LC-MS/MS and LC-HRAM MS for PFAS Analysis

PRESENTED BY
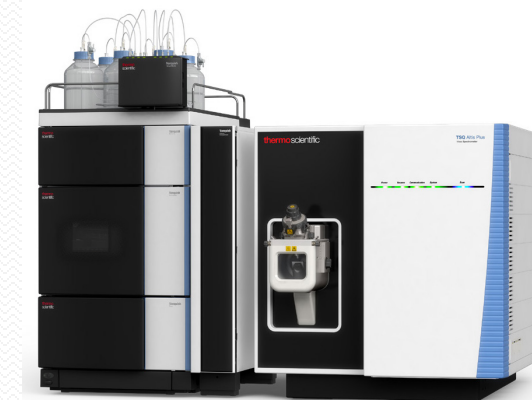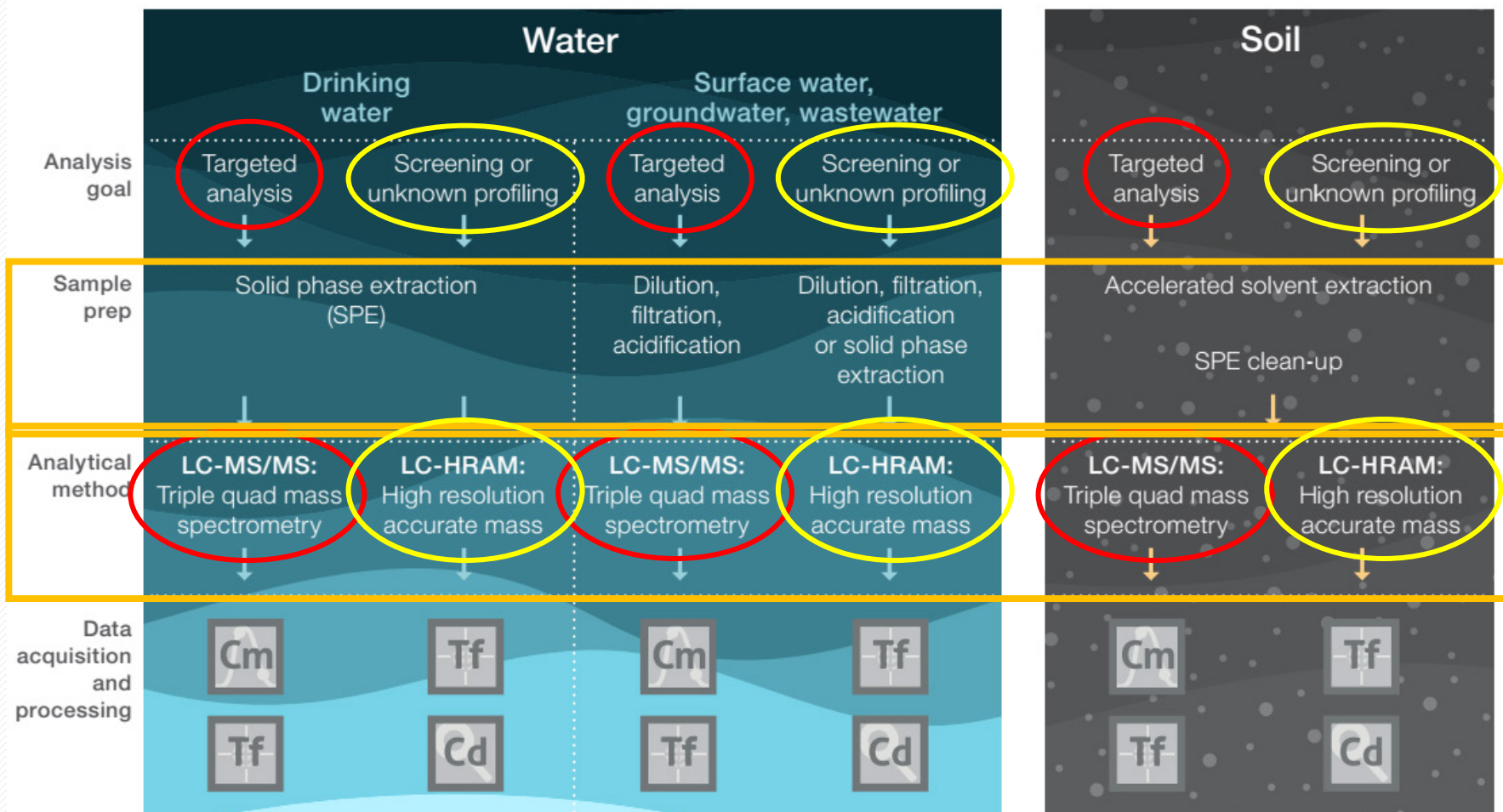
Niramol Jitsommai

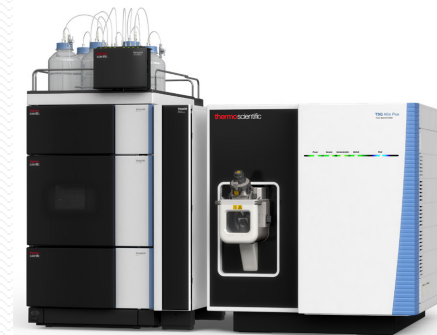Chromatography Product Specialist

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PFAS workflow and analytical instrument

LC-MS/MS
Triple quad mass spectrometer

LC-HRAM
High resolution accurate mass
Q-orbitrap mass spectrometer

2

Targeted analysis

LC-MS/MS Methods for Quantitation of PFAS

TSQ Series LC-MS/MS, Triple quadrupole MS

Method Name	PFAS Targets	Sample Preparation	Reporting Limits
EPA Method 537.1	18	SPE (250 mL drinking water); reconstitute 1 mL 96% MeOH	0.53-6.3 ng/L
EPA Method 533	25	SPE (100-250 mL drinking water); reconstitute 1 mL 80% MeOH	1.4-16 ng/L
EPA 8327	24	5 mL water sample + 5 mL MeOH; filter; add 10 uL HOAc	10-50 ng/L
ASTM D7979-16	21	5 mL water sample + 5 mL MeOH; filter	1.5-107 ng/L
ASTM D7968-14	21	2 g Soil + 10 mL 50% MeOH + 0.1% HOAc; filter	2.4-258 ng/kg

Application note for PFAS analysis

Drinking water

APPLICATION NOTE 73346

Determination of per- and polyfluorinated alkyl substances (PFAS) in drinking water using automated solid-phase extraction and LC-MS/MS

Authors: Changling Qiu, Xin Zhang, Rahmat Ullah, Wei Chen, and Yan Liu
Thermo Fisher Scientific, Sunnyvale, CA

Keywords: Perfluorinated alkyl substances, AutoTrace 280, U.S. EPA Method 537.1, Accucore RP-MS column, Vanquish Flex Duo UHPLC, TSQ Fortis triple quadrupole mass spectrometer

1. Goal
To demonstrate an efficient and reliable solid-phase extraction method with the Thermo Scientific™ Dionex™ AutoTrace™ 280 instrument for the determination of per- and poly-fluorinated compounds in drinking water per U.S. EPA Method 537.1

2. Introduction
Per- and polyfluorinated alkyl substances (PFAS) are a group of man-made chemicals including perfluorooctanoic acid (PFOA), perfluorooctanesulfonic acid (PFOS), and GenX chemicals that have been manufactured and used in a variety of industries globally.[1,2] These compounds have a wide range of commercial product applications including industrial polymers, stain repellents, surfactants, waterproofing products, packaging, and aqueous film forming foams used for firefighting. PFAS are highly soluble in water, chemically stable, persistent in the environment, and can accumulate in the human body over time, leading to adverse human health effects.[3] PFOA and PFOS are no longer manufactured in the U.S. due to their persistence and potential human health risks.

APPLICATION NOTE 73883

Determination of per- and polyfluorinated alkyl substances (PFAS) in drinking water

Using automated solid phase extraction and LC-MS/MS for U.S. EPA Method 533

Authors: Xin Zhang, Changling Qiu, Rahmat Ullah, and Yan Liu

Thermo Fisher Scientific, Sunnyvale, CA

Keywords: Perfluorinated and polyfluorinated alkyl substances, PFAS, AutoTrace 280 PFAS, U.S. EPA Method 533, Acclaim 120 C18 column, Vanquish Flex UHPLC, TSQ Fortis triple quadrupole mass spectrometer

Goal
To demonstrate an efficient and reliable solid phase extraction method with a Thermo Scientific™ Dionex™ AutoTrace™ PFAS, an automated solid-phase extraction (SPE) system, for the determination of per- and poly-fluorinated compounds in drinking water per U.S. EPA Method 533

1. Introduction
Drinking water perfluorinated and polyfluorinated alkyl substances (collectively referred to as PFAS) occurrence studies have typically targeted perfluorooctanoic acid (PFOA) and perfluorooctanesulfonate (PFOS), and as a result, these two are the most commonly detected compounds. This discussion focuses primarily on PFOS and PFOA. However, other compounds including PFBA,

In November 2018, the United States Environmental Protection Agency (U.S. EPA) published Method 537.1 "Determination of selected per- and polyfluorinated alkyl substances in drinking water by solid phase extraction and LC-MS/MS".[4] The method uses an offline solid-phase extraction (SPE) with liquid chromatography tandem mass spectrometry (LC-MS/MS) to extract, enrich, and determine 18 PFAS in drinking water. Currently most testing laboratories perform the sample extraction manually using a vacuum manifold, which is labor-intensive, time-consuming, and the flow rate through the cartridge is difficult to control. There is a high demand for automation of the SPE procedure.

PFHxA, PFHpA, PFNA, PFUnDA, and PFHxS have also been detected in drinking water. In December 2019, the United States Environmental Protection Agency (U.S. EPA) took a key step in implementing the PFAS Action Plan by announcing a new validated method for testing PFAS in drinking water. This new validated test method complements other actions the agency is taking under the Action Plan to help communities address PFAS nationwide. It focuses on "short chain" PFAS, those PFAS with carbon chain lengths of four to twelve, and covers PFOS, PFOA, and other common PFAS that have been detected and reported. Currently most testing laboratories are

Non-drinking water

APPLICATION NOTE 65097

Direct analysis of selected per- and polyfluorinated alkyl substances (PFAS) in ground, surface, and waste water by LC-MS/MS

Authors
Cristina C. Jacob,
Claudia P.B. Martins, Alan R. Atkins,
Richard F. Jack

Thermo Fisher Scientific,
San Jose, CA, USA

Keywords
Perfluorinated organic compounds, PFAA, PFOS, PFOA, GenX, PFCs, environmental contaminants, emerging contaminants, EPA 8327, EPA 537, EPA 537.1

Goal
To demonstrate method performance for the PFAS analysis at low levels (ng/L) in a wide variety of non-drinking water matrices by direct analysis and submit data package for EPA 8327 interlaboratory method validation.

Introduction
Per- and polyfluoroalkyl substances (PFAS) are a group of man-made chemicals that includes perfluorooctanoic (PFOA), perfluorooctyl sulfonic acid (PFOS), and hexafluoropropylene oxide dimer acid (HFPO-DA, which is part of GenX process). PFAS compounds have been manufactured since the 1940s. The most well-known PFAS compounds, PFOA and PFOS, have been the most extensively produced and studied for chemical properties and toxicological effects. Both chemicals are very persistent in the environment and accumulate in the human body over time. It is well documented that exposure to PFAS can lead to adverse human health effects[1,2] and are found in food packaging material as well as food processing equipment. Plants can accumulate PFAS when grown in PFAS-containing soil and/or water. These compounds are also found in a wide variety of consumer products such as

Soil

CUSTOMER APPLICATION NOTE 73937

Extraction and analysis of poly- and perfluoroalkyl substances (PFAS) from soil

Authors: Matthew S. MacLennan[1,2],
Daniel Ng[1], David Hope[1,3]
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[3]Dave@pacificrimlabs.com

Introduction
Recent studies suggest that toxic and highly persistent poly- and perfluorinated alkyl substances (PFAS) are much more prevalent in tissue and soil than in water. The increasing length of perfluoroalkyl chain in PFAS is correlated strongly to lower water solubility/higher adsorption behavior of a particular PFAS molecule in the environment (i.e., migration of PFAS at soil/water/air interfaces) and in remediation/filtration (i.e., choice of filtration media or sorbents). There are over 6,000 PFAS commercially available, many of which have high environmental persistence and have been found in water and soils globally. This poses a significant challenge to developing analytical methods, especially for the extraction of a variety of PFAS from solid matrices such as soil. Previously, we reported unsatisfactory (0–50%) recovery of long-chain PFAS from soil using vortex/sonication.[1]

In the present study, soil was spiked with 24 PFAS (C4-C14 acids, C4-C10 sulfonates, 4:2, 6:2 and 8:2 fluorotelomers, C8 sulfonamide) at 1 ng/g, which were allowed to absorb overnight into the soil samples.

The soil samples were extracted using the Thermo Scientific™ Dionex™ ASE™ 350 Accelerated Solvent Extractor, which produced 70–130% recovery of all PFAS target compounds. Accelerated solvent extraction has outperformed commonly used, manual "shaking" extraction methods under the same conditions.

After ASE extraction, the solution from the ASE sample collection vials underwent clean-up using solid-phase extraction (SPE) and were analyzed on an LC/MS/MS in a 15-min run. Blanks contained no significant amounts of PFAS. Accelerated solvent extraction is demonstrated to be acceptable for the extraction of short- and long-chain PFAS, with a variety of polarities and head-groups, from soil in the range of 1 ng/g to 400 ng/g.
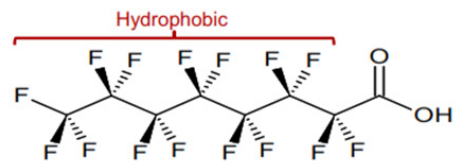
PFAS Analysis Kit

Thermo Scientific™ PFC-free kit (P/N 80100-62142)

Hydrophobic

Perfluorooctanoic acid
- PFOA
- Teflon®

Replacing the wetted Teflon™ surfaces with comparable PEEK components and installing a PFOA trapping column.
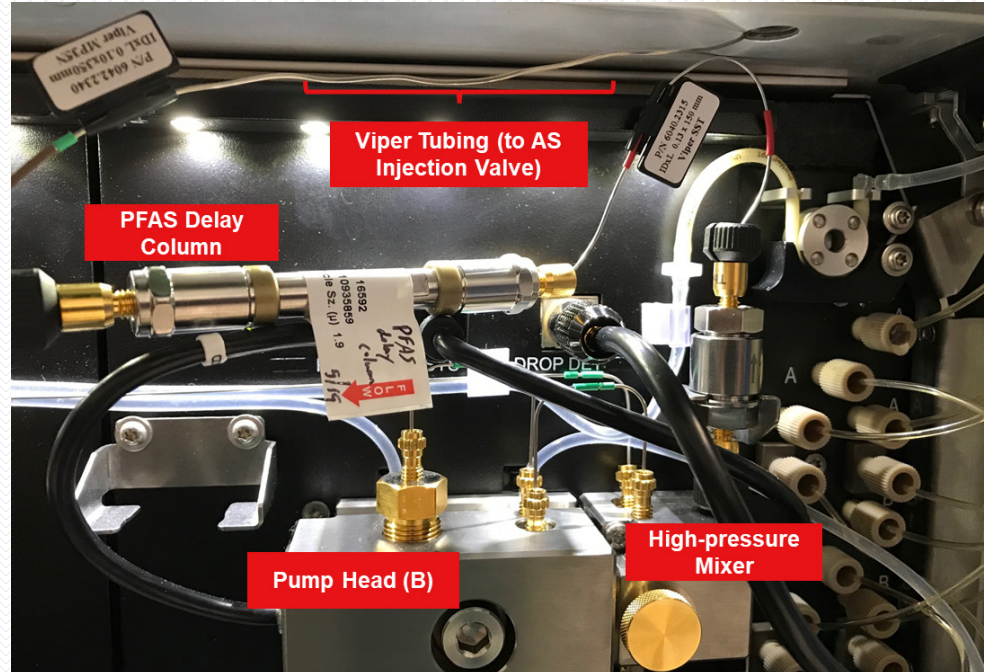
solvent bottle cap

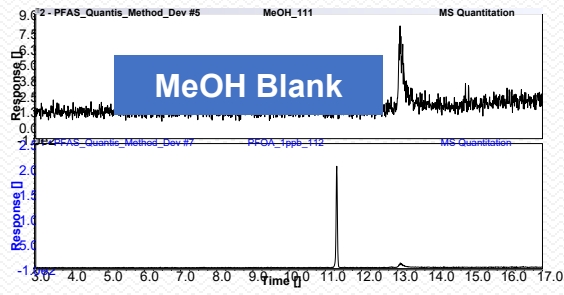PEEK Tubing

HPLC vials to do PFAS analysis

PEEK tubing assemblies

PEEK Tubing

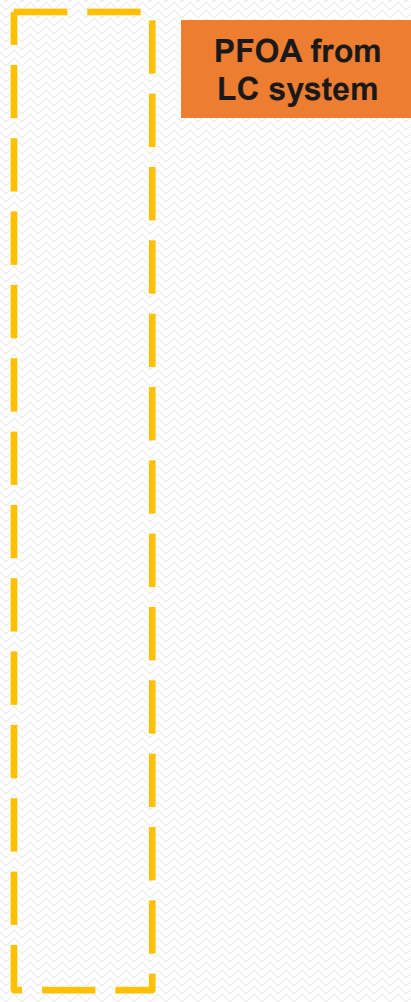PEEK tubing assemblies and the trap column

Viper Tubing (to AS Injection Valve)

PFAS Delay Column

PEEK Tubing (from Mobile Phase Reservoirs to Vacuum Degasser)

Pump Head (B)

High-pressure Mixer

MeOH Blank

1 ppb PFOA
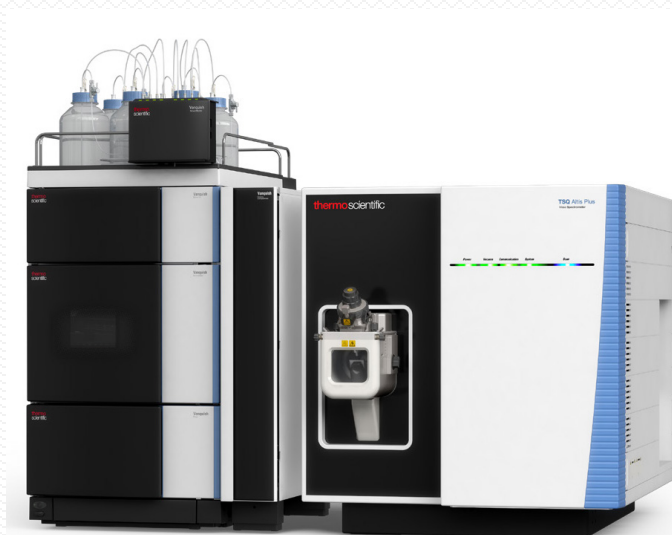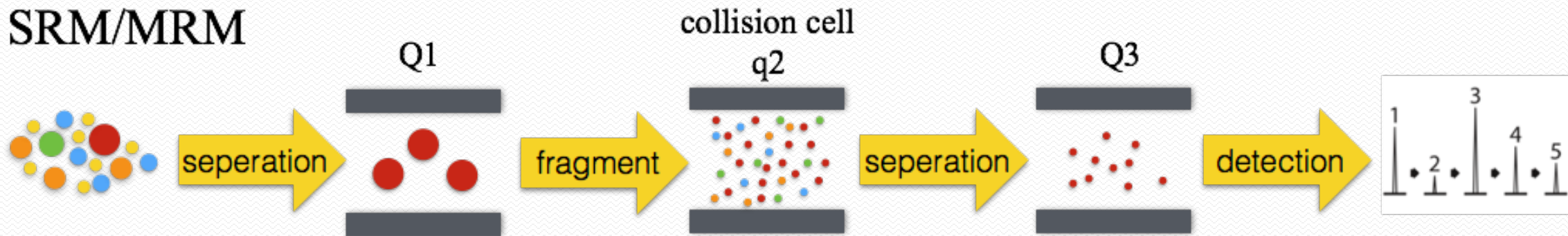
PFOA injected on
analytical column

PFOA from
LC system

Later eluting peak is artifact from LC system

LC-MS/MS method for PFAS analysis

SRM/MRM

collision cell

Q1   q2   Q3

seperation → fragment → seperation → detection

Thermo Scientific™ **Vanquish™ UHPLC system** fitted with **PFC-free kit** and interfaced with Thermo Scientific™ **TSQ mass spectrometer**

LC-MS/MS Condition

Parameter	Value
Analytical column	Accucore RP-MS, 2.1 × 100 mm, 2.6 µm
Isolator column	Hypersil BDS C18, 2.1 × 50 mm, 5 µm. This column was installed prior to the autosampler to remove any contaminants from the mobile phase.
Column temp.	45 °C
Flow rate	0.5 mL/min
Injection volume	5 µL
Autosampler temp.	6 °C
Solvent A	Water containing 0.1% acetic acid
Solvent B	Methanol containing 0.1% acetic acid
Solvent C	20 mM ammonium acetate in water

	Time (min)	%B	%C
Gradient	0	30	5
	1	30	5
	14	95	5
	17	95	5
	18	30	5
	21	30	5

Parameter	Value
Ion source type	H-ESI
Polarity	Negative
Negative ion	2500 V
Sheath gas	50 arbitrary units
Aux gas	10 arbitrary units
Sweep gas	1 arbitrary units
Ion transfer tube temp.	325 °C
Vaporizer temp.	300 °C
Q1 resolution (FWHM*)	0.7
Q3 resolution (FWHM*)	1.2
CID gas	2 mTorr

SRM: Selected Reaction Monitoring

Demonstration of low system background

PFAS LC-MS/MS chromatogram for a method blank sample

Results: 18 Targeted PFAS in drinking water

Linearity & Chromatogram
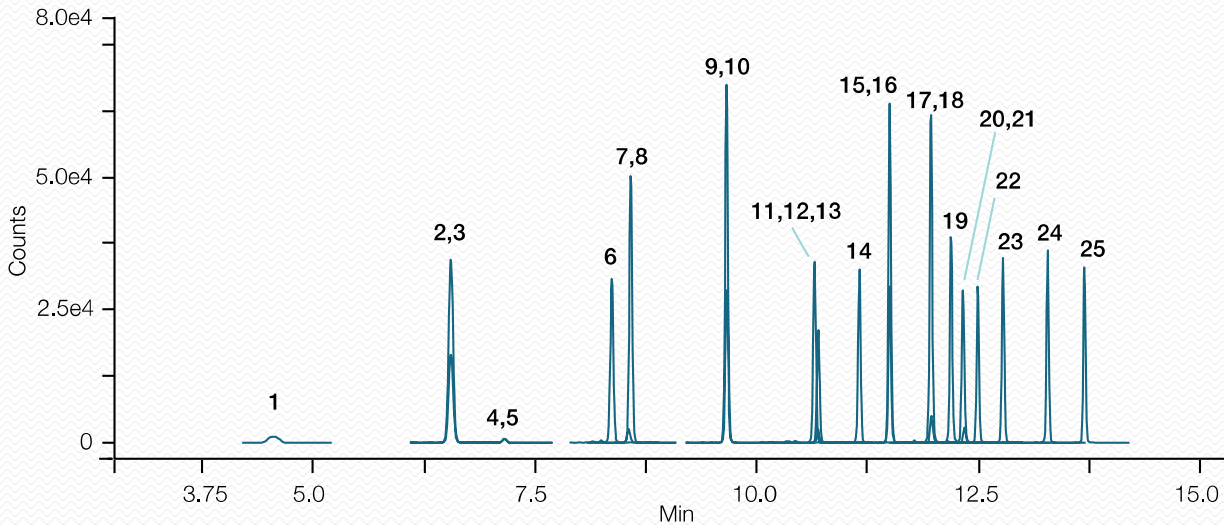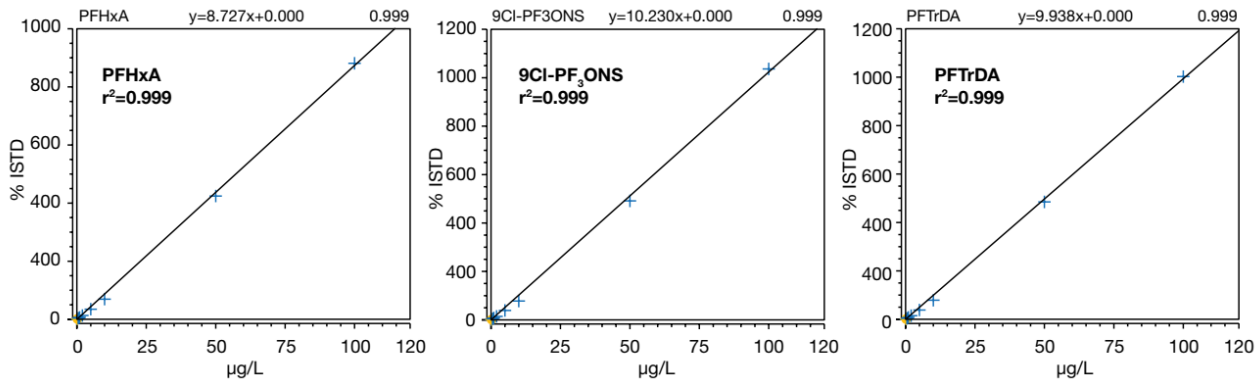
Precision and accuracy (n=6) of PFAS in fortified drinking water

Peak number	Analyte	Fortified Conc. (ng/L)	Mean Recovery (%)	RSD	Fortified Conc. (ng/L)	Mean Recovery (%)	RSD
1	PFBS	16.0	107	3.3	80.0	98.3	3.6
2,3*	PFHxA	16.0	108	2.3	80.0	106	2.6
4,5*	HFPO-DA	16.0	84.1	7.5	80.0	88.6	6.3
6	PFHpA	16.0	113	2.7	80.0	117	1.3
7	PFHxS	16.0	120	3.4	80.0	123	2.1
8	ADONA	16.0	117	2.5	80.0	121	1.1
9,10*	PFOA	16.0	113	2.5	80.0	119	1.6
11	PFNA	16.0	114	2.9	80.0	118	2.1
12,13*	PFOS	16.0	113	4.5	80.0	117	2.9
14	9Cl-PF3ONS	16.0	96.1	4.1	80.0	103	2.6
15*,16	PFDA	16.0	105	3.2	80.0	111	2.1
17*,18	NMeFOSAA	16.0	103	5.2	80.0	110	5.2
19	PFUnA	16.0	96.8	5.0	80.0	103	3.1
20*21	NEtFOSAA	16.0	100	9.9	80.0	104	2.3
22	11Cl-PF3OUdS	16.0	88.5	5.5	80.0	97.1	4.8
23	PFDoA	16.0	89.8	4.4	80.0	97.3	3.4
24	PFTrA	16.0	89.6	3.8	80.0	95.8	3.7
25	PFTA	16.0	89.0	4.8	80.0	98.1	3.3

**Linearity over the range 0.1 – 100 ppb for PFAS compounds R2 > 0.99 for all PFAS compounds**

All measured recovery and precision values surpass regulatory requirements

Recovery test

Compound	Recoveries %							
	Reagent water		Ground water		Surface water		Waste water	
	Low level	High level	Low level	High level	Low level	High level	Low level	High level
PFBA	77%	78%	71%	75%	74%	74%	58%	75%
PFPeA	84%	80%	104%	80%	115%	81%	88%	78%
PFBS	87%	81%	95%	81%	95%	79%	72%	77%
PFHxA	82%	81%	83%	79%	86%	80%	77%	74%
4:2 FTS	81%	82%	90%	78%	87%	79%	76%	91%
PFPeS	80%	80%	82%	79%	85%	78%	80%	83%
PFHpA	84%	81%	88%	80%	89%	80%	74%	81%
PFHxS	81%	81%	87%	78%	94%	81%	85%	85%
6:2 FTS	84%	82%	85%	80%	87%	94%	78%	79%
PFOA	83%	80%	88%	82%	123%	83%	83%	86%
PFHpS	81%	81%	84%	76%	83%	78%	79%	86%
PFNA	79%	81%	84%	80%	86%	80%	79%	82%
PFOS	91%	82%	91%	78%	93%	81%	79%	90%
8:2 FTS	85%	80%	81%	75%	76%	79%	78%	83%
PFNS	85%	75%	89%	79%	81%	76%	72%	78%
PFDA	80%	81%	86%	78%	85%	79%	74%	83%
NMeFOSAA	77%	81%	80%	77%	86%	81%	82%	84%
PFOSA	76%	76%	87%	75%	91%	75%	79%	81%
PFDS	82%	78%	89%	77%	85%	79%	72%	81%
PFUnA	76%	76%	80%	81%	75%	78%	75%	83%
NEtFOSAA	82%	79%	89%	77%	89%	81%	80%	85%
PFDoA	79%	82%	83%	78%	85%	82%	79%	85%
PFTriA	87%	86%	89%	79%	92%	91%	87%	89%
PFTreA	109%	103%	112%	91%	113%	119%	100%	110%

Linearity & Chromatogram

PFOS
y = 3.14x -6.56
R^2 = 0.9955, weighting 1/x

a)

PFOS, 12.14 min

25 fg on column
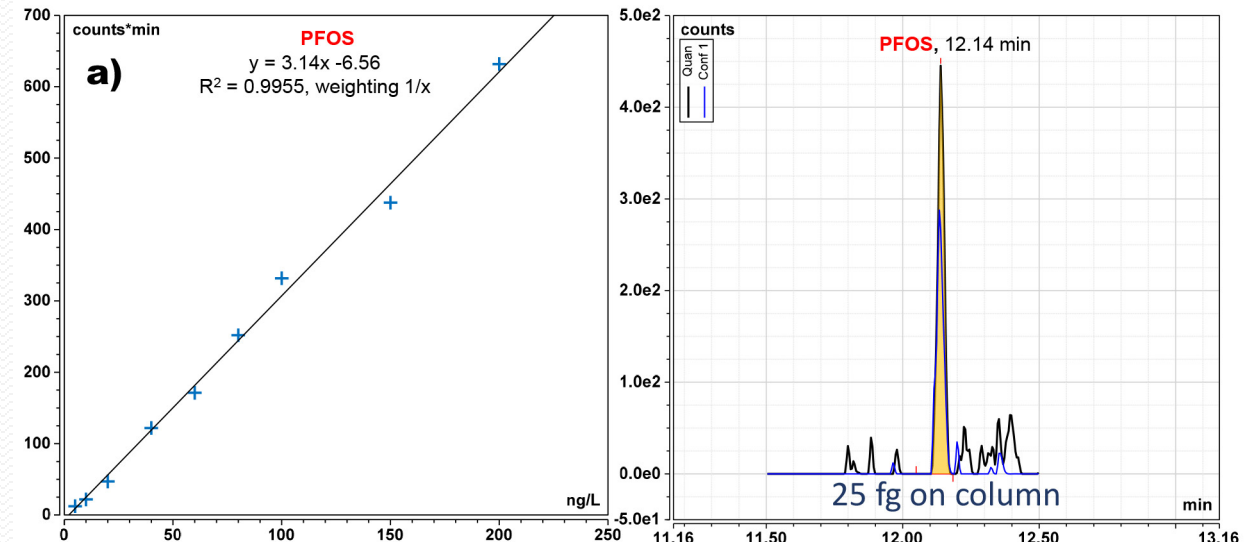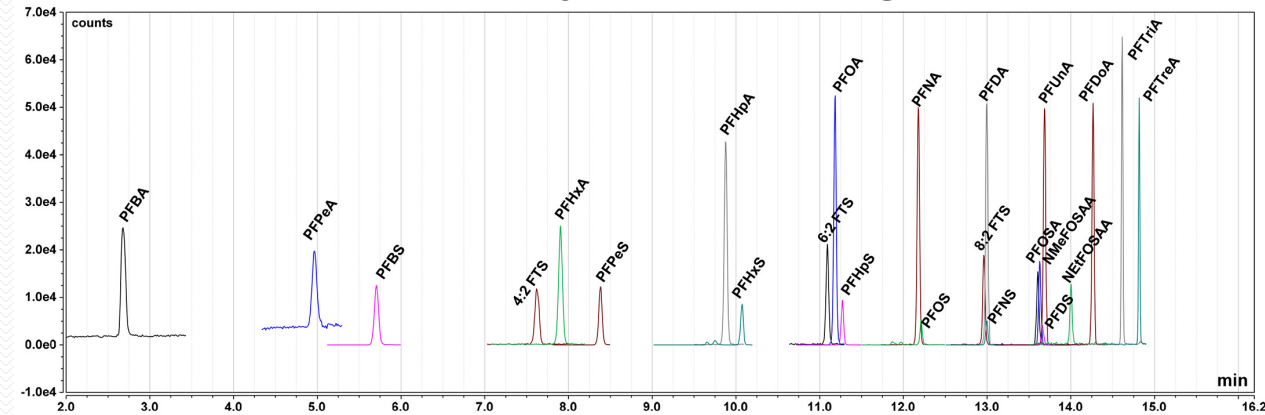
Recovery were within the range of 70% to 130% as required, except for PFBA spiked at low level in wastewater.
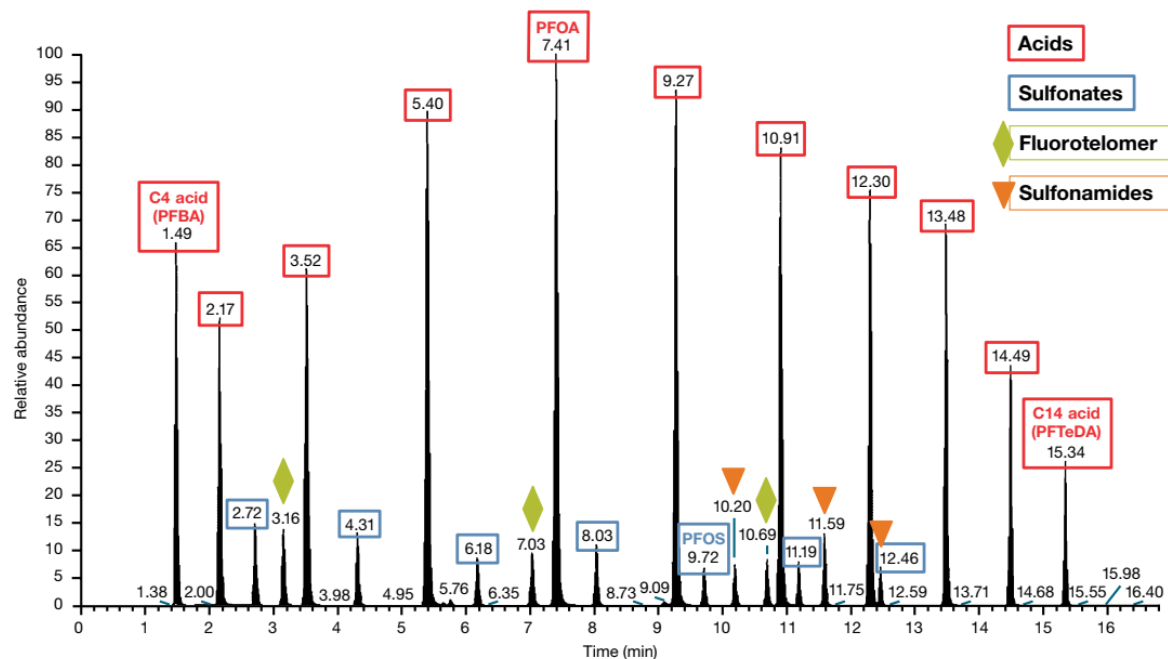
Figure 1. Chromatogram of PFAS compounds analyzed

Recovery test

Compound	Recovery (%)
$^{13}C_4$-PFBA	71
$^{13}C_5$-PFPeA	93
$^{13}C_5$-PFHxA	97
$^{13}C_4$-PFHpA	96
$^{13}C_8$-PFOA	94
$^{13}C_9$-PFNA	104
$^{13}C_6$-PFDA	99
$^{13}C_7$-PFUdA	95
$^{13}C_2$-PFDoA	97
$^{13}C_2$-PFTeDA	108

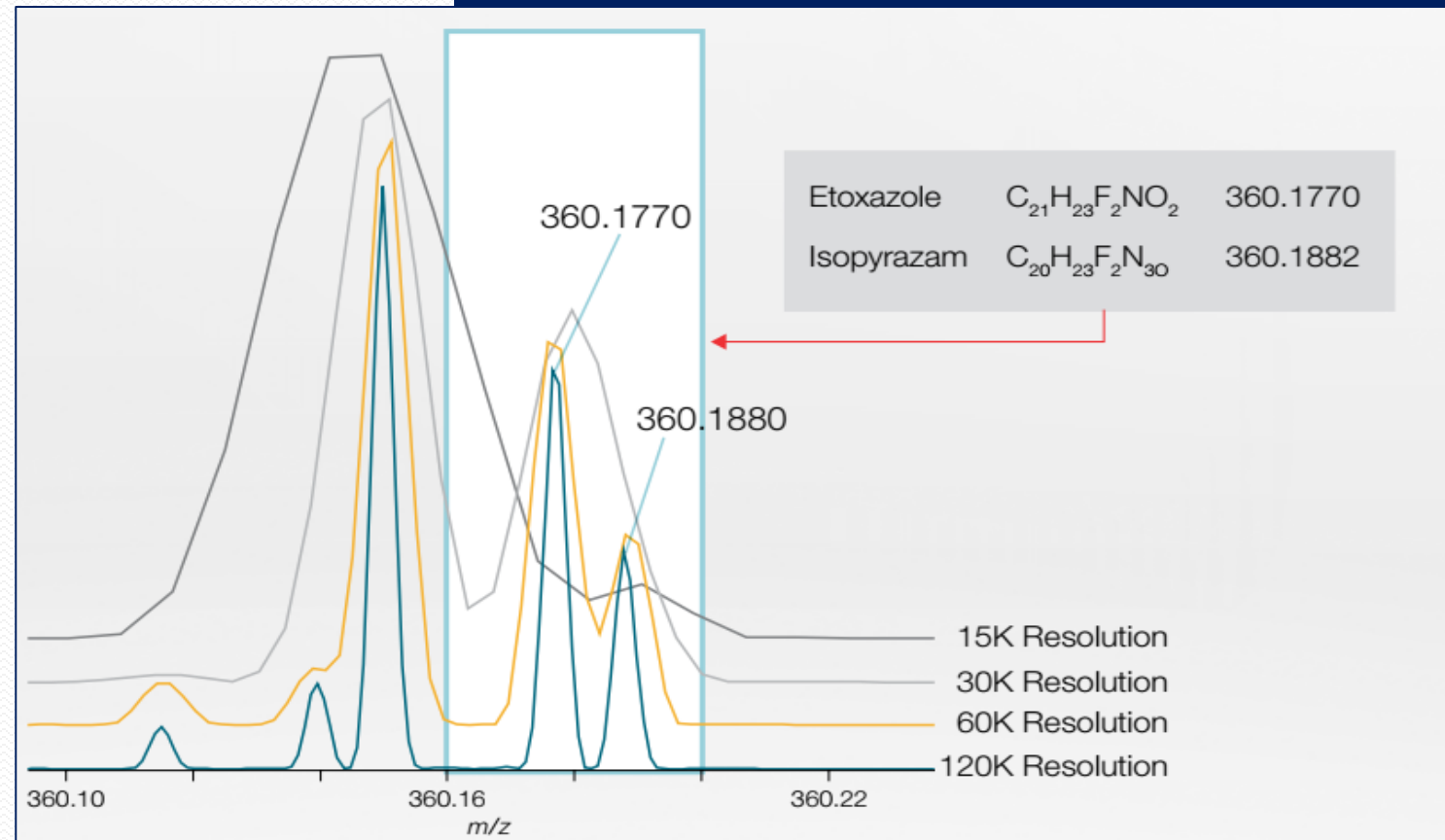| Compound	Recovery (%)
$^{13}C_3$-PFBS	98
$^{13}C_3$-PFHxS	95
$^{13}C_8$-PFOS	91
$^{13}C_3$-HFPODA	56
$^{2}H_3$-NMEFOSAA	93
$^{2}H_3$-NETFOSAA	90
$^{13}C_8$-FOSA	92
$^{13}C_2$-4:2FTS	110
$^{13}C_2$-6:2FTS	93
$^{13}C_2$-8:2FTS	98

Recovery were within the range of 70% to 130% for all PFAS target compound

11

Screening for unknown PFAS

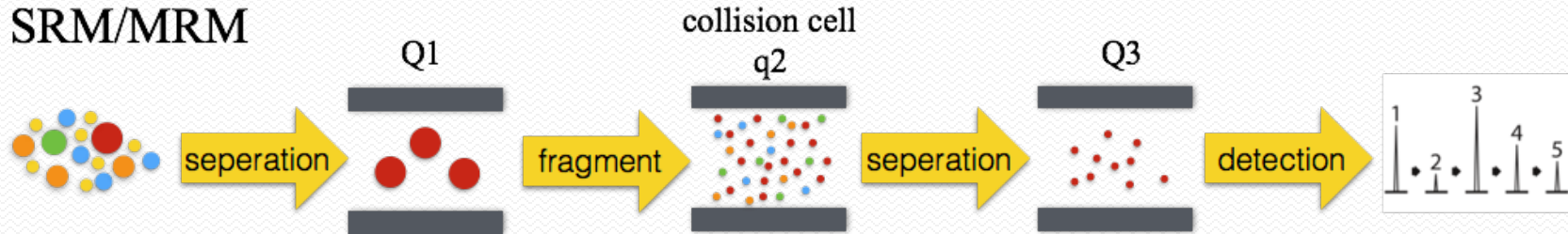Thermo Scientific™ Orbitrap Exploris™ 120 High Resolution, Accurate-Mass (HRAM) Quadrupole Orbitrap MS

High Resolution & High Accuracy

| Etoxazole | $C_{21}H_{23}F_2NO_2$ | 360.1770 |
| Isopyrazam | $C_{20}H_{23}F_2N_3O$ | 360.1882 |

360.1770
360.1880

15K Resolution
30K Resolution
60K Resolution
120K Resolution

360.10   360.16   360.22
m/z

Acquisition mode

Screening for unknown PFAS

Full scan quantitation of target PFASs

+ MS/MS confirmation

+ Screening for other contaminants

- Increase the range of potential targets monitored

- Increase confidence in assignments

- Identify unknowns and emerging contaminants

- Retrospectively analyze data even when the sample no longer exists

Thermo Scientific™ Orbitrap Exploris™ 120 High Resolution, Accurate-Mass (HRAM) Quadrupole Orbitrap MS
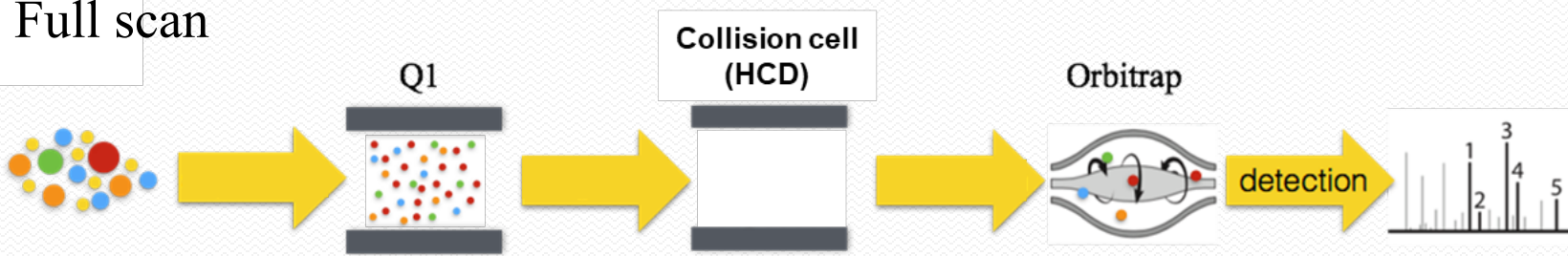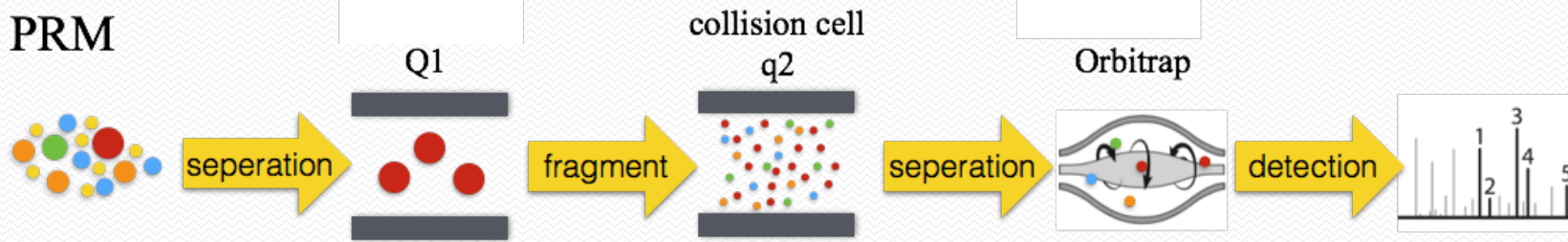
Routine quantitative workflows and non-targeted analysis can be performed in a single analysis.

Full scan quantitation of target PFASs

thermoscientific

APPLICATION NOTE 667

A comparison between HRAM Orbitrap technology and MS/MS for the analysis of polyfluoroalkyl substances by EPA Method 537

Authors
Ali Haghani,[1] Andy Eaton,[1] Richard F. Jack,[2] Ed George,[2] Dipankar Ghosh[2]

[1]Eurofins Eaton Analytical, Inc. Monrovia, CA; [2]Thermo Fisher Scientific, San Jose, CA

Keywords
Contaminants of emerging concern, CEC, perfluorinated compound, perfluoroalkyl acid, PFOA, PFOS, perfluorinated alkyl substances, PFAS, perfluoroalkyl acids, PFAA, persistent organic pollutants, POPs, fire-fighting foam, Orbitrap

Goal
To demonstrate a liquid chromatography – high-resolution, accurate-mass (LC-HRAM) methodology using Orbitrap™ technology as a sensitive, accurate, and reliable quantitative alternative to the use of triple quadrupole mass spectrometers while simultaneously determining unknown perfluorinated compounds in the same drinking water extracts.

Introduction
The unique water-, oil-, grease-, stain- and heat-resistant properties of perfluoroalkyl substances (PFASs) have led to their widespread use in diverse industrial applications and multiple consumer products for over fifty years.

Perfluoroalkyl substances are compounds for which all hydrogens on all carbons (except for carbons associated with functional groups) have been replaced by fluorines, e.g., perfluoroalkyl acids (e.g., PFOA, PFOS). Polyfluoroalkyl substances are compounds for which all hydrogens on at least one (but not all) carbons have been replaced by fluorines, e.g., fluorotelomer-based compounds.[1] The carbon-hydrogen linkages allow for biotic and abiotic degradation in the environment. However, the C–F bond

ThermoFisher SCIENTIFIC

U.S. EPA Method 537 target list

PFAS compound	Critical Level (ng/L)	DL (ng/L)	LCMRL (ng/L)
PFBS	0.15	0.2	<0.5
PFDA	0.15	0.26	<0.5
PFDoA		0.47	0.73
PFHpA	0.09	0.15	<0.5
PFHxA	0.13	0.19	<0.5
PFHxS		1.7	2.4
PFNA	0.11	0.17	<0.5
PFOA		0.22	0.5
PFOS		0.26	0.5
PFTA	0.15	0.2	<0.5
PFTrDA		0.31	0.55
PFuNA		0.38	1

Using a full scan approach, required detection limits or MRLs can be achieved while interrogating for other untargeted PFAS compounds.

The compounds highlighted in blue are additional analytes that are not part of the original U.S. EPA Method 537 list but were found in processed drinking water from the same UCMR3 water extracts.

PFAS compound	Critical Level (ng/L)	DL (ng/L)	LCMRL (ng/L)
PFBA		0.19	0.64
PFODA		0.55	1
PFDS	0.13	0.19	<0.5
PFHxDA		0.12	0.5
PFPA	0.18	0.19	<0.5

HRAM Orbitrap technology provides equal or better quantitation in full scan as compared to traditional triple quadrupole techniques, with the additional capability to screen for unknown PFASs.

Identify unknowns and emerging contaminants

For complex samples with unknown amounts of other PFASs, utilization of Compound Discoverer software can reduce the data processing time and quickly show results
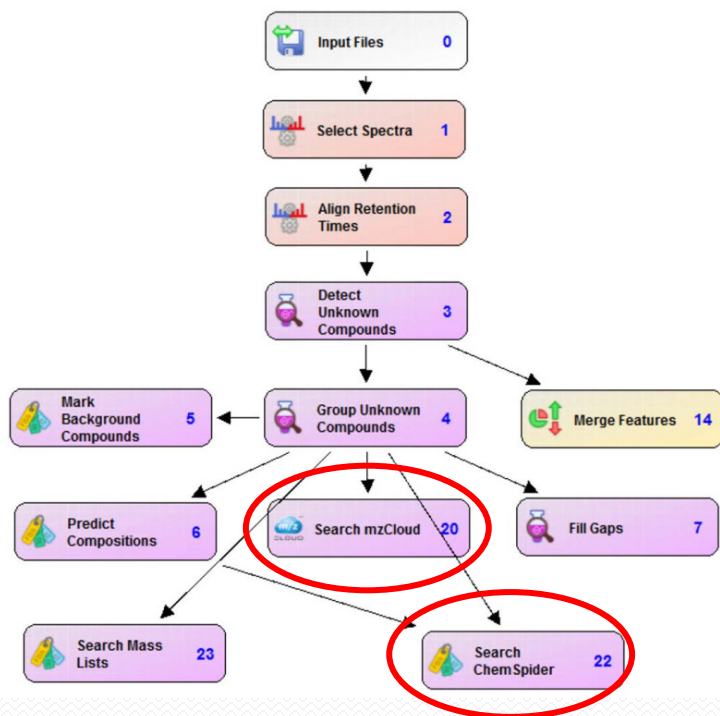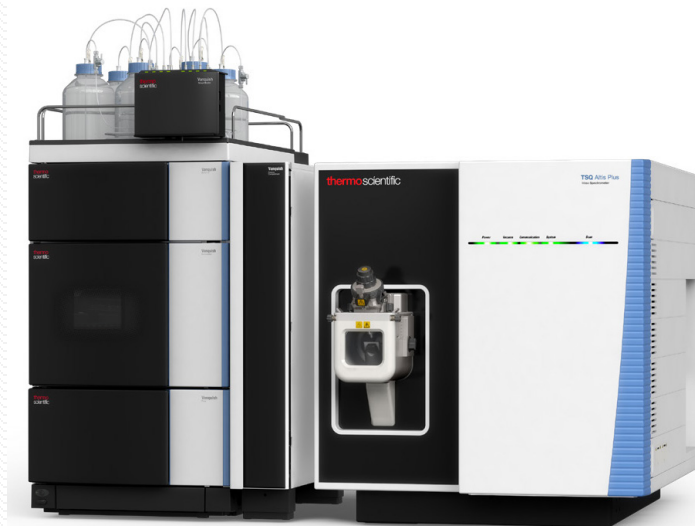

COMPOUND DATABASES
AND SPECTRAL LIBRARIES

Summary

TARGET QUANTITATION

- PFAS analysis following standard methods e.g. US EPA, ASTM

LC-MS/MS

Triple quad mass spectrometer

SCREENING OR UNKNOWN PROFILING

- Identify unknowns and emerging contaminants
- Retrospectively analyze data even when the sample no longer exists

LC-HRAM

High resolution accurate mass Q-orbitrap mass spectrometer