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Application News

High Performance Liquid Chromatograph Mass Spectrometer

Breakthrough Sensitivity and Robustness for PFAS Analysis in Chicken Tissue for EPA Method 1633A

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User Benefits

- LCMS-8065XE achieved up to 80 times lower limit of quantifications than LLOQ concentration in EPA Method 1633A.
- The measurement time was 10 min while meeting the chromatographic requirements by EPA Method 1633A.
- This method showed the excellent robustness across 7 days of over 900 continuous injections of the matrix sample.

■ Introduction

Per- and polyfluoroalkyl substances (PFAS) are a diverse group of synthetic chemicals widely used in industry and consumer products. Owing to their persistence and potential health risks, PFAS have become a major environmental concern. The U.S. Environmental Protection Agency (EPA) has developed standardized analytical methods, including EPA Method 1633A, to monitor PFAS contamination in a wide range of environmental and biological matrices. This study evaluates the performance of LCMS-8065XE for PFAS analysis in accordance with EPA Method 1633A. By assessing calibration stability over the course of one week, we demonstrate the method's suitability for routine PFAS monitoring. All aspects of the workflow including sample extraction, preparation, and analysis, were performed in compliance with EPA Method 1633A guidelines[1]. Our results highlight the system's robustness and minimal downtime, underscoring its value for laboratories responsible for delivering rapid and reliable PFAS determinations.

Fig. 1 LCMS-8065XE. The combination of low-diffusion nebulizer nozzle and IonFocus™ technology achieve both high sensitivity and robustness.

■ Method Overview

This application describes the analysis of 40 native PFAS target compounds using 24 extracted internal standards (EIS), and 7 non-extracted internal standards (NIS). Stock standards were purchased from Wellington Laboratories (Ontario, Canada) as a series of native and mass-labelled PFAS mixtures in methanol (PFAC-MXF, PFAC-MXG, PFAC-MXH, PFAC-MXI, PFAC-MXJ, MPFAC-HIF-ES, and MPFAC-HIF-IS). Three spiking standards were prepared, containing the native targets, EIS and NIS compounds, by diluting the stock solutions in methanol. Calibration curves were generated by preparing methanol containing 4% water, 1% ammonium hydroxide, and 0.625% acetic acid. The stock standards were then diluted to yield concentration ranges of 2.5 to 62,400 ng/L for native PFAS targets, 1.0 to 20.0 µg/L for EIS, and 1.0 to 4.0 µg/L for NIS. All standards were prepared for analysis in 200 µL silanized glass inserts in 1.5 mL amber silanized glass vials, sealed with PE/silicone blue screw caps.

Table 1 EPA Method 1633A compound list

#	Type	Name	#	Type	Name
1	Target	PFBA	1	EIS	13C4-PFBA
2	Target	PFMPA	2	EIS	13C5-PFPeA
3	Target	3:3 FTCA	3	EIS	13C2-4:2 FTS
4	Target	PFPeA	4	EIS	13C5-PFHxA
5	Target	PFMBA	5	EIS	13C3-PFBS
6	Target	4:2 FTS	6	EIS	13C3-HFPO-DA
7	Target	NFDHA	7	EIS	13C4-PFHpA
8	Target	PFHxA	8	EIS	13C2-6:2FTS
9	Target	PFBS	9	EIS	13C8-PFOA
10	Target	HFPO-DA	10	EIS	13C3-PFHxS
11	Target	5:3 FTCA	11	EIS	13C9-PFNA
12	Target	PFEESA	12	EIS	13C2-8:2FTS
13	Target	PFHpA	13	EIS	D3-NMeFOSAA
14	Target	PFPeS	14	EIS	13C6-PFDA
15	Target	ADONA	15	EIS	D5-NEtFOSAA
16	Target	6:2 FTS	16	EIS	13C8-PFOS
17	Target	PFOA	17	EIS	13C7-PFUnA
18	Target	PFHxS	18	EIS	13C2-PFDoA
19	Target	7:3 FTCA	19	EIS	13C8-PFOSA
20	Target	PFNA	20	EIS	13C2-PFTeDA
21	Target	PFHpS	21	EIS	D7-NMeFOSE
22	Target	8:2 FTS	22	EIS	D3-NMeFOSA
23	Target	NMeFOSAA	23	EIS	D9-NEtFOSE
24	Target	PFDA	24	EIS	D5-EtFOSA
25	Target	NEtFOSAA	1	NIS	13C3-PFBA
26	Target	PFOS	2	NIS	13C2-PFHxA
27	Target	PFUnA	3	NIS	13C4-PFOA
28	Target	9Cl-PF3ONS	4	NIS	18O2-PFHxS
29	Target	PFNS	5	NIS	13C5-PFNA
30	Target	PFDOA	6	NIS	13C2-PFDA
31	Target	PFOSA	7	NIS	13C4-PFOS
32	Target	PFDS			
33	Target	PFTrDA			
34	Target	11Cl-PF3OUdS			
35	Target	PFTeDA			
36	Target	PFDOS			
37	Target	NMeFOSE			
38	Target	NMeFOSA			
39	Target	NEtFOSE			
40	Target	NEtFOSA			

■ Sample Preparation and Extraction

Automated extraction was performed using the EDGE PFAS system (see Fig. 2). Sample preparation procedures are described in Fig3. Approximately 2 grams of chicken tissue was weighed into a Q-Cup. Each sample was spiked with 25 µL of MPFAC-HIF-ES and 40 µL of native compounds. Method Blanks (MB) were also prepared, spiked only with EIS. Samples were extracted by solid phase extraction (SPE) using Supelclean™ ENVI-WAX SPE Tube (Millipore Sigma). Silanized glass wool was added to each cartridge prior to extraction, and the cartridges were pre-conditioned with 1% methanolic ammonium hydroxide.

Figure 2. The appearance of EDGE PFAS (CEM) which automates the extraction of PFAS from field samples.

Automated extraction procedures by EDGE PFAS

Cycle 1	・Weigh 2 grams of tissue sample into Q-Cup ・0.05 M KOH in methanol, 10mL, 65 °C, 3 min
Cycle 2	・Acetonitrile, 10mL, 65 °C, 3 min
Wash 1	・IPA, 10 mL
Wash 2	・0.05 M KOH in methanol, 10 mL, 65 °C, 0.5 min
Wash 3	・0.05 M KOH in methanol, 10 mL

Clean-up procedures

Condition	・Insertion of silanized glass wool to the WAX SPE cartridge ・15 mL 1% methanolic ammonium hydroxide ・5mL 0.3 M formic acid
Sample Loading	・5 mL/min
Washing	・5 mL Water (twice) ・5mL 1:1 0.1M formic acid/methanol
Elution	・5mL 1% methanolic ammonium hydroxide
Cleanup	・25 µL Acetic Acid ・Handshake ・Transfer to LC vial

Figure 3. Extraction and clean-up scheme

Samples were loaded onto WAX cartridges at a flow rate of 5 mL/min. The cartridges were rinsed with LC/MS grade water followed by 0.1 mol/L formic acid/methanol and then dried under vacuum for 15 seconds. Elution was performed by rinsing the sample bottles with 1% methanolic ammonium hydroxide and passing the eluate through the WAX cartridge. Acetic acid was added to each extract, which was then hand-shaken for up to five minutes and centrifuged for ten minutes. An aliquot of the supernatant was transferred to a 1 mL silanized amber glass vial and spiked with 0.5 µL MPFAC-HIF-IS this vial was vortexed prior to LC/MS analysis.

■ Instrument and Operational Conditions

LCMS analyses were performed using a Shimadzu triple quadrupole mass spectrometer, LCMS-8065XE, coupled with a Shimadzu Nexera™ 40 series UHPLC. To minimize PFAS background contamination, a delay column was installed between the mixer and high-pressure valve. The LC and MS parameters are summarized in Table 2 and 3. Analyses included a calibration curve, instrument blank, a calibration verification (CV), method blanks, and spiked chicken tissue samples.

A robustness test was conducted by monitoring calibration verification, method blanks, and spiked chicken tissue samples. Prior to each LC-MS/MS run, every vial was vortexed to resuspend PFAS compounds that may have adsorbed to the vial walls. This procedure helped improve the relative standard error (RSE), as PFAS compounds are knows to adsorb to glass surfaces.

Table 2. LC conditions

System	Nexera X3
Delay Column	Shim-pack Scepter™ C18-120, 2.1 × 100 mm, 3 µm (P/N: 227-31035-05)
Analytical Column	Shim-pack Scepter C18-120, 2.1 × 50 mm, 1.9 µm (P/N: 227-31033-03)
Flow rate	0.3 mL/min
Mobile phase A	2mM Ammonium Acetate in Water
Mobile phase B	Acetonitrile
Injection volume	5 µL standard injection
Oven temperature	35 °C
Measurement time	10 min

Table 3. MS conditions

System	LCMS-8065XE
Nebulizing gas	1.1 L/min
Heating gas	15.0 L/min
Drying gas	5.0 L/min
Interface Temperature	225°C
DL Temperature	200°C
Heat Block Temperature	250°C
Interface voltage	-0.5 kV
Focus voltage	0 kV

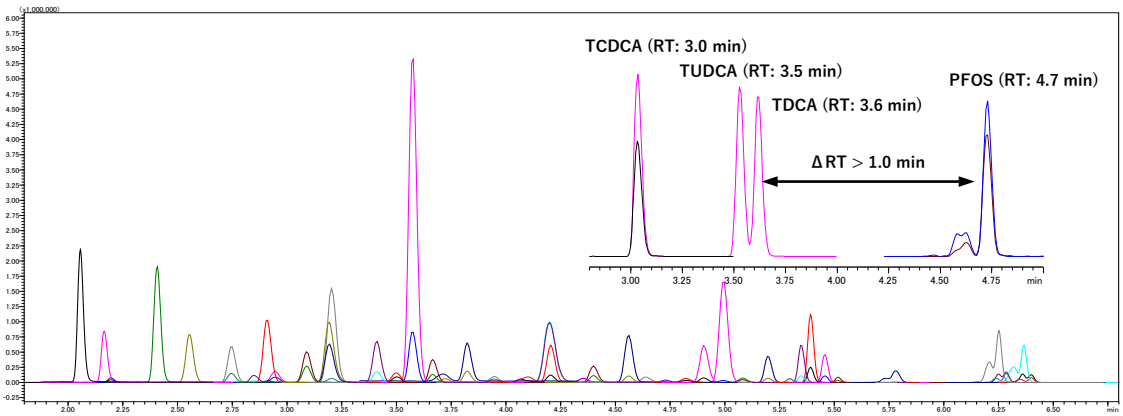

Figure 4. MS chromatogram of 40 PFAS and 3 cholic acids.
Measuring time for 40 PFASs was 10 min.

■ Chromatographic separation

Cholic acids, such as taurodeoxycholic acid (TDCA), taurochenodeoxycholic acid (TCDCA), and tauroursodeoxycholic acid (TUDCA), can interfere with PFOS during the ionization process because their precursor and product ions are similar. These bile acids are present in tissue and wastewater samples. Therefore, one of the requirements of the LC method for EPA Method 1633A is to achieve a separation of at least 1 min between PFOS and these cholic acids. In this study, acetonitrile was used as mobile phase B to meet this requirement. Figure 4 shows the chromatographic separation of forty PFAS listed in EPA Method 1633A. With acetonitrile, the cholic acids eluted much earlier than both branched and linear PFOS. The retention time difference was 1.5 min, which exceeded the required one-minute separation.

■ Calibration Curve Results

The relative standard error (RSE) of all native target PFAS ranged from 3% to 17%, remaining below the maximum limit of 20% specified in the EPA Method 1633A. Table 4 summarizes the concentration ranges, RF RSE values and calibration correlations for 40 PFAS compounds. All calibration curves, blanks and MS chromatograms at the LLOQ are shown in Figure 5. Each calibration curve included at least seven calibration standards within the linear quantitative range. The generated calibration curves exhibited linear regression with R^2>0.995. All accuracy ranges at each calibration points were within 70-130%.

Table 4. Calibration ranges, RF RSE values and correlation R^2 and accuracy ranges for 40 PFAS

#	Name	Calibration Range [ng/L]	EPA LLOQ conc. [ng/L]	RF RSE (curve)	Linearity (R2)	% Accuracy range
1	PFBA	10-10000	800	4	0.998	95-105
2	PFMPA	5-5000	400	7	0.995	92-113
3	3:3 FTCA	62.4-62400	998	7	0.995	90-113
4	PFPeA	5-5000	400	4	0.998	95-106
5	PFMBA	5-5000	400	6	0.996	92-110
6	4:2 FTS	10-10000	800	7	0.995	88-106
7	NFDHA	5-5000	400	7	0.995	89-111
8	PFHxA	2.5-2500	200	6	0.996	94-114
9	PFBS	2.5-2500	200	6	0.997	92-110
10	HFPO-DA	10-10000	800	4	0.998	93-106
11	5:3 FTCA	2.5-2500	4992	5	0.997	92-106
12	PFEESA	5-5000	400	3	0.999	95-104
13	PFHpA	2.5-2500	200	5	0.997	92-109
14	PFPeS	2.5-2500	200	6	0.996	92-108
15	ADONA	10-10000	800	5	0.998	91-105
16	6:2 FTS	2.5-2500	800	7	0.995	94-111
17	PFOA	2.5-2500	200	6	0.996	95-113
18	PFHxS	2.5-2500	200	6	0.996	87-107
19	7:3 FTCA	25-25000	4992	5	0.997	94-106
20	PFNA	2.5-2500	200	6	0.996	94-109
21	PFHpS	2.5-2500	200	7	0.995	92-111
22	8:2 FTS	2.5-2500	800	17	0.995	85-116
23	NMeFOSAA	6.3-2500	200	7	0.995	89-108
24	PFDA	2.5-2500	200	6	0.996	92-109
25	NEtFOSAA	6.3-2500	200	7	0.995	90-109
26	PFOS	6.3-2500	200	6	0.997	94-110
27	PFUnA	2.5-2500	200	4	0.998	94-105
28	9Cl-PF3ONS	10-10000	800	6	0.997	91-109
29	PFNS	2.5-2500	200	7	0.995	90-110
30	PFDOA	2.5-2500	200	5	0.997	94-109
31	PFOSA	2.5-2500	200	5	0.997	95-111
32	PFDS	2.5-2500	200	7	0.995	92-112
33	PFTrDA	2.5-2500	200	5	0.997	90-106
34	11Cl-PF3OUdS	10-10000	800	4	0.998	94-106
35	PFTeDA	2.5-2500	200	6	0.996	93-111
36	PFDOS	6.3-2500	200	9	0.997	84-116
37	NMeFOSE	25-25000	2000	6	0.996	93-113
38	NMeFOSA	2.5-2500	200	5	0.997	92-111
39	NEtFOSE	25-25000	2000	3	0.999	95-110
40	NEtFOSA	6.3-2500	200	5	0.997	96-106

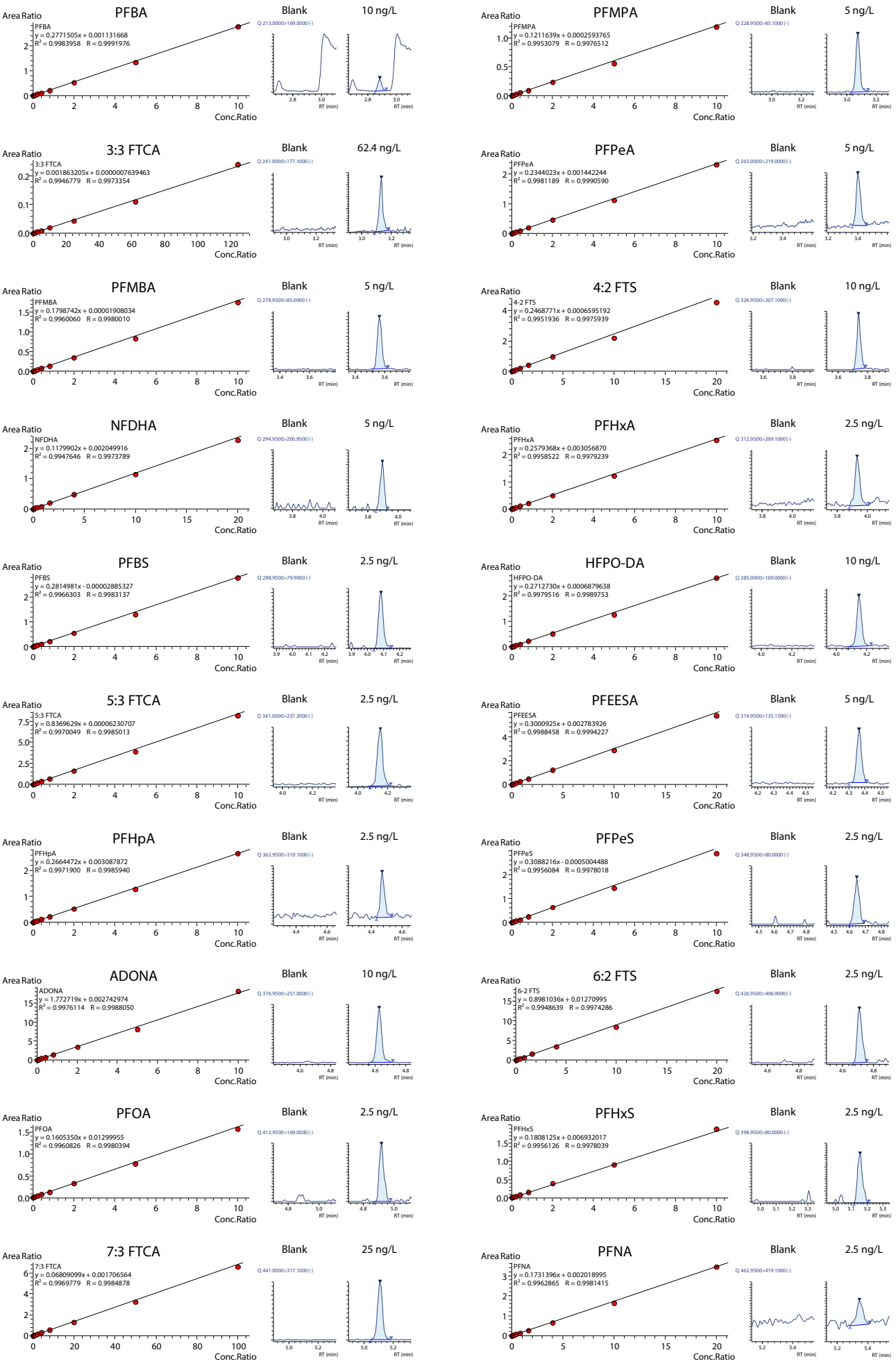

Figure 5. Calibration curves and MS chromatograms of blank and LLOQ for 40 PFAS compounds

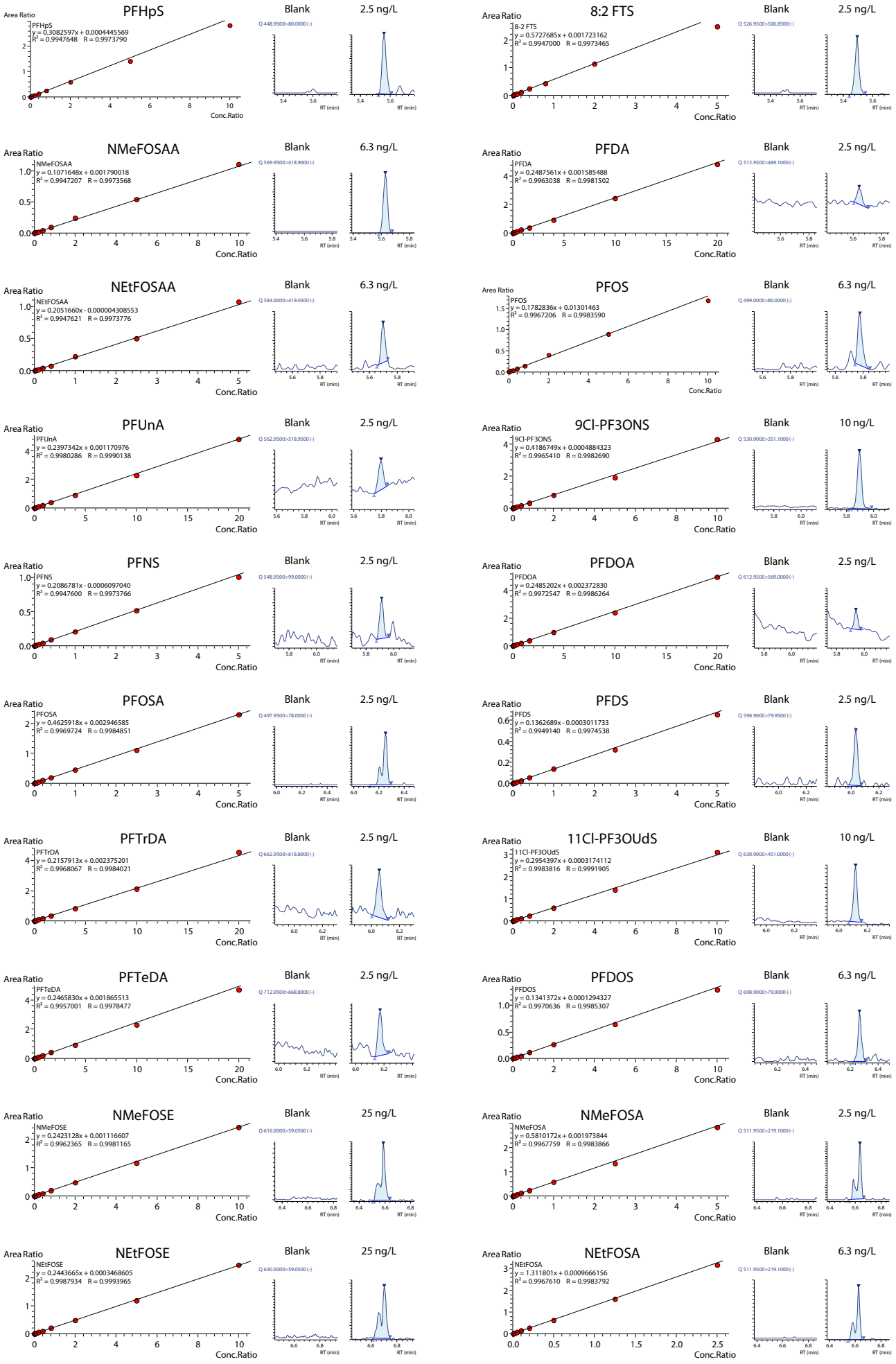

Figure 5 (continued). Calibration curves and MS chromatograms of blank and LLOQ for 40 PFAS compounds

■ Robustness test

The level 5 calibration standard was used as the continuing calibration verification (CCV). This concentration was four times lower than the required LLOQ specified in EPA Method 1633A. CCVs were analyzed to monitor quantitative performance stability. Figure 6 shows the accuracy of representative PFBS, HFPO-DA, PFOA, PFHxS, PFNA, and PFOS in CCV samples. A total of 930 injections were performed, with CCVs injected after the analysis of several chicken tissue samples during the test. All CCVs (n=73), shown in Figure 6, demonstrated accuracies within 70-130%. These results highlight the excellent robustness of the LCMS-8065XE for analysis in complex sample matrices.

■ Conclusion

The LCMS-8065XE was able to detect concentrations up to 80 times lower than the LLOQ required in EPA Method 1633A, using a neat standard solution.

Excellent linearity was achieved with the developed method, as indicated by RSE values below 20% and high R^2 values. This method demonstrates high throughput and robust instrument performance, maintaining accurate quantification in complex chicken tissue matrices without the need for maintenance or cleaning.

■ Reference

(1) Method 1633A, Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/MS

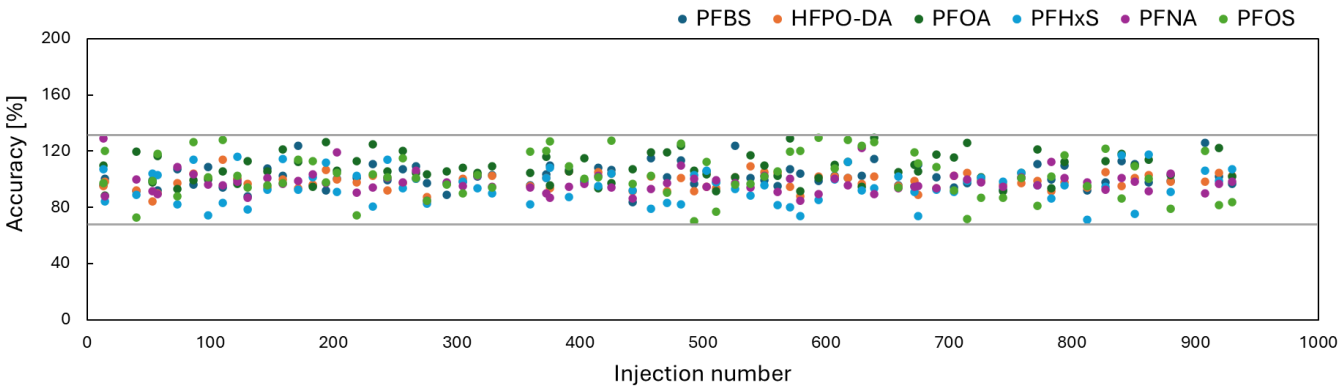

Figure 6. Accuracy plots for PFBS, HFPO-DA, PFOA, PFHxS, PFNA and PFOS in continuing calibration verification (CCV) samples.
CCVs were injected after several chicken tissue samples during robustness test.
CCV accuracies were within ±30% (gray line) from assigned concentration level over 900 injections.

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