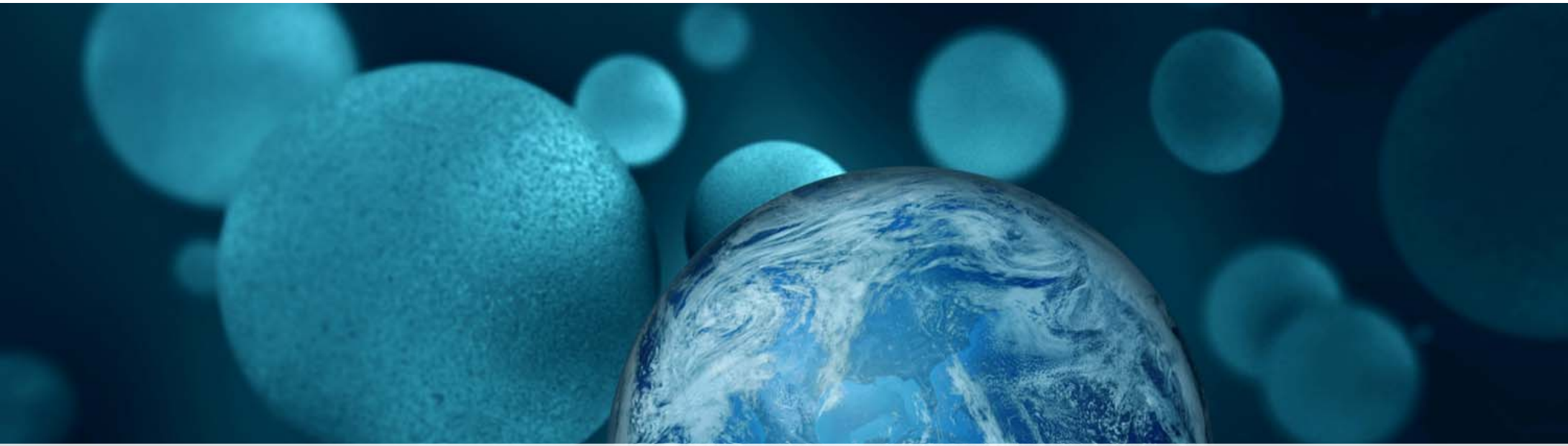

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EPA Method 533 Validation of PFAS Comparing Manual and Automated Offline SPE Followed by LC-MS/MS

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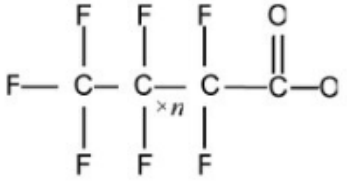
Thermo Fisher Scientific

Outline

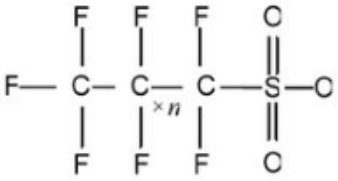
- PFAS: Introduction
- Interlaboratory study results for EPA Method 533
  - Manual SPE LC-MS/MS
  - Calibration ranges, LCMRL, precision and accuracy
- Automatic SPE
  - Automatic SPE LC-MS/MS
  - Calibration ranges, blanks and background, LCMRL, LOQ, precision and accuracy
- Conclusion

PFAS: Who Are They?

- PFAS: per- and poly-fluorinated alkyl substances
  - Hydrophobic perfluoroalkyl backbone and hydrophilic end group
  - > 4000 compounds, with a variety of chain lengths and end groups
  - PFOA (Perfluorooctanoic acid) and PFOS (Perfluorooctanesulfonic acid) most well-known

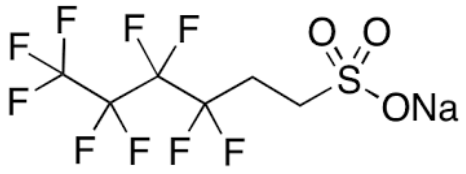


$CF_3(CF_2)_nCOO^-$



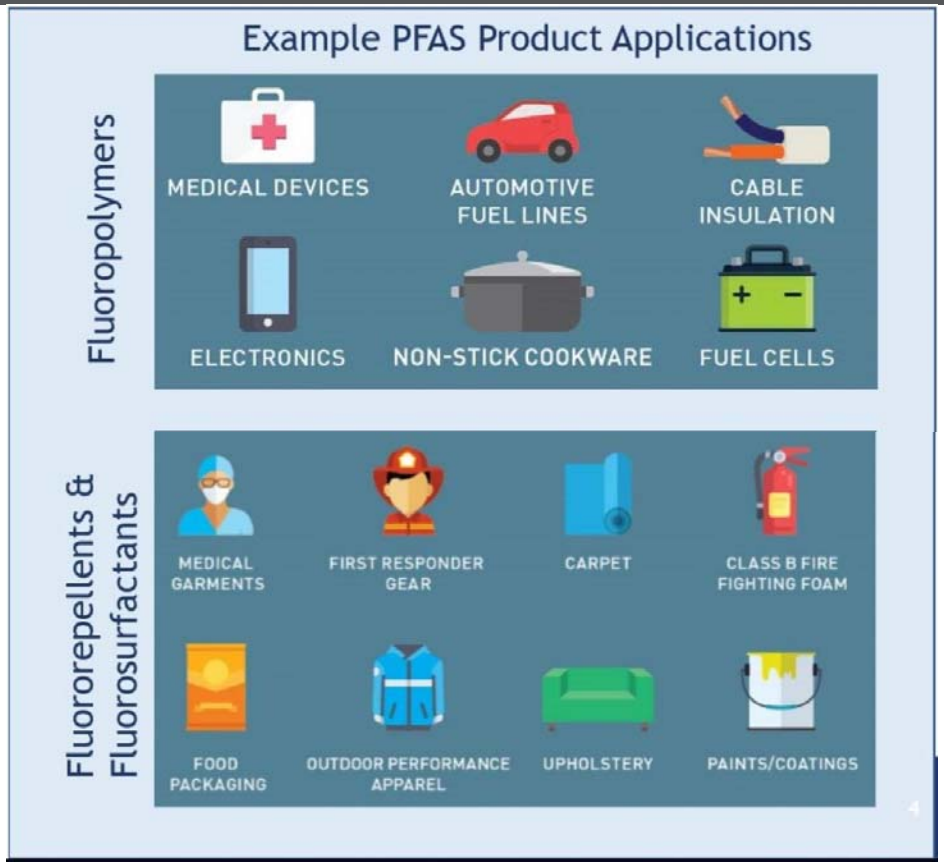
$CF_3(CF_2)_nSO_3^-$

  - Partial fluorinate-substituted alkyl compound: **4**:**2 FTS (4:2** Fluorotelomer sulfonic acid), 8:2 FTS **(8:2** Fluorotelomer sulfonic acid)



PFAS: What Do They Do?

- Thermal stability, chemical stability,
- Industrial polymers, stain repellents firefighting foams, waterproofing products, packaging etc.
- Persistence against biochemical attack and toxicity to mammalian species



Call for regulations on PFAS in environmental monitoring

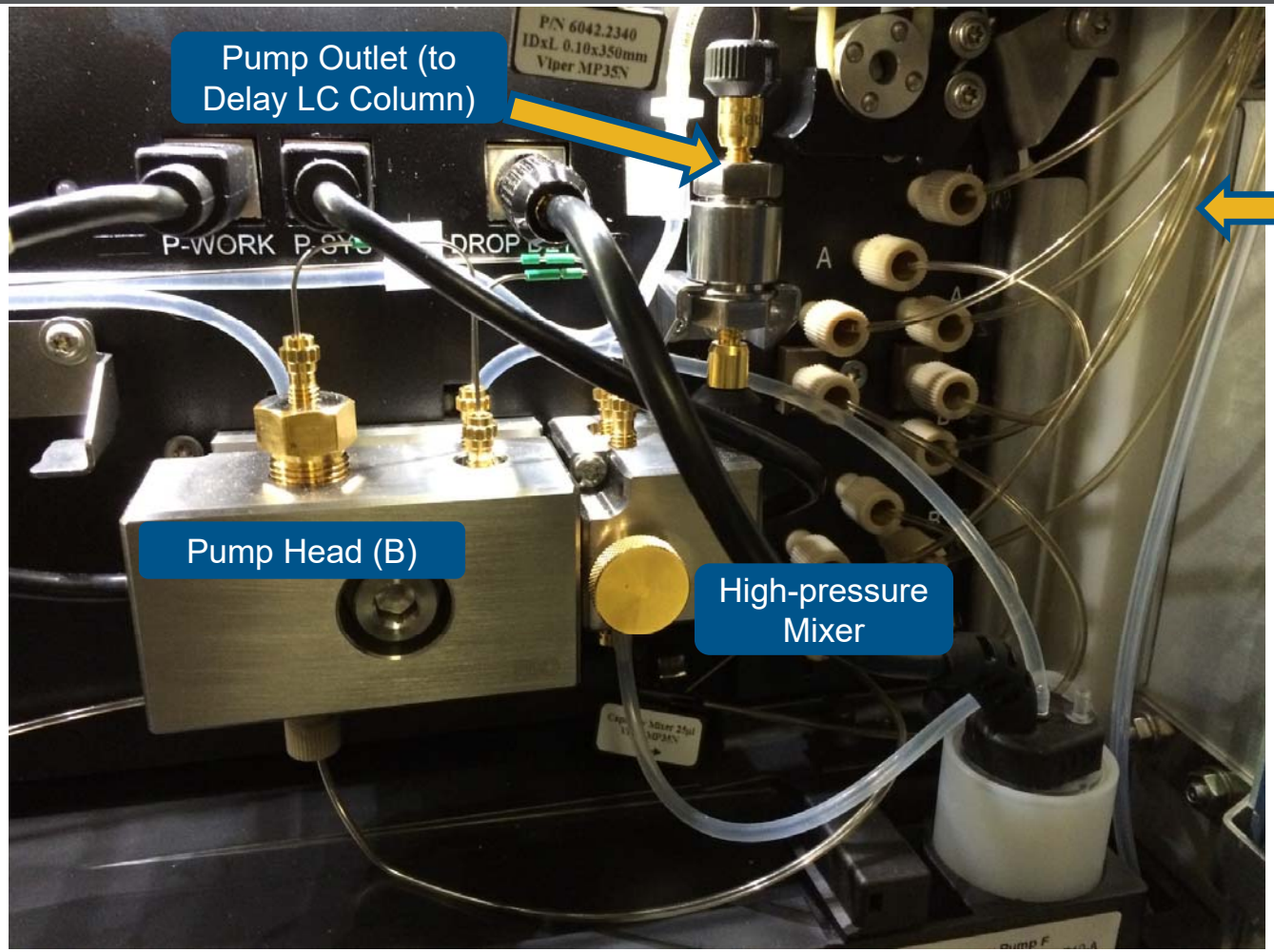
Overview PFAS Regulated Methods in Water

Regulated Method	EPA 537	EPA 537.1	EPA 533	ASTM D7979	EPA 8327
Application	Drinking water			non-drinking water	Ground/waste water
Publish year	2009	2018	2019	2015	2018
# analytes	14	18	25	24	24
Quantitation method	External standard	External standard	Isotope dilution	Isotope dilution	External standard
Test workflow	SPE-LC-MS/MS	SPE-LC-MS/MS	SPE-LC-MS/MS	LC-MS/MS	LC-MS/MS

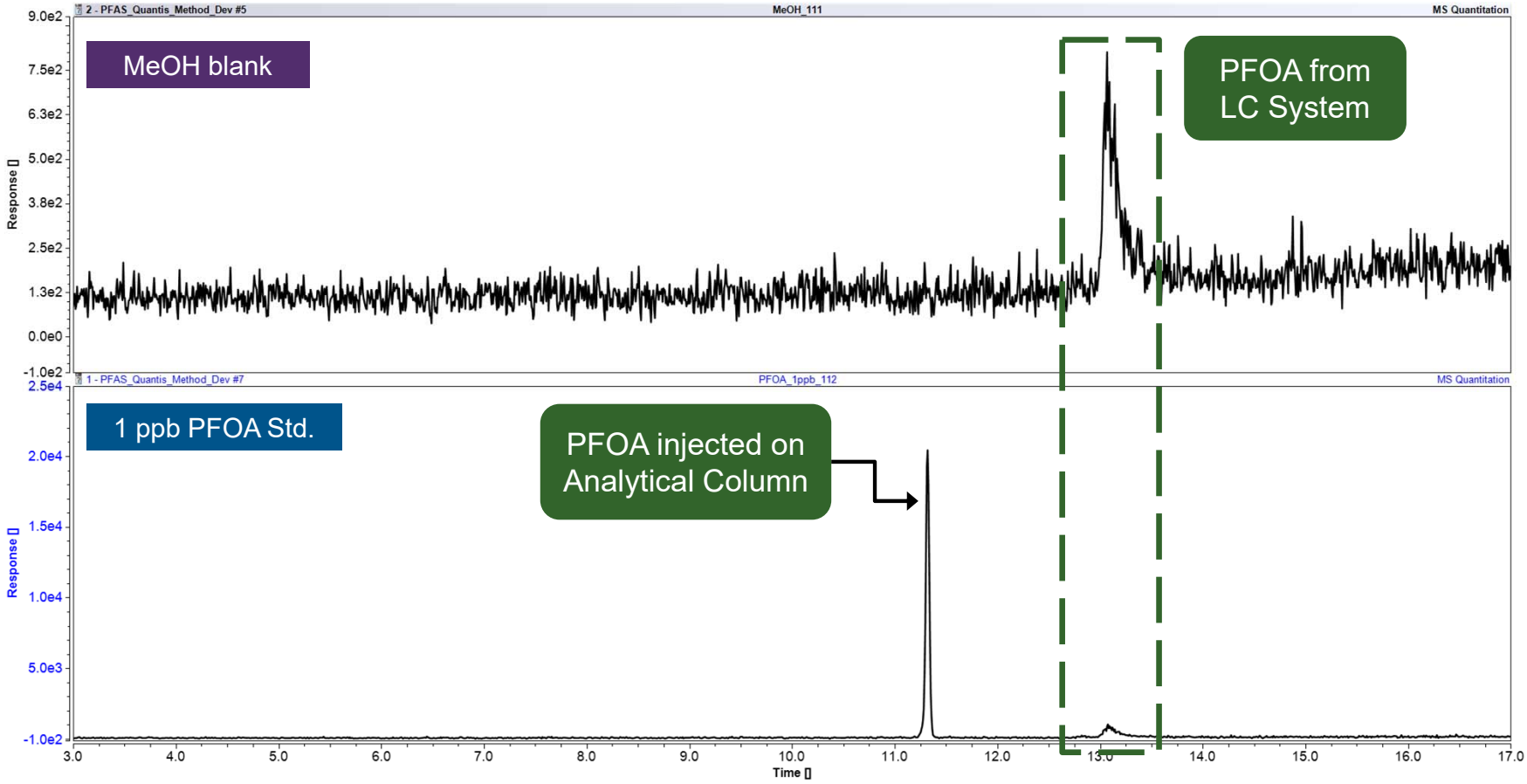
Analytes in Method 537.1 and 533

RP retention order	Name	Analyte	Method 537.1	Method 533
1	Perfluorobutanoic acid	PFBA		√
2	Perfluoro-3-methoxypropanoic acid	PFMPA		√
3	Perfluoropentanoic acid	PFPeA		√
4	Perfluorobutanesulfonic acid	PFBS	√	√
5	Perfluoro-4-methoxybutanoic acid	PFMBA		√
6	Perfluoro(2-ethoxyethane)sulfonic acid	PFEESA		√
7	Nonafluoro-3,6-dioxaheptanoic acid	NFDHA		√
8	1H,1H, 2H, 2H-Perfluorohexane sulfonic acid	4:2FTS		√
9	Perfluorohexanoic acid	PFHxA	√	√
10	Perfluoropentanesulfonic acid	PFPeS		√
11	Hexafluoropropylene oxide dimer acid	HPFO-DA	√	√
12	Perfluoroheptanoic acid	PFHpA	√	√
13	Perfluorohexanesulfonic acid	PFHxS	√	√
14	4,8-Dioxa-3H-perfluorononanoic acid	ADONA	√	√
15	Perfluorooctanoic acid	PFOA	√	√
16	1H,1H, 2H, 2H-Perfluorooctane sulfonic acid	6:2FTS		√
17	Perfluoroheptanesulfonic acid	PFHpS		√
18	Perfluorononanoic acid	PFNA	√	√
19	Perfluorooctanesulfonic acid	PFOS	√	√
20	9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	9Cl-PF3ONS	√	√
21	Perfluorodecanoic acid	PFDA	√	√
22	1H,1H, 2H, 2H-Perfluorodecane sulfonic acid	8:2FTS		√
23	N-methyl perfluorooctanesulfonamidoacetic acid	NMeFOSAA	√	
24	Perfluoroundecanoic acid	PFUnA	√	√
25	N-ethyl perfluorooctanesulfonamidoacetic acid	NEtFOSAA	√	
26	11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	11Cl-PF3OUdS	√	√
27	Perfluorododecanoic acid	PFDoA	√	√
28	Perfluorotridecanoic acid	PFTrDA	√	
29	Perfluorotetradecanoic acid	PFTA	√	

Experimental Design – Liquid Chromatography: PFAS Kit retrofit for Vanquish Flex



Using “Delay” Column - PFOA



Later eluting peak is artefact from LC system, which is held-up by “Delay” column before Autosampler

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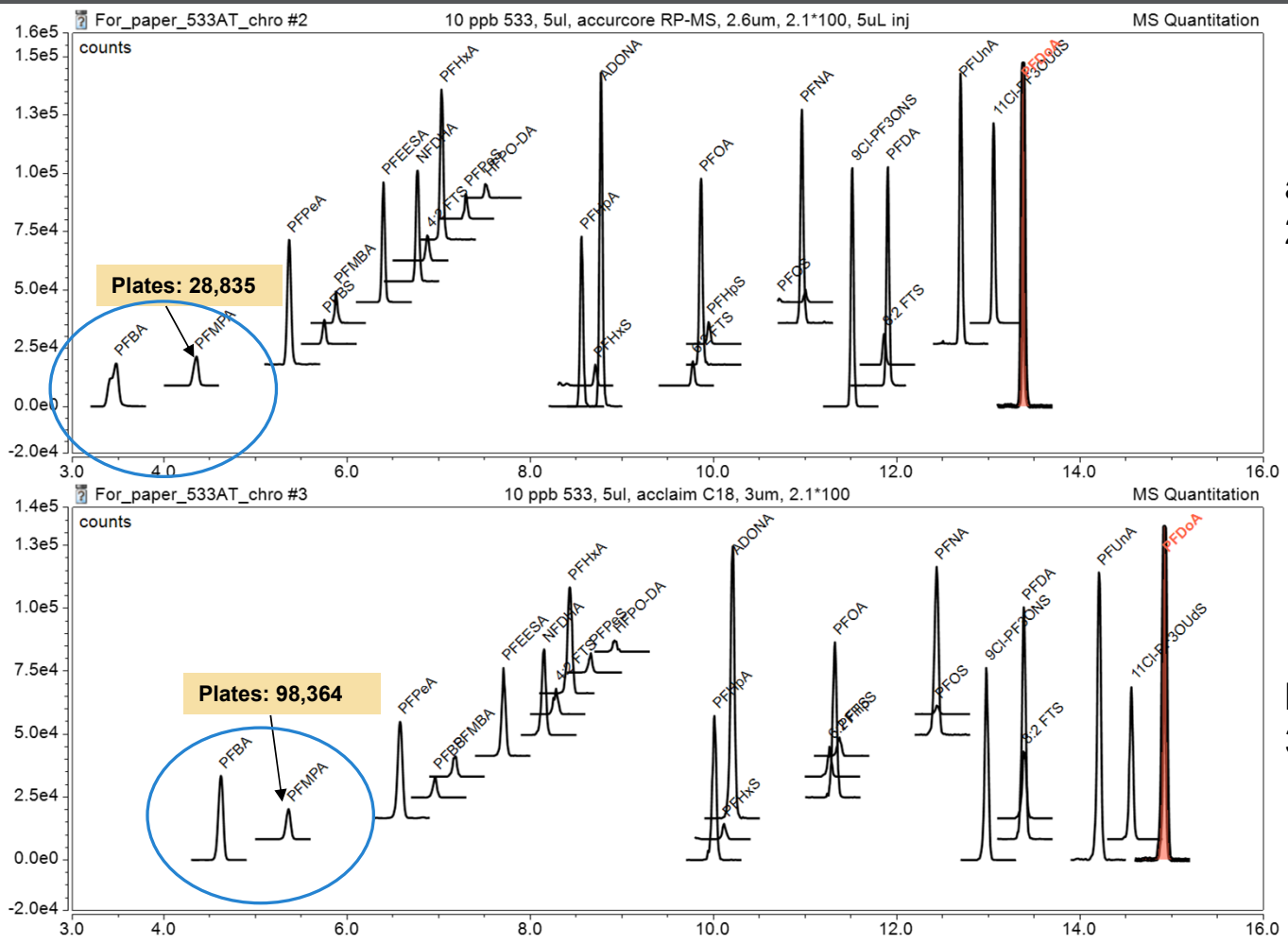
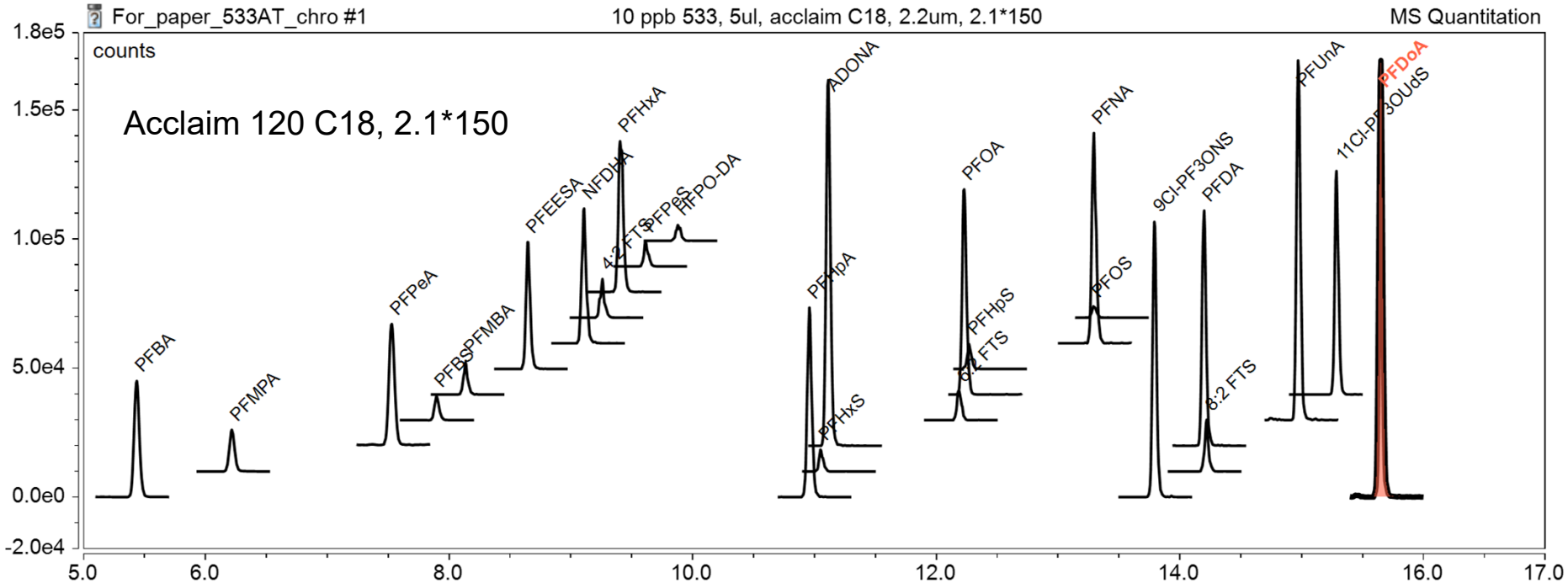
Separation of Twenty-five PFASs in 80% Methanol/Water Solvent



a. Solid core RP C18, 2.6 µm, 2.1*100mm

b. Full porous RP C18, 3 µm, 2.1*100mm

Chromatogram with Acclaim 120 C18, 2.1*150 mm Analytical Column



Asymmetries ranging from 1-1.28) and full width at half maximum (FWHM) at 1.6 - 3 seconds)

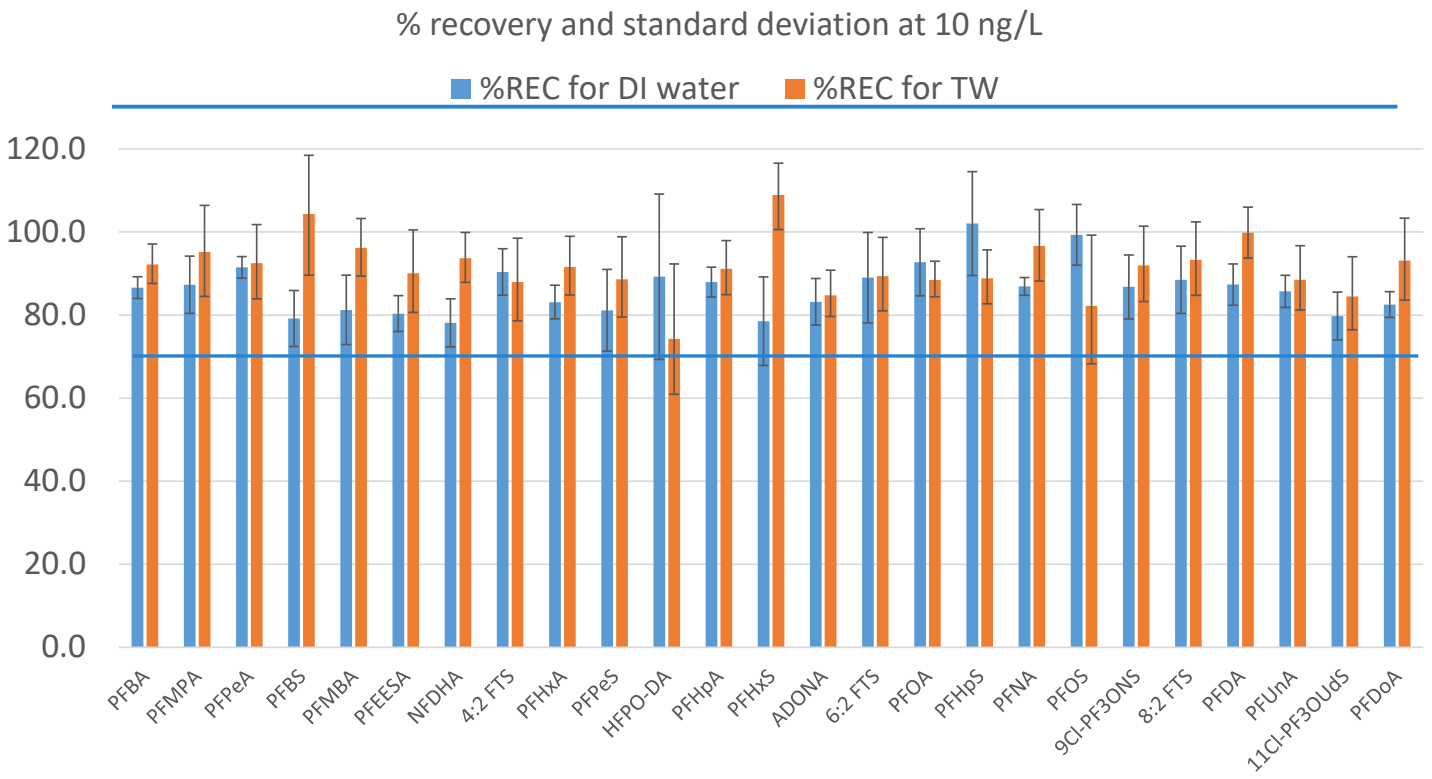
Procedure and Device for Interlaboratory Study

250 mL Sample with 250 mg Ammonium Acetate → Add Isotope Dilution Analogs → Extract Using WAX SPE → Evaporate to Dryness, Reconstitute in 80/20 MeOH/H_2O → Add Isotope Performance Standards → Analyze By LC/MS/MS

LCMRL

Analytes (ng/L)	EPA 533 reported	LCMRL (manual)
PFBA	13	1.6
PFMPA	3.8	3.1
PFPeA	3.9	1.9
PFBS	3.5	5.5
PFMBA	3.7	3.3
PFEESA	2.6	1.8
NFDHA	16	1.2
4:2 FTS	4.7	2
PFHxA	5.3	0.97
PFPeS	6.3	3.4
HFPO-DA	3.7	10
PFHpA	2.6	1.3
PFHxS	3.7	4.6
ADONA	3.4	1.0
6:2 FTS	14	4.6
PFOA	3.4	7.6
PFHpS	5.1	4.8
PFNA	4.8	1.8
PFOS	4.4	4.8
9Cl-PF3ONS	1.4	0.73
8:2 FTS	9.1	3.7
PFDA	2.3	1.0
PFUnA	2.7	0.88
11Cl-PF3OUdS	1.6	7.9
PFDoA	2.2	0.96

Precision and Accuracy @ 10 ng/L



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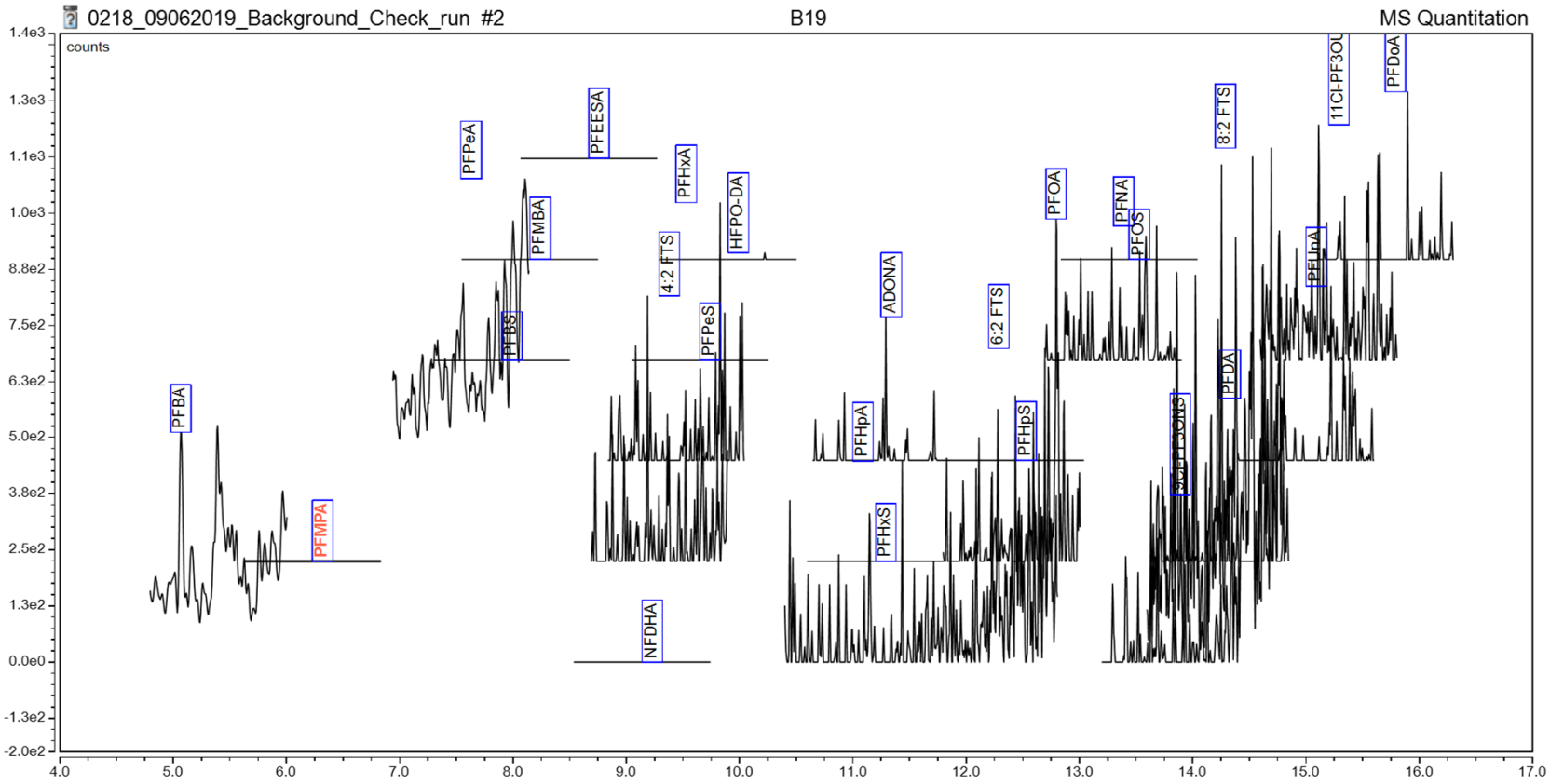
Summary for Interlaboratory Study

- Separation : peak asymmetry within 1-1.3
- Valid LCMRL obtained
- Precision and Accuracy meet EPA requirement with D.I. water and tapping water
- All tested results fall in EPA criteria

EPA 533 Procedure with Auto SPE device

250 mL Sample with 250 mg Ammonium Acetate → Add Isotope Dilution Analogs → Extract Using WAX SPE → Evaporate to Dryness, Reconstitute in 80/20 MeOH/H_2O → Add Isotope Performance Standards → Analyze By LC/MS/MS

Blank Test



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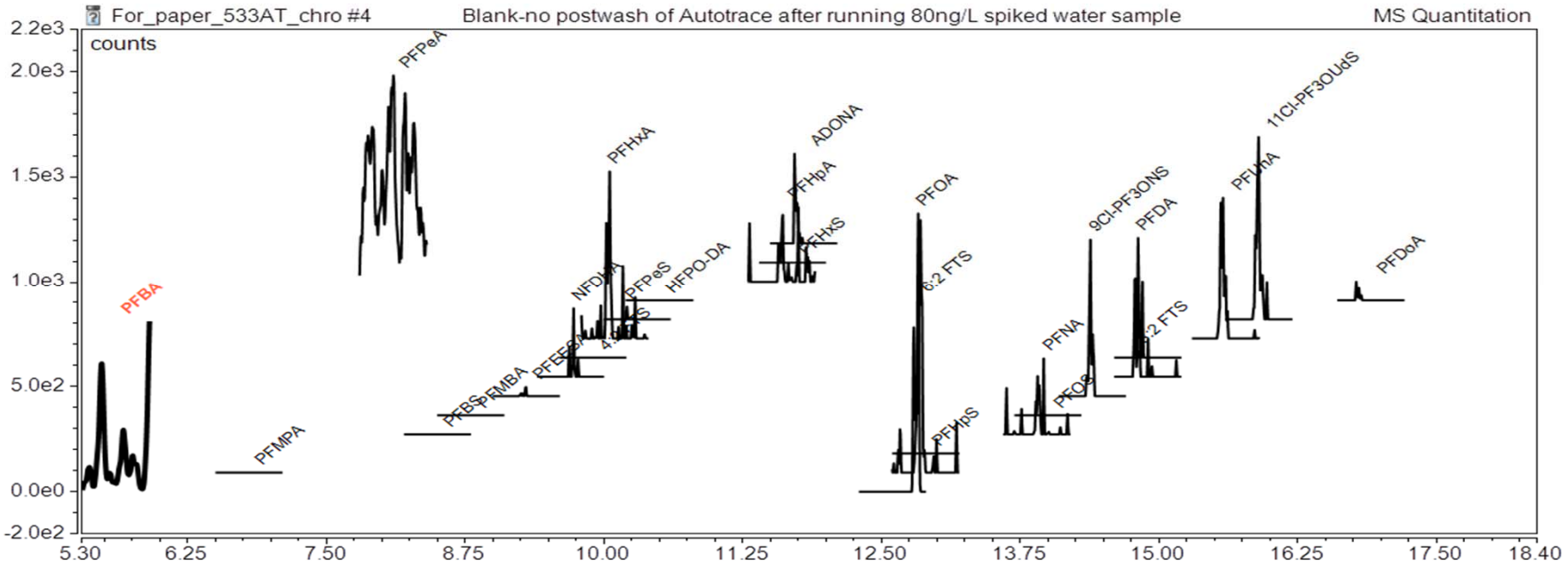
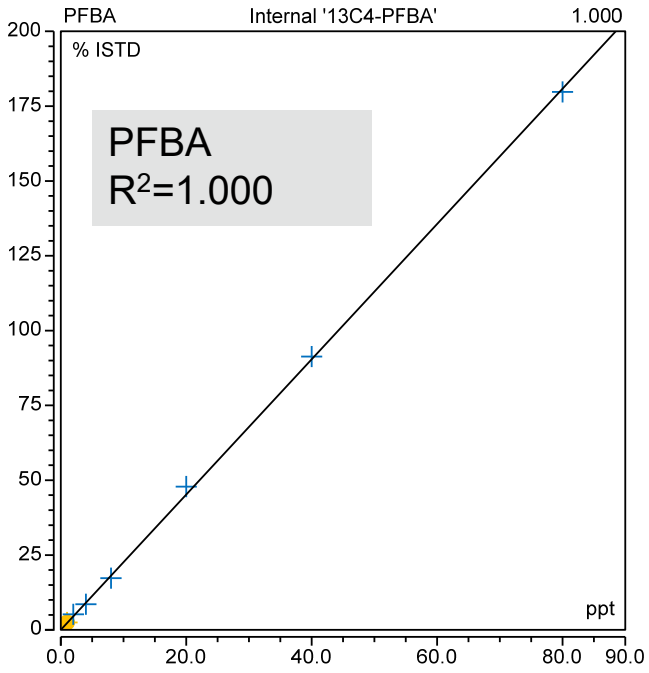
Carry over Test

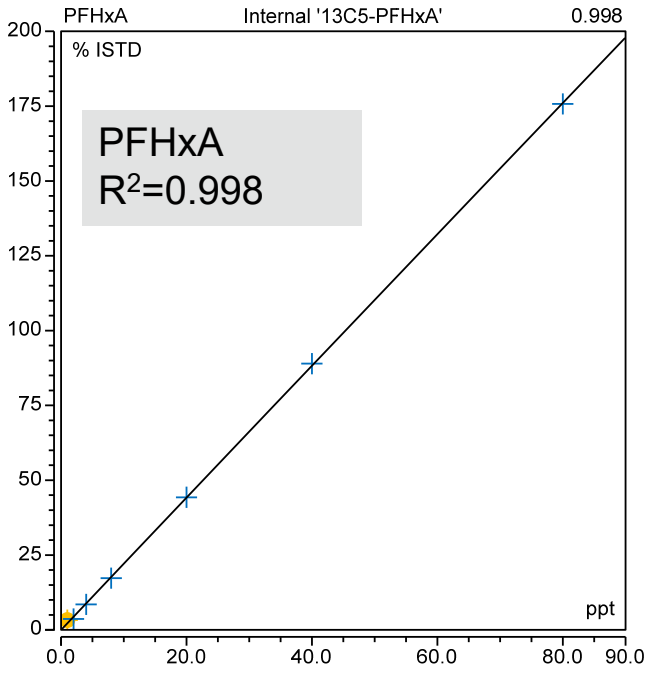
Procedure: Run 80 ng/L PFAS spiked water, followed by reagent water without any clean up

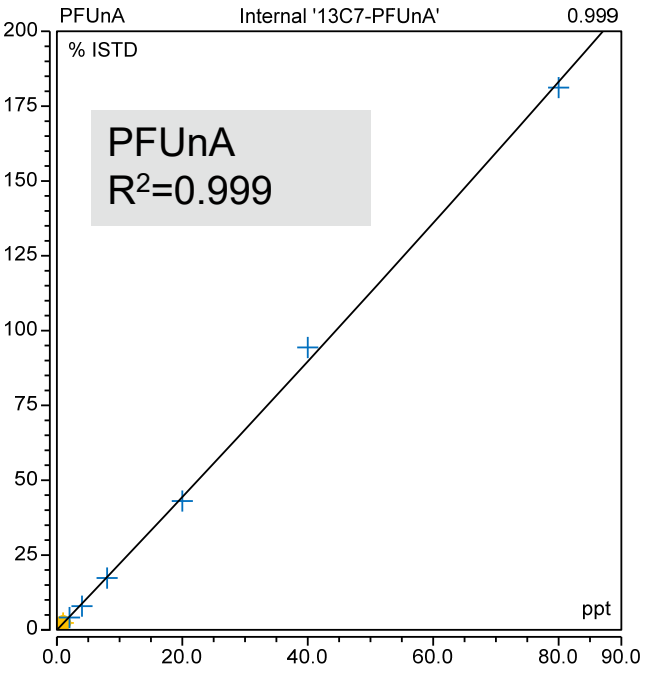


No significant carry over was observed. All the tested PFAS are lower than 1/3 of LCMRL

Calibration Curves







Great calibration curve and coefficient obtained for early, middle and later eluting analytes

Calibration Summary

Analytes	LOQ ($ng \cdot L^{-1}$)	Concentration Range ($ng \cdot L^{-1}$)	R^2	Curve
PFBA	0.25	0.25-50	1.000	Linear
PFMPA	0.25	0.25-50	0.994	Qudratic
PFPeA	0.5	0.5-50	1.000	Qudratic
PFBS	0.25	0.25-20	0.998	Qudratic
PFMBA	0.5	0.5-50	1.000	Linear
PFEESA	0.25	0.25-50	0.997	Linear
NFDHA	0.2	0.5-50	0.996	Qudratic
4:2 FTS	0.25	0.25-20	0.998	Linear
PFHxA	0.25	0.25-50	0.998	Qudratic
PFPeS	0.25	0.25-20	0.999	Qudratic
HFPO-DA	0.25	0.25-20	0.999	Qudratic
PFHpA	0.25	0.25-50	0.998	Linear
PFHxS	0.5	0.5-50	0.994	Linear
ADONA	0.25	0.25-50	0.999	Linear
6:2 FTS	0.25	0.25-20	1.000	Qudratic
PFOA	0.25	0.25-50	0.998	Linear
PFHpS	0.5	0.5-20	0.992	Qudratic
PFNA	0.25	0.25-50	0.998	Qudratic
PFOS	0.25	0.25-20	0.999	Qudratic
9Cl-PF3ONS	0.25	0.25-20	0.997	Qudratic
8:2 FTS	0.25	0.25-50	0.999	Linear
PFDA	0.25	0.25-50	0.994	Linear
PFUnA	0.25	0.25-50	0.999	Linear
11Cl-PF3OUdS	0.25	0.25-20	0.996	Qudratic
PFDoA	0.25	0.25-50	0.995	Qudratic

LCMRL (AT 280, Sol EX WAX (6CC, 500mg))

Analytes	Fortification Concentration levels (ng/L)	LCMRL (ng/L)	MDL (ng/L)
PFBA	1.0, 2.0,4.0, 6.0, 10, 14, 20	8.6	1.4
PFMPA	1.0, 2.0,4.0, 6.0, 10, 14, 20	4.5	0.71
PFPeA	1.0, 2.0,4.0, 6.0, 10, 14, 20	3.9	1.7
PFBS	1.0, 2.0,4.0, 6.0, 10, 14, 20	3.2	1.3
PFMBA	1.0, 2.0,4.0, 6.0, 10, 14, 20	3.9	0.82
PFEESA	1.0, 2.0,4.0, 6.0, 10, 14, 20	3.4	0.78
NFDHA	1.0, 2.0,4.0, 6.0, 10, 14, 20	5.7	0.75
4:2 FTS	1.0, 2.0,4.0, 6.0, 10, 14, 20	7.0	1.5
PFHxA	1.0, 2.0,4.0, 6.0, 10, 14, 20	3.2	0.78
PFPeS	1.0, 2.0,4.0, 6.0, 10, 14, 20	2.6	1.1
HFPO-DA	1.0, 2.0,4.0, 6.0, 10, 14, 20	7.4	1.8
PFHpA	1.0, 2.0,4.0, 6.0, 10, 14, 20	3.4	0.73
PFHxS	1.0, 2.0,4.0, 6.0, 10, 14, 20	6.5	3.3
ADONA	1.0, 2.0,4.0, 6.0, 10, 14, 20	1.6	0.35
6:2 FTS	1.0, 2.0,4.0, 6.0, 10, 14, 20, 40	5.7	5.7
PFOA	1.0, 2.0,4.0, 6.0, 10, 14, 20	3.9	0.8
PFHpS	1.0, 2.0,4.0, 6.0, 10, 14, 20, 40	5.8	1.8
PFNA	1.0, 2.0,4.0, 6.0, 10, 14, 20	2.8	0.34
PFOS	1.0, 2.0,4.0, 6.0, 10, 14, 20	5.2	1.7
9Cl-PF3ONS	1.0, 2.0,4.0, 6.0, 10, 14, 20	2.9	0.62
8:2 FTS	1.0, 2.0,4.0, 6.0, 10, 14, 20, 40	9.5	2.0
PFDA	1.0, 2.0,4.0, 6.0, 10, 14, 20	2.1	0.52
PFUnA	1.0, 2.0,4.0, 6.0, 10, 14, 20	4.1	0.59
11Cl-PF3OUdS	1.0, 2.0,4.0, 6.0, 10, 14, 20	2.4	0.96
PFDoA	1.0, 2.0,4.0, 6.0, 10, 14, 20,40	4.8	1.3

Precision and Accuracy in Reagent Water

		Spiking concentration: 10 ng/L		Spiking concentration: 80 ng/L	
		% Recovery	%RSD	% Recovery	%RSD
1	PFBA	119	4.5%	94.4	4.1%
2	PFMPA	106	9.2%	97.8	4.7%
3	PFPeA	100	5.8%	95.4	8.0%
4	PFBS	85.9	14%	96.2	9.5%
5	PFMBA	103	11%	96.3	5.1%
6	PFEESA	109	3.8%	91.9	4.5%
7	NFDHA	118	11%	104	2.3%
8	4:2 FTS	108	14%	95.8	2.4%
9	PFHxA	110	6.0%	96.0	4.2%
10	PFPeS	97.6	5.0%	109	7.2%
11	HFPO-DA	102	6.0%	100	14%
12	PFHpA	100	6.7%	90.5	6.2%
13	PFHxS	111	16%	96.5	8.4%
14	ADONA	104	1.5%	91.9	5.1%
15	6:2 FTS	99.0	3.0%	93.8	6.0%
16	PFOA	123	9.9%	99.9	4.0%
17	PFHpS	108	5.0%	105	10%
18	PFNA	105	3.2%	95.5	3.8%
19	PFOS	93.4	8.1%	101	8.4%
20	9Cl-PF3ONS	107	10%	94.3	6.5%
21	8:2 FTS	116	5.0%	107	4.4%
22	PFDA	106	4.6%	95.9	1.8%
23	PFUnA	107	9.2%	95.6	1.2%
24	11Cl-PF3OUdS	95.1	6.8%	78.9	9.9%
25	PFDoA	97.9	6.9%	91.4	2.4%

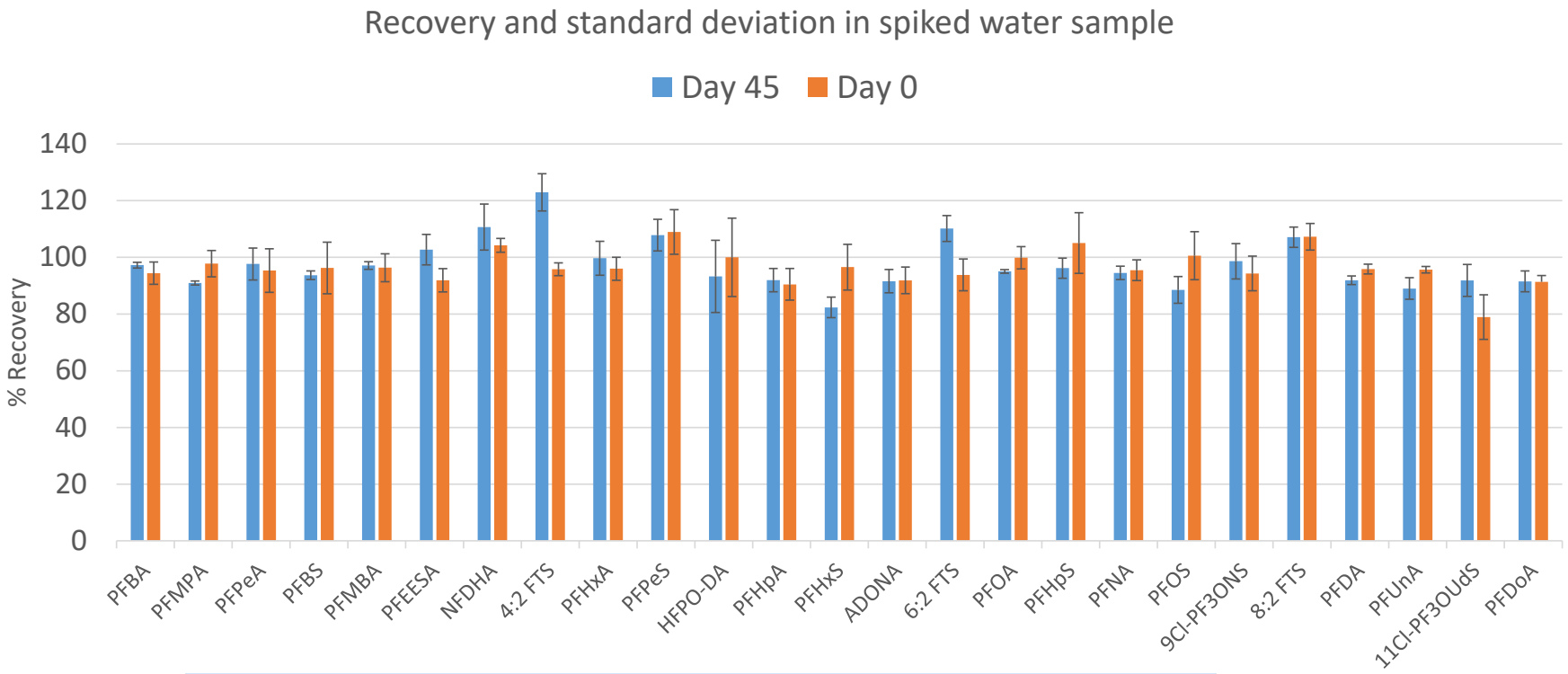
Precision and Accuracy in Reagent water: Isotope Dilution Analogue Recovery Data

	Fortification (ng/L)	% Recovery (10 ng/L)	%RSD (10 ng/L)	% Recovery (80 ng/L)	%RSD (80 ng/L)
13C4-PFBA	40	105	6.1	106	6.7
13C5-PFPeA	40	102	4.8	105	6.0
13C3-PFBS	40	106	2.6	117	3.9
13C2 4:2 FTS	160	110	3.3	121	6.4
13C5-PFHxA	40	95.5	3.9	97.7	4.1
13C3-HFPO-DA	40	109	15	120	13
13C4-PFHpA	40	106	4.8	111	7.4
13C3-PFHxS	40	99.1	6.5	104	5.3
13C2-6:2 FTS	160	102	4.9	115	8.8
13C8-PFOA	40	101	6.7	106	8.4
13C9-PFNA	40	97.9	5.9	106	11
13C8-PFOS	40	92.3	11	105	13
13C2-8:2 FTS	160	97.6	8.3	103	11
13C6-PFDA	40	96.2	7.4	104	11
13C7-PFUnA	40	90.0	9.0	92.8	14
13C2-PFDoA	40	91.7	7.7	94.5	14

Precision and Accuracy in City Drinking Water

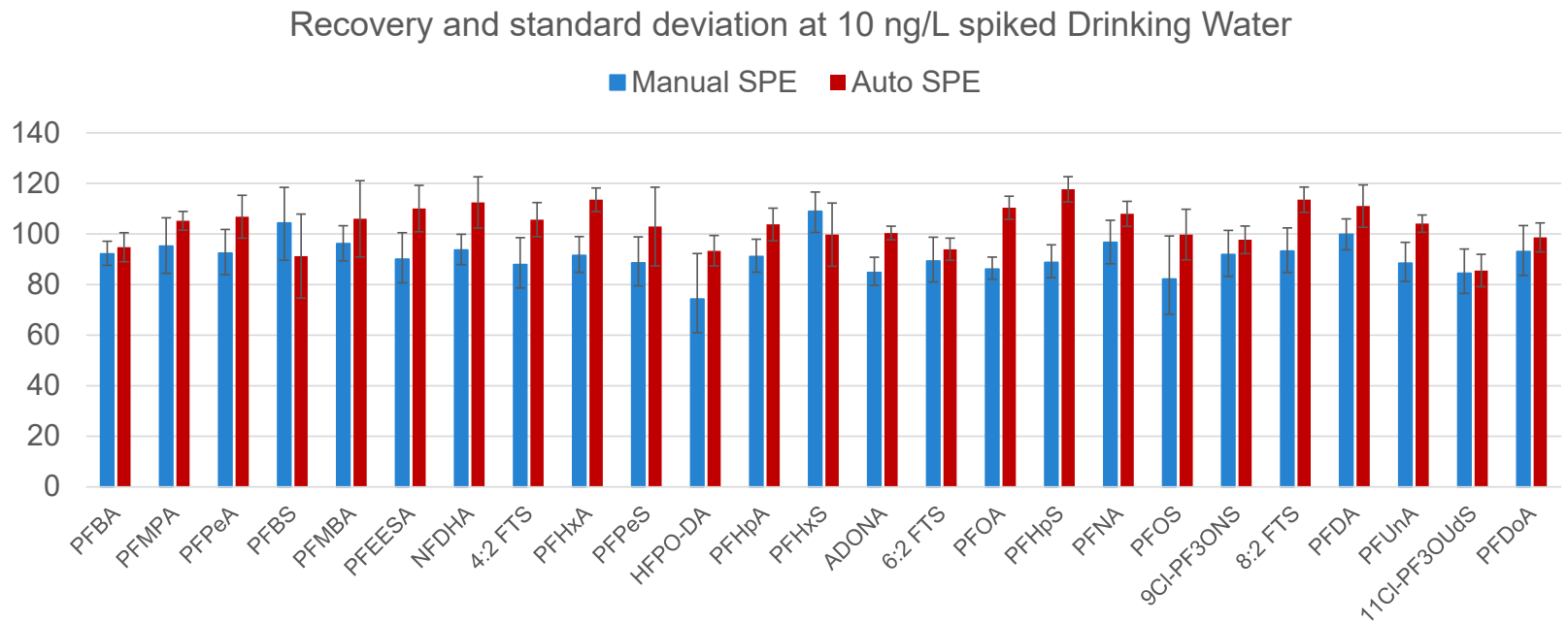
		Spiking concentration: 10 ng/L		Spiking concentration: 80 ng/L	
		Mean %R	%RSD	Mean %R	%RSD
1	PFBA	94.8	6.1%	105.7	4.1%
2	PFMPA	105	3.5%	110.8	6.5%
3	PFPeA	107	7.9%	102.8	8.5%
4	PFBS	91.3	18%	110.7	9.4%
5	PFMBA	106	14%	114.0	3.7%
6	PFEESA	110	8.3%	105.2	7.4%
7	NFDHA	112	9.0%	117.2	4.1%
8	4:2 FTS	106	6.4%	112.4	4.4%
9	PFHxA	114	4.1%	108.5	5.4%
10	PFPeS	103	15%	114.1	8.3%
11	HFPO-DA	93.4	15%	120.5	3.6%
12	PFHpA	104	6.2%	107.7	2.8%
13	PFHxS	99.7	13%	107.2	5.2%
14	ADONA	100	2.7%	108.9	3.5%
15	6:2 FTS	94.0	4.7%	107.2	5.4%
16	PFOA	110	4.2%	113.4	1.6%
17	PFHpS	118	16%	119.6	6.7%
18	PFNA	108	4.5%	112.8	3.1%
19	PFOS	99.8	13%	117.1	5.7%
20	9Cl-PF3ONS	97.7	5.6%	110.5	4.3%
21	8:2 FTS	114	16%	124.1	6.8%
22	PFDA	111	7.5%	111.0	3.0%
23	PFUnA	104	3.3%	107.9	2.3%
24	11Cl-PF3OUdS	85.6	7.5%	95.0	4.0%
25	PFDoA	98.7	5.8%	101.9	2.2%

Inter day Precision and Accuracy Consistency



Comparable results obtained after 45 days of operations

Precision and Accuracy Results in Manual vs. Auto SPE



Overall comparable / slight higher recoveries were obtained with automatic SPE system

Summary

- 25 PFAS Separation: peak well separated with good peak shape
- Method validation meet EPA method 533 requirement using both manual and automatic SPE devices
  - Blank test and carry over test
  - Valid LCMRL obtained
  - Precision and Accuracy with reagent water and city drinking water
    - 25 targeted analytes
    - 16 Isotope dilution analogue
  - Comparable inter day PFAS recoveries and %RSDs obtained

Acknowledgement

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- Thermo Fisher CMD Commercial Organization
  Kevin Mchale

Questions