

**Validation of the Analysis of
PFAS/PFOS in
Wastewater Samples Using
EPA Method 1633 with
Semi-Automated
Solid Phase Extraction**

Purpose

- Sample Preparation Workflows and Tools for EPA Method 1633
- Validation Study Data
- EPA Method 1633 Various Aqueous Matrices

Target Analytes

Method	533	537.1	1633	ISO21675
PFBA	x		x	x
PFPeA	x		x	x
PFHxA	x	x	x	x
PFHpA	x	x	x	x
PFOA	x	x	x	x
PFNA	x	x	x	x
PFDA	x	x	x	x
PFUnA	x	x	x	x
PFDoA	x	x	x	x
PFTrDA		x	x	x
PFTeDA		x	x	x
PFBS	x	x	x	x
PFPeS	x		x	
PFHxS	x	x	x	x

Target Analytes

Method	533	537.1	1633	ISO21675
PFHpS	x		x	x
PFOS	x	x	x	x
PFNS			x	
PFDS			x	x
PFDoS			x	
4:2FTS	x		x	
6:2FTS	x		x	x
8:2FTS	x		x	x
PFOSA			x	x
NMeFOSA			x	x
NEtFOSA			x	x
NMeFOSAA		x	x	x
NEtFOSAA		x	x	x
NMeFOSE			x	

Target Analytes

Method	533	537.1	1633	ISO21675
NETFOSE			x	
HFPO-DA	x	x	x	x
ADONA	x	x	x	x
PFMPA	x		x	
PFMBA	x		x	
NFDHA	x		x	
9CI-PF3ONS	x	x	x	x
11CI-PF3OUdS	x	x	x	
PFEESA	x		x	
3:3 FTCA			x	
5:3 FTCA			x	
7:3 FTCA			x	
PFOcDA				x
PFHxDA				x
8:2 FTUCA				x
8:2 diPAP				x

Optimizing the PFAS Analysis Workflow

- **Automate the Sample Prep Workflow**
 - **Automate the Solid Phase Extraction Step**
 - **Automate the Concentration/Evaporation Step**
- **Automated, Semi-Automated SPE extractions and Concentration**
 - **Reduces Human Error**
 - **Reduces Outside contamination and background**
 - **Reduces Solvent Usage**
 - **Reduces Labor**
- **Use SPE solutions to deliver consistent, reproducible results**
- **Different configurations to handle Matrix type and Budget**
- **No Teflon Components**

Comparison of Manual SPE vs. Automated SPE Methods

Manual SPE

Manually Separate Waste

<10 mls solvent evaporate

Run times are ~ 30 to 90 minutes

Technician Time 25 minutes

Physical transfer and Concentration steps

Concentration step 70 to 200 minutes

Automated/Semi-Auto SPE

Separates Aqueous and Organic Waste

<10 mls solvent to evaporate

Run times are ~20 to 50 minutes or less

Technician time 5 minutes

Automatic and Direct to Concentration
delivery and completion

Concentration step 70 minutes ready for
injection

- **Closed System**
- **Ability to load samples by vacuum or positive pressure consistently.**
- **Handle difficult Matrices**
- **Ability to dry cartridges by vacuum and positive Nitrogen gas pressure.**
- **Easily handle various cartridge designs and sizes without cumbersome modifications.**
- **Easy, Simple Sample delivery**
- **Bottle Rinse**

Automated Solid Phase Extraction front end for LC/MS



EconoTrace® PFC



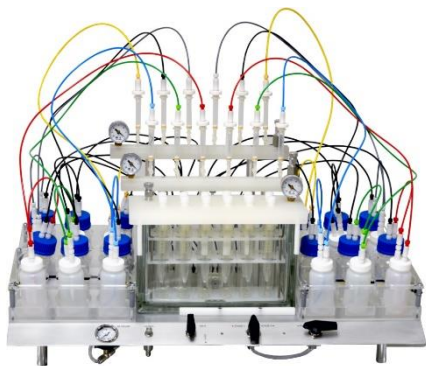
TurboTrace® PFC



TurboTrace®
Parallel
Sequential



LC/MS



EZPFC



LC/MS

Can this Handle Dirty Samples?

Typical Cartridge can have problems!

- **6ml cartridge**
 - Doesn't do well
 - Frit Surface Area is too small

Yes, A Cartridge will work

- **25ml cartridge**
 - Does well
 - 3X the Frit Surface Area



Glass/Plastic Wool

- Irregular random stranding
- Slows Particles to the Uniform Frit
- Prevents Clogging



Sample Bottle Filter



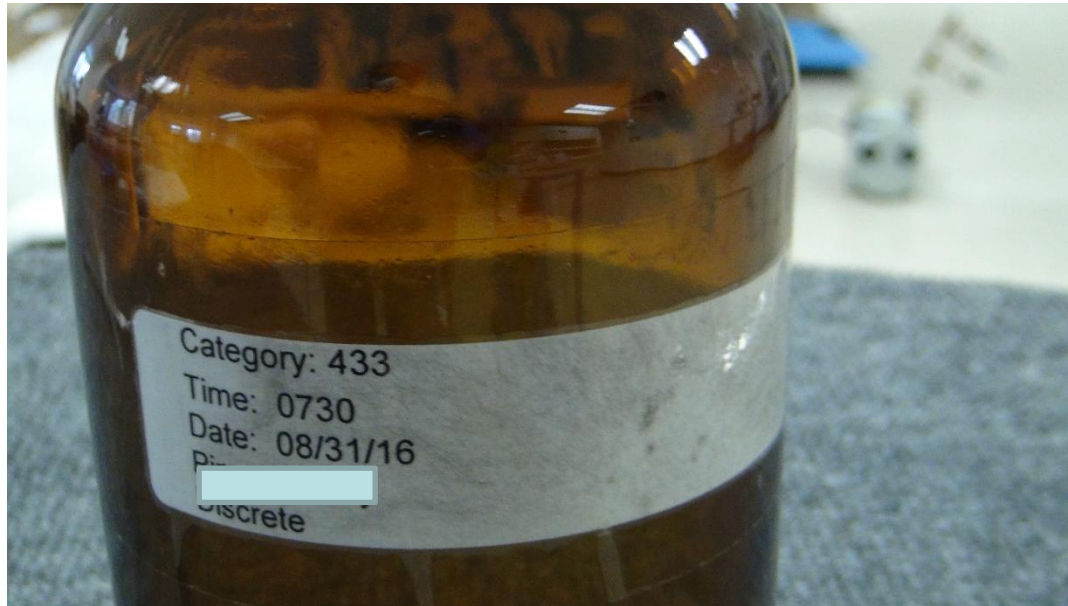
Prepping the 6ml Cartridge with Plastic Filtration Wool

6ml cartridge with Plastic wool

- Take a little and push it into the barrel of the syringe until it touches the cartridge Frit
- The Sample will not clog, it will take longer to process

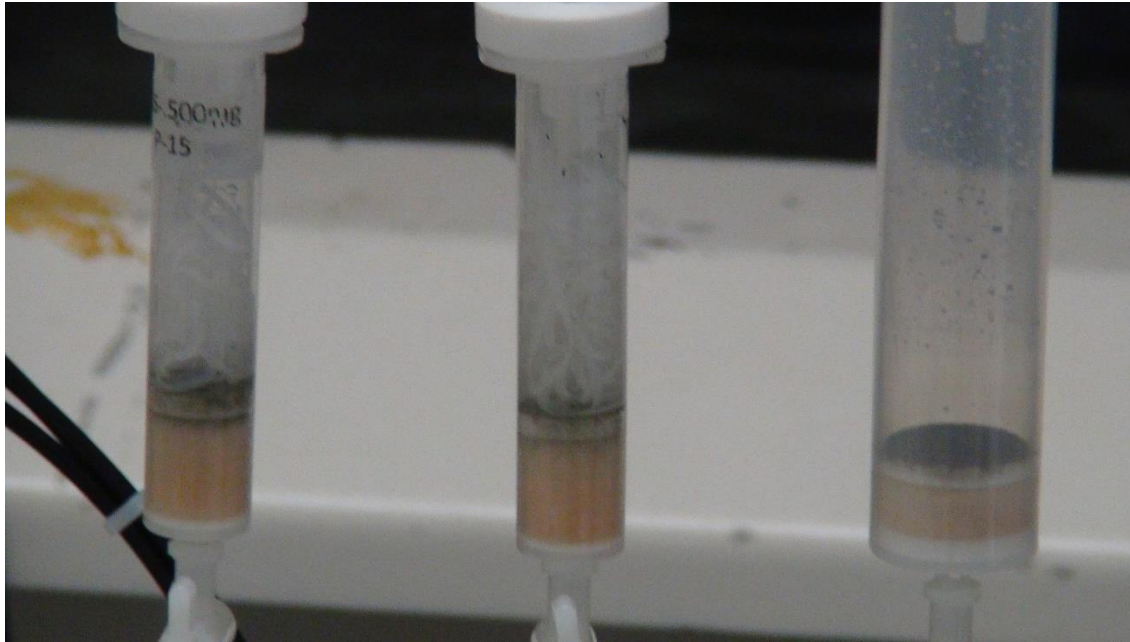


Dirty Sample from a Customer

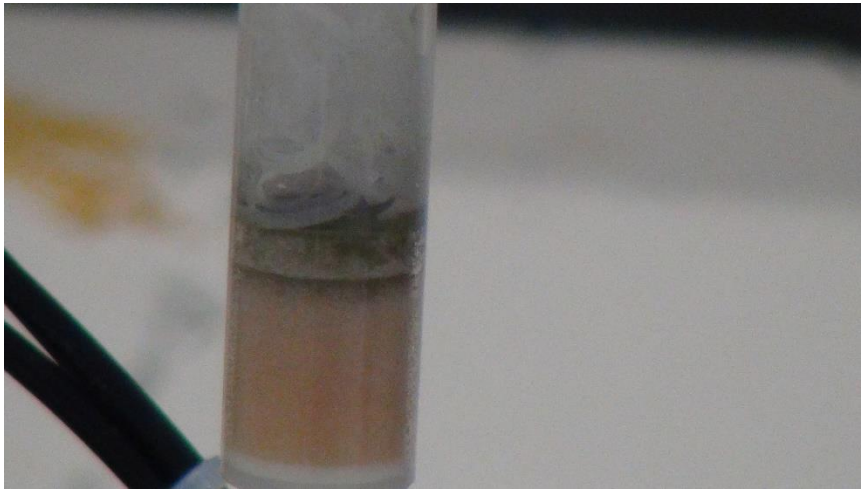




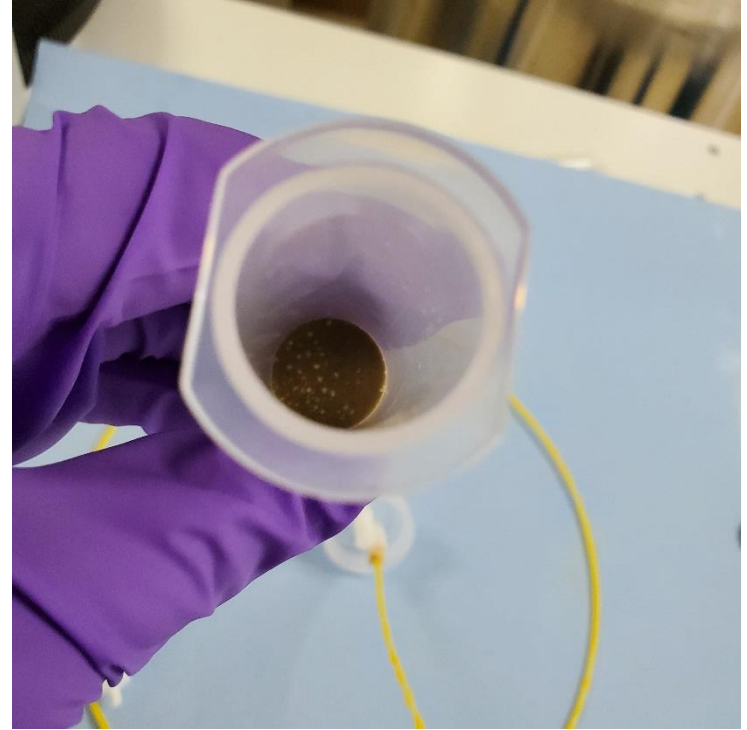
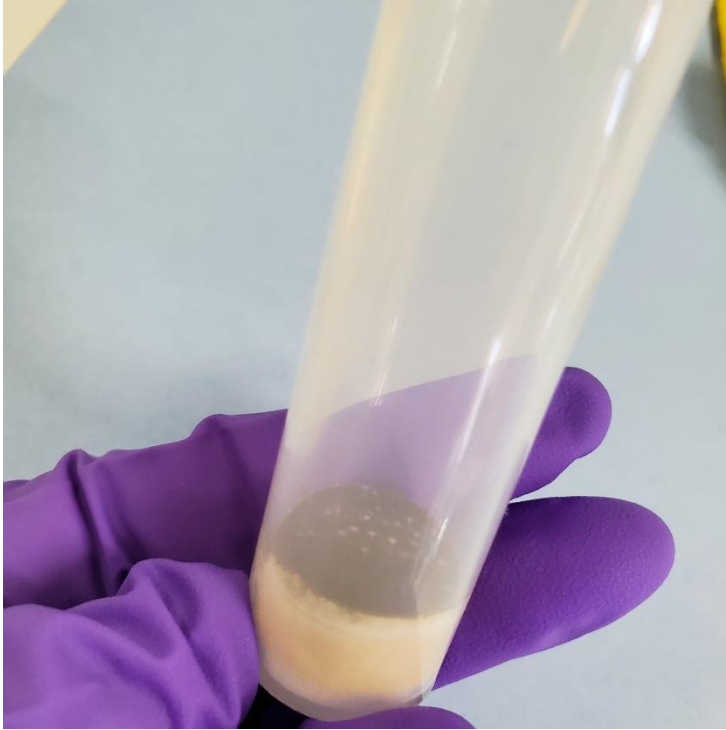
6ml and 25ml Cartridges



500 ml run to completion on 6 ml cartridge with Plastic Wool



500ml run to completion 25ml cartridge



- **Closed System**
- **12 samples in parallel**
- **Reduced Actions / Easy to Use**
- **Simplified procedures**
- **Semi-Automated versus Manual protocols = Reproducibility**
- **Increased Sample Throughput**
- **Low cost compared to Automated solutions**

Objective for Semi Automation

- **Incorporate automated system capabilities to the semi-automated platform.**
- **Our goal is to establish several Standard Operating Procedures (SOPs) for the testing lab through the utilization of a solitary extraction platform.**
- **In order to optimize time and minimize mistakes, it is crucial to decrease the amount of manual steps involved.**

Goal

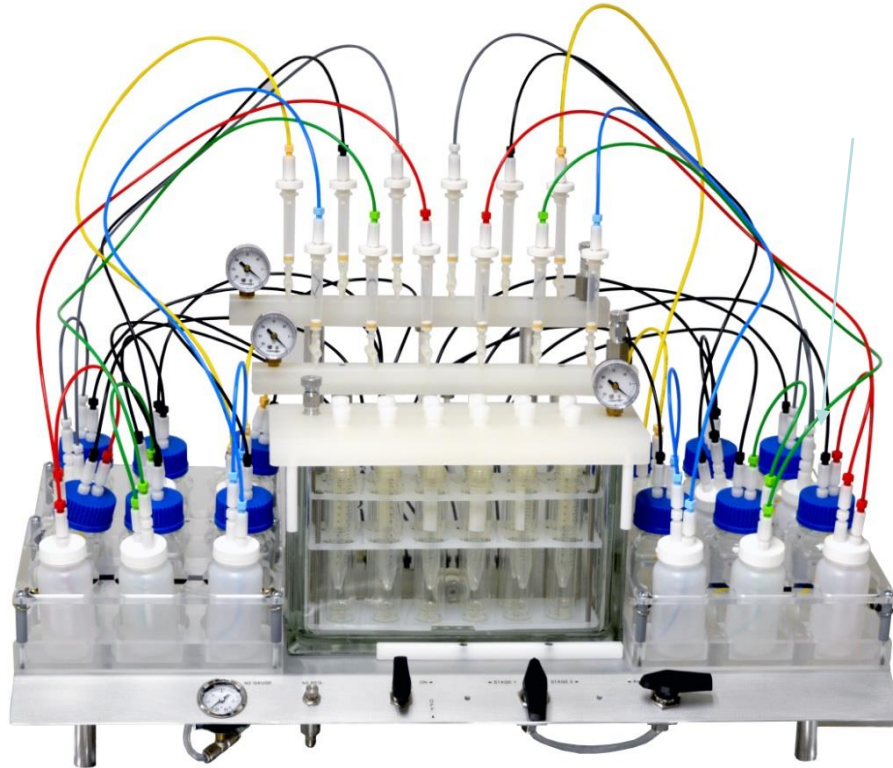
- **Efficient**

- Uses all SPE cartridge sizes
- Dedicated manifold for cartridge conditioning and sample loading
- Dedicated manifold for extraction and extracts
- Separates Organic from Aqueous waste
- Vacuum cartridge drying, Nitrogen cartridge drying or combined
- Automated Bottle Rinse and Elution
- Inline Extract Drying
- Small number of components to clean

- **Low Capital Expense**



Drinking Water,
Wastewater,
Particulate laden samples



EZPFC 12 sample

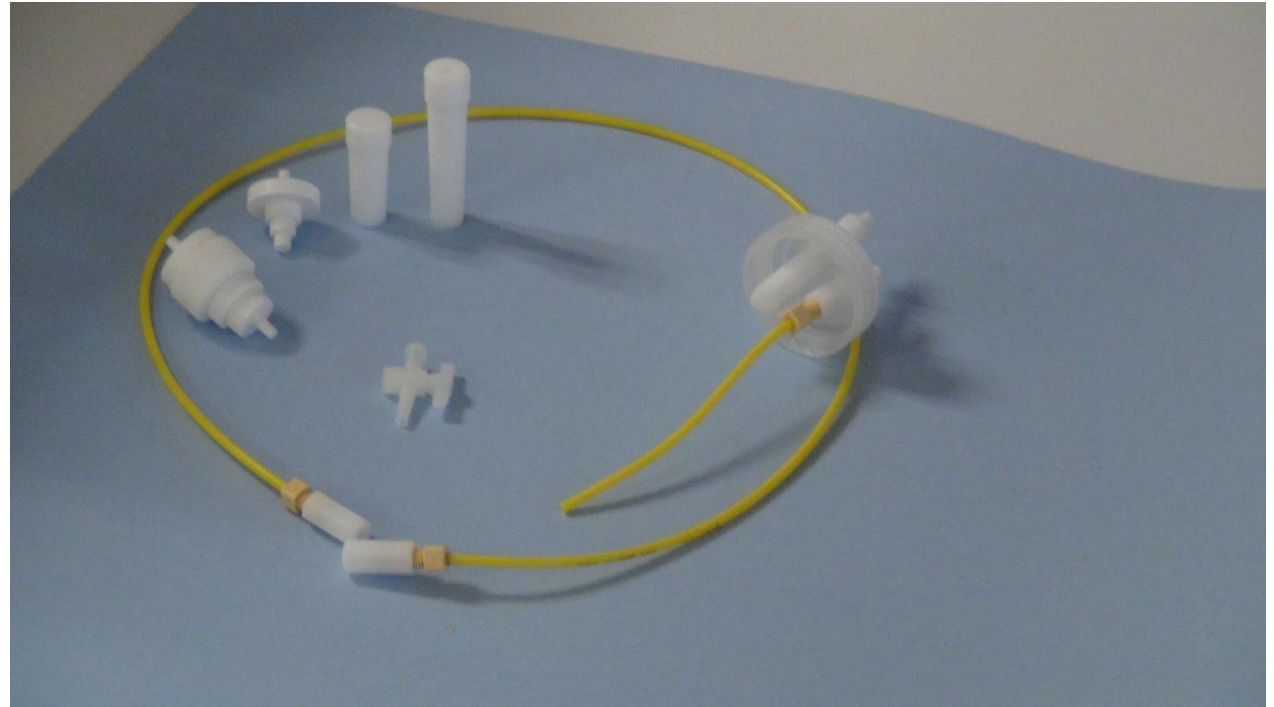
System Components

No Teflon

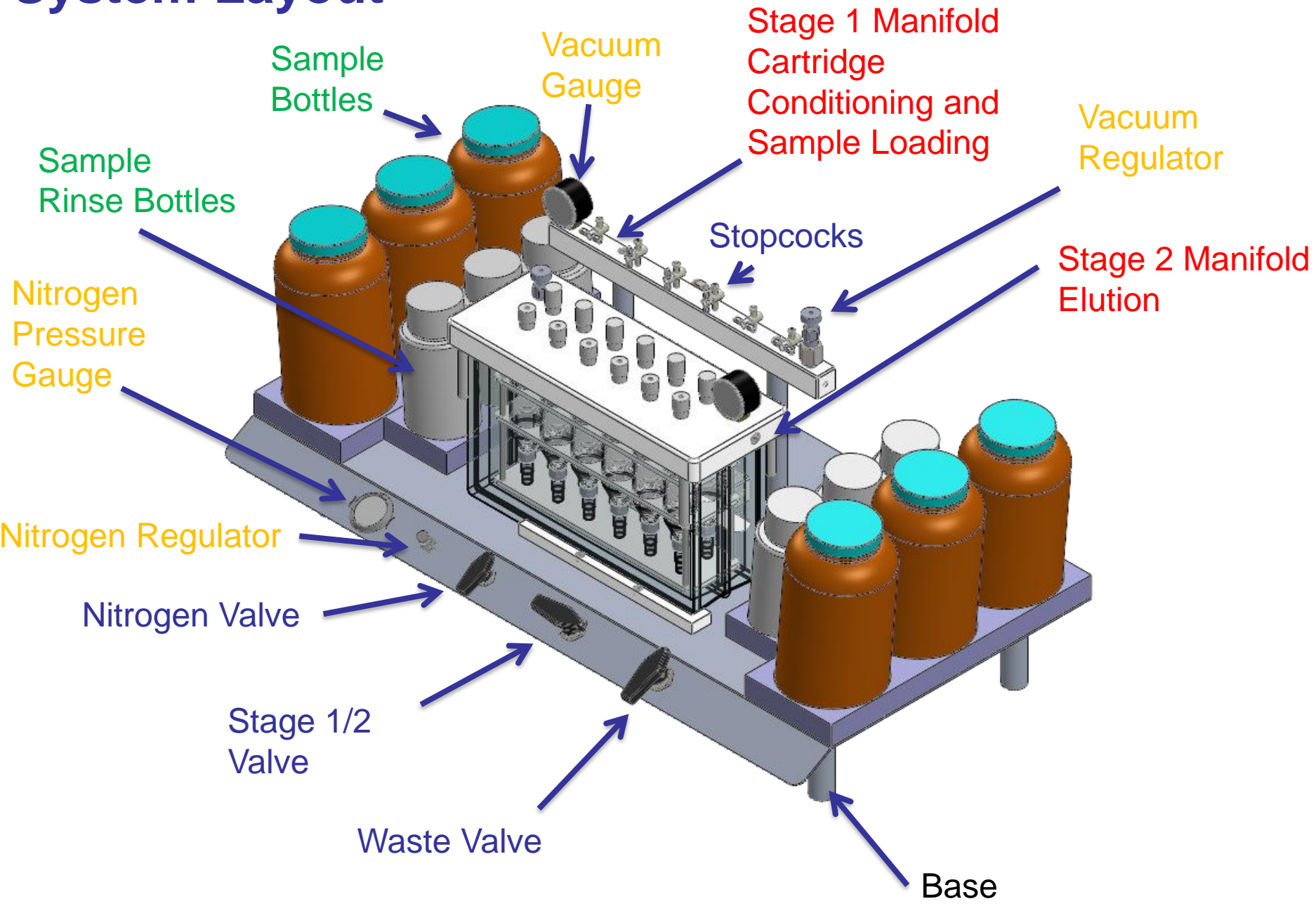
**Tubing - High Density
Polyethylene**

Fittings – Delrin

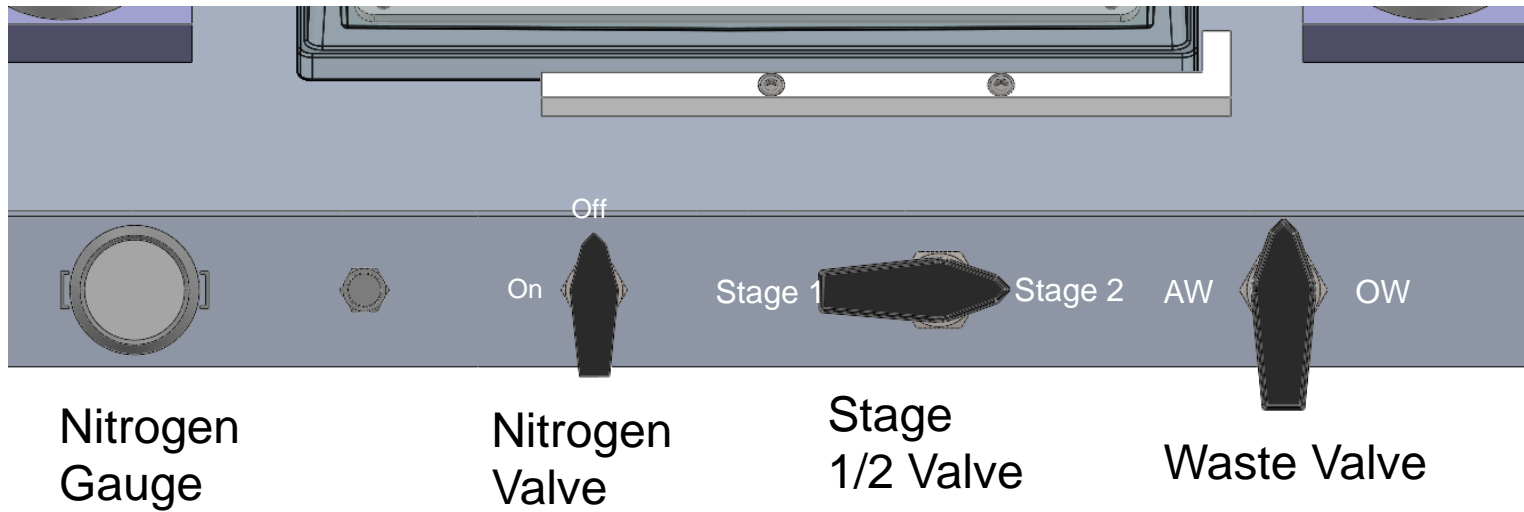
**Cartridge Adapters –
Medical Grade
Polypropylene**



System Layout



Control Valve Layout



Automated Concentration for PFAs

- SuperVap 24 PFC
 - 24 positions
 - 15ml Conical vials
 - Timed Endpoint
- SuperVap 12 PFC
 - 12 positions
 - 50ml Conical vials
 - Timed Endpoint

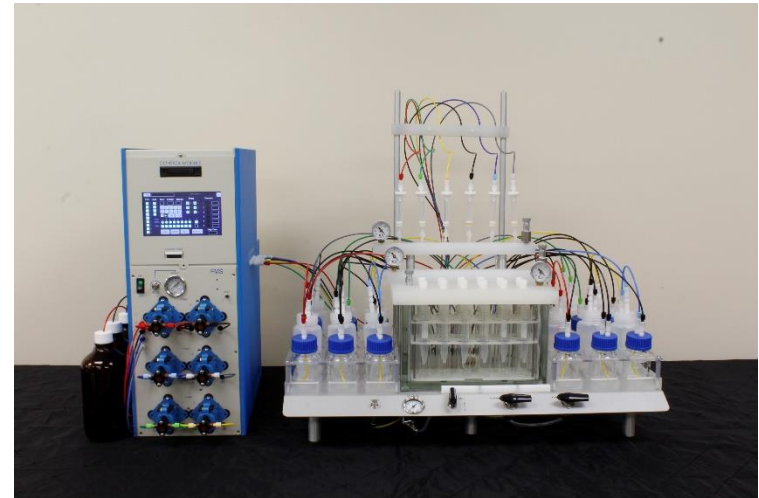


Clean up is easy with no cross contamination

- Back Flush the sample line into the original sample bottle with an IPA non-Teflon squirt bottle.
- Wash the inside of the bottle cap with IPA squirt bottle
- Wash Cartridge Adapters with IPA squirt bottle or sonicate in a beaker
- Ready for the next 12 samples

Multipump for EZPFC

- For delivery of Solvent to 6 cartridges in Parallel
- Used for Conditioning and Elution
- Up to Six solvents
- Nitrogen Drying
- No Teflon



We Learned about Sources of Contamination

- Make sure you test for Background Contamination before you start
 - LC/MS Check
 - Pipette Tips
 - Solvents
 - Water
 - Solvent Bottles

- **Analysis**

- Take aliquot from final 5 mL extract (Method 1633 does not require volume reduction of final extract)
- Agilent 1290 Infinity II LC System
- Agilent 6475 Triple quad LC/MS
- Agilent Zorbax Eclipse Plus C18 column 3.0 x 50 mm, 1.8 μ m
- Column temperature 40 °C
- Injection 5.0 μ L

- **Analysis**

- Mobile phase 5 mM ammonium acetate in 95% water, 5% acetonitrile (A) and methanol (B) Gradient
- 0 min 98% A 2% B
- 0.2 min 98% A 2% B 10 min 5% A 95% B
- Stop time 12.2 min
- Dynamic MRM negative electrospray
- T (gas) = 230 °C

Stage 1

- Condition cartridges with 15 mL 1% methanolic ammonium hydroxide, followed by 5 mL of 0.3M formic acid
- Load samples across the cartridges at 5-10 mL/min (~ 8-inch Hg)
- Sample bottles rinsed with 5 mL reagent water (twice), followed by 5 mL of 1:1 0.1M formic acid/methanol (using nitrogen)
- Rinses loaded across cartridges
- Dry 15 sec under vacuum

Stage 2

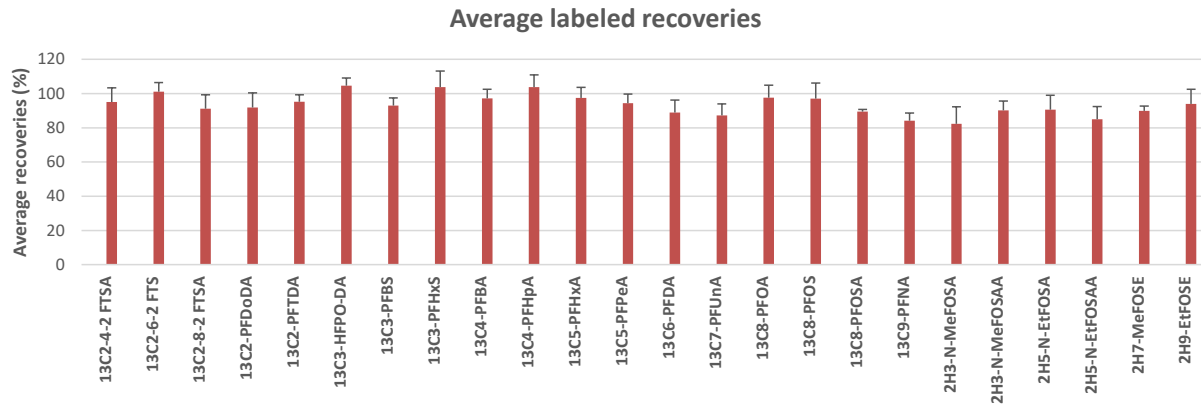
- Rinse sample bottles with 5 mL 1% methanolic ammonium hydroxide
- Load rinses across cartridges and collect in polypropylene tubes
- Cleanup carried out with 10 mg loose carbon
- As per the method no further concentration is carried out.

IDC Data for Labeled

Recoveries (%) and acceptance windows (%) for 24 surrogate PFAS in synthetic wastewater (1633) using EZPFC.

Compound						
Name	Percent	EZPFC-IDC-1	EZPFC-IDC-2	EZPFC-IDC-3	EZPFC-IDC-4	Window
13C2-4-2 FTSA		95.1	93.7	105.3	86.3	40-200
13C2-6-2 FTS		96.9	104.7	106.7	96.0	40-200
13C2-8-2 FTSA		91.1	98.3	94.4	80.8	40-300
13C2-PFDoDA		81.6	97.7	98.4	89.6	10-130
13C2-PFTDA		90.3	96.9	99.3	94.4	10-130
13C3-HFPO-DA		103.0	110.3	106.0	99.3	40-130
13C3-PFBS		89.0	96.9	96.3	90.0	40-135
13C3-PFHxS		95.9	108.0	115.7	95.9	40-130
13C4-PFBA		92.0	102.6	100.7	93.4	5-130
13C4-PFHpA		95.9	112.5	107.0	100.0	40-130
13C5-PFHxA		93.4	102.0	103.2	91.3	40-130
13C5-PFPeA		94.2	98.9	97.2	87.6	40-130
13C6-PFDA		81.8	93.0	95.7	85.3	40-130
13C7-PFU _n A		83.9	86.4	95.9	83.0	30-130
13C8-PFOA		89.0	106.0	99.6	96.1	50-200
13C8-PFOS		93.9	110.4	91.3	92.5	50-200
13C8-PFOSA		90.4	90.0	88.1	89.7	40-130
13C9-PFNA		85.0	89.0	82.6	80.1	40-130
2H3-N-MeFOSA		87.6	91.0	76.8	74.2	10-130
2H3-N-MeFOSAA		95.5	93.0	87.7	84.5	40-170
2H5-N-EtFOSA		95.8	96.6	90.0	80.0	10-130
2H5-N-EtFOSAA		89.6	79.0	91.3	80.3	25-135
2H7-MeFOSE		93.3	89.0	90.1	87.3	10-130
2H9-EtFOSE		92.2	105.8	89.3	88.7	10-130

IDC Labeled



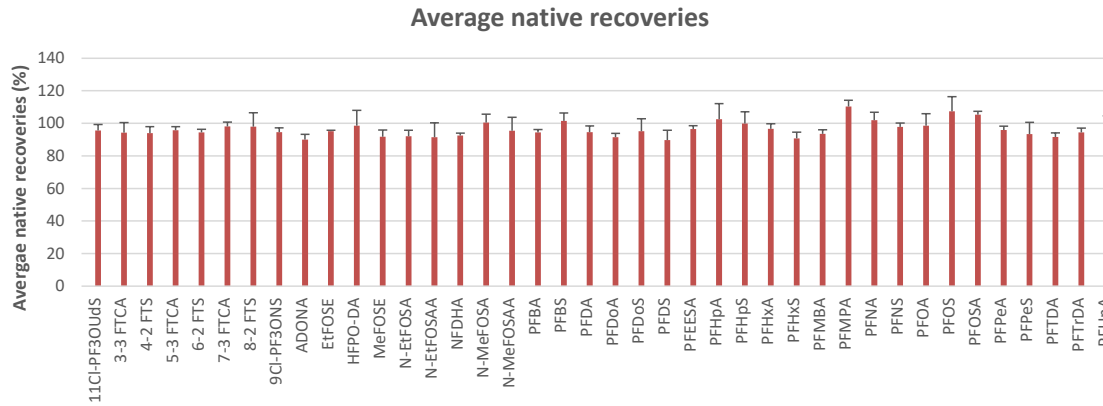
Average labeled recoveries (% , n=4)

IDC Data for Natives

Recoveries (%) and
RSDs (%) for 40 native
PFAS in synthetic
wastewater (1633)
using EZPFC
(spiked with 1-38 ng/L).

Compound		EZPFC-IDC-1	EZPFC-IDC-2	EZPFC-IDC-3	EZPFC-IDC-4	Window	RSD (%)
Name	Percent						
11Cl-PF3OUds		92	93	100	97	50-150	3.62
3-3 FTCA		87	96	93	101	70-130	6.18
4-2 FTS		89	98	94	96	70-135	3.90
5-3 FTCA		96	96	93	98	70-130	2.14
6-2 FTS		92	95	95	96	70-135	1.89
7-3 FTCA		98	97	95	102	55-130	2.79
8-2 FTS		92	91	100	109	70-140	8.52
9Cl-PF3ONS		93	92	97	97	70-145	2.72
ADONA		87	90	94	89	70-135	3.24
EtFOSE		95	95	95	96	70-130	0.57
HFPO-DA		92	111	100	91	70-135	9.42
MeFOSE		89	96	89	94	70-135	4.02
N-EtFOSA		89	93	91	96	70-135	3.57
N-EtFOSAA		93	90	81	101	70-135	8.81
NFDHA		93	92	94	91	65-140	1.57
N-MeFOSA		94	101	100	106	70-135	5.12
N-MeFOSAA		86	95	105	96	65-140	8.21
PFBA		93	96	93	95	70-135	1.68
PFBS		109	98	100	99	70-140	4.88
PFDA		98	94	90	96	65-140	3.83
PFDoA		93	92	88	93	70-130	2.34
PFDoS		93	105	87	96	45-135	7.64
PFDS		85	91	97	86	70-135	6.07
PFEESA		95	99	96	96	70-135	1.99
PFHpA		99	101	94	116	70-135	9.44
PFHpS		91	108	101	99	70-140	7.22
PFHxA		96	97	93	100	70-135	3.07
PFHxS		90	91	87	95	70-135	
PFMBA		91	92	96	95	65-145	2.55
PFMPA		106	107	115	113	60-140	3.83
PFNA		105	100	96	107	70-140	4.85
PFNS		96	100	95	99	70-135	2.44
PFOA		102	89	99	105	65-155	7.24
PFOS		97	109	120	104	70-140	8.89
PFOSA		107	102	105	107	70-135	2.16
PFPeA		93	98	96	96	70-135	2.36
PFPeS		93	91	87	103	70-135	7.17
PFTDA		91	93	94	89	70-145	2.50
PFTrDA		92	98	95	93	60-145	2.52
PFUnA		89	105	93	100	70-135	7.62

IDC Native Recoveries



Initial
Demonstration
of Capability (1-
38 ng/L, n=4)

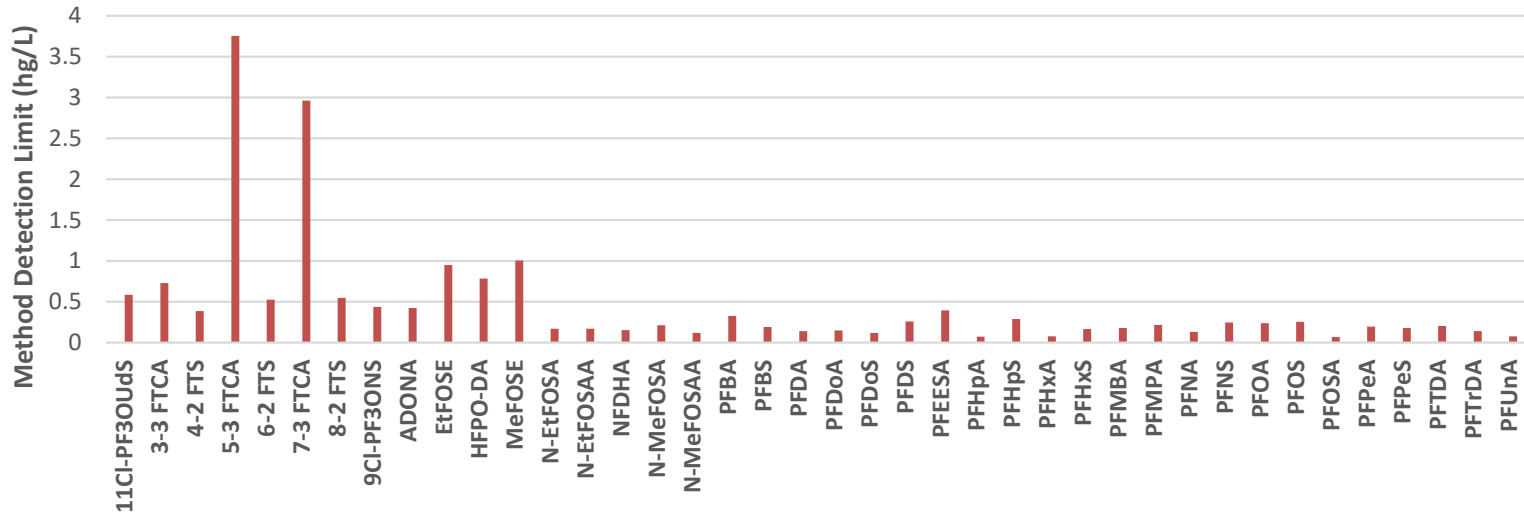
MDL Data

Method Detection Limit values for 40 native PFAS in synthetic wastewater (1633) using EZPFC (spiked with 0.2-9 ng/L).

Compound Name	In ng/L	EZPFC-MDL-1 Final Conc.	EZPFC-MDL-2 Final Conc.	EZPFC-MDL-3 Final Conc.	EZPFC-MDL-4 Final Conc.	EZPFC-MDL-5 Final Conc.	EZPFC-MDL-6 Final Conc.	EZPFC-MDL-7 Final Conc.	STDEV	MDL
11CI-PF3OUds		1.24	1.30	1.47	1.00	1.19	1.30	0.93	0.19	0.58
3-3 FTCA		1.08	1.53	1.71	1.07	1.39	1.29	1.26	0.23	0.73
4-2 FTS		1.46	1.43	1.48	1.44	1.21	1.62	1.50	0.12	0.39
5-3 FTCA		8.16	8.96	10.10	7.16	8.24	9.73	6.98	1.19	3.75
6-2 FTS		1.54	1.61	1.58	1.51	1.30	1.74	1.28	0.17	0.53
7-3 FTCA		8.73	9.36	9.52	8.02	8.94	9.47	6.94	0.94	2.96
8-2 FTS		1.26	1.32	1.51	1.76	1.32	1.56	1.43	0.17	0.55
9CI-PF3ONS		1.02	1.31	1.26	0.98	1.13	1.17	0.95	0.14	0.44
ADONA		0.95	1.07	1.27	0.95	0.97	1.18	0.92	0.13	0.42
EtFOSE		3.21	3.20	3.79	3.46	3.04	3.80	3.24	0.30	0.95
HFPO-DA		0.98	1.22	1.75	1.43	1.34	1.46	1.16	0.25	0.79
MeFOSE		3.41	3.23	3.83	3.28	2.77	3.48	3.36	0.32	1.01
N-EtFOSA		0.24	0.23	0.25	0.28	0.34	0.34	0.37	0.05	0.17
N-EtFOSAA		0.30	0.34	0.35	0.33	0.38	0.21	0.32	0.05	0.17
NFDHA		0.60	0.64	0.63	0.55	0.55	0.64	0.52	0.05	0.16
N-MeFOSA		0.41	0.28	0.27	0.31	0.24	0.35	0.21	0.07	0.21
N-MeFOSAA		0.31	0.33	0.28	0.31	0.25	0.34	0.36	0.04	0.12
PFBA		1.12	1.19	1.35	1.21	1.05	1.30	1.16	0.10	0.33
PFBS		0.26	0.34	0.33	0.33	0.22	0.41	0.31	0.06	0.19
PFDA		0.27	0.31	0.29	0.32	0.22	0.36	0.28	0.04	0.14
PFDoA		0.33	0.31	0.43	0.29	0.30	0.31	0.30	0.05	0.15
PFDoS		0.36	0.25	0.35	0.29	0.28	0.33	0.32	0.04	0.12
PFDS		0.25	0.32	0.49	0.26	0.26	0.31	0.28	0.08	0.26
PFEESA		0.72	0.70	0.86	0.67	0.53	0.90	0.66	0.13	0.39
PFHpA		0.33	0.31	0.30	0.28	0.26	0.29	0.30	0.02	0.07
PFHpS		0.34	0.28	0.49	0.39	0.26	0.33	0.20	0.09	0.29
PFHxA		0.30	0.30	0.35	0.30	0.27	0.32	0.29	0.02	0.08
PFHxS		0.40	0.28	0.32	0.27	0.27	0.34	0.38	0.05	0.17
PFMBA		0.51	0.58	0.61	0.54	0.45	0.61	0.56	0.06	0.18
PFMPA		0.50	0.54	0.58	0.38	0.41	0.49	0.46	0.07	0.22
PFNA		0.29	0.25	0.33	0.31	0.29	0.35	0.23	0.04	0.13
PFNS		0.23	0.21	0.25	0.34	0.22	0.07	0.23	0.08	0.25
PFOA		0.46	0.32	0.28	0.23	0.26	0.30	0.32	0.08	0.24
PFOS		0.40	0.28	0.32	0.52	0.33	0.35	0.30	0.08	0.26
PFOSA		0.30	0.32	0.32	0.28	0.30	0.32	0.26	0.02	0.07
PFPeA		0.55	0.60	0.70	0.63	0.50	0.63	0.57	0.06	0.20
PFPeS		0.27	0.31	0.35	0.23	0.29	0.35	0.40	0.06	0.18
PFTDA		0.21	0.26	0.39	0.23	0.23	0.22	0.31	0.06	0.20
PFTTrDA		0.29	0.24	0.39	0.29	0.28	0.30	0.30	0.04	0.14
PFUnA		0.29	0.28	0.29	0.32	0.30	0.31	0.25	0.02	0.08

MDL Study

Native PFAS 1633 Method Detection Limit



Method Detection Limit
(0.2-9 ng/L, n=7)

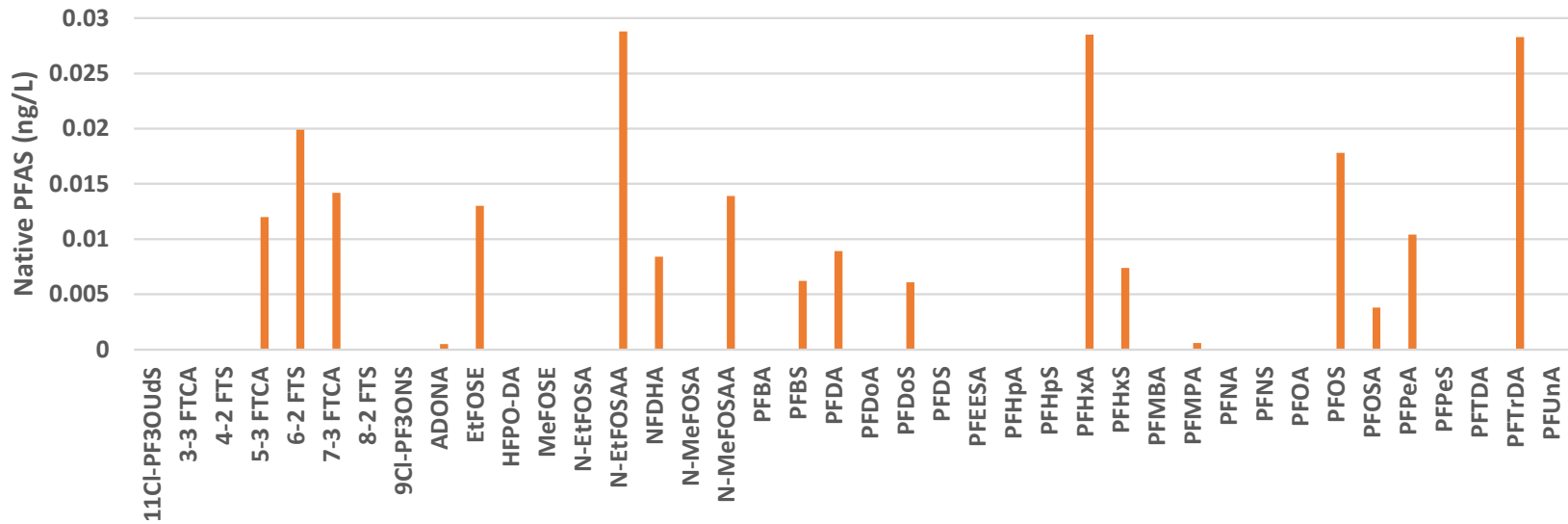
System Background

Native PFAS 1633
background for EZPFC
(ng/L)

11CI-PF3OUdS	0.0000
3-3 FTCA	0.0000
4-2 FTS	0.0000
5-3 FTCA	0.0120
6-2 FTS	0.0199
7-3 FTCA	0.0142
8-2 FTS	0.0000
9CI-PF3ONS	0.0000
ADONA	0.0005
EtFOSE	0.0130
HFPO-DA	0.0000
MeFOSE	0.0000
N-EtFOSA	0.0000
N-EtFOSAA	0.0288
NFDHA	0.0084
N-MeFOSA	0.0000
N-MeFOSAA	0.0139
PFBA	0.0000
PFBS	0.0062
PFDA	0.0089
PFDoA	0.0000
PFDoS	0.0061
PFDS	0.0000
PFEESA	0.0000
PFHpA	0.0000
PFHpS	0.0000
PFHxA	0.0285
PFHxS	0.0074
PFMBA	0.0000
PFMPA	0.0006
PFNA	0.0000
PFNS	0.0000
PFOA	0.0000
PFOS	0.0178
PFOSA	0.0038
PFPeA	0.0104
PFPeS	0.0000
PFTDA	0.0000
PFTTrDA	0.0283
PFUnA	0.0000

System Background

Native PFAS background EZPFC (ng/L)

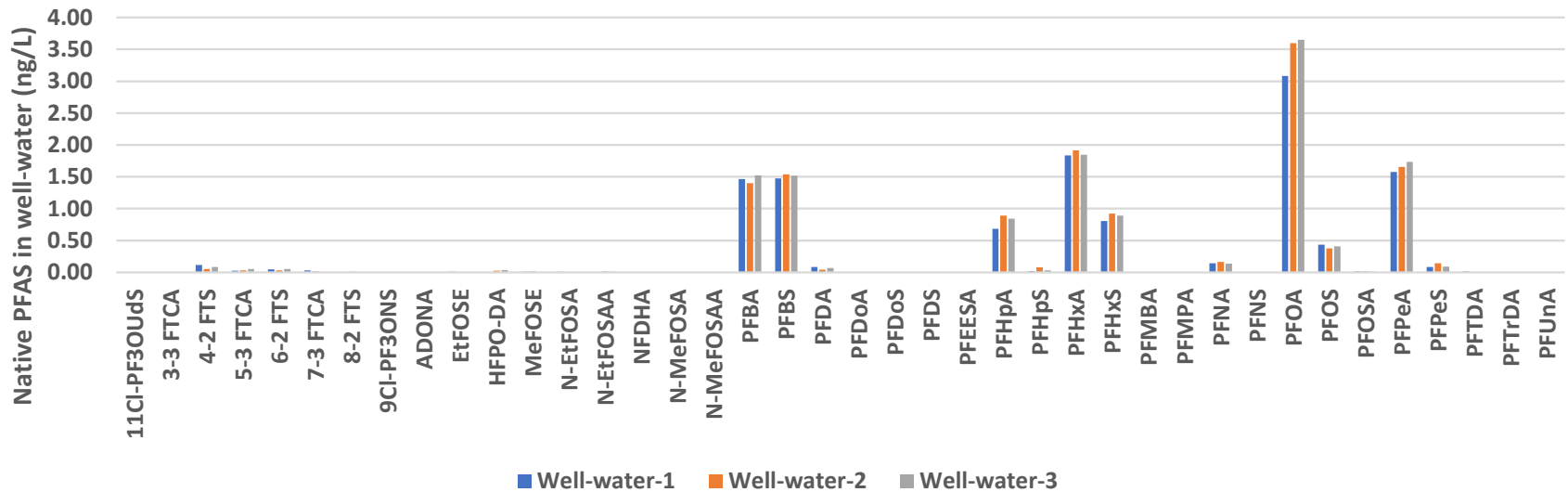


Matrices

Compound	Well-water-1	Well-water-2	Well-water-3	River-water-1	River-water-2	River-water-3	TAP-WATER-1	TAP-WATER-2	TAP-WATER-3
Name In ng/L	Final Conc.	Final Conc.	Final Conc.	Final Conc.	Final Conc.	Final Conc.	Final Conc.	Final Conc.	Final Conc.
11CI-PF3OUdS	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00
3-3 FTCA	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00
4-2 FTS	0.12	0.06	0.09	0.01	0.01	0.03	0.02	0.01	0.01
5-3 FTCA	0.02	0.03	0.05	0.04	0.12	0.02	0.03	0.02	0.02
6-2 FTS	0.05	0.03	0.05	0.09	0.03	0.03	0.04	0.05	0.04
7-3 FTCA	0.03	0.02	0.00	0.00	0.04	0.00	0.00	0.00	0.00
8-2 FTS	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
9CI-PF3ONS	0.01	0.01	0.00	0.00	0.00	0.00	0.01	0.00	0.00
ADONA	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
EtFOSE	0.01	0.00	0.01	0.05	0.01	0.02	0.00	0.01	0.02
HFPO-DA	0.00	0.03	0.04	0.03	0.02	0.05	0.02	0.00	0.05
MeFOSE	0.01	0.01	0.00	0.00	0.00	0.00	0.01	0.01	0.06
N-EtFOSA	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
N-EtFOSAA	0.00	0.01	0.01	0.01	0.02	0.05	0.01	0.01	0.03
NFDHA	0.01	0.00	0.00	0.01	0.00	0.01	0.00	0.01	0.00
N-MeFOSA	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.02
N-MeFOSAA	0.00	0.00	0.00	0.01	0.02	0.01	0.01	0.01	0.01
PFBA	1.46	1.40	1.52	0.77	0.56	0.73	0.60	0.57	0.65
PFBS	1.47	1.54	1.52	1.41	1.04	1.39	0.75	0.80	0.86
PFDA	0.09	0.04	0.07	0.06	0.03	0.06	0.02	0.01	0.00
PFDoA	0.00	0.00	0.00	0.01	0.03	0.01	0.02	0.02	0.02
PFDoS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PFDS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PFEESA	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PFHpA	0.68	0.89	0.84	0.59	0.55	0.62	0.36	0.39	0.42
PFHpS	0.02	0.08	0.03	0.06	0.03	0.03	0.04	0.00	0.07
PFHxA	1.84	1.92	1.85	0.99	0.90	1.03	0.54	0.57	0.61
PFHxS	0.81	0.93	0.89	0.83	0.49	0.74	0.66	0.55	0.59
PFMBA	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PFMPA	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PFNA	0.14	0.17	0.14	0.21	0.17	0.15	0.03	0.06	0.07
PFNS	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00
PFOA	3.08	3.60	3.65	1.43	1.28	1.29	1.06	0.82	1.02
PFOS	0.44	0.38	0.41	1.30	1.38	1.70	0.64	0.55	0.81
PFOSA	0.02	0.01	0.01	0.01	0.01	0.00	0.02	0.01	0.01
PFPeA	1.58	1.65	1.74	0.94	0.80	0.89	0.66	0.63	0.70
PFPeS	0.09	0.14	0.09	0.12	0.10	0.05	0.11	0.11	0.12
PFTDA	0.01	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.02
PFTrDA	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PFUnA	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.00

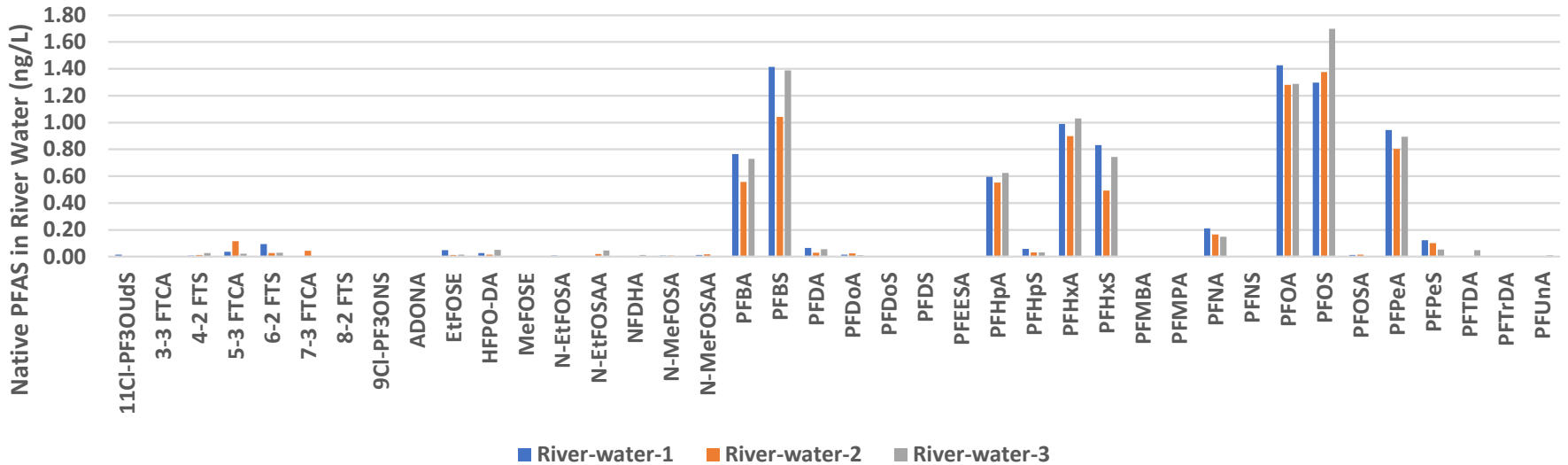
Well Water

Native PFAS 1633 in Well-water



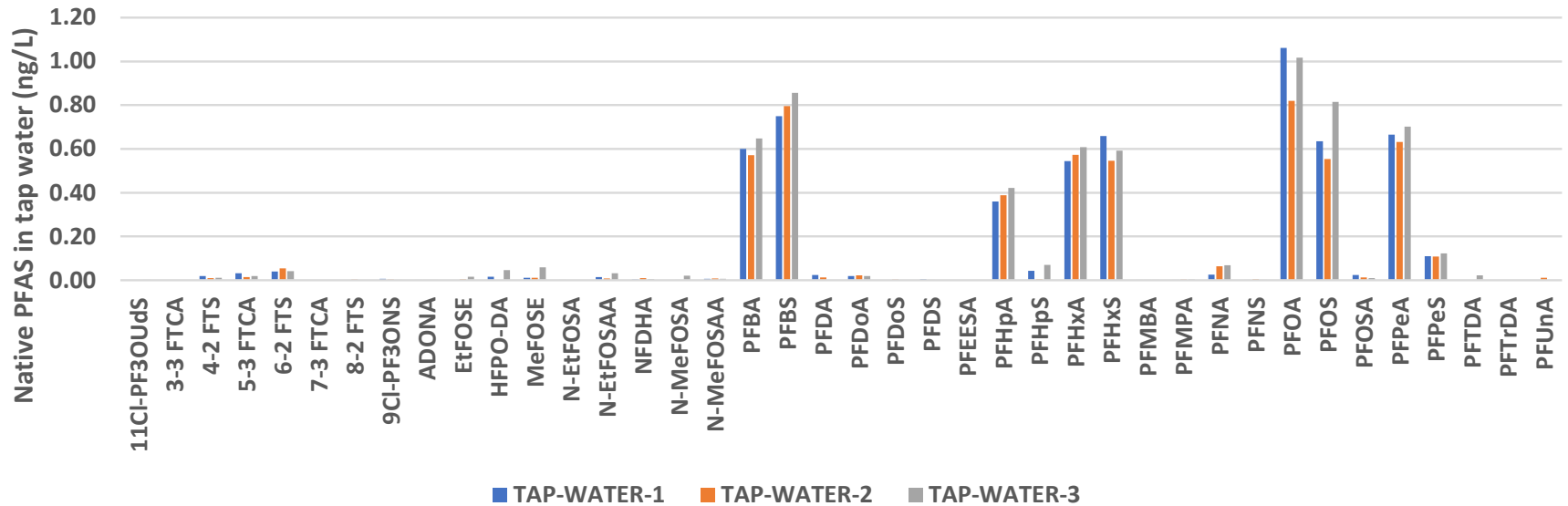
River Water

Native PFAS 1633 in River Water



Tap Water

Native PFAS 1633 in tap water



- **EZPFC and SuperVap systems are easy to use and install**
 - Complete Water Sample Prep Workflow
- **Low cost, High throughput, Low maintenance solution**
- **EZPFC Extractions and Concentration**
 - Closed System Reduces Contamination
 - Less user interactions
 - Reduces Human error
 - Bottle Rinse

- **FMS semi-automated SPE and SuperVap systems deliver consistent, reproducible results**
- **Handles a wide range of Sample sizes and matrix types**
- **Uses all SPE Cartridge sizes**

Semi Automated SPE for PFAS

- **EZPFC**
 - **2 EZPFC 12 position systems runs 24 samples a batch in 90 Minutes**

Questions

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