Achieving exceptional robustness for PFAS analysis in food with the next-generation SCIEX 7500+ system

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This technical note demonstrates >2x robustness improvement for the analysis of PFAS food extracts on the SCIEX 7500+ system. At the end of the study, comprising over 6400 food matrix injections, the majority of PFAS compounds (10 out 13) maintained >70% of the initial sensitivity. Residue analysis in food matrices is challenged by the presence of interfering co-extractables which can result in instrument contamination and system downtime. Here, the robustness of the SCIEX 7500+ system and SCIEX 7500 system was evaluated in an accelerated manner through an aggressive sample preparation procedure and by omitting the diverter valve. The SCIEX 7500+ system features new Mass Guard technology[1] designed to improve instrument robustness while maintaining optimal sensitivity longer.

Key benefits of high-throughput PFAS analysis in food using the SCIEX 7500+ system

- **Enhanced robustness from Mass Guard technology:** Improved hardware components to reduce downstream instrument contamination, maintaining instrument uptime
- **Exceptional instrument stability:** The SCIEX 7500+ system achieved >2x improvement in robustness, as demonstrated by >6400 injections of food matrices, compared to >3000 injections on the SCIEX 7500 system
- **User-accessibility via an extractable DJet+ assembly:** Increased flexibility for user cleaning when required

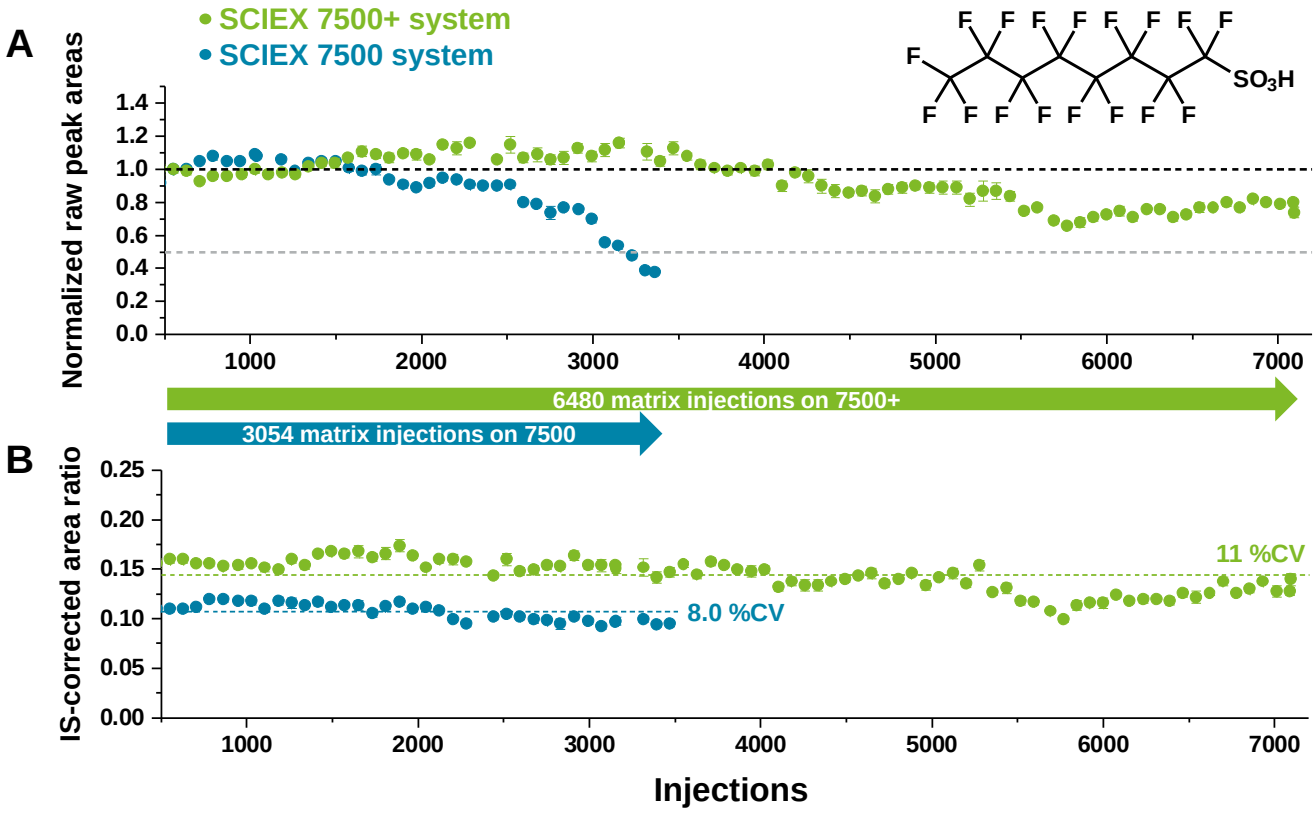

Figure 1. Raw peak areas normalized to initial response (A) and internal standard (IS)-corrected peak area ratios (B) for perfluorooctane sulfonate (PFOS) in solvent quality control (QC) samples on the SCIEX 7500 (blue) and on the SCIEX 7500+ (green) systems. Each datapoint represents the mean with standard error bars. In panel A, the dotted lines represent the 100% and 50% raw peak areas relative to the initial response. Robustness was compared based on the total number of injections before the instrument sensitivity declined to 50% of the maxima. In panel B, the dotted lines represent the overall mean and %CV for each experiment. Food extracts were injected between each solvent QC datapoint.

Introduction

The high-throughput analysis of PFAS in food samples is challenging due to the matrix complexity. Instrument robustness is crucial to maintaining optimal assay performance with minimal downtime. The SCIEX 7500+ system maintains the high sensitivity of the SCIEX 7500 system but also features enhanced robustness using Mass Guard technology.[1] This includes the addition of the T Bar electrodes to the Q0 region, which actively filters out contaminating ions to create a cleaner ion beam (**Figure 2**). Visual examination of the downstream ion optics reveals fewer contamination deposits on the SCIEX 7500+ system, compared to the SCIEX 7500 system on the IQ1 lens. In addition, the SCIEX 7500+ system features improved customer access to the instrument front-end DJet+ assembly to facilitate instrument cleaning.[1] Here, the long-term robustness of the SCIEX 7500+ system and SCIEX 7500 system was evaluated under accelerated conditions.

Methods

To measure the robustness of both systems, the experimental regime was designed to maximize the matrix load injected over consecutive weeks, which included:

- A broad selection of representative food matrices
- A modified sample preparation resulting in extracts containing higher proportions of matrix interferences than typical optimized procedures
- A short runtime to maximize the number of consecutive matrix injections between solvent QCs (**Figure 3**)
- Continuous acquisition without the use of a diverter valve and any interim maintenance on the mass spectrometer

Samples and reagents: Native and mass-labeled PFAS standards were purchased from Wellington Laboratories and used to prepare quality control (QC) samples in solvent and matrix post-spikes in food extracts. Salmon fillets, avocado, five-spice powder and rabbit feed were purchased from local supermarkets.

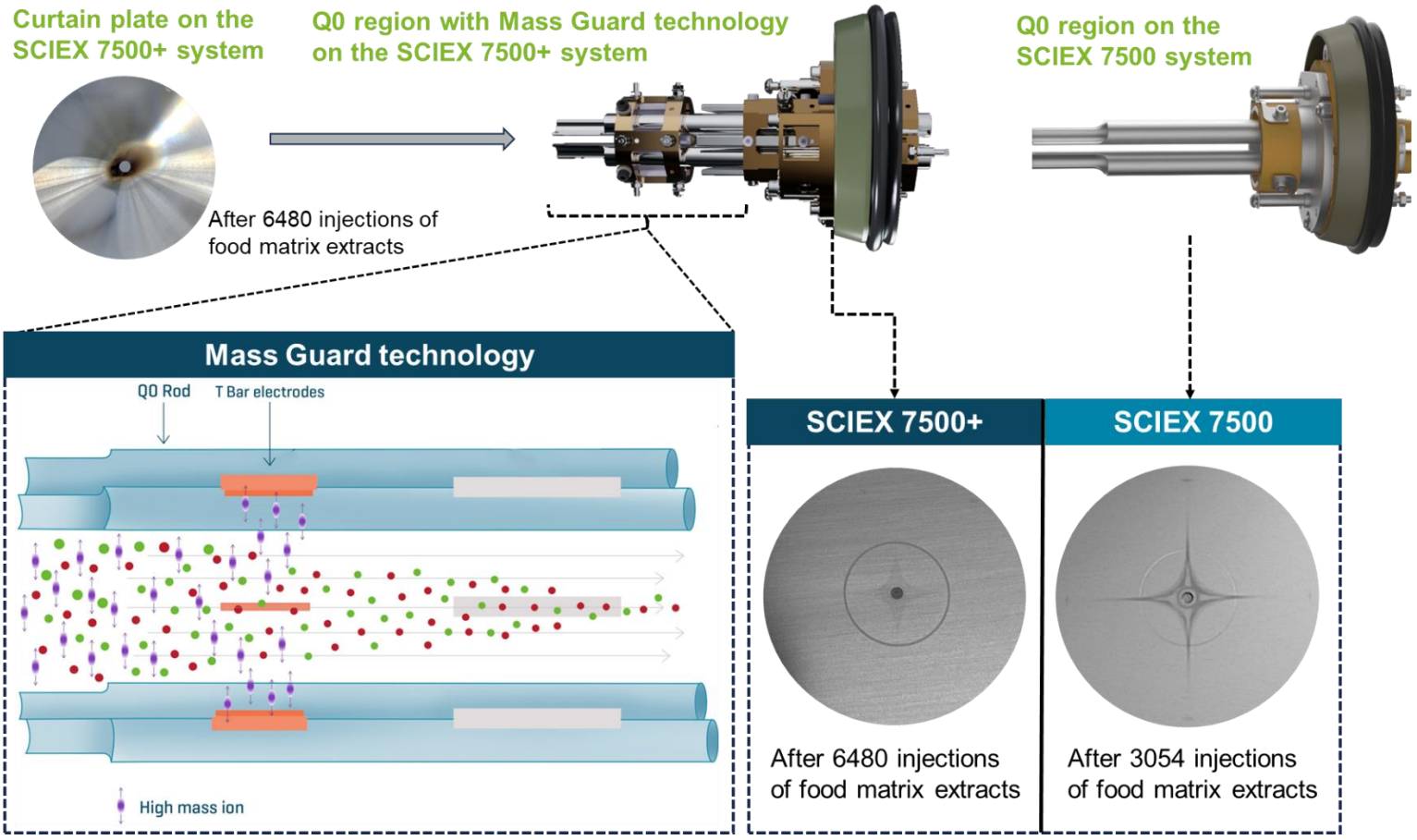

Figure 2. Hardware components of Mass Guard technology. The added T Bar electrodes in the Q0 region of the SCIEX 7500+ system actively remove contaminating ions (purple symbols), resulting in a much cleaner sample plume (red and green symbols) entering the instrument. Visual comparison of the ion optics downstream of the T Bar electrodes showed less impact from matrix contamination despite significant residue deposited on the source curtain plate (top left), when compared against the same component on the SCIEX 7500 system without this protection, as shown on the bottom right.

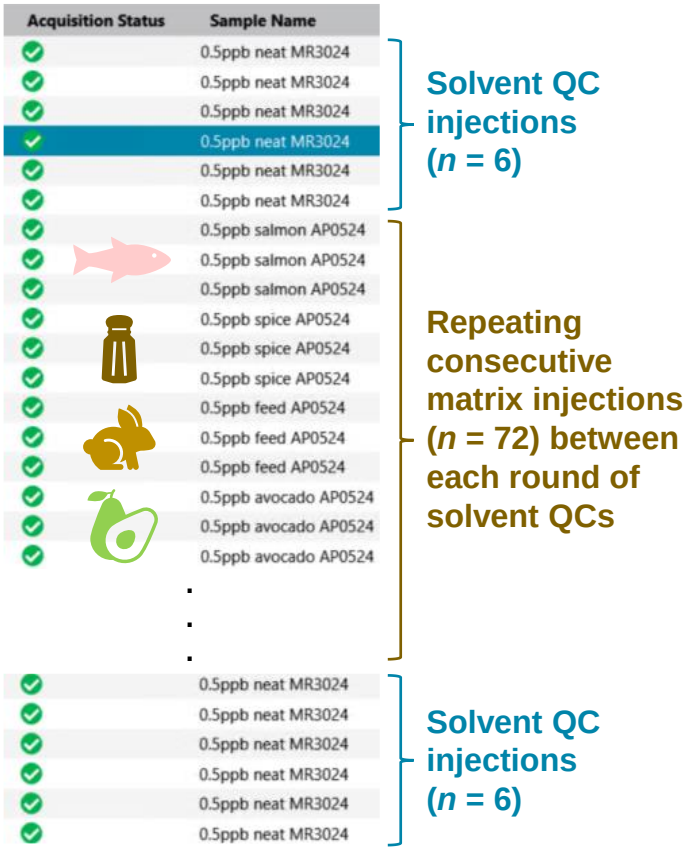

Figure 3. Injection sequence on both systems. Robustness was evaluated by consecutive injections of food matrix extracts that were bracketed by solvent QC replicates at a frequency ratio of 12:1 matrix:solvent, i.e. 72 matrix injections for every 6 solvent QC replicates.

Sample preparation: Prior to extraction, the salmon fillet and avocado (~50 g each) were homogenized using a food processor, while 50 g of rabbit feed was ground into powder using a mortar and pestle. The food samples were extracted using a QuEChERS method that had been developed for PFAS analysis in food,[2] but the final solid-phase extraction (SPE) step was omitted to maximize the impurities in the final matrix extracts. In a 50 mL centrifuge tube, 5 g of homogenized salmon/avocado or 2 g of feed/spice powder was combined with water (5 mL for salmon/avocado, 15 mL for feed/spice powder), then extracted with 12 mL of acetonitrile and 150 µL of formic acid. Upon adding a QuEChERS packet (6000 mg $MgSO_4$, 1.5 g NaCl; Phenomenex P/N AH0-9044), the sample was vortexed and shaken for 5 min at 1500 rpm. After centrifuging at 10000 rcf for 5 min, the supernatant was transferred to a 15 mL dispersive (dSPE) tube (900 mg $MgSO_4$, 150 mg PSA, 45 mg GCB; Phenomenex P/N KS0-9510). After shaking and centrifuging the dSPE tube, the supernatant was evaporated to dryness and reconstituted in 10 mL of methanol. This final methanolic extract was dispensed into equal 1 mL aliquots at a solvent composition of 80/20 (v/v) methanol/water. Solvent QC samples were prepared by spiking with a mixture of native and mass-labeled PFAS standards at the same solvent composition as the food matrix extracts. Instrument robustness was evaluated by monitoring the solvent QCs between large blocks of consecutive matrix injections, as shown in **Figure 3.**

Chromatography: Chromatographic separation was performed on an ExionLC AC system using a Luna Omega PS C18 as the analytical column (100 x 3.0 mm, 3 µm, Phenomenex P/N 00D-4758-Y0) and a Luna Omega PS C18 as the delay column (50 x 3.0 mm, 5 µm, Phenomenex P/N 00B-4753-Y0). A SecurityGuard ULTRA UPLC Fully Porous PS C18 cartridge (Phenomenex P/N AJ0-9508) was used. A flow rate of 0.8 mL/min, an injection volume of 10 µL and a column temperature of 40°C were used. The LC gradient used is shown in Table 1.

Table 1: LC gradient.

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0.00	90	10
0.25	90	10
0.50	45	55
1.50	10	90
3.50	10	90
3.60	90	10
5.00	90	10

Mobile phase A: Water with 10mM ammonium acetate
Mobile phase B: Methanol with 10mM ammonium acetate

Mass spectrometry: Analysis was performed using scheduled multiple reaction monitoring (sMRM). Table 2 shows the source and gas parameters used on both systems. Optimized MRM parameters were used for the target PFAS.

Table 2: Source and gas parameters.

Parameter	Value
Polarity	Negative
Ion source gas 1*	40 psi (7500+), 50 psi (7500)
Ion source gas 2	70 psi
Curtain gas	50 psi
Source temperature	400°C
Ion spray voltage	-2000 V
CAD gas	12

* GS1 was optimized to achieve equivalent sensitivity between both systems.

Data processing: Data acquisition and processing were performed using the SCIEX OS software, version 3.4.0. Instrument contamination was monitored using built-in automated tests for quick and easy troubleshooting in the SCIEX OS software.

Comparison of robustness performance between the SCIEX 7500+ system and SCIEX 7500 system

The foods selected for the matrix injections (salmon, spice powder, avocado and rabbit feed) represent the diversity of food samples analyzed by routine testing laboratories. Rabbit feed served as a proxy for silage, which is known to have high levels of interferences. Initial experiments using the full US FDA extraction and clean-up procedure[2] showed no substantial sensitivity decline for any target analyte after >3000 injections on the SCIEX 7500 system. In an effort to increase the matrix interference load onto the system, the final SPE clean-up was removed. However, these changes represent aggressive conditions, and the use of the optimized extraction and clean-up procedure is recommended for routine food analysis.

Figure 1A depicts the robustness performance based on the uncorrected raw peak areas monitored. Solvent QC monitoring began after the initial 500 matrix injections were performed. The raw peak areas were normalized to the initial stabilized response, as demonstrated by the 100% black dotted line in **Figure 1A**. Robustness was compared based on the total number of injections before the instrument sensitivity declined to 50% of the initial response. Excellent robustness was demonstrated on both instruments, as shown for perfluorooctane sulfonate (PFOS) in **Figure 1A**. On the SCIEX 7500 system, the raw peak areas of PFOS remained stable for ~2500 injections before declining to 50% after >3000 injections. More impressively, the SCIEX 7500+ system surpassed this injection count by >2x whereby the sensitivity of PFOS remained stable at 80% after >7000 injections.

Similar robustness trends were observed for the other target PFAS (**Figure 4**). Of the 13 PFAS tested, 11 analytes retained >90% of the initial sensitivity on the SCIEX 7500+ system at ~3400 injections; whereas, most of the analytes had declined to <50% on the SCIEX 7500 system at this point. This sensitivity performance remained strong on the SCIEX 7500+ system past 7000 injections, with most analytes still at 70% of the initial response. **Figure 4** provides a visual comparison of the superior robustness of the SCIEX 7500+ system compared to the SCIEX 7500 system for four other representative PFAS: PFHxA, PFOA, PFHxS and PFDS.

Isotope-labeled internal standards (IS) are commonly used in PFAS food analysis. However, IS-corrected peak areas can mask the true performance of an instrument due to the identical responses expected from both the native and internal standards to the assay environment**.** For example, the %CVs of the IS-corrected area ratios of PFOS were 8% for >3300 injections on the SCIEX 7500 system and 11% for >7000 injections on the SCIEX 7500+ system (**Figure 1B**). This reproducibility suggests the instrument's performance remained stable throughout the experiment despite the harsh conditions, such as the high matrix load introduced continuously to the ion source without the protection of a diverter valve and any interim maintenance. As such, uncorrected raw peak areas represent a more accurate measure of instrument performance over time and can better inform the user when maintenance is required.

Enhanced software tools for system performance tracking

System suitability tests (SSTs) based on QC samples are critical to ensuring data accuracy and reproducibility in long-term assays. Intermittent infusion-based checks can also provide real-time insights regarding the instrument's performance between acquisition batches. SCIEX OS software provides a built-in automated workflow that enables the user to monitor the detector performance and system charging without manual intervention (**Figure 5**). Any suboptimal performance, as indicated by the SSTs and these contamination tests, would trigger the need for instrument maintenance. The removable DJet+ assembly on the SCIEX 7500+ system improves front-end serviceability by empowering the user with more control over scheduling maintenance and system uptime.

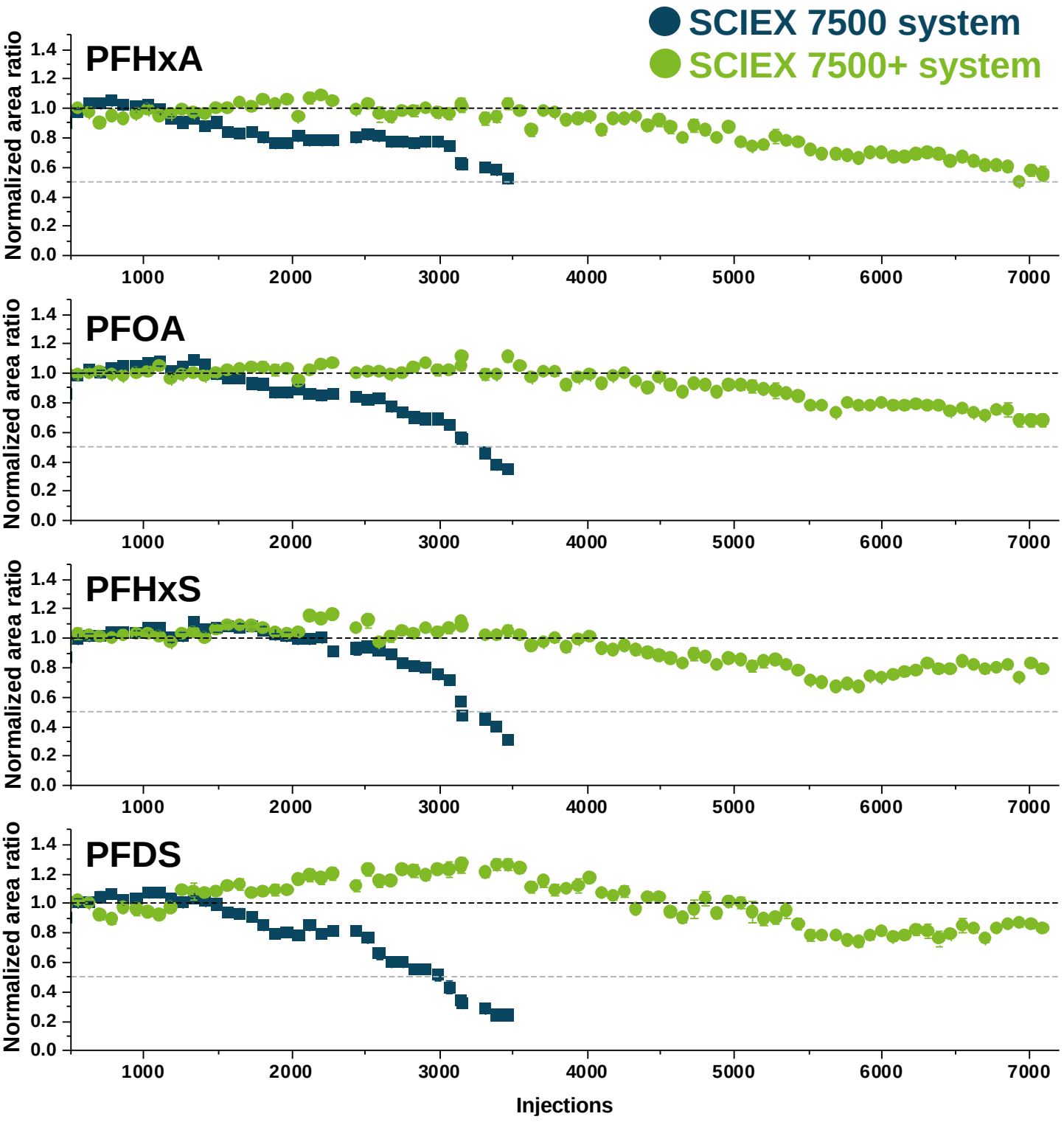

Figure 4. Comparison of robustness trends observed for perfluorohexanoic acid (PFHxA), perfluorooctanoic acid (PFOA), perfluorohexane sulfonate (PFHxS) and perfluorodecane sulfonate (PFDS) in solvent QC samples injected on the SCIEX 7500 system (blue) and SCIEX 7500+ system (green) throughout the experiment. Each datapoint represents the average of replicate injections with its associated standard error bars. The black and grey dotted lines represent the initial stable analyte response at 100% and 50% of that initial response, respectively.

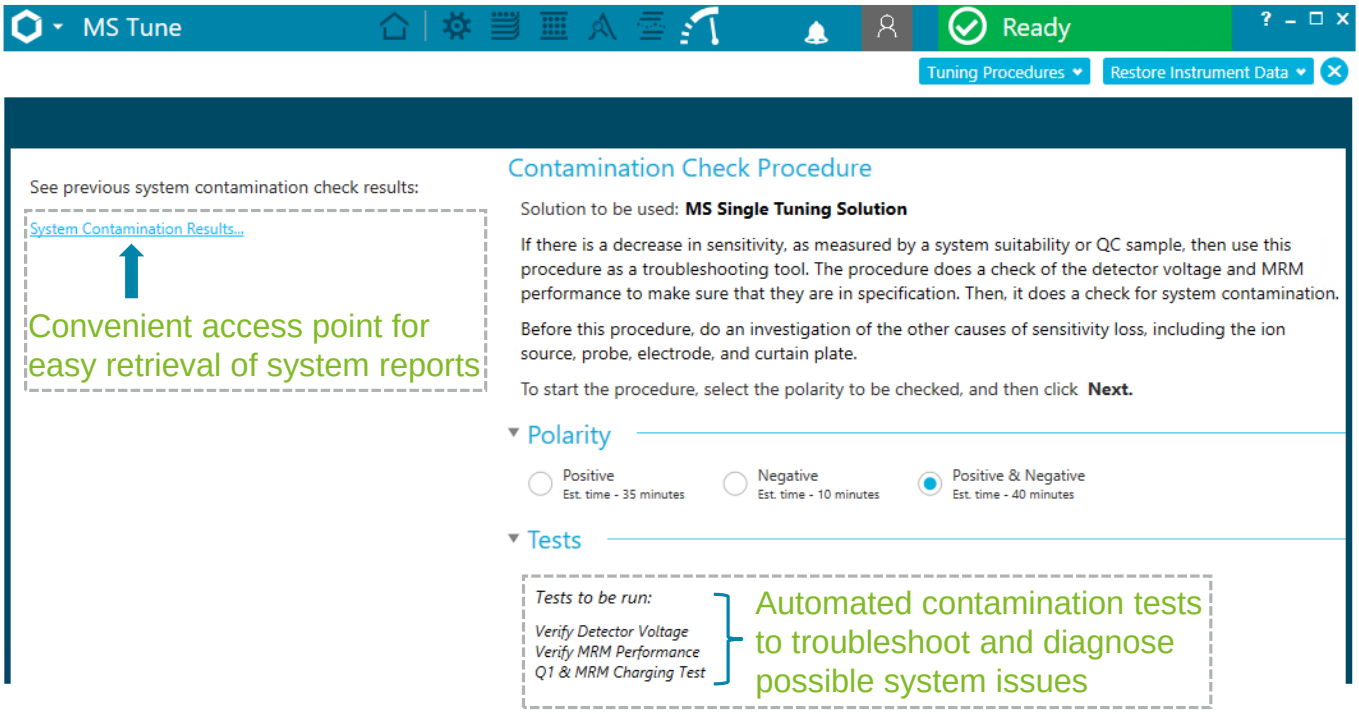

Figure 5. Built-in contamination check procedures in SCIEX OS software for easy troubleshooting. The MS Tune module in SCIEX OS software provides an automated contamination check procedure that allows the user to troubleshoot and monitor instrument performance during sensitivity loss. At the end of the procedure, the software generates a summary report of the instrument health based on the tests ran.

Conclusions

- Mass Guard technology actively removed contaminating ions in complex matrices, which imparted exceptional robustness to the SCIEX 7500+ system
- The SCIEX 7500+ system maintained quantitative performance after >7000 injections of food extracts and solvent QC samples with most PFAS analytes retaining >70% of the initial peak area sensitivity
- The combination of SCIEX OS software enhancements for system performance tracking and the extractable DJet+ assembly offers increased flexibility for user-initiated management of system maintenance and uptime
- Equipped with the renowned sensitivity of the SCIEX 7500 system, the exceptional data stability and robustness demonstrated by the SCIEX 7500+ system deliver a powerful and efficient platform for PFAS analysis in food

References

1. Build resilience with the 7500+ system. SCIEX brochure, MKT-31468-A.
2. Genauldi, S. *et al.* Analyte and matrix method extension of per- and polyfluoroalkyl substances in food and feed. *Anal. Bioanal. Chem.* **2024**, *416*, 627-633.

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