Strategies for Ultra Low-level Detection and Quantification of Short- and Long-Chain Per- and Polyfluoroalkyl Substances (PFAS) by Direct Injection LC-MS/MS

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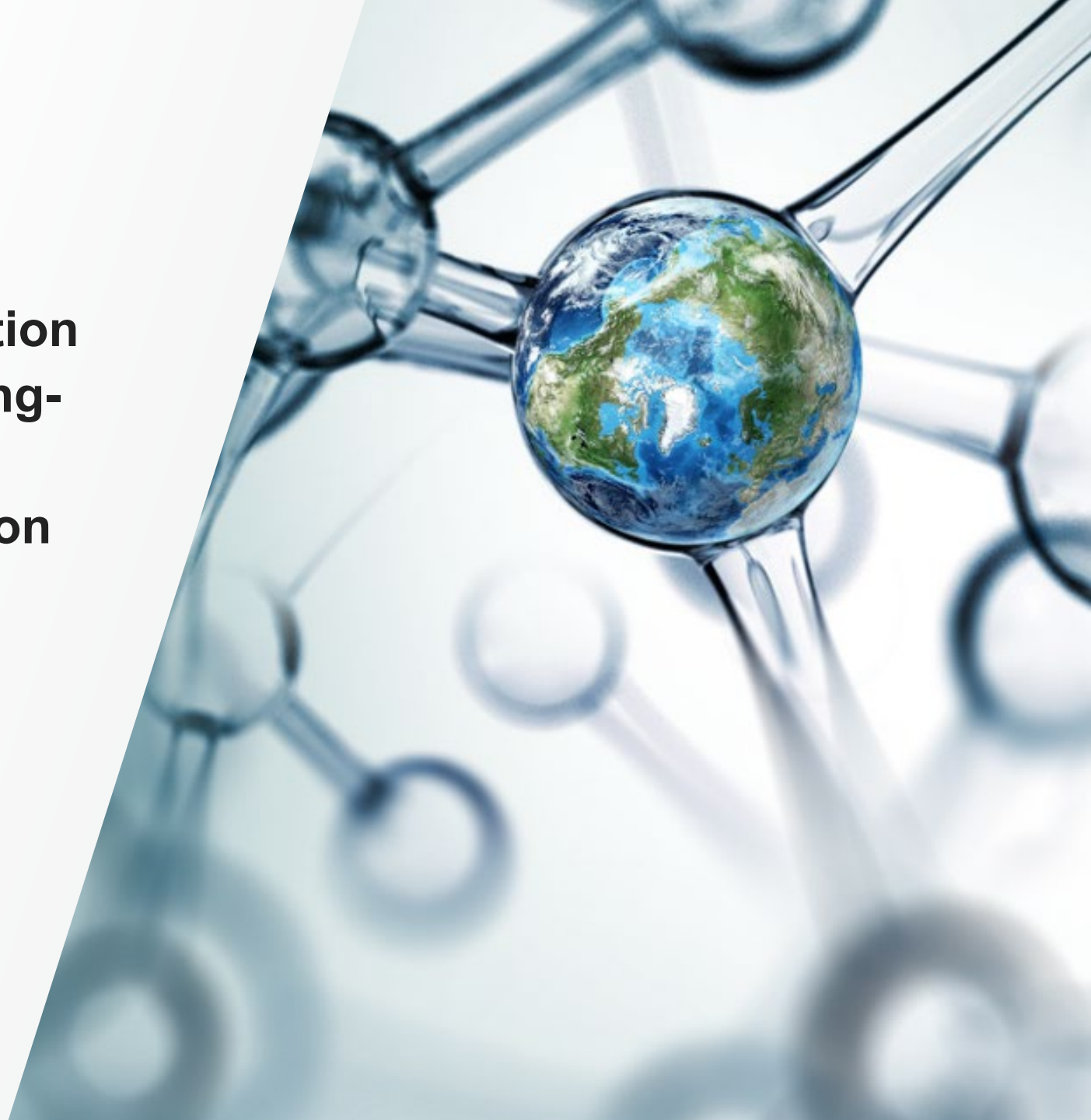
NEMC 2022 Crystal City, VA



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Overview

- Part 1: A single LC-MS/MS method was developed to measure various PFAS classes, including perfluoronated ether acids in surface by direct injection
  - A Thermo Scientific TSQ Altis Plus MS and Vanquish UHPLC system was used to measure quantitative performance of PFAS in surface water samples via a 25 µL direct injection.
  - Method was developed for 43 target PFAS compounds.
  - PFAS calibration curves ranged from 0.5 – 1000 ng/L, using internal calibration.
- Part 2: Method optimization strategies for direct injection for both short and longer chain PFAS- Aqueous samples and samples containing a high concentration of organic solvent (>95%)
  - LC system set-up with large sample loop and solvent sandwich injections
  - LC configurations available for obtaining high throughput and/or improved sensitivity

Experimental – Liquid Chromatography

Thermo Scientific Vanquish Flex Binary UHPLC System

- PFAS Delay Column: 3.0 x 50 mm, 1.9 um Hypersil GOLD (Thermo Scientific)
- Analytical Column: 2.1 x 100 mm, 2.2 um Acclaim RSLC C18 (Thermo Scientific)
- Column Temp: 40 C
- Mobile Phase A: H_2O with 2% MeOH + 2 mM Am. Acetate + 0.1% HOAc
- Mobile Phase B: MeOH with 2% H_2O + 2 mM Am. Acetate + 0.1% HOAc
- Gradient: see table
- Injection Volume: 25 uL
- Sample Temp: 20 C

No	Time	Flow [ml/min]	%B	Curve
1	0.000	Run		
2	0.000	0.400	20.0	5
3	1.000	0.400	50.0	5
4	15.000	0.400	100.0	5
5	17.000	0.400	100.0	5
6	17.200	0.400	20.0	5
7	22.500	0.400	20.0	5
8	*New Row*			
9	22.500	Stop Run		

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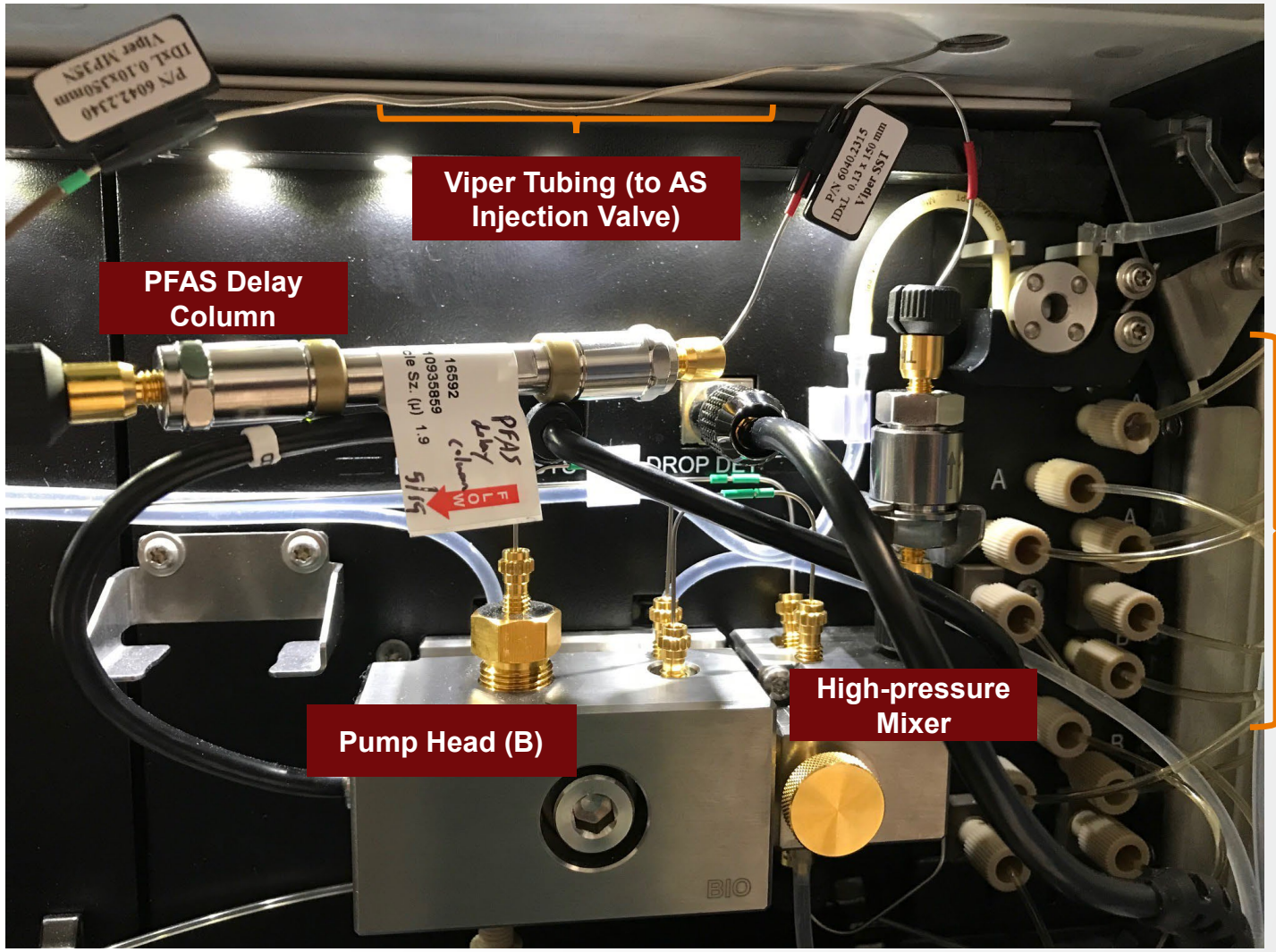
Experimental – Liquid Chromatography

PFAS Kit Retrofit & Delay Column setup



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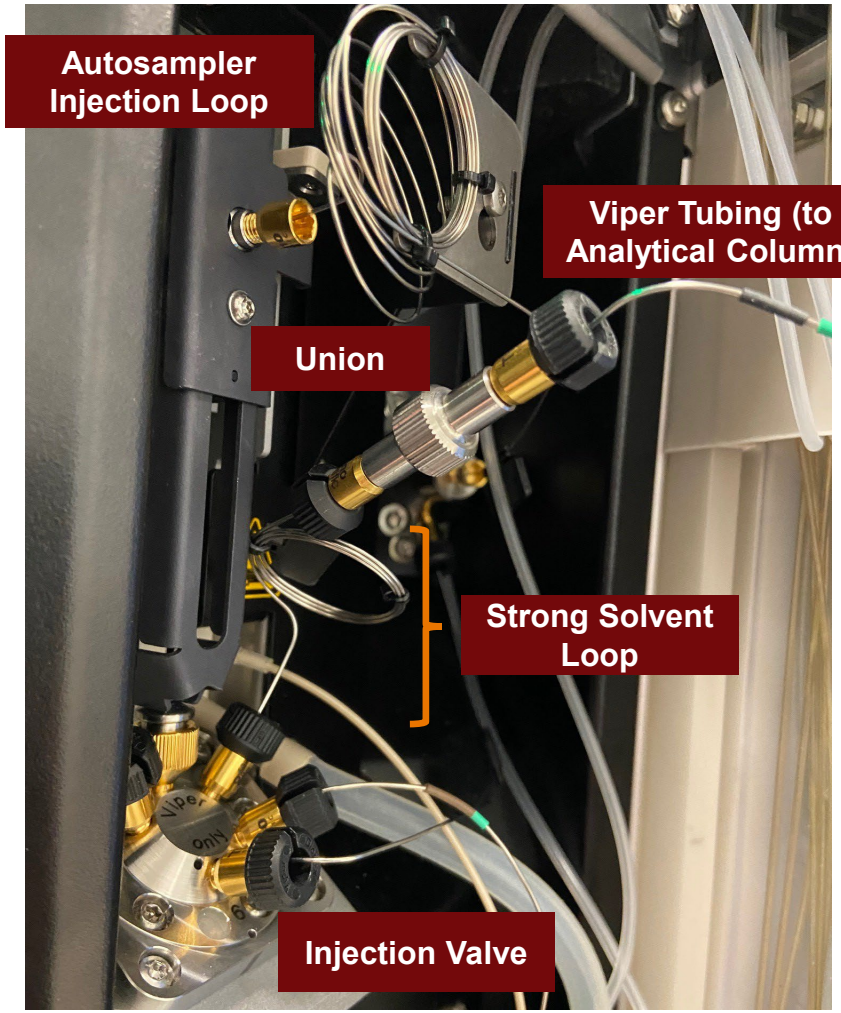
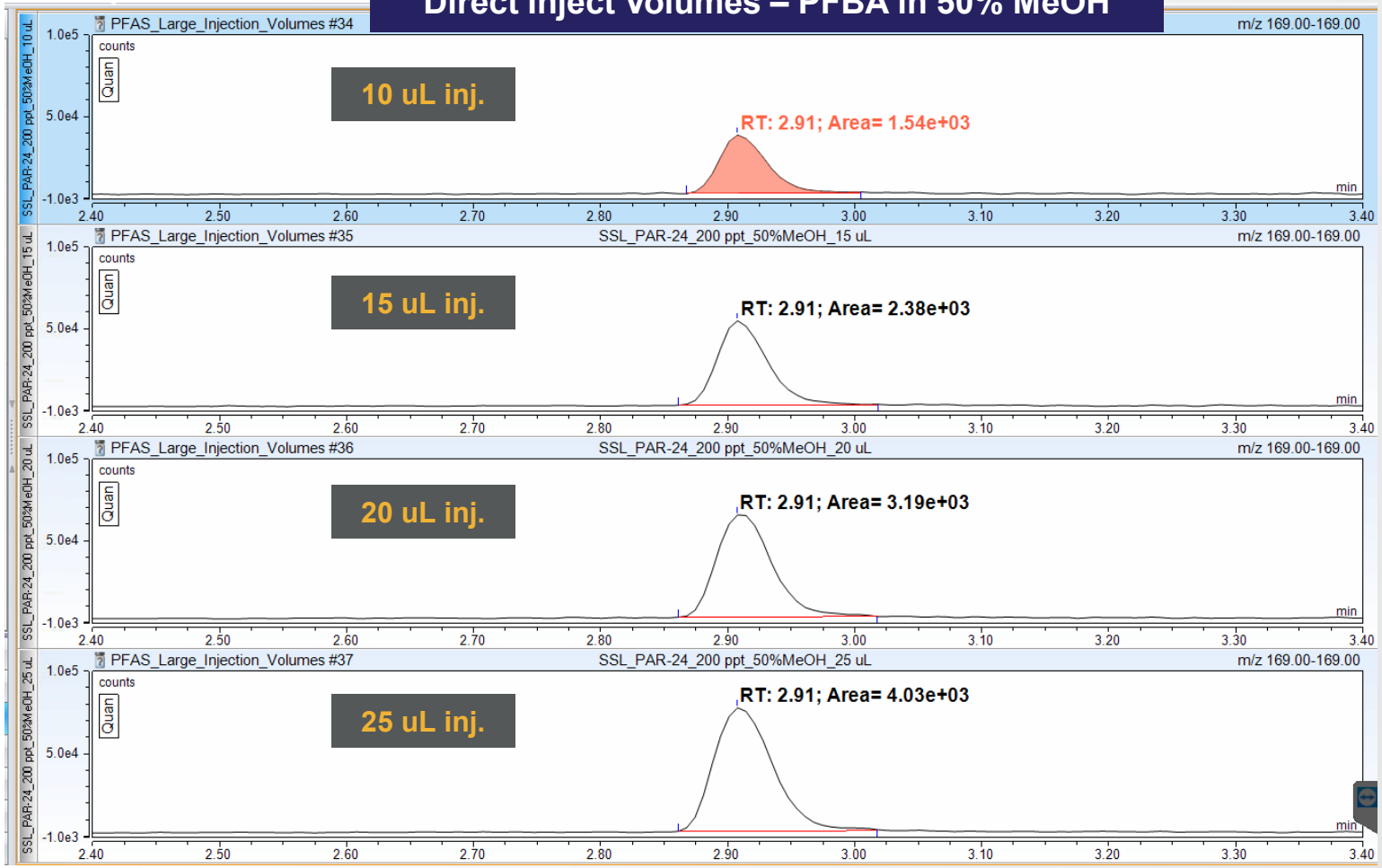
Experimental – Liquid Chromatography

Strong Solvent Loop added in autosampler: Large volume direct injections



Direct Inject Volumes – PFBA in 50% MeOH



Peak shape for PFBA is maintained up to 25 uL inj. of 50% MeOH solution

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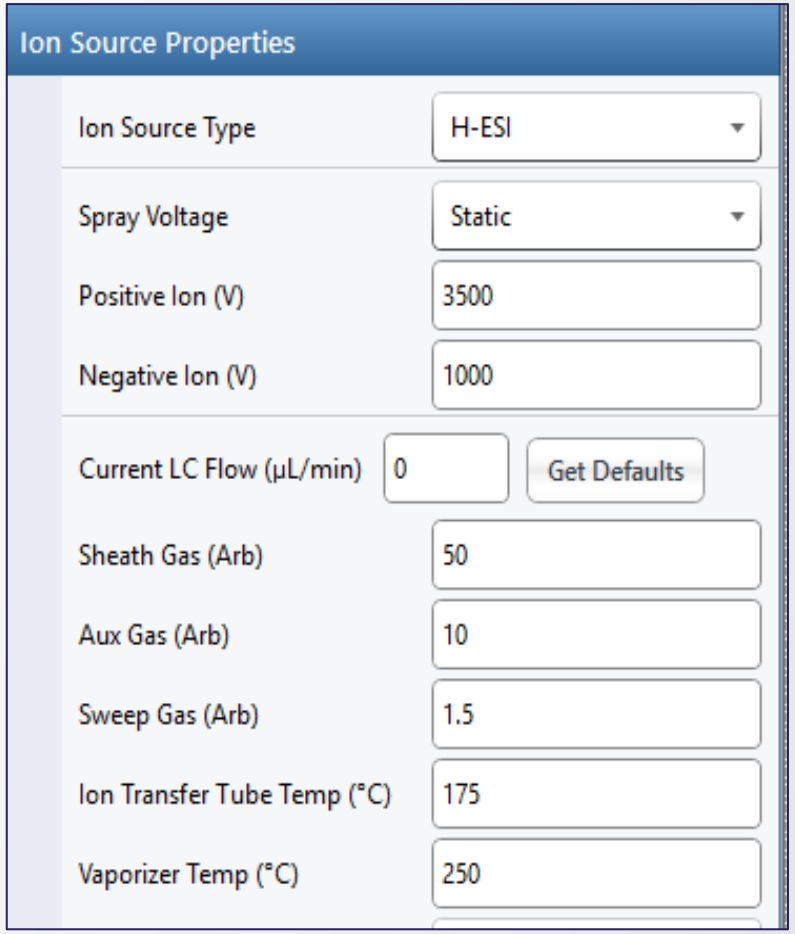
Experimental – Mass Spectrometry

Thermo Scientific TSQ Altis Plus

- Ionization Mode: HESI, Negative ion mode
  - Source Parameters: see figure at right
- MS Acquisition Mode: Timed Selected Reaction Monitoring (SRM)
- SRM Cycle Time: 0.4 s
- Quad Isolation Width: Q1, Q3 = Unit (0.7 Da FWHM)



Experimental – Sample Prep

PFAS Calibration Standard Solutions

- Standard solutions were provided by Wellington Labs. Standards were stored at 4 C until needed.
- Final calibration standard solutions were prepared over a concentration range 0.5-1000 ng/L in 50% MeOH.
- The calibration standards were spiked with isotopically-labeled standards to a final concentration of 50-400 ng/L.
- All calibration solutions were prepared in amber glass autosampler vials with polypropylene caps to prohibit PFAS contamination.
- Final PFAS calibration standards were analyzed by LC-MS/MS shortly after preparation to limit sample adsorption losses.

Experimental – Sample Prep

Surface Water Samples

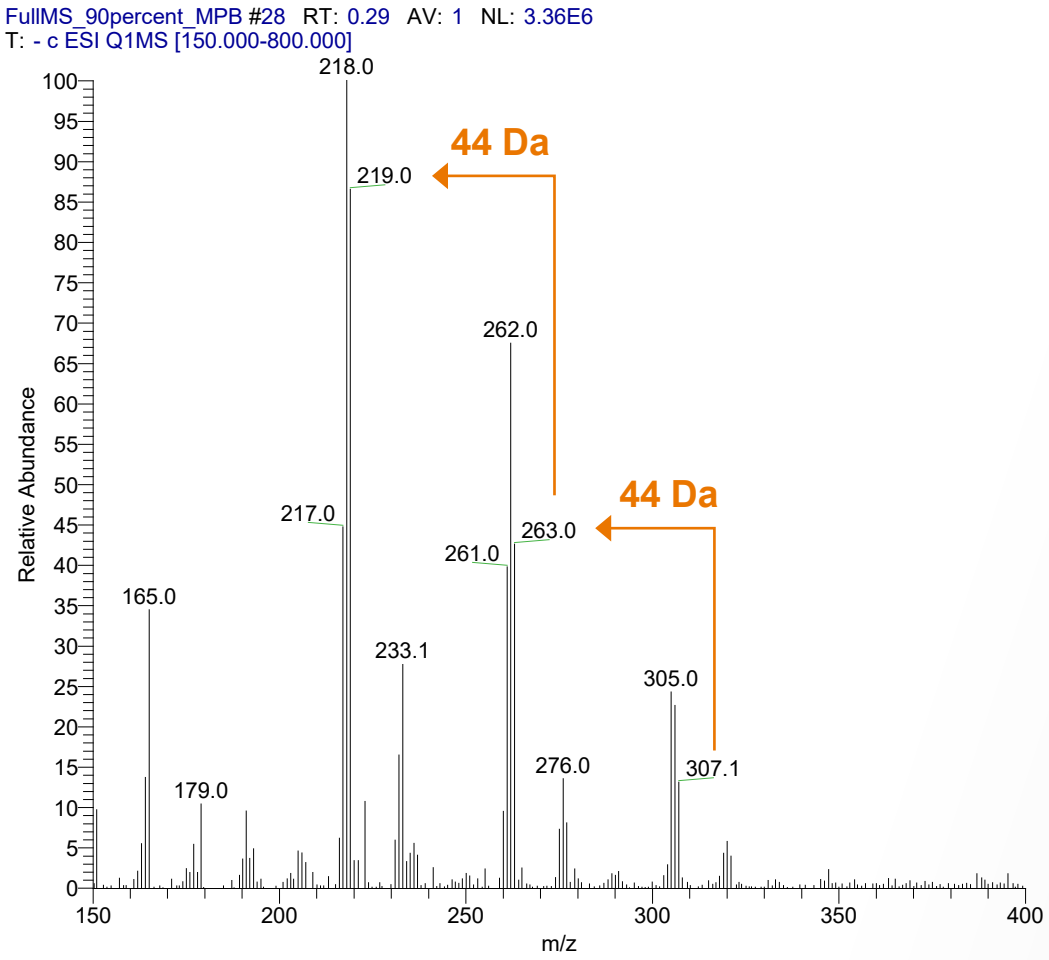
- Volume of surface water samples were determined by subtracting the mass of empty polypropylene centrifuge tube from mass of each water containing tube.
- Prepared 25 mL MeOH solution with 100-800 ng/L isotopically-labeled solution. [Note, this is 2X the concentrations used for calibration standard solutions.]
- This methanolic solution was added to polypropylene tubes at an equal volume to the surface water samples.
- After thoroughly vortexing surface water solutions, ~0.5 mL was transferred to amber glass autosampler vials with polypropylene caps for LC-MS/MS analyses.

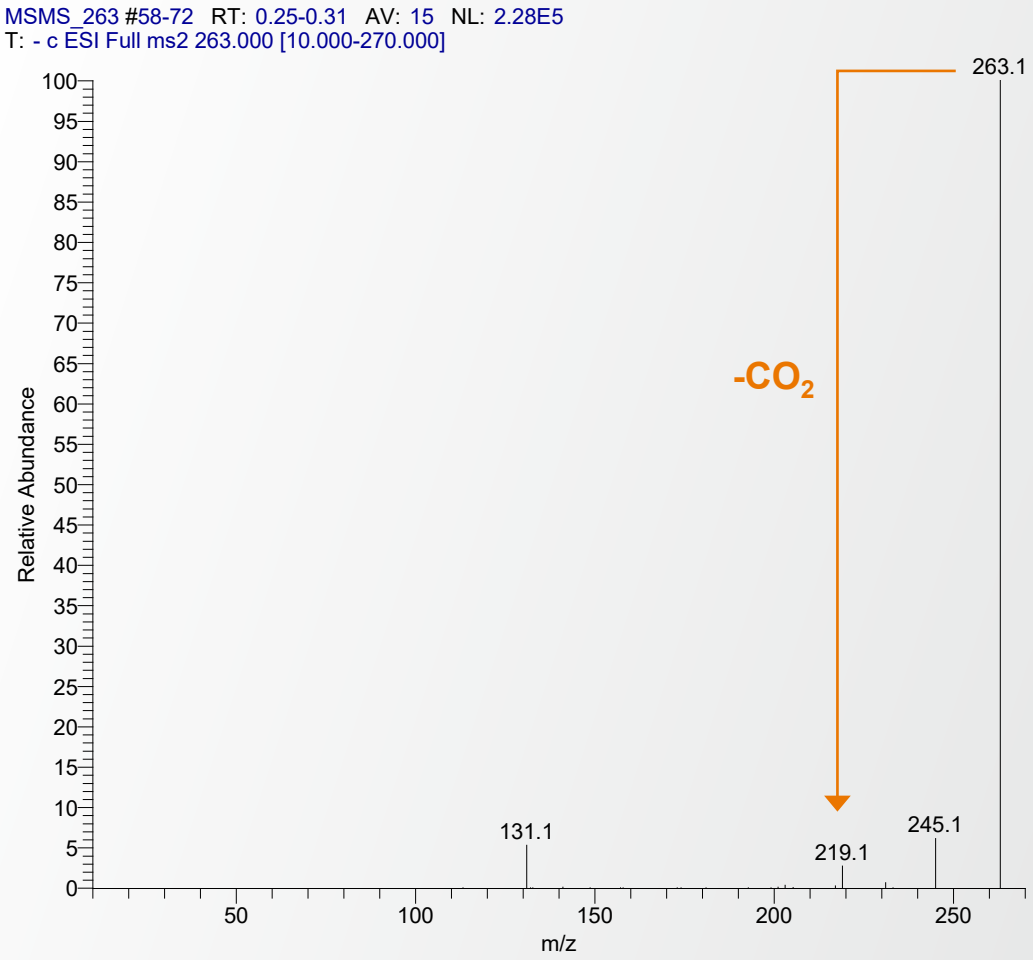
PFAS Method

- **Example PFAS data via direct injection on TSQ Altis Plus**
  - Solvent Blank PFAS contamination
  - Effect of Ion Transfer Tube temperature on PFAS

Full-scan MS & MS/MS (m/z 263, PFPeA isobar)

Background ions detected: ESI(-) at 90% Mobile Phase B

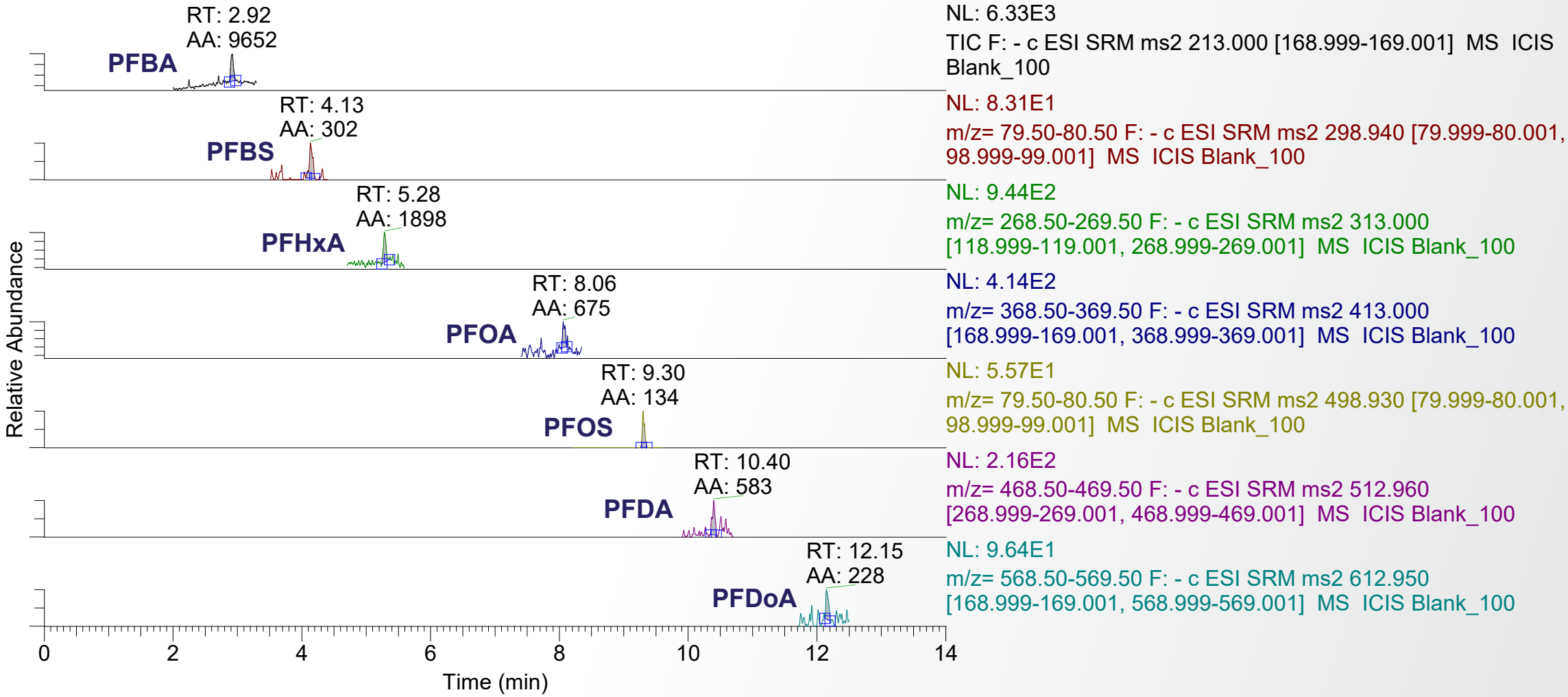




Background has ethylene glycol signature; MS2 of m/z 263 shows loss of CO_2

PFAS Contamination – Solvent Blanks

50% MeOH

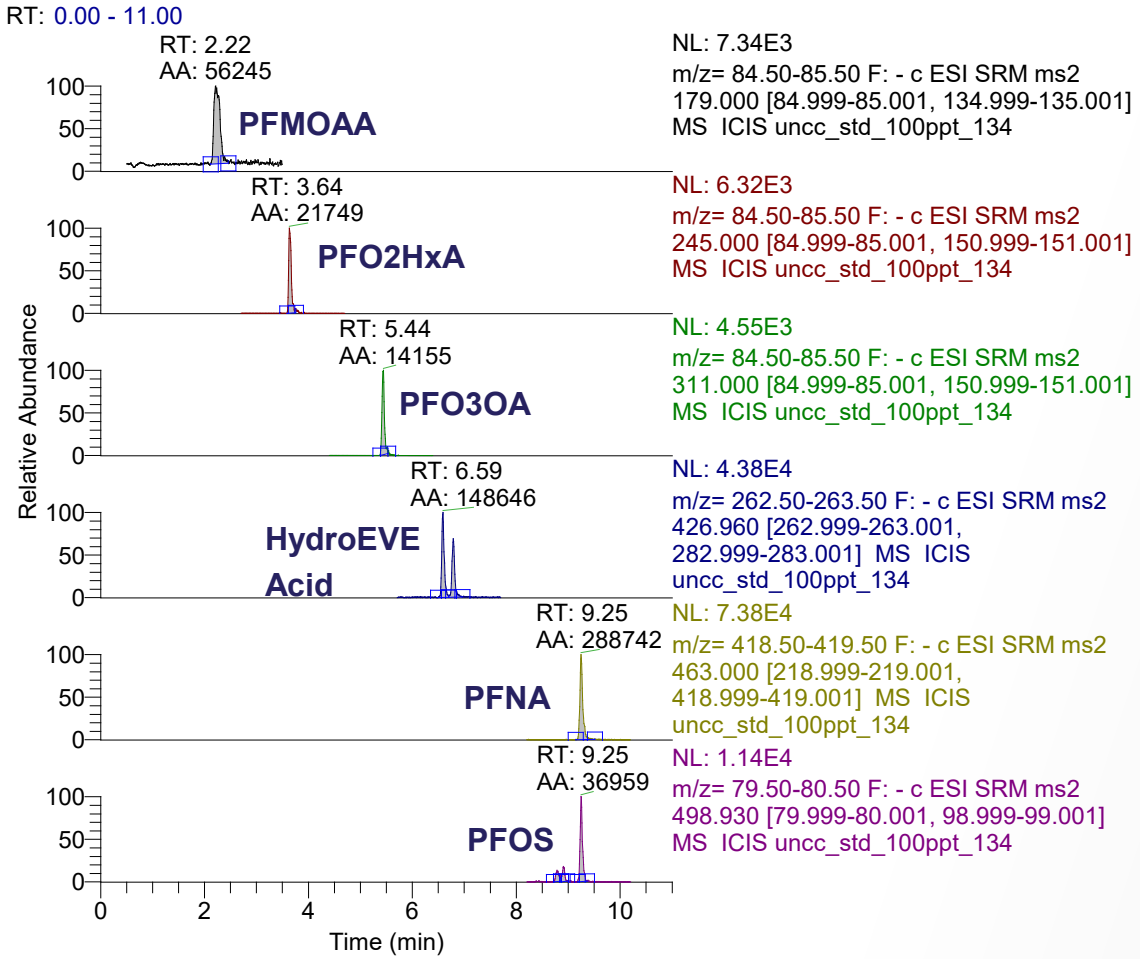


For some PFAS, a "null" response was not achieved (e.g., PFBA, PFHxA)

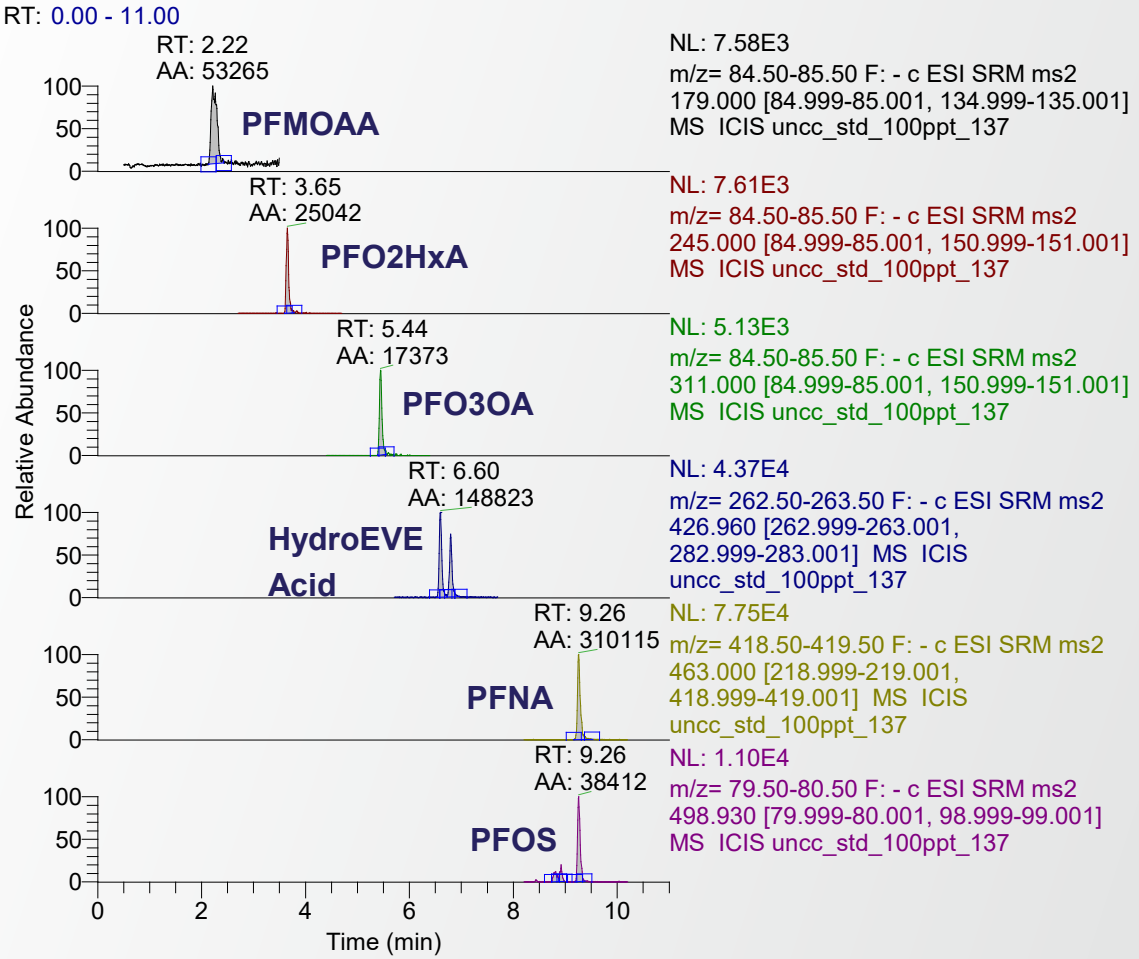
Effect of TSQ Ion Transfer Tube Temperature

100 ng/L PFAS: Perfluoronated ether acids, sulfonates and carboxylic acids

ITT Temp = 225 C



ITT Temp = 175 C



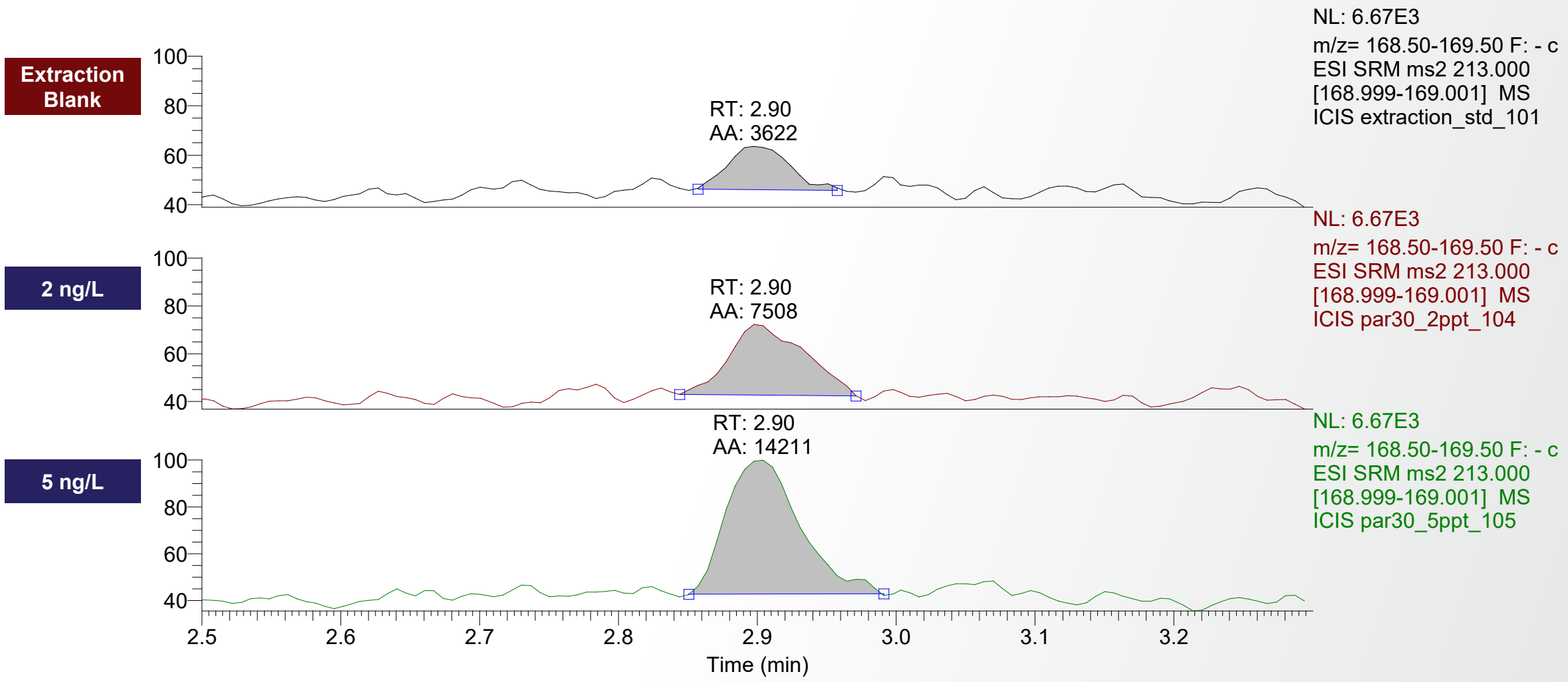
All PFAS classes show comparable or improved response at lower ITT temperature

Quantitation Results – PFAS Stds.

- **Examples of LC-MS/MS data for direct injection of PFAS standards**
  - Chromatogram displays near LODs
  - Calibration curves
  - Final table of estimated LODs

Example Chromatograms near LOD

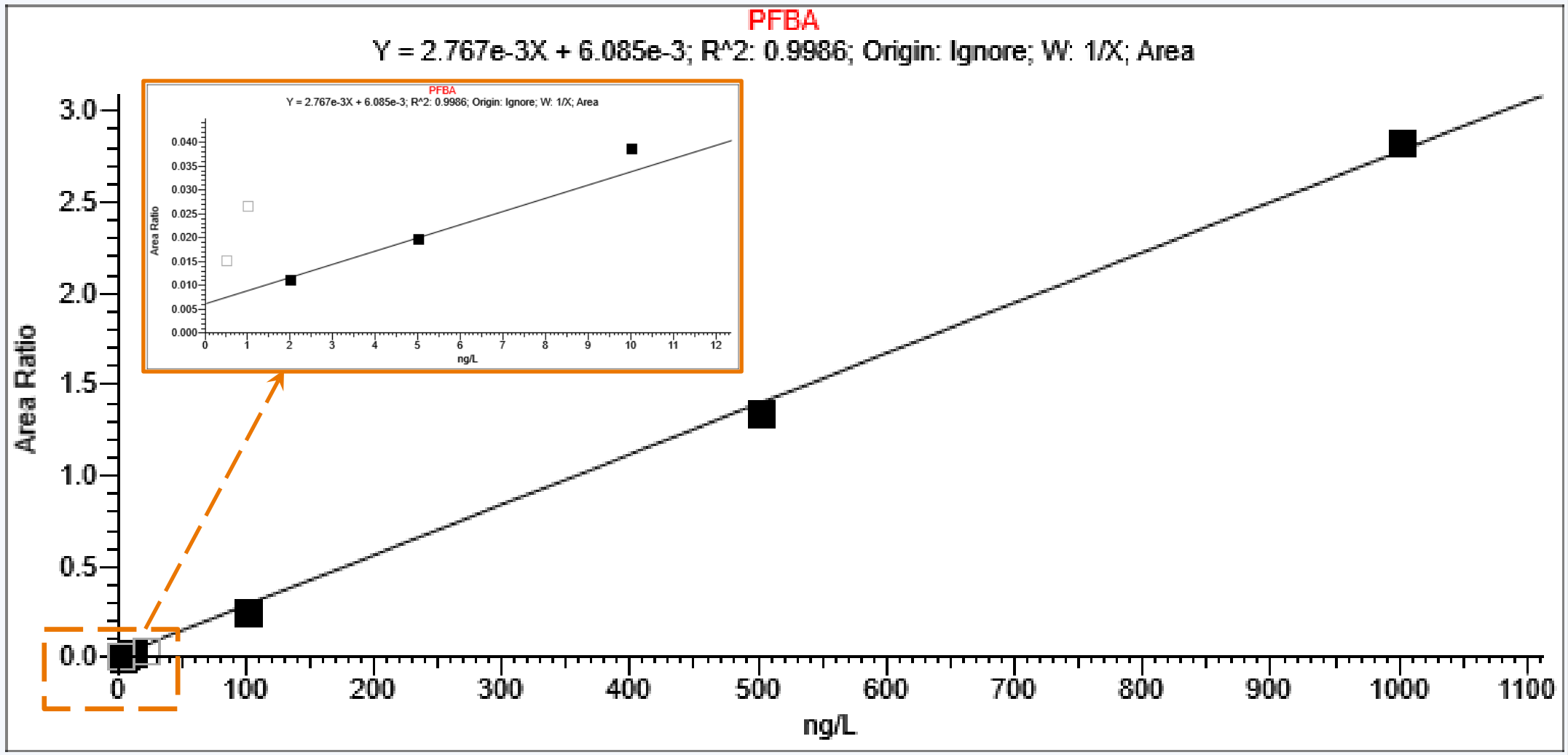
PFBA (in 50% MeOH, 25 uL inj.)



Est. LOD PFBA is 2 ng/L (50 fg on-column); limited by blank contamination

Calibration Curve: PFBA

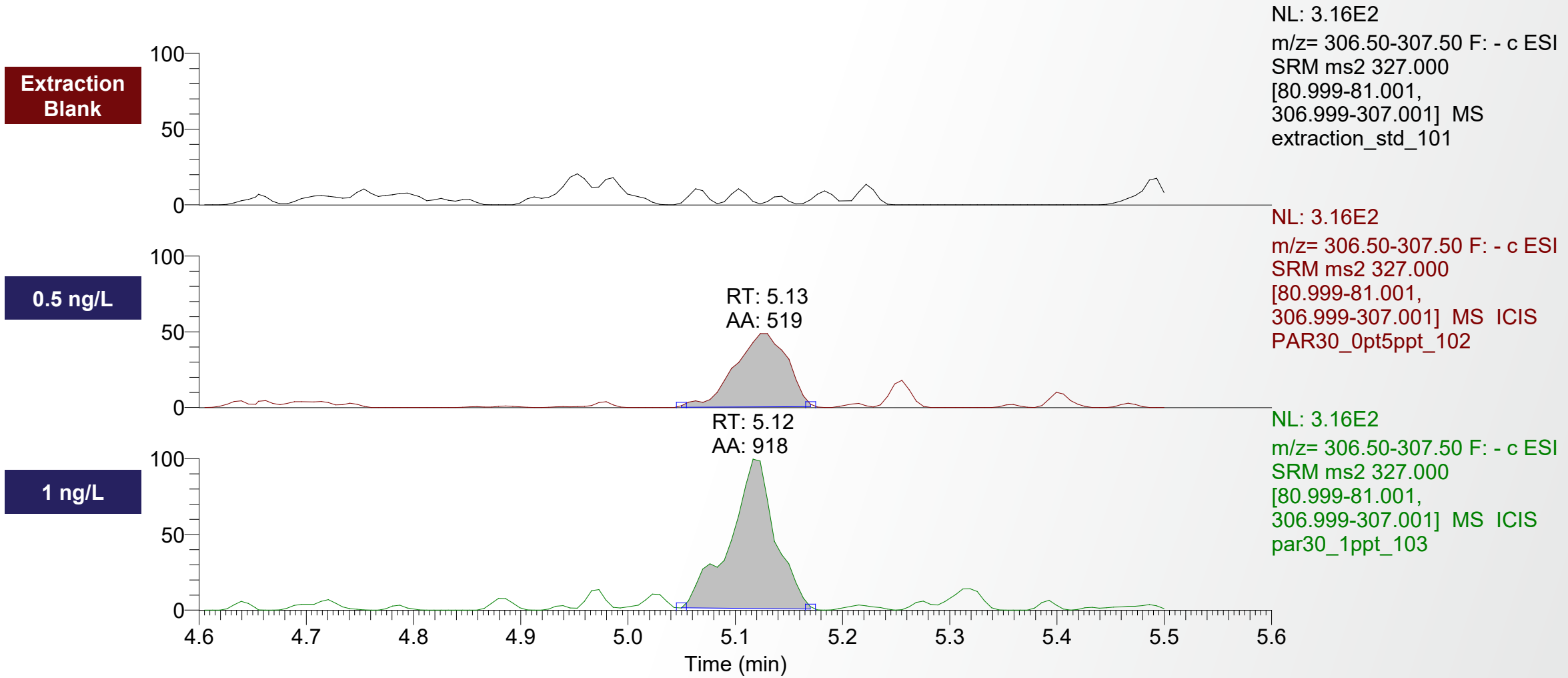
2 – 1000 ng/L, Linear, 1/x weighting



PFBA (PAR30) has linear regression R^2 = 0.9986 over 3 decade dynamic range

Example Chromatograms near LOD

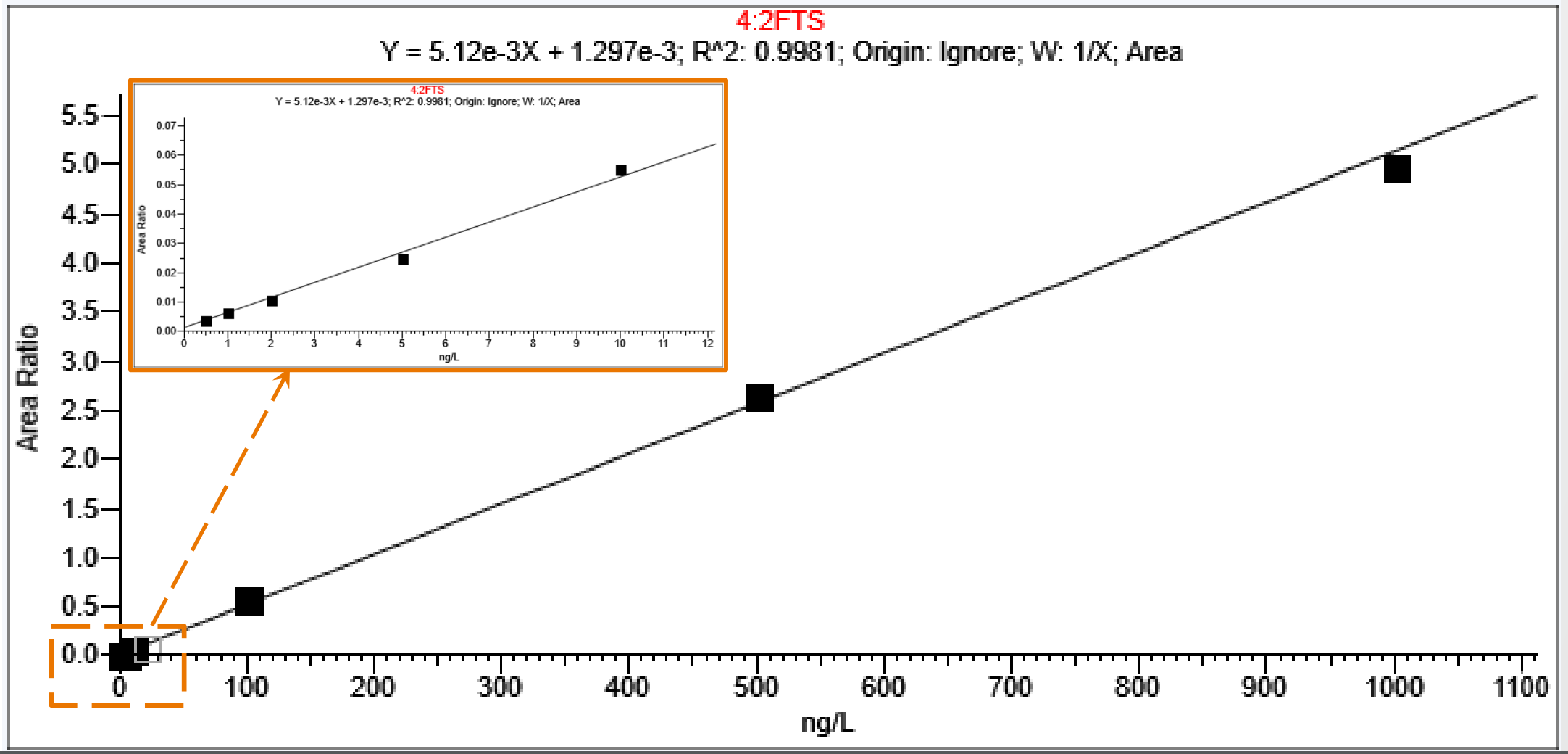
4:2 FTS (in 50% MeOH, 25 uL inj.)



Est. LOD 4:2FTS is 0.25 ng/L (6.25 fg on-column)

Calibration Curve: 4:2FTS

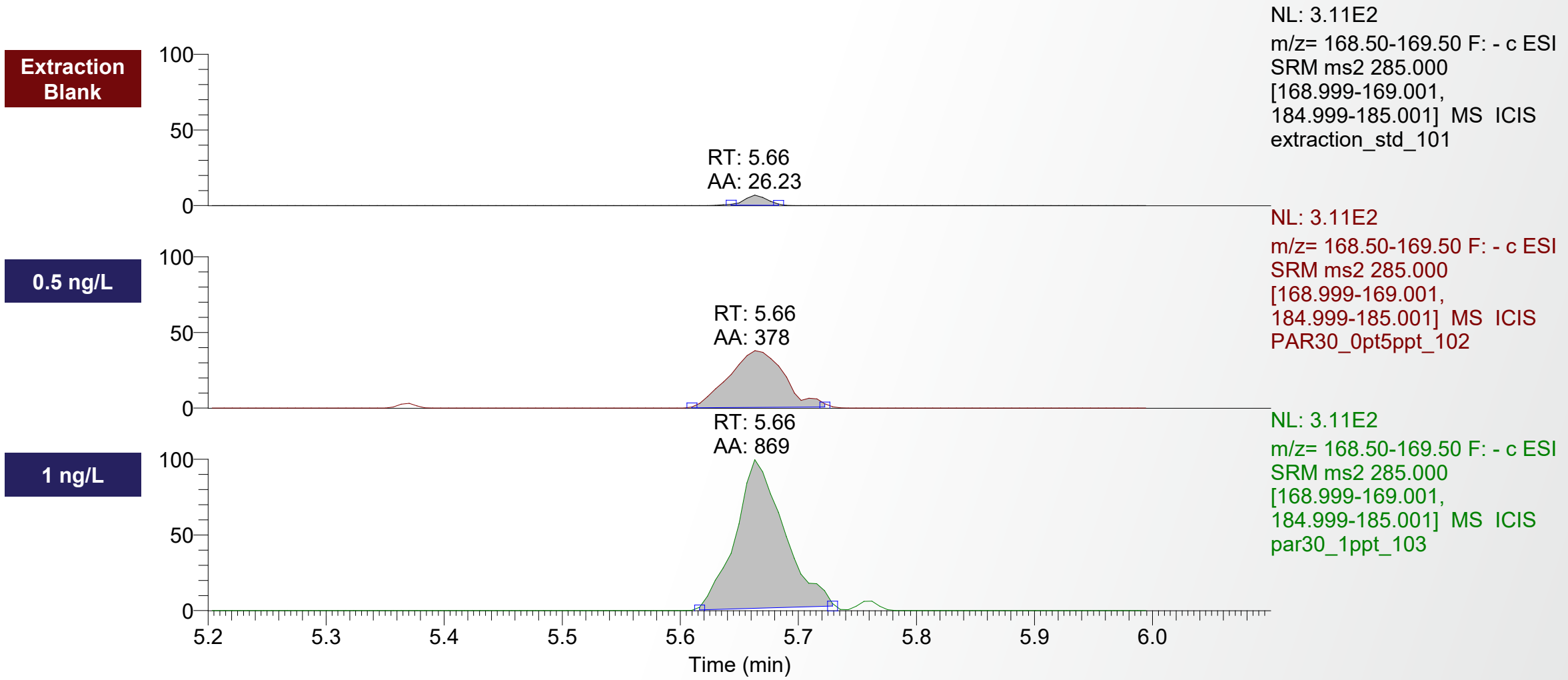
0.5 – 1000 ng/L, Linear, 1/x weighting



4:2FTS (PAR30) has linear regression R^2 = 0.9981 over 3.5 decade dynamic range

Example Chromatograms near LOD

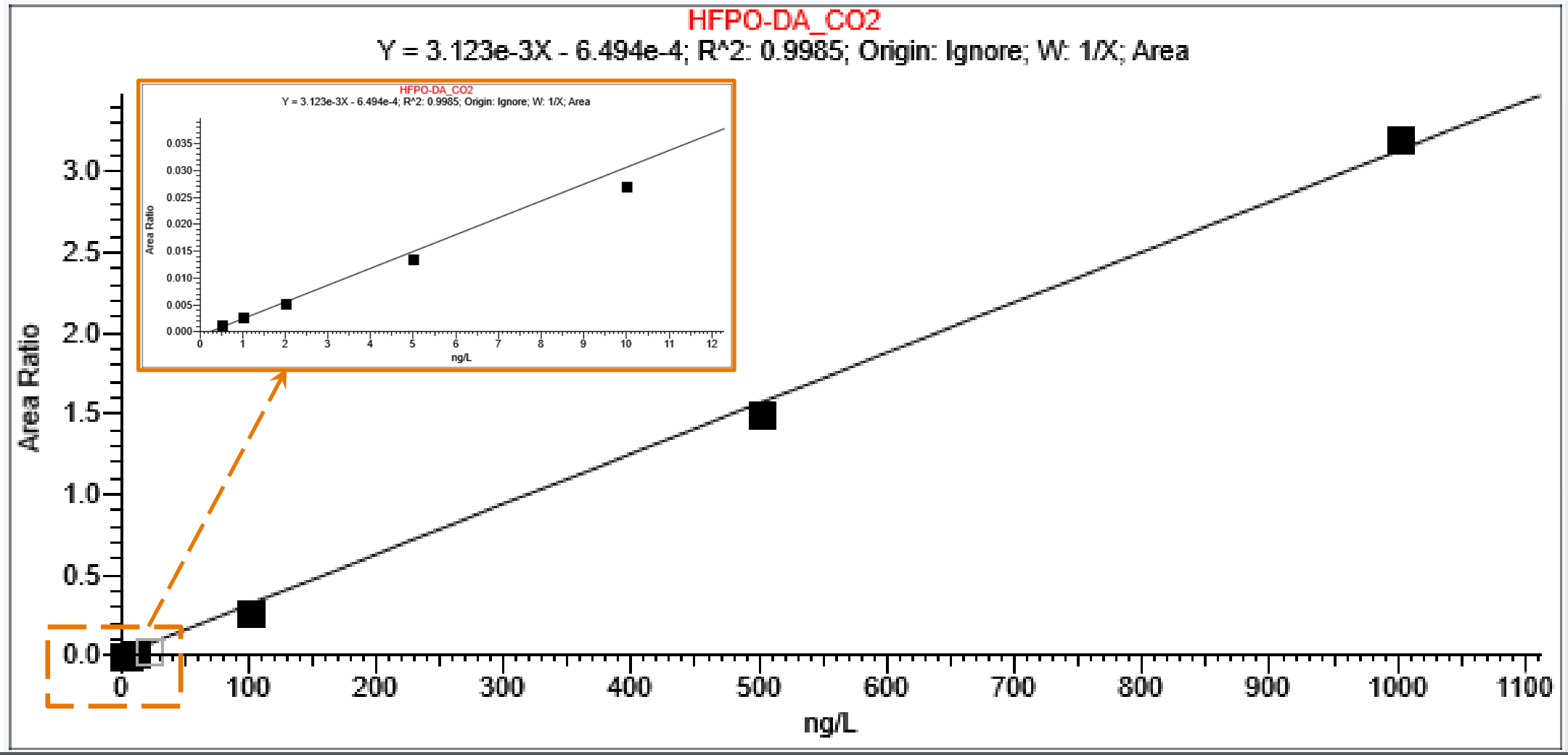
HFPO-DA (in 50% MeOH, 25 uL inj.)



Est. LOD HFPO-DA is 0.25 ng/L (6.25 fg on-column)

Calibration Curve: HFPO-DA

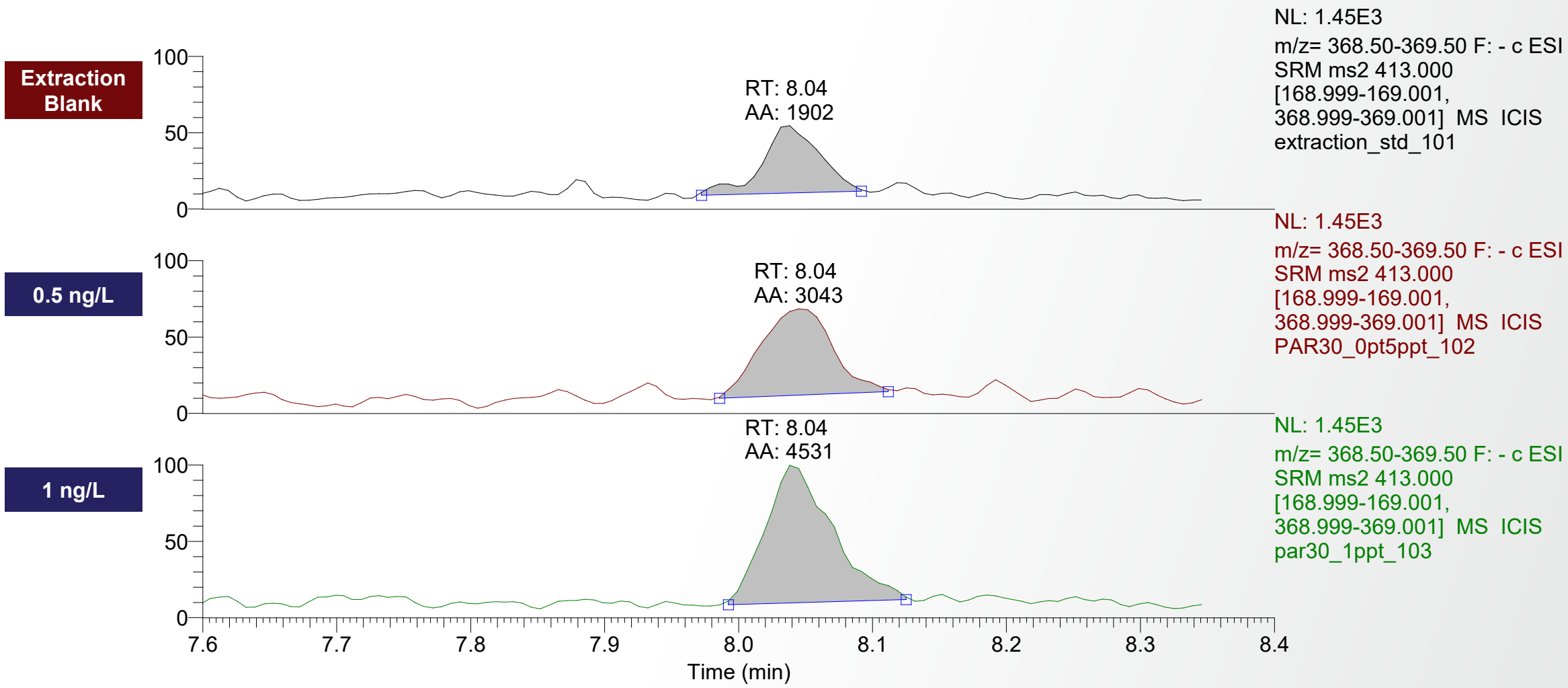
0.5 – 1000 ng/L, Linear, 1/x weighting



HFPO-DA (PAR30) has linear regression R^2 = 0.9985 over 3.5 decade dynamic range

Example Chromatograms near LOD

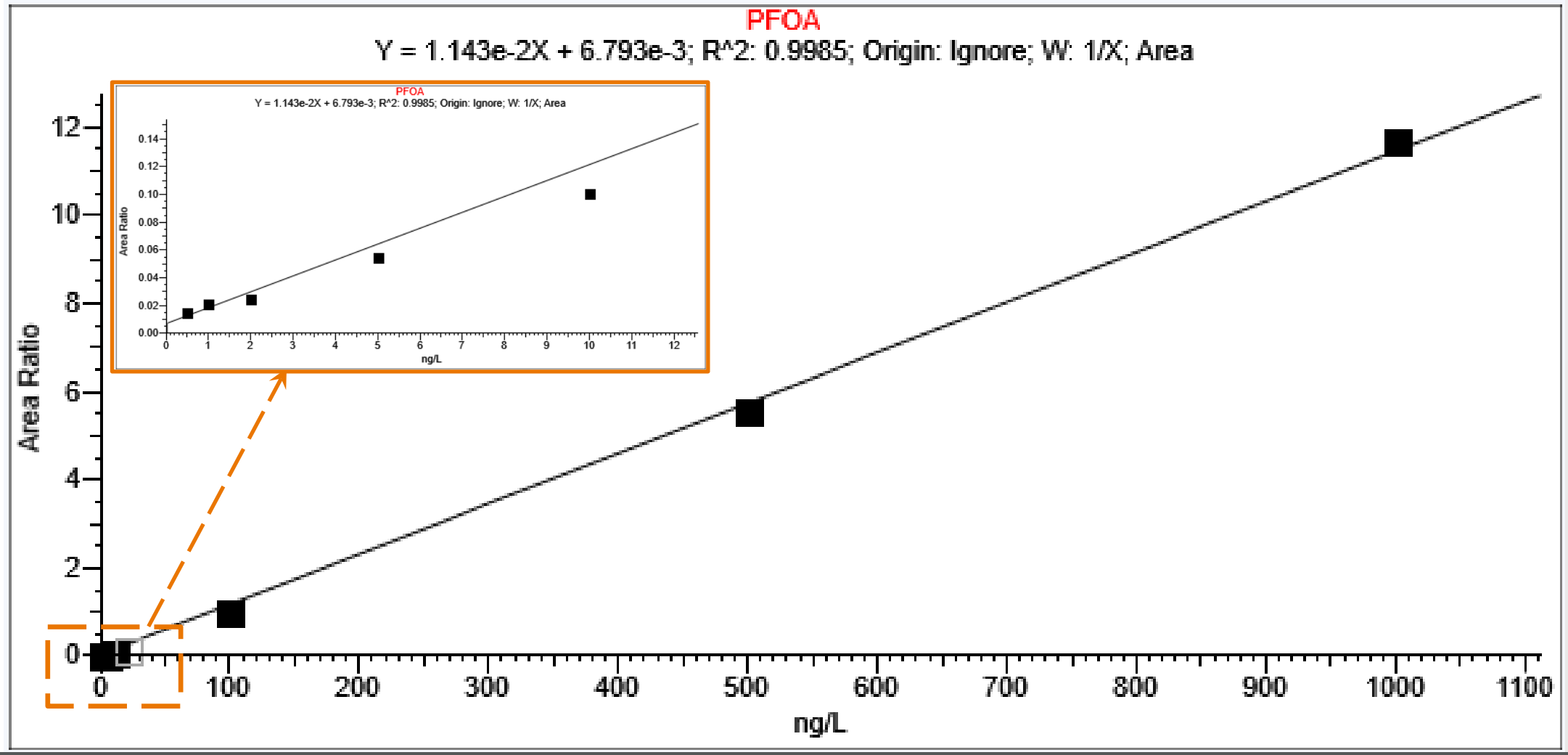
PFOA (in 50% MeOH, 25 uL inj.)



Est. LOD PFOA is 1.0 ng/L (25 fg on-column); limited by blank contamination

Calibration Curve: PFOA

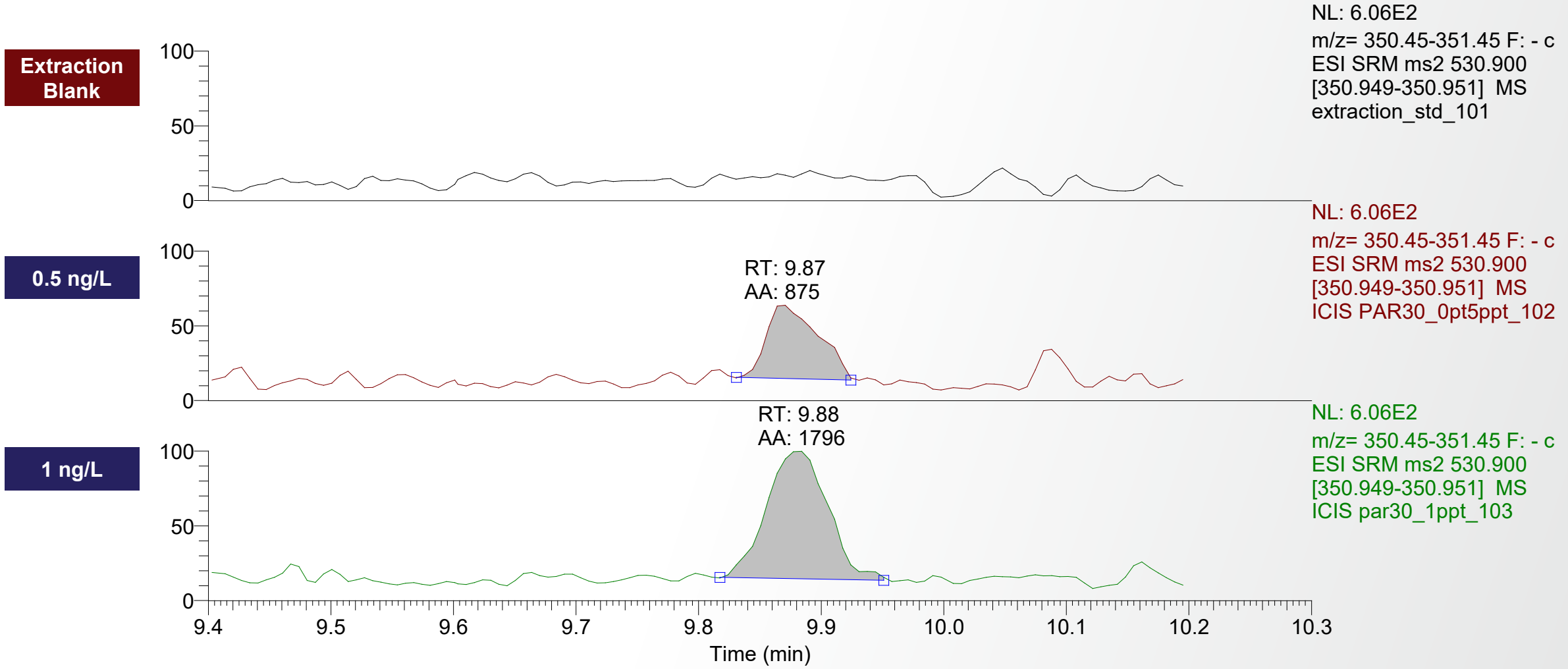
0.5 – 1000 ng/L, Linear, 1/x weighting



PFOA (PAR30) has linear regression R^2 = 0.9985 over 3.5 decade dynamic range

Example Chromatograms near LOD

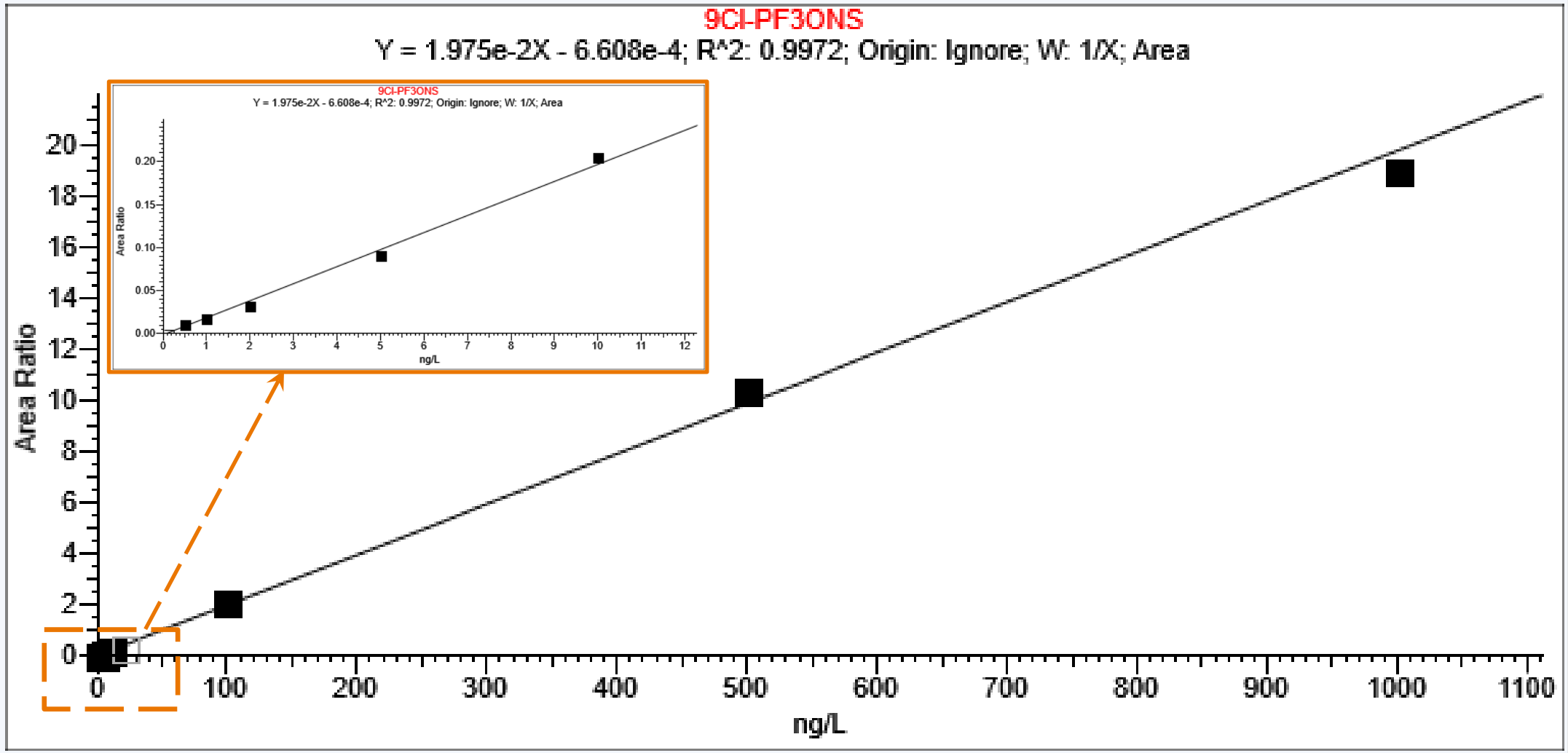
9Cl-PF3ONS, (in 50% MeOH, 25 uL inj.)



Est. LOD 9Cl-PF3ONS is 0.25 ng/L (6.25 fg on-column)

Calibration Curve: 9Cl-PF3ONS

0.5 – 1000 ng/L, Linear, 1/x weighting



9Cl-PF3ONS (PAR30) has linear regression R^2 = 0.9972 over 3.5 decade dynamic range

Table of PFAS Compounds – LODs

Analyte	Acronym	LOD (ng/L)	LOD (fg OC)
N-ethyl perfluorooctanesulfonamidoacetic acid	NEtFOSAA	1	25
N-methyl perfluorooctanesulfonamidoacetic acid	NMeFOSAA	2	50
Perfluorobutanoic acid	PFBA	2 (est.)	50
Perfluorobutanesulfonic acid	PFBS	0.5	12.5
1H,1H,2H,2H-Perfluorodecane sulfonic acid	8:2FTS	1	25
Perfluorodecane sulfonic acid	PFDS	0.5	12.5
Perfluorodecanoic acid	PFDA	0.25 (est.)	6.25 (est.)
Perfluorododecanoic acid	PFDoA	0.25 (est.)	6.25 (est.)
Perfluoroheptane sulfonic acid	PFHpS	0.5	12.5
Perfluoroheptanoic acid	PFHpA	0.5	12.5
1H,1H,2H,2H-Perfluorohexane sulfonic acid	4:2FTS	0.25 (est.)	6.25 (est.)
Perfluorohexanesulfonic acid	PFHxS	0.5	12.5
Perfluorohexanoic acid	PFHxA	1	25
Perfluorononane sulfonic acid	PFNS	0.5	12.5
Perfluorononanoic acid	PFNA	0.25 (est.)	6.25 (est.)
1H,1H,2H,2H-Perfluorooctane sulfonic acid	6:2FTS	0.5	12.5
Perfluoro-1-butanesulfonamide	FBSA	0.25 (est.)	6.25 (est.)
Perfluoro-1-hexanesulfonamide	FHxSA	0.25 (est.)	6.25 (est.)
Perfluoro-1-octanesulfonamide	FOSA	0.5	12.5
Perfluorooctanesulfonic acid	PFOS	0.5	12.5
Perfluorooctanoic acid	PFOA	0.25 (est.)	6.25 (est.)
Perfluoropentanoic acid	PFPeA	5	125
Perfluoropentanesulfonic acid	PFPeS	0.5	12.5
Perfluorotetradecanoic acid	PFTeDA	0.25 (est.)	6.25 (est.)
Perfluorotridecanoic acid	PFTrDA	0.25 (est.)	6.25 (est.)
Perfluoroundecanoic acid	PFUnA	0.25 (est.)	6.25 (est.)
Hexafluoropropylene oxide dimer acid	HFPO-DA	0.25 (est.)	6.25 (est.)
11-chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	11Cl-PF2OUdS	0.25 (est.)	6.25 (est.)
9-chlorohexadecafluoro-3-oxanone-1-sulfonic acid	9Cl-PF3ONS	0.25 (est.)	6.25 (est.)
4,8-dioxa-3H-perfluorononanoic acid	ADONA	N.A.	N.A.

- Data from a 25 uL direct injection of PFAS standards in 50% MeOH
- LODs are defined as lowest concentration where the compound is based on the response difference between the 0.5 ng/L standard and solvent blank.
- Concentrations for PFAS Sulfonates are not corrected for salt form or for presence of branched isomers

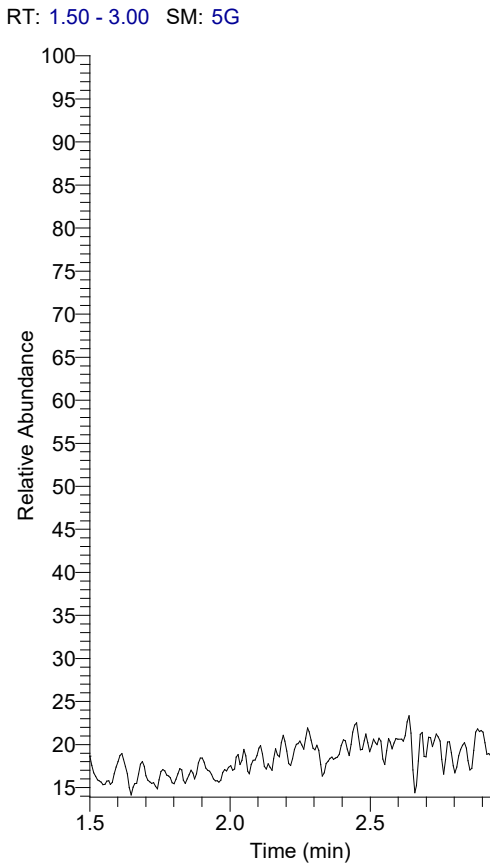
Results

Measurement of PFAS in Surface Water samples

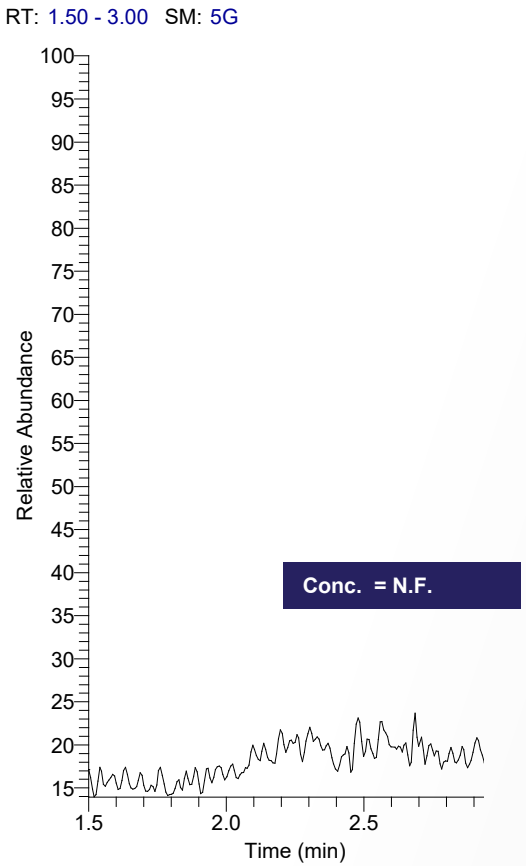
Example Chromatograms – Spiked Surface Water Samples

PFMOAA (in 50% MeOH, 25 uL inj.)

Blank



Sample 1



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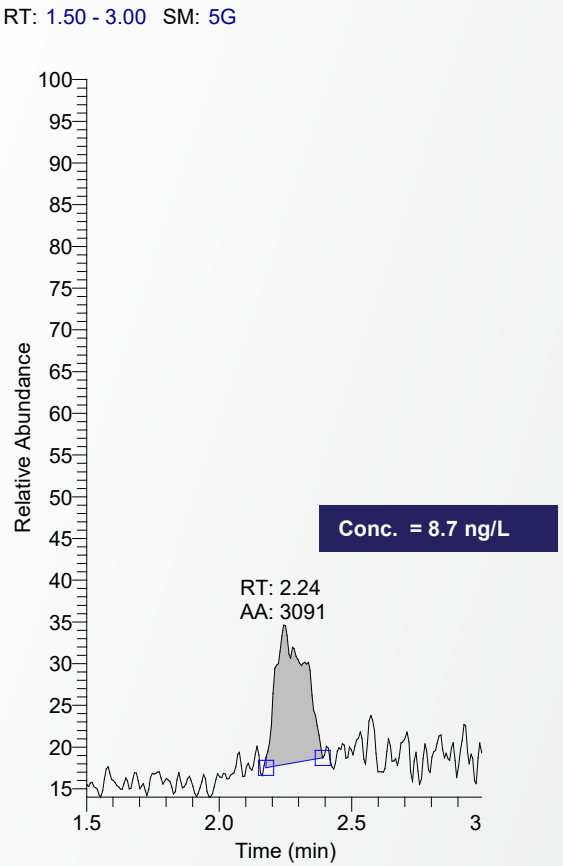

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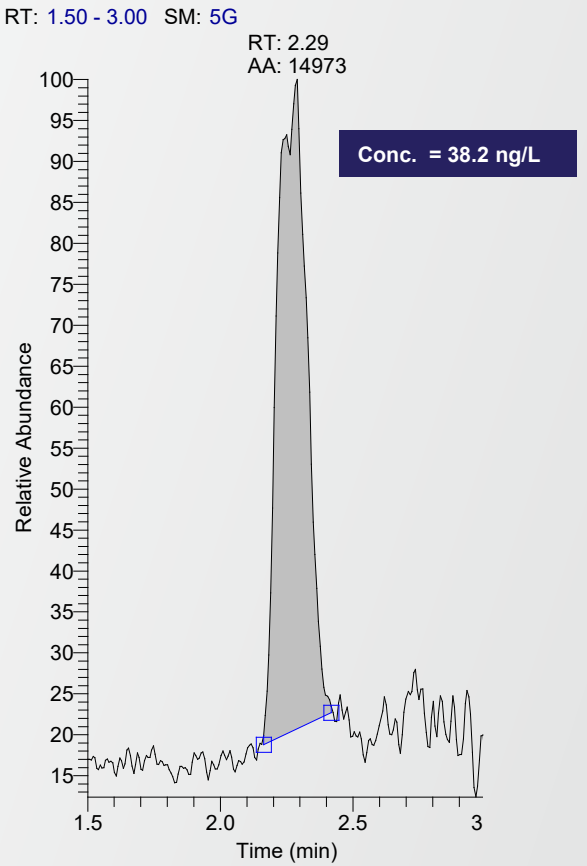
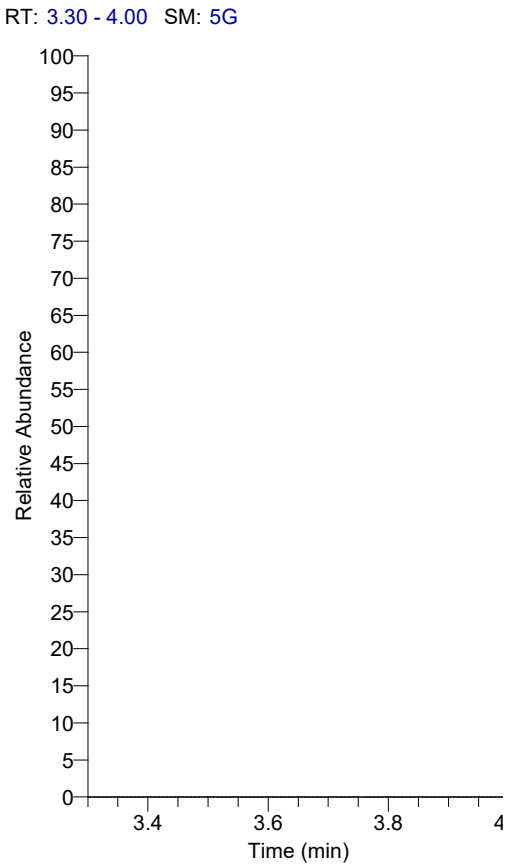


PFMOAA was observed in 2 of 3 surface water samples

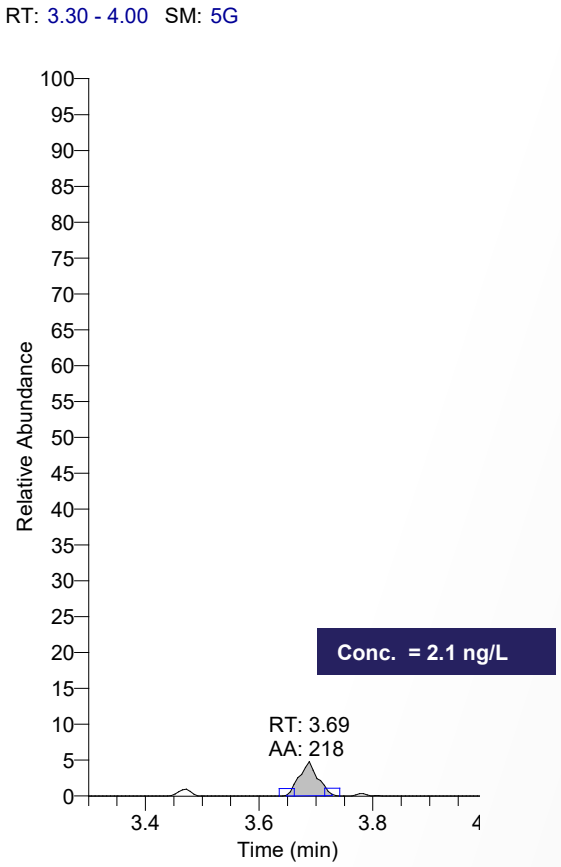
Example Chromatograms – Surface Water Samples

PFO2HxA (in 50% MeOH, 25 uL inj.)

Blank



Sample 1



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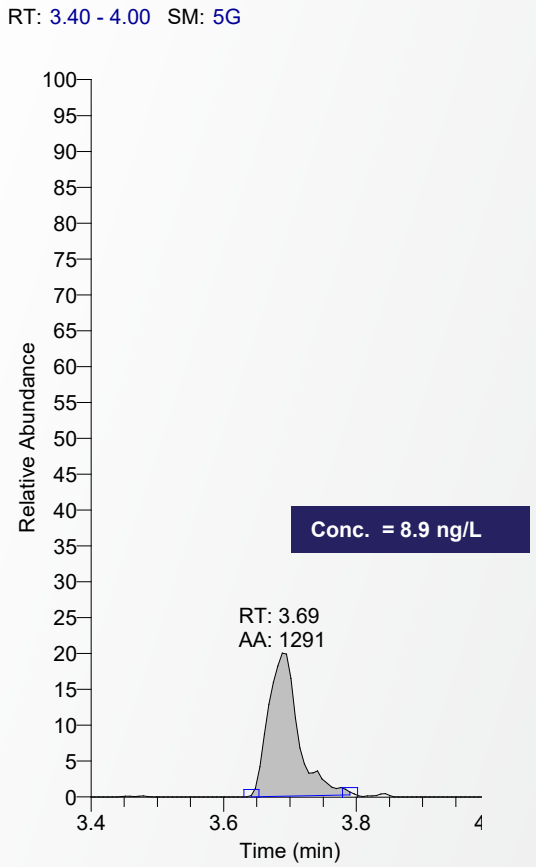

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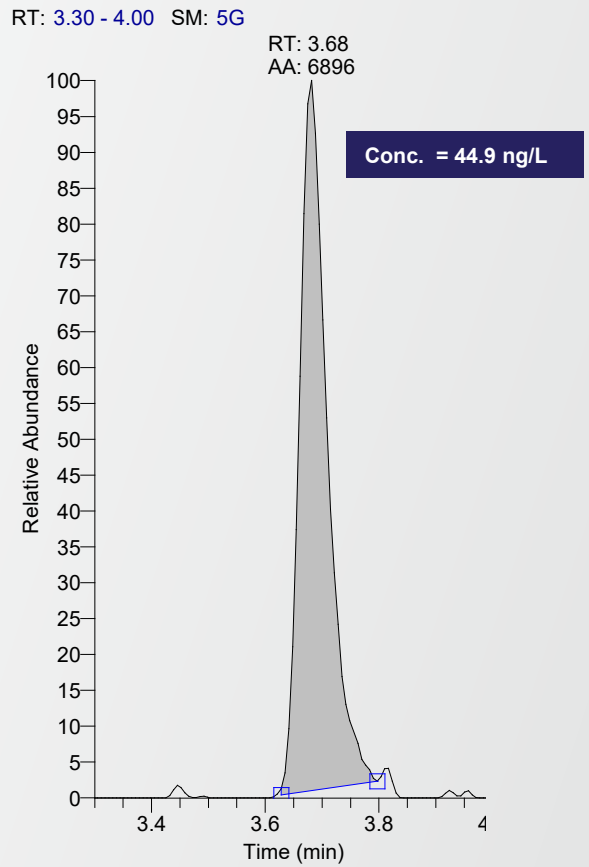
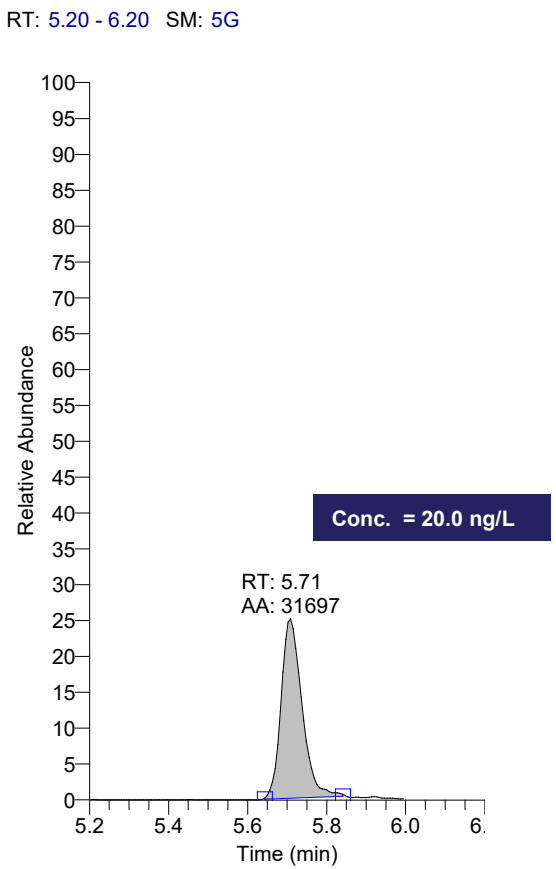


PFO2HxA is observed in 3 of 3 surface water samples

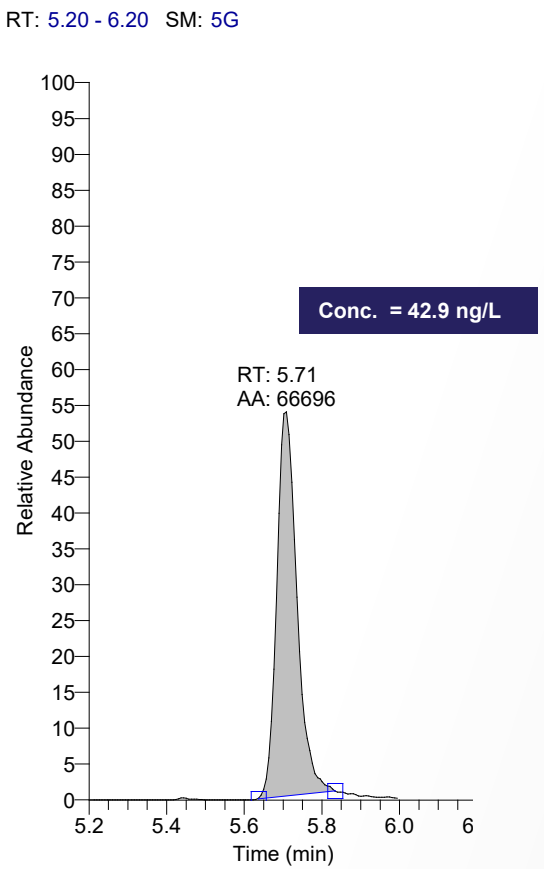
Example Chromatograms – Surface Water Samples

HFPO-DA (in 50% MeOH, 25 uL inj.)

Blank



Sample 1



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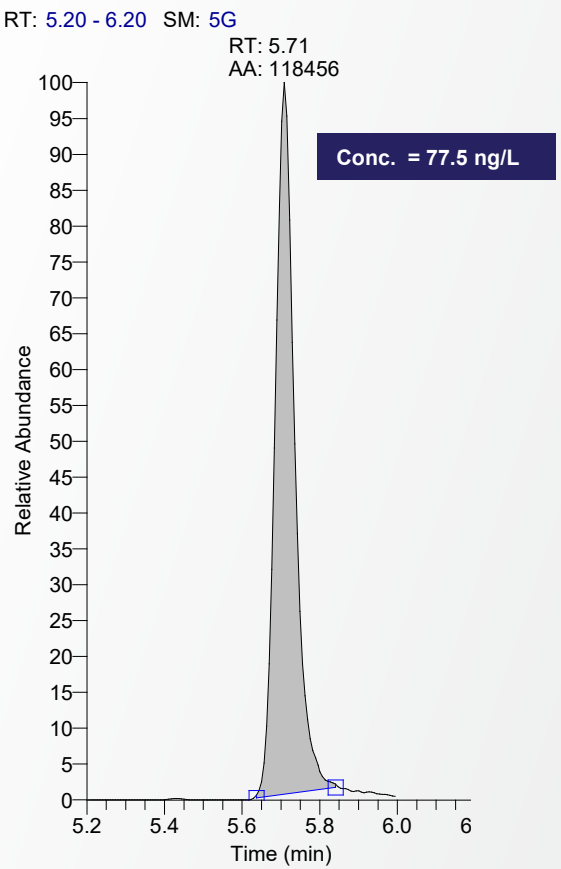

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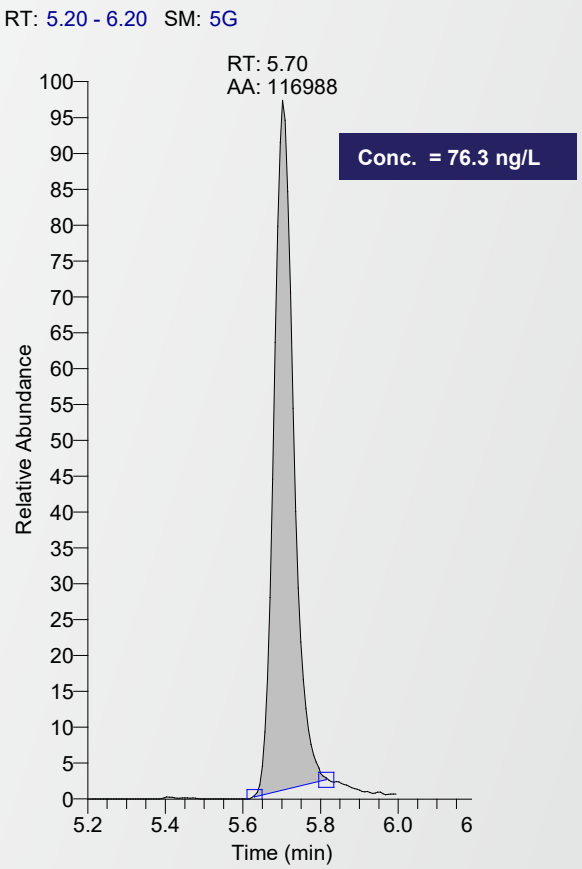
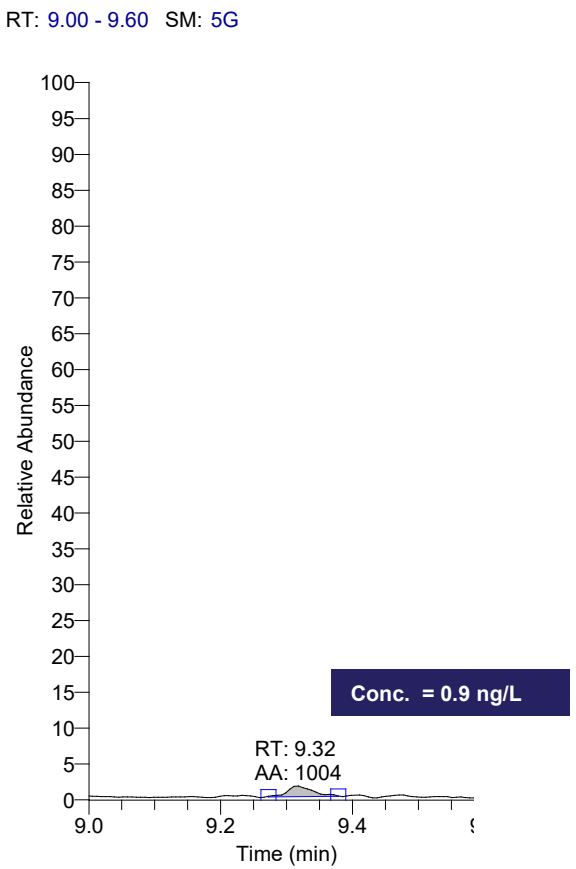


HFPO-DA is observed in all surface water samples, including blank

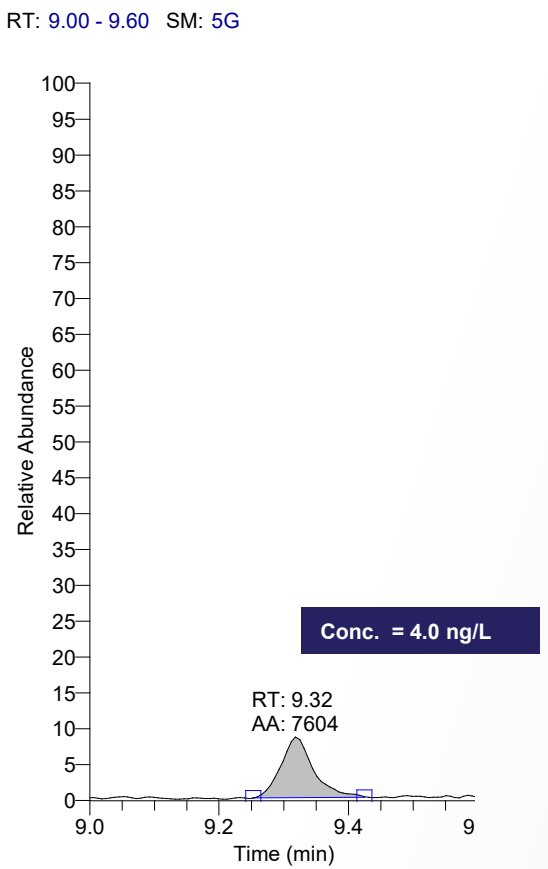
Example Chromatograms – Surface Water Samples

PFNA (in 50% MeOH, 25 uL inj.)

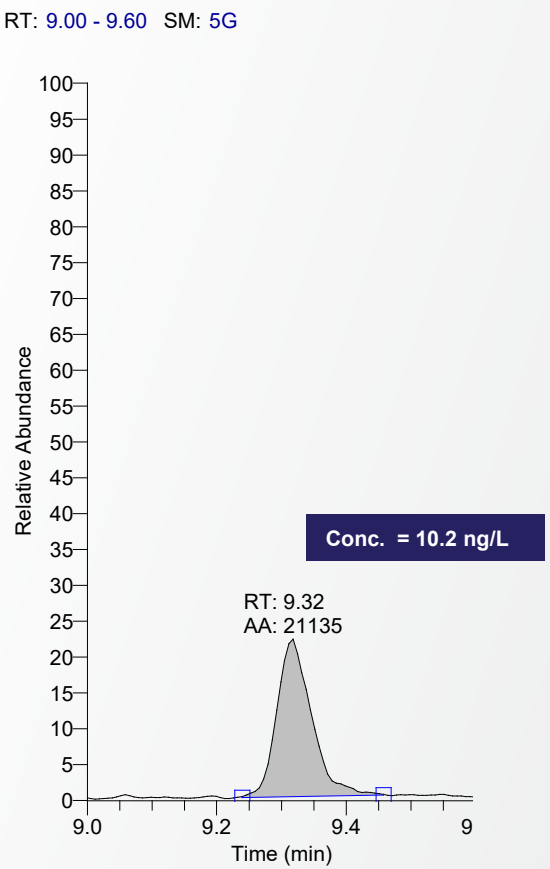
Blank



Sample 1



Sample 2



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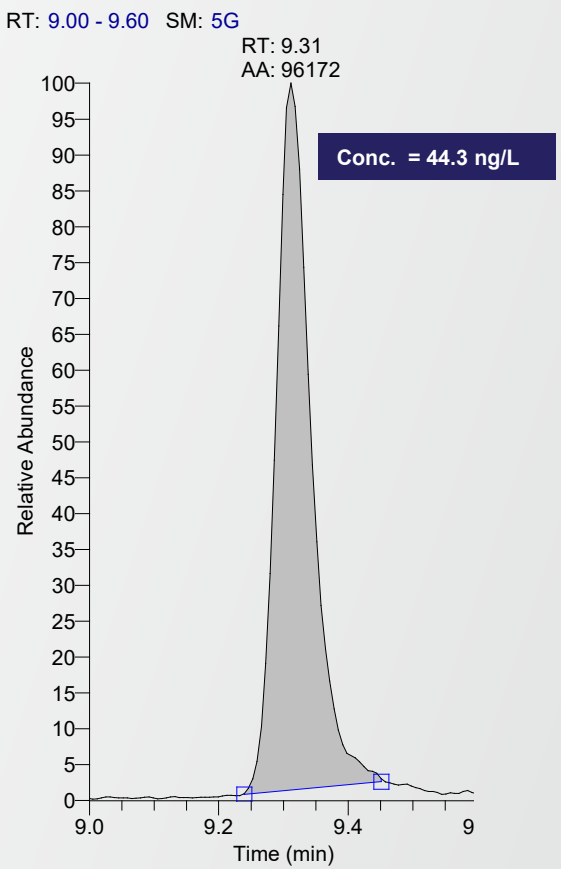


PFNA is observed in all surface water samples, including blank

Conclusions

- Thermo Scientific TSQ Altis Plus MS system provides excellent quantitative performance for measuring PFAS in surface water samples via 25 µL direct injection LC-MS/MS down to low single and or sub- ng/L concentrations.
- A Single LC-MS/MS method was used to measure wide variety PFAS classes, including perfluoronated ether acids.
- Nearly all (40 of 43) targeted PFAS compounds have LODs at or below 1 ng/L for neat PFAS solutions. Lower LODs in several cases (e.g., PFOA) were limited by contamination in solvent blanks.
- PFAS calibration curves from 0.5 – 1000 ng/L, using internal calibration, yielded linear regression calibrations with $r^2 > 0.995$.
- Surface water samples were analyzed by adding equal volume of methanol containing isotopically-labeled PFAS standards. Nearly all targeted PFAS compounds were able to be measured in spiked surface water samples at 1-2 ng/L levels (after correcting for 1:1 dilution).

Addressing the Main PFAS Analytical Challenges

How do I optimize LC injection volumes to obtain highest MS sensitivity ?

- **Heterogenous group of alkyl compounds:**
  - Short-chain (C4-C8)
  - Long-chain (C8-C18)

→ solubility, adsorption, LC retention

- **Ubiquitous occurrence:**
  - Nature, clothes
  - Laboratory equipment
  - (U)HPLC system

→ consumable selection, blank controls

- **Guidelines:**
  - Sample preparation
  - Sample solvents
  - Limit of quantitation

→ different flavours of PFAS analysis

An Alternate Liquid Chromatography Set-Up for Large Volume Injections

Vanquish Core HPLC system setup

Custom Injection Program

- Sample loop: 1000 µL
- Sandwich injection
- In needle mixing

Strong Solvent Loop

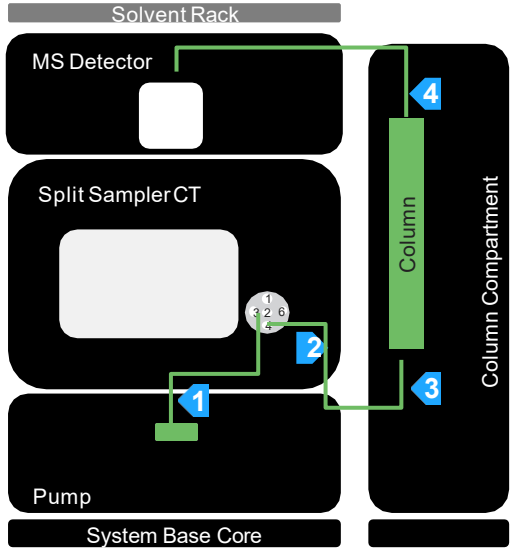
- Volume: 46.2 µL

Capillary

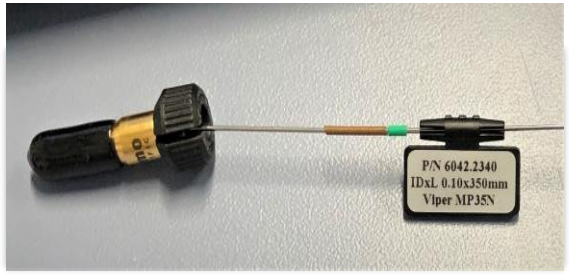
- Sampler-column
- Viper MP35N

Delay Column

- Accucore™ aQ, 3.0 x 50 mm, 2.6 µm)



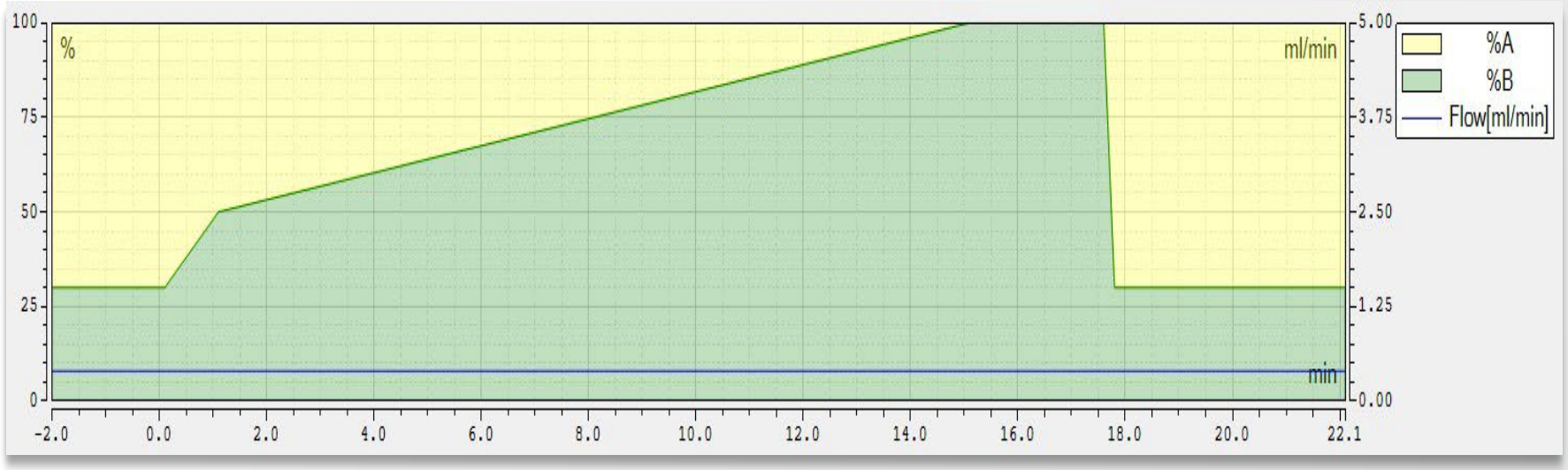
#	Connection between	Description
1	Pump Out – Port 1	0.18 x 350mm, SST, P/N 6040.2375
2	Port 2 – Viper Union	Strong Solvent Loop, SST, P/N 6036.2200
3	Viper Union – Column Inlet	0.10 x 350 mm, MP35N, P/N 6042.2340
4	Column Outlet - Detector	0.10 x 450 mm, MP35N, P/N 6042.2340



LC Method

LC System:	**Vanquish Core Binary HPLC System**
Column:	Acclaim™ Polar Advantage, 2.1 mm x 100 mm, 2.2 µm
Eluent A:	Water, 5 mM ammonium formate, 0.1% formic acid
Eluent B:	Methanol, 5 mM ammonium formate, 0.1% formic acid
Flow rate:	400 µL/min
Injection volume:	**Custom Injection Program (user defined)**

No	Time	Flow	%B	Curve
1	-2.000	Equilibration		
2	-2.000	0.400	30.0	5
3	*New Row*			
4	0.000	Run		
5	0.000	0.400	30.0	5
6	0.100	0.400	30.0	5
7	1.100	0.400	50.0	5
8	15.100	0.400	100.0	5
9	17.600	0.400	100.0	5
10	17.800	0.400	30.0	5
11	22.100	0.400	30.0	5
12	*New Row*			
13	22.100	Stop Run		



Custom Injection Program- High Solvent Content

Vanquish Split SamplerCT

Temperature: 5 °C

Wash solvent: methanol/water (95/5; v/v)

Program: **Solvent sandwich injection- 3 x 25 µL sample**

General Settings | User Defined Program | Method Transfer | Temperature Control

(●) Replace normal injection

( ) Normal injection with liquid handling

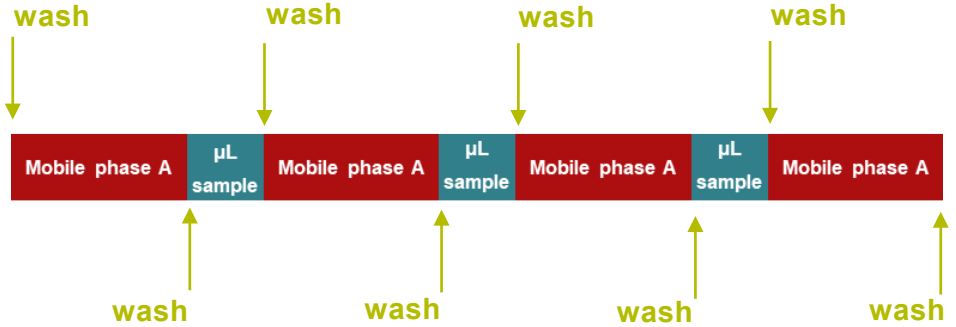
No	Command	Parameters
1	UDP_PrepareLiquidHandling	Volume=250 [µl]
2	UDP_NeedleWash	Duration=5 [s]
3	UDP_Draw	Position=SR:1, Volume=System.Injectio.CustomVariables.UDP_vol_01, Speed=10 [µl/s], NeedleHeight=2000 [µm]
4	UDP_NeedleWash	Duration=5 [s]
5	UDP_Draw	
6	UDP_NeedleWash	Duration=5 [s]
7	UDP_Draw	Position=SR:1, Volume=System.Injectio.CustomVariables.UDP_vol_02, Speed=10 [µl/s], NeedleHeight=2000 [µm]
8	UDP_NeedleWash	Duration=5 [s]
9	UDP_Draw	
10	UDP_NeedleWash	Duration=5 [s]
11	UDP_Draw	Position=SR:1, Volume=System.Injection.CustomVariables.UDP_vol_01, Speed=10 [µl/s], NeedleHeight=2000 [µm]
12	UDP_NeedleWash	Duration=5 [s]
13	UDP_Draw	
14	UDP_NeedleWash	Duration=5 [s]
15	UDP_Draw	Position=SR:1, Volume=System.Injection.CustomVariables.UDP_vol_02, Speed=10 [µl/s], NeedleHeight=2000 [µm]
16	UDP_NeedleWash	Duration=5 [s]
17	UDP_InNeedleMix	Volume=10 [µl], DrawSpeed=10 [µl/s], DispenseSpeed=10 [µl/s], Cycles=5
18	UDP_Wait	10 [s]
19	UDP_PrepareInject	

Injection wash procedure parameters

Wash mode: Both

Wash time: 10.0

Wash speed: 50.0



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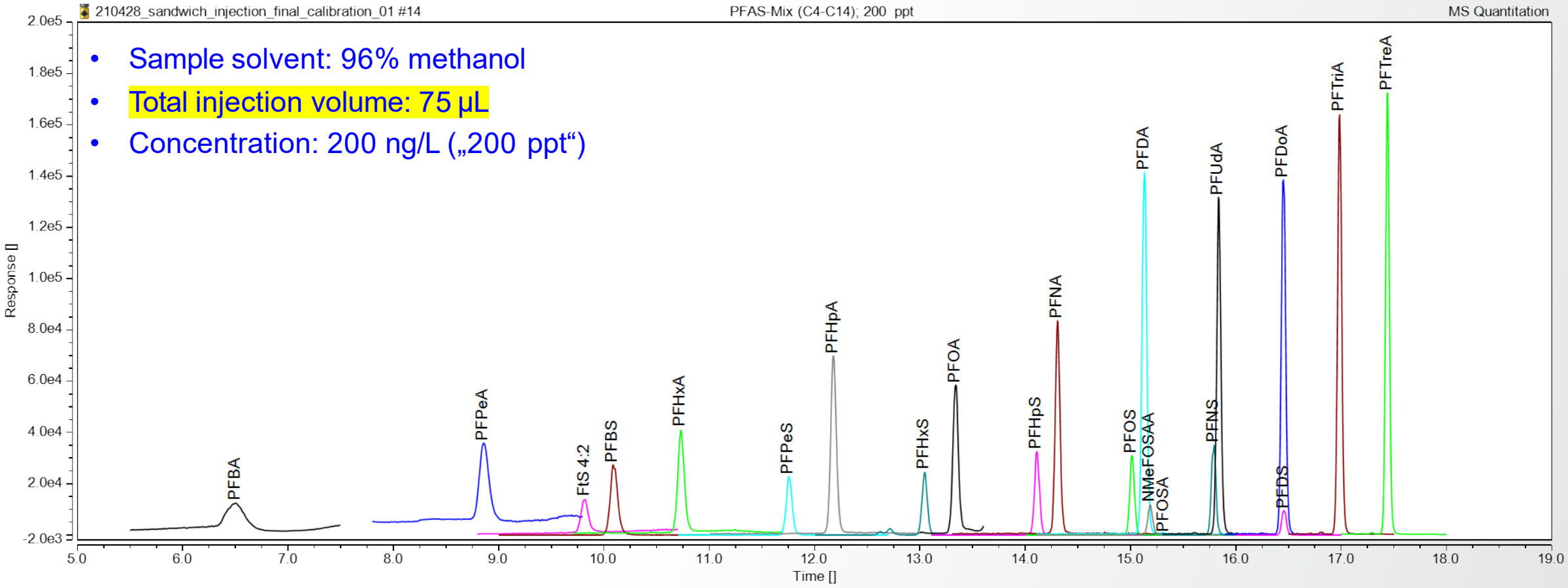
Chromatogram- Solvent Sandwich Injection

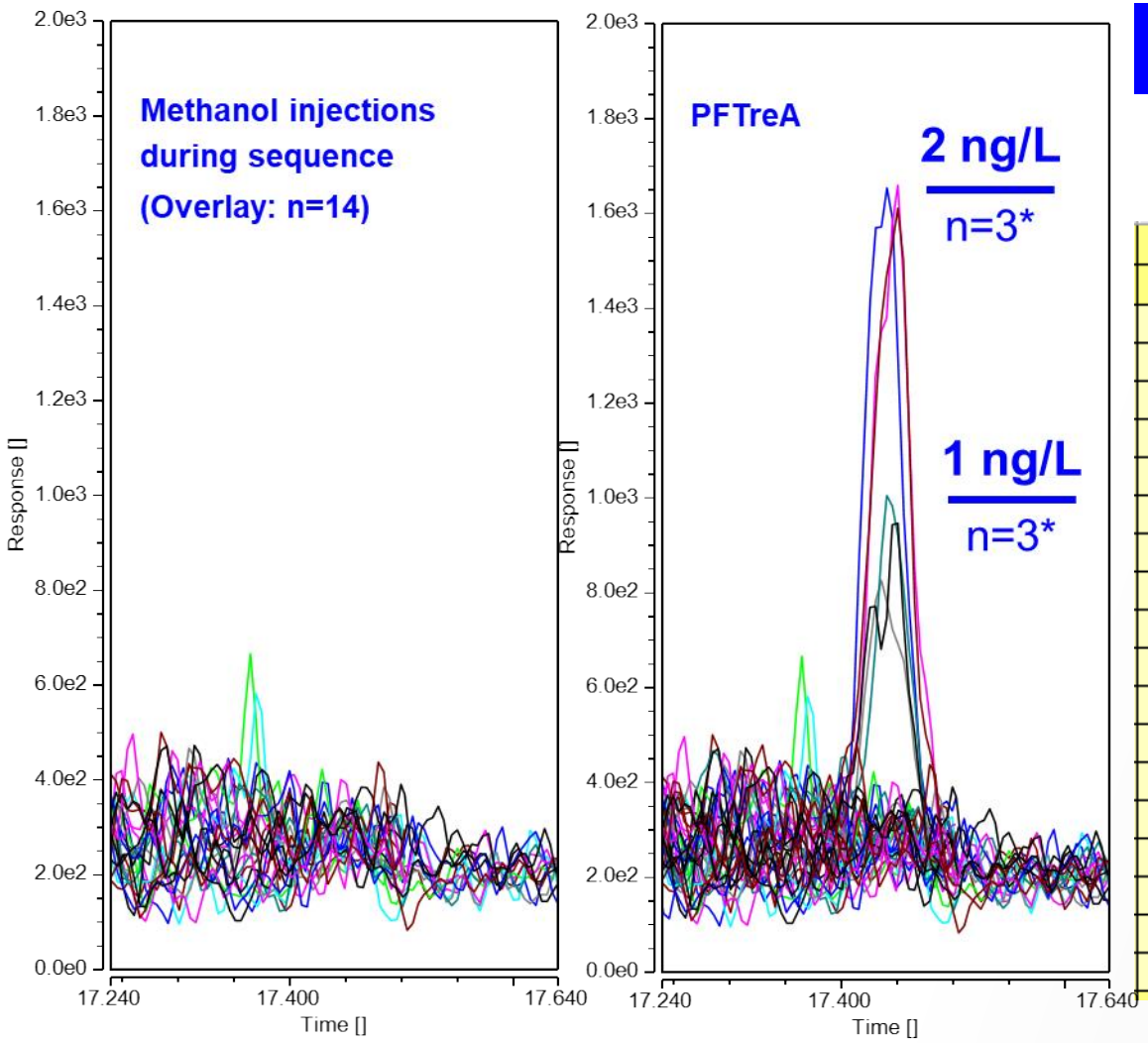
Acclaim™ Polar Advantage, 2.1 mm x 100 mm, 2.2 µm



Long-chain PFAS approach- Requires higher percent organic in sample

Calibration Curves- Sandwich Injection

PFTreA at low end of calibration range



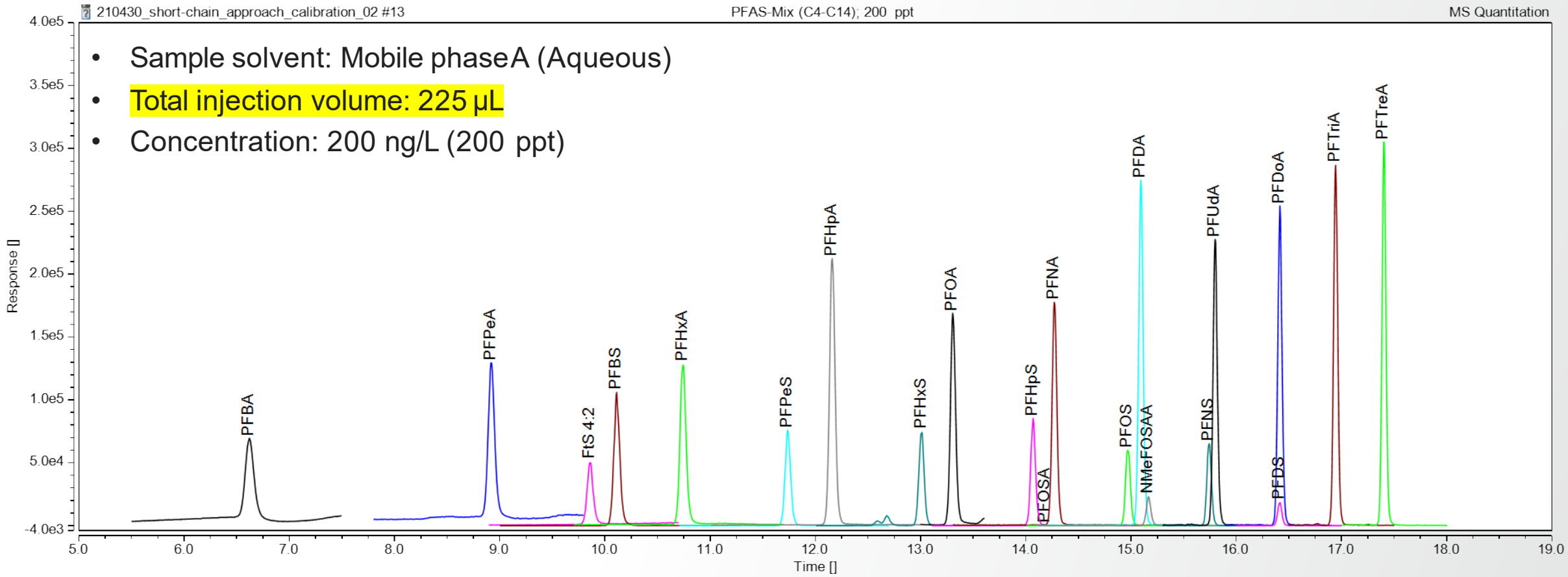
Long-chain approach

Injection volume: Total 75 µL (96% methanol)

Theo Amount	Amnt.Dev.	Cal Point	Mean - AMT	% RSD-AMT
ngL	%	Status		
1.20	2.28	OK		
1.20	14.26	OK		
1.20	13.47	OK	1.32	6.09
11.60	-14.56	OK		
11.60	-5.84	OK	10.42	6.87
116.00	-0.03	OK		
116.00	-4.10	OK	113.60	2.93
2.30	12.91	OK		
2.30	1.34	OK		
2.30	9.23	OK	2.48	5.48
23.20	-3.78	OK		
23.20	-5.28	OK	22.15	1.11
232.00	4.48	OK		
232.00	3.60	OK	241.37	0.60
5.80	-4.29	OK		
5.80	-6.90	OK	5.48	1.96
58.00	-9.20	OK		
58.00	-7.58	OK	53.13	1.26

Chromatogram- Short Chain Aqueous Injection

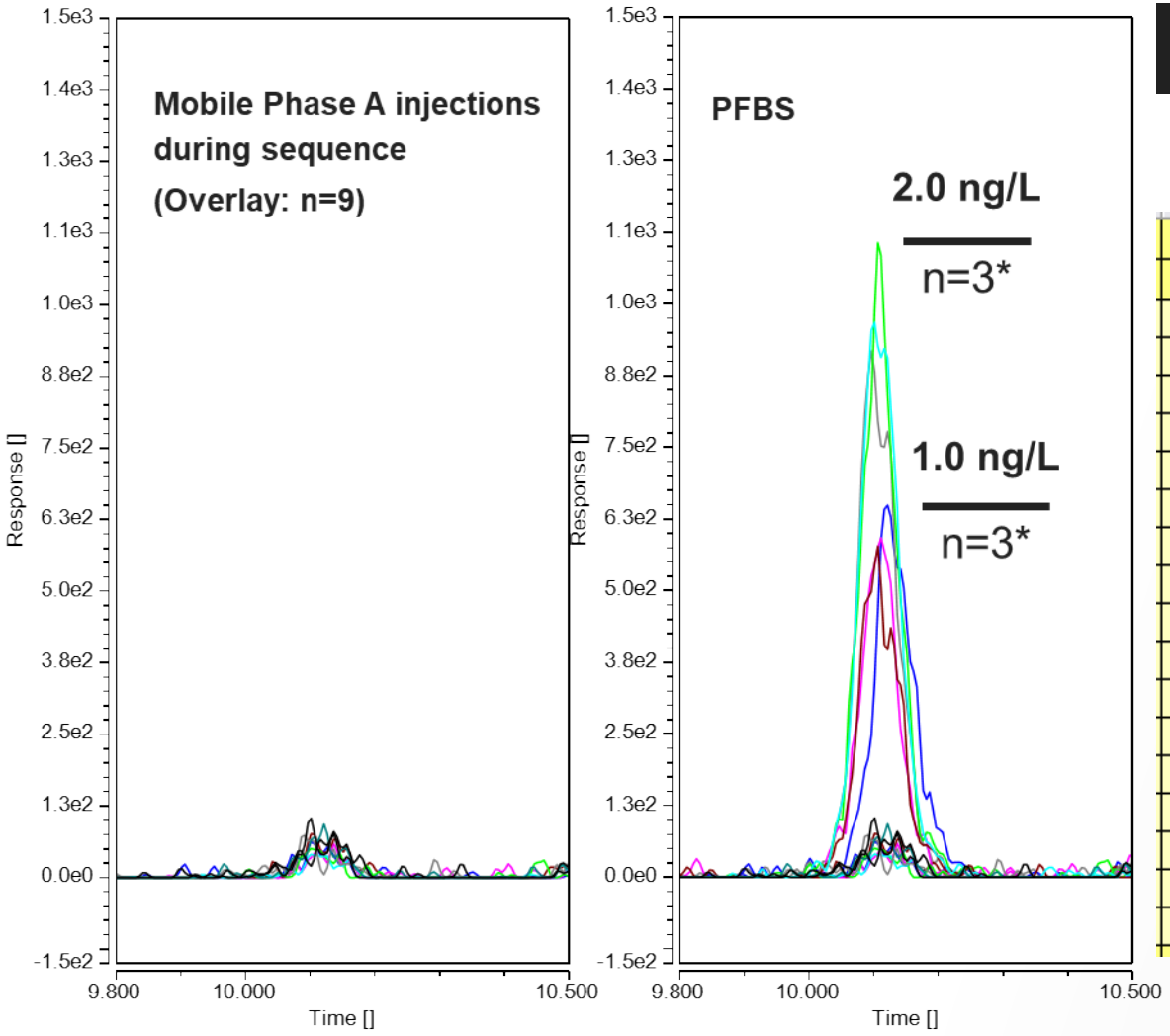
Acclaim™ Polar Advantage, 2.1 mm x 100 mm, 2.2 µm



Short-chain PFASapproach- Highly aqueous sample, longer chains compounds less soluble

Calibration Curves

PFBS at low end of calibration range



Short-chain approach

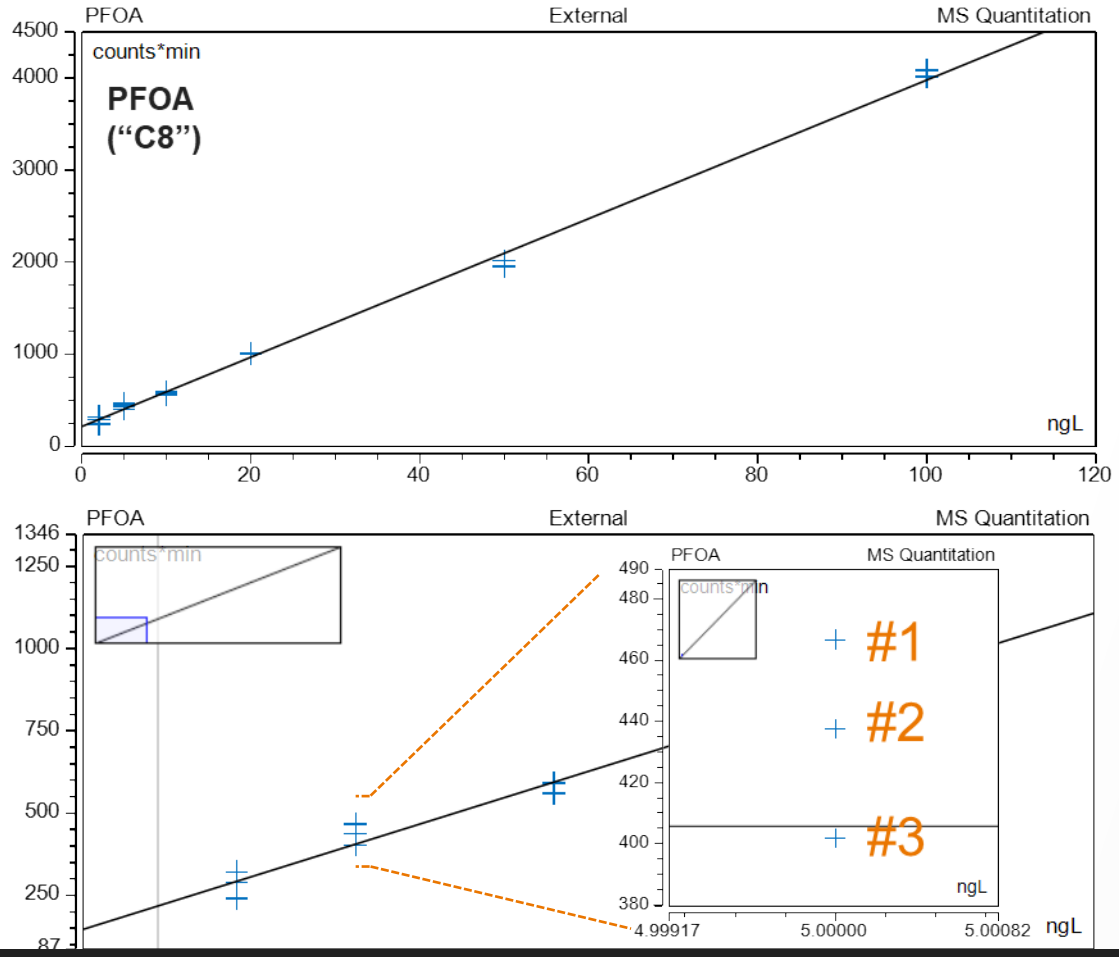
Injection volume: 225 µL (Mobile phase A sample composition)

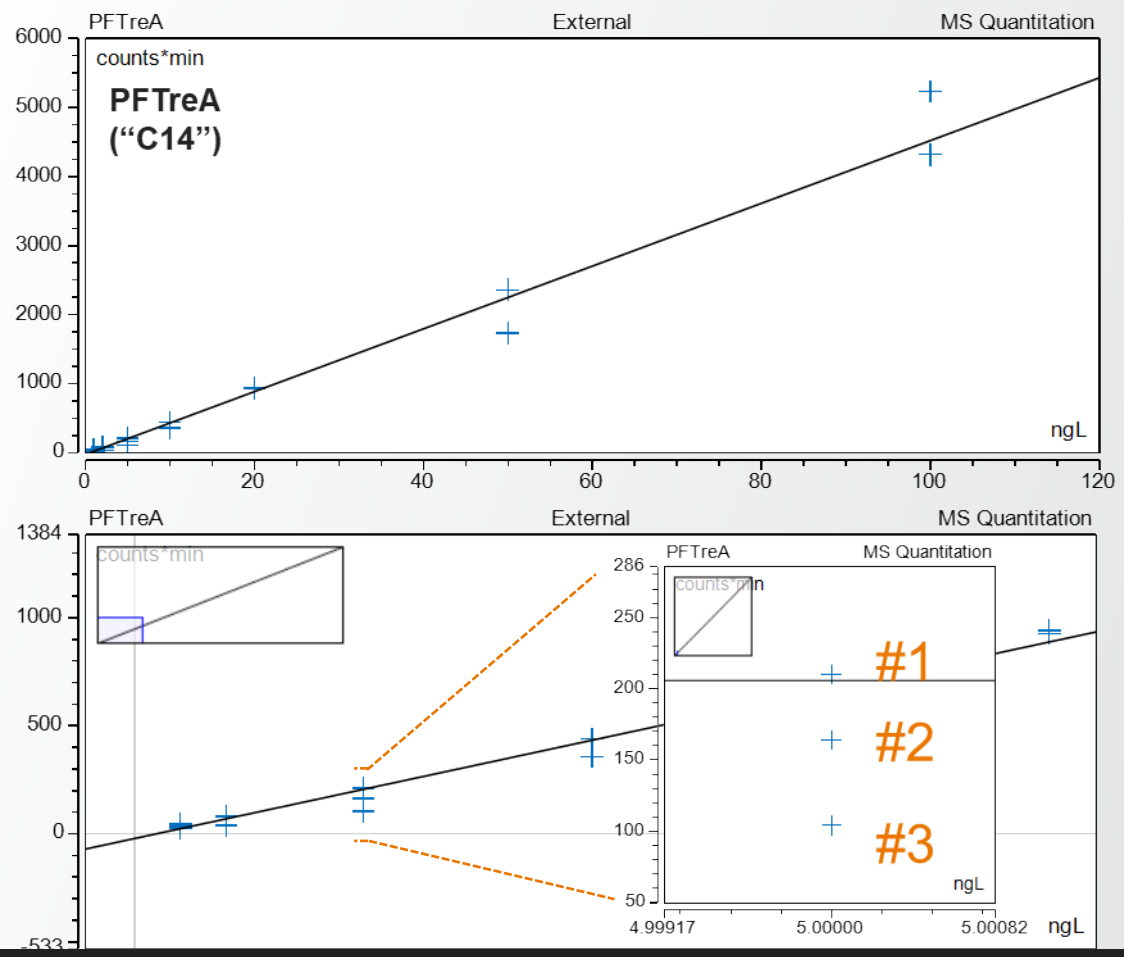
Theo Amount ngL	Amnt.Dev. %	Cal Point Status	Mean - AMT	% RSD-AMT
1.00	42.35	OK		
1.00	27.16	OK		
1.00	22.42	OK	1.31	7.97
10.00	-0.18	OK		
10.00	3.87	OK	10.18	2.81
100.00	2.02	OK		
100.00	-0.60	OK	100.71	1.84
2.00	1.43	OK		
2.00	10.41	OK		
2.00	10.06	OK	2.15	4.74
20.00	-6.99	OK		
20.00	-2.00	OK	19.10	3.70
5.00	6.20	OK		
5.00	6.86	OK		
5.00	6.41	OK	5.32	0.32
50.00	-0.68	OK		
50.00	-3.96	OK	48.84	2.38

* Triplicates were performed for all calibration levels. However, sequence interruption led to n=2 for calibration levels 10-100 ng/L

Limitation

Short-Chain Approach





Expected Vial adsorption of mid- and long-chain PFAS using mobile phase A.

Comparison of Short- and Long-Chain Approach

Standards Only. Limit of Quantitation.

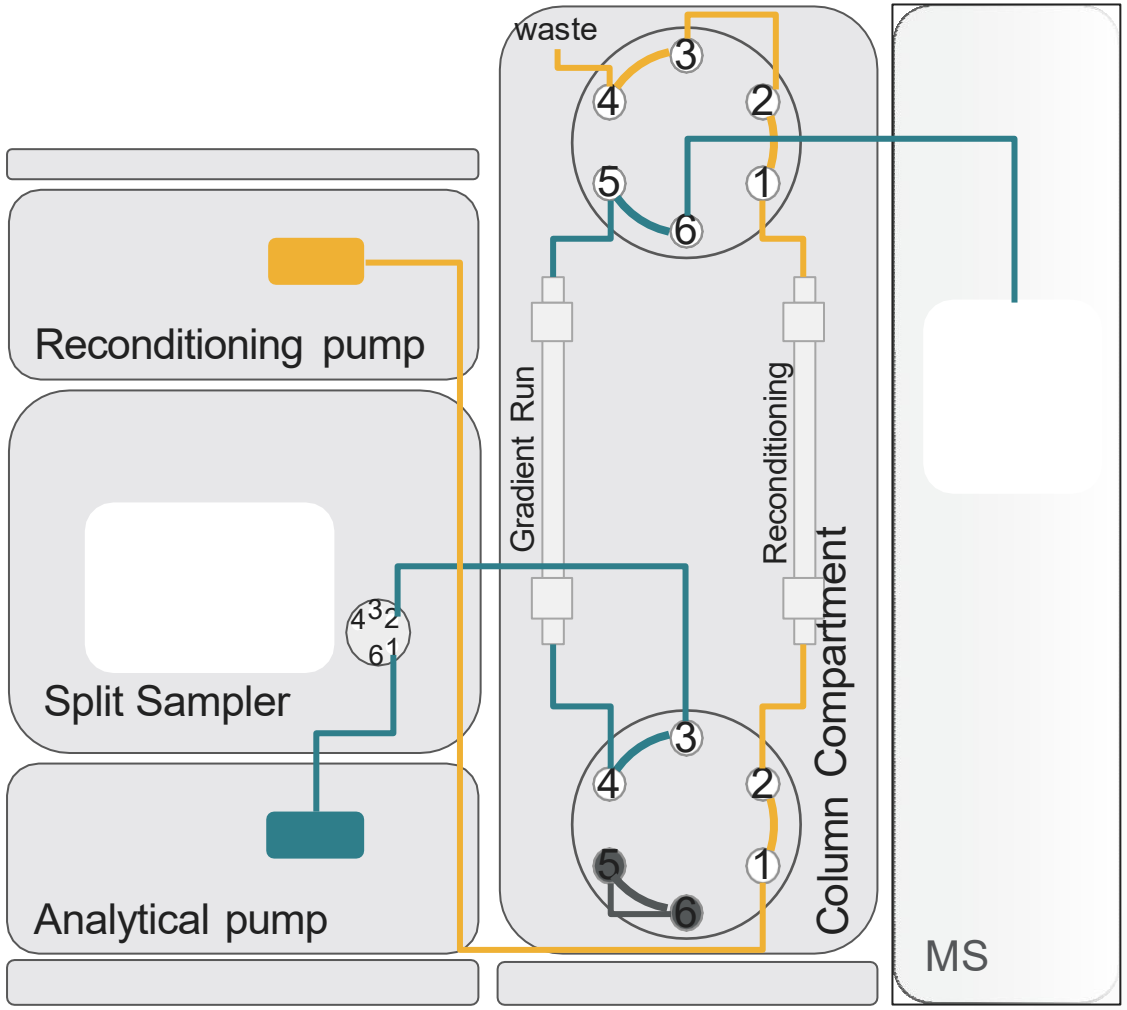
Compound name	Retention time [min]	Limit of quantitation [ng/L] (long-chain approach)	Limit of quantitation [ng/L] (short-chain approach)
PFBA Perfluorobutanoic acid	6.51	20	5
PFPeA Perfluoropentanoic acid	8.86	20	5
FtS 4:2	9.82	10	2
PFBS Perfluorobutanesulfonate	10.09	1	1
PFHxA Perfluorohexanoic acid	10.73	2	1
PFPeS Perfluoropentansulfonate	11.75	1	1
PFHpA Perfluoroheptanoic acid	12.18	2	1
PFHxS Perfluorohexanesulfonate	13.04	1	1
PFOA Perfluorooctanoic acid	13.34	5	2
PFHpS Perfluoroheptanesulfonate	14.11	2	1
PFNA Perfluorononanoic acid	14.31	2	2
PFOS Perfluorooctanesulfonate	15.01	1	2
NMeFOSAA	15.10	5	5
PFDA Perfluorodecanoic acid	15.13	1	2
PFNS Perfluorononanesulfonate	15.79	1	1 (out of range: Amnt.Dev. | RSD-AMT)
PFUdA Perfluoroundecanoic acid	15.83	2	1 (out of range: Amnt.Dev. | RSD-AMT)
PFDoA Perfluorododecanoic acid	16.45	1	1 (out of range: Amnt.Dev. | RSD-AMT)
PFDS Perfluorodecanesulfonate	16.46	2	1 (out of range: Amnt.Dev. | RSD-AMT)
PFTriA Perfluorotridecanoic acid	16.98	1	1 (out of range: Amnt.Dev. | RSD-AMT)
PFTreA Perfluorotetradecanoic acid	17.44	1	1 (out of range: Amnt.Dev. | RSD-AMT)

Mobile phase A
225 µL

96% methanol
75 µL

PFAS Decision Tree – Vanquish UHPLC Systems

Vanquish Duo UHPLC System for Tandem LC-MS

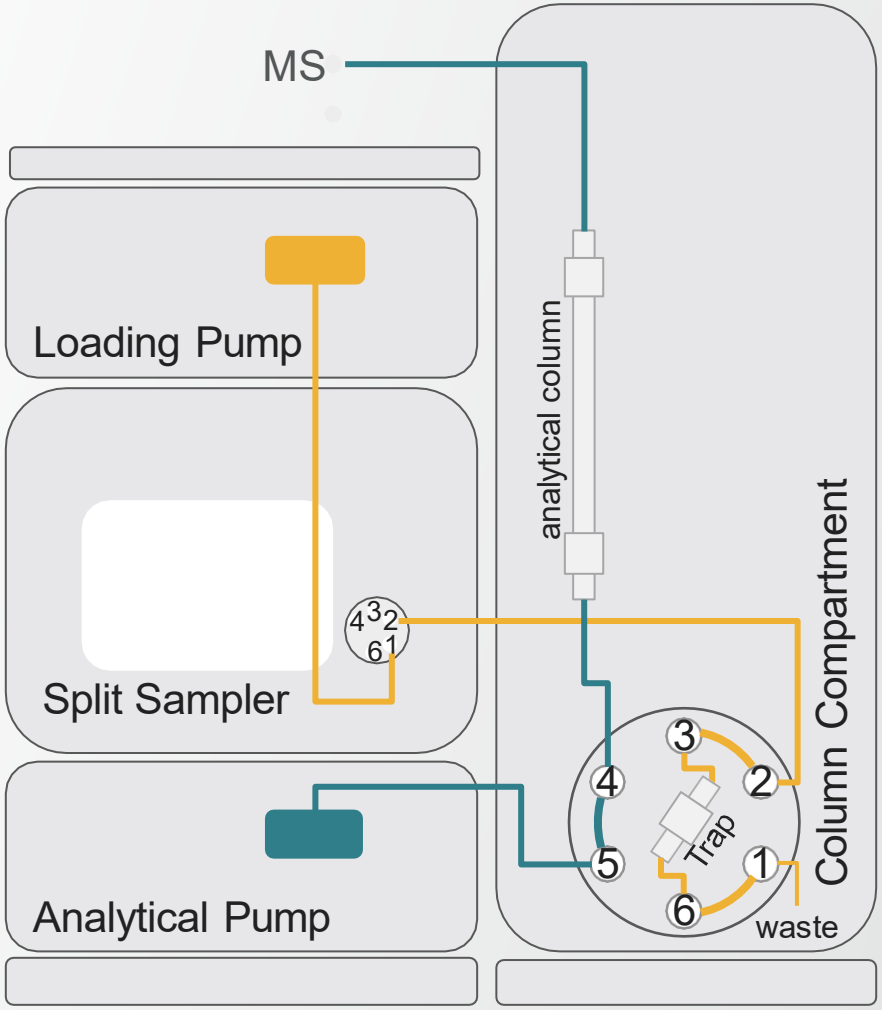


Minor changes in configuration

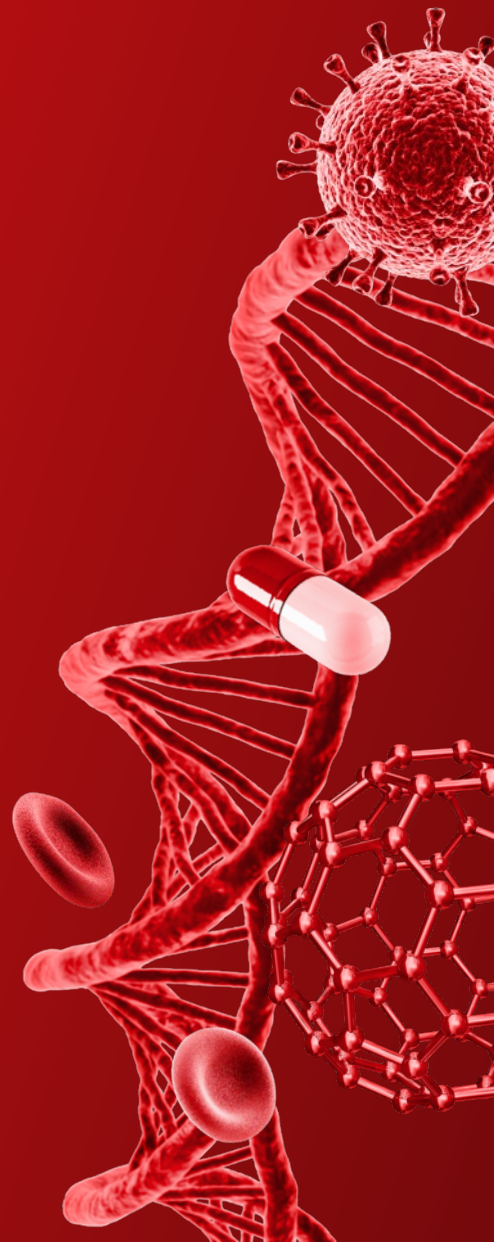
1. Subtract column #2
2. Subtract valve 2-p 6-p #2
3. Add trap column

Adapt capillaries

Vanquish Online SPE HPLC and UHPLC-Systems



Thank you



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