

3M ENVIRONMENTAL LABORATORY

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METHOD

DETERMINATION OF PERFLUOROOCTANE SULFONATE (PFOS), PERFLUOROOCTANE SULFONYLAMIDE (PFOSA), AND PERFLUOROOCTANOATE (POAA) IN WATER BY LIQUID-SOLID EXTRACTION AND HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY/TANDEM MASS SPECTROMETRY (HPLC/MS/MS)

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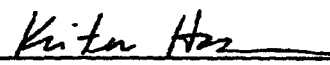
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Author: Kristen J. Hansen/Harold O. Johnson

Approved By: William K. Reagen, Kent R. Lindstrom


William K. Reagen, Laboratory Management

4/28/00
Date


Kristen J. Hansen, Ph.D., Group Leader

04/28/00
Date


Kent R. Lindstrom, Technical Reviewer

04/28/00
Date

1.0 SCOPE AND APPLICATION

- 1.1** This method provides collection, extraction, and analytical procedures for the determination of Perfluorooctane sulfonate (PFOS), Perfluorooctane Sulfonylamide (PFOSA), and Perfluorooctanoate (POAA) in groundwater, surface water, and drinking water samples.
- 1.2** This method was prepared according to the EPA document, "Guidelines and Format for Methods to be Proposed at 40 CFR Part 136 or Part 141" (see Reference 18.1), and is based in part on the report "Method of Analysis for the Determination of Perfluorooctane sulfonate (PFOS), Perfluorooctane sulfonylamide (PFOSA), and Perfluorooctanoate (POAA) in Water" (see Reference 18.2).

2.0 SUMMARY OF METHOD

- 2.1** Water samples are collected from a site of interest and shipped cold to an analytical facility. PFOS, PFOSA, and POAA are extracted from 40mL water samples using C₁₈ solid phase extraction (SPE) cartridges. The compounds are eluted from the C₁₈ cartridge, using methanol. Separation, identification, and measurement are accomplished by high-performance liquid chromatography/tandem mass spectrometry (HPLC/MS/MS) analysis using multiple response monitoring (MRM).

The concentration of each identified component is measured by comparing the MS response of the quantitation ion produced by that compound to the MS response of the quantitation ion produced by the same compound in an extracted calibration standard (external standard).

3.0 DEFINITIONS

- 3.1 Analytical Sample**—A portion of an extracted Laboratory sample prepared for analysis.
- 3.2 Calibration Standard**—A solution prepared from the Working Standard (WS) and extracted according to this method. The calibration standard solutions are used to calibrate the instrument response with respect to analyte concentration.
- 3.3 Duplicate Sample (DS)**—A separate aliquot of a sample, taken in the analytical laboratory and analyzed separately with identical procedures. Analysis of DSs 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.
- 3.4 Field Blank Control Sample (FB)**—Type I 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.

- 3.5 Field Duplicate (FD)**—A sample collected in duplicate at the same time as the sample and placed under identical circumstances and treated exactly the same throughout field and laboratory procedures. Analysis of FD compared to that of the first sample gives a measure of the precision associated with sample collection, preservation and storage, as well as with laboratory procedures.
- 3.6 Field Matrix Spike (FMS)**—A sample collected in duplicate to which known quantities of the target analytes are added in the field at the time of sample collection. The FMS should be spiked at approximately 50–150% of the expected analyte concentration in the sample. The FMS is analyzed to ascertain if any matrix effects, interferences, or stability issues may complicate the interpretation of the sample analysis.
- 3.7 Field Spike Control Sample (FSCS)**—An aliquot of type I water to which known quantities of the target analytes are added in the field at the time of sample collection (at an appropriate concentration to be determined by the project lead). The FSCS is extracted and analyzed exactly like a sample to determine whether a loss of analyte could be attributed to sample storage and/or shipment.
- 3.8 Laboratory Control Sample (LCS)**—An aliquot of type I water to which known quantities of the target analytes are added in the laboratory. Two levels are included, one at the LOQ (approx. 25Pg/mL), the other at a concentration of approx. 100–250Pg/mL or another concentration to be determined by the project lead. The LCS is extracted and analyzed exactly like a laboratory sample to determine whether the methodology is in control, and whether the laboratory is capable of making accurate measurements at the required method detection limit and higher.
- 3.9 Laboratory Sample**—A portion of a sample received from the field for testing.
- 3.10 Limit of Detection (LOD)**—The lowest concentration of an analyte that can be measured and reported with 99% confidence that the analyte concentration is greater than zero. The LOD can be determined in several ways, including signal-to-noise ratio and statistical calculations.
- 3.11 Limit of Quantitation (LOQ)**—The lowest concentration (LLOQ) or highest concentration (ULOQ) that can be reliably achieved within the specified limits of precision and accuracy during routine operating conditions.
- Note:** The LLOQ is generally 5–10 times the LOD. For many analytes, the LLOQ analyte concentration is selected as the lowest non-zero standard in the calibration curve. However, it may be nominally chosen within these stated guidelines to simplify data reporting. Sample LLOQs are matrix-dependent.
- 3.12 Matrix Spike (MS)**—An aliquot of a sample, to which known quantities of target analytes are added in the laboratory. The MS is extracted and analyzed exactly like a laboratory sample to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the MS corrected for background concentrations.

- 3.13 Method Blank**—An aliquot of type I water that is treated exactly like a laboratory sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and surrogates that are used with other 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.
- 3.14 Method Detection Limit (MDL) Determination**—One of several processes that may be used to establish a LOD value. The statistically calculated minimum amount of an analyte that can be measured with 99% confidence that the reported value is greater than zero. This term is usually associated with the EPA definition in 40 CFR Part 136 Appendix B.
- 3.15 Sample**—A sample is a small portion collected from a larger quantity of material intended to represent the original source material.
- 3.16 Spiking Stock Standard (SSS)**—A solution prepared from stock standards used to prepare the working standard.
- 3.17 Stock Standard (SS)**—A concentrated solution of a single analyte prepared in the laboratory with an assayed reference compound.
- 3.18 Working Standard (WS)**—A solution of several analytes prepared in the laboratory from SSs and diluted as needed to prepare calibration standards and other required analyte solutions.

4.0 WARNINGS AND CAUTIONS

4.1 Health and Safety Warnings

- 4.1.1** 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.
- 4.1.2** Unknown samples may contain high concentrations of volatile toxic compounds. Sample containers should be opened in a hood and handled with gloves to prevent exposure.
- 4.1.3** The laboratory is responsible for maintaining a safe work environment and a current awareness of local regulations regarding the handling of the chemicals used in this method. A reference file of material safety data sheets (MSDS) should be available to all personnel involved in these analyses.

5.0 INTERFERENCES

- 5.1** During extraction and analysis, major potential contaminant sources are reagents and liquid-solid extraction devices.
- 5.2** All materials used in the analyses shall be demonstrated to be free from interferences under conditions of analysis by running method blanks.
- 5.3** Teflon® containing materials (e.g. caps, wash bottles) contain fluorocompounds which may cause interferences and should not be used during collection, storage, extraction, or analysis of the samples.

6.0 EQUIPMENT, SUPPLIES, AND MATERIALS

Note: Brand names, suppliers, and part numbers are for illustrative purposes only. Equivalent performance may be achieved using apparatus and materials other than those specified here, but demonstration of equivalent performance that meets the requirements of this method is the responsibility of the laboratory.

6.1 Sampling Equipment

6.1.1 Sample collection bottles—LDPE (e.g., Nalgene™) narrow-mouth bottles with screw cap.

Note: Do not use Teflon bottles or Teflon lined caps.

6.1.2 Coolers for sample shipment.

6.1.3 Ice for sample shipment.

6.1.4 Bottles must be lot-certified to be free of artifacts by running Method blanks according to this method.

6.2 Laboratory Equipment (Extraction and Analytical)

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

6.2.2 Vacuum pump, Büchi.

6.2.3 Visiprep vacuum manifold, Supelco.

6.2.4 Sep Pak Vac 6cc (1g) tC_{18} cartridges (part # WAT 036795), Waters.

6.2.5 50mL disposable polypropylene centrifuge tubes, VWR.

6.2.6 15mL disposable polypropylene centrifuge tubes, VWR.

6.2.7 Disposable micropipettes (50–100 μ L, 100–200 μ L), Drummond.

6.2.8 Class A pipettes and volumetric flasks, various.

6.2.9 Hypercarb drop-in guard column (4mm) (part # 844017–400), Keystone.

6.2.10 Stand-alone drop-in guard cartridge holder, Keystone.

6.2.11 125mL LDPE narrow-mouth bottles, Nalgene.

6.2.12 HPLC pump (LC10AD), Shimadzu.

6.2.13 2mL clear HPLC vial kit (cat # 5181–3400), Hewlett Packard.

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

6.2.15 LC/MS/MS and HPLC systems, as described in section 10.1.

6.3 Equipment Notes

6.3.1 In order to avoid contamination, the use of disposable labware is highly recommended (tubes, pipettes, etc.).

6.3.2 Teflon or Teflon-lined containers or equipment, including Teflon-lined HPLC vials or caps for the HPLC auto sampler must not be used.

6.3.3 Type I water used during the sample and standard extraction should be filtered through a Hypercarb guard column using a HPLC pump. This water is referred to as “filtered type I water”, hereafter in this report.

6.3.4 It is necessary to check the solvents (methanol) for the presence of contaminants (especially POAA) by LC/MS/MS prior to use. Certain lot numbers have been found to be unsuitable for use.

6.3.5 Use disposable micropipettes or pipettes to aliquot standard solutions to make calibration standards and matrix spikes.

7.0 REAGENTS AND STANDARDS

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

7.1 Chemicals

- 7.1.1** Methanol (MeOH), HPLC grade, JT Baker, Catalog No. JT9093-2.
- 7.1.2** Ammonium Acetate, Reagent grade, Sigma-Aldrich, Catalog No. A-7330.
- 7.1.3** Water, type I, prepared in-house.
- 7.1.4** Sodium Thiosulfate, Reagent grade, JT Baker.

7.2 Standards

- 7.2.1** Potassium perfluorooctane sulfonate (see Attachment A, Figure 1).
- 7.2.2** Perfluorooctane sulfonylamide (see Attachment A, Figure 2).
- 7.2.3** Ammonium perfluorooctanoate (see Attachment A, Figure 3).

7.3 Reagent Preparation

- 7.3.1** 250mg/mL sodium thiosulfate solution (Extraction)—Dissolve 25g of sodium thiosulfate in 100mL reagent water.
- 7.3.2** 40% methanol (Extraction)—Measure 400mL methanol and adjust the volume to 1.0L with reagent water.
- 7.3.3** 100mM ammonium acetate solution (Analysis)—Weigh 7.71g of ammonium acetate and dissolve in 1.0L of reagent water. Dilute the 100mM solution by a factor of 50 to make the 2mM ammonium acetate solution used for mobile phase A.

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

7.4 Spiking Stock Standard (SSS) Preparation

- 7.4.1** 100µg/mL each PFOS, PFOSA, and POAA SSSs—Weigh out 10mg of analytical standard (corrected for percent salt and purity—i.e., 10 mg $C_8F_{17}SO_3K$ purity 90% = 8.35mg $C_8F_{17}SO_3-$) and dilute to 100mL with methanol in a 100mL volumetric flask. Transfer to a 125mL LDPE bottle. Prepare a separate solution for each analyte. Store solutions in a refrigerator at $4^{\circ}\pm 2^{\circ}C$ for a maximum period of 6 months from the date of preparation.
- 7.4.2** 1µg/mL mixed SSS—Add 1.0mL each of the 100µg/mL SSSs (from 7.4.1) to a 100mL volumetric flask and bring up to volume with methanol.
- 7.4.3** 0.1µg/mL mixed SSS—Add 10.0mL of the 1.0µg/mL-mixed solution (from 7.4.2) to a 100mL volumetric flask and bring up to volume with methanol.
- 7.4.4** 0.01µg/mL mixed SSS—Add 10.0mL of the 0.1µg/mL-mixed solution (from 7.4.3) to a 100mL volumetric flask and bring up to volume with methanol.
- 7.4.5** Storage Conditions—Store all SSSs in a refrigerator in 125mL LDPE bottles at $4^{\circ}\pm 2^{\circ}C$ for a maximum period of 3 months from the date of preparation.

7.5 Calibration Standards

- 7.5.1** 100µg/mL each PFOS, PFOSA, and POAA stock standard solutions—Weigh out 10mg of analytical standard (corrected for percent salt and purity) and dilute to 100mL with methanol in a 100mL volumetric flask. Transfer to a 125mL LDPE bottle. Prepare a separate solution for each analyte. Store solutions in a refrigerator at 4°±2°C for a maximum period of 6 months from the date of preparation.
- 7.5.2** 1µg/mL Working Standard—Add 1.0mL each of the 100µg/mL SS solutions (from 7.5.1) to a 100mL volumetric flask and bring up to volume with methanol.
- 7.5.3** 0.1µg/mL Working Standard—Add 10.0mL of the 1.0µg/mL mixed solution (from 7.5.2) to a 100mL volumetric flask and bring up to volume with methanol.
- 7.5.4** 0.01µg/mL Working Standard—Add 10.0mL of the 0.1µg/mL mixed solution (from 7.5.3) to a 100mL volumetric flask and bring up to volume with methanol.
- 7.5.5** Storage Conditions—Store all WSs in a refrigerator (in 125mL LDPE bottles) at 4°±2°C for a maximum period of 3 months from the date of preparation.
- 7.5.6** Calibration Standard—Prepare a minimum of five calibration solutions in filtered type I water according to the following table:

Concentration of WS, µg/mL	Volume of WS, µL	Final Calibration Standard Volume, mL	Final Concentration of Calibration Standard, Pg/mL
0.0	0	40	0
0.010	100	40	25
0.010	200	40	50
0.010	400	40	100
0.10	100	40	250
0.10	200	40	500
0.10	300	40	750 ¹
0.10	400	40	1000 ²

¹ May be prepared to extend the range beyond 500Pg/mL.

² May be prepared to extend the range beyond 750Pg/mL.

Note: The absolute volumes of the standards may be varied by the analyst as long as the correct proportions of solute to solvent are maintained.

7.5.7 The standards are processed through the extraction procedure (Section 9.0), identical to the laboratory samples. The extracted concentration of the calibration standard is equal to 8X the initial concentration, due to the concentration of the standard during the extraction process.

7.5.8 Storage Conditions—Store all extracted calibration standards in 15mL polypropylene tubes at 4°±2°C, for a maximum period of two weeks from the date of preparation.

8.0 SAMPLE COLLECTION, PRESERVATION AND STORAGE

Note: Sampling equipment, including automatic samplers, must be free of Teflon tubing, gaskets, and other parts that may leach interfering analytes into the water sample. Automatic samplers that composite samples over time should use refrigerated polypropylene sample containers if possible. Sample bottles should not be rinsed before sample collection.

- 8.1 Tap Water**—Open the tap and allow the system to flush until the water temperature ($15^{\circ}\pm 10^{\circ}\text{C}$) has stabilized (usually about two minutes). Adjust the flow to about 500mL/min and collect samples from the flowing stream.
- 8.2 Ground Water**—Purge the well of standing water using a pump or a bailer. Collect the sample directly from the pump or from the bailer.
- 8.3 Surface Water**—When sampling from an open body of water, fill the sample container with water from a representative area.
- 8.4 Sample Dechlorination**—All samples should be iced or refrigerated at $4^{\circ}\pm 2^{\circ}\text{C}$ and kept in the dark from the time of collection until extraction. Residual chlorine should be reduced by adding 200 μL of a 250mg/mL sodium thiosulfate solution to each water sample, FB, and FSCS (which may be placed in each bottle before leaving for the sampling site.).
- 8.5 Holding Time (HT)**—Results of the time/storage study of all target analytes showed that the three compounds are stable for 14 days in water samples when the samples are dechlorinated and stored as described in section 8.4 (see also reference 18.3). Therefore, laboratory samples must be extracted within 14 days and the extracts analyzed within 30 days of sample collection. If the HT exceeds 14 days, great care is used when evaluating field spikes to avoid misrepresentation of the sample concentration.
- 8.6 Field Blanks**
 - 8.6.1** Process a Field Blank Control Sample (FB) along with each sample set (samples collected from the same general sample site at approximately the same time). At the laboratory, prior to sample collection, fill a sample container with filtered type I water, seal, and ship the FB to the sampling site along with the empty sample containers. Return the FB to the laboratory with the filled sample bottles.
 - 8.6.2** When sodium thiosulfate is added to samples, use the same procedure to preserve the FB.
- 8.7 Field Duplicates**
 - 8.7.1** Collect a Field Duplicate (FD) for every ten (10) samples collected or per each sampling set, if less than 10 samples are collected.
 - 8.7.2** Separate FDs must be collected for each type of water sample (ground, tap, etc.) collected.
 - 8.7.3** Collect the FD immediately after the sample.
 - 8.7.4** Preserve, store and ship FD using the same procedures as used for the samples.

8.8 Field Spike Control Sample (FSCS)

- 8.8.1** A Field Spike Control Sample (FSCS) must be prepared for each sample shipment. If multiple coolers are used to ship a set of samples, each cooler must contain a FSCS.
- 8.8.2** At the laboratory, fill a sample container with 100mL of type I water. Seal and ship to the sampling site along with the empty sample containers and FBs.
- 8.8.3** When sodium thiosulfate is added to samples, use the same procedure to add the same amounts to the FSCS.
- 8.8.4** Seal and gently invert the FSCS to mix. Store and ship the FSCS using the same procedures as used for the samples.

9.0 EXTRACTION PROCEDURE

9.1 Extraction Scheme

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

9.1.2 Measure 40mL of sample into 50mL polypropylene centrifuge tubes (Spike the QC and Matrix spikes as required*, replace lid and mix well).

Note: * Samples may need to be prescreened to determine an appropriate matrix spike level (typically 50–150% of sample concentration).

9.1.3 Condition the C₁₈ SPE cartridges (1g, 6mL) by passing 10mL methanol followed by 5mL filtered type I water (~2drop/sec). Do not let column run dry.

Note: For the following steps, maintain a ~1 drop/sec flow rate. Do not allow the column to run dry at any time.

9.1.4 Load the analytical sample onto the C₁₈ SPE cartridge. Discard eluate.

9.1.5 Wash with ~5mL 40% methanol in water. Discard eluate.

9.1.6 Elute with ~5mL 100% methanol. Collect 5mL of eluate into graduated 15mL polypropylene centrifuge tubes. This is the target elution fraction (final volume = 5mL).

9.1.7 Analyze a portion of the target elution fraction eluent using negative electrospray HPLC/MS/MS (Section 10.2).

Note: Samples are concentrated by a factor of eight during the extraction; Initial Vol = 40mL → Final Vol. = 5mL.

9.1.8 Samples are stable at room temperature for at least 24 hours. Analytical samples may be stored in a refrigerator at 4°±2°C until analysis.

9.1.9 Standardization of C₁₈ SPE columns—If poor recoveries are observed, it may be necessary to standardize the C₁₈ SPE columns in the following manner before analyzing samples.

9.1.9.1 Use a standard with an analyte concentration between 1000 and 4000 Pg/mL. Follow the extraction scheme as outlined from steps 9.1.1 to 9.1.6, except, collect the eluate fraction separately (approx. 5mL), as well as the target elution fraction.

9.1.9.2 After step 9.1.6, collect a post-elution fraction by, eluting with an additional 5mL of 100% methanol.

9.1.9.3 Analyze all three fractions by HPLC/MS/MS. If the target fraction contains a minimum of 85% of the respective analytes, it may be considered acceptable.

9.1.9.4 If the wash contains significant standard (>15%), either the wash volume or percentage of MeOH should be decreased.

9.1.9.5 If the post-elution fraction contains significant standard (>15%), the target elution volume should be increased.

10.0 CALIBRATION AND STANDARDIZATION (ANALYTICAL SETUP)

Note: Other instruments may be used and the equipment and conditions may be very different as long as the method criteria are met. The operator must optimize and document the equipment and settings used.

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

Mass Spec: Micromass Quattro Ultima (Micromass)

Interface: Electrospray (Micromass)

Mode: Electrospray Negative, Multiple Response Monitoring (MRM)

Harvard infusion pump (Harvard Instruments), for tuning

Computer: COMPAQ Professional Workstation AP200

Software: Windows NT, MassLynx 3.3

HPLC: Hewlett Packard (HP) Series 1100

HP Quat Pump

HP Vacuum Degasser

HP Autosampler

HP Column Oven

Note: A 4 x 10mm Hypercarb drop-in guard cartridge (Keystone, part # 844017-400) is attached on-line after the purge valve and before the sample injector port to trap any residue contaminants that may be in the mobile phase and/or HPLC system.

HPLC Column: Genesis C₈ (Jones Chromatography), 2.1mm x 50mm, 4µm

Column Temperature: 35°C

Injection Volume: 15µL

Mobile Phase (A): 2mM Ammonium Acetate in filtered type I water (See 7.3.1)

Mobile Phase (B): Methanol

HPLC Gradient Program:

Time, min	Percent Mobile Phase A	Percent Mobile Phase B	Flow Rate, mL/min
0.0	60	40	0.3
0.4	60	40	0.3
1.0	10	90	0.3
7.0	10	90	0.3
7.5	0	100	0.3
9.0	0	100	0.4
9.5	60	40	0.4
13.5	60	40	0.4
14.0	60	40	0.3

Note: Other HPLC gradients may be used as long as the method criteria 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.

Ions Monitored:

Analyte	Primary Ion	Product Ion	Approximate Retention Time
POAA	413.0	169.0	5.0
PFOS	499.0	99.0	5.2
PFOSA	498.0	78.0	5.8

Other product ions may be chosen at the discretion of the analyst, although m/z 99 is suggested for PFOS. 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 etc. Drift in retention times is acceptable within an analytical run, as long as the drift continues through the entire analysis and the standards are interspersed throughout the analytical run.

10.2 Tune File Parameters

10.2.1 The following values are provided as an example. Actual values may vary from instrument to instrument. Also, these values may be changed from time to time in order to optimize for greatest sensitivity.

Analyte	Dwell, sec	Collision Energy, eV	Cone, V
POAA	0.2-0.4	10-25	20-30
PFOS	0.2-0.4	30-60	50-80
PFOSA	0.2-0.4	20-50	30-60

Source	Set
Capillary	2.56-3.5kV
Hexapole 1	0.5V
Aperture 1	0.2V
Hexapole 2	0.8V
Source Block Temp.	100-150°C
Desolvation Temp.	250-400°C

Analyzer	Set
LM Res 1	12.0-15.0V
HM Res 1	12.0-15.0V
IEnergy 1	0.7V
Entrance	-2V
Exit	1V
LM Res 2	11.0V
HM Res 2	11.0V
IEnergy 2	1.0V
Multiplier	650V

Gas Flows	Set
Cone Gas	150L/hr
Desolvation	700L/hr

Pressures	Set
Gas Cell	3.0e-3mbar

11.0 ANALYTICAL QUALITY CONTROL

- 11.1** Analytical results of the FB, FMS, FD, and FSCS should be evaluated at the conclusion of the study to help interpret the data quality of samples data. Analytical results for these control/duplicate samples must be reported with the sample data.

12.0 ANALYTICAL PROCEDURE

12.1 Sample Analysis

- 12.1.1 Set up analysis sample queue.
- 12.1.2 Inject the same aliquot (between 5–25 µL) of each standard, analytical sample, recovery, control etc. into the LC/MS/MS system.
- 12.1.3 All samples showing a response for one or more analytes above the response of the highest, active calibration curve level must be diluted and reanalyzed.

12.2 Calibration Curve

- 12.2.1 Starting with the standard of lowest concentration, inject the same size aliquot (between 10–25 µL) of each extracted calibration standard according to Section 12.1 and tabulate the response (peak height or area) versus the concentration in the standard. Use linear standard curves for quantitation generated for each analyte by linear regression with 1/x weighting of peak area versus calibration standard concentration. The correlation coefficient (r) for the calibration curves must be ≥ 0.990 ($r^2 \geq 0.980$). If calibration results fall outside these limits, then appropriate steps must be taken to adjust instrument operation and the standards reanalyzed.
- 12.2.2 Curve—The measured value for each curve point must be within $\pm 30\%$ of theoretical values when curve is evaluated over a range appropriate to the data. High or low points may be deactivated to achieve these criteria, but an acceptable curve must contain at least five active curve points.
- 12.2.3 Continuing Curve Verification (CCV)—Mid- and low-level calibration checks should be analyzed every 5–10 injections. The analyte level measured in the CCVs should be within $\pm 30\%$ of theoretical values. If CCVs fall outside of this range, data collected subsequent to the last passing CCV should not be used. Only data collected between acceptable CCVs or the initial curve can be used.

13.0 DATA ANALYSIS AND CALCULATIONS

- 13.1 Calculate the analytical sample (extract) concentration from the standard curve using the following equation:

$$\text{Extract Concentration, pg/mL} = \frac{(\text{Peak area} - \text{intercept})}{(\text{slope})}$$

- 13.2 Calculate the percent recovery of the FSCS using the following equation:

$$\text{FSCS \% rec.} = \frac{(\text{FSCS conc., Pg/mL})}{(\text{Conc. added, Pg/mL})} \times 100$$

- 13.3 Calculate the percent recovery of the MSs using the following equation:

$$\text{MS \% rec.} = \frac{(\text{MS conc., Pg/mL} - \text{Sample Conc., Pg/mL})}{(\text{Conc. added, Pg/mL})} \times 100$$

14.0 METHOD PERFORMANCE PARAMETERS

Note: 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 any reporting of the data.

- 14.1 Linearity**—Linear standard curves for quantitation generated for each analyte by linear regression with 1/x weighting of peak area versus calibration standard concentration. The correlation coefficient (r) for the calibration curves must be ≥ 0.990 ($r^2 \geq 0.980$).
- 14.2 Calibration Curve Standards**—The measured value for each curve point must be within $\pm 30\%$ of theoretical values when curve is evaluated over a range appropriate to the data. High or low points may be deactivated to achieve these criteria, but an acceptable curve must contain at least five active curve points.
- 14.3 CCV Performance**—Mid and low level calibration checks to be analyzed every 5–10 injections. The analyte level measured in the CCVs should be within $\pm 30\%$ of theoretical values. If CCVs fall outside of this range, data collected subsequent to the last passing CCV should not be used. Only data collected between acceptable CCVs can be used.
- 14.4 Limit of Detection (LOD)**—The lowest calibration standard with a peak area at least 2X the peak area of the extraction blank that can be measured at a concentration greater than zero.
- 14.5 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 extraction blank. By definition, the measured value of the LLOQ must be within 30% of the theoretical value.
- 14.6 Matrix Spikes**—Matrix spike percent recoveries must be within $\pm 30\%$ of the spiked concentration.
- 14.7 Solvent Blanks, Method Blanks, and Matrix Blanks**—Values must be below the lowest non-zero active standard in the calibration curve. Matrix blanks are considered compliant if no test substance is detected above the LOD for that analyte.
- 14.8 Reproducibility**—Reproducibility of the method is defined by the results of the matrix spikes and matrix spike duplicates. The MS/MSD should be reproducible to within 20%.
- 14.9 Use of Confirmatory Methods**—None
- 14.10 Demonstration of Specificity**—Specificity is demonstrated by chromatographic retention time (within 3% of standard) and the mass spectral response of unique product ions generated from a characteristic primary ion.
- 14.11 Documentation**
 - 14.11.1** If criteria listed in this method performance section are not met, maintenance may be performed on the system and samples reanalyzed, or other actions taken as determined by the analyst. Document all actions in the appropriate logbook.
 - 14.11.2** 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.

15.0 POLLUTION PREVENTION AND WASTE MANAGEMENT

- 15.1** Sample extract waste and flammable solvent is discarded in high BTU containers, and glass pipette waste is discarded in broken glass containers located in the laboratory.

16.0 RECORDS

- 16.1** Each page generated for a study must have the following information included, either in the header or hand-written on the page: study or project number, acquisition method, integration method, sample name, extraction date, dilution factor (if applicable), and analyst.
- 16.2** Print the tune page, sample list, and acquisition method from MassLynx to include in the appropriate study folder. Copy these pages and tape into the instrument run log.
- 16.3** Plot the calibration curves as described in this method, then print these graphs and store in the study folder.
- 16.4** Print data integration summary, integration method, and chromatograms, from MassLynx, and store in the study folder.
- 16.5** Summarize data using suitable software (MS Excel 97) and store in the study folder.
- 16.6** Back up electronic data to appropriate medium. Record in study notebook the file name and location of backup electronic data.

17.0 ATTACHMENTS

- 17.1** Attachment A: Figures—Fluorochemical Compounds

18.0 REFERENCES

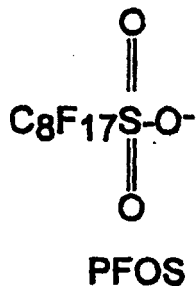
- 18.1** "Guidelines and Format for Methods to be Proposed at 40 CFR Part 136 or Part 141", U.S. Environmental Protection Agency, Office of Science and Technology Office of Water, Washington, D.C. Draft 1996.
- 18.2** "Method of Analysis for the Determination of Perfluorooctane sulfonate (PFOS), Perfluorooctane sulfonylamide (PFOSA), and Perfluorooctanoate (POAA) in Water", E. Wickremesinhe and J. Flaherty, Study Number 023-002, Centre Analytical Laboratories, Inc., State College, Pennsylvania, January 2000.
- 18.3** Validation report for the "Method of Analysis for the Determination of Perfluorooctane sulfonate (PFOS), Perfluorooctane sulfonylamide (PFOSA), and Perfluorooctanoate (POAA) in Water", E. Wickremesinhe and J. Flaherty, Study Number 023-002, Centre Analytical Laboratories, Inc., State College, Pennsylvania, (Approval pending)

19.0 REVISIONS

<u>Revision Number.</u>	<u>Reason For Revision</u>	<u>Revision Date</u>
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Figure 1: PFOS

Chemical Name = Perfluorooctane sulfonate
Molecular ion = 499 ($\text{C}_8\text{F}_{17}\text{SO}_3^-$)



Note: Standards are made from the salt, potassium perfluorooctane sulfonate [$\text{C}_8\text{F}_{17}\text{SO}_3\text{K}$], m/w 538.

Figure 2: PFOSA

Chemical Name = Perfluorooctane sulfonylamide
Molecular ion = 498 ($\text{C}_8\text{F}_{17}\text{SO}_2\text{NH}_2$)

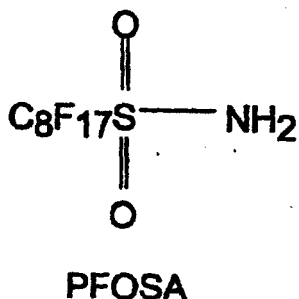
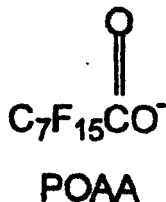


Figure 3: POAA

Chemical Name = Perfluorooctanoate
Molecular ion = 413 ($\text{C}_7\text{F}_{15}\text{COO}^-$)



Note: Standards are made from the salt, ammonium perfluorooctanoate [$\text{C}_7\text{F}_{15}\text{COONH}_4$], m/w 431