Application Brief

Food & Beverage Testing

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Determination of 30 Per- and Polyfluoroalkyl Substances in Bovine Kidney

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 bovine kidney. The method uses QuEChERS extraction followed by enhanced matrix removal (EMR) mixed-mode passthrough cleanup with 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 are listed in a previous application note.[2] The only difference is that the ISTD spiking solution used in this study was 1,000 ng/mL in methanol (MeOH).

Equipment and material

The study was performed using an Agilent 1290 Infinity II LC system coupled to a 6495D LC/TQ equipped with an Agilent Jet Stream iFunnel electrospray (ESI) ion source. Agilent MassHunter Workstation software was used for data acquisition and analysis.

Other equipment used for sample preparation in this study was same as those used in 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, 2.1 × 100 mm, 1.8 µm (part number 959758-902) and an Agilent 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)
- Captiva filter vial, 0.2 µm, nylon, 100/pk (part number 5610-5936)
- Polypropylene (PP) screw cap vials and caps, 2 mL (part number 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 are listed in a previous application note.[2]

Sample preparation

Bovine kidney was purchased from a local grocery store. The fresh sample was washed, cut into small pieces, and frozen at –20 °C overnight. The frozen sample pieces were ground to homogenate using a mechanical blender. The sample homogenate was then used for sample extraction.

For each of the homogenized samples, 2 g of sample homogenate were weighed into a clean 50 mL PP tube for extraction. The native PFAS spiking solutions and ISTD spiking solution (1,000 ng/mL) were added to the quality control (QC) samples appropriately, and only ISTD was added to matrix blanks. The samples were then ready for the sample preparation procedure, which is described in Figure 1.

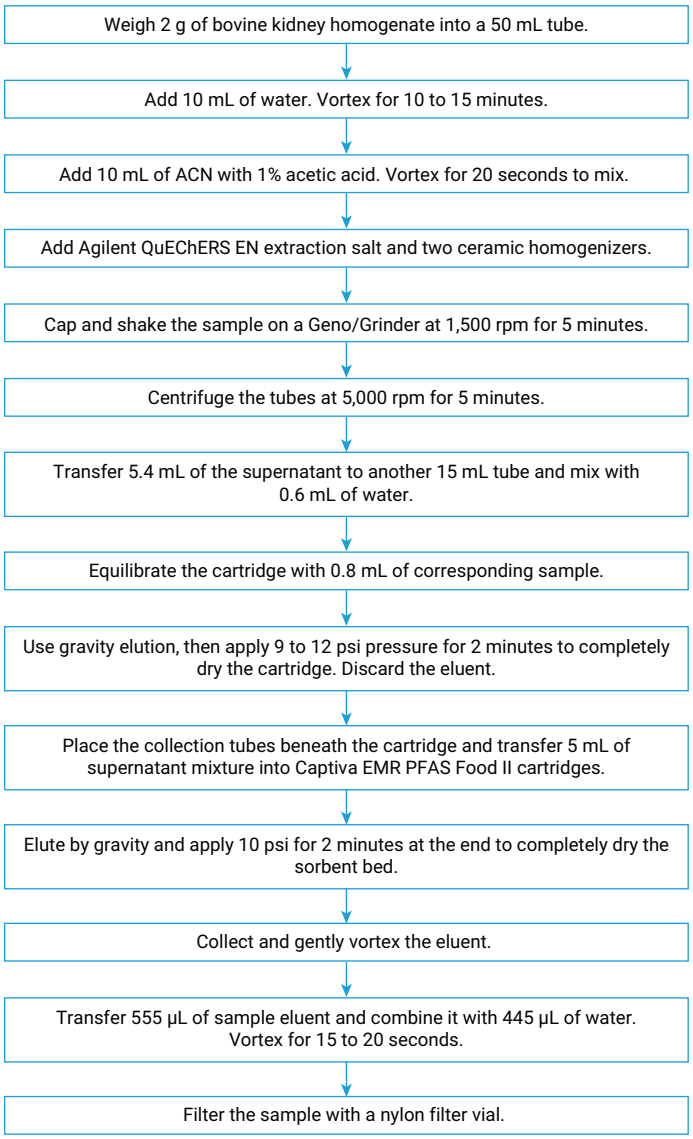

Figure 1. Sample preparation procedure for PFAS analysis in bovine kidney.

Method performance evaluation

The method was validated for method limit of quantitation (LOQ), recovery, and precision. Due to a few factors, including the limited volume of high ISTD spiking solution used in this study, cost considerations, and the higher required LOQs in animal offal matrix, four prespiked QC level samples were prepared in replicates of four at the levels of 0.2, 0.4, 1.0, and 5.0 µg/kg in bovine kidney. The ISTD prespiking level was 10.0 µg/kg in bovine kidney. In addition, the matrix blanks were prepared in replicates of five with 10.0 µg/kg ISTD prespiked for quantitation.

Results and discussion

EMR mixed-mode passthrough cleanup

The EMR mixed-mode passthrough cleanup was compared with traditional dSPE cleanup for PFAS target recovery. Figure 2 shows the PFAS target recovery comparison with spiking of 100 ng/L PFAS in bovine kidney crude extract after QuEChERS extraction.

The results demonstrated that EMR mixed-mode passthrough cleanup provided a significant improvement on PFAS target recovery compared to traditional dSPE cleanup.

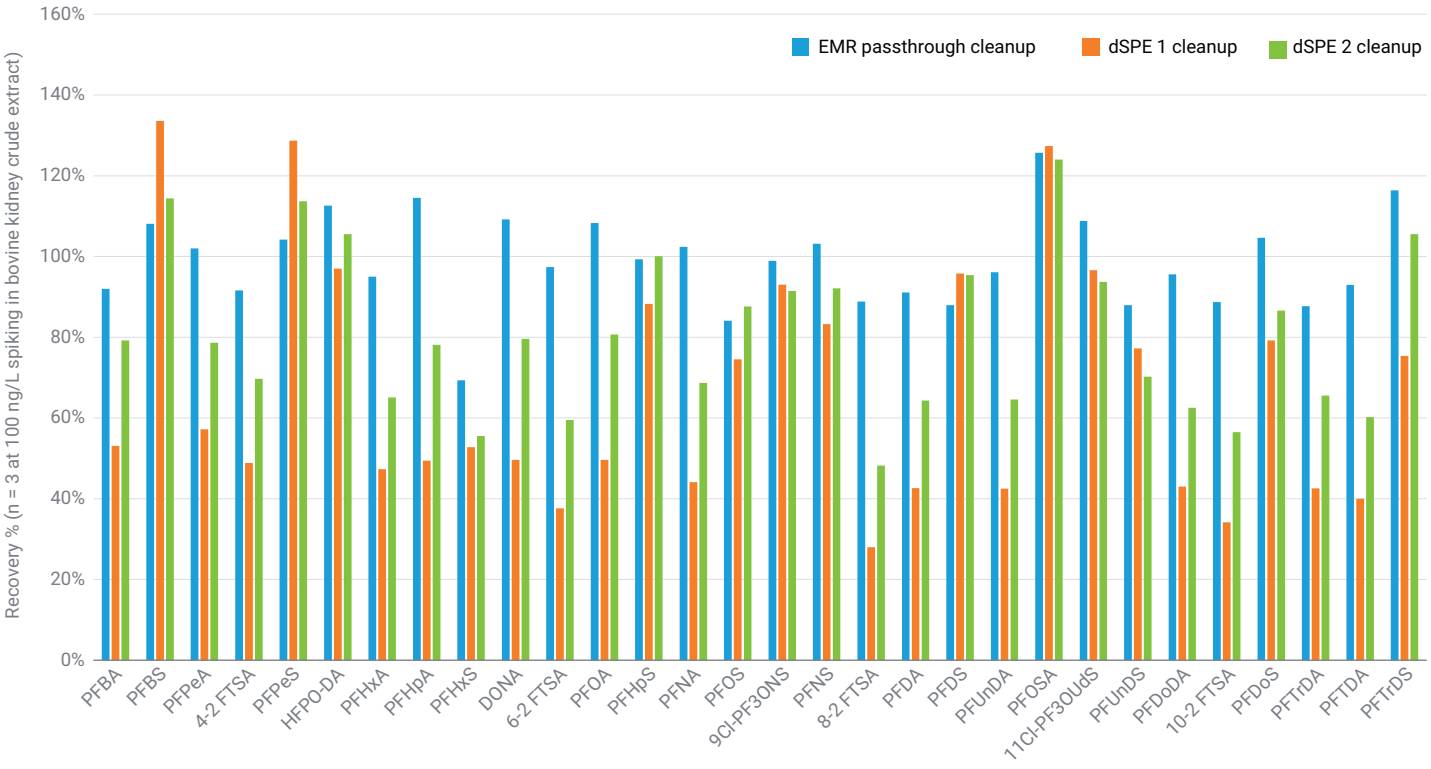

Figure 2. Comparison of EMR passthrough cleanup using the Agilent Captiva EMR PFAS Food II cartridges with traditional dSPE cleanup for PFAS recovery in bovine kidney extract.

Method validation

The newly developed method was validated for the determination of 30 PFAS targets in bovine kidney following the AOAC SMPR guidance. Considering bovine kidney to be in the "edible offal" category, the required LOQs are ≤ 0.4 µg/kg for the four core PFAS targets (PFOS, PFOA, PFNA, and PFHxS) and ≤ 4.0 µg/kg for the remaining PFAS targets.[2] Since bovine kidney is a highly complex food matrix with higher required LOQs, postconcentration treatment is not necessary and postdilution is more suitable. After EMR mixed-mode passthrough cleanup, the sample eluent was diluted with water to 1:1 ACN:water. Therefore, the entire method introduced a 10x dilution. The postdilution treatment allowed the obsoletion of a sample drying step, which saved significant time and made the entire method procedure easier and quicker. The only consideration for the postdilution treatment was the adjustment on the ISTD prespiking concentration. Due to the introduction of the dilution factor during sample preparation, the prespiking ISTD concentration needs to be higher to correct the dilution factor and match the ISTD concentration in neat calibration standards. Since the ISTD concentration in neat calibration curve standards was 1,000 ng/L, the prespiking ISTD concentration in bovine kidney homogenate was adjusted to 10,000 ng/kg. Thus, this adjustment required the use of ISTD spiking solution at a higher concentration level.

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 bovine kidney. The validated method LOQs were all below or equal to the required LOQs in editable offal matrix. Results also showed that more PFAS targets were detected at higher levels in bovine kidney matrix blank. Figure 3 shows the bovine kidney 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 bovine kidney matrix.

Target	Bovine Kidney LOQ (µg/kg)	
	LOQ_{cal}	LOQ_{val}
PFBA	1.183	4.0
PFPeA	0.374	0.4
PFBS	0.109	0.2
4:2 FTS	0.25	0.4
PFPeS	0.207	0.2
PFHxA	0.356	0.4
HFPO-DA	0.174	0.2
PFHpA	0.195	0.4
PFHxS*	0.1	0.2
DONA	0.01	0.2
6:2 FTS	0.608	1.0
PFOA*	0.174	0.4
PFHpS	0.177	0.2
PFNA*	0.176	0.4
PFOS*	0.339	0.4

Target	Bovine Kidney LOQ (µg/kg)	
	LOQ_{cal}	LOQ_{val}
9Cl-PF3ONS	0.024	0.2
8:2 FTS	0.108	0.4
PFNS	0.247	0.2
PFDA	0.172	0.4
PFDS	0.028	0.2
PFUnDA	0.54	1.0
PFOSA	0.009	0.2
11Cl-PF3OUdS	0.007	0.2
PFUnDS	NA	0.2
PFDoDA	0.239	0.4
10:2 FTS	NA	0.2
PFDoS	NA	0.2
PFTrDA	NA	0.2
PFTrDS	NA	0.2
PFTeDA	0.82	1.0

* Core PFAS targets
NA = not applicable

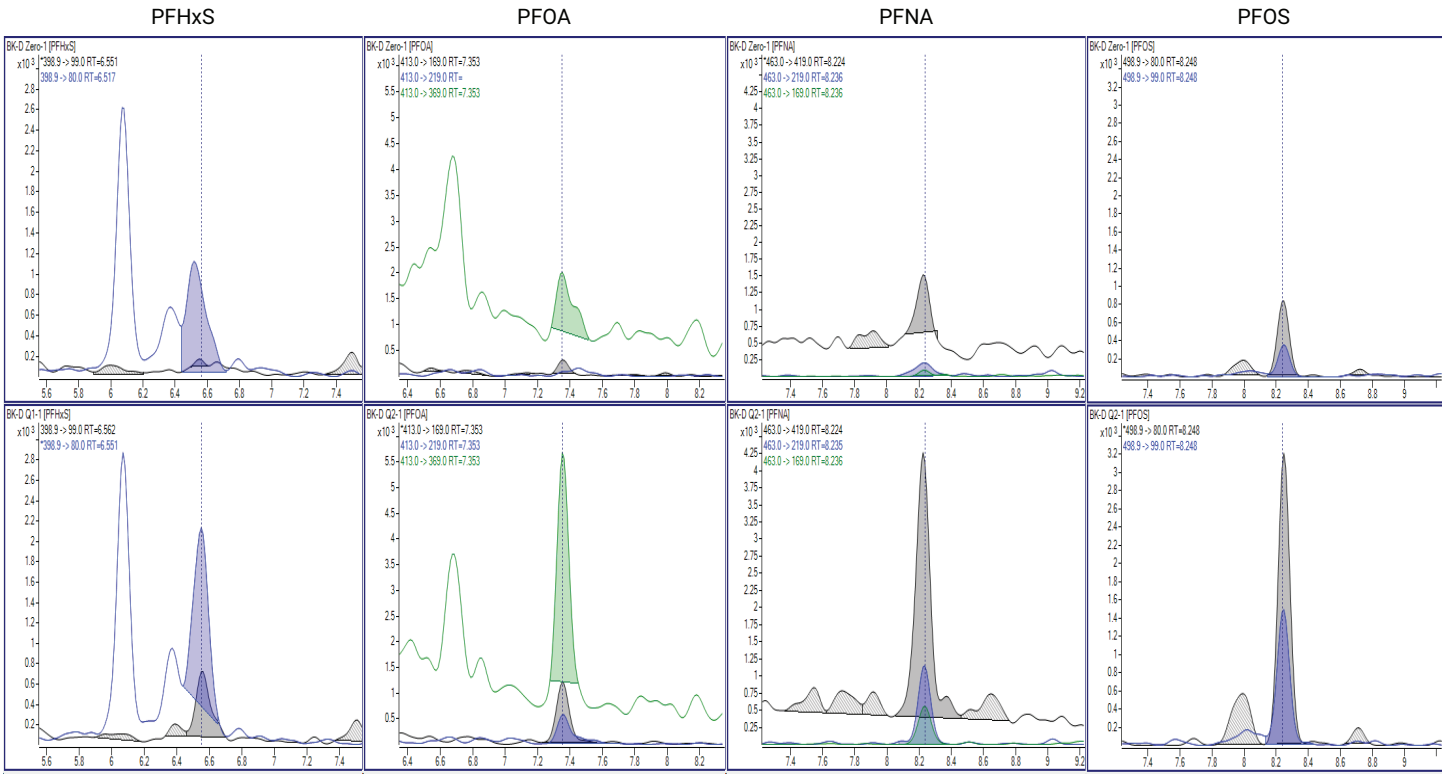

Figure 3. Bovine kidney matrix blanks (top) and LOQ (bottom) chromatograms for the core PFAS targets, PFHxS (0.2 µg/kg), PFOA (0.4 µg/kg), PFNA (0.4 µg/kg), and PFOS (0.4 µg/kg).

Method accuracy and precision

The acceptance criteria for PFAS in edible offal are 80 to 120% recovery and ≤ 20% RSD% for the four core PFAS targets, and 65 to 135% recovery and ≤ 25% RSD% for the remaining PFAS targets with corresponding isotopic ISTDs. For the rest PFAS targets without corresponding isotopic ISTDs, 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 in bovine kidney, including LOQ, mid, and high levels, demonstrating the acceptable method recovery and repeatability for most PFAS targets in bovine kidney. However, some exceptions occurred, including that only two spiking level results were reportable for 6:2 FTS and PFUnDA due to a significant positive detection from the matrix blank, and a higher recovery for 4:2 FTS and PFPeS at one spiking level due to matrix effect.

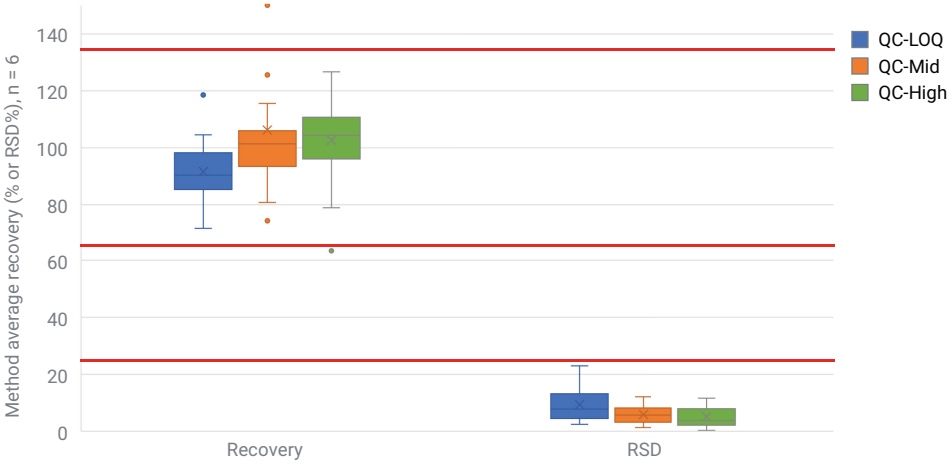

Figure 4. Method validation recovery and repeatability (RSD%) summary for PFAS analysis in bovine kidney.

Conclusion

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

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 Pass-Through Cleanup and LC/MS/MS Detection, *Agilent Technologies application note*, publication number 5994-7366EN, **2024**.

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Printed in the USA, June 1, 2024
5994-7370EN

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