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Quality Assurance Project Plan:

Public Drinking Water Per- and Polyfluoroalkyl Substances (PFAS) Sampling and Analysis

Version 1.2

July 23, 2021

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Quality Assurance Project Plan:

Public Drinking Water Per- and Polyfluoroalkyl Substances (PFAS) Sampling and Analysis

July 23, 2021

Prepared by:
Wisconsin Department of Natural Resources
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SECTION A. PROJECT MANAGEMENT

A1. Approval Sheet

DocuSigned by:
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Darsi Foss, Division Administrator, WI DNR, Environmental Management Division

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Steven Elmore, Program Director, WI DNR Bureau of Drinking Water and Groundwater

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Adam DeWeese
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Adam DeWeese, Project Manager

Chief, WI DNR Public Water Supply Section, Bureau of Drinking Water and Groundwater

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Constantine Tsoris
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Constantine Tsoris, Project QA Officer

WI DNR Public Water Supply Section, Bureau of Drinking Water and Groundwater

KIMBERLY HARRIS

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Kim Harris, U.S. EPA, Multi-Media PFAS Advisor

JACQUELINE RIVERA

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Date:

Jackie Adams, U.S. EPA, Region 5 Quality Program Manager

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A2. Table of Contents

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Abbreviations and Acronyms

CAS – chemical abstracts service

DHS – Wisconsin Department of Health Services

DNR – Wisconsin Department of Natural Resources

DWS – Drinking Water System [database]

FRB – field reagent blank

LDES – Laboratory Data Entry System

MDL – method detection limit

MRL – method reporting limit

PWS – public water system

QAPP – quality assurance project plan

QA – quality assurance

QC – quality control

PFAS – per- and polyfluoroalkyl substances

WSLH – Wisconsin State Laboratory of Hygiene

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A3. Distribution List and Organization

The final QAPP will be provided to appropriate project staff by email. If the plan is updated, each person on the distribution list will be sent an email with the most current document. The most current date of revision will be included in the document name and in the header of the document. The most current document will also be maintained on a SharePoint site accessible to all project staff.

Name/Title	Contact E-mail
Jackie Adams, U.S. EPA, Region 5 Quality Program Manager	Adams.Jacqueline@epa.gov
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Kyle Burke, Organics QA Officer, Wisconsin State Lab of Hygiene	Kyle.Burke@slh.wisc.edu

A4. Project/Task Organization

Project roles and responsibilities are outlined below in the following table and organizational chart.

Role	Name/Title	Contact E-mail
Project Manager	Adam DeWeese	Adam.DeWeese@wisconsin.gov
Project Coordinator	Courtney Botelho	Courtney.Botelho@wisconsin.gov
Response Manager	Constantine Tsoris	constantine.tsoris@wisconsin.gov
Project QA Officer	Constantine Tsoris	constantine.tsoris@wisconsin.gov
Laboratory QA Officer	Kyle Burke	Kyle.Burke@slh.wisc.edu
IT Manager	Kathy Mooney	Kathleen.Mooney@wisconsin.gov

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Project Manager and Project Coordinator

Responsible for oversight and coordination between the project members. Organization of information, data, and project reviews, including all deviations from QAPP and standard operating procedure (SOP) protocols. Distribution and maintenance of QAPP. Coordinates data approval, verification, and distribution to relevant parties. Ensure proper document retention for sampling conducted by WI DNR. Authorized to review and edit QAPP. Immediately notified on any issues with drinking water systems denying access for sampling.

Response Manager

Responsible for reviewing analytical results and recommending response strategies; providing technical assistance to Public Water Systems (PWS), coordination of communication to PWSs and WI DNR regional offices. Assist in response and follow-up sampling coordination/design for public water systems.

IT Manager

Responsible for secure and accurate electronic records and data management. Oversight and troubleshooting of data flow from the laboratory into WI DNR databases.

QA Officers

Responsible for input and maintenance of QA documents, including this QAPP. Review and approval of all QAPP changes and signatures. Notified on all significant deviations of QAPP or protocols that impact data credibility or usability. Review of QA processes and data verification. The laboratory QA officer is Kyle Burke of WSLH, who will not be the analyst processing any of the project samples.

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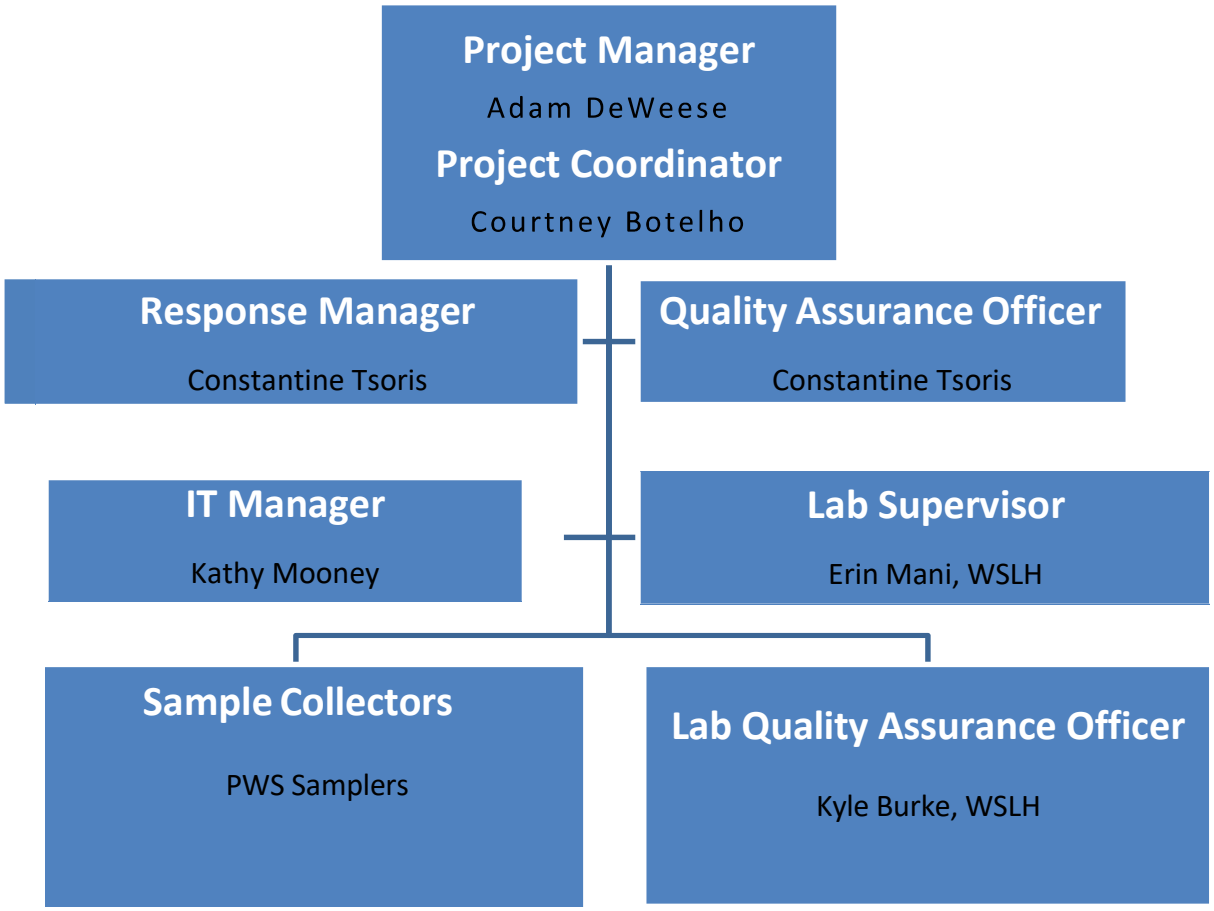
Figure 1: Organizational Chart



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A5. Problem Definition/Background

Per- and polyfluoroalkyl substances (PFAS) are a group of synthetic chemicals that have been in use since the 1940s. PFAS are found in a wide variety of consumer and industrial products. PFAS manufacturing and processing facilities, facilities using PFAS in manufacturing of other products, airports, and military installations are some of the known or suspected contributors of PFAS releases into the air, soil, and water. Due to their widespread use and persistence in the environment, most people in the United States have been exposed to PFAS. There is evidence that continued exposure above specific levels to certain PFAS may lead to adverse health effects.

The Wisconsin Department of Health Services (DHS) has recommended health-based standards for PFAS in groundwater, which includes drinking water uses. This includes recommendations for two PFAS in the Cycle 10 review as well as recommendations for an additional 16 PFAS in the Cycle 11 review. The Wisconsin Department of Natural Resources (DNR) is proceeding with rulemaking processes to regulate these 18 PFAS (Cycle 10 and Cycle 11 combined) in groundwater and drinking water. On December 16, 2020, the Wisconsin PFAS Action Council delivered the Wisconsin PFAS Action Plan to Governor Evers. The plan recommends that the state conduct statewide PFAS sampling of public drinking water systems to determine if PFAS are present in drinking water, a prominent way people may be exposed to PFAS.

Interpreting data from analysis of PFAS in a variety of environmental sample types can be challenging due to variations in analytical protocols, quality control types and criteria, data review procedures across laboratories, and general ubiquity in the environment. Stringent quality control is needed to ensure data quality and reliability to inform decisions regarding site specific actions. This document outlines the level of quality control necessary to conduct PFAS sampling and analysis for public health decision making purposes.

A6. Project Description

The purpose of the sampling project is to begin to characterize the state-wide occurrence of PFAS in drinking water. This will be done by sampling at least 90 municipal public drinking water systems. The possibility of extending the project to additional systems may be considered, pending factors including availability of funds. Information and procedures employed during this project will be provided to municipal systems wishing to voluntarily sample for PFAS.

The project will use EPA Method 537.1 to analyze for PFAS in drinking water. This method tests for 18 PFAS in total, including 13 of the PFAS for which DHS has recommended health-based standards. All results will include PFAS detected at or above method detection limits (MDLs) (Appendix A), which are at least one order of magnitude lower than the respective recommended health-based standard for each substance. This project will use the Wisconsin State Laboratory of Hygiene (WSLH) as the project laboratory.

Sampling efforts will be coordinated by DNR in partnership with the WSLH. WSLH will conduct analyses in accordance with this Quality Assurance Project Plan (QAPP) and EPA Method 537.1. PFAS results will be reported via the state's electronic drinking water reporting application and will be available online in DNR's Drinking Water System (DWS) database to allow for public access to the data.

PWS operators will collect samples at entry points comprising water from all source wells serving the utility, with a detailed protocol provided by DNR. If any PFAS are detected in a field sample, the accompanying field reagent blank (FRB) will be analyzed.

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Samples of drinking water will be collected from finished water entry point sources, consistent with safe drinking water regulatory monitoring requirements, from at least 90 municipal water systems. Selection of the systems to be sampled is described in Appendix C. At the selected municipal water systems, one sample will be collected from every distribution system entry point. Standard protocols are cited within this document to ensure consistent methodologies are followed to minimize variability in results.

Results from the initial round of sampling will be evaluated by DNR and additional sampling at the PWSs may be conducted to explore additional well impacts based on the information gathered in the initial round of sampling. All sampling will be completed in accordance with this QAPP.

General Project Schedule

Activity	Date	Participants
Phase 1 sampling	August 2021 – February 2022	Municipal water systems/WSLH/DNR
Phase 2 sampling (follow-up sampling on health recommendation exceedances)	October 2021 – December 2022	Municipal water systems/WSLH/DNR
Quarterly Reports	November 2021, February 2022, May 2022	DNR
Project Summary Report	July 2023	DNR

A7. Quality Objectives and Criteria

Management decisions regarding PFAS in drinking water are based on the ability to reliably detect and quantify PFAS in drinking water. The possibility of outside contamination of samples is high for PFAS, while the DHS recommended standards vary by compound (Appendix A) with some being as low as tens of parts per trillion. To reliably achieve such low analytical detection and to assure samples are free of outside contamination, sampling and analysis protocols that adhere to strict criteria are required. The generation of quality data therefore is a process that relies on planning at the outset of the sampling project. Data verification may identify potential sampling and analysis errors, such as sample handling procedures, which are out of conformance with data quality objectives.

DNR worked with the WSLH to develop this QAPP and the Planning and Sampling Protocol (Appendix D), which will ensure that samples are collected to accurately represent water quality for the analyzed PFAS at each select municipal water system entry point. WSLH has quality management procedures in place. Quality control data will be generated in conformance with EPA method 537.1 (Appendix B). These control data, along with the results from the data verification checklist (Appendix E), provide measures of accuracy and precision and whether good laboratory management was practiced. WSLH will evaluate the data generated for the project, note any quality system issues, and generate a report that will include measures of quality control that demonstrate the acceptability of the data.

Quality control (QC) samples will be collected, prepared and analyzed in accordance with EPA Method 537.1 (Appendix B). For each field sample collected from a municipal water system entry point, enough sample volume will be collected to allow for the QC analyses specified in EPA Method 537.1 with each extraction batch (i.e. one Field Duplicate or Laboratory Fortified Sample Matrix Duplicate).

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Data will be acceptable if 1) approved protocols (Appendix D) are followed to minimize outside contamination, 2) appropriate QC samples are performed to minimize contamination from either the laboratory or sampling methodology and to regularly check laboratory precision, 3) data generated can be verified or validated through the established procedures listed in Section D of this QAPP, and 4) the method detection limits are below the specified method reporting limits (MRLs).

DNR has identified the number of municipal system entry points to be sampled during the project. Representative drinking water samples will be collected as described in the sampling protocol (Appendix D). The number of identified entry point samples will be evaluated comparatively with the number of validated sample results to measure completeness.

A8. Special Training/Certifications

Samplers from the selected municipal drinking water systems will be trained to ensure they follow established PFAS planning and sampling protocols (Appendix D) to minimize PFAS contamination during sampling. Samplers will be trained via videoconference meeting and online prepared video. If WSLH and DNR staff are not satisfied that QC criteria have been met, additional training will be provided and resampling will occur. The WSLH is Wisconsin certified to conduct PFAS analysis by EPA Method 537.1.

A9. Documents and Records

The final QAPP will be provided to the appropriate project staff by email. If the plan is updated, each person on the distribution list will be sent an email with the most current document. The most current date of revision will be included in the document name and in the header of the document. The most current document will also be maintained on a SharePoint site accessible to all project staff.

The sample submission form will be maintained in its received form by WSLH and copies will be available to DNR upon request. For samples sent to any other project laboratory, the sample submission forms will either be filed as above or sent to and filed by DNR. Records of sample analytical results will be maintained in laboratory and DNR databases. These databases are backed up on secure servers. Analytical results will be submitted to DNR electronically through the Laboratory Data Entry System (LDES). Electronic transmittal will minimize transcription errors that could occur from transferring results manually into databases. Data will be stored in the DWS database, which will ease retrieval.

The format for all data recording will be consistent with the requirements and procedures used for data assessment, verification and validation described in this QAPP. All communications regarding project plan changes or refinements, such as changes to water systems, staff and parameters, will be filed in the SharePoint project file. DNR will provide updates to WSLH and municipal water system samplers as necessary.

Document/record control

The recording media for the project will be a combination of paper and electronic means to document site conditions. Data gathered on paper will be recorded using ballpoint pens or pencil, and changes to such data records will be made by drawing a single line through the error with an initial by the responsible person. Similar methods will be used for electronic data recording.

DNR program management will approve any updates to the QAPP, as needed. Project staff will retain copies of all management reports, memoranda, and all correspondence between team members.

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Retention of records will emphasize any deviations from the approved QAPP, including the rationale for those changes.

Document storage
Project staff will maintain a central project file, uploaded to a SharePoint folder, that will act as a repository for all documents collected or generated as part of this project. The project file will include both hardcopy and electronic data and will be stored at the DNR office. All files will be retained by DNR according to the DNR records retention policy. Municipal systems retain chemical analytical results for a period of 10 years. WSLH will maintain documents and records that demonstrate compliance with Wisconsin administrative code chapter NR 149 for a minimum of three years.

SECTION B. DATA GENERATION AND ACQUISITION

B1. Sampling Process Design

Types and numbers of samples required
The number of samples will vary depending on the municipal water system, specifically by the number of finished water distribution system entry points. Samples will be discrete grab samples from each entry point, with an FRB collected along with each sample. To minimize air-water interface effects on PFAS concentrations, samples will be collected late in the source well pumping cycle for each entry point and sample tap water will be run until water temperature has stabilized as indicated in EPA Method 537.1 Section 8.2.2 (Appendix B). Collected water will be preserved by cold storage and Trizma, as specified in the Planning and Sampling Protocol (Appendix D). Any other type of sample will be documented on the laboratory sample forms.

Sample locations and matrices
Finished water samples will be collected at the designated entry point tap, which is a location in the PWS after treatment or chemical addition, but before the distribution system. The samples will be designated as drinking water. FRBs and other quality control samples will be designated as water.

B2. Sampling Methods

All sampling methods and procedures shall adhere to those specified in EPA Method 537.1 (Appendix B) and the sampling protocol (Appendix D). The monitoring and laboratory sample form (Appendix F) will be filled out to document collection of each sample.

B3. Sample Handling

Samplers will be provided with the Planning and Sampling Protocol document (Appendix D). This document outlines good sampling practices including hand washing, wearing gloves, and how to handle the sampling containers. It gives instructions on how to plan for sampling and properly sample the Field Reagent Blank and the sample. It also lists items to be avoided during sampling to minimize potential sources of contamination. Finally, it instructs the sampler how to send the sample kit back to the designated laboratory. Sample handling at the laboratory is described in the WSLH laboratory SOP *Analysis of PFAS in Drinking Water by HPLC-MS/MS – EPA Method 537.1* (Appendix G).

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B4. Analytical Methods

The WSLH will use EPA Method 537.1 (Appendix B). A 30-day turnaround time is the goal for processing and reporting of sample results. Planning of PWS sampling, including mailing of sampling kits and instructions for sampling, will be done in partnership with WSLH to make use of available capacity for PFAS analysis, without creating a sample backlog.

B5. Quality Control

Quality control samples will be performed in accordance with EPA Method 537.1 (Appendix B).

B6. Instrument/Equipment Testing, Calibration, Inspection and Maintenance

All procedures will conform to EPA Method 537.1 (Appendix B) and manufacturer's calibration, inspection and maintenance recommendations.

B7. Instrument/Equipment Calibration and Frequency

Calibration will be conducted according to EPA Method 537.1. WSLH instrument calibration information is provided in Appendix G (Analysis of PFAS in Drinking Water by HPLC-MS/MS – EPA Method 537.1).

B8. Supplies and Consumables

Supplies and consumables for sampling are described in Appendix D and for laboratory analysis in Appendix G (Analysis of PFAS in Drinking Water by HPLC-MS/MS – EPA Method 537.1). WSLH will be responsible for inspecting and checking supplies and consumables associated with sampling and analytical procedures.

B9. Data Management

Each PFAS data result obtained from a PWS will be identified by PWS ID and sample ID and will be submitted to the laboratory by the samplers. Analytical results will be submitted to DNR electronically and DNR will upload results to the DWS database. The laboratory will strive to report analytical results within 30 days of receiving the sample. **The data generated from this project are not compliance data and will be designated as investigative.**

Field sampling data form entries will include the following information at a minimum:

- Project name
- PWS ID and sampling location
- Sampler name(s)
- PWS contact(s)
- Samples and QC samples collected (Entry Point drinking water samples, FRBs)
- ID, time and date for each sample on the sample collection (lab slip) form
- Stage in the pumping cycle for raw water source(s) to the Entry Point
- Field observations and any issues encountered, including any significant deviations from the sampling protocol or QAPP
- Any site visitors and associated interaction

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Laboratory Data Management
The data will be maintained in an electronic or hard-copy format. All material records will be maintained for the duration of the project.

Data Management Summary
Project staff will maintain the project file in a dedicated folder on SharePoint. The objective is to have a complete record of all decisions about modifications of data collection, assessment, verification, validation, or interpretation between the QAPP signoff and project report completion. Data received via LDES from WSLH will be stored in the DNR DWS database.

SECTION C: ASSESSMENT AND OVERSIGHT

C1. Assessments and Response Actions

Periodic assessment of PWS sample sites, field equipment and laboratory equipment are necessary to ensure that sampling is efficient, and data obtained meet quality objectives. This is an ongoing process that continues every day the project is implemented.

WSLH will prepare and send field kits weekly. DNR staff will review the scheduled sampling with WSLH weekly as well. These routine check-ins will cover topics including: 1) samples processed during the past week, 2) samples currently in the lab and outlook for the next week's analytical activities, and 3) field kits sent out for upcoming sampling. These check-ins will be done by the DNR Project Coordinator and WSLH Organics Supervisor and may also involve other WSLH and DNR project staff. The DNR Project Manager will be notified of any issues that cannot be addressed by procedures or criteria identified in this QAPP and its appendices.

Samples will be collected from Safe Drinking Water Act entry point taps, a controlled sampling environment. Sampling will be conducted or overseen by a certified water system operator. The sampler's name will be indicated on the Monitoring and Laboratory Sample Form (Appendix F) in the box "Sampler Phone/Name/Address". If necessary, an individual pre-sampling meeting will be conducted between the Project QA officer (or his designee) and the sampler.

A few business days after WSLH sends each round of field sample kits, the DNR Project Coordinator will call each municipal water system to which kits were sent for upcoming sampling and conduct a pre-sampling assessment. The pre-sampling assessment with each municipal water system will include the following:

- Verification that the sampler(s) attended the Zoom training session put on by DNR.
- Verification that a certified water system operator will collect the sample(s) or supervise collection of samples.
- Confirmation that the sampler(s) have reviewed site access information and obtained anything needed for site access.
- Confirmation that the sampler(s) have read the Planning and Sampling Protocol (Appendix D); an opportunity will be given to ask questions.
- Discussion of the instructions in the Planning and Sampling Protocol (Appendix D) to collect the samples during the last third of the pumping cycle for all source wells to the entry point, or as close as possible to this time window for all source wells.

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The DNR Project Coordinator will keep a spreadsheet, stored on the project SharePoint site and available to all DNR project staff, to record the date when each pre-sampling sampling assessment was conducted and confirm that all of the above points were addressed.

When samples are received at the lab, WSLH staff will review the Laboratory Sample Form (Appendix F) and assess applicable sample collection information. WSLH staff will also measure and record the sample temperature. If sample temperature does not meet the requirements of Method 537.1 Section 8.4 (Appendix B), the PWS will be sent a new kit by WSLH and asked to resample. The DNR Project Coordinator would follow-up with a phone call to review appropriate measures for cold storage during sample shipment.

After sample analysis, the WSLH analyst will complete the PFAS in Drinking Water Data Verification Checklist (Appendix E). This checklist will be stored at WSLH and available to DNR upon request. Any data qualifiers to the results will be input by the WSLH analyst to Horizon software and contained in the lab report and data upload to DNR's LDES. A second WSLH staff member will review each sample batch before release of the results.

Despite best preparations, assessments may find situations requiring corrective actions. Small day-to-day level assessment findings may commonly be addressed in communication between appropriate staff of the PWS, responsible laboratory and/or DNR.

WSLH staff are aware that response may be necessary (many of these will result in changes to the analytical reporting via data qualifiers and comments) if any of the following occur:

- QC data are outside acceptable limits for precision and accuracy
- Blanks contain target analytes above acceptable levels
- Undesirable trends are detected in spike recoveries or relative percent difference between duplicates
- There are unusual changes in detection limits
- Deficiencies are detected by the laboratory or DNR staff during any internal or external audits or from the results of performance evaluation samples
- Inquiries concerning data quality are received

WSLH analysts will report any of the above actions to the WSLH QA officer, who will consider the likelihood that the situation may affect the quality of the data and determine any necessary corrective action.

Laboratory corrective actions will follow regular laboratory procedures. Any laboratory corrective action with the potential to affect data quality will be communicated in a timely manner to DNR staff. The laboratory will evaluate if data require any additional qualifiers and/or if the data are usable for their originally intended purpose.

Laboratory staff and field samplers will notify DNR staff of any deviations from the QAPP or the sampling protocols.

Data Completeness

Overall success of the project relies on useable data results. Potential data gaps will be monitored as the project progresses, and the schedule will be revised to fill these gaps where they are determined to be significant or to potentially impact the fulfillment of project objectives. Follow-up samples will be utilized if laboratory staff or DNR staff are not confident in the results.

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C2. Reports to Management

Reports will be generated by DNR staff and will include, but not be limited to, sample schedules, summary of laboratory analyses performed and technical support to samplers. Once sampling has commenced, DNR staff will provide periodic updates to the appropriate managers in the Drinking Water and Groundwater Program and Environmental Management Division.

Sample Results

Sample results shall be reported to the MDL and will include the MRL with each result. The results will be qualified when reported between the MDL and MRL as estimated concentrations. Analytical laboratory reports shall report the 18 analytes listed in EPA Method 537.1 (Appendix B). WSLH MDLs and MRLs are provided in Appendix A and Appendix G.

Data Management

Data shall be reported electronically using the LDES system. DNR staff will load the results to the DWS database. Full lab reports (in PDF format) will generally be sent directly to the public water system and DNR. Additionally, data will be submitted electronically directly to DNR for database upload. Data with qualifiers shall be appropriately flagged, particularly when detections are found in blanks at or above the MRL. PDFs of qualified data shall be submitted to the DNR in a timely manner when requested by DNR staff. Raw instrument data shall also be made available when requested.

SECTION D: DATA USABILITY

The analytical data generated during this project must be of sufficient quality to decide whether a PWS exceeds a DHS-recommended standard for any of the 13 PFAS with a Wisconsin groundwater standard recommendation from DHS (Appendix A). DNR and WSLH will implement and adhere to the following requirements:

1. A copy of the current certificate/letter of accreditation, issued to the laboratory by the Wisconsin laboratory accreditation program.

2. The results of the most recent proficiency testing sample study using the WSLH PFAS in Drinking Water SOP *Analysis of PFAS in Drinking Water by HPLC-MS/MS – EPA Method 537.1* (Appendix G). The proficiency testing sample provider must be approved by the Wisconsin laboratory accreditation program.

3. Audit reports from the most recent on-site inspection by the Wisconsin laboratory accreditation program issuing PFAS certification to WSLH. The on-site inspection must be completed by a U.S. EPA-certified Certification Officer.

4. WSLH must comply with the quality control requirements set forth in EPA Method 537.1 (Appendix B).

5. WSLH will provide verification that the data meet the quality control standards provided in EPA Method 537.1 (Appendix B). WSLH will use the data verification checklist provided in Appendix E. This checklist is completed once per analytical batch of samples. The completed checklist will be kept on file at WSLH and will be available to DNR upon request. All data will be compiled and processed according to the WSLH PFAS Drinking Water SOP, *Analysis of PFAS in Drinking Water*

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by HPLC-MS/MS – EPA Method 537.1 (Appendix G). The analyst responsible for sample preparation and data processing is also responsible for initial review of data against all quality control measures and qualification of data. All data are reviewed by a second analyst before they are finalized.

6. If concerns arise regarding data quality that cannot be addressed through the criteria listed above, DNR Bureau of Drinking Water and Groundwater staff will consult with DNR's Laboratory Certification program to resolve the issue and ensure project data quality objectives are met.

DNR staff will evaluate all components of the sampling process and analytical reports to determine whether the data quality objectives have been met and that data are appropriate as a basis for decisions regarding the presence of PFAS in public water systems.

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Appendix A: Analytes and Recommended Health-Based Standards

Compound Name	Abbreviation	CAS #	WSLH MDL (ng/L)	WSLH MRL (ng/L)	Health-based recommendation from DHS (ng/L)
Perfluoro-n-butanesulfonic acid	PFBS	375-73-5	0.576	1.00	450,000
Perfluoro-n-hexanesulfonic acid	PFHxS	355-46-4	0.666	1.00	40
Perfluoro-n-octanesulfonic acid	PFOS	1763-23-1	0.645	1.00	20*
N-methyl perfluorooctanesulfonamidoacetic acid	NMeFOSAA	2355-31-9	0.785	1.00	--
N-ethyl perfluorooctanesulfonamidoacetic acid	NEtFOSAA	2991-50-6	0.839	1.00	20*
Perfluoro-n-hexanoic acid	PFHxA	307-24-4	0.716	1.00	150,000
Perfluoro-n-heptanoic acid	PFHpA	375-85-9	0.733	1.00	--
Perfluoro-n-octanoic acid	PFOA	335-67-1	0.782	1.00	20*
Perfluoro-n-nonanoic acid	PFNA	375-95-1	0.709	1.00	30
Perfluoro-n-decanoic acid	PFDA	335-76-2	0.632	1.00	300
Perfluoro-n-undecanoic acid	PFUnA	2058-94-8	0.721	1.00	3,000
Perfluoro-n-dodecanoic acid	PFDoA	307-55-1	0.612	1.00	500
Perfluoro-n-tridecanoic acid	PFTrDA	72629-94-8	0.580	1.00	--
Perfluoro-n-tetradecanoic acid	PFTA	376-06-7	0.389	1.00	10,000
Hexafluoropropylene oxide dimer acid	HFPO-DA	13252-13-6	0.727	1.00	300
4,8-Dioxa-3H-perfluorononanoic acid	DONA	919005-14-4	0.792	1.00	3,000
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	9Cl-PF3ONS	756426-58-1	0.606	1.00	--
11-chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	11Cl-PF3OUdS	763051-92-9	0.628	1.00	--

Notes

CAS – chemical abstracts service

* DHS recommends that PFOA, PFOS, NEtFOSAA and NEtFOSE (and three other substances that are not EPA Method 537.1 analytes) not exceed 20 ng/L individually or combined.

Dashes (--) indicate that DHS did not recommend a standard for the applicable compound.

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Appendix B: EPA Method 537.1

The EPA Method 537.1 description is provided on the following 50 pages.

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EPA Document #: EPA/600/R-18/352

METHOD 537.1 DETERMINATION OF SELECTED PER- AND POLYFLUORINATED ALKYL SUBSTANCES IN DRINKING WATER BY SOLID PHASE EXTRACTION AND LIQUID CHROMATOGRAPHY/TANDEM MASS SPECTROMETRY (LC/MS/MS)

Version 1.0
November 2018

J.A. Shoemaker and D.R. Tettenhorst, Office of Research and Development

J.A. Shoemaker (Office of Research and Development), P.E. Grimmett (Office of Research and Development), B.K. Boutin (National Council on Aging), Method 537, Rev 1.1 (2009)

NATIONAL EXPOSURE RESEARCH LABORATORY
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METHOD 537.1

DETERMINATION OF SELECTED PER- AND POLYFLUORINATED ALKYL SUBSTANCES IN DRINKING WATER BY SOLID PHASE EXTRACTION AND LIQUID CHROMATOGRAPHY/TANDEM MASS SPECTROMETRY (LC/MS/MS)

1. SCOPE AND APPLICATION

1.1. This is a solid phase extraction (SPE) liquid chromatography/tandem mass spectrometry (LC/MS/MS) method for the determination of selected per- and polyfluorinated alkyl substances (PFAS) in drinking water. Accuracy and precision data have been generated in reagent water and drinking water for the compounds listed in the table below.

Analyte[a]	Acronym	Chemical Abstract Services Registry Number (CASRN)
Hexafluoropropylene oxide dimer acid	HFPO-DA	13252-13-6[b]
N-ethyl perfluorooctanesulfonamidoacetic acid	NEtFOSAA	2991-50-6
N-methyl perfluorooctanesulfonamidoacetic acid	NMeFOSAA	2355-31-9
Perfluorobutanesulfonic acid	PFBS	375-73-5
Perfluorodecanoic acid	PFDA	335-76-2
Perfluorododecanoic acid	PFDoA	307-55-1
Perfluoroheptanoic acid	PFHpA	375-85-9
Perfluorohexanesulfonic acid	PFHxS	355-46-4
Perfluorohexanoic acid	PFHxA	307-24-4
Perfluorononanoic acid	PFNA	375-95-1
Perfluorooctanesulfonic acid	PFOS	1763-23-1
Perfluorooctanoic acid	PFOA	335-67-1
Perfluorotetradecanoic acid	PFTA	376-06-7
Perfluorotridecanoic acid	PFTrDA	72629-94-8
Perfluoroundecanoic acid	PFUnA	2058-94-8
11-chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	11Cl-PF3OUdS	763051-92-9[c]
9-chlorohexadecafluoro-3-oxanone-1-sulfonic acid	9Cl-PF3ONS	756426-58-1[d]
4,8-dioxa-3H-perfluorononanoic acid	ADONA	919005-14-4[e]

[a] Some PFAS are commercially available as ammonium, sodium and potassium salts. This method measures all forms of the analytes as anions while the counterion is inconsequential. Analytes may be purchased as acids or as any of the corresponding salts (see Section 7.2.3 regarding correcting the analyte concentration for the salt content).
[b] HFPO-DA is one component of the GenX processing aid technology.
[c] 11Cl-PF3OUdS is available in salt form (e.g. CASRN of potassium salt is 83329-89-9).
[d] 9Cl-PF3ONS analyte is available in salt form (e.g. CASRN of potassium salt is 73606-19-6)
[e] ADONA is available as the sodium salt (no CASRN) and the ammonium salt (CASRN is 958445-448).

1.2. Minimum Reporting Level (MRL) is the lowest analyte concentration that meets Data Quality Objectives (DQOs) that are developed based on the intended use of this

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method. The single laboratory lowest concentration MRL (LCMRL) is the lowest true concentration for which the future recovery is predicted to fall, with high confidence (99%), between 50 and 150% recovery. Single laboratory LCMRLs for analytes in this method range from 0.53-6.3 ng/L, and are listed in Table 5. The procedure used to determine the LCMRL is described elsewhere.[1]

1.3. Laboratories using this method will not be required to determine the LCMRL for this method, but will need to demonstrate that their laboratory MRL for this method meets requirements described in Section 9.2.6.

1.4. Determining the Detection Limit (DL) for analytes in this method is optional (Sect. 9.2.8). Detection limit is defined as the statistically calculated minimum concentration that can be measured with 99% confidence that the reported value is greater than zero.[2] The DL is compound dependent and is dependent on extraction efficiency, sample matrix, fortification concentration, and instrument performance.

1.5. This method is intended for use by analysts skilled in solid phase extractions, the operation of LC/MS/MS instruments, and the interpretation of the associated data.

1.6. METHOD FLEXIBILITY – In recognition of technological advances in analytical systems and techniques, the laboratory is permitted to modify the evaporation technique, separation technique, LC column, mobile phase composition, LC conditions and MS and MS/MS conditions (Sect. 6.12, 9.1.1, 10.2, and 12.1). **Changes may not be made to sample collection and preservation (Sect. 8), the sample extraction steps (Sect. 11.4), or to the quality control requirements (Sect. 9).** Method modifications should be considered only to improve method performance. Modifications that are introduced in the interest of reducing cost or sample processing time, but result in poorer method performance, should not be used. Analytes must be adequately resolved chromatographically to permit the mass spectrometer to dwell on a minimum number of compounds eluting within a retention time window. Instrumental sensitivity (or signal-to-noise) will decrease if too many compounds are permitted to elute within a retention time window. In all cases where method modifications are proposed, the analyst must perform the procedures outlined in the initial demonstration of capability (IDC, Sect. 9.2), verify that all Quality Control (QC) acceptance criteria in this method (Sect. 9) are met, and that acceptable method performance can be verified in a real sample matrix (Sect. 9.3.6).

NOTE: The above method flexibility section is intended as an abbreviated summation of method flexibility. Sections 4-12 provide detailed information of specific portions of the method that may be modified. If there is any perceived conflict between the general method flexibility statement in Section 1.6 and specific information in Sections 4-12, Sections 4-12 supersede Section 1.6.

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2. SUMMARY OF METHOD

A 250-mL water sample is fortified with surrogates and passed through an SPE cartridge containing polystyrenedivinylbenzene (SDVB) to extract the method analytes and surrogates. The compounds are eluted from the solid phase sorbent with a small amount of methanol. The extract is concentrated to dryness with nitrogen in a heated water bath, and then adjusted to a 1-mL volume with 96:4% (vol/vol) methanol:water and addition of the internal standards. A 10-µL injection is made into an LC equipped with a C_{18} column that is interfaced to an MS/MS. The analytes are separated and identified by comparing the acquired mass spectra and retention times to reference spectra and retention times for calibration standards acquired under identical LC/MS/MS conditions. The concentration of each analyte is determined by using the internal standard technique. Surrogate analytes are added to all Field and QC Samples to monitor the extraction efficiency of the method analytes.

3. DEFINITIONS

3.1. ANALYSIS BATCH – A set of samples that is analyzed on the same instrument during a 24-hour period, including no more than 20 Field Samples, that begins and ends with the analysis of the appropriate Continuing Calibration Check (CCC) standards. Additional CCCs may be required depending on the length of the analysis batch and/or the number of Field Samples.

3.2. CALIBRATION STANDARD (CAL) – A solution prepared from the primary dilution standard solution and/or stock standard solution, internal standard(s), and the surrogate(s). The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.3. COLLISIONALLY ACTIVATED DISSOCIATION (CAD) – The process of converting the precursor ion's translational energy into internal energy by collisions with neutral gas molecules to bring about dissociation into product ions.

3.4. CONTINUING CALIBRATION CHECK (CCC) – A calibration standard containing the method analytes, internal standard(s) and surrogate(s). The CCC is analyzed periodically to verify the accuracy of the existing calibration for those analytes.

3.5. DETECTION LIMIT (DL) – The minimum concentration of an analyte that can be identified, measured, and reported with 99% confidence that the analyte concentration is greater than zero. This is a statistical determination of precision (Sect. 9.2.8), and accurate quantitation is not expected at this level.[2]

3.6. EXTRACTION BATCH – A set of up to 20 Field Samples (not including QC samples) extracted together by the same person(s) during a work day using the same lot of SPE devices, solvents, surrogate, internal standard and fortifying solutions. Required QC samples include Laboratory Reagent Blank, Laboratory Fortified Blank, Laboratory Fortified Sample Matrix, and either a Field Duplicate or Laboratory Fortified Sample Matrix Duplicate.

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3.7. FIELD DUPLICATES (FD1 and FD2) – Two separate samples collected at the same time and place under identical circumstances, and treated exactly the same throughout field and laboratory procedures. Analyses of FD1 and FD2 give a measure of the precision associated with sample collection, preservation, and storage, as well as laboratory procedures.

3.8. FIELD REAGENT BLANK (FRB) – An aliquot of reagent water that is placed in a sample container in the laboratory and treated as a sample in all respects, including shipment to the sampling site, exposure to sampling site conditions, storage, preservation, and all analytical procedures. The purpose of the FRB is to determine if method analytes or other interferences are present in the field environment.

3.9. INTERNAL STANDARD (IS) – A pure chemical added to an extract or standard solution in a known amount(s) and used to measure the relative response of other method analytes and surrogates that are components of the same solution. The internal standard must be a chemical that is structurally similar to the method analytes, has no potential to be present in water samples, and is not a method analyte.

3.10. LABORATORY FORTIFIED BLANK (LFB) – A volume of reagent water or other blank matrix to which known quantities of the method analytes and all the preservation compounds are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements.

3.11. LABORATORY FORTIFIED SAMPLE MATRIX (LFSM) – A preserved field sample to which known quantities of the method analytes are added in the laboratory. The LFSM is processed and analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate sample extraction and the measured values in the LFSM corrected for background concentrations.

3.12. LABORATORY FORTIFIED SAMPLE MATRIX DUPLICATE (LFSMD) – A duplicate of the Field Sample used to prepare the LFSM. The LFSMD is fortified, extracted, and analyzed identically to the LFSM. The LFSMD is used instead of the Field Duplicate to assess method precision when the occurrence of method analytes is low.

3.13. LABORATORY REAGENT BLANK (LRB) – An aliquot of reagent water or other blank matrix that is treated exactly as a sample including exposure to all glassware, equipment, solvents and reagents, sample preservatives, internal standard, and surrogates that are used in the analysis batch. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

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3.14. LOWEST CONCENTRATION MINIMUM REPORTING LEVEL (LCMRL) – The single laboratory LCMRL is the lowest true concentration for which a future recovery is expected, with 99% confidence, to be between 50 and 150% recovery.[1]

3.15. MINIMUM REPORTING LEVEL (MRL) – The minimum concentration that can be reported as a quantitated value for a method analyte in a sample following analysis. This defined concentration can be no lower than the concentration of the lowest calibration standard for that analyte and can only be used if acceptable QC criteria for this standard are met. A procedure for verifying a laboratory's MRL is provided in Section 9.2.6.

3.16. 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*.

3.17. 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.

3.18. PRODUCT ION – For the purpose of this method, a product ion is one of the fragment ions produced in MS/MS by collisionally activated dissociation of the precursor ion.

3.19. QUALITATIVE STANDARD – A qualitative standard is a standard for which either the concentration is estimated or method analyte impurities exist at a concentration >1/3 of the MRL in the highest concentration calibration standard. For the purposes of this method, qualitative standards are used to identify retention times of branched isomers of method analytes and are not used for quantitation purposes.

3.20. QUALITY CONTROL SAMPLE (QCS) – A solution of method analytes of known concentrations that is obtained from a source external to the laboratory and different from the source of calibration standards. The second source SSS is used to fortify the QCS at a known concentration. The QCS is used to check calibration standard integrity.

3.21. QUANTITATIVE STANDARD – A quantitative standard is a standard of known concentration and purity. The quantitative standard must not contain any of the method analytes as impurities at concentrations >1/3 of the MRL in the highest concentration calibration standard.

3.22. SAFETY DATA SHEET (SDS) – Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire, and reactivity data including storage, spill, and handling precautions.

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3.23. STOCK STANDARD SOLUTION (SSS) – A concentrated solution containing one or more method analytes prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source.

3.24. SURROGATE ANALYTE (SUR) – A pure chemical which chemically resembles method analytes and is extremely unlikely to be found in any sample. This chemical is added to a sample aliquot in known amount(s) before processing and is measured with the same procedures used to measure other method analytes. The purpose of the SUR is to monitor method performance with each sample.

4. INTERFERENCES

4.1. All glassware must be meticulously cleaned. Wash glassware with detergent and tap water, rinse with tap water, followed by a reagent water rinse. Non-volumetric glassware can be heated in a muffle furnace at 400 °C for 2 h or solvent 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: Samples and extracts should not come in contact with any glass containers or pipettes as these analytes can potentially adsorb to glass surfaces. PFAS analyte, IS and SUR standards commercially purchased in glass ampoules are acceptable; however, all subsequent transfers or dilutions performed by the analyst must be prepared and stored in polypropylene containers.

4.2. 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 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.[3] All items such as these must be routinely demonstrated to be free from interferences (less than 1/3 the MRL for each method analyte) under the conditions of the analysis by analyzing laboratory reagent blanks as described in Section 9.3.1. **Subtracting blank values from sample results is not permitted.**

4.3. 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.[4-5] 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 total organic carbon (TOC) were not observed.

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4.4. Relatively large quantities of the preservative (Sect. 8.1.2) are added to sample bottles. The potential exists for trace-level organic contaminants in these reagents. Interferences from these sources should be monitored by analysis of laboratory reagent blanks (Sect. 9.3.1), particularly when new lots of reagents are acquired.

4.5. SPE cartridges can be a source of interferences. The analysis of field and laboratory reagent 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.

5. SAFETY

5.1. The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined. Each chemical should be treated as a potential health hazard, and exposure to these chemicals should be minimized. Each laboratory is responsible for maintaining an awareness of OSHA regulations regarding safe handling of chemicals used in this method. A reference file of SDSs should be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available.[6-8]

5.2. PFOA has been described as likely to be carcinogenic to humans.[9] Pure standard materials and stock standard solutions of these method analytes should be handled with suitable protection to skin and eyes, and care should be taken not to breathe the vapors or ingest the materials.

6. EQUIPMENT AND SUPPLIES

(Brand names and/or catalog numbers are included for illustration only, and do not imply endorsement of the product.) Due to potential adsorption of analytes onto glass, polypropylene containers were used for all standard, sample and extraction preparations. Other plastic materials (e.g., polyethylene) which meet the QC requirements of Section 9 may be substituted.

6.1. SAMPLE CONTAINERS – 250-mL polypropylene bottles fitted with polypropylene screw caps.

6.2. POLYPROPYLENE BOTTLES – 4-mL narrow-mouth polypropylene bottles (VWR Cat. No.: 16066-960 or equivalent).

6.3. CENTRIFUGE TUBES – 15-mL conical polypropylene tubes with polypropylene screw caps for storing standard solutions and for collection of the extracts (Thomas Scientific Cat. No.: 2602A10 or equivalent).

6.4. AUTOSAMPLER VIALS – Polypropylene 0.4-mL autosampler vials (ThermoFisher Cat. No.: C4000-11) with polypropylene caps (ThermoFisher Cat. No.: C5000-50 or equivalent).

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NOTE: Polypropylene vials and caps are necessary to prevent contamination of the sample from PTFE coated septa. However, polypropylene caps do not reseal, so evaporation occurs after injection. Thus, multiple injections from the same vial are not possible.

6.5. POLYPROPYLENE GRADUATED CYLINDERS – Suggested sizes include 25, 50, 100 and 1000-mL cylinders.

6.6. MICRO SYRINGES – Suggested sizes include 5, 10, 25, 50, 100, 250, 500 and 1000-µL syringes.

6.7. PLASTIC PIPETS – Polypropylene or polyethylene disposable pipets (Fisher Cat. No.: 13-711-7 or equivalent).

6.8. ANALYTICAL BALANCE – Capable of weighing to the nearest 0.0001 g.

6.9. SOLID PHASE EXTRACTION (SPE) APPARATUS FOR USING CARTRIDGES

6.9.1. SPE CARTRIDGES – 0.5 g, 6-mL SPE cartridges containing styrenedivinyl-benzene (SDVB) single polymer (copolymers not allowed) sorbent phase (Agilent Cat. No.: 1225-5021 or equivalent).

6.9.2. VACUUM EXTRACTION MANIFOLD – A manual vacuum manifold with Visiprep™ large volume sampler (Supelco Cat. No. 57030 and 57275 or equivalent) for cartridge extractions, or an automatic/robotic sample preparation system designed for use with SPE cartridges, may be used if all QC requirements discussed in Section 9 are met. Extraction and/or elution steps may not be changed or omitted to accommodate the use of an automated system. Care must be taken with automated SPE systems to ensure the PTFE commonly used in these systems does not contribute to unacceptable analyte concentrations in the LRB (Sect. 9.3.1).

6.9.3. SAMPLE DELIVERY SYSTEM – Use of a polypropylene transfer tube system, which transfers the sample directly from the sample container to the SPE cartridge, is recommended, but not mandatory. Standard extraction manifolds come equipped with PTFE transfer tube systems. These can be replaced with 1/8" O.D. x 1/16" I.D. polypropylene or polyethylene tubing (Hudson Extrusions LLDPE or equivalent) cut to an appropriate length to ensure no sample contamination from the sample transfer lines. Other types of non-PTFE tubing may be used provided it meets the LRB (Sect. 9.3.1) and LFB (Sect. 9.3.3) QC requirements. The PTFE transfer tubes may be used, but an LRB must be run on each PTFE transfer tube and the QC requirements in Section 9.3.1 must be met. In the case of automated SPE, the removal of PTFE lines may not be feasible; therefore, LRBs will need to be rotated among the ports and must meet the QC requirements of Sections 9.2.2 and 9.3.1.

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6.10. EXTRACT CONCENTRATION SYSTEM – Extracts are concentrated by evaporation with nitrogen using a water bath set no higher than 65 °C (Meyer N-Evap, Model 111, Organomation Associates, Inc. or equivalent).

6.11. LABORATORY OR ASPIRATOR VACUUM SYSTEM – Sufficient capacity to maintain a vacuum of approximately 10 to 15 inches of mercury for extraction cartridges.

6.12. LIQUID CHROMATOGRAPHY (LC)/TANDEM MASS SPECTROMETER (MS/MS) WITH DATA SYSTEM

6.12.1. LC SYSTEM – Instrument capable of reproducibly injecting up to 10-µL aliquots, and performing binary linear gradients at a constant flow rate near the flow rate used for development of this method (0.3 mL/min). The usage of a column heater is optional.

NOTE: During the course of method development, it was discovered that while idle for more than one day, PFAS built up in the PTFE solvent transfer lines. To prevent long delays in purging high levels of PFAS from the LC solvent lines, they were replaced with PEEK™ tubing and the PTFE solvent frits were replaced with stainless steel frits. It is not possible to remove all PFAS background contamination, but these measures help to minimize their background levels.

6.12.2. LC/TANDEM MASS SPECTROMETER – The LC/MS/MS must be capable of negative ion electrospray ionization (ESI) near the suggested LC flow rate of 0.3 mL/min. The system must be capable of performing MS/MS to produce unique product ions (Sect. 3.18) for the method analytes within specified retention time segments. A minimum of 10 scans across the chromatographic peak is required to ensure adequate precision. Data are demonstrated in Tables 5-9 using a triple quadrupole mass spectrometer (Waters XEVO TQMS). **See the Note in Sect. 10.2.3 pertaining to potential limitations of some MS/MS instrumentation in achieving the required MS/MS transitions.**

6.12.3. DATA SYSTEM – An interfaced data system is required to acquire, store, reduce, and output mass spectral data. The computer software should have the capability of processing stored LC/MS/MS data by recognizing an LC peak within any given retention time window. The software must allow integration of the ion abundance of any specific ion within specified time or scan number limits. The software must be able to calculate relative response factors, construct linear regressions or quadratic calibration curves, and calculate analyte concentrations.

6.12.4. ANALYTICAL COLUMN – An LC C_{18} column (2.1 x 150 mm) packed with 5 µm d_p C_{18} solid phase particles (Waters #: 186001301 or equivalent) was used. Any column that provides adequate resolution, peak shape, capacity, accuracy, and precision (Sect. 9) may be used.

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7. REAGENTS AND STANDARDS

7.1. GASES, REAGENTS, AND SOLVENTS – Reagent grade or better chemicals should be used. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. 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.

7.1.1. REAGENT WATER – Purified water which does not contain any measurable quantities of any method analytes or interfering compounds greater than 1/3 the MRL for each method analyte of interest. Prior to daily use, at least 3 L of reagent water should be flushed from the purification system to rinse out any build-up of analytes in the system's tubing.

7.1.2. METHANOL (CH_3OH, CAS#: 67-56-1) – High purity, demonstrated to be free of analytes and interferences (Fisher LC/MS grade or equivalent).

7.1.3. AMMONIUM ACETATE ($NH_4C_2H_3O_2$, CAS#: 631-61-8) – High purity, demonstrated to be free of analytes and interferences (Sigma-Aldrich ACS grade or equivalent).

7.1.4. 20 mM AMMONIUM ACETATE/REAGENT WATER – To prepare 1 L, add 1.54 g ammonium acetate to 1 L of reagent water. This solution is volatile and must be replaced at least once a week. More frequent replacement may be necessary if unexplained loss in sensitivity or retention time shifts are encountered and attributed to loss of the ammonium acetate.

7.1.5. TRIZMA® PRESET CRYSTALS, pH 7.0 (Sigma cat# T-7193 or equivalent) – Reagent grade. A premixed blend of Tris [Tris(hydroxymethyl)aminomethane] and Tris HCL [Tris(hydroxymethyl)aminomethane hydrochloride]. Alternatively, a mix of the two components with a weight ratio of 15.5/1 Tris HCL/Tris may be used. This blend is targeted to produce a pH near 7.0 at 25 °C in reagent water. Trizma® functions as a buffer, and removes free chlorine in chlorinated finished waters (Sect. 8.1.2).

7.1.6. NITROGEN – Used for the following purposes:

7.1.6.1. Nitrogen aids in aerosol generation of the ESI liquid spray and is used as collision gas in some MS/MS instruments. The nitrogen used should meet or exceed instrument manufacturer's specifications.

7.1.6.2. Nitrogen is used to concentrate sample extracts (Ultra High Purity or equivalent).

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7.1.7. ARGON – Used as collision gas in MS/MS instruments. Argon should meet or exceed instrument manufacturer's specifications. Nitrogen gas may be used as the collision gas provided sufficient sensitivity (product ion formation) is achieved.

7.2. STANDARD SOLUTIONS – When a compound purity is assayed to be 96% or greater, the weight can be used without correction to calculate the concentration of the stock standard. PFAS analyte, IS and SUR standards commercially purchased in glass ampoules are acceptable; however, all subsequent transfers or dilutions performed by the analyst must be prepared and stored in polypropylene containers. Solution concentrations listed in this section were used to develop this method and are included as an example. Alternate concentrations may be used as necessary depending on instrument sensitivity and the calibration range used. Standards for sample fortification generally should be prepared in the smallest volume that can be accurately measured to minimize the addition of excess organic solvent to aqueous samples. PDS and calibration standards were found to be stable for, at least, one month during method development. Laboratories should use standard QC practices to determine when standards need to be replaced. The target analyte manufacturer's guidelines may be helpful when making the determination.

NOTE: Stock standards (Sect. 7.2.1.1, 7.2.2.1 and 7.2.3.1) were stored at ≤4 °C. Primary dilution standards (Sect.7.2.1.2, 7.2.2.2 and 7.2.3.2) 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. However, standards may be stored cold provided the standards are allowed to come to room temperature and vortexed well prior to use.

7.2.1. INTERNAL (IS) STOCK STANDARD SOLUTIONS – This method uses three IS compounds listed in the table below. These isotopically labeled IS(s) were carefully chosen during method development because they encompass all the functional groups of the method analytes. Although alternate IS standards may be used provided they are isotopically labeled compounds with similar functional groups as the method analytes, the analyst must have documented reasons for using alternate IS(s). Alternate IS(s) must meet the QC requirements in Section 9.3.4. Note that different isotopic labels of the same IS(s) are acceptable (e.g., $^{13}C_2$-PFOA and $^{13}C_4$-PFOA) but will require modification of the MS/MS precursor and product ions.

Internal Standards	Acronym
Perfluoro-[1,2-$^{13}C_2$]octanoic acid	$^{13}C_2$-PFOA
Sodium perfluoro-1-[1,2,3,4-$^{13}C_4$]octanesulfonate	$^{13}C_4$-PFOS
N-deuteriomethylperfluoro-1-octanesulfonamidoacetic acid	d_3-NMeFOSAA

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7.2.1.1. IS STOCK STANDARD SOLUTIONS (IS SSS) – These IS stocks can be obtained as individual certified stock standard solutions. The ISs can also be purchased as PDSs, making the preparation of individual SSSs unnecessary. Analysis of the IS(s) is less complicated if the IS(s) purchased contains only the linear isomer.

7.2.1.2. INTERNAL STANDARD PRIMARY DILUTION (IS PDS) STANDARD (1-4 ng/µL) – Prepare, or purchase commercially, the IS PDS at a suggested concentration of 1-4 ng/µL. The IS PDS (in methanol with four molar equivalents of sodium hydroxide) was purchased from Wellington Labs. Alternatively, the IS PDS can be prepared in methanol containing 4% reagent water. Use 10 µL of this 1-4 ng/µL solution to fortify the final 1-mL extracts (Sect. 11.5). This will yield a concentration of 10-40 ng/mL of each IS in the 1-mL extracts.

IS	Final Conc. of IS PDS (ng/µL)
$^{13}C_2$-PFOA	1.0
$^{13}C_4$-PFOS	3.0
d_3-NMeFOSAA	4.0

7.2.2. SURROGATE (SUR) STANDARD SOLUTIONS – The four SUR(s) listed in the table below were purchased from Wellington Labs as linear only isomers. These isotopically labeled SUR standards were carefully chosen during method development because they encompass most of the functional groups, as well as the water solubility range of the method analytes. Although alternate SUR standards may be used provided they are isotopically labeled compounds with similar functional groups as the method analytes, the analyst must have documented reasons for using alternate SUR standards. The alternate SUR standards chosen must still span the water solubility range of the method analytes. In addition, alternate SUR standards must meet the QC requirements in Section 9.3.5.

Surrogates	Acronym
Perfluoro-n-[1,2-$^{13}C_2$]hexanoic acid	$^{13}C_2$-PFHxA
Perfluoro-n-[1,2-$^{13}C_2$]decanoic acid	$^{13}C_2$-PFDA
N-deuterioethylperfluoro-1-octanesulfonamidoacetic acid	d_5-NEtFOSAA
Tetrafluoro-2-heptafluoropropoxy-$^{13}C_3$-propanoic acid	$^{13}C_3$-HFPO-DA

7.2.2.1. SUR STOCK STANDARD SOLUTIONS (SUR SSS) – These SUR stocks can be obtained as individual certified stock standard solutions. The SURs can also be purchased as PDSs, making the preparation of individual SSSs

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unnecessary. Analysis of the SUR(s) is less complicated if the SUR(s) purchased contains only the linear isomer.

7.2.2.2. SURROGATE PRIMARY DILUTION STANDARD (SUR PDS) (1-4 ng/µL) – Prepare, or purchase commercially, the SUR PDS at a suggested concentration of 1-4 ng/µL. The SUR PDS (in methanol with four molar equivalents of sodium hydroxide) was purchased from Wellington Labs. Alternatively, the SUR PDS can be prepared in methanol containing 4% reagent water. Use 10 µL of this 1-4 ng/µL solution to fortify all QC and Field Samples. (Sect. 11.5). This will yield SUR concentrations of 40-160 ng/L in the 250 mL aqueous samples.

SUR	Final Conc. of SUR PDS (ng/µL)
$^{13}C_2$-PFHxA	1.0
$^{13}C_2$-PFDA	1.0
d_5-NEtFOSAA	4.0
$^{13}C_3$-HFPO-DA	1.0

7.2.3. ANALYTE STANDARD SOLUTIONS – Analyte standards may be purchased commercially as ampoulized solutions or prepared from neat materials. If commercially available, the method analytes must be purchased as technical grade (linear and branched isomers) standards or neat materials. Standards or neat materials that contain only the linear isomer can be substituted only if technical grade (linear and branched isomers) standards or neat material cannot be purchased as quantitative standards (see note below regarding PFOA). At the time of this method development, PFHxS, PFOS, NEtFOSAA and NMeFOSAA are available as technical grade (containing branched and linear isomers) and therefore must be purchased as technical grade.

A qualitative standard (Sect. 3.19) is available for PFOA that contains the linear and branched isomers (Wellington Labs, Cat. No. T-PFOA, or equivalent). This qualitative PFOA standard must be purchased and used to identify the retention times of the branched PFOA isomers, but the linear only PFOA standard must be used for quantitation (Sect. 12.2) until a quantitative PFOA standard containing the branched and linear isomers becomes commercially available.

PFHxS, PFOS, ADONA, 9Cl-PF3ONS and 11CL-PF3OUdS may not be available as the acids listed in Section 1.1, but rather as their corresponding salts, such as NH_4^+, Na^+ and K^+. These salts are acceptable starting materials for the stock standards provided the measured mass is corrected for the salt content according to the equation below. Prepare the Analyte Stock and Primary Dilutions Standards as described below.

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

where:

MW_{acid} = the molecular weight of PFAS
MW_{salt} = the molecular weight of purchased salt

7.2.3.1. ANALYTE STOCK STANDARD SOLUTION (SSS) – Analyte standards may be purchased commercially as ampoulized solutions prepared from neat materials. Commercially prepared SSSs are available for all method analytes. During method development, mixes or individual stocks were obtained from Accustandard, Absolute, Wellington Labs and Synquest. When using these stock standards to prepare a PDS, care must be taken to ensure that these standards are at room temperature and adequately vortexed.

7.2.3.2. ANALYTE PRIMARY DILUTION STANDARD (PDS) SOLUTION (0.5-2.5 ng/µL) – The analyte PDS contains all the method analytes of interest at various concentrations in methanol containing 4% water (or in methanol containing four molar equivalents of sodium hydroxide). The ESI and MS/MS response varies by compound; therefore, a mix of concentrations may be needed in the analyte PDS. See Tables 5-9 in Section 17 for suggested concentrations for each analyte. During method development, the analyte PDS was prepared such that approximately the same instrument response was obtained for all the analytes. The analyte PDS is prepared by dilution of the combined Analyte Stock Standard Solutions and is used to prepare the CAL standards, and fortify the LFBs, LFSMs, and LFSMDs with the method analytes. If the PDS is stored cold, care must be taken to ensure that these standards are at room temperature and adequately vortexed before usage.

7.2.4. CALIBRATION STANDARDS (CAL) – At least five calibration concentrations are required to prepare the initial calibration curve spanning a 20-fold concentration range (Sect. 10.2). Larger concentration ranges will require more calibration points. Prepare the CAL standards over the concentration range of interest from dilutions of the analyte PDS in methanol containing 4% reagent water. The suggested analyte concentrations found in Tables 5-9 can be used as a starting point for determining the calibration range. The IS and SUR are added to the CAL standards at a constant concentration. During method development, the concentrations of the SUR(s) were 10-40 pg/µL in the standard (40-160 ng/L in the sample) and the IS(s) were 10-40 ng/mL. The lowest concentration CAL standard must be at or below the MRL, which may depend on system sensitivity. The CAL standards may also be used as CCCs (Sect. 9.3.2).

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8. SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1. SAMPLE BOTTLE PREPARATION

8.1.1. Samples must be collected in a 250-mL polypropylene bottle fitted with a polypropylene screw-cap.

8.1.2. The preservation reagent, listed in the table below, is added to each sample bottle as a solid prior to shipment to the field (or prior to sample collection).

Compound	Amount	Purpose
Trizma® (Sect. 7.1.5)	5.0 g/L	buffering reagent and removes free chlorine

8.2. SAMPLE COLLECTION

8.2.1. 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.

8.2.2. Open the tap and allow the system to flush until the water temperature has stabilized (approximately 3 to 5 min). Collect samples from the flowing system.

8.2.3. Fill sample bottles, taking care not to flush out the sample preservation reagent. Samples do not need to be collected headspace free.

8.2.4. After collecting the sample, cap the bottle and agitate by hand until preservative is dissolved. Keep the sample sealed from time of collection until extraction.

8.3. FIELD REAGENT BLANKS (FRB)

8.3.1. A FRB must be handled along with each sample set. The sample set is composed of samples collected from the same sample site and at the same time. At the laboratory, fill the field blank sample bottle with reagent water and preservatives, seal, and ship to the sampling site along with the sample bottles. For each FRB shipped, an empty sample bottle (no preservatives) must also be shipped. At the sampling site, the sampler must open the shipped FRB and pour the preserved reagent water into the empty shipped sample bottle, seal and label this bottle as the FRB. The FRB is shipped back to the laboratory along with the samples and analyzed to ensure that PFAS were not introduced into the sample during sample collection/handling.

8.3.2. The same batch of preservative must be used for the FRBs as for the field samples.

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8.3.3. The reagent water used for the FRBs must be initially analyzed for method analytes as a LRB (using the same lot of sample bottles as the field samples) and must meet the LRB criteria in Section 9.3.1 prior to use. This requirement will ensure samples are not being discarded due to contaminated reagent water or sample bottles rather than contamination during sampling.

8.4. SAMPLE SHIPMENT AND STORAGE – Samples must be chilled during shipment and must not exceed 10 °C during the first 48 hours after collection. Sample temperature must be confirmed to be at or below 10 °C when the samples are received at the laboratory. Samples stored in the lab must be held at or below 6 °C until extraction, but must not be frozen.

NOTE: Samples that are significantly above 10° C, at the time of collection, may need to be iced or refrigerated for a period of time, in order to chill them prior to shipping. This will allow them to be shipped with sufficient ice to meet the above requirements.

8.5. SAMPLE AND EXTRACT HOLDING TIMES – Results of the sample storage stability study (Table 10) indicated that all compounds listed in this method have adequate stability for 14 days when collected, preserved, shipped and stored as described in Sections 8.1, 8.2, and 8.4. Therefore, water samples should be extracted as soon as possible but must be extracted within 14 days. Extracts must be stored at room temperature and analyzed within 28 days after extraction. The extract storage stability study data are presented in Table 11.

9. QUALITY CONTROL

9.1. QC requirements include the Initial Demonstration of Capability (IDC) and ongoing QC requirements that must be met when preparing and analyzing Field Samples. This section describes the QC parameters, their required frequencies, and the performance criteria that must be met in order to meet EPA quality objectives. The QC criteria discussed in the following sections are summarized in Tables 12 and 13. These QC requirements are considered the minimum acceptable QC criteria. Laboratories are encouraged to institute additional QC practices to meet their specific needs.

9.1.1. METHOD MODIFICATIONS – The analyst is permitted to modify LC columns, LC conditions, evaporation techniques, internal standards or surrogate standards, and MS and MS/MS conditions. Each time such method modifications are made, the analyst must repeat the procedures of the IDC. **Modifications to LC conditions should still produce conditions such that co-elution of the method analytes is minimized to reduce the probability of suppression/enhancement effects.**

9.2. INITIAL DEMONSTRATION OF CAPABILITY – The IDC must be successfully performed prior to analyzing any Field Samples. Prior to conducting the IDC, the

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analyst must first generate an acceptable Initial Calibration following the procedure outlined in Section 10.2.

9.2.1. INITIAL DEMONSTRATION OF BRANCHED vs LINEAR ISOMER PROFILE for PFOA IN A QUALITATIVE STANDARD – Prepare and analyze a qualitative standard used for identifying retention times of branch isomers of PFOA. Identify the retention times of branched isomers of PFOA in the purchased technical grade PFOA standard. This qualitative PFOA standard is not used for quantitation (see Section 12.2). This branched isomer identification check must be repeated any time changes occur that affect the analyte retention times.

9.2.2. INITIAL DEMONSTRATION OF LOW SYSTEM BACKGROUND – Any time a new lot of SPE cartridges, solvents, centrifuge tubes, disposable pipets, and autosampler vials are used, it must be demonstrated that an LRB is reasonably free of contamination and that the criteria in Section 9.3.1 are met. If an automated extraction system is used, an LRB should be extracted on each port to ensure that all the valves and tubing are free from potential PFAS contamination.

9.2.3. INITIAL DEMONSTRATION OF PRECISION (IDP) – Prepare, extract, and analyze four to seven replicate LFBs fortified near the midrange of the initial calibration curve according to the procedure described in Section 11.4. Sample preservatives as described in Section 8.1.2 must be added to these samples. The relative standard deviation (RSD) of the results of the replicate analyses must be less than 20%.

9.2.4. INITIAL DEMONSTRATION OF ACCURACY (IDA) – Using the same set of replicate data generated for Section 9.2.3, calculate average recovery. The average recovery of the replicate values must be within ± 30% of the true value.

9.2.5. INITIAL DEMONSTRATION OF PEAK ASYMMETRY FACTOR – Peak asymmetry factors must be calculated using the equation in Section 9.3.9 for the first two eluting peaks (if only two analytes are being analyzed, both must be evaluated) in a mid-level CAL standard. The peak asymmetry factors must fall in the range of 0.8 to 1.5. See guidance in Section 10.2.4.1 if the calculated peak asymmetry factors do not meet the criteria.

9.2.6. MINIMUM REPORTING LEVEL (MRL) CONFIRMATION – Establish a target concentration for the MRL based on the intended use of the method. The MRL may be established by a laboratory for their specific purpose or may be set by a regulatory agency. Establish an Initial Calibration following the procedure outlined in Section 10.2. The lowest CAL standard used to establish the Initial Calibration (as well as the low-level CCC, Section 10.3) must be at or below the concentration of the MRL. Establishing the MRL concentration too low may cause repeated failure of ongoing QC requirements. Confirm the MRL following the procedure outlined below.

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9.2.6.1. Fortify, extract, and analyze seven replicate LFBs at the proposed MRL concentration. These LFBs must contain all method preservatives described in Section 8.1.2. Calculate the mean measured concentration (*Mean*) and standard deviation for these replicates. Determine the Half Range for the prediction interval of results (HR_{PIR}) using the equation below

$$HR_{PIR} = 3.963s$$

where

s = the standard deviation
3.963 = a constant value for seven replicates.[1]

9.2.6.2. Confirm that the upper and lower limits for the Prediction Interval of Result ($PIR = Mean \pm HR_{PIR}$) meet the upper and lower recovery limits as shown below

The Upper PIR Limit must be ≤150% recovery.

$$\frac{Mean + HR_{PIR}}{Fortified\,Concentration} \times 100\% \leq 150\%$$

The Lower PIR Limit must be ≥ 50% recovery.

$$\frac{Mean - HR_{PIR}}{Fortified\,Concentration} \times 100\% \geq 50\%$$

9.2.6.3. The MRL is validated if both the Upper and Lower PIR Limits meet the criteria described above (Sect. 9.2.6.2). If these criteria are not met, the MRL has been set too low and must be determined again at a higher concentration.

9.2.7. CALIBRATION CONFIRMATION – Analyze a QCS as described in Section 9.3.10 to confirm the accuracy of the standards/calibration curve.

9.2.8. DETECTION LIMIT DETERMINATION *(optional) – While DL determination is not a specific requirement of this method, it may be required by various regulatory bodies associated with compliance monitoring. It is the responsibility of the laboratory to determine if DL determination is required based upon the intended use of the data.*

9.2.8.1. Replicate analyses for this procedure should be done over at least three days (i.e., both the sample extraction and the LC/MS/MS analyses should be done over at least three days). Prepare at least seven replicate LFBs at a concentration estimated to be near the DL. This concentration may be estimated by selecting a concentration at 2-5 times the noise level. The DLs

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in Table 5 were calculated from LFBs fortified at various concentrations as indicated in the table. The appropriate fortification concentrations will be dependent upon the sensitivity of the LC/MS/MS system used. All preservation reagents listed in Section 8.1.2 must also be added to these samples. Analyze the seven replicates through all steps of Section 11.

NOTE: If an MRL confirmation data set meets these requirements, a DL may be calculated from the MRL confirmation data, and no additional analyses are necessary.

Calculate the *DL* using the following equation

$$DL = s \times t_{(n-1, 1-\alpha=0.99)}$$

where

s = standard deviation of replicate analyses
$t_{(n\text{-}1,\ 1\text{-}\alpha=0.99)}$ = Student's t value for the 99% confidence level with n-1 degrees of freedom
n = number of replicates.

NOTE: Do not subtract blank values when performing DL calculations. The DL is a statistical determination of precision only.[2] If the DL replicates are fortified at a low enough concentration, it is likely that they will not meet the precision and accuracy criteria for CCCs. Therefore, no precision and accuracy criteria are specified.

9.2.8.2. If a laboratory is establishing their own MRL, the calculated DLs should not be used as the MRL for analytes that commonly occur as background contaminants. Method analytes that are seen in the background should be reported as present in Field Samples, only after careful evaluation of the background levels. It is recommended that a MRL be established at the mean LRB concentrations + 3σ or 3 times the mean LRB concentration, whichever is greater. This value should be calculated over a period of time, to reflect variability in the blank measurements. It is recommended that this value be used as an MRL in order to avoid reporting false positive results.

9.3. ONGOING QC REQUIREMENTS – This section summarizes the ongoing QC criteria that must be followed when processing and analyzing Field Samples.

9.3.1. LABORATORY REAGENT BLANK (LRB) – An LRB is required with each extraction batch (Sect. 3.6) to confirm that potential background contaminants are not interfering with the identification or quantitation of method analytes. If more than 20 Field Samples are included in a batch, analyze an LRB for every 20 samples. If the LRB produces a peak within the retention time window of any analyte that would prevent the determination of that analyte, determine the source of contamination and eliminate the interference before processing samples.

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Background contamination must be reduced to an acceptable level before proceeding. Background from method analytes or other contaminants that interfere with the measurement of method analytes must be below 1/3 of the MRL. Blank contamination is estimated by extrapolation, if the concentration is below the lowest CAL standard. This extrapolation procedure is not allowed for sample results as it may not meet data quality objectives. If the method analytes are detected in the LRB at concentrations equal to or greater than this level, then all data for the problem analyte(s) must be considered invalid for all samples in the extraction batch. Because background contamination is a significant problem for several method analytes, maintaining a historical record of LRB data is highly recommended.

9.3.2. CONTINUING CALIBRATION CHECK (CCC) – CCC Standards are analyzed at the beginning of each analysis batch, after every 10 Field Samples, and at the end of the analysis batch. See Section 10.3 for concentration requirements and acceptance criteria.

9.3.3. LABORATORY FORTIFIED BLANK (LFB) – An LFB is required with each extraction batch (Sect. 3.6). The fortified concentration of the LFB must be rotated between low, medium, and high concentrations from batch to batch. The low concentration LFB must be as near as practical to, but no more than two times, the MRL. Similarly, the high concentration LFB should be near the high end of the calibration range established during the initial calibration (Sect. 10.2). Results of the low-level LFB analyses must be 50-150% of the true value. Results of the medium and high-level LFB analyses must be 70-130% of the true value. If the LFB results do not meet these criteria for method analytes, then all data for the problem analyte(s) must be considered invalid for all samples in the extraction batch.

9.3.4. INTERNAL STANDARDS (IS) – The analyst must monitor the peak areas of the IS(s) in all injections during each analysis day. The IS responses (peak areas) in any chromatographic run must be within 70-140% of the response in the most recent CCC and must not deviate by more than 50% from the average area measured during initial analyte calibration. If the IS areas in a chromatographic run do not meet these criteria, inject a second aliquot of that extract aliquotted into a new capped autosampler vial. Random evaporation losses have been observed with the polypropylene caps causing high IS(s) areas.

9.3.4.1. If the reinjected aliquot produces an acceptable IS response, report results for that aliquot.

9.3.4.2. If the reinjected extract fails again, the analyst should check the calibration by reanalyzing the most recently acceptable CAL standard. If the CAL standard fails the criteria of Section 10.3, recalibration is in order per Section 10.2. If the CAL standard is acceptable, extraction of the sample may need to be repeated provided the sample is still within the holding time.

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Otherwise, report results obtained from the reinjected extract, but annotate as suspect. Alternatively, collect a new sample and re-analyze.

9.3.5. SURROGATE RECOVERY – The SUR standard is fortified into all samples, CCCs, LRBs, LFBs, LFSMs, LFSMDs, FD, and FRB prior to extraction. It is also added to the CAL standards. The SUR is a means of assessing method performance from extraction to final chromatographic measurement. Calculate the recovery (%R) for the SUR using the following equation

$$\%R = \left(\frac{A}{B}\right) \times 100$$

where

A = calculated SUR concentration for the QC or Field Sample
B = fortified concentration of the SUR.

9.3.5.1. SUR recovery must be in the range of 70-130%. When SUR recovery from a sample, blank, or CCC is less than 70% or greater than 130%, check 1) calculations to locate possible errors, 2) standard solutions for degradation, 3) contamination, and 4) instrument performance. Correct the problem and reanalyze the extract.

9.3.5.2. If the extract reanalysis meets the SUR recovery criterion, report only data for the reanalyzed extract.

9.3.5.3. If the extract reanalysis fails the 70-130% recovery criterion, the analyst should check the calibration by injecting the last CAL standard that passed. If the CAL standard fails the criteria of Section 10.3, recalibration is in order per Section 10.2. 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/SUR recovery to inform the data user that the results are suspect due to SUR recovery. Alternatively, collect a new sample and re-analyze.

9.3.6. LABORATORY FORTIFIED SAMPLE MATRIX (LFSM) – Analysis of an LFSM is required in each extraction batch and is used to determine that the sample matrix does not adversely affect method accuracy. Assessment of method precision is accomplished by analysis of a Field Duplicate (FD) (Sect. 9.3.7); however, infrequent occurrence of method analytes would hinder this assessment. If the occurrence of method analytes in the samples is infrequent, or if historical trends are unavailable, a second LFSM, or LFSMD, must be prepared, extracted, and analyzed from a duplicate of the Field Sample. Extraction batches that contain LFSMDs will not require the extraction of a FD. If a variety of different sample matrices are analyzed regularly, for example, drinking water from groundwater and surface water sources, method performance should be

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established for each. Over time, LFSM data should be documented by the laboratory for all routine sample sources.

9.3.6.1. Within each extraction batch (Sect. 3.6), a minimum of one Field Sample is fortified as an LFSM for every 20 Field Samples analyzed. The LFSM is prepared by spiking a sample with an appropriate amount of the Analyte PDS (Sect. 7.2.3.2). Select a spiking concentration that is greater than or equal to the matrix background concentration, if known. Use historical data and rotate through the low, mid and high concentrations when selecting a fortifying concentration.

9.3.6.2. Calculate the percent recovery (%*R*) for each analyte using the equation

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

where

A = measured concentration in the fortified sample
B = measured concentration in the unfortified sample
C = fortification concentration.

9.3.6.3. Analyte recoveries may exhibit matrix bias. For samples fortified at or above their native concentration, recoveries should range between 70-130%, except for low-level fortification near or at the MRL (within a factor of 2-times the MRL concentration) where 50-150% recoveries are acceptable. If the accuracy of any analyte falls outside the designated range, and the laboratory performance for that analyte is shown to be in control in the CCCs, the recovery is judged to be matrix biased. The result for that analyte in the unfortified sample is labeled suspect/matrix to inform the data user that the results are suspect due to matrix effects.

9.3.7. FIELD DUPLICATE OR LABORATORY FORTIFIED SAMPLE MATRIX DUPLICATE (FD or LFSMD) – Within each extraction batch (not to exceed 20 Field Samples, Sect. 3.6), a minimum of one FD or LFSMD must be analyzed. Duplicates check the precision associated with sample collection, preservation, storage, and laboratory procedures. If method analytes are not routinely observed in Field Samples, an LFSMD should be analyzed rather than an FD.

9.3.7.1. Calculate the relative percent difference (*RPD*) for duplicate measurements (*FD1* and *FD2*) using the equation

$$RPD = \frac{|FD1 - FD2|}{(FD1 + FD2)/2} \times 100$$

9.3.7.2. RPDs for FDs should be ≤30%. Greater variability may be observed when FDs have analyte concentrations that are within a factor of 2 of the MRL. At these concentrations, FDs should have RPDs that are ≤50%. If the RPD of

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any analyte falls outside the designated range, and the laboratory performance for that analyte is shown to be in control in the CCC, the recovery is judged to be matrix biased. The result for that analyte in the unfortified sample is labeled suspect/matrix to inform the data user that the results are suspect due to matrix effects.

9.3.7.3. If an LFSMD is analyzed instead of a FD, calculate the relative percent difference (RPD) for duplicate LFSMs (LFSM and LFSMD) using the equation

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

9.3.7.4. RPDs for duplicate LFSMs must be ≤30% for samples fortified at or above their native concentration. Greater variability may be observed when LFSMs are fortified at analyte concentrations that are within a factor of 2 of the MRL. LFSMs fortified at these concentrations must have RPDs that are ≤50% for samples fortified at or above their native concentration. If the RPD of any analyte falls outside the designated range, and the laboratory performance for that analyte is shown to be in control in the CCC, the recovery is judged to be matrix biased. The result for that analyte in the unfortified sample is labeled suspect/matrix to inform the data user that the results are suspect due to matrix effects.

9.3.8. FIELD REAGENT BLANK (FRB) – The purpose of the FRB is to ensure that PFAS measured in the Field Samples were not inadvertently introduced into the sample during sample collection/handling. Analysis of the FRB is required only if a Field Sample contains a method analyte or analytes at or above the MRL. The FRB is processed, extracted and analyzed in exactly the same manner as a Field Sample. If the method analyte(s) found in the Field Sample is present in the FRB at a concentration greater than 1/3 the MRL, then all samples collected with that FRB are invalid and must be recollected and reanalyzed.

9.3.9. PEAK ASYMMETRY FACTOR – A peak asymmetry factor must be calculated using the equation below during the IDC and every time chromatographic changes are made that may affect peak shape. The peak asymmetry factor for the first two eluting peaks in a mid-level CAL standard (if only two analytes are being analyzed, both must be evaluated) must fall in the range of 0.8 to 1.5. Modifying the standard or extract composition to more aqueous content to prevent poor shape is not permitted. See guidance in Section 10.2.4.1 if the calculated peak asymmetry factors do not meet the criteria.

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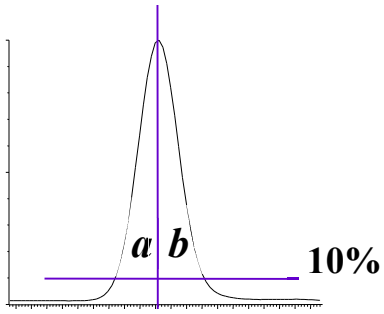
$$A_s = \frac{b}{a}$$



where:

A_s = peak asymmetry factor

B = width of the back half of the peak measured (at 10% peak height) from the trailing edge of the peak to a line dropped perpendicularly from the peak apex

a = the width of the front half of the peak measured (at 10% peak height) from the leading edge of the peak to a line dropped perpendicularly from the apex.

9.3.10. QUALITY CONTROL SAMPLES (QCS) – As part of the IDC (Sect. 9.2), each time a new Analyte PDS (Sect. 7.2.3.2) is prepared, and at least quarterly, analyze a QCS sample from a source different from the source of the CAL standards. If a second vendor is not available, then a different lot of the standard should be used. The QCS should be prepared at a mid-level concentration and analyzed just like a CCC. Acceptance criteria for the QCS are identical to the CCCs; 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.

10. CALIBRATION AND STANDARDIZATION

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

10.2. INITIAL CALIBRATION

10.2.1. ESI-MS/MS TUNE

10.2.1.1. Calibrate the mass scale of the MS with the calibration compounds and procedures prescribed by the manufacturer.

10.2.1.2. Optimize the $[M-H]^-$ or $[M-CO_2]^-$ for each method analyte by infusing approximately 0.5-1.0 µg/mL of each analyte (prepared in the initial mobile phase conditions) directly into the MS at the chosen LC mobile phase flow rate (approximately 0.3 mL/min). This tune can be done on a mix of the

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method analytes. The MS parameters (voltages, temperatures, gas flows, etc.) are varied until optimal analyte responses are determined. The method analytes may have different optima requiring some compromise between the optima. See Table 2 for ESI-MS conditions used in method development.

10.2.1.3. Optimize the product ion (Sect. 3.18) for each analyte by infusing approximately 0.5-1.0 µg/mL of each analyte (prepared in the initial mobile phase conditions) directly into the MS at the chosen LC mobile phase flow rate (approximately 0.3 mL/min). This tune can be done on a mix of the method analytes. The MS/MS parameters (collision gas pressure, collision energy, etc.) are varied until optimal analyte responses are determined. Typically, the carboxylic acids have very similar MS/MS conditions and the sulfonic acids have similar MS/MS conditions. See Table 4 for MS/MS conditions used in method development.

10.2.2. Establish LC operating parameters that optimize resolution and peak shape. Suggested LC conditions can be found in Table 1. The LC conditions listed in Table 1 may not be optimum for all LC systems and may need to be optimized by the analyst (See Sect. 10.2.4.1). Modifying the standard or extract composition to more aqueous content to prevent poor shape is not permitted.

Cautions: LC system components, as well as the mobile phase constituents, contain many of the analytes in this method. Thus, these PFAS will build up on the head of the LC column during mobile phase equilibration. 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). In addition, prior to daily use, flush the column with 100% methanol for at least 20 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.

Mobile phase modifiers other than 20 mM ammonium acetate may be used at the discretion of the analyst, provided that the retention time stability criteria in Sect. 11.7.2 can be met over a period of two weeks. During method development, retention times shifted to shorter and shorter times as days progressed when mobile phases with less than 20 mM ammonium acetate were used.

10.2.3. Inject a mid-level CAL standard under LC/MS conditions to obtain the retention times of each method analyte. Divide the chromatogram into retention time windows each of which contains one or more chromatographic peaks. During MS/MS analysis, fragment a small number of selected precursor ions ($[M-H]^-$; Sect. 3.16) for the analytes in each window and choose the most abundant product ion. The product ions (also the quantitation ions) chosen during method

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development are in Table 4, although these will be instrument dependent. For maximum sensitivity, small mass windows of ±0.5 daltons around the product ion mass were used for quantitation.

NOTE: There have been reports[10] 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.

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

NOTE: Ensure that the retention time window used to collect data for each analyte is sufficient to detect earlier eluting branched isomers.

10.2.4.1. If broad, split or fronting peaks are observed for the first two eluting chromatographic peaks (if only two analytes are being analyzed, both must be evaluated), change the initial mobile phase conditions to higher aqueous content until the peak asymmetry ratio for each peak is 0.8 – 1.5. The peak asymmetry factor is calculated as described in Section 9.3.9 on a mid-level CAL standard. The peak asymmetry factor must meet the above criteria for the first two eluting peaks during the IDC and every time a new calibration curve is generated. Modifying the standard or extract composition to more aqueous content to prevent poor shape is not permitted.

10.2.4.2. Most PFAS are produced by two different processes. One process gives rise to linear PFAS only while the other process produces both linear and branched isomers. Thus, both branched and linear PFAS can potentially be found in the environment. Refer to Section 12.2 for guidance on integration and quantitation of PFAS.

10.2.5. Prepare a set of at least five CAL standards as described in Section 7.2.4. The lowest concentration CAL standard must be at or below the MRL, which may depend on system sensitivity. It is recommended that at least four of the CAL standards are at a concentration greater than or equal to the MRL.

10.2.6. The LC/MS/MS system is calibrated using the IS technique. Use the LC/MS/MS data system software to generate a linear regression or quadratic calibration curve for each of the analytes. This curve **must always** be forced through zero and may

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be concentration weighted, if necessary. Forcing zero allows for a better estimate of the background levels of method analytes.

10.2.7. CALIBRATION ACCEPTANCE CRITERIA – Validate the initial calibration by calculating the concentration of each analyte as an unknown against its regression equation. For calibration levels that are ≤ MRL, the result for each analyte must be within ± 50% of the true value. All other calibration points must calculate to be within ± 30% of their true value. If these criteria cannot be met, the analyst will have difficulty meeting ongoing QC criteria. It is recommended that corrective action is taken to reanalyze the CAL standards, restrict the range of calibration, or select an alternate method of calibration (forcing the curve through zero is still required).

CAUTION: When acquiring MS/MS data, LC operating conditions must be carefully reproduced for each analysis to provide reproducible retention times. If this is not done, the correct ions will not be monitored at the appropriate times. As a precautionary measure, the chromatographic peaks in each window must not elute too close to the edge of the segment time window.

10.3. CONTINUING CALIBRATION CHECK (CCC) – Minimum daily calibration verification is as follows. Verify the initial calibration at the beginning and end of each group of analyses, and after every tenth sample during analyses. In this context, a "sample" is considered to be a Field Sample. LRBs, CCCs, LFBs, LFSMs, FDs FRBs and LFSMDs are not counted as samples. The beginning CCC of each analysis batch must be at or below the MRL to verify instrument sensitivity prior to any analyses. If standards have been prepared such that all low CAL points are not in the same CAL solution, it may be necessary to analyze two CAL standards to meet this requirement. Alternatively, the analyte concentrations in the analyte PDS may be customized to meet these criteria. Subsequent CCCs should alternate between a medium and high concentration CAL standard.

10.3.1. Inject an aliquot of the appropriate concentration CAL standard and analyze with the same conditions used during the initial calibration.

10.3.2. Determine that the absolute areas of the quantitation ions of the IS(s) are within 70-140% of the areas measured in the most recent continuing calibration check, and within 50-150% from the average areas measured during initial calibration. If any of the IS areas has changed by more than these amounts, adjustments must be made to restore system sensitivity. These adjustments may include cleaning of the MS ion source, or other maintenance as indicated in Section 10.3.4. Major instrument maintenance requires recalibration (Sect. 10.2) and verification of sensitivity by analyzing a CCC at or below the MRL (Sect. 10.3). Control charts are useful aids in documenting system sensitivity changes.

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10.3.3. Calculate the concentration of each analyte and SUR in the CCC. The calculated amount for each analyte and SUR for medium and high level CCCs must be within ± 30% of the true value. The calculated amount for the lowest calibration point for each analyte must be within ± 50% and the SUR must be within ± 30% of the true value. If these conditions do not exist, then all data for the problem analyte must be considered invalid, and remedial action should be taken (Sect. 10.3.4) which may require recalibration. Any Field or QC Samples that have been analyzed since the last acceptable calibration verification that are still within holding time must be reanalyzed after adequate calibration has been restored, with the following exception. **If the CCC fails because the calculated concentration is greater than 130% (150% for the low-level CCC) for a particular method analyte, and Field Sample extracts show no detection for that method analyte, non-detects may be reported without re-analysis.**

10.3.4. REMEDIAL ACTION – Failure to meet CCC QC performance criteria may require remedial action. Major maintenance, such as cleaning the electrospray probe, atmospheric pressure ionization source, cleaning the mass analyzer, replacing the LC column, etc., requires recalibration (Sect. 10.2) and verification of sensitivity by analyzing a CCC at or below the MRL (Sect. 10.3)

11. PROCEDURE

11.1. This procedure may be performed manually or in an automated mode using a robotic or automatic sample preparation device. The data presented in Tables 5-11 demonstrate data collected by manual extraction. If an automated system is used to prepare samples, follow the manufacturer's operating instructions, but all extraction and elution steps must be the same as in the manual procedure. Extraction and/or elution steps may not be changed or omitted to accommodate the use of an automated system. If an automated system is used, the LRBs should be rotated among the ports to ensure that all the valves and tubing meet the LRB requirements (Sect. 9.3.1).

11.2. Some of the PFAS adsorb to surfaces, including polypropylene. Therefore, the aqueous sample bottles must be rinsed with the elution solvent (Sect. 11.4.4) whether extractions are performed manually or by automation. The bottle rinse is passed through the cartridge to elute the method analytes and is then collected (Sect. 11.4.4).

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

11.3. SAMPLE PREPARATION

11.3.1. Samples are preserved, collected and stored as presented in Section 8. All Field and QC Samples, including the LRB, LFB and FRB, must contain the dechlorinating agent listed in Section 8.1.2. Before extraction, verify that the

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sample pH is 7 ± 0.5. Determine sample volume. An indirect measurement may be done in one of two ways: by marking the level of the sample on the bottle or by weighing the sample and bottle to the nearest 1 g. After extraction, proceed to Section 11.6 for final volume determination. Some of the PFAS adsorb to surfaces, thus the sample volume may **NOT** be transferred to a graduated cylinder for volume measurement. The LRB, LFB and FRB may be prepared by measuring 250 mL of reagent water with a polypropylene graduated cylinder or filling a 250-mL sample bottle to near the top.

11.3.2. Add an aliquot of the SUR PDS (Sect. 7.2.2.2) to each sample, cap and invert to mix. During method development, a 10-µL aliquot of the 1-4 ng/µL SUR PDS (Sect. 7.2.2.2) was added to 250 mL of sample for a final concentration of 40 ng/L for $^{13}C_2$-PFHxA, $^{13}C_3$-HFPO-DA, and $^{13}C_2$-PFDA and 160 ng/L for d_5-NEtFOSAA.

11.3.3. In addition to the SUR(s) and dechlorination agent, if the sample is an LFB, LFSM, or LFSMD, add the necessary amount of analyte PDS (Sect. 7.2.3.2). Cap and invert each sample to mix.

11.4. CARTRIDGE SPE PROCEDURE

11.4.1. CARTRIDGE CLEAN-UP AND CONDITIONING – DO NOT allow cartridge packing material to go dry during any of the conditioning steps. Rinse each cartridge with 15 mL of methanol. Next, rinse each cartridge with 18 mL of reagent water, 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. Add 2-3 mL of reagent water to each cartridge, attach the sample transfer tubes (Sect. 6.9.3), turn on the vacuum, and begin adding sample to the cartridge.

NOTE: If low recoveries are observed for PFBS and PFHxA during the IDC, recoveries may be improved by allowing a one- or two-minute soak time after each addition of the methanol and water used in the clean-up and conditioning step.

11.4.2. SAMPLE EXTRACTON – Adjust the vacuum so that the approximate flow rate is 10-15 mL/min. Do not allow the cartridge to go dry before all the sample has passed through.

11.4.3. SAMPLE BOTTLE AND CARTRIDGE RINSE – After the entire sample has passed through the cartridge, rinse the sample bottles with two 7.5-mL aliquots of reagent water and draw each aliquot through the sample transfer tubes and the cartridges. Draw air or nitrogen through the cartridge for 5 min at high vacuum (10-15 in. Hg).

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

11.4.4. 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 4 mL of methanol and elute the analytes from the cartridges by pulling the 4 mL of methanol through the sample transfer tubes and the cartridges. Use a low vacuum such that the solvent exits the cartridge in a dropwise fashion. Repeat sample bottle rinse and cartridge elution with a second 4-mL aliquot of methanol.

NOTE: If low recoveries are observed for PFBS and PFHxA during the IDC, recoveries may be improved by allowing a one or two-minute soak time after each five to six mL addition of the methanol and water used in the clean-up and conditioning step.

NOTE: If empty plastic reservoirs are used in place of the sample transfer tubes to pass the samples through the cartridges, these reservoirs must be treated like the transfer tubes. After the reservoirs have been rinsed in Section 11.4.3, 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.

11.5. EXTRACT CONCENTRATION – Concentrate the extract to dryness under a gentle stream of nitrogen in a heated water bath (60-65 °C) to remove all the water/methanol mix. Add the appropriate amount of 96:4% (vol/vol) methanol:water solution and the IS PDS (Sect. 7.2.1.2) to the collection vial to bring the volume to 1 mL and vortex. (10 µL of the 1-4 ng/µL IS PDS for extract concentrations of 10-40 ng/mL were used for method development). Transfer a small aliquot with a plastic pipet (Sect. 6.7) to a polypropylene autosampler vial.

NOTE: It is recommended that the entire 1-mL aliquot not be transferred to the autosampler vial because the polypropylene autosampler caps do not reseal after injection. Therefore, do not store the extracts in the autosampler vials as evaporation losses occur in these autosampler vials. Extracts can be stored in 15-mL centrifuge tubes (Sect. 6.3).

11.6. SAMPLE VOLUME DETERMINATION – If the level of the sample was marked on the sample bottle, use a graduated cylinder to measure the volume of water required to fill the original sample bottle to the mark made prior to extraction. Determine to the nearest 2 mL. If using weight to determine volume, weigh the empty bottle to the

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nearest 1 g and determine the sample weight by subtraction of the empty bottle weight from the original sample weight (Sect. 11.3.1). Assume a sample density of 1.0 g/mL. In either case, the sample volume will be used in the final calculations of the analyte concentration (Sect. 12.3).

11.7. EXTRACT ANALYSIS

11.7.1. Establish operating conditions equivalent to those summarized in Tables 1-4 of Section 17. Instrument conditions and columns should be optimized prior to the initiation of the IDC.

11.7.2. 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 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.

11.7.3. Calibrate the system by either the analysis of a calibration curve (Sect. 10.2) or by confirming the initial calibration is still valid by analyzing a CCC as described in Section 10.3. If establishing an initial calibration for the first time, complete the IDC as described in Section 9.2.

11.7.4. Begin analyzing Field Samples, including QC samples, at their appropriate frequency by injecting the same size aliquots (10 µL was used in method development), under the same conditions used to analyze the CAL standards.

11.7.5. 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 dalton mass range around a single product ion for each method analyte.

11.7.6. The analyst must not extrapolate beyond the established calibration range. If an analyte peak area exceeds the range of the initial calibration curve, the extract may be diluted with 96%:4% (vol/vol) methanol:water solution and the appropriate amount of IS added to match the original concentration. Re-inject the diluted extract. Incorporate the dilution factor into the final concentration calculations. Acceptable SUR performance (Sect. 9.3.5.1) should be determined from the undiluted sample extract. The resulting data must be documented as a dilution and MRLs adjusted accordingly.

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12. DATA ANALYSIS AND CALCULATION

12.1. 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 Table 4. Other ions may be selected at the discretion of the analyst.

12.2. Because environmental samples may contain both branched and linear isomers for method analytes, but quantitative standards that contain the linear and branched isomers do not exist for all method analytes, integration and quantitation of the PFAS is dependent on type of standard available for each PFAS. It is recognized that some of the procedures described below for integration of standards, QC samples and Field Samples may cause a small amount of unavoidable bias in the quantitation of the method analytes due to the current state of the commercially available standards.

12.2.1. During method development, multiple chromatographic peaks were observed for standards of PFHxS, PFOS, NMeFOSAA, and NEtFOSAA using the LC conditions in Table 1 due to chromatographic resolution of the linear and branched isomers of these compounds. For PFHxS, PFOS, NMeFOSAA and NEtFOSAA, 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.

12.2.2. For PFOA, identify the branched isomers by analyzing a qualitative standard 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.

12.2.3. If standards containing the branched and linear isomers cannot be purchased (i.e., only linear isomer is available), only the linear isomer can be identified and quantitated in Field Samples and QC samples using the linear standard because the retention time of the branched isomers cannot be confirmed.

12.3. Calculate analyte and SUR concentrations using the multipoint calibration as described in Section 10.2. Do not use daily calibration verification data to quantitate analytes in samples. Adjust final analyte concentrations to reflect the actual sample volume determined in Section 11.6.

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

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

NOTE: Some data in Section 17 of this method are reported with more than two significant figures. This is done to better illustrate the method performance.

13. METHOD PERFORMANCE

13.1. PRECISION, ACCURACY, AND MINIMUM REPORTING LEVELS – Tables for these data are presented in Section 17. LCMRLs and DLs for each method analyte are presented in Table 5. Precision and accuracy are presented for four water matrices: reagent water (Tables 6); chlorinated (finished) ground water (Table 7); chlorinated (finished) surface water (Table 8); and private well water (Table 9).

13.2. SAMPLE STORAGE STABILITY STUDIES – An analyte storage stability study was conducted by fortifying the analytes into chlorinated surface water samples that were collected, preserved, and stored as described in Section 8. The precision and mean recovery (n=4) of analyses, conducted on Days 0, 8, and 14 are presented in Table 10.

13.3. EXTRACT STORAGE STABILITY STUDIES – Extract storage stability studies were conducted on extracts obtained from a chlorinated surface water fortified with the method analytes. The precision and mean recovery (n=4) of injections conducted on Days 0, 8, 14, 22, and 28 are reported in Table 11.

13.4. MULTI-LABORATORY DEMONSTRATION – The performance of this method was demonstrated by multiple laboratories, with results similar to those reported in Section 17. The authors wish to acknowledge the work of 1) EPA Region 2 in Edison, NJ., 2) Eurofins Eaton Analytical, LLC in Monrovia, CA, and 3) New Jersey Department of Health in Ewing, NJ.

14. POLLUTION PREVENTION

14.1. This method utilizes SPE to extract analytes from water. It requires the use of very small volumes of organic solvent and very small quantities of pure analytes, thereby minimizing the potential hazards to both the analyst and the environment as compared to the use of large volumes of organic solvents in conventional liquid-liquid extractions.

14.2. For information about pollution prevention that may be applicable to laboratory operations, consult "Less is Better: Laboratory Chemical Management for Waste Reduction" available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington, D.C., 20036 or on-line at http://membership.acs.org/c/ccs/pub_9.htm (accessed August 2008).

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15. WASTE MANAGEMENT

The analytical procedures described in this method generate relatively small amounts of waste since only small amounts of reagents and solvents are used. The matrices of concern are finished drinking water or source water. However, laboratory waste management practices must be conducted consistent with all applicable rules and regulations, and that laboratories 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, particularly the hazardous waste identification rules and land disposal restrictions.

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17. TABLES, DIAGRAMS, FLOWCHARTS AND VALIDATION DATA

TABLE 1. LC METHOD CONDITIONS

Time (min)	% 20 mM ammonium acetate	% Methanol
Initial	60.0	40.0
1.0	60.0	40.0
25.0	10.0	90.0
32.0	10.0	90.0
32.1	60.0	40.0
37.0	60.0	40.0
Waters Atlantis® dC_{18} 2.1 x 150 mm packed with 5.0 µm C_{18} stationary phase Flow rate of 0.3 mL/min 10 µL injection into a 50 µL loop		

TABLE 2. ESI-MS METHOD CONDITIONS

ESI Conditions	
Polarity	Negative ion
Capillary needle voltage	-3 kV
Cone gas flow	50 L/hr
Nitrogen desolvation gas	800 L/hr
Desolvation gas temp.	350°C

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TABLE 3. METHOD ANALYTES, RETENTION TIMES (RT) AND SUGGESTED IS REFERENCES

Analyte	Peak # (Fig. 1)	RT (min)	IS# Ref
PFBS	1	7.62	2
PFHxA	2	10.42	1
HFPO-DA	4	11.38	1
PFHpA	6	13.40	1
PFHxS	7	13.58	2
ADONA	8	13.73	1
PFOA	9	15.85	1
PFOS	11	17.91	2
PFNA	13	17.92	1
9Cl-PF3ONS	14	18.91	2
PFDA	15	19.69	1
NMeFOSAA	17	20.50	3
PFUnA	19	21.21	1
NEtFOSAA	20	21.26	3
11CL-PF3OUdS	22	21.84	2
PFDoA	23	22.52	1
PFTrDA	24	23.66	1
PFTA	25	24.64	1
$^{13}C_2$-PFHxA	3	10.42	1
$^{13}C_3$-HFPO-DA	5	11.40	1
$^{13}C_2$-PFDA	16	19.69	1
d_5-NEtFOSAA	21	21.24	3
$^{13}C_2$-PFOA– IS#1	10	15.85	-
$^{13}C_4$-PFOS– IS#2	12	17.91	-
d_3-NMeFOSAA–IS#3	18	20.49	-

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TABLE 4. MS/MS METHOD CONDITIONS[a]

Segment[b]	Analyte	Precursor Ion[c] *(m/z)*	Product Ion[c,d] *(m/z)*	Cone Voltage (v)	Collision Energy[e] (v)
1	PFBS[g]	299	80	42	30
1	PFHxA	313	269	14	10
1	HFPO-DA	285[f]	169	12	8
2	PFHpA	363	319	12	10
2	PFHxS[g,h]	399	80	46	32
2	ADONA	377	251	14	12
3	PFOA	413	369	14	10
3	PFOS[g,h]	499	80	52	42
3	PFNA	463	419	16	12
4	9Cl-PF3ONS	531	351	34	24
4	PFDA	513	469	14	10
4	NMeFOSAA[g]	570	419	30	20
4	PFUnA	563	519	12	10
4	NEtFOSAA[g]	584	419	30	20
4	11CL-PF3OUdS	631	451	40	24
4	PFDoA	613	569	18	10
5	PFTrDA	663	619	14	14
5	PFTA	713	669	14	12
1	$^{13}C_2$-PFHxA	315	270	16	10
1	$^{13}C_3$-HFPO-DA	287	169	10	6
4	$^{13}C_2$-PFDA	515	470	18	10
4	d_5-NEtFOSAA	589	419	28	22
3	$^{13}C_2$-PFOA	415	370	16	10
3	$^{13}C_4$-PFOS	503	80	58	42
4	d_3-NMeFOSAA	573	419	28	14

[a] An LC/MS/MS chromatogram of the analytes is shown in Figure 1.

[b] Segments are time durations in which single or multiple scan events occur.

[c] Precursor and product ions listed in this table are nominal masses. During MS and MS/MS optimization, the analyst should determine precursor and product ion masses to one decimal place by locating the apex of the mass spectral peak place (e.g., *m/z* 498.9→79.9 for PFOS). These precursor and product ion masses (with at least one decimal place) should be used in the MS/MS method for all analyses.

[d] Ions used for quantitation purposes.

[e] Argon used as collision gas at a flow rate of 0.15 mL/min.

[f] HFPO-DA is not stable in the ESI source and the $[M-H]^-$ is not observed under typical ESI conditions. The precursor ion used during method development was $[M-CO_2]^-$.

[g] Analyte has multiple resolved chromatographic peaks due to linear and branched isomers. All peaks summed for quantitation purposes.

[h] To reduce bias regarding detection of branch and linear isomers, the *m/z* 80 product ion must be used for this analyte.

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TABLE 5. DLs AND LCMRLs IN REAGENT WATER

Analyte	Fortified Conc. (ng/L)[a]	DL[b] (ng/L)	LCMRL[c] (ng/L)
PFBS	4.0	1.8	6.3
PFHxA	4.0	1.0	1.7
HFPO-DA	4.0	1.9	4.3
PFHpA	4.0	0.71	0.63
PFHxS	4.0	1.4	2.4
ADONA	4.0	0.88	0.55
PFOA	4.0	0.53	0.82
PFOS	4.0	1.1	2.7
PFNA	4.0	0.70	0.83
9Cl-PF3ONS	4.0	1.4	1.8
PFDA	4.0	1.6	3.3
NMeFOSAA	4.0	2.4	4.3
PFUnA	4.0	1.6	5.2
NEtFOSAA	4.0	2.8	4.8
11CL-PF3OUdS	4.0	1.5	1.5
PFDoA	4.0	1.2	1.3
PFTrDA	4.0	0.72	0.53
PFTA	4.0	1.1	1.2

[a] Spiking concentration used to determine DL.

[b] Detection limits were determined by analyzing seven replicates over three days according to Section 9.2.8.

[c] LCMRLs were calculated according to the procedure in reference 1.

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TABLE 6. PRECISION AND ACCURACY (n=8) OF PFAS IN FORTIFIED REAGENT WATER

Analyte	Fortified Conc. (ng/L)	Mean % Recovery	% RSD	Fortified Conc. (ng/L)	Mean % Recovery	% RSD
PFBS	16.0	90.8	6.8	80.0	85.1	6.7
PFHxA	16.0	101	8.0	80.0	96.5	4.6
HFPO-DA	16.0	97.8	1.8	80.0	96.8	5.1
PFHpA	16.0	105	3.3	80.0	104	2.7
PFHxS	16.0	109	6.7	80.0	107	4.4
ADONA	16.0	108	1.3	80.0	106	3.6
PFOA	16.0	106	1.8	80.0	104	3.1
PFOS	16.0	111	4.7	80.0	107	4.8
PFNA	16.0	110	2.6	80.0	104	3.6
9Cl-PF3ONS	16.0	108	8.8	80.0	101	3.8
PFDA	16.0	111	2.4	80.0	107	3.6
NMeFOSAA	16.0	104	5.2	80.0	102	5.4
PFUnA	16.0	107	2.8	80.0	101	1.3
NEtFOSAA	16.0	97.7	6.8	80.0	101	2.5
11CL-PF3OUdS	16.0	109	3.4	80.0	103	6.1
PFDoA	16.0	101	7.2	80.0	107	3.7
PFTrDA	16.0	108	2.6	80.0	99.1	3.6
PFTA	16.0	110	0.9	80.0	97.2	3.6
$^{13}C_2$-PFHxA	40.0	88.5	6.4	40.0	97.0	4.9
$^{13}C_3$-HFPO-DA	40.0	94.5	3.2	40.0	101	9.9
$^{13}C_2$-PFDA	40.0	99.1	3.4	40.0	106	2.7
d_5-NEtFOSAA	160	90.0	2.6	160	99.5	4.8

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TABLE 7. PRECISION AND ACCURACY (n=4) OF PFAS IN TAP WATER[a] FROM A GROUND WATER SOURCE

Analyte	Fortified Conc. (ng/L)	Mean % Recovery	% RSD	Fortified Conc. (ng/L)	Mean % Recovery	% RSD
PFBS	16.0	104	3.1	80.0	90.2	2.1
PFHxA	16.0	105	3.5	80.0	91.6	3.9
HFPO-DA	16.0	99.6	4.0	80.0	90.6	2.9
PFHpA	16.0	101	3.4	80.0	91.2	4.2
PFHxS	16.0	110.0	3.3	80.0	93.5	4.8
ADONA	16.0	104	3.9	80.0	92.2	4.7
PFOA	16.0	105	2.7	80.0	91.1	4.8
PFOS	16.0	108	3.3	80.0	93.9	3.8
PFNA	16.0	105	2.4	80.0	92.4	6.9
9Cl-PF3ONS	16.0	101	8.1	80.0	92.4	4.9
PFDA	16.0	102	4.5	80.0	92.5	7.7
NMeFOSAA	16.0	92.6	7.4	80.0	87.1	9.4
PFUnA	16.0	104	4.8	80.0	92.8	5.6
NEtFOSAA	16.0	108	18.4	80.0	94.1	6.7
11CL-PF3OUdS	16.0	103	3.4	80.0	95.4	5.4
PFDoA	16.0	99.4	4.6	80.0	92.0	5.0
PFTrDA	16.0	98.8	4.1	80.0	93.1	5.9
PFTA	16.0	102	3.7	80.0	93.9	5.0
$^{13}C_2$-PFHxA	40.0	97.7	3.4	40.0	87.0	6.2
$^{13}C_3$-HFPO-DA	40.0	97.2	3.9	40.0	88.8	6.2
$^{13}C_2$-PFDA	40.0	97.5	5.3	40.0	86.0	10
d_5-NEtFOSAA	160	94.7	8.8	160	80.8	10

[a] TOC = 0.53 mg/L and hardness = 377 mg/L measured as calcium carbonate.

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TABLE 8. PRECISION AND ACCURACY (n=4) OF PFAS IN TAP WATER[a] FROM A SURFACE WATER SOURCE

Analyte	Fortified Conc. (ng/L)	Mean % Recovery	% RSD	Fortified Conc. (ng/L)	Mean % Recovery	% RSD
PFBS	16.0	91.6	3.8	80.0	91.9	7.1
PFHxA	16.0	92.0	5.5	80.0	99.3	4.0
HFPO-DA	16.0	88.6	1.3	80.0	102	2.2
PFHpA	16.0	95.5	3.6	80.0	101	3.3
PFHxS	16.0	99.1	2.5	80.0	102	0.9
ADONA	16.0	95.5	2.9	80.0	102	3.5
PFOA	16.0	97.9	5.2	80.0	98.8	3.9
PFOS	16.0	93.5	5.9	80.0	101	2.4
PFNA	16.0	96.4	3.4	80.0	101	2.8
9Cl-PF3ONS	16.0	93.1	4.6	80.0	102	3.3
PFDA	16.0	95.3	1.7	80.0	99.2	3.3
NMeFOSAA	16.0	99.3	7.2	80.0	94.9	4.5
PFUnA	16.0	99.8	1.7	80.0	100	4.1
NEtFOSAA	16.0	93.3	8.0	80.0	90.5	3.9
11CL-PF3OUdS	16.0	97.6	6.7	80.0	97.5	3.1
PFDoA	16.0	88.0	1.8	80.0	97.0	2.7
PFTrDA	16.0	94.7	2.5	80.0	95.5	1.8
PFTA	16.0	94.1	5.9	80.0	97.8	3.3
$^{13}C_2$-PFHxA	40.0	86.3	2.8	40.0	90.6	4.1
$^{13}C_3$-HFPO-DA	40.0	92.9	2.4	40.0	101	1.8
$^{13}C_2$-PFDA	40.0	89.3	4.3	40.0	95.8	2.2
d_5-NEtFOSAA	160	86.5	5.4	160	83.1	4.4

[a] TOC = 2.4 mg/L and hardness = 103 mg/L measured as calcium carbonate.

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TABLE 9. PRECISION AND ACCURACY (n=4) OF PFAS IN TAP WATER[a] FROM A PRIVATE WELL

Analyte	Fortified Conc. (ng/L)	Mean % Recovery	% RSD
PFBS	80.0	99.7	3.1
PFHxA	80.0	96.3	2.7
HFPO-DA	80.0	94.2	4.3
PFHpA	80.0	97.4	1.9
PFHxS	80.0	99.4	4.0
ADONA	80.0	98.7	2.8
PFOA	80.0	97.2	1.5
PFOS	80.0	100	1.9
PFNA	80.0	99.4	1.3
9Cl-PF3ONS	80.0	101	2.2
PFDA	80.0	98.7	2.3
NMeFOSAA	80.0	93.2	4.6
PFUnA	80.0	98.8	1.7
NEtFOSAA	80.0	94.4	0.6
11CL-PF3OUdS	80.0	99.8	2.5
PFDoA	80.0	99.3	1.9
PFTrDA	80.0	96.2	1.3
PFTA	80.0	97.9	1.2
$^{13}C_2$-PFHxA	40.0	89.9	2.7
$^{13}C_3$-HFPO-DA	40.0	95.7	5.3
$^{13}C_2$-PFDA	40.0	92.3	1.8
d_5-NEtFOSAA	160	86.3	4.5

[a] TOC = 0.56 mg/L and hardness = 394 mg/L measured as calcium carbonate.

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TABLE 10. AQUEOUS SAMPLE HOLDING TIME DATA FOR TAP WATER SAMPLES FROM A SURFACE WATER SOURCE[a], FORTIFIED WITH METHOD ANALYTES AND PRESERVED AND STORED ACCORDING TO SECTION 8 (n=4)

Analyte	Fortified Conc. (ng/L)	Day 0 Mean % Recovery	Day 0 % RSD	Day 8 Mean % Recovery	Day 8 % RSD	Day 14 Mean % Recovery	Day 14 % RSD
PFBS	80.0	91.9	7.1	99.4	4.2	93.4	11
PFHxA	80.0	99.3	4.0	101	5.4	93.4	7.9
HFPO-DA	80.0	102	2.2	101	5.3	100	11
PFHpA	80.0	101	3.3	99.2	2.2	101	3.6
PFHxS	80.0	102	0.9	103	4.0	107	4.5
ADONA	80.0	102	3.5	102	4.7	101	4.4
PFOA	80.0	98.8	3.9	99.8	0.63	100	3.5
PFOS	80.0	101	2.4	101	3.6	106	6.8
PFNA	80.0	101	2.8	101	0.87	105	4.8
9Cl-PF3ONS	80.0	102	3.3	100	2.2	102	4.4
PFDA	80.0	99.2	3.3	99.6	1.6	102	5.5
NMeFOSAA	80.0	94.9	4.5	98.0	3.5	95.4	7.3
PFUnA	80.0	100	4.1	101	4.4	100	6.2
NEtFOSAA	80.0	90.5	3.9	102	5.3	96.5	7.7
11CL-PF3OUdS	80.0	97.5	3.1	101	4.5	102	5.5
PFDoA	80.0	97.0	2.7	98.4	3.5	103	3.8
PFTrDA	80.0	95.5	1.8	99.5	3.2	99.4	3.8
PFTA	80.0	97.8	3.3	102	3.2	96.2	2.1
$^{13}C_2$-PFHxA	40.0	90.6	4.1	93.6	5.5	93.0	8.8
$^{13}C_3$-HFPO-DA	40.0	101	1.8	101	3.1	91.5	12
$^{13}C_2$-PFDA	40.0	95.8	2.2	92.6	6.8	104	2.8
d_5-NEtFOSAA	160	83.1	4.4	87.6	2.6	95.2	4.3

[a] TOC = 2.4 mg/L and hardness = 103 mg/L measured as calcium carbonate.

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TABLE 11. EXTRACT HOLDING TIME DATA FOR TAP WATER SAMPLES FROM A SURFACE WATER SOURCE, FORTIFIED WITH METHOD ANALYTES AND PRESERVED AND STORED ACCORDING TO SECTION 8 (n=4)

Analyte	Fortified Conc. (ng/L)	Day 0 Mean % Recovery	Day 0 % RSD	Day 8 Mean % Recovery	Day 8 % RSD	Day 14 Mean % Recovery	Day 14 % RSD	Day 28 Mean % Recovery	Day 28 % RSD
PFBS	80.0	91.9	7.1	96.9	5.1	90.6	10	99.4	5.3
PFHxA	80.0	99.3	4.0	10	1.3	94.1	2.9	105	2.6
HFPO-DA	80.0	102	2.2	103	1.4	98.7	2.6	103	1.1
PFHpA	80.0	101	3.3	102	2.9	98.3	1.0	104	3.5
PFHxS	80.0	102	0.9	105	2.9	99.7	1.8	107	2.5
ADONA	80.0	102	3.5	104	3.7	98.6	2.5	106	2.5
PFOA	80.0	98.8	3.9	106	3.7	101	1.8	106	2.8
PFOS	80.0	101	2.4	102	1.1	103	1.8	109	2.2
PFNA	80.0	101	2.8	105	1.8	103	2.3	107	2.4
9Cl-PF3ONS	80.0	102	3.3	99.4	3.1	97.6	2.9	107	2.2
PFDA	80.0	99.2	3.3	104	1.9	101.2	0.9	107	3.4
NMeFOSAA	80.0	94.9	4.5	101	3.9	90.5	5.2	105	6.8
PFUnA	80.0	100	4.1	104	5.5	102	4.2	106	3.0
NEtFOSAA	80.0	90.5	3.9	104	3.1	93.6	7.7	102	2.9
11CL-PF3OUdS	80.0	97.5	3.1	103	1.9	97.3	1.6	108	2.7
PFDoA	80.0	97.0	2.7	102	3.7	99.8	3.3	106	2.6
PFTrDA	80.0	95.5	1.8	102	3.0	97.2	1.6	104	3.1
PFTA	80.0	97.8	3.3	105	4.2	98.8	2.1	108	2.5
$^{13}C_2$-PFHxA	40.0	90.6	4.1	101	1.2	101	2.6	114	2.1
$^{13}C_3$-HFPO-DA	40.0	101	1.8	95.5	3.2	96.5	2.7	111	2.5
$^{13}C_2$-PFDA	40.0	95.8	2.2	100	2.7	109	1.9	124	4.4
d_5-NEtFOSAA	160	83.1	4.4	94.7	1.6	91.4	4.8	113	9.1

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TABLE 12. INITIAL DEMONSTRATION OF CAPABILITY QUALITY CONTROL REQUIREMENTS

Method Reference	Requirement	Specification and Frequency	Acceptance Criteria
Sect. 9.2.2	Initial Demonstration of Low System Background	Analyze LRB prior to any other IDC steps.	Demonstrate that all method analytes are below 1/3 the MRL and that possible interferences from extraction media do not prevent the identification and quantification of method analytes.
Sect. 9.2.3	Initial Demonstration of Precision (IDP)	Analyze four to seven replicate LFBs fortified near the midrange calibration concentration.	%RSD must be <20%
Sect. 9.2.4	Initial Demonstration of Accuracy (IDA)	Calculate average recovery for replicates used in IDP.	Mean recovery ± 30% of true value
Sect. 9.2.5	Initial Demonstration of Peak Asymmetry Factor	Calculate the peak asymmetry factor using the equation in Section 9.3.9 for the first two eluting chromatographic peaks in a mid-level CAL standard.	Peak asymmetry factor of 0.8 - 1.5
Sect. 9.2.6	Minimum Reporting Limit (MRL) Confirmation	Fortify, extract and analyze seven replicate LFBs at the proposed MRL concentration. Calculate the Mean and the Half Range (HR). Confirm that the upper and lower limits for the Prediction Interval of Result (Upper PIR, and Lower PIR, Sect. 9.2.6.2) meet the recovery criteria.	Upper PIR ≤ 150% Lower PIR ≥ 50%
Sect. 9.2.7 and 9.3.10	Quality Control Sample (QCS)	Analyze a standard from a second source, as part of IDC.	Results must be within 70-130% of true value.
Sect. 9.2.8	Detection Limit (DL) Determination (optional)	Over a period of three days, prepare a minimum of seven replicate LFBs fortified at a concentration estimated to be near the DL. Analyze the replicates through all steps of the analysis. Calculate the DL using the equation in Sect. 9.2.8.1.	Data from DL replicates are not required to meet method precision and accuracy criteria. If the DL replicates are fortified at a low enough concentration, it is likely that they will not meet precision and accuracy criteria.

NOTE: Table 12 is intended as an abbreviated summary of QC requirements provided as a convenience to the method user. Because the information has been abbreviated to fit the table format, there may be issues that need additional clarification, or areas where important additional information from the method text is needed. In all cases, the full text of the QC in Section 9 supersedes any missing or conflicting information in this table.

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TABLE 13. ONGOING QUALITY CONTROL REQUIREMENTS (SUMMARY)

Method Reference	Requirement	Specification and Frequency	Acceptance Criteria
Sect. 8.1 - Sect. 8.5	Sample Holding Time	14 days with appropriate preservation and storage as described in Sections 8.1-8.5.	Sample results are valid only if samples are extracted within the sample holding time.
Sect. 8.5	Extract Holding Time	28 days when stored at room temperature in polypropylene centrifuge tubes.	Extract results are valid only if extracts are analyzed within the extract holding time.
Sect. 9.3.1	Laboratory Reagent Blank (LRB)	One LRB with each extraction batch of up to 20 samples.	Demonstrate that all method analytes are below 1/3 the MRL, and confirm that possible interferences do not prevent quantification of method analytes. If targets exceed 1/3 the MRL or if interferences are present, results for these subject analytes in the extraction batch are invalid.
Sect. 9.3.3	Laboratory Fortified Blank (LFB)	One LFB is required for each extraction batch of up to 20 Field Samples. Rotate the fortified concentrations between low, medium and high amounts.	Results of LFB analyses must be 70-130% of the true value for each method analyte for all fortified concentrations except the lowest CAL point. Results of the LFBs corresponding to the lowest CAL point for each method analyte must be 50-150% of the true value.
Sect. 9.3.4	Internal Standard (IS)	Internal standards, $^{13}C_2$-PFOA (IS#1), $^{13}C_4$-PFOS (IS#2), and d_3-NMeFOSAA (IS#3), are added to all standards and sample extracts, including QC samples. Compare IS areas to the average IS area in the initial calibration and to the most recent CCC.	Peak area counts for all ISs in all injections must be within ± 50% of the average peak area calculated during the initial calibration and 70-140% from the most recent CCC. If ISs do not meet this criterion, corresponding target results are invalid.
Sect. 9.3.5	Surrogate Standards (SUR)	Surrogate standards, $^{13}C_2$-PFHxA, $^{13}C_3$-HFPO-DA, $^{13}C_2$-PFDA, and d_5-NEtFOSAA, are added to all CAL standards and samples, including QC samples. Calculate SUR recoveries.	SUR recoveries must be 70-130% of the true value. If a SUR fails this criterion, report all results for sample as suspect/SUR recovery.

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TABLE 13. (Continued)

Method Reference	Requirement	Specification and Frequency	Acceptance Criteria
Sect. 9.3.6	Laboratory Fortified Sample Matrix (LFSM)	Analyze one LFSM per extraction batch (20 samples or less) fortified with method analytes at a concentration close to but greater than the native concentration, if known. Calculate LFSM recoveries.	Recoveries at mid and high levels must be within 70-130% and within 50-150% at the low-level fortified amount (near the MRL). If these criteria are not met, results are labeled suspect due to matrix effects.
Sect. 9.3.7	Laboratory Fortified Sample Matrix Duplicate (LFSMD) or Field Duplicates (FD)	Extract and analyze at least one FD or LFSMD with each extraction batch (20 samples or less). A LFSMD may be substituted for a FD when the frequency of detects are low. Calculate RPDs.	Method analyte RPDs for the LFMD or FD must be ≤30% at mid and high levels of fortification and ≤50% near the MRL. If these criteria are not met, results are labeled suspect due to matrix effects.
Sect. 9.3.8	Field Reagent Blank (FRB)	Analysis of the FRB is required only if a Field Sample contains a method analyte or analytes at or above the MRL. The FRB is processed, extracted and analyzed in exactly the same manner as a Field Sample.	If the method analyte(s) found in the Field Sample is present in the FRB at a concentration greater than 1/3 the MRL, then all samples collected with that FRB are invalid and must be recollected and reanalyzed.
Sect. 9.3.9	Peak Asymmetry Factor	Calculate the peak asymmetry factor for the first two eluting chromatographic peaks in a mid-level CAL standard during IDC and when chromatographic changes are made that affect peak shape.	Peak asymmetry factor of 0.8 - 1.5
Sect. 9.3.10	Quality Control Sample (QCS)	Analyze at least quarterly or when preparing new standards, as well as during the IDC.	Results must be within 70-130% of true value.
Sect. 10.2 and Sect. 9.3.2	Initial Calibration	Use IS calibration technique to generate a first or second order calibration curve forced through zero. Use at least five standard concentrations. Check the calibration curve as described in Sect. 10.2.6.	When each CAL standard is calculated as an unknown using the calibration curve, the analyte and SUR results must be 70-130% of the true value for all except the lowest standard, which must be 50-150% of the true value. Recalibration is recommended if these criteria are not met.
Sect. 9.3.2 and Sect. 10.3	Continuing Calibration Check (CCC)	Verify initial calibration by analyzing a low level (at the MRL or below) CCC prior to analyzing samples. CCCs are then injected after every 10 samples and after the last sample, rotating concentrations to cover the calibrated range of the instrument.	Recovery for each analyte and SUR must be within 70-130% of the true value for all but the lowest level of calibration. Recovery for each analyte in the lowest CAL level CCC must be within 50-150% of the true value and the SUR must be within 70-130% of the true value.

NOTE: Table 13 is intended as an abbreviated summary of QC requirements provided as a convenience to the method user. Because the information has been abbreviated to fit the table format, there may be issues that need additional clarification, or areas where important additional information from the method text is needed. In all cases, the full text of the QC in Sections 8-10 supersedes any missing or conflicting information in this table.

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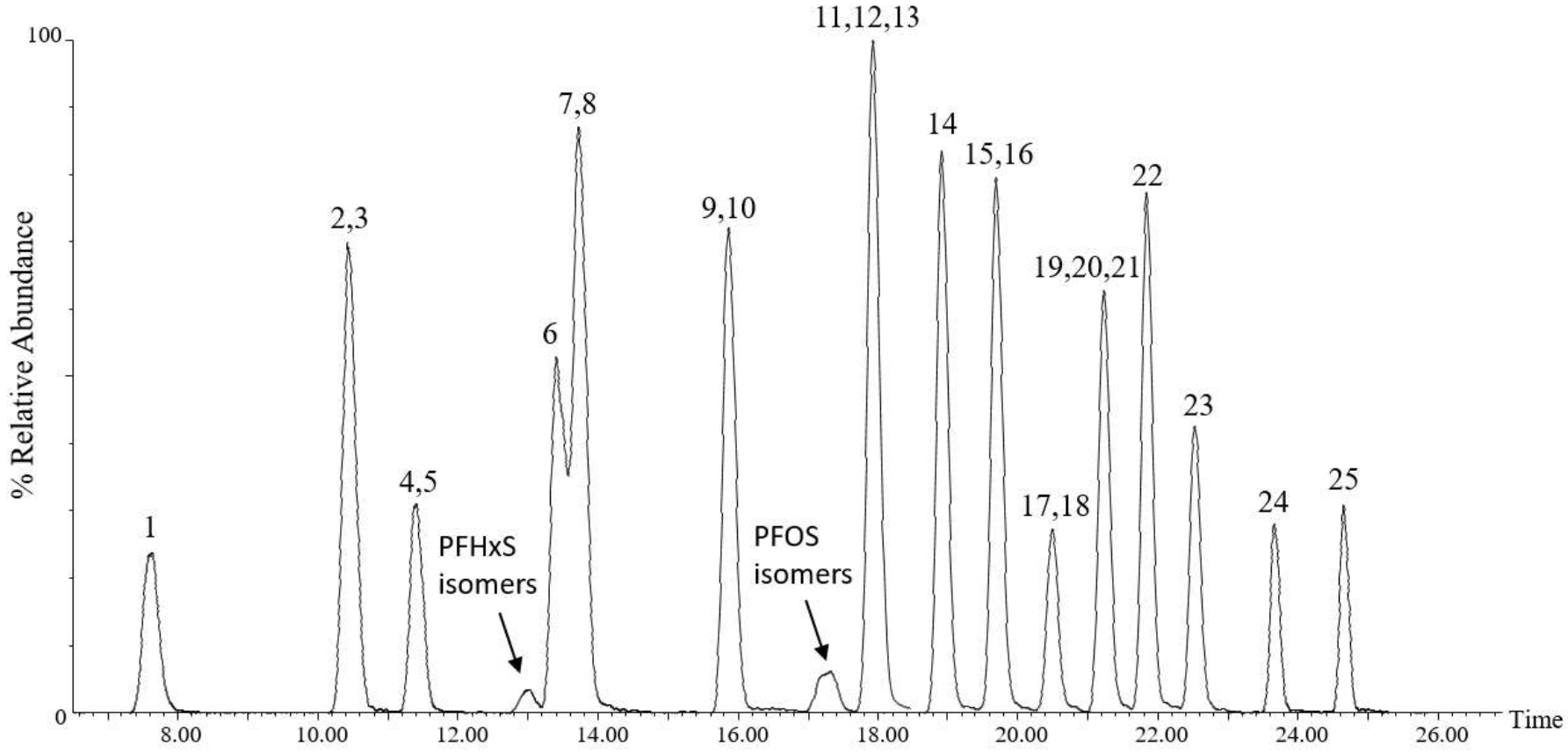
FIGURE 1. EXAMPLE CHROMATOGRAM FOR REAGENT WATER FORTIFIED WITH METHOD 537.1 ANALYTES AT 80 ng/L. NUMBERED PEAKS ARE IDENTIFIED IN TABLE 3.



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Appendix C: Selection of Municipal Systems for Sampling

Municipal systems were selected for sampling in consideration of currently available indications that source water might be impacted by perfluoroalkyl and polyfluoroalkyl substances (PFAS). Selection of systems was necessary because available funding is only enough to collect samples from entry points with source water from about 30% of all municipal groundwater supply wells statewide, while reserving some funds for possible follow-up samples. The criteria described here serve as reasons for sampling the selected systems at this time to determine how the levels of any PFAS in water being delivered to consumers compares to recommended health standards. However, these criteria and the resulting systems selection list does not mean that PFAS will ultimately be found to be present in higher concentrations in these municipal systems compared to others in the state.

A goal of the sampling is to understand the role of different water supply sources within a municipal system to PFAS concentrations in the water supply. Therefore, selection of sample locations was conducted by compiling a list of all municipal water systems and all entry points to each system. All entry points in each selected municipal system will be sampled. Systems were selected using a tiered approach, as described in the following paragraphs, to identify drinking water source wells within municipal water systems for which available information indicates a possibility of PFAS contamination.

First, municipal water supply wells located within one mile of sites with known or potential release of aqueous film forming foam (AFFF) were identified and those systems placed on the list for sampling.

Second, a GIS layer of facilities with primary North American Industrial Classification System (NAICS) codes for which PFAS use is known or suspected was queried, with facilities given risk ranks as follows: 4 – PFAS directly used in industrial applications, 3 – suspected use of PFAS in manufacturing, 2 – secondary sources of PFAS, 1 – possible but less likely PFAS use in manufacturing or industry. Areas within Municipal Systems assigned risk scores by summing the risk ranks of all facilities within each area. While primary NAICS codes were used in the selection as an indicator of density of industrial facilities that might use PFAS in an area, the codes are not definitive in indicating whether PFAS were used and/or discharged to the environment. Environmental scientists and regulators are still in the early stages of understanding where PFAS are present in the environment. More will be learned over time regarding to what degree NAICS codes are useful in identifying PFAS sources.

Third, the scores of all facilities within the designated areas were summed to create risk scores. Systems were characterized by the highest-score area within their system, rather than an average for the system. Then, system selection proceeded by adding systems with areas containing the highest cumulative risk scores to the list. Finally, the list was shared internally to check for the absence of any systems for which there are known PFAS issues—such systems with nearby Remediation & Redevelopment Sites with PFAS contamination.

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Appendix D: Planning and Sampling Protocol

The Planning and Sampling Protocol is provided on the following three pages.

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PFAS in Public Drinking Water:
Planning and Sampling Protocol

Part I: Planning

Before the day of sampling, please make arrangements for overnight shipping of the samples to the Wisconsin State Laboratory of Hygiene (WSLH), including scheduling a package collection time in advance if necessary. WSLH will pay for return shipping with UPS; if a different provider is used, please arrange and pay for overnight shipping. Please plan to collect samples on a Monday, Tuesday, Wednesday or Thursday. Every sample should be shipped the same day as it is collected. The samples must arrive at WSLH at a time when staff are present to receive the samples and transfer them to laboratory cold storage.

PFAS are attracted to air-water interfaces and thus concentrations may fluctuate, especially early in a pumping cycle. Therefore, samples should be collected from each water system entry point during the final third of a pumping cycle (i.e., pumping of the source well(s) at least 67% of the way through the current cycle) to aid in result comparability across samples. Please make a note in the *special instructions* line of the sample form if necessary to plan sample collection time accordingly. If multiple wells serve one finished water distribution system entry point, samples should be collected as late as possible in the pumping cycle for all wells. If it is not possible to sample the entry point at a time when all source wells to that entry point are in operation, please note which wells were not in operation at the time of sample collection.

A WSLH video of the sample collection process is available at www.slh.wisc.edu/environmental/pfas/. This video was developed for private well owners. Most of the video content applies to sampling public drinking water, but a few details are different. Where in doubt, please refer to these instructions and the enclosed sample form and contact WSLH or DNR with any questions.

The sampling kit includes three sample bottles, each containing a preservative called Trizma. Safety Data Sheets (SDS) can be found at http://www.slh.wisc.edu/environmental/water/environmental-test-kit-safety-data-sheets/. The sampling kit also contains two bottles (labeled FB1 and FB2) for the Field Reagent Blank: one bottle filled with PFAS-free water and one empty bottle (see instructions on Page 3).

Many common commercial and consumer products contain PFAS. Please plan ahead to avoid use of the following during sampling:

- Polytetrafluoroethylene (PTFE/Teflon) or polyvinylidene fluoride (PVDF) containing materials (used in some tubing, bailers, tape, plumbing paste) or other materials with substances containing “fluoro”, “perfluoro” or “fluorosurfactant”
- LDPE bags or containers
- Waterproof field books, clipboards, binders, notebooks, sticky notes, glue materials, pens, paper (please begin to fill out the provided field sheets before sampling and finish after sampling is completed, so that handling pens and paper during sampling may be avoided)
- Markers (except to label)
- Blue ice packs
- Decontamination soaps (detergents) that contain fluoro-surfactants
- Water that is not “PFAS-free”, except for the sample water when filling the appropriate sample bottles
- Water resistant, waterproof, stain treated clothing or shoes including Gore-Tex and Tyvek materials; wearing all-cotton clothing that has been washed without fabric softener is preferred

- Field personnel should not use cosmetics, moisturizers, hand cream or other related personal care products
- Some manufactured sunblock and insect repellants contain PFAS
- No food or drink with the exception of bottled water

The following information (source: https://www.michigan.gov/documents/pfasresponse/General_PFAS_Sampling_Guidance_634597_7.pdf) is provided to aid in decisions about which sunscreens and insect repellants might be okay to use on sampling day (avoiding use of sunscreens and insect repellants is preferable) and which should be investigated further or avoided:

Sunscreen and Insect Repellents[1]

■ Allowable Insect Repellants	
Photos	**Insect Repellent Spray**
	• OFF Deep Woods • Sawyer Permethrin
■ Allowable Sunscreens	
Photos	**Sunscreens**
	• Banana Boat Sport Performance Sunscreen Lotion Broad Spectrum SPF 30. • Meijer Sunscreen Lotion Broad Spectrum SPF 30. • Neutrogena Ultra-Sheer Dry-Touch Sunscreen Broad Spectrum SPF 30.

■ Allowable Sunscreens
• Banana Boat for Men Triple Defense Continuous Spray Sunscreen SPF 30
• Banana Boat Sport Performance Coolzone Broad Spectrum SPF 30
• Banana Boat Sport Performance Sunscreen Lotion Broad Spectrum SPF 30
• Banana Boat Sport Performance Sunscreen Stick SPF 50
• Coppertone Sunscreen Lotion Ultra Guard Broad Spectrum SPF 50
• Coppertone Sport High-Performance AccuSpray Sunscreen SPF 30
• Coppertone Sunscreen Stick Kids SPF 55
• L'Oréal Silky Sheer Face Lotion 50+
• Meijer Clear Zinc Sunscreen Lotion Broad Spectrum SPF 15, 30 and 50
• Meijer Wet Skin Kids Sunscreen Continuous Spray Broad Spectrum SPF 70
• Neutrogena Beach Defense Water + Sun Barrier Lotion SPF 70
• Neutrogena Beach Defense Water + Sun Barrier Spray Broad Spectrum SPF 30
• Neutrogena Pure & Free Baby Sunscreen Broad Spectrum SPF 60+
▲ Materials That Require Screening
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, Baby Ganics.
Sunscreen and Insect Repellent: Avon Skin So Soft Bug Guard Plus – SPF 30 Lotion.

[1]This table is not considered to be a complete listing of allowable materials and materials that require screening. All materials should be evaluated before use during sampling. Some of the sunscreen and insect repellent testing has been performed using a PFAS screening Method known as Particle Induce Gamma-Ray Emission (PIGE). The use of approved gloves should always be used, and the sample should never come into contact with any of the sunscreen or insect repellent products. An Equipment Blank sample could also be collected to verify the product as PFAS-free.

●- Prohibited ■- Allowable ▲- Needs Screening

WISCONSIN DEPT. OF NATURAL RESOURCES

Wisconsin State Laboratory of Hygiene
UNIVERSITY OF WISCONSIN-MADISON

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Part II: Sample Collection and Handling

1. Using permanent marker write the following information on the sample label:
   Sampling Site
   Date
   Time

2. IMPORTANT: Prior to handling any of the items in the sampling kit, the sample collector must wash their hands and wear powderless nitrile gloves (provided) while filling and sealing the sample bottles. This will help minimize contamination sources. Change gloves between each sampling point to prevent sample cross-contamination but note that the number of gloves provided is limited to the number of sampling points due to short supply. After putting on the gloves, avoid touching clothing (which can contain PFAS itself or from detergents used) until after the sample bottles are filled and closed.
   Note: Powderless nitrile gloves are provided as the best available low-PFAS gloves for sampling. However, the gloves might contain low levels of a few PFAS. It is therefore important to ensure that the gloves only touch the outside of the sample bottle.

3. Samples must be collected in the provided 250-mL polypropylene bottles (bottles for each sample). Finished water samples should be collected from a designated entry point tap, which is a location in the PWS after treatment or chemical addition, but before the distribution system. Before collecting the sample, open the tap and allow the system to flush until water temperature has stabilized (approximately 3 to 5 min). The sample should be shielded from light to the extent practical.

4. Fill all three sample bottles up to the shoulder of the bottle. The PFAS sample bottles should be filled before any other bottles if you are doing multiple analyses. Do not touch the inside of the cap or around the edge of the bottle. Do not place the cap on any surface when collecting the sample.

5. After collecting the sample, cap each bottle. Do not use any type of tape to close the cap. Keep the bottle sealed until extraction at WSLH.

6. Two bottles are provided for Field Reagent Blanks. One bottle is labeled FB1 and is already full of water. During the sampling event, as soon as possible after the drinking water sample is collected, the sample collector must open FB1 and pour it into the empty bottle labeled FB2. Seal FB2 and return it to the lab. The empty FB1 bottle can be discarded.

7. Store the samples refrigerated until ready to ship to the laboratory. Ship back to WSLH on ice to ensure their temperature does not exceed 6°C.

8. When shipping samples to the laboratory, line the cooler with the large clear plastic bag provided. Ensure samples are tightly capped. Once samples are added, add plenty of regular ice to the bag (do not use ice packs as they may contain PFAS – when in doubt use more ice), and close the large plastic bag and secure with the enclosed zip tie. Place sample submission forms in the provided Zip-Lock bag and place on top of the samples. Dispose of gloves.

9. On the same day of sample collection, ship the samples to WSLH by overnight courier.

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Appendix E: WSLH PFAS in Drinking Water Data Verification Checklist

The WSLH PFAS in Drinking Water Data Verification Checklist is provided on the following page.

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Batch:

Folder Contents	Submittal sheets	
	Batch worklist	
	Data printouts for all samples/QC	
	Calibration curves for all compounds (and confirmation)	
	Extraction log	
	Method	
	Internal standards count worksheet	
QC Requirements	LRB - All compounds must be <1/3 of the MRL	
	FRB - All compounds must be <1/3 of the MRL	
	LCS (high and middle) - All compounds must be within 70-130% of the	
	LCS (low) - All compounds must be within 50-150% of the true value	
	Internal standards - Peak area counts for all IS in all injections must be	
	Surrogates - 70-130%	
	Dup - RPD ± 30%	
	Initial calibration - 70-130% of the true value, 50-150% of the lowest	
	CCC- 70-130% for the high and middle check standards, 50-150% for the	
	Calibration curves for all compounds must be forced through zero and	
	Every QC failure MUST be flagged, unless there is a deviation stated in	
	% Relative Error must be ≤50% for lowest calibration standard, ≤30% for	
Standards	Standard numbers should be listed on the front page of the first	
	All standard forms have been audited by peer analyst or supervisor and	
Horizon	Verify the numbers are inputted correctly	
	Verify that appropriate flags are entered	
	Verify that *REC is complete	
	Dilutions are correctly entered into Horizon	
Analysis	Data printout is attached to its calibration curve	

Reviewed By: _____

Date: _____

Comment: _____

Method	**ESS ORG Method OC12731 PFAS in Drinking Water**	**Rev:**	

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Appendix F: Monitoring and Laboratory Sample Form

The WDNR Monitoring and Laboratory Sample Form is provided on the following two pages.

DocuSign Envelope ID: EC66AF91-D4C0-4E96-9AC1-0D84B2BD2EC2

DNR Drinking Water Program
Central Office Headquarters
101 S. Webster Street PO Box 7921
Madison, WI 53707-7921

PER/POLY-FLUOROALKYL ANALYSES

(ENCLOSE FORM WHEN SENDING SAMPLE TO LAB)

Rev: 01/20

Section I: To be completed by the Department of Natural Resources/SAMPLER

System Name: _____ System Type: (Check One) MC ___ NN ___ OC ___ TN ___

System Address: _____ City: _____ County: _____ Region Code: ___

Pws Id#: _____ Entry Point ID: _____ WI Unique Well No: _____ DNR Contact: _____

Sampler Phone/Name/Address **(Notify DNR Contact of Corrections)**	**Sampler:** Provide information to have results faxed or e-mailed or to change a billing address, if your lab offers these services (leave blank if you don't use these services). Fax number: _____ E-mail: _____ Billing Address: _____

Sample Source:

___ W - Well

___ E - Entry Point

___ D - Distribution System

Sample Type:

___ D - Compliance Sample

___ C - Confirmation Sample

___ I - Investigation Sample

___ W - Raw Water Sample

Special Instructions: _____

Collect sample between: ___ / ___ / _____ **and** ___ / ___ / _____

Section II: To be completed by SAMPLER -- ALL ITEMS REQUIRED

Sample Collection Date: ___ / ___ / _____ Time: ___ : ___ ☐a.m. ☐p.m.

Address where sample was collected: _____

Monitoring Point ID: _____ Sample Point Description: _____

First Initial and Last Name of Sampler: ___ _____ Sampler Phone: _____

Section III: To be completed by LAB. Report results on back and electronically to DNR within 10 days per NR 809.80

☐ **Check here if some or all of the parameters were analyzed by a subcontracted lab.**
NOTE: A separate form must be completed by each lab with data for only the parameters which that lab analyzed.

Laboratory ID Number: _____ Laboratory Name: _____

Date Sample Received: ___ / ___ / _____ Time Sample Received: ___ ___ : ___ ___ Laboratory Sample ID: _____

Signature of Receiving Lab Official: _____ Date Reported to PWS: ___ / ___ / _____

Condition of Sample Upon Receipt: _____

Notice: This form must be submitted with laboratory samples analyzed to determine compliance with ch. NR 809, Wis. Adm. Code, Safe Drinking Water. Completion of this form or a similar form approved by the Department is mandatory. Failure to submit a completed form to the Department is a violation punishable by a forfeiture of no less than $10 nor more than $5000, or by a fine of not less than $10 nor more than $100 or imprisonment of not less than 30 days, or both. Each day of continued violation is a separate offense (ss. 144.99, Wis. Stats.). Authorization for these requirement is under s. 280.13(d), Wis. Stats. and ch. NR 809.80. Personally identifiable information on this form will be used for no other purpose.

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PER/POLY-FLUOROALKYL ANALYSES System Name: _____

To be completed by the laboratory performing analysis. PWS ID: _____ Lab Sample ID: _____

Storet Code		Parameter	Abbrev.	SDWA Method	MDL	Results	MCL	Units
97433		11-CHLOROEICOSAFLUORO-3-OXAUNDECANE-1-SULFONIC ACI	11CL-PF3OUDS					NG/L
97434		4,8-DIOXA-3H-PERFLUORONONANOIC ACID	DONA					NG/L
97415		4:2 FLUOROTELOMER SULFONIC ACID	4:2 FTSA					NG/L
97414		6:2 FLUOROTELOMER SULFONIC ACID	6:2 FTSA					NG/L
97413		8:2 FLUOROTELOMER SULFONIC ACID	8:2 FTSA					NG/L
97432		9-CHLOROHEXADECAFLUORO-3-OXANONANE-1-SULFONIC ACID	9CL-PF3ONS					NG/L
97435		HEXAFLUOROPROPYLENE OXIDE DIMER ACID	HFPO-DA					NG/L
97420		N-ETHYL PERFLUOROOCTANESULFONAMIDE	N-EtFOSA					NG/L
97436		N-ETHYL PERFLUOROOCTANESULFONAMIDO-ACETIC ACID	N-EtFOSAA					NG/L
97416		N-ETHYL PERFLUOROOCTANESULFONAMIDO-ETHANOL	N-EtFOSE					NG/L
97421		N-METHYL PERFLUOROOCTANESULFONAMIDE	N-MeFOSA					NG/L
97437		N-METHYL PERFLUOROOCTANESULFONAMIDO-ACETIC ACID	N-MeFOSAA					NG/L
97417		N-METHYL PERFLUOROOCTANESULFONAMIDO-ETHANOL	N-MeFOSE					NG/L
97423		PERFLUORODODECANESULFONIC ACID	PFDoS					NG/L
99987		PERFLUORO-N-BUTANESULFONIC ACID	PFBS					NG/L
99991		PERFLUORO-N-BUTANOIC ACID	PFBA					NG/L
99990		PERFLUORO-N-DECANESULFONIC ACID	PFDS					NG/L
99996		PERFLUORO-N-DECANOIC ACID	PFDA					NG/L
99998		PERFLUORO-N-DODECANOIC ACID	PFDoA					NG/L
99989		PERFLUORO-N-HEPTANESULFONIC ACID	PFHpS					NG/L
99994		PERFLUORO-N-HEPTANOIC ACID	PFHpA					NG/L
99988		PERFLUORO-N-HEXANESULFONIC ACID	PFHxS					NG/L
99993		PERFLUORO-N-HEXANOIC ACID	PFHxA					NG/L
99995		PERFLUORO-N-NONANOIC ACID	PFNA					NG/L
99598		PERFLUORO-N-OCTANESULFONIC ACID	PFOS					NG/L
99597		PERFLUORO-N-OCTANOIC ACID	PFOA					NG/L
97424		PERFLUORONONANESULFONIC ACID	PFNS					NG/L
99992		PERFLUORO-N-PENTANOIC ACID	PFPeA					NG/L
99924		PERFLUORO-N-TETRADECANOIC ACID	PFTeDA					NG/L
99923		PERFLUORO-N-TRIDECANOIC ACID	PFTRiA					NG/L
99997		PERFLUORO-N-UNDECANOIC ACID	PFuNA					NG/L
97422		PERFLUOROOCTANESULFONAMIDE	FOSA					NG/L
97425		PERFLUOROPENTANESULFONIC ACID	PFPeS					NG/L

Approved By: QA Officer: _____ Date: _____

Laboratory Manager: _____ Date: _____

Comments: _____

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Appendix G: WSLH PFAS Drinking Water SOP

The WSLH laboratory SOP for EPA Method 537.1 is provided on the following 31 pages.

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ESS ORG Method OC12731
Analysis of PFAS in Drinking Water by HPLC-MS/MS – EPA Method 537.1, Version 1.0, November 2018

Matrix: Drinking Water
Acode: OC12731
Method Code (.J)

1. Scope and Application

1.1. This is a high performance liquid chromatographic triple quadrupole mass spectrometric (HPLC-MS/MS) method applicable to the determination of perfluorinated compounds in drinking water following EPA method 537.1.

1.2. The compounds included in this method of analysis and associated method detection limits/minimum reporting limits (MDLs/MRLs) are listed below in ng/L of water. The lowest calibration point used for each compound (Low Cal.) is also included in the table below.

Analyte	MDL (ng/L)	Low Cal. (ng/L)	MRL (ng/L)
Perfluoro-1-octanesulfonate (PFOS)	0.645	0.6	1.00
Perfluoro-1-butanesulfonate (PFBS)	0.576	0.6	1.00
Perflioro-1-hexanesulfonate (PFHxS)	0.666	0.6	1.00
Perfluoro-n-octanoic acid (PFOA)	0.782	0.6	1.00
Perfluoro-n-hexanoic acid (PFHxA)	0.716	0.6	1.00
Perfluoro-n-heptanoic acid (PFHpA)	0.733	0.6	1.00
Perfluoro-n-nonanoic acid (PFNA)	0.709	0.6	1.00
Perfluoro-n-decanoic acid (PFDA)	0.632	0.6	1.00
Perfluoro-n-undecanoic acid (PFUnDA, PFUdA or PFUnA)	0.721	0.6	1.00
Perfluoro-n-dodecanoic acid (PFDoDA or PFDoA)	0.612	0.6	1.00
Perfluoro-n-tridecanoic acid (PFTrDA)	0.580	0.6	1.00
Perfluoro-n-tetradecanoic acid (PFTeDA or PFTA)	0.389	0.6	1.00
N-methyl perfluorooctanesulfonamidoacetic acid (NMeFOSAA)	0.785	0.6	1.00
N-ethyl perfluorooctanesulfonamidoacetic acid (NEtFOSAA)	0.839	0.6	1.00
Dodecafluoro-3H-4,8-dioxanoanoate (DONA)	0.792	0.6	1.00
Potassium 9-chlorohexadecafluoro-3-oxanonane-1-sulfonate (9Cl-PF3ONS)	0.606	0.6	1.00
Potassium 11-chloroeicosafluoro-3-oxaundecane-1-sulfonate (11Cl-PF3OUdS)	0.628	0.6	1.00
2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3-heptafluoropropoxy)-propanoic acid (HFPO-DA)	0.727	0.6	1.00

2. Summary of Method:

2.1. Labeled surrogates are added to a water sample and per and polyfluoroalkyl substances (PFAS) present in water samples are extracted by a solid phase extraction, eluted with

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MeOH, and evaporated to dryness under nitrogen gas. The contents of the tube are brought to exactly 1mL with 96:4 MeOH:H_2O and then an appropriate amount of internal standard mix is added to the collection vial. Prior to analysis by HPLC-MS/MS, the contents are transferred to a polypropylene autosampler vial via polypropylene pipet. Separation of the analytes is achieved using gradient elution chromatography. After elution from the HPLC column, compounds of interest are analyzed using a turbo ion spray triple quadruple mass spectrometer in the negative ionization mode.

3. Safety and Waste Management:

3.1. General safety practices for all laboratory operations are outlined in the Chemical Hygiene Plan for Environmental Sciences located at O:\SOP\Safety\Final\AD_SAFETY_GENOP_102_Chemical Hygiene Plan.doc.

3.2. All laboratory waste, excess reagents and samples will be disposed of in a manner which is consistent with applicable rules and regulations. Waste disposal guidelines are described in

3.2.1. O:\SOP\EHD\Division Wide\Final\EHD GENOP 038 SOP Waste Management.doc.

3.2.2. University of Wisconsin Chemical Safety and Disposal Guide located at http://ehs.wisc.edu/disposal-services/.

4. Sampling Handling and Preservation:

4.1. See Appendix 2, **Rejection of Samples and Sample Results for PFAS by EPA 537.1** for a detailed list of sample and sample result rejection criteria.

4.2. Samples must be collected in a polypropylene container (with Trizma preservative added) and chilled during shipment between 0-10ºC during the first 48 hours after collection. Upon arrival to WSLH, the samples must be confirmed to be between 0-10ºC, if within 48 hours of collection, and 0-6ºC if received after 48 hours. Samples shall be refrigerated below 6 ºC as soon as possible and shielded from light from the time of collection until analysis. PFAS have been shown to be stable under these conditions. If samples are not received in the temperature ranges indicated, then the samples shall be rejected and not analyzed by the laboratory. Sample extracts are stored at room temperature.

4.3. If a sample arrives in a container other than what is sent out by WSLH (with Trizma included), it is considered invalid and shall be rejected and not analyzed by the laboratory.

4.4. Samples must be extracted within 14 days of collection. Extracts must be stored at room temperature and analyzed within 28 days after extraction.

4.5. The pH of all of the samples must be checked prior to extraction. Sample pH must be 6.5 – 7.5. If the pH is not between 6.5 and 7.5, the sample can be extracted and analyzed but a pH failure flag must be added to the final report.

5. Interferences:

5.1. Matrix interference may be caused by contaminants that are present in the sample. The extent of matrix interference is unknown until further sample analysis is completed (see EPA 537.1 §4.3).

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5.2. Since PFAS are known to adsorb onto glass surfaces, any type of glass should not be used in any step in the extraction or standard-making process (see §6.3.1, EPA 537.1 §4.1).

5.3. Extraction equipment and reagents can be a source of contamination and interferences. Trizma is used to preserve all samples and remove free chlorine (see §6.2.2, EPA 537.1 §8.1.2). Each new lot of extraction equipment and reagents must be proven free of contamination before it is used to process samples (see §6.2.3, §8.4.3, §8.5.1, EPA 537.1 §4.2, §4.4, §4.5)

6. Reagents and Standards:

6.1. Reagents

6.1.1. Methanol, Reagent grade

6.1.2. Ammonium Acetate, Reagent grade

6.1.3. 18 Mohm water

6.1.4. Trizma (preservation reagent)

6.2. Reagent and bottle preparation

6.2.1. 2 mM Ammonium Acetate in Water: Add 0.154 g of ammonium acetate to 950 mL of 18 Mohm water and 50 mL of MeOH. This solution will be replaced weekly due to volatility.

6.2.2. 1.25 grams of Trizma is added to all sample bottles to be sent into the field as well as all QC samples (blanks, LCS, etc.). The same lot number of Trizma must be used for the FRBs and field samples and this will be documented in the extraction log.

6.2.3. Sample bottle identification – Every box of bottles that is purchased has a unique lot number associated with it (usually provided by the manufacturer). This lot number will be put on every bottle that goes out into the field, which will be the same lot number bottle that is used in all QC to ensure no contamination from the bottle is seen. This is done by performing a normal lab blank test on a bottle to make sure it passes all QC (see 8.5.1). The results of this test will be kept in the folder -M:\EHD\ESS(4900)\ESS Org(4940)\Method Related Documents\PFAS\QC Bottle Checks. If the Trizma or bottle lot number for the field blank does not match the Trizma or bottle lot numbers for the samples, then the results for the associated sample must be appropriately qualified.

6.2.4. Instructions for sample collection and kit preparation are available. (M:\EHD\ESS(4900)\ESS Org(4940)\Forms and Sampling Instructions\Final\PFAS in Drinking Water)

6.3. Standards

6.3.1. Since PFAS are known to adsorb onto glass surfaces, any type of glass should not be used in any step in the extraction or standard-making process. Polypropylene bottles should be used to store and make all standards. Polypropylene tubes (1.2mL – used for storing samples) and autosampler vials (~300 uL) should be used

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for samples and QC. All standards should be prepared on ice and stored in the smallest container available to limit headspace and the risk of evaporation.

6.3.2. Target compound working stock solution - To make the working stock standard solution, a premade mix that has both branched (for PFOS, PFHxS, NMeFOSAA, and NEtFOSAA) and linear forms of the compounds (EPA-537PDS-R1) is purchased from Wellington laboratories at 2 ppm each. Fifty (50) µL of the premade mix is added to 40 µL water and 910 µL of methanol into a 1-mL polypropylene vial (this results in a final mixture in 96:4 MeOH:H_2O; see Table 1 – This is PFCDWStk in Horizon). Place a mark on the vial at the level of the solution so that solvent loss by evaporation can be detected. Replace the solution if solvent loss has occurred. This mix is used to make the calibration standards as well as spike the samples.

6.3.3. Surrogate working stock solution – To make the surrogate stock solution, four pre-made standards (^{13}C5-Perfluorohexanoic acid, ^{13}C6-Perfluorodecanoic acid, N-ethyl-d5-perfluoro-1-octane-sulfonamidoacetic acid, and Tetrafluoro-2-heptafluoropropoxy-13C3-propanoic acid) at 50 µg/mL are purchased from Wellington Laboratories. These standards are combined (20 µL of each standard) and diluted to 1 mL (880 µL of MeOH + 40 µL water) to get a final concentration of 1000 ng/mL (Table 2). Another dilution is performed (40µL of the 1000ng/mL surrogate standard mix) to 1mL (40 µL water + 920 µL of MeOH) to get a final working stock solution of 40 ng/mL (Table 3). Twenty-five (25) µL of this standard is added to each sample and extracted QC prior to the SPE step in the extraction process; for instrument quality control (calibration standards, CCCs, QCSs), it is added prior to injection. This is PFCDWSStk in Horizon.

6.3.4. Internal standard working stock solution – To make the internal standard stock solution, 20 µL each of three individual compounds at 50 µg/mL (N-methyl-d3-perfluoro-1-octane-sulfonamidoacetic acid, 13C_2-PFOA, and 13C_4-PFOS) are combined with 900 µL methanol and 40 µL water until the final concentration is 1000 ng/mL (Table 4). Forty (40) µL of this solution is then added to 920 µL of methanol and 40 µL of H_2O to get a final concentration of working internal standard solution at 40 ng/mL (Table 5). Twenty-five (25) µL of this standard is added to each sample and extracted QC after the samples are concentrated to dryness and brought up to 1.0 mL using 96:4 MeOH: H_2O. For instrument quality control, it is added prior to injection.

6.3.5. Calibration standards - Combine and dilute the solutions in Sections 6.3.2 through 6.3.4 to produce the calibration solutions in Table 6. These solutions permit the relative response (labeled to target) and response factor to be determined as a function of concentration.

6.3.6. QCS (Second source solution) – A second source standard of all compounds must be used to check the original concentrations of the purchased standards. This can be from either a different supplier or a different lot number from the same supplier (if available). A premade mixture was purchased from Wellington Laboratories (separate lot from the working target mix) that has all 18 compounds in; it will be used for the second source standard (Table 7). This standard will be diluted to 1

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ng/mL in 96:4 MeOH: H_2O and will be used as a QCS in each recalibration run and at least quarterly.

6.3.7. When standards are not being used, store the solutions in the dark at <5ºC. Standards may be stored at <0°C. Before use, make sure each standard is brought up to room temperature and the vial or bottle is vortexed extensively. All stock standards expire when the earliest manufacturer's expiration date occurs. Standards prepared from stocks expire one year after preparation, when the parent standard expires, or when the preparation solvent expires (whichever is soonest).

7. Apparatus:

7.1. Polypropylene sample bottles - do not use Teflon septa/cap liners

7.2. Various sized beakers

7.3. Analytical Balance capable of accurately weighing to the nearest 0.01 g. Capable of weighing samples up to 500 mL

7.4. 15-ml conical screw capped polypropylene centrifuge tubes, graduated

7.5. Promochrom Technologies SPE-03 8-channel cleanup station

7.6. Nitrogen source – to dry SPE cartridges

7.7. SPE cartridge, 500 mg, sytrenedivinylbenzene (SDVB), PN Agilent 1225-5021

7.8. Neutral pH paper (pH accurate from 6-8 in 0.4 pH unit increments)

7.9. Free chlorine check paper, HF Scientific 09940

7.10. Nitrogen blow-down apparatus with heated water bath

7.11. Vortex mixer

7.12. Disposable polypropylene pipettes

7.13. Polypropylene Autosampler vials

7.14. Screw caps that have polypropylene septa

7.15. Waters Acquity UHPLC

7.16. Applied Biosystems/SCIEX Q-Trap 5500 Triple quadrupole mass spectrometer

7.17. HPLC/MS/MS Instrument Conditions

7.17.1. The HPLC-MS/MS method is performed on a Waters Acquity UPLC, followed by an Applied Biosystems/SCIEX Q-Trap 5500 triple quadrupole mass spectrometer (Foster City, CA).

7.18. General Method Parameters

7.18.1. Synchronization Mode: LC Sync

7.18.2. Auto-Equilibration: Off

7.18.3. Acquisition Duration: 11 minutes 30 seconds

7.18.4. Number of Scans: 690

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7.18.5. Period In File: 1

7.18.6. Acquisition Module: Acquisition Method

7.18.7. Software version: Analyst 1.63

7.19. Source height setting-3, Source L/R setting-5

7.20. Waters Acquity Pump Method

7.20.1. Pump Model: Waters Acquity UPLC

7.20.2. Column: Zorbax Rapid Resolution, 3.5 µm, 30 mm long x 2.1 mm I.D. (Part # 873700-902).

7.20.3. Waters Acquity UPLC Pump Method Properties

Minimum Pressure (psi)	0.0
Maximum Pressure (psi)	18000
Left Solvent	A1 (2mM ammonium acetate)/MeOH 95%/5%
Right Solvent	B1 (Methanol)

7.20.4. Step Table

Step	Total Time (min)	Flow Rate (µl/min)	A (%)	B (%)
0	0.00	400	95	5
1	0.50	400	95	5
2	6.00	400	15	85
3	6.10	400	0	100
4	8.00	400	0	100
5	10.5	400	95	5

7.21. MS/MS Method Properties:

7.21.1. Period 1:

7.21.1.1. Scans in Period: 690

7.21.1.2. Relative Start Time: 0.00 msec

7.21.1.3. Experiments in Period: 1

7.21.2. Period 1 Experiment 1:

Scan Type:	MRM (MRM)
Scheduled MRM	Yes
Polarity:	Negative
Scan Mode:	N/A
Ion Source:	Turbo Spray
Resolution Q1:	Unit
Resolution Q3:	Unit
Intensity Thres.:	0.00 cps
Settling Time:	0.0000 msec
MR Pause:	5.0070 msec
MCA:	No
Step Size:	0.00 Da

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7.21.3. MRM Parameters & Internal Standard Assignments (Q1, Q3, DP, CE, & CXP values may vary slightly due to MRM optimization, but Q1 & Q3 values will remain constant to the nearest whole number):

Analyte	Internal Standard	Q1 Mass (Da)	Q3 Mass (Da)	Dwell (msec)	DP	CE	CXP
PFOS-1	PFOS IS-1	498.802	79.9	3-250	-40	-70	-7
PFOS-2	PFOS IS-1	498.802	98.9	3-250	-40	-57	-11
PFOA-1	PFOA IS-1	412.877	368.9	3-250	-55	-14	-17
PFOA-2	PFOA IS-1	412.877	168.9	3-250	-55	-24	-13
PFBS-1	PFOS IS-1	298.839	80.0	3-250	-25	-70	-9
PFBS-2	PFOS IS-1	298.839	99.0	3-250	-25	-36	-9
PFHxS-1	PFOS IS-1	398.841	79.9	3-250	-5	-86	-7
PFHxS-2	PFOS IS-1	398.841	98.9	3-250	-5	-42	-9
PFHxA-1	PFOA IS-1	312.898	269.0	3-250	-45	-14	-13
PFHxA-2	PFOA IS-1	312.898	119.0	3-250	-45	-30	-7
PFHpA-1	PFOA IS-1	362.858	318.9	3-250	-40	-14	-13
PFHpA-2	PFOA IS-1	362.858	169.0	3-250	-40	-24	-15
PFNA-1	PFOA IS-1	462.817	419.0	3-250	-55	-16	-17
PFNA-2	PFOA IS-1	462.817	219.0	3-250	-55	-24	-19
PFDA-1	PFOA IS-1	512.83	469.0	3-250	-40	-16	-13
PFDA-2	PFOA IS-1	512.83	218.9	3-250	-40	-26	-17
PFUdA-1	PFOA IS-1	562.846	519.0	3-250	-60	-18	-13
PFUdA-2	PFOA IS-1	562.846	268.9	3-250	-60	-28	-13
PFDoA-1	PFOA IS-1	612.828	569.0	3-250	-65	-20	-17
PFDoA-2	PFOA IS-1	612.828	168.9	3-250	-65	-36	-19
PFTrDA-1	PFOA IS-1	662.782	619.0	3-250	-75	-18	-17
PFTrDA-2	PFOA IS-1	662.782	169.0	3-250	-75	-36	-11
PFTeDA-1	PFOA IS-1	712.763	668.9	3-250	-35	-20	-25
PFTeDA-2	PFOA IS-1	712.763	168.9	3-250	-35	-36	-11
NMeFOSAA-1	d3-N-MeFOSAA IS	569.863	419.1	3-250	-80	-30	-7
NMeFOSAA-2	d3-N-MeFOSAA IS	569.863	512.0	3-250	-80	-36	-13
NEtFOSAA-1	d3-N-MeFOSAA IS	583.962	419.0	3-250	-120	-37	-10
NEtFOSAA-2	d3-N-MeFOSAA IS	583.962	525.8	3-250	-120	-37	-10
HFPO-DA-1	PFOA IS-1	285.000	168.8	3-250	-5	-8	-5
11Cl-PF3OUdS-1	PFOS IS-1	630.757	450.9	3-250	-95	-38	-19
9Cl-PF3ONS-1	PFOS IS-1	530.725	351.0	3-250	-105	-36	-27
DONA-1	PFOA IS-1	376.770	251.000	3-250	-60	-18	-11

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PFHxA-Surrogate	PFOA IS-1	317.875	272.900	3-250	-20	-14	-5
PFDA-Surrogate	PFOA IS-1	518.898	474.019	3-250	-25	-16	-25
d5-N-EtFOSAA-Surr	d3-N-MeFOSAA IS	588.841	531.041	3-250	-20	-32	-31
13C3-HFPO-DA-Surr	PFOA IS-1	287.000	168.8	3-250	-45	-12	-7
PFOS IS-1	---	502.8	80.0	3-250	-40	-102	-9
PFOA IS-1	---	416.89	372.0	3-250	-65	-18	-47
d3-N-MeFOSAA IS	---	572.777	514.984	3-250	-25	-32	-13

7.22. Parameter Table (Period 1 Experiment 1):

CUR:	30.00
GS1:	30.00
GS2:	30.00
IS:	-4500.00
TEM:	650.00
ihe:	ON
CAD:	Medium
EP	-10.00

7.23. Electron Multiplier Settings

Detector Parameters (Negative):	
CEM	2200.0
DF	200.0

8. Quality Control

8.1. For general quality control, procedures see the Quality Assurance Manual. For specific quality control acceptance limits that apply to laboratory control samples, surrogates, calibration check standards, matrix spikes, and duplicates for this analytical procedure please consult the laboratory's LIMS system. For details, see the standard operating procedure O:\SOP\EHD\ESS\Enviro Organic\Organic and Air Chem\Final\Quality Assurance (QA)\ESS ORG QA 0001_Horizon and QA.docx. See Appendix 2, **Rejection of Samples and Sample Results for PFAS by EPA 537.1** for a detailed list of sample and sample result rejection criteria. See §12 for this method's corrective action procedure.

8.2. The MS/MS detector is required to pass a polypropylene glycol (PPG) tune check at least once annually as part of regularly scheduled maintenance. A standard containing 300 µM of SCIEX Mixed PPG solution is analyzed. The tune results must meet the recommended SCIEX operating criteria before samples are analyzed, and a record of maintenance must include acknowledgement of passing tune results. See example SCIEX tuning criteria from Planned Maintenance Procedure documents used by field technicians (stored in a binder near the instrument PC) in 8.3 below. Further instrument maintenance may be necessary if the tuning criteria are not met.

8.3. SCIEX tuning criteria for PPGs in negative Turbo Ion Spray mode (NOTE: cps = counts per second)

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Target Mass (Da)	Intensity Required (cps)	Peak Width	Mass Shift (Da)
933.6360 (Q1)	**≥1.0x10^7**	**0.6-0.8**	**<0.1**
933.6360 (Q3)	**≥8.0x10^6**	**0.6-0.8**	**<0.1**

8.4. Initial demonstration of capability (IDC)

8.4.1. An IDC will be successfully performed by each analyst before any field samples can be analyzed by that analyst. An initial calibration curve is generated with at least five calibration standards and a linear regression. The curve *must be forced* through zero and may be weighted by concentration (the default weighting is 1/x). Using the generated curves each calibration point for each analyte must calculate between 70-130% of the true value (except for the lowest point, which must calculate between 50-150%).

8.4.2. PFOA qualitative standard – Qualitative analysis has shown that there are branched isomers of PFOA in drinking water samples. A separate qualitative PFOA standard consisting of branched and linear isomers is analyzed to identify the retention time of the branched chain isomer. Only the linear isomer will be used to generate the calibration curve and quantitate all results. Quantitation of field samples includes both linear and branched isomers. This PFOA standard will be analyzed any time there is a significant change in the PFOA retention time.

8.4.3. Initial demonstration of low system background – If a new lot of SPE cartridges, solvents, tubes, pipets or autosampler vials are used, a Lab Reagent Blank (LRB) must be analyzed to show that the equipment is free of contamination.

8.4.4. Initial demonstration of precision (IDP) – Four to seven laboratory fortified blanks (LFBs) will be prepared near the midpoint of the initial calibration curve and extracted like a normal sample. Sample preservative (Trizma) will be added to each LFB. The relative standard deviation of the results of the replicates must be less than 20%.

8.4.5. Initial demonstration of accuracy – Using the same four to seven samples extracted for the IDP, calculate the average recovery. The recovery must be within 70-130% of the true value.

8.4.6. Peak asymmetry factor – Using the equation:

$$A_s = b/a$$

Where a is the width of the front half of the peak measured (at 10% peak height) from the front end of the peak to a line dropped from the apex of the peak. In addition, b is the width of the back half of the peak measured (at 10% peak height) from the back end of the peak to a line dropped from the apex of the peak. The value of A_s will be measured for the first two peaks in a mid-level calibration standard and must fall between 0.8 and 1.5. The peak asymmetry factor must be assessed whenever peak shape has been affected.

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8.4.7. MDL study – Seven LFBs are spiked at a level one to five times the estimated LOD. The estimated LOD is roughly determined using the recoveries of the initial calibration standards. The LFBs (as well as seven lab blanks) are to be extracted using the established method (including the Trizma preservative and the same branched isomers used to report sample results). The MDL is calculated by following the standard operating procedure O:\SOP\EHD\Division Wide\Final\EHD QA 116 LOD Procedures.doc.

8.4.8. MRL confirmation – Calculate the mean measured concentration and the standard deviation of seven LFBs that are spiked at or below the proposed MRL.

8.4.8.1. Determine the Half Range for the prediction interval of results (HR_{PIR}) using the equation:

$$HR_{PIR} = 3.963s$$

Where s is the standard deviation of each compound over seven replicates.

Confirm that the upper and lower limits for the Prediction Interval of Result

(PIR = Mean recovery ± HR_{PIR}) meet the upper and lower recovery limits:

(Mean + HR_{PIR})/fortified concentration *100% ≤ 150%

(Mean - HR_{PIR})/fortified concentration *100% ≥ 50%

8.4.8.1. The MRL is validated if both of these criteria are met. If either of these fails, then the MRL has been set too low and must be determined at a higher concentration.

8.4.8.2. EPA 537.1 §9.2.6.1 states that the seven replicate LFBs for MRL verification are spiked *at* the proposed MRL concentration. This method spikes seven LFB replicates *at or below* the proposed MRL concentration with the understanding that a MRL at a higher concentration than what was verified would meet the same verification criteria, e.g. if a proposed MRL for PFOS is verified at 0.4 ng/L, this method may select a final MRL for PFOS of 1.0 ng/L.

8.5. Ongoing QC requirements

8.5.1. Laboratory reagent blanks (LRB) – The analyst must demonstrate that all equipment and reagent interferences are under control. If the LRB produces a peak that would prevent the determination of an analyte of interest within the retention time window of that analyte, determine the source of contamination and eliminate the interference before processing samples. Background from method analytes or other contaminants that interfere with the measurement of method analytes must be below 1/3 of the MRL. If the target compounds are detected above or equal to this level, the data for the problem analytes will be considered invalid and will not be reported. The source water used for all LRBs, FRBs, and LCSs is from one of the polished water generators (such as the generator in room 218, RO218A). Since a multi-port manifold is being used to extract samples, a lab blank must be rotated

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through all ports of the extraction unit, changing position after each batch, to ensure no blank contamination is coming from the manifold system. The extraction position of the LRB is noted on the Extraction Log (Appendix 1).

8.5.2. Field reagent blanks (FRB) – A FRB will be sent with each sampling event. This is done to ensure that target compounds are not being introduced during sample collection and handling. At the laboratory, a field blank sample bottle (labeled FRB1 and includes the QC lot number of the bottle and the Trizma lot number) is filled with reagent water. This is sent with an additional sample bottle containing only preservative (labeled FRB2 and includes QC lot number of the bottle and the Trizma lot number) to the sampling site. At the site, FRB1 is opened and poured into the FRB2 bottle, which is sealed and returned along with the samples. If the target compounds are detected above or equal to 1/3 the MRL for any compound, then all samples that were collected with the FRB will be considered invalid and will not be reported.

8.5.3. Assessing laboratory performance with laboratory-fortified blanks (LFB) - Laboratory fortified blanks are spiked over the working range of the calibration standards used for this method. An LFB is analyzed with each extraction batch and will be rotated between low (at least 2x the MDL – 1 ng/L), medium (4 ng/L), and high (20 ng/L) concentrations from batch to batch. Results for the low LFB must be within 50-150% of the true value. Results from the medium and high LFB must be within 70-130% of the true value. If the LFB results do not meet these criteria, then all data for the problem analyte(s) will be considered invalid for the entire extraction batch and will not be reported.

LFB (LCS) = 1 ng/L (2.5 µL PFCDWStk => 250 mL)

LFB1 (LCS1) = 4 ng/L (10 µL PFCDWStk => 250 mL)

LFB2 (LCS2) = 20 µg/L (50 µL PFCDWStk => 250 mL)

8.5.4. Assessing analyte recovery with laboratory fortified sample matrix (LFSM) - LFSMs are spiked over the working range of the calibration standards used for this method. An LFSM is performed with each extraction batch (provided sufficient sample was received) and will be rotated between low (at least 2x the MDL – 1 ng/L), medium (4 ng/L), and high (20 ng/L) concentrations from batch to batch. Calculate the percent recovery, P, of the concentration for each analyte, after correcting the analytical result, X, from the fortified sample for the background concentration, b, measured in the unfortified sample, i.e.:

$$P = 100 (X - b) / \text{fortifying concentration}$$

Results for the low LFSM must be within 50-150% of the true value. Results from the medium and high LFSM must be within 70-130% of the true value. If the results fall outside of this criterion, the results for the analyte in the unfortified sample is appropriately flagged as suspect due to matrix effects. If the spike level of the LFSM is not at or above the native level of a particular analyte, any flag for recovery failure does not need to be applied to that compound. e.g. If a sample with a native level of 10 ng/L PFOA is spiked with 1 ng/L PFOA, a recovery of 7 ng/L (64% LFSM recovery) does not require a flag.

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8.5.5. Assessing precision with duplicates - If an extra sample bottle is provided for some samples, the laboratory must analyze either one laboratory fortified sample matrix duplicate (LFSMD) or one field duplicate (FD) per extraction batch. Based upon the analyst's experience, a sample which is expected to contain the analytes above the limit of quantitation should be chosen for the field duplicate. By default, the precision assessment is performed by analysis of a LFSMD due to the generally-low concentration of native analytes in drinking water samples. For concentrations 1ng/L or less, the RPD must be < 50%. For all other concentrations, the RPD must be < 30%. If any analyte falls outside of this criterion, the analyte for the original sample is labeled as suspect due to matrix effects.

8.5.6. Continuing calibration check (CCC) - A calibration standard is analyzed at the beginning of each analysis batch, after every 10 field samples, and at the end of the batch (in this context, a "sample" is considered to be a field sample; LRBs, CCCs, LFBs, LFSMs, LFSMDs, FDs, and FRBs are not counted as samples). The beginning (low) CCC must be at or below the MRL for all compounds. Subsequent CCCs analyzed during analysis will rotate between the medium and high calibration standards. If any of these CCCs fail, reanalyze the check standard before continuing. If this fails (outside of 50-150% of the true value for the low CCC and 70-130% of the true value for the medium and high CCC), then all data for the problem analyte(s) will be considered invalid and a new curve must be generated. The only exception to this is if the CCC fails because the calculated concentration is greater than 130% (150% for the low level CCC) for an analyte and all associated field samples show no detection for that specific analyte. In this case, reanalysis does not have to be performed and a non-detect may be reported as normal. Data associated with unacceptable calibration verifications shall be qualified if reported. Corrective action shall be taken and documented appropriately. (NELAC V1M4 1.7.1.2(f)) See section 12 for corrective action procedures.

CCC (CCV) = 1.0 ng/L (calibration level 2)

CCC1 (CCV1) = 4.0 ng/L (calibration level 4)

CCC2 (CCV2) = 20 ng/L (calibration level 6)

8.5.7. Internal Standards recovery – 25 µL of the internal standard mix (at 40 ppb) is added to all samples and batch quality control after they have been reconstituted with 1.0 mL 96:4 MeOH:H_2O; the same amount is added to all instrument quality control in the cryovials used for their preparation. The peak area of the internal standards must be monitored in all injections during a run. The response must be within 70-140% of the response of the most recent continuing calibration check and must be within 50-150% of the average area measured during initial calibration.

If a samples internal standard area fails, inject a second aliquot of that extract aliquoted in a new-capped autosampler vial.

If the reinjected aliquot produces an acceptable IS response, report results from that aliquot.

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If the reinjected extract fails again, reanalyze the most recently acceptable check standard.

If the check standard passes, then extraction of the sample may need to be repeated if the sample is still within holding time.

If the check standard fails, perform a new initial calibration.

If no additional sample within holding time is available, report results obtained from the reinjected extract, but qualify the data as suspect.

The internal standard recoveries are transferred into a spreadsheet located at M:\EHD\ESS(4900)\ESS Org(4940)\Method Related Documents\PFAS\IS Check Template.xltx

8.5.8. Surrogate recovery – 25 µL of the surrogate mix (at 40 ppb) is added to all samples and batch quality control prior to extraction; the same amount is added to all instrument quality control in the cryovials used for their preparation. Surrogate recovery must be within 70-130% of the true value. If this fails, appropriately flag the data as suspect due to low/high surrogate recovery. Surrogate recovery must be within 70-130% of the true value for all CCCs or all data for that day's samples are considered invalid and will not be reported.

8.5.9. Identification of Analytes - Analytes are identified by retention time, quantitation ion transitions, and confirmation ion transitions (where they exist).

8.5.9.1. After optimization of the instrument, absolute retention times are established by analyzing a mid-level calibration standard with the mass spectrometer (MS) in the nonscheduled multiple reaction monitoring (MRM) mode (i.e., a full "scan" with no limiting MRM detection window). Analytes are identified in this standard by extracting each ion transition (optimized in §7.20.3) and transferring the retention time (RT) of the peak's apex to a scheduled MRM Acquisition Method. For analytes with both linear and branched isomers, the largest peak's retention time is used. In a scheduled MRM Acquisition Method, optimized MRM conditions are limited to a detection window centered on the absolute RTs, allowing for greater sensitivity. A 35 second MRM detection window acts as a RT window and results in no fewer than 10 scans across each chromatographic peak while ensuring that the entire peak (including branched isomers) is captured by the instrument detector (EPA 537.1 §6.12.2). A particular scheduled MRM Acquisition Method may be used for sample analysis as long as each analyte's RT remains within the MRM detection window. If an analyte is no longer detectable, new absolute RTs must be established and added to the Acquisition Method by first analyzing a mid-level standard in nonscheduled MRM mode.

8.5.9.2. The Quantitation Method (QM) establishes an expected RT and RT windows used for automatic peak integration. In contrast to the absolute RTs and MRM detection window in the Acquisition Method, these expected RTs and RT windows are not used for analyte identification; they allow for automatic integration only. A QM uses a

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representative sample to establish expected RTs. On days when the instrument is calibrated, the QM must reference a mid-level standard from the calibration curve; on days when the instrument is not calibrated, the QM must reference the initial CCV. See §13.1 for instructions on updating a QM to reference a particular standard. QM RT windows are centered on the expected RTs and span 30 seconds. This window allows for automatic integration even with slight variations in retention times due to matrix differences (see §8.5.12.3 below).

8.5.9.3. Analyst experience is an important factor in compound identification and quantitation. The QM automatic integration software will often exclude branched isomer peaks, and the RT windows allow for small variations in RT. Peak integration and retention times must be examined closely by the analyst after updating QM integration parameters. Integration must be manually adjusted to include both branched and linear isomer peaks, and all standards and samples must be integrated consistently with the exception of PFOA (the calibration curve for PFOA is generated with a linear isomer standard only, but use of a qualitative standard allows for integration of linear and branched isomers in samples). Compounds with branched isomers are integrated with the baseline extending as a single line from the beginning of the branched isomer peak until the end of the linear isomer peak (see Figure 1 below).

8.5.9.3.1. For analytes with analogous labelled internal standards, a shift in RT for the target analyte is expected to be accompanied by a similar shift in the RT of the internal standard. If a RT shift is observed in an analyte without a labelled analog, the analyst must rely on the presence of a confirmation ion transition to positively identify a compound.

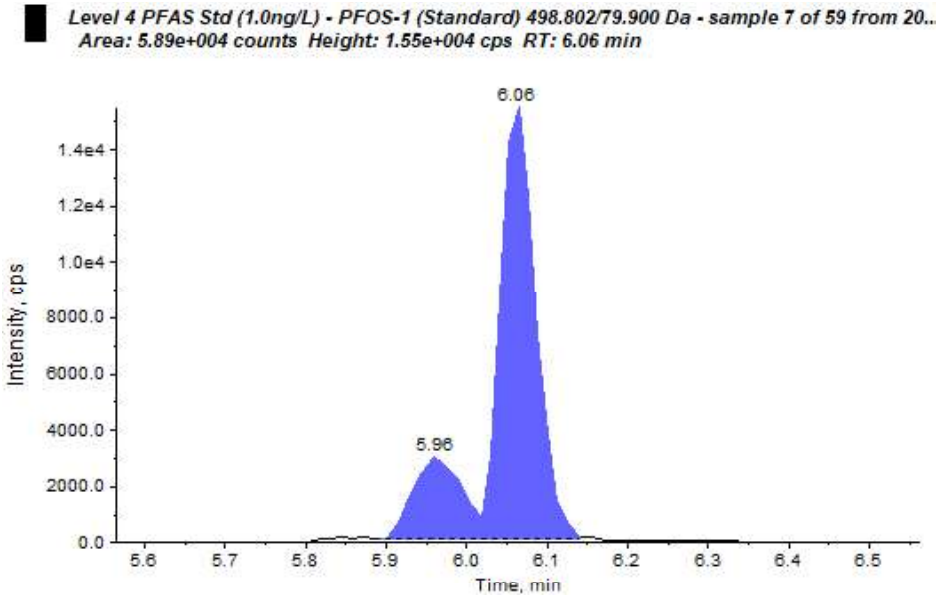

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Figure 1. Integration of PFOS Standard Peaks (Branched and Linear Isomers)

8.5.10. QCS (Second Source Standard)—every time a new working target analyte solution is made (Table 2), a second source standard (Table 7) must be analyzed before any samples to check the validity of the new working target analyte solution. The second source standard must be either from a different vendor or a different lot number if from the same vendor. The value of the second source standard must be within 30% of the expected value.

8.5.11. Proficiency Testing (PT) and Blind Samples—the laboratory treats samples that are known to have very high concentrations of PFAS by diluting them before extraction; the same procedure will be followed if a PT or blind sample is expected to have high levels of PFAS. The Proficiency Testing Reporting Limit (PTRL) for PT and blind samples may be over 10 times higher than that of the laboratory's PFAS reporting limits. It is expected that such PT and blind samples will be diluted before analysis. The PTRL for a PT or blind sample may be obtained from QA personnel prior to analysis of a PT or blind sample.

8.5.12. Up to 20 samples may be analyzed on the same instrument during a 24-hour period as long as one LRB, LFB, and LFSM/LFSMD are extracted. A FRB must be extracted for every sampling site with samples that have a method analyte or analytes that are at or above the MRL.

9. Method Calibration

9.1. Working Standard Preparation Procedure
A minimum of five calibration points are required for each analyte (EPA 537.1 §7.2.4). A linear regression is used to generate a calibration curve. The suggested calibration levels are 0.15, 0.25, 0.5, 1, 2.5, 5, 10, and 25ng/mL (this is equivalent to 0.6, 1, 2, 4, 10, 20, and 40 ng/L of water after adjusting for the 250 mL to 1.0 mL extraction procedure). The Applied Biosystems Analyst data system is used to prepare an internal standard linear calibration curve for each analyte. The curve must be forced through zero, which allows for a better estimate of background levels. When each calibration standard is calculated as an unknown using the calibration curve, the analyte and results must be 70-130% of the true value for all except the lowest standard, which must be 50-150% of the true value (This exceeds the criteria for measurement of relative error in the calibration curve outlined in the 2016 NELAC Standard Vol. 1, Module 4, §1.7.1). The surrogate results in all calibration standards must be 70-130% of the true value.

9.2. The laboratory may remove individual analyte calibration levels from the lowest and/or highest levels of the curve. Multiple levels may be removed, but removal of interior levels is not permitted. The laboratory may remove an entire single standard calibration level from the interior of the calibration curve when the instrument response demonstrates that the standard was not properly introduced to the instrument, or an incorrect standard was analyzed. A laboratory that chooses to remove a calibration standard from the interior of the calibration shall remove that particular standard calibration level for all analytes. Removal of calibration points from the interior of the curve is not to be used to compensate for lack of maintenance or repair to the instrument. The laboratory shall adjust the LOQ/reporting limit and quantitation range of the calibration based on the concentration of the remaining high and low calibration

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standards. The laboratory shall ensure that the remaining initial calibration standards are sufficient to meet the minimum requirements for number of initial calibration points as mandated by the NELAC Standard (five standards, Vol. 1, Module 4, §1.7.1.1f.), the method (five standards, EPA 537.1 §7.2.4), or regulatory requirements. The laboratory may replace a calibration standard provided that:

9.2.1. the laboratory analyzes the replacement standard within twenty-four (24) hours of the original calibration standard analysis for that particular calibration level;

9.2.2. the laboratory replaces all analytes of the replacement calibration standard if a standard within the interior of the calibration is replaced;

9.2.3. and the laboratory limits the replacement of calibration standards to one calibration standard concentration.

The laboratory shall document a technically valid reason for either removal or replacement of any interior calibration point. See 2016 NELAC Standard Vol. 1, Module 4, §1.7.1.1.

9.3. The working calibration curve must be verified on each working day by the injection of one or more calibration standards at the beginning and end of each analytical run, and after the analysis of 10 samples if 10 or more samples are analyzed in an analysis day. See §8.5.6 for CCC criteria.

10. Sample Preparation Procedure

10.1. Samples must only be collected in 250-mL polypropylene bottles that have polypropylene caps. Prior to shipping to the field 1.25 grams of Trizma is added to each sample bottle (and QC). This is a buffering reagent and helps remove free chlorine, which could interfere with the extraction process. The sample is loaded onto a SPE cartridge (SDVB) by using an automated extractor unit (AEU) (Promochrom Technologies SPE-03 8-channel cleanup station) that is programmed to extract PFAS.

10.2. Documenting pH of samples – Each field sample must have the pH measured prior to extraction. This is done by taking a piece of narrow-range pH paper that is specifically designed to measure samples that are around pH of 7. The range on the pH paper is from 6-8 and shows different colors in 0.4 pH increments. Each field sample must be 7.0 ±0.5, or data will be qualified.

10.3. Free chlorine content of samples – The free chlorine content of each field sample must be measured prior to extraction. If the residual chlorine content of a sample is >0.1 mg/L, Trizma is added to the sample until the free chlorine content is <0.1 mg/L. If Trizma is added to a sample, the same amount of Trizma must be added to the associated FRB, LRB, and LFB. If a batch of samples with varying amount of Trizma is analyzed, QC samples corresponding to each Trizma amount must be analyzed alongside the samples. Samples fortified with additional Trizma must be qualified (the analyzed sample would contain greater than 5.0 g/L of Trizma).

10.4. Weighing the samples – Each sample (with cap included) must be weighed prior to and after extraction to obtain the total volume of the sample (assuming the water sample density is 1 g/mL). Calibrate the balance up to at least 500 g before weighing is performed.

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10.5. Vortex all standard mixes before removing aliquots for spiking. Add 25 µL of the surrogate standard mix to each sample and batch QC. Add a proper amount of target-compound stock mix to the LFB and LFSM/LFSMD (if analyzed). The spike volume used for the LFB and LFSM/LFSMD will rotate between a high (50 µL-5 ng/mL), medium (10 µL-1 ng/mL), and low (2.5 µL-0.25 ng/mL) concentration. After spiking, recap the bottles and mix well.

10.6. On the side of the AEU, install empty bottles that can be used for the system clean. These cleaning bottles should be replaced every week to ensure no contamination is coming from the bottles. Check the solvent reservoirs on top of the AEU. Make sure there is enough water and MeOH in the reservoirs. Replace the water using a new bottle at least every week. Leaving the old SPE cartridges in place, use the stylus to select the dropdown menu in the upper left-hand corner. Find the method labeled “clean sys”. Tap the play button to run the method. This cleans out the lines that run from the sample bottles to the plunger reservoirs. This method cleans the lines with two-5 mL rinses of MeOH, followed by two-4.5 mL rinses of water. This ensures that the transfer lines have only water in them and no leftover MeOH. This is followed by a 3 mL and then a 5 mL rinse of the syringe reservoirs with MeOH. This ensures that when the extraction method (EPA 537-10 mL) is run, the syringe reservoirs have only MeOH in them and no water is being added to the new cartridges in the first step.

10.7. Replace the old SPE cartridges with new ones. Label each of the new SPE cartridges with the appropriate ID. Remove the AEU caps and lines from the cleaning bottles, and screw the caps on to the correct samples and QC. Install each bottle in the bottle rack such that each is routed to the correct SPE column. Poke a small hole near the bottom of the bottle where there is air present (since the bottles are inverted for extraction this is the top as it sits in the holder) using a pin to make sure air can get into the bottles as the water is removed.

10.8. Place labeled 15-mL Falcon collection tubes in the slots in the front of the AEU. Using the stylus, tap the AEU’s display and select the dropdown menu in the upper left-hand corner. Find the method labeled “EPA 537”. Click the play button to extract the samples. This method follows the extraction procedure described in EPA Method 537.1. It first rinses the SPE columns with 15 mL of MeOH, followed by 18 mL of water. At this point, an additional 3 mL of water is added to each cartridge. The entire sample is then extracted through the SPE column at 10 mL/min. Two separate 7.5 mL rinses of water are then used to make sure the entire sample is completely rinsed through the SPE column. This is followed by a 5-minute hold where a stream of nitrogen flows through the SPE columns at about 2.5 L/min in order to dry the packing material. After the drying step, two separate 4 mL MeOH rinses are passed through the SPE columns and collected in the collection tubes. These rinses include rinsing of the sample bottle before entering the SPE column. Note: EPA 537.1 §11.4.1 states that the cartridge packing material must not be allowed to go dry during the conditioning phase. The AEU uses positive pressure to push solvent and sample through the cartridge, and no liquid is drawn out of the packing material as it is under vacuum. The AEU manufacturer has weighed SPE cartridges before and after the conditioning phase and demonstrated that the cartridges retain 0.5-1 mL of water. See manufacturer correspondence at M:\EHD\ESS(4900)\ESS Org(4940)\Method Related Documents\PFAS\PFAS in DW\RE SPE-03 Solvent Additions.msg

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10.9. Take the extracts and blow them down to dryness under a gentle stream of nitrogen in a heated water bath at 60-65°C. This temperature will be verified each day of use by a certified thermometer to make sure that the temperature of the water bath is accurate. The serial number of the thermometer will be noted on the extraction log for each sample extracted. Begin evaporating samples at 2 L/min, and increase the flow to 3.5 L/minute after approximately 30 minutes. Continue evaporating at 3.5 L/min for about 60 minutes until samples are dry. Add 1.0 mL of 96:4 MeOH:H_2O and 25 µL of internal standard mix to each tube and mix well. Vortex and transfer the samples using a disposable polypropylene pipet into individual 1.2-mL polypropylene cryovials. Take a portion of this mixture and transfer to a 300-µL polypropylene autosampler vial. Cap with polypropylene caps. Extracts are stored at room temperature. Autosampler vials are single-use only, and remaining extract in an autosampler vial with a pierced septum must be discarded.

11. Calculations: Sample results are calculated using the Applied Biosystems Analyst software, performing a multilevel calibration, and using a linear fit if possible.

11.1. Analyte concentrations are calculated automatically by the Analyst software using the equation below where $Conc_A$ is the analyte concentration in ng/L, PA_A is the analyte peak area, PA_{IS} is the internal standard peak area, $Conc_{IS}$ is the internal standard concentration in ng/L, and m is the slope of the calibration curve.

$$Conc_A = \frac{\frac{PA_A}{PA_{IS}} * Conc_{IS}}{m}$$

11.2. Dilutions—if the peak area of a sample analyte exceeds that of the highest calibration standard, the sample must be diluted appropriately and additional internal standard added to achieve 1ng/L in the injected sample. For adjustment of the result, a dilution factor must be added to either the instrument Results Table or LIMS. MDLs and MRLs will also be adjusted by dilution factors.

11.3. Volume of Extracted Sample—Horizon automatically adjusts final results, MDLs, and MRLs for the amount of extracted sample (based on a default extraction volume of 250 mL).

$$\text{Result, MDL, MRL (ng/L)} = \text{Posted Result, MDL, MRL (ng/L)} * \frac{250\text{ mL}}{\text{Sample Volume (mL)}}$$

11.4. Adjustments for Salt vs. Acid—some PFAS may not be available in their acid forms, but rather as their corresponding salts. These salts must be mass corrected according to the equation below.

$$\text{Mass Acid} = \text{Measured Mass Salt} * \frac{\text{Molecular Weight Acid}}{\text{Molecular Weight Salt}}$$

12. Corrective Action Procedure:

12.1. For a detailed explanation of the corrective action procedure, please refer to: O:\SOP\EHD\Division Wide\Final\NELAC QA Manual rev 18 2020\NELAC QA Manual rev 18 2020 6 Organic Chem.docx. Some corrective actions are addressed in Appendix 2, **Rejection of Samples or Sample Results**

12.2. QC Failures, instrument problems, or analytical problems:

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12.2.1. Corrective action shall be documented either in the instrument logbook, the report generated from the instrument, or in an occurrence management form. Most incidences can be isolated to the instrument. Instruments could require repairing, adjusting, servicing instrument hardware/software, or a combination of actions.

12.2.1.1. The analyst should document on the lab report a concise explanation for resolving the immediate issue(s).

12.2.1.2. The instrument logbook can be found at: M:\EHD\ESS(4900)\ESS Org(4940)\Logbooks\PFAS

12.2.2. Each batch of samples is assessed against the required QC for data reporting integrity. All instrument problems, analytical problems, or QC failures should be addressed by answering the following questions.

12.2.2.1. **What was the source of the problem?**

12.2.2.1.1. The analyst should determine and document what the failure was. Example: blank contamination, lab matrix spike failure, matrix spike failure, precision failure, surrogate spike failure, etc.

12.2.2.1.2. The source of the issue or failure may not always be known, but a comment should be documented stating that the issue was investigated; include a comment of why corrective action cannot be performed. (e.g. checks OK – unknown cause; failure investigated – unknown cause – reanalysis not possible due to… etc.)

12.2.2.2. **What corrective action was performed?** The analyst should formulate and document an immediate assessment as how best to proceed. Some solutions could be: recalibration, check standard assessment, interference assessment, qualify data, reanalysis, instrument maintenance, stock standard response assessment, etc.

12.2.2.3. **What was done to verify the corrective action?** All assumptions of correction must be verified and documented. If possible, reanalysis after corrective action would provide conclusive evidence that corrective action worked. ***Note: Not all QC failures can be readily explained. Analyst experience weighs heavily in determining the proper corrective action.***

13. Data Management: Data is collected, and calculations are made on a PC-based system running SCIEX Analyst Software by the analyst. PFAS analyte data is transcribed onto the sample worksheet, reviewed, and transferred to the LIMS by the analyst (or designee). It is then reviewed by peers or the section supervisor according to ESS ORG QA 0008 before being released.

13.1. Updating a Quantitation Method

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13.1.1. Create a Results Table containing at least the calibration standards or the initial CCV to be used for Quantitation Method (QM) reference; select a previously used QM for initial quantitation parameters.

13.1.2. In the newly created Results Table, begin peak review for the QM reference standard. Select a peak by dragging the cursor across its width (without Manual Integration enabled) and pressing Select Peak. Select Apply and then Accept. In the quantitation parameters, the Expected RT should update to the RT of the selected peak. After selecting each peak, (including IS) in the reference standard (note: the peak selection tool will often not include branched isomers; this integration must be adjusted later), right click in the peak review pane and select Update Method. All samples in the Results Table will be re-integrated with the updated method, and any additions to the same Results Table will be integrated with that method.

13.1.3. If desired, select Tools > Results Table > Export Method… and save the new QM (it is acceptable to save over an old QM to save space). This step is necessary to save the quantitation parameters; otherwise, the QM referencing will apply only to the created Results Table. Because a new QM reference will generally be used for each new Results Table, saving is not necessary.

13.2. Horizon:

13.2.1. When Creating a Batch, include all QC that will be analyzed. This includes MB, FRB, FD, LFB (low, medium and high), LFSM (low, medium, and high), and LFSMD (low, medium, and high).

13.2.2. If a QC sample fails, add an additional one to the batch (giving it a second number) and upload both into Horizon.

13.2.3. OK the failed QC so it will count when new limits established.

13.2.4. These new limits will be entered by a QC person or a LIMS Admin, not by the Chemist.

13.3. Upload into Horizon:

13.3.1. With the results table open in Analyst, right click anywhere on the table to bring up a drop-down menu. Select “Full” to get all compounds and all injections into one table.

13.3.2. Highlight the entire file (click on the small rectangle to the left of the “Sample Name” column to highlight the entire table) and copy it by hitting CTRL + C. Copy the data into an Excel sheet and save it as a .CSV file.

13.3.3. Add the batch number (number only) to cell A1. Make sure that the Sample Name is in column B, Calculated Concentration is in column D, the Acquisition Date is in column H, and the Analyte Peak Name is in column J.

13.3.4. The Sample Name (row B) must contain the Horizon number of any sample or QC or the upload will not work correctly.

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13.3.5. Move a copy of the .CSV file to the appropriate instrument interface folder: WLSH Network > slhhorizprdapp> InstrumentResults> ORG_LCMS > Individual Analyst Folder

13.3.6. Horizon will automatically move the data from the instrument interface folder to the LIMS Results Upload queue. If the .CSV file does not disappear from the interface folder within 60 seconds, check that the file is formatted correctly. Contact a LIMS Administrator with further issues.

13.4. LIMS Results Upload:

13.4.1. In Horizon, select Data > Results Upload

13.4.2. Enter the batch number in the search bar, and hit Enter

13.4.3. Highlight all data for upload and select Upload Results. The data will automatically transfer to the corresponding samples in the batch, and each sample's Active Status should now be "In Review".

13.5. Calculations in Horizon

13.5.1. Horizon cannot perform an appropriate calculation for the expected values of the LFBs using the amounts of standard that are actually spiked into a water sample. Due to the calculation problems with Horizon, the volumes that are used in the LFBs are 250 times lower than the actual spike amount. The Horizon calculations have been manually verified.

13.6. Standards in Horizon for PFC's in water:

13.6.1. PFCDWStk - Perfluorinated Compounds Stock 1 @ 100 ng/mL (Table 2)

13.6.2. PFCDWSStk – Perfluorinated Surrogate Stock 1 @ 40 ng/mL (Table 4)

13.7. Data is reported in ng/L of water.

13.8. Standard logbook information is listed in Tables 1-9 below.

13.9. Add the analyst's initials and sample information to the Sample ID column in the results table so that data on the custom instrument reports is traceable to the analyst.

14. Definitions: General definitions of terms that may be used in this method can be found in the following documents.

14.1. Check reference methods (see §16).

14.2. TNI Standard, EL-V1M2-2016-Rev. 2.1, Section 3.0, Terms and Definitions, The NELAC Institute, 2016 and is located at O:\Teams\EHD QC Team\Accreditation\NELAC\2016 TNI Standard + guidance doc\STD-ELV1-2016-Rev2,1_LabRegs

14.3. Chapter NR 149, LABORATORY ACCREDITATION, Wisconsin State Legislative Reference Bureau, Register February, 2021 No. 782, effective June 29, 2021. See Section NR149.03 Definitions.

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15. Method Performance:

15.1. Where applicable, the laboratory's initial accuracy and precision data (MDLs and IDCs) were generated in compliance with the reference method and the Departments standard operating procedure

15.1.1. MDL procedure is located at O:\SOP\EHD\Division Wide\Final\EHD QA 116 LOD Procedures.doc.

15.1.2. IDC procedure is located at O:\SOP\EHD\Division Wide\Final\EHD QA 115 rev 0_DOCs.docx.

15.2. Data generated within the last two years will be located in filing cabinet across from cubicle SC213. Any data older than two years is archived, stored at Wisconsin State Record faculties and then destroyed after meeting its required retention time.

16. References:

16.1. Determination of selected perfluorinated alkyl acids in drinking water by solid phase extraction and liquid chromatography/tandem mass spectrometry (LC/MS/MS). Method 537.1 EPA/600/R-08/092.

16.2. "Methods for the Determination of Organic Compounds in Drinking Water," US EPA/600/4-88/039, 1995.

16.3. TNI Standard, EL-V1-2016-Rev.2.1, The NELAC Institute, 2016 and is located at O:\Teams\EHD QC Team\Accreditation\NELAC\2016 TNI Standard + guidance docs\STD-ELV1-2016-Rev2.1_LabReqs.pdf

16.4. Chapter NR 149, LABORATORY ACCREDITATION, Wisconsin State Legislative Reference Bureau, Register February, 2021 No. 782, effective June 29, 2021

16.5. “PFAS in Drinking Water Sample Handling Instructions”, located at M:\EHD\ESS(4900)\ESS Org(4940)\Forms and Sampling Instructions\Final\PFAS in Drinking Water\PFAS in Drinking Water Sample Handling Instructions.doc.

16.6. EHD GENOP 034, “Manual Integration Policy and Procedure”

16.7. “How to Prepare PFAS in Drinking Water Kits”, located at M:\EHD\ESS(4900)\ESS Org(4940)\Forms and Sampling Instructions\Final\PFAS in Drinking Water\How to prepare PFAS in Drinking Water.doc

16.8. ESS ORG IOP 0503, “PFAS SPE System”

16.9. ESS ORG QA 0008, “Data Auditing”

16.10. Horizon Laboratory Information Management System (LIMS) and Quality Assurance, O:\SOP\EHD\ESS\Enviro Organic\Organic and Air Chem\Final\Quality Assurance (QA)\ESS ORG QA 0001_Horizon and QA.docx

16.11. QC Bottle Check Template, M:\EHD\ESS(4900)\ESS Org(4940)\Method Related Documents\PFAS\QC Bottle Checks

16.12. “Internal Standard Counts for PFAS in Drinking Water”, M:\EHD\ESS(4900)\ESS Org(4940)\Method Related Documents\PFAS\IS Check Template.xltx

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16.13. Chemical Hygiene Plan, O:\SOP\Safety\Final\AD_SAFETY_GENOP_102_ Chemical Hygiene Plan.doc

16.14. Waste Management, O:\SOP\EHD\Division Wide\Final\EHD GENOP 038 SOP Waste Management.doc

16.15. University of Wisconsin Chemical Safety and Disposal Guide, http://ehs.wisc.edu/disposal-services/

16.16. "LOD/LOQ Procedures", EHD QA 116. O:\SOP\EHD\Division Wide\Final\EHD QA 116 LOD Procedures.doc

16.17. "Initial and Ongoing DOC Procedures". EHD QA 115. O:\SOP\EHD\Division Wide\Final\EHD QA 115 DOCs.docx

16.18. "How to Prepare PFAS in Drinking Water Kits", M:\EHD\ESS(4900)\ESS Org(4940)\Forms and Sampling Instructions\Final\PFAS in Drinking Water\How to prepare PFAS in Drinking Water.doc

16.19. "PFAS in Drinking Water Sample Handling Instructions", M:\EHD\ESS(4900)\ESS Org(4940)\Forms and Sampling Instructions\Final\PFAS in Drinking Water\PFAS in Drinking Water Sample Handling Instructions.doc

16.20. M:\EHD\ESS(4900)\ESS Org(4940)\Method Related Documents\PFAS\PFAS in DW\RE SPE-03 Solvent Additions.msg

16.21. M:\EHD\ESS(4900)\ESS Org(4940)\Logbooks\PFAS

17. Tables, figures, diagrams, charts, checklists, appendices: See following pages

Note: Final concentrations listed in the tables below are examples based on concentrations of initial purchased stocks; concentrations in future preparations may differ slightly.

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Table 1 – Working target stock solution at 0.1 ppm (96% MeOH, PFCDWStk in Horizon)

Mix ID	Compound	Aliquot (µL)	Final Volume (mL)	Concentration (ng/mL)
EPA-537PDS-R1	PFHxA	50	1	100
	PFHpA			100
	PFOA			100
	PFNA			100
	PFDA			100
	PFUnDA			100
	PFDoDA			100
	PFTrDA			100
	PFTeDA			100
	PFBS			88.5
	PFHxS			91.2
	PFOS			92.6
	N-MeFOSAA			100
	N-EtFOSAA			100
	HFPO-DA			100
	11Cl-PF3OUdS			94
	9Cl-PF3ONS			93
	DONA			91.7

Note: See §11.3 for salt adjustment calculation.

Table 2 – Stock Surrogate solution at 1000 ppb (96% MeOH)

Compound	Stock Concentration (µg/mL)	Aliquot (µL)	Final Volume (mL)	Final Concentration (ng/mL)
Perfluoro-13C_5 hexanoic acid	50	20	1	1000
Perfluoro-13C_6 decanoic acid	50	20	1	1000
d_5-NEtFOSAA	50	20	1	1000
$^{13}C_3$-HFPO-DA	50	20	1	1000

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Table 3 – Working surrogate stock solution (96% MeOH, PFCDWSStk in Horizon)

Compound	Stock Concentration (ng/mL)	Aliquot (µL)	Final Volume (mL)	Final Concentration (ng/mL)
Perfluoro-$13C_5$ hexanoic acid	1000	40	1	40
Perfluoro-$13C_6$ decanoic acid	1000		1	40
d_5-NEtFOSAA	1000		1	40
$^{13}C_3$-HFPO-DA	1000		1	40

Table 4 – Dilution of internal standards to 1,000 ppb (96% MeOH)

Compound	Stock Concentration (ug/mL)	Volume to add (µL)	Final Volume (mL)	Final Conc. (ng/mL)
d_3 –N-MeFOSAA	50	20	1	1,000
$13C_2$-PFOA	50	20	1	956
$13C_4$-PFOS	50	20	1	1,000

Table 5 – Working internal standard stock mix (96% MeOH)

Compound	Stock Concentration (ng/mL)	Volume to add (µL)	Final Volume (mL)	Final Conc. (ng/mL)
$13C_4$-PFOS	956	40	1	38.24
$13C_2$-PFOA	1,000		1	40
d_3 –N-MeFOSAA	1,000		1	40

Table 6 – Calibration Standards using 100 ppb mix (96% MeOH)

Level (ng/mL of target compounds)	Concentration of standard in water (ng/L)	Amount of 100 ppb target compound mix (µL)	Amount of surrogate compound mix (µL)	Amount of Internal Standard mix (µL)
Level 7 (10 ng/mL)	40	100	25	25
Level 6 (5 ng/mL)	20	50	25	25
Level 5 (2.5 ng/mL)	10	25	25	25
Level 4 (1 ng/mL)	4	10	25	25
Level 3 (0.5 ng/mL)	2	5	25	25
Level 2 (0.25 ng/mL)	1.0	2.5	25	25
Level 1 (0.15 ng/mL)	0.6	1.5	25	25

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Table 7 – Second source (QCS) stock standard at 1 ppb (96% MeOH)

Compound	Concentration (ppb)
PFHxA	1
PFHpA	1
PFOA	1
PFNA	1
PFDA	1
PFUnDA	1
PFDoDA	1
PFTrDA	1
PFTeDA	1
PFBS	0.885
PFHxS	0.912
PFOS	0.926
N-MeFOSAA	1
N-EtFOSAA	1
HFPO-DA	1
11Cl-PF3OUdS	0.94
9Cl-PF3ONS	0.93
DONA	0.945

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Appendix 1 Method OC12731 PFAS EXTRACTION LOG

Batch: _____ Method: ____OC12731 (EPA 537.1)_____

Extraction:

Start Date/Time: _____ End Date/Time: _____ Analyst: _____

SPE System ID: _____ SPE Cartridge Lot:_____

Spiking Standard Information:

Surrogate Spike Std ID: _____

Laboratory Control Spike ID: _____

Other Sample Information:

QC number on sample/field blank/lab blank bottles _____

Trizma Lot in field blank _____

Trizma Lot in samples _____

Source of water for all QC samples _RO218A_____

Temperature of water bath _____ Serial # of thermometer _____

pH is 7.0 +/- 0.5 prior to extraction ___ pH Lot _____

Free chlorine <0.1mg/L prior to extraction _____ Free chlorine check Lot _____ _____ **ID**	**Horizon ID**	**Extraction Position**	**MS**	**DUP**	**FRB**	**Surrogate Amt. (µL)**	**Spike Amt. (µL)**	**Samp. Vol. (mL)**
MB			---	---	---			250
LCS			---	---	---			250
1.								
2.								
3.								
4.								

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5.								
6.								
7.								
8.								
9.								
10.								

Solvent/Reagent Lot #'s

Methanol: VWR Lot: _____ Received Date/Analyst Initials: _____

Expiration Date/Analyst Initials: _____

Weighing full/empty bottles:

Date: _____ Analyst: _____ Balance ID: _____

ID	Horizon ID	MS	DUP	FRB	Initial Weight (g)	Final Weight (g)	Samp. Vol. (mL)
MB		---	---	---	---	---	250
LCS		---	---	---	---	---	250
1.							
2.							
3.							
4.							
5.							
6.							
7.							
8.							
9.							
10.							

Adding internal standards:

Date: _____ Analyst: _____ Internal Standard ID: _____

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TurboVap ID: 181000417

Additional Extraction Notes:

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Appendix 2

Rejection of Samples or Sample Results for PFAS by EPA 537.1

Rejection of the sample or sample results is defined as:
"**Results are not to be reported to the WDNR**" *with or without data qualifiers or narratives*.
The client is to be contacted and the affected sample re-collected. The laboratory must notify the authority requesting the analyses and ask for a resample. [40 CFR 141.23 (a)(4)(i), SDWA Manual Chapter IV (6.1)]
Samples shall be rejected when the following cases occur:

1. The container received was not a 250 mL polypropylene or HDPE container, fitted with a polypropylene or HDPE screw cap.
[SDWA Manual Chapter IV (6.2), EPA 537.1 (8.1.1)]

2. The container received did not contain Trizma at a concentration of 5.0 g/L.
[SDWA Manual Chapter IV (6.2), EPA 537.1 (8.1.2)]

3. Samples received at the laboratory exceeded 10 °C during the first 48 hours after collection.
[SDWA Manual Chapter IV (6.2), EPA 537.1 (8.4)]

4. Samples received at the laboratory after 48 hours of collection exceeded 6 °C.
[SDWA Manual Chapter IV (6.2), EPA 537.1 (8.4)]

5. Samples exceeded 6 °C before extraction during storage at the laboratory.
[SDWA Manual Chapter IV (6.2), EPA 537.1 (8.4)]

6. Samples were extracted after 14 days of collection.
[SDWA Manual Chapter IV (6.3), EPA 537.1 (8.5)]

7. Samples were analyzed after 28 days of extraction.
[SDWA Manual Chapter IV (6.3), EPA 537.1 (8.5)]
All laboratories analyzing drinking water compliance samples must adhere to any required QC procedures specified in the methods. [SDWA Manual Chapter III (11)]

8. If the method analytes are detected in the LRB at concentrations equal to or greater than 1/3 the MRL, then all data for the problem analyte(s) must be considered invalid for all samples in the extraction batch.
[EPA 537.1 (9.3.1)]

9. If the LFB results do not meet recovery limits for method analytes, then all data for the problem analyte(s) must be considered invalid for all samples in the extraction batch. If the LFB fails because the calculated concentration is greater than 130% (150% for low-level spikes) for a particular analyte, and the field sample extracts show no detection for that analyte, then the non-detects may be reported without re-analysis.
[EPA 537.1 (9.3.3)]

10. If the method analyte(s) found in the Field Sample is present in the FRB at a concentration greater than 1/3 the MRL, then all samples collected with that FRB are invalid and must be recollected and reanalyzed.
[EPA 537.1 (9.3.8)]

11. If the surrogate in the CCC recovers outside of 70-130%, then all data for the problem analyte(s) must be considered invalid.
[EPA 537.1 (10.3.3)]
The laboratory must notify the authority requesting the analyses and ask for a resample.

[40 CFR 141.23 (a)(4)(i), SDWA Manual Chapter IV (6.1)]

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18. Signatory Page:

18.1. Written by: Brandon Shelton
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Date: Revision 1, 02/04/2019

18.2. _____
Updated by: Kyle Burke
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Unit: ESS Organic Chemistry
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Date: Revision 10, 7/22/2021
Rev. 9, 1/13/2021

18.3. Reviewed by: Angela Albrecht
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Date: Revision 10, 7/23/2021
Rev. 9, 1/15/2021