Untargeted PFAS Analysis and Forensics: Promise and Bottlenecks to Widespread Adoption from a Commercial Lab Perspective

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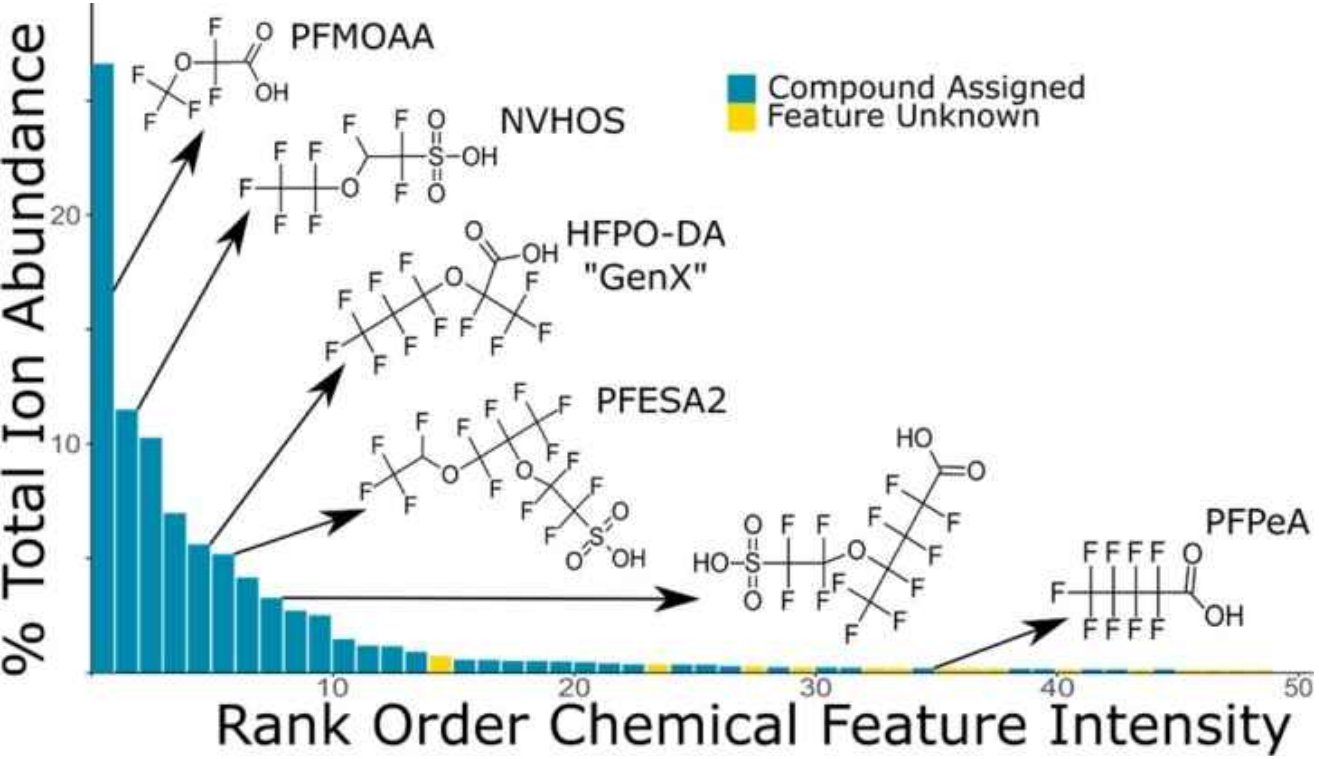
Battelle Chlorinated - 03-June-2024

Non-Target Analysis for PFAS is very exciting!

Identification of Per- and Polyfluoroalkyl Substances in the Cape Fear River by High Resolution Mass Spectrometry and Nontargeted Screening

James McCord[†] and Mark Strynar[*,‡]



Strynar et al. 2017 (US EPA)

ANALYTICAL CHEMISTRY

Nontargeted mass-spectral detection of chloroperfluoropolyether carboxylates in New Jersey soils

John W. Washington[1]*, Charlita G. Rosal[1], James P. McCord[2], Mark J. Strynar[2], Andrew B. Lindstrom[2], Erica L. Bergman[3], Sandra M. Goodrow[4], Haile K. Tadesse[2], Andrew N. Pilant[2], Benjamin J. Washington[5], Mary J. Davis[1], Brittany G. Stuart[6], Thomas M. Jenkins[7]

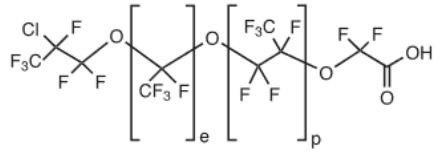
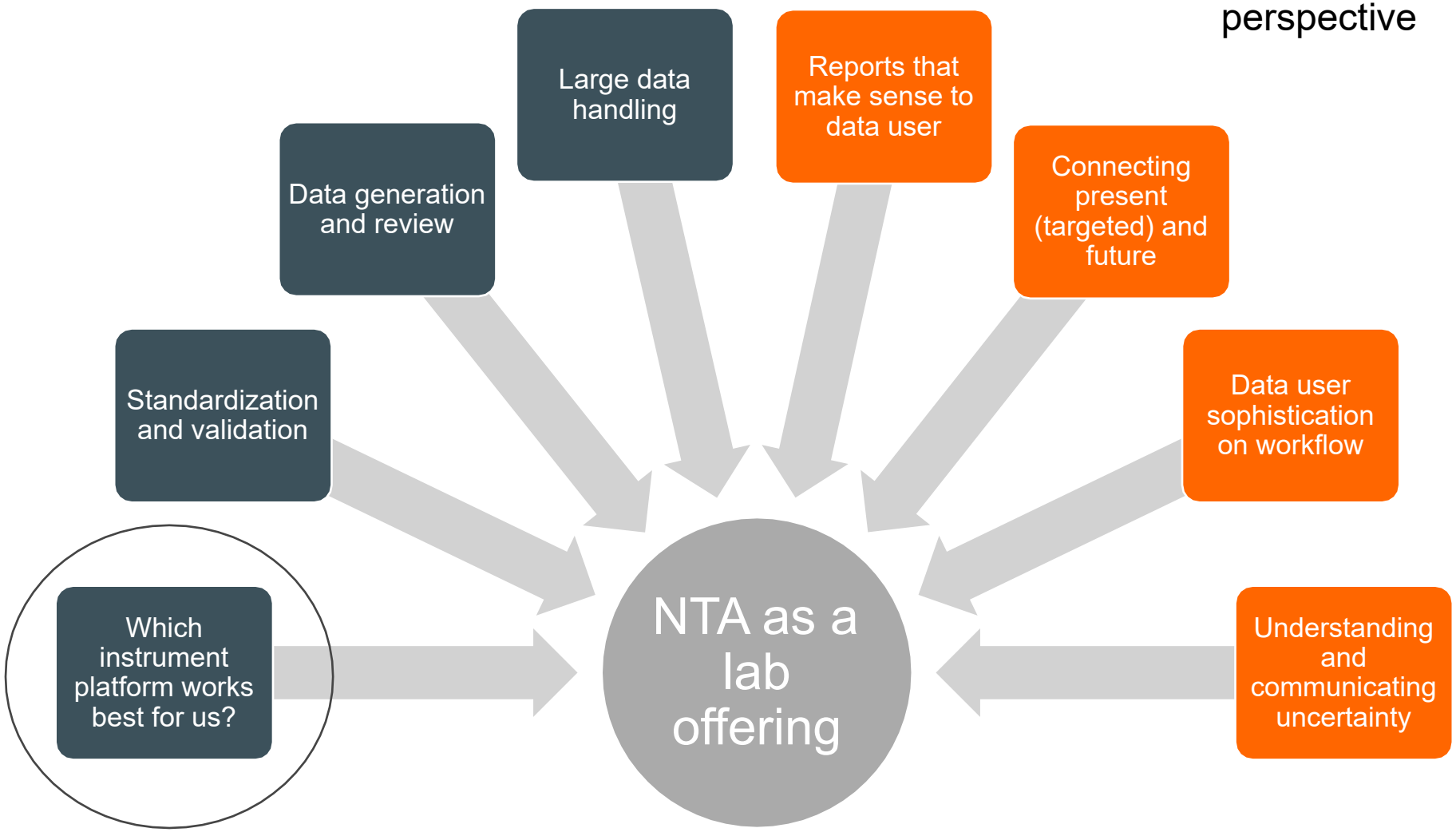


Fig. 1. A chloroperfluoropolyether carboxylate (ClPFPECA) identified by nontargeted MS analyses in soil samples from New Jersey. In the New Jersey samples, perfluoroethyl (e) plus perfluoropropyl (p) groups were observed to range in sum from one to four. The example congener depicted here would be designated (e,p) = 1,1. Isomers likely include an alternative terminal structure of $ClCF_2CF(CF_3)O$– (*13*, *14*) as well as relative positions for the perfluoroethyl and perfluoropropyl groups.

Washington et al. 2020 (US EPA)

The challenges to overcome

Orange: Data user perspective
Grey: Lab perspective



Workflows

Target analysis

- Quantitatively measure knowns
- Uses standards, isotope dilution etc.
- Also generate full-scan data at the same time

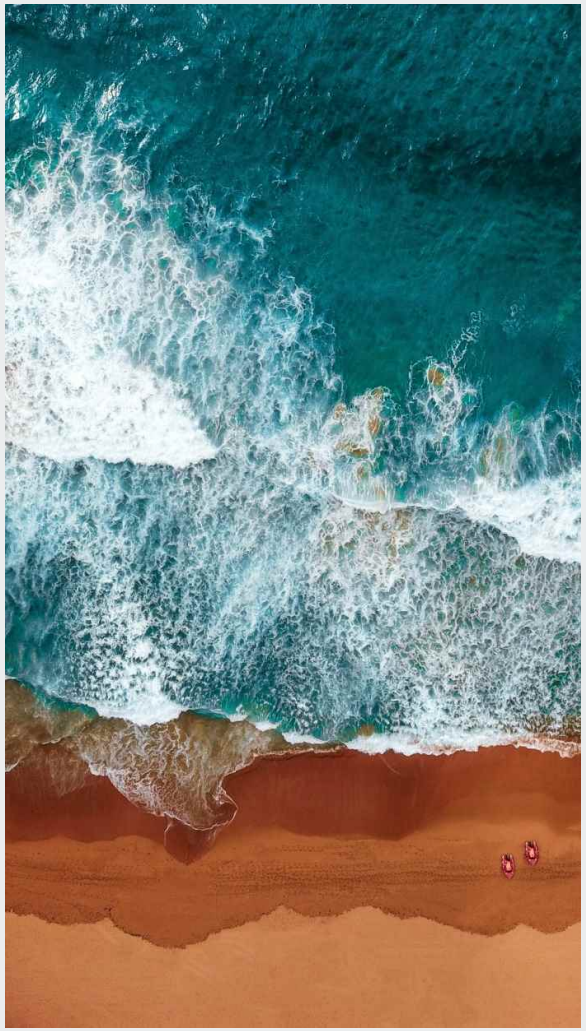
Suspect Screening

- Use library of known PFAS to identify list of “suspects”
- Use standards for relative “quantitation”

Untargeted

- Looking for unknown, uncharacterized
- Uses user-developed, proprietary or public workflows such as FluoroMatch to annotate features
- Qualitative/relative

Instrument Platform Evaluation Objectives



- Sensitivity in target analysis – Can it run 1633 for example?
  - 1633 calibration used as the basis of comparison
- Suspect screening workflow in real samples for PFAS
  - Used extracts from biosolids previously run by 1633
- Unknowns workflow
  - Used extracts from biosolids previously run by 1633
- Software and data use
- Friendliness with current and new tools such as FluoroMatch, NIST, MzCloud
- Experience of team, strength of suspects lists

Two TOFs and a Trap: Meet the candidates

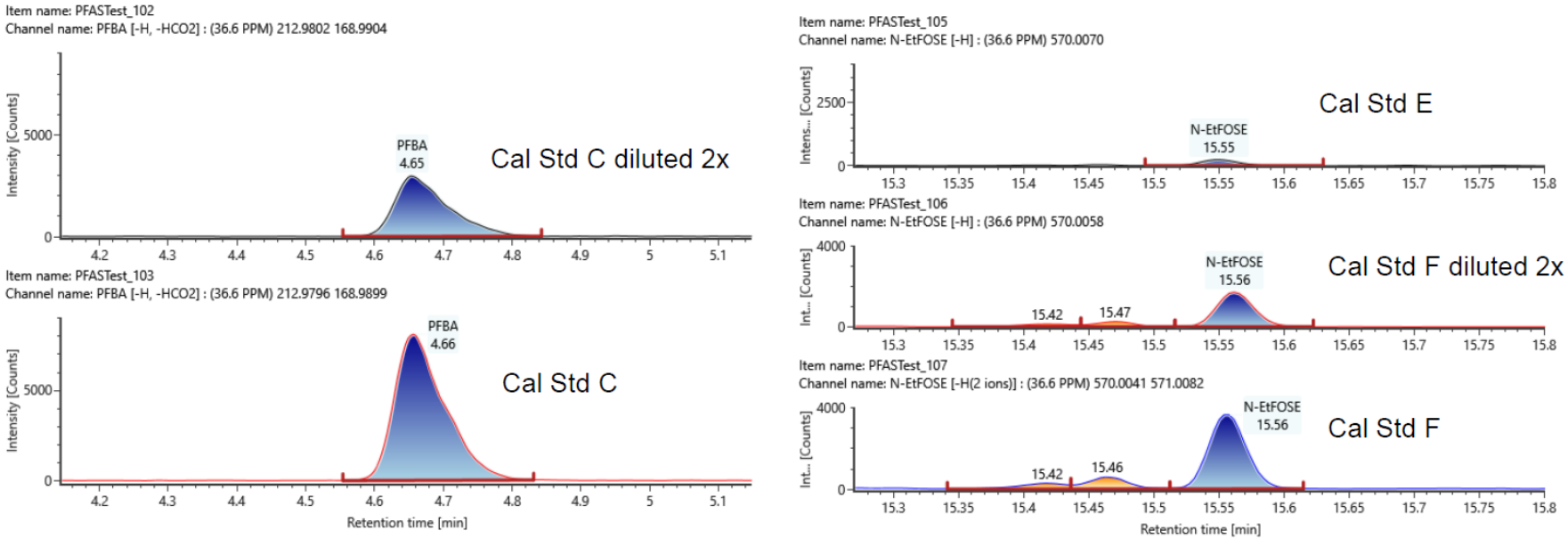
QTOF-1	QTOF-2	Trap-1
• 20-32,000 m/z mass range • Mass accuracy: < 1 ppm • Scan speed 30 Hz	• 20-4000 m/z • Mass accuracy < 1 ppm • Scan speed 50 Hz	• 40-3000 m/z mass range • Mass accuracy: <1-2 ppm • Scan speed 22 hz (resolution dependent)

Results

CAVEAT!!! All instrument work done by vendors. Their workflow and optimization factors, not ours, affect results and so, definitely not for direct comparison

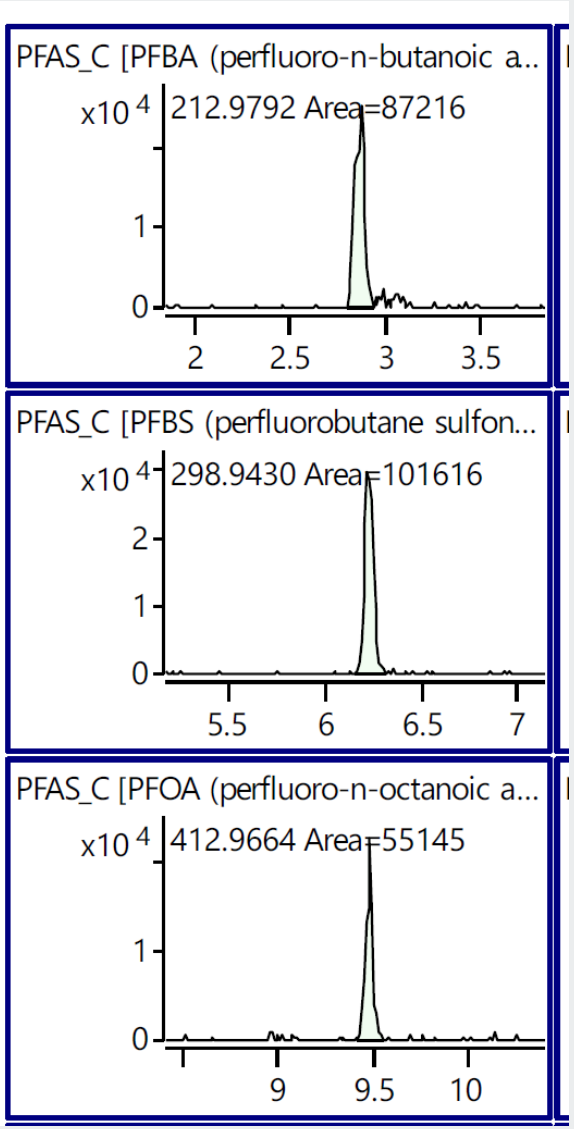
I am definitely not an NTA expert, so this is a learning experience

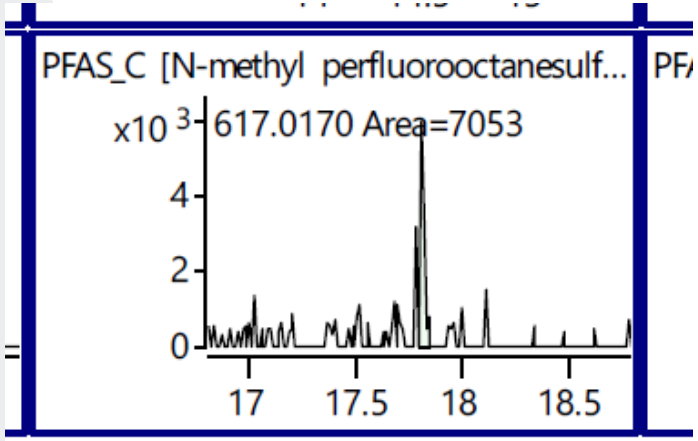
Results: 1633 QTOF-1

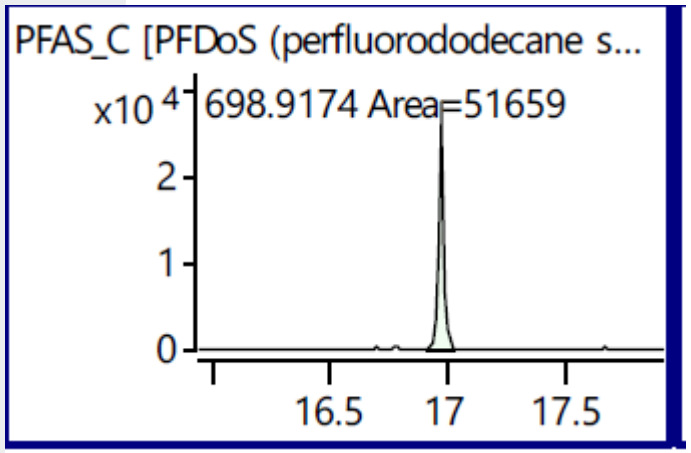


- Sensitive enough for most 1633 targets at the low calibration
- 37 out of 40 picked out
- Missing neutrals N-EtFOSE, N-MeFOSE and long-chain PFSA PFDoS
- Pretty good!

1633 Q-TOF-2



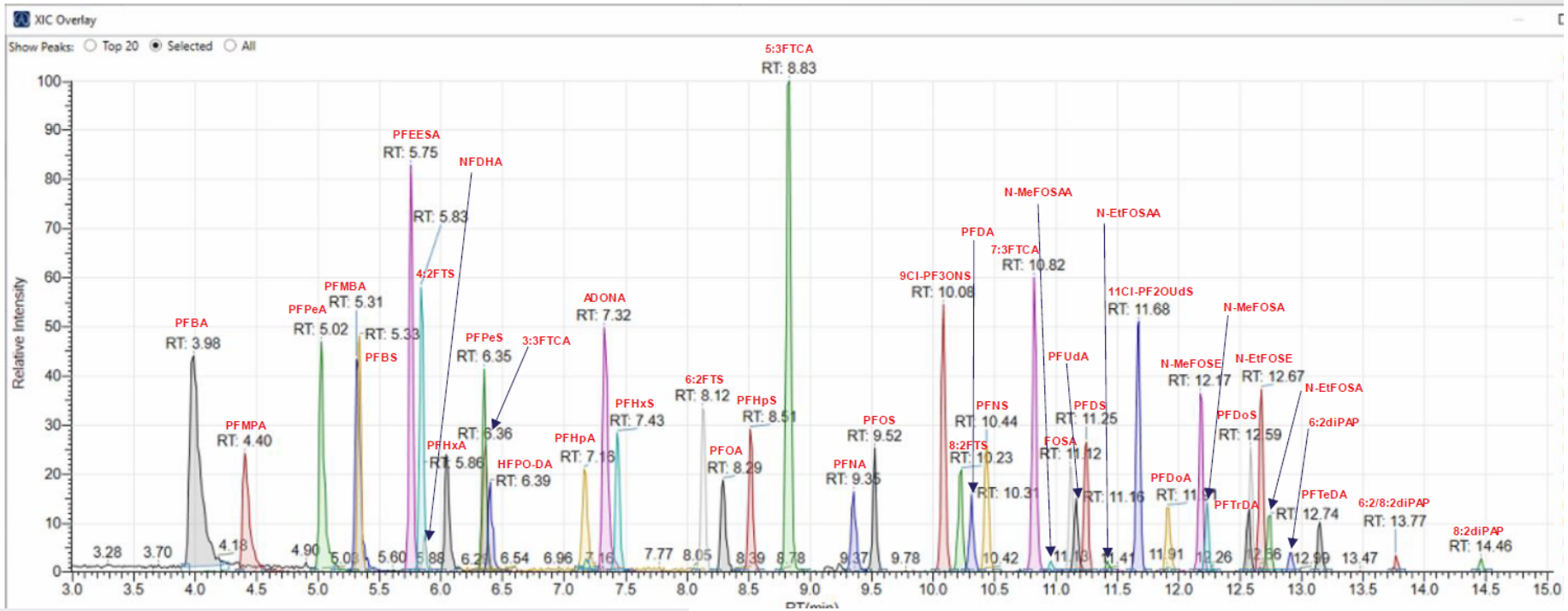




- Sensitive enough for most 1633 targets
- 38 out of 40 provided good enough signal for measurement, all 40 identified.
- N-MeFOSE and N-EtFOSE behaved the worst
- Pretty good!

1633 Trap-1

EPA 1633 PFAS targets + diPAPs: XICs from Full-MS data

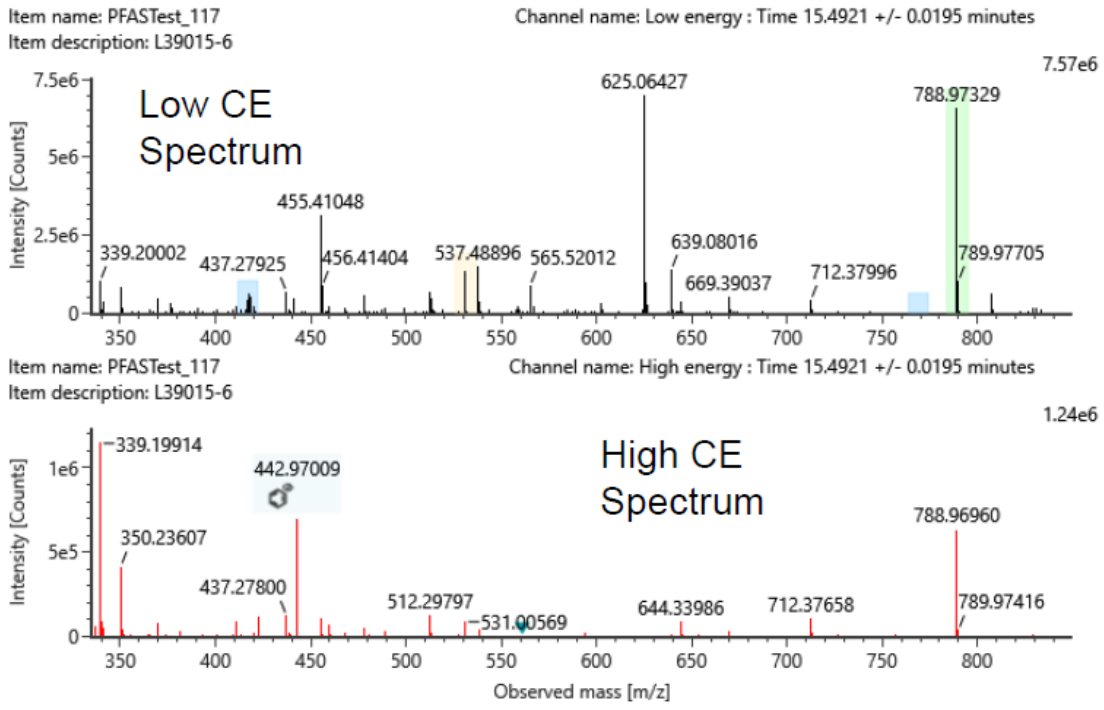


- **Caveat: Only demo to run full calibration set**
- Calibrated 1633 successfully
- All 40 targets detected at or below 1633 limits

Quantitative target analysis takeaways

- Performance can be essentially equivalent to a mid-range unit-resolution LC-MS/MS system while working at high enough resolution to provide interference solutions
- A unit-resolution LC-MS/MS would have a higher dynamic range and is more suitable for day-to-day quantitative analysis
- **Instruments are sensitive enough for quantitative analysis**

Suspect Screening: QTOF-1



High Energy Fragments

6:2diPAP (2 expected fragments) (26 found generated fragments)

	Expected m/z	Status	Observed m/z	Mass error (ppm)	Mass error (mDa)	Detector counts
1	442.97234	Found	442.97009	-5.08	-2.2	21034
2	96.96962	Found	96.96011	-98.10	-9.5	45466

- Data independent acquisition (DIA) at two different energies to increase information
- Vendor software for all processing
- Internally generated PFAS library containing ~100 PFAS
- Suspect list reported was 1633 targets, FOSAA, 6:2 and 8:2 DiPAP

Suspect Screening: QTOF-2

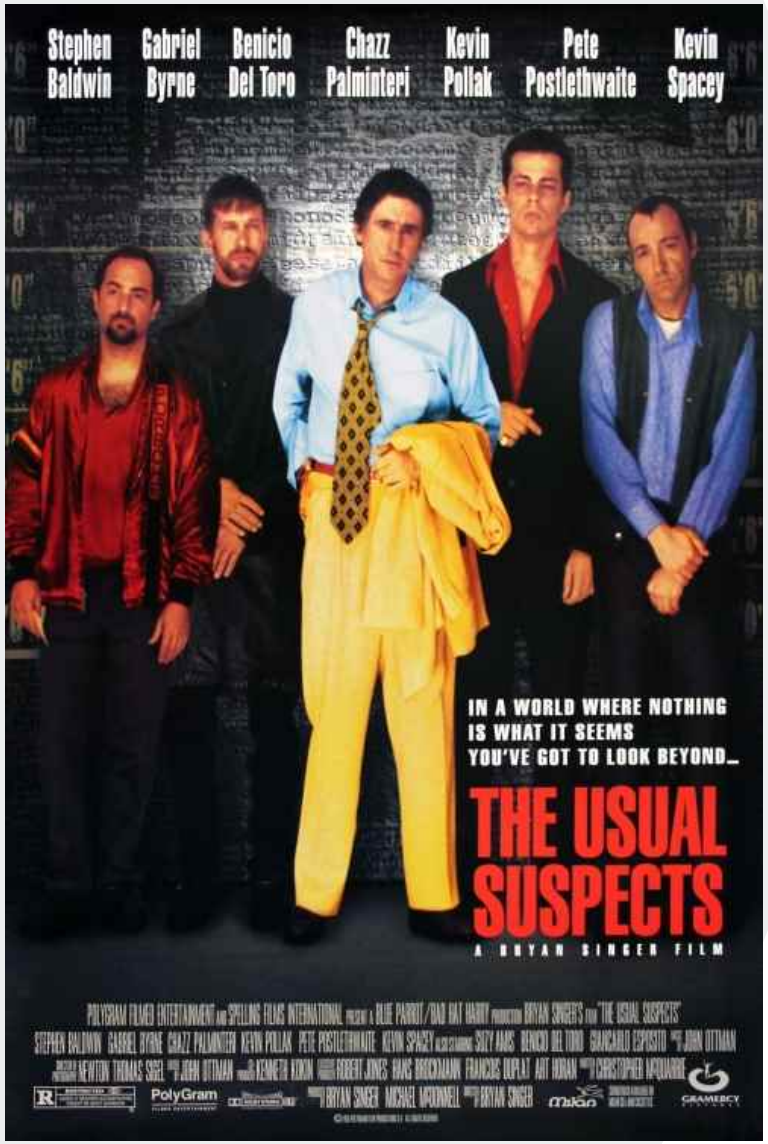
Screening Data Review of 6:2, 6:2 Fluorotelomer phosphate diester

- Full scan DIA approach
- Vendor software for all processing
- Used Getzinger et al. 2021 in-silico database for ~50,000 list of suspects and other sources incl. CompTox
- Detected suspect list was 1633 targets, FOSAA, 6:2 and 8:2 DiPAP, multiple short-chain sulfonamides

Suspect Screening: Trap-1

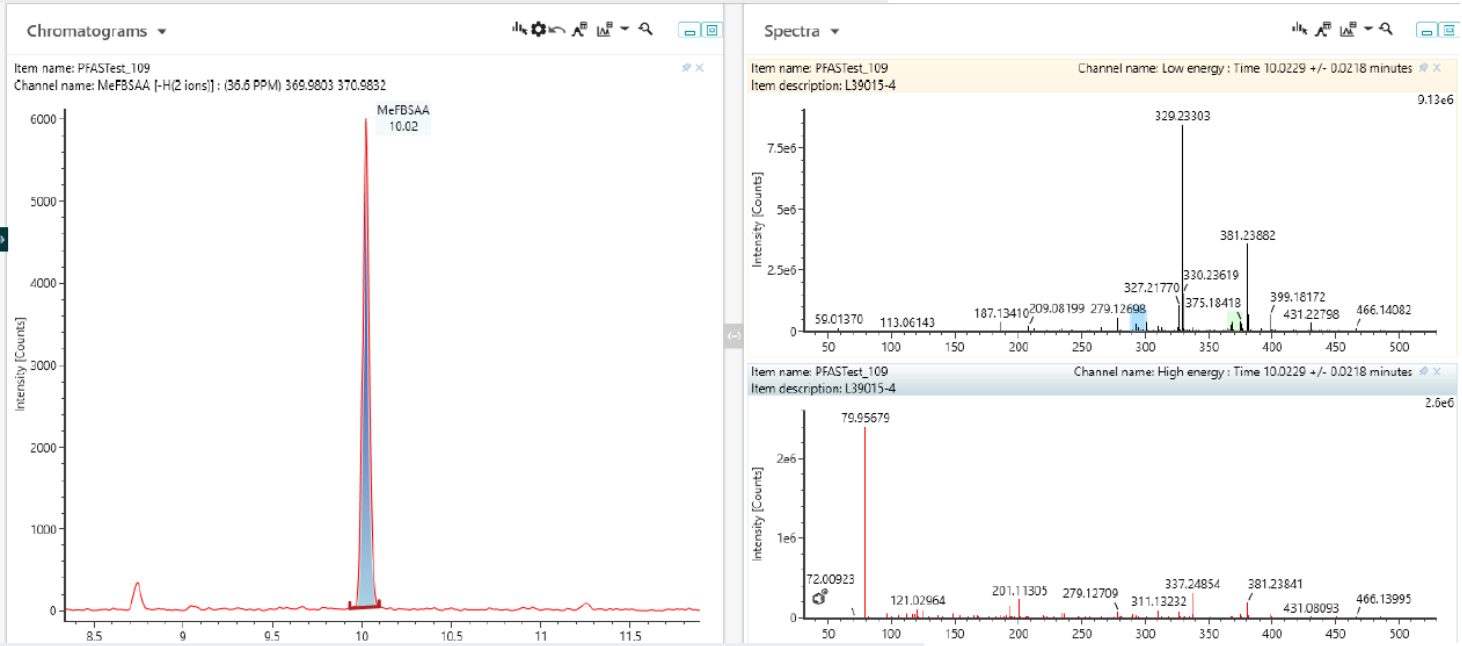
Not specifically performed, integrated into NTA workflow

Suspect Screening takeaways



- This is a useful and relatively easily scalable approach which could be the first candidate for a more universally available standardized commercial approach
- However, standardization of sample preparation, analysis (to an extent) and post-data processing including suspects lists is critical for results comparison
- Add-on relative quantitation requires a labeled standard-based common convention
- Custom/proprietary/inhouse suspect lists will (in my opinion!) hinder this process

NTA QTOF-1



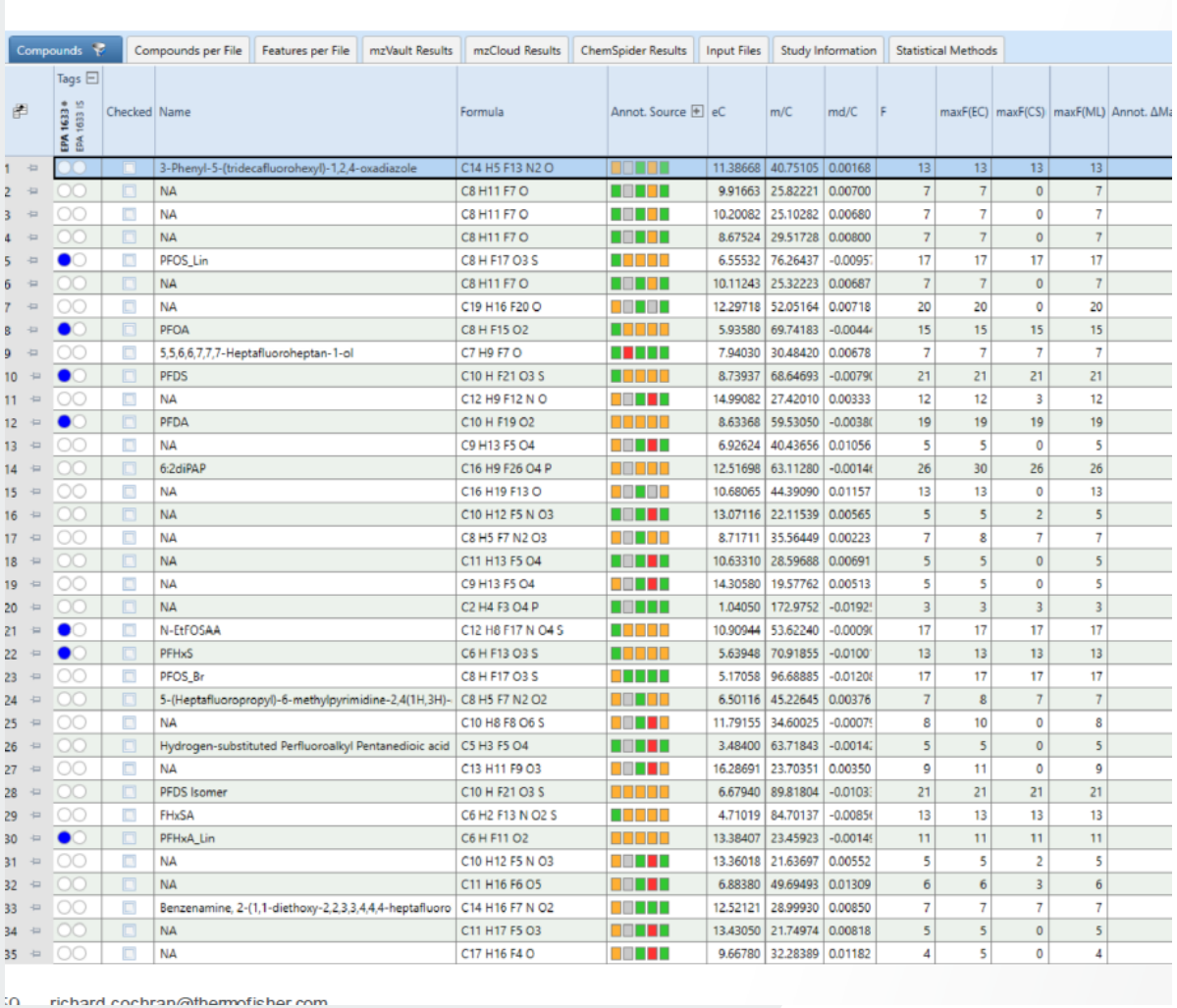
- Performed in DIA mode
- Fully internal approach using proprietary software
  - Common fragments
  - Mass defect filtering
  - Other processing
- Vendor process used info from Chemspider, Toxcast, etc. No direct connect to Fluoromatch
- Vendor standard reporting did not provide list of IDs with Schymanski confidence levels et.

NTA QTOF-2

Score	Formula	Name or Class
A	C12H2F22O2	PFCA-H
A	C10HF19O2	PFCA-perfluoroalkyl_branched
A	C8HF17O3S	PFSA-perfluoroalkyl
A-	C3HF7O	PFOH-perfluoroalkyl_alcohol
B	C11H8F9N	N-Methyl-4-(nonafluorobutyl)aniline
B	C7H9F7O	1,1,1,2,2,3,3-heptafluoroheptan-4-ol
B	C7H2F14O	1,1,2,2,3,3,4,4,5,5,6,6,7,7-Tetradecafluoroheptan-1-ol
B	C17H21F13O	12,12,13,13,14,14,15,15,16,16,17,17,17-Tridecafluoroheptadec-10-en-1-ol
B	C17H21F13O	12,12,13,13,14,14,15,15,16,16,17,17,17-Tridecafluoroheptadec-10-en-1-ol
B	C10H17F5O5	13,13,14,14,14-Pentafluoro-3,6,9,12-tetraoxatetradecan-1-ol
B	C4H4F4O2	2,2,3,3-Tetrafluorobutanoic acid
B	C3H2F4S2	2,3,3,3-Tetrafluoroprop-1-ene-1,1-dithiol
B	C5H8F4O	2-Methyl-3,3,4,4-tetrafluoro-2-butanol
B	C6H5ClF6O2	2-Pentanone, 1-chloro-4-hydroxy-5,5,5-trifluoro-4-(trifluoromethyl)-
B	C6H10F4O3	3-(2,2,3,3-Tetrafluoropropoxy)propane-1,2-diol
B	C6H10F4O3	3-(2,2,3,3-Tetrafluoropropoxy)propane-1,2-diol
B	C6H3F7O3	4,4,5,5,6,6,6-Heptafluoro-3-oxohexanoic acid
B	C7H3F9O	4,4,5,5,6,6,7,7,7-Nonafluorohept-2-yn-1-ol
B	C6H2F6O2	4,4,5,5,6,6-Hexafluorohex-2-ynoic acid
B	C18H17F7N2O2	5-(4-tert-Butylphenyl)-N-(2,2,3,3,4,4,4-heptafluorobutyl)-1,2-oxazole-3-carboxamide
B	C18H17F7N2O2	5-(4-tert-Butylphenyl)-N-(2,2,3,3,4,4,4-heptafluorobutyl)-1,2-oxazole-3-carboxamide
B	C6H2F6N2O2	5-Fluoro-6-(pentafluoroethyl)pyrimidine-2,4(1H,3H)-dione
B	C6H8F4O2	Butanoic acid, 3,3,4,4-tetrafluoro-2,2-dimethyl-

- Performed in DDA mode with iterative exclusion
- Incorporates FluoroMatch for annotation
- Identified > 7165 possible features and worked through Schymanski Scale towards confident matches such as a C12-hydrogen-substituted carboxylate
- Overall, fairly straightforward to implement
- Explaining the Schymanski scale to a data user accustomed to looking at targeted quantitative data will be interesting ☺

NTA Trap-1



Compounds | Compounds per File | Features per File | mzVault Results | mzCloud Results | ChemSpider Results | Input Files | Study Information | Statistical Methods

	Tags	Checked	Name	Formula	Annot. Source	eC	m/C	md/C	F	maxF(EC)	maxF(CS)	maxF(ML)	Annot. ΔMa
1			3-Phenyl-5-(tridecafluorohexyl)-1,2,4-oxadiazole	C14 H5 F13 N2 O		11.38668	40.75105	0.00168	13	13	13	13	
2			NA	C8 H11 F7 O		9.91663	25.82221	0.00700	7	7	0	7	
3			NA	C8 H11 F7 O		10.20082	25.10282	0.00680	7	7	0	7	
4			NA	C8 H11 F7 O		8.67524	29.51728	0.00800	7	7	0	7	
5			PFOS_Lin	C8 H F17 O3 S		6.55532	76.26437	-0.0095	17	17	17	17	
6			NA	C8 H11 F7 O		10.11243	25.32223	0.00687	7	7	0	7	
7			NA	C19 H16 F20 O		12.29718	52.05164	0.00718	20	20	0	20	
8			PFOA	C8 H F15 O2		5.93580	69.74183	-0.0044	15	15	15	15	
9			5,5,6,6,7,7,7-Heptafluoroheptan-1-ol	C7 H9 F7 O		7.94030	30.48420	0.00678	7	7	7	7	
10			PFDS	C10 H F21 O3 S		8.73937	68.64693	-0.0079	21	21	21	21	
11			NA	C12 H9 F12 N O		14.99082	27.42010	0.00333	12	12	3	12	
12			PFDA	C10 H F19 O2		8.63368	59.53050	-0.0038	19	19	19	19	
13			NA	C9 H13 F5 O4		6.92624	40.43656	0.01056	5	5	0	5	
14			6:2diPAP	C16 H9 F26 O4 P		12.51698	63.11280	-0.0014	26	30	26	26	
15			NA	C16 H19 F13 O		10.68065	44.39090	0.01157	13	13	0	13	
16			NA	C10 H12 F5 N O3		13.07116	22.11539	0.00565	5	5	2	5	
17			NA	C8 H5 F7 N2 O3		8.71711	35.56449	0.00223	7	8	7	7	
18			NA	C11 H13 F5 O4		10.63310	28.59688	0.00691	5	5	0	5	
19			NA	C9 H13 F5 O4		14.30580	19.57762	0.00513	5	5	0	5	
20			NA	C2 H4 F3 O4 P		1.04050	172.9752	-0.0192	3	3	3	3	
21			N-EtFOSAA	C12 H8 F17 N O4 S		10.90944	53.62240	-0.0009	17	17	17	17	
22			PFHxS	C6 H F13 O3 S		5.63948	70.91855	-0.0100	13	13	13	13	
23			PFOS_Br	C8 H F17 O3 S		5.17058	96.68885	-0.0120	17	17	17	17	
24			5-(Heptafluoropropyl)-6-methylpyrimidine-2,4(1H,3H)-	C8 H5 F7 N2 O2		6.50116	45.22645	0.00376	7	8	7	7	
25			NA	C10 H8 F8 O6 S		11.79155	34.60025	-0.0007	8	10	0	8	
26			Hydrogen-substituted Perfluoroalkyl Pentanedioic acid	C5 H3 F5 O4		3.48400	63.71843	-0.0014	5	5	0	5	
27			NA	C13 H11 F9 O3		16.28691	23.70351	0.00350	9	11	0	9	
28			PFDS Isomer	C10 H F21 O3 S		6.67940	89.81804	-0.0103	21	21	21	21	
29			FHxSA	C6 H2 F13 N O2 S		4.71019	84.70137	-0.0085	13	13	13	13	
30			PFHxA_Lin	C6 H F11 O2		13.38407	23.45923	-0.0014	11	11	11	11	
31			NA	C10 H12 F5 N O3		13.36018	21.63697	0.00552	5	5	2	5	
32			NA	C11 H16 F6 O5		6.88380	49.69493	0.01309	6	6	3	6	
33			Benzenamine, 2-(1,1-diethoxy-2,2,3,3,4,4,4-heptafluoro	C14 H16 F7 N O2		12.52121	28.99930	0.00850	7	7	7	7	
34			NA	C11 H17 F5 O3		13.43050	21.74974	0.00818	5	5	0	5	
35			NA	C17 H16 F4 O		9.66780	32.28389	0.01182	4	5	0	4	

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- Note: Different biosolids extracts were used for the TOFs and the Trap due to availability, so direct comparison not the goal
- Data-dependent acquisition (DDA) with dynamic exclusion
- Results used proprietary software. Also fully compatible with FluoroMatch workflow (not evaluated)
- More complex and customizable workflow, produced similar data
- 35 Level 2 confidence detects in negative and 9 in positive (35 new)

NTA Takeaways

- Critical to understand and standardize instrument and data processing workflows
- Both vendor-neutral (QTOF-2) and vendor proprietary (Trap-1) produced similar looking data
- Either approach would work
- From a commercial perspective, this will require clear collaboration and communication with the data user on experiment, data limits and more
- Data interpretation will likely start at data user end rather than lab end

Standardization Initiatives

https://nontargetedanalysis.org

NTA Study Reporting Tool (SRT)

Analytical and Bioanalytical Chemistry (2019) 411:853–866
https://doi.org/10.1007/s00216-018-1435-6

RESEARCH PAPER

EPA's non-targeted analysis collaborative trial (ENTACT): genesis, design, and initial findings

Elin M. Ulrich[1] · Jon R. Sobus[1] · Christopher M. Grulke[2] · Ann M. Richard[2] · Seth R. Newton[1] · Mark J. Strynar[1] · Kamel Mansouri[3,4] · Antony J. Williams[2]

- What is being reported?
- What methods are being used?
- Quality controls
- Statistics
- Is the documentation sufficient to understand/reproduce?

https://doi.org/10.1007/s00216-018-1435-6 Ulrich et al. 2019

We have a long way to go

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- Greater commercial availability much needed and 2024-2025 will be a pivot timeframe
- Need for PFAS measurement in complex food/biota and interferences present point to use cases of high-resolution mass spectrometry as a fall-back option for definitive data
- Suspect screening options hold great promise, but needs standardization and greater availability of standard lists
- Discovery work is a whole new frontier for commercial labs and will require much higher level discussion on uncertainty, data use