US EPA SW-846 Method 8327

Multi-Laboratory Validation Study

Quality Control Summary Report

Introduction: This document summarizes quality control (QC) results from the multi-laboratory validation of SW-846 Method 8327 *Per- and Polyfluoroalkyl Substances (PFAS) Using External Standard Calibration and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)* in four water matrices using the dilution preparation method in Appendix B of Method 8327 (future Method 3512). Precision and bias were evaluated for 24 PFAS target chemicals and 19 associated isotopically-labeled surrogates in reagent water (RW), ground water, (GW), surface water (SW) and waste water effluent (WW), and those results are discussed in the Statistical Analysis Report for SW-846 Method 8327 Multi-Lab Validation Study, June 2019 (hereafter referred to as the statistics report). Only the QC results associated with study sample analyses are discussed in this report.

Validation Study Design: The validation of SW-846 Method 8327 (hereafter referred to as the method) was performed in a multi-step process. In Phase I, laboratories performed and submitted initial demonstration of capability (IDOC) information which included initial calibration, lower limit of quantitation (LLOQ), and analyst demonstration of capability using PFAS standards provided. These results are not presented in this document. Phase II of the study was conducted in two steps, with blind replicate spiked and unspiked samples of each matrix, shipped to six internal laboratories in 2017, and similarly prepared blind samples shipped to seven external laboratories in 2018. Each laboratory was tasked with 1) following the method and study instructions for sample preparation and analysis, 2) striving to meet the recommended acceptance criteria for sample preparation and analysis in the method (refer to Table 7) and study instructions, and 3) returning data to the study team in a prescribed format. Of the 13 labs that received study samples, one of the external laboratories did not provide sample results by the submittal deadline.

Aqueous samples (5 mL) were prepared by the EPA Region 5 Laboratory in 15 mL polypropylene containers with screw-cap lids. Five replicates of each sample matrix (RW, GW, SW and WW) were provided to laboratories unspiked (i.e., with no target compounds added) and at spiked concentrations of 60 and 200 ng/L (nom.) in 5 mL water, for a total of 15 replicates per matrix, with a total of 60 sample containers shipped to each laboratory. Trip blanks (2) were also included with shipments of study samples to participating laboratories.

Laboratories were instructed to follow the sample preparation protocols embedded in the method (Appendix B) by dilution (1:1) with a water-miscible organic solvent (methanol) followed by manual filtration through a particle filter (0.2 µm) and addition of acetic acid (0.1% by volume) prior to analysis.

Laboratories were instructed to follow the sample analysis protocols embedded in the method and additional information provided in the study instructions. See these documents for detailed information. In the study instruments from several manufacturers were used to ensure that the range is applicable across a variety of platforms.

To minimize variables, laboratories were provided with PFAS target and surrogate stock standards and supplies for the study, including glass luer-lock syringes, filters, a liquid chromatography column, and autosampler vials.

Data Evaluation: EPA staff evaluated the data from the 12 laboratories who did submit for compliance with the study instructions and overall usability. Data from four laboratories were excluded for not following the specified protocols (more detail about the basis used for exclusion of each laboratory's data is provided in Appendix E of the statistics report. Data verification and validation were performed by contract staff for completeness, correctness, compliance, and analytical quality against criteria provided in the method and study instructions. Statistical analyses were performed on the data from the remaining eight laboratories and are presented in the statistics report.

A summary of laboratory performance for instrumental analysis and sample preparation Quality Controls (QC) is presented in the following sections by QC type. Categories of instrumental QC included initial calibration, continuing calibration verification, and reagent blanks. Categories of QC that addressed both sample preparation and analysis included method blanks, lower limit of quantitation (LLOQ) verification, laboratory control samples (LCS; spiked blank), and surrogates.

Table 1 provides a summary comparison of laboratory performance by QC category, including frequencies at which the QC acceptance criteria were met. Acceptance criteria for all QC types were met at a frequency of >90% by all laboratories except for the coefficient of determination for initial calibration in data from labs 4 and 16 and for LCS recovery in data from lab 5, which are discussed in more detail in the relevant sections below.

Initial Calibration:

The method and study instructions specified analysis of 9 initial calibration standards over a concentration range of 5-200 ng/L, representing 10 - 400ng/L in the samples before dilution. Initial calibration acceptance criteria included minimum acceptance limits for coefficient of determination (r^2); % error, i.e., recalculated concentrations $\leq\pm$50% for the lowest standard and $\leq\pm$30% of the higher standards; and a signal-to-noise (s/n) ≥3 for the calibration standard the LLOQ. (Note: no specification was provided for calculating s/n). For bias measurements, higher importance was placed on meeting % error acceptance followed by r^2 (for the internal validation study, initial calibrations were specified to be linear only with a minimum r^2 ≥0.98, while for the external validation study linear or quadratic regressions were permitted, with a minimum r^2 ≥0.99).

Participating labs were able to identify and calibrate most target analytes and surrogates in standards in the concentration range of 5-200 ng/L (nom.; Table 2). The upper limit of calibration linearity for quantitative analysis was not evaluated as part of the scope of this validation study. Laboratory-reported LLOQs were in the 10-20 ng/L concentration range for most target analytes, with higher ranges (up to 40-80 ng/L). % Error/r^2 and target analyte recoveries in LLOQ verification samples did not always support the LLOQs reported by some laboratories.

Laboratories met % error criteria across 6 or more calibration standards at a higher frequency than the minimum r^2 criteria (whether assessed using a minimum r^2 of 0.98 or 0.99 for linear regressions). Laboratories did not meet r^2 criteria and/or did not meet % error criteria at the lower calibration levels for certain target analytes and their associated surrogates, including the long-chain carboxylic acids (PFTreA, PFTriA, PFDoA, PFUnDA, PFDA), the short-chain carboxylic acids with no or low-abundance qualifier transitions (PFBA, PFPeA, PFHxA), the telomer sulfonates (particularly 8:2 FTS and 6:2 FTS), and the sulfonamidoacetic acids (N-MeFOSAA and N-EtFOSAA). 6:2 FTS met the initial calibration % error criteria across a minimum of six initial calibration standards at the lowest frequency of any target analyte or surrogate (77%), likely due to background contamination reported by multiple laboratories.

Calibration options that could have reduced calibration-related measurement bias (i.e., reduced % error and/or increased r^2) are within the scope of the method, but not all laboratories applied these options to meet the ICAL acceptance criteria. *Note: Recommendations were added to the post validation method to calibrate target analytes with lower signal, more variable performance, or background at higher concentrations relative to other targets, and the chemicals above were provided as examples. Initial calibration acceptance criteria (r^2≥0.99 for linear or quadratic regressions, ≤±50% error at LLOQ and ≤±30% error for higher concentration standards) are retained in the post validation method as these are standard criteria from Method 8000D. 6:2 FTS and associated surrogate are also listed as especially problematic.*

Continuing Calibration Verification (CCV):

The method and study instructions specified analysis of CCV standards at a concentration near the middle of the calibration range (80 ng/L, nom., with some labs using 60 or 100 ng/L). Phase II study instructions provided to internal laboratories specified analysis of CCV standards at the end of the analysis sequence, while instructions for external laboratories specified analysis of CCVs at the beginning (if ICAL standards were not analyzed), after every 10 samples, and at the end of the analysis sequence. The CCV acceptance criterion is ±30% for calculated concentrations of target analytes and surrogates.

CCV criteria were met at high frequency across laboratories (Table 3), with all target analytes and surrogates except 6:2 FTS and M2-6:2 FTS meeting acceptance criteria in ≥95% of CCVs. Laboratories that had problems meeting CCV criteria for 6:2 FTS also had problems meeting the initial calibration acceptance criteria due to background contamination.

Blanks:

The method and study instructions specified the preparation of one reagent blank (RB) and the preparation of two method blanks (MBs) with each batch of 20 or fewer samples to monitor for contamination introduced by reagents and materials during analysis and sample preparation. The acceptance criterion for RBs and MBs was for target analyte concentrations to be <1/2 the LLOQ. *Note: The requirement for two MBs per batch was reduced to one in the post validation draft method. A caution was also added that more than one blank may be needed to evaluate for commonly observed laboratory contaminants (e.g., 6:2 FTS) or very low levels (i.e., at or near the LLOQ) are of interest.*

RB and MB contamination overall was infrequent and generally limited to concentrations near or below laboratory-reported LLOQs (Table 4). The only target analytes found in a MB or RB at a concentration > ½ the LLOQ at a frequency >5% were PFTreA, PFBS, and 6:2 FTS. 6:2 FTS was reported in at least one MB at concentrations > 100 ng/L by two different laboratories, and it was reported in an RB from a third laboratory at a concentration around 30 ng/L. The maximum measured concentration of any target analyte other than 6:2 FTS in any blank was <20 ng/L.

LLOQ Verification

The method and study instructions specified preparation and analysis of one or more LLOQ verification samples with each batch of 20 or fewer samples. LLOQ verification samples were recommended to be prepared at concentrations of 10 and/or 20 ng/L (nom.) in 5 mL water, but some laboratories included LLOQ verification QC samples at 40 and/or 80 ng/L. The recovery criterion for LLOQ verification samples is 50-150% of the expected (prepared) concentrations.

The frequency of target analytes meeting LLOQ verification acceptance criteria was higher at 20 ng/L than at 10 ng/L for all target analytes (Table 5). At a concentration of 20 ng/L, only a few target analytes did not meet the LLOQ verification criteria at a frequency >90%, including PFTriA, 8:2 FTS, 6:2 FTS, N-EtFOSAA, and N-MeFOSAA. Two laboratories did not meet LLOQ verification acceptance criteria for 6:2 FTS in any batch. *Note: Recommendations were added to the post validation method to 1) either prepare LLOQ verification QC samples at multiple concentrations, 2) to calibrate the instrument to below the reported LLOQ, or 3) consider the concentration levels of interest for the project to determine if the LCS (at a higher concentration) would meet requirements for the LLOQ verification.*

LCS

The method and study instructions specified preparation and analysis of Laboratory Control Sample/Laboratory Control Sample Duplicate (LCS/LCSD) QC samples with each batch of 20 or fewer study samples. LCS samples were specified to be prepared at concentrations of 160 ng/L (nominal) in 5 mL water. The acceptance criteria for LCS is 70-130% recovery and ≤30% for the relative percent difference (RPD) between LCS and LCSD concentrations.

LCS recovery and LCS/LCSD RPD (Table 6) acceptance criteria were met for all target analytes at frequencies >90% except for PFTriA, PFBA, and 6:2 FTS. Other target analytes that had higher frequencies of QC failures for ICAL, CCV, and LLOQ verification also met LCS recovery acceptance criteria at lower frequency, including PFTreA, PFDoA, 8:2 FTS, N-EtFOSAA, and N-MeFOSAA, but rates of QC failure were acceptable. *Note: Statistically-derived acceptance limits will be recommended in the post validation method for some targets, including PFTriA, PFBA, and 6:2 FTS, as 70-130% default limits may be too narrow, provided the calculated limits are not more restrictive than current limits.*

Surrogates

The method and study instructions specified addition of 19 isotopically-labeled surrogates to every field sample and QC sample prior to any further preparation steps at a concentration of 160 ng/L (nominal) in 5 mL water. The acceptance criterion for surrogates is 70-130% recovery.

Of the overall study sample surrogate recoveries (Table 7a), 36% had at least 1 of 19 surrogates recovered outside of 70-130%, 13% had 2 or more of 19 out, <5% had 3 or more of 19 out, and <2% had 4 or more of 19 out. In study samples (Table 7b), M2PFTreDA met the acceptance criteria at the lowest frequency (around 90%), and M2-4:2FTS, d5-N-EtFOSAA, and d3-N-MeFOSAA were the only other surrogates that met the recovery criteria at frequencies <95%. Surrogates were recovered within the acceptance criteria at similar frequencies in laboratory-prepared QC samples (method blanks, LLOQ verifications, and LCS), which suggested that the sample matrix was not a strong determinant of surrogate performance in this study.

Note: Statistically-derived acceptance limits will be recommended in the post validation method for some surrogates as 70-130% default limits may be too narrow, provided the calculated limits are not more restrictive than current limits.

Assessment of Method Ruggedness or Robustness:

Three laboratories whose data was not included in this summary demonstrated issues related to method robustness by not directly following study instructions for preparing standards or samples. Two labs prepared spiking solutions in the solvent matrix used for calibration standards and sample extracts (50:50 methanol-water with 0.1% acetic acid) instead of 95:5 acetonitrile-water and stored these solutions in glass containers, and study data submitted by both of these labs exhibited variable performance of the longer chain acids, likely due to loss from solution. *Note: Cautions were included in the post validation draft method regarding the minimum organic solvent content of higher concentration solutions of target compounds and/or surrogates and avoiding storage of calibration standards and sample extracts in glass containers to prevent loss of longer chain PFAS from solution.* Another laboratory subsampled from aqueous sample containers prior to addition of organic solvent or surrogates, which also resulted in apparent loss of some target analytes, particularly the longer chain carboxylic acids, N-MeFOSAA and N-EtFOSAA. *Note: More cautions were added to the post validation draft method to help users understand the target analyte specific biases resulting from sub-sampling.*

Conclusions:

The majority of analytes met acceptance criteria for precision, bias and method DQIs. The determinative method (LC/MS/MS) used is highly selective because multiple reaction monitoring (MS) is used. Interferences are most likely to be seen as suppression or enhancement to ionization rather than a false signal, and these types of matrix effects are monitored with isotopically-labeled surrogates added to every sample.

The principal issues with this analysis are retaining the analytes in solution, background contamination and instrument sensitivity. The 50% aqueous solvent composition of the analytical samples and standards imposes an upper limit on the concentration of the C10 – C14 acids and FOSAAs that will be stable in solution. That limit was not determined in this study.

A general caution was also added to the method for problems observed with multiple QC failures for 6:2 FTS in the validation study, including the enhancement of M262FTS when high concentrations of 6:2FTS are present.

Every data set submitted by the laboratory participants was reviewed carefully and provided valuable insight regarding both flexibilities that could be incorporated in the reference method and additional cautions that might be needed in the method regarding potential sources of measurement bias. The SW-846 methods team is grateful for the effort all participating laboratories invested in this validation study.

EPA will post draft methods for public comment, as soon as possible, through the recently approved SW-846 streamlined process.

Table 1 Summary of study performance across QC types, by laboratory

Lab	Initial calibration (ICAL)[1]		Continuing Calibration Verification (CCV)	Reagent Blank (RB)	Method Blank (MB)	LLOQ Verification				LCS	Surrogates in Study Samples
	% of target analytes and surrogates that met r^2 criteria	% of target analytes and surrogates that met % ICAL error criteria across at least 6 standard concentrations	% of target analytes and surrogates within ±30% % drift	RB % of target analytes concs <½ LLOQ	MB % of target analytes concs <½ LLOQ	% of target analytes in LLOQ verification recovered within 50-150%				% of target analytes that met 70-130% recovery	% within 70-130% recovery
						10 ng/L (nom.)	20 ng/L (nom.)	40 ng/L (nom.)	80 ng/L (nom.)		
2	93% (121/129)	100% (129/129)	99% (128/129)	100% (216/216)	100% (144/144)	58% (42/72)	92% (66/72)	-	-	97% (139/144)	96% (1089/1140)
4	80% (106/129)	98% (126/129)	99% (128/129)	99% (142/144)	96% (92/96)	-	-	100% (48/48)	100% (24/24)	98% (141/144)	93% (1060/1140)
5	97% (125/129)	100% (129/129)	100% (129/129)	98% (141/144)	100% (144/144)	67% (48/72)	88% (63/72)	97% (70/72)	-	88% (127/144)	94% (1074/1140)
6	100% (129/129)	100% (129/129)	100% (129/129)	100% (144/144)	99% (142/144)	99% (143/144)	-	-	-	97% (140/144)	97% (1104/1140)
10	100% (43/43)	100% (43/43)	100% (344/344)	100% (144/144)	97% (140/144)	83% (60/72)	93% (67/72)	-	-	94% (135/144)	98% (1104/1121)
11	98% (127/129)	97% (125/129)	97% (250/258)	92% (66/72)	90% (128/144)	-	96% (69/72)	-	-	96% (138/144)	98% (1122/1140)
12	100% (129/129)	100% (129/129)	100% (258/258)	93% (67/72)	99% (143/144)	99% (71/72)	100% (72/72)	-	-	100% (144/144)	99.6% (1135/1140)
16	79% (102/129)	95% (122/129)	97% (418/430)	99% (427/432)	99.5% (215/216)	89% (64/72)	94% (68/72)	-	-	92% (133/144)	96% (1090/1140)

[1]Minimum r^2 criteria for initial calibration was 0.98 for linear calibrations for Phase II internal laboratory study and 0.99 for linear and quadratic calibrations for Phase II external laboratory study; individual lab data summarized in this table was assessed against the acceptance criteria that was provided to the laboratories for the study.

[2]CCVs were specified to be analyzed at the end of the analysis sequence for the Phase II internal laboratory study and were specified to be analyzed once every 10 samples in the Phase II external laboratory study. Laboratory 10 analyzed opening CCVs instead of initial calibrations for two batches of samples.

Table 2. Initial calibration (ICAL) summary of performance by eight laboratories

Target or Surrogate	Range of LLOQs reported by laboratories in ng/L[1]	% of initial calibrations that met % error acceptance criteria across the stated ranges across all laboratories (n=22)[2]					% of ICALs that met minimum r^2 and minimum number of calibration points, all laboratories (n=22)[2]	
		5 – 200 ng/L	10 – 200 ng/L	20 – 200 ng/L[3]	40 – 200 ng/L	% that met acceptance criteria for range of 6 or more calibration standards[4]	Using $r^2 \geq 0.98$ for linear or 0.99 for quadratic regressions	Using $r^2 \geq 0.99$ for linear and quadratic regressions
PFTreA	10-20	68	23	5	-	95	82	73
PFTriA	10-20	64	27	9	-	100	77	73
PFDoA	10-40	68	27	5	-	100	91	82
PFUnA	10-20	82	14	5	-	100	91	86
PFDA	10-80	82	14	5	-	100	77	73
PFNA	10	95	-	5	-	100	96	77
PFOA	10-20	95	-	5	-	100	100	100
PFHpA	10-20	91	5	5	-	100	100	100
PFHxA	10-40	77	9	14	-	100	91	86
PFPeA	10-40	77	9	14	-	100	96	95
PFBA	10-40	73	18	5	-	95	100	95
PFDS	10-20	77	9	9	5	100	96	82
PFNS	10-20	86	9	5	-	100	96	86
PFOS	10-20	77	5	9	9	100	96	77
PFHpS	10	95	-	5	-	100	96	95
PFHxS	10	91	5	5	-	100	100	95
PFPeS	10-20	91	5	5	-	100	100	100
PFBS	10-20	91	5	5	-	100	100	95
PFOSA	10	95	-	5	-	100	100	100
FtS 8:2	10-20	73	14	9	5	100	96	77
FtS 6:2	10-20	73	-	5	-	77	86	77
FtS 4:2	10-40	91	-	9	-	100	100	95
NEtFOSAA	10-40	59	23	14	5	100	82	73
NMeFOSAA	10-40	55	18	18	9	100	77	64
M2PFTeDA	NA	77	5	5	-	86	82	68
MPFDoA	NA	95	-	5	-	100	96	91
M7PFUdA	NA	95	-	5	-	100	91	82
M6PFDA	NA	91	-	5	5	100	96	91
M9PFNA	NA	91	5	5	-	100	96	91
M8PFOA	NA	95	-	5	-	100	96	95
M4PFHpA	NA	95	-	5	-	100	100	91
M5PFHxA	NA	95	-	5	-	100	100	95
M5PFPeA	NA	95	-	5	-	100	100	100
MPFBA	NA	86	5	5	-	95	96	95
M8PFOS	NA	95	-	5	-	100	100	95
M3PFHxS	NA	95	-	5	-	100	100	100
M3PFBS	NA	95	-	5	-	100	100	100
M8FOSA-I	NA	82	14	5	-	100	100	86
M2-8:2FTS	NA	64	14	9	9	95	96	73
M2-6:2FTS	NA	82	9	-	-	91	96	68
M2-4:2FTS	NA	86	-	9	-	95	86	82
d5-N-EtFOSAA	NA	64	18	14	5	100	82	68
d3-N-MeFOSAA	NA	50	18	18	14	100	82	73

[1]One laboratory did not report LLOQs by target analyte; LLOQs were determined during validation based on meeting acceptance criteria

[2]All laboratories reported three initial calibrations except Laboratory 10, which only reported a single initial calibration, using continuing calibration verification standards to demonstrate the initial calibration was valid. Note that initial calibrations from which calibration points in the middle of the calibration range were removed to meet initial calibration % error or r^2 criteria were re-evaluated by EPA during validation, and some initial calibrations were counted as unacceptable

[3]One laboratory excluded ICAL standards at 5 and 10 ng/L concentrations in one of the reported initial calibrations for all target analytes and surrogates

[4]Sum of percentages in each of the calibration range columns; If an ICAL did not meet the % error criteria across 6 consecutive ICAL standards, this column is <100% (note that some values may not sum to exactly 100% due to rounding)

Table 3. Continuing calibration verification (80 ng/L, nom.) performance summary for eight laboratories (n=42)

target analyte or surrogate	average % drift[1]	Standard Deviation % drift	% of CCVs that met % drift criteria (≤±30%, or 70-130% of expected concentration)
PFTreA	-0.4	13.8	98
PFTriA	0.2	11.7	98
PFDoA	3.2	11.0	98
PFUnA	3.0	11.1	98
PFDA	0.4	11.0	98
PFNA	-1.8	9.9	100
PFOA	-0.5	7.7	100
PFHpA	-1.8	9.9	100
PFHxA	-1.5	10.6	95
PFPeA	-0.1	5.9	100
PFBA	-1.4	10.3	100
PFDS	-1.1	7.2	100
PFNS	0.3	6.3	100
PFOS	-0.1	7.3	100
PFHpS	1.2	5.4	100
PFHxS	1.2	6.8	100
PFPeS	0.4	6.4	100
PFBS	-0.2	12.6	98
PFOSA	0.3	4.3	100
FtS 8:2	1.5	9.6	100
FtS 6:2	27.5	121	86
FtS 4:2	0.1	8.2	100
NEtFOSAA	1.4	6.0	100
NMeFOSAA	-1.3	7.0	100
M2PFTeDA	-2.3	11.0	100

target analyte or surrogate	average % drift[1]	Standard Deviation % drift	% of CCVs that met % drift criteria (≤±30%, or 70-130% of expected concentration)
MPFDoA	-1.4	9.7	100
M7PFUdA	1.8	10.6	100
M6PFDA	-0.2	7.9	100
M9PFNA	0.4	8.5	100
M8PFOA	-3.0	9.2	100
M4PFHpA	-0.3	10.0	100
M5PFHxA	-2.1	10.9	98
M5PFPeA	0.3	5.0	100
MPFBA	-0.9	8.9	100
M8PFOS	1.9	6.3	100
M3PFHxS	0.8	6.3	100
M3PFBS	-1.5	10.2	98
M8FOSA-I	1.7	4.7	100
M2-8:2FTS	1.2	8.7	100
M2-6:2FTS	8.0	26.2	90
M2-4:2FTS	1.6	9.5	98
d5-N-EtFOSAA	1.7	9.2	100
d3-N-MeFOSAA	-1.3	9.9	98

Table 4. Method blank and reagent blank performance summary for eight laboratories

Target Analyte	Method blank (n=49)[1]		Reagent blank (n=54)	
	Maximum reported concentration (ng/L)	% with measured concentration <50% of laboratory-reported LLOQ (ng/L)	Maximum reported concentration (ng/L)	% with measured concentration <50% of laboratory-reported LLOQ (ng/L)
PFTreA	14.3	90	11.9	93
PFTriA	11.1	96	8.2	96
PFDoA	10.4	98	5.0	98
PFUnA	8.0	98	5.1	98
PFDA	9.0	98	9.8	100
PFNA	8.8	98	2.4	100
PFOA	6.8	98	6.3	98
PFHpA	3.4	100	1.7	100
PFHxA	9.7	98	0.6	100
PFPeA	9.2	100	4.6	100
PFBA	8.0	100	7.5	96
PFDS	4.3	100	4.6	100
PFNS	2.7	100	2.3	100
PFOS	9.9	98	1.8	100
PFHpS	3.8	100	3.0	100
PFHxS	7.3	98	12.3	98
PFPeS	1.8	100	2.2	100
PFBS	19.0	92	17.0	96
PFOSA	3.1	100	3.2	100
FtS 8:2	2.0	100	1.8	100
FtS 6:2	116.2	86	29.6	89
FtS 4:2	2.2	100	1.5	100
NEtFOSAA	8.0	98	5.0	98
NMeFOSAA	9.4	98	3.6	100

[1] Laboratory 4 submitted data for spiked blanks instead of method blanks in batch 2; this data was excluded from the method blanks statistical summary

Table 5. LLOQ verification performance summary for eight laboratories

Target Analyte	10 ng/L (nom.) in 5 mL water (n=21 across 6 labs)			20 ng/L (nom.) in 5 mL water (n=18 across 6 labs)			40 ng/L (nom.) in 5 mL water (n=5 across 2 labs)		
	Average % recovery	Standard deviation of % recovery	% that met 50-150% recovery	Average % recovery	Standard deviation of % recovery	% that met 50-150% recovery	Average % recovery	Standard deviation of % recovery	% that met 50-150% recovery
PFTreA	112	25.1	81	110	30.0	94	112	18.2	100
PFTriA	126	53.0	71	118	32.4	83	128	36.6	80
PFDoA	103	29.9	81	109	20.5	94	94.6	11.0	100
PFUnA	108	27.4	81	99.9	16.0	100	88.7	10.1	100
PFDA	96.8	26.3	86	103	19.5	94	100	17.6	100
PFNA	100	26.1	91	99.7	14.1	100	108	9.3	100
PFOA	101	26.8	86	99.5	16.3	100	98.1	6.6	100
PFHpA	93.2	15.7	100	99.5	13.8	100	97.9	6.4	100
PFHxA	99.1	41.6	86	94.9	17.6	100	98.1	19.5	100
PFPeA	103	36.2	86	99.1	13.2	100	102	6.9	100
PFBA	89.1	27.4	86	95.2	20.6	94	94.3	7.9	100
PFDS	105	24.6	81	100	24.2	100	105	24.2	100
PFNS	102	28.5	95	106	18.0	100	112	15.3	100
PFOS	112	23.0	86	106	16.8	100	114	8.2	100
PFHpS	89.1	41.8	91	105	14.5	100	100	9.8	100
PFHxS	99.0	17.7	100	99.3	12.7	100	103	11.4	100
PFPeS	95.8	12.4	100	99.7	12.3	100	100	8.0	100
PFBS	93.1	17.2	95	91.6	12.5	100	108	24.7	100
PFOSA	101	15.0	100	99.7	8.5	100	111	14.7	100
FtS 8:2	112	36.5	67	129	57.9	72	120	32.5	100
FtS 6:2	1470	5540	57	125	152	50	85.6	21.0	100
FtS 4:2	102	16.4	91	96.2	14.3	100	101	14.2	100
NEtFOSAA	122	33.6	71	111	18.9	78	106	25.3	80
NMeFOSAA	109	52.8	71	104	34.1	83	100	25.9	100

Table 6. LCS performance for eight laboratories (160 ng/L nom. expected concentration in 5 mL water)

Target Analyte	LCS % Recovery, All Labs (n=48)			Relative % Difference (RPD) between concentration in LCS and LCSD, All labs (n=24)		
	Average % recovery	Standard deviation of % recovery	% that met 70-130% Recovery	Average RPD	Standard Deviation RPD	% that met RPD (≤±30%)
PFTreA	103	18.9	90	7.1	4.9	100
PFTriA	107	22.7	83	8.9	8.0	96
PFDoA	104	16.7	92	10.3	9.7	96
PFUnA	101	12.1	100	9.0	8.7	100
PFDA	102	11.5	98	8.9	7.4	96
PFNA	103	12.3	96	6.9	7.2	100
PFOA	101	12.1	98	6.8	6.6	96
PFHpA	96.4	8.7	100	5.4	5.3	100
PFHxA	95.8	10.5	100	8.1	7.0	100
PFPeA	94.1	10.1	100	4.1	3.3	100
PFBA	91.5	15.1	88	4.4	4.4	100
PFDS	100	10.2	100	5.5	4.8	100
PFNS	105	12.6	100	6.9	6.3	100
PFOS	99.9	8.9	100	5.1	4.9	100
PFHpS	101	9.1	100	5.2	5.2	100
PFHxS	97.9	8.1	100	4.5	4.7	100
PFPeS	98.0	7.2	100	5.5	5.2	100
PFBS	93.2	9.6	100	3.3	5.5	100
PFOSA	98.7	8.2	100	3.6	2.7	100
FtS 8:2	104	15.0	94	8.2	6.9	100
FtS 6:2	91.1	33.0	65	10.2	8.4	100
FtS 4:2	98.0	12.0	96	8.8	8.9	96
NEtFOSAA	102	15.6	94	9.0	8.6	100
NMeFOSAA	102	15.2	94	9.2	7.7	96

Table 7a. Overall surrogate performance in study samples, by laboratory

Laboratory #	2	4*	5	6*	10**	11	12	16	All
# of study samples with reported results	60	60	60	60	59	60	60	60	479
# of samples with one or more surrogates outside 70-130% recovery	34	25	42	9	14	14	5	29	172
# of samples with two or more surrogates outside 70-130% recovery	12	6	18	6	3	3	0	12	60
# of samples with three or more surrogates outside 70-130% recovery	4	1	5	5	0	1	0	6	22
# of samples with four or more surrogates outside 70-130% recovery	1	1	1	1	0	0	0	3	7
# of surrogates reported across all samples	1140	1140	1140	1140	1121	1140	1140	1140	9101
# of surrogates that met 70-130% recovery across all samples	1089	1060	1074	1104	1104	1122	1135	1090	8778
% of surrogates that met 70-130% recovery across all samples	95.5	93	94.2	96.8	98.5	98.4	99.6	95.6	96.4

*Reported surrogate recoveries were near 200% in a study sample, suggesting it was double-spiked with surrogates. Surrogate recoveries from these samples are included in the summary table above.

**Results for one study sample were rejected due to lack of identified surrogates or target analytes; Surrogate data from this sample was excluded from these summary statistics.

Table 7b. Comparison of surrogate recovery in study samples and clean matrix QC samples (Method blank, LLOQ verification, LCS) across all laboratories and matrices

Surrogate	Surrogates in Study Samples, All Labs (n=477)[1]			Surrogates in Laboratory QC Samples, All Labs (n=143)		
	Average % Recovery	Standard Deviation of % recovery	% that met 70-130% recovery limits	Average % Recovery	Standard Deviation of % recovery	% that met 70-130% recovery limits
M2PFTeDA	96.8	18.8	90	101	19.5	90
MPFDoA	101	14.7	96	101	13.3	97
M7PFUdA	103	11.6	99	101	10.3	99
M6PFDA	104	12.1	98	102	10.5	99
M9PFNA	102	11.6	99	99.7	9.3	100
M8PFOA	101	9.5	100	99.7	9.1	100
M4PFHpA	98.9	10.9	98	97.1	9.9	99
M5PFHxA	97.4	11.8	98	95.3	10.6	98
M5PFPeA	98.7	7.5	100	95.7	8.0	100
MPFBA	95.6	10.9	98	93.7	11.5	94
M8PFOS	104	11.2	99	102	9.3	100
M3PFHxS	102	8.0	100	99.1	7.5	100
M3PFBS	96.9	12.0	98	94.5	9.1	98
M8FOSAI	101	8.9	99	99.8	8.8	99
M282FTS	106	13.9	96	101	14.2	97
M262FTS	100	15.4	98	100	17.3	95
M242FTS	97.8	19.4	92	97.4	18.4	92
d5NEtFOSAA	104	16.0	91	106	14.1	95
d3NMeFOSAA	102	16.1	92	103	14.2	95

[1]Surrogate data for three samples were removed prior to calculating these statistics (one sample had no qualitatively identifiable surrogates or target analytes, and the other two samples had surrogate recoveries around 200% and appear to have been double spiked).

Appendix A. Target analyte abbreviations used for the validation study

Analyte	CAS RN	Abbreviation
PFAS sulfonic acids		
Perfluoro-1-butanesulfonic acid	375-73-5	PFBS
Perfluoro-1-pentanesulfonic acid	2706-91-4	PFPeS
Perfluoro-1-hexanesulfonic acid	355-46-4	PFHxS
Perfluoro-1-heptanesulfonic acid	375-92-8	PFHpS
Perfluoro-1-octanesulfonic acid	1763-23-1	PFOS
Perfluoro-1-nonanesulfonic acid	68259-12-1	PFNS
Perfluoro-1-decanesulfonic acid	335-77-3	PFDS
1H, 1H, 2H, 2H-perfluorohexane sulfonic acid	757124-72-4	4:2 FTS
1H, 1H, 2H, 2H-perfluorooctane sulfonic acid	27619-97-2	6:2 FTS
1H, 1H, 2H, 2H-perfluorodecane sulfonic acid	39108-34-4	8:2 FTS
PFAS carboxylic acids		
Perfluorobutanoic acid	375-22-4	PFBA
Perfluoropentanoic acid	2706-90-3	PFPeA
Perfluorohexanoic acid	307-24-4	PFHxA
Perfluoroheptanoic acid	375-85-9	PFHpA
Perfluorooctanoic acid	335-67-1	PFOA
Perfluorononanoic acid	375-95-1	PFNA
Perfluorodecanoic acid	335-76-2	PFDA
Perfluoroundecanoic acid	2058-94-8	PFUdA (PFUnA)*
Perfluorododecanoic acid	307-55-1	PFDoA
Perfluorotridecanoic acid	72629-94-8	PFTrDA (PFTriA)*
Perfluorotetradecanoic acid	376-06-7	PFTeDA (PFTreA)*
PFAS sulfonamides and sulfonamidoacetic acids		
N-ethylperfluoro-1-octanesulfonamidoacetic acid	2991-50-6	N-EtFOSAA
N-methylperfluoro-1-octanesulfonamidoacetic acid	2355-31-9	N-MeFOSAA
Perfluoro-1-octanesulfonamide (FOSA)	754-91-6	PFOSA

*Two abbreviations were used during the study, both are given here