Polyfluoroalkyl Substances (PFAS) in the Environment
(Session 5)

- **Webinar Starts at 1:30 pm Eastern Daylight Time**
- Sound will be on your computer unless you choose to call in.
  - To "Call In", click on Communicate – then Audio Connection and choose to use "Call In".
  - Be sure to enter your ID number when you call or it will not link the call properly to Webex.
- Contact Ilona or Suzanne with any difficulties.
  - ilona.taunton@nelac-institute.org or suzanne.rachmaninoff@nelac-institute.org

NEMC

Meeting Mechanics

This session is being recorded!

Meeting Mechanics

- Phone lines and computer sound are muted when you join the call. Look for the Q&A feature in Webex and type in a Q&A question at any time during the presentation. **Choose to send the question to All Panelists.** Time permitting, there will be a Q&A session at the end of each presentation.

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Polyfluoroalkyl Substances (PFAS) in the Environment (session 5)

Session Chairs: Charles Neslund, Eurofins Lancaster Laboratories Environmental and Mike Chang, Restek Corporation

- 1:30 Quadrupole HRMS for Quantification and Screening of PFAS in EPA 537.1 and 533
  Emily Parry, Agilent Technologies, Inc.
- 2:00 Innovative Technique for Measuring Total Organic Fluoride by Direct-Injection Combustion Ion Chromatography
  Jay Gandhi, Metrohm USA
- 2:30 Automated Sample Preparation for Determination of Perfluoroalkyl and Polyfluoroalkyl Substances (PFAS)
  Angelika Kopf, LCTech GmbH

3:00 BREAK

- 3:15 Column Chemistry Considerations for Full Coverage of Sample Matrices and Analyte Ranges in PFAS LC-MS/MS Workflows
  J Preston, Phenomenex, Inc.
- 3:45 Multi-Laboratory Validation of SW-846 Method 8327, Per- and Polyfluoroalkyl Substances (PFAS) using External Standard Calibration and Multiple Reaction Monitoring (MRM) Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)
  Troy Strock, USEPA Office of Resource Conservation and Recovery

Quadrupole HRMS for Quantification and Screening of PFAS in EPA 537.1 and 533

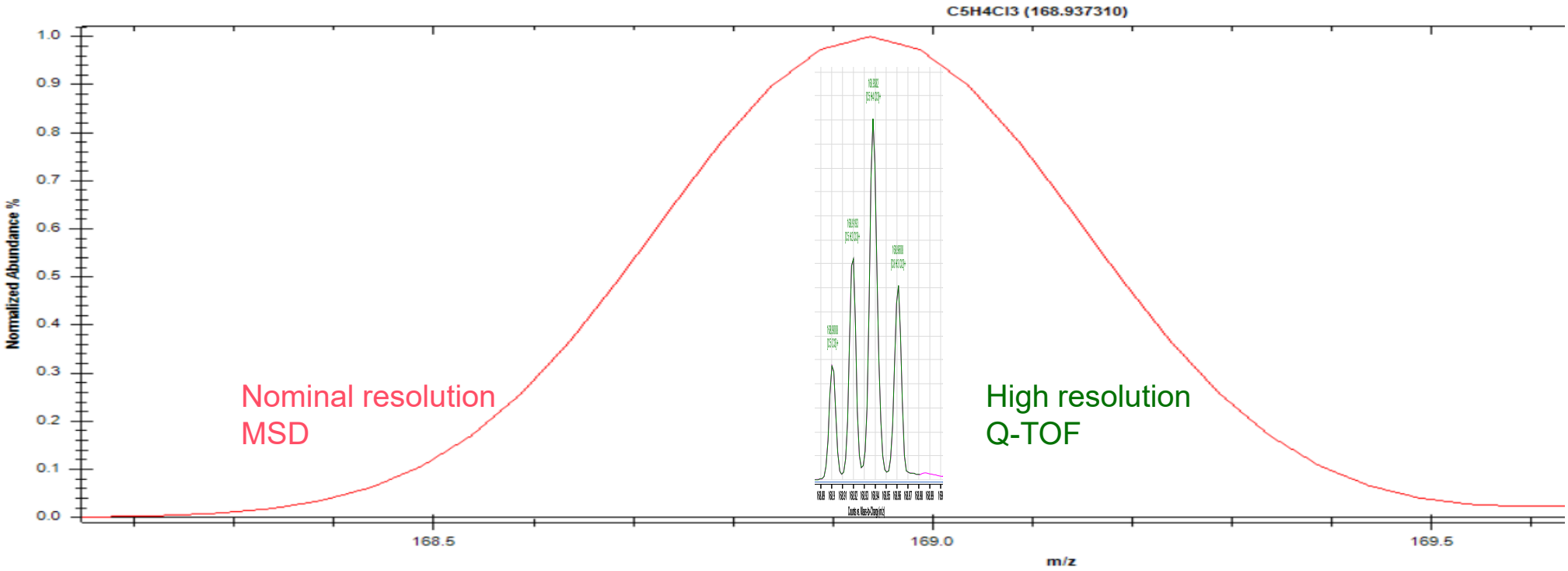
Emily Parry, Tarun Anumol
Agilent Technologies Inc.
Wilmington, DE, USA

Ralph Hindle
Vogon Laboratories
Cochrane, AB, CA

10/31/2020 NEMC 2020 DE.6166319444

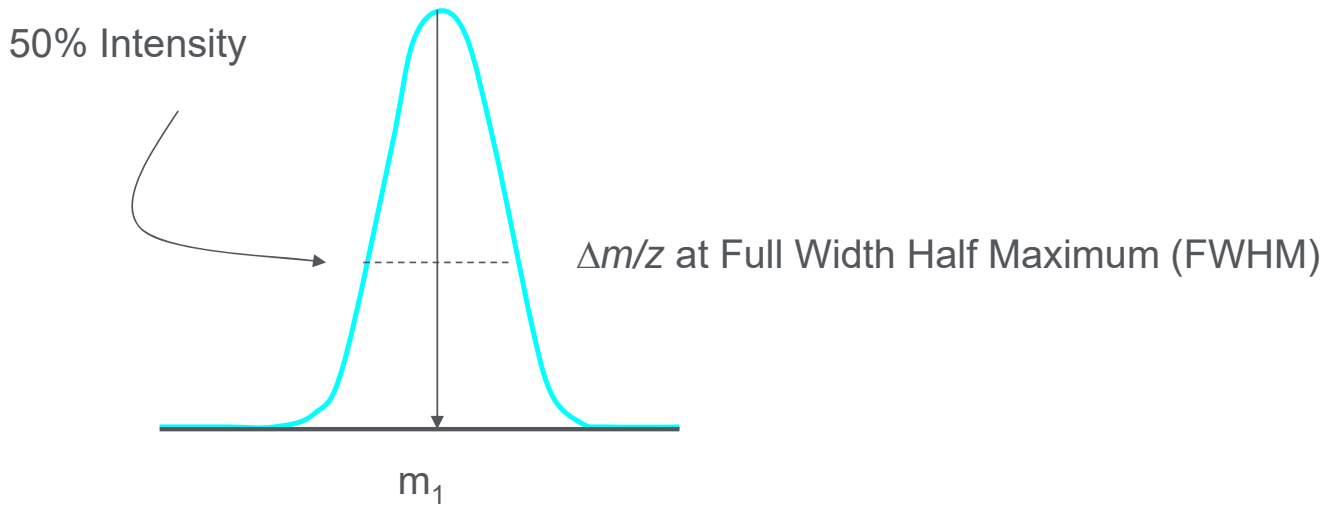
Agilent
Trusted Answers

Why HRMS: Nominal *vs* Accurate Mass



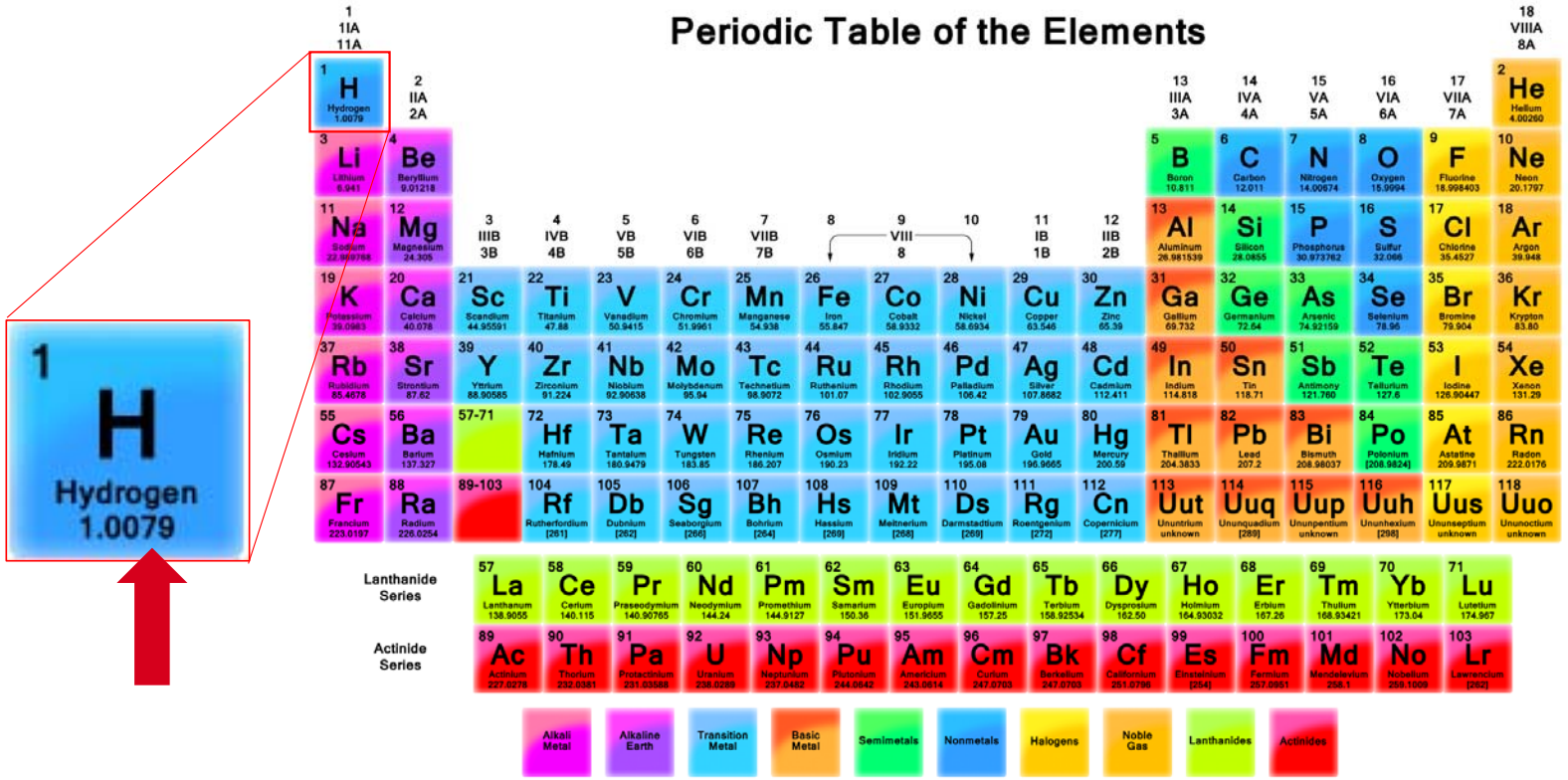
The Definition of Resolution

The definition of resolution for Q-TOF's, Quad's, FT-MS: 50% intensity definition



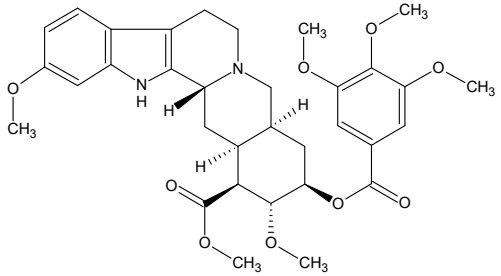
$$\text{Resolution} = m_1/(\Delta m/z)$$

Your High School Chemistry Teacher Lied



Calculation of Exact Mass and Error in Measured Mass

Atom	Mass of Atom	# of Atoms	Sum
Hydrogen	1.00783	40	**40.31300**
Carbon	12.00000	33	**396.00000**
Nitrogen	14.00307	2	**28.00615**
Oxygen	15.99492	9	**143.95424**
Total			**608.27338**
Plus H	1.00783	1	**1.00783**
Total			**609.28121**
Minus e-	0.00055	1	**0.00055**
			609.28066



Reserpine ($C_{33}H_{40}N_2O_9$)

Calculating ppm mass error:

$$= \frac{\text{(Measured Mass – Calculated Mass) X 1,000,000}}{\text{Calculated Mass}}$$

$$= \frac{\text{609.28121 – 609.28066) X 1,000,000}}{\text{609.28066}}$$

= 0.9027039 ppm mass error if the electron was omitted

What is the Benefit of Accurate Mass? Confidence in Compound Identification!

Reserpine ($C_{33}H_{40}N_2O_9$) has a protonated ion at 609.28066

A single quad reports mass to +/- 0.1 = 165 ppm

Number of possible formulae using only C, H, O & N:

- 165 ppm 209 possibilities
- 10 ppm 13
- 5 ppm 7
- 3 ppm 4
- 2 ppm 2

Accurate mass reduces risk of investing effort on the wrong molecule

Accurate Mass + Isotopic Ratios

Accurate Mass

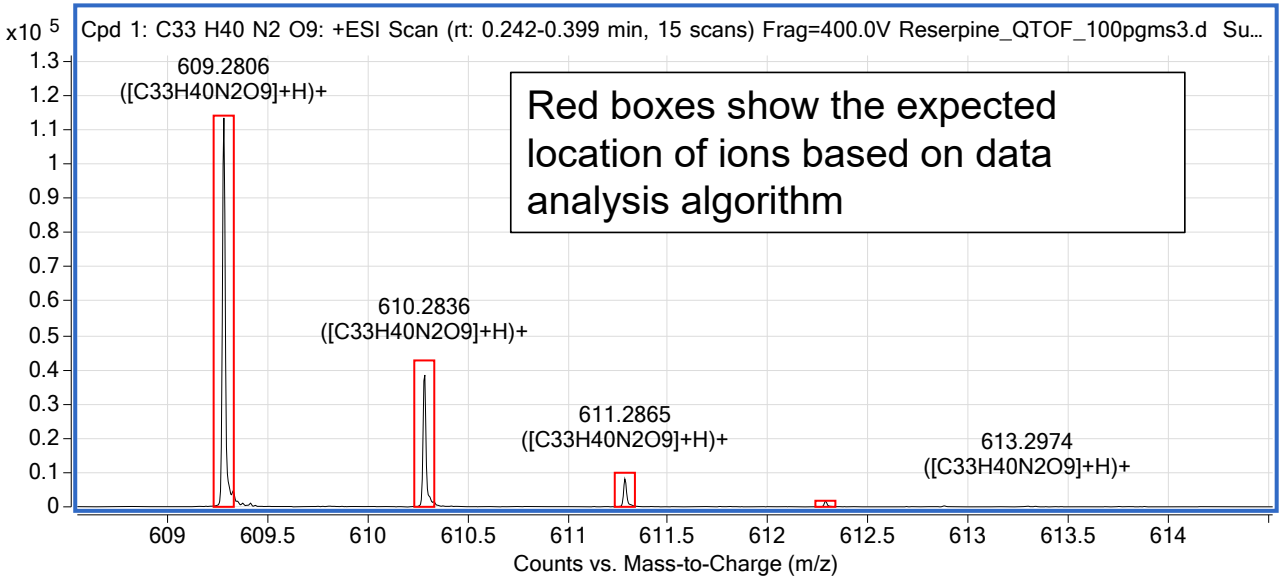
- Highly accurate mass measurement narrows the window of potential compounds possible

Isotopic Fidelity

- Ratios and spacing of ions give clues to what elements are present in the formula

Data Analysis

- Targeted analysis (FbF, DB/Lib Search, etc)
- Untargeted analysis (Formula Generation)

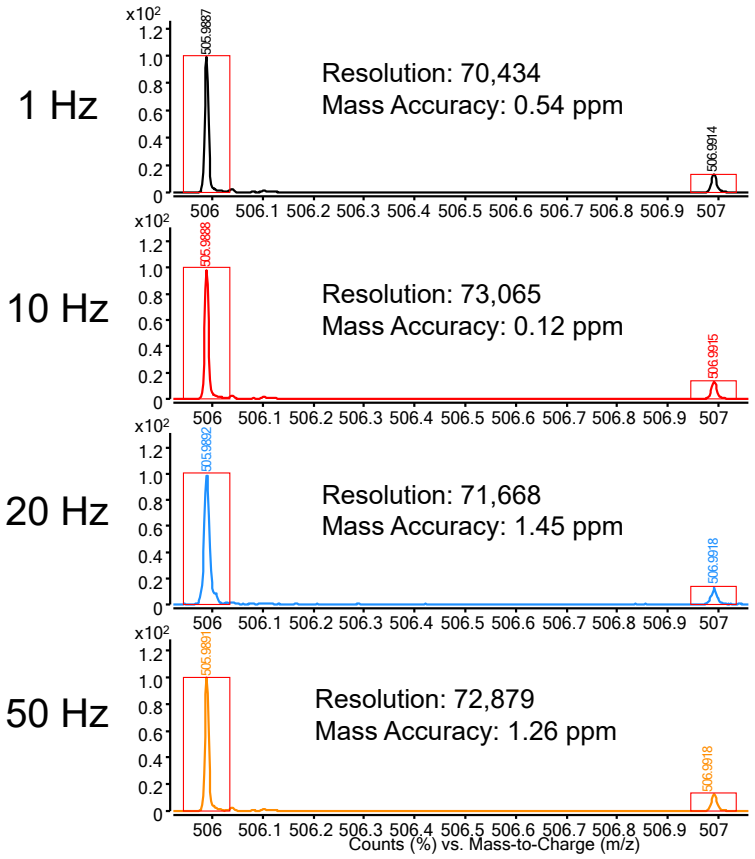


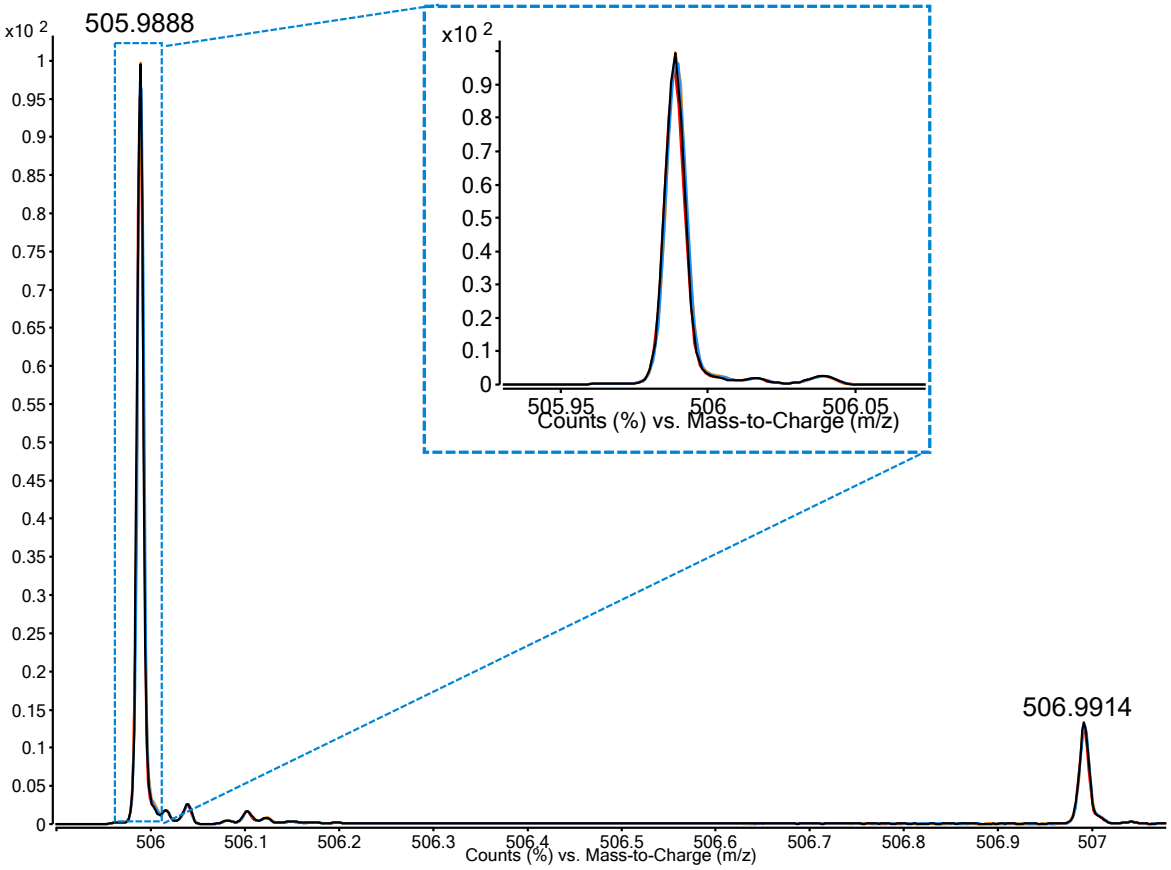
Cpd	File	ID Source	RT	m/z	Diff (Tgt, ppm)	Score
1	Reserpine_QTOF_100pgms3.d	FBF	0.276	609.2806	-0.23	99

Overall fit score based on factors like mass accuracy and isotopic fit

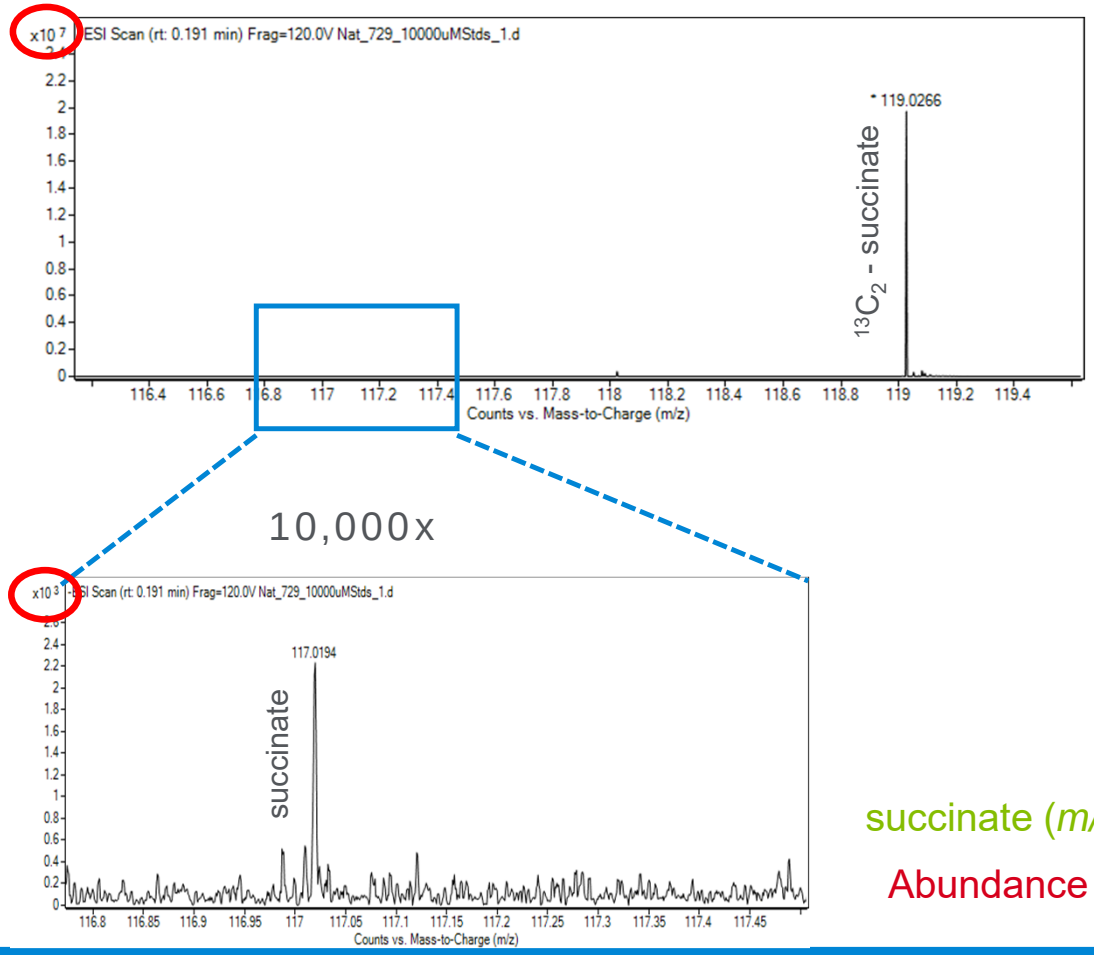
6546 Q-TOF Performance for small molecule analysis

Resolution independent of acquisition rate for ATP





Q-TOF Extended Dynamic Range using two 10GHz channels

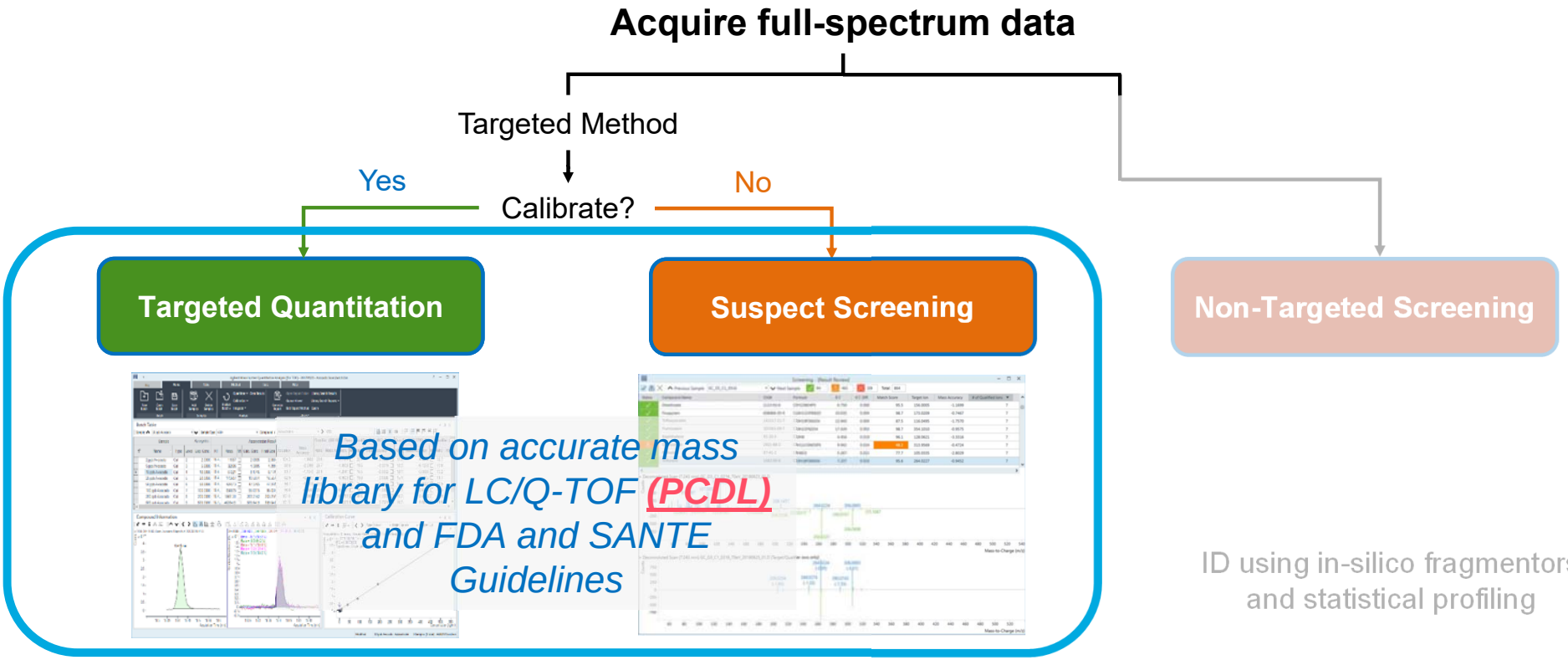


$^{13}C_2$ succinate (*m/z* 119.0259).
Abundance ~ 2 x 10^7

succinate (*m/z* 117.0191)

Abundance ~ 2 x 10^3

Screening Workflow with LC/Q-TOF



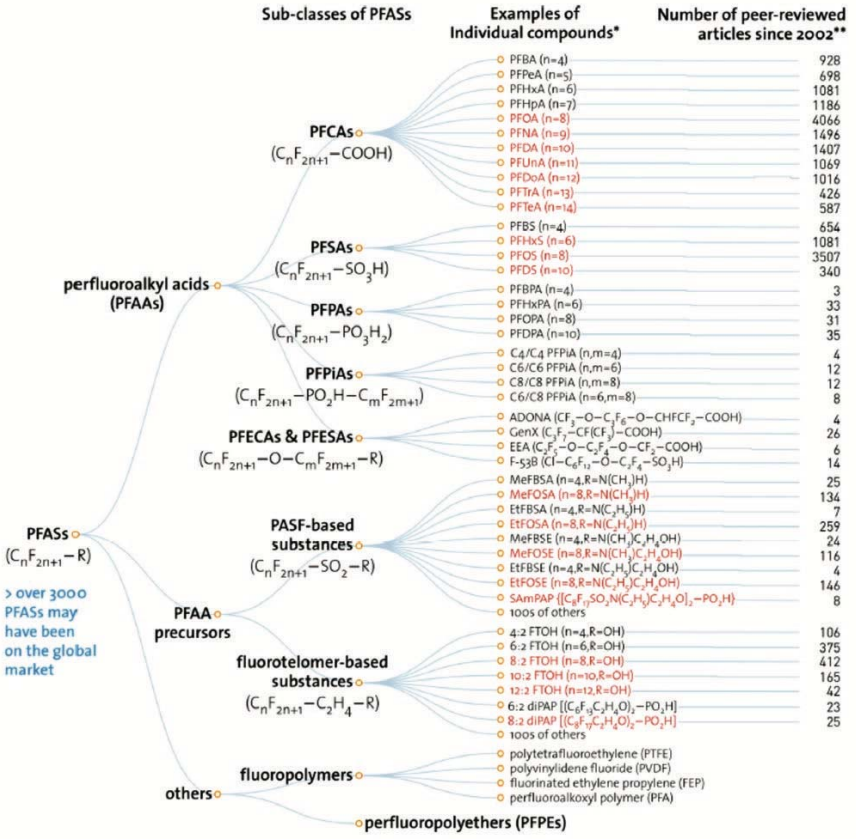
One Software

PFAS Classifications and Terminology

>4000 PFAS compounds in commerce

Common Acronyms

PFCA	Perfluoroalkylcarboxylic acid
PFOA	Perfluorooctanecarboxylic acid
PFAS	Perfluoroalkylsulfonate
PFOS	Perfluorooctanesulfonate
PFASi	Perfluoroalkylsulfinate
FOSA	Perfluorooctanesulfonamide
FOSAA	Perfluorooctanesulfonamidoacetic acid
FOSE	Perfluorooctanesulfonamidoethanol
FTOH	Fluorinated telomer alcohol (-OH functional group)
FTA	Fluorinated telomer acid
FTUA	Fluorinated telomer unsaturated acid
FTS	Fluorinated telomer sulfonate
PFAPA	Perfluoroalkylphosphonic acid
PFPi	Perfluoroalkylphosphinate
PAP	Mono-substituted polyfluoroalkylphosphate ester
diPAP	Di-substituted polyfluoroalkylphosphate ester
PFAI	Perfluoroalkyl iodide
SFA	Semifluorinated alkane
FTI	Fluorinated telomer iodide
FTO	Fluorinated telomer olefin
FTAC	Fluorinated telomer acrylate



Wang, Z *et al.* (2017). *Environ. Sci. Technol.* 51, 2508-2518.

EPA 533 – a method to include “short-chain” PFAS

EPA 533	EPA 537.1
25 Analytes	18 Analytes
SPE with WAX	SPE with SDVB
28 days hold time	28 days hold time (14 days for analysis)
Isotope Dilution	Internal Standard
Final extract in 80% MeOH	Final extract in 96% MeOH
2 ions required for reporting with LC/MS/MS	Confirmation ion not necessary
Drinking Water	Drinking Water Only

Analyte	EPA 537.1	EPA 533	Analyte	EPA 537.1	EPA 533
PFBA		✓	PFBS	✓	✓
PFMPA		✓	PFPeS		✓
PFPeA		✓	PFHxS	✓	✓
PFMBA		✓	PFHpS		✓
PFEESA		✓	PFOS	✓	✓
NFDHA		✓	9Cl-PF3ONS	✓	✓
PFHxA	✓	✓	11Cl-PF3OUdS	✓	✓
HFPO-DA	✓	✓	NEtFOSAA	✓	
PFHpA	✓	✓	NMeFOSAA	✓	
ADONA	✓	✓	PFTeDA	✓	
PFOA	✓	✓	PFTrDA	✓	
PFNA	✓	✓	4:2 FTS		✓
PFDA	✓	✓	6:2FTS		✓
PFUnA	✓	✓	8:2FTS		✓
PFDoA	✓	✓			

EPA 533 – Sample Prep and Chromatography

250 mL water sample

↓

Add isotopically labelled std mix (16 compounds)

↓

SPE extraction (method uses WAX cartridge)

↓

Evaporate to dryness

↓

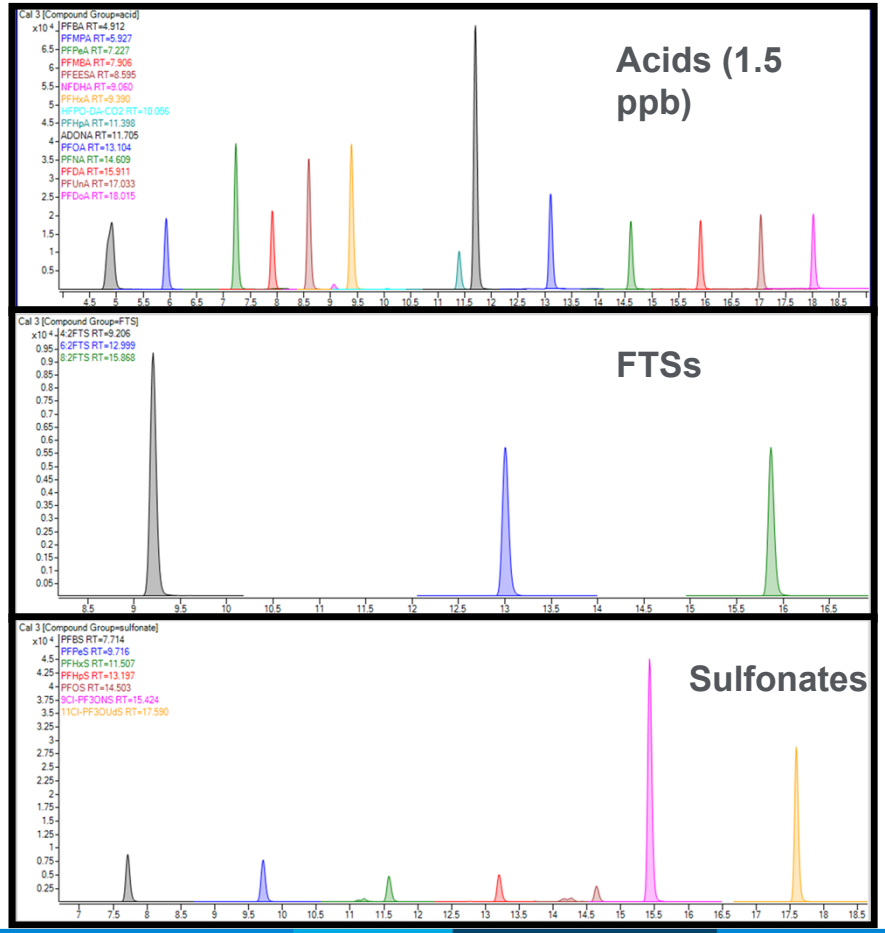
Dissolved in 80:20 Methanol: water

↓

Add IS (3 labeled compounds) – 1 mL final volume

↓

LC-MS/MS analysis



Experimental Study

- Triplicate tap water samples were spiked at 3 levels and taken through offline SPE, as per EPA Method 533 using a weak anion exchange resin
- The final extracts were run from the same vials on both the 6470 triple quadrupole (dMRM mode) and 6545 quadrupole time-of-flight mass spectrometers
- Low level sensitivity was compared with both 6545 QTOF & 6470 MS/MS

LC Conditions

LC Conditions		
Delay Column	Agilent ZORBAX SB-C18, 4.6 × 50 mm, 3.5 µm (p/n 835975-902)	
Analytical Column	Agilent ZORBAX Eclipse Plus C18, 3 × 50 mm; 1.8 µm (p/n 959757-302)	
Column Temperature	50 ºC	
Injection Volume	10 µL	
Mobile Phase	A: 20 mM Ammonium Acetate in water	B: Methanol
Flow Rate	0.40 mL/min	
Gradient program	Time (min)	B (%)
	0.5	5
	0.5	5
	3.0	40
	16	80
	18	80
	20	95
Stop Time	20 min	
Post Time	6 min	

Instrumental Analysis

Targeted Quantification

Ultivo

6470

6495

Suspect Screening/Non-target Analysis

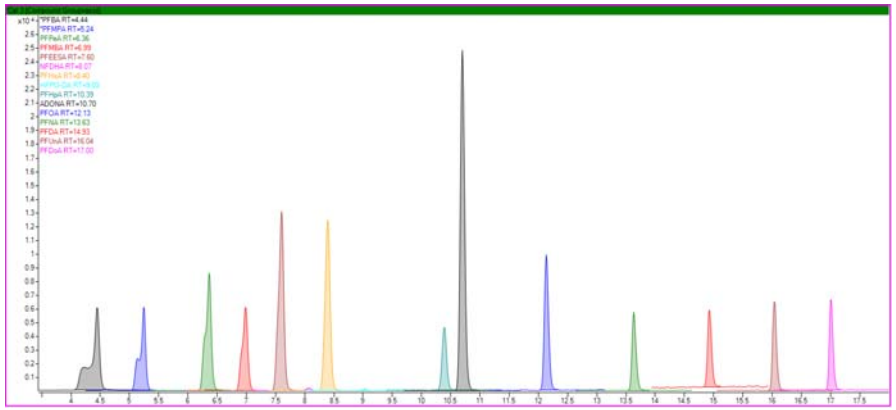
6530

6545

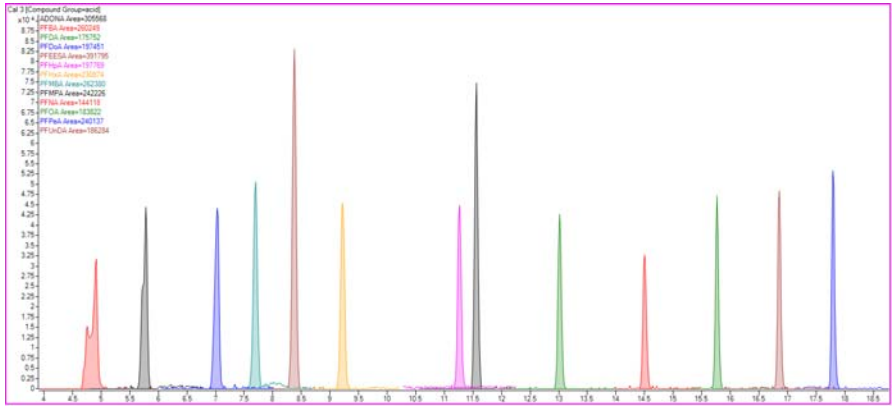
6546

Chromatography – Acids at 1.5 ng/mL (in vial)

6470 QQQ
dMRM



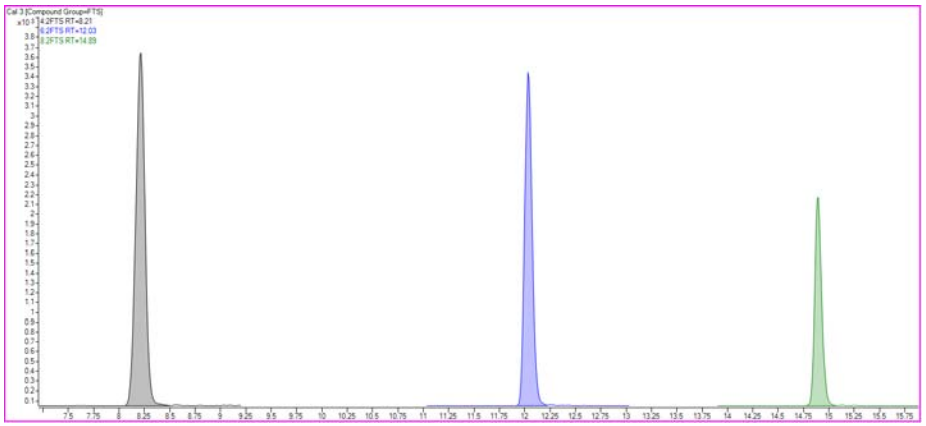
6545 QTOF
All Ions



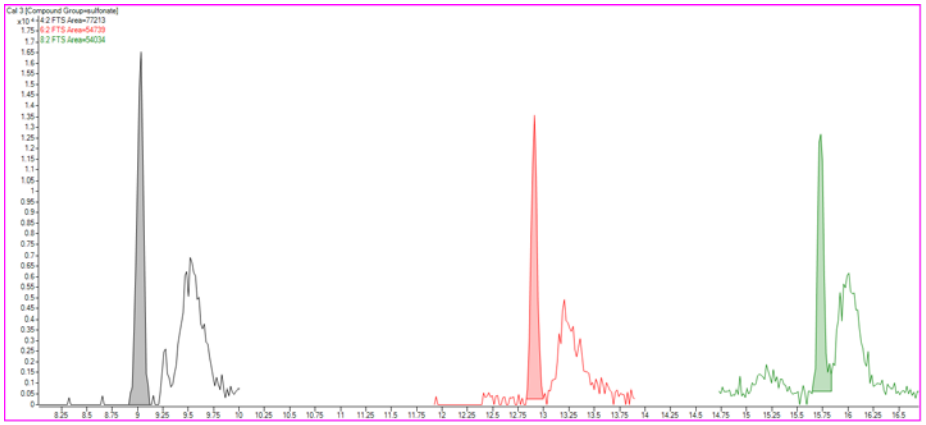
peak shape on early eluters distorted due to 10 uL injection which is not needed for EPA 533 sensitivity levels

Chromatography – FTS's at 1.5 ng/mL (in vial)

6470 QQQ
dMRM

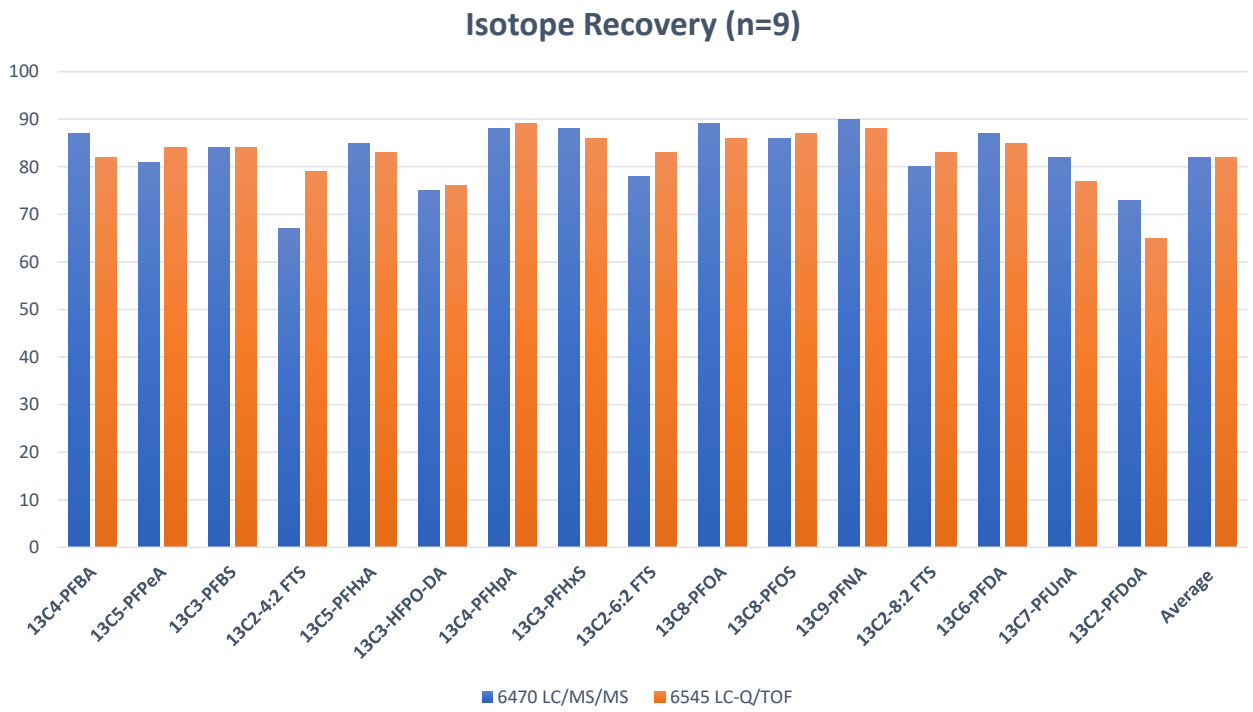


6545 QTOF
All Ions



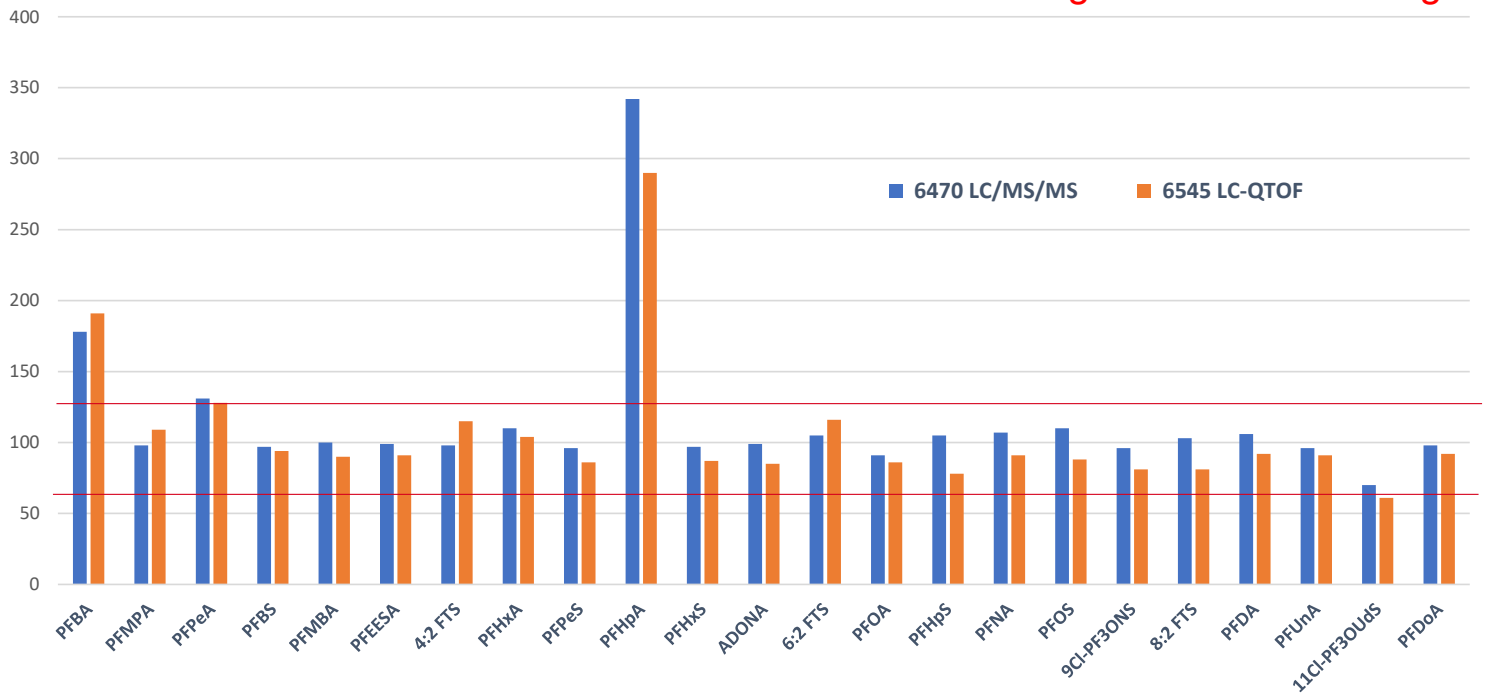
Isotope Dilution Analogs – Precision & Accuracy (n = 9)

Isotope	RT
13C4-PFBA	4.90
13C5-PFPeA	7.00
13C3-PFBS	7.50
13C2-4:2 FTS	9.00
13C5-PFHxA	9.20
13C3-HFPO-DA	9.90
13C4-PFHpA	11.30
13C3-PFHxS	11.40
13C2-6:2 FTS	12.90
13C8-PFOA	13.00
13C8-PFOS	14.50
13C9-PFNA	14.50
13C2-8:2 FTS	15.70
13C6-PFDA	15.80
13C7-PFUnA	16.80
13C2-PFDoA	17.80



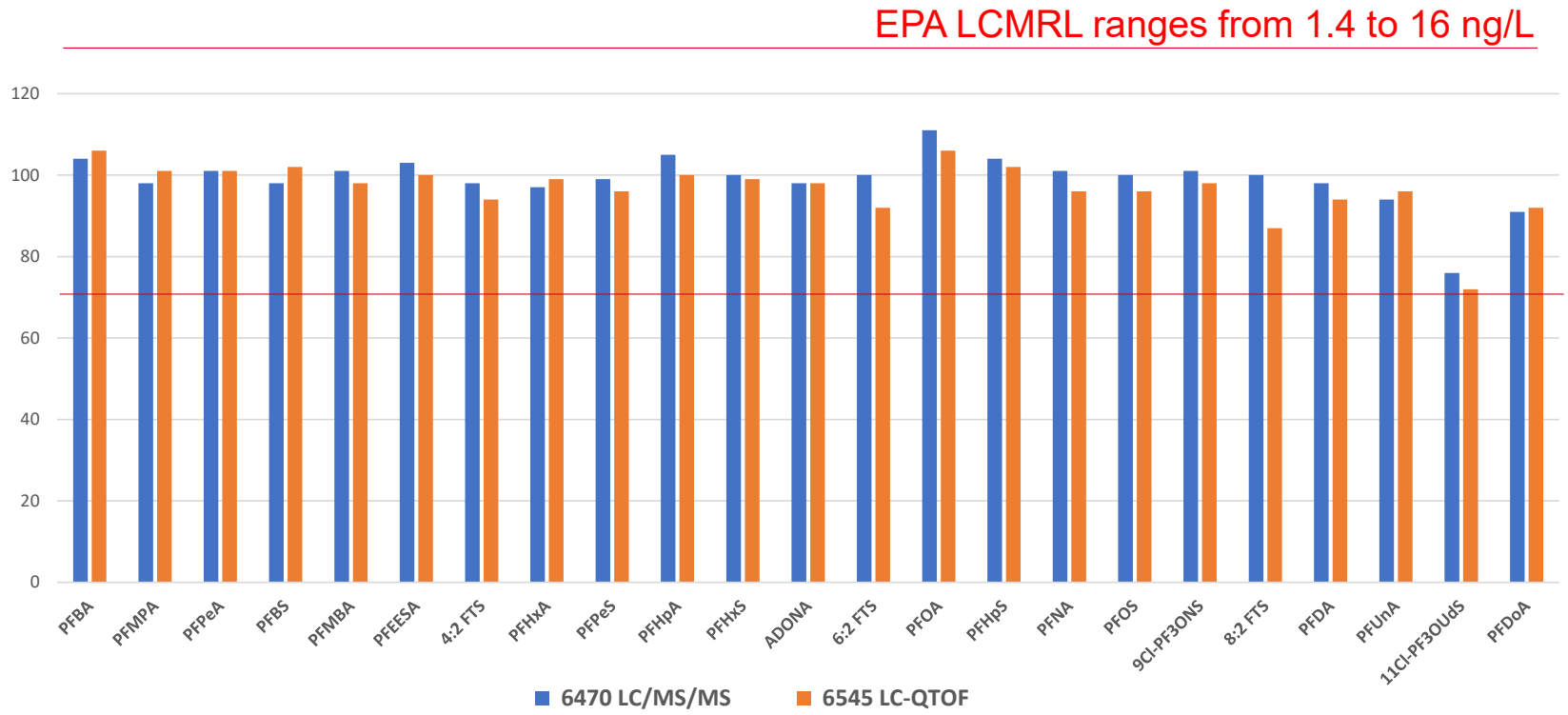
Low level Recovery Comparison (n=3)
1 ng/L drinking water spike

EPA LCMRL ranges from 1.4 to 16 ng/L



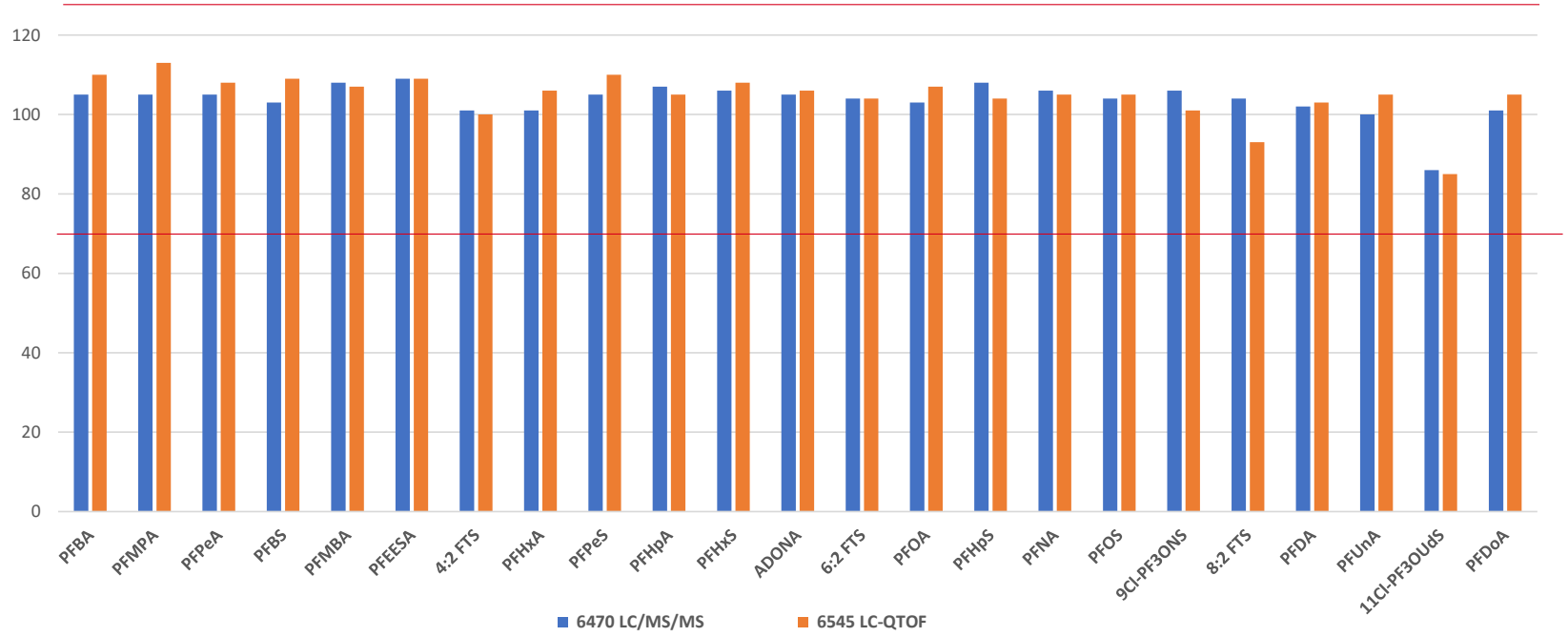
PFBA & PFHpA had low level background that skewed recovery data
Both QTOF & MS/MS give excellent reproducibility at low level spikes too.

Mid level Recovery Comparison (n=3)
15 ng/L drinking water spike

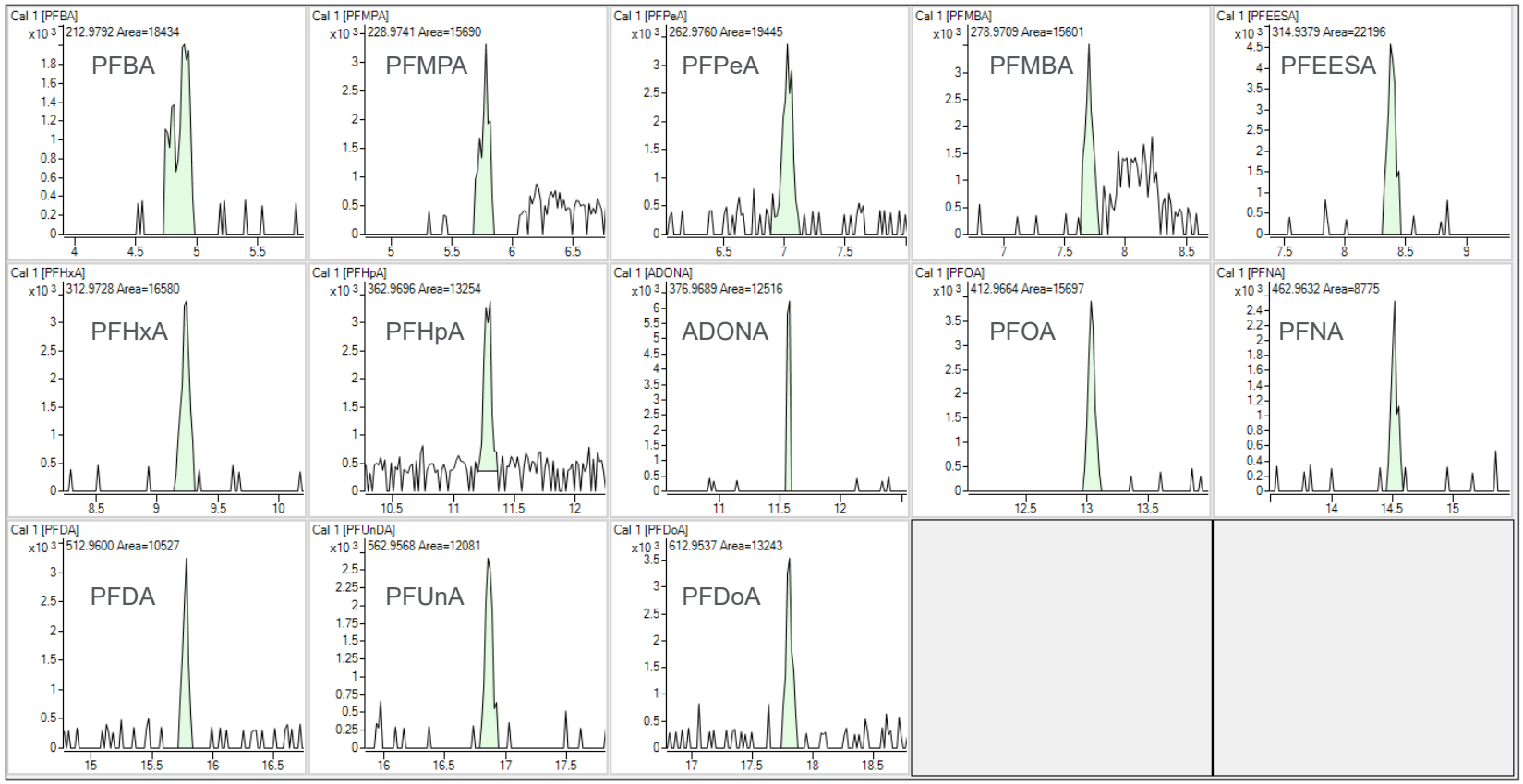


High level Recovery Comparison (n=3)
50 ng/L drinking water spike

EPA LCMRL ranges from 1.4 to 16 ng/L

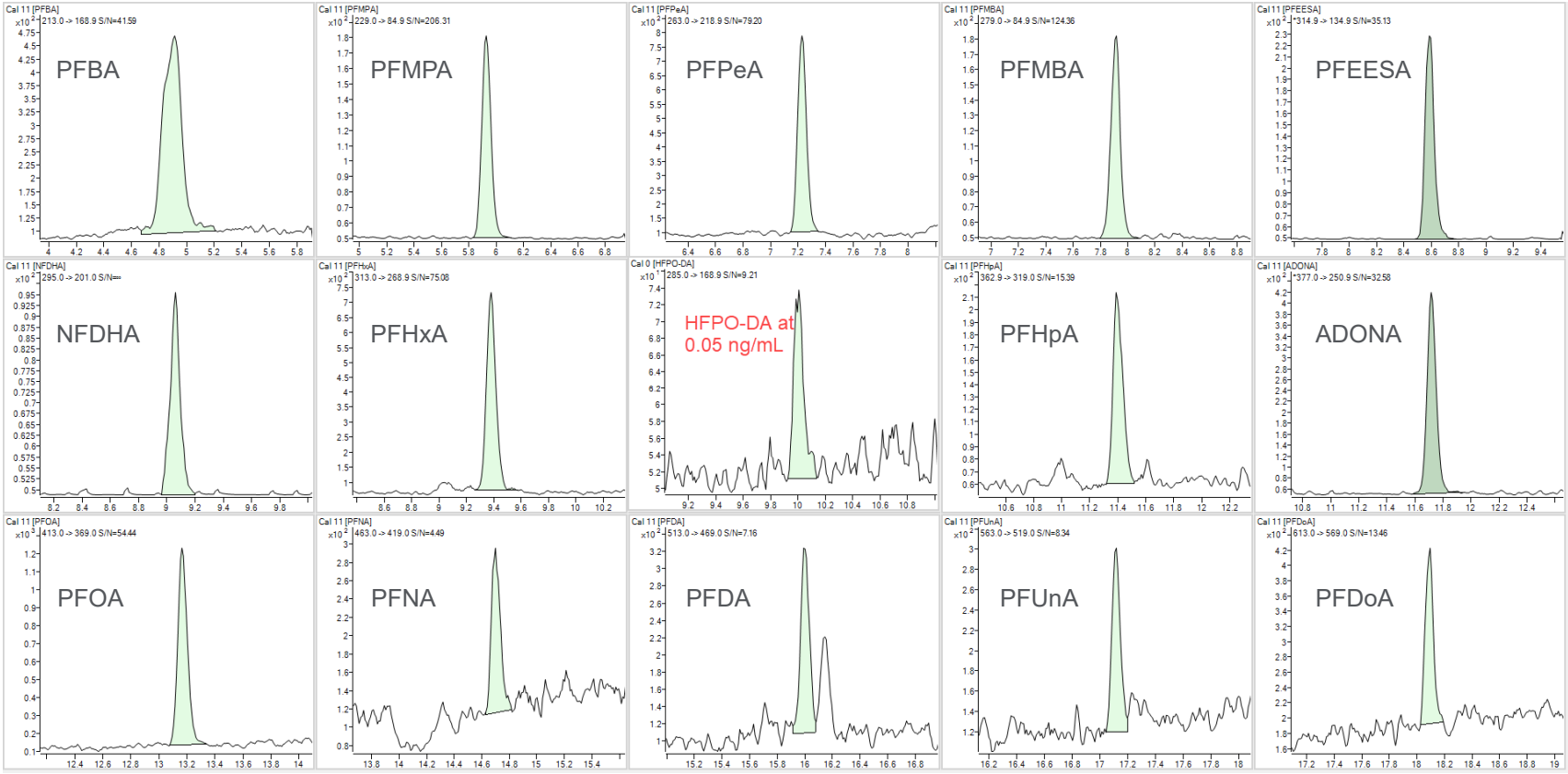


Acids - Low Level Calibrator (0.10 ng/mL)
6545 LC-QTOF

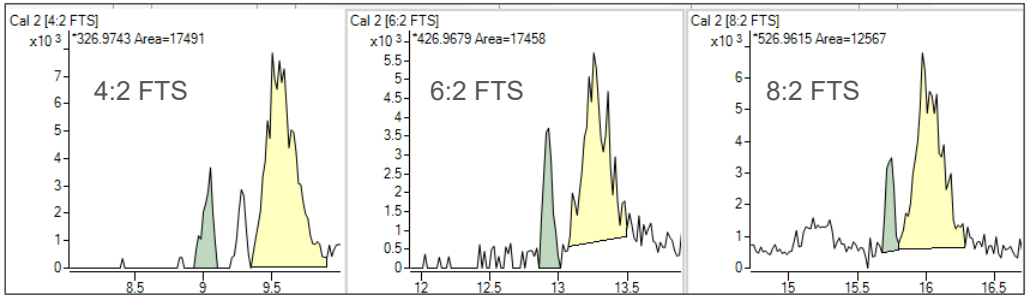


This calibration level would correspond to 0.25 ng/L spike in the water sample (250X concentration through SPE as per EPA 533)

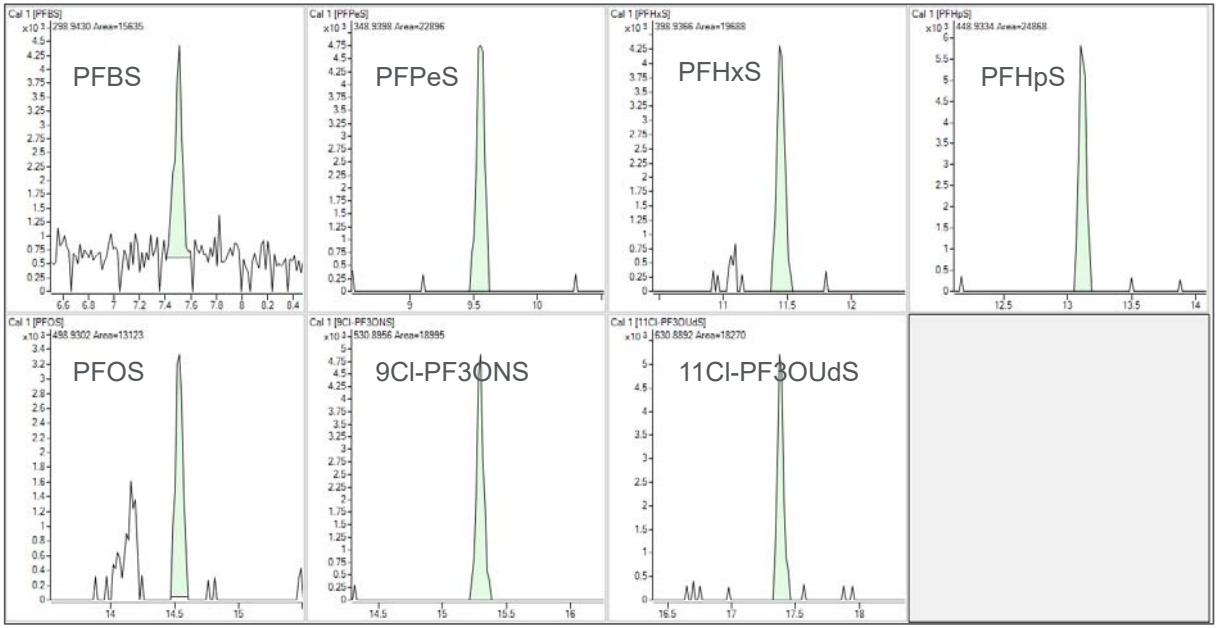
Acids - Low Level Calibrator (0.003 ng/mL)
6470 LC/MS/MS



FTS's – Low Level Calibrator (0.39 ng/mL)
6545 LC-QTOF



Sulfonates – Low Level Calibrator (0.10 ng/mL)
6545 LC-QTOF



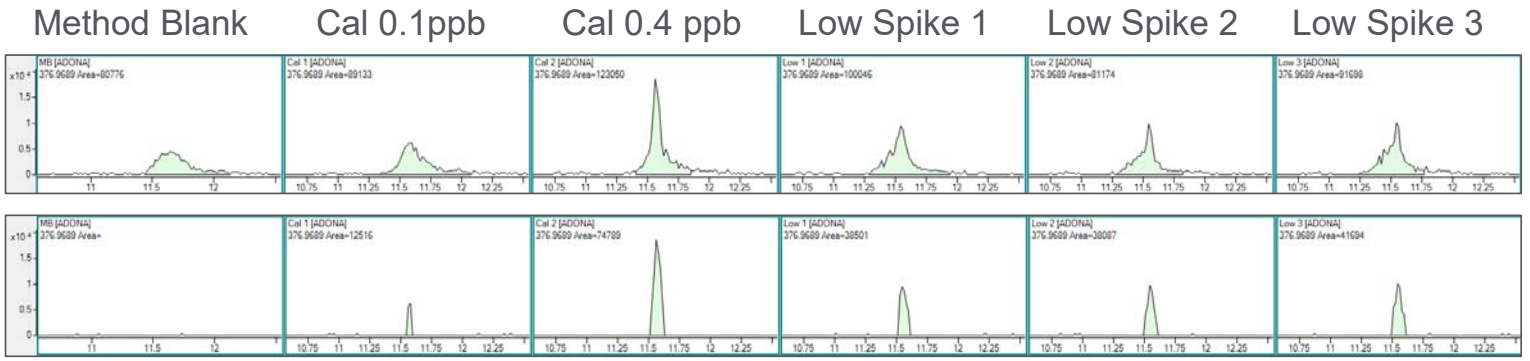
Note the branched isomers for PFHxS and PFOS

Advantages of High Resolution Accurate Mass

Mass Extraction

ADONA
100 ppm

20 ppm



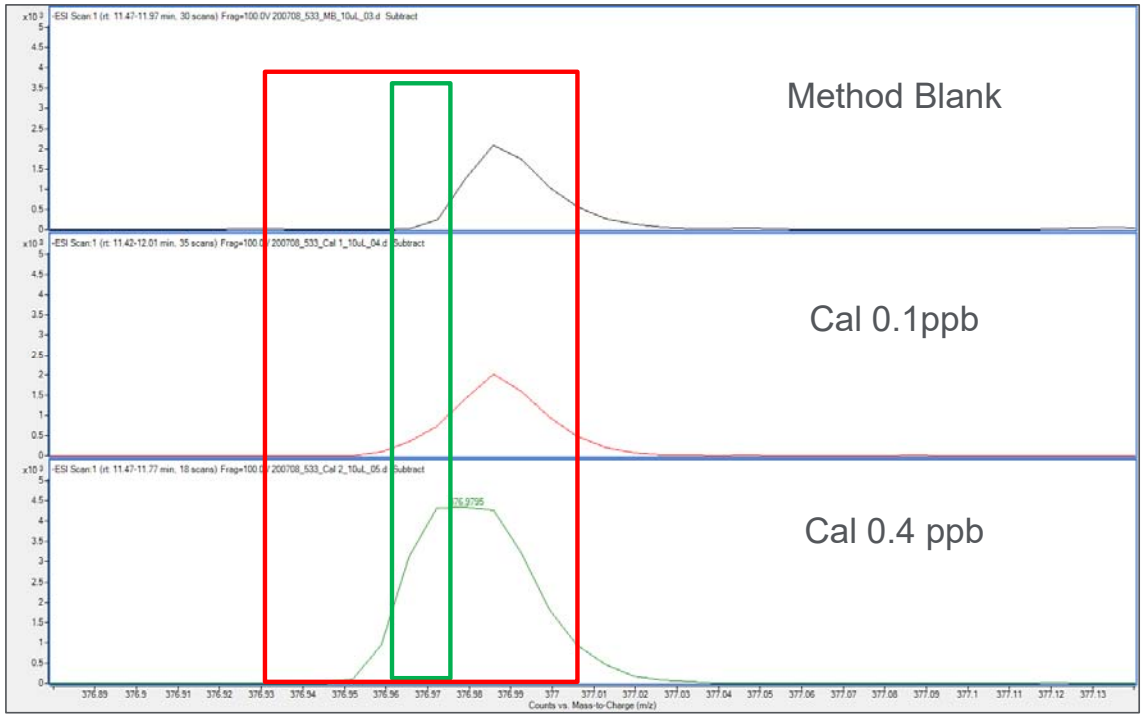
- Reducing the width of the mass extraction window can eliminate background from interferences that have the same nominal mass but different accurate mass (i.e. the compounds have different chemical formulas).

- ADONA = 376.9689 amu
  - 100 ppm (0.0377 amu) extraction from 376.9312 – 377.0066
  - 20 ppm (0.0075 amu) extraction from 376.9614 – 376.9764

Mass Spectrum Extraction Windows - ADONA

Red box = 100 ppm

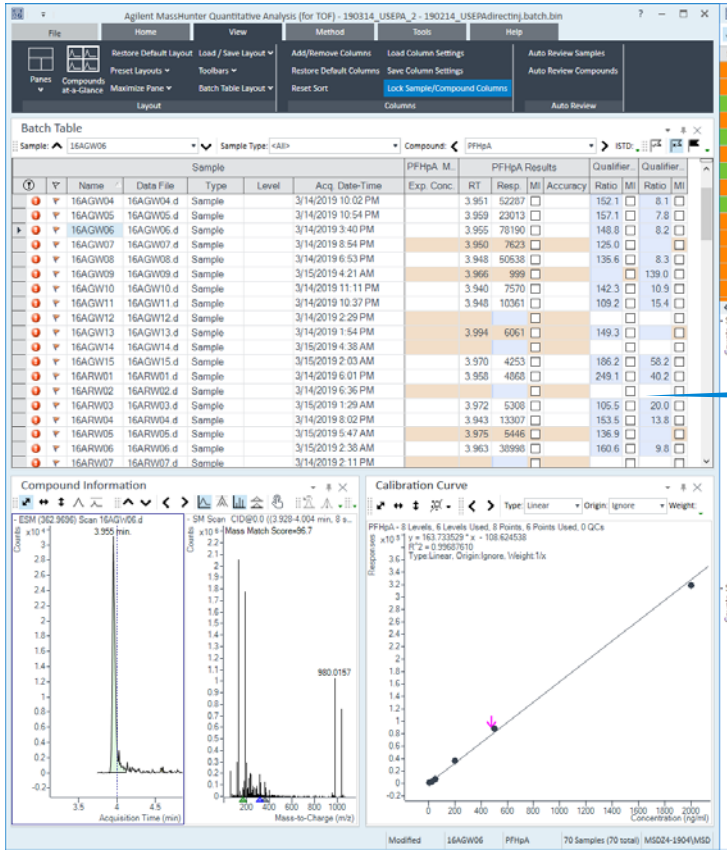
Green box = 20 ppm



- ADONA = 376.9689 amu
  - 100 ppm (0.0377 amu) extraction from 376.9312 – 377.0066
  - 20 ppm (0.0075 amu) extraction from 376.9614 – 376.9764

Advantage of QTOF - Monitoring suspect PFAS

Simultaneous Quantitation and Screening



Filter compounds that are Verified, Needs Review, Not Detected

Filter Targets and Suspects

Set outliers flags according to SANTE guidelines

MassHunter Quantitative ‘Batch-at-a-glance’ view

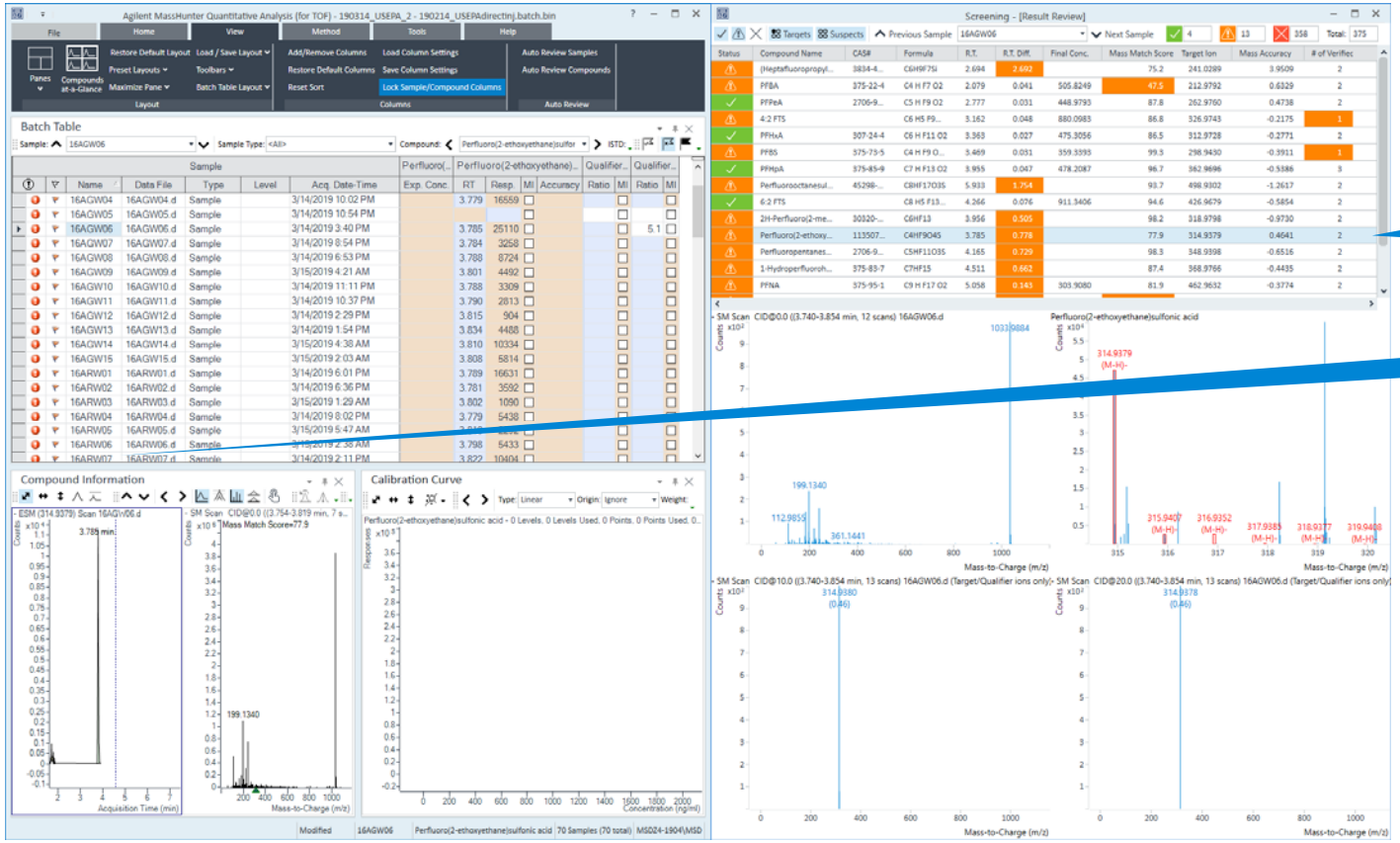
New Screener Tool

Review Isotope pattern match

Review Fragments

Monitoring suspect PFAS

Simultaneous Quantitation and Screening



Monitoring suspect PFAS

Screening summary PDF report

Screening Summary Report

Agilent | Trusted Answers

Sample name: 16AGW06 **Good** 4 **Warning** 17 **Error** 354

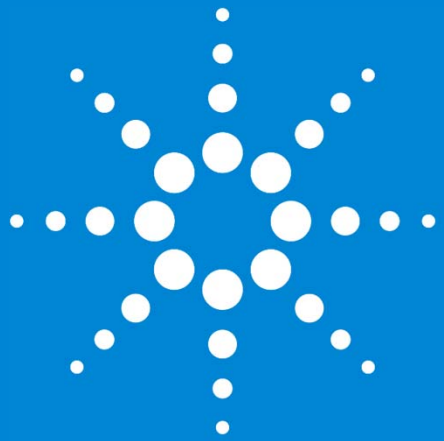
Status	Screening Summary Report	Formula	R.T.	R.T. Diff.	Match Score	Target Ion	Mass Accuracy	# of Qualified Ions	Final Conc.
!	(Heptafluoropropyl)trimethylsilane	C6H9F7Si	2.694	2.692		241.0289	3.95 PPM	2	
+	PFBA	C4 H F7 O2	2.079	0.041		212.9792	0.63 PPM	2	472.7851
+	PFPeA	C5 H F9 O2	2.777	0.031		262.9760	0.47 PPM	2	448.9793
!	4:2 FTS	C6 H5 F9 O3 S	3.162	0.048		326.9743	-0.22 PPM	1	880.0983
+	PFHxA	C6 H F11 O2	3.363	0.036		312.9728	-0.28 PPM	2	475.3056
!	PFBS	C4 H F9 O3 S	3.469	0.031		298.9430	-0.39 PPM	1	359.3393
!	3H-Perfluorobutanoic acid	C4H2F6O2	3.530	0.499		194.9886	-1.07 PPM	1	
!	Perfluorooctanesulfonate	C8HF17O3S	5.933	1.754		498.9302	-1.26 PPM	2	
+	6:2 FTS	C8 H5 F13 O3 S	4.266	0.076		426.9679	-0.59 PPM	2	911.3406
!	2H-Perfluoro(2-methylpentane)	C6HF13	3.956	0.505		318.9798	-0.97 PPM	2	
!	Perfluoro(2-ethoxyethane)sulfonic acid	C4HF9O4S	3.785	0.778		314.9379	0.46 PPM	2	
!	Perfluoropentanesulfonic acid	C5HF11O3S	4.165	0.729		348.9398	-0.65 PPM	2	
!	1-Hydroperfluoroheptane	C7HF15	4.511	0.662		368.9766	-0.44 PPM	2	
!	PFNA	C9 H F17 O2	5.058	0.143		462.9632	-0.38 PPM	2	303.9080
!	2,3,3,3-Tetrafluoro-2-(perfluoropentoxy)propan-1-ol	C8H3F15O2	4.526	0.718		414.9821	0.81 PPM	2	
!	1H-Perfluorohexane	C6HF13	3.956	1.326		318.9798	-0.97 PPM	2	
!	((Perfluorooctyl)ethyl)phosphonic acid	C10H6F17O3P	5.300	0.485		526.9710	4.37 PPM	1	
!	4-[3-(Perfluorobutyl)-1-propyloxy]benzyl alcohol	C14H13F9O2	6.167	0.221		383.0699	2.89 PPM	1	
!	(Perfluorooctyl)propanoyl chloride	C11H4ClF17O	5.927	0.137		508.9606	-2.22 PPM	1	
!	PFOS	C8 H F17 O3 S	5.933	0.167		498.9302	-1.23 PPM	2	63.0760
!	FOSA	[illegible]	[illegible]	0.015		[illegible]	[illegible] PPM	1	0.4484

Flagging RT outlier

Flagging number of verified ions

Conclusions

- The QTOF allows simultaneous quantification and suspect screening
  - 6545 QTOF met the sensitivity goals of EPA 533
  - Spike recovery experiments should reproducibility on both the QTOF and MS/MS
  - QTOF data allows retrospective data mining
- LC-MS/MS still offers best sensitivity which can allow greater sample dilutions and direct aqueous injections
- LC-MS/MS and QTOF are complementary techniques for holistic environmental monitoring



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