Determination of PFAS in Biological Tissue by LC-MS/MS

- Using QuEChERS extraction followed with EMR mixed-mode passthrough cleanup

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Trusted Answers

PFAS Analysis in Tissue

Background Introduction

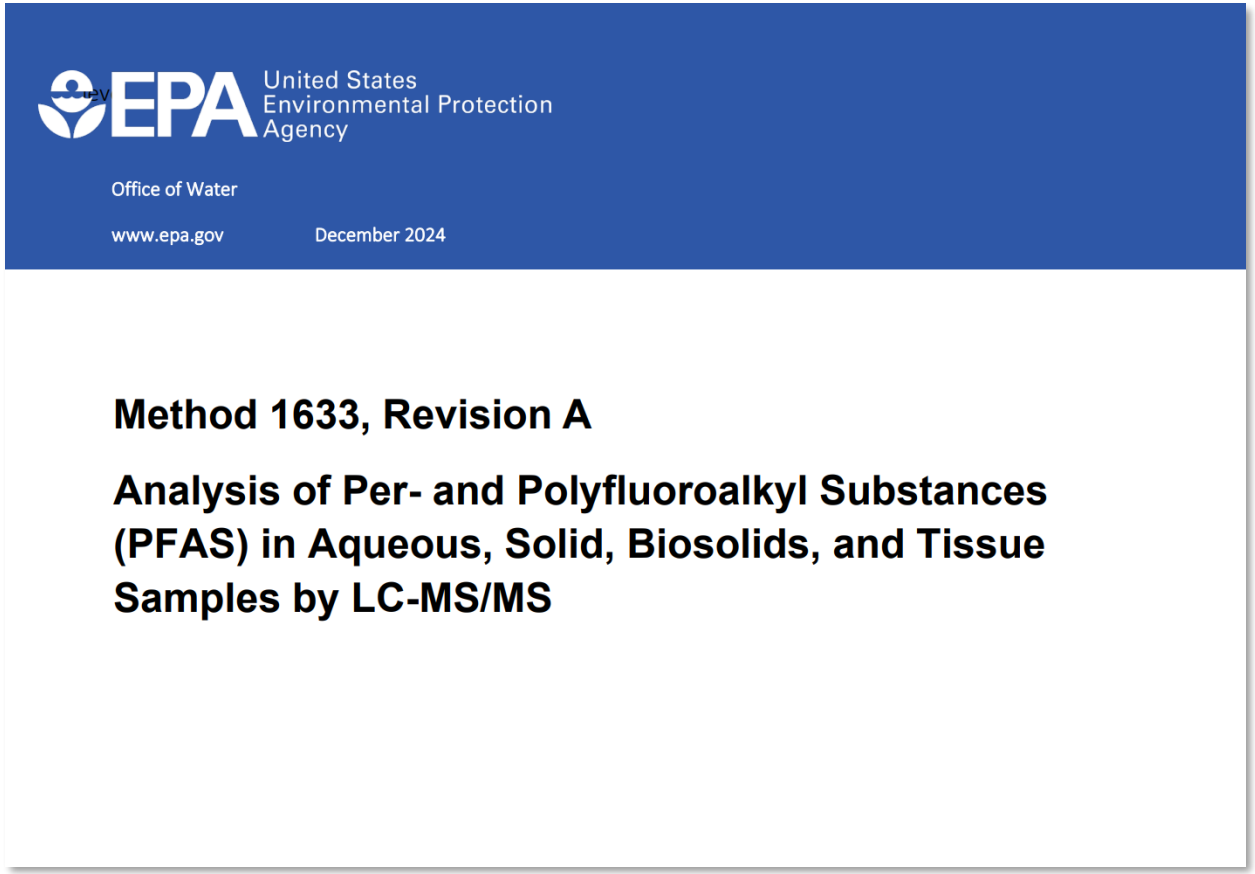
Determination of PFAS in Biological Tissue

- ❑ Environmental monitoring of PFAS residue in wildlife, especially in fish
- ❑ EPA Method 1633
- ❑ 40 PFAS analytes
- ❑ Quantitation based on calibration curve in neat with the use of both EIS and NIS
- ❑ Reported LOQ at 0.4 – 0.5 ng/g or higher
- ❑ Relatively large and various tolerance window on targets recovery and RSD across the 40 analytes

- ❑ Food safety surveillance for PFAS residue in edible fish and meat of terrestrial animals
- ❑ AOAC SMPR 2023.003 & EC Regulation 2023/915
- ❑ 30 PFAS analytes
- ❑ No strict requirements on quantitation method
- ❑ Required LOQ at < 0.1 ng/g for core PFAS compounds, and < 1 ng/g for others
- ❑ 80-120% recovery and < 20% RSD for core PFAS; and 65-135% recovery and < 25% for others.

Targets: PFAS, Matrix: biological tissue, Detection: LC-MS/MS

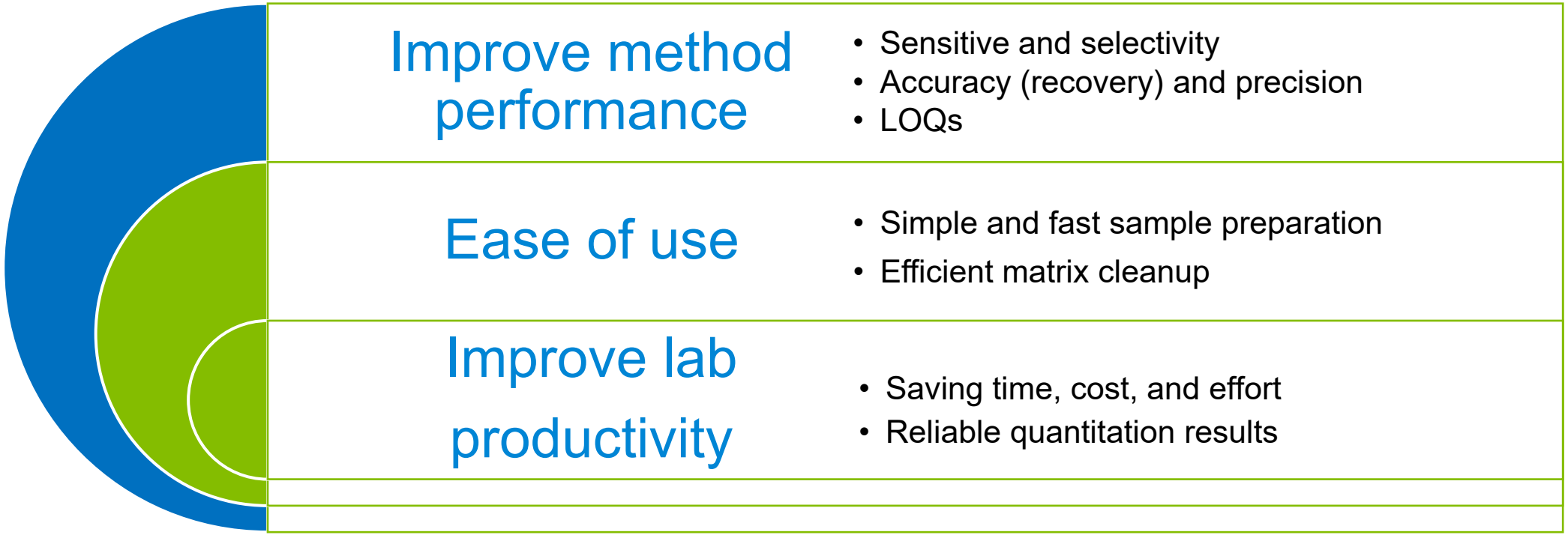
EPA Method 1633 Overview for PFAS Determination in Tissue



- Performance-based EPA method
- Detection ☺
  - LC-MS/MS detection
- Quantitation ☺
  - Extraction ISTD (EIS) and non-extracted ISTD (NIS)
  - Calibration curve standards in solvent
- Sample preparation ☹
  - Three-step solvent extraction using alkaline MeOH and ACN with extensive incubation
  - Carbon/WAX SPE or Carbon dispersive SPE + WAX SPE
  - Long procedure taking > 20 hrs!
  - Multiple steps with high risk of contaminations and deviations
  - Challenging with complex matrices

Project Objectives

An improved sample preparation method for PFAS analysis in biological tissue



Fish tissue

Poultry and terrestrial animal meat

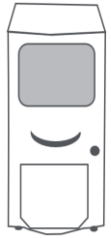
PFAS Analysis in Biological Tissue

Sample Preparation

Sample Preparation Overview

Sample extraction

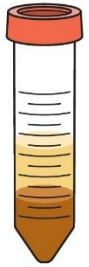
QuEChERS extraction



Mechanical shaking

Centrifuge

Sample cleanup



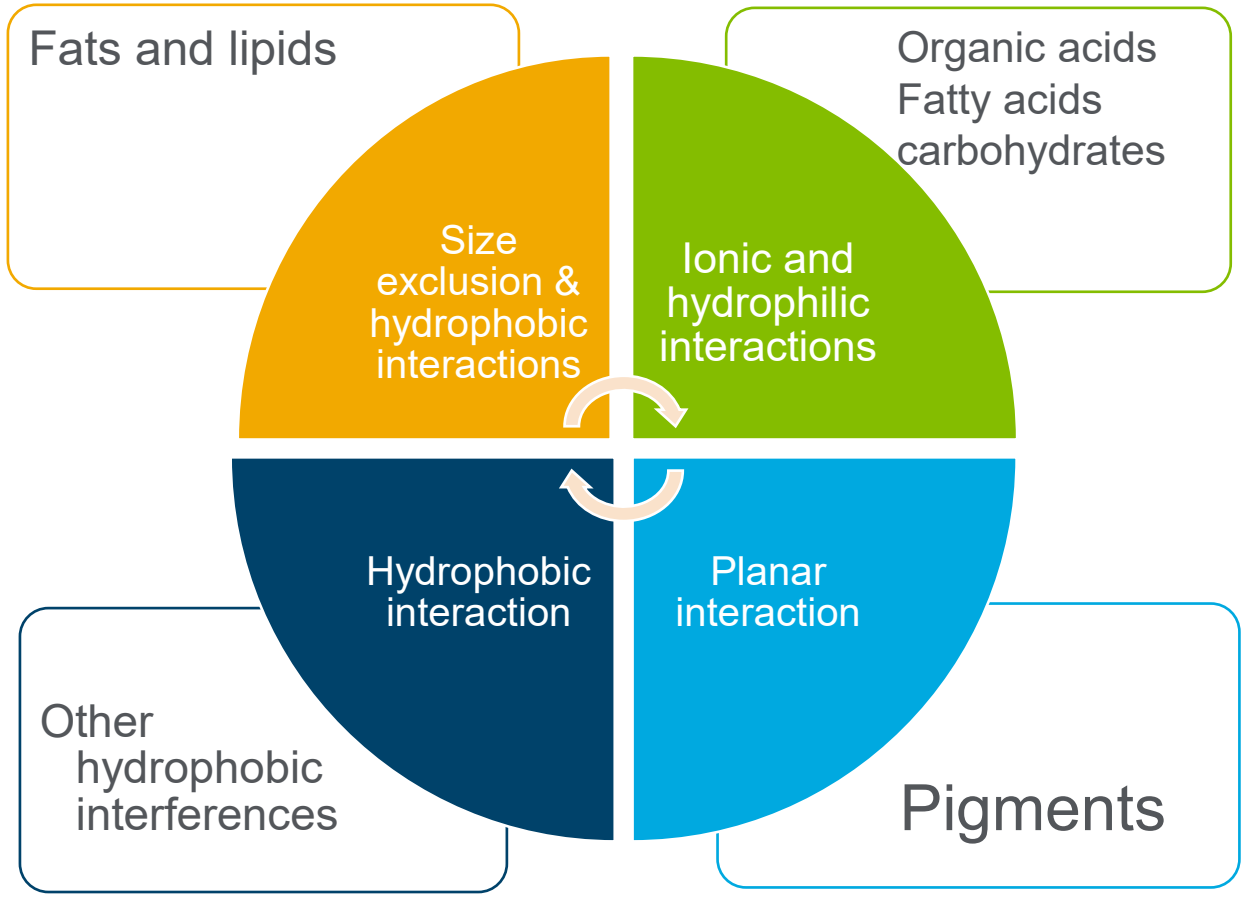
Sample crude extract

Captiva EMR PFAS Food II cartridge

LC/MS/MS

EMR Mixed-mode Passthrough Cleanup

Comprehensive matrix co-extractives removal



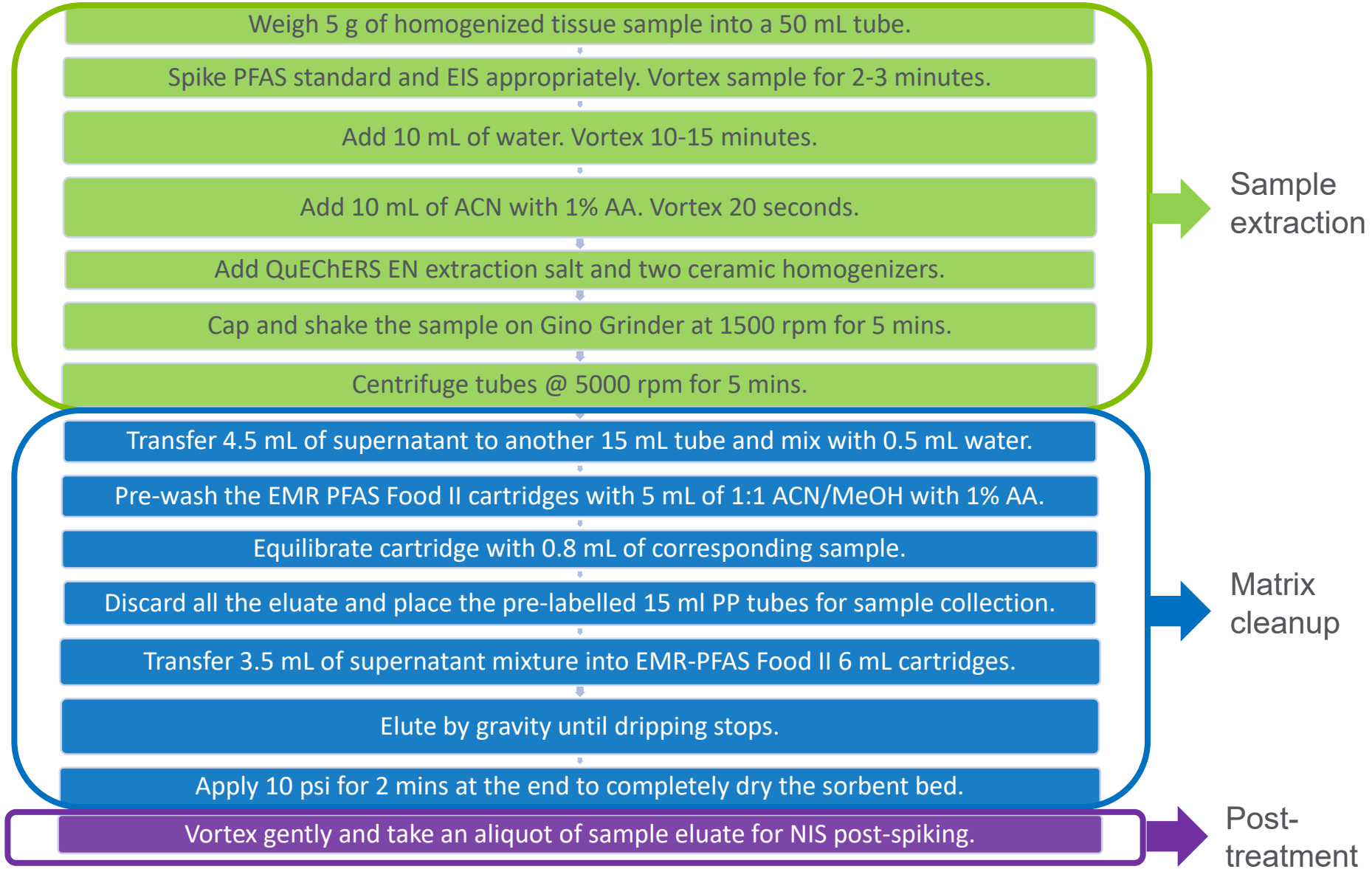
Method features

- ✓ Matrix co-extractives targeted chemical filtration mechanism
- ✓ Minimal impact on PFAS targets recovery
- ✓ Direct compatible with QuEChERS extraction
- ✓ One step cleanup
- ✓ Comprehensive and efficient matrix removal
- ✓ Higher sample volume (>90%)
- ✓ Saving time and effort

Sample Preparation Procedure

Sample prep consumables:

- Bond Elut EN buffered salts and ceramic homogenizers
- Captiva EMR PFAS Food II, 6 mL cartridge
- Polypropylene centrifuge tubes, 50 mL and 15 mL
- Polypropylene vials and caps
- All consumables are either certified or pre-screened for acceptable PFAS cleanliness



Methods Comparison

Method	Novel sample prep method	Traditional EPA 1633 sample prep method	
	$QuEChERS_{ext}$-EMR	$Solvent_{ext}$-Carbon/WAX SPE	$Solvent_{ext}$-Carbon dSPE-WAX SPE
Pre-work	Make 2 reagents	• Make 6 reagents • Pack glass wool in SPE cartridge	• Make 6 reagents • Pack glass wool in SPE cartridge
Sample extraction	One-step QuEChERS extraction	Three-step solvent extraction	Three-step solvent extraction
Transition step	Dilution with 10% water	• Drying and redissolving • pH check and adjustment	• Carbon dSPE cleanup • Drying and redissolving • pH check and adjustment
Matrix cleanup	EMR passthrough cleanup	Carbon/WAX SPE extraction and cleanup	WAX SPE extraction and cleanup
Post treatment	NIS post-spiking	• Sample neutralization • NIS post-spiking • Filtration	• Sample neutralization • NIS post-spiking • Filtration
Total time	2-4 hours	> 20 hours	
Total cost	Low with >50% cost saving	High	

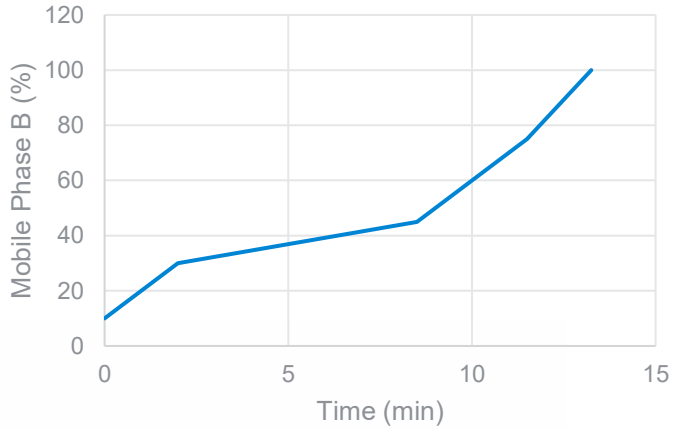
PFAS Analysis in Biological Tissue

LC/MS/MS Instrument Detection and Quantitation

Instrumental Analysis by LC-MS/MS

LC method parameters			MS QQQ Parameters	
Solvent A	5 mM ammonium acetate in water		Ion Source	ESI
Solvent B	ACN		Acquisition	dMRM
Flow	0.4 mL/min		Polarity	Negative
Pump Program	$T_{0.0}$	10% B		
	$T_{2.0}$	30% B	Source Parameters	
	$T_{8.5}$	45% B	Gas Temp	200 ºC
	$T_{11.5}$	75% B	Gas Flow	18 L/min
	$T_{13.25}$	100% B	Nebulizer	15 psi
Stop Time	15.5 min		Sheath Gas Heater	300 ºC
Post Time	2 min		Sheath Gas Flow	11 L\min
Injection Volume & program	5 µL		Capillary	2500 V (-), 0 V (+)
	15 µL water + 5 µL sample + 10 µL water + 10 µL air		Nozzle Voltage	0 V
Needle Wash	Multi-wash program using 1. IPA; 2. ACN; 3. H_2O			
Analytical LC column	RRHD Eclipse Plus C18, 1.8 µm, 2.1 x 100 mm			
Guard column	Eclipse Plus C18, 1.8 µm, 2.1 x 5 mm			
Delay column	InfinityLab PFC delay column, 4.6 x 30 mm			
Column temperature	55°C			

LC Gradient

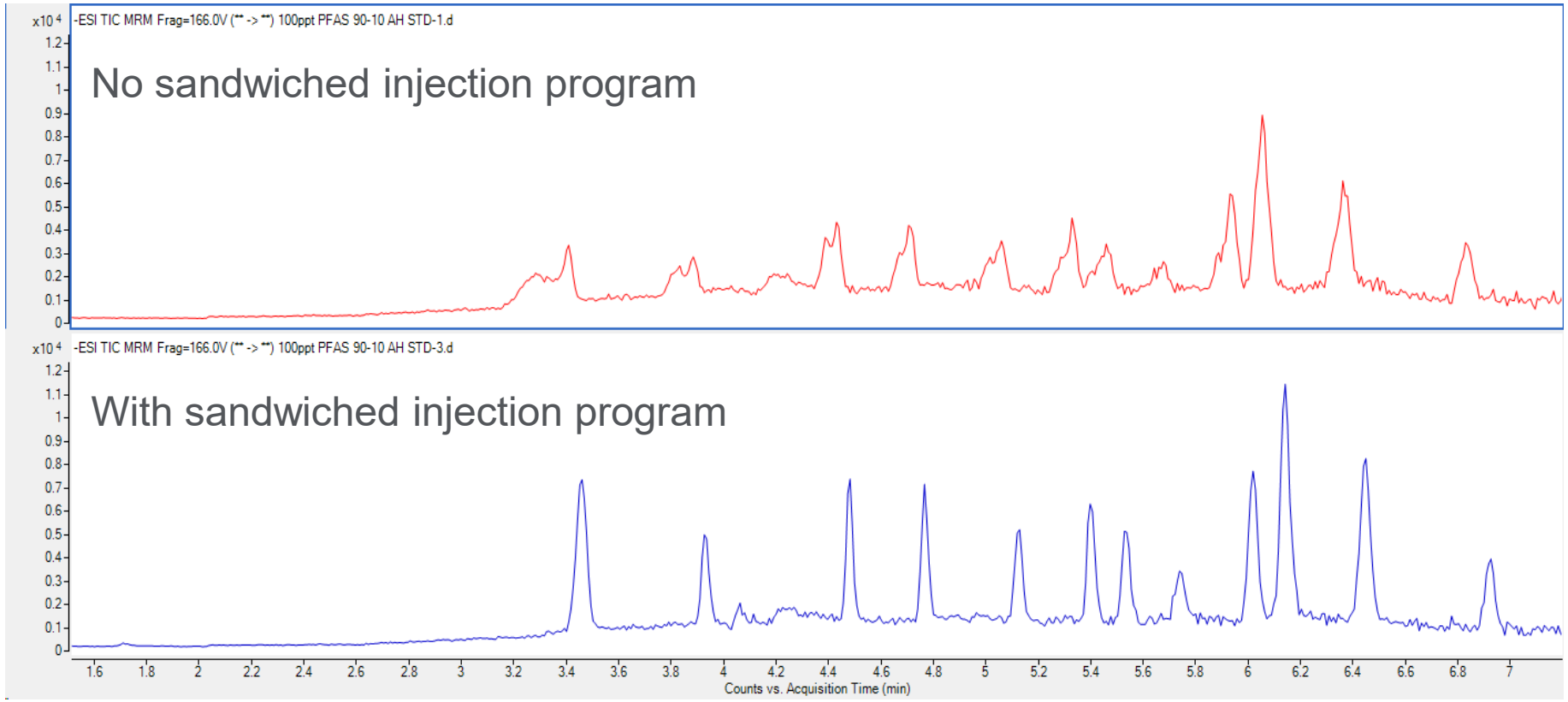


Agilent Triple quadrupole LC/MS system, 6495D mass spectrometer

Sandwich Injection

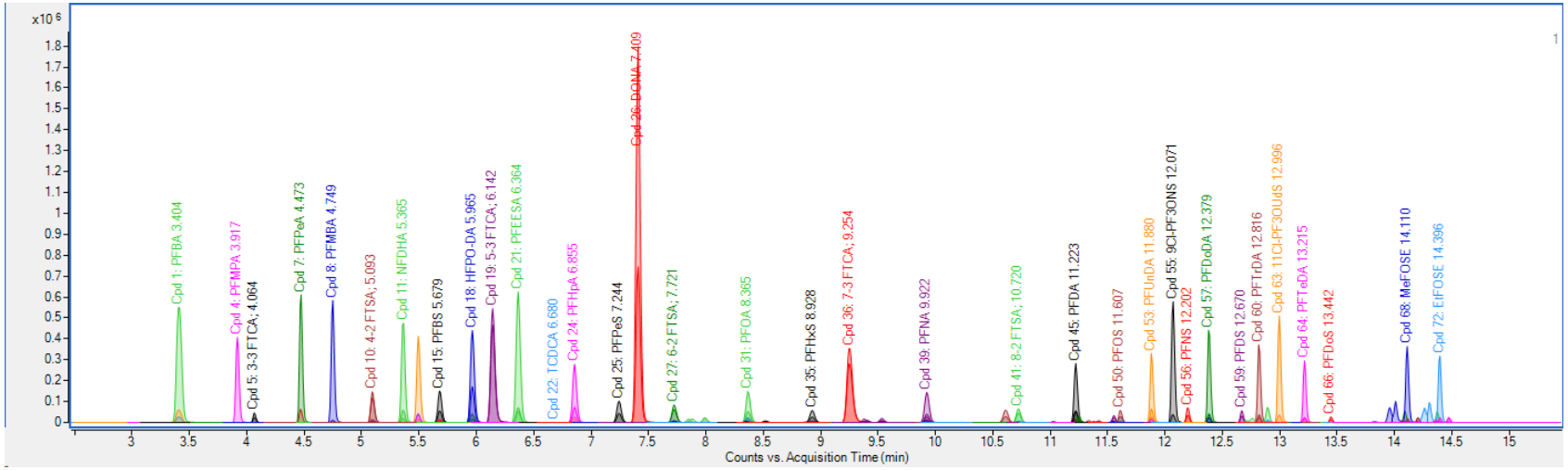
- Injection plug sandwiched between low strength solvent plugs

- Enable the injection of sample eluent after EMR cleanup directly

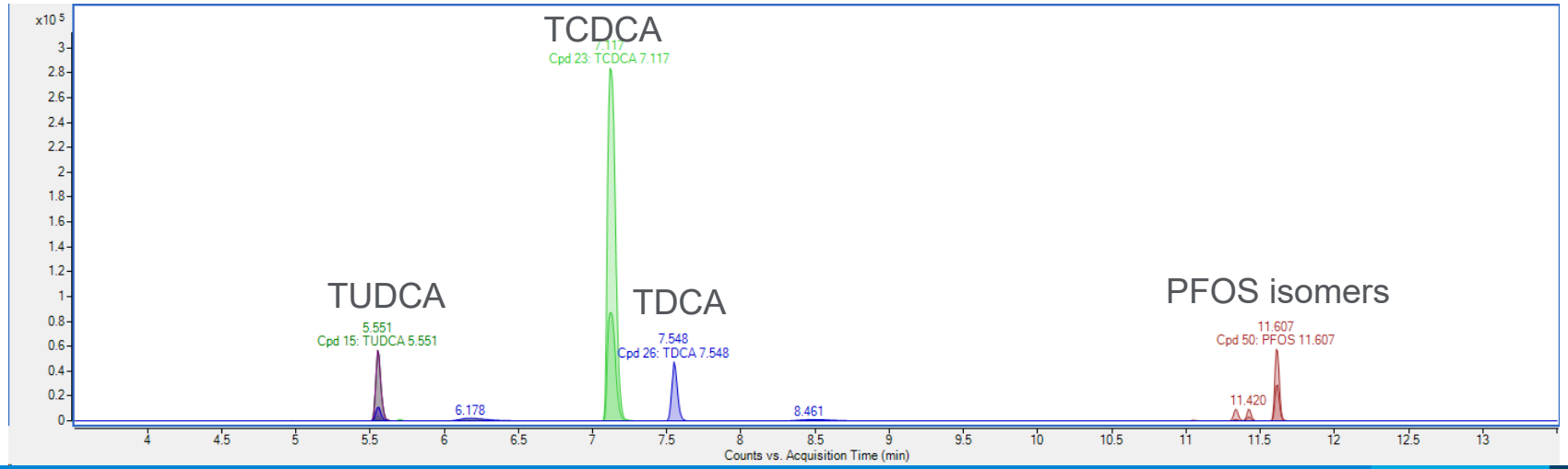


Chromatographic Separation

PFAS analytes, EIS and NIS chromatographic separation and distribution



Baseline separation of PFOS and isobaric cholic acids



Quantitation

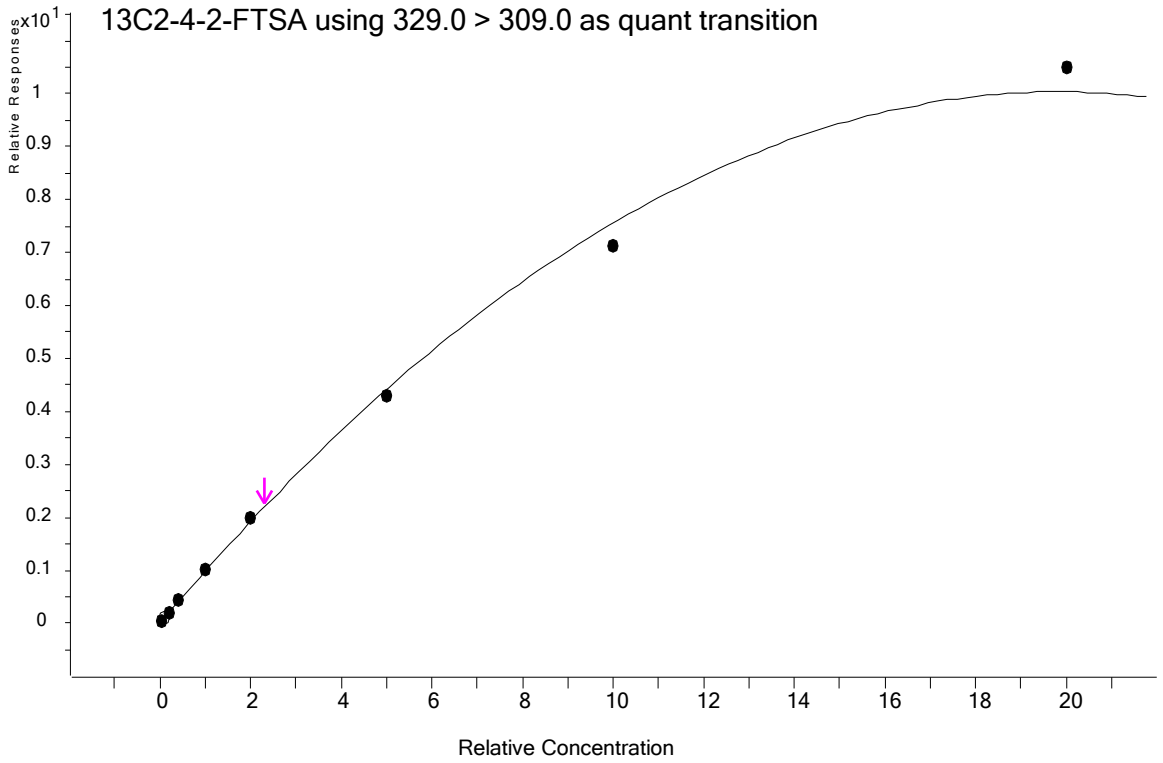
- Calibration standards made in solvent (ACN)
- 400-fold dynamic range
- PFAS analytes quantitation based on the ratio of PFAS analytes and EIS compounds
- EIS assignment was in alignment with EPA Method 1633, with few exceptions.
- Linear regression, $1/x^2$ weight.
- $R^2 > 0.99$ across all analytes' calibration curves.

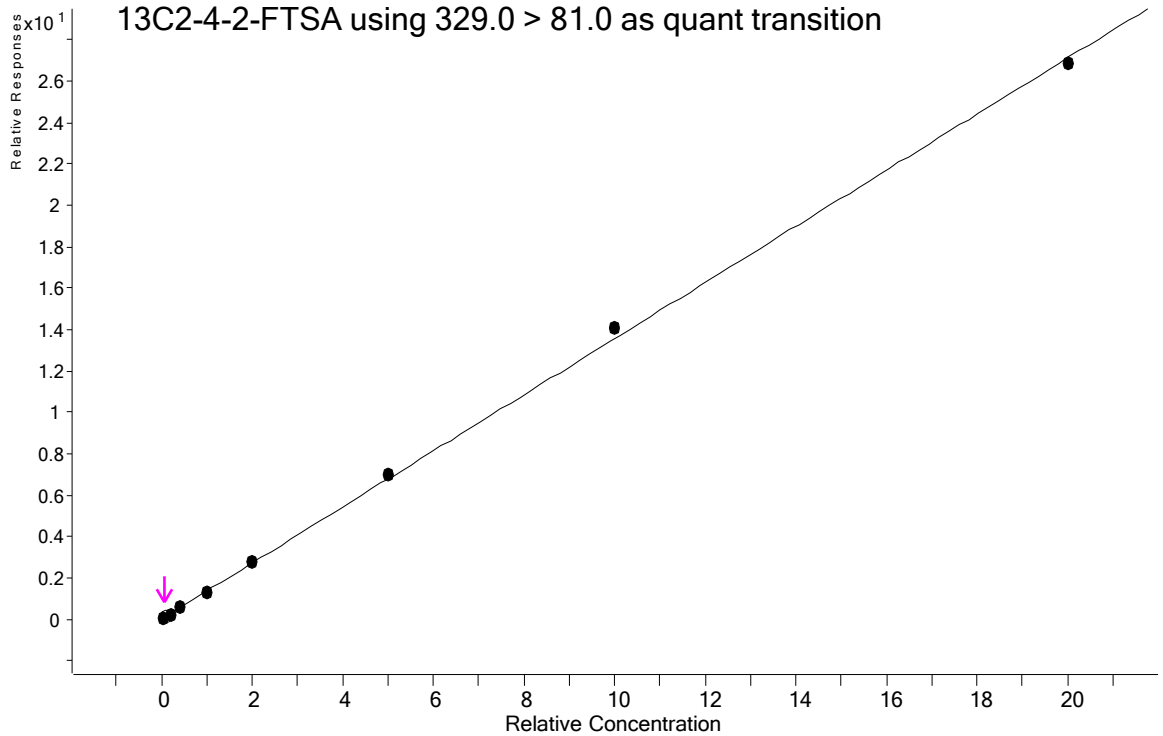
Target	Assigned EIS	Cal. range (ng/g)	Target	Assigned EIS	Cal. range (ng/g)
PFBA	$^{13}C_4$-PFBA	0.2 - 80	**PFHpS**	$^{13}C_9$-PFNA	0.05 - 20
PFMPA	$^{13}C_4$-PFBA	0.1 - 40	**8:2 FTS**	$^{13}C_2$-8:2 FTS	0.2 - 80
3:3 FTCA	$^{13}C_5$-PFPeA	0.25 - 100	**PFDA**	$^{13}C_6$-PFDA	0.05 - 20
PFPeA	$^{13}C_5$-PFPeA	0.1 - 40	**N-MeFOSAA isomers**	D_3-N-MeFOSAA	0.05 - 20
PFMBA	$^{13}C_5$-PFPeA	0.1 - 40	**N-EtFOSAA isomers**	D_5-N-EtFOSAA	0.05 - 20
4:2 FTS	$^{13}C_2$-4:2 FTS	0.2 - 80	**PFOS isomers**	$^{13}C_8$-PFOS	0.05 - 20
NFDHA	$^{13}C_5$-PFHxA	0.1 - 40	**PFUnA**	$^{13}C_7$-PFUdA	0.05 - 20
PFHxA	$^{13}C_5$-PFHxA	0.05 - 20	**9Cl-PF3ONS**	$^{13}C_7$-PFUdA	0.2 - 80
PFBS	$^{13}C_3$-PFBS	0.05 - 20	**PFNS**	$^{13}C_7$-PFUdA	0.05 - 20
HFPO-DA	$^{13}C_2$-HFPO-DA	0.2 - 80	**PFDoA**	$^{13}C_2$-PFDoA	0.05 - 20
5:3 FTCA	$^{13}C_4$-PFHpA	1.25 - 500	**PFDS**	$^{13}C_2$-PFDoA	0.05 - 20
PFEESA	$^{13}C_4$-PFHpA	0.1 - 40	**PFTrDA**	$^{13}C_2$-PFDoA	0.05 - 20
PFHpA	$^{13}C_4$-PFHpA	0.05 - 20	**PFOSA isomers**	$^{13}C_8$-PFOSA	0.05 - 20
PFPeS	$^{13}C_4$-PFHpA	0.05 - 20	**11Cl-PF3OUdS**	$^{13}C_8$-PFOS	0.2 - 80
ADONA	$^{13}C_8$-PFOA	0.2 - 80	**PFTeDA**	$^{13}C_2$-PFTeDA	0.05 - 20
6:2 FTS	$^{13}C_2$-6:2 FTS	0.2 - 80	**PFDoS**	$^{13}C_8$-PFOS	0.05 - 20
PFOA isomers	$^{13}C_8$-PFOA	0.05 - 20	**N-MeFOSE isomers**	D_7-N-MeFOSE	0.5 - 200
PFHxS isomers	$^{13}C_3$-PFHxS	0.05 - 20	**N-MeFOSA isomers**	D_3-N-MeFOSA	0.05 - 20
7:3 FTCA	$^{13}C_3$-PFHxS	1.25 - 500	**N-EtFOSE isomers**	D_9-N-EtFOSE	0.5 - 200
PFNA isomers	$^{13}C_9$-PFNA	0.05 - 20	**N-EtFOSA isomers**	D_5-N-EtFOSA	0.05 - 20

FTS Targets Calibration

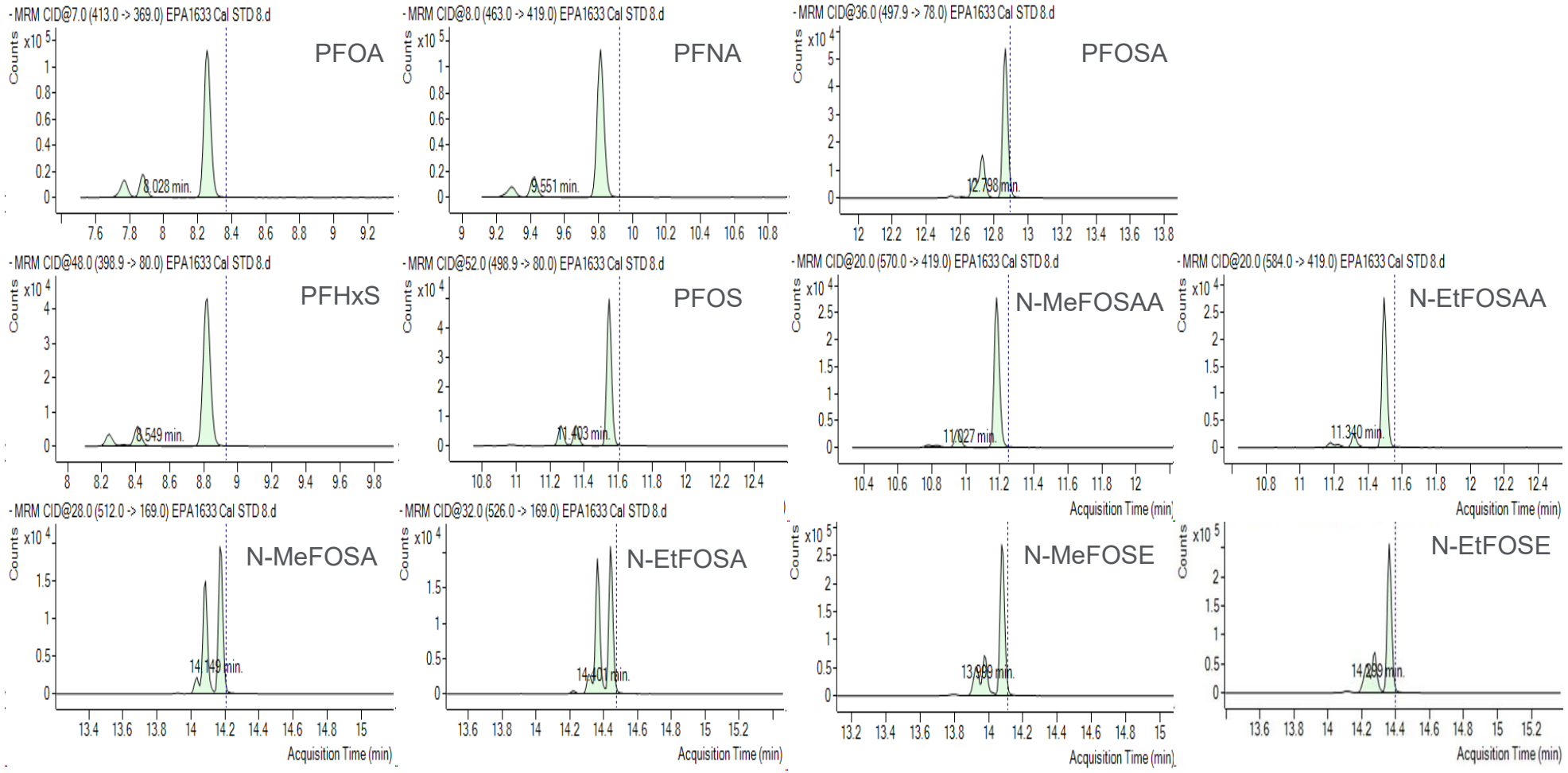
- Isotopic labelled FTS EIS compounds used the less abundant transition with product 80 or 81.
- Applied to all FTS target analytes
- Linear calibration curves were achieved.

Example: 4:2 FTS calibration curves





Quantitation for Targets with Isomers



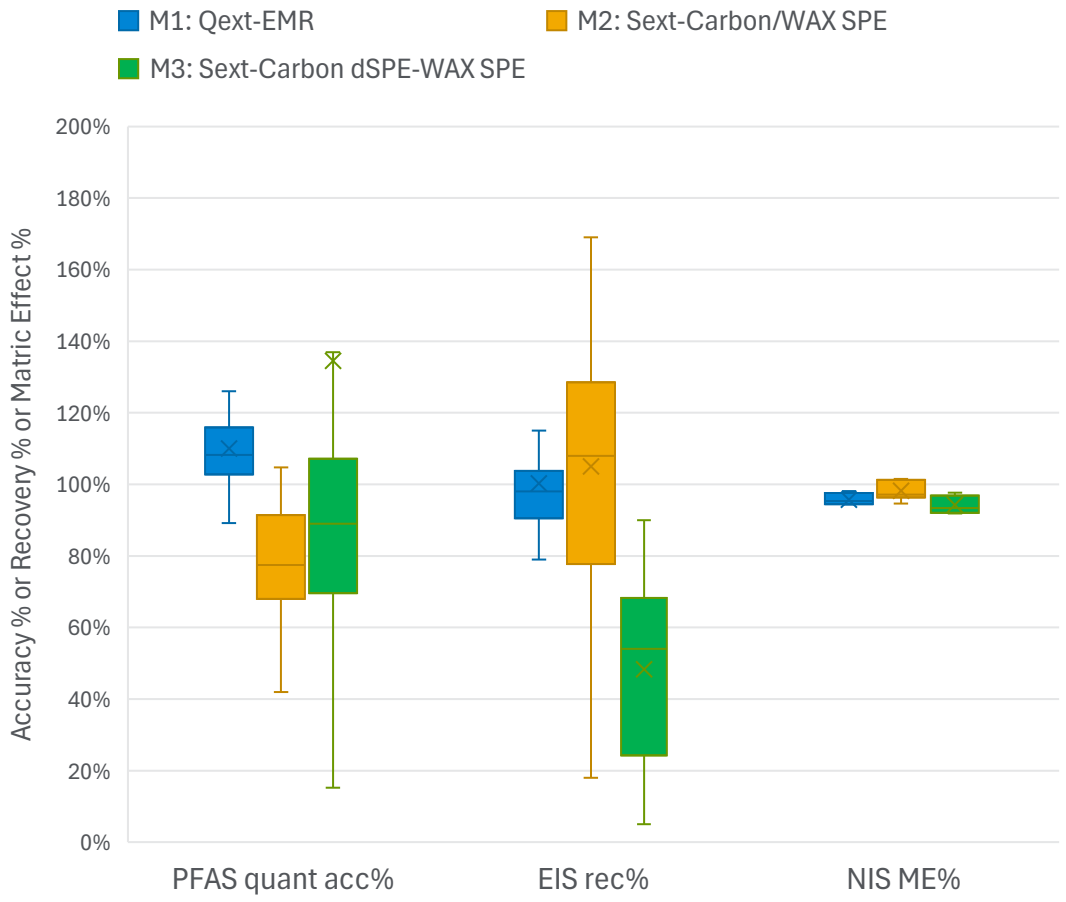
All PFAS analytes with isomers were based on summated integration of all isomers for quantitation.

PFAS Analysis in Biologic Tissue

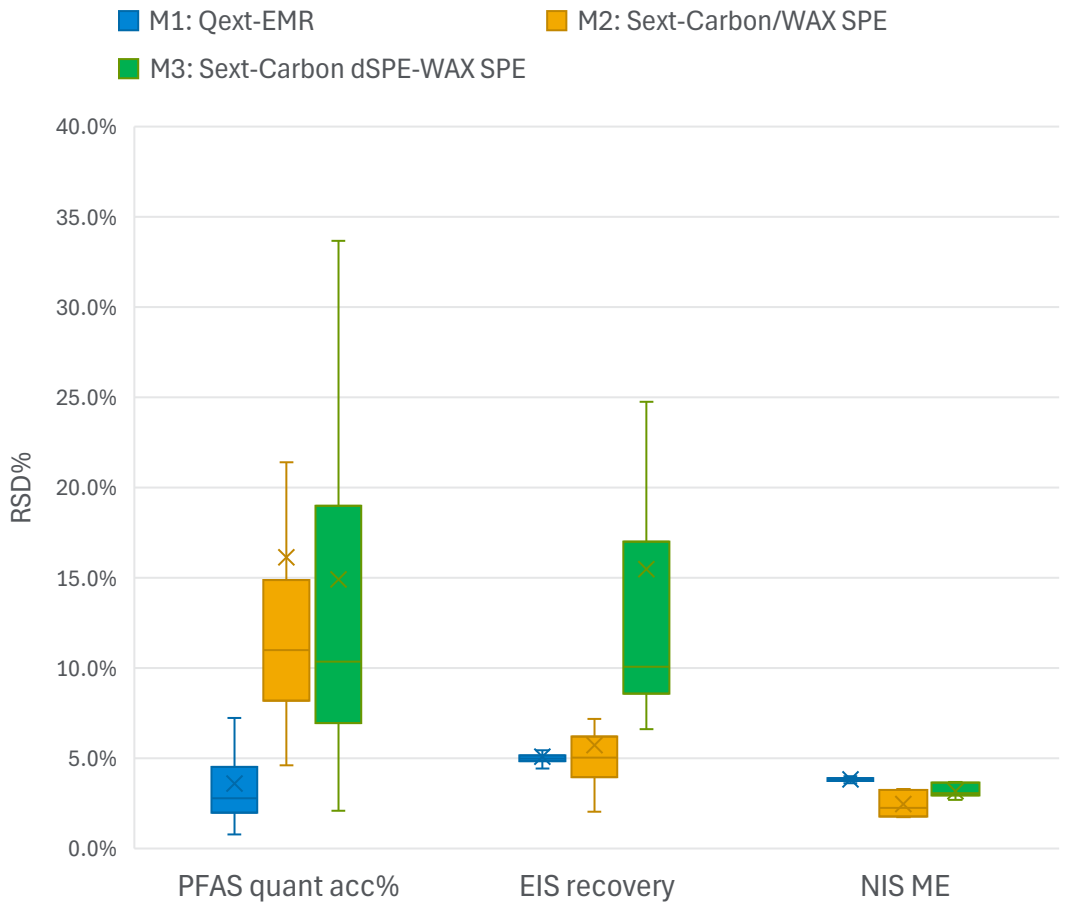
Comparison and Method Final Validation

Methods Comparison – Quantitation Performance

A) Method Comparison for PFAS in Tissue Quantitative Analysis - Targets accuracy, EIS recovery, NIS matrix effect

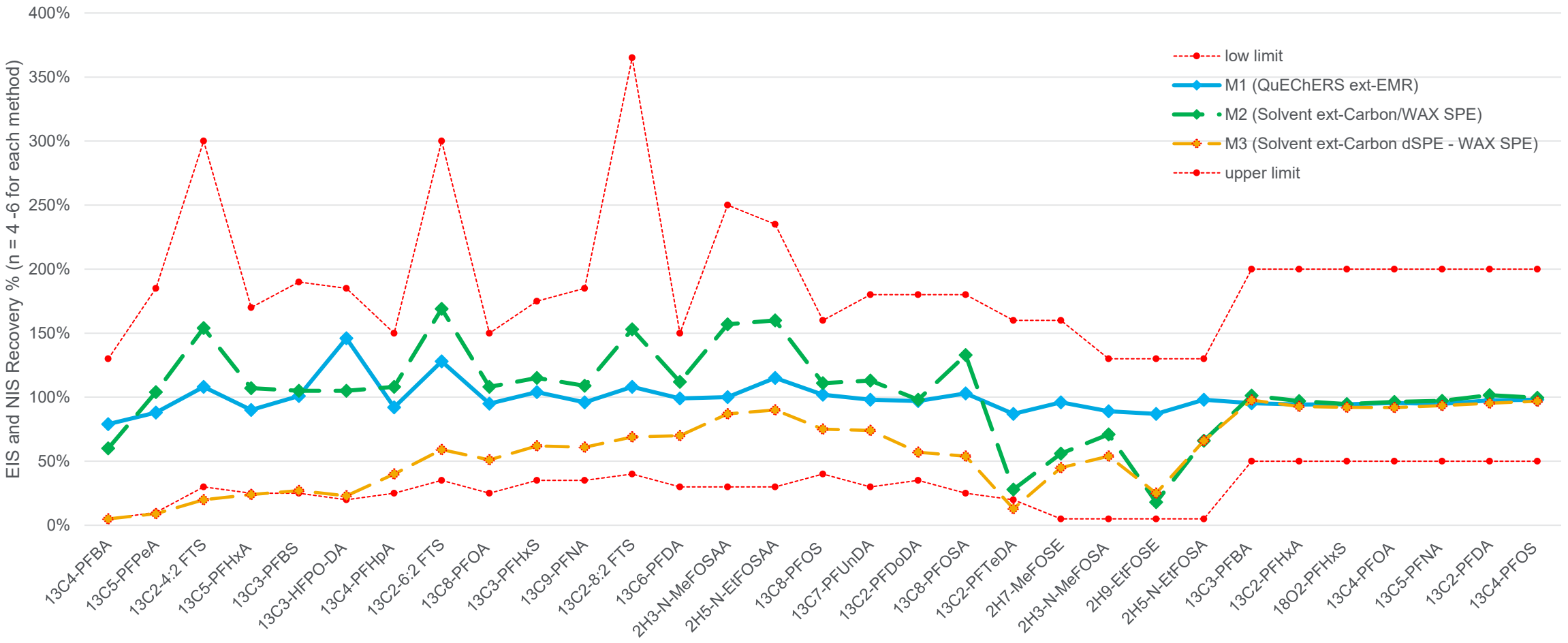


B) Method Comparison for PFAS in Tissue Quantitative Analysis – Targets, EIS, and NIS Repeatability (RSD%)



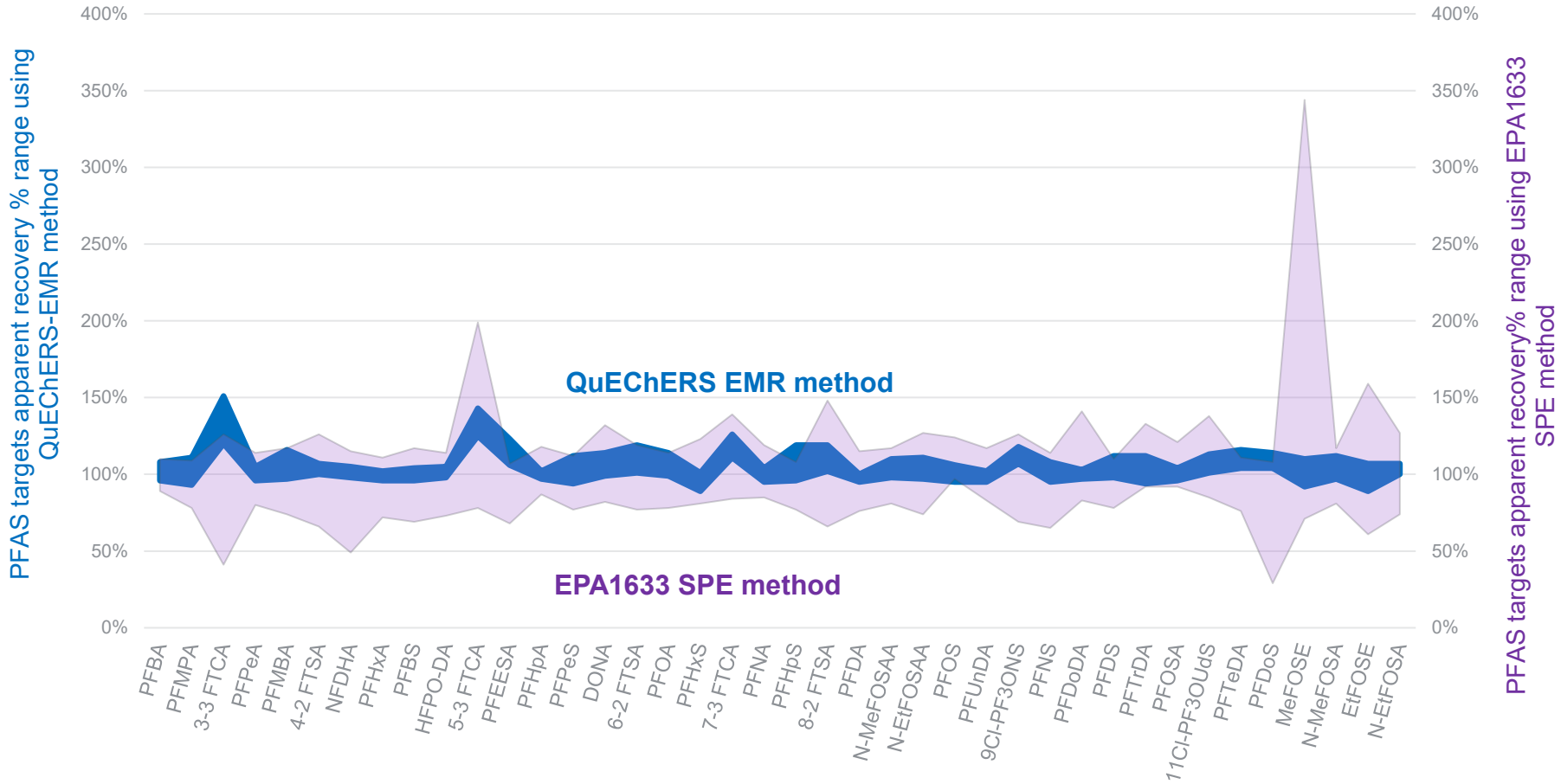
The QuEChERS-EMR method demonstrated improved quantitation performance for PFAS compound recovery and repeatability.

EIS and NIS Recovery Comparison



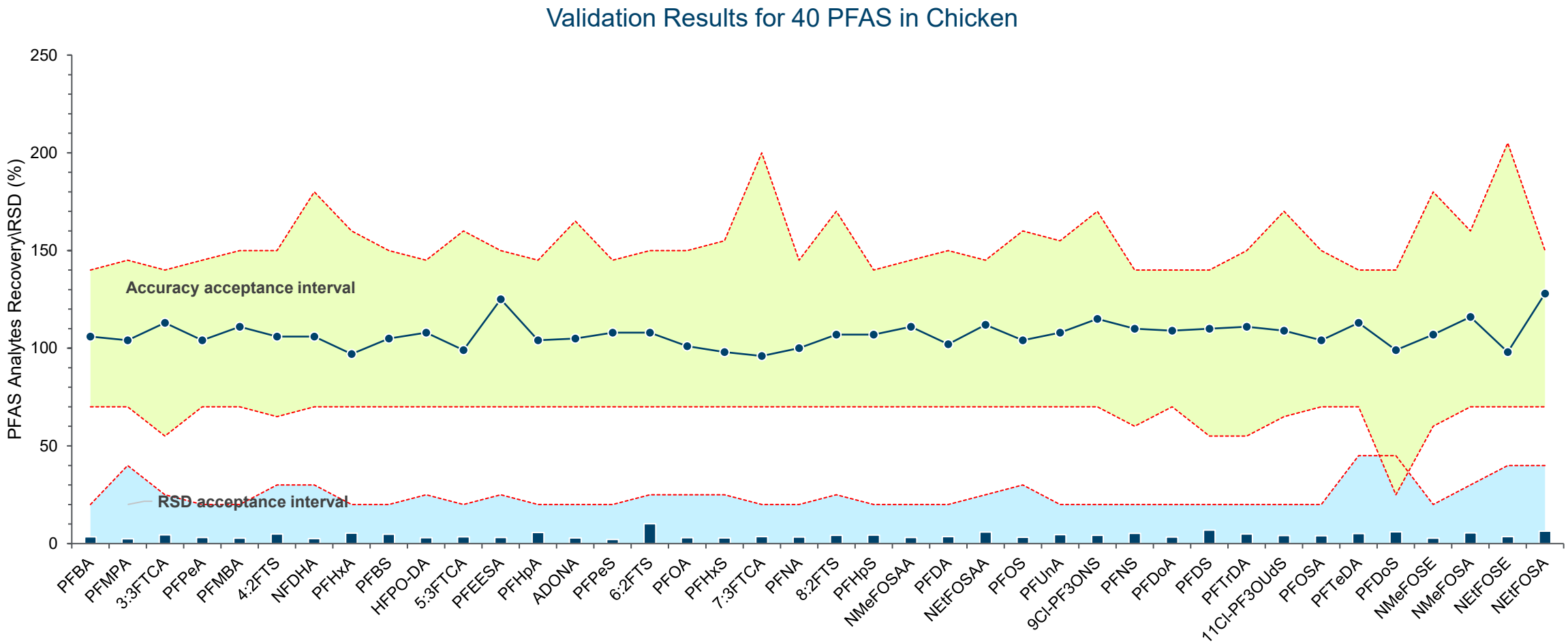
- QuEChERS-EMR protocol presented excelled recovery for all EIS compounds
- Traditional EPA 1633 protocols showed various EIS recoveries across the targets.

Comparison 40 PFAS Targets in Fish Recovery (Single-lab validation)



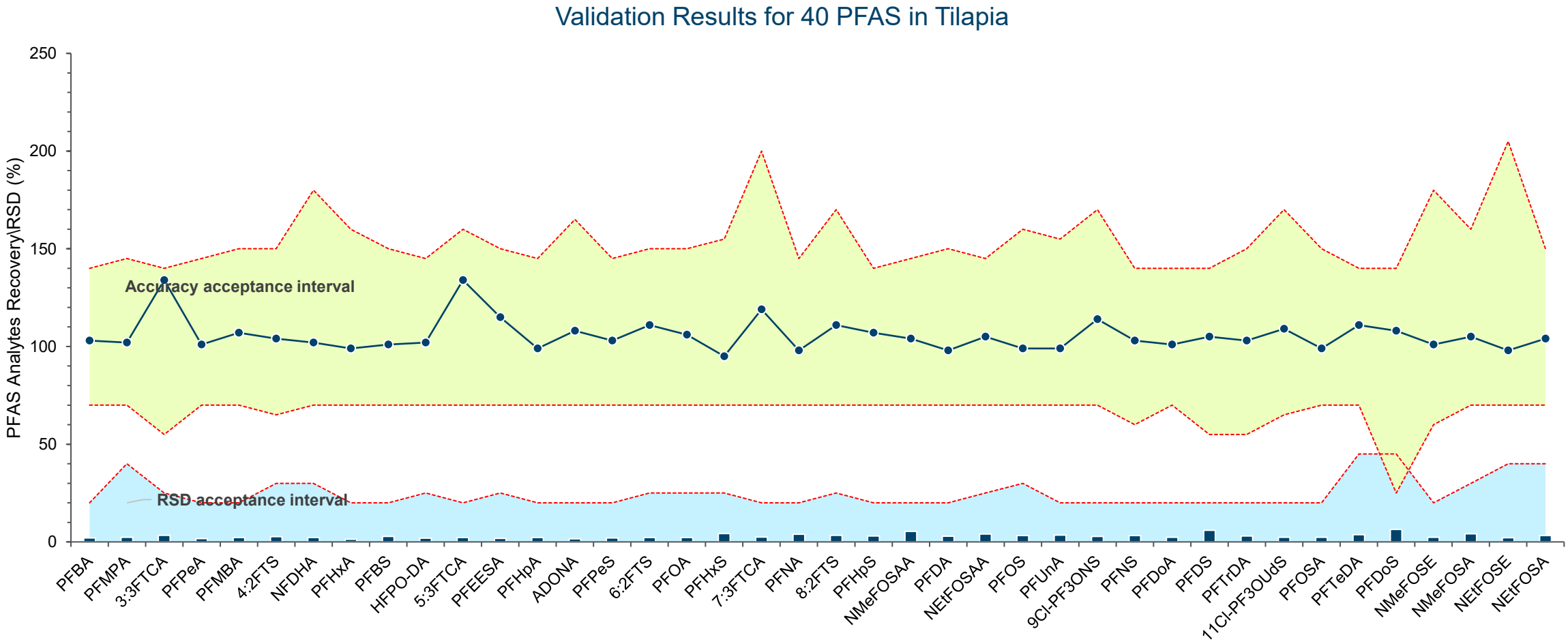
- Results from QuEChERS EMR method were based on full validation results from three spiking levels with six replicates of each level.
- Results from EPA 1633 SPE method was based on single-lab study reported from *EPA draft method 1633 (EPA 1633:2021).*
- QuEChERS-EMR method consistently produced narrower accuracy ranges close to 100% across all PFAS targets.

Validation Results for PFAS in Chicken



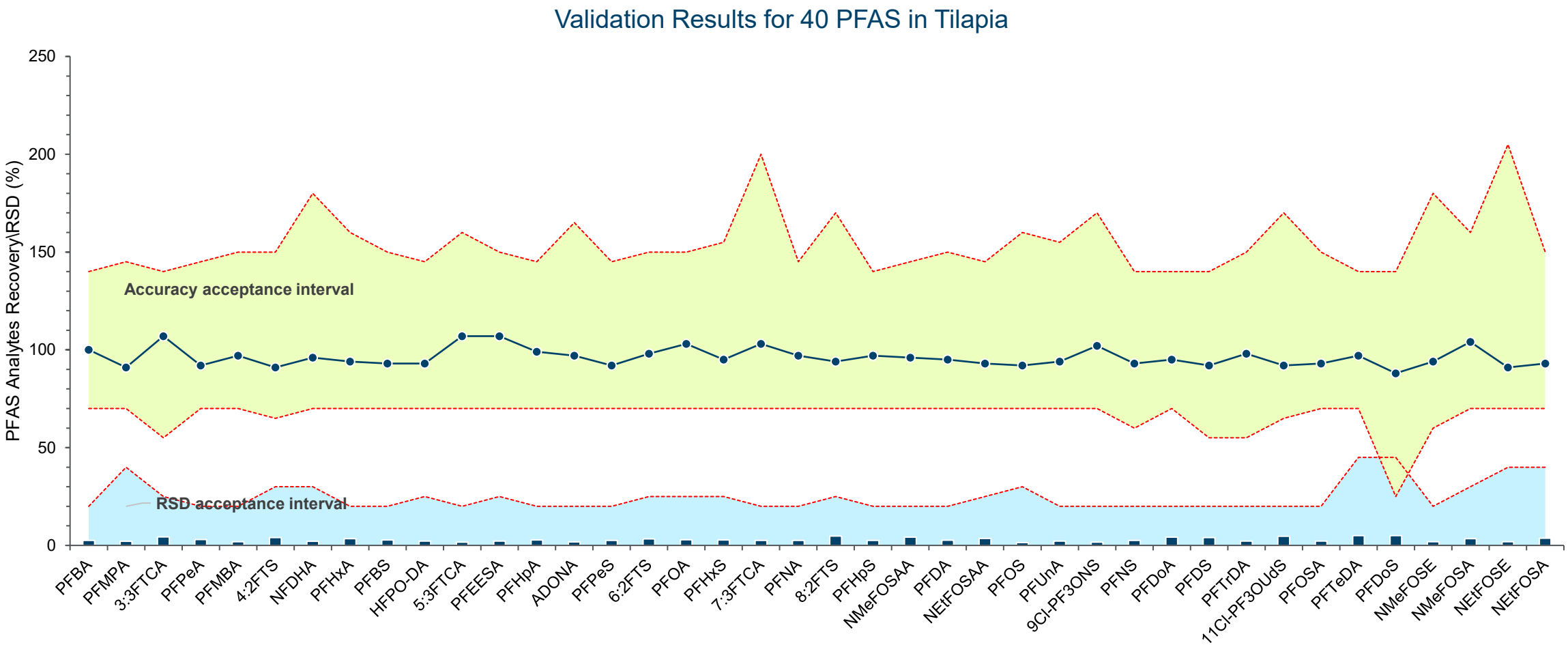
- Summary results are based on average of three spiking levels at LOQ, mid-QC (4x of LOQ), and high-QC (40x LOQ)
- Validated LOQ (spiking): 0.05 – 1.25 µg/kg

Validation Results for PFAS in Tilapia



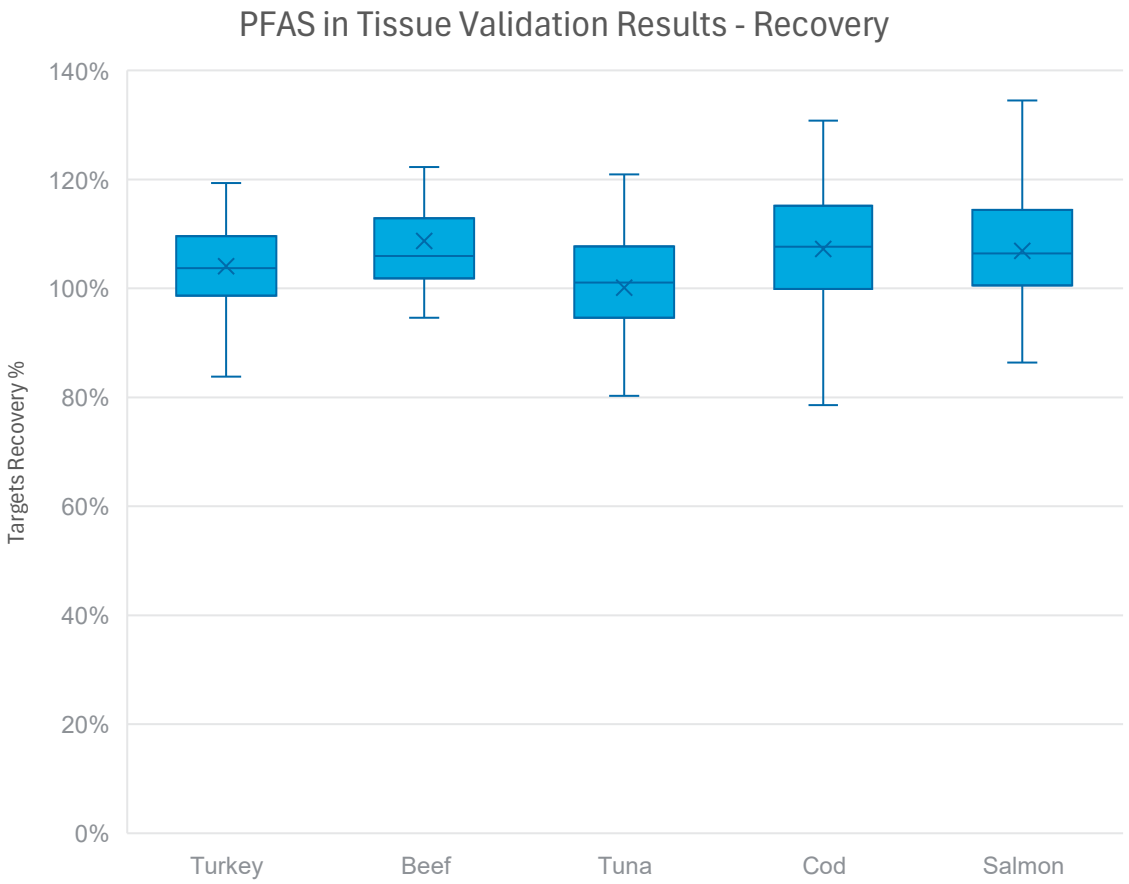
- Summary results are based on average of three spiking levels at LOQ, mid-QC (4x of LOQ), and high-QC (40x LOQ)
- Validated LOQ (spiking): 0.05 – 1.25 µg/kg

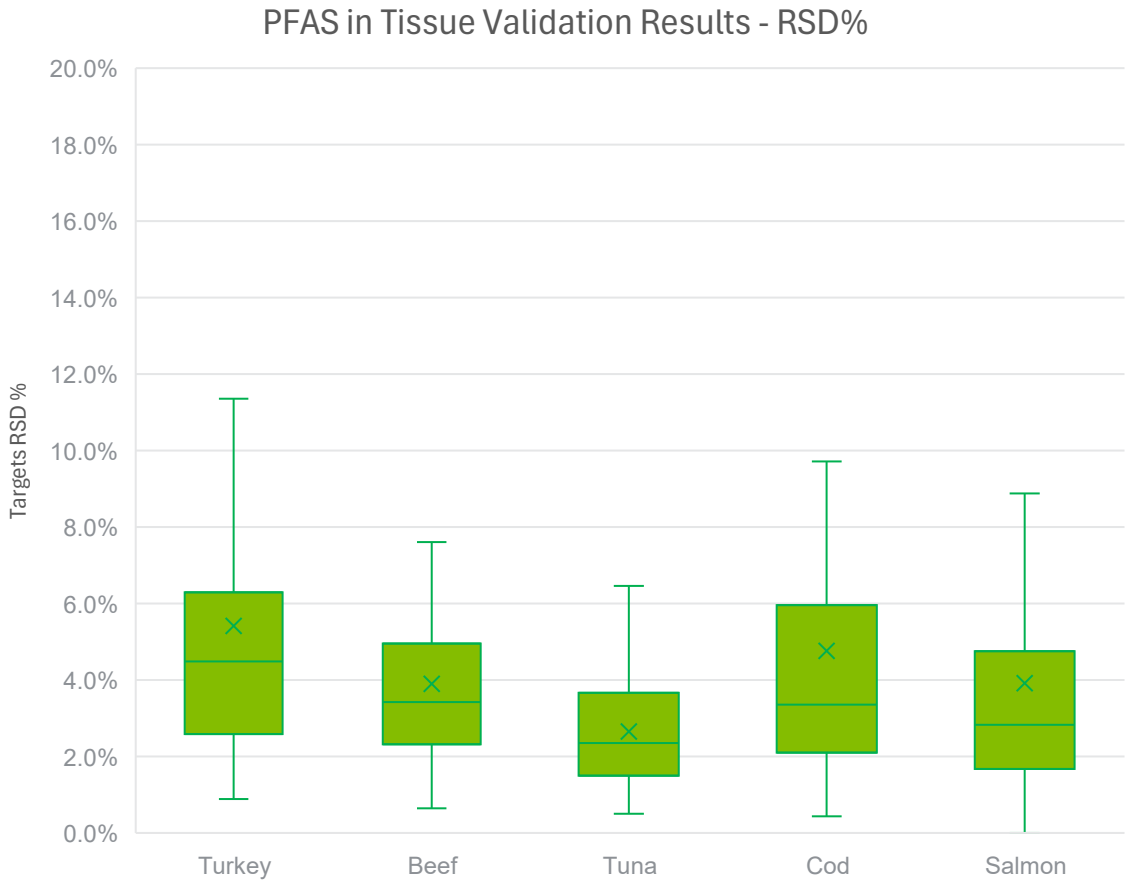
Validation Results for PFAS in Pork



- Summary results are based on average of three spiking levels at LOQ, mid-QC (4x of LOQ), and high-QC (40x LOQ)
- Validated LOQ (spiking): 0.05 – 1.25 µg/kg

Cross-validation Results for PFAS in Cod, Tuna, Salmon, Turkey and Beef





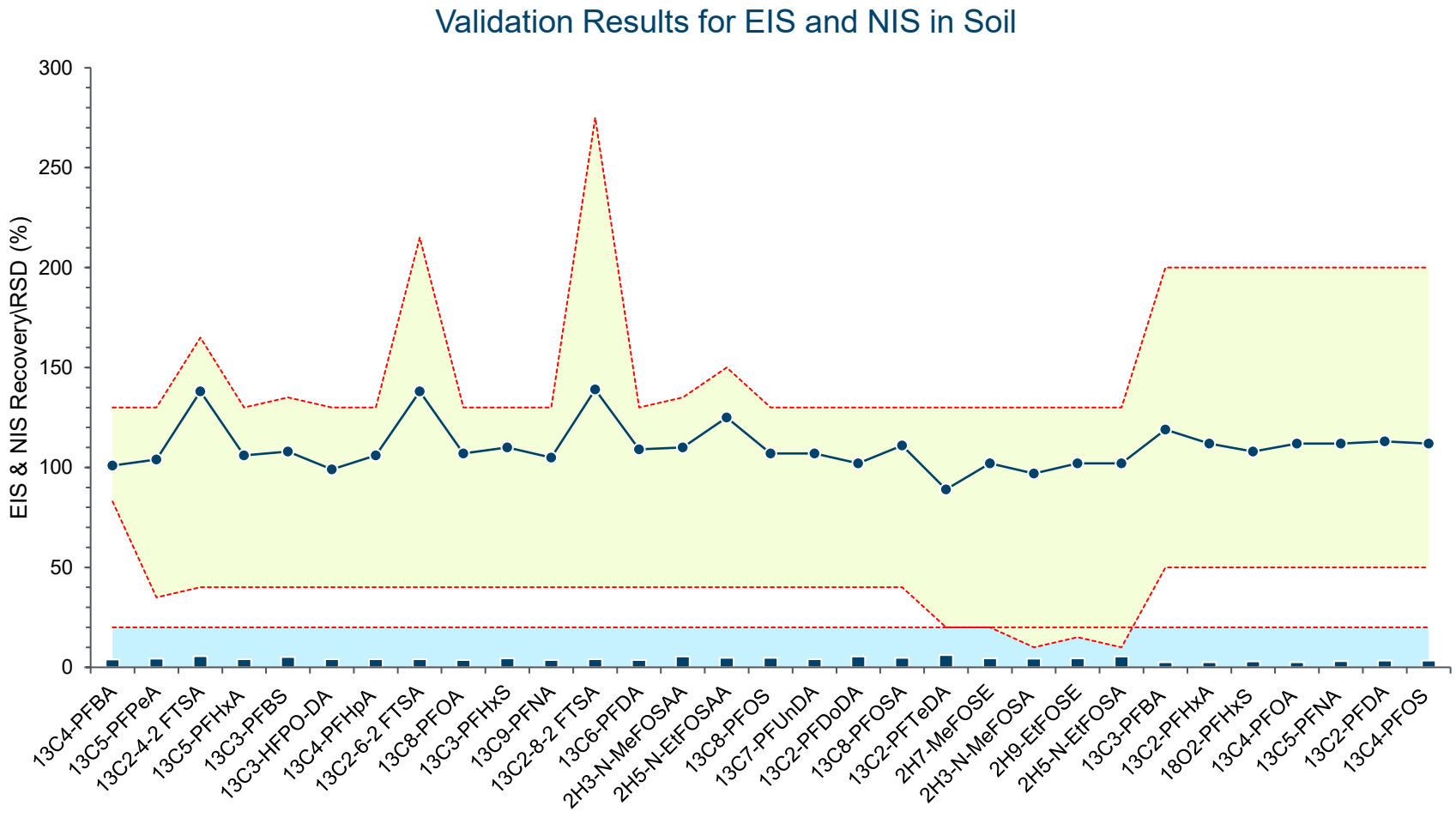
- Summary results are based on LOQ spiking levels in additional five more tissue matrices
- LOQ between 0.05 – 0.2 µg/kg, except 1.25 µg/kg for 5:3 FTCA and 7:3 FTCA

PFAS Analysis in Biosolid and Soil

Extended applications

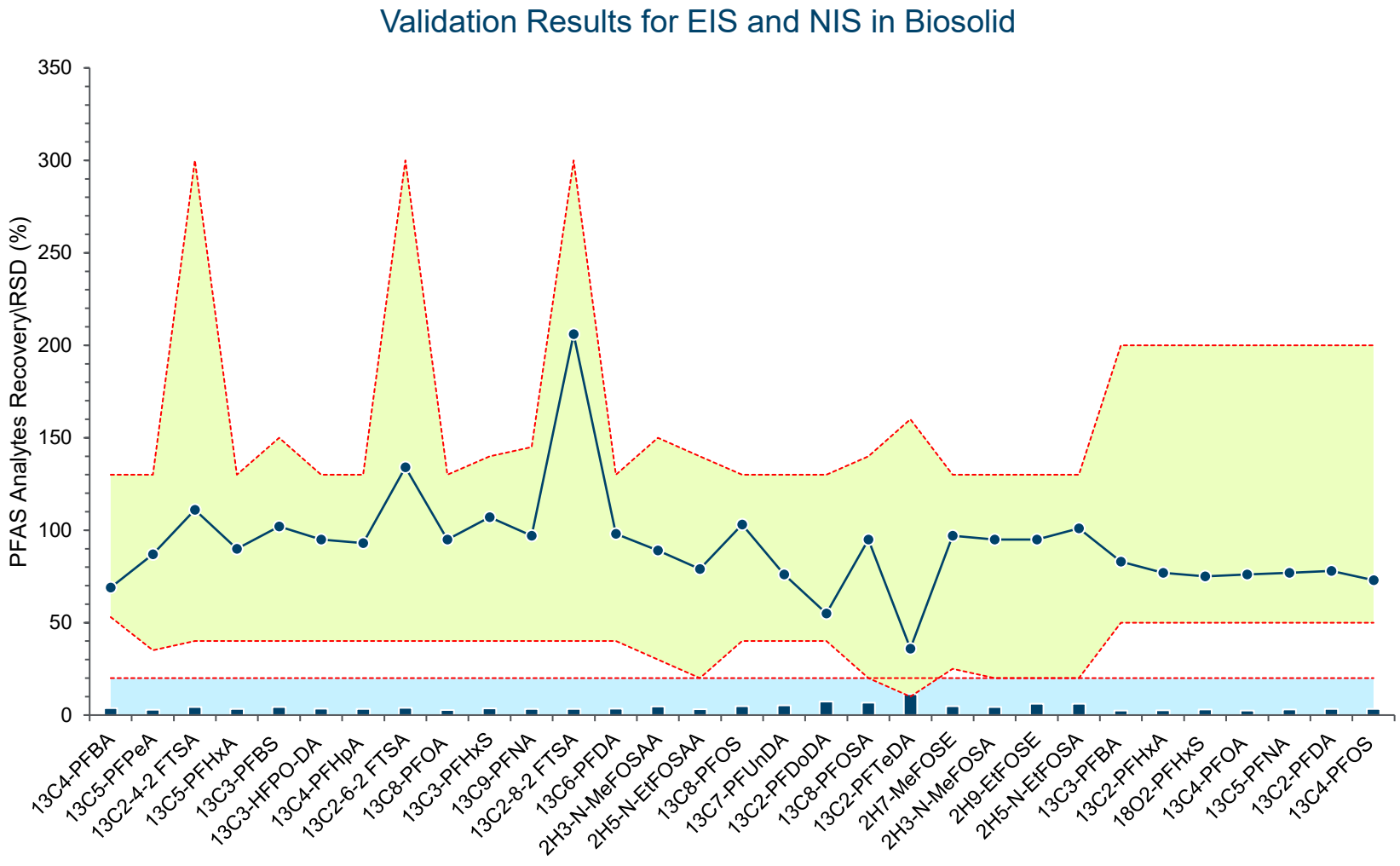
Method Extension to Soil

- Soil is a moderate complex matrix without too much fatty components
- Same instrument method
- Modified sample prep method
  - Captiva EMR PFAS I, 680 mg cartridge
  - Direct loading of QuEChERS crude extract
- LOQs (spiking validated):
  0.05 – 1.25 µg/kg



Method Extension to Biosolid

- Biosolid matrix is significantly complex with high positive background
- Same instrument method
- Modified sample prep method
  - Smaller sample size: 0.5 g
  - Captiva EMR PFAS II cleanup
  - Reduced loading volume: 2 mL
- LOQ (calculated and spiking validated): 0.03 – 136.3 µg/kg



Summary

QuEChERS - EMR

- Validated method for 40 PFAS determination in biological tissues
- Simplified workflow saving time and effort
- High PFAS recovery and matrix removal
- Demonstrated method suitability and selectivity
- Quantitation performance meet both environmental and food analysis requirement
- Demonstrated extension to soil and biosolid
- Journal publication – *J. Chrom. A, 1758 (2025) 466150*

Acknowledgement

Carrie Xu from New Jersey Department of Health

Matthew Giardina, Megan Juck, and other colleagues from Agilent

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Additional related talks:

1. A Semi-Automated Workflow for the Extraction and Analysis of 40 PFAS Targets in Biosolids, Emily Parry, Wednesday 2pm

2. The Evaluation of Novel Weak Anion Exchange and Graphic Carbon Sorbent Blends for PFAS Extraction and Matrix Reduction in Environmental Extracts, Mattew Giardina, Thursday 11 am.