Application Note

Food

Determination of 43 PFAS in Beer and Wine

Using the Agilent 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 43 per- and polyfluoroalkyl substances (PFAS) in beer and wine. The method utilizes solvent extraction followed by enhanced matrix removal (EMR) mixed-mode passthrough cleanup using Agilent Captiva EMR PFAS Food I cartridges, with subsequent LC/MS/MS detection. Key features of the method include streamlined and efficient sample preparation, direct injection using Feed Injection by the Agilent 1290 Infinity III Hybrid Multisampler, sensitive LC/MS/MS detection, and reliable quantitation using neat standard calibration curves. The method was validated in accordance with AOAC Standard Method Performance Requirements (SMPR) 2023.003.

Introduction

Determination of PFAS residues in food has become an increasing concern in recent years. In April 2023, the European Commission (EC) implemented regulations for four PFAS compounds—PFOS, PFOA, PFNA, and PFHxS—across various food categories.[1] In November 2023, AOAC released SMPR 2023.003, establishing performance requirements for the analysis of 30 PFAS compounds in 11 food categories.[2] Although alcoholic beverages are not yet included in either EC regulation or AOAC SMPR guideline, the demand for PFAS analysis in these products—particularly wine and beer—has grown rapidly.[3,4] Studies have reported that PFAS contamination in wine and beer is linked to the use of municipal water and the geographic location of production. Beverages produced in areas with water sources highly contaminated with PFAS tend to show elevated levels of PFAS residues.

Alcoholic beverages are not considered complex matrices, but rather unique ones due to the presence of alcohol as a key component. The alcohol content in these beverages, which ranges from 3 to over 50%, presents challenges for common sample extraction techniques such as QuEChERS and solid-phase extraction (SPE). Specifically, high alcohol percentages can hinder efficient phase separation during the salt partitioning step in QuEChERS extraction. Additionally, alcohol in the sample matrix increases the risk of analyte breakthrough during sample loading in SPE-based approaches. A direct dilute-and-shoot approach may be considered due to the relatively lower matrix complexity. However, the presence of various additives—such as sugars, acids, flavorings, preservatives, emulsifiers, colorants, and even proteins—can compromise the robustness, cleanliness and longer-term durability of LC/MS/MS instrumentation.

To address these challenges, this study developed a direct solvent extraction method for PFAS analysis in alcoholic beverages. Samples were extracted using acidified acetonitrile (ACN), followed by centrifugation. However, the crude extract obtained through this approach may contain more matrix co-extractives than those from QuEChERS extraction, necessitating stronger EMR cleanup in subsequent steps. The EMR mixed-mode passthrough cleanup using Captiva EMR PFAS Food I has previously demonstrated streamlined and efficient matrix cleanup for fresh, plant-based matrices, such as fresh fruits, vegetables, juices[5], and baby food.[6] In this study, matrix cleanup was further improved by using EMR PFAS Food I cartridges with a higher bed mass of 680 mg, providing enhanced cleanup for alcoholic beverage samples.

Additionally, the instrument method was optimized by incorporating Agilent 1290 Infinity III Hybrid Multisampler in feed injection mode, enabling direct injection of a large volume of the final sample extract in high percentage of ACN. This advancement eliminates the need for a drying and reconstitution step while maintaining the desired limit of quantitation (LOQ). The modified protocol not only simplifies the overall workflow but also reduces sample preparation time by approximately 30 to 50%.

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 Millipore Sigma (Burlington, MA, U.S.).

Solutions and standards

Native PFAS and ISTD spiking solutions were prepared by diluting their respective stock solutions with MeOH. Native PFAS spiking solution I was formulated in MeOH with the following concentrations: 25 ng/mL group I analytes (25 compounds); 50 ng/mL for group II analytes (five compounds); 100 ng/mL for group III analytes (eight compounds), 125 ng/mL for 3:3 FTCA, 250 ng/mL for group IV analytes (two compounds), and 625 ng/mL for group V analytes (two compounds). Native PFAS spiking solution II was prepared by diluting Native PFAS spiking solution I 25-fold with MeOH.

ISTD spiking solutions I and II were prepared by diluting the Wellington MPFAC-HIF-ES stock solution 5-fold and 25-fold, respectively, with MeOH.

Neat calibration curve standards were prepared using native PFAS spiking solutions I and II, along with ISTD spiking solution II, diluted in a 3:1 ACN/water mixture containing 1% AA, following the detailed instructions outlined in Table 1.

All STD and ISTD solutions were stored in a refrigerator at 4 ˚C. Solutions were brought to room temperature and vortexed thoroughly before use.

All standards were stored at 4 °C and used within two weeks. For routine calibration testing, aliquots of the calibration solutions were transferred into separate vials equipped with polypropylene (PP) inserts for instrument injection. Prior to injection, it is essential to vortex the sample within the insert to eliminate any air bubbles, which could otherwise lead to injection errors during LC/MS/MS analysis.

Table 1. Preparation of neat calibration curve standards.

Calibration Standard (STD)	Native Spiking Solution	Native Spiking Solution Volume (µL)	ISTD Spiking Solution II Volume (µL)	ISTD Conc. (ng/mL)	Diluent (µL)	Concentration (ng/mL)					
						G1	G2	G3	3:3 FTCA	G4	G6
Calibration STD 10	I	80	10	0.1	910	2	4	8	10	20	50
Calibration STD 9	I	40	10	0.1	950	1	2	4	5	10	25
Calibration STD 8	I	20	10	0.1	970	0.5	1	2	2.5	5	12.5
Calibration STD 7	I	10	10	0.1	980	0.25	0.5	1	1.25	2.5	6.25
Calibration STD 6	I	4	10	0.1	986	0.1	0.2	0.4	0.5	1	2.5
Calibration STD 5	II	50	10	0.1	940	0.05	0.1	0.2	0.25	0.5	1.25
Calibration STD 4	II	25	10	0.1	965	0.025	0.05	0.1	0.125	0.25	0.625
Calibration STD 3	II	10	10	0.1	980	0.01	0.02	0.04	0.05	0.1	0.25
Calibration STD 2	II	5	10	0.1	985	0.005	0.01	0.02	0.025	0.05	0.125
Calibration STD 1	II	2.5	10	0.1	987.5	0.0025	0.005	0.01	0.0125	0.025	0.0625
G1 Analytes	PFHxA, PFBS, PFHpA, PFPeS, PFHxS, PFOA, PFNA, PFHpS, PFDA, PFUnDA, PFDoDA, PFTrDA, PFTDA, PFOS, PFNS, PFDS, PFUnDS, PFDoS, PFTrDS, PFOSA, 10:2 FTS, N-MeFOSA, N-EtFOSA, N-MeFOSAA, N-EtFOSAA										
G2 Analytes	PFPeA, PFMPA, PFMBA, NFDHA, PFEESA										
G3 Analytes	PFBA, HFPO-DA, 4:2 FTS, 6:2 FTS, 8:2 FTS, DONA, 9Cl-PF3ONS, 11Cl-PF3OUdS										
G4 Analytes	N-MeFOSE, N-EtFOSE										
G5 Analytes	5:3 FTCA, 7:3 FTCA										

The extraction solvent (ACN with 1% AA) was prepared by adding 10 mL glacial acetic acid to 990 mL ACN and storing at room temperature. The 3:1 ACN/water diluent was prepared by mixing three parts extraction solvent with one part MilliQ water. For LC/MS/MS analysis, mobile phase A consisted of 5 mM ammonium acetate (NH_4OAc) in water, and mobile phase B was ACN. Needle wash solvents included IPA, MilliQ water, and ACN.

Equipment and material

The study was conducted using an Agilent 1290 Infinity II LC system consisting of an Agilent 1290 Infinity II high speed pump (G7120A), an Agilent 1290 Infinity III Hybrid Multisampler (G7137B), and an Agilent 1290 Infinity II Multicolumn Thermostat (G7116B). The LC system was coupled to an Agilent 6495D Triple Quadrupole LC/MS (LC/TQ) system equipped with an Agilent Jet Stream iFunnel Electrospray ion source. Data acquisition and analysis were performed using Agilent MassHunter Workstation software.

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 repeater (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), which includes the InfinityLab PFC delay column (4.6 × 30 mm; part number 5062-8100) to minimize background PFAS contamination. 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), rated for up to 1200 bar pressure. An Agilent ZORBAX RRHD Eclipse Plus C18 column (2.1 × 5 mm, 1.8 µm; part number 821725-901) was also used to protect the analytical column and extend its lifetime.

The sample preparation and other Agilent consumables used included:

- 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, 680 mg (part number 5610-2231)
- 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/MS/MS instrument conditions

Table 2 lists the LC pump conditions.

Table 2. LC pump conditions for LC/MS/MS.

Parameter	Setting			
Mobile Phase A	5 mM NH_4OAc in water			
Mobile Phase B	ACN			
Gradient	Time (min)	A%	B%	Flow (mL/min)
	0.00	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 injection settings.

Table 3. LC multisampler program for LC/MS/MS.

Parameters	Setting			
Feed Injection	Injection mode: Feed Injection volume: 12.50 µL Feed speed: Adaptive, 10% of pump flow Flush out mode: Automatic			
Injection Path Cleaning	Inner wash mode: Extended Outer wash mode: Extended			
	Step	**Task**	**Solution**	**Duration/ Volume**
	Draw Sample			
	1	Outer wash	S1 = ACN	10 s
	2	Outer wash	S3 = 1:1 ACN/IPA	10 s
	Injection			
	1	Inner wash	S2 = 90:10 MPA/MPB	150 µL
	2	Inner wash	S2 = 90:10 MPA/MPB	150 µL
	3	Seat wash	S1 = ACN	150 µL
	4	Seat wash	S3 = 1:1 ACN/IPA	150 µL
	5	Reconditioning	S2 = 90:10 MPA/MPB	

Mass spectrometer Jet Stream Electrospray ion source settings

The Electrospray ion source settings include drying gas at 200 °C, 18 L/min; sheath gas at 300 °C, 11 L/min; nebulizer gas at 15 psi; capillary voltage at 2,500 V (NEG); and nozzle voltage at 0 V (NEG). Negative ion mode with constant fragmentor setting at 166 V. iFunnel standard mode for all compounds. The MS acquisition conditions for PFAS targets and ISTDs were from the PFAS MRM Database for LC/TQ (G1736AA).

Sample preparation procedure

Six types of alcoholic beverages were selected for method validation, based on their popularity, alcoholic content, and matrix complexity. The chosen beverages included fruit beer (ALC 3.2%), light beer (ALC 4.2%), milk stout (ALC 6%), white wine (ALC 11.5%), red wine (ALC 13.5%), and margarita (ALC 13.9%). All samples were purchased from a local wine store. To minimize foaming during sample preparation, the products were prechilled in a refrigerator for 1 to 2 hours prior to opening the bottles.

An aliquot of 2 g of each alcoholic beverage sample was weighed into a 15 mL PP tube. PFAS standards and ISTDs were appropriately spiked into all prespiked quality control (QC) samples, while only ISTDs were spiked to matrix blanks (MBs). For procedure blanks (PBs), 2 g of water spiked with ISTDs were used. Light beer and red wine were used for full validation using four prespiking levels, whereas the remaining beverages were spiked at the two lowest levels for cross-validation purposes.

Table 4 summarizes the spiking details for prespiked QC samples using native PFAS spiking solutions I and II, along with ISTD spiking solution I. In accordance with the sample preparation protocol, all samples underwent a four-fold dilution. Consequently, the spiking concentrations in the QC samples were calculated by factoring in the dilution and the required LOQs. To ensure accurate quantitation using the previously established calibration curve, it was essential to maintain the theoretical ISTD concentration at 0.1 ng/mL in the final sample extract—matching the concentration used in the calibration standards.

Table 4. Matrix-matched prespiked QC samples and matrix blank preparation for validation and cross-validation batches.

Prespiking for Full Validation in Light Beer and Red Wine								
Prespiked Samples	Matrix Sample (mL)	Native PFAS Spiking Solution	Spiking Volume (µL)	Native Concentration (ng/mL)*		ISTD Spiking I Volume (µL)	ISTD Concentration (ng/mL)**	
				In Sample	In Final Extract		In Sample	In Final Extract
MB	2	--	--	--	--	16	0.4	0.1
QC 1		Native PFAS spiking II	20	0.01	0.0025			
QC 2			80	0.04	0.01			
QC 3		Native PFAS spiking I	8	0.1	0.025			
QC 4			32	0.4	0.1			
Prespiking for Cross-Validation in Five More Alcoholic Drinks								
Zero	2	--	--	--	--	16	0.4	0.1
QC 1		Native PFAS spiking II	20	0.01	0.0025			
QC 2			80	0.04	0.01			

* Native PFAS concentrations were based on group I, with the proportional higher concentrations assigned to the remaining analytes. Refer to Table 1 for the calculation of other analytes' concentrations, determined by the relative ratios among the groups.
** ISTD concentration was based on compounds with lowest concentration in the original stock, with proportionally higher concentrations applied to the remaining ISTD compounds.

After spiking, all samples were vortexed for 3 minutes to ensure equilibrium. Subsequently, the samples were extracted following the developed procedure, as illustrated in Figure 1.

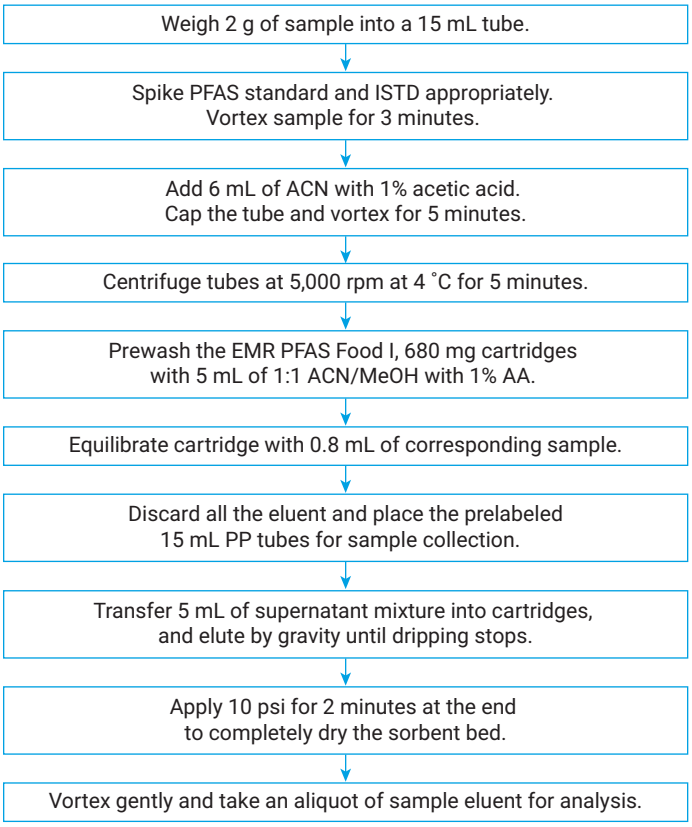

Figure 1. Sample preparation procedure using acidified ACN extraction followed by EMR mixed-mode passthrough cleanup with the Agilent Captiva EMR PFAS Food I, 680 mg cartridge.

Results and discussion

Solvent effect mitigation in liquid chromatography (LC)

The solvent used to dissolve samples for LC/MS/MS analysis can significantly impact analyte peak shape and resolution in liquid chromatography, particularly for early-eluting analytes. When samples are dissolved in a solvent stronger than the mobile phase, peak distortion may occur—manifesting as asymmetric peaks, split peaks, fronting, and tailing. This phenomenon is commonly referred to as the solvent effect in LC. In reversed-phase LC, a strong solvent typically contains a higher percentage of organic solvent (for example ACN, MeOH), while a weak solvent is more aqueous. The solvent effect can compromise chromatographic quality and, consequently, the accuracy and reliability of both qualitative and quantitative analyses.

To address this issue, either offline or online mitigation strategies can be employed:

- Offline approaches include solvent exchange via drying and reconstitution or dilution with water or aqueous buffer. However, these steps may increase preparation time, risk analyte loss and contamination, or compromise method sensitivity.
- Online approaches, such as sandwiched injection using a classic multisampler or feed injection using a hybrid multisampler offer a more convenient solution. Online approaches enable direct injection of sample dissolved in highly organic solvents by performing online dilution with water (sandwiched injection) or mobile phase A (feed injection). Both approaches effectively mitigate solvent effects while maintaining acceptable peak shapes and resolution.

Since final extracts after sample preparation are often dissolved in highly organic solvents, online approaches enable direct injection of these extracts, simplifying post-treatment and reducing preparation time by 30 to 50%. Compared to sandwiched injection, feed injection offers a greater capacity for the injection of larger volumes (> 10 µL) of samples in stronger solvents (such as ACN) while maintaining acceptable chromatographic performance. As a result, this approach can potentially eliminate the need for sample concentration to meet the desired method LOQs.

Another advantage of the feed injection program over the traditional sandwiched injection is its ease of use. The sandwiched injection method relies on weak solvent stored in small sample vials (such as 2 mL) located on the sample tray, which limits the number of samples that can be injected using the diluent from each vial—usually no more than 10 injections per diluent vial. As a result, running a large batch with more than 10 injections often requires multiple methods to accommodate diluent vials in different tray positions. Feed injection using a hybrid multisampler eliminates this limitation by enabling consistent method application across many sample injections, streamlining the workflow and improving operational efficiency.

Figure 2 presents a comparison of chromatograms by injecting PFAS neat standard dissolved in 90:10 ACN/water using different injection programs. In the classic flow-through injection without online dilution, a 5 µL injection of the standard in strong solvent resulted in poor chromatographic performance for over 50% of the analytes (Figure 2A). When the sandwiched injection program was applied using a standard multisampler, the solvent effect was mitigated, delivering acceptable peak shapes for all analytes with a 5 µL injection. However, increasing the injection volume to 10 µL led to noticeable peak distortion in approximately 20% of the analytes that eluted in the early retention time window (Figure 2B). In contrast, the feed injection program using the hybrid multisampler achieved excellent, symmetric, and integrated peak shapes for all analytes with the 10 µL injection volume (Figure 2C). Although the first two eluted analytes showed slightly broader peaks, the shape remained acceptable. The two diagrams on the right side of Figure 2 illustrate the flow paths for a classic flow-through and feed injection program.

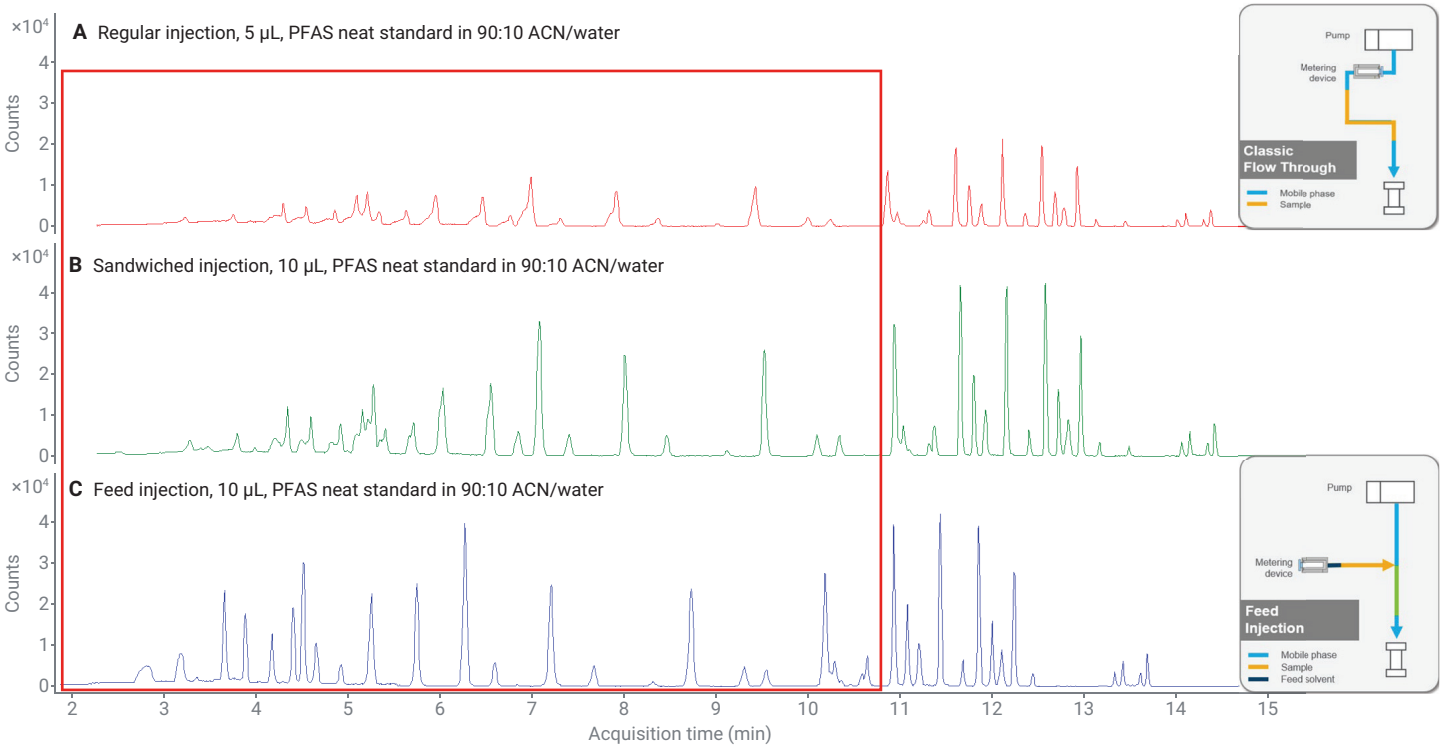

Figure 2. MRM chromatograms of PFAS neat standard dissolved in 90:10 ACN/water using different injection programs: (A) classic injection using a regular multisampler with 5 µL injection; (B) a sandwiched injection program using a regular multisampler with 10 µL injection; (C) a feed injection program using a hybrid multisampler with 10 µL injection.

Sample preparation procedure

Instead of using QuEChERS extraction as in previous methods[5,6], this method applied direct solvent extraction for alcoholic samples due to the unique characteristics of their matrices. Commonly used solvents for PFAS extraction (acidified ACN and MeOH) were evaluated.

Results indicated that acidified ACN, used at a 3:1 ACN/sample ratio, provided higher analyte recovery and cleaner crude extracts. Figure 3A compares the extraction performance of acidified ACN and MeOH for two challenging matrices: milk stout and margarita. When acidified ACN was used, the sample mixture separated into two distinct layers after centrifugation: a top layer containing a homogenous crude extract and a bottom layer consisting of matrix interferences, such as sugars, emulsifiers, and proteins. In contrast, MeOH extraction resulted in minimal or no visible phase separation, suggesting that more matrix interferences were dissolved in the crude sample extract. Based on these findings, acidified ACN was selected as the extraction solvent for this method.

The subsequent matrix cleanup was straightforward. An aliquot of 5 mL of the supernatant was directly loaded on Captiva EMR PFAS Food I cartridge (680 mg) for passthrough cleanup. The use of a higher bed mass was intended to enhance matrix removal efficiency, as crude extracts obtained through direct solvent extraction typically contain more matrix co-extractives than those obtained via QuEChERS extraction. Figure 3B demonstrates the effectiveness of matrix cleanup on a red wine crude extract. The dark-red, cloudy crude extract was transformed into a transparent, colorless solution after EMR mixed-mode cleanup.

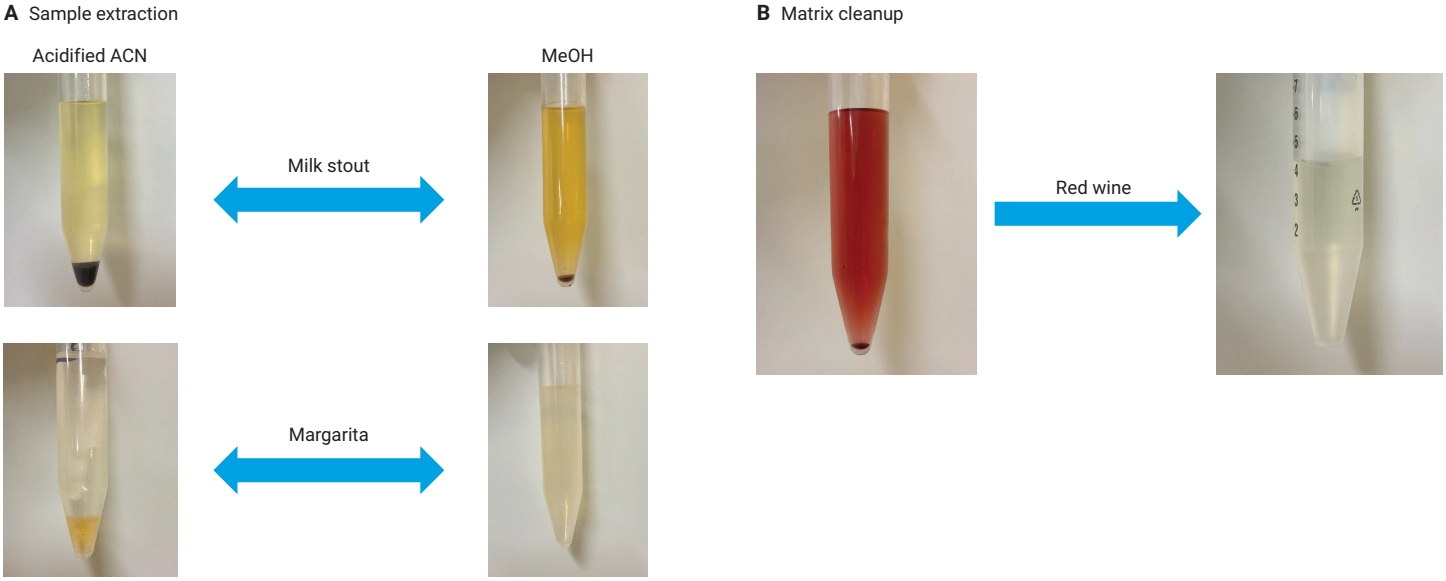

Figure 3. (A) Comparison of solvent extraction performance for milk stout and margarita using acidified ACN (left) versus MeOH (right). (B) Matrix cleanup of red wine using Agilent Captiva EMR PFAS Food I passthrough cleanup.

Method validation

Matrix blank suitability and method LOQs: One of the major challenges for PFAS analysis is the suitability of matrix blanks (MB), as PFAS residues are commonly detected in many MBs. Among the alcoholic beverages tested, none were completely free of PFAS background contamination. This is primarily due to the ultralow detection limits of the analytical method and the widespread presence of PFAS in the environment. Unlike other food matrices, PFAS contamination in alcoholic beverages included more polar, short-chain analytes such as PFBA, PFPeA, and PFHxA.

The method LOQ was defined as the lowest experimental QC spiking level that met all acceptance criteria. These criteria included target identification parameters—such as retention time, signal-to-noise ratio, and the quantifier-to-qualifier ratio—as well as acceptable recovery and repeatability. For matrices demonstrating 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. However, in cases where PFAS detections in MBs exceeded acceptable thresholds for experiment LOQs, the method LOQ was determined based on the next experimental QC level that met all validation requirements and remained below the required LOQ by SMPR. If none of the validated experimental QC levels satisfied the required LOQ, method LOQs were then calculated using the Equation 1, based on standard deviation (SD) derived from the results of seven MBs.

Equation 1. $LOQ_{cal} = SD_{MBs} \times 10$

Where LOQ_{cal} is the calculated LOQ based on PFAS detections in MBs, and SD_{MBs} is the SD of PFAS concentrations detected across seven replicates of MB samples.

Table 2 summarizes the results of matrix blank detections and reported LOQs (whether determined experimentally or calculated) and the LOQ requirements specified by AOAC SMPR for PFAS targets in all six alcoholic beverages. PFAS detections in matrix blanks were confirmed based on retention time and qualifier ratio criteria. The LOQ requirements referenced correspond to the most relevant food category—fruits, vegetables, and beverages—as outlined in AOAC SMPR.[2]

The results demonstrate that the method provided acceptable selectivity and suitability, meeting the required LOQ for all analytes across all six alcoholic beverages. Calculated LOQs were reported for PFBA in red wine and milk stout and PFOA in milk stout due to significantly elevated detections in matrix blanks. For analytes not covered by AOAC SMPR guidance, required LOQs are not available.

Table 5. PFAS detections in matrix blanks, reported LOQs (experimentally determined or calculated), and required LOQs for PFAS analytes in beers and wines. All values are expressed in µg/kg.

	Fruit Beer		Light Beer		Milk Stout		Red Wine		White Wine		Margarita		Required LOQ by AOAC SMPR
	MB	Reported LOQ	MB	Reported LOQ	MB	Reported LOQ	MB	Reported LOQ	MB	Reported LOQ	MB	Reported LOQ	
PFBA	0.021	0.16	0.002	0.04	0.067	0.054	0.122	0.34	0.037	0.16	ND	0.04	≤ 1
PFMPA	ND	0.02	ND	0.02	ND	0.02	ND	0.02	ND	0.02	ND	0.02	NA
3-3 FTCA	ND	0.05	ND	0.05	ND	0.05	ND	0.05	ND	0.05	ND	0.05	NA
PFPeA	0.016	0.08	0.027	0.08	0.005	0.02	0.002	0.02	0.013	0.08	0.010	0.08	≤ 1
PFMBA	ND	0.02	ND	0.02	ND	0.02	ND	0.02	ND	0.02	ND	0.02	NA
4:2 FTS	ND	0.04	ND	0.04	ND	0.04	ND	0.04	ND	0.04	ND	0.04	≤ 0.1
NFDHA	ND	0.02	ND	0.02	ND	0.02	ND	0.02	ND	0.02	ND	0.02	NA
PFHxA	0.001	0.01	0.005	0.04	0.003	0.01	0.013	0.04	0.006	0.04	0.001	0.01	≤ 0.1
PFBS	0.002	0.01	0.001	0.01	0.002	0.01	0.003	0.01	ND	0.01	0.002	0.01	≤ 0.1
HFPO-DA	ND	0.04	ND	0.04	ND	0.04	ND	0.04	ND	0.04	ND	0.04	≤ 0.1
5-3 FTCA	ND	0.25	ND	0.25	ND	0.25	ND	0.25	ND	0.25	ND	0.25	NA
PFEESA	ND	0.02	ND	0.02	ND	0.02	ND	0.02	ND	0.02	ND	0.02	≤ 0.1
PFHpA	ND	0.01	0.001	0.01	ND	0.01	0.002	0.01	ND	0.01	ND	0.01	≤ 0.1
PFPeS	ND	0.01	ND	0.01	ND	0.01	0.002	0.01	ND	0.01	ND	0.01	≤ 0.1
DONA	ND	0.04	ND	0.04	ND	0.04	ND	0.04	ND	0.04	ND	0.04	≤ 0.1
6:2 FTS	0.014	0.04	0.008	0.04	0.012	0.04	0.008	0.04	0.014	0.04	0.011	0.04	≤ 0.1

	Fruit Beer		Light Beer		Milk Stout		Red Wine		White Wine		Margarita		Required LOQ by AOAC SMPR
	MB	Reported LOQ	MB	Reported LOQ	MB	Reported LOQ	MB	Reported LOQ	MB	Reported LOQ	MB	Reported LOQ	
PFOA	ND	0.01	ND	0.01	0.005	0.01	ND	0.01	ND	0.01	ND	0.01	≤ 0.01
PFHxS	ND	0.01	0.001	0.01	ND	0.01	0.003	0.01	0.001	0.01	ND	0.01	≤ 0.01
7-3 FTCA	ND	0.25	ND	0.25	ND	0.25	ND	0.25	ND	0.25	ND	0.25	NA
PFNA	ND	0.01	ND	0.01	ND	0.01	ND	0.01	ND	0.01	ND	0.01	≤ 0.01
PFHpS	ND	0.01	ND	0.01	ND	0.01	ND	0.01	ND	0.01	ND	0.01	≤ 0.1
8:2 FTS	ND	0.01	ND	0.01	ND	0.01	ND	0.01	ND	0.01	ND	0.01	≤ 0.1
N-MeFOSAA	ND	0.01	0.003	0.01	ND	0.01	0.001	0.01	ND	0.01	ND	0.01	NA
PFDA	ND	0.01	ND	0.01	ND	0.01	ND	0.01	ND	0.01	ND	0.01	≤ 0.1
PFOS	ND	0.01	0.001	0.01	ND	0.01	ND	0.01	ND	0.01	ND	0.01	≤ 0.01
N-EtFOSAA	ND	0.01	0.003	0.01	ND	0.01	ND	0.01	ND	0.01	ND	0.01	NA
PFUnDA	ND	0.01	ND	0.01	ND	0.01	ND	0.01	ND	0.01	ND	0.01	≤ 0.1
9Cl-PF3ONS	ND	0.04	ND	0.04	ND	0.04	0.001	0.04	ND	0.04	ND	0.04	≤ 0.1
10:2 FTS	0.001	0.01	0.001	0.01	0.001	0.01	ND	0.01	0.001	0.01	ND	0.01	≤ 0.1
PFNS	ND	0.01	ND	0.01	ND	0.01	ND	0.01	ND	0.01	ND	0.01	≤ 0.1
PFDoDA	ND	0.01	ND	0.01	ND	0.01	ND	0.01	ND	0.01	ND	0.01	≤ 0.1
PFDS	ND	0.01	ND	0.01	ND	0.01	ND	0.01	ND	0.01	ND	0.01	≤ 0.1
PFOSA	ND	0.01	ND	0.01	ND	0.01	ND	0.01	ND	0.01	ND	0.01	≤ 0.1
PFTrDA	ND	0.01	ND	0.01	ND	0.01	ND	0.01	ND	0.01	ND	0.01	≤ 0.1
11Cl-PF3OUdS	ND	0.04	ND	0.04	ND	0.04	0.003	0.04	ND	0.04	ND	0.04	≤ 0.1
PFUnDS	ND	0.01	ND	0.01	ND	0.01	ND	0.01	ND	0.01	ND	0.01	≤ 0.1
PFTeDA	ND	0.01	ND	0.01	ND	0.01	ND	0.01	ND	0.01	ND	0.01	≤ 0.1
PFTrDS	ND	0.01	ND	0.01	ND	0.01	ND	0.01	ND	0.01	ND	0.01	≤ 0.1
PFDoS	ND	0.01	ND	0.01	ND	0.01	0.001	0.01	ND	0.01	ND	0.01	≤ 0.1
MeFOSE	ND	0.1	ND	0.1	ND	0.1	0.004	0.1	ND	0.1	ND	0.1	NA
N-MeFOSA	ND	0.01	ND	0.01	ND	0.01	ND	0.01	ND	0.01	ND	0.01	NA
EtFOSE	ND	0.1	ND	0.1	ND	0.1	ND	0.1	ND	0.1	ND	0.1	NA
N-EtFOSA	ND	0.01	ND	0.01	ND	0.01	ND	0.01	ND	0.01	ND	0.01	NA

ND = Not detectable
NA = Not applicable

Method recovery and repeatability: Method recovery and repeatability were fully validated in light beer and red wine. Final validation results were reported at three QC levels for each matrix: LOQ, low, and mid. When a calculated LOQ was used, the QC level closest to the calculated LOQ was selected for reporting recovery and RSD at the LOQ level. The method was then cross-validated in four additional alcoholic beverages—fruit beer, milk stout, white wine, and margarita—using two lower prespiking QC levels, with results reported at the LOQ level.

Full validation results are summarized in Figure 4 (light beer) and Figure 5 (red wine). Validation in light beer demonstrated acceptable quantitation accuracy and precision for all 30 PFAS analytes required by AOAC SMPR. All other analytes also met acceptance criteria, except for 3-3 FTCA, which showed high recovery due to matrix enhancement and the absence of a corresponding isotopically labeled ISTD. Validation results in red wine demonstrated acceptable quantitation results for all 43 PFAS analytes across all three spiking levels.

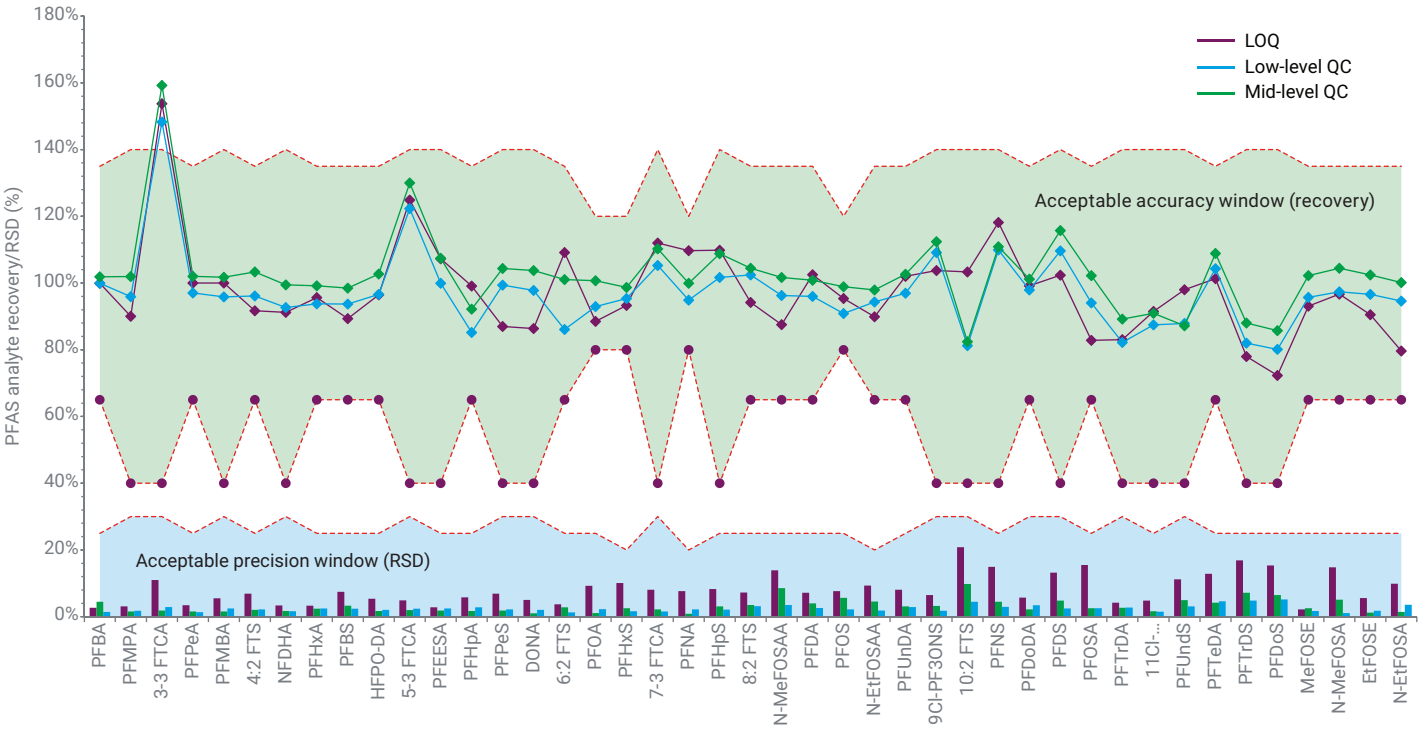

Figure 4. Validation results summary for 43 PFAS in light beer. The three lines in the middle show analyte recovery results, and the three sets of columns at the bottom represent RSD values at three spiking levels. Results are color-coded by spiking levels: purple for LOQ, blue for low, and green for mid-level.

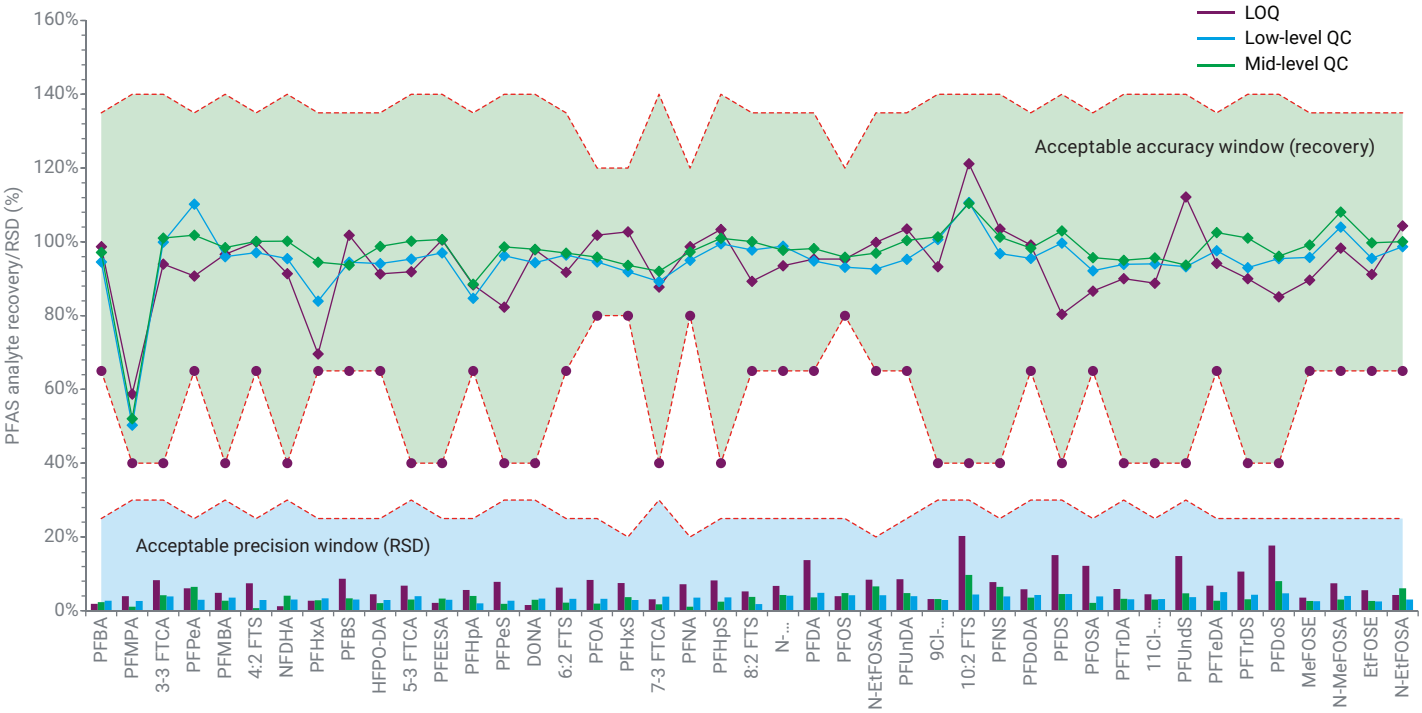

Figure 5. Validation results summary for 43 PFAS in red wine. The three lines in the middle show analyte recovery results, and the three sets of columns at the bottom represent RSD values at three spiking levels. Results are color-coded by spiking levels: purple for LOQ, blue for low, and green for mid-level.

Figure 6 presents the cross-validation results at LOQ spiking level for the remaining four alcoholic beverages. The results confirmed acceptable quantitation performance for all analytes across all four matrices, meeting the acceptance criteria.

The only exception was 3-3 FTCA in milk stout, which showed elevated recovery due to matrix enhancement and the absence of a corresponding ISTD.

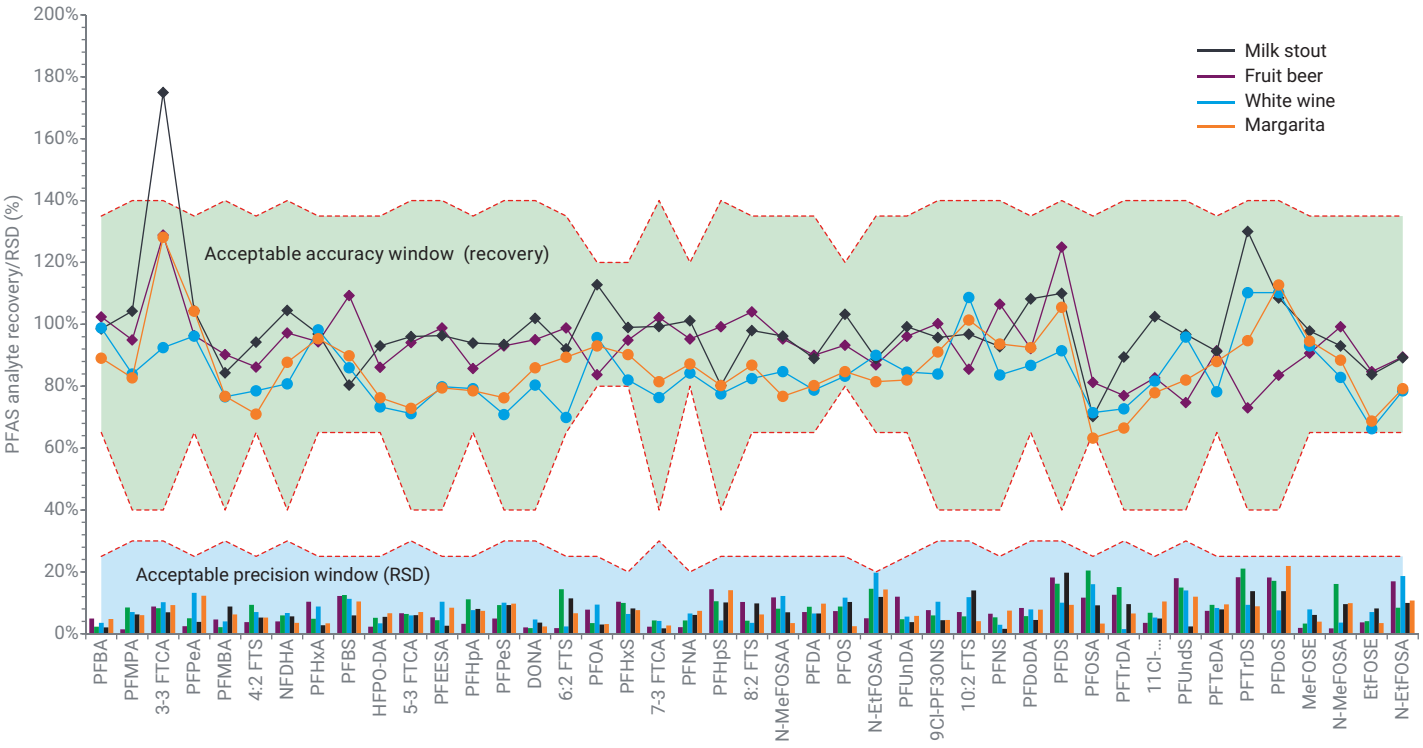

Figure 6. Cross-validation results summary for 43 PFAS in four alcoholic beverages at the LOQ spiking level. Four lines in the middle show analyte recovery results, while four sets of columns at the bottom represent RSD values across the four matrices. Results are color-coded by matrix: black for milk stout, purple for fruit beer, blue for white wine, and orange for margarita.

Conclusion

A simplified, rapid, and reliable method was developed and validated for the quantitative determination of 43 PFAS targets in alcoholic beverages. The method utilized solvent extraction followed by EMR mixed-mode passthrough cleanup using Agilent Captiva EMR PFAS Food I, 680 mg, cartridges, and LC/MS/MS detection. As a result of its simplicity, robustness, and cost-effectiveness, the sample preparation approach offers significant savings in time and resources. The method was validated in accordance with the acceptance criteria outlined in the AOAC SMPR guidelines.

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