Accurate Mass LC/Q-TOF
A New Direction in Quantitative PFAS Analysis

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PFAS

HEALTH | News

Makeup sold in Canada, U.S. may contain potentially toxic chemicals called PFAS: study

Sandee LaMotte
CNN Digital
Contact

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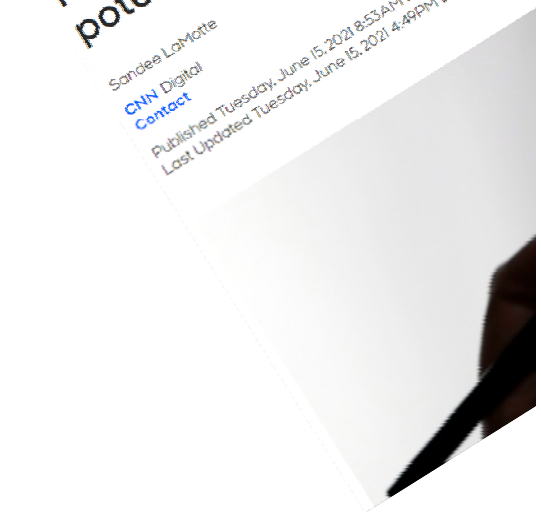
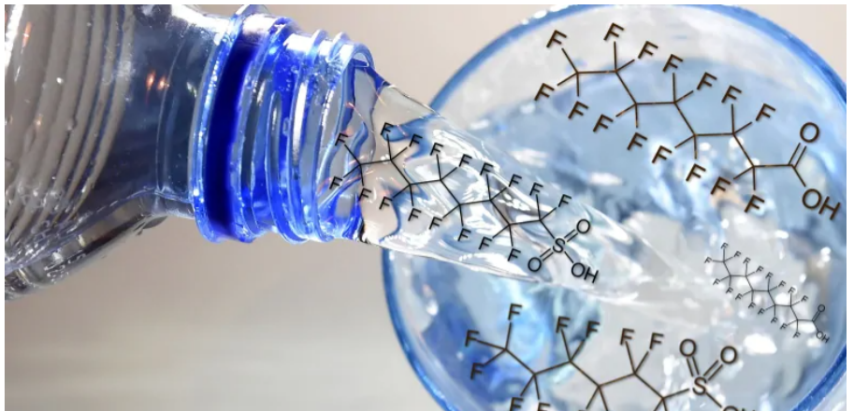


Quirks & Quarks

A new class of 'forever chemicals' is an emerging threat to our health and environment

98% of Canadians have PFAS chemicals in their blood

CBC Radio · Posted: Oct 09, 2020 4:52 PM ET | Last Updated: October 14, 2020



CBC | MENU | Tokyo 2020

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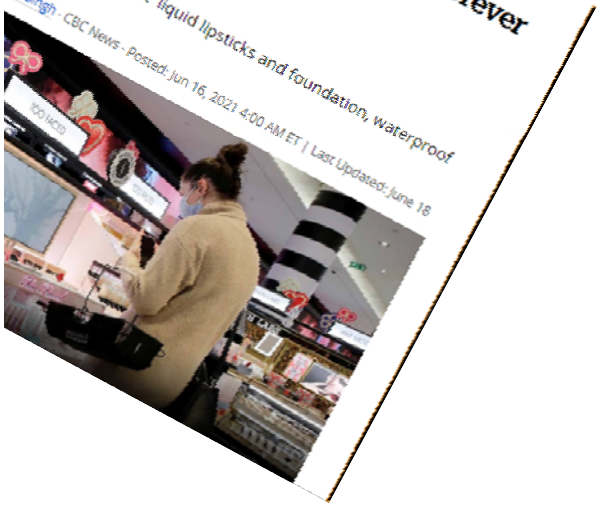
Science

How to make sense of the new findings on 'forever cals' in makeup

ften in 'wear-resistant' liquid lipsticks and foundation, waterproof

yat Singh · CBC News · Posted: Jun 16, 2021 4:00 AM ET | Last Updated: June 18



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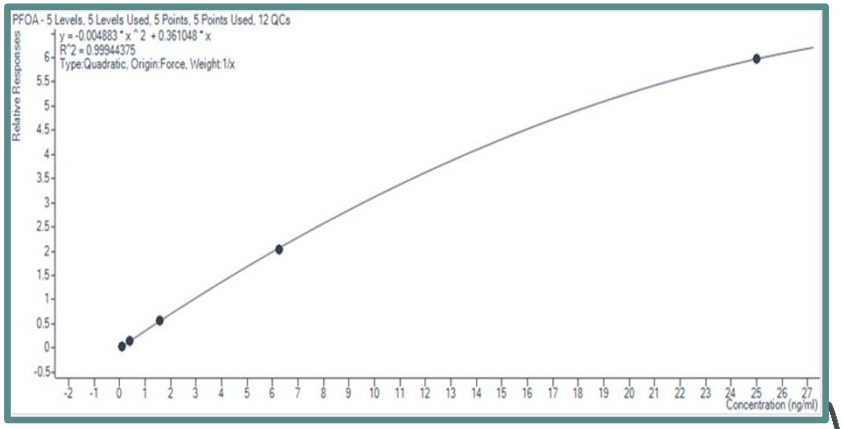
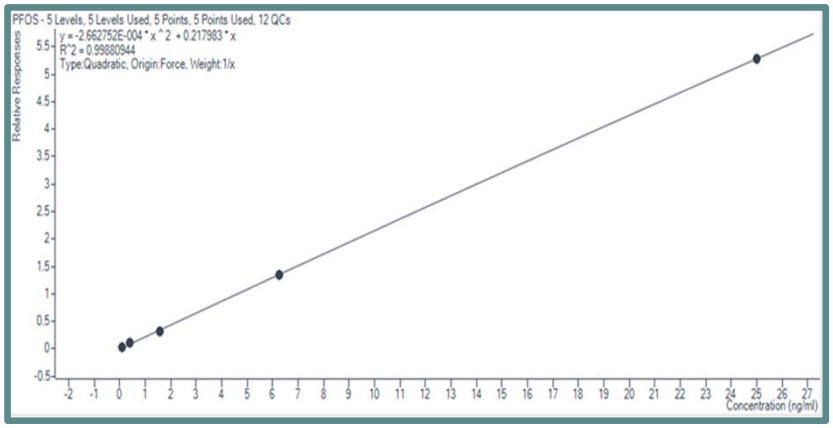
PFAS Quantitation

- Traditionally preformed with liquid chromatography and triple quadrupole mass spectrometry

- Can it be done by LC/Q-TOF instead?
  - What acquisition mode should I use?





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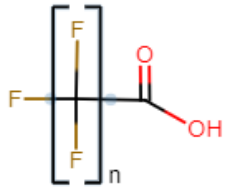
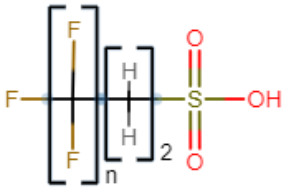
USEPA Method 533 Target Compounds

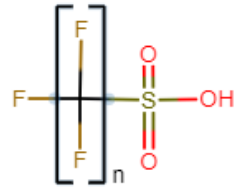


Perfluorinated Carboxylic Acids



Fluorotelomer Sulfonates

Analyte	Abbreviation	Compound Class
Perfluorobutanoic acid	PFBA	acid
Perfluoro-3-methoxypropanoic acid	PFMPA	acid
Perfluoropentanoic acid	PFPeA	acid
Perfluoro-4-methoxybutanoic acid	PFMBA	acid
Nonafluoro-3,6-dioxaheptanoic acid	NFDHA	acid
Perfluorohexanoic acid	PFHxA	acid
Hexafluoropropylene oxide dimer acid	HFPO-DA	acid
Perfluoroheptanoic acid	PFHpA	acid
4,8-Dioxa-3H-perfluorononanoic acid	ADONA	acid
Perfluorooctanoic acid	PFOA	acid
Perfluorononanoic acid	PFNA	acid
Perfluorodecanoic acid	PFDA	acid
Perfluoroundecanoic acid	PFUnA	acid
Perfluorododecanoic acid	PFDoA	acid
1H,1H, 2H, 2H-Perfluorohexane sulfonic acid (4:2 Fluorotelomer sulfonate)	4:2FTS	FTS
1H,1H, 2H, 2H-Perfluorooctane sulfonic acid (6:2 Fluorotelomer Sulfonate)	6:2FTS	FTS
1H,1H, 2H, 2H-Perfluorodecane sulfonic acid (8:2 Fluorotelomer sulfonate)	8:2FTS	FTS
Perfluorobutanesulfonic acid	PFBS	sulfonate
Perfluoropentanesulfonic acid	PFPeS	sulfonate
Perfluorohexanesulfonic acid	PFHxS	sulfonate
Perfluoroheptanesulfonic acid	PFHpS	sulfonate
Perfluorooctanesulfonic acid	PFOS	sulfonate
Perfluoro (2-ethoxyethane) sulfonic acid	PFEESA	sulfonate
9-Chloroehexadecafluoro-3-oxanonane-1-sulfonic acid	9Cl-PF3ONS	sulfonate
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	11Cl-PF3OUdS	sulfonate



Perfluorinated Sulfonates

Next Generation PFAS

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LC Instrument Conditions

Parameter	Value	
LC	**TQ** & **All Ions** work: Agilent 1260 series Infinity binary pump, G1367E Infinity ALS, G1316A Infinity thermostated column compartment **Targeted MS/MS & Q-RAI** work: Agilent 1290 series Infinity II – G7120A High Speed Pump, G7167A Multisampler, G7116B MCT thermostated column compartment	
Analytical Column	Agilent ZORBAX Eclipse Plus C18, 3 × 50 mm; 1.8 µm (p/n 959757-302)	
Delay Column	Agilent ZORBAX SB-C18, 4.6 × 50 mm, 3.5 µm (p/n 835975-902)	
Column Temperature	50°C	
Injection Volume	10 µL	
Mobile Phase	A) 20mM Ammonium Acetate in water (LC Grade) B) MeOH (LC Grade)	
Gradient Flow Rate	0.4 mL/min	
Gradient	Time (min)	%B
	0.0	5
	0.5	5
	3.0	40
	16.0	80
	18.0	80
	20.0	95
Stop Time	20.0 minutes	
Post Time	6.0 minutes	

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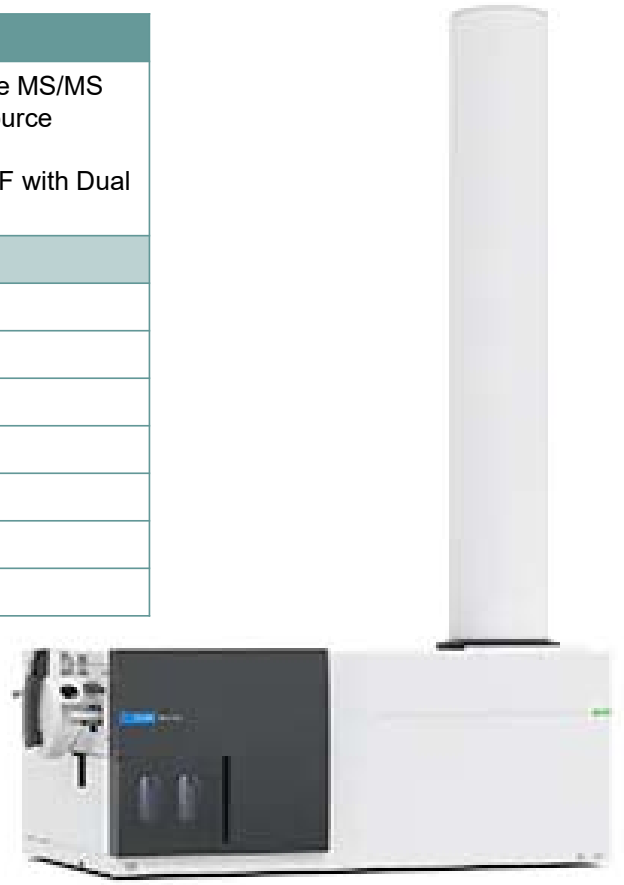
MS – Jet Stream ESI Instrument Source Conditions

Parameter	Value
MS	Agilent 6470 Triple Quadrupole MS/MS with Agilent Jet Stream ESI source Agilent 6545 & 6546 LC/Q-TOF with Dual Agilent Jet Stream ESI source
Source Parameters	
Gas Temperature	230 °C
Gas Flow	4 L/min
Nebulizer	20 psi
Sheath Gas Temperature	375 °C
Sheath Gas Flow	12 L/min
Capillary Voltage (Neg)	2,500 / 2,000 V
Nozzle Voltage (Neg)	0 V



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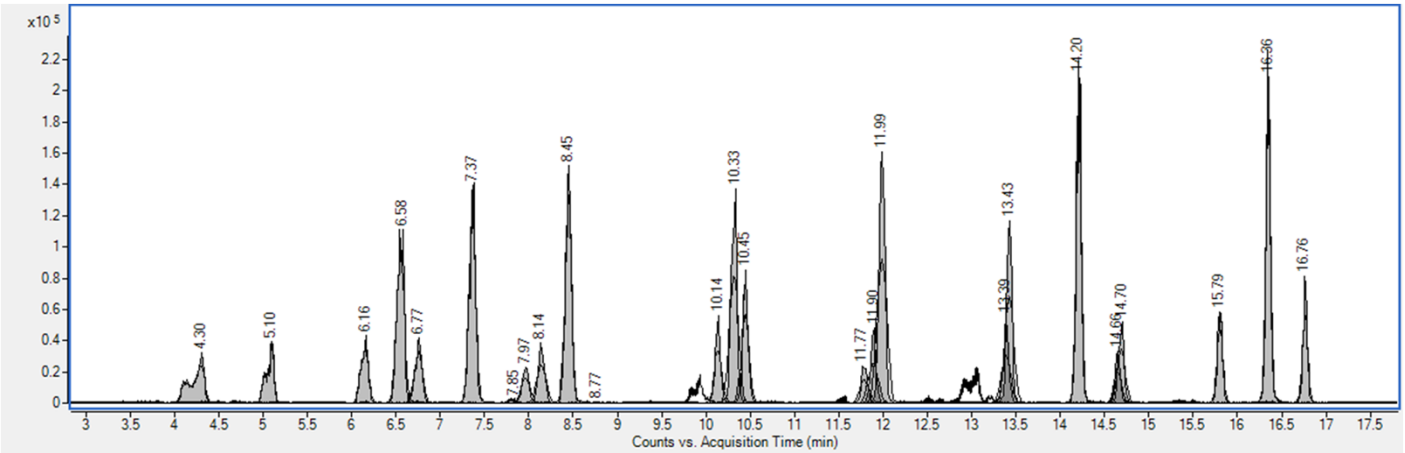
Additional Method-Specific MS Conditions

LC/TQ	d-MRM Transitions
LC/Q-TOF – TOF Acquisition - All Ions	TOF parameters, Acquisition rates & Collision Energies
LC/Q-TOF – Targeted MS/MS Acquisition	TOF parameters, Acquisition rates & Targeted transitions
LC/Q-TOF – Data Independent Acquisition - Q-RAI	TOF parameters, Acquisition rates & Quadrupole Windows



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LC/TQ

- Gold standard for quantitation
- Highest sensitivity, excellent selectivity and specificity
- USEPA approved methodology
- Unique fragmentation and collision energy voltages for each product ion

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LC/Q-TOF

- Why would you want to quantitate PFAS on a LC/Q-TOF rather than an LC/TQ?
  - One instrument lab
  - Able to perform target and non-target analysis
  - Accurate mass for assurance of highest quality compound identification
  - Suspect screening without standards

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LC/Q-TOF – TOF Acquisition - All Ions

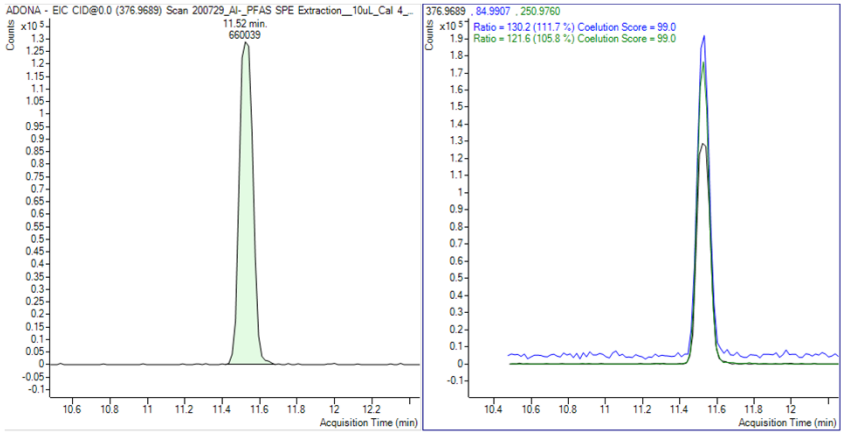
Parameter	Value
MS TOF	
Fragmentor	100 V
Skimmer	65 V
Oct 1 RF Vpp	750 V

Spectral Parameters	
MS	
Mass range	50 – 1000 *m/z*
Acquisition Rate	4 spectra/s

Experiment Table	
Experiment	**Collision Energy**
1	0
2	10
3	20
4	40

Quantitate

Confirm



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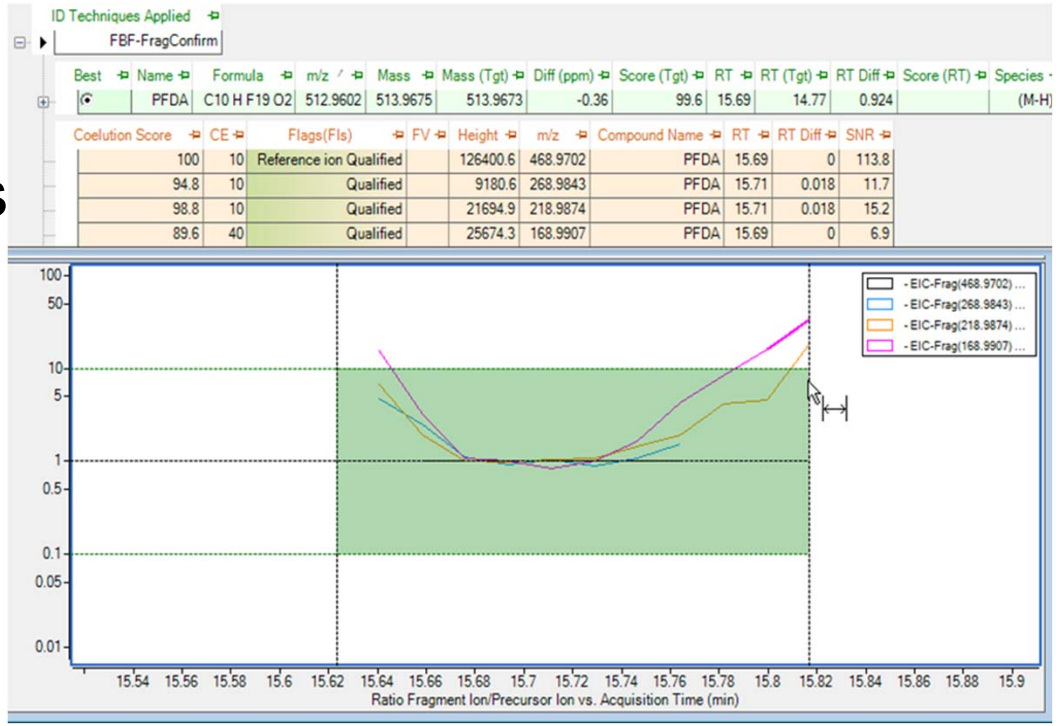
All Ion Acquisition Benefits

- ✓ All compounds detected
- ✓ Precursor and fragment ions
- ✓ Spectral library matching
- ✓ Simple one-time set up



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LC/Q-TOF – Targeted MS/MS Acquisition

Parameter	Value
MS TOF	
Fragmentor	95 V
Skimmer	65 V
Oct 1 RF Vpp	750 V

Spectral Parameters	
MS	
Mass range	100 – 1000 *m/z*
Acquisition Rate	50 spectra/s
MS/MS	
Mass Range	40 – 650 *m/z*
Acquisition Rate	5 spectra/s

Compound	Prec. m	Ret. Time (min)	Delta Ret. Time (min)	Collision Energy
PFBA	212.9813	4.25	0.6	8
13C3-PFBA	215.9893	4.25	0.6	8
13C4-PFBA	216.9926	4.25	0.6	8
PFMPA	228.9765	5.16	0.4	12
PFPeA	262.9788	6.18	0.4	8
13C5-PFPeA	267.9956	6.18	0.4	8
PFMBA	278.9739	6.85	0.4	12
HFPO-DA -CO2	284.9778	8.95	0.4	4
13C3-HFPO-DA -13CO2	286.9845	8.95	0.4	4
PFOA	412.9711	12.16	0.4	4
13C2-PFOA	414.9777	12.16	0.4	4
13C8-PFOA	420.9978	12.16	0.4	4
6:2 FTS	426.9725	12.06	0.4	24
13C2-6:2 FTS	428.9791	12.06	0.4	24
PFHpS	448.9384	12.25	0.4	52
PFNA	462.9683	13.64	0.4	4
13C9-PFNA	471.9985	13.64	0.4	4
PFOS	498.9357	13.67	0.4	50
13C4-PFOS	502.9492	13.67	0.4	50
13C8-PFOS	506.9625	13.67	0.4	50
PFDoA	612.9604	16.94	0.4	5
13C2-PFDoA	614.9604	16.94	0.4	5
11Cl-PF3OUdS	630.8960	16.53	0.4	32

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Targeted MS/MS Acquisition Benefits

- ✓ Familiar methodology
- ✓ Decreased background
- ✓ Specified precursor and fragment ions at unique collision energies
- ✓ Co-eluting compounds with common fragments are isolated based on precursor mass by the quadrupole

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LC/Q-TOF – Data Independent Acquisition - Q-RAI

Parameter	Value
MS TOF	
Fragmentor	95 V
Skimmer	65 V
Oct 1 RF Vpp	750 V

Spectral Parameters	
MS	
Mass range	50 – 1100 *m/z*
Acquisition Rate	2 spectra/s
MS/MS	
Mass Range	25 – 800 *m/z*
Acquisition Rate	11 spectra/s

Quadrupole Resolved All Ions (Q-RAI) List Table			
Start *m/z*	**End *m/z***	**Window Width**	**Collision Energy**
98	198	100	20
196	296	100	20
294	394	100	20
392	492	100	20
490	590	100	20
588	688	100	20
686	786	100	20
210	270	60	5
510	570	60	10
330	410	80	40
440	502	62	40

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Q-RAI Acquisition Benefits

- ✓ Non-targeted method
- ✓ Decreased background
- ✓ Reduced risk of co-eluting compounds with common fragments
- ✓ Generic method is simple to set up

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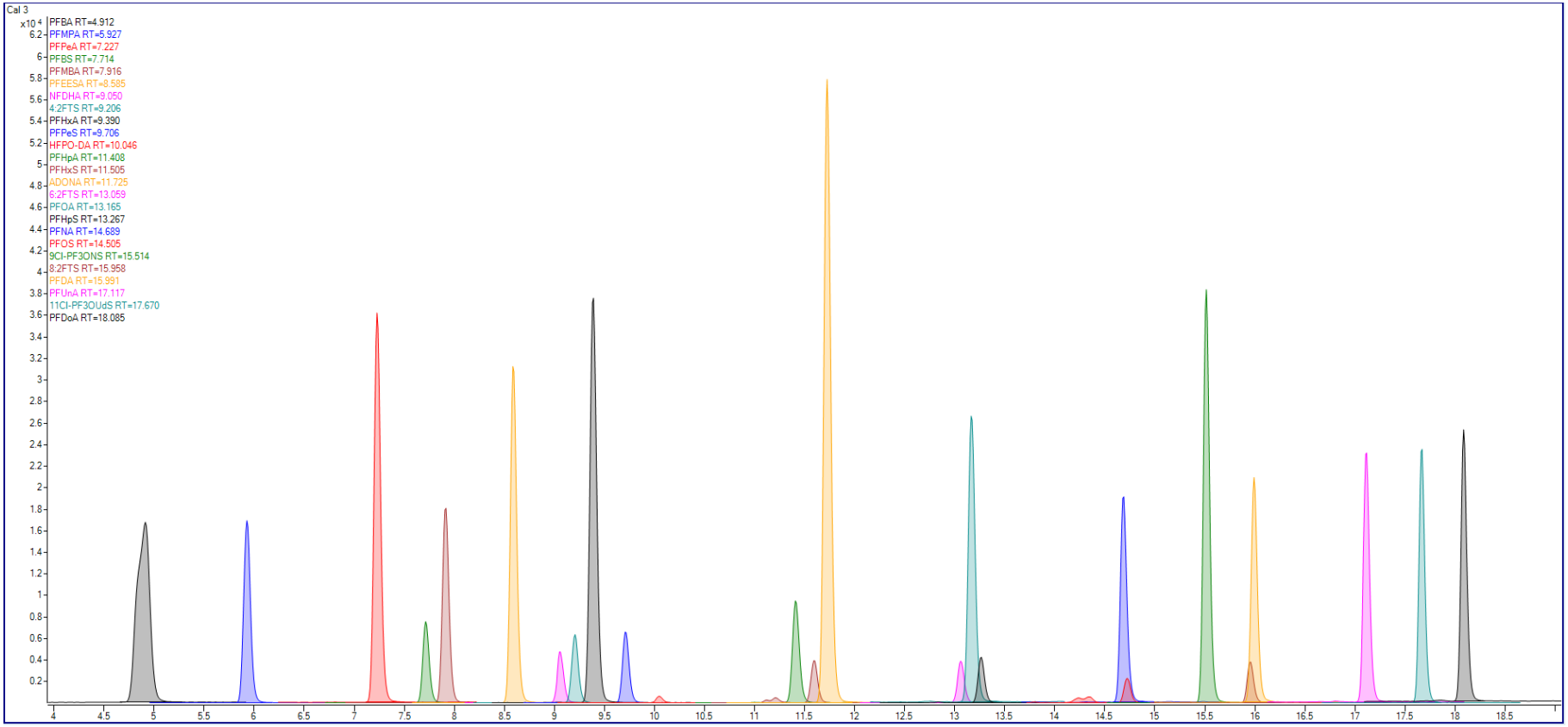
General Chromatography (LC/TQ) – 1.6 ng/mL (6.3 ng/L in Water)



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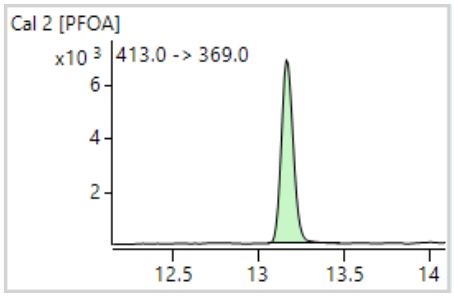
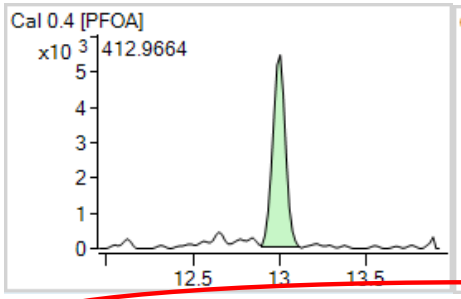
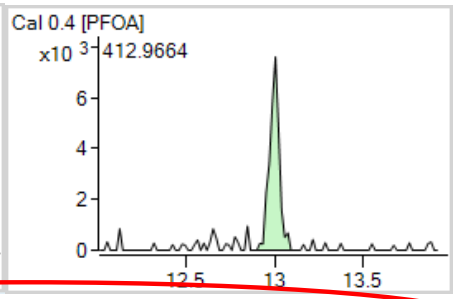
PFOA Chromatography – 0.4 ng/mL (1.6 ng/L in Water) Targets only

LC/TQ



LC/Q-TOF – All Ions

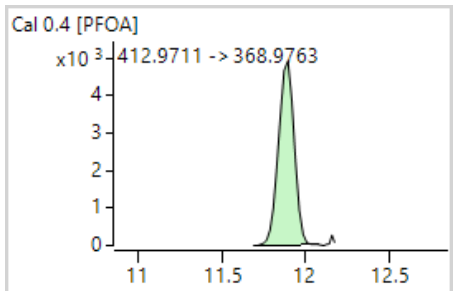




Smoothed Data

Raw Data

LC/Q-TOF – Targeted MS/MS



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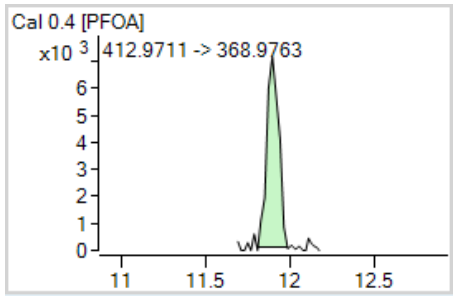
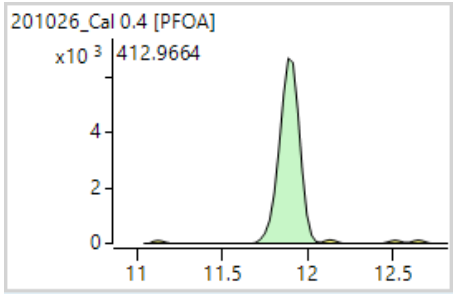


Raw Data

LC/Q-TOF – Q-RAI



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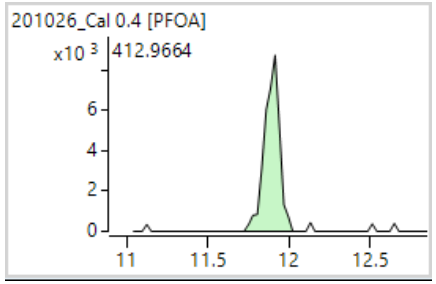

Raw Data

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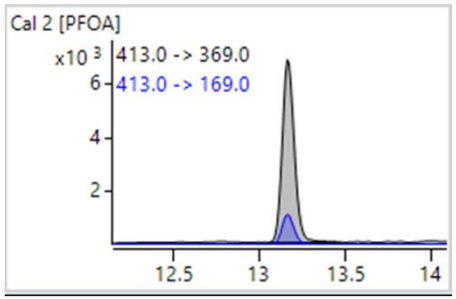
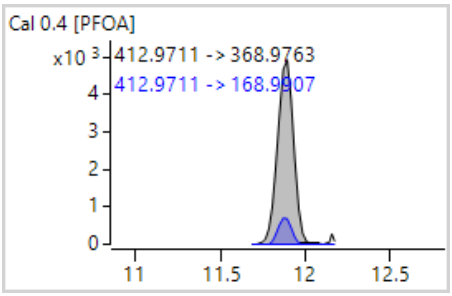
PFOA Chromatography – 0.4 ng/mL (1.6 ng/L in Water) Targets & Qual

LC/TQ



Transition 413.0 -> 369.0 @ CE = 4 V
Transition 413.0 -> 169.0 @ CE = 12 V

LC/Q-TOF – Targeted MS/MS



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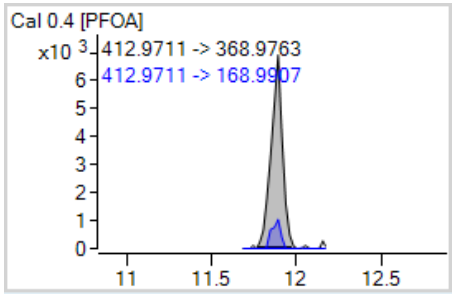

Raw Data

Precursor 412.9711 -> all product ions @ CE = 4 V

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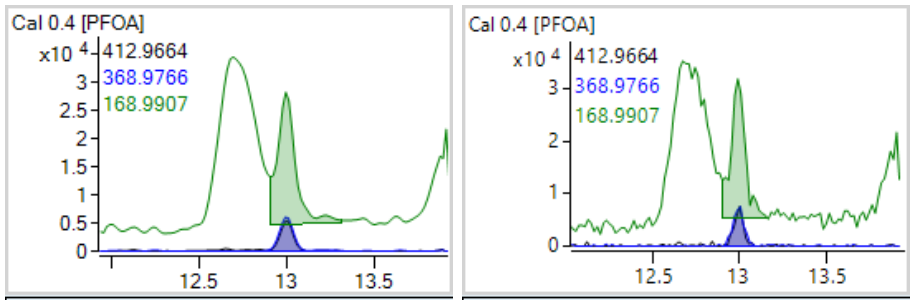
PFOA Chromatography – 0.4 ng/mL (1.6 ng/L in Water) Targets & Qual

LC/Q-TOF - All Ions

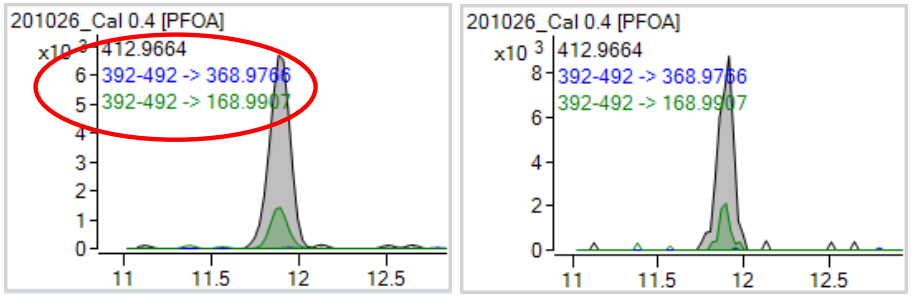


Smoothed Data | Raw Data

All Ions Comments:

- Collision energies collected: 10, 20, 40 V
- Fragment ions 368.9766 & 168.9907 use CE = 10 V

LC/Q-TOF – Q-RAI



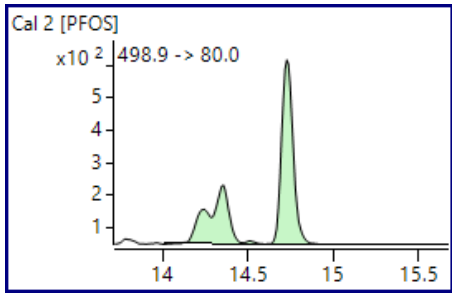
Smoothed Data | Raw Data

Q-RAI Comments:

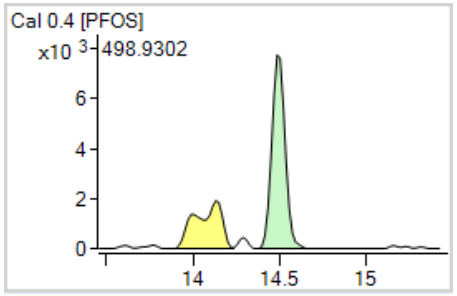
- Precursor quadrupole window collision energy = 20 V
- Fragment ion 368.9766 is largely lost at this collision energy and would require a lower CE to be detected

PFOS Chromatography – 0.4 ng/mL (1.6 ng/L in Water) Targets only

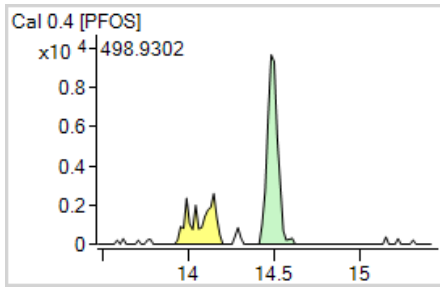
LC/TQ



LC/Q-TOF – All Ions

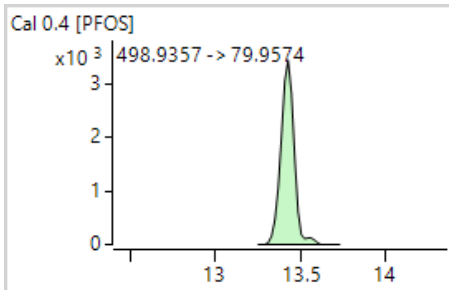


Smoothed Data



Raw Data

LC/Q-TOF – Targeted MS/MS



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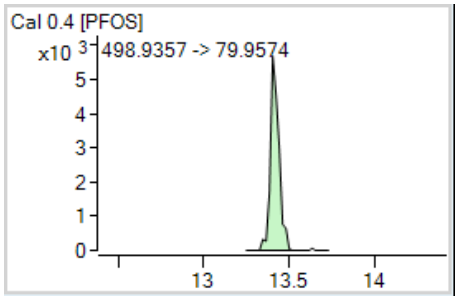
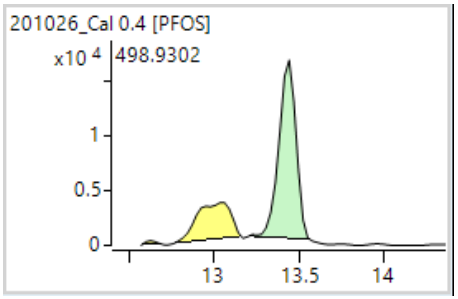


Raw Data

LC/Q-TOF – Q-RAI



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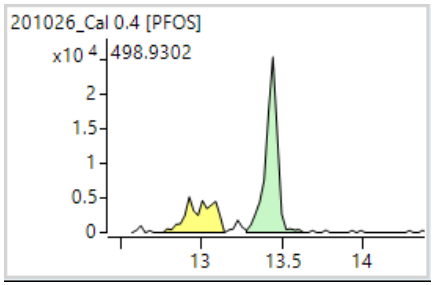

Raw Data

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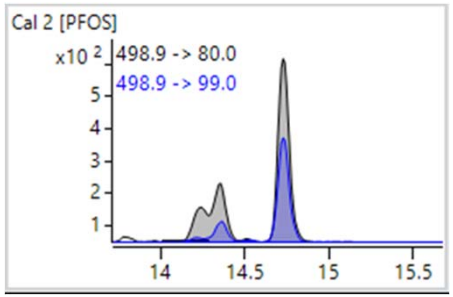
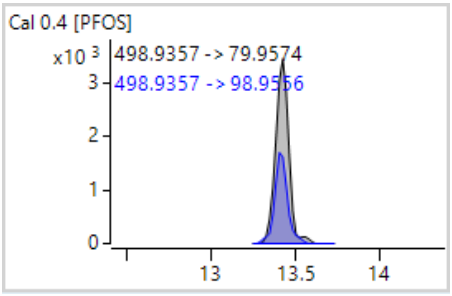
PFOS Chromatography – 0.4 ng/mL (1.6 ng/L in Water) Targets & Qual

LC/TQ

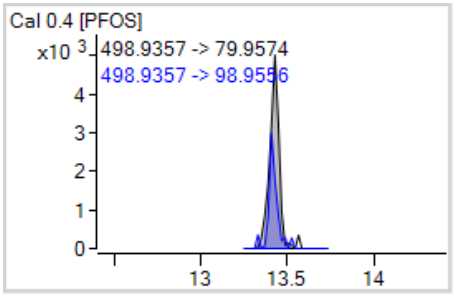


Transition 498.9 -> 80.0 @ CE = 50 V
Transition 498.9 -> 99.0 @ CE = 50 V

LC/Q-TOF – Targeted MS/MS



Smoothed Data

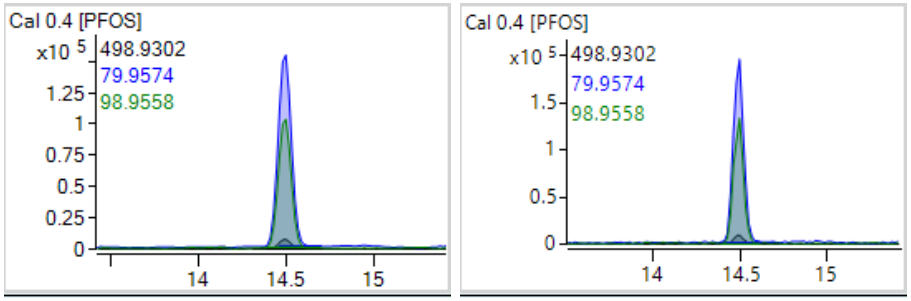


Raw Data

Precursor 498.9357 -> all product ions @ CE = 50 V

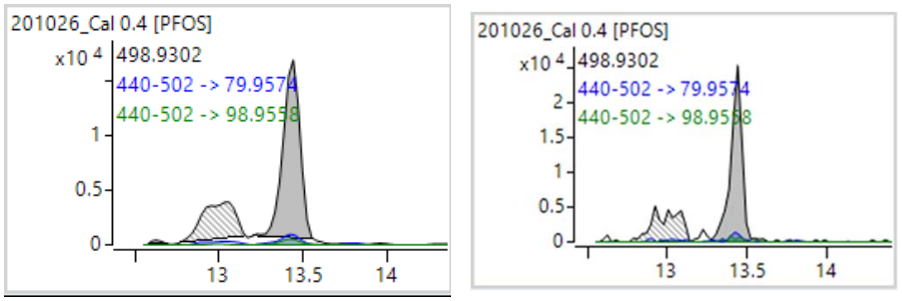
PFOS Chromatography – 0.4 ng/mL (1.6 ng/L in Water) Targets & Qual

LC/Q-TOF - All Ions



Smoothed Data | Raw Data

LC/Q-TOF – Q-RAI



Smoothed Data | Raw Data

All Ions Comments:

- Collision energies collected: 10, 20, 40 V
- Fragment ions 79.9574 & 98.9558 use CE = 40 V
- ISTD/IDA contribution to both fragment ions

Q-RAI Comments:

- Precursor quadrupole window collision energy = 40 V
- No ISTD/IDA fragment ion contribution

Calibration

Method	Quantitation	Calibration Curve Fit
LC/TQ	Quantitation and qualification is done on transitions from quadrupole isolated precursors to product ions	All target compounds have R^2 values > 0.999
LC/Q-TOF – All Ions	Quantitation is done on zero energy (collision cell) accurate mass precursor ions Coeluting higher energy fragment ions are used to qualify target identification	All target compounds have R^2 values > 0.99
LC/Q-TOF – Targeted MS/MS	Quantitation is done on transitions from precursor ions to accurate mass product ions. Additional transitions are used to qualify target identification	All target compounds have R^2 values > 0.98 with the majority > 0.995
LC/Q-TOF – Q-RAI	Quantitation is done on zero energy (collision cell) accurate mass precursor ions Coeluting higher energy fragment ions are used to qualify target identification	All target compounds have R^2 values > 0.997

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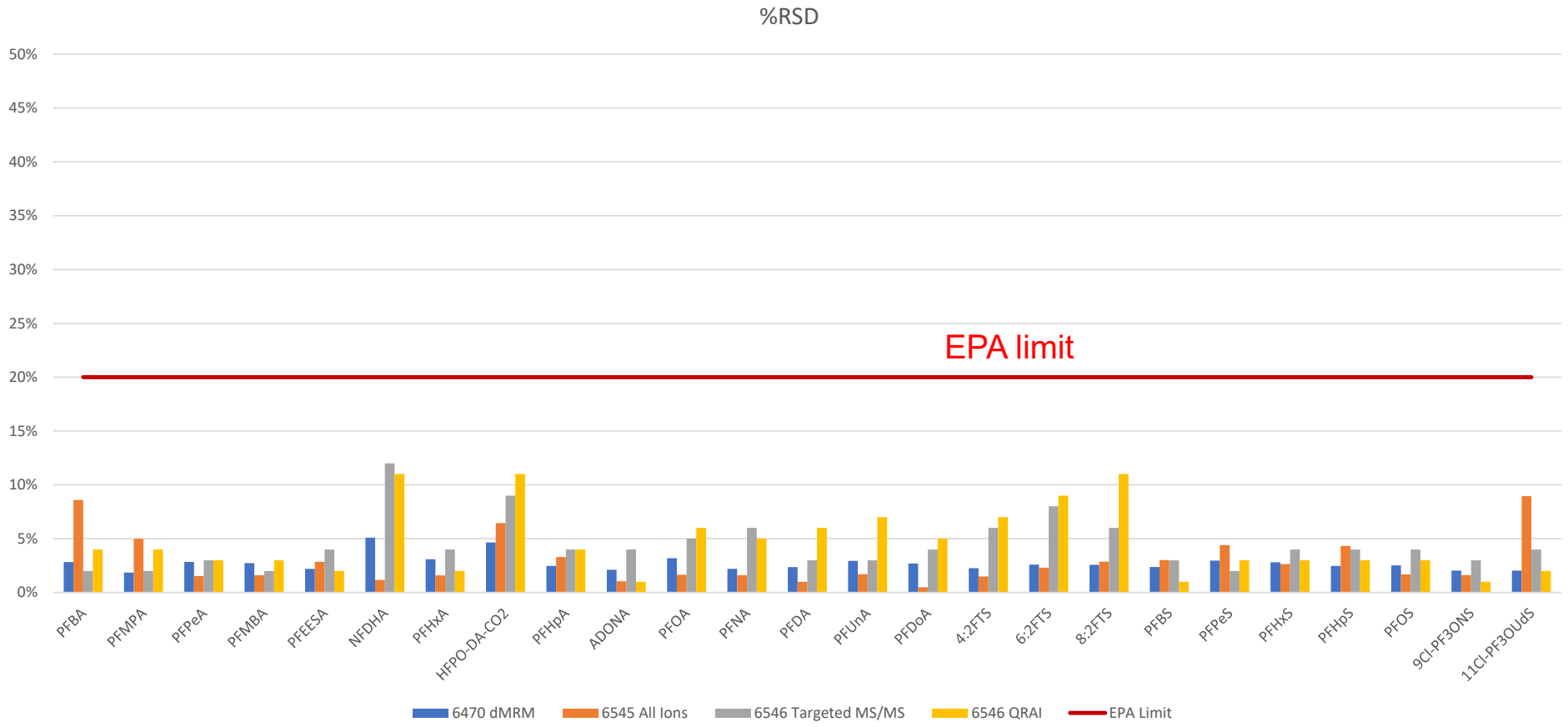
Performance – Low System Background

- All instruments (6470 LC/TQ, 6545 LC/Q-TOF and 6546 LC/Q-TOF) were assessed for system background PFAS contamination.

- Evidence of low system background is demonstrated by injecting a laboratory reagent blank (LRB) immediately following the high calibrator and evaluating the concentration of each analyte in the LRB. The LRB is an aliquot of reagent water fortified with the isotope dilution analogues and processed as a field sample. LRBs are used to determine if method analytes are introduced from the lab equipment, reagents, glassware, or extraction apparatus.

- While low trace levels of PFAS were seen in some of the LRBs, which could be due to contamination from sample preparation, etc. unextracted instrument blanks that were 80% MeOH did not contain any significant PFAS contamination, indicating that all the LC-MS systems were PFAS-free.

Performance - %RSD (Graphic)



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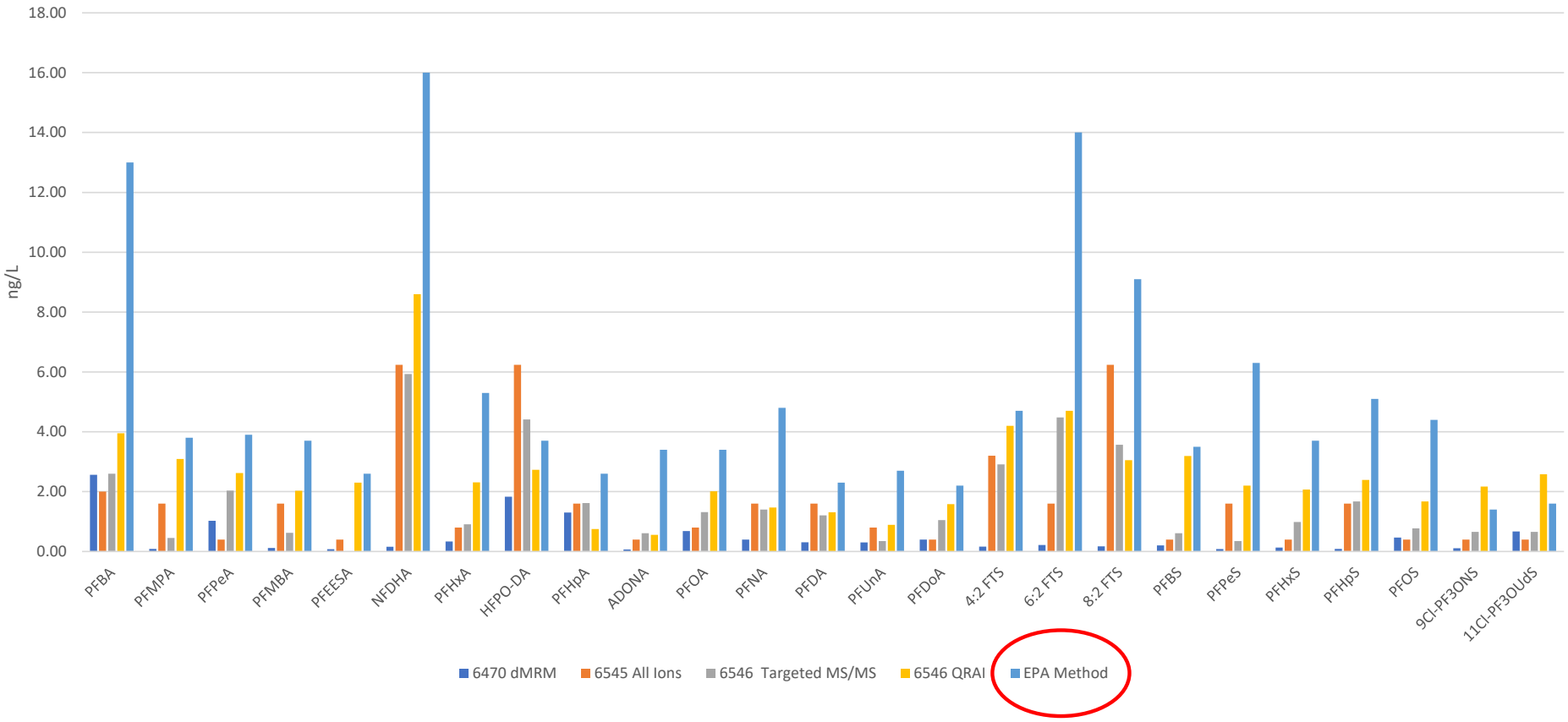
LCMRL (ng/L)

(or Lowest Calibrator where LCMRL was not determined)



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PFAS Acquisition Mode Review

	LC/TQ	Targeted MS/MS	All Ions	Q-RAI
EPA level sensitivity	✓✓✓	✓✓✓	✓✓✓	✓✓✓
Lowest detection limits	✓✓✓	✓	✓	✓
Accurate mass	✘	✓✓✓	✓✓✓	✓✓✓
Fragment ions for confirmation	✓✓✓	✓✓	✓✓✓	✓
Method Setup & ease of adding additional compounds	✓	✓	✓✓✓	✓✓
Background/Noise reduction	✓✓✓	✓✓✓	✓✓	✓✓✓
Labeled ISTD/IDAs	✓✓✓	✓✓✓	?	?
Simultaneous Unknowns Analysis	✘	✓	✓✓✓	✓✓

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Summary

- The instrument conditions are presented for quantitation of USEPA 533 PFAS compounds for LC/TQ (dMRM), and LC/Q-TOF (All Ions, Targeted MS/MS and Q-RAI)

- Agilent 6545 and 6546 LC/Q-TOFs have adequate sensitivity, linearity and reproducibility, relative to EPA Method 533 performance criteria, to quantitate PFAS compounds in multiple acquisition modes

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Acknowledgements

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    - Director, Global Environment & Food Applied Markets
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    - Senior Application Scientist
  - Chris Klein
    - LC/Q-TOF and IM-QTOF Product Manager

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