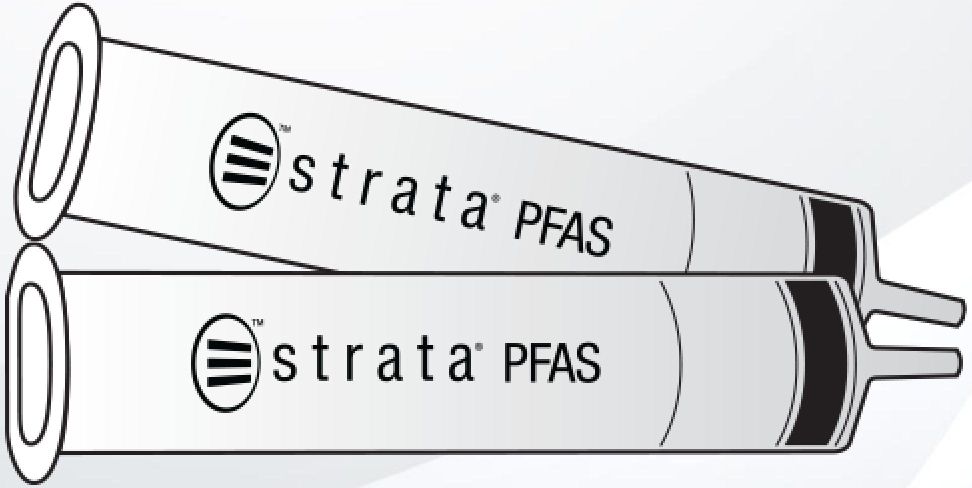
Improved Accuracy, Precision and Laboratory Productivity from Employing a Single Tube SPE-GCB Sample Preparation Configuration for the Analysis of PFAS under DOD QSM 5.3



Presented By **Sam Lodge**
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August 5, 2021

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- BRIEF History of PFAS
- Overview of Current Methodology
- SPE requirements for EPA Methods (Drinking Water)
- Non-DW matrices – DOD requirements
- Future considerations
- Conclusion

Accidental Chemicals

- Viagra
- Penicillin
- Saccharin
- Teflon

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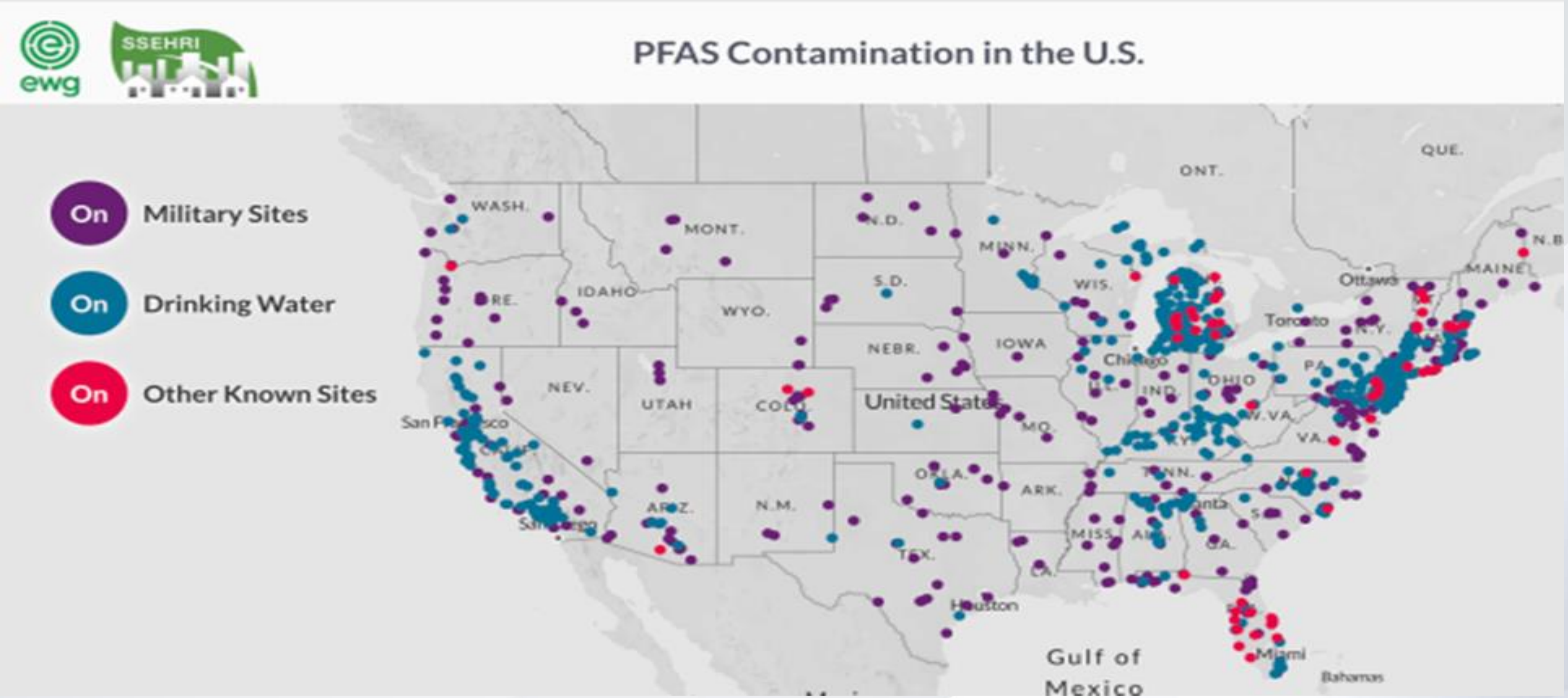
Negative impact of PFAS

- Forever chemicals
  - Biopersistent – hard to destroy (remediation)
  - Bioaccumulative/Biomagnified
  - Highly mobile
  - Toxic???

Negative Impact of PFAS

Toxicity???

- Animal studies show possible health issues
- Lack of any definitive data to link PFAS (esp. specific PFAS compounds)
- Different PFAS have different effects (see Beluga whale study)
- Short chain with shorter half life



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

https://www.pennlive.com/news/2021/02/pa-american-water-sues-manufacturer-of-toxic-pfas-forever-chemicals.html

History of PFAS Analytical methodology

- EPA 537 (2009)
- EPA 8327 (2015) - 2021
- EPA 537.1 (2018)
- EPA 533 (2020)
- DOD QSM (2019 for Ver. 5.3) – NOT A METHOD!

- No official/promulgated methods for non-DW matrices (NOT TRUE AS OF LAST WEEK!)

History of PFAS Analytical methodology

- EPA 537 (Polymer SPE)
- EPA 537.1 (SDVB SPE only)
- ASTM D7979/EPA 8327 (DAI)
- EPA 533 (WAX SPE)
- **DOD (WAX + GCB)**

- No official/promulgated methods for non-DW matrices

Solid Phase Extraction Protocol

Following the procedures of EPA Method 537.1, V2, Sections 6.9 - 6.11 and 11.3 - 11.4

Cartridge: Strata SDB-L, 500 mg/6 mL
Part No.: 8B-S014-HCH
Load: 250 mL sample that has been fortified with surrogates
Elution: 2x 3 mL Methanol
Dry Down: With Nitrogen in a heated water bath
Reconstitute: Adjust final volume to 1 mL with 96:4 Water:Methanol (v/v) and add internal standards

Method Precision and Accuracy from the Analysis of 50 Co Laboratory Control Samples (LCS)

	Mean % Recovery	Standard Deviation
13C2-PFDA	106	15.8
13C2-PFHxA	108	18.2
d5-EtFOSAA	104	19.8
13C2-HFPO-DA	104	17.1
11Cl-PF3OUdS	105	10.9
9Cl-PF3OUNS	104	11.9
ADONA	103	13.6
Et-FOSAA	111	13.8
HFPO-DA	104	15.6
Me-FOSAA	113	18.5
PFBS	104	14.7
PFDA	106	12
PFDoA	105	17.4
PFHpA	111	14.8
PFHxA	109	14.1
PFHxS	108	15.1
PFNA	109	12.7
PFOA	109	12.5
PFOS	111	13
PFTeDA	103	14.6
PFTrDA	104	13.7
PFUnA	107	13.7

Special thanks to Agustin Pierri and Weck Labs for providing this data

Sample Preparation Protocol

Pre-treatment: 100-250 mL sample is fortified with isotopically labeled analogues of the method analytes
Cartridge: Strata-X-AW 500 mg/6 mL
Part No.: 8B-S038-HCH
Load: Pass pre-treated sample through the cartridge
Wash 1: Aqueous Ammonium acetate followed by Methanol
Wash 2: Methanol
Elute: Ammonium hydroxide in Methanol
Dry Down: Under a gentle stream of Nitrogen in a heated water bath
Reconstitute: Adjust the final volume to 1 mL with 20 % Water in Methanol (v/v) before analyzing by LC-MS

Table 6.
EPA 533 Precision and Accuracy Data from a Commercial Laboratory

Analyte	MS	MSD	BS	BSD
11Cl-PF3OUdS	85 %	84 %	95 %	86 %
4-2FTS	113 %	104 %	109 %	100 %
6-2 FTS	94 %	96 %	108 %	102 %
8-2 FTS	97 %	100 %	89 %	101 %
9Cl-PF3OUdS	101 %	107 %	99 %	119 %
ADONA	118 %	116 %	111 %	99 %
HFPO-DA	100 %	97 %	110 %	101 %
NFDHA	117 %	126 %	117 %	114 %
PFBA	102 %	116 %	89 %	95 %
PFBS	117 %	106 %	97 %	105 %
PFDA	102 %	99 %	112 %	104 %
PFDoA	104 %	107 %	108 %	109 %
PFEESA	116 %	109 %	119 %	115 %
PFHpA	112 %	115 %	94 %	97 %
PFHpS	119 %	117 %	119 %	114 %
PFHxA	113 %	107 %	91 %	95 %
PFHxS	96 %	101 %	108 %	110 %
PFMBA	106 %	101 %	111 %	118 %
PFMPA	99 %	100 %	108 %	117 %
PFNA	107 %	104 %	105 %	110 %
PFOA	101 %	104 %	101 %	100 %
PFOS	117 %	115 %	108 %	108 %
PFPeA	97 %	96 %	92 %	88 %
PFPeS	86 %	99 %	103 %	104 %
PFUnA	105 %	103 %	115 %	113 %

Special thanks to Agustin Pierri and Weck Labs for providing this data

Table B-15. Per- and Polyfluoroalkyl Substances (PFAS) Using Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS) With Isotope Dilution or Internal Standard Quantification in Matrices Other Than Drinking Water

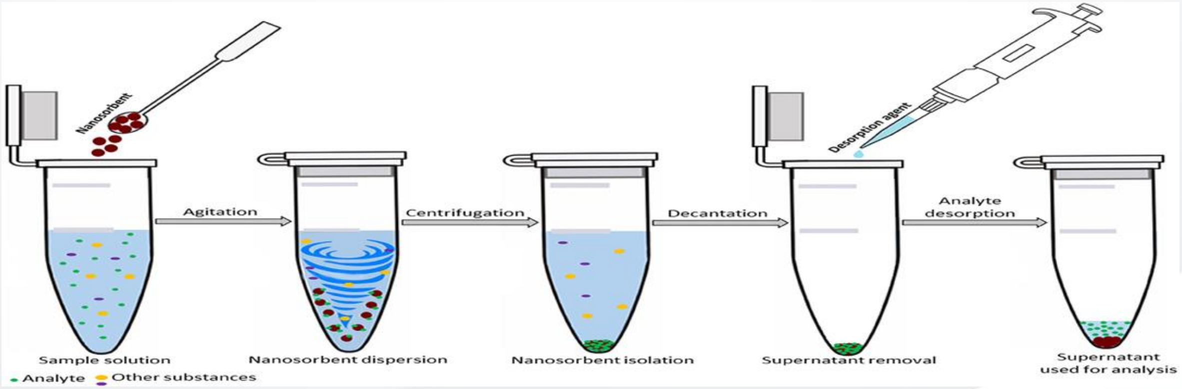
QC Check	Minimum Frequency	Acceptance Criteria	Corrective Action	Flagging Criteria	Comments
AFFF and AFFF Mixture Samples Preparation	Each sample and associated batch QC samples.	Each field sample must be prepared in duplicate (equivalent to matrix duplicate). Serial dilutions must be performed to achieve the lowest LOQ possible for each analyte.	NA.	NA.	Adsorption onto bottle is negligible compared to sample concentration so subsampling is allowed. Multiple dilutions will most likely have to be reported in order to achieve the lowest LOQ possible for each analyte.
Sample Cleanup Procedure	Each sample and associated batch QC samples. Not applicable to AFFF and AFFF Mixture Samples.	ENVI-Carb™ or equivalent must be used on each sample and batch QC sample.	NA.	Flagging is not appropriate.	Cleanup should reduce bias from matrix interferences.

GCB in PFAS sample prep

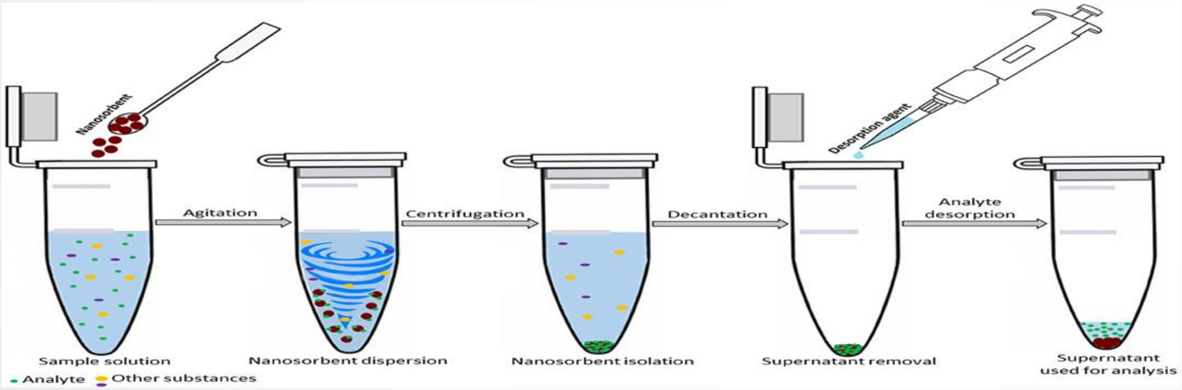
- Only for non-DW matrices
- Removes Organic Acids that can suppress ionization (TDCA and PFOS)
- Table B-15 provides for equivalent to Envi CarbTM
- Can actually improve recovery of long chain/neutral compounds (depends on format)
- Potential loss of analytes unless GCB is re-extracted
- Several ways to skin a cat!

GCB for QSM 5.3, Table B-15

dSPE with Loose GCB (10mg-100mg)



Downside of dSPE



- Time – additional 45-60 minutes per batch of samples
- Precision – more sample manipulation = more variability
- Messy – Loose GCB requires use of PPE and ventilation
- Recovery – potential loss of long chain compounds (unless re-extract the GCB)
- Potential clogging of LC or MS from contamination with GCB from extract

Table 1: Recovery Comparisons of WAX SPE and dSPE using GCB vs Strata PFAS Single Cartridge Method

Analyte	WAX SPE +dSPE GCB % Recovery	Strata PFAS Stacked Cartridge % Recovery
13C2-PFDoDA	77	84.5
13C2-PFTeDA	62	84.0
PFODA	38	78.3
PFHxDA	63	89.3

Special thanks to Eurofins Lancaster Labs for providing this data

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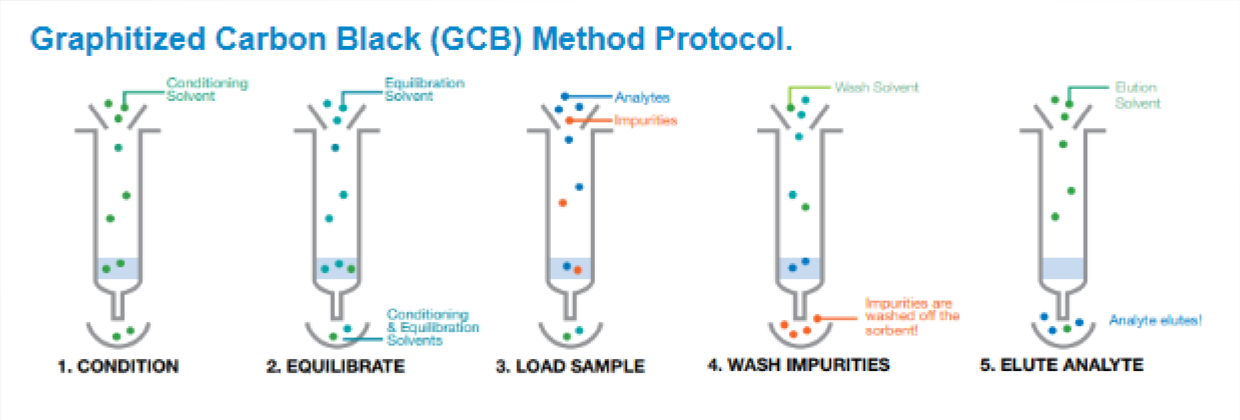
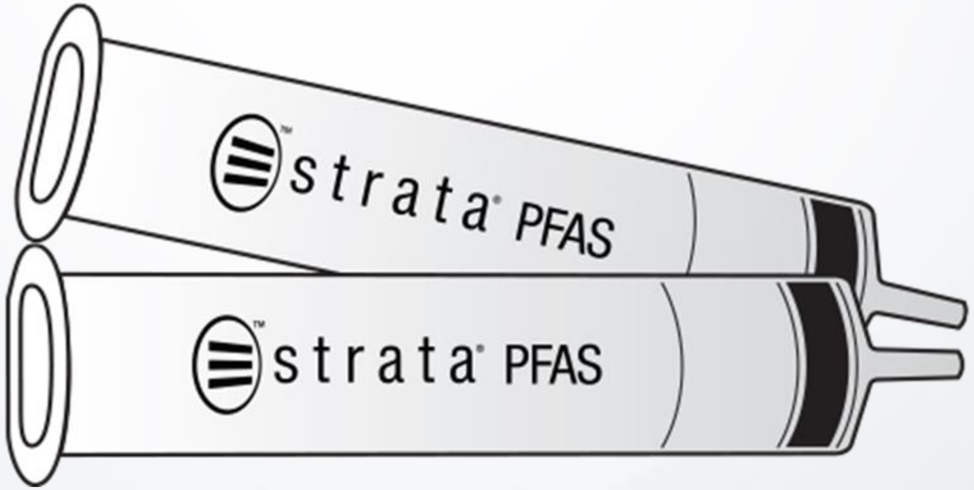
Downside of separate GCB tubes



- Additional Time – approximately 15 minutes per batch of samples
- Cost – solvent and tube ($5-7 per sample)

Incorporate WAX and GCB in a single SPE tube!



- 200mg WAX/50mg GCB
- 500mg WAX/50mg GCB

	dSPE			Stacked Tubes CS0-9207		
Analyte	Spk Conc. (ng/L)	% Rec	RSD	Spk Conc. (ng/L)	% Rec	RSD
10:2 FTS	50.00	80%	6.2%	20.00	94%	11.5%
11Cl-PF3OUdS	50.00	85%	23.6%	20.00	93%	7.0%
3:3 FTCA	50.00	89%	11.6%	20.00	86%	4.2%
4:2 FTS	50.00	100%	2.9%	20.00	103%	2.9%
5:3 FTCA	50.00	86%	0.6%	20.00	94%	3.0%
6:2 FTS	50.00	98%	5.0%	20.00	109%	4.5%
7:3 FTCA	50.00	79%	12.6%	20.00	90%	5.3%
8:2 FTS	50.00	97%	5.8%	20.00	105%	3.4%
9Cl-PF3ONS	50.00	94%	18.6%	20.00	95%	5.9%
ADONA	50.00	99%	3.2%	20.00	100%	3.4%
EtFOSA	50.00	109%	9.6%	20.00	104%	11.3%
EtFOSE	50.00	92%	11.8%	20.00	92%	7.1%
HFPO-DA	50.00	110%	6.9%	20.00	102%	9.9%
MeFOSA	50.00	108%	6.6%	20.00	102%	16.7%
MeFOSE	50.00	93%	11.2%	20.00	109%	8.4%
N-EtFOSAA	50.00	96%	6.1%	20.00	101%	11.2%
N-MeFOSAA	50.00	103%	9.7%	20.00	99%	12.1%
PFBA	50.00	96%	1.4%	20.00	96%	0.6%
PFBS	50.00	97%	3.2%	20.00	98%	4.7%
PFDA	50.00	101%	4.3%	20.00	97%	6.1%
PFDoDA	50.00	100%	1.7%	20.00	98%	3.6%
PFDS	50.00	85%	21.5%	20.00	96%	6.9%
PFHpA	50.00	99%	2.8%	20.00	97%	3.2%
PFHpS	50.00	102%	1.9%	20.00	92%	6.1%
PFHxA	50.00	96%	2.3%	20.00	100%	5.4%
PFHxDA	50.00	73%	15.6%	20.00	97%	1.0%
PFHxS	50.00	97%	0.8%	20.00	95%	7.3%
PFNS	50.00	97%	10.5%	20.00	95%	3.7%
PFOA	50.00	106%	8.0%	20.00	101%	3.8%
PFOcDA	50.00	32%	23.8%	20.00	87%	2.5%
PFOS	50.00	96%	12.5%	20.00	98%	5.0%
PFPeA	50.00	96%	3.6%	20.00	98%	4.0%
PFPeS	50.00	95%	4.2%	20.00	95%	5.7%
PFTeDA	50.00	100%	2.6%	20.00	100%	4.2%
PFTrDA	50.00	96%	12.5%	20.00	94%	2.2%
PFUndA	50.00	104%	5.9%	20.00	97%	0.8%
Average		94%	8%		98%	6%

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Table 2: Recovery of QSM 5.3 Target Analytes from a Laboratory Control Sample Using Strata PFAS SPE (WAX/GCB)

Analyte	Actual Concentration	Sample Result	% Recovery	Method Recommendation Limits	Pass/Fail
PFBA	25.600	22.640	88	84-135	Pass
PFPeA	25.600	22.157	87	75-138	Pass
PFBS	22.640	22.300	99	81-133	Pass
4:2-FTS	23.920	22.078	92	64-134	Pass
PFHxA	25.600	24.644	96	80-137	Pass
PFPeS	24.000	21.699	90	82-132	Pass
HFPODA	25.600	26.336	103	70-130	Pass
PFHpA	25.600	[illegible]018	106	80-140	Pass
PFHxS	24.200	[illegible]713	102	71-131	Pass
DONA	24.120	26.083	108	70-130	Pass
6:2-FTS	24.280	24.217	100	51-155	Pass
PFHpS	24.360	23.015	94	80-129	Pass
PFOA	25.600	25.043	98	83-138	Pass
PFOS	24.480	22.492	92	54-139	Pass
PFNA	25.600	25.872	101	73-140	Pass
9Cl-PF3ONS	23.840	21.863	92	70-130	Pass
PFNS	24.560	21.993	90	71-121	Pass
PFDA	25.600	25.047	98	78-137	Pass
8:2-FTS	24.520	22.231	91	62-133	Pass
PFOSA	25.600	25.714	100	73-121	Pass
NMEFOSAA	25.600	30.906	121	53-136	Pass
PFDS	24.640	22.873	93	69-124	Pass
PFUnDA	25.600	26.353	103	70-134	Pass
NEtFOSAA	25.600	28.765	112	59-145	Pass
11Cl-PF3OUdS	24.120	22.625	94	70-130	Pass
PFDoDA	25.600	27.710	108	75-139	Pass
10:2-FTS	24.680	26.626	108	50-124	Pass
PFDoS	24.800	21.509	87	39-121	Pass
PFTrDA	25.600	25.814	101	67-144	Pass
PFTeDA	25.600	25.446	99	79-134	Pass
PFHxDA	25.600	29.662	116	36-136	Pass
PFODA	25.600	27.373	107	10-124	Pass

Recovery Range: 87 % - 116 %
Average Recovery: 98.8 %
Mean Recovery: 99.0 %

Special thanks to Eurofins Lancaster Labs for providing this data

Percent Recoveries of a 2ng/L for Matrix Spikes (MS) and Matrix Spike Duplicates (MSD) of PFAS compounds spikes in a wastewater Discharge pond, Stormwater Runoff and Two Wastewater effluents.

	WW Effluent 1		WW Discharge Pond		Stormwater Runoff		WW Effluent 2	
	MS	MSD	MS	MSD	MS	MSD	MS	MSD
N-EtFOSAA	94%	92%	86%	92%	109%	114%	118%	123%
N-MeFOSAA	112%	115%	100%	117%	112%	115%	120%	136%
PFBS[a]	209%	219%	239%	218%	162%	182%	2040%	2003%
PFBS[b]	-	-	-	-	-	-	111%	106%
PFBA	104%	79%	123%	123%	103%	113%	108%	114%
PFPeA	107%	70%	133%	139%	106%	119%	114%	115%
PFHxA	125%	76%	166%	163%	102%	103%	111%	119%
PFHpA	107%	82%	106%	107%	93%	114%	122%	112%
PFHxS	103%	96%	145%	160%	117%	88%	112%	101%
PFOA	96%	71%	121%	114%	93%	113%	126%	117%
PFNS	99%	94%	104%	87%	102%	96%	105%	110%
PFNA	109%	94%	102%	108%	96%	102%	109%	114%
PFDA	113%	85%	104%	110%	99%	95%	117%	125%
PFUnDA	110%	90%	97%	95%	109%	114%	113%	114%
PFDoDA	117%	92%	83%	88%	114%	110%	119%	123%
PFOS	105%	88%	110%	114%	129%	133%	102%	106%
PFTrDA	104%	83%	71%	74%	89%	88%	95%	96%
PFPeS	93%	86%	78%	92%	82%	86%	94%	97%
PFTeDA	107%	94%	67%	67%	116%	108%	118%	127%
PFHxDA	104%	110%	54%	56%	100%	103%	116%	125%
PFOcDA	90%	101%	54%	55%	65%	63%	92%	105%
5:3-FTCA	114%	76%	126%	134%	91%	93%	107%	124%
7:3-FTCA	122%	77%	131%	144%	102%	98%	107%	106%
HFPO-DA	109%	80%	104%	99%	87%	101%	115%	86%
DONA	105%	93%	90%	106%	85%	86%	92%	95%
9Cl-PF3ONS	83%	77%	92%	94%	107%	100%	103%	102%
11Cl-PF3OUdS	70%	66%	55%	51%	92%	90%	95%	91%
EtFOSE	102%	100%	92%	72%	120%	102%	142%	161%
MeFOSE	86%	106%	64%	83%	124%	102%	130%	118%
EtFOSA	96%	100%	54%	61%	112%	105%	113%	124%
MeFOSA	100%	97%	67%	72%	110%	112%	137%	146%
PFOSA	127%	95%	116%	101%	120%	110%	120%	114%
4:2-FTS	117%	84%	109%	118%	98%	102%	112%	109%
PFHpS	106%	91%	68%	115%	101%	98%	102%	111%
PFDS	82%	86%	51%	69%	95%	83%	96%	96%
8:2-FTS	119%	93%	109%	111%	107%	104%	114%	111%
6:2-FTS	114%	97%	106%	134%	129%	104%	128%	107%
10:2-FTS	107%	105%	70%	69%	47%	54%	80%	87%
3:3-FTCA	101%	94%	84%	83%	83%	71%	23%	54%
PFEESA	108%	120%	97%	95%	98%	112%	111%	106%
PFMPA	121%	126%	102%	116%	132%	129%	134%	133%
PFMBA	117%	103%	107%	116%	114%	112%	125%	123%
NFDHA	111%	111%	96%	108%	104%	103%	117%	120%

Special thanks to David Scheissel and Babcock Labs for providing this data

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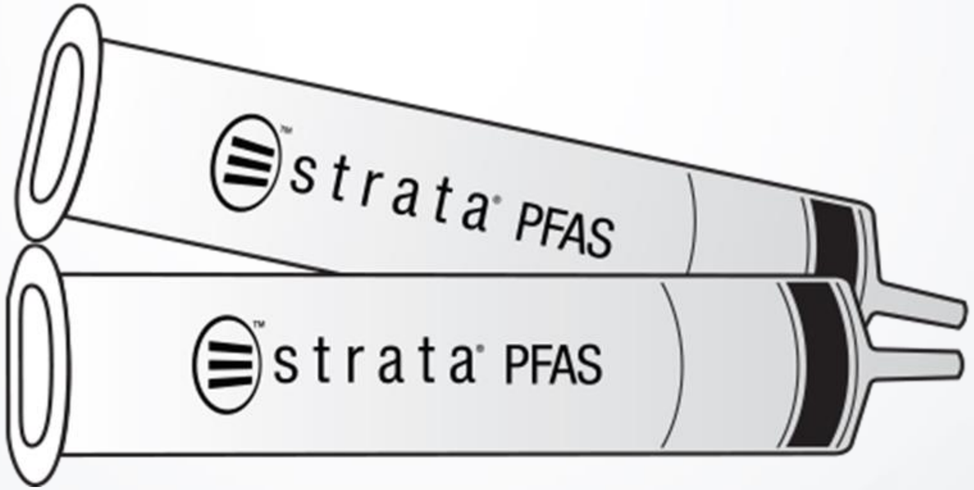
BENEFITS OF STACKED TUBES



- High recoveries that meet DOD requirements
- No need for PPE (N95 Masks or hoods)
- Lower cost of solvents and consumables
- Less sample manipulation
- Consistent formats for reduced variability
- Single step extraction and cleanup to save time and reduce costs

Future considerations

- UCMR5
- GCB Pass Through (50mg or 25mg)
- On-line SPE (Including On-line GCB cartridges)
- Unique SPE formats for dirty matrices (soil, sediment, biota)
- Extended panels and resolution issues – Unique LC chemistry and MP needed to improve resolution of more analytes
- TOP and TOF assays for fingerprinting source and showing transport mechanisms
- Non-targeted assays
- Pending 1600 Series method to replace Table B15

Conclusion

1. PFAS are not going away – and we have just scratched the surface
2. Lots of different ways to analyze PFAS – depending on matrix and analyte list
3. Stacked Tubes offer performance and productivity benefits for DOD compliant work
4. Phenomenex SPE products are demonstrated Fit for Purpose for all published methods
5. New formats and chemistries being considered
6. https://www.phenomenex.com/Info/Page/pfasresources

Special thanks to the following labs and individuals for their invaluable assistance:

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David Scheissel at ES Babcock
Andrew Patterson and Bob Brown at Eurofins Lancaster Labs

Questions?