SHIMADZU Demonstration of LC-MS/MS Performance for EPA 1633A: Robustness and Sensitivity for PFAS Analysis in Water, Soil, and Tissues

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1. Overview

Demonstration of a sensitive and robust LC-MS/MS method for quantifying PFAS over extended time (~1,500 injections) in multi-matrices by EPA 1633A.

2. Introduction

Regulatory agencies, such as EPA, have standardized analytical methods like EPA Method 1633A to monitor Per- and poly-fluoroalkyl substances (PFAS). To meet the quality criteria (QC) outlined in the method, laboratories must ensure accurate and precise results for diverse and challenging matrices such as soil, water, and tissue matrices on a routine basis. This study evaluated the performance of a Shimadzu LCMS-8060RX, including calibration stability and adherence to QC, over an extended period to time by analyzing extracts from diverse environmental matrices. All steps of the workflow were performed following EPA 1633A. Results demonstrate a robust and reliable performance of the LC-MS/MS with minimal downtime, essential for testing laboratories to meet the analytical demand for fast turn-around-time of PFAS monitoring.

3. Method

The quantitative analysis was conducted for 40 PFAS compounds in strict accordance with EPA Method 1633A, using a 13-minute cycle time for each injection. Chromatographic separation of PFAS was achieved on a C18 column utilizing gradient elution (figure 1). The mobile phases consisted of 2 mM ammonium acetate in water (aqueous phase) and acetonitrile (organic phase, no additives). To further minimize background interference from native PFAS potentially present in solvents or the LC system, a C18 delay column was strategically implemented after mixer in the LC system. With this conditions, the chromatographic separation between the TDCA and PFOS linear peaks greater than 1.5 minutes was achieved (see insert in figure 1).



Figure 1. Chromatogram of 40 PFAS and their corresponding internal standards.

requirement.

4. Results and Discussion

A calibration curve covering a range of 0.04 to 100 ppb, with 7 calibration levels, was established, utilizing appropriate internal standard concentrations. The method achieved limits of quantitation (LOQs) up to 21 times lower than EPA Calibration Level 1, while maintaining strict adherence to quality control requirements for precision and accuracy as shown in Table 1. Representative calibration curves are shown in Figure 2.

	Week 1		Week 2			Week 1		Week 2	
Compound Name	RF RSE (curve)	%RSD (curve)	RSE (curve)	%RSD (curve)	Compound Name	RF RSE (curve)	%RSD (curve)	RSE (curve)	%RSD (curve)
PFBA	3	5	10	10	8:2 FTS	10	12	6	5
PFMPA	4	5	3	3	PFHpS	7	10	5	10
3:3 FTCA	5	5	15	14	NMeFOSAA	10	10	4	5
PFPeA	4	7	9	10	PFDA	9	10	6	15
PFMBA	6	7	4	4	NEtFOSAA	7	7	4	4
4:2 FTS	7	7	4	4	PFOS	6	7	9	8
NFDHA	7	8	4	5	PFUnA	7	6	2	7
PFHxA	11	14	7	14	9CIPF3ONS	10	10	7	9
PFBS	6	8	8	7	PFNS	9	11	5	5
HFPODA	5	15	8	14	PFDOA	11	11	5	5
5:3 FTCA	8	9	7	8	PFOSA	7	7	5	4
PFEESA	8	11	5	6	PFDS	9	10	4	4
PFHpA	6	5	15	15	PFTrDA	10	10	5	5
ADONA	8	9	5	5	11ClPF3OUd S	10	10	6	5
6:2 FTS	8	8	4	3	PFTeDA	7	8	2	2
PFPeS	7	10	5	4	NMeFOSE	8	8	3	5
PFOA	7	17	6	13	NMeFOSA	8	8	6	6
7:3 FTCA	9	11	4	5	PFDOS	9	11	6	7
PFHxS	10	14	5	8	NEtFOSE	8	9	4	4
PFNA	9	10	3	18	NEtFOSA	8	8	4	5

Sample matrices-including soil, water, and tissue-were extracted and prepared following the detailed protocols outlined in EPA 1633A. All extracts, as well as standards and QC were provided by Eurofins to ensure the integrity and consistency of the study. LC-MS/MS analysis strictly followed the EPA's 1633A guidelines to maintain compliance with all critical QC requirements. These included analysis of instrument blanks, method blanks, sensitivity checks, calibrators, calibration verification (CV) standards, and representative sample matrices to rigorously monitor system performance and data quality. Chromatographic parameters were optimized for the effective separation of both bile salts (TUDCA, TCDCA and TDCA) and PFOS, in accordance with EPA

Over a two-week period, approximately 1,500 injections were performed on the LCMS-8060RX, encompassing calibration standards, quality control samples. To ensure data reliability and method integrity during this extended analysis, calibration verification was conducted after every 10 sample matrices, while daily Instrument Sensitivity Checks LCMS-8060RX operated continuously and reliably throughout the study without requiring any cleaning or maintenance despite the complexity of the analyzed matrices. This robust performance highlights the system's exceptional suitability for highthroughput, routine analysis in rigorous environmental and biological testing scenarios.

Table 1. RF RSE and % RSD for the calibration curve for week 1 and week 2.



Instrument Sensitivity check (ISC) were analyzed daily in duplicate throughout the study. An overlay of 10 ISC injections for six selected PFAS compounds is presented in Figure 2, demonstrating reproducibility at the LOQ level. For most PFAS compounds at the LOQ (ISC), percent accuracy ranged from 70–130%, with percent relative standard deviations (%RSD) below 20%.CCVs were injected every 10 field samples that included soil, water, and tissue extracts over a two weeks period. All CCVs (n=112), shown in Figure 3, demonstrated accuracy within 70–130%, except for 3:3 FTCA in a limited number of injections. %Accuracy range and %RSD of all targets are listed in Table 2. These results highlight the Shimadzu system's excellent robustness across a variety of sample matrices.

5. Conclusions

The Calibration verification and instrument sensitivity checks consistently met all requirements outlined in EPA method 1633A, demonstrating stable instrument performance throughout the study. The results also confirmed the instrument's ability to maintain accurate measurements when analyzing complex sample matrices such as soil, water, and tissue, without requiring maintenance or Cleaning.





	Week 1		Week 2			Week 1	Week 2		2
Compound Name	% Accuracy Range	% RSD	% Accuracy Range	% RSD	Compound Name	% Accuracy Range	% RSD	% Accuracy Range	% RSD
PFBA	89-100	4	92-104	5	8:2 FTS	102-127	5	84-110	4
PFMPA	104-114	2	96-106	2	PFHpS	105-127	4	97-113	3
3:3 FTCA	103-135	6	80-115	6	NMeFOSAA	78-116	9	83-111	8
PFPeA	103-114	2	88-99	3	PFDA	103-128	5	88-109	4
PFMBA	105-114	2	96-105	2	NEtFOSAA	93-129	8	88-100	3
4:2 FTS	90-110	4	92-104	3	PFOS	99-122	5	86-100	3
NFDHA	92-128	6	90-117	6	PFUnA	99-119	5	94-105	2
PFHxA	103-123	5	89-100	3	9CIPF3ONS	88-113	6	97-120	5
PFBS	101-124	5	89-104	3	PFNS	99-124	5	97-110	3
HFPODA	90-120	6	82-109	6	PFDOA	101-118	4	90-101	3
5:3 FTCA	86-116	9	75-98	9	PFOSA	98-121	5	95-104	2
PFEESA	101-121	5	82-104	7	PFDS	97-125	5	95-112	3
PFHpA	86-108	6	84-110	9	PFTrDA	96-124	6	91-112	5
ADONA	86-116	7	82-108	6	11CIPF3OUdS	96-121	5	86-112	7
6:2 FTS	102-122	4	94-108	3	PFTeDA	101-125	5	93-109	4
PFPeS	101-127	4	88-101	3	NMeFOSE	100-122	4	89-107	5
PFOA	100-122	5	85-95	3	NMeFOSA	90-121	6	85-113	5
7:3 FTCA	90-116	7	82-102	7	PFDOS	99-127	6	91-112	4
PFHxS	81-122	9	93-101	2	NEtFOSE	99-126	5	92-108	4
PFNA	78-116	9	94-107	3	NEtFOSA	91-121	6	90-106	3

6. Reference

EPA Method 1633A. Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Water, Soil, and Tissue Samples by LC-MS/MS. U.S. Environmental Protection Agency, Washington, DC, 2024. All content contained herein resulted solely from Shimadzu and Eurofins, and no conflict of interest exists. Disclaimer: The products and applications in this presentation are intended for Research Use Only (RUO). Not for use in diagnostic procedures.

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Figure 3. % accuracy of 40 PFAS in calibration verification; n=112 during two weeks, every 10 field samples.

Table 2. % Accuracy and Precision (%RSD) of CCV Throughout Weeks 1 and 2.