MicroFlow Solution for PFAS Analysis

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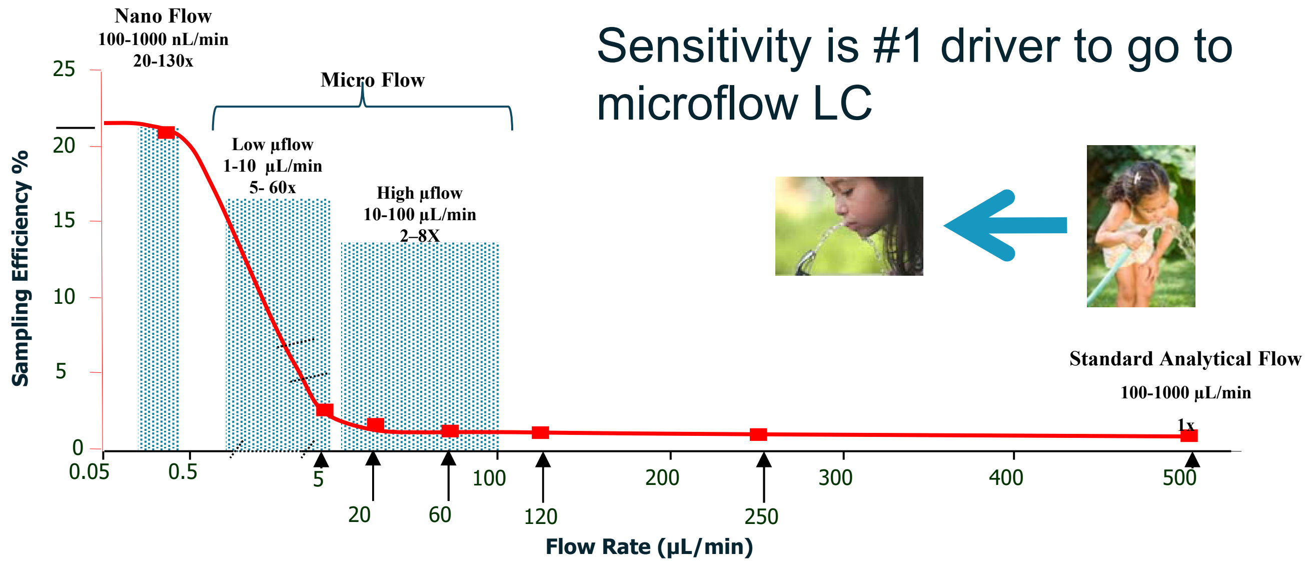
Benefits of Microflow LC-MS

Answers for Science.
Knowledge for Life.™

Microflow LC for Increased Sensitivity



Sensitivity is #1 driver to go to microflow LC

Schneider, Javaheri, Covey. “Ion Sampling Efficiency Under Conditions of Total Solvent Consumption,” 2006, RCM, 20, 1538-1544

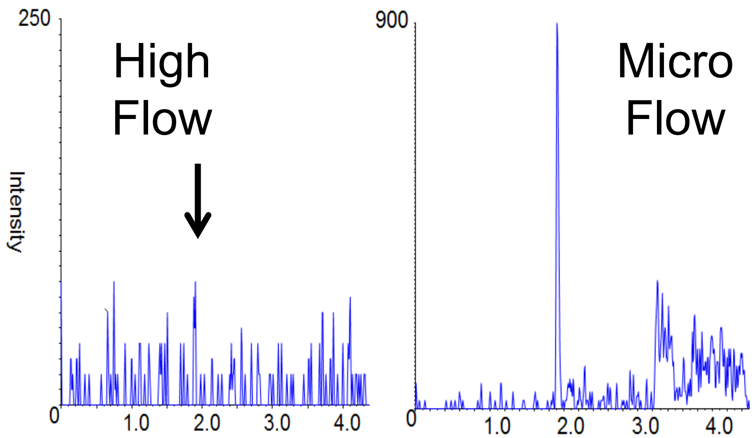
Covey.; Thomson; , Schneider. “Atmospheric Pressure Ion Sources,” Mass Spec. Reviews, 2009, 00, 1-29.

Covey, Schneider, Kovarik, Corr, Javahari, et al. “”The Central Analytical Figures of Merit of ESI, MALDI, and APCI.” In:Cole RB, ed. Electrospray and MALDI mass spectrometry. 2010, Chapter 13. Hoboken: John Wiley & Sons, Inc.

Microflow LC Benefits

SENSITIVITY – SEE THE PREVIOUSLY UNSEEABLE

- More accurate quantitation at lower LLOQs
- Higher signal-to-noise ratio for more distinct peaks → confidence
- More sensitive → use less sample & solvents



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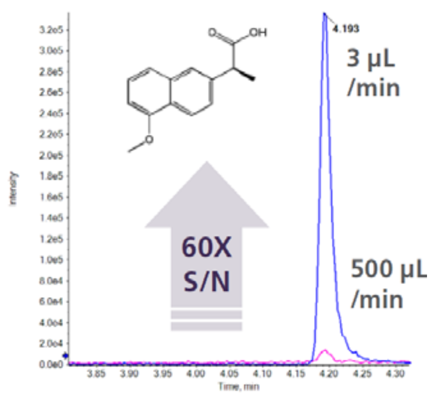
Sensitivity Improvement for Small Molecule Analytes

MICROFLOW VS ANALYTICAL FLOW

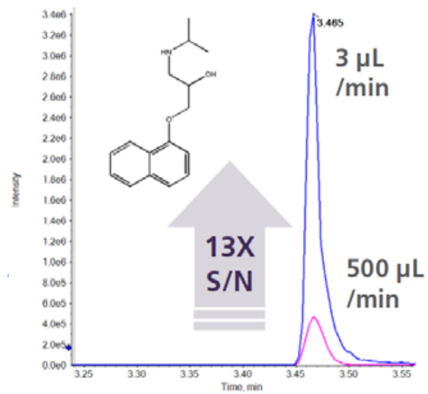
- Analytical flow assay performed at 500 µL/min (2.1 mm i.d. column)
- Microflow assay at 3 µL/min (0.2mm i.d. column)
- Concentration curve in neat solution

Compound	Avg. Area Gain	Avg. Signal-to-Noise Gain
Naproxen	25.9	60.7
Buprenorphine	6.7	13.9
Propranolol	6.1	13.4
Alprazolam	5.3	33.5
Dextromethorphan	5.2	10.7
Buspirone	5.0	8.4
Haloperidol	4.8	9.0

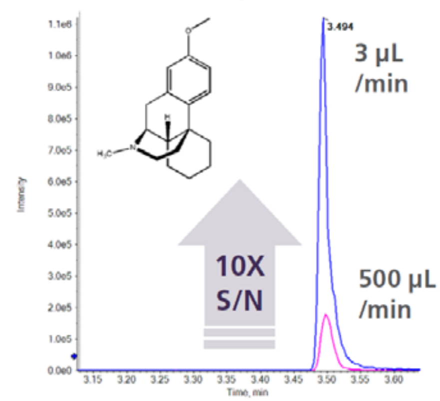
Naproxen



Propranolol



Dextromethorphan



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Why Microflow for PFAS Analysis?

- **Significant solvent savings**
  - ~100x savings
  - LC solvent is often the major cost in sample analysis
- **Lower injection volume**
  - Less matrix injected, cleaner systems, robustness
  - Reduced matrix interference
- **Easy method transfer**
  - Used same gradient used as high flow
- *Untapped potential for high sensitivity sample throughput*

Microflow Applications for PFAS

-What we tried
-How we got it to work

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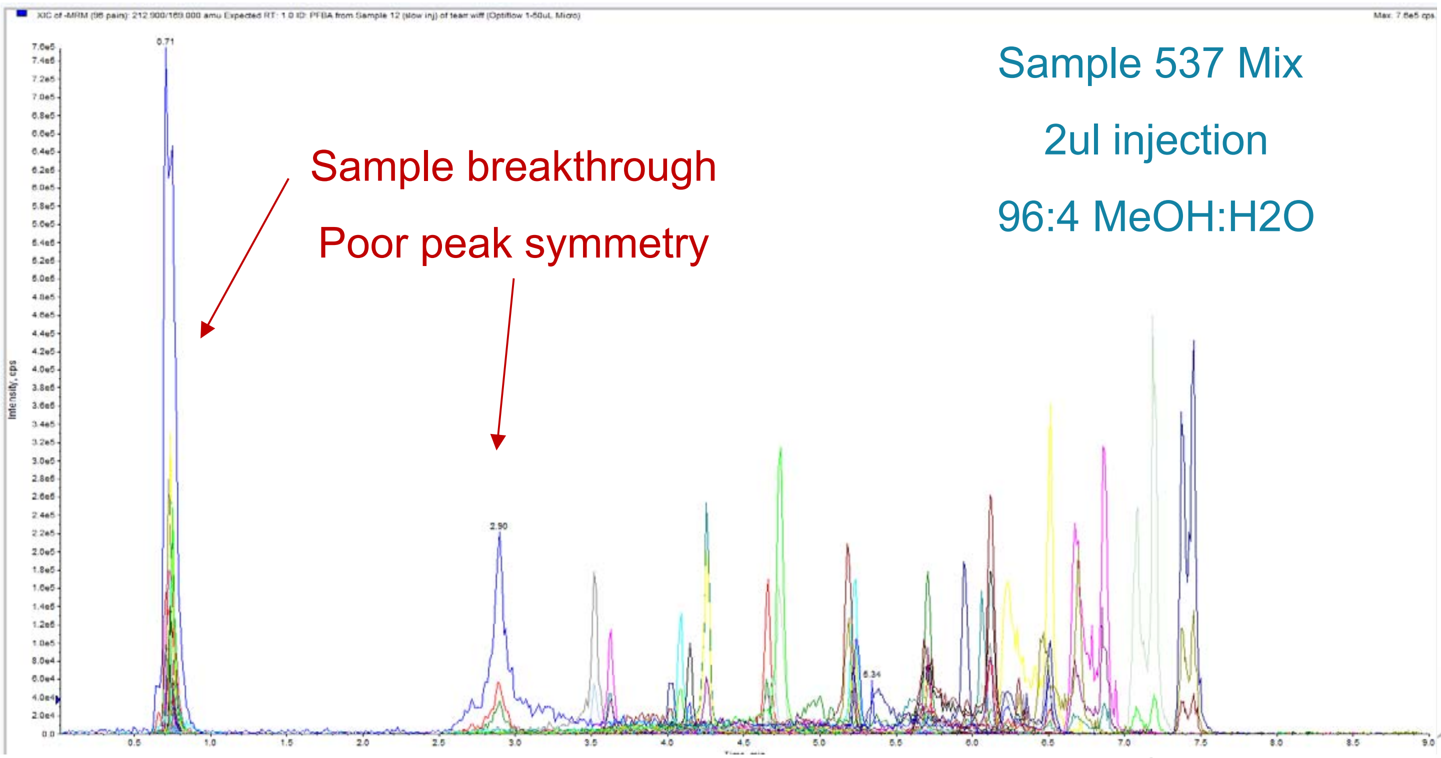
Initial Method Development

DIRECT INJECTION 2UL LOOP



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What we tried: Tee in Aqueous

GOAL: REDUCE SOLVENT STRENGTH HITTING COLUMN

Approaches tried:

1. Mixing aqueous during injection only
   - **Major problems**
     1. **Smearing**
     2. **Backflow**
     3. **Breakthrough still present**

2. Dual gradient (G1 and G2)
   - **Major Problems**
     1. **G2 is only able to go to 20uL/min**
     2. **Breakthrough still present**

Mixing Tee

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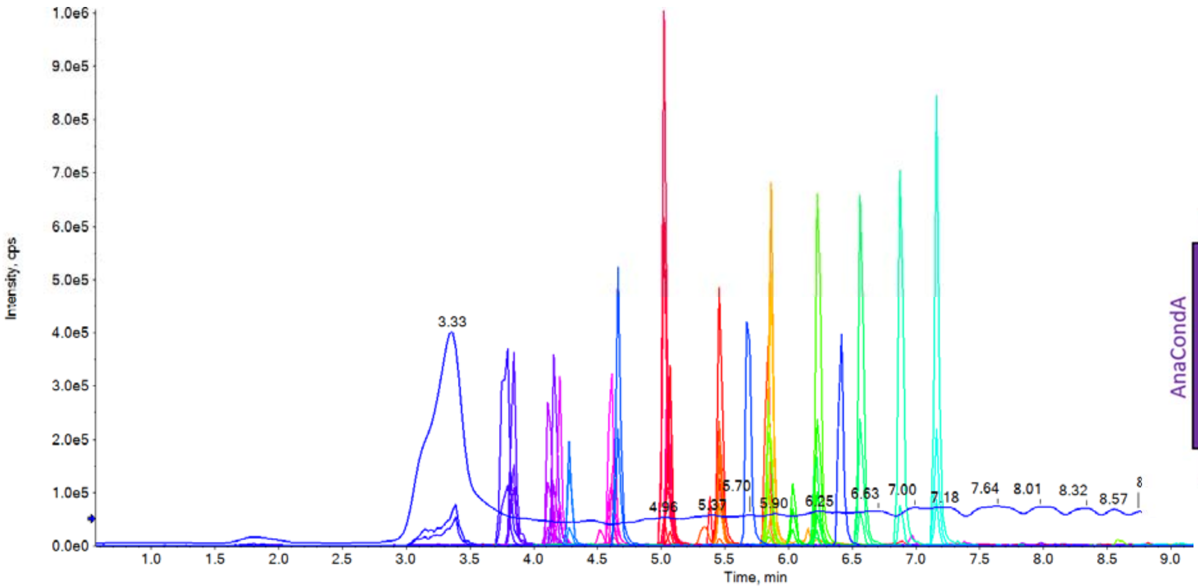
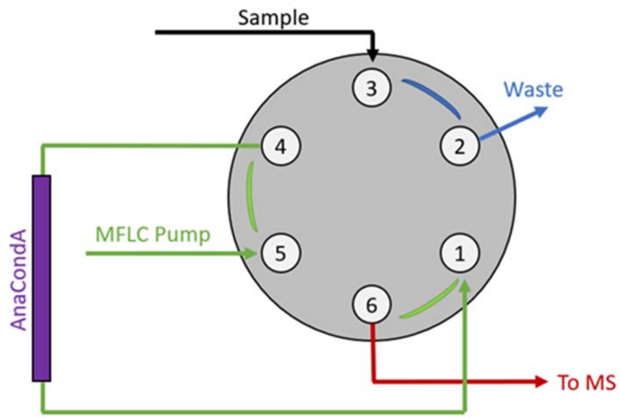
What finally worked: “AnaCondA”
(Analytical Conduit Adapter)

GOAL: DILUTE THE INJECTION “PLUG” WITH AQUEOUS MOBILE PHASE

Use 1/16in yellow stainless steel with reducers





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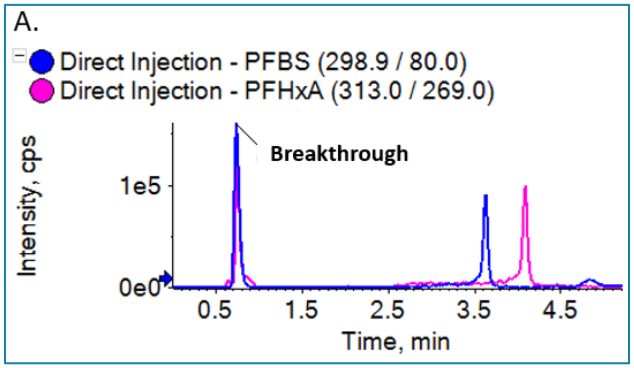
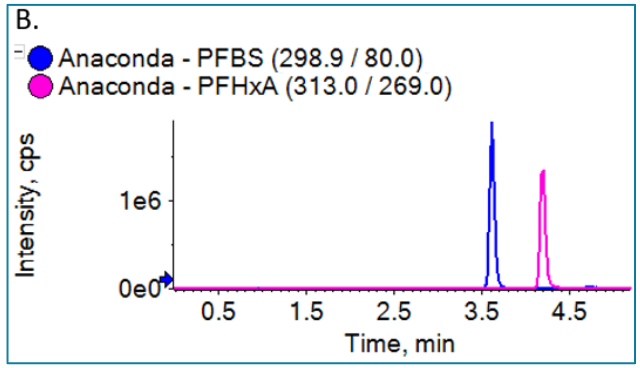
Advantage of using the online analytical conduit adapter mixer





$$Re = \frac{\rho V D}{\mu}$$

Re = Reynolds Number
ρ = Density of mobile phase
V = Velocity
D = Diameter of anaconda
μ = Dynamic viscosity of mobile phase

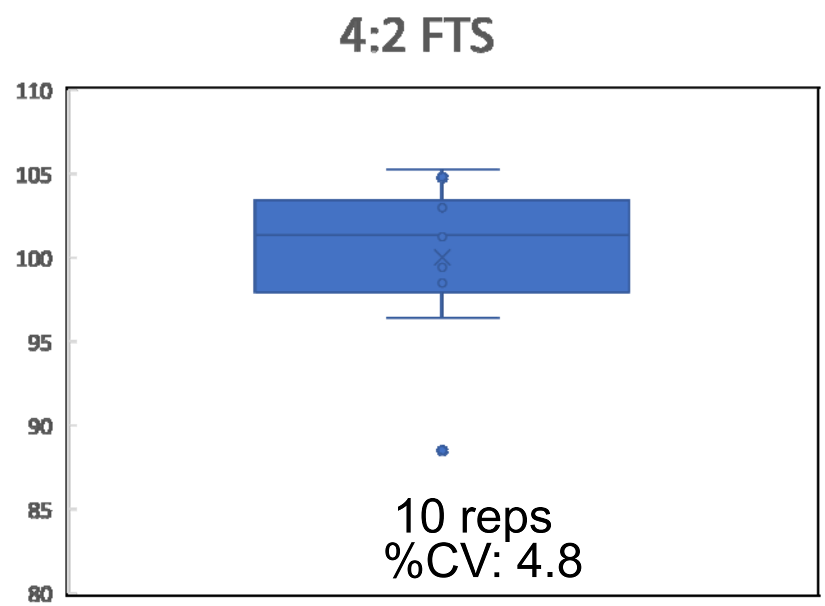
- High injection solvent strength required by EPA Method 537 causes breakthrough, even with a 1 µL injection volume
- This approach works through increasing the Reynolds number and promoting turbulence, therefore creating more mixing upstream of the analytical column

Injection Reproducibility

SLOW ASPIRATION RATE (0.1 UL/SEC) = IMPROVED REPRODUCIBILITY

Aspiration Rate	%CV
0.1 uL/sec	4.8
1uL/sec	19

*3uL injection



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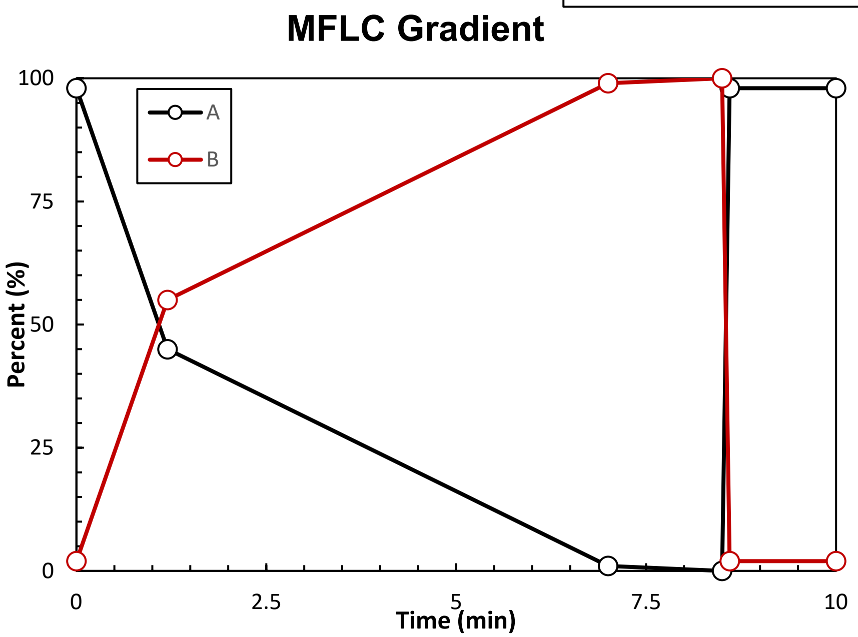
Final Flow Conditions

Same gradient as high flow, ~100x solvent savings



Column: 100 x 0.3 mm, 3 um

Solvent Savings

	Mobile A		Mobile B	
Flow Rate mL/min	0.6	0.01	0.6	0.01
	0.71	0.0118	0.014	0.0002
	1.57	0.0307	1.914	0.0008
	0.01	0.0005	0.891	0.0011
	0.00	0.0000	0.060	0.0001
	0.82	0.0000	0.017	0.0000
TOTAL (mL)	3.1	0.0429	2.9	0.0023
Cost ($)	0.30	0.0041	0.28	0.0002

	Per Sample	Per 100 Sample	Per Year (100 sample/day)
Total High Flow (mL)	6.0	600	60000
Total Low Flow (mL)	0.6	57.6	5760
High Flow Cost ($)	0.58	57.6	5760
Low Flow Cost ($)	0.004	0.43	43

Optima Fisher Water Cost $96 / L
Optima Fisher MeOH Cost $71 / L

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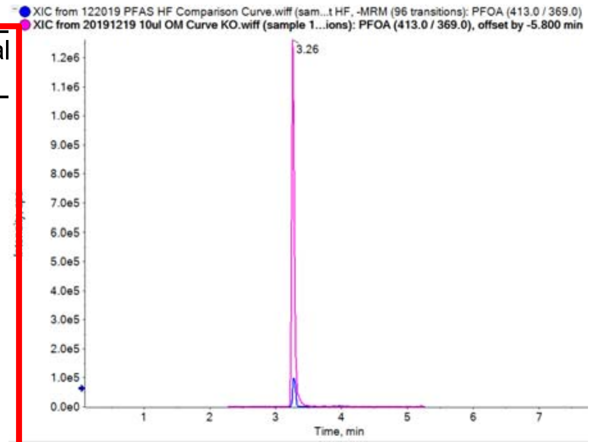
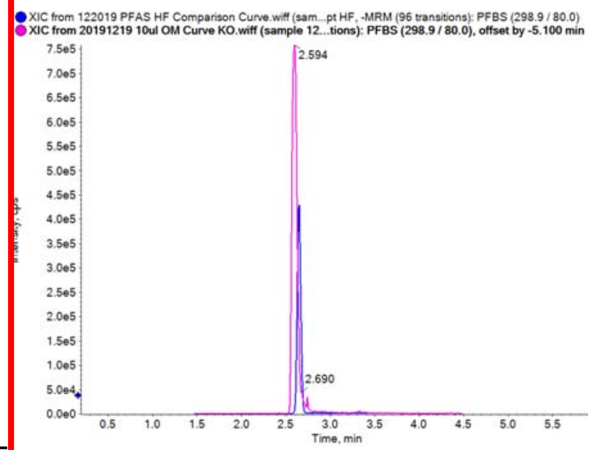
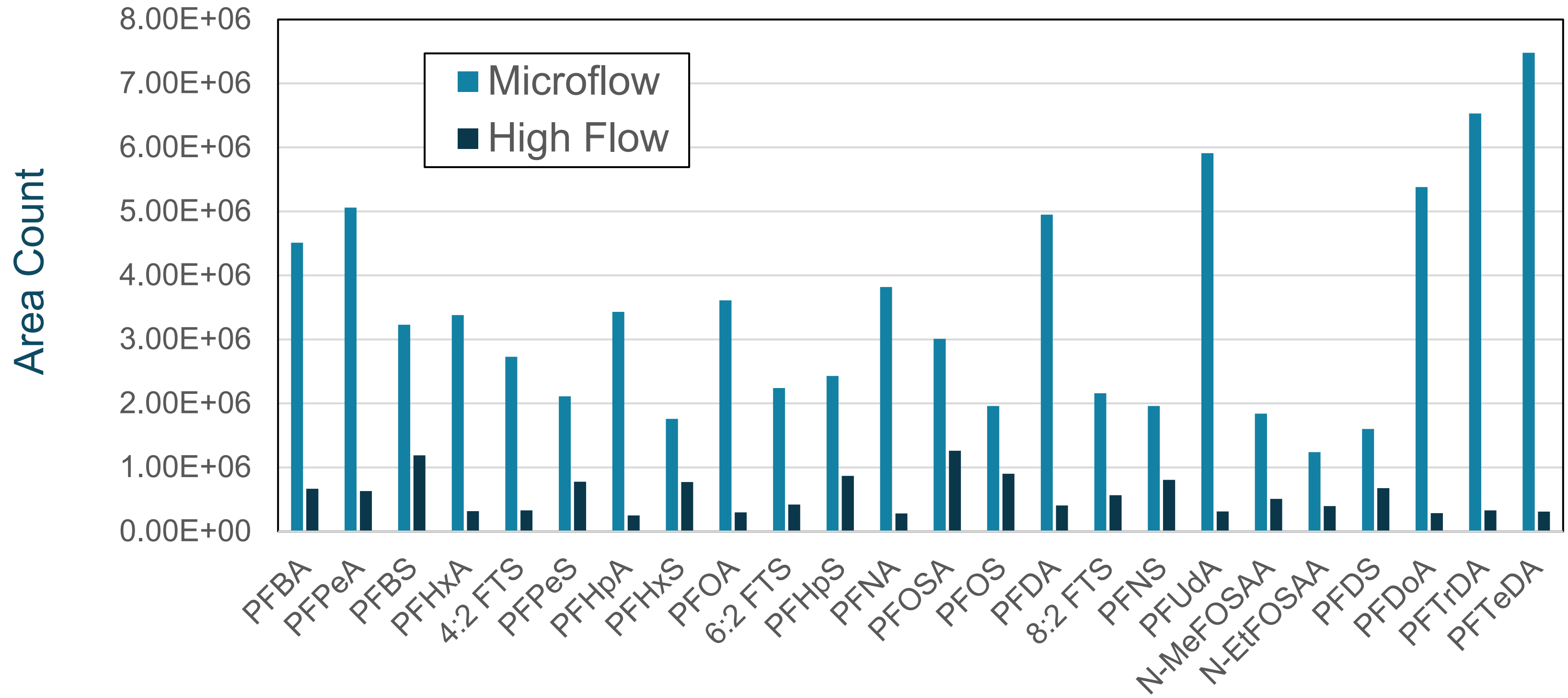
Comparison to Traditional Flow

Component Name	MFLC Area	HF Area	MFLC / Traditional Flow
PFBA	4.51E+06	6.66E+05	6.8
PFPeA	5.06E+06	6.30E+05	8.0
PFBS	3.23E+06	1.19E+06	2.7
PFHxA	3.38E+06	3.17E+05	10.7
4:2 FTS	2.73E+06	3.30E+05	8.3
PFPeS	2.11E+06	7.76E+05	2.7
PFHpA	3.43E+06	2.49E+05	13.7
PFHxS	1.76E+06	7.73E+05	2.3
PFOA	3.61E+06	2.99E+05	12.1
6:2 FTS	2.24E+06	4.20E+05	5.3
PFHpS	2.43E+06	8.67E+05	2.8
PFNA	3.82E+06	2.80E+05	13.6
PFOSA	3.01E+06	1.26E+06	2.4
PFOS	1.96E+06	9.02E+05	2.2
PFDA	4.95E+06	4.05E+05	12.2
8:2 FTS	2.16E+06	5.67E+05	3.8
PFNS	1.96E+06	8.05E+05	2.4
PFUdA	5.91E+06	3.11E+05	19.0
N-MeFOSAA	1.84E+06	5.08E+05	3.6
N-EtFOSAA	1.24E+06	3.96E+05	3.1
PFDS	1.602E+06	6.76E+05	2.4
PFDoA	5.38E+06	2.87E+05	18.7
PFTrDA	6.53E+06	3.28E+05	19.9
PFTeDA	7.48E+06	3.09E+05	24.2

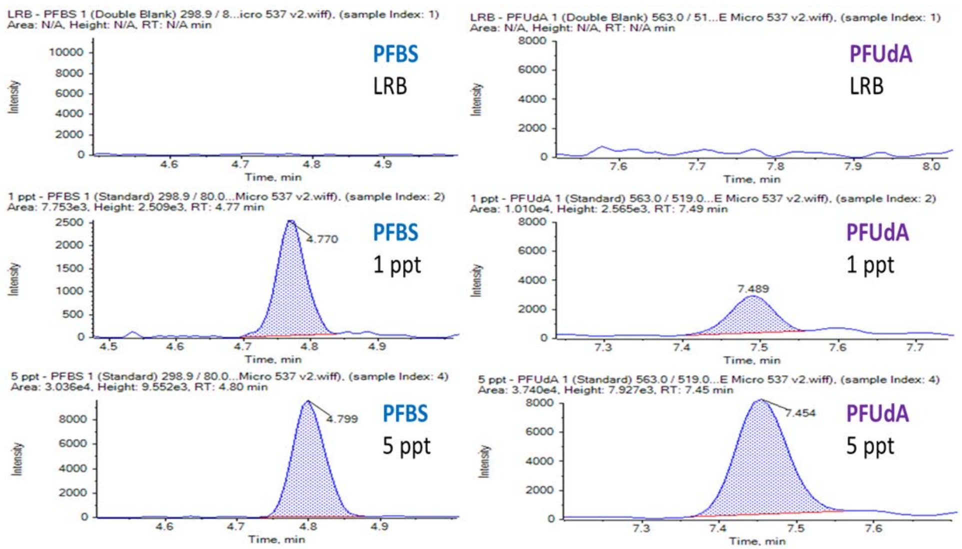




Summary: Gains in Sensitivity Using Microflow



Example LLOQ chromatograms



- Neat standards showed excellent peak profiles down to 1ppt
- Clean baselines for reagent blanks

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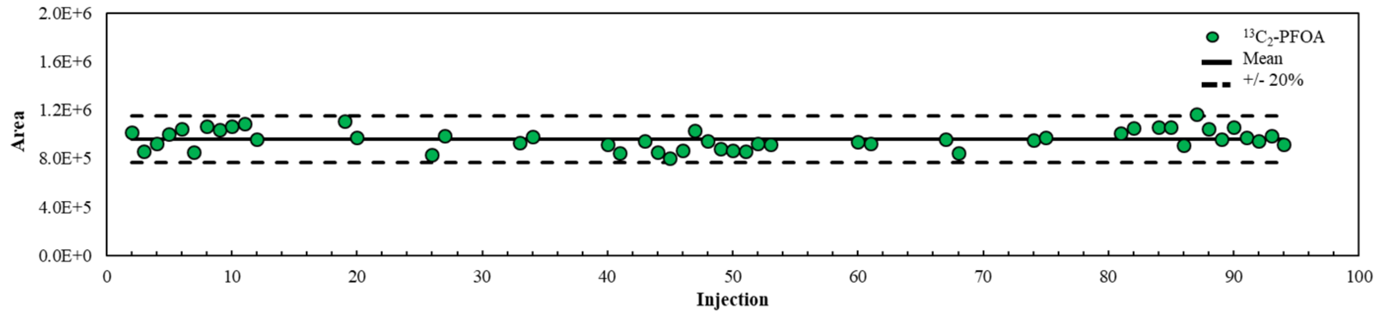
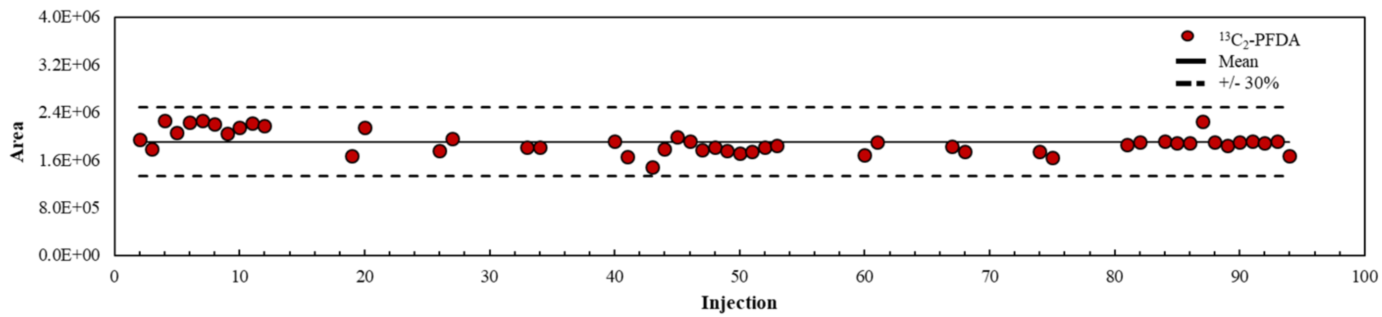
Reproducibility: Response of ISTD





- $^{13}C_2$-PFOA (used as an internal standard, top) and $^{13}C_2$-PFDA (used as a surrogate, bottom) in the analysis were plotted for all standards, QC’s and blanks

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Conclusions

- A sensitive and robust method was developed for microflow analysis of the analytes in EPA Method 537.
- The assay showed reproducibility of internal standards, surrogates, and calculated concentrations of unknown environmental samples over multiple days.
- The increase in sensitivity in this study enabled LLOQs of 1-5 ppt for EPA Method 537 with a 4 µL injection volume.
- A larger injection volume, enabled by the AnaCondA mixing approach, would allow for even lower LLOQs if necessary.

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