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A Comprehensive Workflow for PFAS Analysis in Wastewater with Extended EPA Draft Method 1633 List

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Introduction

Per- and polyfluoroalkyl substances (PFAS) pose an increasing threat to the environment, and animals due to extreme chemical stability and bioaccumulation potential. Their detection at trace level is often interfered with by the environmental matrix and background contaminants.

A comprehensive workflow was developed for PFAS analysis in wastewater, based on the existing EPA draft Method 1633 with additional PFAS of varying size and functional group. This workflow contains off-line solid phase extraction (SPE) and a clean-up step, followed by LC-MS/MS analysis, and automatic reporting. The workflow demonstrates a reliable solution for the targeted analysis of PFAS in complex matrices with high robustness.

Experimental

In total, 57 native and 31 labeled PFAS covering EPA 1633, wastewater, UK, and EU lists were spiked into reagent water at low (CS2 from EPA 1633) or middle (CS4) concentration levels, and wastewater at the middle level. Concentrations of PFAS that are not listed on the EPA 1633 list were optimized prior to spiking. Spiked aqueous and blank samples were extracted according to the theme shown in Figure 1.[1]

Subsequently, the extracts were analyzed using the Infinity II 1290 HPLC equipped with the PFC-Free Conversion Kit, followed by the mass spectrometric detection using the new 6495 LC/TQ (G6495D) mass spectrometer (Figure 2). The LC and TQ conditions are shown in Table 1. This process can be automated by SLIMS.

Sample Preparation
- Aqueous sample size: 500 mL in HDPE bottles
- No preservative
- Add EIS directly into sample bottles – swirl to mix
- Check pH is 6.0 – 7.0

Extraction Setup
- Clean silanized glass wool packed to half height of **Agilent Bond Elut PFAS WAX SPE cartridge (Table 2)**
- Adapters and large volume reservoirs in place

Condition SPE
- 15 mL - 1% methanolic ammonium hydroxide
- 5 mL – 0.3M formic acid

Load Sample
- Pour samples into reservoir
- Pass through cartridge at 5 mL/min

Rinse Reservoir
- 2 x 5 mL reagent water
- 5 mL 1:1 0.1M formic acid/methanol
- Dry under vacuum for 15 seconds

Figure 1. Extraction Method.

Experimental

Elution
- Rinse sample bottle with 5 mL 1% methanolic ammonium hydroxide
- Transfer to SPE cartridge

Carbon Cleanup
- Add 25 µL concentrated acetic acid to each sample eluate & vortex
- Add 10 mg **Carbon S** to each sample
- Hand-shake for < 5 minutes then vortex for 30 seconds
- Centrifuge for 10 minutes at 2800 rpm

Internal Standard
- Add NIS to a clean collection tube

Filter
- Install a **Captiva Premium Nylon Syringe Filter** on a 5 mL polypropylene syringe
- Decant sample supernatant into syringe barrel
- Filter entire extract into NIS collection tube and vortex

Analysis
- Transfer an aliquot into a poly ALS vial for LCTQ analysis
- Store remaining at 0-4 °C

Figure 1. Continued.

Table 1. LC and TQ Conditions.

Column	•ZORBA Eclipse Plus C18, 2.1 x 100 mm, 1,8 µm •ZORBA Eclipse Plus C18, 2.1 x 5 mm, 1,8 µm •PFC Delay Column, 4.6 x 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% ACN B: 100% ACN
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 (Neg)	2500 V
Nozzle voltage (Neg)	0 V

Figure 2. Infinity II 1290 and 6495 LC/TQ.

Results and Discussion

Precision and Accuracy.

Figure 3 shows the recovery of PFAS in reagent water and wastewater at middle level concentration. All native PFAS and EIS are within the acceptance limits from the 3rd EPA 1633 draft method. Most of the native PFAS in spiked reagent water and wastewater had a recovery close to 100%. Most of the RSDs were well blow 5%.

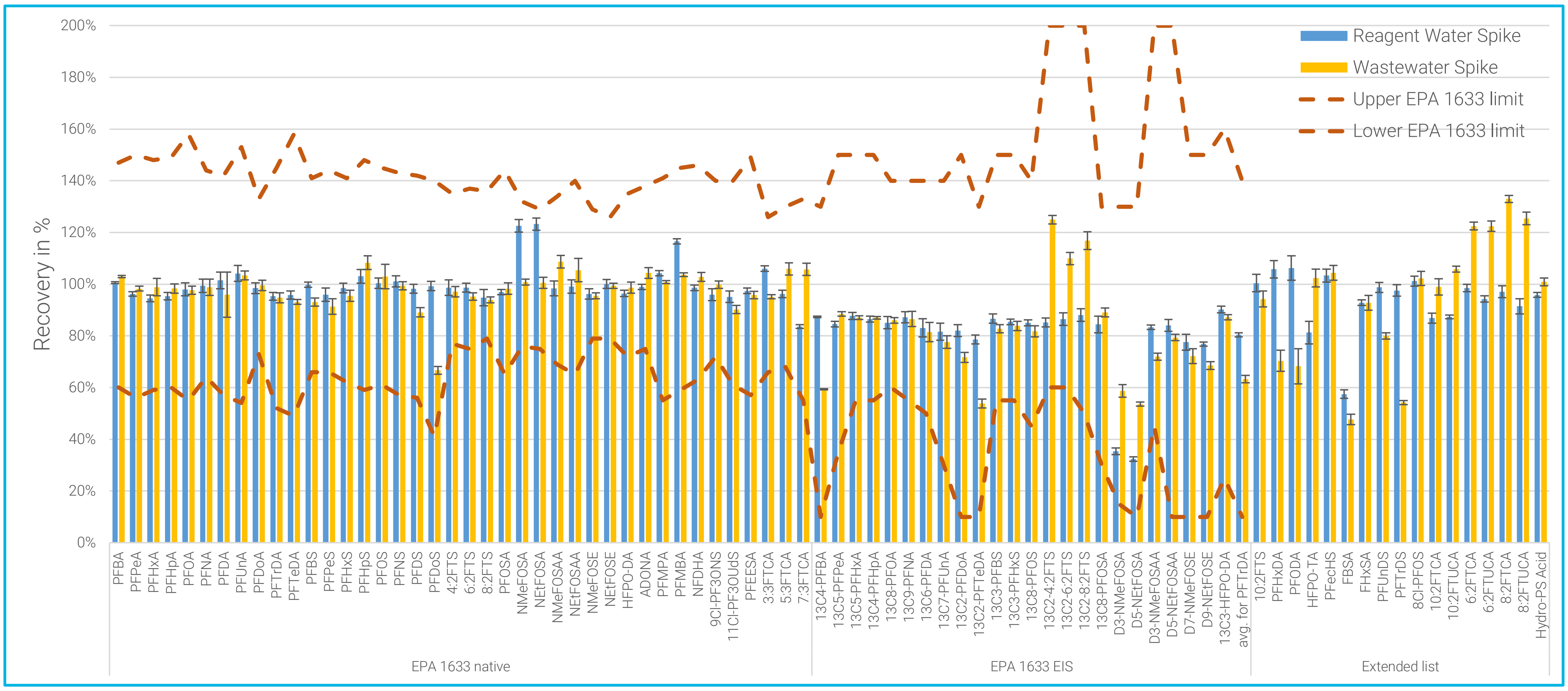


Figure 3. Recovery of PFAS in Spiked Reagent Water and Wastewater at Middle Level Concentration (CS4, n=8).

Calibration Performance

4 examples of calibration curves (7 calibration points, n =8) for selected PFAS are shown in Figure 4. For all native PFAS with the calibration range from the EPA 1633 list the R^2 values were greater than 0.998. The R^2 values of other PFAS were greater than 0.995. The relative standard errors for all EPA 1633 native PFAS were below 10 (Figure 5).

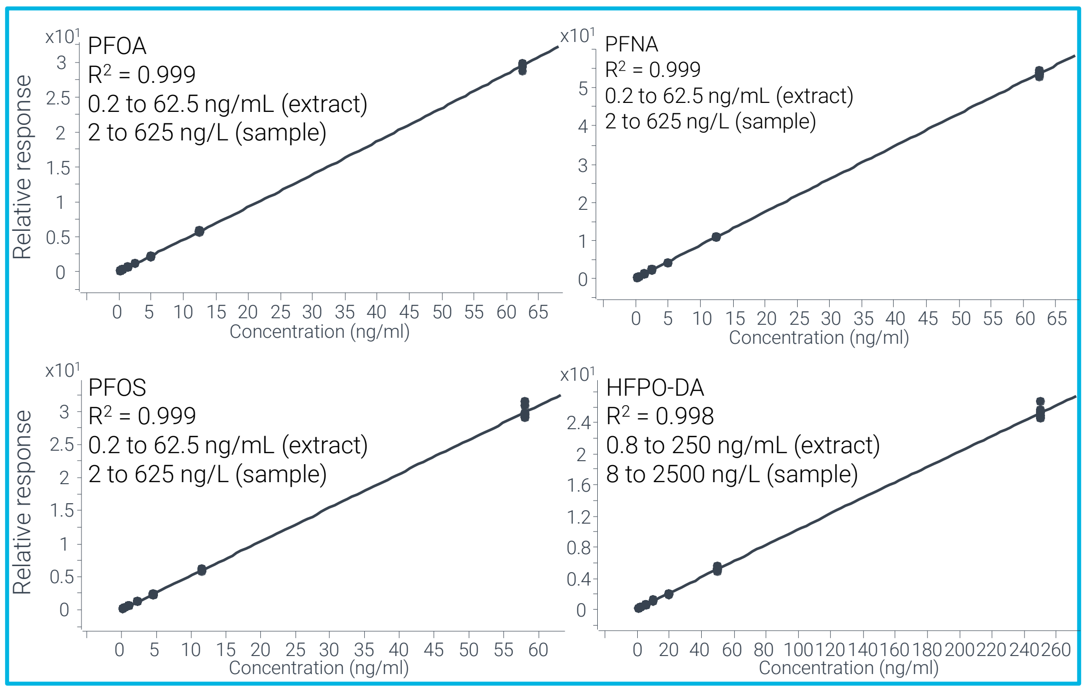


Figure 4. Four Calibration Curves (7 levels, n=8).

Method Detection Limit and Relative Standard Error

The method detection limits shown in Figure 5 were determined according to the MDL procedure in 40 CFR part 136, appendix B. However, samples are all measured within one day instead. The measured MDL for all PFAS were equivalent to or well below the pooled values in the 3rd EPA 1633 draft method.

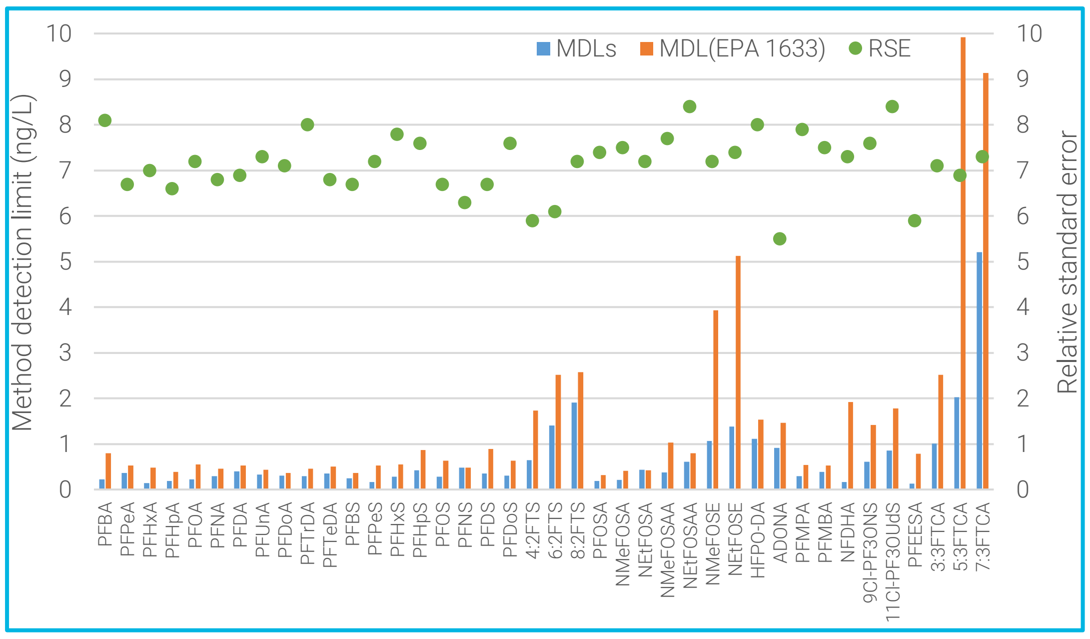


Figure 5. Method Detection Limit and Relative Standard Error for EPA 1633 Native PFAS (n=8).

Results and Discussion

Method Robustness

300 continuous injections (around 80 h) of spiked wastewater samples at middle level concentration were performed for assessing instrument robustness. Excellent reproducibility and robustness with RSDs of the 19 representative PFAS, which cover the whole RT (from 3 to 10 min) and compound classes, lower than 6% were achieved. Figure 6 summarizes the normalized absolute abundances (actual absolute abundance/mean + constant) per each injection.

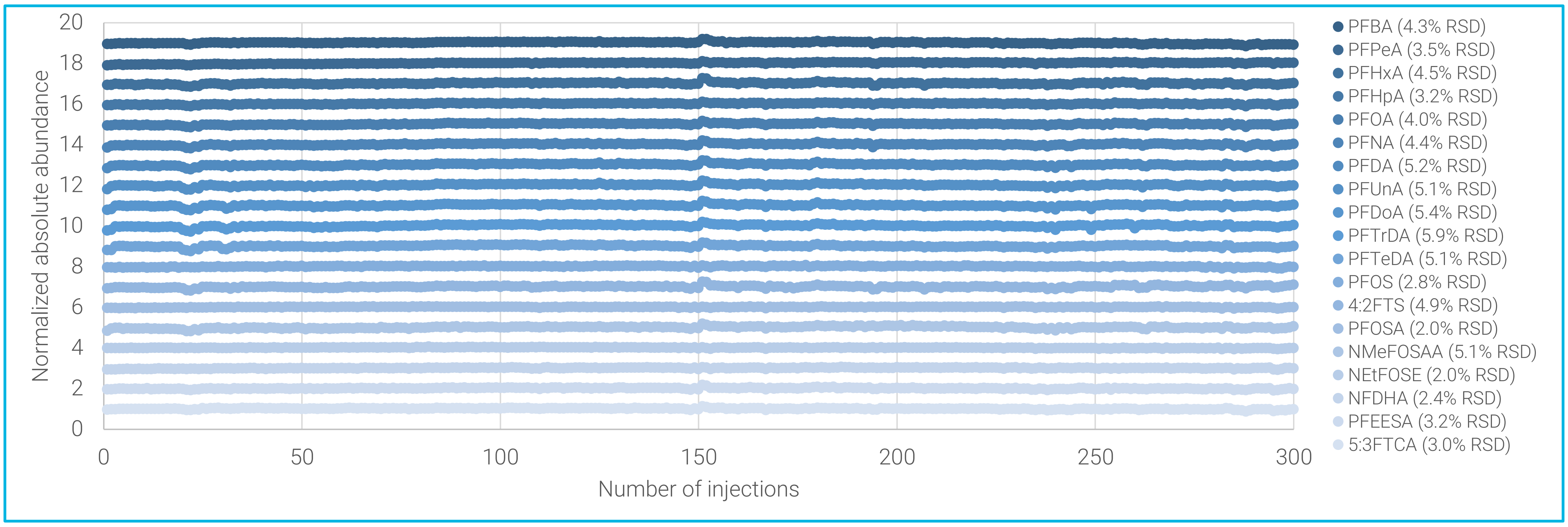
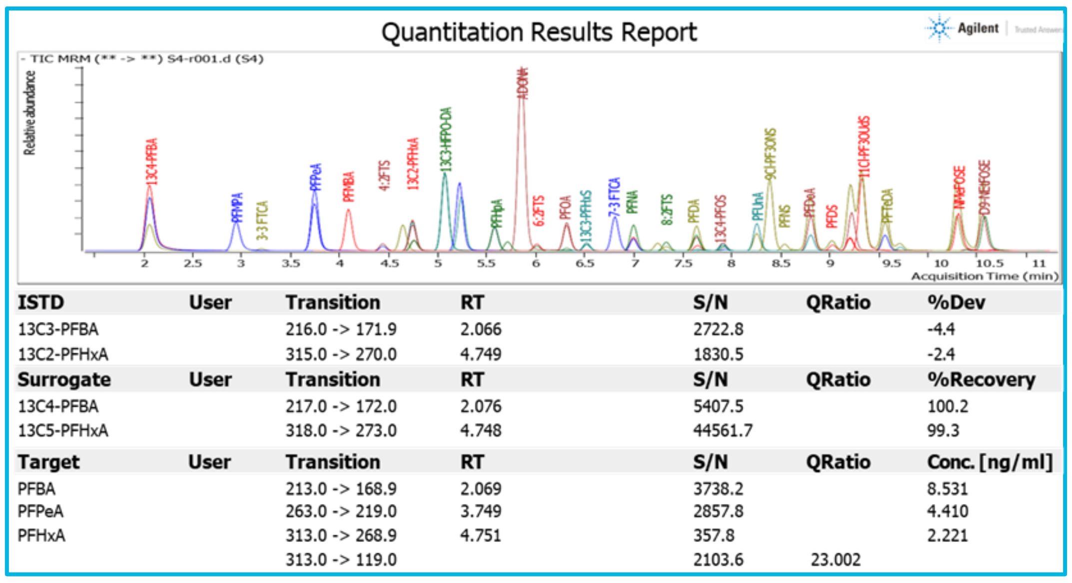


Figure 6. Normalized Absolute Abundance of 19 Representative PFAS Covering the Whole RT and Compound Classes.



Quantitation Results Report

ISTD	**User**	**Transition**	**RT**	**S/N**	**QRatio**	**%Dev**
13C3-PFBA		216.0 -> 171.9	2.066	2722.8		-4.4
13C2-PFHxA		315.0 -> 270.0	4.749	1830.5		-2.4
Surrogate	**User**	**Transition**	**RT**	**S/N**	**QRatio**	**%Recovery**
13C4-PFBA		217.0 -> 172.0	2.076	5407.5		100.2
13C5-PFHxA		318.0 -> 273.0	4.748	44561.7		99.3
Target	**User**	**Transition**	**RT**	**S/N**	**QRatio**	**Conc. [ng/ml]**
PFBA		213.0 -> 168.9	2.069	3738.2		8.531
PFPeA		263.0 -> 219.0	3.749	2857.8		4.410
PFHxA		313.0 -> 268.9	4.751	357.8		2.221
		313.0 -> 119.0		2103.6	23.002	

Figure 7. Example of a Report for the PFAS Analysis.

Table 2. Consumables for EPA Method 1633.

Description	Part Number
PFC-free LC conversion kit	5004-0006
Agilent InfinityLab PFC Delay Column 4.6 × 30 mm	5062-8100
Agilent ZORBAX Eclipse Plus C18, 2.1 × 5 mm, 1.8 µm	821725-901
Agilent ZORBAX Eclipse Plus C18, 2.1 × 100 mm, 1.8 µm	959758-902
Vial, screw, 2 mL, polypropylene, certified for PFAS, 100/pk	5191-8150
Cap, 9 mm, screw, polypropylene/silicone, certified for PFAS, 100/pk	5191-8151
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
Carbon S bulk, 25 g bottle	5610-2093
5 mL disposable syringe, 100/pk	9301-6476
Agilent Captiva Premium Syringe Filter, nylon, 25 mm, 0.2 µm, 100/pk	5190-5092

Reporting

A new reporting template (Figure 7) has been generated to cover all the required calculations for EPA method 1633, such as :

- Ion ratio (Quantifier/Qualifier)
- EIS recovery
- Averaging EIS response e.g., for PFTrDA
- Manual vs. original integration, etc.

Conclusions

- A comprehensive workflow including sample preparation, consumables, data acquisition/analysis, and reporting was developed for PFAS analysis.
- Reliable sample preparation with excellent recovery.
- Outstanding instrumental reproducibility and robustness.
- Workflow managed by SLIMS reduces human errors.

References

[1] Agilent 5994-5226EN. Analysis Of Per- And Polyfluoroalkyl Substances (PFAS) In Aqueous Samples Per EPA Draft Method 1633 (PDF)

https://www.agilent.com/en/promotions/asms

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