

Troubleshooting PFAS Solid Phase Extraction Chemistry for EPA Methodology

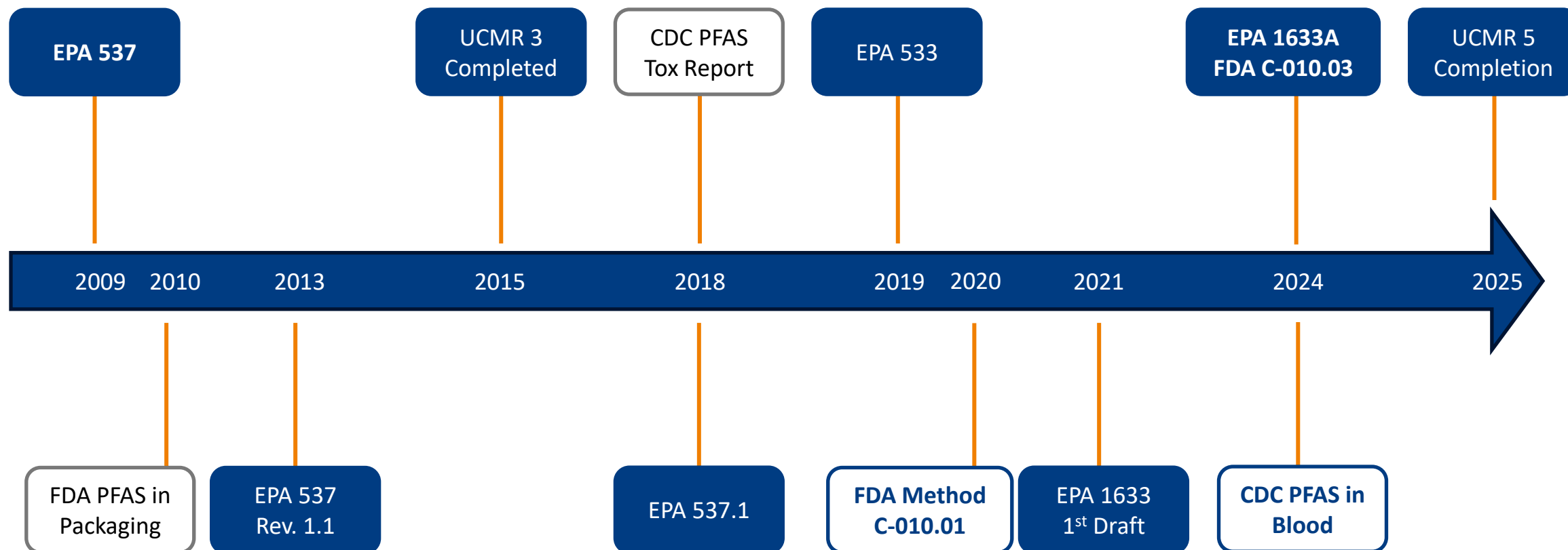
Outline

Troubleshooting SPE Chemistry for EPA Methodology



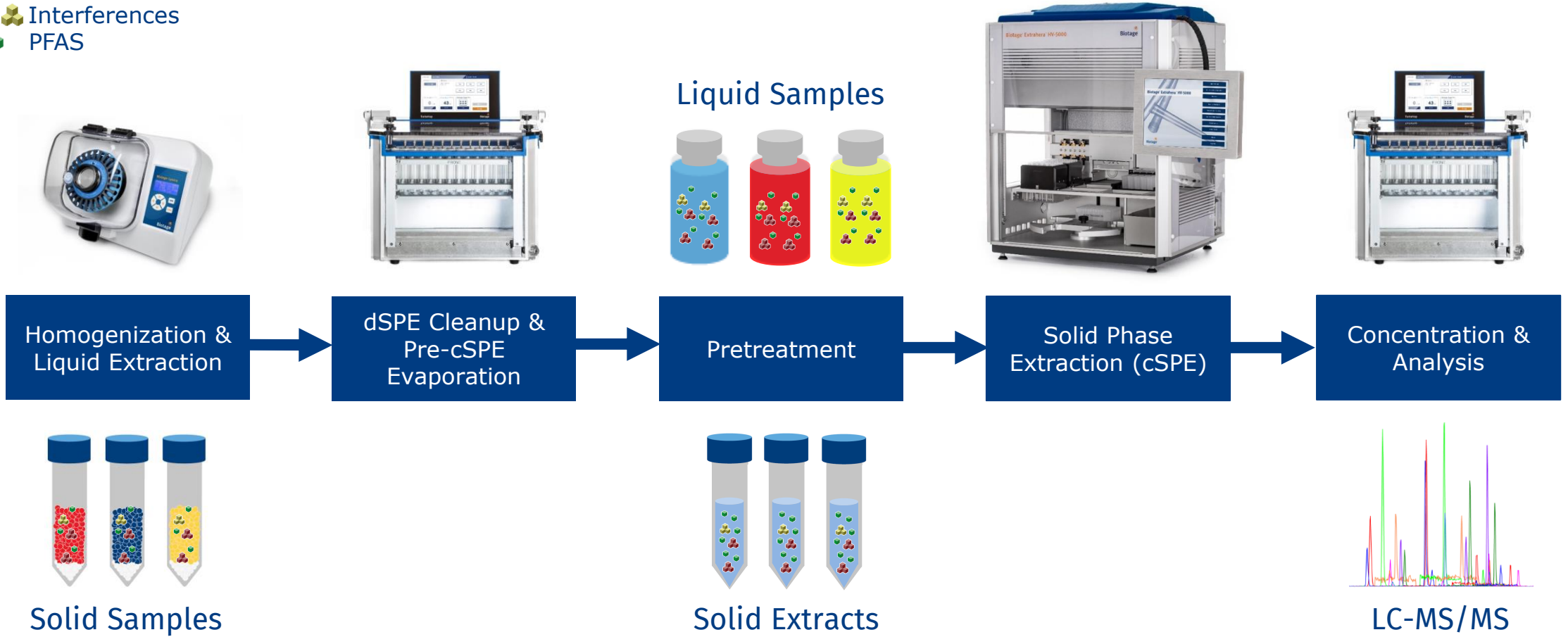
- PFAS Testing Milestones & Sample Preparation Workflow
- Matrix & Target Analyte Chemistry by Method
- Performance of EPA Method Relevant SPE Sorbents relevant
- Initial Demonstration of Capability (IDOC) by Matrix
- Final Conclusions

EPA Milestones for PFAS Testing



PFAS Sample Preparation Workflow

 Interferences
 PFAS



Sample Matrices by EPA Method

Matrix	EPA 537.1	EPA 533	EPA 1633A
Drinking Water	250 mL	100 or 250 mL	
Groundwater			500 - 1000 mL
Surface Water			
Wastewater			
Leachate			100 mL
Soils			5 g
Biosolids			0.5 g
Fish Tissue			1 – 2 g



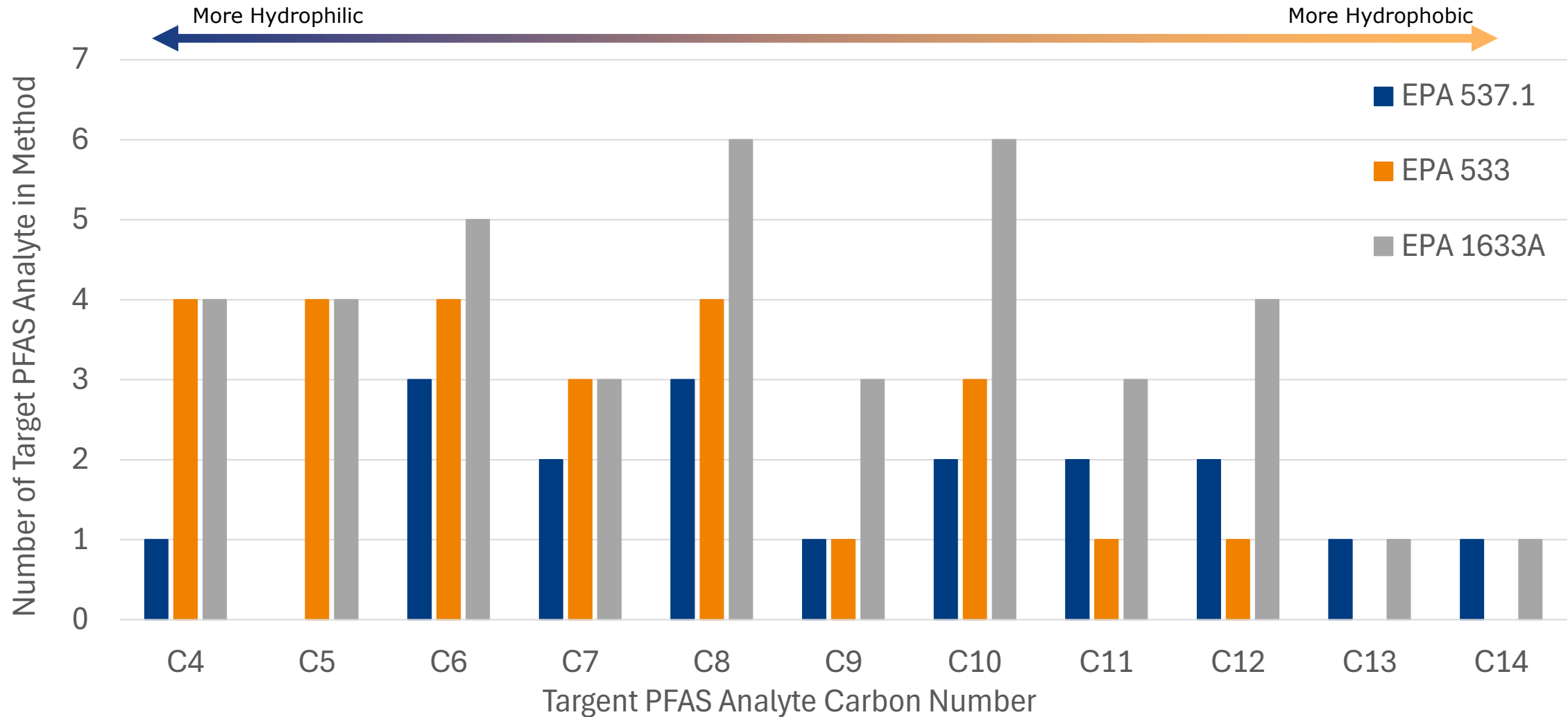
Target Analytes

Abbreviation	EPA 537.1	EPA 533	EPA 1633A
PFBS	X	X	X
PFDA	X	X	X
PFDaA	X	X	X
PFHpA	X	X	X
PFHxS	X	X	X
PFHxA	X	X	X
PFNA	X	X	X
PFOS	X	X	X
PFOA	X	X	X
PFUnA	X	X	X
9Cl-PF3ONS	X	X	X
ADONA	X	X	X
HFPO-DA	X	X	X
11Cl-PF3OUdS	X	X	X
NEtFOSAA	X		X
NMeFOSAA	X		X
PFTA	X		X
PFTTrDA	X		X
PFBA		X	X
PFPeA		X	X
6:2 FTS		X	X
8:2 FTS		X	X
PFHpS		X	X

Abbreviation	EPA 537.1	EPA 533	EPA 1633A
PFPeS		X	X
4:2 FTS		X	X
NFDHA		X	X
PFEESA		X	X
PFMPA		X	X
PFMBA		X	X
3:3 FTCA			X
5:3 FTCA			X
7:3 FTCA			X
NMeFOSE			X
N-MeFOSA			X
PFNS			X
PFDS			X
FOSA			X
PFDoS			X
N-EtFOSA			X
NEtFOSE			X
Total	18	25	40

Target Analyte Chemistry

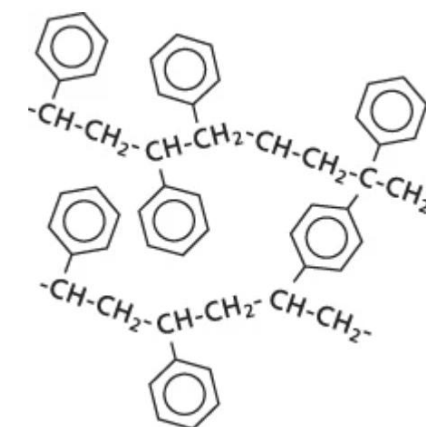
EPA 537.1, 533, 1633A



Solid Phase Extraction & Solvent Evaporation

Extraction	EPA 537.1	EPA 533	EPA 1633A
Technique	SPE	SPE	SPE & dSPE
Format	Cartridge	Cartridge	Cartridge & dSPE
SPE Media	DVB	WAX	WAX/GCB
SPE Mass	500mg	200 or 500mg	150mg WAX 10mg GCB
Sample Load Rate	10-15 mL/min	5 mL/min	3-6 mL/min
Elution	8 mL MeOH	10 mL NH4OH/MeOH (2%, v/v)	5 mL NH4OH/MeOH (1%, v/v)

Evaporation	EPA 537.1	EPA 533	EPA 1633A
Temperature	60-65C	55-60C	55C
Evaporation	Dryness	Dryness	5 mL
Reconstitution	96:4% (vol/vol) MeOH:Water	80:20% (vol/vol) MeOH:Water	n/a
Vortex	Yes	Yes	Yes



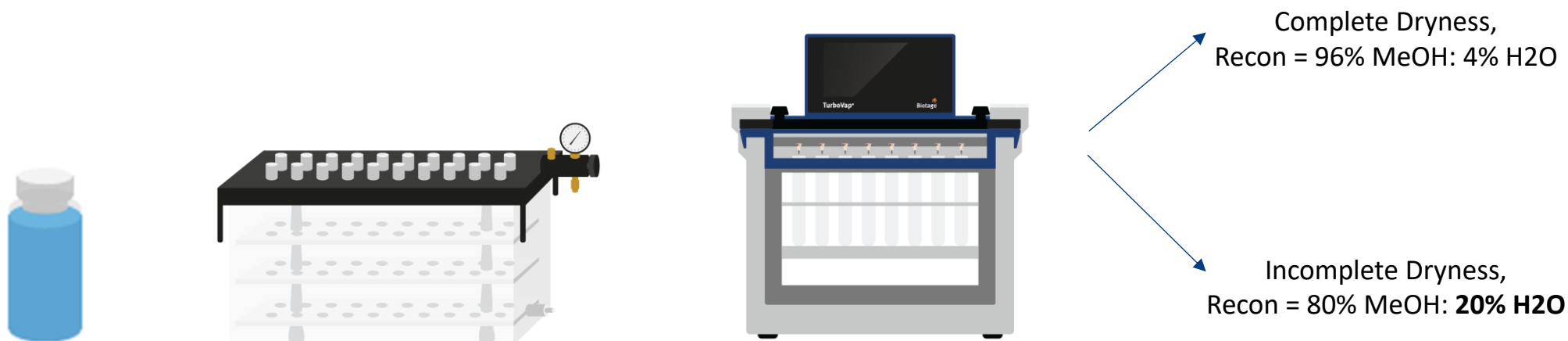
DVB particle size influences extract composition and peak asymmetry in EPA Method 537.1



EPA 537.1 Method Requirement

Extract Concentration

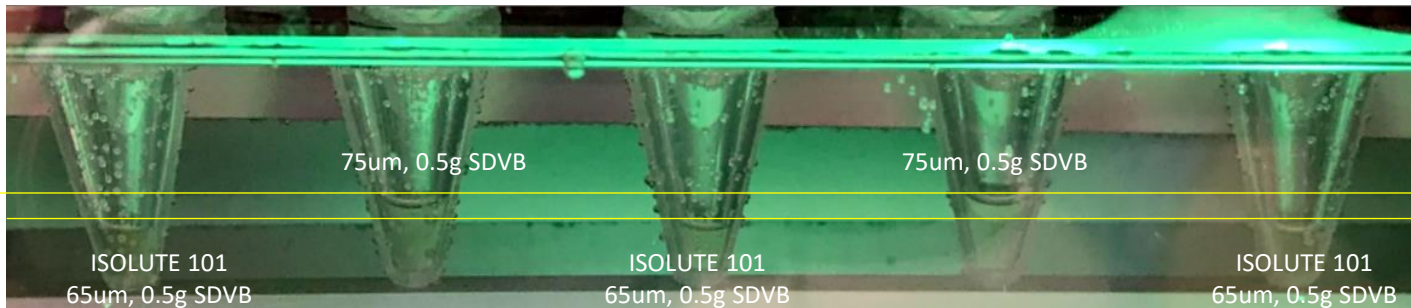
11.5. EXTRACT CONCENTRATION – Concentrate the extract to dryness under N₂ water bath @60-65 °C to remove all the water/methanol. Add 96:**4%** (vol/vol) MeOH:**Water** solution and bring the volume to 1 mL and vortex.



Section 9.3.9., Section 10.2.2., & Section 10.2.4.1. – “Modifying the standard or extract composition to more aqueous content to prevent poor shape is not permitted.”

Incomplete Evaporation

DVB Particle Size Impact on Residual Water



SPE Cartridge	Media	Bed Mass	Particle Size (um)	Pore Size (A)	Estimated Residual Water(mL)	Total Evaporation Time to Dryness (min)
Vendor A	SDVB	500 mg	100	260	0.20	< 120min
Vendor B	SDVB	500 mg	75	300	0.10	< 90min
Biotage	SDVB	500 mg	65	100	0.05	< 75min

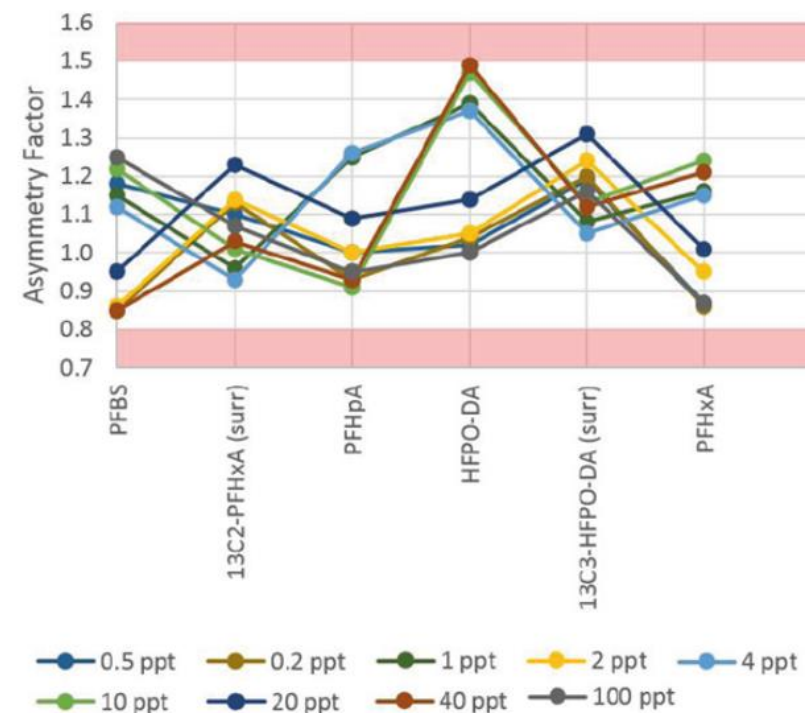
Importance of Removing Water

Peak Asymmetry - EPA 537.1 Method Requirement

9.3.9. PEAK ASYMMETRY FACTOR – A peak asymmetry factor must be calculated....Modifying the standard or extract composition to more aqueous content to prevent poor shape is not permitted

10.2.2. Establish LC operating parameters that optimize resolution and peak shape... The LC conditions listed in Table 1 may not be optimum for all LC systems and may need to be optimized.... Modifying the standard or extract composition to more aqueous content to prevent poor shape is not permitted.

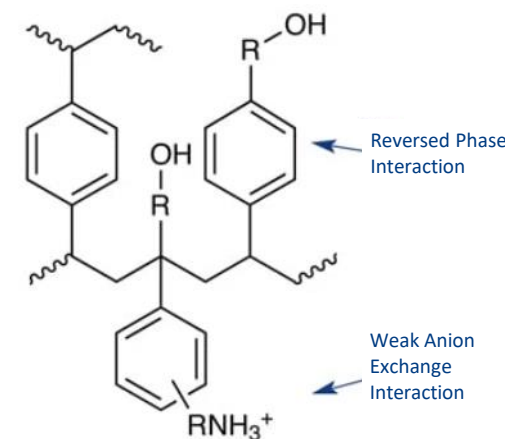
Biotage 537.1 AN949			
Time (min)	% 20 mM Ammonium Acetate in 95:5 H2O/Acetonitrile	% Methanol	Flow Rate Program (ml/min)
Initial	95	5	0.6
0.10	45	55	0.6
4.50	1	99	0.6
4.95	1	99	0.6
5.00	95	5	0.6
6.75	95	5	0.6



Solid Phase Extraction & Solvent Evaporation

Extraction	EPA 537.1	EPA 533	EPA 1633A
Technique	SPE	SPE	SPE & dSPE
Format	Cartridge	Cartridge	Cartridge & dSPE
SPE Media	DVB	WAX	WAX/GCB
SPE Mass	500mg	200 or 500mg	150mg WAX 10mg GCB
Sample Load Rate	10-15 mL/min	5 mL/min	3-6 mL/min
Elution	8 mL MeOH	10 mL NH ₄ OH/MeOH (2%, v/v)	5 mL NH ₄ OH/MeOH (1%, v/v)

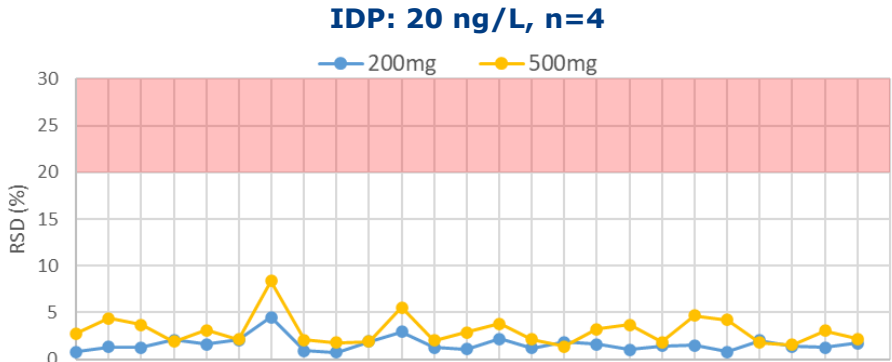
Evaporation	EPA 537.1	EPA 533	EPA 1633A
Temperature	60-65C	55-60C	55C
Evaporation	Dryness	Dryness	5 mL
Reconstitution	96:4% (vol/vol) MeOH:Water	80:20% (vol/vol) MeOH:Water	n/a
Vortex	Yes	Yes	Yes



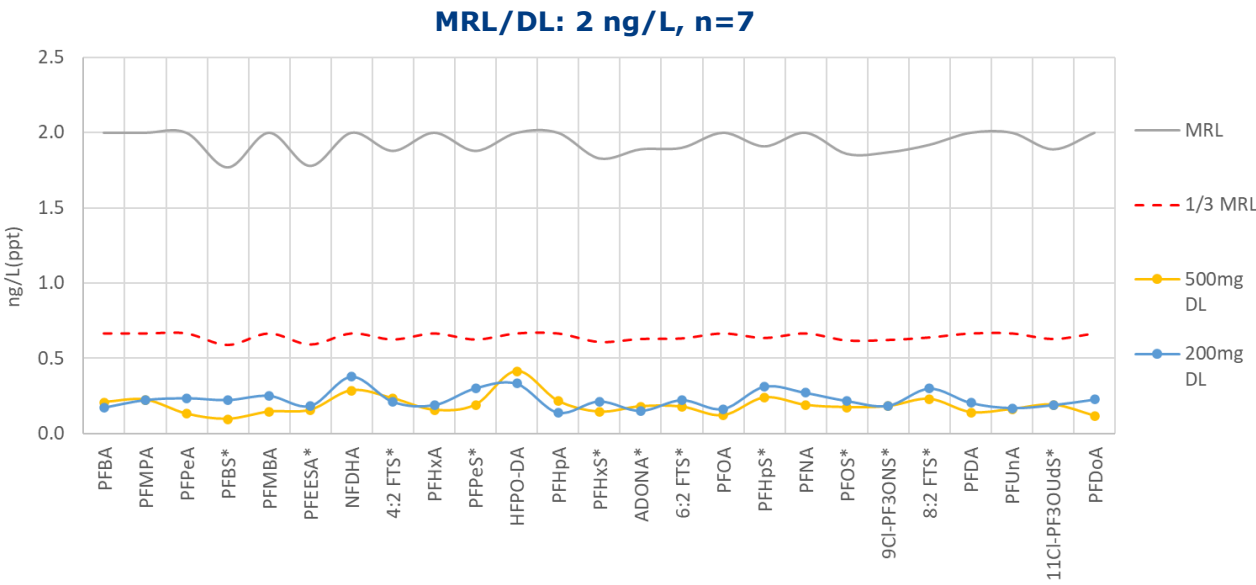
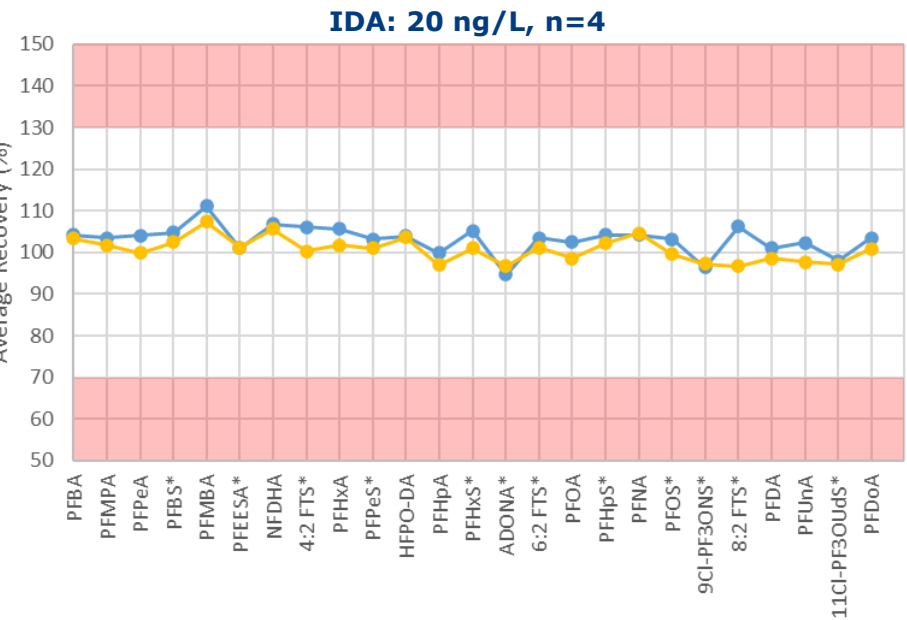
EPA 533 recommends 30µm WAX particle size and SPE bed mass based on sample volume
 100 mL = 200 mg WAX
 250 mL = 500 mg WAX



Extraction Performance – EPA 533

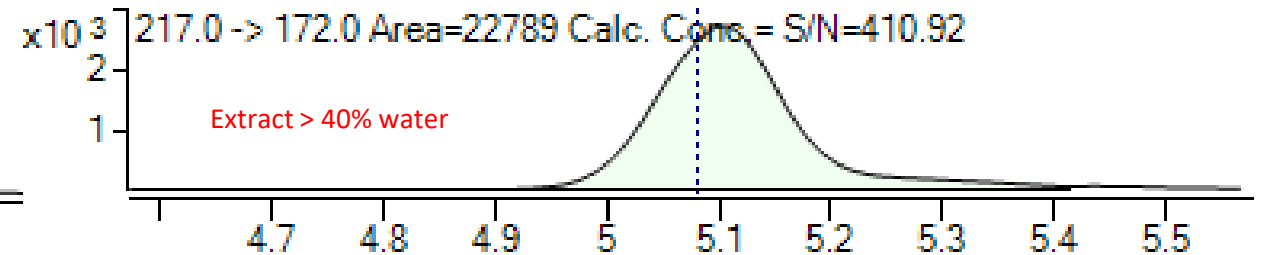
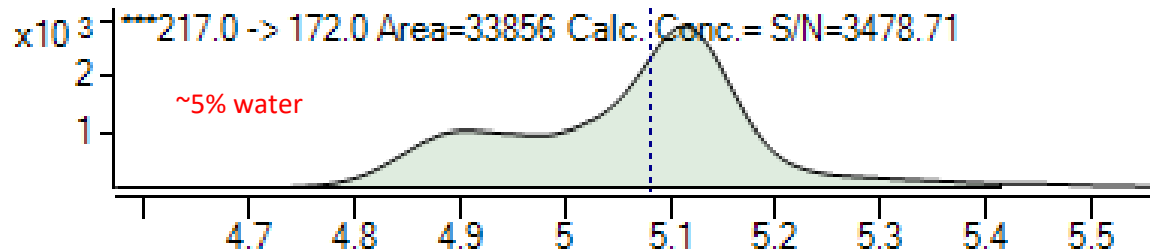
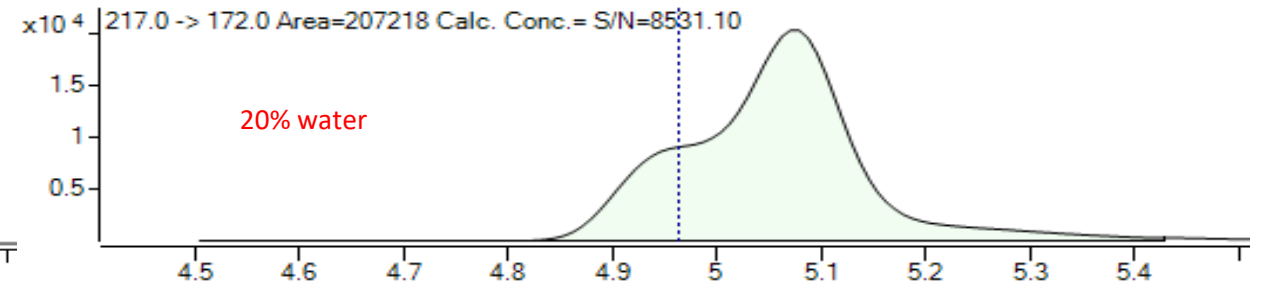
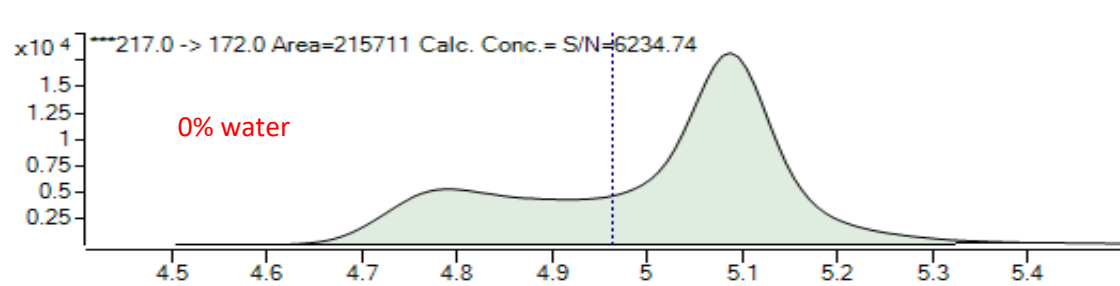


Sample Volume	250mL
Sorbent	200mg & 500mg WAX (30µm)
Manifold	Biotage® VacMaster™ 20
Concentrator	TurboVap® LV



Peak Asymmetry Factor

Inferring Extract Water Content from Peak Shape

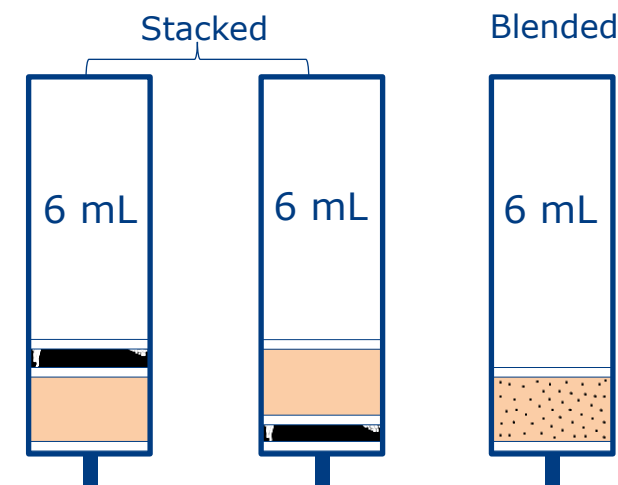


- The labeled internal standard C4-PFBA is common to both EPA 533 and EPA 1633A
- It is the first eluter most sensitive to extract water composition and behaves identically to the unlabeled target PFBA

Solid Phase Extraction & Solvent Evaporation

Extraction	EPA 537.1	EPA 533	EPA 1633A
Technique	SPE	SPE	SPE & dSPE
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SPE Media	DVB	WAX	WAX/GCB
SPE Mass	500mg	200 or 500mg	150mg WAX 10mg GCB
Sample Load Rate	10-15 mL/min	5 mL/min	3-6 mL/min
Elution	8 mL MeOH	10 mL NH ₄ OH/MeOH (2%, v/v)	5 mL NH ₄ OH/MeOH (1%, v/v)

Evaporation	EPA 537.1	EPA 533	EPA 1633A
Temperature	60-65C	55-60C	55C
Evaporation	Dryness	Dryness	5 mL
Reconstitution	96:4% (vol/vol) MeOH:Water	80:20% (vol/vol) MeOH:Water	n/a
Vortex	Yes	Yes	Yes



EPA 1633A recommends WAX & GCB SPE. GCB can be combined with WAX in single column.



Dual Sorbent Cartridge SPE (cSPE)

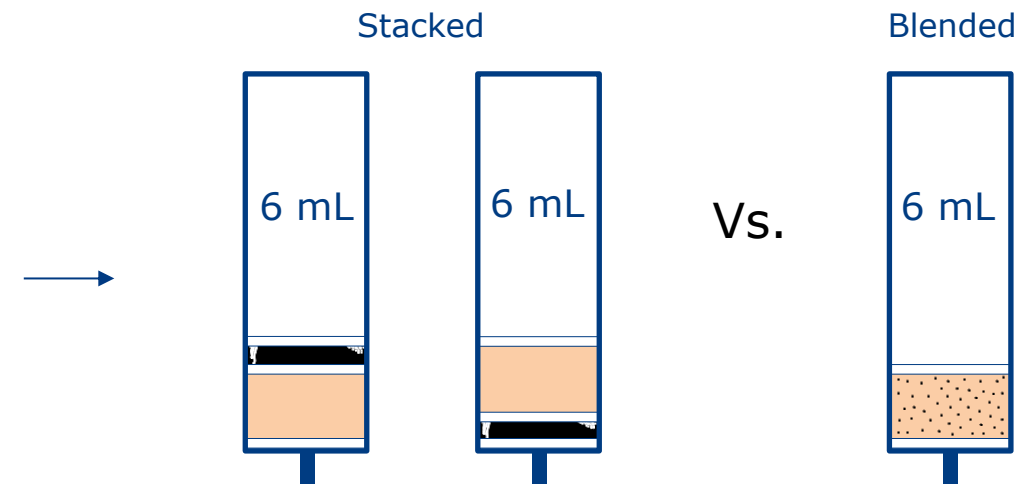
Combining GCB dSPE Sorbent with WAX Column SPE

» Advantages of Dual Sorbent cSPE

- » 2x Increase in Productivity
- » Reduction of Consumables Required
- » Eliminate Transfer Steps

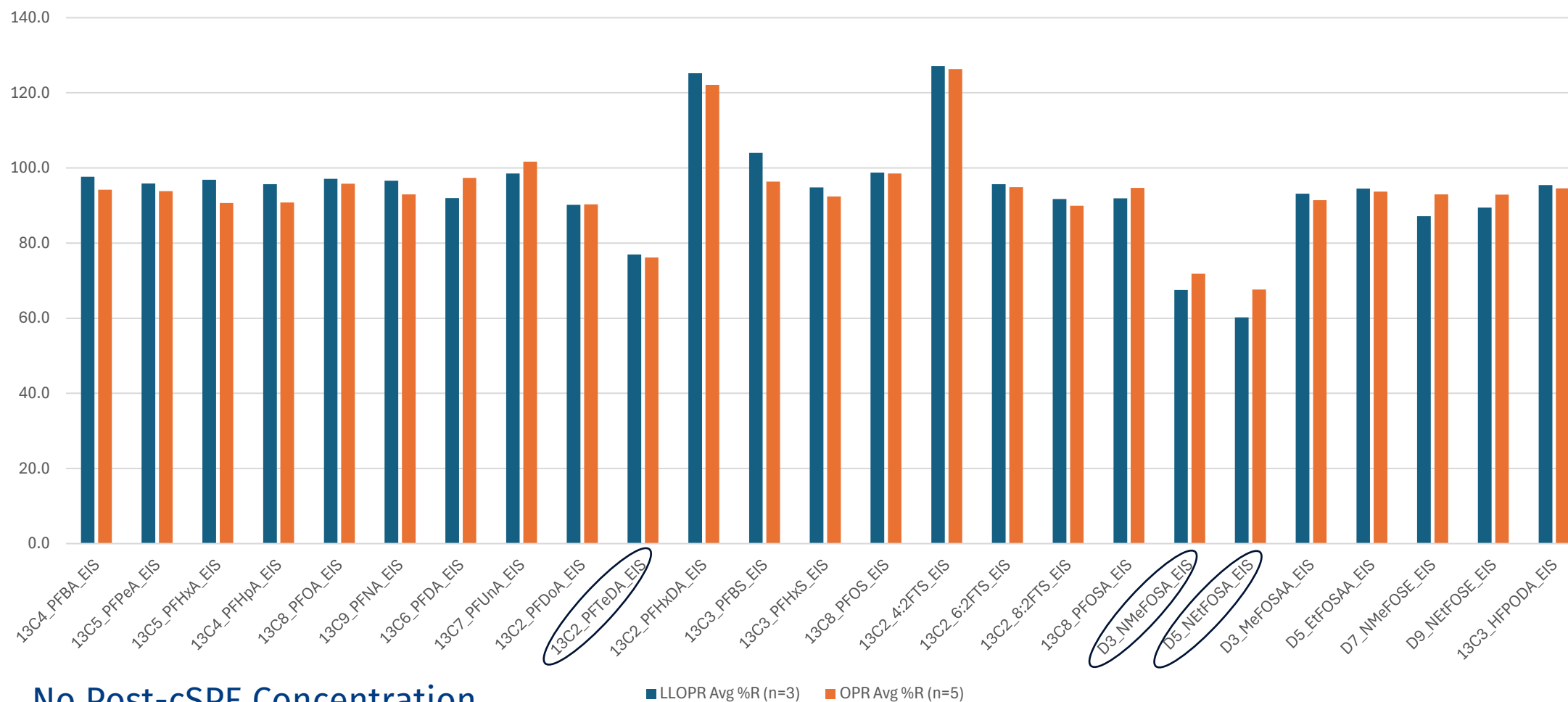
» Advantages of Blended vs. Stacked cSPE

- » Reduce Amount of GCB Needed
 - PFAS compounds stick to GCB
 - Can cause low recoveries
- » Eliminates Voiding
 - Layered format Produces Visible Gaps
 - Reduces precision & accuracy
- » Reduces Cost of Cartridge
 - GCB is expensive
 - Blending allows for 10x less GCB



Blended WAX/GCB cSPE

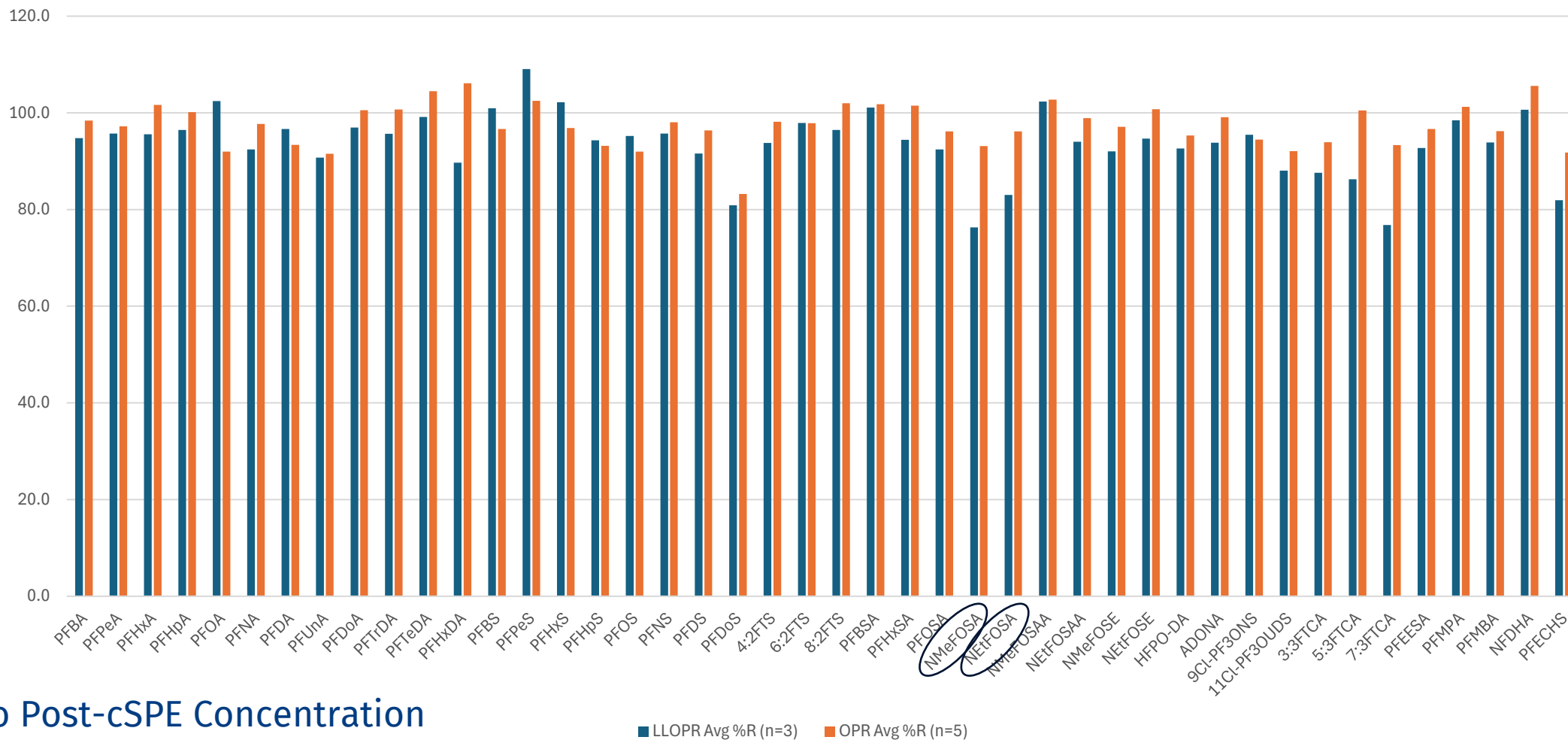
1633A Reagent Water EIS Recoveries



No Post-cSPE Concentration
5 mL Final Volume

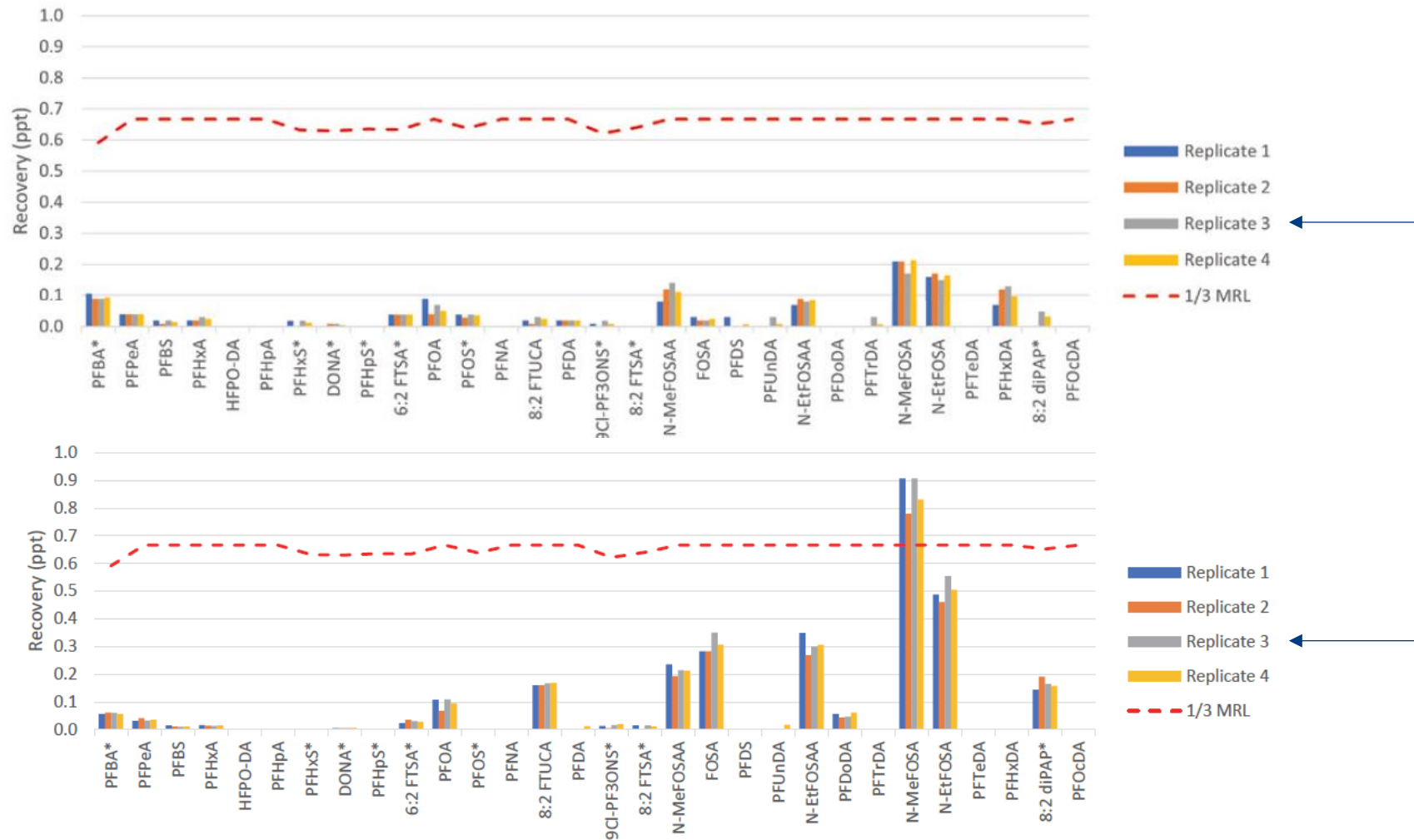
Blended WAX/GCB cSPE

1633A Reagent Water Targets Recoveries



No Post-cSPE Concentration
5 mL Final Volume

Carry Over Study Manual & Automated Systems



8-Channel Automated
Tube System

Initial Demonstration of Capability

Matrix	EPA 537.1	EPA 533	EPA 1633A
Drinking Water	Reagent Water		
Groundwater			Reagent Water
Surface Water			
Wastewater			
Leachate			
Soils			Ottawa (Reagent) Sand
Biosolids			
Fish Tissue			Chicken Breast or Similar Animal Tissue

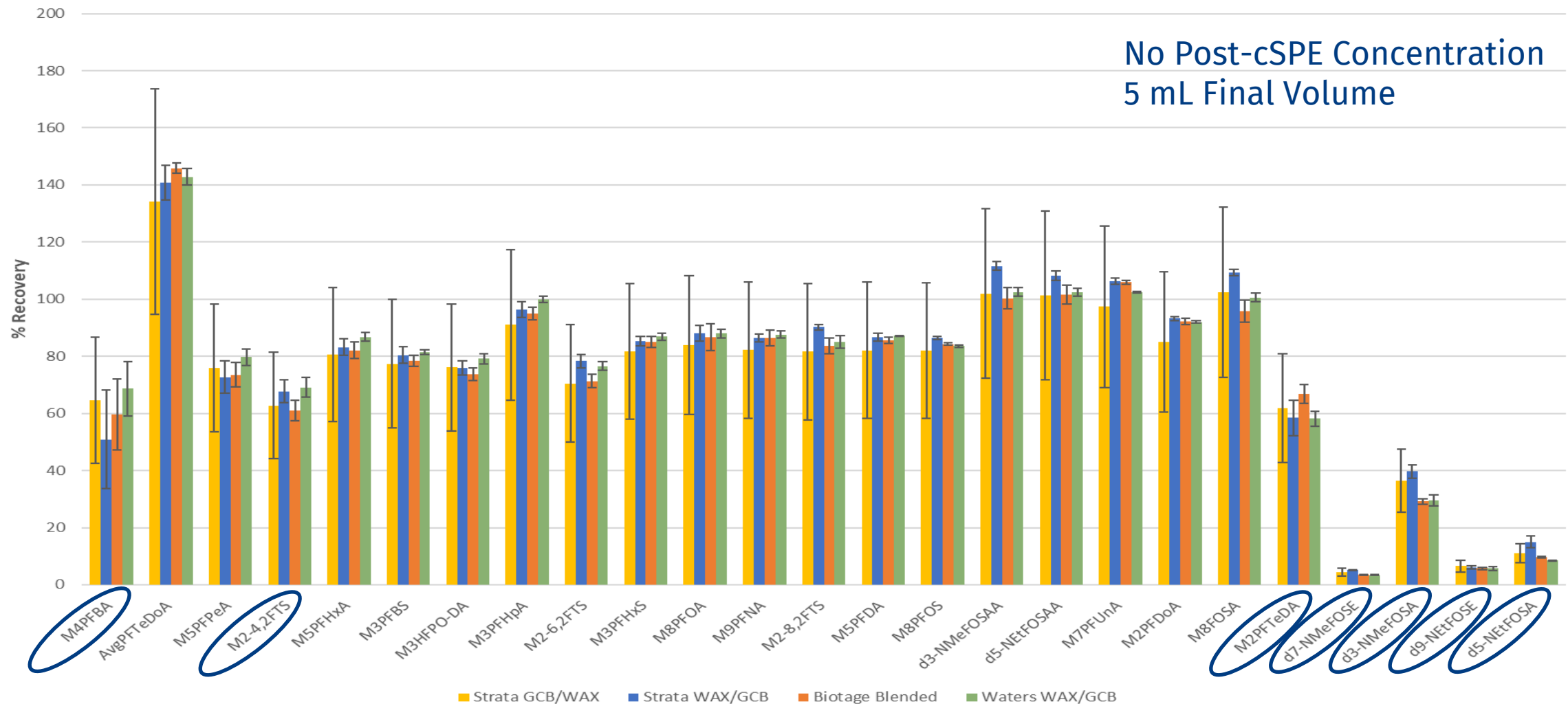
Extraction Comparison

Method (SPE)	EPA 537.1 (DVB)	EPA 533 (WAX)	EPA 1633A (WAX/GCB)
Pre-Treat	Tris(hydroxymethyl)aminomethane	Glacial Acetic Acid	Formic Acid or NH ₄ OH
Pre-Treat	Conc. HCl (pH = 7 ± 0.5)	pH = 7 ± 1.0	pH = 6.5 ± 0.5
Condition	Methanol	Methanol	15mL 0.1% (v/v) NH ₄ OH in MeOH
Condition	n/a	0.1 M Phosphate Buffer	n/a
Equilibrate	Reagent Water	0.1 M Phosphate Buffer	5 mL 0.3M Formic Acid
Equilibrate	Reagent Water	Reagent Water	n/a
Load	250mL Sample	100 or 250mL Sample	50 mL Solids & Tissue 100 mL Leachate 500+ mL Aqueous
Wash	Reagent Water	1 g/L NH ₄ OAc in Reagent Water	Reagent Water
Wash	Reagent Water	Methanol	Reagent Water
Wash	n/a	n/a	1:1 (v:v) 0.1M formic acid/MeOH
Dry	Under vacuum for 5 min	Under vacuum for 5 min	Under vacuum for 15 sec.
Elute	Methanol	NH ₄ OH/Methanol (2%, v/v)	1% NH ₄ OH in MeOH
Elute	Methanol	NH ₄ OH/Methanol (2%, v/v)	n/a

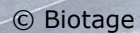
Dual Sorbent Cartridge SPE (cSPE) Evaluation

Tilapia/Salmon Recoveries of EIS

EIS Recovery of Mixed Tilapia/Salmon Fish Samples Using Various SPE Cartridges

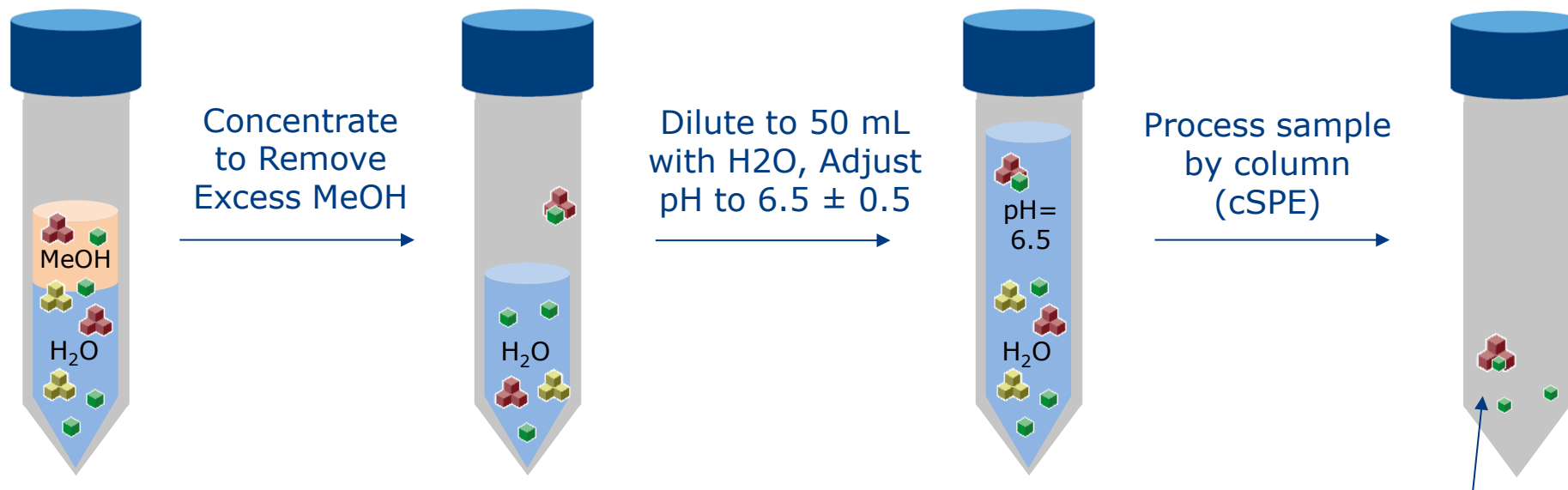


No Post-cSPE Concentration 5 mL Final Volume



Loss of Neutral PFAS

Non-Specific Binding



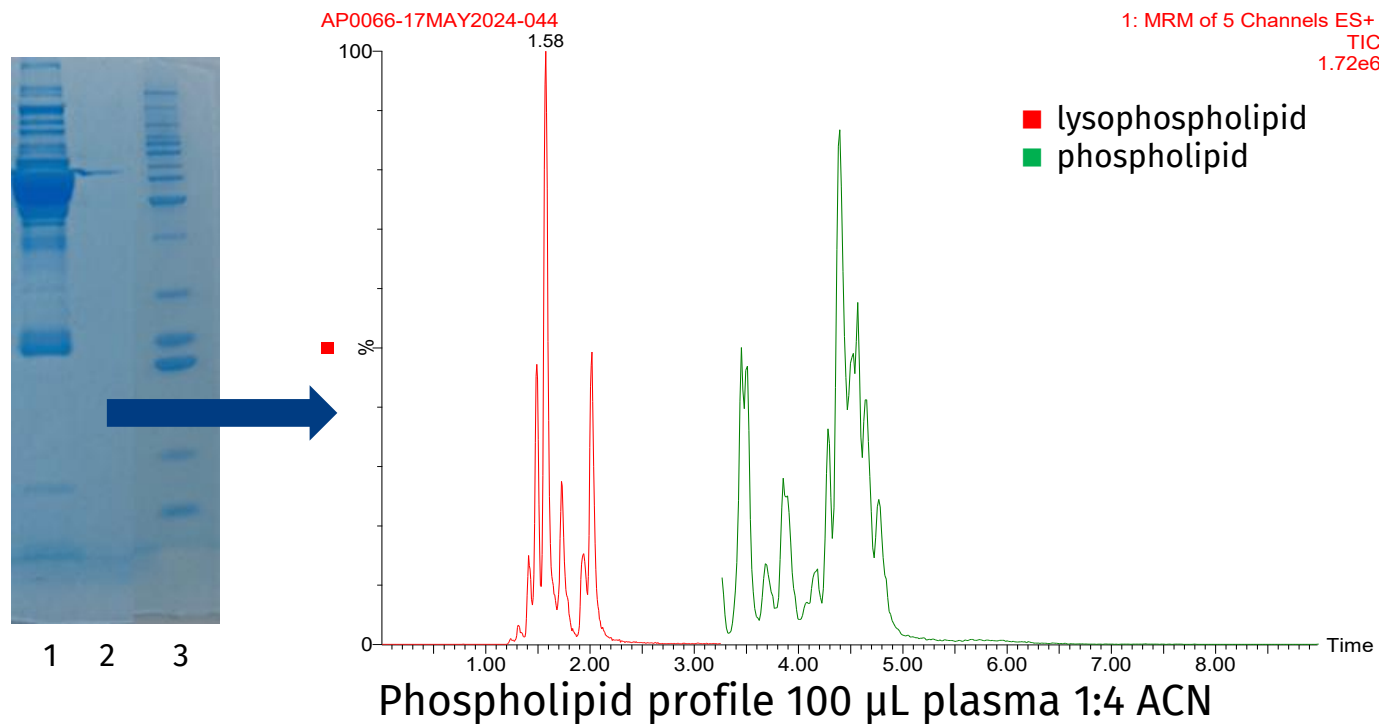
Neutral PFAS stuck to walls of tube or bound to sample components (fats, lipids, ect.)

Challenges with PFAS Analysis in Biological Matrices

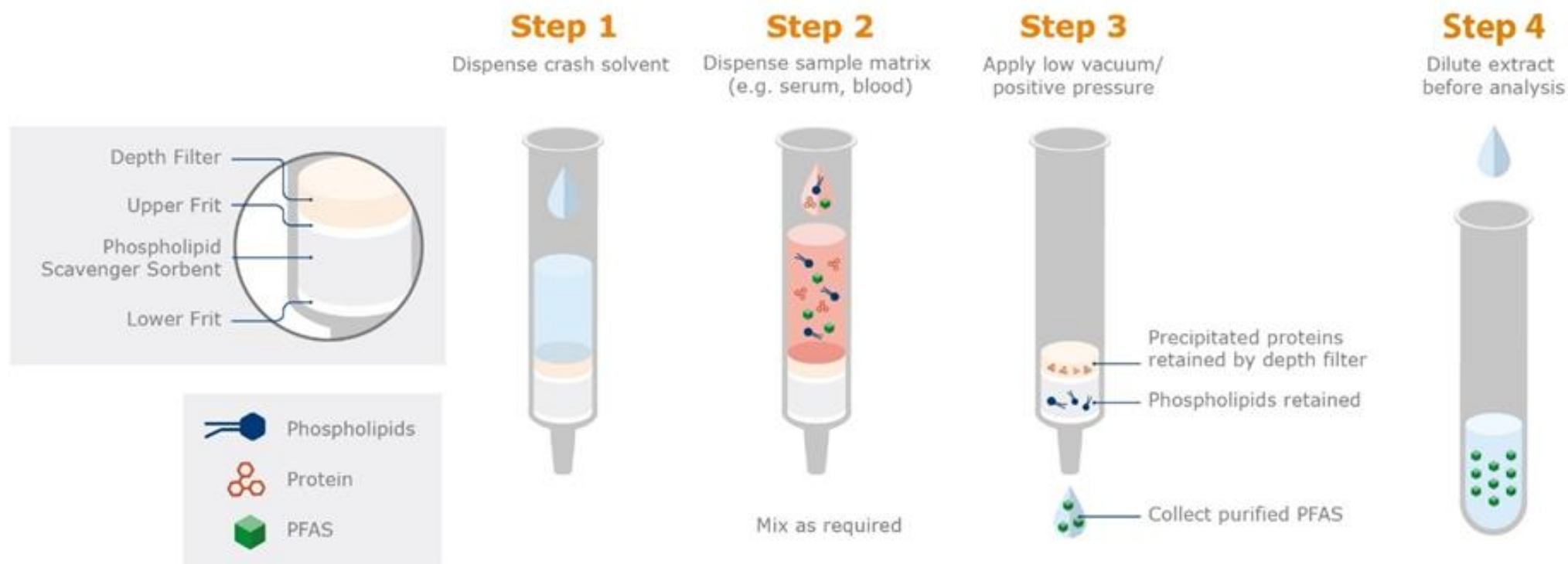
- Protein removal leaves issues with phospholipids

SDS-PAGE:

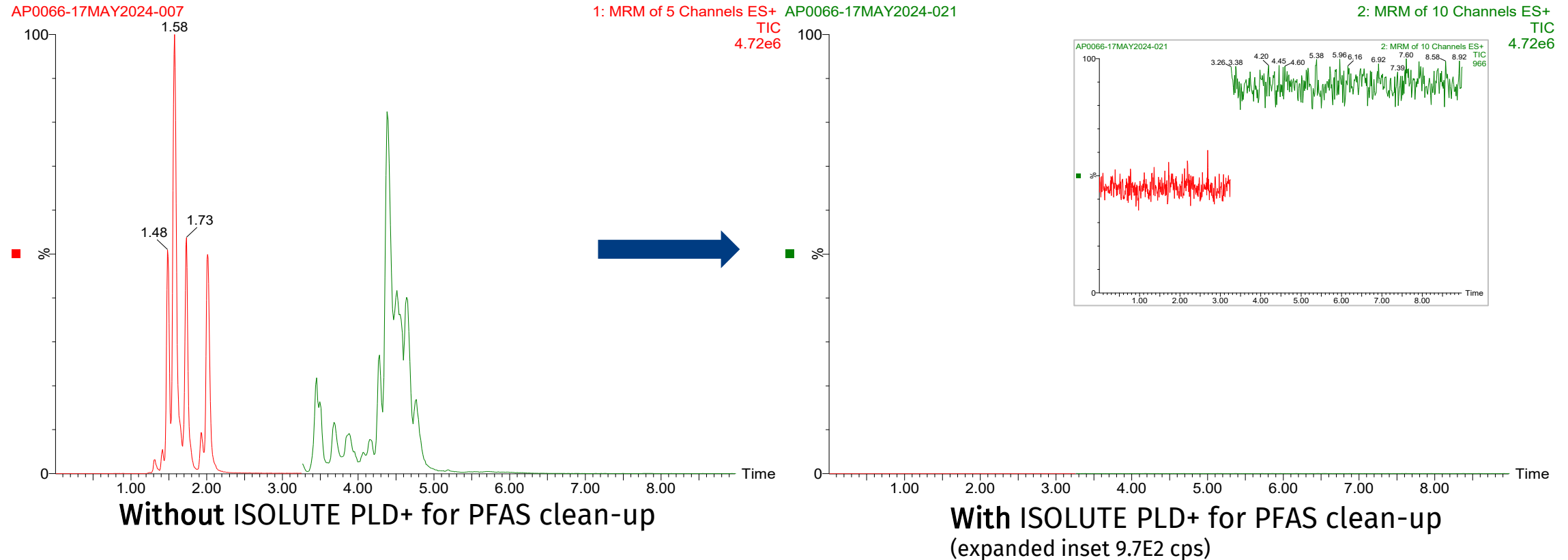
1. raw plasma, 0.16 μL load
2. 100 μL plasma ppt 1:4 ACN, 2.5 μL load
3. 15-220 kDa ladder



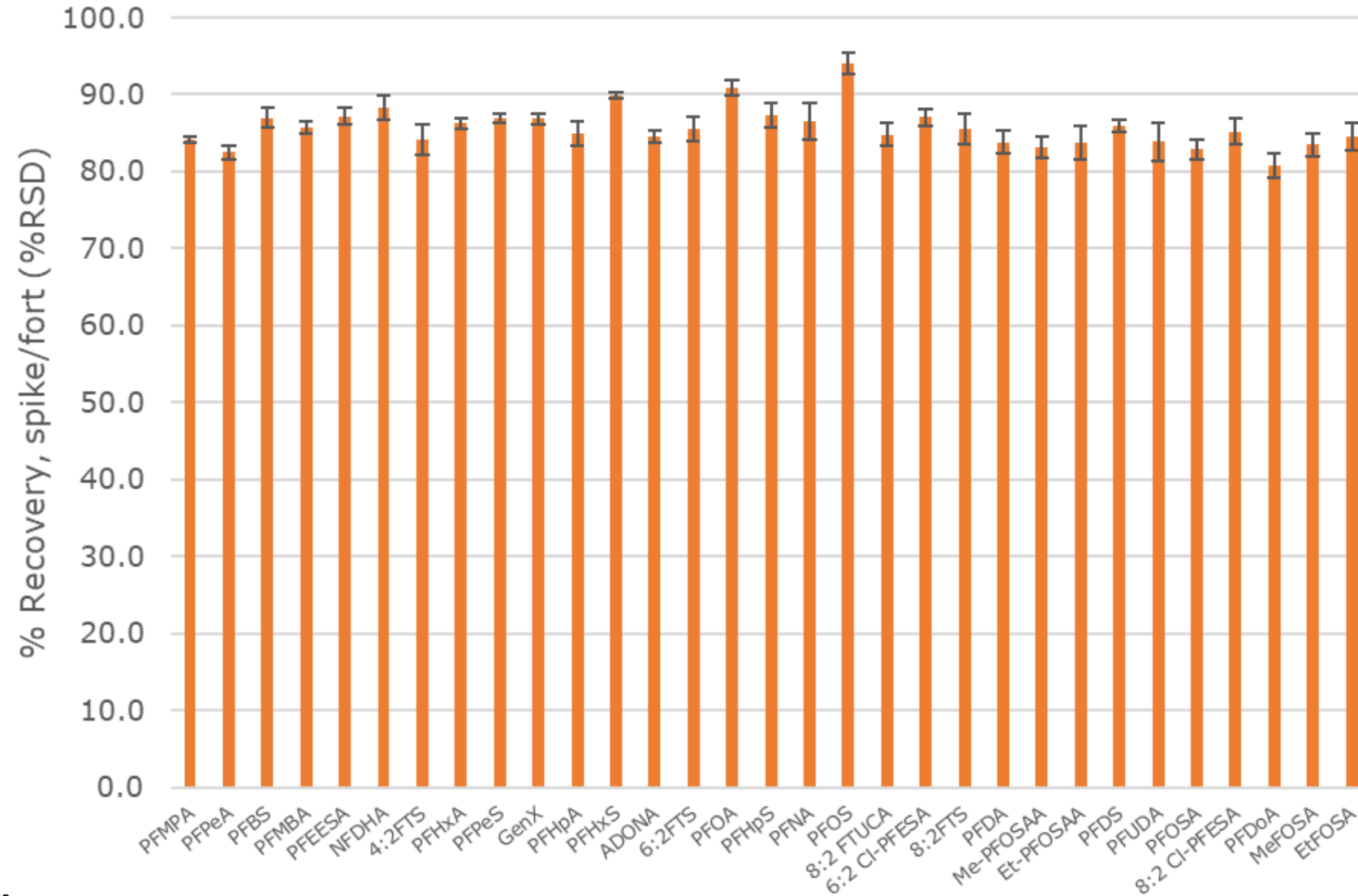
Simple, flow-through extraction mechanism



Reduction of Phospholipids >99.9%



PFAS Recovery ISOLUTE® PLD+ for PFAS



Serum sample, (100 μ L)
1.6 ng/mL spike, n=6

Final Conclusions

Troubleshooting SPE Chemistry for EPA Methodology

- EPA PFAS Testing Milestones & Sample Preparation Workflow
- DVB particle size influences extract composition and peak asymmetry in EPA 537.1
- Acceptable extraction performance for EPA 533 target list can be achieved with 200mg & 500mg WAX(30µm) bed masses.
- Comparisons between WAX/GCB stacked and blended media highlight performance for various matrices and suggest loss of neutral PFAS is likely attributed to non-specific binding to extraction equipment or tissue matrix components for EPA Method 1633
- ISOLUTE[®] PLD+ technique simplifies processing matrices with high protein & lipid content, while also addressing common challenges for low EIS recoveries

Thank You

