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Determination of PFAS in Tissue Using QuEChERS Extraction Followed by Noval Enhanced Matrix Removal Mixed-mode Passthrough Cleanup with LC-MS/MS Detection

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Introduction

Determination of PFAS residues in tissue, especially in fish, has been an important avenue for monitoring and regulating PFAS residues in the environment. The EPA published method 1633 for the quantitative analysis of PFAS in aqueous, solid, biosolid, and tissue samples using LC/MS/MS for 40 PFAS targets. The method applies polymeric WAX SPE for sample extraction. It demonstrates excellent performance for aqueous sample analysis. However, the SPE methodology is challenging for complex biological tissue samples, requiring multiple steps, taking significant longer time, and increasing high risks of contamination. The SPE method also doesn't clean the fatty tissue matrix efficiently, resulting in poor performance for some longer chain PFAS targets.

QuEChERS extraction followed with enhanced matrix removal (EMR) mixed-mode passthrough cleanup significantly simplifies the sample preparation procedure, saving > 80% of preparation time and using approximately 80% less organic solvents. The EMR mixed-mode passthrough cleanup provides comprehensive and effective cleanup for tissue matrices without compromises on PFAS targets recovery.

The objectives of this study were to apply this method to the analysis of 40 PFAS in biological tissue matrices and validate it to meet the acceptance criteria of EPA 1633 method.

LC/MS/MS Detection

LC conditions (Agilent 1290 Infinity II)			
Columns	Agilent ZORBAX Eclipse Plus C18, 2.1 x 100 mm, 1.8 µm column (p/n 959758-902) Agilent InfinityLab PFC delay column, 4.6 x 30 mm,		
Flow Rate	0.4 mL/min		
Column Temp.	55 °C	Injection volume	5 µL (with water sandwiched injection)
Mobile Phase	A: 5 mM ammonium acetate in water B: Acetonitrile		
Needle Wash	IPA, ACN, water		
Gradient	Time (min) %B Flow (mL/min) 0 10 0.4 2 30 0.4 8.5 45 0.4 11.5 75 0.4 13.25 100 0.4		
Stop Time	15.5 min	Post time	2 min

QQQ conditions (Agilent 6495D LC/MS system)					
Drying Gas	200 °C, 18 L/min			Sheath Gas	300 °C, 11 L/min
Nebulizer Gas	15 psi	Polarity	Neg	Acquisition	dMRM
Capillary Voltage	2500 V		Nozzle Voltage		0 V

Sample Preparation

Weigh 5 g of grinded tissue sample into a 50 mL tube

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Add 5-10 mL of water. Vortex 10-15 mins

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Add 10 mL of ACN w/ 1% acetic acid. Vortex 20 sec for mixing.

↓

Add QuEChERS EN salt and two ceramic homogenizers

↓

Cap and shake the sample on a Gino Grinder at 1500 rpm for 5 mins.

↓

Centrifuge tubes at 5000 rpm for 5 mins

↓

Transfer 4.5 mL of supernatant mixture to another 15 mL tube and mix with 0.5 mL water.

↓

Prewash the EMR PFAS Food II cartridges w/ 5 mL of 1:1 ACN/MeOH w/ 1% acetic acid, the equilibrate with 0.8 mL of corresponding sample mixture.

↓

Discard the eluent, dry the cartridge completely and place the pre-labelled 15 mL PP tubes for sample eluent collection

↓

Transfer 3.5 mL sample mixture into cartridge and elute with gravity until dripping stops. Apply 10 psi for 2 mins at the end.

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Vortex the eluent and take an aliquot for analysis.

Figure 1. Sample preparation procedure chart for preparing tissue samples.

LC/MS/MS chromatography for targets distribution and critical separation with cholic acids.

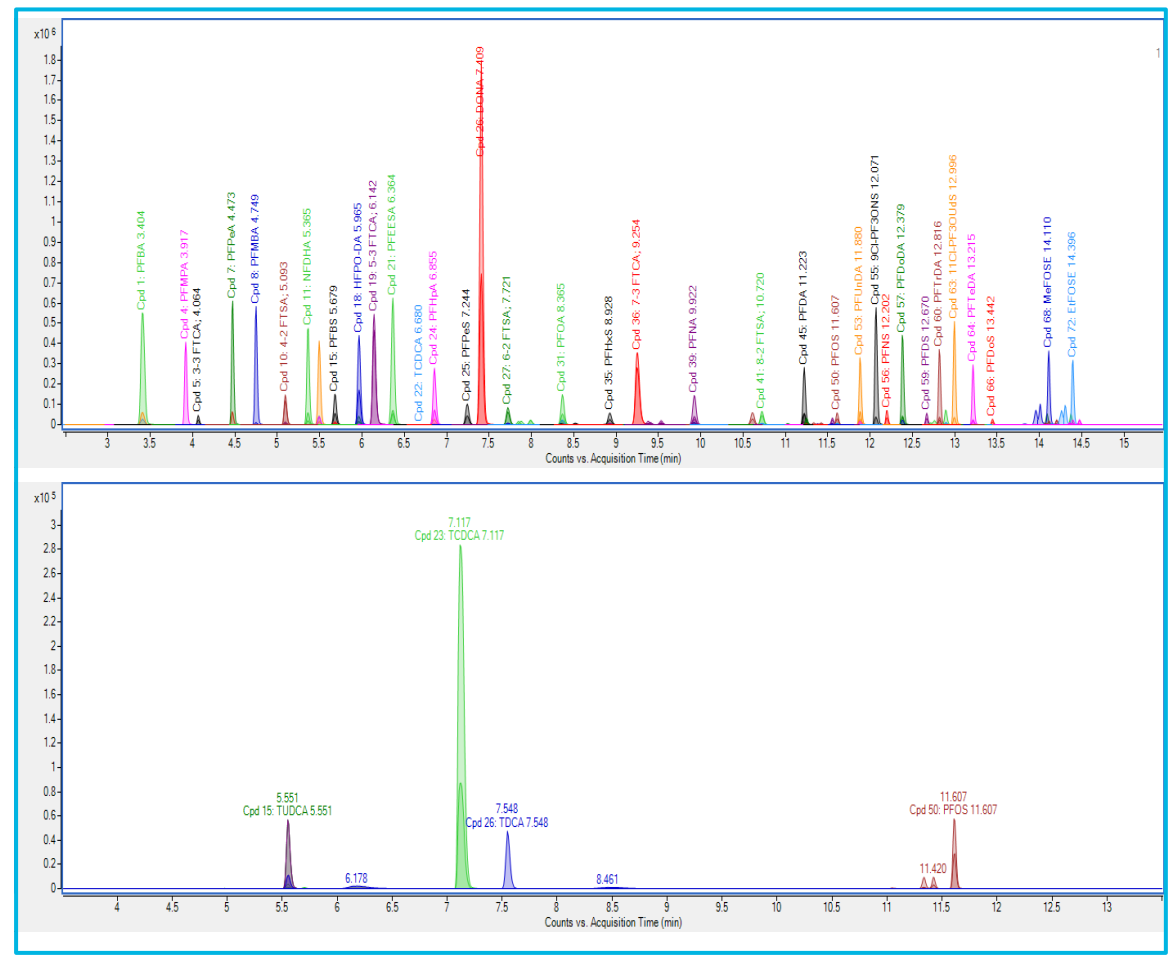


Figure 2. MRM chromatograms for all PFAS targets, EIS, and NIS compounds (top), and PFOS isomers and cholic acids interferences. (B)

Results and Discussion

Sandwiched injection program allows injection of sample in ACN

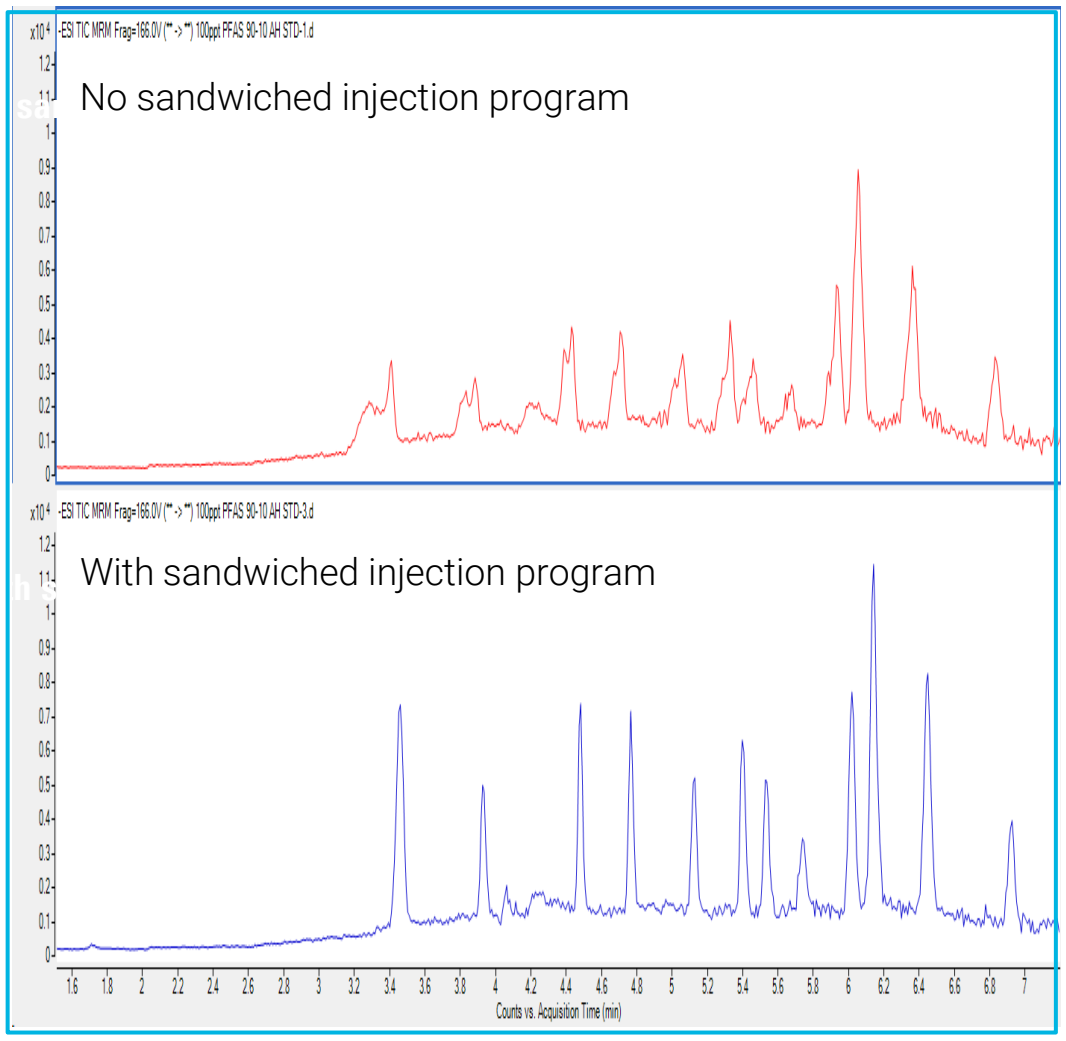


Figure 3. MRM chromatograms comparison without (top) vs. with (bottom) sandwiched injection program.

Simplified sample prep saving time and effort

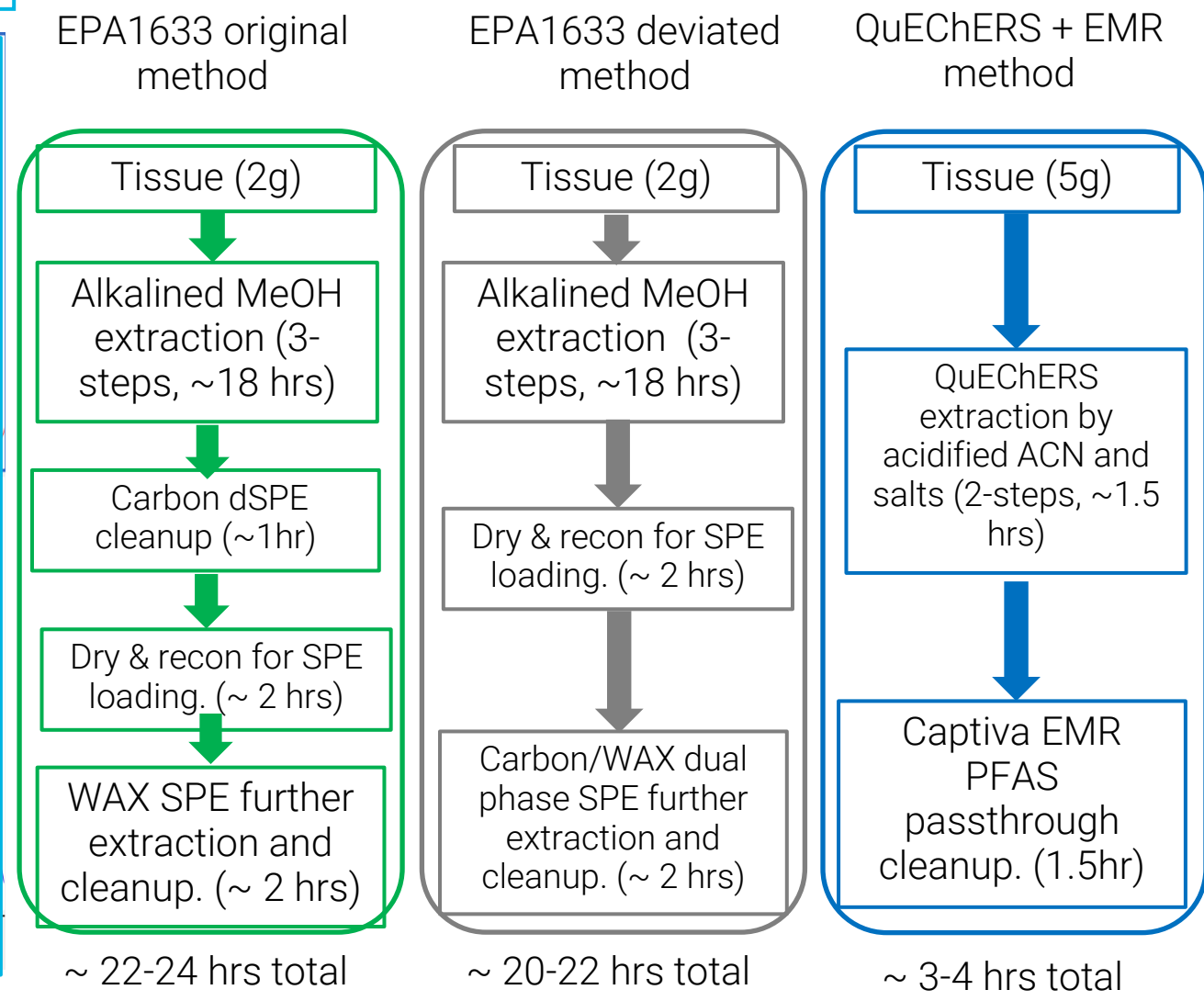
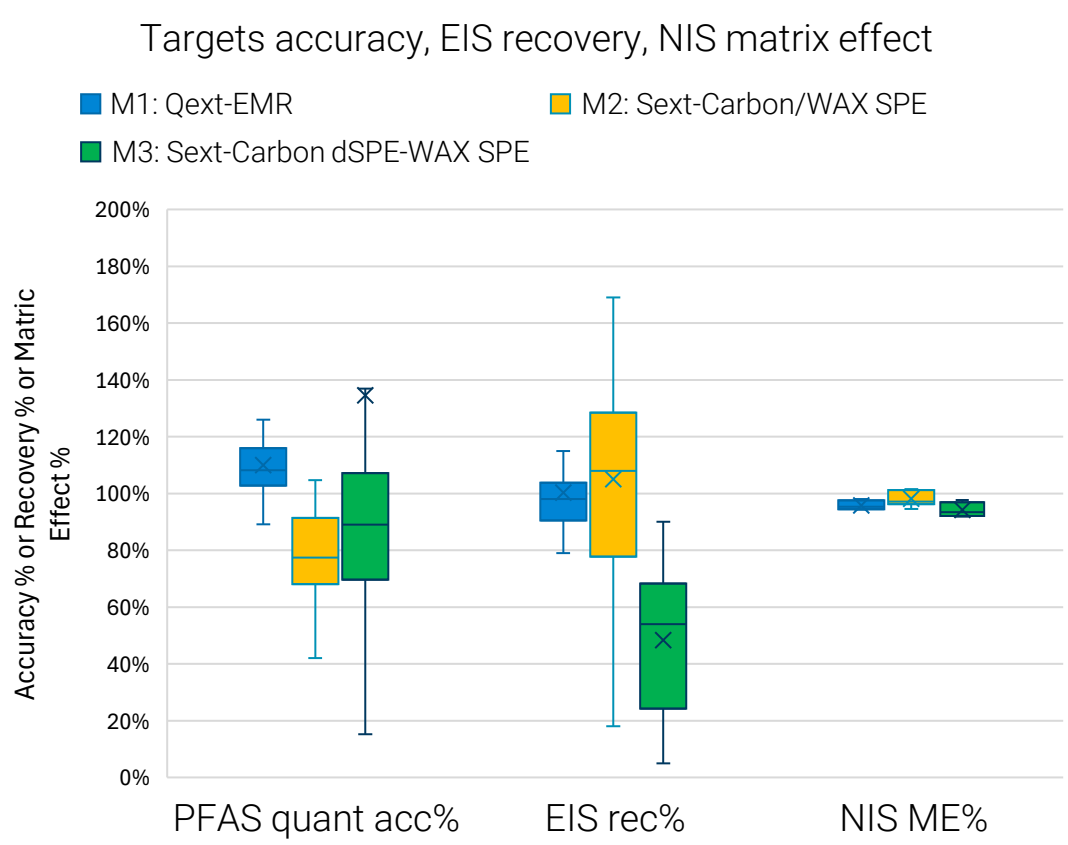


Figure 4. Sample preparation procedure comparison.

Method performance improvement on recovery, repeatability and matrix effect



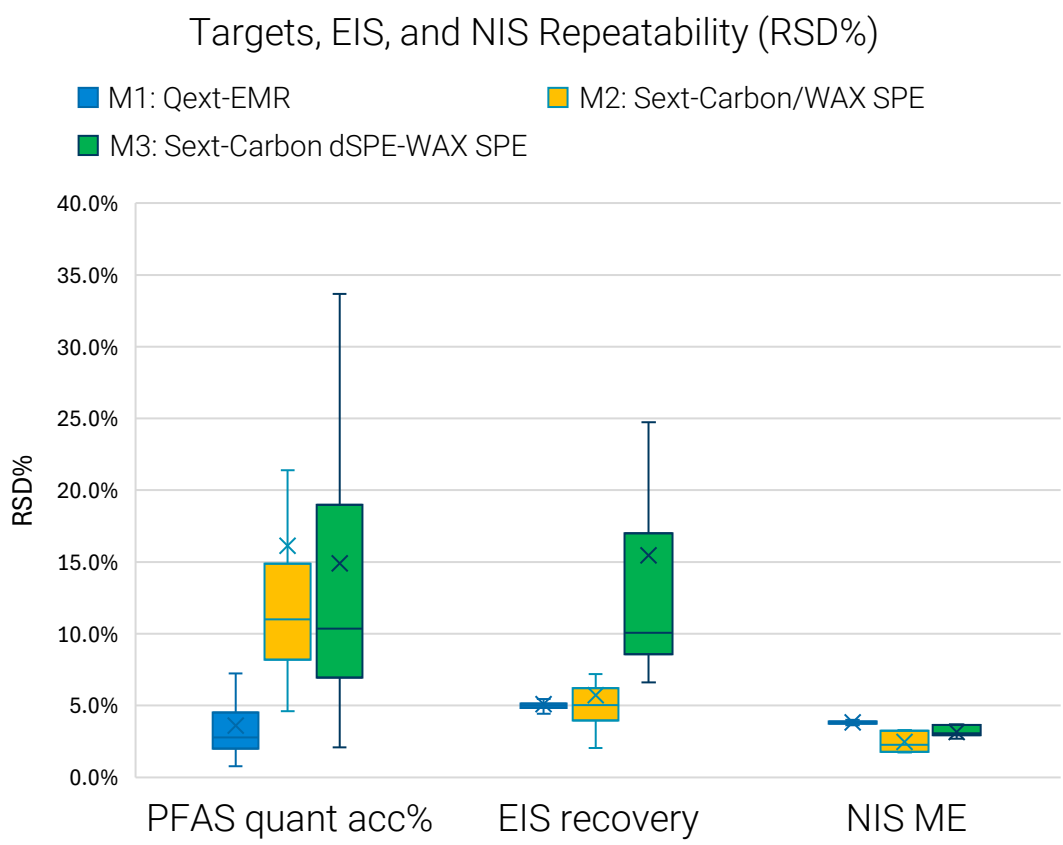


Figure 5. Method statistical comparison for PFAS in tissue quantitative analysis for targets accuracy, EIS recovery and NIS matrix effect (left), and method repeatability (RSD%) (right).

Results and Discussion

Method Validation

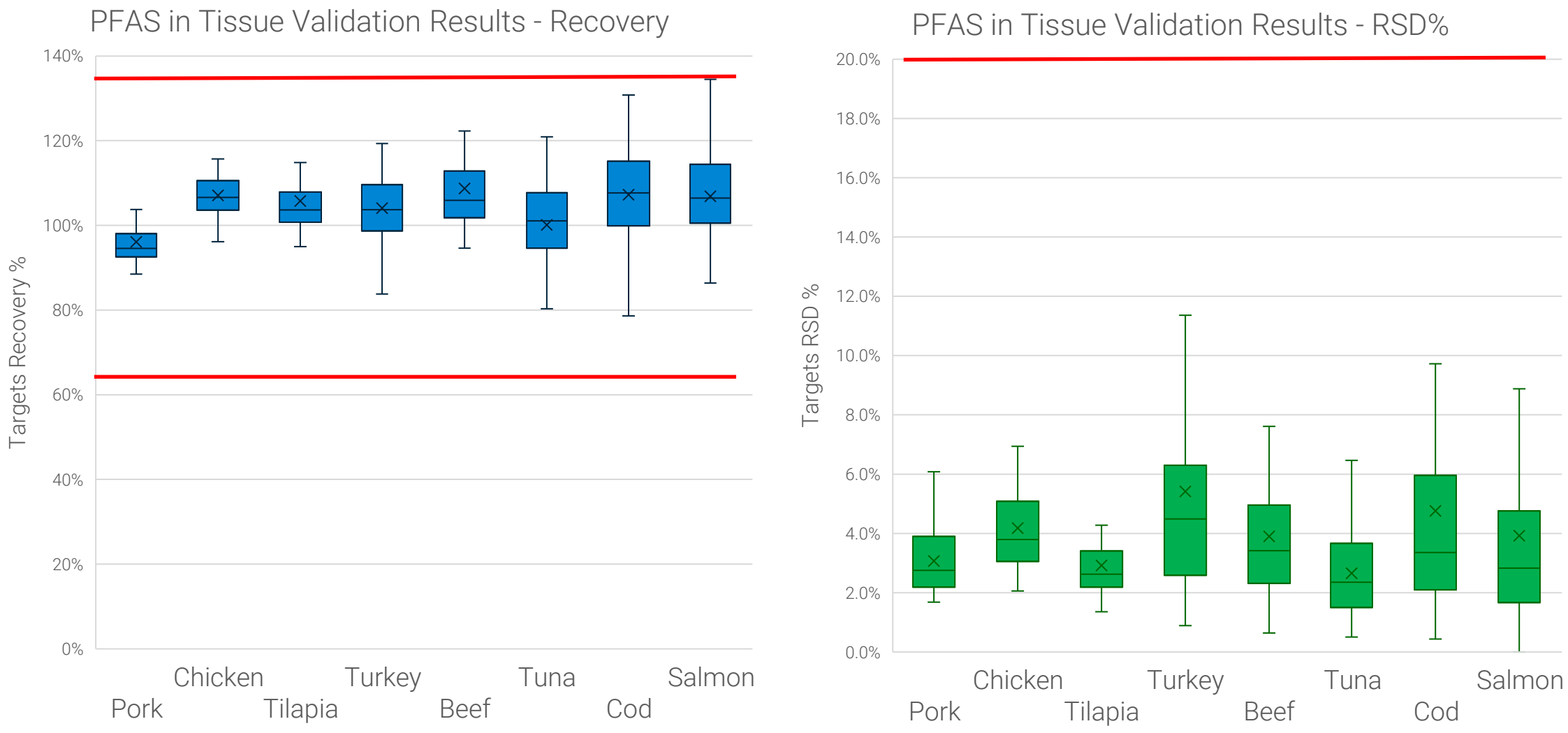


Figure 6. Method validation results for 40 PFAS in eight tissue matrices for targets recovery% (left) and RSD% (right).

Conclusions

- A novel sample preparation method was developed for PFAS in tissue analysis.
- LC/MS/MS instrument method demonstrated excellent chromatography, sensitivity and selectivity.
- Method comparison with EPA 1633 method demonstrated the improvement on method simplicity, performance and sample analysis productivity. .
- Method validation for 40 PFAS in eight tissue matrices with satisfying EPA acceptance criteria.

References

1. U.S. Environmental Protection Agency. Method 1633, Revision A: Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/MS. *EPA 820-R-24-007*, December **2024**.

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