

Long Term Robustness of the Agilent 6495D LC/MS for PFAS Analysis

Sustained stability across
> 13,700 continuous injections

Authors

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Abstract

This technical overview highlights the exceptional robustness and sustained performance of the Agilent 6495D triple quadrupole LC/MS system, paired with the Agilent Jet Stream electrospray ionization (AJS-ESI) source, for the analysis of per- and polyfluoroalkyl substances (PFAS) in complex matrices. Engineered for high-throughput, high-sensitivity workflows, the 6495D incorporates innovations such as Agilent iFunnel technology and heated hyperbolic quadrupoles, which prevent contamination of optical elements and mass filters, ensuring consistent performance even under prolonged, matrix-intensive operation.

In a rigorous stress test, a PFAS mixture was continuously analyzed over 13,700 injections without any cleaning or retuning. Despite the demanding conditions, the system maintained exceptional quantitative precision, with peak area %RSDs of $\leq 7.5\%$ across all analytes without internal standard correction. This level of robustness and reliability makes the 6495D LC/TQ an uncompromising solution for laboratories requiring sustained sensitivity and uptime.

Introduction

Due to the rapidly evolving regulatory environment for PFAS, many analytical laboratories are choosing high-sensitivity triple quadrupole mass spectrometers for the targeted analysis of these compounds to ensure that they can meet present and potential future requirements for sensitivity and PFAS coverage. Assays of PFAS are regularly conducted in a high-throughput manner in complex sample matrices such as food, soil, wastewater or biological tissues. To maintain productivity and profitability, system robustness and uptime are crucial for these applications. In this work, we present results from expedited robustness testing of a high-sensitivity triple quadrupole LC/MS system for the measurement of selected PFAS in salmon – one of the most challenging food matrices to analyze.

Experimental

Chemical and standards

Native PFAS standards were purchased from Wellington Laboratories (Guelph, ON, Canada). Isotopically labelled internal standards were obtained from Agilent Technologies (Santa Clara, CA).

Sample preparation

Salmon was selected as a complex and challenging representative food matrix. Multiple portions of salmon extract were prepared using the following procedure: Fresh salmon was ground to homogenate and 5 g of the sample was vortexed for 10 minutes with 10 mL of water. Next, 10 mL of acetonitrile was added, followed by QuEChERS extraction salt (Agilent part number 5982-5650CH), and two ceramic homogenizers. The sample was shaken at 1,500 rpm for 5 minutes and then centrifuged at 4,000 rpm for 5 minutes. The resulting supernatant was mixed with 10% water and passthrough-cleaned on an Agilent Captiva EMR PFAS Food II Cartridge (Agilent part number 5610-2232).

A portion of the salmon extract was spiked with a mixture of native and isotopically labeled PFAS standards to achieve a final concentration of 250 fg/ μ L, which corresponded to approximately 0.5 ppb relative to the initial mass of salmon. This spiked matrix was used to monitor instrument performance throughout the experiment.

Instrumentation

Data were collected using an **Agilent 1290 Infinity II LC System** coupled to an **Agilent 6495D triple quadrupole mass spectrometer (LC/TQ)**. The LC and MS instrument parameters are summarized in Tables 1 and 2.

The LC parameters were chosen to provide increased throughput to expedite the experiment. Table 3 lists the MRM parameters for the target PFAS.

System performance was monitored by injecting five replicates of the spiked matrix (2 μ L injection volume) after every 100 matrix injections (10 μ L injection volume, total volume of 1 mL). Performance monitoring samples were analyzed in an expedited manner using a short column, high flow rate, and a fast 3-minute gradient. Two blank injections were carried out before and after these samples, respectively, to condition and clean the analytical column. For matrix injections, the LC flow was redirected to go directly to the mass spectrometer without switching to waste. No system maintenance or calibration was carried out during the entire experiment.

Table 1. Agilent 1290 Infinity II LC System method parameters.

Parameter	Value												
Columns	Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 \times 30 mm, 1.8 μ m Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 \times 5 mm, 1.8 μ m Agilent InfinityLab PFC Delay Column, 4.6 \times 30 mm												
Sampler Temperature	4 $^{\circ}$ C												
Mobile Phase A	Water + 2 mM NH_4 -acetate												
Mobile Phase B	ACN												
Flow Rate	0.8 mL/min												
Injection Volume	2 μ L												
Column Temperature	45 $^{\circ}$ C												
Gradient Program	<table><tr><td>Time (min)</td><td>%B</td></tr><tr><td>0.0</td><td>20</td></tr><tr><td>1.0</td><td>50</td></tr><tr><td>1.5</td><td>90</td></tr><tr><td>2.5</td><td>90</td></tr><tr><td>2.51</td><td>20</td></tr></table>	Time (min)	%B	0.0	20	1.0	50	1.5	90	2.5	90	2.51	20
Time (min)	%B												
0.0	20												
1.0	50												
1.5	90												
2.5	90												
2.51	20												
Stop Time	3.0 min												

Table 2. Agilent 6495D LC/TQ method parameters.

Parameter	Value
Ion Source	Agilent AJS-ESI
Polarity	Negative
Gas Temperature	160 $^{\circ}$ C
Drying Gas Flow	18 L/min
Nebulizer	24 psi
Sheath Gas Temperature	390 $^{\circ}$ C
Sheath Gas Flow	11 L/min
Capillary Voltage	2,400 V
Nozzle Voltage	0 V
Scan Type	dMRM
Detector Gain Factor	5
LC Diverter to Waste	Never

Table 3. Agilent 6495D LC/TQ MRM parameters per PFAS.

Compound	Precursor <i>m/z</i>	Product <i>m/z</i>	IFunnel Mode	CAV (V)	CE (V)	Polarity	Measured Retention Time (min)
PFHxS	398.9	99.0	Large Mol.	5	43	Neg.	1.44
PFOA	413.0	369.0	Fragile	5	7	Neg.	1.39
PFOA-13C8	421.0	376.0	Fragile	5	7	Neg.	1.39
PFNA	463.0	419.0	Fragile	5	8	Neg.	1.52
PFOS	498.9	80.0	Large Mol.	5	52	Neg.	1.67
PFOS-13C8	507.0	80.0	Large Mol.	5	52	Neg.	1.67

Results and discussion

During the experiment, 13,700 matrix injections were carried out over approximately three weeks of continuous operation with no maintenance or mass spectrometer calibration. Even though the instrument was exposed to more than 137 mL of salmon extract, which resulted in significant matrix deposition on the inlet of the mass spectrometer (Figure 1), no significant performance loss was observed.

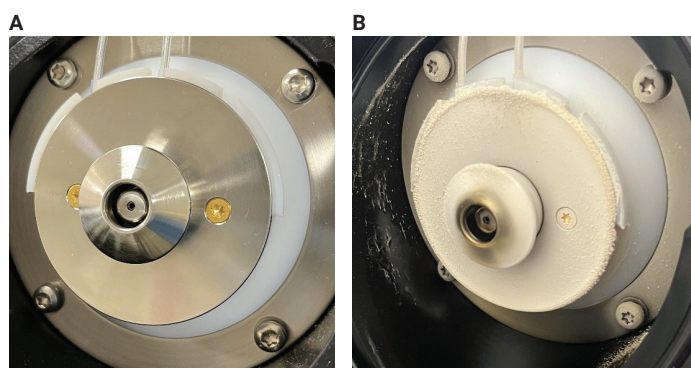


Figure 1. The inlet of the Agilent 6495D LC/TQ before (A) and after the robustness testing (B) with 13,700 matrix injections.

Peak area response

Raw peak area responses from all target analytes remained stable during the experiment as demonstrated by single-digit RSD% values and stayed within the range of $\pm 20\%$ of the mean for all compounds (Figures 2A, B, C, and D). Additionally, signal stability was demonstrated by overlaying the MRM chromatograms for all four target PFAS after every 1,000 matrix injections, which is equivalent to the infusion of 10 mL of undiluted salmon extract into the LC/TQ system (Figure 3).

Applying internal standard correction further decreased the variation in signal, and ISTD-corrected peak area ratios had < 5% RSD for both PFOA and PFOS (Figure 2E).

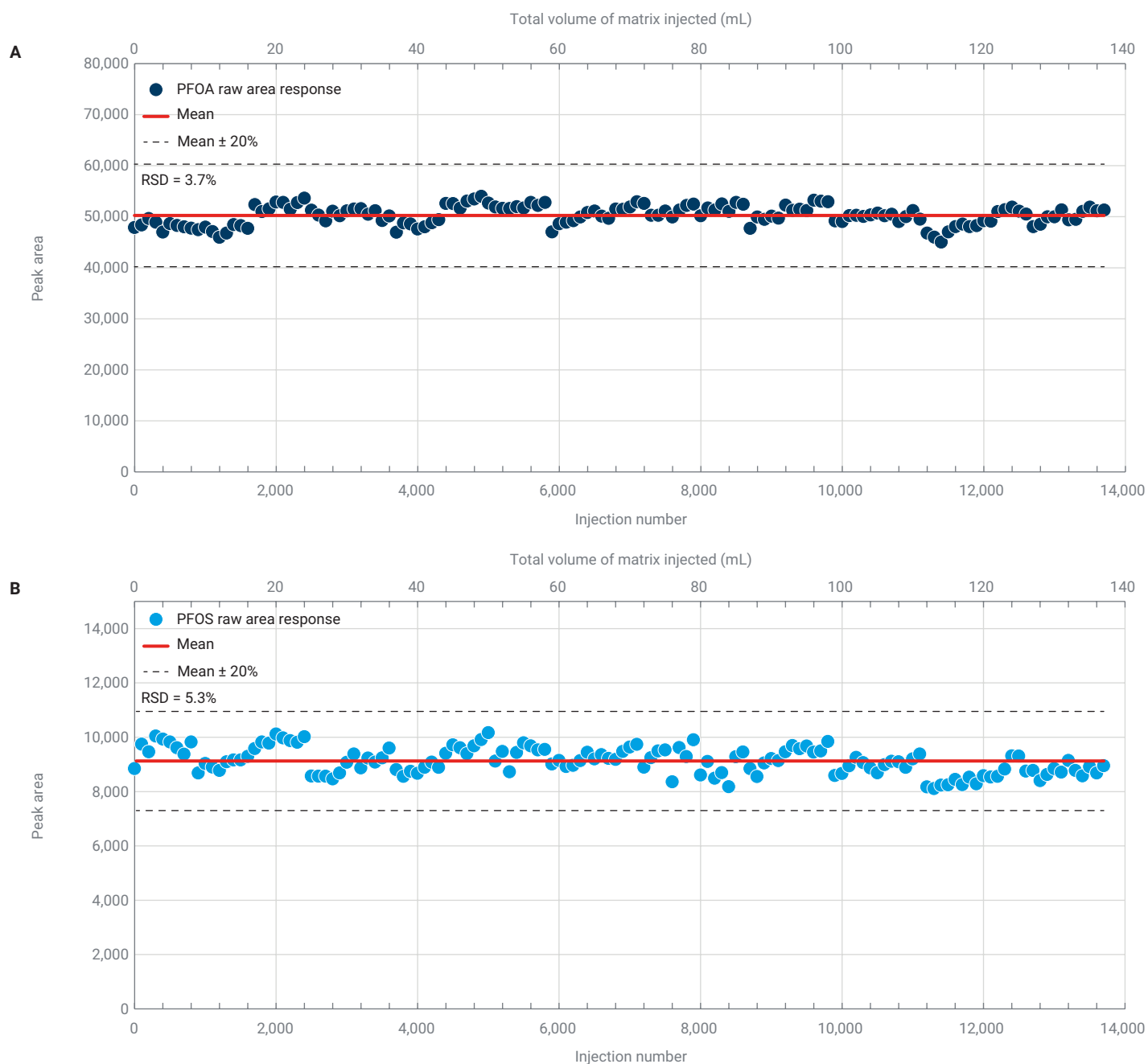


Figure 2A-B. Raw peak area responses for (A) perfluorooctanoic acid (PFOA), (B) perfluorooctanesulfonic acid (PFOS), (C) perfluorononanoic acid (PFNA), and (D) perfluorohexanesulfonic acid (PFHxS) in spiked salmon matrix; (E) Internal-standard-corrected area ratios for PFOA and PFOS in spiked salmon matrix. Each point represents the average data from five replicate injections of the performance monitoring sample for every 1 mL of salmon matrix injected (equivalent to 100 matrix injections).

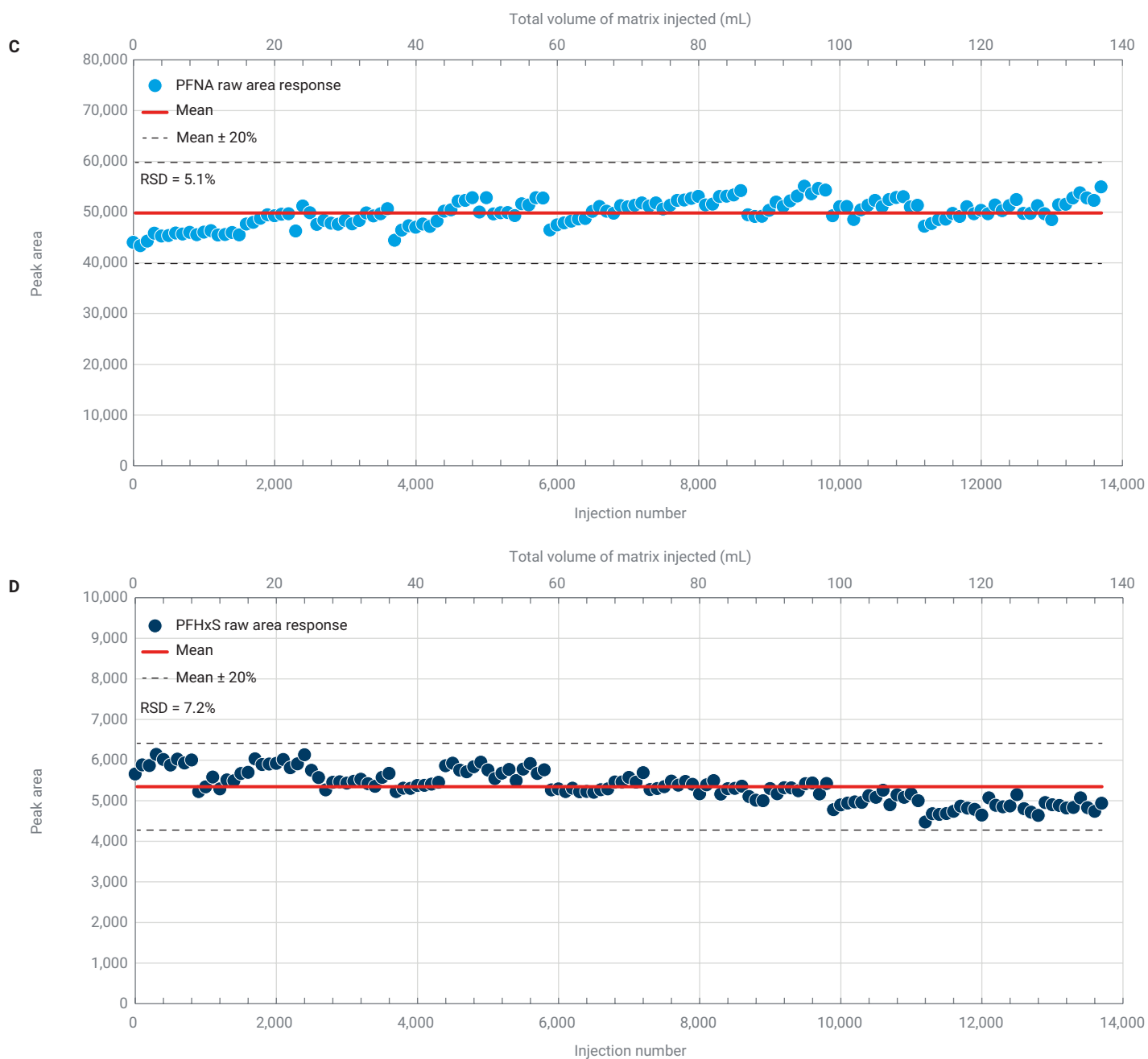


Figure 2C-D. Raw peak area responses for (A) perfluorooctanoic acid (PFOA), (B) perfluorooctanesulfonic acid (PFOS), (C) perfluorononanoic acid (PFNA), and (D) perfluorohexanesulfonic acid (PFHxS) in spiked salmon matrix; (E) Internal-standard-corrected area ratios for PFOA and PFOS in spiked salmon matrix. Each point represents the average data from five replicate injections of the performance monitoring sample for every 1 mL of salmon matrix injected (equivalent to 100 matrix injections).

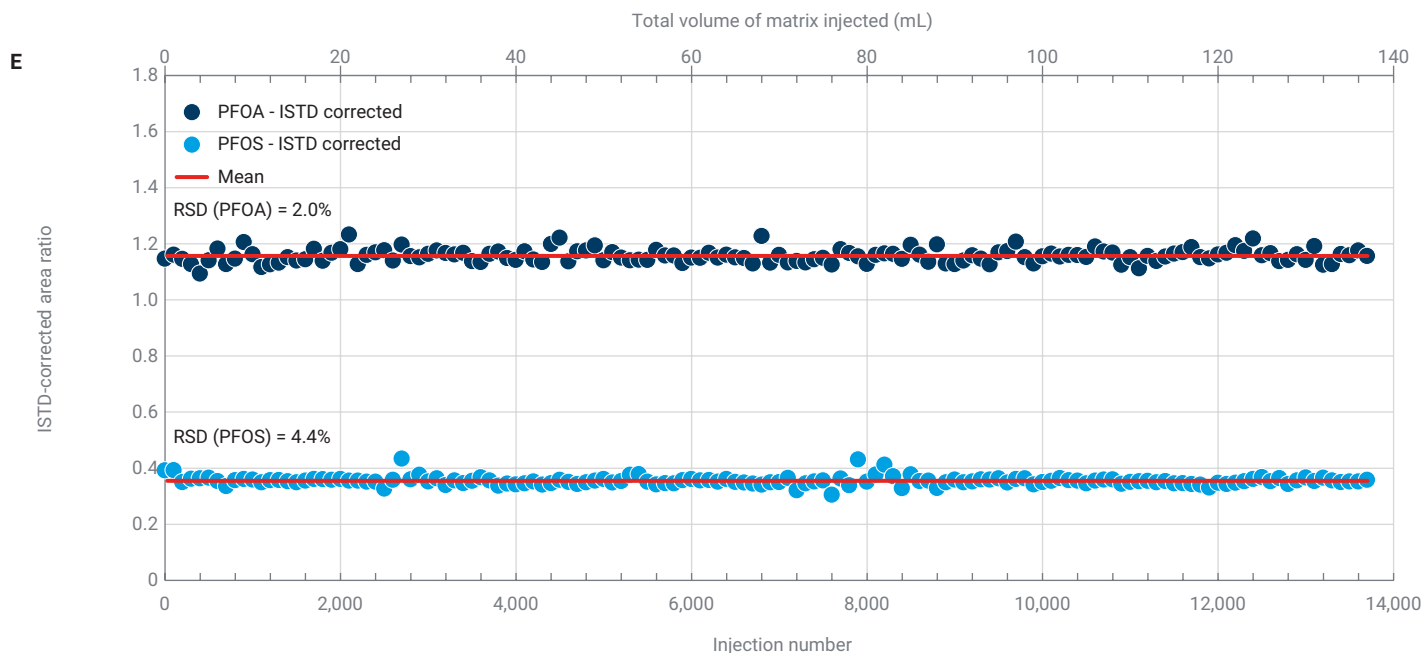


Figure 2E. Raw peak area responses for (A) perfluorooctanoic acid (PFOA), (B) perfluorooctanesulfonic acid (PFOS), (C) perfluorononanoic acid (PFNA), and (D) perfluorohexanesulfonic acid (PFHxS) in spiked salmon matrix; (E) Internal-standard-corrected area ratios for PFOA and PFOS in spiked salmon matrix. Each point represents the average data from five replicate injections of the performance monitoring sample for every 1 mL of salmon matrix injected (equivalent to 100 matrix injections).

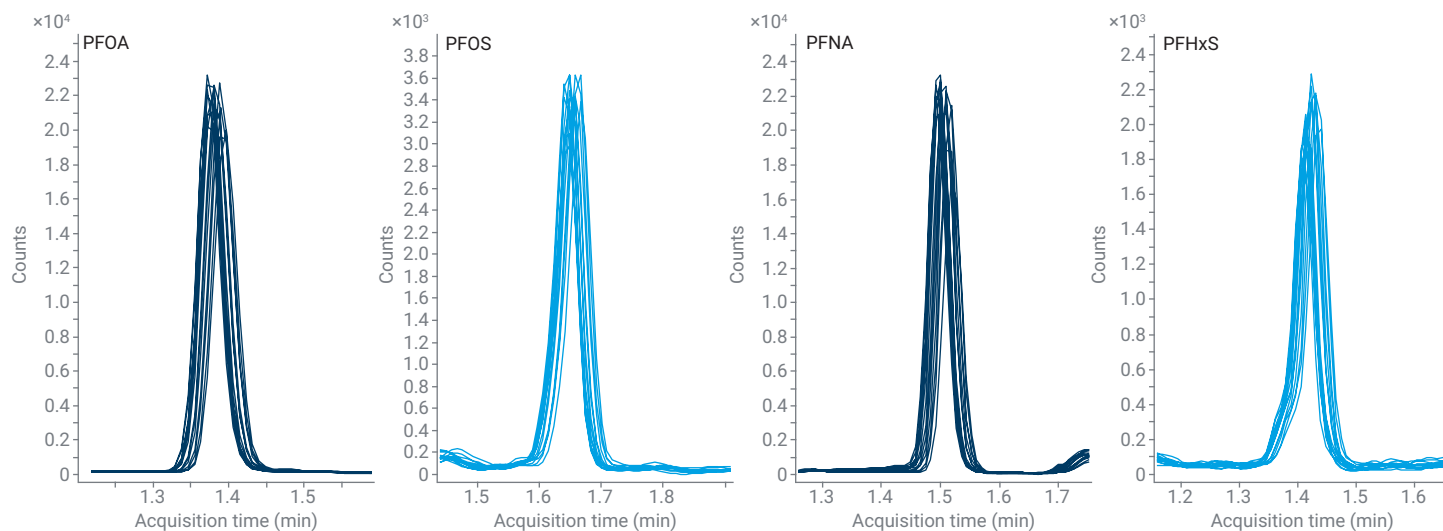


Figure 3. Overlaid MRM chromatograms for the four target PFAS after every 1,000 matrix injections (equivalent to the infusion of 10 mL of undiluted salmon extract).

Mass axis and peak width calibration stability

During the experiment, the mass spectrometer mass axis and peak width calibrations were checked at regular intervals (after approximately every 2,500 to 3,000 matrix injections). The calibration remained within the manufacturer-recommended limits throughout the entire experiment, without need to readjust any tune parameters. Figure 4 shows the passing results after experiment completion.

The experiment was stopped after 13,700 matrix injections because all the matrix and samples (prepared in bulk at the beginning of the experiment) had been used. No obvious performance degradation or significant contamination of inner ion optics elements were observed after the experiment was concluded.

In summary, the instrument was exposed to the following amount of undiluted salmon matrix:

- 690 injections of performance monitoring samples (injection volume of 2 µL each, for a total of 1.38 mL)
- 13,700 matrix injections (injection volume of 10 µL each, for a total of 137 mL)
- Grand total of 138.38 mL of salmon extract, equivalent to 69,190 sample injections of 2 µL each

Conclusion

The robustness of the Agilent 6495D LC/TQ for PFAS analysis was tested using salmon extract, a complex and challenging food matrix. During the experiment, the system was exposed to more than 130 mL of the matrix over approximately 3 weeks of continuous operation, without any maintenance. System performance was monitored using injections of a mixture of native and isotopically labeled PFAS samples. The monitored signals exhibited low variation (RSD < 7.5%) throughout the entire experiment. No obvious performance degradation or significant contamination of the inner ion optics elements of the LC/TQ system were observed after the conclusion of the test.

Although no loss of LC/MS instrument performance was observed during this expedited robustness test, routine maintenance of ion source and optics is recommended to ensure prolonged instrument operation.


			
Summary MS Checktune Report - G6495D			
Instrument Information			
Model	G6495D	Checktune Date	2025-03-23T10:56:39-07:00
Serial Number	SG2305D301	SW/FW Version	3.1.704.4/11.2.20
Ion Source	AJS ESI	Ionization Mode	ESI
Tune Mode	Standard Quadrupole	Last Autotune Date	2025-02-27T20:48:17-07:00
Vacuum Pressure	3.25E+0 [R] (Torr); 3.22E-5 [H] (Torr)	Overall Result	Passed

Figure 4. Instrument calibration check (Checktune) report showing a passed result after the experiment was completed.

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