DoD AFFF01:

DETERMINATION OF PERFLUOROOCTANOIC ACID AND PERFLUOROOCTANESULFONIC ACID IN AQUEOUS FILM FORMING FOAM (AFFF) FOR DEMONSTRATION OF COMPLIANCE TO MIL-PRF-24385

Revision 1.0

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1.0 Scope and Application

This method describes a procedure for the quantitative determination of two method analytes, Perfluorooctanoic acid (PFOA) and Perfluorooctanesulfonic acid (PFOS) (Table 1.1), in Class B aqueous film forming foam (AFFF) concentrates (herein cited as "AFFF samples"). The military performance specification for Fire Extinguishing Agent, Aqueous Film-Forming Foam (AFFF) Liquid Concentrate, for Fresh and Sea Water, MIL-PRF-24385 (Reference 17.2), requires the PFOA and PFOS content of AFFF concentrates be determined (herein cited as "compliance testing") by a laboratory accredited for this method under their Department of Defense Environmental Laboratory Accreditation Program (DoD ELAP) scope of accreditation. Therefore in addition to the requirements contained in this method, the requirements contained in the latest version of the DoD/DOE Quality Systems Manual for Environmental Laboratories (DoD/DOE QSM) (Reference 17.1) also apply, with the exception of those contained in Appendix B, Table B-15. Requirements contained in this method supersede the requirements contained in Table B-15 for compliance testing purposes.

This method utilizes solid phase extraction (SPE) and carbon cleanup techniques to prepare AFFF samples for analysis by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Selectivity is optimized through the use of Multiple Reaction Monitoring (MRM) mode and the monitoring of at least two MS/MS transitions for each method analyte. Isotopically labeled standards of PFOA and PFOS are used to calibrate and quantify PFOA and PFOS by isotope dilution quantitation. Branched and linear isomers are included in the quantitation of both method analytes. Inter- and intra-laboratory studies have generated accuracy and precision data for the determination of PFOA and PFOS in a variety of AFFF concentrates.

This method is restricted to use by or under the supervision of analysts experienced in the use of a liquid chromatography/tandem quadrupole mass spectrometer and in the interpretation of mass spectra. In addition, PFAS analysis requires specific skills and experience in minimizing laboratory background and contamination and it is highly recommended that this method only be performed in laboratories with prior experience in PFAS analysis by LC-MS/MS.

Table 1.1 Method Analyte

Table 1.1 Names, Abbreviations, and CAS Registry Numbers for Method Analytes

Method Analyte Name	Abbreviation	CAS Number
Perfluorooctanoic acid	PFOA	335-67-1
Perfluorooctanesulfonic acid	PFOS	1763-23-1

1.1 Detection of PFAS Isomers

Per- and polyfluoroalkyl substances (PFAS), including PFOA and PFOS, may exist as branched as well as linear isomers in AFFF samples, therefore both branched and linear isomers must be included in the determination of each method analyte. A quantitative standard that contains a mixture of branched and linear isomers of PFOS is used for calibration and quantification of PFOS. This standard must be used for all calibration, calibration verifications, and quality control (QC) samples. No such quantitative standard is currently commercially available for PFOA, therefore a quantitative standard that contains only the linear isomer of PFOA is used for calibration. A qualitative standard containing a mixture of branched and linear isomers of PFOA is analyzed post calibration in order to determine the retention time of the branched isomers of PFOA, to therefore be included in the quantification of PFOA.

1.2 Limit of Quantitation

Single-laboratory and interlaboratory validation studies of this method demonstrated a limit of quantitation (LOQ) of less than 25 ppb for both PFOA and PFOS is routinely achievable. An LOQ of less than 25 ppb must be achieved for both PFOA and PFOS in order for results to be applicable to compliance testing.

1.3 Method Flexibility

The type of LC system (UPLC, HPLC), the LC columns, LC conditions, and MS conditions utilized may be different than those utilized in the development of this method. Changes may not be made to the quality control (QC) (Section 9.0), Calibration (Section 10.0), Sample Preparation (Section 11.0), Instrumental Analysis (Section 12.0), and the Data Analysis, Calculations, and Reporting (Section 13.0) requirements.

2.0 Summary of Method

A 0.02 mL aliquot of the AFFF sample is diluted using PFAS-free reagent water, spiked with an extracted internal standard (EIS) solution containing isotopically labeled PFOA and PFOS compounds (Table 2.1), extracted using SPE, and matrix interferences are reduced using a carbon clean-up procedure. The extract is spiked with non-extracted internal standard (NIS) solution containing other isotopically labeled PFOA and PFOS compounds (Table 2.1) and analysis is conducted by liquid chromatography-tandem mass spectrometry (LC-MS/MS) utilizing negative ion spray and multiple reaction monitoring (MRM) mode.

This method requires PFOA and PFOS to be quantified and reported in their acid form. The quantitation scheme utilized by this method is isotope dilution quantitation, which recovery corrects the results for method analytes using the response of the isotopically labeled PFOA and PFOS compounds (EIS) added to the sample prior to extraction. To assess overall analytical quality, the recovery of the EIS compound is determined through comparison of its response to response of the applicable NIS compound that was added post extraction(see Table 2.2), prior to sample analysis. At a minimum, the transitions listed in Table 2.2 must be utilized for quantitation and confirmation. A third transition may be added for confirmation purposes.

Table 2.1 Names and Abbreviations for Extracted Internal Standard (EIS) and Non-extracted Internal Standard (NIS) Compounds

EIS Compounds	**Abbreviation**
Perfluoro-n-[$^{13}C_8$]octanoic acid	$^{13}C_8$-PFOA
Perfluoro-1-[$^{13}C_8$]octanesulfonic acid	$^{13}C_8$-PFOS
NIS Compounds	**Abbreviation**
Perfluoro-n-[1,2,3,4-$^{13}C_4$]octanoic acid	$^{13}C_4$-PFOA
Perfluoro-n-[1,2,3,4-$^{13}C_4$]octanesulfonic acid	$^{13}C_4$-PFOS

Table 2.2. Analyte Ions Monitored, Extracted Internal Standard, and Non-extracted Internal Standard Used for Quantification

Abbreviation	**Example Retention Time**	**Parent Ion Mass**	**Quantification Ion Mass**	**Confirmation Ion Mass**	**Typical Ion Ratio**	**Quantification Reference Compound**
Analytes						
PFOA	6.16	413.0	369.0	169.0	3.0	$^{13}C_8$-PFOA
PFOS	7.59	498.9	79.9	98.8	2.3	$^{13}C_8$-PFOS
Extracted Internal Standards						

Table 2.2. Analyte Ions Monitored, Extracted Internal Standard, and Non-extracted Internal Standard Used for Quantification						
Abbreviation	**Example Retention Time**	**Parent Ion Mass**	**Quantification Ion Mass**	**Confirmation Ion Mass**	**Typical Ion Ratio**	**Quantification Reference Compound**
$^{13}C_8$-PFOA	6.16	421.1	376.0	NA		$^{13}C_4$-PFOA
$^{13}C_8$-PFOS	7.59	507.1	98.9	79.9		$^{13}C_4$-PFOS
Non-Extracted Internal Standards						
$^{13}C_4$-PFOA	6.16	417.1	172.0	NA		
$^{13}C_4$-PFOS	7.59	502.8	79.9	98.9		

3.0 Definitions

Definitions of terms used in this method are consistent with those published in the DoD/DOE QSM (current version). Additions and deviations from these are provided below.

3.1 AFFF

Aqueous film-forming foam containing fluorinated and hydrocarbon surfactants and other constituents formulated for class B hydrocarbon fires.

3.2 Analysis Batch

A set of samples that are analyzed on the same instrument during a 24-hour period.

3.3 AFFF Concentrate

Formulation of AFFF in concentrate form prior to dilution for end-use firefighting application.

3.4 Military Specification

A United States defense standard used to help achieve standardization objectives by the U.S. Department of Defense.

3.5 PFAS-free

Concentration of PFOA and PFOS each have been verified to be less than ½ the Limit of Quantitation (LOQ). This definition is applicable to materials, supplies, and quality control samples (e.g., instrument and method blanks) contained in this method.

3.6 Qualitative Standards

A standard that contains a mixture of branched and linear isomers of a target analyte that is not of the purity needed to be considered quantitative. These standards are often identified as technical grade mixtures by manufacturers. Qualitative standards are analyzed for comparison to suspected branched isomer peaks in test samples.

4.0 Contamination and Interferences

4.1 Labware, Reagents, Equipment, and Supplies

Materials, and reagents used and components in the analytical instruments should be handled and chosen carefully to prevent contamination with the method analytes and interferences. Documented best practices to accomplish this are available. Samples in aqueous-based solvents should minimize contact with polypropylene to minimize any adsorption effects. Sample containers must be high density polyethylene (HDPE) containers. The use of glassware for transfer procedures and standards preparation is allowed, but standards and sample extracts are to be stored in HDPE containers.

4.1.1 All glass equipment that is used in the preparation or storage of reagents must be appropriately cleaned prior to use. The process must include detergent, reagent water, and solvent rinses followed by baking in a kiln or furnace. After detergent washing, glassware should be rinsed immediately with reagent water. A solvent rinse procedure using methanolic ammonium hydroxide (1%), toluene, and methanol is recommended.

4.1.2 Due to the potential for sample preparation and analysis supplies to introduce PFAS into the sample, the residual PFAS content of disposable plasticware and filters must be verified by batch/lot number. If the residual PFAS content of these materials is at a concentration less than half the limit of quantitation, these materials can be utilized without cleaning.

4.1.3 The SPE manifold can be a significant source of PFAS contamination. All parts of the SPE manifold must be cleaned between samples by sonicating in methanolic ammonium hydroxide (1%) and air drying prior to use. In order to validate the cleaning process used is sufficient, the manifold position of the batch quality control (QC) samples (specifically the method blank (MB) and laboratory control sample (LCS) in each batch must be rotated on a batch by batch basis.

4.2 Samples

4.2.1 AFFF samples can contain high concentrations of PFAS, therefore the risk of PFAS cross-contamination is high. To reduce the potential for cross-contamination, all equipment must be cleaned prior to, and after each use. The cleaning solvents typically used include water, methanol, and methanolic ammonium hydroxide.

4.2.2 Interferences co-extracted from samples will vary considerably sample to sample, depending on the non-PFAS constituents of each AFFF concentrate. Concentrations of these interfering compounds can be several orders of magnitude higher than concentration of the targeted PFAS analytes. Given the goal of this method is to achieve quantitation of low levels of PFAS, it is critical that these interferences are eliminated to the greatest extent possible. The SPE and carbon cleanup procedures contained in this method is included for this purpose.

5.0 Safety

5.1 The toxicity or carcinogenicity of all of the PFAS included in the scope of this method are yet to be determined; therefore they should be treated as a potential health hazard. Requirements contained in the laboratory's Safety Manual and associated standard operating procedures (SOPs) should be followed.

5.2 A safety data sheets (SDS) must accompany each AFFF sample submitted and be retained by the laboratory in accordance with Occupational Safety and Health Administration (OSHA) regulations and laboratory policy.

6.0 Equipment and Supplies

Brand names, suppliers, and part numbers are for illustration purposes only and no endorsement is implied. If equivalent performance can be achieved using apparatus and materials other than those specified here, they can be used.

6.1 Sample bottles and caps –HDPE, with liner-less HDPE or polypropylene caps. Use of PTFE-lined caps is prohibited.

6.2 Nitrile gloves

6.3 Balances

6.3.1 Analytical – Capable of weighing 0.1 mg

6.3.2 Top loading – Capable of weighing 10 mg

6.4 Ultrasonic mixer (sonicator)

6.5 HDPE bottles, with liner-less HDPE or polypropylene caps – 60 mL and 500 mL

6.6 pH Paper, range 0-14 - (Whatman® Panpeha™ or equivalent), 0.5-unit readability

6.7 Analog or digital vortex mixer, single or multi-tube (Fisher Scientific 02-215-452, or equivalent)

6.8 Volumetric flasks, Class A

6.9 Disposable polypropylene collection tubes (13 x 100 mm, 8 mL)

6.10 Variable speed mixing table (Fisherbrand™ Nutating mixer or equivalent)

6.11 Silanized glass wool (Sigma-Aldrich, Cat # 20411 or equivalent) – store in a clean glass jar and rinsed with methanol (2 times) prior to use.

6.12 Disposable syringe filter, 25-mm, 0.2-µm Nylon membrane, PALL/Acrodisc or equivalent

6.13 Glass fiber filter, 47 mm, 1 µm, PALL A/E or equivalent

6.14 Centrifuge (Thermo Scientific Legend RT+, 16 cm rotor, or equivalent), capable of reaching at least 3000 rpm

6.15 Centrifuge tubes – Disposable HDPE or polypropylene centrifuge tubes (15 and 500 mL)

6.16 Norm-Ject® syringe (or equivalent), polypropylene/HDPE, 5 mL

6.17 Variable volume pipettes with disposable HDPE or polypropylene tips (10 µL to 5 mL) – used for preparation of calibration standards and spiked samples.

6.18 Disposable glass pipets

6.19 Calibrated mechanical pipettes or Hamilton graduated syringes

6.20 Solid-phase extraction (SPE) cartridges (Waters Oasis WAX 150 mg, Cat # 186002493 or equivalent). The SPE sorbent must have a pKa above 8 so that it remains positively charged during the extraction.

6.21 Vacuum manifold for SPE Cartridges (Waters™ extraction manifold #WAT200607 or equivalent)

6.22 Automatic or manual solvent evaporation system (TurboVap® LV or equivalent)

6.23 Evaporation/concentrator tubes: 60 mL clear glass vial, 30 x 125 mm, without caps (Wheaton Cat # W226060 or equivalent). Cover with foil if required.

6.24 Snap cap/crimp top vials, 300 µL, polypropylene (12 x 32 mm) – used in sample pre-screening (DWK Life Sciences Cat # 225180 or equivalent)

6.25 Polypropylene crimp/snap vials, 1 mL (Agilent Cat # 5182-0567 or equivalent)

6.26 Clear snap cap, PVDC film/white silicone, 11 mm (American Chromatography Supplies Cat # C299-11 or equivalent)

6.27 Single step filter vials (Restek Thomson SINGLE StEP® Standard Filter Vials, 0.2 µm Nylon membrane, with Black Preslit caps Cat # 25891 or equivalent) – used in sample pre-screening

6.28 10 mg polypropylene or stainless steel scoops

6.29 Ultra high-performance liquid chromatograph (UPLC also called UHPLC) or high- performance liquid chromatograph (HPLC) equipped with tandem quadrupole mass spectrometer (Waters Xevo TQ-S Micro or equivalent capable of electrospray ionization in the negative ion mode.

6.30 C18 column, 1.7 µm, 50 x 2.1 mm (Waters Acquity UPLC® BEH or equivalent)

6.31 Guard column (Phenomenex Kinetex® Evo C18 or equivalent)

6.32 Trap/delay column (Purospher Star RP-18 endcapped [3 µm] Hibar® RT 50-4 or equivalent)

6.33 Bottles, HDPE or glass, with liner-less HDPE or polypropylene caps. Various sizes. To store prepared reagents.

7.0 Reagents and Standards

7.1 Reagents

When prepared by the laboratory, must be stored in either glass or HDPE containers.

7.1.1 Acetic acid - ACS grade or equivalent, store at room temperature

7.1.2 Acetic acid (0.1%) - dissolve acetic acid (1 mL) in reagent water (1 L), store at room temperature, replace after 3 months. This reagent is used for sample extract dilution only.

7.1.3 Acetonitrile – UPLC grade or equivalent, verified before use, store at room temperature

7.1.4 Ammonium acetate - (Caledon Ultra LC/MS grade, or equivalent), store at 2-8° C, replace 2 years after opening date

7.1.5 Ammonium hydroxide - certified ACS+ grade or equivalent, 30% in water, store at room temperature

7.1.6 Aqueous ammonium hydroxide (3%) - add ammonium hydroxide (10 mL, 30%) to reagent water (90 mL), store at room temperature, replace after 3 months

7.1.7 Methanolic ammonium hydroxide (0.3%) - add ammonium hydroxide (1 mL, 30%) to methanol (99 mL), store at room temperature, replace after 1 month

7.1.8 Methanolic ammonium hydroxide (1%) - add ammonium hydroxide (3.3 mL, 30%) to methanol (96.7 mL), store at room temperature, replace after 1 month

7.1.9 Methanolic ammonium hydroxide (2%) - add ammonium hydroxide (6.6 mL, 30%) to methanol (93.4 mL), store at room temperature, replace after 1 month

7.1.10 Methanolic potassium hydroxide (0.05 M) – add 3.3 g of potassium hydroxide to 1 L of methanol, store at room temperature, replace after 3 months

7.1.11 Methanol with 4% water, 1% ammonium hydroxide and 0.625% acetic acid - add ammonium hydroxide (3.3 mL, 30%), reagent water (1.7 mL) and acetic acid (0.625 mL) to methanol (92 mL), store at room temperature, replace after 1 month. This solution is used to prepare the instrument blank (Section 7.3.6).

7.1.12 Eluent A – Acetonitrile, Caledon Ultra LCMS grade or equivalent

7.1.13 Eluent B - 2 mM ammonium acetate in 95:5 water/acetonitrile. Dissolve 0.154 g of ammonium acetate (Section 7.1.4) in 950 mL of water and 50 mL of acetonitrile (Caledon Ultra LCMS grade, or equivalent). Store at room temperature, shelf life 2 months.

7.1.14 Formic acid - (greater than 96% purity or equivalent), verified by lot number before use, store at room temperature

7.1.15 Formic acid (aqueous, 0.1 M) - dissolve formic acid (4.6 g) in reagent water (1 L), store at room temperature, replace after 2 years

7.1.16 Formic acid (aqueous, 0.3 M) - dissolve formic acid (13.8 g) in reagent water (1 L), store at room temperature, replace after 2 years

7.1.17 Formic acid (aqueous, 5% v/v) - mix 5 mL formic acid with 95 mL reagent water, store at room temperature, replace after 2 years

7.1.18 Formic acid (aqueous, 50% v/v) - mix 50 mL formic acid with 50 mL reagent water, store at room temperature, replace after 2 years

7.1.19 Formic acid (methanolic 1:1, 0.1 M formic acid/methanol) - mix equal volumes of methanol and 0.1 M formic acid, store at room temperature, replace after 2 years

7.1.20 Methanol - (HPLC grade or better, 99.9% purity), verified by lot number before use, store at room temperature

7.1.21 Potassium hydroxide – certified ACS or equivalent, store at room temperature, replace after 2 years

7.1.22 Reagent water – Laboratory reagent water, test by lot/batch number for residual PFAS content

7.1.23 Carbon – EnviCarb® 1-M-USP or equivalent, verified by lot number before use, store at room temperature. Loose carbon allows for better adsorption of interferent organics. The use of carbon cartridges is not allowed.

7.1.24 Reference matrix – PFAS-free reagent water, purified water, Type I. Used to prepare the batch QC samples (e.g., method blank, limit of quantitation verification sample, and laboratory control samples)

7.2 Standards

Solutions are prepared by the laboratory using solutions or mixtures (prime stocks) with certification to their purity, concentration, and authenticity. Standard solutions must be stored in the dark at less than 4 °C unless the vendor recommends otherwise in screw-capped vials with foiled-lined caps. Monitor solutions for evaporation. If loss is detected, replace the solution.

7.2.1 Native Spiking Standard Solutions

Prepare the native spiking standard by diluting prime stocks that contain PFOA and PFOS with methanol. Quantitative prime stock solutions must be used to create this standard. As stated in Section 1.1, the quantitative standard used for PFOS must contain a mixture of branched and linear isomers while the quantitative standard used for PFOA contains only the linear isomer. It is used to prepare the calibration, instrument sensitivity check, initial calibration verification and continuing calibration verification standards and to spike preparation batch QC samples (LCS, LCSD, and LOQVER).

7.2.2 Qualitative Standard

As stated in Section 1.1, a qualitative standard that contains a mixture of branched and linear isomers of PFOA must be analyzed prior to sample analysis. Prepare this standard by diluting a qualitative stock standard containing PFOA with a solution that matches the solvent mix of sample extracts, which contain methanol with 4% water, 1% ammonium hydroxide and 0.625% acetic acid. This standard is used for comparison with suspected branched isomer peaks in AFFF samples.

7.2.3 Extracted Internal Standards (EIS) Solutions

Prepare extracted internal standard solutions by diluting, with methanol, prime stock standards containing the isotopically labeled compounds listed in Table 7.1. Table 7.1 provides the volume of EIS solution used to spike samples and the resulting nominal amount of each compound in the sample.

Table 7.1 Nominal Masses of EIS Spike Added to Samples

EIS Compounds	Volume Spiked	Amount Added (ng)
$^{13}C_8$-PFOA	50 µL	10
$^{13}C_8$-PFOS	50 µL	10

7.2.4 Non-Extracted Internal Standard (NIS) Solutions

Prepare non-extracted internal standard solutions by diluting, with methanol, prime stock standards containing the isotopically labeled compounds listed in Table 7.2. Table 7.2 provides the volume of NIS solution used to spike samples and the resulting nominal amount of each compound in the sample.

Table 7.2 Nominal Masses of NIS Spike Added to Extracts		
NIS Compounds	**Volume Spiked**	**Amount Added (ng)**
$^{13}C_4$-PFOA	50 µL	10
$^{13}C_4$-PFOS	50 µL	10

7.2.5 Calibration Standard Solutions

Prepare a minimum of 6 calibration standard solutions by diluting native standards with methanol, methanolic ammonium hydroxide (2%), water, and acetic acid to achieve final concentrations of PFOA and PFOS that encompass the working range of the instrument. NIS and EIS compounds are added to each calibration standard such that the concentration of these compounds remain constant over the series of calibration standards. After dilution, the final solutions will match the solvent mix of sample extracts, which contain methanol with 4% water, 1% ammonium hydroxide and 0.625% acetic acid. Calibration standard solutions do not undergo solid phase extraction or cleanup.

A minimum of five contiguous calibrations standards are required for a valid analysis when using a linear calibration model, with at least five calibration standards within the quantitation range (i.e., from the LOQ to the highest calibration standard). If a second-order calibration model is used, then a minimum of six calibration standards are required, with at least six calibration standards within the quantitation range. The lowest level calibration standard must meet a signal-to-noise ratio of 3:1 and be at a concentration less than or equal to the Limit of Quantitation (LOQ). Table 7.3 provides the concentrations for the eight calibration solutions utilized during method development. If instrument sensitivity allows, additional lower concentration standards may be added to accommodate a lower LOQ.

Table 7.3. Calibration Solutions (ng/mL)								
Analytes	**CS1**	**CS2**	**CS3**	**CS4**	**CS5(CCV)**	**CS6**	**CS7**	**CS8**
PFOA	0.05	0.1	0.2	0.5	2.5	12.5	62.5	250
PFOS	0.05	0.1	0.2	0.5	2.5	12.5	62.5	250
Extracted Internal Standard (EIS) Compounds								
$^{13}C_8$-PFOA	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
$^{13}C_8$-PFOS	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Non-extracted Internal Standard (NIS) Compounds								
$^{13}C_4$-PFOA	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
$^{13}C_4$-PFOS	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5

7.2.6 Instrument Sensitivity Check (ISC) Standard

Prepare an instrument sensitivity check (ISC) standard in the same manner as the calibration standards solutions. The concentration of PFOA and PFOS in the ISC standard must correspond to the LOQ for each method analyte.

7.2.7 Initial Calibration Verification (ICV) Standard

Initial calibration verification standards are to be prepared in the same manner as calibration standards, however, must use a prime stock for a source different than the prime stock used to prepare the calibration standards. This alternative source can be either a different manufacturer or a different lot from the same manufacturer. The concentration of PFOA and PFOS can range from the LOQ to the mid-level calibration standard concentration.

7.2.8 Continuing Calibration Verification (CCV) Standard

Prepare the continuing calibration verification standards in the same manner as the calibration standards. The concentration of PFOA and PFOS can range from the LOQ to the mid-level calibration standard concentration. The mid-level calibration standard solution can serve as the CCV.

7.2.9 Instrument Blank

Prepare instrument blanks (IBs) by fortifying the solution in Section 7.1.11 with EIS and NIS.

8.0 Sample Collection, Preservation, Storage, and Holding Times

8.1 Scope of Sampling Procedure

This sample collection procedure is applicable to sampling performed by the AFFF concentrate vendor prior to submission of the concentrate for consideration, by Naval Research Laboratory (NRL) personnel during qualification inspection, conformance inspection, retention inspection, and by facilities for verification of composition of the formulation of the lot purchased is valid only when it conforms to the requirements herein. All samples, regardless of the entity collecting the sample, must be collected from containers that are compliant with MIL-PRF-24385, section 3.6 requirements.

It is critical that the sampling of current and future AFFF stock concentrates be performed in a safe and consistent manner such that the subsequent analysis is representative of the original concentrate and the concentrations of the constituents within each product meet product specifications.

8.2 Sampling Procedure Summary

Prior to sampling, drums or containers must be inspected and opened by personnel possessing a thorough understanding of MIL-PRF-24385 requirements and PFAS sampling precautions and practices. Inspection involves the observation and recording of visual qualities of each drum/container and any characteristics or identification markings pertinent to the classification of the drum's contents. Full documentation is captured on a chain-of-custody (COC). Sampling should be performed on previously unopened AFFF stock concentrate containers if possible, in order to avoid any question of providence/integrity. Sampling of a small but representative volume of the container is to be performed using a glass drum thief sampler and the contents placed into a high density polyethylene (HDPE) bottle with a polypropylene, unlined cap.

8.3 AFFF Concentrate Stock Containers and Sample Bottles

Sample bottles (and lids) for collection of concentrated AFFF must be virgin HDPE bottles verified to be PFAS-free and supplied by the laboratory performing the analysis. HDPE bottles with unlined polypropylene caps are the only bottles that may be used for these samples. Use of any other types of bottles, especially Teflon™ sample bottles or Teflon-lined lids of any kind will void the analysis and require resampling. Avoid agitating the AFFF drum prior to sampling in order to avoid foaming. AFFF drums should be upright and relatively level. The drum must be easily identifiable and preferably unopened.

8.4 Precautions

All personnel should review the SDS of the specific AFFF concentrate being sampled and be warned of the hazards prior to handling AFFF drums. An adequate volume of absorbent material should be kept near areas where minor spills may occur. Where major spills may occur, a containment berm adequate to contain the entire volume of liquid in the drums should be constructed before any handling takes place. If drum contents spill, personnel trained in spill response should be used to isolate and contain the spill.

8.5 Equipment and Supplies

8.5.1 Personal protection equipment (nitrile gloves, goggles, etc.)

8.5.2 Virgin 75-150 mL, PFAS-fee HDPE sample bottle with un-lined screw cap

8.5.3 Chain of Custody

8.5.4 Virgin glass drum thief sampler (no larger than 12 mm O.D.)

8.5.5 Drum opening devices

8.5.6 Sample Labels

8.5.7 Sampling notebook

8.5.8 Re-sealable Plastic Bags

8.5.9 Paper towels

8.5.10 Sample bottle custody seals

8.6 Preparation and Inspection

AFFF containers/drums should be visually inspected to gain as much information as possible about their contents. The drums should be inspected for general condition, punctures, leaking contents, evidence of broken seals and signs that the drum is under pressure. It is recommended, but not required, that photographs of the drum, including the drum labels and drum opening (prior to breaking the seal) be taken and kept as part of the sampling record. Ensure the equipment and supplies listed in Section 8.5 are readily available and quantities are sufficient for the number of drums to be sampled.

8.7 Sampling Procedure

Since some layering or stratification may occur in any solution left undisturbed and mixing could cause excessive foaming, a drum thief is used to obtain a sample that represents the entire depth of the container. A glass thief sampler is most widely used for sampling container liquids. It is a simple glass tube and is commonly referred to as a drum thief. This tool is cost effective, quick, and disposable. A new drum thief MUST be used for each container in order to decrease the chance for cross contamination. A drum thief that is used for AFFF sampling is not reusable and must be disposed of after sampling. Appropriate personal protective equipment such as nitrile gloves and goggles, at a minimum, must be worn during the sampling process. Follow steps 8.7.1 through 8.7.13 for each AFFF container to be sampled.

8.7.1 Record all identifying information from the AFFF container label including the product name, manufacturer, batch number, date of manufacture, location of manufacture, expiration date (month/year), if applicable, concentrate type (3% or 6%), and the condition of the seal in the sampling notebook.

8.7.2 Create a unique identifier that will be used to track the sample through the sampling and analysis process. Record this identifier, the date of sampling and the sampler's initials in the sampling notebook, on the COC, on a label, and on the original AFFF container. It is recommended, but not required, that photographs be taken of the AFFF container clearly showing all information on the label and the unique identifier and of the opening with the seal intact be taken to further document the sampling event.

8.7.3 Break the seal and open the container very slowly to allow for the gradual release of any built-up pressure.

8.7.4 Remove the cap from one of the laboratory supplied sample bottles.

8.7.5 Slowly insert drum thief as deep into the drum as possible, keeping the drum thief in a completely vertical orientation and allow the AFFF in the container to reach natural level in the drum thief.

8.7.6 Cap the top of the drum thief with a tapered stopper or thumb; ensuring liquid does not come into contact with stopper.

8.7.7 Carefully remove the capped drum thief from the AFFF container. Then insert the bottom of the drum thief into the sample bottle.

8.7.8 Release thumb or stopper and allow the drum thief to completely drain into the sample bottle. Repeat as necessary until the bottle is approximately two-thirds full. Do not allow a subsample to overfill the sample bottle.

8.7.9 Dispose of glass drum thief according to the appropriate procedures specified by the facility management.

8.7.10 Reseal the AFFF drum.

8.7.11 Cap the sample bottle tightly and wipe off any excess AFFF from the outside of the bottle with clean, unused paper towels.

8.7.12 Record the time of sampling in the sampling notebook, on the COC, on the drum, and on the label. Place the label on the sample bottle.

8.7.13 Place a custody seal over the cap/bottle interface and then place the sample bottle into a plastic bag and seal.

8.7.14 After all of the AFFF samples have been taken, ensure all other appropriate information has been recorded on the COC, the information recorded on the COC matches the information on the corresponding sample bottle, and the COC has been signed by the personnel performing the sampling. The sampler must retain a copy of the completed COC; however, the original must be included in the shipping container and follow the sample to the laboratory. Package the samples for transport per the laboratory's instructions.

8.8 Sample Preservation, Shipping, and Holding Times

8.8.1 Sample Preservation and Shipping

AFFF samples collected from containers are in concentrated form and do not require any chemical preservation. In addition, these concentrated samples are not subject to excessive thermal degradation or decomposition and once sealed can be kept and transported without thermal preservation. There is currently limited data on whether the PFAS constituents are photosensitive therefore, the sample should be protected from direct sunlight.

8.8.2 Holding Times

No formal Holding Time Studies of PFAS content in AFFF concentrates has been published to date. This method takes a conservative approach, requiring collected samples be prepared within 90 days of collection. AFFF sample extracts must be stored in the dark at less than 4 ºC until analyzed. If stored in the dark at less than 4 ºC, sample extracts may be stored for up to 30 days prior to analysis.

9.0 Quality Control

9.1 Initial Demonstration of Capability

DoD ELAP accreditation for the analysis of PFOA and PFOS in AFFF concentrates in accordance with this method is required for PFOA and PFOS content compliance testing per MIL-PRF-24385. As such, laboratories performing this method must meet the requirements of the DoD/DOE QSM (current version). These requirements include, but are not limited to, those applying to Initial Demonstration of Capability (IDC), detection limit (DL) determination, Limit of Detection (LOD) verification, LOQ verification, reporting requirements, and proficiency testing. The requirements of this method supersede the PFAS-specific ongoing QC requirements contained in the DoD/DOE QSM, Version 5.3, Appendix B, Table B-15.

9.2 Ongoing QC Requirements

This section describes the ongoing QC elements that must be included when processing and analyzing field samples. Table A.1 in Appendix A provides a summary of the acceptance criteria for each QC sample.

9.2.1 Analytical Batch QC Samples

9.2.1.1 Instrument Blank (IB)

IBs (Section 7.2.9) evaluate the background concentrations of method analytes associated with the analytical system. Background concentrations of method analytes must be less than one-half the LOQ. EIS compounds must recover within 50–200% of their true value, and NIS compounds must recover within 50–200% of their true value. Instrument blanks (IBs) (Section 7.2.9) must be analyzed before analysis of the calibration curve, after the highest-level calibration standard, and after CCVs. In addition, IB(s) must be analyzed following samples whose PFOA and/or PFOS concentrations exceed the quantitation range, until method analyte concentrations are less than or equal to one-half the LOQ in an IB. Any samples analyzed before this criteria is achieved, must be re-analyzed using a new aliquot of the final sample extract, if the exceeding method analyte(s) is at a concentration greater than or equal to the LOQ. Subtraction of IB concentrations from sample results is not permitted.

9.2.1.2 Instrument Calibration Verification (ICV)

An ICV (Section 7.2.7) must be analyzed after the analysis of the IB which follows the calibration curve. The acceptance criterion for the ICV is 70–130% of the true value of PFOA and PFOS, EIS compounds must recover within 50–200% of their true value, and NIS compounds must recover within 50–200% of their true value. If this criteria are not met, a fresh ICV should be prepared and analyzed. Analysis of samples cannot proceed until the analysis of an ICV meeting this criterion.

9.2.1.3 Instrument Sensitivity Check (ISC)

An ISC (Section 7.2.6) must be analyzed after each calibration curve and daily, following the first IB of the analytical sequence. The acceptance criteria for the ISC is 70–130% of the true value of PFOA and PFOS EIS compounds must recover within 50–200% of their true value, and NIS compounds must recover within 50–200% of their true value. If these criteria are not met, a fresh ISC should be prepared and analyzed. Analysis of samples cannot proceed until the analysis of an ISC meeting this criterion.

9.2.1.4 Continuing Calibration Verification (CCV)

A CCV (Section 7.2.8) must be analyzed at the beginning of each analysis batch, after every ten field samples, and at the end of the analysis batch. The acceptance criterion for the CCV is 70–130% of the true value of PFOA and PFOS EIS compounds must recover within 50–200% of their true value, and NIS compounds must recover within 50–200% of their true value. If a CCV exceeds this criterion, immediately analyze two additional consecutive CCVs. If both of these CCVs are within the criteria, samples may be reported without re-analysis. If either exceed the criteria, or if two consecutive CCVs cannot be analyzed immediately after the failing CCV, corrective action must be taken. Once correction has been made and a CCV has been analyzed and has met the criteria, all samples bracketed by the failing CCV must be reanalyzed.

9.2.2 Preparation Batch QC Samples

A preparation batch consists of up to 20 AFFF samples (10 unique AFFF samples and their associated duplicates) and preparation batch QC samples that are extracted together using the same lot of each material used (e.g., solid phase cartridge, fortifying solutions, and solvents). The preparation batch QC samples included in each batch must consist of, at a minimum, a Method Blank (MB), Laboratory Control Sample (LCS), Laboratory Control Sample Duplicate (LCSD), and LOQ Verification (LOQVER), and duplicate samples for each AFFF sample included in the batch. PFAS-free reagent water is used as the reference media for the MB, LCS, LCSD, and LOQVER.

9.2.2.1 Method Blank (MB)

The concentration of PFOA and PFOS each must be < one-half the LOQ. If the concentration of PFOA and/or PFOS in the MB exceeds this criterion and the concentration of that analyte(s) is equal to or greater than the LOQ in the AFFF sample, the corresponding AFFF result is not valid and the AFFF sample must be re-extracted. Subtracting blank values from sample results is not permitted.

9.2.2.2 Laboratory Control Sample/Laboratory Control Sample Duplicate (LCSD/LCSD)

The recoveries of PFOA and PFOS in the LCS and LCSD must be within 70–130% of their true value. The relative percent difference (RPD) for both the PFOA and PFOS results for the LCS and LCSD must be ≤ 30%. If these criteria are not met, then all samples in the associated batch must be re-extracted.

9.2.2.3 Limit of Quantitation (LOQ) Verification Sample

The recoveries of PFOA and PFOS in the LCS and LCSD must be within 70–130% of their true value. If these criteria are not met, then all samples in the associated batch must be re-extracted.

9.2.2.4 Sample Duplicate (SD)

For concentration of PFOA and/or PFOS that are equal to or greater than the LOQ, the relative percent difference (RPD) between the sample and the corresponding SD must be ≤ 30%. If this criterion is exceeded, then the sample and associated SD must be re-extracted.

9.2.2.5 Extracted Internal Standard (EIS) Recoveries

The concentration of Extracted Internal Standard (EIS) compounds are quantitated with respect to the non-extracted internal standard (NIS) response. EIS compounds must recover within 50–200% of their true value. If the recovery of EIS compounds falls outside of this range, the sample and associated SD must be re-extracted. If the exceedance is associated with the MB, LCS, LCSD, and/or LOQVER the entire batch must be extracted. Samples bracketed by a calibration verification (ICV, CCV, or ISC) that fail to meet these criteria must be reanalyzed.

9.2.2.6 Non-extracted Internal Standard (NIS) Recoveries

The recovery of the Non-extracted Internal Standard (NIS) compounds is determined by comparing the NIS compound peak areas with the average area of the corresponding NIS in the calibration curve standards. NIS compounds must recover within 50–200% of this

average. If the recovery of a NIS compound falls outside of this range, the sample (and associated SD) must be re-extracted. If the exceedance is associated with the MB, LCS, LCSD, and/or LOQVER the entire batch must be extracted. Samples bracketed by a calibration verification (ICV, CCV, or ISC) that fail to meet these criteria must be reanalyzed.

10.0 Calibration

10.1 Mass Calibration and Mass Calibration Verification

10.1.1 Mass calibration must be performed at least annually and must be repeated on an as-needed basis (e.g., QC failures, ion masses fall outside of the instrument required mass window, major instrument maintenance, or if the instrument is moved) in accordance with procedures prescribed by the manufacturer. The manufacturer's instructions for confirmation of the mass calibration, mass resolution, and peak relative response must be followed and all criteria must be met. The procedures used must evaluate an ion range that encompasses the ion range (Q1 and Q2 m/z) of the analytes of interest and isotopically labeled compounds of this method (Table 2.2).

10.1.2 While the MS conditions used during the development of this method (Table 10.1) are provided below for guidance, suitable operating conditions must be established in accordance with the manufacturer's instructions.

Table 10.1 Operating Conditions for Waters Acquity UPLC, TQ-S Xevo MS/MS

MS/MS Conditions	Source Temp (ºC)	140
	Desolvation Temp (°C)	500
	Capillary Voltage (kV)	0.70
	Cone Gas (L/h)	~70
	Desolvation gas (L/h)	~800

10.1.3 Each mass calibration performed must be followed by a mass calibration verification prior to standards and samples analysis. These verifications must be performed in accordance with manufacturer's instructions. The ion masses evaluated to the manufacturer's acceptance criteria must bracket the masses of interest (quantitative and qualitative ions).

10.2 Chromatographic Conditions

10.2.1 Chromatographic conditions must be optimized for compound separation and sensitivity. Conditions must be kept the same for the analysis of all standards, blanks, QC samples, and AFFF samples. Table 10.2 provides the instrumentation and chromatographic conditions utilized in the development of this method. Operating conditions are dependent on the instrumentation used. The LC gradient program runtime of 12.0 minutes helps to ensure later eluting components found in AFFF samples do not adversely affect the superseding sample analyses. This capability must be demonstrated for the LC gradient program used as part of the optimization process.

Table 10.2 Instrumentation and General LC Operating Conditions			
Instrument	Waters Acquity UPLC, TQ-S Xevo MS/MS or TQ-S Xevo Micro MS/MS, (or equivalent)		
LC Column	Waters Acquity UPLC ® BEH C18 column, 1.7 µm, 50 x 2.1 mm (or equivalent)		
Guard Column	Guard column: Phenomenex Evo C18 Guard (or equivalent)		
Acquisistion	MRM mode, negative ESI, unit resolution		
Injection Volume	2.0 µL (Note: Injection volume will vary with instrument, this volume is provided for the default method only.)		
General LC Conditions			
Column Temp (°C)	40		
Max Pressure (bar)	1100.0		
LC Gradient Program			
Time (min)	**Flow mixture [1,2]**	**Flow Rate Program**	**Gradient Curve**
0.0	2% eluent A, 98% eluent B	0.35 mL/min	Initial
0.2	2% eluent A, 98% eluent B	0.35 mL/min	2
4.0	30% eluent A, 70% eluent B	0.40 mL/min	7
7	55% eluent A, 45% eluent B	0.40 mL/min	8
9	75% eluent A, 25% eluent B	0.40 mL/min	8
10	95% eluent A, 5% eluent B	0.40 mL/min	6
10.4	2% eluent A, 98% eluent B	0.40 mL/min	10
11.8	2% eluent A, 98% eluent B	0.40 mL/min	7
12.0	2% eluent A, 98% eluent B	0.35 mL/min	1

[1] Eluent A = Acetonitrile

[2] Eluent B = 2 mM ammonium acetate in 95:5 water/acetonitrile

10.2.2 Retention Time Calibration

10.2.2.1 The retention time of PFOA, PFOS, and their associated EIS and NIS compounds are provided in Table 2.2. Since retention time is dependent on the columns/mobile phase combination employed, the elution order of PFOA, PFOS, and their associated NIS and EIS compounds must be verified for the combination employed by injecting a series of solutions, each containing a single compound. On the days an initial calibration (ICAL) is performed, the retention time of each compound is established by the retention time of the mid-level standard of the ICAL. On days an ICAL is not performed, the retention time of the initial CCV of the day or the mid-level standard of the ICAL can be used to establish the retention time.

10.2.2.2 The retention time of method analytes and NIS and EIS compounds must fall within 0.4 minutes of the established retention time. On days an ICAL is performed, the RT for each analyte, EIS compound, and NIS compound shall be set using the midpoint standard of the ICAL curve. On days an ICAL is not performed, the initial daily continuing calibration verification for that day or the midpoint standard of the ICAL curve can be used to establish the RT. All branched isomers identified in the PFOS calibration standards and the qualitative PFOA standard must fall within their windows. PFOA and PFOS must elute within 0.1 minutes of their associated EIS compounds.

10.3 Initial Calibration

10.3.1 Initial Calibration Procedure

The most recent mass calibration must be used. Prior to initial calibration (ICAL), confirm the mass calibration used meets all manufacturer's criterion for confirmation of the mass calibration, mass resolution, and peak relative response. ICAL must be performed after a new mass calibration has been performed, whenever the laboratory has taken corrective actions that might affect the initial calibration criteria, and when the acceptance criterion of calibration verifications (ICV, CCV, and ISC) cannot be met. Following the analysis of an IB (Section 7.2.9), analyze each of the calibration standards that were prepared as described in Section 7.2.5. After the analysis of the highest level calibration standard, analyze another IB followed by an ICV. The same injection volume must be used for all standards, AFFF samples, blanks, and QC samples.

10.3.2 Initial Calibration Calculations

PFOA and PFOS are quantified by isotope dilution quantitation, whereby the response of PFOA and PFOS are compared to the response of their associated EIS compounds. Calculate the response ratio (RR) for PFOA and PFOS in each calibration standard using the equation below.

$$RR = \frac{Area_n\, M_l}{Area_l\, M_n}$$

where:

$Area_n$ = The measured area of the Q1 m/z for the native (unlabeled) PFAS

$Area_l$ = The measured area at the Q1 m/z for the corresponding isotopically labeled PFAS added to the sample before extraction

M_l = The mass of the isotopically labeled compound in the calibration standard

M_n = The mass of the native compound in the calibration standard

Calculate the response factor (RF_s) for each EIS in the calibration standard using the equation below.

$$RF_s = \frac{Area_l\, M_{NIS}}{Area_{NIS}\, M_l}$$

where:

$Area_l$ = The measured area of the Q1 m/z for the isotopically labeled PFAS standard added to the sample before extraction

$Area_{NIS}$ = The measured area at the Q1 m/z for the isotopically labeled PFAS used as the non-extracted internal standard (NIS)

M_{NIS} = The mass of the isotopically labeled compound used as the non-extracted internal standard (NIS) in the calibration standard

M_l = The mass of the isotopically labeled PFAS standard added to the sample before extraction

10.3.3 Instrument Linearity

10.3.3.1 To establish acceptable linearity, the calibration must meet one of the following criteria:

Option 1: The calculated relative standard deviation (RSD) of the RR values of the calibration standards for PFOA, PFOS and EIS and NIS compound each must be ≤ 20%.

Option 2: The calculated relative standard error (RSE) of the calibration standards for PFOA, PFOS and EIS and NIS compound each must be ≤ 20%.

Option 3: Linear or non-linear regression calibration must have a coefficient of determination (r^2) that is ≥ 0.99 for PFOA and PFOS both.

10.3.3.2 In addition, to meeting the criteria stated in one of the options listed above, PFOA and PFOS must recover within 70-130% of their true value for each calibration standard included in the calibration curve. These criteria must be met before any AFFF samples, QC samples, additional blanks, or calibration verifications are analyzed.

10.3.4 Initial Calibration Verification

10.3.4.1 IBs analyzed immediately before and after the initial calibration standards must not contain PFOA or PFOS at a concentration ≥ ½ the LOQ in order the calibration to be used. If the IB analyzed after the highest level calibration does not meet this criterion, the instrument must be recalibrated using a series of calibration standards in which the highest level standard is a lower concentration than the one previously used.

10.3.4.2 The ICV must meet the criteria stated in Section 9.2.1.2. If this criterion is not met, corrective action must be taken and the ICV must be reanalyzed. If the ICV continues to fail, initial calibration must be performed again.

11.0 Sample Preparation

11.1 Precautions

Fluoropolymer articles or *Kim-wipes* must not be used during sample preparation. Only HDPE or polypropylene squeeze bottles and centrifuge tubes are to be used. Reagents and solvents for cleaning syringes may be kept in glass containers.

11.2 Subsample Preparation

11.2.1 To prepare the sample, place a 60 mL PFAS-free HDPE sample bottle onto a four decimal place balance and tare the weight. Place an aliquot of approximately 0.02 g of the AFFF sample into the tared sample bottle and record the weight of the sample. This is the sample size.

11.2.2 Add 60 mL of PFAS-free reagent water to the bottle and swirl to mix.

11.2.3 To prepare the SD, repeat Section 11.2.1 and 11.2.2 using a second aliquot of the sample.

11.2.4 Repeat the process in Sections 11.2.1 through 11.2.3 for each AFFF sample to be included in the preparation batch (up to 20 AFFF samples).

11.2.5 To prepare the MB, repeat Section 11.2.1 and 11.2.2 using a 0.02 g of PFAS-free reagent water as the collected sample. Add 60 mL of PFAS-free reagent water to the bottle and swirl to mix.

11.2.6 To prepare the LCS, LCSD, and LOQVER, for each, repeat the process in Section 11.2.1 using a 0.02 g of PFAS-free reagent water as the collected sample. Using a native spiking standard solution, spike the LCS and LCSD at a concentration that is > LOQ and ≤ mid-level calibration standard and the LOQVER at a concentration ≥ LOQ and ≤ 2 times the LOQ. Add 60 mL of PFAS-free reagent water to the bottle and swirl to mix.

11.2.7 Do not continue processing before at least 3 hours has lapsed to ensure the samples are completely dissolved. Some samples may take additional time to dissolve. The dissolution time for QC samples must be the same as the longest dissolution time required for an AFFF sample in the batch. Inspect the samples to ensure that it has dissolved before proceeding. If it is not completely dissolved, record this and notify the client before continuing. The 60 mL HDPE sample bottle is now considered the AFFF sample container.

11.2.8 After the required dissolution time has lapsed (Section 11.2.7), spike an aliquot of EIS solution directly into each of the 60 mL AFFF sample containers. Swirl the samples to mix.

11.2.9 Verify, using pH paper, that the pH of each sample is 6.5 ± 0.5. Adjust the pH if necessary, with 50% formic acid, ammonium hydroxide, or with 5% formic acid and 3% aqueous ammonium hydroxide.

11.3 Sample Extraction and Cleanup

This section applies to all batch QC samples (MB, LCS, LCSD, LOQVER, and SDs) and AFFF samples. Note that the volumes below are associated with validation of the specific cartridge used in validation. 150 mg WAX cartridges from other manufacturers may on different volumes. In addition, laboratories may implement steps to reduce the final extract volume (e.g., by using nitrogen blowdown techniques) to improve sensitivity. Addition of final extract volume reduction steps and changes to the volumes cited in this section are permitted so long as all of the quality control criteria of this method are met.

11.3.1 Collect the required number (one per sample) of Waters Oasis 150 mg WAX SPE cartridges. Pack solvent rinsed silanized glass wool up to half the height of each cartridge barrel. Label each cartridge with a sample identifier. Load the cartridges in the vacuum manifold and add a reservoir and reservoir adaptor to each cartridge.

11.3.2 To pre-condition the cartridges, without using the vacuum, wash the cartridges with 15 mL of 1% methanolic ammonium hydroxide followed by 5 mL of 0.3 M formic acid. Do not allow the cartridge to go dry. Discard the wash solvents.

11.3.3 Pour each sample into its corresponding reservoir (do not use a pipette) while avoiding splashing the sample. Empty the bottle as much as possible. Adjust the vacuum and pass the sample through the cartridge at approximately 5 mL/min. Retain the emptied sample bottle and allow it to air dry. These containers will be rinsed later (Section 11.3.5). Discard the sample being pulled through the cartridge.

11.3.4 Leaving the reservoir in place until after the sample has been eluted, rinse the walls of the reservoir thoroughly with 5 mL reagent water twice (10 mL total) followed by 5 mL of 1:1 0.1M

formic acid:methanol and pass these rinses through the cartridge at a rate of approximately 5 mL/min using vacuum. Dry the cartridge by pulling air through for 15 seconds. Discard these rinses.

11.3.5 Label a 13 x 100 mm polypropylene collection tube (Section 6.9) for each sample and place them in the manifold rack. Make sure extract delivery needles are positioned inside each collection tube but are not touching the walls of the tubes. Rinse the inside of each of the 60 mL sample bottles that were retained in Section 11.3.3 with a total of 5 mL of elution solvent (1% methanolic ammonium hydroxide). Use a glass pipette to transfer the bottle rinse to its corresponding reservoir, washing the walls of the reservoir. Use vacuum to pull the elution solvent through the cartridges and into their corresponding collection tubes. After this step, the sample bottles no longer need to be retained.

11.3.6 Add 25 µL of acetic acid to each collection tube and vortex to mix. Using a 10 mg scoop, add 10 mg of carbon to a sample. Occasionally hand-shake the sample. Hand-shake for no longer than a total of 5 minutes and immediately vortex for 30 seconds and centrifuge at 2800 rpm for 10 minutes.

11.3.7 Label a fresh set of collection tubes. Use a 5 mL polypropylene syringe equipped with a syringe filter (25 mm filter, 0.2 µm nylon membrane) to filter the entire extract into the prepared collection tubes. Add NIS solution to each collection tube and vortex to mix. Transfer a portion into a 1 mL polypropylene microvial for LC-MS/MS analysis. Cap the collection tube with the remaining extract and store at 4˚C.

12.0 Instrumental Analysis

The analysis of sample extracts for PFAS by LC-MS/MS is performed on a high performance or ultrahigh performance liquid chromatograph (HPLC/UPLC) coupled to a triple quadrupole mass spectrometer, running manufacturer's software. The mass spectrometer is run with unit mass resolution in the multiple reaction monitoring (MRM) mode.

12.1 Perform the mass calibration and mass calibration verification (Section 10.1), establish the operating conditions (Section 10.2), and perform the initial calibration (Section 10.3). Samples can be analyzed only after all associated criteria is met. The injection volume of samples (including batch QC samples) must be the same as that of the standards and blanks. Standards and sample extracts must be brought to room temperature and vortexed prior to aliquoting into an instrument vial in order to ensure their homogeneity. Samples must be sequenced for analysis as follows

1. Instrument Blank (IB)
2. Instrument Sensitivity Check (ISC)
3. Continuing Calibration Verification (CCV)
4. PFOA Qualitative Identification Standard
5. Instrument Blank (IB)
6. Method Blank (MB)
7. Limit of Quantitation Verification Sample (LOQVER)
8. Laboratory Control Sample (LCS)
9. Laboratory Control Sample Duplicate (LCSD)
10. Samples (10 or fewer)

11. Sample Duplicates for samples analyzed in within bracket
12. Continuing Calibration Verification (CCV)
13. Instrument Blank
14. Samples (10 or fewer)
15. Sample Duplicates for samples analyzed in within bracket
16. Continuing Calibration Verification (CCV)
17. Instrument Blank (IB)

12.2 All analytical batch QC standards (IB, ISC, and CCV) must meet the acceptance criteria stated in Section 9.2.1 in order for sample results to be reported.

13.0 Data Analysis, Calculations, and Reporting

13.1 Identification of Peaks

AFFF samples may contain both branched and linear isomers of PFOA and PFOS, therefore all isomers must be included in the quantitation of PFOA and PFOS. Retention times are established by the linear isomer (Section 10.2.2.1), however, the retention time window for PFOS must encompass the retention time of the branched isomers identified in the PFOS calibration standards while the retention time window for PFOA must encompass the retention time of the branched isomers identified in the PFOA qualitative standard analyzed after the initial calibration.

PFOA, PFOS, EIS compounds, and NIS compounds are considered identified in IBs, standards, AFFF samples, and QC samples if the following criteria are met.

13.1.1 The peak response must be at least three times the background noise level (S/N 3:1). This criteria applies to both the quantification and confirmation ion peaks. If the S/N ratio is not met but the background is low, then the analyte is to be considered a non-detect.

13.1.2 AFFF samples may contain both branched and linear isomers of PFOA and PFOS, therefore all isomers must be included in the quantification of PFOA and PFOS. The retention time window for PFOS must encompass the retention time of the branched isomers identified in the PFOS calibration standards while the retention time window for PFOA must encompass the retention time of the branched isomers identified in the PFOA qualitative standard analyzed after the initial calibration.

13.1.3 The RTs of the PFOA, PFOS, EIS compound, and NIS compound peaks must fall within ± 0.4 minutes of the predicted RTs from the midpoint standard of the ICAL or initial daily CCV, whichever was used to establish the RT window position for the analytical batch. In addition, PFOA and PFOS peaks must elute within ± 0.1 minutes of the associated EIS. If this criteria is not met, then the analyte is to be considered a non-detect.

13.1.4 The ion ratio for each method analytes (PFOA and PFOS) in each sample (including preparatory batch QC samples) must be determined as the ratio of the total (branched and linear isomers) quantification ion response of the analyte to the total (branched and linear isomer) confirmation ion response of the analyte. This ratio must fall within ±50% of the ion ratio observed in the mid-level initial calibration standard. If the ion ratio failed to meet this criteria, reanalyze to confirm the failure using a fresh aliquot of the extract. If the preparatory batch QC sample failure is

confirmed and ion ratios criteria is met for an associated sample, that sample may be reported. Confirming failures in preparatory batch QC samples and AFFF samples must report data with a "I" data qualifier and must be discussed in the case narrative.

13.2 Quantitative determination

Concentration of PFOA and PFOS are determined with respect to their corresponding EIS compounds. The EIS compound recoveries are determined with respect to their corresponding NIS compounds.

13.2.1 Results for native compounds are recovery corrected by the method of quantification.

To calculate method analytes (PFOA and PFOS) concentrations:

$$Concentration\ (ng/g) = \frac{Area_n\ M_l}{Area_l(\overline{RR}\ )} \times \frac{1}{W_S}$$

where:

$Area_n$ = The measured area of the Q1 m/z for the native (unlabeled) PFAS

$Area_l$ = The measured area at the Q1 m/z for the isotopically labeled PFAS (EIS).

M_l = The amount of the isotopically labeled compound added (ng)

$\overline{RR}$ = Average response ratio used to quantify method analyte by the isotope dilution method

W_S = Sample weight (g)

13.2.2 Extracted internal standard (EIS) recoveries are determined similarly against the non-extracted internal standard (NIS) and are used as general indicators of overall analytical quality.

To calculate EIS compounds ($^{13}C_8$-PFOA and $^{13}C_8$-PFOS) concentrations:

$$Concentration\ (ng/g) = \frac{Area_l\ M_{nis}}{Area_{nis}\overline{RF_s}} \times \frac{1}{W_S}$$

where:

$Area_l$ = The measured area at the Q1 m/z for the isotopically labeled PFAS (EIS)

$Area_{nis}$= The measured area of the Q1 m/z for the non-extracted internal standard (NIS)

M_{nis} = The amount of the added non-extracted internal standard (NIS) compound (ng)

W_S = Sample weight (g)

$\overline{RF_s}$ = Average response factor used to quantify the isotopically labeled compound

13.2.3 If the response of a method analyte exceeds the quantitation range for any sample, extracts must be diluted to bring the exceeding analyte's response within the calibration range, or the sample must be re-extracted for the exceeding analyte using a smaller aliquot of the AFFF sample. Instrument blanks must be analyzed following quantitation range exceedances per Section 9.2.1.1.

13.3 Reporting

13.3.1 Results must be reported in ppb. All associated QC data must be reported with the sample results. Refer to the DoD/DOE QSM, Appendix A for general reporting requirements. Reports must include all documentation needed to facilitate Stage 4 Validation per the *DoD Data Validation Guidelines Module 3: Data Validation Procedure for Per- and Polyfluoroalkyl Substances Analysis by QSM Table B-15* (Reference 17.3).

13.3.2 Report concentrations ≥ the DL to 3 significant figures. If the result falls between the DL and LOQ, report the data with a "J" data qualifier, indicating it is an estimated value. If the method analyte was not detected or the result falls below the DL, report the DL concentration with a "U" data qualifier.

13.3.3 The DL and LOQ values associated with each sample must take into account the volume of sample prepared and any dilutions made. Report the result for each method analyte from the lowest dilution that is within the quantification range that meets the acceptance criteria for EIS and NIS recoveries. Samples with an associated LOQ that is ≥ 25 ppb for PFOA or PFOS cannot be used to demonstrate compliance to the requirements of MIL-PRF-24385.

13.3.4 Results from tests performed with an analytical system that is not in control (i.e., that does not meet acceptance criteria for any QC tests in this method) must be documented and reported (e.g., as a qualifier on results and discussed in the case narrative). Results with associated ion ratio, EIS compound recovery, NIS compound recovery, and SD % RPD acceptance criteria exceedances may not be used to demonstrate compliance to the requirements of MIL-PRF-24385. Samples with an associated LOQ that is ≥ 25 ppb for PFOA or PFOS cannot be used to demonstrate compliance to the requirements of MIL-PRF-24385. All other failures must be evaluated by NAVSEA to determine if the results are acceptable to demonstrate compliance.

14.0 Method Performance

Routine method performance must be monitored through certified reference materials (CRMs), when commercially available. Currently, no such CRMs are available. When QC samples analyzed with samples monitor ongoing method performance.

This method was validated using data generated by NAVSEA's interlaboratory validation study.

15.0 Pollution Prevention

Application of this method must be compliant with all Federal, Provincial/State and Municipal regulations governing waste management, including land disposal restrictions and sewage discharge regulations. All standards are prepared in volumes consistent with volumes required by the method to minimize the disposal of standards. The laboratory safety manual governs the safe storage, labeling and disposal of laboratory wastes.

16.0 Waste Management

Application of this method must be compliant with all Federal, State, and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions,

and to protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Compliance is also required with any sewage discharge permits and regulations.

17.0 References

17.1 Department of Defense/Department of Energy Consolidated Quality Systems Manual for Environmental Laboratories, https://www.denix.osd.mil/edqw/

17.2 Fire Extinguishing Agent, Aqueous Film-Forming Foam (AFFF) Liquid Concentrate, for Fresh and Sea Water, MIL-PRF-24385, current version,

17.3 DoD Data Validation Guidelines Module 3: Data Validation Procedure for Per- and Polyfluoroalkyl Substances Analysis by QSM Table B-15, https://www.denix.osd.mil/edqw/