

**Liquid Chromatography/
Mass Spectrometry****AUTHOR**

Jingcun Wu

PerkinElmer

Woodbridge, ON, Canada

Unmatched Robustness for Analysis of Per- and Poly-fluoroalkyl Substances in Difficult Food Matrices

Introduction

To realize high-throughput analysis of analytes such as PFAS from complex sample matrices such as various food samples, it is required to have a highly robust instrument that can provide consistent results for a long period of time with no or minimal instrument downtime for maintenance. Recently, an LC/MS manufacturer published data demonstrating exceptional robustness. In this technical note, using the same methodologies, the robustness of the QSight 500 system was evaluated in an accelerated manner with a simple and fast QuEChERS sample preparation procedure without using solid phase extraction (SPE) for clean-up and a diverter valve. Our results clearly demonstrated that QSight LC-MS/MS system had superior robustness compared to the competitors' similar systems. This competitive advantage of QSight system provides the customer a great opportunity to save time, improve their sample throughput and enhance their laboratory productivity.

HIGHLIGHTS

- Complex food sample matrices were used to evaluate PerkinElmer QSight™ LC/MS/MS system's robustness for per- and poly-fluoroalkyl substances (PFAS) analysis.
- Unmatched robustness was achieved with the patented StayClean™ technology in QSight mass detection system.
- Exceptional signal stability was obtained for most PFAS analytes studied even after 21700 injections of difficult food sample matrices.
- **Customer Benefits:** High sample throughput could be realized with minimum instrument downtime for maintenance, thus significantly enhance system efficiency and laboratory productivity.

Experimental

Safety First

Several PFAS, including perfluorooctanoic acid (PFOA), have been described as likely to be carcinogenic to humans. Exposure to these compounds should be reduced to the lowest possible level. Personal protection equipment and safety training must be provided, safety procedures must be implemented before and during work.

Instrumentation

The chromatographic separation was conducted using a PerkinElmer QSight LX 50™ ultra-high-performance liquid chromatography (UHPLC) system. PFAS detection was achieved using a PerkinElmer QSight® 500 triple quadrupole mass spectrometer under negative ESI ionization mode. The LX50 Autosampler was modified by replacing all polytetrafluoroethylene (PTFE) based tubing with polyether ether ketone (PEEK) tubing to reduce any contamination from PFAS compounds introduced by the PTFE tubing. All instrument control, data acquisition and data processing were performed using the Simplicity™ 3Q Software.

Materials and Methods

Standards, Reagents and Samples

The mixed native PFAS standards and isotope labelled PFAS standards were purchased from Wellington Laboratories (Guelph, Ontario) and used to prepare quality control (QC) samples in solvent. LC-MS grade solvents methanol (MeOH), acetonitrile (ACN), and water, and other chemicals such as formic acid and ammonium acetate are obtained from MilliporeSigma. Disposable syringe filters, polypropylene (PP) centrifuge tubes, autosampler vials, delay column, guard column and analytical column were obtained from PerkinElmer (Shelton, CT, USA). Salmon fillets, avocado, multi-purpose spice powders, dog and cat feeds were purchased from local supermarkets (Woodbridge, Ontario, Canada).

Sample Preparation

Before extraction, the salmon fillet and avocado (~100 g each) were homogenized using a food processor, while 50 g each of the cat and dog feeds was ground into powder using a food processor. The food samples were extracted using a QuEChERS method that had been developed for PFAS analysis in food,^{1,2} but the final solid-phase extraction (SPE) step was omitted to maximize sample matrix components 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 MgSO₄, 1500 mg NaCl; ECMSSFCS-MP, UCT, Bristol, PA), the sample was vortexed and shaken for 5 min at 1500 rpm. After centrifuging at 4400 rpm for 10 min, the supernatant was transferred to a 15 mL dispersive (dSPE) tube (900 mg MgSO₄, 300 mg PSA, and 150 mg CGB (ECMPSCB15CT, UCT, Bristol, PA). This tube was then shaken/vortexed and centrifuged using the same conditions as before. The supernatant was transferred to a clean tube and evaporated to dryness and then 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 QC sample responses between large blocks of consecutive matrix injections (20 injections for each matrix, total 100 matrix injections per batch), as shown below in Figure 1.

LC Conditions and MS Parameters

The LC method and MS source parameters are shown in Table 1. Two C18 columns were used in this study: one was used as a delay column to separate possible interferent PFAS coming from the LC system; another was used as an analytical column to separate PFAS compounds and any interfering components. A guard column with the same phase was also used to protect the analytical column. The applied LC gradient program is shown in Table 2. MS Source parameters, including gas flows, source temperature and probe position settings, were optimized for maximum sensitivity. Compound-dependent parameters, such as collision energies (CE), entrance voltages (EV), and lens voltages (CCL2), were optimized for the target PFAS as shown in Table 3. During method development, the retention times for all PFAS peaks were determined, and then the potential interfering components from LC system and mobile phases were identified and separated from analyte peaks using a delay column. Finally, the MS acquisition method was generated using Simplicity™ software in the time-managed-MRM module with the retention times and corresponding retention time windows for all PFASs.

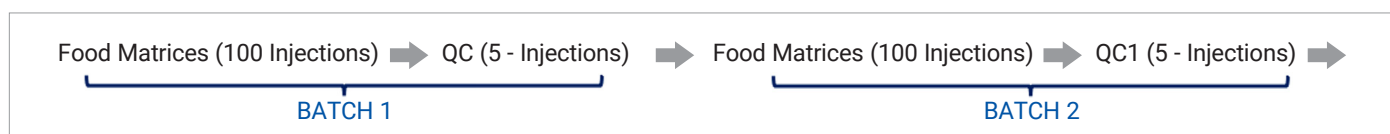


Figure 1. Sample injection sequences: Robustness was evaluated by consecutive injections of food matrix extracts that were bracketed by solvent QC replicates (100 food matrix injections for every 5 solvent QC injections).

Table 1. LC method and MS source conditions.

LC Conditions	
Analytical Column	Brownlee, SPP C18, 75 x 4.6 mm, 2.7 µm (N9308415), Guard (N9308515)
Delay Column	Brownlee, SPP C18, 50 x 3 mm, 2.7 µm (N9308408)
Mobile Phase A	5 mM Ammonium Acetate in Water
Mobile Phase B	LC/MS Grade Methanol
Mobile Phase Gradient	See Table 2
Flow Rate	0.75 mL/min
Column Oven Temperature	40°C
Auto Sampler Temperature	10°C
Injection Volume	5 µL for PFAS QC sample, and 10 µL for food matrices
Needle Wash 1 (Weak Wash)	15% Methanol in Water
Needle Wash 2 (Strong Wash)	100% Methanol
MS Source Conditions	
ESI Voltage (Negative)	-3800 V
Drying Gas	180
Nebulizer Gas	400
Source Temperature	350°C
HSID Temperature	280°C
Detection Mode	Time Managed MRM

Table 2. LC gradient program.

Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
0.00	85	15
0.25	85	15
0.50	35	65
2.50	10	90
3.50	10	90
3.60	85	15
5.00	85	15

Table 3. Optimized MRM parameters.

PFAS Analyte	Precursor Ion (m/z)	Product Ion (m/z)	RT (min)	CE	EV	CCL2	Quantifier /Qualifier	Type
¹³ C ₄ -PFBA	217.0	172.0	3.5	14	-4	40	Quantifier	IS
PFBA-1	213.0	169.0	3.5	13	-9	36	Quantifier	Analyte
PFBA-2	213.1	69.1	3.5	90	-14	124	Qualifier	Analyte
¹³ C ₅ -PFPeA	268.0	223.0	4.26	11	-10	104	Quantifier	IS
PFPeA-1	263.0	219.0	4.26	15	-8	80	Quantifier	Analyte
PFPeA-2	263.0	69.1	4.26	70	-12	88	Qualifier	Analyte
¹³ C ₂ -4:2FTS-1	329.0	81.0	4.56	38	-43	96	Quantifier	IS
¹³ C ₂ -4:2FTS-2	329.0	309.0	4.56	23	-14	120	Qualifier	IS
4:2FTS-1	327.0	81.0	4.56	38	-43	96	Quantifier	Analyte
4:2FTS-2	327.0	307.0	4.56	23	-1	110	Qualifier	Analyte
¹³ C ₅ -PFHxA-1	318.0	273.0	4.75	12	-4	96	Quantifier	IS
¹³ C ₅ -PFHxA-2	318.0	120.1	4.75	32	-10	112	Qualifier	IS
PFHxA-1	313.0	269.0	4.75	17	-10	55	Quantifier	Analyte
PFHxA-2	313.0	119.0	4.75	31	-10	50	Qualifier	Analyte
¹³ C ₃ -PFBS-1	302.0	80.0	4.88	67	-28	80	Quantifier	IS
¹³ C ₃ -PFBS-2	302.0	99.0	4.88	41	-28	80	Qualifier	IS
PFBS-1	299.1	80.1	4.88	63	-36	100	Quantifier	Analyte
PFBS-2	299.1	98.9	4.88	42	-21	104	Qualifier	Analyte
¹³ C ₄ -PFHpA	367.0	322.0	5.16	17	-6	75	Quantifier	IS
PFHpA-1	363.0	319.0	5.16	16	-10	56	Quantifier	Analyte
PFHpA-2	363.0	169.0	5.16	23	-10	92	Qualifier	Analyte
PFPeS-1	349.0	80.0	5.4	73	-6	100	Quantifier	Analyte
PFPeS-2	349.0	99.0	5.4	45	-6	90	Qualifier	Analyte
¹³ C ₂ -6:2FTS-1	429.0	81.0	5.38	43	-16	124	Quantifier	IS
¹³ C ₂ -6:2FTS-2	429.0	409.0	5.38	28	-11	152	Qualifier	IS

Table 3. Optimized MRM parameters CONTINUED..

PFAS Analyte	Precursor Ion (m/z)	Product Ion (m/z)	RT (min)	CE	EV	CCL2	Quantifier /Qualifier	Type
8:2FTS-1	527.0	81.0	6.56	70	-3	100	Quantifier	Analyte
8:2FTS-2	527.0	507.0	6.56	30	-62	220	Qualifier	Analyte
PFHpS-1	449.0	80.0	6.64	86	-20	144	Quantifier	Analyte
PFHpS-2	449.0	99.0	6.64	48	-20	136	Qualifier	Analyte
D ₃ -NMeFOSAA	573.0	419.0	6.72	27	-25	105	Quantifier	IS
NMeFOSAA-1	570.0	419.0	6.72	28	-20	100	Quantifier	Analyte
NMeFOSAA-2	570.2	169.0	6.72	40	-10	168	Qualifier	Analyte
NMeFOSAA-3	570.0	483.0	6.72	19	-20	100	Qualifier	Analyte
¹³ C ₆ -PFDA	519.0	474.0	6.92	17	0	88	Quantifier	IS
PFDA-1	513.1	469.1	6.92	16	-14	170	Quantifier	Analyte
PFDA-2	513.0	219.0	6.92	28	-10	96	Qualifier	Analyte
D ₅ -NETFOSAA-1	589.0	419.0	7.05	28	-20	112	Quantifier	IS
D ₅ -NETFOSAA-2	589.0	531.0	7.05	28	-20	105	Qualifier	IS
NETFOSAA-1	584.0	419.0	7.05	30	-20	100	Quantifier	Analyte
NETFOSAA-2	584.1	169.0	7.05	43	-20	174	Qualifier	Analyte
NETFOSAA-3	584.1	526.2	7.05	25	-20	176	Qualifier	Analyte
¹³ C ₈ -PFOS-1	507.0	80.0	7.16	107	-22	140	Quantifier	IS
¹³ C ₈ -PFOS-2	507.0	99.0	7.16	50	-22	140	Qualifier	IS
PFOS-1	499.1	80.0	7.16	107	-10	179	Quantifier	Analyte
PFOS-2	499.1	99.0	7.16	56	-10	161	Qualifier	Analyte
¹³ C ₇ -PFUnA	570.0	525.0	7.64	15	-14	184	Quantifier	IS
PFUnA-1	563.0	519.0	7.64	19	-10	96	Quantifier	Analyte
PFUnA-2	563.0	269.1	7.64	25	-14	184	Qualifier	Analyte
PFNS-1	549.0	80.0	8.06	96	-34	184	Quantifier	Analyte
PFNS-2	549.0	99.0	8.06	62	-34	184	Qualifier	Analyte
¹³ C ₂ -PFDoA	615.0	570.0	8.27	17	-14	104	Quantifier	IS
PFDoA-1	613.0	569.0	8.27	17	-10	104	Quantifier	Analyte
PFDoA-2	613.0	169.1	8.27	35	-14	180	Qualifier	Analyte
PFDoA-3	613.0	319.0	8.27	30	-14	150	Qualifier	Analyte
PFDS-1	599.1	80.1	8.55	138	-14	230	Quantifier	Analyte
PFDS-2	599.1	99.0	8.55	57	-14	240	Qualifier	Analyte
PFTrDA-1	663.0	619.0	8.7	18	-6	104	Quantifier	Analyte
PFTrDA-2	663.0	169.1	8.7	38	-14	220	Qualifier	Analyte
¹³ C ₂ -PFTeDA	715.0	670.0	9.01	18	-7	140	Quantifier	IS
PFTeDA-1	713.0	669.0	9.01	19	-4	120	Quantifier	Analyte
PFTeDA-2	713.0	168.9	9.01	40	-14	240	Qualifier	Analyte
PFDoS-1	698.9	79.9	9.15	130	-86	184	Quantifier	Analyte
PFDoS-2	698.9	99.0	9.15	69	-86	184	Qualifier	Analyte
¹³ C ₈ -PFOSA	506.0	77.8	8.98	48	-48	144	Quantifier	IS
PFOSA-1	498.0	78.0	8.98	48	-3	110	Quantifier	Analyte
PFOSA-2	498.0	478.0	8.98	30	-27	212	Qualifier	Analyte
¹³ C ₂ -PFTeDA	715.0	670.0	9.01	18	-7	140	Quantifier	IS
PFTeDA-1	713.0	669.0	9.01	19	-4	120	Quantifier	Analyte
PFTeDA-2	713.0	168.9	9.01	40	-14	240	Qualifier	Analyte
PFDoS-1	698.9	79.9	9.15	130	-86	184	Quantifier	Analyte
PFDoS-2	698.9	99.0	9.15	69	-86	184	Qualifier	Analyte
¹³ C ₈ -PFOSA	506.0	77.8	8.98	48	-48	144	Quantifier	IS
PFOSA-1	498.0	78.0	8.98	48	-3	110	Quantifier	Analyte
PFOSA-2	498.0	478.0	8.98	30	-27	212	Qualifier	Analyte

Results and Discussion

Robustness Performance

The food samples selected for the matrix injections (salmon, avocado, spice powder, cat and dog feeds) represent some of the most difficult food/feed matrices analyzed by routine testing laboratories. For routine food analysis, after QuEChERS extraction and d-SPE, additional clean-up by SPE is recommended to reduce matrix components. However, in this study, to increase the matrix components load onto the system, the final SPE clean-up step was removed.

Instrument robustness was evaluated and compared to one of the best competitors' systems based on the total number of food matrix injections before the instrument sensitivity declined to 50% of the initial response. As shown in Figure 2A, excellent robustness was observed on QSight system for perfluorooctanesulfonic acid (PFOS), the normalized raw peak areas of PFOS remained stable over 21700 injections, while the competitor's system remained stable up to 6400 injections (Figure 2A demonstrates superior robustness compared to competitor). Similar results are illustrated in Figures 3 - 8 (for perfluorobutanoic acid (PFBA), perfluorohexanesulfonic acid (PFHxS), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorohexane sulfonic acid (6:2 ETS), and perfluorooctanesulfonamide (PFOSA), respectively).

To obtain accurate analytical results, isotope-labeled internal standards (IS) are commonly used in analysis to compensate for sample matrix effects and variations during analysis due to same or similar responses expected from both the native and internal standards to the assay environment. And therefore, IS-corrected peak areas should be consistent throughout the whole analysis process for each targeted PFAS. For example, as illustrated in Figures 2 - 8B, the %CVs of the IS corrected area ratios of PFOS, PFBA, PFHxS, PFHxA, PFHpA, 6:2ETS, and PFOSA were 7.2%, 3.3%, 3.2%, 5.8%, 7.7%, 4.1%, and 3.0%, respectively, for >21700 injections on the QSight system, demonstrating great stability and consistency of the system and methodology. This reproducibility suggests that 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. However, uncorrected raw peak areas (as shown in Figure 2-8A) represent a more accurate measure of instrument performance robustness over time and can better inform the user when maintenance is required. This study was carried out for several months, of the 25 PFAS tested, most analytes retained >70% of the initial sensitivity on the QSight system past 21700 injections although the ion source was heavily contaminated with dirty sample matrices after these injections as shown in Figure 9.

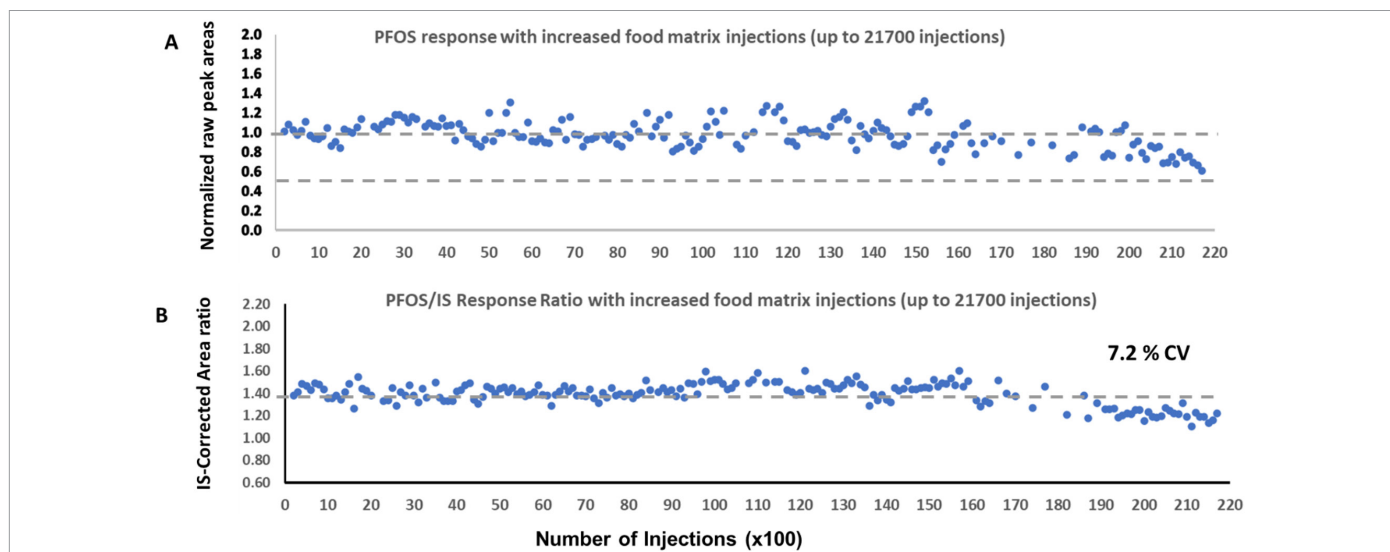


Figure 2. Raw peak areas normalized to initial response (A) and internal standard (IS)-corrected peak area ratios (B) for PFOS in QC samples on QSight system. Each datapoint represents the mean of 5 replicate injections. In panel A, the dotted lines represent the 100% and 50% raw peak areas relative 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 QC datapoint.

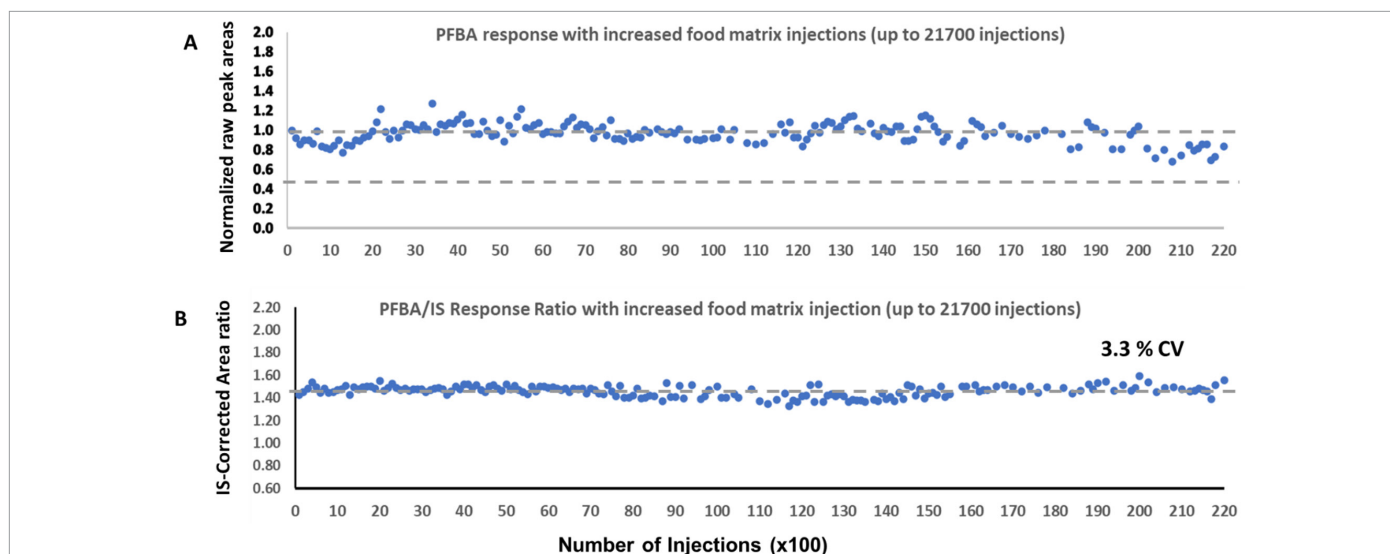


Figure 3. Raw peak areas normalized to initial response (A) and internal standard (IS)-corrected peak area ratios (B) for PFBA in QC samples on QSight system. Each datapoint represents the mean of 5 replicate injections. In panel A, the dotted lines represent the 100% and 50% raw peak areas relative 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 QC datapoint.

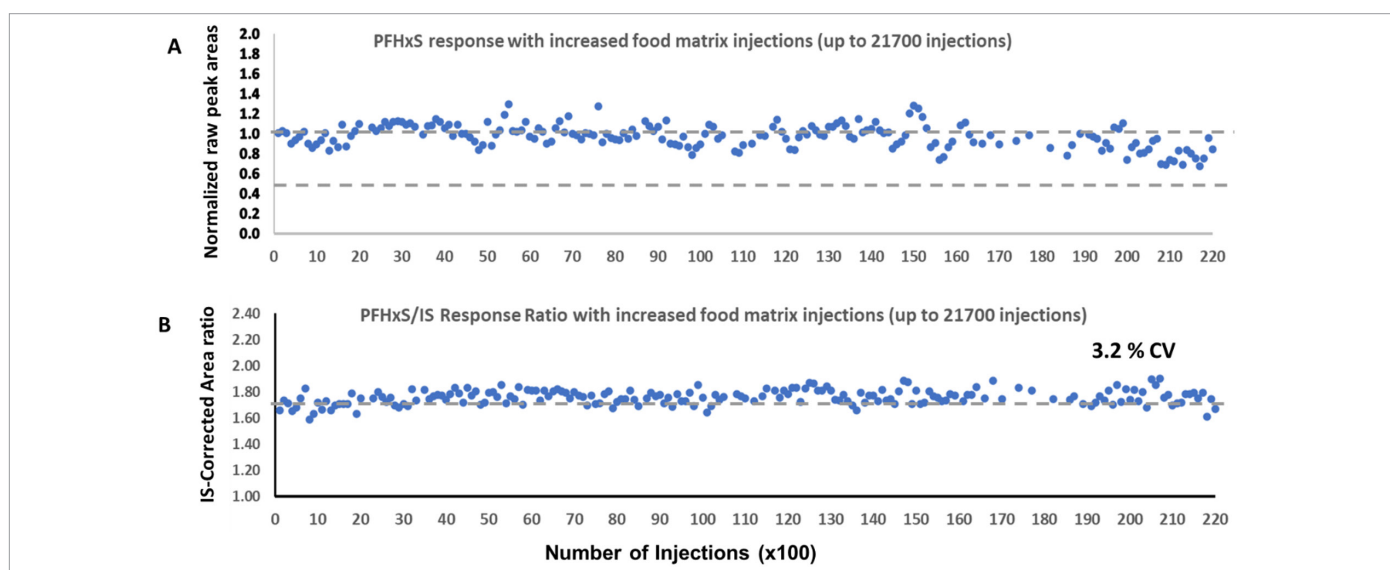


Figure 4. Raw peak areas normalized to initial response (A) and internal standard (IS)-corrected peak area ratios (B) for PFHxS in QC samples on QSight system. Each datapoint represents the mean of 5 replicate injections. In panel A, the dotted lines represent the 100% and 50% raw peak areas relative 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 QC datapoint.

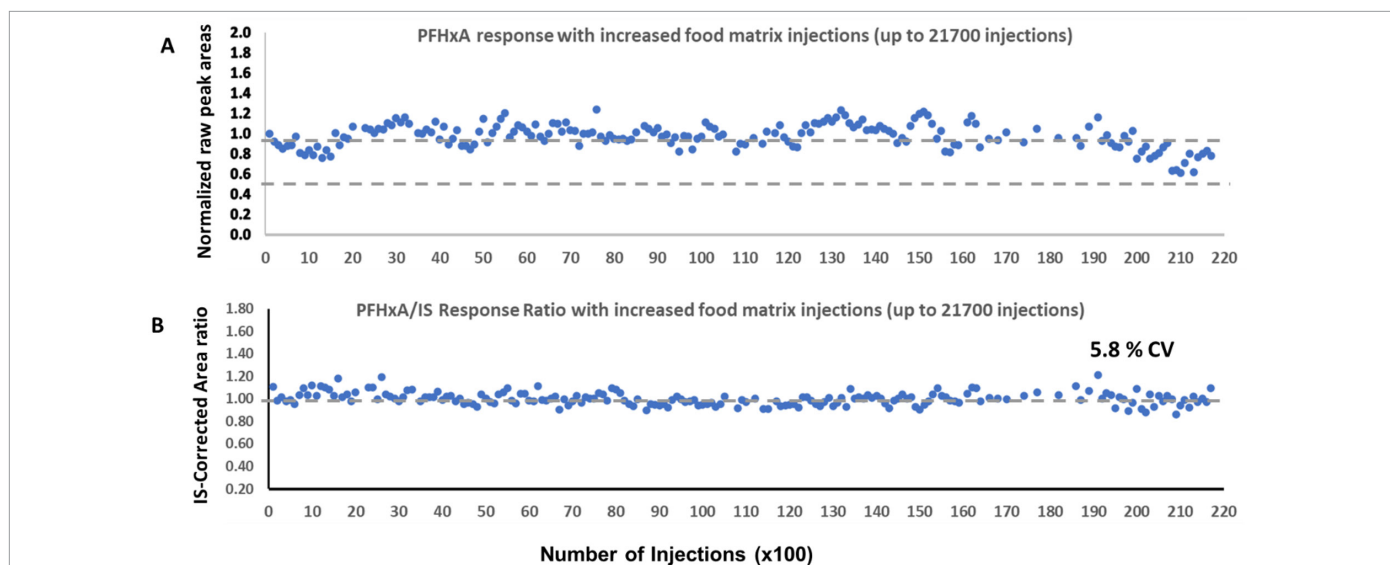


Figure 5. Raw peak areas normalized to initial response (A) and internal standard (IS)-corrected peak area ratios (B) for PFHxA in QC samples on QSight system. Each datapoint represents the mean of 5 replicate injections. In panel A, the dotted lines represent the 100% and 50% raw peak areas relative 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 QC datapoint.

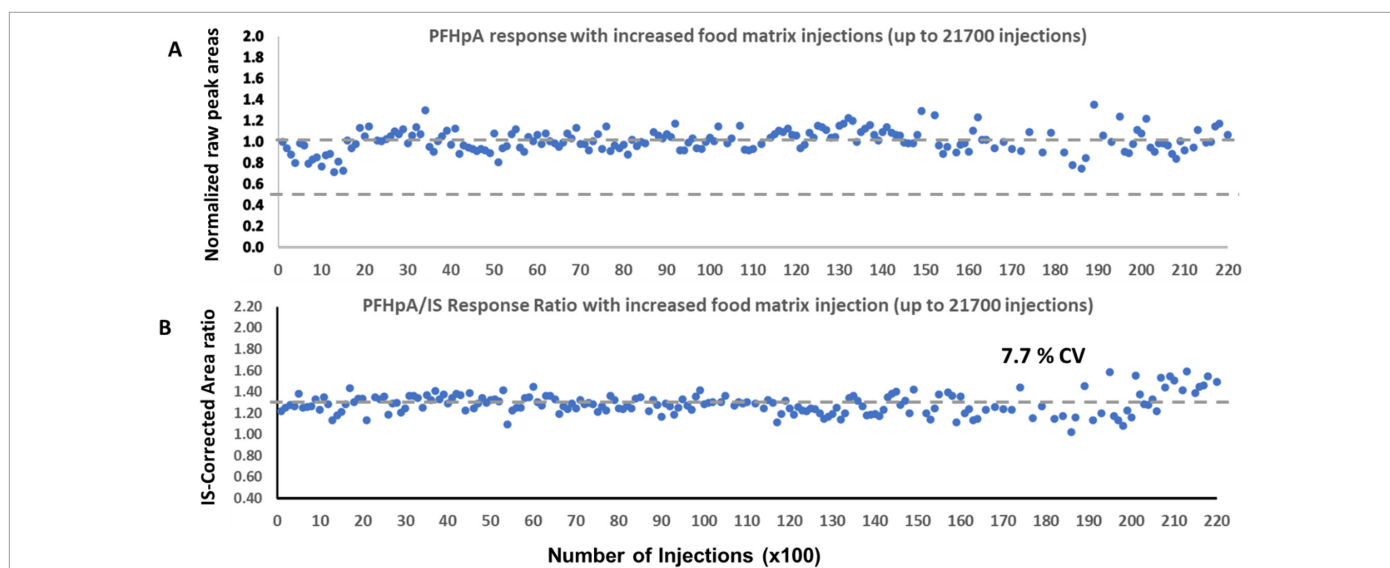


Figure 6. Raw peak areas normalized to initial response (A) and internal standard (IS)-corrected peak area ratios (B) for PFHpA in QC samples on QSight system. Each datapoint represents the mean of 5 replicate injections. In panel A, the dotted lines represent the 100% and 50% raw peak areas relative 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 QC datapoint.

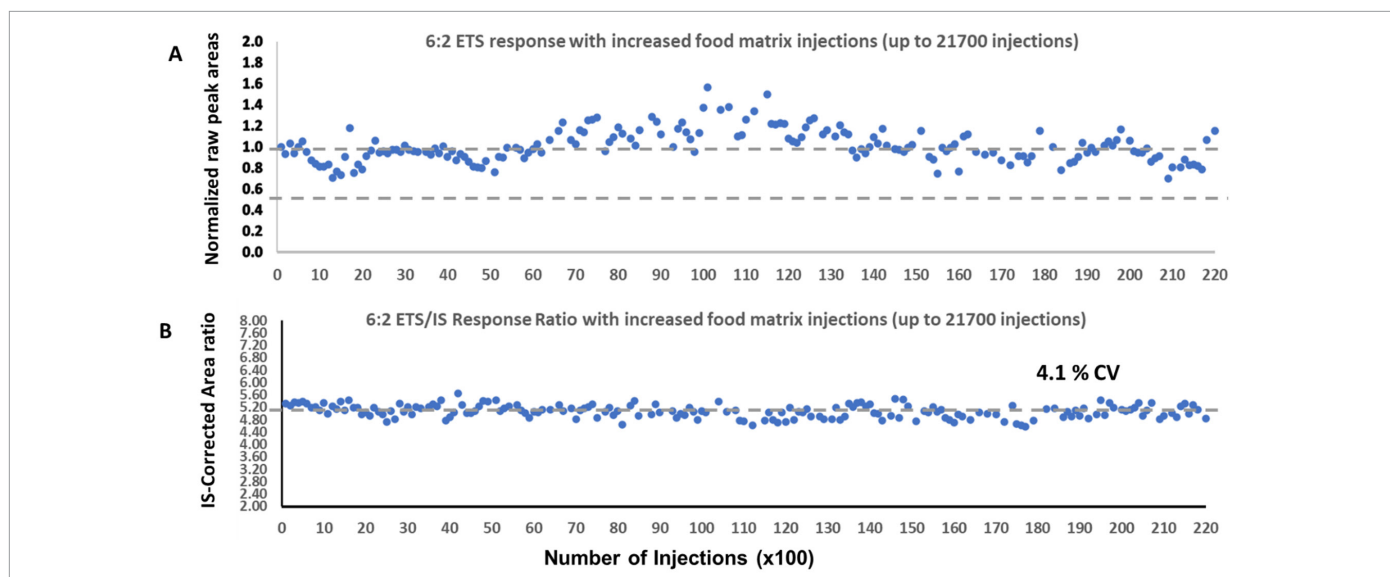


Figure 7. Raw peak areas normalized to initial response (A) and internal standard (IS)-corrected peak area ratios (B) for 6:2ETS in QC samples on QSight system. Each datapoint represents the mean of 5 replicate injections. In panel A, the dotted lines represent the 100% and 50% raw peak areas relative 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 QC datapoint.

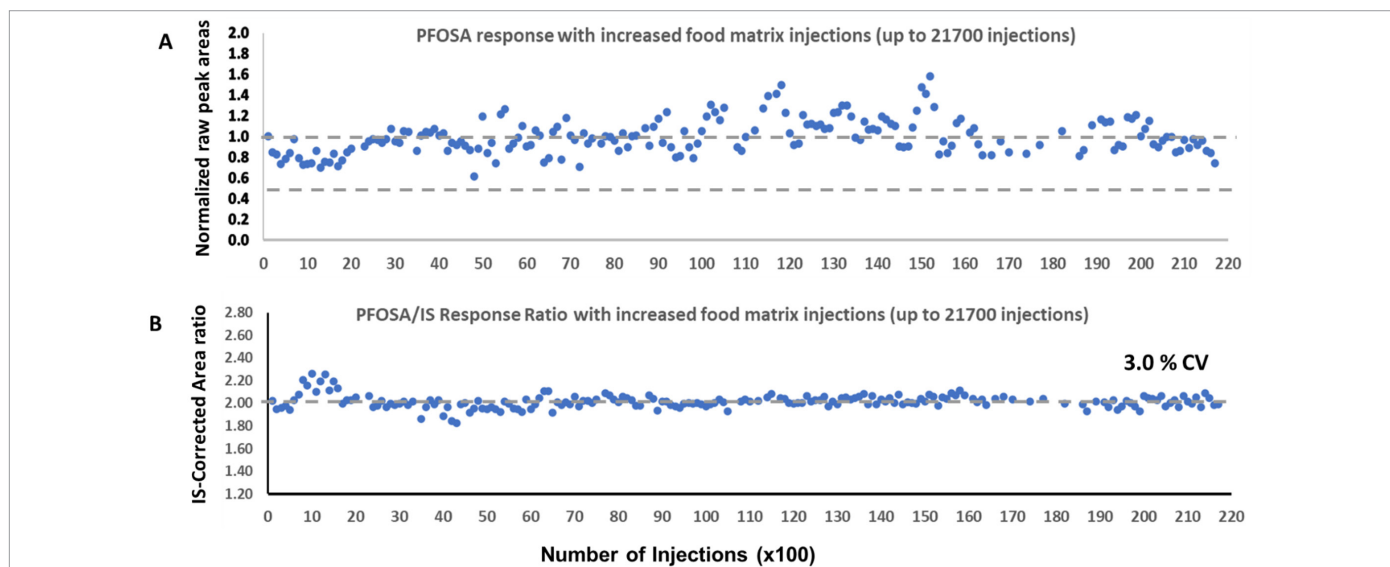


Figure 8. Raw peak areas normalized to initial response (A) and internal standard (IS)-corrected peak area ratios (B) for PFOSA in QC samples on QSight system. Each datapoint represents the mean of 5 replicate injections. In panel A, the dotted lines represent the 100% and 50% raw peak areas relative 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 QC datapoint.

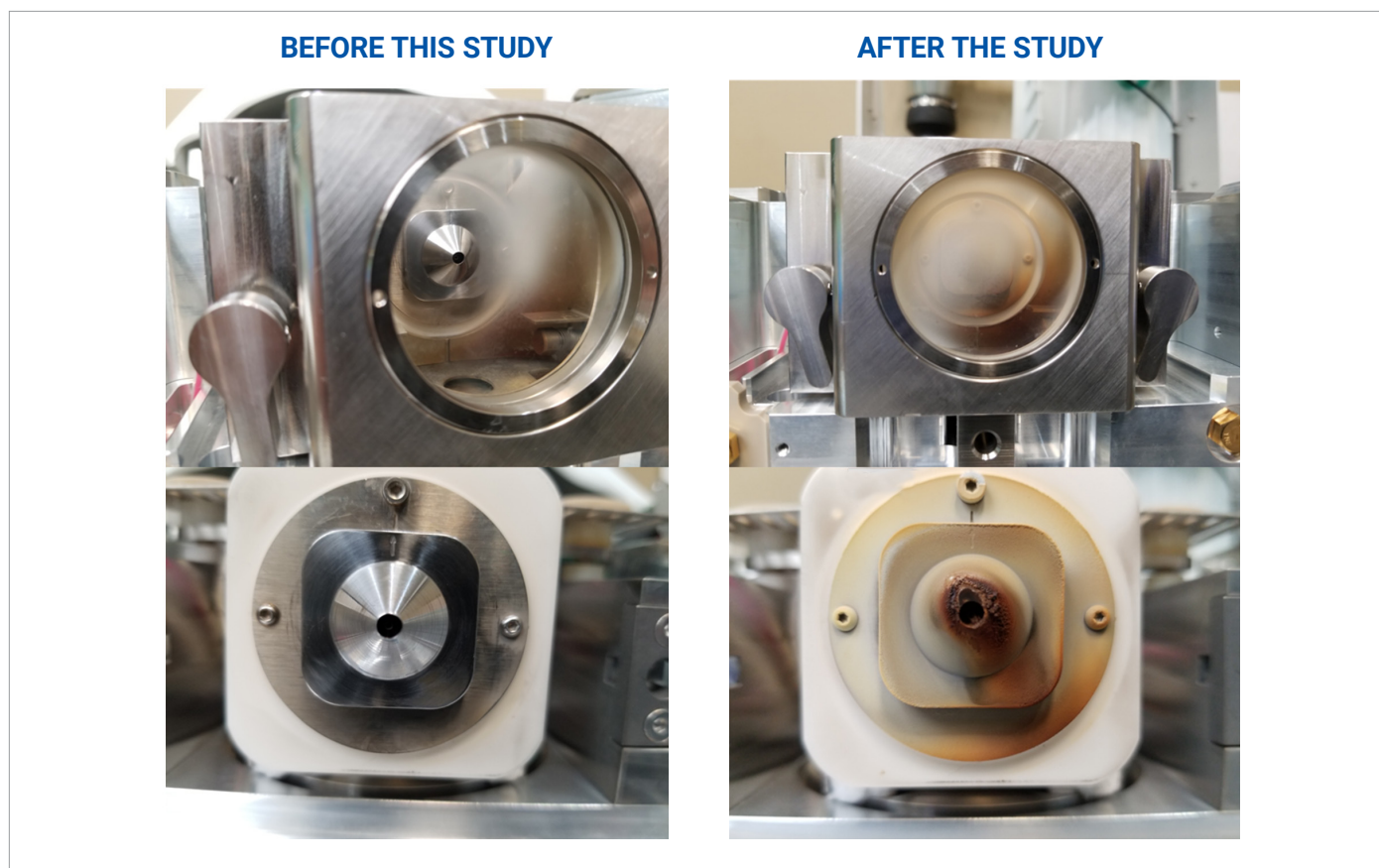


Figure 9. Comparison of the ion source cleanliness/contamination before and after the study.

Conclusions

QSight's StayClean™ technology with a hot-surface-induced desolvation (HSID™) system (which is estimated to provide 15% higher uptime than conventional LC/MS/MS systems) can effectively prevent mass detection system from contamination in complex matrices and thus provide unmatched robustness for analysis of the most difficult sample matrices. The system maintained quantitative performance even after >21700 injections of food extracts and solvent QC samples with most PFAS analytes retaining >70% of the initial peak area sensitivity. This competitive advantage of QSight system over any other systems on the market provides the customers the benefits of time saving, improved sample throughput and enhanced laboratory productivity.

References

1. Susan Genualdi, Wendy Young, Elsie Peprah, Cynthia Srigley, Christine M. Fisher, Brian Ng, Lowri DeJager. "Analyte and matrix method extension of per- and polyfluoroalkyl substances in food and feed". *Anal. Bioanal. Chem.* 2024, 416, 627-633
2. Susan Genualdi, Wendy Young, Lowri DeJager L. "Method development and validation of per- and polyfluoroalkyl substances in foods from FDA's Total Diet Study Program". *J Agric Food Chem.* 2021, 69(20), 5599-606.