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REPORT

September 2021

Connecticut Department of Energy and Environmental Protection
Statewide PFAS Initiative

Sampling Plan – Per- and Polyfluoroalkyl Substances at Publicly Owned Treatment Works – REVISION 2

SECTION I – TITLE AND APPROVAL PAGE

Document Title: Sampling Plan – Per- and Polyfluoroalkyl Substances at Publicly Owned Treatment Works

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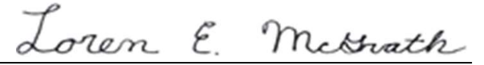

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Loren A. McGrath 09/02/2021

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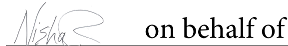
CTDEEP Project Manager:

 on behalf of

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SECTION II – INTRODUCTION

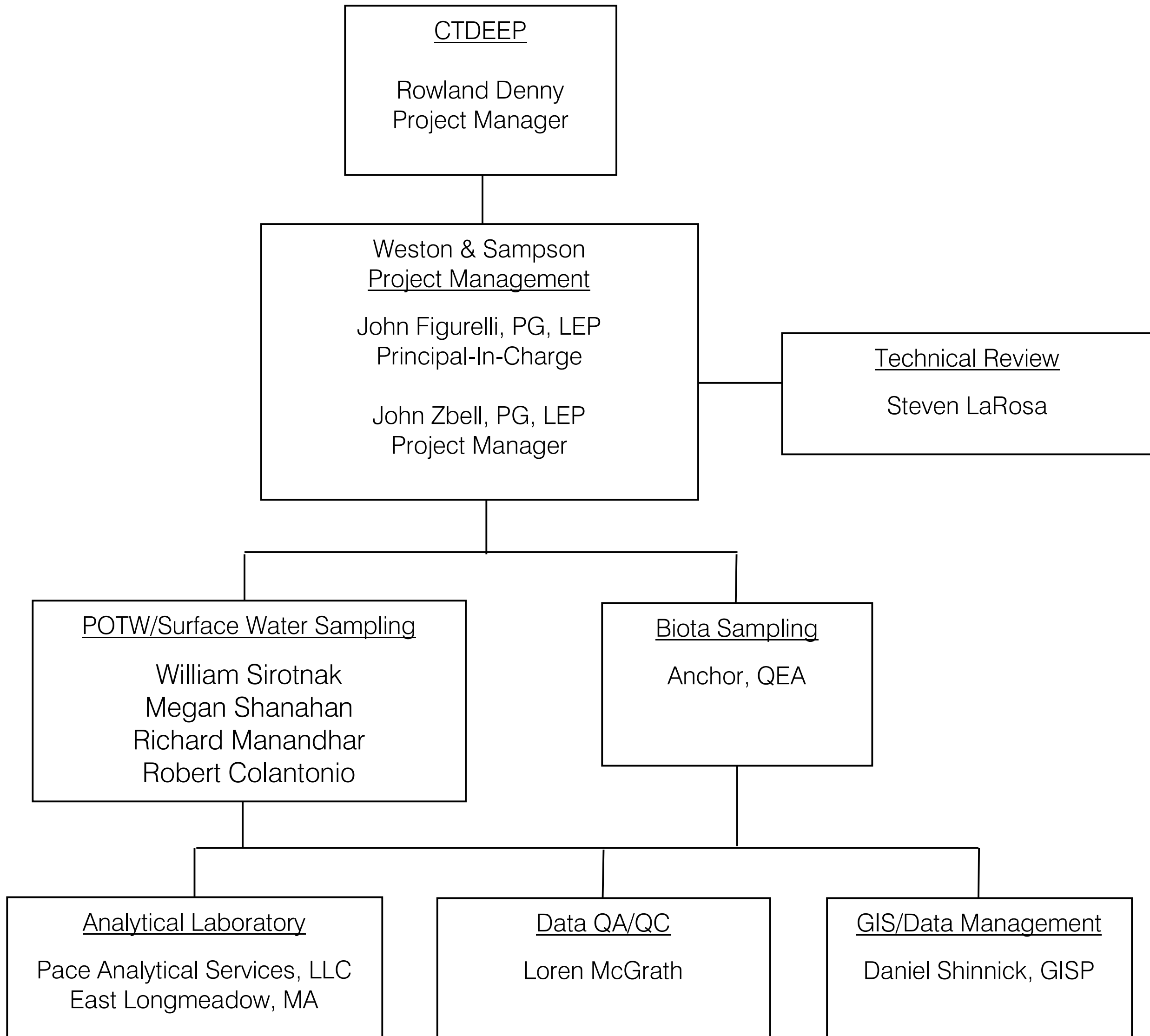
Weston & Sampson has prepared this Sampling Plan on behalf of the Connecticut Department of Energy and Environmental Protection (CTDEEP) for sampling and laboratory analysis of environmental media to evaluate the presence of per- and polyfluoroalkyl substances (PFAS) at publicly owned treatment works (POTWs) throughout Connecticut. This project is being completed with funding provided by the CTDEEP under a statewide PFAS Initiative.

Background

The CTDEEP is conducting an investigation to identify the presence of PFAS at POTWs geographically distributed throughout the State. This project is focused on evaluating the presence of PFAS in POTW influent, effluent, and sludge at 35 POTW locations. In addition, 4 biosolid incinerators will be sampled from the input sludge and incinerator scrubber water. Finally, receiving surface water bodies and select species of fish tissue within these water bodies at 10 locations co-located near sampled POTWs will also be sampled. The results will be used by the CTDEEP to evaluate the general distribution of PFAS at POTWs and the water bodies into which they discharge.

SECTION III – PROJECT ORGANIZATION AND RESPONSIBILITY

Organization and Responsibility



The following briefly describes project responsibilities for personnel involved in this Sampling Plan:

- CTDEEP – Responsible for initial coordination with POTWs, contract and project management, document review, approval, and project changes.

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- Weston & Sampson:
  - Project Management – Responsible for ensuring the project schedule and scope adheres to this Sampling Plan. Responsible for regularly informing the CTDEEP of work progress and for ensuring that project quality control and quality assurance elements adhere to this Sampling Plan.
  - Field Sampling – Responsible for ensuring the project fieldwork is performed in accordance with the Sampling Plan and coordinating field investigations with Anchor, QEA.

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SECTION IV – PROBLEM DEFINITION

In July 2019, the Governor of Connecticut established the Interagency PFAS Task Force to protect Connecticut residents and the environment from the harmful effects of PFAS. PFAS are a large group of human-made chemicals that have been used for decades due to their resistance to heat, oil, stains, grease, and water. PFAS have been documented in surface water, groundwater, soils, food, indoor dust, plants, invertebrates, fish, and mammals. These compounds are stable, persistent, and considered highly mobile.

In November 2019, the Governor released the "PFAS Action Plan", which was prepared by the Task Force. The Plan includes a number of actions items and steps to evaluate and address PFAS in Connecticut. The responsibility for implementing the Plan has transitioned to the CTDEEP, Connecticut Department of Public Health, and other state agencies.

As part of their efforts, the CTDEEP is presently undertaking the development of an interagency geographic information system (GIS) to document sources of PFAS contamination and evaluate the potential exposure to nearby sensitive receptors. The CTDEEP is also interested in identifying likely sources of PFAS contamination. The scope of work addressed under this Sampling Plan, will specifically focus on the assessment of PFAS associated with POTWs. These facilities are believed to be impacted by discharges of PFAS to the sanitary sewer system from industrial and commercial processes.

SECTION V – PROJECT DESCRIPTION/PROJECT TIMELINE

Project Description

The CTDEEP has identified 35 POTWs where they plan to sample and analyze environmental media to evaluate the occurrence of PFAS. In addition, surface water and fish tissue sampling are planned at 10 of the POTWs. The locations of sample collection are summarized on the attached Table 1.

Task 1: Kick-Off Meeting and Sampling Plan Preparation:

On June 11, 2021, staff from Weston & Sampson, Anchor QEA and the CTDEEP held a virtual project kick-off meeting to review the proposed scope of work and schedule.

Weston & Sampson has prepared this Sampling Plan for review and approval by the CTDEEP.

Task 2: Coordinate Access with POTWs:

On March 10 and May 28, 2021, the CTDEEP provided letters to the Superintendents of the 35 POTWs, which had been selected for sampling. The letters, which are attached as Appendix A, outlined the Statewide PFAS initiative, the scope for the proposed sampling and requested access to the facility under Connecticut General Statues Section 22a-6(a)(6).

Upon approval of this Sampling Plan, Weston & Sampson will contact each of the POTWs to reiterate the sampling scope and coordinate dates and times to conduct the sampling.

Task 3: Field Sampling:

Prior to the visiting each POTW, our interactive data collection system, iDataCollect, will be populated with contact information, addresses, and additional information for each POTW. The iDataCollect system integrates with Geographic Information System and EnvrioData8 platforms, which will be used to create databases and maps of the results.

Influent, effluent and sludge samples will be collected at each of the 35 POTWs during two seasonal sampling events: one in the summer and the other in the winter. At each of the 4 facilities that have active sludge treatment, influent sludge and scrubber water samples will also be collected. Samples will be collected at locations already established by the POTW operators. If a sample location has not previously been established, we will work in concert with the POTW operator to determine an appropriate sample location. If the optimal sampling location is not located within a regularly occupied space and/or may pose a health and safety risk, in consultation with our in-house safety administrators we will develop an acceptable method for sampling the location. A digital tablet will be used to collect real-time site-specific sample information (date, time, location, visual observations, etc.). Photographs will be taken at each sampling location to document the condition of the sample and facilitate the collection of samples from the same location during subsequent sampling events.

An attempt will be made to schedule sampling of the POTWs, within a 2-week time timeframe for both the summer and winter sampling events. If possible, sampling of multiple POTWs will be completed on the same day.

Surface water sampling and fish collection will be conducted at the ten locations listed on Table 1. Surface water sampling will be conducted by Weston & Sampson and fish collection will be conducted by Anchor QEA. Sampling locations will extend from the POTW outfalls to 1.5 kilometers (km) downstream. Sampling may also extend upstream of the outfalls but will not extend beyond barriers to fish passage or into major confluence areas. Attempts will be made to avoid fish collection in areas of other potential substantial PFAS discharge. Distances will be measured with a Differential Global

Positioning System (DGPS) and start and end points of the sampling transects will be recorded on the DGPS. Detailed logs of land use on each bank within the sampling reach will be kept noting direct discharges and facilities with the potential to be using PFAS. Prior to initiating fish collection, a data collection permit will be obtained from CTDEEP.

Fish samples will be collected by electrofishing. The method of electrofishing will be dependent on water depth and may include the use of a backpack unit for shallow water, tote barge for wadeable water up to waist deep and a vessel for deeper water (i.e., the Connecticut River sites).

Electrofishing will occur over the entirety of the site (from the outfall downstream 1.5 km, bank to bank), targeting areas of preferred habitat which may include pools, riffles and varying structure. Sampling will generally be conducted starting upstream and moving downstream to aid in netting the fish as the current will keep them in front of the netting crew.

Target species are smallmouth bass (*Micropterus dolomieu*) and white sucker *(Catostomus commersonii)*. These species were selected to include a predatory gamefish species (smallmouth bass) residing in the top trophic level and a bottom dwelling species (white sucker). Substitute species may be considered based on availability. The order of preference for alternate gamefish species are largemouth bass, then yellow perch. The order of preference for alternate bottom fish is catfish, common carp and then fallfish. Stocked species such as trout will not be targeted due to spending less time in the waterbodies and limiting the amount of bioaccumulation over time. Species substitutions and/or size range modifications will be discussed between the field crew and project managers and will need CTDEEP approval before a final determination is made.

At each of the ten locations, three composite samples of gamefish consisting of five fish of each species, will be collected for a total of 30 samples. Composites of gamefish will consist of legal-size fish of similar size (CTDEEP proposed that fish should be within +/- 1 inch in length). If necessary, we will collect composites of more than one species to achieve the 3 composite samples per location (e.g., 2 composites of smallmouth bass and 1 composite of perch) depending upon species abundance.

Three composites of bottom feeding fish will also be collected at each location consisting of similar size fish (within +/- 1 inch) for a total of 30 samples. Each composite will be made up of the same species If necessary, we will collect composites of more than one species to achieve the 3 composite samples per location (e.g., 2 composites of white sucker and 1 composite of carp) depending upon species abundance.

If the target number of fish is not reached at a location, composite samples may consist of a minimum of two fish.

Based on our experience, achieving the proposed size requirement of within 1 inch in length for all fish within a composite may be difficult. Any field modification to target size range will approved by CTDEEP as a field modification, e.g., to allow retained fish to be within 75% of the length of the composite batch, which is a standard approach.

Field processing of fish will be limited to avoid cross-contamination. Stunned fish will be netted and placed in HDPE buckets to recover. Non-target fish and target fish with deformities/abnormalities will be released upon recovery. Target species will be placed in a resealable plastic bag to measure length and inspect for abnormalities. Each bag will be labelled and then placed in a second bag prior to storage on wet ice for transport to Weston & Sampson's Rocky Hill, Connecticut office for pickup by a laboratory courier. In the event the fish need to remain at the office overnight they will be placed in the freezer.

Upon receipt, the laboratory will freeze the fish samples prior to shipping to the processing and analytical laboratory.

Fish will be processed in the analytical laboratory by descaling and filleting prior to homogenization as composite samples. The composite samples will utilize the left-side fillets. It has been assumed that sufficient tissue mass will be available from a single side fillet from 3-5 fish for each composite. The right-side fillets will be utilized if more sample mass is required. If sufficient mass is present from the left-side fillets, the right-side fillets will be archived for future use, if necessary (e.g., QA/QC issues with original sample analysis). Following completion of data validation, unused samples can be archived at the laboratory for 6 months or shipped to CTDEEP for archiving.

Michigan Department of Environmental Quality (MDEQ) has developed a reference guide summarizing procedures and protocols for the sampling of tissue for PFAS which will be adhered to for this sampling event. Further information regarding these procedures is presented in Section VIII.

Task 4: Laboratory Analysis:

All samples will be submitted to Con-Test Analytical Laboratory, a Pace Analytical Laboratory, for analysis. The POTW influent, effluent, incinerator scrubber water and surface water samples will be analyzed in accordance with Con-Test SOP 454, an in-house isotope dilution method for analyzing PFAS in non-drinking water samples that complies with Department of Defense Quality Systems Manual 5.3 B-15. Sludge samples will be analyzed by Contest SOP 466. Fish tissue samples will be analyzed by SOP MIN4-0178. All three methods report up to at least 34 PFAS, including the 18 PFAS specified in EPA Method 537. A summary of the analytes and detection limits by media are included in Appendix E. The results will be reported to Weston & Sampson as an electronic data deliverable (EDD), EPA Level IV data package, within 28 days of sample receipt. Electronic data deliverables will also be formatted for upload to the EPA Water Quality Exchange database.

Data Quality Objectives

The primary data quality objective (DQO) for this project is to provide information regarding overall PFAS loading at POTWs, the distribution of PFAs in the POTW treatment train, contribution of PFAS to receiving surface water bodies, and biota within those surface water bodies.

Applicable Standards

There are currently no applicable standards for PFAS in the media being sampled as part of this project.

Project Timeline

Upon approval of this sampling plan, Weston & Sampson anticipates commencing the first of two rounds of sampling in Late August/early September 2021. The second round of sampling would be completed in February/March 2022. The schedule outlining project tasks, major milestones, anticipated dates, and development of deliverables is included in Appendix B.

SECTION VI – SAMPLING DESIGN

The detailed scope of work addressing sample collection and analysis is provided below.

Sampling - Weston & Sampson will coordinate with the facility operators to collect the designated media samples at the locations shown in Table 1 attached. The table below summarizes the total number of samples to be collected of each media.

<table>
<tr><th>Sample Location</th><th colspan="2">Sample Type</th><th>Number</th><th>Sample Frequency</th><th>Total # of Samples</th></tr>
<tr><td rowspan="5">POTW</td><td colspan="2">Influent</td><td rowspan="3">35</td><td>2 rounds</td><td>70</td></tr>
<tr><td colspan="2">Effluent</td><td>2 rounds</td><td>70</td></tr>
<tr><td colspan="2">Sludge</td><td>2 rounds</td><td>70</td></tr>
<tr><td colspan="2">Incinerator Sludge</td><td rowspan="2">4</td><td>2 rounds</td><td>8</td></tr>
<tr><td colspan="2">Scrubber Water</td><td>2 rounds</td><td>8</td></tr>
<tr><td rowspan="4">Surface Water</td><td colspan="2">Upstream</td><td rowspan="4">10</td><td>1 round</td><td>10</td></tr>
<tr><td colspan="2">Downstream</td><td>1 round</td><td>10</td></tr>
<tr><td rowspan="2">Fish Tissue</td><td>Predator</td><td>3/location</td><td>30</td></tr>
<tr><td>Bottom Feeder</td><td>3/location</td><td>30</td></tr>
</table>

The sampling locations will be dependent on each individual facility design and available sampling ports. Weston& Sampson will utilize the same sampling locations as the facility uses for influent, effluent, and sludge as applicable. Samples of the various media will be collected in accordance with the Field Sampling Standard Operating Procedures (SOPs) provided in Appendix C. Additional information regarding sampling methods is presented in Section VII.

Sample Analyses - Each of the samples will be shipped to Con-Test Analytical under chain-of-custody in sealed coolers. The analytical methods used for PFAS quantification will be dependent upon the media being analyzed.

POTW influent, POTW effluent, POTW scrubber water and surface water samples will be analyzed using a proprietary method following SOP 454, which includes the use of solid phase extraction and internal isotope dilution. Solids/sludges will be analyzed using a proprietary method following SOP 466 Rev 7 using internal isotope dilution. Fish tissue samples will be analyzed via MIN4-0178, which includes the use of internal isotope dilution. All three methods comply with Department of Defense Quality Systems Manual 5.3 B-15 and are included in Appendix D. Additional information regarding analytical methods is presented in Sections VII and VIII.

The list of PFAS to be reported by each method of analysis and the limits of detection and quantification are included in the table in Appendix E.

SECTION VII – SAMPLING AND ANALYTICAL METHOD REQUIREMENTS

The parameters and number of samples associated with each analysis for the different types of media are presented in the following table.

Parameter	Media Type	Sample #	Sampling SOP***	Analytical Method	Analytical SOP	Containers	Preservation	Maximum Holding Time
PFAS	Aqueous*	148 samples + 8 Blanks + 14 Field Duplicates Total=170	SOP-25 SOP-26	DoD QSM 5.3	See Section VIII	(2) 250-mL high-density polyethylene (HDPE) or polypropylene (PP) containers with unlined plastic screw caps	Cool to ≤ 10°C but not frozen	Extracted within 14 days and extracts analyzed within 28 days
PFAS	Solid**	78 samples + 7 Field Duplicates Total=85	SOP-1 SOP-10 SOP-26	DoD QSM 5.3	See Section VIII	(1) 250-mL high-density polyethylene (HDPE) or polypropylene (PP) containers with unlined plastic screw caps	Cool to ≤ 10°C but not frozen	Extracted within 14 days and extracts analyzed within 28 days
PFAS	Surface Water	20 samples + 2 Field Duplicates Total=22	SOP-13 SOP-26	DoD QSM 5.3	See Section VIII	(2) 250-mL high-density polyethylene (HDPE) or polypropylene (PP) containers with unlined plastic screw caps	Cool to ≤ 10°C but not frozen	Extracted within 14 days and extracts analyzed within 28 days
PFAS	Fish Tissue	60 samples + 2 Field Duplicates (second fillet) Total=62	See Lab SOP	DoD QSM 5.3	See Section VIII	Resealable plastic bags (double)	Cool to ≤ 6°C to lab for homogenization****	One year

* Aqueous samples include influent, effluent and scrubber water
**Solid samples include sludges and incinerator sludges
***Sampling SOPs can be found in Appendix C
****Samples will be frozen at processing laboratory

SECTION VIII – METHOD AND SOP REFERENCE TABLE

The following table includes the laboratory SOP references, included in Appendix D.

Matrix	Analytical Method Reference:	Testing Facility	Laboratory Analytical SOPs:
Aqueous	Determination of Selected Perfluorinated Alkyl Substances (PFAS) by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry Isotope Dilution (LC/MS/MS)	Con-Test	SOP ID 454 PFAS Water Isotope Dilution Revision #7 06/30/21
Solid	Determination of Selected Perfluorinated Alkyl Substances (PFAS) Soil/Solid Samples by Liquid Chromatography/Tandem Mass Spectrometry Isotope Dilution (LC/MS/MS)	Con-Test	SOP ID 466 PFAS Water Isotope Dilution Revision #7 06/30/21
Tissue (Preparation)	USEPA National Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories, Volume 1: Fish Sampling and Analysis – 3rd Edition	Con-Test	ENV-SOP-GBAY-0129 Sample Homogenization, Compositing and Sub-Sampling Revision #3 02/09/21
Tissue (Analysis)	Department of Defense Department of Energy Consolidated Quality Systems Manual (QSM) for Environmental Laboratories, Version 5.3, Appendix B, Table B-15, 06/19 DoD Guidance for PFAS Analysis in Biota. 04/20	Con-Test	ENV-SOP-MIN4-0178 Determination of Selected 36 Per- and Polyfluoroalkyl Substances (PFAS) by LC/MS/MS (Isotope Dilution)

SECTION IX – ANALYTICAL SENSITIVITY, PRECISION, AND ACCURACY

The method detection limits and reporting limits related to the analytical testing methods are provided in Appendix E. All reporting limits have been reviewed for accuracy as of the date of this Sampling Plan.

SECTION X – FIELD DATA EVALUATION

Verification and Validation Requirements

This section provides a discussion of the type and extent of quality control evaluation that will be completed in conjunction with the analytical data collected at the POTWs. Results of this evaluation will be used to provide verification for reported sample concentrations.

Verification of Sampling Procedures

The following criteria will be used to evaluate the field sampling data:

- Documentation of field equipment calibration activities;
- reviewing data for technical credibility vs. the sample site setting;
- auditing field sample data records and chain-of-custody;
- auditing of sample handling and preservation procedures; and
- holding times for sample analyses.

Sampling procedures will be evaluated by the Field Lead and/or the Project Manager as appropriate. The results of the evaluation will be included in reports prepared after each of the seasonal sampling rounds and resulting impacts to the data will be discussed.

Data Verification and Validation

Data evaluation reports, in general accordance with EPA Region 1 Functional Guidelines, will be submitted with each of the seasonal reports. Deviations from the standard evaluation process, along with justification for why the changes were made will be described in the reports.

SECTION XI – LABORATORY DATA EVALUATION

Laboratory Data Review Process

Every laboratory report will include a certification pertaining to the analytical procedures, and associated QC criteria and performance standards for all data included in the reports. This Analytical Report Certification Form includes a statement attesting to the validity of the results, a dated signature of a qualified laboratory representative and a set of "yes" or "no" answers pertaining to questions regarding compliance with method-specific QC criteria, performance standards, analytes reported and consistency with COC documentation. Any question answered with a "no" should be fully explained in the project narrative, with additional information and/or documentation attached as necessary.

Data Review Process

When Weston & Sampson receives the laboratory data package, Weston & Sampson will verify the data is complete, correct, and contractually compliant. If Weston & Sampson finds that data are missing or incorrect and the discrepancies are not already noted in the laboratory's case narrative for that data package, Weston & Sampson will request missing data and an explanation from the laboratory. The EPA "New England Environmental Data Review Program Guidance", dated April 22, 2013, will be used as guidance for the data evaluation.

Tier I Evaluation

Data review evaluation will include a review of tabulated quality control results and comparison against EPA Region I validation limits and/or project specific criteria to identify bias or other interferences that could affect the quality of sample results. Specific quality control components to be evaluated include the following:

- Data completeness check
- Holding times
- Sample preservation
- Blank results. The 5x and 10x rules (EPA, 1996) will be used to qualify sample concentrations that have detections in associated blanks
- Surrogate recoveries
- Matrix spike and matrix spike duplicate results
- Field duplicates
- Laboratory control sample results

Tier II Evaluation

If, during data evaluation, significant problems with data are encountered, then a Tier II validation process may be performed. Specific quality control components to be evaluated in the Tier II review include everything from the Tier 1 review plus the following:

- Initial and continuing calibration results
- Internal standard results
- GC/MS tuning results
- Serial dilution results

Review Laboratory Report Narratives

If any QC issues are documented in the Laboratory Report Narrative, which are not included in the data package, then Weston & Sampson, during the data validation process, will contact the laboratory and obtain copies of relevant necessary QC information. Surrogate recovery, laboratory control sample, duplicate, field blank, trip blank and temperature blanks checks are part of the data validation process that Weston & Sampson will complete and are thoroughly reviewed in the Tier II-type data validation/usability assessment process. Data limitations and bias will be identified within the report analytical summary tables.

Verification Summary Report

Weston & Sampson will include a data verification section in the text of the seasonal summary reports that summarizes overall data quality observations and conclusions and explains any limitations of the data.

SECTION XII – DATA USABILITY

Weston & Sampson will review the data in general accordance with the "U.S. Environmental Protection Agency Guidance on Quality Assurance Project Plans CI0 2106-G-05 QAPP". Weston & Sampson will evaluate the data quality objectives (DQOs) in general accordance with the PARCCS (precision, accuracy, representativeness, completeness, comparability and sensitivity) parameters outlined in the above-referenced guidance document.

Data Qualifiers

Based on validation results, qualifiers will be added to reported analyte concentrations to indicate uncertainty or potential bias or interferences. Specific data qualifiers which will be applied to organic sample concentration include the following:

- U - The analyte was not detected above the practical quantitation limit.
- J - The analyte was detected but the associated reported concentration is approximate and is considered estimated.
- R - The reported analyte concentration is rejected due to serious deficiencies with associated quality control results. The presence or absence of the analyte cannot be confirmed.
- UJ - The analyte was not detected above the PQL. However, due to quality control results that did not meet acceptance criteria, the quantitation limit is uncertain and may not accurately represent the actual limit.

Weston & Sampson will evaluate the data to determine if it meets the PARCCS (precision, accuracy, representativeness, completeness, comparability and sensitivity) parameters outlined in the above-referenced guidance document. The PARCCS criteria are described below.

Precision

Precision is a measure of mutual agreement among individual measurements of the same property and is generally expressed as the reproducibility of the analytical result between the initial sample and the field duplicate sample as expressed by the relative percent difference (% RPD). Weston & Sampson will include field duplicate results in the report tables. Duplicate sample results will be listed adjacent to the existing sample results so that a direct analyte by analyte concentration comparison can be made. Weston & Sampson will calculate the RPD for each duplicate sample result by calculating:

$$\frac{|\,(\text{sample concentration}) - (\text{duplicate concentration})\,|}{(\text{simple average of sample and duplicate concentration})} \times 100 = \text{RPD}\ (\%)$$

The acceptable RPD values for POTW influent, effluent, sludges and fish tissue will be $<50\%$ and for surface water will be $<30\%$. Any duplicate results that are above these RPD limits will be flagged on the data tables.

Accuracy

Accuracy is the degree of measurement with an accepted reference or true value. The difference between the measurement and the true value is usually expressed as a percentage ratio. Weston &

Sampson will evaluate accuracy by reviewing the following: laboratory control sample (LCS) results, surrogate results, matrix spike/matrix spike duplicate (MS/MSD) results, calibration QC results, and field and laboratory blank results. Poor accuracy may be the result of laboratory error, field error, or the natural sample matrix. In the data usability reports, Weston & Sampson will evaluate the cause of bias/accuracy problems. For environmental samples, poor accuracy can be due to interferences present in the natural sample.

Continuing calibration accuracy checks are assessed by comparing the true value against the reported concentration. The percent difference between the results is calculated as follows:

Accuracy may be expressed as a percent difference (%D) calculated by the following equation:

$$\%D = (V_t - V_m)/V_t \times 100$$

Where:

V_t = the true or real value expected.

V_m = the measured or observed value.

The degree of accuracy demonstrated for laboratory control and matrix spike samples is expressed as a percent recovery. The percent recovery indicates the amount of known concentration of an analyte that has been detected by the associated instrumentation. The percent recovery (%R) is calculated as follows:

$$\%R = (SSR - SR)/SA \times 100$$

Where:

SSR = the spiked sample result.

SR = the unspiked sample result.

SA = the value of the spike added.

The objective for accuracy of laboratory determinations is to demonstrate that the analytical instrumentation provides consistent measurements, which are within EPA and statistically derived method-specific accuracy criteria. Laboratory data quality objectives for accuracy as measured by "%Recovery" are provided in the laboratory SOPs (see Appendix D).

Representativeness

Representativeness expresses the degree to which data accurately and precisely represent a characteristic of a population, parameter variation, or environmental condition. Weston & Sampson will review data qualitatively for representativeness. Sample representativeness will be reviewed qualitatively through the review of the proper use of the specified sampling SOPs, through internal field sampling audits, and through the use of field QC samples including field duplicates and trip blanks. Any unexpected, unusual, or anomalous results will be mentioned in the report text.

Completeness

Completeness is a measure of the amount of valid data obtained from a measurement system relative to the amount expected under normal conditions. Completeness is expressed as a percentage and is determines as follows:

$$\text{Completeness (A\%)} = \frac{\text{\# of valid values reported for a parameter x 100}}{\text{\# of samples collected for analysis for that particular parameter}}$$

A% = Acceptance Percentage

The project completeness goal is 85-90% percent of the samples being successfully analyzed. If less than 85 percent completeness is obtained, further sampling may need to be performed. Incomplete sampling from critical areas may result in the need for further sampling, even if the overall 85-90% completeness goal is met.

Comparability

Comparability refers to the ability to generate data for each parameter that is both comparable between sampling locations and over time. Comparability is a qualitative parameter that expresses the confidence with which data sets can be compared to one another. Comparable data allows for the ability to combine analytical results (e.g., current data with historic data) acquired from the various media to be sampled at each of the POTWs. Comparability relies upon precision and accuracy within the individual data sets to be acceptable to promote confidence in the data sets.

Data Sensitivity

Sensitivity is a measure of whether the laboratory method was sufficient to report detected contaminants at concentrations at or below the applicable guidance criteria. Prior to laboratory analysis, anticipated laboratory reporting limits will be compared to the most recent and applicable state and EPA guidance levels and procedures will be adjusted as needed to ensure that reporting limits are at or below the required standards. The applicable reporting limits will likely vary based on site conditions, sensitive receptors, etc.

Any non-detected data result that has a quantitation limit above the applicable state and EPA guidance concentration will be flagged on the data tables. For non-detected chemical results for which the project quantitation limit (QL) was not achieved, the risk assessor may determine that the use of one-half of the reported QL is sufficient as a conservative approach in risk calculations.

Usability Summary

Any or all of the following considerations for precision, accuracy, and completeness may be evaluated to determine if DQOs have been met. To meet these requirements, quality control criteria are provided in the standard laboratory methodologies. These criteria include the use of field duplicates and matrix spike samples to assess precision, matrix spikes, laboratory control samples and calibration results to assess accuracy; blank samples to determine representativeness; field duplicates to assess comparability. The amount (percentage) of valid data obtained from validation will be used to determine completeness. The results of the data usability evaluation will be included in the seasonal reports, and impacts and/or limitations on the use of the data will be discussed.

Weston & Sampson will include a data usability section in each report's text that summarizes overall data usability and answers whether the data is usable overall for report findings and conclusions.

Data usability will be performed by the project staff with support from the Project QA Officer and confirmed by the Project Manager.

\\wse03.local\WSE\Projects\CT\CT DEEP\PFAS WWTF Sampling\Sampling Plan\Text_PFAS WWTF_ POTW_Sampling Plan.docx

TABLE 1

CTDEEP STATEWIDE PFAS INITIATIVE
SAMPLING OF PUBLICLY OWNED TREATMENT WORKS

POTWs TO BE SAMPLED

PLANT	Sample Types per Location						
	Influent	Effluent	Sludge	Incinerator Input Sludge	Incinerator Scrubber Water	Surface Water	Fish Tissue
BEACON FALLS WPCF	✓	✓	✓			✓	✓
BRIDGEPORT WEST WPCF	✓	✓	✓				
BRIDGEPORT WPCF	✓	✓	✓				
BRISTOL WPCF	✓	✓	✓			✓	✓
CHESHIRE WPCF	✓	✓	✓				
DANBURY WPCF	✓	✓	✓				
EAST HADDAM WPCF	✓	✓	✓				
FARMINGTON WPCF	✓	✓	✓			✓	✓
GEORGETOWN WPCF	✓	✓	✓				
GRASS ISLAND WPCF	✓	✓	✓				
HARTFORD MDC WPCF	✓	✓	✓	✓	✓	✓	✓
HERITAGE VILLAGE WATER CO	✓	✓	✓				
LEDYARD WPCF	✓	✓	✓				
MATTABASSETT DISTRICT WPCF	✓	✓	✓			✓	✓
MERIDEN WPCF	✓	✓	✓				
MONTVILLE WPCF	✓	✓	✓				
NAUGATUCK WPCF	✓	✓	✓	✓	✓		
NEW HAVEN WPCF	✓	✓	✓	✓	✓		
NEW LONDON WPCF	✓	✓	✓				
NEWTOWN WPCF	✓	✓	✓				
NORWALK WPCF	✓	✓	✓				
SALISBURY WPCF	✓	✓	✓				
SHARON WPCF	✓	✓	✓				
SOMERS WPCF	✓	✓	✓			✓	✓
STAFFORD WPCF	✓	✓	✓				
THOMASTON WPCF	✓	✓	✓				
THOMPSON WPCF	✓	✓	✓				
TORRINGTON WPCF	✓	✓	✓				
UNIVERSITY OF CONNECTICUT WPCF	✓	✓	✓				
VERNON WPCF	✓	✓	✓			✓	✓
WALLINGFORD WPCF	✓	✓	✓			✓	✓
WATERBURY WPCF	✓	✓	✓	✓	✓		
WINDHAM WPCF	✓	✓	✓				
WINDSOR POQUONOCK WPCF	✓	✓	✓			✓	✓
WINSTED WPCF	✓	✓	✓			✓	✓

Weston & Sampson℠

APPENDIX A

79 Elm Street • Hartford, CT 06106-5127 www.ct.gov/deep Affirmative Action/Equal Opportunity Employer

March 10, 2021

RE: Statewide PFAS Initiative

Superintendent:

PFAS are a family of man-made chemicals that contain a high content of fluorine atoms, which gives them many useful properties, including the abilities to repel water, prevent staining, and increase heat resistance. As such, PFAS are used in thousands of industrial processes and consumer products, including coatings for fabrics and nonstick cookware, grease-resistant food packaging materials (e.g., microwave popcorn bags), and firefighting foam used to put out flammable liquid fires. Once released to the environment, PFAS are persistent and do not readily biodegrade or break down and have serious adverse impacts on human health and the environment, even at low levels. Currently, neither the U.S. Environmental Protection Agency (EPA) nor the Connecticut Department of Energy and Environmental Protection (DEEP) has a regulatory water quality standard for PFAS. DEEP has undertaken a statewide initiative to study PFAS, including the levels present in wastewater.

As part of the Statewide PFAS Initiative the DEEP will be working with a contractor to conduct sampling of domestic wastewater treatment plant (POTW) influent, effluent, sludge and/or scrubber water (if applicable) for PFAS compounds from 34 POTWs around the state.

Your facility has been selected for this effort and a separate letter will be sent to inform you who the commissioner's agent is once selected. You will then be contacted by the commissioner's authorized agent to schedule a date for sample collection.

There is no cost to your facility for this effort nor for the testing. When the report is finalized an electronic copy will be provided to you.

Please provide access for this sampling effort as directed by CGS 22a-6(a)(6). Under this statute the commissioner may undertake any studies, inquiries, surveys or analyses she may deem relevant, through the personnel of the department or in cooperation with any public or private agency, to accomplish the functions, powers and duties of the commissioner.

Thank you for your cooperation in our effort to determine the prevalence of PFAS in our state.

If you have any questions or comments please contact Rowland C. Denny of my staff at 860-424-3749 or rowland.denny@ct.gov.

Sincerely,

Jennifer Perry, P.E., Director
Water Planning and Management Division
Water Planning and Land Reuse Bureau

79 Elm Street • Hartford, CT 06106-5127 www.ct.gov/deep Affirmative Action/Equal Opportunity Employer

May 28, 2021

RE: Statewide PFAS Initiative

Superintendent:

As a follow up to my letter to you dated March 8, 2021, the Department of Energy and Environmental Protection (DEEP) has selected Weston & Sampson Engineers to conduct sampling of publicly owned treatment works (POTW) influent, effluent, sludge and scrubber water (if applicable) for PFAS compounds from 34 POTWs around the state.

As part of the Statewide PFAS Initiative, your facility has been selected for this effort. You will be contacted by Weston & Sampson Engineers, the commissioner's authorized agent, to schedule a date(s) for sample collection.

There is no cost to your facility for this effort, including but not limited to, testing and analysis costs. When the report is finalized, an electronic copy will be provided to you.

Please provide access to the necessary sampling locations for this sampling effort as directed by Connecticut General Statutes Section 22a-6(a)(6). Under this statute, the commissioner may undertake any studies, inquiries, surveys or analyses she may deem relevant, through the personnel of the department or in cooperation with any public or private agency, to accomplish the functions, powers and duties of the commissioner.

The intent of this effort is to assist the DEEP in directing our efforts to evaluate the introduction of PFAS into sanitary sewers and determine the extent and degree of PFAS at POTWs at various points in the treatment train.

Thank you for your cooperation in our effort to determine the prevalence of PFAS in our state.

If you have any questions or comments please contact Rowland C. Denny of my staff at 860-424-3749 or rowland.denny@ct.gov.

Sincerely,

Jennifer Perry, P.E., Director
Water Planning and Management Division
Water Protection and Land Reuse Bureau

APPENDIX B

CTDEEP STATEWIDE PFAS INTIATIVE
SAMPLING OF PUBLICLY OWNED TREATMENT WORKS

Project Schedule

ACTIVITY	2021					2022					
	8	9	10	11	12	1	2	3	4	5	6
CTDEEP Review and Comment on Sampling Plan											
Finalize Sampling Plan											
Communication/Scheduling with POTWs											
Sampling of POTWs (24 locations with no surface water or fish tissue sampling)											
Sampling of POTWs (10 locations with surface water and fish tissue sampling)											
Laboratory Analysis (up to 28 days)											
QA/QC and Data Tabulation and Review											
Prepare Draft Report											
CTDEEP Review and Comment on Draft Report											
Finalize Report											
Communication/scheduling with POTWs											
Round 2 Sampling of POTWs (24 locations with no surface water or fish tissue sampling)											
Laboratory Analysis (up to 14 days)											
QA/QC and Data Tabulation and Review											
Draft Report Generation											
CTDEEP Review and Comment on Draft Report											
Finalize Report											

APPENDIX C

SOP 1: Composite Sludge Sampling

Purpose:	A Composite Sludge Sample is a mixture of one or more individual "grab samples" from several discrete locations in a stockpile and/or discrete depths within a tank or lagoon.
Applicable for:	Compositing sludge samples while in the field for laboratory analysis of metals, polychlorinated biphenyls (PCBs), or other suitable analyte.
Not applicable for:	Sludge being collected for volatile organic compounds (VOCs) analysis.

PREREQUISITES	MATERIALS NEEDED
None, unless otherwise noted.	✓ Sludge collection/measuring device (i.e. stainless steel trowel, pre-cleaned/ disposable scoop/bucket auger with "T" handle, "Sludge-Judge"); ✓ Stainless steel mixing bowl or inert plastic sheeting; ✓ Pre-cleaned mixing spoon/device (i.e. wooden tongue depressor); ✓ Pre-cleaned laboratory container; and ✓ Personal protection equipment: at a minimum protective gloves and eye protection.

PROCEDURE

1) Determine the number of "grab" locations to be sampled. This will be dependent on the volume, solids content and storage method of the sludge. Generally, at least 4 "grab" locations should be sampled. The intent is to collect sufficient "grabs" from locations likely representative of the entire sludge mass.
2) Select locations and/or depths from which sludge "grab" samples are desired. Tank samples can be selected from several access points at depths of 1/3 to 2/3 of the tank depth. Lagoon bottom samples should be collected from locations both near and distal to the lagoon inlet.
3) Prepare a sampling sketch showing the general location of each "grab" sample. Label each "grab" location uniquely.
4) Collect a known volume of equal amount of sludge "grab" sample from each location using a collection/measuring device (decontaminated HDPE bottle, sludge-judge, scoop).
5) Empty each sludge "grab" sample into the homogenization container or onto a mixing surface.
6) Thoroughly mix or "composite" sludge using an inert mixing device.
7) Using a pre-cleaned, stainless steel scoop, plastic spoon, or trowel, remove composite soil sample and place required volume into designated pre-cleaned laboratory container for analysis.
8) Label sampling container with a waterproof label using a waterproof ink pen with: unique name, project name, W&S project number, date, time of collection, sampler initials, preservative utilized and required analyses.

9) Transfer sampling label information to the laboratories preferred Chain of Custody.

10) Place sample container into a cooler/box and prepare for transportation to the laboratory (ice, vermiculite, bubble wrap, etc.).

REFERENCES

1. MassDEP. 1991. *Standard References for Monitoring Wells (MassDEP Policy #WSC-310-91).*

2. USACE, 2001*, Requirements for the Preparation of Sampling and Analysis Plans (USACE EM 200-1-3).* February 1

SOP 10: Sampling Soil with a Scoop or Hand Auger

Purpose:	This procedure covers the method and equipment used to collect surface and near-surface samples of soils and physically similar materials using a scoop or hand auger.
Applicable for:	Collecting samples of contaminated soils and similar materials primarily near the surface. Subsurface samples can be obtained by first removing higher layers using a shovel or other suitable equipment and collecting the sample with the scoop.
Not applicable for:	Water samples.

PREREQUISITES	MATERIALS NEEDED
Driller to contact utility clearance Phone (811) at least 72 hours and no more than 10 days prior to any drilling activities and AFTER pre-marking is performed. Additional notification of applicable local municipalities and/or private utilities where required. Refer to SOP 5: Pre-Marking Boring Locations if needed.	✓ Stainless steel hand auger; ✓ Ruler or Measuring tape; ✓ Decontamination equipment; ✓ Sampling Plan; ✓ Soil collection/measuring device (e.g. Encore or Terracore syringe or pre-cleaned stainless steel trowel, disposable scoop); ✓ Pre-cleaned, pre-preserved laboratory containers; ✓ Sample container label; and ✓ Personal Protective Equipment: at a minimum protective gloves and eye protection

PROCEDURE

Non VOC Analysis

1. Samples should be collected in accordance with an appropriate work plan.
2. Remove the top layers of material down to the required sample depth using a shovel or other suitable equipment. A shovel or other suitable equipment can be used for the initial removal of overburden material. This equipment should be manufactured from material that is compatible with the soil or waste to be sampled.
3. Measure to the depth at which the sample will be collected with a ruler or tape measure. Record this information in a field log book.
4. Remove the thin layer of material that was in contact with the overburden removal equipment and discard it using a clean scoop. The project scope will define if the scoop may or may not be reused to collect the actual sample.

5. Collect a suitable volume of sample with the scoop* (the same scoop can be used to collect multiple scoopfuls to obtain sufficient volume to fill the container). Use a new (or decontaminated) scoop for each sample. Transfer the sample into the suitable container. Samples should be contained in plastic, glass, or other non-reactive certified-clean containers.
6. Close the sample container and label with a waterproof label using a waterproof ink pen with: unique name, project name, W&S project number, date, time of collection, sampler initials, preservative utilized and required analyses.
7. Place sample container into a cooler/box and prepare for transportation to the laboratory (ice, vermiculite, bubble wrap, etc.).
8. Complete the field log book with all relevant information and observations about the sample location and chain-of-custody form.
9. Decontaminate the reusable equipment in accordance with the protocol specified in the project scope and refer to SOP 8: Decontaminating Equipment.

VOC Analysis

This method is suitable for collecting soil samples for volatile organic compounds (VOC) for high and low level detection limits via EPA Method 5035. In most cases samples are being collected as a part of an initial subsurface investigation and general VOC concentrations are unknown. Therefore, samples should be collected for both "low concentration" and "high concentration" analysis methods. By collecting samples for both analysis methods you are assured results will be reported with appropriate detection limits. The procedure below collects sufficient samples for both analysis methods.

1. Organize sampling containers near sampling device.
    a. Low Concentration – 40 ml vial with 5ml water and 1g sodium bisulfate
    b. High Concentration – 40 ml vial with 10 mls methanol
    c. Moisture Content – 40 ml vial with no preservative
2. Select desired sampling interval(s) within sampling device.
3. Remove the thin layer of material that was in contact with the overburden removal equipment and discard it using a clean scoop.
4. Using an appropriate sample collection device (preferably Encore or Terracore syringe or clean stainless steel scoop), collect approximately 5g of sample as soon as possible after the surface of the soil or other solid material has been exposed to the atmosphere: generally within a few minutes at most.
5. Transfer sample from syringe/scoop to pre-preserved sample container. Clean thread and secure cap. Repeat Step 3 for each pre-preserved container. Fill remaining unpreserved container nearly full and secure cap.
6. Label each container with a waterproof label using a waterproof ink pen with: unique name, project name, W&S project number, date, time of collection, sampler initials, preservative utilized and required analyses.

7. Place sample containers into a cooler/box and prepare for transportation to the laboratory (ice, vermiculite, bubble wrap, etc.).
8. Complete the field log book with all relevant information and observations about the sample location and chain-of-custody form.
9. Complete the field log book and chain-of-custody form.

Decontaminate the reusable equipment in accordance with the protocol specified in the project scope and refer to SOP 8: Decontaminating Equipment.

REFERENCES

1. USEPA. 2005. *Region 9 – Technical Guidelines for Accurately Determining Volatile Organic Compound (VOC) Concentrations in Soil and Solid Matrices (EPA-R9QA/05.2).* December.
2. USACE. 2001. *Requirements for the Preparation of Sampling and Analysis Plans (USACE EM 200-1-3).* February 1.
3. USACE. 1998. *Sample Collection and Preparation Strategies for Volatile Organic Compounds in Solids (USACE 1998b).*

SOP 13: Surface Water Sampling

Purpose:	Surface water sampling is the process of collecting surface water samples from a body of water for laboratory analysis.
Applicable for:	Surface water sampling while in the field for laboratory analysis of polychlorinated biphenyls (PCBs), volatile organic compounds (VOCs), metals or other suitable analyte.
Not applicable for:	Soil samples and groundwater samples.

PREREQUISITES	MATERIALS NEEDED
None, unless otherwise noted by Project Engineer.	✓ Sampling Plan ✓ Selected Sampling Equipment: Disposable Bailers, Peristaltic Pump, Kemmerer or Von Dorn sampler. ✓ Nitrile disposable gloves ✓ Paper Towels ✓ Pre-cleaned sample containers, preserved as appropriate ✓ Field notebook ✓ Personal protective equipment (i.e., hard hat, waterproof boots, waders, PFD, etc.) ✓ Cooler and ice

PROCEDURE

1) Select locations from which surface water grab samples are desired using Site Sampling Plan. Select the appropriate sampling method for each sample location and data needs.

2) Initiate sampling at the downstream location and proceed up stream to each sampling location.

3) Collect Sample:

Dipping Method

A sample may be collected directly into the sample container when the surface water source is accessible by wading or other means. The sampler should face upstream if there is a current and collect the sample without disturbing the bottom sediment. The surface water sample should always be collected prior to the collection of a sediment sample at the same location. The sampler should be careful not to displace the preservative from a pre-preserved sample container, such as the 40-ml VOC.

Bailer Method

Submerge the bailer into the water column until the bailer is filled with water. Carefully transfer the water from the bailer into the pre-cleaned laboratory sample container, making sure that the bailer does not come in contact with the sample containers.

Peristaltic Pump Method

The peristaltic pump can be used to collect a water sample from any depth if the pump is located at or near the surface water elevation. There is no suction limit for these applications. The use of a metal conduit to which the tubing is attached, allows for the collection of a vertical sample (to about a 25-foot depth) which is representative of the water column. The tubing intake is positioned in the water column at the desired depth by means of the conduit. Using this method, discrete samples may be collected by positioning the tubing intake at one depth or a vertical composite may be collected by moving the tubing intake at a constant rate vertically up and down the water column over the interval to be composited. New tubing is to be used at each sampling location.

Kemmerer/Von Dorn Method

The Kemmerer sampler is a brass cylinder with rubber stoppers that leave the ends of the sampler open while being lowered in a vertical position, thus allowing free passage of water through the cylinder. The Van Dorn sampler is plastic and is lowered in a horizontal position. In each case, a messenger is sent down a rope when the sampler is at the designated depth, to cause the stoppers to close the cylinder, which is then raised. Water is removed through a valve to fill respective sample containers. With a rubber tube attached to the valve, dissolved oxygen sample bottles can be properly filled by allowing an overflow of the water being collected. With multiple depth samples, care should be taken not to disturb the bottom sediment, thus biasing the sample. When metals and organic compounds parameters are of concern, then a double-check valve, stainless steel bailer or Kemmerer sampler should be used to collect the sample. The sampler must be decontaminated after each sample is collected.

4) Transfer surface water sample to appropriate sample containers. For volatile organic compounds (VOC) analysis ensure as little agitation or disturbance as possible.

5) Close the sample container and complete and attach the sample label.

6) Record all relevant information and observations about the sample location.

7) Complete the field log book and chain-of-custody form.

8) Decontaminate the reusable equipment in accordance with the protocol specified in the project scope and refer to SOP 8: Decontaminating Equipment.

REFERENCES

1. USEPA. 2016. *Science and Ecosystem Support Division Operating Procedure – Surface Water Sampling (EPA-SESDPROC-201-R4). Revision 4, Effective December 16*

SOP 26: Per-and Polyfluorinated Alkyl Substance Related Sampling

Purpose:	Guidelines to be applied IN ADDITION to environmental media sampling and laboratory analysis for the presence of per- and polyfluorinated alkyl substances (PFAS).
Applicable for:	PFAS sampling while in the field for laboratory analysis via EPA Method 537 Ver. 1.1, 537.1, 533; DoD QSM 5.2; or lab proprietary PFAS methods.
Not applicable for:	N/A

PREREQUISITES	MATERIALS NEEDED
Media specific SOP, soil, groundwater, surface water etc.	✓ Sampling Plan ✓ Sampling Materials (spoons, bailers, etc) ✓ Laboratory provided bottles and blanks ✓ Field notebook ✓ Paper towels ✓ Sampling Sheet and/or iPad with appropriate iDataCollect^{SM} Form ✓ Nitrile gloves ✓ Cooler and ice

PROCEDURE

PFAS are frequently present in the clothing, sampling materials and PPE used for routine environmental media sampling. PFAS presence should be considered ubiquitous in all consumer materials we use, unless specifically noted as "PFAS free". Therefore, extra care must be taken when collecting samples for PFAS analyses. The following provides guidance to be utilized to minimize the risk of inadvertently contaminating media samples with PFAS. Adherence to "standard"/"good practice" sampling protocols will greatly reduce the risk of inadvertently contaminating samples. These additional measures should be taken to further reduce potential contamination of samples.

1) Sampler must assure that no potential PFAS containing materials are utilized during sampling. No materials containing Teflon, Goretex, or other waterproofing can be utilized while sampling. LDPE products should be avoided (tubing, tarps, spoons, bailers) in favor of HDPE. Aluminum foil should also be avoided as it may have a thin layer of coating with PFAS.

2) Extra care to assure clothing, storage containers, and sampling equipment do not contain potential PFAS must be taken. Clothing should not be "weather proof", "water proof" or contain Teflon. Natural fiber clothing that has been laundered without fabric softener is preferred. If rain gear must be worn, field blanks should be collected at each sample location to determine if PFAS impacts occur during sampling.

3) Use of cosmetics, insect repellant, deodorant, perfumes, and sun block should be minimized if possible. PFAS free products may exist and should be utilized when available. The following products are acceptable:

- Sunscreens - Alba Organics Natural Sunscreen, Yes To Cucumbers, Aubrey Organics, Jason Natural Sun Block, Kiss my face, and baby sunscreens that are "free" or "natural"
- Insect Repellents - Jason Natural Quit Bugging Me, Repel Lemon Eucalyptus Insect repellant, Herbal Armor, California Baby Natural Bug Spray, BabyGanics
- Sunscreen and insect repellant – Avon Skin So Soft Bug Guard Plus – SPF 30 Lotion

4) Decontamination solutions and rinse waters must also be assured to be PFAS free. If deemed necessary samples of the decontamination materials may be needed to confirm lack of PFAS.

5) Record all relevant information and observations about the sample location including an inventory of potential PFAS containing items in the area nearby the sampling point, if any exist. Avoid "Rite-in-the-Rain" notebooks and waterproof paper, sharpies and pencils. Ballpoint pen and loose leaf paper is preferred.

6) Place bottles in the shipping container, add ice and packing materials sufficient to keep samples cool and protected from damage during shipping. DO NOT UTILIZE CHEMICAL ICE PACKS.

References: ITRC PFAS Fact Sheets, 2019

APPENDIX D

Document Information

Document Number: **Revision:**

Document Title:

Department(s):

Date Information

Effective Date:

Notes

Document Notes:

All Dates and Times are listed in:

Signature Manifest

Document Number: ENV-SOP-GBAY-0129 **Revision:** 03

Title: Sample Homogenization, Compositing and Sub-Sampling

All dates and times are in Central Time Zone.

ENV-SOP-GBAY-0129-Rev.03 Sample Homogenization, Compositing and Sub-Sampling

QM Approval

Name/Signature	Title	Date	Meaning/Reason
Kate Verbeten (007119)	Manager - Quality	08 Feb 2021, 07:26:11 PM	Approved

Management Approval

Name/Signature	Title	Date	Meaning/Reason
Nils Melberg (007142)	General Manager 2	09 Feb 2021, 08:41:28 AM	Approved
Christopher Haase (007121)	Manager	09 Feb 2021, 01:28:41 PM	Approved

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1.0 Scope and Application

This standard operating procedure (SOP) describes the laboratory procedure for homogenizing soil, liquid, and biota samples to obtain a representative sample aliquot used for analysis or composite. This procedure is restricted to use by, or under the supervision of, technicians experienced in the preparation of samples.

2.0 Summary of Method

Solid, liquid, or biological samples are thoroughly mixed or blended to ensure that any aliquots taken are representative of the sample as a whole. The samples are mixed in either their original containers or, in the event that original container does not allow for adequate mixing, transferred to an inert container for thorough homogenization.

Necropsy and/or filleting of whole-body animals may be performed to isolate the individual organs or portions of the specimen to be homogenized and utilized for analysis.

This SOP involves instruction to chop, grind, and blend plant materials, biological tissue, sediment and synthetic materials into a homogenized sample compatible with preparation of semi volatile organic extracts and metals digestates.

Analysts must make reasonable judgments when sub-sampling materials in order to obtain a homogenous, representative aliquot of the material. Because of the nature of environmental samples, the analyst may have to treat samples on a case-by-case basis ensuring that all aspects of the analytical method are performed. In the event that the analyst cannot reasonably determine what constitutes a representative sample, the project manager or supervisor must be involved so that the client can help ensure an appropriate representative aliquot is utilized.

3.0 Interferences

Metallic Devices – Samples to be analyzed for metal constituents must not be homogenized using any metallic mixing devices or containers as it may result in contamination of the sample with a variety of metals. Use only glass, plastic or ceramic materials when working with these sample types. This may not be applicable for tissue samples since metallic devices (blenders, etc.) may be necessary for grinding and chopping prior to sample homogenization.

Plastic Devices – Samples to be analyzed for organic constituents must not be homogenized using any plastic mixing devices or containers as it may result in both positive and negative interferences. Use only glass and ceramic devices when working with these sample types. Metal instruments may also be used if analysis for metals is not required from the same sample

Solvents, reagents, glassware, and other sample processing hardware may yield discrete artifacts and/or elevated baselines causing misinterpretation of the analytical results. All these materials must be free from interferences under the conditions of the analysis, demonstrated by performing method blanks.

Any printed copy of this SOP and all copies of this SOP outside of Pace are uncontrolled copies.
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4.0 DEFINITIONS

Refer to the Laboratory Quality Manual for a glossary of common lab terms and definitions.

Biota- the flora or fauna of a region.

Composite- combining the typical or essential characteristics of individuals, making up a group.

Fillet- to cut an edible portion of fish. This may or may not contain the ribcage and belly flap and is dependent upon the regulatory, scientific, and data quality objectives for the project.

Head- the upper or anterior division of the animal body that contains the brain, the chief sense organs, and the mouth.

5.0 HEALTH AND SAFETY

The toxicity or carcinogenicity of each chemical material used in the laboratory has not been fully established. Each chemical should be regarded as a potential health hazard and exposure to these compounds should be as low as reasonably achievable.

The laboratory maintains documentation of hazard assessments and OSHA regulations regarding the safe handling of the chemicals specified in each method. Safety data sheets for all hazardous chemicals are available to all personnel. Employees must abide by the health, safety and environmental (HSE) policies and procedures specified in this SOP and in the Pace Chemical Hygiene / Safety Manual.

Personal protective equipment (PPE) such as safety glasses, gloves, and a laboratory coat must be worn in designated areas and while handling samples and chemical materials to protect against physical contact with samples that contain potentially hazardous chemicals and exposure to chemical materials used in the procedure. Hearing protection should be worn when a blender is in operation, or during sediment processing when applicable.

Liquid Nitrogen presents additional hazards and may cause cryogenic burns or displace oxygen and cause rapid suffocation. Use in a well-ventilated area with additional PPE designed for handing these materials.

Contact your supervisor or local HSE coordinator with questions or concerns regarding safety protocol or safe handling procedures for this procedure.

Any printed copy of this SOP and all copies of this SOP outside of Pace are uncontrolled copies.
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6.0 Sample Collection, Preservation, Holding Time, and Storage

The laboratory does not perform sample collection for this procedure. Samples should be collected in accordance with a sampling plan and procedures appropriate to achieve the regulatory, scientific, and data quality objectives for the project. The laboratory will record any nonconformance to these requirements in the laboratory's sample receipt record.

Details concerning sample shipping, preservation and storage can be found in the applicable extraction and/or analytical SOPs, or as specifically discussed below. Samples must be stored separately from all standards and reagents. Where possible, samples for trace analysis should be segregated from highly contaminated samples to avoid cross contamination. Food or drink products must always be kept away from samples and never stored in the same area with samples.

Biota samples must be kept frozen at ≤-10˚C in their original sample containers until the homogenization process occurs.

Small rodents must undergo a special procedure to destroy any Hantavirus which may be present. Refer to the Pace SOP: ENV-SOP-GBAY-0130 *Small Rodent Handling and Homogenization* (most recent revision or replacement) for details.

After homogenization, biota samples are kept frozen at ≤-10˚C in glass jars sized closest to the prepared homogenate sample volume. Individual jars of samples are grouped together as appropriate and stored in a labeled cardboard box within the freezer.

Sediment samples which will be air-dried are received at ≤6°C. After the dry and grind procedure is completed the samples are retained at room temperature.

Synthetic materials may be kept at room temperature.

7.0 Equipment and Supplies

7.1 Equipment

Table 7.1: General Laboratory Homogenization Equipment and Supplies

Supply	Vendor*	Model / ID*	Catalog #*	Description
Spatula	Fisher	Stainless Steel	S50822	-
Spatula	Fisher	Cooper Surgical Inc	11080	Plastic scrapers
Analytical Balance	Varied	Capable of weighing nearest sensitivity	NA	Analytical or top loading
Sample Mixing Containers	Varied	Metal, plastic, glass or ceramic	NA	Dependent upon sample composition

*Or equivalent

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Table 7.2: Biota Homogenization Equipment and Supplies

Supply	Vendor*	Model / ID*	Catalog #	Description
Spatula	Fisher	Stainless Steel	S50822	-
Spoons	Fisher	Spoonulet	14-375-2	Lab spoon
Cutting Board	Cooking Supply	HDPE Stainless Steel	NA	Inert
Knives	Sharp Hi Carbon	Stainless Steel or Titanium	NA	Inert
Meat Cleaver	Sharp Hi Carbon	Lamson	NA	Stainless Steel
Mallet	Stanley	2lb Mallet	NA	Plastic Face, 2-3lb
Robot Coupe	Robot Coupe	R2UB	NA	Serial# 2471016003J-11
Meat Grinder	Hobart	E-222 / Cast Iron	NA	Serial# 9243-0011-02990
Bell Housing	Berkle	Cast Iron	NA	For Meat Grinder
Blender	Waring	Industrial Grade	NA	Stainless steel blade and blender cup (may use glass)
Scaler	NA	NA	NA	Stainless Steel
Aluminum Foil	NA	Heavy Duty	NA	NA
Pliers	NA	Stainless Steel	NA	NA
Analytical Balance	Mettler Toledo	PE-16	NA	Capable 15000±0.1g
Analytical Balance	Sargent Welch	400DR	NA	Capable of 150±0.001g
Analytical Balance	A&D	GH200	NA	Capable of 50±0.0001g
Vial	C&G	40mL	NA	Amber Glass
Wide-mouth Container	C&G or QEC	2oz CG 4oz AG 9oz AG	Various	Clear or Amber Glass with Teflon lined cap
Ear plugs or Earmuffs	Howard Leight	Foam ear plugs or noise reducing earmuffs	NA	Moldex
Paper Towels	NA	11x8.8inch	NA	Georgia Pacific

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Table 7.3: Sediment Homogenization Equipment and Supplies

Supply	Vendor*	Model / ID	Catalog #	Description
Spatula	Fisher	Stainless Steel	S50822	-
Cutting Board	Cooking Supply	HDPE Stainless Steel	NA	Inert
Mallet	Stanley	2lb Mallet	NA	Plastic Face, 2-3lb
Rolling Pin	Cooking Supply	Marble	NA	NA
Aluminum Foil	NA	Heavy Duty	NA	Reynolds
Scissors	Varies	Stainless Steel	NA	NA
Pliers	NA	Stainless Steel	NA	NA
Weigh Boat	Big Science	Large	80060	Plastic
Analytical Balance	Sargent Welch	Capable 0.001g	NA	NA
Vial	C&G	40mL	NA	Amber Glass
Plastic Bags	NA	Quart size Gallon size	NA	Ziploc®
Ear plugs or Earmuffs	Howard Leight	Foam ear plugs or noise reducing earmuffs	NA	Moldex
Dust Mask	Modex	NA	4240-01-255-7856	NA
Paper Towels	NA	11x8.8inch	NA	Georgia Pacific

*Or equivalent

Table 7.4 Computer Hardware and Software

Hardware/Software*	Description*	Make/ModelVersion*
Computers	Laptop or desktop	Various
Horizon	Data reporting software (LIMS)	See master list for current version.
Electronic Prep Log	Electronic bench sheet software	See master list for current version.

*Or equivalent

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8.0 Reagents And Standards

8.1 Reagents

Table 8.1: Biota Reagents and Standards

Reagent	Concentration/ Description	Requirements/ Vendor/ Item #*	Expiration Date
Liquid Nitrogen	To homogenize biota matrices.	11700	Manufacturer's recommended expiration date or 5 years from receipt, whichever is sooner.
Tuna	Canned / matrix for SVOA parameters as required	Starkist in water or Equivalent	None assigned until blended, then given a 1-year expiration date.
Chicken	Ground / matrix for metals and wetchem parameters as required	NA	
DI Water	Type II ASTM	US Filter 18Ω	NA
Alconox	Cleaning Solution	Fisher / 50-212-165	NA
Bleach	Cleaning Solution / Commercial Grade	Clorox or equivalent	NA
Dry Ice	For shipping purposes	NA	NA

*Or Equivalent

Table 8.2: Sediment Homogenization Reagents and Standards

Reagent	Concentration/ Description	Requirements/ Vendor/ Item #*	Expiration Date
DI Water	Type II ASTM	US Filter 18Ω	NA
Methanol	Pesticide Grade	Fisher / 141.40	Manufacturer's recommended expiration date or 2 years from receipt, whichever is sooner.

*Or equivalent

9.0 Procedure

9.1 Analytical Balance Calibration

9.1.1 Annual Calibration – The balance must be calibrated at least annually by an outside agency and checked daily before each use using Class 1 or 2 weights. Refer to Pace ENV-SOP-GBAY-0115 *Support Equipment* (current revision or replacement).

9.1.2 Daily Calibration Check

9.1.2.1 Clean the balance and surrounding area prior to starting the daily calibration check.

9.1.2.2 Check the sight level on the balance. If it needs adjusting, level the balance.

9.1.2.3 The weight set ID indicated in the logbook is used as the primary set. If an alternate weight set ID is used, that ID must be recorded in the comment section of the balance calibration logbook for that day.

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9.1.2.4 Tare the balance before weighing the NIST certified weights.

9.1.2.5 Use forceps or other means to lift each weight (Do not touch the weights with fingertips as the residue may artificially adjust the true value of the weights). Record the date of the calibration check, the true value of the weight, and the actual measured weight in the logbook. Repeat this procedure for the other certified weights. If calibration weights differ from the certified weights by more than specified in the balance calibration logbook, corrective action must be taken (see Section 9.1.3).

9.1.3 Corrective Action

9.1.3.1 Clean the balance and balance pan. Check the sight level on the balance and adjust if necessary. Re-tare and reweigh all the certified weights.

9.1.3.2 The internal calibration function (if available) of the balance may be used as a means of corrective action.

9.1.3.3 Utilize the internal calibration function and diagnostics. Refer to instrument manual.

9.1.3.4 Contact the QA office for assistance if the balance does not meet the calibration tolerances.

9.1.3.5 If the above action does not correct the problem, the balance should be taken out of service and appropriately labeled to avoid improper usage. A service technician should be contacted.

9.1.3.6 Record any corrective action. Initial and date all entries in the logbook.

9.2 Soil/Solid Sample Homogenization and Sub-sampling

9.2.1 Soil samples that are collected in regulated domestic areas or that are of foreign origin must be handled in accordance with the Pace SOP: ENV-SOP-GBAY-0121, *Regulated Soil Handling* (current revision or replacement).

9.2.2 An analyst examines the sample as received by the laboratory. If standing water is noted on top of a soil or sediment sample, the water is incorporated into the sample. Any foreign material, not subject to analysis, must be removed and discarded. This material may include sticks, leaves, rocks, etc. that are not or cannot be analyzed for that particular procedure. Any questions related to the nature of the sample should be directed to the Project Manager (PM). The PM will work with the client to help determine what should be considered foreign material and how to appropriately qualify the final report, if necessary.

9.2.3 The remaining sample must be homogenized until the point of an even consistency throughout the entire sample. The easiest option, for non-volatile samples, is to mix the sample thoroughly in the original container. The sample is considered thoroughly mixed once all layers, colors, and inconsistencies have been evenly distributed throughout the container. If additional mixing is still required to get an even consistency, proceed to the next step.

9.2.4 If mixing is difficult in the original container, the analyst should transfer the entire sample into a larger, clean container and attempt homogenization there. After mixing, return the homogenized sample to its original container. If homogenization within the container was successful, this step may be omitted.

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9.2.5 After mixing thoroughly, the analyst must take out a representative aliquot to use for preparation or analysis. The analyst must not add or remove minute quantities of sample from the representative aliquot in an attempt to target a specific final weight. If an analyst weighs out slightly more than the required weight of sample, they have not adversely impacted the reporting limit for that sample. If applicable, the analyst should also weigh out portions of the sample for duplicate or spike analysis at this time so that all aliquots are approximately equal to each other. When taking aliquots for QC samples, they must be taken one after the other from the container. A larger amount of sample may not be removed from the container, mixed further, and then subdivided.

9.2.6 For volatile soil samples, the analyst must attempt homogenization as quickly as possible to minimize the loss of volatile analytes. This may limit the amount of mixing that can be done on the sample. The analyst can quickly scrape off the top layer of soil, core down to the center of the jar, and take an aliquot to acquire a sufficiently representative sample. It should also be noted that for sample containers that are received for multiple analyses, the volatiles department must take their aliquot first to minimize loss of volatiles and minimize the chance of contamination from other departments. Then the sample can be processed for percent moisture, organic extractions, etc.

9.2.7 Other options for soil homogenization may be employed if requested by a particular client. When employing a client-requested method, there must be clear documentation in the preparatory and analytical logbooks stating the method used.

9.3 Water/Liquid Homogenization and Sub-sampling

9.3.1 Water samples are homogenized by shaking the sample bottle prior to pouring an aliquot. This applies to non-volatile analytical methods only. If a sample contains distinct layers – either liquid or solid – the department supervisor must be consulted to determine the most appropriate means of homogenizing and splitting the sample.

9.4 Biota (Biological Tissue and Plant) Homogenization:

9.4.1 Clean the work area by wiping the surfaces with a damp cloth. Follow procedure outlined in SOP: ENV-SOP-GBAY-0143 *Labware Cleaning Procedures* (current revision or replacement) to prepare utensils and grinders for use.

9.4.2 Depending on the sample matrix and specific instructions provided by the customer, the method for ensuring homogeneity may vary. Necropsy and/or filleting of whole-body animals may be performed to isolate the individual organs or portions of the specimen to be homogenized and utilized for analysis. The project manager must be contacted for clarification prior to thawing the samples if there are any questions.

9.4.3 Select a set of samples for processing. Depending on the size of the specimen, remove the samples from the freezer to allow the specimen to partially thaw. Large specimens typically need to thaw overnight at room temperature. Small specimens require a shorter amount of time and may be placed in a refrigerator overnight or thawed at room temperature for 2-3 hours during the day of processing. It is important to make sure that each specimen is not touching another specimen during the thawing process.

9.4.4 Record the date that the specimens are removed from the freezer to thaw in the Electronic Biota Homogenization Log.

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9.4.5 Pre-label the appropriate sized sample jars with the LIMS numbers. Samples should be placed in the appropriate sized container dependent upon the sample mass received (40mL, 2oz, 4oz, 9oz). Transport the clean, dry utensils and pre-labeled jars to the countertop work area.

9.4.6 If the client requires an equipment blank to be processed with the samples, the same equipment which is used to process the samples must be Deionized Water (DI) rinsed prior to sample processing. Multiple equipment blanks may be processed with each batch. The equipment blank must be logged into the LIMs system to report with the sample data.

9.4.7 Once the specimen is adequately thawed, processing may begin. Compare the label on the specimen with the pre-labeled jar to verify errors have not occurred.

9.4.8 Small fish, such as minnows, are usually collected as composites and will represent a single composite sample. Large whole fish that require compositing are chopped into cubes and put through the meat grinder together (refer to 9.4.10) and aliquots of the ground tissue are blended with liquid nitrogen (refer to 9.4.11 through 9.4.12).

9.4.9 If the specimen requires filleting prior to homogenization, thaw the fish to the point that it can be cut into with a sharp clean knife. Skinning or scaling may be necessary prior to filleting the fish.

9.4.9.1 Skinning: Catfish, bullheads, and other fish may need to be skinned prior to removing fillets. With a sharp knife slice the skin front to back along the dorsal side of fish. Make another incision from top to bottom just behind the gills. Hold the fish head with one hand and grasp an edge of the skin just behind the gill with pliers. Peel the skin back toward the tail. The skin may also be removed by placing the fillet with skin attached, skin side down on the cutting board. Remove the fillet by running knife along the skin between the skin and fillet.

9.4.9.2 Scaling: If scales are to be removed prior to filleting, lay the fish flat on a cutting board. Grasp the fish with one hand and with the other hand use a scaler to scrape the scales off the fish. Work the scaler from the tail toward the head. Rinse the scales and slime from fish prior to filleting.

9.4.9.3 Filleting: Begin with an incision just behind the gills, cutting through the fish from back to belly. Next, make a clean cut along the dorsal ridge towards the tail. Be careful not to cut into the gut cavity. After cutting through to the tail, separate the fillet from the rib cage, peeling the fillet from the carcass with the non-cutting hand. Pick out any bones. (Rib cage may be removed with the fillet at the client's request).

9.4.10 Chop large whole-body specimens, plant material, or fillets into 2-3 inch cubes using a sharp knife and mallet. Smaller samples of limited quantity must be finely ground using the blender in step 9.4.11.

9.4.11 Grind the cubes in a large commercial meat grinder to coarse texture. Repeat the procedure a minimum of two times to ensure proper texture.

9.4.12 Transfer the course ground sample to a stainless-steel bowl containing liquid nitrogen. Place the frozen sample in a blender cup and blend the frozen tissue to a powder consistency.

9.4.13 Transfer the blended sample into the pre-labeled jars.

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9.4.14 Samples such as eggs, insects, and small individual organs (liver, brain) may be stirred vigorously with a metal spatula in an appropriate sized container without liquid nitrogen to avoid loss of sample. The technician documents that the sample was prepared in the container on the prep worksheet or notebook.

9.4.15 Clean the work area and the utensils in accordance with the procedure outlined in SOP: ENV-SOP-GBAY-0143 *Labware Cleaning Procedures* (current revision or replacement) between samples.

9.4.16 Periodically, canned tuna or chicken is homogenized using this procedure for use as a quality control matrix within the laboratory. Each analysis may require a different quality control matrix, See Section 11 for additional information.

9.4.17 Sample integrity must be maintained throughout the digestion and analytical processes, therefore samples which were blended with liquid nitrogen should be kept within a freezer at ≤-10°C up until the weighing process..

9.5 Sediment Homogenization Procedure (Air Dry-Grind Procedure):

9.5.1 Clean the work area by wiping the surfaces with Methanol. Follow procedure outlined in SOP: ENV-SOP-GBAY-0143 *Labware Cleaning Procedure* (current revision or replacement) to prepare utensils and grinding equipment for use.

9.5.2 Pre-label the appropriate aluminum sample drying tray with 2 removable labels containing the sample LIMS numbers. Double bagged client samples should be placed behind the aluminum sample drying tray. The secondary technician must verify the correct bag is placed behind the correct drying tray.

9.5.3 Remove the interior sample bag and place on top of the exterior client bag. Rip along both side seams of the interior bag. Place the opened bag inside out slightly above the drying tray (the sample should fall from the bag into the tray). Ensure no contact occurs between the outside of the bag and the drying tray. The technician may need to manually transfer the sediment from the bag to the tray with their gloved hand. Once transferred, the sample is spread evenly throughout the bottom of the drying tray.

9.5.3.1 An aliquot of the wet sample must be taken in order to complete the dry weight analysis. Please see SOP: ENV-SOP-GBAY-0004 *Measurement of Percent Moisture in Soils and Solids* (current revision or replacement).

9.5.4 The interior sample bag is then placed bag into the exterior client bag. Both bags are then placed into a 2-gallon plastic bag labeled with the batch workorder number.

9.5.5 Prior to placing samples on the cart for transfer, clean the surface of the sample cart by sweeping it to remove visible particulates. Then wipe the surfaces clean with Methanol.

9.5.6 Place all drying trays on the clean sample cart and deliver to the drying room. Use Methanol to wipe the sample racks prior to placing the drying trays in the drying room and record the drying room temperature, which should not exceed 100 degrees Fahrenheit. Adjust the temperature as needed and log any changes in the temperature logbook.

9.5.7 Samples should dry for a minimum of 8 hours or until moisture content is less than or equal to 10%. Once the sediment is adequately dried, processing may begin.

9.5.8 Clean the work area and all processing equipment with Methanol following SOP: ENV-SOP-GBAY-0143 *Labware Cleaning Procedure* (current revision or replacement).

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9.5.9 Obtain the appropriate number of plastic bags. For each sample, separately transfer the labels on the drying tray to the plastic bag. Immediately transfer the dried sample to the plastic bag by sliding the drying tray into the plastic bag and transferring the dried sediment.

9.5.10 Using a rubber mallet and/or a rolling pin, pulverize the sample until the sample is free flowing in nature.

9.5.11 Transfer the labels on the processing bag to a new pre-labeled plastic bag. Immediately transfer the ground/homogenized sample to the new bag, shifting all sediment to one side of the bag, and cutting one corner off with a methanol cleaned scissors.

9.5.11.1 An aliquot of the air-dried sample must be taken in order to complete the air-dry dry weight analysis, please see SOP: ENV-SOP-GBAY-0004 *Measurement of Percent Moisture in Soils and Solids* (current revision or replacement).

9.5.12 Clean the work area and all processing equipment with Methanol following SOP: ENV-SOP-GBAY-0143 *Labware Cleaning Procedure* (current revision or replacement), between samples.

9.5.13 Dried/grind samples are stored at room temperature in individually labeled boxes by Work order.

9.6 Sample Compositing Procedure for Homogenized Sediment:

9.6.1 Clean the work area by wiping the surfaces with Methanol. Follow procedure outlined in SOP: ENV-SOP-GBAY-0143 *Labware Cleaning Procedure* (current revision or replacement) to prepare the work surface for use.

9.6.2 Pre-label the appropriate sample composite container with the LIMS numbers for the composite sample with one permanent label and one removable label.

9.6.3 Set out all samples that will be used to create the composite sample.

9.6.4 Transfer the Pace work order label from the “parent” sample to the “child” composite sample container prior to the sample aliquot being taken. Weigh 20g of the first sample that will be used to create the composite sample into a large weigh boat. Record the mass of the sample in the composite logbook. (Please see Appendix II).

9.6.5 Immediately after the sample aliquot is measured into the large weigh boat, transfer the 20g aliquot to the pre-labeled plastic Ziploc® bag for the composite sample.

9.6.6 Repeat steps 9.6.4 through 9.6.5 using a new large weigh boat each time, until all sample aliquots have been sub-sampled and placed into the composite sample container.

9.6.7 Thoroughly mix the sample in the container to create a homogenized sample.

9.6.8 Record the composite date and time in the composite logbook. The time should be the end time of the compositing process.

9.6.9 An aliquot of the air-dried sample must be taken in order to complete the air-dry dry weight analysis. Please see SOP: ENV-SOP-GBAY-0004 *Measurement of Percent Moisture in Soils and Solids* (current revision or replacement).

9.6.10 Clean the work area and all processing equipment with Methanol following SOP: ENV-SOP-GBAY-0143 *Labware Cleaning Procedure* (current revision or replacement), between samples.

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9.7 Synthetic Material Homogenization:

9.7.1 With Methanol, clean the countertop inside of the prep hood.

9.7.2 Randomly arrange synthetic material into 4 separate quadrants labeled 1,2,3,4 or A, B, C, D. A dust mask and gloves are required to be worn.

9.7.3 Randomly draw a number or letter that corresponds to a quadrant from the sample square. Diagram and log the selection into the fluff prep logbook.

9.7.4 Breakdown all of the material into “penny” sized pieces by chopping, clipping, cutting or tearing the material within the selected square. The sample pieces should be small enough to fit into an extraction thimble.

9.7.5 Place the prepped pieces into a sample bag labeled with the sample number. Return any unused material to its original bag.

9.7.6 Clean the benchtop and all of the utensils used with methanol. Replace your gloves before proceeding to the next sample.

10.0 DATA ANALYSIS AND CALCULATIONS

Not applicable to this SOP.

11.0 QUALITY CONTROL AND METHOD PERFORMANCE

11.1 Tuna is prepared as outlined in Section 9 to be utilized as the Method Blank (MB) and Laboratory Control Spike (LCS) matrix for organic analysis by EPA 8081A/B, 8082, 8082A, and 8270C-SIM. The tuna is an analyte free biota matrix.

11.2 Chicken is prepared as outlined in Section 9. The Chicken Blank (CB) must be prepared for every biota batch analyzed for metals analysis by EPA 6020/A, EPA 7471B and EPA 245.6. The CB will contain detectable amounts of elements such as K, Ca, Na, Mg, and P etc., and is used to ensure acceptable performance of the laboratory control spike. The chicken is also used as the matrix modifier for the Laboratory Control Spike (LCS) matrix.

11.3 Air drying of sediment samples is a minimum of 8 hours or until moisture content is ≤10%.

12.0 DATA REVIEW AND CORRECTIVE ACTION

12.1 Data Review

Pace’s data review process includes a series of checks performed at different stages of the analytical process by different people to ensure that SOPs were followed, the analytical record is complete and properly documented, proper corrective actions were taken for QC failure and other nonconformance(s), and that test results are reported with proper qualification.

The review steps and checks that occur as employees complete tasks and review their own work is called primary review.

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All data and results are also reviewed by an experienced peer or supervisor. Secondary review is performed to verify SOPs were followed, that calibration, instrument performance, and QC criteria were met and/or proper corrective actions were taken, qualitative ID and quantitative measurement is accurate, all manual integrations are justified and documented in accordance with the Pace ENV's SOP for manual integration, calculations are correct, the analytical record is complete and traceable, and that results are properly qualified.

A third-level review, called a completeness check, is performed by reporting or project management staff to verify the data report is not missing information and project specifications were met.

Refer to laboratory SOP ENV-SOP-GBAY-0120 *Data Review and Final Report Process* (current revision or replacement) for specific instructions and requirements for each step of the data review process.

12.2 Corrective Action

Corrective action is expected any time QC or sample results are not within acceptance criteria. If corrective action is not taken or was not successful, the decision/outcome must be documented in the analytical record. The primary analyst has primary responsibility for taking corrective action when QA/QC criteria are not met. Secondary data reviewers must verify that appropriate action was taken and/or that results reported with QC failure are properly qualified. Please see Section 9 for support equipment corrective actions.

12.3 Trouble Shooting

Not applicable to this SOP.

13.0 Pollution Prevention And Waste Management

Pace proactively seeks ways to minimize waste generated during our work processes. Some examples of pollution prevention include but are not limited to: reduced solvent extraction, solvent capture, use of reusable cycletainers for solvent management, and real-time purchasing. Additional information can be found in the Pace's Chemical Hygiene Plan / Safety Manual (current revision or replacement).

The EPA requires that laboratory waste management practice to be conducted consistent with all applicable federal and state laws and regulations. Excess reagents, samples and method process wastes must be characterized and disposed of in an acceptable manner in accordance with Pace's SOP ENV-SOP-GBAY-0125 *Waste Handling and Management* (current revision or replacement).

Regulated soil samples are to be handled in accordance with Pace SOP: ENV-SOP-GBAY-0121, *Regulated Soil Handling* (current revision or replacement).

14.0 Modifications

A modification is a change to a reference test method made by the laboratory. For example, changes in stoichiometry, technology, quantitation ions, reagent or solvent volumes, reducing digestion or extraction times, instrument runtimes, etc. are all examples of modifications. Refer to Pace ENV corporate SOP ENV-SOP-CORQ-0011 *Method Validation and Instrument Verification* (current revision or replacement) for the conditions under which the procedures in test method SOPs may be modified and for the procedure and document requirements.

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15.0 RESPONSIBILITIES

Employees that perform any step of this procedure must have a completed Read and Acknowledgment Statement for this version of the SOP in their training record. In addition, prior to unsupervised (independent) work on any client sample, analysts that complete dry weight analysis must have successful initial demonstration of capability (IDOC) and must successfully demonstrate on-going proficiency on an annual basis. Successful means the initial and on-going DOC met criteria, documentation of the DOC is complete, and the DOC record is in the employee's training file. Refer to laboratory SOP ENV-SOP-GBAY-0094 *Orientation and Training Procedures* (current revision or replacement) for more information.

Pace supervisors/managers are responsible for training employees on the procedures in this SOP and monitoring the implementation of this SOP in their work area.

16.0 ATTACHMENTS

16.1 Attachment I: Sediment Dry-Grind Tracking Logbook

16.2 Attachment II: Fox River Sediment Composite Logbook

17.0 REFERENCES

17.1 Pace Analytical Services, LLC – Green Bay, WI Quality Assurance Manual- current version.

17.2 TNI Standard, Management and Technical Requirements for Laboratories Performing Environmental Analyses, EL-VI-2016-Rev.2.1.

17.3 Department of Defense (DoD) Quality Systems Manual - current version.

17.4CERCLA Quality Assurance Manual; October 1989.

17.5 USEPA National Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories, Volume I: Fish Sampling and Analysis-Third Edition

17.6 Pace Analytical SOP ENV-SOP-GBAY-0130 *Small Rodent Handling and Homogenization* (current revision or replacement).

17.7 Pace Analytical SOP: ENV-SOP-GBAY-0143 *Labware Cleaning Procedures*, (current revision or replacement).

17.8 Pace Analytical SOP: ENV-SOP-GBAY-0121 *Regulated Soil Handling*, (current revision or replacement).

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18.0 Revision History

This Version: ENV-SOP-GBAY-0129-Rev.03

Section	Description of Change
7.4	Added computer hardware and software.
12.3	Added Trouble shooting
Attachment I, II	Updated to current document.

This document supersedes the following document(s):

Document Number	Title	Version
ENV-SOP-GBAY-0129	Sample Homogenization, Compositing and Sub-Sampling	02
ENV-SOP-GBAY-0129	Sample Homogenization, Compositing and Sub-Sampling	01
ENV-SOP-GBAY-0129	Biological Tissue, Plant, Sediment, and Synthetic Material Preparation	00
ENV-SOP-GBAY-0110	Sample Homogenization and Sub-Sampling	01

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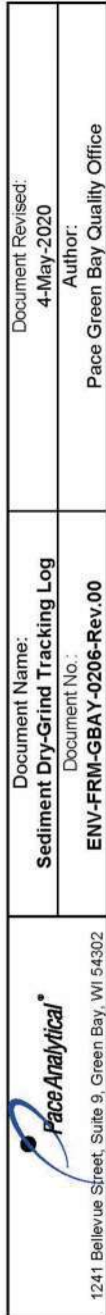
Attachment I: Sediment Dry-Grind Tracking Logbook



Pace Analytical 1241 Bellevue Street, Suite 9, Green Bay, WI 54302	Document Name: Sediment Dry-Grind Tracking Log	Document Revised: 4-May-2020
	Document No.: ENV-FRM-GBAY-0206-Rev.00	Author: Pace Green Bay Quality Office

Sediment Dry-Grind Tracking Logbook

Logbook#: ____________

Sample ID	Initials Set-up Tech	Int. 2° Rev.	Wet Wt Initials (IN)	WET WT (Batch)	Wet Wt Initals (OUT)	Air Dry Room	Air Dry Room Set-up		Initials	Grind Date	Initials (IN)	DRY-DRY WT Batch	Initials (OUT)
							Initials/Date/Time In	Initials/Date/Time Out					
						40DRY01	/ /	/ /					
						40DRY01	/ /	/ /					
						40DRY01	/ /	/ /					
						40DRY01	/ /	/ /					
						40DRY01	/ /	/ /					
						40DRY01	/ /	/ /					
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						40DRY01	/ /	/ /					
						40DRY01	/ /	/ /					
						40DRY01	/ /	/ /					
						40DRY01	/ /	/ /					

Data Entry Instructions: If an error is made while recording information, the error must be corrected by drawing a single line through the mistake, and inserting the date and initials of the person making the change at a minimum. All changes should be made by the person making the original entries to insure there is an understanding of why a change is required. Alternate personnel, such as quality or laboratory management, may make the changes in the event that the original person is not available, but this must be clearly defined.

Peer Review: ____________ Date: ____________

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Attachment II: Fox River Sediment Composite Logbook

Pace Analytical®	Document Name: **FR Sed A/D Composite Logbook**	Document Revised: 5-May-2020
	Document No.: **ENV-FRM-GBAY-0211-Rev.00**	Author: Pace Green Bay Quality Office

Logbook # ______________

Fox River Sediment Air-Dried Composite Logbook

Composite ID:

AFFIX WORKORDER LABEL HERE

Balance ID: 40BALP
Initials of Weighing Technician: ______________
Date/Time of Composite: ______________

Individual Sample ID(s)	Weight of Air-Dried Sample Used to Make Composite (g)	Comments
AFFIX WORKORDER LABEL HERE		
AFFIX WORKORDER LABEL HERE		
AFFIX WORKORDER LABEL HERE		
AFFIX WORKORDER LABEL HERE		
AFFIX WORKORDER LABEL HERE		

Data Entry Instructions: If an error is made while recording information, the error must be corrected by drawing a single line through the mistake, and inserting the date and initials of the person making the change at a minimum. All changes should be made by the person making the original entries to insure there is an understanding of why a change is required. Alternate personnel, such as quality or laboratory management, may make the changes in the event that the original person is not available, but this must be clearly defined.

Peer Review: ______________ Date: ______________

Document Information

Document Number:	Revision:
Document Title:	
Department(s):	

Date Information

Effective Date:

Notes

Document Notes:

All Dates and Times are listed in:

Signature Manifest

Document Number: ENV-SOP-MIN4-0178 **Revision:** 01

Title: Determination of Selected 36 Per- and Polyfluoroalkyl Substances (PFAS) by LC/MS/MS (Isotope Dilution)

All dates and times are in Central Time Zone.

ENV-SOP-MIN4-0178

QM Approval

Name/Signature	Title	Date	Meaning/Reason
Janielle Ward (007319)	Manager - Quality	30 Dec 2020, 07:54:27 PM	Approved

Management Approval

Name/Signature	Title	Date	Meaning/Reason
Adam Haugerud (005828)	General Manager 2	23 Dec 2020, 03:18:11 PM	Approved
Krista Carlson (004514)	Project Manager 1	23 Dec 2020, 03:42:42 PM	Approved
Keith Sturgeon (003603)	Manager	28 Dec 2020, 10:26:58 AM	Approved

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1.0 SCOPE AND APPLICATION

The purpose of this Standard Operating Procedure (SOP) is to document the procedure used for the identification and simultaneous measurement of per- and polyfluoroalkyl substances (PFAS) in non-potable waters, leachate, solid (e.g. soil, sediment, and wipe), and tissue matrices using LC/MS/MS technology based on Table B-15 of the Department of Defense Quality Systems Manual Version 5.3, Appendix B (DoD QSM 5.3), and the Wisconsin PFAS Aqueous (Non-Potable Water) and Non-Aqueous Matrices Method Expectations.

1.1 Target Analyte List and Limit of Quantitation (LOQ)

The target analytes and the normal LOQ that can be achieved with this procedure are provided in Table 1, Appendix A.

LOQ are established in accordance with Pace policy and SOPs for method validation and for the determination of detection limits (DL) and quantitation limits (LOQ). DL and LOQ are routinely verified and updated when needed. The current LOQ for each target analyte that can be determined by this SOP as of the effective date of this SOP is provided in Table 1, Appendix A.

The reporting limit (RL) is the value to which analytes are reported as detected or not detected in the final report. When the RL is less than the lower limit of quantitation (LLOQ), all detects and non-detects at the RL are qualitative. The LLOQ is the lowest point of the calibration curve used for each target analyte.

DL, LOQ, and RL are always adjusted to account for actual amounts used and for dilution.

2.0 SUMMARY OF METHOD

A 250-mL water sample is fortified with a known quantity of isotope dilution extracted internal standards (EIS) and then passed through a solid phase extraction (SPE) cartridge (e.g., Strata™ PFAS, WAX/GCB sorbent, weak anion exchange mixed-mode) to extract the method analytes and EIS. The analytes and EIS are eluted from the cartridge with a small amount of ammonia/methanol solution. The method for the analysis of PFAS in solid materials extracts 5 g of material with a total of 9-mL aliquot of 0.2% ammonia/methanol. The extract is treated with 50 mg ENVI-Carb™ and filtered prior to nitrogen concentration. For tissue samples, 2 g of material is extracted with 7 mL of 1% ammonia acetonitrile for 16 hours. The extract is treated with ENVI-Carb™ and filtered prior to SPE cleanup. The water or solid sample extract is concentrated to ~0.8 mL while the tissue extract is concentrated to ~0.1 mL with nitrogen and spiked with Injection Internal Standards (IIS) and then brought to 1 mL with 96:4% (vol/vol) methanol:H_2O solution prior to LC/MS/MS analysis. A 3-µL injection is made into a Liquid Chromatography (LC) System equipped with a C18 column that is interfaced to a tandem mass spectrometer (MS/MS). The concentration of each analyte is determined by using the isotope dilution and internal standard techniques, depending on target analyte. EIS is added to all calibration standards, field samples, blanks and QC samples to monitor the extraction efficiency of the method analytes.

3.0 INTERFERENCES

3.1 All glassware must be meticulously cleaned. Wash glassware with non-phosphate alkaline detergent and deionized (DI) water, rinse with DI water and reagent water, followed by a methanol rinse. Non-volumetric glassware can be heated in a muffle furnace at 400 °C for 2 h or solvent

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rinsed. Volumetric glassware should be solvent rinsed and not be heated in an oven above 120 °C. Store clean glassware inverted or capped. **Do not cover with aluminum foil because PFAS can be potentially transferred from the aluminum foil to the glassware.**

NOTE: PFAS standards, extracts and samples should not come in contact with any glass containers or pipettes as these analytes can potentially adsorb to glass surfaces. PFAS analytes, EIS and IIS commercially purchased in glass ampoules are acceptable; however, all subsequent transfers or dilutions performed by the analyst must be prepared and stored in polypropylene or equivalent containers

3.2 Method interferences may be caused by contaminants in solvents, reagents (including reagent water), sample bottles and caps, and other sample processing hardware that lead to discrete artifacts and/or elevated baselines in the chromatograms. The method analytes in this method can also be found in many common laboratory supplies and equipment, such as PTFE (polytetrafluoroethylene) products, LC solvent lines, methanol, aluminum foil, SPE sample transfer lines, etc. All items such as these must be routinely demonstrated to be free from interferences (less than 1/2 the RL) under the conditions of the analysis by analyzing method blanks. **Subtracting blank values from sample results is not permitted.**

3.3 Matrix interferences may be caused by contaminants that are co-extracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature of the water. Humic and/or fulvic material can be co-extracted during SPE and high levels can cause enhancement and/or suppression in the electrospray ionization source or low recoveries on the SPE sorbent. Total organic carbon (TOC) is a good indicator of humic content of the sample. Under the LC conditions used during method development, matrix effects due to TOC were not observed.

3.4 SPE cartridges can be a source of interferences. The analysis of field and method blanks can provide important information regarding the presence or absence of such interferences. Brands and lots of SPE devices should be tested to ensure that contamination does not preclude analyte identification and quantitation.

4.0 DEFINITIONS

Refer to the Laboratory Quality Manual for a glossary of common lab terms and definitions.

4.1 **Confirmation Ion –** One of the product ions used to help qualitatively confirm presence of the analytes. The product ion chosen is typically one of the remaining ions with high sensitivity and minimum interferences after the quantitation ion has been chosen. Not all precursor ions provide confirmation ions.

4.2 **Extraction Internal Dilution standards (EIS) –** Isotopically labeled internal standards that undergo the same extraction and analysis as the other analytes in the sample. The EIS are added to the sample at the beginning of the procedure before extraction, centrifugation, filtering, or phase separation. Ideally, there are exact isotopically labeled analogs of the native analytes so that identical behavior can be assumed. The recoveries of these standards are used to adjust the native analyte results.

4.3 **Internal Standard Quantitation –** measurement of native analytes using an alternate analog isotope (one that has the same chemical behavior and is close in retention time to the native

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analyte), thus providing a close approximation of matrix effects and losses that can occur during the preparation and analysis. The native analyte concentration is adjusted for the recovery of the alternate analog isotope. An alternate analog isotope is typically used when an exact analog isotope is not available.

4.4 **Isotope Dilution Quantification –** measurement of native analytes using an exact analog isotope of the native analyte. The native analyte concentration is adjusted for the recovery of the exact analog isotope that has been included in the preparatory and analytical procedure.

4.5 **Precursor Ion –** For the purpose of this method, the precursor ion is the deprotonated molecule ($[M-H]^-$) of the method analyte. In MS/MS, the precursor ion is mass selected and fragmented by collisionally activated dissociation to produce distinctive product ions of smaller *m/z*.

4.6 **Product Ion –** For the purpose of this method, a product ion is one of the fragment ions produced in MS/MS by collisional activated dissociation of the precursor ion.

4.7 **Primary Dilution Standard (PDS) solution –** A solution containing the analytes prepared in the laboratory from stock standard solutions and diluted as needed to prepare calibration solutions and other needed analyte solutions.

4.8 **Preparation Batch –** A group of up to 20 field samples (not including QC samples) extracted together by the same person(s) during a workday (24 hours) using the same lot of SPE devices, solvents, surrogate, internal standard and fortifying solutions. Required QC samples include MB, LCS, MS, and MSD.

4.9 **Quantitation Ion –** One of the product ions used to quantitate analyte concentrations. The product ion chosen is typically one of high sensitivity and minimum interference

5.0 HEALTH AND SAFETY

The toxicity or carcinogenicity of each chemical material used in the laboratory has not been fully established. Each chemical should be regarded as a potential health hazard and exposure to these compounds should be as low as reasonably achievable.

The laboratory maintains documentation of hazard assessments and OSHA regulations regarding the safe handling of the chemicals specified in each method. Safety data sheets for all hazardous chemicals are available to all personnel. Employees must abide by the health, safety and environmental (HSE) policies and procedures specified in this SOP and in the Pace Chemical Hygiene / Safety Manual.

Personal protective equipment (PPE) such as safety glasses, gloves, and a laboratory coat must be worn in designated areas and while handling samples and chemical materials to protect against physical contact with samples that contain potentially hazardous chemicals and exposure to chemical materials used in the procedure.

Concentrated corrosives present additional hazards and are damaging to skin and mucus membranes. Use these acids in a fume hood whenever possible with additional PPE designed for handing these materials. If eye or skin contact occurs, flush with large volumes of water. When working with acids, always add acid to water to prevent violent reactions. Any processes that emit large volumes of solvents (evaporation/concentration processes) must be in a hood or apparatus that prevents employee exposure.

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Contact your supervisor or local HSE coordinator with questions or concerns regarding safety protocol or safe handling procedures for this procedure.

6.0 SAMPLE COLLECTION, PRESERVATION, HOLDING TIME, AND STORAGE

Samples should be collected in accordance with a sampling plan and procedures appropriate to achieve the regulatory, scientific, and data quality objectives for the project.

The laboratory does not perform sample collection or field measurements for this test method. To assure sample collection and field checks and treatment are performed in accordance with applicable regulations. Pace project managers will inform the client of these requirements at the time of request for analytical services when the request for testing is received prior to sample collection. If samples were already collected, the laboratory will record any nonconformance to these requirements in the laboratory's sample receipt record when sufficient information about sample collection is provided with the samples.

The laboratory will provide containers for the collection of samples upon client request for analytical services. Bottle kits are prepared in accordance with laboratory SOP ENV-SOP-MIN4-009 *Bottle Preparation* (current version or equivalent replacement).

Requirements for container type, preservation, and field quality control (QC) for the common list of test methods offered by Pace are included in the laboratory's quality manual.

General Requirements

Matrix	Routine Container	Minimum Sample Amount	Preservation	Holding Time
Aqueous	250 mL HDPE bottle fitted with polyethylene screw-cap lid	250 mL	Thermal: <6 °C but >0 °C Chemical: NA	Collection to Prep: 28 Days Prep to Analysis: 28 Days Extract stored at 0-6ºC
Solid	250 mL HDPE bottle fitted with polyethylene screw-cap lid	5 g	Thermal: <6 °C but >0 °C Chemical: NA	Collection to Prep: 28 Days Prep to Analysis: 28 Days Extract stored at 0-6ºC
Tissue	250 mL HDPE bottle fitted with polyethylene screw-cap lid	2 g	Thermal: Frozen Chemical: NA	Collection to Prep: 1 year Prep to Analysis: 30 Days Extract stored at 0-6ºC

6.1 Sample Collection

6.1.1 The sample handler must wash their hands before sampling and wear nitrile gloves while filling and sealing the sample bottles. PFAS contamination during sampling can occur from a number of common sources, such as food packaging and certain foods and beverages. Proper hand washing and wearing nitrile gloves will aid in minimizing this type of accidental contamination of the samples.

6.1.2 Fill sample bottles. Do not fill aqueous sample containers completely.

6.1.3 Matrix spike (MS) and matrix spike duplicate (MSD) sample. Analysis of a MS is requested in each extraction batch and is used to determine that the sample matrix does not adversely affect method accuracy.

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6.1.4 Field Duplicates. Collect one per sampling event for each sampling site

6.2 Sample Shipment –Aqueous and solid samples must be chilled during shipment and must not exceed 6 °C after collection. Ship tissue samples frozen.

6.3 Sample Receipt and Storage - Thermal preservation is checked and recorded on receipt in the laboratory in accordance with laboratory SOP ENV-SOP-MIN4-0008 *Sample Management* (current version or equivalent replacement).

6.3.1 Aqueous and solid sample temperature must be confirmed to be at or below 6 °C when the samples are received at the laboratory.

6.3.2 Samples stored in the laboratory must be held at or below 6 °C until extraction but should not be frozen.

6.3.3 Tissue samples received frozen can be documented as “frozen” at sample receipt. Store tissue samples at less than or equal to -10ºC at the laboratory.

7.0 EQUIPMENT AND SUPPLIES

7.1 Equipment

7.1.1 Brand names and catalogue numbers represent materials in use at the time of this revision. Due to potential adsorption of analytes onto glass, polypropylene containers were used for all standard, sample and extraction preparations. Other materials which meet the QC requirements may be substituted.

Table 7.1.1 - Equipment and Supplies (Including Computer Software)

Supply	Description	Vendor/Item #/Description
SPE cartridge	Phenomenex Strata™ PFAS, WAX/GCB, 200 mg/50 mg 6 mL	Phenomenex, Cat# CS0-9207 or equivalent
Extraction manifold	An automatic/robotic sample preparation system designed for use with SPE cartridges	Supelco Cat# 57030 and 57275 or equivalent
Analytical column	Gemini® 100 × 3 mm 3 µm C18 reverse phase LC column	Phenomenex Cat# 00D-4439-Y0 or equivalent
HPLC	1100/1290 infinity series/NexeraXR	Agilent/Shimadzu
MS	API 4000/5500 quadrupole	Sciex
Analyst®	Data acquisition software	Version 1.6.3
Multiquant™	Data processing software	Version 3.0.2
Avalon	Data reporting software	See master list for current version
Nitrogen evaporator	N-EVAP™ 112 nitrogen evaporator equivalent nitrogen evaporator/heated waterbath capable of heating 25-60ºC	Oasys Heating system (Berlin, MA, USA)
Balance	Electronic, capable of weighing to 0.001 g or equivalent.	NA

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Supply	Description	Vendor/Item #/Description
Syringe pump	Model # NE-300 or equivalent system capable of delivering variable flow rates.	New Era Pump Systems, Inc
Ultrasonicator	Branson ultrasonicator	Branson Model 8510
Sample container	High density polyethylene (HDPE) or polypropylene, 250 mL, wide mouth, with screw top	C&C Container, Cat# 183277
Centrifuge tube and cap	15-mL and 50-mL conical polypropylene tubes with polypropylene screw caps for collection and storage of the extracts	BD Falcon, P/N 352096 and P/N352070
Polypropylene bottles	4-mL narrow-mouth polypropylene bottles	Thermo Cat# 2006-9125
Polypropylene bottles	15-mLnarrow-mouth polypropylene bottles	Thermo Cat# 2002-9050
Autosampler vials	Polypropylene 0.3-mL autosampler vials with polypropylene caps	Phenomenex Cat# AR0-9995-12-C
Adjustable auto-pipettors	Ranges 10-100 µL, 100-1000 µL, and 1000-5000 µL. Laboratory or aspirator vacuum system	NA
ENVI-Carb	Supelclean™ ENVI-Carb™ SPE Bulk Packing	Sigma Aldrich, Cat# 57210-U.
Vacuum extraction manifold	A manual vacuum manifold with Visiprep volume sampler (Supelco Cat# 57030 and 57275 or equivalent) for extraction, or an automatic/robotic sample preparation system designed for use with SPE cartridges, may be used if all QC requirements are met.	Supelco Cat# 57250-U and 57275 or equivalent

7.1.2 Liquid Chromatography (LC)/ Tandem Mass Spectrometer (MS/MS)

7.1.2.1 LC System – Liquid chromatography (LC) system with binary pump, autosampler, column heater. All solvent lines were replaced with (polyether ether ketone) PEEK tubing. PFAS isolator column (Phenomenex Luna ® 30 × 3 mm 5 µm C18 reverse phase LC column, Cat# 00A-4252-Y0) and stainless steel tubing installed between the mixing chamber and injection port. Other equivalent automated LC system capable of reproducibly injecting up to 5-µL aliquots and performing binary linear gradients at a constant flow rate near the flow rate used for development of this method (0.5 mL/min) may be used.

7.1.2.2 LC/MS/MS – The LC/MS/MS must be capable of negative ion electrospray ionization (ESI) near the suggested LC flow rate of 0.6 mL/min. The system must be capable of performing MS/MS to produce unique product ions for the method analytes within specified retention time segments. A minimum of 10 scans across the chromatographic peak is required to ensure adequate precision.

8.0 REAGENTS AND STANDARDS

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8.1 Gases, Reagents, and Solvents – LC/MS grade or equivalent is used (Fisher equivalent is Optima). Other grades may be used, provided it is first determined that the reagent is of sufficiently high purity to permit its use without lessening the quality of the determination. Fisher solvents are preferred for mobile phases (water, methanol, acetonitrile) as the one liter bottles can be directly loaded on the instrument removing a transfer step with the inherent low level contamination.

Reagent/Standard	Concentration/ Description	Requirements/Vendor/Item #
Ammonium Acetate ($NH_4C_2H_3O_2$, CAS# 631-61-8)	Optima LC/MS grade, demonstrated to be free of analytes and interferences	Fisher or equivalent
Acetonitrile (CH_3CN, CAS# 75-05-8)	Optima HPLC grade	Fisher or equivalent Cat# A955-4
Ammonium Hydroxide (NH_4OH, 28-30% in water)	Certified ACS Plus grade demonstrated to be free of analytes and interferences	Fisher or equivalent
Ammonia/Methanol Solution	Optima HPLC grade, w = 0.2% mass fraction. Mix 0.72 mL of 28-30% ammonia solution with 99.28 mL of methanol	Fisher or equivalent
Glacial Acetic Acid ($C_2H_3CO_2H$, CAS# 64-19-7)	HPLC grade. Demonstrated to be free of analytes and interferences	VWR Analytical Cat# BDH20108
Sodium hydroxide (NaOH, 1310-73-2)	Certified ACS. High purity demonstrated to be free of analytes and interferences	Fisher, or equivalent
Methanol (CH_3OH, CAS# 67-56-1)	Optima HPLC grade, demonstrated to be free of analytes and interferences	Fisher, or equivalent
Reagent water (H_2O, CAS# 7732-18-5)	Optima HPLC grade, demonstrated to be free of analytes and interferences	Fisher or equivalent Cat# W7-4
Aqueous Mobile Phase (20 mM Ammonium acetate)	To prepare 1 L, add 1.54 g ammonium acetate to 1 L of reagent water.	Fisher or equivalent
Acetate Buffer (25 mM, pH 4)	Mix 0.5 mL of acetic acid with 349.5 mL of water. Dissolve 0.116 g of ammonium acetate in 60 mL of water. Mix 200 mL of the diluted acetic acid with 50 mL of the ammonium acetate solution	Fisher or Equivalent
PPG Tuning Solutions	Instrument tuning compound. Using standards chemical kit with low/high concentration is recommend, however solution can be prepared from a neat material.	Sciex P/N 4406127
Ottawa Sand	To prepare method blank, LCSs, for the extraction of soil samples	EMD or equivalent Cat# SX0075-3
Nitrogen (N_2)	Nitrogen aids in aerosol generation of the ESI liquid spray and is used as collision gas in some MS/MS instruments. The nitrogen (Ultra High Purity or equivalent) used should meet or exceed instrument manufacturer's specifications	Ultra-High Purity or equivalent
Canola Oil	Canola oil, or equivalent, for Oil quality control sample matrix,	Local grocery store
Lake Michigan Fish Tissue	Standard reference materials (SRM) for tissue analysis	NIST, 1947

8.2 Stock Standards – non-neat standards purchased from vendors that are used for the preparation of working standards. Standards containing both branched and linear isomers must be used when commercially available. If not available, the total response of the analyte must be integrated, (i.e.

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accounting for peaks that are identified as linear and branched isomers) and quantitated using a calibration curve which includes the linear isomer only for that analyte, i.e. PFOA.

If no expiration date is assigned by the vendor, expiration date is 1 year from the date of receipt. For open stock standards, the expiration is date is 1 year for the open date.

PFBS, PFPeS, PFHxS, PFHpS, PFOS, PFNS, PFDS, 4:2FTS, 6:2FTS, 8:2FTS, DONA, 9Cl-PF3ONS and 11Cl-PF3OUdS are not available as the acid form, but rather as their corresponding salts, such as Na^+ and K^+. These salts are acceptable for use as stock standards as long as the weight is corrected for the salt content according to the equation below:

$$Mass_{acid} = Measured\ Mass_{salt} \times \frac{MW_{acid}}{MW_{salt}}$$

Where: $MW_{(acid)}$ = the molecular weight of PFAS

$MW_{(salt)}$ = the molecular weight of purchased salt

NOTE: All standards purchased are greater than or equal to 98% purity, therefore the weight can be used without correction to calculate the concentration of stock standards. Primary stock standards are stored at ≤ 4 ± 2 ºC. Stock solution is brought to room temperature before using. PFAS may be purchased in glass ampoules however all further solutions and storage is in polypropylene or equivalent containers.

Table 8.2 – Description and Concentration of Each Analyte in Stock Solution

Analyte/Concentration		Used to prepare
Wellington Laboratories PFAC-30PAR (µg/mL)		Cal curve and spiking solution.
PFBA	1	
PFPeA	1	
PFHxA	1	
PFHpA	1	
PFOA	1	
PFNA	1	
PFDA	1	
PFUdA	1	
PFDoA	1	
PFTrDA	1	
PFTeDA	1	
PFOSA	1	
N-EtFOSAA*	1	
N-MeFOSAA*	1	
HFPO-DA	1	
DONA	0.945	
PFBS	0.887	

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Analyte/Concentration		Used to prepare
PFPeS	0.941	
PFHxS*	0.914	
PFHpS	0.953	
PFOS*	0.928	
PFNS	0.962	
PFDS	0.965	
4:2FTS	0.937	
6:2FTS	0.951	
8:2FTS	0.960	
9Cl-PF3ONS	0.933	
11Cl-PF3OUdS	0.943	
Wellington Laboratories PFAC-8Native (µg/mL)		
10:2FTS	48.2	
N-MeFOSA	50	
N-EtFOSA	50	
N-MeFOSE	50	
N-EtFOSE	50	
PFDoS	48.4	
PFHxDA	50	
PFODA	50	
Wellington Laboratories PFAC-24PAR (µg/mL)		Use for the ICV
PFBA	2	
PFPeA	2	
PFHxA	2	
PFHpA	2	
PFOA	2	
PFNA	2	
PFDA	2	
PFUdA	2	
PFDoA	2	
PFTrDA	2	
PFTeDA	2	
PFOSA	2	
N-EtFOSAA	2	
N-MeFOSAA	2	
PFBS	1.77	

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Analyte/Concentration		Used to prepare
PFPeS	1.88	
PFHxS*	1.82	
PFHpS	1.90	
PFOS*	1.86	
PFNS	1.92	
PFDS	1.93	
4:2FTS	1.87	
6:2FTS	1.90	
8:2FTS	1.92	
Wellington Laboratories PFAC-12Native (µg/mL)		Use for the ICV
10:2FTS	48.2	
HFPO-DA	50	
DONA	47.25	
N-MeFOSA	50	
N-EtFOSA	50	
N-MeFOSE	50	
N-EtFOSE	50	
9Cl-PF3ONS	46.6	
11Cl-PF3OUdS	47.1	
PFDoS	48.4	
PFHxDA	50	
PFODA	50	
Wellington Laboratories MPFAC-24ES (µg/mL)		Isotopically labelled Extracted Internal Standards
13C4_PFBA	1	
13C5_PFPeA	1	
13C3_PFBS	0.929	
13C2_4:2FTS	0.935	
13C5_PFHxA	1	
13C4_PFHpA	1	
13C3_PFHxS	0.946	
13C2_6:2FTS	0.949	
13C8_PFOA	1	
13C9_PFNA	1	
13C8_PFOS	0.957	
13C2_8:2FTS	0.958	
13C6_PFDA	1	

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Analyte/Concentration		Used to prepare
d3-MeFOSAA	1	
13C8_FOSA	1	
d5-EtFOSAA	1	
13C7_PFUdA	1	
13C2_PFDoA	1	
13C2_PFTeDA	1	
Wellington Laboratories MPFAC-6ES (µg/mL)		Isotopically labelled Extracted Internal Standards
13C3_HFPO-DA	50	
13C2_PFHxDA	50	
d7-N-MeFOSE	50	
d9-N-EtFOSE	50	
d3-N-MeFOSA	50	
d5-N-EtFOSA	50	
Wellington Laboratories MPFAC-Injection Internal Standards (µg/mL)		Isotopically labelled Injection Internal Standards (IIS)
13C2_PFHxA	50	
13C4_PFOA	50	
13C2_PFDA	50	
13C4_PFOS	50	
Wellington Laboratories T-PFOA (µg/mL)		Qualitative Standard for PFOA (branch isomer of PFOA)*
T-PFOA*	50	

Note(s):
Asterisked (*) analytes indicate the presence of both linear and branch isomers.
See Appendix A for additional information about acronyms variations.

9.0 PROCEDURE

9.1 Equipment Preparation

9.1.1 Instrument

9.1.1.1 Routine Instrument Operating Conditions

Table 9.1.1.1 – LC-MS/MS Operating Conditions

Injector	Syringe Size	100 µL
	Sample Loop vol.	40 µL
	Injection Volume:	3 µL
	Needle Wash 1	100% Methanol
Pump	Flow rate	400 µL/min

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	Flow method	Gradient		
	Mobile Phase 1	20 mM Ammonium Acetate H2O		
	Mobile Phase 2	LCMS Acetonitrile		
	Gradient Program	Time	% Mobile Phase 1	% Mobile Phase 2
		Initial	90	10
		0.5	90	10
		8.0	20	80
		10.0	20	80
		10.1	5	95
		12.0	5	95
		12.1	90	10
		15.0	90	10
Column	Type:	Phenomenex Gemini® (or equivalents)		
	Part Number:	00D-4439-Y0		
	Running temp	40 °C		
	Length:	100 mm		
	Diameter:	3 mm		
	Particle Size	3.0 µm		
Nominal Tune Values	Collision Gas	10 psi		
	Curtain Gas	25 psi		
	Ion Source Gas 1	40 psi		
	Ion Source Gas 2	50 psi		
	IonSpray Voltage	-4500 v		
	Temperature	450 °C		
	ESI polarity	Negative		
	Declustering Potential	Optimized for each analyte (See Appendix C for reference)		
	Collision Energy			
	Collision Cell Exit Potential			

9.1.2 Routine Instrument Maintenance

9.1.2.1 Routine instrument maintenance is critical to achieve optimum method sensitivity. All laboratory materials must be determined to be free of contamination to ensure potential background interferences are minimized.

9.1.2.2 Please refer to the instrument manual for maintenance procedures performed by the lab.

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9.1.2.3 All maintenance activities are listed in maintenance logs that are assigned to each separate instrument.

9.1.2.4 **LC Maintenance –** LC system components, as well as the mobile phase constituents, contain many of the method analytes in this method. Thus, these PFAS will build up on the head of the LC column during mobile phase equilibration.

9.1.2.5 **Column Equilibrate –** To minimize the background PFAS peaks and to keep background levels constant, the time the LC column sits at initial conditions must be kept constant and as short as possible (while ensuring reproducible retention times).

9.1.2.6 **Column Flush –** In addition, prior to daily use, flush the column with 95% methanol for at least 15 min before initiating a sequence. It may be necessary on some systems to flush other LC components such as wash syringes, sample needles or any other system components before daily use.

9.1.3 **MS Maintenance –** Please refer to the instrument manual for maintenance procedures performed by the lab. Common maintenance procedures are listed below

9.1.3.1 **Source Cleaning –** Clean the ion source parts which include curtain plate, orifice plate or skimmer, Q0, and etc. with reagent water and methanol. Tuning or optimizing the instrument followed the instrument manual. Refer to the Operating Instruction – Tune and Calibrate, the ion source operator guide, or the Analyst® software Help system.

9.1.3.2 **Pump Oil –** Check pump oil level and color periodically. Add or change pump oil when necessary followed the manual instruction.

9.1.4 **Troubleshooting**

9.1.4.1 Any deviations from the norm encountered while conducting this analysis must be noted and brought to the attention of the section supervisor. This section contains basic information for troubleshooting basic system issues. Certain activities may be carried out by the Agilent and AB SCIEX trained Qualified Maintenance Person (QMP) in the laboratory. For advanced troubleshooting, contact field service agents of the instruments.

9.1.4.2 **LC Troubleshooting –** Please refer to the instrument manual for troubleshooting procedures performed by the lab. Common LC issues are listed below.

9.1.4.2.1 **Pressure Issue –** Large pressure variation could cause by the presence of air bubble in the system, blockage of the system, column contamination, system leaking, and etc. High pressure issue could be solved through system solvent purge, column rinse, clean or change of column inlet frit, injection valve, needle seat, and etc. Low pressure issue could usually be fixed by tighten or replace the capillary connection or other parts such as pump seals.

9.1.4.2.2 **Peak Shape Issue –** Split peaks, peak tailing, poor efficiency, and inconsistent response are usually associated with issues like column contamination, partially plugged frit, column void, injection solvent

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effects, or sample overload effects. Rinsing or changing the column, preparing fresh mobile solvent, reducing sample injection volume could usually

9.1.4.2.3 **Retention Time –** Deviation of retention time from originally values could cause by column aging or contamination, insufficient system equilibration, mobile phase variation, change in column temperature, or other instrument issues. Cleaning the HPLC system and column could Ammonium acetate is volatile and may cause the shift of retention time over certain period of time. Prepare fresh mobile phase solvent when necessary.

9.1.4.2.4 **Background Contamination –** After multiple injections or long period of operation, background interference may accumulate at the gradient proportional valve, needle seat, or other instrument parts. Rinsing and cleaning corresponding parts with reagent water, methanol or stronger solvents to remove the interference.

9.1.4.2.5 **MS Troubleshooting –** Please refer to the instrument manual for troubleshooting procedures performed by the lab. Common MS issues are listed below:

9.1.4.2.5.1 **Sensitivity Loss –** The possible causes for intensity decrease could be contamination of TurboV ion spray, or the instrument requires tuning and optimization. Clean the ion source including curtain plate, orifice plate or skimmer, Q0. Tune or optimize the instrument following the instrument manual. Refer to the Operating Instruction – Tune and Calibrate, the ion source operator guide, or the Analyst® software Help system.

9.1.4.2.5.2 **Low Vacuum Pressure –** Low pump oil level could cause the vacuum pressure issue. Check the pump oil level and add oil if necessary.

9.2 Initial Calibration

9.2.1 Calibration Design

9.2.1.1 Demonstration and documentation of acceptable initial calibration is required before any samples are analyzed. After the initial calibration is successful, a continuing calibration verification (CCV) is required at the beginning and end of each period in which analyses are performed, and after every tenth field sample. Samples must be bracketed with CCV's passing for all criteria, or the samples should be re-analyzed (with the single exception mentioned in Appendix B).

9.2.1.2 **ESI-MS/MS Tune –** Tuning should occur at least every six months using PPGs for tuning. Tune the system when ICAL won't pass, when the peak shape is significantly off (indicating an MS problem), when major maintenance is performed, or instrument is moved.

9.2.1.3 The instrument tuning with PPGs has its own manufacturing criteria- see the documentation. After PPGs passes, calibration must be verified to be +/- 0.5 amu

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of true values by acquiring a full scan continuum mass spectrum of a PFAS stock standard.

9.2.1.4 Mass calibration range must bracket the ion masses of interest.

9.2.1.5 When done, run the Compound Optimization or Manual Tuning under the Tune and Calibrate tab to optimize response and peak shape.

9.2.1.6 Prepare a set of six CAL standards (Table 9.2.1.20A as an example). Analyze each standard level with the same acquisition method used to analyze samples, changes to retention times or other analytical parameters are saved as part of the local method generated with each analytical sequence, these parameters can be adjusted mid-sequence so long as they are applied to all data.

9.2.1.7 The LC/MS/MS system is calibrated using the isotope dilution and internal standard technique. Use the LC/MS/MS data system software to generate a linear regression calibration curve for each of the relevant analytes. This curve may be concentration weighted, if necessary.

9.2.1.8 A calibration meets criteria when the recovery for each calibration point reads back at ±30% for all calibration points.

9.2.1.8.1 For Wisconsin samples, re-quantitated concentrations for all target compounds at all concentration levels must be within the range 70-130% of their actual concentrations, except for the lowest calibration concentration level, which must be within the range of 50-150% of actual concentration.

9.2.1.9 Provided a minimum of five calibration points are still being used, a point at the top or bottom of the calibration curve may be dropped to achieve recovery requirements across the remaining points. Dropping high concentration points lowers the PQL of the calibration and may require that more dilutions are performed. Dropping low calibration points can potentially elevate the RL for this sequence.

9.2.1.10 An ICV (prepared from a second source standard or by different analyst) is run with every initial calibration curve (ICAL). The acceptance criteria are ±30% of the true value.

9.2.1.11 Additional calibration procedures (where applicable) can be found in ENV-POL-CORQ-0005 Acceptable Calibration Practices for Instrument Testing (or equivalent replacement).

9.2.1.12 **537 Mix –** Mix 40 mL of Optima grade Water with 960 mL of Optima grade Methanol. Expires 1 year from prep.

9.2.1.13 **PFAC_EIS – PFAC (Extracted Internal Standards) (0.05 µg/mL, 25 EIS)**

9.2.1.13.1 Dissolve 40 µL of each MPFAC-6ES standard in 537 Mix. Dilute to 2 mL.

9.2.1.13.2 Dissolve each 1 mL of MPFAC-24ES and 10.3.2.1 mix solution in 537 Mix. Dilute to 20 mL. Added 100 µL to each field sample, standard, blanks and QC samples prior to extraction.

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9.2.1.13.3 Used for curve and sample prep. Store at room temperature. Expires 180 days from prep.

9.2.1.14 PFAC_IIS – PFAC (Injection Internal Standards) (0.05 µg/mL)

9.2.1.14.1 Dissolve 50 µL of each MPFAC-Injection Internal Standards in 537 Mix. Dilute to 50 mL. Added 100 µL to each field sample, standard, blanks and QC samples just prior to analysis.

9.2.1.14.2 Used for curve prep and sample extraction. Store at room temperature. Expires 180 days from prep.

9.2.1.15 PFAC_Native Spike Solution

9.2.1.15.1 Dissolve 100 µL of each PFAC-8Native in 537 Mix. Dilute to 5 mL.

9.2.1.15.2 PFAC_Native Spike 1 (~0.2 µg/mL) – Dissolve 1 mL of PFAC-30PAR and 1 mL of 9.2.1.16.1 mix solution to 537 Mix. Dilute to 5 mL.

9.2.1.15.3 PFAC_Native Spike 2 (~0.050 µg/mL) – Dissolve 2.5 mL of PFAC_Native Spike 1 to 537 Mix. Dilute to 10 mL.

9.2.1.15.4 Used for curve prep and sample extraction. Store at room temperature. Expires 180 days from prep.

9.2.1.16 PFAC_ICV Spike Solution

9.2.1.16.1 Dissolve each of 100 µL PFAC-12Native in 537 Mix. Dilute to 2.5 mL.

9.2.1.16.2 PFAC_ICV Spike 1 (~0.2 µg/mL) – Dissolve 1 mL of PFAC-24PAR and 1 mL of 9.2.1.17.1 mix solution to 537 Mix. Dilute to 10 mL.

9.2.1.16.3 PFAC_ICV Spike 2 (~0.050 µg/mL) – Dissolve 2.5 mL of PFAC_ICV Spike 1 to 537 Mix. Dilute to 10 mL.

9.2.1.16.4 Used for ICV prep. Store at room temperature. Expires 180 days from prep.

9.2.1.17 Isomer Check PDS – Isomer check Qualitative primary standard Spike

9.2.1.17.1 Dissolve 40 µL of T-PFOA stock solution in 537 Mix, dilute to 1 mL. Expires 180 days from prep.

9.2.1.17.2 PFOA qualitative dilution standard spike – Dissolve 50 µL PFOA qualitative primary standard spike in 537 Mix, dilute to 2 mL. Expires 180 days from prep.

9.2.1.18 Calibration Curve – Different volumes of PFAC_Native Spike solutions at various concentrations are added to 1 mL 537 Mix (Table 9.2.1.20A). A known amount of EIS is added into each calibration point. The corresponding concentration in 1 mL final solvent is shown in Table 9.2.1.20B.

9.2.1.19 Table 9.2.1.20A – Example Calibration Curve

Calibration Standard Point	Native std. Soln added (µL)	Native std. Soln conc. (µg/mL)	Extracted IS Soln added (µL)	Extracted IS Soln conc. (µg/mL)	Injection IS Soln added (µL)	Injection IS Soln conc. (µg/mL)
CS-1	10	0.05	100	0.05	100	0.05

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Calibration Standard Point	Native std. Soln added (µL)	Native std. Soln conc. (µg/mL)	Extracted IS Soln added (µL)	Extracted IS Soln conc. (µg/mL)	Injection IS Soln added (µL)	Injection IS Soln conc. (µg/mL)
CS-2	20	0.05	100	0.05	100	0.05
CS-3	40	0.05	100	0.05	100	0.05
CS-4	100	0.05	100	0.05	100	0.05
CS-5	200	0.05	100	0.05	100	0.05
CS-6	100	0.20	100	0.05	100	0.05
CS-7	250	0.20	100	0.05	100	0.05
CS-8	500	0.20	100	0.05	100	0.05
ICV	100	0.05	100	0.05	100	0.05
T-PFOA (Qualitative Calibration)	100	0.05	100	0.05	100	0.05

Table 9.2.1.20B – Concentrations of Each Analyte in 1 mL Final Solvent (ng/L)

Analyte	CS-1	CS-2	CS-3	CS-4	CS-5	CS-6	CS-7	CS-8	ICV
PFBA	0.50	1.00	2.00	5.00	10.00	20.00	50.00	100.00	5.00
PFPeA	0.50	1.00	2.00	5.00	10.00	20.00	50.00	100.00	5.00
PFHxA	0.50	1.00	2.00	5.00	10.00	20.00	50.00	100.00	5.00
PFHpA	0.50	1.00	2.00	5.00	10.00	20.00	50.00	100.00	5.00
PFOA	0.50	1.00	2.00	5.00	10.00	20.00	50.00	100.00	5.00
PFNA	0.50	1.00	2.00	5.00	10.00	20.00	50.00	100.00	5.00
PFDA	0.50	1.00	2.00	5.00	10.00	20.00	50.00	100.00	5.00
PFUdA	0.50	1.00	2.00	5.00	10.00	20.00	50.00	100.00	5.00
PFDoA	0.50	1.00	2.00	5.00	10.00	20.00	50.00	100.00	5.00
PFTrDA	0.50	1.00	2.00	5.00	10.00	20.00	50.00	100.00	5.00
PFTeDA	0.50	1.00	2.00	5.00	10.00	20.00	50.00	100.00	5.00
PFOSA	0.50	1.00	2.00	5.00	10.00	20.00	50.00	100.00	5.00
N-EtFOSAA	0.50	1.00	2.00	5.00	10.00	20.00	50.00	100.00	5.00
N-MeFOSAA	0.50	1.00	2.00	5.00	10.00	20.00	50.00	100.00	5.00
PFBS	0.44	0.89	1.77	4.43	8.85	17.70	44.25	88.50	4.43
PFPeS	0.47	0.94	1.88	4.70	9.40	18.80	47.00	94.00	4.70
PFHxS*	0.46	0.91	1.82	4.55	9.10	18.20	45.50	91.00	4.55
PFHpS	0.48	0.95	1.90	4.75	9.50	19.00	47.50	95.00	4.75
PFOS*	0.47	0.93	1.86	4.65	9.30	18.60	46.50	93.00	4.65
PFNS	0.48	0.96	1.92	4.80	9.60	19.20	48.00	96.00	4.80
PFDS	0.48	0.97	1.93	4.83	9.65	19.30	48.25	96.50	4.83

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Analyte	CS-1	CS-2	CS-3	CS-4	CS-5	CS-6	CS-7	CS-8	ICV
4:2FTS	0.47	0.94	1.87	4.68	9.35	18.70	46.75	93.50	4.68
6:2FTS	0.48	0.95	1.90	4.75	9.50	19.00	47.50	95.00	4.75
8:2FTS	0.48	0.96	1.92	4.80	9.60	19.20	48.00	96.00	4.80
10:2FTS	0.48	0.97	1.93	4.83	9.65	19.30	48.25	96.50	4.83
HFPO-DA	0.50	1.00	2.00	5.00	10.00	20.00	50.00	100.00	5.00
DONA	0.47	0.95	1.89	4.73	9.45	18.90	47.25	94.50	4.73
N-MeFOSA	0.50	1.00	2.00	5.00	10.00	20.00	50.00	100.00	5.00
N-EtFOSA	0.50	1.00	2.00	5.00	10.00	20.00	50.00	100.00	5.00
N-MeFOSE	0.50	1.00	2.00	5.00	10.00	20.00	50.00	100.00	5.00
N-EtFOSE	0.50	1.00	2.00	5.00	10.00	20.00	50.00	100.00	5.00
9Cl-PF3ONS	0.47	0.93	1.86	4.66	9.32	18.64	46.60	93.20	4.66
11Cl-PF3OUdS	0.47	0.94	1.88	4.71	9.42	18.84	47.10	94.20	4.71
PFDoS	0.48	0.97	1.94	4.84	9.68	19.36	48.40	96.80	4.84
PFHxDA	0.50	1.00	2.00	5.00	10.00	20.00	50.00	100.00	5.00
PFODA	0.50	1.00	2.00	5.00	10.00	20.00	50.00	100.00	5.00

9.2.1.20 Store at room temperature. Expires 180 days from prep.

NOTE: Stock standards (Section 8.2) were stored at ≤ 4± 2 °C. Primary dilution standards were stored at room temperature to prevent adsorption of the method analytes onto the container surfaces that may occur when refrigerated. Storing the standards at room temperature will also minimize daily imprecision due to the potential of inadequate room temperature stabilization.

9.2.2 Calibration Sequence

9.2.2.1 ESI-MS/MS Tune – Tuning should occur at least every six months using PPGs for tuning. Tune the system when ICAL won't pass, when the peak shape is significantly off (indicating an MS problem), when major maintenance is performed, or instrument is moved.

9.2.2.1.1 Load a 500 µL syringe filled with PPGs tuning solution in the syringe pump and connect it directly to the probe. Hold syringe pump power button for a few seconds to purge the line. Use the SCIEX Analyst® 1.6.3 software to adjust the parameters under the Tune and Calibrate tab to a relative signal maxima for peaks 44.998, 585.385, 933.636, 1223.845, 1572.097, 1863.306, 2037.431, 2211.557 in negative mode and 59.050, 175.133, 616.464, 906.673, 1254.925, 1545.134, 2010.469, 2242.637 in positive mode.

9.2.2.1.2 Mass assignment of tuning standard within 0.5 amu of true value.

9.2.2.1.3 When done, run the Compound Optimization or Manual Tuning under the Tune and Calibrate tab to optimize response and peak shape.

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9.2.2.2 Optimize the precursor ion and product ion for each target analyte by infusing a standard mix from calibration curve to MS. The MS parameters (voltages, temperatures, gas flows, etc.) and the MS/MS parameters (collision energy, declustering potential, collision cell exit potential, etc.) are determined to achieve optimal analyte responses.

NOTE: There have been reports that not all product ions in the linear PFOS are produced in all branched PFOS isomers. (This phenomenon may exist for many of the PFAS.) Thus, to reduce PFOS, PFBS and PFHxS bias, it is required that the precursor m/z → m/z 80 transition be used as the quantitation transition. Some MS/MS instruments may not be able to scan a product ion with such a wide mass difference from the precursor ion; therefore, if the MS/MS cannot measure the precursor m/z → m/z 80 transition they may not be used for this method if PFOS, PFBS, or PFHxS analysis is to be conducted.

9.2.2.3 Establish LC operating parameters that optimize resolution and peak shape.

9.2.2.4 Inject a mid-level CAL standard under optimized LC/MS/MS conditions to ensure that each target analyte is observed in its retention time window and that there are at least 10 scans across the peak for optimum precision.

9.2.2.5 Prepare a set of at least five calibration point standards for linear fit (Table 9.2.1.20A as an example). Analyze each standard level with the same acquisition method used to analyze samples, changes to retention times or other analytical parameters are saved as part of the local method generated with each analytical sequence, these parameters can be adjusted mid-sequence so long as they are applied to all data. Use the LC/MS/MS data system software to generate a linear regression curve for each of the relevant analytes. This curve may be concentration weighted, if necessary. Forcing zero is not allowed for this analysis.

9.2.2.5.1 Calibration points at the top or the bottom of the curve may be dropped to achieve recovery requirements across the remaining points provided the minimum number of calibration points are still being used based on the curve fit. Dropping high concentration points lowers the upper QL of the calibration and may require that more dilutions are performed. Dropping low calibration points may elevate the reporting limit for samples associated with this calibration. The RL must be met without exception.

9.2.2.6 Analyte quantification uses the isotope dilution technique for the analytes having commercially available isotopically labeled analogs. The internal standard technique is used when a labeled analog is not commercially available for the target analyte. Details in analytes quantification refer to Section 10.5.

9.2.3 ICAL Evaluation

9.2.3.1 Calibration factors have RSD that is ≤ 20% for all analytes

9.2.3.2 Linear regressions have a coefficient of determination that is r^2 ≥0.99 and a minimum of five non-zero concentration standards is used.

9.2.3.3 Do not force linear regression through zero.

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9.2.3.4 For each calibration standard, reprocess the target (native) analyte against the chosen calibration function. The reprocessed recoveries are expected to be within ±30% of true value. For some data uses, the lowest concentration standard reprocessed recoveries are expected to be within ±50% of true value.

9.2.3.5 The lowest concentration ICAL standard must be ≤ reporting level (RL).

9.2.3.6 S/N Ratio: ≥ 10: 1 for all quantification ions and S/N Ratio of ≥ 3:1 for confirmation ions

9.2.3.7 Demonstration and documentation of acceptable initial calibration is required before any samples are analyzed. After the initial calibration is successful, a CCV is required at the beginning and end of each period in which analyses are performed, and after every tenth field sample.

9.2.4 **Relative Standard Error (RSE) –** Percent error between the calculated and expected amounts of an analyte should be ≤ 30% for all standards. For some data uses, ≤ 50% may be acceptable for the lowest calibration point.

9.2.5 Initial Calibration Verification

9.2.5.1 **Initial Calibration Verification (ICV) –** analyze an ICV sample from a source different from the source of the CAL standards with each new ICAL before sample analysis. If a second vendor is not available, then a different lot of the standard should be used. The ICV should be prepared and analyzed just like a CCV. Acceptance criteria for the ICV are identical to the CCV; the calculated amount for each analyte must be ± 30% of the expected value. If measured analyte concentrations are not of acceptable accuracy, check the entire analytical procedure to locate and correct the problem.

9.2.6 Continuing Calibration Verification

9.2.6.1 CCVs are run at the beginning, end, and bracketing every 10 field samples. Blanks, rinses, and spiked QC (LCS/LCSD/MS/MSD) are not considered field samples, and so can be run in addition to 10 field samples in a CCV window.

9.2.6.2 The opening CCV for any batch must be below or at the RL (CS-1), all further CCVs cycle between mid and high level calibration point.

9.2.6.3 Calculate the concentration of each analyte in the CCV. The calculated amount for each analyte must be within ± 30% of the true value. Determine that the absolute areas of the quantitation ions of the EIS and IIS are within ±50% from the mid-point measured during initial calibration. On days when ICAL is not performed, the peak areas must be within ±50% of the peak area measured in daily initial CCV. If any of the EIS and IIS areas has changed by more than these amounts, adjustments must be made to restore system.

9.2.6.3.1 For Wisconsin samples, the calculated amount for each analyte must be within ± 30% of the true value except for the lowest ICAL point, for which the calculated amount for each analyte must be within ± 50% of the true value.

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9.2.6.4 If the CCV fails high for a particular analyte, and the field sample shows no detection for that analyte, samples may be reported without re-analysis.

9.2.7 Laboratory Control Sample/Laboratory Control Sample Duplicate (LCS/LCSD)

9.2.7.1 A LCS is required with each extraction batch. See DoD acceptance criteria for LCS targets in Appendix E for aqueous and solid matrices. If the LCS results do not meet the criteria listed in Appendix E for method analytes, then all data for the problem analyte(s) must be considered invalid for all samples in the extraction batch. For target analytes not included in the DoD Limits for batch control Table B-15 per DoD QSM 5.3, , limits of 70-140% recovery will be used as acceptance criteria. For tissue batches, the recoveries are expected to be within 60-140%.

9.2.7.1.1 For Wisconsin samples, the recoveries are expected to be within 60-135%, except for the low range (1 – 2x RL) where the recoveries are expected to be within 50-150% in aqueous and solid batches. For tissue batches, spike the LCS at midrange. For tissue batches, the recoveries are expected to be within 60-135% with the following exceptions: for PFHxDA, PFODA, and NMeFOSA, the recoveries are expected to be within 50-135%; for PFDS, PFDoS, and 4:2 FTS, the recoveries are expected to be within 40-135%.

9.2.8 EIS Recovery

9.2.8.1 The EIS is fortified into all samples, CCVs, MBs, LCSs, MSs, MSDs, and FD prior to extraction. It is also added to the CAL standards. The EIS is a means of assessing method performance from extraction to final chromatographic measurement.

9.2.8.2 A minimal signal to noise ratio of 10:1 is expected for each EIS. Do not report results with a qualifier if this minimum is not achieved.

9.2.8.3 EIS recovery must be in ±50% of the mid-point ICAL when the day the ICAL was performed. When EIS recovery from a sample, blank, or CCV failed the criteria, check 1) calculations to locate possible errors, 2) standard solutions for degradation, 3) contamination, and 4) instrument performance. Correct the problem and re-analyze the extract.

9.2.8.3.1 For Wisconsin samples, all EIS compounds must recover within the range 25-150%, except 13C8-PFOSA, d3-MeFOSA, d5-EtFOSA, d7-MeFOSE, and d9-EtFOSE, which must recover within the range 10-150%. Recovery will be based on area counts.

9.2.8.4 If the EIS recoveries in a chromatographic run do not meet these criteria, inject a second aliquot of that extract from a new capped auto-sampler vial.

9.2.8.5 If the reinjected aliquot produces an acceptable EIS recoveries, report results for that aliquot.

9.2.8.6 If recoveries are acceptable for QC samples, but not field samples, the field samples must be re-prepped and reanalyzed (greater dilution may be needed). If recoveries are unacceptable for QC samples, correct problem, and reanalyze all associated failed field samples.

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9.2.8.7 Apply Q-flag and discuss in the Case Narrative only if reanalysis confirms failures in exactly the same manner.

9.2.8.8 If the extract re-analysis meets the EIS recovery criterion, report only data for the re-analyzed extract.

9.2.8.9 If the extract re-analysis fails the criterion, the analyst should check the calibration by injecting the last CAL standard that passed. If the CAL standard fails the criteria, re-calibration is in order. If the CAL standard is acceptable, extraction of the sample should be repeated provided the sample is still within the holding time. If the re-extracted sample also fails the recovery criterion, report all data for that sample as suspect recovery to inform the data user that the results are suspect due to EIS recovery. Alternatively, collect a new sample and re-analyze.

9.2.9 IIS Recovery

9.2.9.1 The IIS is fortified into all samples, CCVs, MBs, LCSs, MSs, MSDs, and FD prior to extraction. It is also added to the CAL standards. The IIS is a means of assessing instrument performance.

9.2.9.2 A minimal signal to noise ratio of 10:1 is expected for each IIS. Do not report results with a qualifier if this minimum is not achieved.

9.2.9.3 IIS recovery must be in ±50% of the mid-point ICAL when the day the ICAL was performed. When IIS recovery from a sample, blank, or CCV is failed the criteria, check 1) calculations to locate possible errors, 2) standard solutions for degradation, 3) contamination, and 4) instrument performance. Correct the problem and re-analyze the extract.

9.2.9.4 If the EIS recoveries in a chromatographic run do not meet these criteria, inject a second aliquot of that extract from a new capped auto-sampler vial.

9.2.9.5 If the reinjected aliquot produces an acceptable IIS recovery, report results for that aliquot.

9.2.9.6 If recoveries are acceptable for QC samples, but not field samples, the field samples must be re-prepped and reanalyzed (greater dilution may be needed). If recoveries are unacceptable for QC samples, correct problem, and reanalyze all associated failed field samples.

9.2.9.7 If the extract re-analysis meets the IIS recovery criterion, report only data for the re-analyzed extract.

9.2.10 Additional calibration procedures (where applicable) can be found in ENV-POL-CORQ-0005 Acceptable Calibration Practices for Instrument Testing (or equivalent replacement).

9.3 Sample Preparation

9.3.1 Some of the PFAS adsorb to surfaces, including polypropylene. Therefore, the aqueous sample bottles must be rinsed with the elution solvent. The bottle rinse is passed through the cartridge to elute the method analytes and is then collected.

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NOTE: The SPE cartridges and sample bottles described in this section are designed as single use items and should be discarded after use. They may not be refurbished for reuse in subsequent analyses.

9.3.2 Solid Sample Preparation

9.3.2.1 Homogenize the entire solid sample received in the sample container in which it was collected in by stirring the solid with a clean spatula or other implement.

9.3.2.2 5 g of solid sample is weighed in a tared 50-mL polypropylene bottle

9.3.2.3 Add a 100 µL aliquot of the PFAC_EIS to all field and QC samples at the very beginning of the procedure.

9.3.2.4 QC samples for each batch include a MB, LCS and MS/MSD which are extracted along with each prep batch.

9.3.2.4.1 MB is required for each prep batch. Each batch contains a LCS and a pair of MS/MSD. LCS/MS/MSD spike at concentrations ≥ LOQ and ≤ the mid-level calibration concentration. If insufficient sample is available for a pair of MS/MSD, an MS, Dup, and LCSD at the same level of LCS may be used.

9.3.2.4.2 The LCS/LCSD/MS/MSD is spiked with 20 µL of the PFAC_Native Spike 2.

9.3.2.5 5 mL of 0.2% ammonia/methanol is added to all samples and QC, bottles are sealed and put on an ultrasonicator for 20 minutes and then shake for one hour.

9.3.2.6 Centrifuge the samples and QC for 5 minutes after shake.

9.3.2.7 Decant the supernatant layer in a 50-mL polypropylene bottle with 50 mg of ENVI-Carb powder.

9.3.2.8 Repeat sections 9.3.2.5 with 4 mL of 0.2% ammonia/methanol and centrifuge. All supernatant are collected and combined.

9.3.2.9 The combined supernatant is shaken for one hour and then centrifuge for 5 minutes after shake.

9.3.2.10 Clean the filter with 10 mL of 1% of ammonia/acetonitrile.

9.3.2.11 Condition the pre-cleaned filter with 10 mL methanol. Pass the combined supernatant through the filter. Rinse the filter with additional 1 mL 0.2% ammonia/methanol. Collect the filtrate and turn on the vacuum for 10 minutes.

9.3.3 Aqueous Sample Preparation

9.3.3.1 Sample volume is determined gravimetrically. The full sample bottle is weighed and the empty bottle is weighted after extraction. The sample volume is the difference between the full and empty bottle weights. Sample density is assumed at 1 g/mL. When the sample has significant solids, the laboratory should account for the weight or volume displaced by the solid in the initial sample volume determination and include this information in the report.

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9.3.3.2 pH is taken using strips in the lab. This is accomplished via the use of common laboratory grade pH strips (Whatman Indicator Paper pH 0-14 Type CF Cat. No. 2613-991). Adjust the pH to ~5 with acetic acid or 10 mM sodium hydroxide solution when necessary.

9.3.3.3 Add a 100 µL aliquot of the PFAC_EIS to all field and QC samples at the very beginning of the procedure, before extraction, centrifuging, filtering or phase separation takes place. Cap and invert and mix.

9.3.3.4 Ideally, whole samples will pass through the cartridge as received. If particulates in the sample is greater than one percent, centrifuge the sample and take the liquid phase through the SPE after spiking the PFAC_EIS.

9.3.3.5 QC samples for each batch include a MB, LCS and MS/MSD which are extracted along with each prep batch.

9.3.3.5.1 MB is required for each prep batch.

9.3.3.5.2 Each batch contains a LCS and a pair of MS/MSD. LCS/MS/MSD spike at concentrations ≥ LOQ and ≤ the mid-level calibration concentration. If insufficient sample is available for a pair of MS/MSD, an MS, Dup, and LCSD at the same level of LCS may be used.

9.3.3.5.3 The LCS/LCSD/MS/MSD is spiked with 20 µL of the PFAC_Native Spike 2.

9.3.3.6 Proceed with SPE procedure in section 9.3.6.

9.3.4 Tissue Sample Preparation

9.3.4.1 Homogenization will be performed on the entire tissue sample in accordance with SOP ENV-SOP-GBAY-0129 *Sample Homogenization, Compositing and Sub-Sampling* (or equivalent replacement) by the Pace Green Bay laboratory.

9.3.4.2 2 g of tissue sample is weighed in a tared 50-mL HDPE bottle.

9.3.4.3 Add a 100 µL aliquot of the PFAC_EIS to all field and QC samples (canola oil and SRM) at the very beginning of the procedure.

9.3.4.4 QC samples for each batch include a MB, LCS, MS/MSD, and SRM which are extracted along with each prep batch.

9.3.4.4.1 MB is required for each prep batch. Each batch contains a LCS and a pair of MS/MSD. LCS/MS/MSD spike at concentrations at concentrations ≥ LOQ and ≤ the mid-level calibration concentration. If insufficient sample is available for a pair of MS/MSD, an MS, Dup, and LCSD at the same level of LCS may be used.

9.3.4.4.2 The LCS/LCSD/MS/MSD is spiked with 40 µL of the PFAC_Native Spike 2.

9.3.4.5 7 mL of 1% ammonia/acetonitrile is added to all samples and QC, bottles are sealed and put on a shaker for 16 hours.

9.3.4.6 Centrifuge the samples and QC for 5 minutes after shake.

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9.3.4.7 Decant the supernatant layer in a 50-mL polypropylene bottle with 100 mg of ENVI-Carb powder and shake for 1 hour and then centrifuge for 5 minutes after shake

9.3.4.8 Clean the 250 mg ENVI-Carb cartridge with 10 mL of 1% ammonia/acetonitrile.

9.3.4.9 Condition the pre-cleaned cartridge with 10 mL methanol. Pass the supernatant through the cartridge. Rinse the filter with additional 1 mL 1% ammonia/acetonitrile.

9.3.4.10 Collect the filtrate and dilute the filtrate with 125 mL H2O and adjust pH to ~5. Proceed with SPE procedure in 9.3.5.

9.3.5 Cartridge SPE Procedure

9.3.5.1 **Cartridge Clean-up –** Rinse each cartridge with 20 mL of 1% ammonia/acetonitrile solution.

9.3.5.2 **Cartridge Conditioning –** Do NOT allow cartridge packing material to go dry during any of the conditioning steps. Condition each cartridge with 10 mL of 0.2% ammonia/methanol solution following with 10 mL of methanol. Next, rinse each cartridge with 10 mL of reagent water following with 10 mL of acetate buffer, without allowing the water to drop below the top edge of the packing. If the cartridge goes dry during the conditioning phase, the conditioning must be started over. Turn on the vacuum, and begin adding sample to the cartridge through the attached plastic sample transfer reservoir.

9.3.5.3 **Sample Extraction –** Adjust the vacuum so that the approximate flow rate is 6-10 mL/min. Do not allow the cartridge to go dry before all the sample has passed through.

9.3.5.4 **Sample Bottle and Cartridge Rinse –** Rinse the sample bottles with two 5-mL aliquots of reagent water, then draw each aliquot through the plastic sample transfer reservoir and the cartridges. Draw air through the cartridge for 25 min at high vacuum (10-15 in. Hg).

NOTE: If transfer tubes are used in place of the sample transfer tubes to pass the samples through the cartridges, these reservoirs must be treated like the empty plastic sample transfer reservoirs. After the entire sample has passed through the cartridge, the tubes must be rinsed to waste with reagent water.

9.3.5.5 **Sample Bottle and Cartridge Elution –** Turn off and release the vacuum. Lift the extraction manifold top and insert a rack with collection tubes into the extraction tank to collect the extracts as they are eluted from the cartridges. Rinse the sample bottles with 3 mL of 0.2% ammonia/methanol twice and elute the analytes from the cartridges by pulling the additional 3 mL of 0.2% ammonia/methanol through the sample plastic reservoirs and the cartridges. Turn the vacuum on for 20 minutes between each elution. The elution solvent used to rinse the sample bottles must be swirled down the sides of the reservoirs while eluting the cartridge to ensure that any method analytes on the surface of the reservoirs are transferred to the extract.

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NOTE: After centrifuging, it is expected that the solid phase remains in the bottom of the container when rinsing the container with elution solvent. If rinsing the container disrupts the solid phase significantly, the container can be centrifuged again before removing the solvent for use during the elution step.

9.3.6 **Extract Concentration –** Concentrate the extract to approximately 0.8 mL for water and solid extract and approximate 0.2 mL for tissue extract under a gentle stream of nitrogen without a heated water bath. Add 100 µL of PFAC_IIS and fill the sample vial to 1 mL mark with 537 Mix. Then vortex for 5-10 seconds. Transfer a ~100 µL to a 300 µL polypropylene autosampler vial with a plastic pipette. The remaining extract is stored at 0-6 °C.

9.4 Analysis

9.4.1 Establish operating conditions equivalent to those summarized in Appendix C. Instrument conditions and columns should be optimized prior to the initiation of the IDOC.

9.4.2 Establish an appropriate retention time window for each analyte. This should be based on measurements of actual retention time variation for each method analyte in Calibration (CAL) standard solutions analyzed on the LC over the course of time. A value of plus or minus three times the standard deviation of the retention time obtained for each method analyte while establishing the initial calibration and completing the IDC can be used to calculate a suggested window size. However, the experience of the analyst should weigh heavily on the determination of the appropriate retention window size.

9.4.3 Retention Time (RT) acceptance – RT of each analyte and EIS analyte must fall within 0.4 minutes (±0.2 minutes) of the predicted retention times from the daily CCV or, on days when ICAL is performed, from the midpoint standard of the ICAL. Analytes must elute within 0.1 minute of the associated EIS. This criterion applies only to analyte and labeled analog pairs.

9.4.4 Calibrate the system by either the analysis of a calibration curve or by confirming the initial calibration is still valid by analyzing a CCV. If establishing an initial calibration, complete the IDC.

9.4.5 Begin analyzing field samples, including QC samples, at their appropriate frequency by injecting 3 µL of final sample extractant, under the same conditions used to analyze the ICAL standards.

9.4.6 At the conclusion of data acquisition, use the same software that was used in the calibration procedure to identify peaks of interest in predetermined retention time windows. Use the data system software to examine the ion abundances of the peaks in the chromatogram. Identify an analyte by comparison of its retention time with that of the corresponding method analyte peak in a reference standard. Comparison of the MS/MS mass spectra is not particularly useful given the limited ±0.5 amu mass range around a single product ion for each method analyte.

9.4.7 Dilution – When the concentrations of target analytes exceed the highest concentration of ICAL, dilution analyses are required.

9.4.7.1 An appropriate dilution should be in the upper half of the calibration range, or close to the CCV. The diluted extract must maintain the same methanol/water ratio as the original extract

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9.4.7.2 If an analyte concentration exceeds the range of the initial calibration curve, the extract is diluted with 537 Mix. Re-inject the diluted extract. Incorporate the dilution factor into the final concentration calculations. Acceptable injection internal standard (IIS) performance is determined from the undiluted sample extract. The resulting data is documented as a dilution, with an increased LOQ.

9.4.8 In validating this method, concentrations were calculated by measuring the product ions listed in Appendix C. Two transitions and the ion transition ratio per analyte shall be monitored and documented with the exception of PFBA and PFPeA. In order to avoid biasing results high due to known interferences for some transitions, the following transitions must be used for the quantification of the following analytes: PFOA: 413 —› 369, PFOS: 499 —› 80, PFHxS: 399 —› 80, PFBS: 299 —› 80, 4:2FTS: 327 —› 307, 6:2FTS: 427 —› 407 8:2FTS: 527 —› 507, N-EtFOSAA: 584 —› 419, N-MeFOSAA: 570 —› 419. If these transitions are not used, the reason must be technically justified and documented (e.g., alternate transition was used due to observed interferences).

9.4.9 Calculate analyte concentrations using the multipoint calibration established in Section 9.2.1.20. Do not use daily calibration verification data to quantitate analytes in samples. Adjust final analyte concentrations to reflect the actual sample volume.

9.4.10 Prior to reporting the data, the chromatogram is reviewed for any incorrect peak identification or poor integration. Modify if necessary.

9.4.11 Calculations must utilize all available digits of precision, but final reported concentrations are rounded to an appropriate number of significant figures (one digit of uncertainty), typically two, and not more than three significant figures.

9.4.12 For native analytes, the Signal to Noise (S/N) ratio should be ≥3:1 for both quantitation and confirmation ions. If S/N is not achieved, the analyte would be reported as not detected.

9.4.13 Ion Ratios – For analytes with two ion transitions (quantitation and confirmation) are analyzed, the area ratio between the confirmation and quantitation transitions shall be monitored and documented. The ion ratio for all analytes in each injection should be within ± 50% of the mid ICAL ion ratio for the same analyte in the ICAL. On days ICAL is not performed, the ion ratio should be within ±50% of the initial CCV standard.

9.4.14 Report results in acid form.

9.4.15 Perform a moisture analysis on solid samples (on a subsample different than that used for extraction) and adjust the final concentration of solid sample for the percent moisture.

9.4.16 DoD acceptance criteria for LCS and MS target analytes are listed in Appendix E. If the LCS results do not meet the criteria listed then all data for the problem analyte(s) must be considered invalid for all samples in the extraction batch. For target analytes not included in the DoD Limits for batch control table in Table B-15 per DoD QSM 5.3, limits of 70-140% recovery for water and soil, 60-140% recovery for tissue will be used as acceptance criteria.

9.5 Analytical Sequence

9.5.1 Example analytical sequence

Sequence

Instrument Blank (ICB)

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Initial Calibration (ICAL)
Instrument Blank (ICB)
Initial Calibration Verification (ICV)
Continuing Calibration Curve (CCV)
Continuing Calibration Blank (CCB)
Qualitative Standard for PFOA (T-PFOA)
Method Blank (MB)
Laboratory Control Spike/Dup (LCS/LCSD)
Matrix Spike/Dup (MS/MSD)
Field Samples
Continuing Calibration Curve (CCV)
Continuing Calibration Blank (CCB)
Field Samples
Continuing Calibration Curve (CCV)
Continuing Calibration Blank (CCB)

10.0 Data Analysis And Calculations

10.1 Qualitative Identification

10.1.1 Manual Integration

Manual changes to automated integration is called manual integration. Manual integration is sometimes necessary to correct inaccurate automated integrations but must never be used to meet QC criteria or to substitute for proper instrument maintenance and/or method set-up. To assure that all manual integrations are performed consistently and are ethically justified, all manual integrations must be performed, reviewed, and recorded in accordance with corporate SOP ENV-SOP-CORQ-0006, *Manual Integration.*

10.2 Quantitative Identification

10.2.1 Complete chromatographic resolution is not necessary for accurate and precise measurements of analyte concentrations using MS/MS. In validating this method, concentrations were calculated by measuring the product ions listed in Appendix C. Other ions may be selected at the discretion of the analyst.

10.2.2 Prior to reporting the data, the chromatogram should be reviewed for any incorrect peak identification or poor integration.

10.2.3 For PFHxS, PFOS, N-MeFOSAA and N-EtFOSAA, all the chromatographic peaks observed in the standard must be integrated and the areas summed. Chromatographic peaks in all Field Samples and QC samples must be integrated in the same way as the CAL standard for analytes with quantitative standards containing the branched and linear isomers.

10.2.4 For PFOA, identify the branched isomers by analyzing a qualitative standard (T-PFOA) that includes both linear and branched isomers and compare retention times and tandem mass spectrometry transitions. Quantitate Field Samples and QC samples by integrating the total response (i.e., accounting for peaks that are identified as linear and branched isomers) and relying on the initial calibration with a linear isomer quantitative PFOA standard. This qualitative PFOA standard is not used for quantitation. This branched isomer identification check must be repeated any time changes occur that affect the analyte retention times.

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10.2.5 Peaks that are consistent with branched isomers have been observed with other target analytes, in particular PFOA. Quantitate of PFOA by integrating the total response (i.e. accounting for peaks that are identified as linear and branched isomers) and relying on the initial calibration with the linear-isomer quantitative standard is acceptable.

10.2.6 All analytes are quantified using the isotope dilution or internal standard technique.

10.2.7 The native analytes are quantified by comparison of their responses to the mass-labelled internal standards. Relative response factors are calculated from analyses of standard mixtures containing native analytes at six concentration levels, and the concentration remains at a constant level for each internal standard. The target analytes response factors are calculated by comparing the response from the native ion mass monitored to the response from the ion mass of the corresponding isotopically labelled internal standard (See Appendix D for reference).

10.3 Calculations

See the laboratory SOP ENV-SOP-MIN4-0171 *Laboratory Calculations* (or equivalent replacement) for equations for common calculations.

10.3.1 Linear Calibration Using Average Response Factors

For each target analyte, calculate the response factor of each calibration level as follows:

Equation 1

$$RF_i = (A_a Q_s)/A_s Q_a$$

Where, RF = Response factor

A_a = Sum of integrated areas for analyte

Q_s = Quantity of labeled standard

A_s = Sum of integrated areas for labeled standard

Q_a = Quantity of analyte

10.3.2 The levels of native analytes in the samples are quantified using the following equations:

Equation 2

$$C = (A_n Q_{is})/A_{is} \times W \times RF$$

Where, RF = Response factor

A_n = Sum of integrated areas for target isomer

Q_{is} = Quantity of labeled internal standard added to the sample

A_{is} = Sum of integrated areas for labeled internal standard

W = Sample amount

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C = Concentration of target isomer

Equation 3

$$\text{Average Response Factor} = \overline{RF} = \left(\frac{\sum_{i=1}^{n} RF_i}{n}\right)$$

Where, n = Number of calibration levels

RF_i = Response factor for the ith level

10.3.3 The relative standard deviation (RSD) is calculated as follows:

Equation 4

$$RSD\ (\%) = \frac{SD}{\overline{RF}} \times 100\%$$

Where SD is the standard deviation of the average RF, which is calculated as follows:

$$SD = \sqrt{\frac{\sum_{i=1}^{N}(RF_i - \overline{RF})^2}{N-1}}$$

10.3.4 Linear regression fit: *y* = m*x* + b

Equation 5

$$A_x/A_{is} = m(C_x/C_{is}) + b$$

Where, A_x = Response area for analyte

A_{is} = Response area for the internal standard

C_x = Analyte concentration of calibration standard

C_{is} = Internal standard concentration

m = Slope

b = *y*-intercept

10.3.5 The levels of native analytes in the samples are quantified using the following equation:

Equation 6

$$C_{sx} = (A_x/A_{is} - b) * C_{is}/m$$

Where, C_{sx} = Unknown sample analyte concentration

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A_x = Response area for analyte

A_{is} = Response area for the internal standard

C_{is} = Internal standard concentration

m = Slope

b = y-intercept

10.3.6 For Wisconsin samples, report sample results and all quality control blank results to the MDL and include the RL for each result reported. Quality results reported between the MDL and RL are estimated concentrations and reported with a J-flag.

Example 1

	Laboratory	Report Result as
MDL =	0.6	0.6
MRL =	2.0	2.0
Sample Result =	0.4	< 0.6

Example 2 (Estimated Concentration)

	Laboratory	Report Result as
MDL =	0.6	0.6
MRL =	2.0	2.0
Sample Result =	0.8	0.8 J

11.0 QUALITY CONTROL AND METHOD PERFORMANCE

11.1 Quality Control

The following QC samples are prepared and analyzed with each batch of samples. Refer to Appendix B for acceptance criteria and required corrective action.

QC Item	Frequency
Method Blank (MB)	1 per batch of 20 or fewer samples. If batch exceeds, 20 samples, every 20.
Laboratory Control Sample (LCS)	1 per batch of 20 or fewer samples. If batch exceeds, 20 samples, every 20.
Laboratory Control Sample Duplicate (LCSD)	As needed
Matrix Spike (MS)	1 per batch of 20 or fewer samples. If batch exceeds, 20 samples, every 20.
Matrix Spike Duplicate (MSD)	1 per batch of 20 or fewer samples. If batch exceeds, 20 samples, every 20.
Field Duplicate	1 per batch of 20 or fewer samples. If batch exceeds, 20 samples, every 20.

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QC Item	Frequency
Extraction Internal Standard	All samples and QC
Injection Internal Standard	All samples and QC
Standard Reference Material	1 per batch of 20 or fewer tissue samples. If batch exceeds, 20 samples, every 20.

11.2 Instrument QC

The following Instrument QC checks are performed. Refer to Appendix B for acceptance criteria and required corrective action.

QC Item	Frequency
Tune	Every 6 months or when ICAL won't pass, the peak shape is significantly off, major maintenance is performed, or instrument is moved.
Initial Calibration	At instrument set up, after CCV failure
Initial Calibration Verification	Once per calibration at mid-level of ICAL
Initial Calibration Blank	One following the highest standard analyzed and prior to ICV
Continuing Calibration Verification	At the beginning, end, and bracketing every 10 field samples
Continuing Calibration Blank	1 after each CCV
RT Window	RT of each analyte and EIS analyte must fall within 0.4 minutes (±0.2 minutes) of the predicted retention times from the daily CCV or, on days when ICAL is performed, from the midpoint standard of the ICAL.
Relative Retention Time	Analytes must elute within 0.1 minute of the associated EIS. This criterion applies only to analyte and labeled analog pairs

11.3 Method Performance

11.3.1 Method Validation

11.3.1.1 Detection Limits

Detection limits (DL) and limits of quantitation (LOQ) are established at initial method setup and verified on an on-going basis thereafter. Refer to Pace ENV corporate SOP ENV-SOP-CORQ-0011 *Method Validation and Instrument Verification*.

11.4 Analyst Qualifications and Training

11.4.1 Employees that perform any step of this procedure must have a completed Read and Acknowledgment Statement for this version of the SOP in their training record. In addition, prior to unsupervised (independent) work on any client sample, analysts that prepare or analyze samples must have successful initial demonstration of capability (IDOC) and must successfully demonstrate on-going proficiency on an annual basis. Successful means the initial and on-going DOC met criteria, documentation of the DOC is complete, and the DOC record is in the employee's training file. Refer to laboratory SOP ENV-SOP-MIN4-0165 *Orientation and Training Procedures* (or equivalent replacement) for more information.

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11.4.2 For each analyte, the mean accuracy is true value ±30%. The RSD must be less than 20%. If any target analyte fails to meet this criterion, the source of the problem must be corrected and the test repeated

11.4.2.1 For Wisconsin samples, the mean accuracy is true value ±35%. The RSD must be less than or equal to 30%. If any target analyte fails to meet this criterion, the source of the problem must be corrected and the test repeated

12.0 DATA REVIEW AND CORRECTIVE ACTION

12.1 Data Review

Pace's data review process includes a series of checks performed at different stages of the analytical process by different people to ensure that SOPs were followed, the analytical record is complete and properly documented, proper corrective actions were taken for QC failure and other nonconformance(s), and that test results are reported with proper qualification.

The review steps and checks that occur as employee's complete tasks and review their own work is called primary review.

All data and results are also reviewed by an experienced peer or supervisor. Secondary review is performed to verify SOPs were followed, that calibration, instrument performance, and QC criteria were met and/or proper corrective actions were taken, qualitative ID and quantitative measurement is accurate, all manual integrations are justified and documented in accordance with the Pace ENV's SOP for manual integration, calculations are correct, the analytical record is complete and traceable, and that results are properly qualified.

A third-level review, called a completeness check, is performed by reporting or project management staff to verify the data report is not missing information and project specifications were met.

Refer to laboratory SOP ENV-SOP-MIN4-0092 *Data Review Process* (or equivalent replacement) for specific instructions and requirements for each step of the data review process.

12.2 Corrective Action

Corrective action is expected any time QC or sample results are not within acceptance criteria. If corrective action is not taken or was not successful, the decision/outcome must be documented in the analytical record. The primary analyst has primary responsibility for taking corrective action when QA/QC criteria are not met. Secondary data reviewers must verify that appropriate action was taken and/or that results reported with QC failure are properly qualified.

Corrective action is also required when carryover is suspected and when results are over range.

Samples analyzed after a high concentration sample must be checked for carryover and reanalyzed if carryover is suspected. Carryover is usually indicated by low concentration detects of the analyte in successive samples analyzed after the high concentration sample.

Sample results at concentrations above the upper limit of quantitation must be diluted and reanalyzed. The result in the diluted samples should be within the upper half of the calibration range. Results less than the mid-range of the calibration indicate the sample was over diluted and analysis should be repeated with a lower level of dilution. If dilution is not performed, any result

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reported above the upper range is considered a qualitative measurement and must be qualified as an estimated value.

Refer to Appendix B for a complete summary of QC, acceptance criteria, and recommended corrective actions for QC associated with this test method.

13.0 Pollution Prevention And Waste Management

Pace proactively seeks ways to minimize waste generated during our work processes. Some examples of pollution prevention include but are not limited to: reduced solvent extraction, solvent capture, use of reusable cycletainers for solvent management, and real-time purchasing.

The EPA requires that laboratory waste management practice to be conducted consistent with all applicable federal and state laws and regulations. Excess reagents, samples and method process wastes must be characterized and disposed of in an acceptable manner in accordance with Pace's Chemical Hygiene Plan / Safety Manual.

14.0 Modifications

A modification is a change to a reference test method made by the laboratory. For example, changes in stoichiometry, technology, quantitation ions, reagent or solvent volumes, reducing digestion or extraction times, instrument runtimes, etc. are all examples of modifications. Refer to Pace ENV corporate SOP ENV-SOP-CORQ-0011 *Method Validation and Instrument Verification* for the conditions under which the procedures in test method SOPs may be modified and for the procedure and document requirements.

15.0 Responsibilities

Pace ENV employees that perform any part this procedure in their work activities must have a signed Read and Acknowledgement Statement in their training file for this version of the SOP. The employee is responsible for following the procedures in this SOP and handling temporary departures from this SOP in accordance with Pace's policy for temporary departure.

Pace supervisors/managers are responsible for training employees on the procedures in this SOP and monitoring the implementation of this SOP in their work area.

16.0 Attachments

Appendix A – Target Analyte List and Routine LOQ

Appendix B – QC Summary

Appendix C – Typical MS/MS Method Conditions

Appendix D – PFAS Analyte and Recommended Extracted Internal Standard Used for Quantification

Appendix E –PFAS by LCMSMS Compliant with DoD QSM Batch Control Limits

Appendix F – DoD QSM 5.3, Appendix B, Table B-15 - Per- and Polyfluoroalkyl Substances (PFAS) Using Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS) With Isotope Dilution or Internal Standard Quantification in Matrices Other Than Drinking Water

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17.0 REFERENCES

Department of Defense Department of Energy Consolidated Quality Systems Manual (QSM) for Environmental Laboratories, Version 5.3, Appendix B, Table B-15, June 2019.

Wisconsin Department of Natural Resources, Wisconsin PFAS Aqueous (Non-Potable Water) and Non-Aqueous Matrices Method Expectations, EA-19-0001, December, 2019.

DoD Guidance for PFAS Analysis in Biota. April, 2020.

USEPA, Method 537.1, Version 1.0 "Determination of selected per- and polyfluorinated alkyl substances in drinking water by solid phase extraction and liquid chromatography/tandem mass spectrometry (LC/MS/MS)"; November 2018.

USEPA, Technical Advisory, "Technical Advisory - Laboratory Analysis of Drinking Water Samples for Perfluorooctanoic Acid (PFOA) Using EPA Method 537 Rev. 1.1"; September 2016.

JT Baker, Application Technical Support Group, Endothall extraction using BAKERBOND Speedisk SAX, PN-8058-06, 2006.

Pace Quality Assurance Manual- most current version.

TNI Standard, Management and Technical Requirements for Laboratories Performing Environmental Analyses, EL-V1-2009.

TNI Standard, Management and Technical Requirements for Laboratories Performing Environmental Analyses, EL-VI-2016-Rev.2.1.

USEPA, "Manual for the Certification of Laboratories Analyzing Drinking Water"; Fifth Edition, January 2005.

USEPA, "Supplement 1to the Fifth Edition of the Manual for the Certification of Laboratories Analyzing Drinking Water"; June 2008.

40 CFR Appendix B to Part 136, *Definition and Procedure for the Determination of the Method Detection Limit - Rev 2*, August 28, 2017.

18.0 REVISION HISTORY

This Version:

Section	Description of Change
All	Updated sections with "Wisconsin" reference and removed verbiage following, in regard to "compliance" or "non-compliance samples", etc. Updated short-hand reference throughout SOP to "DoD QSM 5.3"—reference of Table B-15 where applicable.
Header	Updated test method reference.
6.0	Added back some verbiage to section 6.0 that are part of the SOP template. Deleted 6.1.1. Split 6.2 into two sections, 6.3 for receipt and storage.
Table 8.2	Added "Used to Prepare" entry for "Wellington Laboratories MPFAC-6ES" rows. Updated note below table verbiage.
10.0	Fixed/added missing numbering issues. Reworded 10.3.6.

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16.0	Appendix A – added back verbiage from template. Appendix F – updated format and added missing info (First 11 sections/QC Check info from DoD Table B-15 were missing).

This document supersedes the following document(s):

Document Number	Title	Version
ENV-MIN4-SOP-0178	Determination of Selected 36 Per- and Polyfluoroalkyl Substances (PFAS) by LC/MS/MS	00

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Appendix A: Target Analyte List and Routine LOQ[1]

Table 1: Routine Analyte List and Limits of Quantitation (LOQ)[1]

Analyte	Acronym(s)[3]	CAS#	LOQ		
			Water (ng/L)	Solid (ng/kg)	Tissue (ng/kg)
Perfluorobutanoic acid	PFBA	375-22-4	2	100	250
Perfluoropentanoic acid	PFPeA	2706-90-3	2	100	250
Hexafluoropropylene oxide dimer acid	HFPO-DA[2] PFPrOPrA	13252-13-6	2	100	250
Perfluorohexanoic acid	PFHxA	307-24-4	2	100	250
Perfluoroheptanoic acid	PFHpA	375-85-9	2	100	250
Perfluorooctanoic acid	PFOA	335-67-1	2	100	250
Perfluorononanoic acid	PFNA	375-95-1	2	100	250
Perfluorooctanesulfonamide	PFOSAm PFOSA FOSA	754-91-6	2	100	250
N-methylperfluorooctane sulfonamide	MeFOSA[2] N-MeFOSA NMeFOSA	31506-32-8	2	100	250
Perfluorodecanoic acid	PFDA	335-76-2	2	100	250
N-ethylperfluorooctane sulfonamide	EtFOSAm[2] N-EtFOSA NEtFOSA	4151-50-2	2	100	250
Perfluoroundecanoic acid	PFUnDA PFUnA PFUdA	2058-94-8	2	100	250
N-methyl perfluorooctanesulfonamidoacetic acid	NMeFOSAA N-MeFOSAA	2355-31-9	2	100	250
N-ethyl perfluorooctanesulfonamidoacetic acid	NEtFOSAA N-EtFOSAA	2991-50-6	2	100	250
Perfluorododecanoic acid	PFDOA PFDoA PFDoDA	307-55-1	2	100	250
N-methylperfluorooctane sulfonamidoethanol	MeFOSE[2] N-MeFOSE NMeFOSE	24448-09-7	2	100	250
N-ethylperfluorooctane sulfonamidoethanol	EtFOSE[2] N-EtFOSE NEtFOSE	1691-99-2	2	100	250
Perfluorotridecanoic acid	PFTrDA PFTriA PFTrA	72629-94-8	2	100	250

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Analyte	Acronym(s)[3]	CAS#	LOQ		
			Water (ng/L)	Solid (ng/kg)	Tissue (ng/kg)
Perfluorotetradecanoic acid	PFTDA PFTeDA PFTA PFTeA	376-06-7	2	100	250
Perfluorohexadecanoic acid	PFHxDA[2]	67905-19-5	2	100	250
Perfluorooctandecanoic acid	PFODA[2]	16517-11-6	2	100	250
Perfluorobutanesulfonic acid	PFBS	375-73-5	1.77	88.5	221.3
Perfluoropentanesulfonic acid	PFPeS	2706-91-4	1.88	94	235
Perfluorohexanesulfonic acid	PFHxS	355-46-4	1.82	91	227.5
Perfluoroheptanesulfonic acid	PFHpS	375-92-8	1.90	95	237.5
Perfluorooctanesulfonic acid	PFOS	1763-23-1	1.85	92.5	231.3
Perfluorononanesulfonic acid	PFNS	68259-12-1	1.92	96	240
Perfluorodecanesulfonic acid	PFDS	335-77-3	1.93	96.5	241.3
Perfluorododecanesulfonic acid	PFDoS[2] PFDoDS	79780-39-5	1.94	97	242.5
4:2 Fluorotelomer sulfonic acid	4:2 FTS 4:2 FTSA 4:2FTS	757124-72-4	1.87	93.5	233.8
6:2 Fluorotelomer sulfonic acid	6:2 FTS 6:2 FTSA 6:2FTS	27619-97-2	1.90	95	237.5
8:2 Fluorotelomer sulfonic acid	8:2 FTS 8:2 FTSA 8:2FTS	39108-34-4	1.93	96.5	241.3
10:2 Fluorotelomer sulfonic acid	10:2 FTS[2] 10:2 FTSA	120226-60-0	1.93	96.5	241.3
4,8-Dioxa-3H-perfluorononanoic acid	DONA[2] ADONA	919005-14-4	1.89	94.5	236.3
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	9Cl-PF3ONS[2] F-53B Major	756426-58-1	1.86	93	232.5
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	11Cl-PF3OUdS[2] F-53B Minor	763051-92-9	1.88	94	235

[1] Values in place as of effective date of this SOP. LOQs are subject to change. For the most up to date LOQ, refer to the LIMS or contact the laboratory.

[2] DoD currently does not have guidance for the analyte in Table B-15 per DoD QSM 5.3, Appendix B, as of June 2019.

[3] All possible acronym variations are listed as the acronym used and/or referenced may vary depending on the State data is being reported to.

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Appendix B: QC Summary

QC Item	Frequency	Acceptance Criteria		Corrective Action	Qualification
		Wisconsin Guidance	Table B-15 per DoD QSM 5.3		
ICAL	At instrument set up, after CCV failure	Lowest ICAL ± 50% Other points ± 30% For any curve fit other than Average RF (RSD), curve must also pass RSE test at the low and midpoint calibration standard.	All points ±30% of true value. For any curve fit other than Average RF (RSD), curve must also pass RSE test at the low and midpoint calibration standard.	Identify and correct source of problem, repeat	None. Do not proceed with analysis
Curve Refitting	Whenever ICAL is performed	Must meet one of curve fit options presented in Section 10.0.	Must meet one of curve fit options presented in Section 10.0.	Identify and correct source of problem, repeat	None. Do not proceed with analysis
ICV	1 after each ICAL	True value ± 30%	True value ± 30%	Identify source of problem, re-analyze. If repeat failure, repeat ICAL. Analysis may proceed if it can be demonstrated that the ICV exceedance has no impact on analytical measurements. For example, the ICV %R is high, CCV is within criteria, and the analyte is not detected in sample(s).	Qualify analytes with ICV out of criteria.
RT Window Position (Daily)	Once per ICAL and at the beginning of the analytical window.	Position is set using the mid-point of the ICAL on the day ICAL is performed; otherwise mid-point of CCV is used	Position is set using the mid-point of the ICAL on the day ICAL is performed; otherwise mid-point of CCV is used	NA	NA
RT Window Study	At method set-up and after major instrument maintenance	Window is ±0.2 minutes the daily CCV or, on days when ICAL is performed, from	Window is ±0.2 minutes the daily CCV or, on days when ICAL is performed,	Correct problem and reanalyze samples	NA

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QC Item	Frequency	Acceptance Criteria		Corrective Action	Qualification
		Wisconsin Guidance	Table B-15 per DoD QSM 5.3		
		the midpoint standard of the ICAL. Analytes must elute within 0.1 minute of the associated EIS	from the midpoint standard of the ICAL. Analytes must elute within 0.1 minute of the associated EIS		
Continuing Calibration Curve (CCV)	Daily, before sample analysis, after every 10 field samples, and at end of analytical window.	CCV at low level: True value ±50% Other CCV: True value ±30%	True value ±30%	Perform necessary maintenance and demonstrate stability by analyzing an initial calibration before resuming sample analysis. Samples between passing CCV and failing CCV should be re-analyzed.	Qualify analytes with CCV out of criteria.
Extracted Internal Standards (EIS)	Every field sample, standard and QC sample	Must meet criteria specified in Section 9.2.8	Must meet criteria specified in Section 9.2.8	If the CCV fails high for a particular analyte, and the field sample is non-detect for that analyte, samples may be reported without re-analysis.	Qualify outages and explain in case narrative.
Injection Internal Standards (IIS)	Every field sample, standard and QC sample	Must meet criteria specified in Section 9.2.9	Must meet criteria specified in Section 9.2.9	Troubleshoot instrument performance. Reanalyze samples.	Qualify outages and explain in case narrative.
Method Blank (MB)	1 per batch	Analytes $\leq$1/2 the RL or 1/10th the amount measured in any sample	Analytes $\leq$1/2 the RL or 1/10th the amount measured in any sample or 1/10th the regulatory limit, whichever is greater	1) If sample ND, report sample without qualification. 2) If sample result >10x MB detects and sample cannot be reanalyzed, report sample with appropriate qualifier indicating blank contamination. 3) If sample result <10x MB detects, report sample with appropriate qualifier to indicate an estimated value. Client must be alerted to give authorization to report this data. 4) Analyte detection or failure of internal standard fails entire batch.	Qualify outages and explain in case narrative

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QC Item	Frequency	Acceptance Criteria		Corrective Action	Qualification
		Wisconsin Guidance	Table B-15 per DoD QSM 5.3		
LCS/LCSD	1 spiked at a concentration ≥ LOQ and ≤ the mid-level calibration concentration	True Value ≤ 1-2x RL 50-150% True Value > 2x RL 60-135% RPD ≤ 30%	See Appendix E RPD ≤ 30%	Reanalyze and/or re-prepare batch of samples with new LCS. If LCS rec > QC limits and these compounds are non-detect in the associated samples, the sample data may be reported with appropriate data qualifiers. If these criteria are not met, where extra samples are available, a re-extract is analyzed or else data is narrated.	Qualify outages and explain in case narrative
MS/MSD	1 pair/batch spiked at a concentration ≥ LOQ and ≤ the mid-level calibration concentration	True Value ≤ 1-2x RL 50-150% True Value > 2x RL 60-135% RPD ≤ 30%	See Appendix E RPD ≤ 30%	Failures are flagged but do not prevent reporting data if MB and LCS meet criteria.	Qualify outages and explain in case narrative
Field Duplicate (FD)	1 per batch. A MSD may be substituted for a sample duplicate if sample is insufficient	NA	NA	If these criteria are not met, results are labeled suspect due to matrix effects	Qualify outages and explain in case narrative
Tune Standard	Every six month, when ICAL won't pass, when the peak shape is significantly off (indicating an MS problem), when major maintenance is performed, or instrument is moved	See section 9.2.1 for reference	See section 9.2.1 for reference	Refer to manufacture criteria	NA
Instrument Blank (ICB)	1 following the highest standard analyzed	< ½ RL	≤ ½ RL	If acceptance criteria are not met after the highest calibration standard, calibration must be	Flagging is only appropriate in cases when

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QC Item	Frequency	Acceptance Criteria		Corrective Action	Qualification
		Wisconsin Guidance	Table B-15 per DoD QSM 5.3		
				performed using a lower concentration for the highest standard until acceptance criteria is met.	the sample cannot be reanalyzed and when there is no more sample left.
CCB	1 following the CCV and prior to sample analysis	< ½ RL	NA	If acceptance criteria are not met after the CCV. Clean the system and prepare new CCV if needed.	Flagging is only appropriate in cases when the sample cannot be reanalyzed and when there is no more sample left.

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Appendix C: Typical MS/MS Method Conditions

Analyte	Precursor Ion (m/z)	Product Ion (m/z)	RT (min)	Declustering Potential (v)	Collision Energy (v)	Collision Cell Exit Potential (v)
PFBA	212.9	169	2.43	-45	-12	-11
PFPeA	262.9	219	3.33	-25	-12	-11
HFPO-DA	285	169	4.3	-70	-12	-11
HFPO-DA_2	285	185	4.29	-70	-24	-13
PFBS	298.9	80	4.27	-65	-58	-9
PFBS_2	298.9	99	4.27	-65	-40	-9
PFHxA	313	269	4.03	-25	-12	-19
PFHxA_2	313	119	4.02	-25	-28	-19
4:2FTS	327	307	3.75	-65	-28	-13
4:2FTS_2	327	81	3.75	-65	-56	-11
PFPeS	349	80	4.94	-45	-68	-9
PFPeS_2	349	99	4.94	-65	-40	-9
PFHpA	363	319	4.63	-50	-14	-15
PFHpA_2	363	169	4.63	-50	-24	-11
DONA	377	251	4.84	-50	-16	-11
DONA_2	377	85	4.84	-50	-36	-11
PFHxS	399	80	5.52	-55	-84	-9
PFHxS_2	399	99	5.52	-55	-68	-11
PFOA	413	369	5.17	-55	-14	-17
PFOA_2	413	169	5.17	-55	-24	-9
6:2FTS	427	407	4.89	-65	-32	-17
6:2FTS_2	427	81	4.89	-65	-68	-7
PFHpS	449	80	6.07	-105	-92	-9
PFHpS_2	449	99	6.07	-80	-80	-13
PFNA	463	419	5.7	-70	-16	-15
PFNA_2	463	169	5.7	-70	-26	-11
PFOSA	498	78	7.47	-130	-90	-11
PFOS	499	80	6.58	-65	-112	-9
PFOS_2	499	99	6.58	-65	-90	-11
N-MeFOSA	512	169	8.76	-55	-36	-11
N-MeFOSA_2	512	218.9	8.75	-60	-34	-19
PFDA	513	469	6.21	-80	-16	-19
PFDA_2	513	169	6.21	-80	-28	-13
N-EtFOSA	526	169	9.23	-40	-36	-13
N-EtFOSA_2	526	219.15	9.23	-15	-34	-9

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Analyte	Precursor Ion (m/z)	Product Ion (m/z)	RT (min)	Declustering Potential (v)	Collision Energy (v)	Collision Cell Exit Potential (v)
8:2FTS	527	507	5.92	-70	-38	-21
8:2FTS_2	527	81	5.92	-70	-92	-9
9Cl-PF3ONS	530.9	351	6.97	-75	-36	-15
9Cl-PF3ONS_2	530.9	83	6.97	-75	-70	-11
PFNS	549	80	7.08	-65	-118	-13
PFNS_2	549	99	7.08	-85	-96	-11
PFUdA	563	519	6.72	-30	-18	-21
PFUdA_2	563	169	6.71	-30	-32	-11
N-MeFOSAA	570	419	6.11	-125	-28	-33
N-MeFOSAA_2	570	483	6.11	-125	-22	-33
N-EtFOSAA	584	419	6.34	-125	-28	-33
N-EtFOSAA_2	584	526	6.33	-125	-28	-33
PFDS	599	80	7.57	-85	-122	-11
PFDS_2	599	99	7.57	-85	-100	-11
PFDoA	613	569	7.21	-25	-18	-23
PFDoA_2	613	169	7.21	-25	-34	-11
N-MeFOSE	616	59	8.58	-20	-76	-5
10:2FTS	627	607	6.92	-50	-44	-25
10:2FTS_2	627	81	6.91	-50	-108	-9
N-EtFOSE	630	59	9.03	-20	-58	-27
11Cl-PF3OUdS	630.9	451	7.94	-90	-40	-19
11Cl-PF3OUdS_2	630.9	99	7.94	-90	-92	-5
PFTrDA	663	619	7.69	-75	-20	-25
PFTrDA_2	663	169	7.69	-75	-34	-9
PFDoS	699	80	8.46	-30	-134	-9
PFDoS_2	699	99	8.46	-20	-132	-11
PFTeDA	713	669	8.17	-85	-20	-27
PFTeDA_2	713	169	8.17	-85	-36	-13
PFHxDA	813	769	9.23	-30	-22	-33
PFHxDA_2	813	169	9.23	-30	-38	-9
PFODA	913	869	9.72	-5	-22	-35
PFODA_2	913	169	9.72	-5	-42	-11

Note(s):
Analyte_2 Ions used for confirmation purposes.

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Appendix D: PFAS Analyte and Recommended Extracted Internal Standard Used for Quantification

Analyte	EIS Name
PFBA	13C4_PFBA
PFPeA	13C5_PFPeA
HFPO-DA	13C3_HFPO-DA
PFBS	13C3_PFBS
PFHxA	13C5_PFHxA
4:2FTS	13C2_4:2FTS
PFPeS	13C3_PFHxS
PFHpA	13C4_PFHpA
DONA	13C8_PFOA
PFHxS	13C3_PFHxS
PFOA	13C8_PFOA
6:2FTS	13C2_6:2FTS
PFHpS	13C3_PFOS
PFNA	13C9_PFNA
PFOSA	13C8_PFOSA
PFOS	13C8_PFOS
N-MeFOSA	d3-N-MeFOSA
PFDA	13C6_PFDA
N-EtFOSA	d5-N-EtFOSA
8:2FTS	13C2_8:2FTS
9Cl-PF3ONS	13C8_PFOS
PFNS	13C8_PFOS
PFUdA	13C7_PFUdA
N-MeFOSAA	d3-MeFOSAA
N-EtFOSAA	d5-EtFOSAA
PFDS	13C8_PFOS
PFDoA	13C2_PFDoA
N-MeFOSE	d7-N-MeFOSE
10:2FTS	13C2_8:2FTS
N-EtFOSE	d9-N-EtFOSE
11Cl-PF3OUdS	13C8_PFOS
PFTrDA	13C2_PFDoA
PFDoS	13C8_PFOS
PFTeDA	13C2_PFTeDA
PFHxDA	13C2_PFHxDA
PFODA	13C2_PFHxDA

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Appendix E: PFAS by LCMSMS Compliant with DoD QSM Batch Control Limits

CAS#	Analyte Acronym	Aqueous Matrix		Solid Matrix		Tissue Matrix[3]	
		Lower Control Limit (%REC)	Upper Control Limit (%REC)	Lower Control Limit (%REC)	Upper Control Limit (%REC)	Lower Control Limit (%REC)	Upper Control Limit (%REC)
2991-50-6	N-EtFOSAA	61	135	61	139	60	140
2355-31-9	N-MeFOSAA	65	136	63	144	60	140
757124-72-4	4:2 FTS	63	143	62	145	60	140
27619-97-2	6:2 FTS	64	140	64	140	60	140
39108-34-4	8:2 FTS	67	138	65	137	60	140
375-73-5	PFBS	72	130	72	128	60	140
375-22-4	PFBA	73	129	71	135	60	140
335-77-3	PFDS	53	142	59	134	60	140
335-76-2	PFDA	71	129	69	133	60	140
307-55-1	PFDoA	72	134	69	135	60	140
375-92-8	PFHpS	69	134	70	132	60	140
375-85-9	PFHpA	72	130	71	131	60	140
355-46-4	PFHxS	68	131	67	130	60	140
307-24-4	PFHxA	72	129	70	132	60	140
68259-12-1	PFNS	69	127	69	125	60	140
375-95-1	PFNA	69	130	72	129	60	140
754-91-6	PFOSA	67	137	67	137	60	140
1763-23-1	PFOS	65	140	68	136	60	140
335-67-1	PFOA	71	133	69	133	60	140
2706-91-4	PFPeS	71	127	73	123	60	140
2706-90-3	PFPeA	72	129	69	132	60	140
376-06-7	PFTeDA	71	132	69	133	60	140
72629-94-8	PFTrDA	65	144	66	139	60	140
2058-94-8	PFUdA	69	133	64	136	60	140
31506-32-8	N-MeFOSA[1]	68	141	70	140	60	140
4151-50-2	N-EtFOSA[2]	70	140	70	140	60	140
120226-60-0	10:2FTS[2]	70	140	70	140	60	140
13252-13-6	HFPO-DA[2]	70	140	70	140	NA	NA
919005-14-4	DONA[1]	70	140	70	140	60	140
756426-58-1	9Cl-PF3ONS[2]	70	140	70	140	60	140
763051-92-9	11Cl-PF3OUDS[2]	70	140	70	140	60	140

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CAS#	Analyte Acronym	Aqueous Matrix		Solid Matrix		Tissue Matrix[3]	
		Lower Control Limit (%REC)	Upper Control Limit (%REC)	Lower Control Limit (%REC)	Upper Control Limit (%REC)	Lower Control Limit (%REC)	Upper Control Limit (%REC)
24448-09-7	N-MeFOSE[2]	70	140	70	140	60	140
1691-99-2	N-EtFOSE[2]	70	140	70	140	60	140
67905-19-5	PFHxDA[2]	70	140	70	140	60	140
16517-11-6	PFODA[2]	70	140	70	140	60	140
79780-39-5	PFDoS[2]	70	140	70	140	60	140

[1] DoD currently does not have guidance for the analyte in solid matrix in Table B-15 per DoD QSM 5.3, Appendix B, as of June 2019.

[2] DoD currently does not have guidance for the analyte in both aqueous and solid matrix in Table B-15 per DoD QSM 5.3, Appendix B, as of June 2019.

[3] DoD currently does not have guidance for the analyte in tissue matrix in Table B-15 per DoD QSM 5.3, Appendix B, as of June 2019.

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Appendix F: DoD QSM 5.3, Appendix B, Table B-15 - Per- and Polyfluoroalkyl Substances (PFAS) Using Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS) With Isotope Dilution or Internal Standard Quantification in Matrices Other Than Drinking Water

QC Check	Minimum Frequency	Acceptance Criteria	Corrective Action	Flagging Criteria	Comments
Aqueous Sample Preparation	Each sample and associated batch QC samples.	Solid Phase Extraction (SPE) must be used unless samples are known to contain high PFAS concentrations (e.g., Aqueous Film Forming Foam (AFFF) formulations). Inline SPE is acceptable. Entire sample plus bottle rinsate must be extracted using SPE. Known high PFAS concentration samples require serial dilution be performed in duplicate. Documented project approval is needed for samples prepared by serial dilution as opposed to SPE.	NA.	NA.	Samples with >1% solids may require centrifugation prior to SPE extraction. Pre-screening of separate aliquots of aqueous samples is recommended.
Solid Sample Preparation	Each sample and associated batch QC samples.	Entire sample received by the laboratory must be homogenized prior to subsampling.	NA.	NA.	NA.
Biota Sample Preparation	Each sample and associated batch QC samples.	Sample prepared as defined by the project (e.g., whole fish versus filleted fish).	L	NA.	NA.
AFFF and AFFF Mixture Samples Preparation	Each sample and associated batch QC samples.	Each field sample must be prepared in duplicate (equivalent to matrix duplicate). Serial dilutions must be performed to achieve the lowest LOQ possible for each analyte.	NA.	NA.	Adsorption onto bottle is negligible compared to sample concentration so subsampling is allowed. Multiple dilutions will most likely have to be reported in order to achieve the lowest

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QC Check	Minimum Frequency	Acceptance Criteria	Corrective Action	Flagging Criteria	Comments
					LOQ possible for each analyte.
Sample Cleanup Procedure	Each sample and associated batch QC samples. Not applicable to AFFF and AFFF Mixture Samples.	ENVI-Carb^{TM} or equivalent must be used on each sample and batch QC sample.	NA.	Flagging is not appropriate.	Cleanup should reduce bias from matrix interferences.
Mass Calibration	Instrument must have a valid mass calibration prior to any sample analysis. Mass calibration is verified after each mass calibration, prior to initial calibration (ICAL).	Calibrate the mass scale of the MS with calibration compounds and procedures described by the manufacturer. Mass calibration range must bracket the ion masses of interest. The most recent mass calibration must be used for every acquisition in an analytical run. Mass calibration must be verified to be ±0.5 amu of the true value, by acquiring a full scan continuum mass spectrum of a PFAS stock standard.	If the mass calibration fails, then recalibrate. If it fails again, consult manufacturer instructions on corrective maintenance.	Flagging is not appropriate.	Problem must be corrected. No samples may be analyzed under a failing mass calibration. The mass calibration is updated on an as-needed basis (e.g., QC failures, ion masses fall outside of the ±0.5 amu of the true value, major instrument maintenance is performed, or the instrument is moved).
Mass Spectral Acquisition Rate	Each analyte, Extracted Internal Standard (EIS) Analyte.	A minimum of 10 spectra scans are acquired across each chromatographic peak.	NA.	Flagging is not appropriate.	NA.
Calibration, Calibration Verification, and Spiking Standards	All analytes.	Standards containing both branched and linear isomers must be used when commercially available. PFAS method analytes may consist of both branched and linear isomers, but quantitative standards that contain the linear and branched isomers do not exist for all method analytes.	NA.	Flagging is not appropriate.	Standards containing both branched and linear isomers are to be used during method validation and when reestablishing retention times, to ensure the total response is quantitated for that analyte. Technical grade

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QC Check	Minimum Frequency	Acceptance Criteria	Corrective Action	Flagging Criteria	Comments
		For PFAS that do not have a quantitative branched and linear standard, identify the branched isomers by analyzing a qualitative standard that includes both linear and branched isomers and determine retention times, transitions and transition ion ratios. Quantitate samples by integrating the total response (i.e., accounting for peaks that are identified as linear and branched isomers) and relying on the initial calibration that uses the linear isomer quantitative standard.			standards cannot be used for quantitative analysis.
Sample PFAS Identification	All analytes detected in a sample.	The chemical derivation of the ion transitions must be documented. A minimum of two ion transitions (Precursor → quant ion and precursor → confirmation ion) and the ion transitions ratio per analyte are required for confirmation. Exception is made for analytes where two transitions do not exist (PFBA and PFPeA). Documentation of the primary and confirmation transitions and the ion ratio is required. In-house acceptance criteria for evaluation of ion ratios must be used and must not exceed 50-150%. Signal to Noise Ratio (S/N) must be ≥ 10 for all ions used for quantification and must	NA.	PFAS identified, with Ion ratios that fail acceptance criteria, must be flagged. Any quantitation ion peak that does not meet the maximization criteria shall be included in the summed integration and the resulting data flagged as “estimated, biased high”.	For example: Ion Ratio = (quant ion abundance/confirm ion abundance) Calculate the average ratio (A) and standard deviation (SD) using the ICAL standards. An acceptance range of ratio could be within A ±3SD for confirmation of detection.

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QC Check	Minimum Frequency	Acceptance Criteria	Corrective Action	Flagging Criteria	Comments
		be ≥ 3 for all ions used for confirmation. Quant ion and confirmation ion must be present and must maximize simultaneously (±2 seconds).			
Ion Transitions (Precursor-> Product)	Every field sample, standard, blank, and QC sample.	In order to avoid biasing results high due to known interferences for some transitions, the following transitions must be used for the quantification of the following analytes: PFOA: 413 → 369 PFOS: 499 → 80 PFHxS: 399 → 80 PFBS: 299 → 80 4:2 FTS: 327 → 307 6:2 FTS: 427 → 407 8:2 FTS: 527 → 507 NEtFOSAA: 584 → 419 NMeFOSAA: 570 → 419 If these transitions are not used, the reason must be technically justified and documented (e.g., alternate transition was used due to observed interferences).	NA.	Flagging is not appropriate	NA.
Initial Calibration (ICAL)	At instrument set-up and after ICV or CCV failure, prior to sample analysis.	The isotopically labeled analog of an analyte (Extracted Internal Standard Analyte) must be used for quantitation if commercially available (Isotope Dilution Quantitation). Commercial PFAS standards available as salts, are acceptable, providing the measured mass is corrected to the neutral acid concentration. Results shall be reported as the neutral acid with	Correct problem, then repeat ICAL.	Flagging is not appropriate.	No samples shall be analyzed until ICAL has passed. External Calibration is not allowed for any analyte. Calibration can be linear (minimum of 5 standards) or quadratic (minimum of 6 standards); weighting is allowed.

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QC Check	Minimum Frequency	Acceptance Criteria	Corrective Action	Flagging Criteria	Comments
		appropriate CAS number. If a labeled analog is not commercially available, the Extracted Internal Standard Analyte with the closest retention time or chemical similarity to the analyte must be used for quantitation. (Internal Standard Quantitation) Analytes must be within 70-130% of their true value for each calibration standard. ICAL must meet one of the two options below: Option 1: The RSD of the RFs for all analytes must be ≤20%. Option 2: Linear or non-linear calibrations must have r^2 ≥ 0.99 for each analyte.			
Retention Time window position establishment	Once per ICAL and at the beginning of the analytical sequence.	Position shall be set using the midpoint standard of the ICAL curve when ICAL is performed. On days when ICAL is not performed, the initial CCV is used.	NA.	NA.	Calculated for each analyte and EIS.
Retention Time (RT) window width	Every field sample, standard, blank, and QC sample.	RT of each analyte and EIS analyte must fall within 0.4 minutes of the predicted retention times from the daily calibration verification or on days when ICAL is performed, from the midpoint standard of the ICAL. Analytes must elute within 0.1 minutes of the associated EIS. This criterion applies only to analyte and labeled analog pairs.	Correct problem and reanalyze samples.	NA.	Calculated for each analyte and EIS.

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QC Check	Minimum Frequency	Acceptance Criteria	Corrective Action	Flagging Criteria	Comments
Instrument Sensitivity Check (ISC)	Prior to analysis and at least once every 12 hours.	Analyte concentrations must be at LOQ; concentrations must be within ±30% of their true values.	Correct problem, rerun ISC. If problem persists, repeat ICAL.	Flagging is not appropriate.	No samples shall be analyzed until ISC has met acceptance criteria. ISC can serve as the initial daily CCV.
Initial Calibration Verification (ICV)	Once after each ICAL, analysis of a second source standard prior to sample analysis.	Analyte concentrations must be within ±30% of their true value.	Correct problem, rerun ICV. If problem persists, repeat ICAL.	Flagging is not appropriate.	No samples shall be analyzed until calibration has been verified.
Continuing Calibration Verification (CCV)	Prior to sample analysis, after every 10 field samples, and at the end of the analytical sequence.	Concentration of analytes must range from the LOQ to the mid-level calibration concentration. Analyte concentrations must be within ±30% of their true value.	Immediately analyze two additional consecutive CCVs. If both pass, samples may be reported without reanalysis. If either fails, or if two consecutive CCVs cannot be run, perform corrective action(s) and repeat CCV and all associated samples since last successful CCV. Alternately, recalibrate if necessary; then reanalyze all associated samples since the last acceptable CCV.	If reanalysis cannot be performed, data must be qualified and explained in the Case Narrative. Apply Q-flag to all results for the specific analyte(s) in all samples since the last acceptable calibration verification.	Results may not be reported without valid CCVs. Instrument Sensitivity Check (ISC) can serve as a bracketing CCV.
Instrument Blanks	Immediately following the highest standard analyzed and daily prior to sample analysis.	Concentration of each analyte must be ≤ ½ the LOQ. Instrument Blank must contain EIS to enable quantitation of contamination.	If acceptance criteria are not met after the highest calibration standard, calibration must be performed using a lower concentration for the highest standard until acceptance criteria is met. If sample concentrations exceed the highest	Flagging is only appropriate in cases when the sample cannot be reanalyzed and when there is no more sample left.	No samples shall be analyzed until instrument blank has met acceptance criteria. Note: Successful analysis following the highest standard analyzed determines the highest concentration that carryover does not occur.

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QC Check	Minimum Frequency	Acceptance Criteria	Corrective Action	Flagging Criteria	Comments
			allowed standard and the sample(s) following exceed this acceptance criteria (>1/2 LOQ), they must be reanalyzed.		When the highest standard analyzed is not part of the calibration curve, it cannot be used to extend out the calibration range, it is used only to document a higher concentration at which carry over still does not occur.
Extracted Internal Standard (EIS) Analytes	Every field sample, standard, blank, and QC sample.	Added to solid sample prior to extraction. Added to aqueous samples, into the original container, prior to extraction. For aqueous samples prepared by serial dilution instead of SPE, added to final dilution of samples prior to analysis. Extracted Internal Standard Analyte recoveries must be within 50% to 150% of ICAL midpoint standard area or area measured in the initial CCV on days when an ICAL is not performed.	Correct problem. If required, re-extract and reanalyze associated field and QC samples. If recoveries are acceptable for QC samples, but not field samples, the field samples must be re-extracted and analyzed (greater dilution may be needed). Samples may be re-extracted and analyzed outside of hold times, as necessary for corrective action associated with QC failure.	Apply Q-flag and discuss in the Case Narrative only if reanalysis confirms failures in exactly the same manner.	Failing analytes shall be thoroughly documented in the Case Narrative. EIS should be 96% (or greater) purity. When the impurity consists of the unlabeled analyte, the EIS can result in a background artifact in every sample, standard and blank, if the EIS is fortified at excessive concentrations.
Method Blank (MB)	One per preparatory batch.	No analytes detected > ½ LOQ or > 1/10th the amount measured in any sample or 1/10th the regulatory limit, whichever is greater.	Correct problem. If required, re-extract and reanalyze MB and all QC samples and field samples processed with the contaminated blank. Samples may be re-extracted and analyzed outside of hold times, as necessary for corrective action associated with QC failure.	If reanalysis cannot be performed, data must be qualified and explained in the Case Narrative. Apply B-flag to all results for the specific analyte(s) in all samples in the associated preparatory batch.	Results may not be reported without a valid MB. Flagging is only appropriate in cases where the samples cannot be reanalyzed.

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QC Check	Minimum Frequency	Acceptance Criteria	Corrective Action	Flagging Criteria	Comments
			Examine the project-specific requirements. Contact the client as to additional measures to be taken.		
Laboratory Control Sample (LCS)	One per preparatory batch.	Blank spiked with all analytes at a concentration ≥ LOQ and ≤ the mid-level calibration concentration. A laboratory must use the DoD/DOE QSM Appendix C Limits for batch control if project limits are not specified. If the analyte(s) are not listed, use in-house LCS limits if project limits are not specified.	Correct problem, then re- extract and reanalyze the LCS and all samples in the associated preparatory batch for failed analytes if sufficient sample material is available. Samples may be re-extracted and analyzed outside of hold times, as necessary for corrective action associated with QC failure. Examine the project-specific requirements. Contact the client as to additional measures to be taken.	If reanalysis cannot be performed, data must be qualified and explained in the Case Narrative. Apply Q-flag to specific analyte(s) in all samples in the associated preparatory batch.	Results may not be reported without a valid LCS. Flagging is only appropriate in cases where the samples cannot be reanalyzed.
Matrix Spike (MS)	One per preparatory batch. Not required for aqueous samples prepared by serial dilution instead of SPE.	Sample spiked with all analytes at a concentration ≥ LOQ and ≤ the mid-level calibration concentration. A laboratory must use the DoD/DOE QSM Appendix C Limits for batch control if project limits are not specified. If the analyte(s) are not listed, use in-house LCS limits if project limits are not specified.	Examine the project-specific requirements. Contact the client as to additional measures to be taken.	For the specific analyte(s) in the parent sample, apply J-flag if acceptance criteria are not met and explain in the Case Narrative.	For matrix evaluation only. If MS results are outside the limits, the data shall be evaluated to determine the source(s) of difference (i.e., matrix effect or analytical error).
Matrix Spike Duplicate (MSD) or Matrix Duplicate	For MSD: One per preparatory batch.	For MSD: Sample spiked with all analytes at a concentration ≥ LOQ and	Examine the project-specific requirements.	For the specific analyte(s)	The data shall be evaluated to determine

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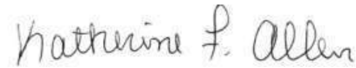
QC Check	Minimum Frequency	Acceptance Criteria	Corrective Action	Flagging Criteria	Comments
(MD)	For MD: Each aqueous sample prepared by serial dilution instead of SPE.	≤ the mid-level calibration concentration. A laboratory must use the DoD/DOE QSM Appendix C Limits for batch control if project limits are not specified. If the analyte(s) are not listed, use in-house LCS limits if project limits are not specified. RPD ≤ 30% (between MS and MSD or sample and MD).	Contact the client as to additional measures to be taken. If the analyte(s) are not listed, use in-house LCS limits if project limits are not specified.	in the parent sample, apply J-flag if acceptance criteria are not met and explain in the Case Narrative.	the source of difference. For Sample/MD: RPD criteria only apply to analytes whose concentration in the sample is ≥LOQ. The MD is a second aliquot of the field sample that has been prepared by serial dilution.
Post Spike Sample	Only applies to aqueous samples prepared by serial dilution instead of SPE that have reported value of <LOQ for analyte(s).	Spike all analytes reported as <LOQ into the dilution that the result for that analyte is reported from. The spike must be at the LOQ concentration to be reported for this sample as <LOQ. When analyte concentrations are calculated as <LOQ, the post spike for that analyte must recover within 70-130% of its true value.	When analyte concentrations are calculated as <LOQ, and the spike recovery does not meet the acceptance criteria, the sample, sample duplicate, and post spike sample must be reanalyzed at consecutively higher dilutions until the criteria is met.	Flagging is not appropriate.	When analyte concentrations are calculated as <LOQ, results may not be reported without acceptable post spike recoveries.

Determination of Selected Per- and Polyfluorinated Alkyl Substances (PFAS) by Solid Phase Extraction & Isotope Dilution by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)

Approved:

Tod Kopyscinski
Laboratory Director



Katherine Allen
QA Officer

Revision Number: 7

DRAFT

Change Record

Revision	Date	Responsible Person	Description of Change
0	06/01/2017	Brianna McLaughlin	Original
1	10/10/2017	Brianna McLaughlin	Changes to Recovery Allowances for specific Compounds; Changes to LC method conditions
2	11/13/2018	Katherine Allen/KAF	Updates from Sept 2018 NH audit: "Effective" date added to header, Sec 3.4 (should replaced with must), Sec 7.3.4 (expansion of Isotope Dilution Technique-note added), Sec 7.3.5 (surrogates should change to surrogates must), Sec 7.5.2 (Blank Spike Duplicate deleted), Sec 7.5.3 (addition of DI water rinse after methanol), Sec 7.6.1.1 (blank extraction replaced with blank subtraction), Sec 7.7.3 (delete to prove system is contamination free), deletion of section 8.1.11 (IDOC), Sec 12.0 (addition of calculations), and Sec 5.7(updated volumes), Sec 5.8 (changed water to methanol), Appendix B(added pH adjustment method). Addition of section 7.3.8 for explanation of isotope dilution technique.
3	03/17/2020	Brianna Mclaughlin/James Constantino	Update to make entire new procedure: Entire SOP edited.
4	07/01/2020	Brianna Henriquez/James Constantino	Update to make entire new procedure: Entire SOP edited and CAS number added.
5	08/17/2020	Brianna Henriquez	Updates from NH 2020 Audit: Section 7.3.5 should to shall, 7.3.7 & 7.3.8 updates to current procedure and clarification. Section 7.5.11 grammatical error fixed. Section 12.5 removed. Section 8.2.4 – MS/MSD spiked at mid-level added, Edit to Appendix C to including 40CFR reference, Addition to section 7.3.4 to include verbiage of using branched and linear isomers, and Sec 12.0 (removal of calc not needed).
6	11/18/2020	Katherine Allen	Update to holding time in Section 3.5 – update from 14 days to 28 days to be the same as EPA 533.
7	06/30/21	Brianna Henriquez	Updates to Procedure: Overall: References to Con-Test changed to Pace. Section 1.1: added specific instruments (6470 and 6495). Section 4: Updated column PNs. Removed envi-carb cartridge that isn't used in this method. Section 5: 20MM mobile phase changed to 5MM, mobile phase expiration changed. Section 7: Sampling instructions changed. Removed section on separate internal standard. Section 7.1: Sampling instructions changed. Section 7.2.6: Prepared and in-use standards stored at room temperature. Added 24PAR and certain individual analyte stocks for separate spike sourcing. Standard prep and calibration tables were changed. CCVs changed to only be run at the mid-level (level 4) after an opening low-level. Section 7.3: Updated calibration method, technique, and requirements (removal of IS/surrogate now functioning as both surrogate and IS). Added section 7.3.5.1. Section. Section 7.4.1: Requirement added of IBL and low-level CCV every 12 hours. Section 7.5.1: pH range changed from ± 0.5 pH units to ± 1.0 pH units. Section 7.5.4: Surrogate and spike step combined, and are now always the same amount. Sec 7.5: changed Dup and MS to MS and MSD and specified balance, Section 7.5.5: Added volume by weight step, and special instructions for sediment-heavy samples (Appendix F). Section 7.5.11: Added cartridge soaking step before solvent collection. Section 7.5.14: Rest of new volume by weight step. Section 7.6.2: N-Me-FOSAA was written incorrectly and changed. Section 7.6.2.1: TPFOA branched check section added. Section 7.7.2: Instrument method conditions changed. Cell accelerator voltage moved to this section. Section 8.2.4: MS/MSD spike levels held constant. 8.2.7: Updated how isotope dilution technique is calculated. Section 8.2.9: Section reworded and changed to reflect proper CCV criteria. Appendices: Removed Appendices A and C, changing other appendices letters. Appendix A: Now has transitions and conditions for both instruments, and qualifier transitions. Added Appendix F with instructions on extracting samples with heavy sediment.

Distribution/Training List

See Employee Training Record File for signed training statements for trained users.

1.0 SUMMARY, SCOPE, AND APPLICATION

1.1 This method is used to analyze water samples for selected per- and polyfluorinated alkyl acids (PFAAs). A water sample of approximately 250mLs (preserved with Trizma if chlorinated source) is fortified with spikes and surrogates and extracted via Solid Phase Extraction (SPE). The sample is then concentrated to near dryness and subsequently brought up to a final volume of 1mL. All samples are analyzed using a Triple Quad LC/MS 6470 or 6495 (LC/MS/MS) system. Target analytes are identified by comparing mass spectra and retention times to reference spectra and retention times of calibration standards. Analytes are quantitated using the isotope dilution technique explained in the initial calibration section. The following compounds can be identified by this method:

Analyte	Acronym	Standard RL (ng/L)	Cas Number
11- 11-chloroeicosafluoro-3-oxanone-1-sulfonic acid	**11Cl-PF3OUdS**	2.0	763051-92-9
9 -Chlorohexadecafluoro-3-oxapentane-1-sulfonic acid	**9Cl-PF3ONS**	2.0	756426-58-1
4,8-Dioxa-3H-perfluorononanoic acid	**ADONA**	2.0	919005-14-4
Hexafluoropropylene oxide dimer acid	**HFPO-DA**	2.0	13252-13-6
Perfluoro-3,6-dioxaheptanoic acid	**NFDHA**	2.0	151772-58-6
Perfluorobutanoic acid	**PFBA**	2.0	375-22-4
Perfluorobutanesulfonic acid	**PFBS**	2.0	375-73-5
8:2 Fluorotelomer sulfonic acid	**8:2FTS**	2.0	39108-34-4
Perfluorodecanoic acid	**PFDA**	2.0	335-76-2
Perfluorododecanoic acid	**PFDoA**	2.0	307-55-1
Perfluoro(2-ethoxyethane)sulfonic acid	**PFEESA**	2.0	113507-82-7
Perfluoroheptanesulfonic acid	**PFHpS**	2.0	375-92-8
Perfluoroheptanoic acid	**PFHpA**	2.0	375-85-9
4:2 Fluorotelomer sulfonic acid	**4:2FTS**	2.0	757124-72-4
Perfluorohexanesulfonic acid	**PFHxS**	2.0	355-46-4
Perfluorohexanoic acid	**PFHxA**	2.0	307-24-4
Perfluoro-3-methoxypropanoic acid	**PFMPA**	2.0	377-73-1
Perfluoro-4-methoxybutanoic acid	**PFMBA**	2.0	863090-89-5
Perfluorononanoic acid	**PFNA**	2.0	375-95-1
6:2 Fluorotelomer sulfonic acid	**6:2FTS**	2.0	27619-97-2

Perfluorooctanesulfonic acid	**PFOS**	2.0	1763-23-1
Perfluorooctanoic acid	**PFOA**	2.0	335-67-1
Perfluoropentanoic acid	**PFPeA**	2.0	2706-90-3
Perfluoropentanesulfonic acid	**PFPeS**	2.0	2706-91-4
Perfluoroundecanoic acid	**PFUnA**	2.0	2058-94-8
N-ethyl perfluorooctanesulfonamidoacetic acid	**NEtFOSAA**	2.0	2991-50-6
N-methyl perfluorooctanesulfonamidoacetic acid	**NMeFOSAA**	2.0	2355-31-9
Perfluoro-1-butanesulfonamide	**FBSA**	2.0	30334-69-1
Perfluoro-1-hexanesulfonamide	**FHxSA**	2.0	41997-13-1
Perfluorotetradecanoic acid	**PFTA**	2.0	376-06-7
Perfluorotridecanoic acid	**PFTrDA**	2.0	72629-94-8
Perfluorooctanesulfonamide	**FOSA**	2.0	754-91-6
Perfluorononanesulfonic acid	**PFNS**	2.0	68259-12-1
Perfluorodecanesulfonic acid	**PFDS**	2.0	335-77-3

2.0 INTERFERENCES

2.1 Standards and samples should not come into contact with glass other than standards purchased in glass ampules. PFAS commonly adsorb to the surface and could result in recovery discrepancies.

2.2 Matrix interferences may be caused by co-extracted contaminants present in the sample.

2.3 Method interferences may be caused by contaminants in solvents, reagents, and other sample processing hardware.

2.3.1 Other common lab supplies that are associated with PFAAs and should be avoided where possible: aluminum foil, permanent marker, and PTFE.

2.3.1.1 To eliminate any residual PTFE from the Agilent LC, an inline filter column has been installed to reduce any background contamination prior to sample introduction into the system. See Equipment and Supplies Section 4.0.

2.3.2 Organic contaminants can pose a threat of interference due to the high quantities of de-chlorinating agent added to samples.

2.3.3 Contamination levels should be monitored and all blanks should be free from interferences (less than 1/2 the MRL) in all Laboratory Reagent Blanks (LRB).

2.3.4 Blank subtraction is not permitted in this method.

2.3.5 There is a possibility of matrix effects due to co-extracted organic material. When high levels of TOC are present, this can affect the ionization of 4:2 FTS considerably

3.0 SAMPLE PRESERVATION/STORAGE/HOLDING TIME

3.1 Samples should be collected in a wide mouth 250-mL polypropylene bottle fitted with a polypropylene screw cap.

3.2 Prior to shipment to the field for sampling, Trizma will be added for any chlorinated sources or drinking water samples.
3.2.1 It is important that no amount of preservative is spilled from the container or overflowed during sampling.

3.3 Samples cannot exceed 10°C during the first 48 hours following sample collection. Samples must be received at or below 10°C OR have ice remaining in the cooler.

3.4 Prior to extraction, samples must be stored at or below 6°C and cannot be frozen.

3.5 Samples collected must be extracted within 28 days. Extracted samples must be run within 28 days of extraction and remain stored at room temperature.

4.0 EQUIPMENT & SUPPLIES

4.1 Triple Quad LC/MS System

4.2 Inline Delay column

4.2.1 Agilent Zorbax Eclipse Plus C18 3.0x50mm 1.8-micron P.N. PFCDELAY or equivalent

4.3 Analytical Column

4.3.1 Agilent Zorbax Eclipse Plus C18 3.0x50mm 1.8-micron P.N. 959757-02 or equivalent

4.4 Auto-pipettors: 0-10uL, 10-100uL, 100-1000uL

4.5 Polypropylene pipet tip: 0-10uL, 10-100uL, 100-1000uL

4.6 Polypropylene transfer pipets

4.7 Polypropylene graduated cylinder: 10mL, 50mL, 100mL, 1000mL

4.8 Vials: 2ml polypropylene vials

4.9 Caps: 11mm polypropylene snap caps

4.10 Sample containers: 250ml wide mouth polypropylene containers

4.11 Polypropylene centrifuge tubes

4.12 SPE System

4.12.1 Supelco Visiprep manifold or equivalent

4.12.2 Non-PTFE SPE Reservoirs and/or sample transfer tubing with adapters

4.12.3 Phenomenex Pre-Stacked WAX/GCB Cartridge or equivalent

4.12.3.1 Each lot should be checked to be free of contamination prior to usage for any field samples or batch QC. This can be done in the form of an additional LRB prior to usage for extraction.

4.12.4 Vacuum pump capable of reaching up to 20" Hg

4.12.5 13L Safety coated Pyrex waste collection container

4.12.6 Polypropylene tubing for vacuum pump and manifold

4.13 N-Evap concentrator system

4.14 Balance: Analytical, capable of accurately weighing 0.0001g

4.15 Vortexer

4.16 Polypropylene inserts

5.0 REAGENTS & STANDARDS

5.1 Reagent Water : interferent free

5.2 Methanol : LC/MS Grade

5.3 Nitrogen : Ultra high purity

5.4 Ammonium Acetate : LC/MS Grade

5.5 Stock Standard Solutions: Purchased as either certified solutions or neat standards.

5.6 Surrogate, Internal Standard, and ESI-L Low concentration tuning mix purchased as certified solutions

5.7 5 mM Ammonium Acetate reagent water: Prepared by adding 1.54 grams of Ammonium Acetate to 1000mL of reagent water and mixing until solids are into solution.

5.8 1 g/L Ammonium Acetate reagent water.

5.9 96:4 Methanol:Water- Made fresh every 2 days.

5.10 Ammonium hydroxide (56.6% w/w)

5.11 0.1 M Sodium Phosphate Monobasic

5.12 0.1 M Sodium Phosphate Dibasic

5.13 0.1 M Phosphate Buffer: Prepared by mixing 500 mL of dibasic sodium phosphate with 275 mL of monobasic sodium phosphate. Verify solution pH of about 7.0.

5.14 Agilent ESI-L Low Concentration Tuning Mix

5.15 Sodium Hydroxide

5.16 Elution Solvent: 1% ammonium hydroxide in methanol (made fresh daily)

6.0 SAFETY

See Material Safety Data Sheets (MSDS) and Pace Chemical Hygiene Plan.

7.0 PROCEDURE

7.1 Sampling

7.1.1 Samples are to be collected in lab-provided plastic containers. Guidance for sampling is obtained through Pace corporate website or preferably local project authorities/municipalities.

7.2 Surrogate/Spike/Isotope Performance Standard Preparation

7.2.1 All standards must be documented in Element and have Certificate of Analysis forms attached electronically. All information should be documented and each standard should be given an Element Standard ID#.

7.2.2 Standards may be received in purchased glass ampoules but any transfer or dilution must be stored in polypropylene vials with Non-PTFE caps.

7.2.3 All standards purchased from Wellington come pre-treated with sodium hydroxide for compound stability. If making standards from solid, standards must be stored under basic condition to prevent esterification of fluorinated carboxylic acids. See calculation 1 in section 12.1.

7.2.4 PFAS Surrogate Preparation

7.2.4.1 All purchased surrogate stock standards are to be stored until expiration date provided by manufacturer at 4°C.

Compound	Abbreviation	PDS, ng/mL
Perfluoro-n-[1,2,3,4-13C4]butanoic acid	MPFBA	1000
Perfluoro-n-[1,2,3,4,5-13C5]pentanoic acid	M5PFPeA	1000
Sodium perfluoro-1-[2,3,4-13C3]butanesulfonate	M3PFBS	929
Sodium 1H,1H,2H,2H-perfluoro-1-[1,2-13C2]hexane sulfonate	M2-4:2FTS	935
Perfluoro-n-[1,2,3,4,6-13C5]hexanoic acid	M5PFHxA	1000
Perfluoro-n-[1,2,3,4-13C4]heptanoic acid	M4PFHpA	1000
Sodium perfluoro-1-[1,2,3-13C3]hexanesulfonate	M3PFHxS	946
Sodium 1H,1H,2H,2H-perfluoro-1-[1,2-13C2]-octane sulfonate	M2-6:2FTS	949
Perfluoro-n-[13C8]octanoic acid	M8PFOA	1000
Perfluoro-n-[13C9]nonanoic acid	M9PFNA	1000
Sodium perfluoro-[13C8]octanesulfonate	M8PFOS	957
Sodium 1H,1H,2H,2H-perfluoro-1-[1,2-13C2]-decane sulfonate	M2-8:2FTS	958
Perfluoro-n-[1,2,3,4,5,6-13C6]decanoic acid	M6PFDA	1000
Perfluoro-n-[1,2,3,4,5,6,7-13C7]undecanoic acid	M7PFUnA	1000
2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy-13C3-propanoic acid	M3HFPO-DA	1000
Perfluoro-n-[1,2-13C2]dodecanoic acid	M2PFDoA	1000
Perfluoro-n-[12-13C2]tetradecanoic acid	M2PFTA	1000

Perfluoro-1-[13C8]octanesulfonamidoacetic acid	**M8FOSA**	**1000**
N-methyl-d3-perfluoro-1-octansulfonamidoacetic acid	**d3-N-MeFOSAA**	**1000**
N-ethyl-d5-perfluoro-1-octansulfonamidoacetic acid	**d5-N-EtFOSAA**	**1000**

Ordered as MPFAC-24ES from Wellington Labs

2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy-13C3-propanoic acid	**M3HFPO-DA**	**1000**

Ordered as M3HFPO-DA from Wellington Labs

7.2.6 Analyte Primary Dilution Standard Preparation

7.2.6.1 All purchased PFAA spike standard stock standards are to be stored until expiration date provided by manufacturer at 4°C.

7.2.6.2 Prepared and in use PFAA stock standard solutions should be stored at room temperature and vortexed prior to usage. These standards will expire 2 months after preparation date or manufacturer's expiration date, whichever comes first.

Analyte Standards

Compound	Vendor	Concentration of Standard (ng/ mL)
PFAC-30PAR	Wellington	1000*
PFAC-24PAR	Wellington	2000*
PFEESA	Wellington	50000*
NFDHA	Wellington	50000
PFMPA	Wellington	50000
PFMBA	Wellington	50000
HPFO-DA	Wellington	50000
NaDONA	Wellington	50000*
9ClPF3ONS	Wellington	50000*
11ClPF3OUdS	Wellington	50000*
FBSA	Wellington	50000
FHxSA	Wellington	50000

*Individual analyte concentration may vary due to amount of anion present in solution. All calculations must use the anion concentration, not the salt concentration. See Calculation 2 in section 12.2.

7.2.6.3 PFEESA, NFDHA, PFMPA, PFMBA, HPFO-DA, NaDONA, 9ClPF3ONS, 11ClPF3OUdS, FBSA, and FHxSA are not included in the 24PAR mixture and are mixed into a separate "supplemental" 1000 ng/ mL stock. See below table for mixture.

7.2.6.4 The Supplemental Stock is used to make a 500ppb spike dilution with 24PAR so that a different lot is used for spiking. The calibration stock is made using 30PAR and directly adding

PFEESA, NFDHA, PFMPA, and PFMBA, which are missing from that mixture. See below table for prep instructions:

Stock Dilution Prep Table

	Volume of Compound/Standard Mixtures (µL)	**Volume of Methanol (µL)**	**Final Volume (µL)**	**Final Concentration (ng/mL)**
Supplemental Stock	100µL – HPFO-DA 100µL – NaDONA 100µL – 9ClPF3ONS 100µL – 11ClPF3OUdS 100µL – PFEESA 100µL – PFMBA 100µL – PFMPA 100µL – NFDHA 100µL – FBSA 100µL – FHxSA	4000µL	5000µL	1000
500 ng/ mL **Spike**	1250µL – PFAC24PAR 2500µL – Supplemental Stock	1250µL	5000µL	500
100 ng/ mL **Spike**	250µL – PFAC24PAR 500µL – Supplemental Stock	4250µL	5000µL	100
100 ng/ mL **Cal Stock**	500µL – PFAC30PAR 10µL – PFEESA 10µL – PFMBA 10µL – PFMPA 10µL – NFDHA	4460µL	5000µL	100

7.2.6.5 The calibration is prepared as follows using the stock dilutions prepared above.

Calibration Table

Volume 100ppb Stock Standard (µL)	**Volume 30PAR Stock Standard (µL)**	**Volume Supplemental Stock Standard (µL)**	**Volume of Surrogate Stock (µL)**	**Volume of M3HFPODA Surrogate Stock**	**Volume of 96:4 Methanol: Water (µL)**	**Final Volume (µL)**	**Final Concentration (ng/mL)**
12.5	0	0	25	25	4937.5	5000	0.25*
25	0	0	25	25	4925	5000	0.5*
50	0	0	25	25	4900	5000	1.0*
125	0	0	25	25	4825	5000	2.5*
250	0	0	25	25	4700	5000	5.0*

0	50	50	25	25	4850	5000	10.0*
0	125	125	25	25	4700	5000	25.0*

*Individual analyte concentration may vary due to amount of anion present in solution. All calculations must use the anion concentration, not the salt concentration. See Calculation 2 in section 12.2.

7.2.6.6 Continuing calibration verification (CCVs) standards are made at the mid-level, identically to the 4th calibration level above. The ICV/QCS is made similarly, just like the 5th calibration level above, except instead of calibration stock 100 ppb Spike is used as shown in the table below:

QCS/ICV Preparation Table

Standard Name	Volume Standards	Volume of 96:4 Methanol:Water (µL)	Final Volume (µL)	Final Concentration (ng/mL)
QCS/ICV	25µL- PFAC-24ES 25µL- M3HPFO-DA Surrogate Dilution 250µL- 100ppb Spike	4700µL	5000µL	5.0

7.3 Initial Calibration Criteria

7.3.1 All analytes must first be product ion optimized with the LC/MS/MS system using the MassHunter Optimizer program to determine optimal fragmentor and collision cell energy for applicable ions. This optimization should be done using a high-level standard for each analyte and using all of the LC parameters used in the analytical method. A level 4 standard must then be run to identify all retention time windows for all compounds of interest (See Section 7.7.2). A minimum of 10 spectra scans are acquired across each chromatographic peak. See Appendix A for further analyte MS/MS conditions.

7.3.2 Prior to initial calibrations, and when the instrument is having difficulty passing regular calibrations, a mass calibration will be performed using Agilent ESI-L Low Concentration Tuning Mix. The MassHunter program has an autotune feature that performs the mass calibration using this mix and verifies it in a report.

7.3.3 A calibration is to be run when continuing calibration checks or surrogates do not pass QC criteria. A calibration should also be performed when any hardware is changed or major instrument maintenance is performed.

7.3.4 The initial calibration must contain a minimum of five points with the lowest point being at or below the MRL. A minimum of six points is required for a quadratic calibration. The total of the branched and linear isomers must be used for calibration for the following target analytes: PFOS, PFHxS, NEtFOSAA, NMeFOSAA.

7.3.5 The LC/MS/MS system is to be calibrated using the isotope dilution technique. Therefore, isotope dilution analogues are added at a constant concentration in all standards prior to injection. Either linear or quadratic regression can be used, but it must always be forced through zero and can be concentration weighted. Forcing through zero allows for more sensitivity to detect background contamination within the system. The calibration shall be done using the same LC conditions as the samples (See Section 7.7.2). Because the isotope dilution analogues are added in equal concentrations, calibrate for them using an average response factor. Not all analytes have an exact mass-labeled analogue, in which case the closest analogue is used (either by chemical properties or retention time). See Appendix A for the list of isotope analogues and corresponding target analytes.

7.3.5.1 The isotope dilution technique utilizes extracted compounds to serve as a traditional internal standard. In this case, the extracted analogues must pass criteria listed in section 8.2.6. The analogue is then used as the internal standard compound for associated target analytes.

7.3.6 Calibration levels for linear or non-linear analyte targets must have a $r^2 \geq 0.99$ for each analyte and the recovery for each analyte must be within 70-130% of the true value. Surrogate and internal standards must have an RSD of the RFs for all analytes of ≤20%.

7.3.7 A quality control standard (QCS) will serve as an initial calibration verification (ICV) and be run following initial calibration and all subsequent calibrations. The ICV shall be prepared from a separate dilution of a different stock standard. This sample must be run following a calibration or quarterly, whichever comes first. The accepted values for the ICV are 70-130% of the true value for each analyte.

7.3.8 If any instrumentation or analytical setpoints are changed to the instrument calibration, an initial demonstration of capability (IDOC) for the procedure and instrumentation shall be performed. See Appendix E.

7.4 Continuing Calibration

7.4.1 An instrument blank (IBL) and a low-level instrument sensitivity check (ISC) must be run at the MRL before any other injections and once every 12 hours. The results must be between 70-130% of the true value for all analytes. The IBL should have no hits greater than ½ the MRL. The ISC can serve as your initial CCV for the day.

7.4.2 Prior to samples analysis, a low-level continuing calibration verification (CCV) must be run. After every ten field samples a subsequent CCV must be at Level 4 (same as calibration point 4 above). A closing CCV must also be run at the end of each analysis. The requirements for the CCVs are 70-130% of the true value for method analytes.

7.4.3 An instrument blank is required to be run following analysis of the highest-level standard analyzed (after a calibration in this case). One is also required daily prior to sample analysis. All analytes must be at >½ the MRL in order to pass.

7.4.4 All isotope dilution analogues (surrogates) must have a recovery between 50-150%

7.4.5 In the case of CCV failure, two consecutive CCVs should be immediately run. If these pass, analysis can continue. Otherwise, a calibration or tuning and re-analysis of affected samples is required.

7.4.6 A checktune will be run as needed to verify MS operating criteria. This is run through the MassHunter program using Agilent ESI-L Low Concentration Tuning Mix. If criteria are out of spec, the parameters set forth in the Agilent 6400 Series Triple Quadrupole LC/MS System Quick Start Guide must be followed to adjust values. If re-run of checktune does not pass, an autotune must be run and the instrument must be recalibrated.

7.5 Sample Extraction Procedure

7.5.1 For each required lab QC sample, fill a clean sample bottle with 250 mL DI water. Check verify pH is 7.0 ± 1.0 pH units with pH paper. If sample pH is not 7.0 ± 1.0 pH units, note on bench sheet and adjust using Trizma.

7.5.2 For every 20 field samples, a blank and a blank spike must be extracted. (Field blanks are considered field samples in this consideration as they are treated as such) Ideally, if adequate sample volume is available, a matrix spike and matrix spike duplicate should be included on every batch.

7.5.3 All polypropylene equipment including graduated cylinders and sample transfer lines/reservoirs should be washed prior to using with extraction solvent (96:4 Methanol:water), followed by a DI water rinse.

7.5.4 Add 5uL of surrogate to each sample and 25ul of 100ppb spike to all BS and MS samples included on the extraction batch. Cap and invert to mix well.

7.5.5 Take initial weight (in grams) of each bottle and sample with the Sartorius Top Loading Balance. Observe if any samples have heavy sediment or are very cloudy, and decide if it seems like the cartridge will clog. These samples can be extracted following a special protocol using a centrifuge outlined in Appendix C.

7.5.6 After SPE system is set up, condition the cartridges first with 5mL methanol, followed by 5mL of 0.1M Sodium Phosphate Buffer.

***Note: The sample cartridges must not be allowed to run dry at any point during conditioning. If they become dry, the conditioning must be started over.**

7.5.7 Next add 2 mL of 0.1M Sodium Phosphate Buffer and attach either sample transfer tube or reservoir to the cartridge and begin transferring sample. The samples should be passed through the cartridge at approximately 5mL/min. This equates to a drop wise fashion eluting from the cartridge.

7.5.8 Rinse sample bottle with 7.5mL of reagent water and pass through tubing or reservoir and cartridge. Repeat once more.

7.5.9 Add 0.5mL of acetonitrile to each cartridge. Remove sample transfer tubes/reservoirs and allow air to pass through the cartridges for a minimum of 5 minutes at approximately 10-15" Hg.

7.5.10 Turn off vacuum and add tray of labeled collection vials to manifold.

7.5.11 Using a pipette, rinse each respective reservoir into the sample container taking care to rinse the sides with 6mL of 1% NH4OH methanol. Pour solvent from sample bottles directly into cartridge and allow to soak in the cartridge for 5 minutes after solvent fully wets the solid phase. Then, allow to elute through the cartridge at a low vacuum elute with an additional 5-6mL of solvent so the final eluent is ~12mL.

7.5.12 Samples can then be concentrated to ~850uL at room temperature

7.5.13 Add 96:4 methanol:water, taking care to rinse the side of the container until the final volume reaches 1mL.

7.5.14 Determine initial volume by taking the weight (in grams) of the empty container following extraction. Subtract this from the weight taken in step 7.5.5 to determine the volume by weight of the sample (it is assumed that 1 gram is equal to 1 mL).

7.6 Data Analysis

7.6.1 The analyst cannot extrapolate beyond the range of the calibration. However, by isotope dilution analysis, the extract cannot be diluted. If an analyte is outside of the determined range, the sample must be re-extracted at an appropriate dilution level.

7.6.1.1 There is extrapolation allowed only to determine if there is blank contamination. Since there is no blank subtraction, any contamination present must be below 1/2 of the MRL for specific analyte.

7.6.1.2 If a sample exceeds the calibration range the sample must be re-extracted. This would involve diluting the sample with reagent water to be within the calibration range and adding ammonium acetate to be at a final concentration of 1 g/L.

7.6.1.3 Additionally, if a sample exceeds the calibration range, one or more LRB must be run until the system meets acceptable criteria. If this occurs during an automated sequence, the samples subsequently must be evaluated. If the over-range analytes are present in the subsequent samples at or above the RL, the samples are considered invalid and must be re-run. If the analyte in question does not exceed the RL, the samples can be reported.

7.6.2 Compounds that have both branched and linear isomers will be reported as total. These compounds include PFOS, PFHxS, N-Et-FOSAA, N-Me-FOSAA and PFOA. PFOS, PFHxS, N-Et-FOSAA, and N-Me-FOSAA have the branched and linear compounds available for quantitation. PFOA is a special case outlined below:

7.6.2.1 PFOA will be quantitated by using a qualitative/semi-quantitative approach per EPA guidance. Since there is no standard available, the calibration will be done using the linear isomer only. A technical grade standard will be run to identify the retention time of the branched isomer. All samples will be quantitated using the area of both the linear and branched isomers of PFOA that may be present within the sample. A branched isomer check for PFOA will be run with every calibration curve to verify the retention times of the branched isomers for PFOA.

7.6.3 All analytes and surrogates will be calculated based off the initial calibration criteria.

7.6.4 All results for analytes shall be reported as the neutral acid.

7.6.5 Retention time windows are established once per ICAL and at the beginning of each sequence. On days when an ICAL is not run, the initial CCV is used to set the times. All retention times of analytes and EIS analytes must fall within 0.4 minutes of the established time. Analytes must also elute within 0.1 minutes of their respective EIS.

7.6.6 In addition to retention time identification criteria, most ions are identified by two ion transitions. (The following ions are exceptions: PFBA, PFPeA, FBSA, FHxSA, PFMPA, PFMBA, 9Cl-PF3ONS, 11Cl-PF3OUdS, ADONA). The secondary, or qualifier ion, must have a signal to noise of 3:1. The ratio between the qualifier and the quantifier ion must be averaged from the calibration. For samples to be valid, the ratio of qualifier to quantifier must be +/-50% from the average ratio from the applicable calibration.

7.7 Instrumentation Procedure

7.7.1 Before any QC or samples can be run, the HPLC must be allowed to purge for at least thirty minutes. This purge can be done using any combination of the mobile phases, but prior to samples running, the initial mobile phase conditions used in the method must be allowed to run for 15 minutes or until pressure has stabilized.

7.7.2 The instrument must be stable in all parameters before a run is started. The following are the HPLC and ESI-MS Method Conditions. Also, See Appendix A for additional MS/MS Method Conditions.

Time (min)	% 5 mM Ammonium Acetate in water	% Methanol	Flow Rate (mL/minute)
0.00	95	5	1.0
0.10	65	35	1.0
2.00	50	50	1.0
3.00	25	75	1.0
4.50	1	99	1.0
4.51	1	99	1.0
5.00	1	99	1.0
5.10	95	5	1.0
6.50	95	5	1.0

Parameter	Value
Injection Volume 6470	10 uL
Injection Volume 6495	5 uL
Column Compartment Temperature	40 °C
Autosampler Compartment Temperature	10 °C
Polarity	Negative
Gas Temperature	250 °C
Gas Flow	11 L/min
Nebulizer	50 psi
Sheath Gas Temperature	300 °C
Sheath Gas Flow	12 L/min

Capillary Needle Voltage (Negative mode)	-3000 V
Cell Accelerator Voltage	5 V

7.7.3 An instrument sequence will be made. It will open with a blank and a low level CCV. After the CCV, the batch can start running. Every 10 field samples (excluding QC and FRBs) a subsequent CCV must be run, at level 4. The sequence must end with a CCV.
7.7.4 The run can end with a script to put the instrument into standby mode.

8.0 QUALITY CONTROL

8.1 Definitions

For definitions and explanations of quality control measures (blanks, LCS/QC Reference, LFB, Duplicates, MS/MSD, etc.) refer to the Contest, A Pace Analytical Lab Quality Assurance Manual.

8.2 Quality Control Measures & Acceptance Criteria

8.2.1 Method Blank

The method blank is matrix specific and extracted with every batch or every 20 samples (whichever is more frequent). The target compounds and ranges must be ≤1/2 the MRL, or <1/10th the amount measured in any sample, or <1/10th the regulatory limit. If any analytes are present above this level, the detected analytes are considered invalid for all samples extracted in that batch.

8.2.2 Field Reagent Blank

It is highly recommended to collect a Field Reagent Blank per every sampling event. If provided, Field reagent blanks only need to be run and reported if there are analytes at or above the MRL in any associated field samples. Any analyte peaks present in field reagent blanks must be below 1/3 of the MRL of that analyte. If any analytes are present above this level, all samples collected with said FRB are invalid and must be recollected and reanalyzed. Data will be reported to client as suspect, noting the field blank contamination.

8.2.3 Laboratory Control Sample/Duplicate (LCS)

A matrix-specific LCS is extracted every 20 samples or per batch. The concentration must be ≥ LOQ and ≤ mid-range of calibration. All analytes recoveries must be within limits specified in Appendix D. If analyte is not listed in table, acceptance criteria is to remain 50-150% until in-house limits can be determined. Samples should be re-extracted if criteria are not met, even if outside of hold. If samples cannot be re-extracted then the failures must be notated in the narrative.

8.2.4 Matrix Spikes

A matrix-specific MS is extracted every 20 samples or per batch. MS spike concentrations will be at the mid-level of the calibration curve. If historical data is available, the sample will be spike at a level similar to expected contaminant levels. All analytes recoveries must be within limits specified in Appendix D. If analyte is not listed in table, acceptance criteria is to remain 50-150% until in-house limits can be determined. See calculation 3.

***Note: Matrix spike samples may display matrix bias. If the CCC and LFB samples are passing, but the MS recoveries are outside the designated range, the recovery is deemed to be matrix biased. A note on the unfortified sample will indicate the possibility of matrix effects being suspect.**

8.2.5 Matrix spike duplicates

Extract a spiked sample duplicate every 20 samples (when enough aliquot is provided). Matrix Spike Duplicate samples should be calculated to have an RPD ≤30%. See calculation 4.

8.2.6 Quality Control Samples

A quality control sample must be run from a second source at least quarterly, or after an initial calibration as an ICV. If a second source is not commercially available, a different lot number from the same vendor is acceptable. The recoveries must be within 70-130% of the true value.

8.2.7 Isotope Dilution Analogue

Isotope dilution analogues are added to all blanks, standards, samples, and spikes. Analogue compounds must have an area of 50-150% of the associated compound in level 4 of the calibration on days when a calibration is run. On days when a calibration is not run, analogue compounds must have an area of 50-150% of the associated compound in the opening instrument sensitivity check/CCV.

If the surrogate is outside these limits, the extract should be re-analyzed. If the re-analysis passes, report re-analyzed sample. If this fails, the associated isotope performance standard should be evaluated. The system may need recalibration or maintenance. If the CCV has surrogate out of range, the instrument needs to be recalibrated.

If the re-analysis fails, re-extract the sample to confirm failure if an aliquot is available. If the re-extract fails, report both results with appropriate flagging criteria .If not enough volume is provided to re-extract the sample then report with appropriate flags.

8.2.8 Calibration Curve

A minimum of a 5-point calibration curve (for linear regression) or a 6-point calibration curve (for quadratic) is used to calibrate the system.

The curve must be verified with an independent standard (QCS) prior to sample analysis, (10 ng/L). The curve will be forced through zero and may or may not be concentration weighted.

If a peak is not properly integrated by the data system, manual integration may be necessary. Manual integrations must comply with the Pace SOP on Chromatographic Integration Procedures. The integration of the peaks for the samples and quality control samples must be as consistent as possible with the integration used with the initial calibration.

8.2.9 Continuing Calibration Checks (CCCs)

The results must be between 70-130% of the true value for all analytes for the initial low level CCV

After every ten field samples, a subsequent CCV must be at level 4. The requirements for the CCVs are 70-130% of the true value. All analogue compound areas must fall within 50-150% of the appropriate calibration point or CCV.

The ending CCV acquisition time must fall within 24 hours of the acquisition starting time of the opening CCV with the associated analysis batch.

8.2.10 Any failures in QC require reanalysis, even if the samples in question are outside of hold.

9.0 CORRECTIVE ACTIONS/ CONTINGENCIES OF HANDLING OUT-OF-CONTROL DATA

9.1 Refer to Pace Quality Assurance Manual
9.2 Refer to Pace Corrective Action SOP.

10.0 POLLUTION PREVENTION

10.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Many opportunities for pollution prevention exist in laboratory operation. EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address waste generation. Standards should be prepared in volumes consistent with laboratory use to minimize the disposal of excess volumes of expired standards.

11.0 WASTE MANAGEMENT

11.1 It is the laboratory's responsibility to comply with all federal, state, and local regulations governing the waste management, particularly the hazardous waste identification rules and land disposal restrictions, and to protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Also, compliance is required with any sewage discharge permits and regulations.

11.2 Acidic samples and waste are dumped into satellite waste containers.

12.0 CALCULATIONS

12.1 Calculation 1: Adding 4 mole equivalents to standards to prevent esterification

$$\frac{Total\ PFAS\ mass\ (g) \times 160(\frac{g}{mol})}{250\ (\frac{g}{mol})} = Mass\ of\ NaOH\ Required\ (g)$$

12.2 Calculation 2: Mass of the anion
$Mass_{acid}$= Measured $Mass_{salt}$ * (Molecular $Weight_{acid}$/Molecular $Weight_{salt}$)

12.3 Calculation 3: Percent Recovery

$$\%R = \frac{(A - B)}{C} \times 100$$

12.4 Calculation 4: Relative percent deviation calculation

$$RPD = \frac{|LFSMD - LFSM|}{(LFSMD + LFSM)/2} \times 100$$

13.0 REFERENCES

13.1 Pace Analytical Chemical Hygiene Plan

13.2 Pace Analytical Quality Assurance Manual.

13.3 Pace Analytical Corrective Action SOP.

13.4 Pace Analytical Controlled Document SOP.

13.5 Agilent 1260 Infinity Binary LC Operators manual

13.6 Agilent MassHunter Study Manager

13.7 Agilent MassHunter Optimizer

13.8 MassHunter Personal Compound Database and Library Manager

13.9 Agilent 6400 Series Triple Quadrupole LC/MS System Quick Start Guide

13.10 MassHunter Data Acquisition Compliance Software Quick Start Guide

13.11 MassHunter Quantitative Analysis Compliance Software Quick Start Guide

13.12 Agilent 6000 Series LC/MS System Maintenance Guide

13.13 EPA Method 537, "Determination of Selected Perfluorinated Alkyl acids in Drinking Water by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)", Version 1.1, September 2009.

13.14 Method ISO 25101:2009, "Determination of perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) – Method for unfiltered samples using solid phase extraction and liquid chromatography/mass spectrometry", April 30, 2009.

13.15 EPA Technical Advisory-Laboratory Analysis of Drinking Water Samples for Perfluorooctanoic Acid (PFOA) using EPA Method 537 Rev. 1.1 EPA 815-B-16-021 September 2016

13.16 Agilent Application note by Peter JW Stone, Linda Cote, Jennifer Gushue, Robert J. Letcher and Shaogang Chu. A Low Femtogram Target Screen Method for Perfluorinated Compounds in Food Matrices and Potable Water Using the Agilent 6460 Triple Quadrupole LC/MS System Equipped with Agilent Jet Stream Technology.

13.17 TNI Standard, The NELAC Institute, EL-V1-2009-ISO, 2009.

13.18 Department of Defense (DoD) Department of Energy (DOE) Consolidated Quality Systems Manual (QSM) for Environmental Laboratories Based on ISO/IEC 17025:2005(E) ISO/IEC 17025:2017(E) and The NELAC Institute (TNI) Standards, Volume 1, (September 2009).

Appendix A

6470 Transitions and MS Conditions:

Analyte	Precursor Ion	Product Ion	Collision Energy Voltage (V)	Fragmentor Voltage (V)	Qualifer/Quantifier
11Cl-PF3OUdS	631	451	24	100	N/A
4-2 FTS	327	307	20	120	Quant
4-2 FTS	327	81	30	120	Qual
6-2 FTS	427	406.9	24	135	Quant
6-2 FTS	427	80	40	125	Qual
8-2 FTS	527	507	28	145	Quant
8-2 FTS	527	80	40	170	Qual
9Cl-PF3ONS	531	351	24	100	N/A
ADONA	377	251	12	100	Quant
ADONA	377	85	12	100	Qual
d3-N-MeFOSAA	573.2	419	20	114	N/A
d5-N-EtFOSAA	589.2	419	20	104	N/A
FBSA	297.99	78	28	115	N/A
FHXSA	398	78.1	30	135	N/A
HFPO-DA	285.1	184.9	5	150	Quant
HFPO-DA	285.1	169	5	150	Qual
M2-4-2-FTS	328.99	309.2	20	135	N/A
M2-6-2-FTS	428.99	409.2	24	160	N/A
M2-8-2-FTS	528.99	509	28	170	N/A
M2PFDA	514.9	469.9	5	102	N/A
M2PFHxA	315	270	4	66	N/A
M2PFOA	415	370	4	69	N/A
M2PFTA	715	670	9	100	N/A
M3HFPO-DA	287	169	2	50	N/A
M3PFBA	216	171.8	4	56	N/A
M3PFBS	301.9	80	45	100	N/A
M3PFHxS	401.9	80	49	100	N/A
M4PFHpA	367	322	4	102	N/A
M5PFHxA	318	273	4	68	N/A
M5PFPeA	268	223	8	120	N/A

M6PFDA	519	474	4	81	N/A
M7PFUnA	570	525	5	73	N/A
M8FOSA	506	78	36	125	N/A
M8PFOA	421	376	5	65	N/A
M8PFOS	506.9	80	50	100	N/A
M9PFNA	472	427	4	85	N/A
MPFBA	217	172	8	60	N/A
MPFDoA	615	570	5	79	N/A
MPFOS	502.9	80	60	180	N/A
N-EtFOSAA	584	525.9	20	115	Qual
N-EtFOSAA	584	418.9	20	115	Quant
NFDHA	201	85	14	115	N/A
N-MeFOSAA	570	482.9	16	115	Qual
N-MeFOSAA	570	418.9	20	115	Quant
PFBA	213	169	8	60	N/A
PFBS	298.9	98.9	29	100	Qual
PFBS	298.9	80	45	100	Quant
PFDA	513	469	4	81	Quant
PFDA	513	169	16	100	Qual
PFDoA	613	569	5	79	Quant
PFDoA	613	268.7	20	100	Qual
PFDS	598.9	99	60	100	Qual
PFDS	598.9	80	80	100	Quant
PFEESA	315	135	24	110	N/A
PFHpA	362.9	319	8	72	Quant
PFHpA	362.9	169	20	72	Qual
PFHpS	448.9	98.7	44	44	Qual
PFHpS	448.9	79.7	52	52	Quant
PFHxA	313	268.9	8	8	Quant
PFHxA	313	119	18	18	Qual
PFHxS	398.9	99	45	100	Qual
PFHxS	398.9	80	49	100	Quant
PFMBA	279	85.1	8	55	N/A
PFMPA	229	85.1	12	55	N/A
PFNA	463	419	4	66	Quant
PFNA	463	169	17	66	Qual
PFNS	548.9	98.9	40	165	Qual
PFNS	548.9	79.9	40	165	Quant
PFOA	413	369	4	69	Quant
PFOA	413	169	12	69	Qual
PFOS	498.9	99	50	100	Qual
PFOS	498.9	80	50	100	Quant
PFOSA	497.9	77.9	36	125	Quant
PFOSA	497.9	47.9	80	100	Qual
PFPeA	263	218.9	8	60	N/A
PFPeS	348.9	98.9	40	135	Qual
PFPeS	348.9	79.9	40	135	Quant
PFTA	713	669	9	100	Quant

PFTA	712.9	169	30	100	Qual
PFTrDA	663	619	9	91	Quant
PFTrDA	663	169	30	100	Qual
PFUnA	563	519	5	73	Quant
PFUnA	563	218.7	20	100	Qual

6495 Transitions and MS Conditions:

Analyte	Precursor Ion	Product Ion	Collision Energy Voltage (V)	Fragmentor Voltage (V)	Qualifer/Quantifier
11Cl-PF3OUdS	631	451	24	166	N/A
4-2 FTS	327	307	20	166	Quant
4-2 FTS	327	81	30	166	Qual
6-2 FTS	427	406.9	24	166	Quant
6-2 FTS	427	80	40	166	Qual
8-2 FTS	527	507	28	166	Quant
8-2 FTS	527	80	40	166	Qual
9Cl-PF3ONS	531	351	24	166	N/A
ADONA	377	251	12	166	Quant
ADONA	377	85	12	166	Qual
d3-N-MeFOSAA	573.2	419	20	166	N/A
d5-N-EtFOSAA	589.2	419	20	166	N/A
FBSA	297.99	78	28	166	N/A
FHXSA	398	78.1	30	166	N/A
HFPO-DA	285.1	184.9	5	166	Quant
HFPO-DA	285.1	169	5	166	Qual
M2-4-2-FTS	328.99	309.2	20	166	N/A
M2-6-2-FTS	428.99	409.2	24	166	N/A
M2-8-2-FTS	528.99	509	28	166	N/A
M2PFDA	514.9	469.9	5	166	N/A
M2PFHxA	315	270	4	166	N/A
M2PFOA	415	370	4	166	N/A
M2PFTA	715	670	9	166	N/A
M3HFPO-DA	287	169	2	166	N/A
M3PFBA	216	171.8	4	166	N/A
M3PFBS	301.9	80	45	166	N/A
M3PFHxS	401.9	80	49	166	N/A
M4PFHpA	367	322	4	166	N/A
M5PFHxA	318	273	4	166	N/A
M5PFPeA	268	223	8	166	N/A
M6PFDA	519	474	4	166	N/A
M7PFUnA	570	525	5	166	N/A
M8FOSA	506	78	36	166	N/A
M8PFOA	421	376	5	166	N/A
M8PFOS	506.9	80	50	166	N/A
M9PFNA	472	427	4	166	N/A
MPFBA	217	172	8	166	N/A
MPFDoA	615	570	5	166	N/A
MPFOS	502.9	80	60	166	N/A

N-EtFOSAA	584	525.9	20	166	Qual
N-EtFOSAA	584	418.9	20	166	Quant
NFDHA	201	85	14	166	N/A
N-MeFOSAA	570	482.9	16	166	Qual
N-MeFOSAA	570	418.9	20	166	Quant
PFBA	213	169	8	166	N/A
PFBS	298.9	98.9	29	166	Qual
PFBS	298.9	80	45	166	Quant
PFDA	513	469	4	166	Quant
PFDA	513	169	16	166	Qual
PFDoA	613	569	5	166	Quant
PFDoA	613	268.7	20	166	Qual
PFDS	598.9	99	60	166	Qual
PFDS	598.9	80	80	166	Quant
PFEESA	315	135	24	166	N/A
PFHpA	362.9	319	8	166	Quant
PFHpA	362.9	169	20	166	Qual
PFHpS	448.9	98.7	44	166	Qual
PFHpS	448.9	79.7	52	166	Quant
PFHxA	313	268.9	8	166	Quant
PFHxA	313	119	18	166	Qual
PFHxS	398.9	99	45	166	Qual
PFHxS	398.9	80	49	166	Quant
PFMBA	279	85.1	8	166	N/A
PFMPA	229	85.1	12	166	N/A
PFNA	463	419	4	166	Quant
PFNA	463	169	17	166	Qual
PFNS	548.9	98.9	40	166	Qual
PFNS	548.9	79.9	40	166	Quant
PFOA	413	369	4	166	Quant
PFOA	413	169	12	166	Qual
PFOS	498.9	99	50	166	Qual
PFOS	498.9	80	50	166	Quant
PFOSA	497.9	77.9	36	166	Quant
PFOSA	497.9	47.9	80	166	Qual
PFPeA	263	218.9	8	166	N/A
PFPeS	348.9	98.9	40	166	Qual
PFPeS	348.9	79.9	40	166	Quant
PFTA	713	669	9	166	Quant
PFTA	712.9	169	30	166	Qual
PFTrDA	663	619	9	166	Quant
PFTrDA	663	169	30	166	Qual
PFUnA	563	519	5	166	Quant
PFUnA	563	218.7	20	166	Qual

Appendix B

Analyte	Acronym	Isotope Dilution Analogue
11- Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	11Cl-PF3OUdS	M8PFOS
9- Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	9Cl-PF3ONS	M8PFOS
4,8-Dioxa-3H-perfluorononanoic acid	ADONA	M4PFHpA
Hexafluoropropylene oxide dimer acid	HFPO-DA	M3HFPO-DA
Nonafluoro-3,6-dioxaheptanoic acid	NFDHA	M5PFHxA
Perfluorobutanoic acid	PFBA	MPFBA
Perfluorobutanesulfonic acid	PFBS	M3PFBS
1H,1H, 2H, 2H-Perfluorodecane sulfonic acid	8:2FTS	M2-8:2FTS
Perfluorodecanoic acid	PFDA	M6PFDA
Perfluorododecanoic acid	PFDoA	MPFDoA
Perfluoro(2-ethoxyethane)sulfonic acid	PFEESA	M3PFBS
Perfluoroheptanesulfonic acid	PFHpS	M8PFOS
Perfluoroheptanoic acid	PFHpA	M4PFHpA
1H,1H, 2H, 2H-Perfluorohexane sulfonic acid	4:2FTS	M2-4:2FTS
Perfluorohexanesulfonic acid	PFHxS	M3PFHxS
Perfluorohexanoic acid	PFHxA	M5PFHxA
Perfluoro-3-methoxypropanoic acid	PFMPA	MPFBA
Perfluoro-4-methoxybutanoic acid	PFMBA	M5PFPeA
Perfluorononanoic acid	PFNA	M9PFNA
1H,1H, 2H, 2H-Perfluorooctane sulfonic acid	6:2FTS	M2-6:2FTS
Perfluorooctanesulfonic acid	PFOS	M8PFOS
Perfluorooctanoic acid	PFOA	M8PFOA
Perfluoropentanoic acid	PFPeA	M5PFPeA
Perfluoropentanesulfonic acid	PFPeS	M3PFHxS
Perfluoroundecanoic acid	PFUnA	M7PFUnA
N-ethyl perfluorooctanesulfonamidoacetic acid	NEtFOSAA	d5-N-EtFOSAA
N-methyl perfluorooctanesulfonamidoacetic acid	NMeFOSAA	d3-N-MeFOSAA
Perfluoro-1-butanesulfonamide	FBSA	M5PFHxA
Perfluoro-1-hexanesulfonamide	FHxSA	M8PFOA
Perfluorotetradecanoic acid	PFTA	M2PFTA
Perfluorotridecanoic acid	PFTrDA	M2PFTA

Perfluorooctanesulfonamide	**FOSA**	**M8FOSA**
Perfluorononanesulfonic acid	**PFNS**	**M8PFOS**
Perfluorodecanesulfonic acid	**PFDS**	**M3PFBS**

Appendix C

Requirement	Specification	Acceptance Criteria
Demonstration of precision	**Extract and analyze 7 replicate laboratory fortified blanks at the mid-range of the calibration.**	**Percent relative standard deviation must be </=20%.**
Demonstration of accuracy	**Calculate mean recovery for replicated used in demonstration of precision.**	**Mean recovery within 70-130% of the true value.**
MDL Confirmation	**Extract and analyze 9 blanks, and 9 laboratory fortified blanks at the proposed reporting limit over three days.**	**Calculated MDL and MDL-b is < Proposed reporting limit. Calculation done using 40CFR.**
Calibration Verification	**Analyze a mid-level QCS after each initial calibration.**	**Results must be within 70-130% of the true value.**

Appendix D

Table C-45. Method PFAS by LCMSMS Compliant with QSM Table B-15 Solid Matrix

CAS ID	Analyte	N Records	Mean	Standard Deviation	Lower Control Limit	Upper Control Limit
2991-50-6	2-(N-Ethylperfluorooctanesulfonamido) acetic acid	249	99.7	12.9	61	139
2355-31-9	2-(N-Methylperfluorooctanesulfonamido) acetic acid	254	103.9	13.3	63	144
757124-72-4	Fluorotelomer sulphonic acid 4:2	266	103.1	13.7	62	145
27619-97-2	Fluorotelomer sulphonic acid 6:2	575	101.9	12.4	64	140

Table C-45. Method PFAS by LCMSMS Compliant with QSM Table B-15 Solid Matrix

CAS ID	Analyte	N Records	Mean	Standard Deviation	Lower Control Limit	Upper Control Limit
39108-34-4	Fluorotelomer sulphonic acid 8:2	544	100.8	11.8	65	137
375-73-5	Perfluorobutanesulfonic acid	624	100.5	9.2	72	128
375-22-4	Perfluorobutanoic acid	333	102.9	10.5	71	135
335-77-3	Perfluorodecanesulfonic acid	336	96.2	12.4	59	134
335-76-2	Perfluorodecanoic acid	569	101.0	10.4	69	133
307-55-1	Perfluorododecanoic acid	565	101.7	10.8	69	135
375-92-8	Perfluoroheptanesulfonic acid	511	101.0	10.3	70	132
375-85-9	Perfluoroheptanoic acid	652	101.2	10.0	71	131
355-46-4	Perfluorohexanesulfonic acid	639	98.5	10.5	67	130
307-24-4	Perfluorohexanoic acid	614	100.8	10.2	70	132
68259-12-1	Perfluorononanesulfonic acid	338	96.8	9.1	69	125
375-95-1	Perfluorononanoic acid	650	100.3	9.3	72	129
754-91-6	Perfluorooctanesulfonamide	377	102.2	11.5	67	137
1763-23-1	Perfluorooctanesulfonic acid	518	101.9	11.3	68	136
335-67-1	Perfluorooctanoic acid	663	101.1	10.4	69	133
2706-91-4	Perfluoropentanesulfonic acid	335	97.9	8.1	73	123
2706-90-3	Perfluoropentanoic acid	588	100.2	10.3	69	132
376-06-7	Perfluorotetradecanoic acid	551	101.3	10.5	69	133
72629-94-8	Perfluorotridecanoic acid	548	102.3	12.1	66	139
2058-94-8	Perfluoroundecanoic acid	587	99.9	12.0	64	136

Appendix E

Table B-15. Per- and Polyfluoroalkyl Substances (PFAS) Using Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS) With Isotope Dilution or Internal Standard Quantification in Matrices Other Than Drinking Water						**Pace SOP Section**
QC Check	**Minimum Frequency**	**Acceptance Criteria**	**Corrective Action**	**Flagging Criteria**	**Comments**	**SOP SECTION**
Aqueous Sample Preparation	Each sample and associated batch QC samples.	Solid Phase Extraction (SPE) must be used unless samples are known to contain high PFAS concentrations (e.g., Aqueous Film Forming Foam (AFFF) formulations). Inline SPE is acceptable. Entire sample plus bottle rinsate must be extracted using SPE. Known high PFAS concentration samples require serial dilution be performed in duplicate. Documented project approval is needed for samples prepared by serial dilution as opposed to SPE.	NA.	NA.	Samples with > 1% solids may require centrifugation prior to SPE extraction. Pre-screening of separate aliquots of aqueous samples is recommended.	7.0 Procedure & Summary, Scope & Application
Solid Sample Preparation	Each sample and associated batch QC samples.	Entire sample received by the laboratory must be homogenized prior to subsampling.	NA.	NA.	NA.	Soil SOP: 7.5.1
Biota Sample Preparation	Each sample and associated batch QC samples.	Sample prepared as defined by the project (e.g., whole fish versus filleted fish).	NA.	NA.	NA.	N/A
QC Check	**Minimum Frequency**	**Acceptance Criteria**	**Corrective Action**	**Flagging Criteria**	**Comments**	**SOP SECTION**

AFFF and AFFF Mixture Samples Preparation	Each sample and associated batch QC samples.	Each field sample must be prepared in duplicate (equivalent to matrix duplicate). Serial dilutions must be performed to achieve the lowest LOQ possible for each analyte.	NA.	NA.	Adsorption onto bottle is negligible compared to sample concentration so subsampling is allowed. Multiple dilutions will most likely have to be reported in order to achieve the lowest LOQ possible for each analyte.	N/A
Sample Cleanup Procedure	Each sample and associated batch QC samples. Not applicable to AFFF and AFFF Mixture Samples.	ENVI-Carb™ or equivalent must be used on each sample and batch QC sample.	NA.	Flagging is not appropriate.	Cleanup should reduce bias from matrix interferences.	Soil:7.5.10
Mass Calibration	Instrument must have a valid mass calibration prior to any sample analysis. Mass calibration is verified after each mass calibration, prior to initial calibration (ICAL).	Calibrate the mass scale of the MS with calibration compounds and procedures described by the manufacturer. Mass calibration range must bracket the ion masses of interest. The most recent mass calibration must be used for every acquisition in an analytical run. Mass calibration must be verified to be ±0.5 amu of the true value, by acquiring a full scan continuum mass spectrum of a PFAS stock standard.	If the mass calibration fails, then recalibrate. If it fails again, consult manufacture r instructions on corrective maintenance .	Flagging is not appropriate.	Problem must be corrected. No samples may be analyzed under a failing mass calibration. The mass calibration is updated on an as-needed basis (e.g., QC failures, ion masses fall outside of the ±0.5 amu of the true value, major instrument maintenance is performed, or the instrument is moved).	7.3.2
QC Check	**Minimum Frequency**	**Acceptance Criteria**	**Corrective Action**	**Flagging Criteria**	**Comments**	**SOP SECTION**
Mass Spectral Acquisition Rate	Each analyte, Extracted Internal Standard (EIS) Analyte.	A minimum of 10 spectra scans are acquired across each chromatographic peak.	NA.	Flagging is not appropriate.	NA.	7.3.1
Calibration, Calibration Verification, and Spiking Standards	All analytes.	Standards containing both branched and linear isomers must be used when	NA.	Flagging is not appropriate.	Standards containing both branched and linear isomers are to be used during method validation and when reestablishing	7.6.2

		commercially available. PFAS method analytes may consist of both branched and linear isomers, but quantitative standards that contain the linear and branched isomers do not exist for all method analytes. For PFAS that do not have a quantitative branched and linear standard, identify the branched isomers by analyzing a qualitative standard that includes both linear and branched isomers and determine retention times, transitions and transition ion ratios. Quantitate samples by integrating the total response (i.e., accounting for peaks that are identified as linear and branched isomers) and relying on the initial calibration that uses the linear isomer quantitative standard.			retention times, to ensure the total response is quantitated for that analyte. Technical grade standards cannot be used for quantitative analysis.	
QC Check	**Minimum Frequency**	**Acceptance Criteria**	**Corrective Action**	**Flagging Criteria**	**Comments**	**SOP SECTION**
Sample PFAS Identification	All analytes detected in a sample.	The chemical derivation of the ion transitions must be documented. A minimum of two ion transitions (Precursor → quant	NA.	PFAS identified with Ion ratios that fail acceptance criteria must be flagged. Any	For example: Ion Ratio = (quant ion abundance/ confirm ion abundance) Calculate the average ratio (A) and standard deviation (SD) using the ICAL standards. An	7.6.6

		ion and precursor → confirmation ion) and the ion transitions ratio per analyte are required for confirmation. Exception is made for analytes where two transitions do not exist (PFBA and PFPeA). Documentation of the primary and confirmation transitions and the ion ratio is required. In-house acceptance criteria for evaluation of ion ratios must be used and must not exceed 50- 150%. Signal to Noise Ratio (S/N) must be ≥ 10 for all ions used for quantification and must be ≥ 3 for all ions used for confirmation. Quant ion and confirmation ion must be present and must maximize simultaneously (±2 seconds).		quantitation ion peak that does not meet the maximization criteria shall be included in the summed integration and the resulting data flagged as "estimated, biased high".	acceptance range of ratio could be within A ±3SD for confirmation of detection.	
QC Check	**Minimum Frequency**	**Acceptance Criteria**	**Corrective Action**	**Flagging Criteria**	**Comments**	**SOP SECTION**
Ion Transitions (Precursor-> Product)	Every field sample, standard, blank, and QC sample.	In order to avoid biasing results high due to known interferences for some transitions, the following transitions must be used for the quantification of the following analytes: PFOA: 413 → 369 PFOS: 499 → 80 PFHxS: 399 → 80 PFBS: 299 → 80 4:2 FTS: 327 → 307	NA.	Flagging is not appropriate	NA.	Appendix A

		6:2 FTS: 427 → 407 8:2 FTS: 527 → 507 NEtFOSAA: 584 → 419 NMeFOSAA: 570 → 419 If these transitions are not used, the reason must be technically justified and documented (e.g., alternate transition was used due to observed interferences).				
QC Check	**Minimum Frequency**	**Acceptance Criteria**	**Corrective Action**	**Flagging Criteria**	**Comments**	**SOP SECTION**
Initial Calibration (ICAL)	At instrument set-up and after ICV or CCV failure, prior to sample analysis.	The isotopically labeled analog of an analyte (Extracted Internal Standard Analyte) must be used for quantitation if commercially available (Isotope Dilution Quantitation). Commercial PFAS standards available as salts are acceptable providing the measured mass is corrected to the neutral acid concentration. Results shall be reported as the neutral acid with appropriate CAS number. If a labeled analog is not commercially available, the Extracted Internal Standard Analyte with the closest retention time or chemical similarity to the analyte must be used for quantitation. (Internal Standard	Correct problem, then repeat ICAL.	Flagging is not appropriate.	No samples shall be analyzed until ICAL has passed. External Calibration is not allowed for any analyte. Calibration can be linear (minimum of 5 standards) or quadratic (minimum of 6 standards); weighting is allowed.	7.6.4 & 7.3.5

		Quantitation) Analytes must be within 70-130% of their true value for each calibration standard. *(continued next page)*				
QC Check	**Minimum Frequency**	**Acceptance Criteria**	**Corrective Action**	**Flagging Criteria**	**Comments**	**SOP SECTION**
Initial Calibration (ICAL) *(Continued)*		ICAL must meet one of the two options below: Option 1: The RSD of the RFs for all analytes must be ≤ 20%. Option 2: Linear or non- linear calibrations must have $r^2 \geq 0.99$ for each analyte.				7.3.6
Retention Time window position establishment	Once per ICAL and at the beginning of the analytical sequence.	Position shall be set using the midpoint standard of the ICAL curve when ICAL is performed. On days when ICAL is not performed, the initial CCV is used.	NA.	NA.	Calculated for each analyte and EIS.	7.6.5
Retention Time (RT) window width	Every field sample, standard, blank, and QC sample.	RT of each analyte and EIS analyte must fall within 0.4 minutes of the predicted retention times from the daily calibration verification or, on days when ICAL is performed, from the midpoint standard of the ICAL. Analytes must elute within 0.1 minutes of the associated EIS. This criterion applies only to analyte and labeled analog pairs.	Correct problem and reanalyze samples.	NA.	Calculated for each analyte and EIS.	7.6.5
QC Check	**Minimum Frequency**	**Acceptance**	**Corrective**	**Flagging**	**Comments**	**SOP SECTION**

		Criteria	**Action**	**Criteria**		
Instrument Sensitivity Check (ISC)	Prior to analysis and at least once every 12 hours.	Analyte concentrations must be at LOQ; concentrations must be within ±30% of their true values.	Correct problem, rerun ISC. If problem persists, repeat ICAL.	Flagging is not appropriate.	No samples shall be analyzed until ISC has met acceptance criteria. ISC can serve as the initial daily CCV.	7.4.1
Initial Calibration Verification (ICV)	Once after each ICAL, analysis of a second source standard prior to sample analysis.	Analyte concentrations must be within ±30% of their true value.	Correct problem, rerun ICV. If problem persists, repeat ICAL.	Flagging is not appropriate.	No samples shall be analyzed until calibration has been verified.	7.3.7
Continuing Calibration Verification (CCV)	Prior to sample analysis, after every 10 field samples, and at the end of the analytical sequence.	Concentration of analytes must range from the LOQ to the mid-level calibration concentration. Analyte concentrations must be within ±30% of their true value.	Immediately analyze two additional consecutive CCVs. If both pass, samples may be reported without reanalysis. If either fails, or if two consecutive CCVs cannot be run, perform corrective action(s) and repeat CCV and all associated samples since last successful CCV. Alternately, recalibrate if necessary; then reanalyze all associated samples since the last acceptable CCV.	If reanalysis cannot be performed, data must be qualified and explained in the Case Narrative. Apply Q-flag to all results for the specific analyte(s) in all samples since the last acceptable calibration verification.	Results may not be reported without valid CCVs. Instrument Sensitivity Check (ISC) can serve as a bracketing CCV.	7.4.2, 7.4.5
QC Check	**Minimum Frequency**	**Acceptance Criteria**	**Corrective Action**	**Flagging Criteria**	**Comments**	**SOP SECTION**

Instrument Blanks	Immediately following the highest standard analyzed and daily prior to sample analysis.	Concentration of each analyte must be ≤ ½ the LOQ. Instrument Blank must contain EIS to enable quantitation of contamination.	If acceptance criteria are not met after the highest calibration standard, calibration must be performed using a lower concentration for the highest standard until acceptance criteria is met. If sample concentrations exceed the highest allowed standard and the sample(s) following exceed this acceptance criteria (>1/2 LOQ), they must be reanalyzed.	Flagging is only appropriate in cases when the sample cannot be reanalyzed and when there is no more sample left.	No samples shall be analyzed until instrument blank has met acceptance criteria. Note: Successful analysis following the highest standard analyzed determines the highest concentration that carryover does not occur. When the highest standard analyzed is not part of the calibration curve, it cannot be used to extend out the calibration range, it is used only to document a higher concentration at which carryover still does not occur.	7.4.3
QC Check	**Minimum Frequency**	**Acceptance Criteria**	**Corrective Action**	**Flagging Criteria**	**Comments**	**SOP SECTION**
Extracted Internal Standard (EIS) Analytes	Every field sample, standard, blank, and QC sample.	Added to solid sample prior to extraction. Added to aqueous samples, into the original container, prior to extraction. For aqueous samples prepared by serial dilution instead of SPE, added to final dilution of samples prior to analysis. Extracted Internal	Correct problem. If required, re-extract and reanalyze associated field and QC samples. If recoveries are acceptable for QC samples, but not field samples, the	Apply Q-flag and discuss in the Case Narrative only if reanalysis confirms failures in exactly the same manner.	Failing analytes shall be thoroughly documented in the Case Narrative. EIS should be 96% (or greater) purity. When the impurity consists of the unlabeled analyte, the EIS can result in a background artifact in every sample, standard and blank, if the EIS is fortified at excessive concentrations.	8.2.7, 8.2.10

		Standard Analyte recoveries must be within 50% to 150% of ICAL midpoint standard area or area measured in the initial CCV on days when an ICAL is not performed.	field samples must be re-extracted and analyzed (greater dilution may be needed). Samples may be re-extracted and analyzed outside of hold times, as necessary for corrective action associated with QC failure.			
Method Blank (MB)	One per preparatory batch.	No analytes detected >½ LOQ or > 1/10th the amount measured in any sample or 1/10th the regulatory limit, whichever is greater.	Correct problem. If required, re-extract and reanalyze MB and all QC samples and field samples processed with the contaminated blank. Samples may be re-extracted and analyzed outside of hold times, as necessary for corrective action associated with QC failure. Examine the project-	If reanalysis cannot be performed, data must be qualified and explained in the Case Narrative. Apply B-flag to all results for the specific analyte(s) in all samples in the associated preparatory batch.	Results may not be reported without a valid MB. Flagging is only appropriate in cases where the samples cannot be reanalyzed.	8.2.1

QC Check	Minimum Frequency	Acceptance Criteria	Corrective Action	Flagging Criteria	Comments	SOP SECTION
			specific requirements. Contact the client as to additional measures to be taken.			
Laboratory Control Sample (LCS)	One per preparatory batch.	Blank spiked with all analytes at a concentration ≥ LOQ and ≤ the mid-level calibration concentration. A laboratory must use the DoD/DOE QSM Appendix C Limits for batch control if project limits are not specified. If the analyte(s) are not listed, use in-house LCS limits if project limits are not specified.	Correct problem, then re-extract and reanalyze the LCS and all samples in the associated preparatory batch for failed analytes if sufficient sample material is available. Samples may be re-extracted and analyzed outside of hold times, as necessary for corrective action associated with QC failure. Examine the project-specific requirements. Contact the client as to additional measures to be taken.	If reanalysis cannot be performed, data must be qualified and explained in the Case Narrative. Apply Q-flag to specific analyte(s) in all samples in the associated preparatory batch.	Results may not be reported without a valid LCS. Flagging is only appropriate in cases where the samples cannot be reanalyzed.	8.2.2
Matrix Spike (MS)	One per preparatory batch. Not required for aqueous samples	Sample spiked with all analytes at a concentration ≥ LOQ	Examine the project-specific	For the specific analyte(s) in the parent	For matrix evaluation only. If MS results are outside the limits, the	8.2.3

	prepared by serial dilution instead of SPE.	and ≤ the mid-level calibration concentration. A laboratory must use the DoD/DOE QSM Appendix C Limits for batch control if project limits are not specified. If the analyte(s) are not listed, use in-house LCS limits if project limits are not specified.	requirements. Contact the client as to additional measures to be taken.	sample, apply J-flag if acceptance criteria are not met and explain in the Case Narrative.	data shall be evaluated to determine the source(s) of difference (i.e., matrix effect or analytical error).	
QC Check	**Minimum Frequency**	**Acceptance Criteria**	**Corrective Action**	**Flagging Criteria**	**Comments**	**SOP SECTION**
Matrix Spike Duplicate (MSD) or Matrix Duplicate (MD)	For MSD: One per preparatory batch. For MD: Each aqueous sample prepared by serial dilution instead of SPE.	For MSD: Sample spiked with all analytes at a concentration ≥ LOQ and ≤ the mid-level calibration concentration. A laboratory must use the DoD/DOE QSM Appendix C Limits for batch control if project limits are not specified. If the analyte(s) are not listed, use in-house LCS limits if project limits are not specified. RPD ≤ 30% (between MS and MSD or sample and MD).	Examine the project-specific requirements. Contact the client as to additional measures to be taken.	For the specific analyte(s) in the parent sample, apply J-flag if acceptance criteria are not met and explain in the Case Narrative.	The data shall be evaluated to determine the source of difference. For Sample/MD: RPD criteria only apply to analytes whose concentration in the sample is ≥ LOQ. The MD is a second aliquot of the field sample that has been prepared by serial dilution.	8.2.4

Post Spike Sample	Only applies to aqueous samples prepared by serial dilution instead of SPE that have reported value of < LOQ for analyte(s).	Spike all analytes reported as < LOQ into the dilution that the result for that analyte is reported from. The spike must be at the LOQ concentration to be reported for this sample as < LOQ. When analyte concentrations are calculated as < LOQ, the post spike for that analyte must recover within 70-130% of its true value.	When analyte concentrations are calculated as < LOQ, and the spike recovery does not meet the acceptance criteria, the sample, sample duplicate, and post spike sample must be reanalyzed at consecutively higher dilutions until the criteria is met.	Flagging is not appropriate.	When analyte concentrations are calculated as < LOQ, results may not be reported without acceptable post spike recoveries.	N/A

Appendix F

PFAS Aqueous sample centrifugation protocol (for DoD work in GW/SW/NPW)

Preliminary considerations:
The DoD QSM5.3, Table B-15, states that "[aqueous] samples with >1% solids may require centrifugation prior to SPE extraction." Samples should only be centrifuged when the suspended solids content appears visually high enough, by chemist inspection, that it would cause the SPE cartridge to clog. It is expected that the solid phase remains in the container when rinsing the container walls with the polar elution solvent. Rinsing the container walls would therefore also include rinsing of the solids. If removing the solvent disrupts the solid phase significantly, the container can be centrifuged again before removing the solvent for use during the elution step of the SPE procedure. When the sample has significant solids, the laboratory should account for the weight or volume displaced by the solids in the initial sample volume determination. One or more rinses of polar solvent can be used for quantitative transfers. Rinse the sample bottle and cap with elution solvent, pour the solvent from each rinse through the SPE cartridge during the elution step, and collect the filtrate for analysis. Bring to a quantitative final volume with the final injection solvent and vortex well. Whether or not an individual sample will require centrifugation for proper preparation will be determined and documented by the preparation analyst.

Procedure:
1. Inspect the sample and consider the necessity of centrifuging. Consider any visible indications of particulate matter including settled solids collected on the bottom of the container, cloudiness and/or dark color of the sample, suspended solids within the sample, increased viscosity, etc. If uncertain, seek a second opinion from another analyst, supervisor, or operations director.

2. If, in the judgement of the preparation analyst, a sample requires centrifugation the analyst will contemporaneously make a note on the prep batch log indicating this fact.

3. Spike samples requiring centrifugation in the same manner and with the same standard volume as samples which will not be centrifuged.

4. Label a 500mL polypropylene centrifuge bottle with the sample ID for each sample that will be centrifuged. Set them in an appropriate rack with the caps removed.

5. Vigorously shake the spiked sample and then quickly pour into the labeled centrifuge bottle. Try to ensure that the original sample bottle is devoid of any solid material. Be careful to avoid spilling sample during the transfer process. Tightly cap each centrifuge bottle after transfers are complete.

6. Transfer capped centrifuge bottles to centrifuge, ensuring that the centrifuge carousel is symmetrically balanced. Close top and centrifuge at 2200 RPM for 20 minutes.

7. Remove centrifuge bottles and decant the centrifuged liquid off of the condensed solids and back into the original sample bottle. Try to avoid transferring any of the condensed solids from the centrifuge bottle back to the original sample bottle, while maximizing the amount of liquid decanted off of the solid portion. Take weight for initial volume of sample without solids in original container.

8. Extract the decanted sample as normal alongside un- centrifuged samples, up to the bottle rinse and elution steps.

9. When the SPE cartridges have been dried, rinse the original sample bottle as normal. Additionally, add 4mL of Methanol (MeOH) to each centrifuge bottle to rinse the inside of the centrifuge bottles as well as the cap. If the condensed solids become re-suspended while rinsing the centrifuge tubes, re-centrifugation may be required. Using a transfer pipet or mechanical pipet, transfer the MeOH rinse from the centrifuge bottle into the SPE cartridge and elute with the original sample bottle rinse into a 15mL conical centrifuge tube.

10. Add an additional volume of MeOH to the elution of all batch QC samples (MB/LCS/LCSD) to match the volume used for elution for any centrifuged sample in the prep batch. Typically, this will mean that 4mL of clean MeOH will be added directly to the SPE reservoir and eluted with the normal bottle rinses.

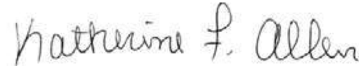
11. Concentrate samples down to ~0.5 mL and reconstitute as outlined in sample extraction procedure.

12. Add a case narrative onto the work order indicating which samples had to be centrifuged.

Determination of Selected Per and Polyfluorinated Alkyl Substances (PFAS) Soil/Solid Samples Isotope Dilution by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)

Approved:

Tod Kopyscinski
Laboratory Director



Katherine Allen
QA Officer

Revision Number: 7

DRAFT

Change Record

Revision	Date	Responsible Person	Description of Change
0	2/16/2018	Brianna McLaughlin Kelly Fellows	Original
1	11/13/2018	Katherine Allen/KAF	Updates from Sept 2018 NH audit: Sec 3.0 (addition pres. requirements added), Sec 7.2.1 (deleted ref. to section 7.7.2), Sec 7.2.4 (deleted ref. to sections 7.2.5.5 and 7.7.2), Sec 7.2.5 (surrogate should changed to surrogate must), Sec 7.4.2 (deletion of blank spike duplicate), Sec 7.4.3 (addition of DI water rinse after methanol), Sec 7.4.4 (changed to vortex for 1 min), Sec. 7.5.1.1 (blank subtraction replaces blank extraction), Sec 7.6.3 (deleted to prove instrument is contamination free), Sec 7.6.6 (deleted ref to App C), and deleted Sec 8.2.12 (deleted response QC check). Sec 3.1(added temperature note), Sec 3.2(added 'of collection'), Sec 3.3(Added Appendix C), added 'Appendix C' Sec 7.2.8 added section on isotope dilution technique.
2	03/12/2020	Brianna McLaughlin James Constantino	Update to make entire new procedure: Entire SOP edited.
3	05/18/2020	Brianna Henriquez	Updates to procedure: Section 1.0 – added CAS #'s to chart, section 7.5: includes clarification on how to use Envi-carb cleanup cartridges. Edited section 8.2.9 to reflect correct limits from previous sections.
4	07/15/2020	Brianna Henriquez	Updates: Removal of reagent (Section 5.0) Change to concentration of CCV standards (Section 7.2.6.4). Multiple changes to extraction procedure 7.5.
5	0817/2020	Brianna Henriquez	Updates from July 2020 NH audit: Edit to section 7.3.7 and 7.3.8 to updated procedure, Section 7.3.5 change should to shall. Edit to Section 8.2.1 and 7.4.3 typo changed all > to <, added Sec 8.2.11 for IDOC and Section 8.2.12 for CDOC.
6	02/26/2021	Katherine Allen	Update for MA DEP approval: Sec 3.5, 8.2.13 and Appendix A (field blank is optional for client), Sec 13.13 (replace EPA 537 with EPA 533), and addition of 13.19 (addition of ASTM D7968-17 ref).
7	06/30/2021	Brianna Henriquez	Updates to procedure: Overall: References to Con-Test changed to Pace. Section 1.1: Updated summary to reflect procedure changes, changed initial WT and FV, added 6495 instrument. Analyte RLs changed. Section 4: Removed pH paper from reagents (in supplies), removed cartridge and filter disc. Section 5: 20MM mobile phase changed to 5MM, 70:30 changed to 96:4, mobile phase expiration changed. Section 7: Removed section on separate internal standard. Section 7.1: Sampling instructions changed. Section 7.2.6: Standards stored at room temperature. Added 24PAR and certain individual analyte stocks for separate spike sourcing. Standard prep and calibration tables were changed. CCVs changed to only be run at the mid-level (level 4) after an opening low-level. Section 7.3: Updated calibration method, technique, and requirements (removal of IS/surrogate now functioning as both surrogate and IS). Section 7.3.4: Branched compound summation added. Section 7.4.1: Requirement added of IBL and low-level CCV every 12 hours. Section 7.5: Extraction method changed completely after the base-addition step. Removed syringe filtering, changed envi-carb instructions, added blow down step. Removed centrifuge step. Surrogate and spike amounts changed and are now always the same. Amount of acid changed. Section 7.5.3: replaced Dup with MSD, Section 7.5.10: changed final to 5mL, Section 7.6.2.1: TPFOA branched check section added. Section 7.7.2: Instrument method conditions changed. Cell accelerator voltage moved to this section. Section 8.2.3: MS/MSD spike levels held constant. 8.2.6: Updated how isotope dilution technique is calculated. Appendices: Removed Appendices A and C, changing other appendices letters. Appendix A: Now has transitions and conditions for both instruments, and qualifier transitions.

Distribution/Training List

See Employee Training Record File for signed training statements for trained users.

1.0 SUMMARY, SCOPE, AND APPLICATION

1.1 This method is used to analyze soil and product/solid samples for selected per- and polyfluorinated alkyl substances (PFAS). A sample of 5.5 grams of soil/solid sample is fortified with spikes and surrogates. A 2 mL aliquot of the sample is removed and pulled through an Envi-Carb cartridge and then concentrated to a final volume of 5mL. All samples are analyzed using an Agilent 6470 or 6495 Triple Quad LC/MS (LC/MS/MS) system. Target analytes are identified by comparing mass spectra and retention times to reference spectra and retention times of calibration standards. Analytes are quantitated using the isotope dilution technique explained in the initial calibration section. The following compounds can be identified by this method.

Analyte	Acronym	Standard RL (ug/kg)	Cas Number
11- 11-chloroeicosafluoro-3-oxanone-1-sulfonic acid	**11Cl-PF3OUdS**	0.5	763051-92-9
9 -Chlorohexadecafluoro-3-oxapentane-1-sulfonic acid	**9Cl-PF3ONS**	0.5	756426-58-1
4,8-Dioxa-3H-perfluorononanoic acid	**ADONA**	0.5	919005-14-4
Hexafluoropropylene oxide dimer acid	**HFPO-DA**	0.5	13252-13-6
Nonafluoro-3,6-dioxaheptanoic acid	**NFDHA**	0.5	151772-58-6
Perfluorobutanoic acid	**PFBA**	0.5	375-22-4
Perfluorobutanesulfonic acid	**PFBS**	0.5	375-73-5
8:2 Fluorotelomer sulfonic acid	**8:2FTS**	0.5	39108-34-4
Perfluorodecanoic acid	**PFDA**	0.5	335-76-2
Perfluorododecanoic acid	**PFDoA**	0.5	307-55-1
Perfluoro(2-ethoxyethane)sulfonic acid	**PFEESA**	0.5	113507-82-7
Perfluoroheptanesulfonic acid	**PFHpS**	0.5	375-92-8
Perfluoroheptanoic acid	**PFHpA**	0.5	375-85-9
4:2 Fluorotelomer sulfonic acid	**4:2FTS**	0.5	757124-72-4
Perfluorohexanesulfonic acid	**PFHxS**	0.5	355-46-4
Perfluorohexanoic acid	**PFHxA**	0.5	307-24-4
Perfluoro-3-methoxypropanoic acid	**PFMPA**	0.5	377-73-1
Perfluoro-4-methoxybutanoic acid	**PFMBA**	0.5	863090-89-5
Perfluorononanoic acid	**PFNA**	0.5	375-95-1
6:2 Fluorotelomer sulfonic acid	**6:2FTS**	0.5	27619-97-2
Perfluorooctanesulfonic acid	**PFOS**	0.5	1763-23-1
Perfluorooctanoic acid	**PFOA**	0.5	335-67-1
Perfluoropentanoic acid	**PFPeA**	0.5	2706-90-3
Perfluoropentanesulfonic acid	**PFPeS**	0.5	2706-91-4

Perfluoroundecanoic acid	**PFUnA**	0.5	2058-94-8
N-ethyl perfluorooctanesulfonamidoacetic acid	**NEtFOSAA**	0.5	2991-50-6
N-methyl perfluorooctanesulfonamidoacetic acid	**NMeFOSAA**	0.5	2355-31-9
Perfluoro-1-butanesulfonamide	**FBSA**	0.5	30334-69-1
Perfluoro-1-hexanesulfonamide	**FHxSA**	0.5	41997-13-1
Perfluorotetradecanoic acid	**PFTA**	0.5	376-06-7
Perfluorotridecanoic acid	**PFTrDA**	0.5	72629-94-8
Perfluorooctanesulfonamide	**FOSA**	0.5	754-91-6
Perfluorononanesulfonic acid	**PFNS**	0.5	68259-12-1
Perfluorodecanesulfonic acid	**PFDS**	0.5	335-77-3

2.0 INTERFERENCES

2.1 Standards and samples should not come into contact with glass other than standards purchased in glass ampules. PFAS commonly adsorb to the surface and could result in recovery discrepancies.

2.2 Matrix interferences may be caused by co-extracted contaminants present in the sample.

2.3 Method interferences may be caused by contaminants in solvents, reagents, and other sample processing hardware.

2.3.1 Other common lab supplies that are associated with PFAAs and should be avoided where possible: aluminum foil, permanent marker, and PTFE.

2.3.1.1 To eliminate any residual PTFE from the Agilent LC, an inline filter column has been installed to reduce any background contamination prior to sample introduction into the system. See Equipment and Supplies Section 4.0.

2.3.2 Organic contaminants can pose a threat of interference due to the high quantities of de-chlorinating agent added to samples.

2.3.3 Contamination levels should be monitored and all blanks should be free from interferences (less than 1/2 the MRL) in all Laboratory Reagent Blanks (LRB).

2.3.4 Blank subtraction is not permitted in this method.

2.3.5 There is a possibility of matrix effects due to co-extracted organic material. When high levels of TOC are present, this can affect the ionization of 4:2 FTS considerably.

3.0 SAMPLE PRESERVATION/STORAGE/HOLDING TIME

3.1 Samples should be collected in a wide mouth polypropylene bottle fitted with a polypropylene screw cap capable of holding at least 50 grams of soil.

3.2 Samples cannot exceed 10°C during the first 48 hours following sample collection. Samples must be received at or below 10°C OR have ice remaining in the cooler.

3.3 Prior to extraction, samples must be stored at or below 6°C and cannot be frozen.

3.4 Samples collected must be extracted within 28 days. Extracted samples must be run within 28 days of extraction and remain stored at room temperature.

3.5 Clients may choose to send a field blank if they want wish to as it is optional. Field reagent blanks only need to be run and reported if there are analytes at or above the MRL in any associated field samples.

4.0 EQUIPMENT & SUPPLIES

4.1 Triple Quad LC/MS System

4.2 Inline Delay column

4.2.1 Agilent Zorbax Eclipse Plus C18 3.0x50mm 1.8-micron P.N. PFCDELAY or equivalent

4.3 Analytical Column

4.3.1 Agilent Zorbax Eclipse Plus C18 3.0x50mm 1.8-micron P.N. 959757-02 or equivalent

4.4 Auto-pipettors: 0-10uL, 10-100uL, 100-1000uL

4.5 Polypropylene pipet tip: 0-10uL, 10-100uL, 100-1000uL

4.6 Polypropylene transfer pipets

4.7 Polypropylene graduated cylinder: 10mL, 50mL, 100mL, 1000mL

4.8 Vials: 2ml polypropylene vials or 2mL glass vials with polypropylene inserts

4.9 Caps: 11mm polypropylene snap caps

4.10 Sample containers: 250ml wide mouth polypropylene containers

4.11 Polypropylene centrifuge tubes

4.12 Hydrion pH paper strips: 1.0-11.0 B

4.13 Balance: Analytical, capable of accurately weighing 0.0001g

4.14 N-Evap concentrator system

4.15 Polypropylene inserts

4.16 Vortex

5.0 REAGENTS & STANDARDS

5.1 Reagent Water: interferent free

5.2 Methanol: LC/MS Grade

5.3 Nitrogen: Ultra high purity

5.4 Ammonium Acetate: LC/MS Grade

5.5 Stock Standard Solutions: Purchased as either certified solutions or neat standards.

5.6 Surrogate and ESI-L Low concentration tuning mix purchased as certified solutions

5.7 5 mM Ammonium Acetate reagent water: Prepared by adding 1 mL of 5 M ammonium acetate to a final volume of 1000 mLs DI water.

5.8 Ammonium Hydroxide

5.9 Acetic acid, glacial

5.10 Agilent ESI-L Low Concentration Tuning Mix

5.11 96:4 Methanol:DI Water- Made fresh every 2 days

5.12 Envi-Carb cartridge or equivalent

6.0 SAFETY

See Material Safety Data Sheets (MSDS) and Pace Chemical Hygiene Plan.

7.0 PROCEDURE

7.1 Sampling

7.1.1 Samples are to be collected in lab-provided plastic containers. Guidance for sampling is obtained through Pace corporate website or preferably local project authorities/municipalities.

7.2 Surrogate/SpikePreparation

7.2.1 All standards must be documented in Element and have Certificate of Analysis forms attached electronically. All information should be documented and each standard should be given an Element Standard ID#.

7.2.2 Standards may be received in purchased glass ampoules but any transfer or dilution must be stored in polypropylene vials with Non-PTFE caps.

7.2.3 All standards purchased from Wellington come pre-treated with sodium hydroxide for compound stability. If making standards from solid, standards must be stored under basic condition to prevent esterification of fluorinated carboxylic acids. See calculation 1 in section 12.1.

7.2.4 PFAS Surrogate Preparation

7.2.4.1 All purchased surrogate stock standards are to be stored until expiration date provided by the manufacturer at 4°C.

Surrogate Standards

Compound	Abbreviation	PDS, ng/mL
Perfluoro-n-[1,2,3,4-13C4]butanoic acid	MPFBA	1000
Perfluoro-n-[1,2,3,4,5-13C5]pentanoic acid	M5PFPeA	1000
Sodium perfluoro-1-[2,3,4-13C3]butanesulfonate	M3PFBS	929
Sodium 1H,1H,2H,2H-perfluoro-1-[1,2-13C2]hexane sulfonate	M2-4:2FTS	935
Perfluoro-n-[1,2,3,4,6-13C5]hexanoic acid	M5PFHxA	1000
Perfluoro-n-[1,2,3,4-13C4]heptanoic acid	M4PFHpA	1000
Sodium perfluoro-1-[1,2,3-13C3]hexanesulfonate	M3PFHxS	946
Sodium 1H,1H,2H,2H-perfluoro-1-[1,2-13C2]-octane sulfonate	M2-6:2FTS	949
Perfluoro-n-[13C8]octanoic acid	M8PFOA	1000
Perfluoro-n-[13C9]nonanoic acid	M9PFNA	1000
Sodium perfluoro-[13C8]octanesulfonate	M8PFOS	957
Sodium 1H,1H,2H,2H-perfluoro-1-[1,2-13C2]-decane sulfonate	M2-8:2FTS	958
Perfluoro-n-[1,2,3,4,5,6-13C6]decanoic acid	M6PFDA	1000
Perfluoro-n-[1,2,3,4,5,6,7-13C7]undecanoic acid	M7PFUnA	1000
2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy-13C3-propanoic acid	M3HFPO-DA	1000
Perfluoro-n-[1,2-13C2]dodecanoic acid	M2PFDoA	1000
Perfluoro-n-[12-13C2]tetradecanoic acid	M2PFTA	1000
Perfluoro-1-[13C8]octanesulfonamidoacetic acid	M8FOSA	1000
N-methyl-d3-perfluoro-1-octansulfonamidoacetic acid	d3-N-MeFOSAA	1000
N-ethyl-d5-perfluoro-1-octansulfonamidoacetic acid	d5-N-EtFOSAA	1000

Surrogate standards are ordered from Wellington Labs part PFAC-24ES

Compound	Abbreviation	PDS, ng/mL
2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy-13C3-propanoic acid	**M3HFPO-DA**	**1000**

Surrogate standard is ordered from Wellington Labs part M3HFPO-DA

7.2.6 Analyte Primary Dilution Standard Preparation

7.2.6.1 All purchased PFAA spike standard stock standards are to be stored until expiration date provided by manufacturer at 4°C.

7.2.6.2 Prepared and in use PFAA stock standard solutions should be stored at room temperature and be vortexed prior to usage. These standards will expire 2 months after preparation date or manufacturer's expiration date, whichever comes first.

Analyte Standards

Compound	Vendor	Concentration of Standard (ng/ mL)
PFAC-30PAR	Wellington	1000*
PFAC-24PAR	Wellington	2000*
PFEESA	Wellington	50000*
NFDHA	Wellington	50000
PFMPA	Wellington	50000
PFMBA	Wellington	50000
HPFO-DA	Wellington	50000
NaDONA	Wellington	50000*
9ClPF3ONS	Wellington	50000*
11ClPF3OUdS	Wellington	50000*
FBSA	Wellington	50000
FHxSA	Wellington	50000

*Individual analyte concentration may vary due to amount of anion present in solution. All calculations must use the anion concentration, not the salt concentration. See Calculation 2 in section 12.2.

7.2.6.3 PFEESA, NFDHA, PFMPA, PFMBA, HPFO-DA, NaDONA, 9ClPF3ONS, 11ClPF3OUdS, FBSA, and FHxSA are not included in the 24PAR mixture and are mixed into a separate "supplemental" 1000 ng/ mL stock. See below table for mixture.

7.2.6.4 The Supplemental Stock is used to make a 500ppb spike dilution with 24PAR so that a different lot is used for spiking. The calibration stock is made using 30PAR and directly adding PFEESA, NFDHA, PFMPA, and PFMBA, which are missing from that mixture. See below table for prep instructions:

Stock Dilution Prep Table

	Volume of Compound/Standard Mixtures (µL)	Volume of Methanol (µL)	Final Volume (µL)	Final Concentration (ng/mL)
Supplemental Stock	100µL – HPFO-DA 100µL – NaDONA 100µL – 9ClPF3ONS 100µL – 11ClPF3OUdS 100µL – PFEESA 100µL – PFMBA 100µL – PFMPA 100µL – NFDHA 100µL – FBSA 100µL – FHxSA	4000µL	5000µL	1000
500 ng/ mL **Spike**	1250µL – PFAC24PAR 2500µL – Supplemental Stock	1250µL	5000µL	500
100 ng/ mL **Spike**	250µL – PFAC24PAR 500µL – Supplemental Stock	4250µL	5000µL	100
100 ng/ mL **Cal Stock**	500µL – PFAC30PAR 10µL – PFEESA 10µL – PFMBA 10µL – PFMPA 10µL – NFDHA	4460µL	5000µL	100

7.2.6.5 The calibration is prepared as follows using the stock dilutions prepared above:

Calibration Table

Volume 100ppb Stock Standard (µL)	Volume 30PAR Stock Standard (µL)	Volume Supplemental Stock Standard (µL)	Volume of Surrogate Stock (µL)	Volume of M3HFPODA Surrogate Stock	Volume of 96:4 Methanol: Water (µL)	Final Volume (µL)	Final Concentration (ng/mL)
12.5	0	0	25	25	4937.5	5000	0.25*
25	0	0	25	25	4925	5000	0.5*
50	0	0	25	25	4900	5000	1.0*
125	0	0	25	25	4825	5000	2.5*
250	0	0	25	25	4700	5000	5.0*

0	50	50	25	25	4850	5000	10.0*
0	125	125	25	25	4700	5000	25.0*
0	250	250	25	25	4450	5000	50.0*

*Individual analyte concentration may vary due to the amount of anion present in solution. All calculations must use the anion concentration, not the salt concentration. See Calculation 2 in section 12.2

7.2.6.6 Continuing calibration verification (CCVs) standards are made at the mid-level, identically to the 4th calibration level above. The ICV/QCS is made similarly, just like the 5th calibration level above, except instead of calibration stock 100 ppb Spike is used as shown in the table below:

QCS/ICV Preparation Table

Standard Name	**Volume Standards**	**Volume of 96:4 Methanol:Water (µL)**	**Final Volume (µL)**	**Final Concentration (ng/mL)**
QCS/ICV	25µL- PFAC-24ES 25µL- M3HPFO-DA Surrogate Dilution 250µL- 100ppb Spike	4700µL	5000µL	5.0

7.3 Initial Calibration Criteria

7.3.1 All analytes must first be product ion optimized with the LC/MS/MS system using the MassHunter Optimizer program to determine optimal fragmentor and collision cell energy for applicable ions. This optimization should be done using a high-level standard for each analyte and using all of the LC parameters used in the analytical method. A level 4 standard must then be run to identify all retention time windows for all compounds of interest (See Section 7.7.2). A minimum of 10 spectra scans are acquired across each chromatographic peak. See Appendix A for further analyte MS/MS conditions.

7.3.2 Prior to initial calibrations, and when the instrument is having difficulty passing regular calibrations, a mass calibration will be performed using Agilent ESI-L Low Concentration Tuning Mix. The MassHunter program has an autotune feature that performs the mass calibration using this mix and verifies it in a report.

7.3.3 A calibration is to be run when continuing calibration checks or surrogates do not pass QC criteria. A calibration should also be performed when any hardware is changed or major instrument maintenance is performed.

7.3.4 The initial calibration must contain a minimum of five points the lowest point being at or below the MRL. A minimum of six points is required for a quadratic calibration. The total of the branched and linear isomers must be used for calibration for the following target analytes: PFOS, PFHxS, NEtFOSAA, NMeFOSAA.

7.3.5 The LC/MS/MS system is to be calibrated using the isotope dilution technique. Therefore, isotope dilution analogues are added at a constant concentration in all standards prior to injection. Either linear or quadratic regression can be used, but it must always be forced through zero and can be concentration weighted. Forcing through zero allows for more sensitivity to detect background contamination within the system. The calibration shall be done using the same LC conditions as the samples (See Section 7.7.2). Because the isotope dilution analogues are added in equal concentrations, calibrate for them using an average response factor. Not all analytes have an exact mass-labeled analogue, in which case the closest analogue is used (either by chemical properties or retention time). See Appendix B for the list of isotope analogues and corresponding target analytes.

7.3.5.1 The isotope dilution technique utilizes extracted compounds to serve as a traditional internal standard. In this case, the extracted analogues must pass criteria listed in section 8.2.6. The analogue is then used as the internal standard compound for associated target analytes.

7.3.6 Calibration levels for linear or non-linear analyte targets must have a $r^2 \geq 0.99$ for each analyte and the recovery for each analyte must be within 70-130% of the true value. Surrogate and internal standards must have an RSD of the RFs for all analytes of ≤20%.

7.3.7 A quality control standard (QCS) will serve as an initial calibration verification (ICV) and be run following initial calibration and all subsequent calibrations. The ICV shall be prepared from a separate lot of stock standard mix. This sample must be run following a calibration or quarterly, whichever comes first. The accepted values for the ICV are 70-130% of the true value for each analyte.

7.3.8 After any changes to calibration instrument parameters, an initial demonstration of capability (IDOC) for the procedure and instrumentation should be performed. See Appendix C.

7.4 Continuing Calibration

7.4.1 An instrument blank (IBL) and a low-level instrument sensitivity check (ISC) must be run before any other injections and once every 12 hours. The results must be between 70-130% of the true value for all analytes. The IBL should have no hits greater than ½ the MRL. The ISC can serve as the opening CCV for the day.

7.4.2 Prior to samples analysis, a low-level continuing calibration verification (CCV) must be run. After every ten field samples a subsequent CCV must be at the same level as the Level 4 calibration point. A closing CCV must also be run at the end of each analysis. The requirements for the CCVs are 70-130% of the true value for method analytes.

7.4.3 An instrument blank is required to be run following analysis of the highest-level standard analyzed (after a calibration in this case). One is also required daily prior to sample analysis. All analytes must be <½ the MRL in order to pass.

7.4.4 All isotope dilution analogues (surrogates) must have a recovery between 50-150%

7.4.5 In the case of CCV failure, two consecutive CCVs should be immediately run. If these pass, analysis can continue. Otherwise, a calibration or tuning and re-analysis of affected samples is required.

7.4.6 A checktune will be run as needed to verify MS operating criteria. This is run through the MassHunter program using Agilent ESI-L Low Concentration Tuning Mix. If criteria are out of spec, the parameters set forth in the Agilent 6400 Series Triple Quadrupole LC/MS System Quick Start Guide must be followed to adjust values. If re-run of checktune does not pass, an autotune must be run and the instrument must be recalibrated.

7.5 Sample Extraction Procedure

7.5.1 Every sample must be fully homogenized prior to weighing out for extraction.

7.5.2 Weigh out 5.5-6.0 grams of soil/solid sample into a 15mL polypropylene conical tube.

7.5.3 For every 20 field samples, a blank and a blank spike must be extracted using sand. Ideally, if adequate sample volume is available, a matrix spike and matrix spike duplicate should be included in every batch.

7.5.4 All polypropylene equipment including graduated cylinders and sample transfer lines/reservoirs should be washed prior to using methanol.

7.5.5 Add 25µL of M3HFPO-DA Surrogate Stock and 25uL of PFAC24-ES to each sample (and 25uL 500 ppb spike to the BS and MS samples as appropriate), recap and vortex for 1 minute to evenly distribute.

7.5.6 Add 10mL 96:4 reagent methanol:water to the sample.

7.5.7 Add 20µL of Ammonium Hydroxide to raise the samples' pH to 9-10 and vortex for 2 minutes.

7.5.8 Prime Envi-Carb cartridge by rinsing twice with straight methanol (approx. 10 mL methanol). Transfer a 2 mL aliquot of filtered sample to Envi-carb and pull through into clean centrifuge tube. Rinse cartridge with 4 mLs of methanol.

7.5.9 To the filtered material, add 10uL of acetic acid and verify the pH to be between 5-6 with pH paper.

7.5.10 Blow the extract down at room temperature to a final volume of 5mL, raising the volume with 96:4 methanol:DI water.

7.5.11 Transfer an aliquot to a 2mL vial with polypropylene insert.

7.6 Data Analysis

7.6.1 The analyst cannot extrapolate beyond the range of the calibration. However, by isotope dilution analysis, the extract cannot be diluted. If an analyte is outside of the determined range, the sample must be re-extracted at an appropriate dilution level.

7.6.1.1 There is extrapolation allowed only to determine if there is blank contamination. Since there is no blank subtraction, any contamination present must be below 1/2 of the MRL for specific analyte.

7.6.1.2 If a sample exceeds the calibration range the sample must be re-extracted. This would involve diluting the sample with reagent water to be within the calibration range and adding ammonium acetate to be at a final concentration of 1 g/L.

7.6.1.3 Additionally, if a sample exceeds the calibration range, one or more LRB must be run until the system meets acceptable criteria. If this occurs during an automated sequence, any subsequent samples must be evaluated. If the over-range analytes are present in the subsequent samples at or above the RL, the samples are considered invalid and must be re-run. If the analyte in question does not exceed the RL, the samples can be reported.

7.6.2 Compounds that have both branched and linear isomers will be reported as total. These compounds include PFOS, PFHxS, N-Et-FOSAA, N-Me-FOSAA and PFOA. PFOS, PFHxS, N-Et-FOSAA, and N-Me-FOSAA have the branched and linear compounds available for quantitation. PFOA is a special case outlined below:

7.6.2.1 PFOA will be quantitated by using a qualitative/semi-quantitative approach per EPA guidance. Until a standard is available, the calibration will be done using the linear isomer only. A technical grade standard will be run to identify the retention time of the branched isomer. All samples will be quantitated using the area of both the linear and branched isomers of PFOA that may be present within the sample. A branched isomer check for PFOA will be run with every calibration curve to verify the retention times of the branched isomers for PFOA.

7.6.3 All analytes and surrogates will be calculated based on the initial calibration criteria.

7.6.4 All results for analytes shall be reported as the acid form of the compound.

7.6.5 Retention time windows are established once per ICAL and at the beginning of each sequence. On days when an ICAL is not run, the initial CCV is used to set the times. All retention times of analytes and EIS analytes must fall within 0.4 minutes of the established time. Analytes must also elute within 0.1 minutes of their respective EIS.

7.6.6 In addition to retention time identification criteria, most ions are identified by two ion transitions. (The following ions are exceptions: PFBA, PFPeA, FBSA, FHxSA, PFMPA, PFMBA, 9Cl-PF3ONS, 11Cl-PF3OUdS, ADONA). The secondary, or qualifier ion, must have a signal to noise of 3:1. The ratio between the qualifier and the quantifier ion must be averaged from the calibration. For samples to be valid, the ratio of qualifier to quantifier must be +/-50% from the average ratio from the applicable calibration.

7.7 Instrumentation Procedure

7.7.1 Before any QC or samples can be run, the HPLC must be allowed to purge for at least thirty minutes. This purge can be done using any combination of the mobile phases, but prior to samples running, the initial mobile phase conditions used in the method must be allowed to run for 15 minutes or until pressure has stabilized.

7.7.2 The instrument must be stable in all parameters before a run is started. The following are the HPLC and ESI-MS Method Conditions. Also, See Appendix A for additional MS/MS Method Conditions.

Time (min)	% 5 mM Ammonium Acetate in water	% Methanol	Flow Rate (mL/minute)
0.00	95	5	1.0
0.10	65	35	1.0
2.00	50	50	1.0
3.00	25	75	1.0
4.50	1	99	1.0
4.51	1	99	1.0
5.00	1	99	1.0
5.10	95	5	1.0
6.50	95	5	1.0

Parameter	Value
Injection Volume 6470	10 uL
Injection Volume 6495	5 uL
Column Compartment Temperature	40 °C
Autosampler Compartment Temperature	10 °C
Polarity	Negative
Gas Temperature	250 °C
Gas Flow	11 L/min
Nebulizer	50 psi
Sheath Gas Temperature	300 °C
Sheath Gas Flow	12 L/min
Capillary Needle Voltage (Negative mode)	-3000 V
Cell Accelerator Voltage	5 V

7.7.3 An instrument sequence will be made. It will open with an instrument blank and a low level CCV. After the CCV, the batch can start running. Every 10 field samples (excluding QC and FRBs) a subsequent level 4 CCV must be run.

7.7.4 The run can end with a script to put the instrument into standby mode.

8.0 QUALITY CONTROL

8.1 Definitions

For definitions and explanations of quality control measures (blanks, LCS/QC Reference, LFB, Duplicates, MS/MSD, etc.) refer to the Contest, A Pace Analytical Quality Assurance Manual.

8.2 Quality Control Measures & Acceptance Criteria

8.2.1 Method Blank

The method blank is matrix specific, and extracted with every batch or every 20 samples (whichever is more frequent). The target compounds must be ≤1/2 the MRL, or <1/10th the amount measured in any sample, or <1/10th the regulatory limit. If any analytes are present above this level, the detected analytes are considered invalid for all samples extracted in that batch.

8.2.2 Laboratory Control Sample/Duplicate (LCS)

A matrix-specific LCS is extracted every 20 samples. The concentration must be ≥ LOQ and ≤ mid-range of calibration . All analytes recoveries must be within limits specified in Appendix D. If analyte is not listed in table, acceptance criteria is to remain 50-150% until in-house limits can be determined. Samples should be re-extracted if criteria are not met, even if outside of hold. If samples cannot be re-extracted then the failures must be notated in the narrative.

8.2.3 Matrix Spikes

A matrix-specific MS is extracted every 20 samples. All analytes recoveries must be within limits specified in Appendix D. If analyte is not listed in table, acceptance criteria is to remain 50-150% until in-house limits can be determined. See calculation 3.
***Note: Matrix spike samples may display matrix bias. If the CCC and LFB samples are passing, but the MS recoveries are outside the designated range, the recovery is deemed to be matrix biased. A note on the unfortified sample will indicate the possibility of matrix effects being suspect.**

8.2.4 Matrix spike duplicates

Extract a spiked sample duplicate every 20 samples. Matrix Spike Duplicate samples should be calculated to have an RPD ≤30%. See calculation 4.

8.2.5 Quality Control Samples

A quality control sample must be run from a second source at least quarterly, or after an initial calibration as an ICV. If a second source is not commercially available, a different lot number from the same vendor is acceptable. The recoveries must be within 70-130% of the true value.

8.2.6 Isotope Dilution Analogue

Isotope dilution analogues are added to all blanks, standards, samples, and spikes. Analogue compounds must have an area of 50-150% of the associated compound in level 4 of the calibration on days when a calibration is run. On days when a calibration is not run, analogue compounds must have an area of 50-150% of the associated compound in the opening instrument sensitivity check/CCV.

If the surrogate is outside these limits, the extract should be re-analyzed. If the re-analysis passes, report re-analyzed sample. If this fails, the associated isotope performance standard should be evaluated. The system may need recalibration or maintenance. If the CCV has surrogate out of range, the instrument needs to be recalibrated.

If the re-analysis fails, re-extract the sample to confirm failure if an aliquot is available. If the re-extract fails, report both results with appropriate flagging criteria. If not enough volume is provided to re-extract the sample then report with appropriate flags.

8.2.7 Calibration Curve

A minimum of a 5-point calibration curve (for linear regression) or a 6-point calibration curve (for quadratic) is used to calibrate the system.

The curve must be verified with an independent standard (QCS) prior to sample analysis, (10 ng/L). The curve will be forced through zero and may or may not be concentration weighted.

If a peak is not properly integrated by the data system, manual integration may be necessary. Manual integrations must comply with the Pace SOP on Chromatographic Integration Procedures. The integration of the peaks for the samples and quality control samples must be as consistent as possible with the integration used with the initial calibration.

8.2.8 Continuing Calibration Verification Checks (CCVs)

The results must be between 70-130% of the true value for all analytes for the initial low level CCV

After every ten field samples, a subsequent CCV must at level 4. The requirements for the CCVs are 70-130% of the true value. All analogue compound areas must fall within 50-150% of the appropriate calibration point or CCV.

The ending CCV acquisition time must fall within 24 hours of the acquisition starting time of the opening CCV with the associated analysis batch.

8.2.10 Any failures in QC require reanalysis, even if the samples in question are outside of hold.

8.2.11 <u>Initial Demonstration of Capability (IDOC):</u> An Initial demonstration of capability (IDOC) must be made prior to performing any test method, and at any time there is a significant change in instrument type, personnel, or test method.

In general, this demonstration does not test the performance of the method in real world samples, but in applicable and available clean matrix (a sample of a matrix in which no target analytes or interferences are present at concentrations that impact the results of a specific test method). Before any results are reported by a new analyst they need to perform and IDOC. See Appendix C.

All demonstrations shall be documented through the use of the IDOC form, which also lists SOP, method associated with the test, certification statement, and authorized signatures.

<u>8.2.12 Continuing Demonstration of Capability (CDOC)</u>: An on-going demonstration of capability must be performed on an annual basis to document the quality of the data produced. On-going data quality checks are compared with established performance criteria to determine if the results of analyses meet the performance standards for the method.

In general, this demonstration does not test the performance of the method in real world samples, but in applicable and available clean matrix (a sample of a matrix in which no target analytes or interferences are present at concentrations that impact the results of a specific test method). Extract and analyze 4 replicate laboratory fortified blanks at level 4 of the calibration with acceptable recoveries between 70-130%.

All demonstrations shall be documented through the use of the CDOC form, which also lists SOP, method associated with the test, certification statement, and authorized signatures.

8.2.13 <u>Field Reagent Blank:</u>

Clients may choose to send a field blank if they want wish to as it is optional. Field reagent blanks only need to be run and reported if there are analytes at or above the MRL in any associated field samples. Any analyte peaks present in field reagent blanks must be below the MRL of that analyte.

9.0 CORRECTIVE ACTIONS/ CONTINGENCIES OF HANDLING OUT-OF-CONTROL DATA

9.1 Refer to Pace Quality Assurance Manual
9.2 Refer to Pace Corrective Action SOP.

10.0 POLLUTION PREVENTION

10.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Many opportunities for pollution prevention exist in laboratory operation. EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address waste generation. Standards should be prepared in volumes consistent with laboratory use to minimize the disposal of excess volumes of expired standards.

11.0 WASTE MANAGEMENT

11.1 It is the laboratory's responsibility to comply with all federal, state, and local regulations governing the waste management, particularly the hazardous waste identification rules and land disposal restrictions, and to protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Also, compliance is required with any sewage discharge permits and regulations.

11.2 Acidic samples and waste are dumped into satellite waste containers.

12.0 CALCULATIONS

12.1 Calculation 1: Adding 4 mole equivalents to standards to prevent esterification

$$\frac{Total\ PFAS\ mass\ (g) \times 160(\frac{g}{mol})}{250\ (\frac{g}{mol})} = Mass\ of\ NaOH\ Required\ (g)$$

12.2 Calculation 2: Mass of the anion

$Mass_{acid}$= Measured $Mass_{salt}$ * (Molecular $Weight_{acid}$/Molecular $Weight_{salt}$)

12.3 Calculation 3: Percent Recovery

$$\%R = \frac{(A - B)}{C} \times 100$$

12.4 Calculation 4: Relative percent deviation calculation

$$RPD = \frac{|LFSMD - LFSM|}{(LFSMD + LFSM)/2} \times 100$$

12.5 Calculation 5: MRL Statistics

Calculate the mean and standard deviation for each analyte in these replicates. Determine the Half Range for the Prediction Interval of Results (HR_{PIR}) using the following equation:

$$HR_{PIR} = 3.963S$$

Where,

S = the standard deviation and 3.963 is a constant value for seven replicates.[1]

Calculate the Upper and Lower Limits for the Prediction Interval of Results ($PIR = Mean \pm HR_{PIR}$) as shown below. These equations are only defined for seven replicate samples.

$$Upper\ PIR\ Limit = \frac{Mean + HR_{PIR}}{Fortified\ Concentration} \times 100$$

$$Lower\ PIR\ Limit = \frac{Mean - HR_{PIR}}{Fortified\ Concentration} \times 100$$

13.0 REFERENCES

13.1 Pace Analytical Chemical Hygiene Plan

13.2 Pace Analytical Quality Assurance Manual.

13.3 Pace Analytical Corrective Action SOP.

13.4 Pace Analytical Controlled Document SOP.

13.5 Agilent 1260 Infinity Binary LC Operators manual

13.6 Agilent MassHunter Study Manager

13.7 Agilent MassHunter Optimizer

13.8 MassHunter Personal Compound Database and Library Manager

13.9 Agilent 6400 Series Triple Quadrupole LC/MS System Quick Start Guide

13.10 MassHunter Data Acquisition Compliance Software Quick Start Guide

13.11 MassHunter Quantitative Analysis Compliance Software Quick Start Guide

13.12 Agilent 6000 Series LC/MS System Maintenance Guide

13.13 EPA Method 533, "Determination of Selected Per- and Polyfluoralkyl Substances in Drinking Water by Isotope Dilution Anion Exchange Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)", November 2019, EPA Document #815-B-19-020.

13.14 Method ISO 25101:2009, "Determination of perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) – Method for unfiltered samples using solid phase extraction and liquid chromatography/mass spectrometry", April 30, 2009.

13.15 EPA Technical Advisory-Laboratory Analysis of Drinking Water Samples for Perfluorooctanoic Acid (PFOA) using EPA Method 537 Rev. 1.1 EPA 815-B-16-021 September 2016

13.16 Agilent Application note by Peter JW Stone, Linda Cote, Jennifer Gushue, Robert J. Letcher and Shaogang Chu. A Low Femtogram Target Screen Method for Perfluorinated Compounds in Food Matrices and Potable Water Using the Agilent 6460 Triple Quadrupole LC/MS System Equipped with Agilent Jet Stream Technology.

13.17 TNI Standard, The NELAC Institute, EL-V1-2009-ISO, 2009.

13.18 Department of Defense (DoD) Department of Energy (DOE) Consolidated Quality Systems Manual (QSM) for Environmental Laboratories Based on ISO/IEC 17025:2005(E) ISO/IEC 17025:2017(E) and The NELAC Institute (TNI) Standards, Volume 1, (September 2009).

13.19 ASTM Method D7968-17a, "Standard Test Method for Determination of Polyfluorinated Compounds in Soil by Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS)", ASTM International, West Conshohocken, PA, 2017.

Appendix A

6470 Transitions and MS Conditions:

Analyte	Precursor Ion	Product Ion	Collision Energy Voltage (V)	Fragmentor Voltage (V)	Qualifer/Quantifier
11Cl-PF3OUdS	631	451	24	100	N/A
4-2 FTS	327	307	20	120	Quant
4-2 FTS	327	81	30	120	Qual
6-2 FTS	427	406.9	24	135	Quant
6-2 FTS	427	80	40	125	Qual
8-2 FTS	527	507	28	145	Quant
8-2 FTS	527	80	40	170	Qual
9Cl-PF3ONS	531	351	24	100	N/A
ADONA	377	251	12	100	Quant
ADONA	377	85	12	100	Qual
d3-N-MeFOSAA	573.2	419	20	114	N/A
d5-N-EtFOSAA	589.2	419	20	104	N/A
FBSA	297.99	78	28	115	N/A
FHXSA	398	78.1	30	135	N/A
HFPO-DA	285.1	184.9	5	150	Quant
HFPO-DA	285.1	169	5	150	Qual
M2-4-2-FTS	328.99	309.2	20	135	N/A
M2-6-2-FTS	428.99	409.2	24	160	N/A
M2-8-2-FTS	528.99	509	28	170	N/A
M2PFDA	514.9	469.9	5	102	N/A
M2PFHxA	315	270	4	66	N/A
M2PFOA	415	370	4	69	N/A
M2PFTA	715	670	9	100	N/A
M3HFPO-DA	287	169	2	50	N/A
M3PFBA	216	171.8	4	56	N/A
M3PFBS	301.9	80	45	100	N/A
M3PFHxS	401.9	80	49	100	N/A
M4PFHpA	367	322	4	102	N/A
M5PFHxA	318	273	4	68	N/A
M5PFPeA	268	223	8	120	N/A
M6PFDA	519	474	4	81	N/A
M7PFUnA	570	525	5	73	N/A
M8FOSA	506	78	36	125	N/A
M8PFOA	421	376	5	65	N/A
M8PFOS	506.9	80	50	100	N/A
M9PFNA	472	427	4	85	N/A
MPFBA	217	172	8	60	N/A
MPFDoA	615	570	5	79	N/A
MPFOS	502.9	80	60	180	N/A
N-EtFOSAA	584	525.9	20	115	Qual

N-EtFOSAA	584	418.9	20	115	Quant
NFDHA	201	85	14	115	N/A
N-MeFOSAA	570	482.9	16	115	Qual
N-MeFOSAA	570	418.9	20	115	Quant
PFBA	213	169	8	60	N/A
PFBS	298.9	98.9	29	100	Qual
PFBS	298.9	80	45	100	Quant
PFDA	513	469	4	81	Quant
PFDA	513	169	16	100	Qual
PFDoA	613	569	5	79	Quant
PFDoA	613	268.7	20	100	Qual
PFDS	598.9	99	60	100	Qual
PFDS	598.9	80	80	100	Quant
PFEESA	315	135	24	110	N/A
PFHpA	362.9	319	8	72	Quant
PFHpA	362.9	169	20	72	Qual
PFHpS	448.9	98.7	44	44	Qual
PFHpS	448.9	79.7	52	52	Quant
PFHxA	313	268.9	8	8	Quant
PFHxA	313	119	18	18	Qual
PFHxS	398.9	99	45	100	Qual
PFHxS	398.9	80	49	100	Quant
PFMBA	279	85.1	8	55	N/A
PFMPA	229	85.1	12	55	N/A
PFNA	463	419	4	66	Quant
PFNA	463	169	17	66	Qual
PFNS	548.9	98.9	40	165	Qual
PFNS	548.9	79.9	40	165	Quant
PFOA	413	369	4	69	Quant
PFOA	413	169	12	69	Qual
PFOS	498.9	99	50	100	Qual
PFOS	498.9	80	50	100	Quant
PFOSA	497.9	77.9	36	125	Quant
PFOSA	497.9	47.9	80	100	Qual
PFPeA	263	218.9	8	60	N/A
PFPeS	348.9	98.9	40	135	Qual
PFPeS	348.9	79.9	40	135	Quant
PFTA	713	669	9	100	Quant
PFTA	712.9	169	30	100	Qual
PFTrDA	663	619	9	91	Quant
PFTrDA	663	169	30	100	Qual
PFUnA	563	519	5	73	Quant
PFUnA	563	218.7	20	100	Qual

6495 Transitions and MS Conditions:

Analyte	Precursor Ion	Product Ion	Collision Energy Voltage (V)	Fragmentor Voltage (V)	Qualifer/Quantifier
11Cl-PF3OUdS	631	451	24	166	N/A
4-2 FTS	327	307	20	166	Quant
4-2 FTS	327	81	30	166	Qual
6-2 FTS	427	406.9	24	166	Quant
6-2 FTS	427	80	40	166	Qual
8-2 FTS	527	507	28	166	Quant
8-2 FTS	527	80	40	166	Qual
9Cl-PF3ONS	531	351	24	166	N/A
ADONA	377	251	12	166	Quant
ADONA	377	85	12	166	Qual
d3-N-MeFOSAA	573.2	419	20	166	N/A
d5-N-EtFOSAA	589.2	419	20	166	N/A
FBSA	297.99	78	28	166	N/A
FHXSA	398	78.1	30	166	N/A
HFPO-DA	285.1	184.9	5	166	Quant
HFPO-DA	285.1	169	5	166	Qual
M2-4-2-FTS	328.99	309.2	20	166	N/A
M2-6-2-FTS	428.99	409.2	24	166	N/A
M2-8-2-FTS	528.99	509	28	166	N/A
M2PFDA	514.9	469.9	5	166	N/A
M2PFHxA	315	270	4	166	N/A
M2PFOA	415	370	4	166	N/A
M2PFTA	715	670	9	166	N/A
M3HFPO-DA	287	169	2	166	N/A
M3PFBA	216	171.8	4	166	N/A
M3PFBS	301.9	80	45	166	N/A
M3PFHxS	401.9	80	49	166	N/A
M4PFHpA	367	322	4	166	N/A
M5PFHxA	318	273	4	166	N/A
M5PFPeA	268	223	8	166	N/A
M6PFDA	519	474	4	166	N/A
M7PFUnA	570	525	5	166	N/A
M8FOSA	506	78	36	166	N/A
M8PFOA	421	376	5	166	N/A
M8PFOS	506.9	80	50	166	N/A
M9PFNA	472	427	4	166	N/A
MPFBA	217	172	8	166	N/A
MPFDoA	615	570	5	166	N/A
MPFOS	502.9	80	60	166	N/A
N-EtFOSAA	584	525.9	20	166	Qual
N-EtFOSAA	584	418.9	20	166	Quant
NFDHA	201	85	14	166	N/A
N-MeFOSAA	570	482.9	16	166	Qual
N-MeFOSAA	570	418.9	20	166	Quant

PFBA	213	169	8	166	N/A
PFBS	298.9	98.9	29	166	Qual
PFBS	298.9	80	45	166	Quant
PFDA	513	469	4	166	Quant
PFDA	513	169	16	166	Qual
PFDoA	613	569	5	166	Quant
PFDoA	613	268.7	20	166	Qual
PFDS	598.9	99	60	166	Qual
PFDS	598.9	80	80	166	Quant
PFEESA	315	135	24	166	N/A
PFHpA	362.9	319	8	166	Quant
PFHpA	362.9	169	20	166	Qual
PFHpS	448.9	98.7	44	166	Qual
PFHpS	448.9	79.7	52	166	Quant
PFHxA	313	268.9	8	166	Quant
PFHxA	313	119	18	166	Qual
PFHxS	398.9	99	45	166	Qual
PFHxS	398.9	80	49	166	Quant
PFMBA	279	85.1	8	166	N/A
PFMPA	229	85.1	12	166	N/A
PFNA	463	419	4	166	Quant
PFNA	463	169	17	166	Qual
PFNS	548.9	98.9	40	166	Qual
PFNS	548.9	79.9	40	166	Quant
PFOA	413	369	4	166	Quant
PFOA	413	169	12	166	Qual
PFOS	498.9	99	50	166	Qual
PFOS	498.9	80	50	166	Quant
PFOSA	497.9	77.9	36	166	Quant
PFOSA	497.9	47.9	80	166	Qual
PFPeA	263	218.9	8	166	N/A
PFPeS	348.9	98.9	40	166	Qual
PFPeS	348.9	79.9	40	166	Quant
PFTA	713	669	9	166	Quant
PFTA	712.9	169	30	166	Qual
PFTrDA	663	619	9	166	Quant
PFTrDA	663	169	30	166	Qual
PFUnA	563	519	5	166	Quant
PFUnA	563	218.7	20	166	Qual

Appendix B

Analyte	Acronym	Isotope Dilution Analogue
11- Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	11Cl-PF3OUdS	M8PFOS
9- Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	9Cl-PF3ONS	M8PFOS
4,8-Dioxa-3H-perfluorononanoic acid	ADONA	M4PFHpA
Hexafluoropropylene oxide dimer acid	HFPO-DA	M3HFPO-DA
Nonafluoro-3,6-dioxaheptanoic acid	NFDHA	M5PFHxA
Perfluorobutanoic acid	PFBA	MPFBA
Perfluorobutanesulfonic acid	PFBS	M3PFBS
1H,1H, 2H, 2H-Perfluorodecane sulfonic acid	8:2FTS	M2-8:2FTS
Perfluorodecanoic acid	PFDA	M6PFDA
Perfluorododecanoic acid	PFDoA	MPFDoA
Perfluoro(2-ethoxyethane)sulfonic acid	PFEESA	M3PFBS
Perfluoroheptanesulfonic acid	PFHpS	M8PFOS
Perfluoroheptanoic acid	PFHpA	M4PFHpA
1H,1H, 2H, 2H-Perfluorohexane sulfonic acid	4:2FTS	M2-4:2FTS
Perfluorohexanesulfonic acid	PFHxS	M3PFHxS
Perfluorohexanoic acid	PFHxA	M5PFHxA
Perfluoro-3-methoxypropanoic acid	PFMPA	MPFBA
Perfluoro-4-methoxybutanoic acid	PFMBA	M5PFPeA
Perfluorononanoic acid	PFNA	M9PFNA
1H,1H, 2H, 2H-Perfluorooctane sulfonic acid	6:2FTS	M2-6:2FTS
Perfluorooctanesulfonic acid	PFOS	M8PFOS
Perfluorooctanoic acid	PFOA	M8PFOA
Perfluoropentanoic acid	PFPeA	M5PFPeA
Perfluoropentanesulfonic acid	PFPeS	M3PFHxS
Perfluoroundecanoic acid	PFUnA	M7PFUnA
N-ethyl perfluorooctanesulfonamidoacetic acid	NEtFOSAA	d5-N-EtFOSAA
N-methyl perfluorooctanesulfonamidoacetic acid	NMeFOSAA	d3-N-MeFOSAA
Perfluoro-1-butanesulfonamide	FBSA	M5PFHxA
Perfluoro-1-hexanesulfonamide	FHxSA	M8PFOA
Perfluorotetradecanoic acid	PFTA	M2PFTA
Perfluorotridecanoic acid	PFTrDA	M2PFTA

Perfluorooctanesulfonamide	**FOSA**	**M8FOSA**
Perfluorononanesulfonic acid	**PFNS**	**M8PFOS**
Perfluorodecanesulfonic acid	**PFDS**	**M3PFBS**

Appendix C

Requirement	Specification	Acceptance Criteria
Demonstration of precision (Requirement of Analyst IDOC and CDOC)	**Extract and analyze 4 replicate laboratory fortified blanks at the level 4 of the calibration.**	**Percent relative standard deviation must be </=20%.**
Demonstration of accuracy (Requirement of Analyst IDOC and CDOC)	**Calculate mean recovery for replicated used in demonstration of precision.**	**Mean recovery within 70-130% of the true value.**
MDL Confirmation	**Extract and analyze 9 blanks, and 9 laboratory fortified blanks at the proposed reporting limit over three days.**	**Calculated MDL and MDL-b is < Proposed reporting limit.**
Calibration Verification	**Analyze a Level 5 QCS after each initial calibration.**	**Results must be within 70-130% of the true value.**

Appendix D

Table C-45. Method PFAS by LCMSMS Compliant with QSM Table B-15 Solid Matrix

CAS ID	Analyte	N Records	Mean	Standard Deviation	Lower Control Limit	Upper Control Limit
2991-50-6	2-(N-Ethylperfluorooctanesulfonamido) acetic acid	249	99.7	12.9	61	139
2355-31-9	2-(N-Methylperfluorooctanesulfonamido) acetic acid	254	103.9	13.3	63	144
757124-72-4	Fluorotelomer sulphonic acid 4:2	266	103.1	13.7	62	145
27619-97-2	Fluorotelomer sulphonic acid 6:2	575	101.9	12.4	64	140

Table C-45. Method PFAS by LCMSMS Compliant with QSM Table B-15 Solid Matrix

CAS ID	Analyte	N Records	Mean	Standard Deviation	Lower Control Limit	Upper Control Limit
39108-34-4	Fluorotelomer sulphonic acid 8:2	544	100.8	11.8	65	137
375-73-5	Perfluorobutanesulfonic acid	624	100.5	9.2	72	128
375-22-4	Perfluorobutanoic acid	333	102.9	10.5	71	135
335-77-3	Perfluorodecanesulfonic acid	336	96.2	12.4	59	134
335-76-2	Perfluorodecanoic acid	569	101.0	10.4	69	133
307-55-1	Perfluorododecanoic acid	565	101.7	10.8	69	135
375-92-8	Perfluoroheptanesulfonic acid	511	101.0	10.3	70	132
375-85-9	Perfluoroheptanoic acid	652	101.2	10.0	71	131
355-46-4	Perfluorohexanesulfonic acid	639	98.5	10.5	67	130
307-24-4	Perfluorohexanoic acid	614	100.8	10.2	70	132
68259-12-1	Perfluorononanesulfonic acid	338	96.8	9.1	69	125
375-95-1	Perfluorononanoic acid	650	100.3	9.3	72	129
754-91-6	Perfluorooctanesulfonamide	377	102.2	11.5	67	137
1763-23-1	Perfluorooctanesulfonic acid	518	101.9	11.3	68	136
335-67-1	Perfluorooctanoic acid	663	101.1	10.4	69	133
2706-91-4	Perfluoropentanesulfonic acid	335	97.9	8.1	73	123
2706-90-3	Perfluoropentanoic acid	588	100.2	10.3	69	132
376-06-7	Perfluorotetradecanoic acid	551	101.3	10.5	69	133
72629-94-8	Perfluorotridecanoic acid	548	102.3	12.1	66	139
2058-94-8	Perfluoroundecanoic acid	587	99.9	12.0	64	136

Appendix E

Table B-15. Per- and Polyfluoroalkyl Substances (PFAS) Using Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS) With Isotope Dilution or Internal Standard Quantification in Matrices Other Than Drinking Water						Pace SOP Section
QC Check	**Minimum Frequency**	**Acceptance Criteria**	**Corrective Action**	**Flagging Criteria**	**Comments**	**SOP SECTION**
Aqueous Sample Preparation	Each sample and associated batch QC samples.	Solid Phase Extraction (SPE) must be used unless samples are known to contain high PFAS concentrations (e.g., Aqueous Film Forming Foam (AFFF) formulations). Inline SPE is acceptable. Entire sample plus bottle rinsate must be extracted using SPE. Known high PFAS concentration samples require serial dilution be performed in duplicate. Documented project approval is needed for samples prepared by serial dilution as opposed to SPE.	NA.	NA.	Samples with > 1% solids may require centrifugation prior to SPE extraction. Pre-screening of separate aliquots of aqueous samples is recommended.	7.0 Procedure & Summary, Scope & Application
Solid Sample Preparation	Each sample and associated batch QC samples.	Entire sample received by the laboratory must be homogenized prior to subsampling.	NA.	NA.	NA.	Soil SOP: 7.5.1
Biota Sample Preparation	Each sample and associated batch QC samples.	Sample prepared as defined by the project (e.g., whole fish versus filleted fish).	NA.	NA.	NA.	N/A
QC Check	**Minimum Frequency**	**Acceptance Criteria**	**Corrective Action**	**Flagging Criteria**	**Comments**	**SOP SECTION**
AFFF and AFFF Mixture Samples Preparation	Each sample and associated batch QC samples.	Each field sample must be prepared in duplicate (equivalent to matrix duplicate).	NA.	NA.	Adsorption onto bottle is negligible compared to sample concentration so subsampling is allowed.	N/A

		Serial dilutions must be performed to achieve the lowest LOQ possible for each analyte.			Multiple dilutions will most likely have to be reported in order to achieve the lowest LOQ possible for each analyte.	
Sample Cleanup Procedure	Each sample and associated batch QC samples. Not applicable to AFFF and AFFF Mixture Samples.	ENVI-Carb™ or equivalent must be used on each sample and batch QC sample.	NA.	Flagging is not appropriate.	Cleanup should reduce bias from matrix interferences.	Soil:7.5.10
Mass Calibration	Instrument must have a valid mass calibration prior to any sample analysis. Mass calibration is verified after each mass calibration, prior to initial calibration (ICAL).	Calibrate the mass scale of the MS with calibration compounds and procedures described by the manufacturer. Mass calibration range must bracket the ion masses of interest. The most recent mass calibration must be used for every acquisition in an analytical run. Mass calibration must be verified to be ±0.5 amu of the true value, by acquiring a full scan continuum mass spectrum of a PFAS stock standard.	If the mass calibration fails, then recalibrate. If it fails again, consult manufacture r instructions on corrective maintenance .	Flagging is not appropriate.	Problem must be corrected. No samples may be analyzed under a failing mass calibration. The mass calibration is updated on an as-needed basis (e.g., QC failures, ion masses fall outside of the ±0.5 amu of the true value, major instrument maintenance is performed, or the instrument is moved).	7.3.2
QC Check	**Minimum Frequency**	**Acceptance Criteria**	**Corrective Action**	**Flagging Criteria**	**Comments**	**SOP SECTION**
Mass Spectral Acquisition Rate	Each analyte, Extracted Internal Standard (EIS) Analyte.	A minimum of 10 spectra scans are acquired across each chromatographic peak.	NA.	Flagging is not appropriate.	NA.	7.3.1
Calibration, Calibration Verification, and Spiking Standards	All analytes.	Standards containing both branched and linear isomers must be used when commercially available. PFAS method analytes may	NA.	Flagging is not appropriate.	Standards containing both branched and linear isomers are to be used during method validation and when reestablishing retention times, to ensure the total response is quantitated for that analyte.	7.6.2

		consist of both branched and linear isomers, but quantitative standards that contain the linear and branched isomers do not exist for all method analytes. For PFAS that do not have a quantitative branched and linear standard, identify the branched isomers by analyzing a qualitative standard that includes both linear and branched isomers and determine retention times, transitions and transition ion ratios. Quantitate samples by integrating the total response (i.e., accounting for peaks that are identified as linear and branched isomers) and relying on the initial calibration that uses the linear isomer quantitative standard.			Technical grade standards cannot be used for quantitative analysis.	
QC Check	**Minimum Frequency**	**Acceptance Criteria**	**Corrective Action**	**Flagging Criteria**	**Comments**	**SOP SECTION**
Sample PFAS Identification	All analytes detected in a sample.	The chemical derivation of the ion transitions must be documented. A minimum of two ion transitions (Precursor → quant ion and precursor → confirmation ion) and the ion transitions ratio per	NA.	PFAS identified with Ion ratios that fail acceptance criteria must be flagged. Any quantitation ion peak that does not meet the maximization	For example: Ion Ratio = (quant ion abundance/ confirm ion abundance) Calculate the average ratio (A) and standard deviation (SD) using the ICAL standards. An acceptance range of ratio could be within A ±3SD for confirmation of detection.	7.6.6

		analyte are required for confirmation. Exception is made for analytes where two transitions do not exist (PFBA and PFPeA). Documentation of the primary and confirmation transitions and the ion ratio is required. In-house acceptance criteria for evaluation of ion ratios must be used and must not exceed 50- 150%. Signal to Noise Ratio (S/N) must be ≥ 10 for all ions used for quantification and must be ≥ 3 for all ions used for confirmation. Quant ion and confirmation ion must be present and must maximize simultaneously (±2 seconds).		criteria shall be included in the summed integration and the resulting data flagged as “estimated, biased high”.		
QC Check	**Minimum Frequency**	**Acceptance Criteria**	**Corrective Action**	**Flagging Criteria**	**Comments**	**SOP SECTION**
Ion Transitions (Precursor-> Product)	Every field sample, standard, blank, and QC sample.	In order to avoid biasing results high due to known interferences for some transitions, the following transitions must be used for the quantification of the following analytes: PFOA: 413 → 369 PFOS: 499 → 80 PFHxS: 399 → 80 PFBS: 299 → 80 4:2 FTS: 327 → 307 6:2 FTS: 427 → 407 8:2 FTS: 527 → 507 NEtFOSAA: 584 → 419	NA.	Flagging is not appropriate	NA.	Appendix A

		NMeFOSAA: 570 → 419 If these transitions are not used, the reason must be technically justified and documented (e.g., alternate transition was used due to observed interferences).				
QC Check	**Minimum Frequency**	**Acceptance Criteria**	**Corrective Action**	**Flagging Criteria**	**Comments**	**SOP SECTION**
Initial Calibration (ICAL)	At instrument set-up and after ICV or CCV failure, prior to sample analysis.	The isotopically labeled analog of an analyte (Extracted Internal Standard Analyte) must be used for quantitation if commercially available (Isotope Dilution Quantitation). Commercial PFAS standards available as salts are acceptable providing the measured mass is corrected to the neutral acid concentration. Results shall be reported as the neutral acid with appropriate CAS number. If a labeled analog is not commercially available, the Extracted Internal Standard Analyte with the closest retention time or chemical similarity to the analyte must be used for quantitation. (Internal Standard Quantitation) Analytes must be within 70-130% of their true value for	Correct problem, then repeat ICAL.	Flagging is not appropriate.	No samples shall be analyzed until ICAL has passed. External Calibration is not allowed for any analyte. Calibration can be linear (minimum of 5 standards) or quadratic (minimum of 6 standards); weighting is allowed.	7.6.4 & 7.4.1

QC Check	Minimum Frequency	Acceptance Criteria	Corrective Action	Flagging Criteria	Comments	SOP SECTION
		each calibration standard. *(continued next page)*				
Initial Calibration (ICAL) *(Continued)*		ICAL must meet one of the two options below: Option 1: The RSD of the RFs for all analytes must be ≤ 20%. Option 2: Linear or non- linear calibrations must have $r^2 \geq 0.99$ for each analyte.				7.3.6
Retention Time window position establishment	Once per ICAL and at the beginning of the analytical sequence.	Position shall be set using the midpoint standard of the ICAL curve when ICAL is performed. On days when ICAL is not performed, the initial CCV is used.	NA.	NA.	Calculated for each analyte and EIS.	7.6.5
Retention Time (RT) window width	Every field sample, standard, blank, and QC sample.	RT of each analyte and EIS analyte must fall within 0.4 minutes of the predicted retention times from the daily calibration verification or, on days when ICAL is performed, from the midpoint standard of the ICAL. Analytes must elute within 0.1 minutes of the associated EIS. This criterion applies only to analyte and labeled analog pairs.	Correct problem and reanalyze samples.	NA.	Calculated for each analyte and EIS.	7.6.5
QC Check	**Minimum Frequency**	**Acceptance Criteria**	**Corrective Action**	**Flagging Criteria**	**Comments**	**SOP SECTION**
Instrument Sensitivity Check (ISC)	Prior to analysis and at least once every 12 hours.	Analyte concentrations must be at LOQ;	Correct problem, rerun ISC. If	Flagging is not appropriate.	No samples shall be analyzed until ISC has met acceptance criteria.	7.4.1

		concentrations must be within ±30% of their true values.	problem persists, repeat ICAL.		ISC can serve as the initial daily CCV.	
Initial Calibration Verification (ICV)	Once after each ICAL, analysis of a second source standard prior to sample analysis.	Analyte concentrations must be within ±30% of their true value.	Correct problem, rerun ICV. If problem persists, repeat ICAL.	Flagging is not appropriate.	No samples shall be analyzed until calibration has been verified.	7.3.7
Continuing Calibration Verification (CCV)	Prior to sample analysis, after every 10 field samples, and at the end of the analytical sequence.	Concentration of analytes must range from the LOQ to the mid-level calibration concentration. Analyte concentrations must be within ±30% of their true value.	Immediately analyze two additional consecutive CCVs. If both pass, samples may be reported without reanalysis. If either fails, or if two consecutive CCVs cannot be run, perform corrective action(s) and repeat CCV and all associated samples since last successful CCV. Alternately, recalibrate if necessary; then reanalyze all associated samples since the last acceptable CCV.	If reanalysis cannot be performed, data must be qualified and explained in the Case Narrative. Apply Q-flag to all results for the specific analyte(s) in all samples since the last acceptable calibration verification.	Results may not be reported without valid CCVs. Instrument Sensitivity Check (ISC) can serve as a bracketing CCV.	7.4.2, 7.4.5
QC Check	**Minimum Frequency**	**Acceptance Criteria**	**Corrective Action**	**Flagging Criteria**	**Comments**	**SOP SECTION**
Instrument Blanks	Immediately following the highest standard analyzed and daily prior to sample	Concentration of each analyte must be ≤ ½ the LOQ. Instrument Blank	If acceptance criteria are not met after	Flagging is only appropriate in cases when	No samples shall be analyzed until instrument blank has met acceptance criteria.	7.4.3

	analysis.	must contain EIS to enable quantitation of contamination.	the highest calibration standard, calibration must be performed using a lower concentration for the highest standard until acceptance criteria is met. If sample concentrations exceed the highest allowed standard and the sample(s) following exceed this acceptance criteria (>1/2 LOQ), they must be reanalyzed.	the sample cannot be reanalyzed and when there is no more sample left.	Note: Successful analysis following the highest standard analyzed determines the highest concentration that carryover does not occur. When the highest standard analyzed is not part of the calibration curve, it cannot be used to extend out the calibration range, it is used only to document a higher concentration at which carryover still does not occur.	
QC Check	**Minimum Frequency**	**Acceptance Criteria**	**Corrective Action**	**Flagging Criteria**	**Comments**	**SOP SECTION**
Extracted Internal Standard (EIS) Analytes	Every field sample, standard, blank, and QC sample.	Added to solid sample prior to extraction. Added to aqueous samples, into the original container, prior to extraction. For aqueous samples prepared by serial dilution instead of SPE, added to final dilution of samples prior to analysis. Extracted Internal Standard Analyte recoveries must be within 50% to 150% of ICAL	Correct problem. If required, re-extract and reanalyze associated field and QC samples. If recoveries are acceptable for QC samples, but not field samples, the field samples must be re-extracted	Apply Q-flag and discuss in the Case Narrative only if reanalysis confirms failures in exactly the same manner.	Failing analytes shall be thoroughly documented in the Case Narrative. EIS should be 96% (or greater) purity. When the impurity consists of the unlabeled analyte, the EIS can result in a background artifact in every sample, standard and blank, if the EIS is fortified at excessive concentrations.	8.2.7, 8.2.10

		midpoint standard area or area measured in the initial CCV on days when an ICAL is not performed.	and analyzed (greater dilution may be needed). Samples may be re-extracted and analyzed outside of hold times, as necessary for corrective action associated with QC failure.			
Method Blank (MB)	One per preparatory batch.	No analytes detected >½ LOQ or > 1/10th the amount measured in any sample or 1/10th the regulatory limit, whichever is greater.	Correct problem. If required, re-extract and reanalyze MB and all QC samples and field samples processed with the contaminated blank. Samples may be re-extracted and analyzed outside of hold times, as necessary for corrective action associated with QC failure. Examine the project-specific requirements. Contact the client as	If reanalysis cannot be performed, data must be qualified and explained in the Case Narrative. Apply B-flag to all results for the specific analyte(s) in all samples in the associated preparatory batch.	Results may not be reported without a valid MB. Flagging is only appropriate in cases where the samples cannot be reanalyzed.	8.2.1

			to additional measures to be taken.			
QC Check	**Minimum Frequency**	**Acceptance Criteria**	**Corrective Action**	**Flagging Criteria**	**Comments**	**SOP SECTION**
Laboratory Control Sample (LCS)	One per preparatory batch.	Blank spiked with all analytes at a concentration ≥ LOQ and ≤ the mid-level calibration concentration. A laboratory must use the DoD/DOE QSM Appendix B Limits for batch control if project limits are not specified. If the analyte(s) are not listed, use in-house LCS limits if project limits are not specified.	Correct problem, then re-extract and reanalyze the LCS and all samples in the associated preparatory batch for failed analytes if sufficient sample material is available. Samples may be re-extracted and analyzed outside of hold times, as necessary for corrective action associated with QC failure. Examine the project-specific requirements. Contact the client as to additional measures to be taken.	If reanalysis cannot be performed, data must be qualified and explained in the Case Narrative. Apply Q-flag to specific analyte(s) in all samples in the associated preparatory batch.	Results may not be reported without a valid LCS. Flagging is only appropriate in cases where the samples cannot be reanalyzed.	8.2.2
Matrix Spike (MS)	One per preparatory batch. Not required for aqueous samples prepared by serial dilution instead of SPE.	Sample spiked with all analytes at a concentration ≥ LOQ and ≤ the mid-level calibration concentration.	Examine the project-specific requirements. Contact the client as to additional	For the specific analyte(s) in the parent sample, apply J-flag if acceptance criteria are not	For matrix evaluation only. If MS results are outside the limits, the data shall be evaluated to determine the source(s) of difference (i.e., matrix effect or analytical error).	8.2.3

		A laboratory must use the DoD/DOE QSM Appendix B Limits for batch control if project limits are not specified. If the analyte(s) are not listed, use in-house LCS limits if project limits are not specified.	measures to be taken.	met and explain in the Case Narrative.		
QC Check	**Minimum Frequency**	**Acceptance Criteria**	**Corrective Action**	**Flagging Criteria**	**Comments**	**SOP SECTION**
Matrix Spike Duplicate (MSD) or Matrix Duplicate (MD)	For MSD: One per preparatory batch. For MD: Each aqueous sample prepared by serial dilution instead of SPE.	For MSD: Sample spiked with all analytes at a concentration ≥ LOQ and ≤ the mid-level calibration concentration. A laboratory must use the DoD/DOE QSM Appendix B Limits for batch control if project limits are not specified. If the analyte(s) are not listed, use in-house LCS limits if project limits are not specified. RPD ≤ 30% (between MS and MSD or sample and MD).	Examine the project-specific requirements. Contact the client as to additional measures to be taken.	For the specific analyte(s) in the parent sample, apply J-flag if acceptance criteria are not met and explain in the Case Narrative.	The data shall be evaluated to determine the source of difference. For Sample/MD: RPD criteria only apply to analytes whose concentration in the sample is ≥ LOQ. The MD is a second aliquot of the field sample that has been prepared by serial dilution.	8.2.4
Post Spike Sample	Only applies to aqueous samples prepared by serial dilution instead of SPE that have reported value of < LOQ for analyte(s).	Spike all analytes reported as < LOQ into the dilution that the result for that analyte is reported from. The spike must be at the LOQ concentration to be reported for this sample as < LOQ. When analyte concentrations are calculated as < LOQ, the post spike	When analyte concentrations are calculated as < LOQ, and the spike recovery does not meet the acceptance criteria, the sample,	Flagging is not appropriate.	When analyte concentrations are calculated as < LOQ, results may not be reported without acceptable post spike recoveries.	N/A

		for that analyte must recover within 70-130% of its true value.	sample duplicate, and post spike sample must be reanalyzed at consecutively higher dilutions until the criteria is met.			

APPENDIX E

CTDEEP STATEWIDE PFAS INITIATIVE
SAMPLING OF PUBLICLY OWNED TREATMENT WORKS

SENSITIVITY TABLE - PFAS

Parameter	Acronym	CAS No.	Aqueous SOP 455 LOQ ng/L	Aqueous SOP 455 DL ng/L	Solid SOP 467 LOQ ug/kg	Solid SOP 467 DL ug/kg	Tissue MIN4-0178 (2 g) LOQ ug/kg	Tissue MIN4-0178 (2 g) DL ug/kg
Perfluoroalkyl carboxylic acid (PFCA)								
Perfluorobutanoic acid	PFBA	375-22-4	2	0.57	1	0.34	0.250	0.0458
Perfluoropentanoic acid	PFPeA	2706-90-3	2	0.66	1	0.08	0.250	0.0490
Perfluorohexanoic acid	PFHxA	307-24-4	2	0.75	1	0.19	0.250	0.0695
Perfluoroheptanoic acid	PFHpA	375-85-9	2	0.62	1	0.23	0.250	0.0906
Perfluorooctanoic acid	PFOA	335-67-1	2	0.38	1	0.15	0.250	0.0581
Perfluorononoic acid	PFNA	375-95-1	2	0.48	1	0.17	0.250	0.0294
Perfluorodecanoic acid	PFDA	335-76-2	2	0.38	1	0.16	0.250	0.106
Perfluoroundecanoic acid	PFUnA	2058-94-8	2	0.49	1	0.22	0.250	0.0644
Perfluorododecanoic acid	PFDoA	307-55-1	2	0.29	1	0.10	0.250	0.0738
Perfluorotridecanoic acid	PFTrDA	72629-94-8	2	1.20	1	0.23	0.250	0.0502
Perfluorotetradecanoic acid	PFTA	376-06-7	2	0.82	1	0.27	0.250	0.0706
Perfluorohexadecanoic acid	PFHxDA	67905-19-5					0.250	0.0317
Perfluorooctandecanoic acid	PFODA	16517-11-6					0.250	0.0542
Perfluoroalkane sulfonic acid (PFSA)								
Perfluorobutanesulfonic acid	PFBS	375-73-5	2	0.32	1	0.15	0.221	0.0429
Perfluoropentanesulfonic acid	PFPeS	2706-91-4	2	0.51	1	0.26	0.235	0.0575
Perfluorohexanesulfonic acid	PFHxS	355-46-4	2	0.58	1	0.22	0.228	0.0437
Perfluoroheptanesulfonic acid	PFHpS	375-92-8	2	1.30	1	0.54	0.238	0.0338
Perfluorooctanesulfonic acid	PFOS	1763-23-1	2	0.38	1	0.18	0.231	0.0338
Perfluorononesulfonic acid	PFNS	68259-12-1	2	0.89	1	0.40	0.240	0.0475
Perfluorodecanesulfonic acid	PFDS	335-77-3	2	0.60	1	0.41	0.241	0.0395
Perfluorododecanesulfonic acid	PFDoS	79780-39-5					0.243	0.0294
Perfluoroalkane sulfomides (FASA) and derivatives								
Perfluorooctanesulfomide	PFOSA	754-91-6	2	0.44	1	0.22	0.250	0.0338
N-ethyl perfluorooctane sulfomidoethanol	NEtFOSE	1691-99-2					0.250	0.0483
N-methyl perfluorooctane sulfomidoethanol	NMeFOSE	24448-09-7					0.250	0.0497
N-ethyl perfluorooctane sulfomide	NEtFOSA	4151-50-2					0.250	0.0338
N-methyl perfluorooctane sulfomide	NMeFOSA	31506-32-8					0.250	0.0377
N-ethyl perfluorooctanesulfomidoacetic acid	NEtFOSAA	2991-50-6	2	0.93	1	0.34	0.250	0.0829
N-methyl perfluorooctanesulfomidoacetic acid	NMeFOSAA	2355-31-9	2	0.96	1	0.25	0.250	0.0251
Fluorotelomer sulfonic acid (FTSA)								
4:2 Fluorotelomer sulfonic acid	4:2 FTS	757124-72-4	2	1.00	1	0.22	0.234	0.0421
6:2 Fluorotelomer sulfonic acid	6:2 FTS	27619-97-2	2	1.10	1	0.24	0.238	0.113
8:2 Fluorotelomer sulfonic acid	8:2 FTS	39108-34-4	2	1.40	1	0.49	0.241	0.0913
10:2 Fluorotelomer sulfonic acid	10:2 FTS	120226-60-0					0.241	0.0410
Perfluoroalkyl ether carboxylic acid (PFECA)								
Perfluoro-3-methoxypropanoic acid	PFMPA	377-73-1	2	0.37	1	0.23		
Perfluoro-4-methoxybutanoic acid	PFMBA	863090-89-5	2	0.65	1	0.09		
Hexafluoropropylene oxide dimer acid [1/]	HFPO-DA	13252-13-6	2	1.60	2	0.95	0.250	0.250
Nofluoro-3,6-dioxaheptanoic acid	NFDHA	151772-58-6	2	0.53	1	0.26		
4,8-dioxa-3H-perfluorononoic acid	ADONA	919005-14-4	2	0.60	1	0.13	0.236	0.0655
Polyfluoroalkyl ether sulfonic acid (PFESA)								
Perfluoro(2-ethoxyethane)sulfonic acid	PFEESA	113507-82-7	2	0.36	1	0.07		
9-Chlorohexadecafluoro-3-oxanone-1-sulfonic acid	9Cl-PF3ONS	756426-58-1	2	0.36	1	0.15	0.233	0.0305
11-chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	11Cl-PF3OUdS	763051-92-9	2	0.54	1	0.20	0.235	0.0358
Perfluoro-1-butanesulfonamide	FBSA	30334-69-1	2	0.55	1	0.12		
Perfluoro-1-hexanesulfonamide	FHxSA	41997-13-1	2	0.70	1	0.16		

(shaded) Compound specified in EPA Method 537.

Notes:
LOQ Limit of Quantitation
DL Detection Limit
ng/L nanograms per liter
ug/kg micrograms per kilogram
1/ The lab will report this compound in biota to the LOQ. Because of the unique biota reference matrix used for batch QC, the labeled EIS used (13C3-HFPO-DA) may exhibit poor recovery in the blank and LCS results. The lab uses canola oil as the reference matrix for batch QC, in which HFPO-DA is known to exhibit poor recovery. We anticipate acceptable recovery of 13C3-HFPO-DA in the actual tissue samples.