Multi-Laboratory Validation of SW-846 Method 8327 Per- and Polyfluoroalkyl Substances (PFAS) Using External Standard Calibration and Multiple Reaction Monitoring (MRM) Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)

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Validation of SW-846 Methods 3512 and 8327 for PFAS in Non-Potable Waters by LC/MS/MS

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Outline

- Overview of test methods
- Summary of interlaboratory validation study:
  - Objectives
  - Experimental design
  - Data for blind samples and quality controls
  - Likely causes of non-conforming data
- Themes in public comments
- Anticipated timeline for completion

The SW-846 Methods Compendium

- >200 test methods
- Published by EPA's Office of Resource Conservation and Recovery
- Method Defined Parameters (MDPs) in 40 CFR Part 260.11: Required
- *SW-846 methods for non-MDPs are guidance and can be modified*, or other reliable analytical methods may be used, as long as:
  - Modifications are acceptable to the end data user
  - Generated data are of sufficient quality for the intended application

https://www.epa.gov/hw-sw846/sw-846-compendium
https://www.epa.gov/sites/production/files/2015-10/documents/abstract.pdf
https://www.epa.gov/sites/production/files/2015-10/documents/policy-statement-federal-register.pdf

Overview of Validated Methods

Method 3512 (Sample Preparation for non-potable waters):

- Add standards (mass-labeled surrogates and any target analytes)
- Dilute sample 1:1 with methanol
- Vortex for 2 min
- Filter through 0.2 µm filter
- Add 0.1% acetic acid by volume

Method 8327 (Determinative):

- External standard calibration
- Calibration standards in 1:1 Methanol-water+0.1% acetic acid
- Spiking solutions in 95:5 ACN-water
- LC Conditions: Acetonitrile-water gradient with ammonium acetate modifier
- ESI negative ionization mode
- Only one monitored product ion for PFBA, PFPeA, PFOSA

Overview of Validated Methods

Advantages	Disadvantages
Small sample size (5 mL) Rapid sample preparation Few process steps	Introduces a small dilution factor (2x) Need modern LC/MS instrument to achieve low ng/L sensitivity Not consistent with current practice in many testing laboratories

Validation study design

Data Quality Objectives:

- Bias/Recovery: 70-130% Recovery (median)
- Precision: ≤50% RSD
- Sensitivity: 10 ng/L Lower Limits of Quantitation (LLOQs)

24 target analytes:

- C4-C14 Perfluorinated carboxylic acids
- C4-C10 Perfluorinated sulfonic acids
- 4:2, 6:2, 8:2 Fluorotelomer sulfonates
- Perfluorooctane sulfonamide
- N-Methyl and N-ethyl perfluorooctane sulfonamidoacetic acids

19 isotopically labeled surrogates:

- Analogs of all targets except PFTriDA, PFPeS, PFHpS, PFNS, PFDS

Validation study design

Study Sample Matrices:	• Reagent Water • Groundwater • Surface Water • Wastewater
Prepared Concentrations (nominal):	• Background (unspiked) • 60 ng/L • 200 ng/L
Replicates:	• 5 of each matrix at each prepared concentration • Total of 60 blind samples for each lab
Preparation and analysis:	• Prepared in 3 batches of 20 samples each • Randomly assigned analysis sequence order

Validation study design

Phase 1 - 2017, 6 USEPA Program, Regional, ORD Labs
Phase 2 – 2018, 7 State and commercial labs, instrument vendors

12 labs submitted data – 6 from each phase

8 labs' data used for statistical analysis – 4 from each phase

4 Excluded labs:

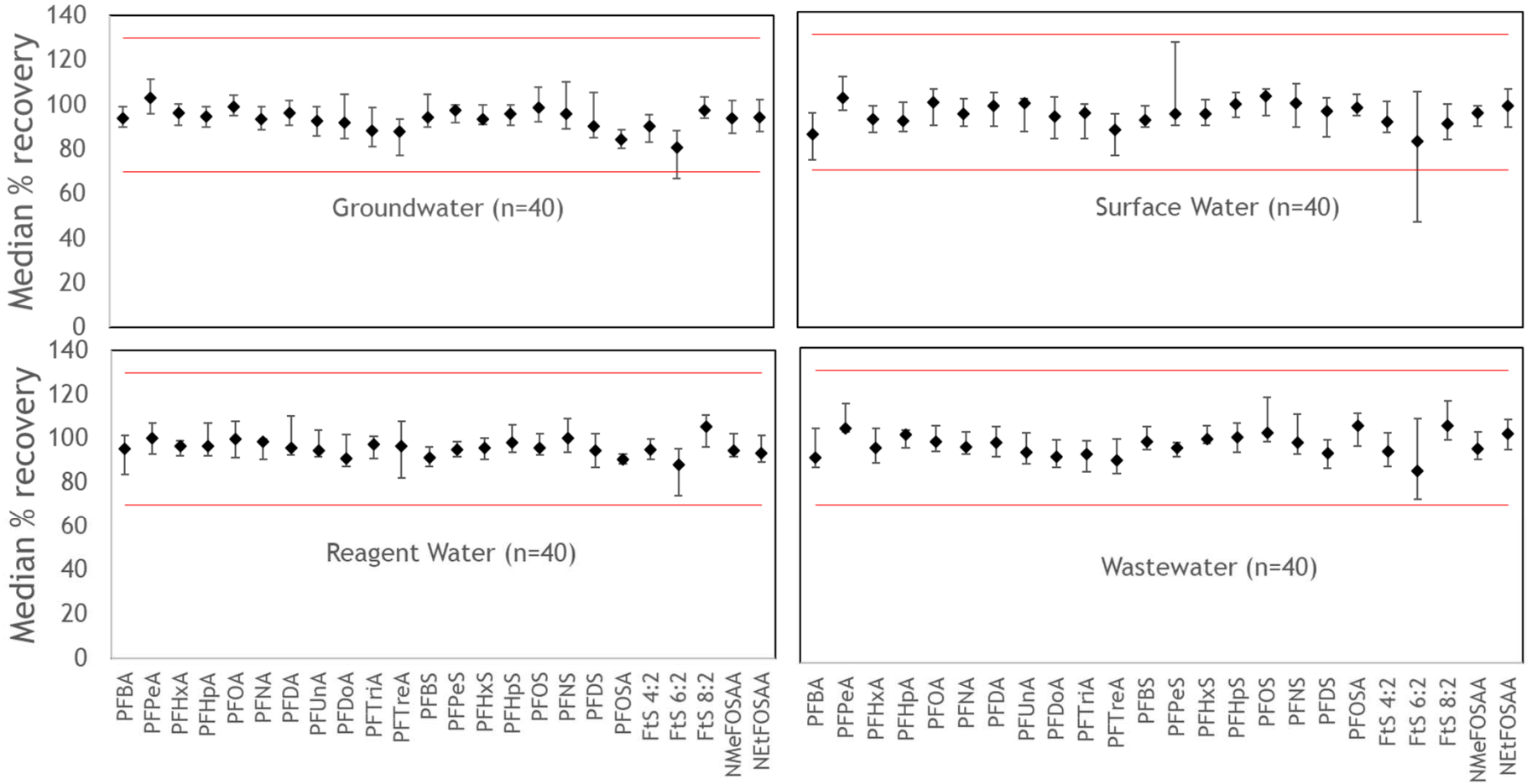
-Subsampled prior to adding solvent, resulting in low recovery of longer-chain target analytes in study samples

-Prepared spiking solutions in 1:1 MeOH-water+0.1% acetic acid and stored in glass resulting in high recovery of longer-chain target analytes in study samples

-One lab identified having instrument stability problems

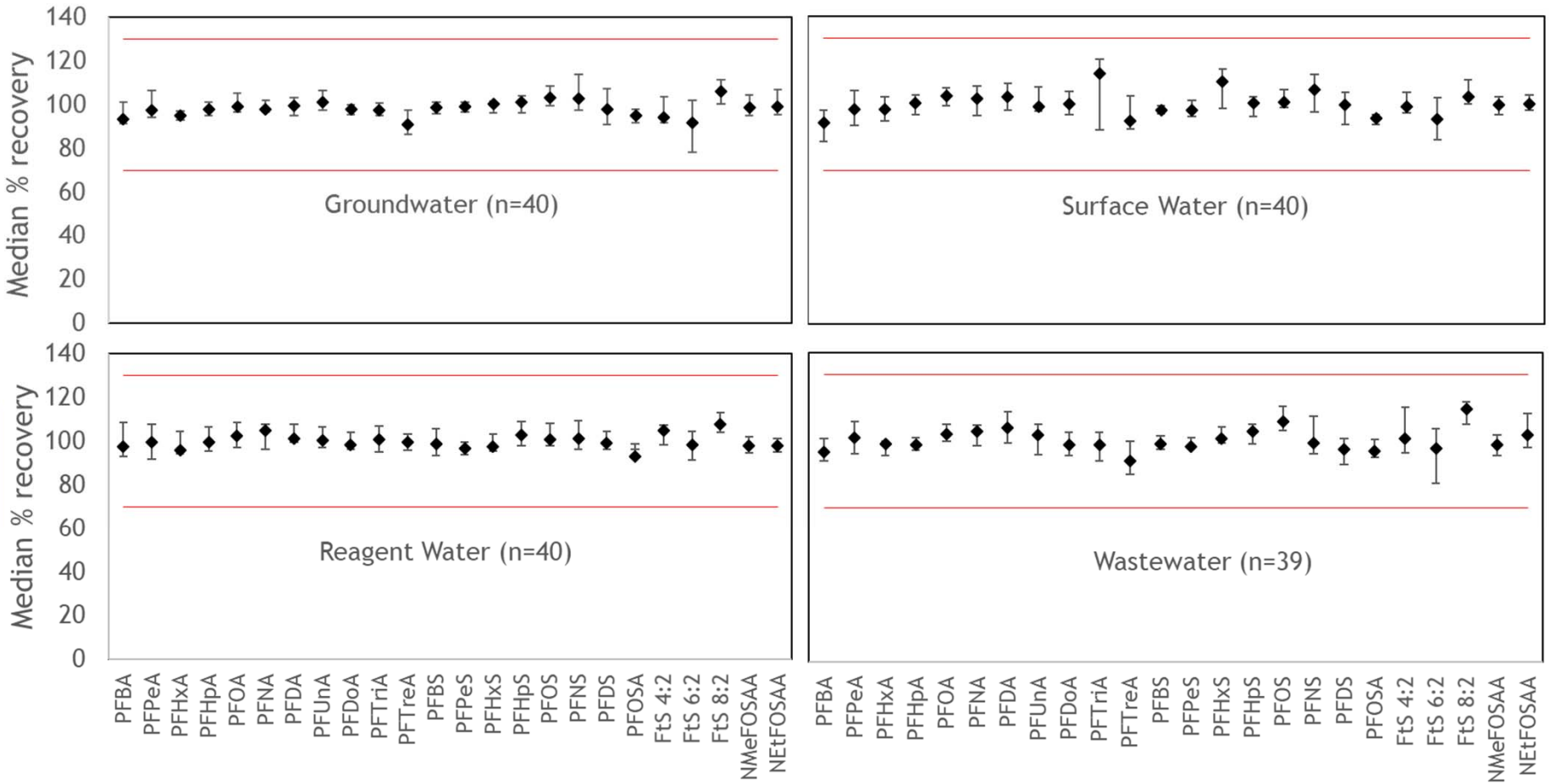
Recovery, by Matrix: Median recovery of 60 ng/L addition (pooled data; error bars show 95% CI)

Red line shows bias data quality objective: 70-130% recovery



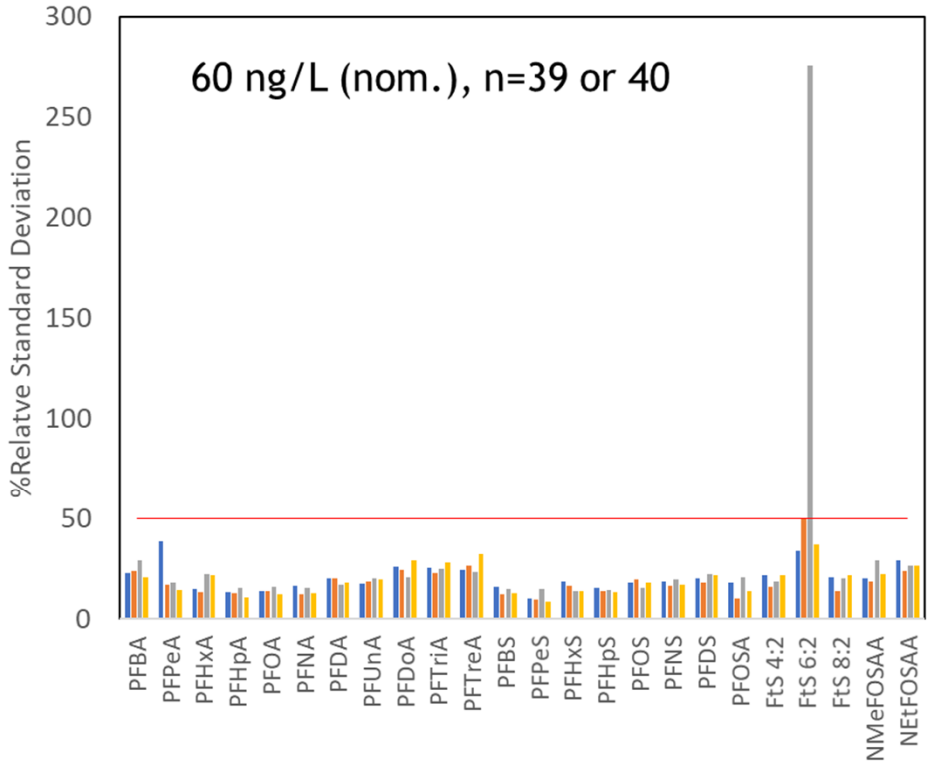
Recovery, by Matrix: Median recovery of 200 ng/L addition (pooled data; error bars show 95% CI)

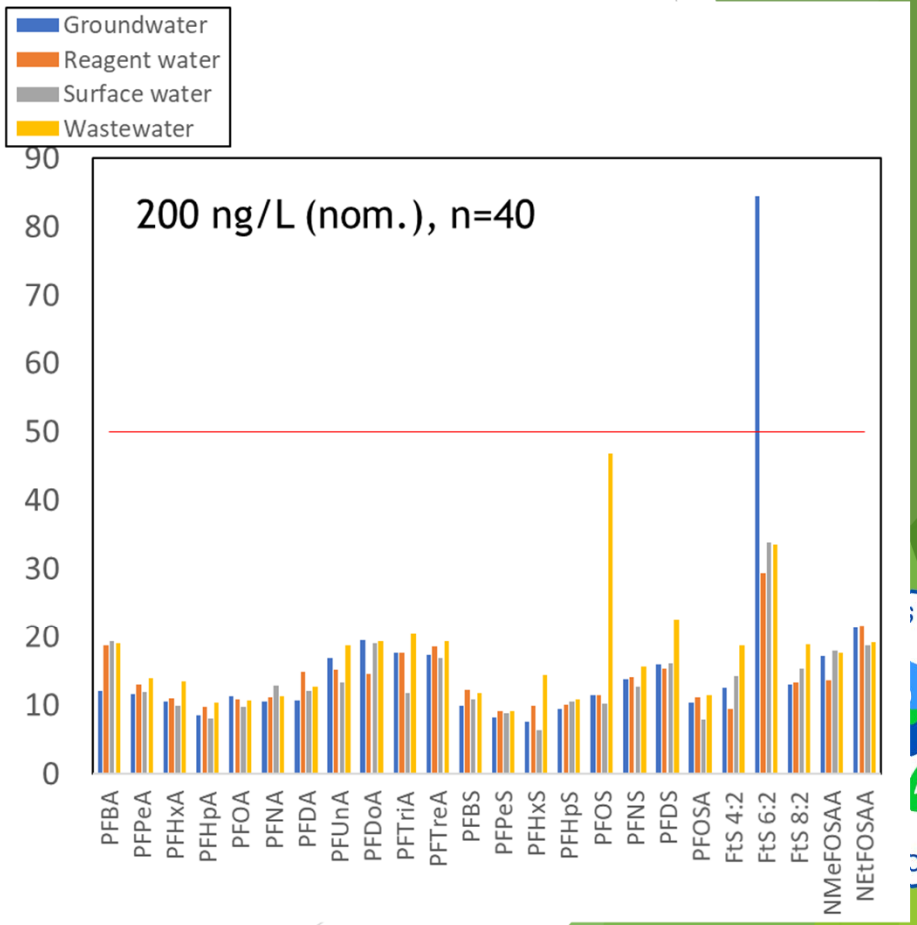
Red line shows bias data quality objective: 70-130%



Precision, by Matrix: %RSD, pooled data

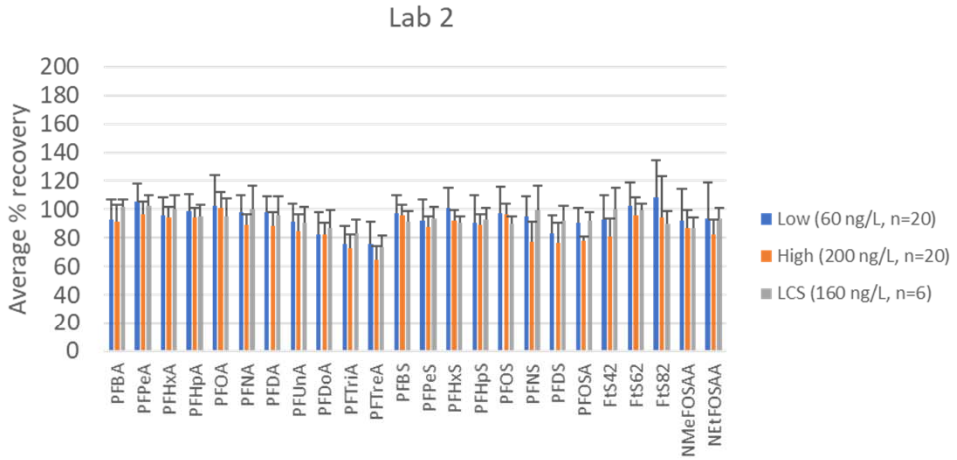
Red line shows precision data quality objective: ≤50% RSD

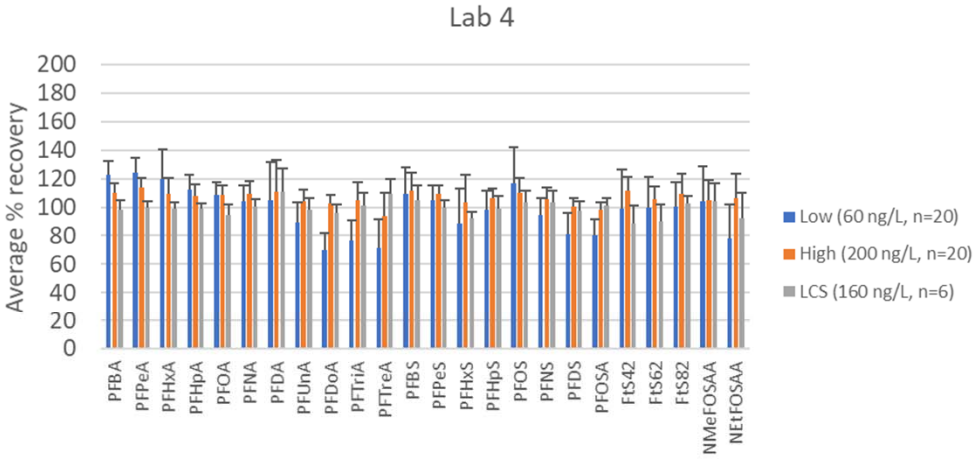


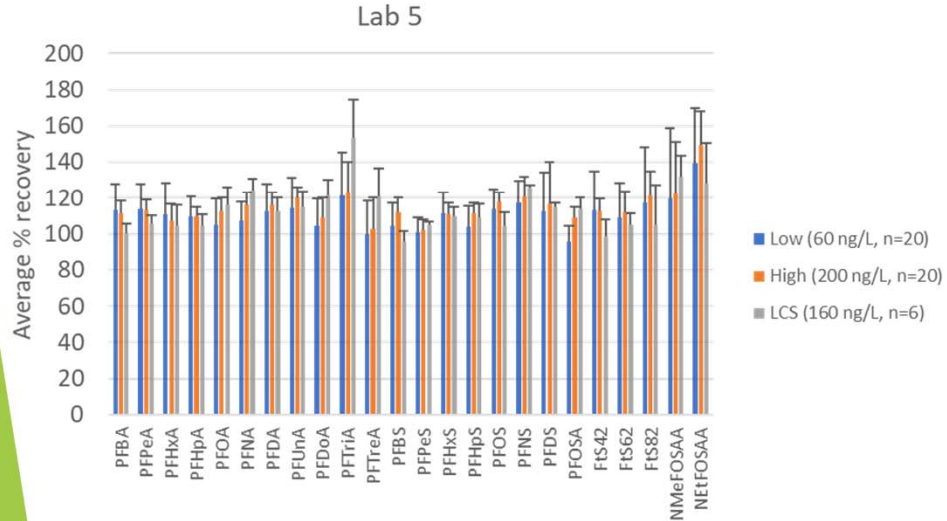


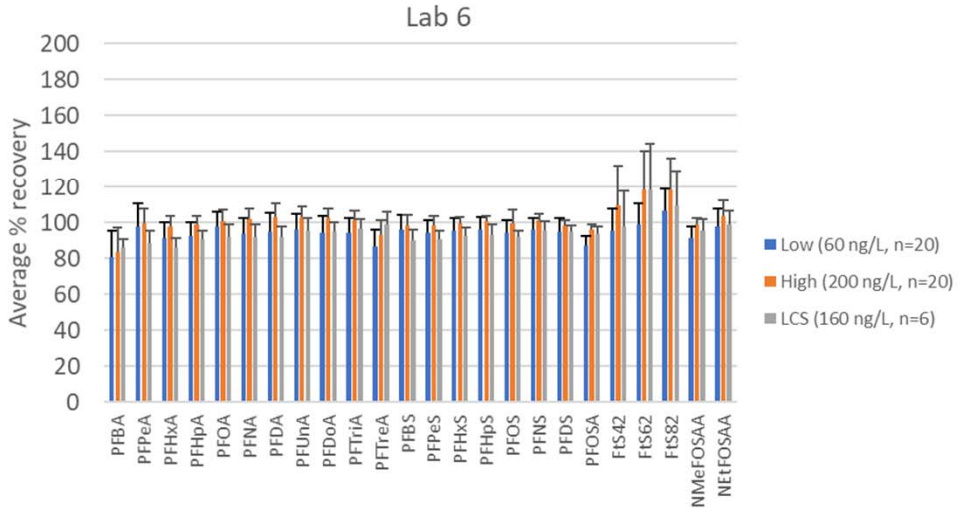
Phase 1 Recovery by Lab and Spike Level Across Matrices and in LCS

Error bars are 1 standard deviation









Phase 2 Recovery by Lab and Spike Level Across Matrices and in LCS

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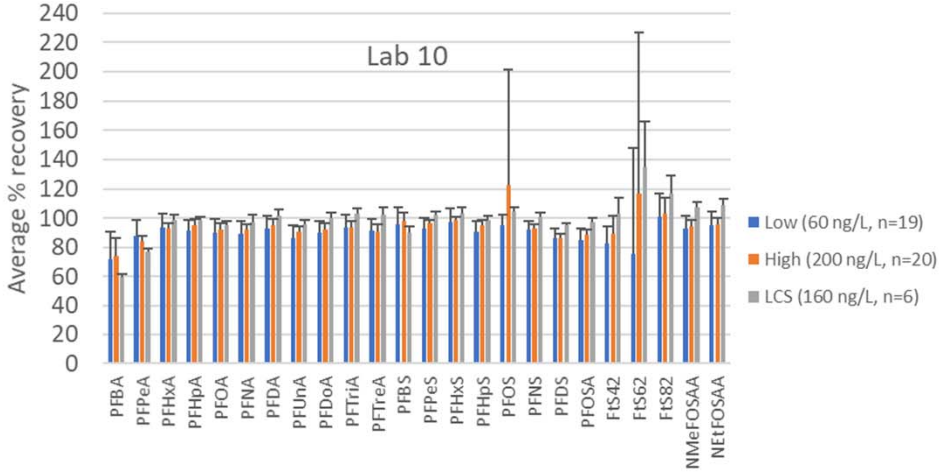
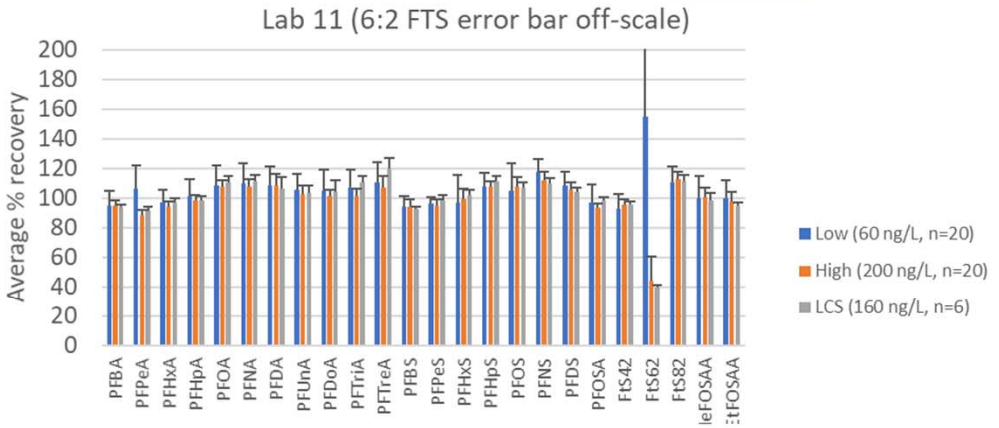
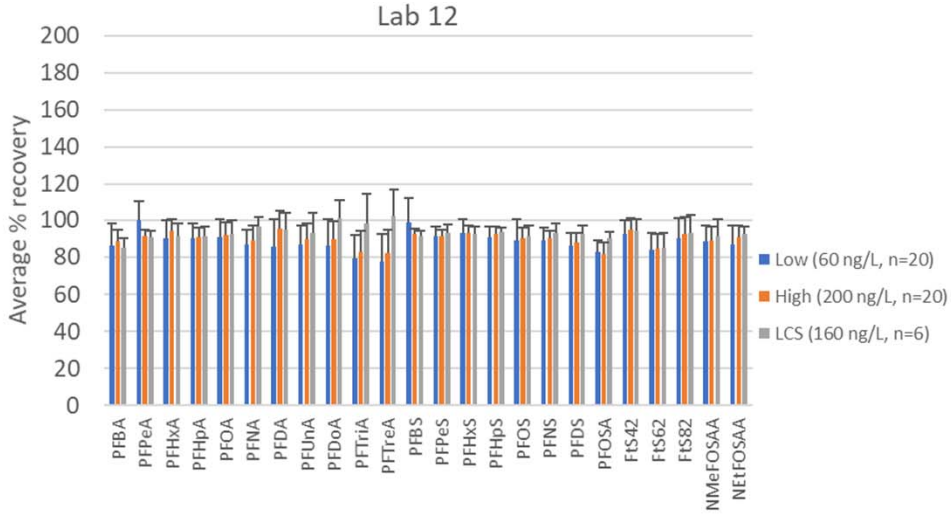
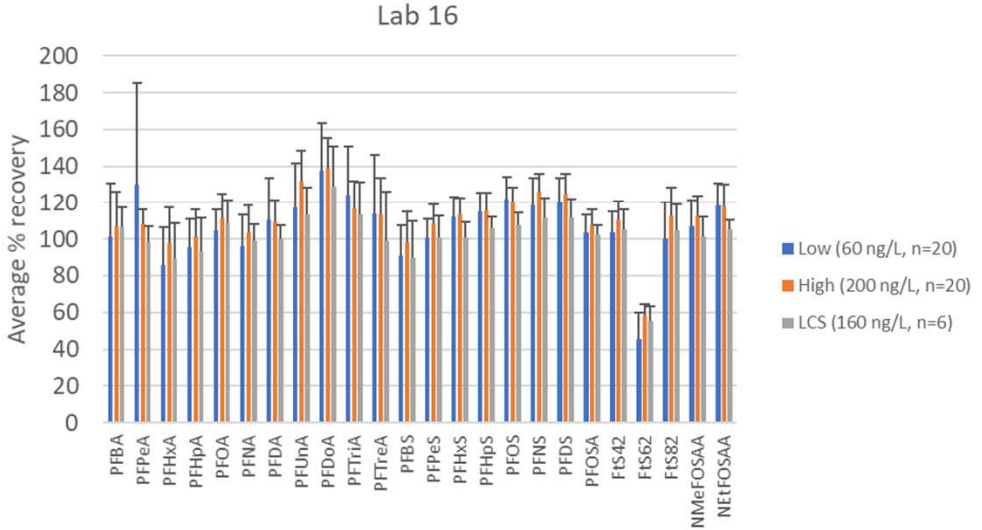


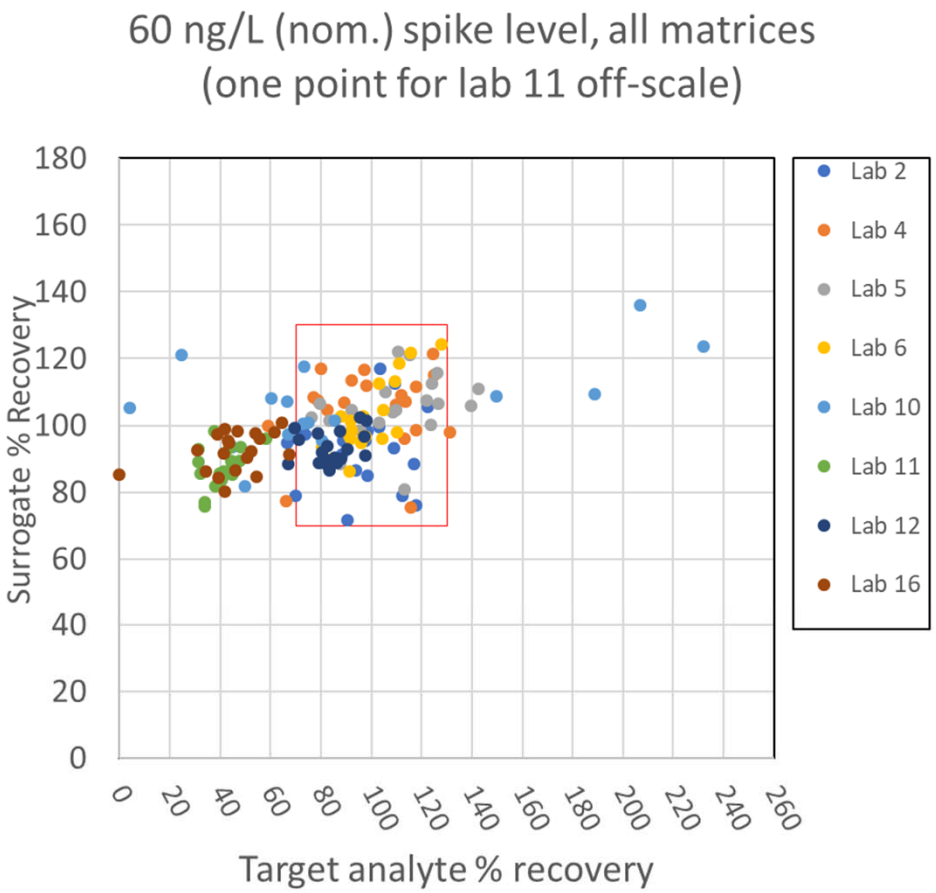


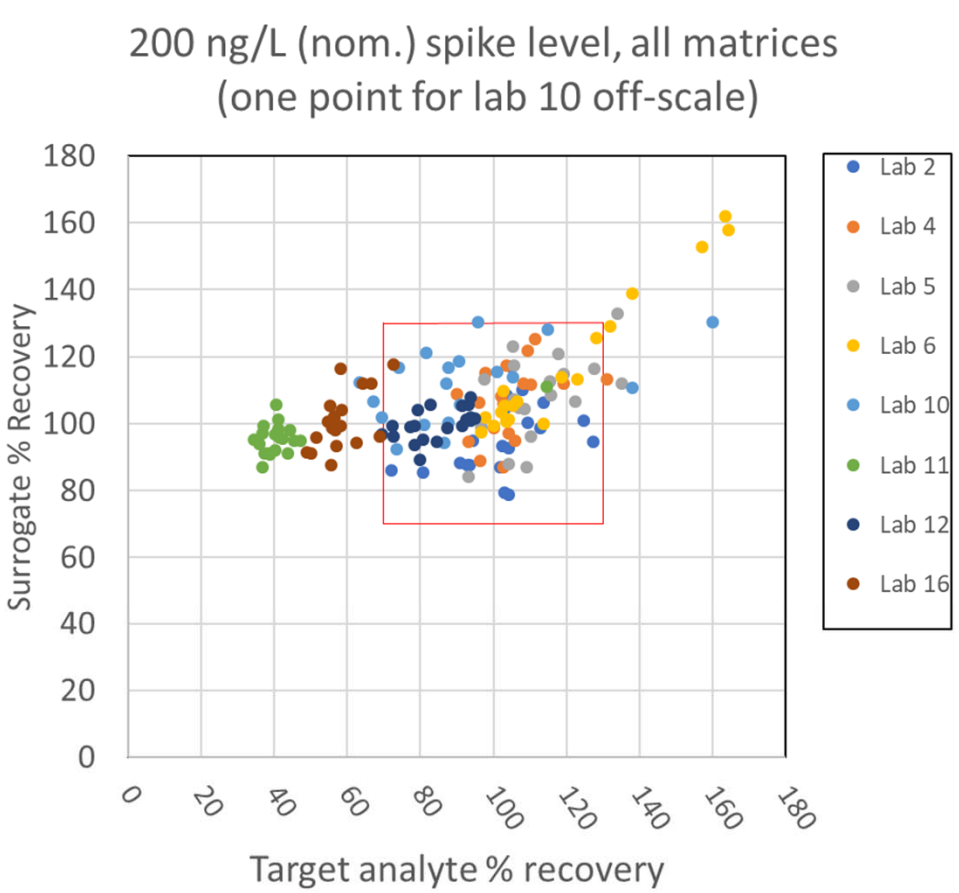




Blind Study Samples: 6:2 Fluorotelomer Sulfonate (6:2 FTS)

Red box shows 70-130% recovery data quality objective



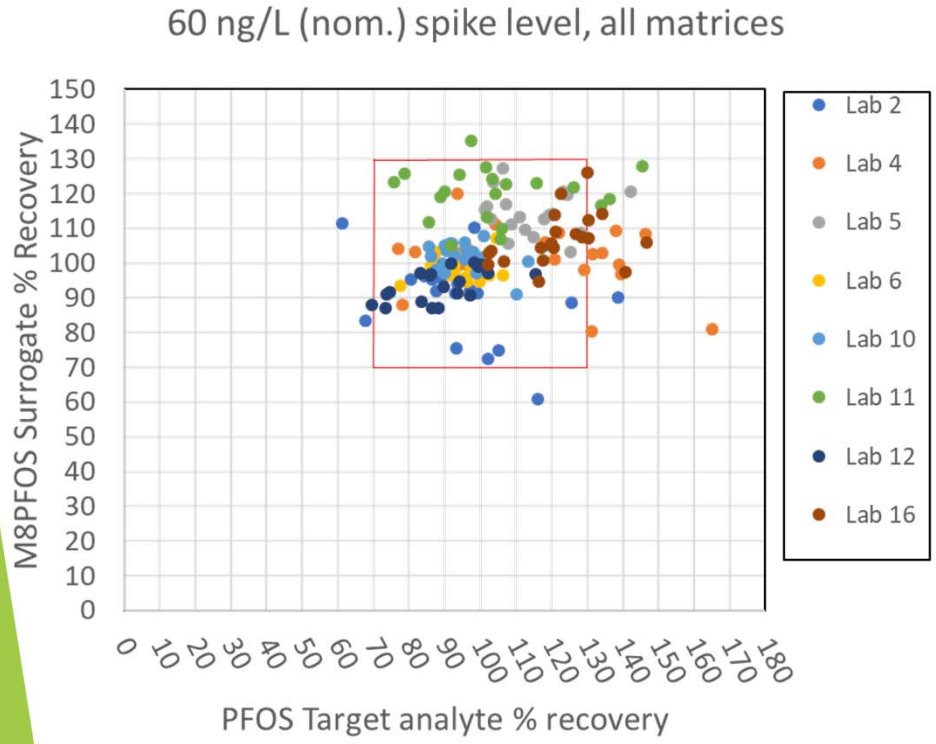


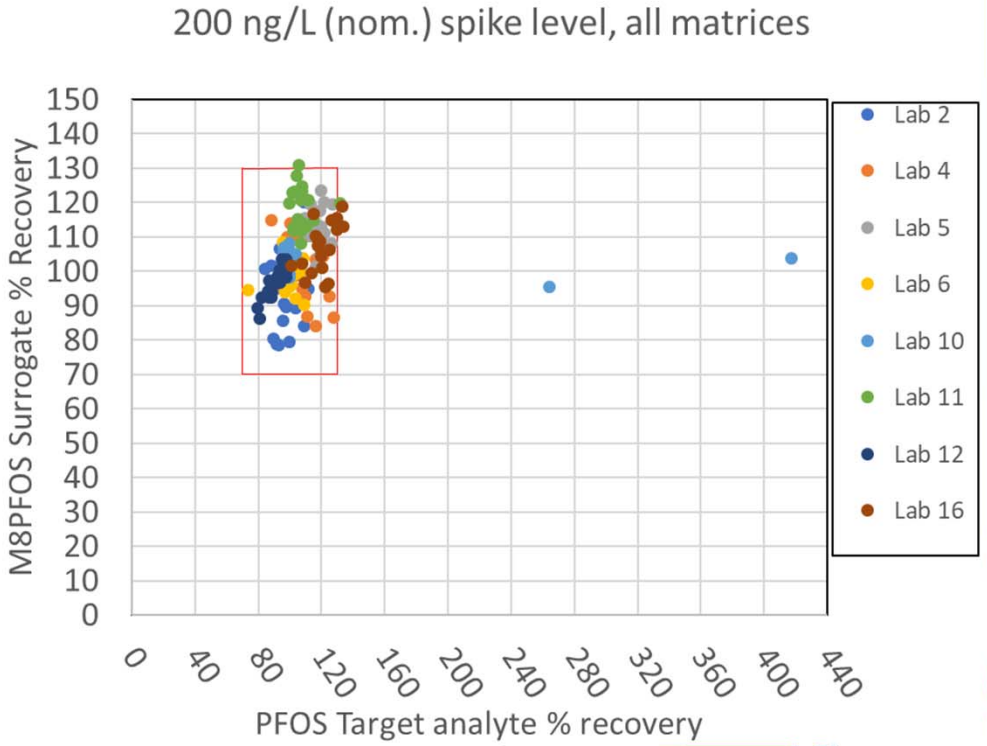
Quality Controls and study data for 6:2 FTS

		Lab 2	Lab 4	Lab 5	Lab 6	Lab 10	Lab 11	Lab 12	Lab 16
Reagent blank	Max conc (ng/L)	< 5	< 5	14.3	< 5	< 5	< 5	< 5	29.6
Method blank	max conc (ng/L)	< 5	< 5	< 5	115	116	< 5	5.5	< 5
CCV % drift (n=3 for labs 2-6; n=6 for labs 10-12; n=10 for lab 16)	# outside ±30% drift	0	0	0	0	0	4	0	2
LLOQ Verification	Prepared conc that met 50-150% REC	10-20 ng/L	40-80 ng/L	10-20 ng/L	10 ng/L	160 ng/L or none	none	20 ng/L	160 ng/L
LCS % Recovery (n=6)	mean	99.9	90.2	105	118	135	40.0	85.0	55.1
	stdev	3.8	11.8	6.0	26.1	31.1	1.5	8.1	8.2
M2-6:2 FTS surrogate % recovery in samples (n=59 or 60)	mean	93.2	107	105	110	107	93.1	99.8	101
	stdev	11.3	17.8	11.7	24.9	13.4	25.1	4.67	8.35
% Recovery in 60 ng/L (nom.) study samples (n=19 or 20)	mean	95.7	100	109	99.3	75.3	155	84.2	45.4
	stdev	16.7	20.9	19.0	11.6	72.8	512	8.9	14.4
% Recovery in 200 ng/L (nom.) study samples (n=20)	mean	102	109	112	118	117	43.9	84.4	58.2
	stdev	13.0	14.4	11.6	22.1	110	17.0	8.3	6.1

Validation study data: PFOS

Red box shows 70-130% recovery data quality objective





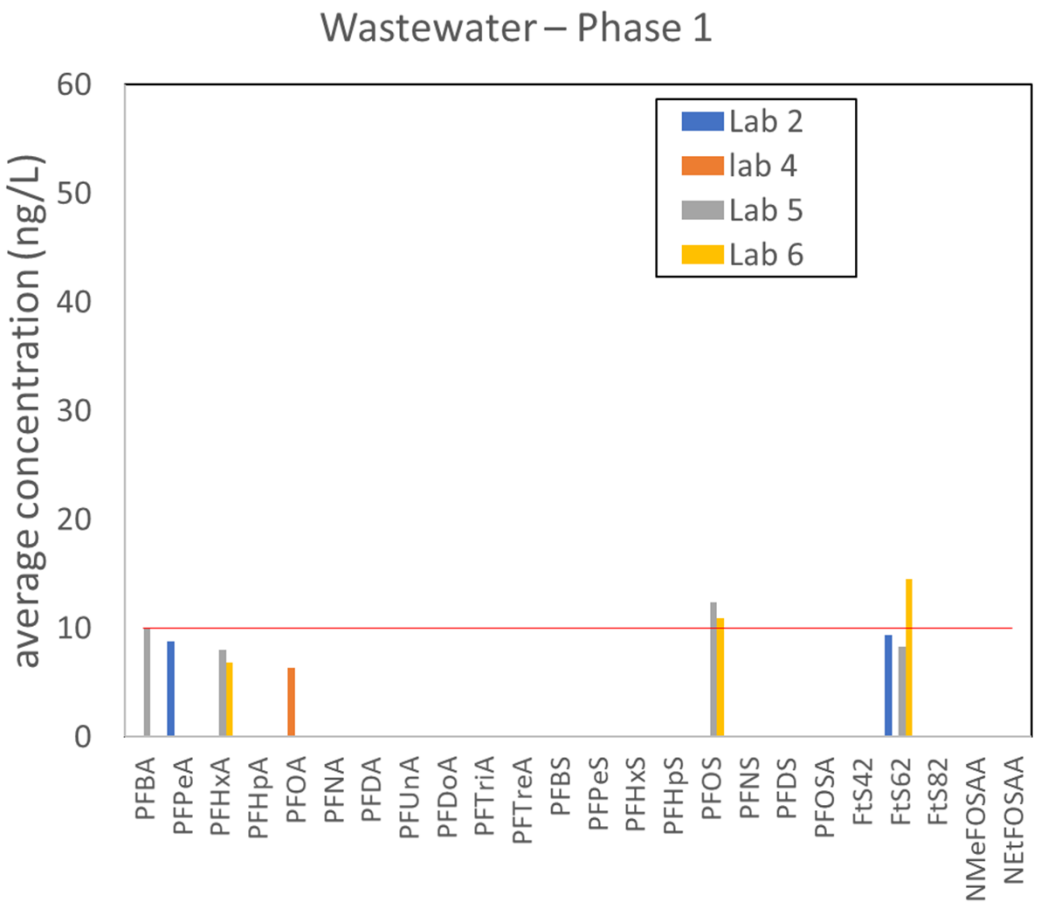
Quality controls and study data for PFOS

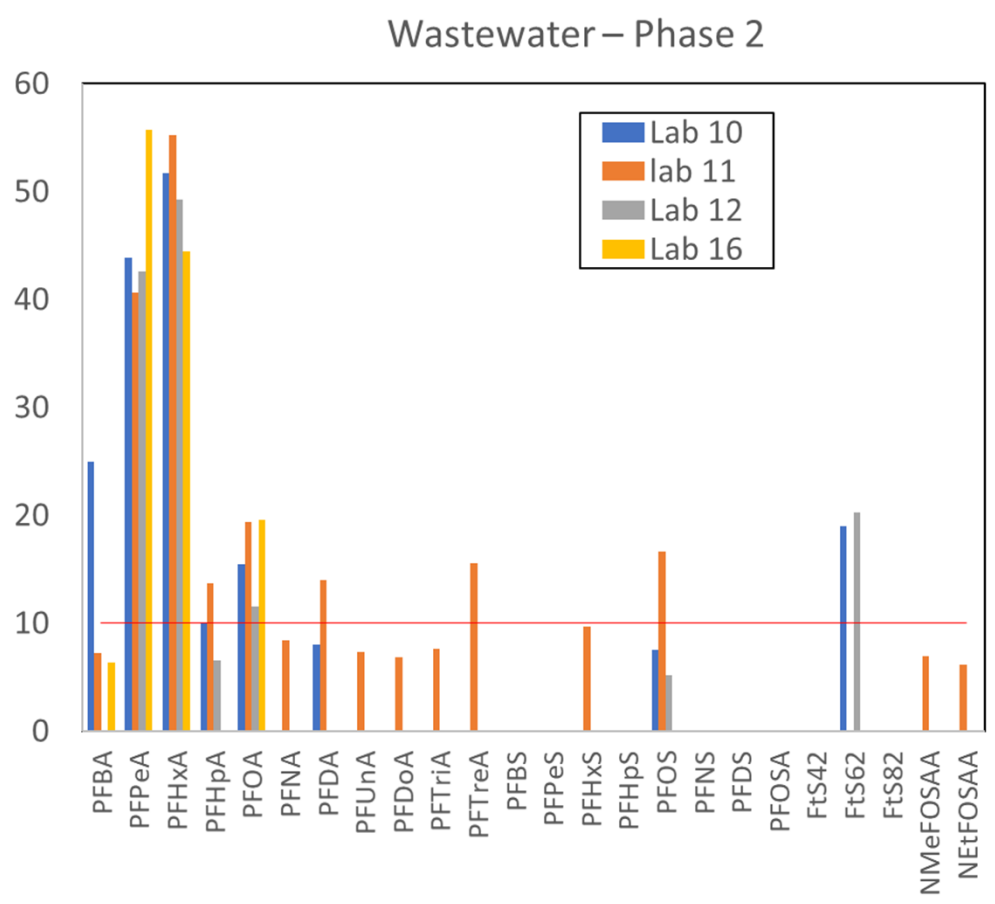
		Lab 2	Lab 4	Lab 5	Lab 6	Lab 10	Lab 11	Lab 12	Lab 16
Reagent blank	Max concentration	<5 ng/L	<5 ng/L	<5 ng/L	<5 ng/L	<5 ng/L	<5 ng/L	<5 ng/L	<5 ng/L
Method blank	Max concentration	<5 ng/L	<5 ng/L	<5 ng/L	<5 ng/L	<5 ng/L	9.9 ng/L	<5 ng/L	<5 ng/L
LLOQ Verification	Prepared conc that met 50-150% REC	10-20 ng/L	40-80 ng/L	10-20 ng/L	10 ng/L	10 ng/L	20 ng/L	10 ng/L	10 ng/L
LCS % REC (n=6)	mean	89.9	103	104	91.8	104	107	91.3	108
	stdev	5.1	8.7	7.7	3.4	2.6	3.9	5.7	6.8
% REC of M8PFOS surrogate in samples (n=59 or 60)	mean	92.3	104	113	101	102	119	95.3	108
	stdev	11.5	17.5	7.0	15.0	6.6	6.9	4.6	8.0
% REC in 60 ng/L blind samples (n=19 or 20)	mean	96.2	117	114	94.5	95.0	105	89.3	122
	stdev	18.3	25.2	11.0	6.8	7.5	18.9	11.4	12.2
% REC in 200 ng/L blind samples (n=20)	mean	97.3	110	118	99.8	123	108	90.2	120
	stdev	7.5	10.5	5.1	7.2	78.7*	6.7	5.5	8.4

*Two replicate high spike wastewaters in different preparation batches had 417% recovery and 264% recovery (extrapolated above high cal standard), respectively; without these two points, the 200 ng/L average % REC was 98.4% and the stdev was 2.5%

Wastewater samples- Background Concentrations

Red line is 10 ng/L, lowest LLOQ evaluated for study





Sample Preparation Quality Controls: Surrogates
Study acceptance limits: 70-130% recovery

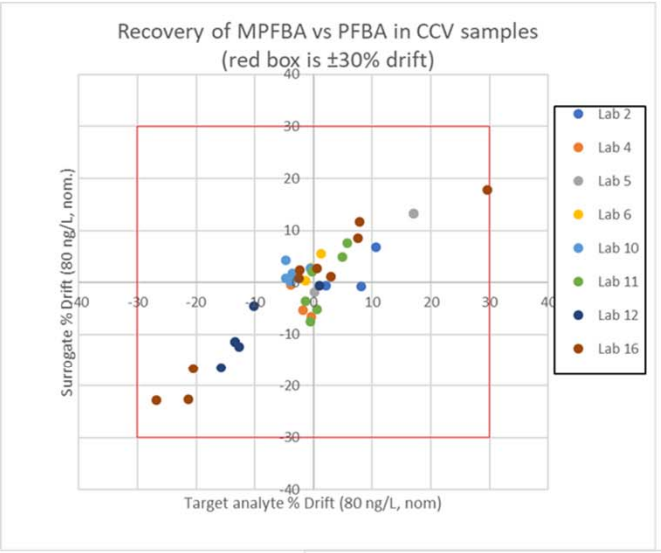
Surrogate Performance across all laboratories

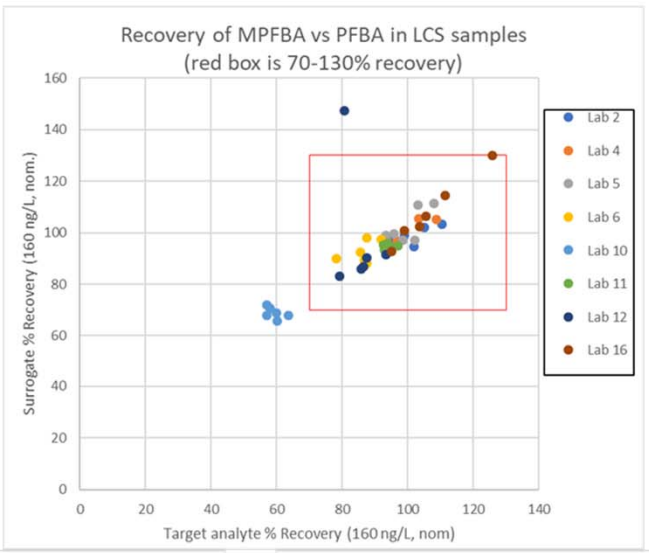
Surrogate	% recovery, all matrices (n=477)			
	mean	stdev	# outside 70-130%	% outside 70-130%
MPFBA	95.6	10.9	11	2.3
M5PFPeA	98.7	7.5	0	0.0
M5PFHxA	97.4	11.8	10	2.1
M4PFHpA	98.9	10.9	8	1.7
M8PFOA	100.9	9.5	1	0.2
M9PFNA	102.2	11.6	4	0.8
M6PFDA	104.5	12.1	8	1.7
M7PFUnDA	103.3	11.6	6	1.3
MPFDoDA	100.8	14.7	21	4.4
M2PFTeDA	96.8	18.8	49	10.3
M3PFBS	96.9	12.0	8	1.7
M3PFHxS	101.5	8.0	0	0.0
M8PFOS	104.0	11.2	5	1.0
M8FOSA	100.6	8.9	5	1.0
M2-8:2FTS	106.0	13.9	21	4.4
M2-6:2FTS	100.4	15.4	11	2.3
M2-4:2FTS	97.8	19.4	38	8.0
d3NMeFOSAA	102.6	16.1	39	8.2
d5NEtFOSAA	104.1	16.0	41	8.6

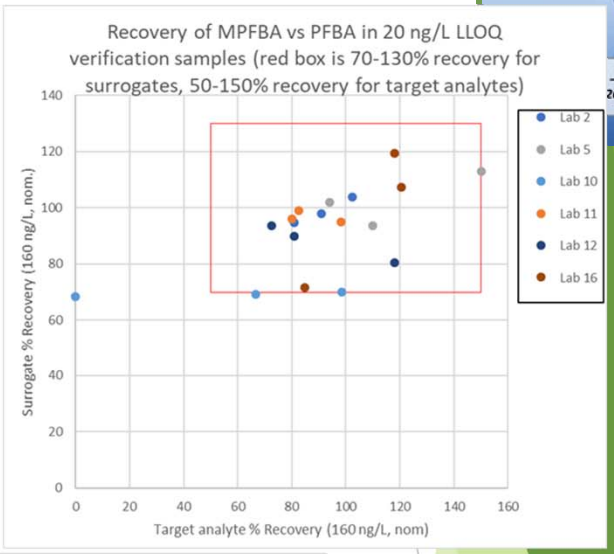
Themes in public comments

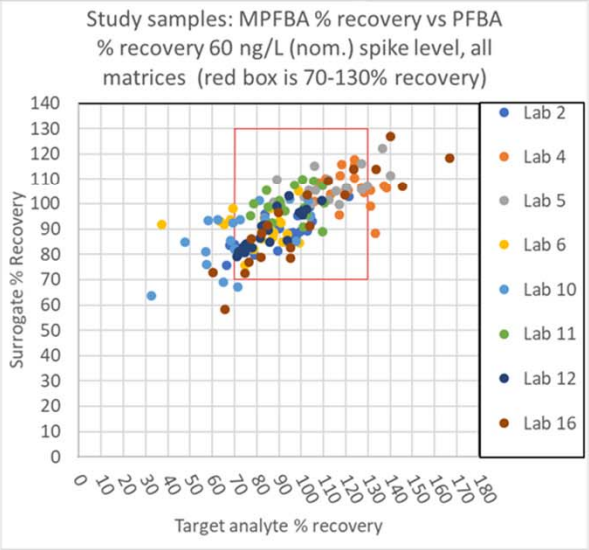
- Use or at least include option for isotope dilution calibration
- Clarify qualitative ID and quantitation of linear and branched isomers
- Need for validated solids preparation method
- Holding time study
- Include additional target analytes (e.g., HFPO-DA, DONA)
- Use statistically derived or (different) fixed limits for standard additions
- CCV frequency and acceptance criteria
- Particle filtration vs centrifuging
- Container materials for samples, sample extracts and standards

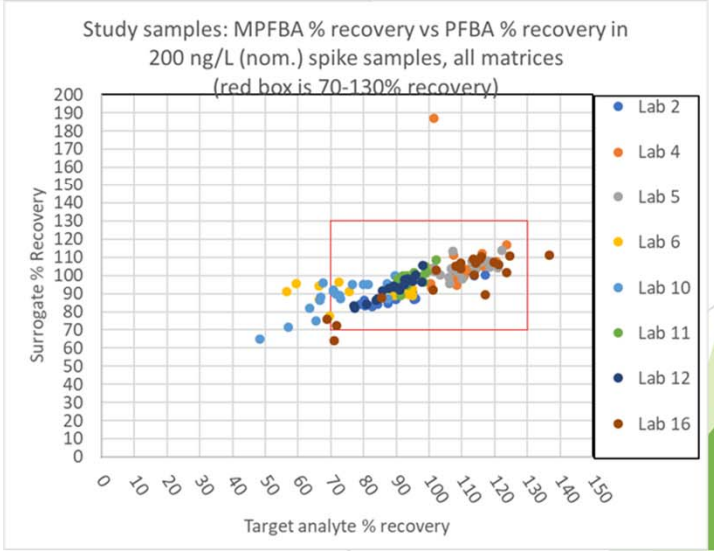
Correlation between PFBA and MPFBA



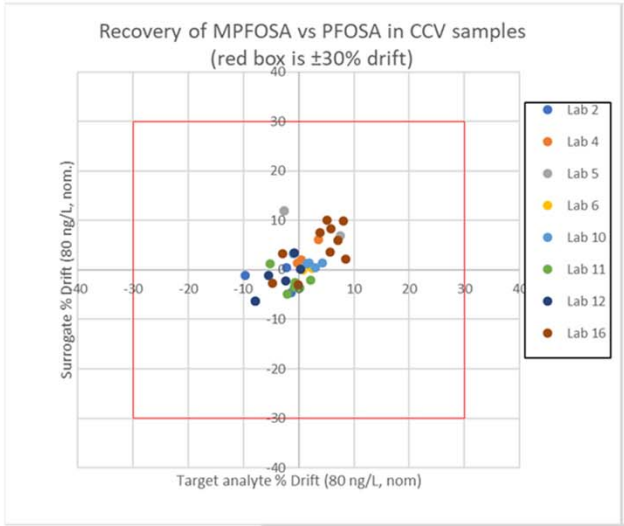


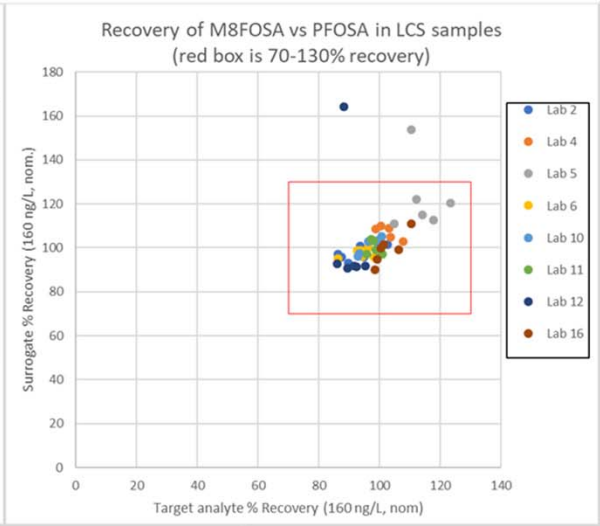


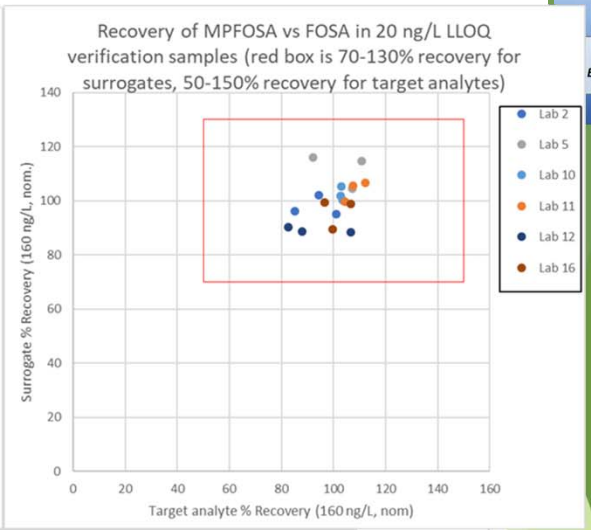


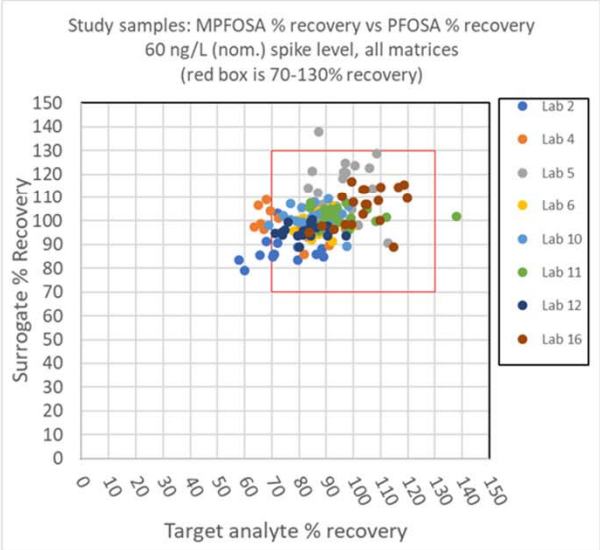


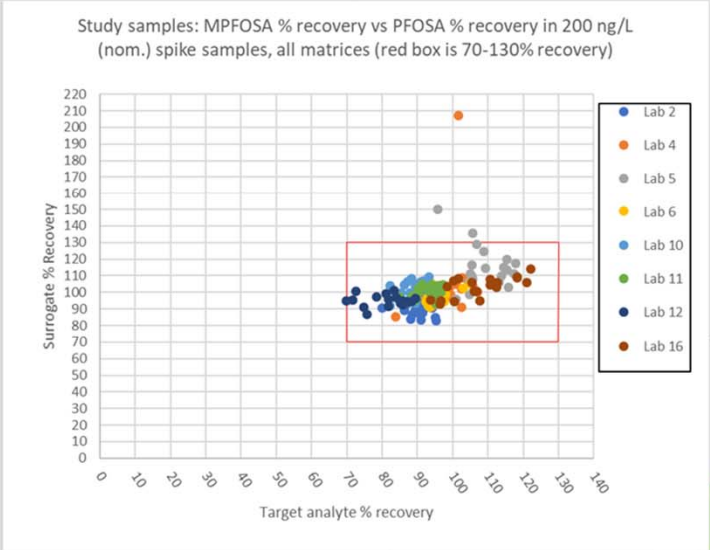
Correlation between PFOSA and MPFOSA





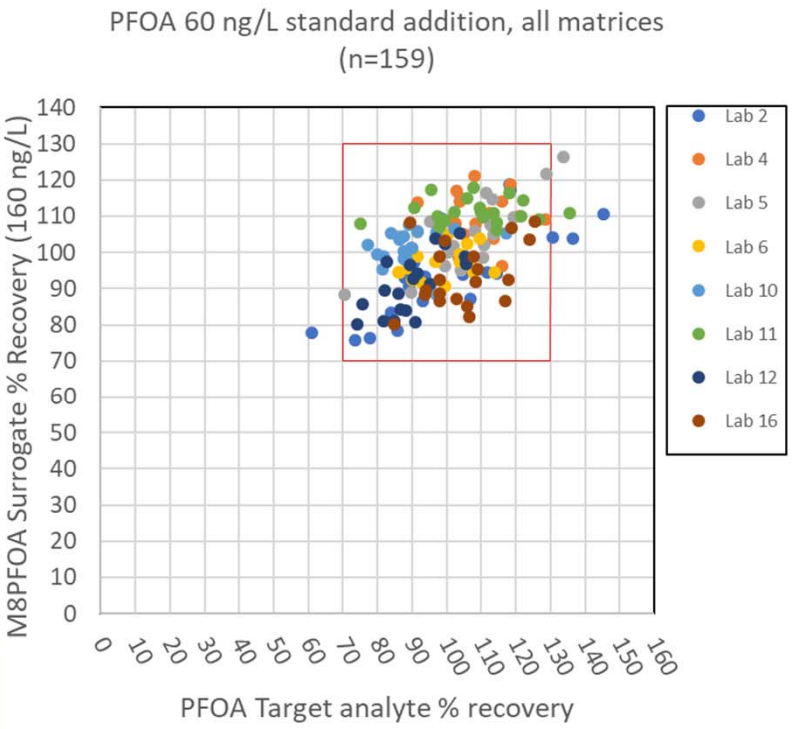


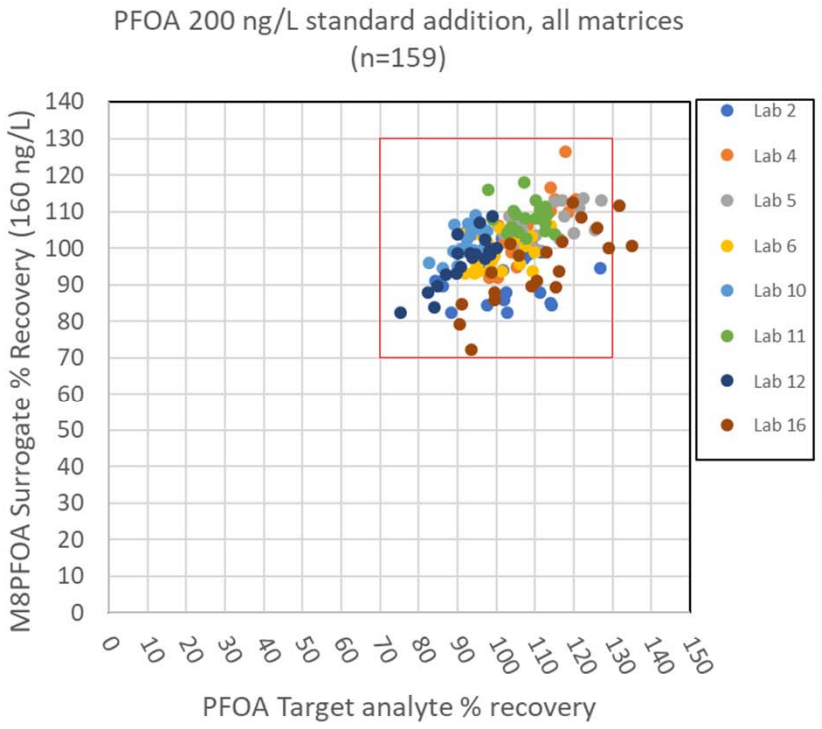




Recovery by Analyte, All Matrices: Perfluorooctanoic acid (PFOA)

Red box shows 70-130% recovery





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Timeline

- Methods workgroup review completed
- Currently finalizing package for ORCR management review, including methods and responses to public comments
- Anticipated publication date: Fall 2020
- Plan to provide more detailed write-up of validation study post-publication

Validation Study Summary and Conclusions

- Data quality objectives for precision, bias and sensitivity were met for all target analytes except 6:2 FTS
  - Note: Labs that consistently met QC criteria for instrument and sample preparation quality controls produced 6:2 FTS data with acceptable precision and bias
- Deviations from critical steps in the study protocol will be identified as cautions in methods
  - Avoid subsampling prior to adding organic solvent
  - Avoid long-term storage of solutions in 1:1 MeOH-water+0.1% acetic acid in glass
- Modern LC/MS systems from multiple instrument vendors achieved LLOQs of 10-20 ng/L for most target analytes, including PFOS, PFOA
- Direct analysis with minimal preparation has a number of advantages, including reduced labor/cost and faster turnaround

Thank you for listening

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