Application Note
Environmental

Agilent 6475 LC/TQ Performance Highlights with EPA Draft Method 1633 for Per and Polyfluoroalkyl Substances (PFAS) in Solid Samples

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Abstract

This application note details the performance of the Agilent 6475 triple quadrupole LC/MS (LC/TQ) coupled with the Agilent 1290 Infinity II liquid chromatograph (LC) for the analysis of per and polyfluoroalkyl substances (PFAS) in accordance with the third draft of EPA 1633.[1] Method transfer between the Agilent 6475 and 6470 B triple quadrupole LC/MS systems was seamless, and the new intelligent source optimization algorithm was tested, as the EPA 1633 analyte list includes thermally sensitive compounds. Calibration linearity and reproducibility were verified, and the results met all method requirements. The same soil extracts were analyzed on both systems (6470 B and 6475 LC/TQ) to demonstrate comparable performance. Finally, instrument robustness was challenged by >1,000 noninterrupted injections of extracted fish samples while monitoring calibration verification standards after every 50 injections of matrix.

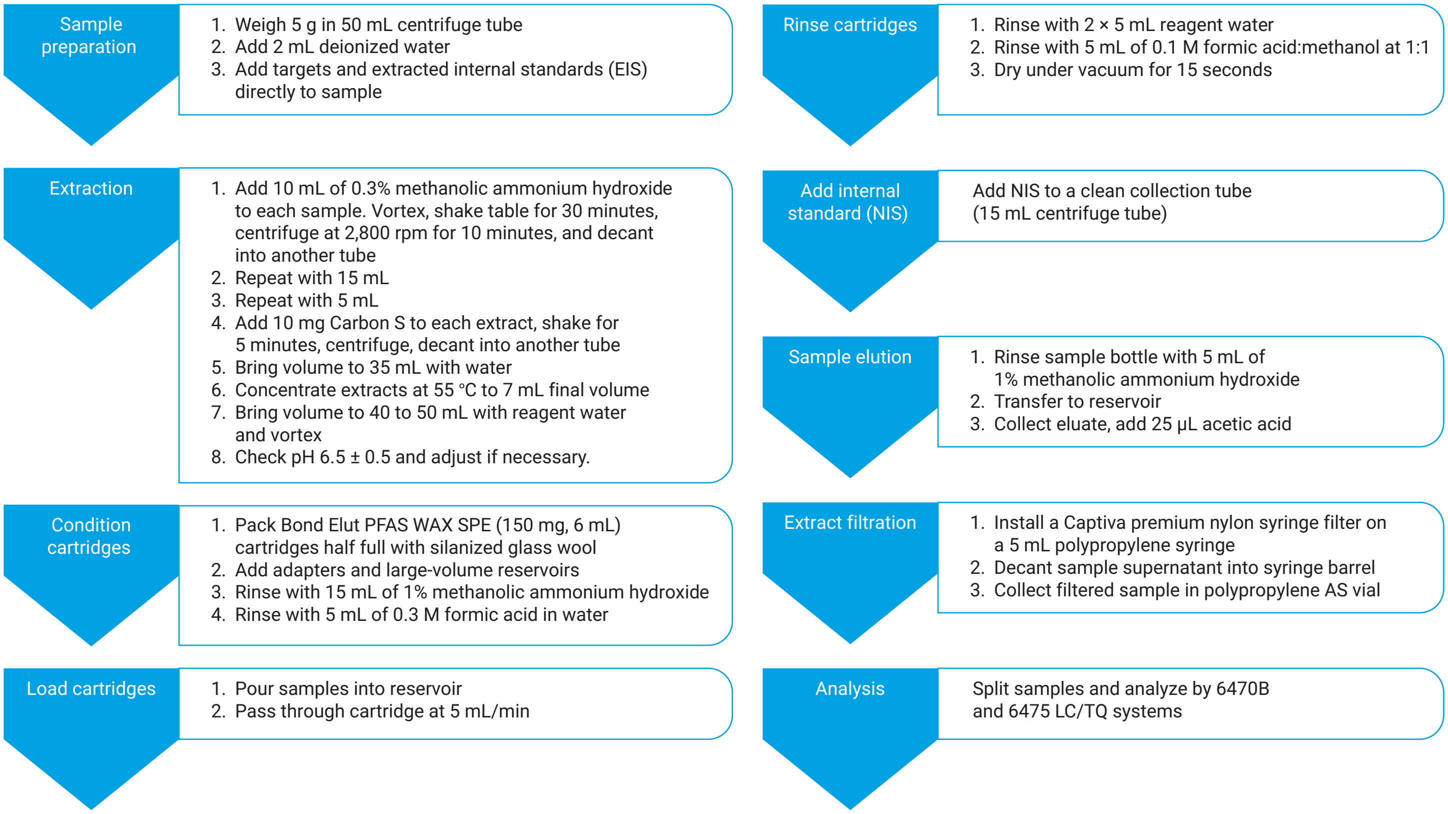
Introduction

PFAS are ubiquitous environmental contaminants that have been found in drinking water, wastewater, soil, and food.[2] The U.S. EPA has two well-established methods (EPA 537.1 and 533) for the analysis of 29 PFAS compounds in potable water. The EPA 1633 third draft was released in December 2022, and details analysis of 40 PFAS compounds in nonpotable water, biosolids, solids, and tissue.[1] It uses isotope dilution quantitation and has matrix-specific extraction protocols. Wastewater testing in accordance with the draft method has previously been detailed on the 6470 B LC/TQ.[3]

This application note shows EPA 1633 method performance in soil using the 1290 Infinity II LC system coupled to a 6475 LC/TQ. The 6475 LC/TQ maintains the robust performance that defined the Agilent 6470 B LC/TQ, while including new intelligence features to simplify tuning and method optimization, such as artificial intelligence (AI)-based tuning, maintenance monitoring and method development automation, and so on.

Specific highlights included here are:

- Ease of method transfer from the 6470 B LC/TQ, and new intelligent optimization features for the 6475 LC/TQ
- Performance of the 6475 LC/TQ with the EPA 1633 analyte list
- Soil quantification comparison between the 6470 B and 6475 LC/TQ systems
- Instrument robustness of >1,000 injections of fish tissue



Sample preparation

1. Weigh 5 g in 50 mL centrifuge tube
2. Add 2 mL deionized water
3. Add targets and extracted internal standards (EIS) directly to sample

Extraction

1. Add 10 mL of 0.3% methanolic ammonium hydroxide to each sample. Vortex, shake table for 30 minutes, centrifuge at 2,800 rpm for 10 minutes, and decant into another tube
2. Repeat with 15 mL
3. Repeat with 5 mL
4. Add 10 mg Carbon S to each extract, shake for 5 minutes, centrifuge, decant into another tube
5. Bring volume to 35 mL with water
6. Concentrate extracts at 55 °C to 7 mL final volume
7. Bring volume to 40 to 50 mL with reagent water and vortex
8. Check pH 6.5 ± 0.5 and adjust if necessary.

Condition cartridges

1. Pack Bond Elut PFAS WAX SPE (150 mg, 6 mL) cartridges half full with silanized glass wool
2. Add adapters and large-volume reservoirs
3. Rinse with 15 mL of 1% methanolic ammonium hydroxide
4. Rinse with 5 mL of 0.3 M formic acid in water

Load cartridges

1. Pour samples into reservoir
2. Pass through cartridge at 5 mL/min

Rinse cartridges

1. Rinse with 2 × 5 mL reagent water
2. Rinse with 5 mL of 0.1 M formic acid:methanol at 1:1
3. Dry under vacuum for 15 seconds

Add internal standard (NIS)

Add NIS to a clean collection tube (15 mL centrifuge tube)

Sample elution

1. Rinse sample bottle with 5 mL of 1% methanolic ammonium hydroxide
2. Transfer to reservoir
3. Collect eluate, add 25 µL acetic acid

Extract filtration

1. Install a Captiva premium nylon syringe filter on a 5 mL polypropylene syringe
2. Decant sample supernatant into syringe barrel
3. Collect filtered sample in polypropylene AS vial

Analysis

Split samples and analyze by 6470B and 6475 LC/TQ systems

Figure 1. Extraction scheme.

Experimental

Samples and calibration standards

Standard mixes were purchased from Wellington Labs (ON, Canada). Seven standards with concentrations similar to those listed in EPA 1633 third draft[1] were prepared. Soil quantification comparison between the 6470 B and 6475 LC/TQ systems are from standards prepared separately.

Soil extracts were spiked with Ottawa sand and extracted in accordance with the methods[1] listed in Figure 1. The Agilent consumables and supplies used for the extraction are listed in Table 1.

The spiking concentrations for the target compounds ranged from 2 to 40 ng/g, extracted internal standard (EIS) ranged from 1 to 20 ng/g, and nonextracted internal standards (NIS) ranged from 1 to 4 ng/g (Appendix A). Four replicate samples were spiked, extracted, and analyzed on both the 6470 B and 6475 LC/TQ systems.

Salmon tissue was sourced from a local supermarket and extracted as described in a previous application note.[5] Extracts were postspiked with a midlevel concentration of PFAS mix. The extract served to represent a challenging matrix to determine the robustness of the 6475 LC/TQ. Fish extract was analyzed with a 5-minute gradient. After 50 injections of fish sample, a midlevel calibration sample was analyzed using the full gradient in Table 1.

Table 1. Agilent consumables and supplies used for extraction.[4]

Consumables and Supplies	Part Number
Bond Elut PFAS WAX Solid Phase Extraction (SPE) Cartridge, 150 mg, 6 mL	5610-2150
Bond Elut Carbon S SPE Bulk Sorbent, 25 g Bottle	5610-2093
Centrifuge Tubes and Caps, 50 mL	5610-2049
Centrifuge Tubes and Caps, 15 mL	5610-2039
Bond Elut Empty SPE Cartridges, 60 mL	12131012
Bond Elut Adapter Cap for 1, 3, and 6 mL Bond Elut Cartridges	12131001
Glass Wool, Silane Treated, 50 g, for GC	8500-1572
Captiva Disposable Syringe, 5 mL	9301-6476
Captiva Premium Syringe Filter, Polypropylene Housing, Nylon Membrane, 25 mm Diameter, 0.2 µm Pore Size	5190-5092
Vac Elut SPS 24 Manifold with Collection Rack for 10 × 75 mm Test Tubes	12234003
Collection Rack and Funnel Set for 12 or 15 mL Conical Tubes, for Vac Elut SPS 24 Manifold	12234027
Stopcock Valve (20 pack)	12234520
Polypropylene Screw-Style Vials, 2 mL	5191-8121
Screw-Style Cap, 9 mm, with Polypropylene/Silicone Screw Septa	5191-8151

Triple quadrupole liquid chromatography/mass spectrometry conditions

LC/TQ analysis was performed with an Agilent 1290 Infinity II LC system coupled to the Agilent 6475 triple quadrupole LC/MS system. The system was controlled by Agilent MassHunter acquisition software 12.0 (LC/TQ), which has new intelligence features and maintains method compatibility with prior versions. The acquisition method was based on the previously published parameters.[3] Full LC parameters are shown in Table 2. MS conditions are displayed in Table 3. The Agilent PFAS MRM Database for triple quadrupole LC/MS (product number G1736AA), which is developed and optimized for the 6470 B LC/TQ, was directly imported into the 6475 LC/TQ with MassHunter acquisition software 12.0.

Table 2. LC instrument conditions.

Parameter	Value
LC	Agilent 1290 Infinity II LC System, consisting of: – Agilent 1290 Infinity II high-speed pump (G7120A) – Agilent 1290 Infinity II multisampler (G7167B) – Agilent 1290 Infinity II multicolumn thermostat (G7116B)
Analytical Column	Agilent ZORBAX Eclipse Plus C18, 2.1 × 100 mm, 1.8 µm (p/n 959758-902)
Delay Column	Agilent InfinityLab PFC delay column, 4.6 × 30 mm (p/n 5062-8100)
Column Temperature	50 °C
Injection Volume	2 µL
Mobile Phase	A) 2 mM ammonium acetate in water B) 95:5 acetonitrile:water
Gradient Flow Rate	0.4 mL/min
Gradient	Time (min) % B 0.0 15 0.5 15 1.5 25 7.0 60 10.0 100 12.0 100 12.1 15.0
Stop Time	12.5 min
Post-Time	2.5 min

Table 3. MS conditions.

Parameter	Value
MS	Agilent 6475 LC/TQ with Agilent Jet Stream Electrospray ion source (p/n G6475A)
Source Parameters	
Gas Temperature	230 °C
Gas Flow	8 L/min
Nebulizer	20 psi
Sheath Gas Temperature	355 °C
Sheath Gas Flow	10 L/min
Capillary Voltage (Negative)	2,500 V
Nozzle Voltage (Negative)	0 V

The 6475 LC/TQ offers new intelligence features, including a fully automated method development algorithm, which updates method settings with the best value without user intervention. Parameter selection for ion source optimization has two algorithm models: extracted ion chromatogram (EIC) and weighted EIC models. The EIC model maximizes total signal response, while the weighted EIC model favors low-responding analytes in a complex mixture with adjustable weights. The 6470B LC/TQ ion source values for EPA 1633 have been previously optimized and published.[6,3] Comparing results from the previous manual process to the newly intelligent software provided an interesting test case. The PFAS in the EPA 1633 analyte list include compounds that are sensitive to source temperature (e.g., NFDHA).

Output can be viewed overall or on an individual compound basis. Figure 2 shows the differences between the two models for drying gas temperature.

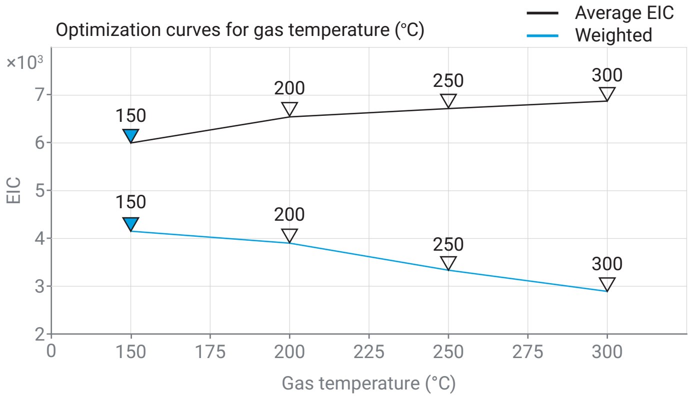


Figure 2. Source optimization curves.

Ion source optimization models started to diverge around 225 °C. Divergence indicated that while the overall EIC went up at the higher temperature, some of the low-abundance compounds showed lower abundance. After considering individual compound results, we confirmed that the best compromise was a temperature of 230 °C, which was selected in our original method.[3] Interestingly, this was the approximate temperature at which the two models diverged, which was helpful for verifying the optimal gas temperature for analysis.

EPA 1633 analyte performance with the 6475 LC/TQ

Calibration standards were injected with seven replicates. Linear or quadratic curves with 1/x weighting were fit for all compounds. The instrument linearity calculation described in EPA 1633 method section 10.3.3.3 was performed, and RSDs for the tested range (Table 4) were all under 14%. The RSDs of the seven replicate injections at the low calibrator were also evaluated. The two compounds with the highest RSDs, near 15%, were MeFOSA and MeFOSAA.

One important consideration for achieving good calibration fits for the fluorotelomer sulfonate (FTS) compounds is the selection of the quantifying transition for the labeled extraction standards. In EPA 533, it is noted that M+2 isotope of the sulfur compound has the same nominal mass as the $^{13}C_2$ labeled isotope.[7] At the lower concentrations examined in EPA 533, this showed minimal interference; however, EPA 1633 has wider concentration range, so it is important to select the lower-abundance product ion: *m/z* 81.

Table 4. Method performance data (continued on next page).

Compound	%RSD of Calibration (Option 1)	%RSE of Calibration (Option 2)	Low Calibration (ng/mL)	High Calibration (ng/mL)	R^2	% RSD at Low Level
11Cl-PF3OUdS	8%	7%	0.20	100.0	0.998	3.2%
3-3 FTCA	10%	10%	0.40	200.0	0.997	5.2%
4:2 FTS	10%	10%	0.38	187.5	0.995	11.8%
5-3 FTCA	10%	10%	2.00	1,000.0	0.998	3.6%
6:2 FTS	9%	11%	0.38	190.0	0.997	6.3%
7-3 FTCA	11%	10%	2.00	1,000.0	0.998	7.1%
8:2 FTS	8%	10%	0.38	192.0	0.996	8.1%
9Cl-PF3ONS	8%	8%	0.20	100.0	0.998	4.2%
ADONA	7%	9%	0.20	100.0	0.998	1.6%
HFPO-DA	9%	10%	0.20	100.0	0.998	4.2%
NEtFOSA	9%	12%	0.10	50.0	0.996	9.5%
NEtFOSAA	13%	13%	0.10	50.0	0.996	11.3%
NEtFOSE	8%	9%	1.00	500.0	0.997	2.5%
NFDHA	9%	10%	0.20	100.0	0.998	8.1%
NMeFOSA	10%	12%	0.10	50.0	0.996	14.1%
NMeFOSAA	12%	14%	0.10	50.0	0.996	15%
NMeFOSE	8%	10%	1.00	500.0	0.997	2.1%
PFBA	8%	10%	0.40	200.0	0.997	3.1%
PFBS	9%	10%	0.09	44.4	0.997	7.3%
PFDA	10%	12%	0.10	50.0	0.997	6.8%
PFDoA	9%	11%	0.10	50.0	0.997	8.3%
PFDoS	9%	11%	0.10	48.5	0.997	10.8%
PFDS	9%	11%	0.10	48.3	0.997	12.8%
PFEESA	8%	8%	0.20	100.0	0.998	2.5%
PFHpA	9%	12%	0.10	50.0	0.996	5.8%
PFHpS	9%	12%	0.10	47.7	0.997	10.4%

Table 4. Method performance data (continued).

Compound	%RSD of Calibration (Option 1)	%RSE of Calibration (Option 2)	Low Calibration (ng/mL)	High Calibration (ng/mL)	R^2	% RSD at Low Level
PFHxA	9%	11%	0.10	50.0	0.997	7.5%
PFHxS	10%	11%	0.09	45.7	0.997	3.5%
PFMBA	8%	10%	0.20	100.0	0.998	3.1%
PFMPA	8%	10%	0.20	100.0	0.998	1.7%
PFNA	10%	12%	0.10	50.0	0.996	9.7%
PFNS	9%	10%	0.10	48.1	0.997	12%
PFOA	9%	12%	0.10	50.0	0.997	11.3%
PFOS	7%	9%	0.09	46.4	0.997	6.7%
PFOSA	8%	10%	0.10	50.0	0.997	3.4%
PFPeA	8%	10%	0.20	100.0	0.997	3.1%
PFPeS	9%	13%	0.09	47.1	0.996	7%
PFTeDA	9%	11%	0.10	50.0	0.996	9.6%
PFTrDA	11%	12%	0.10	50.0	0.996	7%
PFUnA	11%	12%	0.10	50.0	0.996	10.2%

Soil quantification comparisons between the 6470 B and 6475 LC/TQ systems

Extraction recoveries from the four spiked soil extractions were compared between the 6470B and 6475 LC/TQ systems. For all compounds on both instruments, the average recoveries were within 70 and 130% with RSDs less than 20%, as shown in Figures 3 and 4. The overall average recoveries for all targets were 103.2 and 97.7%, with average RSDs of 3.4 and 2.0% for the 6470 B and 6475 LC/TQ systems respectively. This demonstrates excellent and comparable performance between the two instruments.

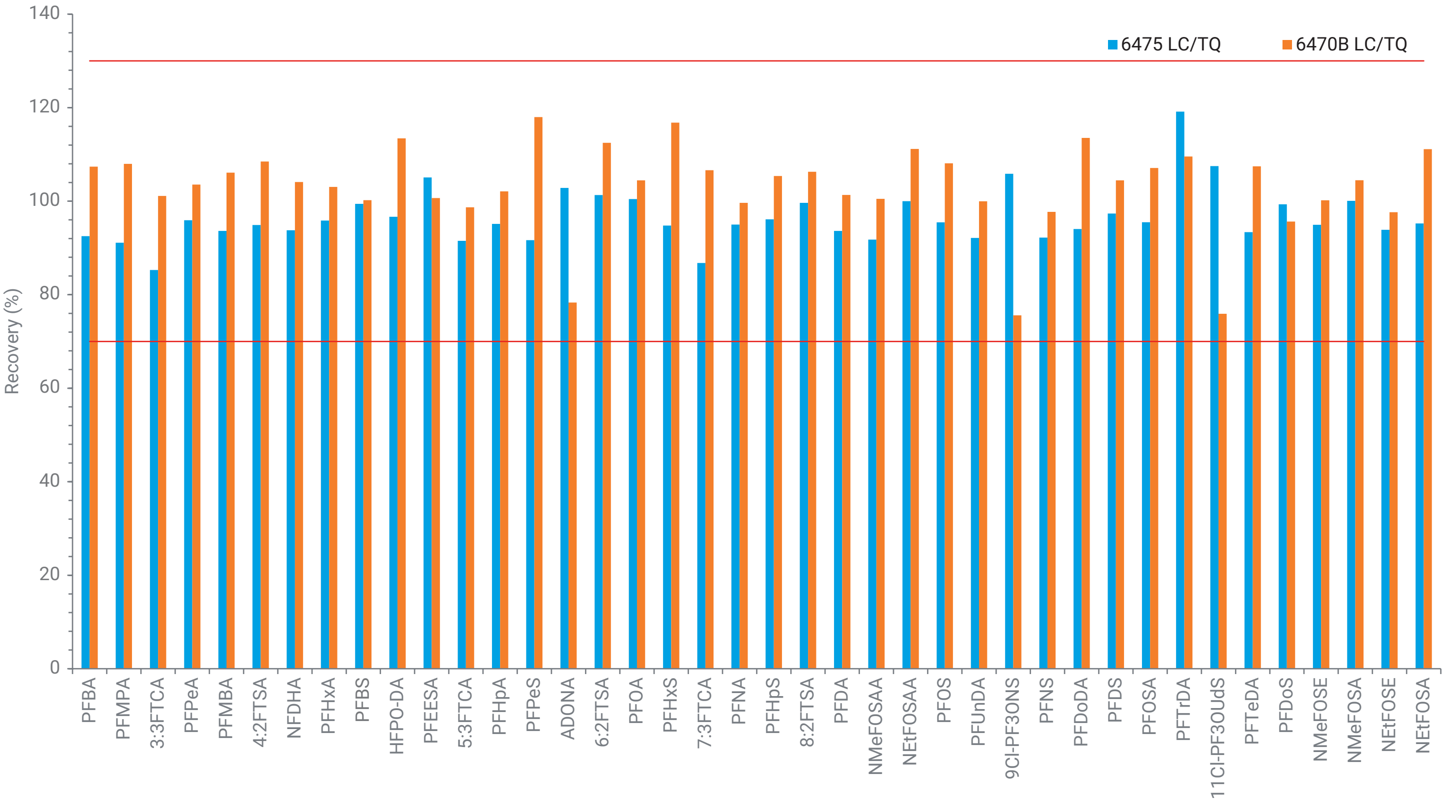


Figure 3. Average target recoveries for four replicate extractions analyzed on the Agilent 6470B and 6475 triple quadrupole LC/MS systems.

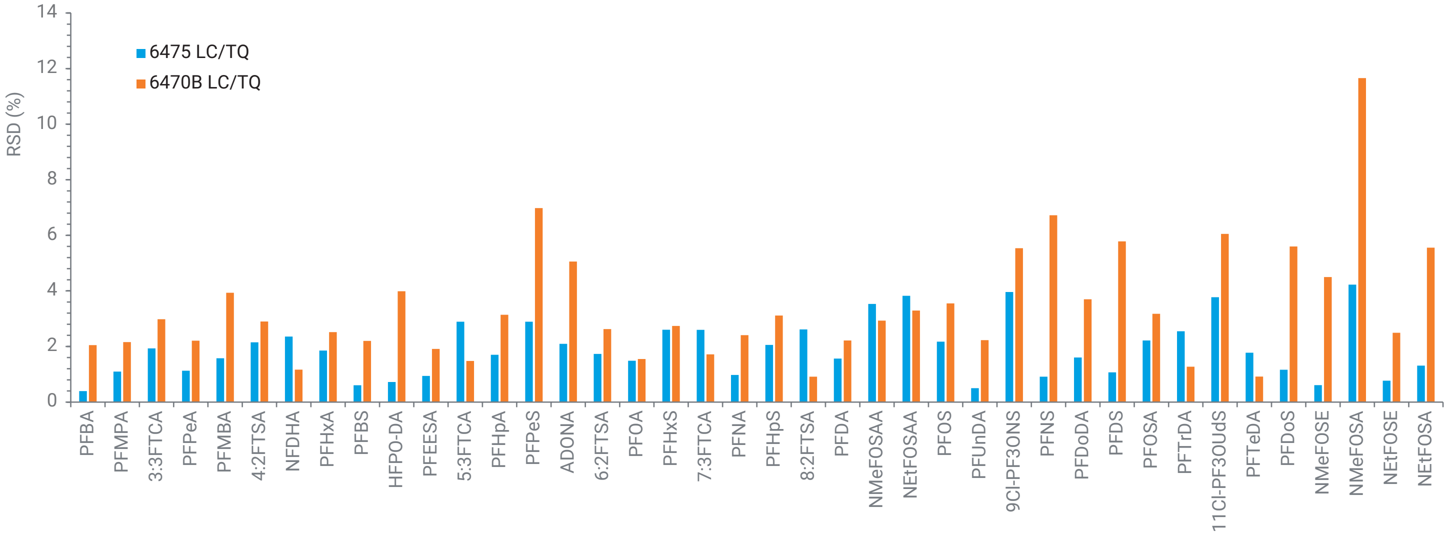


Figure 4. Target %RSDs for four replicate extractions analyzed on the Agilent 6470B and 6475 triple quadrupole LC/MS systems.

Robustness of 6475 LC/TQ

An advantage of the 6475 LC/TQ is its robust performance with matrix samples. Figure 5 shows the performance of PFBA, PFOA, and PFBS across 1,300 injections of salmon matrix. The solid line represents the performance of the CV standards injected every 50 salmon samples, while the colored dots are the replicated injections of the postspiked salmon matrix. For the entire compound list, CV abundances varied between 3 to 22%. Most of the compound list (86%) abundance RSDs were under 10%. The FTS and FOSAA compounds had abundance RSDs higher between 16 and 22%, which is normal for these two classes.

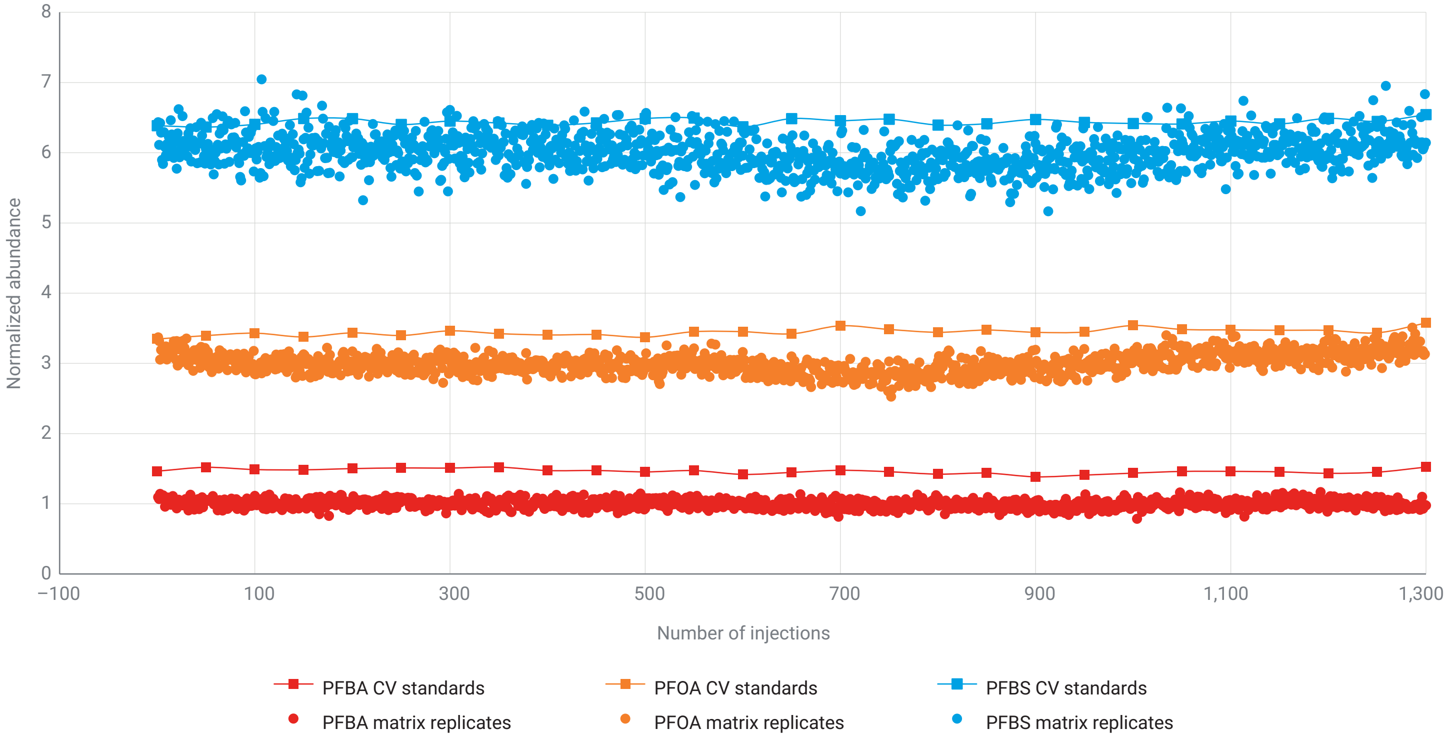


Figure 5. Instrument robustness across 1,300 injections. Abundance was normalized and scaled for visualization. The solid line represents the continuing calibration verification standards with the individual points of the matrix injections.

Conclusion

This application note demonstrates the excellent analytical sensitivity, precision, accuracy, and robustness of the Agilent 6475 triple quadrupole LC/MS system for PFAS analysis. This PFAS analysis of soil samples shows comparable results between the Agilent 6470 B and 6475 LC/TQ systems.

References

1. Office of Water and Office of Science and Technology, Engineering and Analysis Division, United States Environmental Protection Agency. EPA 821-D-21-001. Draft Method 1633, Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/MS. Last revised August **2021**. https://www.epa.gov/system/files/documents/2021-09/method_1633_draft_aug-2021.pdf
2. United States Environmental Protection Agency. Our Current Understanding of the Human Health and Environmental Risks of PFAS. Last updated March 16, **2023**. https://www.epa.gov/pfas/our-current-understanding-human-health-and-environmental-risks-pfas
3. Hunt, K. *et al.* Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous Samples Per EPA Draft Method 1633. *Agilent Technologies application note*, publication number 5994-4926EN, **2022**.
4. Per- and polyfluoroalkyl substances (PFAS) analysis for environmental samples. Agilent Technologies consumables ordering guide, publication number 5994-2357EN, **2023**.
5. Yang, X. A Fast Sample Preparation Workflow for Veterinary Drugs Analysis in Salmon. *Agilent Technologies application note*, publication number 5994-1124EN, **2019**.
6. Giardina, M. Analysis of Per- and Polyfluoroalkyl Substances in Soil Extracts. *Agilent Technologies application note*, publication number 5994-2999EN, **2021**.
7. Rosenblum, L. and Wendelken, S.C., United States Environmental Protection Agency. Method 533: Determination of Per- and Polyfluoroalkyl substances in drinking water by isotope dilution and anion exchange solid phase extraction and liquid chromatography/tandem mass spectrometry, **2019**. https://www.epa.gov/sites/default/files/2019-12/documents/method-533-815b19020.pdf

Appendix A

Table A1. Concentrations of targets, NIS, and EIS.

Targets	Spike Concentration (ng/g)
PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnA, PFDoA, PFTrDA, PFTeDA, PFBS, PFPeS, PFHxS, PFHpS, PFOS, PFNS, PFDS, PFDoS, PFOSA, NMeFOSA, NEtFOSA, NMeFOSAA, NEtFOSSA	2
PFPeA, PFMPA, NFDHA, PFMBA, PFEESA	4
PFBA, 4:2FTS, 6:2FTS, 8:2FTS, HFPO-DA, ADONA, 9Cl-PF3ONS, 11CL-PF3OUdS, 3:3FTCA	8
NMeFOSE, NEtFOSE	20
5:3FTCA, 7:3FTCA	40
EIS	
$^{13}C_2$-PFDoA, $^{13}C_2$-PFTeDA, $^{13}C_6$-PFDA, $^{13}C_7$-PFUnA, $^{13}C_9$-PFNA	1
$^{13}C_3$-PFBS, $^{13}C_3$-PFHxS, $^{13}C_4$-PFHpA, $^{13}C_5$-PFHxA, $^{13}C_8$-PFOA, $^{13}C_8$-PFOS, $^{13}C_8$-PFOSA, D_3-NMeFOSA, D_5-NEtFOSA	2
$^{13}C_2$-4:2FTS, $^{13}C_2$-6:2FTS, $^{13}C_2$-8:2FTS, $^{13}C_5$-PFPeA, D_3-NMeFOSAA, D_5-NEtFOSAA	4
$^{13}C_3$-HFPO-DA, $^{13}C_4$-PFBA	8
D_7-MeFOSE, D_9-EtFOSE	20
NIS	
$^{13}C_5$-PFNA, $^{13}C_2$-PFDA	1
$^{13}C_2$-PFHxA, $^{13}C_4$-PFOA, $^{18}O_2$-PFHxS, $^{13}C_4$-PFOS	2
$^{13}C_3$-PFBA	4

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