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STUDY TITLE

Analysis of Pooled Human Sera and Plasma and Monkey Sera for Fluorocarbons Using Exygen Method ExM-023-071

DATA REQUIREMENTS

OECD Principles of Good Laboratory Practice, ENV/MC/CHEM(98)17, November 26, 1997

STUDY DIRECTOR

Emily R. Decker

STUDY COMPLETED ON

October 30, 2002

PERFORMING LABORATORY / TESTING FACILITY

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PROJECT

Study Plan Number: ExP-023-082
Exygen Study Number: 023-082
Sponsor Study Number: E02-1071

Total Pages: 111

CONTAIN NO CBI

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

Exygen Study Number 023-082, entitled "Analysis of Pooled Human Sera and Plasma and Monkey Sera for Fluorocarbons Using Exygen Method ExM-023-071," conducted for 3M Environmental Laboratory, was performed in compliance with OECD Good Laboratory Practice Standards (as revised in 1997), ENV/MC/CHEM(98)17 by Exygen Research, with the following exceptions:

1. ξ 8.3 (5): The computerized system of data generation did not provide for the retention of a full audit trail to show all changes or to associate all changes to data to a timed and dated electronic signature.
2. ξ 6.2 (4): The stability of the test items under storage or the study test conditions was not known. Also the purity of C6 acid and THPFOS was not known.
3. ξ 5.2 (3): The date of receipt of for the calf serum sample ID 0204718 was not documented.
4. ξ 1.2.2 (g): The instrument used for the analysis has not been qualified.

Emily R. Decker
Study Director
Exygen Research

10/30/02
Date

William K. Reagan, Ph.D.
Sponsor Representative
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10/31/02
Date

QUALITY ASSURANCE STATEMENT

The Quality Assurance Unit of Exygen Research reviewed Exygen Study Number 023-082 entitled, "Analysis of Pooled Human Sera and Plasma and Monkey Sera for Fluorocarbons Using Exygen Method ExM-023-071." All phases were reviewed for conduct according to Exygen Research's Standard Operating Procedures, the Study Protocol, and all applicable Good Laboratory Practice Standards. All findings were reported to the Study Director and to management.

Phase	Date Inspected	Date Reported to Study Director	Date Reported to Exygen Management	Date Reported to Sponsor
1. Protocol Review	10/10/02	10/14/02	10/30/02	10/30/02
2. Extraction, Fortification	10/15/02	10/25/02	10/25/02	10/30/02
3. Raw Data, Draft Report Review	10/25-28/02	10/29/02	10/30/02	10/30/02
4. Final Report Review	10/30/02	10/30/02	10/30/02	10/30/02

Naomi Lovallo
Technical Lead-QA

10/30/02
Date

CERTIFICATION OF AUTHENTICITY

This report, for Exygen Study Number 023-082, is a true and complete representation of the raw data for the study.

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Date

STUDY IDENTIFICATION

Analysis of Pooled Human Sera and Plasma and Monkey Sera for Fluorocarbons Using Exygen Method ExM-023-071

STUDY PLAN NUMBER:	ExP-023-082
EXYGEN STUDY NUMBER:	023-082
SPONSOR STUDY NUMBER:	E02-1071
TYPE OF STUDY:	Residue
TEST SYSTEM:	Human Serum, Human Plasma, and Monkey Serum
TEST ITEMS:	perfluorooctane sulfonate (PFOS), perfluorohexanoic acid (C6), perfluoroheptanoic acid (C7), pentadecafluorooctanoic acid (C8), heptadecafluorononanoic acid (C9), nonadecafluorodecanoic acid (C10), perfluoroundecanoic acid (C11), perfluorododecanoic acid (C12), tetrahydroperfluorooctane sulfonate (THPFOS), and tetrahydroperfluorodecane sulfonate (THPFDS)
SPONSOR:	William K. Reagan- Sponsor Study Monitor 3M Environmental Building 2-3E-09 St. Paul, MN 55133-3331
STUDY DIRECTOR:	Emily R. Decker Exygen Research Phone: (814) 272-1039
TESTING FACILITY:	Exygen Research 3058 Research Drive State College, PA 16801
ANALYTICAL PHASE TIMETABLE:	Study Initiation Date: 10/09/02 Experimental Start Date: 10/15/02 Experimental Termination Date: 10/23/02 Study Completion Date: 10/30/02

PROJECT PERSONNEL

The Study Director for this project at Exygen Research was Emily R. Decker. The following personnel from Exygen Research were associated with various phases of the study:

Name	Title
Paul Connolly	Technical Leader-LC/MS
Emily Decker	Scientist
Xiaoming Zhu	Technician
Rickey Keller	Sample Custodian

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1.0 SUMMARY

Exygen Research conducted a quantitative screening on various human serum, human plasma, and monkey serum samples for the determination of perfluorooctane sulfonate (PFOS), perfluorohexanoate (C6), perfluoroheptanoate (C7), pentadecafluorooctanoate (C8), heptadecafluorononanoate acid (C9), nonadecafluorodecanoate (C10), perfluoroundecanoate (C11), perfluorododecanoate (C12), tetrahydroperfluorooctane sulfonate (THPFOS), and tetrahydroperfluorodecane sulfonate (THPFDS) according to protocol ExP-023-082 (**Appendix A**). This screening was performed on an instrument that had not been used for routine fluorochemical analysis prior to this study. The method used for this study has not been validated at the levels reported for C8 and PFOS and not validated at any level for the other anions. These levels were completely dependent on instrument sensitivity.

Recoveries for fortified samples are given in **Tables I-III**. Residues of each anion in human serum are summarized in **Table IV**. Residues of each anion in human plasma are summarized in **Table V**. Residues of each anion in monkey serum are summarized in **Table VI**.

2.0 OBJECTIVE

The objective of this study was to screen human serum, human plasma, and monkey serum samples and quantitate to the lowest possible level according to instrument sensitivity.

3.0 INTRODUCTION

This report details the results of the analysis for perfluorooctane sulfonate (PFOS), perfluorohexanoate (C6), perfluoroheptanoate (C7), pentadecafluorooctanoate (C8), heptadecafluorononanoate (C9), nonadecafluorodecanoate (C10), perfluoroundecanoate (C11), perfluorododecanoate (C12), tetrahydroperfluorooctane sulfonate (THPFOS), and tetrahydroperfluorodecane sulfonate (THPFDS) in human serum, human plasma, and monkey serum samples.

The study was initiated on October 09, 2002, when the study director signed study plan number ExP-023-082. The experimental start date was October 15, 2002, and the experimental termination date was October 23, 2002.

4.0 TEST SYSTEM

Pooled human serum samples were purchased by the sponsor from Sigma-Aldrich, Milwaukee, WI, Lampire Biological Laboratories, Pipersville, PA, Bioresource

Technology, Inc., Fort Lauderdale, FL, and Golden West Biologicals, Temecula, CA. Pooled monkey serum samples were purchased by the sponsor from Lampire Biological Laboratories, Pipersville, PA. Pooled human plasma samples were purchased by the sponsor from Lampire Biological Laboratories, Pipersville, PA, Bioresource Technology, Inc., Fort Lauderdale, FL, Golden West Biologicals, Temecula, CA, and Innovative Research, Inc. Southield, MI. In addition, blank matrix consisting of pooled human plasma collected in rural China was provided by the sponsor. Also, calf serum was purchased from Sigma-Aldrich by Exygen.

Exgyen ID	Sponsor ID	Matrix	Source
0203963	Lot 020821	Human Serum	BioResource
0203964	Lot 22K0965	Human Serum	Sigma-Aldrich
0203965	Lot G0140604	Human Serum	Golden West Biologicals
0204292	X328-A	Human Serum	Lampire
0204334	TCR-684	Human Plasma	Golden West Biologicals
0204335	TN-A-06332	Monkey Serum	Lampire
0204490	TCR-674	Human Plasma	3M (plasma from rural China)
0204991	TN-A-6337	Human Plasma	Lampire
0204492	TN-A-06333	Monkey Serum	Lampire
0204493	TN-A-06336	Monkey Serum	Lampire
0204718	NA	Bovine (Calf Serum)	Sigma-Aldrich
0204747	TCR-683	Human Plasma	Innovative Research

Samples were received frozen on dry ice and then placed in frozen storage ($\leq$-10°C) until samples were logged in by Exygen personnel. All records concerning sample receipt, processing and storage can be found in the raw data package associated with this study.

5.0 TEST ITEMS

The analytical standards PFOS, C6, C7, C8, C9, C10, C11, C12, THPFOS, and THPFDS were received at Exygen on September 30, 2002 from 3M Environmental Technology and Services. The available information for the reference material is listed below. The reference material was stored frozen.

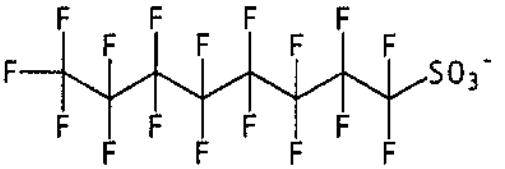
Compound	Exygen Inventory No.	Lot No.	Purity (%)	Expiration Date
PHAA (C6)	SP0002086	NB 117735-32	TBD	01/01/10
TDHA (C7)	SP0002091	PU/07219EU	99.5	01/01/05
PFOA (C8)	SP0002087	210002	>97	07/19/07
PFNA (C9)	SP0002085	H7568	>99	07/19/07
C10	SP0002090	R11K	98	12/01/10
C11	SP0002093	U11N	>99	07/19/07
C12	SP0002089	R24K	96	12/01/10
PFOS	SP0002084	217	86.9	08/31/06
THPFOS	SP0002088	Q75-91	Unknown	06/07/05
THPFDS	SP0002092	PMR-269-83	94.7	08/22/12

The molecular structures of the anions are given below.

Name: PFOS

Chemical Name: Perfluorooctanesulfonate

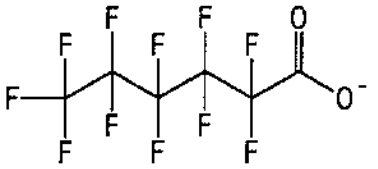
Molecular Weight: 499, as shown



Name: C6

Chemical Name: Perfluorohexanoate

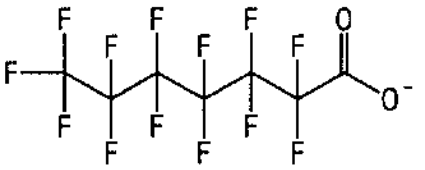
Molecular Weight: 313, as shown



Name: C7

Chemical Name: Perfluoroheptanoate

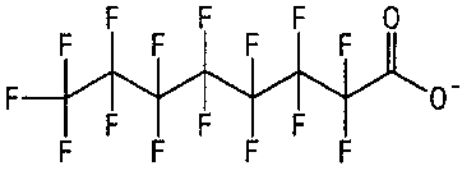
Molecular Weight: 363, as shown



Name: C8

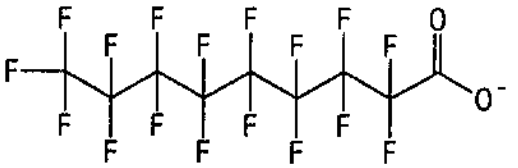
Chemical Name: Pentadecafluorooctanoate

Molecular Weight: 413, as shown



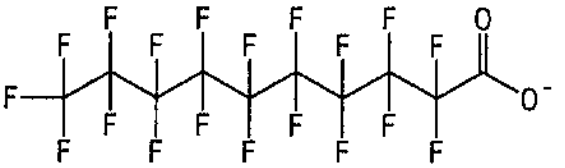
Name: C9

Chemical Name: Heptadecafluorononanoate
Molecular Weight: 463, as shown



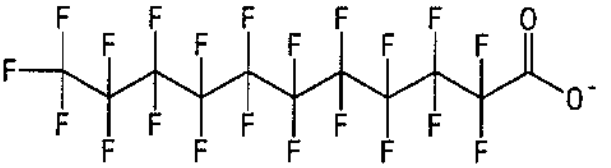
Name: C10

Chemical Name: Nonadecafluorodecanoate
Molecular Weight: 513, as shown



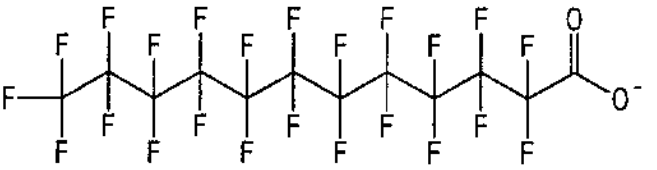
Name: C11

Chemical Name: Perfluoroundecanoate
Molecular Weight: 563, as shown



Name: C12

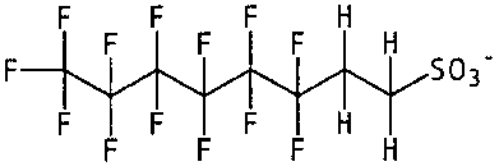
Chemical Name: Perfluorododecanoate
Molecular Weight: 613, as shown



Name: THPFOS

Chemical Name: Tetrahydroperfluorooctane sulfonate

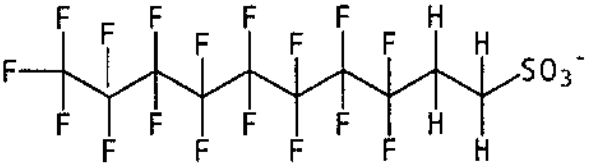
Molecular Weight: 427, as shown



Name: THPFDS

Chemical Name: Tetrahydroperfluorodecane sulfonate

Molecular Weight: 527, as shown



6.0 DESCRIPTION OF ANALYTICAL METHOD

Analytical method entitled "Method of Analysis for the Determination of Perfluorohexanesulfonate (PFHS), Perfluorooctanesulfonate (PFOS) and Pentadecafluorooctanoic Acid (PFOA) in Rat Liver, Serum and Urine" was used for this study. For this study, several modifications were made and have been documented in the protocol/protocol deviations.

6.1 Extraction Procedure

a. Measure 2 mL of serum sample into a 15 mL disposable centrifuge tube and fortify, if appropriate.
b. Add 5 mL of ACN and shake for ~20 minutes on a wrist action shaker.
c. Centrifuge tubes at ~3000 rpm for ~ 5 minutes. Carefully decant supernatant into a 50 mL disposable centrifuge tube and add 35 mL of water.
d. Load the sample onto a conditioned SPE column. Discard the eluate. Any analyte residues will be trapped on the SPE column at this point.
e. Elute with 5 mL of methanol and then evaporate to less than 1 mL using a nitrogen evaporator. Bring final volume up to 1 mL with methanol.
f. Analyze samples using electrospray LC/MS/MS.

The volume of sample used and the volume of methanol used for elution were different than those cited in the method. This was done to allow for lower quantification limits for the anions in this study.

6.2 Preparation of Standards and Fortification Solutions

Individual stock solutions of all of the anions were prepared on October 02, 2002, as specified in method ExM-023-071. The stock standard solutions were prepared at a concentration of ~ 100 µg/mL by dissolving ~10 mg of the standard (corrected for purity and salt content when appropriate) in methanol.

From these solutions, a 1.0 µg/mL mixed fortification standard solution was prepared by transferring the appropriate volume (~0.4 - 1 mL) of each of the stock solutions into a 100-mL volumetric flask and bringing the volume up to the mark with methanol.

The 0.1 µg/mL mixed fortification standard was prepared by transferring 10 mL of the 1.0 µg/mL mixed fortification standard into a volumetric flask and bringing the volume up to 100 mL with methanol.

A set of calibration standards were prepared by dilution in the following manner:

Initial Conc. (ng/mL)	Volume (mL)	Diluted to (mL)	Final Conc. (ng/mL)
100	1	10	10.0
100	0.5	10	5.0
100	0.2	10	2.0
100	0.1	10	1.0
5.0	1	10	0.5
2.0	1	10	0.2
1.0	1	10	0.1

The stock standard solutions and all fortification and calibration standard solutions were stored in a refrigerator (6° ± 2°C) when not in use.

6.3 Chromatography

Quantification was accomplished by electrospray LC/MS/MS analysis. An API 4000 Sciex system was used in this study because of its greater sensitivity and also because it had not been used for fluorochemical analysis prior to this study. Peaks were detected in the control matrices corresponding to some of the target anions, especially for C8.

6.4 Instrument Sensitivity

The smallest standard amount injected during the chromatographic run had a concentration of 0.1 ng/mL, which corresponds to a concentration of 0.05 ng/mL (ppb) in the extracted samples. Residues were calculated below this level where the response of the anion was approximately three times the signal to noise ratio. The results were

reported as Not Detected (ND) if the response was approximately less than three times the signal to noise ratio and Not Quantifiable (NQ) was used for negative results. All other responses were reported.

6.5 Description of Instrument and Operating Conditions

Instrument: PE SCIEX API 4000 Biomolecular Mass Analyzer, (LC/MS/MS #8)
SCIEX Turbo Ion Spray Liquid Introduction Interface
Turbo Ion spray temperature = 350 °C
Auxiliary gas flow = ~ 7.0 L/min
Harvard Infusion Pump

Computer: Dell OptiPlex GX 110

Software: PE Sciex Analyst 1.2

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

HPLC Column: Genesis C-8, 5 cm x 2.1 mm i.d. x 4 µ (Exygen ID: 71A)
(JONESCHROMATOGRAPHY: Part No. FK5962E)

Column Temperature: 35°C

Mobile Phase (A) : 2 mM Ammonium Acetate in Type I Water

Mobile Phase (B) : Methanol

Time (min)	% A	% B	Flow Rate (mL/min)
0.0	90.0	10.0	0.3
2.0	90.0	10.0	0.3
5.0	10.0	90.0	0.3
9.0	10.0	90.0	0.3
9.5	0.0	100.0	0.3
14.0	0.0	100.0	0.3
14.5	90.0	10.0	0.3
20.0	90.0	10.0	0.3

Injected Volume: 15 µL

Ions monitored :

Anion	Parent ion	Daughter ion	Dwell (secs)	Declustering Potential	Collision Energy
C6	313	269	0.1	-20	-10
C7	363	319	0.1	-20	-10
C8	413	369	0.1	-20	-10
C9	463	419	0.1	-20	-10
C10	513	469	0.1	-20	-10
C11	563	519	0.1	-20	-10
C12	613	569	0.1	-20	-10
PFOS	499	80	0.1	-85	-80
THPFOS	427	81	0.1	-65	-60
THPFDS	527	81	0.1	-75	-66

6.6 Quantitation and Example Calculation

Fifteen microliters of sample or calibration standard were injected into the LC/MS/MS. The peak area was measured and the standard curve was generated (using 1/x weighted linear regression) by Analyst software using seven concentrations of standards prepared in methanol. The residue concentration for the samples was determined from the following equations:

Use Equation 1 to calculate the amount of anion found (in ng/mL, based on peak area) using the standard curve (1/x weighted linear regression parameters) generated by the Analyst software program.

Equation 1:

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

Use Equation 2 to calculate the amount of analyte found (in ppb)

Equation 2:

$$\text{Analyte found (ppb = ng/mL)} = \frac{\text{(analyte found (ng/mL) x FV (mL) x DF}}{\text{sample volume (mL)}}$$

FV = final volume
DF = dilution factor

For samples fortified with known amounts of analyte prior to extraction, use Equation 3 to calculate the percent recovery (ppb = ng/mL)

Equation 3:

$$\text{Recovery (\%)} = \frac{\text{[total analyte found (ppb) - analyte found in control or sample (ppb)]}}{\text{analyte added (ppb)}} \times 100$$

Note: Any analyte found in the control was subtracted from analyte found. However, the response for the sample duplicate was not used.

An example of a calculation using an actual sample follows:

Human Serum Sample, Exygen ID 0204491 Spk J (Data Set: 101702A), fortified with 0.5 ng/mL (calculation is using values for C6):

Where:

peak area	=	54636
intercept	=	632.277
slope	=	59435.9
dilution factor	=	1
ng/mL added (fort level)	=	0.5 ng/mL
avg. amt in controls	=	0 (Not detected)
final volume	=	1 mL
sample volume	=	2 mL

From equation 1:

$$\text{Analyte found (ng/mL)} = \frac{[54636 - 632.277]}{59435.9}$$

$$= 0.9 \text{ ng/mL}$$

From equation 2:

$$\text{Analyte found (ppb)} = \frac{0.9 \text{ ng/mL} \times 1 \text{ mL}}{2 \text{ mL}}$$

$$= 0.45 \text{ ppb (ng/mL)}$$

From equation 3:

$$\%\text{ Recovery} = \frac{(0.45 \text{ ng/mL} - 0 \text{ ng/mL}) \times 100}{0.5 \text{ ng/mL}}$$

$$= 90\%$$

Note: This example calculation was done using rounded numbers, and therefore may be slightly different from the values shown in the raw data.

7.0 EXPERIMENTAL DESIGN

For the screening of each sample, duplicate extractions were performed. Also, each sample was fortified at 0.5 ng/mL and 5.0 ng/mL and then taken through the extraction procedure. Two calibration curves were also taken through the extraction procedure, one using calf serum and one using human plasma. These were treated as quality control fortifications in the data set and were not used for the calibration curve. Since there was residue detected in the samples for THPFOS and THPFDS, an additional analysis in which a three-daughter ion confirmation was performed.

8.0 RESULTS

There was no significant residue detected in the reagent blank analyzed with these samples. Also, there was no carry-over present for any of the anions in the instrument blanks (methanol washes) analyzed in the analytical sets, except for C8 and C9, and this is most likely contributed to those analytes being present in the instrumental system, particularly in the mobile phase. This is especially evident with the absence of the anions (except for C8 and C9) in the methanol wash analyzed after the injection of the 10 ng/mL calibration standard. All fortifications were at a level equal to or less than the 10 ng/mL standard. Since there was no carry-over observed after the injection of this standard, the carry-over present after proceeding injections would be minimal. A representative chromatogram of a standard prepared in methanol can be found in **Figure 1**.

Recoveries for fortified samples are given in **Tables I-III**. Recoveries outside the suggested range of 70% to 130% were reported, however this method has not been validated at these low levels and some of the recoveries were outside of this range because the level of residue in the sample was significantly greater than the amount fortified, especially for C8 and PFOS. Example chromatograms of fortified samples are shown in **Figure 2**.

Residues of each anion in human serum are summarized in **Table IV**. Residues of each anion in human plasma are summarized in **Table V**. Residues of each anion in monkey serum are summarized in **Table VI**. Example chromatograms of a human plasma sample are given in **Figure 3**. The detection of THPFDS in some of the samples warranted further investigation. The presence of THPFOS and THPFDS was confirmed with a re-analysis with additional daughter ion confirmation. A chromatogram detailing the three daughter ion confirmation of THPFDS is given in **Figure 4**.

9.0 CONCLUSIONS

The quantitative screening of these serum and plasma samples produced levels of certain analytes at extremely low levels (< 100 ppt). These levels are based solely on the instrument sensitivity and not the method recovery. The results contained in this report should be evaluated as a quantitative screening. Contamination of these samples due to instrument conditions is very limited because the instrument used for the analyses had never been used for routine fluorochemical analysis prior to the initiation of this study. No carry-over was observed throughout the injections of the analytical sets, which was demonstrated with the absence of the target analytes in the methanol washes analyzed after the injection of the highest level of calibration standard (10 ng/mL).

Two people took a set of 64 samples through the sample preparation procedure in approximately 10 hours and the analysis by LC/MS/MS took approximately 48 hours.

10.0 CIRCUMSTANCES THAT MAY HAVE AFFECTED THE DATA

The method used in this study has not been validated for C8 and PFOS at the levels given in this report and at any level for the rest of the anions. Residues were reported lower than the lowest calibration standard.

11.0 RETENTION OF DATA AND SAMPLES

When the final report is complete, all original paper data generated by Exygen Research will be shipped to the sponsor. This does not include facility-specific raw data such as instrument logs. Exact copies of all raw data, as well as a signed copy of the final analytical report and all original facility-specific raw data, will be retained in the archives of Exygen Research for the lifetime of the product. Sponsor permission will be obtained before discarding.

TABLES

Table I Summary of Recoveries for Calibration Curve in Calf Serum Compared to Standards in Methanol

			% Recovery									
Sample ID	Sponsor ID	Fort Level (ng/mL)	C6	C7	C8	C9	C10	C11	C12	PFOS	THPFOS	THPFDS
XC101702-0	NA	0.0	-	-	-	-	-	-	-	**	-	-
XC101702-1	NA	0.2	85	120	163	151	155	96	87	**	148	140
XC101702-2	NA	0.5	98	111	127	132	135	110	111	**	159	144
XC101702-3	NA	1.0	90	113	109	124	112	111	116	**	141	120
XC101702-4	NA	1.5	102	113	121	127	121	120	121	**	157	138
XC101702-5	NA	2.0	102	108	117	122	115	109	110	**	152	129
XC101702-6	NA	2.5	97	99	111	107	103	98	98	**	141	122
XC101702-7	NA	5.0	95	97	109	108	104	106	105	**	141	124
		AVG:	96	109	122	124	121	107	107	**	148	131
		STANDARD DEVIATION:	6.2	8.2	19.1	15.0	18.6	8.2	11.5	**	7.8	9.6
		RELATIVE STANDARD DEVIATION:	6.5	7.5	15.6	12.1	15.4	7.6	10.7	**	5.2	7.3

Table II Summary of Recoveries for Calibration Curve in Human Plasma Compared to Standards in Methanol

			% Recovery									
Sample ID	Sponsor ID	Fort Level (ng/mL)	C6	C7	C8	C9	C10	C11	C12	PFOS	THPFOS	THPFDS
XC101502-8	TCR-674	0.0	-	-	-	-	-	-	-	**	-	-
XC101502-9	TCR-674	0.2	90	120	126	86	33	115	109	**	142	133
XC101502-10	TCR-674	0.5	87	121	146	115	84	132	114	**	160	142
XC101502-11	TCR-674	1.0	96	113	121	104	90	110	108	**	151	123
XC101502-13	TCR-674	2.0	107	114	129	107	112	114	120	**	150	137
XC101502-15	TCR-674	5.0	95	97	106	97	98	101	104	**	127	113
		AVG:	93	113	126	102	81	114	111	**	146	130
		STANDARD DEVIATION:	10.2	9.6	14.4	10.9	29.2	11.3	6.2	**	12.4	11.6
		RELATIVE STANDARD DEVIATION:	10.9	8.5	11.5	10.7	35.8	9.9	5.6	**	8.5	9.0

** Recovery not applicable because the residues detected in sample were significantly greater than the amount fortified.

Table III Summary of Recoveries for Laboratory Fortified Matrix Spikes

Calf Serum

			% Recovery									
Sample ID	Sponsor ID	Fort Level (ng/mL)	C6	C7	C8	C9	C10	C11	C12	PFOS	THPFOS	THPFDS
0204718 Spk A	NA	0.5	98	110	99	126	115	95	105	**	144	126
0204718 Spk B	NA	5.0	84	85	92	97	95	96	100	**	118	108
		AVG:	91	98	96	112	105	96	103	**	131	117
		STANDARD DEVIATION:	9.9	17.7	4.9	20.5	14.1	0.7	3.5	**	18.4	12.7
		RELATIVE STANDARD DEVIATION:	10.9	18.1	5.2	18.4	13.5	0.7	3.4	**	14.0	10.9

Human Serum

			% Recovery									
Sample ID	Sponsor ID	Fort Level (ng/mL)	C6	C7	C8	C9	C10	C11	C12	PFOS	THPFOS	THPFDS
0203963 Spk C	Lot 020821	0.5	114	142	123	133	124	151	160	**	228	198
0203964 Spk D	Lot 22K0965	0.5	44	92	144	91	96	110	107	**	133	117
0203965 Spk E	Lot G0140604	0.5	53	120	376	153	118	139	132	**	265	149
0204292 Spk F	X328-A	0.5	94	125	380	188	129	128	131	**	182	149
0203963 Spk M	Lot 020821	5.0	106	112	164	146	131	137	134	**	321	168
0203964 Spk N	Lot 22K0965	5.0	76	91	106	98	97	107	113	103	118	109
0203965 Spk O	Lot G0140604	5.0	88	109	158	119	110	117	115	**	198	121
0204292 Spk P	X328-A	5.0	88	98	128	105	103	101	109	**	116	113
		AVG:	83	111	197	129	114	124	125	**	195	141
		STANDARD DEVIATION:	24.3	17.6	113.1	32.6	14.0	17.7	17.8	**	73.7	31.4
		RELATIVE STANDARD DEVIATION:	29.3	15.9	57.3	25.3	12.4	14.3	14.2	**	37.8	22.3

** Recovery not applicable because the residues detected in sample were significantly greater than the amount fortified.

Table III (cont') Summary of Recoveries for Laboratory Fortified Matrix Spikes

Human Plasma

			% Recovery									
Sample ID	Sponsor ID	Fort Level (ng/mL)	C6	C7	C8	C9	C10	C11	C12	PFOS	THPFOS	THPFDS
0204334 Spk H	TCR-684	0.5	69	94	211	152	107	93	103	**	122	113
0204490 Spk J	TCR-674	0.5	78	78	73	84	90	91	96	**	107	102
0204491 Spk J	TN-A-6337	0.5	91	97	168	140	110	91	93	**	113	116
0204747 Spk G	TCR-683	0.5	89	124	406	182	143	132	120	**	159	146
0204334 Spk R	TCR-684	5.0	96	100	131	110	110	98	100	**	114	112
0204490 Spk U	TCR-674	5.0	86	85	92	90	92	92	96	65	109	102
0204491 Spk T	TN-A-6337	5.0	97	96	109	101	95	86	88	**	113	106
0204747 Spk Q	TCR-683	5.0	82	85	106	92	90	83	83	**	111	94
		AVG:	86	95	162	119	105	96	97	**	119	111
		STANDARD DEVIATION:	9.4	13.9	108.0	35.3	17.8	15.3	11.1	**	17.0	15.7
		RELATIVE STANDARD DEVIATION:	11.0	14.7	66.7	29.7	17.0	16.0	11.4	**	14.3	14.1

Monkey Serum

			% Recovery									
Sample ID	Sponsor ID	Fort Level (ng/mL)	C6	C7	C8	C9	C10	C11	C12	PFOS	THPFOS	THPFDS
0204335 Spk I	TN-A-06332	0.5	106	91	68	52	88	85	95	**	114	129
0204492 Spk K	TN-A-06333	0.5	122	112	129	134	117	129	125	**	162	155
0204493 Spk L	TN-A-06336	0.5	123	98	97	102	115	93	94	**	132	120
0204335 Spk S	TN-A-06332	5.0	100	93	97	107	108	105	109	**	107	104
0204492 Spk U	TN-A-06333	5.0	88	85	100	94	97	93	101	**	124	116
0204493 Spk V	TN-A-06336	5.0	115	100	116	114	109	106	107	**	141	120
		AVG:	109	97	106	101	106	102	105	**	130	124
		STANDARD DEVIATION:	13.7	9.3	20.6	27.4	11.1	15.5	11.5	**	19.8	17.2
		RELATIVE STANDARD DEVIATION:	12.5	9.6	20.4	27.2	10.5	15.2	10.9	**	15.3	13.9

** Recovery not applicable because the residues detected in sample were significantly greater than the amount fortified.

Table IV Summary of Residues for Human Serum Samples

		Amount Found (ng/mL)									
Sample ID	Sponsor ID	C6	C7	C8	C9	C10	C11	C12	PFOS	THPFOS	THPFDS
0203963	Lot 020821	0.0261	ND	2.85	0.683	0.228	0.371	0.0333	32.0	ND	0.0743
0203963 Dup	Lot 020821	ND	ND	2.93	0.684	0.22	0.373	0.0282	33.3	ND	0.0599
0203964	Lot 22K0965	ND	0.0940	1.45	0.307	0.107	0.0942	0.0122	8.64	ND	0.0402
0203964 Dup	Lot 22K0965	ND	0.0976	1.45	0.276	0.108	0.115	0.0172	8.95	ND	0.0412
0203965	Lot G0140604	0.0687	0.145	5.93	0.508	0.214	0.207	0.0199	29.7	0.0440	0.0846
0203965 Dup	Lot G0140604	0.0884	0.170	6.00	0.557	0.204	0.236	0.0184	29.3	0.0436	0.0944
0204292	X328-A	ND	0.115	3.58	0.634	0.189	0.137	0.0169	16.6	0.0221	0.0791
0204292 Dup	X328-A	ND	0.12	4.21	0.772	0.221	0.149	0.0490	20.6	0.0394	0.0946

Table V Summary of Residues for Human Plasma Samples

		Amount Found (ng/mL)									
Sample ID	Sponsor ID	C6	C7	C8	C9	C10	C11	C12	PFOS	THPFOS	THPFDS
0204334	TCR-684	ND	0.0925	2.57	0.174	0.108	0.0797	ND	14.8	0.0140	0.0539
0204334 Dup	TCR-684	ND	0.0875	2.64	0.198	0.120	0.0695	ND	14.9	ND	0.0609
0204490	TCR-674	ND	ND	0.0887	NQ	0.0252	0.0458	ND	4.78	0.0115	ND
0204490 Dup	TCR-674	ND	ND	0.130	NQ	0.0167	0.0357	ND	4.84	ND	ND
0204491	TN-A-6337	ND	ND	2.23	0.166	0.0840	0.0501	ND	11.4	ND	0.0252
0204491 Dup	TN-A-6337	ND	0.0309	2.38	0.208	0.0958	0.0346	ND	12.6	ND	ND
0204747	TCR-683	ND	0.0897	2.09	0.287	0.121	0.105	ND	14.1	0.0193	0.0843
0204747 Dup	TCR-683	ND	0.0876	2.92	0.416	0.163	0.178	ND	19.4	0.0292	0.109

Table VI Summary of Residues for Monkey Serum Samples

		Amount Found (ng/mL)									
Sample ID	Sponsor ID	C6	C7	C8	C9	C10	C11	C12	PFOS	THPFOS	THPFDS
0204335	TN-A-06332	ND	ND	1.25	3.24	0.286	0.540	0.0365	17.2	ND	0.0141
0204335 Dup	TN-A-06332	ND	ND	1.72	4.37	0.396	0.650	0.0464	22.4	ND	ND
0204492	TN-A-06333	ND	ND	NQ	NQ	0.103	0.137	ND	16.5	ND	0.0130
0204492 Dup	TN-A-06333	ND	ND	NQ	NQ	0.0880	0.114	ND	16.4	ND	ND
0204493	TN-A-06336	ND	ND	NQ	NQ	ND	0.0939	0.0227	14.8	ND	ND
0204493 Dup	TN-A-06336	ND	ND	NQ	NQ	ND	0.106	0.0321	18.1	ND	ND

ND = Not Detected
NQ = Not Quantifiable (negative residue calculated)

FIGURES

Figure 1 Chromatogram Representing 0.1 ng/mL Calibration Standard

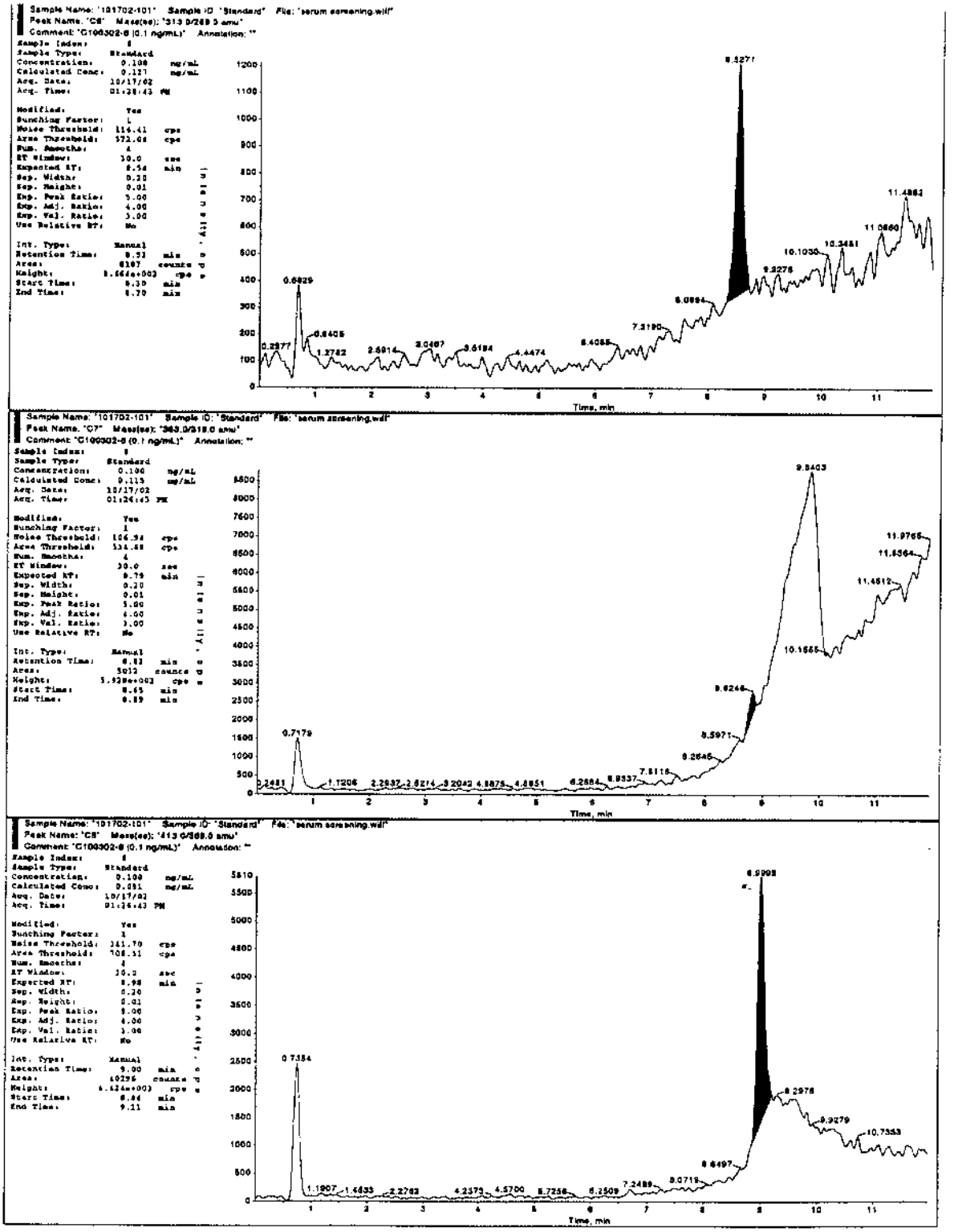


Figure 1 (cont) Chromatogram Representing 0.1 ng/mL Calibration Standard

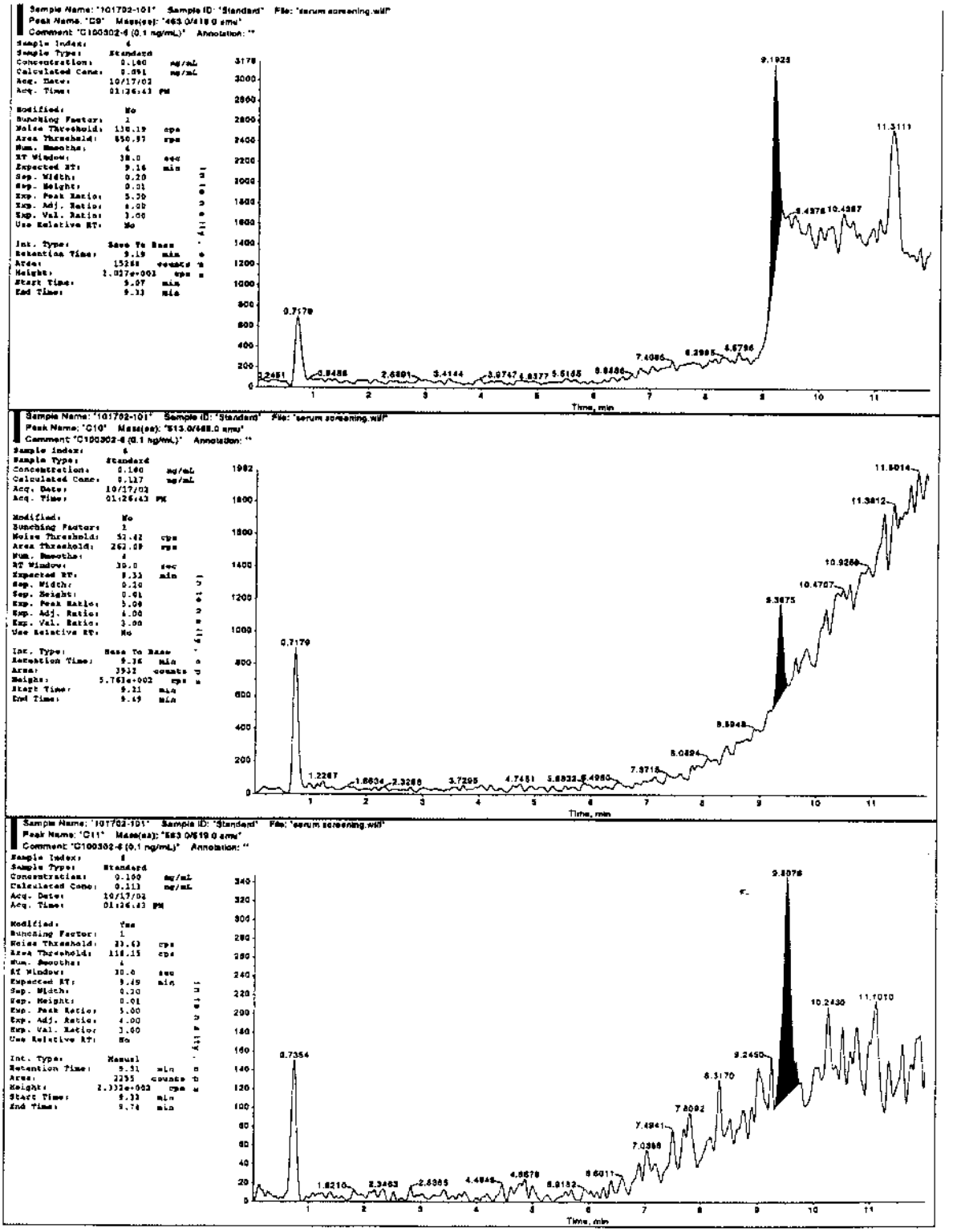


Figure 1 (cont) Chromatogram Representing 0.1 ng/mL Calibration Standard

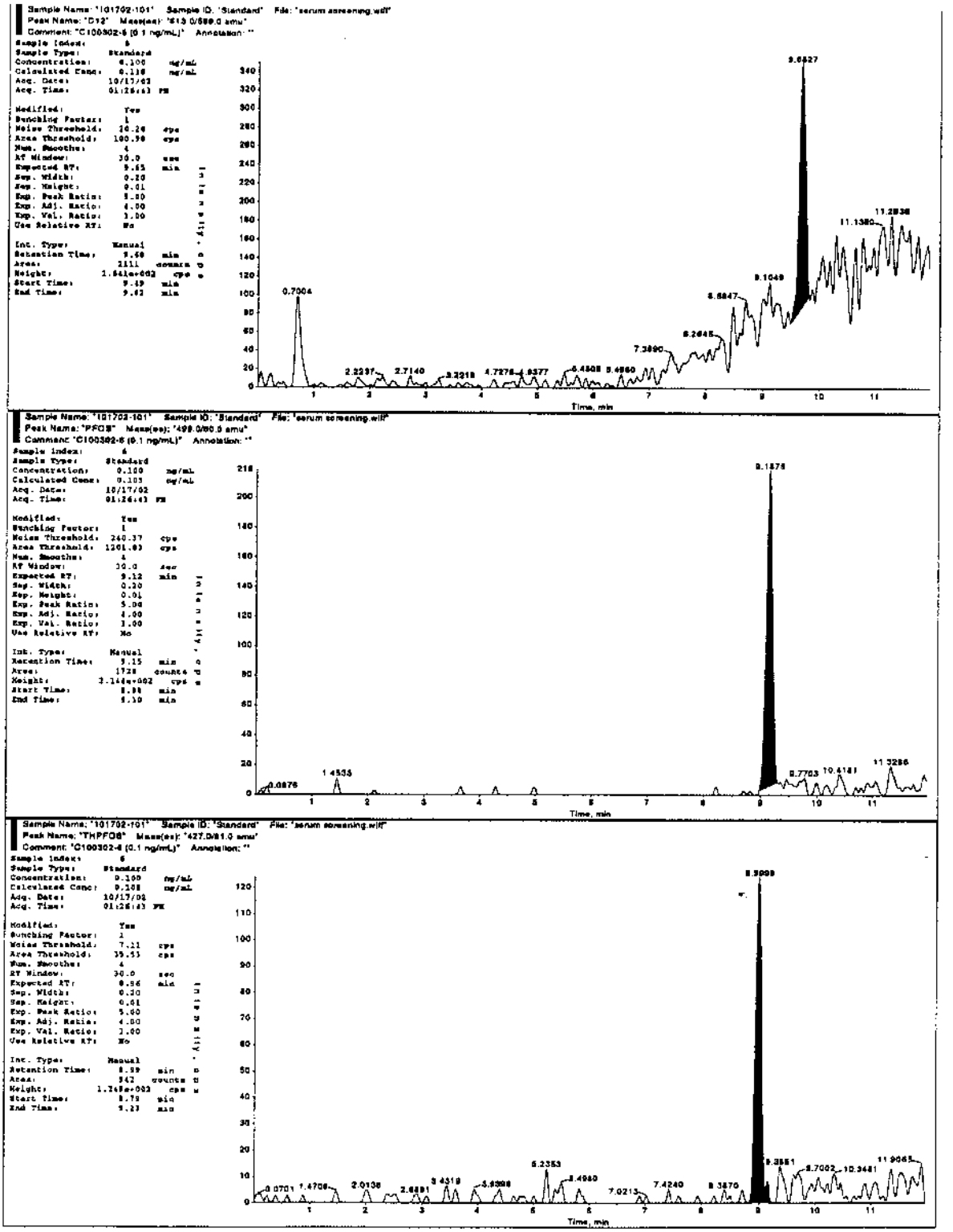


Figure 1 (cont) Chromatogram Representing 0.1 ng/mL Calibration Standard

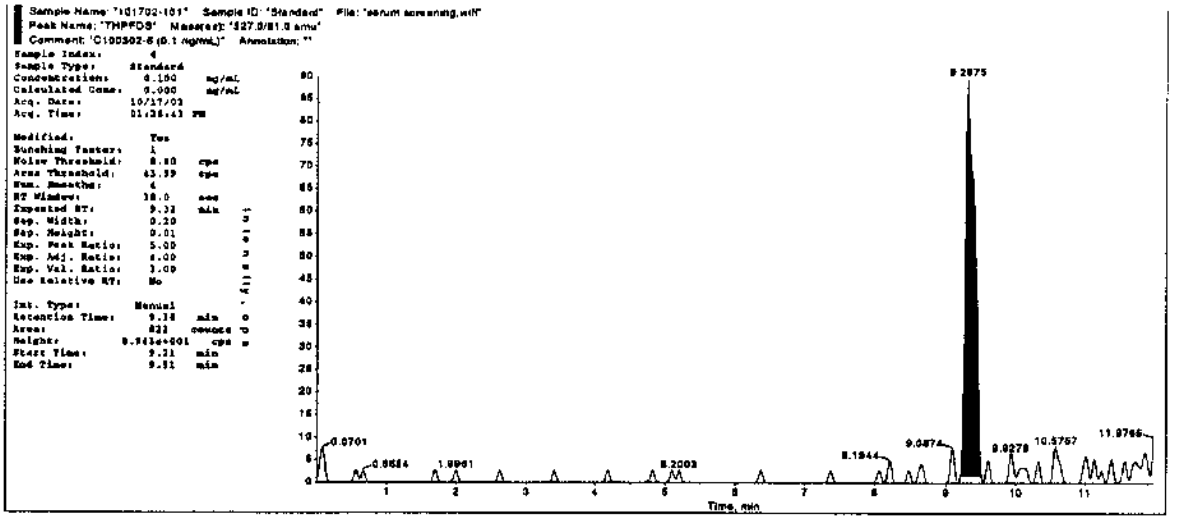


Figure 2 **Chromatogram Representing a Fortified Human Plasma Sample at 0.5 ppb (Exygen ID: 0204490 Spk J, Sponsor ID: TCR-674)**

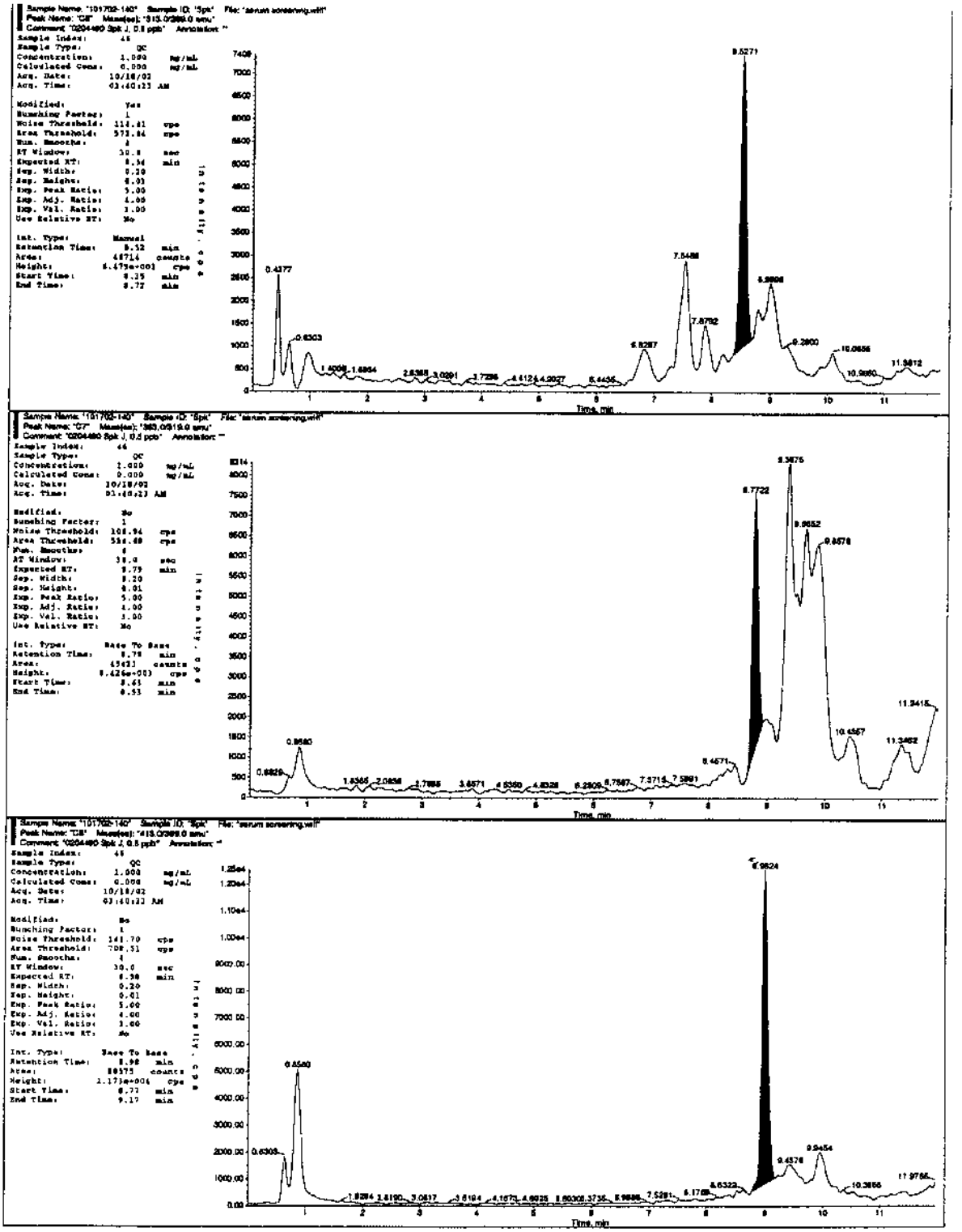


Figure 2 (cont') **Chromatogram Representing a Fortified Human Plasma Sample at 0.5 ppb (Exygen ID: 0204490 Spk J, Sponsor ID: TCR-674)**

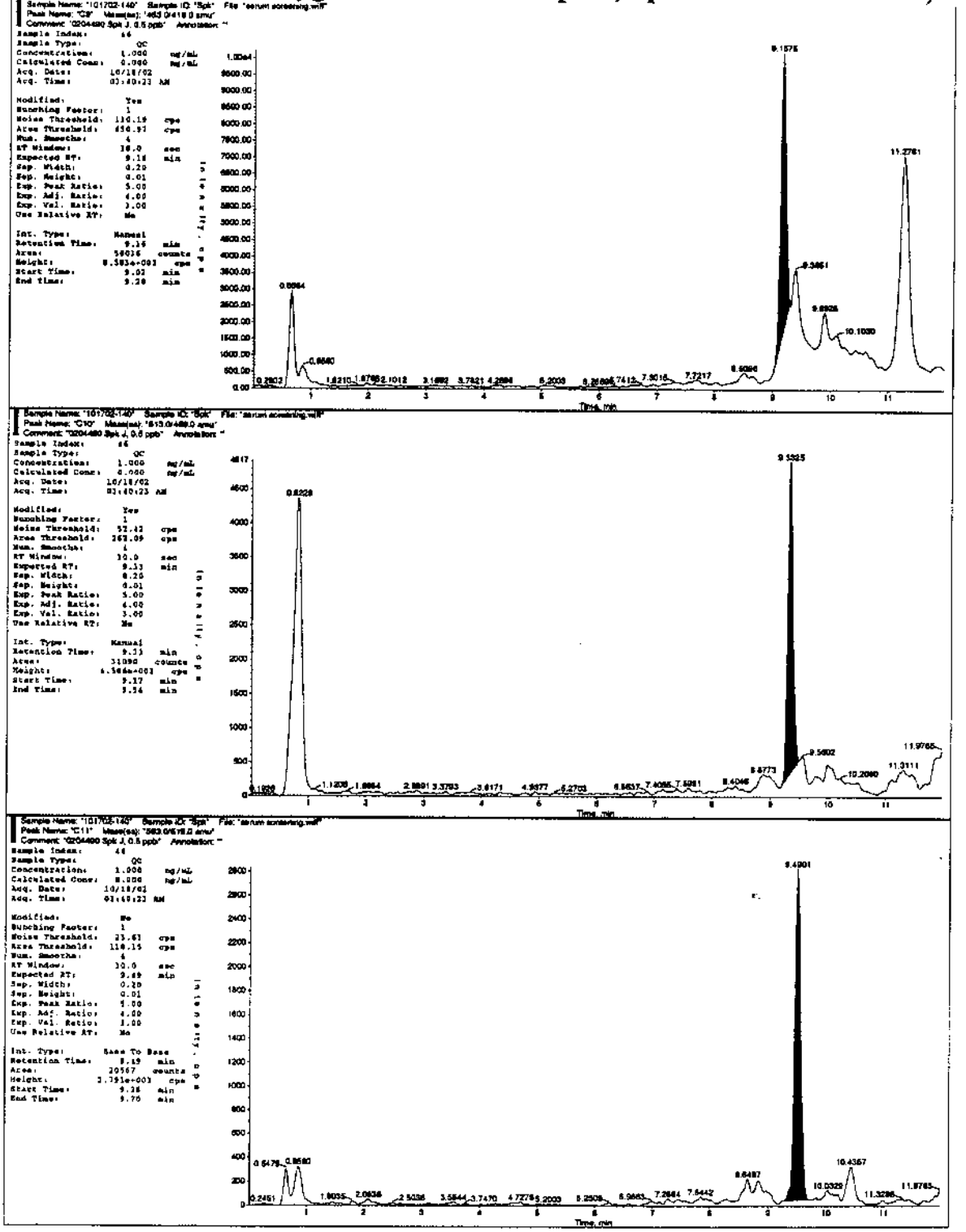


Figure 2 (cont') **Chromatogram Representing a Fortified Human Plasma Sample at 0.5 ppb (Exygen ID: 0204490 Spk J, Sponsor ID: TCR-674)**

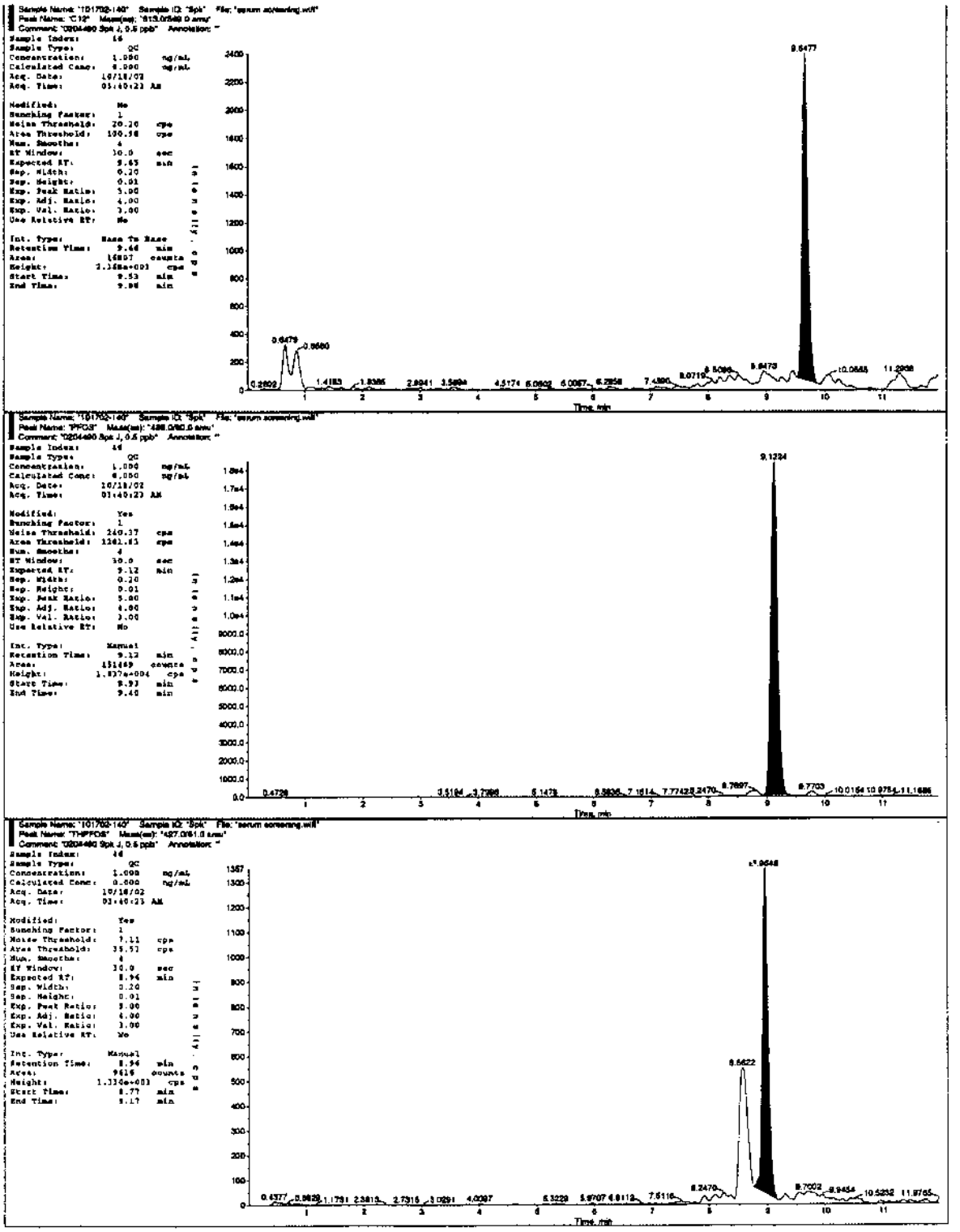


Figure 2 (cont') **Chromatogram Representing a Fortified Human Plasma Sample at 0.5 ppb (Exygen ID: 0204490 Spk J, Sponsor ID: TCR-674)**

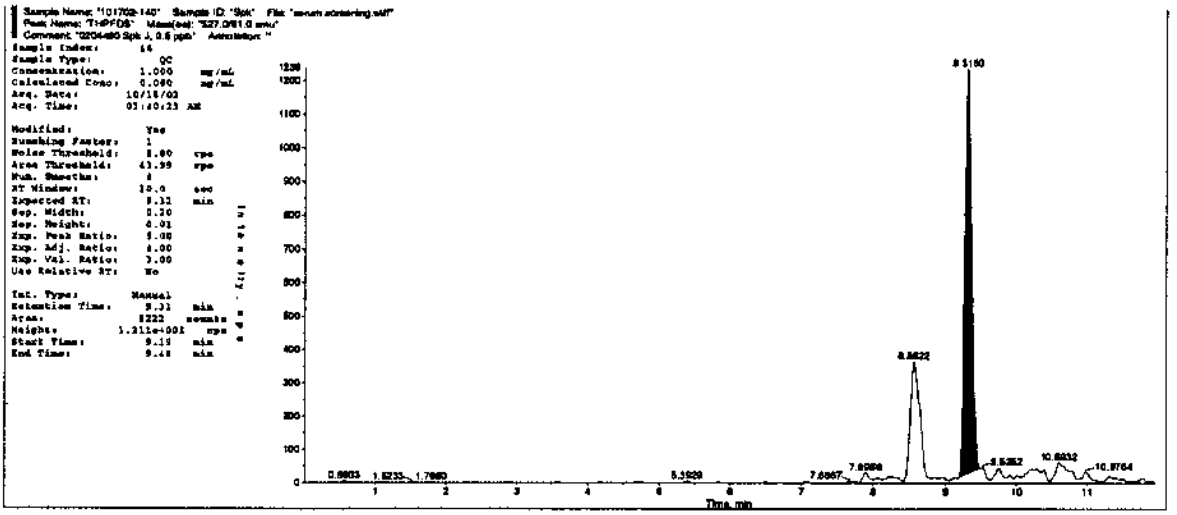


Figure 3 **Chromatogram Representing a Human Plasma Sample (Exygen ID: 0204490, Sponsor ID: TCR-674)**

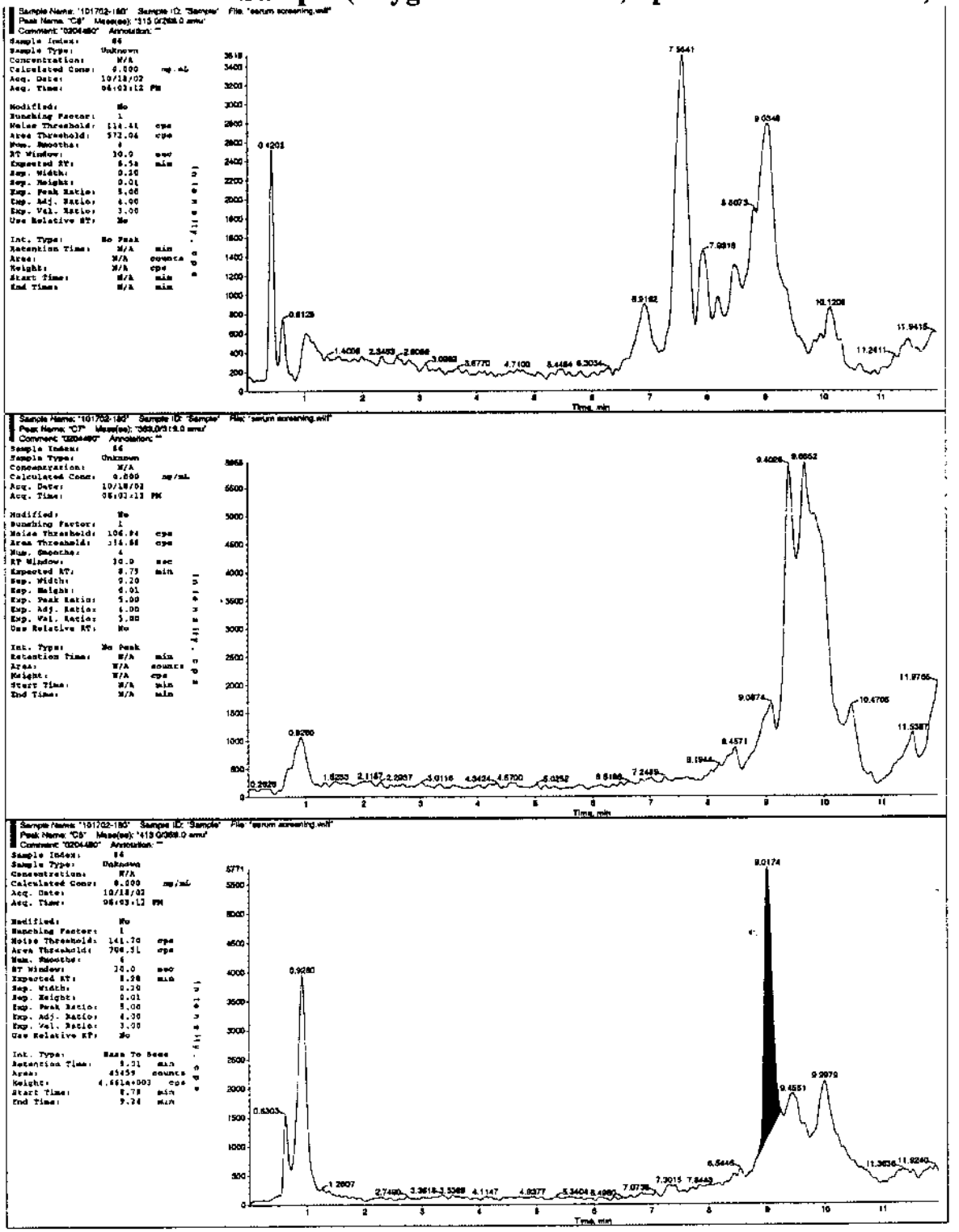


Figure 3 (cont') **Chromatogram Representing a Human Plasma Sample (Exygen ID: 0204490, Sponsor ID: TCR-674)**

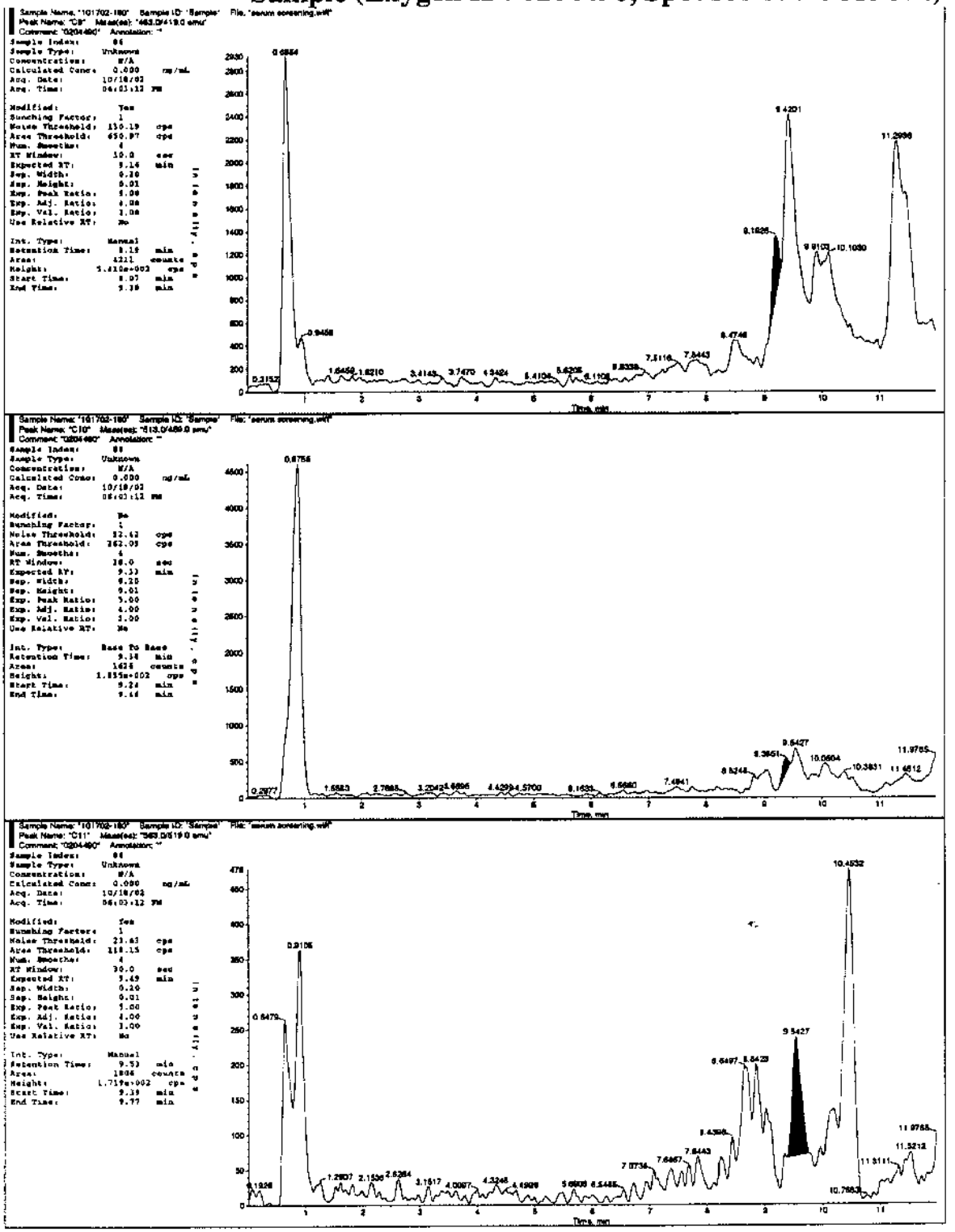


Figure 3 (cont') **Chromatogram Representing a Human Plasma Sample (Exygen ID: 0204490, Sponsor ID: TCR-674)**

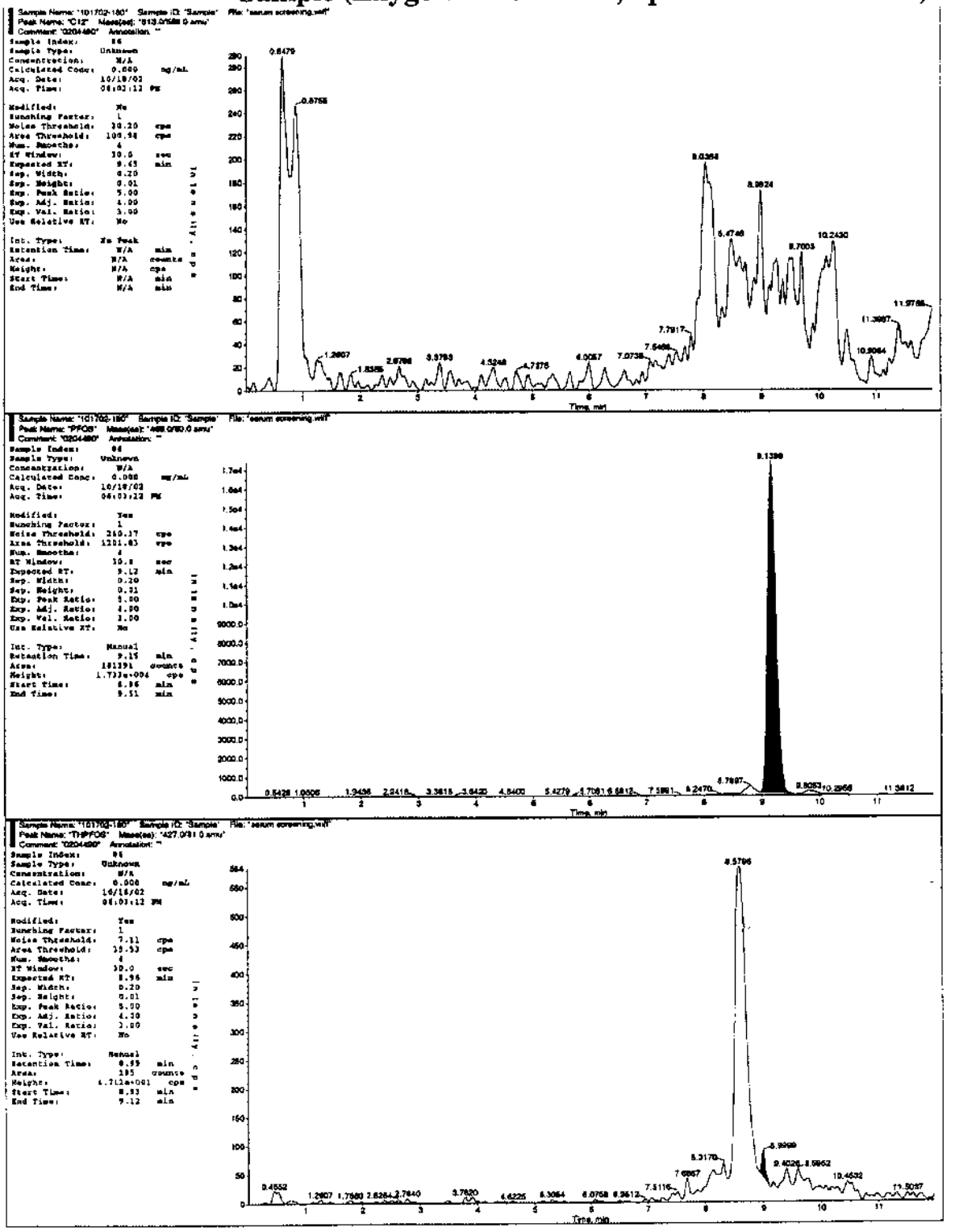


Figure 3 (cont') **Chromatogram Representing a Human Plasma Sample (Exygen ID: 0204490, Sponsor ID: TCR-674)**

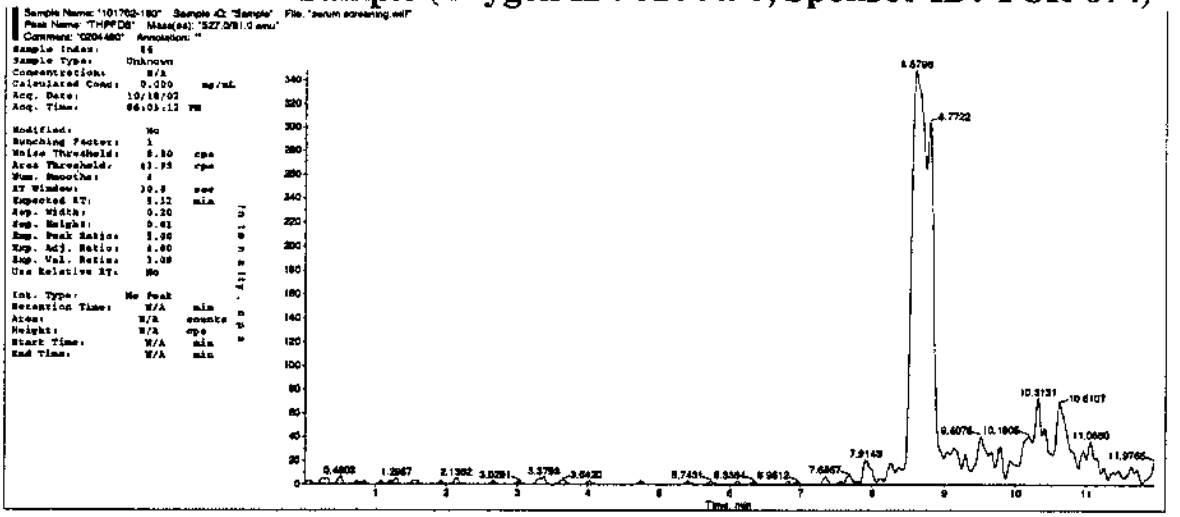
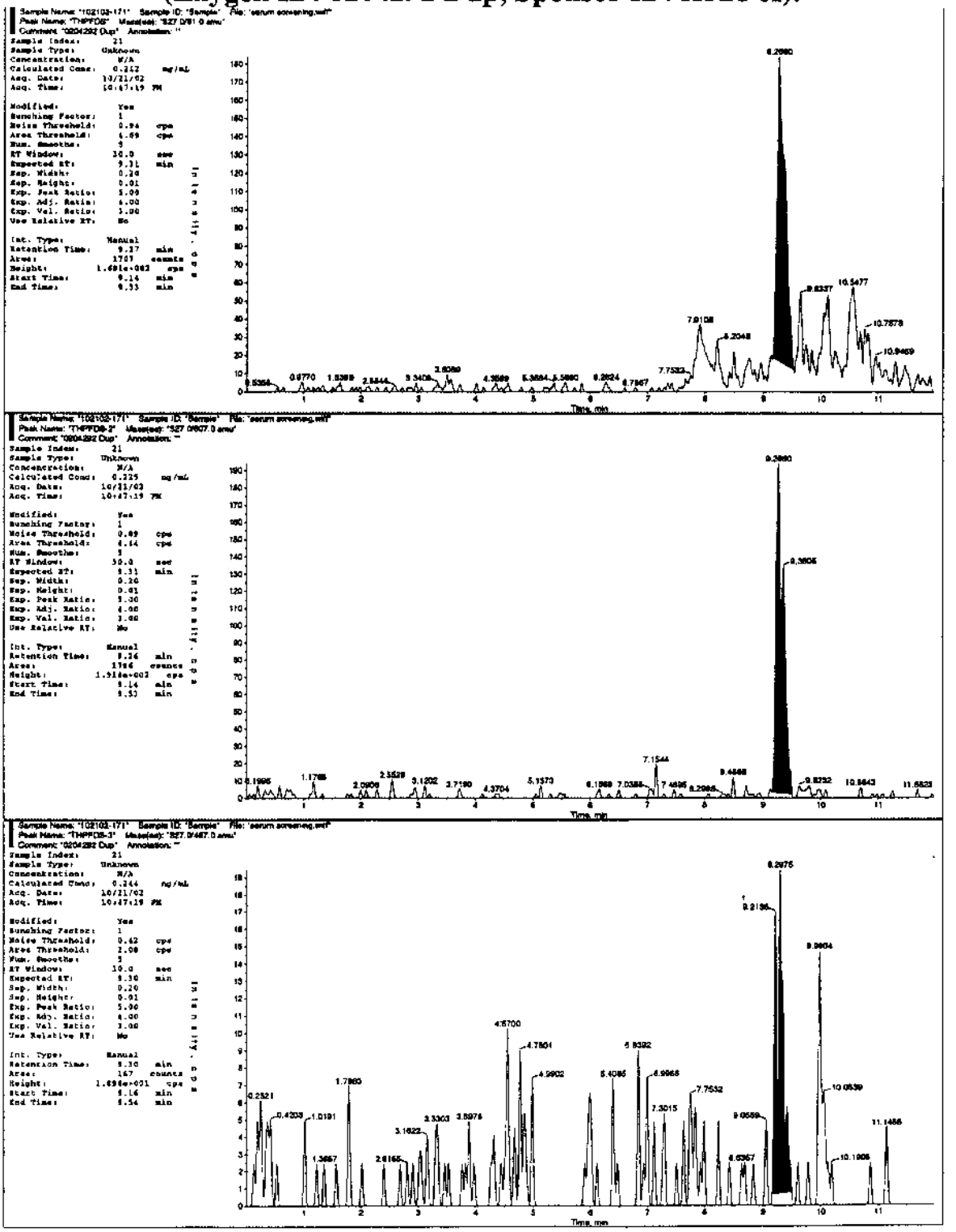


Figure 4 Chromatogram Representing a Sample Analyzed for Three Daughters for THPFDS (Exygen ID: 0204292 Dup, Sponsor ID: X328-A).



APPENDIX A

Exygen Study Plan
ExP-023-082
(Exygen Study No. 023-082)
and
Deviations

STUDY PLAN

Study Title:

Analysis of Pooled Human Sera and Plasma and Monkey Sera for Fluorocarbons Using Exygen Method ExM-023-071

Study Plan Number: ExP-023-082

Exygen Study Number: 023-082

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

Study Sponsor:
3M Environmental Laboratory
Building 2-3E-09
St. Paul, MN 55133-3331
Phone: (651) 778-6565

DISTRIBUTION

1) Emily R. Decker, Study Director, Exygen Research

2) William K. Reagen, Sponsor Study Monitor, 3M

3) Exygen Research Quality Assurance Unit

STUDY PLAN APPROVAL

Study Title: Analysis of Pooled Human Sera and Plasma and Monkey Sera for Fluorocarbons Using Exygen Method ExM-023-071

Study Plan Number: ExP-023-082
Exygen Study Number: 023-082

This study plan was audited by the Quality Assurance Unit of Exygen Research.

Naomi Lovallo
Technical Lead-QA — Date: 10/14/02

APPROVALS

Emily R Decker, Study Director
Exygen — Date: 10/9/02

John Flaherty, Vice President, Facility Management
Exygen — Date: 10/9/02

William Reagen, Sponsor Study Monitor
3M — Date

[redacted]

Study Title: Analysis of Pooled Human Sera and Plasma and Monkey Sera for Fluorocarbons Using Exygen Method ExM-023-071

Study Plan Number: ExP-023-082
Exygen Study Number: 023-082

This study plan was audited by the Quality Assurance Unit of Exygen Research.

Naomi Lovallo
Technical Lead-QA — Date

APPROVALS

Emily R. Decker, Study Director
Exygen — Date

John Flaherty, Vice President, Facility Management
Exygen — Date

William Reagen, Sponsor Study Monitor
3M — 10/09/02 Date

[redacted]

TABLE OF CONTENTS

INTRODUCTION

The purpose of this study is to perform analysis for perfluorooctane sulfonate (PFOS), perfluorohexanoic acid (C6), perfluoroheptanoic acid (C7), pentadecafluorooctanoic acid (C8), heptadecafluorononanoic acid (C9), nonadecafluorodecanoic acid (C10), perfluoroundecanoic acid (C11), perfluorododecanoic acid (C12), tetrahydroperfluorooctane sulfonate (THPFOS), and tetrahydroperfluorodecane sulfonate (THPFDS) in pooled human serum and plasma and monkey sera using Exygen method ExM-023-071 entitled "Method of Analysis for the Determination of Perfluorohexanesulfonate (PFHS), Perfluorooctanesulfonate (PFOS) and Pentadecafluorooctanoic Acid (PFOA) in Rat Liver, Serum and Urine."

The study will be audited for compliance with OECD Principles of Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM(98)17 by the Quality Assurance Unit of Exygen Research.

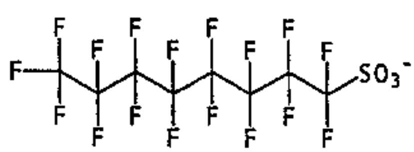
TEST ITEMS

The test items are perfluorooctane sulfonate (PFOS), perfluorohexanoic acid (C6), perfluoroheptanoic acid (C7), pentadecafluorooctanoic acid (C8), heptadecafluorononanoic acid (C9), nonadecafluorodecanoic acid (C10), perfluoroundecanoic acid (C11), perfluorododecanoic acid (C12), tetrahydroperfluorooctane sulfonate (THPFOS), and tetrahydroperfluorodecane sulfonate (THPFDS). All test items were received from the Sponsor.

Name: PFOS

Chemical Name: Perfluorooctanesulfonate

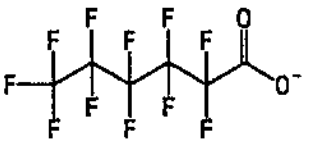
Molecular Weight: 499, as shown



Name: C6

Chemical Name: Perfluorohexanoic acid

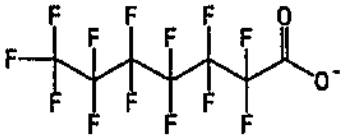
Molecular Weight: 313, as shown



Name: C7

Chemical Name: Perfluoroheptanoic acid

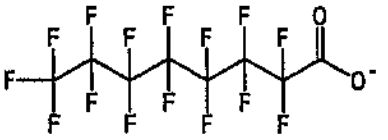
Molecular Weight: 363, as shown



Name: C8

Chemical Name: Pentadecafluorooctanoic acid

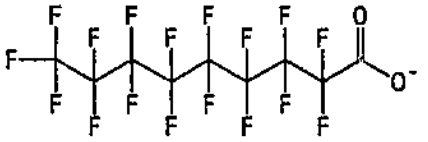
Molecular Weight: 413, as shown



Name: C9

Chemical Name: Heptadecafluorononanoic acid

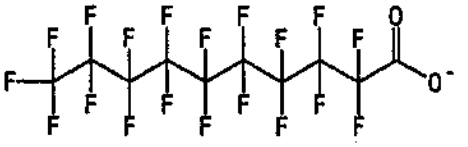
Molecular Weight: 463, as shown



Name: C10

Chemical Name: Nonadecafluorodecanoic acid

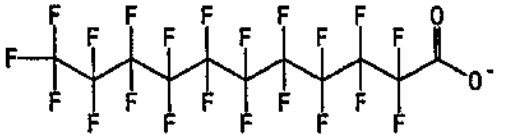
Molecular Weight: 513, as shown



Name: C11

Chemical Name: Perfluoroundecanoic acid

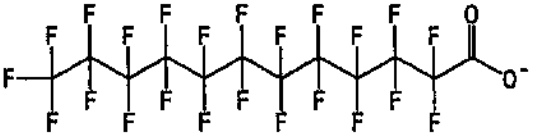
Molecular Weight: 563, as shown



Name: C12

Chemical Name: Perfluorododecanoic acid

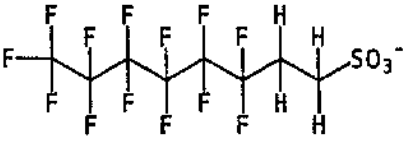
Molecular Weight: 613, as shown



Name: THPFOS

Chemical Name: Tetrahydroperfluorooctane sulfonate

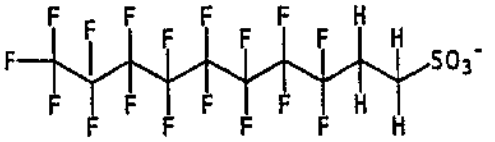
Molecular Weight: 427, as shown



Name: THPFDS

Chemical Name: Tetrahydroperfluorodecane sulfonate

Molecular Weight: 527, as shown



OBJECTIVE

The purpose of this study is to perform analysis on four different lots of pooled human serum, four different lots of pooled human plasma, and three lots of pooled monkey serum for the target fluorocompounds using the analytical method, "Method of Analysis for the Determination of Perfluorohexanesulfonate (PFHS), Perfluorooctanesulfonate (PFOS) and Pentadecafluorooctanoic Acid (PFOA) in Rat Liver, Serum and Urine."

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PROPOSED EXPERIMENTAL START AND TERMINATION DATES

It is proposed that the analytical portion of this study be conducted from October 14 to October 21, 2002. The actual experimental start and termination dates will be included in the final report.

COMMUNICATIONS

All communications between the Testing Facility, method developers, and the Sponsor will be directed through the Study Director (or designate) and the Sponsor Study Monitor. Communications will be fully documented by the Study Director.

IDENTIFICATION AND JUSTIFICATION OF THE TEST SYSTEM

Pooled human sera and plasma and monkey sera are used as the test systems in this study. The matrices will be provided by the sponsor. The matrices will be representative of that for which this analytical method was designed.

SAMPLE PROCUREMENT, RECEIPT AND RETENTION

Pooled human serum samples were purchased by the sponsor from Sigma-Aldrich, Milwaukee, WI, Lampire Biological Laboratories, Pipersville, PA, Bioresource Technology, Inc., Fort Lauderdale, FL, and Golden West Biologicals, Temecula, CA. Pooled monkey serum samples were purchased by the sponsor from Lampire Biological Laboratories, Pipersville, PA. Pooled human plasma samples were purchased by the sponsor from Lampire

In addition to the calibration standards described in Section 3.5.3, a set of calibration standards will be processed through the extraction procedure identical to the samples, using bovine serum and also a set using human plasma. The fortification of the standards before extraction is done according to the following table:

Conc. Of Mixed Fortification Solution (ng/mL)	Fortification Volume (µL)	Volume of Control Sample (mL)	Conc. of Extracted Calibration Standard (ng/mL)
1	400	2.0	0.2
10	100	2.0	0.5
10	200	2.0	1.0
10	300	2.0	1.5
10	400	2.0	2.0
100	50	2.0	2.5
100	100	2.0	5.0

VERIFICATION OF ANALYTICAL PROCEDURE

The testing facility will establish the relationship between the instrument response and the concentration of analyte in order to assess the linearity of the system. A standard curve should be constructed with at least five standards. The testing facility should also verify the endogenous levels of analyte in the matrix control samples. This should be accomplished by analysis of a control sample for each matrix and examination of the region of analyte retention. The potential exists for interference from fluorochemicals introduced from dietary material and other exogenous sources. Samples are fortified with the target analytes. The compounds will be made into solutions as per the method, and added to the matrices via a micropipette. Fortified samples will be processed through the described procedures to ensure method accuracy and to check for bias.

Recoveries should be between 70% and 130% of the fortified levels. The sponsor may accept occasional recoveries outside of this range. The relative standard deviation (RSD) for each fortification level as well as the overall RSD, should be less than or equal to 20%.

Any modifications to the analytical method will be documented in the study raw data. Modifications deemed significant by the Sponsor or Study Director will necessitate revision of the method.

METHOD FOR CONTROL OF BIAS

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

STATISTICAL METHODS

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

GLP STATEMENT

All aspects of this study shall be performed and reported in compliance with OECD Principles of Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM(98)17. 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.

REPORT

A final report will be prepared by the study director 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 Monitor 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.

- Any steps considered critical, i.e. steps where little variation is allowable or directions must be followed precisely.

- Description of the instrumentation used and operating conditions.

- The number of worker-hours or calendar days required to complete one set of samples.

- All results from all sets analyzed. Identify all control and fortified samples, and in the data table include sample number, fortification level, and unique identification number by sample set.

- 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 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 Study Monitor.

SAFETY AND HEALTH

- Laboratory personnel will practice good sanitation and health habits.

- Any health condition of laboratory personnel that may be considered to adversely affect the study will be reported to the Study Director.

- Any injury to laboratory personnel occurring during the conduct of this study will be reported to the study director.

- Every reasonable precaution shall be taken to prevent inadvertent exposure of personnel and the environment to the test or reference substance(s).

AMENDMENTS TO STUDY PLAN

All significant changes to the study plan outlined here will be expressed in writing, signed and dated by the Study Director and Sponsor Study Monitor. 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 Study Monitor.

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, date, Exygen study number, 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 Study Monitor.

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 Sponsor. 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 lifetime of the product and shall obtain permission of the sponsor before discarding.

APPENDIX I

METHOD

"Method of Analysis for the Determination of Perfluorohexanesulfonate (PFHS), Perfluorooctanesulfonate (PFOS) and Pentadecafluorooctanoic Acid (PFOA) in Rat Liver, Serum and Urine "

Study Plan: ExP-023-082
Exygen Study No.: 023-082

TITLE

Method of Analysis for the Determination of Perfluorohexanesulfonate (PFHS), Perfluorooctanesulfonate (PFOS) and Pentadecafluorooctanoic Acid (PFOA) in Rat Liver, Serum and Urine

AUTHORS

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DATE ISSUED

July 30, 2002

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METHOD NUMBER

ExM-023-071

TOTAL NUMBER OF PAGES

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Exygen Study No.: 023-082

Exygen Method No: ExM-023-071

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Sponsor Representative
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1. SUMMARY

This report details a method of analysis for residues of Perfluorohexanesulfonate (PFHS), Perfluorooctanesulfonate (PFOS) and Pentadecafluorooctanoic Acid (PFOA) in Rat Liver, Serum and Urine.

Residues of PFHS, PFOS and PFOA are extracted from each matrix with acetonitrile. The acetonitrile extract is added to water and loaded onto a conditioned C18 solid phase extraction (SPE) cartridge. Analyte residues are eluted with 2 mL of methanol. Quantification of PFHS, PFOS and PFOA is accomplished by liquid chromatography/tandem mass spectrometry (LC/MS/MS) analysis using multiple reaction monitoring (MRM).

The proposed limit of quantitation (LOQ; the lowest fortification specified by the method which gives adequate recovery according to EPA guidelines) for this method is 10 ng/g (parts-per-billion) each for PFHS, PFOS and PFOA.

The theoretical limit of detection (LOD) will be based on the signal to noise ratio and will be at least greater than 3 times the level of noise, based on the instrumentation system used. For all analytes, the lowest analytical standard corresponds to 0.1 ng/mL.

This method was developed using rat liver, serum and urine. Typical percent recoveries ± standard deviations (at 10 and 50 ng/g) are shown below:

Fortification Level (ng/g)	PFHS Recovery in Rat Liver	Fortification Level (ng/mL)	PFHS Recovery in Rat Serum
10	115% ± 9.9% (n=3)	10	108% ± 4.7% (n=3)
50	98% ± 3.5% (n=3)	50	111% ± 9.6% (n=3)

Fortification Level (ng/g)	PFOS Recovery in Rat Liver	Fortification Level (ng/mL)	PFOS Recovery in Rat Serum	PFOS Recovery in Rat Urine
10	96% ± 8.5% (n=3)	10	88% ± 9.8% (n=3)	93% ± 4.7% (n=3)
50	88% ± 1.5% (n=3)	50	120% ± 2.1% (n=3)	79% ± 1.2% (n=3)

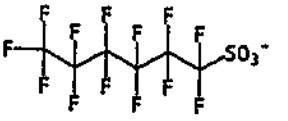
Fortification Level (ng/g)	PFOA Recovery in Rat Liver	Fortification Level (ng/mL)	PFOA Recovery in Rat Serum	PFOA Recovery in Rat Urine
10	98% ± 3.1% (n=3)	10	117% ± 1.5% (n=3)	89% ± 2.5% (n=3)
50	94% ± 2.5% (n=3)	50	111% ± 4.0% (n=3)	87% ± 2.1% (n=3)

Representative calibration curves are shown in Figures 1-3. Representative chromatograms are shown in Figures 4 to 39.

2. EXPERIMENTAL COMPOUNDS

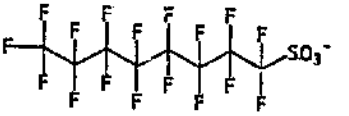
The structures for PFHS, PFOS and PFOA are given below.

PFHS
Chemical Name = Perfluorohexanesulfonate
Molecular weight = 399, as shown



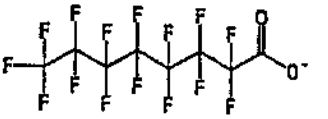
PFHS is supplied as the potassium salt ($C_6F_{13}SO_3^-K^+$), molecular weight = 438

PFOS
Chemical Name = Perfluorooctanesulfonate
Molecular weight = 499, as shown



PFOS is supplied as the potassium salt ($C_8F_{17}SO_3^-K^+$) molecular weight = 538

PFOA
Chemical Name = Pentadecafluorooctanoic Acid
Molecular weight = 413, as shown



3. CHEMICALS AND SUPPLIES

3.1. CHEMICALS

Chemical	Grade	Source	Catalog No.
Methanol (MeOH)	HPLC	EM Science	MX0475-1
Ammonium Acetate	Reagent	JT Baker	0596-01
Water	Type I	Exygen	NA
Acetonitrile	HPLC	EM Science	AX0145-1

Type I water = electrical resistivity, minimum of 16.67 MΩ/cm at 25 °C, from a Labconco Waterpro™ workstation.

3.2. STANDARDS

Standard	TCR Number	Purity (%)	Source
Perfluorohexanesulfonate (PFHS)	SE-036	99.99 all isomers, 84.36 straight chain	3M
Perfluorooctanesulfonate (PFOS)	SD-018	86.9	3M
Pentadecafluorooctanoic Acid (PFOA)	Lot No: 08316DO	96	Aldrich Chem

3.3. EQUIPMENT AND SUPPLIES

Equipment	Supplier
Balance, analytical (display at least 0.0001 g)	Mettler
125-mL LDPE narrow mouth bottles	Nalgene
Disposable glass micropipets (50-100 & 100-200 µL)	Drummond (VWR)
Tissumizer	Tekmar
Wrist action shaker	Burrell Scientific
Sorvall RC 5C plus Centrifuge	Dupont
50 mL disposable polypropylene centrifuge tubes	VWR
15 mL disposable polypropylene centrifuge tubes	VWR
Visiprep vacuum manifold	Supelco
Sep Pak Vac 6 cc (1g) tC18 cartridges (part # WAT 036795	Waters
2-mL clear HPLC vial Kit (cat # 5181-3400)	Hewlett-Packard
Class A pipets and volumetric flasks	various suppliers
Standard lab equipment (graduated cylinders, disposable tubes etc.)	various suppliers
Stand-alone drop-in guard cartridge holder (part #844017-400)	Keystone Scientific
Hypercarb drop-in guard column (4 mm) (part # 844017-400)	Keystone Scientific
HPLC Pump (LC-10AD)	Shimadzu
LC/MS/MS and HPLC systems	As described in section 4.5.

Notes:

1. In order to avoid contamination, the use of disposable labware (containers, tubes, pipettes, etc.) is highly recommended.
2. Teflon or Teflon-lined containers or equipment should not be used.
3. It may be necessary to check the solvents (acetonitrile, methanol) for the presence of contaminants (especially PFOA) by LC/MS/MS before use. Certain lot numbers have been found to be unsuitable for use.
4. Use disposable micropipettes or pipettes to aliquot standard solutions when preparing standards and samples for extraction.
5. Equivalent materials may be substituted for those specified in this method.

3.4. Solutions

(1) 2 mM ammonium acetate in water is prepared by weighing 0.154 g of ammonium acetate and dissolving in 1 L of water.

(2) Hypercarb filtered type I water is prepared by filtering type I water through a Hypercarb guard column using a HPLC pump at ~2-3 mL/min. Before use, wash the guard cartridge with ~25 mL of HPLC grade acetonitrile, then ~ 25 mL of type I water, then begin collecting the filtered type I water eluate for use in the extraction. Repeat the wash after filtering ~2L of water.

Note: The aforementioned example is provided for guidance, alternative volumes may be prepared as long as the appropriate ratios of the solvent to solute are maintained.

3.5. Preparation of Standard Solutions

Analytical standards are used for three purposes:

1. Calibration Standards – These standards are prepared in methanol and are used to calibrate the response of the detector used in the analysis.
2. Laboratory Control Spikes – These fortifications are prepared at concentrations corresponding to the LOQ and 5x LOQ and are used to determine analytical recovery. Laboratory control spikes are prepared in control matrix.
3. Matrix Spikes – These fortifications are prepared by spiking into the field samples at a known concentration. Matrix spikes are used to evaluate the effect of the sample matrix on analytical recovery and are prepared at the client's request.

The analyst may vary the absolute volumes of the standards as long as the correct proportions of solute to solvent are maintained.

3.5.1. Stock solution

Prepare individual stock solutions at 100 µg/mL for PFHS, PFOS and PFOA by weighing out 10 mg of each analytical standard (corrected for purity and if necessary, salt content) and dilute to 100 mL with methanol in separate 100-mL volumetric flasks. The stock solutions (in 125-mL LDPE bottles) are to be stored in a refrigerator at 2°C to 6°C and are stable for a maximum period of one year from the date of preparation.

3.5.2. Fortification Solutions

a. Prepare a mixed fortification standard at 1.0 µg/mL (1000 ng/mL) of PFHS, PFOS and PFOA by adding 1.0 mL of each of the 100 µg/mL stock solutions into a 100-mL volumetric flask and bring up to volume with methanol.
b. Prepare a mixed fortification standard at 0.1 µg/mL (100 ng/mL) of PFHS, PFOS and PFOA by diluting 10.0 mL of the 1.0 µg/mL mixed fortification solution to 100 mL with methanol in a volumetric flask.

Example: one hundred microliters of the 0.1 µg/mL solution spiked into 1 g of liver or 1 mL of serum/urine is equivalent to a 10 ppb (10 ng/mL or ng/g) fortification.

Store all fortification standard solutions in a refrigerator (in 125-mL LDPE bottles) at 2°C to 6°C for a maximum period of one year from the date of preparation. Note also that additional concentrations may be prepared if necessary.

3.5.3. Calibration Standards

LC/MS/MS calibration standards containing PFHS, PFOS and PFOA are prepared at 0.1, 0.2, 0.5, 1.0, 2.0 and 5.0 ng/mL in methanol via dilution of the 0.1 µg/mL mixed fortification solution (section 3.5.2.b).

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

Initial Conc. (ng/mL)	Volume (mL)	Diluted to (mL)	Final Conc. (ng/mL)
100	5.0	100	5.0
100	2.0	100	2.0
100	1.0	100	1.0
5.0	10.0	100	0.5
2.0	10.0	100	0.2
1.0	10.0	100	0.1

The standards may be used for a period of one year (in 125-mL LDPE bottles) when stored refrigerated (at 2°C to 6°C).

4. METHOD

4.1. FLOW DIAGRAM

The flow diagram of the method is given below, followed by a detailed description of each step.

Method Flow Diagram

Weigh 1 g of liver or measure 1 mL of serum or urine
(fortify samples designated as matrix spikes and laboratory control spikes)
↓
Add 9 mL of water to liver, 19 mL of water to serum and urine, homogenize
↓
Remove 1 mL and add 5 mL of ACN, shake
↓
Centrifuge
↓
Decant supernatant into 35 mL of water
↓
Load onto conditioned SPE
↓
Elute with 2 mL methanol
↓
LC/MS/MS Analysis

4.2. SAMPLE PROCESSING

For liver samples, place frozen samples in a food processor and homogenize with dry ice. Then place samples in containers and leave open in frozen storage overnight to allow for CO_2 sublimation. Seal and place the samples in frozen storage below –10°C until time of extraction. No sample processing is needed for serum and urine samples. However, frozen serum and urine samples must be allowed to completely thaw to room temperature before use.

4.3. Batch Set up

a. A batch of samples should not contain more than 20 field samples.

b. Each batch of samples analyzed must include at least one control (method blank using control matrix) and two matrix controls fortified at known concentrations (typically 10 and 50 ng/g for liver or ng/mL for serum and urine) to verify procedural recovery for the batch.

c. At least one field sample in each batch must also be separately fortified at a known concentration and carried through the procedure to verify recovery. Additional samples in the batch may also be fortified if desired.

d. All samples require duplicate injections.

4.4. Sample Extraction

4.4.1. Liver Extraction

a. Weigh 1 g of liver sample into a 50 mL disposable centrifuge tube and fortify, if appropriate.
b. Add water to the sample for a final volume of 9 mL. Cap tightly.
c. Homogenize sample using a tissuemizer for ~1 minute.
d. Transfer 1 mL of the sample using a disposable pipette into 15 mL disposable centrifuge tubes. Add 5 mL of ACN and shake for ~20 minutes on a wrist action shaker.
e. Centrifuge tubes at ~3000 rpm for ~ 5 minutes. Carefully decant supernatant into a 50 mL disposable centrifuge tube and add 35 mL of water.
f. Load the sample onto a conditioned SPE column (for conditioning details, see section 4.4.3.). Discard the eluate. Any analyte residues will be trapped on the SPE column at this point.
g. Elute with 2 mL of methanol. Collect 2 mL of elute into a graduated 15 mL centrifuge tube.
h. Analyze samples using electrospray LC/MS/MS.

4.4.2. Serum and Urine Extraction

a. Measure 1 mL of serum or urine sample into a 50 mL disposable centrifuge tube and fortify, if appropriate.
b. Add 19 mL of water to sample. Cap tightly and vortex for ~1 minute. Then continue with steps d-h in section 4.4.1.

4.4.3. SPE Column Conditioning

Place the unconditioned SPE columns on the vacuum manifold. Condition the SPE columns by passing ~ 10 mL of methanol through the column followed by ~ 5 mL of water. The washes may be pulled through the SPE column using vacuum at a flow rate of ~1 drop/sec or may be allowed to pass through the column unaided. Discard all washes. Do not allow the column to dry.

4.5. Quantitation

4.5.1. LC/MS/MS System and Operating Conditions

Mass Spec: Micromass Quattro Ultima (Micromass)
Interface: Electrospray (Micromass)
Harvard infusion pump (Harvard Instruments), for tuning
Computer: COMPAQ Professional Workstation AP200
Software: Windows NT, Masslynx 3.3

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

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

HPLC Column: Genesis C_8 (Jones Chromatography), 2.1 mm x 50 mm, 4µ
Column Temperature: 35° C
Injection Volume: 15 µL
Mobile Phase (A): 2 mM Ammonium Acetate in Type I water
Mobile Phase (B): Methanol

Time	% A	% B	Flow Rate (mL/min)
0.0	90	10	0.3
2.0	90	10	0.3
5.0	10	90	0.3
9.0	10	90	0.3
9.5	0	100	0.3
14.0	0	100	0.3
14.5	90	10	0.3
20.0	90	10	0.3

It may be necessary to adjust the HPLC gradient in order to optimize instrument performance. Columns with different dimensions (e.g. 2.1 x 30) and also columns

from different manufacturers (Keystone Betasil C_{18} etc.) could be used, provided equivalent chromatography is obtained.

Ions monitored:

Analyte	Mode	Transition Monitored	Approximate Retention Time
PFHS	Negative	399 → 80	~8.2 min.
PFOS	Negative	499 → 99	~8.8 min
PFOA	Negative	413 → 369	~8.6 min

The retention times may vary, on a day to day basis, depending on the batch of mobile phase etc. Drift in retention times (up to ± 4 %) is acceptable within an analytical run, as long as the drift continues through the entire analysis and the standards are included at the beginning and end of the analytical run.

Note: An alternative LC/MS/MS system may be used once demonstrated to be equivalent.

The mass spectrometer is tuned for each analyte by infusing a ~ 1.0 µg/mL standard solution (at 10 µL/min, using an infusion pump) via a "T" into a stream of mobile phase containing 50% methanol and 50% 2mM ammonium acetate in water at 0.2 mL/min flow rate. Each analyte is initially tuned for the parent ion and then tuned for the product ion. Once the instrument is tuned, the optimized parameters are saved as a tune file. This tune file is then used during routine analysis.

4.5.2. Calibration Curve Procedures

a. Inject the same aliquot (between 10 to 50 µL) of each calibration standard (ranging from the lowest level standard to the highest level prepared), into the LC/MS/MS.

b. Use weighted linear standard curves for quantitation. Linear standard curves are generated for each analyte by linear regression using 1/x weighting of peak area versus calibration standard concentration using Masslynx (or equivalent) software system. Any calibration standard found to be a statistical outlier by using an appropriate outlier test, may be excluded from the calculation of the calibration curve. However, the total number of calibration standards that may be excluded must not exceed 20% of the total number of standards injected.

c. The correlation coefficient (R) for calibration curves generated must be ≥0.9925 (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.

Typical calibration curves for PFHS, PFOS and PFOA can be found in Figures 1-3.

4.5.3 Sample Analysis

a. Inject the same aliquot (between 10 to 50 µL) of each standard, sample, recovery, control, etc. into the LC/MS/MS system.

b. Standards corresponding to at least five or more concentration levels (starting with the LOQ level or below) must be included in an analytical set.

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

d. The concentration of each sample/fortification/control is determined from the standard curve, based on the peak area of each analyte. The standard responses should bracket responses of the residue found in each sample set. Results may be quantitated up to 10% outside the curve by extrapolation. If necessary, dilute the samples to give a response within the standard curve range.

e. Fortification recoveries falling within 70 to 130% are considered acceptable.

f. Samples must be stored refrigerated between 2°C to 6°C until analysis.

g. Samples in which either no peaks are detected or peaks less than the lowest concentration of the calibration standards are detected at the corresponding analyte retention time will be reported as ND (not detected). Samples in which peaks are detected at the corresponding analyte retention time that are less than the LOQ and greater than or equal to the lowest concentration of the calibration standards will be reported as NQ (not quantifiable).

The analysis performed during the method development included fortifications at 10 and 50 ng/g of PFHS in rat liver, 10 and 50 ng/mL of PFHS in serum, 10 and 50 ng/g of PFOS and PFOA in rat liver and 10 and 50 ng/mL of PFOS and PFOA in serum and urine. Typical chromatograms can be found in Figures 4-39.

4.6. Acceptance Criteria

The following criteria must be met to ensure the presence of PFHS, PFOS and PFOA:

1. The chromatogram must show a peak of a daughter ion at 80 amu from a parent of 399 amu for PFHS, a daughter ion at 99 amu from a parent of 499 amu for PFOS, and a daughter ion at 369 amu from a parent of 413 amu for PFOA.

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

3. Recoveries of control spikes and matrix spikes (if any) 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.

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.

5. The correlation coefficient (R) for calibration curves generated must be ≥0.9925 (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.

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.

4.7. Performance Criteria

The following two criteria must be performed once as a system suitability test, before the commencement of analysis, when using an instrumentation set-up that has not been used for this method.

First Criterion:
Run a standard solution on LC/MS/MS corresponding to the estimated LOQ (10 ng/mL) in matrix and obtain a signal to noise ratio for the analyte transition of at least 9:1, compared to a reagent blank. If this criterion cannot be met, optimize and change instrument operating parameters (or increase the injection volume, if appropriate).

Second Criterion:
Run a set of standards of five or more concentration levels, from at or below the LOQ, up to the highest concentration level to be included in the analysis. Generate a calibration curve for the analyte and obtain a linear regression with a coefficient of determination (R^2) of at least 0.985 for the analyte. Once this criterion is met, samples may be analyzed with standards interspersed.

4.8. Time Required for Analysis

One person can take a set of 20 samples through the sample preparation procedure in approximately 4 hours. The LC/MS/MS analysis of the set (containing 20 field samples, 1 matrix blank, 2 laboratory control spikes, 1 matrix spike and 12 standard injections) will take approximately 14 hours.

5. CALCULATIONS

a. Use Equation 1 to calculate the amount of analyte found (in ng/mL, based on peak area) using the standard curve (1/x weighted linear regression parameters) generated by the Masslynx software program.

Equation 1:

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

DF = factor by which the final volume was diluted, if necessary.
Aliquot factor= 9 for liver, 20 for serum and urine

b. For samples fortified with known amounts of analyte prior to extraction, use Equation 2 to calculate the percent recovery.

Equation 2:

Recovery (%) =

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

Note: Subtract analyte found in control (ng/mL) from analyte found (ng/mL), if ng/mL in control is greater than LOQ.

c. Use Equation 3 to calculate the amount of analyte found (in ppb)

Equation 3:

$$\text{Analyte found (ng/g or ng/mL)} = \frac{\text{(analyte found (ng/mL) x FV (mL)}}{\text{sample weight (g) or sample volume (mL)}}$$

FV = final volume

For reporting purposes, samples in which either no peaks are detected or peaks less than the lowest concentration of the calibration standards are detected at the corresponding analyte retention time will be reported as ND (not detected). Samples in which peaks are detected at the corresponding analyte retention time that are less than the LOQ and greater than or equal to the lowest concentration of the calibration standards will be reported as NQ (not quantifiable).

6. SAFETY

The analyst should read the material safety data sheets for all standards and reagents before performing this method. Use universal precautions when handling standards and reagents, including working in fume hoods and wearing laboratory coats, safety glasses, and gloves.

Table 1. Recovery Summary of PFHS in Rat Liver and Serum

Recovery Summary of PFHS in Rat Liver

Sample ID	Analyte Added (ng/g)	Percent Recovery (%)
0201684 Spk A	10	108
0201684 Spk B	10	110
0201684 Spk C	10	128
	Average:	115
	Standard Deviation:	9.9

Sample ID	Analyte Added (ng/g)	Percent Recovery (%)
0201684 Spk D	50	101
0201684 Spk E	50	98
0201684 Spk F	50	94
	Average:	98
	Standard Deviation:	3.5

Recovery Summary of PFHS in Rat Serum

Sample ID	Analyte Added (ng/mL)	Percent Recovery (%)
0201682 Spk A	10	112
0201682 Spk B	10	103
0201682 Spk C	10	110
	Average:	108
	Standard Deviation:	4.7

Sample ID	Analyte Added (ng/mL)	Percent Recovery (%)
0201682 Spk D	50	107
0201682 Spk E	50	104
0201682 Spk F	50	122
	Average:	111
	Standard Deviation:	9.6

Table 2. Recovery Summary of PFOS in Rat Liver, Serum and Urine

Recovery Summary of PFOS in Rat Liver

Sample ID	Analyte Added (ng/g)	Percent Recovery (%)
0201684 Spk A	10	88
0201684 Spk B	10	95
0201684 Spk C	10	105
	Average:	96
	Standard Deviation:	8.8

Sample ID	Analyte Added (ng/g)	Percent Recovery (%)
0201684 Spk D	50	90
0201684 Spk E	50	88
0201684 Spk F	50	87
	Average:	88
	Standard Deviation:	1.5

Recovery Summary of PFOS in Rat Serum

Sample ID	Analyte Added (ng/mL)	Percent Recovery (%)
0201682 Spk A	10	80
0201682 Spk B	10	85
0201682 Spk C	10	99
	Average:	88
	Standard Deviation:	9.8

Sample ID	Analyte Added (ng/mL)	Percent Recovery (%)
0201682 Spk D	50	118
0201682 Spk E	50	122
0201682 Spk F	50	121
	Average:	120
	Standard Deviation:	2.1

Recovery Summary of PFOS in Rat Urine

Sample ID	Analyte Added (ng/mL)	Percent Recovery (%)
0201682 Spk A	10	98
0201682 Spk B	10	89
0201682 Spk C	10	91
	Average:	93
	Standard Deviation:	4.7

Sample ID	Analyte Added (ng/mL)	Percent Recovery (%)
0201682 Spk D	50	80
0201682 Spk E	50	78
0201682 Spk F	50	78
	Average:	79
	Standard Deviation:	1.2

Table 3. Recovery Summary of PFOA in Rat Liver, Serum and Urine

Recovery Summary of PFOA in Rat Liver

Sample ID	Analyte Added (ng/g)	Percent Recovery (%)
0201684 Spk A	10	96
0201684 Spk B	10	101
0201684 Spk C	10	99
	Average:	98
	Standard Deviation:	3.1

Sample ID	Analyte Added (ng/g)	Percent Recovery (%)
0201684 Spk D	50	97
0201684 Spk E	50	92
0201684 Spk F	50	94
	Average:	94
	Standard Deviation:	2.5

Recovery Summary of PFOA in Rat Serum

Sample ID	Analyte Added (ng/mL)	Percent Recovery (%)
0201682 Spk A	10	116
0201682 Spk B	10	117
0201682 Spk C	10	115
	Average:	117
	Standard Deviation:	1.5

Sample ID	Analyte Added (ng/mL)	Percent Recovery (%)
0201682 Spk D	50	107
0201682 Spk E	50	112
0201682 Spk F	50	115
	Average:	111
	Standard Deviation:	4.0

Recovery Summary of PFOA in Rat Urine

Sample ID	Analyte Added (ng/mL)	Percent Recovery (%)
0201682 Spk A	10	86
0201682 Spk B	10	91
0201682 Spk C	10	89
	Average:	89
	Standard Deviation:	2.5

Sample ID	Analyte Added (ng/mL)	Percent Recovery (%)
0201682 Spk D	50	89
0201682 Spk E	50	88
0201682 Spk F	50	85
	Average:	87
	Standard Deviation:	2.1

Study Plan: ExP-023-082
Exygen Study No.: 023-082

Exygen Method No: ExM-023-071

Figure 1. Calibration Curve for PFHS

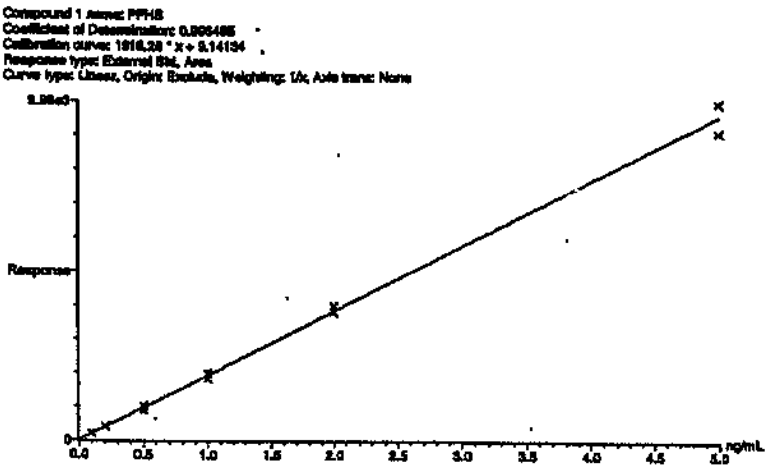


Figure 2. Calibration Curve for PFOS

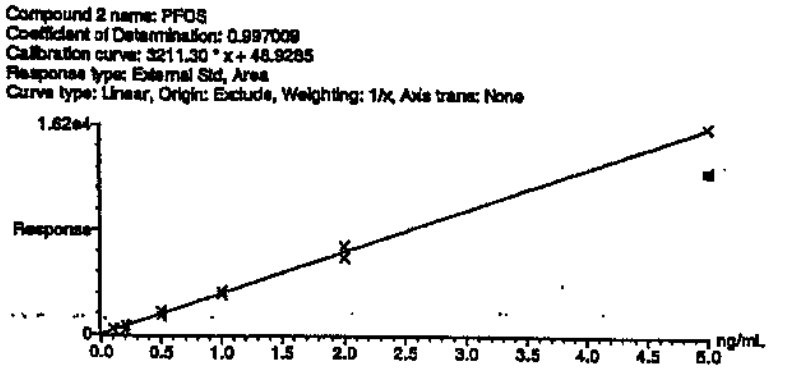


Figure 3. Calibration Curve for PFOA

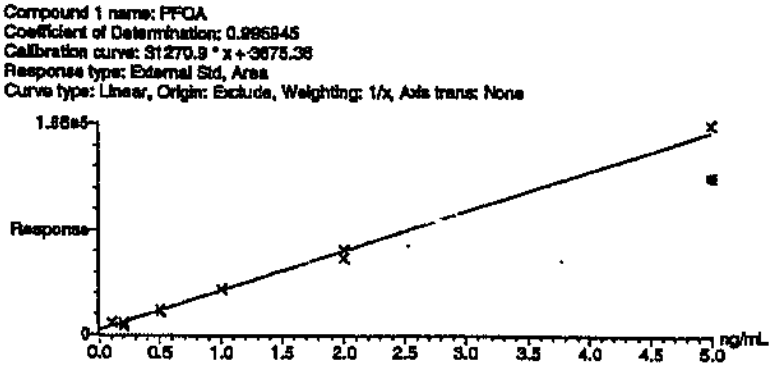


Figure 4. Representative Chromatogram of a 0.1 ng/mL Standard Containing PFHS

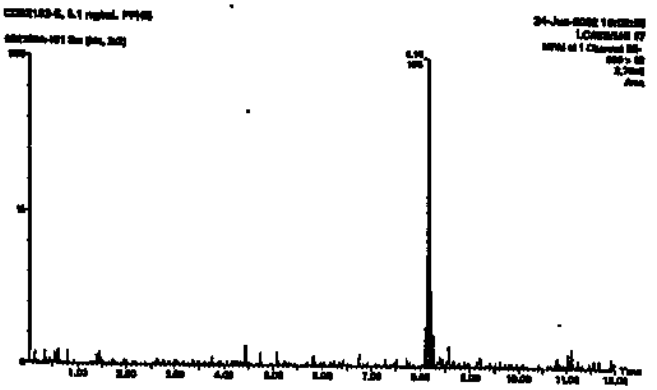


Figure 5. Representative Chromatogram of a 0.1 ng/mL Standard Containing PFOS

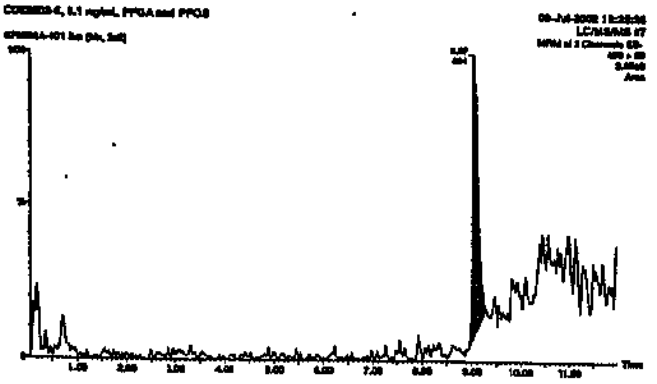


Figure 6. Representative Chromatogram of a 0.1 ng/mL Standard Containing PFOA

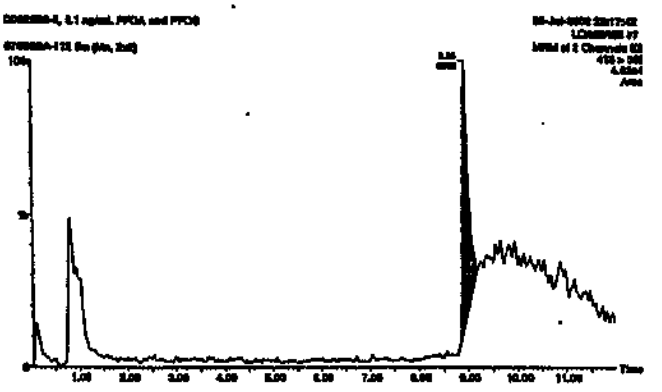


Figure 7. Representative Chromatogram of a 0.5 ng/mL Standard Containing PFHS

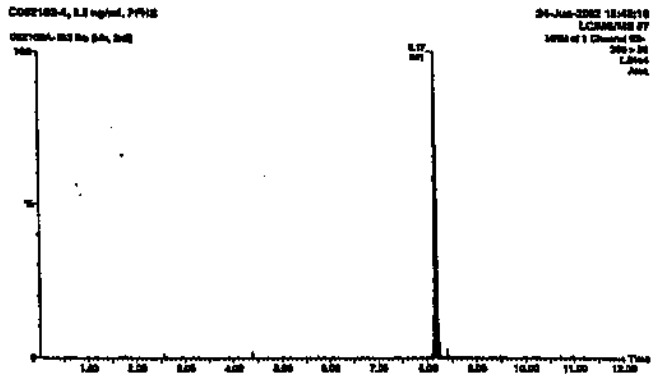


Figure 8. Representative Chromatogram of a 0.5 ng/mL Standard Containing PFOS

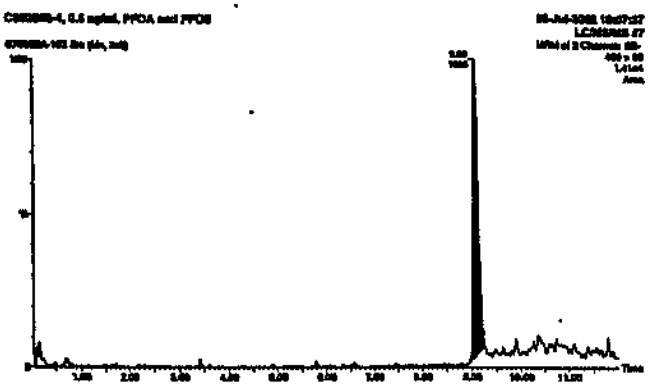


Figure 9. Representative Chromatogram of a 0.5 ng/mL Standard Containing PFOA

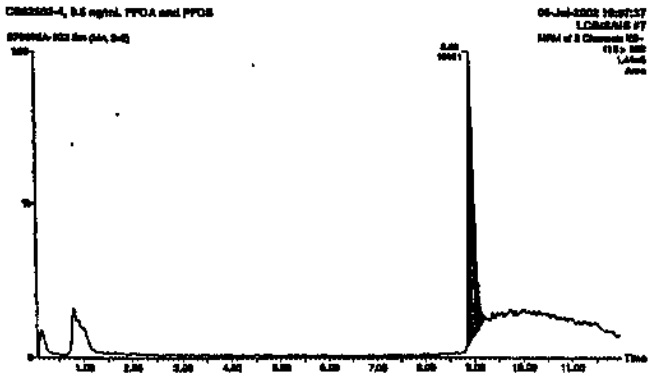


Figure 10. Representative Chromatogram of a 5.0 ng/mL Standard Containing PFHS

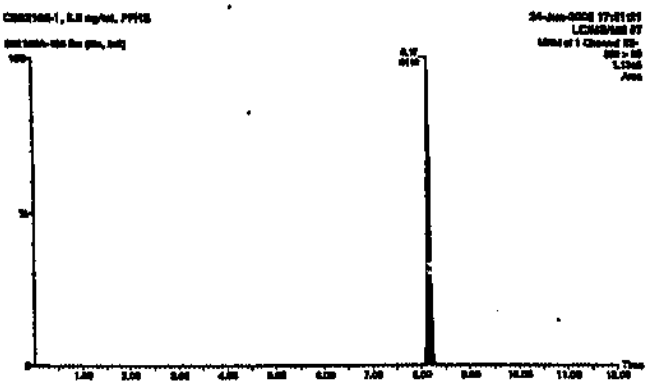


Figure 11. Representative Chromatogram of a 5.0 ng/mL Standard Containing PFOS

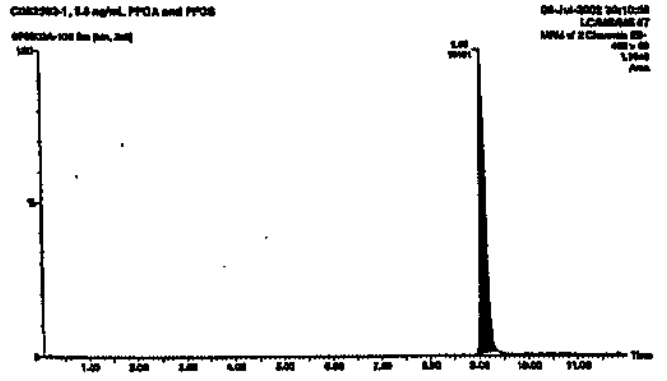


Figure 12. Representative Chromatogram of a 5.0 ng/mL Standard Containing PFOA

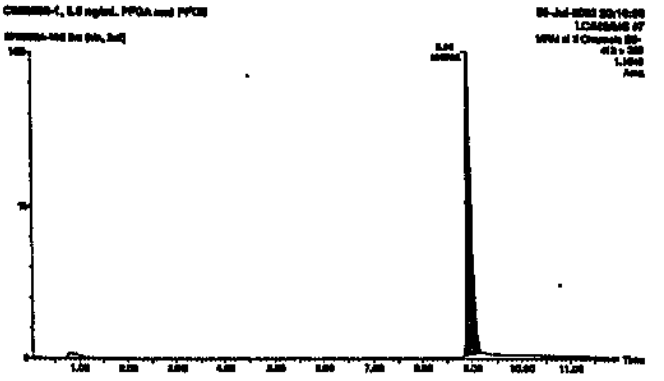


Figure 13. Representative Chromatogram of a Reagent Blank Sample Analyzed for PFHS

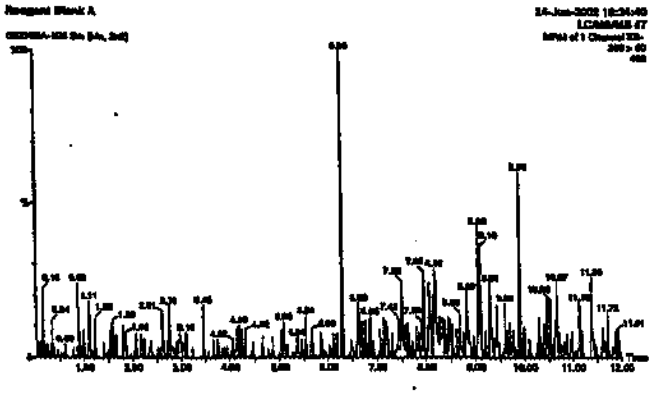


Figure 14. Representative Chromatogram of a Reagent Blank Sample Analyzed for PFOS

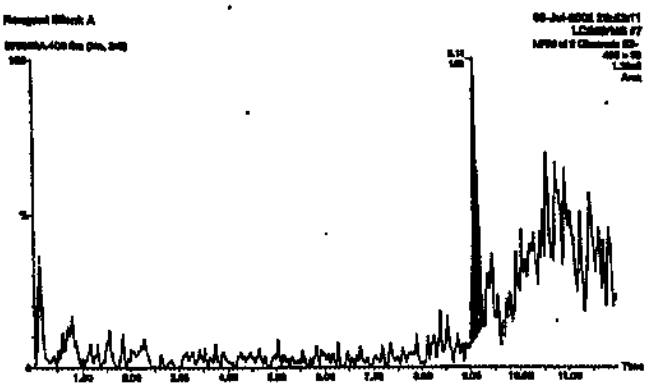


Figure 15. Representative Chromatogram of a Reagent Blank Sample Analyzed for PFOA

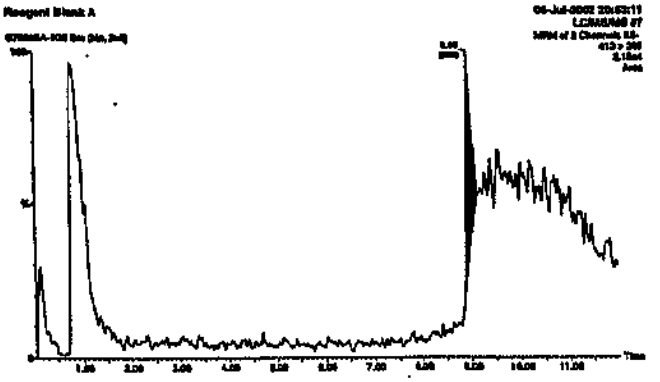


Figure 16. Representative Chromatogram of a Control Liver Sample Analyzed for PFHS

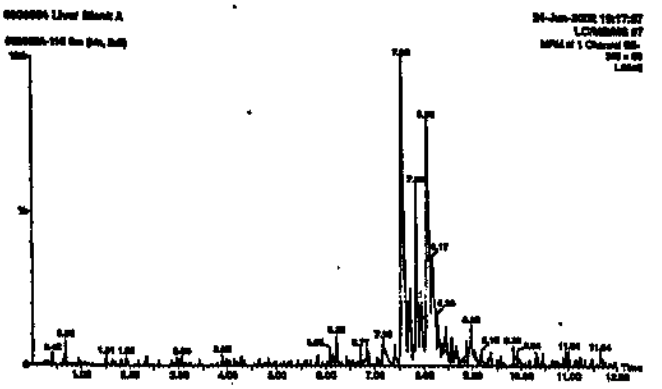


Figure 17. Representative Chromatogram of a Control Liver Sample Analyzed for PFOS

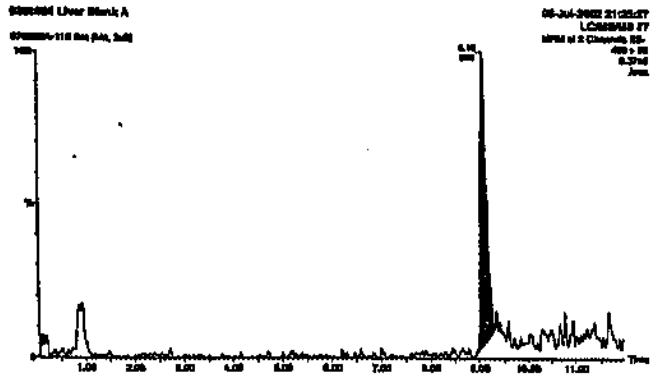


Figure 18. Representative Chromatogram of a Control Liver Sample Analyzed for PFOA

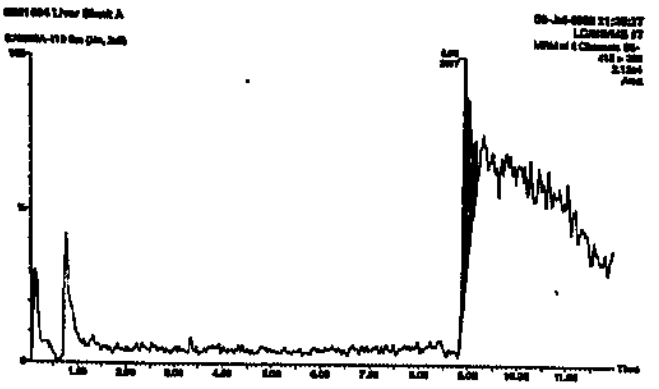


Figure 19. Representative Chromatogram of a Control Serum Sample Analyzed for PFHS

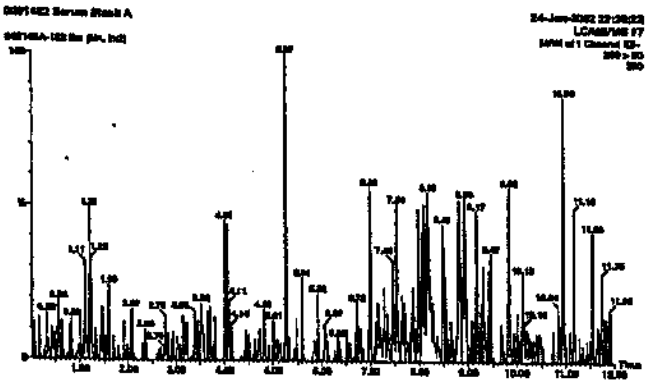


Figure 20. Representative Chromatogram of a Control Serum Sample Analyzed for PFOS

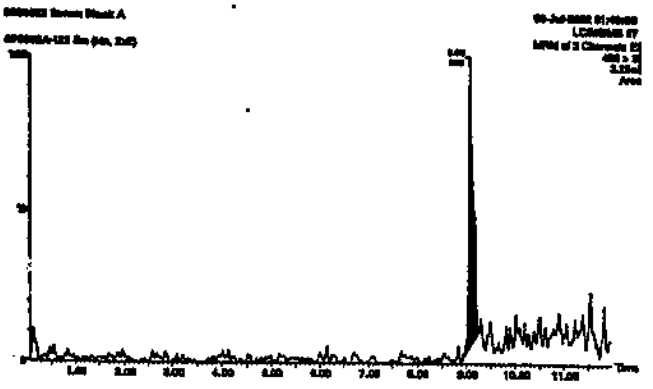


Figure 21. Representative Chromatogram of a Control Serum Sample Analyzed for PFOA

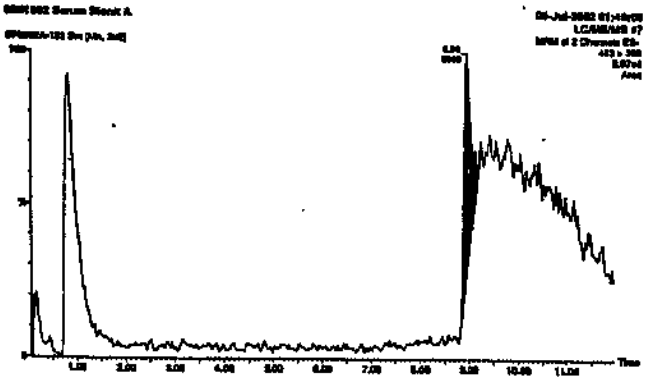


Figure 22. Representative Chromatogram of a Control Urine Sample Analyzed for PFOS

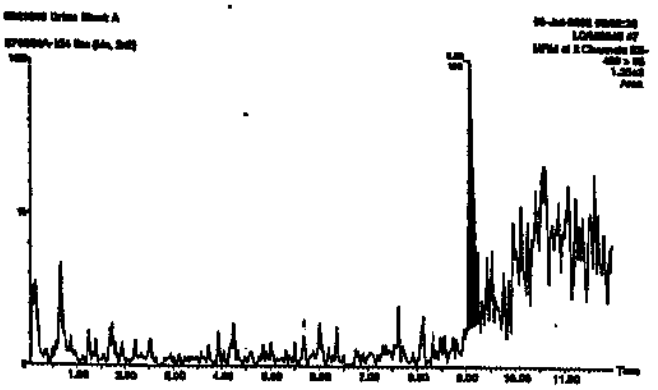


Figure 23. Representative Chromatogram of a Control Urine Sample Analyzed for PFOA

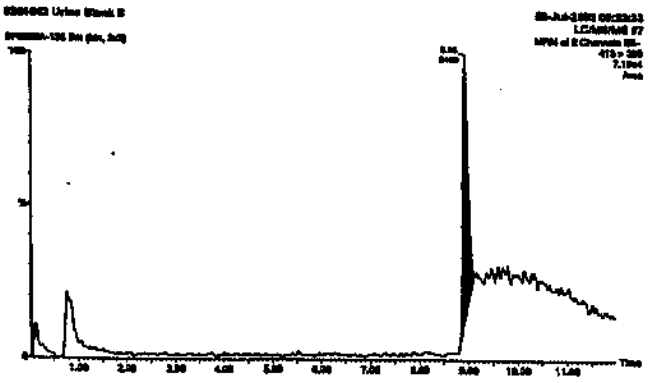


Figure 24. Representative Chromatogram of a Control Liver Sample Fortified at 10 ng/g with PFHS

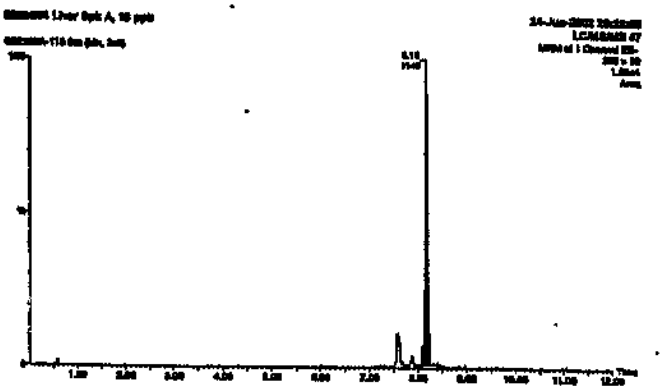


Figure 25. Representative Chromatogram of a Control Liver Sample Fortified at 10 ng/g with PFOS

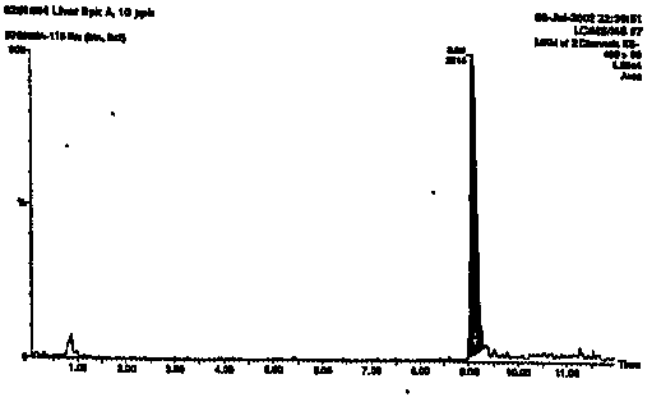


Figure 26. Representative Chromatogram of a Control Liver Sample Fortified at 10 ng/g with PFOA

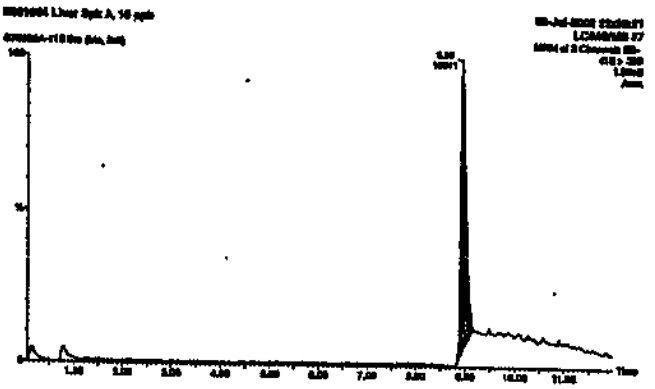


Figure 27. Representative Chromatogram of a Control Liver Sample Fortified at 50 ng/g with PFHS

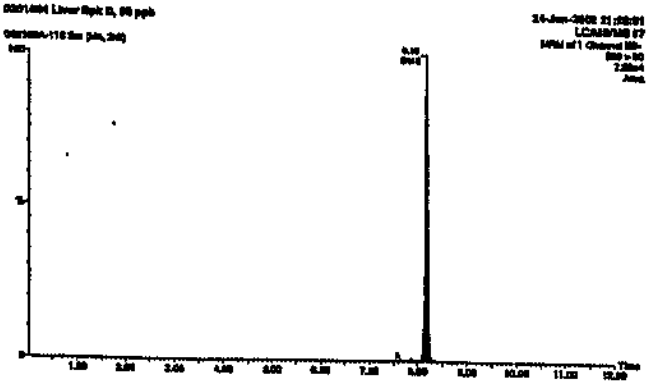


Figure 28. Representative Chromatogram of a Control Liver Sample Fortified at 50 ng/g with PFOS

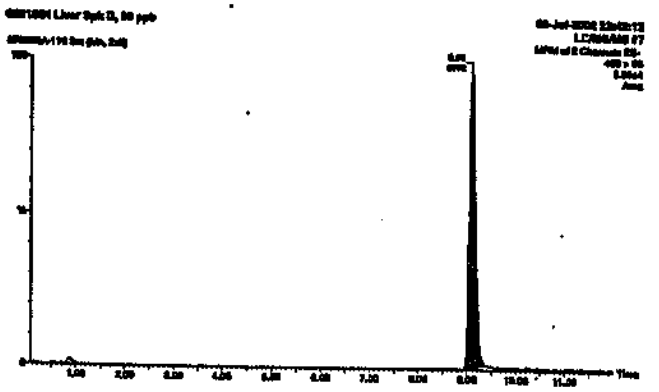


Figure 29. Representative Chromatogram of a Control Liver Sample Fortified at 50 ng/g with PFOA

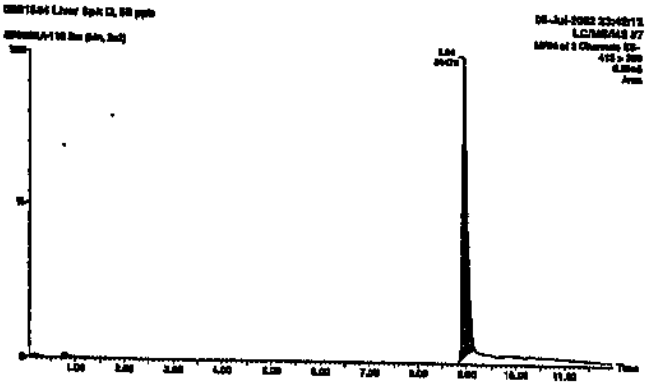


Figure 30. Representative Chromatogram of a Control Serum Sample Fortified at 10 ng/mL with PFHS

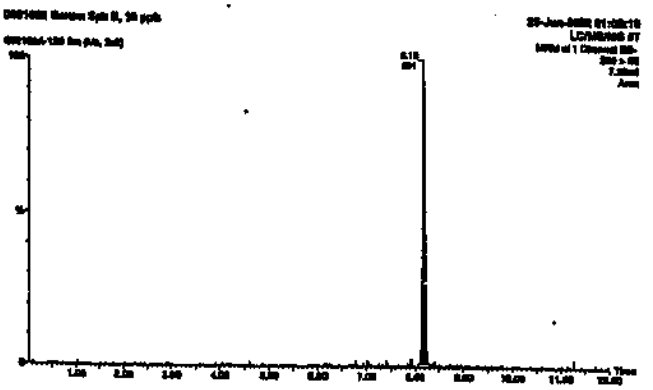


Figure 31. Representative Chromatogram of a Control Serum Sample Fortified at 10 ng/mL with PFOS

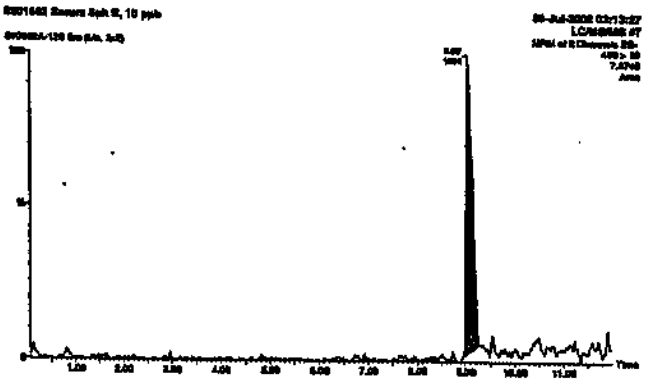


Figure 32. Representative Chromatogram of a Control Serum Sample Fortified at 10 ng/mL with PFOA

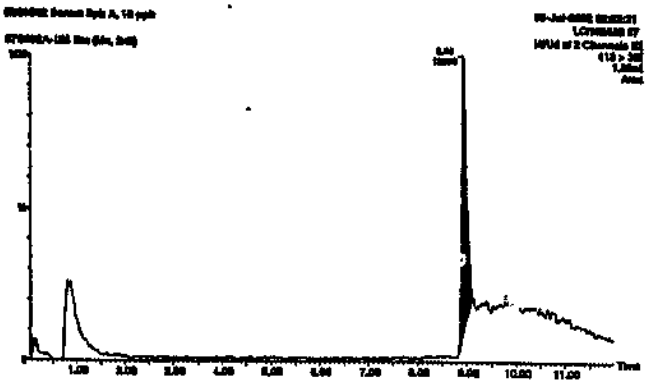


Figure 33. Representative Chromatogram of a Control Serum Sample Fortified at 50 ng/mL with PFHS

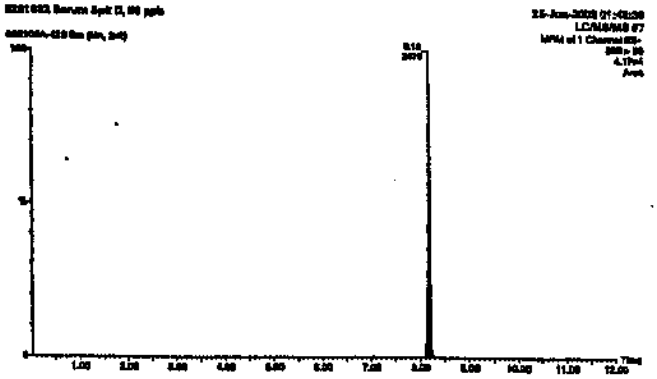


Figure 34. Representative Chromatogram of a Control Serum Sample Fortified at 50 ng/mL with PFOS

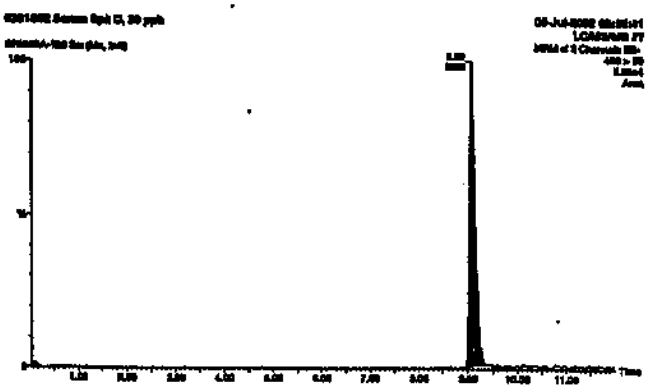


Figure 35. Representative Chromatogram of a Control Serum Sample Fortified at 50 ng/mL with PFOA

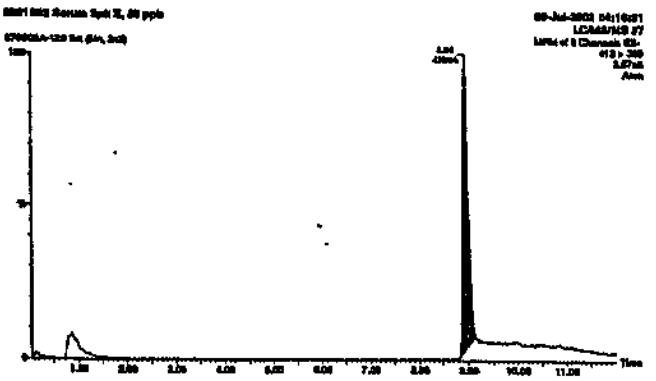


Figure 36. Representative Chromatogram of a Control Urine Sample Fortified at 10 ng/mL with PFOS

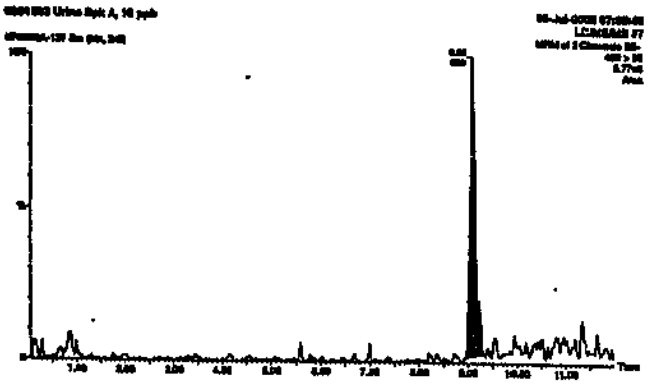


Figure 37. Representative Chromatogram of a Control Urine Sample Fortified at 10 ng/mL with PFOA

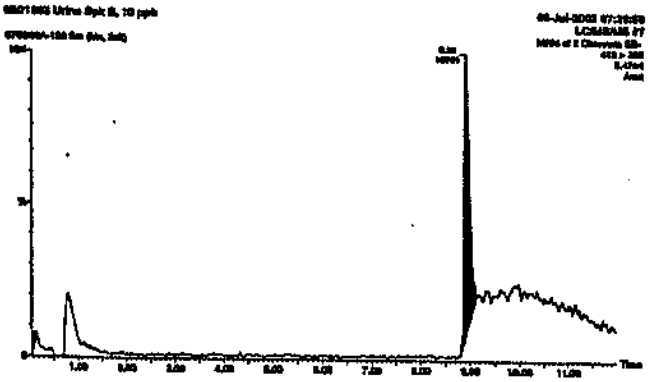


Figure 38. Representative Chromatogram of a Control Urine Sample Fortified at 50 ng/mL with PFOS

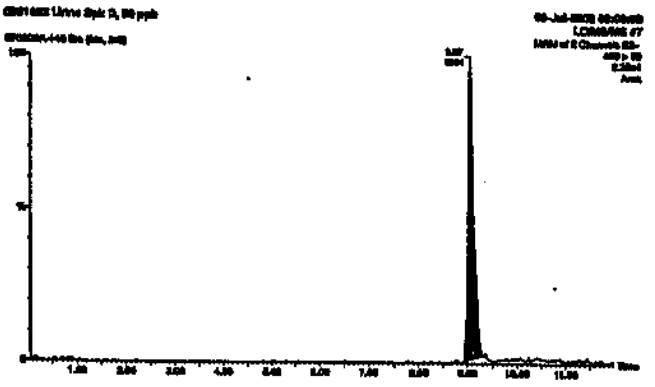
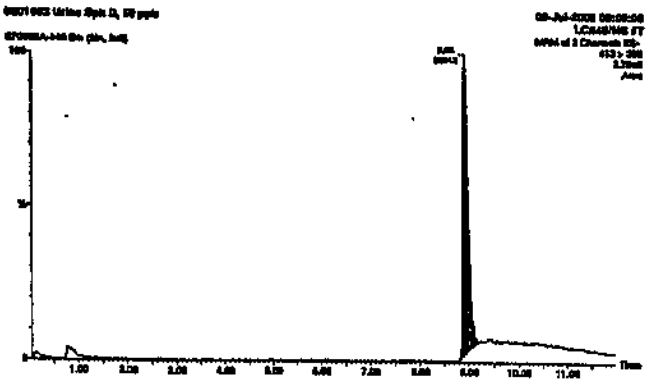


Figure 39. Representative Chromatogram of a Control Urine Sample Fortified at 50 ng/mL with PFOA



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PROTOCOL DEVIATION

Deviation Number: 1
Date of Occurrence: 10/15/02

Exygen Study Number: 023-082 Protocol Number: ExP-023-082

DESCRIPTION OF DEVIATION

Analytical Procedure Summary-II. Calibration Standards

Did not prepare extracted standards in human plasma at 1.5 and 2.5 ppb levels.

ACTIONS TAKEN
for example - deviation issued, SOP revision, etc.

Protocol deviation issued.

Recorded By: Emily R Decker Date: 10/23/02

IMPACT ON STUDY

No negative impact because only had limited sample available and the linear range of the curve was still established without those two levels.

N/A
Principal Investigator Signature — Date

Emily R Decker
Study Director Signature — Date 10/30/02

John M Flaherty
Management Signature — Date 10/30/02

(Sponsor) — Date

Exygen QAU Review NCL 10/31/02

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PROTOCOL DEVIATION

Deviation Number: 2
Date of Occurrence: 10/17/02

Exygen Study Number: 023-082 Protocol Number: ExP-023-082

DESCRIPTION OF DEVIATION

Analytical Procedure Summary-I. Method Summary.e.

Eluted with 5 mL of methanol and evaporated to less than 1 mL using a nitrogen evaporator and then brought final volume up to 1 mL with methanol.

ACTIONS TAKEN
for example - deviation issued, SOP revision, etc.

Protocol deviation issued.

Recorded By: Emily R Decker Date: 10/23/02

IMPACT ON STUDY

No negative impact because final concentration remained the same, but complete elution of the analytes was established.

N/A — Principal Investigator Signature — Date

Emily R Decker — Date 10/30/02
Study Director Signature

John M Flaherty — Date 10/30/02
Management Signature

(Sponsor) — Date

Exygen QAU Review — Date 10/30/02

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PROTOCOL DEVIATION

Deviation Number: 3
Date of Occurrence: throughout study

Exygen Study Number: 023-082 Protocol Number: ExP-023-082

DESCRIPTION OF DEVIATION

1.Analytical Procedure Summary-Method Section 4.5.3.d---several samples were quantified above 10% of the curve.

2.Analytical Procedure Summary-Method Section 4.5.3.e---the acceptance critieria for QC spike recoveries could not always be met.

ACTIONS TAKEN
for example - deviation issued, SOP revision, etc.

1-2.Protocol deviation issued.

Recorded By: Emily R. Decker Date: 10/30/02

IMPACT ON STUDY

1.No negative impact because residue was < than 15% above the curve for C6 and C8 for sample 0204493 Spk V and for C8 for samples 0203965 and 0203965 Dup. Also, the samples that were quantified outside the curve for THPFOS and THPFDS were only fortification recoveries and the majority of the recoveries were > 100%.

2.Impact not determined only documented since this method has not been validated for these anions at these levels.

N/A — Principal Investigator Signature — Date

Emily R. Decker — Date 10/30/02
Study Director Signature

John M Flaherty — Date 10/30/02
Management Signature

(Sponsor) — Date

Exygen QAU Review — Date 10/30/02

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PROTOCOL DEVIATION

Deviation Number: 4
Date of Occurrence: throughout study

Exygen Study Number: 023-082 Protocol Number: ExP-023-082

DESCRIPTION OF DEVIATION

1.Analytical Procedure Summary-Method Section 4.5.3.g---several samples were quantified below the curve and ND was used for when no peak was detected and NQ was used for negative results. All other results were reported.

2.Analytical Procedure Summary-Method Section 4.5.3.c---a second set of calibration standards was not analyzed in a set.

ACTIONS TAKEN
for example - deviation issued, SOP revision, etc.

1-2.Protocol deviation issued.

Recorded By: Emily R Decker Date: 10/30/02

IMPACT ON STUDY

1.No negative impact because no claim of quantitative accuracy has been established.

2.No negative impact because the extracted standards were analyzed throughout the set which provide a QC check on the initial calibration.

N/A
Principal Investigator Signature — Date

Emily R Decker
Study Director Signature — Date 10/30/02

John M Flaherty
Management Signature — Date 10/30/02

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PROTOCOL DEVIATION

Deviation Number: 5
Date of Occurrence: throughout study

Exygen Study Number: 023-082 Protocol Number: ExP-023-082

DESCRIPTION OF DEVIATION

1. Analytical Procedure Summary-Method Section 4.5.1---the transition monitored for PFOS was 499 -> 80

ACTIONS TAKEN
for example - deviation issued, SOP revision, etc.

1. Protocol deviation issued.

Recorded By: Emily R Decker Date: 10/30/02

IMPACT ON STUDY

1. No negative impact.

N/A
Principal Investigator Signature — Date

Emily R Decker
Study Director Signature — Date 10/30/02

John M Rakert
Management Signature — Date 10/30/02

(Sponsor) — Date

Exygen QAU Review NLC 10/30/02

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PROTOCOL DEVIATION

Deviation Number: 1
Date of Occurrence: 10/15/02

Exygen Study Number: 023-082 Protocol Number: ExP-023-082

DESCRIPTION OF DEVIATION

Analytical Procedure Summary-II - Calibration Standards

Did not prepare extracted standards in human plasma at 1.5 and 2.5 ppb levels.

ACTIONS TAKEN
for example: physician issued; SOP revision, etc.

Protocol deviation issued.

Recorded By: Emily R Decker Date: 10/23/02

IMPACT ON STUDY

No negative impact because only had limited sample available and the linear range of the curve was still established without those two levels.

N/A —

Principal Investigator Signature: Emily R Decker Date: 10/30/02

Study Director Signature: John M Flaherty Date: 10/30/02

Management Signature (Sponsor): Date: 10/30/02

Exygen QAU Review: NEC 11/30/02

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PROTOCOL DEVIATION

Deviation Number: 2.
Date of Occurrence: 10/17/02

Exygen Study Number: 023-082 Protocol Number: ExP-023-082

DESCRIPTION OF DEVIATION

Analytical Procedure Summary-i. Method Summary, e.

Eluted with 5 mL of methanol and evaporated to less than 1 mL using a nitrogen evaporator and then brought final volume up to 1 mL with methanol.

ACTIONS TAKEN
(for example - deviation report, SOP revision, etc.)

Protocol deviation issued.

Recorded by: Timothy P. Decker Date: 10/23/02

IMPACT ON STUDY

No negative impact because final concentration remained the same, but complete elution of the analytes was established.

N/A

Principal Investigator Signature: Timothy P. Decker Date: 10/30/02

Study Director Signature: John M. Parker Date: 10/30/02

Management Signature (Sponsor): Date: 10/30/02

Exygen QAU Review: [illegible] 10/30/02

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PROTOCOL DEVIATION

Deviation Number: 3
Date of Occurrence: throughout study

Exygen Study Number: 023-082 Protocol Number: Exp-023-082

DESCRIPTION OF DEVIATION

1.Analytical Procedure Summary-Method Section 4.5.3.d—several samples were quantified above 100% of the curve.

2.Analytical Procedure Summary-Method Section 4.5.3.e—the acceptance criteria for QC spike recoveries could not always be met.

ACTIONS TAKEN
for example - deviation caused SOP revision, etc.

1-2.Protocol deviation issued.

Recorded By: Emily R Decker Date: 10/30/02

IMPACT ON STUDY

1.No negative impact because residue was < than 15% above the curve for C6 and C8 for sample 0204493 Spk V and for C8 for samples 0203963 and 0203963 Dup. Also, the samples that were quantified outside the curve for THPFOS and THPFDS were only fortification recoveries and the majority of the recoveries were > 100%.

2.Impact not determined only documented since this method has not been validated for these anions at these levels.

N/A

Principal Investigator Signature: Emily R Decker Date: 10/30/02

Study Director Signature: John M Rehert Date: 10/30/02

Management Signature (Sponsor): Date: 10/30/02

Exygen QAU Review: QAU 10/30/02

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PROTOCOL DEVIATION

Deviation Number: 4
Date of Occurrence: throughout study

Exygen Study Number: 023-082 Protocol Number: ExP-023-082

DESCRIPTION OF DEVIATION

1. Analytical Procedure Summary-Method Section 4.5.3.g—several samples were quantified below the curve and ND was used for when no peak was detected and NQ was used for negative results. All other results were reported.

2. Analytical Procedure Summary-Method Section 4.5.3.c---a second set of calibration standards was not analyzed in a set.

ACTIONS TAKEN
(for example: deviation noted; SOP revision, etc.

1-2. Protocol deviation issued.

Recorded By: [signature] Date: 10/30/02

IMPACT ON STUDY

1. No negative impact because no claim of quantitative accuracy has been established.

2. No negative impact because the extracted standards were analyzed throughout the set which provide a QC check on the initial calibration.

N/A

Principal Investigator Signature: [signature] Date: 10/30/02

Study Director Signature: [signature] Date: 10/30/02

Management Signature (Sponsor): [signature] Date: 10/30/02

Exygen QAU Review: NLV 10/30/02

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PROTOCOL DEVIATION

Deviation Number: 5
Date of Occurrence: throughout study

Exygen Study Number: 023-082 Protocol Number: ExP-023-082

DESCRIPTION OF DEVIATION

1. Analytical Procedure Summary-Method Section 4.5.1--the transition monitored for PFOS was 499 -> 80

ACTION TAKEN
(re: protocol deviation report, SOP revision, etc.)

1. Protocol deviation issued.

Recorded By: Kevin P Dooley Date: 10/30/02

IMPACT ON STUDY

1. No negative impact.

N/A

Principal Investigator Signature: Kevin P Dooley Date: 10/30/02

Study Director Signature: John M Fickert Date: 10/30/02

Management Signature (Sponsor): Date: 10/30/02

Exygen QAU Review: MLC 10/30/02

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