Application Note

Food & Beverage Testing

Agilent

Trusted Answers

Determination of 30 Per- and Polyfluoroalkyl Substances in Infant Formula, Milk, and Eggs

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

Authors

Limian Zhao,
Matthew Giardina, and
Emily Parry
Agilent Technologies, Inc.

Abstract

This application note presents the development and validation of a multiresidue method for the analysis of per- and polyfluoroalkyl substances (PFAS) in infant formula, milk, and eggs. The method uses QuEChERS extraction followed by EMR mixed-mode passthrough cleanup using an Agilent Captiva EMR PFAS Food II cartridge, then LC/MS/MS detection. The method features a simplified and efficient sample preparation, sensitive LC/MS/MS detection, and reliable quantitation using neat standard calibration curves. The novel Captiva EMR PFAS Food II cartridge was developed and optimized specifically for PFAS analysis in foods of animal origin as well as seeded dry foods of plant origin. The method was validated based on the AOAC Standard Method Performance Requirements (SMPR) 2023.003, including method suitability, sensitivity, accuracy, and precision. The method was demonstrated to meet the required limits of quantitation (LOQs), recovery, and repeatability (RSD) for four core PFAS targets—perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), and perfluorohexane sulfonic acid (PFHxS)—and 26 remaining PFAS targets in infant formula, milk, and eggs.

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]

Methods based on LC/MS/MS for PFAS analysis have been widely applied for environmental water and soil analyses.[3,4] The acidic groups contained in PFAS compounds enable them to be ionized easily and efficiently under negative mode, providing advantages for method sensitivity and selectivity.

For food analysis, the sample preparation method plays a critical role for efficient PFAS extraction, removal of matrix co-extractives, and sample concentration or dilution when needed. 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. However, the SPE methodology is challenging for sample preparation of complex solid food, 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 by typical dispersive SPE (dSPE) cleanup has been reported for PFAS in food sample preparation.[5,6] However, dSPE cleanup does not provide efficient matrix removal for many food matrices, which cannot support the lower LOQ requirement in food. Thus, another cleanup step using WAX SPE is added after dSPE cleanup.[5] This causes the method to be time consuming and labor intensive, which significantly impacts sample process productivity. This type of sample cleanup can also result in loss of PFAS targets.

Agilent Captiva EMR PFAS Food cartridges were developed and optimized specifically for PFAS analysis in food, providing comprehensive mix-mode passthrough cleanup. Two types of cartridges (I and II) were designed to cover the large variety of food matrices. The objective of this study was to develop and validate a complete workflow for the determination of 30 PFAS in infant formula, milk, and eggs. The method uses QuEChERS extraction followed by passthrough cleanup with 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 (AA) 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 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 two times the concentration of the other 28 targets, respectively.

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 isotopic ISTD spiking solutions were used for preparing neat calibration standards at 20, 50, 100, 200, 500, 1,000, 2,000, 5,000, and 10,000 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 two weeks.

The ACN with 1% AA extraction solvent was prepared by adding 10 mL glacial AA into 990 mL of ACN and storing it at room temperature. LC mobile phase A was 5 mM NH_4OAc in water, and mobile phase B was MeOH.

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), 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 (ESI) 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, 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, 750 mg (part number 5610-2232)
- Polypropylene (PP) snap caps and vials, 1 mL (part number 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 binary pump conditions are listed in Table 1 and the multisampler program is listed in Table 2. The column temperature was set at 55 ± 0.8 °C. MS data were acquired in negative ion mode with a constant fragmentor setting of 166 V. The ESI source settings were: drying gas at 150 °C, 18 L/min; sheath gas at 390 °C, 12 L/min; nebulizer gas at 15 psi; capillary voltage at 2,500 V; and nozzle voltage at 0 V.

Table 1. LC pump conditions for LC/MS/MS.

Parameter	Setting			
Mobile Phase A	5 mM NH_4OAc in water			
Mobile Phase B	MeOH			
Gradient	Time (min)	%A	%B	Flow (mL/min)
	0.00	98.00	2.00	0.400
	2.00	98.00	2.00	0.400
	2.50	45.00	55.00	0.400
	6.50	30.00	70.00	0.400
	8.00	20.00	80.00	0.460
	14.20	0.00	100.00	0.460
	17.00	0.00	100.00	0.400
	17.10	98.00	2.00	–
Post Time	3.0 min			

Table 2. LC multisampler program for LC/MS/MS.

Parameter	Setting				
Injection Program	Draw 10 µL water				
	Draw 10 µL				
	Wash needle				
	Draw 50 µL water				
	Mix 10 µL from air five times				
	Inject				
Multiwash	**Step**	**Solvent**	**Time (s)**	**Seat Backflush**	**Needle Wash**
	1	IPA	10	Enabled	Enabled
	2	ACN	10	Enabled	Enabled
	3	Water	10	Enabled	Enabled
	Starting Conditions	Water	NA	Enabled	Enabled

Sample preparation

Infant formula, milk, and egg samples were purchased from local grocery stores. Fresh milk and infant formula samples were used directly for extraction. Fresh eggs were broken and mixed thoroughly in a polypropylene bottle before extraction.

For sample preparation of infant formula, a 5 g sample was used for extraction; for milk and egg samples, a 10 g sample was used for extraction. The native PFAS and ISTD spiking solutions were added to the QC samples appropriately, and only ISTD was added to 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.

Table 3. MS acquisition conditions (on an Agilent 6495D LC/MS system) for PFAS targets, ISTDs, and cholic acid interference monitoring.

Compound	RT (min)	Precursor Ion (*m/z*)	Product Ion (*m/z*)	Collision Energy (V)	Collision Cell Accelerator (V)	iFunnel Mode
PFBA	4.78	213	169	8	2	Standard
PFPeA	5.29	263	219	4	2	Standard
PFHxA	5.93	313	269 119	8 24	2	Standard
PFHpA	6.72	363	319 169	8 16	2	Standard
PFOA	7.6	413	369 219	8 16	2	Standard
PFNA	8.51	463	419 219 169	8 16 20	2	Standard
PFDA	9.3	513	469 269 219	12 16 20	2	Standard
PFUnDA	9.88	563	519 319 269	12 20 20	2	Standard
PFDoA	10.35	613	569 319 269	8 20 24	2	Standard
PFTrDA	10.77	663	619 319 169	12 20 32	2	Standard
PFTeDA	11.17	712.9	669 219 169	12 28 32	2	Standard
PFBS	5.39	298.9	99 80	34 36	2	Standard
PFPeS	5.99	348.9	99 80	40 44	2	Standard
PFHxS	6.76	398.9	99 80	40 56	2	Standard
PFHpS	7.63	448.9	99 80	42 50	2	Standard
PFOS	8.50	498.9	99 79.9	50 54	2	Standard
PFNS	9.29	548.9	99 80	52 56	2	Standard
PFDS	9.86	598.9	99 80	56 60	2	Standard
PFUnDS	10.31	648.9	99 79.8	56 76	2	Standard
PFDoS	10.73	698.9	99 80	62 67	2	Standard
PFTrDS	11.13	748.9	98.9 79.6	64 80	4	Standard
PFOSA	10.0	497.9	169 78 48	36 36 110	3	Standard
9Cl-PF3ONS	9.03	530.9	350.9 83	28 32	3	Standard
11Cl-PF3OUdS	10.14	630.9	450.9 83	36 32	2	Standard
HFPO-DA	6.15	285	185 169 119	20 4 32	5	Fragile
DONA	6.83	377	251 85	8 32	5	Standard
4:2 FTS	5.87	327	307 81 80	20 36 42	2	Standard
6:2 FTS	7.55	427	407 81 80	30 32 58	2	Standard
8:2 FTS	9.29	527	507 81 80	30 46 50	4	Standard
10:2 FTS	10.35	627	606.9 81 80	34 42 54	4	Standard
$^{13}C_2$-4:2 FTS	5.87	329	309	24	2	Standard
$^{13}C_2$-6:2 FTS	7.55	429	409	28	2	Standard
$^{13}C_2$-8:2 FTS	9.29	529	509	28	4	Standard
$^{13}C_2$-PFDoA	10.35	615	570	12	2	Standard
$^{13}C_2$-PFTeDA	11.17	715	670	12	2	Standard
$^{13}C_3$-HFPO-DA	6.15	287	169	4	5	Standard
$^{13}C_3$-PFBS	5.39	302	80	44	2	Standard
$^{13}C_3$-PFHxS	6.76	402	80	48	2	Standard
$^{13}C_4$-PFBA	4.78	217	172	8	2	Standard
$^{13}C_4$-PFHpA	6.72	367	322	8	2	Standard
$^{13}C_5$-PFHxA	5.93	318	273	8	2	Standard
$^{13}C_5$-PFPeA	5.29	268	223	4	2	Standard
$^{13}C_6$-PFDA	9.3	519	474	8	2	Standard
$^{13}C_7$-PFUnDA	9.88	570	525	8	2	Standard
$^{13}C_8$-PFOA	7.6	421	376	8	2	Standard
$^{13}C_8$-PFOS	8.52	507	80	54	2	Standard
$^{13}C_8$-PFOSA	10	506	78	36	3	Standard
$^{13}C_9$-PFNA	8.51	472	427	8	2	Standard
TUDCA	6.8	498	124 80	53 80	4	Standard
TCDCA	8.6	498	124 80	65 80	4	Standard
TDCA	9.0	498	124 80	69 80	4	Standard

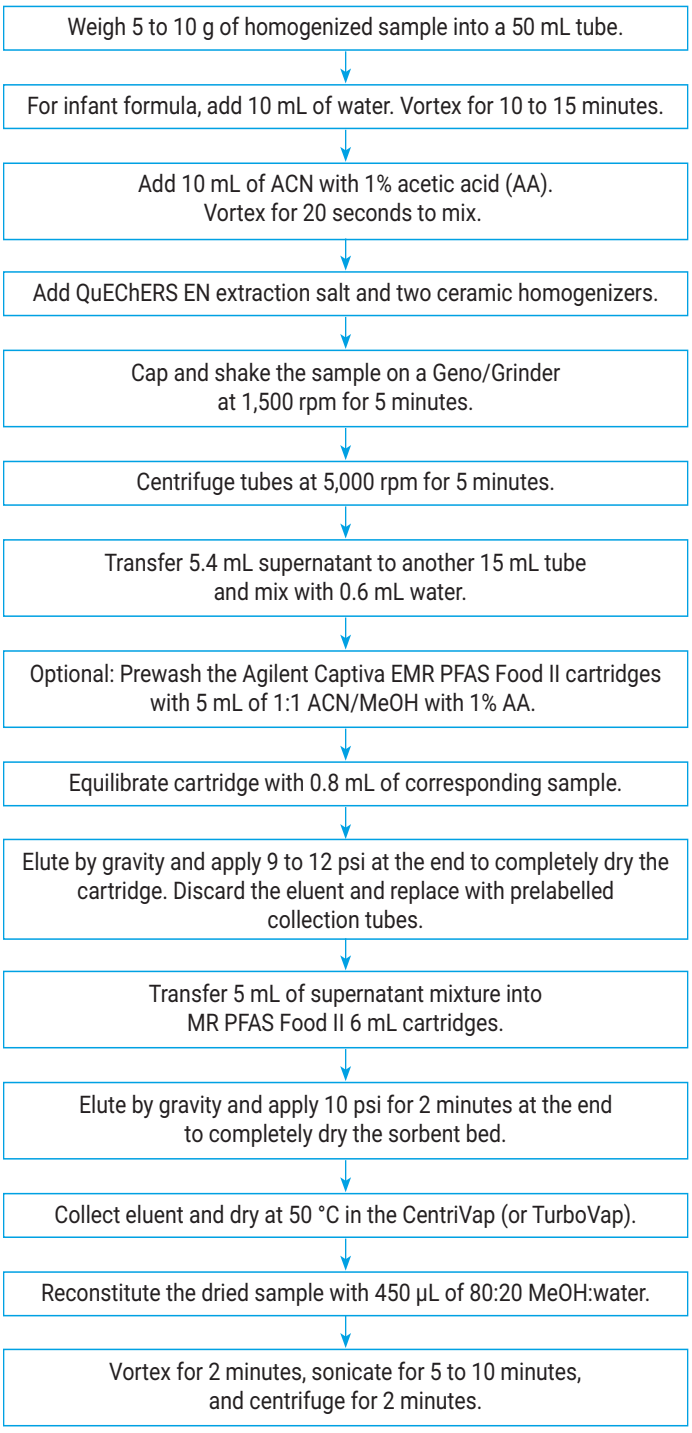

Figure 1. Sample preparation procedure for PFAS analysis in infant formula, milk, and eggs.

Method performance evaluation

The novel passthrough cleanup using Captiva EMR PFAS Food II 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 accuracy and precision. Due to the different requirements of the target LOQs, five 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. Table 4 shows the matrix zero blanks and prespiked QCs with PFAS standard and ISTD spiking. Depending on the different concentration factors introduced through sample preparation, the actual spiking levels in the matrices varied.

Table 4. Matrix-matched QC and matrix-zero samples in group II food matrices.

	Infant Formula		Milk		Eggs	
Sample Size (g)	5		10		10	
Concentration Factor	5x		10x		10x	
Matrix Spiked Samples	Spiking Concentration (µg/kg)					
	STD*	ISTD	STD*	ISTD	STD*	ISTD
Zero	–	0.2	–	0.1	–	0.1
PR-QC 1	0.01	0.2	0.01	0.1	0.01	0.1
PR-QC 2	0.02	0.2	0.02	0.1	0.02	0.1
PR-QC 3	0.1	0.2	0.1	0.1	0.1	0.1
PR-QC 4	0.4	0.2	0.2	0.1	0.2	0.1
PR-QC 5	1.0	0.2	0.5	0.1	0.5	0.1

* Concentrations only indicate 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 through a mixed-mode passthrough cleanup. It is a simple and 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, as well as some foods of plant origin like dry seed feed and food, and oils.

Compared to a traditional dSPE cleanup used after QuEChERS extraction, EMR mixed-mode passthrough cleanup provided significant improvement on PFAS recovery and reproducibility. The PFAS recovery using the EMR passthrough cleanup was evaluated in representative food sample extracts including grape, baby food, infant formula, tuna, eggs, and soybean crude extract after QuEChERS extraction, and was compared to typical dSPE cleanup. Captiva EMR PFAS Food I cartridges were used for baby food and grape extract cleanup, and Captiva EMR PFAS Food II cartridges were used for soybean, infant formula, tuna and egg extract cleanup. Figure 2 shows the comparison results based on the average recovery of each target in each food matrix, demonstrating significant improvement in recovery using EMR mixed-mode passthrough cleanup compared to dSPE cleanup.

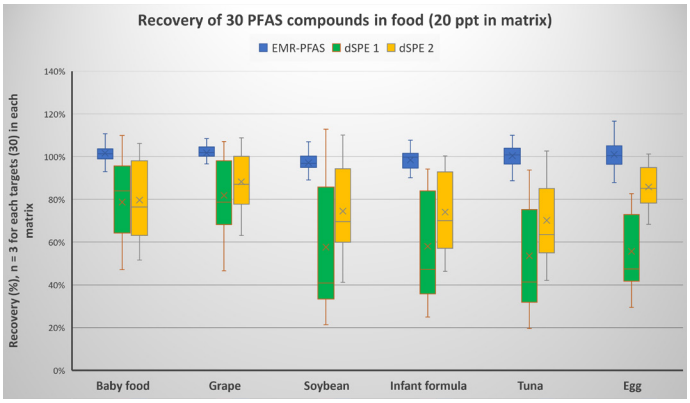

Figure 2. PFAS recovery comparison for food extract cleanup methods using either EMR mixed-mode passthrough cleanup or traditional dSPE cleanup.

The matrix removal during sample cleanup was also evaluated using a GC/MS full scan and an LC/Q-TOF total ion chromatogram (TIC) scan, as shown in the chromatogram comparison in Figures 3 and 4, respectively. The results demonstrate significant improvement in matrix removal using EMR mixed-mode passthrough cleanup.

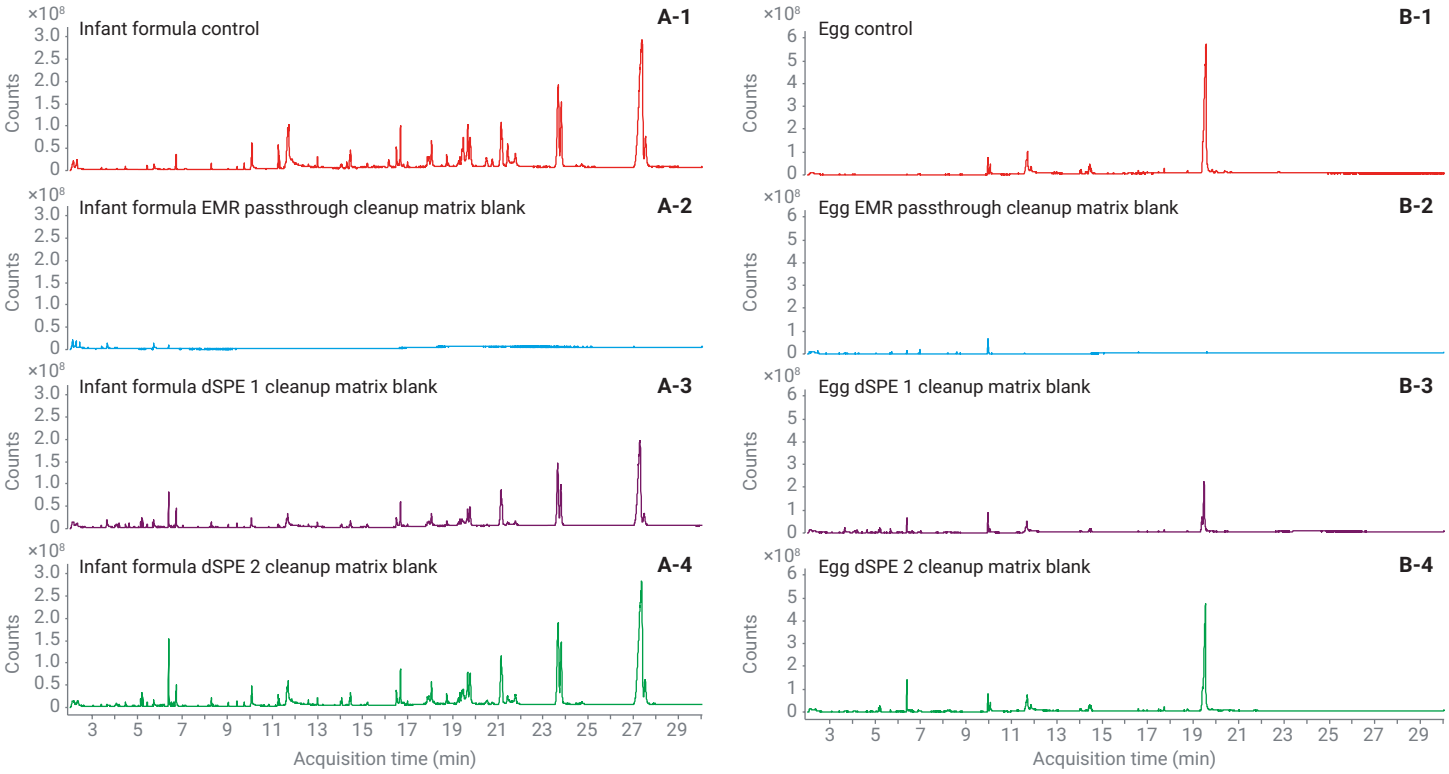

Figure 3. Food matrix removal comparison between EMR mixed-mode passthrough cleanup versus traditional dSPE cleanups using GC/MS full scan for (A) infant formula sample and (B) egg sample for matrix blanks with (1) no cleanup, (2) EMR passthrough cleanup, (3) dSPE 1 cleanup, and (4) dSPE 2 cleanup.

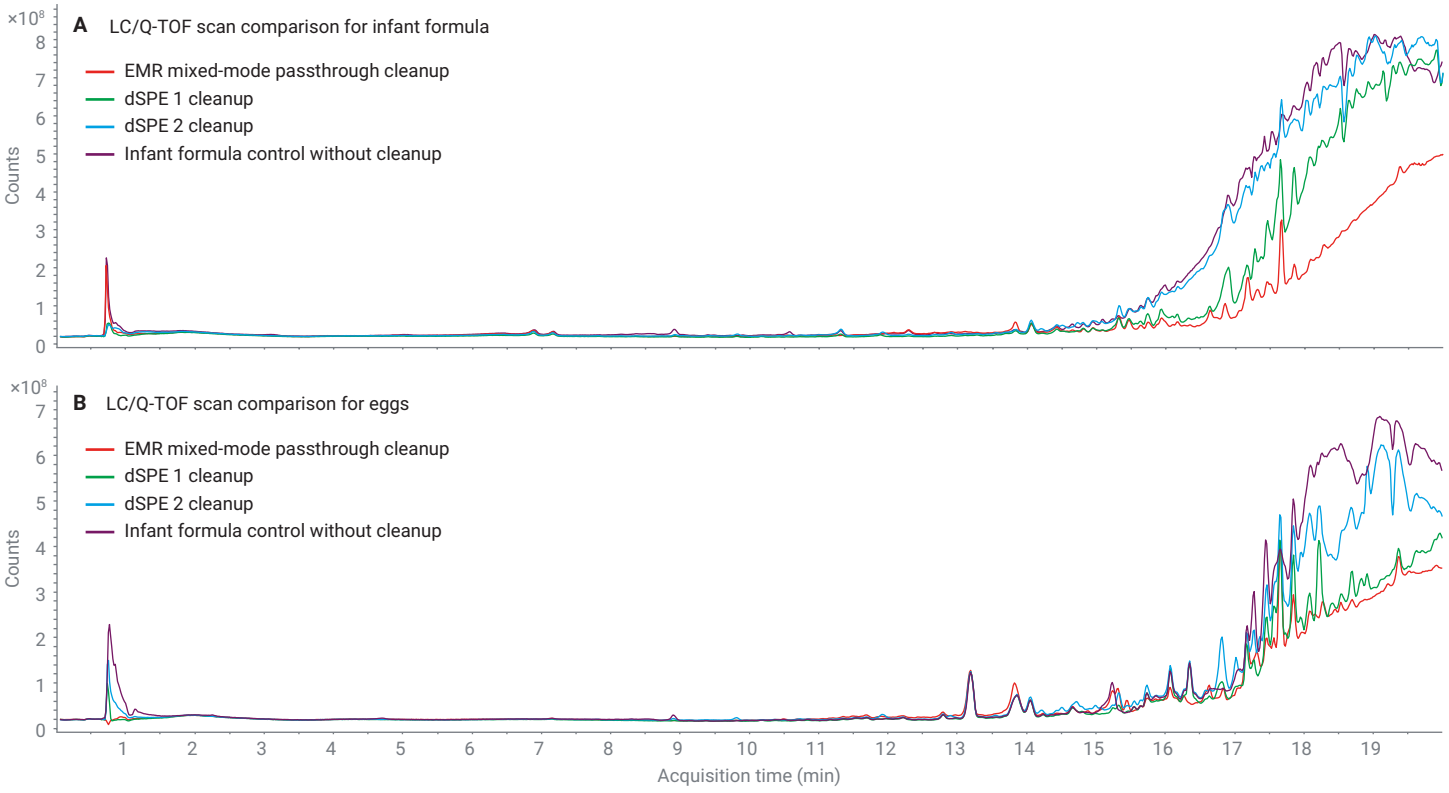

Figure 4. Food matrix removal comparison between EMR mixed-mode passthrough cleanup versus traditional dSPE cleanups using LC/Q-TOF TIC (+) scan for infant formula sample (A) and egg sample (B).

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 pesticide and vet drug 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 the 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% 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.

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 5 shows a comparison of the two sample preparation method procedures. The WAX SPE step used in the traditional method was added to further clean the sample extract after dSPE cleanup. 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 switched in a solution containing at least 90% water before loading on the cartridge. This can be done by either drying and reconstituting in a highly aqueous solution, or by direct dilution with water, which either significantly increases sample loading time or the preparation time for loading in the WAX cartridges. 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, fewer solvents and consumables are used in the new method compared to the traditional. Collectively, these benefits of the new method can improve overall lab productivity.

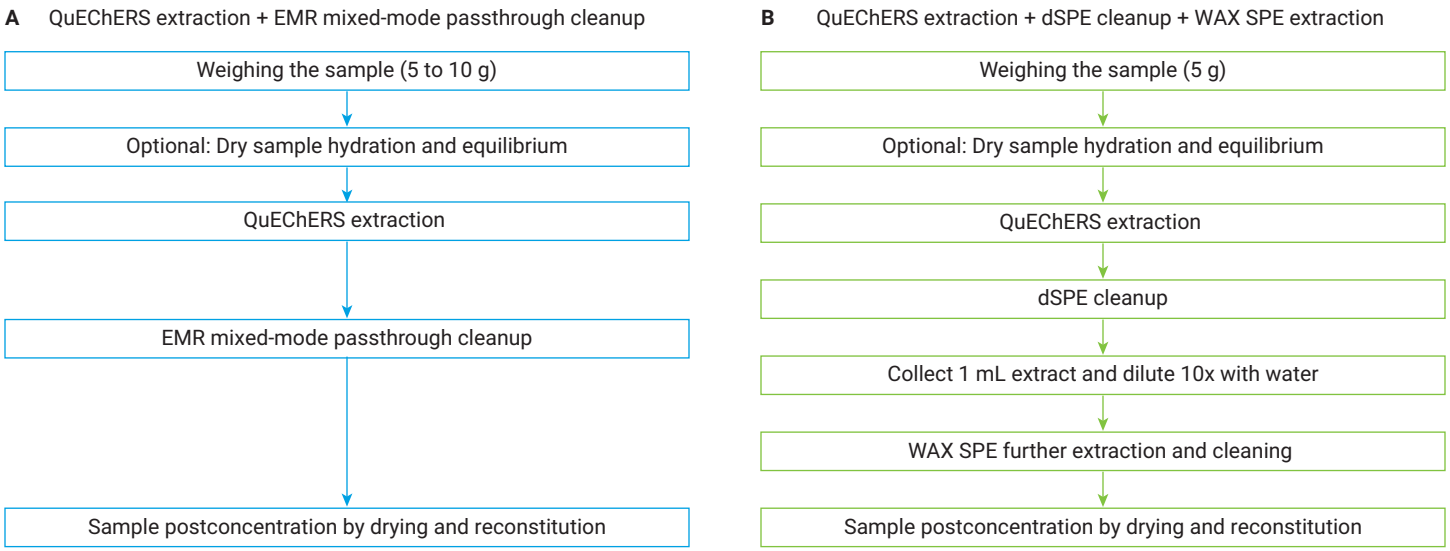

Figure 5. Sample preparation procedure comparison for PFAS in food analysis using the newly developed method (A) versus the traditional method (B).

Entire method validation

The new method was validated for the determination of 30 PFAS targets in infant formula, milk, and eggs following the AOAC SMPR guidance. The requirements for PFAS target LOQs for the tested food matrices are listed in Table 5.

Table 5. AOAC SMPR requirements for LOQs in infant formula, milk, and eggs.

Food Matrix	LOQ (µg/kg)		
	PFHxS, PFOA, PFNA, and PFOS	PFBA and PFPeA	Other PFAS
Infant Formula	≤ 0.01	≤ 1	≤ 0.1
Eggs	≤ 0.3	≤ 3	≤ 3
Whole Milk	≤ 0.01	≤ 1	≤ 0.1

Method LOQs

The three food matrices evaluated in the study all showed positive detection in matrix blanks. As a result, matrix background correction was necessary and was used in method validation for target recovery calculation. Matrix blanks were prepared in five to seven replicates, then the lowest method reportable LOQs 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 (LOQ)
- SD_{MBs} is the standard deviation (SD) of detected incurred targets from five to seven replicates of matrix blanks

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 6 shows the calculated lowest reportable LOQ_{cal} and validated method LOQ_{val} for each target in each matrix.

For the core PFAS targets PFHxS, PFNA, and PFOS, the validated method LOQs were demonstrated to be below or equal to the required LOQs in all three tested matrices. The validated method LOQ for PFOA was below or equal to the required LOQs listed in milk and eggs. In infant formula, it was higher than the required LOQs due to matrix positive occurrence. For other PFAS targets, the validated method LOQs were demonstrated to be below or equal to the required LOQs in all three matrices, except higher LOQs for 6:2 FTS in milk due to positive occurrence. The PFOS LOQ in eggs was higher than other core PFAS targets due to the significant high interference of TCDCA at qualifier transition 498.9 to 79.9, which resulted in the failure of qualifier ratio identification at lower levels. However, the validated method LOQ (0.1 µg/kg) was still below the required 0.3 µg/kg LOQ in eggs.

Figure 6 shows the chromatograms of matrix blanks and validated method LOQs for the core PFAS targets in infant formula, milk, and eggs.

Table 6. Method lowest reportable calculated LOQ (LOQ_{cal}) and validated LOQ (LOQ_{val}) for 30 PFAS targets in infant formula, milk, and eggs.

Target	Infant Formula		Milk		Egg	
	LOQ_{cal}	LOQ_{val}	LOQ_{cal}	LOQ_{val}	LOQ_{cal}	LOQ_{val}
PFBA	NA	0.1	NA	0.1	0.147	0.2
PFPeA	0.005	0.02	0.003	0.02	0.011	0.02
PFBS	0.002	0.01	0.001	0.01	NA	0.01
4:2 FTS	NA	0.01	NA	0.01	NA	0.01
PFPeS	NA	0.02	NA	0.02	0.005	0.01
PFHxA	NA	0.01	NA	0.01	NA	0.01
HFPO-DA	NA	0.01	NA	0.01	NA	0.01
PFHpA	0.001	0.01	0.002	0.01	0.001	0.01
PFHxS*	0.004	0.01	0.002	0.01	0.001	0.01
DONA	0.001	0.01	0.045	0.1	NA	0.01
6:2 FTS	0.007	0.01	0.322	0.5	0.006	0.01
PFOA*	0.016	0.02	0.004	0.01	0.006	0.01
PFHpS	NA	0.01	NA	0.01	NA	0.01
PFNA*	0.005	0.01	0.002	0.01	0.003	0.01
PFOS*	0.002	0.01	0.001	0.01	0.003	0.1
9Cl-PF3ONS	NA	0.01	NA	0.01	NA	0.01
8:2 FTS	NA	0.01	NA	0.01	NA	0.01
PFNS	0.006	0.01	0.002	0.01	0.001	0.01
PFDA	NA	0.01	NA	0.01	NA	0.01
PFDS	0.002	0.01	0.002	0.01	0.001	0.01
PFUnDA	NA	0.01	NA	0.01	NA	0.01
PFOSA	0.001	0.01	0.001	0.01	NA	0.01
11Cl-PF3OUdS	NA	0.01	NA	0.01	NA	0.01
PFUnDS	NA	0.01	NA	0.01	NA	0.01
PFDoDA	0.005	0.01	0.001	0.01	NA	0.01
10:2 FTS	NA	0.01	NA	0.01	NA	0.01
PFDoS	NA	0.01	NA	0.01	NA	0.01
PFTrDA	0.002	0.01	NA	0.01	NA	0.01
PFTrDS	0.004	0.01	NA	0.01	NA	0.01
PFTeDA	NA	0.01	NA	0.01	NA	0.01

* Core PFAS targets

Red text indicates the LOQ_{val} level is above the required LOQ level due to matrix impact.

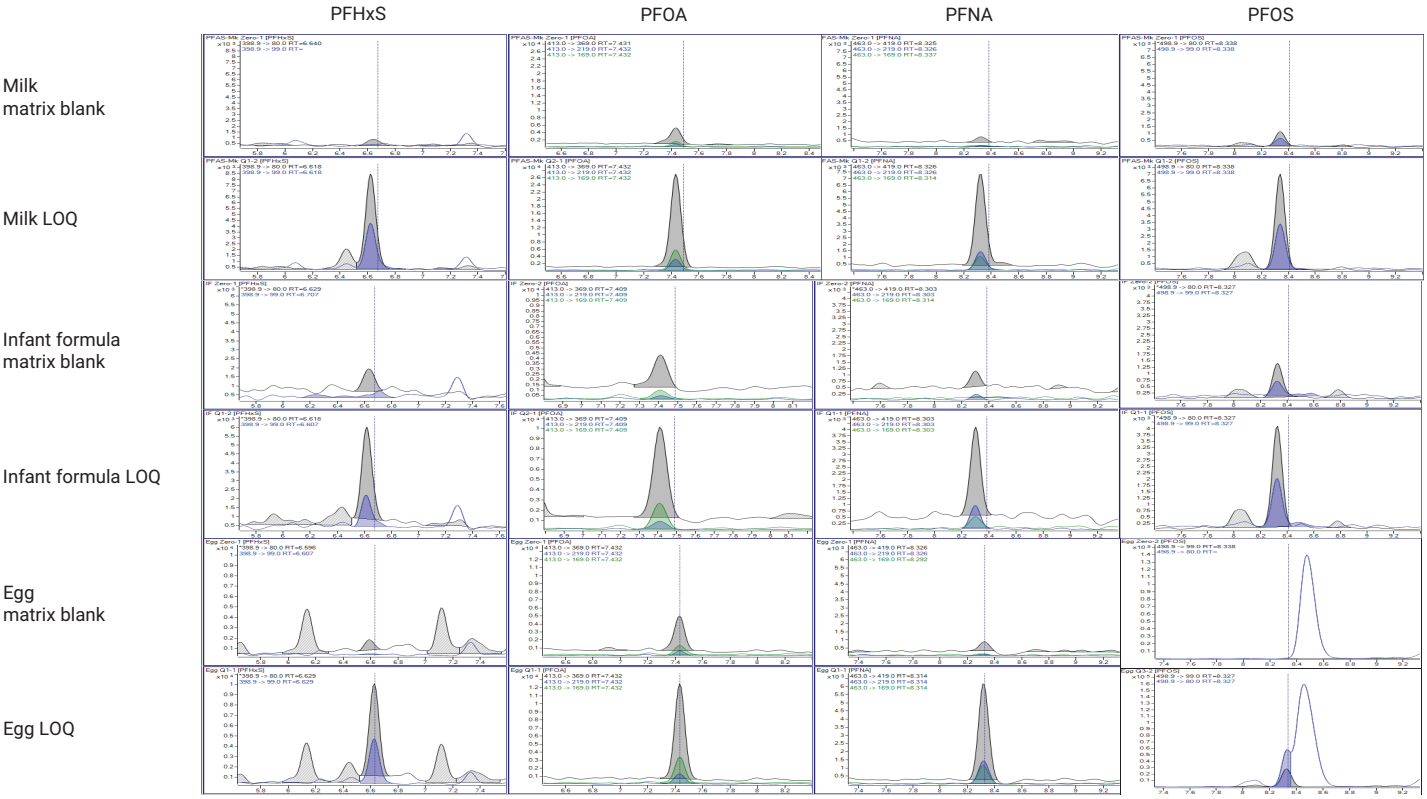

Figure 6. Infant formula (top), milk (middle), and egg (bottom) matrix blanks and LOQ level chromatograms for the core PFAS targets: PFHxS, PFOA, PFNA, and PFOS. LOQ levels in each matrix are listed in Table 6.

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 cost of labor and materials, 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 higher detection levels required for infant formula, milk, and eggs, 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 were validated in infant formula, milk, and eggs. The acceptance criteria for eggs is 80 to 120% recovery and for infant formula and milk is 65 to 135% recovery for PFOS, PFOA, PFHxS, and PFNA.[5] For other PFAS targets in the three matrices, the acceptance recovery criteria is also 65 to 135% for targets with corresponding isotopic ISTD, and 40 to 140% for targets without corresponding ISTD. The repeatability (RSD%) acceptance is ≤ 20% for core PFAS in eggs, ≤ 25% for core PFAS in milk and infant formula, and ≤ 25% for all other PFAS targets with corresponding isotopic ISTD, and ≤ 30% for other PFAS targets without corresponding isotopic ISTD in all three matrices.

The final method validation results included three QC levels in each matrix, including LOQ, mid, and high QC levels. The validated method LOQs are listed in Table 6. The mid-level QCs are reported at 5 to 10 times the LOQ, and the high-level QCs are reported at 20 to 50 times the LOQ. There is one exception for 6:2 FTS in milk, where only one level at 0.5 µg/kg was reportable due to significant high positive detection in sample matrix control.

Figure 7 shows the method validation recovery and repeatability (RSD) summary for PFAS analysis in infant formula, milk, and eggs. Overall, the method delivered acceptable recovery and repeatability results for all 30 targets in tested food matrices that meet the acceptance requirements. The core PFAS targets all generated acceptable recovery (80 to 120%) and RSD (< 20%) for all spiking levels in all matrices. Targets with corresponding isotopically labeled ISTD generated better quantitation results than targets without corresponding isotopically labeled ISTD.

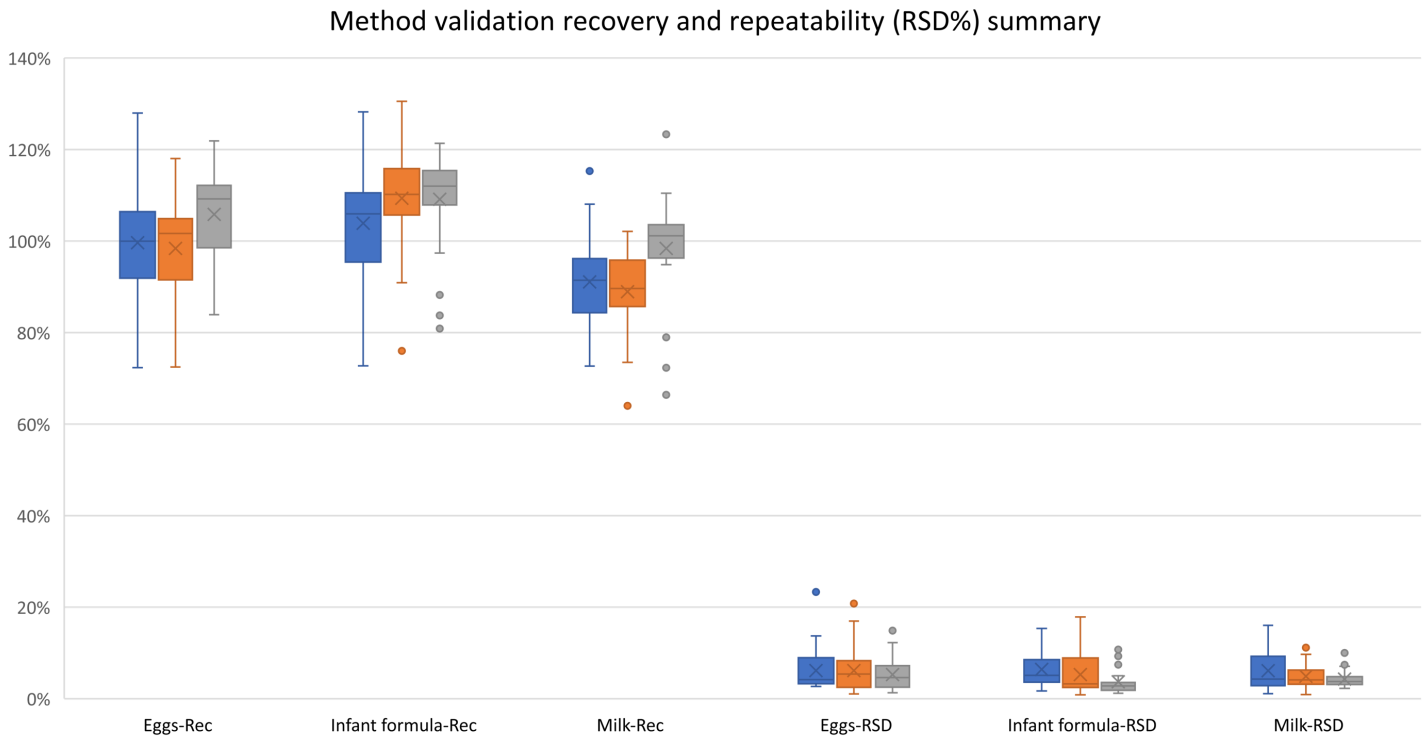

Figure 7. Method validation recovery (Rec) and repeatability (RSD%) summary for PFAS analysis in infant formula, milk, and eggs.

Conclusion

A simplified, rapid, and reliable method using QuEChERS extraction followed by 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 infant formula, milk, and eggs. The novel cleanup method demonstrated a significant improvement over traditional dSPE cleanup in terms of matrix removal, PFAS recovery, and sample volume recovery. This method is also simpler, saving time and effort, and thus 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 on 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. Hwang, S. H.; Ryu, S. Y.; Seo, D.; *et al.* Development of a Method Using QuEChERS and LC-MS/MS for Analysis of Per- and Polyfluoroalkyl Substances in Rice Matrix, *Food Chem.* **2024**, *445*, 138687. doi: 10.1016/j.foodchem.2024.138687.

www.agilent.com

DE90981738

Printed in the USA, June 1, 2024
5994-7366EN

Agilent
Trusted Answers