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PFAS Analysis Based Upon a pH-Variable LC Mobile Phase Gradient

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Outline

- Background
  - CA Investigative Orders
  - Distinct Analyte List (new analytes)
  - Methods Gap
- Analytical Scalability
  - Current analyte chemistry and technique
- Expanded Utility of pH Gradient

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CA Investigative Orders

Recent Timeline

- March 20, 2019 - Landfills and Airports
  - 38 analytes with RLs at 5 – 8ng/L. (23 “required”)
  - New analytes not in any validated method from EPA or ASTM (C16, C18, FOSE, x:3-FTCA)
- October 25, 2019 – Chrome Platers
  - Same 38 analytes. (25 “required”)
  - Nov 22, 2019 – Extension granted
- July 9, 2020 – POTW
  - 4 short chain analytes added (533 add-ons: PFEESA, NFDHA, PFMBA, PFMPA)
- March 12, 2021 – Bulk Fuel Storage / Refineries
  - 31 analytes. (31 required)
  - Removed x:3-FTCA, PFHxDA, PFOcDA, 10:2-FTS, PFNS

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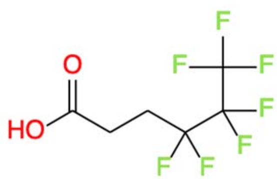
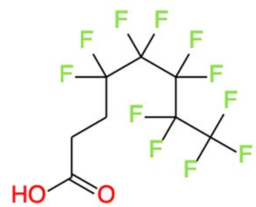
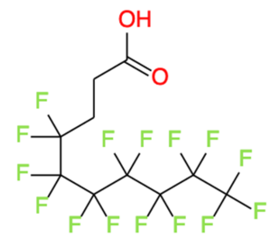
Newer PFAS Chemistry Not In Current Methods



3:3-FTCA

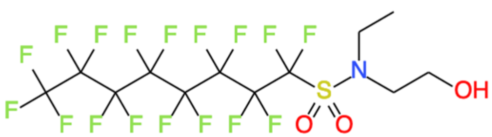


5:3-FTCA

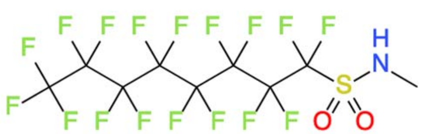


7:3-FTCA

N-MeFOSE (non-ionic)

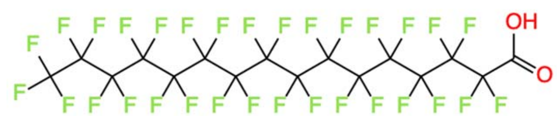


N-EtFOSE (non-ionic)



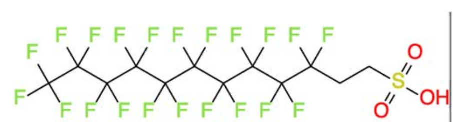
N-MeFOSA (pKa~3.47)

N-EtFOSA (pKa~3.64)



PFHxDA (C16)

PFOcDA (C18)



10:2-FTS

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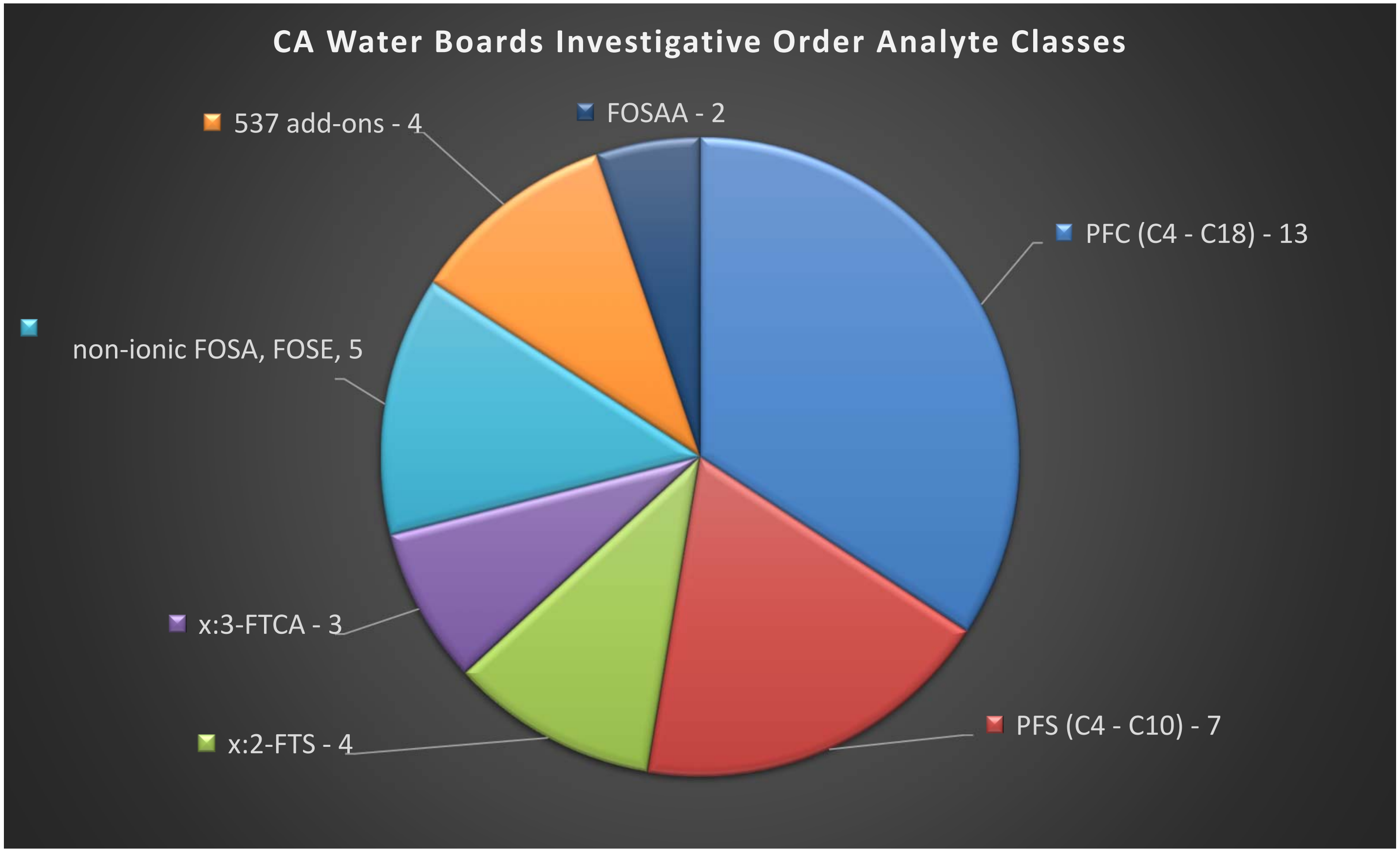
Analyte Chemistry



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Which Method?

- Non-Potable Water and PFAS
  - No standard EPA methods for PFAs in wastewater existed and the first (EPA 8327) was not promulgated yet
  - 537- Mod not standardized / “Mod” not within method scope
  - ASTM methods
    - May have scalability issues with long chain PFAS (D7979)
    - Can’t be used to comply with QSM 5.3 for trace level PFAS
  - Dept of Defense (DoD) released their QSM 5.3 (2019)
    - Has become a gold standard in the United States
    - Requires Solid Phase Extraction (SPE) and cleanup (eg: GCB)
    - Can Scale analyte List
    - **Became the choice of the CA Water Boards**

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Method Chemistry

Method	% Organic	Perfluorocarboxylate Range
EPA 537.1	96% MeOH	C6 to C14
EPA 533	80% MeOH	C4 to C12
EPA 8327	50% MeOH	C4 to C14
ASTM D7979	50% MeOH	C4 to C14

- EPA 533 and 537.1 prescribe extract composition
- Ease of use for EPA 8327 / ASTM D7979 is that it's a 1:1 dilution (50% Organic)
- QSM 5.3 does not require a particular % Organic in the extract although SPE and cleanup is required

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Scalability/Constraints

- Need to do PFBA and PFOcDA (C4 to C18)
  - PFOcDA has solubility issues in >10% water
    - Finding PFOcDA in water samples is unlikely
    - Extract must be 100% organic → can't use ASTM D7979
  - PFBA is difficult to chromatograph in 100% organic
    - Lower LC gradient starting pH to 3-4 to promote protonated form
  - Long chain PFAS stick to column at low pH
    - Increase pH (>9) during LC gradient to minimize tailing
  - Most LC columns not compatible with high pH
    - Use Phenomenex Kinetex EVO product
- All other PFAS fit well into this framework although non-ionic better suited for GC-MS/MS
  - In LC-MS, N-MeFOSE and N-EtFOSE detected as $[M+HOAc-H]^-$
  - Product ion is acetate at mass 59 (not as selective)

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Data Quality Objectives

- Meet DOD QSM 5.3 + Table B-15
  - Calibration/ICV/CCVs (standard)
  - Daily instrument sens. check at LOQ +/-50%
  - Retention Times
    - RT vs Exp RT (+/- 0.4min)
    - RT quant ion vs qual ion (+/- 2sec)
    - RT vs isotope RT (eg: PFOA and 13C-PFOA +/- 0.1min)
  - Ion Ratios 50-150% expected ratios
  - Use specific transitions for specific analytes unless matrix is demonstrated and documented

RT Stability Required

***Not an exhaustive list of all requirements

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Instrument Method /Extraction

Table 1 - MS Conditions

Mass Spec	Thermo TSQ Vantage	
Cap Temp	250	C
Vap Temp	300	C
Sheath gas	40	arb
Aux Gas	15	arb
Sweep Gas	0	arb
CID	1.0	mTorr Argon
Q1 FWHM	0.4	Da

Table 2 - HPLC Conditions

HPLC	Agilent 1100
Column	C18 EVO 100x2.1mm
Delay Column	C18 EVO 50x2.1mm
Column Temp	35C
Inj Vol	10uL
Eluent A	20 mM Acetic Acid
Eluent B	25 mM Ammonium Hydroxide in Methanol

Time	% A	% B
0.00	95	5
1.20	55	45
3.60	35	65
11.00	10	90
13.00	10	90
13.01	95	5
17.00	95	5

Table 3 - SPE Sample Prep

Cartridge	Strata-WAX/GCB 200/50mg
Sample	Check pH. Buffer using phosphate if not between 6-7
Condition	10mL 0.1% NH4OH in MeOH
	10mL MeOH
	10mL Phosphate Buffer pH=7
Load	250mL
Wash	5mL 0.1% Formic Acid in 50:50 Water: Methanol
Dry	2 mins
Elute	4mL 0.1% NH4OH in MeOH
Soak	2 mins
Elute	4mL 0.1% NH4OH in MeOH
Vap	Using N2 just below 2mL
	Adjust to 2mL using 100% MeOH

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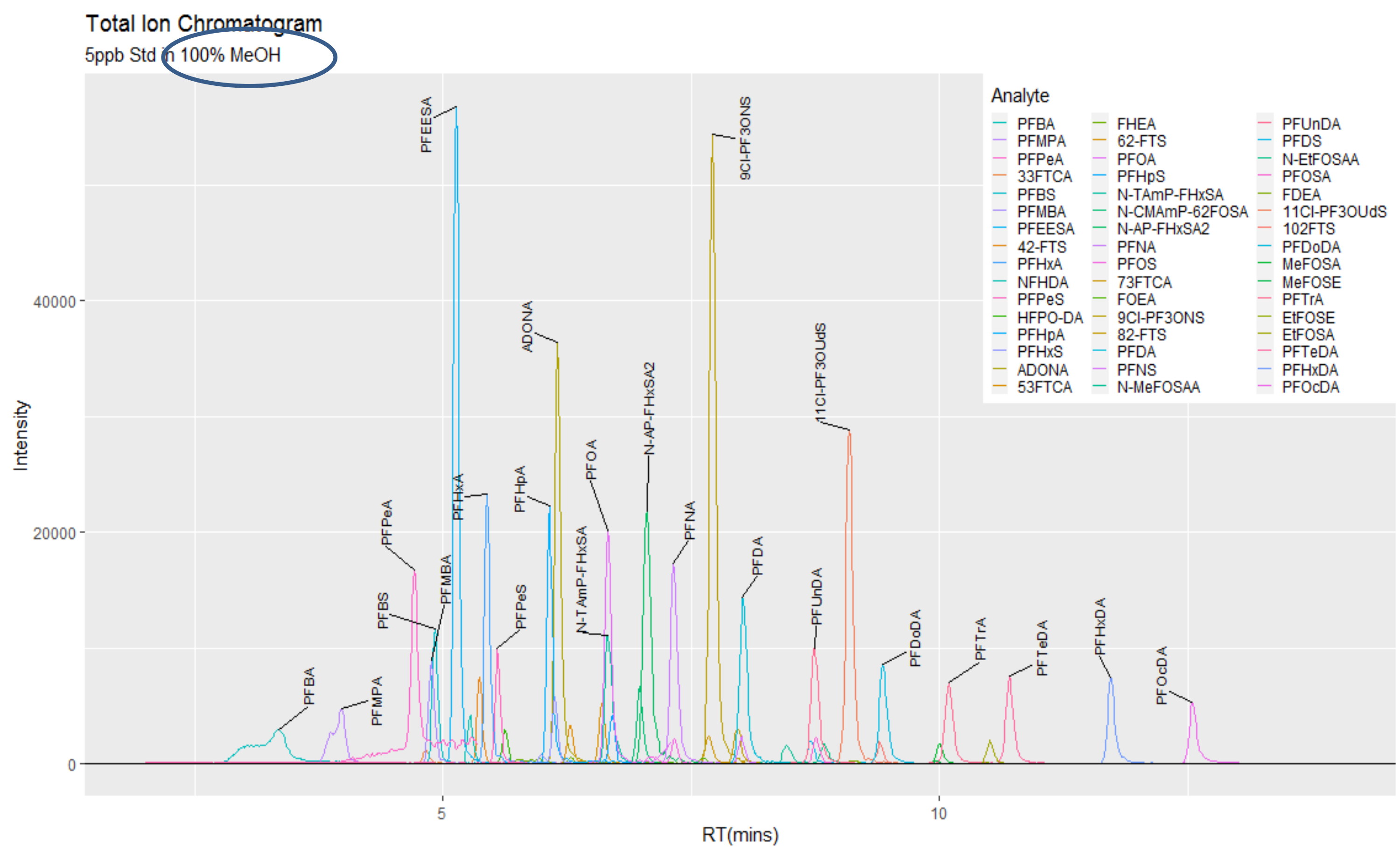
20mM Ammonium Acetate



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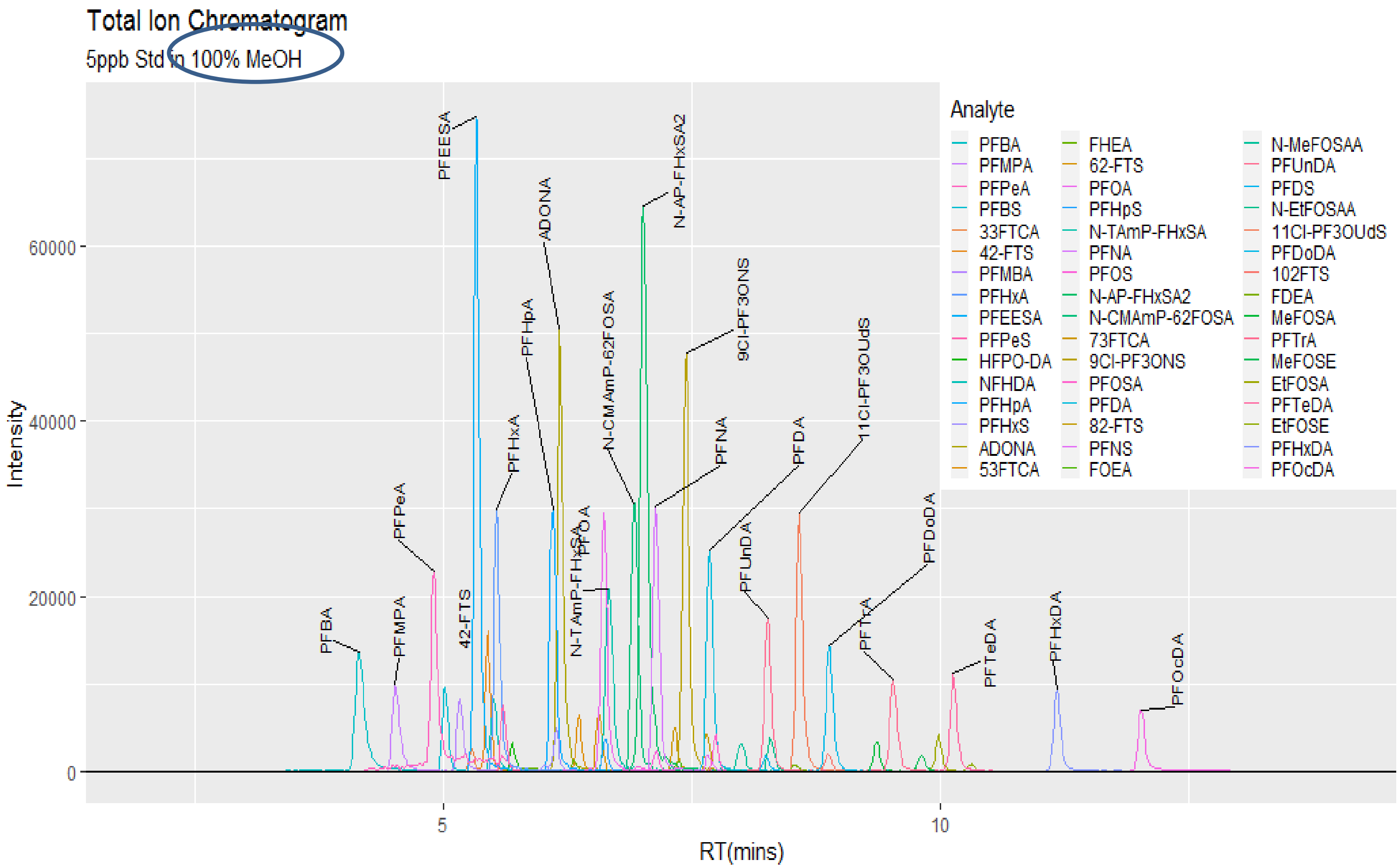
pH Gradient Elution



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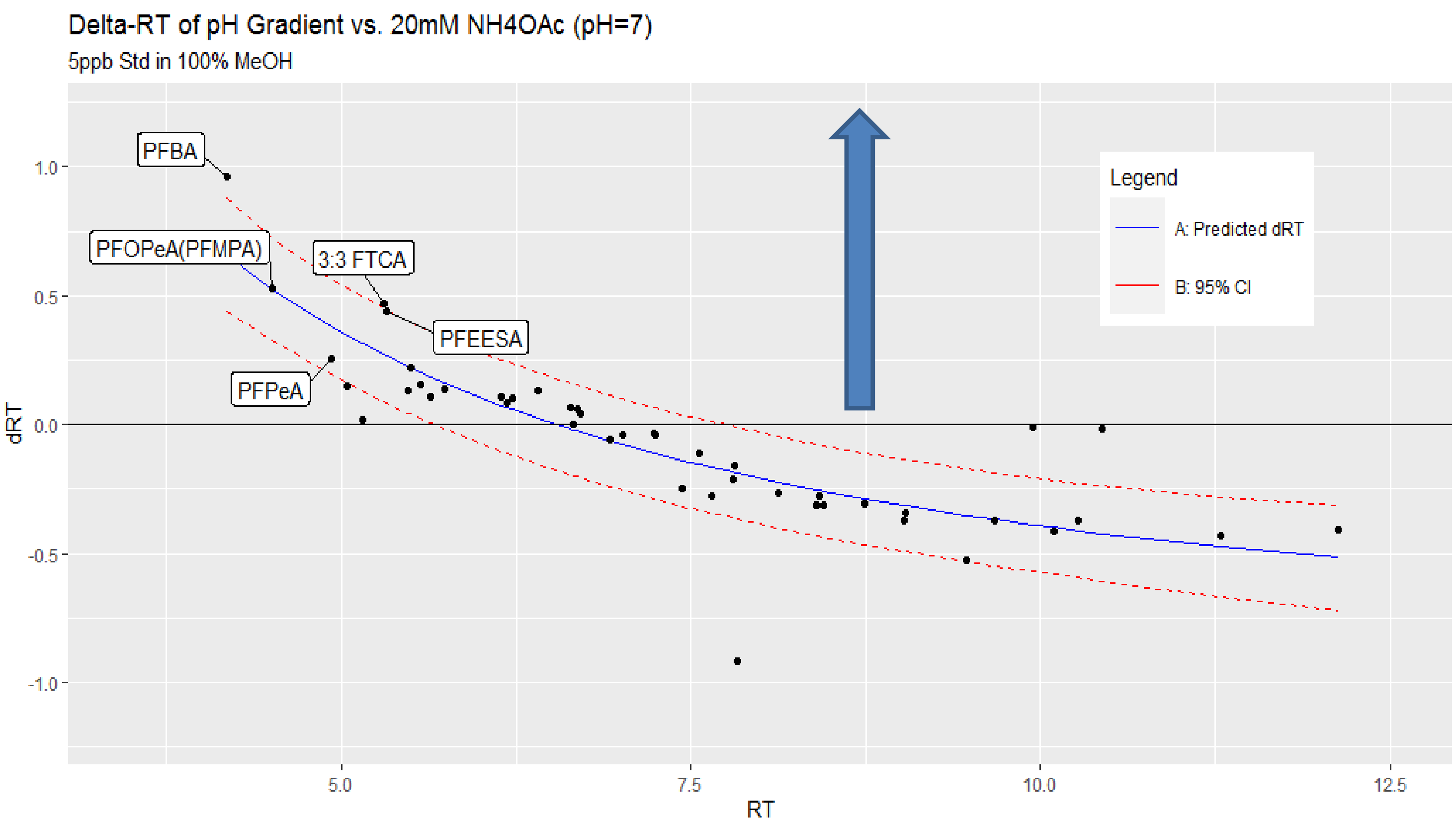
Details of pH Gradient

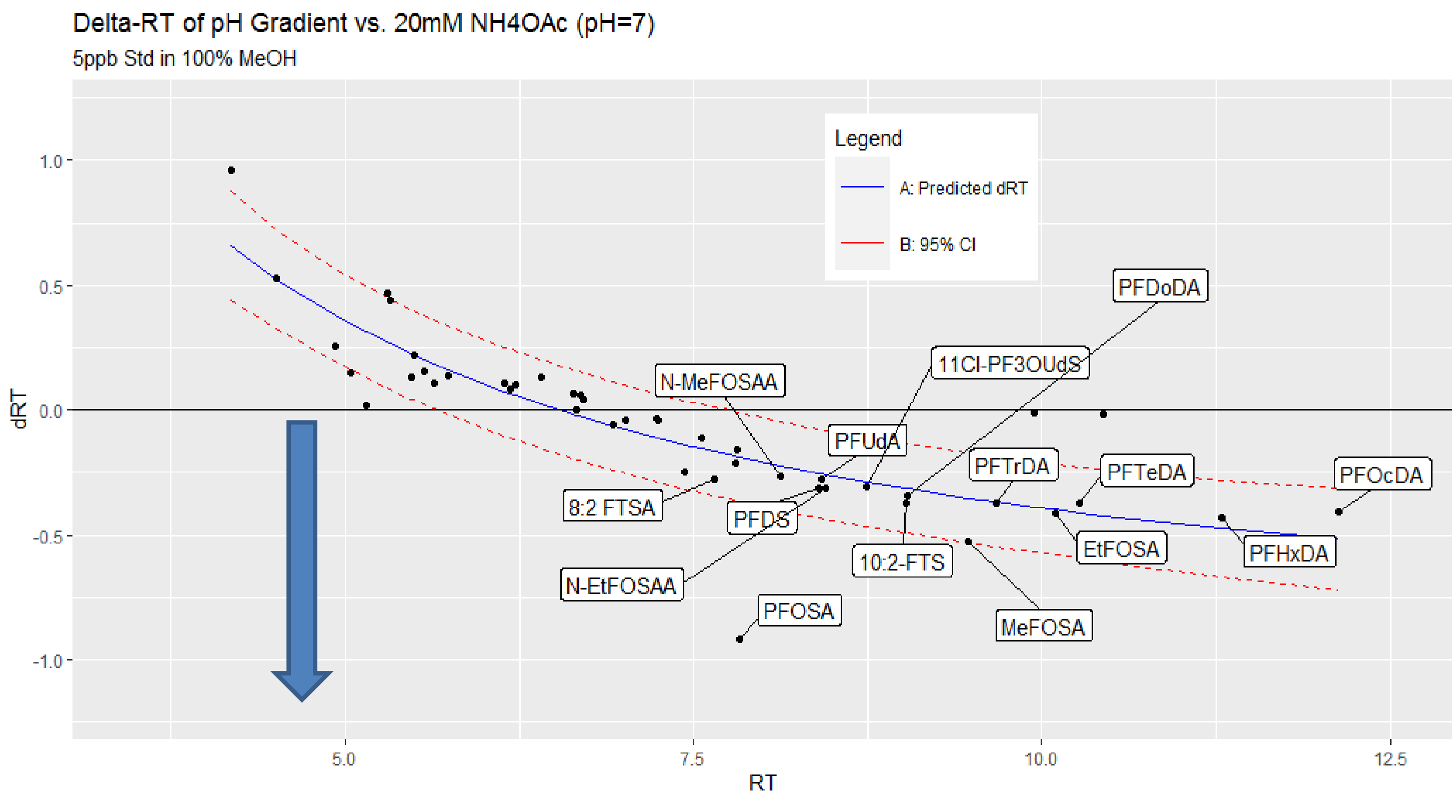
Table 4 - Measured pH of a Binary Mixture of Eluents		
20mM HOAc in Water	25mM NH4OH in MeOH	
%A	% B	Actual pH
100%	0%	3.6
95%	5%	3.9
90%	10%	4.2
80%	20%	4.6
70%	30%	5.1
60%	40%	5.8
50%	50%	6.4
40%	60%	7.1
35%	65%	8.2
30%	70%	8.5
20%	80%	9.0
10%	90%	9.3
0	99.5%	>10

- Ammonium Acetate dissolved in water ***not*** a buffer
- Actual pH during run subject to delay volume
- Still provides stable RTs (meets QSM 5.3)

Retention Increase – ΔRT > 0.25



Retention Decrease – ΔRT < 0.25



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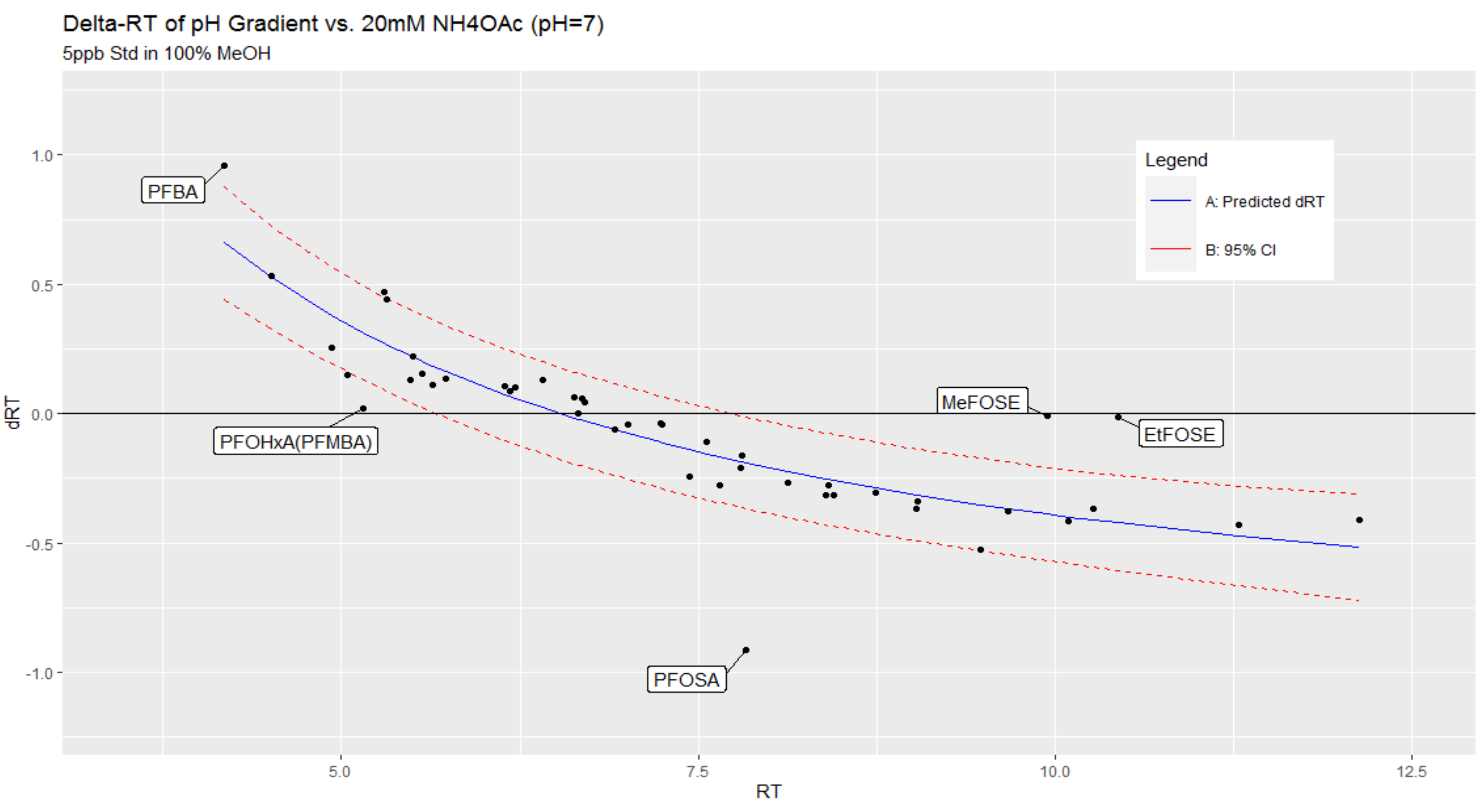
Significant Selectivity Changes Using GVLMA Method



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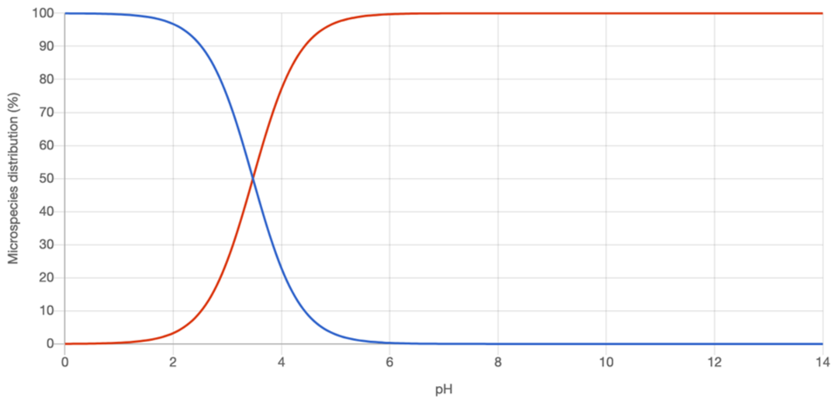
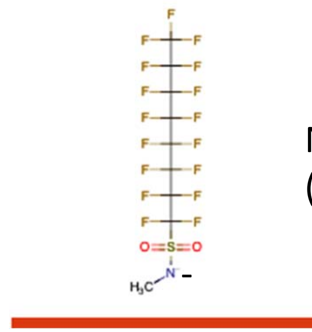
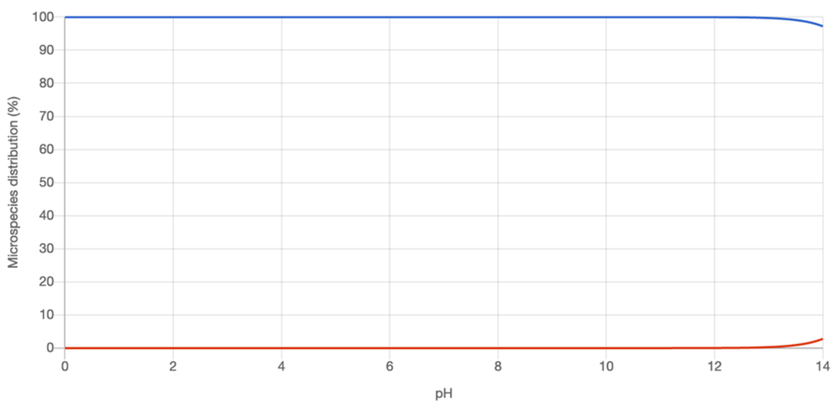
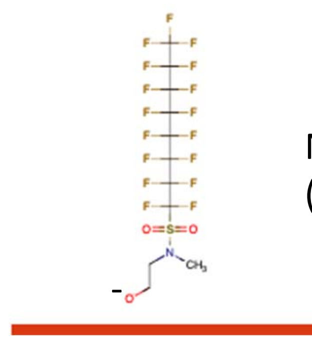
N-MeFOSA vs. N-MeFOSE Ionization and pH





N-MeFOSA
(pKa~3.47)





N-MeFOSE
(non-ionic)

Data from Chemicalize.com

New/Old Column – RT Stability

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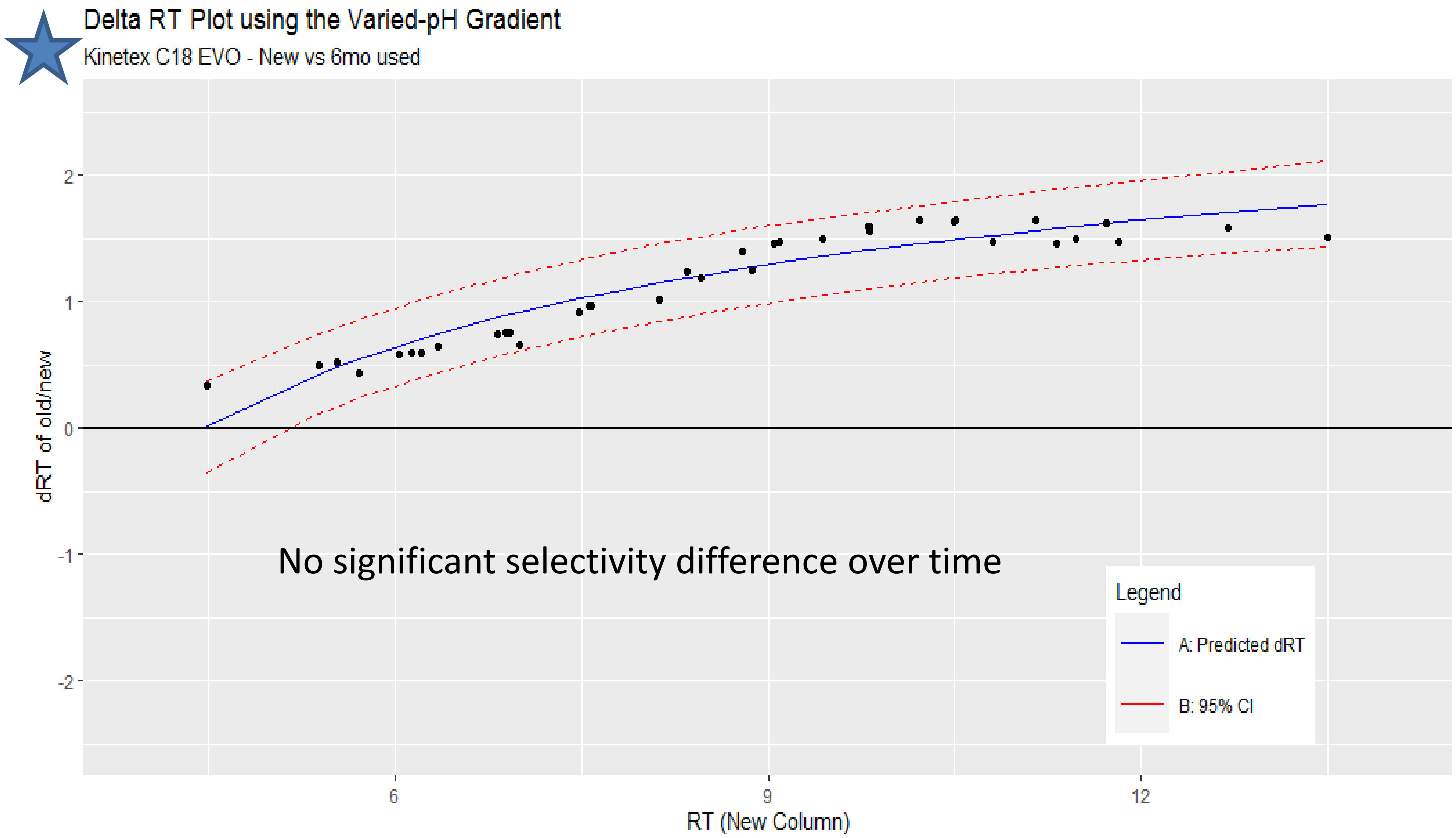

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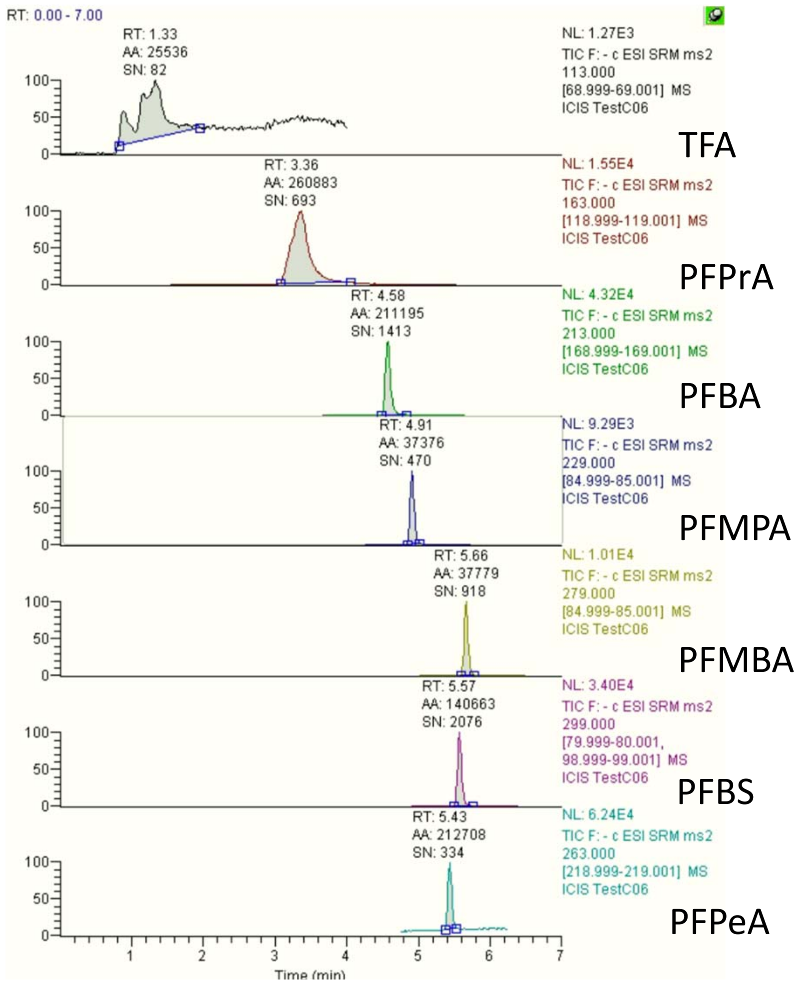
Short Chain PFAS
(< C6)



- Ultra-Short short chain PFAS retention and sensitivity unacceptable for TFA (C2)
- TFA was lost in standards due to volatility and may have analytical precision issues

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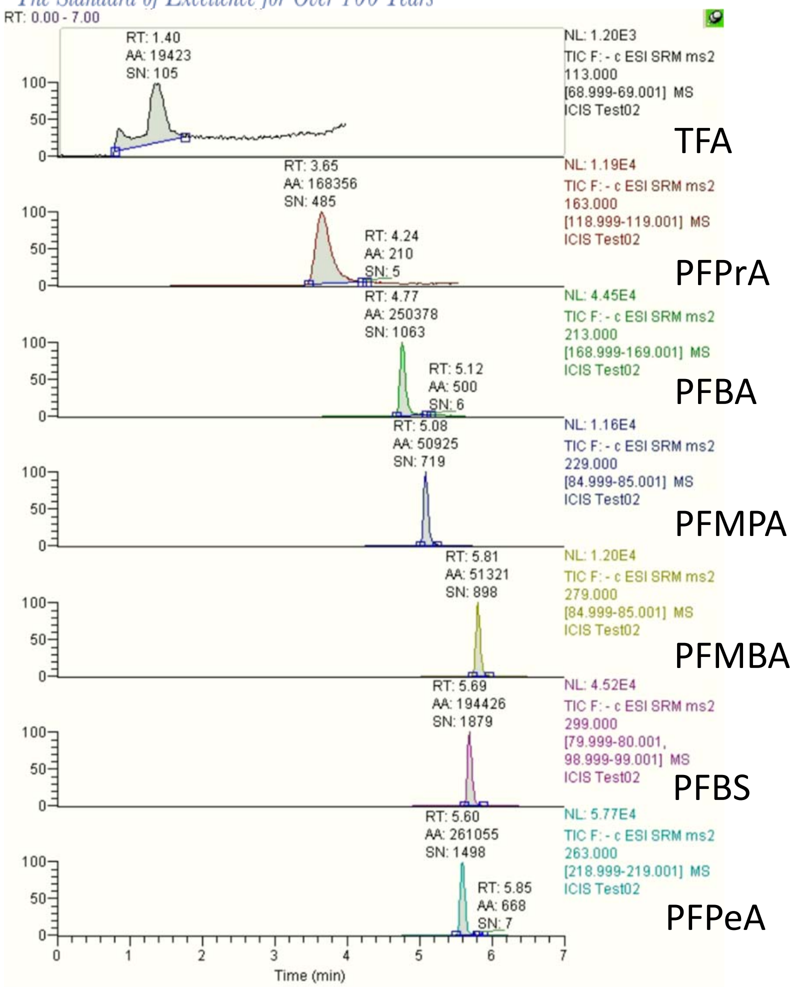
What about Formic Acid

(20mM HOFo)



Lower pKa of Formic acid provides increased retention of short chain however, MeFOSE and EtFOSE not as sensitive because they are detected as adducts of Formate

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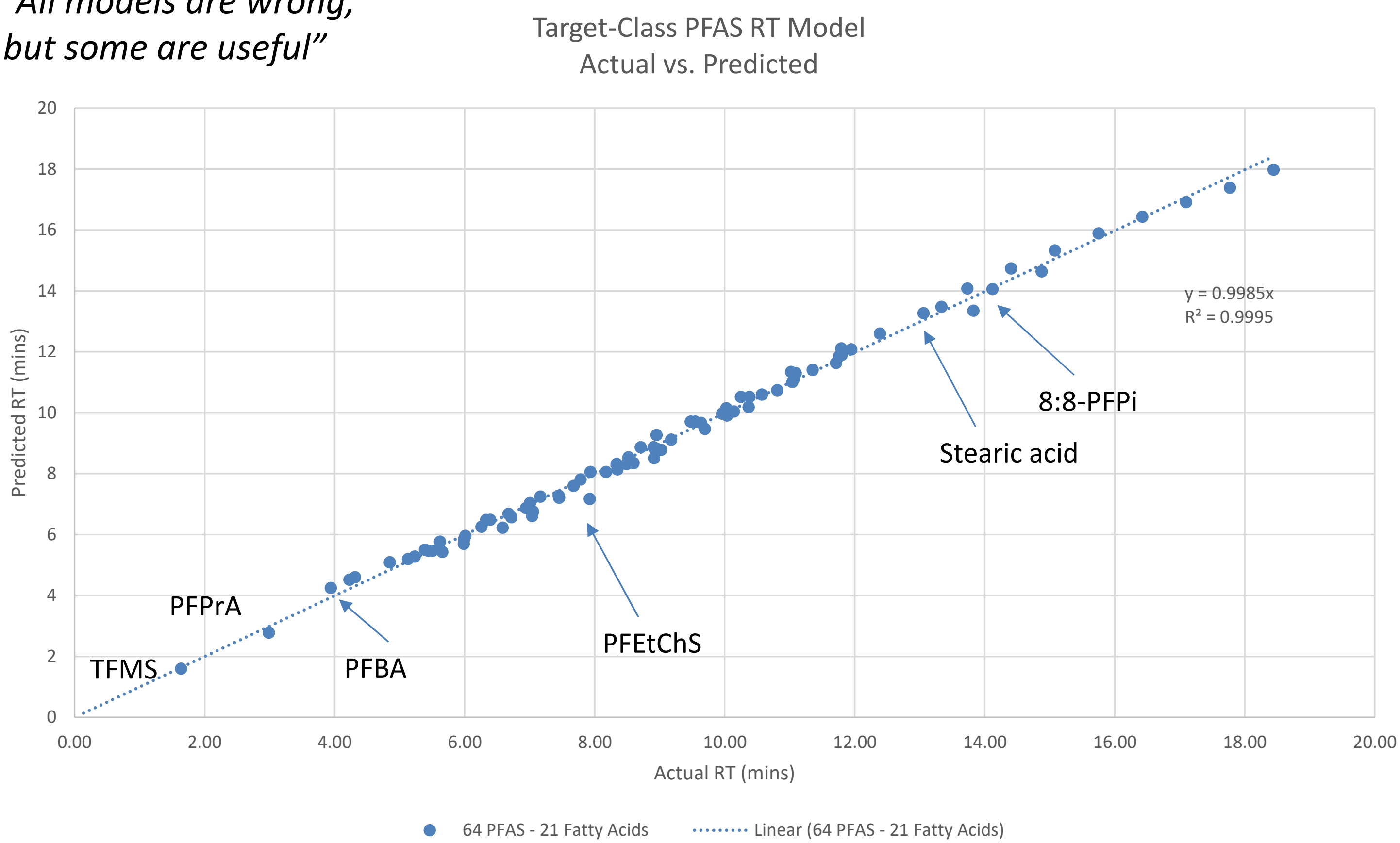
Useful for RT Prediction

Proprietary QSRR Model

"All models are wrong, but some are useful"



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Utility of a pH Gradient

- Increased Retention of Short Chain PFAS
- Was validated under QSM 5.3 (see other work)
- Better Retention Time Prediction of Short Chain PFAS
  - More accurate models based on computationally predicted values (e.g. SLogP)
  - Help Qualitative ID in Targeted Screening using High-Res MS
    - Must know structure to compute SLogP

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More Technical Info

fluoroalkyl
(PFAS) Extraction by

PFAS Analysis Based Upon a pH-Variable LC Mobile Phase Gradient

- GVLMA Statistical method to determine selectivity difference
- Release Full Method Validation soon

phenomenex.com/pfas

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Acknowledgements

Contributors/Reviewers

- Richard Jack - Phenomenex
- Sam Lodge - Phenomenex
- David Kennedy - Phenomenex

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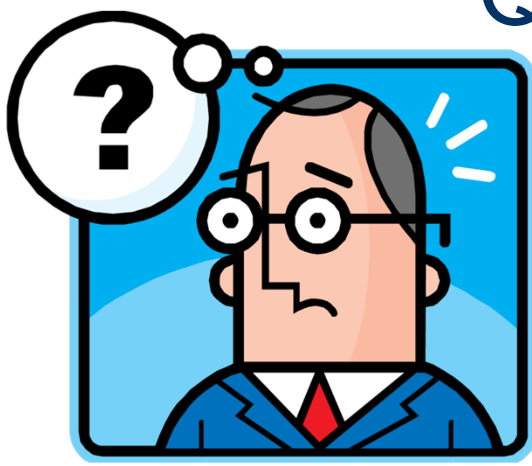
Questions?



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Thank You!