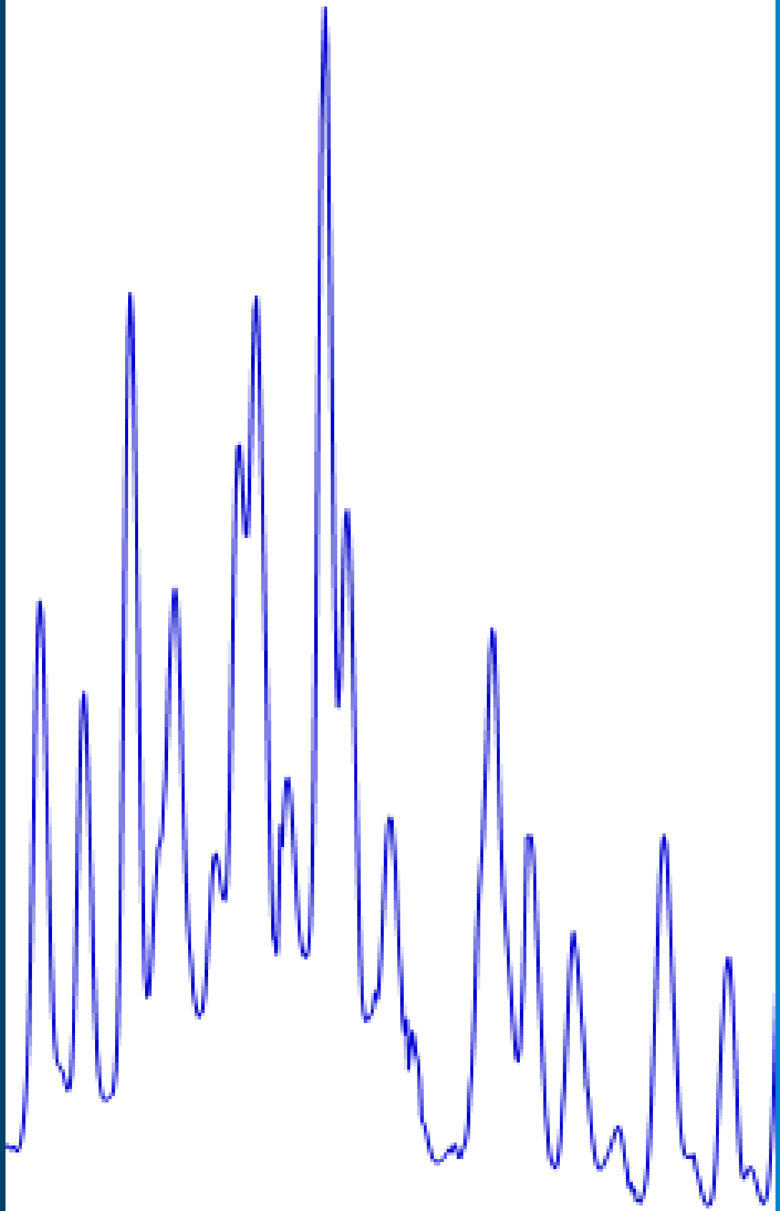
Optimizing Large Volume Injections to Achieve Ultra-Low level Detection while Maintaining Method Robustness



Emily Parry, PhD
LCMS Application Scientist

NEMC 2023

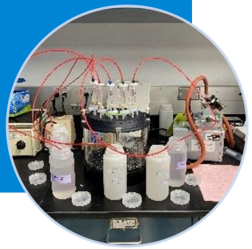
Challenges associated with PFAS Testing

- ❑ ***Increased Scope***
  - 2? Or 6? Or 14? Or 25 or….. 4000 possible PFAS, How many to measure?
  - More volatile PFAS, smaller PFAS, different structures and end groups
  - Drinking Water, Wastewater, Food, Air, Soil, Materials… How many more matrices?
- ❑ ***Extremely low detection levels and background issues***
  - Low and even sub part per trillion levels to be detected
  - PFAS are nearly ubiquitous in work environments and in lab products
- ❑ ***Fast evolving regulations***
  - New PFAS to be measured, different matrices
  - New Standard methods
  - Different Audits and Accreditations – Data Integrity, Security & Compliance
- ❑ ***Increased Throughput***
  - Faster turnaround times demanded
  - Better methodologies to quickly gauge PFAS contamination
- ❑ **Identifying new PFAS and forensic evaluation**
  - Source characterization and other tools to ID PFAS not on target lists.

Strategies to Increase Sensitivity

- Larger sample volume
- Time consuming and laborious
- Potential for increased matrix effect

Sample Preparation



- Most sensitive instrument
- Capital Purchase

Instrument Sensitivity

- Delivers more Mass on column to increase sensitivity
- Solvent Effect

Large Volume Injections

Effects of Injection solvent

Most targeted PFAS assays require at least ≥ 50% organic solvent

Method	Injection Solvent
EPA 537.1	96 : 4 Methanol : water
EPA 533	80: 20 Methanol : water
EPA 1633	96:4 Methanol : water + 1% ammonium hydroxide + 0.6% acetic acid
EPA 8327	1:1 Methanol : water + 0.1% Acetic Acid

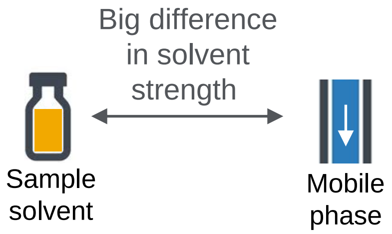
Acetonitrile is a stronger solvent = greater solvent effects

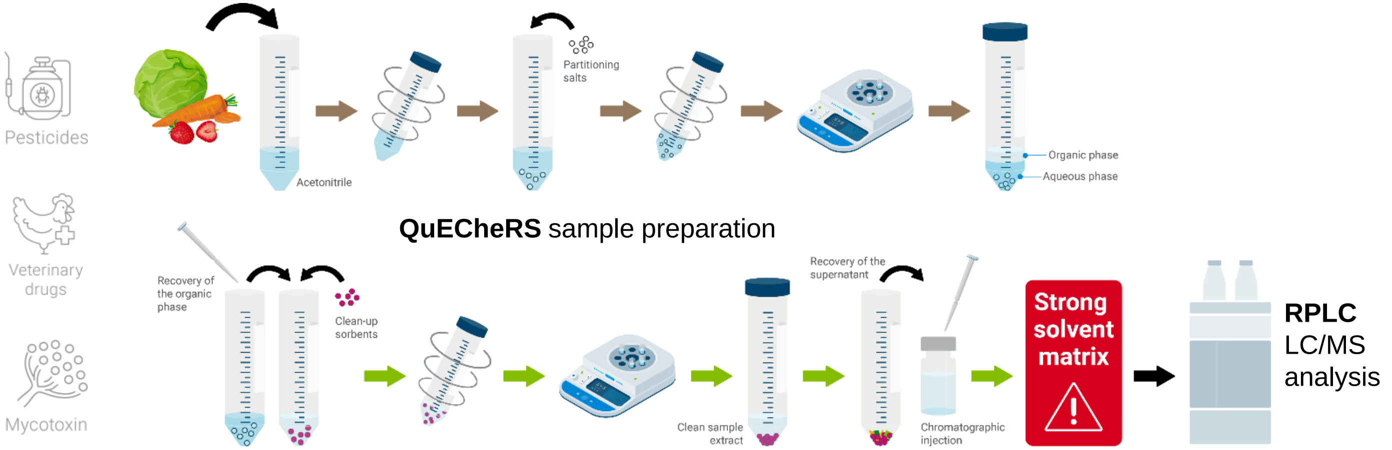
- Biological Fluids (protein precipitation
- Food extractions (QuEChERS)
  - EU recommendations in ug/kg

Foodstuff	PFOS	PFOA	PFNA	PFHxS
Fruits, Veg, Baby Food	0.002	0.001	0.001	0.004

Pain point – Challenge of Solvent Effect

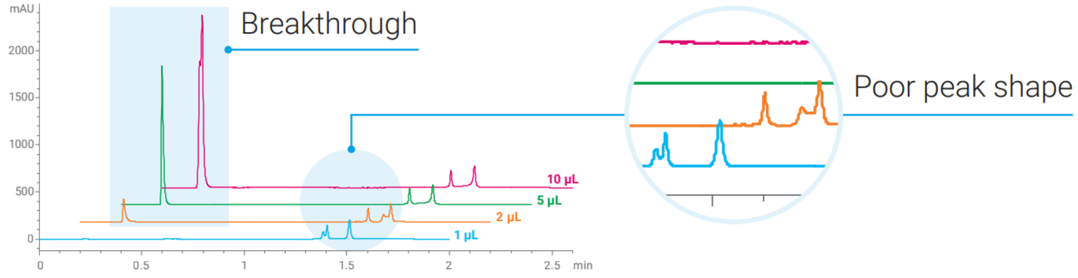
When do they happen?





What do they look like?

- Breakthrough
- Poor peak shape



What impact do they have?

- Not enough sensitivity for the analysis
- Difficult manual peak integration
- Extra sample preparation: dilution, solvent exchange

Strategies to address chromatography issues from potential sample overloading

Focusing Guard Cartridge

Injection Program

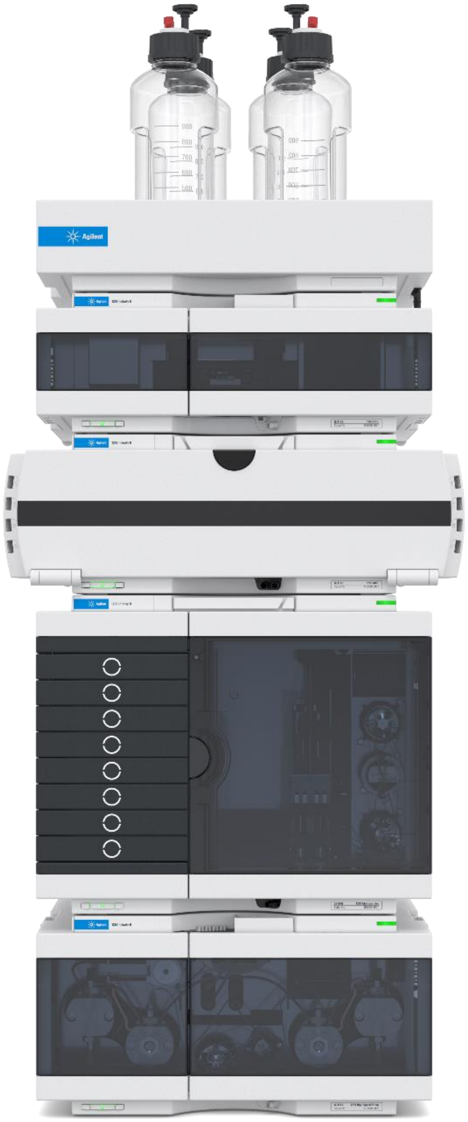
Post injection Mixing

Feed Injection

LC Conditions

Agilent 1290 Binary pump, 1290 multisampler, thermostatted column compartment

LC Conditions			
Column	Delay Column: PFC Free Column, 4.6 x 30 mm (PN 5062-8100) Analytical column: Poroshell EC-C18, 2.1 x 100, 2.7 um or Zorbax Eclipse Plus C18, 2.1 x 100		
Column Temperature	55 ºC		
Injection Volume	20-50 uL		
Mobile Phase	A: Water + 2 mM Ammonium Acetate	B: 95:5 Acetonitrile: Water	
Gradient program	Time (min)	B (%)	Flow
	Varied by Method		



EPA 1633 Analyte List

Focusing Guard Cartridge

Benefits

Good peak shape with high volume of strong solvent in sample

Reusable cartridges

Pressure limit is < 400 bar

Limited to HPLC columns

Possible background introduction

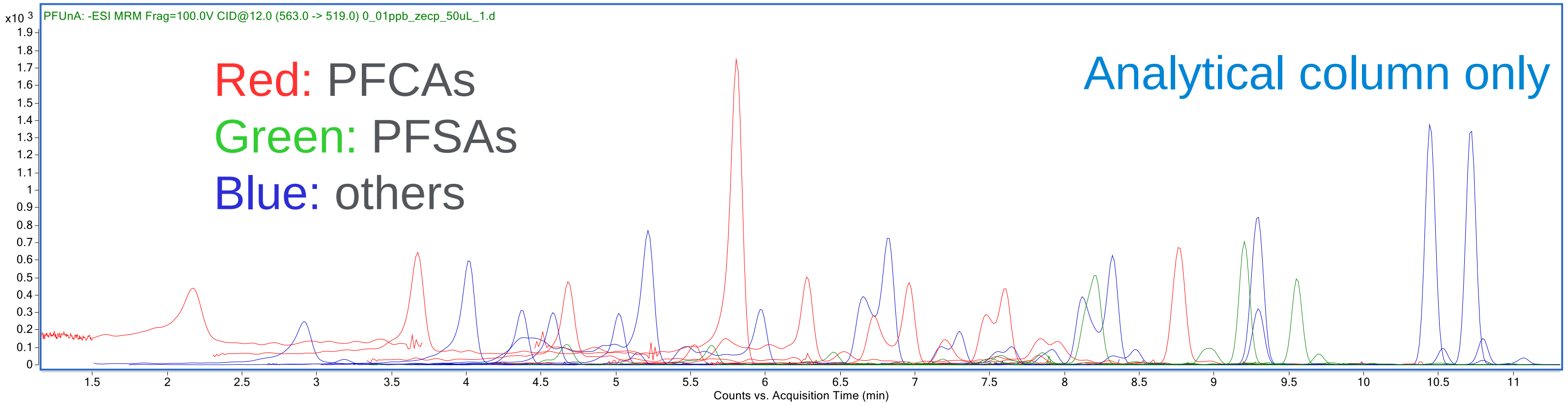
Recommendation:

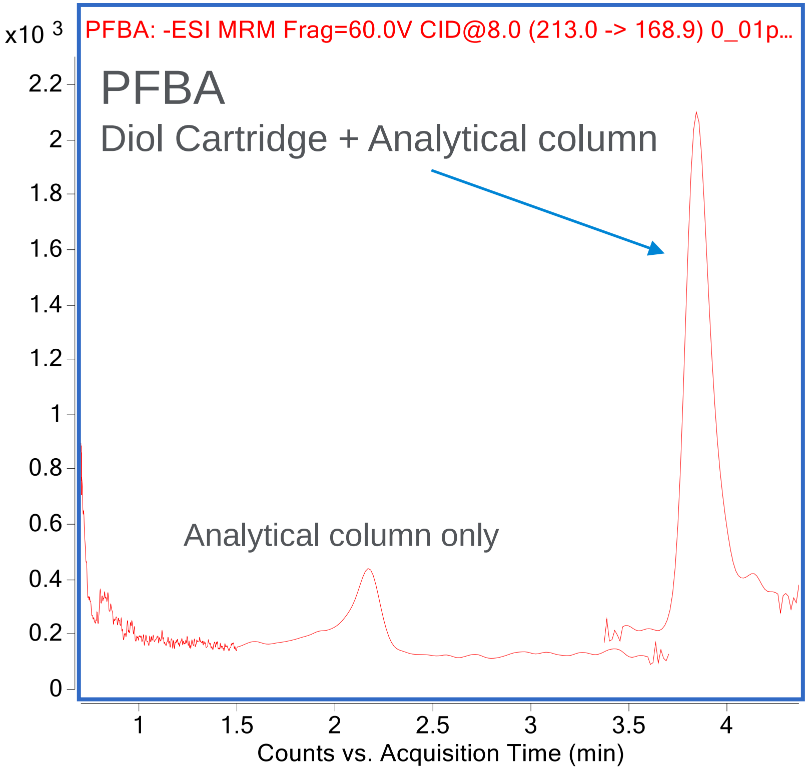
Inline filter between guard cartridge and analytical column to maintain column lifetime

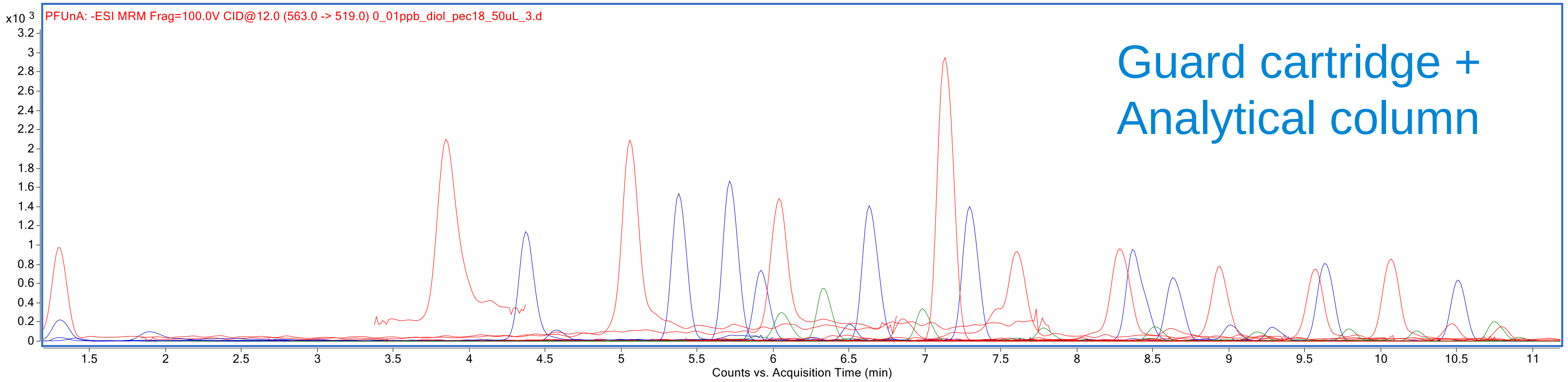
Zorbax Diol, 4.6 x 12.5
PN 820950-911

Focusing Guard Cartridge

50 uL injection of 80:20 methanol : water







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Agilent Injection Program

Sandwich Injection

Benefit

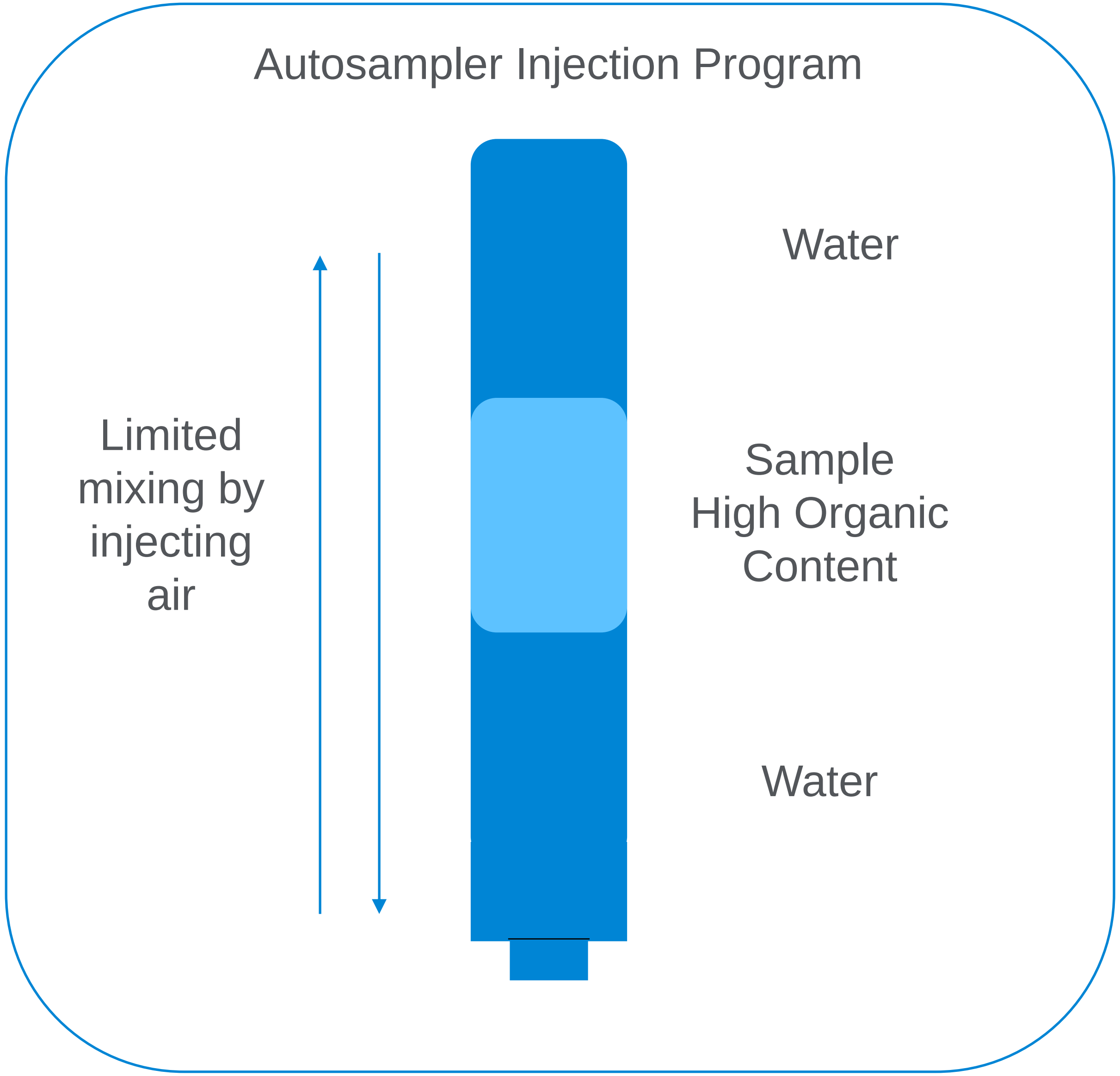
Easy to implement and improves peak shape

Requires a mixing solvent (usually water) maintained in a vial

Adds injection time

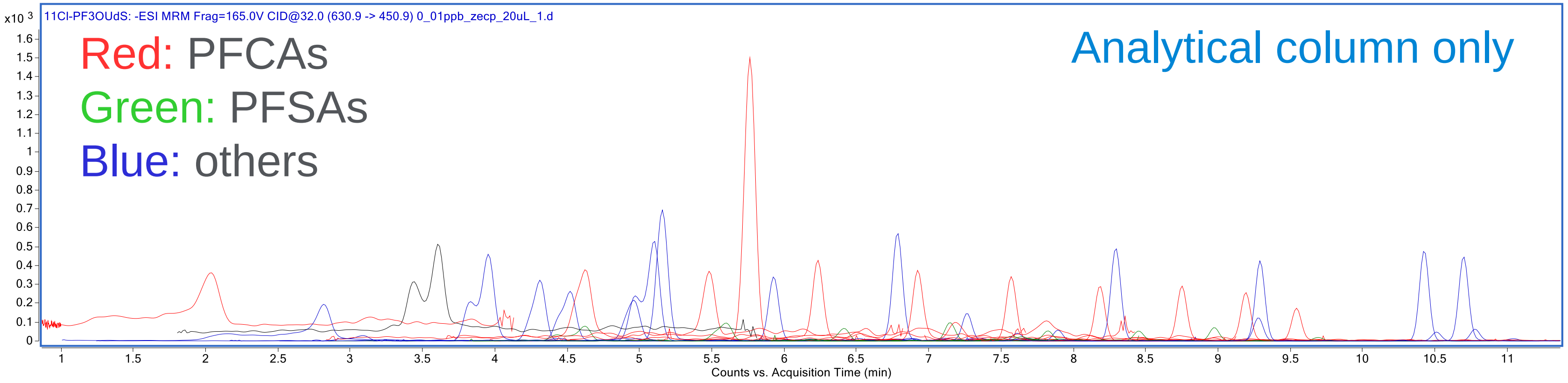
Requires a large loop and analytical head volume

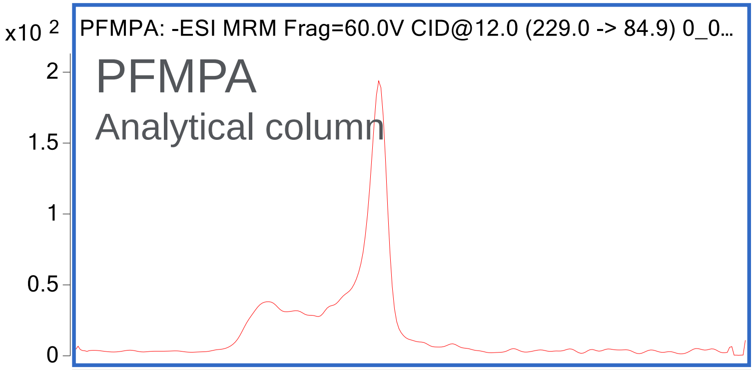
Limited Injection Volume

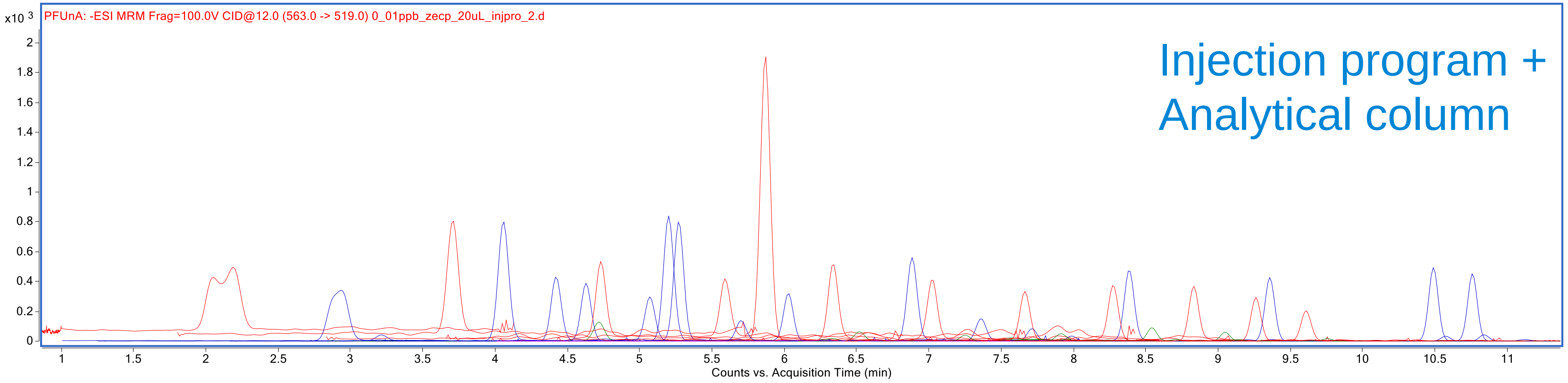


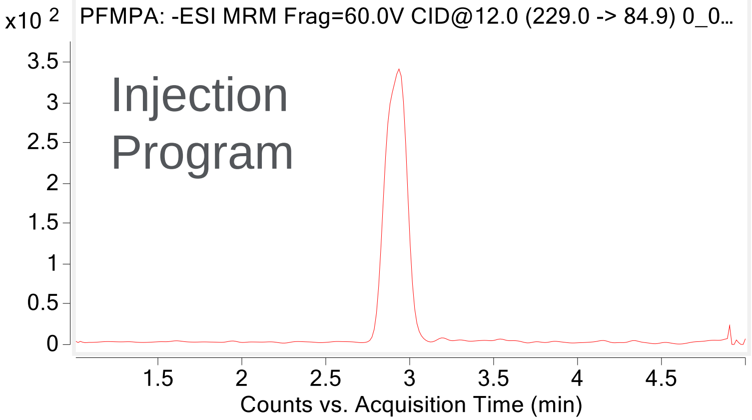
Injection Program

20 uL injection of 80:20 Methanol : water









Post Injection Mixing

<u>**Benefits**</u>

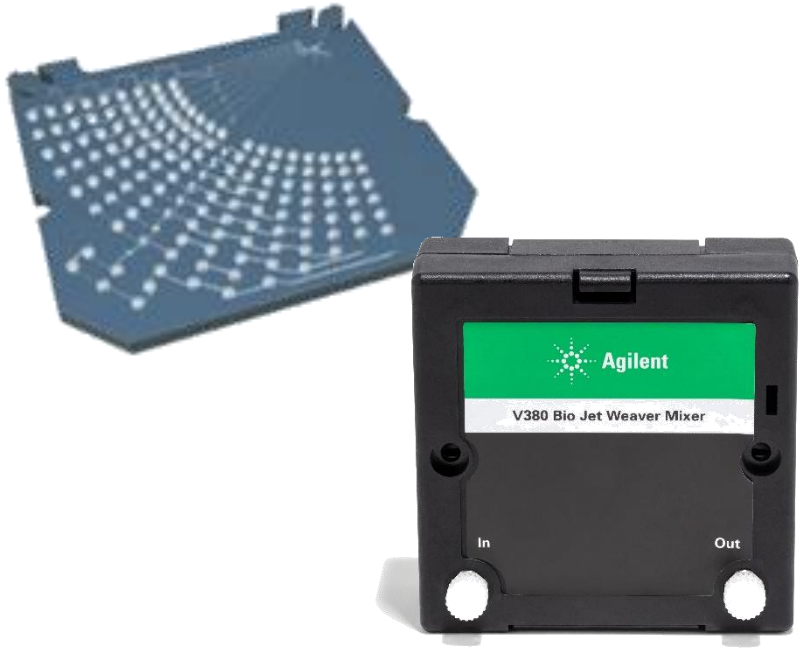
Mediates solvent effects

Requires an additional mixer or capillary length to facilitate the mixing

Adds delay volume, longer retention time

Adds dispersion, leads to peak broadening

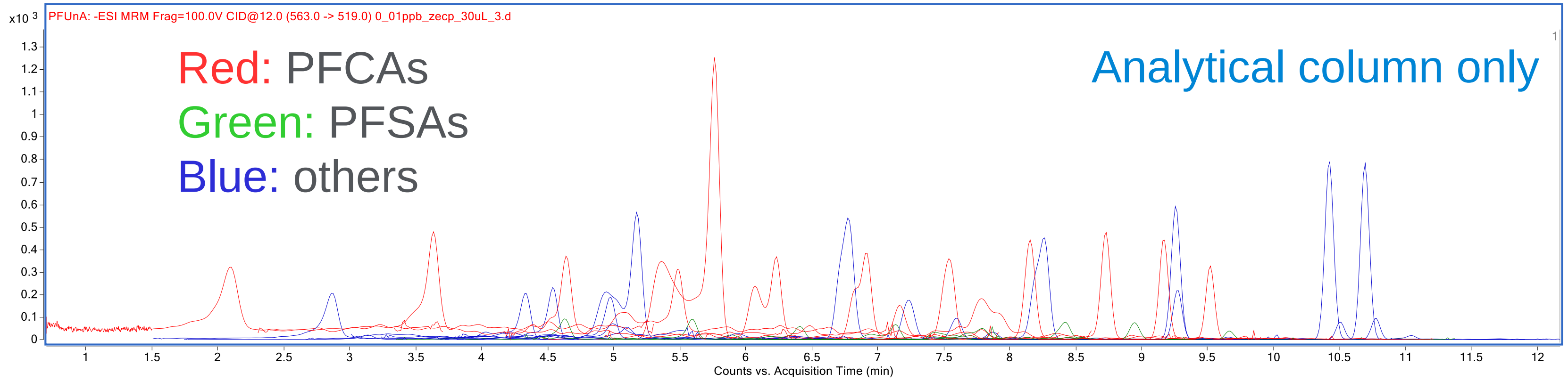
Manual instrument modification needed

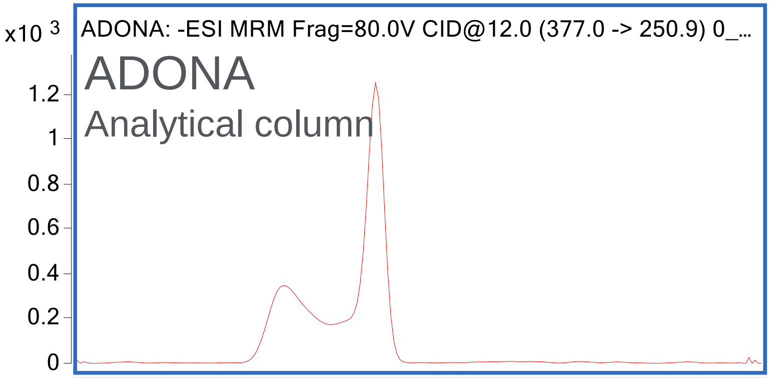


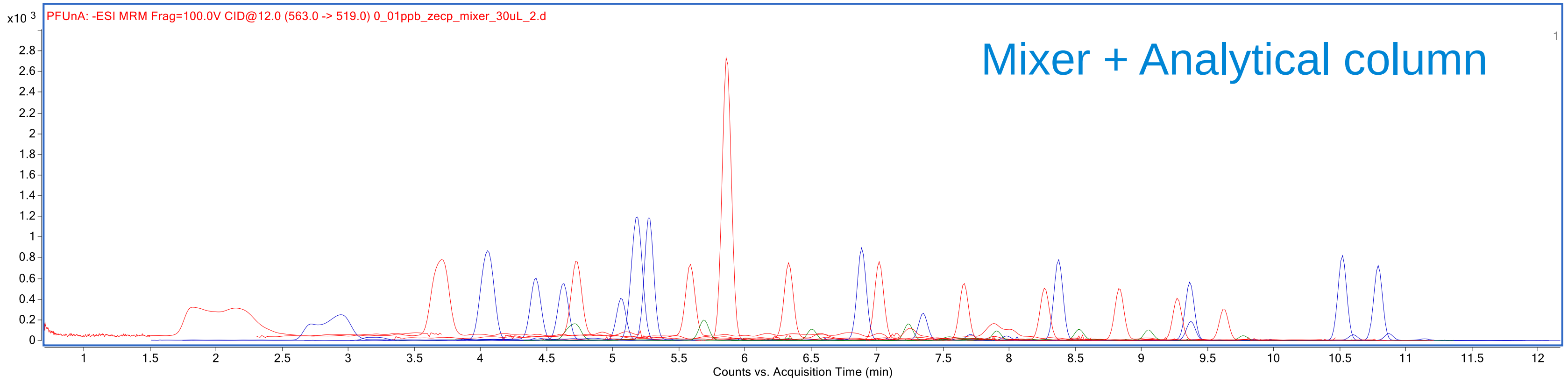
Mixing added after injection and before the analytical column

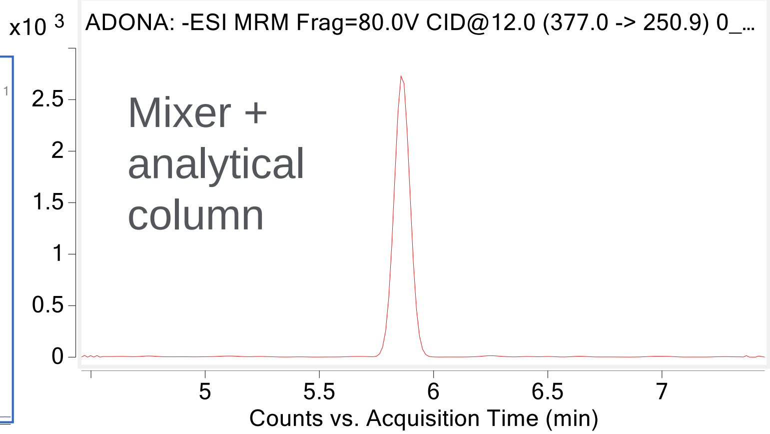
Post Injection Mixing

30 uL injection of 80 :20 Methanol : water







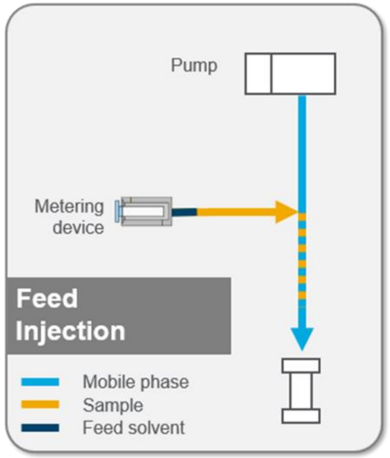


Hybrid Multisampler

Feed Injection

Credit: Matthias Kamuf, Agilent Applications Scientist

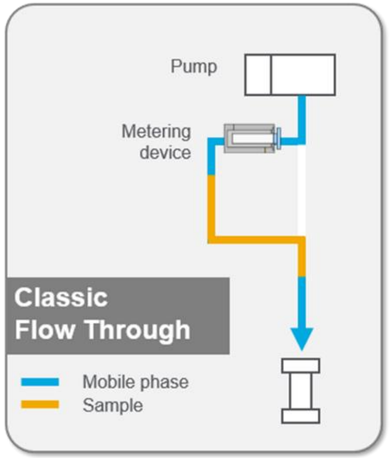
Solution – 1260 Infinity II Hybrid Multisampler Feed Injection



Solution for solvent effect

- Feed Injection
- Mediate strong solvent effect
- Agilent unique feature

- Strong sample (organic solvent)
- Low component concentration
- Requirement of high Signal-to-Noise



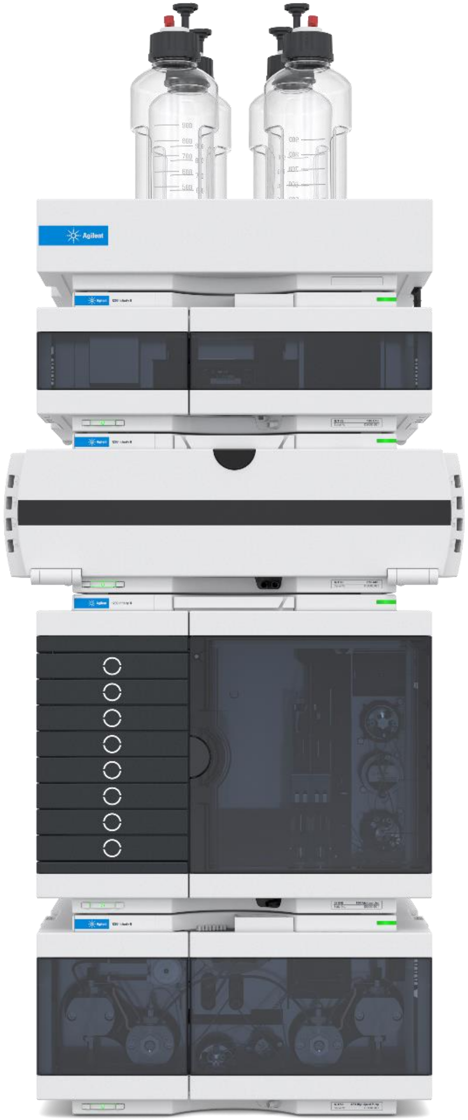
- Flow Through Injection
- Minimized dispersion
- All other autosamplers

Run legacy methods

LC Conditions

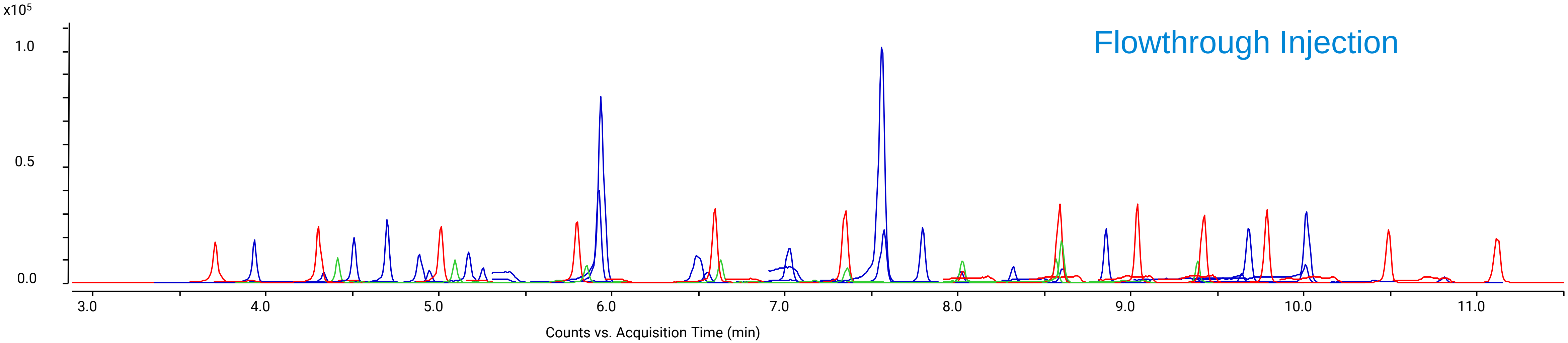
Agilent 1290 Binary pump, 1260 Hybrid multisampler, thermostatted column compartment

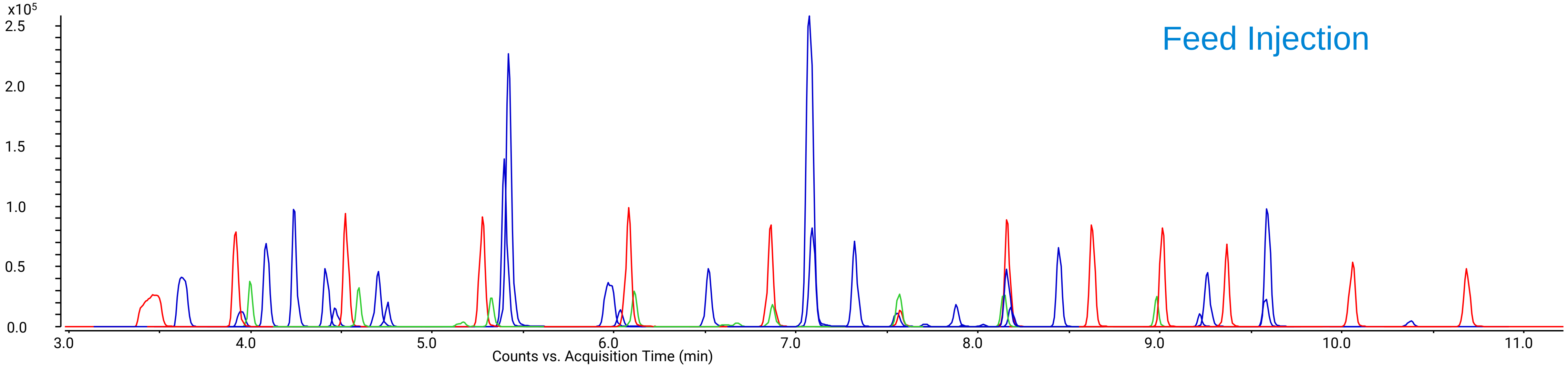
LC Conditions			
Column	Delay Column: PFC Free Column, 4.6 x 30 mm (PN 5062-8100) Analytical column: Poroshell Aq-C18, 2.1 x 100, 2.7 um (PN 695775-742)		
Column Temperature	55 ºC		
Injection Volume	5 - 40 uL		
Feed Speed	10% of flow (adaptive)		
Flushout Solvent	Mobile Phase A		
Mobile Phase	A: Water + 5 mM Ammonium Acetate	B: Methanol	
Gradient program	Time (min)	B (%)	Flow
	1.0	0	0.4
	2.0	50	
	6.0	70	
	7.5	80	
	12.5	100	
	14.9	100	
	15.0	0	
Stop Time	17.5		



Feed Injection

40 uL injection of 99.6% Methanol, mix of 47 PFAS





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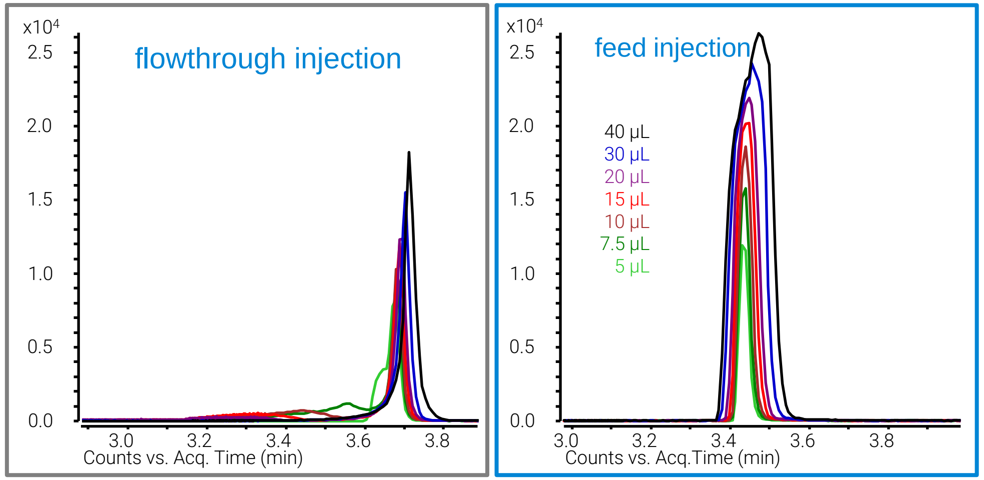
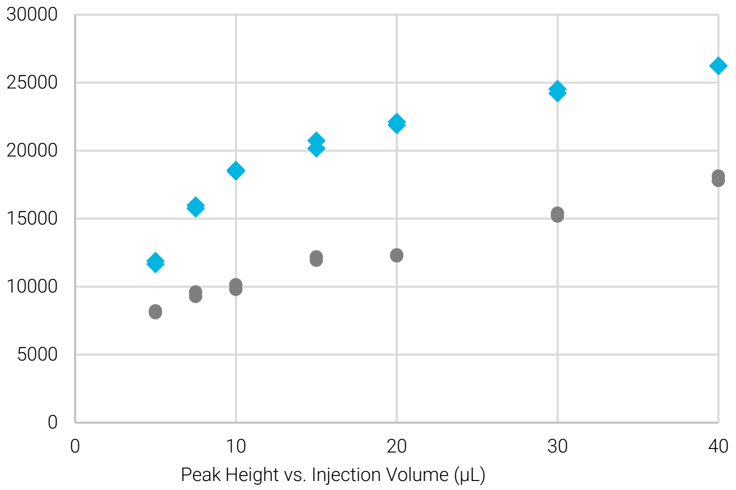
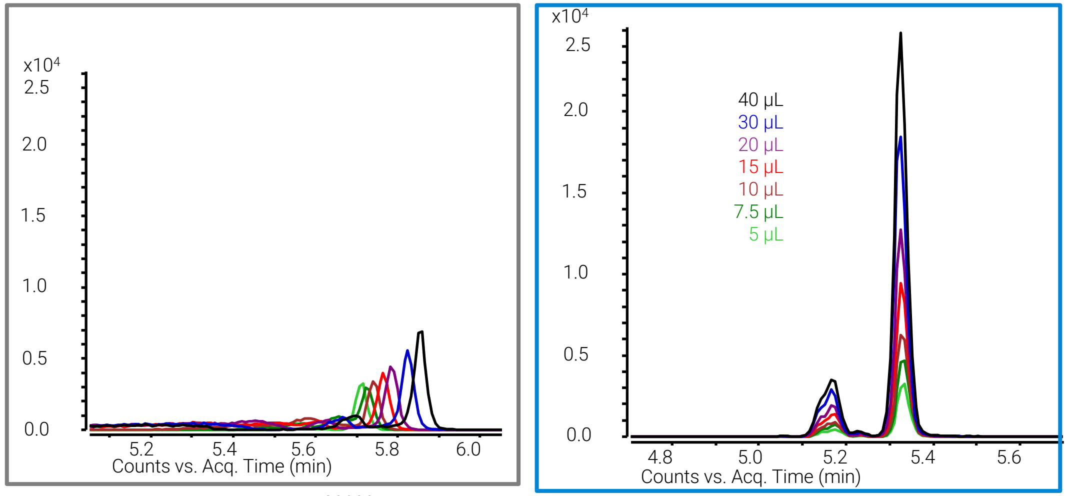
Detailed Comparison

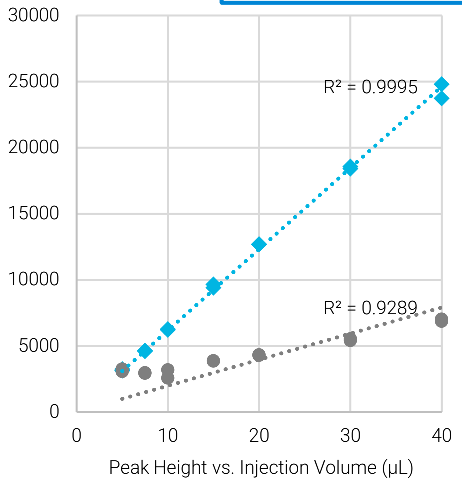
PFBA





PFHxS





Detailed Comparison

Analyte (retention time (min))	Retention factor k' at 10% MP B isocratic elution	Linearity R^2 peak height vs. injection volume, forced through zero; feed injection (flowthrough injection)	Linearity R^2 peak area vs. injection volume, forced through zero; feed injection (flowthrough injection)	Peak area %RSD 40µL feed injection n=6 (40µL flowthrough injection n=6 / 5µL flowthrough injection n=6)	Peak height (x 10^3) at 40µL feed injection (at 40µL flowthrough / at 5µL flowthrough)	Peak height increase factor 40µL feed injection vs. 40µL flowthrough injection / 40µL feed vs. 5µL flowthrough injection
PFBA (3.47)	4.8	**0.866 (0.899)**	0.999 (0.909)	0.49 (0.54 / 0.26)	26.6 (17.6 / 8.28)	1.5 / **3.2**
PFMPA (3.62)	8.9	**0.927 (0.908)**	1.000 (0.915)	0.61 (0.28 / 0.82)	40.6 (17.8 / 8.32)	2.3 / **4.9**
PFPeA (3.92)	24	**1.000 (0.908)**	1.000 (0.916)	0.41 (0.94 / 0.53)	78.1 (24.0 / 11.4)	3.3 / **6.8**
3:3 FTCA (3.95)	20	**0.971 (0.913)**	1.000 (0.879)	0.43 (3.26 / 1.62)	12.6 (4.11 / 1.97)	3.1 / **6.4**
PFBS (3.99)	45	**0.996 (0.926)**	1.000 (0.924)	0.82 (0.99 / 1.32)	37.6 (10.4 / 4.43)	3.6 / **8.5**
PFMBA (4.09)	39	**0.995 (0.918)**	1.000 (0.914)	0.59 (1.18 / 0.69)	68.2 (19.3 / 8.61)	3.5 / **7.9**
PFEESA (4.24)	91	**0.999 (0.924)**	1.000 (0.923)	0.77 (1.12 / 0.79)	100 (26.6 / 11.6)	3.8 / **8.6**
NFDHA (4.41)	n. d.	**0.999 (0.916)**	1.000 (0.928)	1.26 (0.89 / 1.02)	48.4 (12.9 / 5.80)	3.7 / **8.3**
4:2 FTS (4.46)	n. d.	**0.993 (0.907)**	0.997 (0.921)	0.99 (1.29 / 1.61)	16.1 (5.01 / 2.40)	3.2 / **6.7**
PFHxA (4.52)	n. d.	**0.999 (0.913)**	1.000 (0.929)	0.54 (1.57 / 0.71)	92.8 (23.9 / 11.4)	3.9 / **8.2**
All others (4.59 – 10.69)	n. d.	**0.988 - 1.000; MeFOSE: 0.969 (0.884 - 0.980; MeFOSE: 0.805)**	0.984 - 1.000 ; MeFOSE: 0.975 (0.826 - 0.950, MeFOSE: 0.781)	0.24 - 1.31 (NFDHA: 5.27) (0.37 - 6.70; MeOFSE: 10.6 / 0.36 - 3.26 ; HFPO-DA: 4.19)	4.81 - 259 (2.29 - 100 / 0.44 - 41.3)	2.1 - 3.7 / **3.8 - 10.9**

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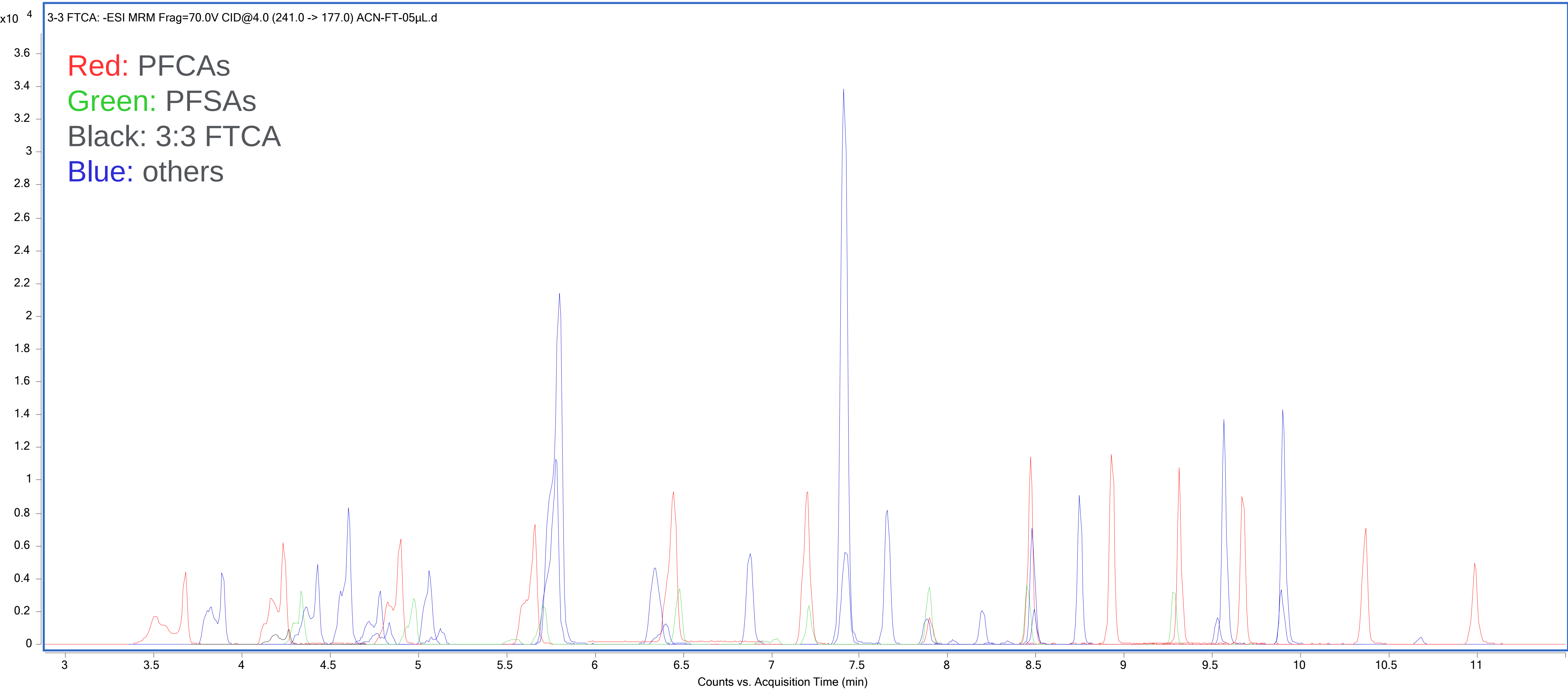
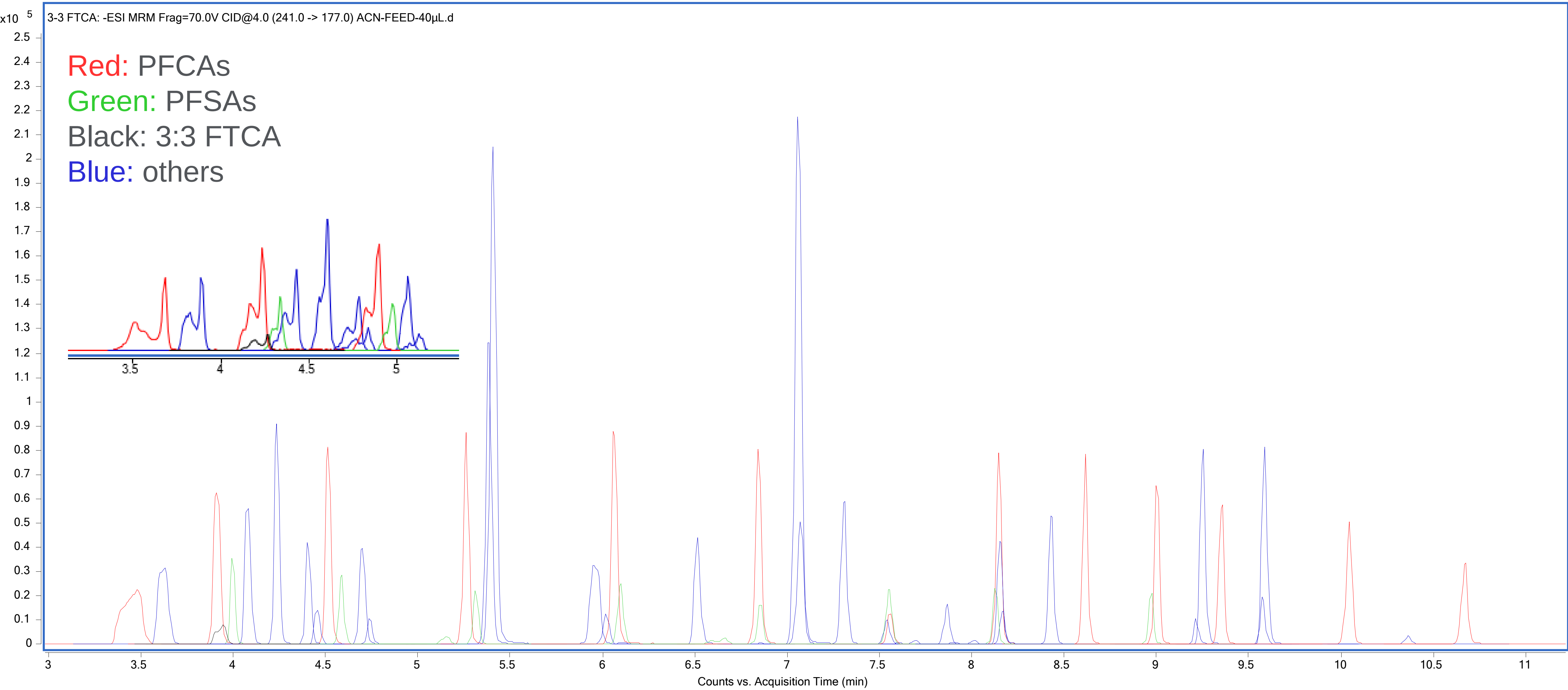
Flowthrough injection 5µL

PFAS in 90% ACN, 9.6% MeOH, 0.4% Water



Feed injection 40µL

PFAS in 90% ACN, 9.6% MeOH, 0.4% Water



Conclusions

- Focusing guard cartridge is very effective but causes method limitations
  - Inline filter recommend
  - < less than 400 bar

- Injection Program is cost effective with limitations
  - Only Limited injection volume possible

- Post injection mixing provided some improvement eliminating fronting, but overall was least effective

- Feed injection offers an efficient solution to increase sensitivity with a straightforward approach.
  - Works well with high percentage of methanol or acetonitrile in the injection solvent
  - Heavy fronting of early eluting compounds mitigated by feed injection

Acknowledgments

Agilent

Matthias Kamuf

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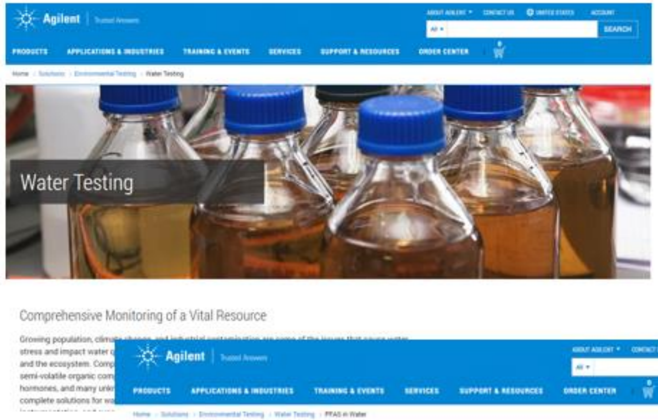
Bill Long

Tarun Anumol

David Weil

>60 NEW webpages on PFAS Testing and Water Testing added to Agilent.com

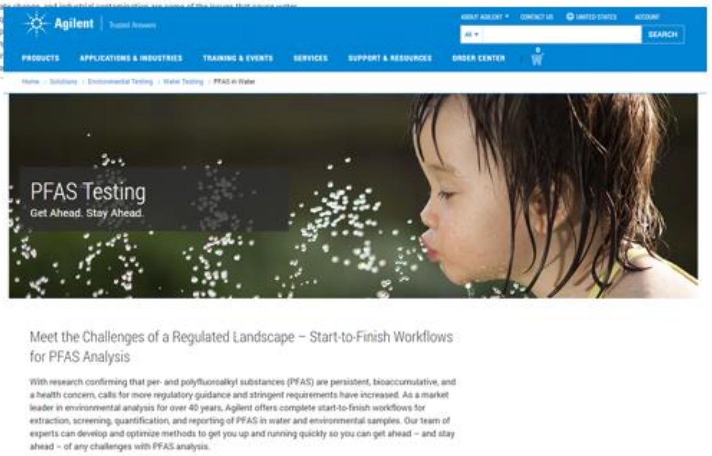
Visit the website to keep up with all PFAS information & collateral

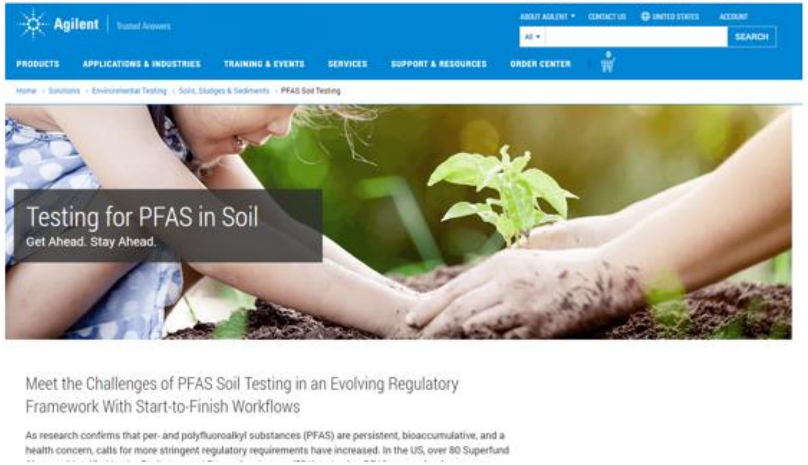


Water Testing | Agilent

PFAS analysis in Water | Agilent

PFAS Soil Testing | Agilent





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Trusted Answers