSD) summary for PFAS analysis in baby food. Overall, the method delivered acceptable recovery and repeatability results for all 30 targets in tested food matrices that meet the acceptance criteria. Targets with corresponding isotopically labeled ISTDs generated better quantitation results than targets without corresponding labeled ISTDs.

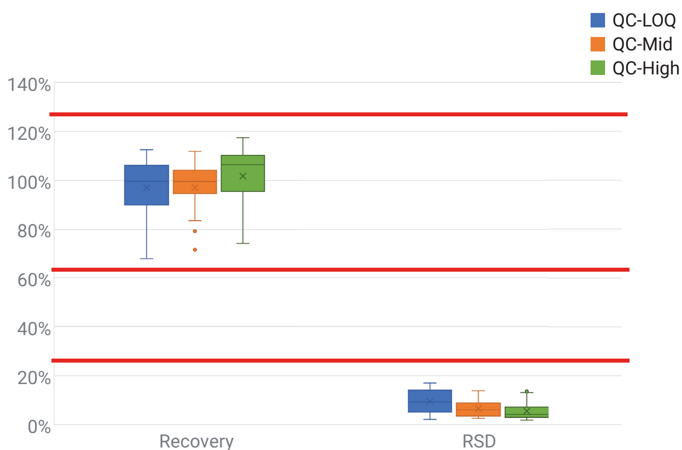


**Figure 7.** Method validation recovery and repeatability (RSD%) summary for PFAS analysis in baby food.

## Conclusion

A simplified, rapid, and reliable method using QuEChERS extraction followed by EMR mixed-mode passthrough cleanup with the Agilent Captiva EMR PFAS Food I cartridge and LC/MS/MS detection was developed and validated for 30 PFAS targets in baby food. The novel cleanup method demonstrated significant improvement on traditional dSPE cleanup in terms of matrix removal, PFAS recovery, and sample volume recovery over the traditional dSPE cleanup. It also features a simplified sample cleanup method, saving time and effort, and thus improves overall lab productivity. The entire method was validated with acceptance performance that meets 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. EPA Method 533: Determination of Per- and Polyfluoroalkyl Substances in Drinking Water by Isotope Dilution Anion Exchange Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (EPA 533:2019).
4. EPA Method 1633: Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/MS (EPA 1633:2024).
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.
6. Determination of 30 Per- and Polyfluoroalkyl Substances (PFAS) in Infant Formula, Milk, and Eggs Using Captiva EMR PFAS Food II Passthrough Cleanup by LC/MS/MS Detection, *Agilent Technologies*, publication number 5994-7366EN.


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