3M Environmental Laboratory

Method

Determination of Perfluorinated Compounds In Water By Solid Phase Extraction and High Performance Liquid Chromatography/Mass Spectrometry

Method Number: ETS-8-154.4

Adoption Date: 28 Apr 2000

Effective Date: 5/26/11

Approved By:

William K. Reagen
Laboratory Department Manager

26 MAY 2011
Date

1 Scope and Application

This method describes the extraction of perfluorinated compounds (PFCs) from water matrices using solid-phase extraction (SPE) followed by separation, identification, and quantitation using high-performance liquid chromatography tandem mass spectrometry (HPLC/MS/MS). The method is generally applicable but not limited to the measurement of perfluoroalkyl sulfonamides and perfluorinated alkyl acids (PFAAs) such as perfluorosulfonic acids (PFSAs) and perfluorocarboxylic acids (PFCAs) (Table 1). The method is supported by validation by both external standard[1] and internal standard[2] calibration.

Table 1. Representative Target Analytes

Acronym	*Analyte*	*Chemical Abstract Services Registry Number (CASRN)*
PFBA (C4 Acid)	Perfluorobutanoic acid	375-22-4
PFPeA (C5 Acid)	Perfluoropentanoic acid	2706-90-3
PFHxA (C6 Acid)	Perfluorohexanoic acid	307-24-4
PFHpA (C7 Acid)	Perfluoroheptanoic acid	375-85-9
PFOA (C8 Acid)	Perfluorooctanoic acid	335-67-1
PFNA (C9 Acid)	Perfluorononanoic acid	375-95-1
PFDA (C10 Acid)	Perfluorodecanoic acid	335-76-2
PFUnA (C11 Acid)	Perfluoroundecanoic acid	2058-94-8
PFDoA (C12 Acid)	Perfluorododecanoic acid	307-55-1
PFTrDA (C13 Acid)	Perfluorotridecanoic acid	72629-94-8
PFBS (C4 Sulfonate)	Perfluorobutanesulfonic acid	375-73-5
PFHS (C6 Sulfonate)	Perfluorohexanesulfonic acid	355-46-4
PFOS (C8 Sulfonate)	Perfluorooctanesulfonic acid	1763-23-1
FBSA (C4 Sulfonamide	Perfluorobutanesulfonamide	30334-69-1
FOSA (C8 Sulfonamide)	Pefluorooctanesulfonamide	754-91-6

The Minimum Reporting Level (MRL) is the Limit of Quantitation (LOQ) that meets Data Quality Objectives (DQOs) that are developed based on the intended use of this method. The MRLs, established in laboratory Milli Q water as practical limits of quantitation by internal standard calibration for 28 day sample holding times, are 0.050 ng/mL for PFBA, PFPeA and 0.025ng/mL for PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnA, PFDoA, PFBS, PFHS, PFOS.[3] The MRLs, established in laboratory Milli Q water as practical limits of quantitation by internal standard calibration for 14 day sample holding times, are 0.0025 ng/mL for PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnA, PFDoA, PFBS, PFHS, PFOS.[4]

Method Flexibility – This is a performance-based method and may be generally applied to the determination of perfluorinated compounds in water matrices when analysis batch quality control (QC) criteria are met.[5] Each set of samples are prepared in an analysis batch with calibration standards, LCSs, blanks, and continuing calibration check standards analyzed on the same instrument during a time period that begins and ends with the analysis of the appropriate continuing calibration check standards. The laboratory is permitted to modify the SPE technique,

[1] The method is supported by validation with external calibration for perfluorooctane sulfonate (PFOS), perfluorooctane sulfonylamide (FOSA), and perfluorooctanoate (PFOA) in groundwater, surface water, Type I laboratory water, and drinking water. 3M method validation report E01-0454 and analytical method validation E04-0735.

[2] The method is supported by validation with internal standard calibration for C4-C12 PFCAs, C4, C6, and C8 PFSAs, and C8 perfluoroalkane sulfonamide in laboratory Milli-Q water, manufacturing treated effluent water, and ground water. 3M method validation report E09-0321.

the LC column, mobile phase composition, LC conditions, and MS/MS conditions. Method modifications should be considered to improve method performance or to meet data quality objectives for the study. In all cases where method modifications are implemented, the batch analytical QCs (section 9) must be completed and pass QC acceptance criteria (section 13) if the data from the analytical batch are to be reported.

2 Method Summary

Water samples are collected in sample bottles containing appropriate internal standards (ISs) and surrogate recovery standards (SRSs). A 40 mL sample aliquot is loaded onto a Sep Pak Vac 6cc (1g) tC18 solid phase extraction (SPE) cartridge pre-conditioned with methanol and laboratory reagent water.[6] The sample is passed through the SPE cartridge and the cartridge eluted with 5 mL of 100% methanol. An aliquot of the methanol extract is then transferred to an autovial and analyzed using high performance liquid chromatography tandem mass spectrometry (LC/MS/MS). Quantitation is by extracted (SPE) stable isotope internal standard calibration in laboratory reagent water. All PFC target analyte concentrations are reported as anions and corrected for their salt or free acid forms. Alternatively, quantitation may be performed by extracted (SPE) external calibration standard calibration.

3 Definitions

3.1 Analysis Batch

A set of study samples that are prepared with calibration standards, laboratory control samples, and procedural blanks, and analyzed on the same instrument during a time period that begins and ends with the analysis of the appropriate continuing calibration check standards.

3.2 Analytical Sample

A portion of an extracted laboratory sample prepared for analysis.

3.3 Calibration Standard

An aqueous solution prepared by spiking a known volume of the Working Standard (WS) into a predetermined amount of ASTM type I water, HPLC grade reagent water, or other suitable water (i.e. matrix water), and extracting the solution according to this method. The calibration standard solutions are used to calibrate the instrument response with respect to analyte concentration.

3.4 Field Blank (FB)/Trip Blank (TB)

ASTM Type I water, HPLC grade reagent water, or other suitable water, is placed in a sample container in the laboratory and treated as a sample in all respects, including exposure to sampling site conditions, storage, preservation and all analytical procedures. The purpose of the TB is to

[3] MRL section 9.2.5 of EPA Method 537. 3M method validation report E09-0321.
[4] Method modified with 50 ml sample aliquots and 2.5 mL SPE methanol eluant to achieve lower limits of quantitation (0.005 ng/mL).
[5] Guidance for establishing method QC Criteria based on a.) FDA May 2001, "Guidance for Industry, Bioanalytical Method Validation", b.) EPA Method 537, and c.) European Commission: Guidance for Generating and Reporting Methods of Analysis in Support of Pre-registration Data Requirements for Annex II (Part A, section 4) and Annex III (Part A,section 5) of Directive 91/414, SANCO/3029/99 rev. 4 (11/07/00).
[6] This method can be modified with 50 ml sample aliquots and 2.5 mL SPE methanol eluant to achieve lower limits of quantitation (0.0025 ng/mL).

determine if test substances or other interferences are present in the field environment. This sample is referred to as a Trip Blank or Field Blank.

3.5 Field Duplicate Sample (FDS)

A sample collected in duplicate at the same time from the same location as the sample. The FDS is handled under identical circumstances and treated exactly the same throughout field and laboratory procedures. Analysis of the FDS compared to that of the first sample gives a measure of the precision associated with sample collection, preservation and storage, as well as with laboratory procedures.

3.6 Field Matrix Spike (FMS)

A sample to which known quantities of appropriate target analytes, ISs, and SRSs are added to the sample bottle in the field or in the laboratory before the sample bottles are sent to the field. Sample quantities are determined volumetrically or gravimetrically.

3.7 Internal Standard (IS)

A compound added to each study sample, calibration standard, laboratory control samples, and procedural blanks at a consistent level prior to SPE (typically around 1 ng/mL). The internal standard(s) are stable isotope labeled versions of the target analytes. The area count ratio of the target analyte to the internal standard is used for calibration. Surrogate ISs are applied when stable isotope ISs of target analytes are unavailable. A surrogate IS is not necessarily a stable isotope labeled version of the target analyte, but is treated as an internal standard for quantitation.

3.8 Laboratory Control Sample (LCS)

An aliquot of ASTM Type I water (HPLC grade reagent water or other suitable water may used) to which known quantities of the appropriate target analytes, ISs, and SRSs are added in the laboratory prior to SPE. At least two levels in triplicate are included, one generally at the low end of the calibration curve and one near the mid to upper range of the curve. The LCSs are extracted and analyzed exactly like a laboratory sample to determine whether the method is in control. LCSs should be prepared each day samples are extracted.

3.9 Laboratory Duplicate Sample (LDS)

A laboratory duplicate sample is a separate aliquot of a sample, taken in the analytical laboratory that is extracted and analyzed separately with identical procedures. Analysis of LDSs compared to that of the first aliquot give a measure of the precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.

3.10 Laboratory Matrix Spike (LMS)

A laboratory matrix spike is an aliquot of a study sample to which known quantities of appropriate target analytes, ISs, and SRSs are added in the laboratory. The LMS is extracted and analyzed exactly like a laboratory (study) sample to determine whether the sample matrix contributes bias to the analytical results. The endogenous concentrations of the target analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LMS corrected for these concentrations.

3.11 Limit of Quantitation (LOQ)

The lower limit of quantitation (LLOQ) for an analytical batch is the lowest concentration that can be reliably quantitated within the specified limits of precision and accuracy. The LLOQ is generally selected as the lowest non-zero standard in the calibration curve that meets method acceptance criteria. The LLOQ for each target analyte is established for each analysis batch as

the lowest calibration standard with area counts at least twice that of the average area counts of the procedural blanks.

The upper limit of quantitation (ULOQ) for an analytical batch is the highest concentration that can be reliably quantitated within the specified limits of precision and accuracy. The highest standard in the calibration curve that meets method acceptance criteria is defined as the ULOQ.

3.12 Procedural Blank

An aliquot of ASTM Type I water (HPLC grade reagent water or other suitable water may used) that is treated exactly like a laboratory sample including exposure to all glassware, equipment, solvents, and reagents that are used with other laboratory samples. The procedural blank is used to determine if test substances or other interferences are present in the laboratory environment, the reagents, ISs, SRSs, the apparatus, or any part of the extraction and analysis procedure. Procedural blanks may include applicable ISs and SRSs.

3.13 Quality Control Sample (QC)

Appropriate LCSs, FMSs, TBMSs, FDSs, LMSs, procedural blanks, and trip blanks included in the analysis batch.

3.14 Sample

A representative portion or aliquot of a sample received from the field for testing. If applicable, a sample may include known quantities of appropriate ISs and SRSs added to the sample bottle in the field or in the laboratory before the bottles are sent to the field.

3.15 Solid Phase Extraction (SPE) cartridge

A column containing an open solvent reservoir, retaining frit, sorbent bed, retaining frit, and luer tip. The sorbent bed is bonded silica which is designed to selectively retain or elute the compounds of interest depending on the solvent conditions. The compounds of interest can be separated from the water matrices and introduced into an appropriate solvent for analysis.

3.16 Stock Standard Solution (SSS)

A concentrated solution of a single analyte prepared in the laboratory with an assayed reference compound.

3.17 Surrogate Internal Standard

An IS that is not necessarily a stable isotopically labeled target analyte, but is treated as an internal standard for quantitation. Surrogate ISs are used when isotopically labeled counterparts of the target analyte are not commercially or readily available.

3.18 Surrogate Recovery Standard (SRS)

An isotopically labeled standard, not used as an internal standard, that is added to each sample and appropriate QC sample as a means to evaluate the method performance for a chemical class of compounds (e.g., PFSAs, PFCAs).

3.19 Time Period

The Mass Spectrometer Time Period refers to a period of time, defined by the user, in which the mass spectrometer will collect a signal for defined MRM transitions. A single LC/MS/MS run may consist of a singe MRM transition or several MRM transitions. Multiple Time Periods may be used in a single LC/MS/MS run if there are a large number of MRM transitions being monitored. The Time Period is analogous to a retention time window in which an analyte is expected to elute from

the LC column. The number of MRMs within a Time Period must be balanced to ensure suitable peak shape and sensitivity for the analysis.

3.20 Trip Blank Matrix Spike (TBMS)

An aliquot of ASTM Type I water (HPLC grade reagent water or other suitable water may used) to which known quantities of the appropriate target analytes, ISs, and SRSs are added in the laboratory prior to the shipment of the collection bottles. The TBMS is extracted and analyzed exactly like a sample to determine whether a loss of analyte or analytical bias could be attributed to sample holding time, sample storage, and/or shipment issues. A low and high TBMS may be appropriate when expected sample concentrations are not known.

3.21 Working Standard (WS)

A solution of several analytes prepared in the laboratory from SSSs and diluted as needed to prepare calibration standards and other required analyte solutions.

4 Warnings and Cautions

4.1 Health and Safety

The acute and chronic toxicity of the standards for this method have not been precisely determined; however, each should be treated as a potential health hazard.

Unknown samples may contain toxic compounds. Sample containers may be opened in a hood and handled with gloves to prevent exposure.

The laboratory is responsible for maintaining a safe work environment and a current awareness of local regulations regarding the handling of the chemicals used in this method. A reference file of material safety data sheets (MSDS) should be available to all personnel involved in these analyses. Alternatively, MSDS sheets may be located in the corporate or similar commercial database.

4.2 Cautions

The analyst must be familiar with the laboratory equipment and potential hazards including, but not limited too, the use of solvents, pressurized gas and solvent lines, high voltage, and vacuum systems. Refer to the appropriate equipment procedure or operator manual for additional information and cautions.

5 Interferences

During extraction and analysis, be aware of potential contamination sources from reagents and solid-phase extraction devices.

All materials used in the analyses shall be demonstrated to be free from interferences under conditions of analysis by running procedural blanks.

Parts and supplies that contain fluorinated materials or Teflon® should be avoided or minimized due to the possibility of interference and/or contamination. These may include, but are not limited to: wash bottles, Teflon® lined caps, autovial caps, HPLC parts, etc.

The use of disposable micropipettes or pipettes to aliquot standard solutions is recommended to make calibration standards and matrix spikes.

6 Instrumentation, Supplies, and Materials

Note: Brand names, suppliers, and part numbers are for illustrative purposes only. Equivalent performance may be achieved using apparatus and materials other than those specified here. Demonstration of equivalent performance (quality control samples meeting method acceptance criteria) is the responsibility of the laboratory performing the analysis.

6.1 Instrumentation

Balance, analytical (display at least 0.0001g), Mettler

HPLC/MS/MS or HPLC/MS system, as described in Section 10.

6.2 Supplies and Materials.

Sample collection bottles—HDPE (e.g., Nalgene™) wide-mouth bottles with screw cap. **Note:** Do not use fluorinated or Teflon® bottles or lined caps.

Coolers or boxes for sample shipment.

AutoTrace automated SPE workstation, Caliper Life Sciences. A manual SPE manifold with vacuum pump may be used if desired. Supplies for the manual SPE manifold are included below.)

Vacuum pump, Bűchi., or house vacuum supply

Visiprep vacuum manifold, Supelco.

15mL disposable polypropylene centrifuge tubes, VWR.

50mL disposable polypropylene centrifuge tubes, VWR

SPE Cartridges: Sep Pak Vac 6cc (1g) tC18 cartridges (part # WAT 036795), Waters, Water's Oasis HLB 6cc (0.2g) (part # WAT 106202), Phenomenex Strata-S 6 mL (200 mg) (part # 8B-S100FCH) (Cartridges with other amount or types of sorbent materials may be used depending on the data quality objectives of the project.)

15 mL disposable culture tubes (17 x 100 mm), VWR (Cat. No. 60818-626).

Disposable micropipettes (50–100µL, 100–200µL), Drummond.

Class A pipettes and volumetric flasks, various.

HDPE wide-mouth bottles, Nalgene (volumes may vary depending on analytical needs).

2 mL clear HPLC vial kit (cat # 5181–3400), Agilent.

Standard lab equipment (graduated cylinders, disposable tubes, etc.) various.

7 Reagents and Standards

Note: Suppliers and catalog numbers are for illustrative purposes only. Equivalent performance may be achieved using chemicals obtained from other suppliers. Do not use a lesser grade of chemical than those listed.

7.1 Chemicals

Methanol (MeOH), HPLC grade, JT Baker, Catalog No. JT9093–2.

Ammonium Acetate, Reagent grade, Sigma-Aldrich, Catalog No. A–7330.

ASTM Type I Water, prepared in-house (HPLC grade reagent water or other suitable water may also be used)

Sodium Thiosulfate, Reagent grade, JT Baker.

7.2 Representative Target Analytes, ISs, and SRSs

PFBA, Heptafluorobutyric Acid, (C_4 Perfluorinated Acid)

PFPeA, Nonafluoropentanoic Acid (C_5 Perfluorinated Acid)

PFHxA, Perfluorohexanoic Acid (C_6 Perfluorinated Acid)

PFHpA, Tridecafluoroheptanoic Acid, (C_7 Perfluorinated Acid)

PFOA, Ammonium perfluorooctanoate, (C_8 Perfluorinated Acid)

PFNA, Heptadecafluorononanoic Acid, (C_9 Perfluorinated Acid)

PFDA, Nonadecafluorodecanoic Acid (C_{10} Perfluorinated Acid)

PFUnA, Perfluoroundecanoic Acid, (C_{11} Perfluorinated Acid)

PFDoA, Perfluorododecanoic Acid, (C_{12} Perfluorinated Acid)

PFTrDA, Perfluorotridecanoic Acid, (C_{13} Perfluorinated Acid)

FBSA, Perfluorobutanesulfonamide

FOSA, Perfluorooctanesulfonylamide

PFBS, Potassium Perfluorobutanesulfonate

PFHS, Perfluorohexanesulfonate

PFOS, Potassium perfluorooctanesulfonate

PFOA [1,2, 3, 4-^{13}C], $^{13}C_4$-isotopically labeled perfluorooctanoic acid (SRS)

PFOS [1,2, 3, 4-^{13}C], $^{13}C_4$-isotopically labeled Perfluorooctanesulfonate (SRS)

PFUnA [1,2-^{13}C], $^{13}C_2$-isotopically labeled Perfluoroundecanoic acid (SRS)

A custom mix of ISs in a methanolic solution containing ([1,2,3,4-$^{13}C_4$]PFBA, [1,2 -$^{13}C_2$]PFHxA, [1,2,3,4,5,6,7,8-$^{13}C_8$]PFOA, [1,2,3,4,5,6,7,8,9-$^{13}C_9$]PFNA, [1,2 -$^{13}C_2$]PFDA, [1,2,3,4,5,6,7 -$^{13}C_7$]PFUnA, [1,2 -$^{13}C_2$]PFDoA, [1,2,3 -$^{13}C_3$]PFHS, [1,2,3,4,5,6,7,8-$^{13}C_8$]PFOS, and [1,2,3,4,5,6,7,8-$^{13}C_8$]PFOSA (Wellington Laboratories, Guelph, ON) in combination with added [$^{18}O_2$]PFBS can be used to prepare a stock IS solution. Alternatively, individual stable isotope ISs can be used to prepare a stock IS mixture.

Other ISs can be applied.

7.3 Reagent Preparation

250 mg/mL sodium thiosulfate solution — Dissolve 25 g of sodium thiosulfate in 100 mL reagent water.

2 mM ammonium acetate solution (Analysis)—Weigh 0.3 g of ammonium acetate and dissolve in 2.0 L of reagent water.

Note: Alternative volumes may be prepared as long as the ratios of the solvent to solute ratios are maintained.

7.4 Stock Standard Solution (SSS) and Working Standard Solution Preparation

Note: Due to the likely possibility of ester formation between carboxylic acids and alcohols, when preparing stock solutions in methanol, as in this method, it is important that the methanol be treated with an excess of base to catalyze the hydrolysis of any ester products. In this method, when preparing the primary stock solution of 100 ppm of perfluorocarboxylic acid in methanol, the methanol needs to be treated with base to an approximate concentration of 10 uM. If stock solutions are created at a higher concentration, then the concentration of NaOH should be scaled up appropriately. Upon making dilutions of the primary stock solutions, it is not necessary to use caustic methanol, as the base will still be present in excess to the acids.

Note: At the time of writing of this SOP, reference material solution available from Wellington Labs are prepared in caustic methanol. Solutions prepared from these purchased solutions do not need to be treated with NaOH.

The following standard preparation procedure serves as an example. Weighed amounts and final volumes may be changed to suit the needs of a particular study. For example, µL volumes may be spiked into volumetric flasks when diluting stock solutions to appropriate levels.

100 µg/mL target analyte SSSs—Weigh out 10 mg of analytical standard ***(corrected for percent salt, acid [ETS-4-031] and purity*)** and dilute to 100 mL with methanol or other suitable solvent, in a 100 mL volumetric flask. Transfer to a 125 mL HDPE bottle or other suitable container. Prepare a separate solution for each analyte. Expiration dates and storage conditions of stock solutions should be assigned in accordance with laboratory standard operating procedure. An example of purity and salt correction is given below for PFOS.

$$\text{salt correction factor} = \frac{\text{molecular weight of anion}}{\text{moclecular weight of salt}}$$

$$\text{PFOS } (K^+)\text{salt correction factor} = \frac{499}{538} = 0.9275$$

10 mg $C_8F_{17}S0_3^-K^+$ with purity 90% = 8.35 mg $C_8F_{17}S0_3^-$ (10 mg*0.90*0.9275=8.35 mg)

Note: For ease of solution preparation and calculations, it may be advisable to calculate the mass of reference material needed to result in a final SSS of 100 ug/mL after applying all relevant salt, acid and purity correction factors.

10 µg/mL (10,000 ng/mL) mixed working standard—Add 5.0 mL each of the 100 µg/mL SSSs to a 50 mL volumetric flask and bring up to volume with solvent.

1 µg/mL (1,000 ng/mL) mixed working standard—Add 0.5 mL of the 100 µg/mL SSSs to a 50 mL volumetric flask and bring up to volume with solvent.

0.1 µg/mL (100 ng/mL) mixed standard—Add 0.05 mL of the 100 µg/mL SSSs to a 50 mL volumetric flask and bring up to volume with solvent.

Storage Conditions—Store all SSSs and working standards in accordance with laboratory standard operating procedure or in a refrigerator at 4°± 2°C for a maximum period of 6 months from the date of preparation.

7.5 Calibration Standards

Calibration can be performed by SPE extracted IS or external calibration. Using the working standards described above, prepare calibration solutions in ASTM Type I water, or other suitable water, using the following table as a guideline. Note: Volumes of water and working standards

may be adjusted to meet the data quality objectives addressed in the general project outline. Table 1 provides examples of spike concentrations and volumes used to achieve a multi-point extracted calibration curve. Calibration levels other than those listed in Table 1 can be prepared as needed.

Table 1. Example Preparation of Extracted Calibration Curve

Concentration of WS, µg/mL	*Volume of WS, µL*	*Final Volume of Calibration Standard (mL of ASTM Type I Water, or other suitable water)*	*Final Concentration of Calibration Standard, ng/mL (ppb) in ASTM Type I Water, or other suitable water*
0.1	10	40	0.025
0.1	20	40	0.050
0.1	40	40	0.100
0.1	100	40	0.250
1.0	20	40	0.500
1.0	40	40	1.00
1.0	100	40	2.50
10.0	20	40	5.00
10.0	40	40	10.0
10.0	60	40	15.0
10.0	100	40	25.0

The calibration standards are processed through the entire SPE procedure (Section 11), identical to the laboratory samples. The concentration of the calibration standard in the final extract depends on the volume extracted and the final elution volume.

Final sample concentration factor = Volume extracted (mL)/Elution Volume (mL)

Storage Conditions— Store all extracted calibration standards in 15 mL polypropylene tubes or in labeled autovials at 4°±2°C. After analysis, archive all extracted standards with the sample extracts in accordance with laboratory standard operating procedures.

7.5.1 Internal Standard (IS) and Surrogate Recovery Standard (SRS)

For IS calibration, stable isotope internal standards of each target analyte or appropriate surrogate ISs should be spiked prior to SPE at the same level in all samples, calibration standards, procedural blanks, and LCSs. Once the calibration standards have been prepared as stated above in Section 7.5, all calibration standards are spiked with a separate internal standard spiking solution. Typically the concentration of the internal standard is consistent with the internal standard concentration expected in the samples being prepared, usually 1 ng/mL. The concentration of the internal standard spiking solution is typically 2 µg/mL.

If the sample being extracted was pre-spiked with SRSs, the calibration curve prepared in Section 7.5 is spiked with a separate SRS spiking solution. Typically, the sample bottles are spiked with a SRS at 0.1 ng/mL. The final calibration curve must consist of at least six calibration points after analysis. The following table provides an example of spike concentrations and volumes used to achieve a multi-point extracted calibration curve with internal standard and surrogate standard.

Table 2 lists recommended stable isotope internal standards for several PFSA and PFCA target compounds. A custom mix of isotopically labeled target analytes in a methanolic solution containing ([1,2,3,4-$^{13}C_4$]PFBA, [1,2 -$^{13}C_2$]PFHxA, [1,2,3,4,5,6,7,8-$^{13}C_8$]PFOA, [1,2,3,4,5,6,7,8,9-

$^{13}C_9$]PFNA, [1,2,3,4,5,6 -$^{13}C_6$]PFDA, [1,2,3,4,5,6,7 -$^{13}C_7$]PFUnA, [1,2 -$^{13}C_2$]PFDoA, [1,2,3-$^{13}C_3$]PFHS, [1,2,3,4,5,6,7,8-$^{13}C_8$]PFOS, and [1,2,3,4,5,6,7,8-$^{13}C_8$]FOSA (Wellington Laboratories, Guelph, ON) in combination with added ([1,2,3,4,5-$^{13}C_5$]PFPeA, ([1,2,3,4-$^{13}C_4$]PFHpA, and [$^{18}O_2$]PFBS can be used to prepare a stock IS solution. Alternative sources of certified stable isotope labeled target analytes are applicable. Alternatively, individual stable isotope ISs can be used to prepare a stock IS mixture. The table below lists the recommended stable isotope ISs and SRSs applied in the method. Other stable isotope ISs and SRSs of target analytes not listed in the table may be used if supported by validation and/or analysis batch QCs meeting method acceptance criteria (e.g., [$^{13}C_2$]-PFOA). The same internal standard should be used for a given analyte throughout the entire project/study. Note: some of the compounds listed below are appropriate to use as surrogate ISs when a stable isotope IS of a target analyte is not available. Generally, surrogate isotopically labeled PFCAs are used for PFCAs, and surrogate isotopically labeled PFSAs are used for PFSAs.

Table 3 provides examples of spike concentrations and volumes used to achieve a multi-point SPE extracted calibration curve with ISs and SRSs.

Table 2. Stable Isotope PFCAs and PFSAs used for ISs and SRSs

Compound Name	*Synonym or Acronym*	*Analytical Purpose*	*Reference Standard Source*
$^{13}C_4$-Perfluorobutanoic acid	[1,2,3,4-$^{13}C_4$]PFBA	IS for PFBA	Wellington Labs (Mix or Individual)
$^{13}C_4$-Perfluoropentanoic acid	[1,2,3,4,5-$^{13}C_5$]PFPeA	IS for PFPeA	Wellington Labs (Mix or Individual)
$^{13}C_2$-Perfluorohexanoic acid	[1,2 -$^{13}C_2$]PFHxA	IS for PFHxA	Wellington Labs (Mix or Individual)
$^{13}C_4$-Perfluoroheptanoic acid	[1,2,3,4-$^{13}C_4$]PFHpA	IS for PFHpA	Wellington Labs (Mix or Individual)
$^{13}C_8$-Perfluorooctanoic acid	[1,2,3,4,5,6,7,8-$^{13}C_8$]PFOA	IS for PFOA and [1,2,3,4 $^{13}C_4$]PFOA	Wellington Labs (Mix or Individual)
$^{13}C_9$-Perfluorononanoic acid	[1,2,3,4,5,6,7,8,9-$^{13}C_9$]PFNA	IS for PFNA	Wellington Labs (Mix or Individual)
$^{13}C_6$-Perfluorodecanoic acid	[1,2,3,4,5,6 -$^{13}C_6$]PFDA	IS for PFDA	Wellington Labs (Mix or Individual)
$^{13}C_7$-Perfluoroundecanoic acid	[1,2,3,4,5,6,7 -$^{13}C_7$]PFUnA	IS for PFUnA	Wellington Labs (Mix or Individual)
$^{13}C_2$-Perfluorododecanoic acid	[1,2 -$^{13}C_2$]PFDoA	IS for PFDoA, *PFTA	Wellington Labs (Mix or Individual)
$^{18}O_2$-Ammonium Perfluorobutane sulfonate	[$^{18}O_2$]PFBS	IS for PFBS	RTI International (Individual)
$^{13}C_3$-Ammonium Perfluorohexane sulfonate	[1,2,3-$^{13}C_3$]PFHS	IS for PFHS	Wellington Labs (Mix or Individual)
$^{13}C_8$-Sodium Perfluorooctane sulfonate	[1,2,3,4,5,6,7,8-$^{13}C_8$]PFOS	IS for PFOS and PFOS[1,2,3,4 $^{13}C_4$],	Wellington Labs (Mix or Individual)
$^{13}C_8$-Perfluorooctanesulfonamide	[1,2,3,4,5,6,7,8-$^{13}C_8$]FOSA	IS for FOSA	Wellington Labs (mix) RTI International (Individual)
$^{13}C_4$-Perfluorooctanoic acid	[1,2,3,4-$^{13}C_4$]PFOA	SRS for all PFCAs C4-C8	Wellington
$^{13}C_2$-Perfluoroundecanoic acid	[1,2 -$^{13}C_2$]PFUnA	SRS for all PFCAs C9-C13	Wellington
$^{13}C_8$-Perfluorooctane sulfonate	[1,2,3,4-$^{13}C_4$]PFOS	SRS for all PFSAs C4, C6, and C8, and FOSA	Wellington

*No isotopically labeled counterpart for PFTrDA is currently commercially available. The labeled PFDoA can be used as the surrogate IS for PFTrDA.

Table 3. Example Preparation of SPE Extracted Calibration Curve with ISs and SRSs

Sample Description	*Concentration of WS, µg/mL*	*Volume of WS, µL*	*Volume of IS (2 µg/mL), µL*	*Concentration of Surrogate, µg/mL*	*Volume of Surrogate, µL*	*Volume of ASTM Type I Water, or other suitable water)*
0.025 ppb extracted curve point	0.1	10	20	0.1	10	40
0.03 ppb extracted curve point	0.1	12	20	0.1	12	40
0.05 ppb extracted curve point	0.1	20	20	0.1	20	40
0.1 ppb extracted curve point	0.1	40	20	0.1	40	40
0.25 ppb extracted curve point	0.1	100	20	0.1	100	40
0.5 ppb extracted curve point	1.0	20	20	1.0	20	40
1.0 ppb extracted curve point	1.0	40	20	1.0	40	40
2.5 ppb extracted curve point	1.0	100	20	1.0	100	40
5.0 ppb extracted curve point	10.0	20	20	10.0	20	40
10 ppb extracted curve point	10.0	40	20	10.0	40	40
15 ppb extracted curve point	10.0	60	20	N/A	N/A	40
25 ppb extracted curve point	10.0	100	20	N/A	N/A	40

N/A - Not Applicable

8 Sample Collection and Bottle Preparation

Sample collection bottles are prepared by 3M Environmental Laboratory (or subcontract supplier) personnel for shipment at ambient temperature to the collection site. Typically, four separate collection bottles are associated with a single collection site: sample, field duplicate sample, low field matrix spike, and high field matrix spike. Alternatively, the sample and field duplicate sample may contain SRSs in lieu of additional target analyte low field matrix spike and target analyte high field matrix spike samples. Depending on the scope of the project, additional replicates of the field sample and field matrix spikes may be added. Also, it is not uncommon for additional mid-level field matrix spikes to be collected if the expected sample concentrations are truly unknown or could span a large concentration range.

High-density polyethylene (HDPE) wide-mouth Nalgene bottles are used for the sample collection containers. (Volumes of the bottles may vary depending on how much sample is required to meet data quality objectives.) The interiors of the Nalgene bottles may be rinsed multiple times with acetone and methanol and allowed to dry before adding the appropriate spikes. Note: rinsing of the bottles is optional and is not required. Sample collection volumes are project specific and based on data quality objectives. Typically, placement of a sample bottle volumetric "fill to here" line is done by using a sample bottle marker template. Alternatively, bottles may be weighed prior to bottle preparation and weighed again after samples have been collected.

All bottles should be clearly labeled to indicate its intended use as a sample, field sample duplicate, low field matrix spike, high field matrix spike, sample/SRS field matrix spike, field duplicate sample/SRS field matrix spike, trip blank, or trip blank matrix spike. If each location has different designated spike levels, the label should also clearly indicate the sample location designation. Generally, a set of bottles for a given collection site are prepared then grouped together in plastic bags for organizational purposes. For each sample collection event, at least one set of trip blank and trip blank matrix spikes are prepared.

Bottle preparation should be documented in a Note to File or on a sample preparation worksheet and should include the following information: date prepared, total number of bottles prepared, number of sample sites, the standard identification numbers and spike volumes used to prepare spiked bottles, the "fill to here" volume, and any other pertinent information needed for reconstructibility of the data. The Note to File will be included in the final data package for the project.

Samples are collected in the field and shipped to the laboratory at ambient temperature.

8.1 Field Matrix Spike Sample (FMS)

Field matrix spike samples are a requirement of the method. A FMS sample is defined as a QC sample to which known quantities of appropriate target analytes are added to the sample bottle in the field or in the laboratory before the bottles are sent to the field. The sample and field duplicate sample may contain appropriate SRSs in lieu of target analyte FMS samples. Sample quantities are determined volumetrically or gravimetrically. A known, specific volume or weight of sample is added to the sample container without rinsing. Volumetric sample measurements may be acquired by a laboratory applied "fill to this level" line on the outside of the sample container. Target analyte FMS samples should be spiked at approximately 0.5-10 times the expected analyte concentration in the sample. If the expected range of analyte concentrations is unknown, multiple spikes at varying levels may be prepared to increase the likelihood that a spike at an appropriate level is made. Typically a low and a high target analyte spike are prepared for each sampling location. In those instances where SRSs are to be used in lieu of target analyte FMS samples, the sample and field duplicate sample are spiked at approximately 2-5 times the target LOQ. The FMS is analyzed to ascertain if matrix effects or sample holding time contributes bias to the analytical results. For the sample bottles designated for matrix spikes, an appropriate

volume of matrix spiking solution is added to the empty bottle prior to sampling. The volume of spike solution added should produce the desired final concentration of target analytes once the bottle is filled with sample to the "fill to here line". The matrix spiking solution(s) should be prepared in a suitable solvent and contain all of the appropriate target analytes, ISs, and SRSs. The target analyte matrix spiking solution is often the same as the working standards used to create the calibration standards. An example of a bottle spike is given below.

"Fill to here" volume = 200 mL (A 250 mL Nalgene bottle is used)

Desired Field Spike Concentration = 0.25 ng/mL

500 μL of a 0.1 μg/mL spiking solution (containing the target analytes) is added to the bottle and the bottle cap promptly sealed.

8.2 Internal Standard and Surrogate Recovery Standard

If analysis of a surrogate recovery standard (SRS) is included in the project objectives, an appropriate volume of a surrogate standard solution is added to all the bottles prior to sampling and SPE. Typically sample bottles are spiked with surrogate recovery standards at a final desired spike concentration of 0.1 ng/mL.

If quantitation by internal standard (IS) is included in the project objective, an appropriate volume of internal standard solution is added to all the bottles prior to sampling and SPE. Typically sample bottles are spiked with internal standard at a final desired spike concentration of 1 ng/mL.

For the trip blank, the SRS spike and IS spike is added to the bottle and then ASTM Type I water (HPLC grade reagent water or other suitable water may used) is added to the "fill to here" line. The bottle is capped and sealing tape may be placed around the outer edge of the cap. Trip blank matrix spikes are prepared by adding the appropriate volume of target analyte spiking solution, IS, and SRS spiking solutions and filling the bottle to the desired volume with the appropriate water and capping and sealing the cap.

9 Quality Control and Data Quality Objectives

Analytical results of QC samples (i.e.TBMS, FMS, LMS, and FDS) should be evaluated at the conclusion of the study to help interpret the quality and acceptability of sample data. Analytical results for these QCs must be reported with the sample data.

9.1 Solvent Blanks

Solvent blanks are analyzed with each sample set to determine contamination or carryover. In general, solvent blanks should have area counts that are less than 50% of the area count of the lowest calibration standard.

Solvent blanks should be analyzed prior to and following each calibration curve, each set of system suitability samples, and after no more than 10 unknown sample extracts. If instrument carryover is a problem, consecutive solvent blanks may be necessary. In this case, the area counts of the solvent blanks should return to <50% of the lowest calibration standard prior to the injection of further standards or samples.

9.2 Procedural blanks

A procedural blank consists of an aliquot of ASTM Type I water (HPLC grade reagent water, or other suitable water may be used) equal in volume to the samples, and extracted in the same manner as the samples. At least five procedural blanks should be prepared and analyzed each day that extractions are performed for an analysis batch. Procedural blanks must be interspersed throughout the analysis batch and analyzed interspersed throughout the analytical sequence. For

IS calibration, procedural blanks should include ISs. Procedural blanks may include appropriate SRSs.

The average area counts or area ratios when using internal standard calibration, for each analyte must be less than 50% of the area counts or area ratios when using internal standard calibration, of the LOQ standard. The standard deviation of the area counts, or area ratios when using internal standard calibration, should be calculated. A specific %RSD acceptance criteria is not specified but is assessed on an analytical batch basis. If the mean area counts or area ratios when using internal standard calibration, of the procedural blank exceed 50% of the LOQ standard, then the LOQ must be raised to the first standard level in the curve that meets criteria. Procedural blanks may be eliminated if technical justification can be provided (e.g. the procedural blank was analyzed after an unexpectedly high level sample). If any procedural blanks are removed from the LOQ determination, document in the raw data and report as appropriate.

9.3 Laboratory Sample Replicates / Field Duplicate Sample

Depending on the scope of the project, all or selected samples may be extracted in duplicate, and in triplicate if difficulties were encountered in the sampling and/or holding conditions of the samples. If field sample replicates are collected, duplicate and triplicate extractions of an individual sample may not be required. The relative percent difference (RPD) of duplicate samples or relative standard deviation (RSD), should be less than 20% for the precision of sample preparation and analysis to be considered in control. Replicate samples not meeting the 20% RPD criteria are flagged and reported as outside of QC acceptance criteria.

9.4 Laboratory Matrix Spikes (LMSs)

LMSs may be performed in lieu of FMSs if FMSs have previously been performed for the sample matrix. Additionally, LMSs may be performed in lieu of FMSs for a sample matrix if the FMS levels were not appropriate for determining spike recoveries relative to endogenous levels of target analytes and appropriate SRSs. Generally, each sample location represents a different sample and sample matrix. LMSs are prepared for each sample and analyzed to determine the matrix effect on spike recovery efficiency of each target analyte and appropriate SRSs. LMSs should be prepared at a minimum of one level and in duplicate. LMS concentrations should be prepared at approximately 0.5-10 times the endogenous concentration or approximately 4-10 times the LOQ concentration of each target analyte.

Lab matrix spike recoveries should fall within ± 30% of expected values. Sample data with LMS recovery outside of ±30% but within ±50% of the expected value are flagged and reported as outside of QC acceptance criteria. Data with LMS recovery outside of ±50% of the expected value are reported as NR, where NR is defined as “Not Reportable” data outside of QC acceptance criteria.

9.5 Laboratory Control Spike (LCS)

Lab control spikes are prepared for each analysis batch to determine method accuracy and precision. LCSs should be prepared at three levels in triplicate for each target analyte and at a minimum of two levels in triplicate for appropriate SRSs. Low lab control spikes should be prepared at a concentration in the range of approximately four to ten times higher than the targeted lower LOQ, the mid lab control spikes should be prepared at a concentration near the mid-point of the calibration curve and the high lab control spikes at approximately 80% of the upper LOQ. For each target analyte and SRSs, the percent relative standard deviation (method precision) for each control spike level must be less than or equal to 20% and the average recovery (method accuracy) for each control spike level must be 80-120%. Sample data for target analytes outside of the laboratory control spike acceptance criteria will be handled as follows:

If the average recovery of a spiking level falls outside method acceptance, but at least 67% (6 out of 9) of LCS samples are within 20% of their respective nominal value (33% of the QC samples, not all replicates at the same concentration, may be outside 20% of nominal value), the average recovery will be flagged as outside method acceptance criteria. All LCS samples will be control charted as per ETS-12-012. If the average recovery of one of the spiking levels exceeded the analytical method uncertainty as determined by ETS-12-012, that analytical batch uncertainty will be expanded for that particular study.

If more than 33% of the LCS samples fail to meet method acceptance criteria, the data will not be reported.

For PFOS/PFOA target analytes, at least one level of triplicate LCSs should be prepared using PFOS/PFOA which contains a mix of linear and branched isomers. These LCSs will be used to demonstrate quantitative equivalency (or quantitative bias) of the isomeric mix when using a predominantly linear standard for calibration. The mixed linear and branched isomer PFOS/PFOA LCSs recoveries should fall within ± 30% of expected values. Alternatively, mixed branched and linear isomer PFOS/PFOA calibration may be applied. Alternatively, in lieu of mixed branched and linear isomer PFOS/PFOA LCSs, mixed branched and linear isomer PFOS/PFOA TBMSs may be applied to demonstrate method accuracy and precision.

9.6 Field Matrix Spikes (FMSs)

FMSs are prepared for each sampling location and analyzed to determine the matrix effect and sample holding time on the spike recovery of each target analyte and/or appropriate SRS. Generally, each sample location represents a different sample and sample matrix.

FMSs are QC samples to which known quantities of appropriate target analytes are added to the sample bottle in the laboratory before the bottles are sent to the field. Typically a low and a high target analyte FMS are prepared for each sampling location. The sample and field duplicate sample may contain appropriate SRSs in lieu of target analyte low field matrix spike and target analyte high field matrix spike samples. The ratio of endogenous analyte to field spike concentration that is appropriate to assess accuracy is defined as approximately 0.5 to 10 times the expected sample concentration. For example, if the endogenous level of analyte in the sample is expected to be 1.0 ng/mL, the appropriate range for target analyte FMS used to assess accuracy of results would be approximately 0.5 ng/mL to 10 ng/mL. For samples that are expected to have endogenous analytes present at or below the targeted LOQ, the appropriate range for target analyte FMS would be approximately 4 to 10 times the LOQ concentration. For example, if the analyte LOQ is 0.025 ng/mL, the appropriate range for low level FMS would be 0.1 ng/mL to 0.25 ng/mL. If the expected range of analyte concentrations is unknown, multiple spikes at varying levels may be prepared to increase the likelihood that a spike at an appropriate level is made. In those instances where SRSs are to be used in lieu of target analyte FMS samples, the sample and field duplicate sample are spiked at approximately 2-5 times the target LOQ.

The concentration range for a target analyte FMS of 0.5 to 10 times the expected sample concentration is only a guideline, with more emphasis being placed on the 0.5 times the expected concentration spike. If the spike level is greater than 10 times the expected concentration, consideration will be given on a case-by-case basis for acceptance of the spike level. Documentation in the raw data and report are sufficient, without need of an SOP deviation.

Field matrix spike method acceptance criteria are recoveries within ±30% of the expected value. If FMS recovery (target analyte or SRS spike) is outside of ±30% of the expected value or could not be assessed because the FMS (target analyte) was spiked at an inappropriate level, the sample result is reported as follows:

1.) If target analyte FMS recovery could not be assessed because the FMS's were at an inappropriate level, then Laboratory Matrix Spikes (LMS) may be substituted. If LMS recoveries

are within ±30% the data are reportable and flagged to indicate that the FMS spikes levels were inappropriate.

2.) If multiple target analyte FMS's were prepared on a sample and the closest FMS level to the reported sample meets the ±30% acceptance criteria but additional FMS's are outside the ±30% acceptance range, the data are reportable and flagged to indicate that while there were failing FMS's, the uncertainty will not be expanded since the most appropriate spike level passed.

3.) If the target analyte FMS recoveries are outside of the ±30% acceptance range but at least 30 acceptable historical reportable FMS sample results are available, the data may be reported but flagged with an expanded uncertainty and as not meeting FMS criteria.

4.) Sample data with FMS recovery outside of ±30% but within ±50% of the expected value are flagged and reported as outside of QC acceptance criteria with an expanded uncertainty.

5.) If FMS recovery is outside of ±50%, the sample result is reported as NR, where NR is defined as "Not Reportable" due to noncompliant QC results.

For data reportability, a sample may be re-extracted and re-analyzed or an alternate analytical method may be applied to the sample. Alternatively, re-sampling and re-analysis of a new sample may be completed. If re-extraction, re-sampling, and re-analysis fail to meet the FMS acceptance criteria, the sample result will be reported as "NR" (not reported due to noncompliant QC results).

Note: It is possible for bottles utilized for Field Matrix Spike samples to be under-filled or over-filled during sample collection. Since this scenario will effect the actual concentration of the FMS sample (surrogate and internal standard concentrations will also be effected, if used), it is important that any obvious under-filling or over-filling of sample bottles be documented in the data package and taken into account in the FMS, ISs, or SRSs recovery calculations. Study E07-0813 determined the effect of under-filling and over-filling of sample bottles on the true volume of these samples. From this study, when measured utilizing a ruler, we conclude that for 200 mL samples collected in 250 mL bottles, a deviation of 0.5 cm from the top of the "fill-to-here" line results in an error of approximately 10%. For 450 mL samples collected in 500 mL bottles, an error of 1.0 cm from the top of the "fill-to-here" line results in an error of approximately 11%.

Therefore, based on these results, the analyst will need to measure with a ruler, from the top of the "fill-to-here" line, the level of each sample upon receipt. Documentation of this will be made on either the sample chain of custody or on the sample preparation worksheet. As there is no effect on concentration on study samples that are not spiked in the original sample collection bottle, there is no need to monitor the sample volumes in any non-spiked samples. For 200 mL samples collected in 250 mL bottles and 450 mL samples collected in 500 mL bottles, if the deviation is ± 0.5 cm or ± 1.0 cm, respectively, it will be necessary to accurately determine the true volume of the sample. A linear curve plotting sample filling height (in cm) versus volume of sample in a 250 mL bottle was determined in study E07-0813. The true volume can be determined by applying the equation of the line to the fill height. The true volume will be used to correct these FMS, ISs and SRSs actual concentrations when calculating recovery results for any sample overfilled by more than 10%. Alternatively, bottles may be weighed prior to bottle preparation and weighed again after samples have been collected.

10 Calibration and Standardization

10.1 Instrument Setup

Note: In this example, an Applied Biosystems Sciex API 4000 (API 5000 or API 5500) Tandem Mass Spectrometer (LC/MS/MS) is used. Other brands/models of LC/MS/MS instruments as well as single quadrupole mass spectrometers (LC/MS) may be used as long as the method

acceptance criteria are met. Brand names, suppliers, part numbers, and models are for illustrative purposes only. Equivalent performance may be achieved using apparatus and materials other than those specified here, but demonstration of equivalent performance that meets the requirements of this method is the responsibility of the laboratory. The operator must optimize and document the equipment and settings used.

Establish the LC/MS/MS system and operating conditions equivalent to the following:

Mass Spec: Applied Biosystems API 4000, API 5000, or API 5500

Ion Source: Turbo Ion Spray (ABS)

Mode: Electrospray Negative

Scan Type: MRM (Multiple Reaction Monitoring)

Harvard infusion pump (Harvard Instruments), for tuning

Computer: Dell DHM

Software: Windows 2000 or Windows XP, Analyst 1.4.2 or higher versions

HPLC: Agilent Series 1100,1200, or 1290

Agilent Quaternary Pump

Agilent Vacuum Degasser

Agilent Autosampler

Agilent Column Oven

Note: One or more C18 HPLC analytical columns (2.1 mm x 100 mm, 5μm or 2.1 mm x 50 mm, 5μm) may be attached on-line after the purge valve and before the sample injection port to retard and separate any residue contaminants that may be in the mobile phase and/or HPLC system.

HPLC Column: Betasil C18, 2.1mm x 100mm, 5μm (ThermoElectron Corporation)

Column Temperature: 35°C

Injection Volume: 5μL

Mobile Phase (A): 2mM Ammonium Acetate in ASTM Type I water (See 7.3)

Mobile Phase (B): Methanol

Table 4. **Liquid Chromatography Gradient Program**.

Step Number	*Total Time (min)*	*Flow Rate (μL/min)*	*Percent A (2 mM ammonium acetate)*	*Percent B (Methanol)*
0	0	300	90.0	10.0
1	2.0	300	90.0	10.0
2	14.5	300	10.0	90.0
3	15.5	300	10.0	90.0
4	16.5	300	90.0	10.0
5	20.0	300	90.0	10.0

Note: Other HPLC gradients may be used as long as the method criteria and project data quality objectives are met.

It may be necessary to adjust the HPLC gradient in order to optimize instrument performance. Columns with different dimensions (e.g. 2.1mm x 30mm) and columns from different manufacturers (Keystone Betasil C18 etc.) may be used.

Table 5. Suggested MRM Transitions for Target Analytes, Surrogates, and Internal Standards

Analyte	*Analyte Description*	*Mass Transition Q1 (amu)*	*Mass Transition Q3 (amu)*
PFBA (C4 Acid)	Target	213	169
PFPeA (C5 Acid)	Target	263	219
PFHxA (C6 Acid)	Target	313	269, 119
PFHpA (C7 Acid)	Target	363	319, 169
PFOA (C8 Acid)	Target	413	369, 219, 169
PFNA (C9 Acid)	Target	463	419, 169, 219
PFDA (C10 Acid)	Target	513	469, 269, 219
PFUnA (C11 Acid)	Target	563	519, 269, 219
PFDoA (C12 Acid)	Target	613	569, 169, 319
PFTA (C13 Acid)	Target	663	619, 369, 319
FBSA (C4 Sulfonamide)	Target	298	78
FOSA (C8 Sulfonamide)	Target	498	78
PFBS (C4 Sulfonate)	Target	299	99, 80
PFHS (C6 Sulfonate)	Target	399	99, 80
PFOS (C8 Sulfonate)	Target	499	80, 99, 130
THPFOS	Target	427	407, 81
THPFDS	Target	527	507, 81
[1,2,3,4 -$^{13}C_4$]PFBA	IS for PFBA	217	172
[1,2,3,4,5 -$^{13}C_5$]PFPeA	IS for PFPeA	268	223
[1,2 -$^{13}C_2$]PFHxA	IS for PFHxA	315	270
[1,2,3,4- $^{13}C_4$]PFHpA	IS for PFHpA	367	322
[1,2,3,4,5,6,7,8-$^{13}C_8$]PFOA	IS for PFOA	421	376
[1,2,3,4,5,6,7,8,9-$^{13}C_9$]PFNA	IS for PFNA	472	427
[1,2,3,4,5,6 -$^{13}C_6$]PFDA	IS for PFDA	519	474
[1,2,3,4,5,6,7 -$^{13}C_7$]PFUnA	IS for PFUnA	570	525
[1,2 -$^{13}C_2$]PFDoA	IS for PFDoA and PFTA	615	570
[$^{18}O_2$]PFBS	IS for PFBS	303	84
[1,2,3-$^{13}C_3$]PFHS	IS for PFHS	402	80
[1,2,3,4- $^{13}C_4$]PFOS	IS for PFOS	503	80
[1,2,3,4,5,6,7,8-$^{13}C_8$]FOSA	IS for FOSA	507	80
[1,2,3,4-$^{13}C_4$]PFOA	Surrogate (C4-C8 Acids)	417	372
[1,2,3,4- $^{13}C_4$]PFOS	Surrogate(Sulfonates, FOSA)	503	80
[1,2 -$^{13}C_2$]PFUnA	Surrogate (C9-C13 Acids)	565	520

Multiple transitions for monitoring the analytes is an option. The use of one daughter ion is acceptable if data sensitivity and selectivity is achieved and provided that retention time criteria are met to assure adequate specificity. While the daughter ions may be chosen at the discretion of the analyst, mass transition 99 is suggested for PFOS. Quantitation may be performed using the total ion chromatogram (TIC, or summed MRMs) for a given analyte. For example, the PFOA TIC would sum all three of the monitored transitions. Use of the suggested primary ion is recommended. Retention times may vary slightly, on a day-to-day basis, depending on the batch of mobile phase and the gradient, column, guard column(s) used etc. Drift in retention times is acceptable within an analytical run, as long as the drift continues through the entire analysis and the standards are interspersed throughout the analytical run.

When quantitating many analytes, the number of required MRMs for these analytes may become too large. To alleviate the effect of monitoring a large number of MRMs during the entire LC run, it is advisable to break the run into multiple time periods. This allows the analyst to specify a retention window in which only certain MRMs are monitored, corresponding to the expected elution of a group of analytes. A second time period is then added to monitor different MRMs during the time window in which a different set of analytes are expected to elute.

However, care must be taken to set the time period width sufficient to accommodate a reasonable shift in retention times, or the software may not be monitoring the correct MRM when an analyte elutes from the LC column.

10.2 Method Acquisition Parameters

The following values are provided as an example of method acquisition parameters for a single period, single experiment method using the Sciex instrumentation. Actual values may vary from instrument to instrument. Also, these values may be changed from time to time in order to optimize for greatest sensitivity. If a multiple period is used, each period may have different gas, temperature, and ion spray voltages.

Curtain Gas (CUR)	15.0
Collision Gas (CAD)	High
IonSpray Voltage (IS)	-4500
Temperature (TEM)	450.0
Gas 1 (Nebulizer) GS1	35.0
Gas 2 (Turbo Gas) GS2	45.0
Interface Heater (Ihe)	ON
Entrance Potential (EP)	-10

10.3 Calibration Curve

Quantitation is by internal standard or external standard extracted water calibration. If internal standard calibration does not meet calibration acceptance criteria, external calibration can be applied. See Table 2 for recommended application of available internal standards. Quantitation of PFOA and PFOS is by summed analyte-specific mass transitions.

The standard curve is analyzed in the run sequence prior to each set of samples. If internal standards were added to the calibration standards area ratios are used to generate the calibration curve.

The standard curve may be plotted by linear regression ($y = mx + b$) or a quadratic fit ($y = ax^2 + bx + c$); weighted $1/x$, $1/x^2$, or unweighted, using suitable software. The coefficient of determination

(r^2) value for the calibration curve must be greater than or equal to 0.990 (or a correlation coefficient (r) of 0.995). Each calibration standard must be verified for accuracy. A standard is considered acceptable if the back-calculated concentration falls within ±25% of the known concentration, except for the lowest calibration standard, which must be within ±30% of the expected value. Back-calculated calibration standards that fall outside the accuracy limits of 100±25% (30% at the low end) must be discarded. A minimum of six standards must be included that meet the accuracy requirement.

If the calibration curve does not meet acceptance criteria, perform routine maintenance or prepare a new standard curve (if necessary) and reanalyze.

For purposes of accuracy when quantitating low levels of analyte, it may be necessary to use the low end of the calibration curve rather than the full range. For example, when attempting to quantitate approximately 0.05 ng/mL of analyte, generate a calibration curve consisting of the standards from 0.025 ng/mL to 10.0 ng/mL rather than the full range of the curve (0.025 ng/mL to 25.00 ng/mL). This will reduce inaccuracy attributed to linear regression weighting of high concentration standards. A minimum of six standards must be included that meet the accuracy requirement.

Points may be excluded from the calibration curves to provide a better fit over the range appropriate to the data or because they did not meet the pre-determined acceptance criteria. Low-level curve points should also be excluded if their area counts (or area ratio if quantitating by IS) are not at least twice that of the average area counts (or area ratio if quantitating by IS) of the procedural blanks. Reasons for excluding calibration curve points will be documented in the raw data.

10.4 Continuing Calibration Verification (CCV)

Continuing calibration verifications (CCV) are analyzed to verify the accuracy of the calibration curve. Analyze a mid-range calibration standard, one of the same standards used to construct the calibration curve, at a minimum after every tenth sample, not including solvent blanks, with a minimum of one per sample set. Calibration verification injections must be within ±25% to be considered acceptable. Samples must be bracketed by passing CCVs or the calibration curve and a passing CCV to be reportable. Multiple CCV levels may be used.

10.5 System Suitability

A minimum of three system suitability samples should be injected at the beginning of each analytical run, prior to the analysis of the calibration curve. Typically these samples are at a concentration near the mid-level of the calibration curve and are repeated injections from one autosampler vial. It is suggested that the system suitability injections have area counts or area ratios when using internal standard calibration, with a target RSD of ≤5% and a target retention time RSD of ≤2%. There is no defined acceptability limit on these results as the %RSD value is dependant on the number of MRM transitions being monitored in the LC/MS/MS run or time period. Ultimately, any effects on these parameters for the System Suitability samples will also be evident on all standards and QC samples analyzed as part of the analysis batch. Any effect of system suitability is incorporated within QC acceptance criteria.[7]

[7] 3M Environmental Laboratory study E08-0096 evaluated the effect on these results as a function of the number of MRMs being monitored.

11 Procedures

11.1 Analysis Batch Scheme

The following steps represent a typical analysis batch scheme. Sample extraction volumes and final elution volumes may be adjusted to meet data quality objectives.

Allow samples to equilibrate to room temperature. Thoroughly mix samples by gently inverting the sample bottle.

Measure a 40mL representative aliquot of the well-mixed sample into a 50mL polypropylene centrifuge tubes (Spike the lab matrix spikes as required*, replace lid and mix well). Alternate volumes may be used depending on the scope of the project. Use a consistent sample volume for all SPEs and document on the sample prep sheet.

Add 40µL of 250 mg/mL sodium thiosulfate solution to 40mL of sample. Adjust the amount of sodium thiosulfate solution added if alternate sample volumes are used. Thoroughly mix sample.

Note: * Samples may need to be prescreened to determine an appropriate matrix spike level (typically approximately 0.5 to 10 times the sample concentration). Alternatively the samples could be spiked at more than one level, allowing for the inappropriate spike level to be eliminated.

Condition the SPE cartridges (1g, 6mL) by passing approximately 10mL methanol (at a minimum) followed by approximately 50mL (at a minimum) ASTM Type I water. Do not let column run dry. If column does run dry, recondition. SPE cartridges cannot be over conditioned.

Load the analytical sample onto the SPE cartridge. Once the entire sample has been loaded onto the cartridge, isolate the cartridge from the vacuum and wait until all samples on the vacuum manifold have been loaded. Discard eluate. Open manifold valves and pull a vacuum on the SPE cartridges for approximately 3 minutes to remove residual water from the SPE cartridge.

Elute with exactly 5 mL of 100% methanol. Collect eluate into graduated 15mL polypropylene centrifuge tubes. This is the target elution fraction (final volume approximately 4.5 mL as not all of the solvent will leave the SPE column. This will not affect the calculations in any way since the calibration curve is also extracted). Note: the elution volume may be altered to meet project needs. The same elution volume needs to be used for all study samples, calibration standards, and QC samples.

Intersperse the procedural blanks throughout the analysis batch. Solvent blanks are recommended after calibration curve and potential high level samples.

Transfer well-mixed aliquots of the final extract to labeled autovials.

Analyze a portion of the SPE elution fraction using HPLC/MS/MS or HPLC/MS.

Note: Samples are typically concentrated by a factor of eight during the SPE;

Initial Vol = 40mL → Final Vol. = 5mL.

11.2 SPE Scheme for the Low-Level Determination PFCs

A modification of this method has been shown to be applicable to the low-level (~1 pg/mL, 1 ppt) determination of perfluorocarboxylic acids (PFCAs) including perfluorooctanoic acid (PFOA) and perfluoroalkyl sulfonates (PFASs), including perfluorooctane sulfonate (PFOS). This modification may be applicable to other PFCs, provided method QC acceptance criteria are met.

Allow samples to equilibrate to room temperature. Thoroughly mix samples by gently inverting the sample bottle.

Measure a 50mL representative aliquot of the well-mixed sample into a 50mL polypropylene centrifuge tubes (Spike the lab matrix spikes as required*, replace lid and mix well). Alternate volumes may be used depending on the scope of the project. Use a consistent sample volume for all SPEs and document on the sample prep sheet.

Add 40µL of 250 mg/mL sodium thiosulfate solution to 50mL of sample. Thoroughly mix sample.

Note: * Samples may need to be prescreened to determine an appropriate matrix spike level (typically approximately 0.5 to 10 times the sample concentration). Alternatively the samples could be spiked at more than one level, allowing for the inappropriate spike level to be eliminated.

Condition the SPE cartridges (0.5g, 3 mL) by passing approximately 10mL methanol (at a minimum) followed by approximately 50 mL (at a minimum) ASTM Type I water. Do not let column run dry. If column does run dry, recondition. SPE cartridges cannot be over conditioned.

Load the analytical sample onto the SPE cartridge. Once the entire sample has been loaded onto the cartridge, isolate the cartridge from the vacuum and wait until all samples on the vacuum manifold have been loaded. Discard eluate. Open manifold valves and pull a vacuum on the SPE cartridges for approximately 3 minutes to remove residual water from the SPE cartridge.

Elute with exactly 2.5 mL of 100% methanol. Collect eluate into graduated 15mL polypropylene centrifuge tubes. This is the target elution fraction (final volume approximately 2 mL as not all of the solvent will leave the SPE column. This will not affect the calculations in any way since the curve is also extracted). Note: the elution volume may be altered to meet project needs. The same elution volume needs to be used for all study samples, calibration standards, and QC samples.

Intersperse the procedural blanks throughout the analysis batch. Solvent blanks are recommended after calibration curve and potential high level samples.

Transfer well-mixed aliquots of the final extract to labeled autovials.

Analyze a portion of the target elution fraction eluent using negative electrospray HPLC/MS/MS or HPLC/MS.

Note: Samples are concentrated by a factor of twenty during the SPE:

Initial Vol = 50 mL → Final Vol. = 2.5 mL.

11.3 Sample Analysis and QCs

For each analysis batch, the instrument analysis run sequence should include an initial calibration curve, samples, FDSs, interspersed blanks, interspersed CCVs, appropriate QCs (i.e., LCSs, LMSs, FMSs, TBMSs, and TBs), and a final CCV or calibration curve bracketing samples and appropriate QCs. Procedural blanks should be interspersed throughout the analytical sequence. Solvent blanks are recommended after the initial calibration curve, CCVs, and potential high level samples.

Inject the same volume (between 2–5µL) of each standard, analytical sample and blank into the instrument (unless an on-instrument sample dilution is desired).

All sample extracts with a concentration > ULOQ must be diluted and reanalyzed. If dilution of the final extract fails to meet method acceptance criteria, the original sample may be diluted and re-extracted for analysis.

12 Data Analysis and Calculations

The chromatography analysis software will typically calculate the amount of target analyte in the sample extracts using the established calibration curve.

Calculate the percent recovery of the LCS using the following equation:

$$\text{LCS\% recovery} = \frac{\text{LCS Concentration } (\frac{ng}{mL})}{\text{Spike Concentration } (\frac{ng}{mL})} * 100\%$$

Calculate the percent recovery of the LMS using the following equation:

$$\text{LMS \% recovery} = \frac{\text{LMS Concentration } (\frac{ng}{mL}) - \text{Concentration of Sample } (\frac{ng}{mL})}{\text{Spike Concentration } (\frac{ng}{mL})} * 100\%$$

Calculate the percent recovery of the FMS using the following equation:

$$\text{FMS \% recovery} = \frac{\text{FMS Concentration } (\frac{ng}{mL}) - \text{Average Concentration of Sample/Sample Duplicate } (\frac{ng}{mL})}{\text{Spike Concentration } (\frac{ng}{mL})} * 100\%$$

13 Analysis Batch Method Performance Criteria

Any method performance parameters that are not achieved must be considered in the evaluation of the data. Nonconformance to any specified parameters must be described in the report.

If criteria listed in this method performance section are not met, maintenance may be performed on the system and samples reanalyzed, or other actions taken as determined by the analyst/project lead. Document all actions in the raw data.

If data are to be reported when performance criteria have not been met, the data must be footnoted on tables and discussed in the text of the report.

13.1 System Suitability – Analysis Batch

A minimum of three system suitability samples should be injected at the beginning of each analytical run. These samples are run prior to the calibration curve. It is suggested that the system suitability injections have area counts with a target RSD of ≤5% and a target retention time RSD of ≤2%. There is no defined acceptability limit on these results as the %RSDs are dependant on the number of MRM transitions being monitored in the LC/MS/MS run or time period. Any effect of system suitability is incorporated in the QC acceptance criteria.

13.2 Calibration and Limit of Quantitation (LOQ) – Analysis Batch

Calibration Curve: The coefficient of determination (r^2) value for the calibration curve must be greater than or equal to 0.990, corresponding to a correlation coefficient (r) = 0.995. Each point in the curve must back-calculate within ±25% of the theoretical concentration with the exception of the LLOQ, which may be within ±30%.

CCV Performance: Calibration verification injections must be within ±25% to be considered acceptable. The calibration curve and the last passing CCV will then bracket acceptable samples. Multiple CCV levels may be used. CCV recoveries should be within ±25% to be considered acceptable and to verify that the method is in control on a given day.

Limits of Quantitation (LOQ): The lower LOQ (LLOQ) is the lowest non-zero active standard in the calibration curve; the peak area of the LLOQ must be at least 2X that of the average area counts for all prepared procedural blank(s). By definition, the measured value of the LLOQ must be within 30% of the theoretical value.

13.3 Blanks – Procedural, Solvent, Trip

Procedural Blanks: Multiple procedural blanks should be interspersed throughout the analysis batch and the analytical sequence. The area counts (or area ratios when using IS calibration) for each analyte must be less than 50% of the area count of the LOQ standard. If the area counts of the procedural blanks exceed 50% of the LOQ standard, then the LOQ must be raised to the first standard level that meets criteria.

Solvent Blanks: Solvent blanks are analyzed with each analysis batch to determine contamination or carryover. In general, solvent blanks should have area counts for each analyte that are less than 50% of the area count of the lowest calibration standard. When successive solvent blanks are analyzed, the area counts of the solvent blanks should return to <50% of the lowest calibration standard prior to the injection of further samples.

Trip Blank: A trip blank of ASTM Type I water (or lab equivalent) is prepared in a sample container in the laboratory and treated as a sample, including exposure to shipping, sampling site conditions, storage, preservation and all analytical procedures. The trip blanks results for each analyte are included with the reported sample results.

13.4 Data Accuracy and Precision – Analysis Batch

Lab Control Spikes: The average recovery at each LCS level for each target analyte and appropriate SRS should be within 80-120% and the percent relative standard deviation of the recoveries must be less than or equal to 20%. If the average recovery of a spiking level falls outside method acceptance, but at least 67% (6 out of 9) of LCS samples are within 20% of their respective nominal value (33% of the QC samples, not all replicates at the same concentration, may be outside 20% of nominal value), the average recovery will be flagged as outside method acceptance criteria. All LCS samples will be control charted as per ETS-12-012. If the average recovery of one of the spiking levels exceeded the analytical method uncertainty as determined by ETS-12-012, that analytical batch uncertainty will be expanded for that particular study. The average recovery at each LCS level for mixed branched/linear isomer PFOA and PFOS should be within 70-130% and the percent relative standard deviation of the recoveries must be less than or equal to 20%.

Field Duplicates: The relative percent difference (RPD) of duplicate samples should be less than 20% for the precision of sample preparation and analysis to be considered in control. Replicate samples not meeting the 20% RPD criteria are flagged and reported as outside of QC acceptance criteria.

Field Matrix Spikes: FMS acceptance criteria are recoveries within ±30% of the expected value for each target analyte and appropriate SRS. Sample data with FMS recovery outside of ±30% but within ±50% of the expected value are flagged and reported as outside of QC acceptance criteria. Data with FMS recovery outside of ±50% of the expected value are reported as NR, where NR is defined as “Not Reportable” data outside of QC acceptance criteria. If FMS recovery could not be assessed because FMSs were at an inappropriate level, then Laboratory Matrix Spikes (LMSs) may be substituted. If LMS recoveries are within ±30% for each target analyte and SRSs the data are reportable but flagged as not meeting the FMS method acceptance criteria.

13.5 Analytical Method Uncertainty

Analytical method uncertainty for each target analyte and SRS is determined with control charted historical analysis batch LCS data for the method and reported with each analysis batch.[8] Uncertainty determinations are based on INTERNATIONAL ANS/ISO/IED STANDARD 17025 reference (GUM, Guide to the Expression of Uncertainty in Measurement) and described in ETS-12-012. At least thirty data points are required for determining analytical method uncertainty. The method uncertainty is defined as 2x the standard deviation of the percent recoveries of the pooled lab control spikes. While all LCS data points are control charted, only the most recent fifty data points are used for determining the method uncertainty.

When less than twenty LCS data points have been generated for a given analyte, the analysis batch LCSs are used to determine the data uncertainty. If FMSs meet the ±30% recovery criteria at a level appropriate to the endogenous level, and the LCS meet the ±20% recovery criteria, then the uncertainty of the data is determined as within 100±20%.

Analysis batch sample data with FMS recovery outside of ±30% but within ±50% of the expected value are flagged and reported as outside of QC acceptance criteria with expanded uncertainties. Data with FMS recovery outside of ±50% of the expected value are reported as NR, where NR is defined as "Not Reportable" data outside of QC acceptance criteria. If FMS recovery could not be assessed because FMSs were at an inappropriate level, then Laboratory Matrix Spikes (LMSs) may be substituted. If LMS recoveries are within ±30% for each target analyte and appropriate SRSs the data are reportable but flagged as not meeting the FMS method acceptance criteria with uncertainties of ±30%. If FMS do not meet the ±30% recovery criteria, and historical FMS data does not exist, the analytical uncertainty is evaluated on a sample-by-sample basis, the data may be reported with expanded uncertainty and are flagged.

The analytical batch uncertainty may be expanded if the average recovery of a LCS spiking level falls outside method acceptance and exceeded the analytical method uncertainty as determined by ETS-12-012.

13.6 Quantitation of PFOA/PFOS – Analysis Batch

For PFOA and PFOS target analytes, the method requires the addition of LCSs of mixed branched/linear isomer PFOS/PFOA. The purpose of including these LCSs is to demonstrate quantitative equivalency (or quantitative bias) of the isomeric mix when using a predominantly linear PFOS or PFOA standard for calibration. Alternatively, mixed branched and linear isomer PFOS/PFOA calibration may be applied. Alternatively, in lieu of mixed branched and linear isomer PFOS/PFOA LCSs, mixed branched and linear isomer PFOS/PFOA TBMSs may be applied to demonstrate method accuracy and precision.

An alternate method of quantitation can be performed whereby only the linear isomer of PFOS/PFOA is integrated and used for generating the calibration curve. The LCS and samples are then quantitated by integrating the linear and branched isomers separately (requires separate analytical results files) and quantitating the resulting peak against the linear calibration curve. The results from both integrations are then summed to produce the final result. Integrating the linear and branched isomers separately reduces the on-column concentration for those samples that contain both linear and branched isomers of PFOA/PFOS. This ensures that the concentration

[8] Method uncertainty based on INTERNATIONAL ANS/ISO/IED STANDARD 17025 reference (GUM, Guide to the Expression of Uncertainty in Measurement). Method application demonstrated in ETS-12-012, citing references: a.) EURACHEM/CITAC Guide, "Quantifying Uncertainty in Analytical Measurement," Second Edition; Editors: S.L.R. Ellison, M. Rosslein, and A. Williams. b.)Georgian, Thomas, "Estimation of Laboratory Analytical Uncertainty Using Laboratory Control Samples," Environmental Testing & Analysis, November/December 2000. c.)Taylor, B.N. and CE. Kuyatt, NIST Technical Note 1297, 1994 Edition: "Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results."d.)Adams, T.M., "A2LA Guide for the Estimation of Measurement Uncertainty in Testing", July 2002..

detected is within the a range of the calibration curve that is comparable regardless of whether the calibration curve was generated using predominantly linear isomers of PFOS/PFOA or linear plus branched isomers of PFOS/PFOA.

14 Pollution Prevention and Waste Management

Waste and flammable solvent is discarded in high BTU containers, and glass pipette waste is discarded in broken glass containers located in the laboratory.

Sample and standard extracts are to be stored with the residual samples following applicable corporate and/or perfluorochemical litigation directives.

15 Records

Each data package generated for a study must include all supporting information for reconstructibility of the data. Information for the data package must include, but is not limited to the following items: study or project number, sample and standard prep sheets/records, instrument run log (instrument batch records, instrument acquisition method, summary pages), instrument results files, chromatograms, calibration curves, and data calculations.

16 Attachments

None.

17 References

E07-0779 Sample Preparation of Water Samples for the Analysis of PFBA, NFPA, PFHA, PFOA, PFBS, PFHS and PFOS Using Water's tC-18, Sep-Pak and Water's Oasis HLB Solid Phase Extraction Cartridges by 3M Method ETS-8-154, Feb. 19, 2008.

Pace Life Sciences, Q15 Report Suitability of Phenomenex Strata-X SPE cartridges for use with 3M Environmental Labs Method ETS-8-154.3., Dec. 12, 2007.

"Method of Analysis for the Determination of Perfluorooctane sulfonate (PFOS), Perfluorooctane sulfonylamide (PFOSA), and Perfluorooctanoate (POAA) in Water", E. Wickremesinhe and J. Flaherty, Study Number 023–002, Centre Analytical Laboratories, Inc., State College, Pennsylvania, January 2000.

E01-0454: Validation report for the "Method of Analysis for the Determination of Perfluorooctane sulfonate (PFOS), Perfluorooctane sulfonylamide (PFOSA), and Perfluorooctanoate (POAA) in Water", E. Wickremesinhe and J. Flaherty, Study Number 023–002, Centre Analytical Laboratories, Inc., State College, Pennsylvania.

E08-0273: Analysis of Low-Level PFOS in Aqueous Samples from Oakdale Granular Activated Carbon (GAC) System, June 2, 2008.

E08-0275: Analysis of Low-Level PFOS in Aqueous Samples from 3M Cottage Grove; Building 116 - April 2008, June 2, 2008.

E07-0813: Determine the Accuracy of Field Samplers Filling 250 mL Sample Bottles Marked with a "Fill to Here" Line.

E08-0096: Evaluation of the Analytical Precision of System Suitability Samples as a Function of the Number of Mass Transitions Monitored in a LC/MS/MS Experiment.

ETS-12-012: Estimation of Uncertainty of Measurements

INTERNATIONAL ANS/ISO/IED STANDARD 17025

EPA Method 537: DETERMINATION OF SELECTED PERFLUORINATED ALKYL ACIDS IN DRINKING WATER BY SOLID PHASE EXTRACTION AND LIQUID CHROMATOGRAPHY/TANDEM MASS SPECTROMETRY (LC/MS/MS); EPA Document#: EPA/600/R-08/092; http://www.epa.gov/nerlcwww/ordmeth.htm

Groundwater Sampling SOP C-4 excerpted from:

WESTON. 2004. Phase 2 Work Plan for Sampling Environmental Media, 3M Company, Decatur, Alabama Plant. October 2004.

The Phase 2 Work Plan is Appendix B to the Memorandum of Understanding between the U.S. Environmental Protection Agency and 3M Company and Dyneon LLC for a Perfluoroctanoic Acid (PFOA) Site-Related Environmental Assessment Program dated October 25, 2004.

The entire MOU including the Phase 2 Work Plan is located in the OPPT-2004-0112 docket at http://www.regulations.gov as OPPT-2004-0112-002.pdf

18 Affected Documents

None.

19 Revisions

Revision Number	Summary of Changes
1	*Updated to the new format. Changed Title.* *Section 1: States the validation of 3 analytes, removes reference to EPA document that's no longer applicable.* *Section 2: Provided for the extraction of more than the 3 validated analytes, allows the use of a LC/MS system, not only the LS/MS/MS previously mentioned.* *Section 3: Revised definitions for field matrix spike, field control spike, LLOQ, method blank, and MDL.* *Section 5: Reworded the interferences, added recommendation to use disposable pipettes.* *Section 6: Re-categorized and pared down.* *Section 7: Changed storage time to 6 months. Added more calibration points to the table.* *Section 8:Added statement addressing labeling requirements and spiking procedures. Expanded section 8.8.* *Section 9: New Section* *Section 9.6 was modified to include a section on measuring the fill level of FMS samples and the need to correct true concentrations for under-filled and over-filled bottles.* *Section 10: Changed some of the parameters in the tables. Allowed for use of different instrumentation. Added information from section 12 of previous version, extensively revised.* *Section 11 (section 9 in previous version): Clarification of wash step, stated exact volume of eluate is 5 mL, revised standardization process, removed requirement to*

use LC/MS/MS.
Section 12 (section 13 in previous version: no changes
Section 13 (section 14 in previous version): Extensively rewritten.
Section 14 (section 15 in previous version): no changes
Section 15 (section 16 in previous version): Minor changes to recording requirements.
Section 16 (section 17 in previous version): Removed attachment.
Section 17 (section 18 in previous version): Removed reference to EPA document that no longer applied to this SOP.
Section 18: New section.

2 *Section 1: Added emphasis that the method is a performance-based method. Added statement that this method does not cover sample collection.*
Section 2: Removed statement that samples are shipped cold as this is no longer the practice. Removed reference to specific C18 SPE cartridge. Added statement that other extraction and elution volumes may be used.
Section 3. Minor edits/clarifications to several definitions. Added surrogate and SPE cartridge as a definition. Removed references to LOD and MDL as this laboratory does not report LCD and/or MDL values.
Section 6. Added reference to automated SPE workstation.
0Section 7. Added additional analytes and updated standard preparation examples.
Section 8. Removed verbiage that pertained to sample collection as the 3M Environmental Lab personnel typically do not collect samples. Addressed sample bottle preparation.
Section 9. Changed method blank criteria, LMS and FMS acceptance criteria from 100±25% to 100±30%, and LCS criteria where pooled recoveries are used to determine acceptance.
Section 10. Updated all example instrument conditions for Sciex instrumentation – removed Micromass references
Section 11. Clarified extraction procedure – removed inclusion of a 40% methanol wash step.
Section 12. Minor edits.
Section 13. Added section on determination of analytical uncertainty and changed performance criteria as appropriate.

3 *Section 11.1. Added use of sodium thiosulfate*

4 *Corrected typographical and spelling errors throughout document.*
Section 3. Added definition of Time Period for MS data acquisition. Indicated that the use of SRSs may be used in lieu of target analyte FMS.
Section 4.1 Added wording that MSDS sheets may be found in a corporate of commercial database.
Section 6.2. Changed bottle types to wide-mouth High Density Polyethylene (HDPE). Changed LC manufacturer from Hewlett-Packard to Agilent. Added acceptability of house vacuum for use in SPE preparation. Added acceptability of Waters Oasis and Phenomenex Strata-X SPE cartridges for sample preparation.
Section 7 Corrected short names of several of the perfluorinated acids for consistency. Changed bottle types to High Density Polyethylene (HDPE).

Added the use of caustic methanol for preparation of stock solutions to inhibit the formation of methyl ester of reference carboxylic acid. Added the use of internal standards and surrogates to the preparation of calibration standards.
Section 8 Changed bottle types to High Density Polyethylene (HDPE). Added bottle preparation to include the addition of internal standard and surrogate.
Section 9 Added verbage to indicated that the high FMS spike concentration of greater than 10X the expected concentration will be evaluated and may be acceptable, with documentation in raw data and report and without the need of an SOP deviation. Clarified the reporting of FMS outside method acceptance criteria.
Section 10 The use of multiple time periods was added when quantitating a large number of analytes within a single acquisition. System Suitability is modified to remove the requirement of peak area count RSD of ≤5% and a retention time RSD of ≤2%. Reference is made to E08-0096 in the effect of System Suitability samples when utilizing a large number of MRM transitions. Include IS and surrogate MRMs to the table.
Section 11.2 Added this section to describe the modifications for low-level determination of PFOS. Renumbered previous Section 11.2 to Section 11.3.
Section 13. System Suitability is modified to remove the requirement of peak area count RSD of ≤5% and a retention time RSD of ≤2%. Reference is made to E08-0096 in the effect of System Suitability samples when utilizing a large number of MRM transitions. Added the acceptability of a correlation coefficient of (r)= 0.995. Modified the requirement to specify that the average of the method blank area counts must be <50% or the LLOQ area counts. Added alternate quantiation procedure for linear + branched PFOA/PFOS
Section 14. Remove wording to discard sample extracts, as these may be required to be kept due to outstanding FC litigation requirements.
Section 17. Several references added to this section.