3M

Interim Report #5: Analysis of West Morgan/East Lawrence Municipal 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 Requirement

EPA TSCA Good Laboratory Practice Standards 40 CFR 792

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Interim Report Completion Date

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Project Identification

E05-0210

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GLP COMPLIANCE STATEMENT

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

Interim Study: Analysis of West Morgan/East Lawrence Municipal Water Samples

Study Identification Number: E05-0210

This study was conducted in compliance with Toxic Substances Control Act (TSCA) Good Laboratory Practice (GLP) Standards, 40 CFR 792, with the exceptions listed below:

Exceptions to GLP compliance:

5/26/05

Jaisimha Kesari P. E., DEE, Study Director Date

6/22/05

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

QUALITY ASSURANCE STATEMENT

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

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Interim Study Identification Number: E05-0210

This study was audited by the 3M Environmental Laboratory Quality Assurance Unit (QAU), as indicated in the following table. The findings were reported to the study director and laboratory management.

		Date Reported to	
		Management	Study Director
May 6, 2005	In-Phase	5-17-2005	5-17-2005
May 13, 2005 & May 16, 2005	Data/Final Report	5-17-2005	5-17-2005

Casey Howell 5-18-05

QAU Representative Date

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Study Dates

Study Initiation: 11/5/2004

Interim Experimental Initiation: May 4, 2005

Interim Experimental Completion: May 8, 2005

Interim Study Completion:

Location of Archives

All original raw data, protocol, and analytical report have been archived at the 3M Environmental Laboratory according to 40 CFR Part 792. The test substance and analytical reference standard reserve samples are archived at the 3M Environmental Laboratory according to 40 CFR Part 792.

SUMMARY AND INTRODUCTION

The 3M Environmental Laboratory extracted and analyzed municipal (raw and finished) water samples collected by Weston Solutions personnel on May 3, 2005 in Decatur, Alabama for 3M Environmental Laboratory project number E05-0210 (3M Decatur Fluorochemical GLP Monitoring Program).

Water samples were analyzed for perfluorobutanesulfonate (PFBS), perfluorohexanesulfonate (PFHS), and perfluorooctanesulfonate (PFOS) using 3M Environmental Laboratory Method ETS 8-154.1 "Determination of Perfluorinated Acids, Alcohols, Amides, and Sulfonates in Water by Solid Phase Extractions and High Performance Liquid Chromatography/Mass Spectrometry" in accordance with Exygen Research Protocol P0001131 (Attachment C). The analytical start date for this interim report was May 4, 2005 and the analytical completion date was May 8, 2005.

Sample containers were prepared at the 3M Environmental Laboratory. Sample containers for each sampling location included a field sample, field sample duplicate, low field spike (0.10 ng/mL) and high field spike (1.0 ng/mL). Each empty container was marked with a "fill to here" line and was fortified with a surrogate spike or an appropriate matrix spike solution containing the surrogate and the target analytes prior to being sent to the field for sample collection.

Table 1 below summarizes the sample results. All results for quality control samples prepared and analyzed with the samples will be reported and discussed elsewhere in this report. Quality control samples were used to establish the analytical uncertainty of the results presented below.

Table 1. Sample Results Summary

Description	*Sample Location*	*3M LIMS ID*	*(1)PFBS Concentration (ng/mL)*	*(2)PFHS Concentration (ng/mL)*	*(3)PFOS Concentration (ng/mL)*
Field Sample	WMEL PW RW01	82756	0.0192	0.0104	0.116
Field Sample Dup	WMEL PW RW01	82757	0.0210	0.00990	0.0693
Average Concentration WMEL PW RW01			***0.0201***	***0.0102***	***0.0926***
Field Sample	WMEL PW FW01	82760	0.0263	0.00955	0.0727
Field Sample Dup	WMEL PW FW01	82761	0.0295	0.00926	0.0782
Average Concentration WMEL PW FW01			***0.0279***	***0.00940***	***0.0754***

(1) The analytical uncertainty of the PFBS results is 100±15% based on method accuracy and precision. The lower limit of quantitation (LOQ) for PFBS is 0.00506 ng/mL.

(2) The analytical uncertainty of the PFHS results is 100±9% based on method accuracy and precision. The lower limit of quantitation (LOQ) for the samples is 0.00525 ng/mL for PFHS.

(3) The analytical uncertainty of the PFOS results is 100±12% based on method accuracy and precision. The lower limit of quantitation (LOQ) for the samples is 0.0103 ng/mL for PFOS.

TEST SAMPLES

Water samples from two locations (3M LIMS ID # 82756-82766) were received on ice on May 4, 2005 from Tim Frinak of Weston Solutions, Inc. A total of eleven containers were submitted for analysis. The samples were logged in by 3M Environmental Laboratory professional services personnel and placed in refrigerated storage after extraction on May 5, 2005.

REFERENCE SUBSTANCES

Table 2 lists the pertinent information regarding the reference substance used for this study.

Table 2. Study Reference Substance.

Reference Substance	*PFBS*	*PFHS*	*PFOS*
Chemical Name	Perfluorobutanesulfonate	Perfluorohexanesulfonate	Perfluorooctanesulfonate
Chemical Formula	$C_4F_9SO_3^-K^+$	$C_6F_{13}SO_3^-K^+$	$C_8F_{17}SO_3^-K^+$
Identifier	CAS # 29420-49-3	CAS # 3871-99-6	CAS # 2795-39-3
(1)Source	3M	3M	3M
Expiration Date	10/17/2006	10/18/2006	8/31/2006
Storage Conditions	Frozen	Frozen	Frozen
Chemical Lot Number	101	NB# 120067-69	171
TCR Number	TCR-116 (99030-023)	TCR-83 (SE036)	TCR-696
Physical Description	White Powder	White Powder	White powder
Purity	96.7%	98.6%	86.4%
(2)Solubility	54,400 ppm	No Information available	680 ppm

(1) Documentation regarding synthesis of the test substances is located at the source.

(2) All test substances are believed to be soluble in water at the levels to be investigated. No visual precipitates observed.

CONTROL SUBSTANCE

Radiolabelled perfluorooctanoic acid (PFOA [1,2 ^{13}C]) was analyzed as a surrogate against a multi-level extracted calibration curve. A known amount of PFOA [1,2 ^{13}C] was spiked into each sample container in the laboratory prior to sample collection. Table 3 lists the pertinent information regarding the study control substance.

Table 3. Study Control Substance (Surrogate).

Reference Substance	*PFOA [1,2 ^{13}C]*
Chemical Name	Perfluorooctanoic Acid
Chemical Formula	$C_6F_{15}[^{13}C]F_2[^{13}C]OOH$
Identifier	N/A
Source	Perkin Elmer
Expiration Date	03/29/2009
Storage Conditions	Frozen
Chemical Lot Number	3507-195
TCR Number	TCR-744
Physical Description	White powder
Purity	>97%

METHOD SUMMARIES

Sample Collection

Samples were collected on May 3, 2005 in pre-rinsed Nalgene™ (low-density polyethylene) bottles prepared at the 3M Environmental Laboratory on May 2, 2005. Prior to sample collection, all bottles were spiked in the laboratory with a known volume of a surrogate solution and appropriate matrix spiking solution if applicable. Table 4 below details the samples collected and final spike concentrations added to each bottle.

Table 4. Sample Collection Information

3M LIMS Number	*Sample Description*	*Sample Comment*	*Nominal Final Volume Collected (mL)*	*Final Nominal Spike Concentration (ng/mL)*	
				PFOA [1,2 ^{13}C] (Surrogate)	*PFBS, PFHS, PFOS*
82756	WMEL PW RW01 0 050503	Location 1 Sample	200	0.050	NA
82757	WMEL PW RW01 DP 050503	Location 1 Sample Duplicate	200	0.050	NA
82758	WMEL PW RW01 LS 050503	Location 1 Low Field Spike	200	0.050	0.10
82759	WMEL PW RW01HS 050503	Location 1 High Field Spike	200	0.050	1.0
82760	WMEL PW FW01 0 050503	Location 2 Sample	200	0.050	NA
82761	WMEL PW FW01 DP 050503	Location 2 Sample Duplicate	200	0.050	NA
82762	WMEL PW FW01 LS 050503	Location 2 Low Spike	200	0.050	0.10
82763	WMEL PW FW01HS 050503	Location 2 High Spike	200	0.050	1.0
82764	WMEL W TRIP 0 050503	Trip Blank	200	0.050	NA
82765	WMEL W TRIP LS 050503	Trip Blank Low Spike	200	0.050	0.10
82766	WMEL W TRIP HS 050503	Trip Blank High Spike	200	0.050	1.0

Preparatory and Analytical Methods

Extraction

All samples, calibration standards, and associated quality control samples were extracted using a modified procedure of ETS-8-154.1 "Determination of Perfluorinated Acids, Alcohols, Amides, and Sulfonates in Water by Solid Phase Extractions and High Performance Liquid Chromatography/Mass Spectrometry". Briefly, approximately eighty mL of sample were loaded onto a pre-conditioned Waters C18 solid phase extraction (SPE) cartridge (Sep-Pak, 6 cc) using a Caliper Life Sciences Autotrace automated SPE workstation. The same workstation then used positive pressure to elute the loaded cartridges with 2 mL of methanol This extraction procedure concentrates the samples by a factor of forty. (Initial volume = 80 mL, final volume = 2 mL). As written, ETS-8-154.1 calls for a 40 mL and 5 mL extraction and elution volume, respectively.

Analysis

All sample and quality control extracts were analyzed for PFBS, PFHS, PFOS, and PFOA [1,2 ^{13}C] using high performance liquid chromatography/ tandem mass spectrometry (HPLC/MS/MS). Pertinent instrument parameters, the liquid chromatography gradient program, and the specific mass transitions analyzed are described in the tables below.

Table 5. Instrument Parameters

Instrument Name	ETSOllie
Liquid Chromatograph	Agilent 1100
Guard column	Betasil C18 2X50, 5 µm
Analytical column	Betasil C18 (2.1 mm X 100 mm), 5 µm
Injection Volume	5 µL
Mass Spectrometer	Applied Biosystems API 4000 Q Trap
Electrode	Z-spray
Ion Source	Turbo Spray
Polarity	Negative
Software	Analyst 1.4.1

Table 6. Liquid Chromatography Gradient Program

Step Number	*Total Time (min)*	*Flow Rate (µL/min)*	*Percent A (2 mM ammonium acetate)*	*Percent B (Methanol)*
0	0	300	80.0	20.0
1	1.0	300	80.0	20.0
2	14.5	300	10.0	90.0
3	15.5	300	10.0	90.0
4	16.5	300	80.0	20.0
5	20.0	300	80.0	20.0

Table 7. Mass Transitions

Analyte	*Mass Transition Q1/Q3*	*Dwell Time (msec)*
(1)PFBS	299/80	100
	299/99	100
(1)PFHS	399/130	100
	399/99	100
	399/80	100
(1)PFOS	499/130	100
	499/99	100
	499/80	100
PFOA [1,2 ^{13}C]	415/370	100

(1) Individual transitions were summed to produce a "total ion chromatogram" (TIC). The TIC was used for quantitation.

ANALYTICAL RESULTS

Calibration

Calibration standards were prepared by spiking known amounts of stock solutions containing PFBS, PFHS, PFOS, and PFOA [1,2 ^{13}C] into 80 mL of ASTM type I water. Each spiked water standard was then extracted in the same manner as the collected samples. A total of ten spiked standards ranging from 0.005 ng/mL to 2.5 ng/mL (nominal) were prepared. A quadratic, 1/x weighted, calibration curve was used to fit the data for each

analyte. The data was not forced through zero during the fitting process. Calculating the standard concentration using the peak area counts and the resultant calibration curve confirmed the accuracy of each curve point. Each extracted calibration standard used to generate the final calibration curve met the method calibration accuracy requirement of 100±25%. Coefficients of determination (r^2) were greater than 0.996 for all analytes.

Limit of Quantitation (LOQ)

The LOQ for this analysis, as defined in ETS-8-154.1, is the lowest non-zero calibration standard in the curve in which the area counts are twice those of the method blank(s). For PFOS, the LOQ was 0.0103 ng/mL as it was the lowest calibration standard to fulfill the area count requirement for *all* method blanks analyzed. For PFBS, PFHS, and PFOA [1,2 ^{13}C], the LOQs were 0.00506, 0.00525, and 0.00499 ng/mL, respectively.

Blanks

Three types of blanks were prepared and analyzed with the samples: method blanks, solvent blanks, and field/trip blanks. Each blank type is described below.

Solvent Blanks

Several methanol solvent blanks were analyzed to assess system contamination and/or instrument carryover. Analyte peak area counts in all blank samples were less than half the area counts of the calibration standard used to establish the LOQ.

Method Blanks

Over the two days in which samples, calibration standards, and quality control samples were extracted, several method blanks were prepared by loading 80 mL of ASTM Type I water onto a C18 SPE cartridge and eluting with 2 mL of methanol using the same extraction procedure as the samples. Method blanks were prepared to evaluate the levels of background contamination in the overall extraction process (glassware, SPE cartridges, etc.) Table 8 lists the area counts for the method blanks.

Table 8. Method Blank Area Counts

Sample Description	*PFBS Area Counts*	*PFHS Area Counts*	*PFOS Area Counts*	*PFOA [1,2 ^{13}C] Area Counts*
Day 1 Method Blank -1	5212	5036	19444	1907
Day 1 Method Blank -2	3285	1352	17140	793
Day 1 Method Blank -3	2983	1463	17746	670
Day 1 Method Blank -4	3110	1603	15230	485
Day 1 Method Blank -5	2239	984	12305	572
Day 1 Method Blank -6	5510	5152	20970	1237
Day 1 Method Blank -7	3385	2132	28896	666
Day 1 Method Blank -8	4119	5640	37615	3283
Day 2 Method Blank -1	1582	2931	34842	1250
Day 2 Method Blank -2	4036	2460	19020	979
Day 2 Method Blank -3	2847	2761	21958	641
Average Area Counts	**3483**	**2865**	**22288**	**1135**
LOQ Area Count	**81616 (0.00506 ng/mL)**	**75010 (0.00525 ng/mL)**	**80616 (0.0103 ng/mL)**	**25953 (0.00499 ng/mL)**

Trip Blanks

Prior to sample collection, one sample container was filled with 200 mL of ASTM Type I water, spiked with surrogate, sealed, and shipped to the sample collection site along with the empty containers. This sample was analyzed as the field/trip blank. The trip blank serves as an additional method blank that accounts for any storage conditions and/or holding time issues that the samples may experience. The resultant field blank concentration for all analytes was than the reported LOQ.

System Suitability

A 25 ng/mL solvent (unextracted) standard was analyzed in triplicate at the beginning and end of the analytical sequence to demonstrate overall system suitability. For PFBS, PFHS, and PFOS, the relative standard deviation (RSD) of the analyte peak area counts and the RSD of the peak retention times was less than 5% and 2%, respectively, for both opening and closing sets of system suitability samples which met method criteria. For PFOA [1,2 ^{13}C], the opening set of system suitabilities met method criteria; however, the peak area RSD was 7.9% for closing set. A method deviation was written for this failure.

Continuing Calibration

During the course of the analytical sequence, several continuing calibration verification samples (CCVs) were analyzed to confirm that the instrument response and the initial calibration curve was still in control. All CCVs, for all analytes, produced recoveries within ±25%, which met method criteria.

Lab Control Spikes (LCSs)

Replicate low (0.025-0.031 ng/mL nominal concentration) and high (1.25 ng/mL nominal concentration) lab control spikes were prepared and extracted each day samples or calibration standards were extracted. LCSs were prepared by spiking known amounts of the analytes into 80 mL of ASTM Type I water to produce the desired concentration. The spiked water samples were then extracted and analyzed in the same manner as the samples. Table 9 provides the LCS recovery results. LCSs were used to evaluate method accuracy and precision which was then used to determine analytical uncertainty for each of the analytes reported with the results in Table 1. Information on how the analytical uncertainty was determined is provided at the end of this report in the "Statistical Methods and Calculations" section. Two low level PFBS LCSs were outside the method acceptance criteria of 100±25% (698% and 134% recovery). The PFBS LCS with 698% is considered an anomaly as only one spiking solution was used and the other analytes for that same sample produced acceptable results. Therefore, this value was excluded when the PFBS LCS statistics were calculated. The sample with 134% recovery was included in statistical calculations as its exceedance of method criteria was not as severe. When the high and low spikes are considered collectively for PFBS, the overall average recovery was 101% with a relative standard deviation (RSD) of 14%, which meets method acceptance criteria for accuracy and precision (100±25%, RSD<15%). For PFHS, PFOS, and PFOA [1,2 ^{13}C], the LCSs show excellent accuracy and good precision, at both individual levels, and when considered collectively.

Table 9. Lab Control Spike Results.

	(1) PFBS			(1) PFHS		
Sample Description	Spiked Concentration (ng/mL)	Calculated Concentration (ng/mL)	%Recovery	Spiked Concentration (ng/mL)	Calculated Concentration (ng/mL)	%Recovery
LCS Low-1 (Day 1)	0.0316	0.221	(2)698	0.0328	0.0343	105
LCS Low-2 (Day 1)	0.0316	0.0307	97.2	0.0328	0.0323	98.5
LCS Low-3 (Day 1)	0.0316	0.0422	134	0.0328	0.0382	116
LCS Low-1 (Day 2)	0.0253	0.0240	95	0.0263	0.0261	99.4
LCS Low-2 (Day 2)	0.0253	0.0248	98	0.0263	0.0248	94.3
Average Low (Day 1 & Day2)		***106% ±18%***			***103% ± 8.2%***	
LCS High-1 (Day 1)	1.26	1.32	104	1.31	1.37	105
LCS High-2 (Day 1)	1.26	1.22	96.3	1.31	1.32	101
LCS High-1 (Day 2)	1.26	1.15	90.9	1.31	1.35	103
LCS High-2 (Day 2)	1.26	1.16	92.1	1.31	1.35	103
Average High (Day 1 & Day 2)		***95.8% ± 6.2%***			***103% ± 1.6%***	
Average All Samples		***101±14%***			***103±5.8%***	

	(1) PFOS			(1) PFOA [1,2 ^{13}C]		
Sample Description	Spiked Concentration (ng/mL)	Calculated Concentration (ng/mL)	%Recovery	Spiked Concentration (ng/mL)	Calculated Concentration (ng/mL)	%Recovery
LCS Low-1 (Day 1)	0.0321	0.0294	91.7	0.0313	0.0291	93
LCS Low-2 (Day 1)	0.0321	0.0264	82.3	0.0313	0.0276	88.3
LCS Low-3 (Day 1)	0.0321	0.0338	105	0.0313	0.0365	116
LCS Low-1 (Day 2)	0.0257	0.0263	102	0.0249	0.0256	103
LCS Low-2 (Day 2)	0.0257	0.0235	91.4	0.0249	0.0236	94.8
Average Low (Day 1 & Day2)		***94.5% ±9.6%***			***99.0% ±11%***	
LCS High-1 (Day 1)	1.28	1.4	109	1.25	1.21	96.6
LCS High-2 (Day 1)	1.28	1.28	100	1.25	1.32	106
LCS High-1 (Day 2)	1.28	1.26	98.7	1.25	1.23	98.4
LCS High-2 (Day 2)	1.28	1.13	88.5	1.25	1.14	91.3
Average High (Day 1 & Day 2)		***99.1% ± 8.5%***			***98.1%±6.2%***	
Average All Samples		***96.5% ±8.9%***			***98.6 ±8.7%***	

(1) Table displays rounded values for all concentration and percent recovery values (3 significant figures). Recovery and RSD values may vary slightly from the values in the raw data.

(2) Value excluded when calculating LCS statistics. Presumed laboratory anomaly.

Sample Duplicates

Because a field sample duplicate (separate container) was collected at each sampling location, duplicate/replicate extractions of a given sample were not performed. Overall method precision was determined using surrogate spikes and LCSs.

Surrogates

Although not specified in the ETS 8-154.1, PFOA [1,2 ^{13}C] was added to all samples and sample spikes as a surrogate to evaluate overall method performance. The final PFOA [1,2 ^{13}C] concentration was 0.0499 ng/mL. Surrogate recoveries are reported in the next section with sample data.

Field Matrix Spikes

Low (nominal concentration of 0.1 ng/mL) and high (nominal concentration 1.0 ng/mL) field matrix spikes were collected at each sampling point to verify that the analytical method is applicable to the collected matrix. Field matrix spike recoveries within method acceptance criteria of 100±25% confirm that "unknown" components in the sample matrix do not interfere with the extraction and analysis of the analytes of interest. Field matrix spikes will be presented in the next section with the sample data.

DATA SUMMARY

The tables below summarize the sample results for the two locations (Table 10: WMEL PW RW01; Table 11: WMEL PW FW01) as well as the Trip Blank (Table 12). An average concentration was calculated for the field sample and field sample duplicate. The relative percent difference (%RPD) of the two measurements is also provided.

For Location #1 (WMEL PW RW01), both the low and high field spike recoveries for PFBS and PFHS met method criteria of 100±25%. Reproducibility of the field sample and field sample duplicate (%RPD) for both of these analytes was less than 10%. Furthermore, the field sample, field sample duplicate, and field spikes produced acceptable surrogate recoveries. However, a low recovery was observed for the low field spike for PFOS (36.4%). This low recovery can be partially explained by the high RPD (50%) between the field sample and the field sample duplicate. Because LCS results for PFOS (Table 9) demonstrate excellent precision for PFOS, the discrepancy between the field sample and the field sample duplicate for Location #1 is attributed to variability in the collected matrix and not the analytical method. If the low field spike for PFOS is calculated using only the field duplicate concentration, then the field spike recovery becomes 59.2% which agrees well with the low PFOS field spike recovery (62.9%) observed for Location #2 (WMEL PW FW01). The high field spike at Location #1 for PFOS did meet method criteria with 87.3% recovery.

Similar to Location #1, both the low and high field spikes for PFBS and PFHS and the high spike for PFOS produced acceptable recoveries for Location #2 (WMEL PW FW01). As mentioned previously, the low field spike recovery for PFOS was 62.9% which was outside the method acceptance criteria of 100±25%. The low level PFOS field spike for the Trip Blank (Table 12) was 79.4%. Although this recovery technically meets method acceptance criteria, it is lower than that PFBS and PFHS recoveries for the same sample. This result may indicate a holding time issue with PFOS at the low end. If the low level Trip Blank recovery is used to correct the low level field spikes, the recoveries for PFOS increase to 74.7% and 79.2% for Locations #1 (WMEL PW RW01) and #2(WMEL PW FW01), respectively. Overall accuracy and precision of the surrogate (excluding Location #2 field duplicate) was 99.8% with a RSD of 16%.

Table 10. Sample Results Location #1: WMEL PW RW01

			(1)PFBS		(1)PFHS	
Description	3M LIMS ID	DataFile	Concentration (ng/mL)	%Recovery	Concentration (ng/mL)	%Recovery
Field Sample	82756	O050506a057	0.0192	NA	0.0104	NA
Field Dup	82757	O050506a058	0.021	NA	0.0099	NA
Field Spike-Low	82758	O050506a059	0.136	114	0.115	99.8
Field Spike-High	82759	O050506a060	1.20	116	1.17	110
Average Sample Concentration (ng/mL) ±%RPD			**0.0201 ± 9%**		**0.0102 ±4.9%**	
			(1)PFOS		(1) PFOA [1,2 ^{13}C] Surrogate	
Description	***3M LIMS ID***	***DataFile***	***Concentration (ng/mL)***	***%Recovery***	***Concentration (ng/mL)***	***%Recovery***
Field Sample	82756	O050506a057	0.116	NA	0.0445	89.2
Field Dup	82757	O050506a058	0.0693	NA	0.0504	101
Field Spike-Low	82758	O050506a059	0.130	(2)36.4	0.0485	97.2
Field Spike-High	82759	O050506a060	0.989	87.3	0.0485	97.2
Average Sample Concentration (ng/mL) ±%RPD			**0.0926 ±50%**		**NA**	

(1) Table displays rounded values for all concentration and percent recovery values (3 significant figures). Recovery and RPD values may vary slightly from the values in the raw data.

(2) If only the field duplicate is used to calculate field spike recovery, the low PFOS spike recovery is 59.2%. If this value is then corrected for the trip blank (holding time) low spike recovery, the recovery is 74.7%.

Table 11. Sample Results Location #2: WMEL PW FW01

			(1)PFBS		(1)PFHS	
Description	***3M LIMS ID***	***DataFile***	***Concentration (ng/mL)***	***%Recovery***	***Concentration (ng/mL)***	***%Recovery***
Sample	82760	O050506a061	0.0263	NA	0.00955	NA
Field Dup	82761	O050506a063	0.0295	NA	0.00926	NA
Field Spike-Low	82762	O050506a064	0.142	113	0.113	98.7
Field Spike-High	82763	O050506a065	1.12	108	1.05	99.1
Average Sample Concentration (ng/mL) ±%RPD			***0.0279 ±11%***		***0.00940 ±3.1%***	
			(1)PFOS		(1)PFOA [1,2 ^{13}C] Surrogate	
Description	***3M LIMS ID***	***DataFile***	***Concentration (ng/mL)***	***%Recovery***	***Concentration (ng/mL)***	***%Recovery***
Sample	82760	O050506a061	0.0727	NA	0.0504	101
Field Dup	82761	O050506a063	0.0782	NA	0.0134	(2)26.9
Field Spike-Low	82762	O050506a064	0.140	(3)62.9	0.0672	135
Field Spike-High	82763	O050506a065	0.896	79.9	0.0596	120
Average Sample Concentration (ng/mL) ±%RPD			***0.0754 ±7.3%***		***NA***	

(1) Table displays rounded values for all concentration and percent recovery values (3 significant figures). Recovery and RPD values may vary slightly from the values in the raw data.

(2) Surrogate result not included in overall statistics as it is not representative of the LCS data. Laboratory anomaly or artifact suspected.

(3) PFOS low spike recovery = 79.2% when corrected for the recovery of the Trip Blank (holding time) low level matrix spike.

Table 12. Sample Results: Trip Blank

			(1)PFBS		(1)PFHS	
Description	3M LIMS ID	DataFile	Concentration (ng/mL)	%Recovery	Concentration (ng/mL)	%Recovery
Trip Blank	82764	O050506a070	<0.00506	NA	<0.00525	NA
Trip Blank Low Spike	82765	O050506a071	0.102	101	0.0985	93.8
Trip Blank High Spike	82766	O050506a073	0.913	90.2	1.03	98.1
			(1)PFOS		(1)PFOA [1,2 ^{13}C] Surrogate	
Description	3M LIMS ID	DataFile	Concentration (ng/mL)	%Recovery	Concentration (ng/mL)	%Recovery
Trip Blank	82764	O050506a070	<0.0103	NA	0.0416	83.4
Trip Blank Low Spike	82765	O050506a071	0.0816	79.4	0.0431	86.4
Trip Blank High Spike	82766	O050506a073	0.933	90.8	0.0439	88.0

(1) Table displays rounded values for all concentration and percent recovery values (3 significant figures). Recovery values may vary slightly from the values in the raw data.

STATISTICAL METHODS AND CALCULATIONS

Statistical methods used to interpret sample results include averages and standard deviations. The Analyst software program calculated sample concentrations using resultant analyte peak areas and the established quadratic, 1/x weighted, calibration curve. None of the samples analyzed for this interim report required dilution. Sample calculations and equations used to report method accuracy and precision are described below.

Accuracy and Precision Equations

$$\text{LCS/Surrogate Percent Recovery} = \frac{\text{Calculated Concentration}}{\text{Spike Concentration}} * 100\%$$

$$\text{Sample Spike Recovery} = \frac{(\text{Spiked Sample Concentration} - \text{Average Concentration : Field Sample and Field Sample Dup.})}{\text{Spike Concentraton}} * 100\%$$

$$\%\text{ RSD (Relative Standard Deviation)} = \frac{\text{standard deviation of replicates}}{\text{replicate average}} * 100\%$$

$$\%\text{ RPD (Relative Percent Difference)} = \frac{\text{Absolute difference between sample duplicates}}{\text{average sample concentration}} * 100\%$$

Determination of Analytical Uncertainty

Both the accuracy (percent recovery) and precision (%RSD) of the lab control spikes are used to estimate the analytical uncertainty for a given analyte. For example, the overall accuracy and precision for PFOS based on LCS results was 96.5%±8.9%. The measured precision (%RSD) is then used to determine the spread of the accuracy.

Example:

96.5*(0.089) = 8.59.

96.5 +8.59 = 105; 96.5 – 8.59 = 87.91

Thus, LCS accuracy results range from 87.9% to 105%. The absolute difference of the low and high ends of this range, when compared 100%, are then calculated.

100%-87.9% = 12.1%

105%-100 = 5%.

The most conservative (largest) absolute difference is then used as the analytical uncertainty for the given analyte. Therefore, the analytical uncertainty for PFOS is given as 100±12% for this set of data.

STATEMENT OF CONCLUSION

Sample results listed in Table 1 are considered accurate within the analytical uncertainties listed. Spike recoveries for low level (0.100 ng/mL) PFOS field matrix spikes may indicate a holding time issue; however method accuracy and precision demonstrated by lab control spike data indicate the method is control and results for the two sampling locations are valid for all three analytes.

LIST OF ATTACHMENTS

- **Attachment A: Sample Chromatograms and Calibration Curves**
- **Attachment B: Extraction and Analytical Methods**
- **Attachment C: Protocol, Protocol Amendments and Deviations**

SIGNATURE PAGE

We certify that this report is a true and complete representation of the data for this study:

Jaisimha Kesari, P.E., DEE ,	Study Director	5/26/05 Date
Michelle D. Malinsky, Ph.D.,	3M Senior Chemist	5/18/2005 Date
William K. Reagen, Ph.D.,	Testing Facility Management	5/16/05 Date
Michael A. Santoro,	Sponsor Representative	6/02/05 Date

ATTACHMENT A. SAMPLE CHROMATOGRAMS AND CALIBRATION CURVES

Figure 1. Calibration Curve: Extracted PFBS Calibration Standards in ASTM Type I Water.

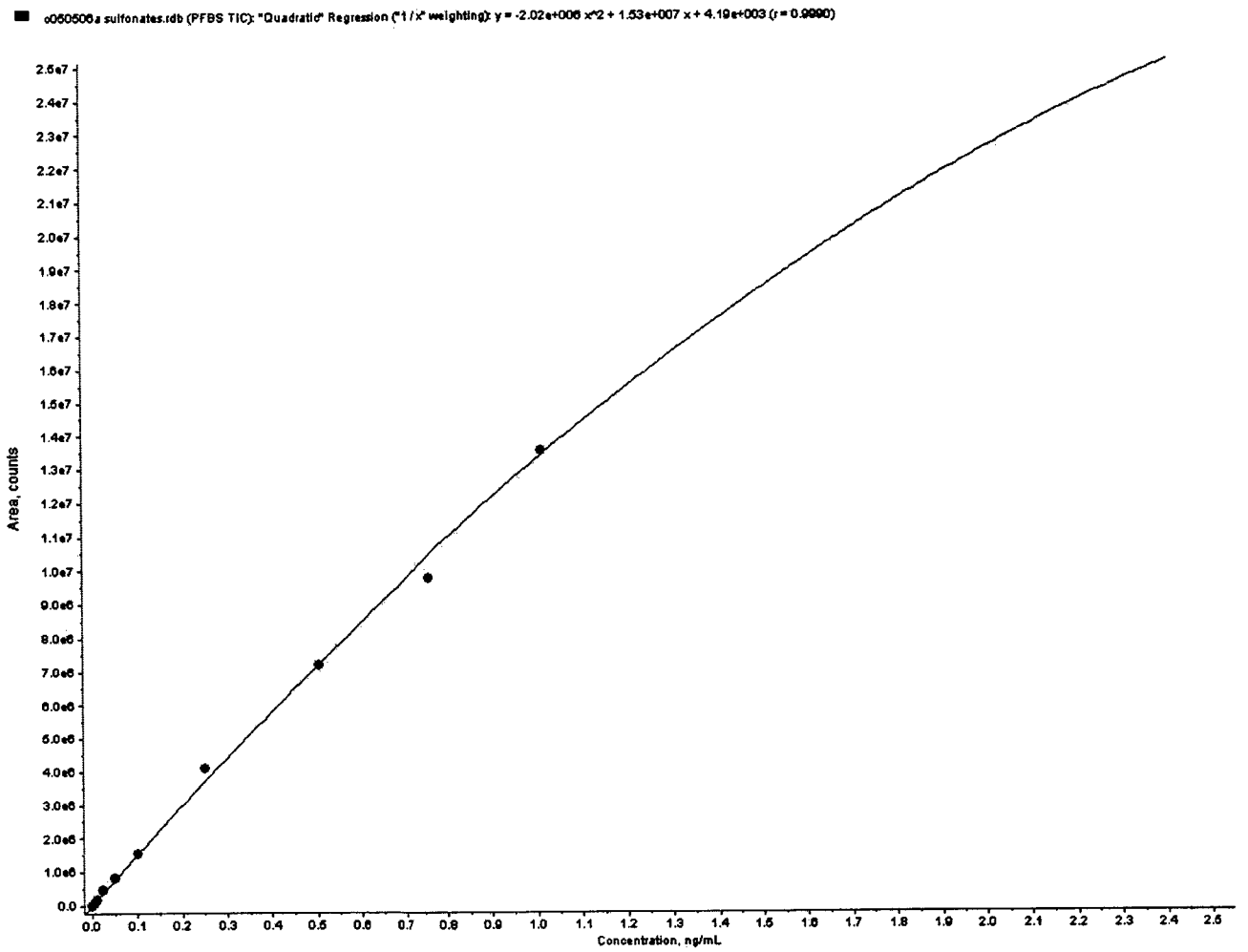


Figure 2. Calibration Curve: Extracted PFHS Calibration Standards in ASTM Type I Water.

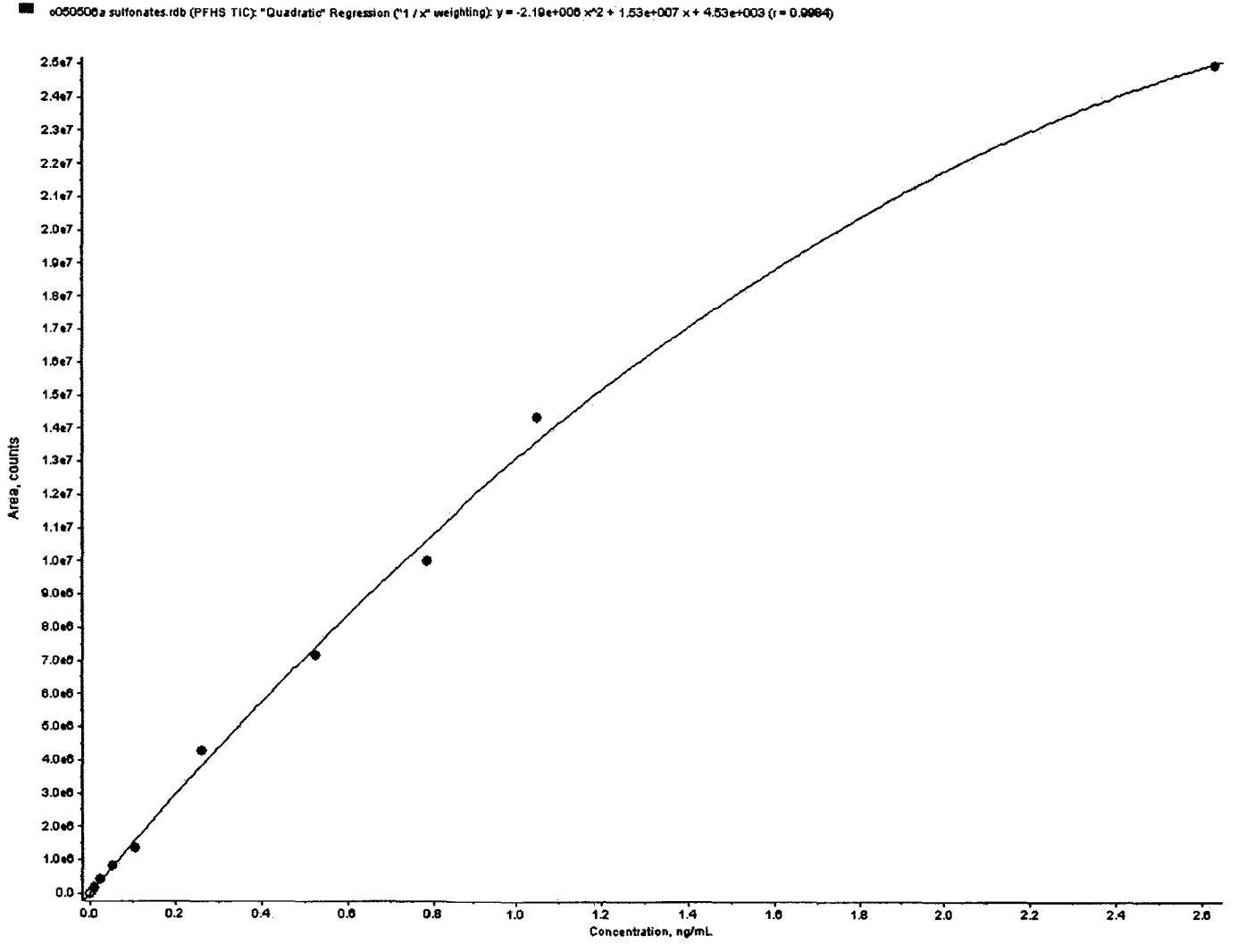


Figure 3. Calibration Curve: Extracted PFOS Calibration Standards in ASTM Type I Water.

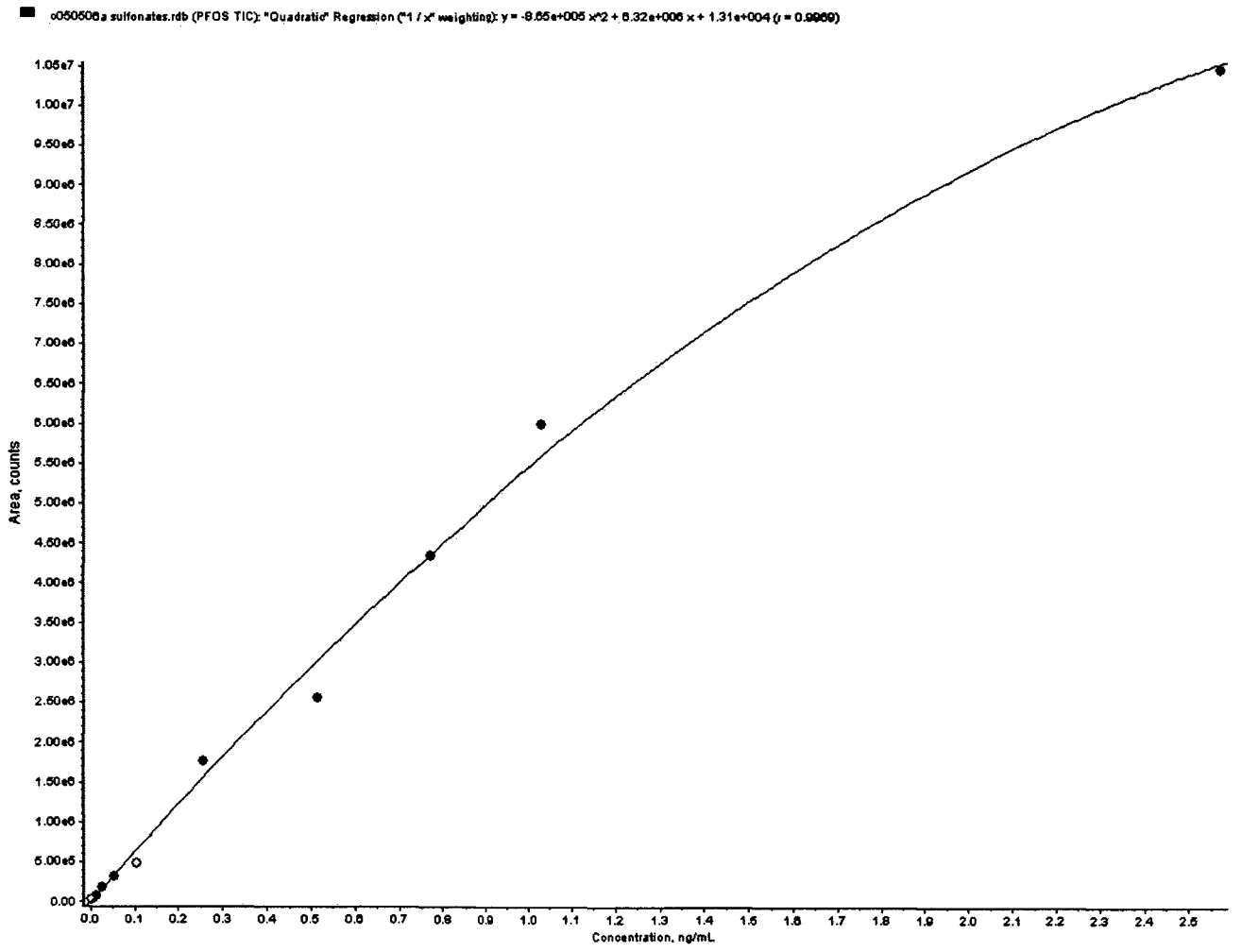


Figure 4. Calibration Curve: Extracted PFOA [1,2 ^{13}C] Calibration Standards in ASTM Type I Water.

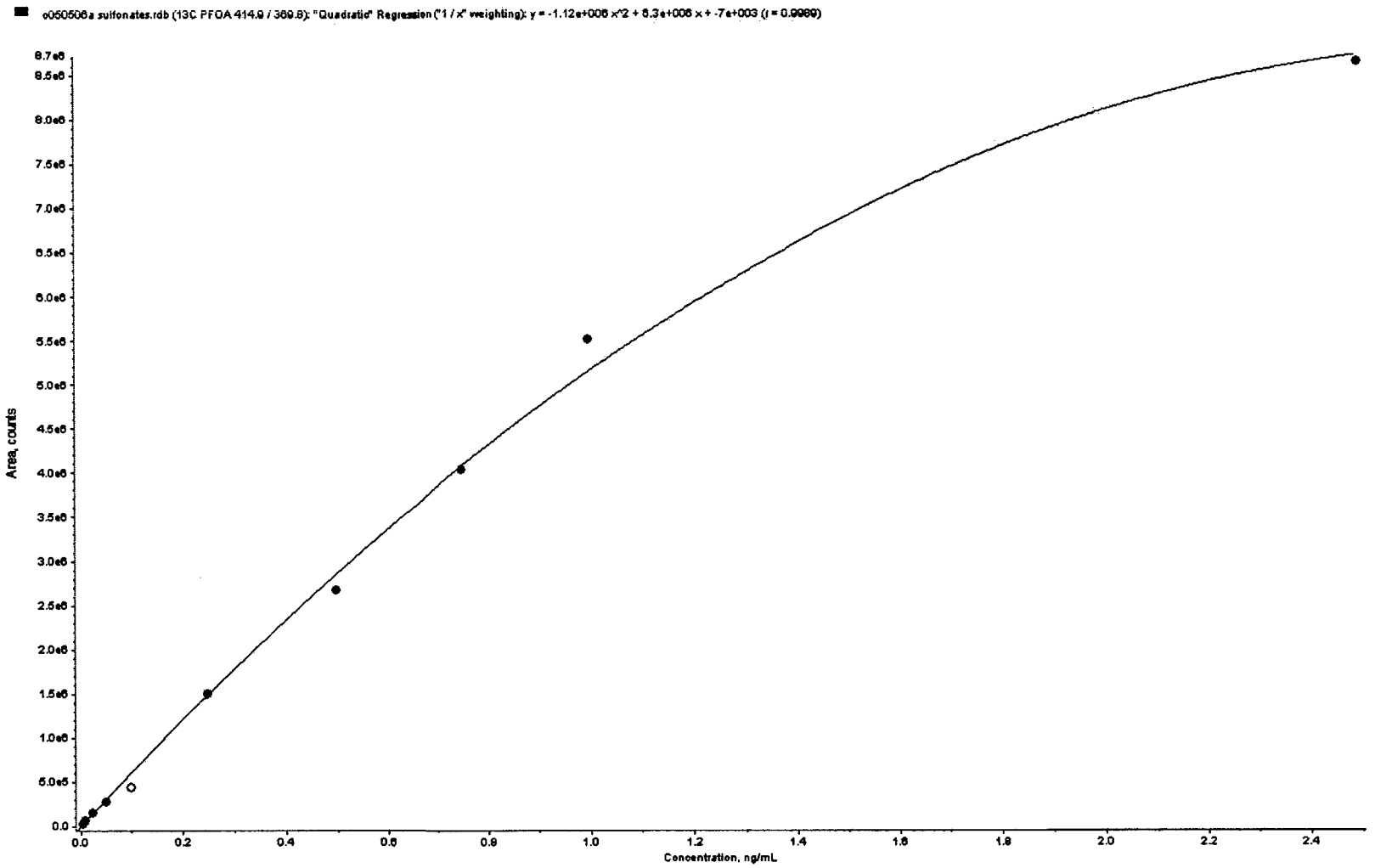


Figure 5. Total Ion Chromatogram for PFBS: 0.0253 ng/mL Extracted Calibration Standard.

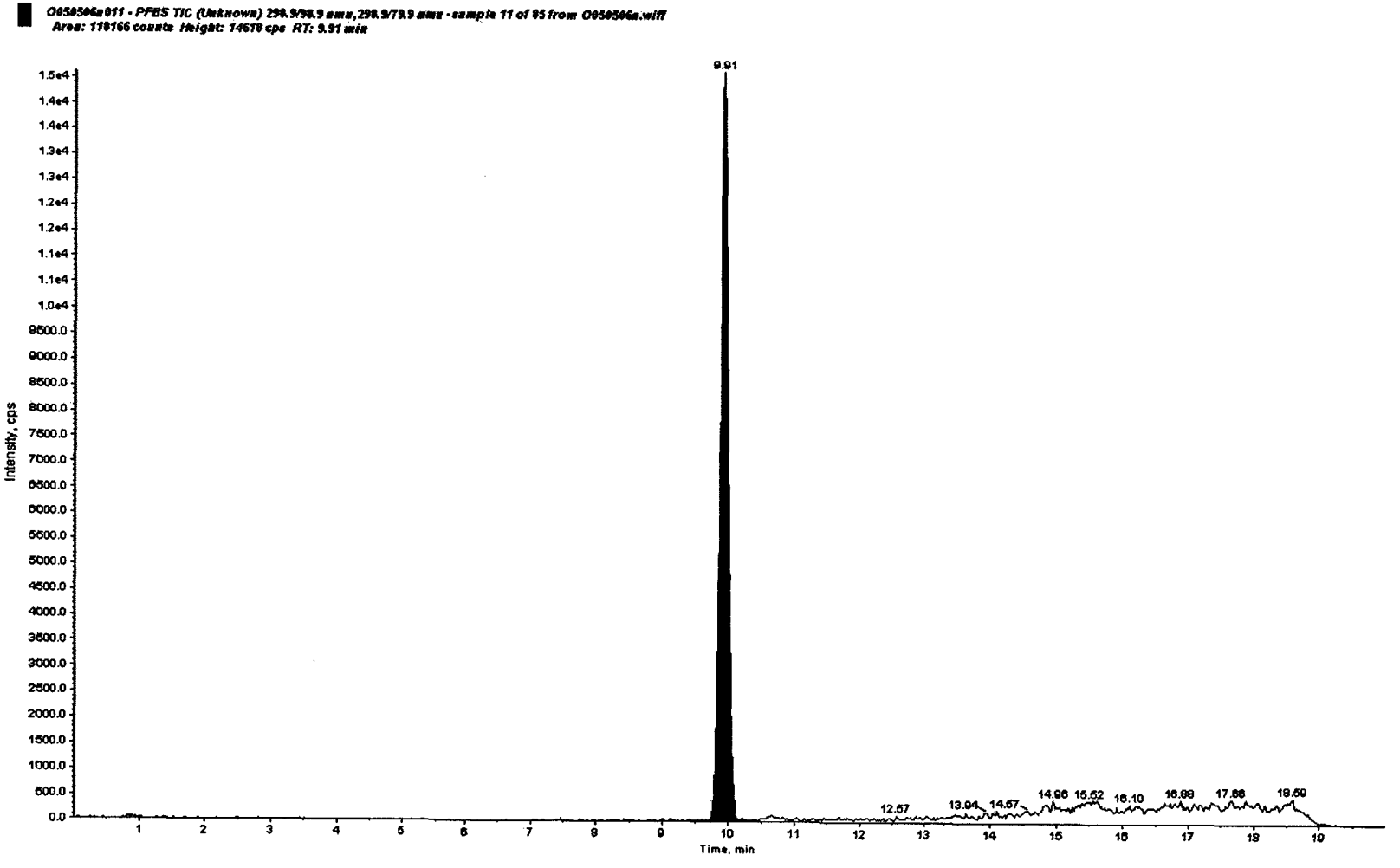


Figure 6. Total Ion Chromatogram for PFHS: 0.0263 ng/mL Extracted Calibration Standard.

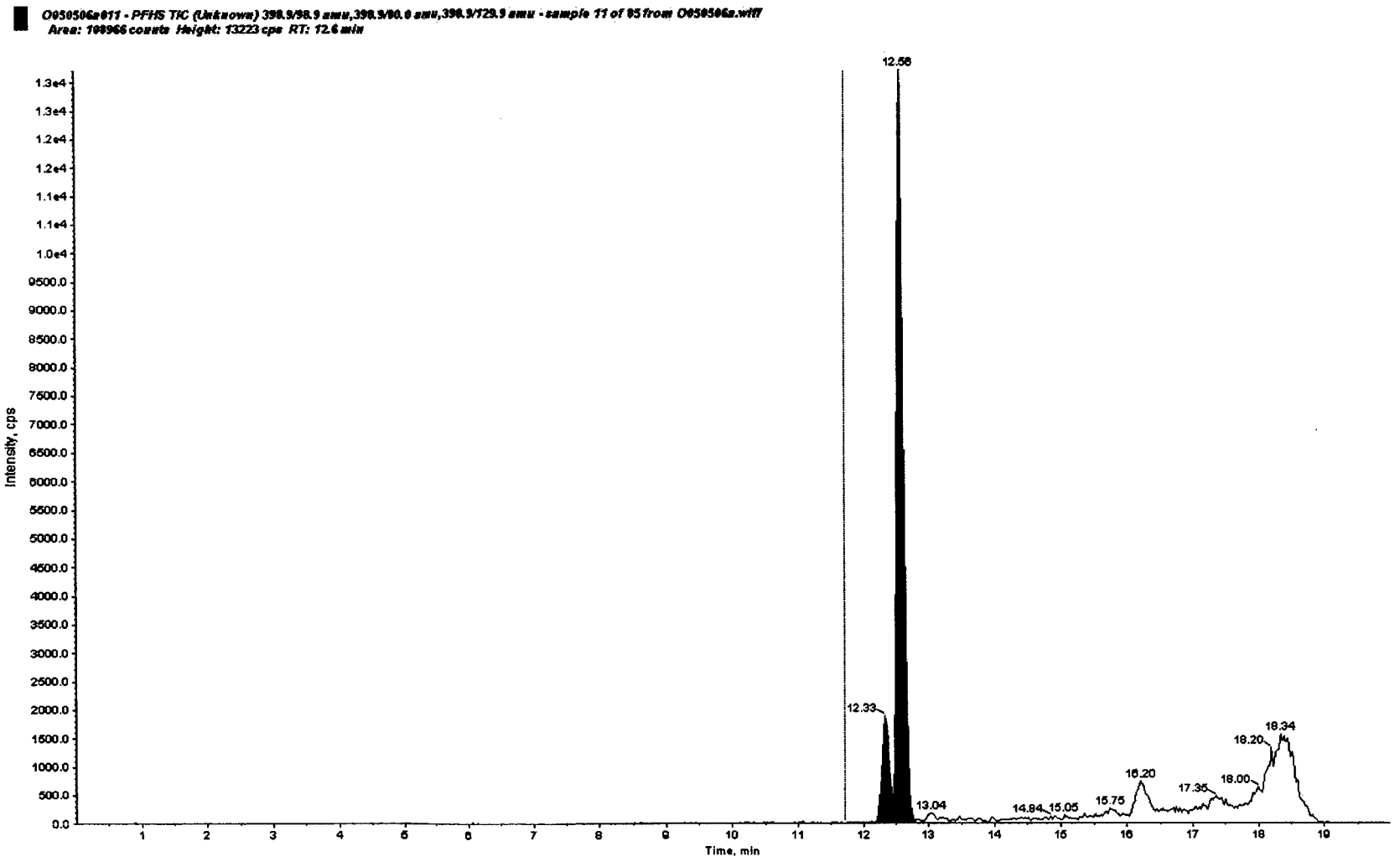


Figure 7. Total Ion Chromatogram of PFOS: 0.0257 ng/mL Extracted Calibration Standard.

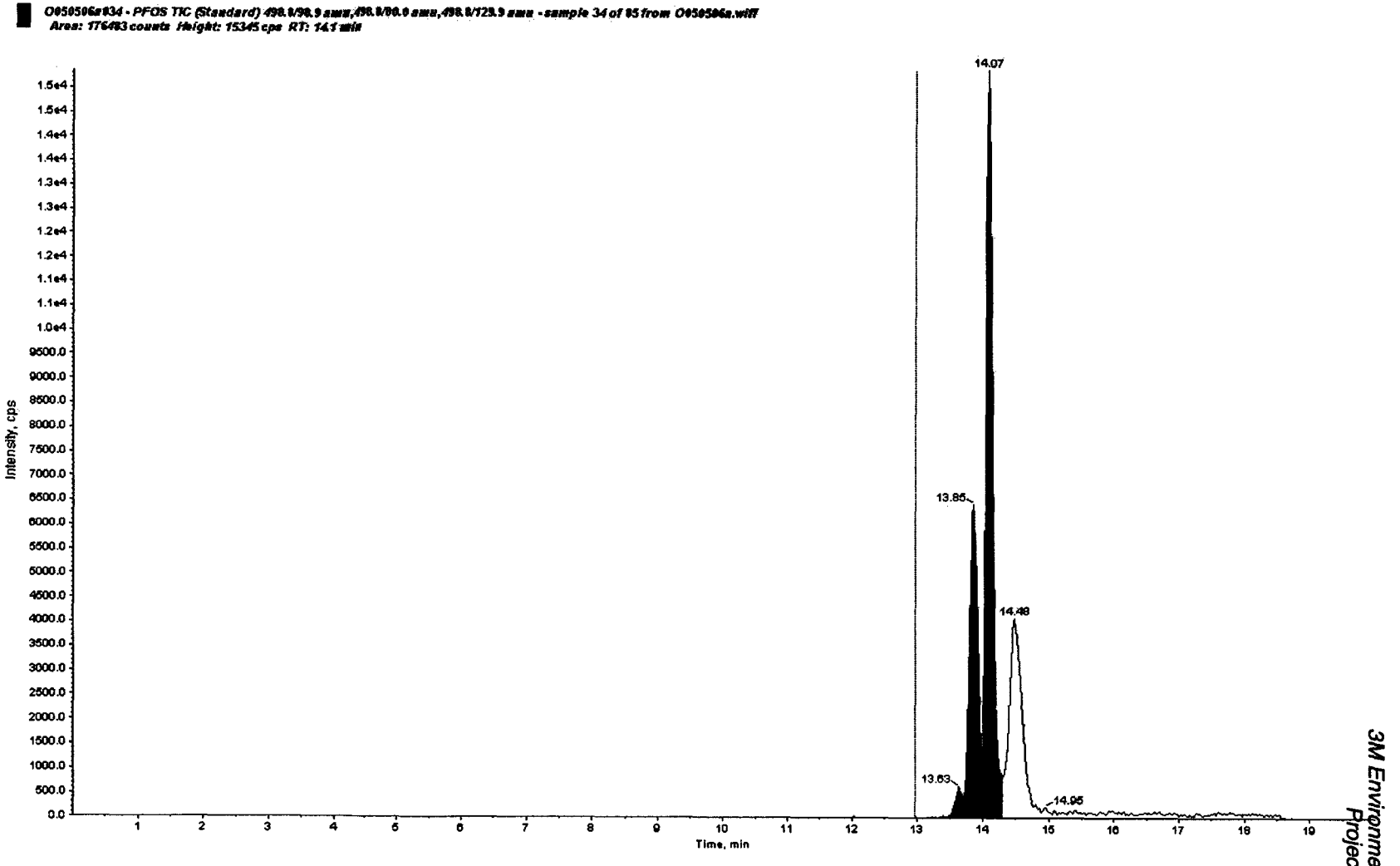


Figure 8. Total Ion Chromatogram of PFOA [1,2 ^{13}C]: 0.0249 ng/mL Extracted Calibration Standard.

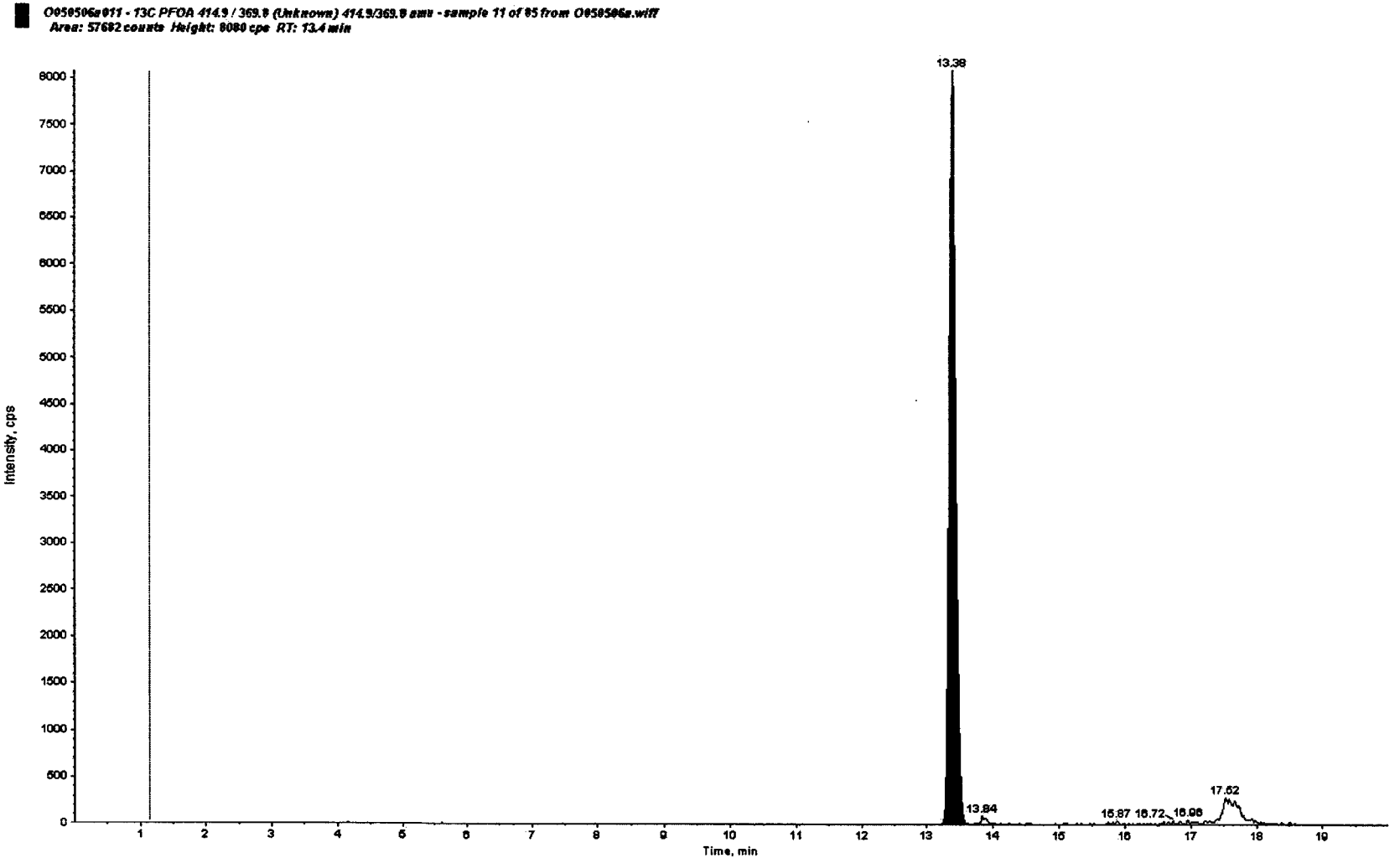


Figure 9. PFOS Total Ion Chromatogram: Representative Method Blank (050506a072; Day 2 Method Blank 3)

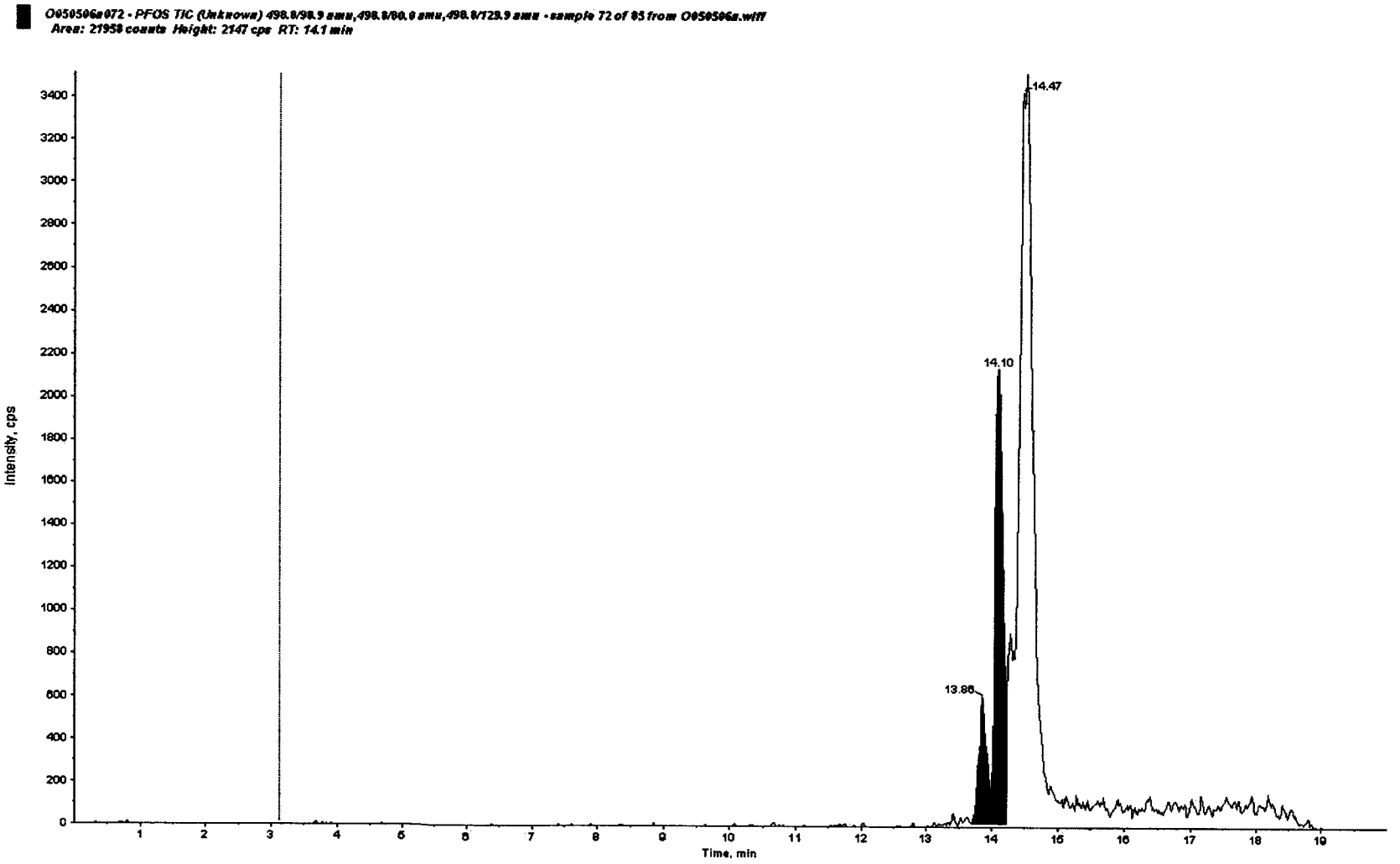


Figure 10. PFOS Total Ion Chromatogram: Representative Methanol Solvent Blank (o050506a052).

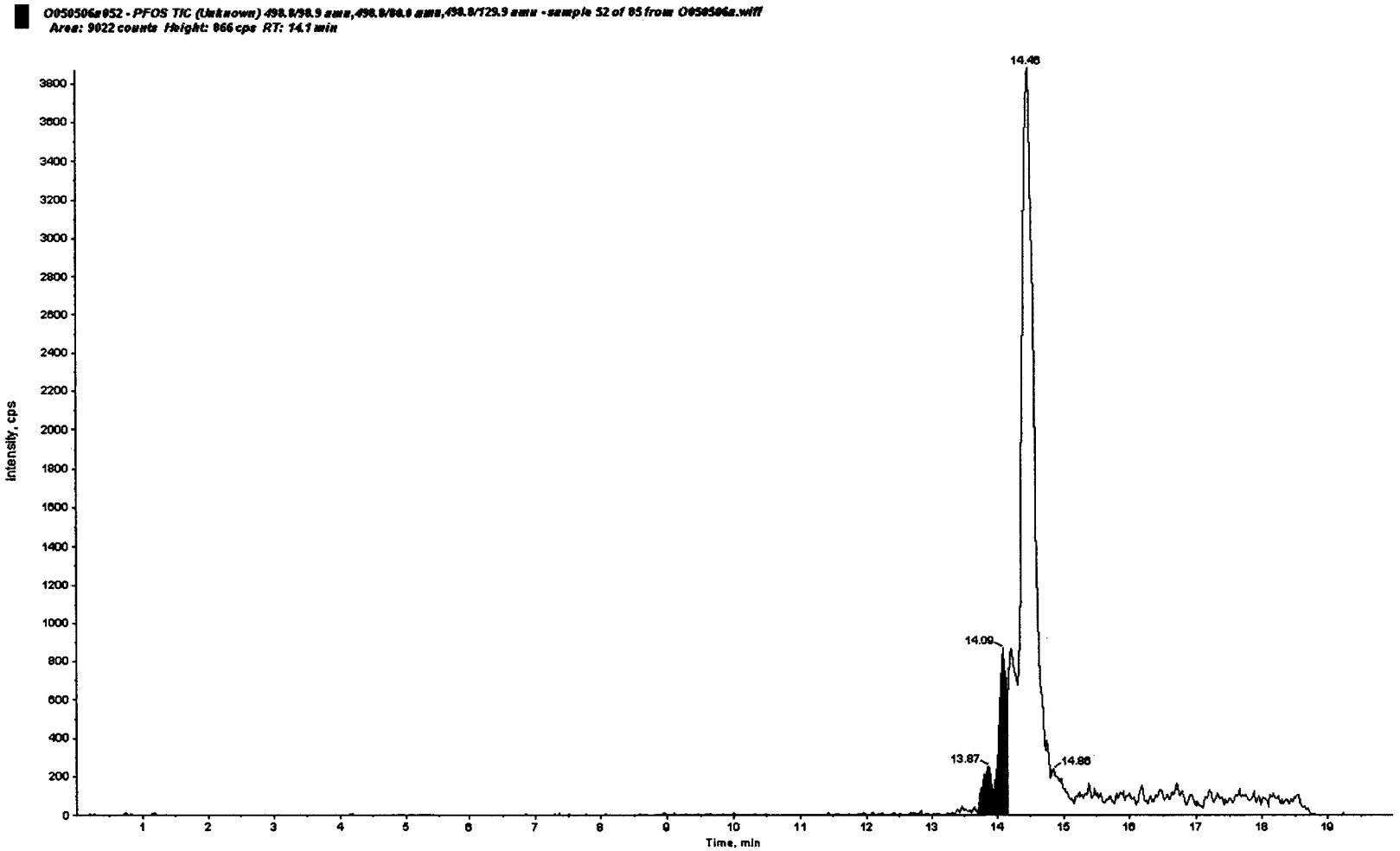


Figure 11. PFBS Total Ion Chromatogram: (Location #2; WMEL PW FW 01, Sample ID82438, o050506a061).

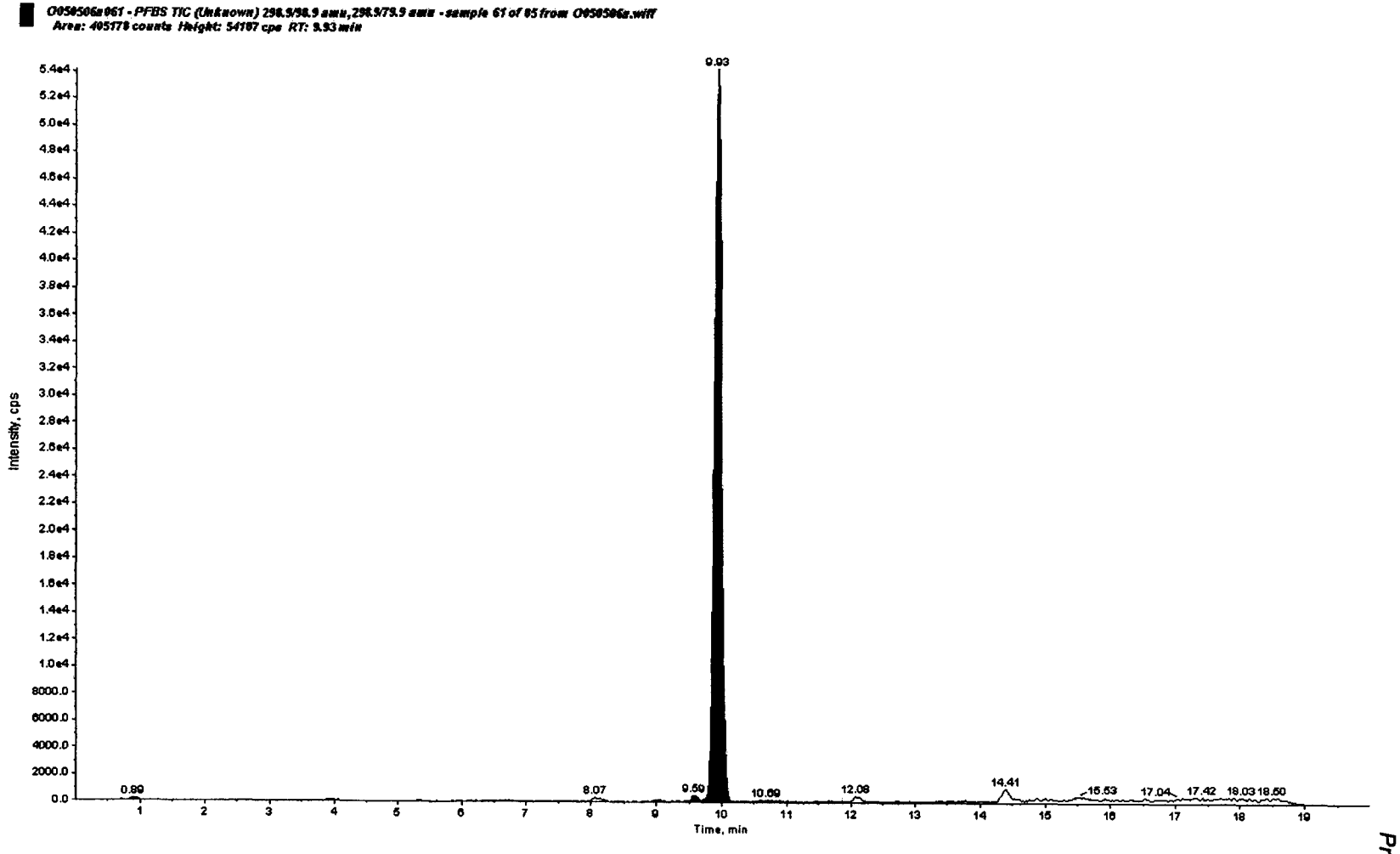


Figure 12. PFHS Total Ion Chromatogram: (Location #2; WMEL PW FW 01, Sample ID82438, o050506a061).

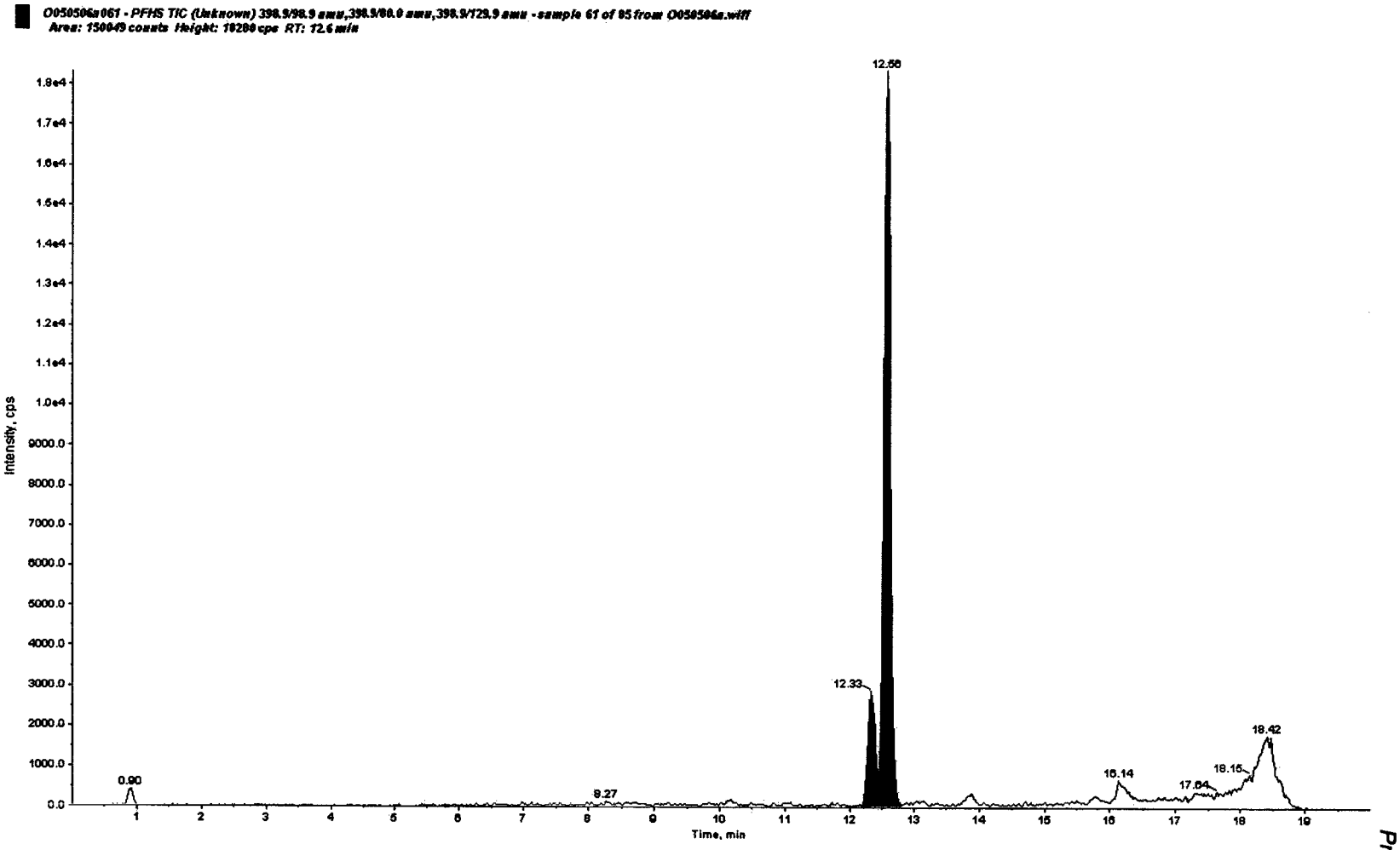


Figure 13. PFOS Total Ion Chromatogram (Location #2; WMEL PW FW 01, Sample ID82438; o050506a061).

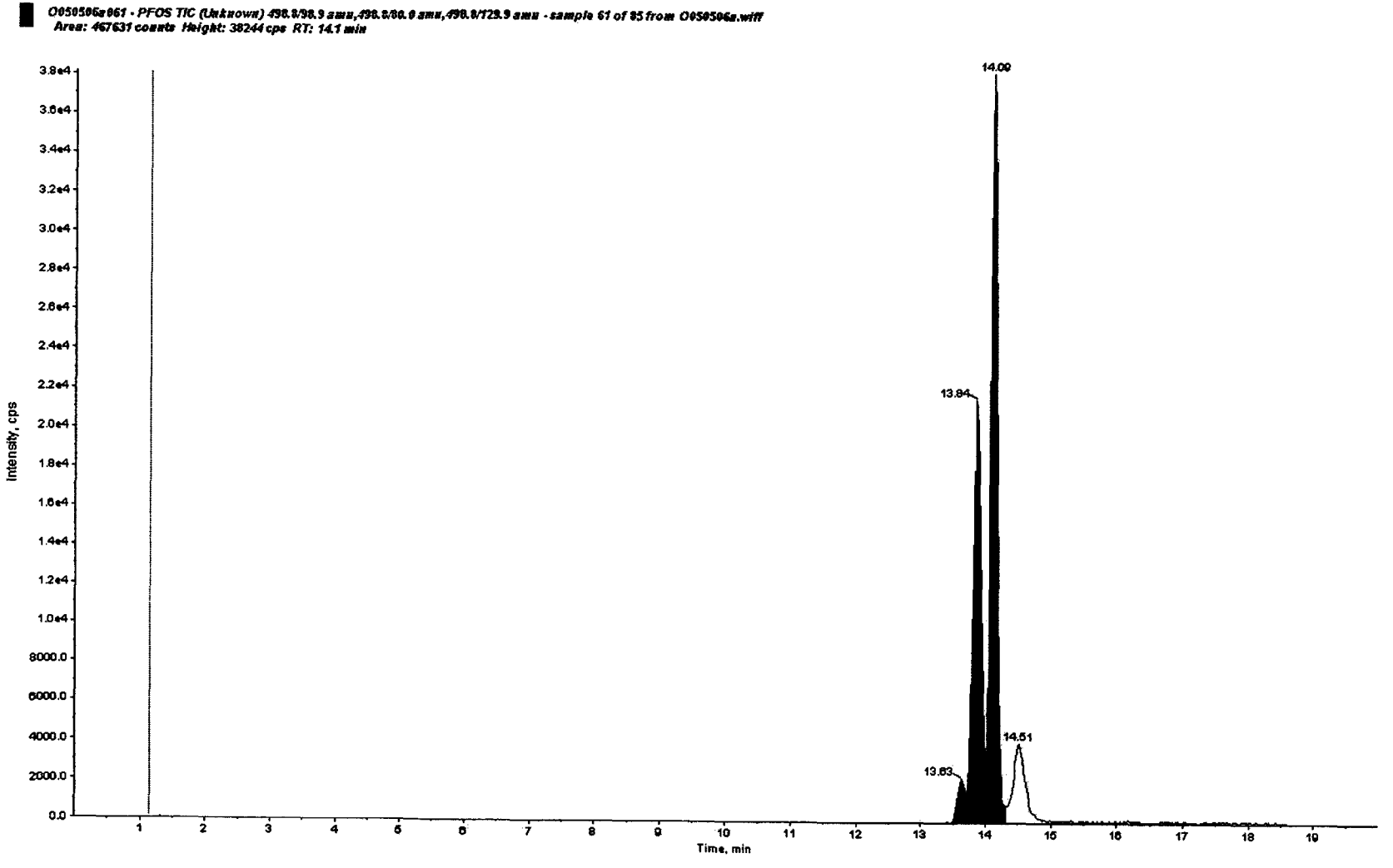
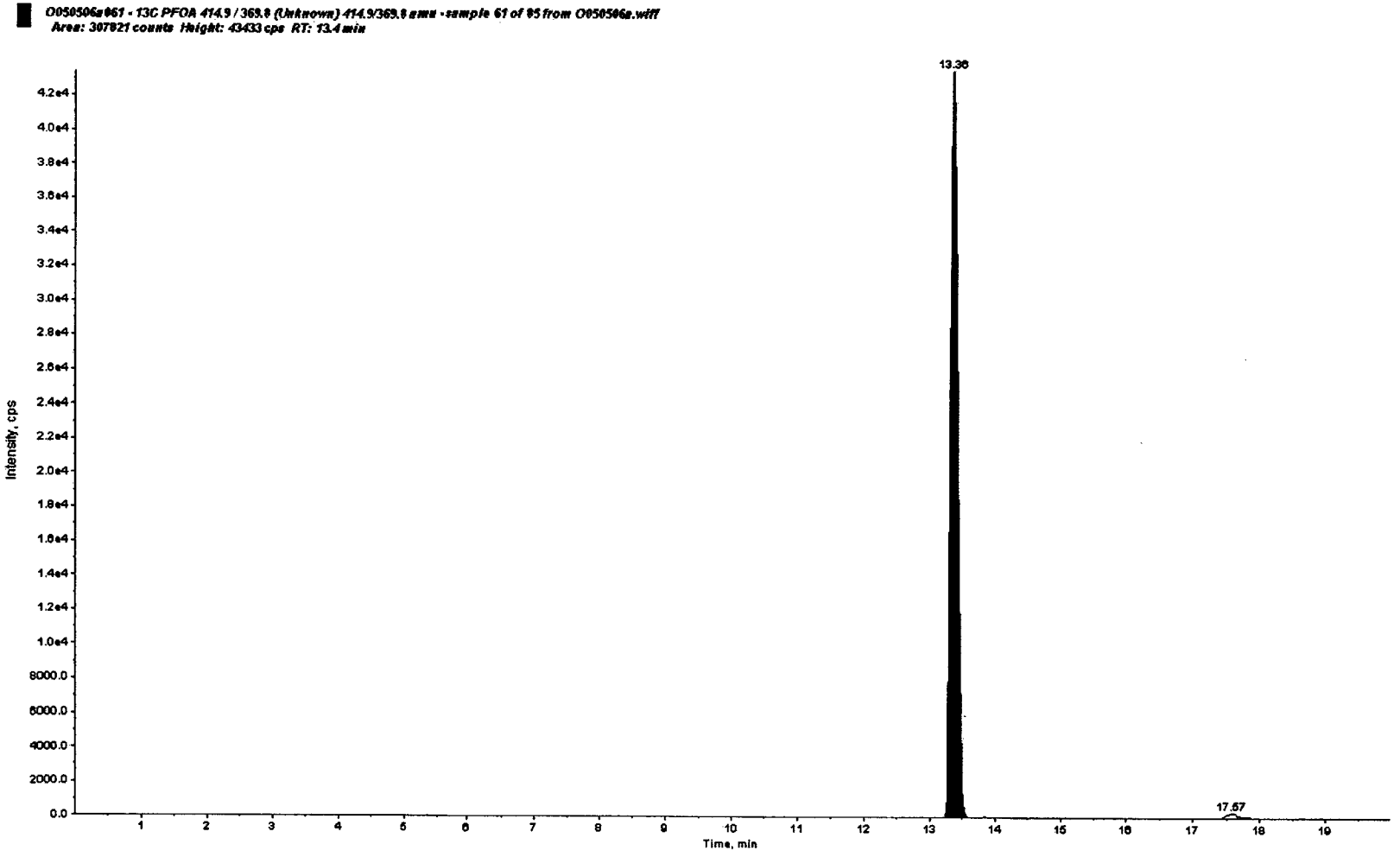


Figure 14. PFOA [1,2 ^{13}C] – Surrogate Chromatogram: (Location #2; WMEL PW FW 01, Sample ID82438, o050506a061).



ATTACHMENT B. EXTRACTION AND ANALYTICAL METHOD

3M Environmental Laboratory

Method

Determination of Perfluorinated Acids, Alcohols, Amides, and Sulfonates In Water By Solid Phase Extraction and High Performance Liquid Chromatography/Mass Spectrometry

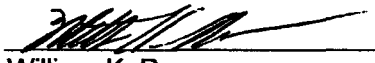
Method Number: ETS-8-154.1

Adoption Date: 28 Apr 2000

Revision Date: 5 May, 2003

Effective Date: 5 May, 2003

Approved By:



05/05/03

William K. Reagen
Manager

Date

1 Scope and Application

This method was validated for the collection, extraction, and analytical procedure for the determination of Perfluorooctane sulfonate (PFOS), Perfluorooctane sulfonylamide (FOSA), and Perfluorooctanoate (PFOA) in groundwater, surface water, and drinking water samples. This method may also be applied to the determination of other perfluorinated acids, alcohols, amides, and sulfonates in similar matrices, as long as the defined QC elements are satisfied and with the understanding that the method is not validated for compounds outside the scope of the original protocol

This method is based in part on the report "Method of Analysis for the Determination of Perfluorooctane sulfonate (PFOS), Perfluorooctane sulfonylamide (PFOSA), and Perfluorooctanoate (POAA) in Water" (see Section 17), as developed and validated by Exygen Research (formerly Centre Analytical Laboratories, Inc.).

2 Method Summary

Water samples are collected from a site of interest and shipped cold to an analytical facility. Perfluorinated acids, alcohols, amides, and sulfonates are extracted from 40mL water samples using C18 solid phase extraction (SPE) cartridges. The compounds are eluted from the C18 cartridge, using methanol. Separation, identification, and measurement are accomplished by high-performance liquid chromatography/ tandem mass spectrometry (HPLC/MS/MS) analysis. High-performance liquid chromatography/mass spectrometry (HPLC/MS) may be used if the defined QC elements are satisfied.

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

3 Definitions

3.1 Analytical Sample

A portion of an extracted Laboratory Sample prepared for analysis.

3.2 Calibration Standard

A solution prepared from the Working Standard (WS) and extracted according to this method. The calibration standard solutions are used to calibrate the instrument response with respect to analyte concentration.

3.3 Duplicate Sample (DS)

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

3.4 Field Blank Control Sample (FB)

ASTM Type I water placed in a sample container in the laboratory and treated as a sample in all respects, including exposure to sampling site conditions, storage, preservation and all analytical procedures. The purpose of the FB is to determine if test substances or other interferences are present in the field environment.

3.5 Field Duplicate (FD)

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

3.6 Field Matrix Spike (FMS)

A sample collected in duplicate to which known quantities of the target analytes are added in the field at the time of sample collection. Alternatively, the known quantity of target analytes may be added to the sample bottle in the laboratory before the bottles are sent to the field. A known, specific volume of sample must be added to sample container without rinsing. This may be accomplished by making a "fill to this level" line on the outside of the sample container. The FMS should be spiked at approximately 50–150% of the expected analyte concentration in the sample. If the expected range of analyte concentrations is unknown, a low and a high spike may be prepared to increase the likelihood that a spike at an appropriate range is made. The FMS is analyzed to ascertain if any matrix effects, interferences, or stability issues may complicate the interpretation of the sample analysis.

3.7 Field Spike Control Sample (FSCS)

An aliquot of ASTM Type I water to which known quantities of the target analytes are added in the field at the time of sample collection (at an appropriate concentration to be determined by the project lead) or in the laboratory prior to the shipment of the collection bottles. The FSCS is extracted and analyzed exactly like a sample to determine whether a loss of analyte could be attributed to sample storage and/or shipment. A low and high FSCS may be appropriate when expected sample concentrations are not known.

3.8 Laboratory Control Sample (LCS)

An aliquot of ASTM Type I water to which known quantities of the target analytes are added in the laboratory. Two levels are included, one at the LLOQ (approx. 25 pg/mL), the other at a concentration of approx. 100–250 pg/mL or another concentration to be determined by the project lead. The LCS is extracted and analyzed exactly like a laboratory sample to determine whether the methodology is in control, and whether the laboratory is capable of making accurate measurements at the required method detection limit and higher.

3.9 Laboratory Sample

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

3.10 Limit of Detection (LOD)

The LOD is the lowest concentration of an analyte that can be measured and reported with 99% confidence that the analyte concentration is greater than zero. If required, the LOD may be determined in several ways, including signal-to-noise ratio and statistical calculations.

3.11 Limit of Quantitation (LOQ)

The LOQ for a dataset is the lowest concentration (LLOQ) or highest concentration (ULOQ) that can be reliably achieved within the specified limits of precision and accuracy during routine operating conditions.

Note: For many analytes, the LLOQ analyte concentration is selected as the lowest non-zero standard in the calibration curve to simplify data reporting. Sample LLOQs are matrix-dependent.

3.12 Matrix Spike (MS)

A matrix spike is an aliquot of a sample, to which known quantities of target analytes are added in the laboratory. The MS is extracted and analyzed exactly like a laboratory sample to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the MS corrected for background concentrations.

3.13 Method Blank

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

3.14 Method Detection Limit (MDL) Determination

A MDL is the statistically calculated minimum amount of an analyte that can be measured with 99% confidence that the reported value is greater than zero. One of several processes that may be used to establish a LOD value is found in 40 CFR Part 136 Appendix B.

3.15 Sample

A sample is a small portion collected from a larger quantity of material intended to represent the original source material.

3.16 Spiking Stock Standard (SSS)

A solution prepared from stock standards used to prepare the working standard.

3.17 Stock Standard (SS)

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

3.18 Working Standard (WS)

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

4 Warnings and Cautions

4.1 Health and Safety

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

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

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

4.2 Cautions

None

5 Interferences

During extraction and analysis, major potential contaminant sources are reagents and solid phase extraction devices.

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

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

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

6 Instrumentation, Supplies, and Materials

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

6.1 Instrumentation

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

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

6.2 Supplies and Materials.

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

Coolers for sample shipment.

Ice for sample shipment.

Vacuum pump, Büchi.

Visiprep vacuum manifold, Supelco.

Sep Pak Vac 6cc (1g) tC18 cartridges (part # WAT 036795),Waters.

50mL disposable polypropylene centrifuge tubes, VWR.

15mL disposable polypropylene centrifuge tubes, VWR.

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

Class A pipettes and volumetric flasks, various.

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

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

125mL LDPE narrow-mouth bottles, Nalgene.

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

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

7 Reagents and Standards

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

7.1 Chemicals

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

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

ASTM Type I Water, prepared in-house.

Sodium Thiosulfate, Reagent grade, JT Baker.

7.2 Standards

Potassium perfluorooctane sulfonate

Perfluorooctane sulfonylamide

Ammonium perfluorooctanoate

Others as required.

7.3 Reagent Preparation

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

40% methanol wash solution – Measure 400mL methanol and adjust volume to 1.0L with reagent water.

100mM ammonium acetate solution (Analysis)—Weigh 7.71g of ammonium acetate and dissolve in 1.0L of reagent water. Dilute the 100mM solution by a factor of 50 to make the 2mM ammonium acetate solution used for mobile phase A.

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

7.4 Spiking Stock Standard (SSS) Preparation

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

100µg/mL each PFOS, PFOSA, and POAA SSSs—Weigh out 10mg of analytical standard (corrected for percent salt and purity—i.e., 10 mg $C_8F_{17}SO_3K$ purity 90% = 8.35mg $C_8F_{17}SO_3^-$) and dilute to 100mL with methanol in a 100mL volumetric flask. Transfer to a 125mL LDPE bottle or other suitable container. Prepare a separate solution for each analyte. Solutions may be stored in a refrigerator at 4°±2°C for a maximum period of 6 months from the date of preparation.

1µg/mL mixed SSS—Add 1.0mL each of the 100µg/mL SSSs (from 7.4.1) to a 100mL volumetric flask and bring up to volume with methanol.

0.1µg/mL mixed SSS—Add 10.0mL of the 1.0µg/mL-mixed solution (from 7.4.2) to a 100mL volumetric flask and bring up to volume with methanol.

0.01µg/mL mixed SSS—Add 10.0mL of the 0.1µg/mL-mixed solution (from 7.4.3) to a 100mL volumetric flask and bring up to volume with methanol.

Storage Conditions—Store all SSSs in a refrigerator at 4°±2°C for a maximum period of 6 months from the date of preparation.

7.5 Calibration Standards

The following standard preparation procedure serves as an example and may be changed to suit the needs of a particular study, provided the concentrations are calculated correctly.

100µg/mL each PFOS, PFOSA, and POAA stock standard solutions—Weigh out 10mg of analytical standard (corrected for percent salt and purity) and dilute to 100mL with methanol in a 100mL volumetric flask. Transfer to a 125mL LDPE bottle or other suitable container. Prepare a separate solution for each analyte. Store solutions in a refrigerator at 4°±2°C for a maximum period of 6 months from the date of preparation.

1µg/mL Working Standard—Add 1.0mL each of the 100µg/mL SS solutions (from 7.5.1) to a 100mL volumetric flask and bring up to volume with methanol.

0.1µg/mL Working Standard —Add 10.0mL of the 1.0µg/mL mixed solution (from 7.5.2) to a 100mL volumetric flask and bring up to volume with methanol.

0.01µg/mL Working Standard —Add 10.0mL of the 0.1µg/mL mixed solution (from 7.5.3) to a 100mL volumetric flask and bring up to volume with methanol.

Storage Conditions—Store all WSs in a refrigerator at 4°±2°C for a maximum period of 6 months from the date of preparation.

Calibration Standard—Prepare calibration solutions in ASTM Type I using the following table as a guideline:

Concentration of WS, µg/mL	Volume of WS, µL	Final Calibration Standard Volume, mL , of ASTM Type I Water	Final Concentration of Calibration Standard, pg/mL, in ASTM Type I Water
0.0	0	40	0
0.010	100	40	25
0.010	200	40	50
0.010	400	40	100
0.10	100	40	250
0.10	200	40	500
0.10	300	40	750
0.10	400	40	1000
1.0	100	40	2500
1.0	400	40	10000
1.0	1000	40	25000

The standards are processed through the extraction procedure (Section 11), identical to the laboratory samples. The concentration of the calibration standard in the final extract is equal to 8X the initial concentration, due to the concentration of the standard during the extraction process.

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

8 Sample Collection and Handling

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

Labeling: Each sample bottle must display information regarding the collection of that sample, the individual collecting the sample, and any matrix spike that has been added to the sample.

This includes the volume and concentration of any spiking solution added and the volume and identification of any preservatives added in the field.

Spiking: The spiking scheme will be clearly outlined in the sampling plan, including whether the samples will be spiked in the field or in the laboratory prior to the shipment of the bottles to the site. If spiking is to be performed in the field, materials and specific instructions will be included in the sampling kit. Be sure to clearly label each bottle with spiking information if applicable.

Tap Water: Open the tap and allow the system to flush until the water temperature (15°±10°C) has stabilized (usually about two minutes). Adjust the flow to about 500mL/min and collect samples from the flowing stream.

Ground Water: Purge the well of standing water using a pump or a bailer. Collect the sample directly from the pump or from the bailer.

Surface Water: When sampling from an open body of water, fill the sample container with water from a representative area.

Sample Dechlorination: All samples should be iced or refrigerated at 4°±2°C and kept in the dark from the time of collection until extraction. Residual chlorine should be eliminated by adding 200µL of a 250mg/mL sodium thiosulfate solution to each tap-water sample and associated FB and FSCS (which may be placed in each bottle before leaving for the sampling site or done in the field.).

Holding Time (HT): Results of the time/storage study of all target analytes showed that the three compounds are stable for 14 days in water samples when the samples are dechlorinated and stored as described in the previous section (see also references in section 17). Therefore, laboratory samples must be extracted within 14 days and the extracts analyzed within 30 days of sample collection. If the HT exceeds 14 days, great care is used when evaluating field spikes to avoid misrepresentation of the sample concentration.

8.1 Field Blanks

Process a Field Blank Control Sample (FB) along with each sample set (samples collected from the same general sample site at approximately the same time). At the laboratory, prior to sample collection, fill a sample container with ASTM Type I water, seal, and ship the FB to the sampling site along with the empty sample containers. Return the FB to the laboratory with the filled sample bottles.

When sodium thiosulfate is added to samples, use the same procedure to preserve the FB.

8.2 Field Duplicates

Collect a Field Duplicate (FD) for every ten (10) samples collected or per each sampling set, if less than 10 samples are collected.

Separate FDs must be collected for each type of water sample (ground, tap, etc.) collected.

Collect the FD immediately after the sample.

Preserve, store and ship FD using the same procedures as used for the samples.

8.3 Field Spike Control Sample (FSCS)

A Field Spike Control Sample (FSCS) must be prepared for each sample shipment. If multiple coolers are used to ship a set of samples, each cooler must contain a FSCS.

At the laboratory, fill a sample container with 100mL of ASTM Type I water. Seal and ship to the sampling site along with the empty sample containers and FBs. Samples may either be spiked in the field or in the laboratory prior to shipment. The method employed should be consistent throughout the study. If the samples are to be spiked in the field, be sure to send appropriate supplies and instructions for the field personnel to follow.

Seal and gently invert the FSCS to mix. Store and ship the FSCS using the same procedures as used for the samples

Provide information on sample collection, preservation, shipment and storage. List applicable holding times. Include sample stability and extract storage requirements. Reference the method used for sample preparation, if applicable.

8.4 Field Matrix Spike (FMS)

A Field Matrix Spike (FMS) must be prepared for each sampling location. One unspiked sample from the same location must accompany the FMS to determine endogenous levels in the sample. The samples should be clearly identifiable as being from the same location.

Samples may either be spiked in the field or in the laboratory prior to shipment. The method employed should be consistent throughout the study. If the samples are to be spiked in the field, be sure to send appropriate supplies and instructions for the field personnel to follow.

9 Quality Control and Data Quality Objectives

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

9.1 Solvent Blanks

Solvent blanks are analyzed with each sample set to determine contamination or carryover. Aliquots of methanol represent the solvent used for the standard curve and the sample extraction. Solvent blanks should have area counts that are less than 50% of the area count of the lowest calibration standard.

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

9.2 Method Blanks

A method blank consists of an aliquot of ASTM Type I water, equal in volume to the samples, and extracted in the same manner as the samples. At least two method blanks should be prepared and analyzed each day that extractions are performed for a particular study or project. When analyzed the area counts of these samples must be less than 50% of the area count of the lowest calibration standard.

9.3 Sample Replicates

All samples, including field spikes, trip blanks, etc., should be extracted at least in duplicate, and in triplicate if difficulties were encountered in the sampling and/or holding conditions of the samples. The relative percent difference (RPD) of duplicate samples or relative standard deviation (RSD) should be less than 15% for the precision of sample preparation and analysis to be considered in control.

9.4 Matrix Spike

Matrix spikes are prepared for each sample type and analyzed to determine the matrix effect on the recovery efficiency. Matrix spike recoveries should fall within ± 25% of expected values. If the matrix spikes fail, evaluate the lab control spikes. If the LCS are within acceptance criteria there may be matrix issues in the samples. Discuss these in the final report.

Matrix spike duplicates are prepared periodically to measure the precision associated with the analysis.

Analyze a matrix spike and matrix spike duplicate (if prepared) in the same run as the original sample.

Matrix spike and matrix spike duplicate concentrations should fall in the mid-range of the initial calibration curve or should be prepared at 1.5-5 times the endogenous concentration of the analyte. Spike concentrations should fall in the low-range of the initial calibration curve if extremely low-levels are expected. Generally two or more levels are prepared, one in the low range of the curve and one in the mid-range. This avoids the need to pre-screen unknown samples prior to preparation.

9.5 Laboratory Control Spike

Lab control spikes are prepared for each study to ensure recovery of the target analytes. These should be prepared at a minimum of 2 levels and in duplicate or triplicate. Recovery of these samples should be within ± 25% of expected values, and the RPD (or RSD) be ≤ 15%. If recoveries fall outside these limits the samples should be addressed in the final report.

10 Calibration and Standardization

10.1 Instrument Setup

Note: In this example, a MicroMass Ultima™ Liquid Chromatography Tandem Mass Spectrometer (LC/MS/MS) is used. Other brands of LC/MS/MSs as well as single quadrupole mass spectrometers (LC/MS) may be used as long as the method criteria are met. Brand names, suppliers, part numbers, and models are for illustrative purposes only. Equivalent performance may be achieved using apparatus and materials other than those specified here, but demonstration of equivalent performance that meets the requirements of this method is the responsibility of the laboratory. The operator must optimize and document the equipment and settings used.

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

Mass Spec: Micromass Ultima (Micromass)

Interface: Electrospray (Micromass)

Mode: Electrospray Negative, Multiple Response Monitoring (MRM)

Harvard infusion pump (Harvard Instruments), for tuning

Computer: COMPAQ Professional Workstation AP200

Software: Windows NT, MassLynx 3.3

HPLC: Hewlett Packard (HP) Series 1100

HP Quaternary Pump

HP Vacuum Degasser

HP Autosampler

HP Column Oven

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

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

Column Temperature: 35°C

Injection Volume: 15µL

Mobile Phase (A): 2mM Ammonium Acetate in ASTM Type I water (See 7.3.1)

Mobile Phase (B): Methanol

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

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

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

Ions Monitored:

Analyte	Primary Ion	Product Ion	Approximate Retention Time (minutes)
PFOA	413	169	5.0
PFOS	499	99	5.2
FOSA	498	78	5.8

Other product ions may be chosen at the discretion of the analyst, although m/z 99 is suggested for PFOS. Use of the suggested primary ion is recommended. Retention times may vary slightly, on a day-to-day basis, depending on the batch of mobile phase etc. Drift in retention times is acceptable within an analytical run, as long as the drift continues through the entire analysis and the standards are interspersed throughout the analytical run.

10.2 Tune File Parameters

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

Analyte	Dwell, sec	Collision Energy, eV	Cone, V
PFOA	0.2–0.4	10–25	20–30
PFOS	0.2–0.4	30–60	50–80
FOSA	0.2–0.4	20–50	30–60

Source	Set
Capillary	2.6–3.5kV
Hexapole 1	0.5V
Aperture 1	0.2V
Hexapole 2	0.8V
Source Block Temp.	100–150°C
Desolvation Temp.	250–400°C

Analyzer	Set
LM Res 1	12.5–15.0V
HM Res 1	12.5–15.0V
IEnergy 1	0.7V
Entrance	–2V
Exit	1V
LM Res 2	11.0V
HM Res 2	11.0V
IEnergy 2	1.0V
Multiplier	650V

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

Pressures	Set
Gas Cell	3.0e–3mbar

10.3 Calibration Curve

Analyze the standard curves prior to each set of samples. The validated method specifies that the standard curve should be plotted using a linear fit, weighted 1/x or unweighted. However, the standard curve may also be plotted by quadratic fit ($y = ax^2 + bx + c$), weighted 1/x or unweighted, using suitable software. The calibration curves may include but should not be forced through zero. The mathematical method used to calculate the calibration curve should be applied consistently throughout a study. Any change should be thoroughly documented in the raw data.

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

For purposes of accuracy when quantitating low levels of analyte, it may be necessary to use the low end of the calibration curve rather than the full range. For example, when attempting to quantitate approximately 50 pg/mL of analyte, generate a calibration curve consisting of the standards from 25 pg/mL to 1000 pg/mL rather than the full range of the curve (25 pg/mL to 25000 pg/mL). This will reduce inaccuracy attributed to linear regression weighting of high concentration standards.

High and/or low points may be excluded from the calibration curves to provide a better fit over the linear range appropriate to the data or because they did not meet the pre-determined acceptance criteria. Low-level curve points should also be excluded if their area counts are not at least twice that of the method and/or solvent blanks. Any curve point may be rejected due to a bad injection or failing to meet accuracy requirements of ± 25% (and ± 30% for the LLOQ). Justification for exclusion of calibration curve points will be noted in the raw data. A minimum of 6 points will be used to construct the calibration curve.

10.4 Continuing Calibration Verification (CCV)

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

10.5 System Suitability

A minimum of three system suitability samples will be injected at the beginning and end of each analytical run. Typically these samples are run prior to the calibration curve. The system suitability injections must have area counts with an RSD of ≤5% and a retention time RSD of ≤2% when evaluated independently.

11 Procedures

11.1 Extraction Scheme

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

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

Note: * Samples may need to be prescreened to determine an appropriate matrix spike level (typically 50–150% of sample concentration). Alternatively the samples could be spiked at more than one level, allowing for the inappropriate spike level to be eliminated.

Condition the C18 SPE cartridges (1g, 6mL) by passing approximately 10mL methanol followed by approximately 50mL ASTM Type I water (flow rate approximately 2 drop/sec). Do not let column run dry.

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

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

Ten mL of the 40% methanol in water wash mixture is passed through the C18 SPE cartridge to rinse away potential interferences and then discarded. This step must be omitted if perfluorinated compounds with chain lengths less than C8 are targeted since these will be lost during this wash step.

Elute with exactly 5mL of 100% methanol. Collect eluate into graduated 15mL polypropylene centrifuge tubes. This is the target elution fraction (final volume approximately 4.5 mL as not all of the solvent will leave the SPE column. This will not affect the calculations in any way since the curve is also extracted).

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

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

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

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

Use a standard with an analyte concentration between 1000 and 4000 pg/mL. Repeat the extraction scheme from the beginning up through the eluting with ~5mL 100% methanol.

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

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

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

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

11.2 Sample Analysis

Set up analysis sample queue.

Inject the same volume (between 5–25µL) of each standard, analytical sample and blank into the instrument.

All samples with a concentration > ULOQ must be diluted and reanalyzed. If dilution of the final extract fails to produce acceptable results (e.g. poor MS recoveries) dilute the original sample and re-extract.

12 Data Analysis and Calculations

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

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

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

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

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

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

13 Method Performance

Note: Any method performance parameters that are not achieved must be considered in the evaluation of the data. Nonconformance to any specified parameters must be described and discussed in any reporting of the data.

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

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

13.1 System Suitability

A minimum of three system suitability samples will be injected at the beginning and end of each analytical run. Typically these samples are run prior to the calibration curve. The system suitability injections must have area counts with an RSD of ≤5% and a retention time RSD of ≤2% when evaluated independently.

13.2 Quantitation

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

Demonstration of Specificity: Specificity is demonstrated by chromatographic retention time (within 3% of standard) and the mass spectral response of unique ions.

13.3 Sensitivity

Solvent Blanks and Method Blanks: Solvent and method blank area counts must be < 50% that of the lowest standard used in the calibration curve.

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

13.4 Accuracy

CCV Performance: Calibration verification injections must be within ±25% to be considered acceptable. The calibration curve and the last passing CCV will then bracket acceptable samples. Multiple CCV levels may be used.

Matrix Spikes: Matrix spike percent recoveries must be within ± 25% of the spiked concentration. If matrix effects are suspected, evaluate the LCS results to determine if a matrix effects are present and if the method is in control based on compliant LCS results. Discuss all results in the analytical report.

13.5 Precision

Reproducibility: Reproducibility of the method is defined by the results of duplicate or triplicate analysis of samples. A RPD or RSD of ≤ 15% will be considered acceptable.

System Suitability: The system suitability injections must have area counts with an RSD of ≤5% and a retention time RSD of ≤2% when evaluated independently.

14 Pollution Prevention and Waste Management

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

15 Records

Each data package generated for a study must have the following information included: study or project number, acquisition method, integration method, sample name, extraction date, dilution factor (if applicable), and analyst.

Print the tune page, sample list, and acquisition method to include in the appropriate study folder. Copy these pages and tape into the instrument run log.

Plot the calibration curves as described in this method, then print these graphs and store in the study folder.

Print data integration summary, integration method, and chromatograms and store in the study folder.

Summarize data using suitable software and store in the study folder.

16 Attachments

None.

17 References

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

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

18 Affected Documents

None.

19 Revisions

Revision Number	Revision Number	Revision Date
1	*Updated to the new format. Changed Title.* *Section 1: States the validation of 3 analytes, removes reference to EPA document that's no longer applicable.* *Section 2: Provided for the extraction of more than the 3 validated analytes, allows the use of a LC/MS system, not only the LS/MS/MS previously mentioned.* *Section 3: Revised definitions for field matrix spike, field control spike, LLOQ, method blank, and MDL.* *Section 5: Reworded the interferences, added recommendation to use disposable pipettes.* *Section 6: Recategorized and pared down.* *Section 7: Changed storage time to 6 months. Added more calibration points to the table.* *Section 8:Added statement addressing labeling requirements and spiking procedures. Expanded section 8.8.* *Section 9: New Section* *Section 10: Changed some of the parameters in the tables. Allowed for use of different instrumentation. Added information from section 12 of previous version, extensively revised.* *Section 11 (section 9 in previous version): Clarification of wash step, stated exact volume of eluate is 5 mL, revised standardization process, removed requirement to use LC/MS/MS.* *Section 12 (section 13 in previous version: no changes* *Section 13 (section 14 in previous version): Extensively rewritten.* *Section 14 (section 15 in previous version): no changes* *Section 15 (section 16 in previous version): Minor changes to recording requirements.* *Section 16 (section 17 in previous version): Removed attachment.* *Section 17 (section 18 in previous version): Removed reference to EPA document that no longer applied to this SOP.* *Section 18: New section.*	7

ATTACHMENT C. PROTOTOL AND PROTOCOL AMENDMENTS

Exygen Protocol Number: P0001131

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
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Sponsor Representative:
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Exygen Protocol Number: P0001131

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

Exygen Protocol Number: P0001131

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

Exygen Protocol Number: P0001131

TABLE OF CONTENTS

Exygen Protocol Number: P0001131

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.

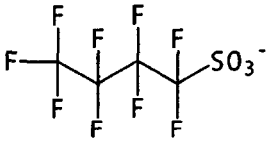
The study will be audited for compliance with EPA TSCA Good Laboratory Practice Standards 40 CFR 792 by the Quality Assurance Unit of Exygen Research.

TEST MATERIALS

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

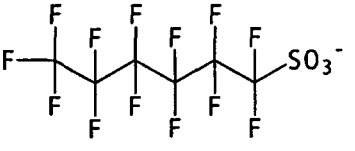
PFBS

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

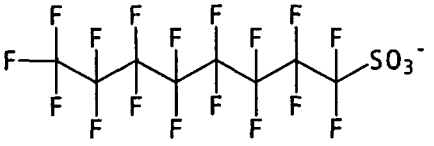
Chemical Name: Perfluorohexanesulfonate
Molecular Weight: 438 supplied as the potassium salt ($C_6F_{13}SO_3^-K^+$)
Lot Number: SE036
Purity: 98.6%
Transitions Monitored: 399 → 80
Structure:



Exygen Protocol Number: P0001131

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

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Exygen Protocol Number: P0001131

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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.

Exygen Protocol Number: P0001131

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"

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"

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).

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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.

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APPENDIX I

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"

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

Total Pages: 7

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.

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ANALYTICAL METHOD

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

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.

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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.

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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

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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 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 (%) =

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

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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

Exygen Research Method Number V0001781

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.

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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.

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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.

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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

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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.

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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:

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

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.

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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.

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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

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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.

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Exygen Research | Method Number V0001782

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 ($R^2 \geq 0.985$). 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.

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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:

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

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.

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

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

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

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

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

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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
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Approved By:

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10/26/04
Date

John Flaherty
Vice President, Operations, Exygen Research

10/26/04
Date

Total Pages: 8

Exygen Protocol Number: P0001131

Exygen Research Method Number V0001783

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.

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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.

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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.

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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.

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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.

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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 ($R^2 \geq 0.985$). 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/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.

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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

11/6/04
Date

Total Pages: 7

Exygen Protocol Number: P0001131

Exygen Research Method Number V0001784

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.

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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.

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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.

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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.

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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.

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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.

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

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

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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.

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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.

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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.

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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 ($R^2 \geq 0.985$). 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/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

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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.

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

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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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.

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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.

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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.

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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 ($R^2 \geq 0.985$). 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/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.

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

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

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

Protocol Exygen P0001131; 3M Study Number E05-0210
Amendment 3

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

PROTOCOL AMENDMENT NO. 3

Amendment Date:
May 16, 2005

Performing Laboratory
3M Environmental, Health, and Safety Operations
3M Environmental Laboratory
935 Bush Avenue
St. Paul, MN 55106

Laboratory Project Identification
E05-0210

Protocol Exygen P0001131; 3M Study Number E05-0210
Amendment 3

This amendment modifies the following portion(s) of the protocol:

PROTOCOL READS:
TESTING FACILITY
Exygen Research
3058 Research Drive
State College, PA 15801
Phone: (814) 272-1039

AMEND TO READ:

TESTING FACILITIES
Exygen Research
3058 Research Drive
State College, PA 15801
Phone: (814) 272-1039

3M Environmental Laboratory
Building 2-3E-09
935 Bush Avenue
St. Paul, MN 55106

REASON:
Addition of 3M Environmental Laboratory as a testing facility allows selected water samples to be sent to the 3M facility for analysis of PFBS, PFHS, and PFOS using the most current version of ETS 8-154 "Determination of Perfluorinated Acids, Alcohols, Amides, and Sulfonates in Water by Solid Phase Extractions and High Performance Liquid Chromatography/Mass Spectrometry".

The following modifications to ETS 8-154.1 will be incorporated into the study:

(1) The sample volume extracted and the final SPE elution volume may be adjusted, at the analyst's discretion, to better achieve the detection limits specified in study objectives. As written, ETS 8-154.1 calls for a 40 mL extraction volume with a 5 mL elution volume which results in an eight-fold sample concentration. The actual volumes used and the resulting overall concentration factor will be given in the final report. Associated method quality control samples will demonstrate that the volume modifications do not impact method accuracy and precision.
(2) A solvent (unextracted) calibration curve may be analyzed with the extracted calibration curve to ascertain extraction efficiency. Samples will be analyzed using the extracted curve unless otherwise stated in the final report. Data from the solvent calibration curve will not be included or discussed in the final report unless it is used to strengthen experimental observations/explanations.
(3) ETS 8-154.1 makes no mention of sample surrogate spikes; however, all samples will be spiked with at least one of the following radiolabeled surrogates, PFOA [1,2 ^{13}C] (perfluorooctanoic acid), PFOS [^{18}O2] and/or PFNA [1,2 ^{13}C] (perfluorononanoic acid – C9).The concentration of the surrogate spike may vary depending on the collection event, but typical samples are spiked with a nominal concentration of 0.05 ng/mL. Surrogate spike recoveries will be documented and discussed in the final report. Surrogate recoveries between 100±25% will be deemed as meeting method criteria for accuracy.
(4) Several method blanks may be prepared and analyzed to better determine a "representative" area count value for the method blank. The method blank area count is instrumental in determining the method limit of quantitation and understanding the average and range of area counts possible is critical. If a method blank value is used as a "zero point" in the final calibration curve, then a brief explanation in the final report or in the raw data as a Note to File must be

included to describe why that specific method blank was selected as the zero point (closest to the average value, most conservative (largest) value, etc.)

(5) The overall analytical uncertainty for each target (non-surrogate) analyte will be estimated using both the method accuracy and precision. The method accuracy and precision will be calculated using data from laboratory contorl spike (LCS) samples prepared and analyzed with the sample set(s). Replicate LCSs must be prepared each day samples and/or other quality control samples are prepared. A sample calculation of how the analytical uncertainty was determined will be provided in the final report and/or raw data.

A new revision of ETS 8-154 may incorporate all or several of the modifications listed above. If a new version of ETS 8-154 is issued during the course of this study, sample results must meet the new method requirements regardless if they are listed above.

3M Environmental Laboratory management will approve all documented deviations to the 3M Environmental Laboratory quality system (SOPs, methods, etc.) that occur during the course of this investigation.

Protocol Exygen P0001131; 3M Study Number E05-0210
Amendment 3

Amendment Approval

Michael A. Santoro, 3M,	Sponsor Representative	Date 6/02/05
William K. Reagen, Ph.D.	3M Environmental Laboratory Manager	Date 05/19/05
Jaisimha Kesari, Weston Solutions,	Study Director	Date 5/26/05