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Evaluating System Robustness of a High-Sensitivity Triple Quadrupole LC/MS for PFAS Analysis in Food Matrix Over an Extended Period

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Introduction

Due to the rapidly evolving regulatory environment of per- and polyfluoroalkyl substances (PFAS), many analytical laboratories are opting to use high-sensitivity triple quadrupole mass spectrometers for the targeted analysis of such compounds to ensure meeting present and potential future requirements for sensitivity and PFAS coverage. These assays are regularly conducted in a high-throughput manner involving complex matrices such as food, soil, wastewater or biological samples. Therefore, system robustness and instrument uptime are crucial for such applications to maintain productivity and profitability. In this work, we present results from an expedited robustness testing of a high-sensitivity triple quadrupole LC/MS system using select PFASs in one of the most challenging food matrices.

Experimental

Samples and Methods

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

Salmon was selected as a complex and challenging representative food matrix. Multiple portions of salmon extract were prepared by repeating 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. Then, 10 mL acetonitrile was added, followed by the addition of QuEChERS extraction salt (PN#5982-5650CH) and two ceramic homogenizers. The sample was shaken at 1500 rpm for 5 minutes and then centrifuged at 4000 rpm for 5 minutes. The resulting supernatant was mixed with 10% water and passthrough cleaned on a Captiva EMR PFAS Food II cartridge (PN#5610-2232).

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

MS acquisition and source setpoints (Table 1) for system performance monitoring were based on a prior work (TP287, ASMS 2024) whereas LC parameters (Table 2) were modified to increase throughput and expedite the experiment.

System performance was monitored by injecting 5 replicates of the spiked matrix (2 µL injection volume) after every 100 matrix injections (10 µL injection volume, total volume of 1 mL). Performance monitoring samples were analyzed in an expedited manner using a short column, high flow rate and fast 3 minutes 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 the MS without switching to waste. No system maintenance or calibration was carried out during the entire experiment.

Instrumentation

Data was collected using an Agilent 1290 Infinity II LC system coupled to an **Agilent 6495D LC/TQ** mass spectrometer. Instrument parameters are summarized in Tables 1 and 2.

Table 1. TQ parameters

Parameter	Value
Ion Source	AJS
Polarity	Negative
Drying Gas Temp. & Flow	160°C @ 18 L/min
Sheath Gas Temp. & Flow	390°C @ 11 L/min
Nebulizer Pressure	24 psi
Capillary/Nozzle Voltage	2400V/0V
Detector Gain Factor	5

Table 2. LC conditions for performance monitoring

Parameter	Value
Columns	• Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 x 30 mm, 1.8 µm • Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 x 5 mm, 1.8 µm • Agilent InfinityLab PFC Delay Column, 4.6 x 30 mm
Injection Vol.	2 µL
Mobile Phase	A: Water + 2mM NH_4-acetate B: ACN
Flow Rate	0.8 mL/min @ 45°C
Gradient	Time (min) %B 0.0 20 1.0 50 1.5 90 2.5 90 2.51 20
Stop-time	3.0 min

Results and Discussion

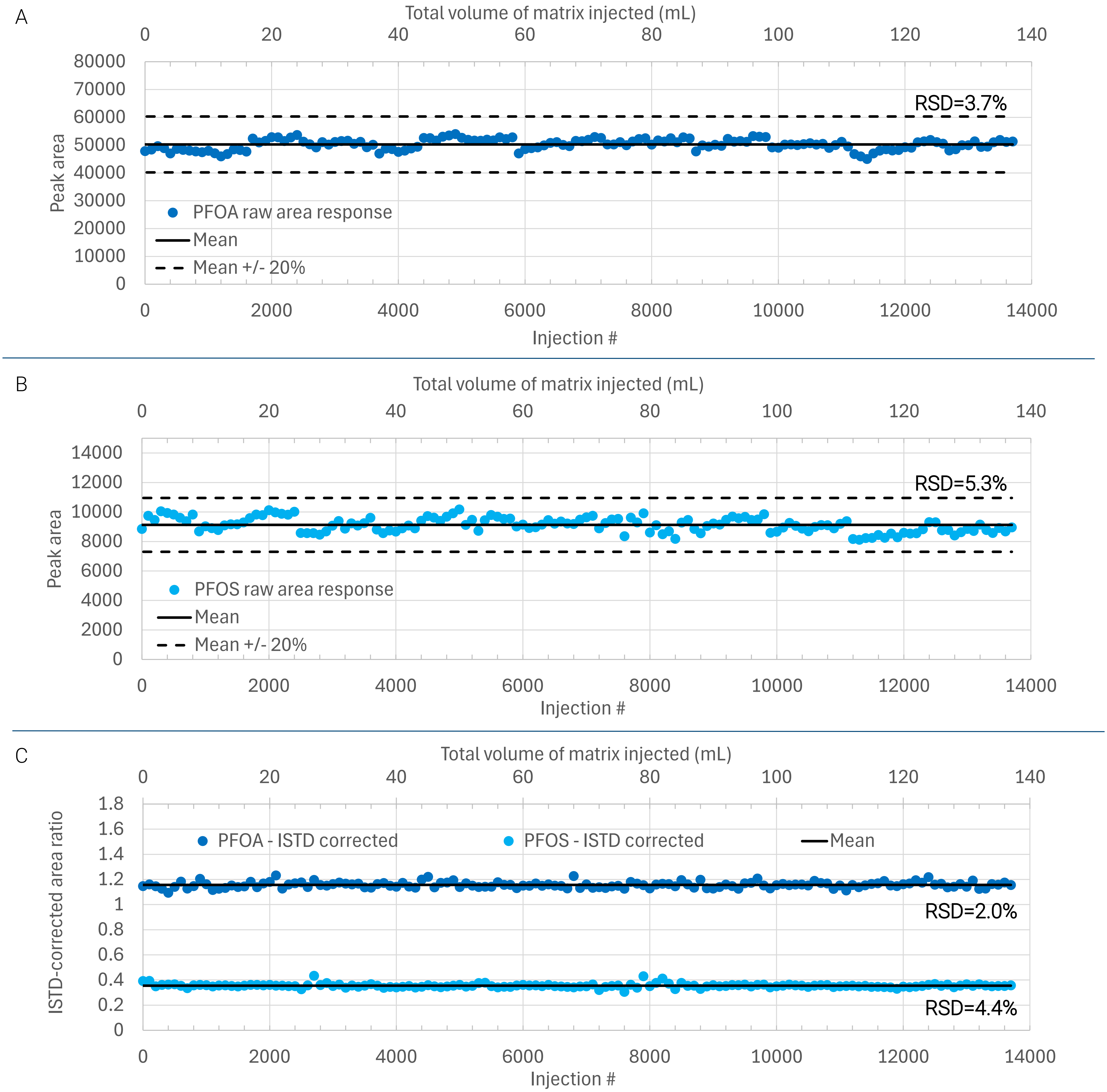


Figure 1. A & B: Raw peak area responses for perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) in spiked salmon matrix; C: Internal standard corrected area ratios for PFOA and PFOS in spiked salmon matrix. Each point represents the average data from five replicate injections of the performance monitoring sample for every 1 mL of salmon matrix injected (equivalent to 100 matrix injections).

Results and Discussion

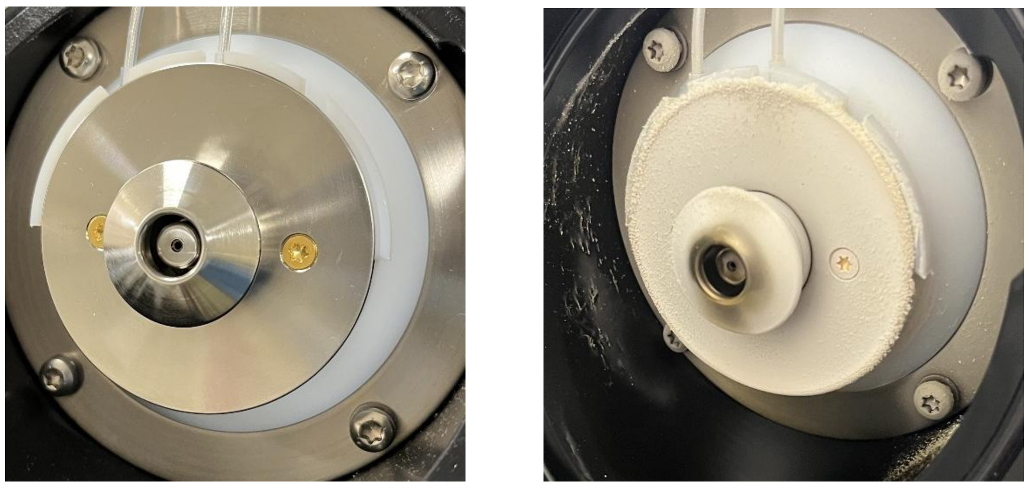


Figure 2. The inlet of the 6495D LC/TQ before (left) and after the robustness testing (right)

During the experiment, a total number of **13,700 matrix injections** were carried out over the period of ~3 weeks of continuous operation with no maintenance events or calibration of the MS. Even though the instrument was exposed to more than **137.00 mL of undiluted salmon extract**, resulting in significant matrix deposition on the inlet of the mass spectrometer (Figure 2), no significant performance loss was observed.

Raw peak area responses from PFOA and PFOS remained stable during the experiment, with single digit RSD% values, and stayed within the range of +/-20% of the mean for both compounds (Figure 1. A & B). Additionally, signal stability is demonstrated by overlaying MRM chromatograms for PFOA and PFOS after every 1000 matrix injections, equivalent to the infusion of 10 mL of undiluted salmon extract into the LC/TQ system (Figure 3).

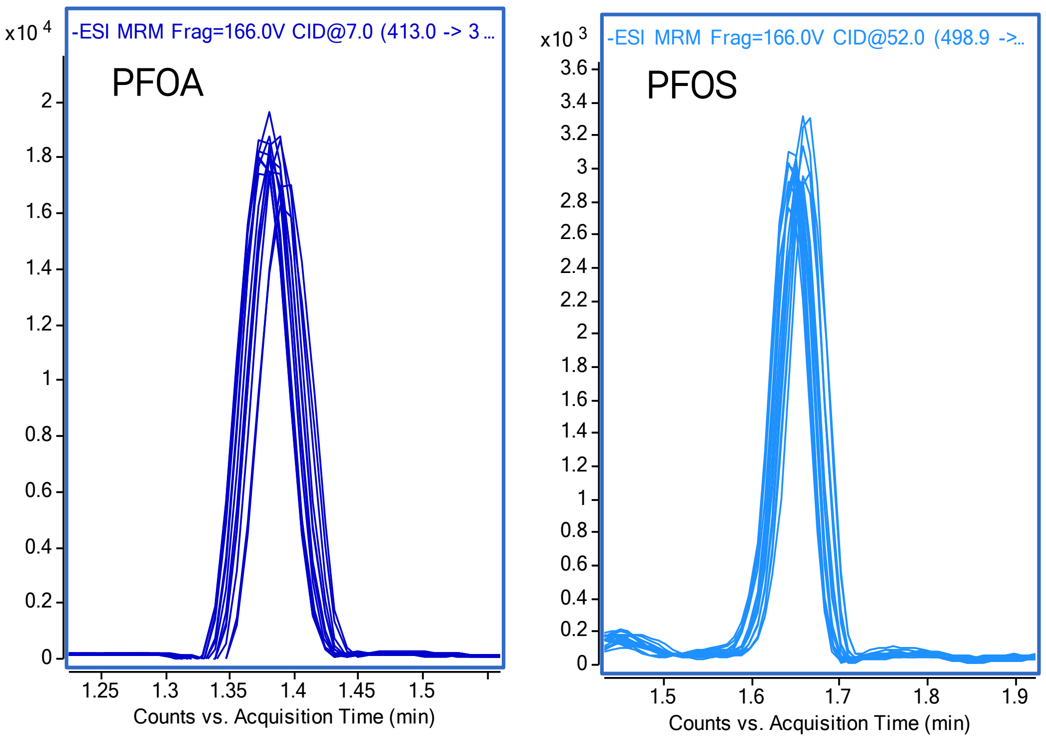
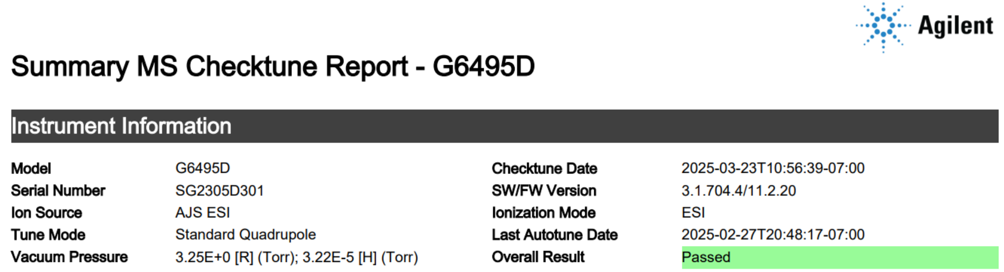


Figure 3. Overlaid MRM chromatograms for PFOA (left) and PFOS (right) after every 1000 matrix injections (equivalent to the infusion of 10 mL undiluted salmon extract)

Using internal standard correction further decreased the variation in signal, and ISTD-corrected peak area ratios had <5% RSD for both PFOA and PFOS (Figure 1. C).



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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) passed after completion of the experiment

Mass axis and peak width calibrations of the mass spectrometer were checked at regular intervals during the experiment (after approximately every 2500-3000 matrix injections). Calibration remained within the limits recommended by the manufacturer throughout the entire experiment, without the need of readjusting any tune parameters. Figure 4 shows the passing results after completion of the experiment.

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

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

- 690 injections of performance monitoring samples (Inj. volume: 2 µL, total of 1.38 mL)
- 13,700 matrix injections (Inj. volume: 10 µL, total of 137.00 mL)
- Grand total of 138.38 mL → equivalent of 69,190 sample injections at 2 µL injection volume

Conclusions

The robustness of a 6495D LC/TQ was tested using undiluted salmon extract, a complex and challenging food matrix. During the experiment, the system was exposed to more than 130 mL of the matrix over a period of ~3 weeks of continuous operation, without any maintenance. System performance was monitored by injections of a mixture of native and isotopically labelled PFAS samples. The monitored signals exhibited low variations (RSD<6%) throughout the entire experiment. No obvious performance degradation or significant contamination of the inner ion optics elements were observed after the conclusion of the test.

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

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