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A Comprehensive Workflow for the Analysis of PFAS in Wastewater per EPA Method 1633

Using the Agilent 6495D triple quadrupole LC/MS

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Abstract

Per- and polyfluoroalkyl substances (PFAS) pose an increasing threat to the
environment and to animals because of their extreme chemical stability and
bioaccumulation potential. Their detection at trace level is often impeded by
the environmental matrix and background contamination. Accuracy, reliability,
robustness, and interferant elimination are the key success factors for the
analytical method.

In this study, a comprehensive workflow was developed to achieve a highly robust
detection of more than 57 legacy and emerging PFAS in wastewater. A range of
performance criteria, including method detection limit, recovery, precision, linearity,
and reproducibility, were investigated. The reproducibility was demonstrated by
examining 300 continuous injections of real wastewater samples spiked with PFAS.

This workflow features a sample preparation protocol that follows U.S.
Environmental Protection Agency (EPA) Method 1633, Draft 4, LC/MS/MS data
acquisition and analysis, and automatic report generation. Sample preparation can
be managed by an Agilent workflow management software.

Introduction

PFAS are a class of several thousand synthetic compounds that are of growing concern because of their bioaccumulative and toxic character.[1] The fourth draft of EPA Method 1633 (July 2023) outlines the quantification of 40 native PFAS in aqueous, solid, and tissue samples by liquid chromatography/tandem mass spectrometry (LC/MS/MS).[2] The targeted analysis of PFAS at ultralow concentration (from low parts per trillion to parts per quadrillion) is usually challenging because of background contamination and interference from the environmental matrices. Sources of background contamination include reagents and equipment used during sample preparation and analysis.

A comprehensive workflow was developed for PFAS analysis in wastewater. The workflow was demonstrated for the 40 compounds listed in EPA Method 1633 Draft 4, plus an extended list of additional PFAS of varying sizes and functional groups. The additional analytes included compounds listed in European Union (EU) and United Kingdom (UK) regulations on PFAS. This workflow consists of (1) offline solid phase extraction (SPE) using the Agilent Bond Elut PFAS WAX SPE cartridge followed by a cleanup step with Agilent Carbon S, (2) LC/MS/MS analysis using the Agilent 6495D triple quadrupole LC/MS system, and (3) automatic reporting. Sample preparation steps can be managed using the Agilent SLIMS sample management system. The workflow provides a reliable solution for targeted analysis of PFAS in complex matrices with high robustness.

Experimental

In total, 57 native and 31 isotopically labeled PFAS were analyzed. This set of analytes covered the 40 compounds listed in EPA Method 1633 Draft 4, as well as 17 compounds not included in the EPA method but listed in UK and EU regulations on such substances. The analytes were spiked into reagent water at the low or middle concentration levels from EPA Method 1633 (calibration solution (CS)2 or CS4, respectively) and into wastewater at the middle concentration level (CS4). Concentrations of PFAS that are not listed in EPA Method 1633 were optimized before being spiked. Native and labeled PFAS standards were purchased as individual stock solutions or solution mixtures from Wellington Laboratories Inc. (Guelph, ON, Canada), Accustandard, Inc. (New Haven, CT, USA), Cambridge Isotopes Laboratories, Inc. (Tewksbury, MA, USA), and ChemScene LLC (Monmouth Junction, NJ, USA). Spiked aqueous and blank samples were extracted according to the protocol defined in EPA Method 1633 (Figure 1).[3]

Briefly, in this workflow, a 500 mL aqueous sample spiked with extracted internal standards (EISs) is concentrated using a conditioned weak anion exchange SPE cartridge (Bond Elut PFAS WAX SPE cartridge) half-filled with silanized glass wool. Samples are eluted using 1% methanolic ammonium hydroxide. The eluate is acidified using acetic acid and cleaned up with Agilent Carbon S. After centrifugation and filtration, the sample is mixed with nonextracted internal standards (NISs). Samples are stored at 4 °C until LC/MS/MS analysis is performed.
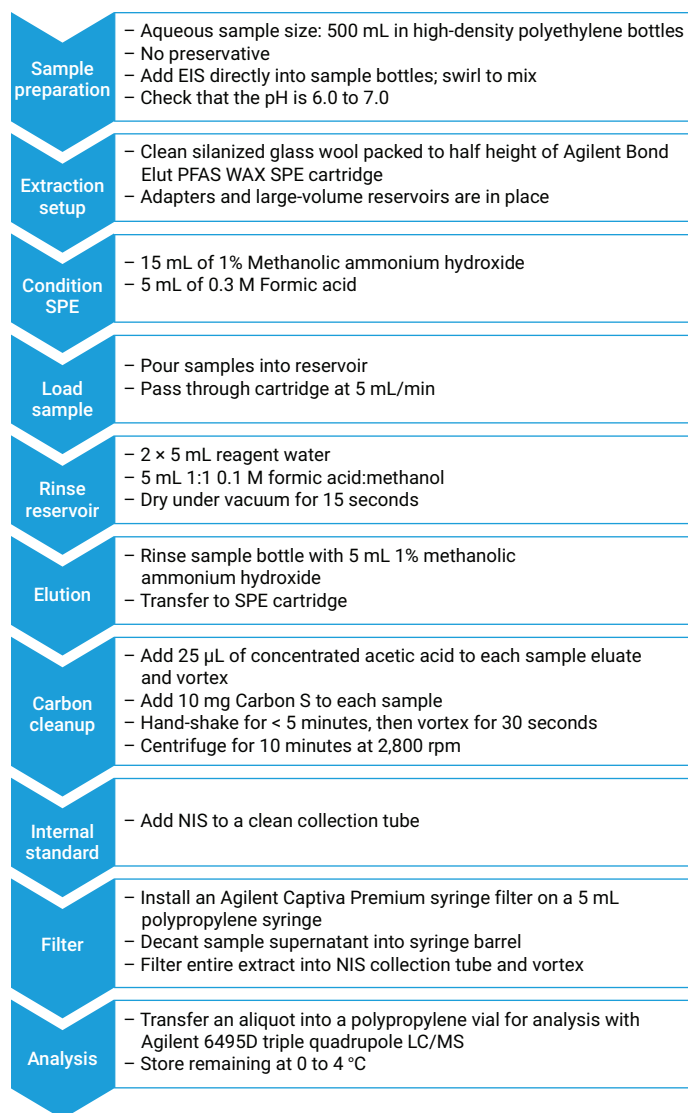

Figure 1. Protocol for extraction of PFAS following EPA Method 1633 Draft 4 (July 2023).

The extracts are first analyzed using the Agilent 1290 Infinity II LC system equipped with the Agilent InfinityLab PFC-free HPLC conversion kit. Next, mass spectrometric detection is performed with optimized measurement conditions using the 6495D triple quadrupole LC/MS mass spectrometer. Agilent offers a database with optimized multiple reaction monitoring parameter settings, intrinsic properties, and identifiers for 72 native and 36 isotopically labeled PFAS (PFAS MRM Database for LC/TQ, part number G1736AA). The use of the PFC-free conversion kit reduces background from HPLC to a minimum.

The liquid chromatography and triple quadrupole mass spectrometry conditions are shown in Table 1. All Agilent consumables used during sample preparation and data acquisition are listed in Table 2. Analytical results and required quality control data are combined in a method-specific reporting template that is automatically generated.

Table 1. Liquid chromatography and triple quadrupole mass spectrometry conditions for analysis of PFAS-containing samples.

Parameter	Value
Columns	− Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 100 mm, 1.8 µm − Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 5 mm, 1.8 µm − Agilent InfinityLab PFC delay column, 4.6 × 30 mm
Flow Rate	0.4 mL/min
Column Temperature	40 °C
Injection Volume	2 µL
Mobile Phase	A) 2 mM CH_3COONH_4 in 95% water + 5% acetonitrile B) 100% Acetonitrile
Gradient	Time (min)　　%B 0.0　　　　　2 0.2　　　　　2 10　　　　　95
Stop Time	12.2 min
Gas Temperature	230 °C
Gas Flow	11 L/min
Nebulizer	20 psi
Sheath Gas Temperature	355 °C
Sheath Gas Flow	10 L/min
Capillary Voltage (−)	2,500 V
Nozzle Voltage (−)	0 V

Table 2. Consumables for EPA Method 1633 Draft 4.

Description	Part Number
Agilent InfinityLab PFC-Free HPLC Conversion Kit	5004-0006
Agilent InfinityLab PFC Delay Column 4.6 × 30 mm	5062-8100
Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 5 mm, 1.8 µm	821725-901
Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 100 mm, 1.8 µm	959758-902
Vial, Screw Style, 2 mL, Polypropylene, Certified for PFAS, 100/pk	5191-8150
Cap, 9 mm, Screw Style, Polypropylene/Silicone, Certified for PFAS, 100/pk	5191-8151
Agilent Bond Elut PFAS WAX SPE Cartridges, 150 mg, 6 mL, 30/pk	5610-2150
Glass Wool, Silane-Treated, 50 g	8500-1572
Centrifuge Tubes and Caps, 15 mL, 50/pk	5610-2039
Agilent Carbon S, Bulk, 25 g Bottle	5610-2093
Agilent Captiva Disposable Syringe, 5 mL, 100/pk	9301-6476
Agilent Captiva Premium Syringe Filter, Nylon, 25 mm, 0.2 µm, 100/pk	5190-5092

Results and discussion

Calibration performance

For each PFAS, a calibration curve with seven calibration points was generated. For each of the 40 native PFAS listed in EPA Method 1633 Draft 4, the EIS and NIS specified in the method were adopted, along with their corresponding concentrations for each calibration point (CS1 to CS7). For native PFAS not listed in EPA Method 1633 Draft 4, concentrations were optimized. Calibration curves (n = 8) for four representative PFAS are shown in Figure 2. For all 40 native PFAS listed in the method, the relative standard error (RSE) values were below 10% (Figure 3). To establish instrument linearity, the RSE must be less than or equal to 20%.

Method detection limit and relative standard error

The method detection limits (MDL) shown in Figure 3 were determined according to the MDL procedure in 40 CFR part 136, appendix B[4], with the exception that all samples were run across one day instead of the required 3 days. For all PFAS, the measured MDL was equivalent to or well below the pooled values for aqueous samples given in EPA Method 1633 Draft 4. For all native PFAS listed in the method, the relative standard error was below 10.
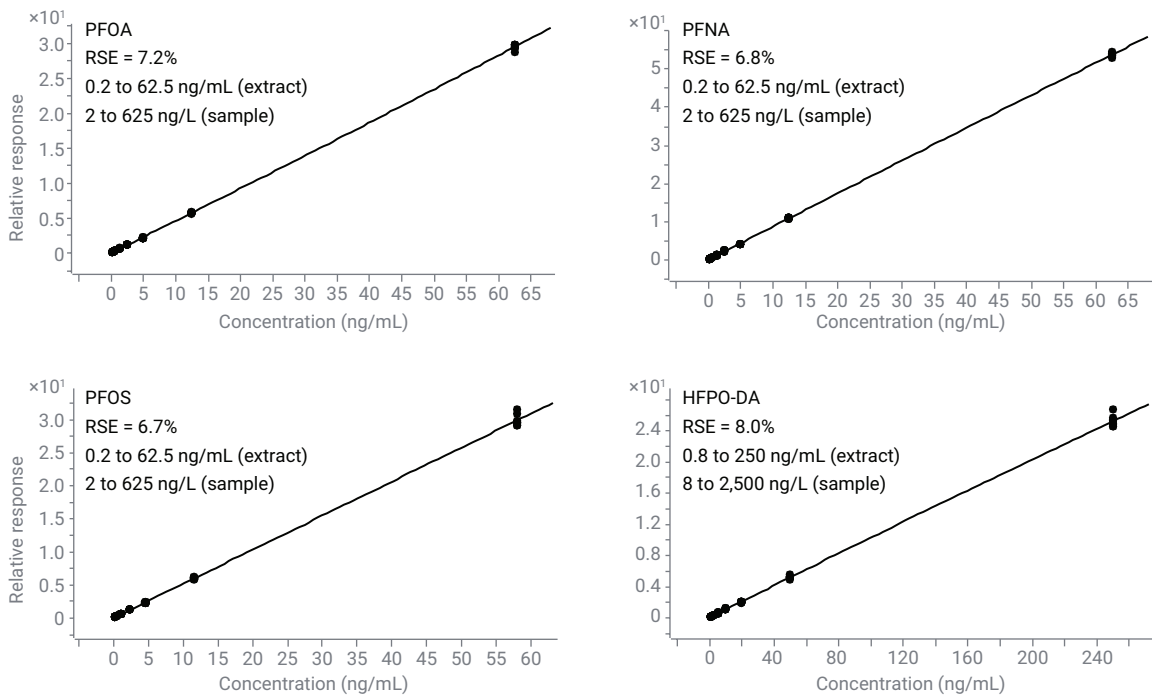
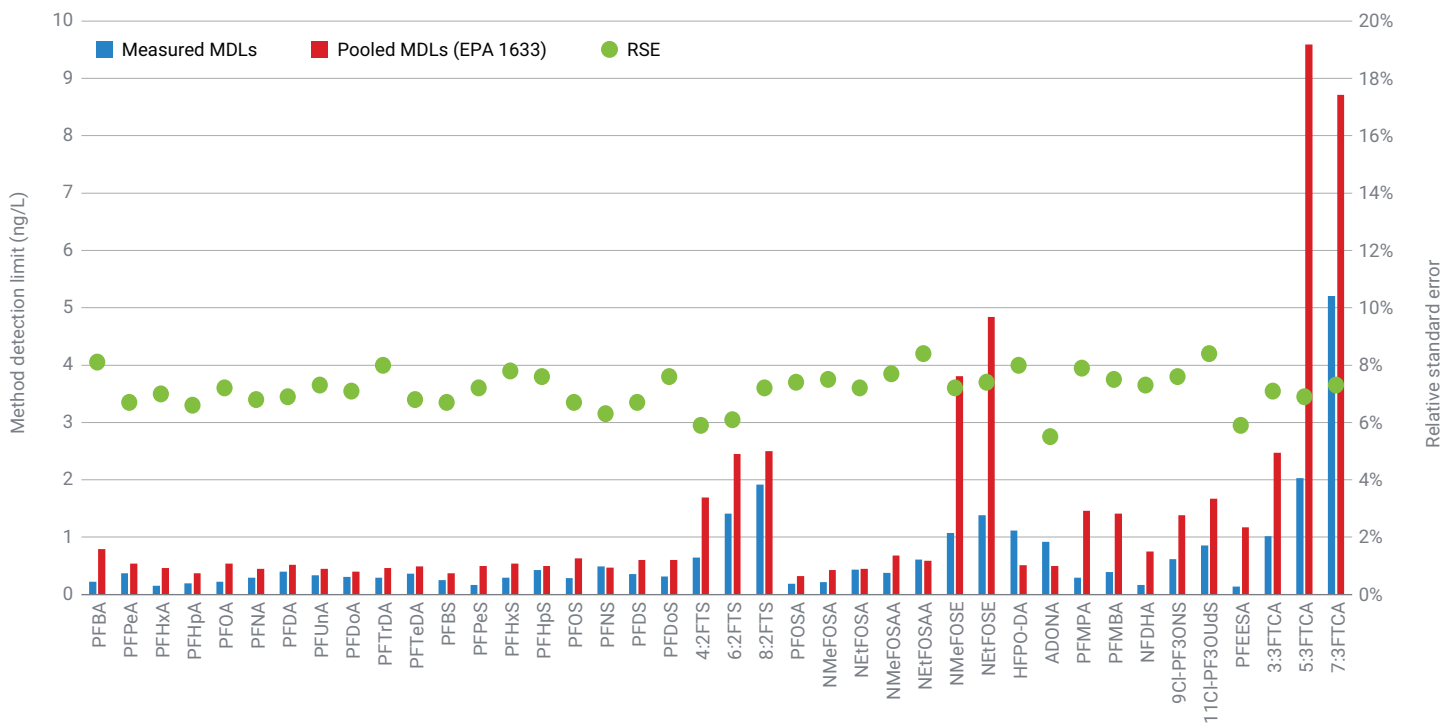**Figure 2.** Calibration curves for four representative PFAS (seven levels, n = 8).

Figure 3. Method detection limit (MDL) and relative standard error (RSE) for the 40 native PFAS listed in EPA Method 1633 Draft 4 (n = 8).

Precision and accuracy

Figure 4 shows the recovery of PFAS in reagent water and wastewater at middle-level concentration (CS4). Recovery percentages for all native PFAS and EISs are within the acceptance limits specified in EPA Method 1633 Draft 4. Most of the native PFAS in spiked reagent water and wastewater had a recovery close to 100%. Most of the relative standard deviations were well below 5%.

Method robustness

To assess the robustness of the method, 300 continuous injections (approximately 80 hours) were performed for spiked wastewater samples at middle-level concentration (CS4) of 19 PFAS. These compounds were selected to be representative of the whole chromatographic run (from 3 to 10 minutes) and compound classes. Excellent reproducibility and robustness were achieved, with relative standard deviations lower than 6%. Figure 5 summarizes the normalized absolute abundances (actual absolute abundance/mean + constant) for each injection.

Reporting

A new reporting template (Figure 6) has been created to cover all the calculations required by EPA Method 1633. This report can be generated automatically in MassHunter Quantitative Analysis software using the built-in PDF-based reporting method. The report includes, among other, calculations for:

– Ion ratio (quantifier/qualifier)

– EIS recovery

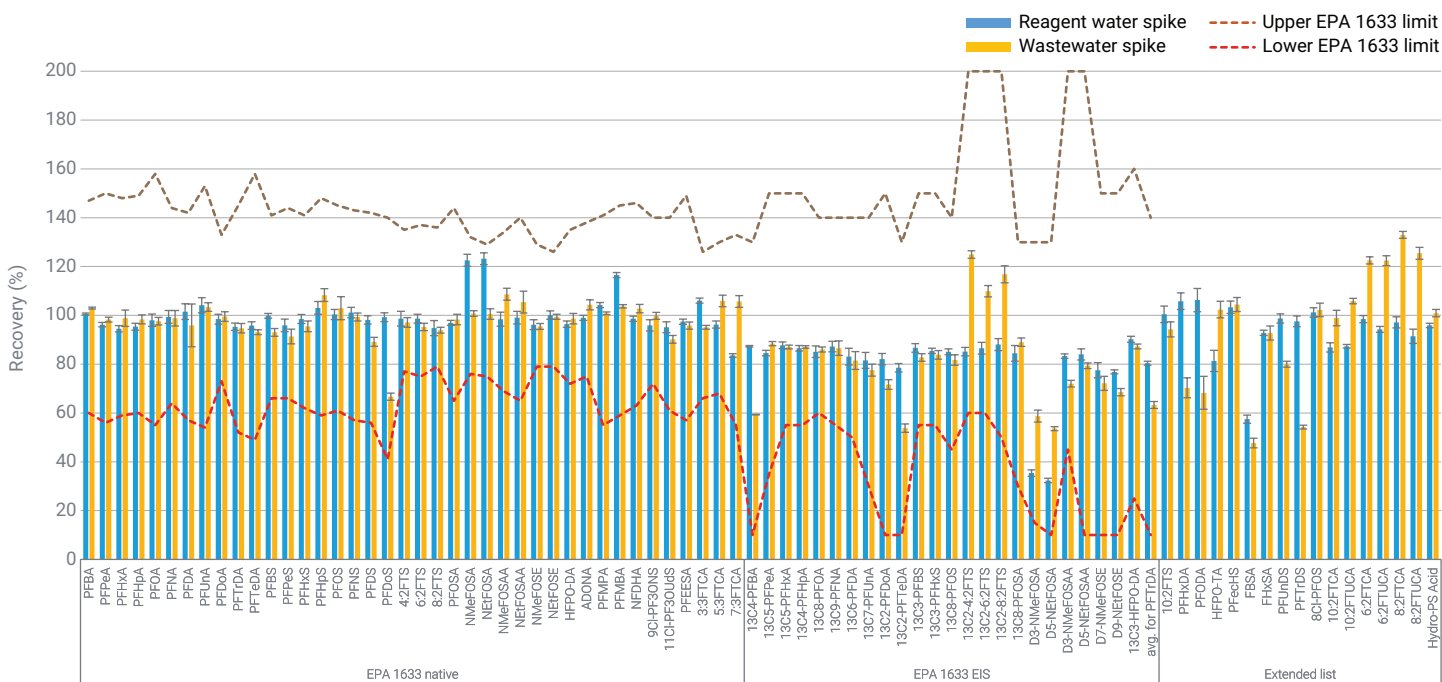– Average EIS response, e.g., for PFTrDA

– Manual versus original integration

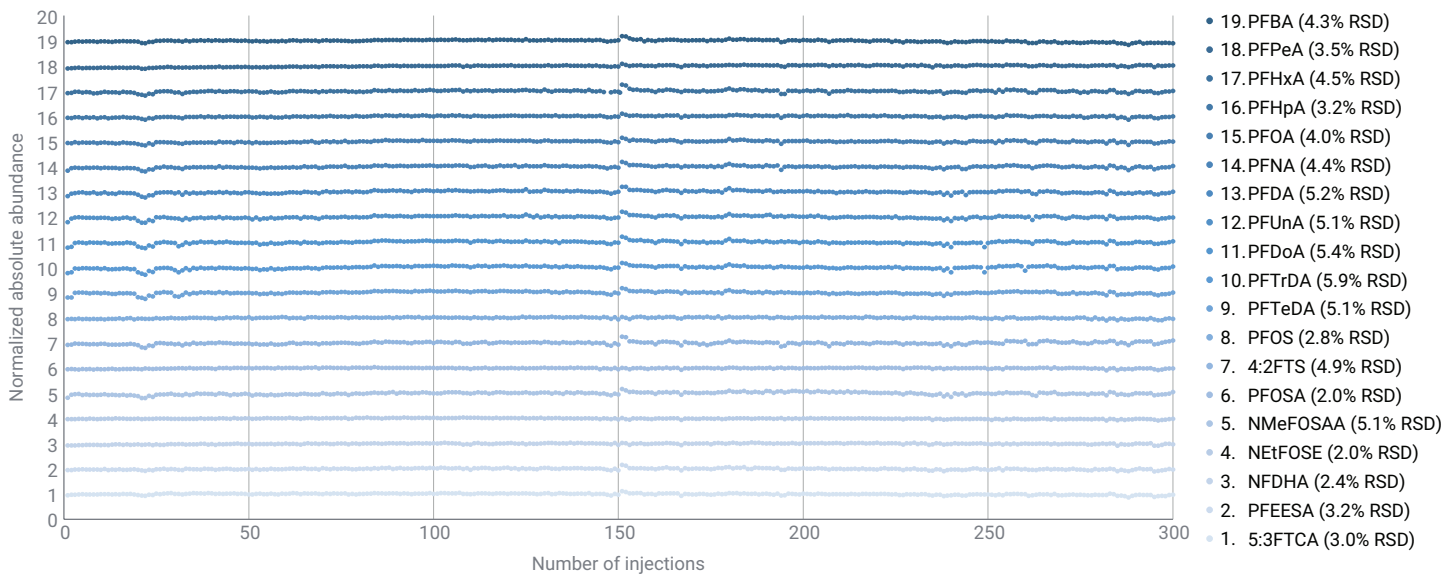**Figure 4.** Recovery of PFAS in spiked reagent water and wastewater at middle-level concentration (CS4, n = 8).

Figure 5. Normalized absolute abundance of 19 representative PFAS covering the whole retention time and compound classes.
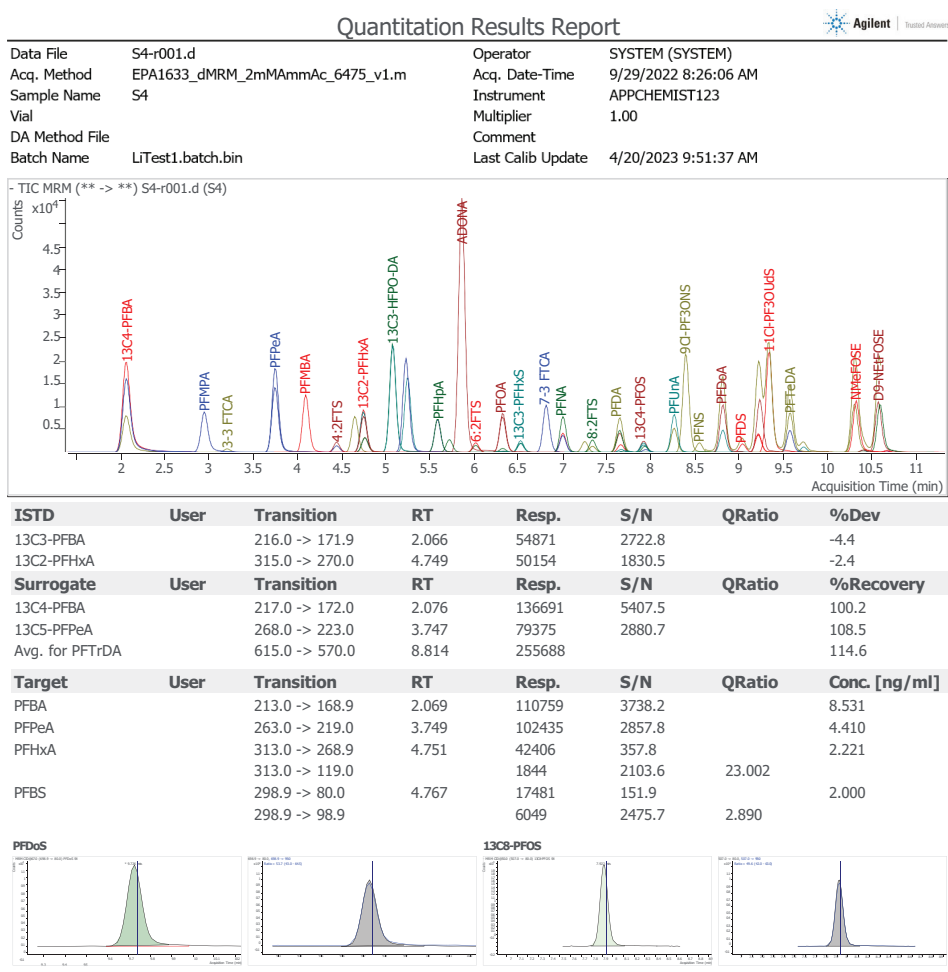
- 19. PFBA (4.3% RSD)
- 18. PFPeA (3.5% RSD)
- 17. PFHxA (4.5% RSD)
- 16. PFHpA (3.2% RSD)
- 15. PFOA (4.0% RSD)
- 14. PFNA (4.4% RSD)
- 13. PFDA (5.2% RSD)
- 12. PFUnA (5.1% RSD)
- 11. PFDoA (5.4% RSD)
- 10. PFTrDA (5.9% RSD)
- 9. PFTeDA (5.1% RSD)
- 8. PFOS (2.8% RSD)
- 7. 4:2FTS (4.9% RSD)
- 6. PFOSA (2.0% RSD)
- 5. NMeFOSAA (5.1% RSD)
- 4. NEtFOSE (2.0% RSD)
- 3. NFDHA (2.4% RSD)
- 2. PFEESA (3.2% RSD)
- 1. 5:3FTCA (3.0% RSD)

Figure 6. Selected sections of a report generated from the PFAS analysis.

Conclusion

This application note demonstrates a comprehensive workflow, including sample preparation, consumables, data acquisition/analysis, and reporting for PFAS listed in EPA Method 1633 Draft 4 and in EU and UK regulations.

Reliable sample preparation with excellent recovery was achieved using Agilent Bond Elut PFAS WAX SPE cartridges. Use of an Agilent InfinityLab PFC-free HPLC conversion kit maximally reduces background contamination by replacing fluorinated polymers present in the original HPLC system and delaying PFAS that originate from the solvent. Analysis was performed using the Agilent 1290 Infinity II LC system coupled to the new Agilent 6495D triple quadrupole LC/MS system, which features an ion source and method optimizer and artificial intelligence-based SWARM autotuning.

The workflow provided outstanding instrumental reproducibility and robustness. Use of Agilent SLIMS, which combines a laboratory information management system and an electronic notebook, enables an end-to-end solution for PFAS analysis.

References

1. Our Current Understanding of the Human Health and Environmental Risks of PFAS. https://www.epa.gov/pfas/our-current-understanding-human-health-and-environmental-risks-pfas (last updated 2023-07-07; accessed 2023-09-18).

2. Method 1633: Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/MS; 4th draft. *United States Environmental Protection Agency*, July **2023**.

3. Hunt, K.; Hindle, R.; Anumol, T.; Giardina, M.; Juck, M.; Parry, E. Analysis of Per- And Polyfluoroalkyl Substances (PFAS) In Aqueous Samples per EPA Draft Method 1633, *Agilent Technologies application note*, publication number, 5994-5226EN, **2022**.

4. Definition and Procedure for the Determination of the Method Detection Limit—Revision 2, *[U.S.] Code of Federal Regulation*, Title 40, Appendix B to Part 136.

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