Comparing Extraction Protocols, Analytical Requirements and Results from Methods Utilized to Test for PFAS in Aqueous Samples

EPA 537.1, EPA 533, ISO 21675

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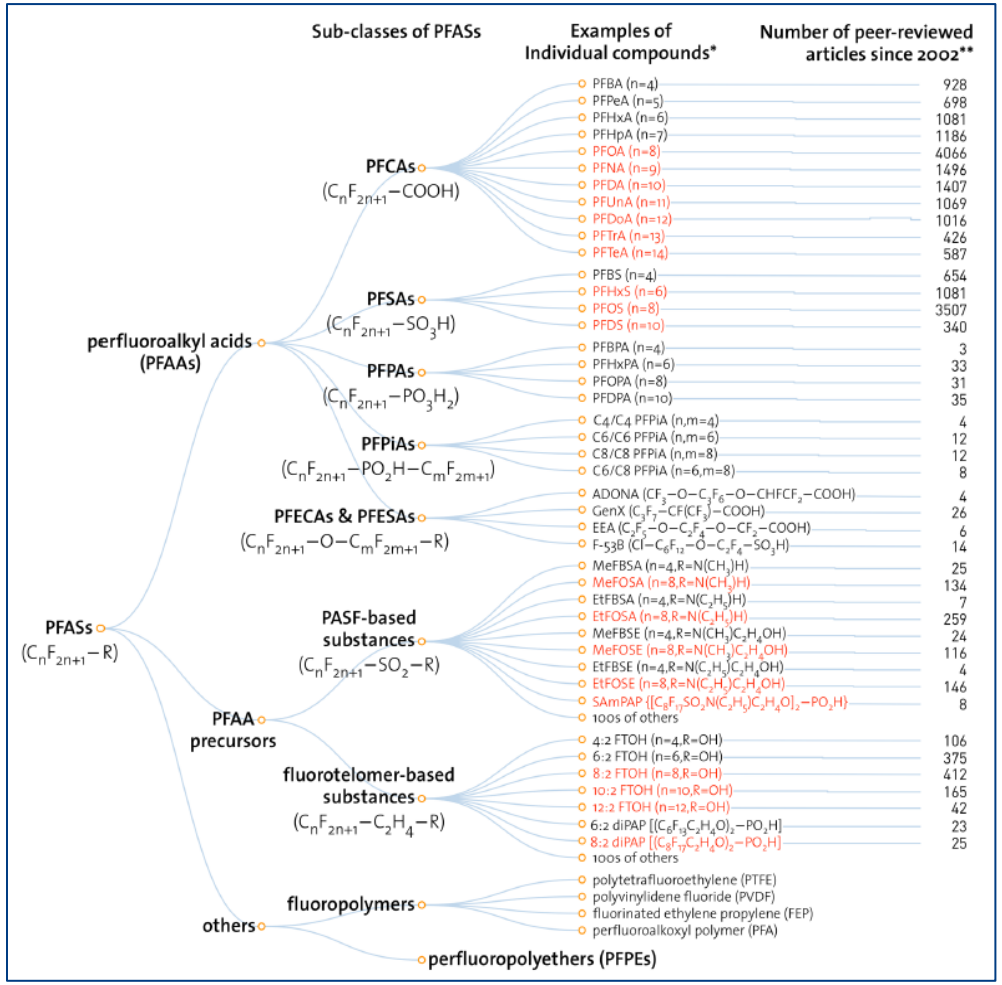
Outline

- The Problem of PFAS
- Navigating Analytical Methods
- Evaluation of Analytical Results
- Summary
- The Future of PFAS Testing

What are PFAS?



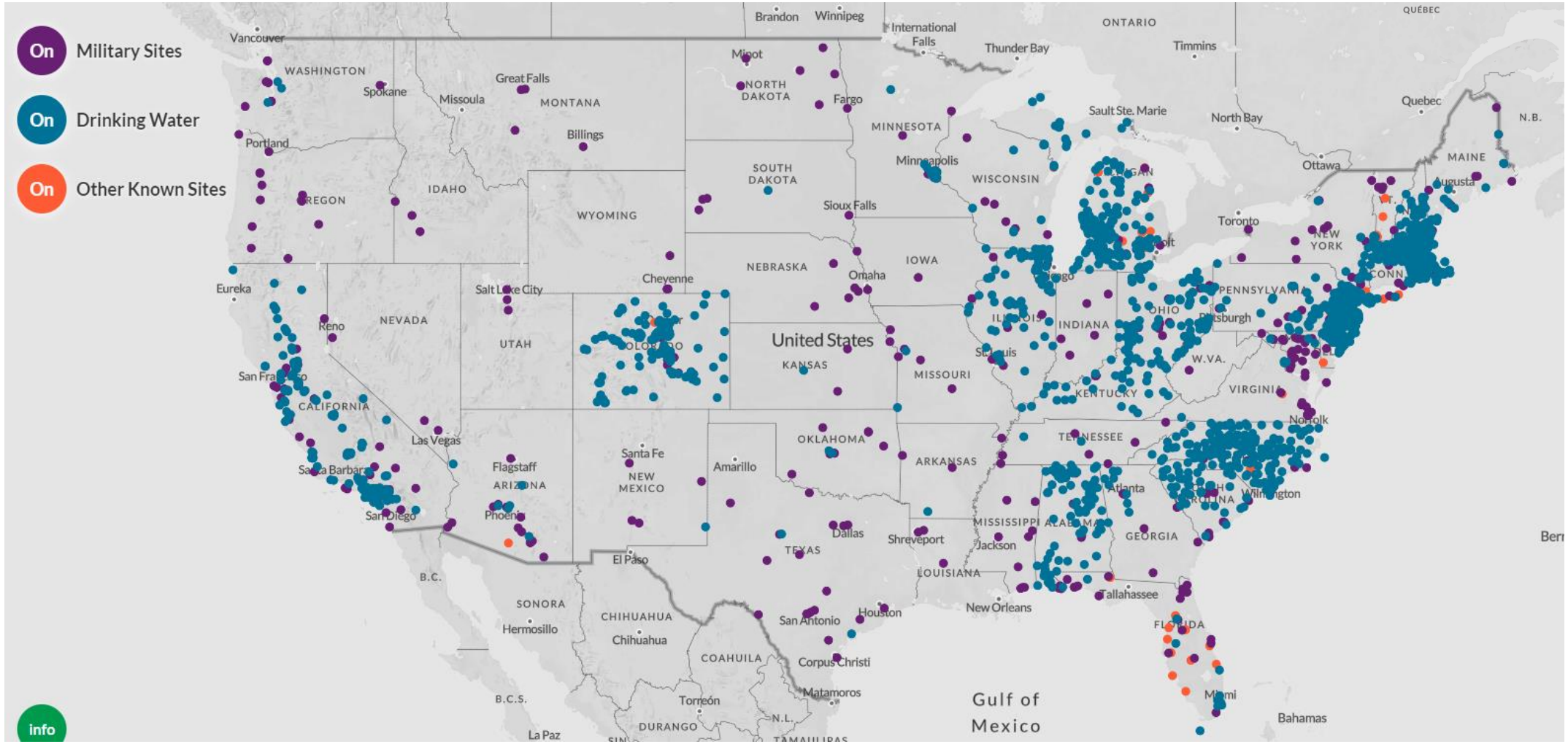
DOI: 10.1021/acs.est.6b04806 Environ. Sci. Technol. 2017, 51, 2508−2518 Wang et. Al A Never-Ending Story of Per- and Polyfluoroalkyl Substances (PFASs)?

PFAS = Per- and polyfluoroalkyl substances are part of a broader class of polyfluorinated compounds (PFCs)

- Resistant to degradation
- Bioaccumulative
- Linked to Health Hazards
  - PFOA and PFOS likely Carcinogenic

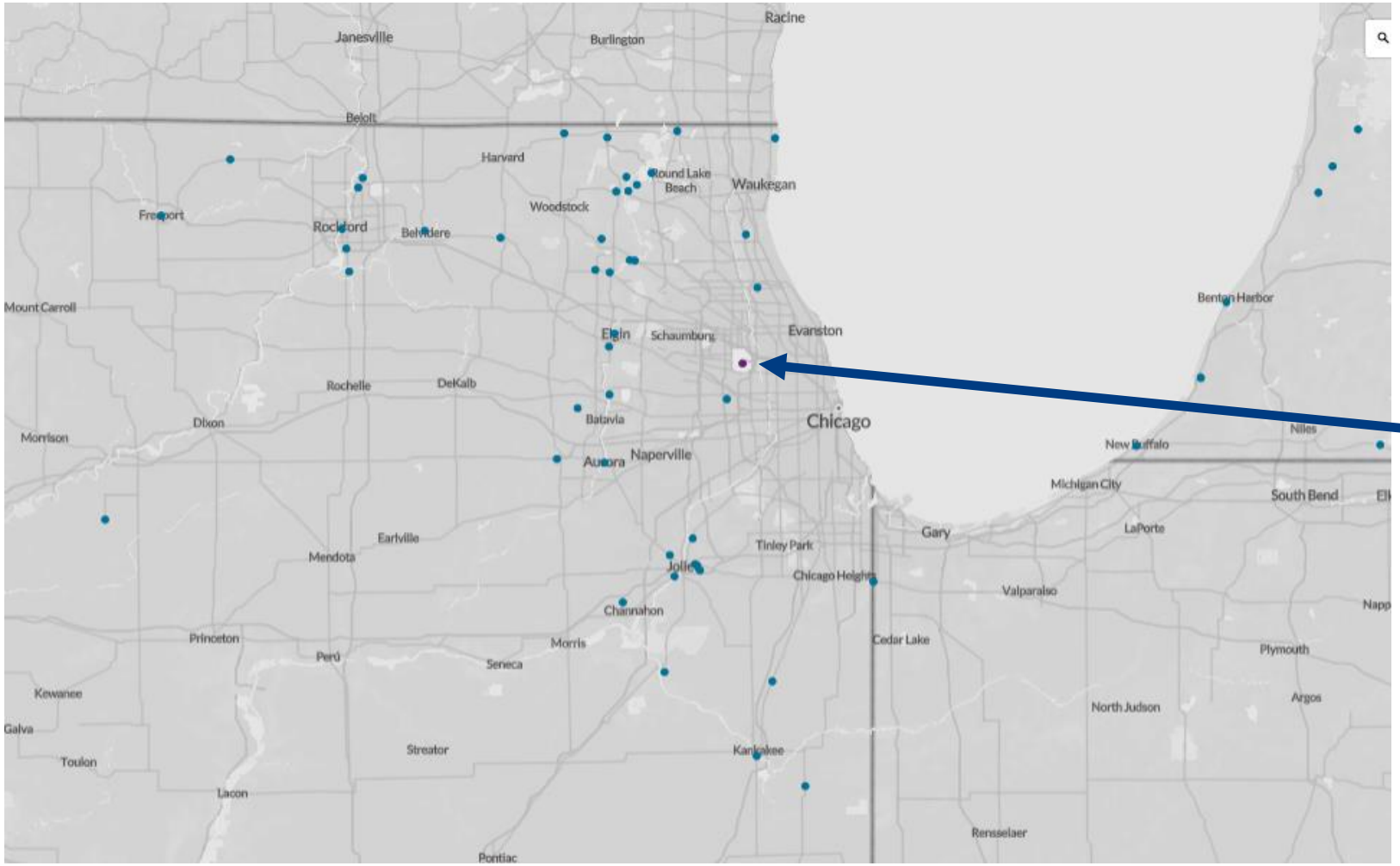
PFAS Contamination in the U.S.

Biotage



https://www.ewg.org/interactive-maps/pfas_contamination/map/

PFAS Contamination in Chicago-Land Area



https://www.ewg.org/interactive-maps/pfas_contamination/map/

Test Results:

PFA	Location detected	Maximum Level (ppt) Years tested
PFOS	Groundwater On-base	1,520 2018-
PFOA	Groundwater On-base	13,600 2018-
PFOS+PFOA	Groundwater On-base	13,800 2018-
PFBS	Groundwater On-base	2,600 2018-
NETFOSAA	Groundwater On-base	0.0 2018-
NMEFOSAA	Groundwater On-base	0.0 2018-
PFDA	Groundwater On-base	0.0 2018-
PFDoA	Groundwater On-base	0.0 2018-
PFHpA	Groundwater On-base	1,480 2018-
PFHxS	Groundwater On-base	10,500 2018-
PFHxA	Groundwater On-base	14,400 2018-
PFNA	Groundwater On-base	10 2018-
PFTeDA	Groundwater On-base	0.0 2018-
PFTrDA	Groundwater On-base	0.0 2018-
PFUnA	Groundwater On-base	0.0 2018-

Detection > 10,000ppt

Testing for PFAS

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	US EPA 537.1	US EPA 533	ISO 21675:2019
Applicable Matrices			
Drinking Water	X	X	X
Groundwater			X
Surface Water			X
Wastewater			<2 g/L SPM
Target Analytes			
Number of Target PFAS	18	25	30
Number of Labeled PFAS	7	19	31
Sample Volumes			
Sampling			
Volume	250 mL	100-250 mL	<=1000 mL
Extraction			
Technique	SPE	SPE	SPE
Format	Tube	Tube	Tube or Disk
Media	SDVB	WAX	WAX
Bed Mass	500 mg	200-500 mg	50-1000 mg
Loading Rate	10-15 mL/min	5 mL/min	3-6 mL/min
Evaporation			
Style	Water Bath	Water Bath	Blowdown
Sparge Gas	N_2	N_2	N_2
Reconstitution	Yes	Yes	No
Analysis			
Analytical			
System	LC-MS/MS	LC-MS/MS	LC-MS/MS
Column	2.1 x 150 mm C18	2 x 50 mm C18	C18 (Various)
Delay Column	Not Specified	Yes	Not Specified
Scan Type	MRM	MRM	MRM
Quantification Method	Internal Standard Correction	Isotope Dilution	Internal Standard Correction

Sample Matrices & Volumes

Matrix	EPA 537.1	EPA 533	ISO 21675
Drinking Water	X	X	X
Groundwater			X
Surface Water			X
Wastewater			X

← Drinking Water

Volume	EPA 537.1	EPA 533	ISO 21675
100mL		X	X
250mL	X	X	X
1000mL			X

← 250mL

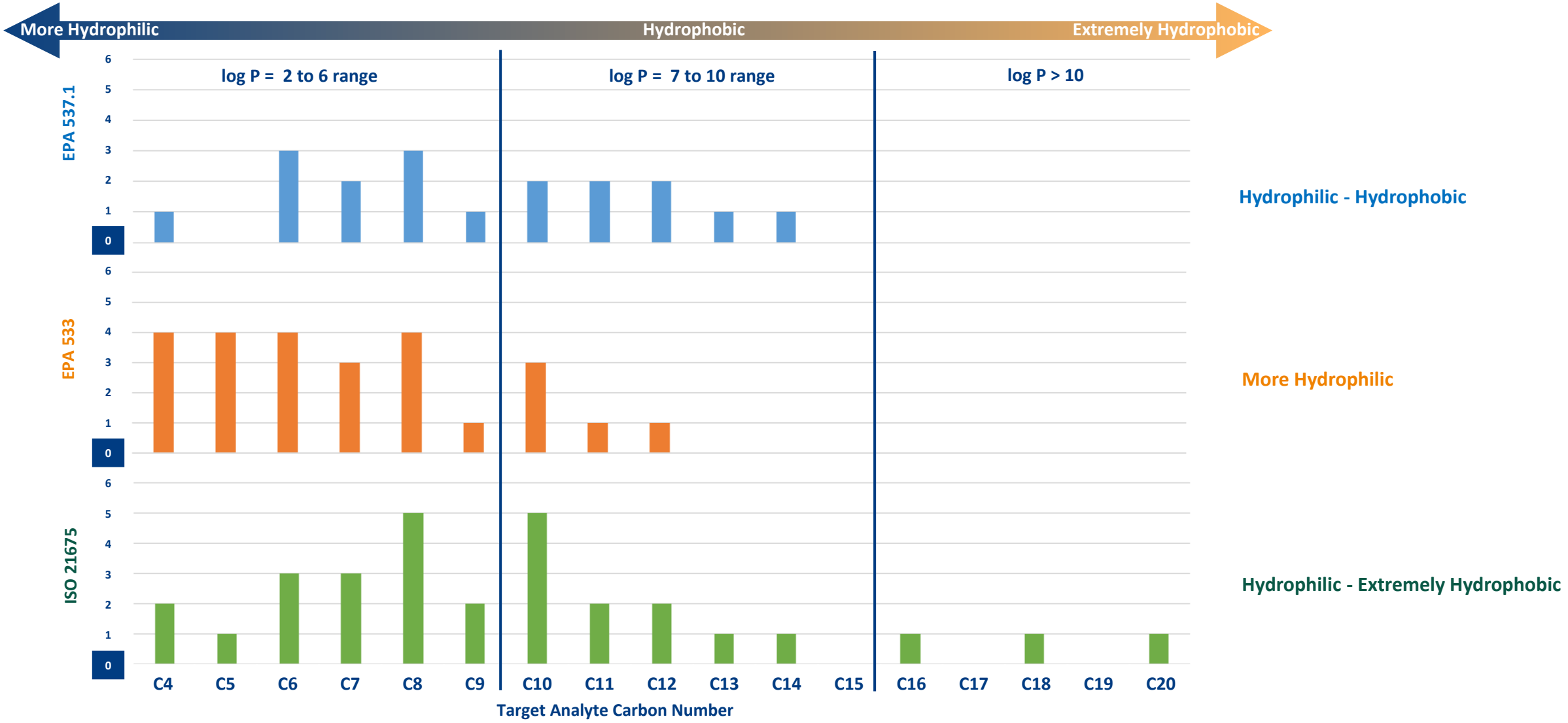
Target Analytes

Abbreviation	EPA 537.1	EPA 533	ISO 21675
PFBS	X	X	X
PFDA	X	X	X
PFDoA	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	
NEtFOSAA	X		X
NMeFOSAA	X		X
PFTA	X		X
PFTrDA	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	ISO 21675
PFPeS		X	
4:2 FTS		X	
NFDHA		X	
PFEESA		X	
PFMPA		X	
PFMBA		X	
PFDS			X
N-EtFOSA			X
FOSA			X
N-MeFOSA			X
PFOcDA			X
PFHxDA			X
8:2 FTUCA			X
8:2 diPAP			X
Total	**18**	**25**	**30**

37 Unique Target PFAS Analytes

Target Analytes Water Solubility



Hydrophilic - Hydrophobic

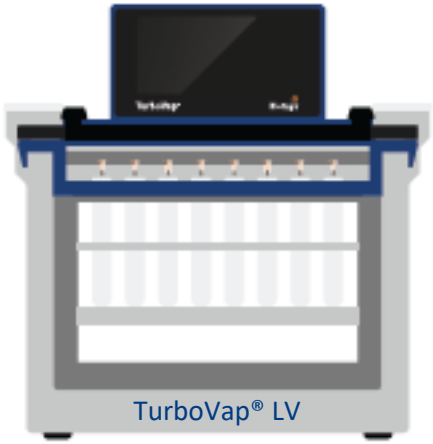
More Hydrophilic

Hydrophilic - Extremely Hydrophobic

Extraction & Evaporation

Extraction	EPA 537.1	EPA 533	ISO 21675
Technique	SPE	SPE	SPE
Format	Tube	Tube	Tube or Disk
Media	SDVB	WAX	WAX
Bed Mass	500mg	200 or 500mg	50-1000mg
Loading Rate	10-15 mL/min	5 mL/min	3-6 mL/min

Evaporation	EPA 537.1	EPA 533	ISO 21675
Style	Water Bath	Water Bath	Blowdown
Sparge Gas	N2	N2	N2
Reconstitution	Yes	Yes	No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Extraction Comparison

Step	EPA 537.1	EPA 533	ISO 21675
Pre-Treat	Tris(hydroxymethyl)aminomethane	Glacial Acetic Acid	Glacial Acetic Acid
Pre-Treat	Concentrated Hydrochloric Acid	n/a	n/a
Condition	Methanol	Methanol	0.1% (v/v) NH4OH in Methanol
Condition	n/a	0.1 M Phosphate Buffer	Methanol
Equilibrate	Reagent Water	0.1 M Phosphate Buffer	Reagent Water
Equilibrate	Reagent Water	Reagent Water	n/a
Load	250mL Sample	250mL Sample	250mL Sample
Wash	Reagent Water	1 g/L NH4OAc in Reagent Water	Acetate Buffer
Wash	Reagent Water	n/a	Reagent Water
Dry	Under vacuum for 5 min	Under vacuum for 5 min	Under vacuum for 5 min.
Elute	Methanol	NH4OH/Methanol (2%, v/v)*	Methanol
Elute	Methanol	NH4OH/Methanol (2%, v/v)*	0.1% (v/v) NH4OH in Methanol
Evaporate	Bring to dryness.	Bring to dryness.	Bring to 1 mL
Reconstitute	Methanol/Water (96/4, v/v)	Methanol/Water (20%, v/v)	n/a
Analyze	LC-MS/MS	LC-MS/MS	LC-MS/MS

← Removes PFAS contamination from SPE

← Basic Methanol not required for 537.1 since method uses SDVB Media

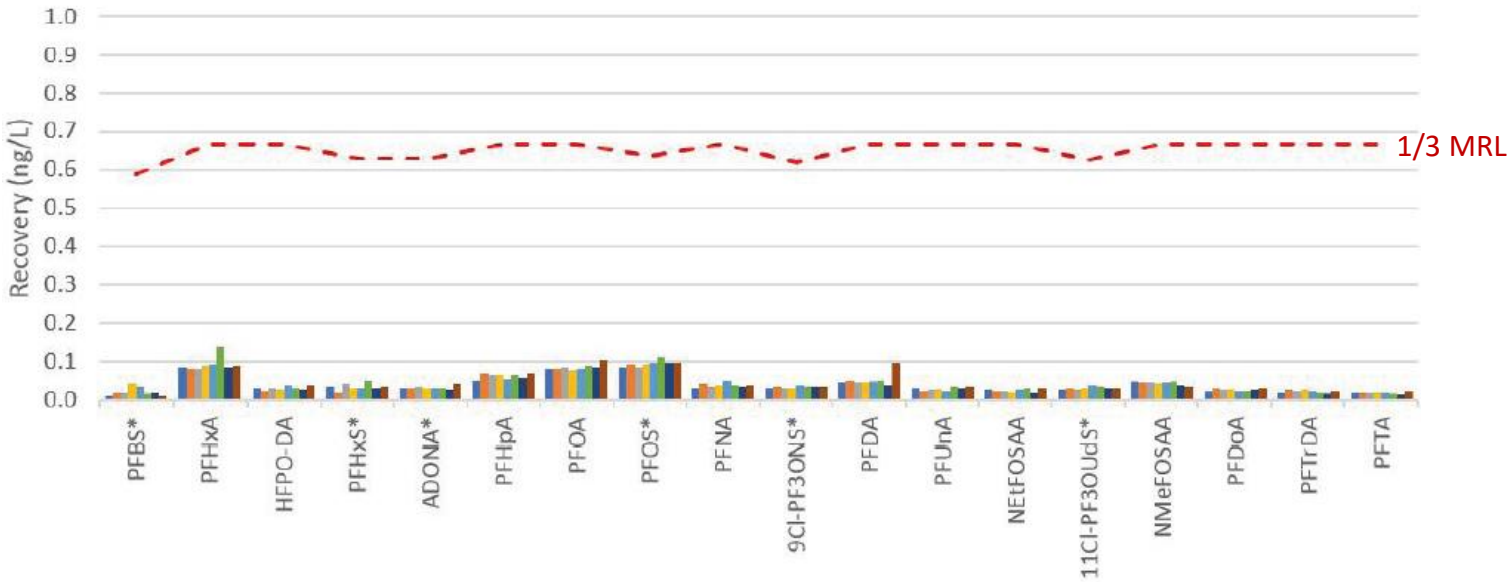
← Excessive water in final extract can cause long chain PFAS to “crash out” leading to poor recoveries

Analysis

Analytical	537.1	533	ISO 21675
System	LC-MS/MS	LC-MS/MS	LC-MS/MS
Column	2.1 x 150mm C18	2 x 50mm C18	C18 (Various)
Delay Column	Not Specified	Yes	Not Specified
Scan Type	MRM	MRM	MRM
Quantification Method	Internal Standard Correction	Isotope Dilution	Internal Standard Correction

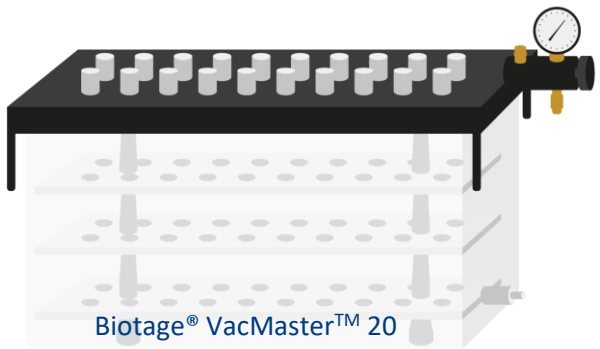
Background Levels – EPA 537.1

ISOLUTE® 101 = SDVB

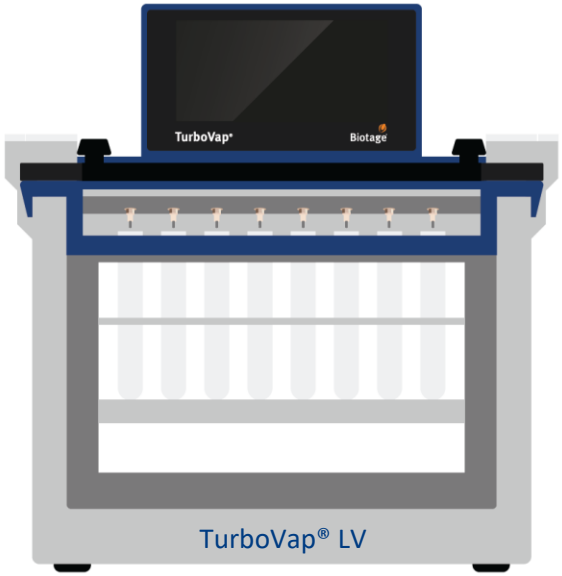


Sample Volume	250mL
Sorbent	500mg SDVB
Manifold	Biotage® VacMaster™ 20
Concentrator	TurboVap® LV

ISOLUTE® 101 = SDVB

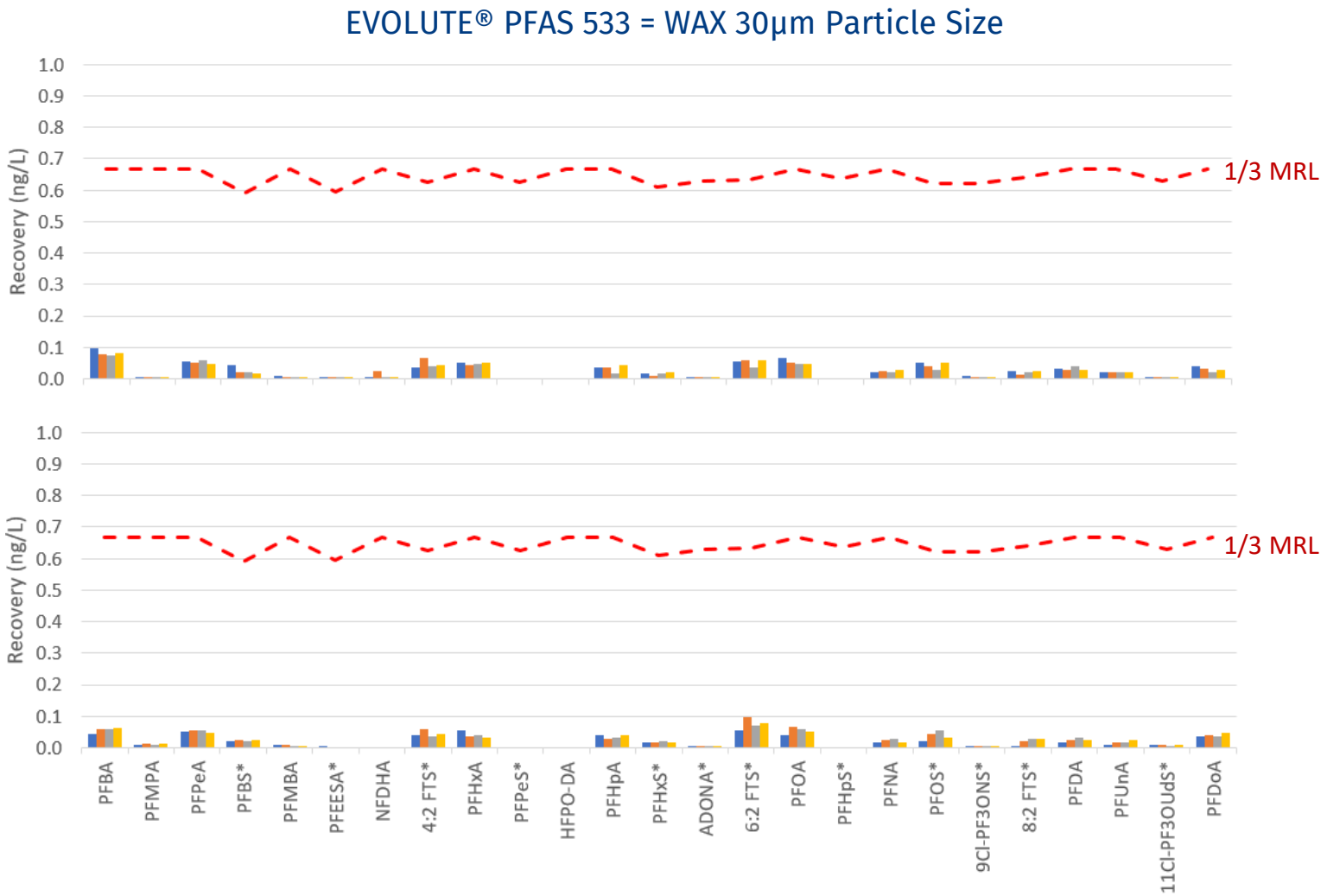


Biotage® VacMaster™ 20



TurboVap® LV

Background Levels – EPA 533

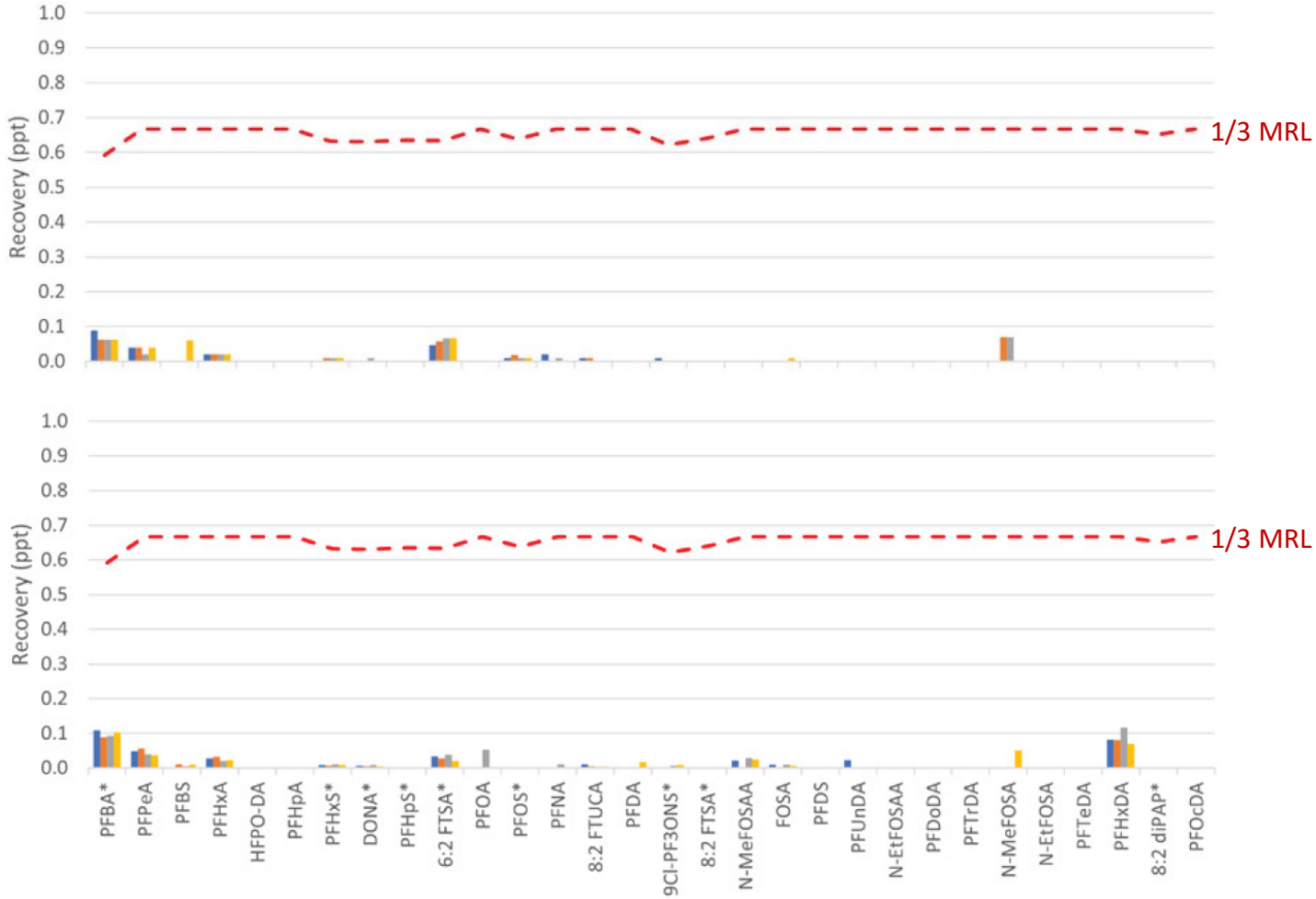


Sample Volume	250mL
Sorbent	500mg WAX
Manifold	Biotage® VacMaster™ 20
Concentrator	TurboVap® LV

Sample Volume	250mL
Sorbent	200mg WAX
Manifold	Biotage® VacMaster™ 20
Concentrator	TurboVap® LV

Background Levels – ISO 21675

EVOLUTE® PFAS = WAX 50µm Particle Size



Sample Volume	250mL
Sorbent	500mg WAX (50µm)
Manifold	Biotage® VacMaster™ 20
Concentrator	TurboVap® LV

Sample Volume	250mL
Sorbent	150mg WAX (50µm)
Manifold	Biotage® VacMaster™ 20
Concentrator	TurboVap® LV

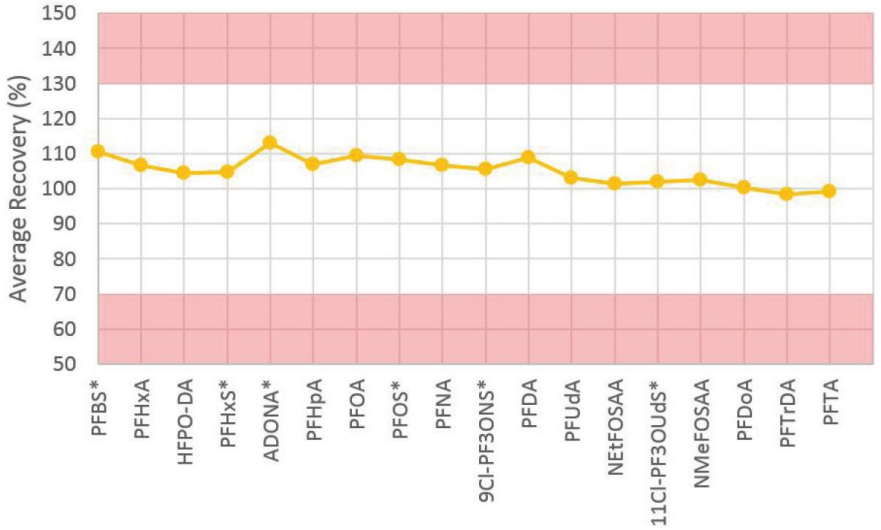
Extraction Performance – EPA 537.1

Biotage®

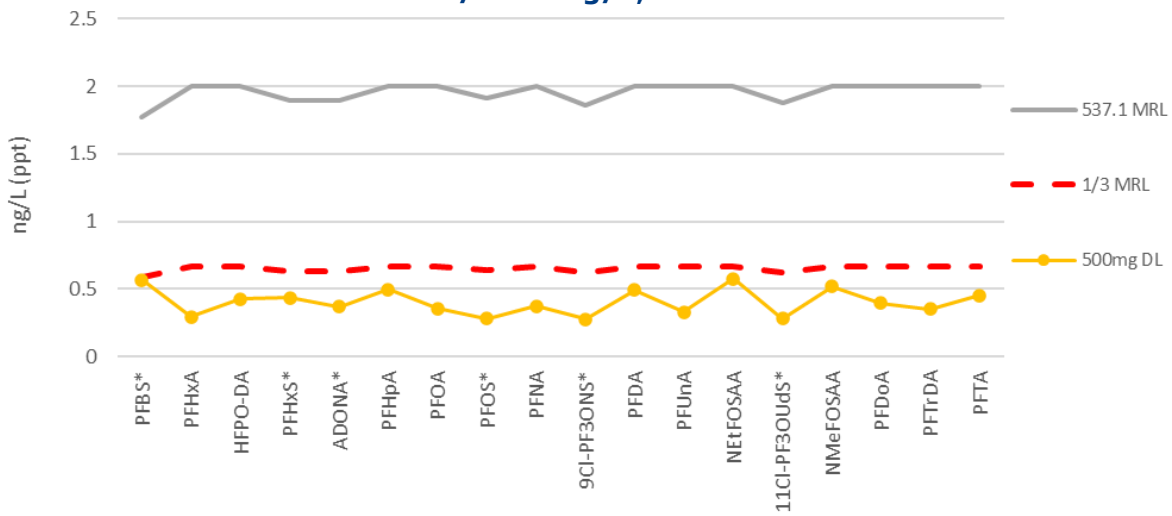
IDP: 20 ng/L, n=8

Sample Volume	250mL
Sorbent	500mg SDVB
Manifold	Biotage® VacMaster™ 20
Concentrator	TurboVap® LV

IDA: 20 ng/L, n=8

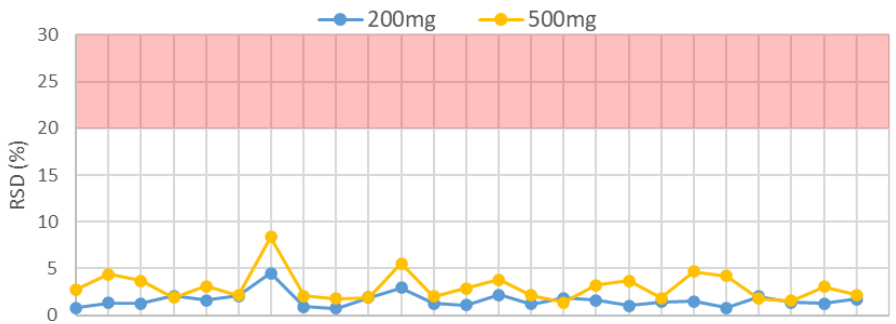


MRL/DL: 2 ng/L, n=8



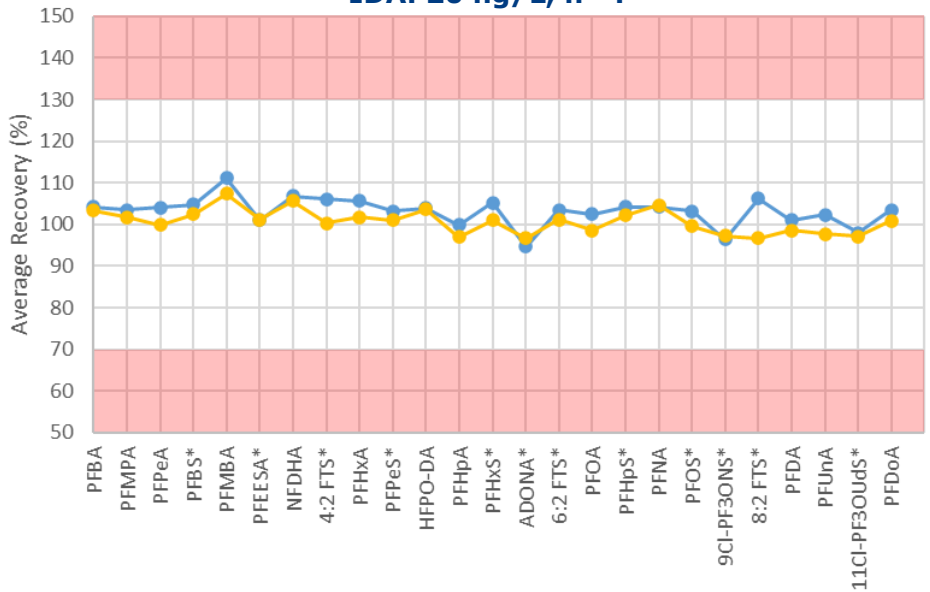
Extraction Performance – EPA 533

IDP: 20 ng/L, n=4

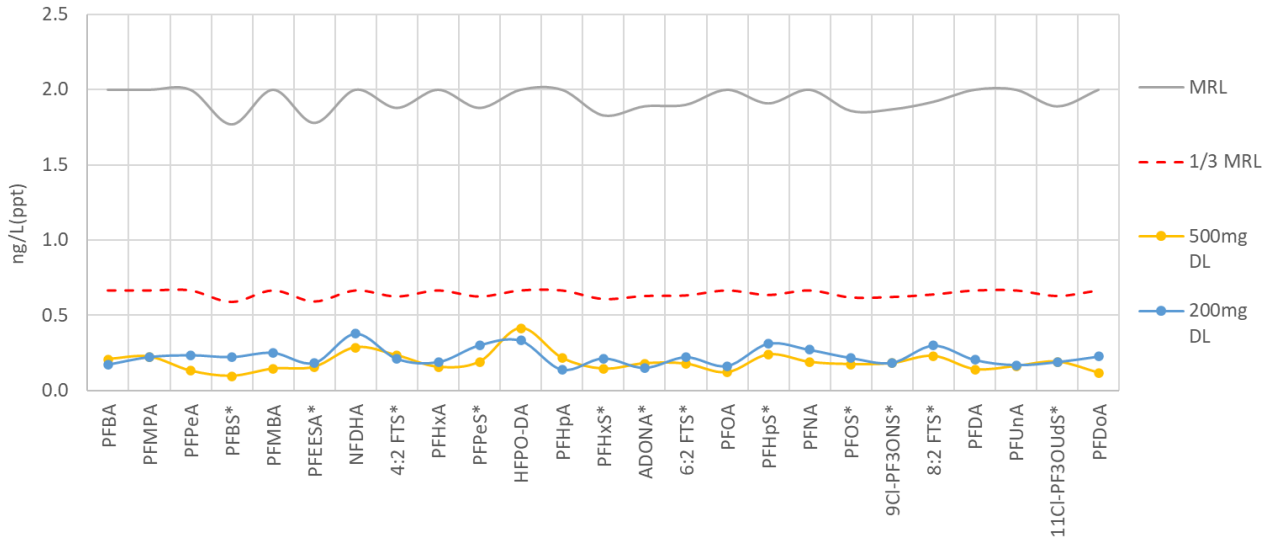


Sample Volume	250mL
Sorbent	200mg & 500mg WAX (30µm)
Manifold	Biotage® VacMaster™ 20
Concentrator	TurboVap® LV

IDA: 20 ng/L, n=4

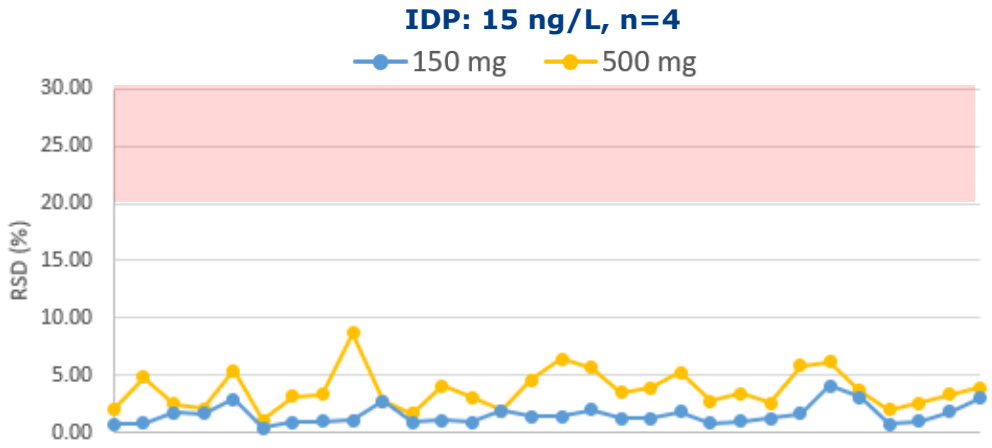


MRL/DL: 2 ng/L, n=7

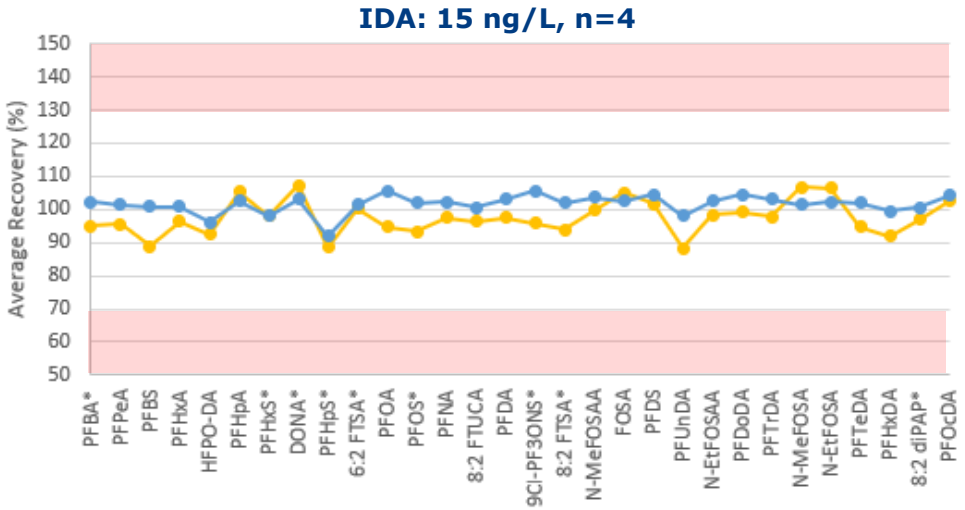


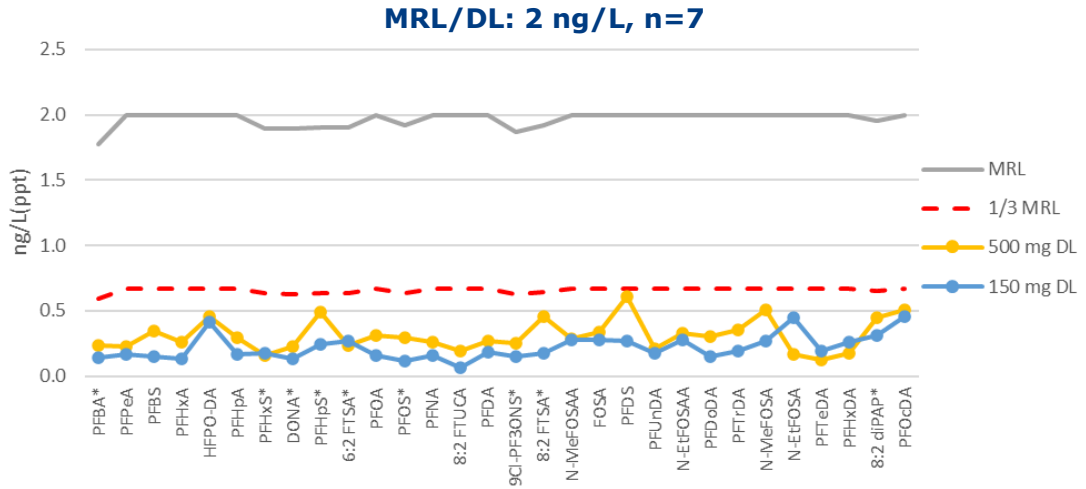
Extraction Performance – ISO 21675

Biotage®



Sample Volume	250mL
Sorbent	150 & 500mg WAX (50µm)
Manifold	Biotage® VacMaster™ 20
Concentrator	TurboVap® LV



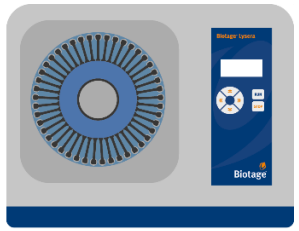


Summary

- We covered three different PFAS analytical methods
  - US EPA 537.1
  - US EPA 533
  - ISO 21675
- In total, there are 37 unique target PFAS analytes, some of them are prescribed in each method
- Understanding water solubility (logP) of each target analyte can help improve extraction performance
- For EPA 533, WAX Sorbent cleanliness is important as there is not an initial cleaning step in the extraction method, as seen in ISO 21675
- ISO 21675 does not require the extract to be concentrated to dryness and reconstituted. This can cause issues with recoveries of longer carbon chain PFAS analytes, as they may “crash out” of solution if too much residual water is in the extract.
- Acceptable extraction performance for each method can be achieved at 250mL sample volumes
- Acceptable extraction performance for EPA 533 target analyte list can be achieved with 200mg & 500mg 30µm WAX bed masses
- Acceptable extraction performance for ISO 21675 target analyte list can be achieved with 150mg & 500mg 50µm WAX bed masses

The Future of PFAS Testing

Matrix	Description	Volume/Mass	% Solids Determination	Homogenization	Liquid Solid Extraction	Concentration	Solid Phase Extraction
Aqueous	Water, sludges, and similar materials containing < 50mg solids/sample	% Solids (500mL)	X			X	X
		Analysis (125 - 250mL)					
		Leachate (100mL)					
Solid	Soils, sediments, and biosolids that contain > 50mg solids	% Solids (5 – 10g)	X	X	X	X	X
		Soil & Sediment (5g)					
		Biosolids (0.5g)					
Tissues	Whole fish, fish fillets, and other tissues	Tissue (1-2g)		X	X	X	X



Biotage® Lysera

Biotage® Extrahera™ HV-5000

Biotage® TurboVap® LV