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6/3/2024

Challenges and Lessons Learned in Validating a PFAS Suspect Screening Workflow

2024 Chlorinated Conference

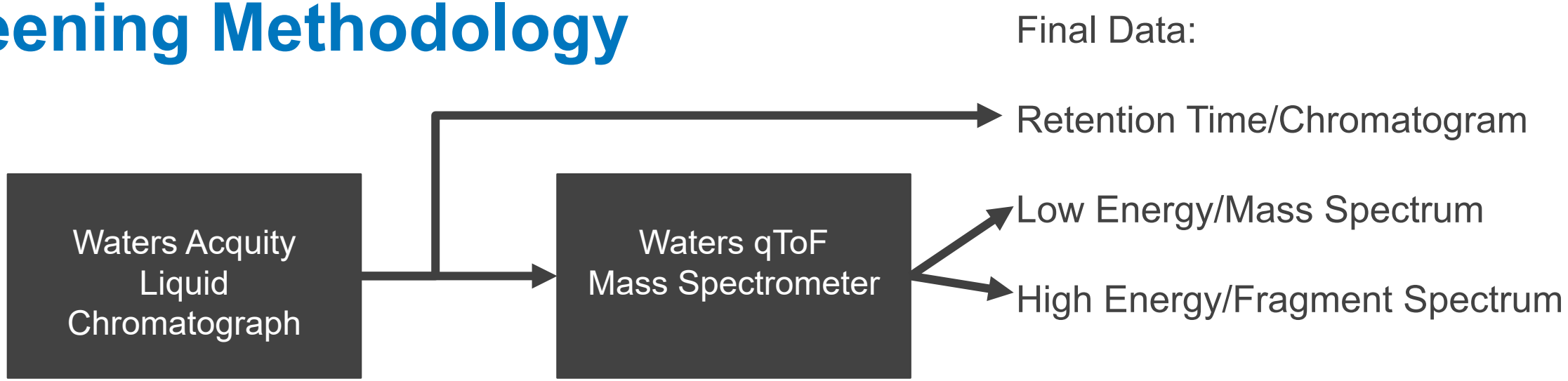
June 2-6, 2024 | Denver, Colorado

Agenda

- Overview of Battelle's Suspect Screening Methodology
- Building a Proprietary Analyte Library
- Ensuring Extraction Robustness
- Optimizing Data Filtration
- Validating Semiquantitation
- Looking Forward

The Team:
PI: Kavitha Dasu
Lab Chemist: Larry Mullins
Data Analyst: Cameron Orth

Overview of Battelle's Suspect Screening Methodology



Key Takeaway
Think of the instrument as two black boxes with 3 outputs

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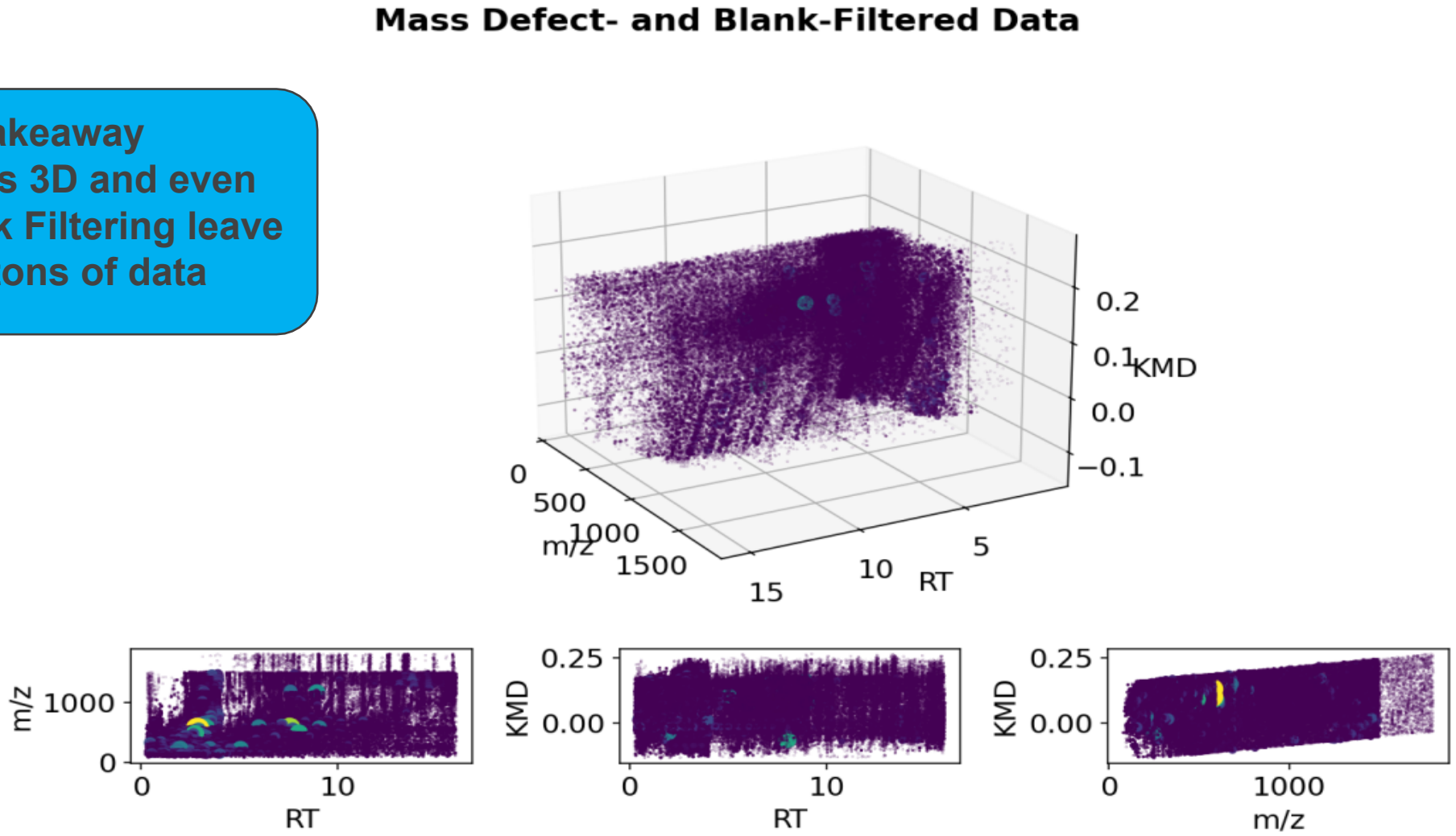
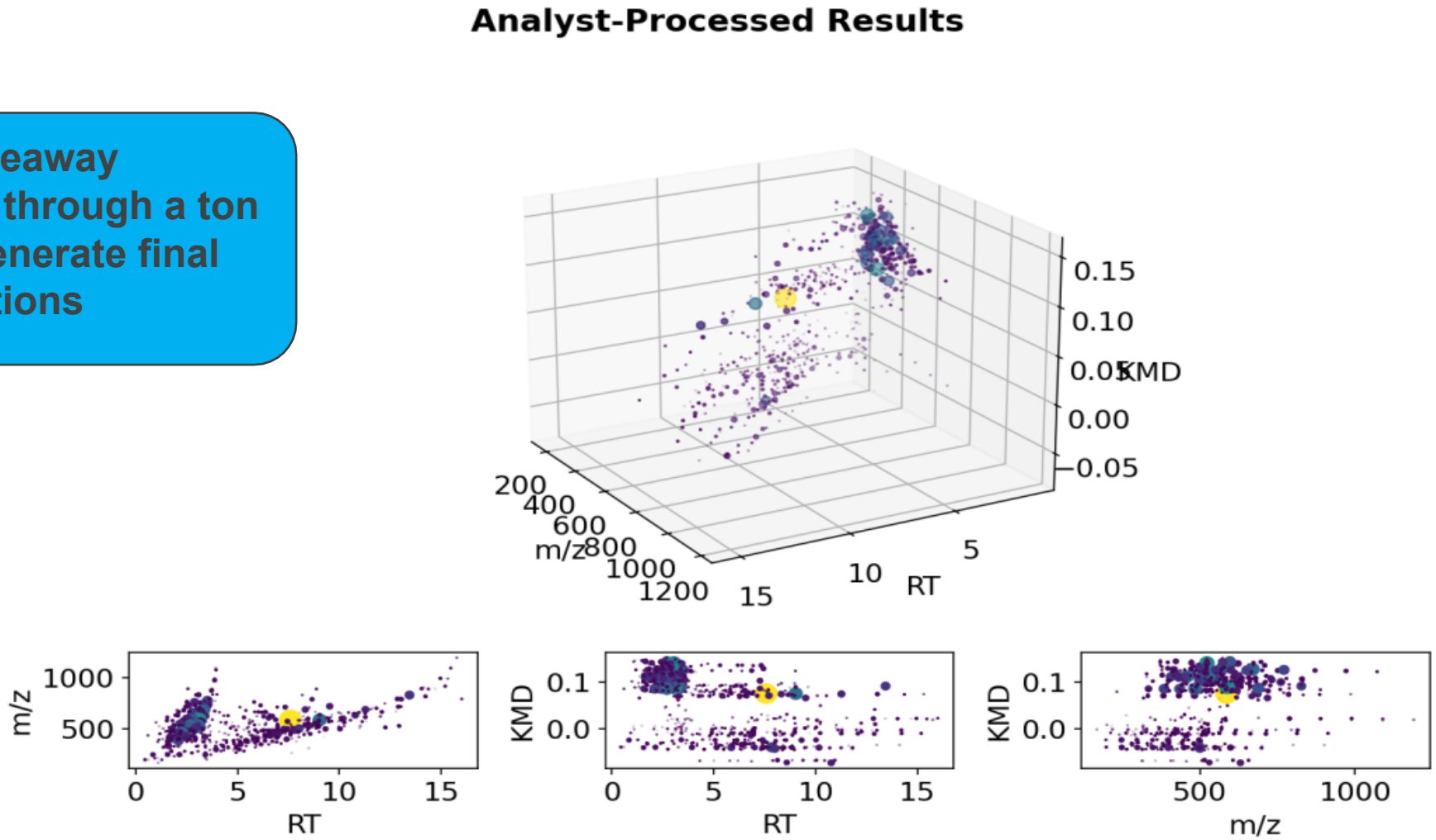
Data Prior to Analyst Processing

Key Takeaway
HRMS data is 3D and even MD- and Blank Filtering leave you with tons of data

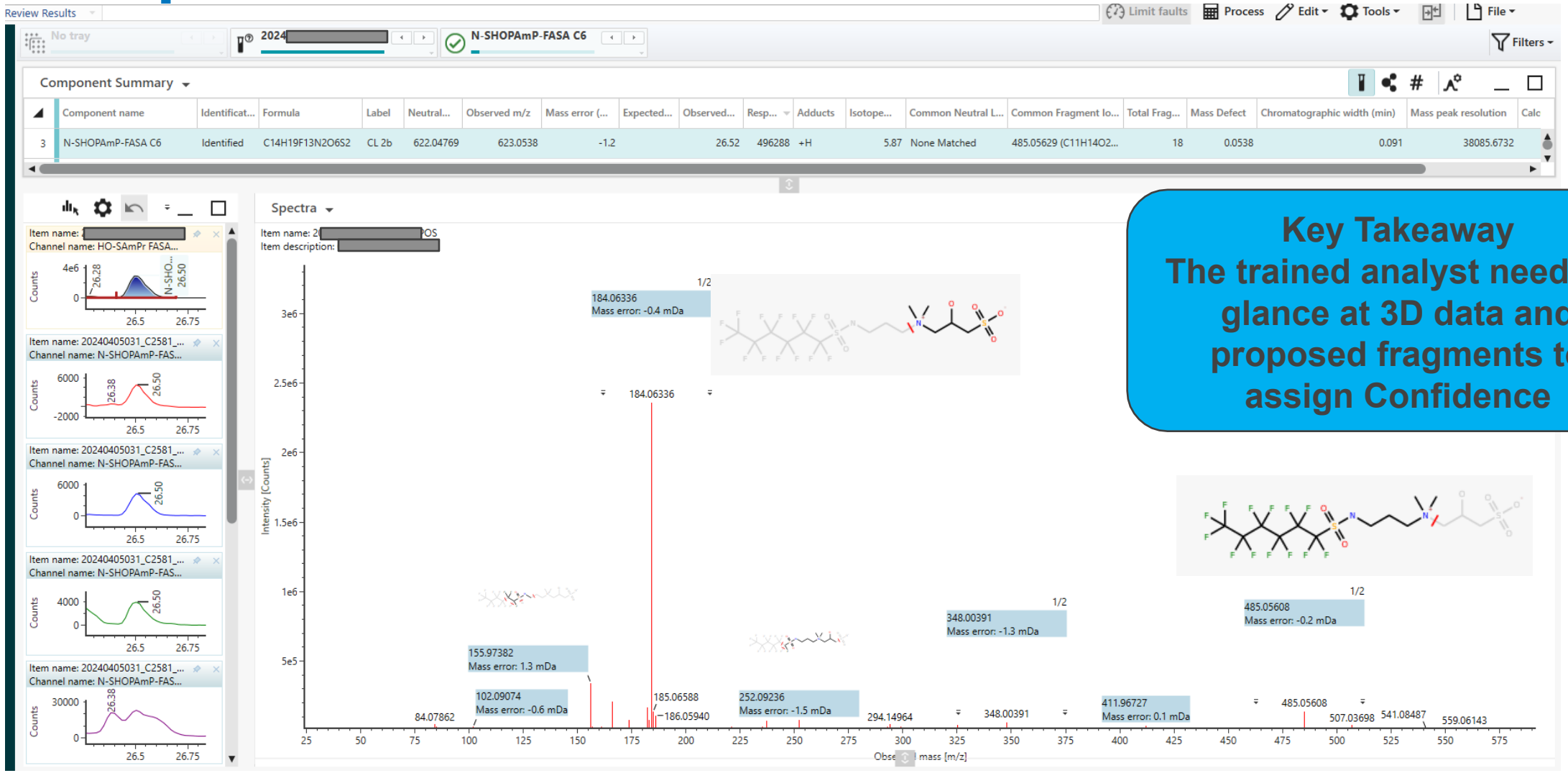


Data After Analyst Processing

Key Takeaway
Analysts comb through a ton of noise to generate final detections



An Example: N-SHOPAmP-FASA C6



Key Takeaway
The trained analyst needs to glance at 3D data and proposed fragments to assign Confidence

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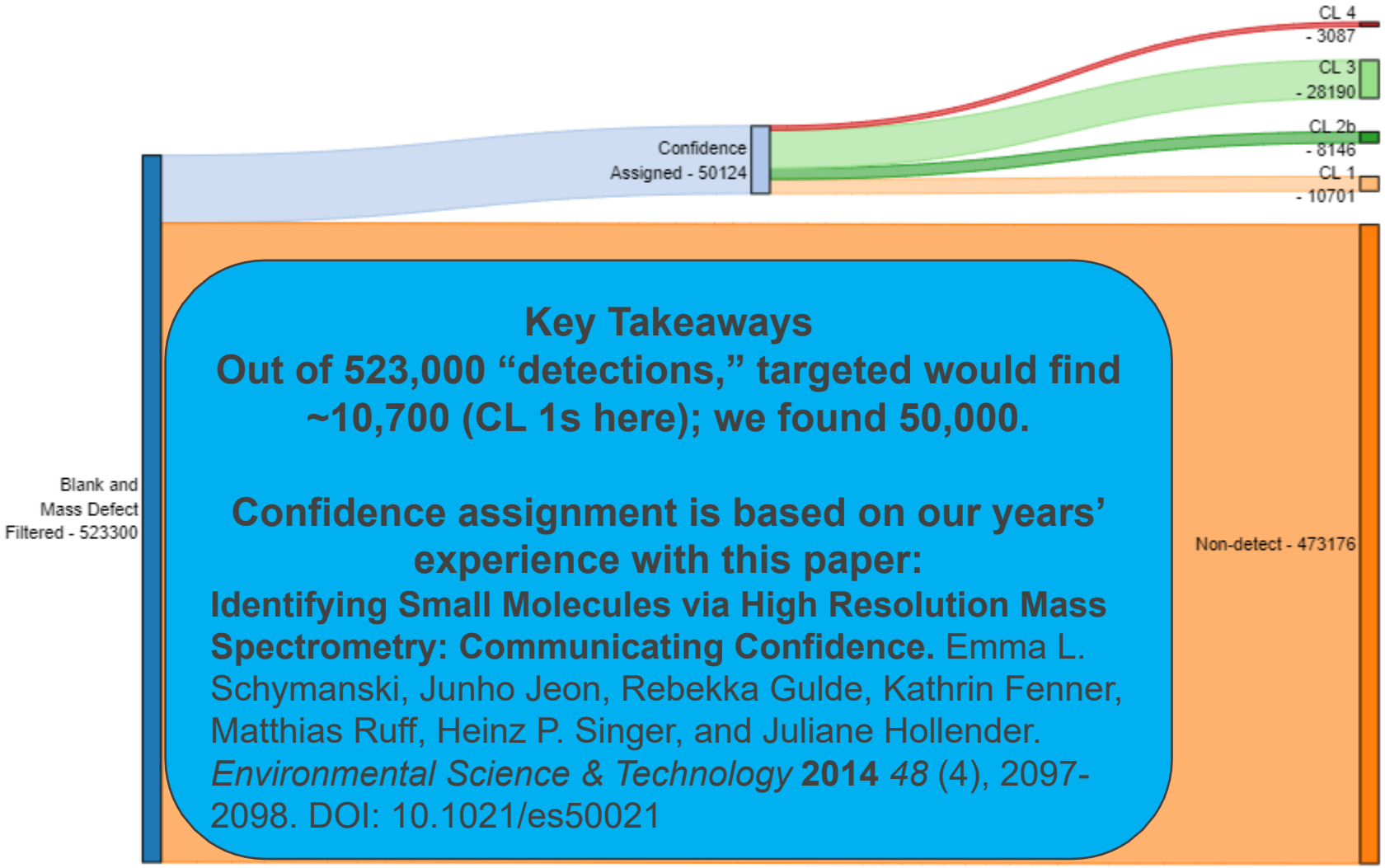
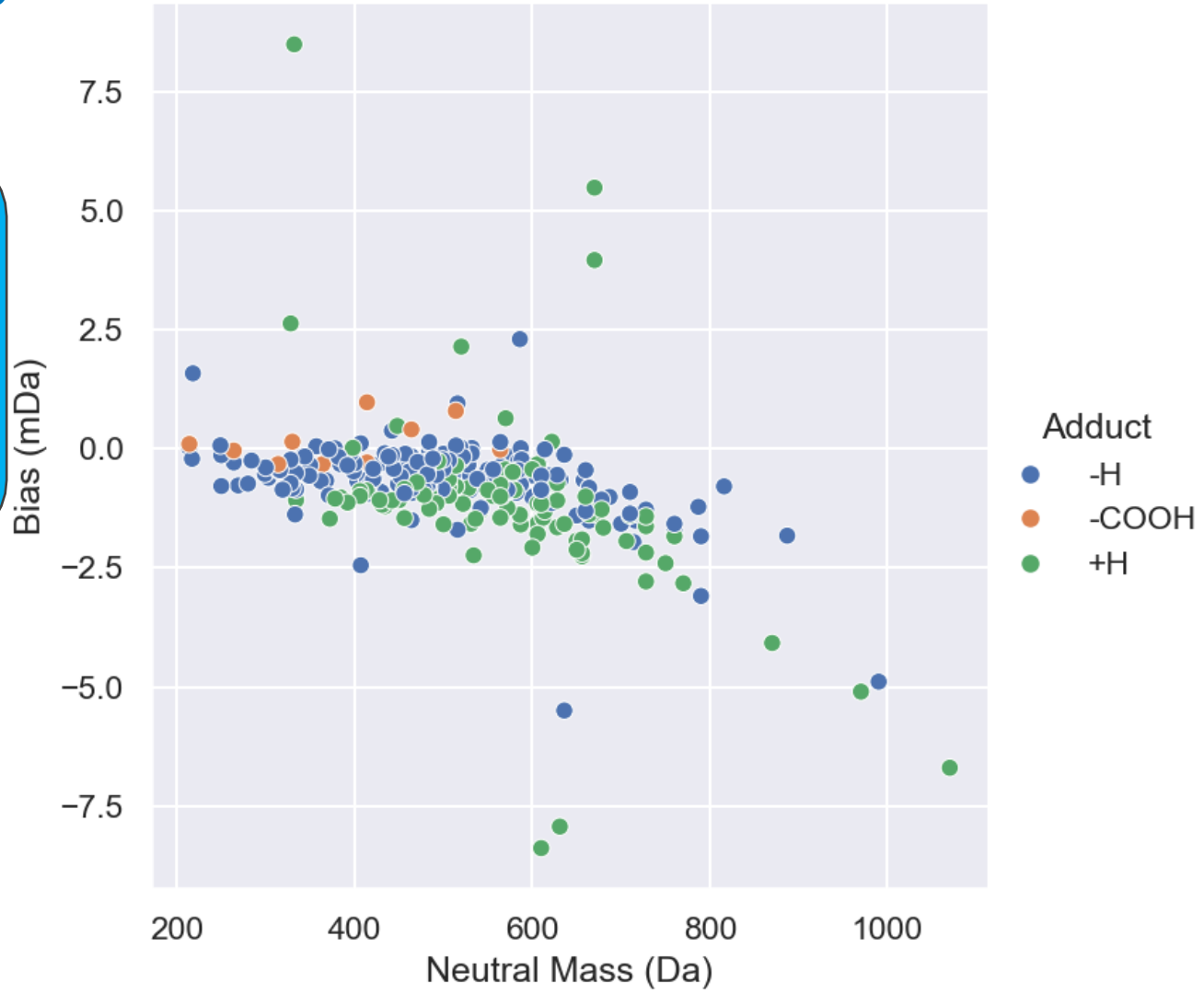
Sankey of Today's Data



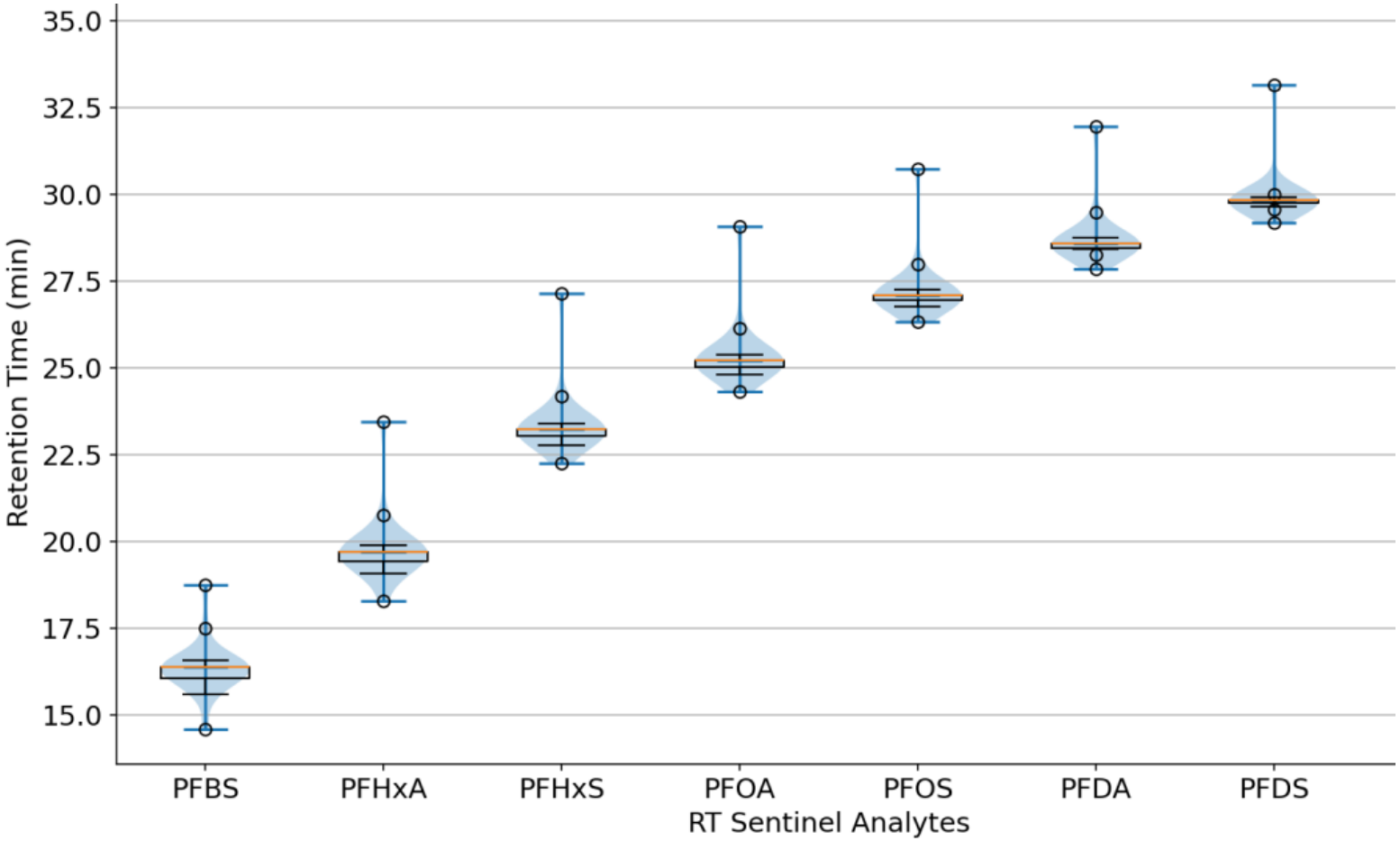
Mass Accuracy

Key Takeaway
Confidence Assignment relies on low-to-no bias; the vast majority here are within 0.0025 Da (an electron is ~0.0005)

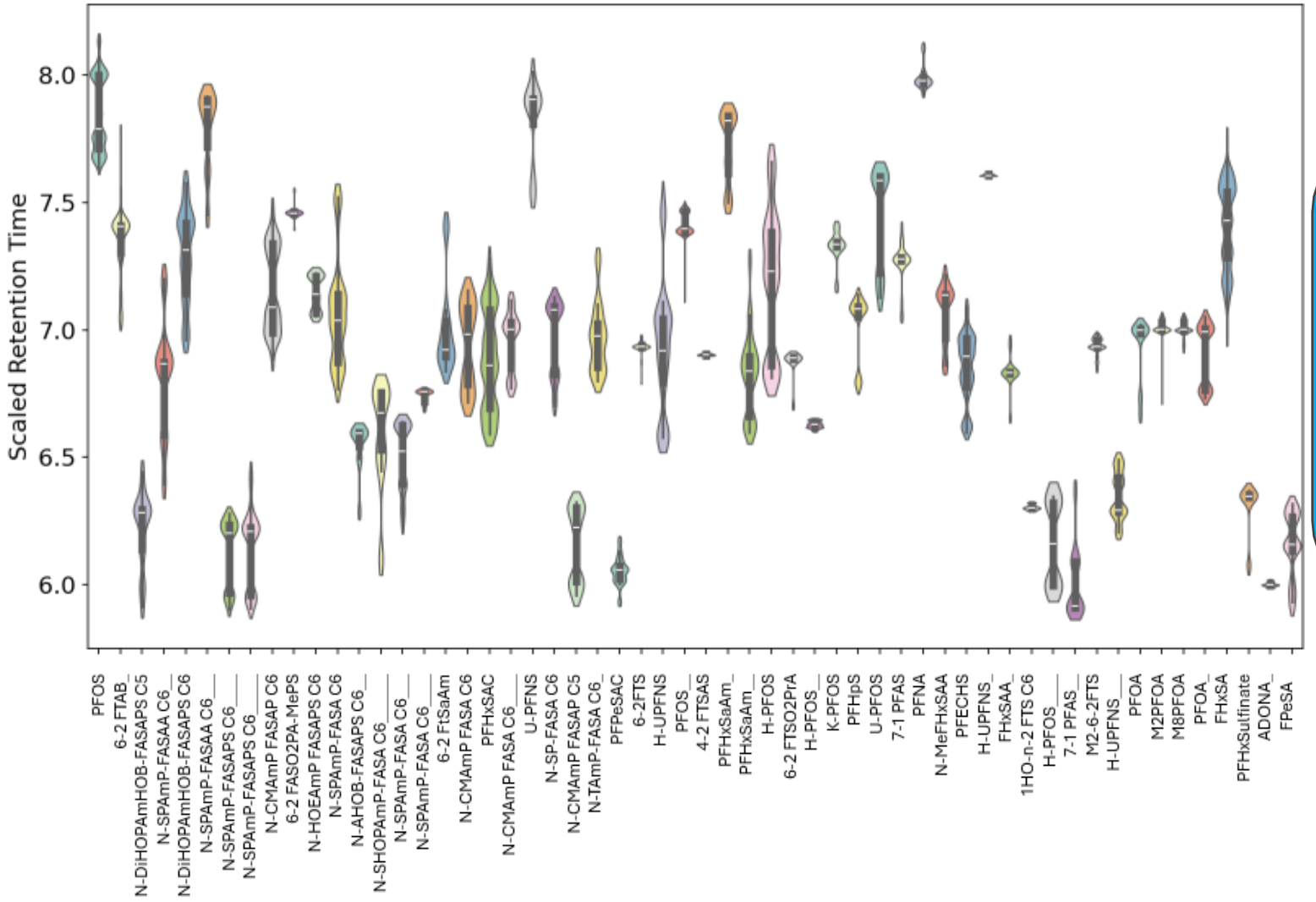


Retention Time Stability

Key Takeaway
Including 3+ years of data, RT is stable for these standard analytes, so let's use them to describe the RT of suspect analytes



Retention Time Scaling



Key Takeaway
This is just a small segment of our data, but you can see how many analytes show up as points, meaning constant scaled RT

Building a Proprietary Analyte Library

Key Takeaway
We've spent 5 years developing an in-house library of suspect analytes and their fragmentation patterns. Current numbers include 620 analytes, spread across negative and positive ionization modes

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Library of Structure Files

Key Takeaway
Our library doesn't just look at monoisotopic mass, we draw the structures for the software to interpret and give a list of expected fragments

Manage Components

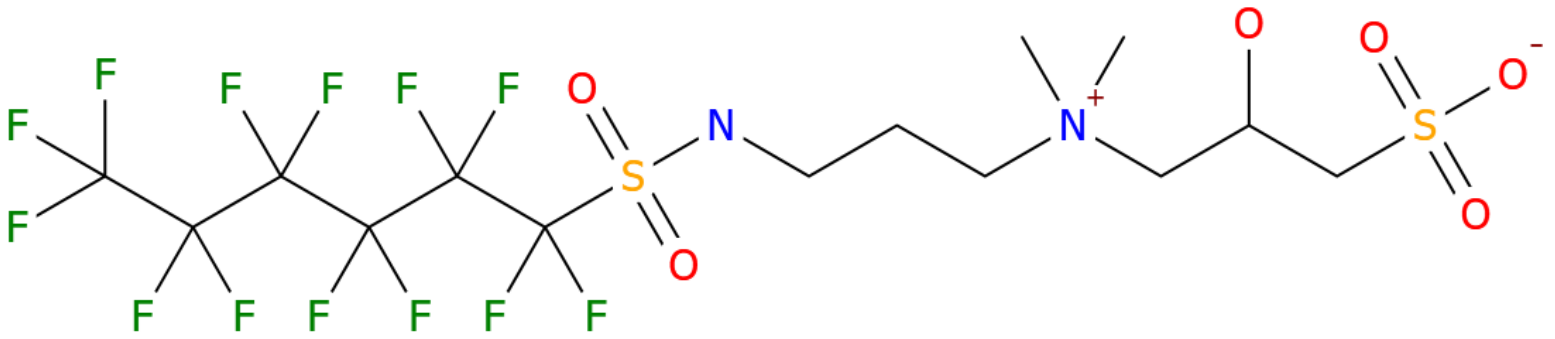
Create Import Paste Results Delete Edit Fragments... Edit Adducts... Add To Comr

	Component name	Label	Item tags	Expected RT (min)	Expected neutral mass (Da)	Expected fragment (m/z)	Adducts	Excluded	Generate transformations	Formula
16	N-SHOPAmP-FASA C6				622.0477	58.0651, 85.0886, 102.0913, 159.1492, 166.0532, 184.0638, 348.0052, 411.9671, 485.0563		☐	☐	C14H19F13N2O6S2

N-SHOPAmP-FASA C6 [PFAS - All Components]

Tools

Property	Value
Item type	Compound
Item description	
IUPAC name	
Formula	C14H19F13N2O6S2
Hill formula	C14H19F13N2O6S2
Average molar mass	622.4197
Monoisotopic mass	622.0477
Item tag	
InChI	1S/C14H19F13N2O6S2/c1-29(2,6-8(30)7-36(31,32)33)5-3-4-28-37(34,35)14(26,27)12(21,22)10(17,18)9(15,16)11(19,20)13(23,24)25/h8,28,30H,3-7H2,1-2H3



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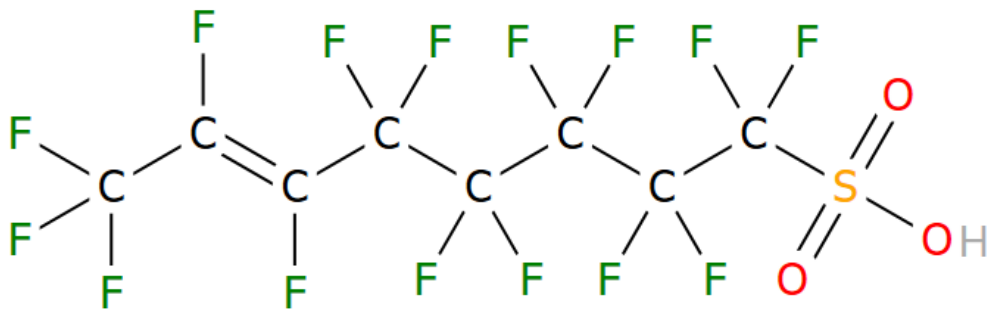
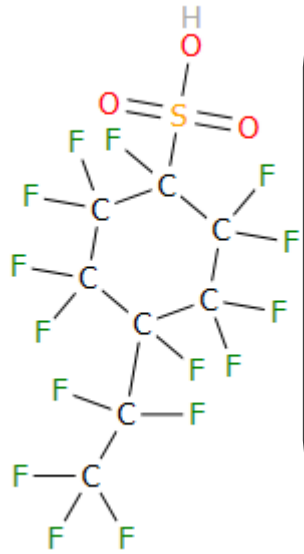
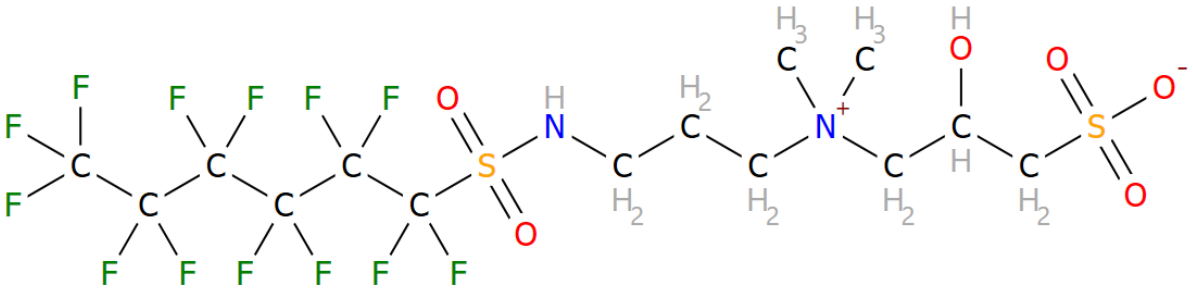
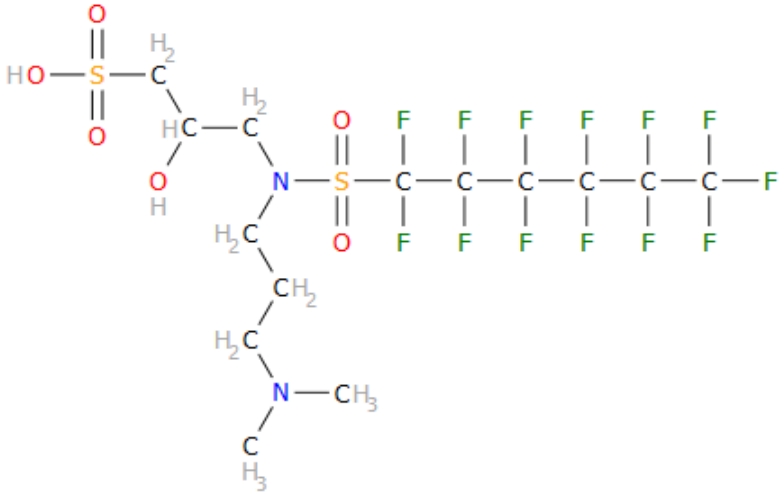
Pitfall 1: Isomers





Key Takeaway
Isomers can only be differentiated by established RT or fragmentation differences, so they require a standard for at least one isomer

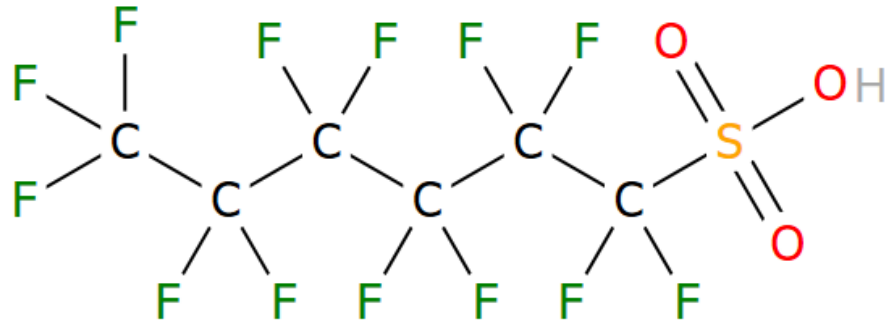




Pitfall 2: Isobaric Suspects

PFHxS [PFAS - All Components]

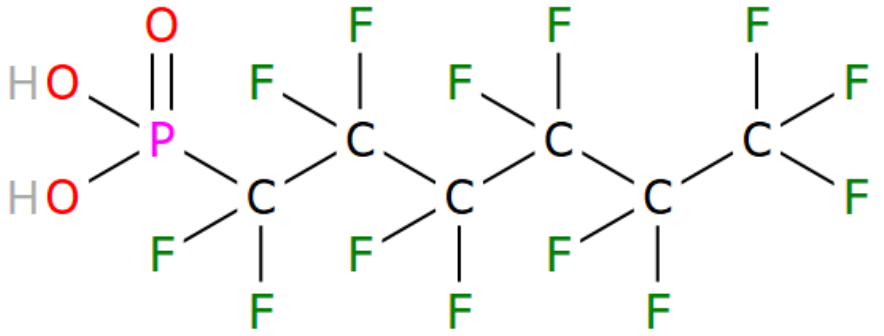
Property	Value
Item type	Compound
Item description	
IUPAC name	
Formula	C6HF13O3S
Hill formula	C6HF13O3S
Average molar mass	400.1146
Monoisotopic mass	399.9439
Item tag	
InChI	1S/C6HF13O3S/c7-1(8,3(11,12)5(15,16)17)2(9,10)4(13,14)6(18,19)23(20,21)22/h(H,20,21,22)



C6 PFPIA [PFAS - All Components]

Property	Value
Item type	Compound
Item description	
IUPAC name	
Formula	C6H2F13O3P
Hill formula	C6H2F13O3P
Average molar mass	400.0313
Monoisotopic mass	399.9534
Item tag	
InChI	1S/C6H2F13O3P/c7-1(8,3(11,12)5(15,16)17)2(9,10)4(13,14)6(18,19)23(20,21)22/h(H2,20,21,22)

Key Takeaway
These two luckily have standards available, but their mass difference is <0.01Da, so a computer may tag the wrong one without RT data.



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Ensuring Extraction Robustness

Key Takeaway

Battelle has launched two in-house Single Lab Validation Studies:

1. **In 2019, we used aqueous matrices to establish precision/bias/matrix effects.**
2. **In 2023, we examined a published extraction method claiming better recoveries of certain analytes.**

A manuscript on these is in process.

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Sensitivity and Selectivity for HRMS

Key Takeaway
Even False Positive and False Negative Rates get more complex with more complex data.

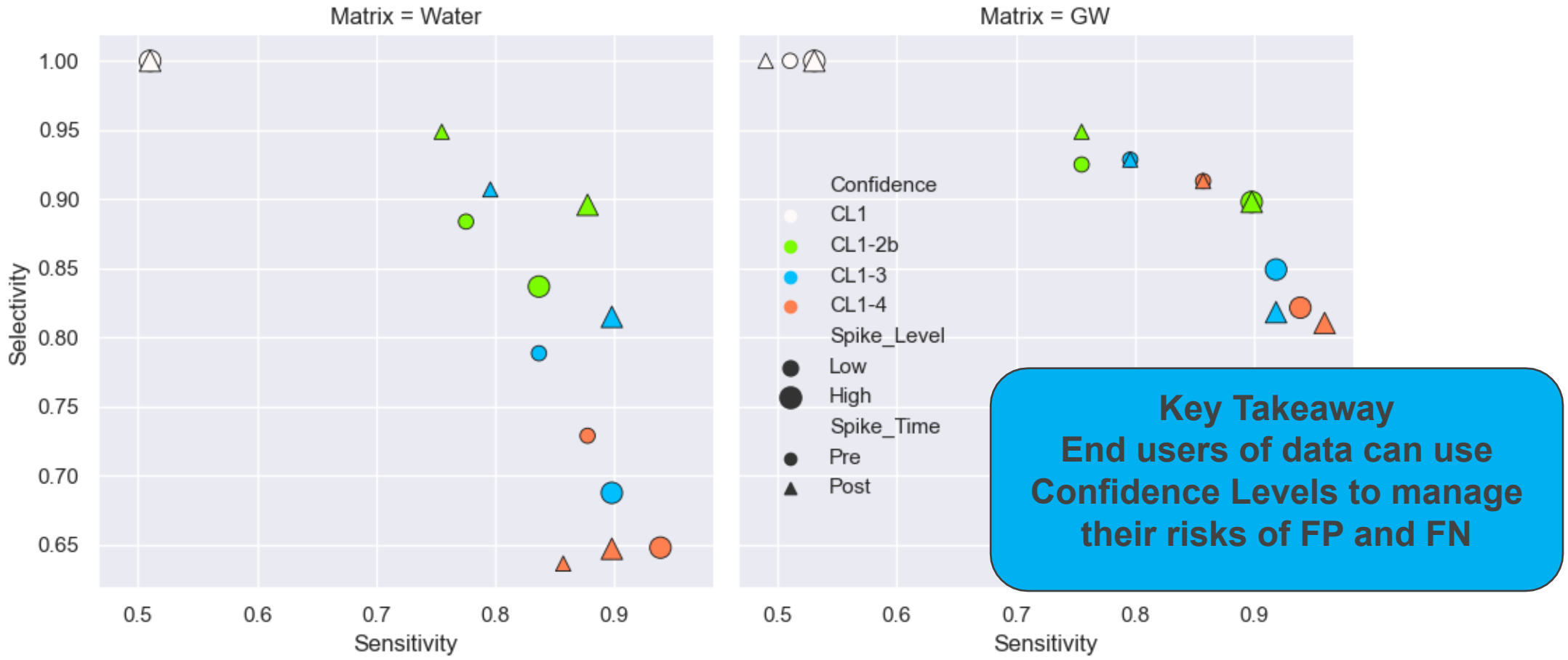
$$\text{ensitivity}\% = \frac{\#\{TP\}}{\#\{TP + FN\}}$$

$$\text{Selectivity}\% = \frac{\#\{TP\}}{\#\{TP + FP}$$

Inspiration for this approach:

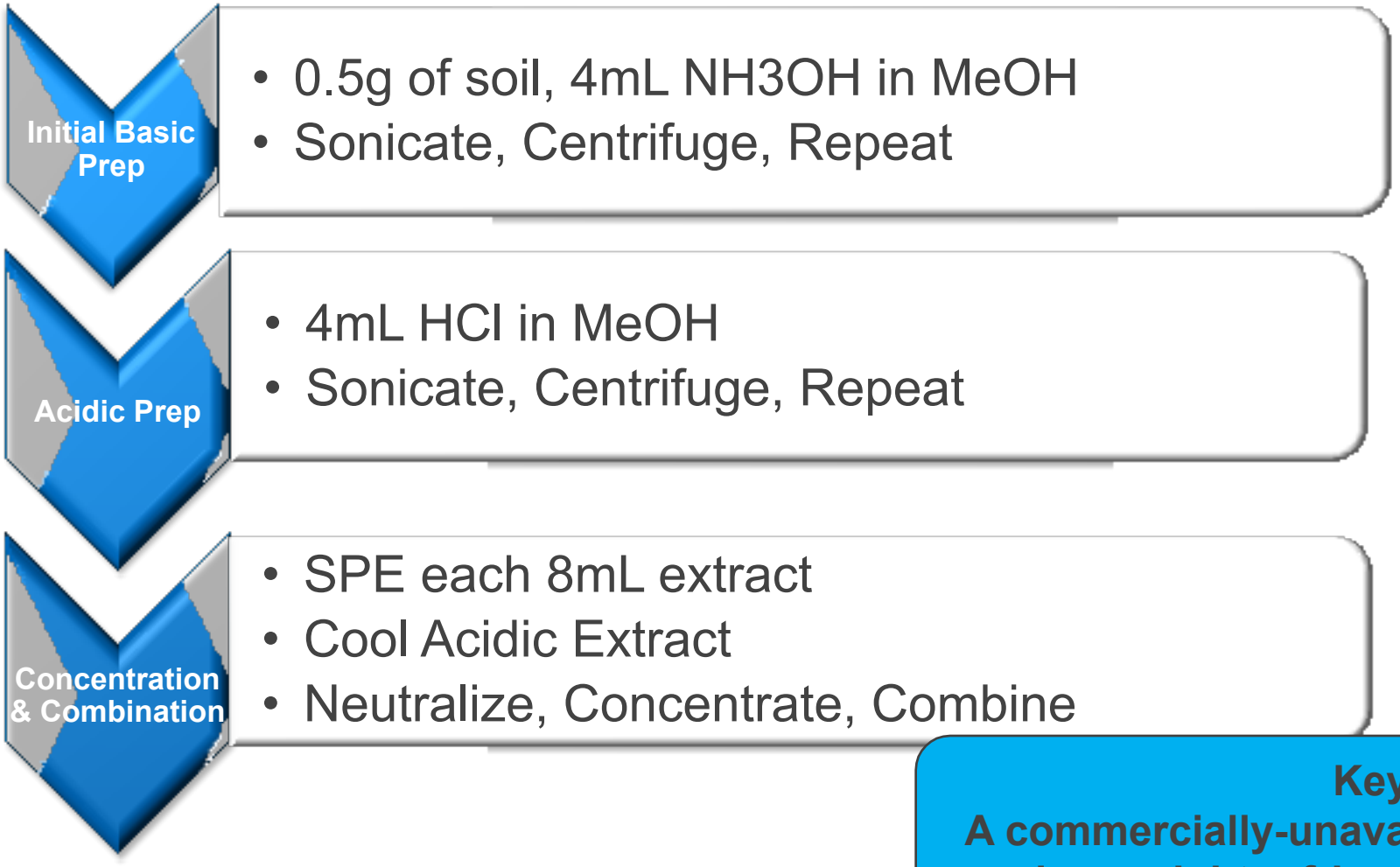
Evaluation, optimization, and application of three independent suspect screening workflows for the characterization of PFASs in water. Paige Jacob, Ri Wang, Casey Ching, Damian E. Helbling. ***Environ. Sci.: Processes Impacts***, 2021, **23**, 1554-1565. https://doi.org/10.1039/D1EM00286D

2021 Sensitivity and Selectivity – Aqueous Data



Key Takeaway
End users of data can use Confidence Levels to manage their risks of FP and FN

Solid Validation: EPA 1633 vs Published Extraction



Enhanced Extraction of AFFF-Associated PFASs from Source Zone Soils

Anastasia Nickerson, Andrew C. Maizel, Poonam R. Kulkarni, David T. Adamson, John J. Kornuc, and Christopher P. Higgins

Environmental Science & Technology **2020** *54* (8), 4952-4962

DOI: 10.1021/acs.est.0c00792

Key Takeaway
A commercially-unavailable method with 4 extracts and overnight refrigeration is tough on production

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2023 Soil Extraction Study:

Negative Detections

Positive Detections

Key Takeaway
We are safe to continue using Method 1633 preparation, even for Positive Mode analytes

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Optimizing Data Filtration

Key Takeaway
In HRMS PFAS, you'll often hear of mass defect filters from -.2 or -.15 up to +.15. Unfortunately, any MD filter will miss some PFAS.

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Some Basic Mass Defect Stats

Key Takeaway
There is a large range in Mass Defects depending on your list of PFAS Suspects.

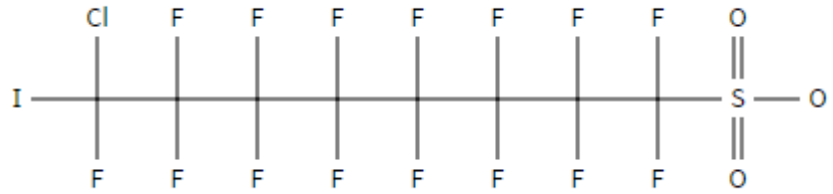
List Source:	Battelle		NIST		GHDB	
# of Analytes:	620		4951		39369	
Mode/Adduct:	(-H)	(+H)	(-H)	(+H)	(-H)	(+H)
Lowest MD:	-0.1682	-0.1033	-0.2643	-0.2487	-0.4186	-0.4030
Q1 MD:	-0.0620	0.0482	-0.0397	-0.0241	-0.0396	-0.0240
Median MD:	-0.0335	0.0673	-0.0007	0.0149	0.0030	0.0186
Q3 MD:	-0.0023	0.0835	0.0464	0.0620	0.0405	0.0561
Highest MD:	0.1057	0.1214	0.3955	0.4111	0.4188	0.4344

NIST: PDR: Suspect List of Possible Per- and Polyfluoroalkyl Substances (PFAS) (nist.gov)
Getzinger-Higgins: DOI:10.7924/r4q23zg65 Dataset

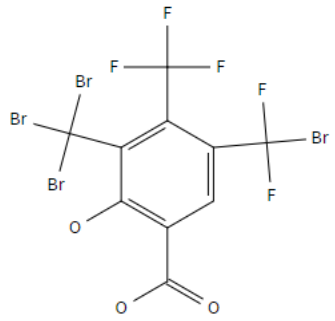
Mass Defect Filter Breakers (Assuming ± 0.15 Naively)

Key Takeaway
Know what suspects are of interest to your end users.

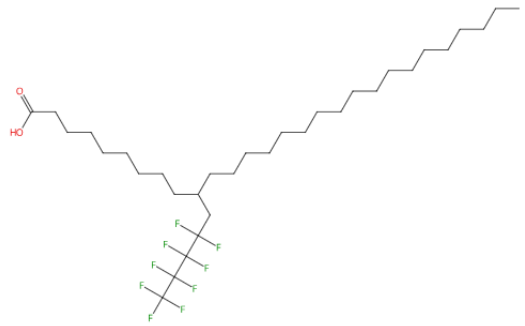
I-Cl-PFOS @ 622.80674



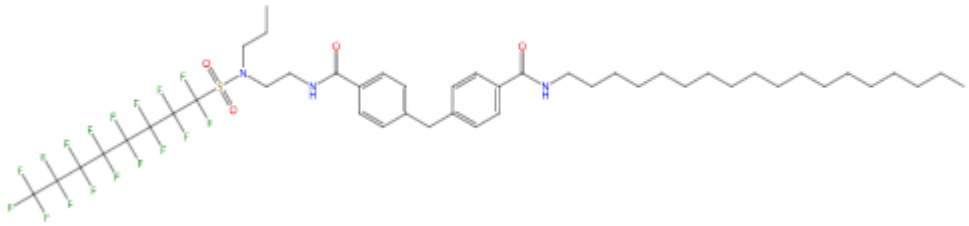
PolyBr-PolyF Hydroxy-Benzoic Acid @ 580.66631



PFPe-Octasanoic Acid @ 646.34378



C46 Benzamide w/ PFO chain @ 1072.39603



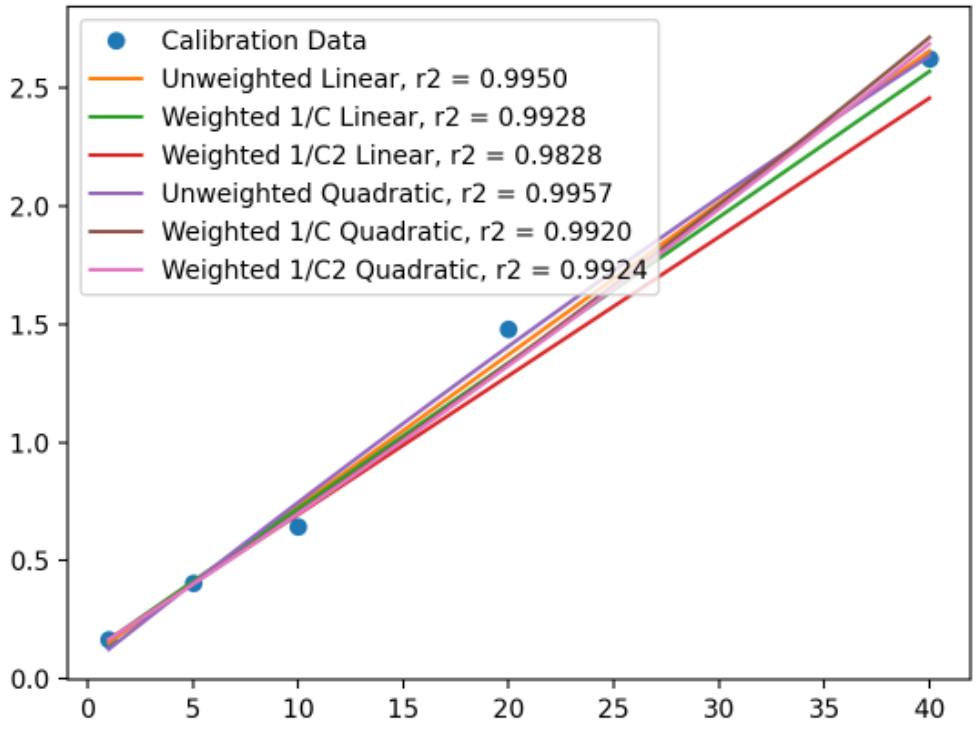
Validating Semiquantitation

Key Takeaway
Without commercially-available reference standards and with less-quantitative instrumentation, semiquantitation will always yield approximate values, but they can still have data quality.

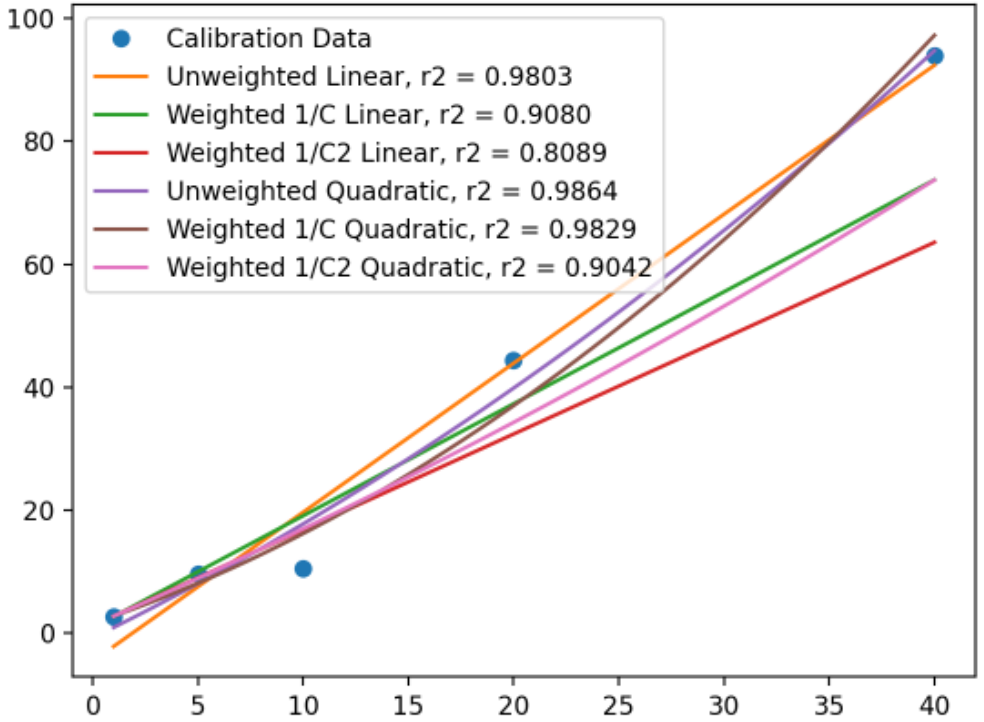
Example Calibration Curves

Key Takeaway
Different analytes will need very different curves and even the type of calibration will vary

- 6:2 FTS (has IS)



- 6:8 PFPiA (no IS)

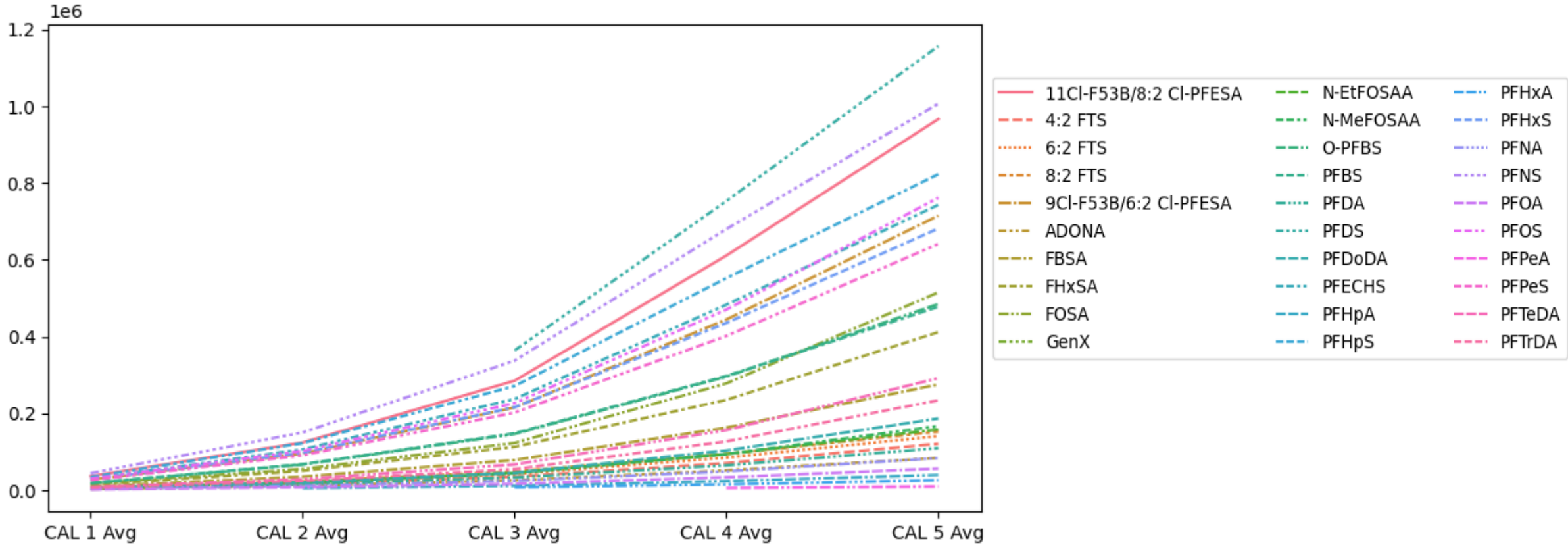


Calibration Recovery Stability

	Avg CAL 1 Rec %	Avg CAL 2 Rec %	Avg CAL 3 Rec %	Avg CAL 4 Rec %	Avg CAL 5 Rec %
Data Set 1	99%	102%	99%	99%	101%
Data Set 2	110%	86%	99%	107%	98%
Data Set 3	137%	103%	84%	108%	100%

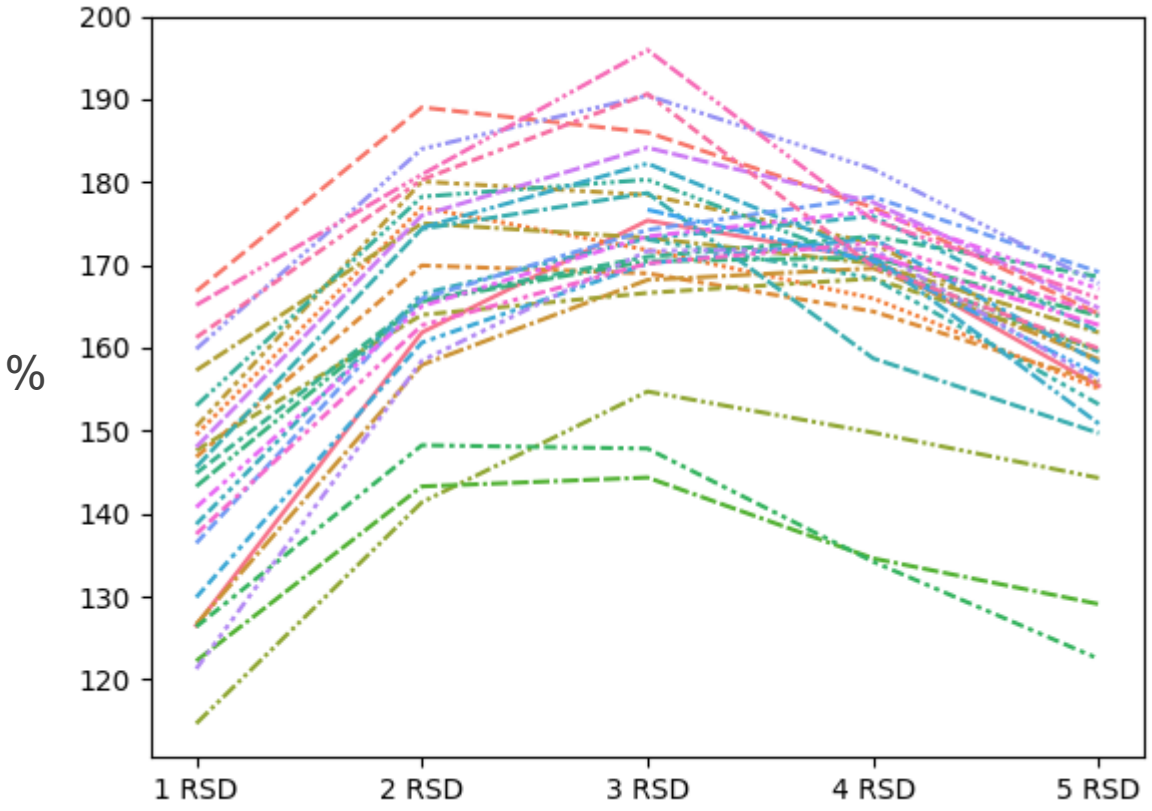
Key Takeaway
These read-back recoveries of calibration standards are similar to other methods' and show no inherent bias.

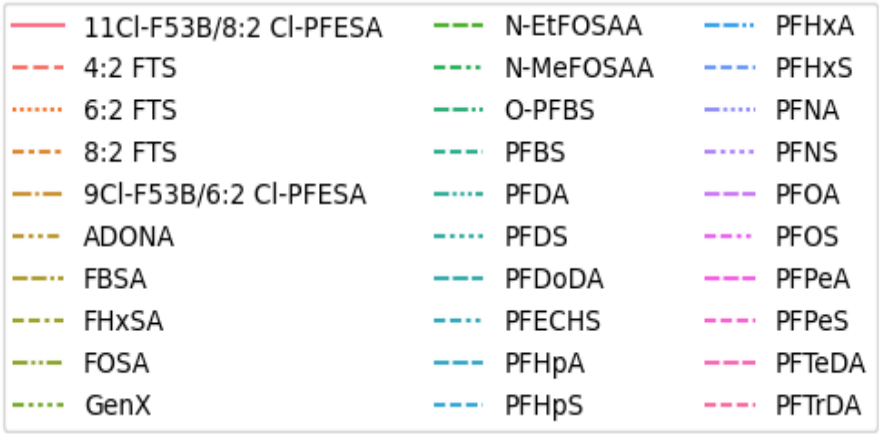
Calibration Average Response



Calibration Response Stability

Key Takeaway
Room for improvement!
Response was not as stable as we'd like, so measures like detection limit vary. New, more frequent data should solve this.





Looking Forward

DOCs for Complex Analyses

Key Takeaway
Complex methods need complex Demonstrations of Capability to ensure analyst are fully trained.

Gold Standard Confidence Assigned						
		CL 1	CL 2b	CL 3	CL 4	ND/CL 6
Trainee Confidence Assigned	CL 1	10	0	1	1	0
	CL 2b	0	10	1	0	1
	CL 3	1	0	10	1	0
	CL 4	1	0	0	10	1
	ND/CL 6	0	1	1	2	20

	Count	% of total
Total Overall:	72	100.0%
Total Correct :	60	83.3%
Missed CL1s	2	16.7%
FPs of any	2	2.8%
FPs of CL1-3	1	1.4%
FNs of any	4	5.6%
FNs of CL1-3	2	2.8%

Scoring:	From Assigning that CL
CL 1	5
CL 2b	5
CL 3	3
CL 4	5
ND/CL 6	11
Total	29

Example Criteria	Passing
Total Correct:	>80%
Missed CL1s:	<10%
FPs of CL1-3:	<5%
FNs of CL1-3:	<3%
Total Score:	<40

Next Steps

Expanding Analyte Library

- Already offer an MS-only (no fragments) NIST list method
- Similar method could use the *in-silico* library of Getzinger et al.

Tackling New Chemistries

- Mixed Mode LC offers ultra-short-chain PFAS
- Derivatization offers new classes like PAS
  - Nontarget Discovery of Per- and Polyfluoroalkyl Sulfonyl Halides in Soils by Integration of Derivatization and Specific Fragment-Based Liquid Chromatography-High Resolution Mass Spectrometry Screening. Kun Wang, Xin Xiao, Zhengzheng Liu, Jing Wang, Xiangyu Zhu, Enhui Wu, Christopher P. Higgins, and Baoliang Chen. *Environmental Science & Technology* **Article ASAP**. DOI: 10.1021/acs.est.4c01610

Improving Semiquantitation

- Add non-PFAS internal standards into positive mode?
- Correct using 1633 results?

Automating the Process?

- Already have filtering and post-processing in Python
- Automate (initial) confidence level assignments?
  - Requires either vendor API or
  - Computer vision to look for co-maximizing peaks

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It can be done

www.battelle.org/pfas