Application Note

Food

Sensitive Measurement of Per- and Polyfluoroalkyl Substances in Drinking Water

Using time-saving Agilent Feed Injection technology with the Agilent 1290 Infinity III Hybrid Multisampler

Authors

Susanne Soelter,
Melanie Behling, and
Edgar Naegele
Agilent Technologies, Inc.

Abstract

This application note demonstrates the use of Agilent Feed Injection using the Agilent 1290 Infinity III Hybrid Multisampler for quantitative per- and polyfluorinated alkyl substance (PFAS) analysis using triple quadrupole LC/MS. In this application note, we compare the classic approach of using a low-volume injection via a common flow-through mode to a high-volume injection using the Agilent Feed Injection capability of the 1290 Infinity III Hybrid Multisampler. High-volume injection eliminates the time-consuming solvent evaporation and reconstitution steps, resulting in significant time and cost savings.

Introduction

The analysis of PFAS often requires sample preparation techniques, such as solid phase extraction (SPE), whenever low detection limits are required. The final samples ready for LC/MS/MS analysis are therefore usually dissolved in 80 to 100% organic solvents.[1] Additionally, recommended concentration limits have gotten even lower in recent years in the respective EU directive.[2] This EU directive comprises 20 PFAS compounds for analysis with a required parametric value of 100 ng/L in total and 5 ng/L per compound, with a required limit of quantification (LOQ) of < 30 % of the parametric value (1.5 ng/L). Injecting high sample volumes can improve sensitivity and enable lower detection limits or avoid evaporation and reconstitution. However, this is limited by undesirable solvent effects due to the high elution strength of the sample solvent in common reversed-phase LC.

This application note presents the use of the new Agilent Feed Injection technology as an alternative to the common flow-through injection. With the Agilent InfinityLab PFAS analysis HPLC conversion kit, the PFAS background of the used Agilent 1290 Infinity III Hybrid Multisampler can be reduced.[3] Feed Injection allows for much higher injection volumes without a negative impact on peak shape, even when the sample is dissolved in 100% organic solvents.[4] This improvement is achieved by infusing the sample into the mobile phase stream with a special valve, resulting in an online dilution (Figure 1). By means of this approach, a significant reduction in sample preparation time can be achieved by avoiding drying down the sample and reconstituting in a solvent comprised of a reduced organic solvent ratio.

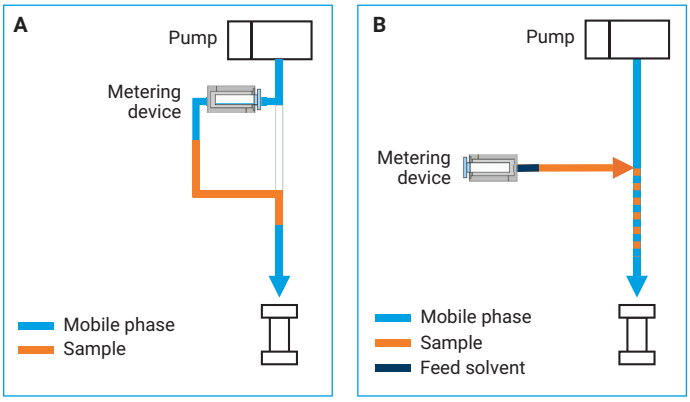

Figure 1. Classic flow-through injection (A) versus Agilent Feed Injection (B).

Experimental

Instrumentation

The following instrumentation was used:

- Agilent InfinityLab Assist
- Agilent 1290 Infinity III Binary Pump (G7120A)
- Agilent 1290 Infinity III Hybrid Multisampler (G7167B)
- Agilent 1290 Infinity III Multi Column Compartment (G7116B) with InfinityLab Quick-Connect heat exchanger, standard (G7116-60015)
- Agilent 6495D LC/TQ with Agilent Jet Stream Electrospray

Software

The following software was used:

- Agilent MassHunter acquisition software (version 12.2)
- Agilent MassHunter Qualitative Analysis software (version 12.0)
- Agilent MassHunter Quantitative Analysis software (version 12.1)

LC parameters

The LC parameters are outlined in Table 1.

Table 1. LC parameters.

Parameter	Value
Eluent A	2 mM NH_4Ac in water
Eluent B	Methanol (MeOH)
Flow Rate	400 µL/min
Gradient	Time (min) %B 0 25 5 70 7 70 10 90 11 90 11.50 25
Stop Time	11.50 min
Post Time	4 min
Column Temperature	40 °C
Flow-Through Injection Volume	2 µL for enriched samples
Feed Injection Volume	20 µL
Feed Injection Feed Speed	10% of flow rate (adaptive)
Feed Injection Flush Out	Automatic
Feed Injection Flush-Out Solvent	Mobile phase A
Needle Wash	3 s, methanol

Columns

The following columns were used:

- Analytical column: Agilent InfinityLab Poroshell 120 Aq-C18 3.0 × 100 mm, 2.7 µm (part number 695675-742)
- Delay column: Agilent InfinityLab PFC delay column, 4.6 × 30 mm (part number 5062-8100)

MS parameters

The MS parameters are outlined in Table 2.

Table 2. MS parameters.

Parameter	ESI AJS-Source
Drying Gas Temperature	200 °C
Drying Gas Flow	12 L/min
Nebulizer Pressure	45 psi
Sheath Gas Temperature	350 °C
Sheath Gas Flow	11 L/min
Capillary Voltage	2,500 V
Nozzle Voltage	0 V

Data were acquired in dMRM mode with a cycle time of 500 milliseconds (see Appendix).

MS settings: Agilent PFAS MRM Database for triple quadrupole LC/MS (G1736AA) was used for all LC/MS settings, without modifications, except retention time settings.

Calibration

The following calibration solutions were prepared for the analysis:

- **Solution I (ISTD, 1 µg/L):** 15 µL surrogate spiking mix + 15 mL 80% MeOH
- **Solution E (ISTD, 200 µg/L):** 200 µL surrogate spiking mix + 800 µL 80% MeOH
- **Solution A (SL1, 40 µg/L):** 20 µL EU Drinking Water Directive PFAS Mixture + 4,980 µL 80% MeOH
- **Solution B (SL2, 400 ng/L):** 50 µL Solution A + 4,950 µL 80% MeOH

Table 3. Dilution scheme for creation of calibration points for the 2 µL flow-through injection.

Name	Std. 1	Std. 2	Std. 3	Std. 4	Std. 5	Std. 6	Std. 7	Std. 8	Std. 10
V (A/B) (µL)	50 B	125 B	250 B	500 B	12.5 A	25 A	50 A	250 A	500 A
V (E)	5	5	5	5	5	5	5	5	5
V (Solvent) (µL)	945	870	745	495	982.5	970	945	745	495
	20 (ng/L)	50 (ng/L)	100 (ng/L)	200 (ng/L)	500 (ng/L)	1 (µg/L)	2 (µg/L)	10 (µg/L)	20 (µg/L)

The finally achieved calibration standards were diluted 1/10 to obtain the calibration standards necessary for the calibration of the 20 µL Feed Injection.

Sample preparation

Sample preparation was carried out as per the following:

- **Sample with ISTD:** 100 mL sample + 500 µL solution I
- **0.5 ng/L spiking:** 100 mL sample + 125 µL solution B
- **5 ng/L spiking:** 100 mL sample + 12.5 µL solution A
- **0.5 ng/L spiking + 5 ng/L ISTD:** 100 mL sample + 125 µL solution B + 500 µL solution I
- **5 ng/L spiking + 5 ng/L ISTD:** 100 mL sample + 12.5 µL solution A + 500 µL solution I

The sample preparation workflow is outlined in Figure 2 together with the time consumption of the individual steps.

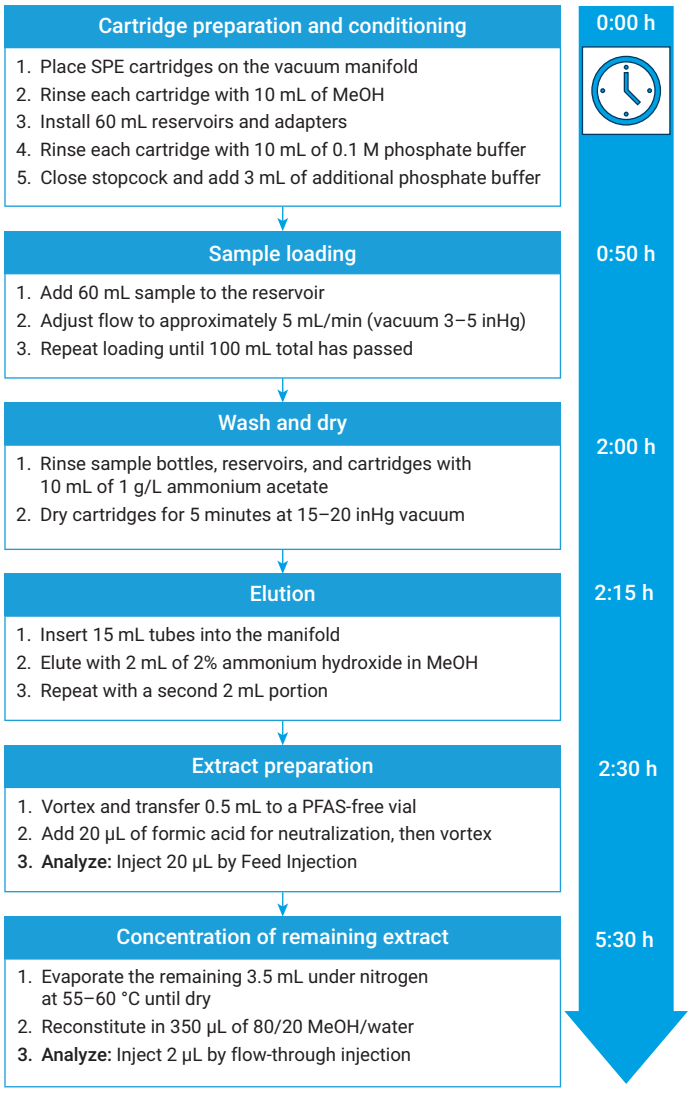

Figure 2. Sample preparation workflow and time consumption.

Samples and analytical sequence

The following samples were prepared: cartridge blank, ultrapure water, and tap water samples. The low spike of 0.5 ng/L is factor 3 below the required LOQ of the Drinking Water Directive and factor 10 below the parametric value. The high spike of 5 ng/L represents the parametric value. The sample types are summarized and described in Table 4.

Table 4. Sample types, their descriptions, and replicates measured in the worklist.

Sample Type	Description	Replicates		
			Ultrapure Water	Tap Water
Cartridge Blank	Each step of the protocol without loading of sample	2		
Double Blank	Enrichment of 100 mL without addition of internal standard (IS)		2	2
Blank	Enrichment of 100 mL with addition of IS		3	6
0.5 ng/L Spike	Enrichment of 100 mL spiked with 0.5 ng/L PFAS and IS		3	6
5 ng/L Spike	Enrichment of 100 mL with 5 ng/L PFAS and IS		3	6

Chemicals and reagents

The following chemicals and reagents were used:

- 0.1 M phosphate buffer pH 7 (500 mL of 0.1 M dibasic sodium phosphate buffer + 275 mL of 0.1 M monobasic sodium phosphate buffer)
- 1 g/L ammonium acetate
- 2% ammonium hydroxide in MeOH
- Solvent: 80/20 MeOH/water
- EU Drinking Water Testing PFAS mix (part number PFS-EU20-DWTM)
- Surrogate Spiking Mix (part number PFS-8327-SSM)
- Agilent InfinityLab acetonitrile for LC/MS (part number 5191-5101-001)
- Agilent InfinityLab water for LC/MS (part number 5191-5121-001)
- Agilent InfinityLab methanol for LC/MS (part number 5191-5111-001)

Consumables

The following consumables were used:

- 100 mL polypropylene or glass volumetric flasks
- Agilent Bond Elut PFAS WAX, 200 mg, 6 mL (part number 5610-2151)
- Agilent polypropylene vial, 2 mL, screw style, clear, optimized for use in PFAS-related applications (part number 5191-8123)
- Agilent caps, 9 mm, screw style, clear, polypropylene, with thin membrane polypropylene / silicone septa, optimized for use in PFAS applications (part number 5191-8151)
- Agilent centrifuge tubes and caps, 15 mL (part number 5610-2039)
- Agilent empty SPE cartridge, 60 mL, large volume reservoir (part number 12131012)
- Agilent adapter cap for 1, 3, and 6 mL Bond Elut cartridges (part number 12131001)
- Agilent Vac Elut 12 Manifold with collection rack (part number 5982-9110)

Results and discussion

For the measurement of the samples, a nine-point calibration was performed for the 2 µL flow-through injection and the 20 µL Feed Injection in the range from 20 to 20,000 ng/L and 2 to 2,000 ng/L, respectively. These correspond to sample concentrations of 0.08 to 80 ng/L (Table 5). The concentration factors of 250 and 25, with and without evaporation/reconstitution, respectively, can be found in the sample preparation scheme (Figure 2). The calibration curves obtained for all PFAS compounds showed R^2 values ≥ 0.998.

Table 5. Measured sample concentrations and corresponding standard concentrations used for flow-through injection with 2 µL injection volume and Feed Injection of 20 µL.

Sample		Corresponding Standard Concentration for 20 µL Injection		Corresponding Standard Concentration for 2 µL Injection	
Conc. (ng/L)	IS Conc. (ng/L)	Conc. (ng/L)	IS Conc. (ng/L)	Conc. (ng/L)	IS Conc. (ng/L)
0.08	4	2	100	20	1,000
0.20	4	5	100	50	1,000
0.4	4	10	100	100	1,000
0.8	4	20	100	200	1,000
2.0	4	50	100	500	1,000
4.0	4	100	100	1,000	1,000
8.0	4	200	100	2,000	1,000
40	4	1,000	100	10,000	1,000
80	4	2,000	100	20,000	1,000

Together with the calibration and the spiked samples, different types of blanks were analyzed in the same worklist. The cartridge blank comprised each step of the sample preparation protocol but without loading sample onto the cartridge. The double blank comprised 100 mL ultrapure water but without adding the internal standard. The blank comprised ultrapure water and internal standard.

The cartridge blank showed very low amounts of PFBS and PFHxA, as well as a low but still significant amount of PFBA (Figure 3). All other PFAS compounds showed no residues in the cartridge blank and the double blank. Amounts of identified PFAS residues in the blank samples were subtracted respectively for later data analysis.

The aim of this work was to compare a 2 µL flow-through injection versus a 20 µL Feed Injection to evaluate whether the evaporation and reconstitution step in WAX SPE enrichment can be avoided by injecting a larger volume of the SPE eluate. The resulting measured concentration for six replicates of tap water spiked at 0.5 and 5 ng/L are displayed in Figure 4 for all PFAS compounds. All compounds met the expected concentration, except for PFBA and PFBS. Figure 5 shows the average recoveries for the six replicates at both spike levels.

A more detailed evaluation shows that the 20 µL injection of the low-level spike provided slightly improved results. Reproducibility (area RSDs; Figure 6), and consequently variation in recovery, were better for PFBA, PFDS, and PFDoS. For all compounds, the 20 µL injection yielded recoveries within 80 to 120% and %RSD values for the calculated concentrations below 10%. Overall, no significant differences were observed between the 20 µL Feed Injection of the eluate and the 2 µL classic flow-through injection of the concentrated eluate.

In the drinking water samples, several PFAS compounds were detected at low concentrations, typically below 0.2 ng/L, except for PFBA and PFBS, which were found at higher levels between 0.8 and 1.0 ng/L. Figure 7 compares the two methods: a classic 2 µL flow-through injection including evaporation/reconstitution, and a 20 µL Feed Injection without this step. No significant differences in the measured PFAS concentrations were observed between the two approaches.

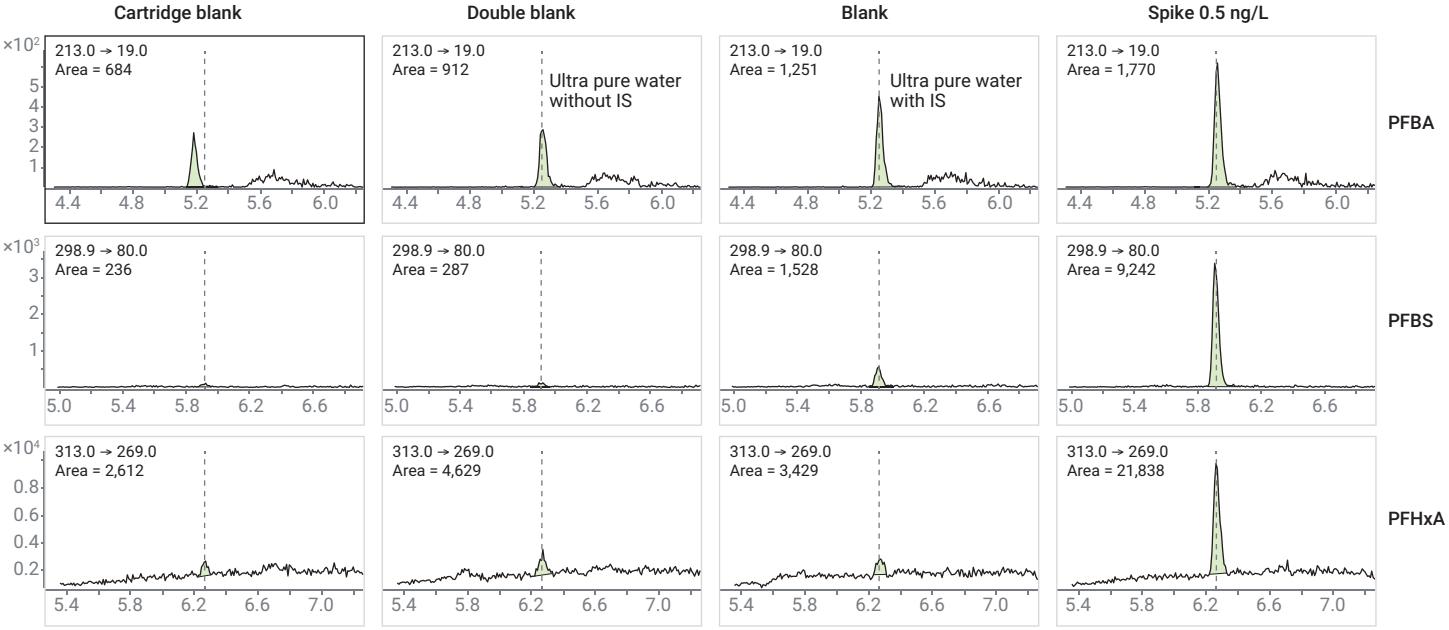

Figure 3. Different blanks analyzed within the worklist together with the calibration and the spiked samples.

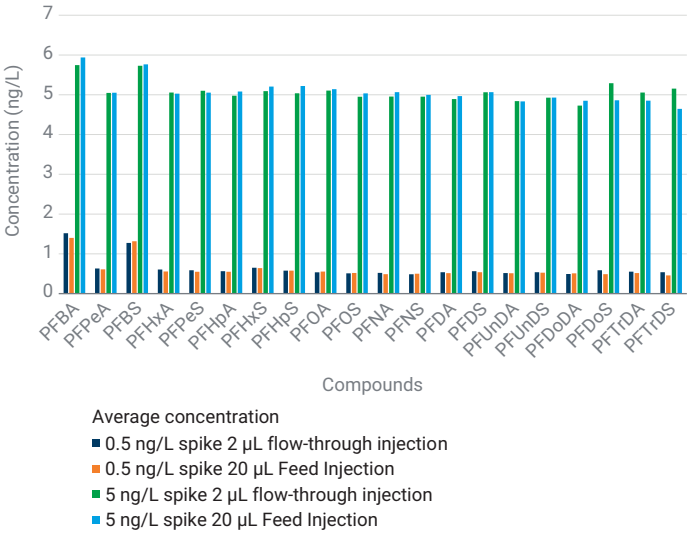

Figure 4. Results of average measured concentration of all PFAS compounds for a 0.5 and 5 ng/L tap water spike of six replicates.

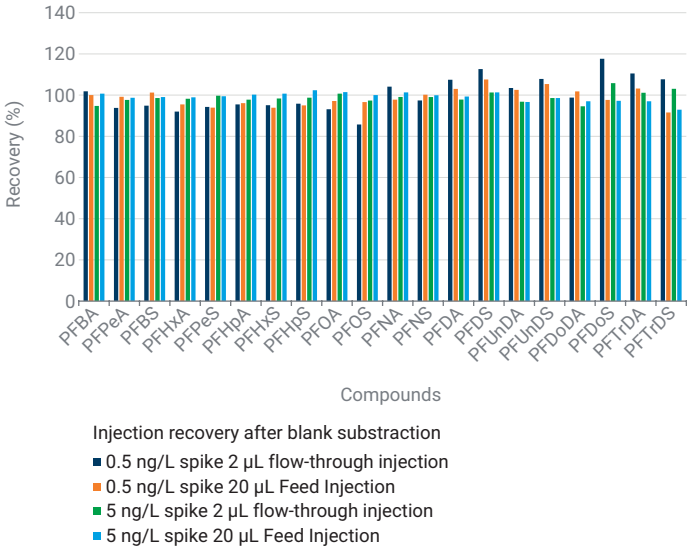

Figure 5. Average recovery percentage of all PFAS compounds for a 0.5 and 5 ng/L tap water spike of six replicates after blank subtraction.

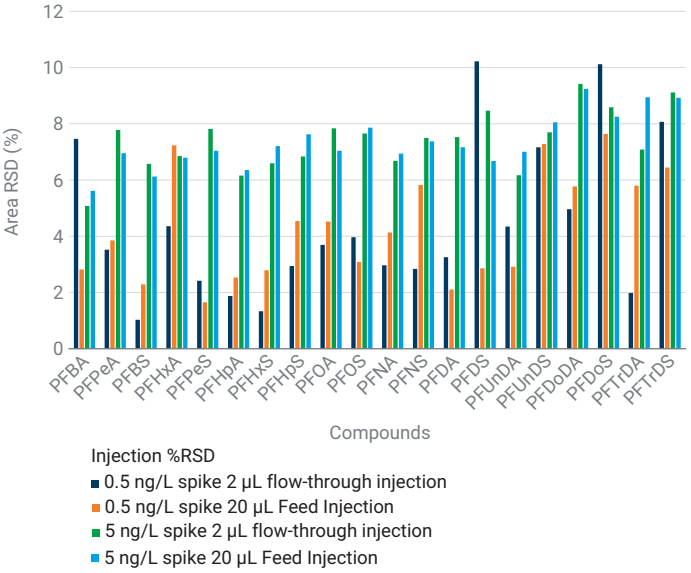

Figure 6. Average area RSD of all PFAS compounds for a 0.5 and 5 ng/L tap water spike of six replicates.

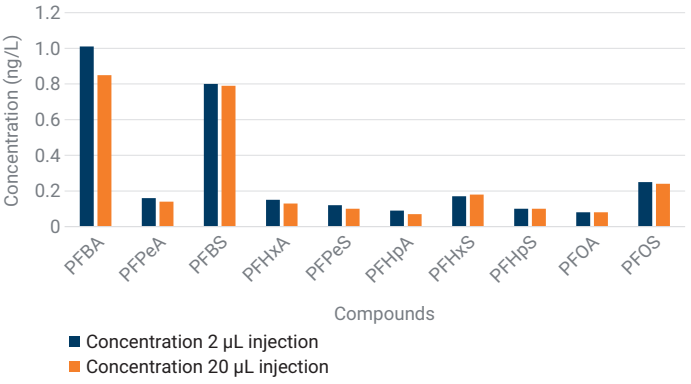

Figure 7. Comparison of analysis results of drinking water for quantified PFAS compounds achieved with sample preparation with evaporation/reconstitution and 2 µL injection by classic flow-through injection and sample preparation without evaporation/reconstitution and injection of 20 µL by means of Feed Injection.

Although both methods performed equally, the high-volume Feed Injection presents the following advantages:

- The sample preparation time can be reduced by 65%.
- The risk of contamination or analyte loss in the concentration step is eliminated.
- Sample preparation can be further simplified. Currently, the procedure is limited by the need for a reconstitution step. If this step is avoided, enrichment of a 25 mL sample with two elution steps of 0.5 mL MeOH (resulting in a final volume of 1 mL) should be possible. This results in a more environmentally friendly method.

A comparison of the time required for each step in both methods shows that a significant amount of time can be saved by eliminating the evaporation/reconstruction step through the use of Feed Injection (Figure 8). In addition, this approach contributes to a greener analytical workflow, which was recently discussed in a publication assessing the environmental impact of standard methods.[5]

Conclusion

This application note demonstrates the benefit of the Agilent 1290 Infinity III Hybrid Multisampler for the analysis of PFAS in drinking water after SPE enrichment. By means of Agilent Feed Injection technology, high volumes of the organic eluent directly from enrichment cartridge elution can be injected. This saves significant time in the sample preparation process by avoiding an evaporation/reconstitution step and reduces errors in sample preparation. The results obtained were compared to the classic approach comprising the evaporation/reconstitution process and a low-volume flow-through injection. Overall, all the results obtained were identical for both approaches.

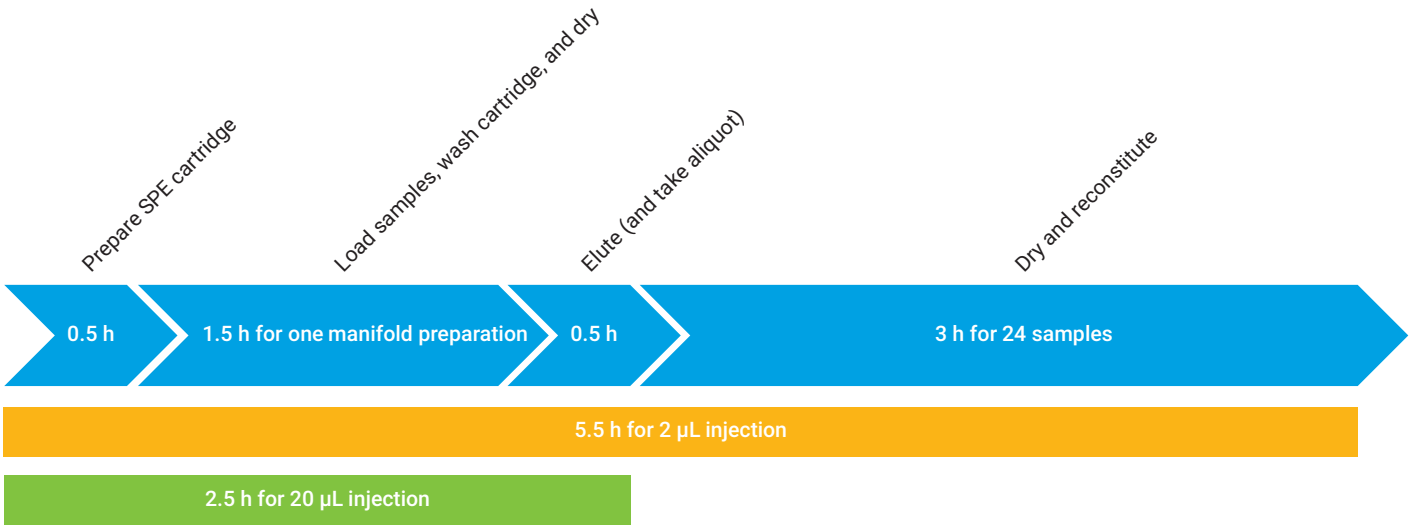

Figure 8. Time needed for sample preparation, with and without the evaporation/reconstitution step.

References

1. Zarębska, M.; Bajkacz, S. Poly- and Perfluoroalkyl Substances (PFAS) - Recent Advances in the Aquatic Environment Analysis. *Trends Anal. Chem.* **2023**, DOI: https://doi.org/10.1016/j.trac.2023.117062
2. *Per- and Polyfluoroalkyl Substances (PFAS): Final PFAS National Primary Drinking Water Regulation,* U.S. Environmental Protection Agency (EPA). https://www.epa.gov/sdwa/and-polyfluoroalkyl-substances-pfas (accessed 2026-05-28)
3. Kamuf, M.; Wälz, M.; Borowiak, A. Reduce PFAS Background with the Agilent PFC-Free HPLC Conversion Kit. *Agilent Technologies technical overview*, publication number 5994-2291EN, **2024**.
4. Giardina, M. Determination of Per and Polyfluoroalkyl Substances in Drinking Water Using Agilent Bond Elut PFAS WAX SPE and LC/MS/MS, *Agilent Technologies application note*, publication number 5994-4960EN, **2025**
5. Bende, J. L.; *et al.* Greenness assessment of 174 CEN, ISO, and pharmacopeia standard methods and their sub-methods used for environmental, food, trace elemental and pharmaceutical analysis, *Advances in Sample Preparation 14* (**2025**) 100180.

Appendix

Table A1. MRM conditions for PFAS compounds.

Compound	Precursor *m/z*	MS 1 Resolution	Product *m/z*	MS 2 Resolution	CE	CAV	Ion Funnel Mode	Polarity
PFBA	213	Unit	169	Unit	7	2	Fragile	Negative
PFBA	213	Unit	19	Unit	20	2	Fragile	Negative
PFBA 13C3	216	Unit	172	Unit	7	2	Fragile	Negative
PFBS	298.9	Unit	99	Unit	36	2	Standard	Negative
PFBS	298.9	Unit	80	Unit	43	2	Standard	Negative
PFBS 13C4	303	Unit	80	Unit	43	2	Standard	Negative
PFDA	513	Unit	469	Unit	8	2	Standard	Negative
PFDA	513	Unit	269	Unit	16	2	Standard	Negative
PFDA	513	Unit	219	Unit	16	2	Standard	Negative
PFDA 13C9	522	Unit	477	Unit	8	2	Standard	Negative
PFDoDA	613	Unit	569	Unit	11	4	Fragile	Negative
PFDoDA	613	Unit	319	Unit	20	4	Fragile	Negative
PFDoDA	613	Unit	269	Unit	20	4	Fragile	Negative
PFDoDA	613	Unit	169	Unit	28	4	Fragile	Negative
PFDoDA 13C12	625	Unit	580	Unit	11	4	Fragile	Negative
PFDoS	698.9	Unit	99	Unit	63	4	Standard	Negative
PFDoS	698.9	Unit	80	Unit	67	4	Standard	Negative
PFDS	598.9	Unit	99	Unit	55	4	Standard	Negative
PFDS	598.9	Unit	80	Unit	63	4	Standard	Negative
PFHpA	363	Unit	319	Unit	7	2	Standard	Negative
PFHpA	363	Unit	169	Unit	19	2	Standard	Negative
PFHpA 13C7	370	Unit	325	Unit	7	2	Standard	Negative
PFHpS	448.9	Unit	99	Unit	44	3	Standard	Negative
PFHpS	448.9	Unit	80	Unit	48	3	Standard	Negative
PFHxA	313	Unit	269	Unit	7	2	Fragile	Negative
PFHxA	313	Unit	119	Unit	23	2	Fragile	Negative
PFHxA 13C6	319	Unit	274	Unit	7	2	Standard	Negative

Compound	Precursor *m/z*	MS 1 Resolution	Product *m/z*	MS 2 Resolution	CE	CAV	Ion Funnel Mode	Polarity
PFHxS	398.9	Unit	99	Unit	43	3	Standard	Negative
PFHxS	398.9	Unit	80	Unit	48	3	Standard	Negative
PFHxS 13C6	405	Unit	80	Unit	48	3	Standard	Negative
PFNA	463	Unit	419	Unit	8	2	Standard	Negative
PFNA	463	Unit	219	Unit	16	2	Standard	Negative
PFNA	463	Unit	169	Unit	20	2	Standard	Negative
PFNA 13C9	472	Unit	427	Unit	8	2	Standard	Negative
PFNA 13C9	472	Unit	223	Unit	16	2	Standard	Negative
PFNS	548.9	Unit	99	Unit	51	4	Standard	Negative
PFNS	548.9	Unit	80	Unit	59	4	Standard	Negative
PFOA	413	Unit	369	Unit	7	2	Standard	Negative
PFOA	413	Unit	219	Unit	15	2	Standard	Negative
PFOA	413	Unit	169	Unit	19	2	Standard	Negative
PFOA 13C8	421	Unit	376	Unit	7	2	Standard	Negative
PFOA 13C8	421	Unit	172	Unit	19	2	Standard	Negative
PFOS	498.9	Unit	99	Unit	48	4	Standard	Negative
PFOS	498.9	Unit	80	Unit	52	4	Standard	Negative
PFOS 13C8	507	Unit	99	Unit	48	4	Standard	Negative
PFOS 13C8	507	Unit	80	Unit	52	4	Standard	Negative
PFPeA	263	Unit	219	Unit	7	2	Fragile	Negative
PFPeA	263	Unit	19	Unit	18	2	Fragile	Negative
PFPeA 13C5	268	Unit	223	Unit	7	2	Fragile	Negative
PFPeS	348.9	Unit	99	Unit	36	2	Standard	Negative
PFPeS	348.9	Unit	80	Unit	40	2	Standard	Negative
PFTrDA	663	Unit	619	Unit	11	4	Standard	Negative
PFTrDA	663	Unit	319	Unit	20	4	Standard	Negative
PFTrDA	663	Unit	169	Unit	32	4	Standard	Negative
PFTrDS	748.9	Unit	98.9	Unit	66	4	Standard	Negative
PFTrDS	748.9	Unit	80	Unit	70	4	Standard	Negative
PFUnDA	563	Unit	519	Unit	11	2	Fragile	Negative
PFUnDA	563	Unit	319	Unit	16	2	Fragile	Negative
PFUnDA	563	Unit	269	Unit	19	2	Fragile	Negative
PFUnDA 13C9	572	Unit	528	Unit	11	2	Fragile	Negative
PFUnDS	648.9	Unit	99	Unit	58	4	Standard	Negative
PFUnDS	648.9	Unit	80	Unit	62	4	Standard	Negative

www.agilent.com

DE-014893

Printed in the USA, July 16, 2026
5994-9250EN

Agilent
Trusted Answers