

Benefits of Using the PFAS-Optimized Agilent 1290 Infinity III Hybrid Multisampler

Including the Agilent 1290 Infinity III High-Speed Pump



Author

Lisa Engelbart
Agilent Technologies, Inc.

Abstract

This technical overview demonstrates the benefits of using the PFAS-optimized Agilent 1290 Infinity III High-Speed Pump and Agilent 1290 Infinity III Hybrid Multisampler for the analysis of per- and polyfluorinated alkyl substances (PFAS). Reduction of PFAS-containing parts in the flow path of both pump and sampler reduces PFAS-background, therefore enhancing sensitivity. Furthermore, by using Agilent Feed Injection mode of the Agilent 1290 Infinity III Hybrid Multisampler, large volume injections of high organic samples, e.g. methanolic PFAS-extracts, are possible without loss in separation performance by omitting solvent effects, which also increases sensitivity. The combination of improved peak shapes and high sensitivity enables faster and more reliable data evaluation, which is essential in view of the increasing regulatory requirements for PFAS analysis.

Introduction

Analysis of PFAS compounds is growing in importance, as PFAS have been widely used in industry and manufacturing due to their unique chemical properties which make them heat-resistant, water-repellent, and long-living. They can be found in products such as nonstick cookware, stain-repellent clothes, and in detergents, cleaning products, and firefighting foams. As more insight is gained regarding their problematic moieties such as bioaccumulation and persistence in the environment, new regulations regarding limit concentrations of different PFAS groups are widely being adopted. For this reason, sensitive, reliable, and fast PFAS analysis is needed for a vast variety of sample matrices such as environmental water samples, soil, drinking water, clothing, food, and food contact material. The Agilent 1290 Infinity III High-Speed Pump and Agilent 1290 Infinity III Hybrid Multisampler PFAS optimized modules offer a great solution for sensitive and reliable PFAS analysis.

Experimental

Instrumentation

- Agilent 1290 Infinity III High-Speed Pump (G7120A) option #047, solvent lines connected directly to degasser, SSV bypassed
- Agilent 1290 Infinity III Hybrid Multisampler (G7137B)
- Agilent 1290 Infinity III MCT (G7116B) with Heat Exchanger (G7116-60015)
- Agilent 6495D triple quadrupole LC/MS (G6495D) with Agilent Jet Stream source

Columns

- Agilent InfinityLab PFC delay column, 4.6 × 30 mm (part number 5062-8100)
- Agilent InfinityLab Poroshell 120 Aq-C18 column, 2.1 × 5 mm, 2.7 µm, UHPLC Guard Column (part number 821725-955)
- Agilent InfinityLab Poroshell 120 Aq-C18 column, 2.1 × 100 mm, 2.7 µm (part number 695775-742)

Software

- Agilent MassHunter Workstation software, version 12.3 (or later versions)
- Agilent MassHunter Qualitative Analysis software, version 13.0 (or later versions)
- Agilent MassHunter Quantitative Analysis software, version 12.0 (or later versions)

Samples

46 PFAS analytes (PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFTeDA, PFHxDA, PFODA, PFBS, PFPeS, PFHxS, PFHpS, PFOS, PFNS, PFDS, PFDoS, FBSA, FHxSA, FOSA, HFPO-DA, MeFOSAA, EtFOSAA, 4:2FTS, 6:2FTS, 8:2FTS, ADONA, 9CI-PF3ONS, 11CI-PF3OUnS, PFMPA, PFMBA, NFDHA, PFEESA, MeFOSA, EtFOSA, MeFOSE, EtFOSE, 3:3FTCA, 5:3FTCA, 7:3FTCA, PFecHxS, and 8:2diPAP) dissolved in 96% MeOH:4% water

Note: A 2 mL polypropylene vial, screw style, clear, optimized for use in PFAS-related applications (part number 5191-8121) and 9 mm caps, screw style, clear, polypropylene, with thin membrane polypropylene/silicone septa, optimized for use in PFAS applications (part number 5191-8151) were used throughout the experiments to reduce analyte sorption on glass surfaces and reduce PFAS-background from vials.

Chemicals

- Agilent InfinityLab Methanol for LC/MS (part number 5191-5111)*
- Agilent InfinityLab Acetonitrile for LC/MS (part number 5191-5101)*
- Agilent InfinityLab Water for LC/MS (part number 5191-5121)*
- Supelco Isopropanol LC-MS Grade
- Ammonium acetate was purchased from VWR, Germany
- PFAS standards were purchased from Wellington Laboratories, Canada

* Only available in selected countries

Chromatographic conditions and MS settings

LC/MS/MS Method			
Mobile Phase	A) Water with 5 mM ammonium acetate B) Methanol		
Flow Rate	0.4 mL/min		
Gradient	Time (min)	B%	
	0.0 to 1.0	0	
	1.0 to 2.0	0 to 50	
	2.0 to 6.0	50 to 70	
	6.0 to 7.5	70 to 80	
	7.5 to 12.5	80 to 100	
	12.5 to 14.9	100	
	14.9 to 15.0	100 to 0	
	Run time: 17.5 min Post time: Off		
Flow-Through Injection	Draw speed: 100 µL/min Injection volumes (µL): 5, 7.5, 10, 20, 30, 40		
	S2: Mobile Phase A S3: Mobile Phase B		
	Task	Solvent	Wash volume/time
	Inner wash	S2	150 µL
	Reconditioning	S2	
	Outer wash	S3	5 s
Agilent Feed Injection	Feed speed: 10% of flow (adaptive) Injection volumes (µL): 5, 7.5, 10, 20, 30, 40 Automatic overfeed volume		
	S1: Acetonitrile/Isopropanol (1:1) S2: Mobile Phase A S3: Mobile Phase B		
	Task	Solvent	Wash volume/time
	Inner wash	S1	150 µL
	Inner wash	S3	150 µL
	Seat wash	S1	150 µL
	Seat wash	S3	150 µL
	Reconditioning	S2	
	Outer wash	S1	10 s
	Outer wash	S3	10 s
Column Temperature	55 °C		
MS Settings			
Drying Gas	250 °C/ 11 L/min		
Sheath Gas	375 °C/ 11 L/min		
Nebulizer Pressure	25 psi		
Capillary Voltage	3,000 V		
Nozzle Voltage	0 V		
Polarity	Negative		
iFunnel mode	Standard		

Results and discussion

Flow-through injection

Commonly, organic solvents such as methanol and acetonitrile are used to extract PFAS from e.g. environmental samples resulting in high organic samples for LC analysis.¹ Injection of large volumes of high organic samples leads to loss in chromatographic performance due to solvent effects and breakthrough by means of classical flow-through injection. This limits injection volume, and for this reason, often makes enrichment steps such as SPE necessary.

Injection of 5 μL of a PFAS standard solution dissolved in 96% MeOH resulted in chromatograms with a good peak shape for the analytes considered (Figure 2) and acceptable area precisions (Table 1). Injection volume increase led to minor increase in peak height for injection volumes above 5 μL for most analytes, while the observed peak shapes were severely affected by solvent effects for larger injection volumes. In Figure 3, strong fronting and peak shoulders can be observed for an injection volume of 40 μL , preventing reliable quantification of analytes. Due to these disoriented peak shapes, no automated integration was possible, and all analytes needed manual integration, which is error prone and time consuming. Furthermore, due to solvent effects, no clear separation or quantification of branched PFAS isomers such as N-EtFOSAA was possible.

Agilent Feed Injection

The 1290 Infinity III Hybrid Multisampler can be operated not only in classical flow-through injection mode but also in Feed Injection mode. When using Feed Injection mode, the sample is infused into the mobile phase stream during injection (Figure 1).⁴ When using the InfinityLab Poroshell 120 Aq-C18 column, starting the gradient with 100% aqueous eluent was possible.

A slow feed speed is recommended to omit solvent effects.^{3,4} For a feed speed of 10%, a 1:10 dilution of the sample with the 100% aqueous starting conditions during injection is achieved.

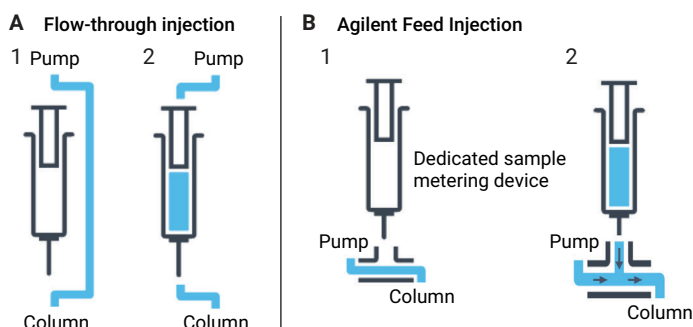


Figure 1. Classical flow-through injection (A) and Agilent Feed Injection (B) functional principles of the Agilent 1290 Infinity III Hybrid Multisampler.

This function enables omitting the solvent effects when injecting high organic samples and leads to analyte trapping and focusing when entering the column even for higher injection volumes such as 40 μL (see Figure 4). This results in a strong increase in sensitivity compared to classical flow-through injection which allows lower detection limits. A good peak area RSD was achieved and peak height was greatly increased compared to classical flow through injection (see Table 1).

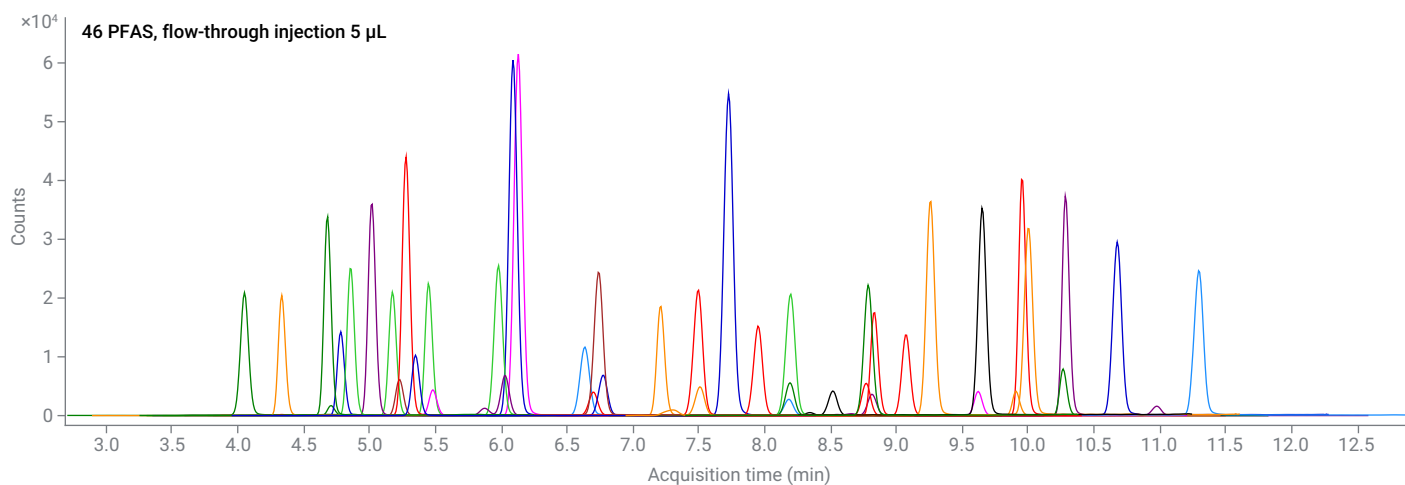


Figure 2. Chromatogram of an injection by means of flow-through injection (5 μ L) of a mixture of 46 PFAS at concentrations between 732 and 10,000 fg/ μ L. Quantifier transitions are shown.

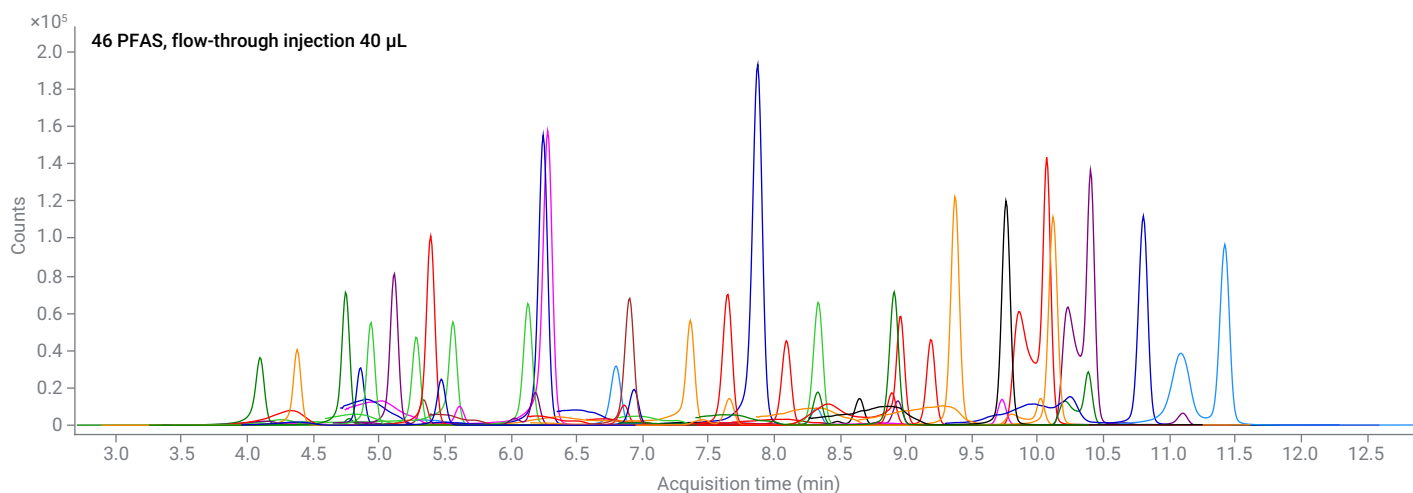


Figure 3. Chromatogram of an injection by means of flow-through Injection (40 μ L) of a mixture of 46 PFAS at concentrations between 732 and 10,000 fg/ μ L. Quantifier transitions are shown.

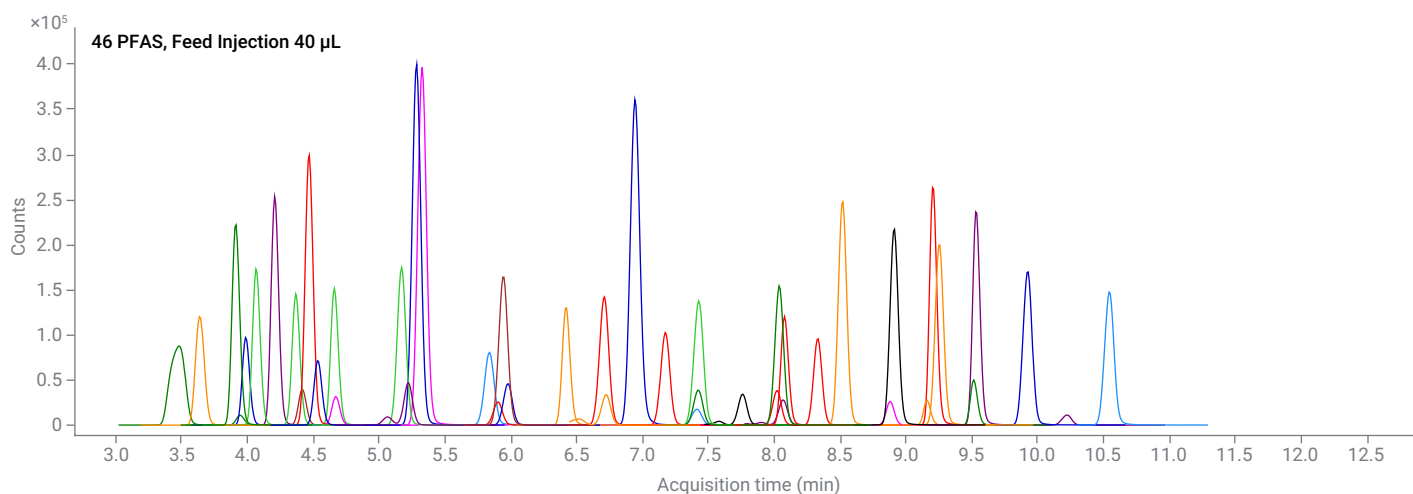


Figure 4. Chromatograms of a mixture of 46 PFAS at concentrations between 732 and 10,000 fg/ μ L. Quantifier transitions are shown. Injection by means of Agilent Feed Injection (40 μ L).

Detailed comparison of selected analytes

For comparison, four PFAS analytes were selected: The first eluting compound in the mixture used (PFBA), two compounds with branched isomers (PFHxS and EtFOSAA), and the last eluting compound (PFDoDA). A strong breakthrough was observed for the most polar (PFBA, see Figure 6) and least polar (PFDoDA, see Figure 8) compound with flow-through injection mode when more than 10 μL sample were injected. Using Feed Injection, good peak shape was achieved for both compounds even at large injection volumes, enabling automated, more reliable, and faster quantification.

Some PFAS compounds have branched isomers. As they have the same m/z ratio, separation is necessary for quantification of isomeric compounds. This is needed as they can have different problematic moieties such as bioaccumulation.² Due to analyte breakthrough and peak broadening, no clear separation of branched isomers was possible with high injection volumes, and quantification with lower injection volumes is difficult due to much lower concentrations. Using Feed Injection, good separation of branched isomers was achieved at high injection volumes, demonstrated in Figures 5 and 7.

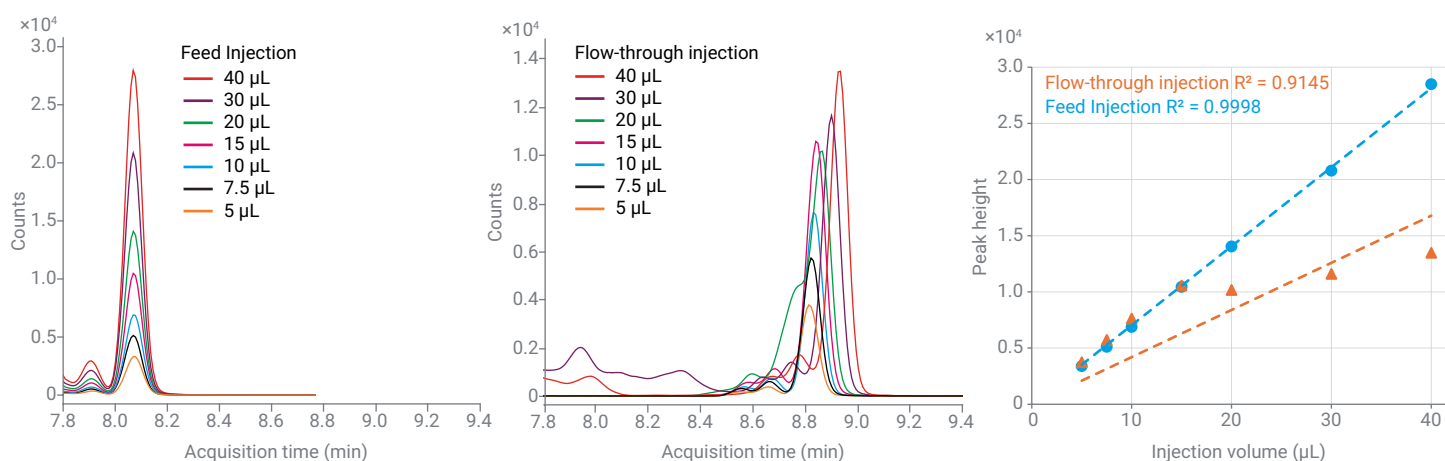


Figure 5. N-EtFOSAA (N-ethylperfluorooctanesulfonamidoacetic acid, comparison of flow-through injection (orange) and Feed Injection (blue)).

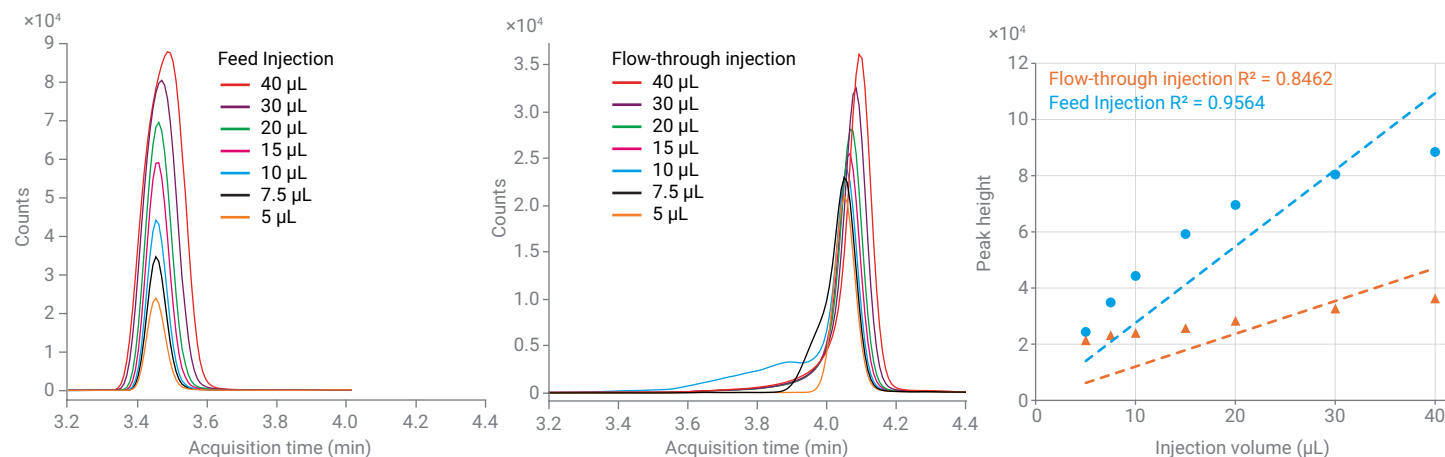


Figure 6. PFBA (comparison of flow-through injection (orange) and Feed Injection (blue)).

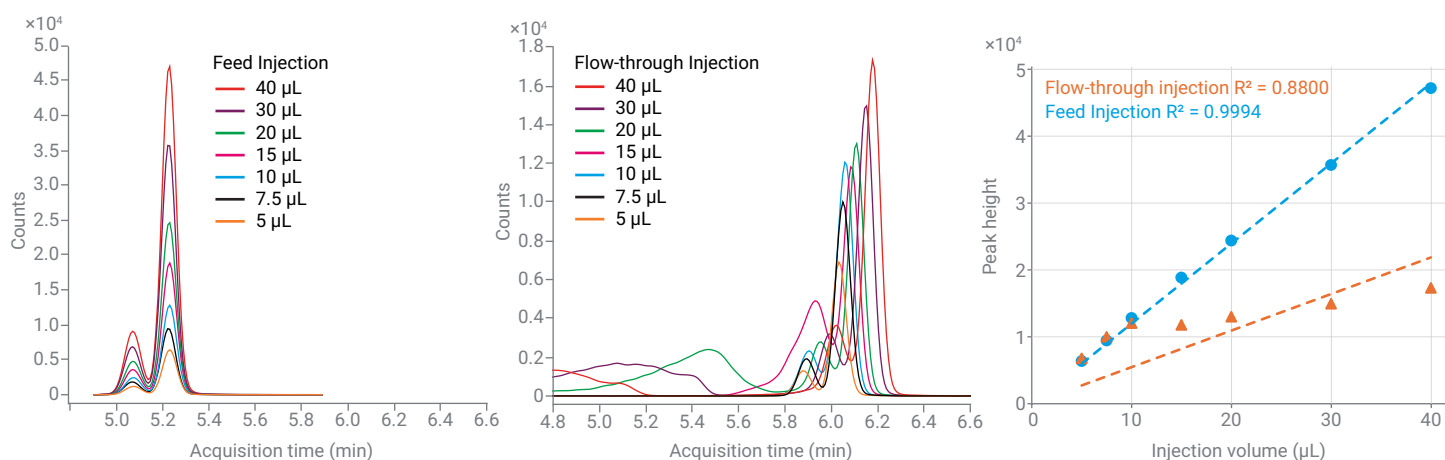


Figure 7. PFHxS (perfluorohexanesulfonic acid, comparison of flow-through injection (orange) and Feed Injection (blue)).

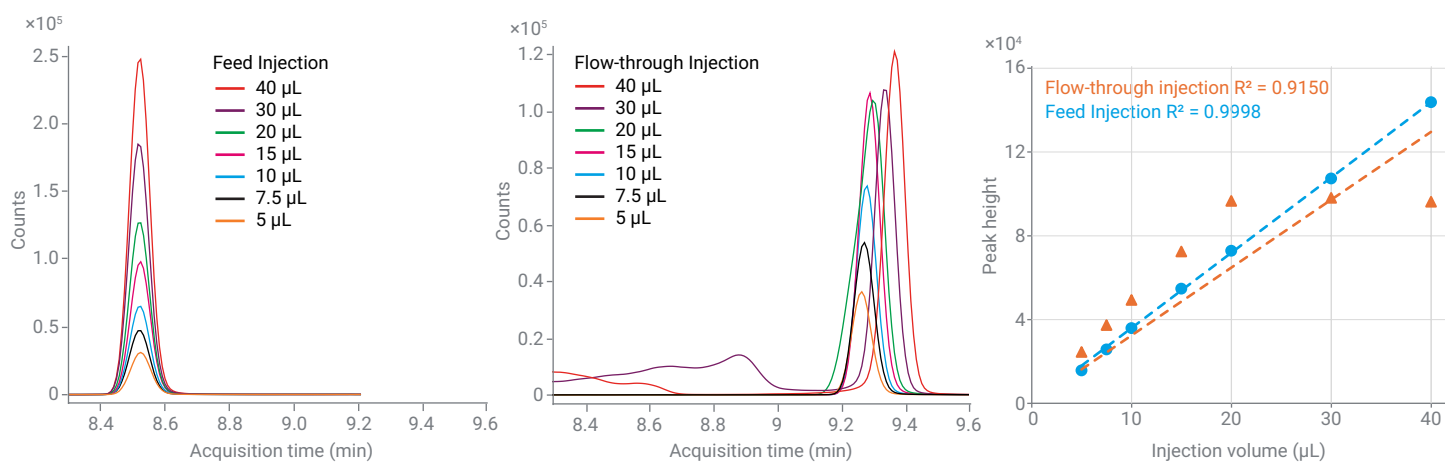


Figure 8. PFODA (perfluorooctadecanoic acid, comparison of flow-through injection (orange) and Feed Injection (blue)).

A linear increase of peak height in correlation to the injected volume on column (Table 1) in comparison to flow through injection was possible with Feed Injection. This is further demonstrated by the linear regressions visible in Figures 5 through 8 for the example compounds. The correlation for the earliest eluting compound, PFBA, is not as good as for later-eluting compounds, which may be explained by weaker trapping and focusing effects during the longer injection time. However, the performance is still clearly better when compared to flow-through injection (see Figure 6).

PFAS background and carryover

The system performs well in terms of carryover. Even at a high injection volume and analyte concentration, no signal was noticeable for most analytes when measuring a solvent blank after sample injection, as shown for DONA in Figure 9. For a few strong sorbing analytes, low carryover was observed, but disappeared after the second solvent blank. The multiwash function of the 1290 Infinity III Hybrid Multisampler ensures cleaning of the injection pathway with up to three solvents and adjustable time/volume settings for each washing step. This enables adaption on the chemical properties of the investigated PFAS group or when analyzing a broad polarity range cleaning with e.g. both water and isopropanol/acetonitrile mixture.

Reduction of PFAS-leaching components in the flow path of the 1290 Infinity III High-Speed Pump and 1290 Infinity III Hybrid Multisampler ensures a low PFAS background. This enables good peak shape and signal even at low concentrations in combination with e.g. the Agilent 6495D triple quadrupole in this case. A concentration of 12.8 fg PFNA and PFHpA on column is clearly visible with good peak shape in comparison to the baseline visible for a solvent blank as demonstrated in the chromatograms in Figure 10.

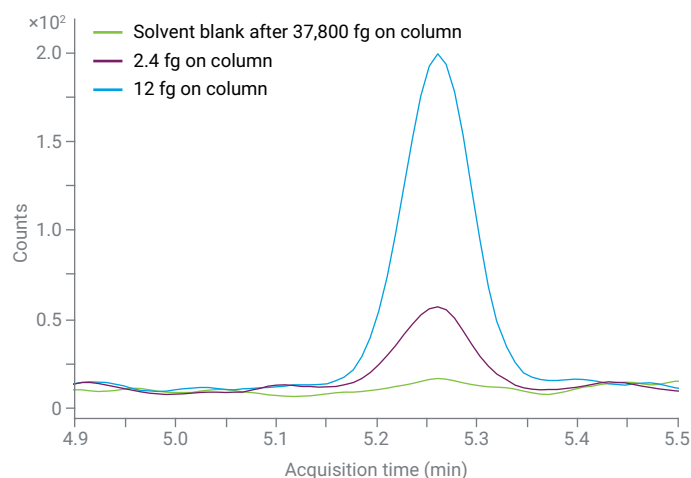


Figure 9. Chromatogram of DONA: Solvent blank after injection of the highest calibration point in comparison to the lowest calibration points 1 and 2 (2.4 and 12 fg on column).

Table 1. Summary of results obtained from typical PFAS compounds.

Analyte Retention Time (min)	Linearity R ² Peak Height Versus Injection Volume, (Forced Through Zero)		Linearity R ² Peak Area Versus Injection Volume (Forced Through Zero)		Peak Area %RSD		Peak Height Increase Factor	
	Feed Injection	Flow-Through Injection	Feed Injection	Flow-Through Injection	Feed Injection (40 µL, n = 6)	Flow-Through Injection (40/5 µL, n = 6)	Feed Injection Versus Flow-Through Injection (40 µL)	Feed Injection Versus Flow-Through Injection (5 µL)
PFBA (3.77)	0.956	0.846	1.000	0.873	0.44	1.60/1.73	2.4	4.3
PFMPA (4.00)	0.993	0.867	1.000	0.867	0.40	2.56/1.43	3.0	5.9
PFPeA (4.31)	0.999	0.877	1.000	0.842	0.40	2.25/0.86	3.1	6.6
3-3 FTCA (4.34)	0.997	0.872	1.000	0.836	0.48	1.33/1.43	3.1	6.5
PFBS (4.40)	0.999	0.884	1.000	0.835	0.59	2.15/1.02	3.1	6.8
PFMBA (4.48)	0.999	0.882	1.000	0.831	0.60	2.49/0.86	3.1	6.9
PFEESA (4.64)	1.000	0.892	1.000	0.872	0.71	3.18/0.52	3.1	7.0
NFDHA (4.80)	1.000	0.891	1.000	0.891	0.41	3.77/1.14	3.1	7.0
4-2 FTSA (4.85)	0.997	0.881	0.998	0.893	1.18	6.62/0.79	2.8	6.2
PFHxA (4.90)	1.000	0.889	1.000	0.890	0.38	3.93/1.04	3.0	6.9
All Others (4.98–10.95)	0.995–1.000	0.896–0.948	0.996–1.000	0.858–1.000	0.16–5.74	0.85–17.45/ 0.35–7.76	0.6–7.8	1.9–24.3

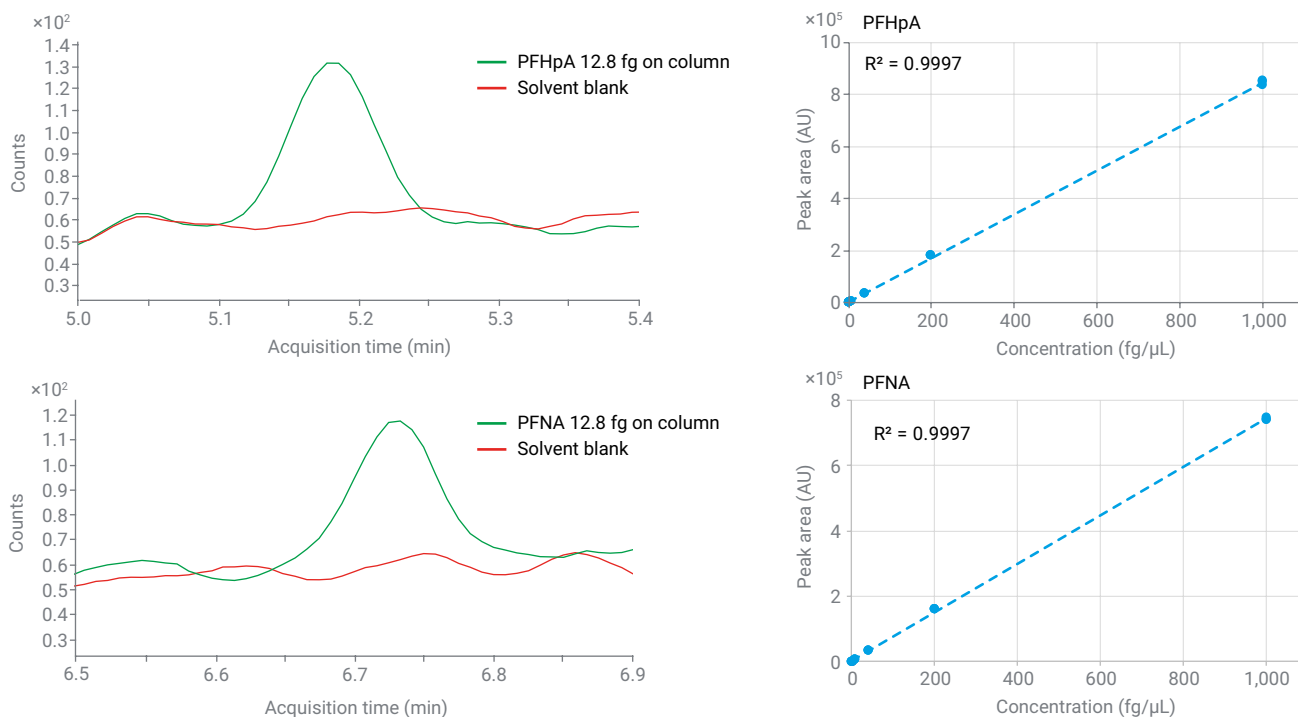


Figure 10. Calibration curves of PFHpA and PFNA using Feed Injection with an injection volume of 40 μL . Chromatograms of calibration level 2 (0.32 $\text{fg}/\mu\text{L}$ equals 12.8 fg on column) of both compounds in comparison to a solvent blank. Calibration curves for each analyte were generated based on at least five calibration points ($n = 3$ each point) with an injection volume of 40 μL using Feed Injection.

Conclusion

As more new regulations and laws regarding PFAS in multiple matrices are passed, fast and reliable PFAS quantification is necessary. The combination of the Agilent 1290 Infinity III High-Speed Pump with the Agilent 1290 Infinity III Hybrid Multisampler is an ideal solution as it is clear that the system shows improved performances compared to non-PFAS-optimized instruments.^{4,5} This is enabled by the great sensitivity achieved by reduced PFAS background, as well as by increasing the injection volume. Automated peak integration enabled by maintaining great peak shapes makes quantification faster and more reliable, which is necessary considering the increasing regulatory requirements for PFAS analysis.

References

1. Iannone, A.; Carriera, F.; Di Fiore, C.; Avino, P. Poly- and Perfluoroalkyl Substance (PFAS) Analysis in Environmental Matrices: an Overview of the Extraction and Chromatographic Detection Methods. *Analytica* **2024**, *5*, 187–202.
2. Ahmed, E.; Kabir, K. M. M.; Wang, H.; Xiao, D.; Fletcher, J.; Donald, W. A. Rapid Separation of Isomeric Perfluoroalkyl Substances by High-Resolution Differential Ion Mobility Mass Spectrometry. *Anal. Chim. Acta* **2019**, *1058*, 127–135.
3. Performance Characteristics of the Agilent 1260 Infinity II Hybrid Multisampler. *Agilent Technologies technical overview*, publication number 5994-5952EN, **2023**.
4. More Sensitive Quantification of PFAS by LC/MS with the Agilent 1260 Infinity II Hybrid Multisampler. *Agilent Technologies application note*, publication number 5994-6994EN, **2024**.
5. Reduce PFAS Background with the Agilent PFAS Analysis HPLC Conversion Kit. *Agilent Technologies application note*, publication number 5994-2291EN, **2026**.

Appendix

dMRM conditions

Analyte	Transition	CAV (V)	CE (V)
3-3 FTCA	241 → 117	2	40
	241 → 63	2	4
4-2 FTSA	327 → 307	2	19
	327 → 81	2	39
4-PFecHS	460.9 → 381	2	28
	460.9 → 99	2	32
5-3 FTCA	341 → 237	3	12
	341 → 217	3	28
6-2 FTSA	427 → 407	2	27
	427 → 81	2	40
7-3 FTCA	441 → 337	4	11
	441 → 317	4	23
8-2 diPAP	989 → 543	3	27
	989 → 97	3	43
8-2 FTSA	527 → 507	2	31
	527 → 81	2	43
9Cl-PF3ONS	530.9 → 350.9	3	27
	530.9 → 83	3	27
11Cl-PF3OUdS	630.9 → 450.9	3	35
	630.9 → 83	3	35
DONA	377 → 251	5	11
	377 → 85	5	35
EtFOSE	630 → 59	5	44
FBSA	298 → 78	3	24
	298 → 48	3	80
FHxSA	397.9 → 78	3	32
	397.9 → 48	3	80
HFPO-DA	285 → 185	5	19
	285 → 169	5	3
MeFOSE	616 → 59	5	11

Analyte	Transition	CAV (V)	CE (V)
N-EtFOSA	526 → 219	4	28
	526 → 169	4	32
N-EtFOSAA	584 → 526	2	20
	584 → 419	2	20
NFDHA	201 → 135	3	8
	201 → 85	3	12
N-MeFOSA	512 → 219	4	28
	512 → 169	4	28
N-MeFOSAA	570 → 482.9	2	12
	570 → 419	2	20
PFBA	213 → 169	2	7
PFBS	298.9 → 99	2	36
	298.9 → 80	2	43
PFDA	513 → 469	2	8
	513 → 219	2	16
PFDODA	613 → 569	4	11
	613 → 319	4	20
PFDoS	698.9 → 99	4	63
	698.9 → 80	4	67
PFDS	598.9 → 99	4	55
	598.9 → 80	4	63
PFEESA	314.9 → 135	5	23
	314.9 → 69	5	63
PFHpA	363 → 319	2	7
	363 → 169	2	19
PFHpS	448.9 → 99	3	44
	448.9 → 80	3	48
PFHxA	313 → 269	2	7
	313 → 119	2	23

Analyte	Transition	CAV (V)	CE (V)
PFHxDA	812.9 → 769	4	12
	812.9 → 219	4	28
PFHxS	398.9 → 99	3	43
	398.9 → 80	3	48
PFMBA	279 → 235	3	3
	279 → 85	3	11
PFMPA	229 → 185	3	3
	229 → 85	3	11
PFNA	463 → 419	2	8
	463 → 219	2	16
PFNS	548.9 → 99	4	51
	548.9 → 80	4	59
PFOA	413 → 369	2	7
	413 → 169	2	19
PFODA	912.9 → 868.9	4	16
	912.9 → 169	4	32
PFOS	498.9 → 99	4	48
	498.9 → 80	4	52
PFOSA	497.9 → 78	3	36
	497.9 → 48	3	80
PFPeA	263 → 219	2	7
PFPeS	348.9 → 99	2	36
	348.9 → 80	2	40
PFTDA	712.9 → 669	4	12
	712.9 → 169	4	36
PFTTrDA	663 → 619	4	11
	663 → 169	4	32
PFUnDA	563 → 519	2	11
	563 → 269	2	19

www.agilent.com

DE-014493

This information is subject to change without notice.

© Agilent Technologies, Inc. 2026
Printed in the USA, May 18, 2026
5994-9202EN