A single LC-MS/MS platform for EPA PFAS regulated methods with a novel dual channel liquid chromatography option to improve productivity and flexibility

Presented By: Ed George

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The world leader in serving science

Thermo strategies around PFAS Analysis


Application note | 002902

Environmental

Direct injection of drinking water for the analysis of 54 PFAS compounds by LC-MS/MS aligned with current and evolving global regulations

Authors
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Keywords
Direct injection, PFAS, drinking water, European regulation

Application benefits
- A simple, reproducible, and robust sample preparation based on the dilution of the sample with mobile phase B followed by direct injection for reducing labor, errors, and potential contamination issues compared to standard methods requiring pre-concentration with solid phase extraction.
- A complete PFAS workflow solution using direct injection of 100 µL of water samples that meets challenging regulatory detection and reporting limits within the EU and UK.
- Dedicated instrumental setup and appropriate sample handling procedures have been established to overcome the main challenges of this application which are sensitivity, contamination, and carryover handling.

Goal
To demonstrate the quantitative measurement of 54 per- and polyfluoroalkyl substances (PFAS) in water samples by direct injection with precision and accuracy that meets current European regulatory reporting requirements on the Thermo Scientific™ TSQ Altis™ Plus triple quadrupole mass spectrometer.

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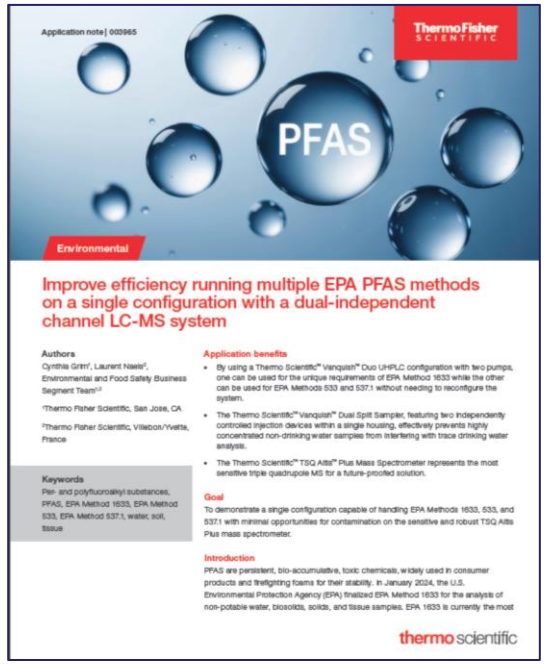
Application note | 002965

PFAS

Environmental

Improve efficiency running multiple EPA PFAS methods on a single configuration with a dual-independent channel LC-MS system

Authors
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[1]Thermo Fisher Scientific, San Jose, CA

[2]Thermo Fisher Scientific, Villebon/Yvette, France

Keywords
Per- and polyfluoroalkyl substances, PFAS, EPA Method 1633, EPA Method 533, EPA Method 537.1, water, soil, tissue

Application benefits
- By using a Thermo Scientific™ Vanquish™ Duo UHPLC configuration with two pumps, one can be used for the unique requirements of EPA Method 1633 while the other can be used for EPA Methods 533 and 537.1 without needing to reconfigure the system.
- The Thermo Scientific™ Vanquish™ Dual Split Sampler, featuring two independently controlled injection devices within a single housing, effectively prevents highly concentrated non-drinking water samples from interfering with trace drinking water analysis.
- The Thermo Scientific™ TSQ Altis™ Plus Mass Spectrometer represents the most sensitive triple quadrupole MS for a future-proofed solution.

Goal
To demonstrate a single configuration capable of handling EPA Methods 1633, 533, and 537.1 with minimal opportunities for contamination on the sensitive and robust TSQ Altis Plus mass spectrometer.

Introduction
PFAS are persistent, bio-accumulative, toxic chemicals, widely used in consumer products and firefighting foams for their stability. In January 2024, the U.S. Environmental Protection Agency (EPA) finalized EPA Method 1633 for the analysis of non-potable water, biosolids, solids, and tissue samples. EPA 1633 is currently the most

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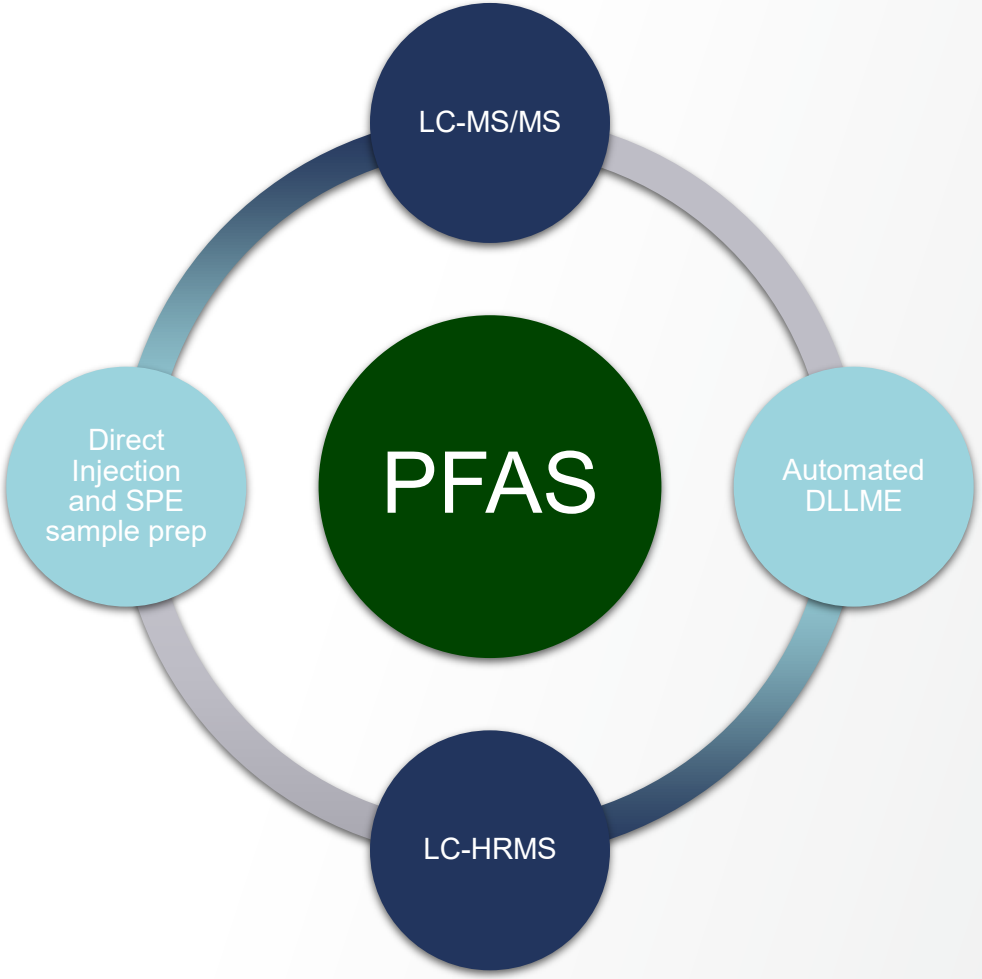

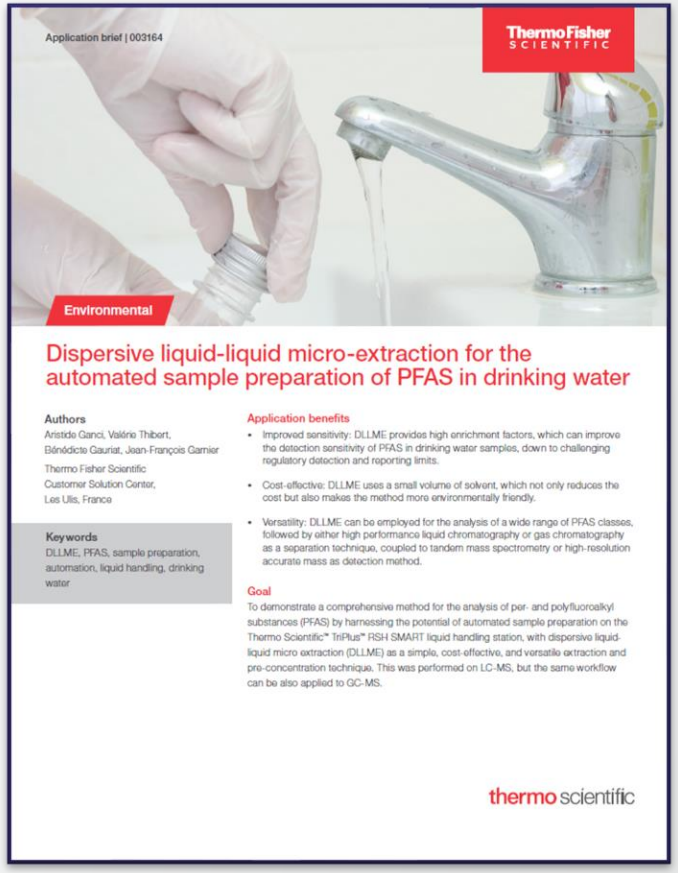
Application brief | 003164

Environmental

Dispersive liquid-liquid micro-extraction for the automated sample preparation of PFAS in drinking water

Authors
Aristide Ganci, Valérie Thibert, Bénédicte Gauriat, Jean-François Garnier

Thermo Fisher Scientific
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Les Ulis, France

Keywords
DLLME, PFAS, sample preparation, automation, liquid handling, drinking water

Application benefits
- Improved sensitivity: DLLME provides high enrichment factors, which can improve the detection sensitivity of PFAS in drinking water samples, down to challenging regulatory detection and reporting limits.
- Cost-effective: DLLME uses a small volume of solvent, which not only reduces the cost but also makes the method more environmentally friendly.
- Versatility: DLLME can be employed for the analysis of a wide range of PFAS classes, followed by either high performance liquid chromatography or gas chromatography as a separation technique, coupled to tandem mass spectrometry or high-resolution accurate mass as detection method.

Goal
To demonstrate a comprehensive method for the analysis of per- and polyfluoroalkyl substances (PFAS) by harnessing the potential of automated sample preparation on the Thermo Scientific™ TriPlus™ RSH SMART liquid handling station, with dispersive liquid-liquid micro extraction (DLLME) as a simple, cost-effective, and versatile extraction and pre-concentration technique. This was performed on LC-MS, but the same workflow can be also applied to GC-MS.

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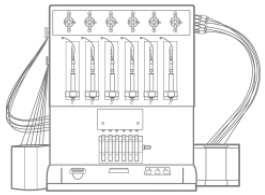
PFAS analysis – Summary of Available Workflows

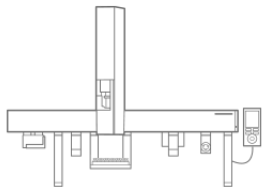
Workflow strategies

Sample collection ▶ **Sample preparation** ▶ **Analysis** ▶ **Data Processing**

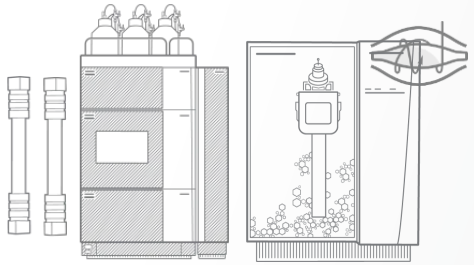


(Automated) SPE
AutoTrace 280

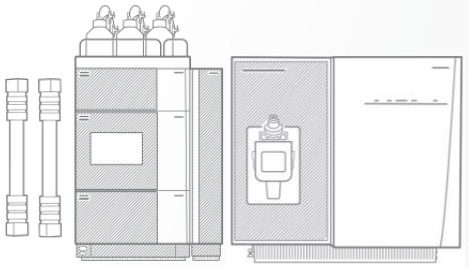
Direct Injection



TriPlus RSH SMART
Automated DLLME



Vanquish Flex / Duo UHPLC +
Orbitrap Exploris MX
Mass Spectrometer



Vanquish Flex / Duo UHPLC +
TSQ Altis Plus Series Triple
Quadrupole Mass Spectrometer

Chromeleon CDS software
• Targeted quantification

2 Channel Tandem LC system

- LC-MS/MS 533, 537.1, 1633, (soon EPA 563 and 534)
- Complete methods and reporting templates
- 2 Independent Flow Paths- single point control
- Automatic or on-demand switching from one channel to the other
- One LC to save capital and warranty costs

Ready Solutions for EPA Regulated PFAS

The National Primary Drinking Water Regulations (NPDWR) have set MCLs (maximum contaminant levels) for PFOA and PFOS in drinking water at 4 ppt.

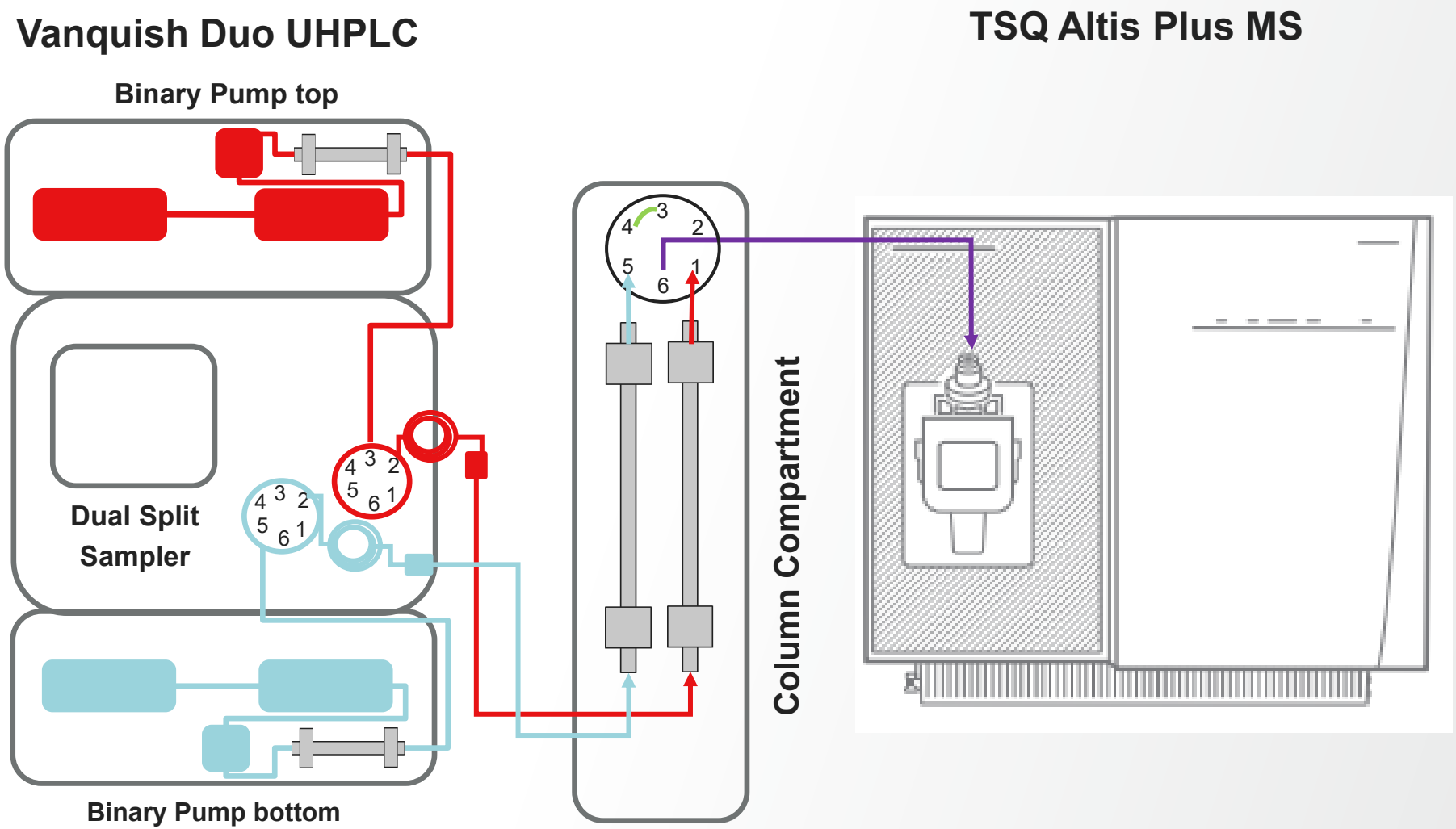
CAS	Name	EPA Method
763051-92-9	11Cl-PF2OUdS	533/537.1/1633
151772-58-6	3,6-OPFHpA (NFDHA)	533/1633
356-02-5	3:3 FTCA	1633
757124-72-4	4:2FTS	533/1633/8327
914637-49-3	5:3 FTCA	1633
27619-97-2	6:2FTS	533/1633/8327
812-70-4	7:3 FTCA	1633
39108-34-4	8:2FTS	533/1633/8327
756426-58-1	9Cl-PF3ONS	533/537.1/1633
919005-14-4	ADONA (DONA)	533/537.1/1633
754-91-6	FOSA (PFOSA)	1633/8327
13252-13-6	HFPO-DA (Gen X)	533/537.1/1633
4151-50-2	N-EtFOSA	1633
2991-50-6	N-EtFOSAA	537.1/1633/8327
1691-99-2	N-EtFOSE	1633
31506-32-8	N-MeFOSA	1633
2355-31-9	N-MeFOSAA	537.1/1633/8327
24448-09-7	N-MeFOSE	1633
377-73-1	PF4OPeA (PFMPA)	533/1633
863090-89-5	PF5HxA (PFMBA)	533/1633
375-22-4	PFBA	533/1633/8327
375-73-5	PFBS	533/537.1/1633/8327
335-76-2	PFDA	533/537.1/1633/8327
307-55-1	PFDoA (PFDoDA)	533/537.1/1633
79780-39-5	PFDoS (PFDoDS)	1633
335-77-3	PFDS	1633/8327
113507-82-7	PFEESA	533/1633
375-85-9	PFHpA	533/537.1/1633/8327
375-92-8	PFHpS	533/1633/8327
307-24-4	PFHxA	533/537.1/1633/8327
355-46-4	PFHxS	533/537.1/1633/8327

CAS	Name	EPA Method
375-95-1	PFNA	537.1/1633/8327
68259-12-1	PFNS	1633/8327
335-67-1	PFOA	533/537.1/1633/8327
1763-23-1	PFOS	533/537.1/1633/8327
2706-90-3	PFPeA	533/1633/8327
2706-91-4	PFPeS	533/1633/8327
376-06-7	PFTeDA (PFTeA)	1633/8327
72629-94-8	PFTrDA (PFTrA)	537.1/1633/8327
2058-94-8	PFUdA (PFUnDA; PFUnA)	533/537.1/1633/8327
120226-60-0	10:2FTS	
34455-29-3	6:2 FTAB (Capstone B)	
647-42-7	6:2 FTOH (FHET)	
943913-15-3	6:2/8:2diPAP	
57677-95-9	6:2diPAP	
1546-95-8	7HPFHpA	
678-39-7	8:2 FTOH (FOET)	
70887-84-2	8:2 FTUCA (FOUEA)	
678-41-1	8:2diPAP	
34598-33-9	8:3FTCA	
30334-69-1	FBSA	
41997-13-1	FHxSA	
27854-31-5	FOEA (8:2 FTCA)	
13252-14-7	HFPO-TA	
68298-12-4	N-MeFBSA	
159381-10-9	N-MeFBSAA	
646-83-3	PFECHS	
67905-19-5	PFHxDA	
16517-11-6	PFOcDA (PFODA)	
791563-89-8	PFTrDS	
749786-16-1	PFUnDS	

CAS	Name	EPA Method
375-95-1	TFSI	563
68259-12-1	PFEtS	563
335-67-1	PFMOAA	563
1763-23-1	PFPrA	563
2706-90-3	PFPrS	563
2706-91-4	TFA	563
376-06-7	TFMS	563
13252-13-6	HFPO-DA (Gen X)	534
375-73-5	PFBS	534
335-76-2	PFDA	534
375-85-9	PFHpA	534
355-46-4	PFHxS	534
307-24-4	PFHxA	534
375-95-1	PFNA	534
335-67-1	PFOA	534
1763-23-1	PFOS	534

Newer EPA Methods in Draft

Advanced Dual Channel Configuration



Overview of the method

Dual Channel LC system applied to EPA methods 1633, 533, and 537.1

Parameter	EPA 533/537.1/1633
Analytical column	Thermo Scientific™ Hypersil GOLD™, 2.1 x 50 mm, 1.9 µm
Delay column	Thermo Scientific™ Hypersil GOLD™, 2.1 x 50 mm, 1.9 µm
Column temperature	40 °C
Injection volume	5 µL
Autosampler temperature	22 °C
Flow rate	0.4 mL/min

Parameter	Value
Spray voltage (V)	-500
Sheath gas	40
Aux gas	10
Sweep gas	0
Ion transfer tube (°C)	200
Vaporizer temperature (°C)	300
Q1 resolution (FWHM)	0.7
Q3 resolution (FWHM)	0.7
CID gas (mTorr)	2.5

- Mobile Phase for EPA Method 1633
  - ACN ensures proper bile acids separation from PFOS to meet method requirement

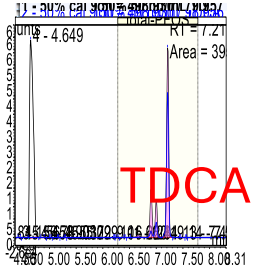
A: H2O + 2% ACN + 2 mM ammonium acetate + 0.1% acetic acid
B: ACN + 2% H2O + 2 mM ammonium acetate + 0.1% acetic acid

- Mobile Phase for EPA 533/537.1
  - Best performance when using MeOH in the mobile phase

H2O + 2% MeOH + 2 mM ammonium acetate + 0.1% acetic acid
MeOH + 2% H2O + 2 mM ammonium acetate + 0.1% acetic acid

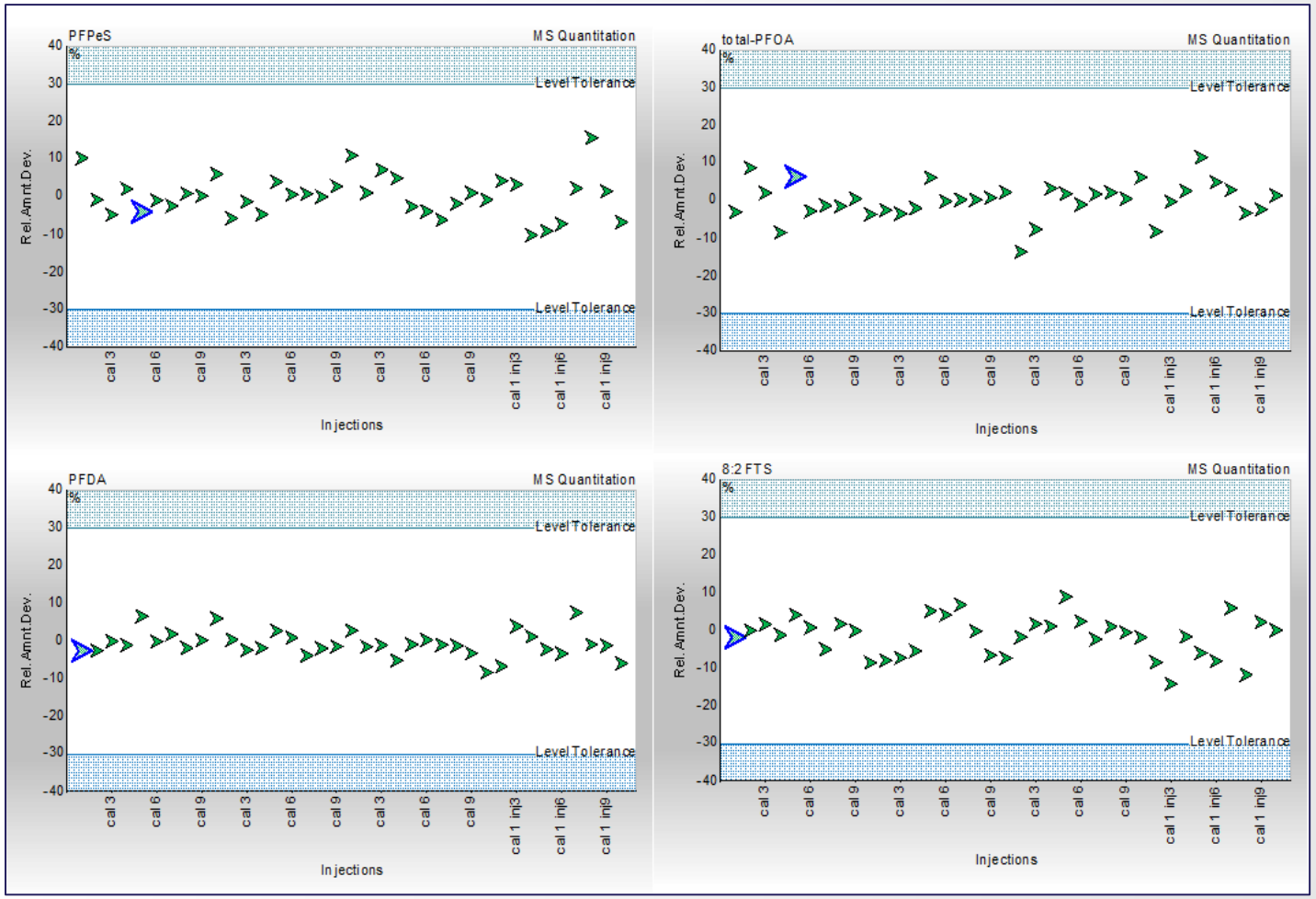
Results

1633 Bile acid and PFOS separation



TDCA PFOS (total)

Separation of PFOS and TDCA



Error diagrams for selected analytes in EPA 1633. Shaded areas on the top and bottom represent ±30% error.

Results

EPA 1633 % RSD at the LOQ levels, N= 10

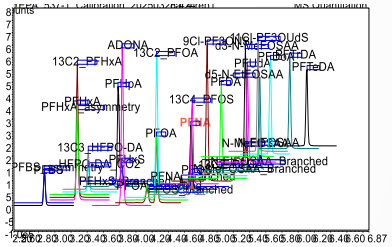
Analyte	Cal 1 (ng/mL)	%RSE	%RSD (n=10) LOQ injections
PFBA	0.2	3.7%	3.26
PFMPA	0.1	3.5%	5.44
PFPeA	0.1	4.1%	9.77
PFMBA	0.1	4.0%	7.23
4:2 FTS	0.2	6.0%	5.99
NFDHA	0.1	5.0%	6.72
PFHxA	0.05	4.9%	4.19
PFBS	0.05	3.9%	10.29
HFPO-DA	0.05	3.7%	6.88
PFEESA	0.1	6.5%	5.7
3:3 FTCA	0.25	8.2%	15.95
PFHpA	0.05	4.1%	5.41
PFPeS	0.05	4.7%	7.97
ADONA	0.2	7.9%	2.98
6:2 FTS	0.2	4.8%	3.21
PFOA (total)	0.05	5.6%	5.42
PFHxS (total)	0.05	3.3%	7.2
PFNA (total)	0.05	5.1%	10.1
PFHpS	0.05	4.7%	9.45
5:3 FTCA	1.25	4.8%	1.98
8:2 FTS	0.2	3.0%	6.68
PFDA	0.05	3.0%	5
PFOS (total)	0.05	5.3%	6.67
PFUdA	0.05	2.4%	4.18
^{9}Cl-PF$_3$ONS	0.2	2.1%	1.6
PFNS	0.05	4.3%	13.21
N-MeFOSAA (total)	0.05	17.9%	23.68
7:3 FTCA	1.25	9.0%	3.42
PFDoA	0.05	7.3%	10.63
PFDS	0.05	7.3%	14.16
N-EtFOSAA (total)	0.05	7.9%	40.58
PFOSA (total)	0.05	10.1%	9.45
PFTrDA	0.05	9.6%	7.69
^{11}Cl-PF$_3$OUdS	0.2	6.6%	3.9
N-MeFOSE (total)	0.5	3.7%	20.97
PFTeDA	0.05	7.4%	8.96
N-MeFOSA (total)	0.05	3.6%	10.1
PFDoS	0.05	7.9%	10.63
N-EtFOSE (total)	0.5	4.3%	14.6
N-EtFOSA (total)	0.05	7.4%	5.23

Calibration levels for this method were achieved four times lower than those specified in EPA 1633. The percent relative standard error (%RSE) and limit of quantitation (LOQ) are shown for each analyte.

Results

EPA 533 and 537.1 Results, Linearity and %RSD at the LOQ

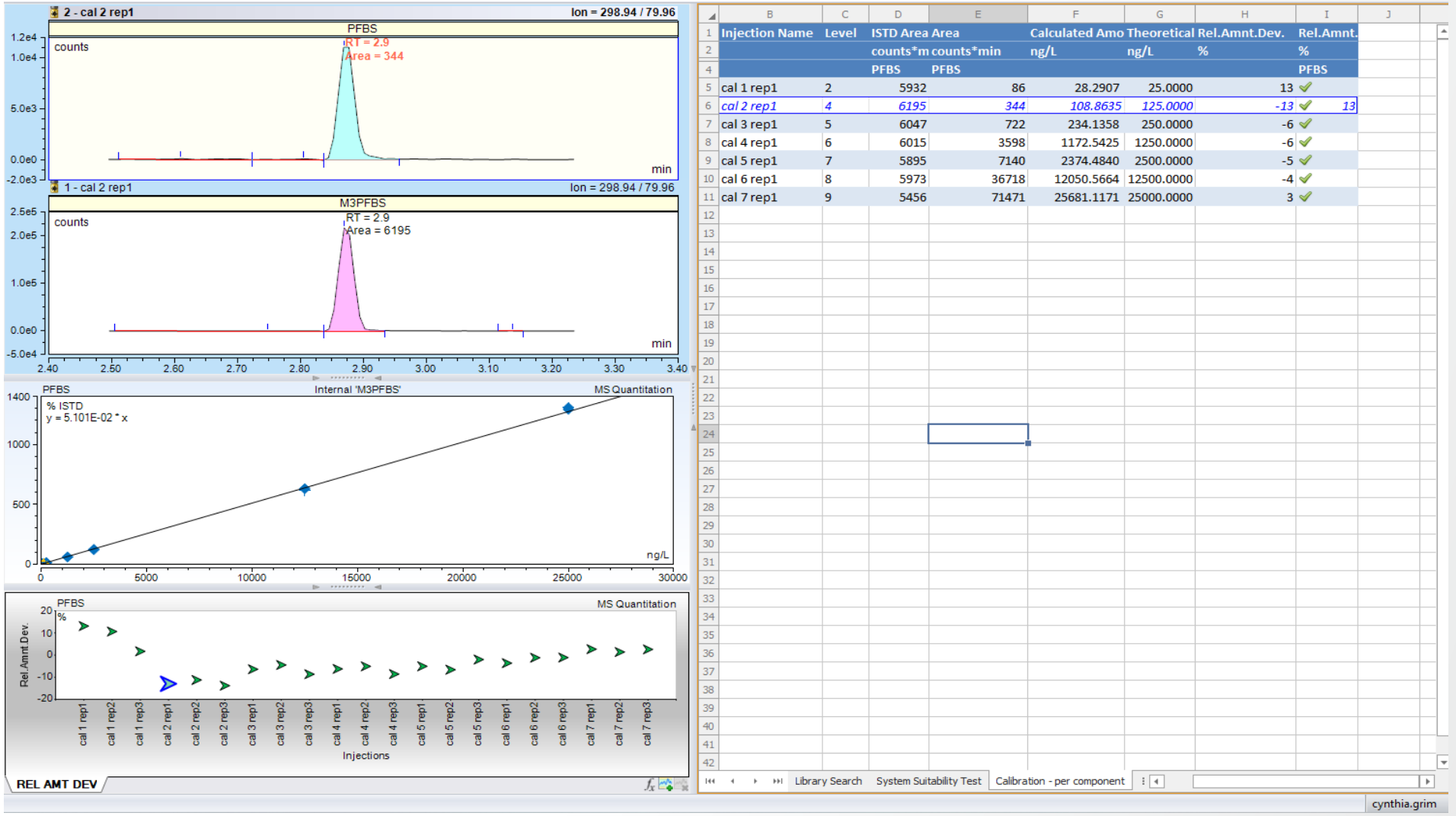
	EPA 533		EPA 537.1	
Analyte	Linearity (R^2)	%RSD at LOQ (n=10)	Linearity (R^2)	%RSD at LOQ (n=10)
PFBA	0.9994	3.4	-	-
PFPeA	0.9996	5.0	-	-
PFHxA	0.9993	6.0	0.9997	2.9
PFHpA	0.9988	4.9	0.9999	6.5
PFOA (total)	0.9992	3.6	0.9990	4.3
PFNA (total)	0.9995	3.3	0.9987	10.1
PFDA	0.9999	4.0	0.9996	5.3
PFUdA	0.9992	4.6	0.9998	4.6
PFDoA	0.9995	5.6	0.9999	2.8
PFTrDA	-	-	0.9998	4.4
PFTeDA	-	-	0.9998	2.3
PFBS	0.9988	8.8	0.9999	6.8
PFPeS	0.9975	9.1	-	-
PFHxS (total)	0.9983	7.0	0.9997	4.2
PFHpS	0.9984	4.7	-	-
PFOS (total)	0.9995	7.3	0.9990	11.5
4:2 FTS	0.9989	10.5	-	-
6:2 FTS	0.9993	11.0	-	-
8:2 FTS	0.9996	8.4	-	-
PFMPA	0.9995	4.5	-	-
PFMBA	0.9990	4.7	-	-
NFDHA	0.9990	10.3	-	-
PFEESA	0.9995	5.3	-	-
HFPO-DA	0.9998	6.3	0.9995	9.3
ADONA	0.9997	3.9	0.9997	4.4
^{9}Cl-PF$_3$ONS	0.9999	5.0	0.9998	5.8
^{11}Cl-PF$_3$OUdS	0.9989	4.1	0.9998	6.1
N-MeFOSAA (total)	-	-	0.9974	7.8
N-EtFOSAA (total)	-	-	0.9990	14.1



Chromatogram of a calibration standard at 0.25 ng/mL for EPA 537

EPA Method Templates

Software has complete method information from acquisition to reporting

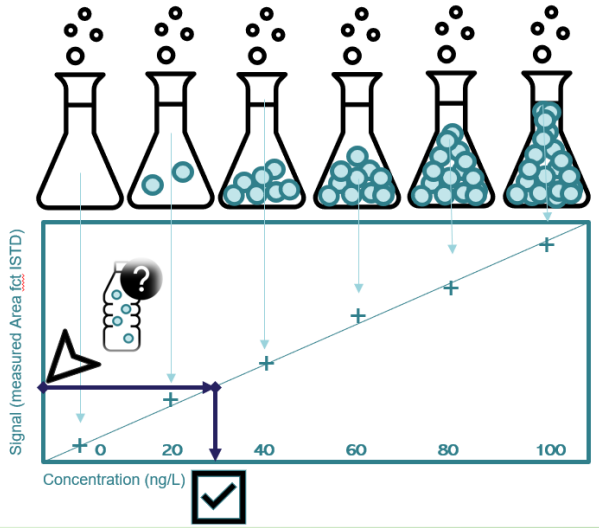


Advantages of HRMS for PFAS analysis

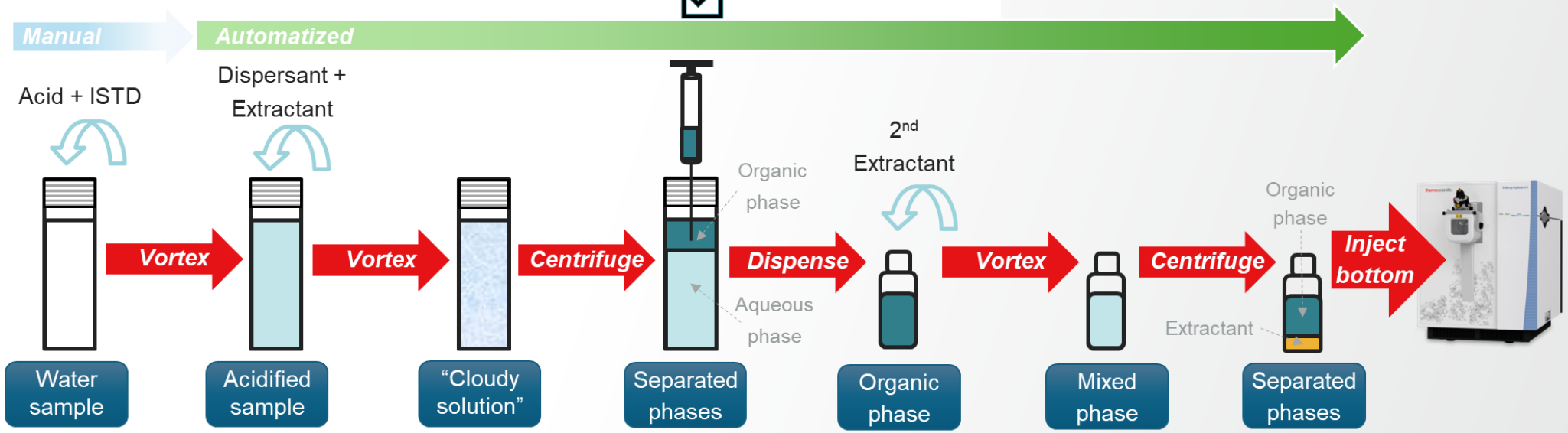
Ease of use	Fully controlled by a single CDS software- same as all other MS in the lab No RT windows with special scan modes to set up Single method: Full Scan operation with defined set of in-source fragmentation energies
Productivity	Excellent mass accuracy across large MW range and for extended periods of time Modern MS systems maintain exceptional mass accuracy, typically <5 ppm Single run versatility: pos/neg, full scan with fragmentation, all at high resolution
Reliability	Accuracy and precision independent of resolution or signal intensity Sensitivity in Full Scan around ppt level Large dynamic range similar to LC-MS/MS (5-6 decades)

Dispersive Liquid-Liquid Microextraction (DLLME)

Initial method: Drinking water (App Brief 003164)



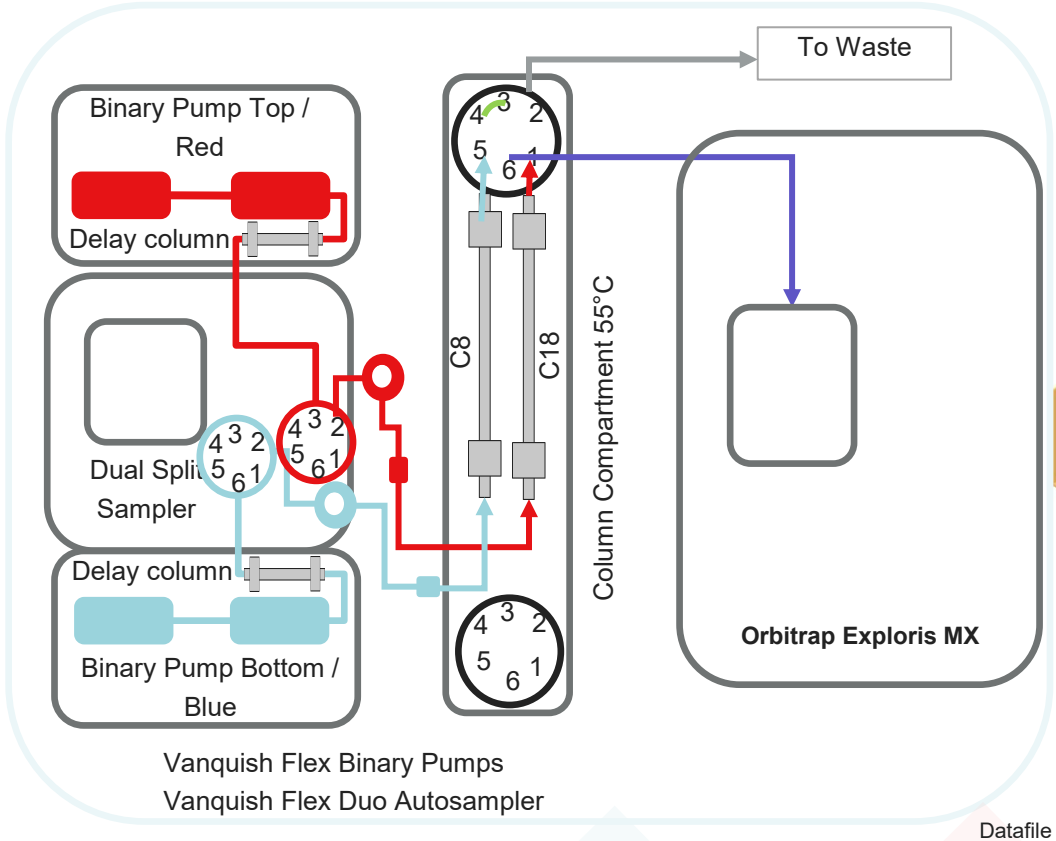
- PFAS
- Individually quantified from 0.1 ng/L to 100 ng/L
- 2 samples in 18 minutes
- LOQ ranging from 0.1 to 1 ng/L (ppt) by HRMS

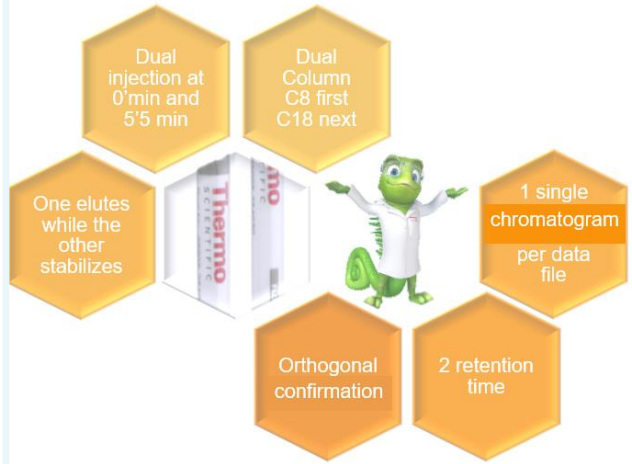


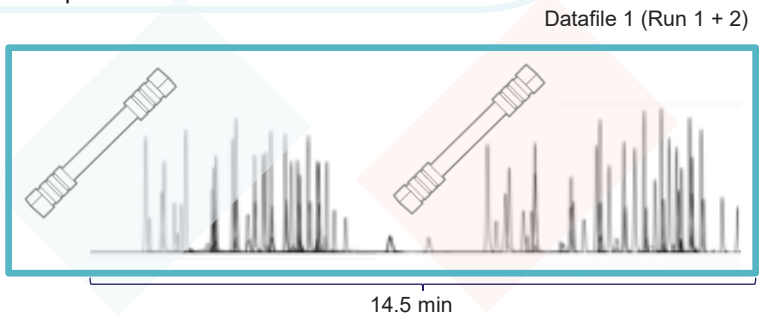
Dual LCMS Method applied to HRMS

Vanquish Duo UHPLC System

PARAMETERS	
Column 1	Hypersil GOLD C8™ 100*2.1mm, 1.9 µm
Column 2	Hypersil GOLD™ 100*2.1mm, 1.9 µm
Mobile Phase A	Water at 0.1M of Ammonium Fluoride
Mobile Phase B	Methanol at 0.1M of Ammonium Fluoride
Total Run Time	14.5 min
Injection Volume	5 µL
Acquisition type	Fullscan (+2 ion-source fragmentation FS)
Scan range (m/z)	70-1000
Resolution	60 000
Source Type	HESI
Polarity mode	Negative

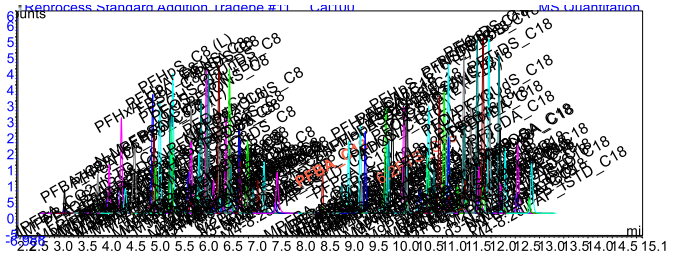






On going confirmation option in a single data file

61 PFAS Mix, injected on two different column types providing 2 retention times per compound



PFAS on 1st column

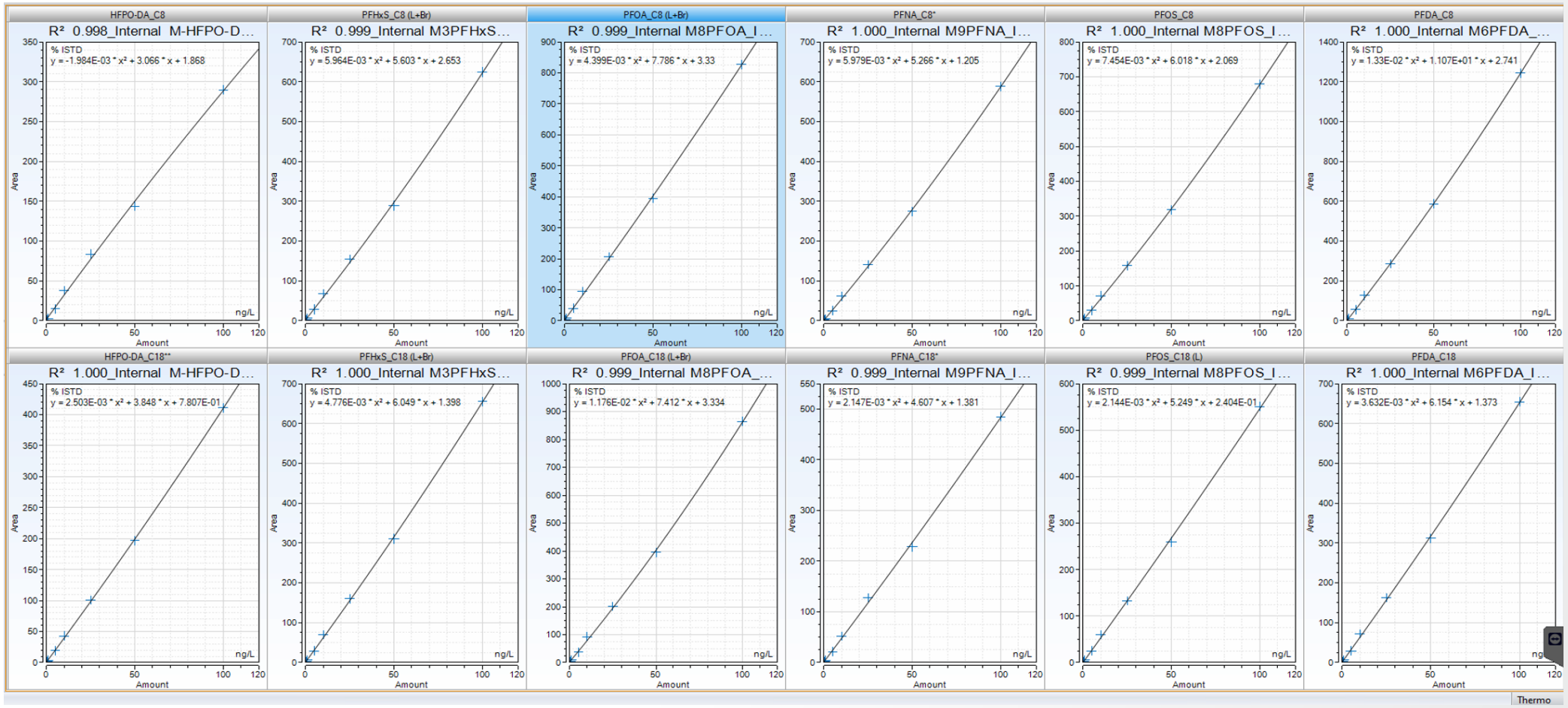
Quantitation

PFAS on 2nd column

Confirmation

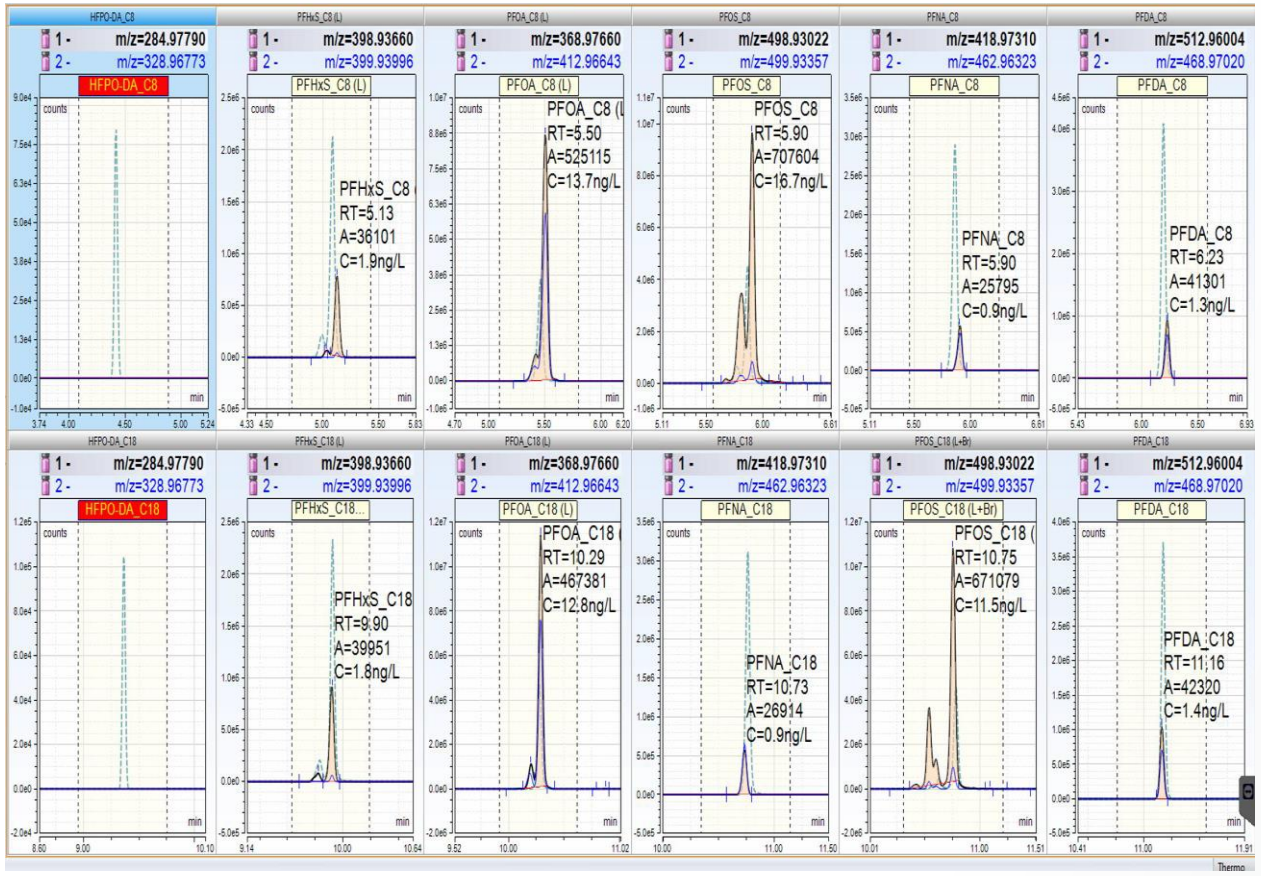
Calibration Curves HRMS- DLLME

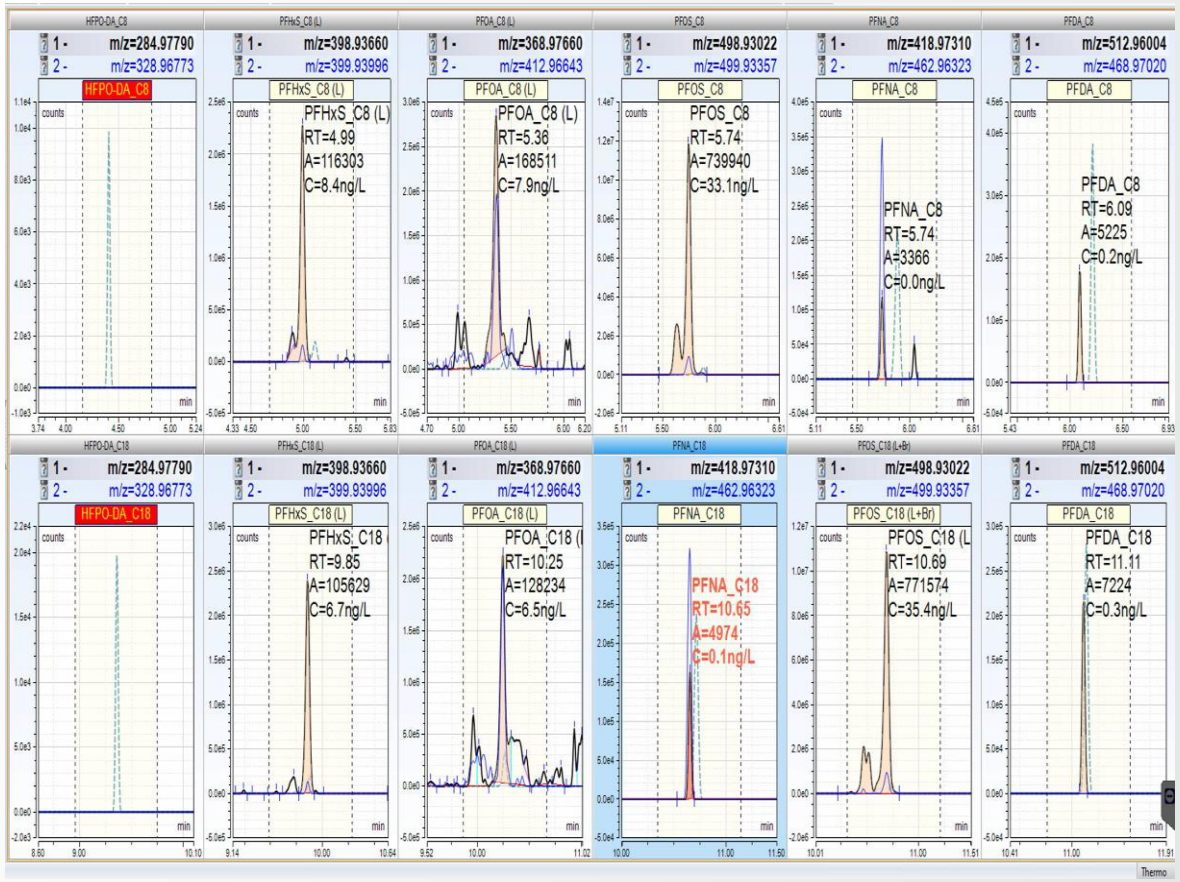
Extracted calibration curves (using internal standards) ranging from 0.1 ng/L to 100



HRMS Extracted Ion Chromatograms

C8 (Top) and C18 (bottom) column types shown



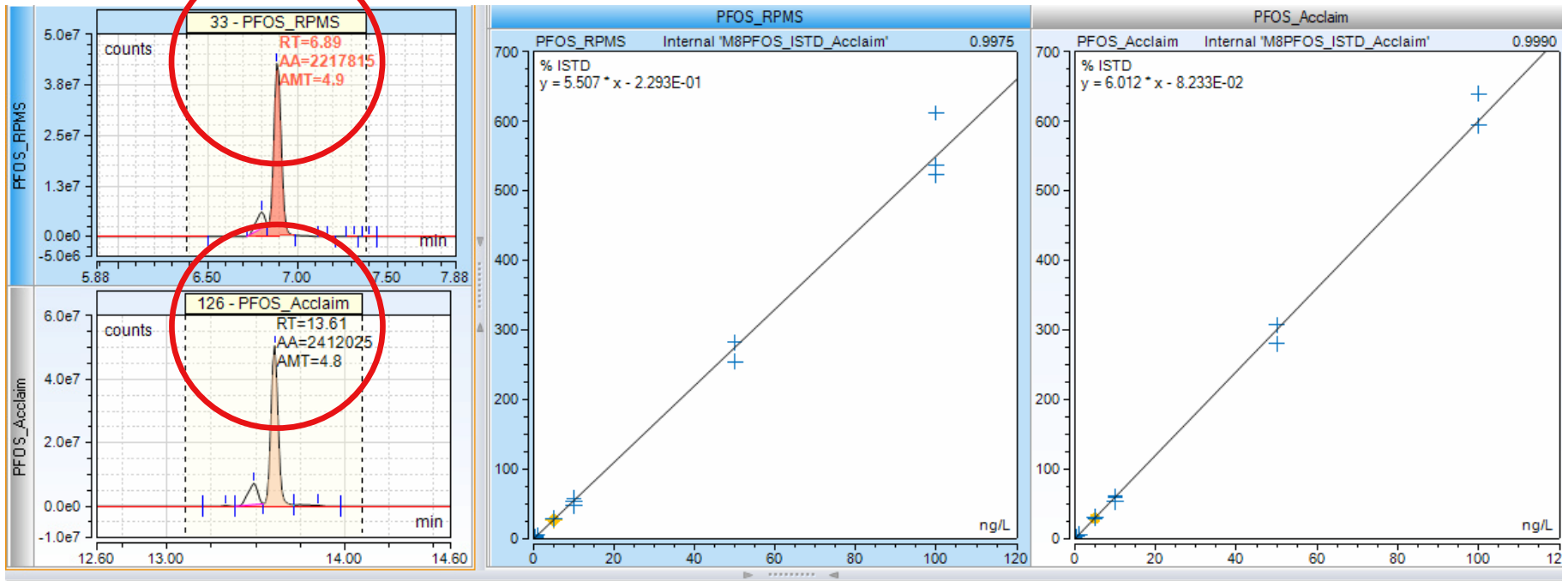


Left: Groundwater sample results, overlaid m/z measurements at 60K resolution and 5 ppm accuracy,

Right: soil sample results, overlaid m/z measurements at 60Kresolution and 5 ppm accuracy.

Dual Column – easily visualize your result

View Settings in the CDS software can show comparative views



J10

	A	B	C	D	E	F	G
1	*Group*	*Peak Name*	*Abs.Ret.Dev. min*	*Amount ng/L*	*Average Amount*	*Std Dev*	
2							
5	*PFOS*	*PFOS_RPMS*	*0.00*	*4.9*			
6	*PFOS*	*PFOS_Acclaim*	*0.00*	*4.8*	*4.8*	*0.7*	

Dual Column – gain confidence in your result

ThermoFisher SCIENTIFIC

View Settings to facilitate quantitation comparisons

	A	B	C	D	E	F	G	H	I	J
1	Compound	Peak Name	Retention (DETECTED)	Abs.Ret.Dev.	CheckRT	Amount	Average (peak.amount)	Rel.Std.Dev. (peak.amount)	Check Amount %RSD>15%	Global Review
2			min	min		ng/L		%		
3	CRM 3	CRM 3	CRM 3	CRM 3	CRM 3	CRM 3	CRM 3	CRM 3	CRM 3	CRM 3
5		*N-MeFOSAA_RPMS*	*7.52*	*0.107*	*issue RT*	*5.3*	*5.3*	*n.a.*		*Only 1 peak*
6		PFBA_RPMS	3.22	-0.008		5.4				
7	PFBA	PFBA_Acclaim	9.67	-0.004		5.4	5.4	0.2		Validated
8		PFBS_RPMS	5.06	0.002		5.3				
9	PFBS	PFBS_Acclaim	11.27	-0.005		5.4	5.4	1.2		Validated
10		PFDA_RPMS	7.14	0.004		2.9				
11	PFDA	PFDA_Acclaim	13.96	0.000		2.7	2.8	3.4		Validated
12		PFDoA_RPMS	7.53	0.010		2.2				
13	PFDoA	PFDoA_Acclaim	14.50	-0.010		2.3	2.2	3.6		Validated
14		PFDS_RPMS	7.33	0.003		1.2				
15	PFDS	PFDS_Acclaim	14.22	0.002		1.1	1.2	7.5		Validated
16		PFHpA_RPMS	6.17	0.009		3.5				
17	PFHpA	PFHpA_Acclaim	12.60	-0.003		3.3	3.4	4.6		Validated
18		PFHpS_RPMS	6.54	-0.004		3.0				
19	PFHpS	PFHpS_Acclaim	13.21	-0.001		3.2	3.1	3.2		Validated
20		PFHxA_RPMS	5.51	0.010		6.5				
21	PFHxA	PFHxA_Acclaim	11.98	-0.007		6.4	6.5	1.1		Validated
22		PFHxS_RPMS	6.18	0.009		3.8				
23	PFHxS	PFHxS_Acclaim	12.64	0.007		3.9	3.8	2.1		Validated
24		PFNA_RPMS	6.90	-0.005		3.3				
25	PFNA	PFNA_Acclaim	13.63	0.005		3.1	3.2	3.7		Validated
26		PFOA_RPMS	6.48	0.007		5.4				
27	PFOA	PFOA_Acclaim	13.22	-0.001		6.1	5.7	8.9		Validated
28		PFOS_RPMS	6.89	0.006		9.8				
29	PFOS	PFOS_Acclaim	13.60	-0.004		9.2	9.5	4.3		Validated
30		PFUdA_RPMS	7.35	0.003		2.7				
31	PFUdA	PFUdA_Acclaim	14.25	0.000		2.6	2.7	2.3		Validated

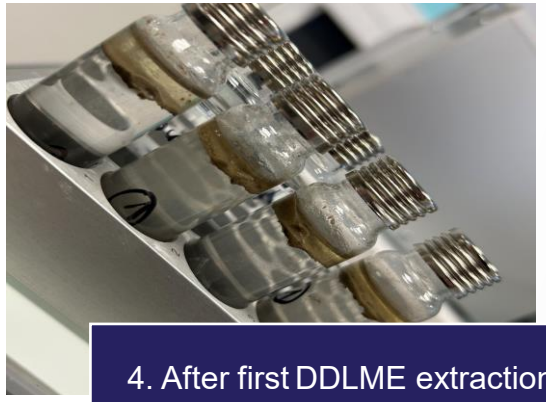
- ✓ **Filtering: > LOQ**
- ✓ **RT deviation**
- ✓ **Amount deviation**
- ✓ **Detected on both columns**

DLLME applicable to other matrices: Soil samples

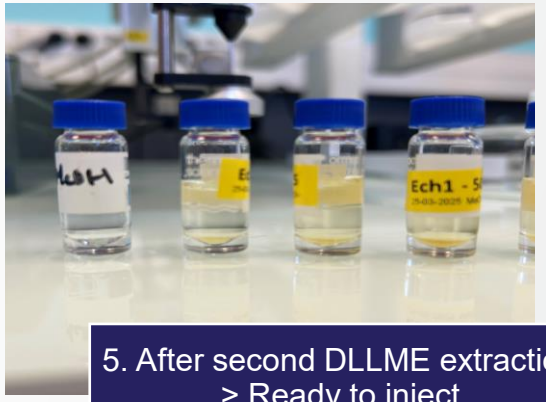
1. Pure Sample

2. Add Water to Sample Aliquot

3. Add Acid and Vortex



4. After first DDLME extraction



5. After second DLLME extraction
> Ready to inject

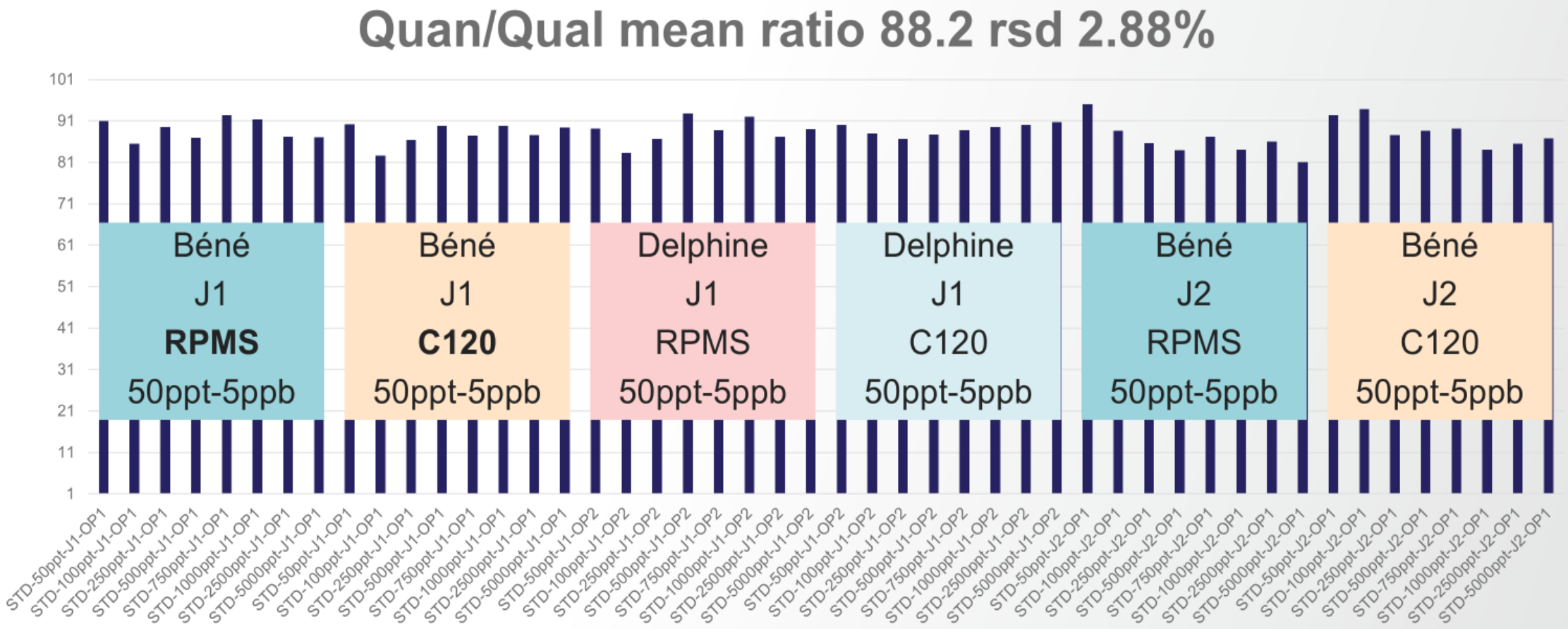
Comparison to SPE

DLLME-HRMS offers a an easier way to extract and analyze complex samples for PFAS

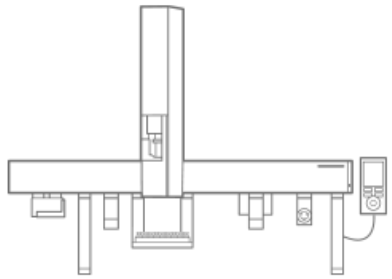
Peak Name	CAS	Column Type	Groundwater			Soil	
			DDLME % recovery at 1 ng/L	DLLME (ng/L)	SPE (ng/L)	DDLME % recovery at 750 ng/kg	SPE (ng/kg)
HFPO-DA_C18	13252-13-6	Hypersil GOLD™	93	ND	ND	107	ND
HFPO-DA_C8	13252-13-6	Hypersil GOLD C8™	✓	ND	ND	✓	ND
PFDA_C18	335-76-2	Hypersil GOLD™	90	1.4	1.04	124	3.25
PFDA_C8	335-76-2	Hypersil GOLD C8™	✓	confirmed	n.a.	✓	confirmed
PFHxS_C18 (L+Br)	355-46-4	Hypersil GOLD™	83	1.9	1.47	98	113.63
PFHxS_C8 (L)	355-46-4	Hypersil GOLD C8™	✓	confirmed	n.a.	✓	confirmed
PFNA_C18	375-95-1	Hypersil GOLD™	97	0.9	ND	72	1.07
PFNA_C8	375-95-1	Hypersil GOLD C8™	✓	confirmed	n.a.	✓	confirmed
PFOA_C18 (L+Br)	335-67-1	Hypersil GOLD™	85	13.8	8.20	95	101.32
PFOA_C8 (L)	335-67-1	Hypersil GOLD C8™	✓	confirmed	n.a.	✓	confirmed
PFOS_C18 (L+Br)	1763-23-1	Hypersil GOLD™	100	11.5	12.30	117	416.72
PFOS_C8	1763-23-1	Hypersil GOLD C8™	✓	confirmed	n.a.	✓	confirmed

ADONA : from 50ppt to 5ppb

8 different concentrations, 2 different operators, 2 different days, 2 different columns

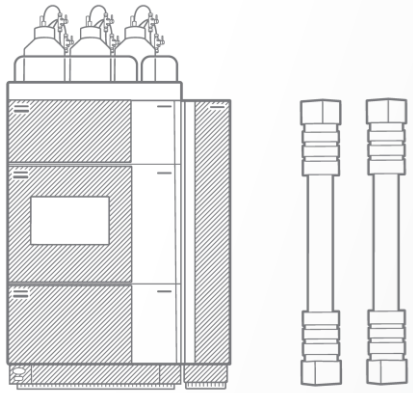


Summary of hardware and benefits



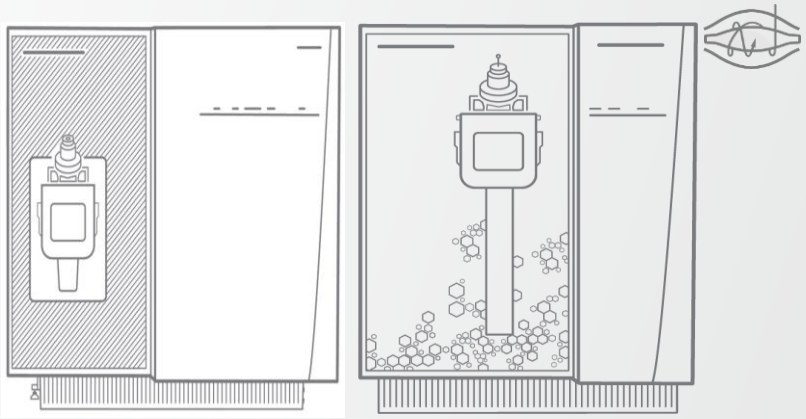
DLLME Option with the TriPlus RSH SMART

- Dispersive Liquid Liquid Micro-Extraction (DLLME)
  - **High enrichment factors**
  - **Reduced sample volume**
  - **Low solvent consumption**
  - **Cost effective**
  - **Versatile (applicable to several chemical classes, and GC-MS)**



2-Channel UHPLC system

- 2 independent channels
- Separate methods or combine runs from different columns
- Save capital and warranty costs
- → **increased confidence and versatility**



TSQ or HRMS Mass Detector

- **EPA Multiple methods ready (TSQ)**
  - **EPA 533, 537.1, 1633**
- **Simple, single method (HRMS)**
  - **Confident confirmation through 2 RT**
  - In-source fragmentation for further confirmation
  - Retrospective analysis- see the ‘whole sample’
  - Affordable technology

Acknowledgements

Thermo Fisher Scientific

- Cynthia Grim
- Bénédicte Gauriat
- Valérie Thibert
- Aristide Ganci
- Julie Moriceau
- Laurent Naels
- Delphine Thomas
- Maryline Carvalho
- Jean-François Garnier

Thank you