Targeted LC-MS/MS Quantitation of Legacy and Emerging Per- and Polyfluoroalkyl Substances (PFAS) in Water Matrices

Tarun Anumol, Ph.D.
Director, Global Environment & Food Markets
Agilent Technologies, Inc.

Agilent
Trusted Answers

Per- and Polyfluoroalkyl substances (PFAS)

Useful or harmful?

- Thermal and chemical stability
  - ➢ Grease-proof food packaging and stain repellents
- Zwitterionic properties
  - ➢ Surfactants
- Surface-tension lowering
  - ➢ Fire-fighting foams

- Presence of PFAS in drinking water and water sources is of emerging concern globally due to widespread usage, environmental persistence and bioaccumulative tendency

Driving PFAS Analysis – Continued Media/Regulatory Focus

These Everyday Toxins May Be Hurting Pregnant Women and Their Babies

PFAS, industrial chemicals used to waterproof jackets and grease-proof fast-food containers, may disrupt pregnancy with lasting effects.

https://www.nytimes.com/2020/09/23/parenting/pregnancy/pfas-toxins-chemicals.html

Europe To Adopt Sweeping Tap Water Limits for PFAS, Other Toxic Contaminants

https://www.ewg.org/release/europe-adopt-sweeping-tap-water-limits-pfas-other-toxic-contaminants

'Canary in a coal mine': Scientists test alligators for PFAS chemical compounds

Kristen Johnson The Fayetteville Observer

Published 6:30 a.m. ET May 6, 2021 | Updated 9:37 a.m. ET May 6, 2021

View Comments

Researchers are studying the effect of per- and polyfluoroalkyl substances, or PFAS, on alligators. *Associated Press*

https://www.fayobserver.com/story/news/2021/05/06/north-carolina-alligators-found-have-autoimmune-response-pfas-chemicals/4945275001/

Emerging chemical risks in Europe — 'PFAS'

It is currently not possible to perform in-depth environmental and health risk assessments of all chemical substances in use in Europe because of the great variety of chemicals and their diverse uses. New and legacy chemicals continue to be released into Europe's environment, adding to the total chemical burden on Europe's citizens and ecosystems. Early identification of emerging risks is one of the activities of the European Environment Agency (EEA). This briefing summarises the known and potential risks to human health and the environment in Europe posed by a group of very persistent chemicals, the per- and polyfluorinated alkyl substances (PFAS).

Published 12 Dec 2019 — Last modified 09 Mar 2021 — 12 min read — Photo: © imani vDQ e3RtaoE / Unsplash PDF

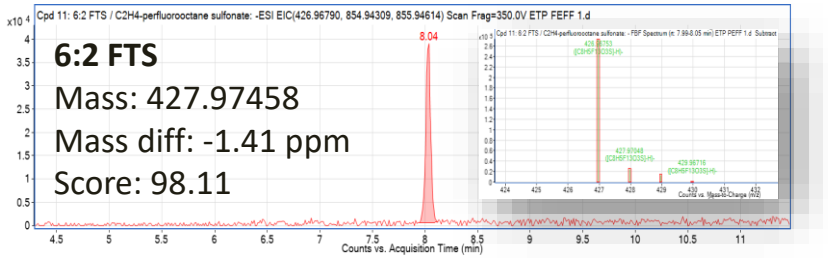
https://www.eea.europa.eu/publications/emerging-chemical-risks-in-europe

Complete PFAS Analytical Workflow

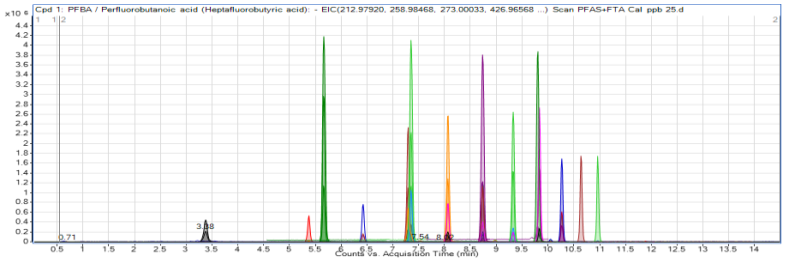
Known knowns
Expanded targeted list
(~50-100 compounds)

LC/TQ

Known unknowns
Expanded PFAS database



Continually refining
targeted method



LC/Q-TOF

Unknown unknowns
Adding identified
compounds to database list

WED, Aug 11 11:30 AM Accurate Mass QTOF – A new Direction in PFAS Quantification, Kathy Hunt, Vogon Labs

Challenges associated with PFAS Testing

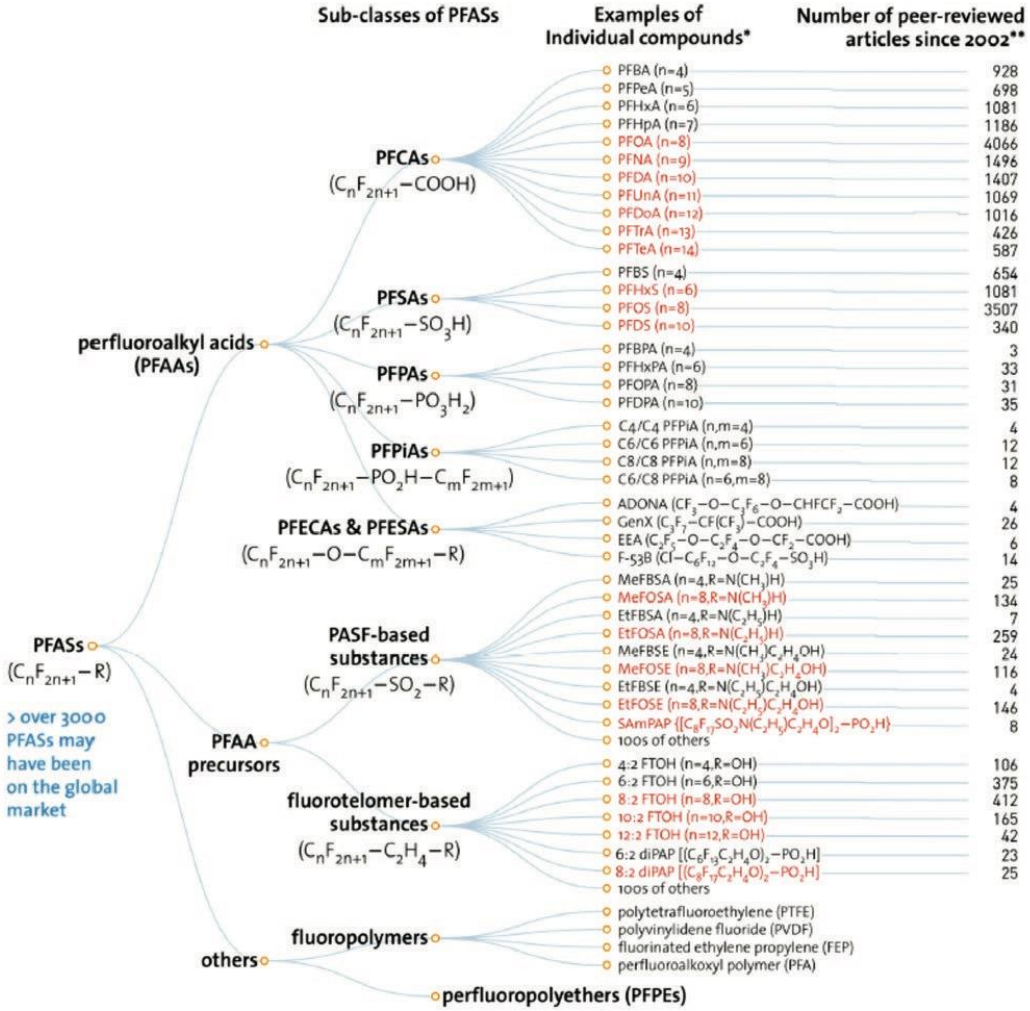
- ***Increased Scope***
  - 2? Or 6? Or 14? Or 25 or….. 4000 possible PFAS, How many to measure
  - More volatile PFAS, smaller PFAS, different structures and end groups
- ***Increased Throughput***
  - Faster turnaround times demanded
  - Better methodologies to quickly gauge PFAS contamination
- ***Extremely low detection levels and background issues***
  - Low and even sub part per trillion levels to be detected
  - PFAS are nearly ubiquitous in work environments and in lab products
- ***Fast evolving regulations***
  - New PFAS to be measured, different matrices
  - New Standard methods
  - Different Audits and Accreditations – Data Integrity, Security & Compliance

PFAS Classifications and Terminology

>4000 PFAS compounds in commerce

Common Acronyms

PFCA	Perfluoroalkylcarboxylic acid
PFOA	Perfluorooctanecarboxylic acid
PFAS	Perfluoroalkylsulfonate
PFOS	Perfluorooctanesulfonate
PFASi	Perfluoroalkylsulfinate
FOSA	Perfluorooctanesulfonamide
FOSAA	Perfluorooctanesulfonamidoacetic acid
FOSE	Perfluorooctanesulfonamidoethanol
FTOH	Fluorinated telomer alcohol (-OH functional group)
FTA	Fluorinated telomer acid
FTUA	Fluorinated telomer unsaturated acid
FTS	Fluorinated telomer sulfonate
PFAPA	Perfluoroalkylphosphonic acid
PFPi	Perfluoroalkylphosphinate
PAP	Mono-substituted polyfluoroalkylphosphate ester
diPAP	Di-substituted polyfluoroalkylphosphate ester
PFAI	Perfluoroalkyl iodide
SFA	Semifluorinated alkane
FTI	Fluorinated telomer iodide
FTO	Fluorinated telomer olefin
FTAC	Fluorinated telomer acrylate



Wang, Z *et al.* (2017). *Environ. Sci. Technol.* 51, 2508-2518.

Where to go with PFAS Quantification

Been there, Done that … ?

History with Pesticides Analysis

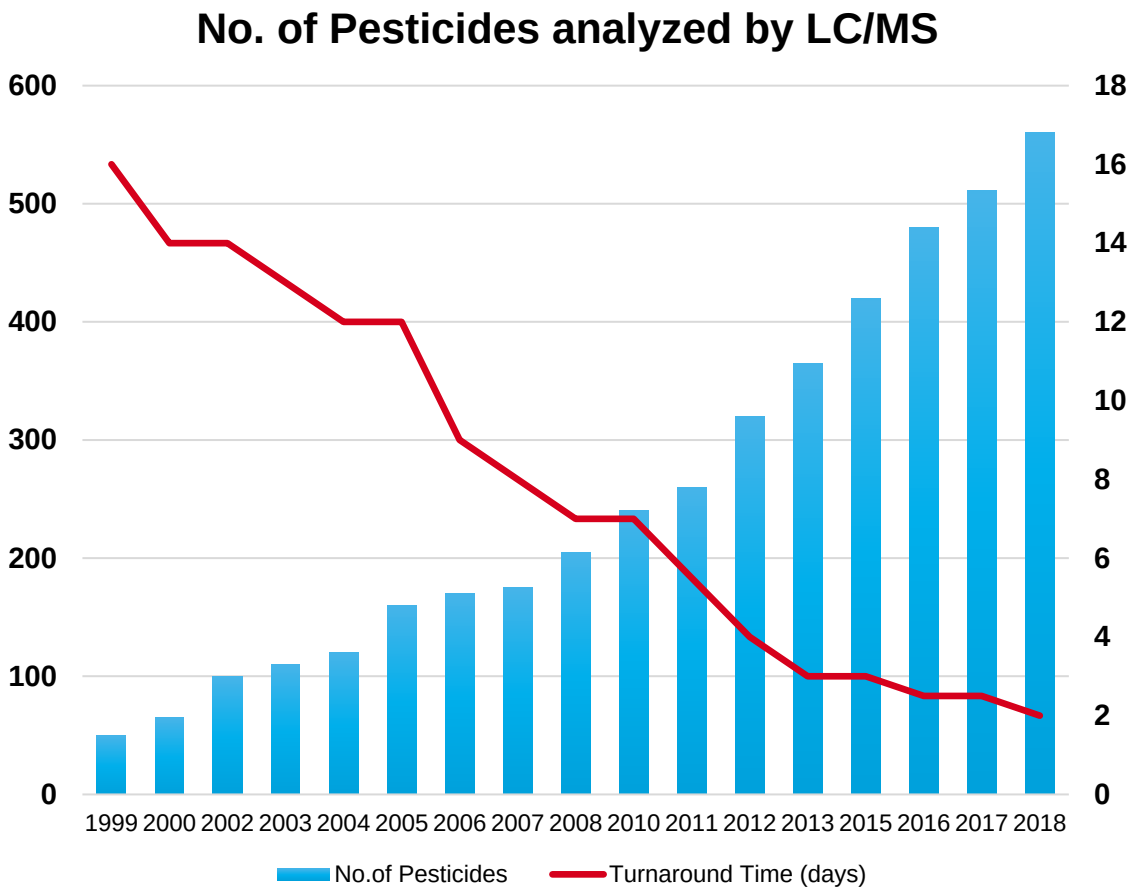
Sample Turnaround Time from 2 weeks to 2 days

Cost per sample drastically reduced

Analysis time on instrument reduced from >80 min to 16 min

Analytical methods reduced to multi-residue mega method

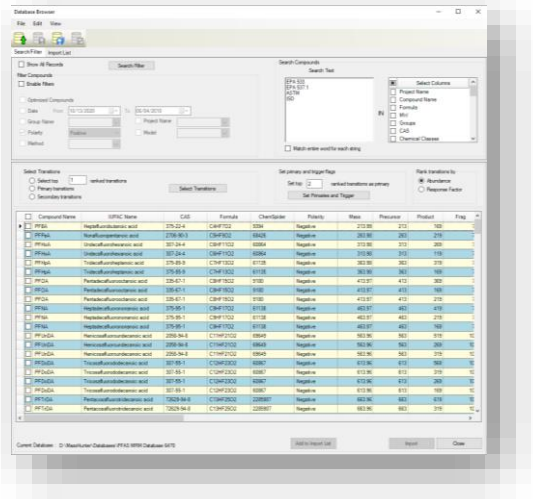
Commercial Food Lab



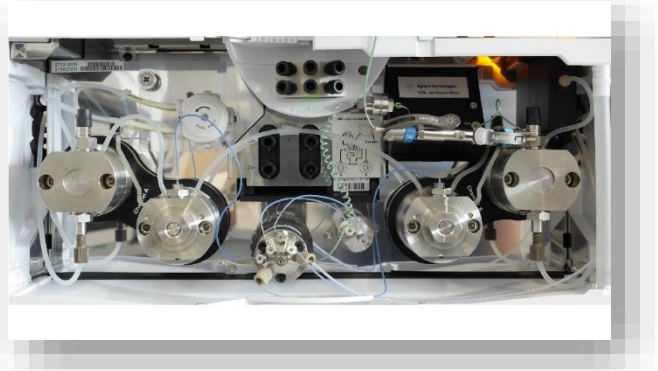
PFAS analysis needs many aspects to Meet Various Laboratory Needs

Individual Products and Services for Regulatory and Individual Method Development

LC-MS/MS instrumentation



PFAS MRM Database



PFC-Free HPLC Conversion Kit

Application Services & Support

PFC-Free Columns and Supplies

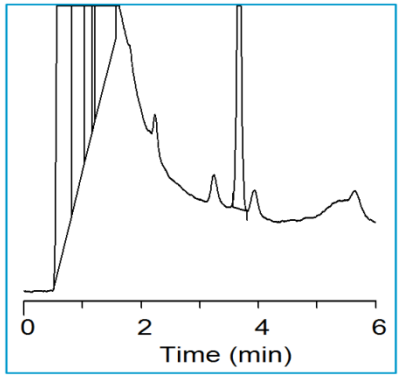
Sample Preparation

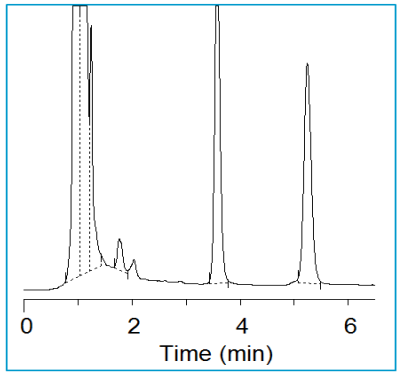
Sample preparation refers to the ways in which samples being treated prior to their analysis. Target analytes are the needle in the haystack of matrix, sample prep helps find the needle in the haystack.

Sample Prep

Sample *without* Sample Prep

Sample with Sample Prep





Why sample prep is important for sample analysis?

- ✓ Extraction
- ✓ Sample clean-up
- ✓ Dilution or concentration
- ✓ To protect the instrument detection system from contamination
- ✓ Improve the detection method robustness and reliability

SKIPPING SAMPLE PREP IS GREAT!!!!!
……. UTIL IT'S NOT

Sampling Handling & Storage Advances
‘PFAS’ specific vials and caps

- PTFE lining in Caps can have PFAS contamination
- This leads to use of PP style snap top vials that have very poor sealability on piercing or longer-term storage with organic solvent
- This can result in use of 2 or more caps per sample

- Glass vials are thought to adsorb certain PFAS, hence PP vials are preferred

Unique bi-layer of PP and silicone that allows resealing once pierced

No PTFE and free of 26 measured PFAS

1.7 mL fill volume and standard screw top

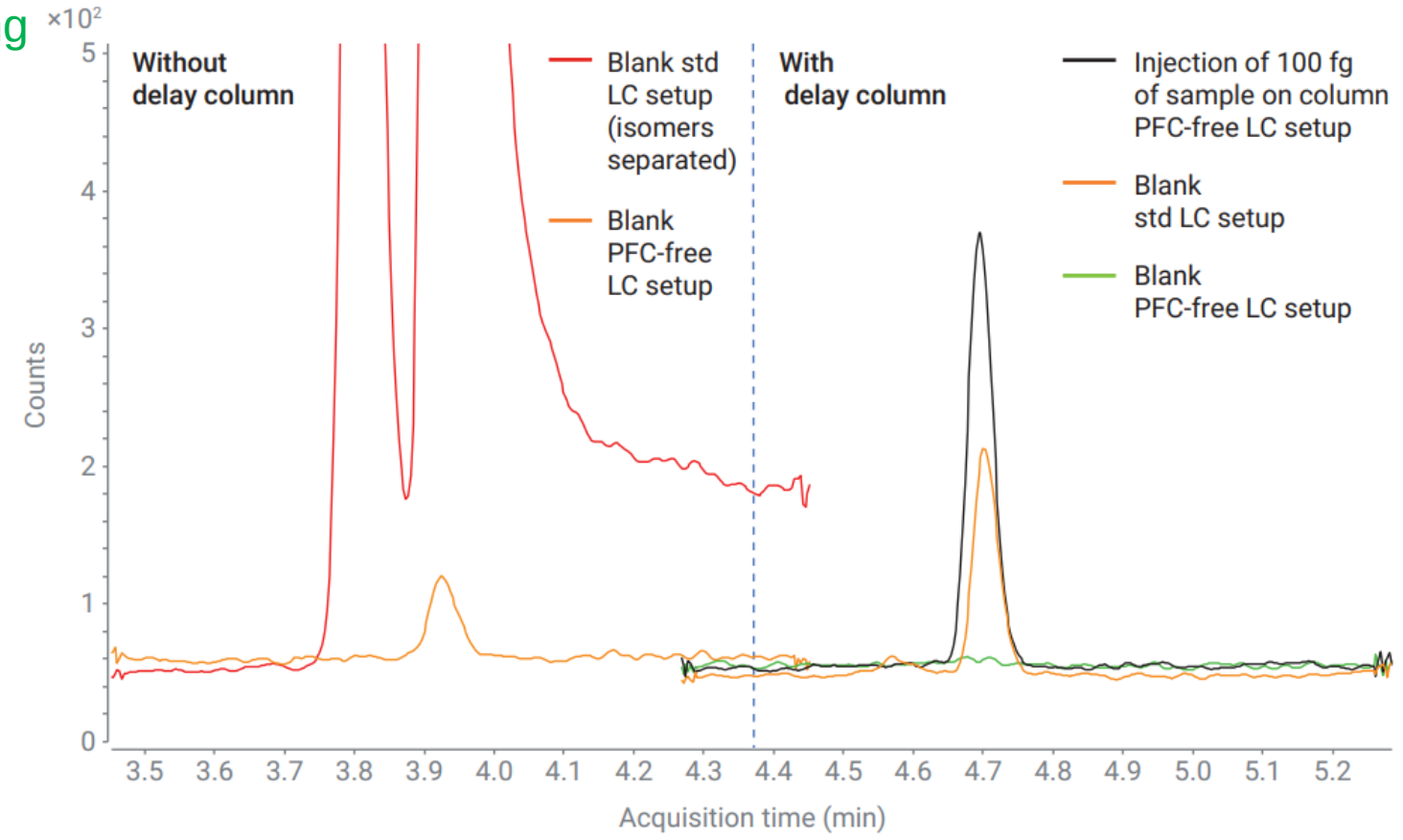
Description	Part number
2 mL screw style clear polypropylene vial (100pk)	5191-8150
9 mm screw style clear polypropylene cap with thin membrane polypropylene / silicone septa (100pk)	5191-8151

PFC Free Kit
Eliminate Background Contamination

LC Configuration	PFHpA Background (fg)	PFNA Background (fg)
Standard LC Setup	>3,000	>500
Standard LC Setup With Delay Column	48	48
PFC-free LC Setup	20	37
PFC-free LC Setup With Delay Column	<2 (below detection limit)	<7 (below detection limit)

Potential Contamination Sources

- Solvents
- Filtration apparatus
- Teflon lined tubing



(P/n: 5004-0006)

Analytical choices for PFAS Quantification
LC-MS/MS still seen as gold standard for quantification

LESS SAMPLE PREP

SENSITIVITY

Direct injection

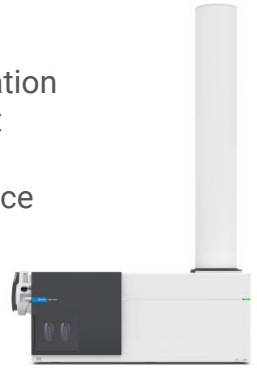
6470 LC/MS/MS
- ✓ Robustness
- ✓ Enhanced sensitivity for Environmental work
- ✓ Gate valve for no-vent capillary cleaning

‘Dilute & shoot’
Large volume injection

6495 LC/MS/MS
- ✓ Ultimate Sensitivity
- ✓ Gate valve for no-vent capillary cleaning

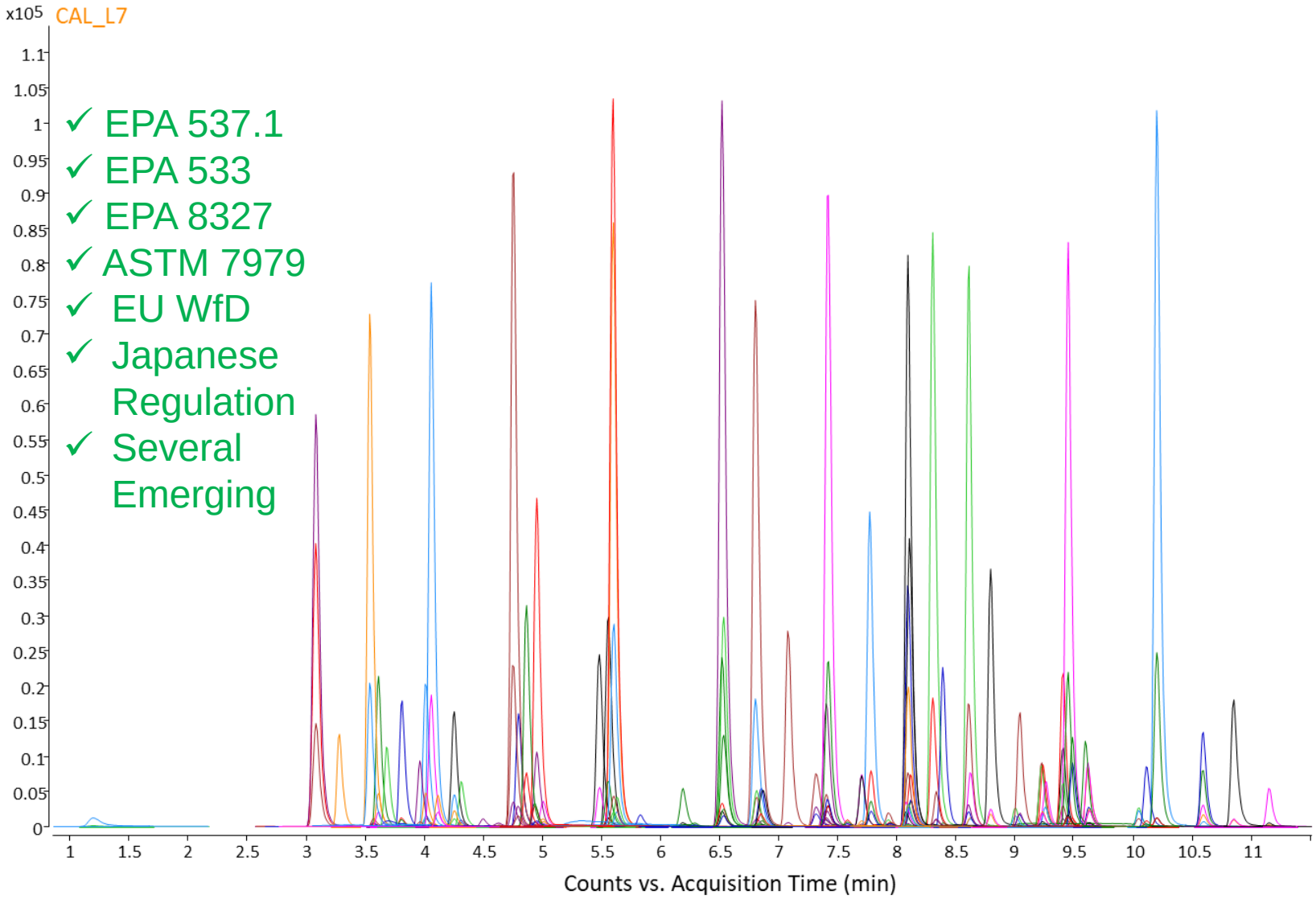
Sample enrichment suggested

ULTIVO
- ✓ Routine Quantification
- ✓ Smallest Footprint LC/MS/MS
- ✓ Guided maintenance



6546 LC-Q/TOF
- ✓ High resolution accurate mass
- ✓ Suspect screening and unknown PFAS

Comprehensive Method – Over 100 PFAS Compounds



Native Analytes:

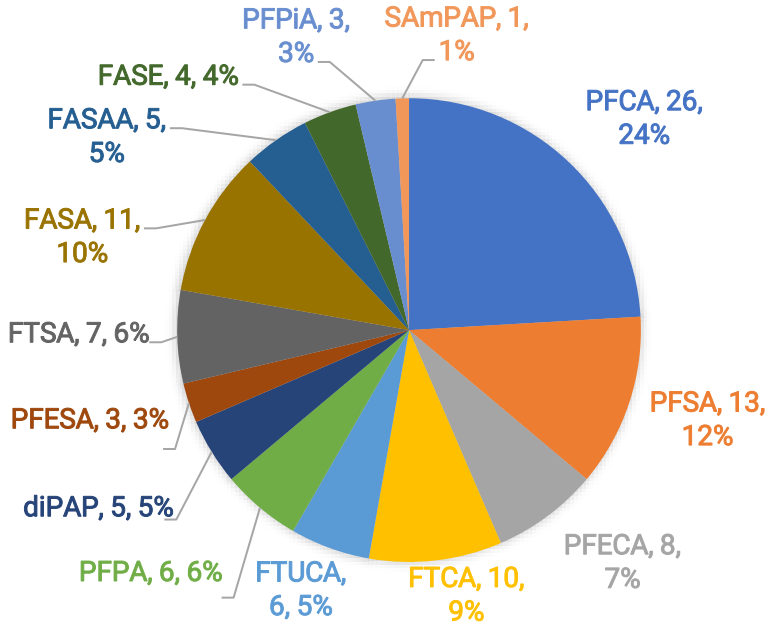
- 71 native PFAS analytes

Surrogates:

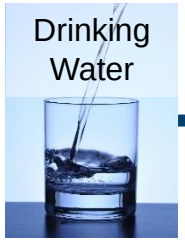
- 33 isotopically labeled PFAS
- 1 native, Cl-PFOPA is used as ISTD for PFPAs

Isotope performance standards:

- 3 isotopically labeled PFAS



PFAS eMethod Workflow overview



250 mL per sample

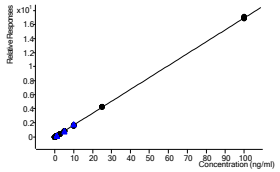
+ SUR

Solid phase extraction (SPE) using SampliQ Weak Anion Exchange (WAX), 6mL, 150 mg

+ IPS

SPE extracts (250x concentration)

Calibration standards (CAL) in 80:20/MeOH:H_2O



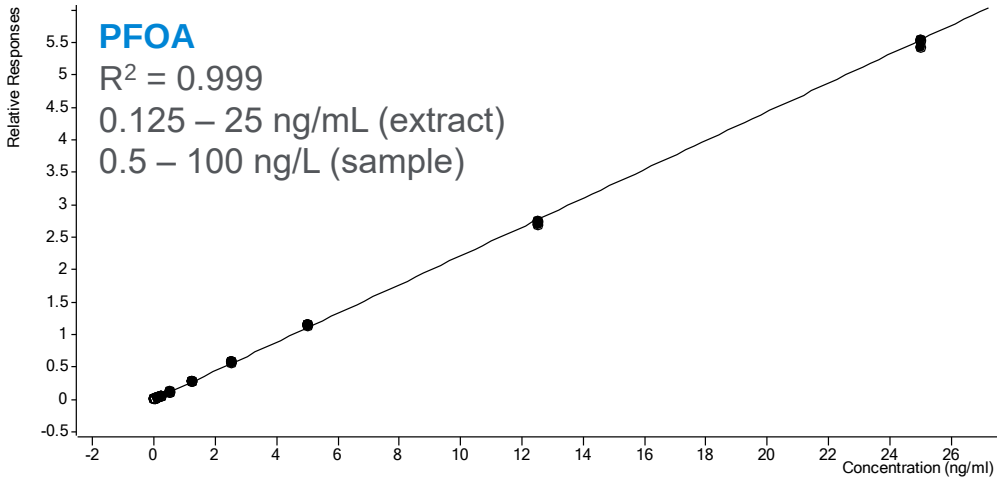
Agilent PFC-Free HPLC Conversion Kit

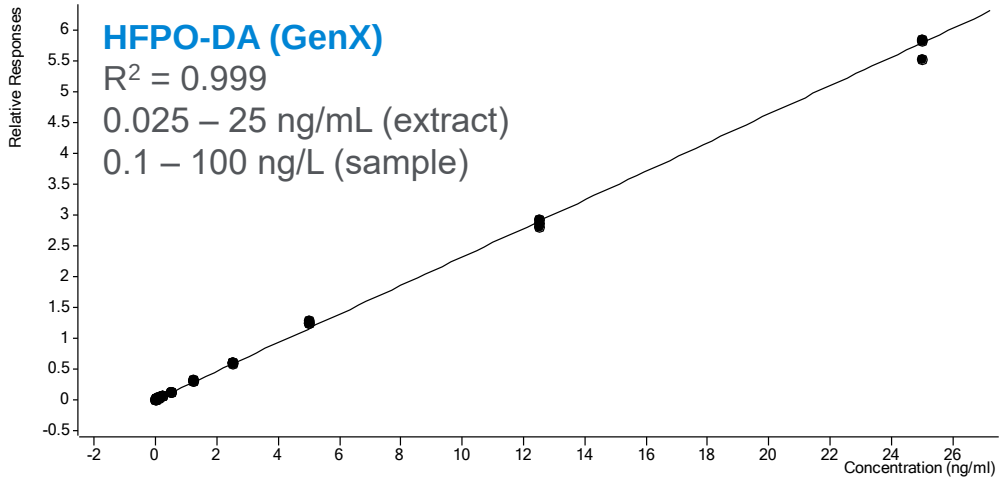
- Agilent 1290 Infinity II UHPLC
- Agilent 6470 LC/TQ

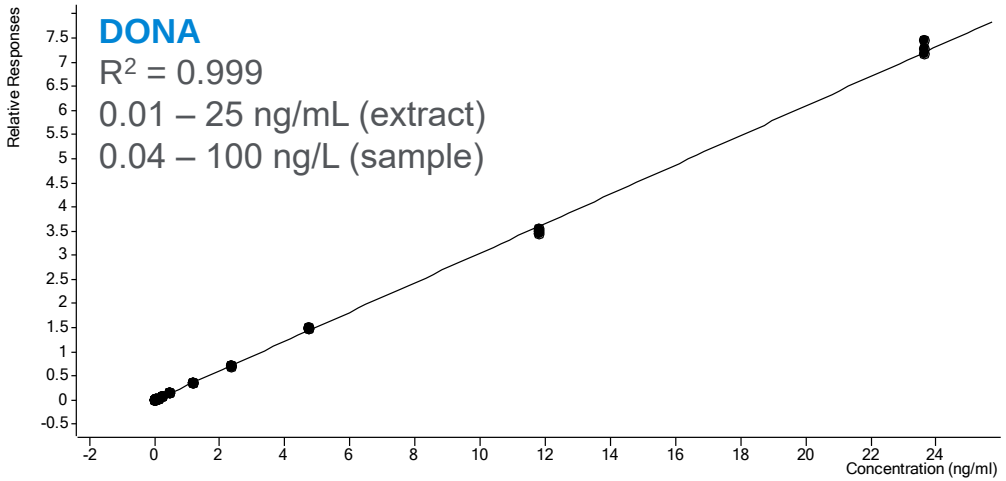
Method assessed on:
- CAL analytical range
- Method sensitivity
- Method recovery
- Method precision

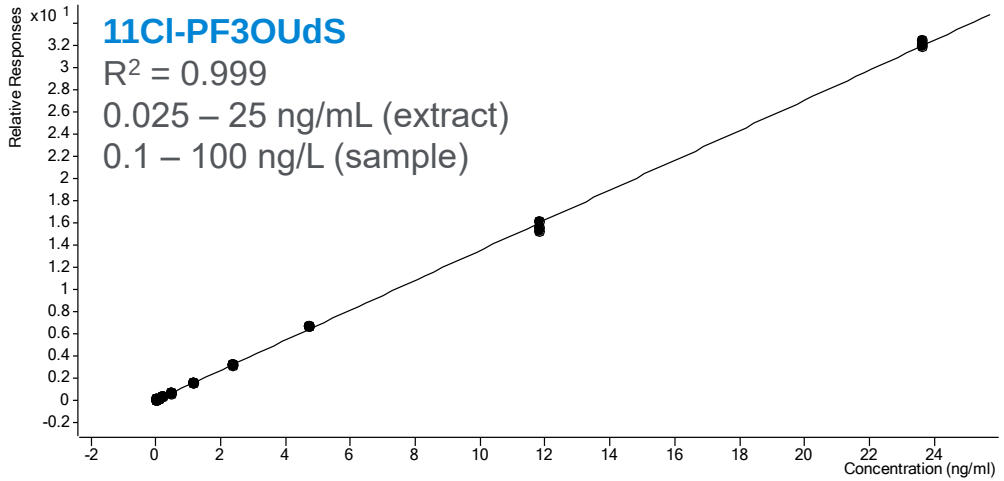
Analytical range and accuracy

Wide analytical range with R^2 > 0.99 with good accuracy; RSE <10% for low cal





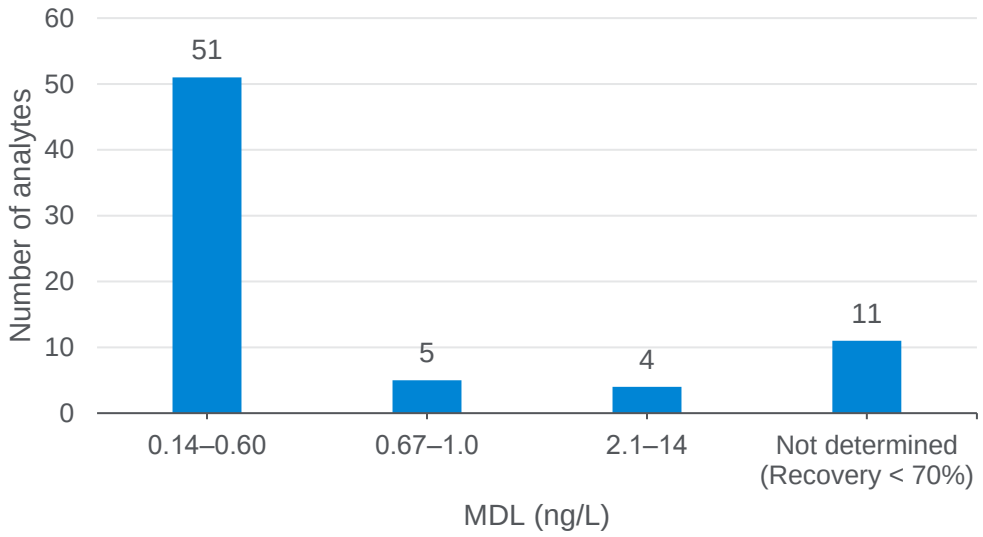




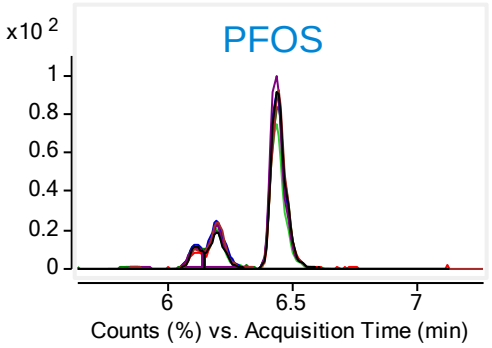
Method detection limit (MDL)

Matrix: Reagent water (Milli-Q water)

- Calculated following procedure described in 40 CFR Part 136 Appendix Revision 2:
  - 7 replicates of reagent water samples spiked at 1 to 25 ng/L and extracted using the SPE protocol
  - MDL was calculated using the following formula: MDL = S.D. × Student's t-test value (3.143)
- MDLs were determined for 60 out of 71 analytes.
- The method demonstrated good sensitivity with MDLs less than 0.6 ng/L for 51 out of 60 analytes.

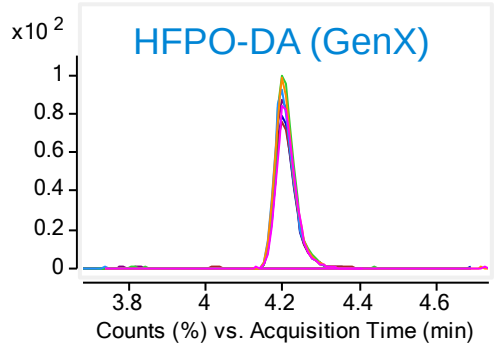


Example 1:



MDL	0.27 ng/L
RSD (n = 7)	8.5%
Recovery	109%

Example 2:



MDL	0.24 ng/L
RSD (n = 7)	7.4%
Recovery	102%

Inter-day Reproducibility Study – Method recovery and precision

Study was:

- Conducted by 2 different analysts using 2 different units of 6470 LC/TQs on 2 separate calendar dates
- Assessed by spiking and extracting 250 mL of drinking water at “low spike” and “high spike”

Drinking Water	Inter-day Recovery (%)	Inter-day Precision (%RSD)
Low spike (n = 8)[a]	76 to 116	2.9 to 16.7
High spike (n = 6)[b]	79 to 119	2.2 to 11.7

60 compounds meet acceptable recovery and precision limits

Surface Water	Inter-day Recovery (%)	Inter-day Precision (%RSD)
Low spike (n = 8)[a]	73 to 116	3.0 to 19.9
High spike (n = 6)[b]	73 to 113	1.6 to 13.7

57 compounds meet acceptable recovery and precision limits

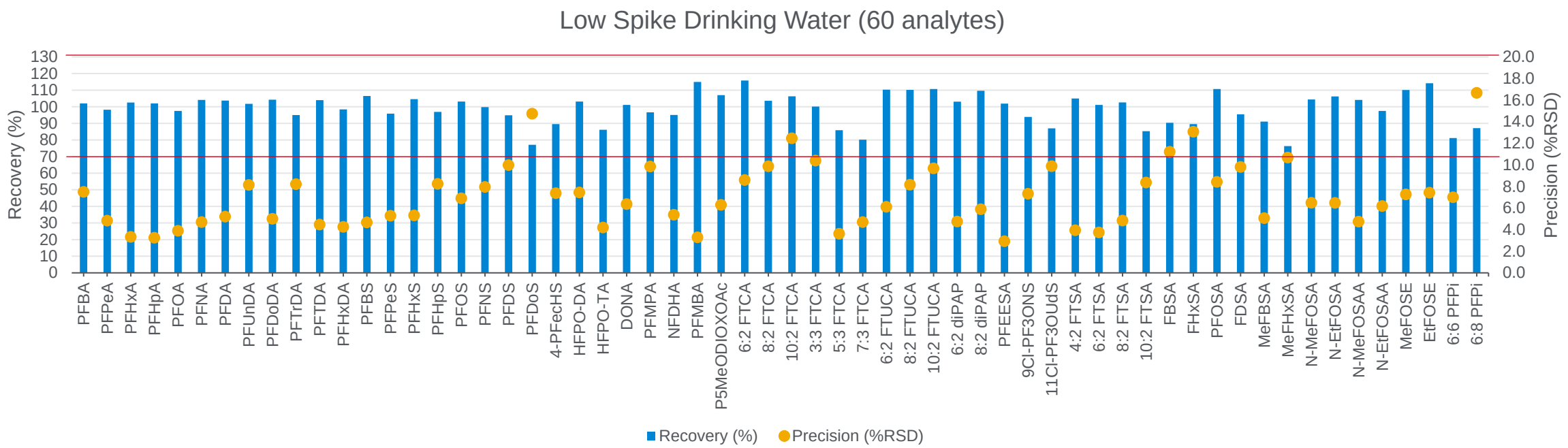
[a] Low spike concentration: 5, 10, 20 or 50 ng/L
[b] High spike concentration: 20, 40, 80 or 200 ng/L

Drinking Water – Method recovery and precision

Good recovery and precision for 60 PFAS across 2 batches

Sample Type	Interbatch Recovery (%)	Interbatch Precision (%RSD)
Low spike (n = 8)[a]	76 to 116	2.9 to 16.7
High spike (n = 6)[b]	79 to 119	2.2 to 11.7

Meet the limits of 70-130% and RSD ≤ 20% for recovery and precision, respectively



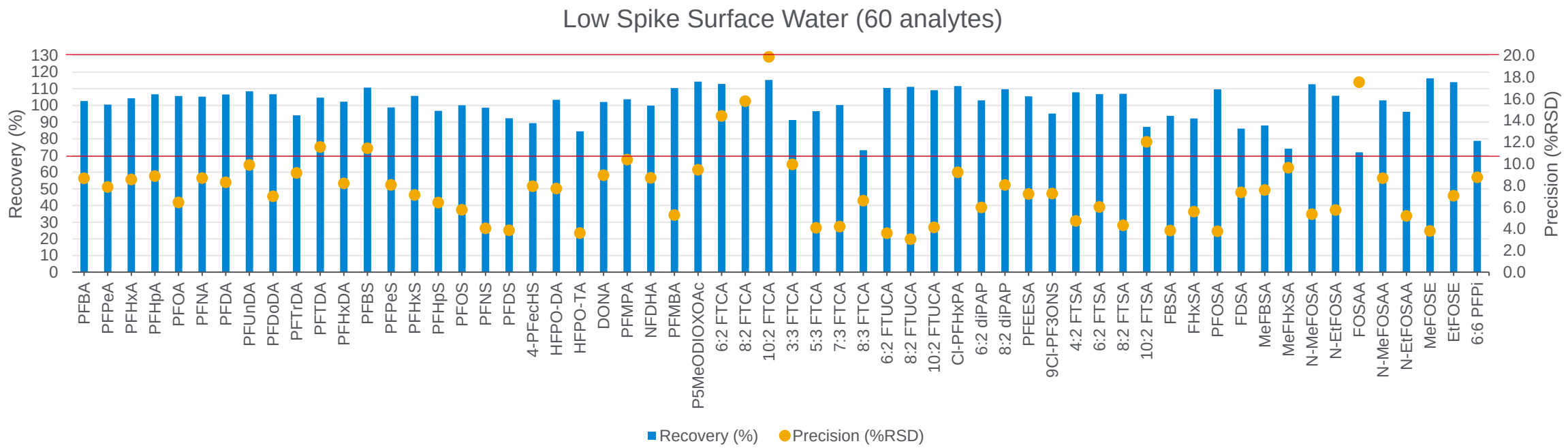
[a] Low spike concentration: 5, 10, 20 or 50 ng/L; [b] High spike concentration: 20, 40, 80 or 200 ng/L

Surface Water – Method recovery and precision

Good recovery and precision for 60 PFAS across 2 batches

Sample Type	Interbatch Recovery (%)	Interbatch Precision (%RSD)
Low spike (n = 8)[a]	72 to 116	3.0 to 19.9
High spike (n = 6)[b]	73 to 120	1.6 to 16.5

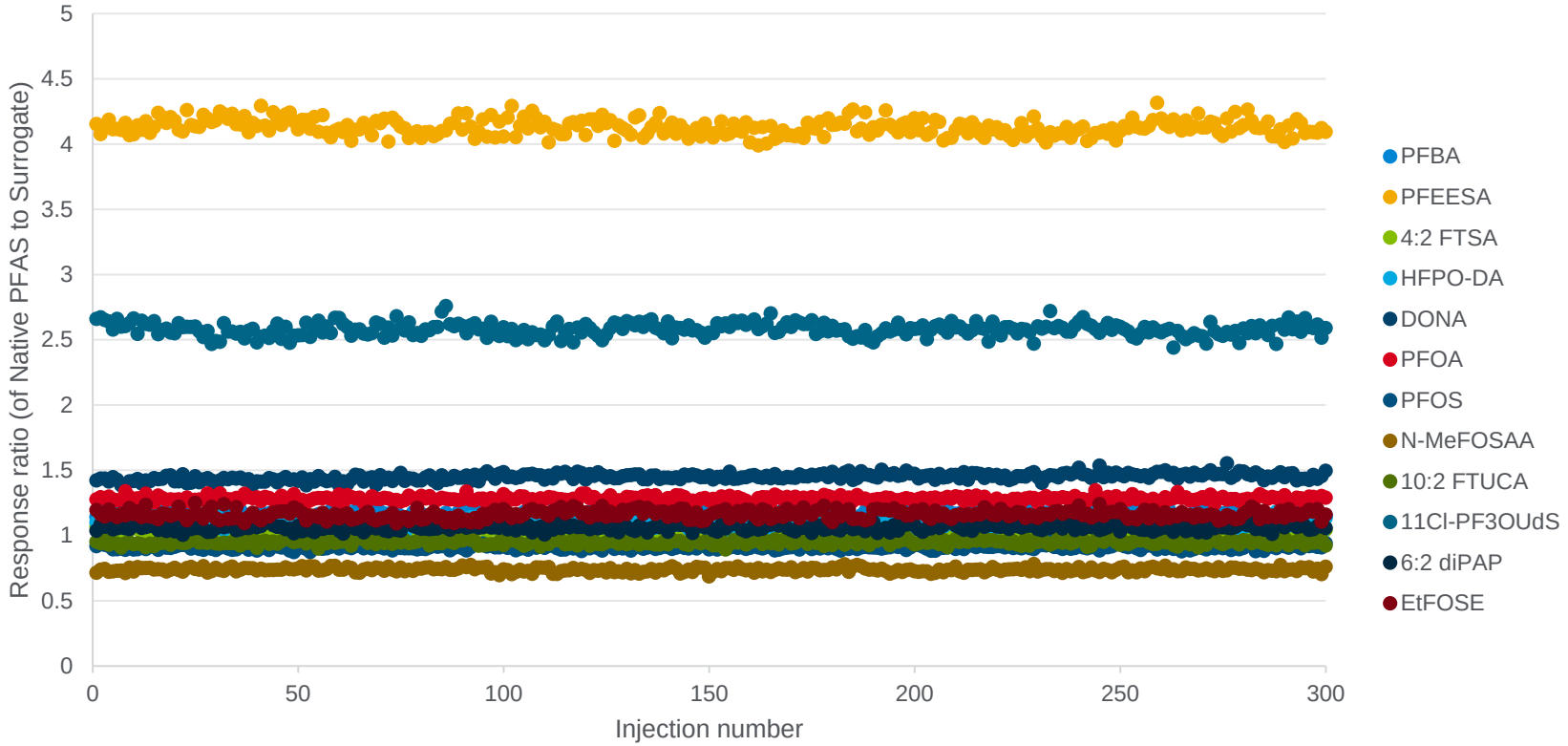
Meet the limits of 70-130% and RSD ≤ 20% for recovery and precision, respectively



[a] Low spike concentration: 5, 10, 20 or 50 ng/L; [b] High spike concentration: 20, 40, 80 or 200 ng/L

Method robustness

300 continuous injections of high spike Surface Water samples



No.	Analyte	RT (min)	Response Ratio %RSD
1	PFBA	3.05	1.8
2	PFEESA	3.75	1.5
3	4:2 FTSA	3.95	1.3
4	HFPO-DA	4.17	3.1
5	DONA	4.75	1.7
6	PFOA	5.46	1.4
7	PFOS	6.37	1.6
8	N-MeFOSAA	7.62	2.1
9	10:2 FTUCA	8.15	1.9
10	11Cl-PF3OUdS	8.25	1.9
11	6:2 diPAP	9.24	2.0
12	EtFOSE	9.63	2.7

Response ratio reproducibility of RSD ≤ 3.1% for 12 analytes using continuous operation of 6470 LC/TQ (93 h)

Note: High spike concentration: 20, 40, 80 or 200 ng/L

Method Validation Study for PFAS eMethod

Sample preparation by 2nd operator and analysis using 2nd LC/MS/MS

Activity	#	Test	Procedure	Acceptance criteria	Results
Calibration in neat solvent	1	Identification	Analyte peaks will be identified using Dynamic MRM transitions	All native compounds included in method identified with minimum of 2 MRM transitions except where 2 MRM transitions do not exist.	Pass • 62 out of the 71 compounds have a minimum of 2 MRM transitions. • 9 compounds have 1 MRM transition
	2	Accuracy	Calculated concentration from calibration curve as percentage with respect to expected concentration	70–130%	Pass
	3	Linearity	Use at least 5 standard concentrations to generate a linear or quadratic calibration curve	$R^2 \geq 0.99$	Pass
Interference check (Milli-Q water)	4	System background	Analyze a Lab Reagent Blank (LRB), i.e. unspiked Milli-Q water, after the highest standard in the calibration range.	Demonstrate that the concentration of the native compounds are less than the method detection limit (MDL)	Pass

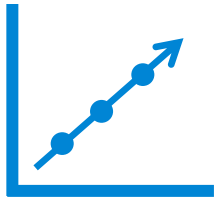
Method Validation Study for PFAS eMethod

Sample preparation by 2nd operator and analysis using 2nd LC/MS/MS

Activity	#	Test	Procedure	Acceptance criteria	Result
Drinking Water – Precision and Recovery	5	Precision	RSD of calculated concentrations of replicate extractions	RSD ≤ 20%	Pass • 68 out of 71 compounds had RSD ≤ 20%
	6	Recovery	Calculated concentration of each analyte with respect to its spiked concentration, expressed in percentage	70–130%	Pass • 60 out of 71 compounds had recoveries within 70–130%
Surface Water – Precision and Recovery	8	Precision	RSD of calculated concentrations of replicate extractions	RSD ≤ 20%	Pass • 69 out of 71 compounds had RSD ≤ 20%
	9	Recovery	Calculated concentration of each analyte with respect to its spiked concentration, expressed in percentage	70–130%	Pass • 61 out of 71 compounds had recoveries within 70–130%

Summary

Final optimized method covers 71 analytes from 14 different PFAS groups



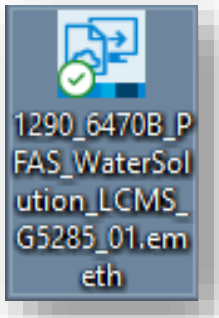
Linear or quadratic calibration with $R^2 \geq 0.99$

Sensitivity: MDLs are sufficient to meet most drinking water standard methods or regulatory limits

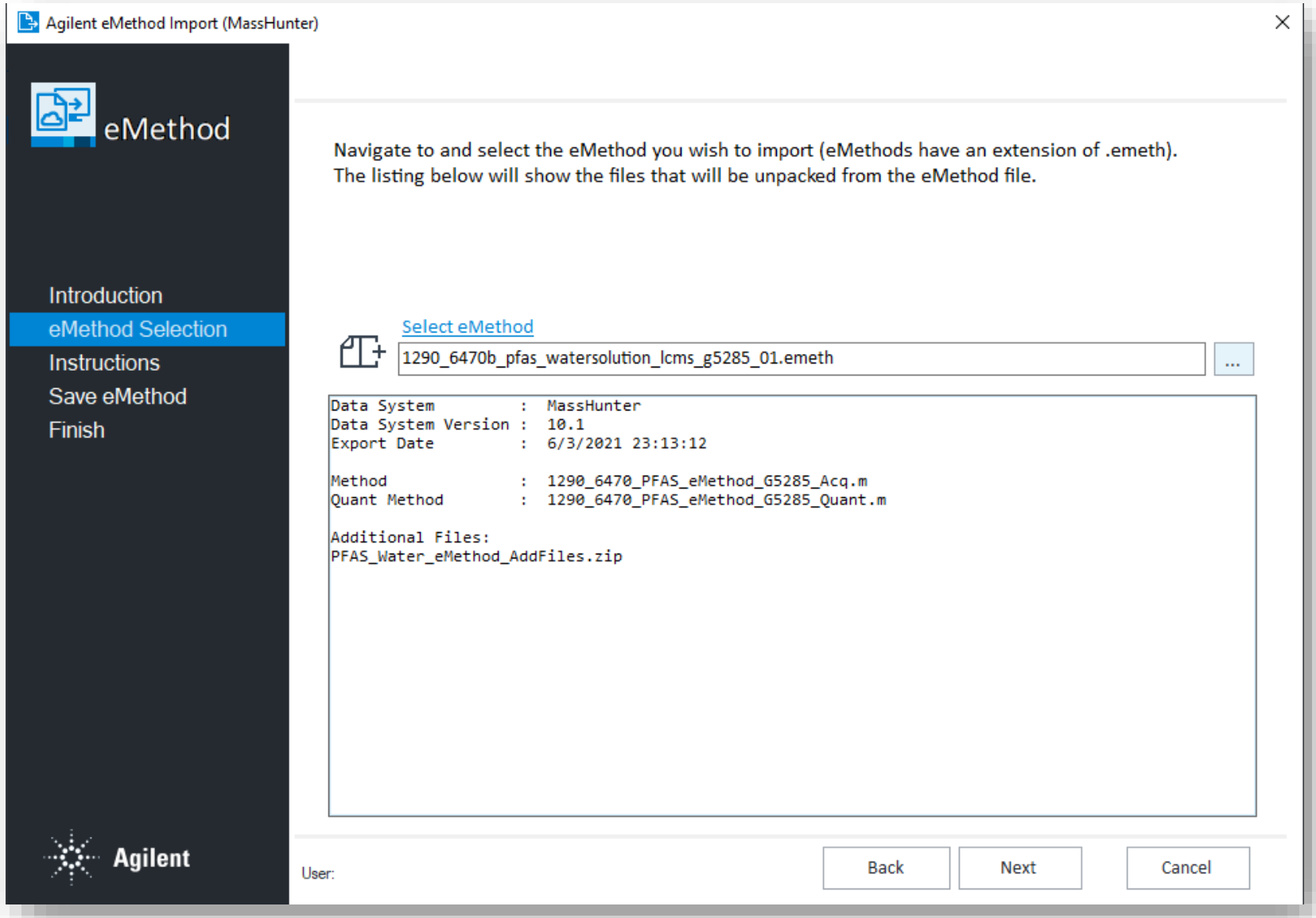
Interbatch recovery and precision confirms method robustness

Method verified for Drinking Water and Surface Water

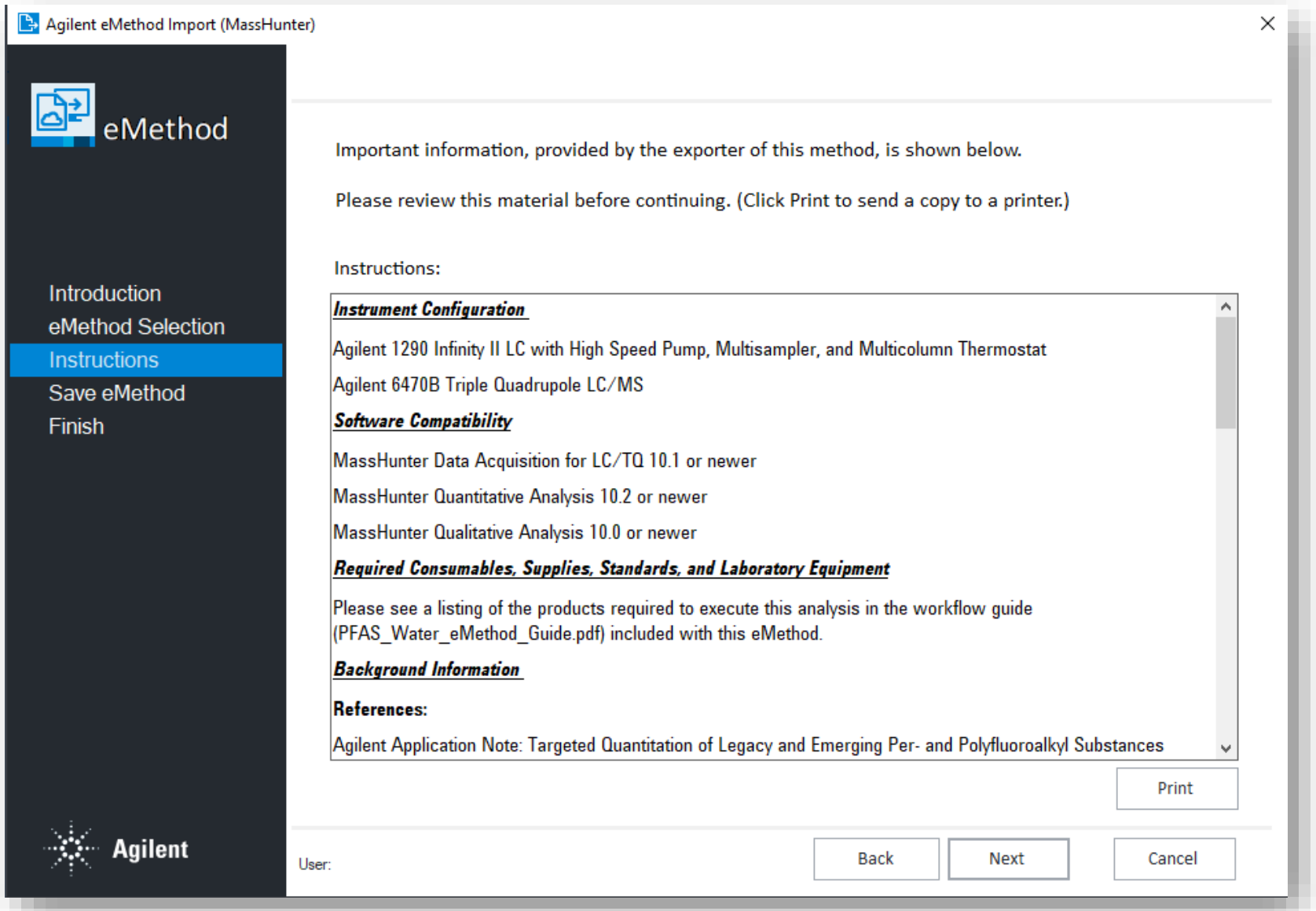
Method robustness: Good response ratio reproducibility for 300 continuous injections of spiked Surface Water samples



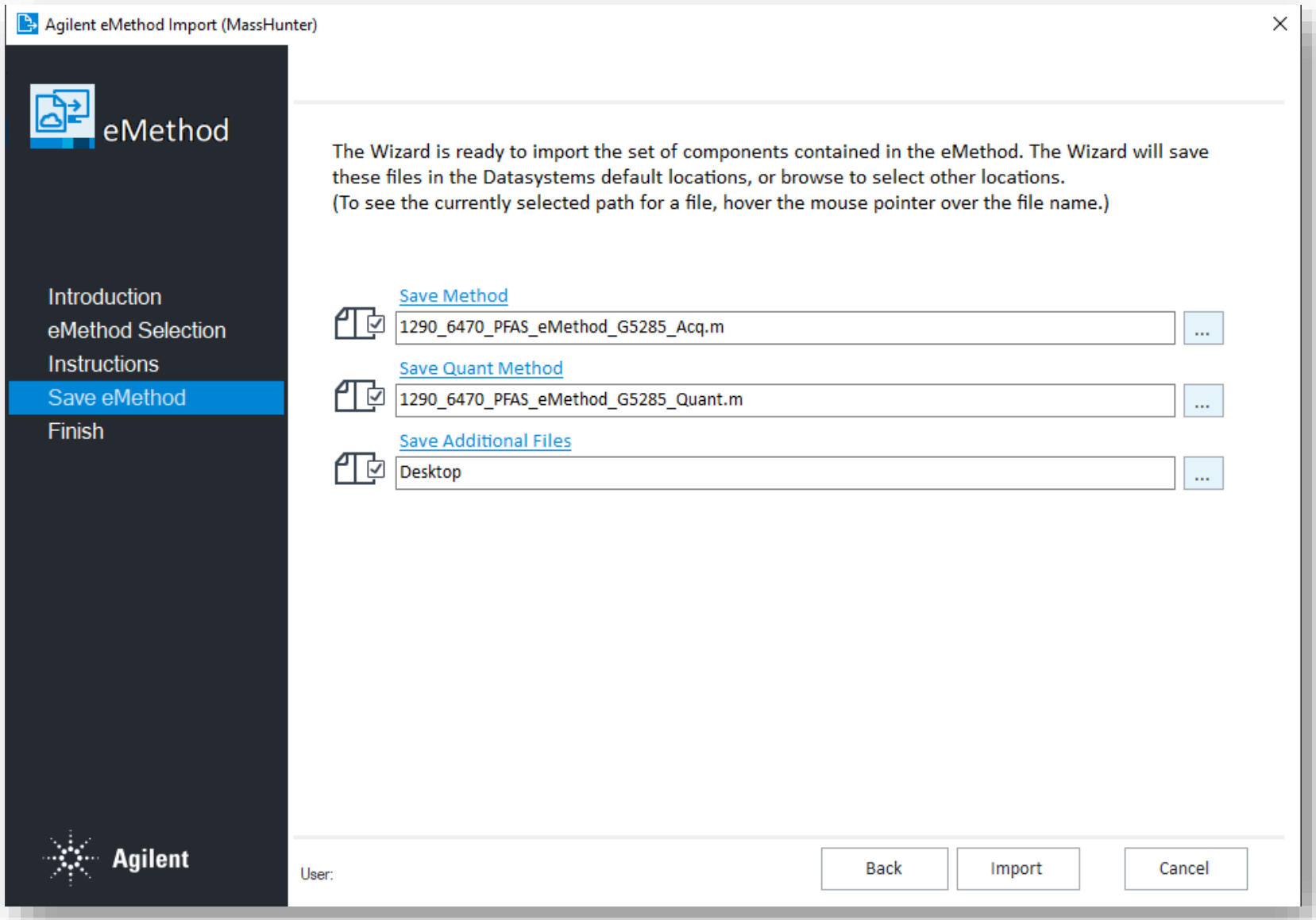
1. Double click eMethod icon



2. Review eMethod detail, click Next

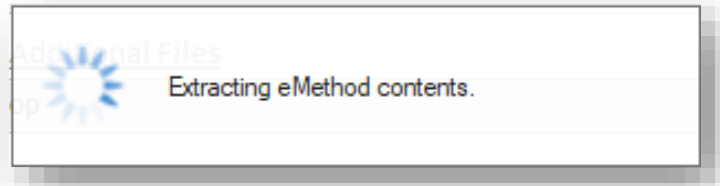


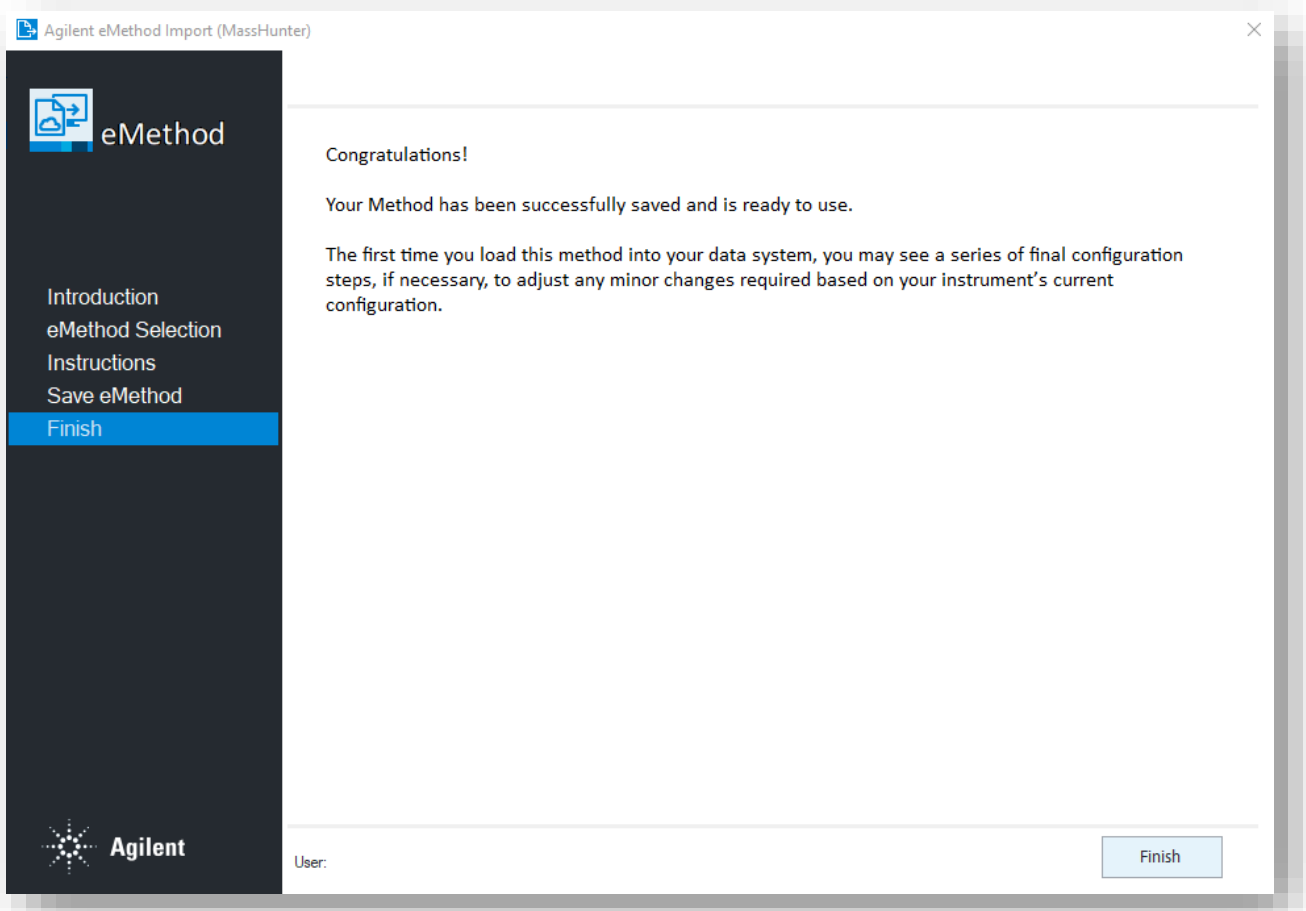
2. Review the import instructions, print as necessary, click Next.



3. Review eMethod method content and review/assign installation locations as necessary, click Import.

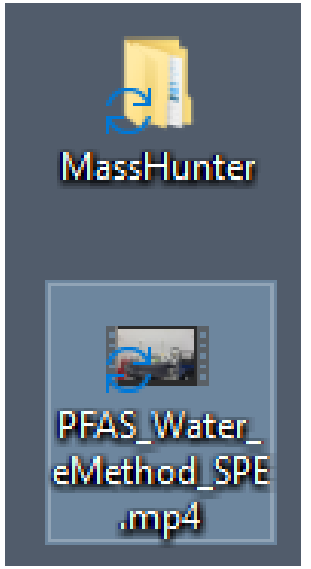
Progress indicator will appear:





4. Import is now complete, click Finish to close wizard.

Additional files should appear on the Desktop (unless location was manually altered



5. Drag/Drop MassHunter folder to the MassHunter directory to incorporate the data and documentation into the correct locations. View the sample preparation training video as necessary.

Agilent PFAS Solutions

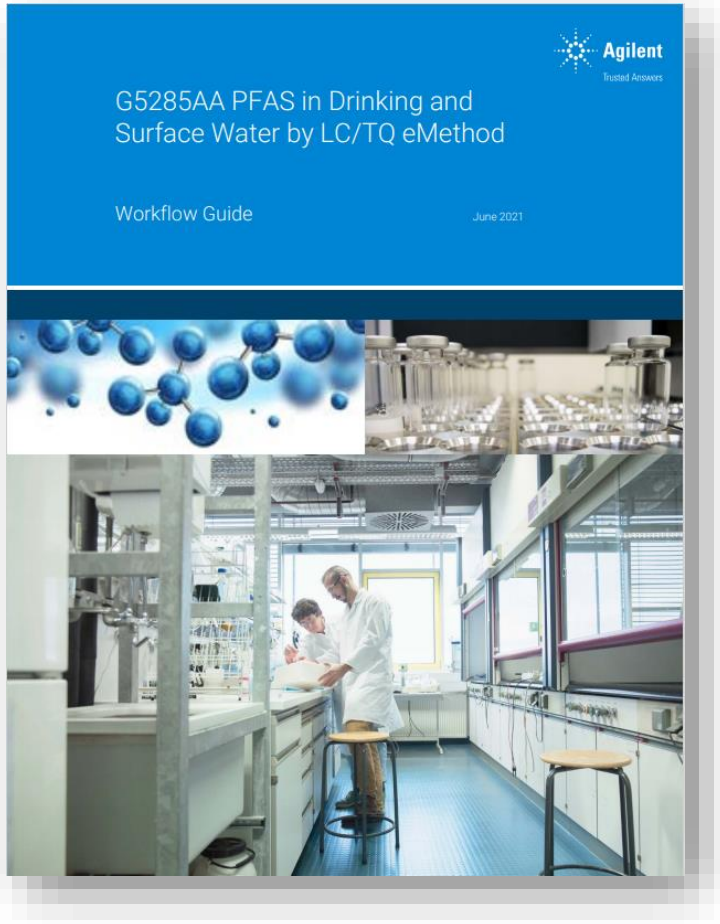
End-to-end Verified Workflow: Turn-key Solution Ready for Immediate Use

PFAS Drinking & Surface Water eMethod

An end-to end, verified solution for the analysis of >100 native & isotopically labeled PFAS in **drinking water** and **surface water** without extensive method development or technical investigation

eMethod Includes:

- Full analysis protocol, from sample prep through reporting
- Optimized MassHunter Acquisition and Quant methods
- Best practices
- Sample preparation training video
- Example calibration data
- Comprehensive ordering information with part number details



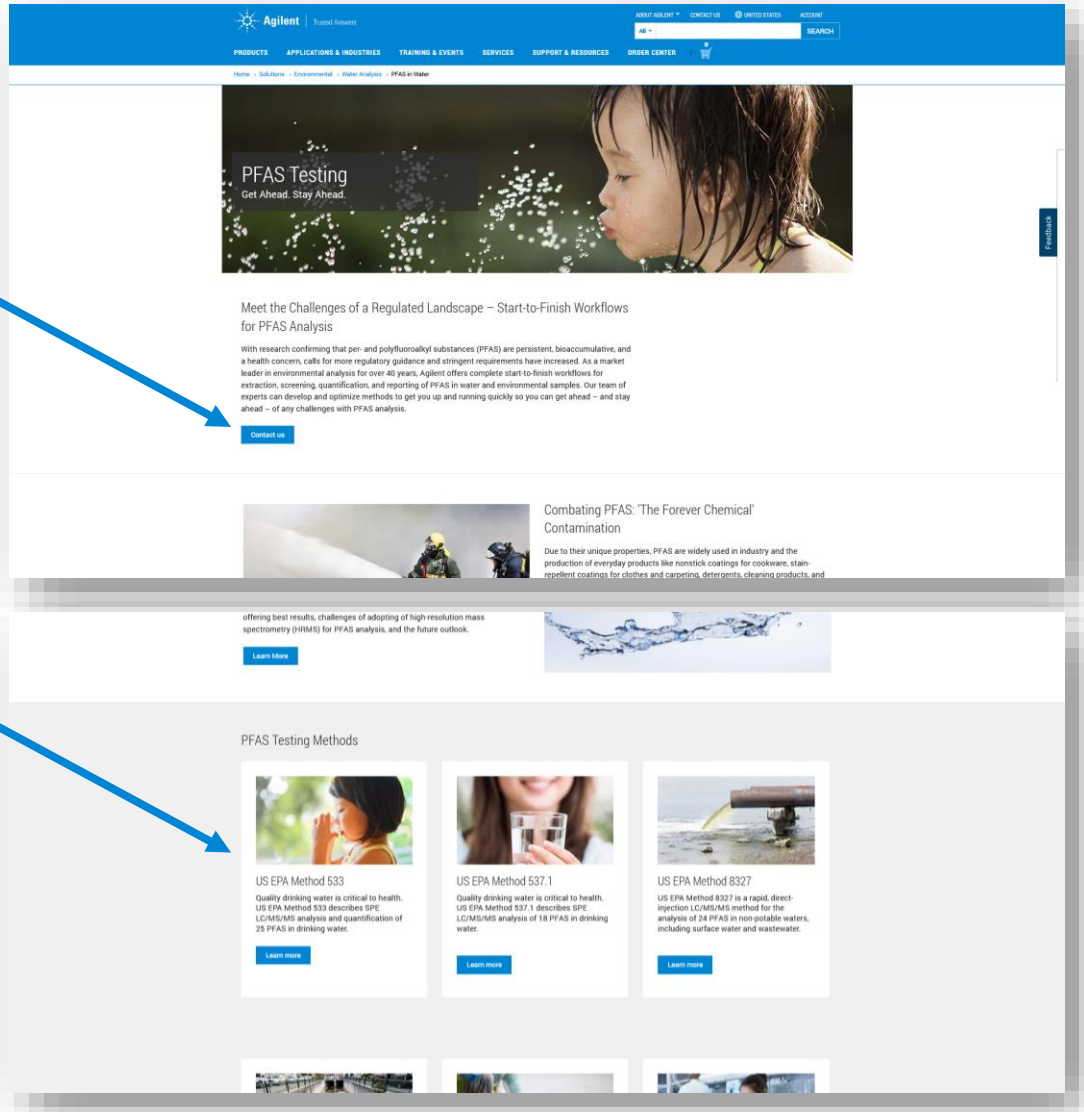
Compatible with1290 + 6470 LC/TQ

LATEST PFAS TESTING INFORMATION

A one-stop for all PFAS info on regulatory and emerging methods

Links to informational content

Links to individual method details



[Link to PFAS A&I page]