

Analysis of Perfluorinated Alkyl Acids Specified in EPA M537 and Beyond in Drinking Water Using Triple Quadrupole LC/MS/MS System.

Brahm Prakash¹ Tairo Ogura¹, Jerry Byrne¹ and William Lipps²

1. Shimadzu Scientific Instruments, Inc., Columbia MD USA

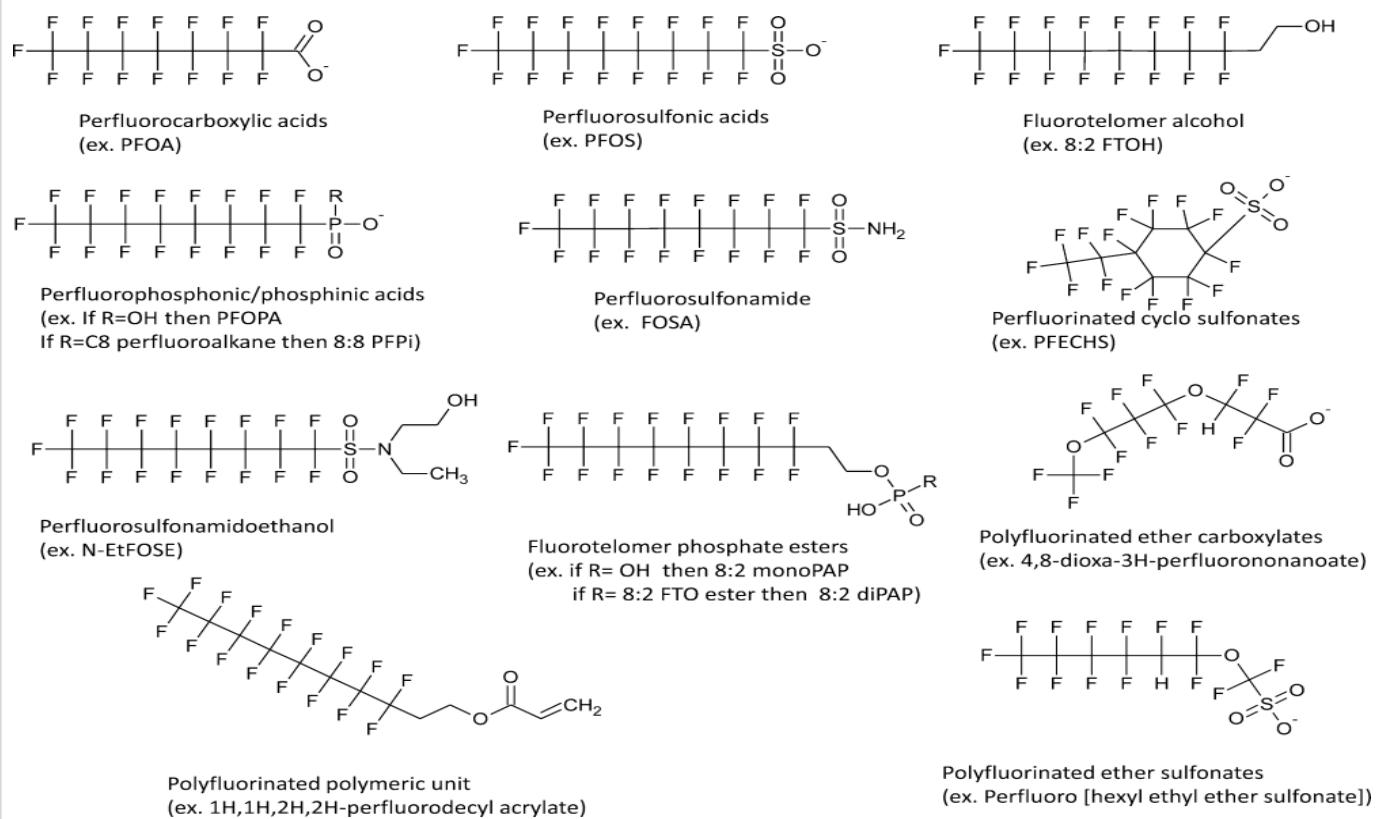
2. Eurofins Eaton Analytical, Inc., Monrovia CA USA

August 9, 2018

Per- and Polyfluoroalkyl Ether Acids (PFAS) in Drinking Water Sources and Finished Drinking Water: Brief Environmental Background

- Produced in the largest amounts in the U.S since 1950, production increased rapidly in 1970s.
- Long-chain PFASs ($C \geq 6$) have been widely used and released into the environment
- Long-chain PFASs ($C \geq 6$) are being phased out (2000 -2015) because of their
 - Toxicity
 - Carcinogenicity
 - Persistence
- Production is still ongoing in other countries, and thus, importation of products containing both compounds is possible
- Continued study of their toxicology is warranted as they will continue to persist in the environment for decades to come

What are Per- and Polyfluoroalkyl Ether Acids (PFAS) Substances



PFAS Environmental Occurrence Examples

Perfluorooctane sulfonic acid (PFOS) – Used in making carpets, leathers, textiles, fabrics for furniture, paper packaging, and other materials that are resistant to water, grease, or stains. It is also used in firefighting foams at airfields.

Perfluorooctanoic acid (PFOA) - Used in making carpets, leathers, textiles, fabrics for furniture, paper packaging, and other materials that are resistant to water, grease, or stains. It is also used in firefighting foams at airfields..

Contamination

Fountain, Colorado: Recently, the New York Times published an article describing perfluorinated chemical contamination in drinking water in Fountain, Colorado.

- The suspected source is firefighting foam used on a nearby military base.
- The airforce had examined nine military bases to date and found contamination at all of them.

Feb 20, 2018: The manufacturer of Scotchgard, 3M, settles with the State of Minnesota for 850 million over groundwater contamination from PFC's

February 2018: DuPont and Chemours paid 670 million, Ohio River Valley settlement involving C8 (GenX) water contamination

Regulatory and also a National Priority:

Per- and Polyfluoroalkyl substances (PFAS) June 18,2018: EPA made *announcement of funding opportunity*, Anticipated Funding Amount: Approximately \$3,968,800 in Grant money for developing methods to identify unknown PFAS in Environmental samples due to transformations, degradation, new formulations, etc.

EPA Method 537: 2008- Lowest Concentration Minimum Reporting Level (LCMRL) was Determined from multiple Labs in Initial Method Validation Study for 14 Analytes

Analyte	LCMRL (ng/L)
PFBS	3.7
PFHxA	2.9
PFHpA	3.8
PFHXs	8
PFOA	5.1
PFNA	5.5
PFOS	6.5
PFDA	3.8
NMeFOSAA	14
NEtFOSAA	14
PFUnA	6.9
PFDoA	3.5
PFTrDA	3.8
PFTA	4.7

[2009 CCL3 Health Reference Level \(HRL\)](#)

PFOA - 1100 ng/L

PFOS - 200 ng/L

[2016 EPA Drinking Water Health Advisory](#)

When both PFOA and PFOS are found in drinking water, the combined concentration of PFOA and PFOS should be compared with the **70 ng/L** health advisory level.

- The Large variation in LCMRLs among labs resulted in a high “national” MRL for UCMR3 because these data are used for the simulation to determine the national **MRL** (Minimum Reporting Level)...[Next Slide](#)
- Since May 2016 USEPA Lifetime Health Advisory Level for both PFOA and PFOS in drinking water was lowered to 70 ng/L
- More than 16 million residents receive drinking water with PFOA+PFOS levels above 70 ng/L
- Ecowatch Group’s (EWG) analysis suggests that up to 110 million Americans May Be Drinking PFAS-Contaminated Water

MRLs (minimum reporting levels) from Multiple Labs in Initial Method Validation (2008)

- EPA M537

- **Method Analytes in UCMR 3** (Unregulated Contaminant Monitoring Rule): EPA Method 537 was used to gather nationwide occurrence data for only 6 PFAAs out of total 14 Analytes.

● Analyte	UCMR3 MRL
PFHpA – perfluoroheptanoic acid	10
PFOA - perfluorooctanoic acid	20
PFNA - perfluorononanoic acid	20
PFBS – perfluorobutanesulfonic acid	90
PFHxS- perfluorohexane sulfonic acid	30
PFOS – perfluorooctane sulfonic acid	40

- MRLs were established based on the LCMRL data obtained from multiple Labs in Initial Method Validation (2008)
- The Large variation in LCMRLs among labs resulted in a high “national” MRL for UCMR3 because these data are used for the simulation to determine the national MRL
- The combined PFOS – PFOA UCMR3 MRL is 60, barely below the HA level of 70 ng/L

Health Effects of PFAS Compounds

- 2005 Study - U.S. Agency for Toxic Substances and Disease Registry (ATSDR) -PFOS exposed rats showed decreased body weight of rat pups, and also delayed eye opening after birth
- 2016 Study – PFOA exposure affected the skeletal development in mice, at lower doses than the EPA's 70 ng/L health advisories level
- PFAS related Health effects
 - included liver damage, elevated cholesterol, risk of thyroid diseases, decreased immune function, increase asthma, and fertility and developments effects
 - Increase in testicular and kidney cancer have been observed in highly exposed humans
- The combined PFOS – PFOA UCMRL3 MRL is 60, barely below the HA level of 70 ng/L, **EPA is expected to revisit its science to announce a lower HA level in the future**
- HA levels are not a federal standard that can be legally enforced and could change as new information becomes available.

Current Updates On PFAS from USEPA Office of Water (OW)

- Evaluating PFOA and PFOS for regulatory determination under the Safe Drinking Water Act (SDWA)
- November 2016 – EPA included PFOA and PFOS in the fourth Contaminant Candidate List (CCL4) to gather information against the three SDWA regulatory determination criteria:
 - May have an adverse effect on the health of persons
 - Is known to occur or there is a substantial likelihood that it will occur in public water systems with a frequency and or at levels of public health concern
 - In the sole judgment of the Administrator, regulating the contaminant presents a meaningful opportunity for health risk reductions for persons served by public water systems
- EPA must decide whether or not to regulate at least five CCL4 contaminants by January 2021
- Public comments on regulatory determination is expected in 2019 (to enable final regulatory determinations by January 2021)

Analytical Methods: PFAS Current LC-MS/MS Methods Available



Method	EPA 537	ASTM D7968 (Needs Multi Labs Validation study)	ASTM D7979 (Needs Multi Labs Validation study)
Analytes	14 targets 3 surrogates 3 ISTDs	21 targets 9 surrogates	21 targets 9 surrogates
Sample materials	Drinking water	Soil	Sludge, Influent, Effluent and Wastewater
Sample prep	SPE	Dilution w/ MeOH	Extraction w/ H ₂ O:MeOH
Injection vol.	10 µL injection	30 µL injection	30 µL injection
Min Reporting Level	5.1 ng/L (PFOA) 1.275 ng/L in vial	10 ng/L (PFOA) 5 ng/L in vial	10 ng/L (PFOA)
Shimadzu TQ (Triple Quad LCMS)	8045/8050	8050/8060	8050/8060



LC-MS/MS Methods Recognized by EPA



Method	EPA M537	M8327
Analytes	14 targets 3 surrogates 3 ISTDs	37 targets 3 surrogates 3 ISTDs
Sample materials	Drinking water	Groundwater, Surfacewater, Influent, Effluent and Wastewater
Sample prep	SPE	Extraction w/ H ₂ O:MeOH
Injection vol.	10 µL injection	30 µL injection
Min Reporting Level	5.1 ng/L (PFOA) 1.275 ng/L in vial	10 ng/L (PFOA) 5 ng/L in vial
Shimadzu TO	8045/8050	8050/8060

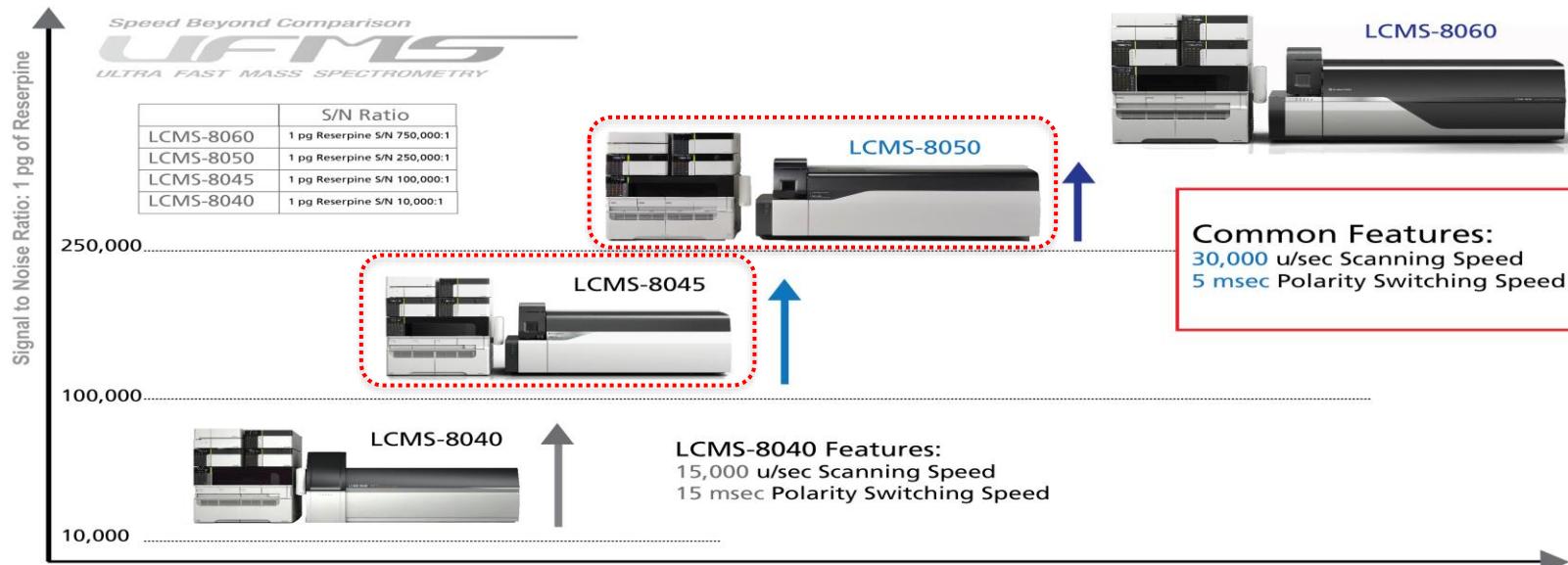


EPA SW-846 method 8327

- Shimadzu has been involved in round robin.

EPA Method 537 is only valid for 14 PFAs and can only be used to analyze drinking water

Shimadzu Instrumentation Used to Conduct M537 PFAS Studies



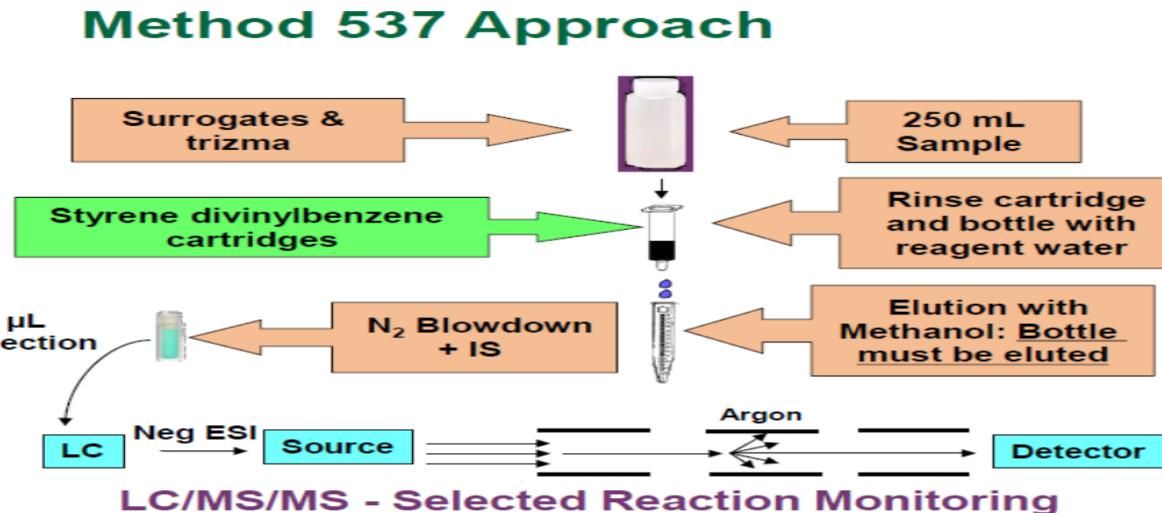
Overview of EPA M537: A Drinking Water Method, Uses Solid Phase Extraction (SPE) and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) to Determine Selected PFCs



Preservative
trizma

Surrogates
 ^{13}C -PFHxA
 ^{13}C -PFDA
 d_5 -NEtFOSAA

Internal Standards
 ^{13}C -PFOA
 ^{13}C -PFOS
 d_3 -NMeFOSAA



Office of Research and Development
National Exposure Research Laboratory

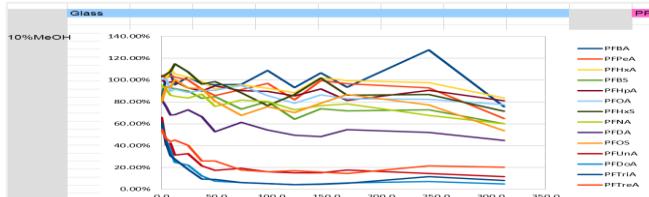
- Source US EPA - % recoveries can be effected through adsorption or extraction loses
- High volume extraction kits tend to have PTFE tubing. All PTFE tubing should be replaced with PEEK tubing

Standard Stability Study: Glass Vials Vs Polypropylene Vials

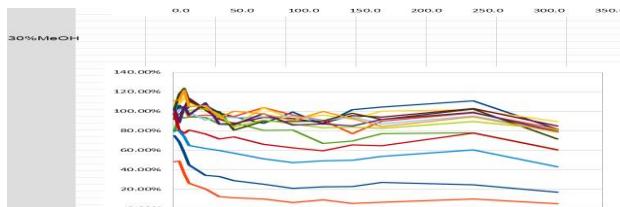
10% Methanol 90% water and 30% Methanol 70% water

- M537 requires the use of polypropylene (PP) vial/caps to prevent adsorption and contamination losses.
 - PP caps do not re-seal**, evaporation occurs, multiple injections not possible
- All PTFE (polytetrafluoroethylene) tubing's in the system were replaced with PEEK (polyether ether ketone) tubing
- Below is the brief observations of the Study performed at Shimadzu

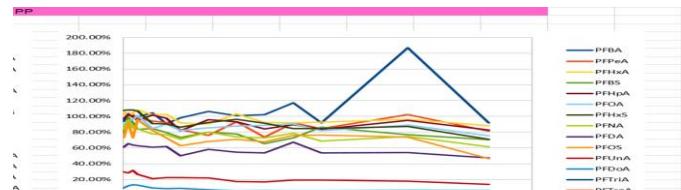
Glass Vial



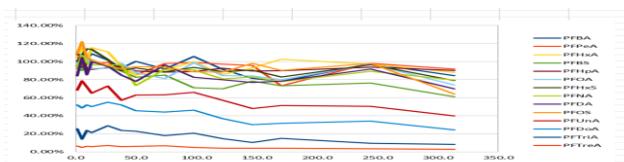
10% MeOH



Polypropylene



10% MeOH

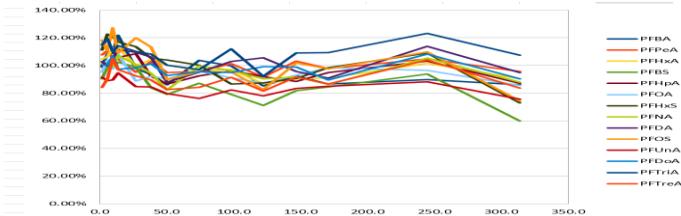


30% MeOH

Standard Stability Study Glass Vials Vs Polypropylene Vials

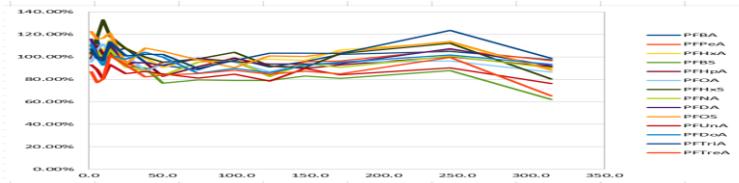
50% Methanol 50% water and 90% Methanol 10% water

Glass Vials

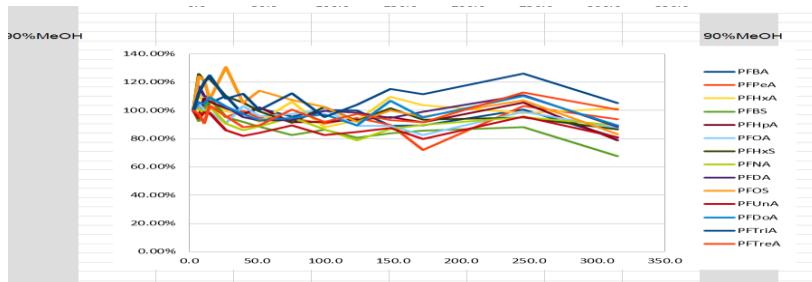


50% MeOH

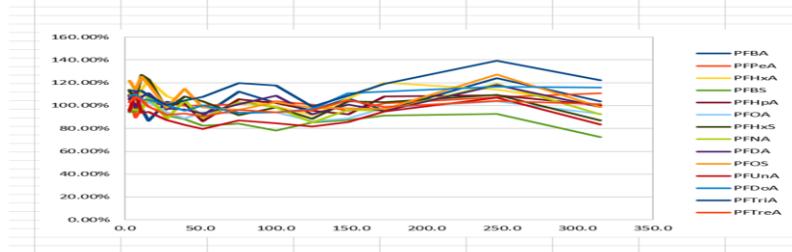
Polypropylene Vials



50% MeOH

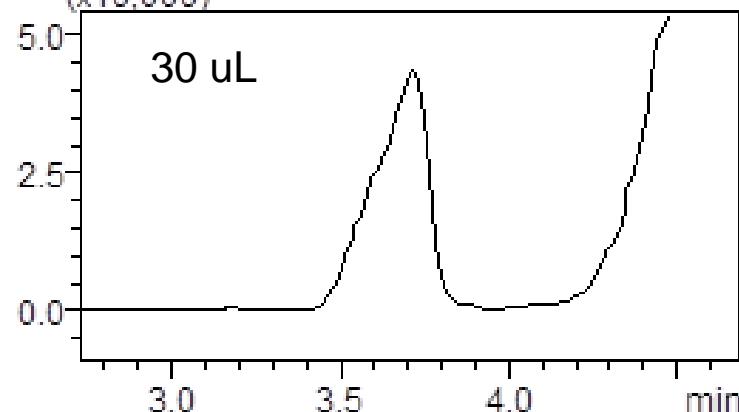
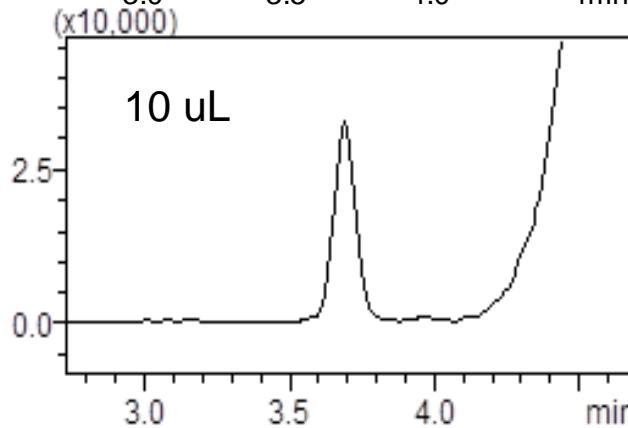
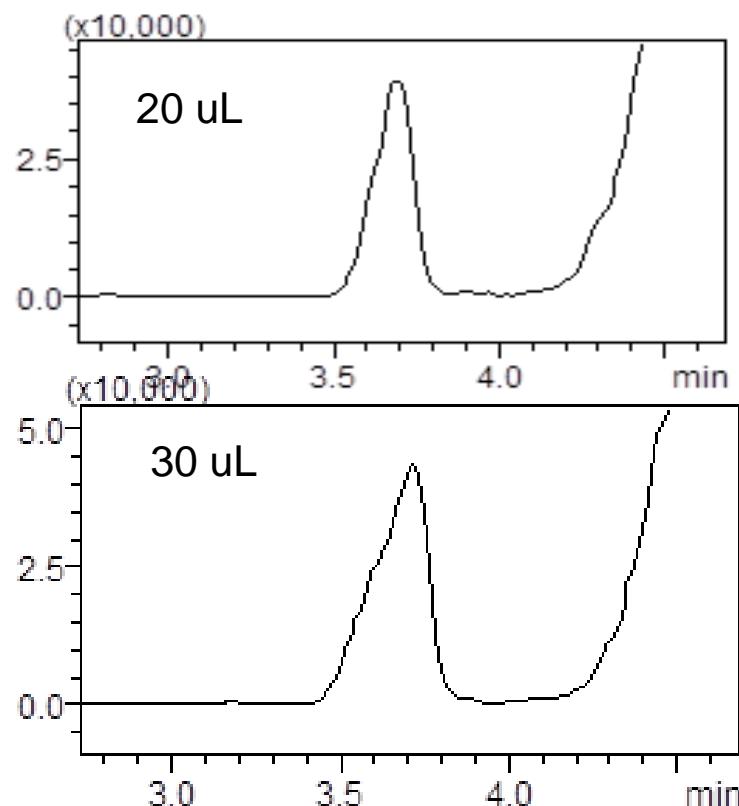
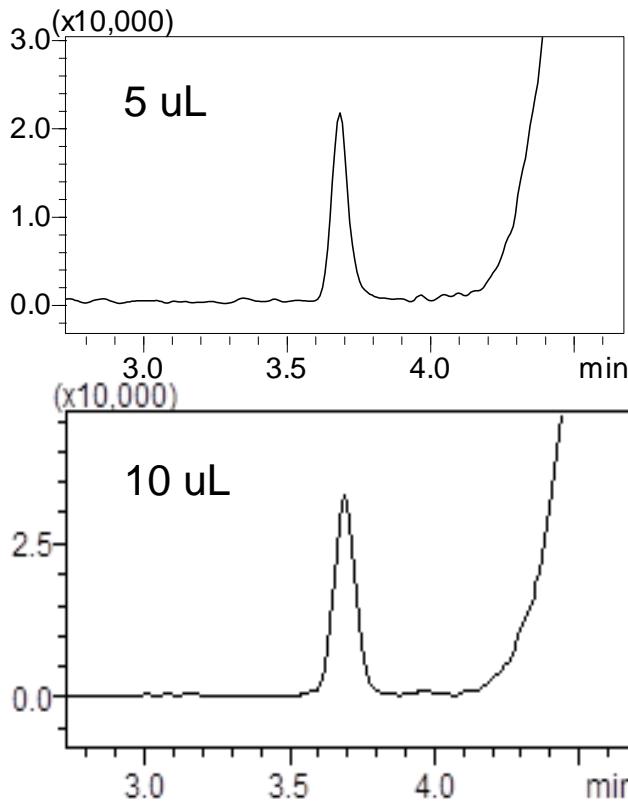


90% MeOH

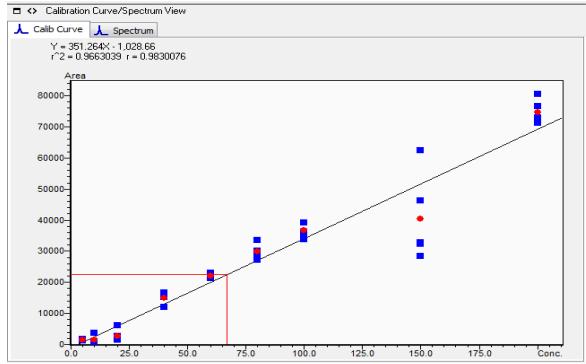


90% MeOH

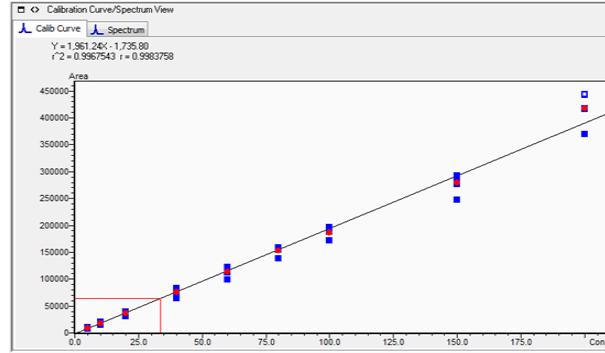
Excessive Methanol Effect



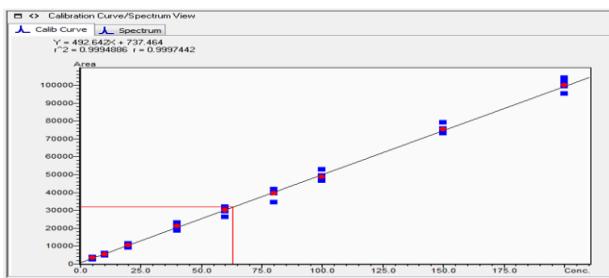
PFAS: Floating Effect



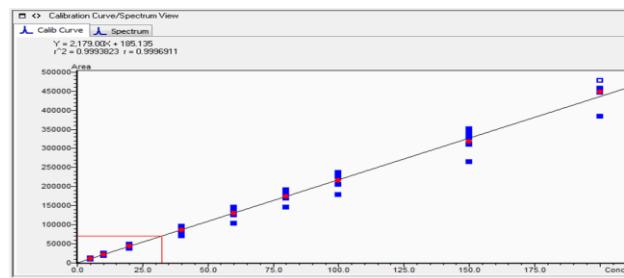
PFTA (No Vortex, n=5 ICAL)



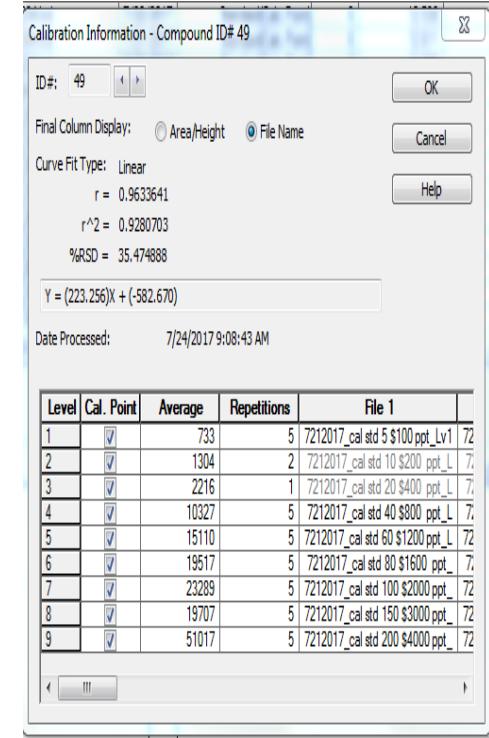
PFTA (After Vortex, 12hrs or before New ICAL)



PFNA (No Vortex, n=5 ICAL)

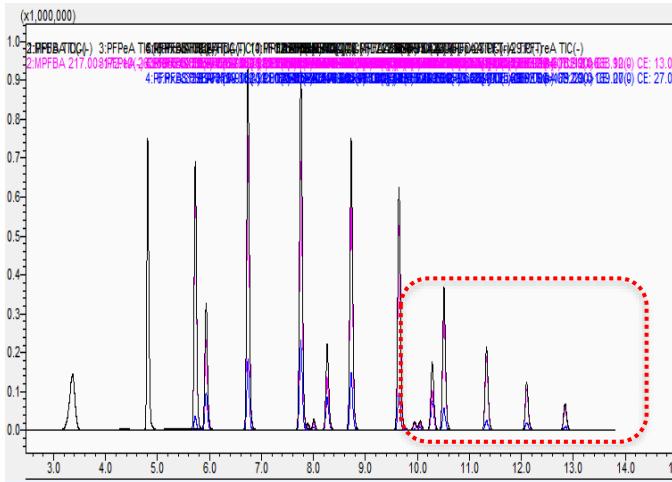


PFNA (After Vortex, 12hrs or before New ICAL)

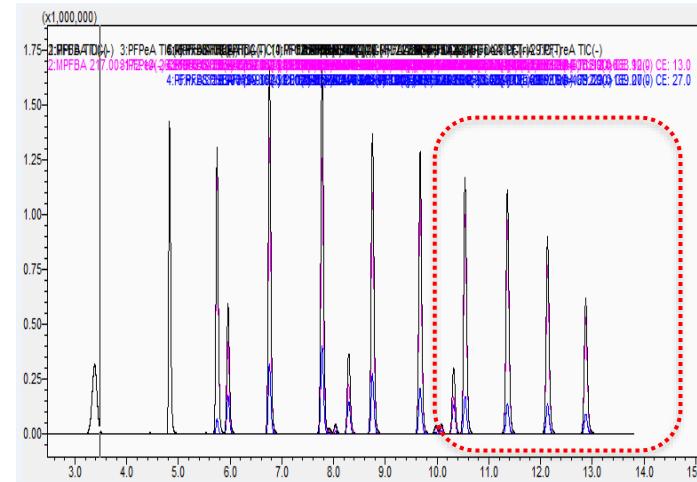


PFAS : Sample Vials Vortex Effect

- PFCs could float on the top of sample in the vials and be not injected... resulting in poor recoveries or could go as non detect.



Before Vortex



Same Vial After Vortex

- It is recommended to vortex all vials at the beginning of the shift to ensure consistent results

PFAS Analysis: Optimized Instrument Method Conditions

- LC-MS Systems: Shimadzu LCMS-8045 and LCMS-8050
- LC parameters
- Analytical Column: Restek Raptor ARC-18 150 mm x 2.1 mm x 2.7 um
- Solvent Delay Column: Shim-pack XR-ODS II (75 mm x 2mm x 2.2 um d_p)
- Mobile Phase A: 20 mM Ammonium acetate in LCMS grade H₂O
- Mobile Phase B: Methanol
- Rinse Phase: Methanol

- MS Parameters
- Nebulizing Gas: 3 L/min
- Heating Gas: 15 L/min
- Interface Temperature: 300 C
- Desolvation Line Temperature: 100 C
- Heat Block Temperature: 200 C
- Drying Gas: 5 L/min

Target List Including Newly Added Compounds To M537 List -

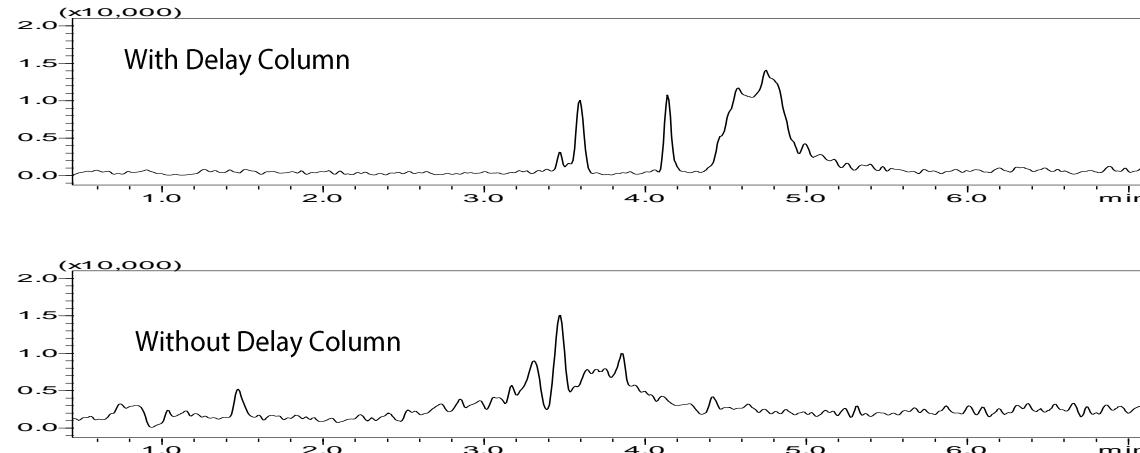
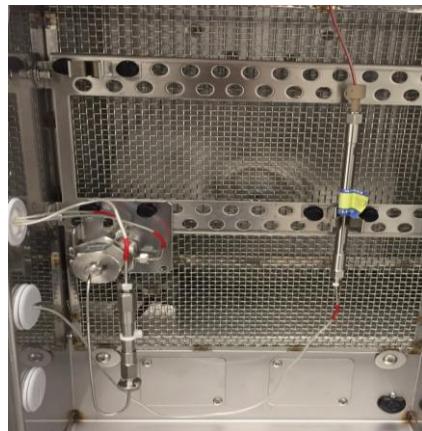
Internal Standards and Surrogates Used as per EPA M537

ID	Compound	Quant Ions	Reference ions
1	PFBA	212.90>169.00	
2	PFPeA	263.00>219.00	
3	PFBS	298.90>80.10	298.90>99.10
4	4-2 FTS	327.00>307.00	327.00>80.90
5	PFHxA	312.90>269.00	322.90>119.10
7	PFPeS	348.90>79.90	348.90>98.90
8	PFHpA	362.90>319.00	362.90>169.00
9	PFHxS	398.90>80.10	398.90>99.10
10	6-2 FTS	427.00>406.90	427.00>80.90
11	PFOA	412.90>369.00	412.90>169.00
13	PFHpS	448.90>79.90	448.90>98.90
14	PFOS	498.90>80.10	498.90>99.10
15	PFNA	262.90>418.80	462.90>219.00
17	8-2 FTS	526.90>506.90	526.90>80.90
18	PFNS	548.90>79.90	548.90>98.90
20	PFDA	512.90>468.90	512.90>219.00
22	N-MeFOSAA	569.90>419.00	569.90>482.90
24	N-EtFOSAA	583.90>419.00	583.90>482.90
25	PFDS	598.90>79.90	598.90>98.90
26	PFUnA	563.90>519.00	562.90>269.00
27	PFDoA	612.90>568.90	612.90>169.00
28	PFTrDA	662.90>618.90	662.00>169.00
29	PFTA	712.90>668.90	7812.90>169.00

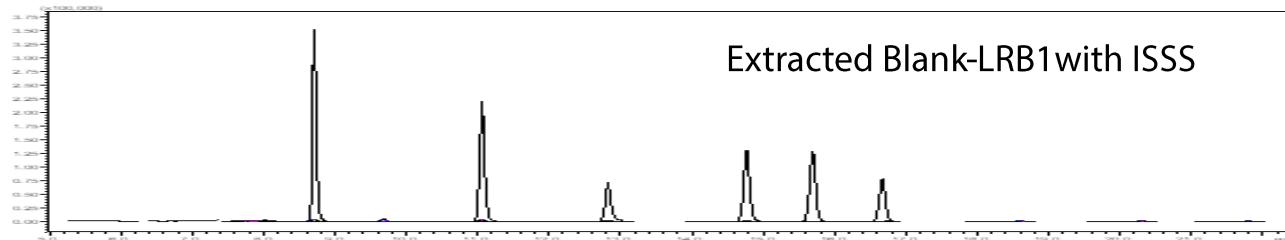
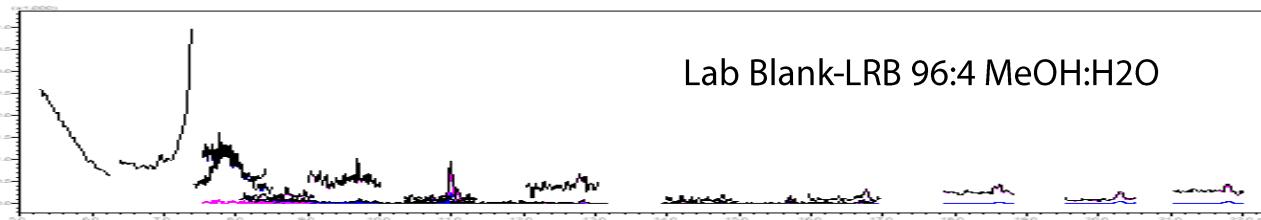
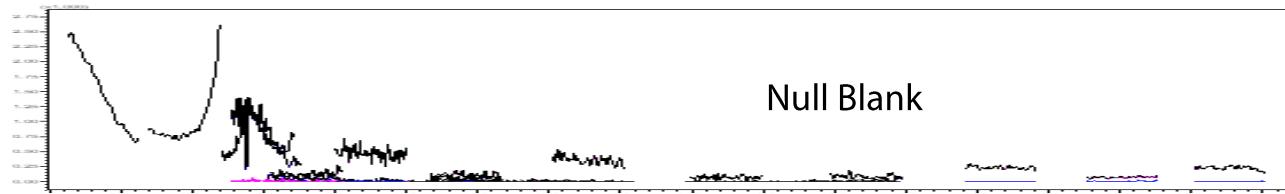
ID	Compound	Quant Ion
6	MPFHxA_Surr	317.00>270.00
12	M2PFOA_IS	415.00>370.00
16	MPHOS_IS	503.00>80.00
19	MPFDA_Surr	515.00>469.95
21	N-MeFOSAA_IS	572.90>419.00
23	MN-EtFoSAA_Surr	588.90>419.00

Isolator or Delay Column – To Overcome Background Contamination

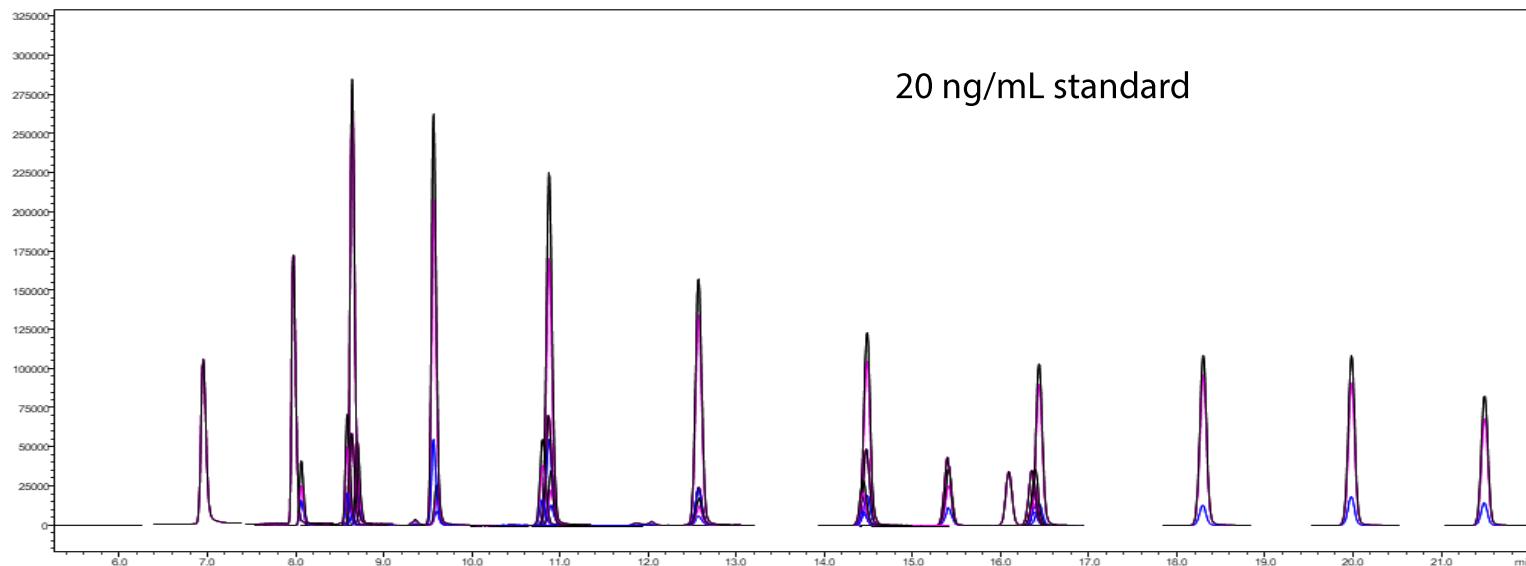
- Depending on the analytical conditions, PFOA exiting in mobile phase, in the online degasser, as well in the flow line can become concentrated in the analytical column.
- To overcome, a delay column installed between the mixer and the injector helps delaying the elution of the impurity, called the Impurity Delay method.



PFAS Analysis: Chromatograms: Null blank, Laboratory Reagent blank and Extracted blank for EPA M537 Using Shimadzu LCMS-8050

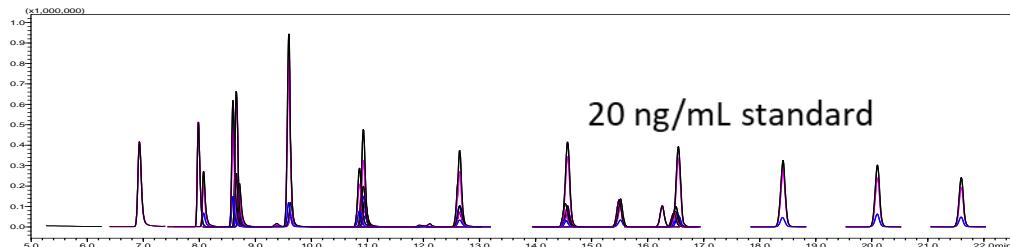


PFAS Analysis : Chromatogram for PFASs fortified at 20 ng/mL for EPA M537 Using Shimadzu LCMS-8045

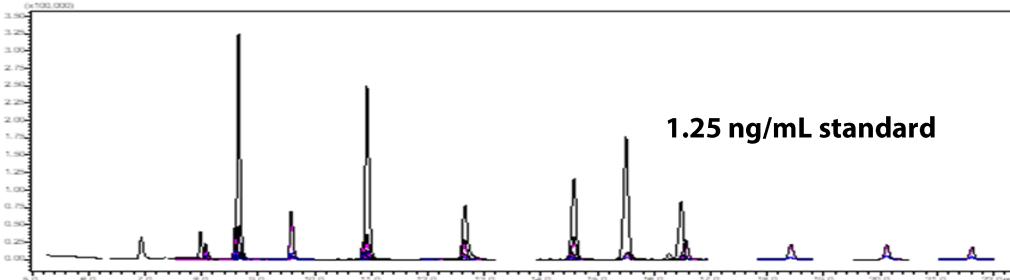


Chromatogram for PFASs fortified at 20 ng/mL standard

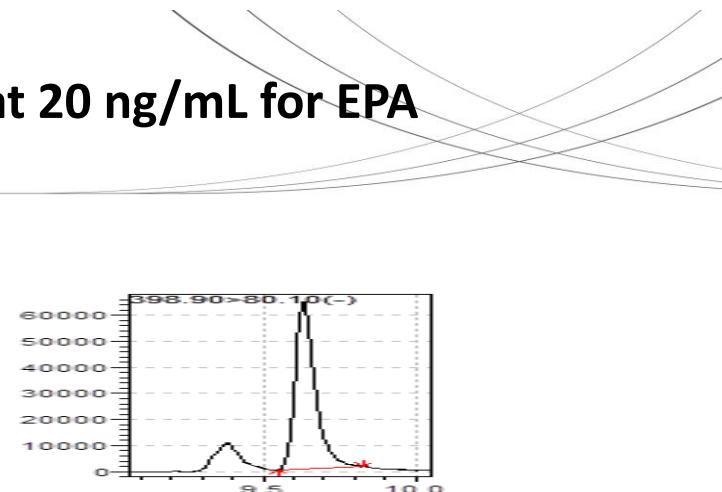
PFAS Analysis : Chromatogram for PFASs fortified at 20 ng/mL for EPA M537 Using Shimadzu LCMS-8050



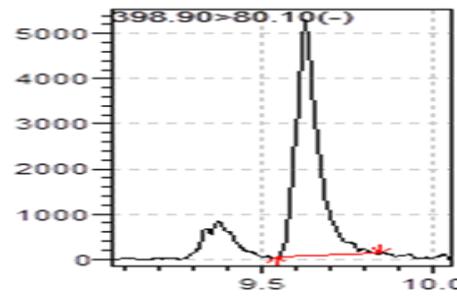
Chromatogram for PFASs fortified at 20 ng/mL standard



Chromatogram for PFASs fortified at 1.25 ng/mL standard

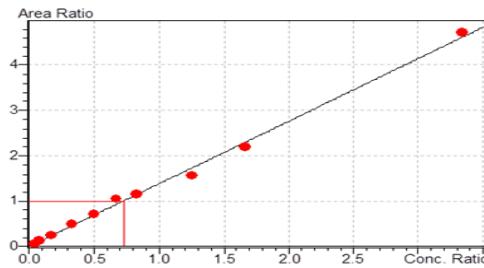
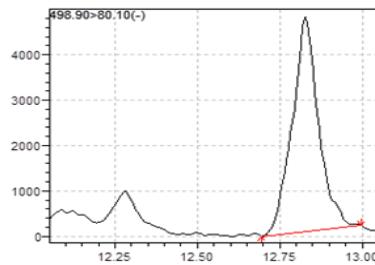


Analyte: PFHxS with branched Isomer Separation

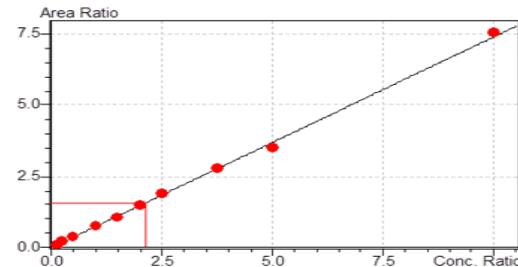
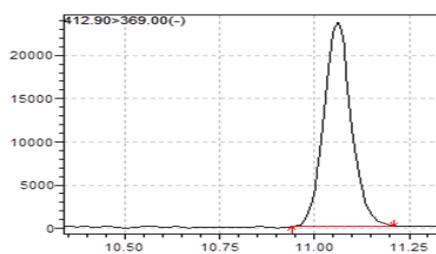


Analyte: PFHxS with branched Isomer Separation

Chromatogram and Calibration curve Method EPA 537 Using LCMS-8050



Chromatogram and calibration curve for 1.25 ng/mL of PFOS



Chromatogram and calibration curve for 1.25 ng/mL of PFOA

Calibration curves were produced in the range of 1.25 ng/L to 100 ng/L for the PFCs.

Level	Concentration
1	1.25 ppb
2	2.5 ppb
3	5.0 ppb
4	10 ppb
5	15 ppb
6	20 ppb
7	25 ppb
8	37.5 ppb
9	50 ppb
10	100 ppb

All curves had a regression coefficient higher than 0.9900. Curves for a selection of target compounds are plotted.

MDL's M537 Vs Shimadzu LCMS-8045 and LCMS-8050

Shimadzu: Minimum Reporting Level (ng/L) n=8				EPA		
Compound	Spiked Conc. (ng/L)	LCMS-8045 MDL (ng/L)	LCMS-8050 MDL (ng/L)	EPA 537 Spike (ng/L)	EPA M537 DL (ng/L)	EPA M537 LCMRL(ng/L)
PFBS	5	1.5	1.2	9.1	3.1	3.7
4-2FTS	5	2.1	1.3			
PFHxA	5	1.2	1.0	1.6	2.9	5
PFPeS	5	1.5	1.4			
PFHpA	5	1.1	1.0	4.1	0.5	3.8
PFHxS	5	0.7	1.2	11	2.0	8.0
6-2FTS	5	2.3	1.1			
PFOA	5	1.6	1.0	4.6	1.7	5.1
PFHpS	5	1.0	1.6			
PFOS	5	1.7	0.8	9.6	1.4	6.5
PFNA	5	0.8	0.7	4.8	0.7	5.5
8-2FTS	5	3.3	1.7			
PFNS	5	1.1	1.1			
PFDA	5	1.0	1.4	3.7	0.7	3.8
N-MeFOSAA	5	1.7	1.2	20	6.5	14
N-EtFOSAA	5	1.2	1.3	21	4.2	14
PFDS	5	2.2	1.4			
PFUnA	5	1.5	1.4	5.4	2.8	6.9
PFDoA	5	1.6	1.2	3.7	1.1	3.5
PFTrDA	5	1.4	1.3	5.5	2.2	3.8
PFTA	5	1.0	1.0	4.4	1.7	4.7

A 1uL injection was used for Shimadzu's study compared to 10 uL injection used in EPA M537

MRL validation for M537- Shimadzu LCMSMS-8050

Minimum Reporting Level (ng/L) n=8			Prediction Interval of Result			
Compound	Spiked Conc. (ng/L)	Calculated Conc. (ng/L)	Half Range Prediction Interval of Results (HR _{PIR})	Upper PIR Limit test <150% recovery	Lower PIR Limit test >50% recovery	MRL Validation
PFBS	5	5.1	1.4939	131.8	72.1	Pass
4-2FTS	5	4.9	1.554	129.2	66.9	Pass
PFHxA	5	4.7	1.290	118.7	67.1	Pass
PFPeS	5	4.8	1.689	130.4	62.8	Pass
PFHpA	5	4.7	1.192	117.6	69.9	Pass
PFHxS	5	4.8	1.500	126.2	66.1	Pass
6-2 FTS	5	4.8	1.309	121.9	69.6	Pass
PFOA	5	4.7	1.264	119.7	69.2	Pass
PFHpS	5	4.7	1.953	133.8	55.7	Pass
PFOS	5	4.6	1.001	112.3	72.3	Pass
PFNA	5	4.8	0.854	113.9	79.8	Pass
8-2 FTS	5	5.3	2.140	148.7	63.0	Pass
PFNS	5	4.4	1.402	118.8	62.7	Pass
PFDA	5	4.8	1.746	130.0	60.2	Pass
N-MeFOSAA	5	4.6	1.534	122.0	60.7	Pass
N-EtFOSAA	5	4.4	1.809	133.2	60.8	Pass
PFDS	5	4.6	1.669	121.3	54.6	Pass
PFUnA	5	4.4	1.721	126.5	57.6	Pass
PFDoA	5	4.3	1.764	122.8	52.2	Pass
PFTrDA	5	4.4	1.514	116.6	56.1	Pass

Results from Initial Calibration and repeat injections LCMS-8045: System Stability

Calibration Curve Repeat Injections (n=4)								
Compound	Retention Time	R ²	Low (20 ppb)		Mid (50 ppb)		High (100 ppb)	
			Conc	%RSD	Conc.	%RSD	Conc.	%RSD
PFBS	8.046	0.9977	21	2	46	3	103	2
4-2FTS	8.558	0.9928	22	3	45	7	94	1
PFHxA	8.614	0.9968	21	4	48	7	102	4
PFPeS	8.666	0.9985	21	2	46	2	100	1
PFHpA	9.512	0.9974	21	5	46	5	101	2
PFHxS	9.558	0.9968	21	3	46	5	104	3
6-2FTS	10.77	0.9968	21	4	44	4	95	2
PFOA	10.84	0.9967	21	5	47	7	103	3
PFHpS	10.859	0.9982	21	4	45	8	104	6
PFOS	12.55	0.9986	20	6	44	7	103	12
PFNA	12.545	0.9975	21	10	47	2	100	3
8-2FTS	14.436	0.994	23	14	45	13	94	13
PFNS	14.469	0.9978	21	2	46	6	100	5
PFDA	14.486	0.9969	21	3	47	3	98	2
N-MeFOSAA	15.423	0.9979	21	3	47	1	100	3
N-EtFOSAA	16.411	0.998	22	4	48	2	102	5
PFDS	16.397	0.997	21	4	45	11	103	5
PFUnA	16.449	0.9973	21	4	48	4	100	6
PFDoA	18.339	0.9975	21	4	48	3	103	6
PFTrDA	20.035	0.9967	20	5	45	5	101	5
PFTA	21.549	0.9966	21	5	47	4	103	3

Precision and Accuracy Study Results LCMS-8045 and LCMS-8050

LCMS-8045: P&A at 60ng/L (n=8)					LCMS-8050: P&A at 20 ng/L, 60 ng/L (n=7)							
Compound	Spk. Conc. (ng/L)	Avg. Conc. (ng/L)	% Rec	%RSD	Spk. Conc. (ng/L)	Avg. Conc. (ng/L)	% Rec	%RSD	Spk. Conc. (ng/L)	Avg. Conc. (ng/L)	% Rec	%RSD
PFBS	60	52	87	13	20	20	100	15	60	54	90	6
4-2FTS	60	54	90	13	20	20	101	14	60	56	94	8
PFHxA	60	54	87	12	20	18	88	13	60	52	87	9
PFPeS	60	52	91	14	20	20	100	12	60	54	90	9
PFHpA	60	54	88	16	20	18	90	11	60	52	87	10
PFHxS	60	53	89	13	20	20	98	13	60	54	90	8
6-2FTS	60	54	92	15	20	20	100	13	60	55	92	9
PFOA	60	55	86	14	20	18	91	12	60	53	88	11
PFHpS	60	52	90	13	20	19	97	14	60	53	89	9
PFOS	60	54	89	17	20	20	98	12	60	51	85	12
PFNA	60	53	86	16	20	22	111	25	60	64	107	21
8-2FTS	60	51	86	19	20	21	106	13	60	56	93	9

Precision and Accuracy Study Results LCMS-8045

LCMS-8045: P&A at 60ng/L (n=8)					LCMS-8050: P&A at 20 ng/L, 60 ng/L & 100ng/L (n=7)							
Compound	Spk. Conc. (ng/L)	Avg. (ng/L)	% Rec	%RSD	Spk. Conc. (ng/L)	Avg. (ng/L)	% Rec	%RSD	Spk. Conc. (ng/L)	Avg. (ng/L)	% Rec	%RSD
PFNS	60	51	89	15	20	18	91	21	60	55	92	11
PFDA	60	54	87	13	20	18	91	12	60	52	87	10
N-MeFOSAA	60	53	88	15	20	18	91	15	60	53	88	9
N-EtFOSAA	60	54	90	15	20	20	99	17	60	56	93	10
PFDS	60	52	86	17	20	19	95	17	60	53	89	9
PFUnA	60	51	85	11	20	18	92	17	60	53	88	10
PFDoA	60	51	86	14	20	17	87	16	60	51	85	9
PFTrDA	60	49	82	14	20	17	87	15	60	51	85	9
PFTA	60	49	82	14	20	17	85	16	60	49	81	8
Surr. Rec Results LCMS-8045												
MPFx A	40	43	107	14	40	40.46	101	10	40	43	107	9
MPFDA	40	44	109	12	40	42.41	106	13	40	43	109	10
MNEt-FOSAA	160	175	109	14	160	160.03	100	12	160	175	109	9

Summary and Conclusions

- Under optimized conditions, the Shimadzu LCMS-8045 and the LCMS-8050 will meet and exceed the requirements for EPA M537
- The Biotage ISOLUTE 101 cartridges meets and exceeds the demands of EPA M537 by allowing for analysis of additional PFC's. Cartridges from other vendors were not evaluated.
- Results demonstrated that high-sensitivity analysis with high repeatability is possible with Shimadzu's triple quadrupole instruments
- If multiple EPA methods need to be run on the same system, then the LCMS-8050 is recommended.

Method Development on PFCs...Continues

Thank You for
your kind attention

Brahm Prakash

brprakash@shimadzu.com Phone:410-910-0903