3M

Interim Report #18: Analysis of Water Samples from Northern Alabama Potable Water Systems, December 2005

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

Author

Michelle D. Malinsky, Ph.D
3M Environmental Laboratory

Interim Report Completion Date

Date of signing

Performing Laboratory

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

Project Identification

E05-0210

Total Number of Pages

122

A2LA ACCREDITED
Certificate #2052-01

The testing reported herein meet the requirements of ISO/IEC 17025-1999 "General Requirements for the Competence of Testing and Calibration Laboratories", in accordance with the A2LA Certificate #2052-01. Testing that complies with this International Standard also operate in accordance with ISO 9001/ISO 9002 (1994).

This page has been reserved for specific country requirements.

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 Water Samples from Northern Alabama Potable Water Systems, December 2005

Interim Study Identification Number: E05-0210 Interim Report #18

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:

None

Michael A. Santoro, 3M Company,	Sponsor Representative	3/23/06 Date
Jaisimha Kesari P. E., DEE,	Study Director	3/21/06 Date

ACCREDITED

Certificate #2052-01

The testing reported herein meet the requirements of ISO/IEC 17025-1999 "General Requirements for the Competence of Testing and Calibration Laboratories", in accordance with the A2LA Certificate #2052-01. Testing that complies with this International Standard also operate in accordance with ISO 9001/ISO 9002 (1994).

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

Interim Study: Analysis of Water Samples from Northern Alabama Potable Water Systems, December 2005

Study Identification Number: E05-0210 Interim Report #18

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.

Inspection Dates	*Phase*	*Date Reported to*	
		Management	*Study Director*
February 15, 2006	Data/Report	March 17, 2006	March 17, 2006

Casey Howell mar 17 2006

QAU Representative Date

TABLE OF CONTENTS

LIST OF TABLES

STUDY INFORMATION

Sponsor

3M Company

Sponsor Representative

Michael A. Santoro
3M Company
3M Building 0236-01-B-10
St. Paul, MN 55144

Study Director

Jaisimha Kesari, P.E., DEE
Weston Solutions, Inc.
1400 Weston Way
West Chester, PA 19380

Study Location

Testing Facilities

Exygen Research
3058 Research Drive
State College, PA 16801

3M Environmental Laboratory
3M Building 2-3E-09
935 Bush Avenue
St. Paul, MN 55144

Study Dates

Study Initiation: 11/5/2004

Interim Experimental Initiation: January 11, 2006

Interim Experimental Completion: January 27, 2006

Interim Study Completion: Date of interim report signing

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 water samples collected from six Northern Alabama potable water systems by Weston Solutions personnel on December 13 and 14, 2005. Samples were submitted for analysis as part of 3M Environmental Laboratory project number E05-0210 (3M Decatur Fluorochemical GLP Monitoring Program, Interim Report #18: Analysis of Water Samples from Northern Alabama Potable Water Systems, December 2005).

Water samples were analyzed for perfluorobutane sulfonate (PFBS), perfluorohexane sulfonate (PFHS), and perfluorooctane sulfonate (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). ETS 8-154.1 falls under the 3M Environmental Laboratory's current ISO/IEC 17025-1999 scope of accreditation and the results reported herein were compliant with this accreditation. The analytical start date for this interim report was January 11, 2006 and the analytical termination date was January 27, 2006.

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 (0.1 ng/mL) and high field spike (1.0 ng/mL). Each empty container was marked with a "fill to here" line to produce a final sample volume of 450 mL. Containers designated for field matrix samples were fortified with an appropriate matrix spike solution containing the three target analytes prior to being sent to the field for sample collection. For the samples and field matrix spikes collected and analyzed for this interim report, PFOA [1,2 ^{13}C] was not included as a surrogate. This is a deviation from Protocol P0001130 Amendment #3. A record of deviation has been issued as is included in Attachment C. Table 1 below summarizes the sample results. The average between the sample and the sample duplicate is provided along with the relative percent difference (%RPD), if applicable. The limit of quantitation (LOQ) of PFBS, PFHS, and PFOS for the sample set was 0.0248 ng/mL, 0.0252 ng/mL, and 0.0249 ng/mL, respectively. All results for quality control samples prepared and analyzed with the samples will be reported and discussed elsewhere in this report.

Table 1. Sample Results Summary

3M LIMS ID	*Sample Description*	*[1]PFBS Concentration (ng/mL)*	*[2]PFHS Concentration (ng/mL)*	*[3]PFOS Concentration (ng/mL)*
E05-0210-92250	DPWS PW FW02 0 051213	<0.0248	<0.0252	<0.0249
E05-0210-92251	DPWS PW FW02 DB 051213	<0.0248	<0.0252	<0.0249
Average Sample/Sample Duplicate		***<0.0248***	***<0.0252***	***<0.0249***
%RPD		***[4]NA***	***[4]NA***	***[4]NA***
E05-0210-92254	WMEL PW FW02 0 051213	0.0350	<0.0252	0.0367
E05-0210-92255	WMEL PW FW02 DB 051213	0.0321	<0.0252	0.0367
Average Sample/Sample Duplicate		***0.0336***	***<0.0252***	***0.0367***
%RPD		***8.6***	***[4]NA***	***0.0***
E05-0210-92262	MSTP PW FW02 0 051214	<0.0248	<0.0252	<0.0249
E05-0210-92263	MSTP PW FW02 DB 051214	<0.0248	<0.0252	<0.0249
Average Sample/Sample Duplicate		***<0.0248***	***<0.0252***	***<0.0249***
%RPD		***[4]NA***	***[4]NA***	***[4]NA***
E05-0210-92266	FWTP PW FW02 0 051214	<0.0248	<0.0252	<0.0249
E05-0210-92267	FWTP PW FW02 DB 051214	<0.0248	<0.0252	<0.0249
Average Sample/Sample Duplicate		***<0.0248***	***<0.0252***	***<0.0249***
%RPD		***[4]NA***	***[4]NA***	***[4]NA***
E05-0210-92270	TVATP PW FW02 0 051214	0.0344	<0.0252	<0.0249
E05-0210-92271	TVATP PW FW02 DB 051214	0.0347	<0.0252	<0.0249
Average Sample/Sample Duplicate		***0.0346***	***<0.0252***	***<0.0249***
%RPD		***0.87***	***[4]NA***	***[4]NA***
E05-0210-92258	SWTP PW FW02 0 051214	<0.0248	<0.0252	<0.0249
E05-0210-92259	SWTP PW FW02 DB 051214	<0.0248	<0.0252	<0.0249
Average Sample/Sample Duplicate		***<0.0248***	***<0.0252***	***<0.0249***
%RPD		***[4]NA***	***[4]NA***	***[4]NA***

(1) The analytical uncertainty of the PFBS results is 100±5.3% based on method accuracy and precision. All results and averages are presented with three significant figures, %RPD to two significant figures. Sample concentrations, averages, and %RPD values may vary slightly from the raw data. Samples with reported results less than the LOQ of 0.0248 ng/mL may or may not have PFBS present. Results less than the LOQ are not quantified.

(2) The analytical uncertainty of the PFHS results is 100±9.3% based on method accuracy and precision. All results and averages are presented with three significant figures, %RPD to two significant figures. Samples with reported results less than the LOQ of 0.0252 ng/mL may or may not have PFHS present. Results less than the LOQ are not quantified.

(3) The analytical uncertainty of the PFOS results is 100±11% based on method accuracy and precision. All results and averages are presented with three significant figures, %RPD to two significant figures. Sample concentrations, averages, and %RPD values may vary slightly from the raw data. Samples with reported results less than the LOQ of 0.0249 ng/mL may or may not have PFOS present. Results less than the LOQ are not quantified.

(4) NA: Not applicable. %RPD values not determined when concentrations for the sample and/or sample duplicate are below the stated LOQ.

TEST SAMPLES

Water samples from six locations were received at the 3M Environmental Laboratory on December 16, 2005 from Tim Frinak of Weston Solutions, Inc. Sample containers were received in satisfactory condition at ambient temperatures. A total of twenty-seven containers were submitted for analysis. The samples were logged in by 3M Environmental Laboratory professional services personnel and placed in refrigerated storage on December 16, 2005 until they were removed for extraction on January 11, 2006. Table 2 below provides the codes for the given sample descriptions.

Table 2. Sample Description Codes.

Sample Description Abbreviation	*Definition*
DPWS	Decatur Public Water System
WMEL	West Morgan/East Lawrence Water Treatment Plant, Lawrence County, AL
SWTP	Sheffield Water Treatment Plant, Sheffield AL
MSTP	Muscle Shoals Treatment Plant, Muscle Shoals, AL
FWTP	Florence Water Treatment Plant, Florence, AL
TVATP	Tennessee Valley Authority Treatment Plant, Muscle Shoals, AL
PW	Potable Water
FW	Finished Water
0	Matrix Sample
DB	Duplicate
LS	Low Spike
HS	High Spike

REFERENCE SUBSTANCES

Table 3 lists the pertinent information regarding the reference substance used for this study.

Table 3. Study Reference Substances.

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	1/17/2007	10/18/2006	8/31/2006
Storage Conditions	Frozen	Frozen	Frozen
Chemical Lot Number	2	NB# 120067-69	171
TCR Number	TCR-281 (99131-039)	TCR-83 (SE036)	TCR-696
Physical Description	White Powder	White Powder	White powder
Purity	97.3%	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.

METHOD SUMMARIES

Sample Collection

Samples were collected on December 13-14, 2005 in rinsed Nalgene™ (low-density polyethylene) bottles prepared at the 3M Environmental Laboratory on December 9, 2005. Containers designated for field matrix spike samples were spiked in the laboratory with a known volume of an appropriate matrix spiking solution containing the target analytes. Four collection bottles were associated with each individual sampling location: sample, sample duplicate, low level field matrix spike (LS), and high level field matrix spike (HS). Additionally, one "Trip Blank" sample set was prepared. A Trip Blank set consisted of three individual bottles: trip blank sample, trip blank low spike, and trip blank high spike. The matrix spike levels for the trip blanks were the same as the samples. Table 4 below details the spike amounts for the four bottles comprising the sample location set.

Table 4. Sample Collection Information

[1]Bottle Description	Nominal Final Volume Collected (mL)	Final Concentration (ng/mL)		
		PFBS	PFHS	PFOS
Sample	450	NA	NA	NA
Sample Dup	450	NA	NA	NA
Low Field Matrix Spike (LS)	450	0.0992	0.101	0.0997
High Field Matrix Spike (HS)	450	0.992	1.01	0.997

(1) The sample description was assigned in the field by Weston Solutions personnel.

Preparatory and Analytical Methods

Extraction

All samples, calibration standards, and associated quality control samples were extracted using the procedure outlined in 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, 40 mL of sample was loaded onto a pre-conditioned Waters C18 solid phase extraction (SPE) cartridge (Sep-Pak, 6 cc) using a vacuum manifold. The loaded cartridges were then eluted with 5 mL of methanol. This extraction procedure concentrates the samples by a factor of eight. (Initial volume = 40 mL, final volume = 5 mL).

Samples were initially extracted on January 11, 2006 and analyzed on January 14, 2006. Because associated laboratory quality control samples did not meet method criteria, samples were reextracted along with a new calibration curve and all associated quality control samples on January 23, 2006. The second set of extracts were analyzed on January 26, 2006. All results reported here are from the second extraction set.

Analysis

All sample and quality control extracts were analyzed for PFBS, PFHS, and PFOS 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	Two Betasil C18 (2.1 mm X 100 mm), 5 µm linked in series before the injection port
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	150
	299/99	150
[1]PFHS	399/130	150
	399/99	150
	399/80	150
[1]PFOS	499/130	150
	499/99	150
	499/80	150

(1) The individual transitions were summed to produce a "total ion chromatogram" (TIC) for the given analyte. The resultant TIC was used for quantitation.

ANALYTICAL RESULTS

Calibration

Calibration standards were prepared by spiking known amounts of stock solutions containing the target analytes into 40 mL of ASTM type I water. Each spiked water standard was then extracted in the same manner as the collected samples. A total of twelve spiked standards ranging from 0.025 ng/mL to 25 ng/mL (nominal) were prepared. A quadratic, 1/x weighted, calibration curve was used to fit the data. 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 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% (100±30% for the LOQ standard). The correlation coefficient was greater than 0.999 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 at least twice those of the method blank(s). This will be presented and discussed in more detail in the section below regarding method blanks. The LOQ for PFBS, PFHS, and PFOS was 0.0248 ng/mL, 0.0252 ng/mL, and 0.0249 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 Blank

Several methanol solvent blanks were analyzed to assess system contamination and/or instrument carryover. One methanol solvent blank analyzed immediately after the highest calibration standard produced PFOS area counts greater than half the LOQ standard. However, the solvent blank analyzed immediately after the high blank demonstrated that the likely instrument carryover observed in the preceding injection had diminished. Analyte peak area counts in all other solvent blank samples were less than half the area counts of the calibration standard used to establish the LOQ for all analytes.

Method Blank

Eight method blanks were prepared by loading 40 mL of ASTM Type I water onto a C18 SPE cartridge and eluting with 5 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 PFOA area counts for the method blanks and the corresponding LOQ standard.

Table 8. Method Blank Area Counts.

		PFBS	PFHS	PFOS
Sample ID	*Sample Comment*	*Area Counts*		
MB-060123-1	Method Blank-1	7923	8472	21276
MB-060123-2	Method Blank-2	18778	25105	25856
MB-060123-3	Method Blank-3	8061	8900	16771
MB-060123-4	Method Blank-4	6404	11382	19740
MB-060123-5	Method Blank-5	5688	7332	(1)55137
MB-060123-6	Method Blank-6	14035	13232	21206
MB-060123-7	Method Blank-7	5693	4863	15055
MB-060123-8	Method Blank-8	4299	7087	18065
Area counts of the highest method blank multiplied by two		37556	50210	(2)51712
Area Counts of the LOQ standard		179926	162287	102452

(1) PFOS area counts for Method Blank-5 determined to be a statistical outlier using Dixon's Q-test.

(2) Highest method blank area count for PFOS is 25856 (Method Blank-2) when Method Blank-5 is excluded as on outlier.

Trip Blank

Prior to sample collection, one sample container was filled with 450 mL of ASTM Type I water, 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 account for any storage conditions and/or holding time issues that the samples may experience. The resultant field blank concentrations for PFBS, PFHS, and PFOS were <0.0248 ng/mL, <0.0252 ng/mL, and <0.0249 ng/mL, respectively.

System Suitability

Five replicate injections of the 1.0 ppb (nominal concentration) extracted calibration standard were analyzed at the beginning and end of the analytical sequence to demonstrate overall system suitability. The relative standard deviation (RSD) of the peak area counts and the RSD of the peak retention times were less than 5% and 2%, respectively, for all analytes which met method criteria.

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 were still in control. ETS-8-154.1 states that all CCV recoveries must be within ±25% for the data to be acceptable. All analyte CCV recoveries met method criteria of ±25%.

Lab Control Spikes (LCSs)

Replicate low (0.05 ng/mL nominal concentration) and high (0.5 ng/mL nominal concentration) lab control spikes were prepared and extracted on the same day as the samples. LCSs were prepared by spiking known amounts of the analytes into 40 mL of ASTM Type I water to produce the desired concentration. The spiked water samples were extracted and analyzed in the same manner as the samples. Individual LCS results, along with the average and percent RSD, for the extraction set are presented in the data table below. All LCS recoveries met method criteria for both accuracy and precision.

Table 9. Lab Control Spike Results.

	[1]PFBS			[1]PFHS			[1]PFOS		
Lab ID	*Spiked Conc. (ng/mL)*	*Calc. Conc. (ng/mL)*	*% Recovery*	*Spiked Conc. (ng/mL)*	*Calc. Conc. (ng/mL)*	*% Recovery*	*Spiked Conc. (ng/mL)*	*Calc. Conc. (ng/mL)*	*% Recovery*
LCS-060123-1	0.0496	0.0469	94.6	0.0503	0.0459	91.2	0.0499	0.0453	90.8
LCS-060123-2	0.0496	0.0473	95.4	0.0503	0.0456	90.8	0.0499	0.0469	94.0
LCS-060123-3	0.0496	0.0483	97.4	0.0503	0.0465	92.4	0.0499	0.0429	86.0
LCS-060123-4	0.496	0.485	97.8	0.503	0.500	99.4	0.499	0.477	95.6
LCS-060123-5	0.496	0.473	95.4	0.503	0.472	93.8	0.499	0.462	92.6
LCS-060123-6	0.496	0.497	100	0.503	0.488	97.0	0.499	0.475	95.2
Average %Recovery ± %RSD	*96.8% ± 2.2%*			*94.1% ± 3.6%*			*92.3%±3.9%*		

(1) Table displays rounded values for all concentration and percent recovery values (3 significant figures) and %RSD (2 significant figures) using EPA rounding rules. Reported values may vary slightly from the raw data.

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 LCSs.

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 100±30% 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 AND DISCUSSION

Table 10 below summarizes the sample results and field matrix spike recoveries for all of the sampling locations plus the trip blank. All field matrix spikes produced recoveries within 100±30% demonstrating that the analytical method was appropriate for the matrices analyzed.

Table 10. Sample and QC Results.

Location #1 DPWS PW FW01		[1]PFBS		[2]PFHS		[3]PFOS	
3M LIMS ID	Description	Conc. (ng/mL)	%Recovery	Conc. (ng/mL)	%Recovery	Conc. (ng/mL)	%Recovery
E05-0210-92250	DPWS PW FW02 0 051213	<0.0248	NA	<0.0252	NA	<0.0249	NA
E05-0210-92251	DPWS PW FW02 DB 051213	<0.0248	NA	<0.0252	NA	<0.0249	NA
E05-0210-92252	DPWS PW FW02 LS 051213	0.0949	95.6	0.0971	96.5	0.0853	85.5
E05-0210-92253	DPWS PW FW02 HS 051213	0.978	98.6	1.02	101	0.918	92.0
Average Sample Concentration (ng/mL) ±%RPD[4]		<0.0248		<0.0252		<0.0249	

Location #2 WMEL PW FW01		[1]PFBS		[2]PFHS		[3]PFOS	
3M LIMS ID	Description	Conc. (ng/mL)	%Recovery	Conc. (ng/mL)	%Recovery	Conc. (ng/mL)	%Recovery
E05-0210-92254	WMEL PW FW02 0 051213	0.0350	NA	<0.0252	NA	0.0367	NA
E05-0210-92255	WMEL PW FW02 DB 051213	0.0321	NA	<0.0252	NA	0.0367	NA
E05-0210-92256	WMEL PW FW02 LS 051213	0.130	97.2	0.0990	98.8	0.121	84.5
E05-0210-92257	WMEL PW FW02 HS 051213	1.02	99.4	0.950	94.4	0.883	84.8
Average Sample Concentration (ng/mL) ±%RPD[4]		0.0336 ±8.6%		<0.0252		0.0367 ±0.0%	

Location #3 MSTP PW FW01		[1]PFBS		[2]PFHS		[3]PFOS	
3M LIMS ID	Description	Conc. (ng/mL)	%Recovery	Conc. (ng/mL)	%Recovery	Conc. (ng/mL)	%Recovery
E05-0210-92262	MSTP PW FW02 0 051214	<0.0248	NA	<0.0252	NA	<0.0249	NA
E05-0210-92263	MSTP PW FW02 DB 051214	<0.0248	NA	<0.0252	NA	<0.0249	NA
E05-0210-92264	MSTP PW FW02 LS 051214	0.115	116	0.0930	92.3	0.0922	92.4
E05-0210-92265	MSTP PW FW02 HS 051214	0.934	94.1	0.958	95.2	0.826	82.8
Average Sample Concentration (ng/mL) ±%RPD[4]		<0.0248		<0.0252		<0.0249	

Table 10 Continued.

Location #4 FWTP PW FW01		*(1)PFBS*		*(2)PFHS*		*(3)PFOS*	
3M LIMS ID	*Description*	*Conc. (ng/mL)*	*%Recovery*	*Conc. (ng/mL)*	*%Recovery*	*Conc. (ng/mL)*	*%Recovery*
E05-0210-92266	FWTP PW FW02 0 051214	<0.0248	NA	<0.0252	NA	<0.0249	NA
E05-0210-92267	FWTP PW FW02 DB 051214	<0.0248	NA	<0.0252	NA	<0.0249	NA
E05-0210-92268	FWTP PW FW02 LS 051214	0.118	119	0.0900	89.3	0.1	100
E05-0210-92269	FWTP PW FW02 HS 051214	0.962	96.9	0.927	92.1	0.811	81.3
Average Sample Concentration (ng/mL) ± %RPD(4)		*<0.0248*		*<0.0252*		*<0.0249*	

Location #5 TVATP PW FW01		*(1)PFBS*		*(2)PFHS*		*(3)PFOS*	
3M LIMS ID	*Description*	*Conc. (ng/mL)*	*%Recovery*	*Conc. (ng/mL)*	*%Recovery*	*Conc. (ng/mL)*	*%Recovery*
E05-0210-92270	TVATP PW FW02 0 051214	0.0344	NA	<0.0252	NA	<0.0249	NA
E05-0210-92271	TVATP PW FW02 DB 051214	0.0347	NA	<0.0252	NA	<0.0249	NA
E05-0210-92272	TVATP PW FW02 LS 051214	0.132	98.2	0.0909	90.4	0.104	104
E05-0210-92273	TVATP PW FW02 HS 051214	0.910	88.2	0.900	89.5	0.868	87.0
Average Sample Concentration (ng/mL) ± %RPD(4)		*0.0346±0.87%*		*<0.0252*		*<0.0249*	

		(1)PFBS		*(2)PFHS*		*(3)PFOS*	
3M LIMS ID	*Description*	*Conc. (ng/mL)*	*%Recovery*	*Conc. (ng/mL)*	*%Recovery*	*Conc. (ng/mL)*	*%Recovery*
E05-0210-92258	SWTP PW FW02 0 051214	<0.0248	NA	<0.0252	NA	<0.0249	NA
E05-0210-92259	SWTP PW FW02 DB 051214	<0.0248	NA	<0.0252	NA	<0.0249	NA
E05-0210-92260	SWTP PW FW02 LS 051214	0.110	111	0.0836	83.1	0.0917	91.9
E05-0210-92261	SWTP PW FW02 HS 051214	0.91	91.5	0.927	92.1	0.837	83.9
Average Sample Concentration (ng/mL) ± %RPD(4)		*<0.0248*		*<0.0252*		*<0.0249*	

Table 10 Continued.

Trip Blank		*(1)PFBS*		*(2)PFHS*		*(3)PFOS*	
3M LIMS ID	*Description*	*Conc. (ng/mL)*	*%Recovery*	*Conc. (ng/mL)*	*%Recovery*	*Conc. (ng/mL)*	*%Recovery*
E05-0210-92247	DPWS PW TRIP 0 051213	< 0.0248	NA	<0.0252	NA	< 0.0249	NA
E05-0210-92248	DPWS PW TRIP LS 051213	0.0921	92.8	0.0921	91.6	0.0837	83.9
E05-0210-92249	DPWS PW TRIP HS 051213	0.946	95.3	0.977	97.1	0.898	90.0

(1) The analytical uncertainty of the PFBS resuts is 100±5.3% based on method accuracy and precision. All results and averages are presented with three significant figures, %RPD to two significant figures. Sample concentrations, averages, and %RPD values may vary slightly from the raw data. Samples with reported results less than the LOQ of 0.0248 ng/mL may or may not have PFBS present. Results less than the LOQ are not quantified.

(2) The analytical uncertainty of the PFHS resuts is 100±9.3% based on method accuracy and precision. All results and averages are presented with three significant figures, %RPD to two significant figures. Sample concentrations, averages, and %RPD values may vary slightly from the raw data. Samples with reported results less than the LOQ of 0.0252 ng/mL may or may not have PFHS present. Results less than the LOQ are not quantified. .

(3) The analytical uncertainty of the PFOS resuts is 100±11% based on method accuracy and precision. All results and averages are presented with three significant figures, %RPD to two significant figures. Sample concentrations, averages, and %RPD values may vary slightly from the raw data. Samples with reported results less than the LOQ of 0.0249 ng/mL may or may not have PFOS present. Results less than the LOQ are not quantified.

(4) NA: Not applicable. %RPD values not determined when concentrations for the sample and sample duplicate are below the stated LOQ.

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

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

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

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

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

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. The measured precision (%RSD) is then used to determine the range of the accuracy. The following example demonstrates how the analytical uncertainty, as defined here, is calculated for PFOS.

Example:

First, the accuracy range is determined by multiplying the average recovery by the precision.

92.35 (average accuracy)*0.0389 (precision) = 3.59 (accuracy range)

Next, the accuracy range is added and subtracted from the average accuracy.

92.35 + 3.59 = 95.94; 92.35 –3.59 = 88.76

Thus, LCS accuracy results range from 88.76% to 95.94%. The absolute difference of the low and high ends of this range, when compared 100%, are then calculated.

100%-95.94 = 4.06%

100%-88.76%= 11.23%.

The most conservative (largest) absolute difference is then used as the analytical uncertainty for the given analyte. Therefore, the analytical uncertainty for PFOS as defined here, is given as 100±11% (2 significant figures) for the data associated with this project.

STATEMENT OF CONCLUSION

Sample results for this project were presented in Table 1. Laboratory control spikes were used to determine the method accuracy and precision for the target analytes for this data set. The accuracy and precision were then used to estimate the analytical uncertainty for each of the analytes (PFBS: 100±5.3%; PFHS: 100±9.3%, and PFOS: 100±11%). Recoveries of field matrix spiked samples demonstrated that the overall analytical method was appropriate for the matrix collected.

REFERENCES

Rorabacher, David B. "Statistical Treatment for Rejection of Deviant Values: Critical Values of Dixon's 'Q' Parameter and Related Subrange Ratios at the 95% Confidence Level." *Anal. Chem.* **1991**, *63*, 139-146.

LIST OF ATTACHMENTS

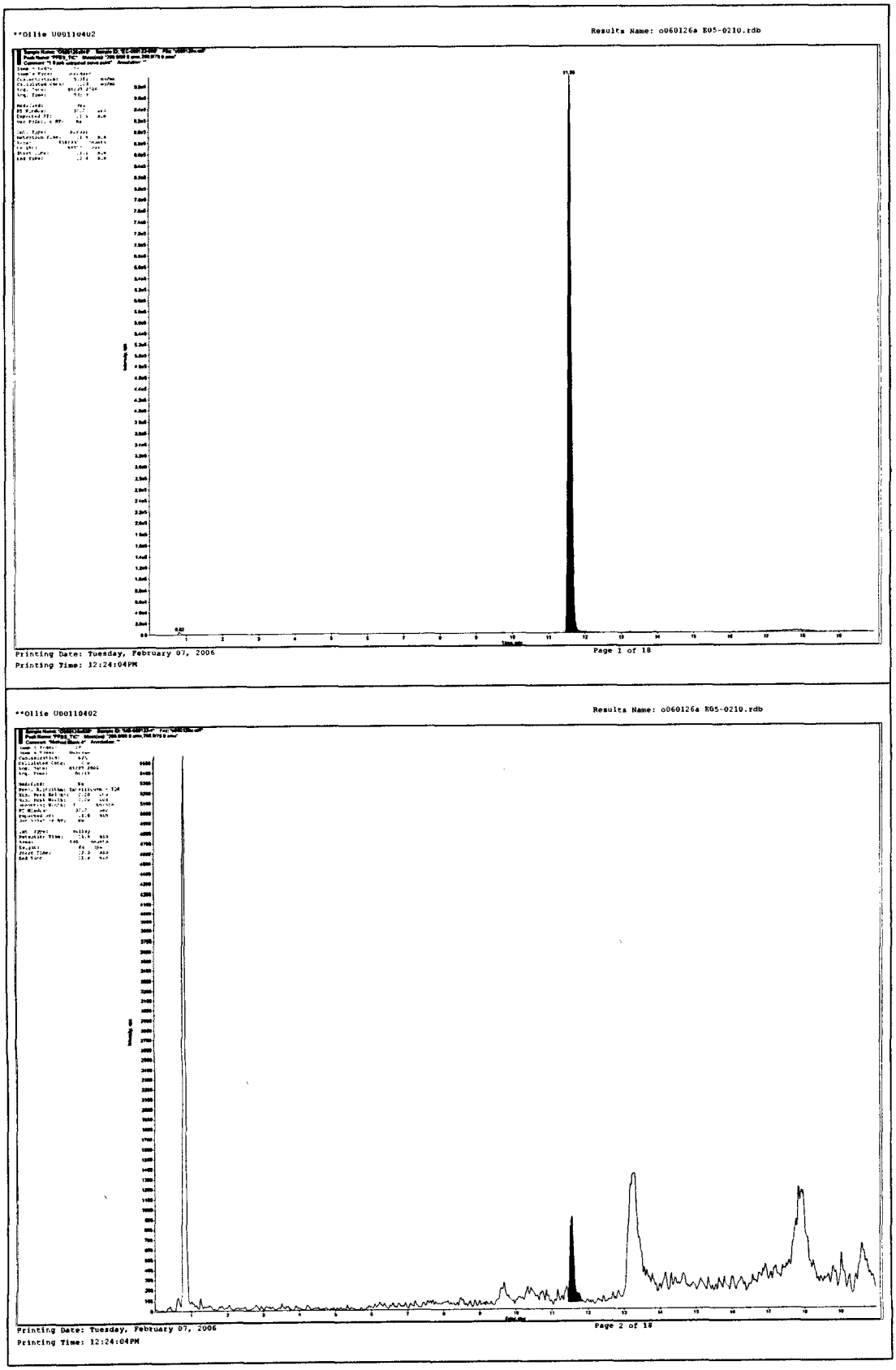
- **Attachment A: Selected Chromatograms and Calibration Curves**
- **Attachment B: Extraction and Analytical Methods**
- **Attachment C: Protocol and Protocol Amendments**

SIGNATURE PAGE

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

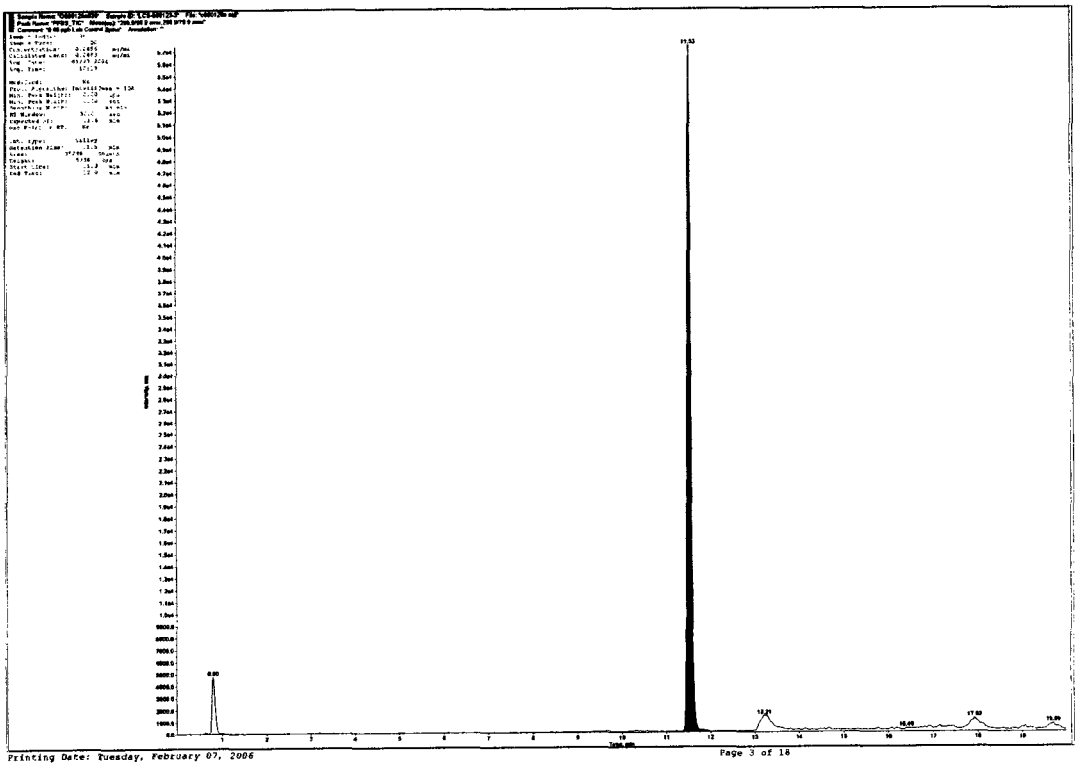
Jaisimha Kesari, P.E., DEE ,	Study Director	3/21/06 Date
Michelle D. Malinsky, Ph.D.,	3M Senior Chemist	3/17/2006 Date
William K. Reagen, Ph.D.,	3M Environmental Laboratory Manager	03/20/06 Date
Michael A. Santoro,	Sponsor Representative	3/23/06 Date

ATTACHMENT A: SAMPLE CHROMATOGRAMS AND CALIBRATION CURVES


**Ollie U00110402
Results Name: o060126a E05-0210.rdb
Printing Date: Tuesday, February 07, 2006
Printing Time: 12:24:04PM
Page 1 of 18
**Ollie U00110402
Results Name: o060126a E05-0210.rdb
Printing Date: Tuesday, February 07, 2006
Printing Time: 12:24:04PM
Page 2 of 18

**Ollie U00110402

Results Name: o060126a E05-0210.rdb

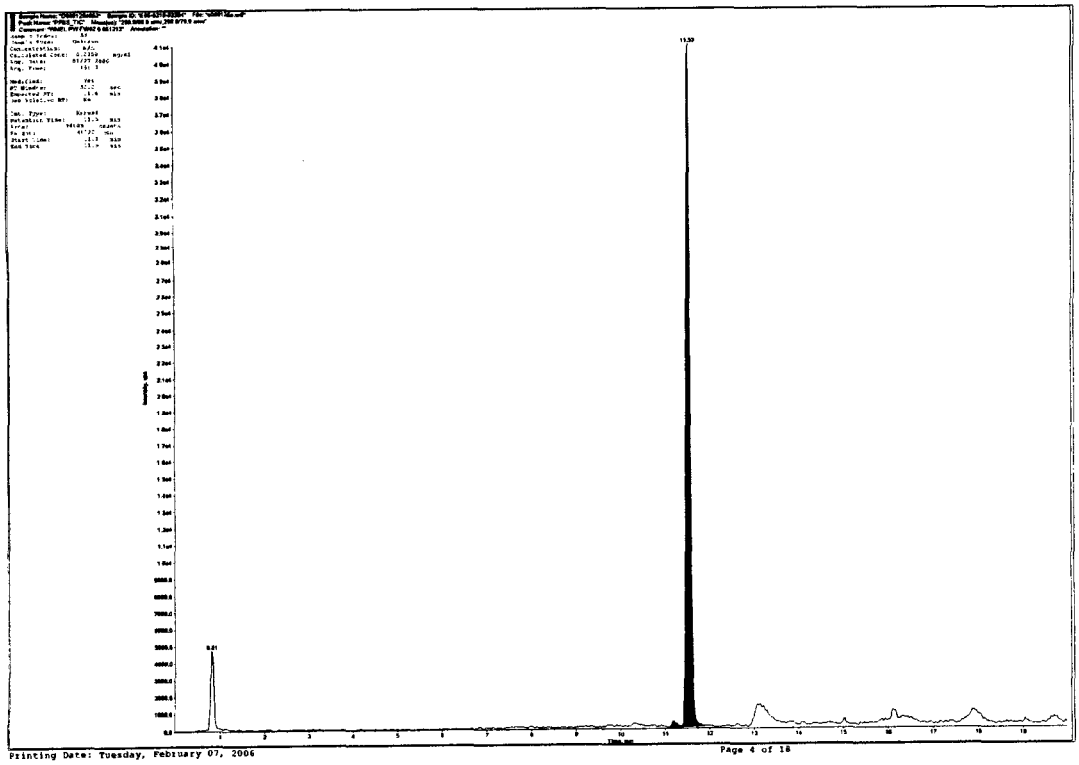


Printing Date: Tuesday, February 07, 2006
Printing Time: 12:24:04PM

Page 3 of 18

**Ollie U00110402

Results Name: o060126a E05-0210.rdb

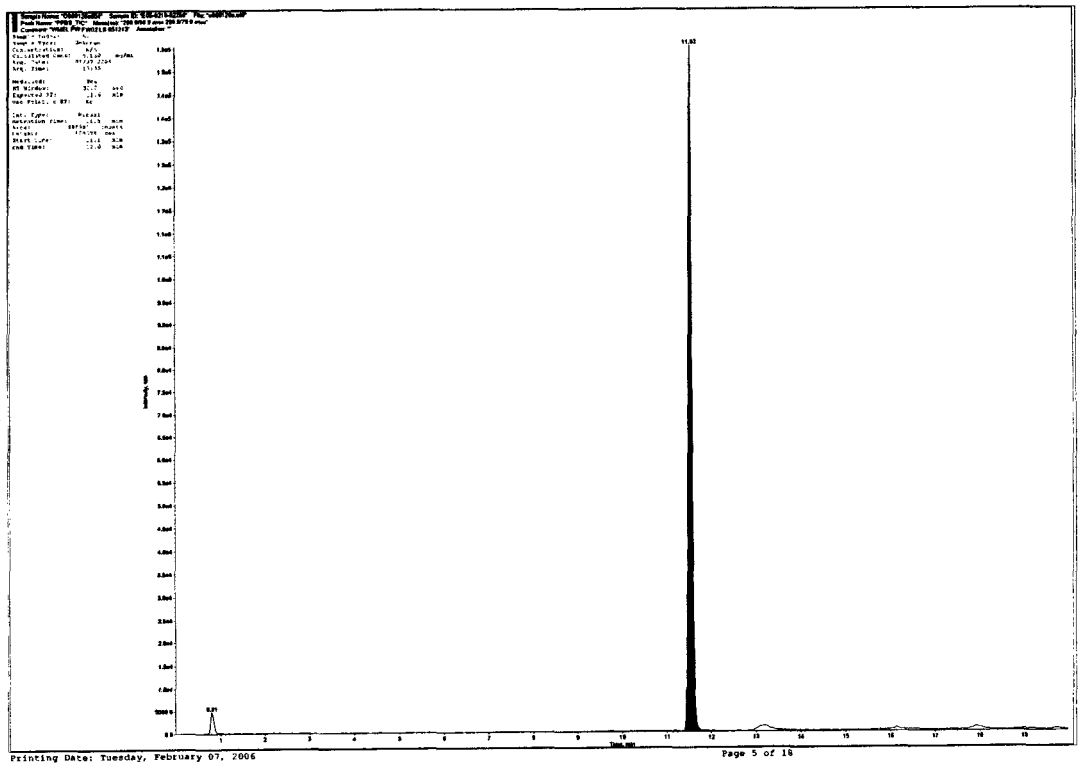


Printing Date: Tuesday, February 07, 2006
Printing Time: 12:24:04PM

Page 4 of 18

**Ollie U00110402

Results Name: o060126a E05-0210.rdb

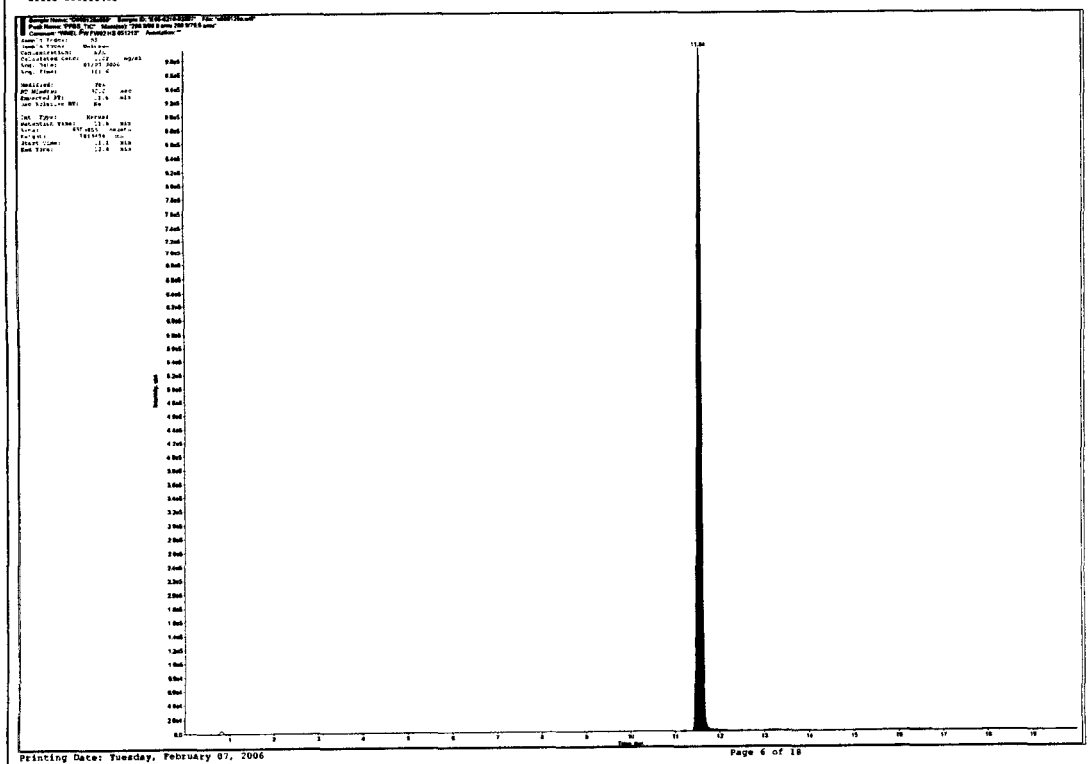


Printing Date: Tuesday, February 07, 2006
Printing Time: 12:24:04PM

Page 5 of 18

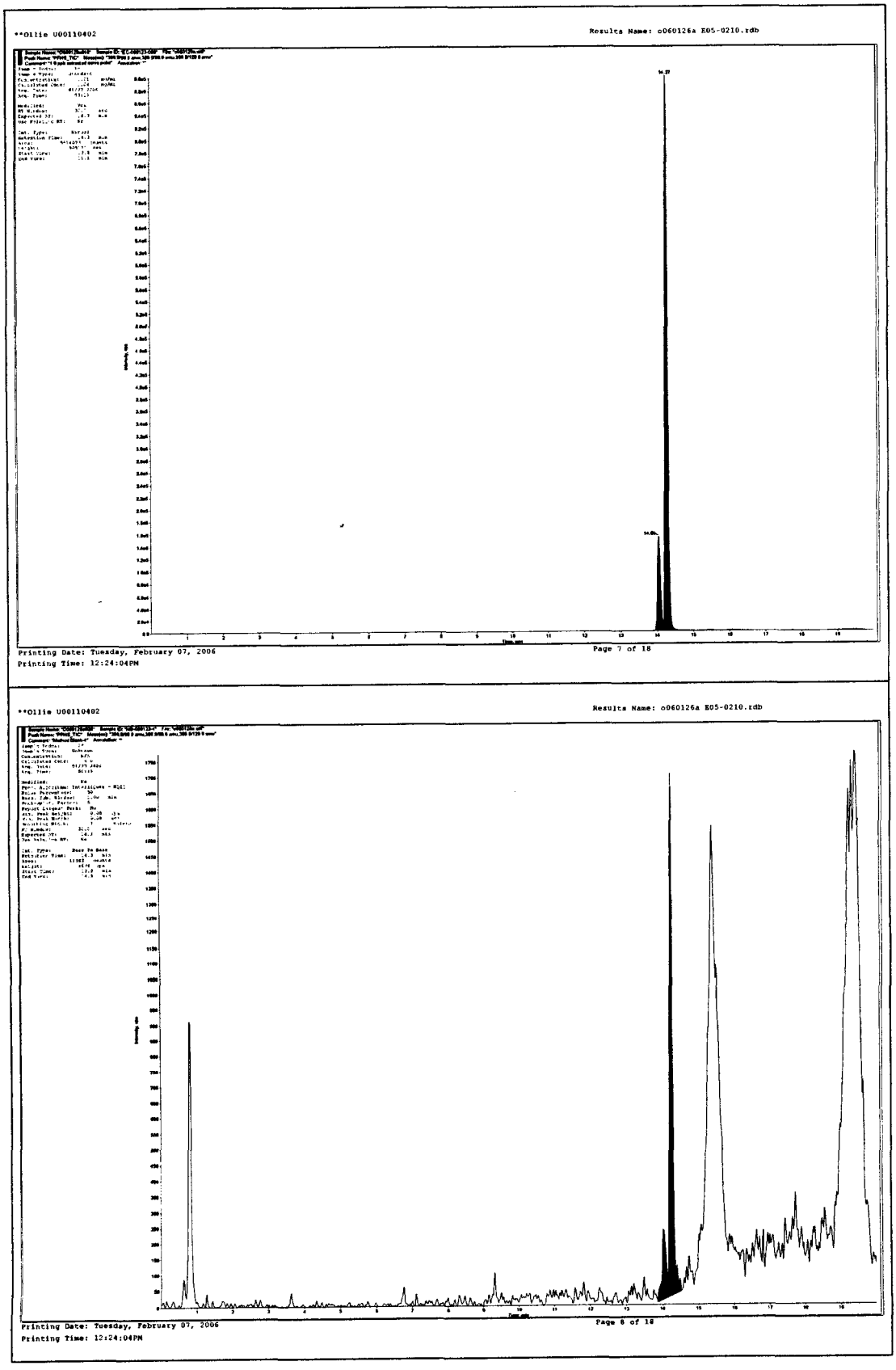
**Ollie U00110402

Results Name: o060126a E05-0210.rdb

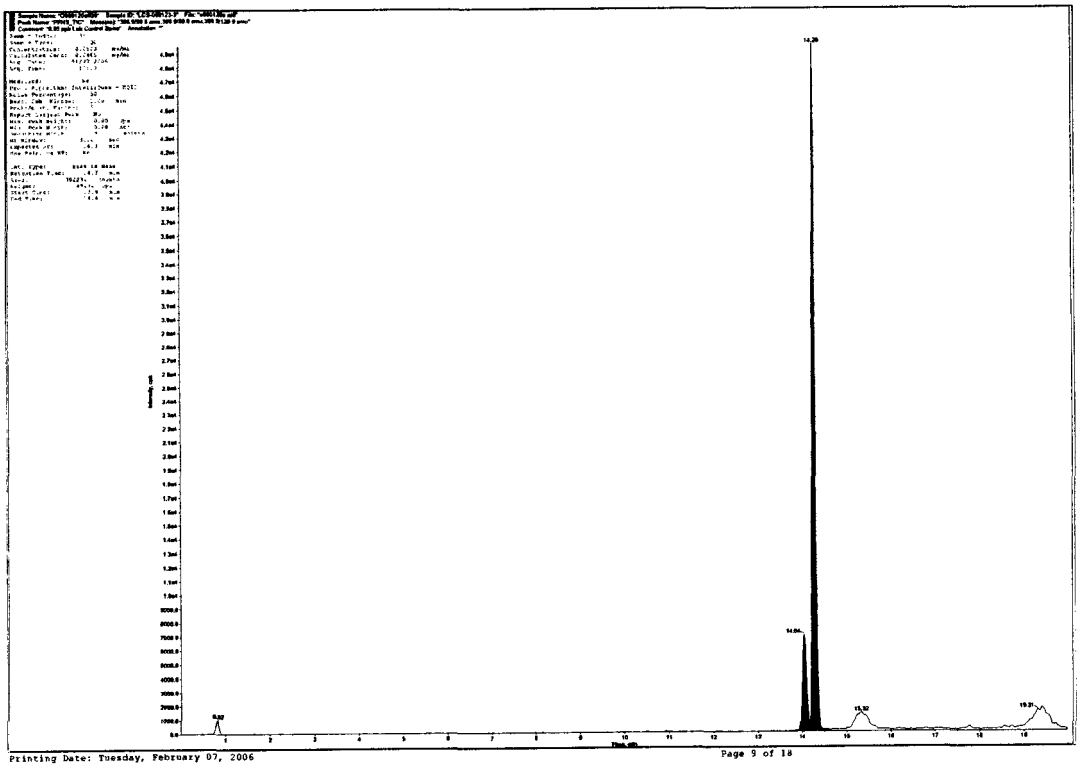


Printing Date: Tuesday, February 07, 2006
Printing Time: 12:24:04PM

Page 6 of 18

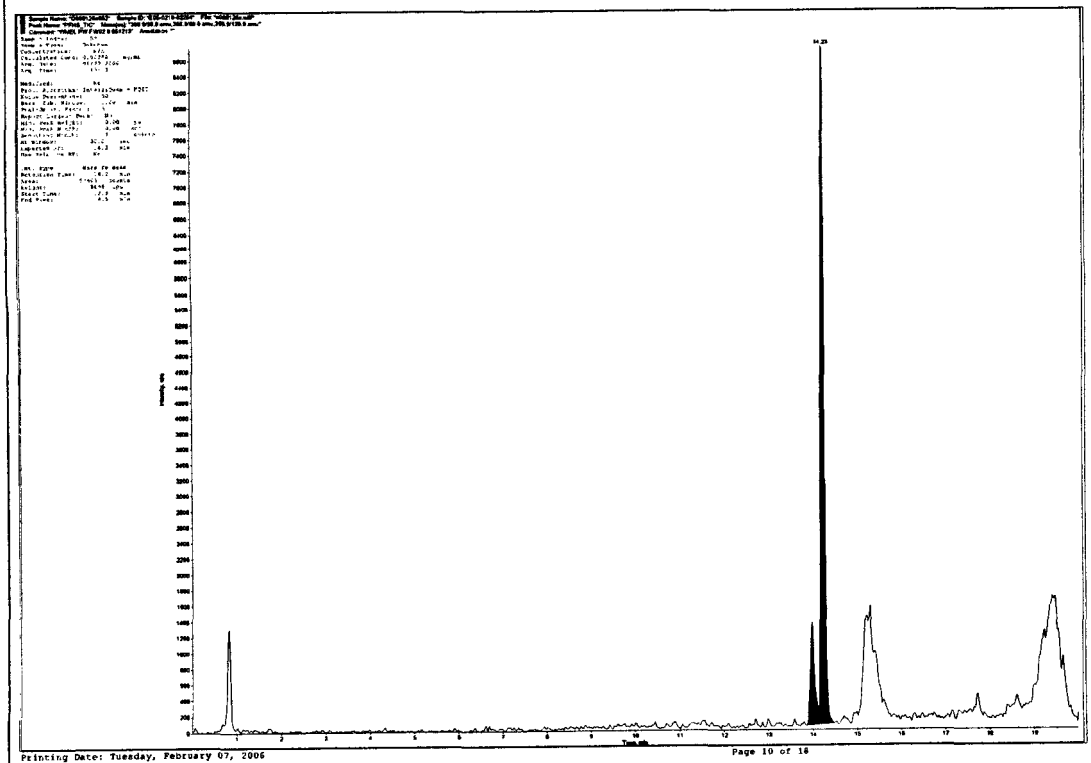

**Ollie U00110402
Results Name: o060126a E05-0210.rdb
Printing Date: Tuesday, February 07, 2006
Printing Time: 12:24:04PM
Page 7 of 18
**Ollie U00110402
Results Name: o060126a E05-0210.rdb
Printing Date: Tuesday, February 07, 2006
Printing Time: 12:24:04PM
Page 8 of 18

**Ollie U00110402 Results Name: o060126a E05-0210.rdb



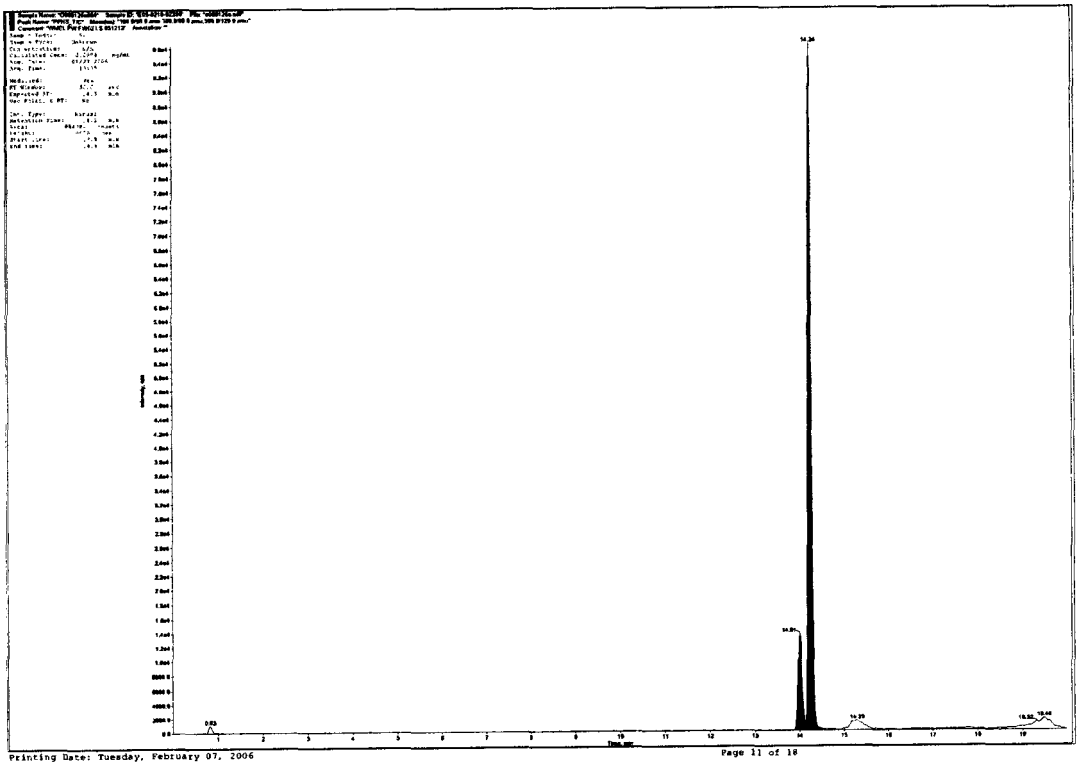
Printing Date: Tuesday, February 07, 2006
Printing Time: 12:24:04PM
Page 9 of 18

**Ollie U00110402 Results Name: o060126a E05-0210.rdb



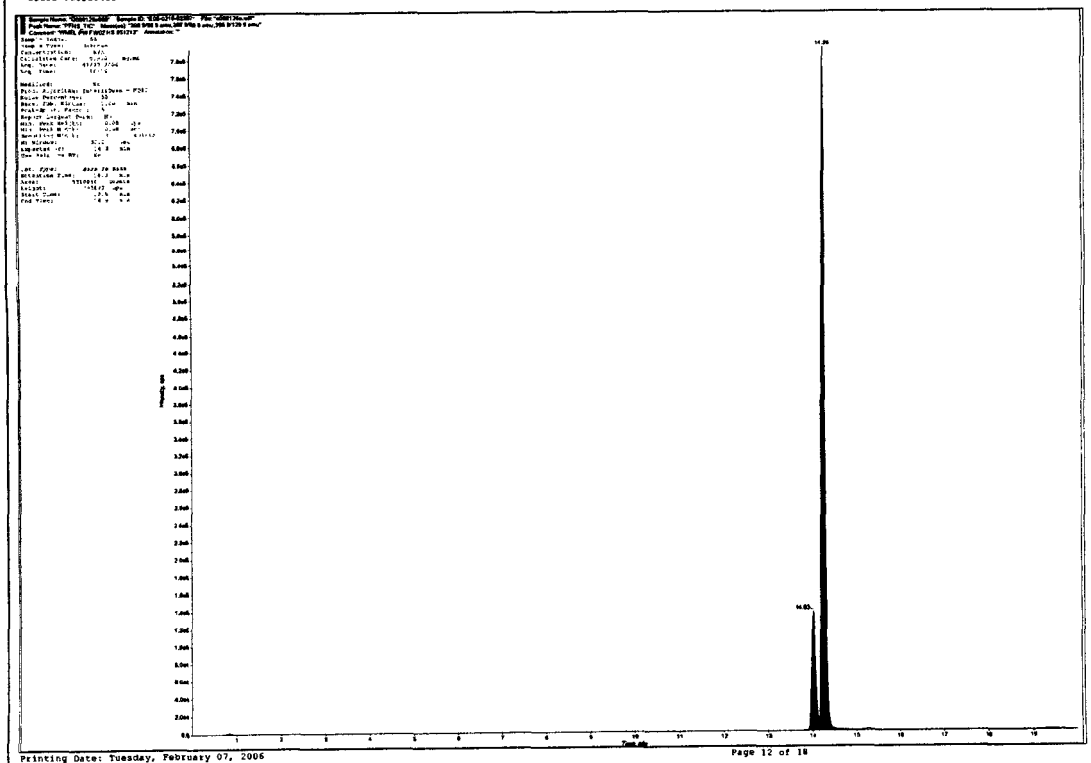
Printing Date: Tuesday, February 07, 2006
Printing Time: 12:24:04PM
Page 10 of 18

**Ollie U00110402 Results Name: o060126a E05-0210.rdb

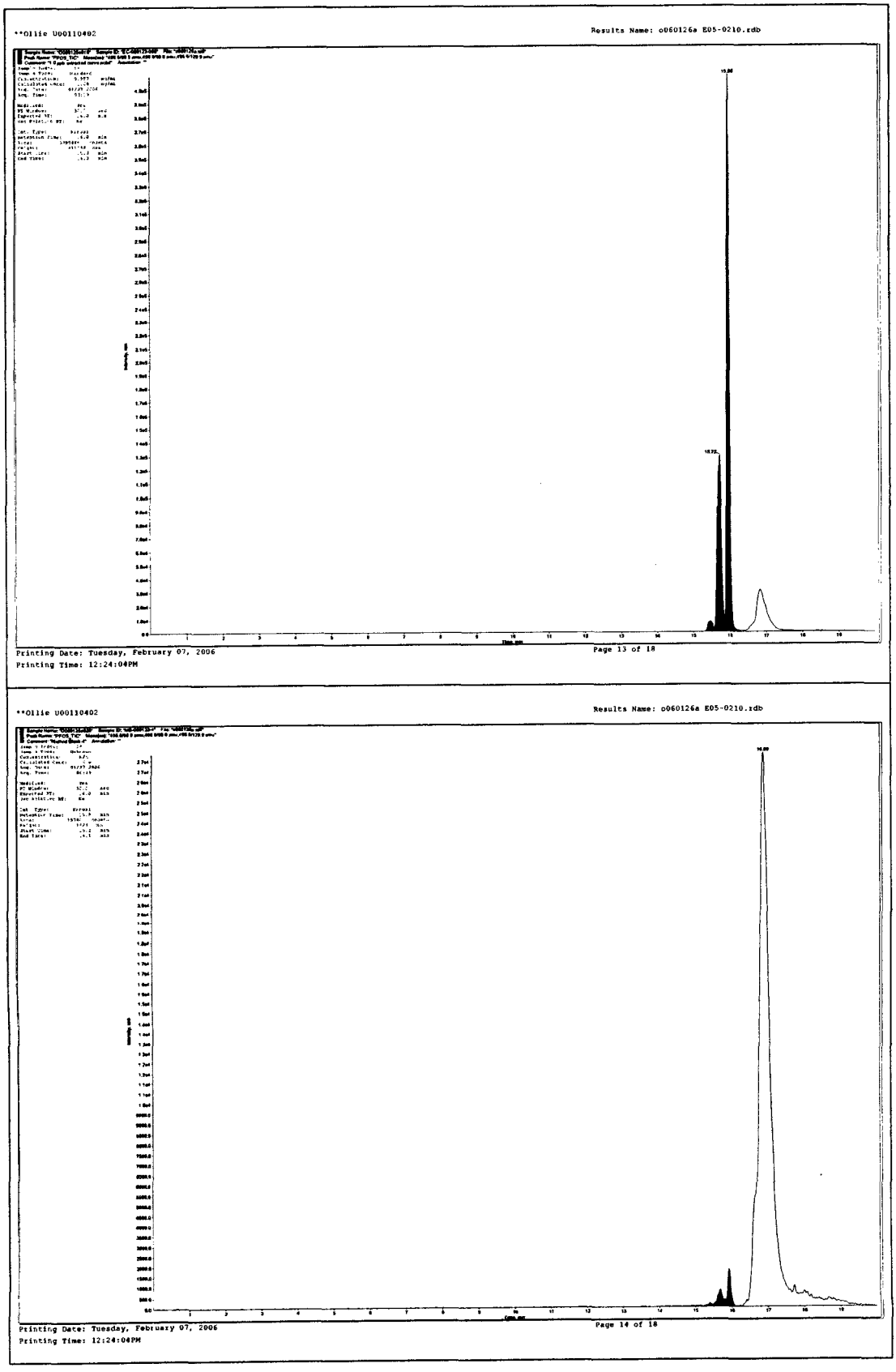


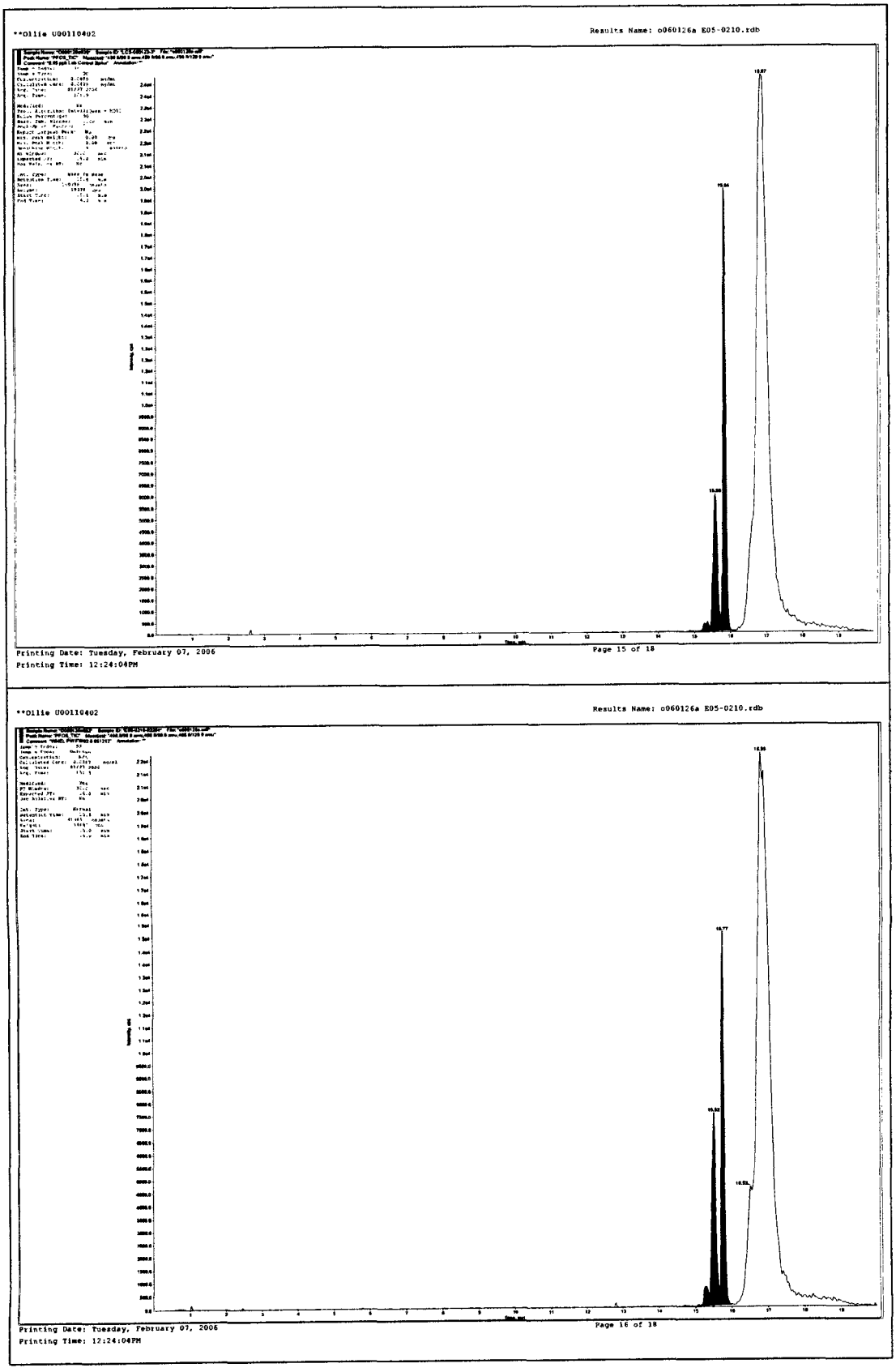
Printing Date: Tuesday, February 07, 2006 Page 11 of 18
Printing Time: 12:24:04PM

**Ollie U00110402 Results Name: o060126a E05-0210.rdb



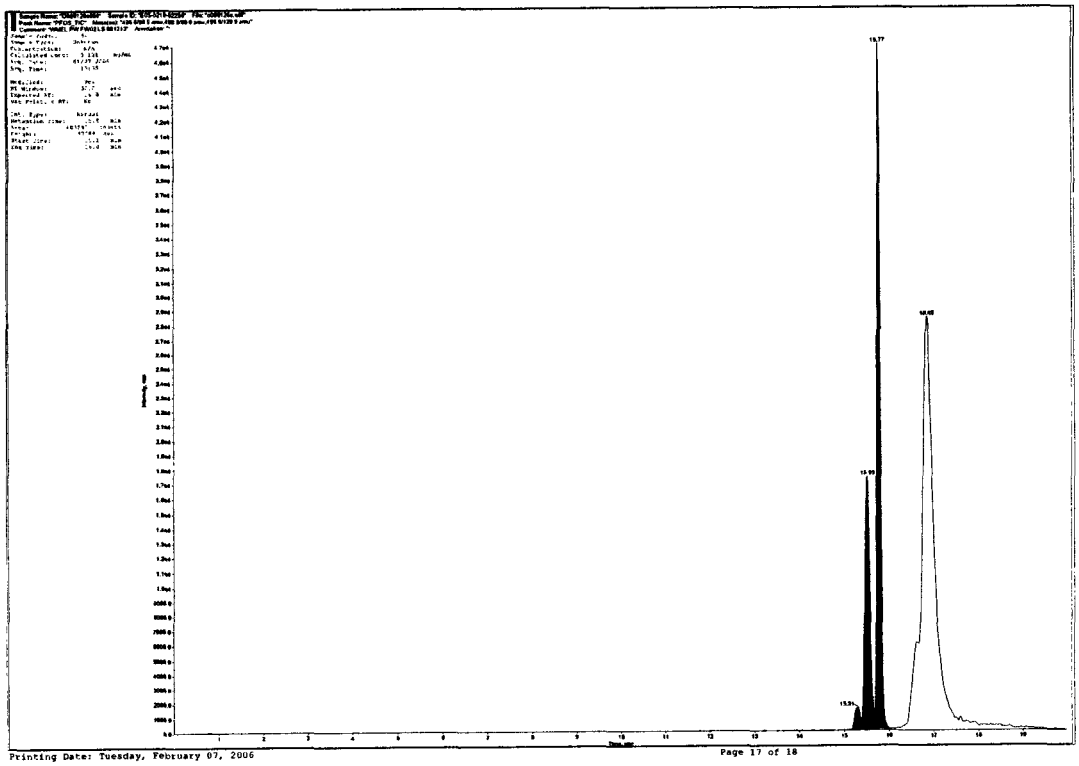
Printing Date: Tuesday, February 07, 2006 Page 12 of 18
Printing Time: 12:24:04PM


**Ollie U00110402
Results Name: o060126a E05-0210.rdb
Printing Date: Tuesday, February 07, 2006
Printing Time: 12:24:04PM
Page 13 of 18
**Ollie U00110402
Results Name: o060126a E05-0210.rdb
Printing Date: Tuesday, February 07, 2006
Printing Time: 12:24:04PM
Page 14 of 18


**Ollie U00110402
Results Name: o060126a E05-0210.rdb
Printing Date: Tuesday, February 07, 2006
Printing Time: 12:24:04PM
Page 15 of 18
**Ollie U00110402
Results Name: o060126a E05-0210.rdb
Printing Date: Tuesday, February 07, 2006
Printing Time: 12:24:04PM
Page 16 of 18

**Ollie U00110402

Results Name: o060126a E05-0210.rdb

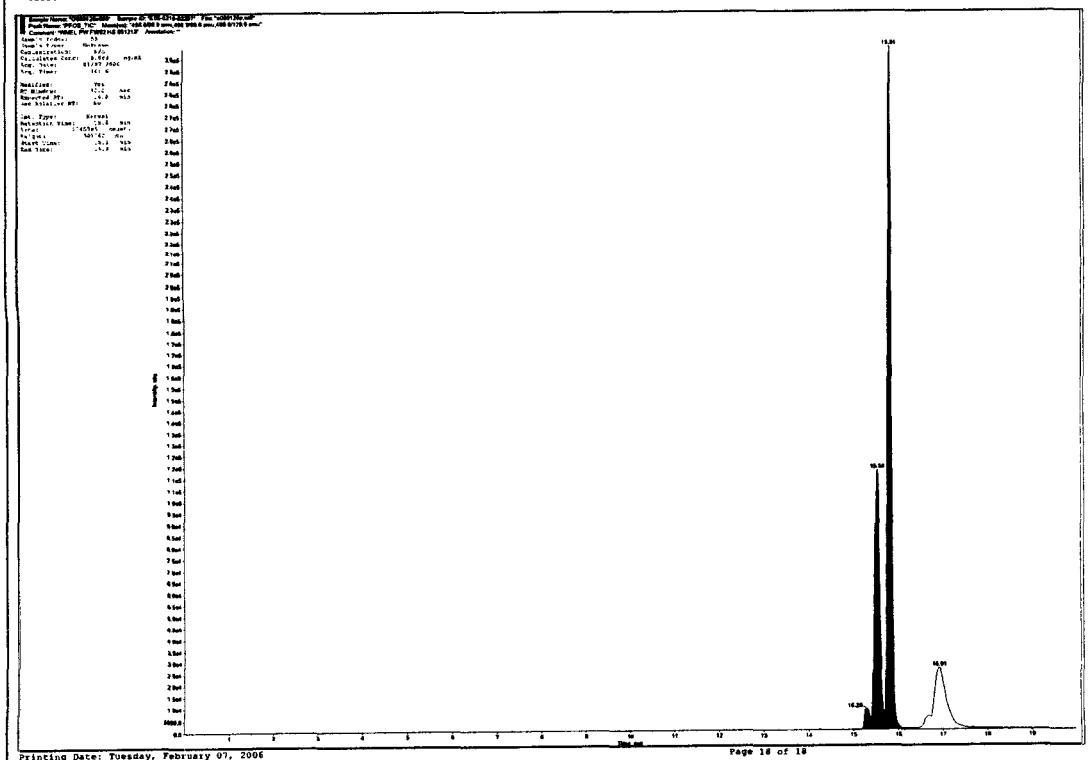


Printing Date: Tuesday, February 07, 2006
Printing Time: 12:24:04PM

Page 17 of 18

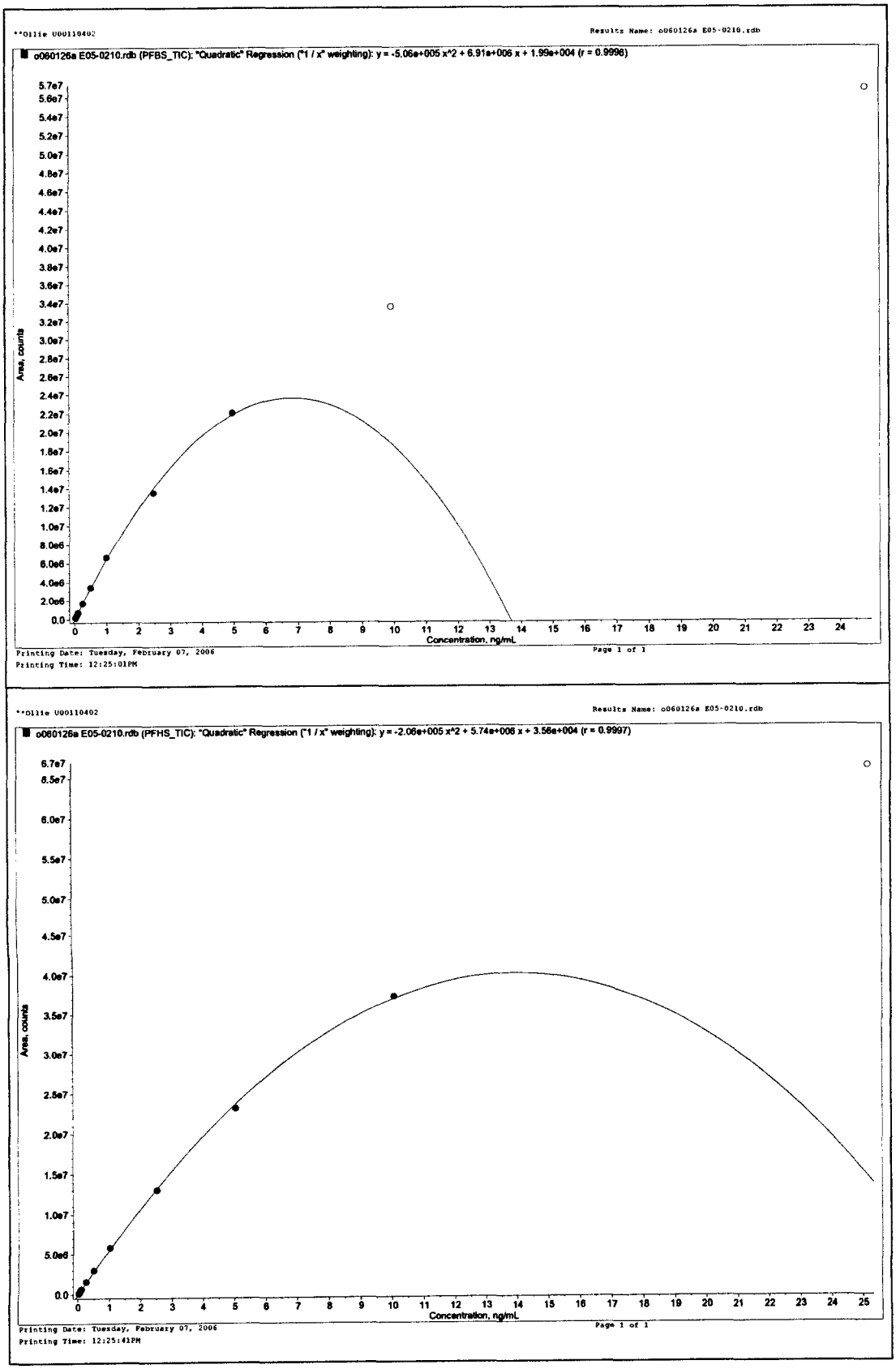
**Ollie U00110402

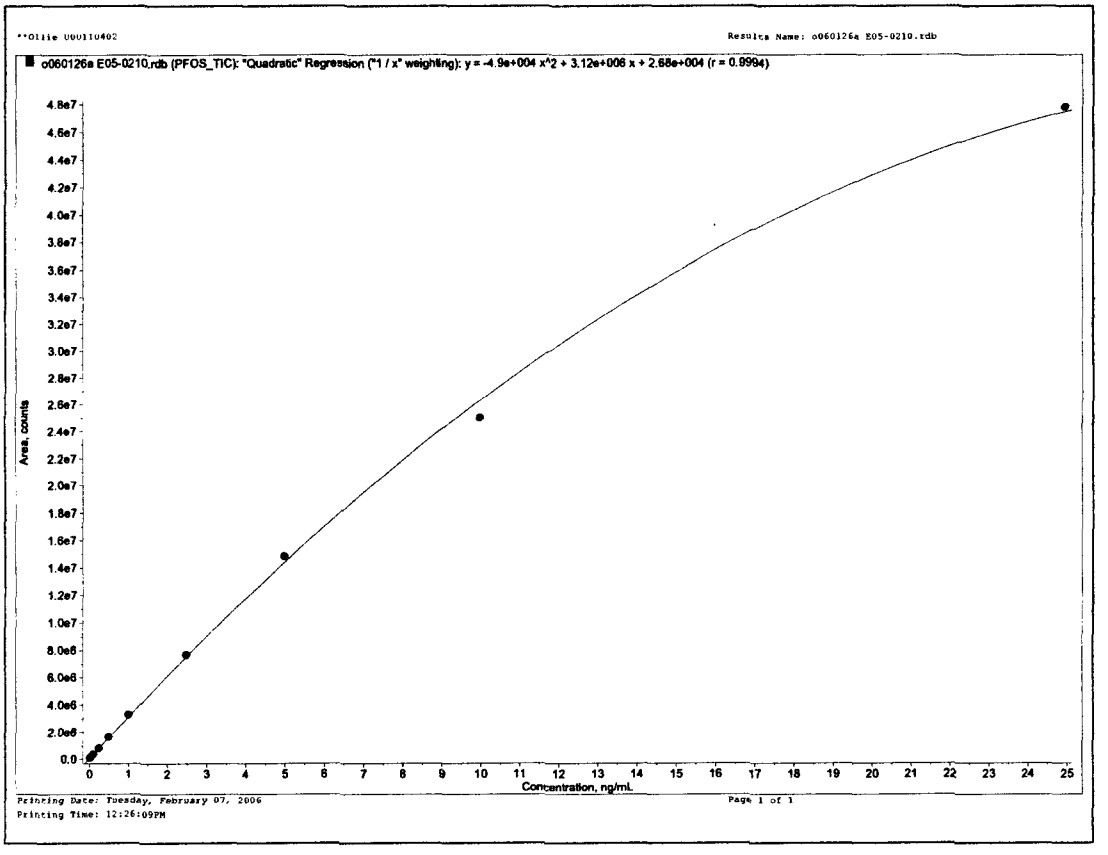
Results Name: o060126a E05-0210.rdb



Printing Date: Tuesday, February 07, 2006
Printing Time: 12:24:04PM

Page 18 of 18


**Ollie U00110402
Results Name: o060126a E05-0210.rdb
o060126a E05-0210.rdb (PFBS_TIC): "Quadratic" Regression ("1 / x" weighting): y = -5.06e+005 x^2 + 6.91e+006 x + 1.99e+004 (r = 0.9996)
Area, counts
Concentration, ng/mL
Printing Date: Tuesday, February 07, 2006
Printing Time: 12:25:01PM
Page 1 of 1
**Ollie U00110402
Results Name: o060126a E05-0210.rdb
o060126a E05-0210.rdb (PFHS_TIC): "Quadratic" Regression ("1 / x" weighting): y = -2.06e+005 x^2 + 5.74e+006 x + 3.56e+004 (r = 0.9997)
Area, counts
Concentration, ng/mL
Printing Date: Tuesday, February 07, 2006
Printing Time: 12:25:41PM
Page 1 of 1


**Ollie 000110402
Results Name: o060126a E05-0210.rdb
o060126a E05-0210.rdb (PFOS_TIC): "Quadratic" Regression ("1 / x" weighting): y = -4.9e+004 x^2 + 3.12e+006 x + 2.88e+004 (r = 0.9994)
Area, counts
Concentration, ng/mL
Printing Date: Tuesday, February 07, 2006
Printing Time: 12:26:09PM
Page 1 of 1

ATTACHMENT B: EXTRACTION AND ANALYTICAL METHODS

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

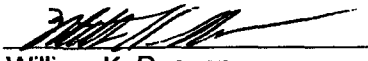
Method Number: ETS-8-154.1

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

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

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.

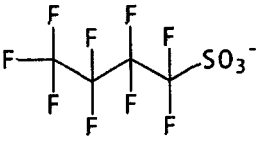
The study will be audited for compliance with EPA TSCA Good Laboratory Practice Standards 40 CFR 792 by the Quality Assurance Unit of Exygen Research.

TEST MATERIALS

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

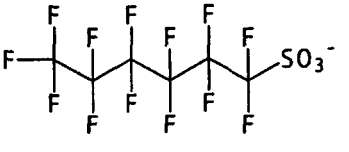
PFBS

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

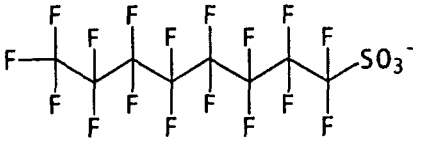
Chemical Name: Perfluorohexanesulfonate
Molecular Weight: 438 supplied as the potassium salt ($C_6F_{13}SO_3^-K^+$)
Lot Number: SE036
Purity: 98.6%
Transitions Monitored: 399 → 80
Structure:



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

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

Exygen Protocol Number: P0001131

STUDY DIRECTOR

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

SPONSOR REPRESENTATIVE

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

PRINCIPAL INVESTIGATOR

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

PROPOSED EXPERIMENTAL START AND TERMINATION DATES

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

Exygen Protocol Number: P0001131

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).

Exygen Protocol Number: P0001131

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.

Exygen Protocol Number: P0001131

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.

Exygen Protocol Number: P0001131

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.

Exygen Protocol Number: P0001131

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.

Exygen Protocol Number: P0001131

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

Exygen Protocol Number: P0001131

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:

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

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

Exygen Protocol Number: P0001131

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

Recovery (%) =

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

Exygen Protocol Number: P0001131

ANALYTICAL METHOD
Method Number: V0001781

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

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

Approved By:

Paul Connolly
Technical Leader, LC-MS, Exygen Research

10/26/04
Date

John Flaherty
Vice President, Operations, Exygen Research

10/26/04
Date

Total Pages: 7

Exygen Protocol Number: P0001131

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.

Exygen Protocol Number: P0001131

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.

Exygen Protocol Number: P0001131

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.

Exygen Protocol Number: P0001131

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

Exygen Protocol Number: P0001131

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.

Exygen Protocol Number: P0001131

14.0 Calculations

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

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

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

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

Recovery (%) =

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

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

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

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

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

Exygen Protocol Number: P0001131

ANALYTICAL METHOD
Method Number: V0001782

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

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

Approved By:

Paul Connolly
Technical Leader, LC-MS, Exygen Research

10/26/04
Date

John Flaherty
Vice President, Operations, Exygen Research

10/26/04
Date

Total Pages: 7

Exygen Protocol Number: P0001131

Exygen Research | Method Number V0001782

ANALYTICAL METHOD

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

1.0 Scope

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

2.0 Safety

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

3.0 Sample Requirement

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

4.0 Reagents and Standards

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

5.0 Instrument and Equipment

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

Exygen Protocol Number: P0001131

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.

Exygen Protocol Number: P0001131

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

Exygen Protocol Number: P0001131

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.

Exygen Protocol Number: P0001131

ANALYTICAL METHOD

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

12.0 Chromatography

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

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

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

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

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

13.0 Acceptance Criteria

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

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

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

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

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

Exygen Protocol Number: P0001131

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.

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(%)]

Exygen Protocol Number: P0001131

ANALYTICAL METHOD
Method Number: V0001783

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

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

Approved By:

Paul Connolly
Technical Leader, LC-MS, Exygen Research

10/26/04
Date

John Flaherty
Vice President, Operations, Exygen Research

10/26/04
Date

Total Pages: 8

Exygen Protocol Number: P0001131

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.

Exygen Protocol Number: P0001131

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.

Exygen Protocol Number: P0001131

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.

Exygen Protocol Number: P0001131

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.

Exygen Protocol Number: P0001131

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.

Exygen Protocol Number: P0001131

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.

Exygen Protocol Number: P0001131

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

Recovery (%) =

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

Exygen Protocol Number: P0001131

ANALYTICAL METHOD
Method Number: V0001784

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

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

Approved By:

Paul Connolly
Technical Leader, LC-MS, Exygen Research

10/26/04
Date

John Flaherty
Vice President, Operations, Exygen Research

10/26/04
Date

Total Pages: 7

Exygen Protocol Number: P0001131

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.

Exygen Protocol Number: P0001131

ANALYTICAL METHOD

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

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.

Exygen Protocol Number: P0001131

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.

Exygen Protocol Number: P0001131

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.

Exygen Protocol Number: P0001131

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.

Exygen Protocol Number: P0001131

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

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

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

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

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

14.0 Calculations

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

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

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

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

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

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

Recovery (%) =

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

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

Exygen Protocol Number: P0001131

Exygen Research Method Number V0001785

ANALYTICAL METHOD

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

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.

Exygen Protocol Number: P0001131

ANALYTICAL METHOD

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

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.

Exygen Protocol Number: P0001131

8.0 Preparation of Solutions

8.1 Mobile Phase

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

Alternate volumes may be prepared.

9.0 Standard Preparation

9.1 Standard Stock/Fortification Solution

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

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

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

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

9.2 Standard Calibration Solutions

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

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

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

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

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

Exygen Protocol Number: P0001131

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

Exygen Protocol Number: P0001131

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:

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

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

Exygen Protocol Number: *P0001131*

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).

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

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.

Exygen Protocol Number: P0001131

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.

Exygen Protocol Number: P0001131

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.

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

11.0 Sample Extraction

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

12.0 Chromatography

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

Exygen Protocol Number: P0001131

13.0 Acceptance Criteria

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

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

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

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

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

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

14.0 Calculations

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

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

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

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

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

Exygen Protocol Number: P0001131

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 06/02/05
William K. Reagen, Ph.D.	3M Environmental Laboratory Manager	Date 05/17/05
Jaisimha Kesari, Weston Solutions,	Study Director	Date 5/26/05

3M Confidential

RECORD OF NONCONFORMANCE / DEVIATION

I. Identification

Study / Project No.	Date(s) of Occurrence:	Document Number:
E05-0210 Interim Report #18	12/9/2005-January 26, 2006	Exygen Protol #P0001131 Amendment #3 (3M)

Deviation type (*Check one*): ☐ SOP ☐ Equipment Procedure ☐ Method ☑ Protocol ☐ GPO ☐ Other:

II. Description

Protocol Requirements:

(3) ETS 8-154.1 makes no mention of sample surrogate spikes; however, all samples will be spiked with a radiolabeled surrogate, PFOA [1,2 ^{13}C]. 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.

Actual procedure/process:

PFOA[1,2 ^{13}C] was not included as a surrogate spike in any of the sample/sample duplicates, or field matrix spikes collected for this project. Surrogate recoveries from the other water projects and previous interim reports for E05-0210 indicate that there are solubility/stability issues with this surrogate in water. The 3M Environmental Lab has started an official investigation (which is not part of E05-0210) to determine the root cause for the low recoveries. Until this situation is fully understood and a method modification has been incorporated to eliminate or circumvent the issue, PFOA[1,2 ^{13}C] will not be added as surrogate.

III. Actions Taken

(such as amendment issued, SOP revision, etc.)

Corrective Action (☐ Yes ☑ No) Reference:

Acceptability of the nonconforming work:

Surrogates were originally used as an additional quality control element in conjunction with lab control spikes and field matrix spikes. The surrogate recovery was never intended to be the sole confirmation of that the analytical method was appropriate for the given matrix. Non-inclusion of the surrogate spike has no impact on the quality and integrity of the data presented here.

Actions: ☐ Halting of Work ☐ Client Notification ☐ Work Recall ☐ Withholding of Report
☑ Other: Non-inclusion of the surrogate will be addressed in the final report.

Recorded by: Date:

Authorization: Date:

Sponsor Approval (GLP Protocols): Date:

Technical Manager Approval (if halting of work or work recall are indicated): Date:

IV. Authorization to Resume Work

Where halting of work occurred, resumption of work must first be approved by Technical Management

Technical Manager Approval: Date:

Deviation No. _____
(assigned by Study Director or Project Lead at the end of study or project)

Protocol Deviation Page 1 of 1