3M Environmental Laboratory

Method

Method of Analysis for the Determination of Perfluorinated Compounds in Water by LC/MS/MS; Direct Injection Analysis

Method Number: ETS-8-044.3

Adoption Date: 4/12/07

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Quality Assurance

1 Scope and Application

This method describes the direct injection analysis of perfluorinated compounds (PFCs) from water matrices 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**). Water samples containing heavy particulate may require preparation by an alternate method such as ETS-8-154 "Determination of Perfluorinated Acids, Alcohols, Amides, and Sulfonates In Water By Solid Phase Extraction and High Performance Liquid Chromatography/Mass Spectrometry". The method is applicable to both external standard and internal standard calibration[1].

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
N-MeFOSAA	N-methylperfluoro-1-octanesulfonamidoacetic acid	2355-31-9
N-EtFOSAA	N-etthylperfluoro-1-octanesulfonamidoacetic acid	2991-50-6

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.

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[2]. 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 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

[1] The method is supported by validation with internal standard calibration for C4-C13 PFCAs, C4, C6, and C8 PFSAs, and C8 perfluoroalkane sulfonamide in laboratory control samples under 3M method validation E11-0667.

[2] 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).

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 analyzed as neat aqueous samples or as solvent diluted aqueous samples by direct injection using LC/MS/MS. Samples containing heavy particulate may not be suitable for analysis by this method. Samples containing suspended particulate should be centrifuged or filtered prior to removing a sample aliquot or diluting with solvent. The water sample is mixed well prior to removing an aliquot or diluting, if necessary, with ASTM Type I water, HPLC water, other suitable water, or with solvent such as methanol.

Quantitation is by stable isotope internal standard calibration in laboratory reagent water. All perfluorinated compounds (PFCs) target analyte concentrations of perfluorosulfonic acids (PFSAs) and perfluorocarboxylic acids (PFCAs) are reported as anions and corrected for their salt or free acid forms. Alternatively, quantitation may be performed by external standard calibration.

This is a performance-based method. Method uncertainty for each target analyte is determined using multiple laboratory control spikes at multiple concentrations from each analytical batch. This method also requires that the precision and accuracy for each sample be determined using field matrix spikes or SRSs to verify that the method is applicable to each sample matrix.

Calibration standards for PFUnA, PFDoA, PFTrDA, and FOSA have been found to be unstable for more than 2 days in 100% water. Samples requiring analysis for these compounds by this method should be diluted 1:1 with methanol and analyzed against a calibration curve prepared in 1:1 laboratory reagent water:MeOH.

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 appropriate continuing calibration check standards.

3.2 Analytical Sample

A portion of a laboratory sample prepared for analysis.

3.3 Calibration Standard

A solution prepared by spiking a known volume of the Working Standard (WS) into a predetermined amount of ASTM Type I water, HPLC grade water, other suitable water (i.e. matrix water), or solvent such as methanol, and analyzed according to this method. Calibration standards are used to calibrate the instrument response with respect to analyte concentration.

3.4 Laboratory Duplicate Sample (LDS, or Lab Dup)

A laboratory duplicate sample is a separate aliquot of a sample taken in the analytical laboratory that is 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.

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

ASTM Type I, HPLC grade water, or other suitable water, 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 FB is to determine if test substances or other interferences are present in the field environment. This sample is also referred to as a Trip Blank.

3.6 Field Duplicate Sample (FDS, Field Dup)

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 primary sample gives a measure of the precision associated with sample collection, preservation and storage, as well as with laboratory procedures.

3.7 Field Matrix Spike (FMS)

A sample to which known quantities of the target analytes, and/or SRSs and ISs are, added to the sample bottle in the laboratory before the bottles are sent to the field for collection of aqueous samples. A known, specific volume of sample must be added to the sample container without prior rinsing of the sample bottle. This may be accomplished by making a “fill to this level” line on the outside of the sample container. The FMS is analyzed to ascertain if any matrix effects, interferences, or stability issues may complicate the interpretation of the sample analysis.

3.8 Trip Blank Matrix Spike (TBMS)

An aliquot of ASTM Type I water, HPLC grade water, or other suitable water, to which known quantities of the target analytes, ISs and SRSs, are added in the laboratory prior to the shipment of the collection bottles. The TBMS is analyzed exactly like a study sample to help determine if the method is in control and 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 are appropriate when expected sample concentrations are not known or may vary.

3.9 Internal Standard (IS)

A compound added to study samples, calibration standards, laboratory control samples, and procedural blanks at a consistent level (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.

3.10 Laboratory Control Sample (LCS)

An aliquot of control matrix to which known quantities of the target analytes, ISs and SRSs (when applicable), are added in the laboratory at the time when samples are prepared. At least three levels (two levels for SRSs) in triplicate are included, one generally at the low end of the calibration curve and one near the mid-range and the upper end of the curve. The LCSs are analyzed exactly like a laboratory sample and evaluate the performance of the method for that batch. LCSs should be included with each batch of prepared samples.

3.11 Laboratory Matrix Spike (LMS)

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

3.12 Laboratory Sample

A portion or aliquot of a sample received from the field for testing.

3.13 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 (or area ratio when quantitating samples using internal standards) at least twice that of the average area counts (or area ratio when quantitating samples using internal standards) of the method/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.

3.14 Method Blank

An aliquot of control matrix that is treated exactly like a laboratory sample including exposure to all glassware, equipment, solvents, and reagents that are used to prepare the laboratory samples. The method blank is used to determine if test substances or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.15 Sample

A sample is an aliquot removed from a larger quantity of material intended to represent the original source material.

3.16 Stock Standard Solution (SSS)

A concentrated solution of a single-analyte prepared in the laboratory or purchased from a vendor and has an associated Certificate of Analysis.

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 samples and appropriate QC samples as a means to evaluate the method performance for a chemical class of compounds (e.g., PFSAs, PFCAs).

3.19 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. The analyst should wear gloves, a lab coat, and safety glasses to prevent exposure to chemicals that might be present.

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. Material safety data sheets (MSDS) are available to all laboratory personnel and can be found in the laboratory's LIM system.

4.2 Cautions

The analyst must be familiar with the laboratory equipment and potential hazards including, but not limited to, 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 sample preparation and analysis, reagents and glassware can be sources of potential contaminant. All materials used in the analyses shall be demonstrated to be free from interferences under conditions of analysis by running method blanks.

Parts and supplies that contain 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 when preparing calibration standards and matrix spikes.

6 Instrumentation, Supplies, and Materials

6.1 Instrumentation

Analytical balance capable of reading to 0.0001g

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.

15-mL and 50-mL disposable polypropylene centrifuge tubes.

Class A pipettes and volumetric flasks, various.

2 mL HPLC glass autovials or 1 mL plastic autovials

Disposable pipettes, polypropylene or glass as appropriate

Repeat pipettor and tips

Centrifuge capable of spinning 15-mL and 50-mL polypropylene tubes at 3000 rpm.

7 Reagents and Standards

Note: Suppliers listed 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

Water – ASTM Type 1 (i.e. Milli-Q™), HPLC grade, or other suitably appropriate sources

Ammonium Acetate – A.C.S. Reagent Grade

Calcium Acetate - A.C.S. Reagent Grade

Magnesium Acetate - A.C.S. Reagent Grade

Acetonitrile – HPLC grade

Methanol – HPLC grade

7.2 Representative Target Analytes, ISs, and SRSs

PFBA, Heptafluorobutyric Acid, (C_4 Perfluorinated Acid)

PFPeA (other possible acronyms include NFPA), Nonafluoropentanoic Acid (C_5 Perfluorinated Acid)

PFHxA, Perfluorohexanoic Acid (C_6 Perfluorinated Acid)

PFHpA, Tridecafluoroheptanoic Acid, (C_7 Perfluorinated Acid)

PFOA, Perfluorooctanoic Acid, (C_8 Perfluorinated Acid)

PFNA, Heptadecafluorononanoic Acid, (C_9 Perfluorinated Acid)

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

PFUnA (other possible acronyms include PFUdA), Perfluoroundecanoic Acid, (C_{11} Perfluorinated Acid)

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

PFTrDA (other possible acronym: PFTrA), Perfluorotridecanoic Acid, (C_{13} Perfluorinated Acid)

FBSA, Perfluorobutanesulfonamide

FOSA (other possible acronym: PFOSA), Perfluorooctanesulfonamide

PFBS, Perfluorobutanesulfonate

PFHS, Perfluorohexanesulfonate

PFOS, Perfluorooctanesulfonate

N-MeFOSAA, N-methylperfluoro-1-octanesulfonamidoacetic acid

N-EtFOSAA, N-ethylperfluoro-1-octanesulfonamidoacetic acid

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

([1,2,3,4,5-$^{13}C_5$]PFPeA, ([1,2,3,4-$^{13}C_4$]PFHpA, [$^{18}O_2$]PFBS, d_3-N-MeFOSAA, and d_5-N-EtFOSAA 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

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

Synthetic Groundwater (containing 25 ppm Ca and Mg) – Weigh 0.61 g of Calcium Acetate and 0.92 g of Magnesium Acetate and dissolve in 6.0 L of reagent water.

50:50 Synthetic Groundwater:Methanol – 100mL of synthetic groundwater is diluted with 100mL of methanol.

90:10 Method:Water – Add 450mL of methanol to a 500mL volumetric flask and bring up to volume with ASTM Type 1 (Milli-Q™) water.

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

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

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.

4000 µg/mL target analyte SSSs—Weigh out 100 mg of analytical standard ***(corrected for percent salt, acid [ETS-4-031] and purity*)** and dilute to 25 mL with acetonitrile or other suitable solvent, in a 25 mL volumetric flask. Transfer to a 45 mL I-Chem vial 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 ETS-4-027. An example of purity and salt correction is given below for PFOS.

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

Equation 1, Salt Correction Factor

Example:

$$\boldsymbol{PFOS\ (K^+) salt\ correction\ factor} = \frac{499}{538} = 0.927$$

100 mg $C_8F_{17}S0_3$-K^+ with purity 90% = 83.4 mg $C_8F_{17}S0_3^-$ (100 mg*0.90*0.927=83.4 mg)

Purified mixed linear and branched solutions of PFOA and PFOS are available from Wellington Laboratories at a concentration of 50 µg/mL. These solutions may be used in the preparation of the mixed working standards in lieu of technical grade PFOA and PFOS.

10 µg/mL (10,000 ng/mL) mixed working standard—Add 62.5 µL each of the nominal 4000 µg/mL SSSs to a 25 mL volumetric flask and bring up to volume with solvent.

1 µg/mL (1,000 ng/mL) mixed working standard—Add 1.0 mL of the 10µg/mL WS to a 10 mL volumetric flask and bring up to volume with solvent.

0.1 µg/mL (100 ng/mL) mixed working standard—Add 1.0 mL of the 1 µg/mL WS to a 10 mL volumetric flask and bring up to volume with solvent.

0.01 µg/mL (10 ng/mL) mixed working standard—Add 1.0 mL of the 0.1 µg/mL WS to a 10 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 IS or external standard calibration. Using the working standards described above, prepare calibration standards in ASTM Type I water, HPLC water, other suitable water, or a mixture of solvent and water. The calibration matrices typically used for this method include but are not limited to; synthetic groundwater with ISs and SRSs, 50:50 mixture of synthetic groundwater and methanol with ISs and SRSs, and a 90:10 mixture of methanol and ASTM Type I water (Milli Q™) with SRSs. The curves prepared using synthetic groundwater or 50:50 mixture of synthetic groundwater and methanol, may also be used for external standard calibration. Guidance on preparation of the calibration curves identified above, including the calibration range, is provided in **Table 3**. Note: Volumes of water or water/solvent mixtures and working standards may be adjusted to meet the data quality objectives addressed in the general project outline. Calibration levels other than those listed below can be prepared as needed.

For the quantitation of PFOA and PFOS, reference materials of certified mixed linear and branched isomer are preferred. Alternately, reference materials of primarily linear isomers of PFOA and/or PFOS may be used, however, when quantitating with predominantly linear reference standards, additional LCS samples containing both linear and branched isomers of PFOA and PFOS are required[3].

Calibration standards should be integrated based on the how the purity of the target analyte reference standard was determined (i.e. branched isomer(s) integrated when purity of the reference standard used to prepare the calibration standard was determined from both linear and branched isomers).

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 at the same level in all calibration standards. 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. A separate zero point or method blank is typically prepared at the same time as the calibration standards, using the same solution used to prepare the standards (ASTM Type I water, HPLC water, other suitable water, or a solvent/water mixture), and is spiked with the internal standard at the same concentration as the calibration curve, typically at 1 ng/mL for the synthetic groundwater and 0.5 ng/mL for the curve in a 50:50 mixture of synthetic groundwater and methanol.

If the samples being analyzed were 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 SRSs at 0.1 ng/mL. The final calibration curve must consist of at least six calibration points after analysis.

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, [$^{18}O_2$]PFBS, d_3-N-MeFSOAA, and d_5-N-EtFOSAA can be used to prepare a stock IS solution. Alternative sources of certified stable isotope labeled target analytes are applicable.

[3] A report summarizing an assessment of the use of reference standards containing certified linear and branched isomers of PFOA/PFOS can be found in 3M report E11-0560.

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 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 and [1,2,3 $^{13}C_3$]PFBA	Wellington Laboratories (Mix or Individual)
$^{13}C_4$-Perfluoropentanoic acid	[1,2,3,4,5-$^{13}C_5$]PFPeA	IS for PFPeA	Wellington Laboratories (Mix or Individual)
$^{13}C_2$-Perfluorohexanoic acid	[1,2 -$^{13}C_2$]PFHxA	IS for PFHxA	Wellington Laboratories (Mix or Individual)
$^{13}C_4$-Perfluoroheptanoic acid	[1,2,3,4-$^{13}C_4$]PFHpA	IS for PFHpA	Wellington Laboratories (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 Laboratories (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 Laboratories (Mix or Individual)
$^{13}C_6$-Perfluorodecanoic acid	[1,2,3,4,5,6 -$^{13}C_6$]PFDA	IS for PFDA	Wellington Laboratories (Mix or Individual)
$^{13}C_7$-Perfluoroundecanoic acid	[1,2,3,4,5,6,7 -$^{13}C_7$]PFUnA	IS for PFUnA	Wellington Laboratories (Mix or Individual)
$^{13}C_2$-Perfluorododecanoic acid	[1,2 -$^{13}C_2$]PFDoA	IS for PFDoA, PFTrDA (1)	Wellington Laboratories (Mix or Individual)
$^{18}O_2$-Ammonium Perfluorobutane sulfonate	[$^{18}O_2$]PFBS	IS for PFBS and FBSA (1)	RTI International (Individual)
$^{13}C_3$-Ammonium Perfluorohexane sulfonate	[1,2,3-$^{13}C_3$]PFHS	IS for PFHS	Wellington Laboratories (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 Laboratories (Mix or Individual)
$^{13}C_8$-Perfluorooctanesulfonamide	[1,2,3,4,5,6,7,8-$^{13}C_8$]FOSA	IS for FOSA	Wellington Laboratories (Mix) RTI International (Individual)
N-deuteriomethylperfluoro-1-octanesulfonamidoacetic acid	d_3-N-MeFOSAA	IS for N-MeFOSAA	Wellington Laboratories
N-deuterioethylperfluoro-1-octanesulfonamidoacetic acid	D_5-N-EtFOSAA	IS for N-EtFOSAA	Wellington Laboratories
$^{13}C_3$-Perfluorobutanoic acid	[1,2,3-$^{13}C_3$]PFBA	SRS for all PFCAs: C4-C5	Wellington Laboratories
$^{13}C_4$-Perfluorooctanoic acid	[1,2,3,4-$^{13}C_4$]PFOA	SRS for all PFCAs: C6-C8	Wellington Laboratories
$^{13}C_2$-Perfluoroundecanoic acid	[1,2 -$^{13}C_2$]PFUnA	SRS for all PFCAs C9-C13	Wellington Laboratories
$^{13}C_8$-Perfluorooctane sulfonate	[1,2,3,4-$^{13}C_4$]PFOS	SRS for all PFSAs: C4, C6, and C8	Wellington Laboratories

(1) At this time an isotopically-labeled PFTrDA or FBSA are not available.

Table 3. Example Preparation of Calibration Curves Typically Used

Synthetic Groundwater Curve with Internal Standards (IS) and Surrogate Recovery Standards (SRSs)						
Sample Description	**Concentration of WS, µg/mL**	**Volume of WS, µL**	**Volume of IS (2 µg/mL), µL**	**Concentration of SRSs, µg/mL**	**Volume of Surrogate, µL**	**Volume of synthetic groundwater [(1)], mL**
0.025 ng/mL curve point	0.10	25	50	0.10	25	100
0.030 ng/mL curve point	0.10	30	50	0.10	30	100
0.040 ng/mL curve point	0.10	40	50	0.10	40	100
0.050 ng/mL curve point	0.10	50	50	0.10	50	100
0.10 ng/mL curve point	0.10	100	50	0.10	100	100
0.25 ng/mL curve point	1.0	25	50	1.0	25	100
0.50 ng/mL curve point	1.0	50	50	1.0	50	100
1.0 ng/mL curve point	1.0	100	50	1.0	100	100
2.5 ng/mL curve point	1.0	250	50	1.0	250	100
5.0 ng/mL curve point	1.0	500	50	1.0	500	100
10 ng/mL curve point	1.0	1000	50	1.0	1000	100
15 ng/mL curve point	10.0	150	50	N/A	N/A	100
25 ng/mL curve point	10.0	250	50	N/A	N/A	100
50 ng/mL curve point	10.0	500	50	N/A	N/A	100
75 ng/mL curve point	10.0	750	50	N/A	N/A	100
100 ng/mL curve point	10.0	1000	50	N/A	N/A	100

N/A - Not Applicable

(1) The synthetic groundwater curve is not suitable for samples requiring analysis for PFNA, PFDA, PFUnA, PFDoA, PFTrDA, and FOSA.

Table 4 continued. Example Preparation of Calibration Curves Typically Used

Curve in a 50:50 mixture of Synthetic Groundwater and Methanol with Internal Standards (IS) and Surrogate Recovery Standards (SRSs)						
Sample Description	**Concentration of WS, µg/mL**	**Volume of WS, µL**	**Volume of IS (2 µg/mL), µL**	**Concentration of SRSs, µg/mL**	**Volume of Surrogate, µL**	**Volume of 50:50 mixture of synthetic groundwater and methanol, mL**
0.0125 ng/mL curve point	0.01	62.5	12.5	0.01	62.5	50
0.0250 ng/mL curve point	0.10	12.5	12.5	0.10	12.5	50
0.0500 ng/mL curve point	0.10	25	12.5	0.10	25	50
0.10 ng/mL curve point	0.10	50	12.5	0.10	50	50
0.25 ng/mL curve point	0.10	125	12.5	0.10	125	50
0.50 ng/mL curve point	1.0	25	12.5	1.0	25	50
1.0 ng/mL curve point	1.0	50	12.5	1.0	50	50
2.5 ng/mL curve point	10.0	125	12.5	1.0	125	50
5.0 ng/mL curve point	10.0	250	12.5	1.0	250	50
10 ng/mL curve point	10.0	50	12.5	1.0	500	50
15 ng/mL curve point	10.0	75	12.5	N/A	N/A	50
25 ng/mL curve point	10.0	125	12.5	N/A	N/A	50
50 ng/mL curve point	10.0	250	12.5	N/A	N/A	50

Table 5 continued. Example Preparation of Calibration Curves Typically Used

Curve in a 90:10 mixture of methanol and Milli Q ™ with Surrogate Recovery Standards (SRSs)					
Sample Description	**Concentration of WS, µg/mL**	**Volume of WS, µL**	**Concentration of SRSs, µg/mL**	**Volume of Surrogate, µL**	**Volume of 90:10 mixture of methanol and Milli Q™ water , mL**
0.020 ng/mL curve point	0.10	10	0.10	10	50
0.050 ng/mL curve point	0.10	25	0.10	25	50
0.10 ng/mL curve point	0.10	50	0.10	50	50
0.25 ng/mL curve point	0.10	125	0.10	125	50
0.50 ng/mL curve point	0.10	250	0.10	250	50
1.0 ng/mL curve point	1.0	50	1.0	50	50
2.5 ng/mL curve point	1.0	125	1.0	125	50
5.0 ng/mL curve point	1.0	250	1.0	250	50
10 ng/mL curve point	10.0	50	10.0	50	50
25 ng/mL curve point	10.0	125	N/A	N/A	50
50 ng/mL curve point	10.0	250	N/A	N/A	50
100 ng/mL curve point	10.0	500	N/A	N/A	50
150 ng/mL curve point	10.0	750	N/A	N/A	50

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.) Sample collection volumes are project specific and based on data quality objectives. The Nalgene™ bottles do not require any pretreatment prior to use. 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 spike level designation. Generally, a set of bottles for a given collection site are prepared and 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, 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 or on ice.

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 pre-rinsing of the sample bottle. 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. However, it may not be possible to select a spike level that is appropriate for all target analytes. The reporting of recoveries for target analyte field matrix spike sample that are spiked greater than 10 time the analyte concentration may be reported but should be flagged as being spiked outside of the recommended spiking range. 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, or 0.1 ng/mL. 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.

Example: ((500 μL*(1mL/1000μL)*(0.1μg/mL*(1000ng/1μg))/200 mL = 0.25 ng/mL

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 analysis. Typically sample bottles are spiked with surrogate recovery standards at a final desired spike concentration of 0.1 ng/mL, which is typically 2-5 times the LLOQ.

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 sample preparation. 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 be 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

9.1 Data Quality Objectives

This method and required quality control samples is designed to generate data accurate to ±30% with a targeted LOQ of 0.025 ng/mL. Any deviations from the quality control measures spelled out below will be documented in the raw data and footnoted in the final report.

Note: For each sampling location, a QC element must be prepared and reported with the sample results. This may include one or more of the following: a FMS sample (target analyte and/or SRS) or a LMS sample (target analytes and/or SRSs).

9.2 Method/Procedural Blanks

The method/procedural blanks (which include ISs) are analyzed on a regular basis with each analysis batch. At a minimum, method blanks are analyzed prior to instrument calibration, prior to the analysis of CCV samples, after every 10 sample injections, and at the end of the analytical run. The mean area counts (or area ratios when using IS calibration), for each analyte in the method blanks must be less than 50% of the area counts or area ratios of the LOQ standard. The standard deviation of the area counts or area ratios of these method blanks 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 of the method blanks exceed 50% of the LOQ standard, then the LOQ must be raised to the first standard level in the curve that meets criteria. Method 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

Typically, samples are collected in duplicates in the field. The relative percent difference (RPD) of duplicate samples should be ≤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 and were found to be within acceptance criteria. 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 location 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. LMS concentrations should be prepared at approximately 0.5-10 times the endogenous concentration or approximately 4-10 times the LOQ concentration of the 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 with an expanded uncertainty. 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 Lab Control Sample

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 70% of the ULOQ. For each target analyte and SRS, the 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%.

LCSs are typically prepared in a 15-mL disposable polypropylene centrifuge tube to a final volume of 10 mL. LCSs being analyzed against the calibration curve prepared in a synthetic groundwater are prepared by spiking the target analytes into 10mLs of synthetic groundwater. LCSs being analyzed against the calibration curve prepared in a mixture of 50:50 synthetic groundwater and methanol are prepared by spiking the target analytes into 10 mLs of synthetic groundwater and then diluting 1:1 with methanol in the same manner as the samples. LCSs being analyzed against the calibration curve prepared in a mixture of 90:10 methanol and Milli Q™ water are typically prepared by spiking the target analytes into 10 mL of Milli Q™ water followed by the appropriate dilution with methanol[4]. When using the 50:50 synthetic groundwater:methanol curve or the 90:10 methanol:Milli Q™ water curve, the LCS spike levels should be selected based on the concentration that when diluted with solvent, will produce on-column concentrations within the calibration range. When preparing samples that require varying dilution levels (i.e 10X and 100X dilution), the LCS spiking level will be determined based on the study samples requiring the greatest dilution.

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-4-026. 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 reporting of the data will be evaluated on a case by case basis. Consideration will be given to the concentration of the project samples in relation to the non-compliant LCS samples, matrix spike samples prepared with the project samples, and the data quality objects of the project. Reporting of the data will be documented in the raw data as a deviation and final report. The non-compliant LCSs will be used in the evaluation of the analytical method uncertainty.

There may be instances where low or high curve points may be disabled from the calibration curve and the resulting spike level of the low of high level LCSs will no longer be within the calibration range and a recovery value cannot be calculated. It should be noted in the raw data when these instances occur, but it is not necessary to document this as a method deviation. However if the spike level is within the calibration range and the

[4] The cross validation for the preparation of LCSs for the 90:10 methanol and Milli Q™ water curve can be found in 3M Report E14-0727.

resulting concentration falls outside of the calibration range, a deviation should be documented if the data is going to be reported.

Calibration standards consisting of mixed branched and linear isomer PFOS/PFOA are preferred. However, for PFOS/PFOA target analytes, if the calibration standards are comprised of predominantly linear isomers only, 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, 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) / Surrogate Recovery Standards (SRSs)

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 field matrix spike samples.

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 either not prepared, or were prepared but not at an appropriate level, and the sample set was pre-spiked with appropriate SRSs, the recoveries of the SRSs may be used to assess method accuracy. Alternately, Laboratory Matrix Spikes (LMS) of the target analytes or SRSs may be substituted. If LMS recoveries are within ±30%, the data are reportable and a discussion is included in the final report to indicate that the FMS spikes levels were not appropriate.

2.) If multiple target analyte FMS's were prepared on a sample and the closest appropriate 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 a discussion is included in the final report to indicate that while there were noncompliant FMS's, the uncertainty will not be expanded since the most appropriate spike level met method acceptance criteria. In general, the most appropriate spike level will be the spike at approximately 0.5-10 times the endogenous concentration.

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.

6.) For sampling locations where SRSs are used in lieu of FMSs, and a surrogate recovery is outside the ±30% acceptance criteria, the average surrogate recovery of the sample and field sample duplicate is calculated. If the average surrogate recovery is greater than ±30%, then the method uncertainty for the sampling location is expanded accordingly.

The targeted fortification levels should be at least 50% of the endogenous level and less than 10 times the endogenous level to be used without justification to determine the statement of accuracy for analytical results. The average of the sample and the field duplicate should be used to calculate the recovery.

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 affect 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 raw data and taken into account in the FMS, ISs, or SRSs recovery calculations. This also applies to any field sample that was spiked prior to sample collections with internal standards and/or surrogate recovery standards. Samples over-filled or under-filled by more than 10% will require recalculation of the FMS, ISs, and SRS true values. The following procedure will be used to determine the final fill volume for any sample bottle that is under-filled or over-filled and will require re-calculation of any pre-spiked value.

If the sample bottle appears to be under-filled or over-filled as compared to the "fill to here" line marked on the sample container, the bottom of the water meniscus line is measured with a ruler and the height, in cm, is recorded on the sample preparation sheet.

The following equation is then used to determine the total sample volume, where x is the water height in cm and y is the calculated sample volume in mL. The equation was generated at sample volumes between 161 mL and 261 mL[5].

$$y = 27.428x - 7.1632$$

Equation 2, Bottle Filling Volume, mL[5]

If the sample bottle is under-filled or over-filled outside of the measurements used to generate equation 1 (e.g. the bottle is significantly under-filled or it is over-filled beyond the shoulder of the bottle) the following procedure may be used. Weigh four separate bottles made from the same manufacturer as the sample bottle(s). Record the values on the sample preparation sheet. Place the sample bottle on the balance, record the weight. Assuming the density of water is 1.0 g/mL, subtract the average weight of the four bottles from the weight of the sample bottle, and use the measured weight as the final volume[6].

10 Procedures

10.1 Water Sample Preparation

This method is applicable to water samples. Samples containing heavy particulate may not be suitable for analysis by this method. Samples containing suspended particulate should be centrifuge prior to removing a sample aliquot, or filtered.

- Assess whether or not the sample container has been under-filled or over-filled using the procedure described above in section 9.6, using Equation 2.
- Thoroughly mix sample before removing an aliquot and placing in a labeled autovial.
- Dilute sample, if necessary, with ASTM Type I water, HPLC water, other suitable water, or solvent (i.e. methanol).
- 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 70% of the ULOQ. For IS quantitation, stable isotope internal standards of each target analyte or appropriate surrogate ISs should be spiked in all LCSs at the same level as the samples being analyzed.

[5] A report summarizing the determination of the equation used to calculate the bottle fill volume can be found in 3M report E14-0002.

[6] Based on empty sample bottle data from E12-0283 and E12-0430 (28 bottles weighed in sets of 4 over 7 days), sample bottles are not expected to differ by more than 0.8 g (sample bottle range was <0.6 g and preparing a bottle by adding a line and labels adds <0.2 g). This is only a 0.4% difference in potential error for a 200 mL filled sample bottle which is within the error of the above procedure using water height.

- If LCSs are being prepared using synthetic groundwater, it is recommended that the LCSs samples are allowed to equilibrate for a minimum of 4 hours before aliquoting for analysis or diluting with solvent (i.e. methanol).
- All sample preparation steps must be documented on a sample preparation sheet.

11 Sample Analysis - LC/MS/MS

11.1 Instrument Setup

Note: In this example, an AB Sciex TripleQuad 5500 Tandem Mass Spectrometer (LC/MS/MS) is used. Other brands/models of LC/MS/MS instruments 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: AB Sciex API 4000, API 5000, Triple Quad 5500, or Triple Quad 6500

- Ion Source: Turbo Ion Spray (ABS)
- Mode: Electrospray Negative
- Scan Type: MRM (Multiple Reaction Monitoring)

Computer: Dell DHM

- Software: Windows 7, Analyst 1.6.1 or higher versions

HPLC: Agilent Series 1100,1200, 1260, or 1290, with the following components:

- Agilent Binary Pump
- Agilent Vacuum Degasser
- Agilent Autosampler
- Agilent Column Oven with an internal solvent selection valve

Note: One or more Betasil C18 HPLC analytical columns (2.1 mm x 100 mm, 5μm or 4.6 mm x 100 mm, 5μm) or Prism RP (2.1 mm x 50 mm, 5μm) should 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 HPLC, 4.6mm x 100mm or 2.1mm x 100mm, 5μm (ThermoElectron Corporation)

Column Temperature: 25°C

Injection Volume: 5 - 100µL

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

Mobile Phase (B): Methanol

Table 6. **Liquid Chromatography Gradient Program**

Step Number	Total Time (min)	Flow Rate (µL/min)	Percent A (2 mM ammonium acetate)	Percent B (Methanol)
0	0	750	90.0	10.0
1	0.50	750	90.0	10.0
2	4.00	750	70.0	30.0
3	6.00	750	70.0	30.0
4	11.0	750	20.0	80.0
5	13.0	750	20.0	80.0
6	13.5	750	10.0	90.0
7	16.0	750	10.0	90.0
8	16.5	750	90.0	10.0
9	19.0	750	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 7. Suggested MRM Transitions for Target Analytes, Surrogate Recovery Standards, 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
PFTrA (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
N-MeFOSAA	Target	570	419, 169, 83, 219
N-EtFOSAA	Target	584	419, 169, 83, 219
[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
[1,2,3,4,5,6,7,8- $^{13}C_8$]PFOS	IS for PFOS	507	80
[1,2,3,4,5,6,7,8-$^{13}C_8$]FOSA	IS for FOSA	506	78
d_3N-MeFOSAA	IS for N-MeFOSAA	573	419, 169, 83, 219
d_5N-EtFOSAA	IS for N-EtFOSAA	584	419, 169, 83, 219
[1,2,3 -$^{13}C_3$]PFBA	SRS for PFBA	216	172
[1,2,3,4-$^{13}C_4$]PFOA	SRS (C4-C8 Acids)	417	372
[1,2,3,4- $^{13}C_4$]PFOS	SRS (Sulfonates, FOSA)	503	80
[1,2 -$^{13}C_2$]PFUnA	SRS (C9-C13 Acids)	565	520

Multiple transitions for monitoring the analytes is recommended where applicable, and required for PFOA and PFOS. 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 continuing calibration standards are interspersed throughout the analytical run.

11.2 Calibration Curve

Quantitation is by internal standard or external standard calibration. Calibration standards may be prepared in ASTM Type I, HPLC water, other suitable water, or a solvent/water mixture. 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.

Analyze the standard curve 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 using a linear regression ($y = mx + b$), weighted 1/x or unweighted, or by quadratic fit ($y = ax^2 + bx + c$), weighted 1/x or unweighted, using suitable software. The mathematical method used to calculate the calibration curve should be applied consistently throughout a study. Any change should be thoroughly documented in the raw data.

High and/or low 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. On occasion, it may also be necessary to disable a mid-level curve point that does not meet the pre-determined acceptance criteria (i.e. contamination). However, if two or more consecutive mid-level curve points require disabling for the same analyte, the calibration curve should be re-analyzed or re-prepared. 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 of method and/or solvent blanks. 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 point in the curve must be within ±25% of the theoretical concentration with the exception of the LLOQ, which may be within ±30%. Justification for exclusion of calibration curve points will be noted in the raw data. A minimum of 6 points will be used to construct the calibration curve.

In general, when disabling standards that do not meet the accuracy acceptance criteria, standards should be disabled starting with the highest %bias or based on the need of the calibration range. There may be instances where the standards used to create the calibration curve meet method acceptance criteria, however; the r value for the curve does not meet method acceptance criteria. If during the review of the curve it is noted that a curve point may be causing the r value to be non-compliant, and disabling the curve point would bring the r value within acceptance criteria, the curve point may be disabled. As noted above, justification for exclusion of any calibration curve points needs to be included in the raw data.

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

11.3 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. The calibration curve and the last passing CCV will then bracket acceptable samples. Multiple CCV levels may be used. Samples must be bracketed by passing CCVs or the calibration curve and a passing CCV to be reportable.

11.4 System Suitability

A minimum of three system suitability samples should be injected at the beginning of each analytical run, and 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 a single autosampler vial. If the instrument has been inactive, the analyst should consider injecting several method blanks and possibly a calibration standard prior to the analysis of the system suitability samples, to ensure that the instrument is stable and operating properly.

It is suggested that the system suitability injections have area counts (or area ratios when using IS 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 dependent 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] Non-compliant system suitability samples should be noted in the final report; however, a method deviation is not required. Repeated non-compliance of the system suitability samples should not be disregarded, since it may be an indication that the instrument is not operating properly.

11.5 Sample Analysis and QCs

For each analysis batch, the instrument analysis run sequence should include the following: system suitability samples, 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

Inject the same volume (between 5 - 100µL) of each standard, analytical sample and blank into the instrument. Samples containing analytes that are quantitated above the concentration of the highest standard in the curve should be further diluted and reanalyzed. The injection volume selected should take into consideration the size of the column being used. Many of the target analytes associated with this method are known to consist of one or more isomers. Injecting too much sample onto the column may result in the inability to differentiate between these linear and branched isomers.

Often times the analyst will work up the analytical data and review the sample results as the analytical run is in progress. During this time, the analyst may notice that the results for a field sample or LCS sample(s) are outside of the expected value. The analyst may choose to re-aliquot the sample(s) in question and insert them onto the end of the analytical run. This procedure is acceptable with justification for the re-aliquoted samples documented in the raw data, such as a "Note to File". Alternately, the analyst may choose to re-prepare a set of LCS samples if for example, the analyst suspects that one of the solutions used to prepare the LCS sample was contaminated or had degraded. This procedure is also acceptable with justification for the preparation of a new set of LCS samples documented in the raw data. In those instances where re-aliquoted or re-prepared sample(s) are inserted onto the end of an analytical run, the analytical case narrative (required with each analytical batch) must include a discussion regarding the reporting of re-aliquoted or re-prepared samples, and reviewed with the principal analytical investigator or laboratory management prior to the reporting of the sample results.

When analyzing diluted samples, there may be occasions where the analyst will note during sample analysis, that and analyte may require further dilution. The analyst should never re-inject the prepared sample at a lesser volume to produce an on-instrument sample dilution, as results from a cross-validation study have shown that this method of sample dilution will not produce accurate results[4].

11.6 Manual Integrations

Due to the nature of the samples, the wide range of concentrations found in the samples, and the environmental occurrence of multiple isomers of the laboratory's analytes of interest, the software used for processing the analytical results is not able to consistently integrate the analytical peak; therefore, manual integration of the analytical peak is necessary. All manual integrations are performed following the procedures outlined in method ETS-12-010.1. The consistency of the laboratory's integration is ensured through the training of laboratory personnel, the peer review process required for all manual integrations, the review of manual integrations by the QAU, and where necessary the review of manual integrations by laboratory management.

For project samples, any presence of a target analyte branched isomer peak(s) should be integrated in addition to the linear isomer peak. If there are specific reporting needs for a project in regards to branched versus linear quantitation in the sample (e.g. reporting branched and linear contributions separately, reporting only linear concentrations, etc.) then the specific quantitation requirements for that study should be documented in the project's GPO.

12 Data Analysis and Calculations

Once all the sample data has been processed through the Analyst software (i.e. calibration standard true values entered and calibration curves reviewed for accuracy and linearity, internal standard concentrations entered for all

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

samples, LCS sample concentrations entered, chromatograms integrated properly, etc.), the analyst will sort the data by analyte name and export the data as a .txt file to the appropriate project folder.

12.1 Data Summary Spreadsheet

The .txt results exported from Analyst will be uploaded into an Excel spreadsheet template and the QC elements of the method evaluated against the method acceptance criteria (i.e. regression results, system suitability samples, CCVs, method blanks, LCSs, sample/sample duplicate RPD results and FMS recovery). An example of a data summary excel spreadsheet is included in Attachment A. The .txt results may also be uploaded into LABWARE LIMS.

The first series of worksheets in the data summary Excel spreadsheet contain the target analyte data by analyte. VLOOKUP tables are used to populate the various worksheets summarizing the QC elements associated with this method (system suitability samples, CCV samples, method blank samples, LCS samples, tabulated field sample / sample duplicate average results and RPD values, as well as any field QC samples such as FMS or SRSs). VLOOKUP is an argument function in Excel that looks for a value in the left most column of a table of data, and then returns a value in the same row from a column the analyst specifies. By default, the table must be sorted in an ascending order. The lookup value can be a value, a reference, or a text string. Help on the use of VLOOKUP tables can be found in the Excel software.

12.2 Analytical Case Narrative

Once the analytical batch data has been summarized in the data summary Excel spreadsheet, an analytical case narrative is completed. An example of an analytical case narrative is included in Attachment B. The analytical case narrative documents the following elements of the analysis:

- **Project Scope and Data Quality Objective;** allows the analyst to designative if a GPO for the work exists and whether or not the work is being done under GLPs.
- **Sample Container Preparation;** allows the analyst to indicate how the sample containers were prepared (if applicable).
- **Sample Analysis;** a review of all pertinent elements of the analysis including the method acceptance criteria for system suitability samples, calibration, LOQ determination, and QC samples. Also included is a section to document peer reviewed manual integrations.
- **Additional analytical notes;** allow the analyst to include a statement on what data will be reported and any other relevant information regarding sample analysis.

12.3 Sample Reportability

There are occasions when using internal standards, where the instrument software will produce a calculated result but upon further review of the data, the calculated sample result is not reportable. This typically occurs when analyte concentrations are at or near the LOQ. Below are examples and guidance to be followed when reviewing any data generated using internal standard calibration.

12.3.1 Sample concentration greater than the LOQ, but the target analyte area counts are below the area count of the LOQ standard.

When quantitating samples using internal standard quantitation, the calibration curve is generated using the ratio of the target analyte peak area counts for the over the peak area counts for the internal standard. There will be occasions where the analyte or IS area response will be lower, either due to signal suppression or analyte adsorption. In these instances, the sample analyte peak area counts and internal standard area counts may produce an area ratio that is within the calibration range, however, the sample will have analyte peak area count value that will be less than the peak area count value for the LOQ standard. When this occurs, further evaluation of the sample is required to determine if the sample result is reportable. To test for this scenario, a True / False logical test is applied to the test data in the data summary excel spreadsheet. The following are examples of a logical test that is applied to the sample data.

Example 1:

IF: The sample has an analyte/IS area count ratio greater or equal to the analyte/IS area count ratio of the LOQ standard = **FALSE**, and

IF: The sample analyte area counts are greater than the analyte area counts for the LOQ standard = **FALSE**,

The value returned will be '**BLOQ**'.

Example 2:

IF: The sample has an analyte/IS area count ratio greater or equal to the analyte/IS area count ratio of the LOQ standard = **TRUE**, and

IF: The sample analyte area counts are greater than the analyte area counts for the LOQ standard = **FALSE**,

The value returned will be '**FALSE**' indicating that further evaluation of the sample is required.

Following guidance similar for determining the LOQ, the average analyte area counts for the method blanks are calculated and multiplied by a factor of two. If the sample analyte area counts are greater than 2 times the average analyte area counts for the method blanks, the sample result is reportable. If the sample analyte area counts are less than 2 times the average analyte area counts for the method blanks, the sample result is reported as 'NR', not reportable and footnoted in the final report as such. An example of the application of the True / False logical test can be found in the data summary Excel spreadsheet included in Attachment A.

12.3.2 Sample has a calculated concentration but an area ratio less than the area ratio for the LOQ standard.

It is possible for the Analyst software to generate a concentration value greater than the LOQ for a sample that has an area ratio less than the area ratio for the LOQ standard. This seldom occurs but has been seen when either the lowest curve point has been disabled, or the LOQ standard accuracy value is near 130%. When applying the True / False logical test outlined in 12.3.1, the value returned for such a sample will be "BLOQ". However, since most of the water PFC data is worked up in Excel using VLOOKUP tables, any summary table that utilize the VLOOKUP value will return a result greater than the LOQ standard. Any sample that has an analyte area ratio value less than the LOQ standard is outside of the calibration range and must be reported as BLOQ. The analyst should be aware of this possible discrepancy when reviewing the analytical results.

Note regarding the use of internal standard calibration: EPA Method 537 includes guidance on the reporting of analytical data generated using internal standards. The method states that the internal standard response (peak area counts) for a sample must not deviate by more than 50% from the average area counts for the internal standard measured during the initial analyte calibration. In 3M method validation report E11-0667, it was demonstrated that internal standard area counts for field samples analyzed for PFUnA, PFDoA, and FOSA, are typically less than 50% of the average internal standard response for the calibration standards, due to analyte adsorption to the walls of the sample container. However, the report also shows that recovery for these analytes over the calibration range, including at the LOQ, were within the method acceptance criteria, even when the IS area response deviated by more than 50%. The analyst should review the sample internal standard area response, as it may provide useful information on possible matrix interferences or errors that may have occurred during sample bottle preparation or field sampling. In general, if sample QC are within method acceptance criteria, and if the peak area response is evaluated using the procedures stated above, the data are reportable. When in doubt, review the data in question with the project lead or laboratory management.

12.4 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:

$$= \frac{\boldsymbol{LCS\ Concentration}\ (\frac{\boldsymbol{ng}}{\boldsymbol{mL}})}{\boldsymbol{Spike\ Concentration}\ (\frac{\boldsymbol{ng}}{\boldsymbol{mL}})} \times \mathbf{100}\%$$

Equation 3: LCS % Recovery

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

$$= \frac{\boldsymbol{LMS\ Concentration}\ (\frac{\boldsymbol{ng}}{\boldsymbol{mL}}) - \boldsymbol{Concentration\ of\ Sample}\ (\boldsymbol{ng/mL}}{\boldsymbol{Spike\ Concentration}\ (\frac{\boldsymbol{ng}}{\boldsymbol{mL}})} \times \mathbf{100}\%$$

Equation 4: LMS % Recovery

For samples fortified with known amounts of analyte prior to sample collection, use the following equation to calculate the percent recovery:

$$= \frac{\boldsymbol{Total\ Analyte\ Concentration}\ (\frac{\boldsymbol{ng}}{\boldsymbol{mL}}) - \boldsymbol{Average\ Analyte\ Concentration\ in\ Sample}\ (\frac{\boldsymbol{ng}}{\boldsymbol{mL}})}{\boldsymbol{Concentration\ of\ Analyte\ Added}\ (\boldsymbol{ng/mL}} \times \mathbf{100}\%$$

Equation 5: Field Matrix Spike Recovery

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 and discussed in the final report if the Technical Manager (non-GLP study) or Study Director (GLP study) chooses to report the data.

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 appropriate. 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. Documentation as a method deviation (ETS-4-008) is not required provided that the procedure for documenting the out of compliant performance criteria is followed in this section (e.g. uncertainty expanded for LCS outside ± 20% or FMS outside ± 30%, a sample/sample duplicate %RPD >20%). If there are additional procedures outside method acceptance criteria, where guidance for reporting non-compliance is not provided (e.g. non LLOQ CCV outside ±25%) then a method deviation is required.

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 dependent 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 be within ±25% of the theoretical concentration with the exception of the LLOQ, which may be within ±30%.

CCV Performance: The calibration standards that are interspersed throughout the analytical sequence are evaluated as continuing calibration verifications in addition to being part of the calibration curve. The accuracy of each curve point must be within 25% of the theoretical value (within 30% for lowest curve point). Samples that are bracketed by CCVs not meeting these criteria must be reanalyzed.

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.

Demonstration of Specificity: Specificity is demonstrated by chromatographic retention time (within 4% of standard) and the mass spectral response of unique ions.

13.3 Blanks – Method/Procedural Blanks and Trip

Method/Procedural Blanks: Multiple procedural blanks should be interspersed throughout the analysis batch and the analytical sequence. At a minimum, method blanks are analyzed prior to instrument calibration, prior to the analysis of CCV samples, after every 10 sample injections, and at the end of the analytical run.

The mean 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 or area ratios of the procedural blanks exceed 50% of the LOQ standard, then the LOQ must be raised to the first standard level that meets criteria.

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 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-4-026. If the average recovery of one of the spiking levels does not meet method acceptance criteria of 80-120%, and it exceeds the analytical method uncertainty as determined by ETS-12-012, the analytical method uncertainty will be expanded for that analytical batch for that particular study.

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 the analytical uncertainty is expanded for the sample as appropriate. Data with FMS recovery outside of ±50% of the expected value are reported as NR, where NR is defined as “Not Reportable” due to data outside of QC acceptance criteria. If FMS recovery could not be assessed because FMSs were either not prepared, or were prepared but not at an appropriate level, then Laboratory Matrix Spikes (LMS) of the target analytes or SRSs may be substituted. If LMS recoveries are within ±30%, the data are reportable and a discussion is included in the final report.

For sampling locations where SRSs were prepared in lieu of FMSs, if a surrogate recovery is outside the ±30% acceptance criteria, the average surrogate recovery of the sample and field sample duplicate is calculated. If the average surrogate recovery is greater than ±30%, then the method uncertainty for the sampling location is expanded accordingly.

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. While all LCS data points are control charted, only the most recent fifty data points are used for determining the method uncertainty. Separate control charts are generated for LCSs analyzed by internal standard calibration and external standard calibration, and with or without solvent dilution.

When less than thirty LCS data points have been generated for a given analyte, the method uncertainty is calculated as described in ETS-12-012.

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 either not prepared, or were prepared but not at an appropriate level, then Laboratory Matrix Spikes (LMS) of the target analytes or SRSs may be substituted. If LMS recoveries are within ±30% for each target analyte and appropriate SRSs, the data are reportable and a discussion is included in the final report. If LMS recoveries 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 flagged and reported with an expanded uncertainty.

13.6 Quantitation of PFOA/PFOS – Analysis Batch

Calibration standards consisting of mixed branched and linear isomer PFOS/PFOA are preferred. Quantitation is performed by integrating the linear and branched isomers together. Alternately, the linear and branched isomers can be integrated separately, applying the appropriate true value to each calibration curve point for each isomer. 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 or branched calibration curve. The results from both integrations are then summed to produce the final result. Integrating the linear and branched isomers separately may be helpful for those samples where the linear/branched ratios do not closely match those of the reference standards.

However, for PFOS/PFOA target analytes, if the calibration standards are comprised of predominantly linear isomers only, 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, 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 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.

[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.

14 Pollution Prevention and Waste Management

Waste generated when performing this method will be disposed of appropriately. The original samples will be archived at the 3M Environmental Laboratory in accordance with internal procedures.

15 Records

Each data package generated for a study must include all supporting information for reconstruction 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, data calculations, and the study report.

16 Affected Documents

None.

17 Attachments

Attachment A: Example of a Data Summary Spreadsheet

Attachment B: Example of an Analytical Case Narrative

18 Revisions

Revision Number	Summary of Changes
1	*Section 1. Included the use of internal standard calibration by this method.* *Section 2. Included the use of internal standard calibration by this method. Included the use of a solvent/water mixture when analyzing for PFUnA, PFDoA, PFTrDA, and FOSA.* *Section 3. Added definitions for internal standard, surrogate internal standard, and surrogate recovery standard.* *Section 6.Removed the details regarding the instrument parameters to section 10 of the method.* *Section 7. Updated reference standards to include internal standards and surrogates. Changed concentration levels for working standards and included the use of internal standards and surrogates.* *Section 8. Inserted a new section on sample bottle preparation.* *Section 9 Quality Control. This section was previously section 10 in ETS-8-044.0. Updated QC criteria to be consistent with method ETS-8-154.4.* *Section 10 Procedures. This section was previously section 8 (Sample Handling) in ETS-8-044.0. Added detail regarding the preparation of LCSs. Included the use of methanol as a dilution solvent.* *Section 11 Sample Analysis. This section was previously section 10 in ETS-8-044.0. Included the details regarding the instrument parameters.* *Section 12 Data Analysis and Calculations. This section was previously section 11 in ETS-8-044.0. Removed the equation for calculating the analytes concentration, indicating that this is done by the instrument software.*

	Section 13 Method Performance. This section was previously section 12 in ETS-8-044.0. Updated QC criteria to be consistent with ETS-8-154.4. Added information on the determination of analytical method uncertainty and quantitation of PFOA/PFOS. *Section 14 Pollution Prevention. This section was previously section 13 in ETS-8-044.0.* *Section 15 Records. This section was previously section 14 in ETS-8-044.0.* *Section 16 Affected Documents. This section was previously section 15 in ETS-8-044.0.* *Section 17 Revisions. This section was previously section 16 in ETS-8-044.0.*
2	*Section 7.4. Changed concentration levels for stock standard solutions and updated preparation procedure SSS and WSs.* *Section 7.5.Identified the calibration curve matrices typically used for this method* *Section 7, Table 2. Updated preparation procedure for the calibration curves typically used.* *Section 9.5. Updated the procedure used for preparing LCSs for the various calibration curve matrices.* *Section 9.6. Updated and clarified the reporting criteria for FMS samples to be consistent with current practices. Included the procedure for determining the final fill volume for sample containers under-filled or over-filled in the field.* *Section 11.1 Added additional instruments to the list specified. Modified Table 3 with more current values.* *Section 11.2 Include the ability to deactivate a mid-level standard and provided guidance on the procedure to follow when disable standards that do not meet acceptance criteria.* *Section 11.5. Include the procedure for re-aliquoting or re-preparing samples analyzed in an analytical batch.* *Section 12. Expanded section on Data Analysis and Calculation to include a discussion on the use of data summary spreadsheets and analytical case narratives, with reference to examples of each (included as Attachments A and B). Included guidance on assessing the reporting of sample results at or near the LOQ. Updated the calculations included in this section, using the insert equation feature in Microsoft Word.* *Section 13.4. Clarified the data accuracy and precision requirement for an analysis batch to be consistent with current practices.* *Section 13.5. Analytical Method Uncertainty. Updated the criteria to be consistent with the SOP ETS-12-012.* *Section 17. New section added for attachments.*

3	*Section 9.5 and 10.1. Changed the required concentration level of the high lab control spikes to approximately 70% of the ULOQ. Provided clarification on the reporting of data when more than 33% of the LCS samples fail to meet method acceptance criteria. Provided clarification on the reporting for LCSs when the spiking level falls outside of the calibration range.* *Section 7.5. Indicated that branched isomers will be included in the integration of the target analyte calibration standards if the purity of the target analyte reference standard is based on the sum of linear and branched isomers.* *Section 11.6. Indicated that the integration of project samples will include any branched isomer(s) in addition to the linear isomer, unless noted otherwise in the general project outline.*