INTERIM REPORT # 17 - Analysis of Sediment and Water Samples

STUDY TITLE

Analysis of Perfluorobutanesulfonate (PFBS), Perfluorohexanesulfonate (PFHS), and Perfluorooctanesulfonate (PFOS) in Water, Soil, Sediment, Fish, Clams, Vegetation, Small Mammal Liver and Small Mammal Serum Using LC/MS/MS for the 3M Decatur Monitoring Program

DATA REQUIREMENTS

EPA TSCA Good Laboratory Practice Standards 40 CFR 792

STUDY DIRECTOR

Jaisimha Kesari P.E., DEE
Weston Solutions, Inc.
1400 Weston Way
West Chester, PA 19380
Phone: 610-701-3761

INTERIM REPORT COMPLETION DATE

June 29, 2007

PERFORMING LABORATORY

Exygen Research
3058 Research Drive
State College, PA 16801
Phone: 814-272-1039

STUDY SPONSOR

3M Company
3M Building 0236-01-B-10
St. Paul, MN 55144
Phone: 651-733-6374

PROJECT

Protocol Number: P0001131
Exygen Study Number: P0001131

Total Pages: 127

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

Exygen Study Number P0001131, entitled "Analysis of Perfluorobutanesulfonate (PFBS), Perfluorohexanesulfonate (PFHS), and Perfluorooctanesulfonate (PFOS) in Water, Soil, Sediment, Fish, Clams, Vegetation, Small Mammal Liver and Small Mammal Serum Using LC/MS/MS for the 3M Decatur Monitoring Program," conducted for 3M Company, is being performed in compliance with EPA TSCA Good Laboratory Practice Standards 40 CFR 792 by Exygen Research.

Charles Simons
Principal Investigator
Exygen Research

6/29/07
Date

Jaisimha Kesari P.E., DEE
Study Director
Weston Solutions, Inc.

7/2/07
Date

Michael A. Santoro
Sponsor Representative
3M Company

07/03/07
Date

QUALITY ASSURANCE STATEMENT

Exygen Research's Quality Assurance Unit reviewed Exygen Study Number P0001131, entitled, "Analysis of Perfluorobutanesulfonate (PFBS), Perfluorohexanesulfonate (PFHS), and Perfluorooctanesulfonate (PFOS) in Water, Soil, Sediment, Fish, Clams, Vegetation, Small Mammal Liver and Small Mammal Serum Using LC/MS/MS for the 3M Decatur Monitoring Program". All reviewed phases[1] were inspected for conduct according to Exygen Research's Standard Operating Procedures, the Study Protocol, and all applicable Good Laboratory Practice Standards. All findings were reported to the Exygen Principal Investigator and Management and to the Study Director.

Phase	Date Inspected	Date Reported to Principal Investigator	Date Reported to Exygen Management	Date Reported to Study Director
23. Raw Data and Interim Report Review	02/23-24/06	03/09/06	03/10/06	04/03/06
52. Final Interim Report Review	06/07/07	06/12/07	06/15/07	06/18/07

Lynann Porter — 6/18/07

Lynann Porter
Quality Assurance Group Leader, Quality Assurance Unit

Date

[1]Note: All in-lab inspections will be documented in the QA statement for the final analytical report at the conclusion of the study. This QA statement involves only the review of the interim report and associated raw data.

CERTIFICATION OF AUTHENTICITY

This interim report, for Exygen Study Number P0001131, is a true and complete representation of the raw data.

Submitted by: Exygen Research
3058 Research Drive
State College, PA 16801
(814) 272-1039

Principal Investigator, Exygen:

Charles Simons
Director, Analytical Laboratory Operations
Exygen Research

6/29/07
Date

Exygen Research Facility Management:

Richard A. Grazzini
Executive Director of Analytical Sciences
Exygen Research

29-JUN-07
Date

Study Director, Weston Solutions, Inc.

Jaisimha Kesari P.E., DEE
Weston Solutions, Inc.

7/2/07
Date

Sponsor Representative, 3M Company:

Michael A. Santoro
Director of Regulatory Affairs

07/03/07
Date

STUDY IDENTIFICATION

Analysis of Perfluorobutanesulfonate (PFBS), Perfluorohexanesulfonate (PFHS), and Perfluorooctanesulfonate (PFOS) in Water, Soil, Sediment, Fish, Clams, Vegetation, Small Mammal Liver and Small Mammal Serum Using LC/MS/MS for the 3M Decatur Monitoring Program

PROTOCOL NUMBER:	P0001131
EXYGEN STUDY NUMBER:	P0001131
TYPE OF STUDY:	Residue
SAMPLE MATRIX:	Sediment and Water
TEST SUBSTANCE:	Perfluorobutanesulfonate (PFBS), Perfluorohexanesulfonate (PFHS), and Perfluorooctanesulfonate (PFOS)
SPONSOR:	3M Company 3M Building 0236-01-B-10 St. Paul, MN 55144
STUDY DIRECTOR:	Jaisimha Kesari P.E., DEE Weston Solutions, Inc. 1400 Weston Way West Chester, PA 19380
STUDY MONITOR:	Michael A. Santoro 3M Company 3M Building 0236-01-B-10 St. Paul, MN 55144
PERFORMING LABORATORY:	Exygen Research 3058 Research Drive State College, PA 16801
ANALYTICAL PHASE TIMETABLE:	Study Initiation Date: 11/05/04 Interim Analytical Start Date: 11/11/05 Interim Analytical Termination Date: 12/09/05 Interim Report Completion Date: 06/29/07

PROJECT PERSONNEL

The Study Director for this project is Jaisimha Kesari at Weston Solutions, Inc. The following personnel from Exygen Research were associated with various phases of this interim portion of the study:

Name	Title
John Flaherty	Vice President
Charles Simons	Director, Analytical Laboratory Operations
Karen Risha	Scientist
Eric Edwards	Sample Custodian
Christine Edwards	Technician
Mark Ammerman	Sample Custodian
Amy Sheehan	Associate Scientist
Mindy Cressley	Technician
Brittany Kravets	Technician
Krista Gallant	Technician

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1.0 SUMMARY

Exygen Research extracted and analyzed sediment samples for the determination of perfluorobutanesulfonate (PFBS), perfluorohexanesulfonate (PFHS), and perfluorooctanesulfonate (PFOS) according to Exygen Method V0001782 and water samples for the determination of perfluorobutanesulfonate (PFBS), perfluorohexanesulfonate (PFHS), and perfluorooctanesulfonate (PFOS) according to Exygen Method V0001780 (**Appendix A**).

The limit of quantitation for PFBS, PFHS, and PFOS in sediment was 0.2 ng/g. The LOQ provided is assuming wet weight values, and the results for the actual samples are reported based on dry weight.

The limit of quantitation for PFBS, PFHS, and PFOS in water was 25 ng/L.

Analytical results and assessed accuracies for the analysis of PFBS, PFHS, and PFOS in sediment samples based on dry weight are summarized in **Table I**. Fortification recoveries for the analysis of PFBS, PFHS, and PFOS in sediment samples based on wet weight are summarized in **Table III**. The overall average percent recoveries ± standard deviations for PFBS, PFHS, and PFOS in sediment samples based on wet weight are 79 ± 9%, 98 ± 14%, and 84 ± 24%, respectively. Analytical results and assessed accuracies for the analysis of PFBS, PFHS, and PFOS in water samples are summarized in **Table II**. Fortification recoveries for the analysis of PFBS, PFHS, and PFOS in water samples are summarized in **Table IV.** The overall average percent recoveries ± standard deviations for PFBS, PFHS, and PFOS in water samples are 88 ± 18%, 105 ± 19%, and 87 ± 22%, respectively.

Quantitative analytical results were obtained for all samples except for PFBS in three water samples, PFHS in one water sample, and PFOS in two sediment and two water samples that are designated as not reported (NR) due to quality control failures.

2.0 OBJECTIVE

The objective of the analytical part of this study was to determine levels of perfluorobutanesulfonate (PFBS), perfluorohexanesulfonate (PFHS), and perfluorooctanesulfonate (PFOS) in sediment and water according to Protocol P0001131 (**Appendix A**).

3.0 INTRODUCTION

This report details the results of the analysis for the determination of PFBS, PFHS, and PFOS in sediment using the analytical method entitled, "V0001782: Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Sediment by LC/MS/MS" and for the determination of PFBS, PFHS, and PFOS in water using the analytical method entitled, "V0001780: Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Water by LC/MS/MS."

The study was initiated on November 05, 2004, when the study director signed protocol number P0001131. The analytical start date for this interim report was November 11, 2005, and the analytical termination date for this interim report was December 9, 2005.

4.0 ANALYTICAL TEST SAMPLES

Twelve sediment (SD) samples and one hundred and three water samples (Exygen ID C0091529 – C0091656) representing twelve sampling sites for sediment, surface water and interstitial (pore) water samples and associated trip blanks, trip blank spikes, and equipment rinseate blank samples were received on wet ice on September 2, 2005 from Tim Frinak at Weston Solutions, Inc. Please note that rinseate blank samples do not have associated field spike samples. Also note that due to the limited volume of sample available at some of the low-yielding pore water sampling locations, not all interstitial water samples include complete field spike series. The samples were logged in by Exygen personnel and placed in refrigerated storage.

Sample identification (ID) codes are of the form DNS-xx-STA0xx-x(x)-05082x and are composed of the strings described below:

The first string defines the general sampling area where DNS = Decatur near shore.

The second string defines the matrix where SW = surface water, IW = interstitial (pore) water and SD = sediment.

The third string indicates the sampling station number (1-12).

The fourth string describes the sample aliquot where 0 = primary sample volume, DB = duplicate sample volume, LS = low spike, HS = high spike and RB = rinseate blank.

The final string is the sample collection date in YYMMDD format.

Sample login and chain of custody information is located in the raw data package associated with this interim report. Storage records will be kept at Exygen Research.

5.0 REFERENCE MATERIAL

The analytical standards, PFBS and PFHS, were supplied by 3M. PFBS was received from 3M at Exygen on May, 13, 2005. PFHS was received from 3M at Exygen on January, 20, 2003. PFOS was purchased from Fluka Corporation and was received at Exygen on April, 23, 2003.

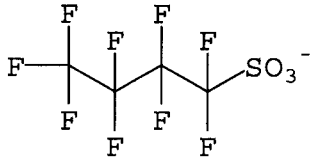
The available information for the reference materials is listed below. PFBS and PFHS were stored frozen and PFOS was stored refrigerated.

Compound	Exygen Inventory No.	Lot #	Purity (%)	Expiration Date
PFBS	SP0005726	101	96.7	12/04/06
PFHS	SP0002401	SE036	98.6	10/18/06
PFOS	SP0002694	430180-1	101.2	10/31/07

The molecular structures of PFBS, PFHS and PFOS are given below:

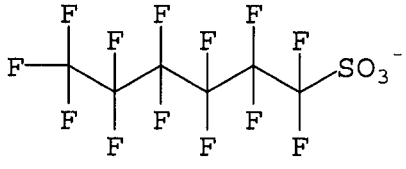
PFBS

Chemical Name: Perfluorobutanesulfonate
Molecular Weight: 338 supplied as the potassium salt ($C_4F_9SO_3^-K^+$)
Transitions Monitored: 299 → 99
Structure:



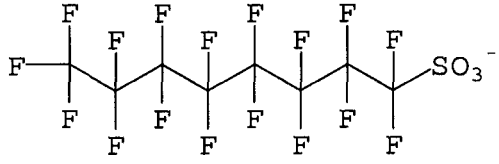
PFHS

Chemical Name: Perfluorohexanesulfonate
Molecular Weight: 438 supplied as the potassium salt ($C_6F_{13}SO_3^-K^+$)
Transitions Monitored: 399 → 80
Structure:



PFOS

Chemical Name: Perfluorooctanesulfonate
Molecular Weight: 538 supplied as the potassium salt ($C_8F_{17}SO_3^-K^+$)
Transitions Monitored: 499 → 80
Structure:



6.0 DESCRIPTION OF ANALYTICAL METHOD

The analytical methods "V0001782: Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Sediment by LC/MS/MS" and "V0001780: Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Water by LC/MS/MS" were used for this study.

6.1. Extraction Procedure For Sediment

Before the samples were weighed for the extraction, they were mixed thoroughly by vigorously shaking the container. A 5 gram portion of sediment was weighed into a fifty milliliter centrifuge tube for the extraction. After fortification of appropriate samples, 35 mL of 1% acetic acid in water was added to the samples. The samples were vortexed and allowed to shake on a wrist action shaker for ~60 minutes. The samples were centrifuged for ~20 minutes at ~3000 rpm. The supernatant was then loaded onto a C_{18} SPE cartridge conditioned with 10 mL of methanol and 20 mL of water. The eluate was discarded. Twenty milliliters of methanol was added to the sediment samples left in the centrifuge tube. The samples were vortexed and allowed to shake on a wrist action shaker for another 30 minutes. The samples were centrifuged again for ~20 minutes at ~3000 rpm. The supernatant was then loaded onto the same C_{18} SPE cartridge. The eluate was collected into a 500 mL Nalgene Bottle. The column was washed with 4 mL of methanol. The wash was collected in the same bottle as the eluate. Approximately two hundred milliliters of water was added to the bottles. The samples were mixed by shaking and loaded onto another C_{18} SPE cartridge conditioned with 10 mL of methanol and 20 mL of water. The eluate was discarded. Approximately five milliliters of methanol was added to the cartridge. Five milliliters of eluate was collected into a graduated 15 mL polypropylene centrifuge tube. Each sample was analyzed by LC/MS/MS electrospray.

6.2 Percent Solids Procedure For Sediment

Percent solids were determined using the procedure indicated in Exygen method V0000427. Approximately 20 grams of sample was weighed into a pan. The weight of the sample plus the pan was recorded. The samples were then dried in an oven overnight at 104 ± 2 °C. Then the samples were transferred to a dessicator and allowed to cool for ~15 minutes. Each sample was then weighed again, including the weight of the pan. The percent solid for each sample was then calculated. See **Table V** for percent solids results.

6.3 Extraction Procedure for Water

A 40 mL aliquot of the water sample was used for the extraction procedure. After fortification of appropriate samples, the samples were loaded onto a C_{18} SPE cartridge conditioned with 10 mL of methanol and 5 mL of water. The eluate was discarded. Approximately five milliliters of methanol was added to the cartridge. Five milliliters of eluate was collected into a graduated 15 mL polypropylene centrifuge tube. Each sample was analyzed by LC/MS/MS electrospray.

6.4 Preparation of Standards and Fortification Solutions

A mixed stock standard solution of PFBS, PFHS, and PFOS was prepared at a concentration of 1000 µg/mL by dissolving 10 mg of each of the standards (corrected for purity and salt content) in methanol. From this solution, a mixed 100 µg/mL fortification standard solution was prepared by taking 10 mL of the appropriate stock solution and bringing the volume up to 100 mL with methanol. By taking 10 mL of the mixed 100 µg/mL fortification standard and bringing the volume up to 100 mL with methanol, a mixed 10 µg/mL fortification standard was prepared. By taking 10 mL of the mixed 10 µg/mL fortification standard and bringing the volume up to 100 mL with methanol, a mixed 1.0 µg/mL fortification standard was prepared. By taking 10 mL of the mixed 1.0 µg/mL fortification standard and bringing the volume up to 100 mL with methanol, a mixed 0.1 µg/mL fortification standard was prepared. By taking 10 mL of the mixed 0.1 µg/mL fortification standard and bringing the volume up to 100 mL with methanol, a mixed 0.01 µg/mL fortification standard was prepared.

A set of non-extracted standards containing PFBS, PFHS and PFOS was prepared in methanol as specified in Exygen method V0001780. The following concentrations were prepared:

Conc. of Fort Solution (ng/mL)[1]	Fort Volume (mL)	Volume of Fortified Sample (mL)	Final Conc. of Calibration Std. (ng/mL)
100	10	100	10
100	5.0	100	5.0

100	2.0	100	2.0
10	10	100	1.0
5.0	10	100	0.5
2.0	10	100	0.2

[1] of PFBS, PFHS and PFOS

The stock standard solution and all fortification and calibration standard solutions were stored in a refrigerator (4° ± 2°C) when not in use. Documentation of standard preparation is located in the raw data package associated with this interim report.

6.5 Chromatography

Quantification of PFBS, PFHS and PFOS was accomplished by LC/MS/MS electrospray. The retention times of PFBS, PFHS and PFOS were ~0.5 mins, ~8.4 mins, and ~11.0 mins, respectively. Peaks above the LOQ were not detected in any of the reagent blank samples corresponding to the analyte retention time.

6.6 Instrument Sensitivity

The smallest standard amount injected during the chromatographic run had a concentration of 0.2 ng/mL of PFBS, PFHS and PFOS.

6.7 Description of LC/MS/MS Instrument and Operating Conditions

Instrument: API 4000 Biomolecular Mass Analyzer

Interface: Turbo Ion Spray Liquid Introduction Interface

Computer: DELL OptiPlex GX400

Software: Windows NT, Analyst 1.4.1

HPLC: Hewlett Packard (HP) Series 1100
HP Quat Pump
HP Vacuum Degasser
HP Autosampler
HP Column Oven

HPLC Column: Thermo Fluophase RP, 50 mm x 2.1 mm
Column Temp.: ~30° C
Injection Vol.: 15 µL
Mobile Phase (A): 2 mM Ammonium Acetate in water
Mobile Phase (B): Methanol

Time (min)	% A	% B
0.0	65	35
1.0	65	35
8.0	25	75
10.0	25	75
11.0	65	35
18.0	65	35

Total run time: ~18 min

Flow Rate: 0.3 mL/min

Ions monitored:

Analyte	Mode	Transition Monitored	Approximate Retention Time (min)
PFBS	negative	299 → 99	~0.5 min.
PFHS	negative	399 → 80	~8.4 min.
PFOS	negative	499 → 80	~11.0 min.

6.8 Quantitation and Example Calculation

Fifteen microliters of sample or calibration standard were injected into the LC/MS/MS. The peak area was measured and the standard curve was generated (using 1/x fit weighted linear regression) by Analyst software using six concentrations of standards. The concentration was determined from the following equations.
Equation 1 calculated the amount of analyte found (in ng/mL, based on peak area) using the standard curve (linear regression parameters) generated by the Analyst software program.

Equation 1:

$$\text{Analyte found (ng/mL)} = \frac{(\text{Peak area} - \text{intercept})}{\text{slope}} \times \text{DF}$$

Where: DF = Dilution Factor, factor by which the final volume was diluted, if necessary.

For samples fortified with known amounts of PFBS, PFHS and PFOS prior to extraction, Equation 2 was used to calculate the percent recovery.

Equation 2:

$$\text{Recovery (\%)} = \frac{(\text{analyte found (ng/mL)} - \text{analyte in control (ng/mL)})}{\text{amount added (ng/mL)}} \times 100\%$$

Note: For the PFBS, PFHS, and PFOS recovery calculations, the "control" is the unspiked aliquot of the primary field sample. Recovery is based on ng/mL for water and on ng/g (ppb) for sediment.

Equation 3 was used to convert the amount of PFBS, PFHS and PFOS found in ng/mL to ng/g (ppb).

Equation 3:

$$\text{Analyte found (ppb)} = \frac{[\text{Analyte found (ng/mL)} \times \text{final volume (5 mL)}]}{\text{sample weight (5 g)}}$$

Equation 4 was then used to calculate the amount of PFBS, PFHS and PFOS found in ppb based on dry weight.

Equation 4:

Analyte found (ppb) dry weight = Analyte found (ppb) x [100% / total solids(%)]

An example of a calculation using an actual sample follows (calculation is for PFHS only):

Sediment sample Exygen ID: C0091582 Spk E (Set: 112105B), fortified at 4 ng/mL with where:

peak area	=	547626
intercept	=	3300
slope	=	119000
dilution factor	=	1
ng/g PFHS added (fort level)	=	4
amt in corresponding sample	=	0.491
final volume (mL)	=	5
sample weight (g)	=	5
total solids (%)	=	56.01

From equation 1:

$$\text{Analyte found (ng/L)} = \frac{[547626 - 3300] \times 1}{119000}$$

$$= 4.57 \text{ ng/mL}$$

From equation 2:

$$\text{\% Recovery} = \frac{(4.57 \text{ ng/g} - 0.491 \text{ ng/g})}{4 \text{ ng/g}} \times 100\%$$

$$= 102 \%$$

From equation 3:

$$\text{Analyte found (ppb)} = \frac{(4.57 \text{ ng/mL} \times 5 \text{ mL})}{5 \text{ g}}$$

$$= 4.57 \text{ ppb}$$

From equation 4:

Analyte found (ppb) dry weight = 4.57 ppb x (100% / 56.01%)

= 8.16 ppb

NOTE: This value may be slightly different than that of the raw data due to rounding.

7.0 EXPERIMENTAL DESIGN

For sediment samples designated as laboratory matrix spikes, PFBS, PFHS, and PFOS were added at a known concentration to the samples in the laboratory after the samples were weighed for extraction. For water samples designated as field matrix spikes, PFBS, PFHS, and PFOS were added at a known concentration to the sample bottles in the laboratory before being shipped to the field. The samples were filled to a 200 mL volumetric fill line in the field.

The sediment samples were extracted in four sets, one of which was a re-extraction. Each set included one reagent blank and two reagent blanks fortified at known concentrations. Two sets contained five sample sites and one set contained two sample sites. One sample was collected for each sample site. For each site, two laboratory matrix spikes and a laboratory duplicate were extracted.

The water samples were extracted in ten sets, two of which contained re-extractions. Each set included one reagent blank, two reagent blanks fortified at known concentrations. Set one contained pore water and surface water for one sample site. Set two contained pore water for two sample sites and surface water for one sample site. Set three contained pore water for one sample site and surface water for two sample sites. Sets four and five each contained pore water for two sample sites and surface water for two sample sites. Set six contained pore water for two sample sites and surface water for one sample site. Set seven contained pore water for one sample site and surface water for two sample sites. Set eight contained pore water for one sample site, surface water for one sample site, two rinseate blanks, and three trip blanks with their associated field spikes. Set nine was a re-extraction of pore water for one sample site and surface water for two sample sites. Set ten was a re-extraction of pore water for two sample sites and surface water for two sample sites. For each site and type of water, a sample, a field duplicate and two-matrix field spikes were collected. For each site and type of water, a laboratory duplicate was extracted and two laboratory matrix spikes were also extracted.

8.0 RESULTS

Analytical results and assessed accuracies for the analysis of PFBS, PFHS, and PFOS in sediment samples based on dry weight are summarized in **Table I**. Fortification recoveries for the analysis of PFBS, PFHS, and PFOS in sediment samples based on wet

weight are summarized in **Table III**. The overall average percent recoveries ± standard deviations for PFBS, PFHS, and PFOS in sediment samples based on wet weight are 79 ± 9%, 98 ± 14%, and 84 ± 24%, respectively. Analytical results and assessed accuracies for the analysis of PFBS, PFHS, and PFOS in water samples are summarized in **Table II**. Fortification recoveries for the analysis of PFBS, PFHS, and PFOS in water samples are summarized in **Table IV**. The overall average percent recoveries ± standard deviations for PFBS, PFHS, and PFOS in water samples are 88 ± 18%, 105 ± 19%, and 87 ± 22%, respectively. Quantitative analytical results were obtained for all samples except for PFBS in three water samples, PFHS in one water sample, and PFOS in two sediment and two water samples that are designated as not reported (NR) due to quality control failures.

Accuracies were assessed for each sample by reviewing the individual QC results obtained for each sample site. For the sediment samples, there were two laboratory matrix spike recovery results available for each sample that were used to assess the accuracy. For the water samples, there were two laboratory and two field spike recovery results available for each sample site that were used to assess the accuracy. In instances of failed laboratory or field spike recoveries, the samples were not reported due to quality control failure, and recoveries associated with other spikes were used to assess sample accuracy.

9.0 CONCLUSIONS

Except as noted above, the sediment and water samples were successfully extracted and analyzed for PFBS, PFHS and PFOS according to analytical methods V0001782 and V0001780, respectively. There were no circumstances that would have affected the quality or integrity of the data.

10.0 RETENTION OF DATA AND SAMPLES

When the final analytical report is complete, all original paper data generated by Exygen Research will be shipped to the study director. This does not include facility-specific raw data such as instrument or temperature logs. Exact copies of all raw data, as well as a signed copy of the final analytical report and all original facility-specific raw data, will be retained in the Exygen Research archives for the period of time specified in EPA TSCA Good Laboratory Practice Standards 40 CFR 792.

TABLES

Table I. Summary of PFBS, PFHS and PFOS in Sediment Samples

		C4 Sulfonate PFBS Perfluorobutanesulfonate		C6 Sulfonate PFHS Perfluorohexanesulfonate		C8 Sulfonate PFOS Perfluorooctanesulfonate	
Exygen ID	Client Sample ID	Analyte Found (ppb, ng/g) Dry Weight	Assessed Accuracy (+/- %)	Analyte Found (ppb, ng/g) Dry Weight	Assessed Accuracy (+/- %)	Analyte Found (ppb, ng/g) Dry Weight	Assessed Accuracy (+/- %)
C0091547	DNS-SD-STA001-0-050824	0.979	30	1.29	30	21.1	30
C0091547 Rep	DNS-SD-STA001-0-050824*	0.787	30	1.42	30	21.3	30
C0091557	DNS-SD-STA002-0-050824	0.452	30	0.435	30	NR	-
C0091557 Rep	DNS-SD-STA002-0-050824*	0.494	30	0.427	30	NR	-
C0091566	DNS-SD-STA003-0-050824	0.519	30	ND	30	10.1	30
C0091566 Rep	DNS-SD-STA003-0-050824*	0.617	30	ND	30	11.7	30
C0091579	DNS-SD-STA004-0-050825	ND	30	ND	30	1.22	30
C0091579 Rep	DNS-SD-STA004-0-050825*	ND	30	ND	30	1.41	30
C0091580	DNS-SD-STA005-0-050825	ND	30	2.08	30	88.0	40
C0091580 Rep	DNS-SD-STA005-0-050825*	ND	30	2.03	30	88.0	40
C0091581	DNS-SD-STA006-0-050825	ND	30	ND	30	0.810	30
C0091581 Rep	DNS-SD-STA006-0-050825*	ND	30	ND	30	0.810	30
C0091582	DNS-SD-STA007-0-050825	ND	30	0.877	30	26.1	30
C0091582 Rep	DNS-SD-STA007-0-050825*	0.357	30	1.25	30	25.7	30
C0091583	DNS-SD-STA008-0-050825	ND	30	0.421	30	16.4	40
C0091583 Rep	DNS-SD-STA008-0-050825*	ND	30	0.428	30	16.1	40
C0091584	DNS-SD-STA009-0-050825	4.13	30	6.20	50	24.7	30
C0091584 Rep	DNS-SD-STA009-0-050825*	4.72	30	6.66	50	26.5	30
C0091585	DNS-SD-STA010-0-050825	0.392	30	1.30	30	NR	-
C0091585 Rep	DNS-SD-STA010-0-050825*	0.430	30	1.21	30	NR	-
C0091586	DNS-SD-STA011-0-050825	0.582	30	ND	30	4.67	40
C0091586 Rep	DNS-SD-STA011-0-050825*	ND	30	0.393	30	4.53	40
C0091587	DNS-SD-STA012-0-050825	0.822	30	0.553	30	15.7	30
C0091587 Rep	DNS-SD-STA012-0-050825*	0.743	30	0.905	30	15.6	30

*Laboratory Duplicate
ND = Not detected at or above the Limit of Quantitation (LOQ) of 0.2 ng/g (wet weight).
NR = Not reported due to quality control failure.

Table II. Summary of PFBS, PFHS and PFOS in Water Samples

		C4 Sulfonate PFBS Perfluorobutanesulfonate		C6 Sulfonate PFHS Perfluorohexanesulfonate		C8 Sulfonate PFOS Perfluorooctanesulfonate	
Exygen ID	Client Sample ID	Analyte Found (ppt, ng/L)	Assessed Accuracy (+/- %)	Analyte Found (ppt, ng/L)	Assessed Accuracy (+/- %)	Analyte Found (ppt, ng/L)	Assessed Accuracy (+/- %)
C0091529	DNS-SW-TRIP01-0-050824	ND	30	ND	30	ND	30
C0091533	DNS-SW-TRIP02-0-050824	ND	30	ND	30	ND	30
C0091536	DNS-SW-TRIP03-0-050824	ND	30	ND	30	ND	30
C0091539	DNS-IW-STA001-0-050823	682	30	790	50	20000	50
C0091539 Rep	DNS-IW-STA001-0-050823*	690	30	832	50	19100	50
C0091540	DNS-IW-STA001-DB-050823	666	30	793	50	18500	50
C0091543	DNS-SW-STA001-0-050823	111	30	24.0	30	164	30
C0091543 Rep	DNS-SW-STA001-0-050823*	110	30	22.4	30	148	30
C0091544	DNS-SW-STA001-DB-050823	101	30	18.8	30	128	30
C0091548	DNS-SD-STA001-RB-050824	ND	30	ND	30	ND	30
C0091549	DNS-IW-STA002-0-050824	364	30	620	40	2860	40
C0091549 Rep	DNS-IW-STA002-0-050824*	368	30	633	40	3170	40
C0091550	DNS-IW-STA002-DB-050824	353	30	632	40	3140	40
C0091553	DNS-SW-STA002-0-050825	140	30	59.2	30	403	30
C0091553 Rep	DNS-SW-STA002-0-050825*	130	30	48.5	30	346	30
C0091554	DNS-SW-STA002-DB-050825	134	30	50.6	30	358	30
C0091558	DNS-IW-STA003-0-050824	76.4	30	51.1	30	645	30
C0091558 Rep	DNS-IW-STA003-0-050824*	79.2	30	44.4	30	593	30
C0091559	DNS-IW-STA003-DB-050824	77.0	30	64.5	30	633	30
C0091562	DNS-SW-STA003-0-050824	58.7	30	42.7	30	180	30
C0091562 Rep	DNS-SW-STA003-0-050824*	59.5	30	43.1	30	165	30
C0091563	DNS-SW-STA003-DB-050824	58.1	30	47.3	30	200	30
C0091588	DNS-IW-STA004-0-050824	75.8	30	45.2	30	184	50
C0091588 Rep	DNS-IW-STA004-0-050824*	77.1	30	45.5	30	151	50
C0091589	DNS-IW-STA004-DB-050824	61.9	30	30.4	30	131	50
C0091592	DNS-SW-STA004-0-050824	134	30	178	30	555	30
C0091592 Rep	DNS-SW-STA004-0-050824*	133	30	168	30	478	30
C0091593	DNS-SW-STA004-DB-050824	131	30	180	30	555	30
C0091596	DNS-IW-STA005-0-050823	86.9	30	21.1	30	167	30
C0091596 Rep	DNS-IW-STA005-0-050823*	90.1	30	22.8	30	158	30
C0091597	DNS-IW-STA005-DB-050823	86.2	30	21.3	30	167	30
C0091600	DNS-SW-STA005-0-050823	64.5	30	16.9	30	124	30
C0091600 Rep	DNS-SW-STA005-0-050823*	61.4	30	15.8	30	99.8	30
C0091601	DNS-SW-STA005-DB-050823	66.9	30	15.8	30	136	30
C0091604	DNS-IW-STA006-0-050824	58.2	30	57.4	30	263	30
C0091604 Rep	DNS-IW-STA006-0-050824*	56.1	30	57.4	30	249	30
C0091605	DNS-IW-STA006-DB-050824	59.6	30	61.0	30	320	30

*Laboratory Duplicate
ND = Not detected at or above the Limit of Quantitation (LOQ) of 25 ng/L.

Table II. Summary of PFBS, PFHS and PFOS in Water Samples Continued

		C4 Sulfonate PFBS Perfluorobutanesulfonate		C6 Sulfonate PFHS Perfluorohexanesulfonate		C8 Sulfonate PFOS Perfluorooctanesulfonate	
Exygen ID	Client Sample ID	Analyte Found (ppt, ng/L)	Assessed Accuracy (+/- %)	Analyte Found (ppt, ng/L)	Assessed Accuracy (+/- %)	Analyte Found (ppt, ng/L)	Assessed Accuracy (+/- %)
C0091608	DNS-SW-STA006-0-050824	NR	-	39.6	40	NR	-
C0091608 Rep	DNS-SW-STA006-0-050824*	NR	-	36.6	40	NR	-
C0091609	DNS-SW-STA006-DB-050824	NR	-	41.6	40	NR	-
C0091612	DNS-IW-STA007-0-050825	578	30	703	30	16200	30
C0091612 Rep	DNS-IW-STA007-0-050825*	564	30	678	30	15700	30
C0091613	DNS-IW-STA007-DB-050825	500	30	675	30	18100	30
C0091615	DNS-SW-STA007-0-050825	132	30	56.9	30	388	30
C0091615 Rep	DNS-SW-STA007-0-050825*	132	30	56.1	30	386	30
C0091616	DNS-SW-STA007-DB-050825	131	30	54.5	30	387	30
C0091619	DNS-IW-STA008-0-050825	6020	30	5190	30	901	30
C0091619 Rep	DNS-IW-STA008-0-050825*	5860	30	4960	30	857	30
C0091620	DNS-IW-STA008-DB-050825	5700	30	5230	30	1130	30
C0091623	DNS-SW-STA008-0-050825	217	30	158	30	NR	-
C0091623 Rep	DNS-SW-STA008-0-050825*	218	30	163	30	NR	-
C0091624	DNS-SW-STA008-DB-050825	220	30	163	30	NR	-
C0091627	DNS-IW-STA009-0-050826	8580	30	11800	30	3260	30
C0091627 Rep	DNS-IW-STA009-0-050826*	8650	30	11900	30	3090	30
C0091628	DNS-IW-STA009-DB-050826	8710	30	12600	30	4220	30
C0091631	DNS-SW-STA009-0-050826	53.4	30	40.7	30	134	30
C0091631 Rep	DNS-SW-STA009-0-050826*	48.1	30	36.8	30	107	30
C0091632	DNS-SW-STA009-DB-050826	46.0	30	36.2	30	106	30
C0091635	DNS-IW-STA010-0-050826	NR	-	846	40	19200	30
C0091635 Rep	DNS-IW-STA010-0-050826*	NR	-	818	40	17700	30
C0091636	DNS-IW-STA010-DB-050826	NR	-	837	40	21500	30
C0091638	DNS-SW-STA010-0-050826	73.2	40	53.6	30	139	40
C0091638 Rep	DNS-SW-STA010-0-050826*	73.5	40	52.1	30	122	40
C0091639	DNS-SW-STA010-DB-050826	79.2	40	57.5	30	177	40
C0091642	DNS-IW-STA011-0-050826	1880	30	844	30	850	30
C0091642 Rep	DNS-IW-STA011-0-050826*	1830	30	807	30	756	30
C0091643	DNS-IW-STA011-DB-050826^	1630	30	750	30	1320	30
C0091644	DNS-SW-STA011-0-050826	NR	-	39.4	30	96.4	50
C0091644 Rep	DNS-SW-STA011-0-050826*	NR	-	47.0	30	110	50
C0091645	DNS-SW-STA011-DB-050826	NR	-	42.4	30	131	50
C0091648	DNS-IW-STA012-0-050826	8730	30	NR	-	1330	40
C0091648 Rep	DNS-IW-STA012-0-050826*	9140	30	NR	-	1220	40
C0091649	DNS-IW-STA012-DB-050826	8850	30	NR	-	1230	40
C0091652	DNS-SW-STA012-0-050826	53.3	30	42.2	30	142	30
C0091652 Rep	DNS-SW-STA012-0-050826*	56.5	30	40.0	30	144	30
C0091653	DNS-SW-STA012-DB-050826	57.3	30	41.4	30	187	30
C0091656	DNS-IW-STA012-RB-050825	ND	30	ND	30	ND	30

*Laboratory Duplicate
^ Sample contained only 33 mL of water when received.
ND = Not detected at or above the Limit of Quantitation (LOQ) of 25 ng/L.
NR = Not reported due to quality control failure.

Table III. Matrix Spike Recovery of PFBS, PFHS and PFOS in Sediment Samples

		C4 Sulfonate PFBS Wet Weight			C6 Sulfonate PFHS Wet Weight			C8 Sulfonate PFOS Wet Weight		
Sample Description	Amount Spiked (ng/g)	Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)
DNS-SD-STA001-0-050824 (C0091547 Spk C, 4 ppb Spike)	4	0.565	3.49	73	0.743	4.95	105	12.2	34.8	*
DNS-SD-STA001-0-050824 (C0091547 Spk D, 40 ppb Spike)	40	0.565	22.7	55	0.743	36.3	89	12.2	48.8	92
DNS-SD-STA002-0-050824 (C0091557 Spk E, 4 ppb Spike)	4	0.236	3.05	70	0.227	4.03	95	NR	NR	NR
DNS-SD-STA002-0-050824 (C0091557 Spk F, 40 ppb Spike)	40	0.236	27.6	68	0.227	39.9	99	NR	NR	NR
DNS-SD-STA003-0-050824 (C0091566 Spk G, 4 ppb Spike)	4	0.238	3.15	73	ND	4.16	104	4.61	8.31	93
DNS-SD-STA003-0-050824 (C0091566 Spk H, 40 ppb Spike)	40	0.238	31.1	77	ND	38.9	97	4.61	28.8	60
DNS-SD-STA004-0-050825 (C0091579 Spk I, 4 ppb Spike)	4	ND	2.84	71	ND	4.04	101	0.855	4.74	97
DNS-SD-STA004-0-050825 (C0091579 Spk J, 40 ppb Spike)	40	ND	28.7	72	ND	38.1	95	0.855	34.5	84
DNS-SD-STA005-0-050825 (C0091580 Spk K, 4 ppb Spike)	4	ND	3.33	83	1.23	5.49	107	52.0	51.1	*
DNS-SD-STA005-0-050825 (C0091580 Spk L, 40 ppb Spike)	40	ND	30.5	76	1.23	37.8	91	52.0	77.5	64
DNS-SD-STA006-0-050825 (C0091581 Spk C, 4 ppb Spike)	4	ND	3.63	91	ND	4.32	108	0.584	5.38	120
DNS-SD-STA006-0-050825 (C0091581 Spk D, 40 ppb Spike)	40	ND	33.6	84	ND	39.4	99	0.584	36.7	90
DNS-SD-STA007-0-050825 (C0091582 Spk E, 4 ppb Spike)	4	ND	3.47	87	0.491	4.59	102	14.6	16.1	*
DNS-SD-STA007-0-050825 (C0091582 Spk F, 40 ppb Spike)	40	ND	29.8	75	0.491	38.8	96	14.6	47.4	82
DNS-SD-STA008-0-050825 (C0091583 Spk G, 4 ppb Spike)	4	ND	3.40	85	0.251	4.43	104	9.77	11.0	31
DNS-SD-STA008-0-050825 (C0091583 Spk H, 40 ppb Spike)	40	ND	30.9	77	0.251	38.4	95	9.77	37.4	69
DNS-SD-STA009-0-050825 (C0091584 Spk I, 4 ppb Spike)	4	2.54	6.30	94	3.81	9.64	146	15.2	34.5	*
DNS-SD-STA009-0-050825 (C0091584 Spk J, 40 ppb Spike)	40	2.54	37.9	88	3.81	43.2	98	15.2	55.3	100
DNS-SD-STA010-0-050825 (C0091585 Spk K, 4 ppb Spike)	4	0.227	3.81	90	0.755	4.10	84	NR	NR	NR
DNS-SD-STA010-0-050825 (C0091585 Spk L, 40 ppb Spike)	40	0.227	36.5	91	0.755	31.3	76	NR	NR	NR

*Sample residue exceeds the spiking level significantly (3x spiking level); therefore, an accurate recovery value cannot be calculated
ND = Not detected at or above the Limit of Quantitation (LOQ) of 0.2 ng/g (wet weight).
NR = Not reported due to quality control failure.
Note: Since this summary table shows rounded results, recovery values may vary slightly from the values in the raw data.

Table III. Matrix Spike Recovery of PFBS, PFHS and PFOS in Sediment Samples Continued

		C4 Sulfonate PFBS Wet Weight			C6 Sulfonate PFHS Wet Weight			C8 Sulfonate PFOS Wet Weight		
Sample Description	Amount Spiked (ng/g)	Amt Found in Sample (ng/g)	Amount Recovered (ng/g)	Recovery (%)	Amt Found in Sample (ng/g)	Amount Recovered (ng/g)	Recovery (%)	Amt Found in Sample (ng/g)	Amount Recovered (ng/g)	Recovery (%)
DNS-SD-STA011-0-050825 (C0091586 Spk C, 4 ppb Spike)	4	0.329	3.52	80	ND	4.03	101	2.64	8.00	134
DNS-SD-STA011-0-050825 (C0091586 Spk D, 40 ppb Spike)	40	0.329	33.8	84	ND	37.5	94	2.64	34.6	80
DNS-SD-STA012-0-050825 (C0091587 Spk E, 4 ppb Spike)	4	0.385	3.70	83	0.259	3.81	89	7.34	10.8	87
DNS-SD-STA012-0-050825 (C0091587 Spk F, 40 ppb Spike)	40	0.385	27.5	68	0.259	28.9	72	7.34	33.1	64
			Average:	**79**		**Average:**	**98**		**Average:**	**84**
			Standard Deviation:	**9**		**Standard Deviation:**	**14**		**Standard Deviation:**	**24**

*Sample residue exceeds the spiking level significantly (3x spiking level); therefore, an accurate recovery value cannot be calculated
ND = Not detected at or above the Limit of Quantitation (LOQ) of 0.2 ng/g (wet weight).
Note: Since this summary table shows rounded results, recovery values may vary slightly from the values in the raw data.

Table IV. Matrix Spike Recovery of PFBS, PFHS and PFOS in Water Samples

		C4 Sulfonate PFBS			C6 Sulfonate PFHS			C8 Sulfonate PFOS		
Sample Description	Amount Spiked (ng/L)	Amt Found in Sample (ng/L)	Amount Recovered (ng/L)	Recovery (%)	Amt Found in Sample (ng/L)	Amount Recovered (ng/L)	Recovery (%)	Amt Found in Sample (ng/L)	Amount Recovered (ng/L)	Recovery (%)
DNS-SW-TRIP01-LS-050824 (C0091530, 1.0 ppb Field Spike)	1000	ND	913	91	ND	970	97	ND	1200	120
DNS-SW-TRIP01-HS-050824 (C0091531, 10 ppb Field Spike)	10000	ND	8980	90	ND	11100	111	ND	11700	117
DNS-SW-TRIP02-LS-050824 (C0091534, 1.0 ppb Field Spike)	1000	ND	960	96	ND	1160	116	ND	1180	118
DNS-SW-TRIP02-HS-050824 (C0091535, 10 ppb Field Spike)	10000	ND	10800	108	ND	11100	111	ND	10500	105
DNS-SW-TRIP03-LS-050824 (C0091537, 1.0 ppb Field Spike)	1000	ND	843	84	ND	1090	109	ND	977	98
DNS-SW-TRIP03-HS-050824 (C0091538, 10 ppb Field Spike)	10000	ND	10900	109	ND	10800	108	ND	10600	106
DNS-IW-STA001-0-050823 (C0091539 Spk C, 1.0 ppb Lab Spike)	1000	682	1710	103	790	2290	150	20000	18700	*
DNS-IW-STA001-0-050823 (C0091539 Spk D, 10 ppb Lab Spike)	10000	682	10200	95	790	13000	122	20000	25900	59
DNS-IW-STA001-LS-050823 (C0091541, 1.0 ppb Field Spike)	1000	682	1410	73	790	2170	138	20000	18400	*
DNS-IW-STA001-HS-050823 (C0091542, 10 ppb Field Spike)	10000	682	8520	78	790	12400	116	20000	25100	51
DNS-SW-STA001-0-050823 (C0091543 Spk E, 10 ppb Lab Spike)	10000	111	8550	84	24.0	9470	94	164	8870	87
DNS-SW-STA001-0-050823 (C0091543 Spk F, 100 ppb Lab Spike)	100000	111	80600	80	24.0	90800	91	164	85200	85
DNS-SW-STA001-LS-050823 (C0091545, 1.0 ppb Field Spike)	1000	111	815	70	24.0	924	90	164	859	70
DNS-SW-STA001-HS-050823 (C0091546, 10 ppb Field Spike)	10000	111	7970	79	24.0	8610	86	164	7230	71
DNS-IW-STA002-0-050824 (C0091549 Spk C, 1.0 ppb Lab Spike)	1000	364	1120	76	620	1320	70	2860	4230	137
DNS-IW-STA002-0-050824 (C0091549 Spk D, 10 ppb Lab Spike)	10000	364	8690	83	620	9950	93	2860	9740	69
DNS-IW-STA002-LS-050824 (C0091551, 1.0 ppb Field Spike)	1000	364	1120	76	620	1240	62	2860	3970	111
DNS-IW-STA002-HS-050824 (C0091552, 10 ppb Field Spike)	10000	364	7740	74	620	8940	83	2860	8850	60

*Sample residue exceeds the spiking level significantly (3x spiking level); therefore, an accurate recovery value cannot be calculated
ND = Not detected at or above the Limit of Quantitation (LOQ) of 25 ng/L.
Note: Since this summary table shows rounded results, recovery values may vary slightly from the values in the raw data.

Table IV. Matrix Spike Recovery of PFBS, PFHS and PFOS in Water Samples Continued

		C4 Sulfonate PFBS			C6 Sulfonate PFHS			C8 Sulfonate PFOS		
Sample Description	Amount Spiked (ng/L)	Amt Found in Sample (ng/L)	Amount Recovered (ng/L)	Recovery (%)	Amt Found in Sample (ng/L)	Amount Recovered (ng/L)	Recovery (%)	Amt Found in Sample (ng/L)	Amount Recovered (ng/L)	Recovery (%)
DNS-SW-STA002-0-050825 (C0091553 Spk E, 1.0 ppb Lab Spike)	1000	140	889	75	59.2	976	92	403	1310	91
DNS-SW-STA002-0-050825 (C0091553 Spk F, 10 ppb Lab Spike)	10000	140	8820	87	59.2	9850	98	403	9530	91
DNS-SW-STA002-LS-050825 (C0091555, 1.0 ppb Field Spike)	1000	140	989	85	59.2	988	93	403	1670	127
DNS-SW-STA002-HS-050825 (C0091556, 10 ppb Field Spike)	10000	140	7330	72	59.2	7610	76	403	8040	76
DNS-IW-STA003-0-050824 (C0091558 Spk G, 1.0 ppb Lab Spike)	1000	76.4	913	84	51.1	814	76	645	1510	87
DNS-IW-STA003-0-050824 (C0091558 Spk H, 10 ppb Lab Spike)	10000	76.4	8130	81	51.1	8820	88	645	9080	84
DNS-IW-STA003-LS-050824 (C0091560, 1.0 ppb Field Spike)	1000	76.4	849	77	51.1	1090	104	645	1880	124
DNS-IW-STA003-HS-050824 (C0091561, 10 ppb Field Spike)	10000	76.4	8830	88	51.1	9450	94	645	8350	77
DNS-SW-STA003-0-050824 (C0091562 Spk C, 1.0 ppb Lab Spike)	1000	58.7	949	89	42.7	1140	110	180	1050	87
DNS-SW-STA003-0-050824 (C0091562 Spk D, 10 ppb Lab Spike)	10000	58.7	9040	90	42.7	11400	114	180	8950	88
DNS-SW-STA003-LS-050824 (C0091564, 1.0 ppb Field Spike)	1000	58.7	860	80	42.7	1280	124	180	1380	120
DNS-SW-STA003-HS-050824 (C0091565, 10 ppb Field Spike)	10000	58.7	8850	88	42.7	12100	121	180	10900	107
DNS-IW-STA004-0-050824 (C0091588 Spk E, 10 ppb Lab Spike)	10000	75.8	8140	81	45.2	9630	96	184	9030	88
DNS-IW-STA004-0-050824 (C0091588 Spk F, 100 ppb Lab Spike)	100000	75.8	60900	61	45.2	77100	77	184	73800	74
DNS-IW-STA004-LS-050824 (C0091590, 1.0 ppb Field Spike)	1000	75.8	783	71	45.2	810	76	184	764	58
DNS-IW-STA004-HS-050824 (C0091591, 10 ppb Field Spike)	10000	75.8	7010	69	45.2	7920	79	184	5710	55
DNS-SW-STA004-0-050824 (C0091592 Spk G, 1.0 ppb Lab Spike)	1000	134	1030	90	178	1350	117	555	1540	99
DNS-SW-STA004-0-050824 (C0091592 Spk H, 10 ppb Lab Spike)	10000	134	8770	86	178	11600	114	555	9240	87
DNS-SW-STA004-LS-050824 (C0091594, 1.0 ppb Field Spike)	1000	134	1010	88	178	1470	129	555	1630	108
DNS-SW-STA004-HS-050824 (C0091595, 10 ppb Field Spike)	10000	134	8480	83	178	12500	123	555	8790	82

*Sample residue exceeds the spiking level significantly (3x spiking level); therefore, an accurate recovery value cannot be calculated
ND = Not detected at or above the Limit of Quantitation (LOQ) of 25 ng/L.
Note: Since this summary table shows rounded results, recovery values may vary slightly from the values in the raw data.

Table IV. Matrix Spike Recovery of PFBS, PFHS and PFOS in Water Samples Continued

		C4 Sulfonate PFBS			C6 Sulfonate PFHS			C8 Sulfonate PFOS		
Sample Description	Amount Spiked (ng/L)	Amt Found in Sample (ng/L)	Amount Recovered (ng/L)	Recovery (%)	Amt Found in Sample (ng/L)	Amount Recovered (ng/L)	Recovery (%)	Amt Found in Sample (ng/L)	Amount Recovered (ng/L)	Recovery (%)
DNS-IW-STA005-0-050823 (C0091596 Spk C, 1.0 ppb Lab Spike)	1000	86.9	945	86	21.1	1120	110	167	995	83
DNS-IW-STA005-0-050823 (C0091596 Spk D, 10 ppb Lab Spike)	10000	86.9	9450	94	21.1	11900	119	167	9020	89
DNS-IW-STA005-LS-050823 (C0091598, 1.0 ppb Field Spike)	1000	86.9	987	90	21.1	1270	125	167	1010	84
DNS-IW-STA005-HS-050823 (C0091599, 10 ppb Field Spike)	10000	86.9	8470	84	21.1	9370	93	167	9980	98
DNS-SW-STA005-0-050823 (C0091600 Spk E, 1.0 ppb Lab Spike)	1000	64.5	845	78	16.9	990	97	124	777	65
DNS-SW-STA005-0-050823 (C0091600 Spk F, 10 ppb Lab Spike)	10000	64.5	9920	99	16.9	11900	119	124	8800	87
DNS-SW-STA005-LS-050823 (C0091602, 1.0 ppb Field Spike)	1000	64.5	909	84	16.9	1200	118	124	863	74
DNS-SW-STA005-HS-050823 (C0091603, 10 ppb Field Spike)	10000	64.5	9870	98	16.9	13100	131	124	10600	105
DNS-IW-STA006-0-050824 (C0091604 Spk G, 1.0 ppb Lab Spike)	1000	58.2	940	88	57.4	1150	109	263	1130	87
DNS-IW-STA006-0-050824 (C0091604 Spk H, 10 ppb Lab Spike)	10000	58.2	9070	90	57.4	11000	109	263	8510	82
DNS-IW-STA006-LS-050824 (C0091606, 1.0 ppb Field Spike)	1000	58.2	1020	96	57.4	1360	130	263	1320	106
DNS-IW-STA006-HS-050824 (C0091607, 10 ppb Field Spike)	10000	58.2	10200	101	57.4	12800	127	263	10300	100
DNS-SW-STA006-0-050824 (C0091608 Spk I, 10 ppb Lab Spike)	10000	NR	NR	NR	39.6	10700	107	NR	NR	NR
DNS-SW-STA006-0-050824 (C0091608 Spk J, 100 ppb Lab Spike)	100000	NR	NR	NR	39.6	87000	87	NR	NR	NR
DNS-SW-STA006-LS-050824 (C0091610, 1.0 ppb Field Spike)	1000	NR	NR	NR	39.6	696	66	NR	NR	NR
DNS-SW-STA006-HS-050824 (C0091611, 10 ppb Field Spike)	10000	NR	NR	NR	39.6	9680	96	NR	NR	NR
DNS-IW-STA007-0-050825 (C0091612 Spk C, 1.0 ppb Lab Spike)	1000	578	1540	96	703	1720	102	16200	18100	*
DNS-IW-STA007-0-050825 (C0091612 Spk D, 10 ppb Lab Spike)	10000	578	10100	95	703	11800	111	16200	26200	100
DNS-IW-STA007-LS-050825 (C0091614, 1.0 ppb Field Spike)	1000	578	1410	83	703	1670	97	16200	17400	*

*Sample residue exceeds the spiking level significantly (3x spiking level); therefore, an accurate recovery value cannot be calculated
ND = Not detected at or above the Limit of Quantitation (LOQ) of 25 ng/L.
NR = Not reported due to quality control failure.
Note: Since this summary table shows rounded results, recovery values may vary slightly from the values in the raw data.

Table IV. Matrix Spike Recovery of PFBS, PFHS and PFOS in Water Samples Continued

Sample Description	C4 Sulfonate PFBS				C6 Sulfonate PFHS			C8 Sulfonate PFOS		
	Amount Spiked (ng/L)	Amt Found in Sample (ng/L)	Amount Recovered (ng/L)	Recovery (%)	Amt Found in Sample (ng/L)	Amount Recovered (ng/L)	Recovery (%)	Amt Found in Sample (ng/L)	Amount Recovered (ng/L)	Recovery (%)
DNS-SW-STA007-0-050825 (C0091615 Spk E, 1.0 ppb Lab Spike)	1000	132	979	85	56.9	1100	104	388	986	60
DNS-SW-STA007-0-050825 (C0091615 Spk F, 10 ppb Lab Spike)	10000	132	9300	92	56.9	9920	99	388	9060	87
DNS-SW-STA007-LS-050825 (C0091617, 1.0 ppb Field Spike)	1000	132	995	86	56.9	1280	122	388	1290	90
DNS-SW-STA007-HS-050825 (C0091618, 10 ppb Field Spike)	10000	132	8960	88	56.9	11000	109	388	7570	72
DNS-IW-STA008-0-050825 (C0091619 Spk G, 1.0 ppb Lab Spike)	1000	6020	6810	*	5190	6930	*	901	1810	91
DNS-IW-STA008-0-050825 (C0091619 Spk H, 10 ppb Lab Spike)	10000	6020	15400	94	5190	17100	119	901	8370	75
DNS-IW-STA008-LS-050825 (C0091621, 1.0 ppb Field Spike)	1000	6020	6180	*	5190	7310	*	901	1900	100
DNS-IW-STA008-HS-050825 (C0091622, 10 ppb Field Spike)	10000	6020	14500	85	5190	16300	111	901	6950	60
DNS-SW-STA008-0-050825 (C0091623 Spk I, 1.0 ppb Lab Spike)	1000	217	1160	94	158	1320	116	NR	NR	NR
DNS-SW-STA008-0-050825 (C0091623 Spk J, 10 ppb Lab Spike)	10000	217	9440	92	158	11900	117	NR	NR	NR
DNS-SW-STA008-LS-050825 (C0091625, 1.0 ppb Field Spike)	1000	217	934	72	158	1090	93	NR	NR	NR
DNS-SW-STA008-HS-050825 (C0091626, 10 ppb Field Spike)	10000	217	8200	80	158	10600	104	NR	NR	NR
DNS-IW-STA009-0-050826 (C0091627 Spk C, 1.0 ppb Lab Spike)	1000	8580	11100	*	11800	12800	*	3260	4920	*
DNS-IW-STA009-0-050826 (C0091627 Spk D, 10 ppb Lab Spike)	10000	8580	22400	138	11800	27600	158	3260	12400	91
DNS-IW-STA009-LS-050826 (C0091629, 1.0 ppb Field Spike)	1000	8580	11700	*	11800	13500	*	3260	5500	*
DNS-IW-STA009-HS-050826 (C0091630, 10 ppb Field Spike)	10000	8580	19500	109	11800	24700	129	3260	14500	112
DNS-SW-STA009-0-050826 (C0091631 Spk E, 1.0 ppb Lab Spike)	1000	53.4	909	86	40.7	1130	109	134	932	80
DNS-SW-STA009-0-050826 (C0091631 Spk F, 10 ppb Lab Spike)	10000	53.4	18700	186	40.7	11500	115	134	8850	87
DNS-SW-STA009-LS-050826 (C00915633, 1.0 ppb Field Spike)	1000	53.4	958	90	40.7	1290	125	134	1080	95
DNS-SW-STA009-HS-050826 (C0091634, 10 ppb Field Spike)	10000	53.4	9100	90	40.7	12600	126	134	8500	84

*Sample residue exceeds the spiking level significantly (3x spiking level); therefore, an accurate recovery value cannot be calculated
ND = Not detected at or above the Limit of Quantitation (LOQ) of 25 ng/L.
NR = Not reported due to quality control failure.
Note: Since this summary table shows rounded results, recovery values may vary slightly from the values in the raw data.

Table IV. Matrix Spike Recovery of PFBS, PFHS and PFOS in Water Samples Continued

Sample Description	C4 Sulfonate PFBS				C6 Sulfonate PFHS			C8 Sulfonate PFOS		
	Amount Spiked (ng/L)	Amt Found in Sample (ng/L)	Amount Recovered (ng/L)	Recovery (%)	Amt Found in Sample (ng/L)	Amount Recovered (ng/L)	Recovery (%)	Amt Found in Sample (ng/L)	Amount Recovered (ng/L)	Recovery (%)
DNS-IW-STA010-0-050826 (C0091635 Spk G, 1.0 ppb Lab Spike)	1000	NR	NR	NR	846	2130	128	19200	19200	*
DNS-IW-STA010-0-050826 (C0091635 Spk H, 10 ppb Lab Spike)	10000	NR	NR	NR	846	12500	117	19200	28800	96
DNS-IW-STA010-LS-050826^ (C0091637, 1.0 ppb Field Spike)	10526	NR	NR	NR	846	7900	67	19200	27500	*
DNS-SW-STA010-0-050826 (C0091638 Spk C, 10 ppb Lab Spike)	10000	73.2	16800	167	53.6	12800	127	139	18400	183
DNS-SW-STA010-0-050826 (C0091638 Spk D, 100 ppb Lab Spike)	100000	73.2	63600	64	53.6	86500	86	139	66500	66
DNS-SW-STA010-LS-050826 (C0091640, 1.0 ppb Field Spike)	1000	73.2	758	68	53.6	1080	103	139	832	69
DNS-SW-STA010-HS-050826 (C0091641, 10 ppb Field Spike)	10000	73.2	6990	69	53.6	10200	101	139	6580	64
DNS-IW-STA011-0-050826 (C0091642 Spk E, 1.0 ppb Lab Spike)	1000	1880	2840	96	844	2020	118	850	1730	88
DNS-IW-STA011-0-050826 (C0091642 Spk F, 10 ppb Lab Spike)	10000	1880	10700	88	844	11900	111	850	7860	70
DNS-SW-STA011-0-050826 (C0091644 Spk G, 10 ppb Lab Spike)	10000	NR	NR	NR	39.4	9310	93	96.4	7110	70
DNS-SW-STA011-0-050826 (C0091644 Spk H, 100 ppb Lab Spike)	100000	NR	NR	NR	39.4	97500	97	96.4	78500	78
DNS-SW-STA011-LS-050826 (C0091646, 1.0 ppb Field Spike)	1000	NR	NR	NR	39.4	746	71	96.4	607	51
DNS-SW-STA011-HS-050826 (C0091647, 10 ppb Field Spike)	10000	NR	NR	NR	39.4	6640	66	96.4	4700	46
DNS-IW-STA012-0-050826 (C0091648 Spk C, 1.0 ppb Lab Spike)	1000	8730	9060	*	NR	NR	NR	1330	2090	76
DNS-IW-STA012-0-050826 (C0091648 Spk D, 10 ppb Lab Spike)	10000	8730	19300	106	NR	NR	NR	1330	10800	95
DNS-IW-STA012-LS-050826 (C0091650, 1.0 ppb Field Spike)	1000	8730	8840	*	NR	NR	NR	1330	2030	70
DNS-IW-STA012-HS-050826 (C0091651, 10 ppb Field Spike)	10000	8730	16100	74	NR	NR	NR	1330	7650	63

*Sample residue exceeds the spiking level significantly (3x spiking level); therefore, an accurate recovery value cannot be calculated
^Sample bottle was received with only 19 mL of water.
ND = Not detected at or above the Limit of Quantitation (LOQ) of 25 ng/L.
NR = Not reported due to quality control failure.
Note: Since this summary table shows rounded results, recovery values may vary slightly from the values in the raw data.

Table IV. Matrix Spike Recovery of PFBS, PFHS and PFOS in Water Samples Continued

	C4 Sulfonate PFBS				C6 Sulfonate PFHS			C8 Sulfonate PFOS		
Sample Description	Amount Spiked (ng/L)	Amt Found in Sample (ng/L)	Amount Recovered (ng/L)	Recovery (%)	Amt Found in Sample (ng/L)	Amount Recovered (ng/L)	Recovery (%)	Amt Found in Sample (ng/L)	Amount Recovered (ng/L)	Recovery (%)
DNS-SW-STA012-0-050826 (C0091652 Spk E, 1.0 ppb Lab Spike)	1000	53.3	886	83	42.2	1160	112	142	1000	86
DNS-SW-STA012-0-050826 (C0091652 Spk F, 10 ppb Lab Spike)	10000	53.3	9030	90	42.2	10500	105	142	7860	77
DNS-SW-STA012-LS-050826 (C0091654, 1.0 ppb Field Spike)	1000	53.3	870	82	42.2	1100	106	142	971	83
DNS-SW-STA012-HS-050826 (C0091655, 10 ppb Field Spike)	10000	53.3	7620	76	42.2	10300	103	142	7500	74
			Average:	88		Average:	105		Average:	87
			Standard Deviation:	18		Standard Deviation:	19		Standard Deviation:	22

*Sample residue exceeds the spiking level significantly (3x spiking level); therefore, an accurate recovery value cannot be calculated
ND = Not detected at or above the Limit of Quantitation (LOQ) of 25 ng/L.
Note: Since this summary table shows rounded results, recovery values may vary slightly from the values in the raw data.

Table V. Total Percent Solids in Sediment Samples

Exygen ID	Client Sample ID	Total Percent Solids (%)
C0091547	DNS-SD-STA001-0-050824	57.70
C0091557	DNS-SD-STA002-0-050824	52.23
C0091566	DNS-SD-STA003-0-050824	45.84
C0091579	DNS-SD-STA004-0-050825	70.28
C0091580	DNS-SD-STA005-0-050825	59.06
C0091581	DNS-SD-STA006-0-050825	72.13
C0091582	DNS-SD-STA007-0-050825	56.01
C0091583	DNS-SD-STA008-0-050825	59.64
C0091584	DNS-SD-STA009-0-050825	61.43
C0091585	DNS-SD-STA010-0-050825	57.91
C0091586	DNS-SD-STA011-0-050825	56.54
C0091587	DNS-SD-STA012-0-050825	46.84

FIGURES

Figure 1. Typical Non-Extracted Calibration Curve for PFBS in Methanol

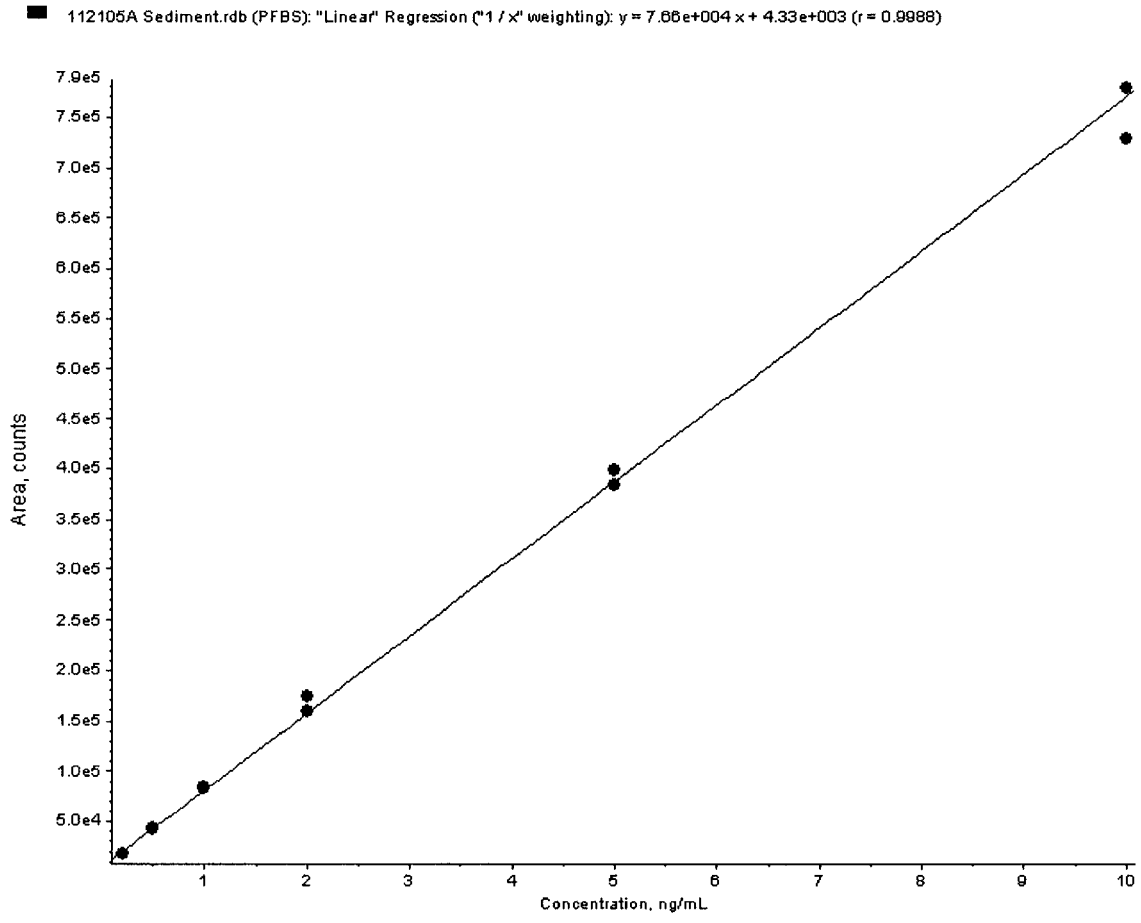


Figure 2. Typical Extracted Calibration Curve for PFBS in Methanol

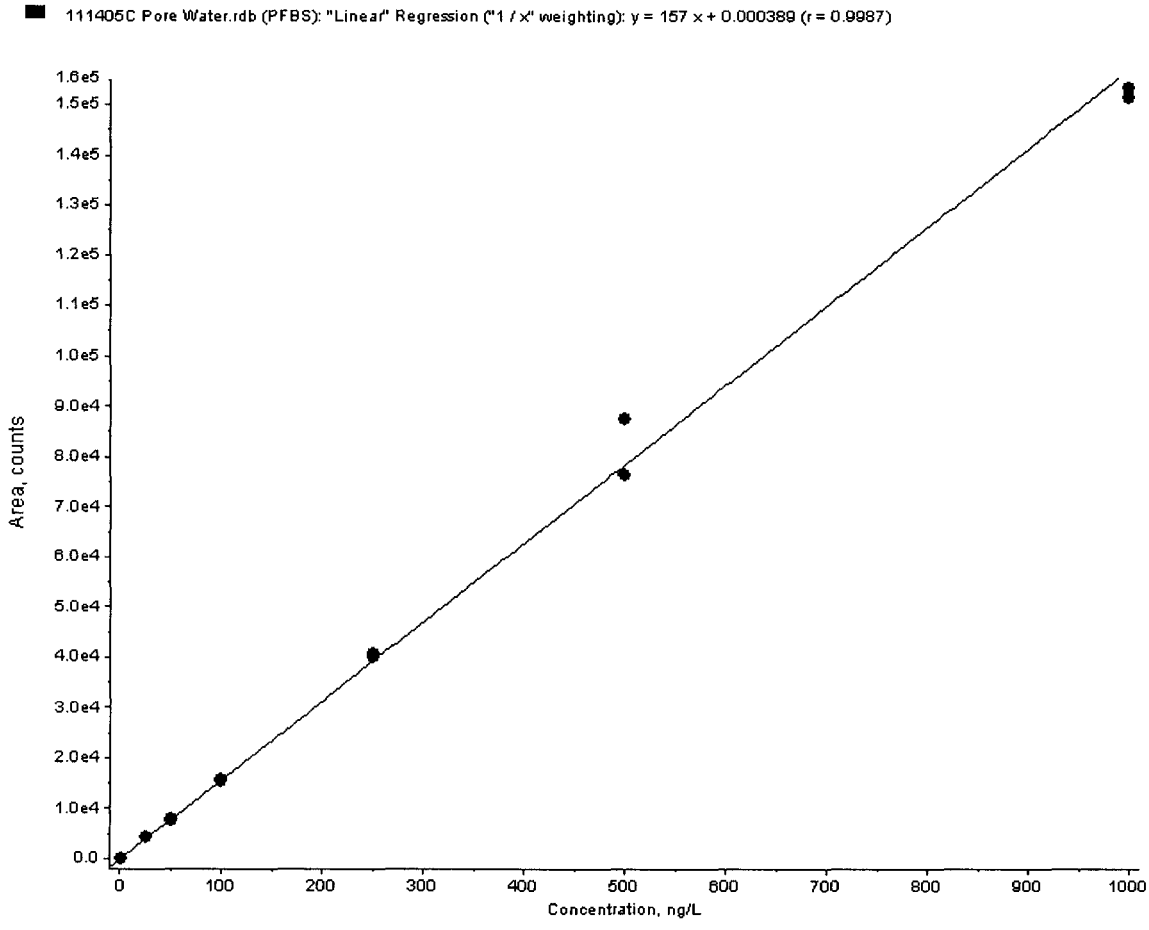


Figure 3. Non-Extracted Standards of PFBS in Methanol, 0.2 ng/mL and 0.5 ng/mL, Respectively

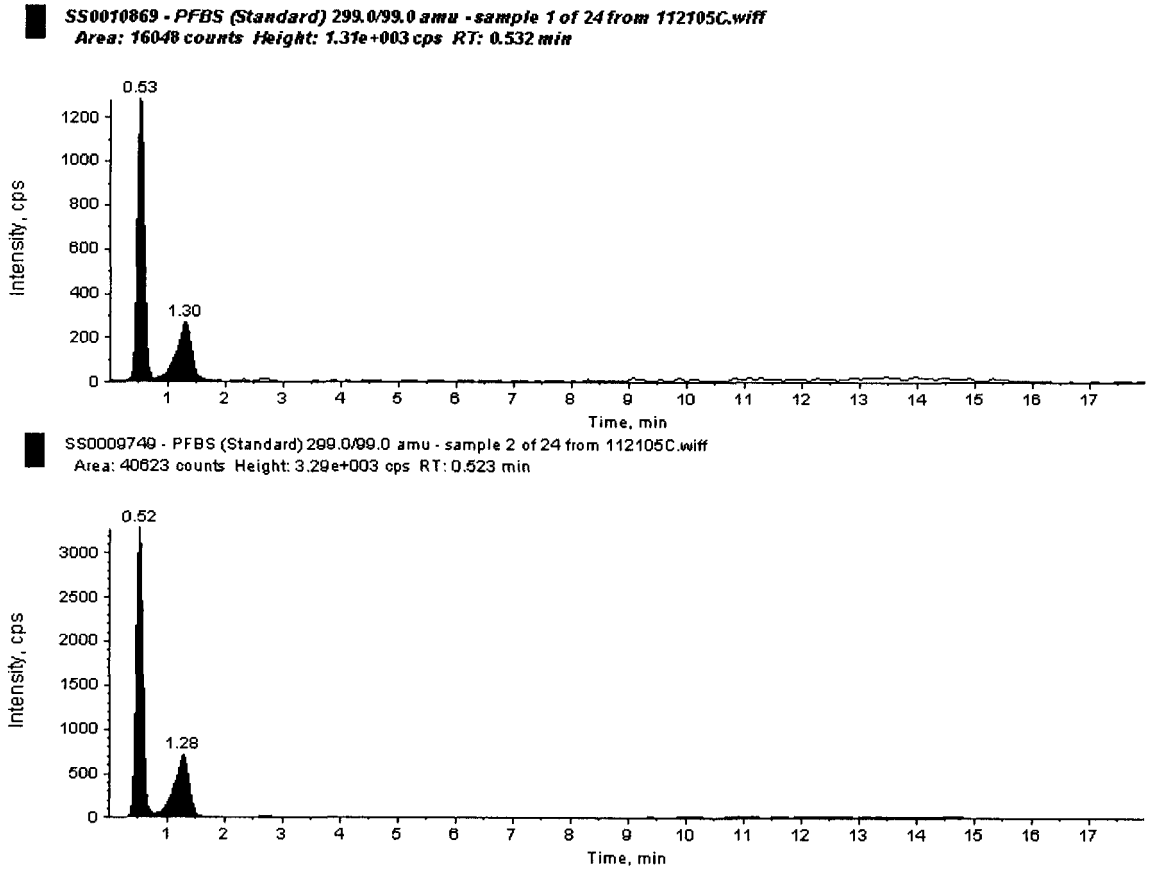


Figure 4. Extracted Standards of PFBS in Methanol, 25 ng/L and 50 ng/L, Respectively

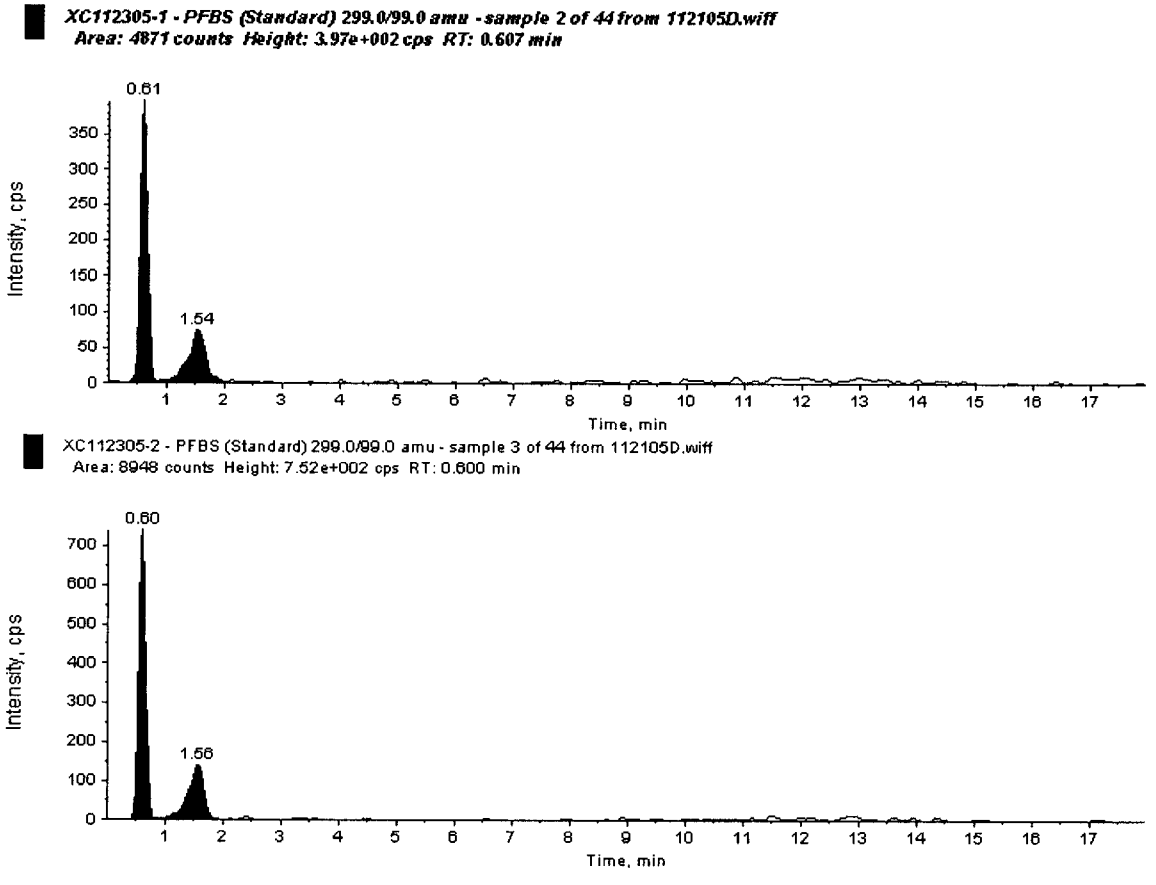


Figure 5. PFBS in 1% Acetic Acid Reagent Blank, 0.4 ng/mL Fortified Reagent Spk A, and 4.0 ng/mL Fortified Reagent Spk B, Respectively

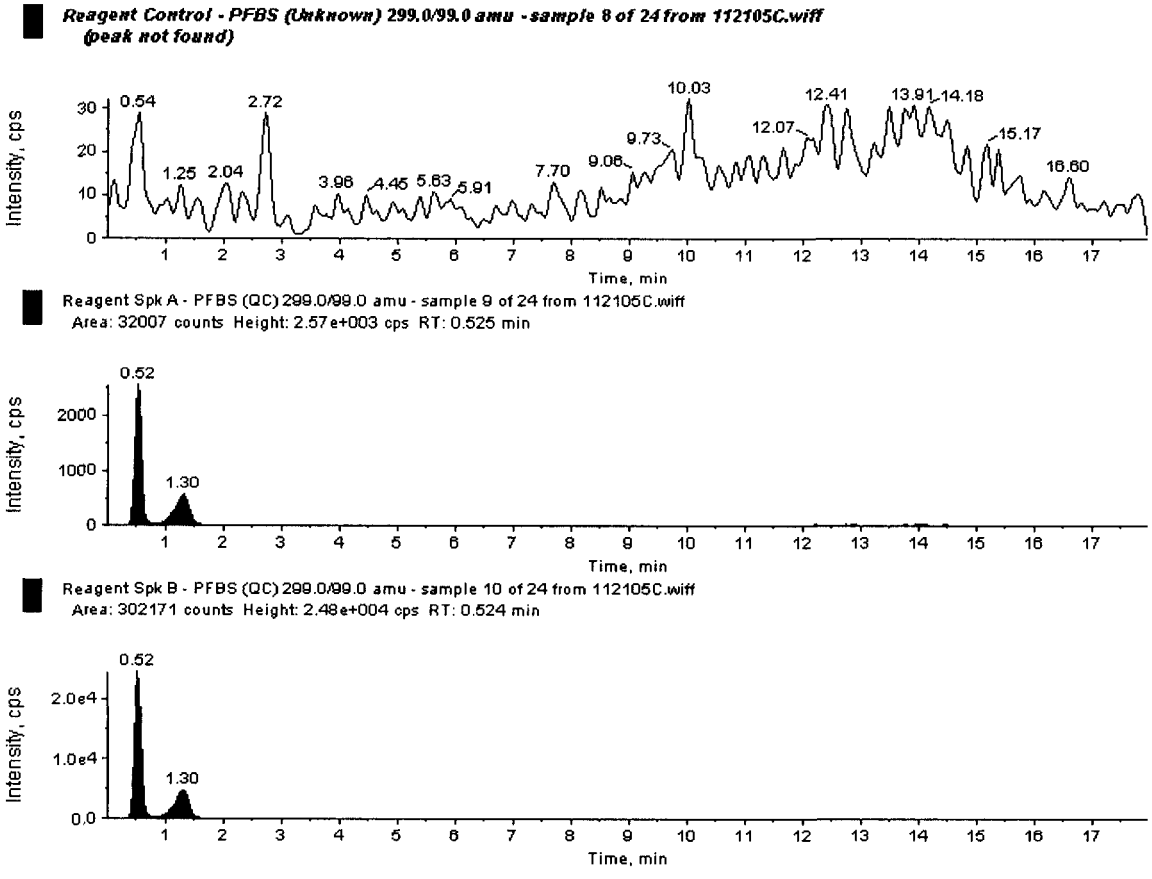


Figure 6. PFBS in Water Reagent Blank, 50 ng/L Fortified Reagent Spk A, and 500 ng/L Fortified Reagent Spk B, Respectively

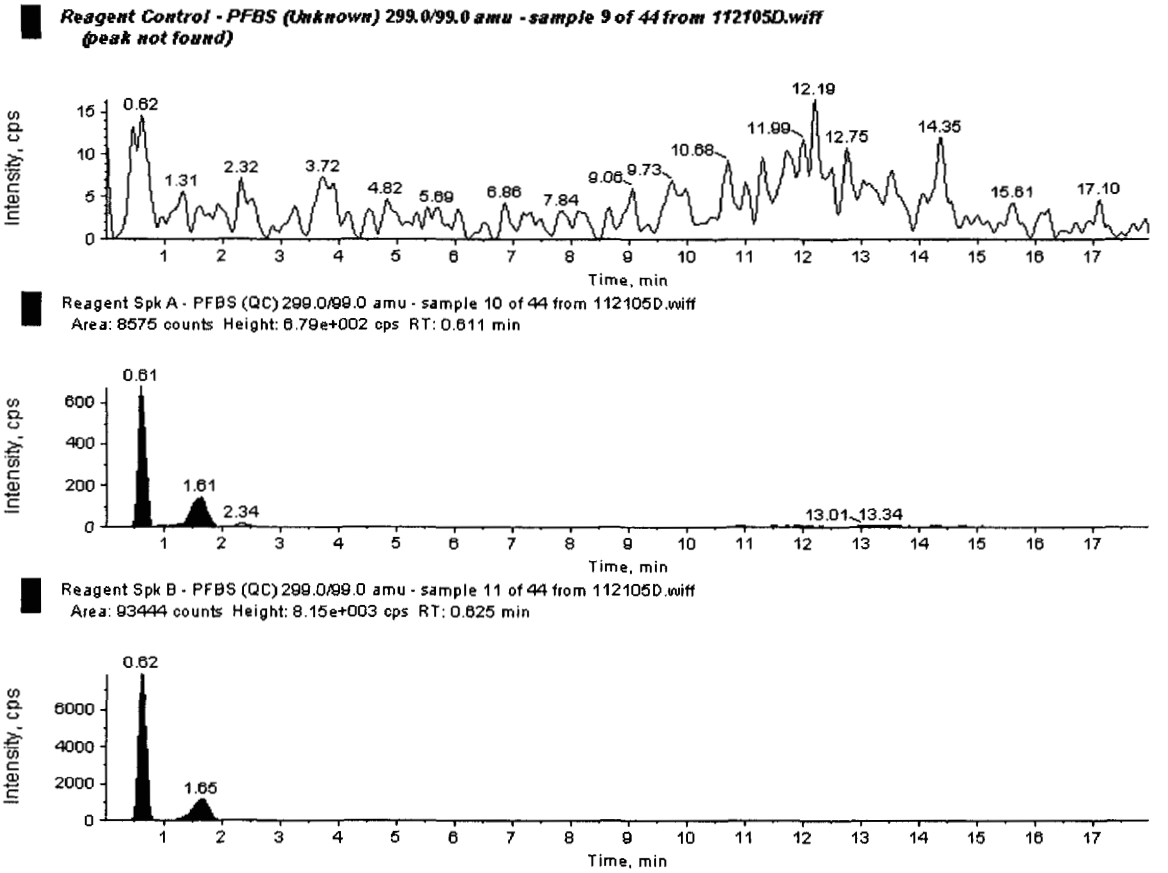


Figure 7. Chromatogram Representing a Sediment Sample Analyzed for PFBS (Exygen ID: C0091586, Data Set: 112105C)

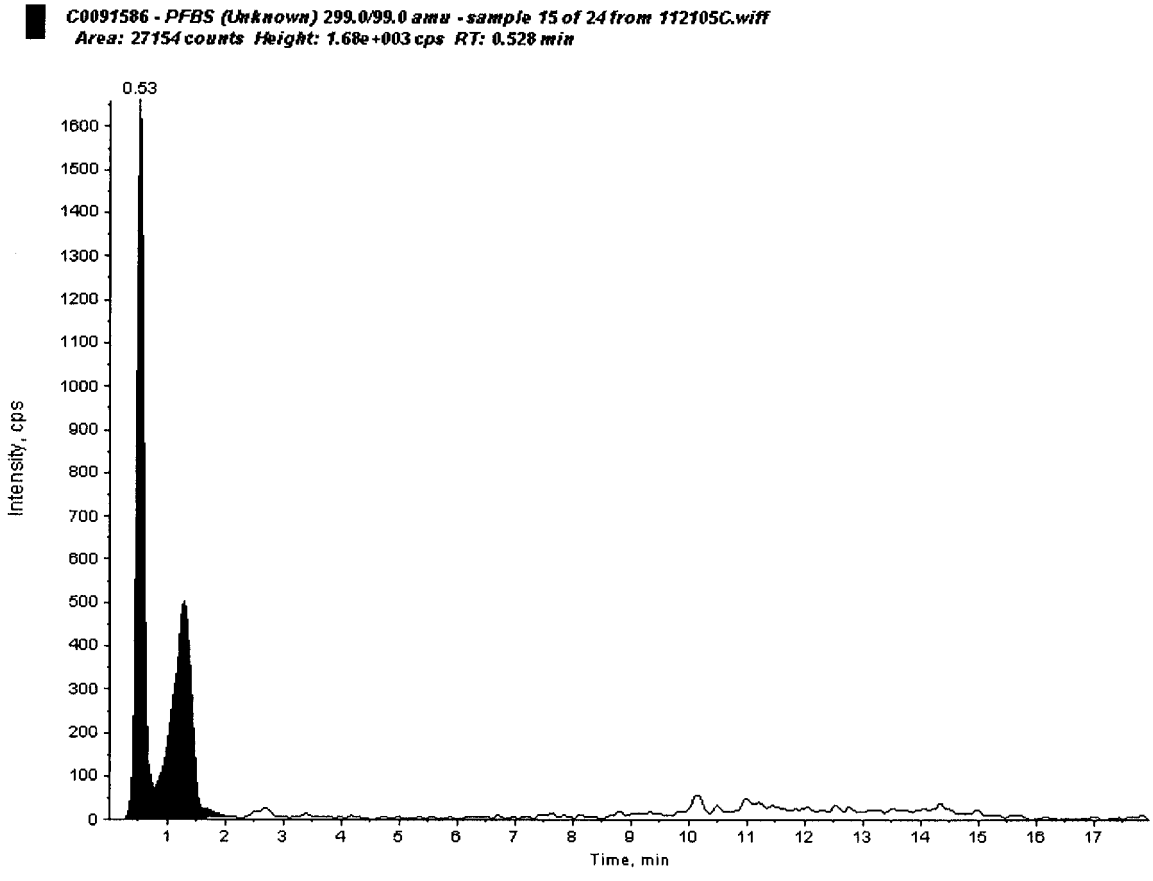


Figure 8. Chromatogram Representing a Water Sample Analyzed for PFBS (Exygen ID: C0091652, Data Set: 112105D)

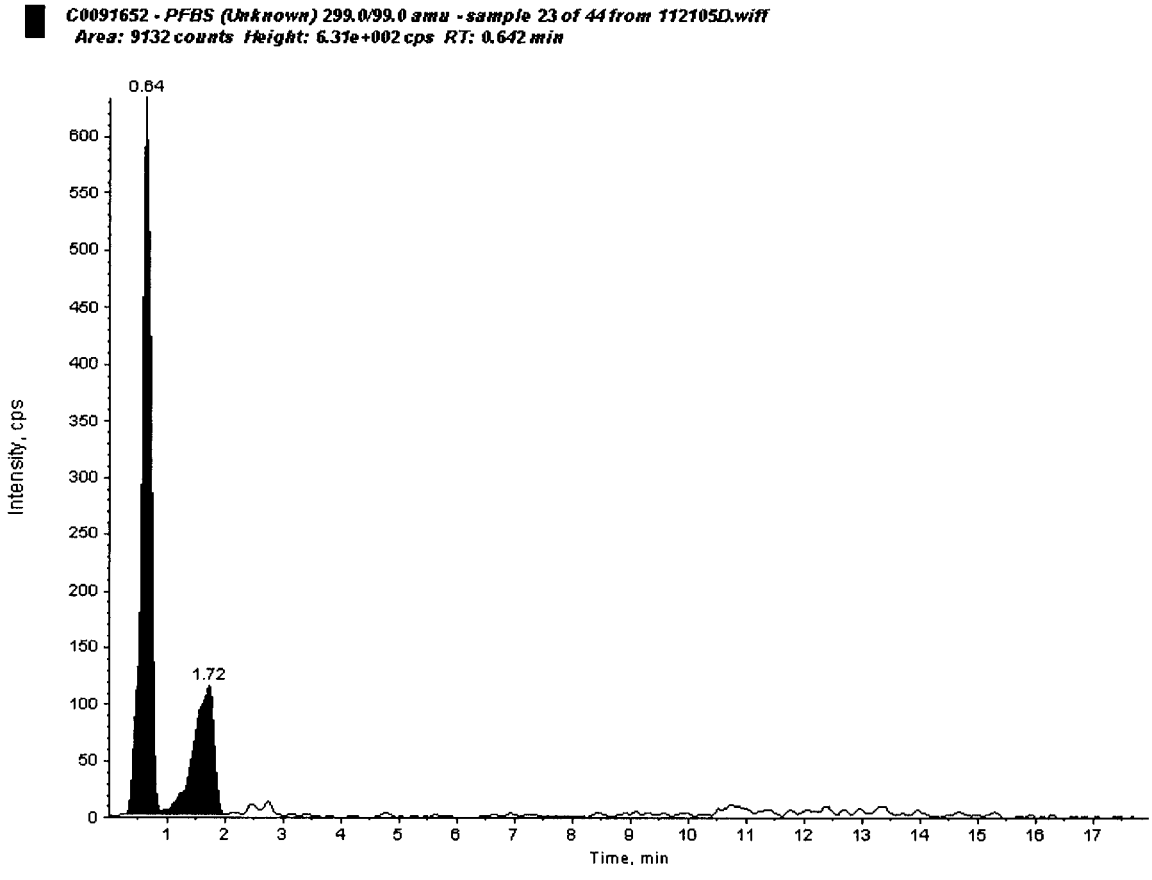


Figure 9. Typical Non-Extracted Calibration Curve for PFHS in Methanol

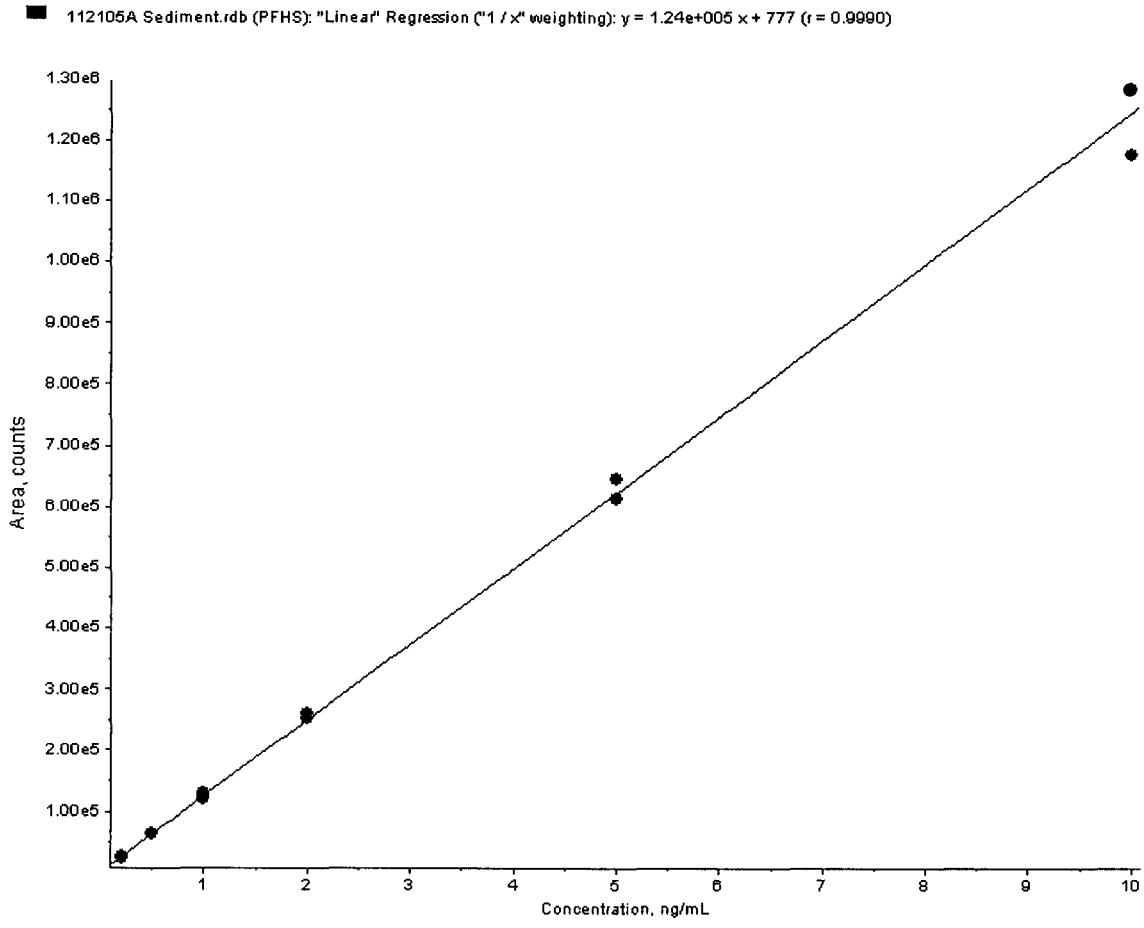


Figure 10. Typical Extracted Calibration Curve for PFHS in Methanol

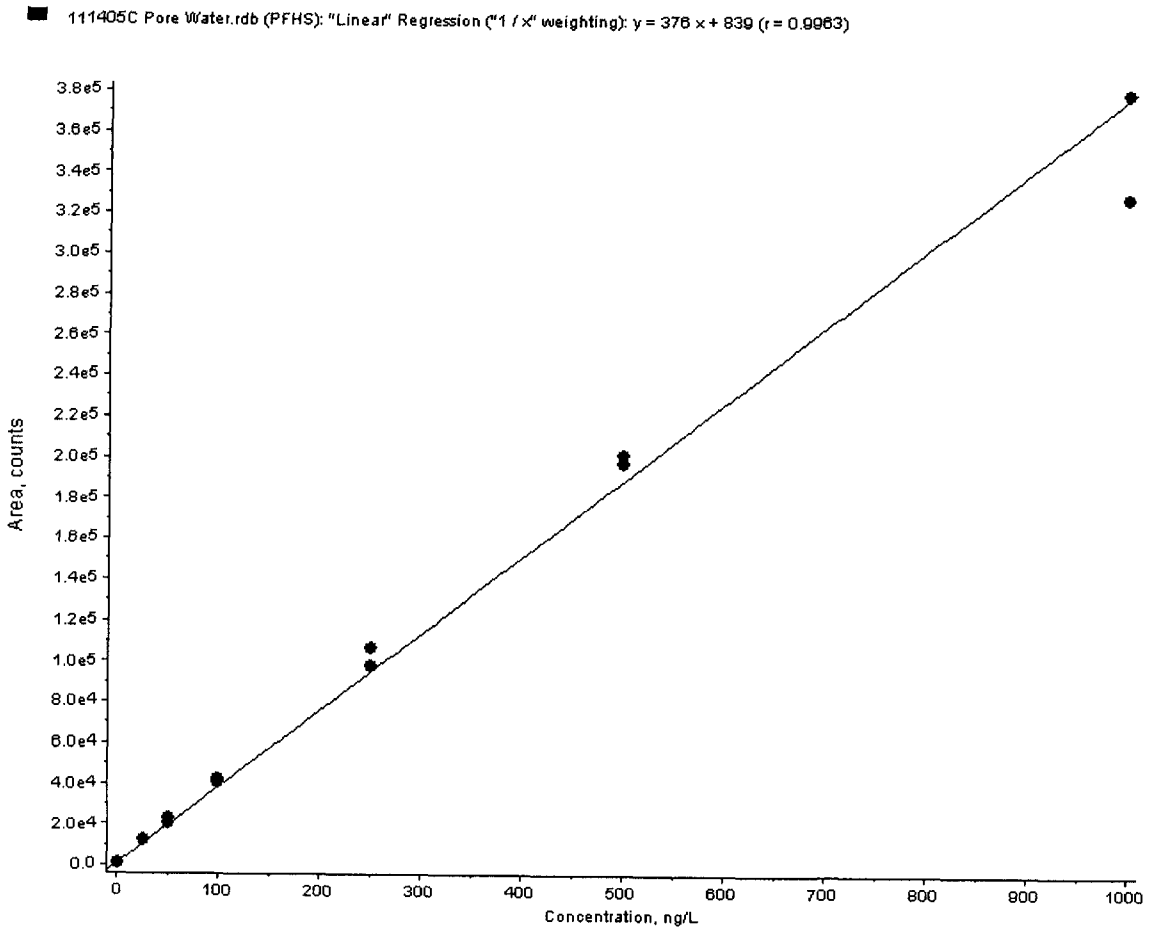


Figure 11. Non-Extracted Standards of PFHS in Methanol, 0.2 ng/mL and 0.5 ng/mL, Respectively

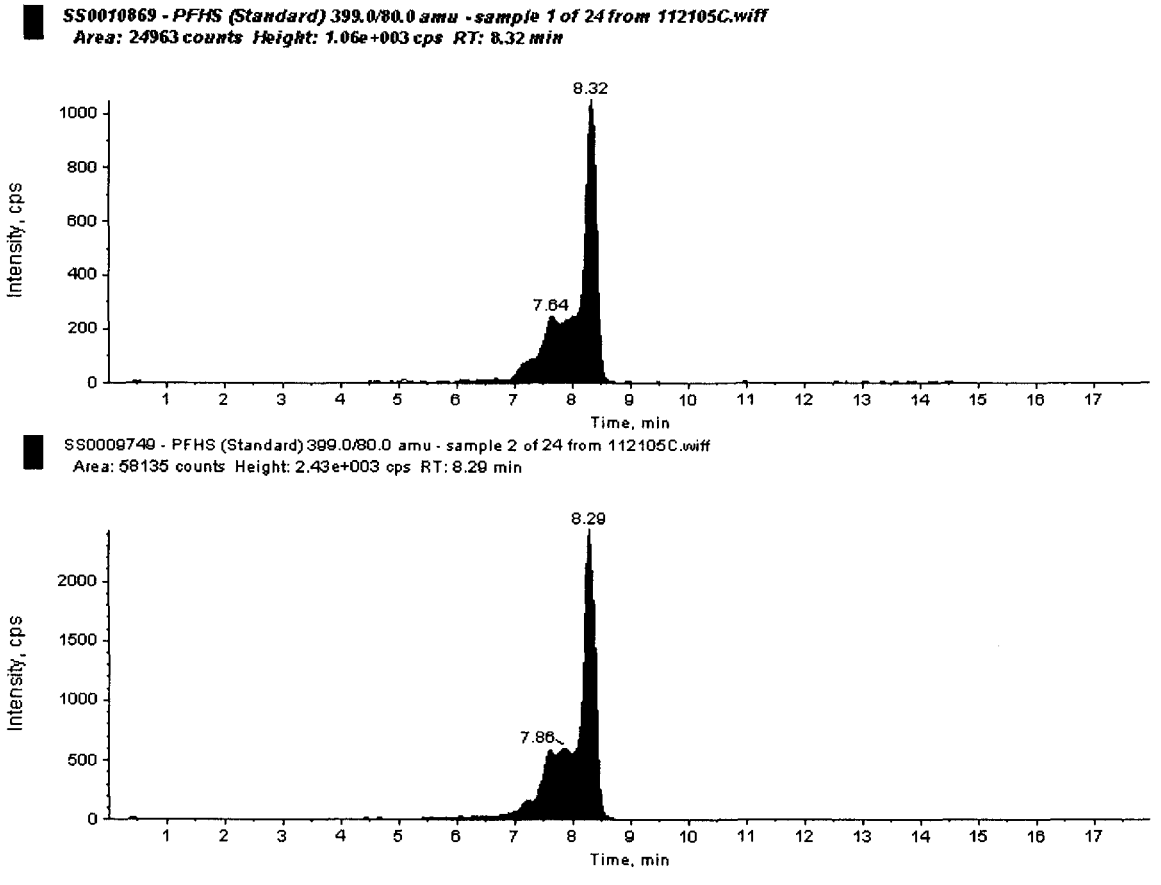


Figure 12. Extracted Standards of PFHS in Methanol, 25 ng/L and 50 ng/L, Respectively

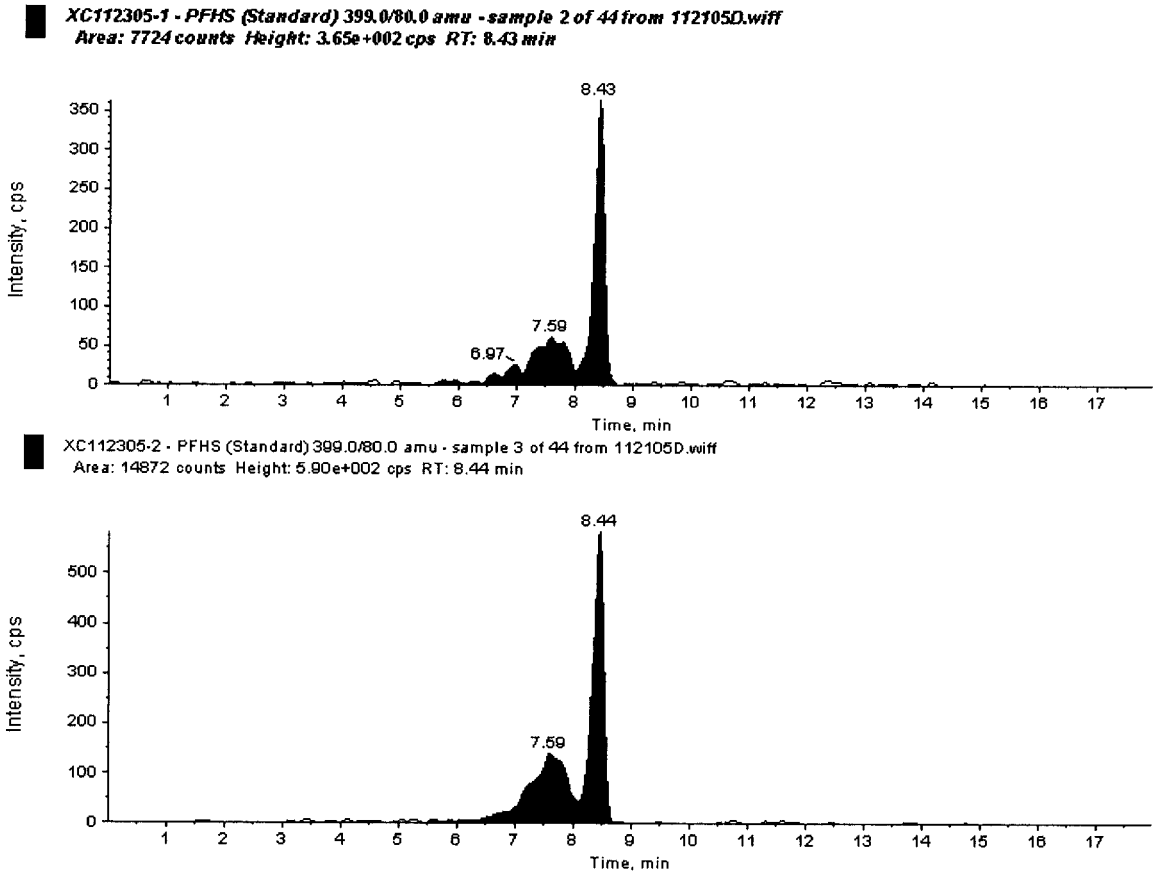


Figure 13. PFHS in 1% Acetic Acid Reagent Blank, 0.4 ng/mL Fortified Reagent Spk A, and 4.0 ng/mL Fortified Reagent Spk B, Respectively

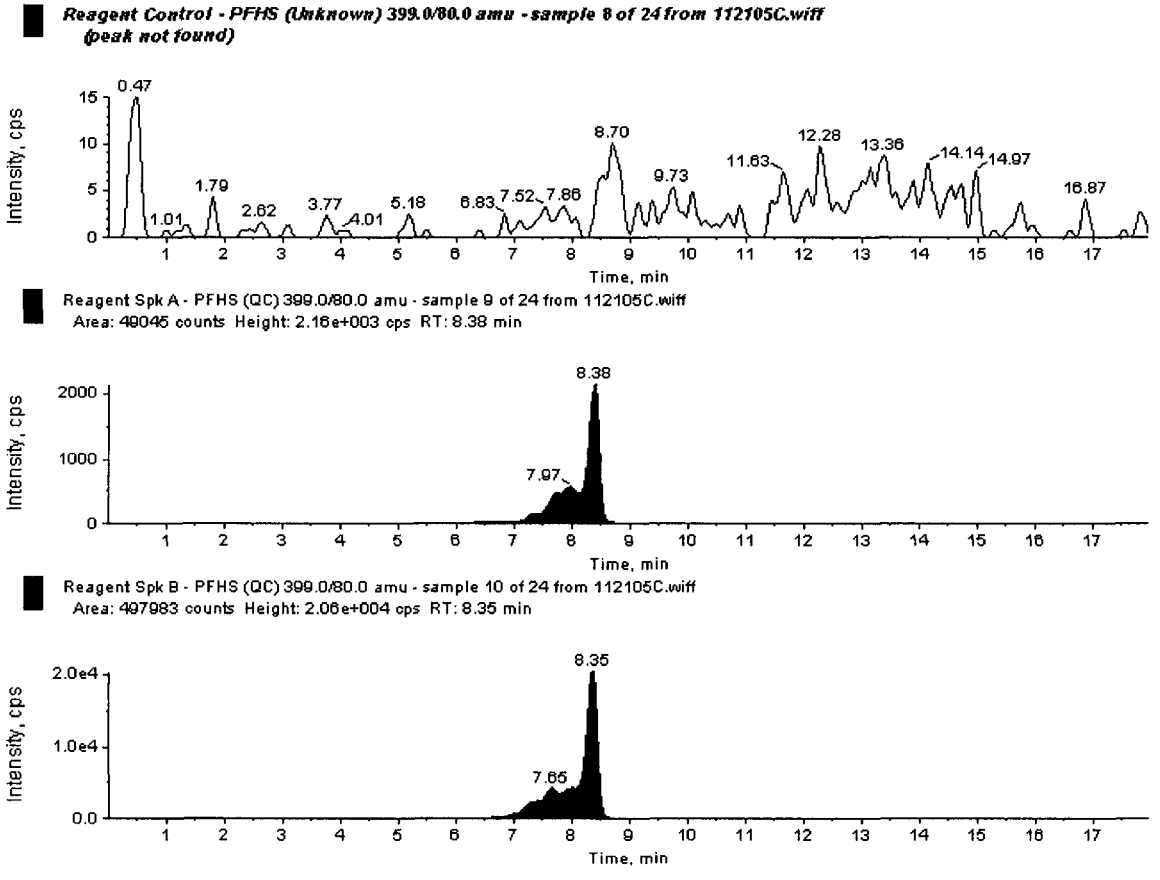


Figure 14. PFHS in Water Reagent Blank, 50 ng/L Fortified Reagent Spk A, and 500 ng/L Fortified Reagent Spk B, Respectively

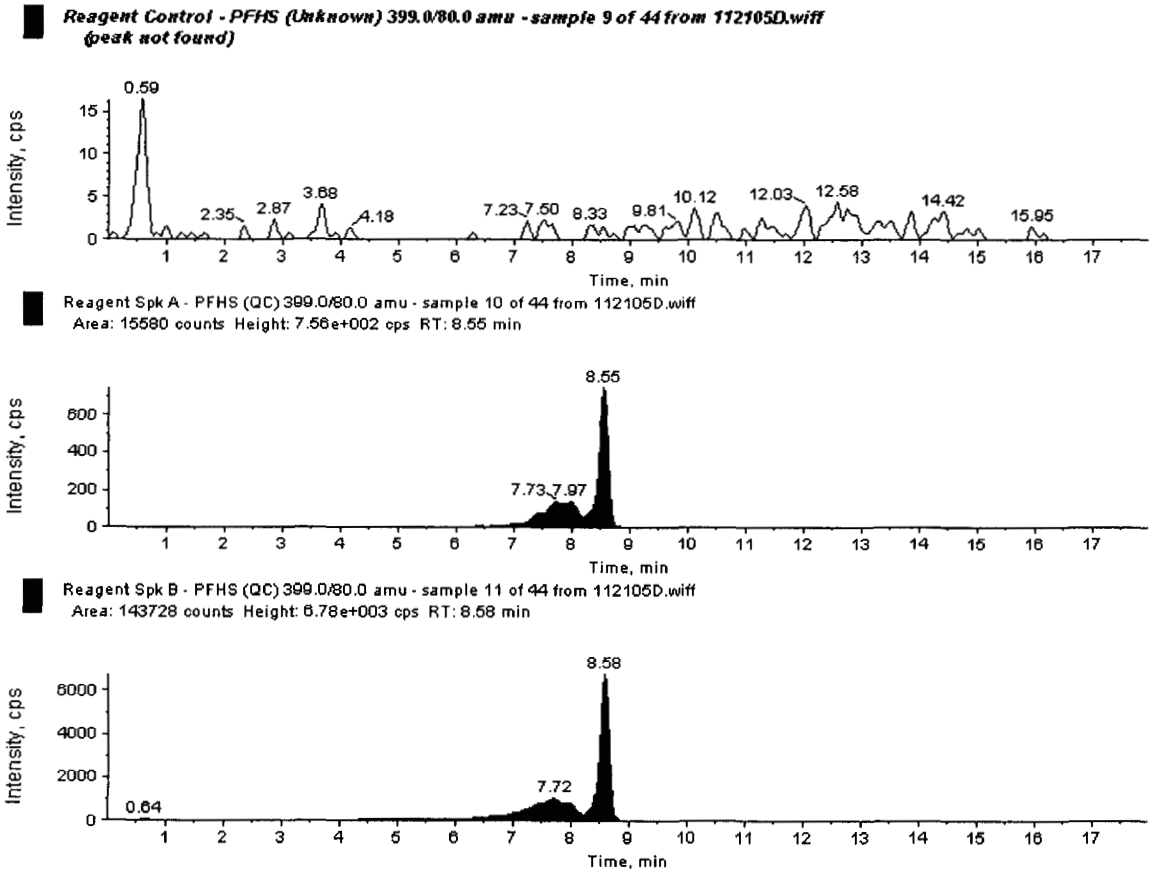


Figure 15. Chromatogram Representing a Sediment Sample Analyzed for PFHS (Exygen ID: C0091587, Data Set: 112105C)

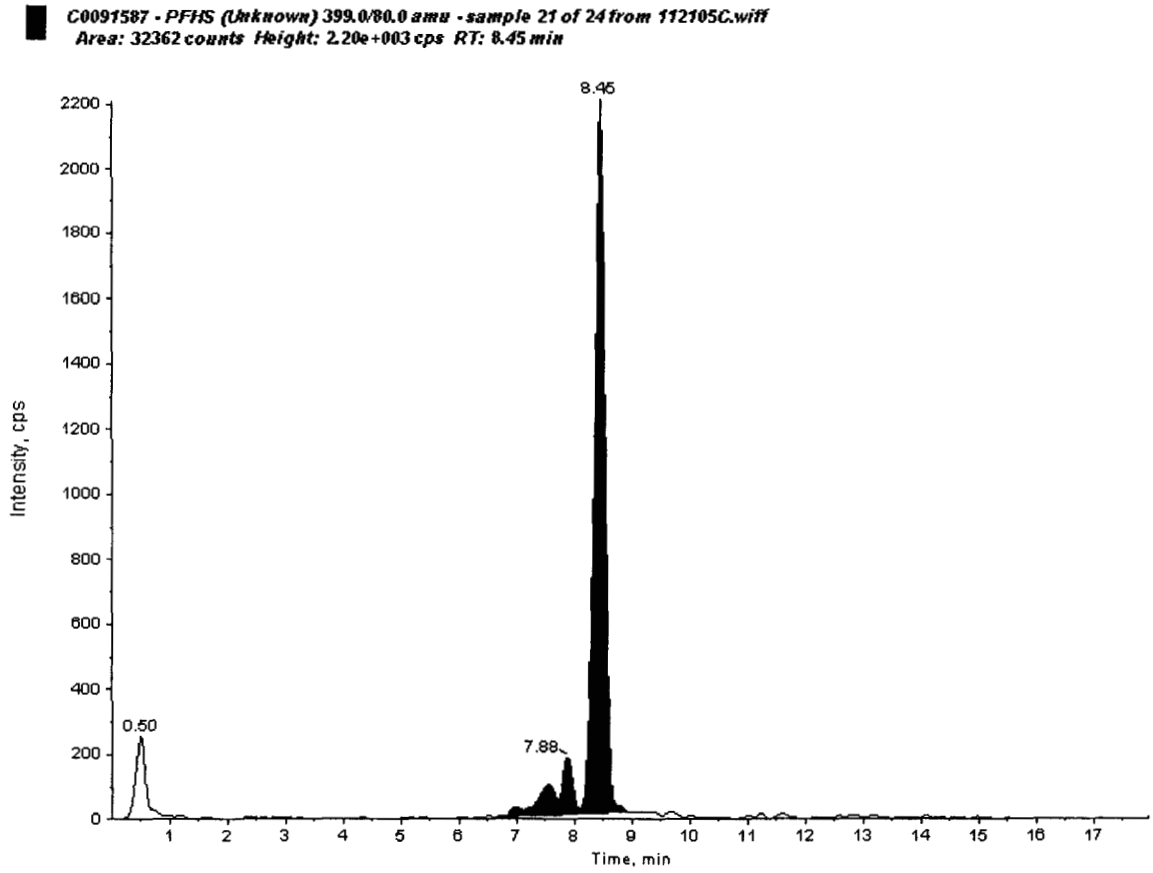


Figure 16. Chromatogram Representing a Water Sample Analyzed for PFHS (Exygen ID: C0091549, Data Set: 111405C)

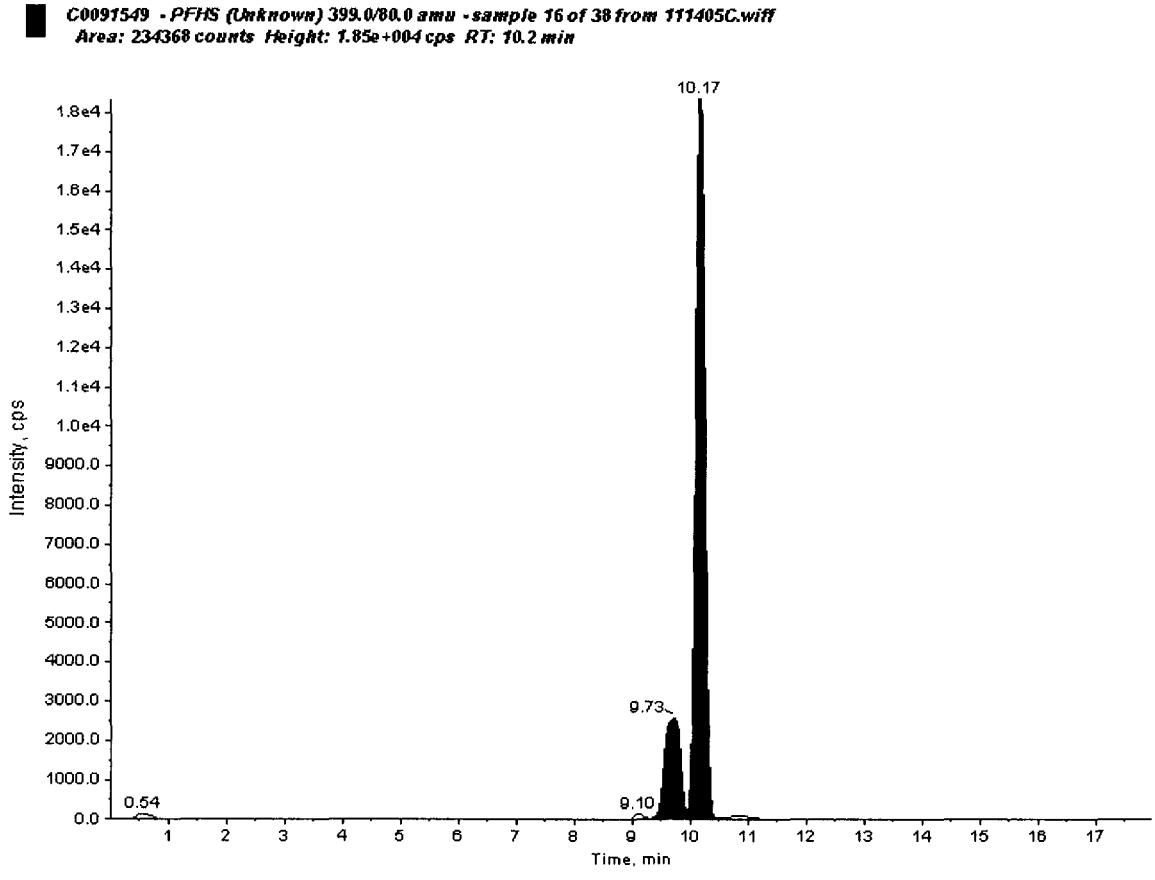


Figure 17. Typical Non-Extracted Calibration Curve for PFOS in Methanol

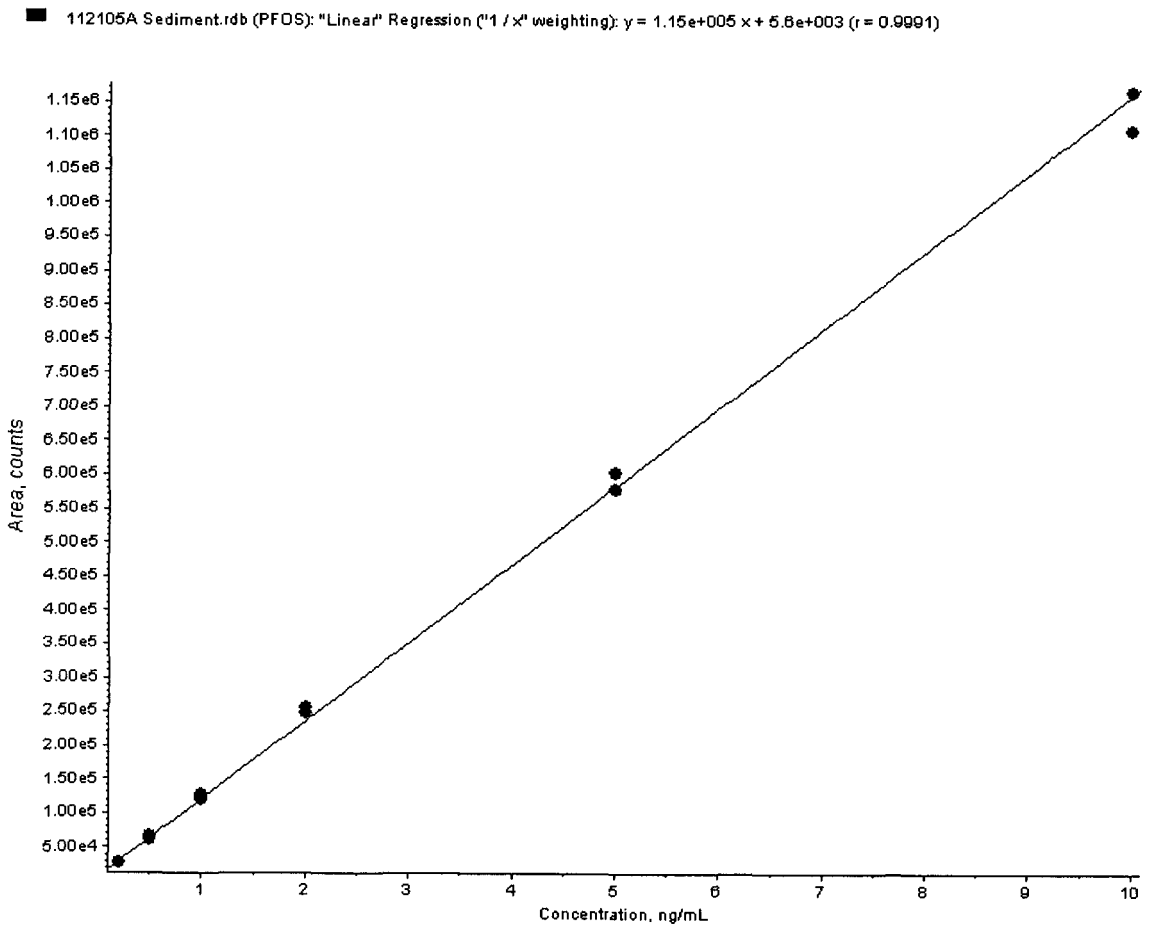


Figure 18. Typical Extracted Calibration Curve for PFOS in Methanol

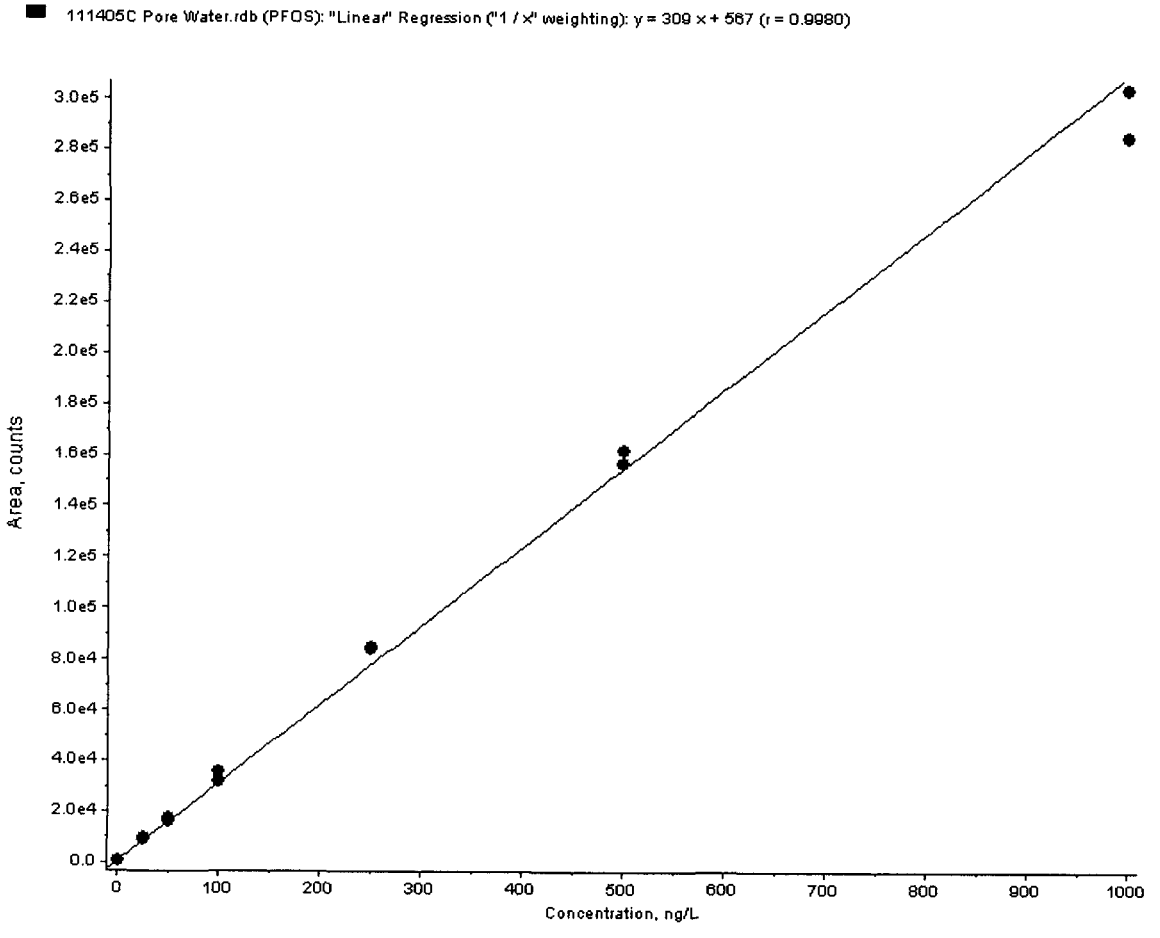


Figure 19. Non-Extracted Standards of PFOS in Methanol, 0.2 ng/mL and 0.5 ng/mL, Respectively

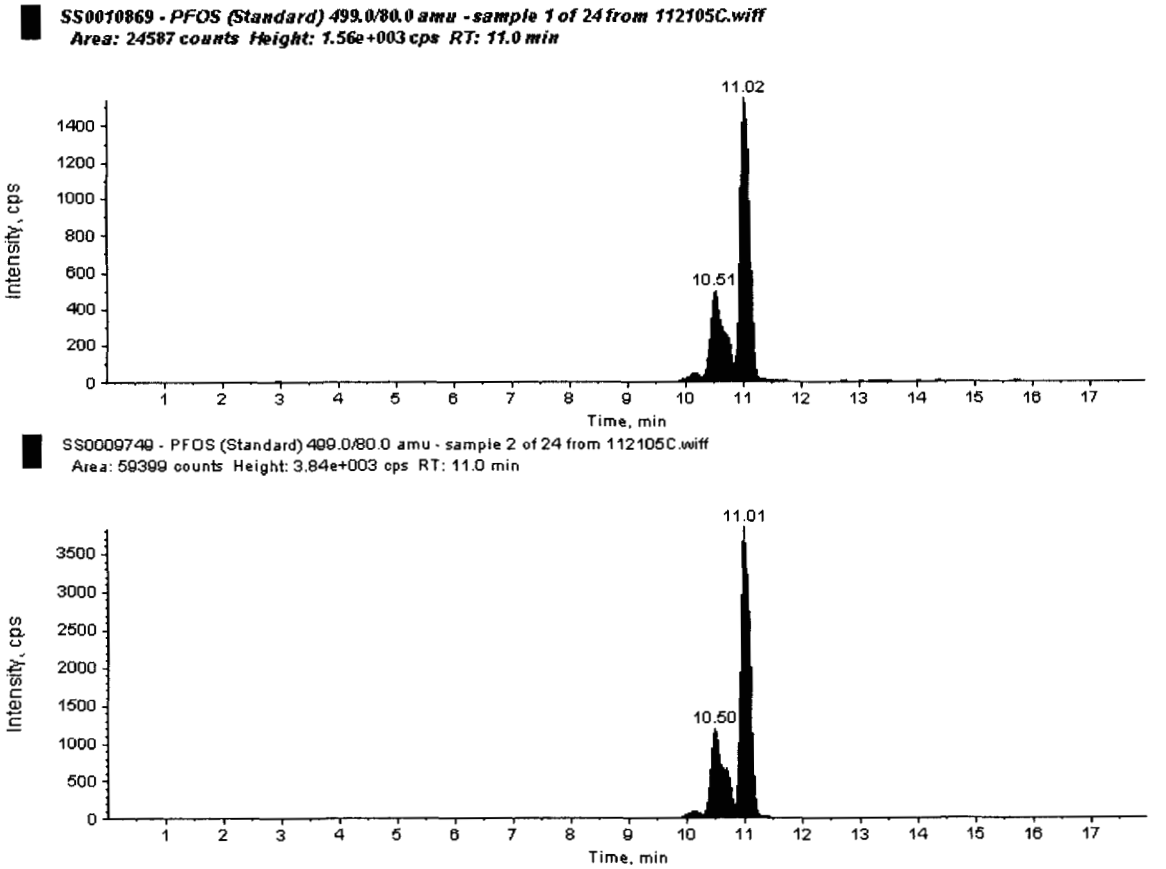


Figure 20. Extracted Standards of PFOS in Methanol, 25 ng/L and 50 ng/L, Respectively

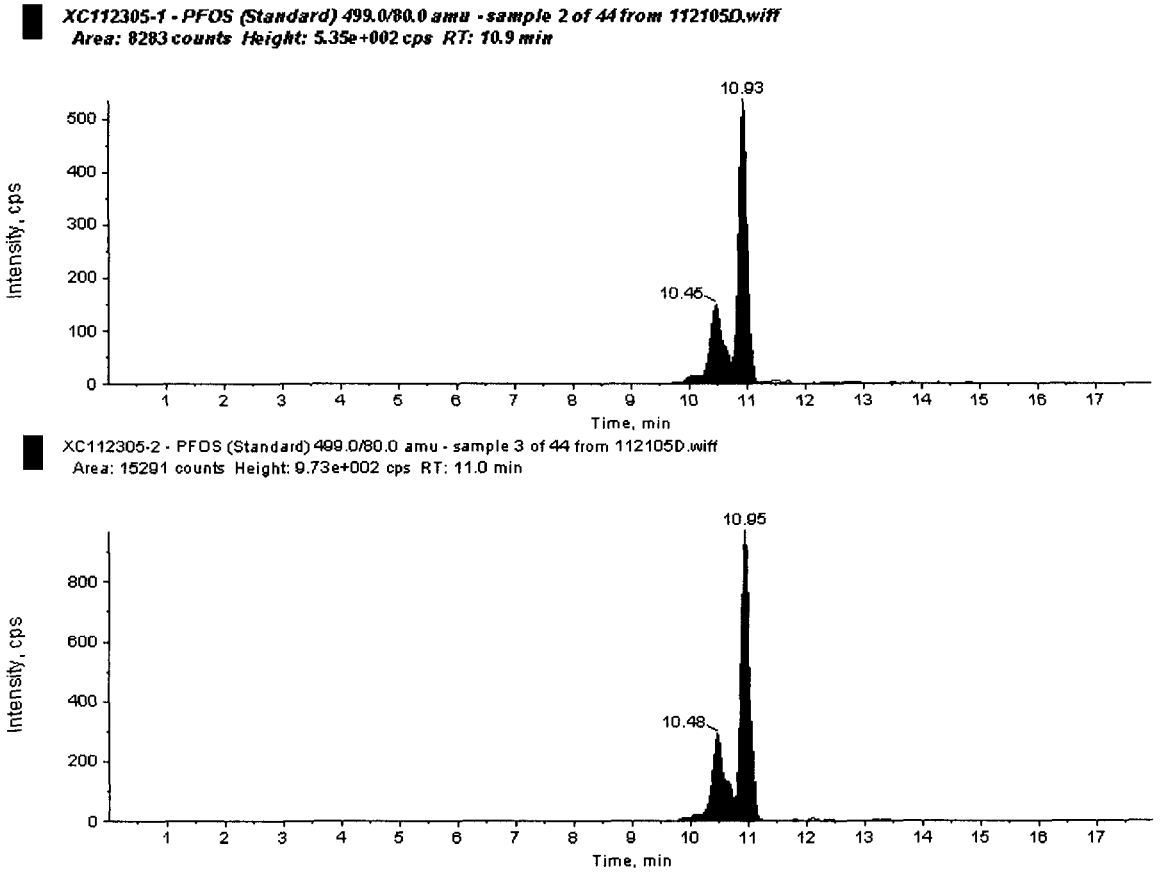


Figure 21. PFOS in 1% Acetic Acid Reagent Blank, 0.4 ng/mL Fortified Reagent Spk A, and 4.0 ng/mL Fortified Reagent Spk B, Respectively

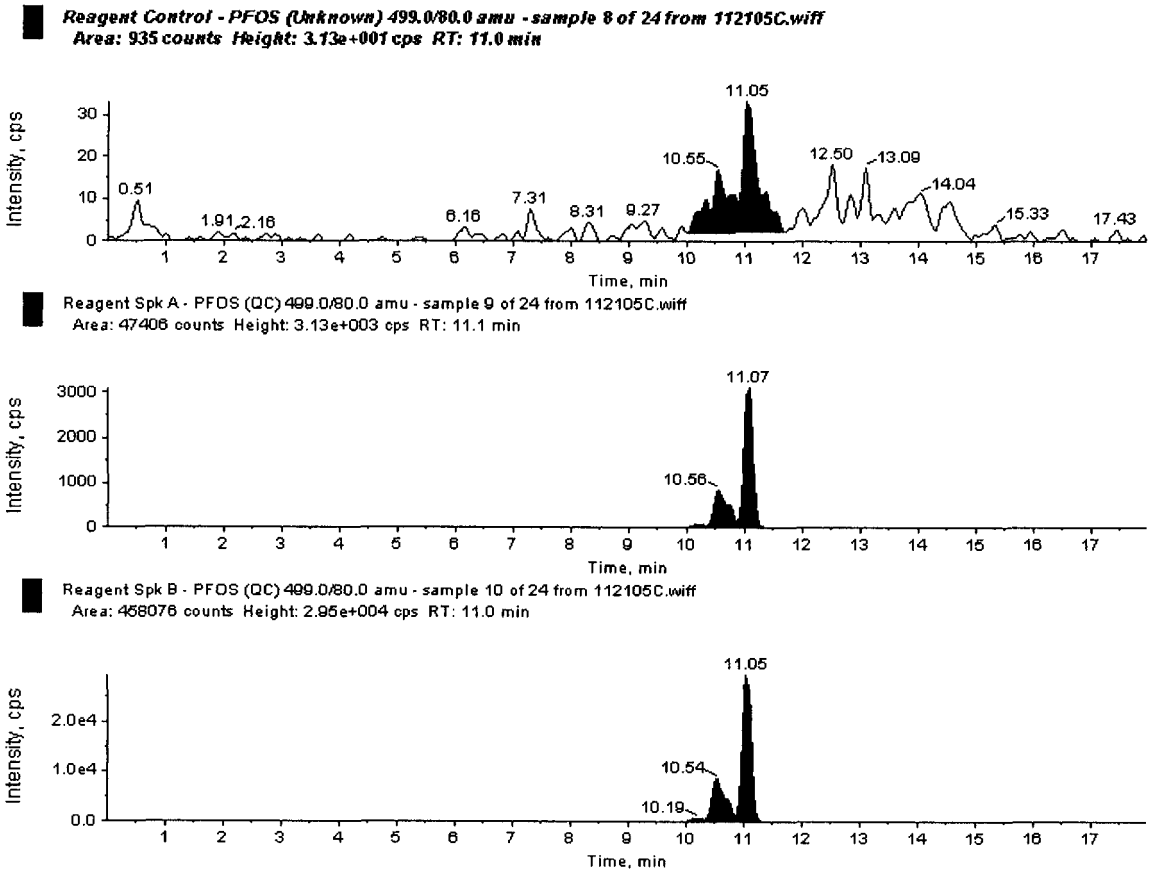


Figure 22. PFOS in Water Reagent Blank, 50 ng/L Fortified Reagent Spk A, and 500 ng/L Fortified Reagent Spk B, Respectively

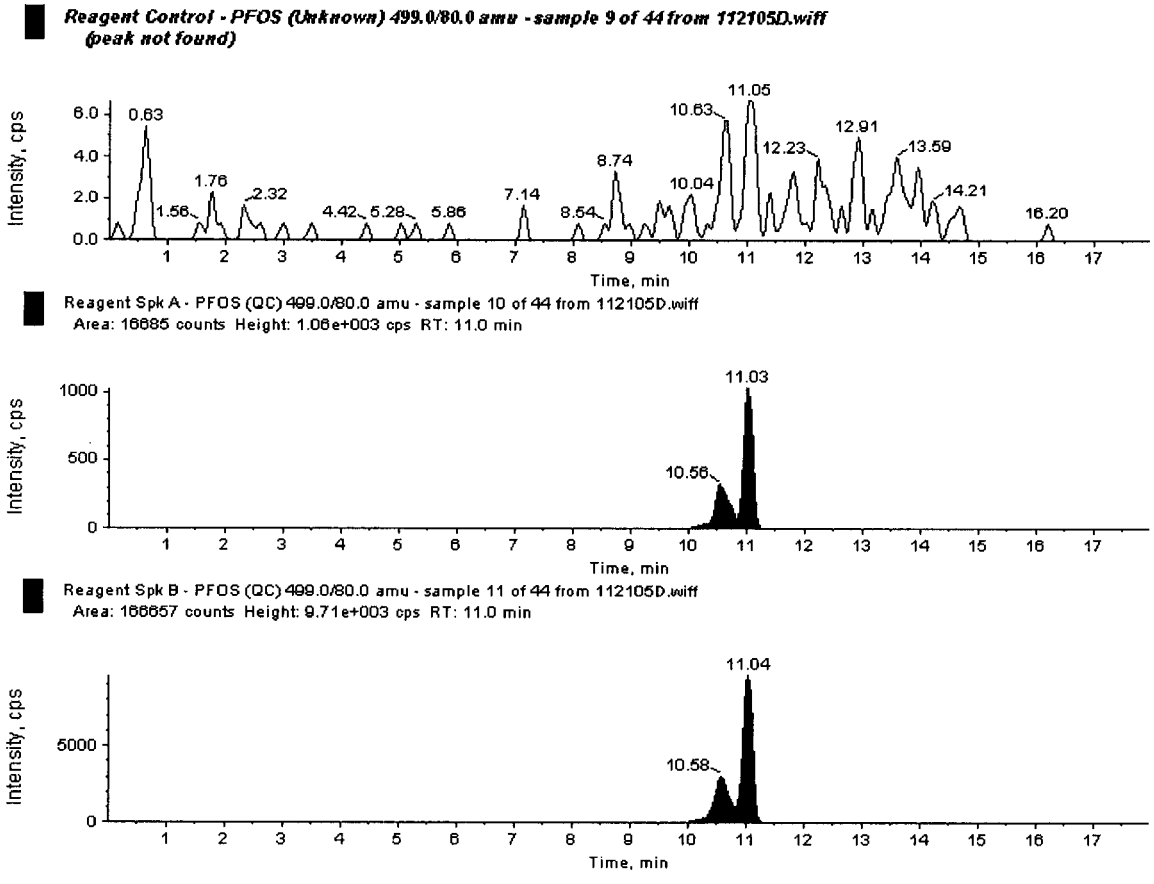


Figure 23. Chromatogram Representing a Sediment Sample Analyzed for PFOS (Exygen ID: C0091566, Data Set: 112105A)

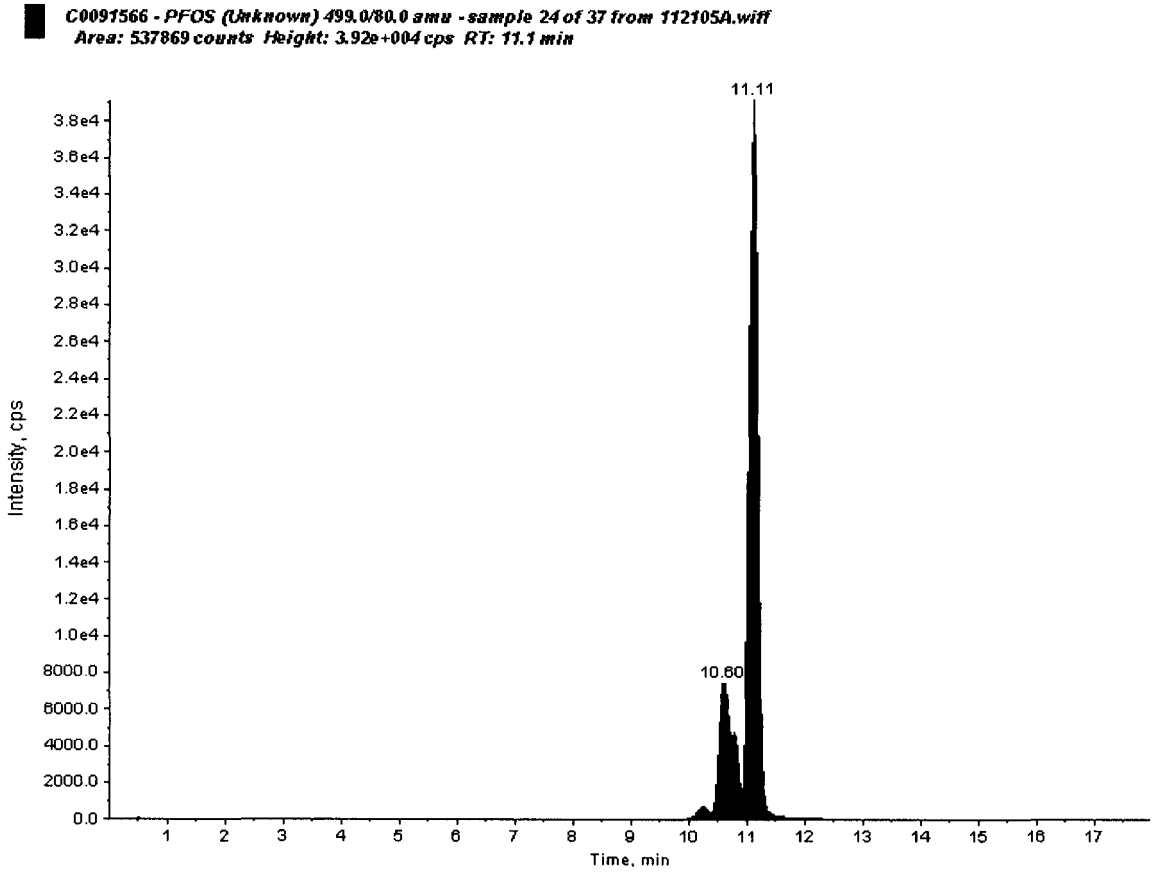
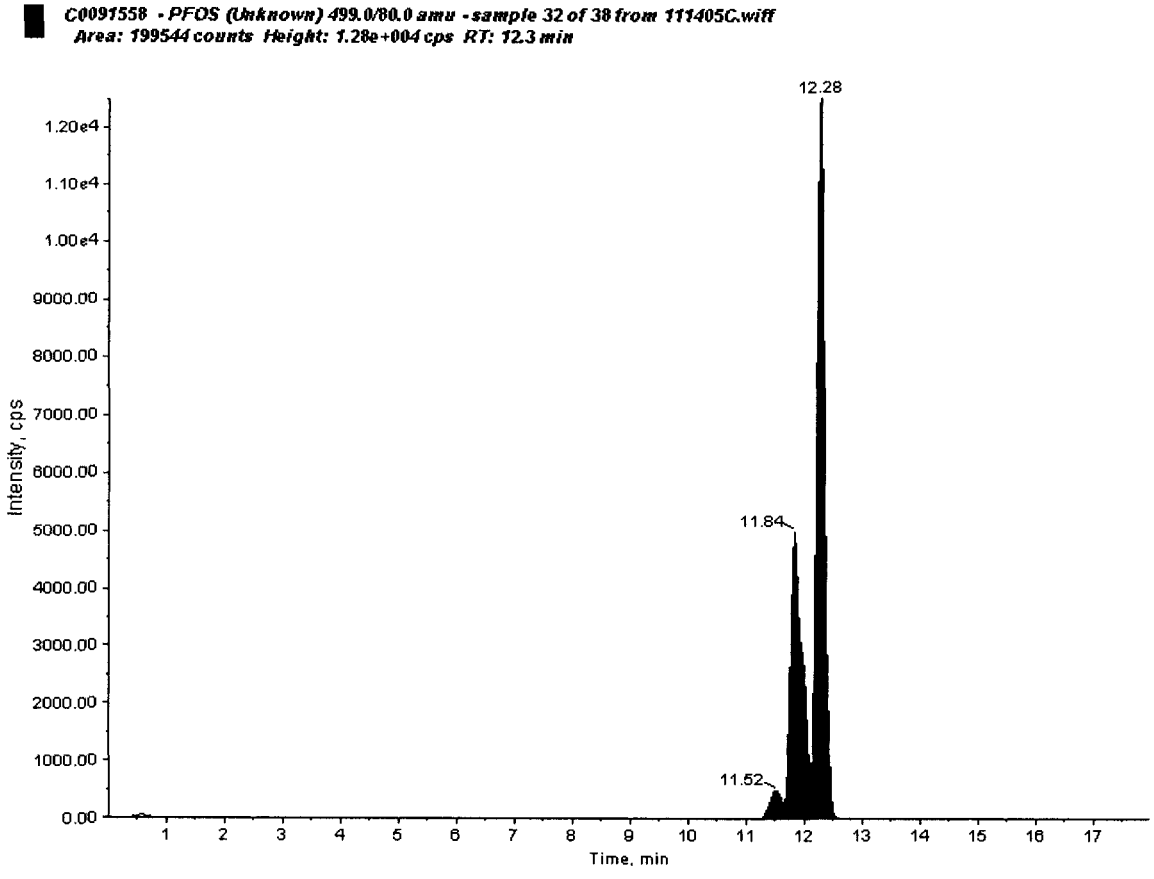


Figure 24. Chromatogram Representing a Water Sample Analyzed for PFOS (Exygen ID: C0091558, Data Set: 111405C)



APPENDIX A

Study Protocol
P0001131
(Exygen Study No. P0001131) with Analytical Methods
and
Protocol Amendments 1 and 2

STUDY PROTOCOL

Study Title:

Analysis of Perfluorobutanesulfonate (PFBS), Perfluorohexanesulfonate (PFHS), and Perfluorooctanesulfonate (PFOS) in Water, Soil, Sediment, Fish, Clams, Vegetation, Small Mammal Liver and Small Mammal Serum Using LC/MS/MS for the 3M Decatur Monitoring Program

Exygen Protocol Number: P0001131

Performing Laboratory:
Exygen Research
3058 Research Drive
State College, PA 16801
Phone: (814) 272-1039

Sponsor Representative:
Michael A. Santoro
Director of Regulatory Affairs
3M Building 0236-01-B-10
St. Paul, MN 55144
Phone: (651) 733-6374

DISTRIBUTION:

1) Jaisimha Kesari, Study Director, Weston Solutions

2) John M. Flaherty, Principal Investigator, Exygen Research

3) Michael A. Santoro, Sponsor Representative, 3M Company

4) Exygen Research Quality Assurance Unit

PROTOCOL APPROVAL

Study Title: Analysis of Perfluorobutanesulfonate (PFBS), Perfluorohexanesulfonate (PFHS), and Perfluorooctanesulfonate (PFOS) in Water, Soil, Sediment, Fish, Clams, Vegetation, Small Mammal Livers and Small Mammal Serum Using LC/MS/MS for the 3M Decatur Monitoring Program

Exygen Protocol Number: P0001131

APPROVALS

Jaisimha Kesari, Study Director
Weston Solutions — 11/5/04 Date

Michael A. Santoro, Sponsor Representative
3M Company — 11/11/04 Date

John M. Flaherty, Principal Investigator
Exygen Research — 10/29/04 Date

Richard A. Grazzini, President, Facility Management
Exygen Research — 29-OCT-2004 Date

Lydia Shaffer, Technical Lead, Quality Assurance Unit
Exygen Research — 10/29/04 Date

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INTRODUCTION

The purpose of this study is to perform analysis for perfluorobutanesulfonate (PFBS), perfluorohexanesulfonate (PFHS) and perfluorooctanesulfonate (PFOS) in water, soil, sediment, fish, clams, vegetation, small mammal livers and small mammal serum using LC/MS/MS for the 3M Decatur Monitoring Program.

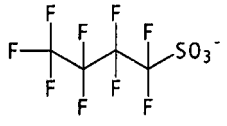
The study will be audited for compliance with EPA TSCA Good Laboratory Practice Standards 40 CFR 792 by the Quality Assurance Unit of Exygen Research.

TEST MATERIALS

The test materials are perfluorobutanesulfonate (PFBS), perfluorohexanesulfonate (PFHS) and perfluorooctanesulfonate (PFOS) and are all supplied by 3M.

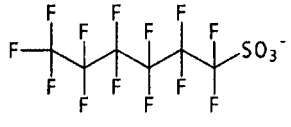
PFBS

Chemical Name: Perfluorobutanesulfonate
Molecular Weight: 338 supplied as the potassium salt ($C_4F_9SO_3^-K^+$)
Lot Number: 101
Purity: 96.7%
Transitions Monitored: 299 → 99
Structure:



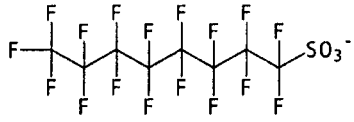
PFHS

Chemical Name: Perfluorohexanesulfonate
Molecular Weight: 438 supplied as the potassium salt ($C_6F_{13}SO_3^-K^+$)
Lot Number: SE036
Purity: 98.6%
Transitions Monitored: 399 → 80
Structure:



PFOS

Chemical Name: Perfluorooctanesulfonate
Molecular Weight: 538 supplied as the potassium salt ($C_8F_{17}SO_3^-K^+$)
Lot Number: 217
Purity: 86.9%
Transitions Monitored: 499 → 99
Structure:



OBJECTIVE

The purpose of this study is to perform analysis for perfluorobutanesulfonate (PFBS), perfluorohexanesulfonate (PFHS) and perfluorooctanesulfonate (PFOS) in water, soil, sediment, fish, clams, vegetation, small mammal livers and small mammal serum for the 3M Decatur Monitoring Program using the current versions of the following Exygen analytical methods:

V0001780: "Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Water by LC/MS/MS"
V0001781: "Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Soil by LC/MS/MS"
V0001782: "Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Sediment by LC/MS/MS"
V0001783: "Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Fish and Clams by LC/MS/MS"
V0001784: "Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Vegetation by LC/MS/MS"
V0001785: "Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small Mammal Liver by LC/MS/MS"
V0001786: "Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small Mammal Serum by LC/MS/MS"

TESTING FACILITY

Exygen Research
3058 Research Drive
State College, PA 16801
Phone: (814) 272-1039

STUDY DIRECTOR

Jaisimha Kesari P.E., DEE
Weston Solutions, Inc.
1400 Weston Way
West Chester, PA 19380
Phone: (610) 701-3761
Fax: (610) 701-7401
j.kesari@westonsolutions.com

SPONSOR REPRESENTATIVE

Michael A. Santoro
3M Company
Director of Regulatory Affairs
3M Building 0236-01-B-10
St. Paul, MN 55144
Phone: (651) 733-6374

PRINCIPAL INVESTIGATOR

John M. Flaherty
Exygen Research
3058 Research Drive
State College, PA 16801
Phone: (814) 272-1039
john.flaherty@exygen.com

PROPOSED EXPERIMENTAL START AND TERMINATION DATES

It is proposed that the analytical portion of this study be conducted from October 01, 2004 to December 31, 2005. The actual experimental start and termination dates will be included in the final report.

IDENTIFICATION AND JUSTIFICATION OF THE TEST SYSTEM

The following are the test systems for this study:

- Water (groundwater and surface water)
- Soil
- Sediment
- Fish
- Clams
- Vegetation
- Small Mammal Liver
- Small Mammal Serum

The samples will be collected by Weston Solutions. The control samples will be purchased and prepared by the testing facility. Purchase and processing details for the control samples will be included in the final report associated with this study.

The test systems were chosen to access the environmental impact of PFBS, PFHS and PFOS in the Decatur, Alabama area.

SAMPLE PROCUREMENT, RECEIPT AND RETENTION

Water, soil, sediment, fish, clam, vegetation, small mammal liver and small mammal serum samples will be received at Exygen directly from Weston Solutions. The details of sample procurement for this study are outlined in the 3M work plan entitled "Phase 2 Work Plan for Sampling Environmental Media." The number and types of samples collected will vary depending availability in the field. The total number of samples received and analyzed for each matrix will be documented in the final report associated with this study.

Water, soil, and sediment samples will be used as received without further processing at Exygen. These samples will be stored refrigerated at 2°C-8°C. Fish, clam, vegetation and small mammal liver samples will be processed according to the appropriate analytical method (see Appendix I). These samples will be stored frozen at ≤ -10°C. Small mammal whole blood samples will be centrifuged in the field at the time of collection and the serum fraction will be used for the study. Small mammal serum will be stored frozen at ≤ -10°C.

The receipt and processing of the samples will be documented in the final report and raw data associated with the study.

SAMPLE IDENTIFICATION

Prior to analysis, each sample will be assigned a laboratory sample reference number. The reference number will be unique and will distinguish each laboratory sample that is processed throughout the analytical procedure. Chromatographic data will be identified by the laboratory sample reference number.

Sample storage conditions and locations will be documented throughout the study.

ANALYTICAL PROCEDURE SUMMARY

References:

V0001780: "Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Water by LC/MS/MS"

V0001781: "Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Soil by LC/MS/MS"

V0001782: "Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Sediment by LC/MS/MS"

V0001783: "Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Fish and Clams by LC/MS/MS"

V0001784: "Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Vegetation by LC/MS/MS"

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V0001786: "Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small Mammal Serum by LC/MS/MS"

The above methods use analytical conditions capable of separating the isomers of PFBS, PFHS and PFOS. The final report will include the isomers summed into total PFBS, total PFHS, and total PFOS found.

VERIFICATION OF ANALYTICAL PROCEDURE

A laboratory control sample will be used for the preparation of fortified control samples. The test substance will be made into solutions as per the method, and added to the matrices via a micropipette.

For water sampling, Exygen will supply one bottle per sample collected. The bottles will be 500 mL precleaned Sci/Spec Premier wide mouth HDPE bottles. These bottles have been routinely used for fluorochemical sample

collection at the testing facility and have been shown to be free of PFBS, PFHS and PFOS. Samples will be added to each container to a volumetric fill line at 200 mL. A field duplicate, a low field spike and a high field spike of each sample will be collected. The low and high field spike bottles will contain PFBS, PFHS and PFOS as well as perfluorooctanoic acid (PFOA) and 1.2-13C perfluorooctanoic acid (^{13}C PFOA). PFOA and ^{13}C PFOA are included in the solutions used to spike the samples. The results for PFOA and ^{13}C PFOA will not be reported in this study. Exygen will supply one field blank (control water) and two field blank spikes (control water fortified with PFBS, PFHS and PFOS at a low and high level) for every twenty samples collected. At the testing facility, each water sample (excluding field duplicates and field spikes) will be extracted in duplicate and will also be fortified at a low and high concentration with PFBS, PFHS and PFOS and processed through the described procedure to determine method accuracy and to check for bias.

For soil, sediment, clams, and vegetation, Exygen will supply one 500 mL precleaned Sci/Spec Premier wide mouth HDPE bottle per sample collected or a zip-seal bag. All containers/bags used for sample collection will be shipped to the sample location. Samples will be added to each container or bag in the field. At the testing facility, each sample will be extracted in duplicate and will also be fortified at a known concentration with PFBS, PFHS and PFOS at both a low and high level and processed through the described procedure to determine method accuracy and to check for bias.

For small mammal liver, Exygen will supply a 50 mL polypropylene centrifuge tube. For small mammal serum, Exygen will supply a collection kit for each sample containing serum separator tubes (red top), vacutainers, needle holders and needles, transfer pipettes, and polypropylene tubes. At the testing facility, each liver and serum sample will be extracted in duplicate and will also be fortified at a known concentration with PFBS, PFHS and PFOS at both a low and high level and processed through the described procedure to determine method accuracy and to check for bias.

Low and high spiking levels for each matrix are defined below:

Matrix	Low Spiking Level	High Spiking Level
Water	500 ng/L	5000 ng/L
Soil	4 ng/g	40 ng/g
Sediment	4 ng/g	40 ng/g
Fish	10 ng/g	100 ng/g
Clams	10 ng/g	100 ng/g
Vegetation	10 ng/g	100 ng/g
Small Mammal Liver	10 ng/g	100 ng/g
Small Mammal Serum	10 ng/mL	100 ng/mL

Recoveries are anticipated to be between 70% and 130% of the fortified levels; however, the exact precision and accuracy will be determined by the analysis of the quality control samples described above. A statement of accuracy will be included in the final report.

METHOD FOR CONTROL OF BIAS

Control of bias will be addressed by taking representative sub-samples from a homogeneous mixture of each matrix from untreated control samples, and by analyzing at least two levels of fortifications.

STATISTICAL METHODS

Statistics will be limited to those specified in the subject methods and to the calculation of average recoveries, as applicable.

GLP STATEMENT

All aspects of this study shall be performed and reported in compliance with EPA TSCA Good Laboratory Practice Standards 40 CFR 792. The final report or data package (supplied to the Sponsor) shall contain a statement that the study was conducted in compliance with current and applicable GLP standards and will outline any deviations in the study from those standards. This statement will be signed by the Study Director and Sponsor Representative.

REPORT

A final report will be prepared by the principal investigator or their designee at the conclusion of the study. The report will include, but will not be limited to, the following:

- The name and address of the Study Director, Sponsor Representative, and of the testing facility.
- A statement of GLP compliance (any related documentation, such as chain-of-custody records, must be in the study records).

- The signed and dated statement by the Exygen Research Quality Assurance Unit regarding dates of study inspections and dates findings were reported to the Study Director and Management.
- A description of the exact analytical conditions employed in the study. If the subject method was followed exactly, it is necessary to include only a copy of the analytical method. Any modifications to this method will be incorporated into the report. If the method is photo-reduced, the project number and page number must be included on each page.
- Description of the instrumentation used and operating conditions.
- All results from all sets analyzed. Control and fortified samples will be identified and the data table will include sample number and fortification level.
- Representative chromatograms for each analyte in each matrix, including chromatograms of a standard and a control sample, and a chromatogram at a fortification level. The location of the analyte peaks will be clearly identified in all chromatograms.
- All circumstances that may have affected the quality or integrity of the data will be documented in the report.
- Locations where raw data and the final report are to be archived.
- Additions or corrections to the final report shall be in the form of an amendment signed by the Study Director. The amendment shall clearly identify that part of the report that is being altered and the reasons for the alterations. The amendment will be signed and dated by the Study Director and the Sponsor Representative.
- All applicable requirements for reporting of study results as per 40 CFR 792.185.

SAFETY AND HEALTH

- Laboratory personnel will practice good sanitation and health habits.
- Every reasonable precaution shall be taken to prevent inadvertent exposure of personnel and the environment to the test or reference substance(s).

AMENDMENTS TO PROTOCOL

All significant changes to the analytical protocol outlined here will be expressed in writing, signed and dated by the Study Director and Sponsor Representative. Amendments usually will be issued prior to initiation of study plan change. However, when a change is required without sufficient time for the issue of a written amendment, that change may be effected verbally with supporting documentation signed and dated by the Study Director and followed with a written amendment as soon as possible. In this case, the effective date of the written amendment will be the date of the documented change. Copies of the signed amendments will be appended to all distributed study plan copies. The original amendment will be maintained with the original study plan. Any deviations from the study plan or from the analytical method as provided will be documented and reported promptly to the Sponsor Representative.

DATA RECORD KEEPING

Records to be maintained include the following (as appropriate):

- Sample tracking sheet(s)
- Sample receipt records, storage history, and chains of custody
- History and preparation of standards (stock, fortification, calibration)
- Description of any modifications to the method
- Instrument run sheets, bench-sheets or logs
- Analytical data tables
- All chromatographic and instrumental conditions
- Sample extraction and analysis dates
- A complete listing of study personnel, signatures and initials
- Chronological presentation of all study correspondence
- Any other documentation necessary for the reconstruction of the study

Chromatograms- All chromatograms will contain the following:

- Sample identification, injection date, arrow or other indication of the area of interest, and injection number corresponding to the run.
- Additionally, fortifications will include the amount of analyte added and the sample number of the sample that was fortified.
- Analytical standard chromatograms will additionally include the concentration (e.g., µg/mL).

- As part of the documentation the following sheets will be included in each analytical set: a run sheet listing the samples to be run in the set, and an instrument conditions sheet describing the instrument type and operating conditions.

QUALITY ASSURANCE

The QA Unit of Exygen Research will inspect the study at intervals adequate to assure compliance with GLP's, and will report the findings of audits to the Study Director, Exygen Management, and the Sponsor Representative.

RETENTION OF DATA AND ARCHIVING

All hard copy raw data, including, but not limited to, the original chromatograms, worksheets, correspondence, and results shall be included with the data package submitted to the Study Director. These will be archived with the original study plan, amendments, final report, and all pertinent information from the Sponsor.

The testing facility shall keep all electronic raw data and any instrument, equipment, and storage logs for the period of time specified in 40 CFR 792.195. An exact copy of the materials submitted to the study director will also be kept at Exygen Research.

Exygen will obtain permission from the study director before discarding or returning samples.

APPENDIX I

ANALYTICAL METHODS

V0001780: "Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Water by LC/MS/MS"
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V0001783: "Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Fish and Clams by LC/MS/MS"
V0001784: "Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Vegetation by LC/MS/MS"
V0001785: "Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small Mammal Liver by LC/MS/MS"
V0001786: "Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small Mammal Serum by LC/MS/MS"

Exygen Protocol Number: P0001131

ANALYTICAL METHOD
Method Number: V0001780

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Water by LC/MS/MS

Analytical Testing Facility: Exygen Research
3058 Research Drive
State College, PA 16801

Approved By:

Paul Connolly
Technical Leader, LC-MS, Exygen Research

10/26/04
Date

John Flaherty
Vice President, Operations, Exygen Research

10/26/04
Date

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Exygen Protocol Number: P0001131

Exygen Research Method Number V0001780

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Water by LC/MS/MS

1.0 Scope

This method is to be employed for the isolation and quantitation of perfluorooctanoic acid by High Performance Liquid Chromatography coupled to a tandem Mass Spectrometric Detector (LC/MS/MS) in water.

2.0 Safety

2.1 Always observe safe laboratory practices.
2.2 Consult the appropriate MSDS before handling any chemical for proper safety precautions.

3.0 Sample Requirement

3.1 At least 40 mL of test sample for extraction.
3.2 No sample processing is needed for water samples.
3.3 Samples stored refrigerated should be allowed to equilibrate to room temperature.
3.4 All samples must be thoroughly mixed before being sampled for extraction.
3.5 Any samples containing particles should be centrifuged at ~3000 rpm for ~5 minutes and the supernatant used for the extraction.
3.6 Sample collection procedures will be specified in the sampling plan for this project.

4.0 Reagents and Standards

4.1 Water – HPLC grade
4.2 Methanol – HPLC grade
4.3 Ammonium Acetate – A.C.S. Reagent Grade
4.4 Perfluorooctanoic Acid – Sigma-Aldrich

5.0 Instrument and Equipment

5.1 A high performance liquid chromatograph capable of pumping up to 2 solvents equipped with a variable volume injector capable of injecting 5-200 µL connected to a tandem Mass Spectrometer (LC/MS/MS).
5.2 A device to collect raw data for peak integration and quantitation.
5.3 Analytical balance capable of reading to 0.00001 g.
5.4 50 mL disposable polypropylene centrifuge tubes.
5.5 15 mL disposable polypropylene centrifuge tubes.
5.6 Disposable micropipets (50-100uL, 100-200uL).
5.7 125-mL LDPE narrow-mouth bottles.
5.8 2 mL clear HPLC vial kit.
5.9 Disposable pipettes.
5.10 Autopipettes (100-1000 µL and 10-100 µL), with disposable tips.
5.11 Waters Sep Pak Vac 6 cc (1g) tC18 SPE cartridges.

5.12 SPE vacuum manifold.
5.13 Centrifuge capable of spinning 50 mL polypropylene tubes at 3000 rpm.

6.0 Chromatographic System

6.1 Analytical Column: Fluophase RP (Keystone Scientific), 2.1 mm x 50 mm, 5µ (P/N: 82505-052130)
6.2 Temperature: 30°C
6.3 Mobile Phase (A): 2 mM Ammonium Acetate in Water
6.4 Mobile Phase (B): Methanol
6.5 Gradient Program:

Time (min)	% A	% B	Flow Rate (mL/min)
0.0	65	35	0.3
1.0	65	35	0.3
8.0	25	75	0.3
20.0	25	75	0.3
22.5	65	35	0.3

6.6 Injection Volume: 15 µL (can be increased to as much as 50 µL).
6.7 Quantitation: Peak Area – external standard calibration curve.
6.8 Run Time: ~ 23 minutes.

The above conditions are intended as a guide and may be changed in order to optimize the HPLC system.

7.0 MS/MS System

7.1 Mode: Electrospray Negative MRM mode, monitoring 413 → 369 m/z.

The above conditions are intended as a guide and may be changed in order to optimize the MSMS system.

8.0 Preparation of Solutions

8.1 Mobile Phase

8.1.1 2 mM ammonium acetate in water is prepared by adding 0.154 g of ammonium acetate to 1000 mL of water.

Alternate volumes may be prepared.

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Water by LC/MS/MS

9.0 Standard Preparation

9.1 Standard Stock/Fortification Solution

9.1.1 Prepare a stock solution of ~100 µg/mL of PFOA by weighing 10 mg of analytical standard (corrected for purity) and dilute to 100 mL with methanol in a 125-mL LDPE bottle.

9.1.2 A 10 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 100 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.3 A 1.0 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 10 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.4 A 0.1 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 1.0 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.5 A 0.01 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 0.1 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.6 The stock and fortification solutions are to be stored in a refrigerator at approximately 4°C and are stable for a maximum period of 6 months from the date of preparation.

9.2 Standard Calibration Solutions

9.2.1 LC/MS/MS calibration standards are prepared in HPLC water. The calibration standards are processed through the extraction procedure, identical to samples.

9.2.2 The following is a typical example: additional concentrations may be prepared as needed.

Concentration of Fortification Solution (ppb)	Fortification Volume (µL)	Volume of Fortified Control Sample (mL)	Final Concentration of Calibration Standard (ppt)*	Calibration Standard ID (example)
0	0	40	0	XCmmddyy-0
10	100	40	25	XCmmddyy-1
10	200	40	50	XCmmddyy-2
10	400	40	100	XCmmddyy-3
100	100	40	250	XCmmddyy-4
100	200	40	500	XCmmddyy-5
100	400	40	1000	XCmmddyy-6

* The extracted concentration of the calibration standard is equal to 8× its initial concentration, due to the concentration of the standard during the extraction (SPE).
XC = extracted calibration standard.

9.2.3 A zero standard solution (reagent blank) must be prepared with each set of standards extracted.

9.2.4 Store all extracted calibration standards in 15-mL polypropylene tubes at 2°C to 6°C, up to two weeks.

9.2.5 Alternate volumes and concentrations of standards may be prepared as needed.

10.0 Batch Set Up

10.1 Each batch of samples extracted (typically 20 or less) must include at least one reagent control (method blank using HPLC water) and two reagent controls fortified at known concentrations (lab control spike) to verify procedural recovery for the batch.

10.2 Requirements for field and laboratory duplicates and spikes will be specified in the quality assurance plan for this project.

11.0 Sample Extraction

11.1 Measure 40 mL of sample or a portion of sample diluted to 40 mL with water into 50 mL polypropylene centrifuge tubes (fortify as needed, replace lid and mix well).

11.2 Condition the C_{18} SPE cartridges (1 g, 6 mL) by passing 10 mL methanol followed by 5 mL of HPLC water (~ 2 drop/sec). Do not let column run dry

11.3 Load sample on conditioned C_{18} SPE cartridge. Discard eluate.

11.4 Elute with ~5 mL 100% methanol. Collect 5 mL of eluate into graduated 15 mL polypropylene centrifuge tubes (final volume = 5 mL).

11.5 Analyze samples using electrospray LC/MS/MS.

12.0 Chromatography

12.1 Inject the same amount of each standard, sample and fortified sample into the LC/MS/MS system. A calibration standard must precede and follow all analyzed samples.

12.2 Standards of PFOA corresponding to at least five or more concentration levels must be included in an analytical set.

12.3 An entire set of extracted calibration standards must be included at the beginning and at the end of a sample set. Extracted standards must be interspersed between every 5-10 samples. As an alternative, an entire set of extracted calibration standards may be injected at the beginning of a set followed by extracted calibration standards interspersed every 5-10 samples (to account for a second set of extracted standards). In either case, extracted calibration standards must be the first and last injection in a sample set.

12.4 Use linear standard curves for quantitation. Linear standard curves are generated for the analyte by linear regression using 1/x weighting of peak area

versus calibration standard concentration using MassLynx 3.3 (or equivalent) software system.

12.5 Sample response should not exceed standard responses. Any samples that exceed standard responses should be further diluted and reanalyzed.

13.0 Acceptance Criteria

13.1 Chromatogram must show a peak of a daughter ion at 369 amu from a parent of 413 amu. The 413 amu parent corresponds to the PFOA anion, while the daughter ion (369 amu) represents the loss of carbon dioxide.

13.2 Method blanks must not contain PFOA at levels greater than the LOQ. If a blank contains PFOA at levels greater than 50 ng/L, then a new blank sample must be obtained and the entire set must be re-extracted.

13.3 Recoveries of control spikes and matrix spikes must be between 70-130% of their known values. If a control spike falls outside the acceptable limits, the entire set of samples should be re-extracted. Any matrix spike outside 70-130% should be evaluated by the analyst to determine if re-extraction is warranted.

13.4 Any calibration standard found to be a statistical outlier by using the Huge Error Test, may be excluded from the calculation of the calibration curve. However, the total number of extracted calibration standards that could be excluded must not exceed 20% of the total number of extracted standards injected.

13.5 The correlation coefficient (R) for calibration curves generated must be ≥ 0.992 ($R^2 \geq 0.985$). If calibration results fall outside these limits, then appropriate steps must be taken to adjust instrument operation, and the standards or the relevant set of samples should be reanalyzed.

13.6 Retention times between standards and samples must not drift more than ± 4 % within an analytical run. If retention time drift exceeds this limit within an analytical run then the set must be reanalyzed.

14.0 Calculations

14.1 Use the following equation to calculate the amount of PFOA found (in ng/L, based on peak area) using the standard curve (linear regression parameters) generated by the Mass Lynx software program:

$$\text{PFOA found (ng/L)} = \frac{(\text{Peak area} - \text{intercept})}{\text{slope}} \times \text{DF}$$

DF = factor by which the final volume was diluted, if necessary.

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14.2 For samples fortified with known amounts of PFOA prior to extraction, use the following equation to calculate the percent recovery.

Recovery (%) =

$$\frac{[\text{total analyte found (ng/L)} - \text{analyte found in control (ng/L)}]}{\text{analyte added (ng/L)}} \times 100$$

Exygen Protocol Number: P0001131

ANALYTICAL METHOD
Method Number: V0001781

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Soil by LC/MS/MS

Analytical Testing Facility: Exygen Research
3058 Research Drive
State College, PA 16801

Approved By:

Paul Connolly
Technical Leader, LC-MS, Exygen Research

10/26/04
Date

John Flaherty
Vice President, Operations, Exygen Research

10/26/04
Date

Total Pages: 7

Exygen Protocol Number: P0001131

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Soil by LC/MS/MS

1.0 Scope

This method is to be employed for the isolation and quantitation of perfluorooctanoic acid by High Performance Liquid Chromatography coupled to a tandem Mass Spectrometric Detector (LC/MS/MS) in soil.

2.0 Safety

2.1 Always observe safe laboratory practices.
2.2 Consult the appropriate MSDS before handling any chemical for proper safety precautions.

3.0 Sample Requirement

3.1 At least 15 g of test sample for extraction.
3.2 No sample processing is needed for soil samples.
3.3 Samples stored refrigerated should be allowed to equilibrate to room temperature.
3.4 All samples must be thoroughly mixed before being sampled for extraction.
3.5 Sample collection procedures will be specified in the sampling plan for this project.

4.0 Reagents and Standards

4.1 Water – HPLC grade
4.2 Methanol – HPLC grade
4.3 Ammonium Acetate – A.C.S. Reagent Grade
4.4 Perfluorooctanoic Acid – Sigma-Aldrich

5.0 Instrument and Equipment

5.1 A high performance liquid chromatograph capable of pumping up to 2 solvents equipped with a variable volume injector capable of injecting 5-200 µL connected to a tandem Mass Spectrometer (LC/MS/MS).
5.2 A device to collect raw data for peak integration and quantitation.
5.3 Analytical balance capable of reading to 0.00001 g.
5.4 50 mL disposable polypropylene centrifuge tubes.
5.5 15 mL disposable polypropylene centrifuge tubes.
5.6 Disposable micropipets (50-100uL, 100-200uL).
5.7 125-mL LDPE narrow-mouth bottles.
5.8 2 mL clear HPLC vial kit.
5.9 Disposable pipettes.
5.10 Autopipettes (100-1000 µL and 10-100 µL), with disposable tips.
5.11 Waters Sep Pak Vac 6 cc (1g) tC18 SPE cartridges.
5.12 SPE vacuum manifold.
5.13 Ultrasonic bath.

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Soil by LC/MS/MS

5.14 Wrist-action shaker.
5.15 Centrifuge capable of spinning 50 mL polypropylene tubes at 5000 rpm.

6.0 Chromatographic System

6.1 Analytical Column: Fluophase RP (Keystone Scientific), 2.1 mm x 50 mm, 5µ (P/N: 82505-052130)
6.2 Temperature: 30°C
6.3 Mobile Phase (A): 2 mM Ammonium Acetate in Water
6.4 Mobile Phase (B): Methanol
6.5 Gradient Program:

Time (min)	% A	% B	Flow Rate (mL/min)
0.0	65	35	0.3
1.0	65	35	0.3
8.0	25	75	0.3
20.0	25	75	0.3
22.5	65	35	0.3

6.6 Injection Volume: 15 µL (can be increased to as much as 50 µL).
6.7 Quantitation: Peak Area – external standard calibration curve.
6.8 Run Time: ~ 23 minutes.

The above conditions are intended as a guide and may be changed in order to optimize the HPLC system.

7.0 MS/MS System

7.1 Mode: Electrospray Negative MRM mode, monitoring 413 → 369 m/z for PFOA.

The above conditions are intended as a guide and may be changed in order to optimize the MSMS system.

8.0 Preparation of Solutions

8.1 Mobile Phase

8.1.1 2 mM ammonium acetate in water is prepared by adding 0.154 g of ammonium acetate to 1000 mL of water.

Alternate volumes may be prepared.

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Soil by LC/MS/MS

9.0 Standard Preparation

9.1 Standard Stock/Fortification Solution

9.1.1 Prepare a stock solution of ~100 µg/mL of PFOA by weighing 10 mg of analytical standard (corrected for purity) and dilute to 100 mL with methanol in a 125-mL LDPE bottle.

9.1.2 A 10 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 100 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.3 A 1.0 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 10 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.4 A 0.1 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 1.0 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.5 A 0.01 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 0.1 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.6 The stock and fortification solutions are to be stored in a refrigerator at approximately 4°C and are stable for a maximum period of 6 months from the date of preparation.

9.2 Standard Calibration Solutions

9.2.1 LC/MS/MS calibration standards are prepared in HPLC water. The calibration standards are processed through the extraction procedure. identical to samples.

9.2.2 The following is a typical example: additional concentrations may be prepared as needed.

Concentration of Fortification Solution (ppb)	Fortification Volume (µL)	Volume of Fortified Control Sample (mL)	Final Concentration of Calibration Standard (ppt)*	Calibration Standard ID (example)
0	0	40	0	XCmmddyy-0
10	100	40	25	XCmmddyy-1
10	200	40	50	XCmmddyy-2
10	400	40	100	XCmmddyy-3
100	100	40	250	XCmmddyy-4
100	200	40	500	XCmmddyy-5
100	400	40	1000	XCmmddyy-6

* The extracted concentration of the calibration standard is equal to 8× its initial concentration, due to the concentration of the standard during the extraction (SPE). XC = extracted calibration standard.

9.2.3 A zero standard solution (reagent blank) must be prepared with each set of standards extracted.
9.2.4 Store all extracted calibration standards in 15-mL polypropylene tubes at 2°C to 6°C, up to two weeks.
9.2.5 Alternate volumes and concentrations of standards may be prepared as needed.

10.0 Batch Set Up

10.1 Each batch of samples extracted (typically 20 or less) must include at least one reagent control (method blank using 5 mL of methanol) and two reagent controls fortified at known concentrations (lab control spike) to verify procedural recovery for the batch.
10.2 Requirements for field and laboratory duplicates and spikes will be specified in the quality assurance plan for this project.

11.0 Sample Extraction

11.1 Weigh 5 g of sample into 50 mL polypropylene centrifuge tubes (fortify as needed, replace lid and mix well).
11.2 Add 5 mL of methanol and shake on a wrist action shaker for ~15 minutes.
11.3 Transfer the tubes to an ultrasonic bath and sonicate for ~15 minutes.
11.4 Bring the volume up to 40 mL with water in the 50 mL polypropylene centrifuge tube.
11.5 Centrifuge for ~10 minutes at ~3000 rpm.
11.6 Condition the C_{18} SPE cartridges (1 g, 6 mL) by passing 10 mL methanol followed by 5 mL of HPLC water (~ 2 drop/sec). Do not let column run dry.
11.7 Load (decant) the sample on the conditioned C_{18} SPE cartridge. Discard eluate.
11.8 Elute with ~5 mL 100% methanol. Collect 5 mL of eluate into graduated 15 mL polypropylene centrifuge tubes (final volume = 5 mL).
11.9 Analyze samples using electrospray LC/MS/MS.

12.0 Chromatography

12.1 Inject the same amount of each standard, sample and fortified sample into the LC/MS/MS system. A calibration standard must precede and follow all analyzed samples.
12.2 Standards of PFOA corresponding to at least five or more concentration levels must be included in an analytical set.
12.3 An entire set of extracted calibration standards must be included at the beginning and at the end of a sample set. Extracted standards must be interspersed between every 5-10 samples. As an alternative, an entire set of

extracted calibration standards may be injected at the beginning of a set followed by extracted calibration standards interspersed every 5-10 samples (to account for a second set of extracted standards). In either case, extracted calibration standards must be the first and last injection in a sample set.

12.4 Use linear standard curves for quantitation. Linear standard curves are generated for the analyte by linear regression using 1/x weighting of peak area versus calibration standard concentration using MassLynx 3.3 (or equivalent) software system.

12.5 Sample response should not exceed standard responses. Any samples that exceed standard responses should be further diluted and reanalyzed.

13.0 Acceptance Criteria

13.1 Chromatogram must show a peak of a daughter ion at 369 amu from a parent of 413 amu. The 413 amu parent corresponds to the PFOA anion, while the daughter ion (369 amu) represents the loss of carbon dioxide.

13.2 Method blanks must not contain PFOA at levels greater than the LOQ. If a blank contains PFOA at levels greater than 50 ng/L, then a new blank sample must be obtained and the entire set must be re-extracted.

13.3 Recoveries of control spikes and matrix spikes must be between 70-130% of their known values. If a control spike falls outside the acceptable limits, the entire set of samples should be re-extracted. Any matrix spike outside 70-130% should be evaluated by the analyst to determine if re-extraction is warranted.

13.4 Any calibration standard found to be a statistical outlier by using the Huge Error Test, may be excluded from the calculation of the calibration curve. However, the total number of extracted calibration standards that could be excluded must not exceed 20% of the total number of extracted standards injected.

13.5 The correlation coefficient (R) for calibration curves generated must be ≥0.992 (R^2 ≥0.985). If calibration results fall outside these limits, then appropriate steps must be taken to adjust instrument operation, and the standards or the relevant set of samples should be reanalyzed.

13.6 Retention times between standards and samples must not drift more than ± 4 % within an analytical run. If retention time drift exceeds this limit within an analytical run then the set must be reanalyzed.

14.0 Calculations

14.1 Use the following equation to calculate the amount of PFOA found (in ng/L, based on peak area) using the standard curve (linear regression parameters) generated by the Mass Lynx software program:

$$\text{PFOA found (ng/L)} = \frac{(\text{Peak area} - \text{intercept})}{\text{slope}} \times \text{DF}$$

DF = factor by which the final volume was diluted, if necessary.

14.2 For samples fortified with known amounts of PFOA prior to extraction, use the following equation to calculate the percent recovery.

Recovery (%) =

$$\frac{[\text{total analyte found (ng/L)} - \text{analyte found in control (ng/L)}]}{\text{analyte added (ng/L)}} \times 100$$

14.3 Use the following equation to convert the amount of PFOA found in ng/L to ng/g (ppb).

$$\text{PFOA found (ppb)} = \frac{[\text{PFOA found (ng/L)} \times \text{volume extracted (0.04L)}]}{\text{sample weight (5 g)}}$$

14.4 Use the following equation to calculate the amount of PFOA found in ppb based on dry weight.

PFOA found (ppb) dry weight = PFOA found (ppb) x [100% / total solids(%)]

Exygen Protocol Number: P0001131

ANALYTICAL METHOD
Method Number: V0001782

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Sediment by LC/MS/MS

Analytical Testing Facility: Exygen Research
3058 Research Drive
State College, PA 16801

Approved By:

Paul Connolly
Technical Leader, LC-MS, Exygen Research

10/26/04
Date

John Flaherty
Vice President, Operations, Exygen Research

10/26/04
Date

Total Pages: 7

Exygen Protocol Number: P0001131

Exygen Research Method Number V0001782

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Sediment by LC/MS/MS

1.0 Scope

This method is to be employed for the isolation and quantitation of perfluorooctanoic acid by High Performance Liquid Chromatography coupled to a tandem Mass Spectrometric Detector (LC/MS/MS) in sediment.

2.0 Safety

2.1 Always observe safe laboratory practices.
2.2 Consult the appropriate MSDS before handling any chemical for proper safety precautions.

3.0 Sample Requirement

3.1 At least 30 g of test sample for extraction.
3.2 No sample processing is needed for sediment samples.
3.3 Samples stored refrigerated should be allowed to equilibrate to room temperature.
3.4 All samples must be thoroughly mixed before being sampled for extraction.
3.5 Sample collection procedures will be specified in the sampling plan for this project.

4.0 Reagents and Standards

4.1 Water – HPLC grade
4.2 Methanol – HPLC grade
4.3 Acetic Acid – Reagent grade
4.4 Ammonium Acetate – A.C.S. Reagent Grade
4.5 Perfluorooctanoic Acid – Sigma-Aldrich

5.0 Instrument and Equipment

5.1 A high performance liquid chromatograph capable of pumping up to 2 solvents equipped with a variable volume injector capable of injecting 5-200 µL connected to a tandem Mass Spectrometer (LC/MS/MS).
5.2 A device to collect raw data for peak integration and quantitation.
5.3 Analytical balance capable of reading to 0.00001 g.
5.4 50 mL disposable polypropylene centrifuge tubes.
5.5 15 mL disposable polypropylene centrifuge tubes.
5.6 Disposable micropipets (50-100uL, 100-200uL).
5.7 125-mL LDPE narrow-mouth bottles.
5.8 2 mL clear HPLC vial kit.
5.9 Disposable pipettes.
5.10 Autopipettes (100-1000 µL and 10-100 µL), with disposable tips.
5.11 Waters Sep Pak Vac 6 cc (1g) tC18 SPE cartridges.
5.12 SPE vacuum manifold.

5.13 Vortexer.
5.14 Wrist-action shaker.
5.15 Centrifuge capable of spinning 50 mL polypropylene tubes at 3000 rpm.

6.0 Chromatographic System

6.1 Analytical Column: Fluophase RP (Keystone Scientific), 2.1 mm x 50 mm, 5µ (P/N: 82505-052130)
6.2 Temperature: 30°C
6.3 Mobile Phase (A): 2 mM Ammonium Acetate in Water
6.4 Mobile Phase (B): Methanol
6.5 Gradient Program:

Time (min)	% A	% B	Flow Rate (mL/min)
0.0	65	35	0.3
1.0	65	35	0.3
8.0	25	75	0.3
20.0	25	75	0.3
22.5	65	35	0.3

6.6 Injection Volume: 15 µL (can be increased to as much as 50 µL).
6.7 Quantitation: Peak Area – external standard calibration curve.
6.8 Run Time: ~ 23 minutes.

The above conditions are intended as a guide and may be changed in order to optimize the HPLC system.

7.0 MS/MS System

7.1 Mode: Electrospray Negative MRM mode, monitoring 413 → 369 m/z for PFOA.

The above conditions are intended as a guide and may be changed in order to optimize the MSMS system.

8.0 Preparation of Solutions

8.1 Mobile Phase

8.1.1 2 mM ammonium acetate in water is prepared by adding 0.154 g of ammonium acetate to 1000 mL of water.

8.2 Extraction Solutions

8.2.1 1% acetic acid in water is prepared by adding 10 mL of acetic acid to 1000 mL of water.

Alternate volumes may be prepared.

9.0 Standard Preparation

9.1 Standard Stock/Fortification Solution

9.1.1 Prepare a stock solution of ~100 µg/mL of PFOA by weighing 10 mg of analytical standard (corrected for purity) and dilute to 100 mL with methanol in a 125-mL LDPE bottle.

9.1.2 A 10 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 100 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.3 A 1.0 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 10 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.4 A 0.1 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 1.0 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.5 A 0.01 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 0.1 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.6 The stock and fortification solutions are to be stored in a refrigerator at approximately 4°C and are stable for a maximum period of 6 months from the date of preparation.

9.2 Standard Calibration Solutions

9.2.1 LC/MS/MS calibration standards are prepared in methanol via dilution of the 0.1 µg/mL fortification solution.

9.2.2 The following is a typical example: additional concentrations may be prepared as needed.

Concentration of Fortification Solution (ng/mL)	Volume (mL)	Diluted to (mL)	Final Concentration (ng/mL)
100	10	100	10.0
100	5	100	5.0
100	2	100	2.0
10	10	100	1.0
5	10	100	0.5
2	10	100	0.2

9.2.3 Store all calibration standards in 125-mL LDPE narrow-mouth bottles at 2°C to 6°C, up to six months.

9.2.4 Alternate volumes and concentrations of standards may be prepared as needed.

10.0 Batch Set Up

10.1 Each batch of samples extracted (typically 20 or less) must include at least one untreated control and two untreated controls fortified at known concentrations (lab control spike) to verify procedural recovery for the batch.

10.2 Requirements for field and laboratory duplicates and spikes will be specified in the quality assurance plan for this project.

11.0 Sample Extraction

11.1 Weigh 5 g of sample into 50 mL polypropylene centrifuge tubes (fortify as needed, replace lid and mix well).

11.2 Add 35 mL of 1% acetic acid, cap, vortex and shake on a wrist action shaker for ~60 minutes.

11.3 Centrifuge the tubes at ~3000 rpm for ~20 minutes.

11.4 Condition the C_{18} SPE cartridges (1 g, 6 mL) by passing 10 mL methanol followed by 20 mL of HPLC water (~ 2 drop/sec). Do not let column run dry

11.5 Load (decant) the sample on the conditioned C_{18} SPE cartridge. Discard eluate.

11.6 Add 20 mL of methanol to the sediment left in the bottom of the 50 mL centrifuge tube. Cap, vortex and shake on a wrist action shaker for ~30 minutes.

11.7 Centrifuge the tubes at ~3000 rpm for ~20 minutes.

11.8 Decant the methanol onto the same SPE cartridge. Collect the eluate.

11.9 Wash the column with 4 mL of methanol. Collect the eluate and add it to the eluate collected in step 11.8.

11.10 Condition a second C_{18} SPE cartridge (1 g, 6 mL) by passing 10 mL methanol followed by 20 mL of HPLC water (~ 2 drop/sec). Do not let column run dry

11.11 Add the methanol to ~200 mL of water and load on the second conditioned SPE cartridge.

11.12 Elute with ~5 mL 100% methanol. Collect 5 mL of eluate into graduated 15 mL polypropylene centrifuge tubes (final volume = 5 mL).

11.13 Analyze samples using electrospray LC/MS/MS.

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Sediment by LC/MS/MS

12.0 Chromatography

12.1 Inject the same amount of each standard, sample and fortified sample into the LC/MS/MS system. A calibration standard must precede and follow all analyzed samples.

12.2 Standards of PFOA corresponding to at least five or more concentration levels must be included in an analytical set.

12.3 An entire set of extracted calibration standards must be included at the beginning and at the end of a sample set. Standards must be interspersed between every 5-10 samples. As an alternative, an entire set of calibration standards may be injected at the beginning of a set followed by calibration standards interspersed every 5-10 samples (to account for a second set of standards). In either case, calibration standards must be the first and last injection in a sample set.

12.4 Use linear standard curves for quantitation. Linear standard curves are generated for the analyte by linear regression using 1/x weighting of peak area versus calibration standard concentration using MassLynx 3.3 (or equivalent) software system.

12.5 Sample response should not exceed standard responses. Any samples that exceed standard responses should be further diluted and reanalyzed.

13.0 Acceptance Criteria

13.1 Chromatogram must show a peak of a daughter ion at 369 amu from a parent of 413 amu. The 413 amu parent corresponds to the PFOA anion, while the daughter ion (369 amu) represents the loss of carbon dioxide.

13.2 Method blanks must not contain PFOA at levels greater than the LOQ. If a blank contains PFOA at levels greater than 0.2 ng/mL, then a new blank sample must be obtained and the entire set must be re-extracted.

13.3 Recoveries of control spikes and matrix spikes must be between 70-130% of their known values. If a control spike falls outside the acceptable limits, the entire set of samples should be re-extracted. Any matrix spike outside 70-130% should be evaluated by the analyst to determine if re-extraction is warranted.

13.4 Any calibration standard found to be a statistical outlier by using the Huge Error Test, may be excluded from the calculation of the calibration curve However, the total number of extracted calibration standards that could be excluded must not exceed 20% of the total number of extracted standards injected.

13.5 The correlation coefficient (R) for calibration curves generated must be ≥0.992 (R^2 ≥0.985). If calibration results fall outside these limits, then appropriate steps must be taken to adjust instrument operation, and the standards or the relevant set of samples should be reanalyzed.

13.6 Retention times between standards and samples must not drift more than ± 4 % within an analytical run. If retention time drift exceeds this limit within an analytical run then the set must be reanalyzed.

14.0 Calculations

14.1 Use the following equation to calculate the amount of PFOA found (in ng/mL, based on peak area) using the standard curve (linear regression parameters) generated by the Mass Lynx software program:

$$\text{PFOA found (ng/mL)} = \frac{(\text{Peak area} - \text{intercept})}{\text{slope}} \times \text{DF}$$

DF = factor by which the final volume was diluted, if necessary.

14.2 For samples fortified with known amounts of PFOA prior to extraction, use the following equation to calculate the percent recovery.

$$\text{Recovery (\%)} = \frac{[\text{total analyte found (ng/mL)} - \text{analyte found in control (ng/mL)}]}{\text{analyte added (ng/mL)}} \times 100$$

14.3 Use the following equation to convert the amount of PFOA found in ng/mL to ng/g (ppb).

$$\text{PFOA found (ppb)} = \frac{[\text{PFOA found (ng/mL)} \times \text{final volume (5 mL)}]}{\text{sample weight (5 g)}}$$

14.4 Use the following equation (if necessary) to calculate the amount of PFOA found in ppb based on dry weight.

$$\text{PFOA found (ppb) dry weight} = \text{PFOA found (ppb)} \times [100\% / \text{total solids(\%)}]$$

Exygen Protocol Number: P0001131

ANALYTICAL METHOD
Method Number: V0001783

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Fish and Clams by LC/MS/MS

Analytical Testing Facility: Exygen Research
3058 Research Drive
State College, PA 16801

Approved By:

Paul Connolly
Technical Leader, LC-MS, Exygen Research

10/26/04
Date

John Flaherty
Vice President, Operations, Exygen Research

10/26/04
Date

Total Pages: 8

Exygen Protocol Number: P0001131

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Fish and Clams by LC/MS/MS

1.0 Scope

This method is to be employed for the isolation and quantitation of perfluorooctanoic acid by High Performance Liquid Chromatography coupled to a tandem Mass Spectrometric Detector (LC/MS/MS) in fish and clams.

2.0 Safety

2.1 Always observe safe laboratory practices.
2.2 Consult the appropriate MSDS before handling any chemical for proper safety precautions.

3.0 Sample Requirement

3.1 At least 20 g of test sample for extraction.
3.2 Samples should be processed before extraction. Place the frozen sample in a food processor and homogenize with dry ice. Place the samples in containers and leave open in frozen storage overnight to allow for carbon dioxide sublimation. Seal and place the samples in frozen storage until time of analysis.
3.3 Sample collection procedures will be specified in the sampling plan for this project.

4.0 Reagents and Standards

4.1 Water – HPLC grade
4.2 Acetonitrile – HPLC grade
4.3 Carbon (120-400 mesh) – Reagent grade
4.4 Methanol – HPLC grade
4.5 Silica gel (60-200 mesh) – Reagent grade
4.6 Florisil (60-100 mesh) – Reagent grade
4.7 Superclean LC-NH_2 – Reagent grade
4.8 1-Octanol – HPLC grade
4.9 L-Ascorbic acid – Reagent grade
4.10 Dimethyldichlorosilane – Reagent grade
4.11 Toluene – Reagent grade
4.12 Ammonium Acetate – A.C.S. Reagent Grade
4.13 Perfluorooctanoic Acid – Sigma-Aldrich

5.0 Instrument and Equipment

5.1 A high performance liquid chromatograph capable of pumping up to 2 solvents equipped with a variable volume injector capable of injecting 5-200 µL connected to a tandem Mass Spectrometer (LC/MS/MS).
5.2 A device to collect raw data for peak integration and quantitation.
5.3 Analytical balance capable of reading to 0.00001 g.

5.4 Rotary evaporator.
5.5 Tissumizer.
5.6 125 mL pear-shaped flasks.
5.7 50 mL disposable polypropylene centrifuge tubes.
5.8 15 mL disposable polypropylene centrifuge tubes.
5.9 Disposable micropipets (50-100uL, 100-200uL).
5.10 125-mL LDPE narrow-mouth bottles.
5.11 2 mL clear HPLC vial kit.
5.12 Disposable pipettes.
5.13 Autopipettes (100-1000 µL and 10-100 µL), with disposable tips.
5.14 SPE tubes (20mL) (Supelco cat. no. N057177).
5.15 Wrist action shaker.
5.16 Centrifuge capable of spinning 50 mL polypropylene tubes at 2000 rpm.

6.0 Chromatographic System

6.1 Analytical Column: Fluophase RP (Keystone Scientific), 2.1 mm x 50 mm, 5µ (P/N: 82505-052130)
6.2 Temperature: 30°C
6.3 Mobile Phase (A) : 2 mM Ammonium Acetate in Water
6.4 Mobile Phase (B) : Methanol
6.5 Gradient Program:

Time (min)	% A	% B	Flow Rate (mL/min)
0.0	65	35	0.3
1.0	65	35	0.3
8.0	25	75	0.3
20.0	25	75	0.3
22.5	65	35	0.3

6.6 Injection Volume: 15 µL (can be increased to as much as 50 µL).
6.7 Quantitation: Peak Area ~ external standard calibration curve.
6.8 Run Time: ~ 23 minutes.

The above conditions are intended as a guide and may be changed in order to optimize the HPLC system.

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Fish and Clams by LC/MS/MS

7.0 MS/MS System

7.1 Mode: Electrospray Negative MRM mode, monitoring 413 → 369 m/z for PFOA.

The above conditions are intended as a guide and may be changed in order to optimize the MSMS system.

8.0 Preparation of Solutions

8.1 Mobile Phase

8.1.1 2 mM ammonium acetate in water is prepared by adding 0.154 g of ammonium acetate to 1000 mL of water.

8.2 Extraction Solutions

8.2.1 2% ascorbic acid in methanol is prepared by dissolving 2 g of ascorbic acid in 100 mL of methanol.

8.2.2 30% Dimethyldichlorosilane in toluene is prepared by bringing 3 mL of dimethyldichlorosilane to a final volume of 10 mL with toluene.

Alternate volumes may be prepared.

9.0 Standard Preparation

9.1 Standard Stock/Fortification Solution

9.1.1 Prepare a stock solution of ~100 µg/mL of PFOA by weighing 10 mg of analytical standard (corrected for purity) and dilute to 100 mL with methanol in a 125-mL LDPE bottle.

9.1.2 A 1.0 µg/mL fortification solution of PFOA is prepared by bringing 1 mL of the 100 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.3 A 0.1 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 1.0 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.4 A 0.01 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 0.1 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.5 The stock and fortification solutions are to be stored in a refrigerator at approximately 4°C and are stable for a maximum period of 6 months from the date of preparation.

9.2 Standard Calibration Solutions

9.2.1 LC/MS/MS calibration standards are prepared in methanol via dilution of the 1.0 µg/mL fortification solution.

9.2.2 The following is a typical example: additional concentrations may be prepared as needed.

Concentration of Fortification Solution (µg/mL)	Volume (mL)	Diluted to (mL)	Final Concentration (µg/mL)
1.0	5.0	100	0.05
1.0	2.5	100	0.025
1.0	1.0	100	0.01
0.05	10	100	0.005
0.025	10	100	0.0025
0.1	10	100	0.001
0.005	10	100	0.0005

9.2.3 Store all calibration standards in 125-mL LDPE narrow-mouth bottles at 2°C to 6°C, up to six months.

9.2.4 Alternate volumes and concentrations of standards may be prepared as needed.

10.0 Batch Set Up

10.1 Each batch of samples extracted (typically 20 or less) must include at least one untreated control and two untreated controls fortified at known concentrations (lab control spike) to verify procedural recovery for the batch.

10.2 Requirements for field and laboratory duplicates and spikes will be specified in the quality assurance plan for this project.

11.0 Sample Extraction

11.1 Weigh 5 g of frozen sample into 50 mL polypropylene centrifuge tubes (fortify as needed, replace lid and mix well).

11.2 Add 30 mL of acetonitrile and shake on a wrist action shaker for ~15 minutes.

11.3 Place the tubes in a freezer for ~1 hour.

11.4 Pack and condition the SPE tubes and silanize the pear-shaped flasks.

11.5 Pack the 20 mL SPE tubes in sequence with 2 g florisil, 2 g silica gel, 2 g carbon, and 1 g LC-NH_2. Condition the columns with 20 mL of methanol, then 20 mL of acetonitrile. Discard all washes. Do not allow the column to dry.

11.6 Silanize the 125 mL pear-shaped flasks by rinsing with the 30% dimethyldichlorosilane in toluene solution. Rinse the flask with toluene once, followed by methanol (three times). Dry the flasks completely before use, either by air-drying or with a stream of nitrogen.

11.7 Centrifuge the 50 mL polypropylene tubes containing sample at ~2000 rpm for ~10 minutes.

11.8 Decant the extract on to a conditioned SPE column fitted inside the mouth of the pear-shaped flask. Collect the eluate in the 125 mL silanized pear-shape flask.

11.9 Add 10 mL of acetonitrile to the sample in the 50 mL centrifuge tube. Homogenize the frozen fat phase using a tissumizer for ~30 seconds and rinse the tissumizer with ~10 mL of acetonitrile into the tube.

11.10 Shake the sample again for ~10 minutes on a wrist-action shaker.

11.11 Place the tubes in a freezer for ~ 1 hour more.

11.12 Centrifuge the 50 mL polypropylene tubes containing sample at ~2000 rpm for ~10 minutes.

11.13 Decant the extract onto the same SPE column. Collect the eluate into the same pear-shaped flask and combine with the eluent from the initial extraction.

11.14 Pass 20 mL of acetonitrile through the SPE column and combine the eluate in the same pear-shaped flask.

11.15 Add 3-4 drops of 1-octanol to the extract in the pear-shaped flask and evaporate at reduced pressure using a rotary evaporator (at < 40°C).

11.16 Make the final volume, by adding 2 mL of 2% ascorbic acid in methanol to the pear-shaped flask and swirl to mix/dissolve.

11.17 Transfer the extracts to HPLC vials using disposable pipets.

11.18 Analyze samples using electrospray LC/MS/MS.

12.0 Chromatography

12.1 Inject the same amount of each standard, sample and fortified sample into the LC/MS/MS system. A calibration standard must precede and follow all analyzed samples.

12.2 Standards of PFOA corresponding to at least five or more concentration levels must be included in an analytical set.

12.3 An entire set of calibration standards must be included at the beginning and at the end of a sample set. Standards must be interspersed between every 5-10 samples. As an alternative, an entire set of calibration standards may be injected at the beginning of a set followed by calibration standards interspersed every 5-10 samples (to account for a second set of standards). In either case, calibration standards must be the first and last injection in a sample set.

12.4 Use linear standard curves for quantitation. Linear standard curves are generated for the analyte by linear regression using 1/x weighting of peak area versus calibration standard concentration using MassLynx 3.3 (or equivalent) software system.

12.5 Sample response should not exceed standard responses. Any samples that exceed standard responses should be further diluted and reanalyzed.

13.0 Acceptance Criteria

13.1 Chromatogram must show a peak of a daughter ion at 369 amu from a parent of 413 amu. The 413 amu parent corresponds to the PFOA anion, while the daughter ion (369 amu) represents the loss of carbon dioxide.

13.2 Method blanks must not contain PFOA at levels greater than the LOQ. If a blank contains PFOA at levels greater than 0.5 ppb, then a new blank sample must be obtained and the entire set must be re-extracted.

13.3 Recoveries of control spikes and matrix spikes must be between 70-130% of their known values. If a control spike falls outside the acceptable limits, the entire set of samples should be re-extracted.

13.4 Any calibration standard found to be a statistical outlier by using the Huge Error Test, may be excluded from the calculation of the calibration curve. However, the total number of calibration standards that could be excluded must not exceed 20% of the total number of standards injected.

13.5 The correlation coefficient (R) for calibration curves generated must be ≥0.992 (R^2 ≥0.985). If calibration results fall outside these limits, then appropriate steps must be taken to adjust instrument operation, and the standards or the relevant set of samples should be reanalyzed.

13.6 Retention times between standards and samples must not drift more than ± 4 % within an analytical run. If retention time drift exceeds this limit within an analytical run then the set must be reanalyzed.

14.0 Calculations

14.1 Use the following equation to calculate the amount of PFOA found (in ng/mL, based on peak area) using the standard curve (linear regression parameters) generated by the Mass Lynx software program:

$$\text{PFOA found (ng/mL)} = \frac{\text{(Peak area - intercept)}}{\text{slope}}$$

14.2 Use the following equation to convert the amount of PFOA found in ng/mL to ng/g (ppb).

$$\text{PFOA found (ppb)} = \frac{\text{[PFOA found (ng/mL) x final volume (mL) x DF]}}{\text{sample weight (g)}}$$

DF = factor by which the final volume was diluted, if necessary.

14.3 For samples fortified with known amounts of PFOA prior to extraction, use the following equation to calculate the percent recovery.

Recovery (%) =

$$\frac{[\text{total analyte found (ng/g)} - \text{analyte found in control (ng/g)}]}{\text{analyte added (ng/g)}} \times 100$$

Exygen Protocol Number: P0001131

ANALYTICAL METHOD
Method Number: V0001784

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Vegetation by LC/MS/MS

Analytical Testing Facility: Exygen Research
3058 Research Drive
State College, PA 16801

Approved By:

Paul Connolly
Technical Leader, LC-MS, Exygen Research

10/26/04
Date

John Flaherty
Vice President, Operations, Exygen Research

10/26/04
Date

Total Pages: 7

Exygen Protocol Number: P0001131

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Vegetation by LC/MS/MS

1.0 Scope

This method is to be employed for the isolation and quantitation of perfluorooctanoic acid by High Performance Liquid Chromatography coupled to a tandem Mass Spectrometric Detector (LC/MS/MS) in vegetation.

2.0 Safety

2.1 Always observe safe laboratory practices.
2.2 Consult the appropriate MSDS before handling any chemical for proper safety precautions.

3.0 Sample Requirement

3.1 At least 20 g of test sample for extraction.
3.2 Samples should be processed before extraction. Place the frozen sample in a food processor and homogenize with dry ice. Place the samples in containers and leave open in frozen storage overnight to allow for carbon dioxide sublimation. Seal and place the samples in frozen storage until time of analysis.
3.3 Sample collection procedures will be specified in the sampling plan for this project.

4.0 Reagents and Standards

4.1 Water – HPLC grade
4.2 Acetonitrile – HPLC grade
4.3 Carbon (120-400 mesh) – Reagent grade
4.4 Methanol – HPLC grade
4.5 Silica gel (60-200 mesh) – Reagent grade
4.6 Florisil (60-100 mesh) – Reagent grade
4.7 Superclean LC-NH_2 – Reagent grade
4.8 1-Octanol – HPLC grade
4.9 L-Ascorbic acid – Reagent grade
4.10 Dimethyldichlorosilane – Reagent grade
4.11 Toluene – Reagent grade
4.12 Ammonium Acetate – A.C.S. Reagent Grade
4.13 Perfluorooctanoic Acid – Sigma-Aldrich

5.0 Instrument and Equipment

5.1 A high performance liquid chromatograph capable of pumping up to 2 solvents equipped with a variable volume injector capable of injecting 5-200 µL connected to a tandem Mass Spectrometer (LC/MS/MS).
5.2 A device to collect raw data for peak integration and quantitation.
5.3 Analytical balance capable of reading to 0.00001 g.

5.4 Rotary evaporator.
5.5 125 mL pear-shaped flasks.
5.6 50 mL disposable polypropylene centrifuge tubes.
5.7 15 mL disposable polypropylene centrifuge tubes.
5.8 Disposable micropipets (50-100uL, 100-200uL).
5.9 125-mL LDPE narrow-mouth bottles.
5.10 2 mL clear HPLC vial kit.
5.11 Disposable pipettes.
5.12 Autopipettes (100-1000 µL and 10-100 µL), with disposable tips.
5.13 SPE tubes (20mL) (Supelco cat. no. N057177).
5.14 Wrist action shaker.
5.15 Centrifuge capable of spinning 50 mL polypropylene tubes at 2000 rpm.

6.0 Chromatographic System

6.1 Analytical Column: Fluophase RP (Keystone Scientific), 2.1 mm x 50 mm, 5µ (P/N: 82505-052130)
6.2 Temperature: 30°C
6.3 Mobile Phase (A) : 2 mM Ammonium Acetate in Water
6.4 Mobile Phase (B) : Methanol
6.5 Gradient Program:

Time (min)	% A	% B	Flow Rate (mL/min)
0.0	65	35	0.3
1.0	65	35	0.3
8.0	25	75	0.3
20.0	25	75	0.3
22.5	65	35	0.3

6.6 Injection Volume: 15 µL (can be increased to as much as 50 µL).
6.7 Quantitation: Peak Area – external standard calibration curve.
6.8 Run Time: ~ 23 minutes.

The above conditions are intended as a guide and may be changed in order to optimize the HPLC system.

7.0 MS/MS System

7.1 Mode: Electrospray Negative MRM mode, monitoring 413 → 369 m/z for PFOA.

The above conditions are intended as a guide and may be changed in order to optimize the MSMS system.

8.0 Preparation of Solutions

8.1 Mobile Phase

8.1.1 2 mM ammonium acetate in water is prepared by adding 0.154 g of ammonium acetate to 1000 mL of water.

8.2 Extraction Solutions

8.2.1 2% ascorbic acid in methanol is prepared by dissolving 2 g of ascorbic acid in 100 mL of methanol.

8.2.2 30% Dimethyldichlorosilane in toluene is prepared by bringing 3 mL of dimethyldichlorosilane to a final volume of 10 mL with toluene.

Alternate volumes may be prepared.

9.0 Standard Preparation

9.1 Standard Stock/Fortification Solution

9.1.1 Prepare a stock solution of ~100 µg/mL of PFOA by weighing 10 mg of analytical standard (corrected for purity) and dilute to 100 mL with methanol in a 125-mL LDPE bottle.

9.1.2 A 1.0 µg/mL fortification solution of PFOA is prepared by bringing 1 mL of the 100 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.3 A 0.1 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 1.0 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.4 A 0.01 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 0.1 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.5 The stock and fortification solutions are to be stored in a refrigerator at approximately 4°C and are stable for a maximum period of 6 months from the date of preparation.

9.2 Standard Calibration Solutions

9.2.1 LC/MS/MS calibration standards are prepared in methanol via dilution of the 1.0 µg/mL fortification solution.

9.2.2 The following is a typical example: additional concentrations may be prepared as needed.

Concentration of Fortification Solution (µg/mL)	Volume (mL)	Diluted to (mL)	Final Concentration (µg/mL)
1.0	5.0	100	0.05
1.0	2.5	100	0.025
1.0	1.0	100	0.01
0.05	10	100	0.005
0.025	10	100	0.0025
0.1	10	100	0.001
0.005	10	100	0.0005

9.2.3 Store all calibration standards in 125-mL LDPE narrow-mouth bottles at 2°C to 6°C, up to six months.

9.2.4 Alternate volumes and concentrations of standards may be prepared as needed.

10.0 Batch Set Up

10.1 Each batch of samples extracted (typically 20 or less) must include at least one untreated control and two untreated controls fortified at known concentrations (lab control spike) to verify procedural recovery for the batch.

10.2 Requirements for field and laboratory duplicates and spikes will be specified in the quality assurance plan for this project.

11.0 Sample Extraction

11.1 Weigh 5 g of frozen sample into 50 mL polypropylene centrifuge tubes (fortify as needed, replace lid and mix well).

11.2 Add 30 mL of acetonitrile and shake on a wrist action shaker for ~15 minutes.

11.3 Centrifuge the 50 mL polypropylene tubes containing sample at ~2000 rpm for ~10 minutes.

11.4 Pack and condition the SPE tubes and silanize the pear-shaped flasks.

11.5 Pack the 20 mL SPE tubes in sequence with 2 g florisil, 2 g silica gel, 2 g carbon, and 1 g LC-NH_2. Condition the columns with 20 mL of methanol, then 20 mL of acetonitrile. Discard all washes. Do not allow the column to dry.

11.6 Silanize the 125 mL pear-shaped flasks by rinsing with the 30% dimethyldichlorosilane in toluene solution. Rinse the flask with toluene once, followed by methanol (three times). Dry the flasks completely before use, either by air-drying or with a stream of nitrogen.

11.7 Decant the extract on to a conditioned SPE column fitted inside the mouth of the pear-shaped flask. Collect the eluate in the 125 mL silanized pear-shape flask.

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Vegetation by LC/MS/MS

11.8 Add 20 mL of acetonitrile to the sample in the 50 mL centrifuge tube.
11.9 Shake the sample again for ~10 minutes on a wrist-action shaker.
11.10 Centrifuge the 50 mL polypropylene tubes containing sample at ~2000 rpm for ~5 minutes.
11.11 Decant the extract onto the same SPE column. Collect the eluate into the same pear-shaped flask and combine with the eluent from the initial extraction.
11.12 Repeat steps 11.8 through 11.11 again.
11.13 Add 3-4 drops of 1-octanol to the extract in the pear-shaped flask and evaporate at reduced pressure using a rotary evaporator (at < 40°C).
11.14 Make the final volume, by adding 2 mL of 2% ascorbic acid in methanol to the pear-shaped flask and swirl to mix/dissolve.
11.15 Transfer the extracts to HPLC vials using disposable pipets.
11.16 Analyze samples using electrospray LC/MS/MS.

12.0 Chromatography

12.1 Inject the same amount of each standard, sample and fortified sample into the LC/MS/MS system. A calibration standard must precede and follow all analyzed samples.
12.2 Standards of PFOA corresponding to at least five or more concentration levels must be included in an analytical set.
12.3 An entire set of extracted calibration standards must be included at the beginning and at the end of a sample set. Extracted standards must be interspersed between every 5-10 samples. As an alternative, an entire set of extracted calibration standards may be injected at the beginning of a set followed by extracted calibration standards interspersed every 5-10 samples (to account for a second set of extracted standards). In either case, extracted calibration standards must be the first and last injection in a sample set.
12.4 Use linear standard curves for quantitation. Linear standard curves are generated for the analyte by linear regression using 1/x weighting of peak area versus calibration standard concentration using MassLynx 3.3 (or equivalent) software system.
12.5 Sample response should not exceed standard responses. Any samples that exceed standard responses should be further diluted and reanalyzed.

13.0 Acceptance Criteria

13.1 Chromatogram must show a peak of a daughter ion at 369 amu from a parent of 413 amu. The 413 amu parent corresponds to the PFOA anion, while the daughter ion (369 amu) represents the loss of carbon dioxide.

13.2 Method blanks must not contain PFOA at levels greater than the LOQ. If a blank contains PFOA at levels greater than 0.5 ppb, then a new blank sample must be obtained and the entire set must be re-extracted.

13.3 Recoveries of control spikes and matrix spikes must be between 70-130% of their known values. If a control spike falls outside the acceptable limits, the entire set of samples should be re-extracted.

13.4 Any calibration standard found to be a statistical outlier by using the Huge Error Test, may be excluded from the calculation of the calibration curve. However, the total number of calibration standards that could be excluded must not exceed 20% of the total number of standards injected.

13.5 The correlation coefficient (R) for calibration curves generated must be ≥0.992 (R^2 ≥0.985). If calibration results fall outside these limits, then appropriate steps must be taken to adjust instrument operation, and the standards or the relevant set of samples should be reanalyzed.

13.6 Retention times between standards and samples must not drift more than ± 4 % within an analytical run. If retention time drift exceeds this limit within an analytical run then the set must be reanalyzed.

14.0 Calculations

14.1 Use the following equation to calculate the amount of PFOA found (in ng/mL, *based on peak area) using the standard curve (linear regression parameters)* generated by the Mass Lynx software program:

$$\text{PFOA found (ng/mL)} = \frac{\text{(Peak area - intercept)}}{\text{slope}}$$

14.2 Use the following equation to convert the amount of PFOA found in ng/mL to *ng/g (ppb).*

$$\text{PFOA found (ppb)} = \frac{\text{[PFOA found (ng/mL) x final volume (mL) x DF]}}{\text{sample weight (g)}}$$

DF = factor by which the final volume was diluted, if necessary.

14.3 For samples fortified with known amounts of PFOA prior to extraction, use the following equation to calculate the percent recovery.

Recovery (%) =

$$\frac{\text{[total analyte found (ng/g) - analyte found in control (ng/g)]}}{\text{analyte added (ng/g)}} \times 100$$

Exygen Protocol Number: P0001131

ANALYTICAL METHOD
Method Number: V0001785

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small Mammal Liver by LC/MS/MS

Analytical Testing Facility: Exygen Research
3058 Research Drive
State College, PA 16801

Approved By:

Paul Connolly
Technical Leader, LC-MS, Exygen Research

10/26/04
Date

John Flaherty
Vice President, Operations, Exygen Research

10/26/04
Date

Total Pages: 7

Exygen Research Method Number V0001785

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small Mammal Liver by LC/MS/MS

1.0 Scope

This method is to be employed for the isolation and quantitation of perfluorooctanoic acid by High Performance Liquid Chromatography coupled to a tandem Mass Spectrometric Detector (LC/MS/MS) in small mammal liver.

2.0 Safety

2.1 Always observe safe laboratory practices.
2.2 Consult the appropriate MSDS before handling any chemical for proper safety precautions.

3.0 Sample Requirement

3.1 At least 5 g of test sample for extraction.
3.2 Samples should be processed before extraction. Place the frozen sample in a food processor and homogenize with dry ice. Place the samples in containers and leave open in frozen storage overnight to allow for carbon dioxide sublimation. Seal and place the samples in frozen storage until time of analysis. Alternately, if there is an insufficient amount of sample (~less than 5 g), then no processing is necessary and the sample can be used as supplied.
3.3 Sample collection procedures will be specified in the sampling plan for this project.

4.0 Reagents and Standards

4.1 Water – HPLC grade
4.2 Methanol – HPLC grade
4.3 Acetonitrile – HPLC grade
4.4 Ammonium Acetate – A.C.S. Reagent Grade
4.5 Perfluorooctanoic Acid – Sigma-Aldrich

5.0 Instrument and Equipment

5.1 A high performance liquid chromatograph capable of pumping up to 2 solvents equipped with a variable volume injector capable of injecting 5-200 µL connected to a tandem Mass Spectrometer (LC/MS/MS).
5.2 A device to collect raw data for peak integration and quantitation.
5.3 Analytical balance capable of reading to 0.00001 g.
5.4 50 mL disposable polypropylene centrifuge tubes.
5.5 15 mL disposable polypropylene centrifuge tubes.
5.6 Disposable micropipets (50-100uL, 100-200uL).
5.7 125-mL LDPE narrow-mouth bottles.
5.8 2 mL clear HPLC vial kit.

5.9 Disposable pipettes.
5.10 Autopipettes (100-1000 µL and 10-100 µL), with disposable tips.
5.11 Waters Sep Pak Vac 6 cc (1g) tC18 SPE cartridges.
5.12 SPE vacuum manifold.
5.13 Tissuemizer.
5.14 Wrist-action shaker.
5.15 Centrifuge capable of spinning 15 mL polypropylene tubes at 3000 rpm.

6.0 Chromatographic System

6.1 Analytical Column: Fluophase RP (Keystone Scientific), 2.1 mm x 50 mm, 5µ (P/N: 82505-052130)
6.2 Temperature: 30°C
6.3 Mobile Phase (A): 2 mM Ammonium Acetate in Water
6.4 Mobile Phase (B): Methanol
6.5 Gradient Program:

Time (min)	% A	% B	Flow Rate (mL/min)
0.0	65	35	0.3
1.0	65	35	0.3
8.0	25	75	0.3
20.0	25	75	0.3
22.5	65	35	0.3

6.6 Injection Volume: 15 µL (can be increased to as much as 50 µL).
6.7 Quantitation: Peak Area – external standard calibration curve.
6.8 Run Time: ~ 23 minutes.

The above conditions are intended as a guide and may be changed in order to optimize the HPLC system.

7.0 MS/MS System

7.1 Mode: Electrospray Negative MRM mode, monitoring 413 → 369 m/z for PFOA.

The above conditions are intended as a guide and may be changed in order to optimize the MSMS system.

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small Mammal Liver by LC/MS/MS

8.0 Preparation of Solutions

8.1 Mobile Phase

8.1.1 2 mM ammonium acetate in water is prepared by adding 0.154 g of ammonium acetate to 1000 mL of water.

Alternate volumes may be prepared.

9.0 Standard Preparation

9.1 Standard Stock/Fortification Solution

9.1.1 Prepare a stock solution of ~100 µg/mL of PFOA by weighing 10 mg of analytical standard (corrected for purity) and dilute to 100 mL with methanol in a 125-mL LDPE bottle.

9.1.2 A 1.0 µg/mL fortification solution of PFOA is prepared by bringing 1 mL of the 100 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.3 A 0.1 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 1.0 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.4 The stock and fortification solutions are to be stored in a refrigerator at approximately 4°C and are stable for a maximum period of 6 months from the date of preparation.

9.2 Standard Calibration Solutions

9.2.1 LC/MS/MS calibration standards are prepared in methanol via dilution of the 0.1 µg/mL fortification solution.

9.2.2 The following is a typical example: additional concentrations may be prepared as needed.

Concentration of Fortification Solution (ng/mL)	Volume (mL)	Diluted to (mL)	Final Concentration (ng/mL)
100	5.0	100	5.0
100	2.0	100	2.0
100	1.0	100	1.0
5.0	10	100	0.5
2.0	10	100	0.2
1.0	10	100	0.1

9.2.3 Store all calibration standards in 125-mL LDPE narrow-mouth bottles at 2°C to 6°C, up to six months.

9.2.4 Alternate volumes and concentrations of standards may be prepared as needed.

Exygen Protocol Number: P0001131

Exygen Research Method Number V0001785

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small Mammal Liver by LC/MS/MS

10.0 Batch Set Up

10.1 Each batch of samples extracted (typically 20 or less) must include at least one untreated control and two untreated controls fortified at known concentrations (lab control spike) to verify procedural recovery for the batch.

10.2 Requirements for field and laboratory duplicates and spikes will be specified in the quality assurance plan for this project.

11.0 Sample Extraction

11.1 Weigh 1 g of sample into a 50 mL polypropylene centrifuge tubes (fortify as needed, replace lid and mix well). Note that alternate weights of liver may be measured depending on the sample size available for use.

11.2 Add water to the sample for a final volume of 10 mL.

11.3 Homogenize sample using a tissuemizer for ~1 minute.

11.4 Transfer 1 mL of the sample using a disposable pipette into a 15 mL disposable centrifuge tube.

11.5 Add 5 mL of acetonitrile and shake for ~20 minutes on a wrist-action shaker.

11.6 Centrifuge the tubes at ~3000 rpm for ~5 minutes.

11.7 Decant the supernatant into a 50 mL disposable centrifuge tube and add 35 mL of water.

11.8 Condition the C_{18} SPE cartridges (1 g, 6 mL) by passing 10 mL methanol followed by 5 mL of HPLC water (~ 2 drop/sec). Do not let column run dry

11.9 Load the sample on conditioned C_{18} SPE cartridge. Discard eluate.

11.10 Elute with ~2 mL of methanol. Collect 2 mL of eluate into a graduated 15 mL polypropylene centrifuge tube (final volume = 2 mL).

11.11 Analyze samples using electrospray LC/MS/MS.

12.0 Chromatography

12.1 Inject the same amount of each standard, sample and fortified sample into the LC/MS/MS system. A calibration standard must precede and follow all analyzed samples.

12.2 Standards of PFOA corresponding to at least five or more concentration levels must be included in an analytical set.

12.3 An entire set of calibration standards must be included at the beginning and at the end of a sample set. Standards must be interspersed between every 5-10 samples. As an alternative, an entire set of calibration standards may be injected at the beginning of a set followed by calibration standards interspersed every 5-10 samples (to account for a second set of standards). In either case, calibration standards must be the first and last injection in a sample set.

12.4 Use linear standard curves for quantitation. Linear standard curves are generated for the analyte by linear regression using 1/x weighting of peak area

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versus calibration standard concentration using MassLynx 3.3 (or equivalent) software system.

12.5 Sample response should not exceed standard responses. Any samples that exceed standard responses should be further diluted and reanalyzed.

13.0 Acceptance Criteria

13.1 Chromatogram must show a peak of a daughter ion at 369 amu from a parent of 413 amu. The 413 amu parent corresponds to the PFOA anion, while the daughter ion (369 amu) represents the loss of carbon dioxide.

13.2 Method blanks must not contain PFOA at levels greater than the LOQ. If a blank contains PFOA at levels greater than 10 ng/g, then a new blank sample must be obtained and the entire set must be re-extracted.

13.3 Recoveries of control spikes and matrix spikes must be between 70-130% of their known values. If a control spike falls outside the acceptable limits, the entire set of samples should be re-extracted. Any matrix spike outside 70-130% should be evaluated by the analyst to determine if re-extraction is warranted.

13.4 Any calibration standard found to be a statistical outlier by using the Huge Error Test, may be excluded from the calculation of the calibration curve However, the total number of calibration standards that could be excluded must not exceed 20% of the total number of standards injected.

13.5 The correlation coefficient (R) for calibration curves generated must be ≥0.992 (R^2 ≥0.985). If calibration results fall outside these limits, then appropriate steps must be taken to adjust instrument operation, and the standards or the relevant set of samples should be reanalyzed.

13.6 Retention times between standards and samples must not drift more than ± 4 % within an analytical run. If retention time drift exceeds this limit within an analytical run then the set must be reanalyzed.

14.0 Calculations

14.1 Use the following equation to calculate the amount of PFOA found (in ng/mL, based on peak area) using the standard curve (linear regression parameters) generated by the Mass Lynx software program:

$$\text{PFOA found (ng/mL)} = \frac{\text{(Peak area - intercept)}}{\text{slope}} \times \text{DF} \times \text{aliquot factor}$$

DF = factor by which the final volume was diluted, if necessary.
Aliquot factor = 10

14.2 For samples fortified with known amounts of PFOA prior to extraction, use the following equation to calculate the percent recovery.

$$\text{Recovery (\%)} = \frac{[\text{total analyte found (ng/mL)} - \text{analyte found in control (ng/mL)}]}{\text{analyte added (ng/mL)}} \times 100$$

14.3 Use the following equation to convert the amount of PFOA found in ng/mL to ng/g (ppb).

$$\text{PFOA found (ppb)} = \frac{[\text{PFOA found (ng/mL)} \times \text{final volume (mL)}]}{\text{sample weight (g)}}$$

Exygen Protocol Number: P0001131

ANALYTICAL METHOD
Method Number: V0001786

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small Mammal Serum by LC/MS/MS

Analytical Testing Facility: Exygen Research
3058 Research Drive
State College, PA 16801

Approved By:

Paul Connolly
Technical Leader, LC-MS, Exygen Research

10/26/04
Date

John Flaherty
Vice President, Operations, Exygen Research

10/26/04
Date

Total Pages: 7

Exygen Protocol Number: P0001131

Exygen Research Method Number V0001786

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small Mammal Serum by LC/MS/MS

1.0 Scope

This method is to be employed for the isolation and quantitation of perfluorooctanoic acid by High Performance Liquid Chromatography coupled to a tandem Mass Spectrometric Detector (LC/MS/MS) in small mammal serum.

2.0 Safety

2.1 Always observe safe laboratory practices.
2.2 Consult the appropriate MSDS before handling any chemical for proper safety precautions.

3.0 *Sample Requirement*

3.1 At least 1 mL of test sample for extraction.
3.2 No sample processing is needed for serum samples. However, frozen serum samples must to allowed to completely thaw to room temperature before use.
3.3 Sample collection procedures will be specified in the sampling plan for this project.

4.0 Reagents and Standards

4.1 Water – HPLC grade
4.2 Methanol – HPLC grade
4.3 Acetonitrile – HPLC grade
4.4 Ammonium Acetate – A.C.S. Reagent Grade
4.5 Perfluorooctanoic Acid – Sigma-Aldrich

5.0 Instrument and Equipment

5.1 A high performance liquid chromatograph capable of pumping up to 2 solvents equipped with a variable volume injector capable of injecting 5-200 µL connected to a tandem Mass Spectrometer (LC/MS/MS).
5.2 A device to collect raw data for peak integration and quantitation.
5.3 Analytical balance capable of reading to 0.00001 g.
5.4 50 mL disposable polypropylene centrifuge tubes.
5.5 15 mL disposable polypropylene centrifuge tubes.
5.6 Disposable micropipets (50-100uL, 100-200uL).
5.7 125-mL LDPE narrow-mouth bottles.
5.8 2 mL clear HPLC vial kit.
5.9 Disposable pipettes.
5.10 Autopipettes (100-1000 µL and 10-100 µL), with disposable tips.
5.11 Waters Sep Pak Vac 6 cc (1g) tC18 SPE cartridges.
5.12 SPE vacuum manifold.
5.13 Vortexer.

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ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small Mammal Serum by LC/MS/MS

5.14 Wrist-action shaker.
5.15 Centrifuge capable of spinning 15 mL polypropylene tubes at 3000 rpm

6.0 Chromatographic System

6.1 Analytical Column: Fluophase RP (Keystone Scientific), 2.1 mm x 50 mm, 5µ (P/N: 82505-052130)
6.2 *Temperature:* 30°C
6.3 Mobile Phase (A) : 2 mM Ammonium Acetate in Water
6.4 Mobile Phase (B) : Methanol
6.5 Gradient Program:

Time (min)	% A	% B	Flow Rate (mL/min)
0.0	65	35	0.3
1.0	65	35	0.3
8.0	25	75	0.3
20.0	25	75	0.3
22.5	65	35	0.3

6.6 Injection Volume: 15 µL (can be increased to as much as 50 µL).
6.7 Quantitation: Peak Area – external standard calibration curve.
6.8 Run Time: ~ 23 minutes.

The above conditions are intended as a guide and may be changed in order to optimize the HPLC system.

7.0 MS/MS System

7.1 Mode: Electrospray Negative MRM mode, monitoring 413 → 369 m/z for PFOA.

The above conditions are intended as a guide and may be changed in order to optimize the MSMS system.

8.0 Preparation of Solutions

8.1 Mobile Phase

8.1.1 2 mM ammonium acetate in water is prepared by adding 0.154 g of ammonium acetate to 1000 mL of water.

Alternate volumes may be prepared.

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small Mammal Serum by LC/MS/MS

9.0 Standard Preparation

9.1 Standard Stock/Fortification Solution

9.1.1 Prepare a stock solution of ~100 µg/mL of PFOA by weighing 10 mg of analytical standard (corrected for purity) and dilute to 100 mL with methanol in a 125-mL LDPE bottle.

9.1.2 A 1.0 µg/mL fortification solution of PFOA is prepared by bringing 1 mL of the 100 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.3 A 0.1 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 1.0 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.4 The stock and fortification solutions are to be stored in a refrigerator at approximately 4°C and are stable for a maximum period of 6 months from the date of preparation.

9.2 Standard Calibration Solutions

9.2.1 LC/MS/MS calibration standards are prepared in methanol via dilution of the 0.1 µg/mL fortification solution.

9.2.2 The following is a typical example: additional concentrations may be prepared as needed.

Concentration of Fortification Solution (ng/mL)	Volume (mL)	Diluted to (mL)	Final Concentration (ng/mL)
100	5.0	100	5.0
100	2.0	100	2.0
100	1.0	100	1.0
5.0	10	100	0.5
2.0	10	100	0.2
1.0	10	100	0.1

9.2.3 Store all calibration standards in 125-mL LDPE narrow-mouth bottles at 2°C to 6°C, up to six months.

9.2.4 Alternate volumes and concentrations of standards may be prepared as needed.

10.0 Batch Set Up

10.1 Each batch of samples extracted (typically 20 or less) must include at least one untreated control and two untreated controls fortified at known concentrations (lab control spike) to verify procedural recovery for the batch.

10.2 Requirements for field and laboratory duplicates and spikes will be specified in the quality assurance plan for this project.

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small Mammal Serum by LC/MS/MS

11.0 Sample Extraction

11.1 Measure 1 mL of sample into a 50 mL polypropylene centrifuge tubes (fortify as needed, replace lid and mix well). Note that alternate volumes of serum may be measured depending on the sample size available for use.
11.2 Add water to the sample for a final volume of 20 mL. Cap tightly
11.3 Vortex for ~1 minute.
11.4 Transfer 1 mL of the sample using a disposable pipette into a 15 mL disposable centrifuge tube.
11.5 Add 5 mL of acetonitrile and shake for ~20 minutes on a wrist-action shaker.
11.6 Centrifuge the tubes at ~3000 rpm for ~5 minutes.
11.7 Decant the supernatant into a 50 mL disposable centrifuge tube and add 35 mL of water.
11.8 Condition the C_{18} SPE cartridges (1 g, 6 mL) by passing 10 mL methanol followed by 5 mL of HPLC water (~ 2 drop/sec). Do not let column run dry
11.9 Load the sample on conditioned C_{18} SPE cartridge. Discard eluate.
11.10 Elute with ~2 mL of methanol. Collect 2 mL of eluate into a graduated 15 mL polypropylene centrifuge tube (final volume = 2 mL).
11.11 Analyze samples using electrospray LC/MS/MS.

12.0 Chromatography

12.1 Inject the same amount of each standard, sample and fortified sample into the LC/MS/MS system. A calibration standard must precede and follow all analyzed samples.
12.2 Standards of PFOA corresponding to at least five or more concentration levels must be included in an analytical set.
12.3 An entire set of calibration standards must be included at the beginning and at the end of a sample set. Standards must be interspersed between every 5-10 samples. As an alternative, an entire set of calibration standards may be injected at the beginning of a set followed by calibration standards interspersed every 5-10 samples (to account for a second set of standards). In either case, calibration standards must be the first and last injection in a sample set.
12.4 Use linear standard curves for quantitation. Linear standard curves are generated for the analyte by linear regression using 1/x weighting of peak area versus calibration standard concentration using MassLynx 3.3 (or equivalent) software system.
12.5 Sample response should not exceed standard responses. Any samples that exceed standard responses should be further diluted and reanalyzed.

Exygen Protocol Number: P0001131

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small Mammal Serum by LC/MS/MS

13.0 Acceptance Criteria

13.1 Chromatogram must show a peak of a daughter ion at 369 amu from a parent of 413 amu. The 413 amu parent corresponds to the PFOA anion, while the daughter ion (369 amu) represents the loss of carbon dioxide.

13.2 Method blanks must not contain PFOA at levels greater than the LOQ. If a blank contains PFOA at levels greater than 10 ng/mL, then a new blank sample must be obtained and the entire set must be re-extracted.

13.3 Recoveries of control spikes and matrix spikes must be between 70-130% of their known values. If a control spike falls outside the acceptable limits, the entire set of samples should be re-extracted. Any matrix spike outside 70-130% should be evaluated by the analyst to determine if re-extraction is warranted.

13.4 Any calibration standard found to be a statistical outlier by using the Huge Error Test, may be excluded from the calculation of the calibration curve However, the total number of calibration standards that could be excluded must not exceed 20% of the total number of standards injected.

13.5 The correlation coefficient (R) for calibration curves generated must be ≥0.992 (R^2 ≥0.985). If calibration results fall outside these limits, then appropriate steps must be taken to adjust instrument operation, and the standards or the relevant set of samples should be reanalyzed.

13.6 Retention times between standards and samples must not drift more than ± 4 % within an analytical run. If retention time drift exceeds this limit within an analytical run then the set must be reanalyzed.

14.0 Calculations

14.1 Use the following equation to calculate the amount of PFOA found (in ng/mL, based on peak area) using the standard curve (linear regression parameters) generated by the Mass Lynx software program:

$$\text{PFOA found (ng/mL)} = \frac{\text{(Peak area - intercept)}}{\text{slope}} \times \text{DF} \times \text{aliquot factor}$$

DF = factor by which the final volume was diluted, if necessary.
Aliquot factor = 20

14.2 For samples fortified with known amounts of PFOA prior to extraction, use the following equation to calculate the percent recovery.

$$\text{Recovery (\%)} = \frac{[\text{total analyte found (ng/mL)} - \text{analyte found in control (ng/mL)}]}{\text{analyte added (ng/mL)}} \times 100$$

14.3 Use the following equation to convert the amount of PFOA found in ng/mL to ppb.

$$\text{PFOA found (ppb)} = \frac{[\text{PFOA found (ng/mL)} \times \text{final volume (mL)}]}{\text{sample volume (mL)}}$$

Exygen RESEARCH
3058 Research Drive Phone: 814-272-1039
State College, PA 16801 Fax: 814-231-1580

PROTOCOL AMENDMENT

Amendment Number: 1

Effective Date: 01/19/05

Exygen Study Number: P0001131 Client Study Number: None

Page 1 of 1

DESCRIPTION OF AMENDED SECTION

1) Analytical Procedure Summary V0001780:Section 9.1
2) Verification of Analytical Procedure

AMENDED TO

1) Add to Section 9.1: Section 9.1.6, Alternate weights of standards may be used to prepare alternate concentrations of stock solutions as necessary. Alternate levels of fortification solutions may also be prepared.
2) Low and high spiking levels of the analytes for each matrix may be altered depending on sample size available for extraction and/or to cover analyte concentrations expected in the samples.

RATIONALE

1) Higher concentrations of standards need to be prepared in order to spike the sample bottles at higher levels.
2) The sample size available for small mammal liver and serum was smaller than expected. Spiking at the pre-determined levels in the protocol puts the spiked concentration lower than the detection limit. Also, the analyte levels in the ground water samples are expected to greatly exceed the pre-determined spiking levels listed in the protocol. When the levels in the samples greatly exceed the spiking levels, an accurate recovery value cannot be calculated for the QC sample. Higher spiking levels in the bottles will cover the analyte concentrations expected in the water samples.

IMPACT ON STUDY

The LOQ is 100 ng/g for a 0.1 g sample of small mammal liver and is 1000 ng/mL for a 0.01 mL sample of small mammal serum.
Higher levels of spiking for the water samples will ensure that more QC recovery data can be used.

Study Director Signature	Date 3/9/05
Principal Investigator Signature	Date 2/2/05
Study Director Management Signature N/A	Date N/A
Sponsor Signature (if required) Mary Rotherstein for Michael A. Santoro	Date 3/10/05

Exygen QAU Review LJS 02/01/05

LIBRARY ID: VV0001226-6 ADMINISTRATIVE FORM

Exygen RESEARCH
3058 Research Drive Phone: 814-272-1039
State College, PA 16801 Fax: 814-231-1580

PROTOCOL AMENDMENT

Amendment Number: 2 Page 1 of 1
Effective Date: 03/07/05
Exygen Study Number P0001131 Client Study Number: None

DESCRIPTION OF AMENDED SECTION

1) Report, page 11 of 65
2) Test Materials, page 6 of 65: PFOS transition monitored 499 -> 99.

AMENDED TO

1) Instead of one final report, interim reports will be issued.
2) PFOS transition monitored may also be 499 -> 80.

RATIONALE

1) Due to the excessive sizes of the data sets, interim reports will be issued to allow the client to receive data in a timeller ~~manor~~ manner 03/04/05 RRK
2) The API 4000 LC/MS/MS systems detect the 499 -> 80 PFOS transition with greater sensitivity than the 499 -> 99 transition.

IMPACT ON STUDY

1) The client will be able to receive and review the data more quickly.
2) The 499 -> 80 transition can be detected with greater sensitivity; therefore, giving better chromatography.

Study Director Signature — Date: 3/9/05
Principal Investigator Signature — Date: 3/8/05
Study Director Management Signature: MA — Date:
Exygen Management Signature — Date: 8-MAR-05
Sponsor Signature (if required) — Date: 3/10/05

Exygen QAU Review LJJ 03/08/05

LIBRARY ID: V0001226-8 ADMINISTRATIVE FORM