Application Brief

Food & Beverage Testing

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Determination of 30 Per- and Polyfluoroalkyl Substances in Dry Soybeans

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

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Introduction

Agilent Captiva EMR PFAS Food cartridges were developed and optimized specifically for per- and polyfluoroalkyl substance (PFAS) analysis in food. The objective of this study was to develop and validate a complete workflow for the determination of 30 PFAS in dry soybeans. The method uses QuEChERS extraction followed by enhanced matrix removal (EMR) mixed-mode passthrough cleanup using a Captiva EMR PFAS Food II cartridge and detection with an Agilent 6495D triple quadrupole LC/MS (LC/TQ). The method was validated to meet AOAC Standard Method Performance Requirements (SMPR) 2023.003[1], including method suitability, sensitivity, accuracy, and precision.

Experimental

Chemicals and reagents

Native PFAS and isotopically labeled internal standard (ISTD) solutions were purchased from Wellington Laboratories (Guelph, Ontario, Canada).

Solutions and standards

The preparation of standard solutions and other reagents were listed in a previous application note.[2]

Equipment and materials

The study was performed using an Agilent 1290 Infinity II LC system coupled to an 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 in this study was the same as those used in a previous study.[2]

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, 95 Å, 2.1 × 100 mm, 1.8 µm (part number 959758-902) and a ZORBAX RRHD Eclipse Plus C18, 2.1 mm, 1.8 µm, 1,200-bar pressure limit, UHPLC guard (part number 821725-901).

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 II cartridges, 6 mL, 750 mg (part number 5610-2232)
- 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 of 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 were listed in a previous application note.[2]

Sample preparation

Dry soybeans were purchased from a local grocery store and was ground into a fine powder using a mechanical blender. The soybean powder was then used for sample extraction. A 5 g sample was weighed into a clean PP 50 mL tube for extraction. The native PFAS spiking and ISTD spiking solutions were added to the quality control (QC) samples appropriately, and only ISTD to matrix blanks. The samples were then ready for the sample preparation procedure, which is described in Figure 1.

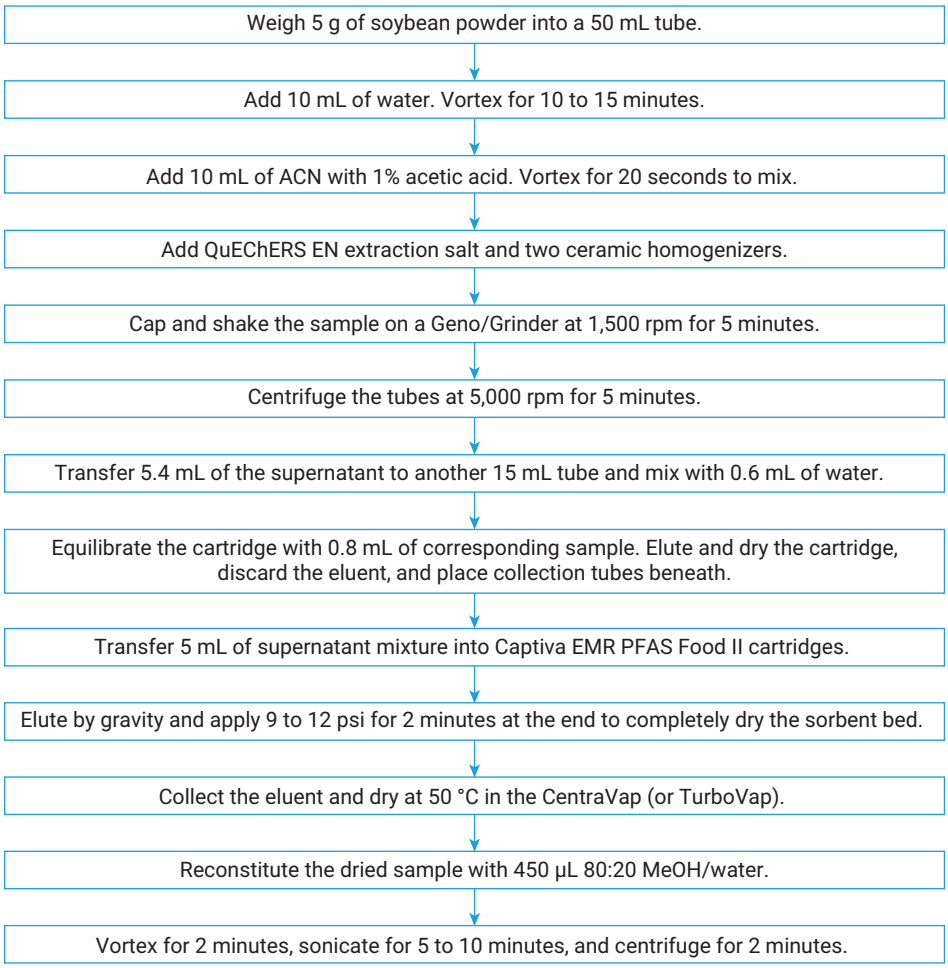

Figure 1. Sample preparation procedure for PFAS analysis in dry soybeans.

Method performance evaluation

The method was validated for method limit of quantitation (LOQ), determination, and recovery accuracy and precision. Five prespiked QC level samples were prepared in replicates of 4 or 5 at each level for 0.05, 0.1, 0.5, 1.0, and 2.0 µg/kg in soybean powder. The ISTD prespiking level was 1.0 µg/kg in soybean powder. In addition, the matrix blanks were prepared in replicates of 5 to 7 with 1.0 µg/kg ISTD prespiked for quantitation.

Results and discussion

EMR mixed-mode passthrough cleanup

The EMR mixed-mode passthrough cleanup was compared with traditional dispersive solid phase extraction (dSPE) cleanup for PFAS target recovery and soybean matrix removal, which was evaluated using an LC/Q-TOF total ion chromatogram (TIC) scan. Figure 2 shows (A) the PFAS target recovery comparison and (B) a TIC scan matrix sample background on LC/Q-TOF.

Results demonstrated that EMR mixed-mode passthrough cleanup with the Captiva EMR PFAS Food II cartridge provided a significant improvement in PFAS target recovery and soybean matrix removal compared to traditional dSPE cleanup.

Method validation

The newly developed method was validated for the determination of 30 PFAS targets in soybean following the AOAC SMPR guidance. Considering dry soybean as the "feed" matrix category, the required LOQs were ≤ 0.5 µg/kg for four core PFAS targets (PFOS, PFOA, PFNA, and PFHxS) and ≤ 5.0 µg/kg for the remaining PFAS targets.[2]

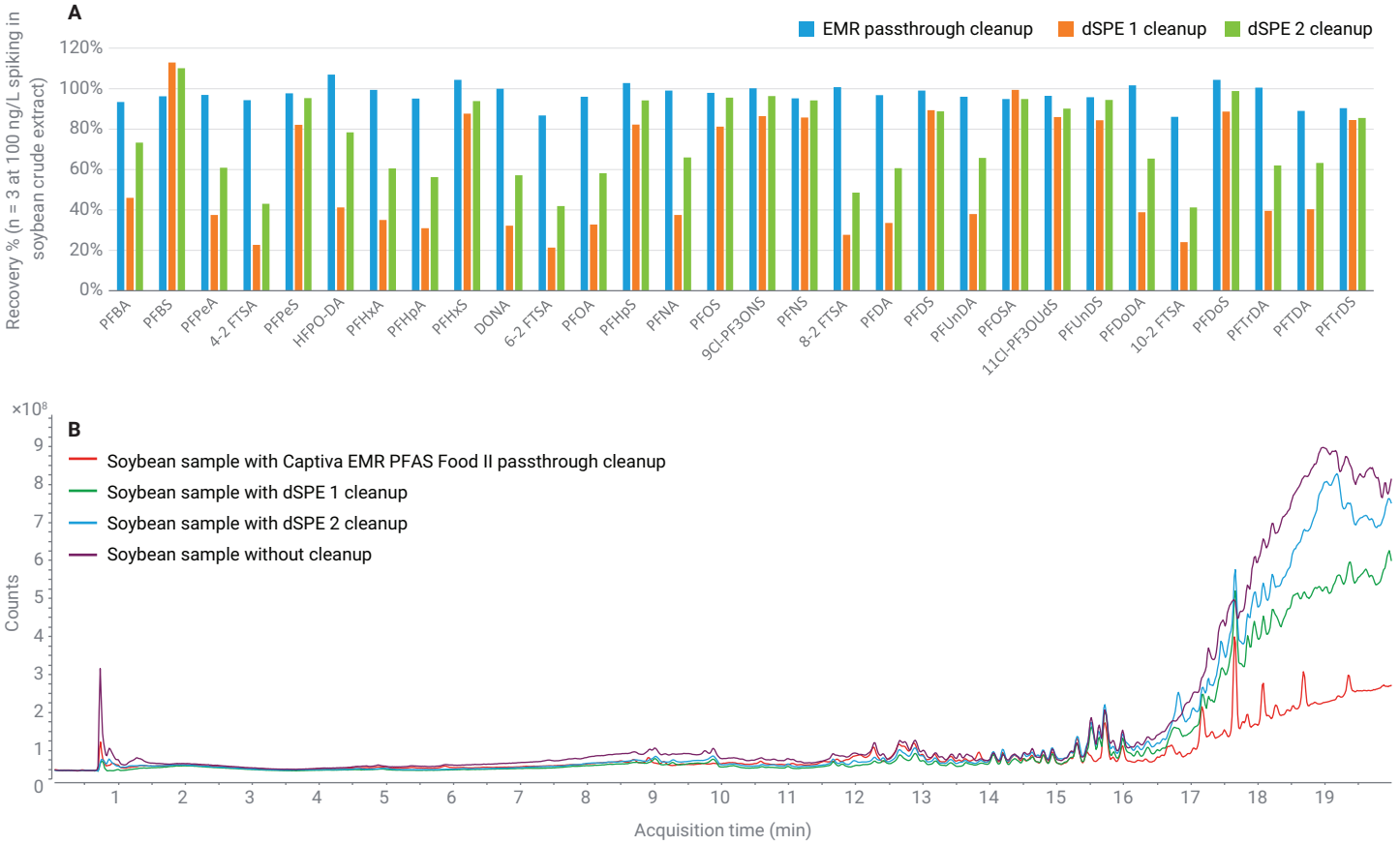

Figure 2. Comparison of EMR passthrough cleanup using Agilent Captiva EMR PFAS Food II cartridges with traditional dSPE cleanup for (A) PFAS recovery and (B) matrix cleanliness using LC/Q-TOF and ESI TIC scan.

Method LOQs

The method LOQs were determined based on the methodology described in a previous application note.[2] Table 1 shows the calculated lowest reportable (LOQ_{cal}) and validated method (LOQ_{val}) for each target in soybean. The validated method LOQs were all below or equal to the required LOQs for feed matrix. Figure 3 shows the soybean matrix blank and validated LOQ-level chromatograms for the four core PFAS targets.

Table 1. Method lowest reportable calculated (LOQ_{cal}) and validated (LOQ_{val}) for 30 targets in the soybean matrix.

Target	Soybean LOQ (µg/kg)	
	LOQ_{cal}	LOQ_{val}
PFBA	2.191	5
PFPeA	0.011	0.1
PFBS	0.008	0.05
4:2 FTS	NA	0.05
PFPeS	NA	0.05
PFHxA	0.03	0.05
HFPO-DA	NA	0.05
PFHpA	0.029	0.05
PFHxS*	0.007	0.05
DONA	0.002	0.5
6:2 FTS	0.072	0.1
PFOA*	0.031	0.05
PFHpS	NA	0.05
PFNA*	0.007	0.05
PFOS*	0.003	0.05
9Cl-PF3ONS	0.001	0.05

Target	Soybean LOQ (µg/kg)	
	LOQ_{cal}	LOQ_{val}
8:2 FTS	0.002	0.05
PFNS	NA	0.05
PFDA	0.007	0.05
PFDS	NA	0.05
PFUnDA	NA	0.05
PFOSA	0.003	0.05
11Cl-PF3OUdS	0.001	0.05
PFUnDS	0.001	0.05
PFDoDA	0.004	0.05
10:2 FTS	NA	0.05
PFDoS	NA	0.05
PFTrDA	NA	0.05
PFTrDS	NA	0.05
PFTeDA	0.015	0.05

* Core PFAS targets
NA = not applicable

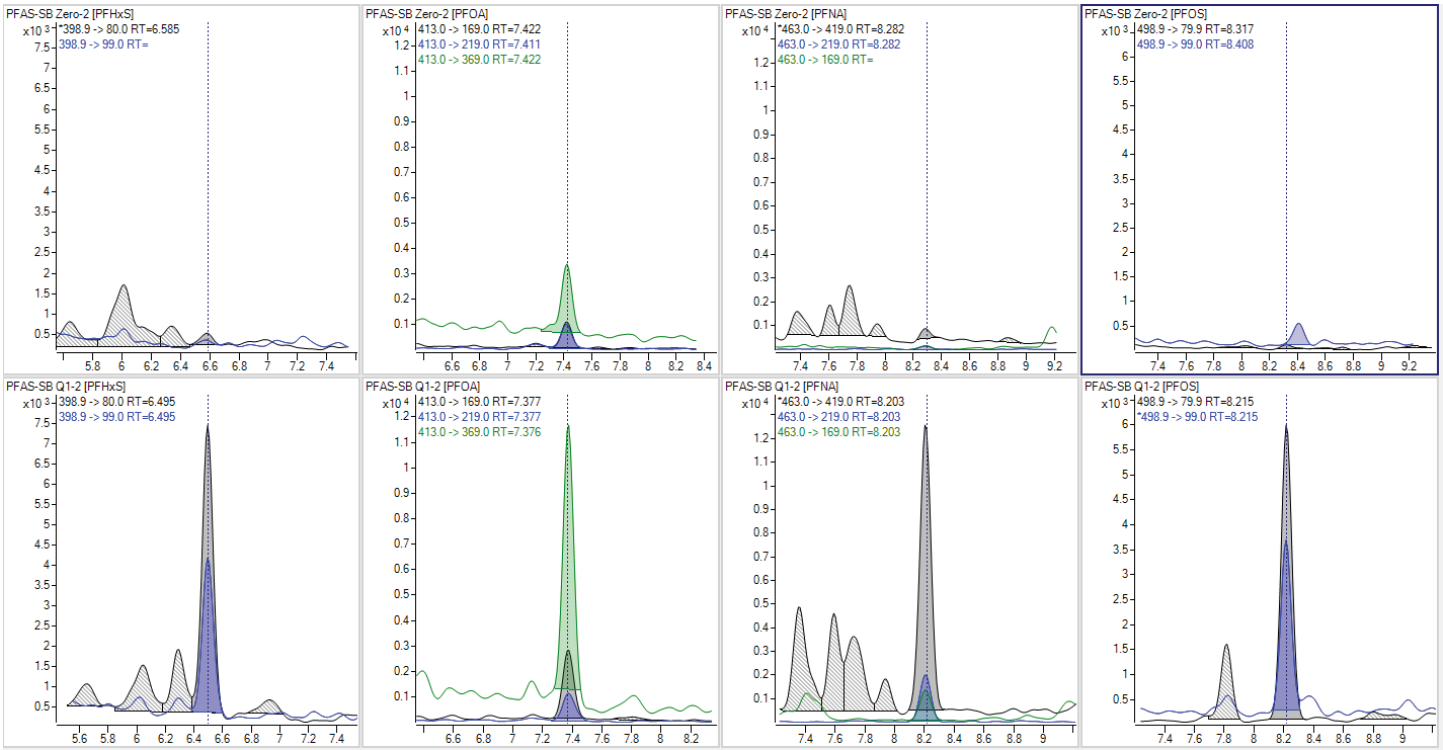

Figure 3. Soybean matrix blanks (top) and LOQ (0.05 µg/kg) samples (bottom) chromatograms for the core PFAS targets: PFHxS, PFOA, PFNA, and PFOS (from left to right).

Method accuracy and precision

The acceptance criteria for PFAS targets with corresponding ISTDs in feed are 65 to 135% for method recovery and ≤ 25% RSD% for method repeatability. For PFAS targets without a corresponding isotopic ISTD, the criteria are 40 to 140% for recovery and ≤ 30% for RSD%. The final reporting validation results shown in Figure 4 include three QC levels, LOQ, mid, and high levels in soybean, demonstrating the acceptable method recovery and repeatability for all 30 PFAS targets in dry soybean.

Conclusion

A simplified, rapid, and reliable method using QuEChERS extraction followed by EMR mixed-mode passthrough cleanup using an Agilent Captiva EMR PFAS Food II cartridge and LC/MS/MS detection was developed and validated for 30 PFAS targets in soybeans. The method was validated with acceptance performance, meeting the requirements described in AOAC SMPR 2023.003.

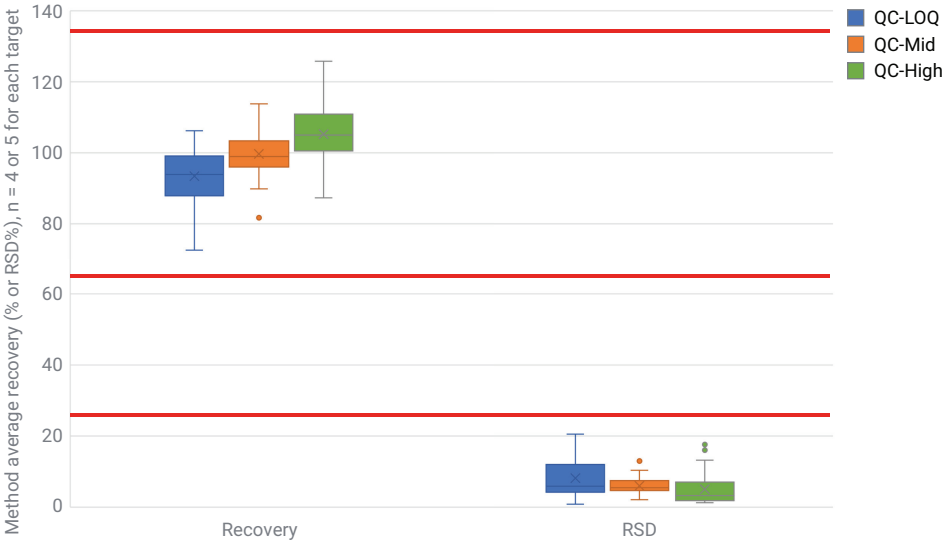

Figure 4. Method validation recovery and repeatability (RSD%) summary for PFAS analysis in dry soybean.

References

1. 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).
2. Zhao, L.; Giardina, M.; Parry, E. Determination of 30 Per- and Polyfluoroalkyl Substances (PFAS) in Infant Formula, Milk, and Eggs Using Agilent 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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