

Ultra-Trace Quantification of Per- and Polyfluoroalkyl Substances (PFAS) in Drinking Water

Achieve part-per-quadrillion detection limits with the Agilent 6495C LC/TQ

Author

Tarun Anumol, Patrick Batoon,
and Emily Parry
Agilent Technologies, Inc.

Abstract

The US EPA has released interim health advisory limits (HALs) of 4 and 20 pg/L in drinking water for the PFAS perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS), respectively. This application note addresses PFAS testing at these challenging new levels in aqueous samples using optimized solid-phase sample extraction and LC/MS/MS analysis with an Agilent 1290 Infinity II liquid chromatography (LC) system coupled to an Agilent 6495C triple quadrupole (TQ) mass spectrometer. The study verified method development and performance for PFOA, PFOS, perfluorobutane sulfonate (PFBS), and GenX (trade name for processing aid used to manufacture fluoropolymers without PFOA) in the study samples, which included real drinking water samples. The study also evaluated two LC setup approaches: a Diol guard cartridge, installed before a C18 column with 100 μ L injection, and a 30 μ L sandwich injection onto a C18 column.

Introduction

PFAS are a group of man-made compounds that have been used since the 1950s and that have become a concern in the last decade due to their persistence in the environment and possible adverse effects on humans and animals.¹ Their persistence and possible adverse effects have led to several restrictions, guidance levels, and regulation of subsets of PFAS in drinking water, wastewater, soil, and other matrices. In the United States, the Environmental Protection Agency (US EPA) has four standard methods for the analysis of PFAS in water: EPA 537.1 and EPA 533 for drinking water, and EPA 8327 and EPA Draft 1633 for wastewater and soil, which analyze between 14 and 40 PFAS. As part of its PFAS Action Plan, set in 2020, the US EPA has committed to moving forward with a regulatory process to determine maximum contaminant levels (MCLs) for PFOA and PFOS, and to set lifetime health advisory levels in drinking water. In June 2022, the US EPA issued interim drinking water HALs for PFOA at 0.004 nanogram per Liter (ng/L), PFOS at 0.02 ng/L, GenX at 10 ng/L, and PFBS at 2,000 ng/L to reduce public health risks due to exposure. While HALs are currently unenforceable, several states and water agencies are interested in monitoring PFAS at these levels in drinking water to achieve baseline occurrence data.

Similarly, in the EU, a proposed revision to the EC Directives 2000/60/EC, 2006/118/EC, and 2008/105/EC attempts to include an environmental quality standard for the sum of 24 PFAS at 4.4 ng/L in surface and ground waters.² Guidance for PFOA and PFOS in the low ng/L range in drinking water also exists in Australia, Japan, South Korea, and other parts of the world.

To measure the ultralow levels for PFOA, PFOS, PFBS, and GenX that are listed in the US EPA HALs and proposed EU limits, an extremely sensitive mass spectrometer is needed, along with stringent method protocols including cleanliness in sample preparation, extraction, and consumable products. This application note describes a procedure that achieves unparalleled parts-per-quadrillion sensitivity using Agilent sample preparation and consumables, combined with robust analysis on the 1290 Infinity II LC and 6495C TQ LC/MS.

Experimental

Consumables and supplies

To assist laboratories in preparing to run the method, a list of all Agilent consumables used in the production of this application note is provided in the Appendix.

Extraction procedure

The extraction procedure for the drinking water samples followed EPA method 533 with minor modifications to reduce PFAS background. Briefly, unpreserved 250 mL aqueous samples were fortified with surrogates (extracted internal standards [EIS]). Weak anion exchange (WAX) solid-phase extraction cartridges (Agilent Bond Elut PFAS WAX) were conditioned with methanol followed with phosphate buffer. Samples were then loaded and eluted with a mixture of ammonium hydroxide and methanol solution. The resulting extract was evaporated to dryness and reconstituted to 1 mL, placed in a 2 mL polypropylene vial (p/n 5191-8150) and closed with a polypropylene/silicone screw cap septum that did not contain PTFE (p/n 5191-8151). Details of the sample preparation protocol and the consumables used to achieve low PFAS background can be found in previously published application notes.^{3,4}

LC/TQ instrument conditions

LC/TQ analysis was performed using a 1290 Infinity II LC system coupled to a 6495C TQ LC/MS. The LC configuration and method parameters are shown in Table 1. A PFAS-specific delay column was placed between the pump and multisampler to separate background contaminants from compounds originating in the sample vial without significantly increasing backpressure. The Agilent PFC-Free LC kit was installed on the LC to reduce PFAS background and eliminate PFAS contamination. The mobile phases used for the method were optimized for maximum sensitivity for PFOA, PFOS, PFBS, and GenX, and contained water with 2 mM ammonium acetate (A) and 95:5 (v:v) acetonitrile:water (B). A 100 or 30 μ L injection was used for analysis in the two different approaches explained below. The final LC conditions used in the method are shown in Table 1.

Table 1. LC instrument conditions.

Parameter	Value																
LC	Agilent 1290 Infinity II LC System with high speed pump (p/n G7120A), Agilent 1290 Infinity II G7167B multisampler, multicolumn thermostat (MCT) (p/n G7116B), and thermostatted column compartment																
Guard Cartridge	Agilent ZORBAX Diol, 4.6 × 12.5 mm (p/n 820950-911)																
Analytical Column	Agilent Poroshell EC C18, 2.1 × 100 mm, 2.7 μm (p/n 695775-902)																
Delay Column	Agilent InfinityLab PFC Delay Column, 4.6 × 30 mm (p/n 5062-8100)																
Column Temperature	50 °C																
Injection Volume	30 μL/100 μL																
Mobile Phase	A) 2 mM ammonium acetate in water B) 95:5 acetonitrile:water																
Gradient Flow Rate	0.4 mL/min																
Gradient	<table border="1"> <thead> <tr> <th>Time (min)</th> <th>% B</th> </tr> </thead> <tbody> <tr> <td>0.0</td> <td>15</td> </tr> <tr> <td>1.0</td> <td>15</td> </tr> <tr> <td>1.5</td> <td>25</td> </tr> <tr> <td>7.0</td> <td>60</td> </tr> <tr> <td>10</td> <td>100</td> </tr> <tr> <td>12</td> <td>100</td> </tr> <tr> <td>12.1</td> <td>15</td> </tr> </tbody> </table>	Time (min)	% B	0.0	15	1.0	15	1.5	25	7.0	60	10	100	12	100	12.1	15
Time (min)	% B																
0.0	15																
1.0	15																
1.5	25																
7.0	60																
10	100																
12	100																
12.1	15																
Stop Time	12.5 min																
Post Time	3.0 min																

Table 2. Agilent 6495C TQ instrument conditions.

Parameter	Value
MS	Agilent 6495C TQ LC/MS with Agilent Jet Stream ESI source
Source Parameters	
Gas Temperature	150 °C
Gas Flow	18 L/min
Nebulizer	25 psi
Sheath Gas Temperature	390 °C
Sheath Gas Flow	11 L/min
Capillary Voltage (Neg)	2,500 V
Nozzle Voltage (Neg)	0 V
Funnel	90/80

The 6495C TQ LC/MS system is ideally suited for applications that require utmost, low-level sensitivity. iFunnel design, superior ion optics and detection, and highly synchronized electronics control enhance analytical sensitivity for better detectability and improved precision at low analyte levels.

The optimized mass spectrometer parameters are shown in Table 2. The optimized MRM parameters were obtained from the Agilent PFAS MRM database without need for additional user optimization. Dynamic multiple reaction monitoring (dMRM) in negative electrospray ionization mode was used for data acquisition. Data acquisition and processing were performed using Agilent MassHunter software.

Calibration standards

Analytical standard mixes that included PFOA, PFOS, PFBS, and GenX were purchased from Wellington Laboratories. The standards were diluted to create six calibration levels in 80:20 methanol:water at 0.1, 0.5, 1, 2, 5, and 10 pg/mL for injection onto the TQ LC/MS instrument. These levels corresponded to 0.0004, 0.002, 0.004, 0.008, 0.02, and 0.04 ng/L in the initial water sample considering the 250x concentration factor applied through sample extraction and concentration.

Results and discussion

LC method setups tested

In this study, two different LC columns and options were evaluated:

- A Diol guard cartridge was installed before the C18 analytical column and a 100 μL injection was made to obtain high sensitivity.
- A standard C-18 column was used with a 30 μL sandwich injection to increase loading and improve peak shape, while reducing the matrix entering the mass spectrometer without sacrificing detection limits.

Practical considerations for large volume injections

Large-volume injection allows increased sensitivity without increasing the sample extraction volume by loading more analyte mass onto the LC/MS. However, injecting large volumes of high organic solvents, such as the 80:20 methanol mixture (as needed for EPA 533), on a reversed-phase column can result in poor chromatographic peak shapes. Strategies to mitigate adverse solvent effects include using a focusing guard cartridge or a sandwich injection.

The Diol guard cartridge provided optimal peak shape (Figures 1 and 2, top row). However, in this case it was the source of low-level PFOA in the background. The Diol guard cartridge was designed for low-pressure applications, therefore exceeding 400-bar backpressure is not recommended. Also, to maintain analytical column life, installation of an inline filter (see Appendix for part number) between the guard cartridge and the analytical column is recommended to eliminate particulates or precipitates.

Another approach is to use a sandwich injection, which involves injecting a plug of water before and after injection of the highly organic sample. This approach improves peak shapes while allowing use of lower injection volumes without sensitivity loss. This approach also reduces the amount of matrix entering the mass spectrometer, providing better longer term robustness. In this experiment, two

plugs of 10 μ L water + 0.1% acetic acid sandwiched the sample and were mixed three times by injecting air. The sandwich injection resulted in less background and poorer peak shapes than the Diol cartridge. However, even the diminished peak shapes observed were acceptable for use in routine quantification.

Calibration performance

Calibration samples were prepared in 80:20 methanol:water to match the final composition in the sample. The concentrations shown in Table 3 are those reported in the vial. The calibration standards for the four compounds all resulted in an $R^2 > 0.99$ and an average relative standard error (RSE) of 8 to 20% (Table 3), indicating good linearity and precision even at extremely low analyte levels. The new US EPA and PFAS methods require that the RSEs of all individual calibration points must be $\leq 20\%$ to establish instrument linearity.

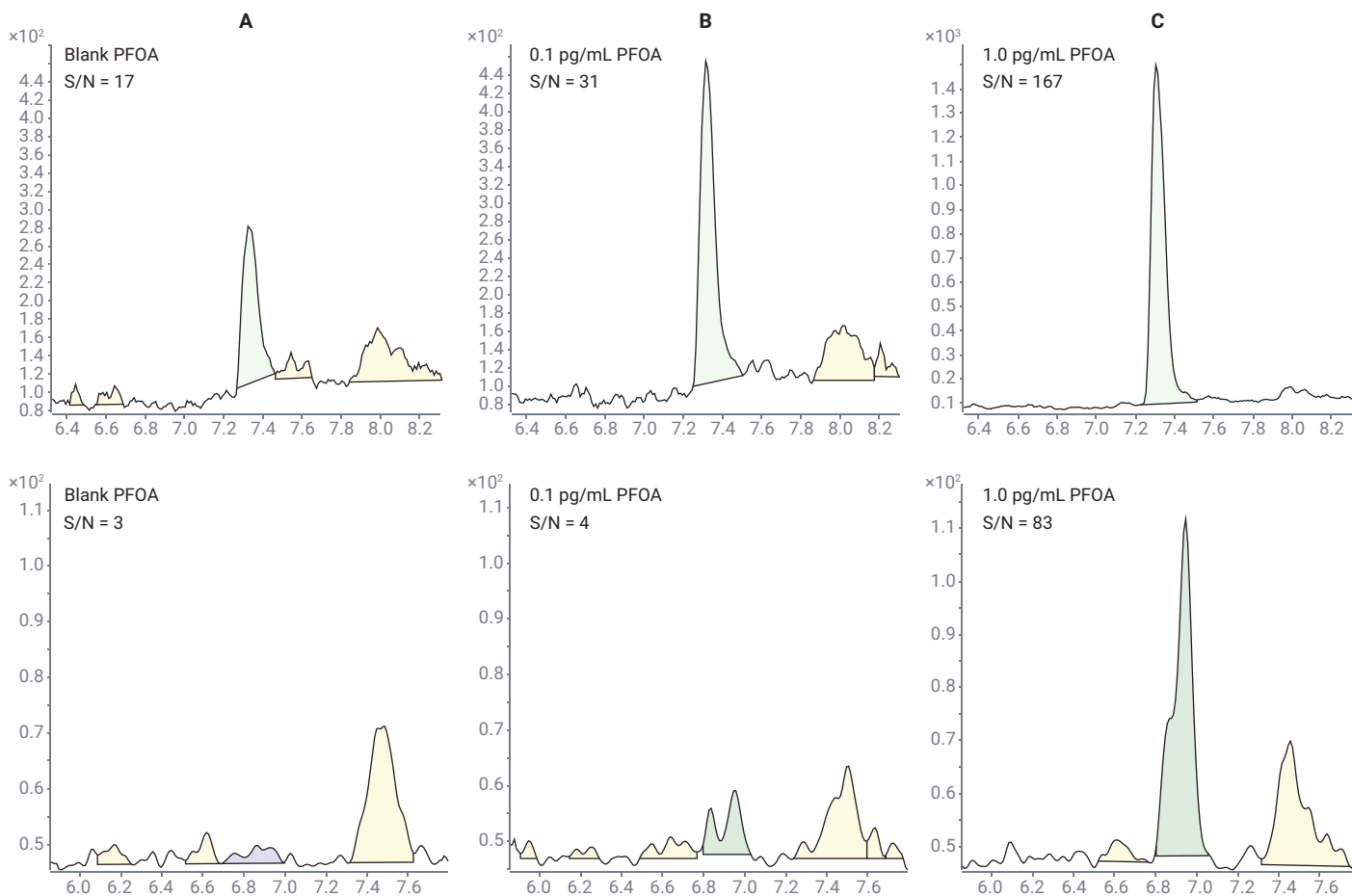


Figure 1. MRM chromatogram of PFOA in 80:20 methanol/water at low calibrator concentrations. Top row: Diol guard cartridge method. Bottom row: sandwich injection method. (A) Blank. (B) 0.1 pg/mL = 0.0004 ng/L with a 250x concentration factor. (C) 1 pg/mL = 0.004 ng/L with a 250x concentration factor.

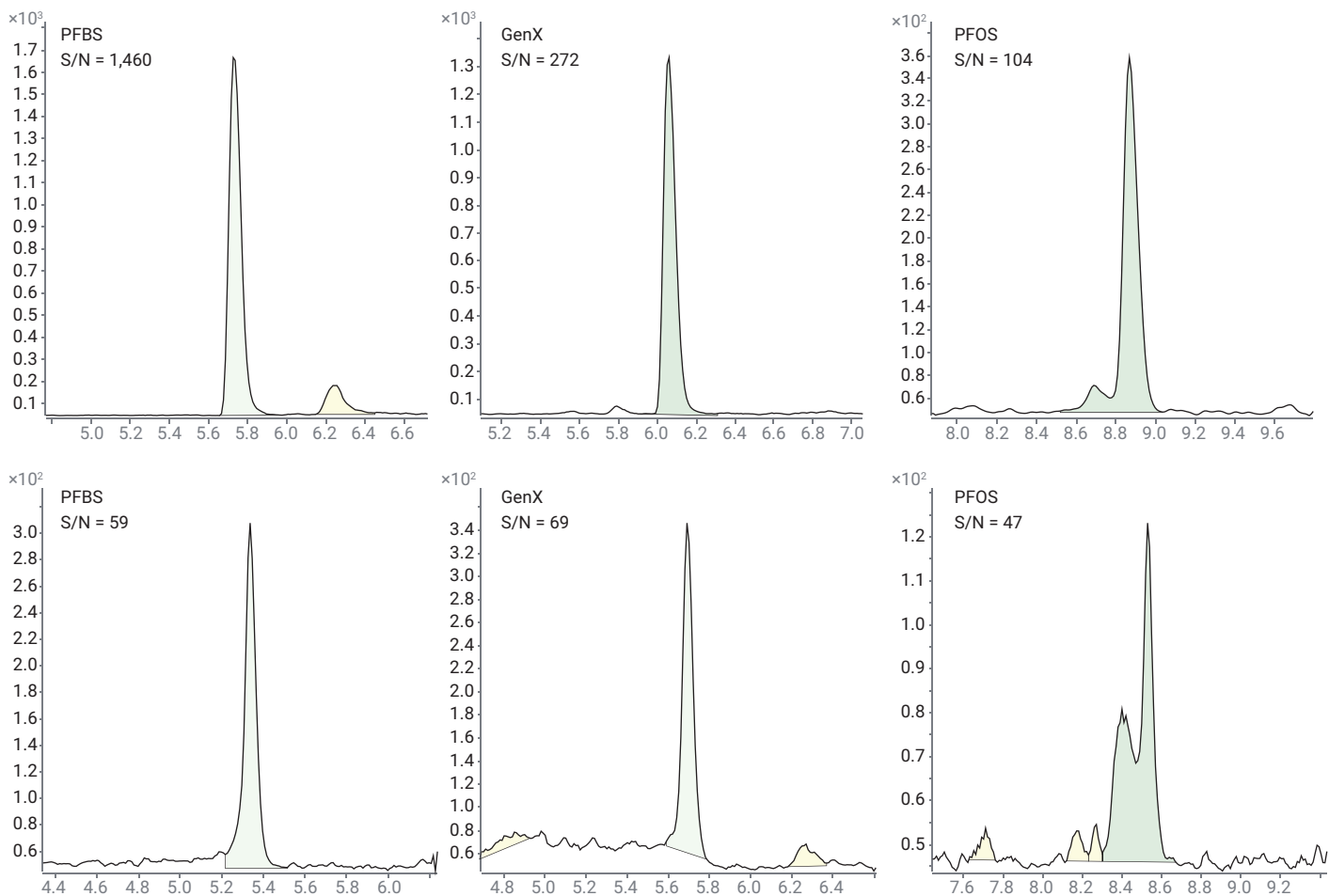


Figure 2. MRM chromatograms of PFBS, GenX, and PFOS at 20 pg/L with a 250x concentration factor. Top row: Diol guard cartridge method. Bottom row: sandwich injection method.

Table 3. Calibration performance for PFAS analyzed.

PFAS	Diol Calibration (Conc. In Vial, pg/mL)			Sandwich Calibration (Conc. In Vial, pg/mL)		
	Low Standard	High Standard	RSE	Low Standard	High Standard	RSE
PFBS	0.1	10	8%	0.1	10	16%
GenX	0.1	10	18%	0.5	10	10%
PFOA	0.1	10	20%	0.1	10	12%
PFOS	0.1	10	13%	0.1	5	16%

Drinking water samples

To demonstrate its real-world applicability, the method was tested with Millipore Water (MQ), LC/MS-grade bottled water (RW) and two different types of bottled water samples (SW and DP). The authors were also interested in determining whether the MQ sample had a significant amount of PFOA. In fact, all samples had PFOA above 4 pg/L (Figure 3), indicating that PFOA background from the SPE cartridge or chemicals used was present. However, one of the bottled water samples had significantly more PFOA, more than 35 pg/L, indicating that its PFOA level exceeded current HALs. PFOS, PFBS, and GenX were not detected in the blanks or bottled water samples.

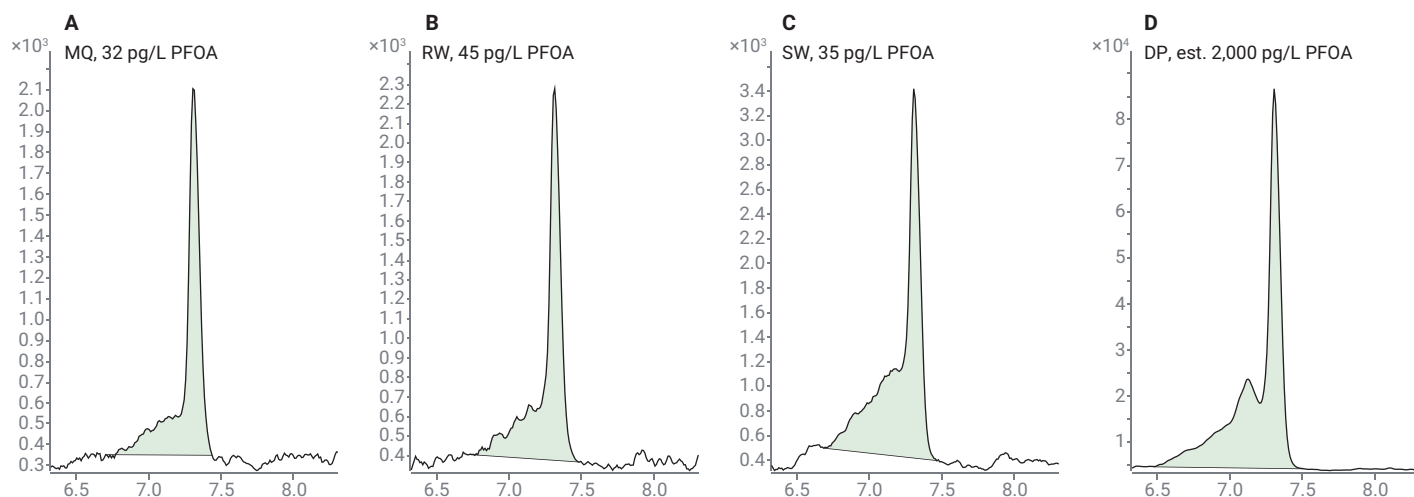


Figure 3. PFOA detected in water samples. (A) MQ, 32 pg/L; (B) RW, 45 pg/L; (C) SW, 35 pg/L; (D) DP exceeded the calibration range, with an estimated value of 2,000 pg/L.

Conclusion

This application note evaluated PFAS testing in aqueous samples at the levels outlined in the US EPA-issued interim drinking water HALs for PFOA (0.004 ng/L), PFOS (0.02 ng/L), GenX (10 ng/L), and PFBS (2,000 ng/L) using optimized solid-phase sample extraction and LC/MS/MS analysis with an Agilent 1290 Infinity II LC system coupled to an Agilent 6495C TQ LC/MS. Method development and performance were verified for all compounds studied. Robust, parts-per-quadrillion sensitivity was achieved using Agilent sample preparation and consumables in conjunction with the 1290 Infinity II LC and 6495C TQ LC/MS. Calibration standards for the four compounds produced an $R^2 > 0.99$ and an average relative standard error (RSE) of 8 to 20%, demonstrating good linearity and precision, even at the extremely low analyte levels studied. A Diol guard cartridge provided optimal peak shapes but was a source of low-level PFOA background. The sandwich injection approach resulted in less background and poorer (but acceptable) peak shapes than the Diol guard cartridge. Real-world applicability of the method was demonstrated by analyzing Millipore Water, LC/MS-grade bottled water and two different types of bottled drinking water samples.

References

1. United States Environmental Protection Agency, Per- and Polyfluoroalkyl Substances (PFAS). Updated January 5, **2023**. <https://www.epa.gov/pfas> (accessed February 7, 2023).
2. European Union. Proposal for a DIRECTIVE OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL. Amending Directive 2000/60/EC establishing a framework for Community action in the field of water policy, Directive 2006/118/EC on the protection of groundwater against pollution and deterioration and Directive 2008/105/EC on environmental quality standards in the field of water policy. October 26, **2022**. (accessed February 7, 2023)
3. **Per- and Polyfluoroalkyl Substances (PFAS) Analysis for Environmental Samples**. *Agilent Technologies consumables ordering guide*, publication number 5994-2357EN, **2022**.
4. Hindle, R.; Hunt, K.; Anumol, T. **EPA Method 533 for Analysis of PFAS in Drinking Water Using Agilent 6470 LC/MS/MS**. *Agilent Technologies application note*, publication number 5994-1628EN, **2020**.

Appendix

Table 4. Agilent consumables and supplies for EPA draft Method 1633.

Description	Specifics	Part Number
PFC Delay Column	InfinityLab PFC Delay Column, 4.6 × 30 mm	5062-8100
Analytical Column	Poroshell ECC18, 2.1 × 100 mm; 2.7 μm	695775-902
Autosampler Vials	Vial, screw, 2 mL, polypropylene, certified for PFAS, 100/pk	5191-8150
Autosampler Caps	Cap, 9 mm, screw, clear, thin membrane polypropylene/ silicone septa, certified for PFAS, 100/pk	5191-8151
SPE Cartridges	Bond Elut PFAS WAX SPE cartridges, 500 mg, 6 mL, 30/pk	5610-2152
Sample Reservoir	Bond Elut Empty SPE cartridge 60 mL (100/pk)	12131012
Centrifuge Tubes	Centrifuge tubes and caps, 15 mL, 50/pk	5610-2039
Guard Cartridge	ZORBAX Diol, 4.6 × 12.5 mm	820950-911
Guard Cartridge Hardware	Hardware kit	820999-901
Inline Filter	InfinityLab Quick Change inline filter assembly	5067-1603

www.agilent.com

DE46039694

This information is subject to change without notice.

© Agilent Technologies, Inc. 2023
Printed in the USA, March 16, 2023
5994-5797EN