Analytical Methodologies for Per/Poly Fluoroalkyl Substances (PFAS) Measurement in Environmental Waters by LC/MS/MS

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Trusted Answers

‘Fit for Purpose’ Analysis

Factors in deciding analytical method selection

Considerations when selecting analytical technique to employ

I. Types of analytes

II. Concentrations required (ppm, ppb, ppt etc.)

III. Type of matrix (drinking water, surface water, wastewater, sludge, food etc.)

IV. Type of sample clean-up required

V. Regulatory considerations (EPA methods, EU & ASTM methods etc.)

Terminology

- **Terminology of these compounds can be confusing but it is *critical* to understand the differences**

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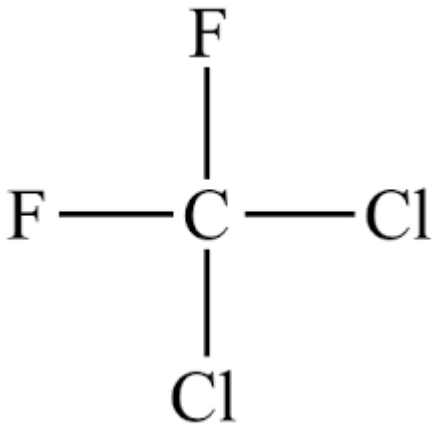
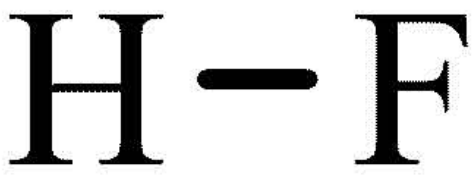
Perfluoroalkyl and Polyfluoroalkyl Substances in the Environment: Terminology, Classification, and Origins

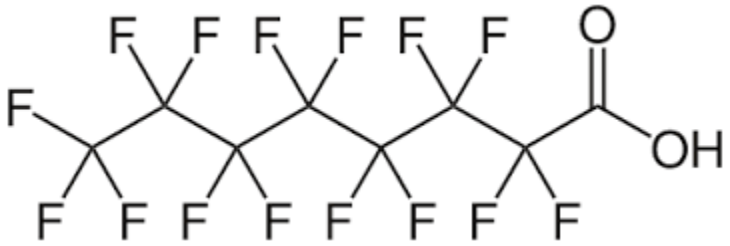
*Robert C Buck, † James Franklin, *‡ Urs Berger, § Jason M Conder, ‖ Ian T Cousins, § Pim de Voogt, # Allan Astrup Jensen, †† Kurunthachalam Kannan, ‡‡ Scott A Mabury, §§ and Stefan PJ van Leeuwen ‖‖*

Terminology
Fluorinated Compounds

Any organic or inorganic compound that has a fluorine atom incorporated in it

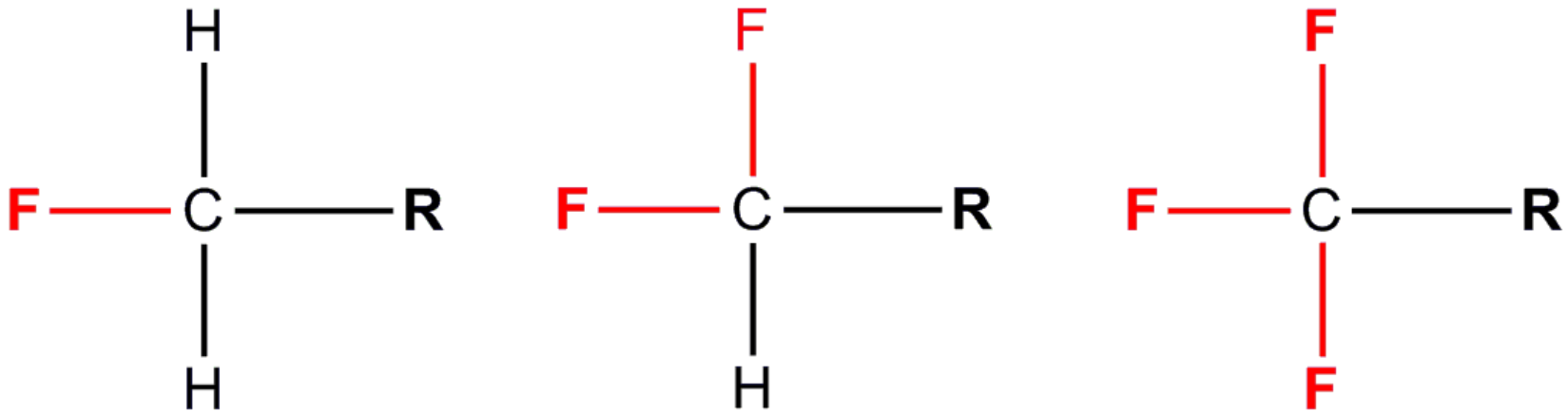






Terminology
Per/PolyfluoroAlkyl Substances (PFAS)

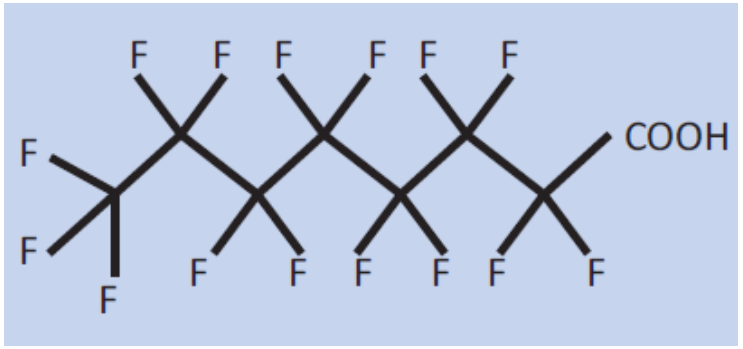
When one or more Carbons (C) in an alkyl chain have the Hydrogen (H) replaced by Fluorine (F)



R: Continuation of alkyl chain or functional group

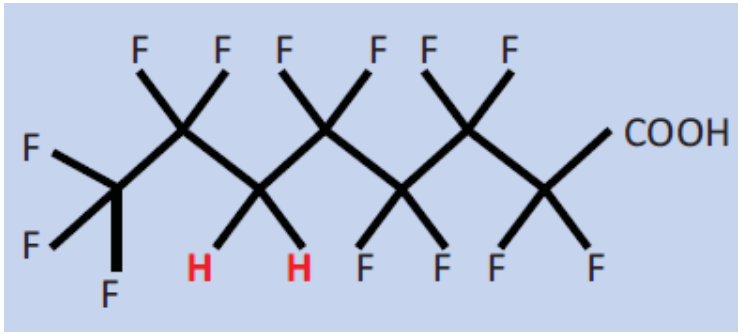
Terminology

Perfluoroalkyl substance



***ALL* H atoms linked to C in alkyl chain are substituted with F**

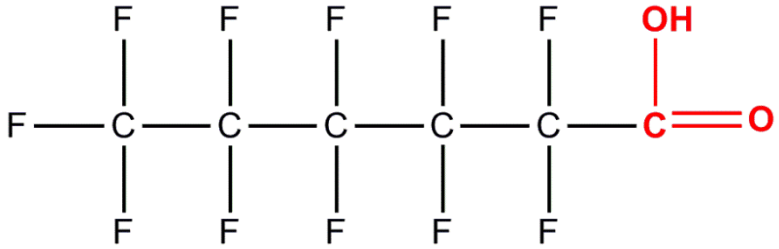
Polyfluoroalkyl substance



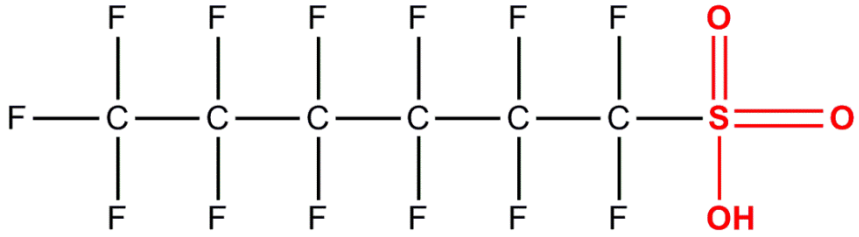
***SOME (but not all)* H atoms linked to C in alkyl chain are substituted with F**

Terminology
Perfluoroalkyl acids (PFAAs)

- Perfluorocarboxylic acids (PFCAs)



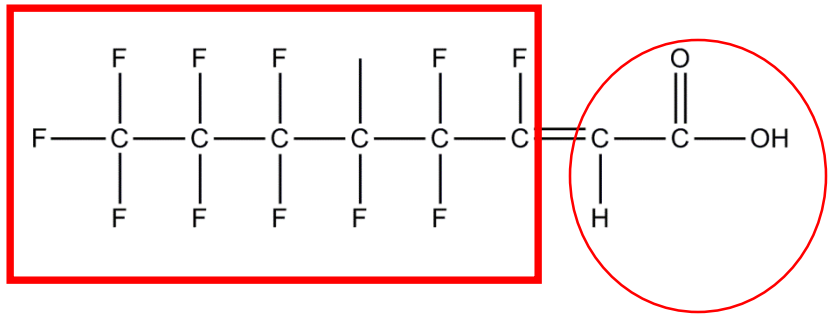
- Perfluorosulfonic acids (PFSAs)



Terminology
Polyfluorinated compounds - Fluorotelomers

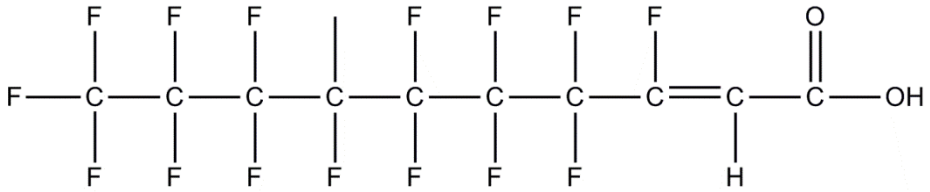
- **Formed during the telomerization process in manufacturing**

6:2 FTUCA



6-C alkyl chain: Hydrophobic **2-C: Hydrophilic**

8:2 FTUCA



Per/Polyfluoroalkyl substances
Unique Properties

- Thermal & Chemical stability: grease-proof food packaging, stain repellents
- Zwitterionic properties: surfactants
- Surface-tension lowering: fire-fighting foams




Per/Polyfluoroalkyl substances
Toxicology & Regulations in US

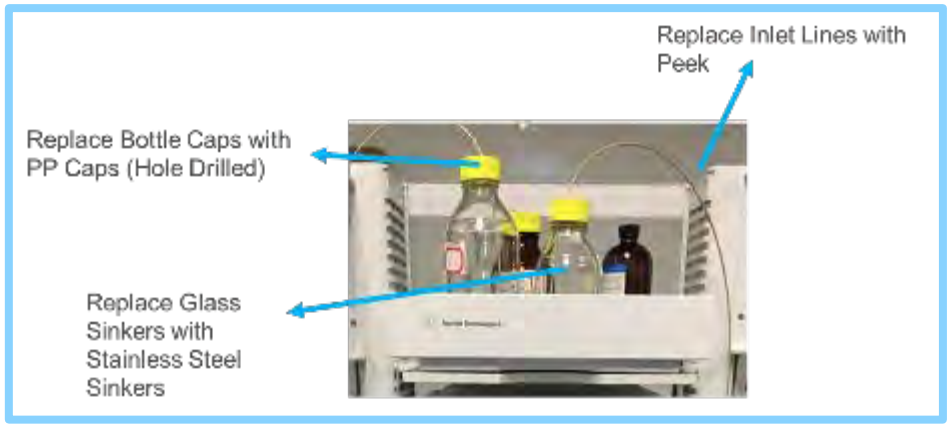
Toxicological data:

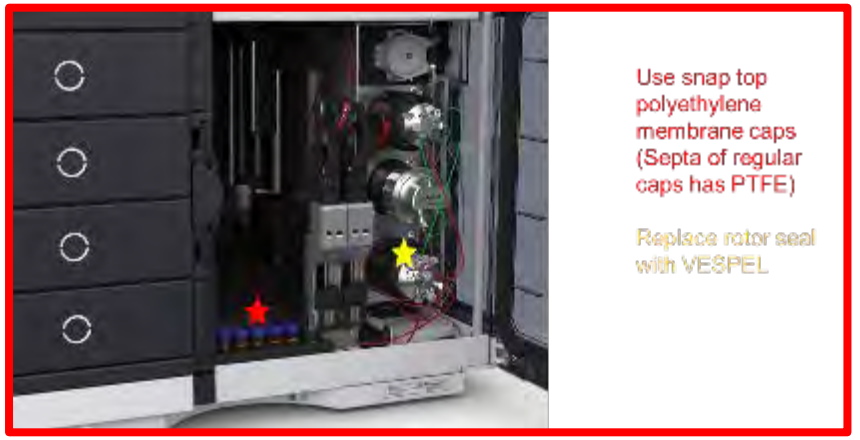
- **PFAS exposure can have adverse effects to the neurological and reproductive systems**
- **Long chain (>7-C chain) PFASs are bio accumulative, and persistent**
- **Long half-lives for longer chain PFAS**
- **Very difficult to degrade the final products (PFCAs, PFSAs) in the environment**

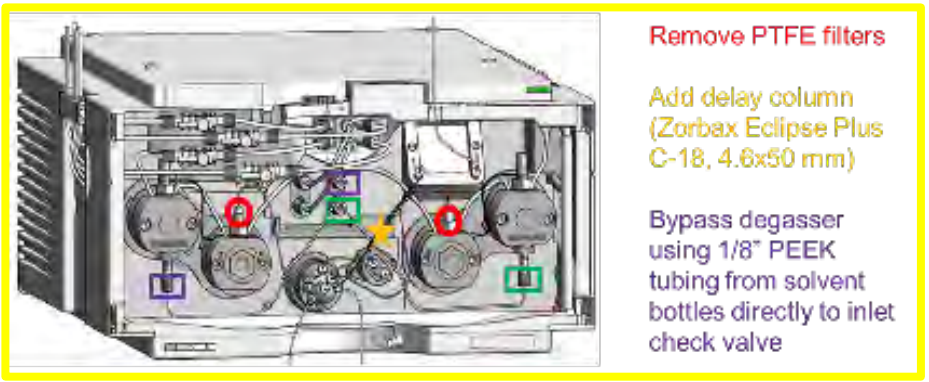
Regulatory Overview:

- **USEPA has a public health guidance for PFOA and PFOS at 70 ng/L in drinking water**
- **Many states have their own health guidelines ranging from 20-400 ng/L for PFOS, PFOA, PFNA and PFHxA**

PFAS Analysis – LC Instrument Setup
Eliminate Background Contamination

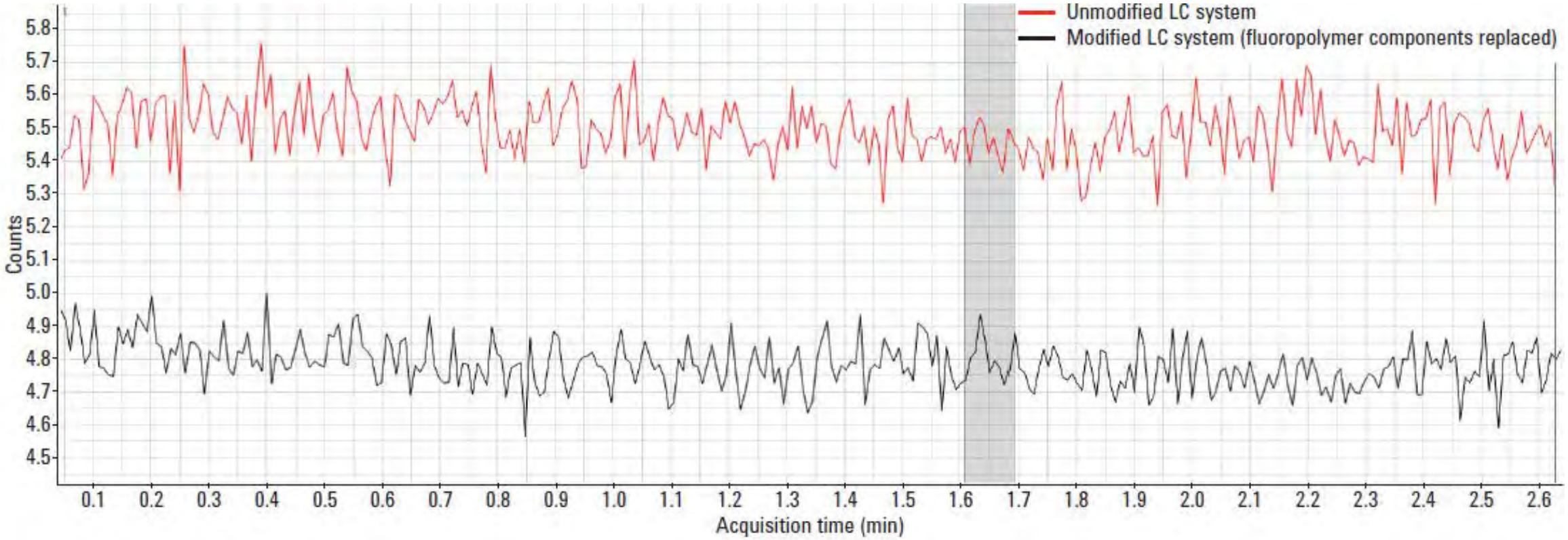




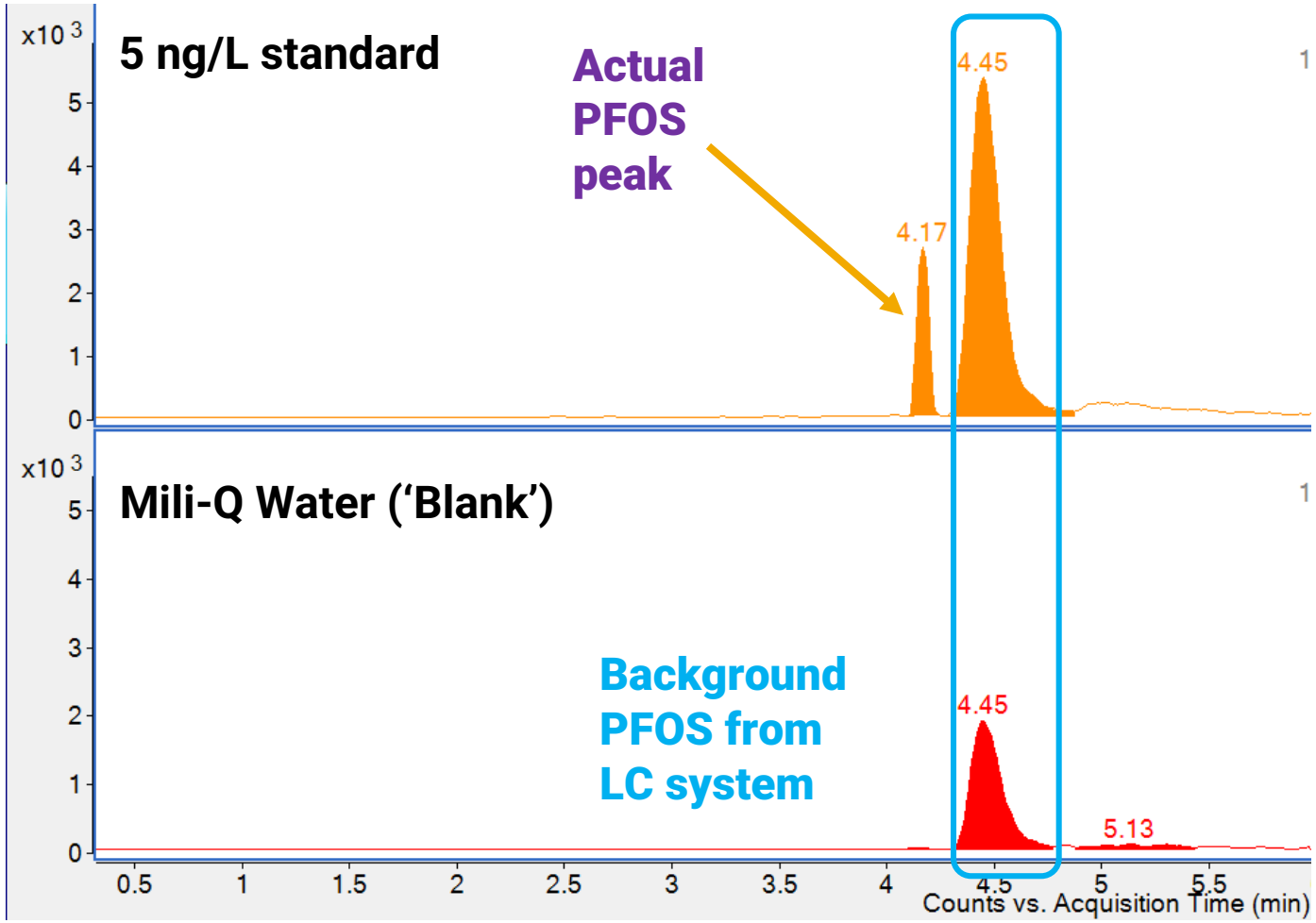


Application Note: Recommended Plumbing Configurations for Reduction in Per/Polyfluoroalkyl Substance Background with Agilent 1260/1290 Infinity (II) LC's (5991-7863EN)

PFAS Analysis – LC Setup
Eliminate Background Contamination



PFAS Instrument Setup
Background Contamination



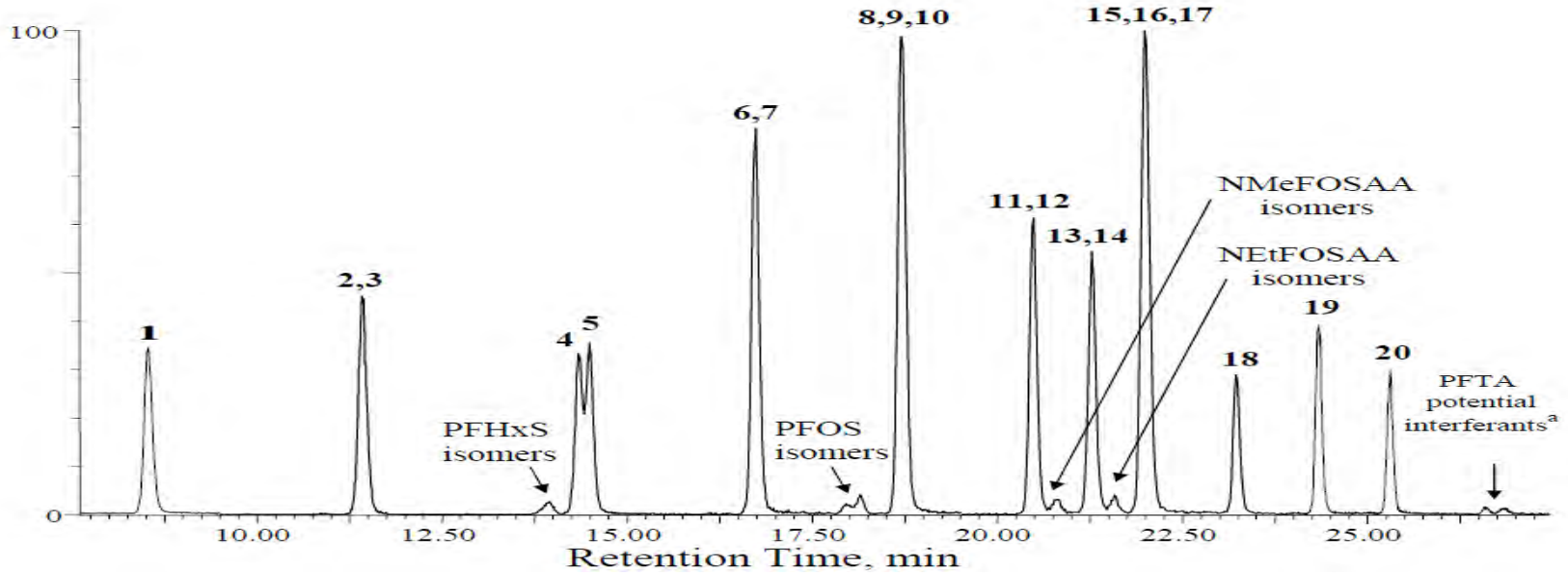
- The use of a Delay column is convenient when running multiple methods on the same instrument.
- "Delay" column and not a "Trapping" column. I.e. the background peak will be retention time separated but will be present.
- The delay column does not account for any contamination after the pump (the autosampler)

EPA 537: Perfluorinated Alkyl Acids in Drinking Water by SPE-LC/MS/MS

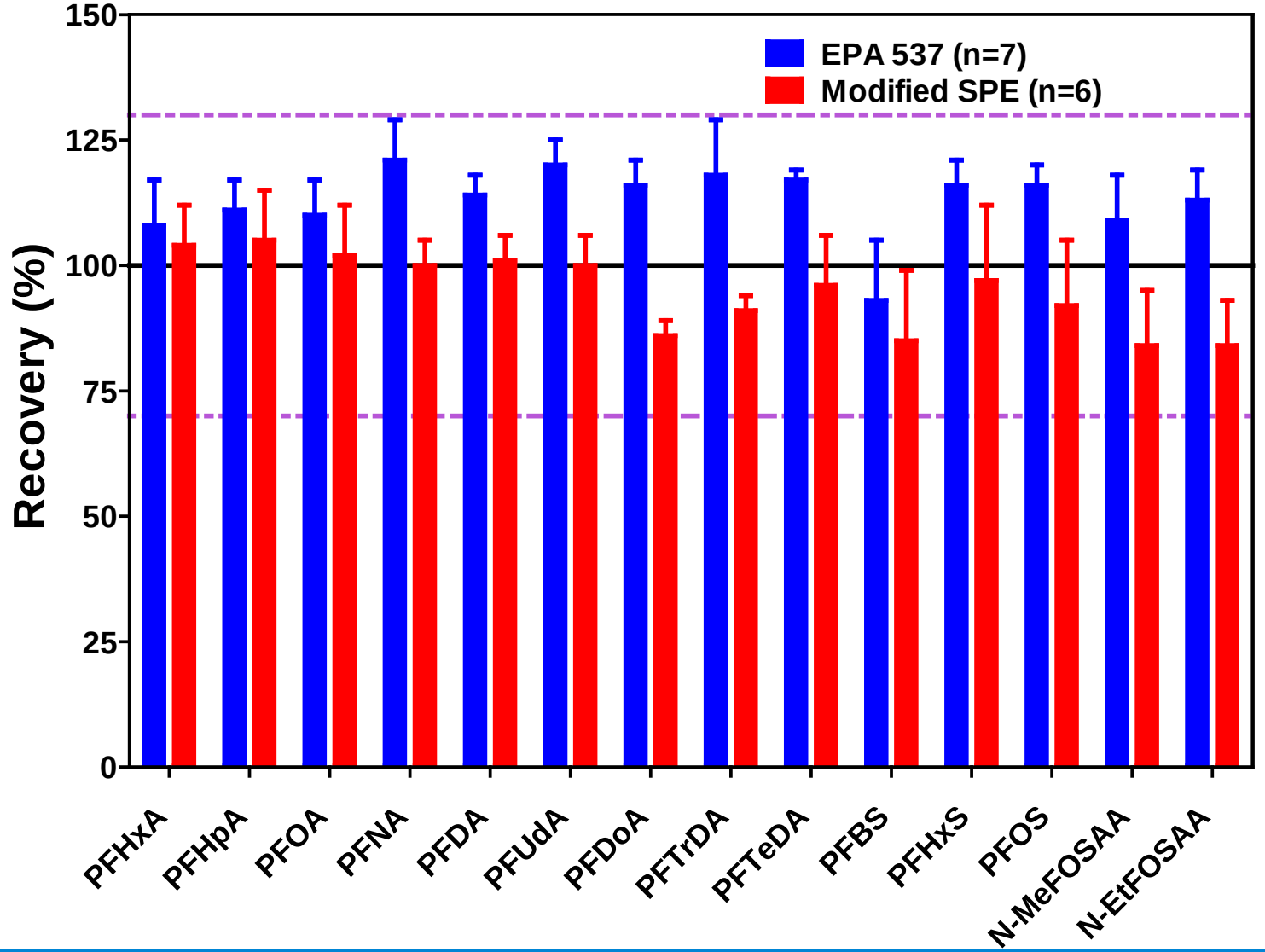
250 mL sample collected in Polypropylene Bottles with 5 g/L Trizma (to quench residual chlorine)

Solid Phase Extraction and evaporation to final 1 mL extract in ~96/4 MeOH/Water

Analysis by LC-MS/MS in Negative Electrospray mode for 14 analytes (9 PFCAs, 3 PFSAs, 2 FOSAAs) – 37 min analysis (10 µL injection)



Solid Phase Extraction
USEPA 537 vs Modified



- Recoveries calculated in Reagent Water
- Modified SPE cartridge: Agilent SampliQ WAX cartridge (6 cc, 150 mg)
- All recoveries between 70-130 %

Spiking concentrations:

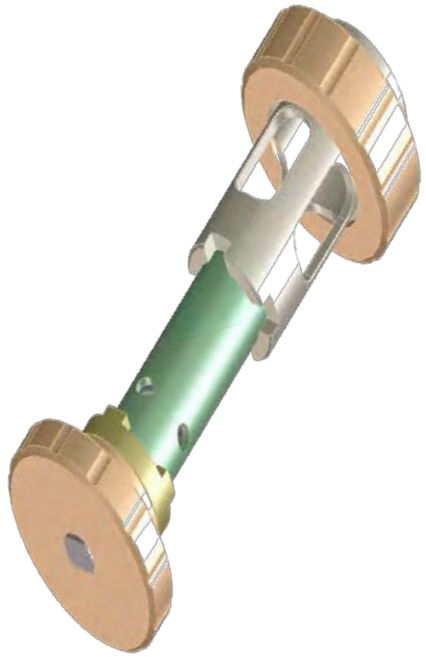
Modified SPE: 4 ng/L in 250 mL
EPA 537: 5-21 ng/L in 250 mL

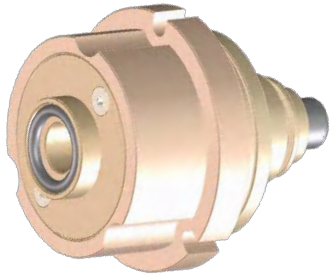
Final extract: 1 mL ~95% MeOH

Typical LC/TQ Lab Layout

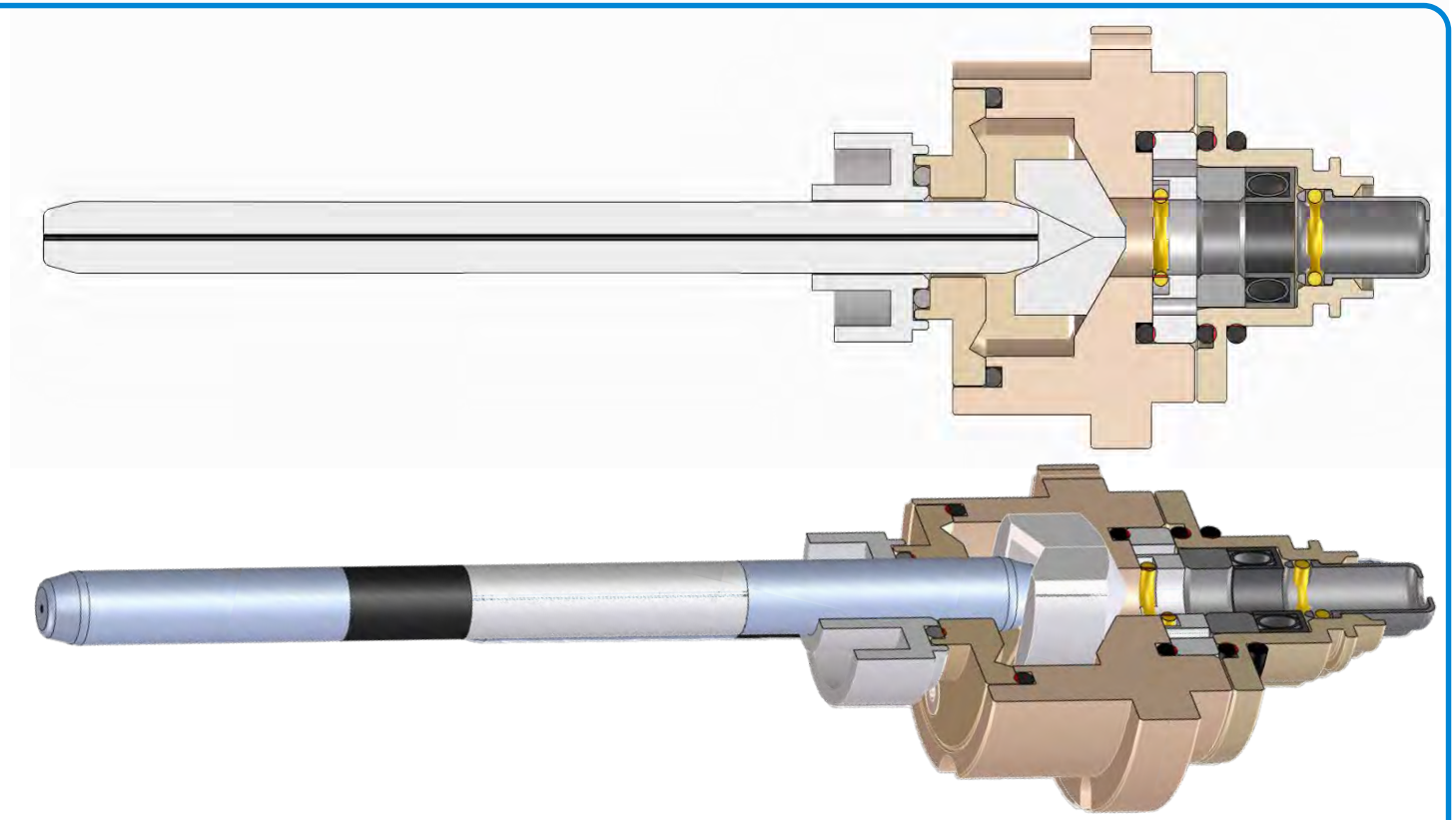
Quick Change Ion Injector

Fast Ion Injector exchange without system venting



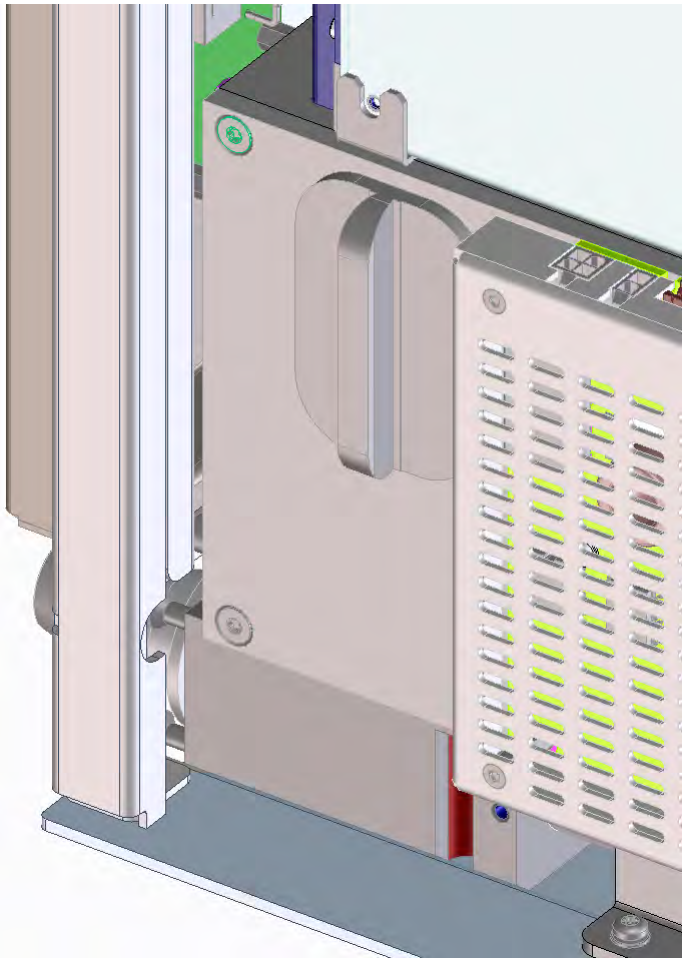


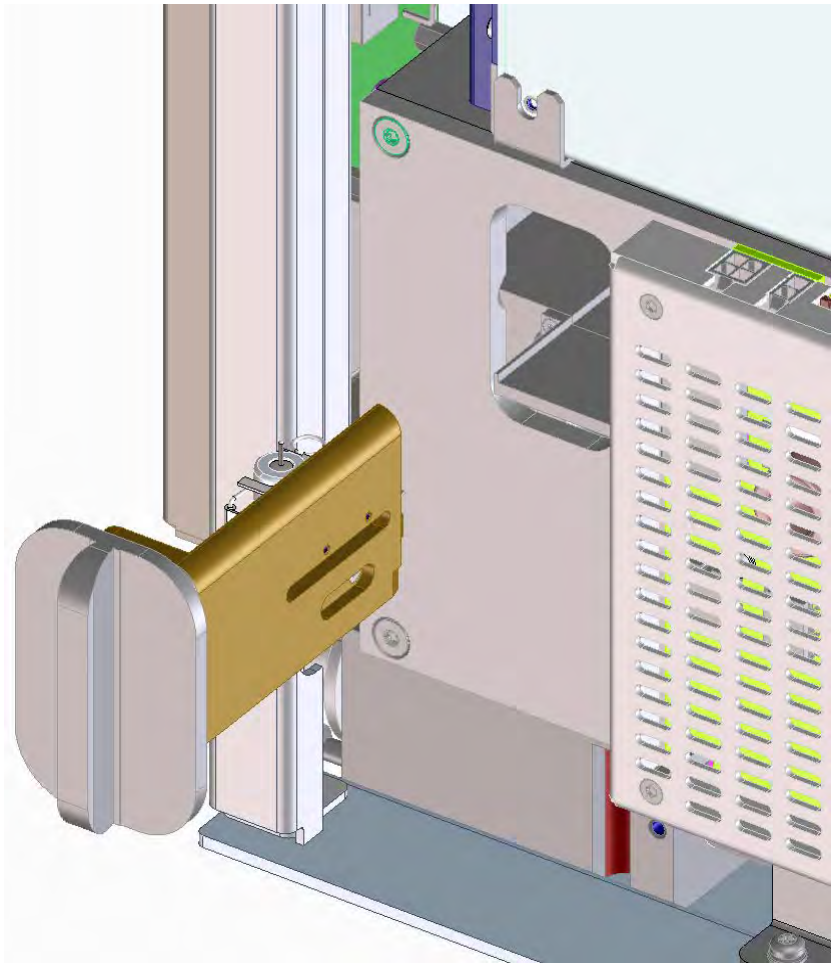
Ion Injector Tool ***VacShield***

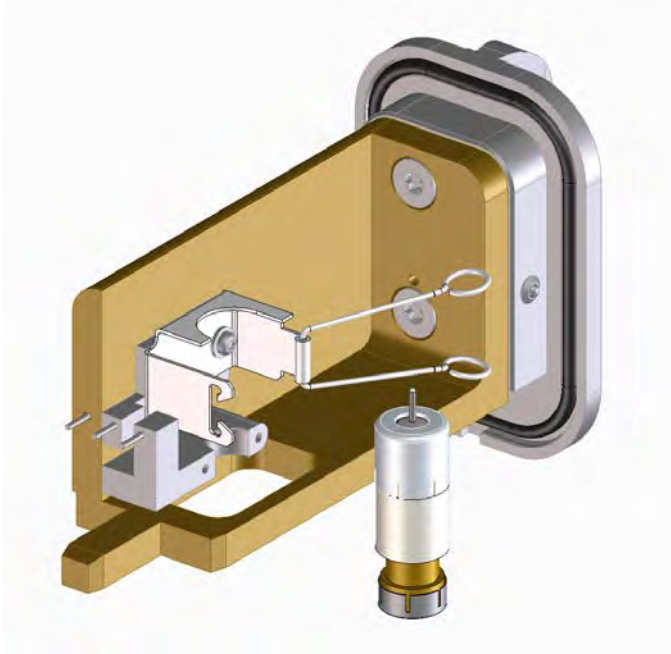


Easy to use, increased throughput, less downtime for maintenance

Easy Change Detector Assembly

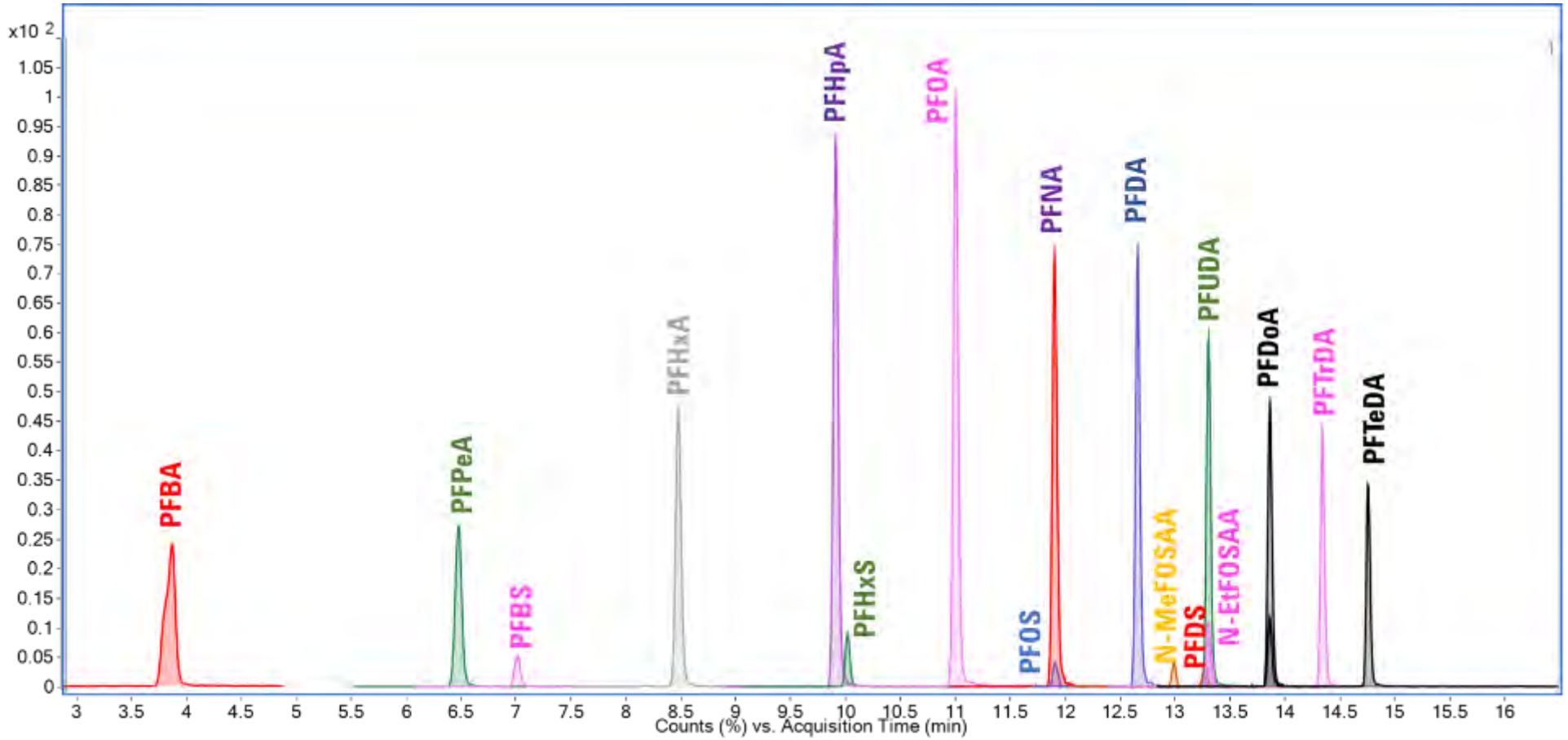






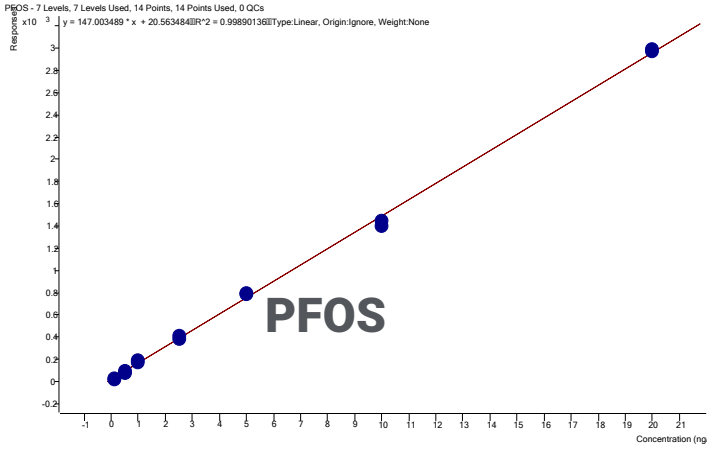
Analysis of PFAS in Drinking Water with Ultivo
EPA Method 537

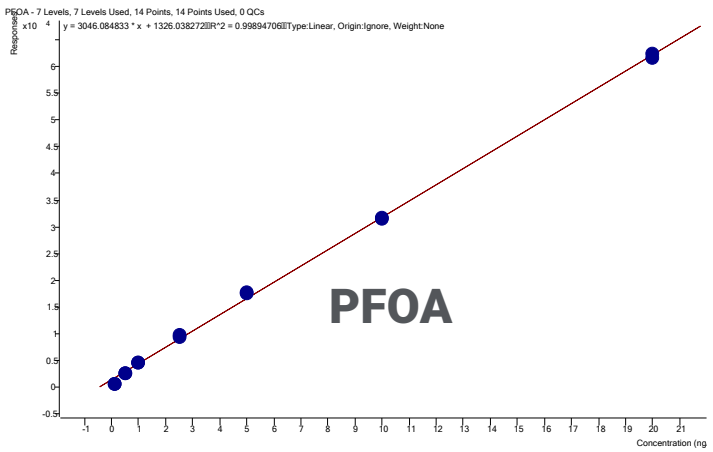
Analysis of 17 PFASs in drinking water with <0.1 ng/mL DLs in extract (includes all comps in EPA 537)



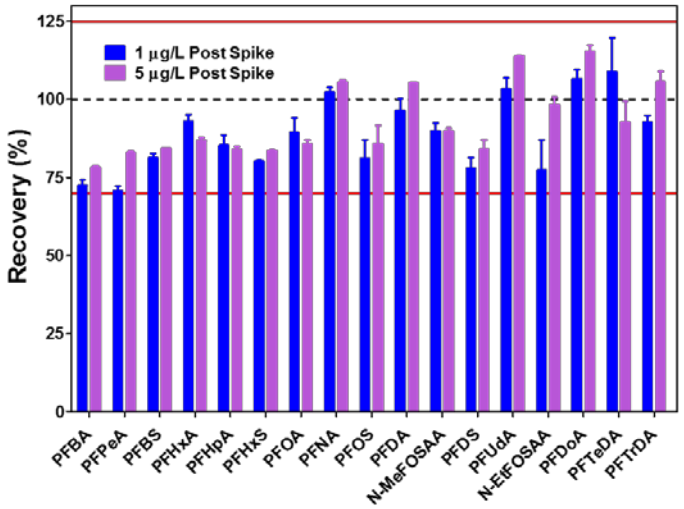
Analysis of PFAS in Drinking Water with Ultivo EPA Method 537

- All 17 PFASs had linear calibration curves, with $R^2 > 0.99$
- Overall recoveries were between 70–125% for both spiking levels
- %RSD was 0.3–10.8 % for all compounds at both spiking levels (1.0 µg/L and 5.0 µg/L)





Calibration Curves from 0.1-20 ng/mL for PFOA and PFOS

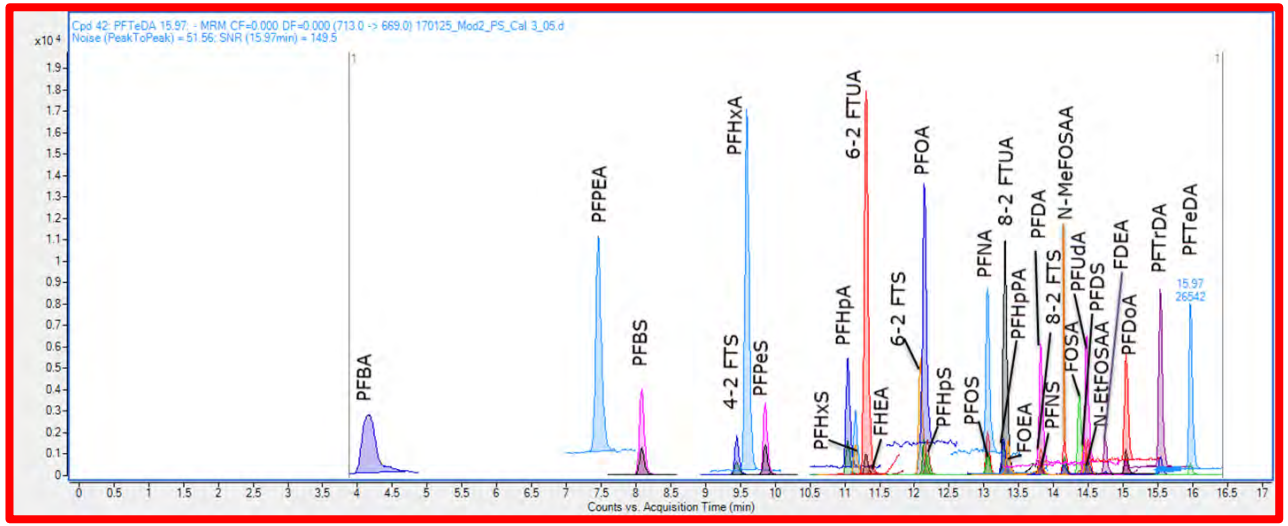


Post-spike recoveries of 17 PFASs in a water sample extracted according to EPA 537 with Agilent SampliQ WAX cartridges

Ultivo Triple Quad LC/MS

Analysis of 30 PFASs in Drinking Water
Agilent 1260 Infinity II + 6470 Triple Quadrupole LC-MS

Compound	Acronym	Class	Fluorinated C chain	EPA 537 or Additional
Perfluorobutanoate	PFBA	Acid	C4	Additional
Perfluoropentanoate	PFPeA	Acid	C5	Additional
Perfluorohexanoate	PFHxA	Acid	C6	537
Perfluoroheptanoate	PFHpA	Acid	C7	537
Perfluorooctanoate	PFOA	Acid	C8	537
Perfluorononanoate	PFNA	Acid	C9	537
Perfluorodecanoate	PFDA	Acid	C10	537
Perfluoroundecanoate	PFUdA	Acid	C11	537
Perfluorododecanoate	PFDoA	Acid	C12	537
Perfluorotridecanoate	PFTrDA	Acid	C13	537
Perfluorotetradecanoate	PFTeDA	Acid	C14	537
Perfluorooctanesulfonamide	FOSA	FOSA	C8	Additional
N-Ethyl-N-((heptadecafluorooctyl)sulfonyl)glycine	N-EtFOSAA	FOSAA	C8	537
N-((Heptadecafluorooctyl)sulfonyl)-N-methylglycine	N-MeFOSAA	FOSAA	C8	537
2-Perfluorohexyl ethanoic acid	FHEA	FTA-e	C6	Additional
2-Perfluorooctyl ethanoic acid	FOEA	FTA-e	C8	Additional
2-Perfluorodecyl ethanoic acid	FDEA	FTA-e	C10	Additional
3-Perfluoroheptyl propanoic acid (FHpPA)	PFHpPA	FTA-p	C7	Additional
4:2 Fluorotelomer sulfonate	4-2 FTS	FTS	C4	Additional
6:2 Fluorotelomer sulfonate	6-2 FTS	FTS	C6	Additional
8:2 Fluorotelomer sulfonate	8-2 FTS	FTS	C8	Additional
2H-Perfluoro-2-octanoic acid (FHUEA)	6-2 FTUA	FTUA	C6	Additional
2H-Perfluoro-2-decanoic acid (FOUEA)	8-2 FTUA	FTUA	C8	Additional
Perfluorobutylsulfonate	PFBS	Sulfonate	C4	537
Perfluoropentylsulfonate	PFPeS	Sulfonate	C5	Additional
Perfluorohexylsulfonate	PFHxS	Sulfonate	C6	537
Perfluoroheptylsulfonate	PFHpS	Sulfonate	C7	Additional
Perfluorooctylsulfonate	PFOS	Sulfonate	C8	537
Perfluorononylsulfonate	PFNS	Sulfonate	C9	Additional
Perfluorodecylsulfonate	PFDS	Sulfonate	C10	Additional

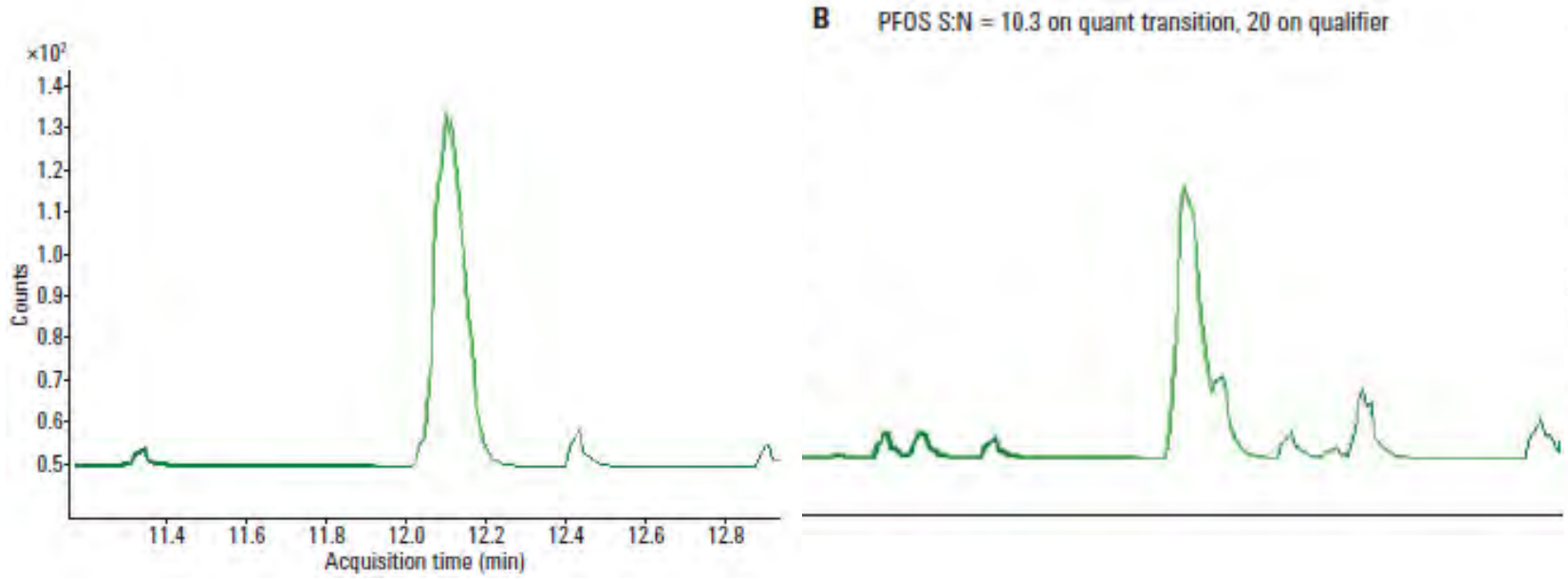


Chromatogram depicting MRM transitions of 30 PFASs post-spiked in a drinking water extract at 1 µg/L

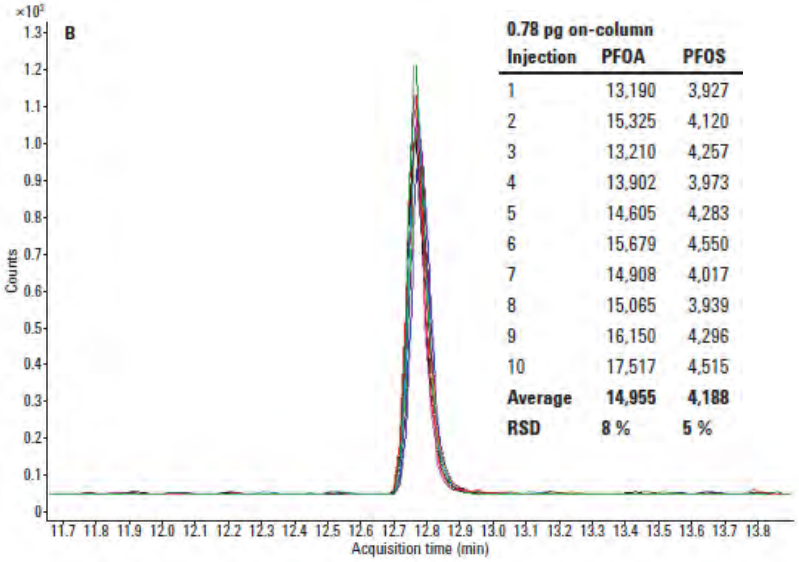
Application Note: Analysis of Per/Polyfluoroalkyl Substances in Water Using an Agilent 6470 Triple Quadrupole LC/MS (5991-7951EN)

Analysis of 30 PFASs in Drinking Water
Agilent 1260 Infinity II + 6470 Triple Quadrupole LC-MS

Sensitivity (0.08 ng/L equil. In water)



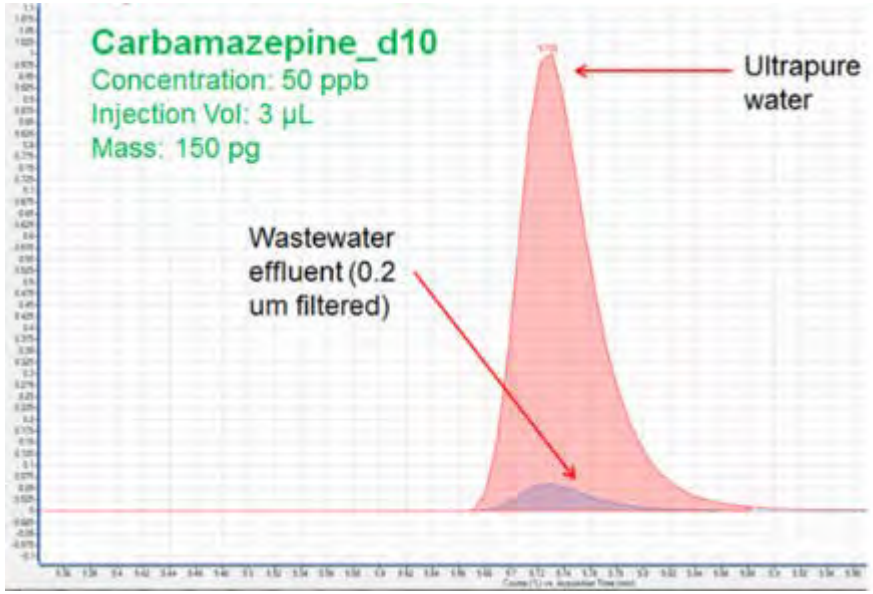
Precision



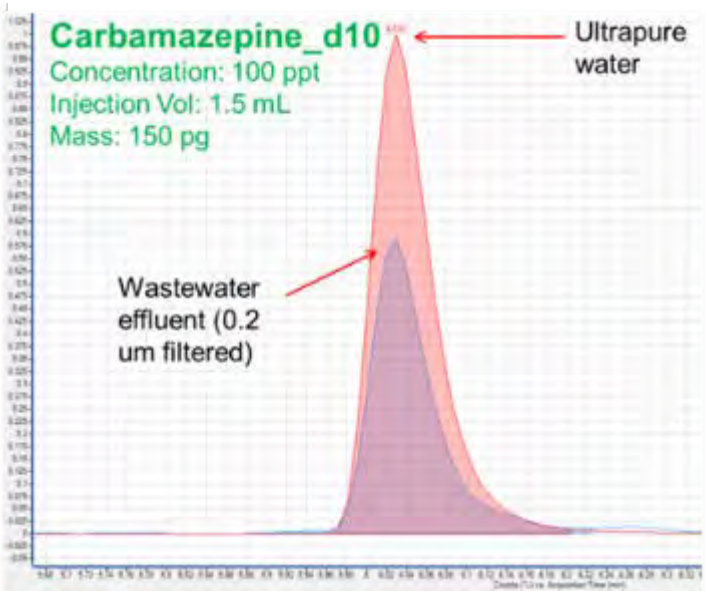
0.78 pg on-column

Injection	PFOA	PFOS
1	13,190	3,927
2	15,325	4,120
3	13,210	4,257
4	13,902	3,973
5	14,605	4,283
6	15,679	4,550
7	14,908	4,017
8	15,065	3,939
9	16,150	4,296
10	17,517	4,515
Average	**14,955**	**4,188**
RSD	**8 %**	**5 %**

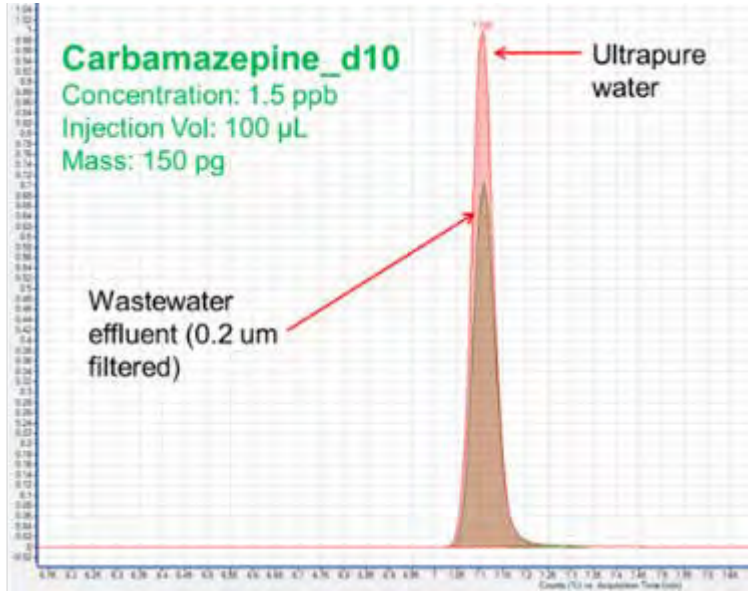
Analysis of Environmental Contaminants by LC-MS/MS
Ion Suppression Comparison by Sample Prep Method



Conventional SPE
Mass on Column :150 pg



Online SPE
Mass on Column :150 pg

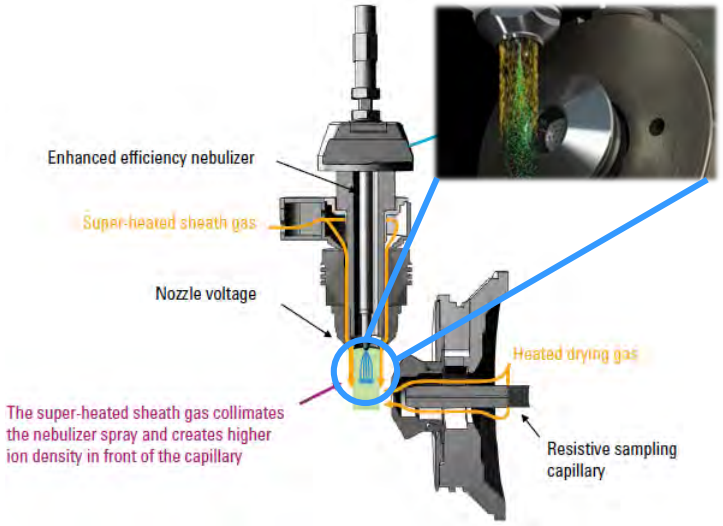


Direct Injection
Mass on Column :150 pg

Agilent 6495 Triple Quadrupole LC/MS
Ultimate Sensitivity, Extreme Reproducibility

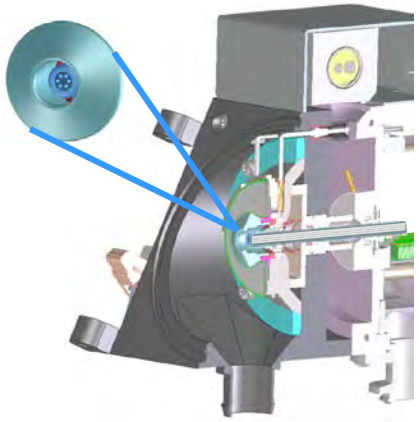
Agilent 6495 LC/TQ

Agilent Jet Stream



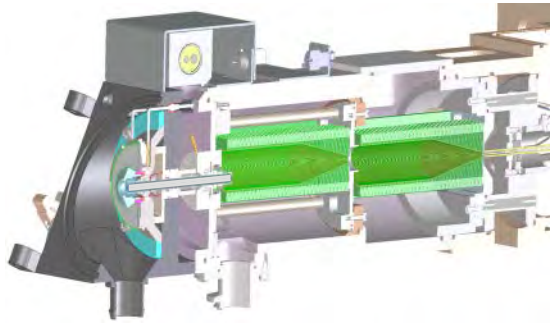
- **Thermal gradient focusing**
- **Efficient desolvation**
- **Creates an ion rich zone**

Hexabore Capillary



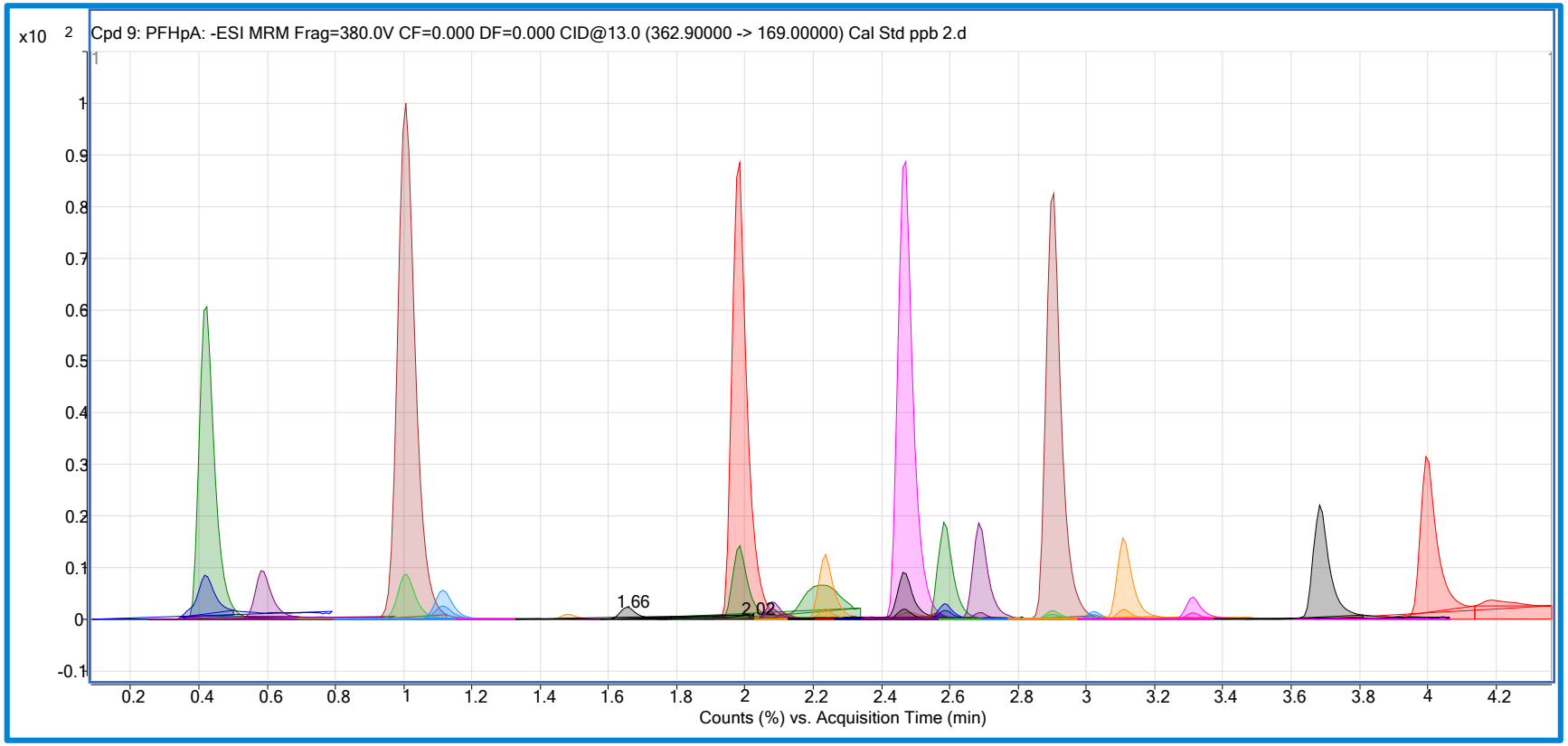
- **Six capillary inlets**
- **Samples x10 times more ion rich gas**

Dual Ion Funnel



- **Removes the gas but captures the ions**
- **Removes neutral noise**

Perfluorinated Alkyl Acids in Drinking Water by LC/TQ
Collaborative work with Suffolk County Water Authority



System

- 1290 Infinity LC coupled to a 6490 LC-MS/MS
- 14 PFAAs in 6 min

MDLs:

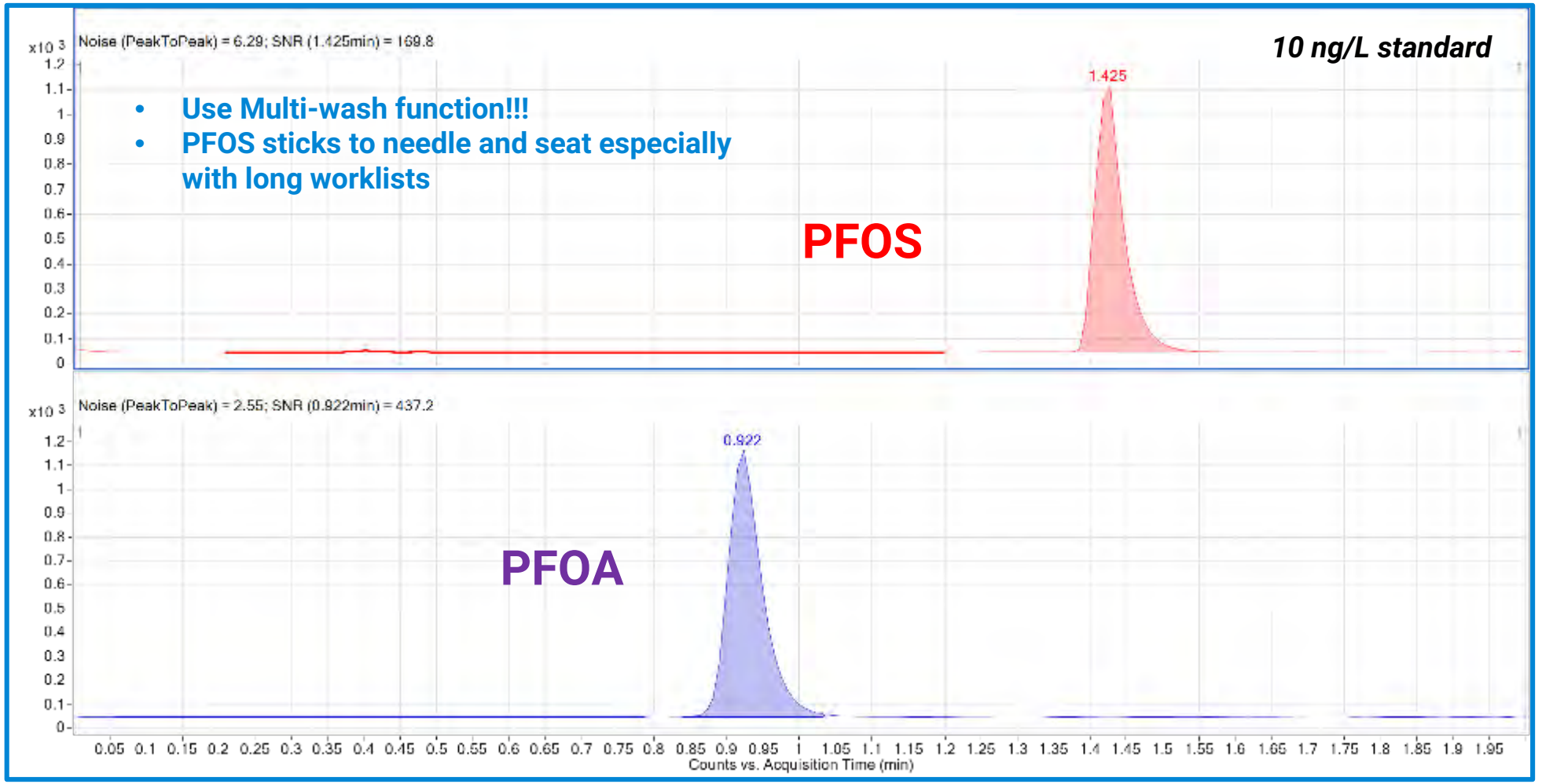
- PFOA, PFOS: <1 ng/L
- Others: 1-10 ng/L
- 80 µL aqueous injection

Matrices:

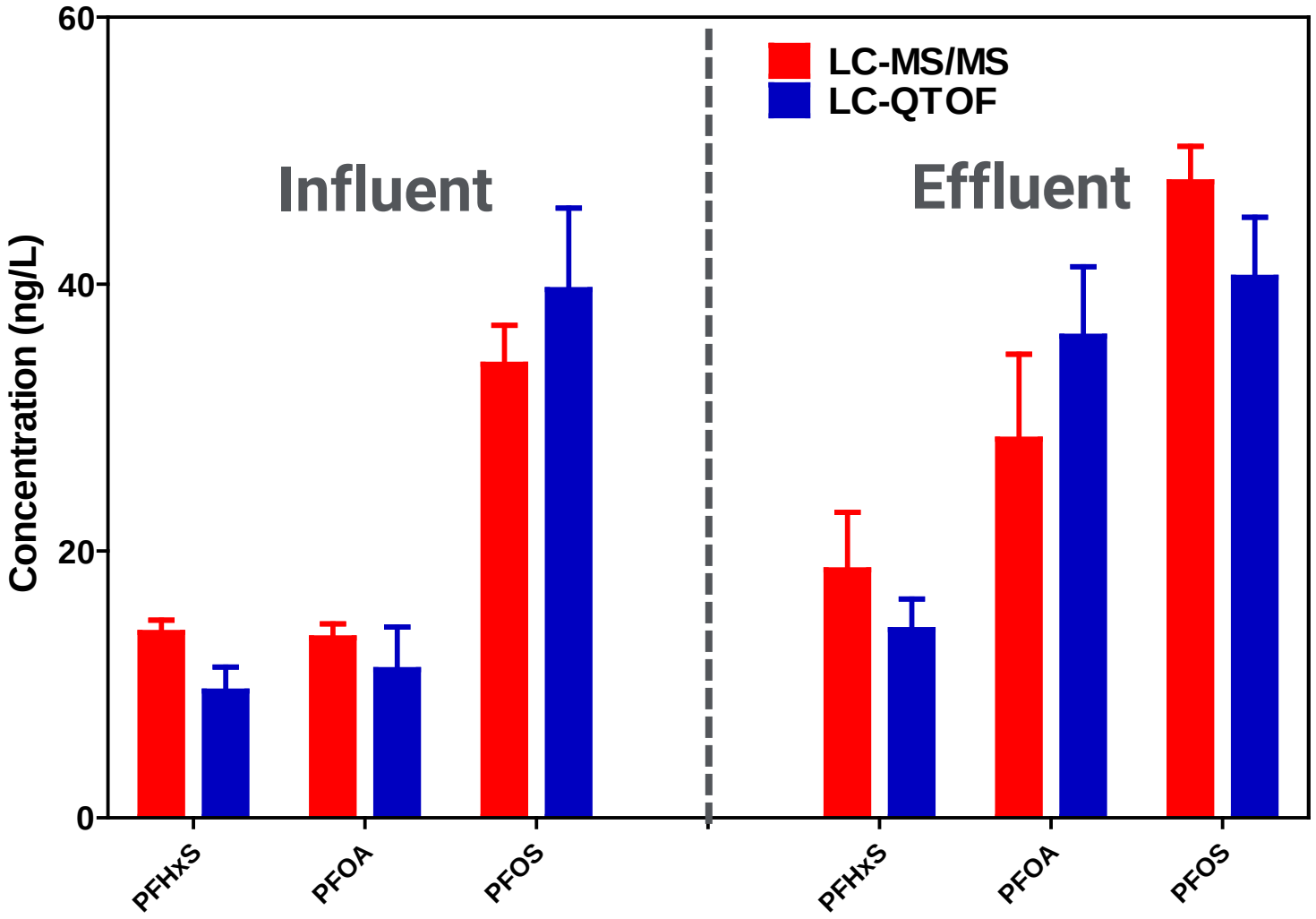
- Drinking Water
- Surface Water
- Ground Water

Analysis of PFOA/PFOS
Direct Injection; Rapid Analysis (1290 II UHPLC + 6495 LC-MS/MS)

Liquid Chromatograph	Agilent 1290 Infinity II Binary Pump
Mass Spectrometer	Agilent 6495 triple quadrupole LC-MS
Analytical Column	Agilent Poroshell 120 EC-C18; 3.0 x 50mm; 2.7um
Delay Column	Agilent Eclipse Plus C18, 4.6 x 50mm; 3.5um
Mobile Phase	A: Water+5mM Amm. Acetate B: Acetonitrile
Run Time	2 min
Injection Volume	80 uL (Water)



Targeted Analysis
LC-MS/MS vs LC-Q/TOF

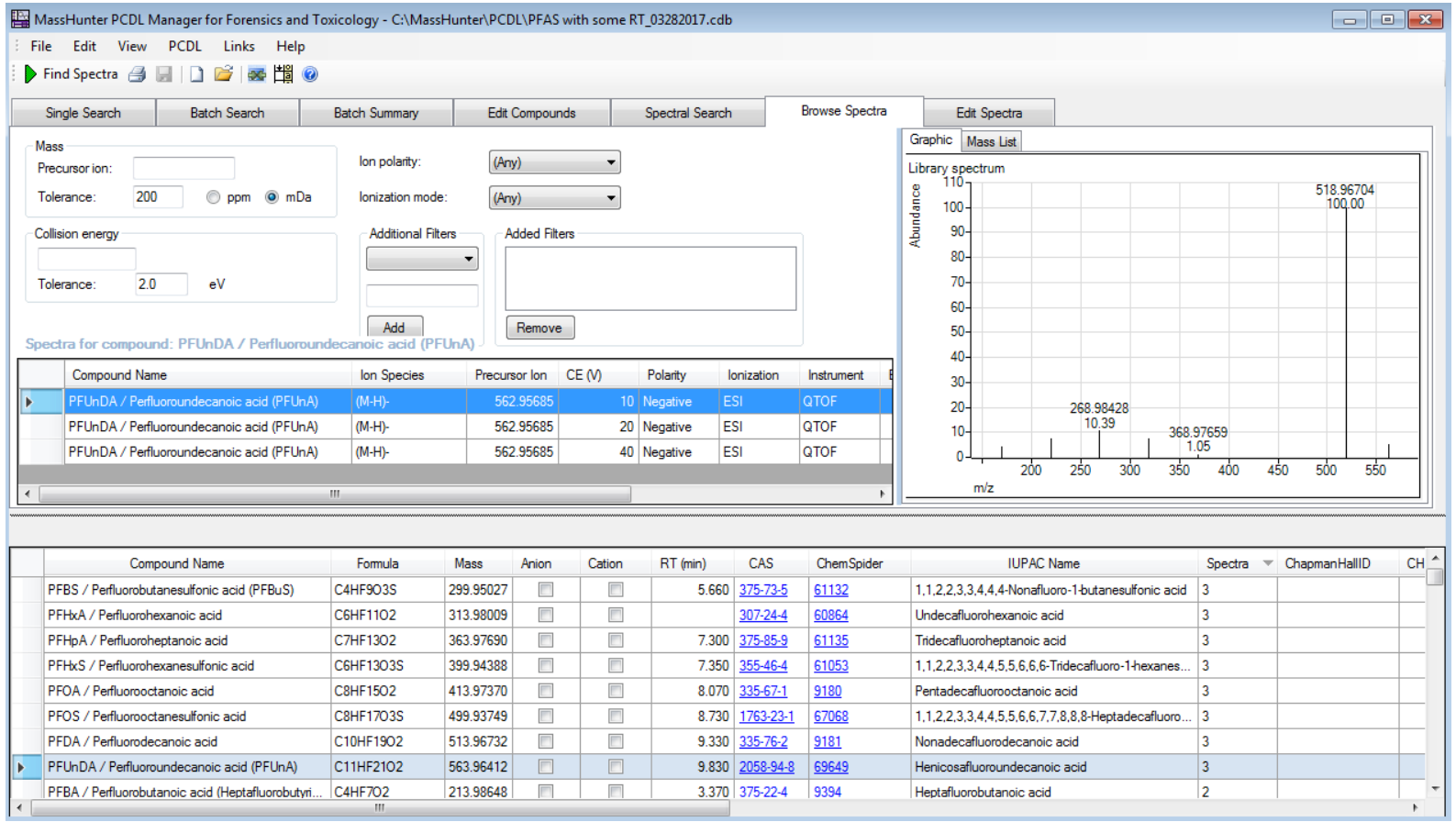


Agilent 6550 LC-Q/TOF

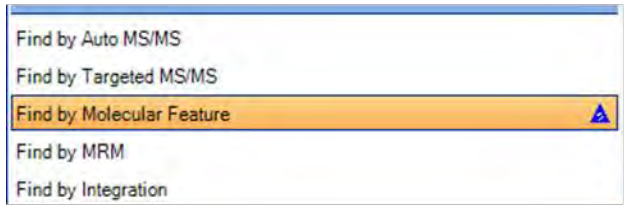
Collaborative project with RMIT University, Melbourne (Timothy Coggan ; Prof. Bradley Clarke)

Suspect Screening
Personal Compounds Database and Library with Spectra

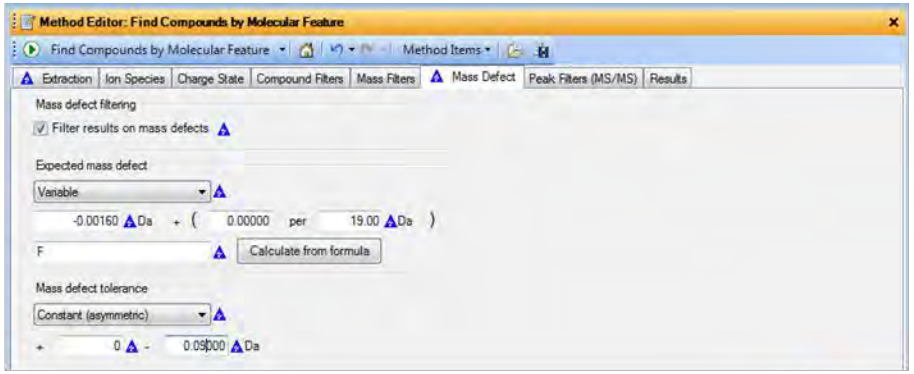
- Custom PFAS database with 106 compounds
- MS/MS spectra and Retention time data available for a subset of compounds



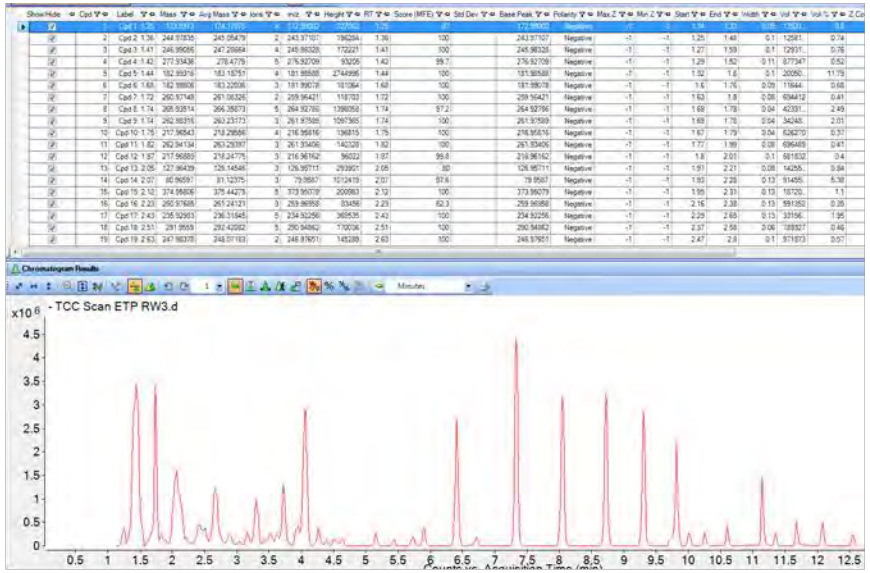
Untargeted Analysis



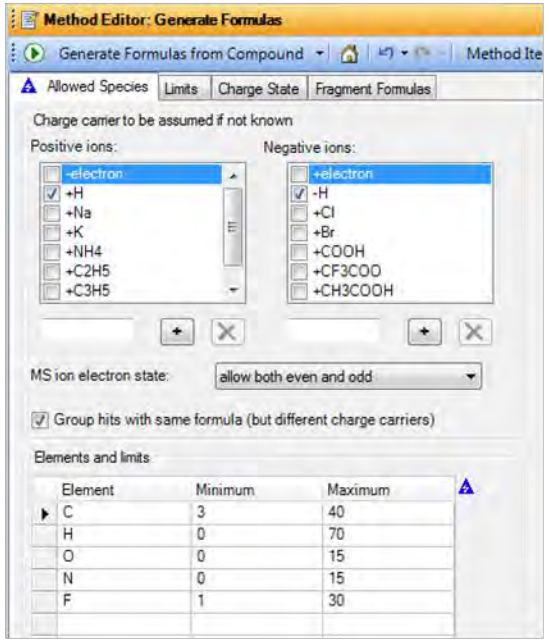
Feature Identification



Mass Defect for Fluorinated substances



Feature list with mass defect filters



Formula Generation

Untargeted Analysis
Identification of Novel PFAS

Show/Hide	Cpd	Label	Name	Formula	Score	Mass	Avg Mass	Ions	m/z	Height	RT
☑	73	Cpd 73: C9...		C9 H5 F13...	97.81	565.96073	566.05939	3	564.95342	870578	9.82
☑	64	Cpd 64: C8...		C8 H4 F11...	92.3	372.99366	373.03874	3	371.98639	1991326	8.05
☑	69	Cpd 69: C9...		C9 H F15...	90.92	505.94803	506.00974	3	504.94081	201819	8.72
☑	78	Cpd 78: C1...		C11 H2 F2...	85.79	615.95987	616.05175	2	614.95267	126557	10.25
☑	77	Cpd 77: C9...		C9 H2 F17...	84.95	570.96636	571.06182	2	569.95916	137880	10.25
☑	19	Cpd 19: C7...		C7 H3 F N...	84.42	247.98374	248.07183	2	246.97651	145289	2.63
☑	61	Cpd 61: C6...		C6 H2 F8...	83.91	401.94769	402.01514	2	400.94045	574068	7.33
☑	41	Cpd 41: C3...		C3 H F O3	82.62	103.9908	104.14269	3	102.98394	84059	3.95
☑	59	Cpd 59: C7...		C7 H2 F6...	81.12	405.94909	406.00834	2	404.94184	212333	7.33
☑	1	Cpd 1: C6...		C6 H3 F O5	80.14	173.99683	174.17875	4	172.99002	327062	1.25
☑	2	Cpd 2: C5...		C5 H2 F3...	79.17	244.97817	245.05479	2	243.97107	196284	1.36
☑	68	Cpd 68: C6...		C6 H7 F9...	79.14	423.99377	424.03414	2	422.9865	1705215	8.72
☑	5	Cpd 5: C4...		C4 H4 F O7	77.73	182.99288	183.18751	4	181.98588	2744996	1.44
☑	7	Cpd 7: C8...		C8 H F2 N...	76.3	260.97134	261.08326	2	259.96421	118703	1.72
☑	32	Cpd 32: C1...		C12 H4 F2...	75.8	329.98252	330.32489	4	328.97589	98662	3.43
☑	42	Cpd 42: C5...		C5 H F3 N...	74.04	257.97381	258.25562	4	256.9671	184002	3.96
☑	36	Cpd 36: C4...		C4 H4 F O6	74.03	166.99785	167.16441	3	165.99097	135303	3.72
☑	43	Cpd 43: C7...		C7 H F3 N...	74	219.98596	220.24417	4	218.97938	136151	4.03
☑	40	Cpd 40: C1...		C10 H F3...	73.9	329.98497	330.32165	4	328.97832	112221	3.93
☑	39	Cpd 39: C1...		C13 H F2...	73.49	302.98512	303.26718	3	301.9786	114865	3.91
☑	9	Cpd 9: C6...		C6 H2 F N...	71.71	262.98287	263.23173	3	261.97589	1097365	1.74
☑	3	Cpd 3: C8...		C8 H4 F O8	69.64	246.9896	247.28664	4	245.98328	172221	1.41
☑	48	Cpd 48: C8...		C8 H2 F N...	68.78	290.96606	291.26063	3	289.95962	89006	4.39
☑	16	Cpd 16: C5...		C5 H F4 N...	68.15	260.97674	261.24121	3	259.96958	83456	2.23
☑	12	Cpd 12: C4...		C4 H F3 O7	65.37	217.96735	218.24775	3	216.96162	96022	1.87
☑	21	Cpd 21: C7...		C7 H2 F N...	64.49	294.9623	295.31824	5	293.95612	353418	2.67
☑	79	Cpd 79: C3...		C3 H F5 O3	64.01	179.9837	180.27145	4	178.97717	161022	10.6
☑	52	Cpd 52: C7...		C7 H5 F2...	63.42	332.97801	333.3129	3	331.97118	101205	5.44
☑	15	Cpd 15: C8...		C8 H2 F3...	62.58	374.95645	375.44275	5	373.95079	200983	2.12
☑	87	Cpd 87: C4...		C4 H3 F N...	60.74	179.99326	180.24644	3	178.98673	85689	12.55
☑	6	Cpd 6: C7...		C7 H2 F N...	59.54	182.99709	183.22036	3	181.99078	181064	1.68
☑	18	Cpd 18: C5...		C5 H F3 N...	59.02	291.95427	292.42082	5	290.94862	170036	2.51
☑	75	Cpd 75: C3...		C3 H F N O7	52.07	181.97636	182.0593	2	180.96904	133909	9.86
☑	49	Cpd 49: C4...		C4 H F3 N...	51.82	263.95848	264.38963	5	262.95261	104212	4.52
☑	63	Cpd 63: PF...	PFOA 13...	C4 [13C]4...	49.76	417.98671	418.04076	4	416.97945	886238	8.05
☑	67	Cpd 67: PF...	PFNA 13...	C4 [13C]5...	49.56	468.98666	469.03418	4	467.9794	978955	8.72

Inclusion list for targeted MS/MS and structural elucidation using fragment information using software tools (Molecular structure correlator)

Conclusions

- ✓ Background contamination of PFASs is prevalent in ALL systems but can be fixed through systematic elimination of fluoropolymers in the LC.
- ✓ The Ultivo Triple Quad LC/MS offers significantly more sensitivity than EPA 537 analysis while using a smaller footprint through several innovative technologies.
- ✓ The sensitive analysis of several newer classes of PFASs is possible using the Ultivo and 6470.
- ✓ Rapid analysis of PFOA/PFOS and PFAAs can be performed by direct large volume aqueous injection using the 6495 with <1 ng/L MDLs.
- ✓ Identification, screening and quantification of ‘emerging’ PFASs has been shown using the Agilent LC-QTOF

References

Analysis of Per/Polyfluoroalkyl Substances in Water Using an Agilent 6470 Triple Quadrupole LC/MS

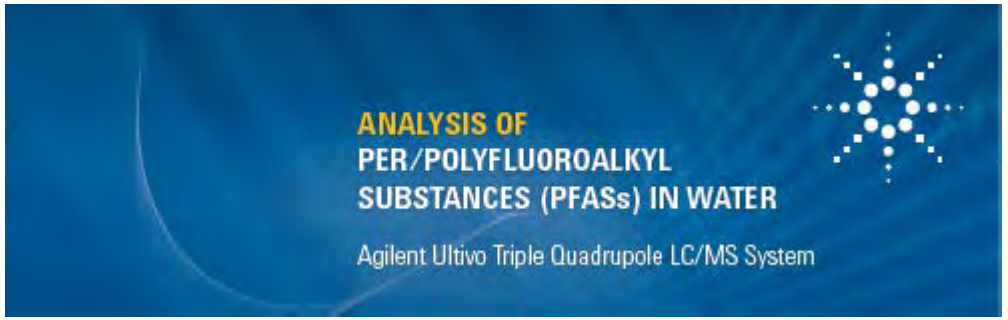
Application Note

5991-7951EN

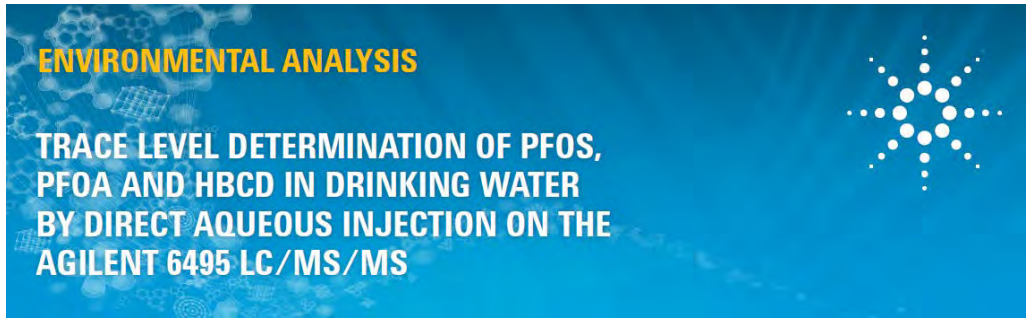
Recommended Plumbing Configurations for Reduction in Per/Polyfluoroalkyl Substance Background with Agilent 1260/1290 Infinity (II) LC Systems

Application Note

5991-7863EN



5991-8156EN



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