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Determination of PFAS in Drinking Water Using Automated Solid Phase Extraction and LC-MS/MS for U.S. EPA Method 533

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Bellevue, WA

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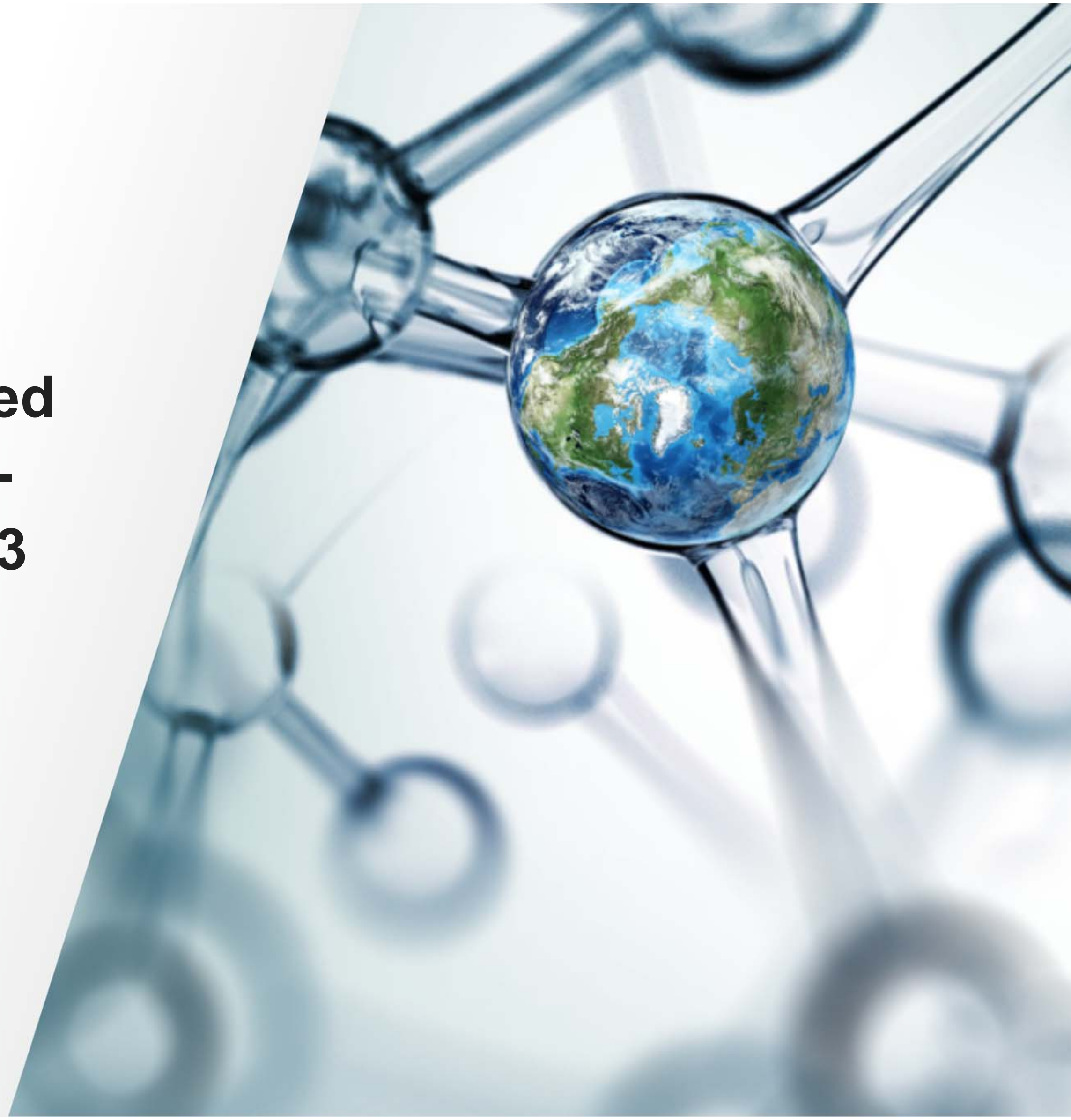

Sources and Where PFAS is Found in the Environment

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PFAS Uses

Food Contact Materials
- Cookware
- Microwave popcorn
- Fast food wrappers

Fabrics
- Stain resistant carpets and fabrics
- Waterproof or resistant clothing

Household Products
- Cleaning products
- Personal care products

Industrial Uses
- Firefighting foam – Major groundwater source
- Manufacturing processes

PFAS in the environment

Water
- Drinking water
- Groundwater
- Wastewater

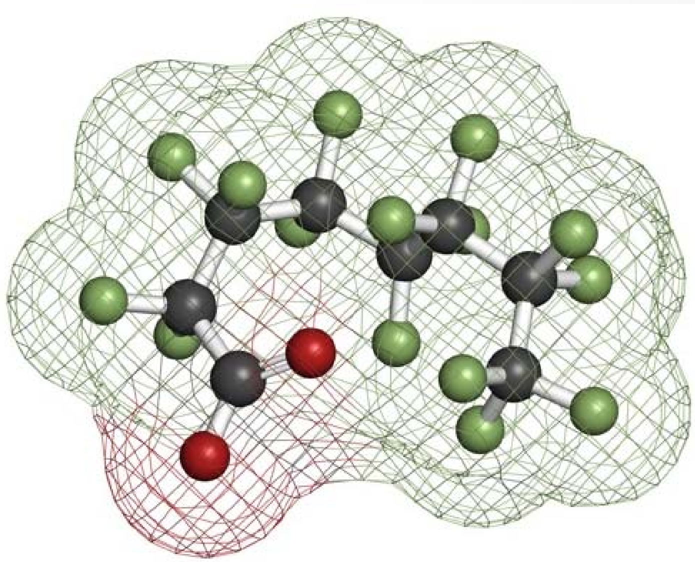
Soil – Runoff

Foods
- Produce
- Fish and Meats

Why PFAS Analysis is Important

Persistence

- C-F bonds very strong; do not break down
- Called “Forever Chemicals”



Bioaccumulation

- Cannot be metabolized
- Build up in biological systems over time

Health Concerns

- Affects growth, learning, behavior
- Endocrine interference
- Increase cholesterol levels
- Affect the immune system
- Increase the risk of cancer
- Infertility

1 – CDC ASTDR https://www.atsdr.cdc.gov/pfas/health-effects.html

PFAS Hotspots

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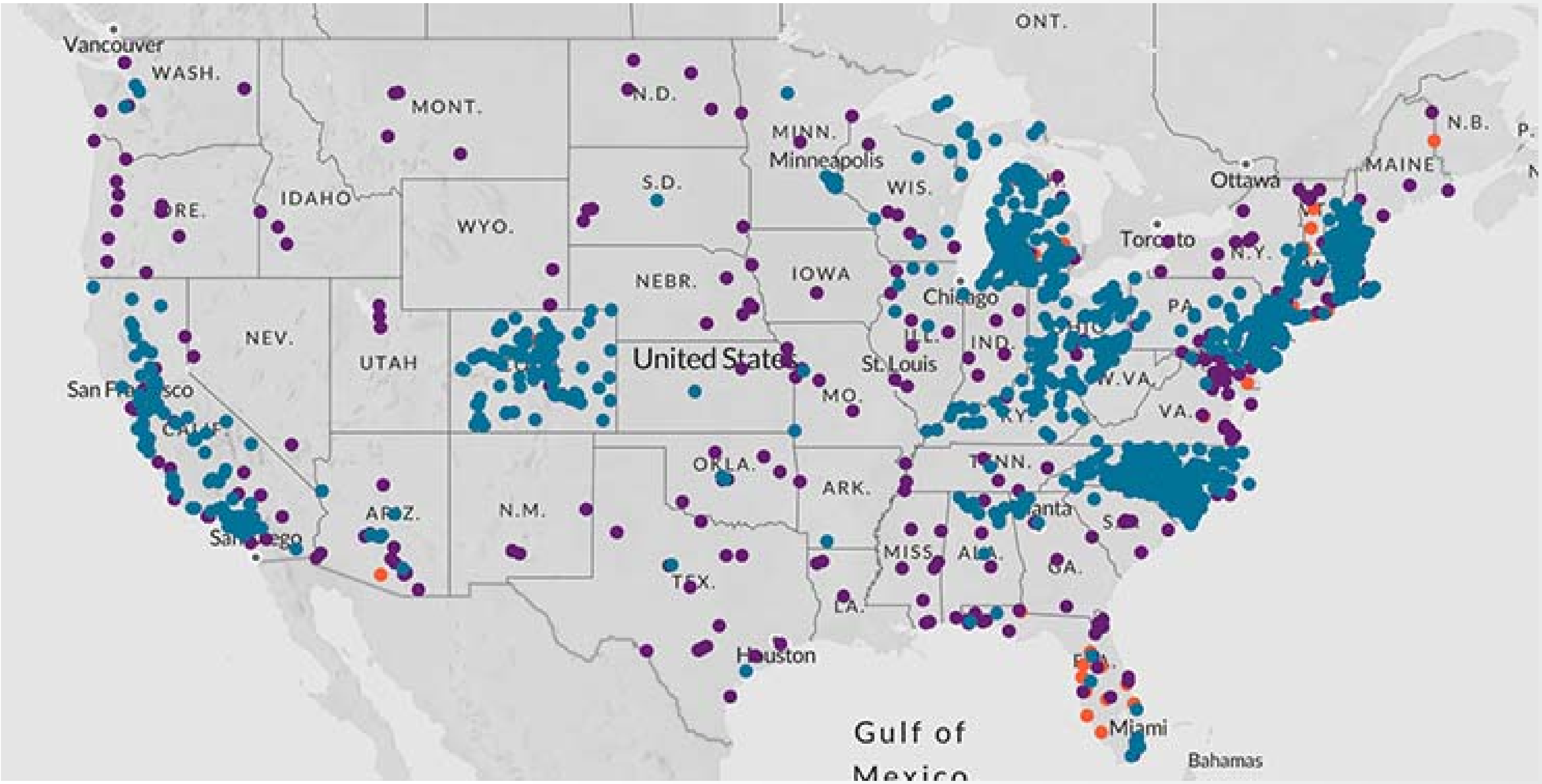

https://www.ewg.org/interactive-maps/2019_pfas_contamination/map/

Drinking Water Methods and Regulation

- US EPA 537, 537.1 and 533 drinking water
- EPA 8327 water, soil, biota
- ASTM 7979 water, sludge and wastewater

- The maximum contaminate level (MCL) for the 18 PFAS compounds is 0.53 - 6.3 ng/L (ppt).
  - National total PFAS MCL is 70 ppt in drinking water
  - Individual states have set lower limits down to 10 ppt

- Background interference
  - Must be maintained below 1/3 of the MRL value
  - Example sources: Reagents, containers, clothing, labware and laboratory instrumentation (PTFE tubing)

PFAS Analysis Considerations

- Background contribution
  - Personal care products and clothing
  - Sample containers and devices – Must be polypropylene
  - Waterproof documents and markers
  - Adhesive products and shipping materials
  - Laboratory items – Especially gloves and other lab ware
  - Analytical Instrumentation
- Sample preparation
  - Multiple manual steps in collection and sample prep
  - Target compounds can adhere to surfaces
  - Extraction efficiency vary by compound
  - Recovery and reproducibility is a challenge

Why a second targeted PFAS Method

- Focus on short chain PFAS (Carbon lengths 4 to 12)
- Adds an additional 11 PFAS compounds over EPA 537.1
- Does not cover 4 of the compounds in EPA 537.1

US EPA 533 was made to target the more water soluble PFAS molecules

Analyte	Abbreviation	CASRN	Method 533	Method 537.1
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	11Cl-PF3OUdS	763051-92-9	x	x
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acd	9Cl-PF3ONS	756426-58-1	x	x
4,8-Dioxa-3H-perfluorononanoic acid	ADONA	919005-14-4	x	x
Hexafluoropropylene oxide dimer acid	HFPO-DA	13252-13-6	x	x
Perfluorobutanesulfonic acid	PFBS	375-73-5	x	x
Perfluorodecanoic acid	PFDA	335-76-2	x	x
Perfluorododecanoic acid	PFDoA	307-55-1	x	x
Perfluoroheptanoic acid	PFHpA	375-85-9	x	x
Perfluorohexanoic acid	PFHxA	307-24-4	x	x
Perfluorohexanesulfonic acid	PFHxS	355-46-4	x	x
Perfluorononanoic acid	PFNA	375-95-1	x	x
Perfluorooctanoic acid	PFOA	335-67-1	x	x
Perfluorooctanesulfonic acid	PFOS	1763-23-1	x	x
Perfluoroundecanoic acid	PFUnA	2058-94-8	x	x
1H,1H, 2H, 2H-Perfluorohexane sulfonic acid	4:2FTS	757124-72-4	x	
1H,1H, 2H, 2H-Perfluorooctane sulfonic acid	6:2FTS	27619-97-2	x	
1H,1H, 2H, 2H-Perfluorodecane sulfonic acid	8:2FTS	39108-34-4	x	
Nonafluoro-3,6-dioxaheptanoic acid	NFDHA	151772-58-6	x	
Perfluorobutanoic acid	PFBA	375-22-4	x	
Perfluoro(2-ethoxyethane)sulfonic acid	PFEESA	113507-82-7	x	
Perfluoroheptanesulfonic acid	PFHpS	375-92-8	x	
Perfluoro-4-methoxybutanoic acid	PFMBA	863090-89-5	x	
Perfluoro-3-methoxypropanoic acid	PFMPA	377-73-1	x	
Perfluoropentanoic acid	PFPeA	2706-90-3	x	
Perfluoropentanesulfonic acid	PFPeS	2706-91-4	x	
N-ethyl perfluorooctanesulfonamidoacetic acid	NEtFOSAA	2991-50-6		x
N-methyl perfluorooctanesulfonamidoacetic acid	NMeFOSAA	2355-31-9		x
Perfluorotetradecanoic acid	PFTA	376-06-7		x
Perfluorotridecanoic acid	PFTrDA	72629-94-8		x

US EPA 537.1 Versus US EPA 533 Workflow

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EPA 537.1

250 mL sample water with 1.25g Trizma → Add surrogates → Extract with AutoTrace 280 PFAS → Evaporate to dryness and reconstitute in 96% MeOH → Add Internal Standards → Analyze by LC-MS/MS

SDVB SPE Cartridge

EPA 533

250 mL sample with 250 mg ammonium acetate → Add isotope dilution analogues → Extract using WAX SPE → Evaporate to dryness and reconstitute in 80:20 MeOH:H_2O → Add internal standards → Analyze by LC-MS/MS

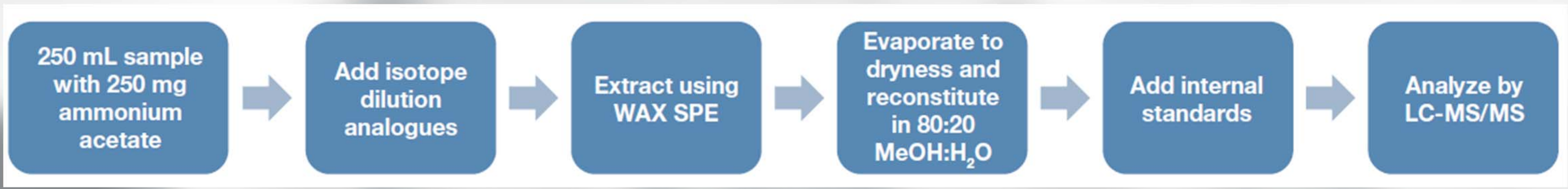
Different preservative

Isotope dilution in 533

WAX SPE Cartridge

Higher conc. H2O in recon

US EPA 533 Workflow



Solid Phase Extraction (SPE) Process

Sample Prep Devices

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Sample Extraction and Clean up

Vacuum Manifold

Thermo Scientific ™ Dionex™ AutoTrace™ 280 PFAS Solid-Phase Extraction Instrument

Solid Phase Extraction – EPA Method 533

Keeping cartridge sorbent wet is very important

Cartridge Conditioning
with methanol and phosphate buffer

Time ~ 10 to 15 min

Flow rate is critical, too quickly will cause loss of analytes

Sample Loading
Load 250 mL drinking water

Time ~ 17 to 25 min

Elution speed must be controlled otherwise analytes will stay on the sorbent

Cartridge Elution
Rinse sample bottles and elute SPE cartridges with 2% ammonium

Time ~ 10 min

Dry and Reconstitute
80:20 MeOH/H_2O

Vacuum Manifold

- 100% manual process – Analyst must dedicate time to do the extraction
- Manual interaction leads to PFAS background
- Extraction quality is very technique dependent
- Differential pressure across the manifold
  - Stronger pull-on cartridges closest to the outlet
  - Causes variable flow rates from cartridge to cartridge
    - Flow rate control is important
  - Individual cartridge valve needs to be set properly

- Different manifold housing needed for each step
- Multiple cartridge loading required at each step
  - 15 and 18 mL into 6 mL cartridge when conditioning
  - 250 mL into 6 mL cartridge

Thermo Scientific Dionex AutoTrace 280 PFAS Instrument Extraction Method

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System Start-up and AutoTrace Prep

- Prime system and clean flow paths using pre-loaded methods
- Attach SPE cartridges, Add samples and collection rack

↓

Select method from instrument panel and start

↓

Rinse sample bottles and start elution method

↓

Evaporate, reconstitute, and load samples on LC-MS/MS

Automated Solid Phase Extraction for Liquid Samples

- Automation
  - No constant attendance necessary - Walk up operation
  - Method stored on the AutoTrace 280 PFAS – No software required
- PEEK sample/solvent lines and other flow path components
  - Ensures automation without the risk of background contamination
- Uses a positive pressure pump to deliver samples and solvents
  - Consistent flow rates – precise control at every step
  - Improved extraction performance
- Achieve lower detection limits
  - Can load up to 4L of sample

Thermo Scientific Dionex AutoTrace 280 PFAS Instrument vs Manual Methods

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Lowers background contamination risk

- Non-fluoropolymer-based materials
- Fewer manual touch points

Better Data Quality

- Improved reproducibility and recoveries
- Lower risk of rerunning or resampling

Positive pressure instead of vacuum

- Allows for more precise control over the process
- Ensures proper flow rates at critical steps
- Sorbents stay wet when required

Significant labor savings

- Minimal user interaction required
- Analysts can accomplish other lab tasks
- Lower costs due to increased efficiency

Vacuum SPE vs Thermo Scientific Dionex AutoTrace 280 PFAS Instrument

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- Vacuum SPE
  - Problems maintaining consistent flow
  - Cannot let it run dry – affects the flow rate and performance
  - Inconsistent volumes run to run
  - Requires constant attendance

- AutoTrace 280 PFAS
  - Pumps are set at a constant flow
  - Ensures cartridges do not dry on critical steps
  - Precise consistency run to run
  - Push “start” and have a cup of coffee

LC-MS/MS EPA 537.1

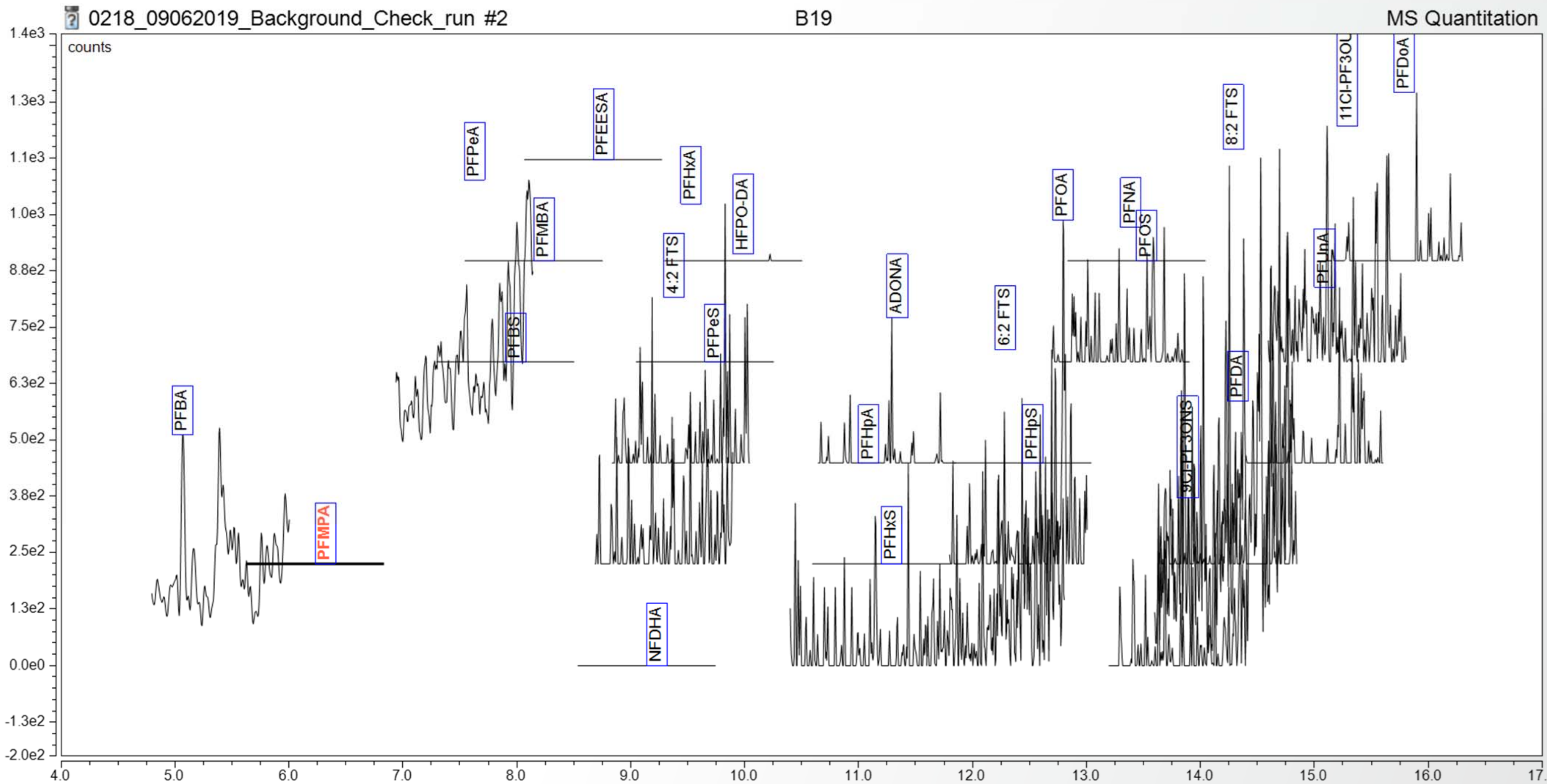
- 250 mL sample with 250 mg ammonium acetate
- Add isotope dilution analogues
- Extract using WAX SPE
- Evaporate to dryness and reconstitute in 80:20 MeOH:H_2O
- Add internal standards
- Analyze by LC-MS/MS

LC-MS/MS Analysis

Thermo Scientific™
Vanquish™ Duo UHPLC Systems
With PFC-Free Kit

Thermo Scientific™
TSQ Fortis™ Triple
Quadrupole Mass
Spectrometer

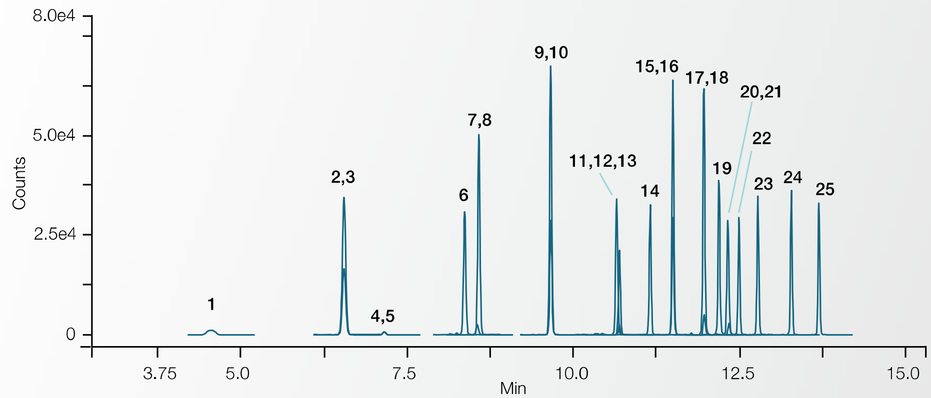
Background Test



Well below the 1/3 of the method MRL requirement

EPA 537.1 Performance Data using Thermo Scientific Dionex AutoTrace 280 PFAS

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



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

All recoveries and precision are well with in the method requirements

- Recovery must be 70 – 130%
- RSD < 20%

EPA 533 Performance Data

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	Fortification (ng/L)	REC (%) (P@A Low)	RSD (P@A Low)	REC (%) (P@A High)	RSD (P@A High)
$^{13}C_4$-PFBA	40	105	6.1	106	6.7
$^{13}C_5$-PFPeA	40	102	4.8	105	6.0
$^{13}C_3$-PFBS	40	106	2.6	117	3.9
$^{13}C_2$ 4:2 FTS	160	110	3.3	121	6.4
$^{13}C_5$-PFHxA	40	95.5	3.9	97.7	4.1
$^{13}C_3$-HFPO-DA	40	109	15	120	13
$^{13}C_4$-PFHpA	40	106	4.8	111	7.4
$^{13}C_3$-PFHxS	40	99.1	6.5	104	5.3
$^{13}C_2$-6:2 FTS	160	102	4.9	115	8.8
$^{13}C_8$-PFOA	40	101	6.7	106	8.4
$^{13}C_9$-PFNA	40	97.9	5.9	106	11
$^{13}C_8$-PFOS	40	92.3	11	105	13
$^{13}C_2$-8:2 FTS	160	97.6	8.3	103	11
$^{13}C_6$-PFDA	40	96.2	7.4	104	11
$^{13}C_7$-PFUnA	40	90.0	9.0	92.8	14
$^{13}C_2$-PFDoA	40	91.7	7.7	94.5	14

Recovery range 91% - 110%

%RSD for all PFAS <20

Number	Analytes	Fortification levels (ng/L)	LCMRL (ng/L)
1	PFBA	1.0, 2.0,4.0, 6.0, 10, 14, 20	8.6
2	PFMPA	1.0, 2.0,4.0, 6.0, 10, 14, 20	4.5
3	PFPeA	1.0, 2.0,4.0, 6.0, 10, 14, 20	3.9
4	PFBS	1.0, 2.0,4.0, 6.0, 10, 14, 20	3.2
5	PFMBA	1.0, 2.0,4.0, 6.0, 10, 14, 20	3.9
6	PFEESA	1.0, 2.0,4.0, 6.0, 10, 14, 20	3.4
7	NFDHA	1.0, 2.0,4.0, 6.0, 10, 14, 20	5.7
8	4:2 FTS	1.0, 2.0,4.0, 6.0, 10, 14, 20	7.0
9	PFHxA	1.0, 2.0,4.0, 6.0, 10, 14, 20	3.2
10	PFPeS	1.0, 2.0,4.0, 6.0, 10, 14, 20	2.6
11	HFPO-DA	1.0, 2.0,4.0, 6.0, 10, 14, 20	7.4
12	PFHpA	1.0, 2.0,4.0, 6.0, 10, 14, 20	3.4
13	PFHxS	1.0, 2.0,4.0, 6.0, 10, 14, 20	6.5
14	ADONA	1.0, 2.0,4.0, 6.0, 10, 14, 20	1.6
15	6:2 FTS	1.0, 2.0,4.0, 6.0, 10, 14, 20, 40	5.7
16	PFOA	1.0, 2.0,4.0, 6.0, 10, 14, 20	3.9
17	PFHpS	1.0, 2.0,4.0, 6.0, 10, 14, 20, 40	5.8
18	PFNA	1.0, 2.0,4.0, 6.0, 10, 14, 20	2.8
19	PFOS	1.0, 2.0,4.0, 6.0, 10, 14, 20	5.2
20	^{9}Cl-PF_3ONS	1.0, 2.0,4.0, 6.0, 10, 14, 20	2.9
21	8:2 FTS	1.0, 2.0,4.0, 6.0, 10, 14, 20, 40	9.5
22	PFDA	1.0, 2.0,4.0, 6.0, 10, 14, 20	2.1
23	PFUnA	1.0, 2.0,4.0, 6.0, 10, 14, 20	4.1
24	^{11}Cl-PF_3OUdS	1.0, 2.0,4.0, 6.0, 10, 14, 20	2.4
25	PFDoA	1.0, 2.0,4.0, 6.0, 10, 14, 20,40	4.8

Lowest concentration minimum reporting limits

PFAS in Drinking Water Workflow

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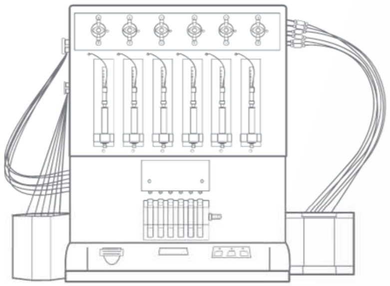
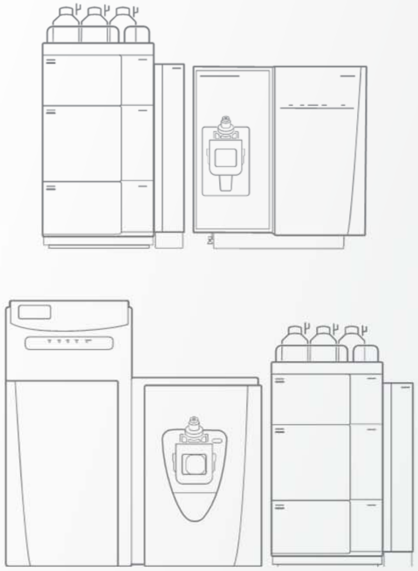
Complete service and support from Thermo Fisher Scientific

Sample collection, processing → Sample extraction and clean up → Analysis → Data processing





Conclusions

The Dionex AutoTrace 280 PFAS Instrument

- Improves lab efficiency
- Requires less user interaction to accomplish solid phase extraction steps
- Operates well within the method requirements
- Greatly reduces risk of errors
    - Gives better assurance of high data quality
    - Fewer occurrences of rerunning or resampling
- Helps to keep background at a minimum
    - Reduces manual touch points which can introduce background

Learn More

Quantification and Screening for PFAS Analysis

- thermofisher.com/pfas

- Download our complete PFAS kit to learn more:
  - View webinars
  - How to perform PFAS analysis for targeted and non-targeted analysis
  - Database information for unknowns

Critical information for robust PFAS analysis

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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

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

Thank you!

Thank you for your time and attention!

Questions?