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December 16, 2002

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Office of Pollution Prevention and Toxics (OPPT)
US Environmental Protection Agency
EPA East, Room 6428
1201 Constitution Avenue, NW
Washington, DC 20460



FYI-00-001378

Attention: Docket No. AR-226 and the FYI Docket

Dear Sir or Madam:

This continues 3M's voluntary submissions of data on perfluorooctane sulfonates and related compounds, as part of our ongoing dialogue with EPA regarding fluorochemicals.

Included in this submission are the following:

- "Biodegradation Study Report: Biodegradation Screen Study for Telomer Type Alcohol", Pace Analytical Services, Inc. Study Number CA085, 3M Sponsor Study Number E01-0684.
- "Comparative Analysis of Fluorochemicals in Human Serum Samples Collected by 3M and Obtained Commercially", 3M Environmental Laboratory Study Number E02-1053.
- "Analysis of Endogenous Fluorochemicals in Normal Pooled Human Serum and Plasma", 3M Environmental Laboratory Study Number E02-1039.
- "Analysis of Pooled Human Sera and Plasma and Monkey Sera for Fluorocarbons Using Exygen Method ExM-023-071", Exygen Research Study Number 023-082, 3M Sponsor Study.

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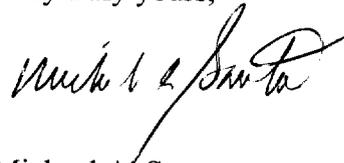
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Page 2
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The analytical method used for the respective analytical studies has been fully validated for PFOS in rat liver and plasma matrices down to 50 ppb. Limited cross validation for PFOS, PFOA and the other perfluorinated carboxylates in human sera and plasma down to 0.1 ppb was performed. Additional validation is recommended.

We will continue to provide information as it becomes available. Please feel free to contact me if you have any questions.

Very truly yours,



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226 - 1150

Analytical Laboratory Report Title

Comparative Analysis of Fluorochemicals in Human Serum Samples Obtained Commercially

Data Requirement

Not Applicable

Author

Lisa Stevenson

Study Completion Date

11/13/02

Performing Laboratory

Extractions and Analyses

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

Project Identification

Analytical Report: E02-1053

Total Number of Pages

52

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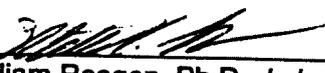
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Compliance Statement

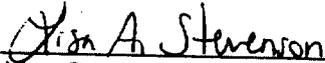
Analytical Laboratory Report Title: Comparative Analysis of Fluorochemicals in
Human Serum Samples Obtained Commercially

Study Identification Number: E02-1053

This study was not conducted under Good Laboratory Practices.



William Reagen, Ph.D., *Laboratory Management, Sponsor Representative* 11/13/02
Date



Lisa Stevenson, *Principal Analytical Investigator* 11/13/02
Date

Quality Assurance Statement

Analytical Laboratory Report Title: Comparative Analysis of Fluorochemicals in Human Serum Samples Obtained Commercially

Study Identification Number: E02-1053

This study has been inspected by the 3M Environmental Laboratory Quality Assurance Unit (QAU) as indicated in the following table. The findings were reported to the Principal Analytical Investigator (PAI) and laboratory management.

Inspection Dates	Phase	Date Reported to	
		Management	PAI
10/17/02	Sample Spiking	10/18/02	10/18/02
10/22/02	Analysis	10/23/02	10/23/02
10/30/02, 10/31/02, 11/07-08/02	Data	11/01/02, 11/08/02	11/01/02, 11/08/02
11/01/02, 11/07-08/02	Draft report	11/01/02, 11/08/02	11/01/02, 11/08/02

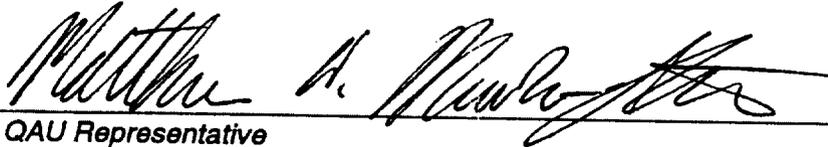

11/13/02
 QAU Representative Date

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Study Personnel and Contributors

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Analytical Chemistry Laboratory

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3M Environmental Laboratory (3M Lab)

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*Contract lab professional service employee

Study Initiation: 10/16/02

Study Completion: 11/13/02

Location of Archives

All original raw data and analytical report have been archived at the 3M Environmental Laboratory according to 3M Standard Operating Procedures. The analytical reference standard reserve samples are archived at the 3M Environmental Laboratory according to 3M Standard Operating Procedures. Remaining specimens pertaining to the analytical phase of this study will be archived at 3M Environmental Laboratory for as long as the quality of the preparation affords evaluation.

Executive Summary

A screening study was undertaken to compare branched and linear isomers of perfluorooctanoate (PFOA - $C_7F_{15}COO^-$) in 4 lots of commercial pooled human sera with concentrations ranging from 0.65 – 5.6 ng/mL PFOA.

Results from this study showed a wide distribution of the percentage of branched isomers of PFOA compared to the linear isomer of PFOA in the commercial pooled populations (Table 1). Since this is a preliminary screening study a larger sample size would be needed to determine if a significant statistical difference exists between pooled human sera samples.

As shown in Table 1, two of the lots of commercial pooled sera showed the presence of branched isomers of PFOA while the other two lots showed a much lower percentage (by at least 40x) of PFOA branched isomers.

Table 1. PFOA Data Summary of Pooled Serum

Sample	Identification	Branched Isomer Area	Linear Isomer Area	% Branched/ Sum Branched + Linear
Pooled Human Serum	TCR-687-Bioresource Lot 020821	<361*	114603	<0.31
	TCR-688-Lampire Lot X324B	8059	41466	16
	TCR-689-Sigma Lot 022K0965	8824	55100	14
	TCR-690-Golden West Lot G01406042	<361*	168154	<0.21

* Area threshold found as 361 in the extracted PFOA-Acid 0.534 ng/mL standard.

Introduction and Purpose

The purpose of the study is to determine the relative isomer ratios of PFOA fluorochemical in 4 lots of commercial pooled human sera with concentrations ranging from 0.65 – 5.6 ng/mL PFOA. The PFOA concentrations were determined in study E02-1039. Analyses of sera extracts for determining the relative isomer ratios of PFOA were completed by the 3M Environmental Laboratory under study number E02-1053, and the results of these analyses are presented in this report. The analytical portion of this study was initiated on 16 October 2002.

Specimen Receipt and Maintenance

The 3M Environmental Laboratory received pooled human sera samples collected from various commercial vendors in July and August, 2002. All specimens were received frozen in good condition on dry ice. All specimens were immediately transferred to storage at $-20^{\circ}\text{C} \pm 10^{\circ}\text{C}$, and maintained at that temperature except when removed for extraction and analysis as described in the method. The samples were kept isolated from the test materials (analytical standards) during storage.

Table 2. Pooled human serum samples received from commercial vendors in July and August, 2002

Samples	Identification	Lot #
Pooled Human Serum	TCR-687-Bioresource	020821
	TCR-688-Lampire	X324B
	TCR-689-Sigma	022K0965
	TCR-690-Golden West	G01406042

The control matrix used in sera analyses performed during E02-1053 was obtained from a commercial source and is presented in Appendix A. Samples analyzed at the 3M Environmental Laboratory will be stored and maintained following 3M Standard Operating Procedures.

Chemical Characterization of the Reference Substances

Perfluorooctanoate Ammonium Salt (PFOA-NH4)

CAS Number: 3825-26-1

Chemical Formula: $C_7F_{15}CO_2^-NH_4^+$

Molecular Weight: 431

This chemical is a 3M electrochemical fluorination production lot and contains, as determined by NMR, approximately 20% branched:80% linear isomers by weight.

Perfluorooctanoate-Acid (PFOA-Acid)

CAS Number: 335-67-1

Chemical Formula: $C_7F_{15}CO_2H$

Molecular Weight: 414

This chemical is a commercial product obtained from Oakwood Products and contains approximately 1% branched:99% linear isomers.

The molecular ion 413 was selected as the primary ion for PFOA. This ion was fragmented further during analysis to produce ions 119, 169, 219, and 369. The total ion current (TIC) was monitored for analysis.

Chemical characterization information on the reference substances used in this study is presented in tabular form below.

Table 3. Characterization of the Analytical Reference Substances in Study E02-1053

Location	3M Lab	
	PFOA-NH4 TCR-99131-037	PFOA-Acid TCR-617
Source	3M	Oakwood Products
Expiration Date	12/15/2006	NA
Storage Conditions	-20°C ± 10°C	Room Temperature
Chemical Lot Number	332	210002
Physical Description	White powder	White crystal
Purity	95.2%*	99.51%**

NA—Not available

*See Certificate of Analysis from Centre Analytical Laboratories in Appendix F.

**See Certificate of Analysis from 3M.

Sample Preparation and Analysis

Human serum samples were analyzed in this study. Sera samples were extracted beginning on 17 October 2002 using a solid phase extraction (SPE) procedure. Sample extracts were analyzed using high-performance liquid chromatography-electrospray/tandem mass spectrometry (HPLC-ES/MS/MS) in the multiple reaction mode versus extracted rabbit sera standards.

Qualitative analysis of branched and linear isomers of PFOA was accomplished using NMR certified standards of a linear isomer PFOA standard (PFOA-Acid) and a mixed branched and linear isomer PFOA standard (PFOA-NH₄). It was determined that the branched PFOA isomers elute within an approximate 0.2 minute retention time window from the linear PFOA isomer.

Method Summaries

Following is a brief description of the method used during this analytical study by the 3M Environmental Laboratory. A detailed description of the method used in this study is located in Appendix B.

3M Environmental Laboratory

PREPARATORY AND ANALYTICAL METHOD

- **ETS-8-231.1**, "Solid Phase Extraction and Analysis of Fluorochemical Compounds from Biological Matrices"
- Human sera was prepared using 2.0 mL of serum then diluted to 10 mL with reagent grade water. The diluted serum/water was spiked with the appropriate analyte mixture. Acetonitrile (ACN) was added as an extraction solvent, which also served to precipitate the proteins. The sample was capped, mixed, and put on the centrifuge to clarify the supernatant. The supernatant was transferred to a clean tube, diluted with water, and passed through a pre-conditioned C₁₈ SPE cartridge.

The analyte(s) of interest were eluted from the SPE cartridge with 2.0 mL of methanol and analyzed. Analyses were performed by monitoring two or more product ions selected from a single primary ion characteristic of PFOA using HPLC-ES/MS/MS. For example, the molecular anion 413 (C₇F₁₅COO⁻), selected as the primary ion for analysis, was fragmented further to produce characteristic daughter ions 119, 169, 219, and 369. The total ion current (TIC) peak areas are the sum of the signal for each daughter ion at specific retention times for each target analyte isomer that were monitored for analysis. Daughter ions may also be referred to as product ions.

ANALYTICAL EQUIPMENT

The following is representative of the settings used during the analytical phase of this study.

Liquid Chromatograph: Hewlett-Packard® Series 1100 Liquid Chromatograph system

Analytical column: Keystone® Betasil™ C₁₈ 2x100 mm (5 µm)

Column temperature: 40°C

Mobile phase components:

Component A: 2mM ammonium acetate

Component B: methanol

Flow rate: 300 µL/min

Injection volume: 1-30 µL

Solvent Gradient: 16.0 minutes

<i>Time (minutes)</i>	<i>%B</i>
0.0	40%
10.0	90%
11.0	90%
11.5	100%
12.5	100%
13.0	40%
16.0	40%

Mass Spectrometer: Micromass® API/Mass Spectrometer Quattro Ultima Triple

Quadrupole system

Software: Mass Lynx™ 3.5

Cone Voltage: 20–60 V

Collision Gas Energy: 20–50 eV

Mode: Electrospray Negative

Source Block Temperature: 150°C ±10°C

Electrode: Z-spray

Analysis Type: Multiple Reaction Monitoring (MRM)

Table 4. Target Ions Monitored in 3M Laboratory Analyses and Observed Retention Times

Target Analyte	Primary Ion (AMU)	Product Ion (AMU)	Isomer retention time	
			Branched	Linear
PFOA	413.0	119, 169, 219, 369	~7.9 min.	~8.1 min.

Data Quality Objectives and Data Integrity

The following data quality objectives (DQOs) were indicated for this study:

Calibration: Calibration curves were not a component of this study. Isomer ratios of extracted rabbit matrix standards (labeled as RBS-date of extraction-concentration) were evaluated to determine instrument response.

Limits of Peak Area Threshold: The limit of peak area threshold was the lowest standard point that had a signal to noise ratio of at least 2 times that of the baseline noise.

Acceptance Criteria: The isomer ratio of branched:linear PFOA in the extracted continuing verification (QC) sample (labeled as RBS-date of extraction-QC-concentration) is required to meet $\pm 30\%$ agreement versus the extracted initial standard at the same concentration.

Confirmatory Methods: No confirmatory method will be used.

Demonstration of Specificity: Isomer identification will be substantiated by chromatographic retention times of the total ion current (TIC).

Data Summary, Analyses, and Results

Data quality objectives for the analytical phase of this study outlined above were met with the exceptions noted in this report.

Summary of Data Results

Table 5. PFOA Data Summary of Pooled Serum

Sample	Identification	Branched Isomer Area	Linear Isomer Area	% Branched/ Sum Branched + Linear
Pooled Human Serum	TCR-687-Bioresource Lot 020821	<361*	114603	<0.31
	TCR-688-Lampire Lot X324B	8059	41466	16
	TCR-689-Sigma Lot 022K0965	8824	55100	14
	TCR-690-Golden West Lot G01406042	<361*	168154	<0.21

* Area threshold found as 361 in the extracted PFOA-Acid 0.534 ng/mL standard

Summary of Quality Control Analyses Results

Calibration: Quantitation was not a component of this study.

Comparison of branched:linear ratio in the extracted rabbit matrix standard was based on the total ion current (TIC) of peak areas at retention times consistent for the target analyte (i.e. 7.9 and 8.1 minutes for PFOA-NH₄, and 7.9 and 8.1 minutes for PFOA-Acid).

Limits of Peak Area Threshold: The peak area threshold was determined based on the extracted PFOA-Acid 0.534 ng/mL standard, using the branched isomer TIC peak area at ~2 times the baseline noise.

Table 6. Limit of Peak Area Threshold in the Analyses of Sera Extracts

Analyte	Concentration ng/mL	Branched Isomer TIC Peak Area	Instrument
PFOA-Acid	0.534	361*	Quattro Ultima

* Based on the extracted PFOA-Acid 0.534 ng/mL standard, branched isomer area at retention time 7.9 minutes, with a TIC peak area ~2 times the baseline noise.

PFOA-Acid – standard spiked with PFOA-Acid standard mix

Blanks: All blanks were below the limit of peak area threshold for the compounds of interest.

Acceptance Criteria: The isomer ratio of branched:linear PFOA in the extracted continuing verification (QC) sample was within +/- 10% for all QC data. Refer to Appendix C for detailed information regarding QC data.

Precision: Precision was not a component of this study.

Matrix Spikes: Matrix spike data were not a component of this study.

Spike Recoveries: Spike recoveries were not determined for the continuing verifications (QCs).

Surrogates: Surrogates were not a component of this study.

Statement of Data Quality

The ratio of isomers observed in the standards throughout this study were stable and a reliable identifier of the source product.

Statistical Methods and Calculations

Statistical methods were limited to the calculation of means, standard deviations, and percent difference. See Appendix E for example calculations used in E02-1053.

Statement of Conclusion

Results from this study showed a wide distribution of the percentage of branched isomers of PFOA compared to the linear isomer of PFOA in the commercial pooled populations (Table 1). Since this is a preliminary screening study a larger sample size would be needed to determine if a significant statistical difference exists between pooled human sera samples.

As shown in Table 1, two of the lots of commercial pooled sera showed the presence of branched isomers of PFOA while the other two lots showed a much lower percentage (by at least 40x) of PFOA branched isomers.

References

3M Environmental Laboratory Study # E02-1039, November 2002

Appendix A: Characterization of the Control Matrix**Table 7. Characterization of the Control Matrix Used for Analyses in Study E02-1053**

Control Matrix	Rabbit Serum TN-A-4511
Source	Sigma
Expiration Date	09/26/2005
Storage Conditions	-20C ± 10C
Chemical Lot #	99H8400
Physical Description	Rabbit Serum

Appendix B: Extraction and Analytical Method

This appendix includes the following method:

ETS-8-231.1, Solid Phase Extraction and Analysis of Fluorochemical Compounds
from Biological Matrices, (19 pages)

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3M Environmental Laboratory

Method

Solid Phase Extraction and Analysis of Fluorochemical Compounds from Biological Matrices

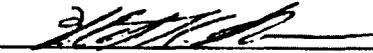
Method Number: ETS-8-231.1

Adoption Date: 11/13/01

Revision Date: 2/18/02

Effective Date: 2/18/02

Approved By:


William K. Reagen
Laboratory Manager

02/18/02
Date

Exact Copy of Original

LAS 11/07/02
Initial Date

ETS-8-231.1
Solid Phase Extraction and Analysis of Fluorochemical
Compounds from Biological Matrices

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1 Scope and Application

This method describes the extraction of target analytes from fish, rat liver, rat sera, mouse liver, and mouse sera using solid phase extraction (SPE). This method may also be extended to other biological matrices provided that the data quality objectives are met.

2 Method Summary

An amount of biological material, determined by the analyst, is prepared (fluids diluted and tissues homogenized) at a 1/6 dilution, or other dilution as determined by the analyst using reagent grade water. An aliquot of the dilution/homogenate is spiked with the appropriate surrogate or analyte mixture. Acetonitrile (ACN) is added as an extraction solvent and also serves to precipitate the proteins. The sample is capped, mixed, and put on the centrifuge to clarify the supernatant. The supernatant is transferred to a clean tube, diluted with water, and passed through a pre-conditioned C₁₈ SPE cartridge. Finally, the analytes of interest are eluted from the SPE cartridge and analyzed by high performance liquid chromatography-electrospray tandem mass spectrometry (HPLC-ES/MS/MS).

3 Definitions

3.1 Dilution

A dilution expressed as 1:5 or 1/6 is defined as: 1 mL of sample + 5 mLs of diluent for a total of 6 mLs combined, unless otherwise noted.

3.2 SPE cartridge

A column containing an open solvent reservoir at one end and packed with bonded silica sorbents at the other end. It is designed to retain the compounds of interest under some solvent conditions and elute them under others. A separation is thus achieved; compounds can be removed from difficult biological matrices and introduced into appropriate solvents for analysis.

3.3 Reagent grade water

Water with no detectable concentration(s) of the target analyte(s).

3.4 Quality control sample

Sample used to monitor the extraction efficiency (as a matrix spike) and to verify the continued accuracy of the initial calibration curve (as a continuing calibration verification).

4 Warnings and Cautions

4.1 Health and Safety Warnings

Always wear appropriate gloves, eyewear, and clothing when working with solvents, samples and/or equipment.

Use caution with the voltage cables for the probe. When engaged, the probe employs a voltage of approximately 5000 volts.

4.2 Cautions

Take care not to allow the SPE column to run to dryness after the methanol and water washes. After washing is complete, add sample then allow all of the liquid to pass through the SPE column to dryness.

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Do not operate solvent pumps above capacity of 400 bar (5800 psi) back pressure. If the back pressure exceeds 400 bar, the HPLC will initiate automatic shutdown.

Do not run solvent pumps to dryness.

5 Interferences

To minimize interferences, Teflon should not be used for sample storage or any part of instrumentation that comes in contact with the sample or extract.

6 Instrumentation, Supplies, and Materials

The following instrumentation, supplies, and materials are used while performing this method. Equivalent instrumentation, supplies, and materials may be used in place of those listed.

6.1 Instrumentation

Vortex mixer, VWR, Vortex Genie 2

Ultra-Turrax T25 tissue homogenizer

Vacuum Pump

SPE Extraction Manifold

Centrifuge, Mistral 1000 or IEC

Shaker, Eberbach or VWR

Balance (+/- 0.1000 g)

Micromass, Quattro II or Ultima triple quadrupole Mass Spectrometer equipped with an electrospray ionization source
HP1100 or Agilent low pulse solvent pumping system, solvent degasser, column compartment, and autosampler

6.2 Supplies and Materials

Eppendorf or disposable pipettes, plastic or glass

Dissecting scalpels

Polypropylene bottles, capable of holding 50 mL to 1 L (Nalgene)

Volumetric flasks, glass, type A

40 mL glass vials (ICHEM)

Plastic sample vials, Wheaton, 6 mL (or other appropriate size)

Centrifuge tubes, polypropylene, 15 mL and 50 mL

Labels

Graduated pipettes, glass

Syringes, capable of measuring 5 µL to 1000 µL

Bottle-Top Dispenser (capable of dispensing 5mL of solvent)

SPE extraction cartridge, 1 g, Sep-Pak 6 cc tri-functional C₁₈ (Waters)

75 mL sample reservoir (or other appropriate size)

Crimp cap glass autovials and caps

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Crimpers

HPLC analytical column, specifics to be determined by the analyst and documented in the raw data.

7 Reagents and Standards

Reagent grade water, Milli-Q™, Nanopure II, or equivalent

Acetonitrile, HPLC grade or equivalent

Methanol, HPLC grade or equivalent

Ammonium acetate, reagent grade or equivalent

Biological fluids or tissues, frozen from supplier

7.1 Reagents preparation

2.0 mM ammonium acetate solution: Weigh approximately 0.300 g ammonium acetate. Pour into a 2000 mL volumetric container containing reagent grade water, mix until all solids are dissolved, bring to volume using reagent grade water. Store at room temperature.

Note: When preparing different volumes than those listed in reagents preparation, target analyte standard preparation, and surrogate standard preparation, adjust accordingly.

7.2 Target analyte standard preparation

Prepare target analyte standard(s) for the standard curve. Multicomponent analyte standards are acceptable. The following is an example only and may or may not be appropriate for all standard preparations.

Weigh approximately 100 mg of target analyte into a 100 mL volumetric flask and record the actual weight in the standard logbook or other appropriate location.

Bring to volume with methanol for a stock standard of approximately 1000 ppm ($\mu\text{g/mL}$).

Dilute the stock solution with methanol for a working standard 1 solution of approximately 50 ppm. Example calculation: $1000 \mu\text{g/mL} \times 5 \text{ mL}/100 \text{ mL} = 50 \mu\text{g/mL}$.

Dilute working standard 1 with methanol to produce a working standard 2 solution of approx. 5.0 ppm. Example calculation: $50 \mu\text{g/mL} \times 10 \text{ mL}/100\text{mL} = 5.0 \mu\text{g/mL}$.

Dilute working standard 1 with methanol to produce a working standard 3 solution of approx. 0.50 ppm. Example calculation: $50 \mu\text{g/mL} \times 1.0 \text{ mL}/100 \text{ mL} = 0.5 \mu\text{g/mL}$.

7.3 Surrogate standard preparation

Prepare surrogate standard(s). The following is an example only and may or may not be appropriate for all surrogate standard preparations.

Weigh approximately 90-110 mg of surrogate standard into a 100-mL volumetric flask and record the actual weight.

Bring to volume with methanol for a surrogate standard stock of approximately 900 - 1100 ppm.

Prepare a surrogate standard working standard. Transfer approximately 1 mL of surrogate standard stock to a 10-mL volumetric flask and bring to volume with methanol for a working standard of 90-110ppm. Record the actual volume transferred and standard concentrations in the standards logbook or other appropriate location.

7.4 Internal standard preparation

Prepare internal standard(s). The following is an example only and may or may not be appropriate for all internal standard preparations.

Weigh approximately 90-110 mg of internal standard into a 100-mL volumetric flask and record the actual weight.

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Bring to volume with methanol for an internal standard stock of approximately 900 - 1100 ppm.

Prepare an internal standard working standard. Transfer approximately 1 mL of internal standard stock to a 10-mL volumetric flask and bring to volume with methanol for a working standard of 90-110ppm. Record the actual volume transferred and standard concentrations in the standards logbook or other appropriate location.

8 Sample Handling

All samples are received frozen and must be kept frozen until the extraction is performed.

Allow samples to thaw to room temperature prior to extraction.

Typically fresh matrix standards are prepared with each analysis. Extracted standards and samples are stored in capped autovials until analysis.

If analysis will be delayed, extracted standards and samples may be refrigerated at approximately 4°C indefinitely or may be stored at room temperature until analysis can be performed.

9 Quality Control

9.1 Blanks

9.1.1 Solvent Blank

An aliquot of methanol is used as a solvent blank. Solvent blanks are not extracted.

9.1.2 Method Blank

An aliquot of 1.0 mL of water, or other appropriate amount, is used as a method blank. Four method blanks are extracted and analyzed with each set following this procedure (two are spiked with surrogate and two are not spiked).

9.1.3 Matrix Blank

An aliquot of 1.0 mL or 1.0 g of matrix (diluted or homogenized) is used as a matrix blank. Other amounts may be used, as appropriate. Matrix blanks are prepared from one of three sources: 1) a study control matrix from a study control animal received with a sample set; 2) a commercially obtained sample of the same species as the study animals; or 3) a surrogate matrix, also obtained commercially, but of a different species than the study animal. (eg. if rat is used to generate standard curves and CCVs for a mouse study). The matrix to use is dependent on the matrix used for the curve.

9.1.3.1 Study control matrix curve - if the study control matrix is used for the curve, prepare four (4) matrix blanks using the study control matrix (two spiked with surrogate and two not spiked).

9.1.3.2 Commercially obtained (same species) matrix curve - if the commercially obtained matrix is used for the curve, prepare four (4) matrix blanks using the same commercially available matrix (two spiked with surrogate and two not spiked).

9.1.3.3 Surrogate matrix curve - if a surrogate matrix is used for the curve, prepare four (4) matrix blanks using the same commercially available matrix and prepare four (4) matrix blanks using a commercially available matrix of the same species as the study animals (two spiked with surrogate and two not spiked).

9.1.3.4 If limited matrix is available, the number of method and matrix blanks may be adjusted and will be noted in the study protocol or in the raw data.

9.2 Sample Replicate

Samples replicates are prepared according to each study protocol or project outline.

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9.3 Surrogate standard

If surrogate standard is a component of the study, all samples are spiked with surrogate standard prior to extraction to obtain a concentration in the mid-range of the calibration curve, with the exception of blank samples as described above.

Typically surrogate standard is spiked into the 1.0 mL diluted/homogenized sample removed for extraction. However, surrogate may be spiked directly into the matrix prior to diluting with water, into the diluted/homogenized sample prior to removing the 1.0 mL sample, or into the 1.0 mL diluted/homogenized sample removed for extraction.

9.4 Internal standard

If internal standard is a component of the study, all samples are spiked with internal standard after extraction to obtain a concentration in the mid-range of the calibration curve.

Typically internal standard is spiked into the 2.0 mL of extract in the 15 mL centrifuge tube, before transferring to the autovial.

9.5 Lab Control Sample

Lab control samples are not a component of this method.

9.6 Quality Control (QC) Sample

Prepare quality control (QC) samples to monitor extraction efficiency and to verify the continued accuracy of the initial calibration curve. Typically 1.0 mL, or other appropriate amount, of the same matrix used to prepare the initial calibration curve is used for each QC sample.

Twelve (12) quality control samples (QC) will be prepared for each matrix during the course of a study. A minimum of 3 QC samples must be prepared (one at each level) on each day of sample extraction. (e.g. If the study is such that samples will be extracted on three different days then four QC samples must be prepared on each day of extraction for a total of twelve.)

QC samples will consist of four samples at each of three levels of analyte. The levels listed below may be used and may represent sample concentrations diluted into the range of the calibration curve:

Low level: 3X to 5X the LLOQ,

Mid-level: equivalent to a point near the middle of the calibration curve,

High level: 80% of the ULOQ

Two QC sample levels are analyzed after every tenth sample injection starting after the last calibration standard injection, with a minimum of three QC per analysis. Solvent blanks are not considered samples but may be included as such for determining when QC samples will be analyzed.

QC samples extracted with a particular sample set must be analyzed in the same analytical run. Any QC samples extracted during the course of the study may be included in subsequent analyses.

If samples from multiple extraction dates are analyzed in one analytical run, then QC samples from the same sample extraction dates must be included in that analysis.

Each QC is expected to show an accuracy of 75-125% of expected. A minimum of 2/3 of all QC samples must meet this criteria, and a minimum of 1/2 of the QC samples at each level must meet this criteria. If not, the set must either be re-analyzed or re-extracted.

9.7 Sample Dilution

Any sample with an area greater than that of the highest acceptable standard will need to be diluted into the range of the calibration curve. If samples are diluted into the range of the curve during analyses and enough sample remains, a post-run dilution validation will be performed to verify sample values.

To perform the dilution validation, one sample will be separated into two representative samples (i.e. two 1.0 mL aliquots for fluid samples or two 1.0 gram amounts for tissue samples, or other amount as determined by the analyst

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and documented in a note to file) then diluted using two procedures. The first procedure consists of diluting the sample with additional matrix prior to extraction (fluid adding fluid), while the second procedure consists of diluting the extract with solvent post-extraction (methanol extract adding additional methanol solvent.)

If the relative percent difference is not within 15% for these two samples; additional testing will be required to determine which value is a correct representation of the sample concentration.

10 Calibration and Standardization

10.1 Instrument Calibration

One calibration curve will be prepared from extracted matrix standards, in the same matrix as the samples, per study. It will consist of a minimum of nine (9) levels. Additional calibration curves may be extracted on separate sample extraction dates, as determined by the analyst and documented in a note to file.

Transfer 1.0 mL, or other appropriate amount, of diluted control fluid or homogenized control tissue to a 15 mL centrifuge tube using a disposable plastic pipette. This will be repeated while preparing aliquots for the standard curve. Be sure to mix or shake the control matrix container between aliquots to ensure a homogenous sample is removed.

Record each standard volume on the weight/volumes sheet or extraction worksheet, as appropriate.

Four 1.0 mL aliquots, or other appropriate amount, of control matrix serve as matrix blanks.

The standard concentrations and spiking amounts listed in Table 1 may be used, when appropriate, to spike one standard curve. A total of 9 standards, four matrix blanks, and four method blanks are prepared in addition to the QC samples and test samples. The number of standards and blanks may be adjusted as determined by the analyst and documented in a note to file.

Use Attachment C, or other appropriate form, as an aid in calculating the concentrations of the working standards. Refer to section 12 to calculate the actual concentration of analyte in each calibration standard and QC sample.

Typically the target analyte standard is spiked into the 1.0 mL diluted/homogenized sample removed for extraction. However, it may be spiked directly into the matrix prior to diluting with water, into the diluted/homogenized sample prior to removing the 1.0 mL sample, or into the 1.0 mL diluted/homogenized sample removed for extraction.

Analyze the extracted matrix standard curve prior to each set of extracts. The curve equation will be determined by regression analysis using the peak areas of the target analyte(s) using MassLynx or other suitable software.

Any level outside 75% - 125% of nominal must be deactivated, and regression re-calculated, except the LLOQ which must be within 30% of nominal. All levels must show a response greater than twice that of the blank. A maximum of three (3) levels may be deactivated in any one set, or the set will be re-analyzed.

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TABLE I APPROXIMATE SPIKING AMOUNTS FOR STANDARDS AND SPIKES USING 1.0 mL OF MATRIX			
Working standard (approximate concentration)	μL	Approximate final concentration of analyte in Matrix diluted 1:5	Approximate final concentration of analyte in Final 2.0 mL volume
-	-	Blank	Blank
0.500 ug/mL	1.5	5.00 ng/g or ng/mL	0.375 ng/mL
0.500 ug/mL	3.0	10.0 ng/g or ng/mL	0.750 ng/mL
0.500 ug/mL	8.0	25.0 ng/g or ng/mL	2.00 ng/mL
0.500 ug/mL	16	50.0 ng/g or ng/mL	4.00 ng/mL
0.500 ug/mL	32	100 ng/g or ng/mL	8.00 ng/mL
5.00 ug/mL	5.6	175 ng/g or ng/mL	14.0 ng/mL
5.00 ug/mL	8.0	250 ng/g or ng/mL	20.0 ng/mL
5.00 ug/mL	16	500 ng/g or ng/mL	40.0 ng/mL
5.00 ug/mL	24	750 ng/g or ng/mL	60.0 ng/mL
5.00 ug/mL	32	1000 ng/g or ng/mL	80.0 ng/mL
5.00 ug/mL	40	1250 ng/g or ng/mL	100 ng/mL
50.0 ug/mL	5.0	1500 ng/g or ng/mL	125 ng/mL
50.0 ug/mL	6.0	1750 ng/g or ng/mL	150 ng/mL
Surrogate Std 100 ug/mL	10	6500 ng/g or ng/mL	500 ng/mL

11 Procedures

11.1 Tissue Sample Preparation

Obtain frozen tissue samples

Cut approximately 1.0000 g of tissue (+/- 0.1000 g), or other appropriate amount, using a dissecting scalpel. This part of the procedure is best performed quickly, not allowing the tissue to thaw.

Weigh the tissue directly into a tared plastic sample vial.

Record the weight on the weight/volume sheet, extraction worksheet, or other appropriate location.

Return unused tissue to the freezer after extraction amounts have been removed.

Add 2.5 mL of reagent water to sample vial, or other volume as determined by the analyst and documented in a note to file.

Homogenize the sample. Put the Ultra-Turrax grinder probe in the sample and grind for approximately 2 minutes, or until the sample is homogeneous.

Rinse the probe into the tube containing the sample with 2.5 mL of reagent grade water, or other volume as determined by the analyst and documented in a note to file, using a pipette.

Take the grinder apart and clean it with methanol after each sample. Refer to ETS-9-52 for more information.

If an amount other than 1.0000 g (not within +/- 0.1000 g) is removed for an initial weight, adjust the water volume accordingly to maintain a 1/6 dilution. (e.g. if 0.5 g is removed for extraction, add a total of 2.5 mL of water.), or other ratio as determined by the analyst and documented in a note to file.

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11.2 Fluid Sample Preparation

Obtain frozen fluid sample and allow it to thaw at room temperature or in lukewarm water.

Label a 15 mL polypropylene centrifuge tube with the study number, sample ID, extraction date and analyst initials. See attached worksheet (Attachment A or similar worksheet) for documenting the remaining steps.

Vortex mix the fluid sample for approximately 15 seconds, then transfer 1.0 mL of fluid, or other appropriate amount to a plastic sample vial, or other appropriate container.

Return unused samples to freezer after extraction amounts have been removed.

Add 5.0 mL of reagent water to the 1.0 mL of fluid for a 1/6 dilution, or other dilution as determined by the analyst and documented in a note to file.

If a volume other than 1.0 mL is removed for an initial volume, adjust the water volume accordingly to maintain the same dilution as above.

11.3 Tissue and Fluid Sample Extraction

After tissue or fluid samples have been prepared according to sections 11.1 and 11.2, vortex mix or shake by hand the diluted/homogenized sample for approximately 15 seconds then transfer 1.0 mL, or other appropriate volume, to a clean 15 mL polypropylene centrifuge tube.

Return unused diluted/homogenized portions to the freezer after extraction amounts have been removed.

Record the volume removed on the extraction worksheet, (Attachment A or similar worksheet).

Spike blanks, samples, and standards, ready for extraction with surrogate standard as described in this method.

Spike each calibration standard matrix with the appropriate amount of standard as described in this method for the calibration curve standards and each QC sample.

Vortex mix the standard curve samples and QC samples for approximately 5 seconds.

To each sample and standard, add 5.0 mL of acetonitrile, cap, and vortex mix or shake by hand approximately 15 seconds.

Place all samples on the shaker at an appropriate speed for 20 minutes to adequately mix (a setting of approximately 300 rpm on the models listed in section 6.1).

Remove from the shaker and centrifuge at an appropriate speed for 10 minutes to adequately pellet the precipitate (a setting of approximately 2000 rpm on the models listed in section 6.1).

Add 40.0 mL of reagent grade water to a clean 50 mL centrifuge tube. Remove samples from the centrifuge and decant the supernatant into the water in the 50 mL tube, taking care not to introduce any of the matrix solids into the solution. Cap and mix by inverting several times. In this step the order of addition may be changed (i.e. the sample may be put into the centrifuge tube and then the water added).

Attach the reservoir to the SPE cartridge and attach this reservoir/cartridge unit to a vacuum manifold.

NOTE: When running the vacuum, set the vacuum chamber at approximately 15 kPA - to give an approximate elution flow of 5-7 mL/min. Flows may vary through cartridges and the kPA may be raised for slow tubes and drying after most have been drawn down.

Prepare the SPE cartridge by washing twice with approximately 5.0 mL of methanol, followed by approximately two 5.0 mL aliquots of water, taking care not to allow the column to run to dryness after each wash.

After washing is complete, pour the sample into the reservoir/cartridge unit and allow all of the liquid to pass through the column to dryness.

Run the vacuum on high for approximately 5 minutes to adequately dry each SPE cartridge.

Place a collection 15 mL polypropylene centrifuge tube under each cartridge and elute with 2.0 mL of methanol.

Spike extracted blanks, samples, and standards with internal standard as described in this method.

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Label each glass autovial, as appropriate, with the study number, vial file archive number, animal number/gender/timepoint or LIMS number, matrix, final solvent, analyte components (if needed), extraction type, extraction date, and analyst(s) performing the extraction.

Transfer each eluant to a glass autovial and cap.

11.4 Extract Analysis

11.4.1 Software set-up

On the MassLynx main page, set up a sample list name. Save the list as instrument designator letter, last 2 digits of test year-month-day, and a letter that will increase through the alphabet with each additional list for that day.

Example Sample List: IYYMMDDa or A020204a

I = Initial of the instrument name (A = "Amelia")

YY = Test year (02)

MM = Test month (02)

DD = Test day (04)

a = First sample list (run) of the day (the next sample list will end with 'b', the next 'c', and so on.)

Assign a filename using the instrument designator letter, the last 2 digits of the test year-month-day, and a 3-digit sequential file number that starts with 1 and increases by one for each filename.

Example filename: IYYMMDD### or A020204001

I = Initial of instrument name

YY = Test year

MM = Test month

DD = Test day

= 3-digit sequential file number starting with 1 through 999 (001)

Also, as part of the samplelist, assign a method (MS) for acquiring, an inlet file, a bottle number, an injection volume, and sample descriptions.

To create a method, click on Method Editor button in the MS Status Pane and select SIR (Single Ion Recording) or MRM (Multiple Reaction Monitoring). Set Ionization Mode as appropriate and mass to 499 or other appropriate mass(es). Also set the acquisition start and stop times. Save acquisition method. If MS/MS instruments are employed, additional product ion fragmentation information may be collected. See Micromass MassLynx "Guide to Data Acquisition" for additional information on MRM.

Typically the analytical batch run sequence begins with system suitability, solvent blanks, and a set of extracted matrix standards.

Sample extracts are analyzed with two QC samples injected after every tenth sample injection. Solvent blanks should be analyzed periodically to monitor possible analyte carryover and are not considered sample extracts but may be included as such.

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11.4.2 HPLC set-up

Set up sample tray according to the sample list prepared above.

Set up the HPLC to the following conditions or at conditions the analyst considers appropriate for optimal response. Record actual conditions in the instrument logbook, or other appropriate location:

Sample size = 10 μ L injection

Inject/sample = 1

Cycle time = 10.0 minutes

Flow rate = 300 μ L/min

Mobile phase: Solvent A = 2 mM Ammonium Acetate, Solvent B = Methanol

Solvent gradient program:

Time	% Solvent B
0.00	10%
1.00	10%
5.50	95%
7.50	95%
8.00	10%

11.4.3 Instrument set-up

Refer to ETS-9-24, "Operation and Maintenance of the Micromass Quattro II Triple Quadrupole Mass Spectrometer Fitted with an Atmospheric Pressure Ionization Source," for details.

Check the solvent level in HPLC reservoirs and refill if necessary.

Check the stainless steel capillary at the end of the probe. Use an eyepiece to check the tip. The tip should be flat with no jagged edges. If the tip is found to be unsatisfactory, disassemble the probe and replace the stainless steel capillary.

Turn on the nitrogen.

Open the tune page. Click on operate to initiate source block and desolvation heaters.

Open the Inlet Editor.

Download the HPLC method and initiate solvent flow to begin system equilibrium.

Set the flow to 10–500 μ L/min or as appropriate

Set HPLC pump to "On"

Observe droplets or mist coming out of the tip of the probe. A fine mist should be expelled with no nitrogen leaking around the tip of the probe. Readjust the tip of the probe if no mist is observed

Allow to equilibrate for approximately 10 minutes.

Typical instrument parameters include:

Drying gas 250–400 liters/hour

ES nebulizing gas 10–15 liters/hour

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HPLC constant flow mode, flow rate 10–500 $\mu\text{L}/\text{min}$

Pressure <400 bar (this parameter is not set, it is a guide to ensure the HPLC is operating correctly.)

Source block temperature approximately 150°C

Desolvation temperature approximately 250°C

These settings may change in order to optimize the response

Print the tune page, sample list, and acquisition method from MassLynx and store it in the study binder with a copy taped into the instrument log.

Click on start button in the Acquisition Control Panel (the location of the start button may vary among MassLynx versions, refer to appropriate MassLynx User's Guide).

12 Data Analysis and Calculations

12.1 Calculations

If other calculations are used than those listed, they will be documented in the raw data.

Calculate the matrix amount contained in the initial dilution using the following equation:

$$\text{Matrix Amount (g/mL or mL/mL)} = \frac{\text{IW (g) (or IV (mL))}}{(\text{IW (g) (or IV (mL))} + \text{DV (mL)})}$$

Calculate actual concentrations of analyte in calibration standards using the following equation:

$$\text{Concentration (ng/g or ng/mL)} = \frac{\text{Spike Concentration (ug/mL)} \times \text{Spiked Amount (mL)}}{\text{SV (mL)} \times \text{Matrix Amount (g/mL or mL/mL)}} \times \frac{1000 \text{ ng}}{1 \text{ ug}}$$

IW = Initial weight (where 1.0 g = 1.0 mL)

IV = Initial volume

DV = Diluent volume (reagent grade water)

SV = Sample volume removed for extraction (typically 1.0 mL)

AR = Analytical result from MassLynx summary

DF = Dilution factor

FV = Final volume

MA = Matrix amount

∂ curve = MA of tissue/fluid standard curve, assumed to be 1 g or 1 mL/5 mL water

∂ sample = MA of tissue/fluid sample (___g or mL of sample/5 mL water)

Calculate spike percent recoveries using the following equation:

$$\% \text{ Recovery} = \frac{\text{Observed Result} - \text{Matrix Blank Result}}{\text{Spiking Level}} \times 100$$

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Calculate relative standard deviation using the following equation:

$$\text{Relative Standard Deviation} = \frac{\text{Standard Deviation}}{\text{Mean}} \times 100$$

Calculate percent deviation using the following equation:

$$\% \text{ Deviation} = \frac{\text{Expected Conc.} - \text{Calculated Conc.}}{\text{Expected Conc.}} \times 100$$

Calculate actual concentration of analyte in fluid ($\mu\text{g/mL}$):

$$\text{AR (ng/mL)} \times \text{DF} \times \frac{\partial \text{ curve (mL/mL)} \times \text{FV (mL) in Curve}}{\partial \text{ sample (mL/mL)} \times \text{FV (mL) in Matrix}} \times \frac{1.0 \mu\text{g}}{1000 \text{ ng}} = (\mu\text{g/g})$$

Calculate actual concentration of analyte in tissue ($\mu\text{g/g}$):

$$\text{AR (ng/g)} \times \text{DF} \times \frac{\partial \text{ curve (g/mL)} \times \text{FV (mL) in Curve}}{\partial \text{ sample (g/mL)} \times \text{FV (mL) in Matrix}} \times \frac{1.0 \mu\text{g}}{1000 \text{ ng}} = (\mu\text{g/g})$$

13 Method Performance

13.1 System Suitability

System suitability will be determined prior to the start and at the completion of each analytical run. Prior to the calibration curve and after the last sample of the run three (3) mid-level unextracted calibration standards will be analyzed. As applicable, the peak area precision, retention time precision, resolution, and peak asymmetry will be monitored at the beginning and the end of the run separately. The peak area precision must be equal to or less than 5.0% RSD, the precision of the retention time must be equal to or less than 2.5% RSD, the resolution must be > 2.0 , and the peak asymmetry (fronting or tailing) must be $0.5 < \text{AF} < 2.0$, where AF is the asymmetry factor.

If any item of the system suitability fails, system maintenance must be completed prior to running a second set of system suitability samples and the system suitability must pass before starting the calibration. If system suitability fails at the completion of a run, the sample set must be reanalyzed.

13.2 Quantitation

The coefficient of determination value for the calibration curve, plotted by regression using the peak areas of the analyte(s), must be 0.990 or better.

All active calibration curve points must be within 25% of the theoretical value with the exception of the LOQ point, which may deviate up to 30%.

Calibration standards with peak areas less than two times the curve matrix blank will be deactivated to disqualify a data range that may be affected by background levels of the analyte.

A valid calibration curve must contain at least 6 active points above and including the LOQ.

If the curve cannot meet these criteria, the sample set must be reanalyzed or reextracted.

13.3 Accuracy

Two thirds of all quality control samples and 1/2 of each quality control sample at each level are expected to show an accuracy of 75-125%.

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Surrogates and internal standards must have a percent deviation < 50%. Deviations outside this range will be reanalyzed to confirm. If the second analysis confirms the original, the deviation will be documented in the raw data. If the second analysis is within 50%, then the second value will replace the original value.

14 Pollution Prevention and Waste Management

Sample waste is disposed of in noninfectious biohazard waste containers.

Flammable solvent waste is disposed of in high BTU containers.

Glass pipette waste is disposed of in broken glass containers located in the laboratory.

15 Records

Complete the extraction worksheet attached to this method, or other applicable worksheet, and store with the study raw data.

Each page generated for a study must contain the following information (if applicable): study/project or instrument number, acquisition method, integration method, sample name, extraction date, dilution factor (if applicable), and analyst. Other information may be added if applicable to the study.

Print the tune page, sample list, and acquisition method from MassLynx to include with the study raw data. Copy these pages and tape into the instrument runlog.

Plot the calibration curve by the appropriate regression. Print these graphs and store with the study raw data.

Print data integration summary, integration method, and chromatograms from MassLynx, and store with the study raw data.

Summarize data using suitable software (Excel 7.0 or LIMS) and store in the study folder.

Back up electronic data to appropriate medium. Record in study notebook the file name and location of backup electronic data.

16 Attachments

Attachment A: Extraction Worksheet

Attachment B: Sample Weight/Volume Worksheet

Attachment C, Calibration Standard Concentration Worksheet

Attachment D, Dilutions Summary Worksheet

17 References

ETS-9-24, "Operation and Maintenance of the Micromass Quattro II Triple Quadrupole Mass Spectrometer Fitted with an Atmospheric Pressure Ionization Source"

ETS-9-52, "Operation and Maintenance of a Tissue Grinder"

18 Affected Documents

None

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19 Revisions

<u>Revision Number</u>	<u>Revision Description</u>	<u>Revision Date</u>
1	Minor formatting changes. Added detailed information to all sections concerning the extraction procedure, analytical procedure, and calculations. Added attachments and references.	02/18/02

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Attachment C: Calibration Standard Concentration Worksheet

Prep date(s):

Standard number:

Analyte(s):

Equipment number:

Sample matrix:

Final solvent and TN:

Method/revision:

Blank Tissue or Fluid/Identifier:

Analyte mix std approx. 0.500 ug/mL:

Analyte mix std approx. 5.00 ug/mL:

Analyte mix std approx. 50.0 ug/mL:

Surrogate std approx. 100 ug/mL:

Actual concentrations of standards in the analyte mix

Analyte Std conc ug/mL	All Am't spiked mL	All Final Volume: mL	All Initial Fluid Dilution mL/mL	All Initial Tissue Density g/mL
0.500	0.0015	2.00	0.1667	0.1600
0.500	0.0030	2.00	0.1667	0.1600
0.500	0.0080	2.00	0.1667	0.1600
0.500	0.0160	2.00	0.1667	0.1600
0.500	0.0320	2.00	0.1667	0.1600
5.00	0.0056	2.00	0.1667	0.1600
5.00	0.0080	2.00	0.1667	0.1600
5.00	0.0160	2.00	0.1667	0.1600
5.00	0.0240	2.00	0.1667	0.1600
5.00	0.0320	2.00	0.1667	0.1600
5.00	0.0400	2.00	0.1667	0.1600
50.0	0.005	2.00	0.1667	0.1600
50.0	0.006	2.00	0.1667	0.1600

Calculated concentrations of standards in relation to the final 2.0 mL solvent and initial matrix

2.0 mL Final Volume		Fluid Matrix		Tissue Matrix	
Analyte Final conc. ng/mL	Surrogate Std conc ng/mL	Analyte Final conc. ng/mL	Surrogate Std conc ng/mL	Analyte Final conc. ng/g	Surrogate Std conc ng/mL
0.375	100	5.00	100	5.00	100
0.750		10.0		10.0	
2.00	Surrogate	25.0	Surrogate	25.0	Surrogate
4.00	Final conc	50.0	Final conc	50.0	Final conc
8.00	ng/mL	100	ng/mL	100	ng/g
14.0	0.500	175	6500	175	6500
20.0		250		250	
40.0		500		500	
60.0		750		750	
80.0		1000		1000	
100		1250		1250	
125		1500		1500	
150		1750		1750	

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Appendix C: QC Data Summary Tables

Table 8. Acceptance Criteria Summary of PFOA-NH4 QC samples Analyzed 11/01/02

Sample Identification	PFOA-NH4 % Branched/ Sum Branched + Linear	% Difference
RBS101702-10 ng/mL-1	26.02	NA
RBS101702-QC-10 ng/mL-1-1	25.35	3
RBS101702-QC-10 ng/mL-1-2	24.93	4

% Difference: The difference between the total ion current peak area ratios of the branched:linear isomers for PFOA in the extracted standard (labeled RBS-date of extraction-concentration) at the same level as the extracted QC (labeled RBS-date of extraction-QC-concentration).

Table 9. Acceptance Criteria Summary of PFOA-Acid QC samples Analyzed 11/01/02

Sample Identification	PFOA-Acid % Branched/ Sum Branched + Linear	% Difference
RBS101702-10 ng/mL-1	2.22	NA
RBS101702-QC-10 ng/mL-1-1	2.24	-1
RBS101702-QC-10 ng/mL-1-2	2.04	8

% Difference: The difference between the total ion current peak area ratios of the branched:linear isomers for PFOA in the extracted standard (labeled RBS-date of extraction-concentration) at the same level as the extracted QC (labeled RBS-date of extraction-QC-concentration).

Appendix D: Data Spreadsheets

ANALYSIS 11/01/02

Identification	TIC			Filename D021101a	Branched Ret Time 7.9/ Sum of Branched:Linear * 100 Branched/Linear
	Branched Peak Ret Time 7.6 Branched PFOA-NH4 Area**	Branched Peak Ret Time 7.9 Branched PFOA-NH4 Area	Linear Peak Ret Time 8.1 Linear PFOA-NH4 Area		
Standards					
RBS101702-10 ng/mL-1	0	93029	264494	D021101003	26.02
RBS101702-QC-10 ng/mL-1-1	0	81108	238850	D021101027	25.35
RBS102802-QC-10 ng/mL-1-1	0	65454	195485	D021101029	25.08
RBS101702-QC-10 ng/mL-1-2	0	59384	178832	D021101055	24.93
RBS102802-QC-10 ng/mL-1-2	0	56858	168536	D021101057	25.23
WB102802-H2O Blk-1	0	355	2873	D021101043	11.00
RBS102802-Sera Blank-1*	0	0	3846	D021101045	0.00
0.5 ng/mL-1	0	1566	6752	D021101063	18.83
Pooled					
TCR-687-Bioresource*	0	361	114603	D021101007	0.314
TCR-688-Lampire	0	8059	41466	D021101009	16.27
TCR-689-Sigma	0	8824	55100	D021101011	13.80
TCR-690-Golden West*	0	361	168154	D021101013	0.214

* Below Limit of Peak Area Threshold

** Branched peak at 7.6 minutes observed only in PFOA-NH4 standards at concentrations > 25 ng/mL. Not included in any calculations.

ANALYSIS 11/01/02

PFOA-Acid Pooled data were not included in the scope of the study and were not reported

TIC				
Identification	Branched Peak Ret Time 7.9 Branched PFOA-Acid Area	Linear Peak Ret Time 8.1 Linear PFOA-Acid Area	Filename D021101a2	Branched Ret Time 7.9/ Sum of Branched:Linear * 100 Branched/Linear
Standards				
RBS101702-10 ng/mL-2	7971	351306	D021101005	2.22
RBS101702-QC-10 ng/mL-2-1	5022	219508	D021101031	2.24
RBS102802-QC-10 ng/mL-2-1	2733	225367	D021101033	1.20
RBS101702-QC-10 ng/mL-2-2	4416	211715	D021101059	2.04
RBS102802-QC-10 ng/mL-2-2	1909	198898	D021101061	0.951
WB102802-H2O Blk-1	260	2873	D021101043	8.30
RBS102802-Sera Blank-1	545	3787	D021101045	12.58
0.5 ng/mL-2	361	6473	D021101065	5.28
Pooled				
TCR-687-Bioresource*	361	113845	D021101007	0.316
TCR-688-Lampire	8207	41469	D021101009	16.52
TCR-689-Sigma	8823	55095	D021101011	13.80
TCR-690-Golden West*	361	168119	D021101013	0.214

* Below Limit of Peak Area Threshold

TIC

11/01/02 Analysis Identification	Branched Peak Ret Time 7.6 PFOA-NH4 Area**	Branched Peak Ret Time 7.9 PFOA-NH4 Area	Linear Peak Ret Time 8.1 PFOA-NH4 Area	Filename	Ret Time 7.9/ Sum of Branched:Linear * 100	% Diff
Standards & CCVs						
RBS101702-10 ng/mL-1	0	93029	264494	D021101003	26.02	NA
RBS101702-QC-10 ng/mL-1-1	0	81108	238850	D021101027	25.35	3
RBS102802-QC-10 ng/mL-1-1*	0	65454	195485	D021101029	25.08	4
RBS101702-QC-10 ng/mL-1-2	0	59384	178832	D021101055	24.93	4
RBS102802-QC-10 ng/mL-1-2*	0	56858	168536	D021101057	25.23	3

* Data not used/reported. Not extracted on the same date as the samples and therefore not included in tables 8 and 9.

** Branched peak at 7.6 minutes observed only in PFOA-NH4 standards at concentrations > 25 ng/mL. Not included in any calculations.

TIC

11/01/02 Analysis Identification	Branched Peak Ret Time 7.9 PFOA-Acid Area	Linear Peak Ret Time 8.1 PFOA-Acid Area	Filename	Ret Time 7.9/ Sum of Branched:Linear * 100	% Diff
Standards & CCVs					
RBS101702-10 ng/mL-2	7971	351306	D021101005	2.22	NA
RBS101702-QC-10 ng/mL-2-1	5022	219508	D021101031	2.24	-1
RBS102802-QC-10 ng/mL-2-1*	2733	225367	D021101033	1.20	46
RBS101702-QC-10 ng/mL-2-2	4416	211715	D021101059	2.04	8
RBS102802-QC-10 ng/mL-2-2*	1909	198898	D021101061	0.951	57

* Data not used/reported. Not extracted on the same date as the samples and therefore not included in tables 8 and 9.

Appendix E: Example Calculations

Calculations used for Analyses in Study E02-1053

Percentage of Branched:Linear Isomer (also referred to as ratio in the report)

$$\text{Percentage} = \frac{\text{Branched TIC Peak Area}}{(\text{Branched TIC Peak Area} + \text{Linear TIC Peak Area})} * 100$$

Sample TCR-688 PFOA Percentage: $(8059/(8059+41466)) * 100 = 16\%$

$$\% \text{ Difference} = \frac{(\text{Expected TIC \% Branched/Sum Initial Std} - \text{Observed TIC \% Branched/Sum QC})}{\text{Expected TIC \% Branched/Sum Initial Std}} * 100$$

Sum = Branched peak area + Linear peak area

% Difference of PFOA RBS102202-QC-10 ng/mL-1-1 sample analyzed 10/22/02

Initial Std = RBS101702-10 ng/mL-1 TIC % Branched/Sum = 22

QC = RBS102202-QC-10 ng/mL-1-1 TIC % Branched/Sum = 24

$$\% \text{ Difference} = |(22 - 24) / 24| = 9$$

Appendix F: Interim Certificate(s) of Analysis



Centre Analytical Laboratories, Inc.

3048 Research Drive
Phone: (814) 231-8032

State College, PA 16801

www.centrelab.com

Fax: (814) 231-1253 or (814) 231-1580

INTERIM CERTIFICATE OF ANALYSIS

Centre Analytical Laboratories COA Reference #: 023-033 (Revision 1)

3M Product: Ammonium Perfluorooctanoate

Test Control Reference #: TCR-99131-37, Lot #: 332

Purity: 95.2%

Test Name	Specifications	Result
Purity ¹		95.2%
Appearance	White, crystalline solid	Conforms
Identification		
NMR		Positive
Metals (ICP/MS)		
1. Calcium		1. 0.001 wt./wt.%
2. Magnesium		2. <0.001 wt./wt.%
3. Sodium		3. 0.005 wt./wt.%
4. Potassium		4. <0.001 wt./wt.%
5. Nickel		5. <0.001 wt./wt.%
6. Iron		6. <0.001 wt./wt.%
7. Manganese		7. <0.001 wt./wt.%
Total % Impurity (NMR)		0.34 wt./wt.%
Total % Impurity (LC/MS)		4.49 wt./wt.%
Total % Impurity (GC/MS)		None Quantified
Residual Solvents (TGA)		None Detected
Purity by DSC		99.7%
Inorganic Anions (IC)		
1. Chloride		1. <0.015 wt./wt.%
2. Fluoride		2. <0.005 wt./wt.%
3. Bromide		3. <0.040 wt./wt.%
4. Nitrate		4. <0.009 wt./wt.%
5. Nitrite		5. <0.006 wt./wt.%
6. Phosphate		6. <0.006 wt./wt.%
7. Sulfate		7. <0.040 wt./wt.%
Organic Acids ² (IC)		
1. TFA		1. <0.1 wt./wt.%
2. PFPA		2. <0.1 wt./wt.%
3. HFBA		3. <0.1 wt./wt.%
4. NFPA		4. <0.25 wt./wt.%
Elemental Analysis ³ :		
1. Carbon	Theoretical Value = 22.3%	1. 18.9 wt./wt.%
2. Hydrogen	Theoretical Value = 0.935%	2. 1.31 wt./wt.%
3. Nitrogen	Theoretical Value = 3.25%	3. 3.75 wt./wt.%
4. Sulfur	Theoretical Value = 0%	4. 4.34 wt./wt.%
5. Fluorine	Theoretical Value = 66.1%	5. 63.2 wt./wt.%
Ammonium Analysis ⁴		
Ion Selective Electrode	Theoretical Value = 4.18%	3.49 wt./wt. %



Centre Analytical Laboratories, Inc.

3048 Research Drive State College, PA 16801 www.centrelab.com
 Phone: (814) 231-8032 Fax: (814) 231-1253 or (814) 231-1580

INTERIM CERTIFICATE OF ANALYSIS

Centre Analytical Laboratories COA Reference #: 023-033 (Revision 1)

3M Product: Ammonium Perfluorooctanoate

Test Control Reference #: TCR-99131-37, Lot #: 332

Date of Last Analysis: 12/15/00

Expiration Date: 12/15/06

Storage Conditions: < -10 °C

Re-assessment Date: 12/15/06

¹Purity = 100% - (Total Metal impurities, 0.006% + Total NMR impurities, 0.34% + Total LC/MS impurities, 4.49%)

Total impurity from all tests = 4.84%

Purity = 100% - 4.84% = 95.2%

² TFA Trifluoroacetic acid
 HFBA Heptafluorobutyric acid
 NFPA Nonafluoropentanoic acid
 PFPA Pentafluoropropanoic acid

³Theoretical value calculations based on the empirical formula, C₈F₁₅O₂⁽⁻⁾NH₄⁽⁺⁾
 (MW=431.1)

LC/MS Purity Profile:

Peak #	Retention Time (min)	Mass(s)	Identity	Area	% Area
1	12.140	269	C ₈	167596	0.73
2	13.504	331, 319	C ₇ homologues/F ₁₃	860991	3.76
3	14.099	369	PFOA	21861700	-
Total	-	-	-	22890287	4.49

This work was conducted under EPA Good Laboratory Practice Standards (40 CFR 160).

Prepared By: Charles Simons
 Charles Simons
 Scientist, Centre Analytical Laboratories

12/9/01
 Date

Reviewed By: John M. Flaherty
 John Flaherty
 Laboratory Manager, Centre Analytical Laboratories

12/9/01
 Date

3M ENVIRONMENTAL LABORATORY

Note to File

Project or Study Number: FACT-TCR-005
Associated Study Number: LIMS # E00-1762

The expiration dates for PFOA lots HU 14401DU and 332 (TCR-99131-018 and TCR-99131-037) may be extended 5 years (12/15/06) as stability was demonstrated by studies E00-1851 (hydrolysis), E00-2192 (photolysis), and E01-0415 (biodegradation).

[Large area of the form is crossed out with a diagonal line.]

LAC 10/04/01

Recorded By: Lisa Clemen <i>Lisa A. Clemen</i>	Date 10/04/01 <i>10/04/01</i>
--	---

Form ETS-4-15.0

Exact Copy of Original
LAC 10/05/01
Initial Date

Exact copy
of copy
LAC 10/19/01

3M SPECIALTY MATERIALS & MANUFACTURING DIVISION ANALYTICAL LABORATORYRequest No. 62631Study No. FACT-TCR-005To: William Reagen - (8-6565) – Environmental Lab- 2-3E-09From: Tom Kestner - (3-5633) - SMMD Analytical Lab - 236-2B-11Subject: **Chemical Characterization of PFOA, Lot 332, TCR-99131-37 by $^1\text{H-NMR}$, $^{19}\text{F-NMR}$, and IR Spectroscopy**Date: December 11, 2000**SAMPLE DESCRIPTION:**

- PFOA, lot 332, TCR-99131-37; Nominal product $=\text{C}_7\text{F}_{15}\text{-CO}_2^{(-)}\text{NH}_4^{(+)}$ (white powder). The sample was stored in a $-20\text{ }^\circ\text{C}$ freezer at all times except when aliquots were removed for analysis.

OBJECTIVE:

This sample was subjected to $^1\text{H-NMR}$ and $^{19}\text{F-NMR}$ spectral analyses to determine the purity of the nominal product and to characterize as many impurity components as possible. An FT-IR spectrum was also acquired for the purpose of confirming the nominal perfluorinated carboxylate salt functional group.

EXPERIMENTAL:**FT-NMR**

A portion of the thawed sample was accurately weighed, spiked with a known amount of 1,4-bis(trifluoromethyl)benzene (p-HFX), and then totally dissolved in acetone- d_6 for subsequent analysis by NMR. An initial 400 MHz $^1\text{H-NMR}$ spectrum (# h62631.401) and a 376 MHz $^{19}\text{F-NMR}$ spectrum (# f62631.401) were acquired using a Varian UNITYplus 400 FT-NMR spectrometer. A second portion of the sample was also accurately weighed, spiked with a known amount of p-HFX, and then totally dissolved in deuterated trifluoroacetic acid ($\text{CF}_3\text{CO}_2\text{D} = \text{TFAD}$). An additional 400 MHz $^1\text{H-NMR}$ spectrum (#h62631.402) and a 376 MHz $^{19}\text{F-NMR}$ spectrum (#f62631.402) were collected in the TFAD solvent. The primary purpose of using the TFAD solvent was to help minimize the interferences associated with broad $\text{NH}_4^{(+)}$ resonance that had been observed in $^1\text{H-NMR}$ spectrum of the acetone- d_6 solution. The sample preparation method described above was intended to permit the use of the p-HFX as an internal standard for absolute weight percent measurements in either solution.

FT-IR

A portion of the sample was prepared for FT-IR spectral analysis using the KBr disk technique. A transmission FT-IR spectrum (#A62631) was then acquired using a Digilab FTS-40 FT-IR spectrophotometer.

RESULTS:

The combined NMR spectral data indicated the sample of PFOA, lot 332, TCR-99131-37 consisted of a high purity form of the nominal isomeric product mixture, $\text{C}_n\text{F}_{2n+1}\text{-CO}_2^{(-)}\text{NH}_4^{(+)}$, where 'n' was mainly 7. A few trace-level impurity components were also observed, tentatively assigned, and quantified from the NMR spectral data.

December 11, 2000

3M SMD Analytical Lab Request # 62631
3M SMD Analytical Lab: Bldg. 236-2B-11**RESULTS (cont.):**

The qualitative and quantitative compositional results that were derived from the single trial $^1\text{H-NMR}$ and $^{19}\text{F-NMR}$ spectral analyses are summarized in **TABLE-1**. Any water that may have been present in the sample was ignored for calculation purposes. The $^{19}\text{F-NMR}$ relative weight percent concentrations shown in **TABLE-1** should be very close to their respective absolute weight percent values with the stated assumptions. In order to perform the relative weight percent calculations, I assumed all of the fluorocarbon chains contained 8 carbon atoms except where noted. In general, the $^{19}\text{F-NMR}$ technique is not particularly well suited for characterizing small amounts of potential fluorochemical homolog impurity components unless the chains are very short. A more complete characterization of any other impurity homologs would require analysis by electrospray MS or a similar technique. Trace amounts of other unidentified impurities are also detected in the NMR spectra, but additional work would be required in an effort to identify or quantify these other materials.

The FT-IR spectrum was used to verify the nominal perfluorinated carboxylate salt functional group.

Copies of the NMR and IR spectra are attached for your reference. If you have any questions about these results, or if any further work is needed, please let me know.

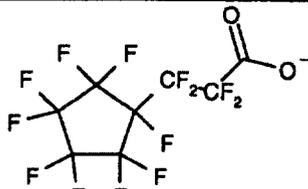
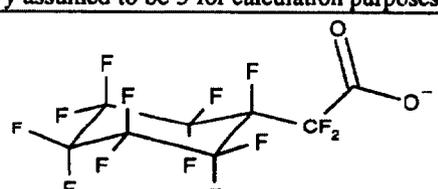
Tom Kestner

c: Lisa Clemen - ET&S - 2-3E-09
Tanya Rude - QAI
Rick Payfer - SA&C Analytical Lab - 236-2C-11

File Reference: wr62631.studyFACTTCR005.doc/78

December 11, 2000

3M SMD Analytical Lab Request # 62631
3M SMD Analytical Lab: Bldg. 236-2B-11**TABLE-1**Sample: PFOA, lot 332, TCR-99131-37
Overall Compositional Results by ^{19}F -NMR Analysis & ^1H -NMR Internal Standardization Analysis

Structural Assignments ¹	^{19}F -NMR Relative Wt. % Concentrations
Normal chain isomer $\text{CF}_3(\text{CF}_2)_x\text{-CO}_2^{(-)}\text{NH}_4^{(+)}$ (where x assumed to be 6 for calculation purposes)	$\approx 77.7\%$
Internal monomethyl branched isomers $\text{CF}_3(\text{CF}_2)_x\text{-CF}(\text{CF}_3)\text{-(CF}_2)_y\text{-CO}_2^{(-)}\text{NH}_4^{(+)}$ (where x+y assumed to be 4 for calculation purpose, and $x \neq 0, y \neq 0$)	$\approx 12.5\%$
Isopropyl branch isomer $(\text{CF}_3)_2\text{CF}(\text{CF}_2)_x\text{-CO}_2^{(-)}\text{NH}_4^{(+)}$ (where x assumed to be 4 for calculation purposes)	$\approx 9.0\%$
t-butyl branch isomer $(\text{CF}_3)_3\text{C}(\text{CF}_2)_x\text{-CO}_2^{(-)}\text{NH}_4^{(+)}$ (where x assumed to be 3 for calculation purposes)	$\approx 0.24\%$
 Possible $\text{NH}_4^{(+)}$	$\approx 0.19\%$
Internal gem-dimethyl branch isomers $\text{CF}_3\text{-(CF}_2)_x\text{-C}(\text{CF}_3)_2\text{-(CF}_2)_y\text{-CO}_2^{(-)}\text{NH}_4^{(+)}$ (where x+y assumed to be 3 for calculation purposes: $x \neq 0$)	$\approx 0.13\%$
 Possible $\text{NH}_4^{(+)}$	$\approx 0.11\%$
Possible alpha branch isomer $\text{C}_x\text{F}_{2x+1}\text{-CF}(\text{CF}_3)\text{-CO}_2^{(-)}\text{NH}_4^{(+)}$ (where x assumed to be 5 for calculation purposes)	$\approx 0.085\%$
Probable $\text{F-SF}_4\text{-R}_f$, possibly as $\text{F-SF}_4\text{-C}_n\text{F}_{2n}\text{-CO}_2^{(-)}\text{NH}_4^{(+)}$ (where n assumed to be 7 for calculation purposes)	$\approx 0.029\%$
Possible $\text{CF}_3\text{CF}_2\text{CO}_2^{(-)}\text{NH}_4^{(+)}$	$\approx 0.009\%$
$\text{CF}_3\text{-O-CF}_2\text{-R}_f$, where -R_f is undefined	Trace
	^1H-NMR Absolute Wt. % Concentration
Total unassigned aliphatic materials	$\approx 0.002\text{-}0.003\%$

1) Trace amounts of other unidentified components are also detected in the NMR spectra.

Certificate of Analysis

Nominal Product: $\text{CF}_3(\text{CF}_2)_n\text{-CO}_2\text{H}$, where average $n \approx 6$

Pentadecafluorooctanoic acid

Product Code: TCR-617, Lot 210002

October 28, 2002

Tom Kestner and Joel Miller

The sample of TCR-617, lot 210002 was analyzed using a combination of ^{19}F -NMR, ^1H -NMR, and LC/MS analysis techniques. The overall qualitative and quantitative compositional results that were derived from these combined analyses are summarized below in TABLE-1.

TABLE-1

Sample: TCR-617, Lot 210002

Quantitative and Qualitative Compositional Results by Combined $^{19}\text{F}/^1\text{H}$ -NMR and LC/MS Analyses

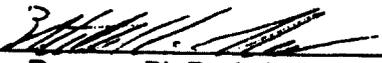
Component Structures ¹	$^1\text{H}/^{19}\text{F}$ -NMR Relative Weight% Concentrations (single trial analysis)
$\text{CF}_3(\text{CF}_2)_n\text{-CO}_2\text{H}$ where average $n = 6.02$ by ^{19}F -NMR. LC/MS showed $n=6$ (major), $n=5$, $n=4$ (minors).	$\leq 99.51\%$ Purity
Probable $(\text{CF}_3)_2\text{-CF-(CF}_2)_n\text{-CO}_2\text{H}$ assume $n=4$ for calculation purposes	0.39%
Probable $(\text{C}_6\text{F}_{13}\text{O})\text{-CO}_2\text{H}$ acyclic ether acid as possible $\text{CF}_3\text{CF}_2\text{-O-CF(CF}_3\text{)-CF}_2\text{CF}_2\text{-CO}_2\text{H}$	0.057%
Possible $\text{CF}_3(\text{CF}_2)_x\text{-CF(CF}_3\text{)-(CF}_2)_y\text{-CO}_2\text{H}$ where $x \neq 0$, $y \neq 0$ and assume $x+y = 4$ for calculation purposes	0.019%
Possible $(\text{CF}_3)_3\text{-C-(CF}_2)_n\text{-CO}_2\text{H}$ assume $n=3$ for calculation purposes	0.013%
Possible $\text{C}_n\text{H}_{2n+2}$ saturated aliphatic hydrocarbons	0.0079%

1. Trace amounts of other unassigned components were also detected in the NMR spectra.

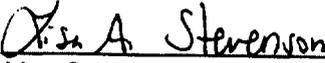
Thomas A. Kestner
10-28-02

Joel Miller
10/28/02

Appendix G: Report Signature Page



William Reagen, Ph.D., *Laboratory Management, Sponsor Representative* 11/13/02
Date



Lisa Stevenson, *Principal Analytical Investigator* 11/13/02
Date



226-1151

MR#64776

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CORP

2002 DEC 17 11:11:03

Study Title

ANALYSIS OF ENDOGENOUS FLUOROCEMICALS IN NORMAL POOLED HUMAN SERUM AND PLASMA

Data Requirement

40 CFR Part 792

Author

Mark E. Ellefson

Study Completion Date

13 November, 2002

Revised Report

9 December, 2002

Performing Laboratory

3M Environmental Laboratory

Building 2-3E-09

935 Bush Avenue

St. Paul, MN 55106

Project Identification

3M Environmental Laboratory Study Number E02-1039

Total Number of Pages

225

CONTAIN NO CBI

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This page has been reserved for specific country requirements.

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GLP COMPLIANCE STATEMENT

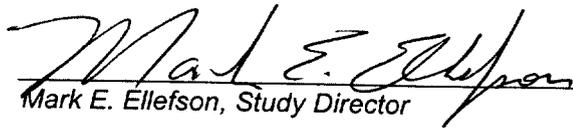
Study Title: Analysis of Endogenous Fluorochemicals in Normal Pooled Human Serum and Plasma

Study Identification Number: 3M Environmental Laboratory study Number E02-1039

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

Exceptions to GLP compliance:

40 CFR 792.130(e): There is not an electronic audit trail of corrections. The authenticated hardcopy printouts are considered the original raw data.


Mark E. Ellefson, Study Director

12/9/02
Date


William K. Reagen, Testing Facility Management

12/09/02
Date

000095

QUALITY ASSURANCE STATEMENT

Study Title: Analysis of Endogenous Fluorochemicals in Normal Pooled Human Serum and Plasma

Study Identification Number: 3M Environmental Laboratory Study Number E02-1039

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

INSPECTION DATES	PHASE	DATE REPORTED TO	
		MANAGEMENT	STUDY DIRECTOR
10/10/02	Protocol	10/10/02	10/10/02
10/11/02	In-phase	10/11/02	10/11/02
10/29/02 - 10/30/02	Data	10/30/02	10/30/02
10/31/02	Final Report	11/1/02	11/1/02

Tampa K. Rude
QAU Representative

12/6/02
Date

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STUDY INFORMATION

Sponsor

William K. Reagen
3M Environmental Laboratory
Bldg. 2-3E-09
935 Bush Avenue
St. Paul, MN 55106
651-778-6565

Study Director

Mark E. Ellefson
3M Environmental Laboratory
Bldg. 2-3E-09
935 Bush Avenue
St. Paul, MN 55106
651-778-5405

Study Location

Testing Facility

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

William K. Reagen, *Laboratory Manager*
Stacy R. A. Hanson, *Analytical Chemist*
Marlene M. Heying, *Analytical Chemist*
Cindy M. Carlson, *Analytical Chemist*
Ognjenka Krupljanin, *Analytical Chemist*

Study Dates

Study Initiation: 10 October 2002
Experimental Initiation: 10 October 2002
Experimental Completion: 31 October 2002
Study Completion: 01 November 2002

Location of Archives

All original raw data and the report have been archived at the 3M Environmental Laboratory according to 3M Standard Operating Procedures. Remaining specimens pertaining to the analytical phase of this study will be archived at 3M Environmental Laboratory for as long as the quality of the preparation affords evaluation.

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EXECUTIVE SUMMARY

A screening study of three lots of commercial pooled human plasma, one lot of pooled human plasma from central China, and four lots of commercial pooled human serum was undertaken to quantify endogenous levels of perfluoroheptanoate (C7), pentadecafluorooctanoate (C8) (PFOA), heptadecafluorononanoate (C9), nonadecafluorodecanoate (C10), perfluoroundecanoate (C11), perfluorododecanoate (C12), and perfluorooctane sulfonate (PFOS).

Results from this study showed quantifiable levels of the linear isomers of C7, C9-C12 ranging from < 0.010 – 0.9 ng/mL (Tables 1a and 1b). Low to non-detect levels of branched isomers were observed for the C7 and C9-C12 compounds detected (Figure 1 – 10). PFOS was present in the screened lots of commercial sera and plasma in concentrations ranging from 2.5 – 27 ng/mL and was present as branched and linear isomers. PFOA was present in the screened lots of commercial sera and plasma in concentrations ranging from 0.65 – 5.6 ng/mL and was present as branched and linear isomers.

000100

SUMMARY

Quantitative screenings were conducted on four lots of pooled human serum and four lots of pooled human plasma to determine endogenous levels of perfluoroheptanoate (C7), pentadecafluorooctanoate (C8), heptadecafluorononanoate (C9), nonadecafluorodecanoate (C10), perfluorodecanoate (C11), perfluorododecanoate (C12), and perfluorooctane sulfonate (PFOS) as described by the 3M Environmental Laboratory Study #E02-1039 protocol. The analytical screening data is summarized in tables 1.a. and 1.b.

Table 1.a. Endogenous levels of test substance in normal pooled human serum

Test Substance	Sigma TCR-689 (ng/mL)	Lampire TCR-688 (ng/mL)	Bioresource TCR-687 (ng/mL)	Golden West TCR-690 (ng/mL)
PFOS	4.56	[2.49]	17.0	27.0
C ₁₂	[0.018]	[< 0.010] ¹	[0.144]	[0.040]
C ₁₁	[0.076]	[< 0.010] ¹	0.295	0.320
C ₁₀	[0.058]	[0.031]	[0.327]	0.203
C ₉	0.265	[0.068]	0.605	0.900
C ₈	1.60	0.650	2.95	5.60
C ₇	[0.013]	[0.025]	[< 0.010] ¹	0.190

[] Denotes values determined from concentrated spe method. ¹Value < LOQ.

Table 1.b. Endogenous levels of test substance in normal pooled human Plasma

Analyte	Chinese TCR-674 (ng/mL)	Lampire TCR-685 (ng/mL)	Innovative Research TCR-683 (ng/mL)	Golden West TCR-684 (ng/mL)
PFOS	< 1.0 ¹	11.1	15.6	18.3
C ₁₂	< 0.10 ¹	[0.036]	[0.022]	[0.024]
C ₁₁	< 0.10 ¹	[0.049]	0.135	[0.071]
C ₁₀	< 0.10 ¹	[0.127]	0.170	0.160
C ₉	< 0.25 ¹	0.435	0.585	0.535
C ₈	< 0.52 ¹	2.61	3.07	3.87
C ₇	< 0.10 ¹	0.100	[0.016]	0.290

[] Denotes values determined from concentrated spe method. ¹Value < LOQ.

Accurate mass measurements and elemental compositions were obtained for endogenous C7-C11, and PFOS from a concentrated SPE extract of normal pooled human serum (see Table 6).

Characteristic daughter ions were observed for endogenous C7-C12, and PFOS from a concentrated SPE extract of normal pooled human plasma (see Table 7).

Qualitative analysis of chromatographically resolved branched and linear isomers of PFOA was accomplished using NMR certified standards of a linear isomer PFOA standard and of a mixed branched and linear isomer PFOA mixed standard. It was determined that the branched PFOA isomers elute within an approximate 0.2 minute retention time window and are baseline resolved from the linear PFOA isomer. This finding of C8 isomer retention time resolution was

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extrapolated to determine qualitatively the presence or absence of branched isomers for the higher homologues of C9-C12, and PFOS (see Figures 1 – 8).

Results from this study showed quantifiable levels of the linear isomers of C7, C9-C12 ranging from < 0.010 – 0.9 ng/mL (Tables 1a and 1b). Low to non-detect levels of branched isomers were observed for the C7 and C9-C12 compounds detected (Figures 1 – 8). PFOS was present in the screened lots of commercial sera and plasma in concentrations ranging from 2.5 – 27 ng/mL and was present as branched and linear isomers. PFOA was present in the screened lots of commercial sera and plasma in concentrations ranging from 0.65 – 5.6 ng/mL and was present as branched and linear isomers.

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INTRODUCTION

The purpose of this study is to perform quantitative screening for perfluoroheptanoate (C7), pentadecafluorooctanoate (C8), heptadecafluorononanoate (C9), nonadecafluorodecanoate (C10), perfluorodecanoate (C11), perfluorododecanoate (C12), and perfluorooctanesulfonate (PFOS) in normal pooled human serum and plasma. This study did not have a typical test substance that is dosed onto a specific controlled test system as per a conventional GLP study. The study design is in compliance with US EPA Toxic Substances Control Act (TSCA) 40 CFR Part 792.

TEST SUBSTANCE

Preliminary screening indicated that the test substances listed below are present as endogenous material in normal pooled human serum and plasma. Information pertaining to traceability, source, physical description, and storage conditions is not available for these compounds as they exist in biological matrices.

Table 2. Characterization of the Test Substances

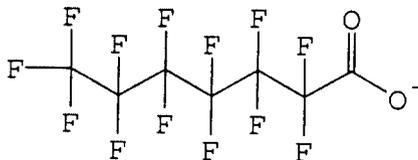
TEST SUBSTANCE	FORMULA
Tridecafluoroheptanoate (C7)	$C_7F_{13}COO^-$
Pentadecafluorooctanoate (C8)	$C_8F_{15}COO^-$
Heptadecafluorononanoate (C9)	$C_9F_{17}COO^-$
Nonadecafluorodecanoate (C10)	$C_{10}F_{19}COO^-$
Perfluoroundecanoate (C11)	$C_{11}F_{21}COO^-$
Perfluorododecanoate (C12)	$C_{12}F_{23}COO^-$
Perfluorooctane sulfonate (PFOS)	$C_8F_{17}SO_3^-$

The molecular structures are given below.

Name: C7

Chemical Name: Perfluoroheptanoate

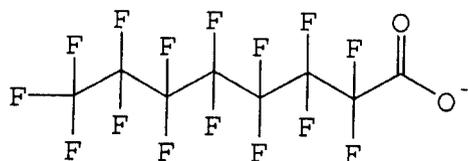
Molecular Weight: 363, as shown



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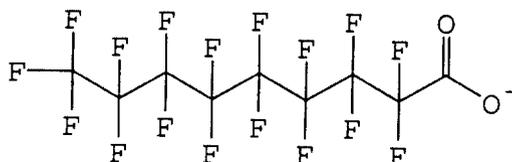
Name: C8

Chemical Name: Pentadecafluorooctanoate
Molecular Weight: 413, as shown



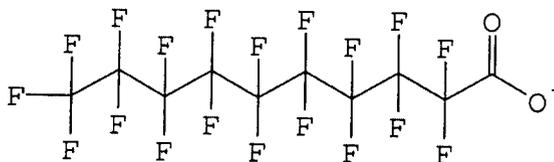
Name: C9

Chemical Name: Heptadecafluorononanoate
Molecular Weight: 463, as shown



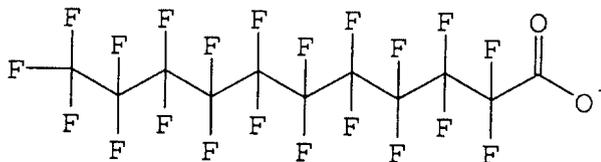
Name: C10

Chemical Name: Nonadecafluorodecanoate
Molecular Weight: 513, as shown



Name: C11

Chemical Name: Perfluoroundecanoate
Molecular Weight: 563, as shown



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REFERENCE SUBSTANCE

Table 3. Characterization of the Reference Substances

Reference Substance	Formula	Traceability #	Source	Physical Description	Purity	Storage Conditions
Tridecafluoro heptanoic Acid	$C_6F_{13}COOH$	TCR-267	Aldrich	Clear Crystals	98.2%**	Frozen
Pentadecafluoro octanoic Acid	$C_7F_{15}COOH$	TCR-617	Oakwood Products	White Crystals	99.5%**	Ambient Temperature
Heptadecafluoro nonanoic Acid	$C_8F_{17}COOH$	TCR-618	Oakwood Products	White Crystals	98.02%**	Ambient Temperature
Nonadecafluoro decanoic Acid	$C_9F_{19}COOH$	TCR-036	Oakwood Products	White Solid	98%**	Frozen
Perfluoroun decanoic Acid	$C_{10}F_{21}COOH$	TCR-619	Oakwood Products	White Crystals	96.4%**	Ambient Temperature
Perfluorodo decanoic Acid	$C_{11}F_{23}COOH$	TCR-037	Oakwood Products	White Powder	99.7%**	Frozen
Potassium Perfluorooctane sulfonate	$C_8F_{17}SO_3K^+$	TCR-018	SMM* 236-1B-10	White Powder	86.9%	Frozen

*Documentation of the method of synthesis is located at the source.

**Reference substances confirmed as linear isomers by NMR.

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TEST SYSTEMS

Table 4. Description of Test Systems Used in this Study

TEST SYSTEM:	SOURCE	TRACEABILITY
Pooled Human Serum	Sigma-Aldrich, Milwaukee, WI	TCR-689, Lot 022K0965
Pooled Human Serum	Lampire Biological Laboratories, Pipersville, PA	TCR-688, Lot X324B
Pooled Human Serum	Bioresource Technology, Inc., Fort Lauderdale, FL	TCR-687, Lot 020821
Pooled Human Serum	Golden West Biologicals, Temecula, CA	TCR-690, Lot G01406042
Pooled Human Plasma	Lampire Biological Laboratories, Pipersville, PA	TCR-685, Lot 22-60824A
Pooled Human Plasma	Golden West Biologicals, Temecula, CA	TCR-684, Lot G01410002
Pooled Human Plasma	Innovative Research, Inc., Southfield, MI	TCR-683, Lot IR02-014
Pooled Human Plasma	Central China	TCR-674, Lot N087P27

Justification of the Test System. Based on preliminary testing, normal pooled serum and plasma contain endogenous levels of the test substances.

Identification of Test System. Samples were identified by the TCR number.

Collection of Test Systems. The commercial vendors indicated that three of the four lots of commercial pooled human serum and three lots of commercial pooled human plasma purchased for this study were collected from subjects residing in close proximity to the individual vendors (Sigma-Aldrich purchases blood products from blood banks located throughout the US). Hence, although limited to single lots of serum or plasma per location, analytical data from the purchased serum and plasma provide a benchmark of endogenous levels of fluorochemicals from different regions of the country.

METHOD SUMMARIES

Preparatory and Analytical Method

ETS-8-231.1, "Solid Phase Extraction and Analysis of Fluorochemical Compounds from Biological Matrices." A 2.0 mL aliquot of serum or plasma is transferred to a 50 mL screw-capped polyethylene centrifuge tube. Spiking solution is added as appropriate, followed by the addition of 8.0 mL of ASTM Type I water. The mixture is shaken and 40.0 mL of acetonitrile are added. The sample container was capped, mixed for 20 minutes, and centrifuged at 3,500 rpm for 20 minutes to clarify the supernatant. Following centrifugation, the supernatant is transferred to a 500 mL Nalgene® container, diluted with 350 mL ASTM Type I water, and passed through a pre-conditioned C₁₈ solid phase extraction (SPE) cartridge. The analytes of interest are eluted from the SPE cartridge using 2.0 mL of methanol.

Concentrated SPE Method, A modified form of the SPE method described by ETS-8-231.1, "Solid Phase Extraction and Analysis of Fluorochemical Compounds from Biological Matrices." used to concentrate analytes present at concentrations below the LOQ. A five-fold concentration is achieved by using 5 times more serum or plasma while maintaining the final elution volume at 2.0 mL methanol. The method consists of a 10.0 mL aliquot of serum or plasma transferred to a 250 mL Nalgene® container. Spiking solution was added as appropriate, followed by the addition of 40.0 mL of ASTM Type I water. The mixture is shaken and 200.0 mL of acetonitrile are added. The sample container is capped, mixed for 20 minutes, and centrifuged at 3,500 rpm for 20 minutes to clarify the supernatant. Following centrifugation, the supernatant is transferred to a 2.0 L Nalgene® container, diluted with 1750 mL ASTM Type I water, and passed through a pre-conditioned C₁₈ solid phase extraction

000107

(SPE) cartridge. The analytes of interest are eluted from the SPE cartridge using 2.0 mL of methanol. Further sensitivity was achieved by doubling the injection volume of the standards used to calibrate the run.

Analytical Method

Liquid Chromatograph: Hewlett-Packard® Series 1100 Liquid Chromatograph system
 Analytical column: Keystone® Betasil™ C₁₈ 2x100mm, 5µm particle size
 Column temperature: 40 °C
 Cycle Time: 17.0 minutes
 Flow rate: 300µL/min
 Injection volume: 10 µL (20 µL for concentrated SPE method only)
 Mobile phase components:
 Solvent A: 2.0 mM ammonium acetate in water
 Solvent B: Methanol

Solvent Gradient:	<u>Time (min.)</u>	<u>% B</u>
	0.00	40%
	10.00	90%
	11.00	90 %
	11.50	100 %
	12.50	100 %
	13.00	40 %
	16.00	40 %

Mass Spectrometer: Micromass Quatro Ultima triple quadrupole mass spectrometer
 Software: Masslynx version 3.5.

Mass Spectrometer Acquisition Parameters:

(C₇)

<u>Channel</u>	<u>Parent Ion (m/z)</u>	<u>Daughter Ion (m/z)</u>	<u>Collision Energy (eV)</u>	<u>Cone (V)</u>
1	363.00	119.00	20	20
2	363.00	169.00	20	20
3	363.00	219.00	20	20
4	363.00	319.00	20	20

(C₈)

<u>Channel</u>	<u>Parent Ion (m/z)</u>	<u>Daughter Ion (m/z)</u>	<u>Collision Energy (eV)</u>	<u>Cone (V)</u>
1	413.00	119.00	20	20
2	413.00	169.00	20	20
3	413.00	219.00	20	20
4	413.00	369.00	20	20

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(C₉)

<u>Channel</u>	<u>Parent Ion (m/z)</u>	<u>Daughter Ion (m/z)</u>	<u>Collision Energy (eV)</u>	<u>Cone (V)</u>
1	463.00	119.00	20	20
2	463.00	169.00	20	20
3	463.00	219.00	20	20
4	463.00	419.00	20	20

(C₁₀)

<u>Channel</u>	<u>Parent Ion (m/z)</u>	<u>Daughter Ion (m/z)</u>	<u>Collision Energy (eV)</u>	<u>Cone (V)</u>
1	513.00	119.00	20	20
2	513.00	169.00	20	20
3	513.00	219.00	20	20
4	513.00	469.00	20	20

(C₁₁)

<u>Channel</u>	<u>Parent Ion (m/z)</u>	<u>Daughter Ion (m/z)</u>	<u>Collision Energy (eV)</u>	<u>Cone (V)</u>
1	563.00	119.00	20	20
2	563.00	169.00	20	20
3	563.00	219.00	20	20
4	563.00	519.00	20	20

(C₁₂)

<u>Channel</u>	<u>Parent Ion (m/z)</u>	<u>Daughter Ion (m/z)</u>	<u>Collision Energy (eV)</u>	<u>Cone (V)</u>
1	613.00	119.00	20	20
2	613.00	169.00	20	20
3	613.00	219.00	20	20
4	613.00	569.00	20	20

000109

PFOS

<u>Channel</u>	<u>Parent Ion (m/z)</u>	<u>Daughter Ion (m/z)</u>	<u>Collision Energy (eV)</u>	<u>Cone (V)</u>
1	499.00	80.00	45	60
2	499.00	99.00	45	60
3	499.00	130.00	45	60

Capillary Voltage: 4000 V
Gain = 1.0 EMV
Mode: Electrospray Negative
Gas Temperature: 250 °C
Drying Gas: 8.0 L /min.
Nebulizer Pressure: 30 psig
Analysis Type: Multiple Reaction Monitoring (MRM)

ANALYTICAL RESULTS

Data quality objectives outlined in the 3M Environmental Laboratory method were met (see Appendix A).

Regressions. Quadratic curve fit weighted 1/x was applied to calibration standards and sample data to improve quantitation over the concentration range appropriate to the data. All calibration curves had a coefficient of determination of 0.998 or greater.

Calibration Standards. Calibration curves were prepared from extracted matrix standards in Chinese plasma for all plasma and serum quantitations. Reported values were not corrected for endogenous levels of test substance in the Chinese plasma calibration curve (levels of all endogenous test substances in the Chinese plasma were determined to be < LOQ). The curves consisted of a minimum of nine (9) points. The equation was determined by regression analysis using the peak areas of the analyte. The accuracy of each level was verified. Any level outside 70% - 130% of nominal was deactivated, and regression re-calculated. All levels showed a response greater than twice that of the blank.

Continuing Calibration Verification. For quantitative determinations, a mid-level matrix calibration check was analyzed at least every ten samples to monitor instrumental drift, with a limit of ±25% deviation of the target concentrations.

Limit of Quantitation (LOQ). The LOQ was equal to the lowest standard in the calibration curve, with a level of accuracy within ±30%. The level of analyte in the LOQ was also greater than two times the response of analyte in the blank samples.

Demonstration of Specificity. The identification of analytes was substantiated by chromatographic retention times, characteristic primary ions, characteristic daughter ions, and isomeric proportions (where applicable).

Control of Bias. Two levels of matrix fortifications, prepared at known concentrations of the test substance and bracketing the anticipated range of the method were evaluated to determine recovery and to evaluate method performance. In serum samples, PFOS and C12 exhibited matrix effects identified by high (>150% recovery) or low (< 75% recovery). For all other analytes, and for all analytes in plasma samples, recoveries ranged from 75% to 105%. Reagent and matrix blanks were run with each set to evaluate the level of background interferences.

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Blanks. Method blanks and matrix blanks were evaluated in the course of this study. The method blanks showed no evidence of background contamination introduced in the sample preparation stage. The matrix blanks did show endogenous levels of the analytes. This was expected and such instances were noted in the raw data.

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DATA SUMMARY

Quantitative screenings were conducted on four lots of normal pooled human serum and four lots of normal pooled human plasma for the determination of endogenous levels of perfluoroheptanoate (C7), pentadecafluorooctanoate (C8), heptadecafluorononanoate (C9), nonadecafluorodecanoate (C10), perfluorodecanoate (C11), perfluorododecanoate (C12), and perfluorooctane sulfonate (PFOS) as described by the 3M Environmental Laboratory Study #E02-1039 protocol. The screening data is summarized in tables 5.a. and 5.b. Matrix spike recovery information is contained in Appendix B.

Table 5.a. Endogenous levels of test substance in normal pooled human serum

Test Substance	Sigma TCR-689 (ng/mL)	Lampire TCR-688 (ng/mL)	Bioresource TCR-687 (ng/mL)	Golden West TCR-690 (ng/mL)
PFOS	4.56	[2.49]	17.0	27.0
C ₁₂	[0.018]	[< 0.010] ¹	[0.144]	[0.040]
C ₁₁	[0.076]	[< 0.010] ¹	0.295	0.320
C ₁₀	[0.058]	[0.031]	[0.327]	0.203
C ₉	0.265	[0.068]	0.605	0.900
C ₈	1.60	0.650	2.95	5.60
C ₇	[0.013]	[0.025]	[< 0.010] ¹	0.190

[] Denotes values determined from concentrated spe method. ¹Value < LOQ.

Table 5.b. Endogenous levels of test substance in normal pooled human Plasma

Analyte	Chinese TCR-674 (ng/mL)	Lampire TCR-685 (ng/mL)	Innovative Research TCR-683 (ng/mL)	Golden West TCR-684 (ng/mL)
PFOS	< 1.0 ¹	11.1	15.6	18.3
C ₁₂	< 0.10 ¹	[0.036]	[0.022]	[0.024]
C ₁₁	< 0.10 ¹	[0.049]	0.135	[0.071]
C ₁₀	< 0.10 ¹	[0.127]	0.170	0.160
C ₉	< 0.25 ¹	0.435	0.585	0.535
C ₈	< 0.52 ¹	2.61	3.07	3.87
C ₇	< 0.10 ¹	0.100	[0.016]	0.290

[] Denotes values determined from concentrated spe method. ¹Value < LOQ.

Accurate Mass Determination, Accurate mass measurements and elemental compositions were obtained for endogenous C7-C11 and PFOS in a concentrated SPE extract of normal pooled human serum purchased from Golden West Biologicals (TCR-690). Accurate mass measurements are presented in Table 6.

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Table 6. Accurate Mass Determination of Endogenous Test Substances

Test Substance	Accurate Mass	Theoretical Mass	Mass Deviation (ppm)	Probable Formula
C7	362.9739	362.9691	13.3	C ₇ O ₂ F ₁₃
C8	412.9637	412.9659	-5.4	C ₈ O ₂ F ₁₅
C9	462.9613	463.9627	-3.0	C ₉ O ₂ F ₁₇
PFOS	498.9280	498.9297	-3.3	C ₈ O ₃ F ₁₇ S
C10	512.9615	512.9595	3.9	C ₁₀ O ₂ F ₁₉
C11	562.9542	562.9563	-3.8	C ₁₁ O ₂ F ₂₁

Daughter Ions of Endogenous Test Substances, Specific daughter ions were observed at characteristic retention times for endogenous C7-C11 and PFOS in a concentrated SPE extract of normal pooled human plasma purchased from Golden West Biologicals (TCR-684). Daughter ions verified in analytical standards of linear isomers. A summary of daughter ions observed is presented in Table 7.

Table 7. Daughter Ions of Endogenous Test Substances

Test Substance	Parent Ion (m/z)	Daughter Ions (m/z)
C ₇	363	319, 119
C ₈	413	369, 219, 169, 119
C ₉	463	419, 269, 219, 169, 119
C ₁₀	513	469, 319, 269, 219, 169
C ₁₁	563	519, 319, 219, 169, 119
C ₁₂	613	569, 419, 319, 269, 219, 169, 119
PFOS	499	130, 99, 80

Isomers, The presence or absence of distributions of branched and linear isomers of endogenous fluorochemicals were qualitatively determined in concentrated SPE extracts of commercial lots of pooled human serum and plasma (Figures 1 – 10). Qualitative analysis of chromatographically resolved branched and linear isomers of PFOA was accomplished using NMR certified standards of a linear isomer PFOA standard and a mixed branched and linear isomer PFOA standard. It was determined that the branched PFOA isomers elute within an approximate 0.2 minute retention time window and are baseline resolved from the linear PFOA isomer. This finding of C8 retention time resolution was extrapolated to determine qualitatively the presence of branched and linear isomers for C7, the higher homologues of C9-C12, and PFOS.

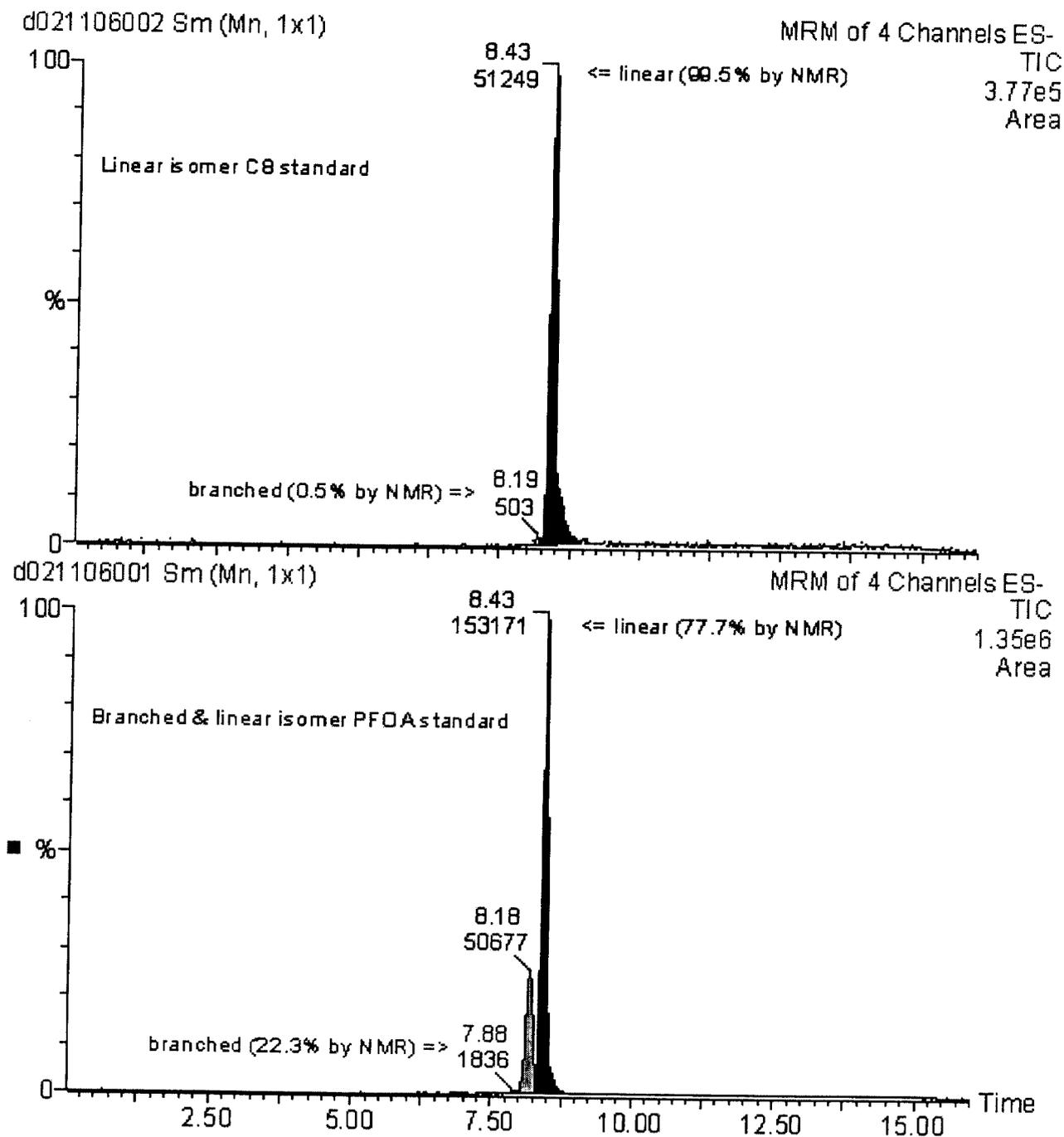
Low to non-detect levels of branched isomers were observed for the C7 and C9-C12 compounds detected. PFOS and PFOA were present in the screened lots of sera and plasma as branched and linear isomer distributions. Figure 1 contains chromatograms of NMR certified linear isomer PFOA and mixed branched and linear isomer PFOA standards. Figures 2 – 8 contain chromatograms of the corresponding standards of C7 – C12 and PFOS in solvent, an extracted calibration curve point (extracted calibration curve point consists of pooled human plasma from central China spiked with 10 ng of test substance/mL of plasma), and three concentrated SPE

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extracts of commercial pooled human serum. Pooled human plasma collected in central China was used as the blank matrix to construct an extracted calibration curve because preliminary screening results indicated it did not contain quantifiable levels of endogenous test substances.

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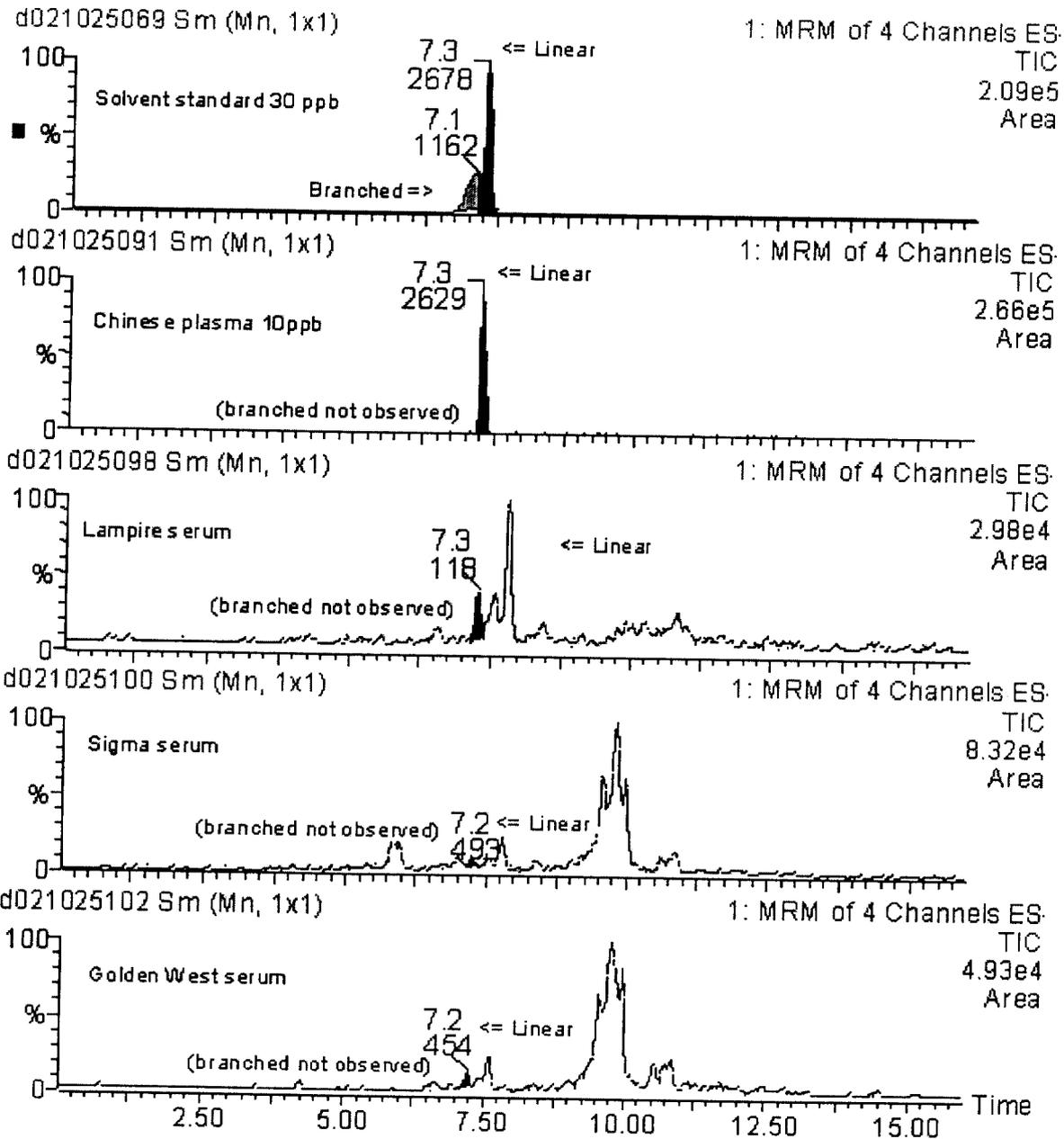
Figure 1: NMR Certified 99.5% Linear Isomer PFOA and Mixed Branched and Linear Isomer PFOA Standards



Summary of PFOA Standards: NMR certified standard of the linear isomer PFOA contains 0.5% branched and 99.5% linear isomers. NMR certified standard of mixed branched and linear isomer PFOA contains 22.3% branched and 77.7% linear isomers.

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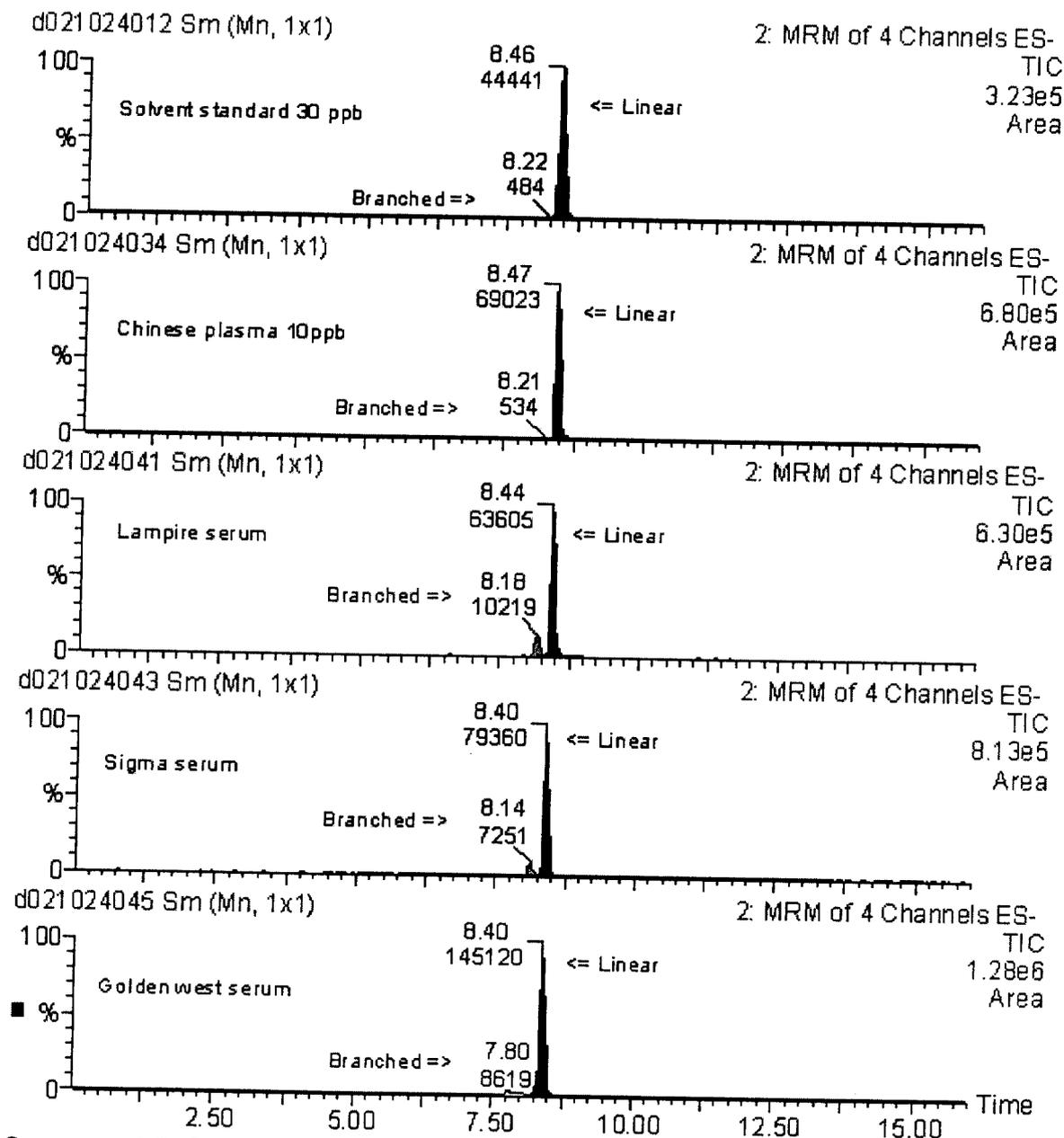
Figure 2: C₇ Isomer Distribution



Summary of C₇ Acid Chromatograms: Low to non-detect levels of the branched isomers of C₇ acid were observed in these lots of commercial pooled human serum.

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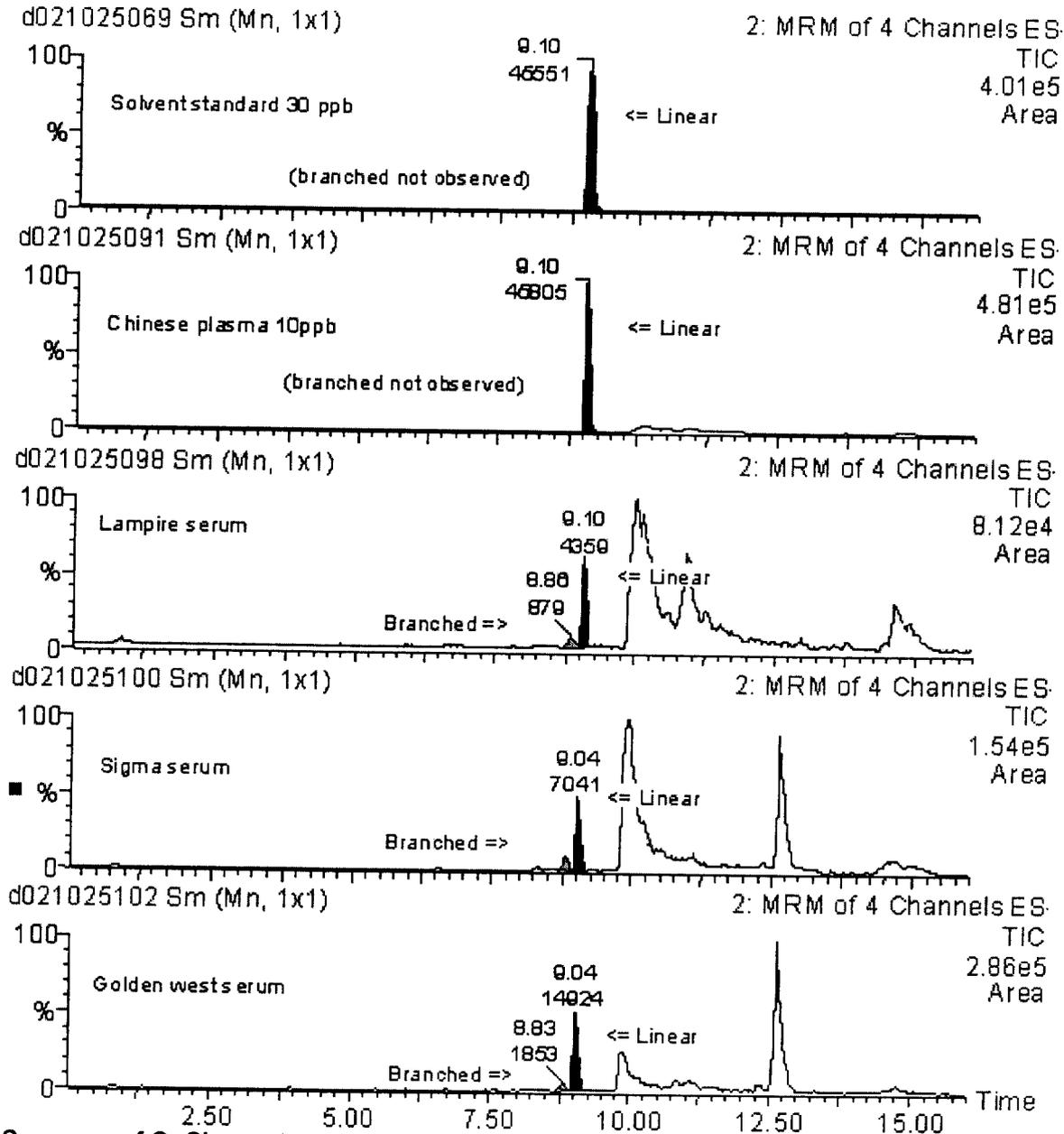
Figure 3: C₈ Isomer Distribution



Summary of C₈ Chromatograms: Low to non-detect levels of branched isomers of C₈ were observed in these lots of commercial pooled human serum.

000117

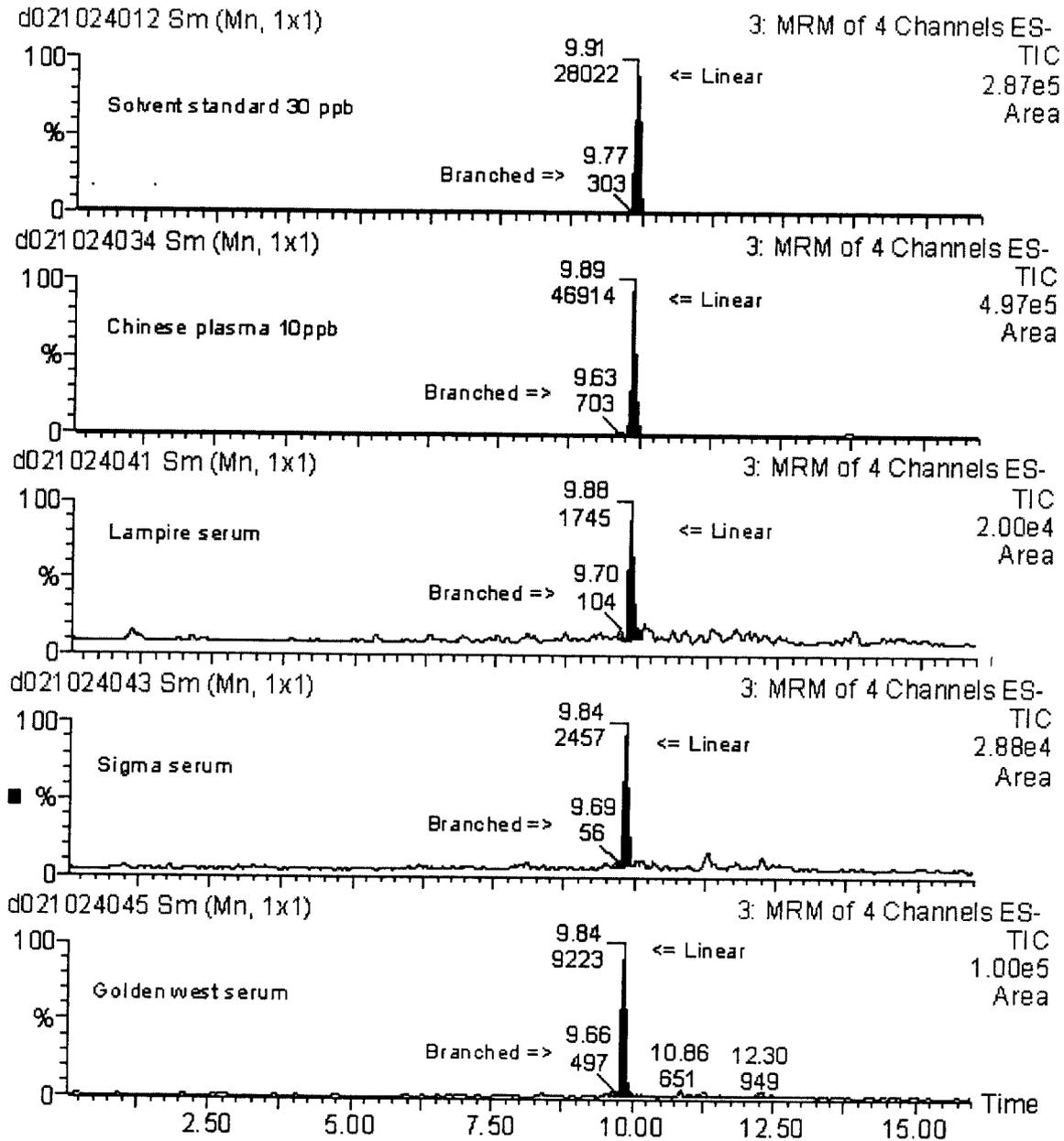
Figure 4: C₉ Isomer Distribution



Summary of C₉ Chromatograms: Low to non-detect levels of branched isomers of C₉ were observed in these lots of commercial pooled human serum.

000118

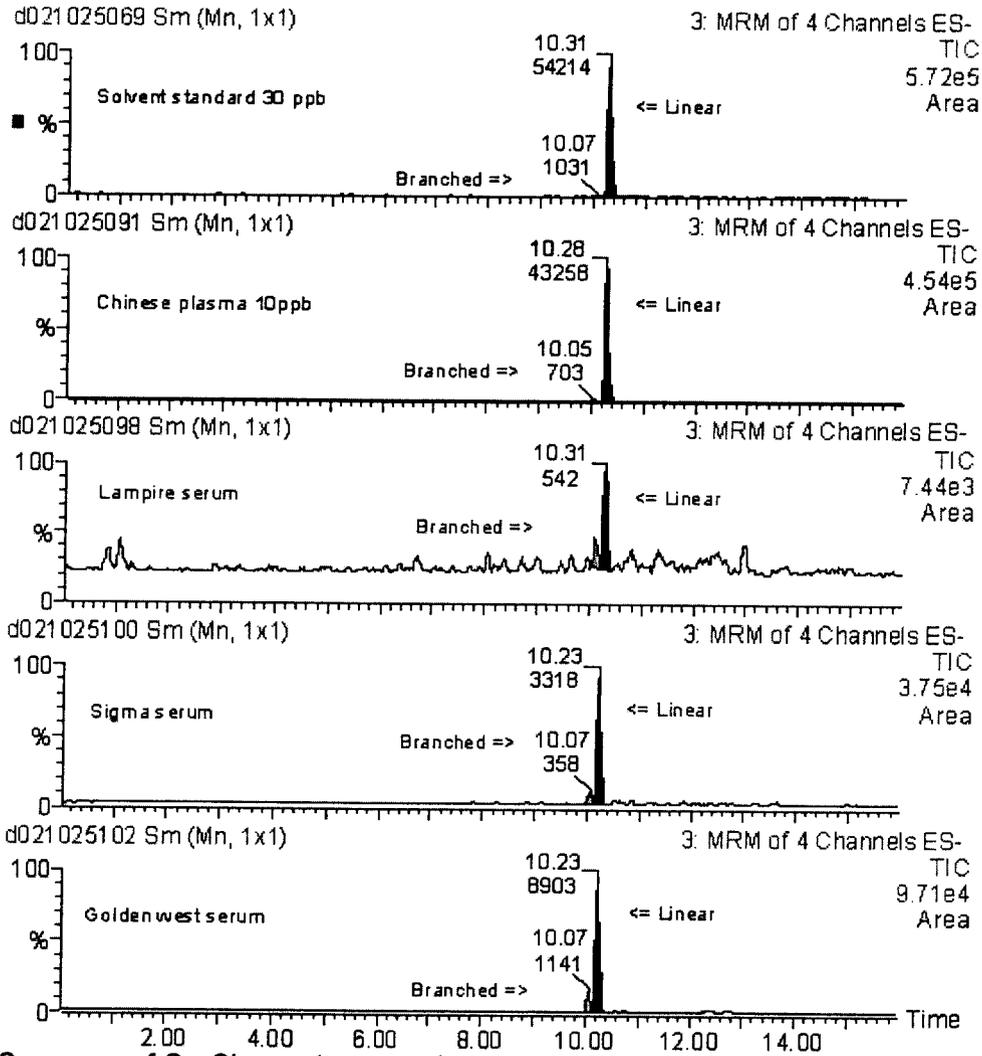
Figure 5: C₁₀ Isomer Distribution



Summary of C₁₀ Chromatograms: Low to non-detect levels of branched isomers of C₁₀ were observed in these lots of commercial pooled human serum.

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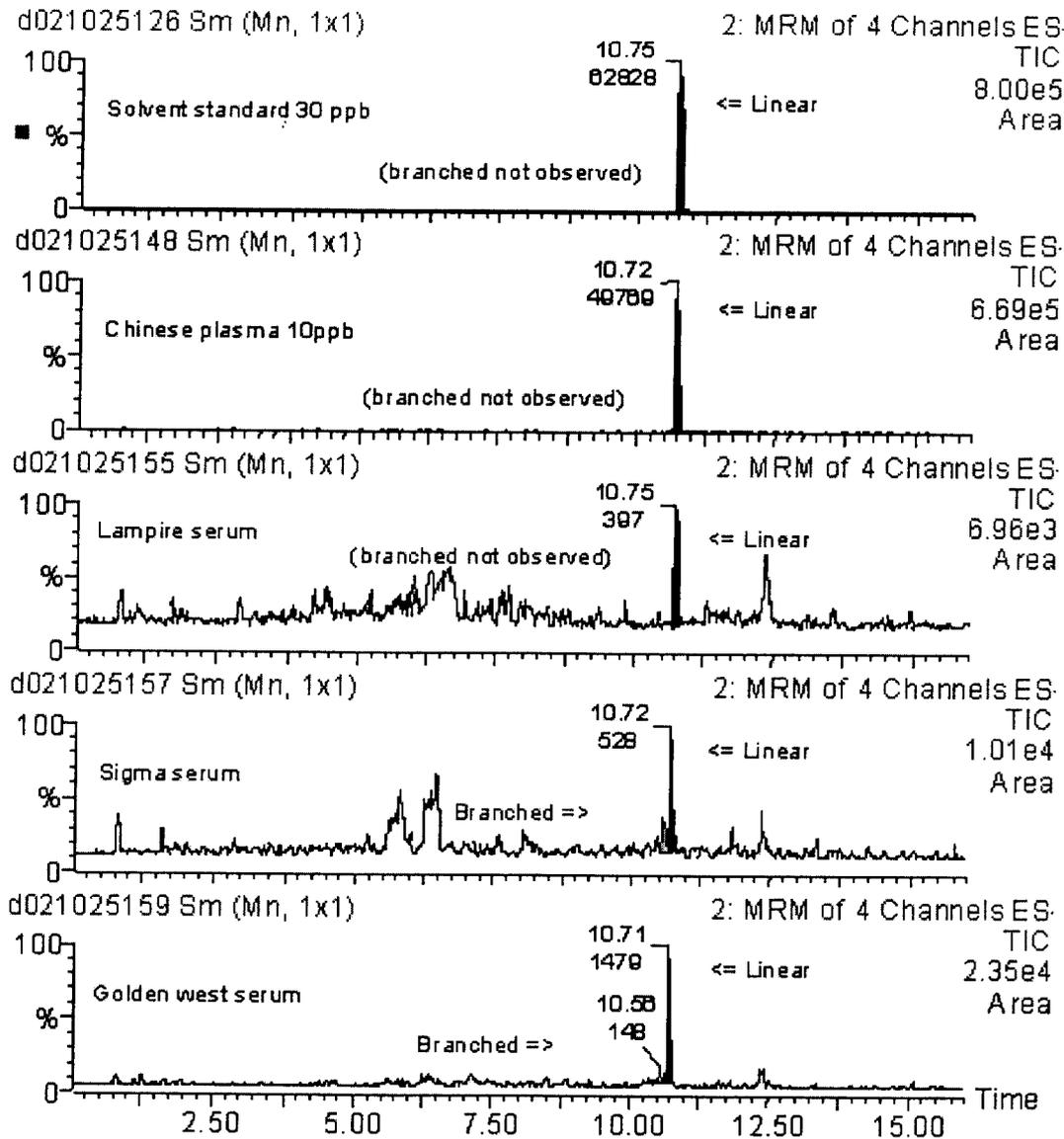
Figure 6: C₁₁ Isomer Distribution



Summary of C₁₁ Chromatograms: Low to non-detect levels of branched isomers of C₁₁ were observed in these lots of commercial pooled human serum.

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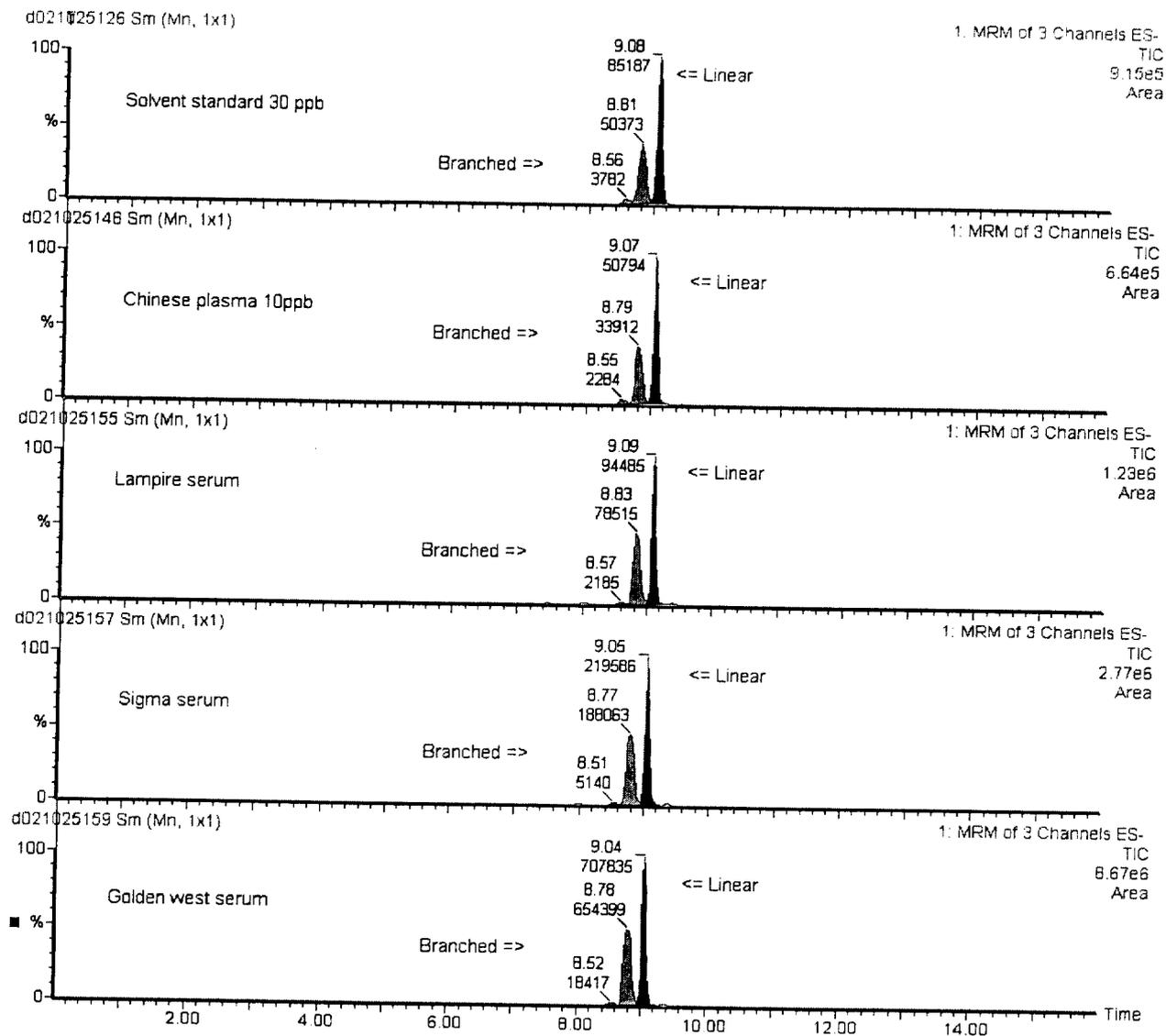
Figure 7: C₁₂ Isomer Distribution



Summary of C₁₂ Chromatograms: Low to non-detect levels of branched isomers of C₁₂ were observed in these lots of commercial pooled human serum.

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Figure 8: PFOS Isomer Distribution



Summary of PFOS Chromatograms: Branched isomers of PFOS were observed in these lots of commercial pooled human serum.

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STATISTICAL METHODS AND CALCULATIONS

Theoretical concentrations of analyte in final eluate:

Concentration = (Concentration of Analytical Standard x Amount of standard added) / EV

Observed result to original sample result:

Original sample result = Observed result x DF

Spike percent recoveries:

$$\% \text{ Recovery} = \frac{\text{Observed Result} - \text{Same Sample, Unspiked}}{\text{Theoretical Concentration}} \times 100$$

EV = Eluate volume

DF = Dilution factor

Relative standard deviation:

$$\text{Relative Standard Deviation} = \frac{\text{Standard Deviation}}{\text{Mean}} \times 100$$

Percent deviation:

$$\% \text{ Deviation} = \frac{\text{Theoretical Conc.} - \text{Calculated Conc.}}{\text{Theoretical Conc.}} \times 100$$

Means and standard deviations were calculated using Excel software.

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STATEMENT OF CONCLUSION

Results from this study showed quantifiable levels of the linear isomers of C7, C9-C12 ranging from < 0.010 – 0.9 ng/mL (Tables 1a and 1b). Low to non-detect levels of branched isomers were observed for the C7 and C9-C12 compounds detected (Figures 1 –10). PFOS was present in the screened lots of commercial sera and plasma in concentrations ranging from 2.5 – 27 ng/mL and was present as branched and linear isomers. PFOA was present in the screened lots of commercial sera and plasma in concentrations ranging from 0.65 – 5.6 ng/mL and was present as branched and linear isomers.

Accurate mass measurements and elemental compositions were obtained for endogenous C7-C11, and PFOS that are consistent with theoretical mass and elemental composition.

The presence of confirmatory daughter ions at characteristic retention times of target analytes in matrix are consistent with the linear isomer analytical standards.

000124

REFERENCES

None

LIST OF ATTACHMENTS

- Attachment A: Extraction and Analytical Methods
- Attachment B: Data Summary Tables
- Attachment C: Sample Chromatograms
- Attachment D: Sample Prep Sheets, Test Substance Information and Notes to File
- Attachment E: Protocol, Protocol Amendments and Deviations

REPORT REVISIONS

Semi-quantitative estimates for THPFOS and THPFDS were removed from tables 1a, 1b, 5a, and 5b because the data was intended as quantitative and only screening estimates could be obtained. References to THPFOS and THPFDS were removed from the list of test substances table 2 (page 11), chemical structures (page 13), reference substances table 3 (page 14), mass spectrometer acquisition parameters (page 18), and control of bias (page 19). References to THPFOS and THPFDS were also removed from the accurate mass determination discussion (page 20) and accurate mass determination data table 6 (page 21), daughter ion discussion (page 21) and daughter ion data table 7 (page 21), isomer discussion (page 21), and isomer figures 8 and 9 (pages 30 and 31). Wording in the Executive Summary (page 8), Summary (page 9), Introduction (page 11), and Statement of Conclusion (page 34) was modified to reflect the removal of the semi-quantitative estimates for THPFOS and THPFDS. Several typographical errors were also corrected, including William K. Reagen's phone number (page 7), formulas for the anionic forms of THPFOS and THPFDS in table 2 (page 11), and the units designator for mass deviation, table 6 (page 21).

A protocol amendment was written to address the removal of THPFOS and THPFDS from the study.

000125

SIGNATURE PAGE

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

 12/09/02
Mark E. Ellefson Date
Study Director

 12/09/02
William K. Reagen Date
Testing Facility Management

000126

ATTACHMENT A: METHOD

000127

Record of Deviation

I. Identification	
Study / Project No. E02-1039	
Deviation Type: <i>(Check one)</i>	<input type="checkbox"/> SOP <input type="checkbox"/> Protocol <input checked="" type="checkbox"/> Method <input type="checkbox"/> Equipment Procedure <input type="checkbox"/> Other:
Document Number: ETS-8-231.1	Date(s) of occurrence: 10 Oct 02 to 25 Oct 02
II. Description:	
Required Procedure/process: ETS-8-231.1 section 9.6, "Quality Control (QC) Sample" see method for details.	
Actual Procedure/process: The continued accuracy of the calibration curve was monitored through the re-injection of a curve point after no more than 10 samples and at the end of the run, not by preparing additional samples. The QC samples described in the method were prepared to monitor extraction efficiency. The QC samples were only spiked at 2 levels, 0.5 and 5ppb. In addition, only 2 QC samples were prepared for each matrix. It was possible, therefore, to distinguish between the continued accuracy of the calibration curve and the extraction efficiency in each matrix tested.	
During the course of the study 2/3 of the QC samples prepared in the same matrix as the calibration curve were within $\pm 25\%$ of expected values. All of the 5ppb spikes passed, but only 3 of 9 low spikes passed.	
III. Actions Taken:	
<i>(such as amendment issued, SOP revision, etc.)</i>	
No additional action will be needed.	
Recorded By:	Date:
<i>Cindy Carlson</i>	11/1/02
IV. Impact on Study / Project	
This deviation does not adversely affect the quality of the data in the context of the current study.	
Authorized By: <i>(Study Director / Project Lead)</i>	Date:
<i>[Signature]</i>	11/1/02

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 Initial CM Date 11/1/02

3M Environmental Laboratory

Method

**Solid Phase Extraction and Analysis of Fluorochemical
Compounds from Biological Matrices**

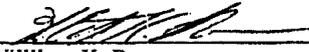
Method Number: ETS-8-231.1

Adoption Date: 11/13/01

Revision Date: 2/18/02

Effective Date: 2/18/02

Approved By:



William K. Reagen
Laboratory Manager

02/18/02

Date

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ETS-8-231.1
Solid Phase Extraction and Analysis of Fluorochemical
Compounds from Biological Matrices

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1 Scope and Application

This method describes the extraction of target analytes from fish, rat liver, rat sera, mouse liver, and mouse sera using solid phase extraction (SPE). This method may also be extended to other biological matrices provided that the data quality objectives are met.

2 Method Summary

An amount of biological material, determined by the analyst, is prepared (fluids diluted and tissues homogenized) at a 1/6 dilution, or other dilution as determined by the analyst using reagent grade water. An aliquot of the dilution/homogenate is spiked with the appropriate surrogate or analyte mixture. Acetonitrile (ACN) is added as an extraction solvent and also serves to precipitate the proteins. The sample is capped, mixed, and put on the centrifuge to clarify the supernatant. The supernatant is transferred to a clean tube, diluted with water, and passed through a pre-conditioned C₁₈ SPE cartridge. Finally, the analytes of interest are eluted from the SPE cartridge and analyzed by high performance liquid chromatography-electrospray tandem mass spectrometry (HPLC-ES/MS/MS).

3 Definitions

3.1 Dilution

A dilution expressed as 1:5 or 1/6 is defined as: 1 mL of sample + 5 mLs of diluent for a total of 6 mLs combined, unless otherwise noted.

3.2 SPE cartridge

A column containing an open solvent reservoir at one end and packed with bonded silica sorbents at the other end. It is designed to retain the compounds of interest under some solvent conditions and elute them under others. A separation is thus achieved; compounds can be removed from difficult biological matrices and introduced into appropriate solvents for analysis.

3.3 Reagent grade water

Water with no detectable concentration(s) of the target analyte(s).

3.4 Quality control sample

Sample used to monitor the extraction efficiency (as a matrix spike) and to verify the continued accuracy of the initial calibration curve (as a continuing calibration verification).

4 Warnings and Cautions

4.1 Health and Safety Warnings

Always wear appropriate gloves, eyewear, and clothing when working with solvents, samples and/or equipment.

Use caution with the voltage cables for the probe. When engaged, the probe employs a voltage of approximately 5000 volts.

4.2 Cautions

Take care not to allow the SPE column to run to dryness after the methanol and water washes. After washing is complete, add sample then allow all of the liquid to pass through the SPE column to dryness.

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Do not operate solvent pumps above capacity of 400 bar (5800 psi) back pressure. If the back pressure exceeds 400 bar, the HPLC will initiate automatic shutdown.

Do not run solvent pumps to dryness.

5 Interferences

To minimize interferences, Teflon should not be used for sample storage or any part of instrumentation that comes in contact with the sample or extract.

6 Instrumentation, Supplies, and Materials

The following instrumentation, supplies, and materials are used while performing this method. Equivalent instrumentation, supplies, and materials may be used in place of those listed.

6.1 Instrumentation

Vortex mixer, VWR, Vortex Genie 2

Ultra-Turrax T25 tissue homogenizer

Vacuum Pump

SPE Extraction Manifold

Centrifuge, Mistral 1000 or IEC

Shaker, Eberbach or VWR

Balance (+/- 0.1000 g)

Micromass, Quattro II or Ultima triple quadrupole Mass Spectrometer equipped with an electrospray ionization source

HP1100 or Agilent low pulse solvent pumping system, solvent degasser, column compartment, and autosampler

6.2 Supplies and Materials

Eppendorf or disposable pipettes, plastic or glass

Dissecting scalpels

Polypropylene bottles, capable of holding 50 mL to 1 L (Nalgene)

Volumetric flasks, glass, type A

40 mL glass vials (ICHEM)

Plastic sample vials, Wheaton, 6 mL (or other appropriate size)

Centrifuge tubes, polypropylene, 15 mL and 50 mL

Labels

Graduated pipettes, glass

Syringes, capable of measuring 5 µL to 1000 µL

Bottle-Top Dispenser (capable of dispensing 5mL of solvent)

SPE extraction cartridge, 1 g, Sep-Pak 6 cc tri-functional C₁₈ (Waters)

75 mL sample reservoir (or other appropriate size)

Crimp cap glass autovials and caps

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Crimpers

HPLC analytical column, specifics to be determined by the analyst and documented in the raw data.

7 Reagents and Standards

Reagent grade water, Milli-Q™, Nanopure II, or equivalent

Acetonitrile, HPLC grade or equivalent

Methanol, HPLC grade or equivalent

Ammonium acetate, reagent grade or equivalent

Biological fluids or tissues, frozen from supplier

7.1 Reagents preparation

2.0 mM ammonium acetate solution: Weigh approximately 0.300 g ammonium acetate. Pour into a 2000 mL volumetric container containing reagent grade water, mix until all solids are dissolved, bring to volume using reagent grade water. Store at room temperature.

Note: When preparing different volumes than those listed in reagents preparation, target analyte standard preparation, and surrogate standard preparation, adjust accordingly.

7.2 Target analyte standard preparation

Prepare target analyte standard(s) for the standard curve. Multicomponent analyte standards are acceptable. The following is an example only and may or may not be appropriate for all standard preparations.

Weigh approximately 100 mg of target analyte into a 100 mL volumetric flask and record the actual weight in the standard logbook or other appropriate location.

Bring to volume with methanol for a stock standard of approximately 1000 ppm ($\mu\text{g/mL}$).

Dilute the stock solution with methanol for a working standard 1 solution of approximately 50 ppm. Example calculation: $1000 \mu\text{g/mL} \times 5 \text{ mL}/100 \text{ mL} = 50 \mu\text{g/mL}$.

Dilute working standard 1 with methanol to produce a working standard 2 solution of approx. 5.0 ppm. Example calculation: $50 \mu\text{g/mL} \times 10 \text{ mL}/100 \text{ mL} = 5.0 \mu\text{g/mL}$.

Dilute working standard 1 with methanol to produce a working standard 3 solution of approx. 0.50 ppm. Example calculation: $50 \mu\text{g/mL} \times 1.0 \text{ mL}/100 \text{ mL} = 0.5 \mu\text{g/mL}$.

7.3 Surrogate standard preparation

Prepare surrogate standard(s). The following is an example only and may or may not be appropriate for all surrogate standard preparations.

Weigh approximately 90-110 mg of surrogate standard into a 100-mL volumetric flask and record the actual weight.

Bring to volume with methanol for a surrogate standard stock of approximately 900 - 1100 ppm.

Prepare a surrogate standard working standard. Transfer approximately 1 mL of surrogate standard stock to a 10-mL volumetric flask and bring to volume with methanol for a working standard of 90-110 ppm. Record the actual volume transferred and standard concentrations in the standards logbook or other appropriate location.

7.4 Internal standard preparation

Prepare internal standard(s). The following is an example only and may or may not be appropriate for all internal standard preparations.

Weigh approximately 90-110 mg of internal standard into a 100-mL volumetric flask and record the actual weight.

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Bring to volume with methanol for an internal standard stock of approximately 900 - 1100 ppm.

Prepare an internal standard working standard. Transfer approximately 1 mL of internal standard stock to a 10-mL volumetric flask and bring to volume with methanol for a working standard of 90-110ppm. Record the actual volume transferred and standard concentrations in the standards logbook or other appropriate location.

8 Sample Handling

All samples are received frozen and must be kept frozen until the extraction is performed.

Allow samples to thaw to room temperature prior to extraction.

Typically fresh matrix standards are prepared with each analysis. Extracted standards and samples are stored in capped autovials until analysis.

If analysis will be delayed, extracted standards and samples may be refrigerated at approximately 4°C indefinitely or may be stored at room temperature until analysis can be performed.

9 Quality Control

9.1 Blanks

9.1.1 Solvent Blank

An aliquot of methanol is used as a solvent blank. Solvent blanks are not extracted.

9.1.2 Method Blank

An aliquot of 1.0 mL of water, or other appropriate amount, is used as a method blank. Four method blanks are extracted and analyzed with each set following this procedure (two are spiked with surrogate and two are not spiked).

9.1.3 Matrix Blank

An aliquot of 1.0 mL or 1.0 g of matrix (diluted or homogenized) is used as a matrix blank. Other amounts may be used, as appropriate. Matrix blanks are prepared from one of three sources: 1) a study control matrix from a study control animal received with a sample set; 2) a commercially obtained sample of the same species as the study animals; or 3) a surrogate matrix, also obtained commercially, but of a different species than the study animal. (eg. if rat is used to generate standard curves and CCVs for a mouse study). The matrix to use is dependent on the matrix used for the curve.

9.1.3.1 Study control matrix curve - if the study control matrix is used for the curve, prepare four (4) matrix blanks using the study control matrix (two spiked with surrogate and two not spiked).

9.1.3.2 Commercially obtained (same species) matrix curve - if the commercially obtained matrix is used for the curve, prepare four (4) matrix blanks using the same commercially available matrix (two spiked with surrogate and two not spiked).

9.1.3.3 Surrogate matrix curve - if a surrogate matrix is used for the curve, prepare four (4) matrix blanks using the same commercially available matrix and prepare four (4) matrix blanks using a commercially available matrix of the same species as the study animals (two spiked with surrogate and two not spiked).

9.1.3.4 If limited matrix is available, the number of method and matrix blanks may be adjusted and will be noted in the study protocol or in the raw data.

9.2 Sample Replicate

Samples replicates are prepared according to each study protocol or project outline.

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9.3 Surrogate standard

If surrogate standard is a component of the study, all samples are spiked with surrogate standard prior to extraction to obtain a concentration in the mid-range of the calibration curve, with the exception of blank samples as described above.

Typically surrogate standard is spiked into the 1.0 mL diluted/homogenized sample removed for extraction. However, surrogate may be spiked directly into the matrix prior to diluting with water, into the diluted/homogenized sample prior to removing the 1.0 mL sample, or into the 1.0 mL diluted/homogenized sample removed for extraction.

9.4 Internal standard

If internal standard is a component of the study, all samples are spiked with internal standard after extraction to obtain a concentration in the mid-range of the calibration curve.

Typically internal standard is spiked into the 2.0 mL of extract in the 15 mL centrifuge tube, before transferring to the autovial.

9.5 Lab Control Sample

Lab control samples are not a component of this method.

9.6 Quality Control (QC) Sample

Prepare quality control (QC) samples to monitor extraction efficiency and to verify the continued accuracy of the initial calibration curve. Typically 1.0 mL, or other appropriate amount, of the same matrix used to prepare the initial calibration curve is used for each QC sample.

Twelve (12) quality control samples (QC) will be prepared for each matrix during the course of a study. A minimum of 3 QC samples must be prepared (one at each level) on each day of sample extraction. (e.g. If the study is such that samples will be extracted on three different days then four QC samples must be prepared on each day of extraction for a total of twelve.)

QC samples will consist of four samples at each of three levels of analyte. The levels listed below may be used and may represent sample concentrations diluted into the range of the calibration curve:

Low level: 3X to 5X the LLOQ,

Mid-level: equivalent to a point near the middle of the calibration curve,

High level: 80% of the ULOQ

Two QC sample levels are analyzed after every tenth sample injection starting after the last calibration standard injection, with a minimum of three QC per analysis. Solvent blanks are not considered samples but may be included as such for determining when QC samples will be analyzed.

QC samples extracted with a particular sample set must be analyzed in the same analytical run. Any QC samples extracted during the course of the study may be included in subsequent analyses.

If samples from multiple extraction dates are analyzed in one analytical run, then QC samples from the same sample extraction dates must be included in that analysis.

Each QC is expected to show an accuracy of 75-125% of expected. A minimum of 2/3 of all QC samples must meet this criteria, and a minimum of 1/2 of the QC samples at each level must meet this criteria. If not, the set must either be re-analyzed or re-extracted.

9.7 Sample Dilution

Any sample with an area greater than that of the highest acceptable standard will need to be diluted into the range of the calibration curve. If samples are diluted into the range of the curve during analyses and enough sample remains, a post-run dilution validation will be performed to verify sample values.

To perform the dilution validation, one sample will be separated into two representative samples (i.e. two 1.0 mL aliquots for fluid samples or two 1.0 gram amounts for tissue samples, or other amount as determined by the analyst

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and documented in a note to file) then diluted using two procedures. The first procedure consists of diluting the sample with additional matrix prior to extraction (fluid adding fluid), while the second procedure consists of diluting the extract with solvent post-extraction (methanol extract adding additional methanol solvent.)

If the relative percent difference is not within 15% for these two samples; additional testing will be required to determine which value is a correct representation of the sample concentration.

10 Calibration and Standardization

10.1 Instrument Calibration

One calibration curve will be prepared from extracted matrix standards, in the same matrix as the samples, per study. It will consist of a minimum of nine (9) levels. Additional calibration curves may be extracted on separate sample extraction dates, as determined by the analyst and documented in a note to file.

Transfer 1.0 mL, or other appropriate amount, of diluted control fluid or homogenized control tissue to a 15 mL centrifuge tube using a disposable plastic pipette. This will be repeated while preparing aliquots for the standard curve. Be sure to mix or shake the control matrix container between aliquots to ensure a homogenous sample is removed.

Record each standard volume on the weight/volumes sheet or extraction worksheet, as appropriate.

Four 1.0 mL aliquots, or other appropriate amount, of control matrix serve as matrix blanks.

The standard concentrations and spiking amounts listed in Table 1 may be used, when appropriate, to spike one standard curve. A total of 9 standards, four matrix blanks, and four method blanks are prepared in addition to the QC samples and test samples. The number of standards and blanks may be adjusted as determined by the analyst and documented in a note to file.

Use Attachment C, or other appropriate form, as an aid in calculating the concentrations of the working standards. Refer to section 12 to calculate the actual concentration of analyte in each calibration standard and QC sample.

Typically the target analyte standard is spiked into the 1.0 mL diluted/homogenized sample removed for extraction. However, it may be spiked directly into the matrix prior to diluting with water, into the diluted/homogenized sample prior to removing the 1.0 mL sample, or into the 1.0 mL diluted/homogenized sample removed for extraction.

Analyze the extracted matrix standard curve prior to each set of extracts. The curve equation will be determined by regression analysis using the peak areas of the target analyte(s) using MassLynx or other suitable software.

Any level outside 75% - 125% of nominal must be deactivated, and regression re-calculated, except the LLOQ which must be within 30% of nominal. All levels must show a response greater than twice that of the blank. A maximum of three (3) levels may be deactivated in any one set, or the set will be re-analyzed.

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TABLE I
APPROXIMATE SPIKING AMOUNTS FOR STANDARDS AND SPIKES
USING 1.0 mL OF MATRIX

Working standard (approximate concentration)	μL	Approximate final concentration of analyte in Matrix diluted 1:5	Approximate final concentration of analyte in Final 2.0 mL volume
-	-	Blank	Blank
0.500 ug/mL	1.5	5.00 ng/g or ng/mL	0.375 ng/mL
0.500 ug/mL	3.0	10.0 ng/g or ng/mL	0.750 ng/mL
0.500 ug/mL	8.0	25.0 ng/g or ng/mL	2.00 ng/mL
0.500 ug/mL	16	50.0 ng/g or ng/mL	4.00 ng/mL
0.500 ug/mL	32	100 ng/g or ng/mL	8.00 ng/mL
5.00 ug/mL	5.6	175 ng/g or ng/mL	14.0 ng/mL
5.00 ug/mL	8.0	250 ng/g or ng/mL	20.0 ng/mL
5.00 ug/mL	16	500 ng/g or ng/mL	40.0 ng/mL
5.00 ug/mL	24	750 ng/g or ng/mL	60.0 ng/mL
5.00 ug/mL	32	1000 ng/g or ng/mL	80.0 ng/mL
5.00 ug/mL	40	1250 ng/g or ng/mL	100 ng/mL
50.0 ug/mL	5.0	1500 ng/g or ng/mL	125 ng/mL
50.0 ug/mL	6.0	1750 ng/g or ng/mL	150 ng/mL
Surrogate Std 100 ug/mL	10	6500 ng/g or ng/mL	500 ng/mL

11 Procedures

11.1 Tissue Sample Preparation

Obtain frozen tissue samples

Cut approximately 1.0000 g of tissue (+/- 0.1000 g), or other appropriate amount, using a dissecting scalpel. This part of the procedure is best performed quickly, not allowing the tissue to thaw.

Weigh the tissue directly into a tared plastic sample vial.

Record the weight on the weight/volume sheet, extraction worksheet, or other appropriate location.

Return unused tissue to the freezer after extraction amounts have been removed.

Add 2.5 mL of reagent water to sample vial, or other volume as determined by the analyst and documented in a note to file.

Homogenize the sample. Put the Ultra-Turrax grinder probe in the sample and grind for approximately 2 minutes, or until the sample is homogeneous.

Rinse the probe into the tube containing the sample with 2.5 mL of reagent grade water, or other volume as determined by the analyst and documented in a note to file, using a pipette.

Take the grinder apart and clean it with methanol after each sample. Refer to ETS-9-52 for more information.

If an amount other than 1.0000 g (not within +/- 0.1000 g) is removed for an initial weight, adjust the water volume accordingly to maintain a 1/6 dilution. (e.g. if 0.5 g is removed for extraction, add a total of 2.5 mL of water.), or other ratio as determined by the analyst and documented in a note to file.

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11.2 Fluid Sample Preparation

Obtain frozen fluid sample and allow it to thaw at room temperature or in lukewarm water.

Label a 15 mL polypropylene centrifuge tube with the study number, sample ID, extraction date and analyst initials. See attached worksheet (Attachment A or similar worksheet) for documenting the remaining steps.

Vortex mix the fluid sample for approximately 15 seconds, then transfer 1.0 mL of fluid, or other appropriate amount to a plastic sample vial, or other appropriate container.

Return unused samples to freezer after extraction amounts have been removed.

Add 5.0 mL of reagent water to the 1.0 mL of fluid for a 1/6 dilution, or other dilution as determined by the analyst and documented in a note to file.

If a volume other than 1.0 mL is removed for an initial volume, adjust the water volume accordingly to maintain the same dilution as above.

11.3 Tissue and Fluid Sample Extraction

After tissue or fluid samples have been prepared according to sections 11.1 and 11.2, vortex mix or shake by hand the diluted/homogenized sample for approximately 15 seconds then transfer 1.0 mL, or other appropriate volume, to a clean 15 mL polypropylene centrifuge tube.

Return unused diluted/homogenized portions to the freezer after extraction amounts have been removed.

Record the volume removed on the extraction worksheet, (Attachment A or similar worksheet).

Spike blanks, samples, and standards, ready for extraction with surrogate standard as described in this method.

Spike each calibration standard matrix with the appropriate amount of standard as described in this method for the calibration curve standards and each QC sample.

Vortex mix the standard curve samples and QC samples for approximately 5 seconds.

To each sample and standard, add 5.0 mL of acetonitrile, cap, and vortex mix or shake by hand approximately 15 seconds.

Place all samples on the shaker at an appropriate speed for 20 minutes to adequately mix (a setting of approximately 300 rpm on the models listed in section 6.1).

Remove from the shaker and centrifuge at an appropriate speed for 10 minutes to adequately pellet the precipitate (a setting of approximately 2000 rpm on the models listed in section 6.1).

Add 40.0 mL of reagent grade water to a clean 50 mL centrifuge tube. Remove samples from the centrifuge and decant the supernatant into the water in the 50 mL tube, taking care not to introduce any of the matrix solids into the solution. Cap and mix by inverting several times. In this step the order of addition may be changed (i.e. the sample may be put into the centrifuge tube and then the water added).

Attach the reservoir to the SPE cartridge and attach this reservoir/cartridge unit to a vacuum manifold.

NOTE: When running the vacuum, set the vacuum chamber at approximately 15 kPA - to give an approximate elution flow of 5-7 mL/min. Flows may vary through cartridges and the kPA may be raised for slow tubes and drying after most have been drawn down.

Prepare the SPE cartridge by washing twice with approximately 5.0 mL of methanol, followed by approximately two 5.0 mL aliquots of water, **taking care not to allow the column to run to dryness after each wash.**

After washing is complete, pour the sample into the reservoir/cartridge unit and **allow all of the liquid to pass through the column to dryness.**

Run the vacuum on high for approximately 5 minutes to adequately dry each SPE cartridge.

Place a collection 15 mL polypropylene centrifuge tube under each cartridge and elute with 2.0 mL of methanol.

Spike extracted blanks, samples, and standards with internal standard as described in this method.

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Label each glass autovial, as appropriate, with the study number, vial file archive number, animal number/gender/timepoint or LIMS number, matrix, final solvent, analyte components (if needed), extraction type, extraction date, and analyst(s) performing the extraction.

Transfer each eluant to a glass autovial and cap.

11.4 Extract Analysis

11.4.1 Software set-up

On the MassLynx main page, set up a sample list name. Save the list as instrument designator letter, last 2 digits of test year-month-day, and a letter that will increase through the alphabet with each additional list for that day.

Example Sample List: IYYMMDDa or A020204a

I = Initial of the instrument name (A = "Amelia")

YY = Test year (02)

MM = Test month (02)

DD = Test day (04)

a = First sample list (run) of the day (the next sample list will end with 'b', the next 'c', and so on.)

Assign a filename using the instrument designator letter, the last 2 digits of the test year-month-day, and a 3-digit sequential file number that starts with 1 and increases by one for each filename.

Example filename: IYYMMDD### or A020204001

I = Initial of instrument name

YY = Test year

MM = Test month

DD = Test day

= 3-digit sequential file number starting with 1 through 999 (001)

Also, as part of the samplelist, assign a method (MS) for acquiring, an inlet file, a bottle number, an injection volume, and sample descriptions.

To create a method, click on Method Editor button in the MS Status Pane and select SIR (Single Ion Recording) or MRM (Multiple Reaction Monitoring). Set Ionization Mode as appropriate and mass to 499 or other appropriate mass(es). Also set the acquisition start and stop times. Save acquisition method. If MS/MS instruments are employed, additional product ion fragmentation information may be collected. See Micromass MassLynx "Guide to Data Acquisition" for additional information on MRM.

Typically the analytical batch run sequence begins with system suitability, solvent blanks, and a set of extracted matrix standards.

Sample extracts are analyzed with two QC samples injected after every tenth sample injection. Solvent blanks should be analyzed periodically to monitor possible analyte carryover and are not considered sample extracts but may be included as such.

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11.4.2 HPLC set-up

Set up sample tray according to the sample list prepared above.

Set up the HPLC to the following conditions or at conditions the analyst considers appropriate for optimal response. Record actual conditions in the instrument logbook, or other appropriate location:

Sample size = 10 μ L injection

Inject/sample = 1

Cycle time = 10.0 minutes

Flow rate = 300 μ L/min

Mobile phase: Solvent A = 2 mM Ammonium Acetate, Solvent B = Methanol

Solvent gradient program:

Time	% Solvent B
0.00	10%
1.00	10%
5.50	95%
7.50	95%
8.00	10%

11.4.3 Instrument set-up

Refer to ETS-9-24, "Operation and Maintenance of the Micromass Quattro II Triple Quadrupole Mass Spectrometer Fitted with an Atmospheric Pressure Ionization Source," for details.

Check the solvent level in HPLC reservoirs and refill if necessary.

Check the stainless steel capillary at the end of the probe. Use an eyepiece to check the tip. The tip should be flat with no jagged edges. If the tip is found to be unsatisfactory, disassemble the probe and replace the stainless steel capillary.

Turn on the nitrogen.

Open the tune page. Click on operate to initiate source block and desolvation heaters.

Open the Inlet Editor.

Download the HPLC method and initiate solvent flow to begin system equilibrium.

Set the flow to 10–500 μ L/min or as appropriate

Set HPLC pump to "On"

Observe droplets or mist coming out of the tip of the probe. A fine mist should be expelled with no nitrogen leaking around the tip of the probe. Readjust the tip of the probe if no mist is observed

Allow to equilibrate for approximately 10 minutes.

Typical instrument parameters include:

Drying gas 250–400 liters/hour

ES nebulizing gas 10–15 liters/hour

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HPLC constant flow mode, flow rate 10–500 $\mu\text{L}/\text{min}$
Pressure <400 bar (this parameter is not set, it is a guide to ensure the
HPLC is operating correctly.)
Source block temperature approximately 150°C
Desolvation temperature approximately 250°C
These settings may change in order to optimize the response

Print the tune page, sample list, and acquisition method from MassLynx and store it in the study binder with a copy taped into the instrument log.

Click on start button in the Acquisition Control Panel (the location of the start button may vary among MassLynx versions, refer to appropriate MassLynx User's Guide).

12 Data Analysis and Calculations

12.1 Calculations

If other calculations are used than those listed, they will be documented in the raw data.

Calculate the matrix amount contained in the initial dilution using the following equation:

$$\text{Matrix Amount (g/mL or mL/mL)} = \frac{\text{IW (g) (or IV (mL))}}{(\text{IW(g) (or IV(mL))} + \text{DV (mL)})}$$

Calculate actual concentrations of analyte in calibration standards using the following equation:

$$\text{Concentration (ng/g or ng/mL)} = \frac{\text{Spike Concentration (ug/mL)} \times \text{Spiked Amount (mL)} \times \frac{1000 \text{ ng}}{1 \text{ ug}}}{\text{SV (mL)} \times \text{Matrix Amount (g/mL or mL/mL)}}$$

IW = Initial weight (where 1.0 g = 1.0 mL)

IV = Initial volume

DV = Diluent volume (reagent grade water)

SV = Sample volume removed for extraction (typically 1.0 mL)

AR = Analytical result from MassLynx summary

DF = Dilution factor

FV = Final volume

MA = Matrix amount

∂ curve = MA of tissue/fluid standard curve, assumed to be 1 g or 1 mL/5 mL water

∂ sample = MA of tissue/fluid sample (___g or mL of sample/5 mL water)

Calculate spike percent recoveries using the following equation:

$$\% \text{ Recovery} = \frac{\text{Observed Result} - \text{Matrix Blank Result}}{\text{Spiking Level}} \times 100$$

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Calculate relative standard deviation using the following equation:

$$\text{Relative Standard Deviation} = \frac{\text{Standard Deviation}}{\text{Mean}} \times 100$$

Calculate percent deviation using the following equation:

$$\% \text{ Deviation} = \frac{\text{Expected Conc.} - \text{Calculated Conc.}}{\text{Expected Conc.}} \times 100$$

Calculate actual concentration of analyte in fluid ($\mu\text{g/mL}$):

$$\text{AR (ng/mL)} \times \text{DF} \times \frac{\text{Slope (mL/mL)}}{\text{Slope (mL/mL)}} \times \frac{\text{FV (mL) in Curve}}{\text{FV (mL) in Matrix}} \times \frac{1.0 \mu\text{g}}{1000 \text{ ng}} = (\mu\text{g/g})$$

Calculate actual concentration of analyte in tissue ($\mu\text{g/g}$):

$$\text{AR (ng/g)} \times \text{DF} \times \frac{\text{Slope (g/mL)}}{\text{Slope (g/mL)}} \times \frac{\text{FV (mL) in Curve}}{\text{FV (mL) in Matrix}} \times \frac{1.0 \mu\text{g}}{1000 \text{ ng}} = (\mu\text{g/g})$$

13 Method Performance

13.1 System Suitability

System suitability will be determined prior to the start and at the completion of each analytical run. Prior to the calibration curve and after the last sample of the run three (3) mid-level unextracted calibration standards will be analyzed. As applicable, the peak area precision, retention time precision, resolution, and peak asymmetry will be monitored at the beginning and the end of the run separately. The peak area precision must be equal to or less than 5.0% RSD, the precision of the retention time must be equal to or less than 2.5% RSD, the resolution must be > 2.0 , and the peak asymmetry (fronting or tailing) must be $0.5 < \text{AF} < 2.0$, where AF is the asymmetry factor.

If any item of the system suitability fails, system maintenance must be completed prior to running a second set of system suitability samples and the system suitability must pass before starting the calibration. If system suitability fails at the completion of a run, the sample set must be reanalyzed.

13.2 Quantitation

The coefficient of determination value for the calibration curve, plotted by regression using the peak areas of the analyte(s), must be 0.990 or better.

All active calibration curve points must be within 25% of the theoretical value with the exception of the LOQ point, which may deviate up to 30%.

Calibration standards with peak areas less than two times the curve matrix blank will be deactivated to disqualify a data range that may be affected by background levels of the analyte.

A valid calibration curve must contain at least 6 active points above and including the LOQ.

If the curve cannot meet these criteria, the sample set must be reanalyzed or reextracted.

13.3 Accuracy

Two thirds of all quality control samples and 1/2 of each quality control sample at each level are expected to show an accuracy of 75-125%.

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Surrogates and internal standards must have a percent deviation < 50%. Deviations outside this range will be reanalyzed to confirm. If the second analysis confirms the original, the deviation will be documented in the raw data. If the second analysis is within 50%, then the second value will replace the original value.

14 Pollution Prevention and Waste Management

- Sample waste is disposed of in noninfectious biohazard waste containers.
- Flammable solvent waste is disposed of in high BTU containers.
- Glass pipette waste is disposed of in broken glass containers located in the laboratory.

15 Records

- Complete the extraction worksheet attached to this method, or other applicable worksheet, and store with the study raw data.
- Each page generated for a study must contain the following information (if applicable): study/project or instrument number, acquisition method, integration method, sample name, extraction date, dilution factor (if applicable), and analyst. Other information may be added if applicable to the study.
- Print the tune page, sample list, and acquisition method from MassLynx to include with the study raw data. Copy these pages and tape into the instrument runlog.
- Plot the calibration curve by the appropriate regression. Print these graphs and store with the study raw data.
- Print data integration summary, integration method, and chromatograms from MassLynx, and store with the study raw data.
- Summarize data using suitable software (Excel 7.0 or LIMS) and store in the study folder.
- Back up electronic data to appropriate medium. Record in study notebook the file name and location of backup electronic data.

16 Attachments

- Attachment A: Extraction Worksheet
- Attachment B: Sample Weight/Volume Worksheet
- Attachment C, Calibration Standard Concentration Worksheet
- Attachment D, Dilutions Summary Worksheet

17 References

- ETS-9-24, "Operation and Maintenance of the Micromass Quattro II Triple Quadrupole Mass Spectrometer Fitted with an Atmospheric Pressure Ionization Source"
- ETS-9-52, "Operation and Maintenance of a Tissue Grinder"

18 Affected Documents

None

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19 Revisions

<u>Revision Number</u>	<u>Revision Description</u>	<u>Revision Date</u>
1	Minor formatting changes. Added detailed information to all sections concerning the extraction procedure, analytical procedure, and calculations. Added attachments and references.	02/18/02

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Attachment C: Calibration Standard Concentration Worksheet

Prep date(s):
Analyte(s):
Sample matrix:
Method/revision:

Standard number:
Equipment number:
Final solvent and TN:
Blank Tissue or Fluid/identifier:

Analyte mix std approx. 0.500 ug/mL:
Analyte mix std approx. 5.00 ug/mL:
Analyte mix std approx. 50.0 ug/mL:
Surrogate std approx. 100 ug/mL:

Actual concentrations of standards in the analyte mix

Analyte Std conc ug/mL	All Am't spiked mL	All Final Volume: mL	All Initial Fluid Dilution mL/mL	All Initial Tissue Density g/mL
0.500	0.0015	2.00	0.1667	0.1600
0.500	0.0030	2.00	0.1667	0.1600
0.500	0.0080	2.00	0.1667	0.1600
0.500	0.0160	2.00	0.1667	0.1600
0.500	0.0320	2.00	0.1667	0.1600
5.00	0.0056	2.00	0.1667	0.1600
5.00	0.0080	2.00	0.1667	0.1600
5.00	0.0160	2.00	0.1667	0.1600
5.00	0.0240	2.00	0.1667	0.1600
5.00	0.0320	2.00	0.1667	0.1600
5.00	0.0400	2.00	0.1667	0.1600
50.0	0.005	2.00	0.1667	0.1600
50.0	0.006	2.00	0.1667	0.1600

Calculated concentrations of standards in relation to the final 2.0 mL solvent and initial matrix

2.0 mL Final Volume		Fluid Matrix		Tissue Matrix	
Analyte Final conc. ng/mL	Surrogate Std conc ng/mL	Analyte Final conc. ng/mL	Surrogate Std conc ng/mL	Analyte Final conc. ng/g	Surrogate Std conc ng/mL
0.375	100	5.00	100	5.00	100
0.750		10.0		10.0	
2.00	Surrogate	25.0	Surrogate	25.0	Surrogate
4.00	Final conc	50.0	Final conc	50.0	Final conc
8.00	ng/mL	100	ng/mL	100	ng/g
14.0	0.500	175	6500	175	6500
20.0		250		250	
40.0		500		500	
60.0		750		750	
80.0		1000		1000	
100		1250		1250	
125		1500		1500	
150		1750		1750	

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ATTACHMENT B: DATA TABLES

000148

**Human Serum Data
Quantitated with Chinese Plasma Calibration Curve**

All data is in units ng/mL

	Lampire Serum			Sigma Serum			Golden West Serum			Bioresource Serum						
	sample 1	sample 2	low spike	High spike	sample 1	sample 2	low spike	High spike	sample 1	sample 2	low spike	High spike	sample 1	sample 2	low spike	High spike
PFOS	< LOQ	< LOQ	< LOQ	5.47	4.74	4.38	4.75	9.06	25.64	28.41	24.16	26.10	16.79	17.26	16.44	16.78
C ₁₂	< LOQ	< LOQ	0.65	5.70	< LOQ	< LOQ	0.45	3.74	< LOQ	< LOQ	0.46	3.65	< LOQ	< LOQ	2.65	< LOQ
C ₁₁	< LOQ	< LOQ	0.82	7.02	< LOQ	< LOQ	0.69	5.45	0.31	0.33	0.85	6.42	0.28	0.31	4.94	0.96
C ₁₀	< LOQ	< LOQ	0.68	6.34	< LOQ	< LOQ	0.60	5.17	< LOQ	< LOQ	0.60	5.20	< LOQ	< LOQ	4.32	0.73
C ₉	< LOQ	< LOQ	0.79	5.91	0.19	0.34	0.84	5.91	0.96	0.84	1.30	6.62	0.63	0.58	4.95	1.11
C ₈	0.73	0.57	1.39	6.81	1.60	1.59	1.86	6.64	5.54	5.65	4.64	9.12	2.83	3.06	7.39	4.03
C ₇	< LOQ	< LOQ	0.75	6.51	< LOQ	0.15	0.65	5.78	0.23	0.15	0.41	4.91	< LOQ	< LOQ	1.47	0.18

Chinese Plasma, Serum Data Cal Curves

Analyte	Calibration Range, ng/mL	System Suitability		CCV #1 recovery, %	CCV #2 recovery, %	CCV #3 recovery, %	Matrix Blanks
		Samples, %RSD	R ²				
PFOS	1.01 - 30.2	0.999596	2.48	52%	3%	**	< LOQ
C ₁₂	0.104 - 31.1	0.999621	0.99	77%	52%	**	< LOQ
C ₁₁	0.103 - 30.9	0.999767	2.46	105%	105%	99%	< LOQ
C ₁₀	0.252 - 30.3	0.999471	1.45	86%	90%	**	< LOQ
C ₉	0.101 - 30.4	0.9997	2.94	91%	105%	102%	< LOQ
C ₈	0.258 - 31.0	0.998816	6.73	94%	87%	**	< LOQ
C ₇	0.102 - 30.7	0.999569	2.37	87%	95%	93%	< LOQ

**None performed

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**Human Plasma Data
Quantitated with Chinese Plasma Calibration Curve**

All data is in units ng/mL

	Chinese Plasma			Lampire Plasma			Golden West Plasma			Innovative Research Plasma		
	sample 1	sample 2	High spike	sample 1	sample 2	High spike	sample 1	sample 2	High spike	sample 1	sample 2	High spike
PFOS	< LOQ	< LOQ	4.78	9.89	12.34	16.19	16.20	20.32	20.34	15.61	15.55	17.32
C ₁₂	< LOQ	0.52	5.02	< LOQ	< LOQ	4.53	< LOQ	< LOQ	4.80	< LOQ	< LOQ	5.66
C ₁₁	< LOQ	0.65	5.18	< LOQ	< LOQ	4.42	< LOQ	0.10	4.95	0.13	0.14	6.16
C ₁₀	< LOQ	0.40	4.88	< LOQ	< LOQ	4.01	0.15	0.17	5.29	0.19	0.15	6.08
C ₉	< LOQ	0.59	4.91	0.37	0.50	4.88	0.42	0.65	5.11	0.68	0.49	6.00
C ₈	< LOQ	< LOQ	4.71	2.30	2.91	7.31	3.46	4.28	7.94	3.04	3.09	9.16
C ₇	< LOQ	0.28	5.28	0.20	< LOQ	5.43	0.45	0.13	5.34	< LOQ	< LOQ	6.14

Chinese Plasma, Plasma Data Cal Curves

Analyte	Calibration Range, ng/mL	R ²	System Suitability		CCV #1 recovery, %	CCV #2 recovery, %	Matrix Blanks
			Samples, %RSD	recovery, %			
PFOS	1.0 - 30.0	0.999721	2.20	102%	84%	< LOQ	
C ₁₂	0.10 - 31.0	0.999759	5.87	101%	84%	< LOQ	
C ₁₁	0.10 - 30.9	0.999585	1.21	90%	76%	< LOQ	
C ₁₀	0.10 - 30.3	0.999695	2.76	91%	83%	< LOQ	
C ₉	0.25 - 30.4	0.999647	5.07	97%	80%	< LOQ	
C ₈	0.52 - 31.0	0.999736	2.85	81%	89%	< LOQ	
C ₇	0.10 - 30.7	0.999695	5.85	102%	75%	< LOQ	

000150

Human Serum Data, 5x Sample Scale-Up with 20uL Injection Volume

Quantitated with Chinese Plasma Calibration Curve

All data is in units of ng/mL

	Lampire Serum		Sigma Serum		Golden West Serum		Bioresource Serum	
	Sample 1, on-Column Concentration	Sample 1, Corrected for Concentration Factor	Sample 1, on-Column Concentration	Sample 1, Corrected for Concentration Factor	Sample 1, on-Column Concentration	Sample 1, Corrected for Concentration Factor	Sample 1, on-Column Concentration	Sample 1, Corrected for Concentration Factor
PFOS	24.859	2.486	> ULOQ (30.2)	> ULOQ (30.2)	> ULOQ (30.2)	> ULOQ (30.2)	> ULOQ (30.2)	> ULOQ (30.2)
C ₁₂	< LOQ	< LOQ	0.177	0.018	0.402	0.040	1.444	0.144
C ₁₁	< LOQ	< LOQ	0.760	0.076	2.473	0.247	2.856	0.286
C ₁₀	0.313	0.031	0.575	0.058	2.025	0.203	3.272	0.327
C ₉	0.675	0.068	1.234	0.123	3.533	0.353	2.657	0.266
C ₈	11.734	1.173	13.396	1.340	26.245	2.625	26.245	2.625
C ₇	0.250	0.025	0.126	0.013	< LOQ	< LOQ	< LOQ	< LOQ

Human Plasma Data, 5x Scale-Up with 20uL Injection Volume

Quantitated with Chinese Plasma Calibration Curve

All data is in units of ng/mL

	Lampire Plasma		Golden West Plasma		Innovative Research Plasma	
	Sample 1, on-Column Concentration	Sample 1, Corrected for Concentration Factor	Sample 1, on-Column Concentration	Sample 1, Corrected for Concentration Factor	Sample 1, on-Column Concentration	Sample 1, Corrected for Concentration Factor
PFOS	> ULOQ (30.2)	> ULOQ (30.2)	> ULOQ (30.2)	> ULOQ (30.2)	> ULOQ (30.2)	> ULOQ (30.2)
C ₁₂	0.364	0.036	0.236	0.024	0.219	0.022
C ₁₁	0.486	0.049	0.711	0.071	1.400	0.140
C ₁₀	1.270	0.127	2.112	0.211	2.169	0.217
C ₉	3.799	0.380	3.436	0.344	4.016	0.402
C ₈	36.207	3.621	> ULOQ (31.0)	> ULOQ (31.0)	37.170	3.717
C ₇	< LOQ	< LOQ	< LOQ	< LOQ	0.160	0.016

Chinese Plasma Calibration Curve for Scale-Up data, 10uL Injection Volume

Analyte	Calibration Range, ng/mL	R ²	System Suitability Samples, %RSD	CCV #1 recovery, %	Matrix Blanks
PFOS	1.01 - 30.2	0.999539	4.21	155%	< LOQ
C ₁₂	0.104 - 31.1	0.999696	3.94	114%	< LOQ
C ₁₁	0.103 - 30.9	0.999526	6.34	95%	< LOQ
C ₁₀	0.101 - 30.3	0.999907	4.02	94%	< LOQ
C ₉	0.254 - 30.4	0.999812	4.16	92%	< LOQ
C ₈	0.103 - 31.0	0.999441	5.99	92%	< LOQ
C ₇	0.102 - 30.7	0.999903	6.21	106%	< LOQ

**Matrix Spike Recovery for Human Serum Data
Quantitated with Chinese Plasma Calibration Curve**

Lampire Serum				Sigma Serum		
Analyte	Average Endogenous Concentration, ng/mL	Low Spike Recovery, %	High Spike Recovery, %	Average Endogenous Concentration, ng/mL	Low Spike Recovery, %	High Spike Recovery, %
PFOS	< LOQ	< LOQ	< LOQ	4.56	39%	90%
C ₁₂	< LOQ	129%	114%	< LOQ	90%	75%
C ₁₁	< LOQ	164%	140%	< LOQ	138%	109%
C ₁₀	< LOQ	136%	127%	< LOQ	120%	103%
C ₉	< LOQ	158%	118%	0.27	115%	113%
C ₈	0.65	148%	123%	1.60	53%	101%
C ₇	< LOQ	150%	130%	0.15	100%	113%

Golden West Serum				Bioresource Serum		
Analyte	Average Endogenous Concentration, ng/mL	Low Spike Recovery, %	High Spike Recovery, %	Average Endogenous Concentration, ng/mL	Low Spike Recovery, %	High Spike Recovery, %
PFOS	27.02	-573%	-19%	17.02	0%	3288%
C ₁₂	< LOQ	92%	73%	< LOQ	530%	< LOQ
C ₁₁	0.32	106%	122%	0.30	93%	6%
C ₁₀	< LOQ	120%	104%	< LOQ	864%	146%
C ₉	0.90	80%	114%	0.61	86%	49%
C ₈	5.60	-191%	71%	2.95	91%	624%
C ₇	0.19	44%	94%	< LOQ	294%	36%

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**Matrix Spike Recovery for Human Serum Data
Quantitated with Chinese Plasma Calibration Curve**

Chinese Plasma				Lampire Plasma		
Analyte	Average Endogenous Concentration, ng/mL	Low Spike Recovery, %	High Spike Recovery, %	Average Endogenous Concentration, ng/mL	Low Spike Recovery, %	High Spike Recovery, %
PFOS	< LOQ	260%	96%	11.12	407%	102%
C ₁₂	< LOQ	104%	100%	< LOQ	96%	91%
C ₁₁	< LOQ	130%	104%	< LOQ	92%	88%
C ₁₀	< LOQ	80%	98%	< LOQ	98%	80%
C ₉	< LOQ	< LOQ	98%	0.44	69%	89%
C ₈	< LOQ	< LOQ	94%	2.61	147%	94%
C ₇	< LOQ	56%	106%	0.20	64%	105%

Golden West Plasma				Innovative Research Plasma		
Analyte	Average Endogenous Concentration, ng/mL	Low Spike Recovery, %	High Spike Recovery, %	Average Endogenous Concentration, ng/mL	Low Spike Recovery, %	High Spike Recovery, %
PFOS	18.26	-940%	42%	15.58	348%	121%
C ₁₂	< LOQ	88%	96%	< LOQ	116%	113%
C ₁₁	0.10	84%	97%	0.14	141%	121%
C ₁₀	0.16	82%	103%	0.17	122%	118%
C ₉	0.54	37%	92%	0.59	51%	108%
C ₈	3.87	-106%	81%	3.07	159%	122%
C ₆	< LOQ	< LOQ	125%	< LOQ	1438%	183%

000153

PFOS Telomer Analysis Summary
3M Pharmaceuticals Analytical Research and Development

LC/MS analysis of a pooled human sera sample obtained from Golden West Biologicals (#E02-1039) was performed using an Agilent 1100 quaternary HPLC system coupled to a Micromass Q-ToF 2 quadrupole time-of-flight mass spectrometer. The sample was run as submitted using the following parameters listed in Table 1:

Table 1

		Run Time (min.)	H ₂ O w/ 2mM NH ₄ CH ₃ CO ₂	MeOH
Inject:	20 µL			
Analog wavelength output:	NA	0	90	10
PDA Wavelength Range:	190-600 nm	7	0	100
Recorded Mass Range:	100-800 Da.	12.5	0	100
Flow Rate:	0.3 ml/min	13.0	90	10
Column Temp:	ambient	17.0	90	10
Column:	Betasil C18, 5 µm, 50x2mm			
Ionization Mode:	ESI (-)			
Resolution:	7,000			

Accurate mass measurements and elemental compositions obtained in this analysis are summarized in Table 2:

Table 2

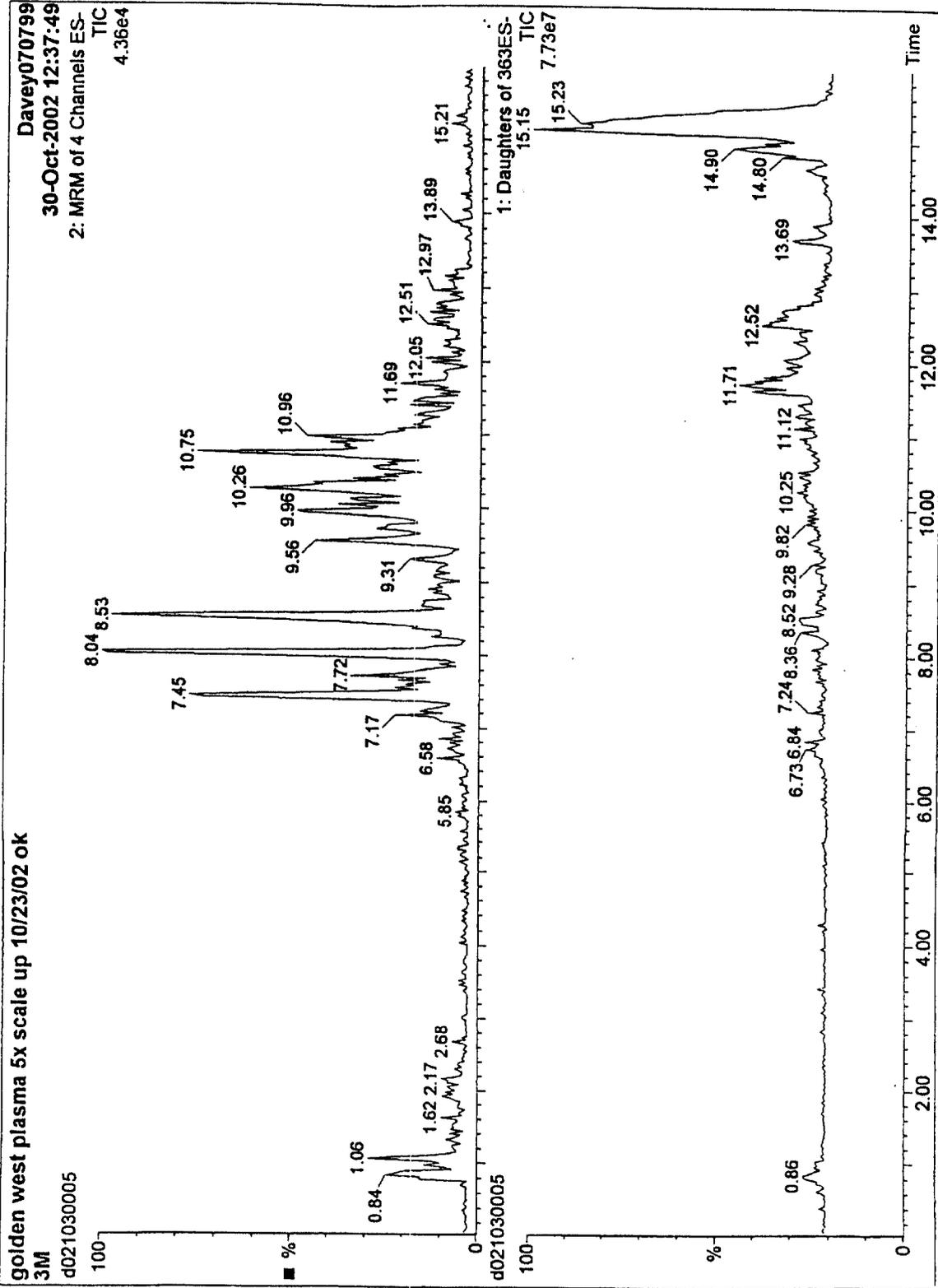
Retention Time	Accurate Mass	Theoretical Mass	Mass Deviation (ppm)	Probable Formula
5.8 min	362.9739	362.9691	13.3	C ₇ O ₂ F ₁₃
6.3 min	412.9637	412.9659	-5.4	C ₈ O ₂ F ₁₅
6.5 min	526.9693	526.9610	15.8	C ₁₀ H ₄ O ₃ F ₁₇ S
6.69 min	462.9613	463.9627	-3.0	C ₉ O ₂ F ₁₇
6.71 min	498.9280	498.9297	-3.3	C ₈ O ₃ F ₁₇ S
6.72 min	512.9615	512.9595	3.9	C ₁₀ O ₂ F ₁₉
6.8 min	426.9734	426.9674	14.1	C ₈ H ₄ O ₃ F ₁₃ S
7.1 min	562.9542	562.9563	-3.8	C ₁₁ O ₂ F ₂₁

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ATTACHMENT C: CHROMATOGRAMS

000155

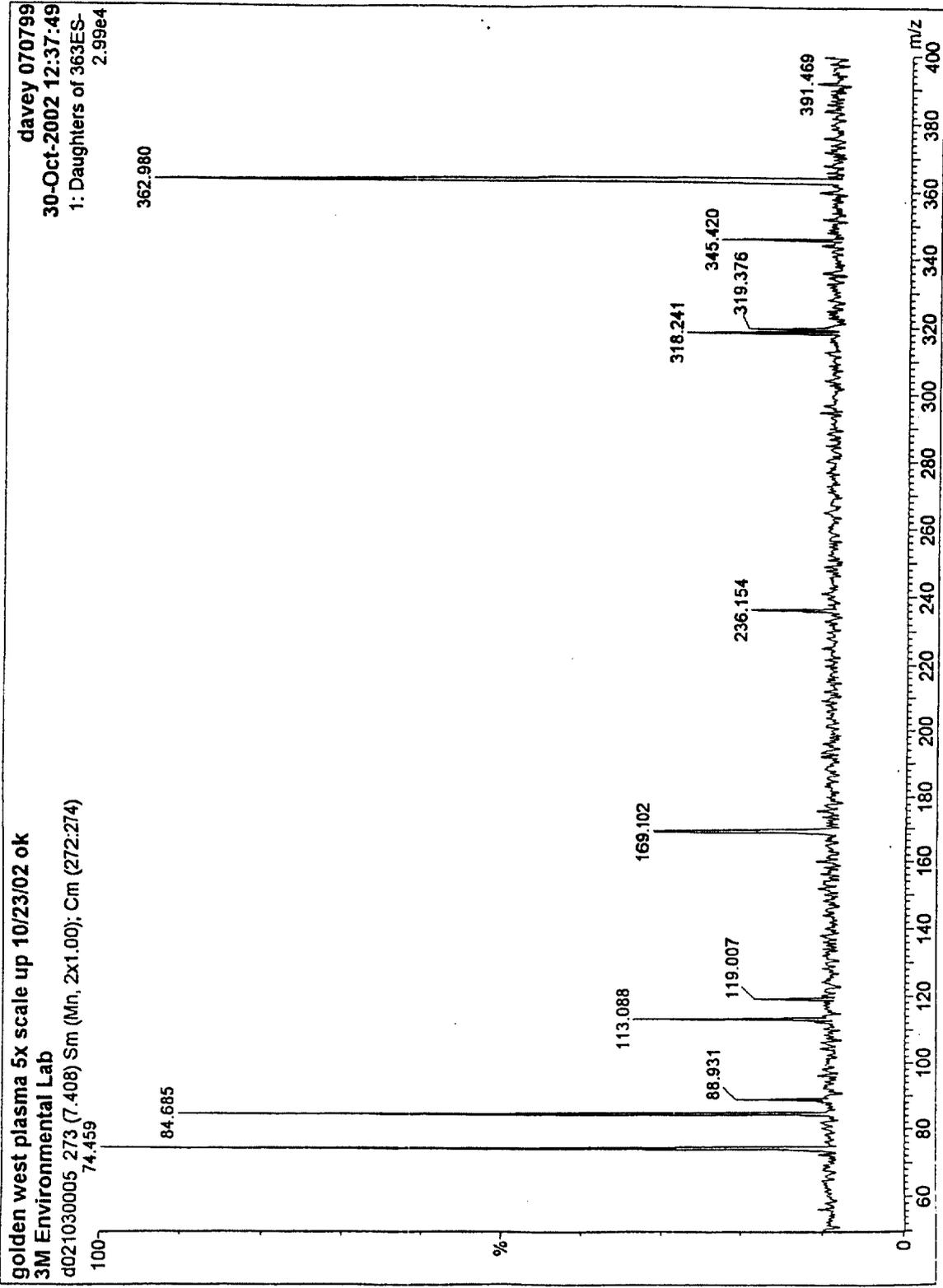
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C7ACID

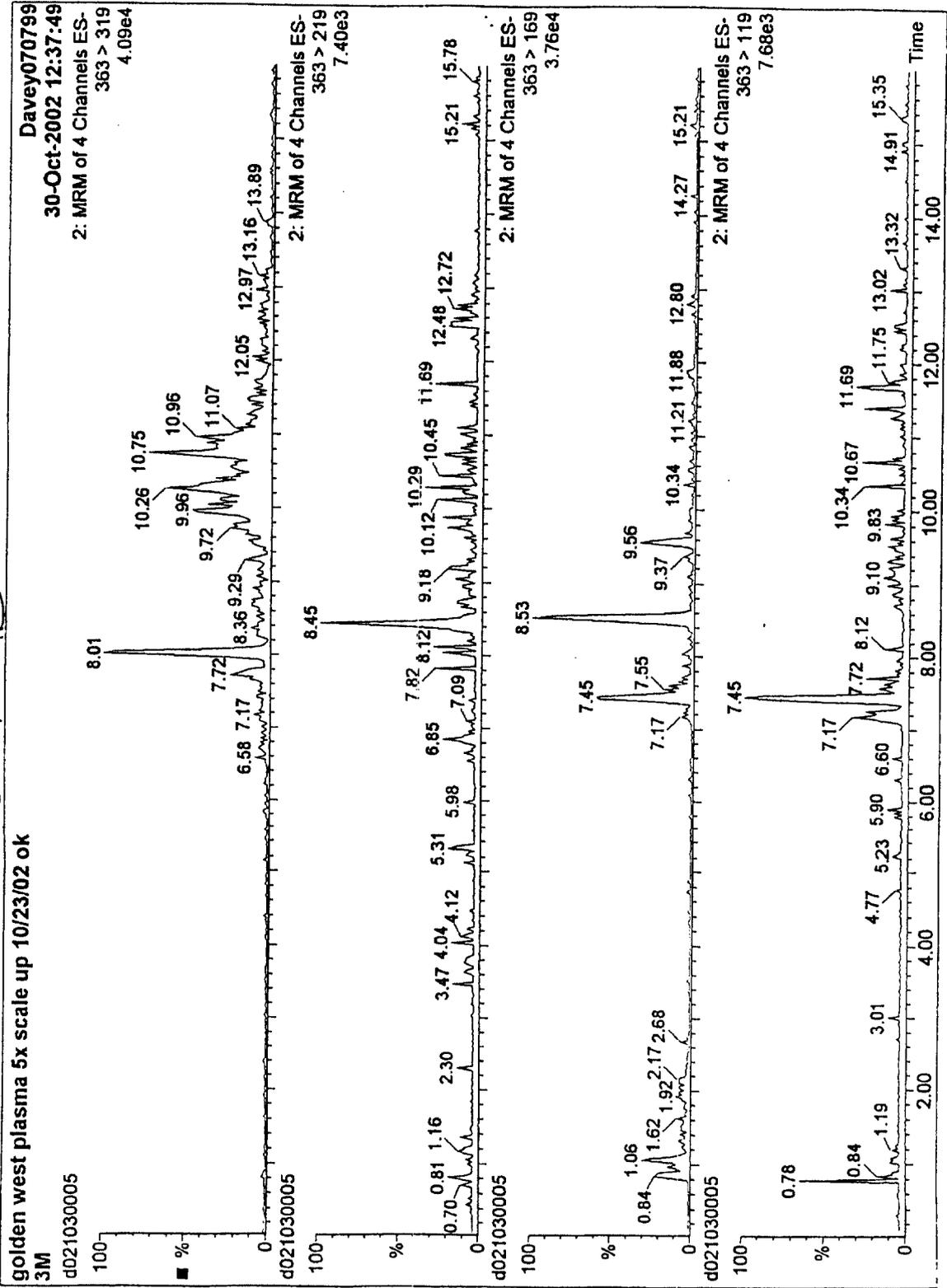


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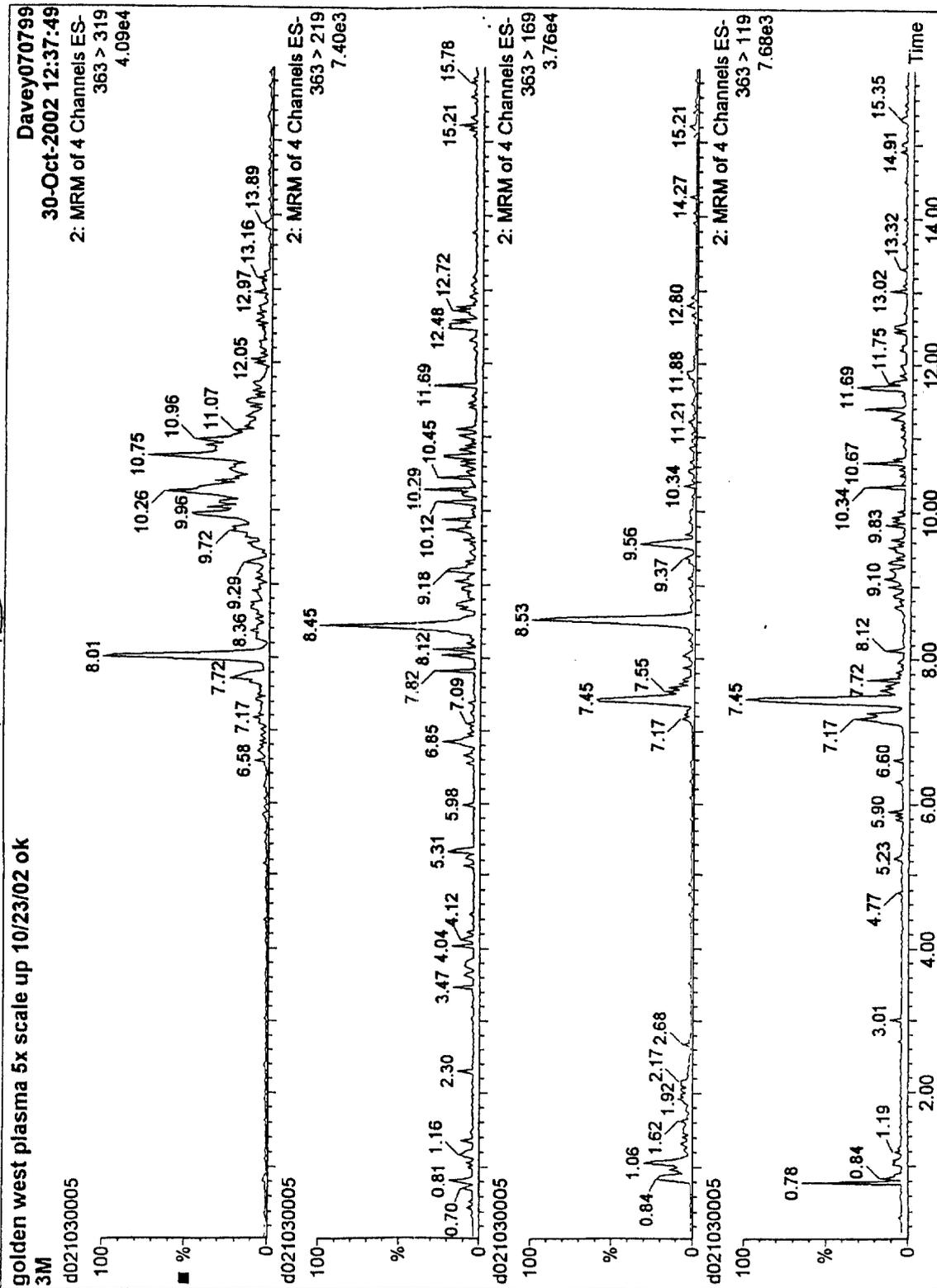
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C7AC10

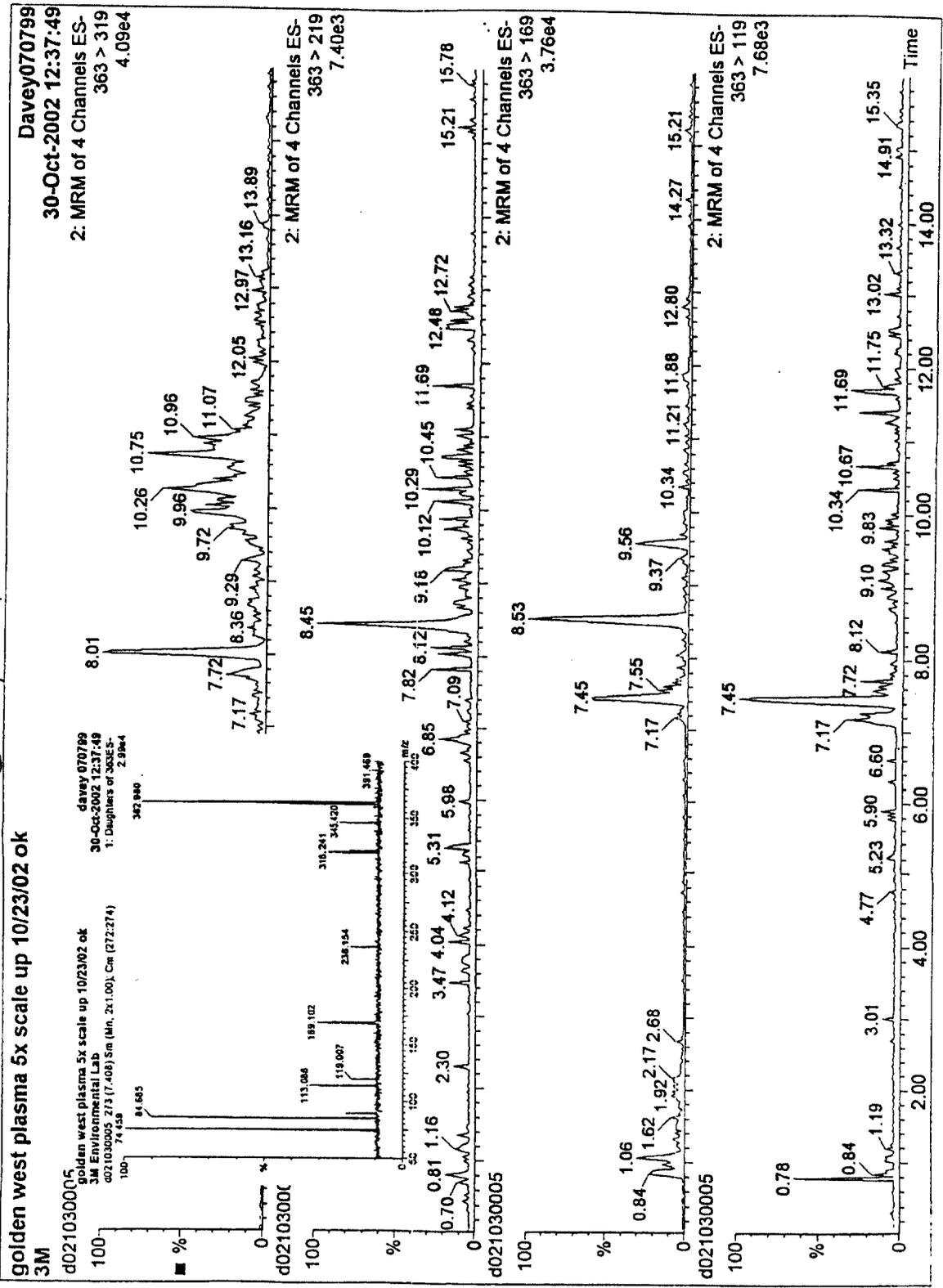


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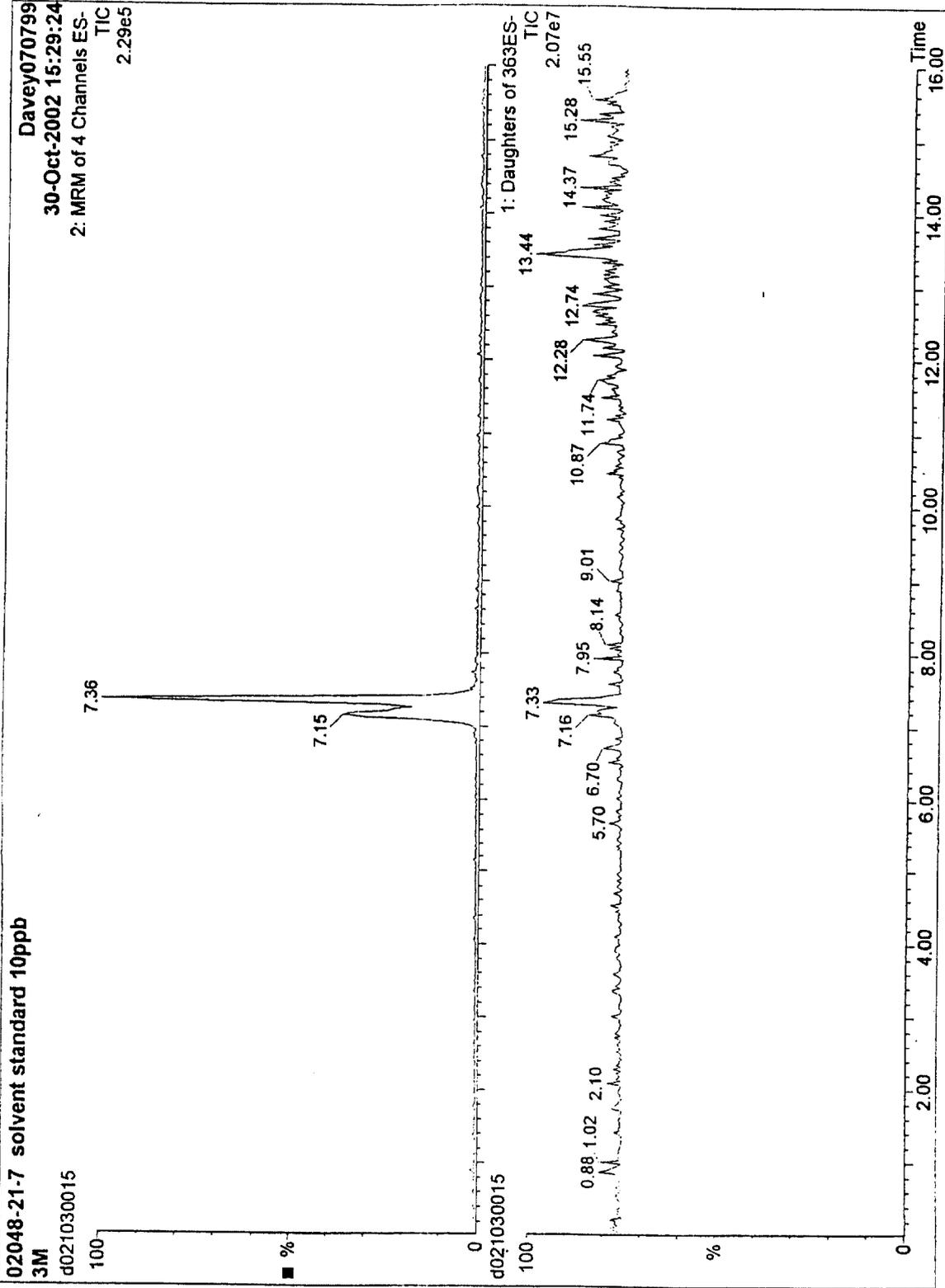
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C7 ACID



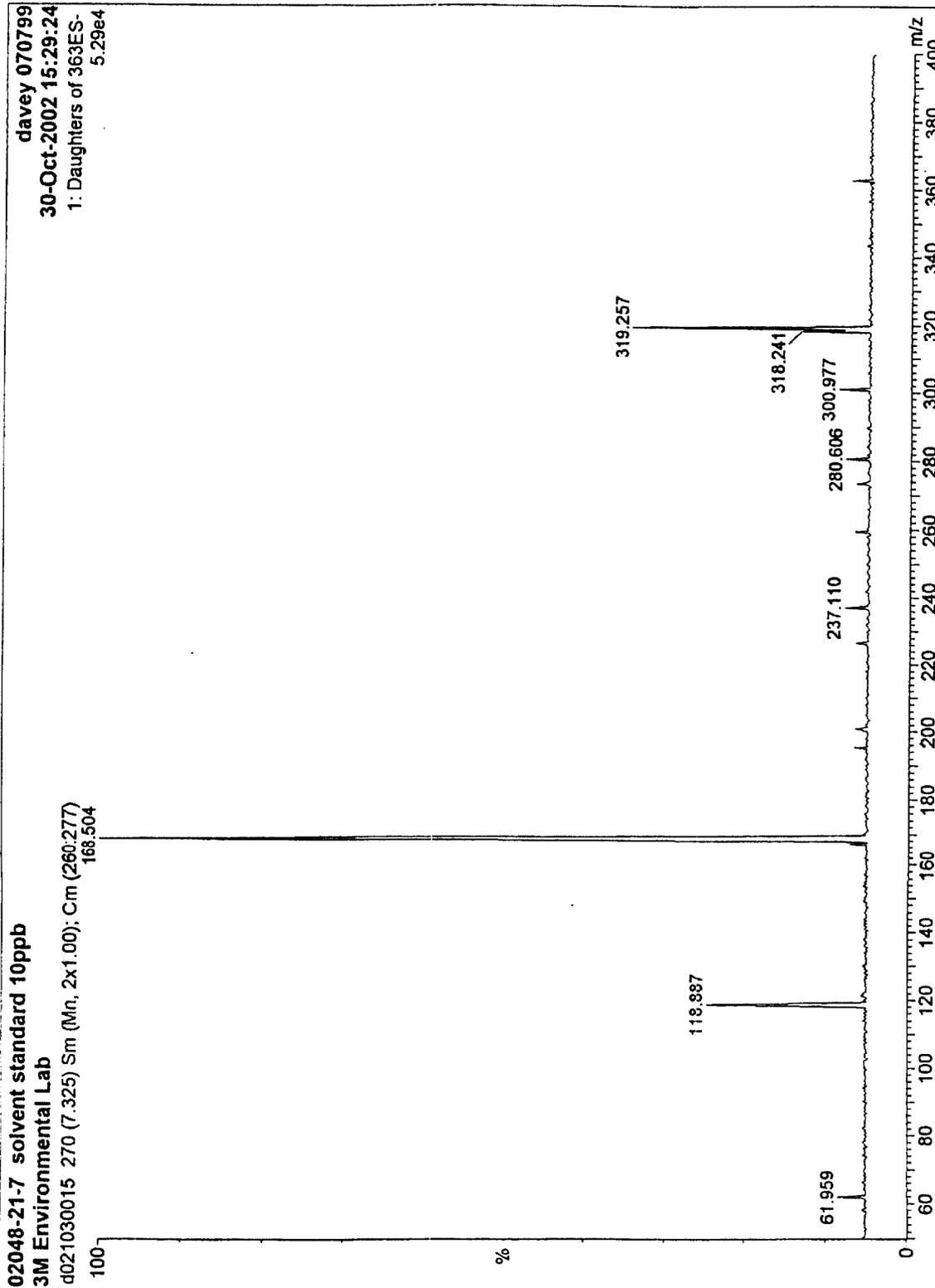
Davey070799
30-Oct-2002 15:29:24
2. MRM of 4 Channels ES-
TIC
2.29e5

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cm
Initial W. L. L.
Date

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C7 ACID

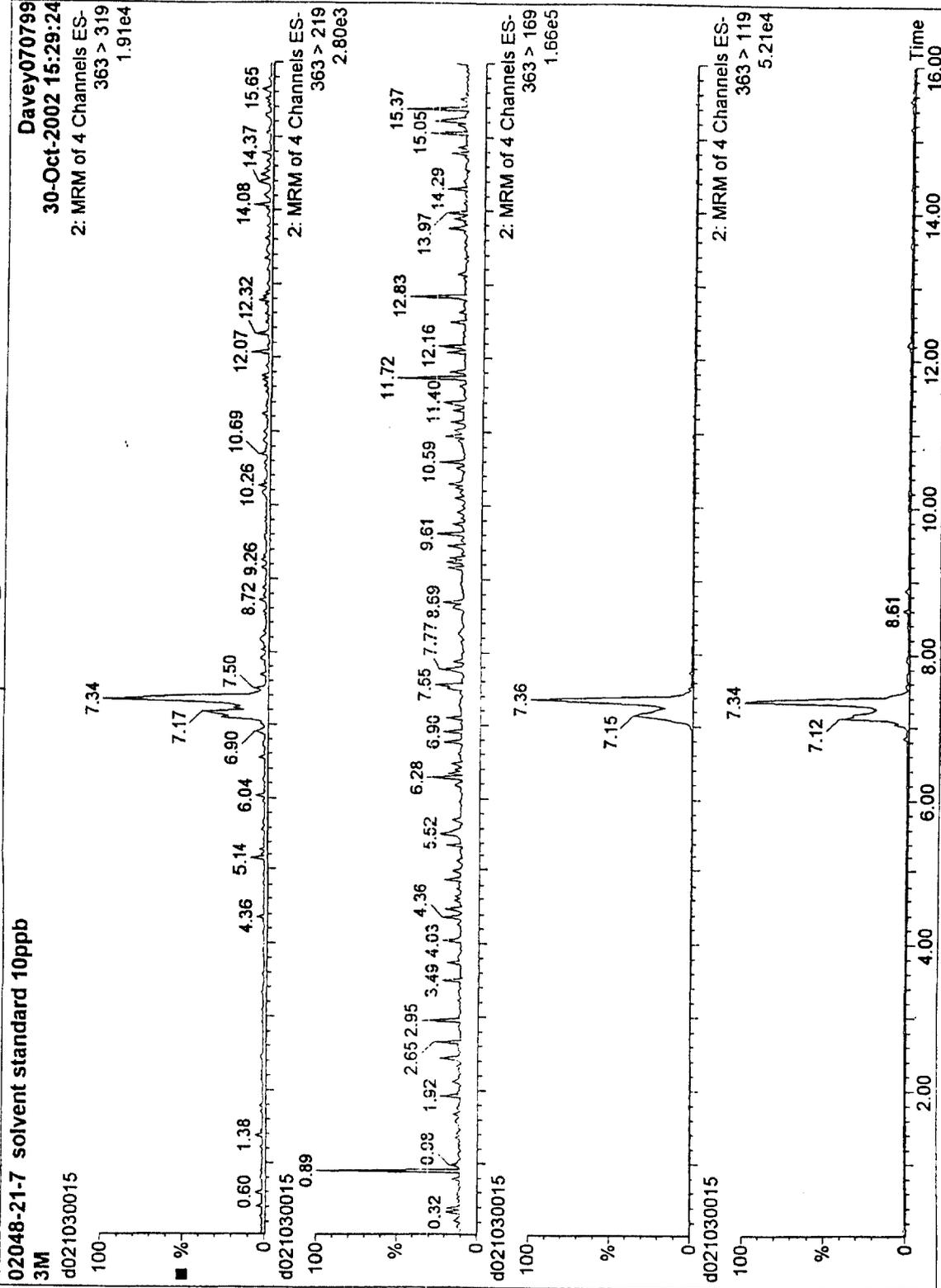


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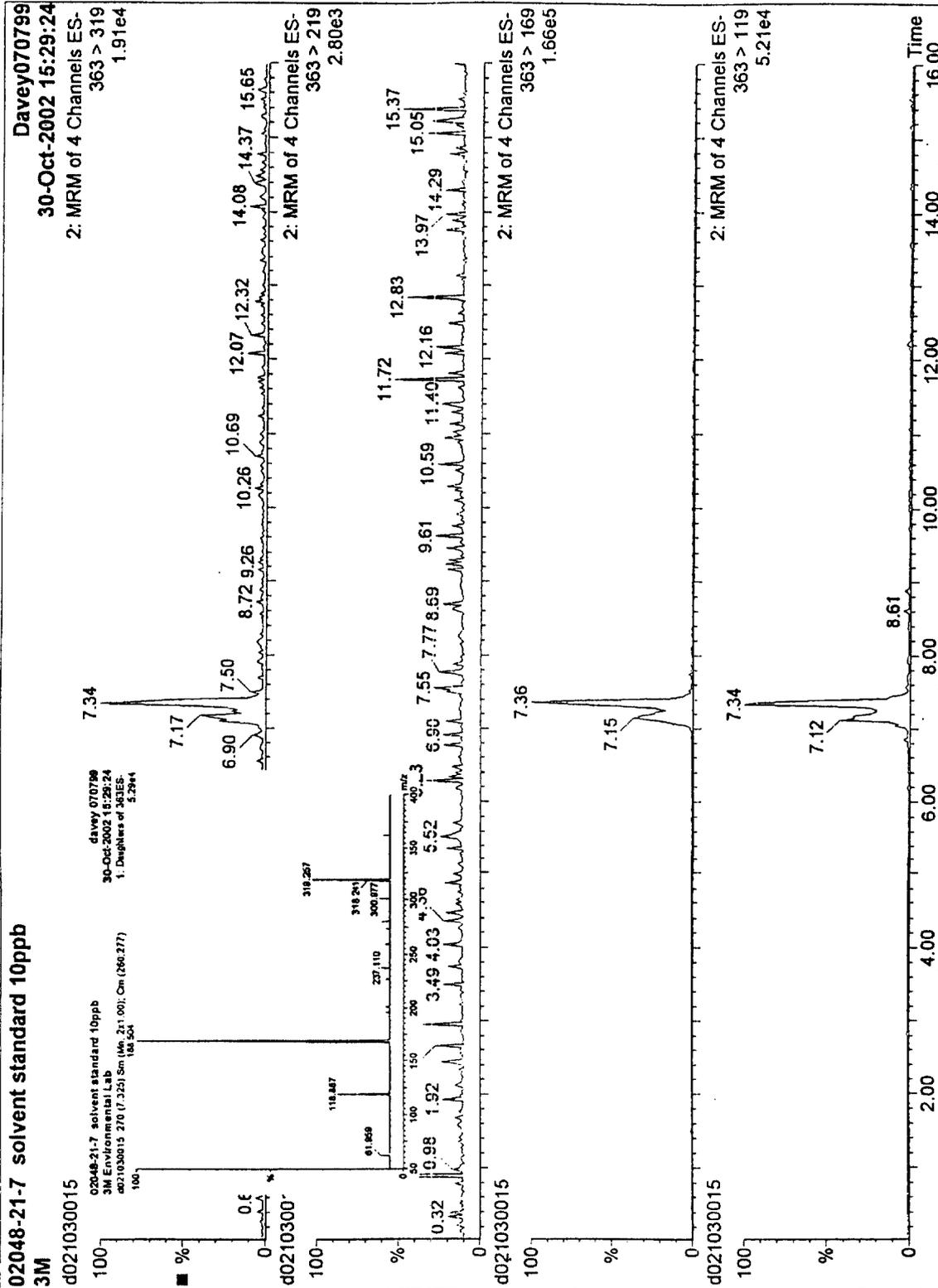


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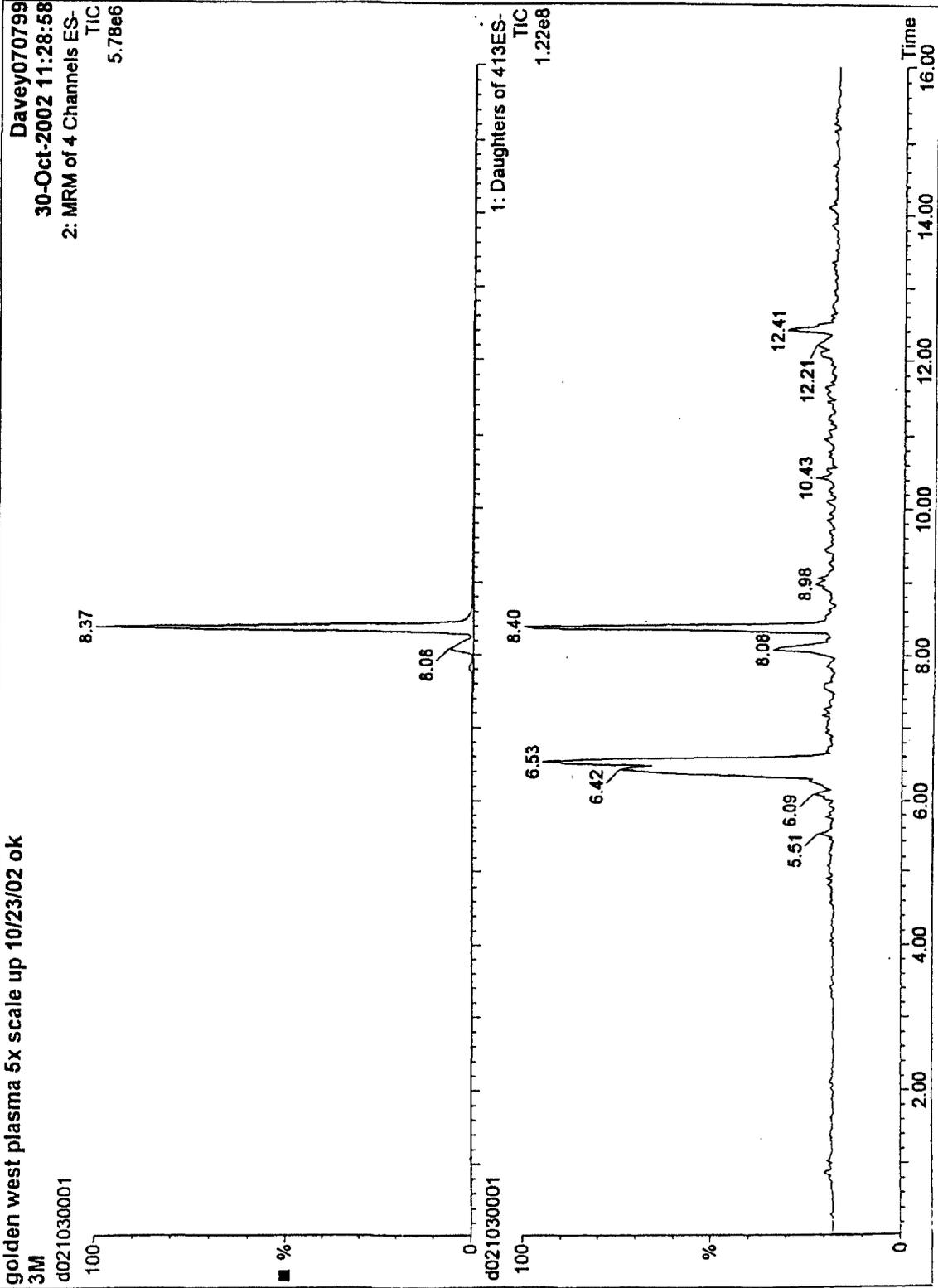


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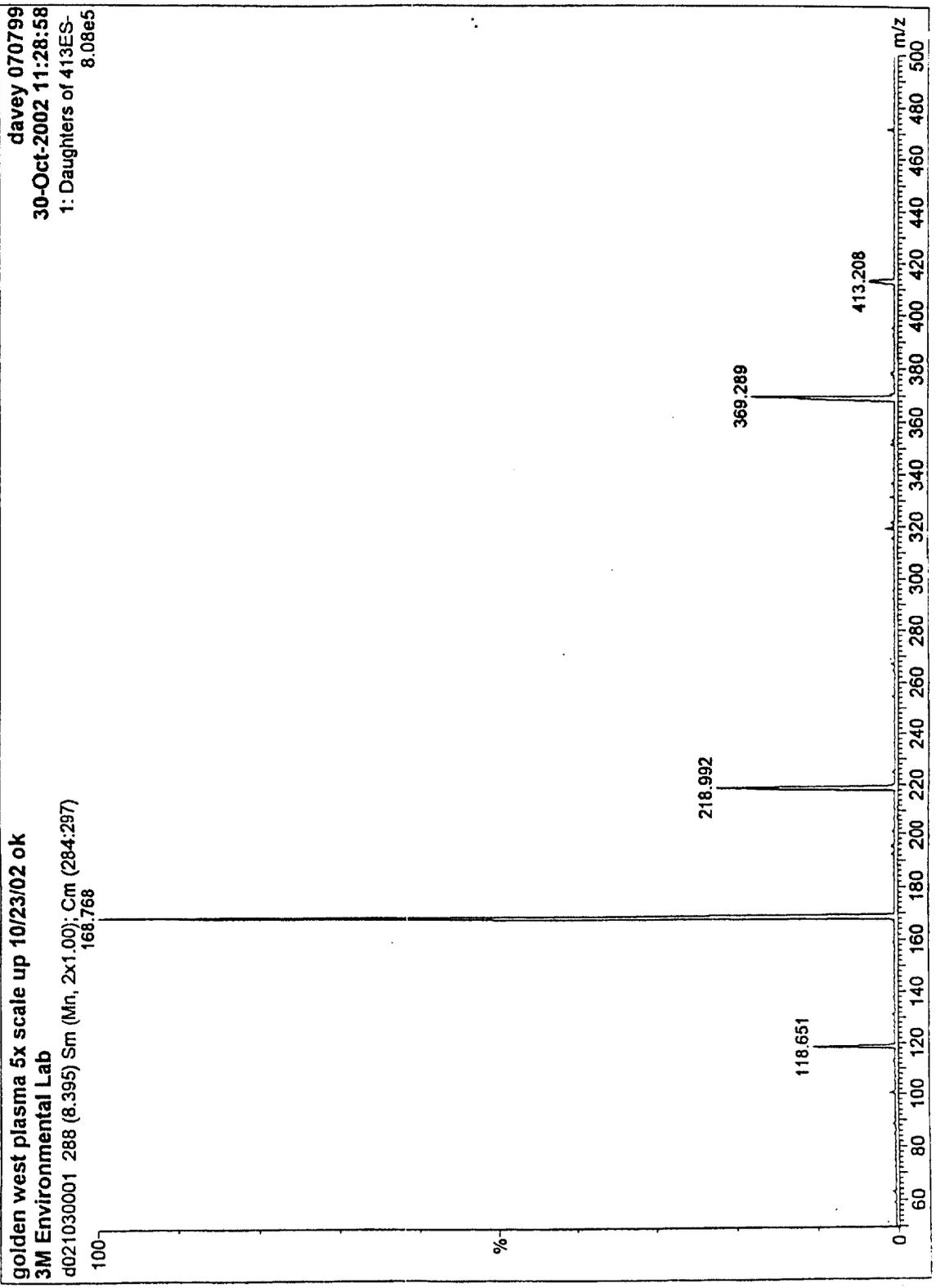


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000165

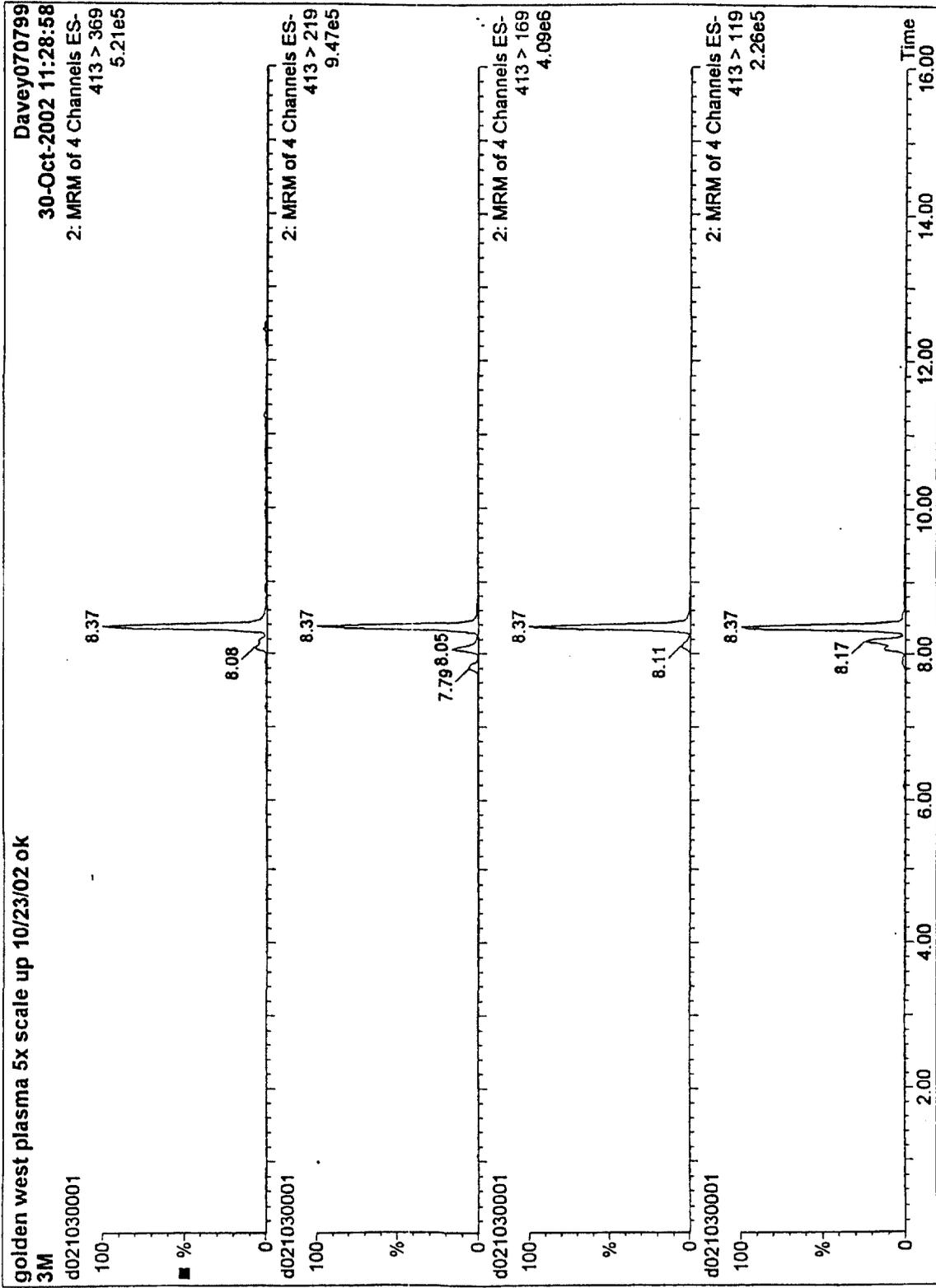
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CML 10/31/02
Initial Date

000166

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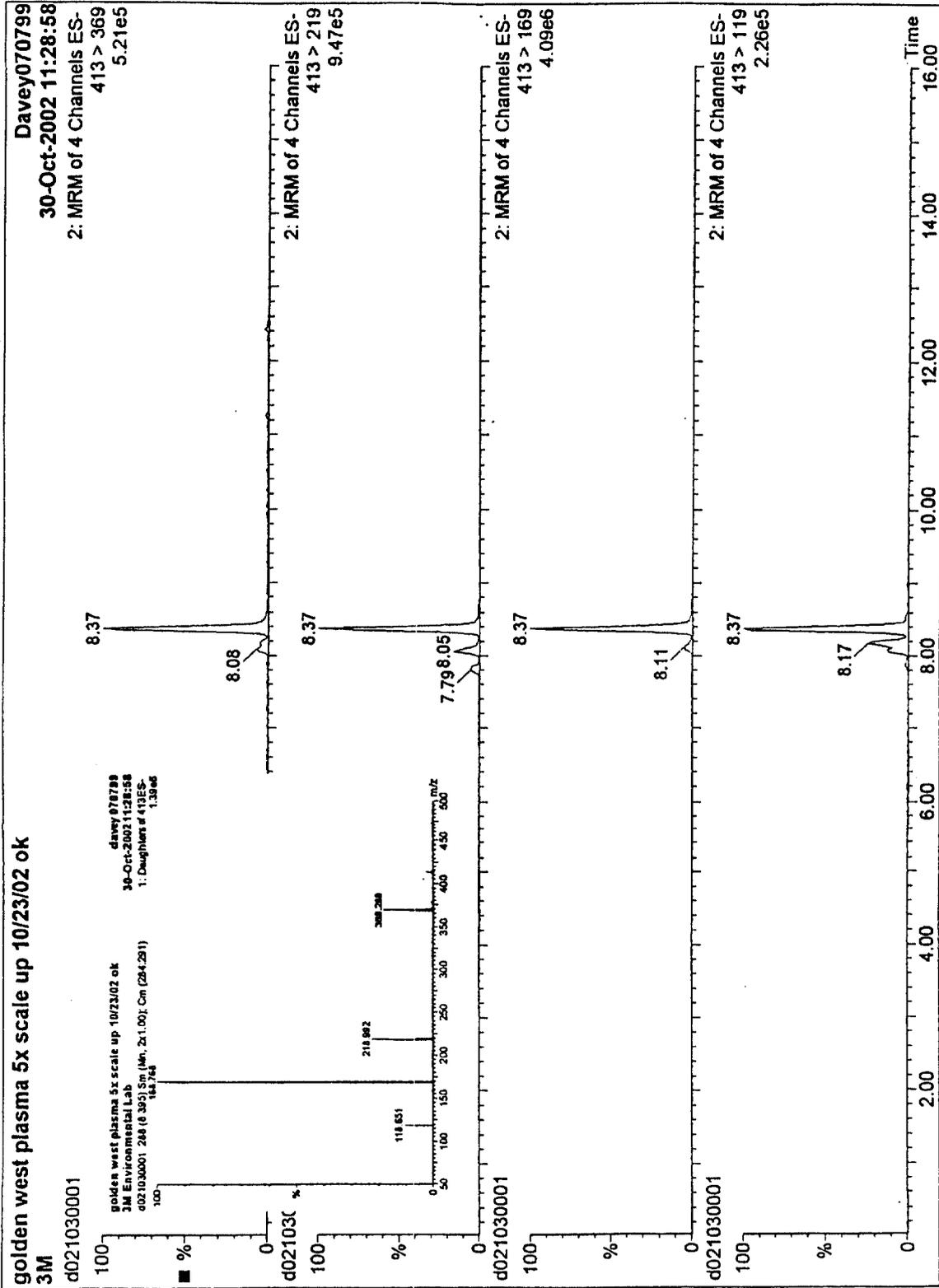


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CMC Initial Date 10/31/02

000167

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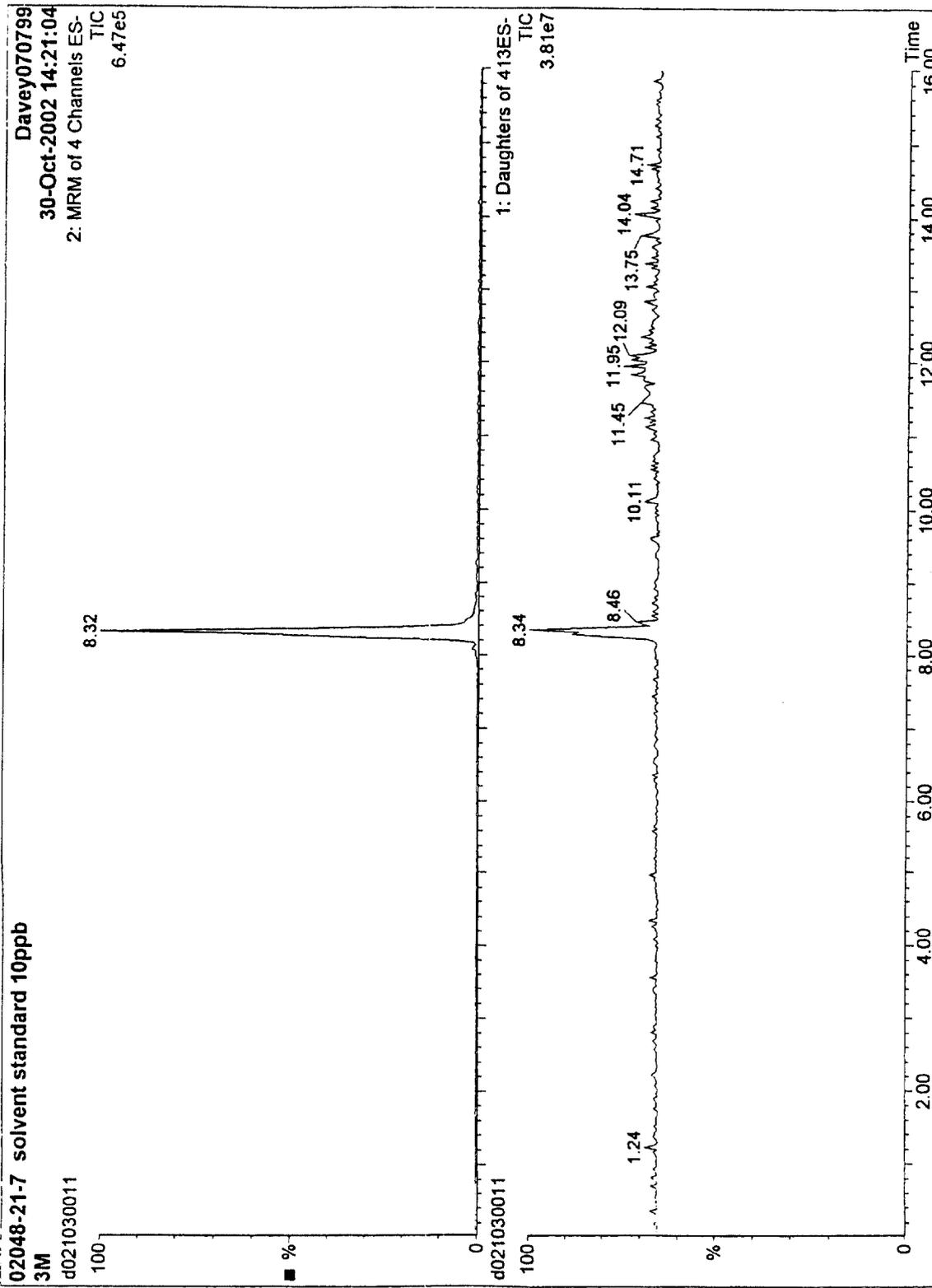


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CMK 10/31/02
Initial Date

000168

CSACID

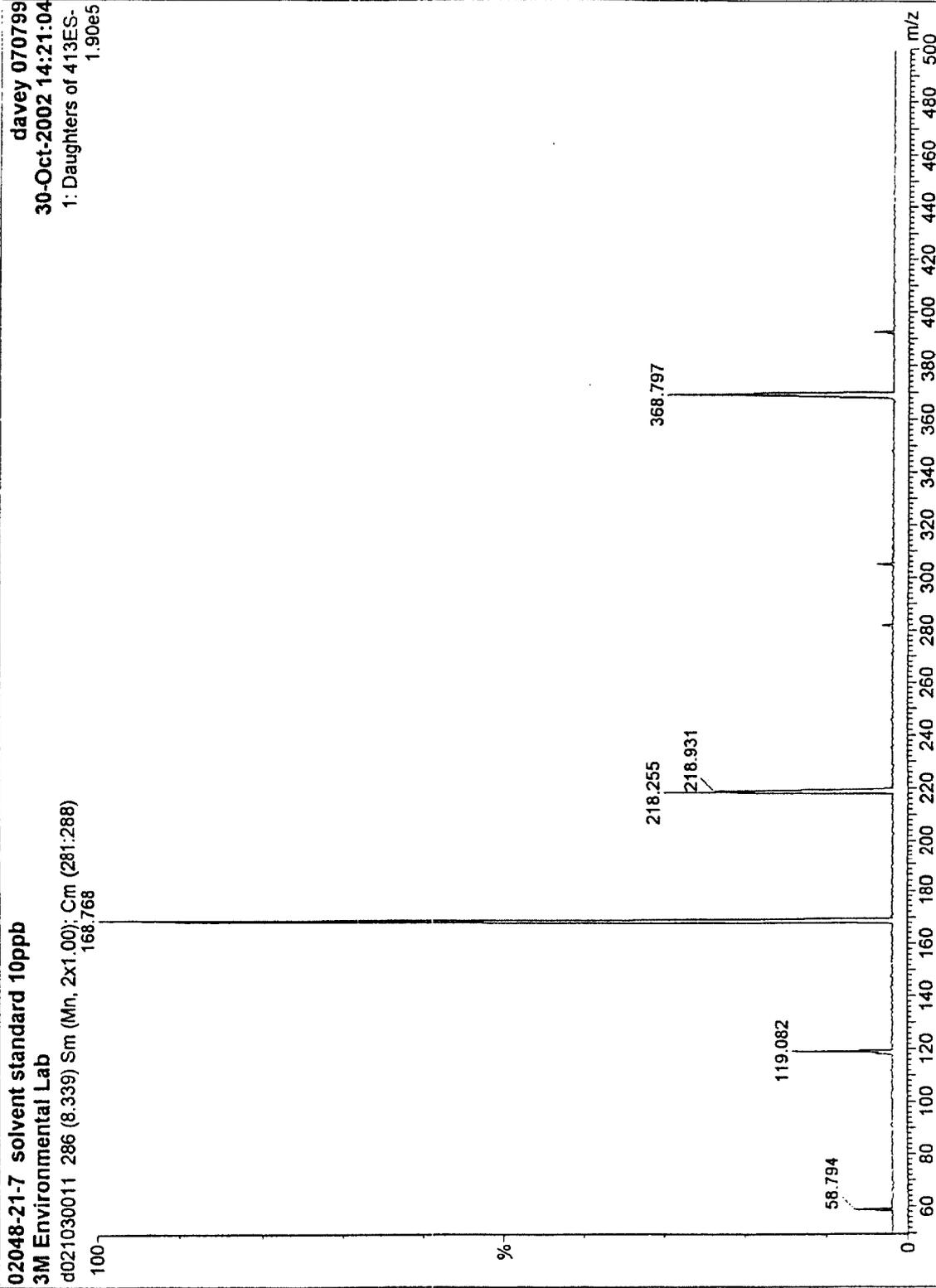


Exact Copy of Original

Initial LLV/02
Date

000169

C8 ACID



davey 070799
30-Oct-2002 14:21:04
1: Daughters of 413ES-
1.90e5

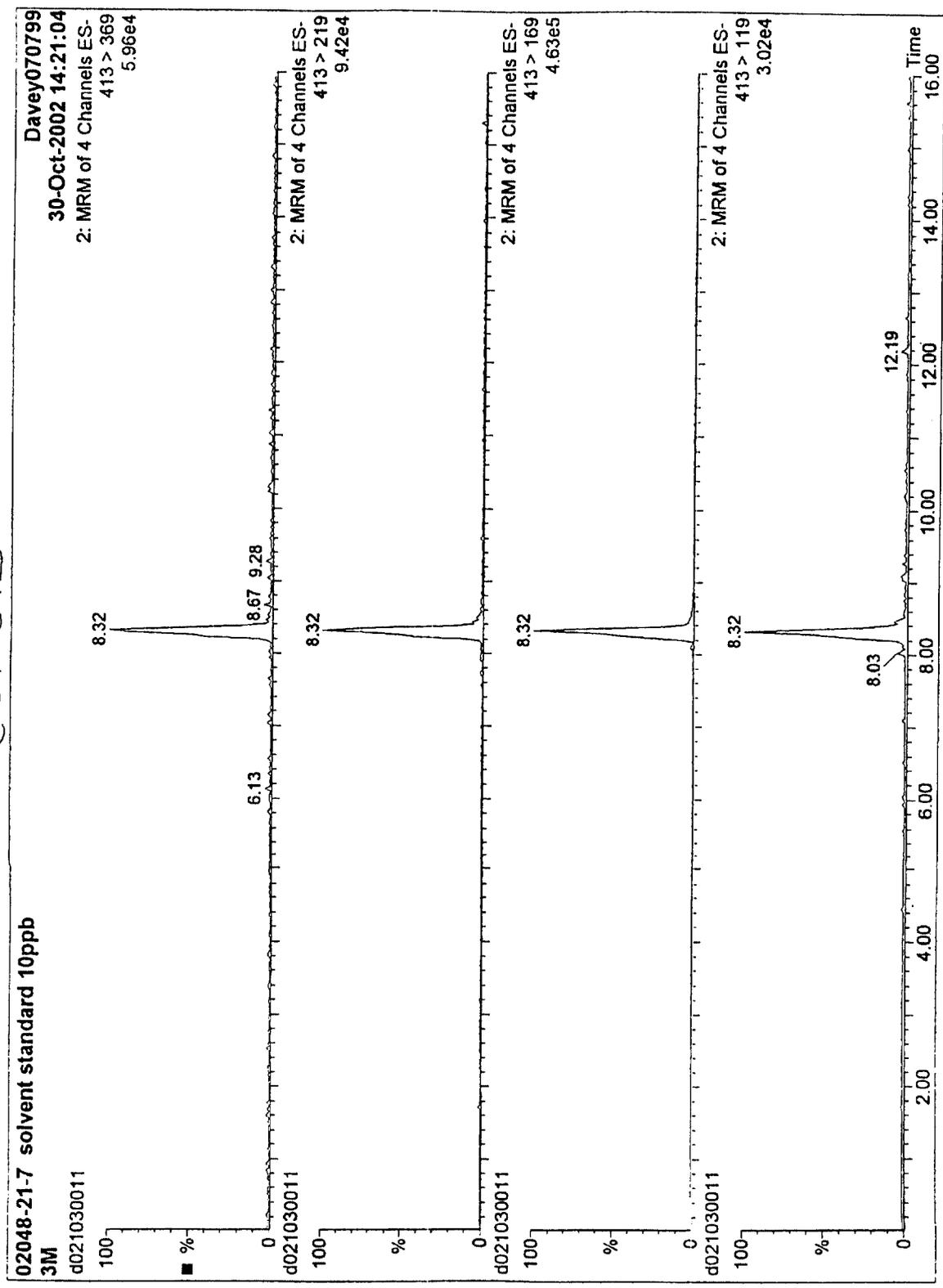
02048-21-7 solvent standard 10ppb
3M Environmental Lab
d021030011 286 (8.339) Sm (Mn, 2x1.00); Cm (281.288)
168.768

Exact Copy of Original

Initial CMS Date 11/1/07

000170

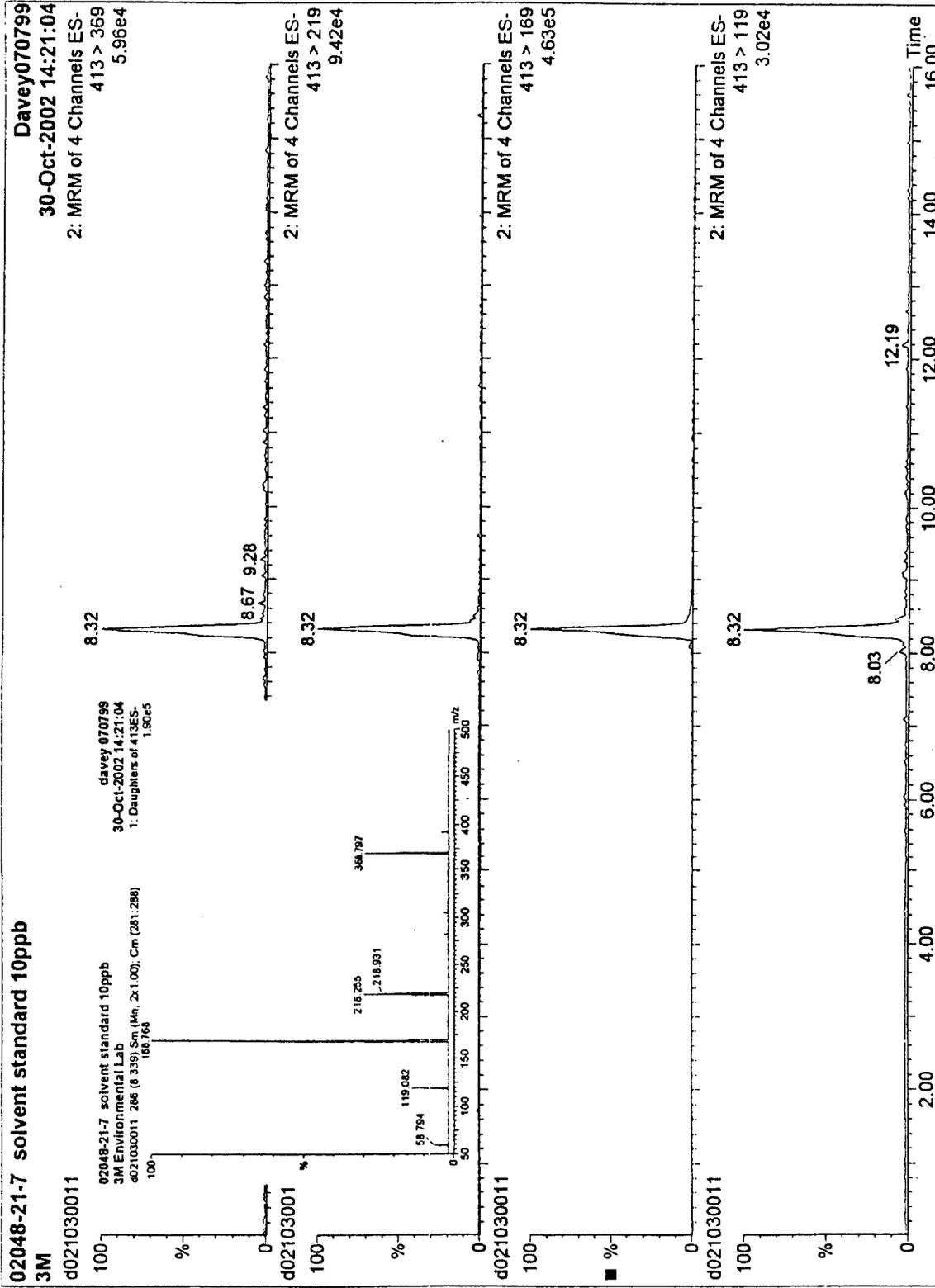
C8ACID



Exact Copy of Original
Initial JK/10 Date

000171

CS ACID

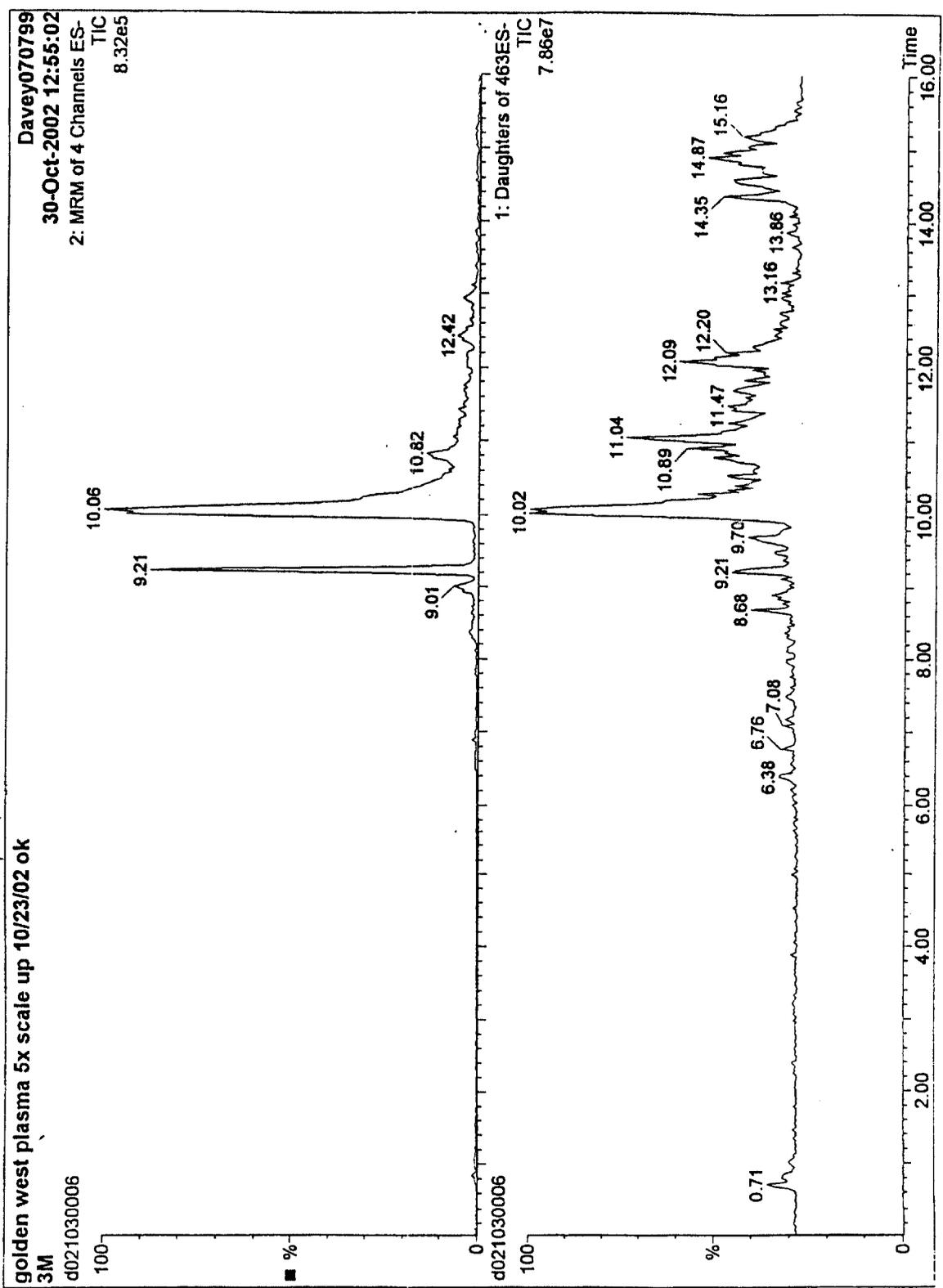


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Initial LLX Date 11/1/02

000172

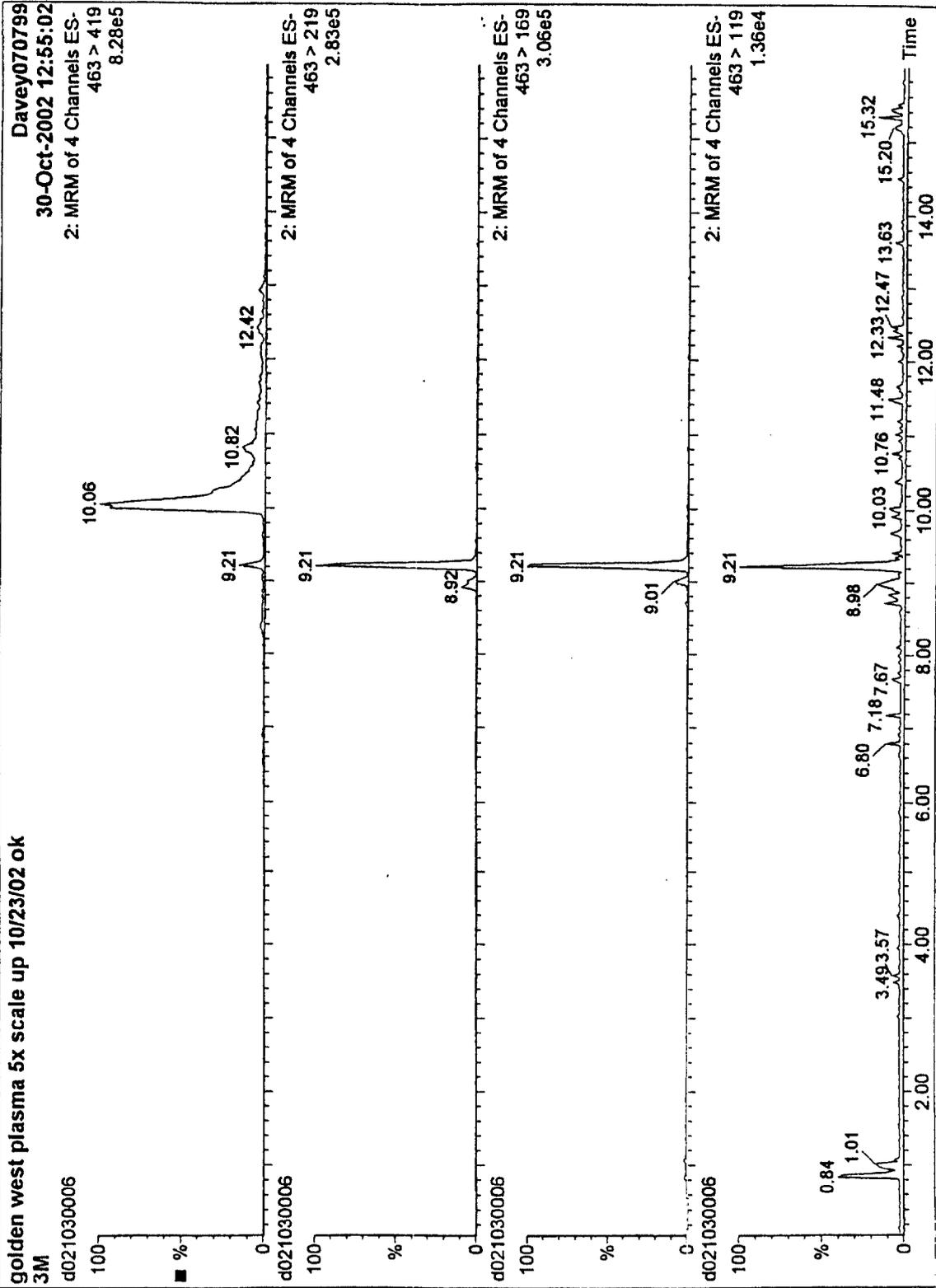
C9 acids



Exact Copy of Original
SMC 10/31/02
Initial Date

000173

Q19 ACID

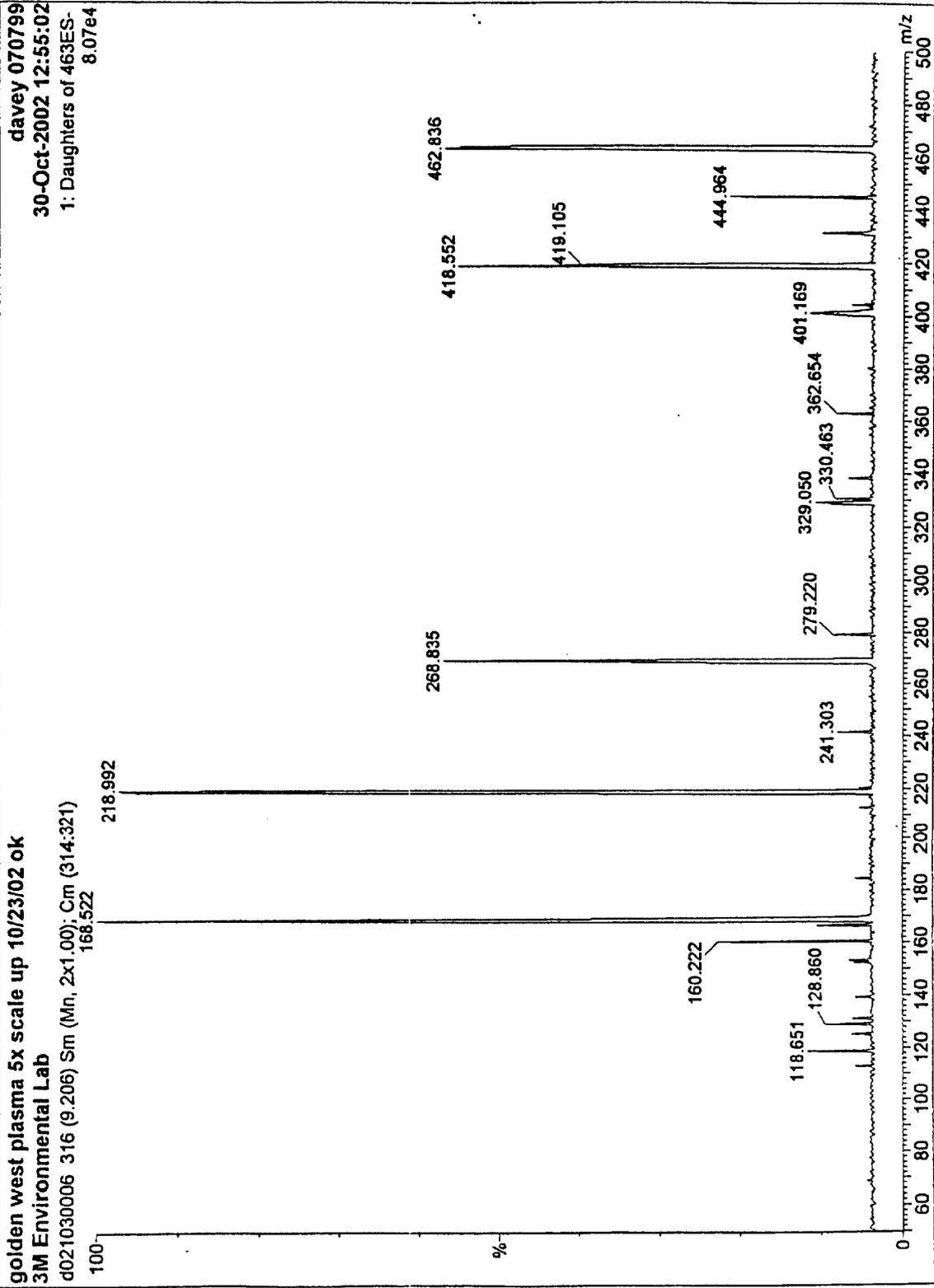


Exact Copy of Original

LM
Initial 10/31/02
Date

000174

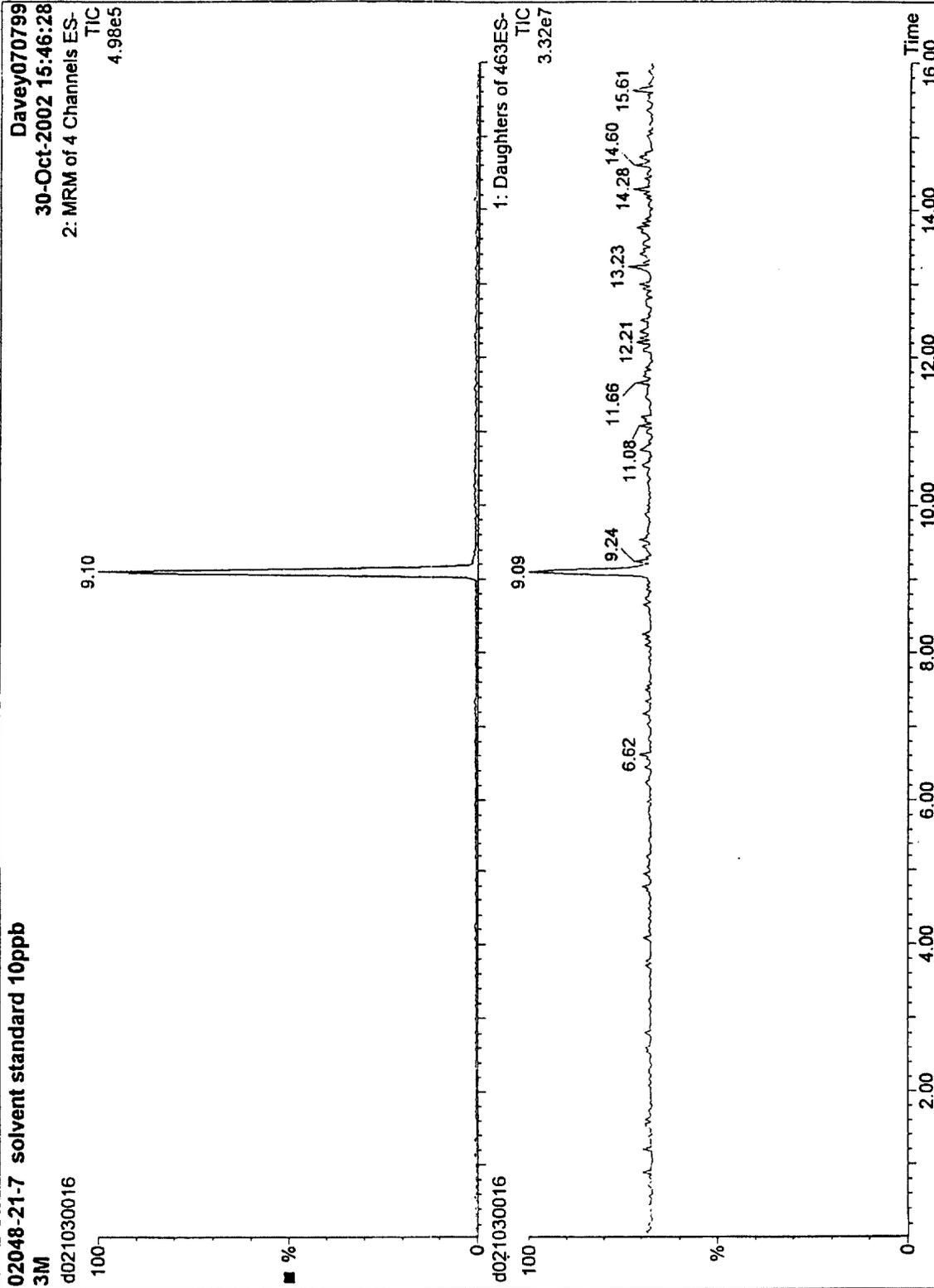
C9 ACD



Exact Copy of Original
C.M.C. 10/31/02
Initial Date

000175

C9 ACID

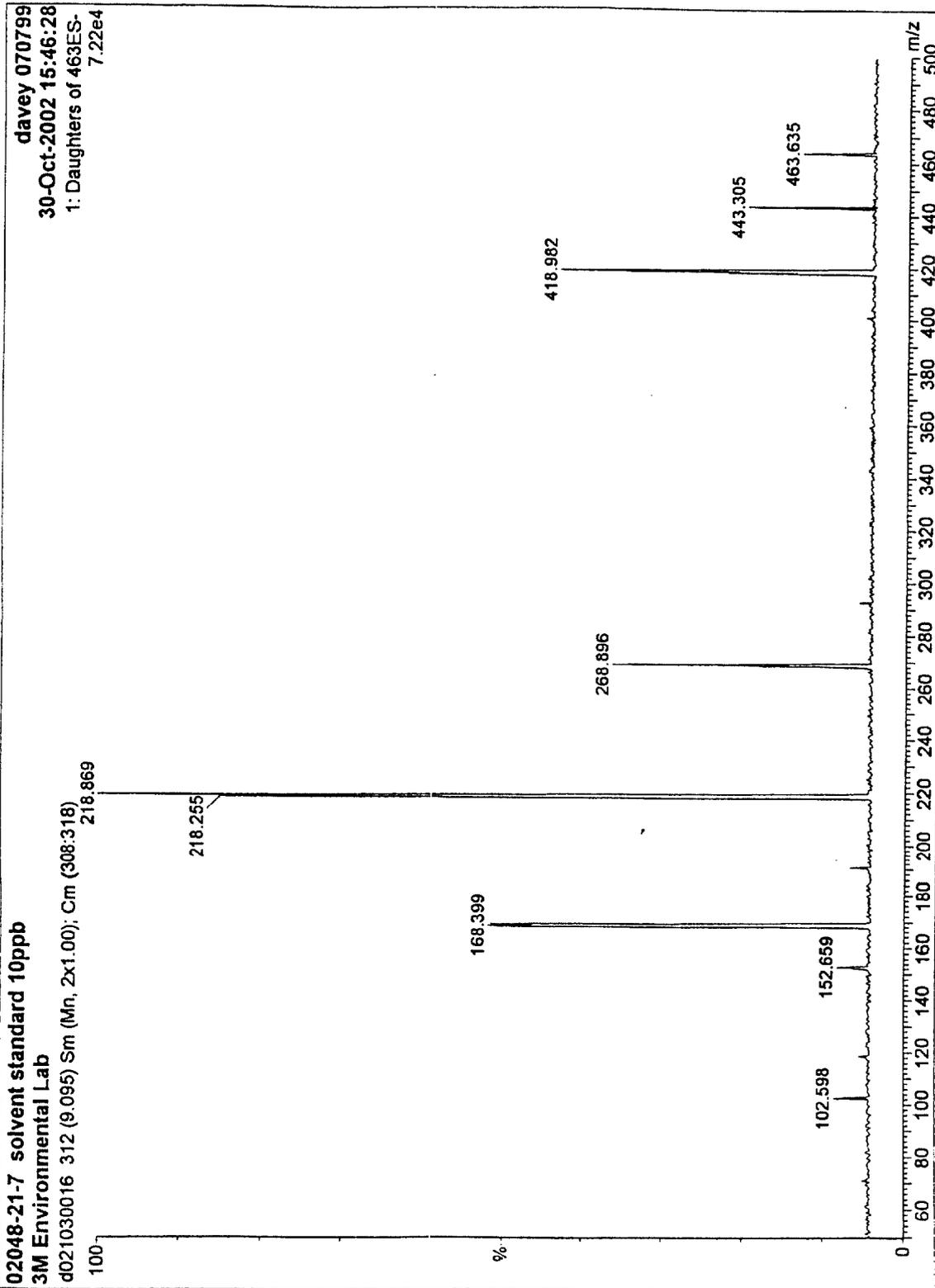


Exact Copy of Original

CMC Initial
11/1/02 Date

000177

CG ACID



davey 070799
30-Oct-2002 15:46:28
1: Daughters of 463ES-
7.22e4

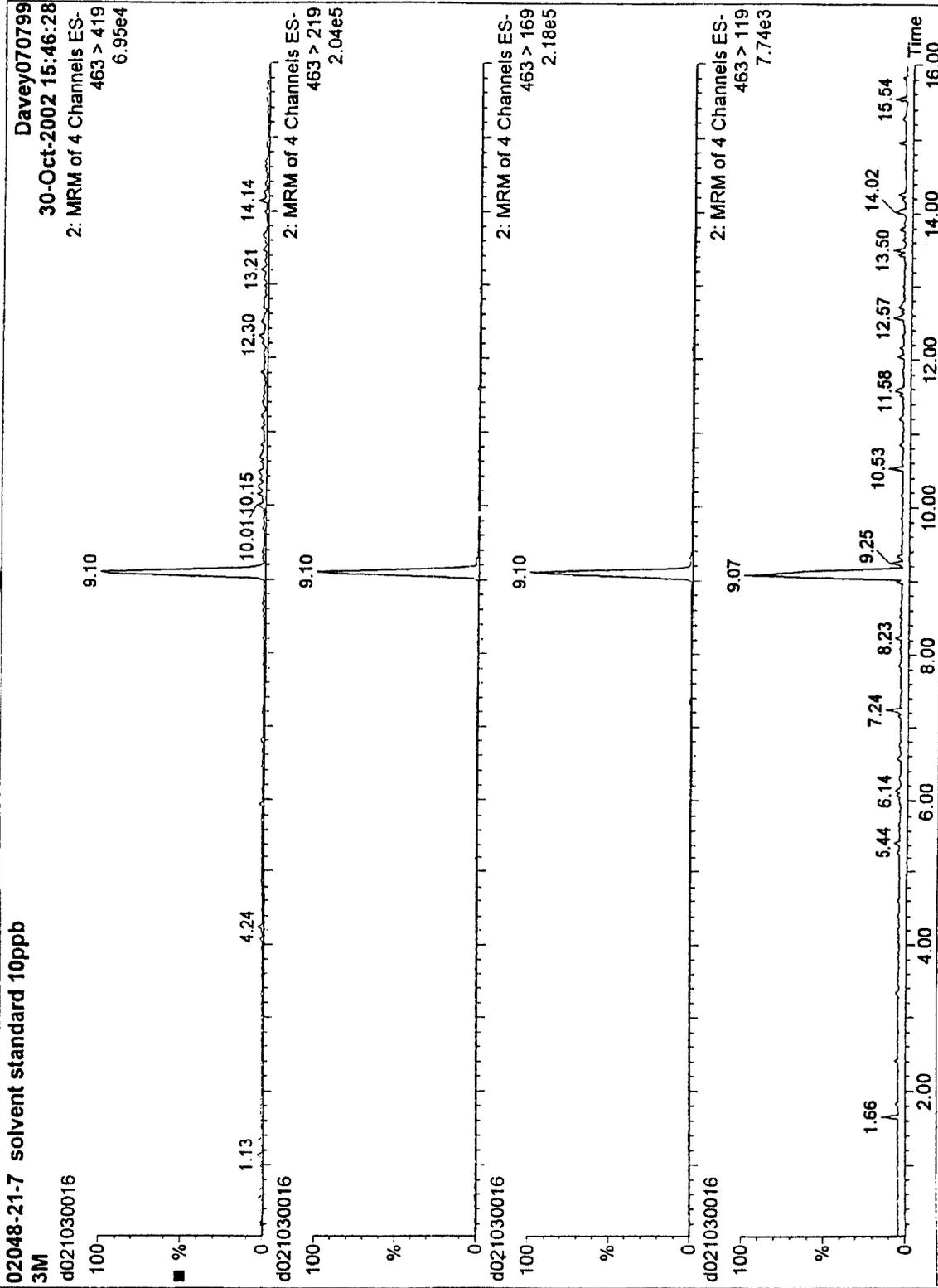
02048-21-7 solvent standard 10ppb
3M Environmental Lab
d021030016 312 (9.095) Sm (Mn, 2x1.00); Cm (308:318)

Exact Copy of Original

Initial Date
Cmi 11/1/02

000178

CA ACID

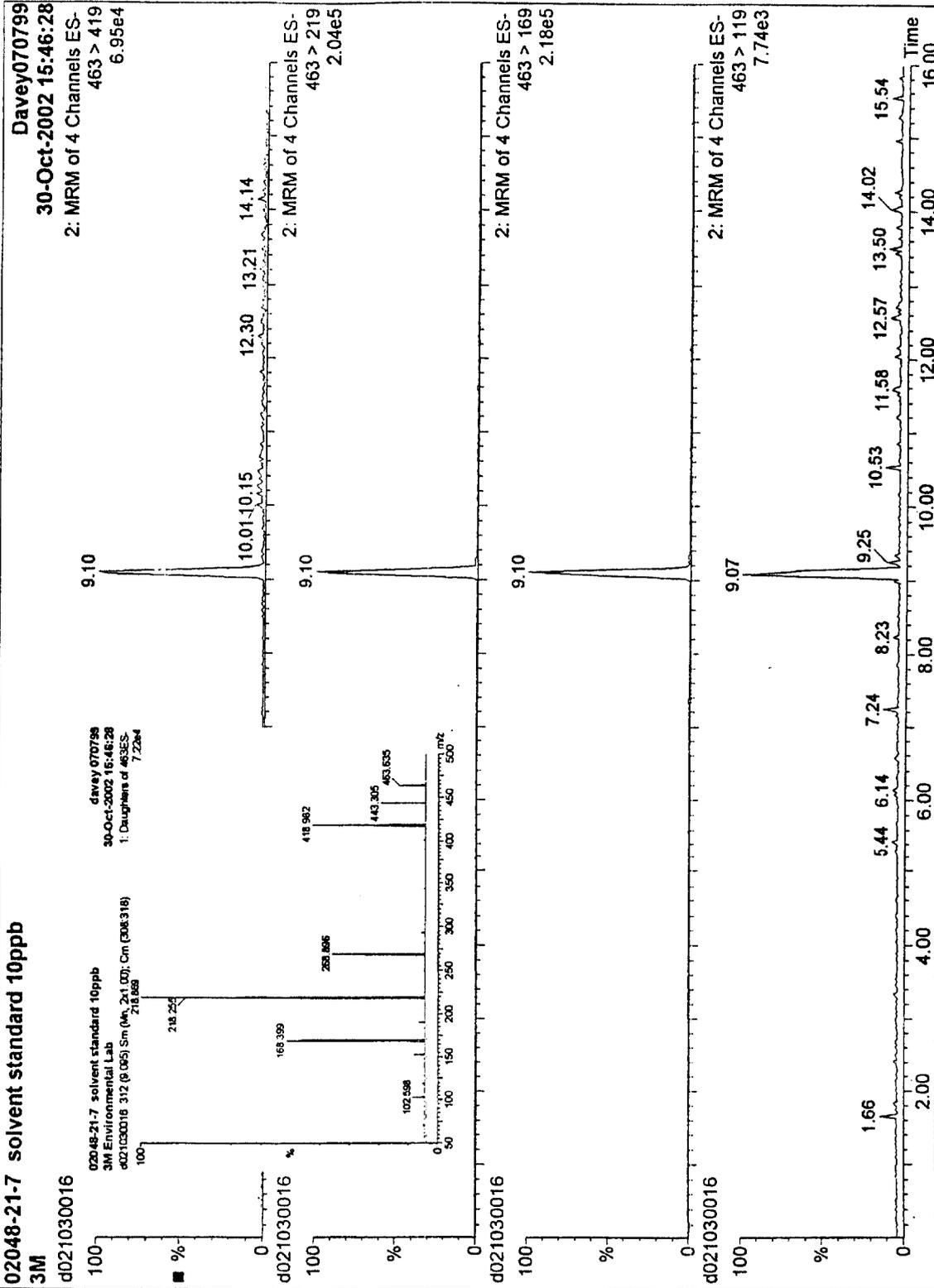


Exact Copy of Original

LM
Initial
11/10
Date

000179

CG ACID

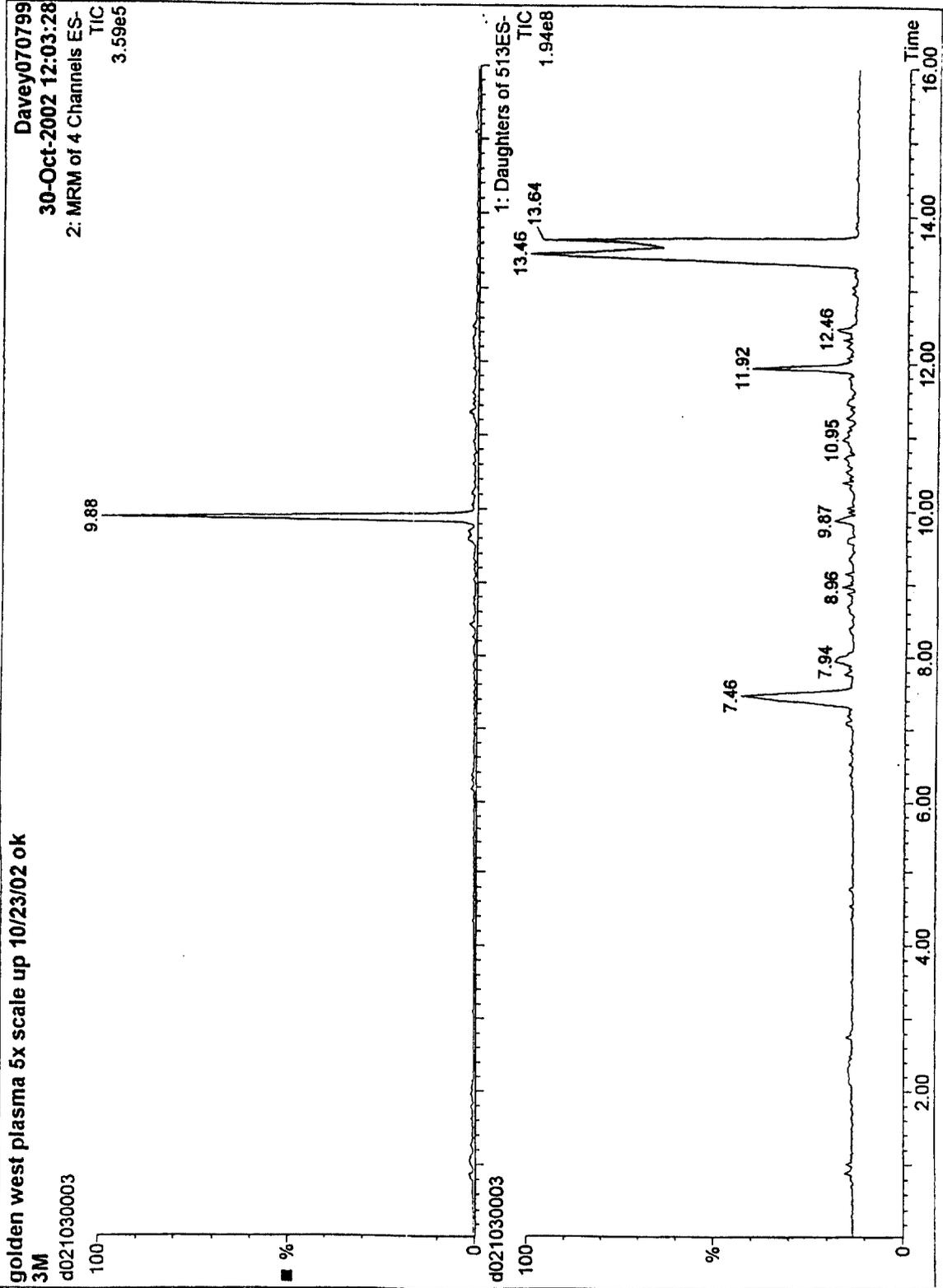


Exact Copy of Original

Initial CMC Date 10/11/02

000180

C10 ACD

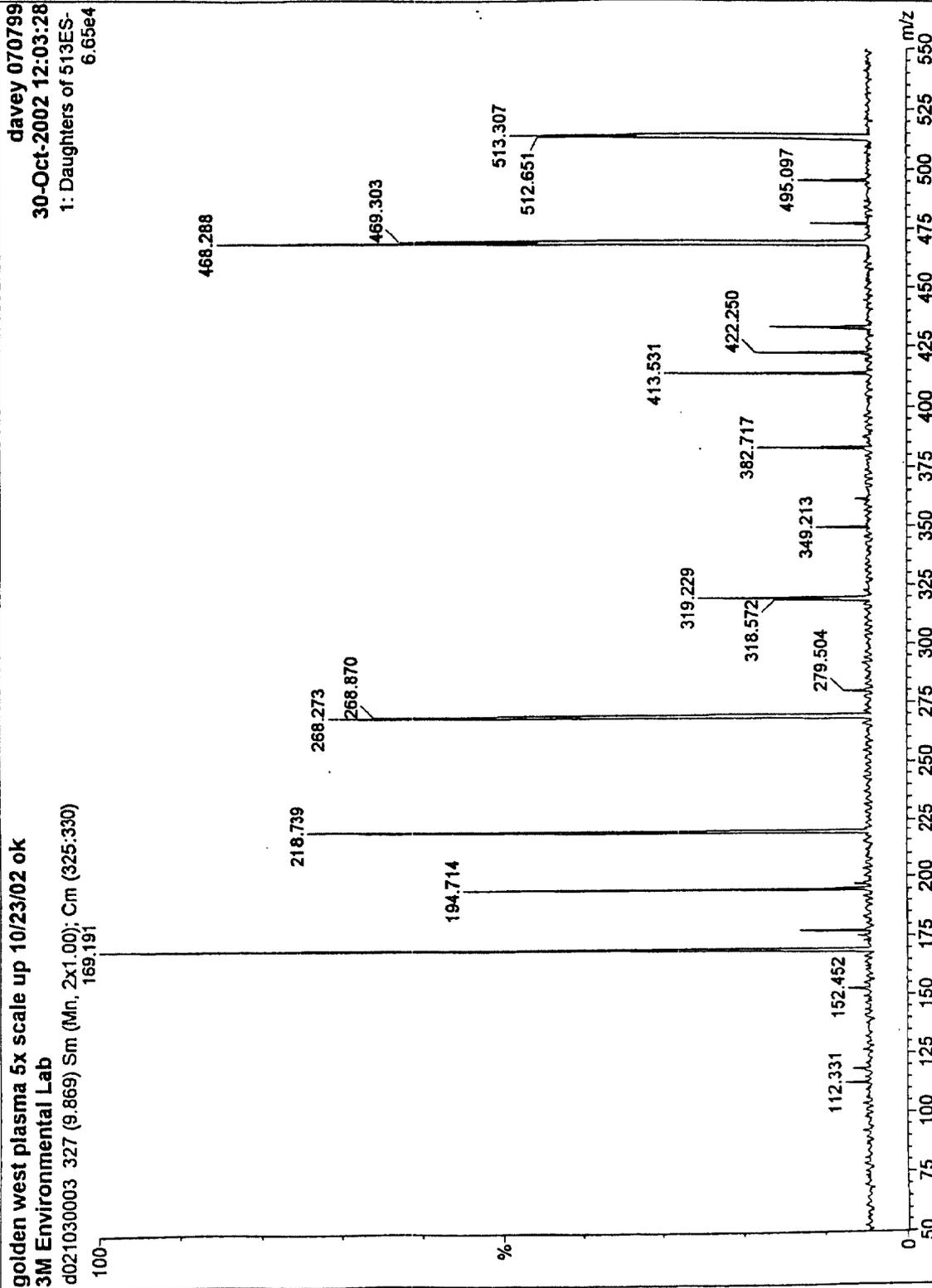


Exact Copy of Original

Initial CLL Date 10/31/02

000181

C10 Acid

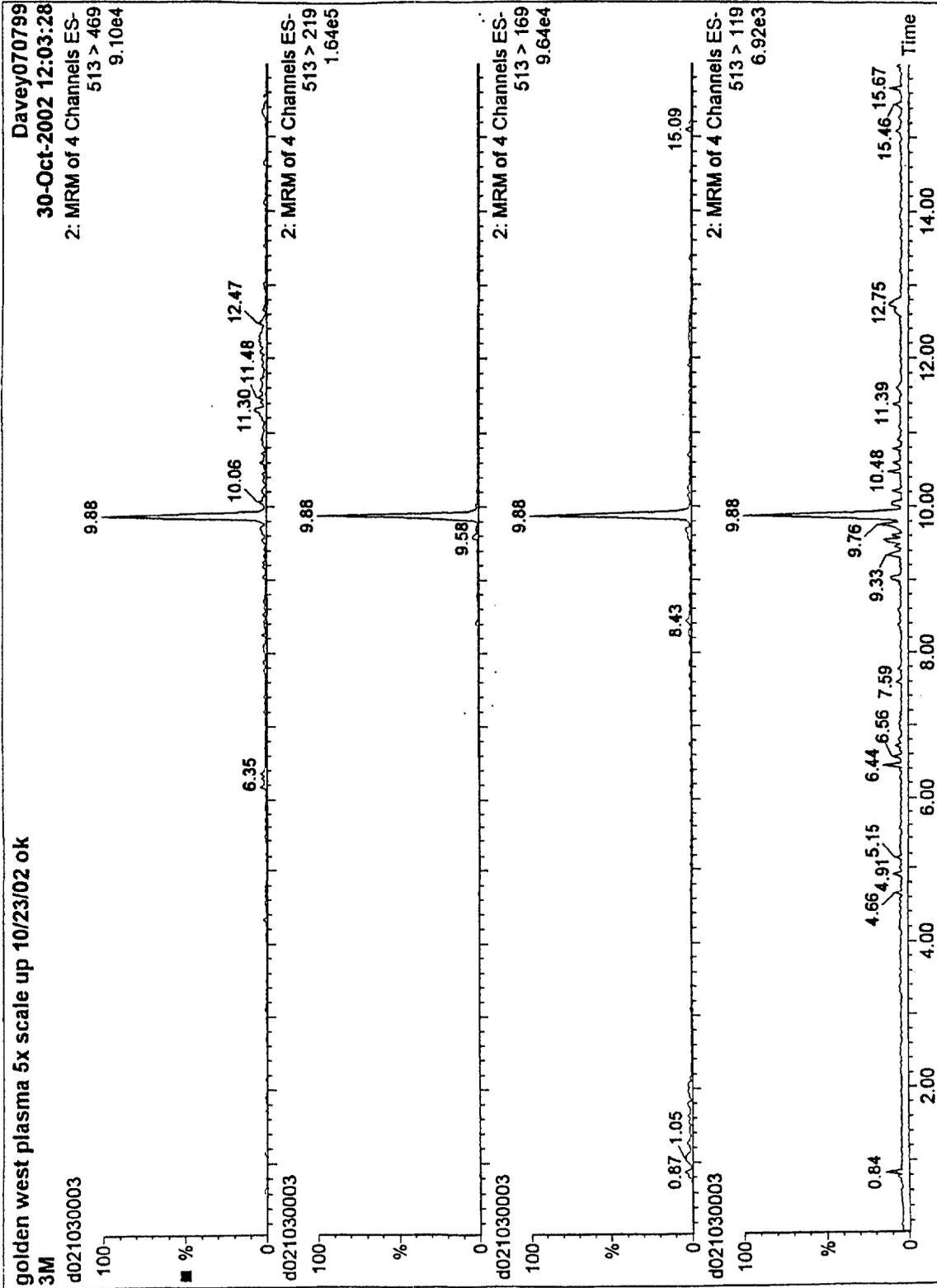


Exact Copy of Original

CM 10/31/02
Initial Date

000182

C10 ACID

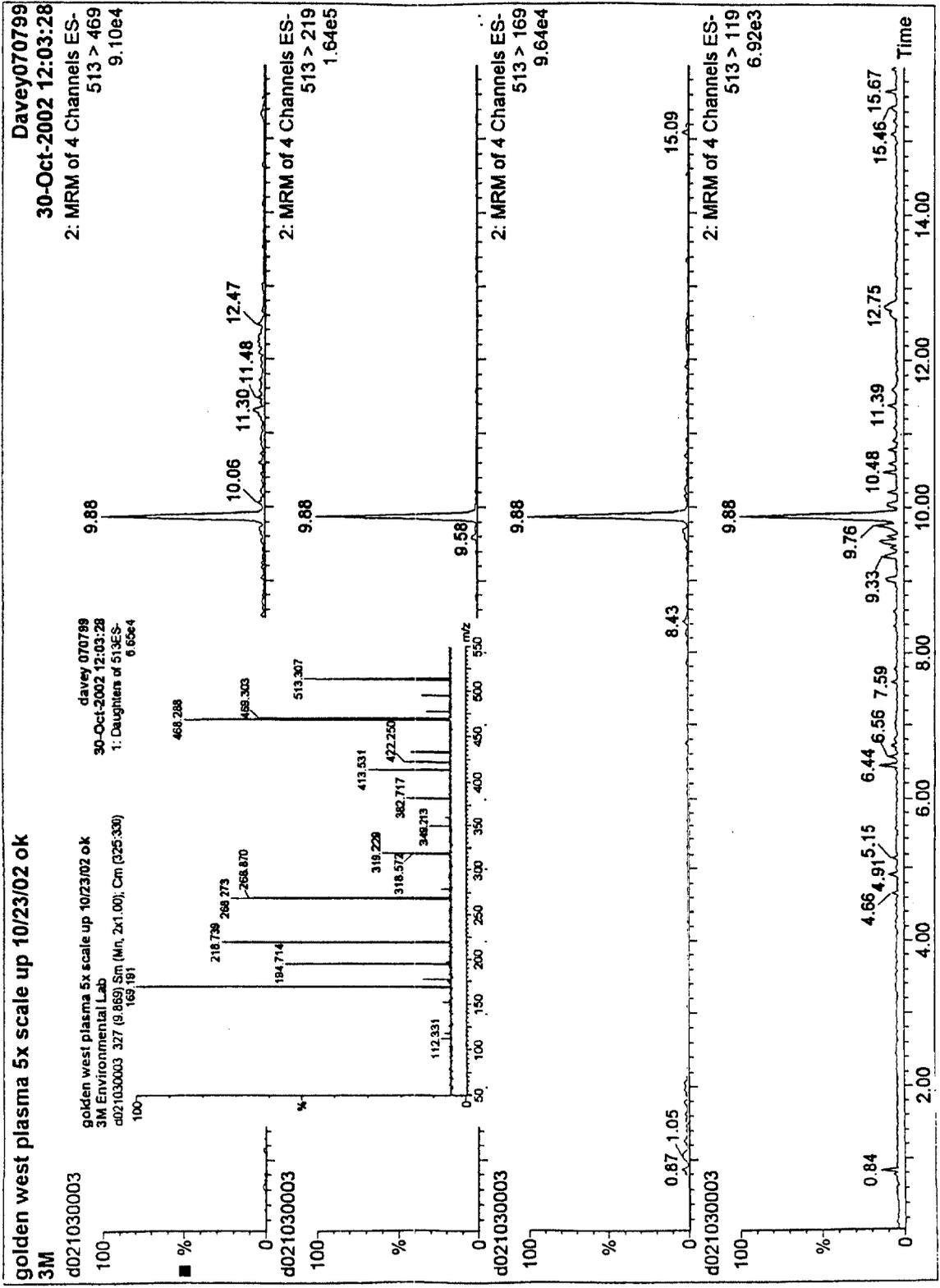


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Initial CMC Date 10/31/02

000183

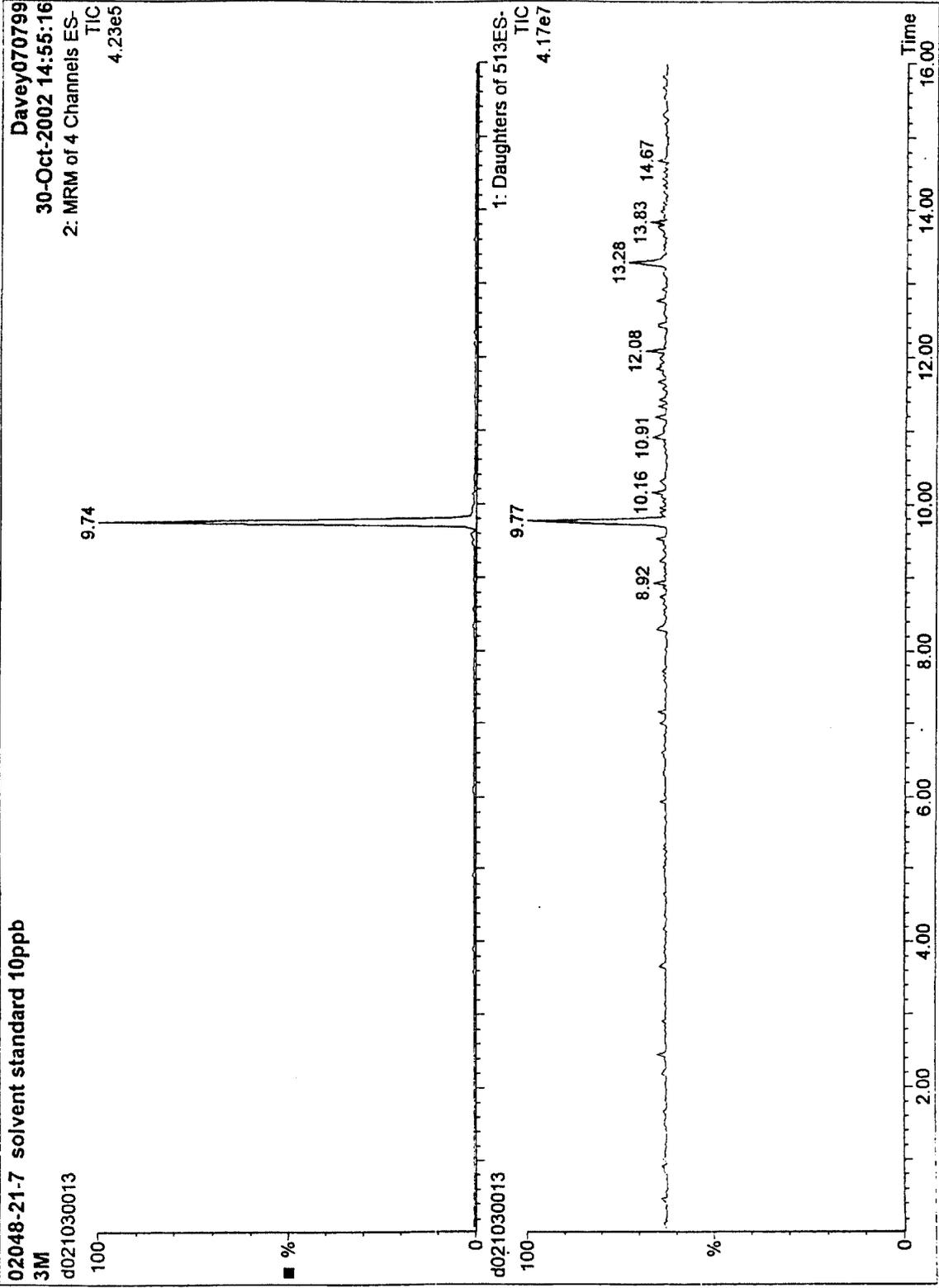
CID ACID



Exact Copy of Original
Initial CMC Date 10/31/02

000184

CID ACID

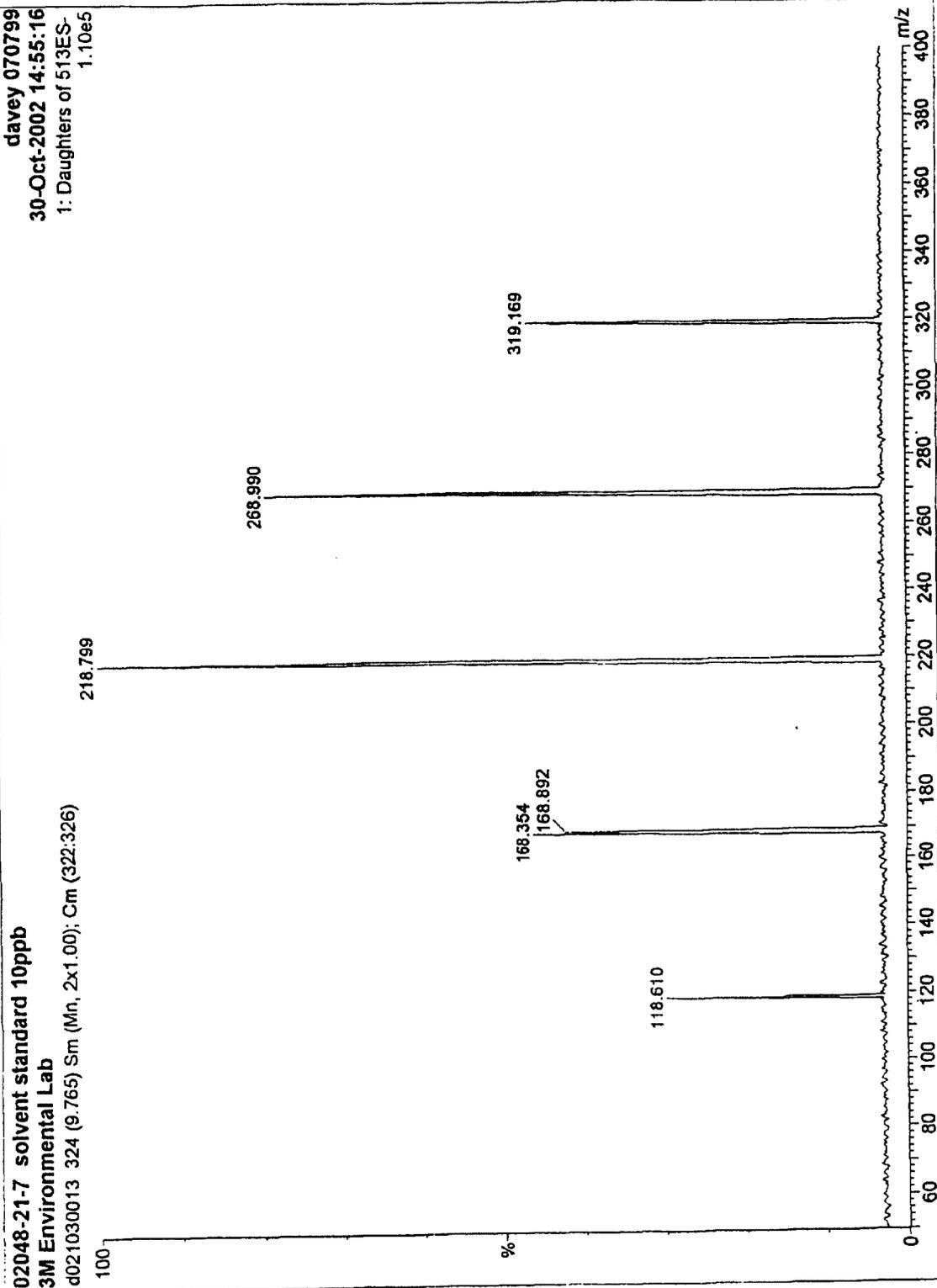


Exact Copy of Original

Initial CMK Date 11/1/02

000185

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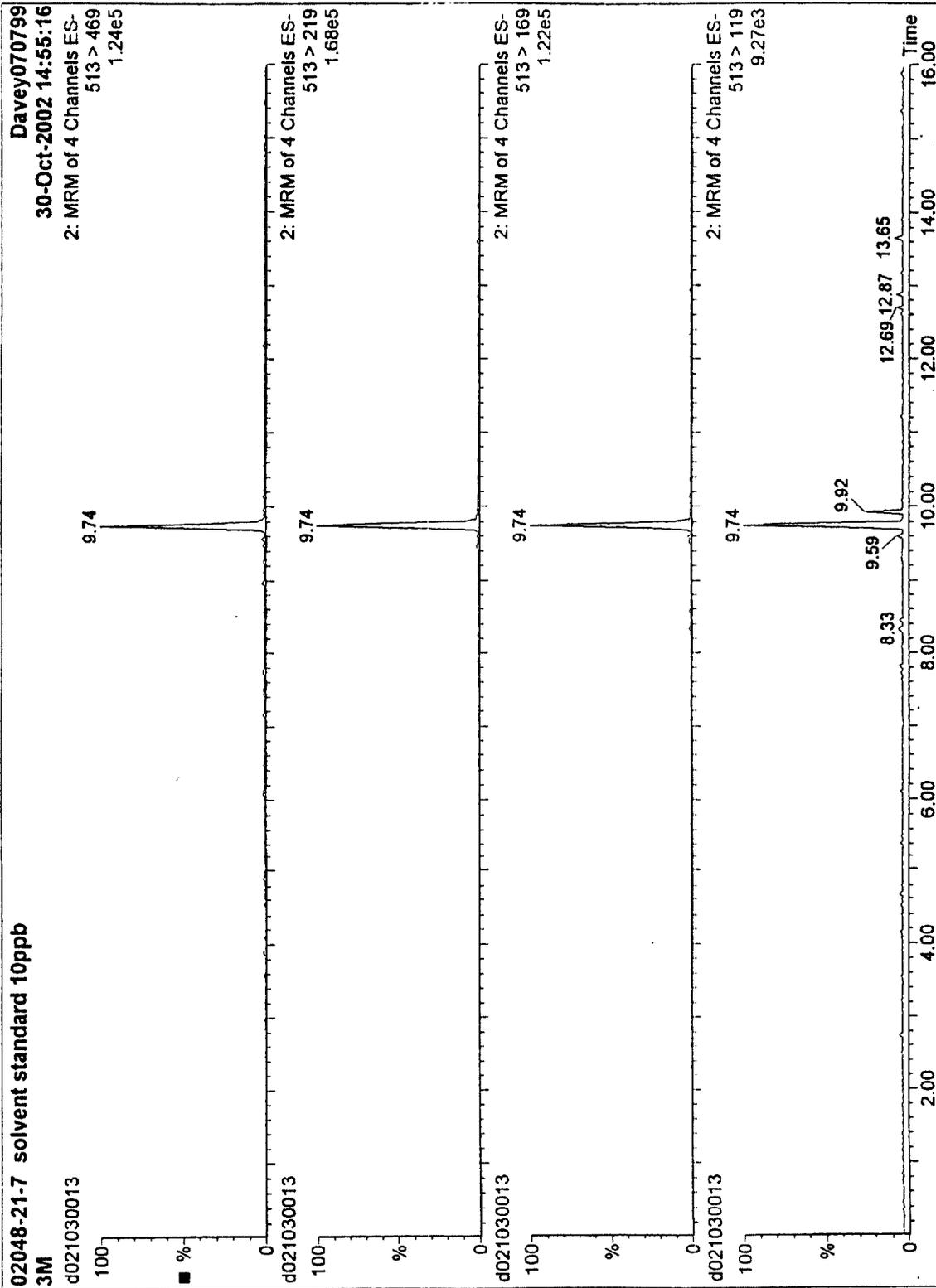


Exact Copy of Original:

Initial: CMC
Date: 11/1/02

000186

CID FID

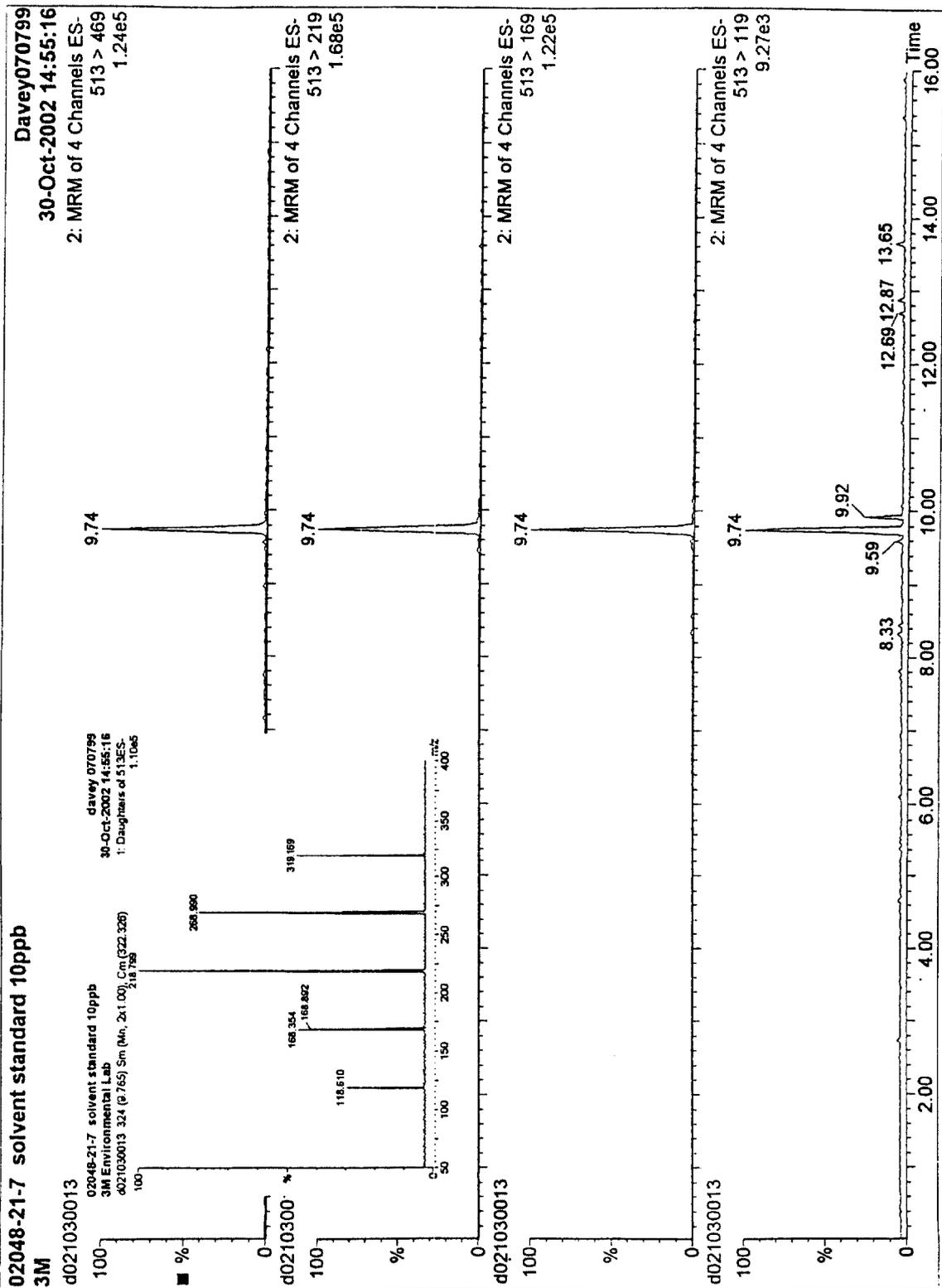


Exact Copy of Original

Initial WV/LB Date

000187

CID ACID

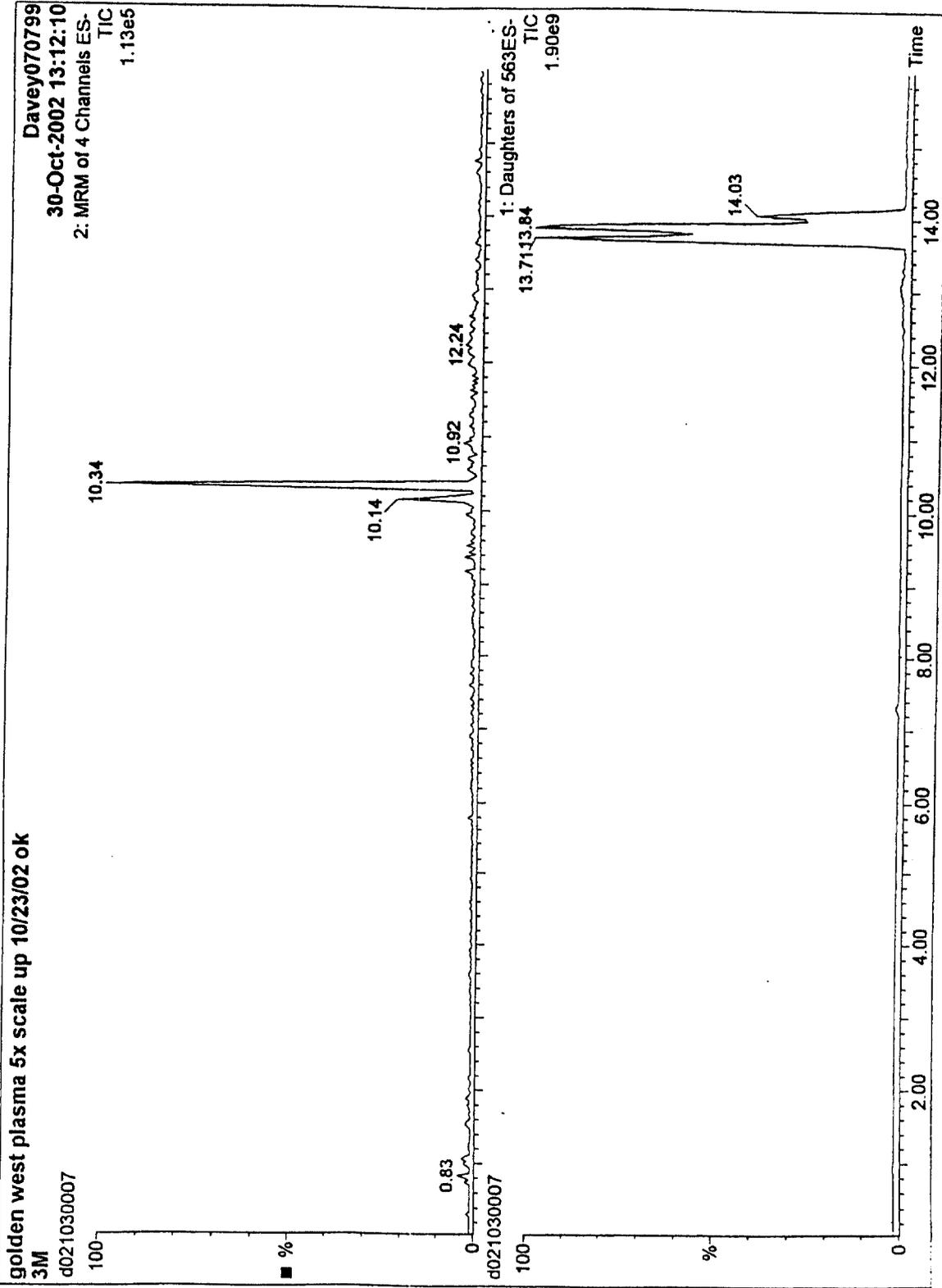


Exact Copy of Original

Initial WV Date 11/10/02

000188

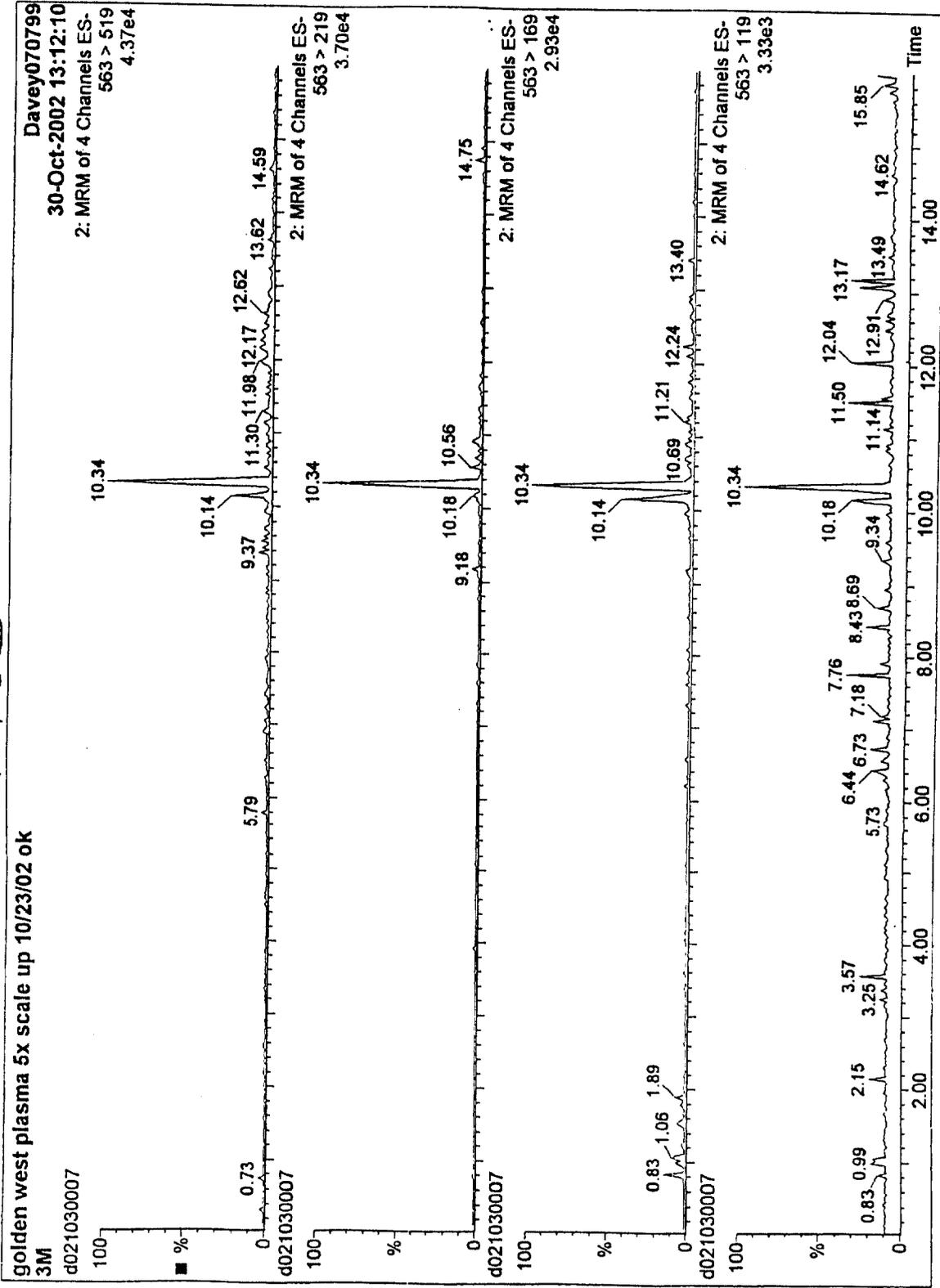
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Exact Copy of Original
Cmx / 10/31/02
Initial Date

000189

CII ACID

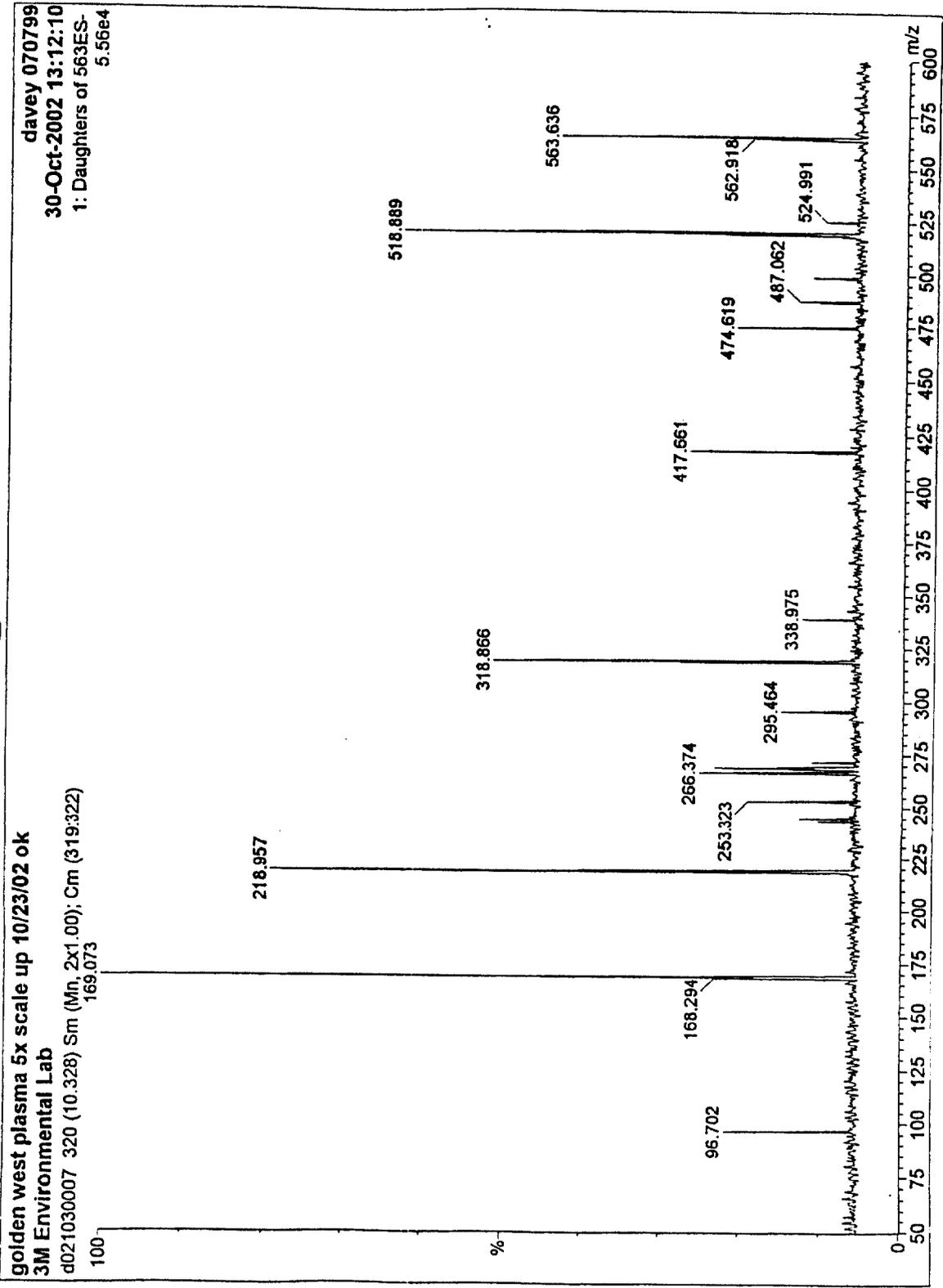


Exact Copy of Original

Initial CMC Date 10/31/02

000190

C11ACID

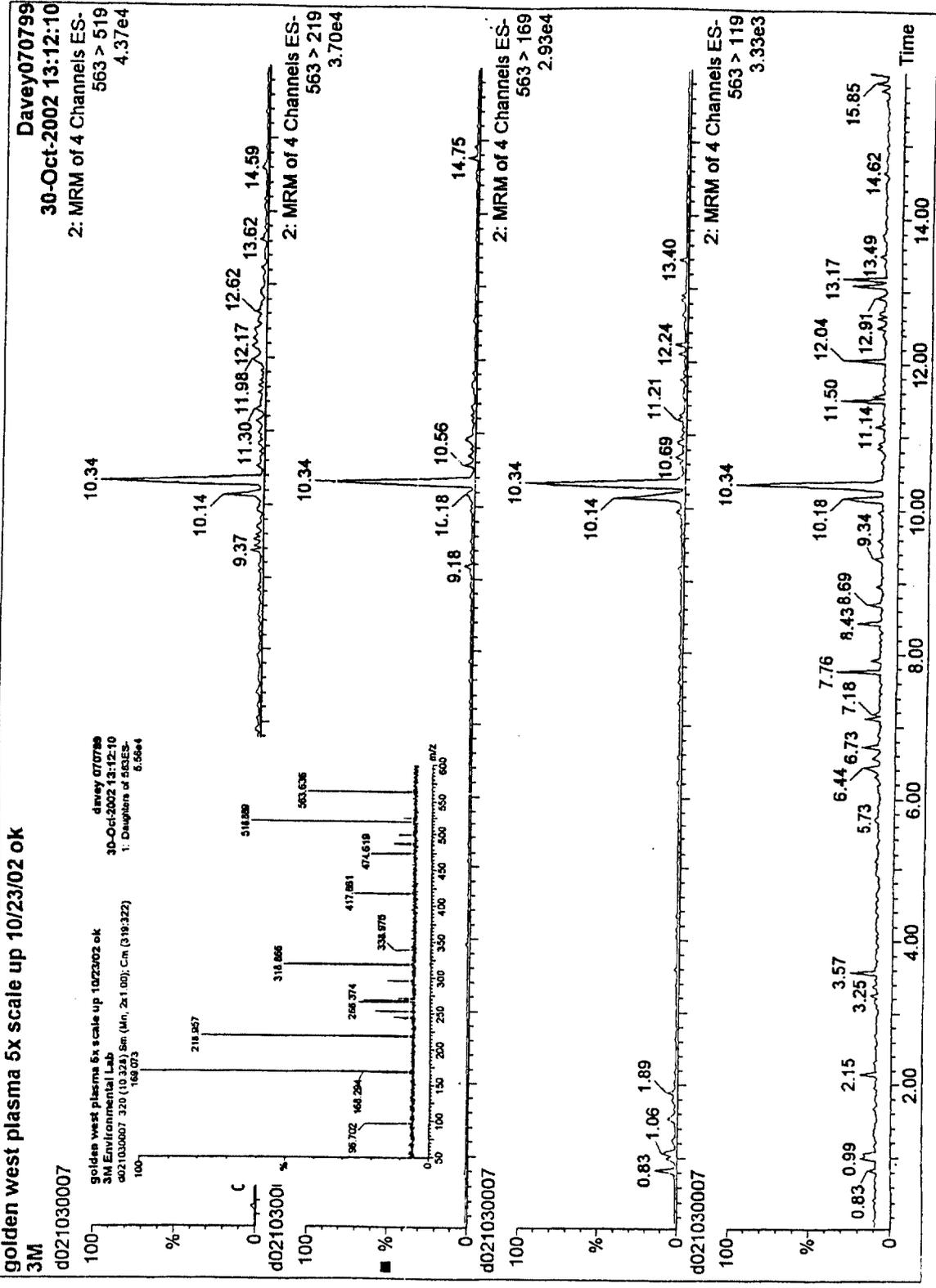


Exact Copy of Original

C11AC
Initial 10/31/02
Date

000191

Handwritten: CHL ACID



golden west plasma 5x scale up 10/23/02 ok

3M

d021030007

golden west plasma 5x scale up 10/23/02 ok

3M Environmental Lab

30-Oct-2002 13:12:10

1: Daughters of 563ES- 5.56e4

davey 070799

30-Oct-2002 13:12:10

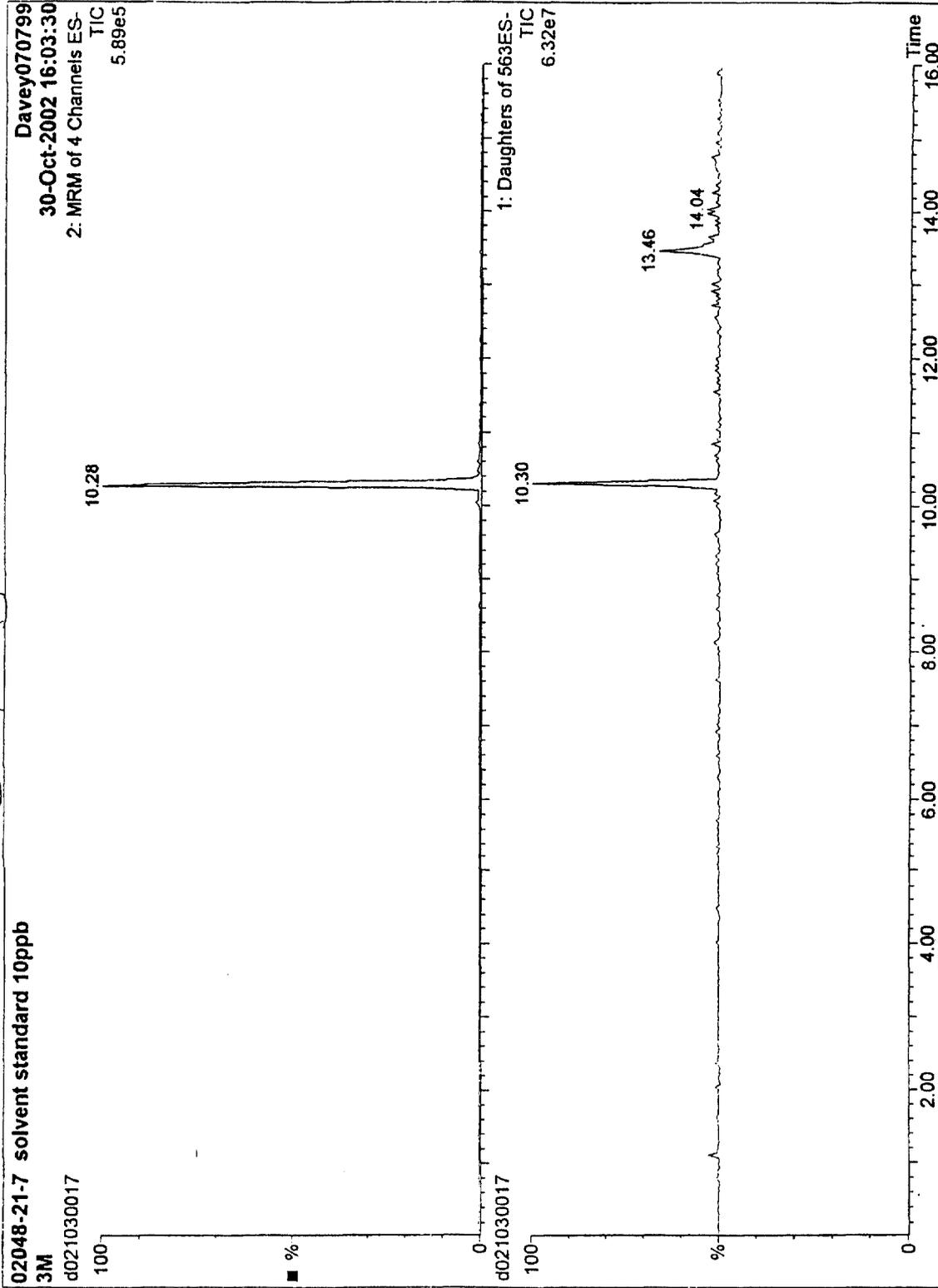
1: Daughters of 563ES- 5.56e4

Exact Copy of Original

Handwritten: CHL
Initial Date

000192

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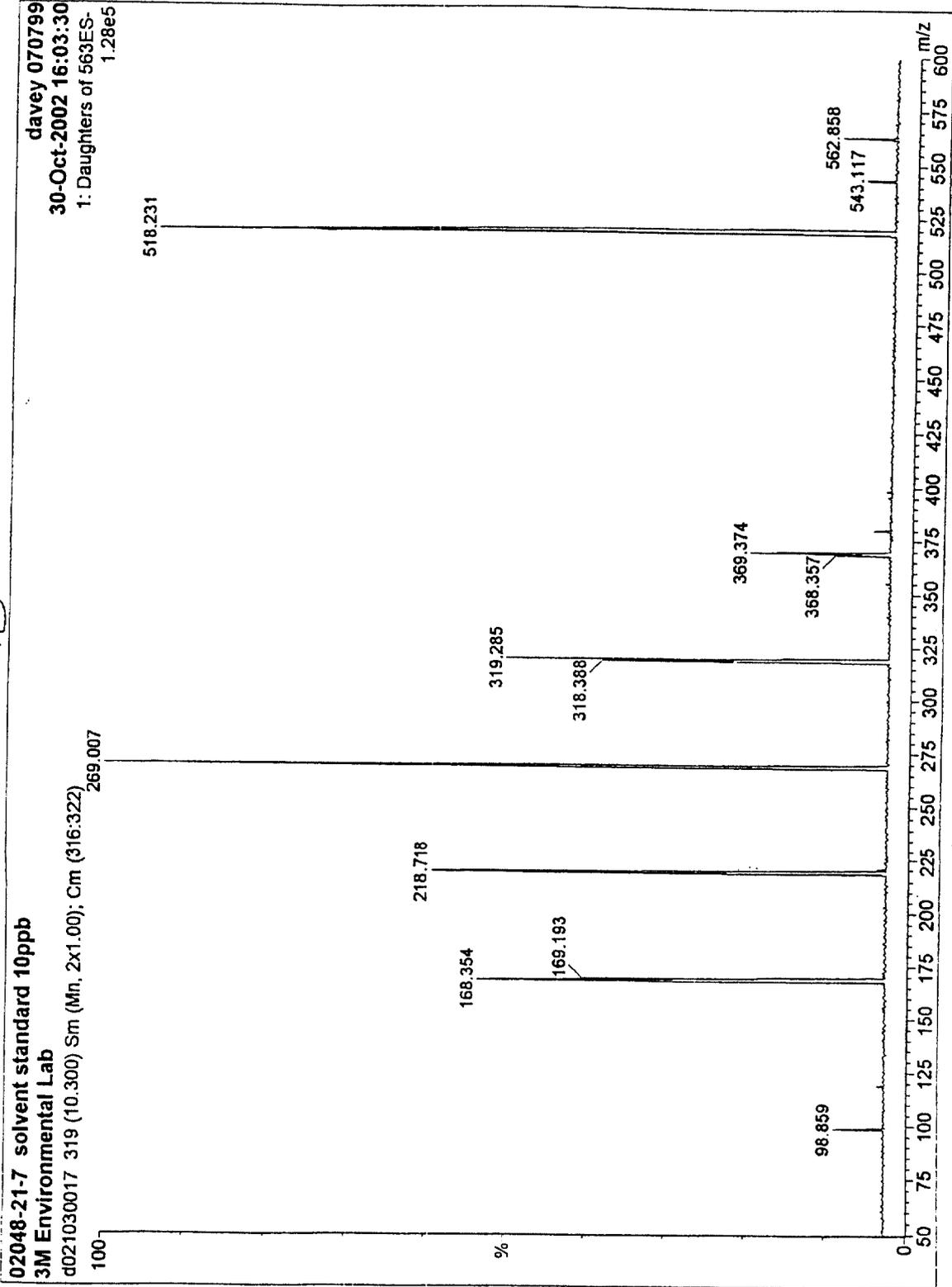


Exact Copy of Original

Initial Date

000193

CII ACID



02048-21-7 solvent standard 10ppb
3M Environmental Lab
d021030017 319 (10.300) Sm (Mn, 2x1.00); Cm (316:322)
1: Daughters of 563ES-1.28e5

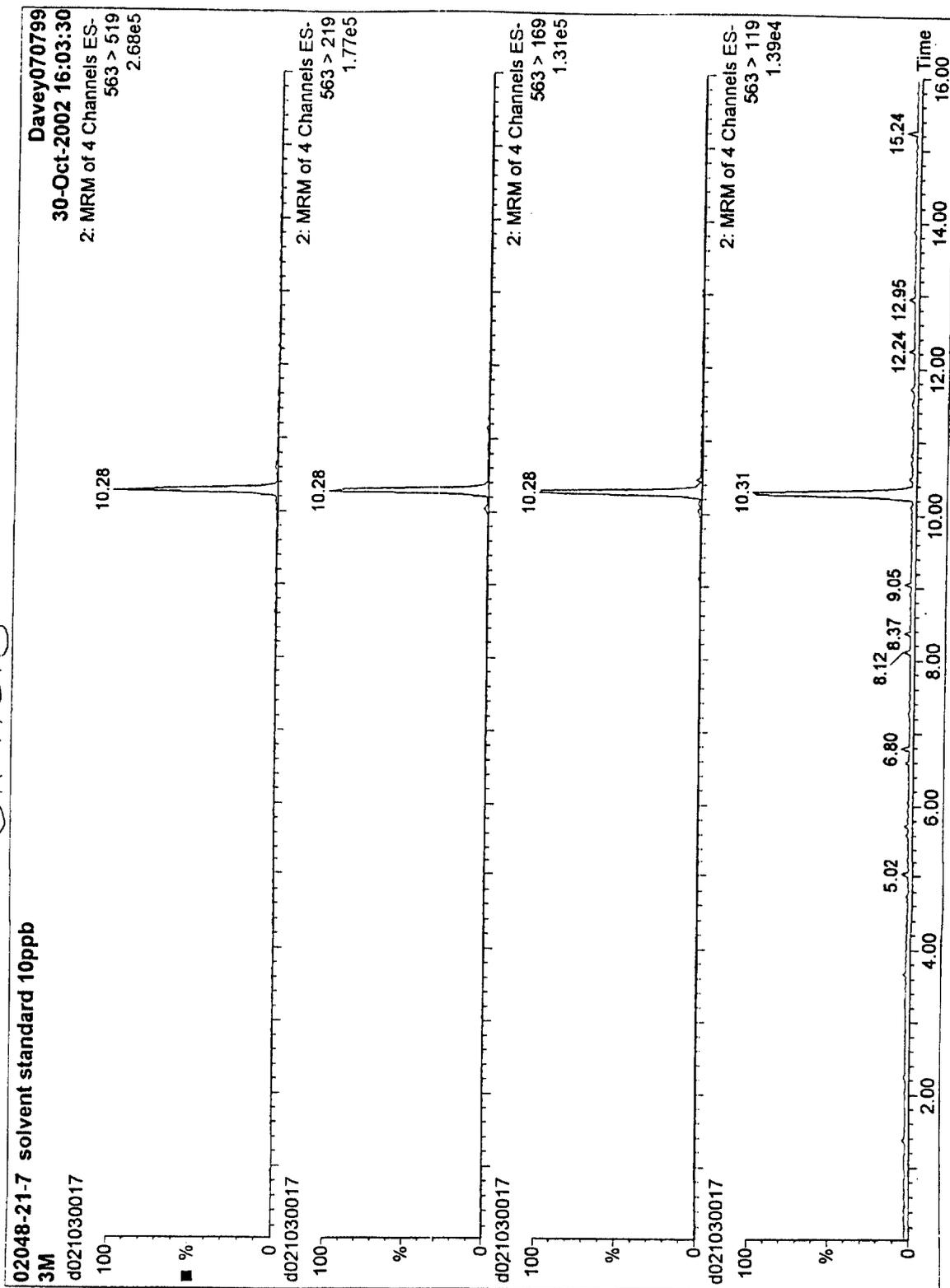
davey 070799
30-Oct-2002 16:03:30

Exact Copy of Original

CMC Initial Date
11/1/02

000194

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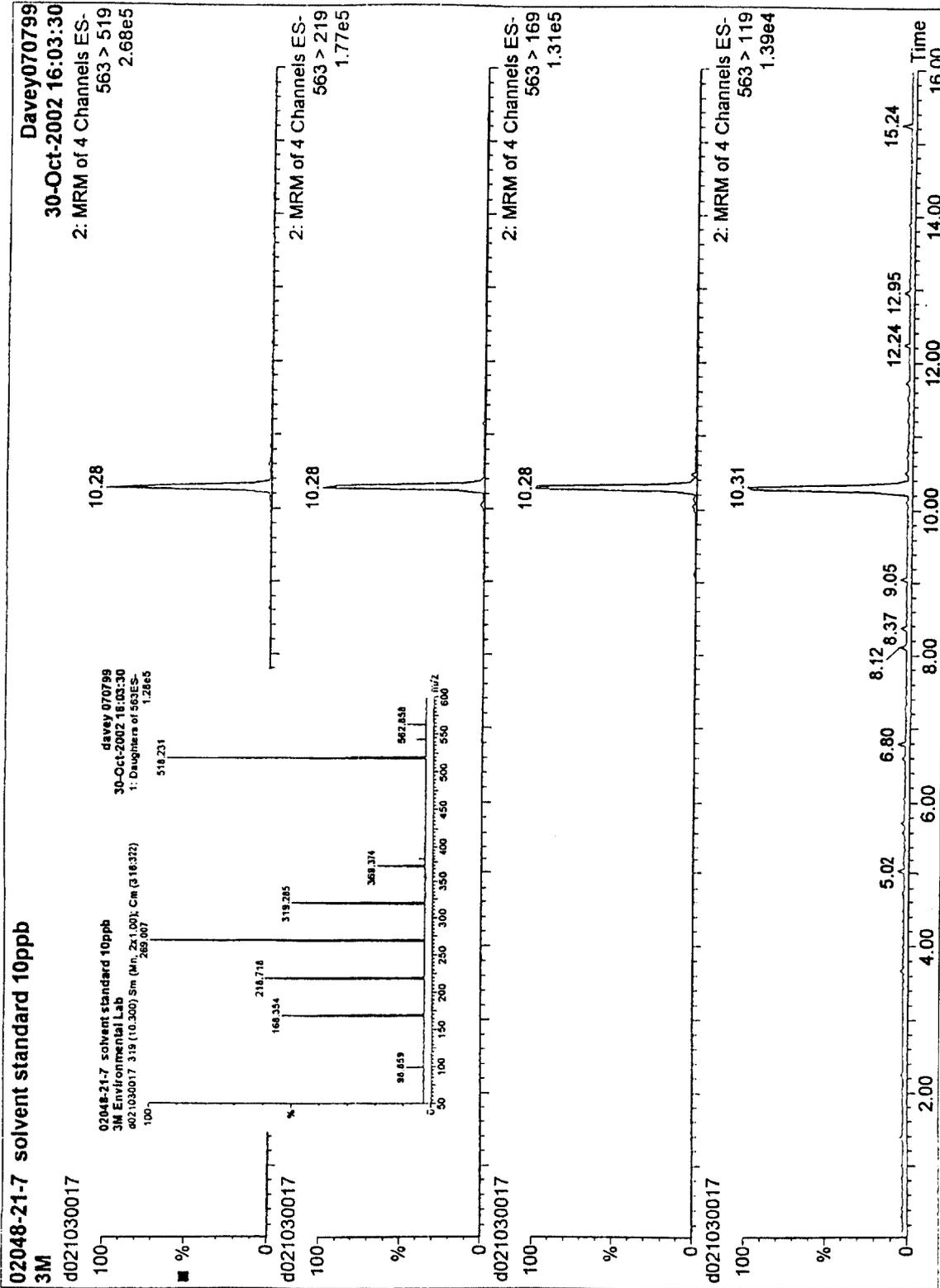


Exact Copy of Original

Initial CAC Date 11/1/02

000195

CII ACID

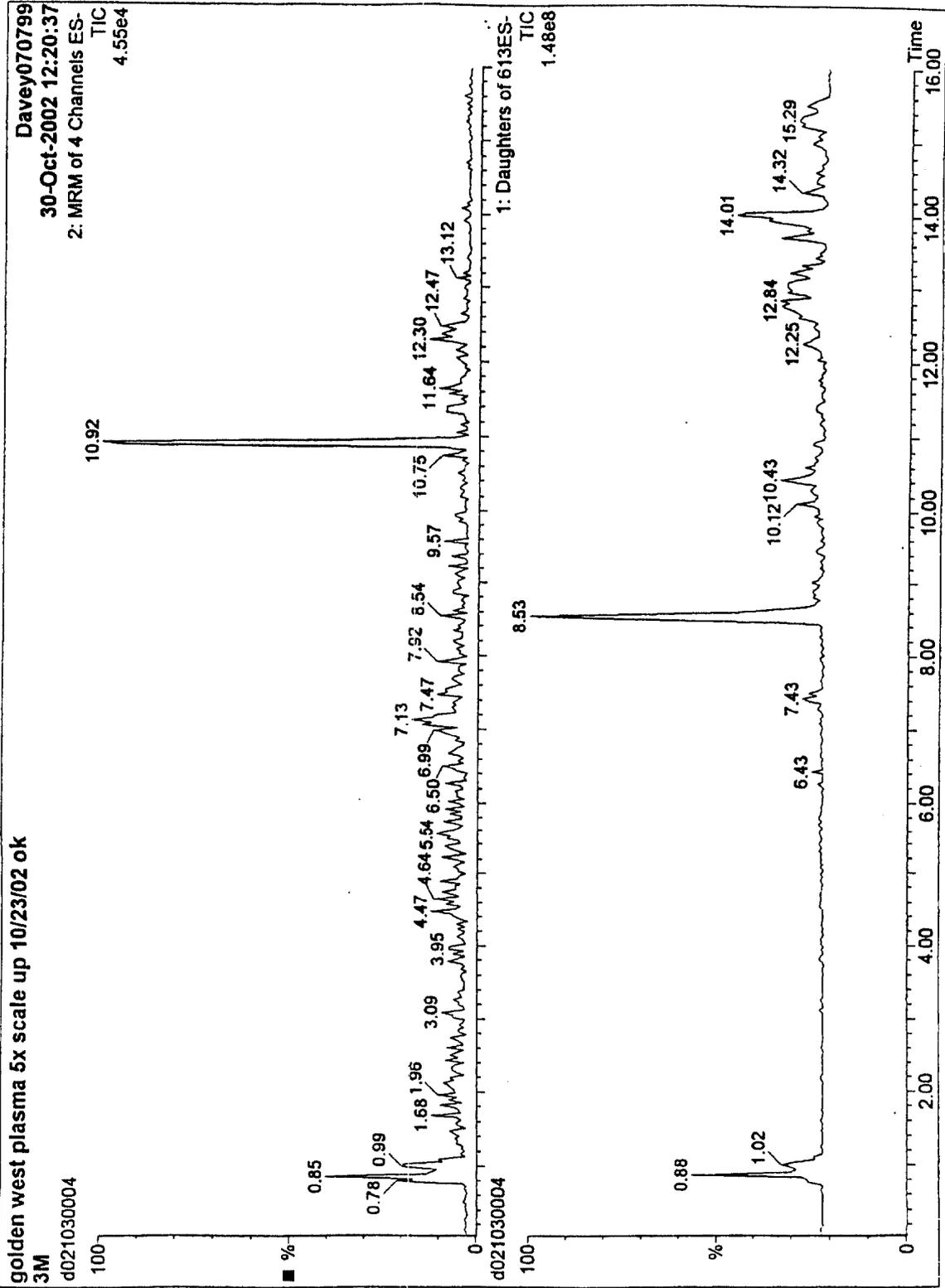


Exact Copy of Original:

CM
Initial
11/10/02
Date

000196

C12 HCl P

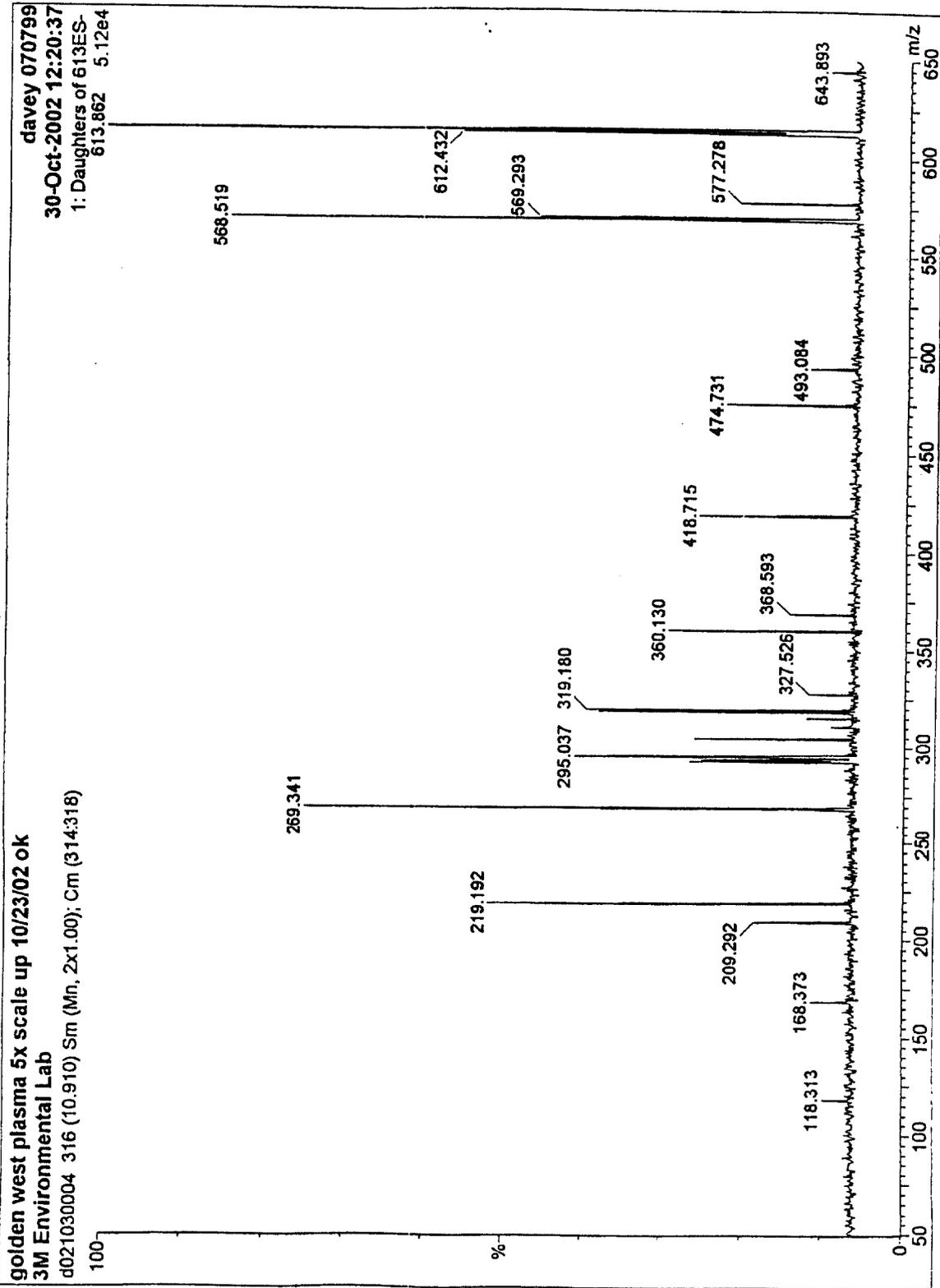


Exact Copy of Original

Initial Date
CWX 10/31/02

000197

Cl2 Acid

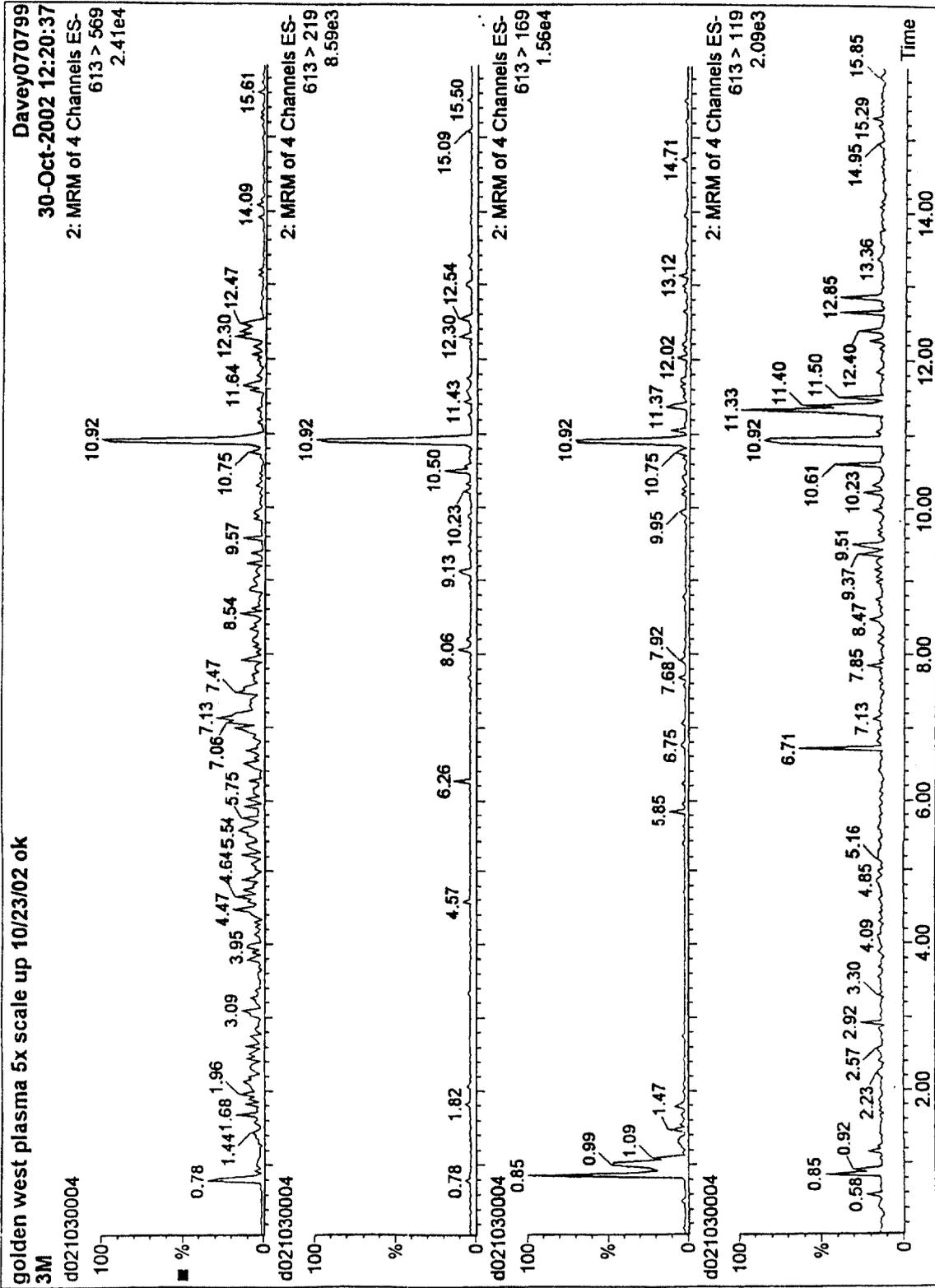


Exact Copy of Original

CDL
Initial *10/31/02*
Date

000198

C12 ACID

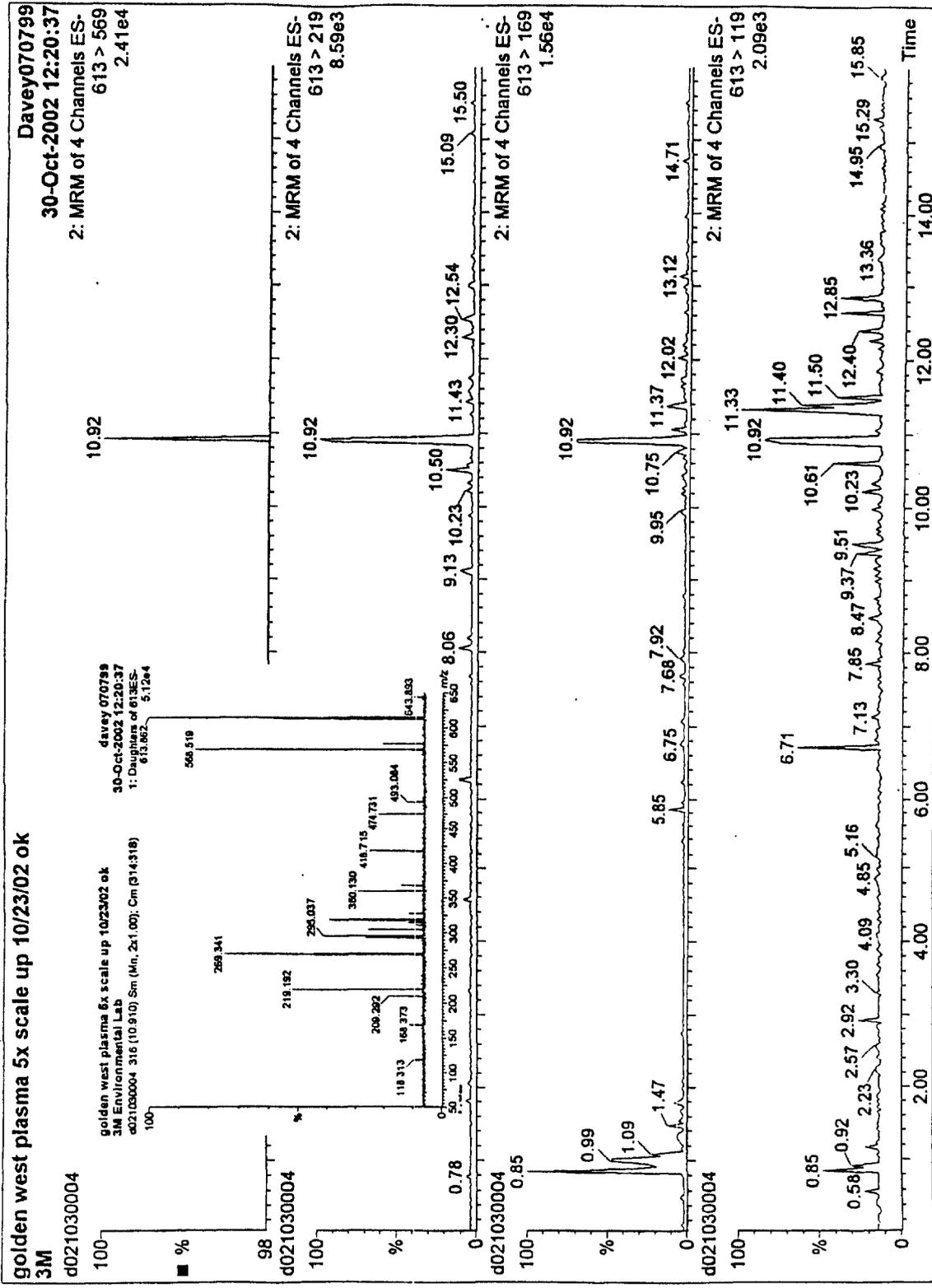


Exact Copy of Original

Initial Date
CML 10/31/02

000199

Cl2 ACID

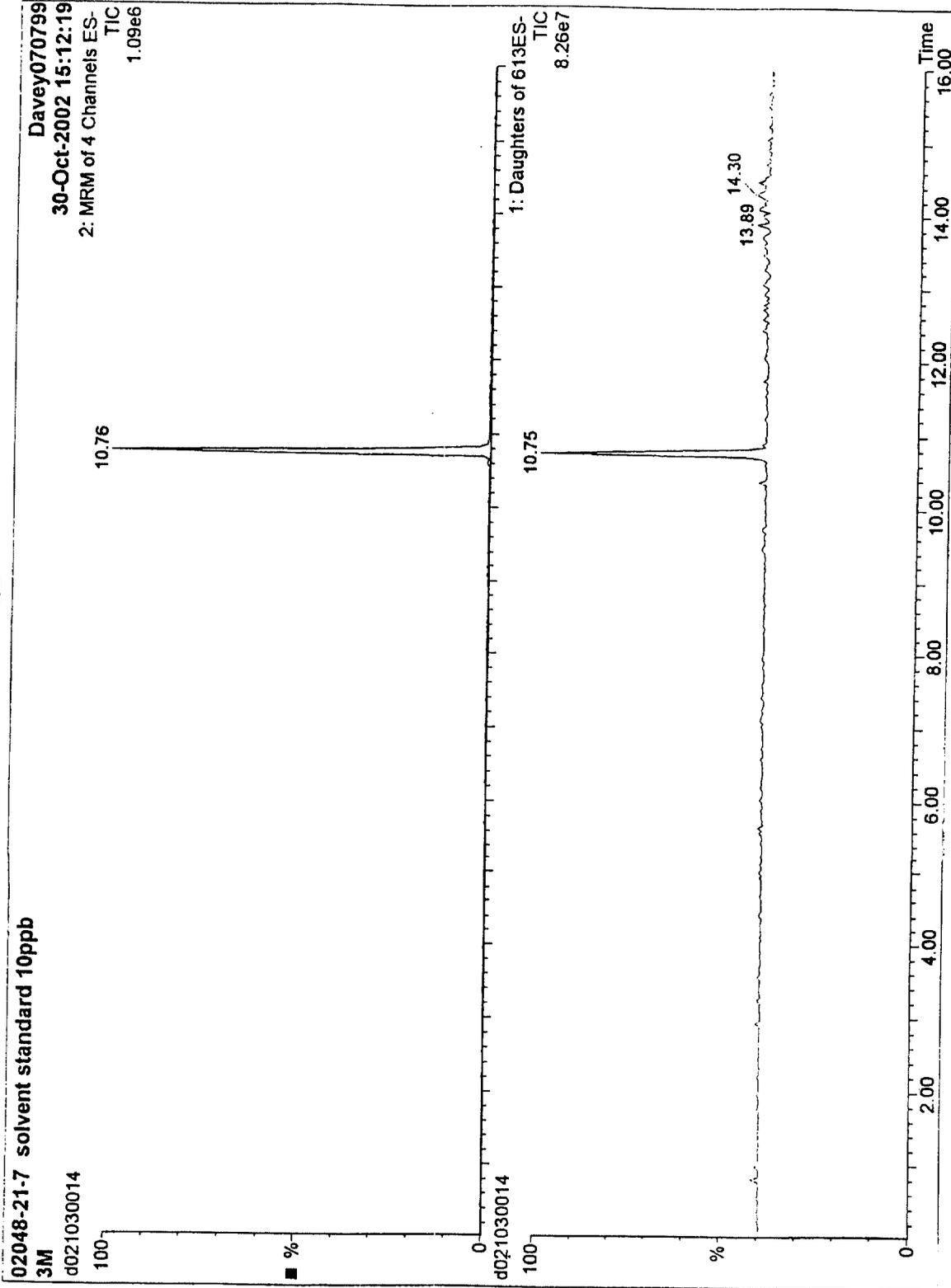


Exact Copy of Original

SMC 10/31/02
Initial Date

000200

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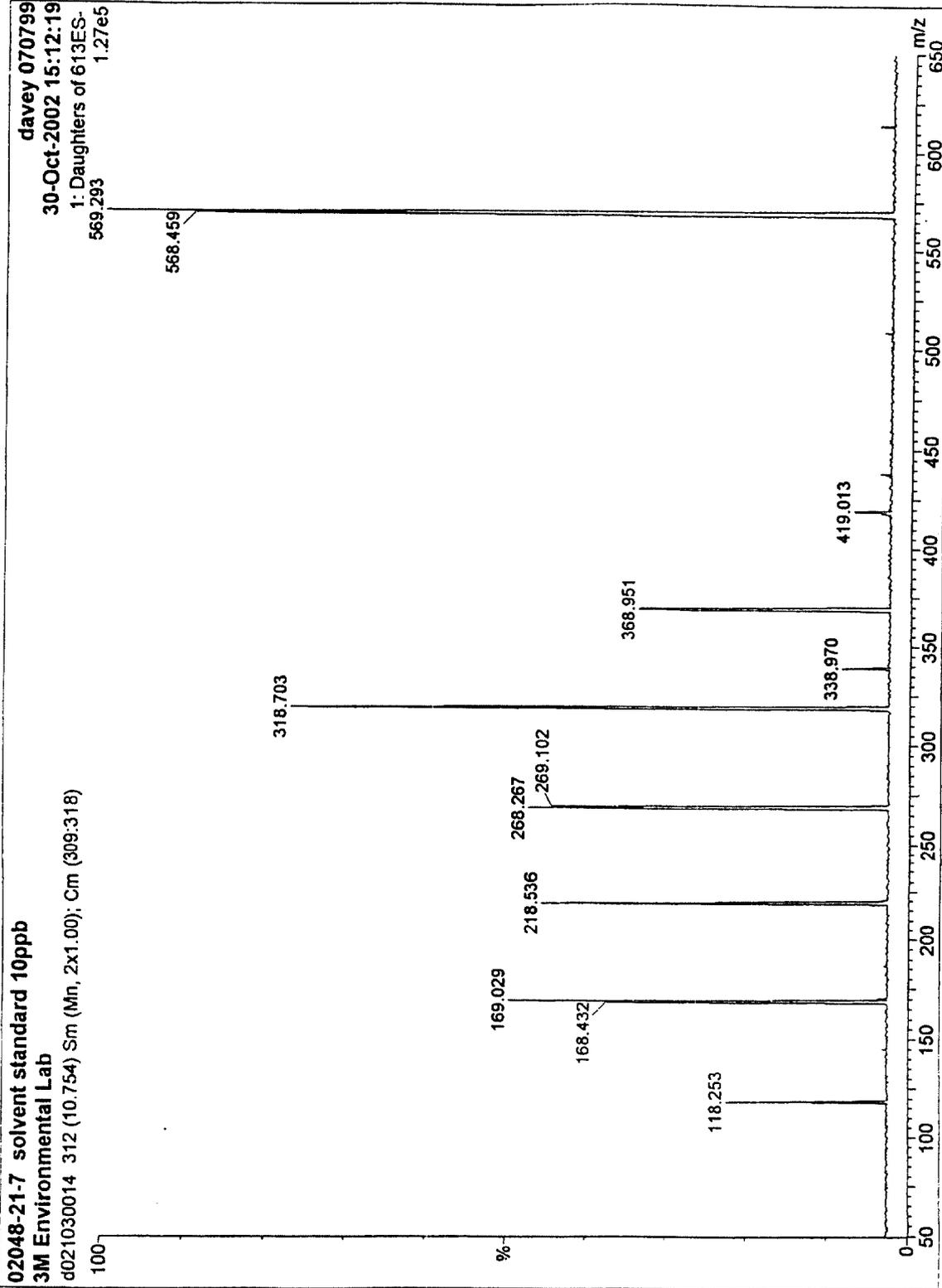


Exact Copy of Original

Initial Date
CM 11/10

000201

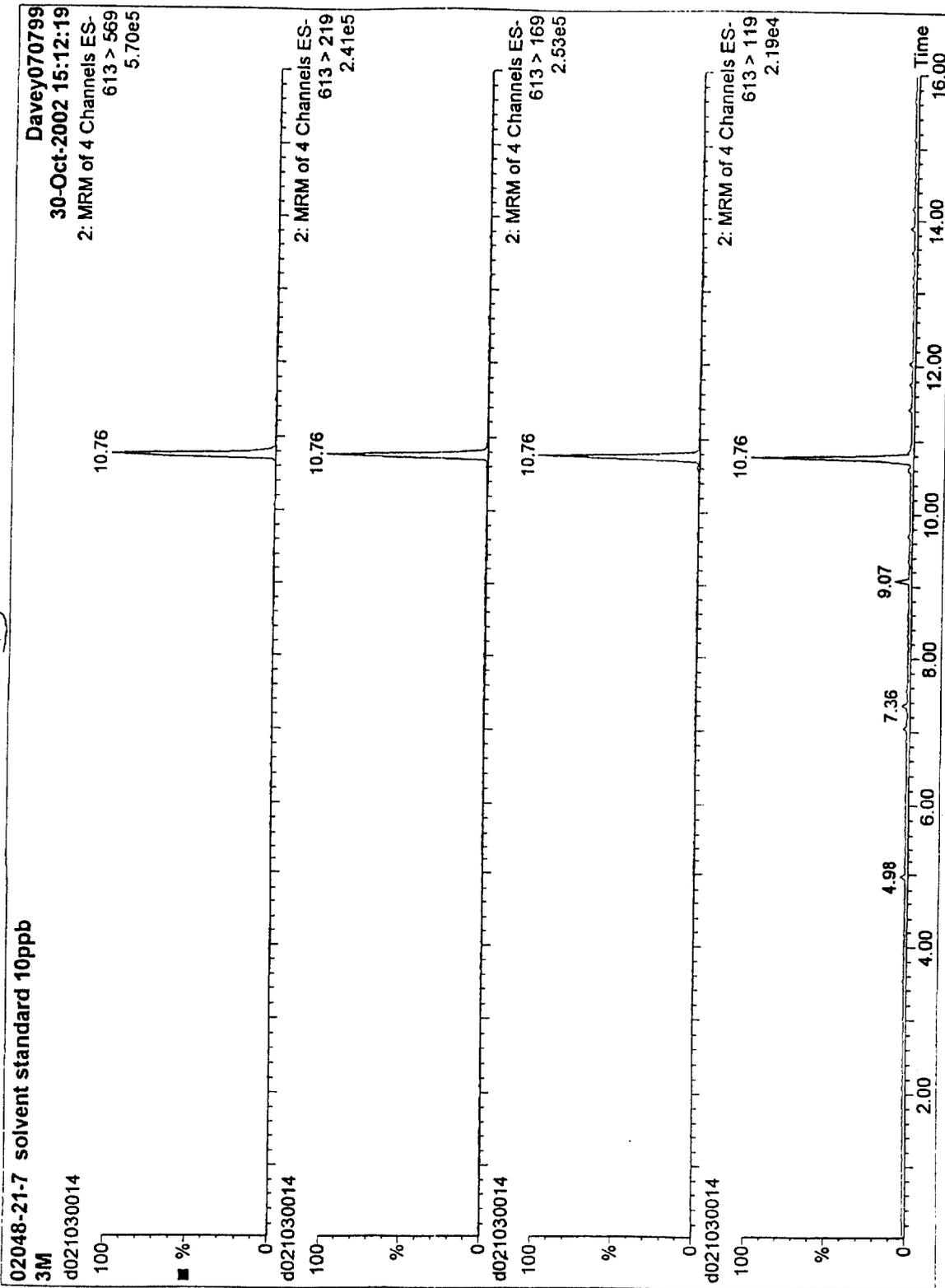
Cla Acid



Exact Copy of Original
CMC *WV/LR*
initial Date

000202

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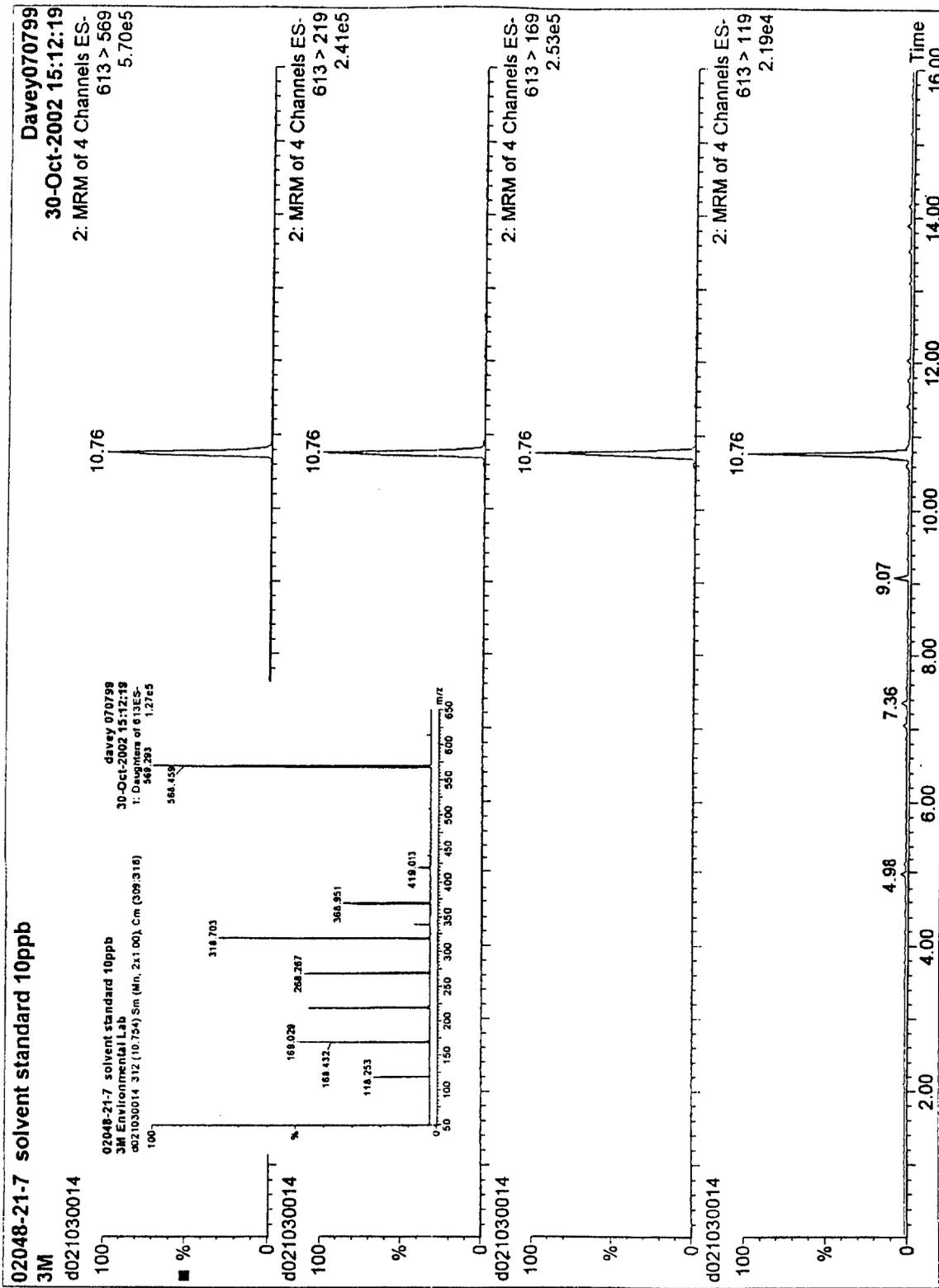


Exact Copy of Original

CMC 11/10
Initial Date

000203

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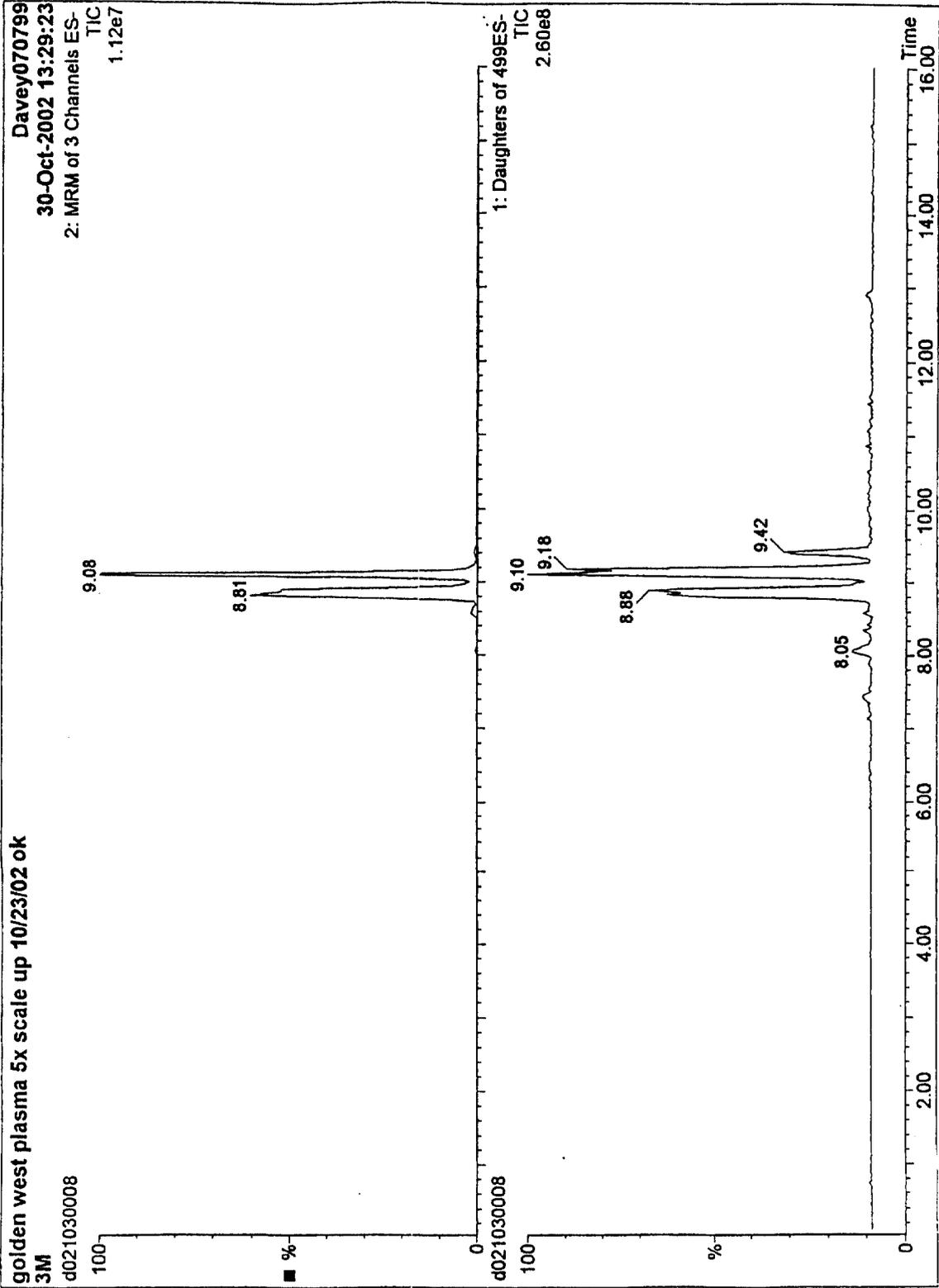


Exact Copy of Original:

Initial LL/LD Date

000204

PFOS

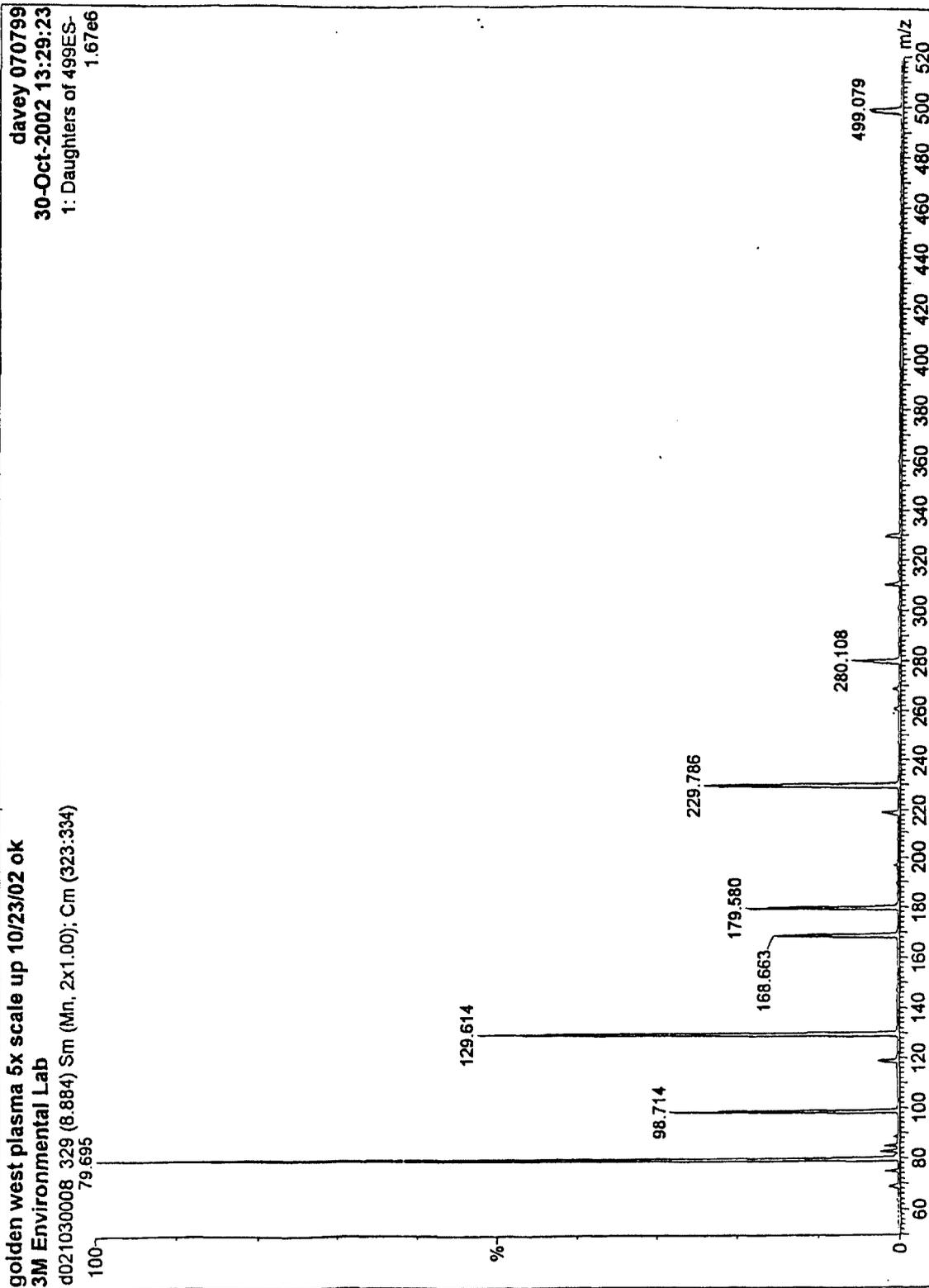


Exact Copy of Original

Initial CLM Date 10/31/02

000205

PFS

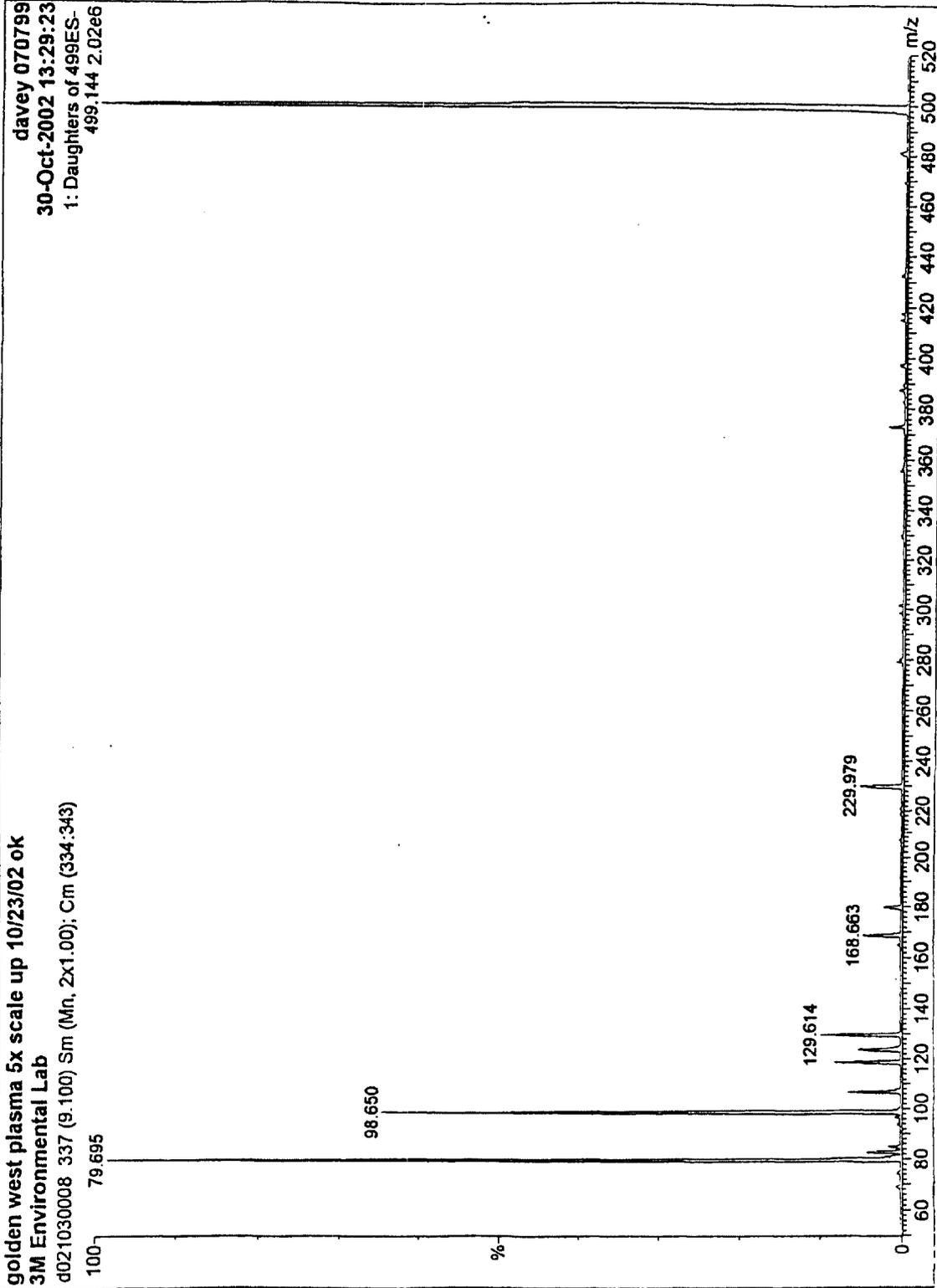


Exact Copy of Original

Initial CM Date 10/31/02

000206

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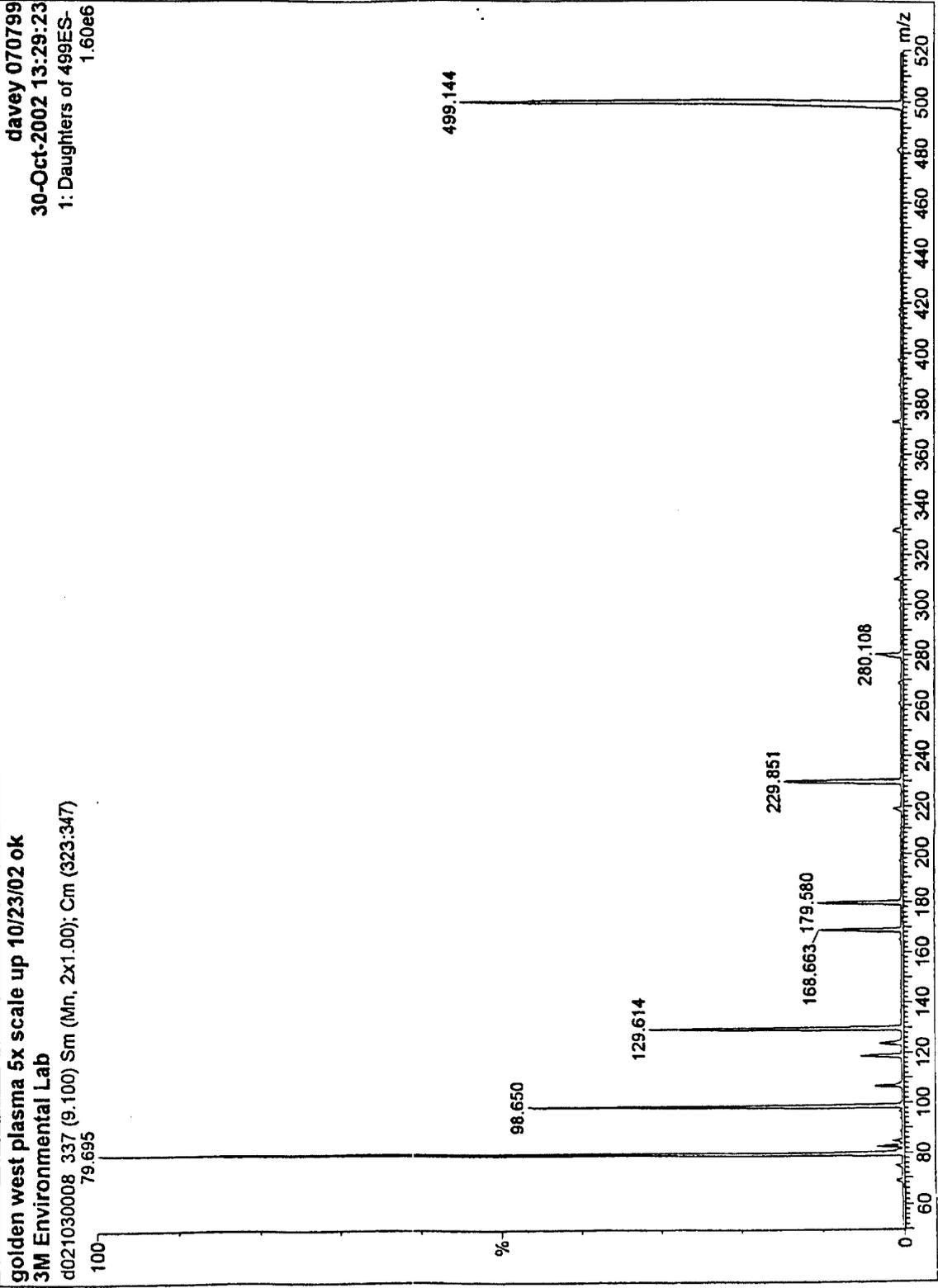


Exact Copy of Original

CMC 10/31/02
Initial Date

000207

PFOS



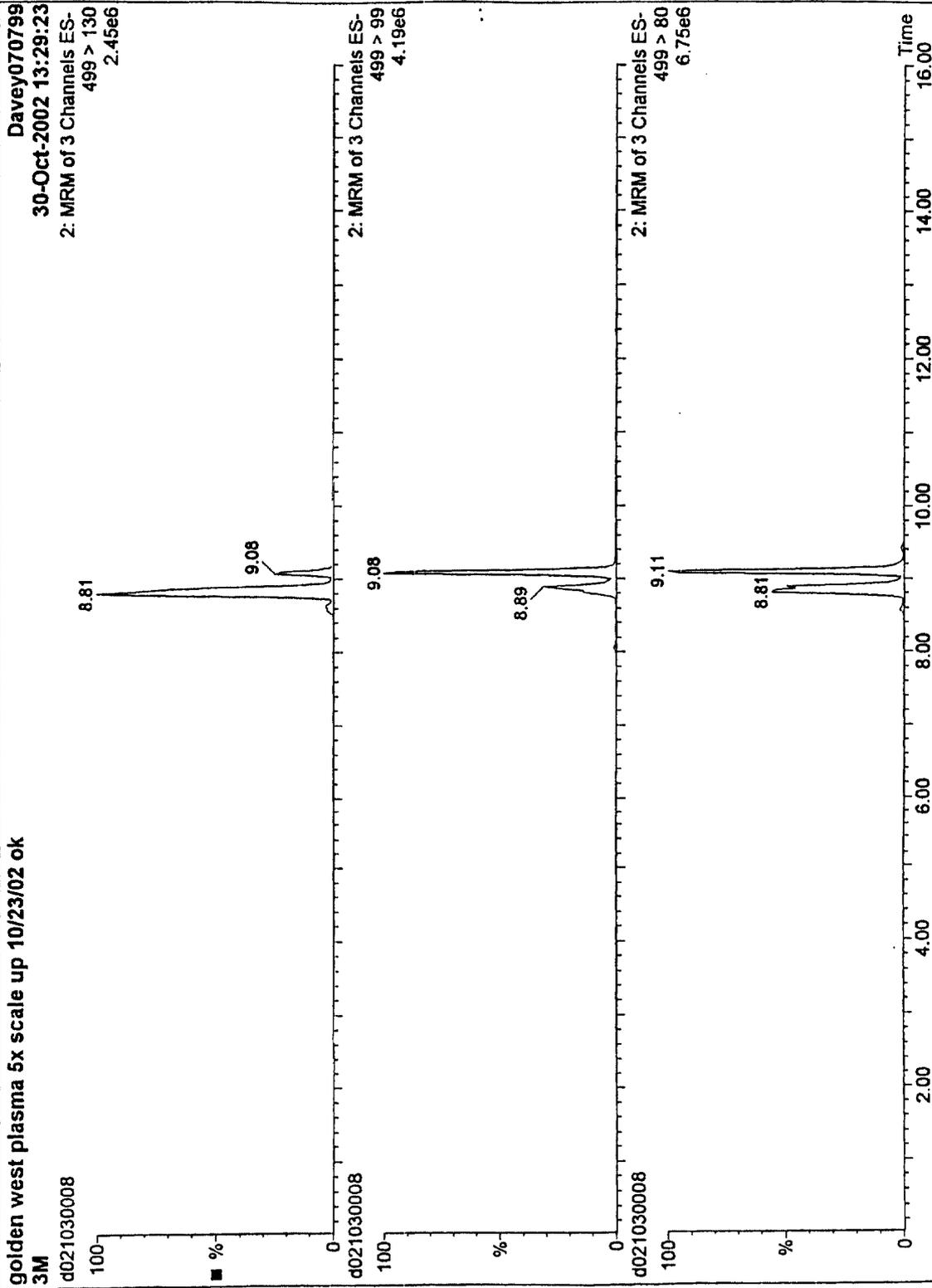
davey 070799
30-Oct-2002 13:29:23
1: Daughters of 499ES-

Exact Copy of Original

Initial Date
CAN 10/31/02

000208

PFS

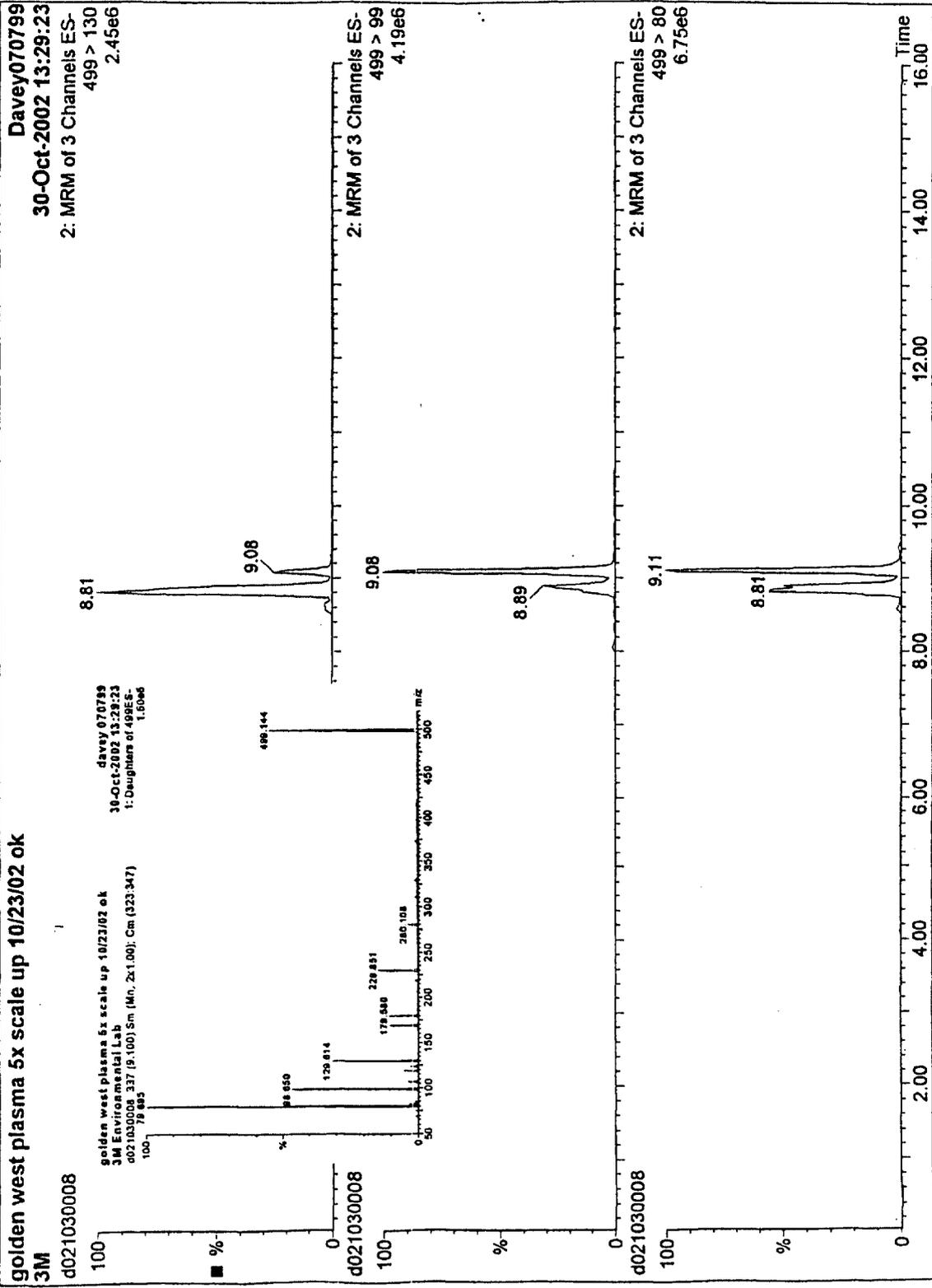


Exact Copy of Original

Initial CAK Date 10/31/02

000209

PFOS

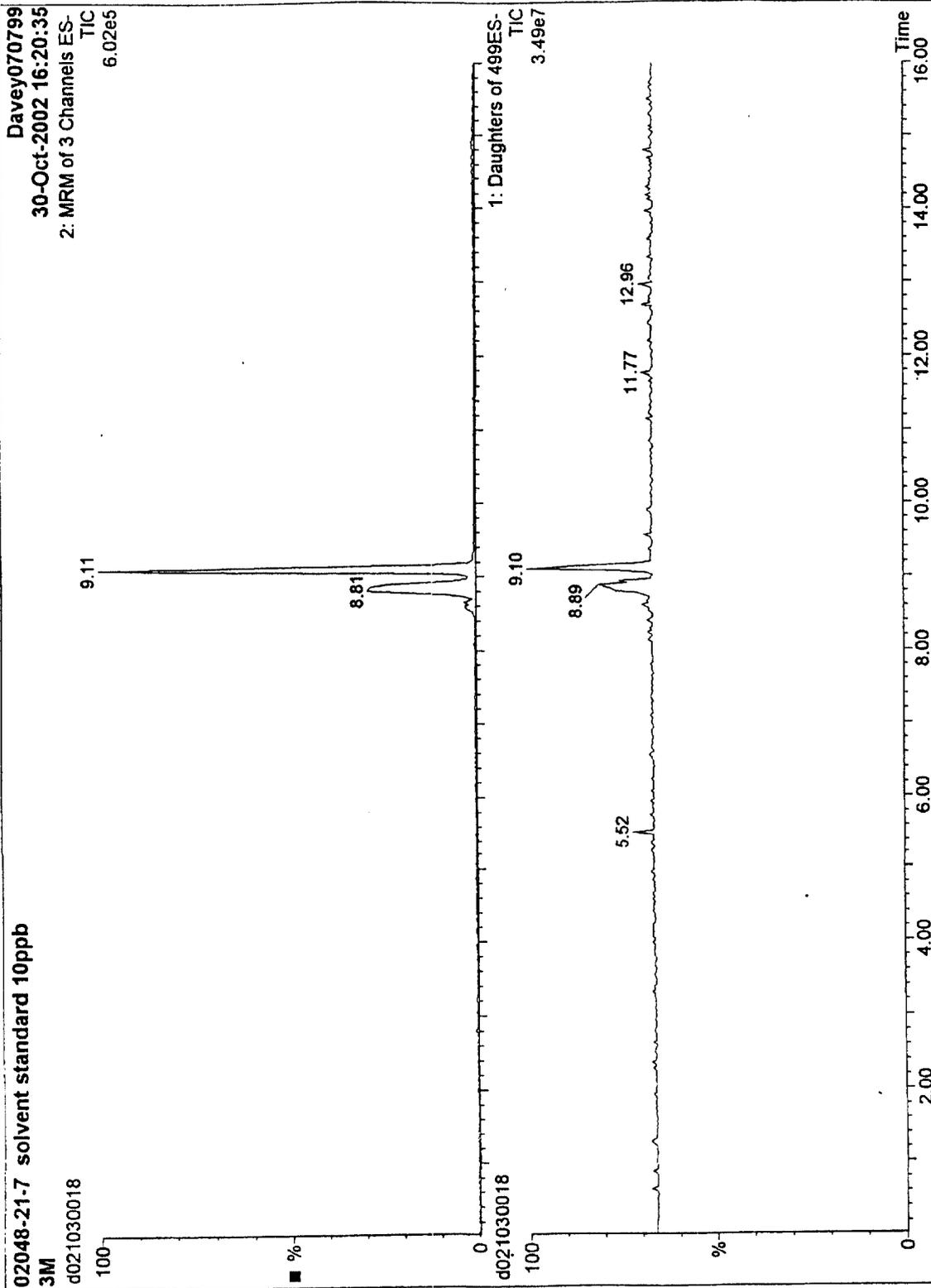


Exact Copy of Original

Initial Date
CAN 10/31/02

C00210

FOS

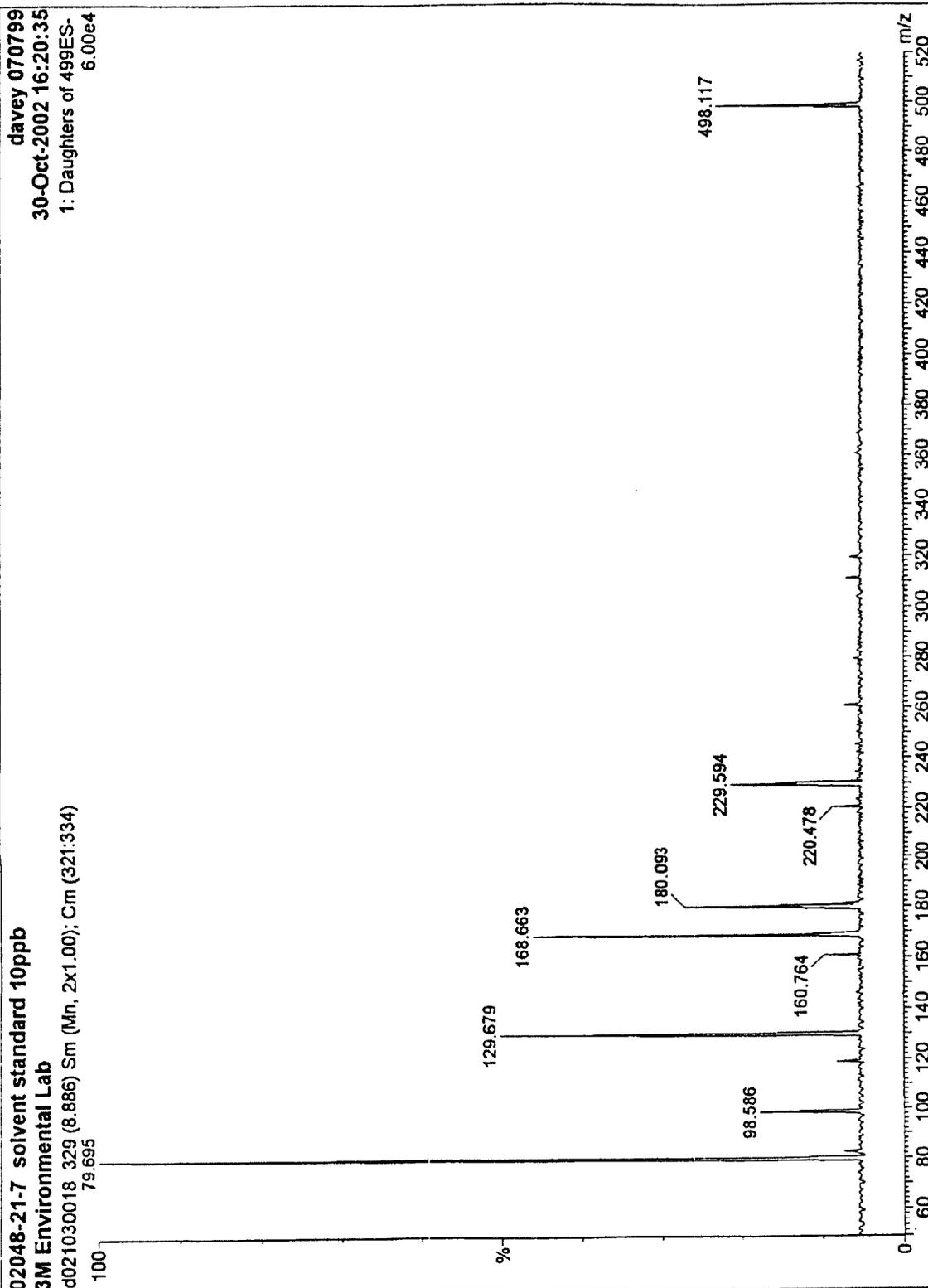


Exact Copy of Original

Initial: CAN
Date: 11/11/02

000211

PFOS



davey 070799
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1: Daughters of 499ES-
6.00e4

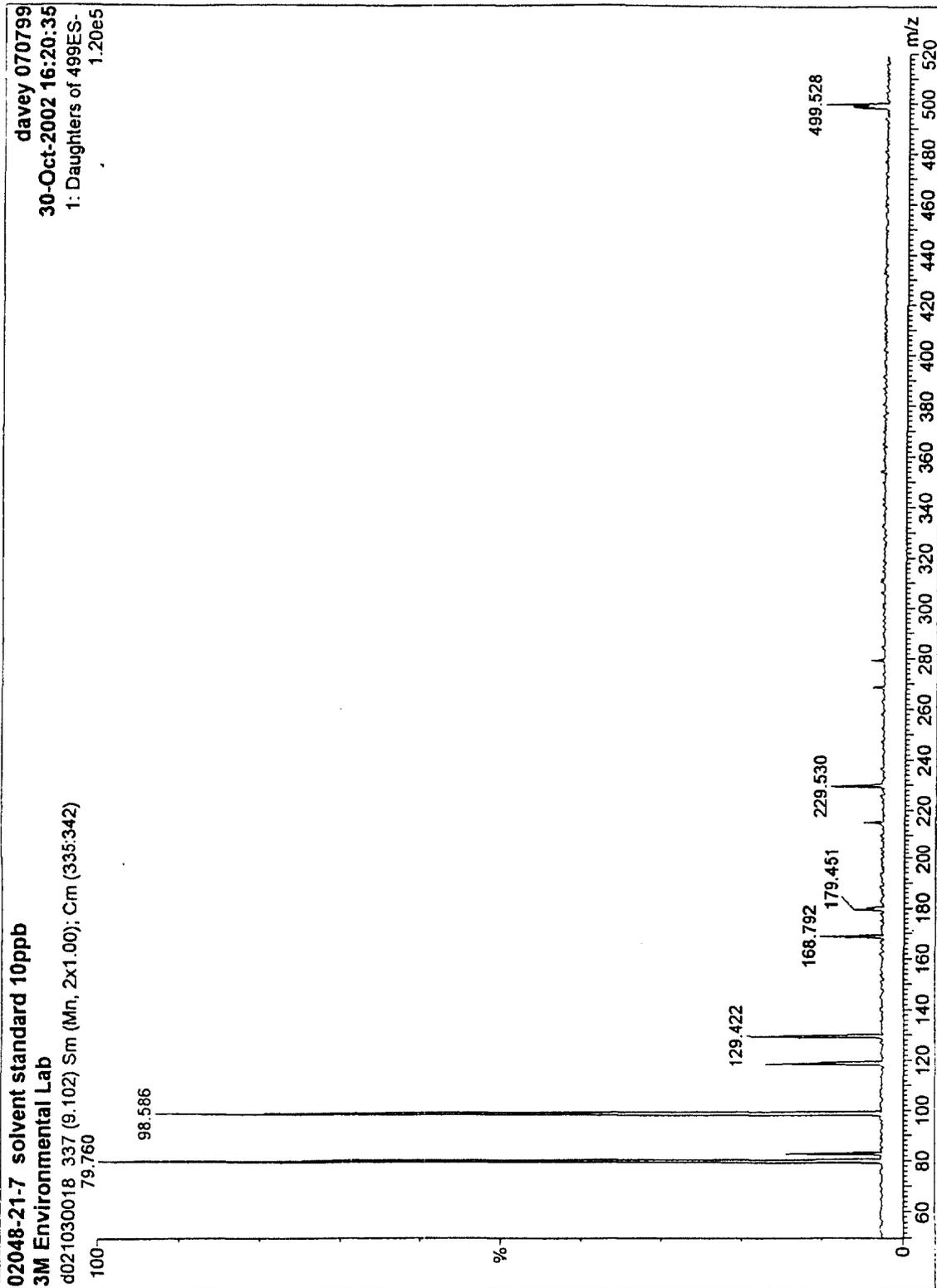
02048-21-7 solvent standard 10ppb
3M Environmental Lab
d021030018 329 (8.886) Sm (Mn, 2x1.00); Cm (321:334)

000212

Exact Copy of Original

CM 11/1/02
Initial Date

PFOS

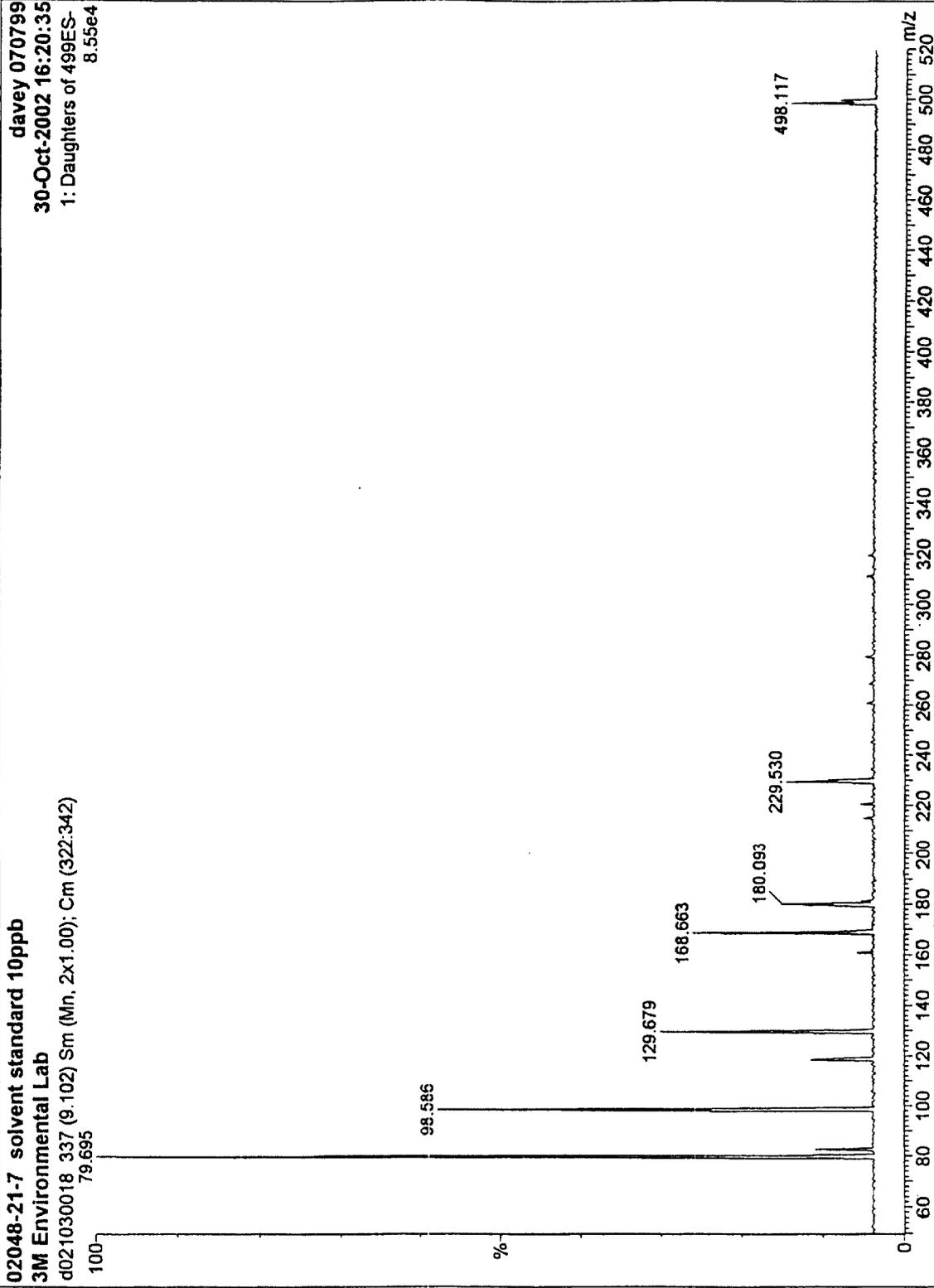


davey 070799
30-Oct-2002 16:20:35
1: Daughters of 499ES-
1.20e5

02048-21-7 solvent standard 10ppb
3M Environmental Lab
d021030018 337 (9.102) Sm (Mn, 2x1.00); Cm (335:342)

Exact Copy of Original
Initial WV Date 11/1/02
000213

PFOS



davey 070799
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1: Daughters of 499ES-
8.55e4

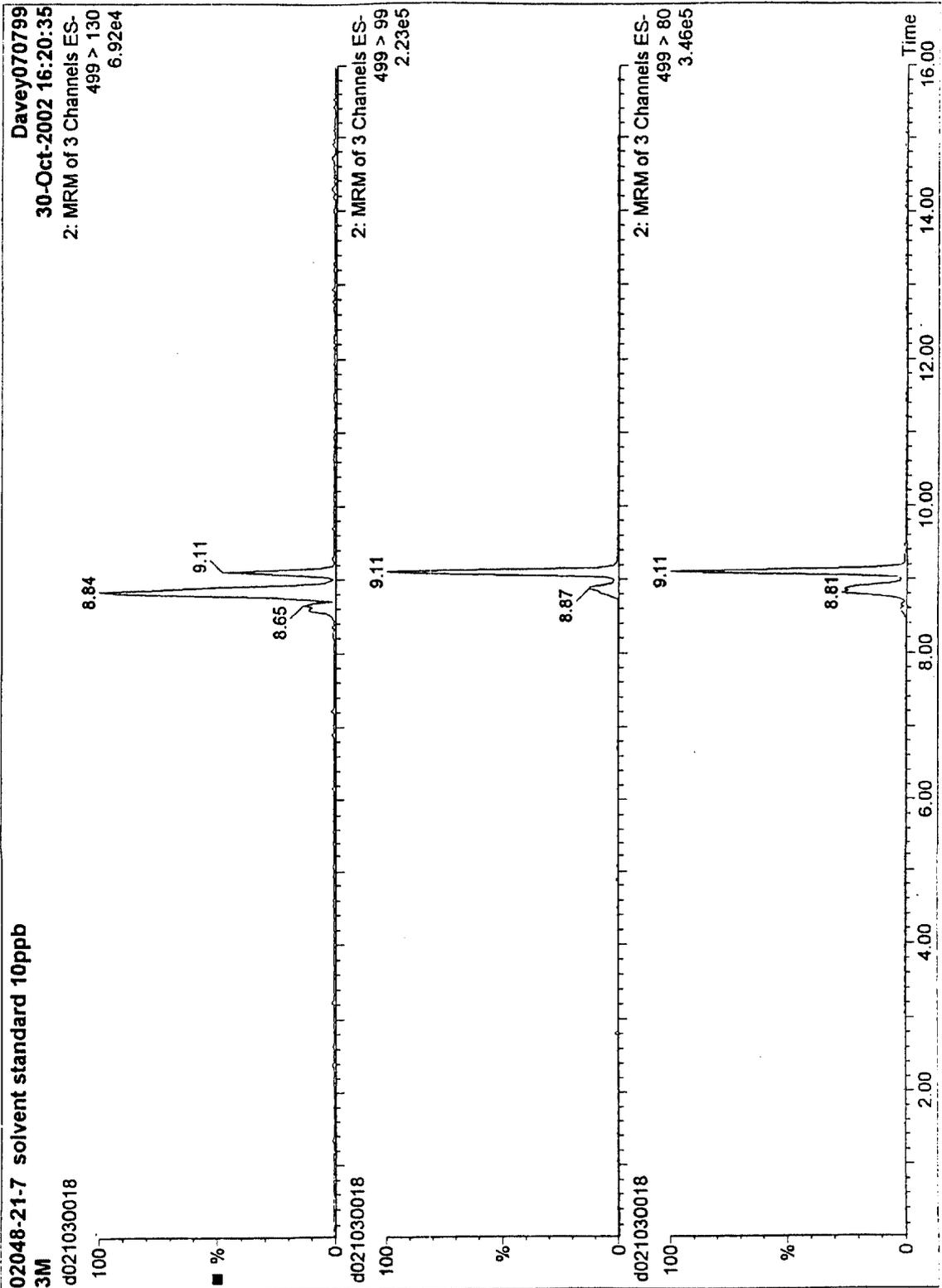
02048-21-7 solvent standard 10ppb
3M Environmental Lab
d021030018 337 (9.102) Sm (Mn, 2x1.00); Cm (322:342)

Exact Copy of Original

Initial Date
CML 11/1/02

000214

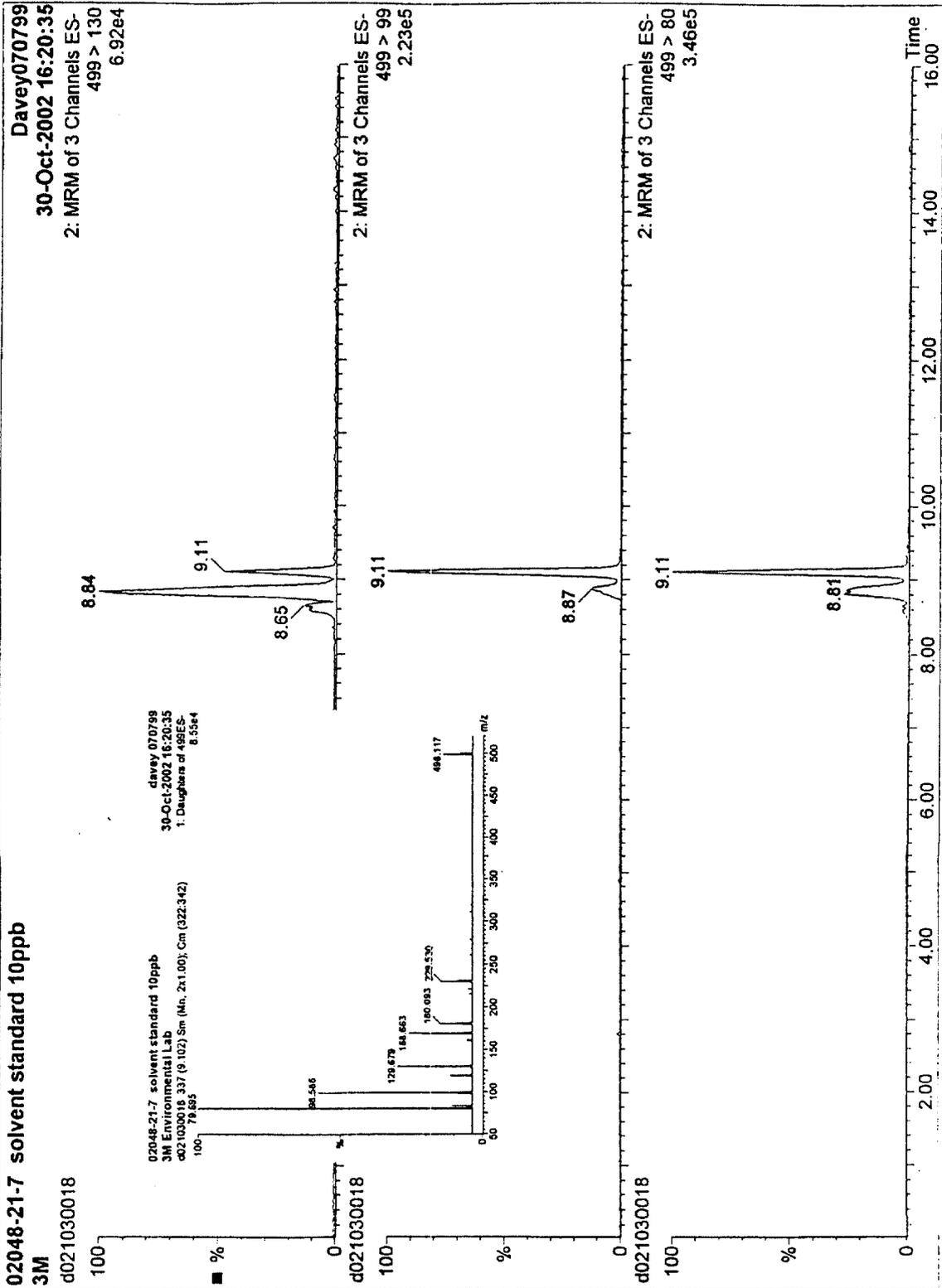
RFOS



Copy of Original
Initial CMC Date 11/10/02

000215

DFOS



Exact Copy of Original
CMC
Initial
JKL
Date

000216

ATTACHMENT D: PREP SHEETS AND TRACEABILITY INFORMATION

SPE Columns Extraction Worksheet

Prep Date:
Analysts initials:

10/23/2002
OK

102302
OK

Method Revision: ETS-8-231.1
Study Number: E02-1039

Sample Number or description	Volume of sample filtered (ml)	Type of column used	Amount and spike mix used	Elution solvent and volume	Comments
Blank milli-q	2000 ml	Waters, 1g, 6ml	NA	2ml of MeOH TN-A-8313	
Lampire Serum	2000 ml	Waters, 1g, 6ml	NA	2ml of MeOH TN-A-8313	TCR-888
Sigma Serum	2000 ml	Waters, 1g, 6ml	NA	2ml of MeOH TN-A-8313	TCR-889
Golden West Serum	2000 ml	Waters, 1g, 6ml	NA	2ml of MeOH TN-A-8313	TCR-890
Bioresource Serum	2000 ml	Waters, 1g, 6ml	NA	2ml of MeOH TN-A-8313	TCR-887
Lampire Plasma	2000 ml	Waters, 1g, 6ml	NA	2ml of MeOH TN-A-8313	TCR-885
Golden West Plasma	2000 ml	Waters, 1g, 6ml	NA	2ml of MeOH TN-A-8313	TCR-884
Innovative Resources Plasma	2000 ml	Waters, 1g, 6ml	NA	2ml of MeOH TN-A-8313	TCR-883

Extraction Steps (initial when/if done):

condition column with MeOH; wash with milli-q water; filter sample; ;
 vacuum dry the column; elute column with solvent;

ACN TN-A-8 ^{OK} 10/23/02 4305 ; LOT OF COLUMN: W 27931

Additional comments:

10ml of sera diluted with 40ml of milli-q and 200ml of ACN. Samples were shaken for 20 min at 300rpm, then centrifuged for 10 min @ 3500rpm/4C
Sample was transferred into 1750ml of milli-q and filtered through conditioned SPE

Samples were transferred onto column using Tygon tubing - OK 10/23/02

Exact Copy of Original
Cmc Initial 10/31/02 Date

Non GLP SPE Columns Extraction Worksheet

Prep Date:
Analysts initials:

MCC
10/24/02 10/11/2002 *10.11.02*
OK OK

Method Revision: *ETS 8231 1101102* ETS-231
Study Number: E 02-1039

Sample Number description	or	Volume of sample filtered (ml)	Type of column used	Amount and spike mix used	Elution solvent and volume	Comments
Blank milli-q-1		400 ml	Waters, 1g, 6ml	NA	2 ml of MeOH TN-A-6310	
Blank milli-q-2		400 ml	Waters, 1g, 6ml	NA	2 ml of MeOH TN-A-6310	
rabbit serum blank		400 ml	Waters, 1g, 6ml	NA	2 ml of MeOH TN-A-6310	TCR-888
rabbit serum curve-0.1ppb		400 ml	Waters, 1g, 6ml	2ul of 02050-14	2 ml of MeOH TN-A-6310	TCR-888
rabbit serum curve-0.25ppb		400 ml	Waters, 1g, 6ml	5ul of 02050-14	2 ml of MeOH TN-A-6310	TCR-888
rabbit serum curve-0.5ppb		400 ml	Waters, 1g, 6ml	10ul of 02050-14	2 ml of MeOH TN-A-6310	TCR-888
rabbit serum curve-0.75ppb		400 ml	Waters, 1g, 6ml	15ul of 02050-14	2 ml of MeOH TN-A-6310	TCR-888
rabbit serum curve-1ppb		400 ml	Waters, 1g, 6ml	20ul of 02050-14	2 ml of MeOH TN-A-6310	TCR-888
rabbit serum curve-2.5ppb		400 ml	Waters, 1g, 6ml	50ul of 02050-14	2 ml of MeOH TN-A-6310	TCR-888
rabbit serum curve-5ppb		400 ml	Waters, 1g, 6ml	100ul of 02050-14	2 ml of MeOH TN-A-6310	TCR-888
rabbit serum curve-10ppb		400 ml	Waters, 1g, 6ml	200ul of 02050-14	2 ml of MeOH TN-A-6310	TCR-888
rabbit serum curve-30ppb		400 ml	Waters, 1g, 6ml	600ul of 02050-14	2 ml of MeOH TN-A-6310	TCR-888
Lampire serum blank-1		400 ml	Waters, 1g, 6ml	NA	2 ml of MeOH TN-A-6310	TCR-888
Lampire serum blank-2		400 ml	Waters, 1g, 6ml	NA	2 ml of MeOH TN-A-6310	TCR-888
Lampire serum MS-0.5ppb		400 ml	Waters, 1g, 6ml	10ul of 02050-14	2 ml of MeOH TN-A-6310	TCR-888
Lampire serum MS-5ppb		400 ml	Waters, 1g, 6ml	100ul of 02050-14	2 ml of MeOH TN-A-6310	TCR-888
Sigma serum blank-1		400 ml	Waters, 1g, 6ml	NA	2 ml of MeOH TN-A-6310	TCR-889
Sigma serum blank-2		400 ml	Waters, 1g, 6ml	NA	2 ml of MeOH TN-A-6310	TCR-889
Sigma serum MS-0.5ppb		400 ml	Waters, 1g, 6ml	10ul ^{CF} of 02050-14	2 ml of MeOH TN-A-6310	TCR-889
Sigma serum MS-5ppb		400 ml	Waters, 1g, 6ml	100ul ^{CF} of 02050-14	2 ml of MeOH TN-A-6310	TCR-889
Golden West serum blank-1		400 ml	Waters, 1g, 6ml	NA	2 ml of MeOH TN-A-6310	TCR-890
Golden West serum blank-2		400 ml	Waters, 1g, 6ml	NA	2 ml of MeOH TN-A-6310	TCR-890
Golden West serum MS-0.5ppb		400 ml	Waters, 1g, 6ml	10ul of 02050-14	2 ml of MeOH TN-A-6310	TCR-890
Golden West serum MS-5ppb		400 ml	Waters, 1g, 6ml	100ul of 02050-14	2 ml of MeOH TN-A-6310	TCR-890
Bioresearch research serum blank-1		400 ml	Waters, 1g, 6ml	NA	2 ml of MeOH TN-A-6310	TCR-887
Bioresearch research serum blank-2		400 ml	Waters, 1g, 6ml	NA	2 ml of MeOH TN-A-6310	TCR-887
Bioresearch research serum MS-0.5ppb		400 ml	Waters, 1g, 6ml	10ul of 02050-14	2 ml of MeOH TN-A-6310	TCR-887
Bioresearch research serum MS-5ppb		400 ml	Waters, 1g, 6ml	100ul of 02050-14	2 ml of MeOH TN-A-6310	TCR-887

Extraction Steps (initial when/if done):

_x_condition column with MeOH; _x_wash with milli-q water; _x_filter sample; ;
_x_vacuum dry the column; _x_elute column with solvent;

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Initial Date

Additional comments:

ACN TN-A-6194 OK 10/10/02
2ml of sera/plasma diluted with 8ml of milli-q and 40ml of ACN. Samples were shaken for 20 min at 300rpm, then centrifuged for 10 min @ 3500rpm/4C
Sample was transferred into 350ml of milli-q and filtered through conditioned SPE
SHAKER VOR #041 094 CENTRIFUGE HM 3 #610P# 021123-55-

000219

Non GLP SPE Columns Extraction Worksheet

Prep Date:
Analysts initials:

MCC
10/24/02 10/10/2002 10/10/02
OK CK

Method Revision: SPE validation
Study Number: E 02-1039

Sample Number description	or	Volume of sample filtered (ml)	Type of column used	Amount and spike mix used	Elution solvent and volume	Comments
Blank milli-q-1		400 ml	Waters, 1g, 6ml	NA	2 ml of MeOH TN-A-6310	
Blank milli-q-2		400 ml	Waters, 1g, 6ml	NA	2 ml of MeOH TN-A-6310	
Chinese plasma curve-0.1ppb		400 ml	Waters, 1g, 6ml	2ul of 02050-14	2 ml of MeOH TN-A-6310	TCR-674
Chinese plasma curve-0.25ppb		400 ml	Waters, 1g, 6ml	5ul of 02050-14	2 ml of MeOH TN-A-6310	TCR-674
Chinese plasma curve-0.5ppb		400 ml	Waters, 1g, 6ml	10ul of 02050-14	2 ml of MeOH TN-A-6310	TCR-674
Chinese plasma curve-0.75ppb		400 ml	Waters, 1g, 6ml	15ul of 02050-14	2 ml of MeOH TN-A-6310	TCR-674
Chinese plasma curve-1ppb		400 ml	Waters, 1g, 6ml	20ul of 02050-14	2 ml of MeOH TN-A-6310	TCR-674
Chinese plasma curve-2.5ppb		400 ml	Waters, 1g, 6ml	50ul of 02050-14	2 ml of MeOH TN-A-6310	TCR-674
Chinese plasma curve-5ppb		400 ml	Waters, 1g, 6ml	100ul of 02050-14	2 ml of MeOH TN-A-6310	TCR-674
Chinese plasma curve-10ppb		400 ml	Waters, 1g, 6ml	200ul of 02050-14	2 ml of MeOH TN-A-6310	TCR-674
Chinese plasma curve-30ppb		400 ml	Waters, 1g, 6ml	600ul of 02050-14	2 ml of MeOH TN-A-6310	TCR-674
Chinese plasma blank-1		400 ml	Waters, 1g, 6ml	NA	2 ml of MeOH TN-A-6310	TCR-674
Chinese plasma blank-2		400 ml	Waters, 1g, 6ml	NA	2 ml of MeOH TN-A-6310	TCR-674
Chinese plasma MS-0.5ppb		400 ml	Waters, 1g, 6ml	10ul of 02050-14	2 ml of MeOH TN-A-6310	TCR-674
Chinese plasma MS-5ppb		400 ml	Waters, 1g, 6ml	100ul of 02050-14	2 ml of MeOH TN-A-6310	TCR-674
Lampire Plasma blank-1		400 ml	Waters, 1g, 6ml	NA	2 ml of MeOH TN-A-6310	TCR-685
Lampire Plasma blank-2		400 ml	Waters, 1g, 6ml	NA	2 ml of MeOH TN-A-6310	TCR-685
Lampire plasma MS-0.5ppb		400 ml	Waters, 1g, 6ml	10ul	2 ml of MeOH TN-A-6310	TCR-685
Lampire plasma MS-5ppb		400 ml	Waters, 1g, 6ml	100ul	2 ml of MeOH TN-A-6310	TCR-685
Golden West Plasma blank-1		400 ml	Waters, 1g, 6ml	NA	2 ml of MeOH TN-A-6310	TCR-684
Golden West Plasma blank-2		400 ml	Waters, 1g, 6ml	NA	2 ml of MeOH TN-A-6310	TCR-684
Golden West Plasma MS-0.5ppb		400 ml	Waters, 1g, 6ml	10ul of 02050-14	2 ml of MeOH TN-A-6310	TCR-684
Golden West Plasma MS-5ppb		400 ml	Waters, 1g, 6ml	100ul of 02050-14	2 ml of MeOH TN-A-6310	TCR-684
Innovative Research Plasma blank-1		400 ml	Waters, 1g, 6ml	NA	2 ml of MeOH TN-A-6310	TCR-683
Innovative Research Plasma blank-2		400 ml	Waters, 1g, 6ml	NA	2 ml of MeOH TN-A-6310	TCR-683
Innovative Research Plasma MS-0.5ppb		400 ml	Waters, 1g, 6ml	10ul of 02050-14	2 ml of MeOH TN-A-6310	TCR-683
Innovative Research Plasma MS-5ppb		400 ml	Waters, 1g, 6ml	100ul of 02050-14	2 ml of MeOH TN-A-6310	TCR-683

Extraction Steps (initial when/if done):

_x_condition column with MeOH; _x_wash with milli-q water; _x_filter sample; ;
_x_vacuum dry the column; _x_elute column with solvent;

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Initial Date

Additional comments:

CRND.F0000 NMS # 021148557 ; dates: VWS SA: SER# 041694

2ml of sera/plasma diluted with 8ml of milli-q and 40ml of ACN. Samples were shaken for 20 min at 300rpm, then centrifuged for 10 min @ 3500rpm/4C
Sample was transferred into 350ml of milli-q and filtered through conditioned SPE

ACN TN-A: 6194

000220

3M ENVIRONMENTAL LABORATORY

Note to File

Project or Study Number: E02-1039
Associated Study Number:

For samples that were prepped on 10/11/02, that are Bioresource Research Serum MS 0.5ppb and 5ppb, labels on vials were accidentally switched.

Recorded By: *[Signature]* Date: *10-30-02*

Exact Copy of Original
[Signature] 11/1/02
Initial Date

Test Control and Reference Substance Log

Substance trade name or reference #	Human plasma from China	TCR Substance #	TCR-674
Substance/chemical name:	Human plasma	TCR #	TCR-674
Lot/batch #:	N087P27	Received from:	Northwest Bioanalytical
3M #			
Expiration date:		Amount received (wt. or vol):	7x~10ml
Initials:	OK	Date:	09/25/2002
Number/size of containers:	7-15 ml centrifuge tubes	Shipper:	FedEx
Condition:	liquid, Biohazard	MSDS (y/n)	<input type="radio"/> Y <input checked="" type="radio"/> N
Retain		Date of Retain	

Archived/Substance Not Available:

Purity:	
Records received:	
Location of synthesis, fabrication, or derivation records:	
Std Location/Storage:	Frozen F19
Molecular Formula:	
Comments	Sera came in 7 separate tubes. Please mark which tube was used for sample, by number marked on the tube (4,5,6,7,8,11 or 12)
Attachment(s)	

Exact Copy of Original

cmc 11/1/02
Initial Date

USE LOG							
Human plasma from China Human plasma TCR-674							
Gross Wt./Vol. Before withdrawal Balance ID 1	Amnt. withdrawn (mass or vol) Balance ID 2	Gross wt/vol after withdrawal Balance ID 1	Purpose (enter standard number or reason for removal)	Balance ID 1	Balance ID 2	Initials	Date
-	26ml	-	E02-1039, validation (all tubes were combined)	na	na	OK	10/09/2002

Exact Copy of Original

cmc 11/1/02
Initial Date

Test Control and Reference Substance Log

Substance trade name or reference #	Pooled Human Plasma in Sodium Citrate	TCR Substance #	TCR-683
Substance/chemical name:	Pooled human plasma	TCR #	TCR-683
Lot/batch #:	IR02-014	Received from:	Innovative Research, MI
3M #			
Expiration date:	09/30/2007	Amount received (wt. or vol):	10x10ml
Initials:	OK	Date:	10/02/2002
Number/size of containers:	10x10ml plastic tubes	Shipper:	
Condition:	good	MSDS (y/n)	<input type="radio"/> Y <input checked="" type="radio"/> N
Retain		Date of Retain	

Archived/Substance Not Available

Purity:	
Records received:	CofA
Location of synthesis, fabrication, or derivation records:	Southfield, MI
Std Location/Storage:	Frozen, F24
Molecular Formula:	
Comments	
Attachment(s)	

Exact Copy of Original

Cmc 11/102
Initial Date

USE LOG							
Pooled Human Plasma Pooled human plasma TCR-683							
Gross Wt./Vol. Before withdrawal Balance ID 1	Amnt. withdrawn (mass or vol) Balance ID 2	Gross wt/vol after withdrawal Balance ID 1	Purpose (enter standard number or reason for removal)	Balance ID 1	Balance ID 2	Initials	Date
-	8ml	-	E02-1039 validation	-	-	OK	10/14/2002

Exact Copy of Original

Cmc 11/1/02
Initial Date

USE LOG							
Pooled Human Plasma							
Pooled human plasma							
TCR-683							

Gross Wt./Vol. Before withdrawal Balance ID 1	Amnt. withdrawn (mass or vol) Balance ID 2	Gross wt/vol after withdrawal Balance ID 1	Purpose (enter standard number or reason for removal)	Balance ID 1	Balance ID 2	Initials	Date
-	10ml	-	E02-1039, validation	-	-	OK	10/23/2002

Exact Copy of Original

Cmc 11/1/02
Initial Date

000226

Test Control and Reference Substance Log

Substance trade name or reference #	Sodium Citrate Pooled Human Plasma	TCR Substance #	TCR-684
Substance/chemical name:	Pooled human plasma	TCR #	TCR-684
Lot/batch #:	G01410002	Received from:	Golden West Biologicals, Inc.
3M #			
Expiration date:	10/03/2007	Amount received (wt. or vol):	100ml
Initials:	OK	Date:	10/03/2002
Number/size of containers:	1x100ml plastic bottle	Shipper:	UPS
Condition:	Frozen	MSDS (y/n)	Y ● N
Retain		Date of Retain	

Archived/Substance Not Available

Purity:	
Records received:	CofA
Location of synthesis, fabrication, or derivation records:	Golden West Biologicals
Std Location/Storage:	Frozen F19
Molecular Formula:	
Comments	
Attachment(s)	

Exact Copy of Original
CMC 11/1/02
 Initial Date

USE LOG							
Sodium Citrate Pooled Human Plasma							
Pooled human plasma							
TCR-684							

Gross Wt./Vol. Before withdrawal Balance ID 1	Amt. withdrawn (mass or vol) Balance ID 2	Gross wt/vol after withdrawal Balance ID 1	Purpose (enter standard number or reason for removal)	Balance ID 1	Balance ID 2	Initials	Date
-	8ml	-	E02-1039, validation	na	na	OK	10/10/2002

Exact Copy of Original
CMC 10/10/02
 Initial Date

USE LOG							
Sodium Citrate Pooled Human Plasma							
Pooled human plasma							
TCR-684							

Gross Wt./Vol. Before withdrawal Balance ID 1	Amnt. withdrawn (mass or vol) Balance ID 2	Gross wt/vol after withdrawal Balance ID 1	Purpose (enter standard number or reason for removal)	Balance ID 1	Balance ID 2	Initials	Date
-	10ml	-	E02-1039	NA	NA	OK	10/23/2002

Exact Copy of Original.

LMC 10/1/02
Initial Date

Test Control and Reference Substance Log

Substance trade name or reference #	Pooled Human Plasma From Lampire	TCR Substance #	TCR-685
Substance/chemical name:	Pooled Human Plasma From Lampire	TCR #	TCR-685
Lot/batch #:	22-60824A	Received from:	Lampire
3M #			
Expiration date:	10/08/2007	Amount received (wt. or vol):	100ml
Initials:	OK	Date:	10/10/2002
Number/size of containers:	1-250ml plastic bottle	Shipper:	UPS
Condition:	Frozen	MSDS (y/n)	Y ● N
Retain		Date of Retain	

Archived/Substance Not Available

Purity:	
Records received:	Disease Screen
Location of synthesis, fabrication, or derivation records:	Lampire
Std Location/Storage:	Frozen F19
Molecular Formula:	
Comments	
Attachment(s)	

Exact Copy of Original

CMC 10/10/02
Initial Date

USE LOG							
Pooled Human Plasma From Lampire							
Pooled Human Plasma From Lampire							
TCR-685							

Gross Wt./Vol. Before withdrawal Balance ID 1	Amnt. withdrawn (mass or vol) Balance ID 2	Gross wt/vol after withdrawal Balance ID 1	Purpose (enter standard number or reason for removal)	Balance ID 1	Balance ID 2	Initials	Date
-	10ml	-	E02-1039	na	na	OK	10/23/2002

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CINC *10/23/02*
 Initial Date

000231

USE LOG							
Pooled Human Plasma From Lampire							
Pooled Human Plasma From Lampire							
TCR-685							

Gross Wt./Vol. Before withdrawal Balance ID 1	Amnt. withdrawn (mass or vol) Balance ID 2	Gross wt/vol after withdrawal Balance ID 1	Purpose (enter standard number or reason for removal)	Balance ID 1	Balance ID 2	Initials	Date
-	8ml	-	E02-1039, validation	na	na	OK	10/10/2002

Exact Copy of Original
CMC 10/10/02
 Initial Date

000232

Test Control and Reference Substance Log

Substance trade name or reference #	Pooled Human Serum From Bioresource	TCR Substance #	TCR-687
Substance/chemical name:	Pooled Human Serum	TCR #	TCR-687
Lot/batch #:	020821	Received from:	Bioresource
3M #			
Expiration date:	08/27/2007	Amount received (wt. or vol):	500ml
Initials:	OK	Date:	10/11/2002
Number/size of containers:	500ml plastic bottle	Shipper:	UPS
Condition:	Frozen	MSDS (y/n)	<input type="radio"/> Y <input checked="" type="radio"/> N
Retain		Date of Retain	

Archived/Substance Not Available

Purity:	
Records received:	
Location of synthesis, fabrication, or derivation records:	Bioresource
Std Location/Storage:	Frozen F19
Molecular Formula:	
Comments	Also assigned TN-A-6284 10/11/02 OK
Attachment(s)	

Exact Copy of Original
cmc 11/1/02
 Initial Date

000233

USE LOG
Pooled Human Serum From Bioresource
Pooled Human Serum
TCR-687

Gross Wt./Vol. Before withdrawal Balance ID 1	Amnt. withdrawn (mass or vol) Balance ID 2	Gross wt/vol after withdrawal Balance ID 1	Purpose (enter standard number or reason for removal)	Balance ID 1	Balance ID 2	Initials	Date
-	10ml	-	E02-1039	-	-	OK	10/23/2002

Exact Copy of Original

CMC 11/1/07
Initial Date

USE LOG							
Pooled Human Serum From Bioresource							
Pooled Human Serum							
TCR-687							
Gross Wt./Vol. Before withdrawal Balance ID 1	Amnt. withdrawn (mass or vol) Balance ID 2	Gross wt/vol after withdrawal Balance ID 1	Purpose (enter standard number or reason for removal)	Balance ID 1	Balance ID 2	Initials	Date
-	8ml	-	E02-1039, validation	-	-	OK	10/11/2002

Exact Copy of Original
CMC 10/11/02
 Initial Date

000235

Test Control and Reference Substance Log

Substance trade name or reference #	Pooled Human serum from Lampire	TCR Substance #	TCR-688
Substance/chemical name:	Pooled Human serum	TCR #	TCR-688
Lot/batch #:	X324B	Received from:	Lampire
3M #			
Expiration date:	08/29/2007	Amount received (wt. or vol):	250ml
Initials:	OK	Date:	10/11/2002
Number/size of containers:	250 ml Nalgene bottle	Shipper:	UPS
Condition:	Frozen	MSDS (y/n)	Y ● N
Retain		Date of Retain	

Archived/Substance Not Available

Purity:	
Records received:	
Location of synthesis, fabrication, or derivation records:	Lampire
Std Location/Storage:	Frozen F19
Molecular Formula:	
Comments	Also assinged TN-A-6286 10/11/02 ok
Attachment(s)	

Exact Copy of Original
CML 10/11/02
 Initial Date

USE LOG							
Pooled Human serum from Lampire							
Pooled Human serum							
TCR-688							

Gross Wt./Vol. Before withdrawal Balance ID 1	Amnt. withdrawn (mass or vol) Balance ID 2	Gross wt/vol after withdrawal Balance ID 1	Purpose (enter standard number or reason for removal)	Balance ID 1	Balance ID 2	Initials	Date
-	8ml	-	E02-1039, validation	-	-	OK	10/11/2002

Exact Copy of Original
CMC 10/11/02
 Initial Date

000237

USE LOG							
Pooled Human serum from Lampire							
Pooled Human serum							
TCR-688							

Gross Wt./Vol. Before withdrawal Balance ID 1	Amtt. withdrawn (mass or vol) Balance ID 2	Gross wt/vol after withdrawal Balance ID 1	Purpose (enter standard number or reason for removal)	Balance ID 1	Balance ID 2	Initials	Date
-	10ml	-	E02-1039	-	-	OK	10/23/2002

Exact Copy of Original
CMS *11/11/02*
 Initial Date

000238

Test Control and Reference Substance Log

Substance trade name or reference #	Male Human Serum from Sigma	TCR Substance #	TCR-689
Substance/chemical name:	Male Human serum	TCR #	TCR-689
Lot/batch #:	022K0965	Received from:	Sigma
3M #			
Expiration date:	07/24/2007	Amount received (wt. or vol):	100ml
Initials:	OK	Date:	10/11/2002
Number/size of containers:	1-100ml plastic bottle	Shipper:	UPS
Condition:	frozen	MSDS (y/n)	() Y ● N
Retain		Date of Retain	

Archived/Substance Not Available

Purity:	
Records received:	
Location of synthesis, fabrication, or derivation records:	Sigma
Std Location/Storage:	Frozen F19
Molecular Formula:	
Comments	Also assigned TN-A-6250 10/11/02 OK
Attachment(s)	

Exact Copy of Original
CMC *11/11/02*
 Initial Date

000239

USE LOG							
Male Human Serum from Sigma							
Male Human serum							
TCR-689							

Gross Wt./Vol. Before withdrawal Balance ID 1	Amnt. withdrawn (mass or vol) Balance ID 2	Gross wt/vol after withdrawal Balance ID 1	Purpose (enter standard number or reason for removal)	Balance ID 1	Balance ID 2	Initials	Date
-	8ml	-	E02-1039, validation	-	-	OK	10/11/2002

Exact Copy of Original

LMC 11/10
Initial Date

000240

USE LOG							
Male Human Serum from Sigma							
Male Human serum							
TCR-689							

Gross Wt./Vol. Before withdrawal Balance ID 1	Amnt. withdrawn (mass or vol) Balance ID 2	Gross wt/vol after withdrawal Balance ID 1	Purpose (enter standard number or reason for removal)	Balance ID 1	Balance ID 2	Initials	Date
-	10ml	-	E02-1039	-	-	OK	10/23/2002

Exact Copy of Original
CME 10/23/02
 Initial Date

000241

Test Control and Reference Substance Log

Substance trade name or reference #	Pooled Human Serum from Golen West	TCR Substance #	TCR-690
Substance/chemical name:	Pooled Human Serum	TCR #	TCR-690
Lot/batch #:	G01406042	Received from:	Golden west
3M #			
Expiration date:	07/25/2007	Amount received (wt. or vol):	500ml
Initials:	OK	Date:	10/11/2002
Number/size of containers:	1-500ml blastic bottle	Shipper:	UPS
Condition:	frozen	MSDS (y/n)	Y ● N
Retain		Date of Retain	

Archived/Substance Not Available

Purity:	
Records received:	
Location of synthesis, fabrication, or derivation records:	Golden West
Std Location/Storage:	Frozen F19
Molecular Formula:	
Comments	Also assigned TN-A-6251 10/11/02 ok
Attachment(s)	

Exact Copy of Original

CMC 11/1/02
Initial Date

000242

USE LOG							
Pooled Human Serum from Golen West							
Pooled Human Serum							
TCR-690							

Gross Wt./Vol. Before withdrawal Balance ID 1	Amt. withdrawn (mass or vol) Balance ID 2	Gross wt/vol after withdrawal Balance ID 1	Purpose (enter standard number or reason for removal)	Balance ID 1	Balance ID 2	Initials	Date
-	10ml	-	E02-1039	-	-	OK	10/23/2002

Exact Copy of Original
cmc *11/1/02*
 Initial Date

000243

USE LOG							
Pooled Human Serum from Golen West							
Pooled Human Serum							
TCR-690							

Gross Wt./Vol. Before withdrawal Balance ID 1	Amnt. withdrawn (mass or vol) Balance ID 2	Gross wt/vol after withdrawal Balance ID 1	Purpose (enter standard number or reason for removal)	Balance ID 1	Balance ID 2	Initials	Date
-	8ml	-	E02-1039, validation	-	-	OK	10/11/2002

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 Initial Date

000244

Standard curve tracking sheet

Prep date: 10/17/2002
 Analyst: OK
 Type of the curve: Solvent curve for SPE sera validation
 Target analytes: C6, C7, C8, C9, C10, C11, C12, THPFOS, THPFDS, PFOS
 Standard mix used for preparing the curve: 02002-63

Study Number: 10702
 Final Solvent & TN-A #: MeOH/6308
 Final Volume of each point: 25

Curve point number	Amount of mix used (ul)	Analyte concentration in every point (ppb)										
		C6	C7	C8	C9	C10	C11	C12	THPFOS	THPFDS	PFOS	
02048-21-1	5	0.100	0.102	0.103	0.101	0.101	0.103	0.104	0.102	0.100	0.101	
02048-21-2	25	0.500	0.511	0.517	0.507	0.505	0.515	0.518	0.512	0.501	0.503	
02048-21-3	50	1.00	1.02	1.03	1.01	1.01	1.03	1.04	1.02	1.00	1.01	
02048-21-4	125	2.50	2.55	2.58	2.54	2.53	2.58	2.59	2.56	2.51	2.52	
02048-21-5	250	5.00	5.11	5.17	5.07	5.05	5.15	5.18	5.12	5.01	5.03	
02048-21-6	375	7.50	7.66	7.75	7.61	7.58	7.73	7.77	7.68	7.52	7.55	
02048-21-7	500	10.00	10.21	10.33	10.14	10.10	10.31	10.36	10.24	10.02	10.06	
02048-21-8	750	15.00	15.32	15.50	15.22	15.15	15.46	15.54	15.37	15.03	15.09	
02048-21-9	1000	20.01	20.43	20.66	20.29	20.20	20.61	20.72	20.49	20.04	20.12	
02048-21-10	1500	30.01	30.64	31.00	30.43	30.30	30.92	31.08	30.73	30.06	30.18	

Signature: *[Handwritten Signature]*
 Verified by: *[Handwritten Signature]* 10/31/02

Exact Copy of Original
 Initial: *[Handwritten Initials]*
 Date: *[Handwritten Date]*

000245

Standard mix tracking sheet

Sera Super mix for SPE 10X MDV

Prep date: 10/7/2002 Exp. 11/13/02 Study Number: Sera SPE validation
 Analyst: OK *OK 10-7-02* Final Solvent&TN-A #: MeOH/6308
 Type of the mix: sera super mix Final Volume of the mix: 25
 Target analytes: C6, C7, C8, C9, C10, C11, C12, THPFOS, THPFDS, PFOS

Standard number for the mix: **02002-63**

Analyte	Analyte standard number	concentration of analyte in standard mg/ml (ppm)	amount of standard used (ul)	concentration of the analyte in the mix ng/ml (ppb)
C6	02040-44	1042	12	500
C7	02040-58	1344	9.5	511
C8	02040-45	1230	10.5	517
C9	02040-46	1266	10	507
C10	02022-90	1010	12.5	505
C11	02040-65	1356	9.5	515
C12	02040-55	1126	11.5	518
THPFOS	02040-42	1348	9.5	512
THPFDS	02040-49	1002	12.5	501
PFOS	02022-56	1048	12	503

Signature: *Rajesh Kugian*

Verified by: *Cindy Carlson 10/31/02*

Exact Copy of Original
CRC 10/31/02
 Initial Date

000246

SINGLE COMPONENT PREPARATION LOG

Date: 10.10.02

Book No. 02 050

Analyst: OKJ

Page No. 14

Description: 100 PPb SUPER MIX FOR STE

Stock Number: 02002-63

Weight or Volume Used: 5 ml

Balance ID: NA

Concentration or Purity: 500 PPb

Other Correction Factors: NA

Corrected Weight: NA

Solvent and TN-A Number: MeOH / TN-A-6310

Final Volume: 25 ml

Final Concentration: 100 PPb

Storage Location: 2nd fl.

Expiration Date: 11.13.02

Exact Copy of Original

Linc 10/31/02
Initial Date

Reviewed by: [Signature] 10/11/02
Signature Date

000247

SINGLE COMPONENT PREPARATION LOG

Date: 10/10/02 Book No. 02 050
Analyst: OK Page No. 17
Description: 0.75 ppb SUPERMIX
Stock Number: 02002-63
Weight or Volume Used: 37.5 μ l Balance ID: NA
Concentration or Purity: 500 ppb Other Correction Factors: NA
Corrected Weight: N/A Solvent and TN-A Number: MeOH / TNA 6310
Final Volume: 25 ml Final Concentration: 0.75 ppb
Storage Location: 2nd fl. Expiration Date: 11/3/02

Exact Copy of Original

cmc 10/31/02
Initial Date

Reviewed by: [Signature] 10/11/02
Signature Date

SINGLE COMPONENT PREPARATION LOG

Date: 10/10/02
Analyst: OKJ

Book No. 02 050
Page No. 16

Description: 0.25 ppb SUPERMIX

Stock Number: 02002-63

Weight or Volume Used: 12.5 μ l

Balance ID: NA

Concentration or Purity: ^{OK} 50 ppb

Other Correction Factors: NA

Corrected Weight: N/A

Solvent and TN-A Number: MeOH / TN-A-6310

Final Volume: 25 μ l

Final Concentration: 0.25 ppb

Storage Location: 2nd fl.

Expiration Date: 11/3/02

Exact Copy of Original

CMC 10/31/02
Initial Date

Reviewed by: [Signature] 10/11/02
Signature Date

MULTI-LEVEL PREPARATION LOG
Book No. 02 048
Page No. 21
Solvent: MeOH
3M Trace #: N.A.G.30A

Description: CURVE FOR SERSA SPE - SURF-MIX
Date Prepared: 10/31/02
Prepared By: CK
Storage Location: 10702
Expiration Date: 11/13/02

Standard ID #	02 048-21-1	02 048-21-2	02 048-21-3	02 048-21-4	02 048-21-5	02 048-21-6	02 048-21-7	02 048-21-8
Description	ppb							
Conc	0.1	0.5	1	2.5	5	7.5	10	15
Expiration Date	11-13-02							
Component Standards								
ID #	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7	Std 8
Description	SURF-MIX FOR SERSA SPE							
Conc	5	25	50	125	250	375	500	750
Expiration Date	11-13-02							
Amount of Component Standard Added μ l								
Final Volume	25							
Final Concentration	0.1	0.5	1	2.5	5	7.5	10	15

Standard ID #	02 048-21-9	02 048-21-10	02 048-21-11	02 048-21-12	02 048-21-13	02 048-21-14	02 048-21-15	02 048-21-16
Description	ppb							
Conc	20	30						
Expiration Date	11/13/02							
Component Standards								
ID #	Std 9	Std 10	Std 11	Std 12	Std 13	Std 14	Std 15	Std 16
Description	SURF-MIX FOR SERSA SPE							
Conc	1000	1500						
Expiration Date	11/13/02							
Amount of Component Standard Added μ l								
Final Volume	25							
Final Concentration	20	30						

Reviewed by: *[Signature]*
10/11/02

Exact Copy of Original
Initial CK Date 10/31/02

000250

MULTI-COMPONENT PREPARATION LOG

Book No. 02 002
Page No. 63

Description: SUPERMIX FOR GRASTE
Date Prepared: 10-6-02
Prepared By: OK
Storage Location: 2nd fl.
Expiration Date: 11-13-02

Solvent: MeOH
3M Trace #: TN-A-6308

Standard ID # 02 002-63

Component Standard						Corrected Weight	Final Concentration
Description	ID #	Conc. or Purity	Expiration Date	Weight or Volume	Balance ID		
C ₆ ACID	02040-44	1042 PPM	2-15-03	12 ml	NA	NA	500
C ₇ ACID	02040-58	1344 PPM	2-29-03	9.5 µl			511
C ₈ ACID	02040-45	1230 PPM	2-15-03	10.5 µl			517
C ₉ ACID	02040-46	1268 PPM	2-15-03	10 µl			507
C ₁₀ ACID	02022-90	1010 PPM	1-2-03	12.5 µl			505
C ₁₁ ACID	02040-65	1356 PPM	2-29-03	9.5 µl			515
C ₁₂ ACID	02040-55	1126 PPM	2-29-03	11.5 µl			518
THPFOS	02040-42	1348 PPM	2-15-03	9.5 µl			512
THPFDS	02040-49	1002 TPU	2-26-03	12.5 µl			501
PTOS	02040-56 02022-56	1048 PPM	11-15-02	12 µl			503
Final Volume				ml	25	Approximate Concentration of the mix	500 ppb

OK
10602

Reviewed by: Mal ELL 10/11/02
Signature, Date

Exact Copy of Original
Date 10/31/02

000251

Test Control and Reference Substance Log

Substance trade name or reference #	TDHA	TCR Substance #	TCR-99131-025
Substance/chemical name:	Tridecafluoroheptanoic Acid	TCR #	TCR-267
Lot/batch #:	PU/07219EU	Received from:	Aldrich
3M #	NA		
Expiration date:	01/01/2005	Amount received (wt. or vol):	25 g, 37.1357 g gross wt.
Initials:	SRP	Date:	12/21/1999
Number/size of containers:	1/ 30 mL plastic container	Shipper:	Courier
Condition:	clear crystals	MSDS (y/n)	<input checked="" type="radio"/> Y <input type="radio"/> N
Retain	0.3994 g	Date of Retain	02/15/2000
<input type="radio"/> Archived/Substance Not Available			
Purity:	99.5% LAC 4/24/01		
Records received:	MSDS KJD 3/1/00		
Location of synthesis, fabrication, or derivation records:	NA		
Std Location/Storage:	F19, Frozen		
Molecular Formula:	C ₆ F ₁₃ COOH		
Comments	<p>Standard has been moved to Freezer 19 in room 347 KJD 06/07/00 Standard was stored at room temperature prior to 06/07/00 LAC 12/22/00</p> <p>Shipping comments: Corrosive, No 3M ID# - must ship ground small quantity exception (30 mL/g or less per vial). LAS 10/02/02</p>		
Attachment(s)	<div style="text-align: center;">   </div> <p style="text-align: center;">tcr99131-25msds.pdf tcr99131-25cofa.pdf</p>		

Exact Copy of Original
CJC 12/31/02
 Initial Date

000252

CERTIFICATE OF ANALYSIS

3M ENVIRONMENTAL
SCOTT POST
451 778 3890

PO NBR:

PRODUCT NUMBER: 34204-1

LOT NUMBER: 07219EU

PRODUCT NAME: TRIDECAFLUOROHEPTANOIC ACID, 99K

FORMULA: C7HF13O2

FORMULA WEIGHT: 364.06

APPEARANCE

WHITE CRYSTALLINE SOLID

INFRARED SPECTRUM

CONFORMS TO STRUCTURE AND STANDARD AS ILLUSTRATED ON PAGE 798B OF EDITION I, VOLUME 1 OF "THE ALDRICH LIBRARY OF FT-IR SPECTRA".

FLUORINE NMR

CONFORMS TO STRUCTURE.

GAS LIQUID
CHROMATOGRAPHY

99.5 %

QUALITY CONTROL
ACCEPTANCE DATE

JUNE 1999

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CMC 10/31/02
Initial Date

ALDRICH CHEMICAL COMPANY
DAVID SWESSEL
JANUARY 26, 2000



chemists helping chemists in research & industry

aldrich chemical co.

P.O. Box 398, Milwaukee, Wisconsin 53201 USA • (414) 273-3060 • FAX (414) 273-4079

ALDRICH warrants that its products conform to the information contained in this and other Aldrich publications. Purchaser must determine the suitability of the product for its particular use. See reverse side of invoice or packing slip for additional terms and conditions of sale.

015 KEY-11.02

** TOTAL PAGE.04 **

000253

USE LOG							
TDHA							
Tridecafluoroheptanoic Acid							
TCR-99131-025							

Gross Wt./Vol. Before withdrawal Balance ID 1	Amnt. withdrawn (mass or vol) Balance ID 2	Gross wt/vol after withdrawal Balance ID 1	Purpose (enter standard number or reason for removal)	Balance ID 1	Balance ID 2	Initials	Date
35.9819g	0.0865g	35.8954g	standard 02040-58	914	-	RWW	08/29/2002

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Cmc 10/31/02
Initial Date

000254

SINGLE COMPONENT PREPARATION LOG

Date: 8/29/02 Book No. 02 040
Analyst: RWV Page No. 58
Description: Tridecafluoroheptanoic Acid (TDHA)
Stock Number: 99131-25
Weight or Volume Used: 0.0675g Balance ID: 914
Concentration or Purity: 99.5% Other Correction Factors: NA
Corrected Weight: 0.0672g Solvent and TN-A Number: MeOH-TN-A-6244
Final Volume: 50ml Final Concentration: 1344 ppm ^{3 @ 100-10/1/02}
Storage Location: 2nd Floor - Rm Temp. Expiration Date: 2/29/03

Exact Copy of Original
cmc 11/1/02
Initial Date

Reviewed by: Vicki A. Stevenson 10/01/02
Signature Date

000255

SINGLE COMPONENT PREPARATION LOG

Date: 8/29/02 Book No. 02 040
Analyst: RW4 Page No. 58
Description: Tridecafluoroheptanoic Acid (TDHA)
Stock Number: 99131-25
Weight or Volume Used: 0.0675g Balance ID: 914
Concentration or Purity: 99.5% Other Correction Factors: NA
Corrected Weight: 0.0672g Solvent and TN-A Number: Meth-TN-A-6244
Final Volume: 50ml Final Concentration: 3 @ RW-10/102
1344 ppm
Storage Location: 2nd Floor - Rm Temp. Expiration Date: 2/29/03

Exact Copy of Original
cmc 11/1/02
Initial Date

Reviewed by: Mike A. Stevenson 10/01/02
Signature Date

000256

3M SPECIALTY MATERIALS MANUFACTURING DIVISION ANALYTICAL LABORATORY

Request # **GID:71638**

To: Lisa Stevenson - (8-5568) - ET&SS - 2-3E-09
From: Tom Kestner - (3-5633) - SMMD Analytical Lab - 236-2B-11
Subject: Characterization of TCR-99131-025 by ¹H-NMR and ¹⁹F-NMR Spectroscopy
Date: October 30, 2002

SAMPLE DESCRIPTION:

- TCR-99131-025, lot PU/07219EU from the Telomer project.
 Nominal product = CF₃(CF₂)_n-CO₂H, where average n ≈ 5 (white solid).

Sample	Spectra #'s	Experiment Descriptions
TCR-99131-025	H71638.GID.401	400 MHz 1D ¹ H-NMR in a CFCl ₃ /acetone-d ₆ solvent mixture + p-HFX cross integration/internal std.
TCR-99131-025	F71638.GID.401	376 MHz 1D ¹⁹ F-NMR in a CFCl ₃ /acetone-d ₆ solvent mixture + p-HFX cross integration/internal std.
TCR-99131-025	F71638.GID.COSY.502	470 MHz 2D ¹⁹ F-NMR COSY (correlated spectroscopy) experiment in a CFCl ₃ /acetone-d ₆ solvent mixture.

OBJECTIVE:

This sample was subjected to a combination of ¹H-NMR and ¹⁹F-NMR spectral analyses to determine the purity of the nominal product. Special emphasis was also placed on attempting to identify and quantify any impurity components.

EXPERIMENTAL:

A portion of the sample was accurately weighed, spiked with a known amount of 1,4-bis(trifluoromethyl)benzene (p-HFX), and then totally dissolved in a CFCl₃/deuterated acetone (acetone-d₆) solvent mixture for subsequent analysis by NMR. Initial one-dimensional (1D) 400 MHz ¹H-NMR and 376 MHz ¹⁹F-NMR spectra were acquired at room temperature using a Varian UNITYplus 400 FT-NMR spectrometer. A two-dimensional (2D) ¹⁹F-NMR correlated spectroscopy (COSY) experiment was also acquired using a Varian UNITY INOVA 500 FT-NMR spectrometer to facilitate assignment of ¹⁹F signals associated with various impurity components. This sample preparation method permitted the p-HFX to be used as either 1) a ¹H/¹⁹F-NMR internal standard to allow the calculation of the absolute weight percent concentrations of specific components, or 2) a ¹H/¹⁹F-NMR cross integration standard to permit the cross correlation of the relative ¹H and ¹⁹F signal intensities for evaluation of the overall sample composition.

RESULTS:

The ¹H-NMR and ¹⁹F-NMR spectral data indicated this sample was a relatively high purity form of the nominal product, CF₃(CF₂)_n-CO₂H, where the average value of n = 5.05. Small amounts of a few impurity components, including probable isomers, were also assigned. A ¹H/¹⁹F-NMR cross integration analysis technique was then used to calculate the relative weight percent concentrations of the identified components. The qualitative and quantitative compositional results that were derived from the single trial ¹H/¹⁹F-NMR cross integration analysis are summarized below in TABLE-1. The relative weight percent concentrations shown in TABLE-1 should be very close to their respective absolute weight percent values assuming no water was present in the sample. Trace amounts of numerous other unassigned components were also detected in the NMR spectra, but additional work would be needed in an effort to identify or quantify some of these other components.

Exact Copy of Original

mk 10/31/02
Initial Date

October 30, 2002

3M SMMD Analytical Lab Request # GID:71638
TCR-99131-025, Lot PU/07219EU: Telomer Project

Copies of the NMR spectra are attached with the paper copy of this report for your reference. If you have any questions about these results, please let me know.

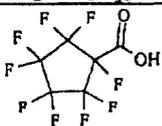
Tom Kestner

c: Mark Ellefson
Rick Payfer
Ron Purcell
William Reagen

File Reference: Is71638.GID.TCR-99131-025_Lot PU/07219EU_Telomer Project.DOC/101

TABLE-1

Sample: TCR-99131-025, Lot PU/07219EU from the Telomer project.
Overall Compositional Results by $^1\text{H}/^{19}\text{F}$ -NMR Cross Integration Analysis

Component Structures ¹	$^1\text{H}/^{19}\text{F}$ -NMR Relative Weight% Concentrations (single trial analysis)
$\text{CF}_3(\text{CF}_2)_n\text{-CO}_2\text{H}$ (where average $n = 5.05$)	98.24%
Probable internal branched isomers $\text{CF}_3(\text{CF}_2)_x\text{-CF}(\text{CF}_3)\text{-(CF}_2)_y\text{-CO}_2\text{H}$ (where $x \neq 0$ and assume $x+y = 3.05$ for calculation purposes)	0.87%
Probable terminal isopropyl branched isomers $(\text{CF}_3)_2\text{-CF-(CF}_2)_n\text{-CO}_2\text{H}$ (assume $n=3.05$ for calculation purposes)	0.52%
A probable ether/acid as possible $\text{O}-(\text{CF}_2\text{CF}_2\text{-CO}_2\text{H})_2$	0.13%
Probable 	0.11%
$\text{C}_n\text{H}_{2n+2}$ saturated aliphatic hydrocarbons and functional aliphatic components, $\text{C}_n\text{H}_{2n+1}\text{-X}$, where -X can be possible ether and ester functional groups.	$\approx 0.11\%$
Possible $\text{CF}_3(\text{CF}_2)_n\text{-CO}_2\text{CH}_3$ (where average $n = 5.05$)	0.021%

1. Trace amounts of numerous other unassigned components were also detected in the ^{19}F -NMR spectrum.

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Cmc 10/31/02
Initial Date

Certificate of Analysis

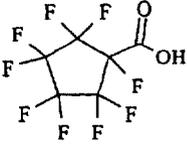
Nominal Product: $\text{CF}_3(\text{CF}_2)_n\text{-CO}_2\text{H}$, where average $n \approx 5$
Tridecafluoroheptanoic acid

Product Code: TCR-99131-025, Lot PU/07219EU

October 30, 2002

The sample of TCR-99131-025, Lot PU/07219EU was analyzed using a combination of ^{19}F -NMR and ^1H -NMR spectral analysis techniques. The overall qualitative and quantitative compositional results that were derived from these combined analyses are summarized below in TABLE-1.

TABLE-1
Sample: TCR-99131-025, Lot PU/07219EU
Overall Compositional Results by Combined $^{19}\text{F}/^1\text{H}$ -NMR Spectral Analyses

Component Structures ¹	$^1\text{H}/^{19}\text{F}$ -NMR Relative Weight% Concentrations (single trial analysis)
$\text{CF}_3(\text{CF}_2)_n\text{-CO}_2\text{H}$ (where average $n = 5.05$)	$\leq 98.2\%$ Purity
Probable internal branched isomers $\text{CF}_3(\text{CF}_2)_x\text{-CF}(\text{CF}_3)\text{-(CF}_2)_y\text{-CO}_2\text{H}$ (where $x \neq 0$ and assume $x+y = 3.05$ for calculation purposes)	0.87%
Probable terminal isopropyl branched isomers $(\text{CF}_3)_2\text{-CF-(CF}_2)_n\text{-CO}_2\text{H}$ (assume $n=3.05$ for calculation purposes)	0.52%
A probable ether/acid as possible $\text{O-}[\text{CF}_2\text{CF}_2\text{-CO}_2\text{H}]_2$	0.13%
Probable 	0.11%
$\text{C}_n\text{H}_{2n+2}$ saturated aliphatic hydrocarbons and functional aliphatic components, $\text{C}_n\text{H}_{2n+1}\text{-X}$, where -X can be possible ether and ester functional groups.	$\approx 0.11\%$
Possible $\text{CF}_3(\text{CF}_2)_n\text{-CO}_2\text{CH}_3$ (where average $n = 5.05$)	0.021%

1. Trace amounts of numerous other unassigned components were also detected in the NMR spectra.

Tom Kestner

Exact Copy of Original

cmc 10/31/02
Initial Date

Test Control and Reference Substance Log

Substance trade name or reference #	Pentadecafluorooctanoic acid	TCR Substance #	TCR-617
Substance/chemical name:	Pentadecafluorooctanoic acid	TCR #	TCR-617
Lot/batch #:	210002	Received from:	Oakwood Products
3M #			
Expiration date:		Amount received (wt. or vol):	5g
Initials:	OK	Date:	07/19/2002
Number/size of containers:	1-10ml amber glass vial	Shipper:	Courier
Condition:	white crystals	MSDS (y/n)	● Y () N
Retain		Date of Retain	

Archived/Substance Not Available

Purity:	99.51%, updated 10/30/02 OK, NMR analysis
Records received:	MSDS Certificate of Analysis LAS 07/25/02
Location of synthesis, fabrication, or derivation records:	Oakwood products
Std Location/Storage:	Room Temp, TCR-C01
Molecular Formula:	C8HF15O2
Comments	Corrosive Solid LAS 07/19/02 Shipping comments: No 3M ID# - must ship ground small quantity exception (30 mL/g or less per vial). LAS 10/02/02
Attachment(s)	 TCR-617.pdf

Exact Copy of Original

CMC 10/31/02
Initial Date

000260

Oakwood Products, Inc.

1741 Old Dunbar Road
West Columbia, SC 29172
Phone (803) 739-8600
Fax (803) 739-6957

CERTIFICATE OF ANALYSIS

Date: 15-Jul-02

Material: Pentadecafluorooctanoic acid

Cat.No.: 1319

Cas No.: [335-67-1]

Lot No.: 210002

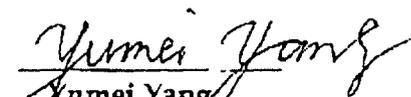
Assay: 97+% by NaOH titration

Appearance: White solid

Melting Point: 59-61°C

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CINC 10/31/02
Initial Date


Yumei Yang
QC Manager

000261

USE LOG							
Pentadecafluorooctanoic acid							
Pentadecafluorooctanoic acid							
TCR-617							

Gross Wt./Vol. Before withdrawal Balance ID 1	Amnt. withdrawn (mass or vol) Balance ID 2	Gross wt/vol after withdrawal Balance ID 1	Purpose (enter standard number or reason for removal)	Balance ID 1	Balance ID 2	Initials	Date
15.5486g	0.0617g	15.4869g	standard 02040-45	914	-	RWW	08/15/2002

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cmc 10/31/02
Initial Date

000262

SINGLE COMPONENT PREPARATION LOG

Date: 8/15/02

Book No. 02 040

Analyst: RWW

Page No. 45

Description: Pentadecafluorooctanoic Acid Stock C7 F15COOH

Stock Number: TCR-617

Weight or Volume Used: 0.0615g

Balance ID: 914

Concentration or Purity: NA

Other Correction Factors: NA

Corrected Weight: 0.0615g

Solvent and TN-A Number: MeOH/TN-A-6243

Final Volume: 50ml

Final Concentration: 1230ppm

Storage Location: 2nd Floor - Room Temp

Expiration Date: 2/15/03

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cmc 10/31/02
Initial Date

Reviewed by: Jim A. Stevenson 08/15/02
Signature Date

000263

3M SPECIALTY MATERIALS MANUFACTURING DIVISION ANALYTICAL LABORATORY

Request # **GID:71638**

To: Lisa Stevenson - (8-5568) - ET&SS - 2-3E-09
From: Tom Kestner - (3-5633) - SMMD Analytical Lab - 236-2B-11
Subject: Characterization of TCR-617 by ¹H-NMR, ¹⁹F-NMR, and LC/MS Analyses
Date: October 28, 2002: Updated Report - LC/MS Analysis for Impurities

SAMPLE DESCRIPTION:

- TCR-617, lot 210002 from the Telomer project.
 Nominal product = CF₃(CF₂)_n-CO₂H, where average n ≈ 6 (white powder).

Sample	Spectra #'s	Experiment Descriptions
TCR-617	H71638.GID.501	500 MHz ¹ H-NMR in acetone-d ₆ solvent + p-HFX cross integration/internal std.
TCR-617	F71638.GID.501	470 MHz ¹⁹ F-NMR in acetone-d ₆ solvent + p-HFX cross integration/internal std.

UPDATE:

After the initial NMR compositional analysis report was issued to you on 10-24-02, Joel Miller performed a qualitative LC/MS analysis to assist in the assignment of some of the impurity components in the sample of TCR-617. This updated report summarizes the new information regarding the identities and concentrations for some of the impurity components. You will notice that the tentatively assigned olefin structure from the original report is now replaced with a probable (C₆F₁₃O)-CO₂H ether acid.

OBJECTIVE:

This sample was subjected to a combination of ¹H-NMR and ¹⁹F-NMR spectral analyses to determine the purity of the nominal product. Special emphasis was also placed on attempting to identify and quantify any impurity components. The sample was also given to Joel Miller for a qualitative LC/MS analysis to assist in the assignment of impurity components. Joel asked that I incorporate the qualitative information from his LC/MS analysis in this updated report.

FT-NMR EXPERIMENTAL:

A portion of the sample was accurately weighed, spiked with a known amount of 1,4-bis(trifluoromethyl)benzene (p-HFX), and then totally dissolved in deuterated acetone (acetone-d₆) for subsequent analysis by NMR. A 500 MHz ¹H-NMR spectrum and a 470 MHz ¹⁹F-NMR spectrum were acquired at room temperature using a Varian UNITY INOVA 500 FT-NMR spectrometer. This sample preparation method permitted the p-HFX to be used as either 1) a ¹H/¹⁹F-NMR internal standard to allow the calculation of the absolute weight percent concentrations of specific components, or 2) a ¹H/¹⁹F-NMR cross integration standard to permit the cross correlation of the relative ¹H and ¹⁹F signal intensities for evaluation of the overall sample composition.

RESULTS:

The combined ¹H-NMR, ¹⁹F-NMR, and LC/MS analyses indicated this sample was a high purity form of the nominal product, CF₃(CF₂)_n-CO₂H, where the average value of n = 6.02. Small amounts of a few impurity components, including probable isomers, were also assigned. A ¹H/¹⁹F-NMR cross integration analysis technique was then used to calculate the relative weight percent concentrations of the identified components. The qualitative and quantitative compositional results that were derived from the combined single trial ¹H/¹⁹F-NMR cross integration analysis, and the qualitative LC/MS analysis, are summarized below in TABLE-1. The relative weight percent concentrations shown in TABLE-1 should be very close to their respective absolute weight percent values assuming no water was present in the sample.

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CMC 10/31/02
Initial Date

000264

October 28, 2002

3M SMMD Analytical Lab Request # GID:71638
Updated Report - LC/MS Analysis for Impurities in TCR-617, Lot 210002

RESULTS (cont.):

Trace amounts of a few other unassigned components were also detected in the NMR spectra, but additional work would be needed in an effort to identify or quantify these other components.

Copies of the NMR spectra are attached with the paper copy of this report for your reference. If you have any questions about these updated results, please let me know.

Tom Kestner

c: Joel Miller
Rick Payfer
Ron Purcell
William Reagen

File Reference: Is71638.GID.Updated Results_TCR-617_Lot 210002_Telomer Project.DOC/101

TABLE-1

Sample: TCR-617, Lot 210002 from the Telomer project.
Overall Compositional Results by ¹H/¹⁹F-NMR Cross Integration and LC/MS Analyses

Component Structures ¹	NMR Relative Weight% Concentrations (single trial measurement)
CF ₃ (CF ₂) _n -CO ₂ H where average n = 6.02 by ¹⁹ F-NMR. LC/MS showed n=6 (major), n=5, n=4 (minors).	99.52%
Probable (CF ₃) ₂ -CF-(CF ₂) _n -CO ₂ H assume n=4 for calculation purposes	0.39%
Probable (C ₆ F ₁₃ O)-CO ₂ H acyclic ether acid as possible CF ₃ CF ₂ -O-CF(CF ₃)-CF ₂ CF ₂ -CO ₂ H	0.057%
Possible CF ₃ (CF ₂) _x -CF(CF ₃)-(CF ₂) _y -CO ₂ H where x≠0, y≠0 and assume x+y = 4 for calculation purposes	0.019%
Possible (CF ₃) ₃ -C-(CF ₂) _n -CO ₂ H assume n=3 for calculation purposes	0.013%
Possible C _n H _{2n+2} saturated aliphatic hydrocarbons	0.0079%

1. Trace amounts of other unassigned components were also detected in the NMR spectra.

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CME 10/31/02
Initial Date

Certificate of Analysis

Nominal Product: $\text{CF}_3(\text{CF}_2)_n\text{-CO}_2\text{H}$, where average $n \approx 6$

Pentadecafluorooctanoic acid

Product Code: TCR-617, Lot 210002

October 28, 2002

Tom Kestner and Joel Miller

The sample of TCR-617, lot 210002 was analyzed using a combination of ^{19}F -NMR, ^1H -NMR, and LC/MS analysis techniques. The overall qualitative and quantitative compositional results that were derived from these combined analyses are summarized below in TABLE-1.

TABLE-1

Sample: TCR-617, Lot 210002

Quantitative and Qualitative Compositional Results by Combined $^{19}\text{F}/^1\text{H}$ -NMR and LC/MS Analyses

Component Structures ¹	$^1\text{H}/^{19}\text{F}$ -NMR Relative Weight% Concentrations (single trial analysis)
$\text{CF}_3(\text{CF}_2)_n\text{-CO}_2\text{H}$ where average $n = 6.02$ by ^{19}F -NMR. LC/MS showed $n=6$ (major), $n=5$, $n=4$ (minors).	$\leq 99.51\%$ Purity
Probable $(\text{CF}_3)_2\text{-CF-(CF}_2)_n\text{-CO}_2\text{H}$ assume $n=4$ for calculation purposes	0.39%
Probable $(\text{C}_6\text{F}_{13}\text{O})\text{-CO}_2\text{H}$ acyclic ether acid as possible $\text{CF}_3\text{CF}_2\text{-O-CF(CF}_3\text{)-CF}_2\text{CF}_2\text{-CO}_2\text{H}$	0.057%
Possible $\text{CF}_3(\text{CF}_2)_x\text{-CF(CF}_3\text{)-(CF}_2)_y\text{-CO}_2\text{H}$ where $x \neq 0$, $y \neq 0$ and assume $x+y = 4$ for calculation purposes	0.019%
Possible $(\text{CF}_3)_3\text{-C-(CF}_2)_n\text{-CO}_2\text{H}$ assume $n=3$ for calculation purposes	0.013%
Possible $\text{C}_n\text{H}_{2n+2}$ saturated aliphatic hydrocarbons	0.0079%

1. Trace amounts of other unassigned components were also detected in the NMR spectra.

Exact Copy of Original

Cmc 10/31/02
Initial Date

Test Control and Reference Substance Log

Substance trade name or reference #	Heptadecafluorononanoic acid	TCR Substance #	TCR-618
Substance/chemical name:	Heptadecafluorononanoic acid	TCR #	TCR-618
Lot/batch #:	H7568	Received from:	Oakwood Products
3M #	NA		
Expiration date:		Amount received (wt. or vol):	5g
Initials:	OK	Date:	07/19/2002
Number/size of containers:	1-50ml plastic jar	Shipper:	Courier
Condition:	white crystals	MSDS (y/n)	<input checked="" type="radio"/> Y <input type="radio"/> N
Retain		Date of Retain	

Archived/Substance Not Available

Purity:	98.02% updated 10/30/02 OK, NMR analysis
Records received:	MSDS Certificat of Analysis LAS 07/25/02
Location of synthesis, fabrication, or derivation records:	Oakwood Products
Std Location/Storage:	Room Temp, TCR-C01
Molecular Formula:	C9HF17O2
Comments	Shipping comments: Corrosive solid, No 3M ID# - must ship ground small quantity exception (30 mL/g or less per vial). LAS 10/02/02
Attachment(s)	 TCR-618.pdf

Exact Copy of Original
Cnc 10/31/02
Initial Date

000267

Oakwood Products, Inc.
1741 Old Dunbar Road
West Columbia, SC 29172
Phone (803) 739-8800
Fax (803) 739-6957

CERTIFICATE OF ANALYSIS

Date: 27-Nov-01

Material: Heptadecafluorononanoic acid

Cat.No.: 2263

Cas No.: [375-95-1]

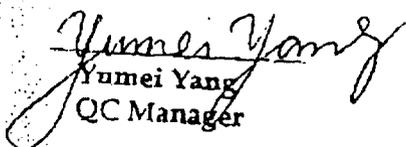
Lot No.: H7568

Assay: 99+% by NaOH titration

Appearance: Off-white solid

Melting Point: 57-62°C

Exact Copy of Original
Cinc 10/2/01
Initial Date


Yumei Yang
QC Manager

000268

USE LOG							
Heptadecafluorononanoic acid							
Heptadecafluorononanoic acid							
TCR-618							

Gross Wt./Vol. Before withdrawal Balance ID 1	Amnt. withdrawn (mass or vol) Balance ID 2	Gross wt/vol after withdrawal Balance ID 1	Purpose (enter standard number or reason for removal)	Balance ID 1	Balance ID 2	Initials	Date
20.8649g	0.0677g	20.7972g	standard 02040-46	914	-	RWW	05/18/2002

Exact Copy of Original

CMS *10/31/02*
Initial Date

000269

SINGLE COMPONENT PREPARATION LOG

Date: 8/15/02
Analyst: RW

Book No. 02 040
Page No. 46

Description: Heptadeca Mercaptanoic Acid Stock CR F176004

Stock Number: TCR-618

Weight or Volume Used: 0.0634g

Balance ID: 919

Concentration or Purity: NA

Other Correction Factors: NA

Corrected Weight: 0.0634g

Solvent and TN-A Number: MeOH/TN-A-6293

Final Volume: 50ml

Final Concentration: 1268 ppm

Storage Location: 2nd Floor - Room Temp

Expiration Date: 2/15/03

Exact Copy of Original

Cmc 10/31/02
Initial Date

Reviewed by: Vin A. Stevenson 08/15/02
Signature Date

000270

3M SPECIALTY MATERIALS MANUFACTURING DIVISION ANALYTICAL LABORATORY

Request # **GID:71638**

To: Lisa Stevenson - (8-5568) - ET&SS - 2-3E-09
From: Tom Kestner - (3-5633) - SMMD Analytical Lab - 236-2B-11
Subject: Characterization of TCR-618 by ¹H-NMR and ¹⁹F-NMR Spectroscopy
Date: October 28, 2002

SAMPLE DESCRIPTION:

- TCR-618, lot H7568 from the Telomer project.
 Nominal product = CF₃(CF₂)_n-CO₂H, where average n ≈ 7 (white solid).

Sample	Spectra #'s	Experiment Descriptions
TCR-618	H71638.GID.402	400 MHz ¹ H-NMR in acetone-d ₆ solvent + p-HFX cross integration/internal std.
TCR-618	F71638.GID.402/403	376 MHz ¹⁹ F-NMR in acetone-d ₆ solvent + p-HFX cross integration/internal std.

OBJECTIVE:

This sample was subjected to a combination of ¹H-NMR and ¹⁹F-NMR spectral analyses to determine the purity of the nominal product. Special emphasis was also placed on attempting to identify and quantify any impurity components.

EXPERIMENTAL:

A portion of the sample was accurately weighed, spiked with a known amount of 1,4-bis(trifluoromethyl)benzene (p-HFX), and then totally dissolved in deuterated acetone (acetone-d₆) for subsequent analysis by NMR. A 400 MHz ¹H-NMR spectrum and 376 MHz ¹⁹F-NMR spectra were acquired at room temperature using a Varian UNITYplus 400 FT-NMR spectrometer. This sample preparation method permitted the p-HFX to be used as either 1) a ¹H/¹⁹F-NMR internal standard to allow the calculation of the absolute weight percent concentrations of specific components, or 2) a ¹H/¹⁹F-NMR cross integration standard to permit the cross correlation of the relative ¹H and ¹⁹F signal intensities for evaluation of the overall sample composition.

RESULTS:

The ¹H-NMR and ¹⁹F-NMR spectral data indicated this sample was a high purity form of the nominal product, CF₃(CF₂)_n-CO₂H, where the average value of n = 6.955. Small amounts of a few impurity components were also assigned. A ¹H/¹⁹F-NMR cross integration analysis technique was then used to calculate the relative weight percent concentrations of the identified components. The qualitative and quantitative compositional results that were derived from the single trial ¹H/¹⁹F-NMR cross integration analysis are summarized below in TABLE-1. The relative weight percent concentrations shown in TABLE-1 should be very close to their respective absolute weight percent values assuming no water was present in the sample. Trace amounts of a few other unassigned components were also detected in the NMR spectra, but additional work would be needed in an effort to identify or quantify these other components.

Copies of the NMR spectra are attached with the paper copy of this report for your reference. If you have any questions about these results, please let me know.

Exact Copy of Original

CKK 10/31/02
 Initial Date

October 28, 2002

3M SMMD Analytical Lab Request # GID:71638
TCR-618, Lot H7568: Telomer Project

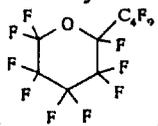
Tom Kestner

c: Rick Payfer
Ron Purcell
William Reagen

File Reference: Is71638.GID.TCR-618_Lot H7568_Telomer Project.DOC/100

TABLE-1

Sample: TCR-618, Lot H7568 from the Telomer project.
Overall Compositional Results by ¹H/¹⁹F-NMR Cross Integration Analysis

Component Structures ¹	NMR Relative Weight% Concentrations (single trial measurement)
CF ₃ (CF ₂) _n -CO ₂ H where average n = 6.955	98.02%
Probable H-(CF ₂) _n -CO ₂ H assume n = 8 for calculation purposes	1.10%
Probable CF ₃ (CF ₂) _n -CO ₂ CH ₃ where average n = 6.955	0.65%
A probable cyclic ether  as possible or similar	0.20%
Total inorganic fluoride (at least 3-types), including some possible HF	0.021%
Possible C _n H _{2n+2} saturated aliphatic hydrocarbons	0.014%

1. Trace amounts of other unassigned components were also detected in the NMR spectra.

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Cinc 10/31/02
Initial Date

Certificate of Analysis

Nominal Product: $\text{CF}_3(\text{CF}_2)_n\text{-CO}_2\text{H}$, where average $n \approx 7$

Heptadecafluorononanoic acid

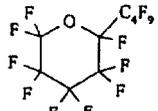
Product Code: TCR-618, Lot H7568

October 28, 2002

Tom Kestner

The sample of TCR-618, lot H7568 was analyzed using a combination of ^{19}F -NMR and ^1H -NMR spectral analysis techniques. The overall qualitative and quantitative compositional results that were derived from these combined analyses are summarized below in TABLE-1.

TABLE-1
Sample: TCR-618, Lot H7568
Quantitative Compositional Results by Combined $^{19}\text{F}/^1\text{H}$ -NMR Spectral Analyses

Component Structures ¹	$^1\text{H}/^{19}\text{F}$ -NMR Relative Weight% Concentrations (single trial analysis)
$\text{CF}_3(\text{CF}_2)_n\text{-CO}_2\text{H}$ where average $n = 6.955$	$\leq 98.02\%$ Purity
Probable $\text{H}-(\text{CF}_2)_n\text{-CO}_2\text{H}$ assume $n = 8$ for calculation purposes	1.10%
Probable $\text{CF}_3(\text{CF}_2)_n\text{-CO}_2\text{CH}_3$ where average $n = 6.955$	0.65%
A probable cyclic ether  as possible or similar	0.20%
Total inorganic fluoride (at least 3-types), including some possible HF	0.021%
Probable $\text{C}_n\text{H}_{2n+2}$ saturated aliphatic hydrocarbons	0.014%

1. Trace amounts of other unassigned components were also detected in the NMR spectra.

Exact Copy of Original
cmc 10/31/02
Initial Date

Test Control and Reference Substance Log

Substance trade name or reference #	Nonadecafluorodecanoic acid	TCR Substance #	SD036
Substance/chemical name:	Nonadecafluorodecanoic acid	TCR #	TCR-36
Lot/batch #:	R11K	Received from:	Oakwood Products Inc 4/2/99
3M #	ID#2264		
Expiration date:	12/01/2010	Amount received (wt. or vol):	25g, 43.3264 g gross wt.
Initials:	JCP	Date:	04/27/1999
Number/size of containers:	1/120 mL plastic bottle	Shipper:	N/A
Condition:	white solid MCH 07/22/99	MSDS (y/n)	Y () N
Retain	0.1874g	Date of Retain	10/18/1999

Archived/Substance Not Available

Purity:	98.01%, updated 10/30/02 OK NMR analysis
Records received:	MSDS, Certificate of Analysis
Location of synthesis, fabrication, or derivation records:	Unknown
Std Location/Storage:	F19, Frozen
Molecular Formula:	C9F19COOH
Comments	TN-A-2451 prior to 4/27/99 JCP 04/27/99 Standard has been moved to Freezer 19 in room 347 KJD 06/06/00 Standard was stored at room temperature prior to 06/06/00. LAC 12/19/00
Attachment(s)	  sd036msds.pdf sd036cofa.pdf

Exact Copy of Original

CJK 10/31/02
Initial Date

000274

3M SPECIALTY MATERIALS MANUFACTURING DIVISION ANALYTICAL LABORATORY

Request # **GID:71638**

To: Lisa Stevenson - (8-5568) - ET&SS - 2-3E-09
From: Tom Kestner - (3-5633) - SMMD Analytical Lab - 236-2B-11
Subject: Characterization of SD036 by ¹H-NMR and ¹⁹F-NMR Spectroscopy
Date: October 28, 2002

SAMPLE DESCRIPTION:

- SD036, lot R11K from the Telomer project.
Nominal product = CF₃(CF₂)_n-CO₂H, where average n ≈ 8 (white powder).

Sample	Spectra #'s	Experiment Descriptions
SD036	H71638.GID.403	400 MHz ¹ H-NMR in acetone-d ₆ solvent + p-HFX cross integration/internal std.
SD036	F71638.GID.404	376 MHz ¹⁹ F-NMR in acetone-d ₆ solvent + p-HFX cross integration/internal std.

OBJECTIVE:

This sample was subjected to a combination of ¹H-NMR and ¹⁹F-NMR spectral analyses to determine the purity of the nominal product. Special emphasis was also placed on attempting to identify and quantify any impurity components.

EXPERIMENTAL:

A portion of the sample was accurately weighed, spiked with a known amount of 1,4-bis(trifluoromethyl)benzene (p-HFX), and then totally dissolved in deuterated acetone (acetone-d₆) for subsequent analysis by NMR. A 400 MHz ¹H-NMR spectrum and a 376 MHz ¹⁹F-NMR spectrum were acquired at room temperature using a Varian UNITYplus 400 FT-NMR spectrometer. This sample preparation method permitted the p-HFX to be used as either 1) a ¹H/¹⁹F-NMR internal standard to allow the calculation of the absolute weight percent concentrations of specific components, or 2) a ¹H/¹⁹F-NMR cross integration standard to permit the cross correlation of the relative ¹H and ¹⁹F signal intensities for evaluation of the overall sample composition.

RESULTS:

The ¹H-NMR and ¹⁹F-NMR spectral data indicated this sample was a relatively high purity form of the nominal product, CF₃(CF₂)_n-CO₂H, where the average value of n = 7.85. Small amounts of a few impurity components, including probable isomers, were also assigned. A ¹H/¹⁹F-NMR cross integration analysis technique was then used to calculate the relative weight percent concentrations of the identified components. The qualitative and quantitative compositional results that were derived from the single trial ¹H/¹⁹F-NMR cross integration analysis are summarized below in TABLE-1. The relative weight percent concentrations shown in TABLE-1 should be very close to their respective absolute weight percent values assuming no water was present in the sample. Trace amounts of a **numerous** other unassigned components were also detected in the ¹⁹F-NMR spectrum, but additional work would be needed in an effort to identify or quantify some of these other components.

Copies of the NMR spectra are attached with the paper copy of this report for your reference. If you have any questions about these results, please let me know.

Exact Copy of Original
LM Initial 10/31/02 Date

October 28, 2002

3M SMMD Analytical Lab Request # GID:71638
SD036, Lot R11K: Telomer Project

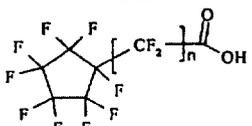
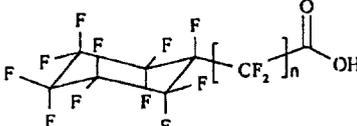
Tom Kestner

c: Rick Payfer
Ron Purcell
William Reagen

File Reference: Is71638.GID.SD036_Lot R11K_Telomer Project.DOC/101

TABLE-1

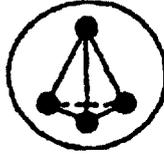
Sample: SD036, Lot R11K from the Telomer project.
Overall Compositional Results by $^1\text{H}/^{19}\text{F}$ -NMR Cross Integration Analysis

Component Structures ¹	NMR Relative Weight% Concentrations (single trial measurement)
$\text{CF}_3(\text{CF}_2)_n\text{-CO}_2\text{H}$ where average $n = 7.85$	98.01%
Probable $\text{CF}_3(\text{CF}_2)_x\text{-CF}(\text{CF}_3)\text{-(CF}_2)_y\text{-CO}_2\text{H}$ where $x \neq 0$ and assume $x+y = 5.85$ for calculation purposes	1.41%
Probable $(\text{CF}_3)_2\text{-CF-(CF}_2)_n\text{-CO}_2\text{H}$ assume $n=5.85$ for calculation purposes	0.36%
Probable  assume $n=3.85$ for calculation purposes	0.12%
Probable  assume $n=2.85$ for calculation purposes	0.060%
Probable $\text{C}_n\text{H}_{2n+2}$ saturated aliphatic hydrocarbons	0.026%
Total inorganic fluoride	$\geq 0.0095\%$

1. Trace amounts of numerous other unassigned components were also detected in the ^{19}F -NMR spectrum.

Exact Copy of Original

RPK 10/31/02
Initial Date



Oakwood Products, Inc.
1741 Old Dunbar Road
West Columbia, SC 29172
Phone (803) 739-8800
Fax (803) 739-6957

CERTIFICATE OF ANALYSIS

Date: 11-Feb-00

Material: Nonadecafluorodecanoic acid

Cas No.: [335-76-2]

Lot No.: R11K

Assay: Products more volatile than Perfluorodecanoic acid <1%
Perfluorodecaonic acid 98% min.
Products less volatile than Perfluorodecanoic acid <1%

Appearance: White solid

Melting Point: 83-85°C

Exact Copy of Original

CHK 10/31/02
Initial Date

Yumei Yang
Yumei Yang
Quality Control

USE LOG

Nonadecafluorodecanoic acid
Nonadecafluorodecanoic acid
SD036

Gross Wt./Vol. Before withdrawal Balance ID 1	Amnt. withdrawn (mass or vol) Balance ID 2	Gross wt/vol after withdrawal Balance ID 1	Purpose (enter standard number or reason for removal)	Balance ID 1	Balance ID 2	Initials	Date
33.9944g	0.1196g	33.8784g	standard 02022-90	914	-	RWW	07/02/2002

Exact Copy of Original

CWC 10/31/02
Initial Date

SINGLE COMPONENT PREPARATION LOG

Date: 7/2/02 Book No. 02 022
Analyst: RW Page No. 90
Description: PFDA stock
Stock Number: SD036
Weight or Volume Used: 103.1 mg Balance ID: 914
Concentration or Purity: 98.7% Other Correction Factors: NA
Corrected Weight: 101.0 mg Solvent and TN-A Number: Methanol - TN-A-06205
Final Volume: 100 ml Final Concentration: 1010 ppm
Storage Location: 2nd Floor Expiration Date: 1/2/03

Exact Copy of Original

CNC 10/31/02
Initial Date

Reviewed by: Cindy Carlson 7/17/02
Signature Date

Test Control and Reference Substance Log

Substance trade name or reference #	Perfluoroundecanoic acid	TCR Substance #	TCR-619
Substance/chemical name:	Perfluoroundecanoic acid	TCR #	TCR-619
Lot/batch #:	U11N	Received from:	Oakwood products
3M #	NA		
Expiration date:		Amount received (wt. or vol):	5 g
Initials:	OK	Date:	07/19/2002
Number/size of containers:	1-10ml amber glass vial	Shipper:	Courier
Condition:	white crystals	MSDS (y/n)	<input checked="" type="radio"/> Y <input type="radio"/> N
Retain		Date of Retain	

Archived/Substance Not Available

Purity:	>99% LAS 07/25/02
Records received:	MSDS Certificate of Analysis LAS 07/25/02
Location of synthesis, fabrication, or derivation records:	Oakwood products
Std Location/Storage:	Room Temp, TCR-C01
Molecular Formula:	C11HF21O2
Comments	Shipping comments: Harmful solid, No 3M ID# - must ship ground small quantity exception (30 mL/g or less per vial). LAS 10/02/02
Attachment(s)	 TCR-619.pdf

Exact Copy of Original

CML *10/31/02*
Initial Date

000280

3M SPECIALTY MATERIALS MANUFACTURING DIVISION ANALYTICAL LABORATORY

Request # **GID:71638**

To: Lisa Stevenson - (8-5568) - ET&SS - 2-3E-09
From: Tom Kestner - (3-5633) - SMMD Analytical Lab - 236-2B-11
Subject: Characterization of TCR-619 by ¹H-NMR and ¹⁹F-NMR Spectroscopy
Date: October 29, 2002

SAMPLE DESCRIPTION:

- TCR-619, lot U11N from the Telomer project.
 Nominal product = CF₃(CF₂)_n-CO₂H, where average n ≈ 9 (white powder).

Sample	Spectra #'s	Experiment Descriptions
TCR-619	H71638.GID.404	400 MHz ¹ H-NMR in acetone-d ₆ solvent + p-HFX cross integration/internal std.
TCR-619	F71638.GID.405	376 MHz ¹⁹ F-NMR in acetone-d ₆ solvent + p-HFX cross integration/internal std.

OBJECTIVE:

This sample was subjected to a combination of ¹H-NMR and ¹⁹F-NMR spectral analyses to determine the purity of the nominal product. Special emphasis was also placed on attempting to identify and quantify any impurity components.

EXPERIMENTAL:

A portion of the sample was accurately weighed, spiked with a known amount of 1,4-bis(trifluoromethyl)benzene (p-HFX), and then totally dissolved in deuterated acetone (acetone-d₆) for subsequent analysis by NMR. A 400 MHz ¹H-NMR spectrum and a 376 MHz ¹⁹F-NMR spectrum were acquired at room temperature using a Varian UNITYplus 400 FT-NMR spectrometer. This sample preparation method permitted the p-HFX to be used as either 1) a ¹H/¹⁹F-NMR internal standard to allow the calculation of the absolute weight percent concentrations of specific components, or 2) a ¹H/¹⁹F-NMR cross integration standard to permit the cross correlation of the relative ¹H and ¹⁹F signal intensities for evaluation of the overall sample composition.

RESULTS:

The ¹H-NMR and ¹⁹F-NMR spectral data indicated this sample was a relatively high purity form of the nominal product, CF₃(CF₂)_n-CO₂H, where the average value of n = 8.63. Small amounts of a few impurity components, including probable isomers, were also assigned. A ¹H/¹⁹F-NMR cross integration analysis technique was then used to calculate the relative weight percent concentrations of the identified components. The qualitative and quantitative compositional results that were derived from the single trial ¹H/¹⁹F-NMR cross integration analysis are summarized below in TABLE-1. The relative weight percent concentrations shown in TABLE-1 should be very close to their respective absolute weight percent values assuming no water was present in the sample. Trace amounts of numerous other unassigned components were also detected in the ¹⁹F-NMR spectrum, but additional work would be needed in an effort to identify or quantify some of these other components.

Copies of the NMR spectra are attached with the paper copy of this report for your reference. If you have any questions about these results, please let me know.

Exact Copy of Original
 CLK 10/31/02
 Initial Date

October 29, 2002

3M SMD Analytical Lab Request # GID:71638
TCR-619, Lot U11N: Telomer Project

Tom Kestner

c: Mark Ellefson
Rick Payfer
Ron Purcell
William Reagen

File Reference: Is71638.GID.TCR-619_Lot U11N_Telomer Project.DOC/101

TABLE-1

Sample: TCR-619, Lot U11N from the Telomer project.
Overall Compositional Results by ¹H/¹⁹F-NMR Cross Integration Analysis

Component Structures ¹	NMR Relative Weight% Concentrations (single trial measurement)
CF ₃ (CF ₂) _n -CO ₂ H (where average n = 8.63)	96.4%
Probable internal branched isomers CF ₃ (CF ₂) _x -CF(CF ₃)-(CF ₂) _y -CO ₂ H (where x≠0 and assume x+y = 6.63 for calculation purposes)	2.6%
Probable trans-olefins of general form: trans-CF ₃ (CF ₂) _x -CF=CF-(CF ₂) _y -CO ₂ H (assume x+y = 6.63 for calculation purposes)	0.89%
Possible terminal isopropyl branched isomers (CF ₃) ₂ -CF-(CF ₂) _n -CO ₂ H (assume n=6.63 for calculation purposes)	0.12%
Probable C _n H _{2n+2} saturated aliphatic hydrocarbons	0.032%
Total inorganic fluoride	≥0.0017%

1. Trace amounts of numerous other unassigned components were also detected in the ¹⁹F-NMR spectrum.

Exact Copy of Original

cmc 10/31/02
Initial Date

Certificate of Analysis

Nominal Product: $\text{CF}_3(\text{CF}_2)_n\text{-CO}_2\text{H}$, where average $n \approx 9$

Perfluoroundecanoic acid

Product Code: TCR-619, Lot U11N

October 29, 2002

The sample of TCR-619, lot U11N was analyzed using a combination of ^{19}F -NMR and ^1H -NMR spectral analysis techniques. The overall qualitative and quantitative compositional results that were derived from these combined analyses are summarized below in TABLE-1.

TABLE-1
Sample: TCR-619, Lot U11N
Overall Compositional Results by Combined $^{19}\text{F}/^1\text{H}$ -NMR Spectral Analyses

Component Structures ¹	$^1\text{H}/^{19}\text{F}$ -NMR Relative Weight% Concentrations (single trial analysis)
$\text{CF}_3(\text{CF}_2)_n\text{-CO}_2\text{H}$ (where average $n = 8.63$)	$\leq 96.4\%$ Purity
Probable internal branched isomers $\text{CF}_3(\text{CF}_2)_x\text{-CF}(\text{CF}_3)\text{-(CF}_2)_y\text{-CO}_2\text{H}$ (where $x \neq 0$ and assume $x+y = 6.63$ for calculation purposes)	2.6%
Probable trans-olefins of general form: $\text{trans-CF}_3(\text{CF}_2)_x\text{-CF=CF-(CF}_2)_y\text{-CO}_2\text{H}$ (assume $x+y = 6.63$ for calculation purposes)	0.89%
Possible terminal isopropyl branched isomers $(\text{CF}_3)_2\text{-CF-(CF}_2)_n\text{-CO}_2\text{H}$ (assume $n=6.63$ for calculation purposes)	0.12%
Probable $\text{C}_n\text{H}_{2n+2}$ saturated aliphatic hydrocarbons	0.032%
Total inorganic fluoride	$\geq 0.0017\%$

1. Trace amounts of numerous other unassigned components were also detected in the ^{19}F -NMR spectrum.

Tom Kestner

Exact Copy of Original
Cmk 10/31/02
Initial Date

Oakwood Products, Inc.

1741 Old Dunbar Road
West Columbia, SC 29172
Phone (803) 739-8800
Fax (803) 739-6957

CERTIFICATE OF ANALYSIS

Date: 15-Jul-02

Material: Perfluoroundecanoic acid

Cat.No.: 2265

Cas No.: [4234-23-5]

Lot No.: U11N

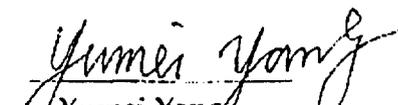
Assay: 99+% by NaOH titration

Appearance: White solid

Melting Point: 92-98°C

Exact Copy of Original

Cmc 10/31/02
Initial Date


Yumei Yang
QC Manager

000284

USE LOG
Perfluoroundecanoic acid Perfluoroundecanoic acid TCR-619

Gross Wt./Vol. Before withdrawal Balance ID 1	Amnt. withdrawn (mass or vol) Balance ID 2	Gross wt/vol after withdrawal Balance ID 1	Purpose (enter standard number or reason for removal)	Balance ID 1	Balance ID 2	Initials	Date
15.5264g	0.0691g	15.4573g	standard 02040-65	914	-	RWW	08/29/2002

Exact Copy of Original

CWC 10/3/02
Initial Date

000285

SINGLE COMPONENT PREPARATION LOG

Date: 8/29/02
Analyst: RWW

Book No. 02 040
Page No. 65

Description: Pec Fluoroundecanoic Acid

Stock Number: TCR-619

Weight or Volume Used: 0.0678g

Balance ID: 914

Concentration or Purity: NA

Other Correction Factors: NA

Corrected Weight: 0.0678g

Solvent and TN-A Number: MeOH/TN-A-6244

Final Volume: 50ml

Final Concentration: 1356 ppm

Storage Location: 2nd Floor - Am Temp

Expiration Date: 2/29/03

Exact Copy of Original

CMC 11/1/02
Initial Date

Reviewed by: Mike A Stevenson 10/01/02
Signature Date

000286

Test Control and Reference Substance Log

Substance trade name or reference #	Perfluorododecanoic acid	TCR Substance #	SD037
Substance/chemical name:	Perfluorododecanoic acid	TCR #	TCR-37
Lot/batch #:	R24K	Received from:	Oakwood Products Inc 4/2/99
3M #	ID#2266		
Expiration date:	12/01/2010	Amount received (wt. or vol):	25g, 43.4548 g gross wt.
Initials:	JCP	Date:	04/27/1999
Number/size of containers:	1/120 mL plastic bottle	Shipper:	N/A
Condition:	white powder MCH 07/22/99	MSDS (y/n)	<input checked="" type="radio"/> Y <input type="radio"/> N
Retain	0.2159g	Date of Retain	10/18/1999

Archived/Substance Not Available

Purity:	99.65% updated 10/30/02 OK, NMR analysis
Records received:	MSDS, Certificate of Analysis
Location of synthesis, fabrication, or derivation records:	Unknown
Std Location/Storage:	F19, Frozen
Molecular Formula:	C11F23COOH
Comments	TN-A-2453 prior to 4/27/99 JCP 04/27/99 Standard has been moved to Freezer 19 in room 347 KJD 06/06/00 Standard was stored at room temperature prior to 06/06/00. LAC 12/19/00 Shipping comments: Irritant, no shipping information - ship as small quantity exception. LAS 10/02/02
Attachment(s)	   sd037msds.pdf sd037cofa.pdf NMR SD037.pdf

Exact Copy of Original

JMC 10/31/02
Initial Date

3M SPECIALTY MATERIALS MANUFACTURING DIVISION ANALYTICAL LABORATORY

Request # **GID:71638**

To: Lisa Stevenson - (8-5568) – ET&SS – 2-3E-09
From: Tom Kestner - (3-5633) - SMMD Analytical Lab - 236-2B-11
Subject: Characterization of SD037 by ¹H-NMR and ¹⁹F-NMR Spectroscopy
Date: October 28, 2002

SAMPLE DESCRIPTION:

- SD-037, lot R24K from the Telomer project.
 Nominal product = CF₃(CF₂)_n-CO₂H, where average n ≈ 10 (white powder).

Samples	Spectra #'s	Experiment Descriptions
SD037	H71638.GID.405	400 MHz ¹ H-NMR in acetone-d ₆ solvent + p-HFX cross integration/internal std.
SD037	F71638.GID.406	376 MHz ¹⁹ F-NMR in acetone-d ₆ solvent + p-HFX cross integration/internal std.

OBJECTIVE:

This sample was subjected to a combination of ¹H-NMR and ¹⁹F-NMR spectral analyses to determine the purity of the nominal product. Special emphasis was also placed on attempting to identify and quantify any impurity components.

EXPERIMENTAL:

A portion of the sample was accurately weighed, spiked with a known amount of 1,4-bis(trifluoromethyl)benzene (p-HFX), and then totally dissolved in deuterated acetone (acetone-d₆) for subsequent analysis by NMR. A 400 MHz ¹H-NMR spectrum and a 376 MHz ¹⁹F-NMR spectrum were acquired at room temperature using a Varian UNITYplus 400 FT-NMR spectrometer. This sample preparation method permitted the p-HFX to be used as either 1) a ¹H/¹⁹F-NMR internal standard to allow the calculation of the absolute weight percent concentrations of specific components, or 2) a ¹H/¹⁹F-NMR cross integration standard to permit the cross correlation of the relative ¹H and ¹⁹F signal intensities for evaluation of the overall sample composition.

RESULTS:

The ¹H-NMR and ¹⁹F-NMR spectral data indicated this sample was a high purity form of the nominal product, CF₃(CF₂)_n-CO₂H, where the average value of n = 10.042. Small amounts of a few impurity components, including probable isomers, were also assigned. A ¹H/¹⁹F-NMR cross integration analysis technique was then used to calculate the relative weight percent concentrations of the identified components. The qualitative and quantitative compositional results that were derived from the single trial ¹H/¹⁹F-NMR cross integration analysis are summarized below in TABLE-1. The relative weight percent concentrations shown in TABLE-1 should be very close to their respective absolute weight percent values assuming no water was present in the sample. Trace amounts of a few other unassigned components were also detected in the NMR spectra, but additional work would be needed in an effort to identify or quantify these other components.

Copies of the NMR spectra are attached with the paper copy of this report for your reference. If you have any questions about these results, please let me know.

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CKM 10/31/02
 Initial Date

October 28, 2002

3M SMMD Analytical Lab Request # GID:71638
SD037, Lot R24K: Telomer Project

Tom Kestner

c: Rick Payfer
Ron Purcell
William Reagen

File Reference: ls71638.GID.SD037_Lot R24K_Telomer Project.DOC/101

TABLE-1
Sample: SD037, Lot R24K from the Telomer project.
Overall Compositional Results by ¹H/¹⁹F-NMR Cross Integration Analysis

Component Structures ¹	NMR Relative Weight% Concentrations (single trial measurement)
CF ₃ (CF ₂) _n -CO ₂ H where average n = 10.02	99.65%
Probable (CF ₃) ₂ -CF-(CF ₂) _n -CO ₂ H assume n=8 for calculation purposes	0.13%
Possible CF ₃ (CF ₂) _x -CF(CF ₃)-(CF ₂) _y -CO ₂ H where x≠0, y≠0 and assume x+y = 8 for calculation purposes	0.088%
Probable chlorinated impurity, Cl-CF ₂ CF ₂ -R _f , possibly as Cl-(CF ₂) ₁₁ -CO ₂ H	0.049%
Possible methyl ester impurity as CF ₃ (CF ₂) _n -CO ₂ CH ₃ where average n = 10.02	0.048%
Probable C _n H _{2n+2} saturated aliphatic hydrocarbons	0.016%
Toluene	0.015%

1. Trace amounts of other unassigned components were also detected in the NMR spectra.

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Certificate of Analysis

Nominal Product: $\text{CF}_3(\text{CF}_2)_n\text{-CO}_2\text{H}$, where average $n \approx 10$
perfluorododecanoic acid

Product Code: SD037, Lot R24K

October 28, 2002

Tom Kestner

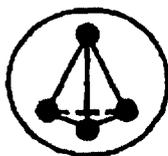
The sample of **SD037, lot R24K** was analyzed using a combination of ^{19}F -NMR and ^1H -NMR spectral analysis techniques. The overall qualitative and quantitative compositional results that were derived from these combined analyses are summarized below in TABLE-1.

TABLE-1
Sample: SD037, Lot R24K
Quantitative Compositional Results by Combined $^{19}\text{F}/^1\text{H}$ -NMR Spectral Analyses

Component Structures ¹	$^{19}\text{F}/^1\text{H}$ -NMR Relative Weight% Concentrations (single trial analysis)
$\text{CF}_3(\text{CF}_2)_n\text{-CO}_2\text{H}$ where average $n = 10.02$	$\leq 99.65\%$ Purity
Probable $(\text{CF}_3)_2\text{-CF-(CF}_2)_n\text{-CO}_2\text{H}$ assume $n=8$ for calculation purposes	0.13%
Possible $\text{CF}_3(\text{CF}_2)_x\text{-CF(CF}_3)\text{-(CF}_2)_y\text{-CO}_2\text{H}$ where $x \neq 0, y \neq 0$ and assume $x+y = 8$ for calculation purposes	0.088%
Probable chlorinated impurity, $\text{Cl-CF}_2\text{CF}_2\text{-R}_f$, possibly as $\text{Cl-(CF}_2)_{11}\text{-CO}_2\text{H}$	0.049%
Possible methyl ester impurity as $\text{CF}_3(\text{CF}_2)_n\text{-CO}_2\text{CH}_3$ where average $n = 10.02$	0.048%
Probable $\text{C}_n\text{H}_{2n+2}$ saturated aliphatic hydrocarbons	0.016%
Toluene	0.015%

1. Trace amounts of other unassigned components were also detected in the NMR spectra.

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Initial Data



Oakwood Products, Inc.

1741 Old Dunbar Road
West Columbia, SC 29172
Phone (803) 739-8800
Fax (803) 739-6957

CERTIFICATE OF ANALYSIS

Date: 11-Feb-00

Material: Perfluorododecanoic acid

Cat.No.: 2266

Lot No.: R22K

Assay: Products more volatile than Perfluorododecanoic acid < 2%
Perfluorododecanoic acid 96% min.
Products less volatile than Perfluorododecanoic acid < 2%

Appearance: White solid

Melting Point: 107-109°C

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Initial Date

Yumei Yang
Yumei Yang
Quality Control

000291

USE LOG

Perfluorododecanoic acid
Perfluorododecanoic acid
SD037

Gross Wt./Vol. Before withdrawal Balance ID 1	Amnt. withdrawn (mass or vol) Balance ID 2	Gross wt/vol after withdrawal Balance ID 1	Purpose (enter standard number or reason for removal)	Balance ID 1	Balance ID 2	Initials	Date
42.5752g	0.0796g	42.4956g	standard 02040-55	914	-	RWW	08/29/2002

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Initial Date

000292

SINGLE COMPONENT PREPARATION LOG

Date: 8/29/02
Analyst: RWW

Book No. 02 040
Page No. 55

Description: Pentfluorodecanoic Acid - C₁₀F₁₃COOH

Stock Number: SD037

Weight or Volume Used: 0.0563g

Balance ID: 914

Concentration or Purity: NA

Other Correction Factors: NA

Corrected Weight: 0.0563g

Solvent and TN-A Number: MeOH-TNA-6244

Final Volume: 50ml

Final Concentration: 1126 ppm

Storage Location: 2nd Floor - Rm Temp

Expiration Date: 2/29/03

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CMC 11/1/02
Initial Date

Reviewed by: Chris A. Stinson 10/01/02
Signature Date

Test Control and Reference Substance Log

Substance trade name or reference #	PFOS	TCR Substance #	SD018
Substance/chemical name:	Potassium Perfluorooctane Sulfonate FC-95	TCR #	TCR-18
Lot/batch #:	217	Received from:	Jo Dickes 8/10/98
3M #	98-0211-0888-5		
Expiration date:	08/31/2006	Amount received (wt. or vol):	160.0706g gross wt.
Initials:	PMR	Date:	04/07/1999
Number/size of containers:	1/175 mL glass container	Shipper:	Unknown
Condition:	white powder MCH-07/22/99	MSDS (y/n)	<input checked="" type="radio"/> Y <input type="radio"/> N
Retain	0.1733g	Date of Retain	10/18/1999

Archived/Substance Not Available

Purity:	86.9% LAC 09/19/00
Records received:	NMR Results/Characterization #53030, MSDS KJD 02/29/00 Interim final report from Centre and Certificate of Analysis. LAC 04/26/01
Location of synthesis, fabrication, or derivation records:	98-0211-0888-5
Std Location/Storage:	F19, Frozen
Molecular Formula:	C8F17SO3-K+
Comments	Environmental Lab Traceability number was TN-A-2130 PMR 04/07/99 Moved to cold storage (< 0C) on 05/16/00 LAC 05/16/00 Standard has been moved to Freezer 19 in room 347 KJD 06/06/00 Standard was stored at room temperature prior to 05/16/00. LAC 12/19/00 Shipping Codes: FC-S000-0185-6(<30 g on dry ice) LAC 06/14/01 Shipping Comments: Dangerous goods in excepted quantity of Class 6, UN2811. REGULATED-TOXIC 98-0211-0888-5 (>30 g (1 oz sample)) Not on dry ice. LAC 01/08/02
Attachment(s)	   sd018msds.pdf sd018nmrreq53030.pdf sd018nmrreq61517.pc  E00-1682-COA-SD018-PFOS-Rev3

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cmx 10/31/02
Initial Date



Centre Analytical Laboratories, Inc.

3048 Research Drive State College, PA 16801 www.centrelab.com
Phone: (814) 231-8032 Fax: (814) 231-1253 or (814) 231-1580

INTERIM CERTIFICATE OF ANALYSIS

Revision 3

Centre Analytical Laboratories COA Reference #: 023-018A

3M Product: PFOS, Lot 217

Reference #: SD-018

Purity: 86.9%

Test Name	Specifications	Result
Purity ¹		86.9%
Appearance	White Crystalline Powder	Conforms
Identification NMR		Positive
Metals (ICP/MS)		
1. Calcium		1. 0.005 wt./wt.%
2. Magnesium		2. 0.001 wt./wt.%
3. Sodium		3. 1.439 wt./wt.%
4. Potassium ²		4. 6.849 wt./wt.%
5. Nickel		5. <0.001 wt./wt.%
6. Iron		6. 0.005 wt./wt.%
7. Manganese		7. <0.001 wt./wt.%
Total % Impurity (NMR)		1.91 wt./wt.%
Total % Impurity (LC/MS)		8.41 wt./wt.%
Total % Impurity (GC/MS)		None Detected
Related Compounds – POAA		0.33 wt./wt.%
Residual Solvents (TGA)		None Detected
Purity by DSC		Not Applicable ³
Inorganic Anions (IC)		
1. Chloride		1. <0.015 wt./wt.%
2. Fluoride		2. 0.59 wt./wt.%
3. Bromide		3. <0.040 wt./wt.%
4. Nitrate		4. <0.009 wt./wt.%
5. Nitrite		5. <0.006 wt./wt.%
6. Phosphate		6. <0.007 wt./wt.%
7. Sulfate ⁴		7. 8.76 wt./wt.%
Organic Acids ⁵ (IC)		
1. TFA		1. <0.1 wt./wt.%
2. PFPA		2. <0.1 wt./wt.%
3. HFBA		3. 0.10 wt./wt.%
4. NFPA		4. 0.28 wt./wt.%
Elemental Analysis ⁶ :		
1. Carbon	1. Theoretical Value = 17.8%	1. 12.48 wt./wt.%
2. Hydrogen	2. Theoretical Value = 0%	2. 0.244 wt./wt.%
3. Nitrogen	3. Theoretical Value = 0%	3. 1.74 wt./wt.%
4. Sulfur	4. Theoretical Value = 5.95%	4. 8.84 wt./wt.%
5. Fluorine	5. Theoretical Value = 60%	5. 54.1 wt./wt.%

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CNC Initial Date
10/31/02



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Phone: (814) 231-8032 Fax: (814) 231-1253 or (814) 231-1580

INTERIM CERTIFICATE OF ANALYSIS

Revision 3

Centre Analytical Laboratories COA Reference #: 023-018A

Date of Last Analysis: 08/31/00

Expiration Date: 08/31/06

Storage Conditions: Frozen $\leq 10^{\circ}\text{C}$

Re-assessment Date: 08/31/06

¹Purity = 100% - (sum of metal impurities, 1.45% +LC/MS impurities, 8.41%+Inorganic Fluoride, 0.59%+NMR impurities, 1.905%+organic acid impurities, 0.38%+POAA, 0.33%)

Total impurity from all tests = 13.07%

Purity = 100% - 13.07% = 86.9%

²Potassium is expected in this salt form and is therefore not considered an impurity.

³Purity by DSC is generally not applicable to materials of low purity. No endotherm was observed for this sample.

⁴Sulfur in the sample appears to be converted to SO_4 and hence detected using the inorganic anion method conditions. The anion result agrees well with the sulfur determination in the elemental analysis, lending confidence to this interpretation. Based on the results, the SO_4 is not considered an impurity.

⁵ TFA	Trifluoroacetic acid
HFBA	Heptafluorobutyric acid
NFPA	Nonafluoropentanoic acid
PFPA	Pentafluoropropanoic acid

⁶Theoretical value calculations based on the empirical formula, $\text{C}_8\text{F}_{17}\text{SO}_3\text{K}^+$ (MW=538)

This work was conducted under EPA Good Laboratory Practice Standards (40 CFR 160).

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3048 Research Drive State College, PA 16801 www.centrelab.com
Phone: (814) 231-8032 Fax: (814) 231-1253 or (814) 231-1580

INTERIM CERTIFICATE OF ANALYSIS

Revision 3

Centre Analytical Laboratories COA Reference #: 023-018A

LC/MS Purity Profile:

Impurity	wt./wt. %
C4	1.22
C5	1.33
C6	4.72
C7	1.14
Total	8.41

Note: The C4 and C6 values were calculated using the C4 and C6 standard calibration curves, respectively. The C5 value was calculated using the average result from the C4 and C6 standard curves. Likewise, the C7 value was calculated using the average result from the C6 and C8 standard curves.

Prepared By: Charles Simons
Charles Simons
Scientist, Centre Analytical Laboratories

10/11/01
Date

Reviewed By: John M Flaherty
John Flaherty
Laboratory Manager, Centre Analytical Laboratories

10/11/01
Date

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CMC
Initial 10/31/01
Date

USE LOG

PFOS
Potassium Perfluorooctane Sulfonate FC-95
SD018

Gross Wt./Vol. Before withdrawal Balance ID 1	Amnt. withdrawn (mass or vol) Balance ID 2	Gross wt/vol after withdrawal Balance ID 1	Purpose (enter standard number or reason for removal)	Balance ID 1	Balance ID 2	Initials	Date
140.4480g	0.0814g	140.3666g	standard 02022-56	914	-	RWW	05/13/2002

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Initial Date /

SINGLE COMPONENT PREPARATION LOG

Date: 5/13/02 Book No. 02 022
Analyst: RWN Page No. 56
Description: PFO5 Stock
Stock Number: SD018
Weight or Volume Used: 65.0mg Balance ID: 914
Concentration or Purity: 86.9% Other Correction Factors: 0.9275
Corrected Weight: 52.59mg Solvent and TN-A Number: M.OH/TN-A-06034
Final Volume: 50ml Final Concentration: 1048ppm
Storage Location: R16 Expiration Date: 11/13/02

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Initial Date

Reviewed by: Jim A. Stevenson 06/26/02
Signature Date

000299

ATTACHMENT E: PROTOCOL AND AMENDMENT

000300



STUDY PROTOCOL

STUDY TITLE

Analysis of Endogenous Fluorochemicals in Normal Pooled Human Serum and Plasma

SPONSOR

William K. Reagen, Ph.D

DATA REQUIREMENT

40 CFR Part 792

TESTING FACILITY

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

LABORATORY STUDY IDENTIFICATION

3M Environmental Study Number E02-1039

NUMBER OF PAGES

10

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Initial Date

000301

Analysis of Endogenous Fluorochemicals

E02-1039

STUDY IDENTIFICATION

Analysis of Endogenous Fluorochemicals in Normal Pooled Human Serum and Plasma

SPONSOR

William Reagen, Ph.D.

3M Environmental Laboratory
935 Bush Avenue, Building 2-3E-09
St. Paul, MN 55106
(651)778-6565

STUDY DIRECTOR

Mark E. Ellefson

3M Environmental Laboratory
935 Bush Avenue, Building 2-3E-09
St. Paul, MN 55106
(651)778-5405

TEST FACILITY

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

PROPOSED STUDY TIMETABLE

Experimental Start Date
Experimental Termination Date

10 October 2002
01 November 2002

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1.0 Introduction and Purpose/Objective

- 1.1 The purpose of this study is to quantify perfluorohexanoic acid (C6), perfluoroheptanoic acid (C7), pentadecafluorooctanoic acid (C8), heptadecafluorononanoic acid (C9), nonadecafluorodecanoic acid (C10), perfluorodecanoic acid (C11), perfluorododecanoic acid (C12), tetrahydroperfluorooctane sulfonate (THPFOS), tetrahydroperfluorodecane sulfonate (THPFDS), and perfluorooctanesulfonate (PFOS) in normal pooled human serum and plasma. This study does not have a typical test substance that is dosed onto a specific controlled test system as per a conventional GLP study.

2.0 Regulatory Compliance

- 2.1 This study will be conducted in accordance with the United States Environmental Protection Agency Good Laboratory Practice Regulations for the Toxic Substances Control Act, 40 CFR 792.

3.0 Test Substances

- 3.1 Preliminary screening indicates that the test compounds listed below are present as endogenous material in normal pooled human serum and plasma. Information pertaining to traceability, source, physical description, and storage conditions is not available for these compounds as they exist in biological matrices.

Test Substances

Test Substance	Formula
Perfluorohexanoic Acid	C ₆ F ₁₁ COOH
Tetradecafluoroheptanoic Acid	C ₈ F ₁₃ COOH
Pentadecafluorooctanoic Acid	C ₇ F ₁₅ COOH
Heptadecafluorononanoic Acid	C ₈ F ₁₇ COOH
Nonadecafluorodecanoic Acid	C ₈ F ₁₉ COOH
Perfluoroundecanoic Acid	C ₁₀ F ₂₁ COOH
Perfluorododecanoic Acid	C ₁₁ F ₂₃ COOH
1H,2H,3H,4H-perfluorooctanesulfonate	C ₈ H ₉ F ₁₃ O ₃ S
1H,2H,3H,4H-perfluorodecane sulfonate	C ₁₀ H ₉ F ₁₇ O ₃ S
Potassium Perfluorooctanesulfonate	C ₈ F ₁₇ SO ₃ K

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4.0 Reference Substances

Reference Substances

Reference Substance	Formula	Traceability #	Source	Physical Description	Purity	Storage Conditions
Perfluorohexanoic Acid	C ₆ F ₁₁ COOH	TCR-047	SMM* 236-1B-10	Colorless Liquid	TBD**	Frozen
Tetradecafluoro heptanoic Acid	C ₆ F ₁₃ COOH	TCR-267	Aldrich	Clear Crystals	99.5%	Frozen
Pentadecafluoro octanoic Acid	C ₇ F ₁₅ COOH	TCR-617	Oakwood Products	White Crystals	> 97%	Ambient Temperature
Heptadecafluoro nonanoic Acid	C ₈ F ₁₇ COOH	TCR-618	Oakwood Products	White Crystals	> 99%	Ambient Temperature
Nonadecafluoro decanoic Acid	C ₉ F ₁₉ COOH	TCR-036	Oakwood Products	White Solid	98%	Frozen
Perfluoroundecanoic Acid	C ₁₀ F ₂₁ COOH	TCR-619	Oakwood Products	White Crystals	> 99%	Ambient Temperature
Perfluorododecanoic Acid	C ₁₁ F ₂₃ COOH	TCR-037	Oakwood Products	White Powder	96%	Frozen
1H,2H,3H,4H-perfluorooctane sulfonate	C ₈ H ₆ F ₁₃ O ₂ S	TCR-343	SynQuest Labs	White Powder	TBD	Frozen
1H,2H,3H,4H-perfluorodecane sulfonate	C ₁₀ H ₈ F ₁₇ O ₂ S	TCR-627	Pace Analytical	White Crystals	94.7%	Ambient Temperature
Potassium Perfluorooctane sulfonate	C ₈ F ₁₇ SO ₃ K*	TCR-018	SMM* 236-1B-10	White Powder	86.9%	Frozen

*Documentation of the method of synthesis is located at the source.

**A sample of the reference substance has been sent for characterization

5.0 Test System

Test Systems

TEST SYSTEM:	SOURCE	TRACEABILITY
Pooled Human Serum	Sigma-Aldrich, Milwaukee, WI	TCR-689
Pooled Human Serum	Lampire Biological Laboratories, Pipersville, PA	TCR-688
Pooled Human Serum	Bioresource Technology, Inc., Fort Lauderdale, FL	TCR-687
Pooled Human Serum	Golden West Biologicals, Temecula, CA	TCR-690

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TEST SYSTEM:	SOURCE	TRACEABILITY
Pooled Human Plasma	Lampire Biological Laboratories, Pipersville, PA	TCR-685
Pooled Human Plasma	Golden West Biologicals, Temecula, CA	TCR-684
Pooled Human Plasma	Innovative Research, Inc., Southfield, MI	TCR-683
Pooled Human Plasma	Central China	TCR-674

- 5.1 **Justification of the test system.** Based on preliminary testing, normal pooled serum and plasma contain endogenous levels of the test analytes.
- 5.2 **Identification of Test System.** Samples shall be identified by the study number, date of initial preparation, test substance or test system, sample number, replicate number (if applicable), analyst(s), and project leader.

6.0 Surrogate Matrix

Surrogate Matrix

SURROGATE MATRIX:	SOURCE	TRACEABILITY
Rabbit Serum	Sigma-Aldrich	TCR-686

- 6.1 **Justification of the Surrogate Matrix.** Based on preliminary testing, normal pooled rabbit serum contains very low endogenous levels of the test analytes and is thereby suitable for use as a surrogate matrix.
- 6.2 **Identification of Surrogate Matrix.** Samples shall be identified by the study number, date of initial preparation, test substance or surrogate matrix, sample number, replicate number (if applicable), analyst(s), and project leader.

7.0 Analytical Methods

- 7.1 Analysis of the test and reference substances will be conducted in the 3M Environmental Laboratory. The analyses will be conducted as described by 3M Environmental Laboratory Method ETS-8-231, "Solid Phase Extraction and Analysis of Fluorochemical Compounds from Biological matrices".

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Initial Date

8.0 Data Quality Objectives

8.1 Absolute Recovery:

8.1.1 The absolute recovery of the method will be evaluated separately in human serum and plasma and rat serum. For each matrix, the samples will be fortified at two levels of (500ppt and 5ppb). All samples will be extracted through the method, and analyzed by comparison with external calibration of non-extracted standards. The non-extracted standard calibration curves will be prepared in methanol, and will consist of a minimum of nine (9) levels, including a methanol blank. The best appropriate regression will be used to describe this curve (for best accuracy at all levels of the standards).

8.1.2 The accuracy (% recovery) and precision (%CV of the recoveries) will be determined at each level, and for all levels combined. There is no control limit for accuracy; precision must be better than 15% at each level.

8.2 Calibration: Calibration curves will be prepared from extracted matrix standards, in Chinese plasma and rabbit serum. The curves will consist of a minimum of nine (9) levels and a matrix blank. The equation will be determined by regression analysis using the peak areas of the analyte. The accuracy of each level will be verified. Any level outside 75% - 125% of nominal must be deactivated, and regression re-calculated, except the LLOQ which must be within 30% of nominal. All levels must show a response greater than twice that of the blank. A maximum of four (4) levels may be deactivated in any one set, or the set will be re-analyzed.

8.3 Limits of Quantitation (LOQ): The lower limit of quantitation (LLOQ) will be determined for each analyte. The level determined as the LLOQ must show a recovery within 75% - 125% for the analytes and must show a response greater than twice that of the blank. These limits will be determined during the course of the study and documented in the raw data. Should the LLOQ level calibration be de-activated in a particular set, the practical limit of quantitation for this set will be raised to the next acceptable level. Samples below the practical LOQ of that set will be reanalyzed until quantitated in a set including the validated LLOQ.

The upper limit of quantitation (ULOQ) will be determined in serum and plasma. The level determined as the ULOQ must show a recovery within 75% - 125% for the analytes. These limits will be determined during the course of the study and documented in the raw data. Should the ULOQ level calibration standard be de-activated in a particular set, the practical limit of quantitation for this set will be lowered to the next highest acceptable level.

Any sample with an area greater than 110% of the highest acceptable standard will need to be diluted into the range of the calibration curve. If samples are diluted into the range of the curve during analyses and enough sample remains, a post-run dilution validation will be performed to verify sample values. To

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perform the dilution validation, one sample will be separated into two representative samples (i.e. two 1 mL aliquots for fluid samples or two 1 gram amounts for tissue samples) then diluted using two procedures. The first procedure consists of diluting the sample with additional matrix prior to extraction (sera adding sera), while the second procedure consists of diluting the extract with solvent post-extraction (methanol extract adding additional methanol solvent).

If the values are not within 15% of each other additional testing will be required to determine which value is a correct representation of the sample concentration.

8.4 Use of Confirmatory Methods

Confirmatory methods are typically not needed with LC/MS/MS analysis.

8.5 Demonstration of Specificity

8.5.1 The identification of analytes will be substantiated by chromatographic retention time, by the characteristic primary ion, the characteristic product ion, and isomeric proportions (where applicable).

7.6 Control of Bias

Two levels of matrix fortifications, prepared at known concentrations of the test substance and bracketing the anticipated range of the method will be evaluated to determine recovery and to evaluate method performance. Reagent and matrix blanks will be run with each set to evaluate the level of background interferences.

9.0 Statistical Methods and Calculations

9.1 Statistical methods for the analytical results will be limited to the calculations of means, standard deviations, and relative standard deviations (as appropriate).

10.0 Report

A report of the results of the study will be prepared by 3M Environmental Laboratory. The report will include, but not be limited to, the following, when applicable:

- 10.1.1 Name and address of the facilities performing the study,
- 10.1.2 Dates upon which the study was initiated and completed.
- 10.1.3 A statement of compliance by the Study Director addressing any exceptions to Good Laboratory Practice Standards.
- 10.1.4 A copy of the protocol, and any amendments and deviations.
- 10.1.5 A description of the methods used to conduct the test(s). The report will

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CML 10/31/07
Initial Date

- contain updated methods incorporating any changes or improvements.
- 10.1.6 A description of the test system.
 - 10.1.7 A description of any circumstances that may have affected the quality or the integrity of the data.
 - 10.1.8 The name of the Study Director and the names of other scientists, professionals, and supervisory personnel involved in the study.
 - 10.1.9 A description of the transformations, calculations, or operations performed on the data, a summary and analysis of the analytical chemistry data, and a statement of the conclusions drawn from the analyses.
 - 10.1.10 Statistical methods used to evaluate the data, if applicable.
 - 10.1.11 The signed and dated reports of each of the individual scientists or other professionals involved in the study, if applicable.
 - 10.1.12 The location where raw data and the final report are to be stored.
 - 10.1.13 A statement prepared by the quality assurance unit listing the dates that study inspections and audits were made and the dates of any findings reported to the Study Director and Management.
- 10.2 If it is necessary to make corrections or additions to a final report after it has been accepted, the changes will be made in the form of an amendment issued by the Study Director. The amendment will clearly identify the part of the final report that is being amended, provide the reasons for the amendment, and will be signed by the Study Director.

11.0 Quality Assurance

- 11.1 The 3M Environmental Laboratory Quality Assurance Unit will review the protocol and audit study conduct, data, and the final report to determine compliance with Good Laboratory Practice Standards and with 3M Environmental Laboratory Standard Operating Procedures. The Quality Assurance Unit will report all findings to the Sponsor Representative and the Study Director.

12.0 Location of Raw Data, Records, and Final Report

- 12.1 Original data or copies thereof, will be available at 3M Environmental Laboratory. When the final report is completed all original paper data, including those items listed below, will be retained in the archives of 3M Environmental Laboratory following signing of the final report.
- 12.2 The following raw data and records will be retained in the study folder in the archives according to 3M Environmental Laboratory SOPs.
- 12.2.1 Approved protocol and amendments

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Initial Date

- 12.2.2 Study correspondence
- 12.2.3 Shipping records
- 12.2.4 Raw data
- 12.2.5 Approved final report (original signed copy)
- 12.2.6 Electronic copies of data
- 12.3 The following supporting records will be retained separately from the study folder in the archives according to 3M Environmental Laboratory SOPs:
 - 12.3.1 Training records
 - 12.3.2 Calibration records
 - 12.3.3 Instrument maintenance logs
 - 12.3.4 Standard operating procedures, equipment procedures, and methods

13.0 Sample Retention

- 13.1 A portion of the reference substances used in the study will be retained in the laboratory for a period of not less than 2 years after a report is issued.
- 13.2 Sample extracts will be retained and refrigerated for a period of not less than three months from the time of analysis.

14.0 Protocol Amendments and Deviations

- 14.1 Amendments and deviations to the protocol will be in the form of written amendments signed by the Study Director and the Sponsor Representative. Amendments will be considered as part of the protocol and will be attached to the final protocol. All changes to the protocol will be indicated in the final report. Any other changes will be in the form of written deviations, signed by the Study Director and filed with the raw data.

15.0 Attachments

None

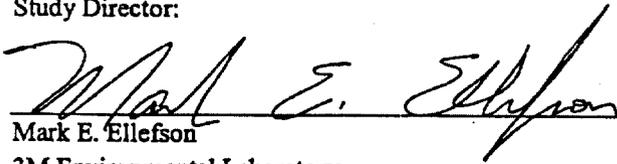
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Initial Date

Analysis of Endogenous Fluorochemicals

E02-1039

Protocol Signature

Study Director:

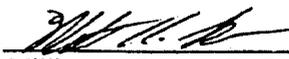


Mark E. Ellefson
3M Environmental Laboratory

10/10/02
Date:

Protocol Approval

Sponsor:



William K. Reagen, Ph.D.
3M Environmental Laboratory

10/10/02
Date:

Exact Copy of Original

cmc 10/31/02
Initial Date

Study Title

Analysis of Endogenous Fluorochemicals in Normal Pooled Human Serum and Plasma

PROTOCOL AMENDMENT NO. 1

Amendment Date:

October 31, 2002

Performing Laboratory

3M Environmental Laboratory
Building 2-3E-09
935 Bush Avenue
St. Paul, MN 55144-1000

Laboratory Project Identification

3M Environmental Laboratory Study LIMS #E02-1039

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cm 10/1/02
Initial Date

Protocol #E00-1311
Amendment 1

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

1. PROTOCOL READS: SECTION 8.1.1: The absolute recovery of the method will be evaluated separately in human serum and plasma and rat serum. For each matrix, the samples will be fortified at two levels of (500ppt and 5ppb). All samples will be extracted through the method, and analyzed by comparison with external calibration of non-extracted standards. The non-extracted standard calibration curves will be prepared in methanol, and will consist of a minimum of nine (9) levels, including a methanol blank. The best appropriate regression will be used to describe this curve (for best accuracy at all levels of the standards).

AMEND TO READ: The absolute recovery of the method will not be evaluated as the matrix effects render this type of comparison limited in its usefulness.

REASON: A planned change in the study's direction.

2. PROTOCOL READS: SECTION 8.2: Calibration: Calibration curves will be prepared from extracted matrix standards, in Chinese plasma and rabbit serum. The curves will consist of a minimum of nine (9) levels and a matrix blank.

AMEND TO READ: Calibration: Calibration curves will be prepared from extracted matrix standards in Chinese plasma. The curves will consist of a minimum of nine (9) levels. Reasons for not using one or more of these standards in the construction of a calibration curve are up to the discretion of the analyst and will be documented in the raw data.

REASON: The samples were run against 9 extracted standards without a blank, but not all of these standards were used to construct the curve. Additionally rabbit curves were not included in the data for this study.

3. PROTOCOL READS: SECTION 7.1: Analysis of the test and reference substances will be conducted in the 3M Environmental Laboratory. The analyses will be conducted as described by 3M Environmental Laboratory Method ETS-8-231, "Solid Phase Extraction and Analysis of Fluorochemical Compounds from Biological matrices".

AMEND TO READ: Analysis of the test and reference substances will be conducted in the 3M Environmental Laboratory. The analyses will be conducted as described by 3M Environmental Laboratory Method ETS-8-231, "Solid Phase Extraction and Analysis of Fluorochemical Compounds from Biological matrices". Selected ion monitoring will be used to detect the presence of the target analytes. The masses scanned will be documented in the raw data and in the final report.

REASON: Add clarity.

4. PROTOCOL READS: SECTION 8.3: Any sample with an area greater than 110% of the highest acceptable standard will need to be diluted into the range of the calibration curve. If samples are diluted into the range of the curve during analyses and enough sample remains, a post-run dilution validation will be performed to verify sample values. To perform the dilution validation, one sample will be separated into two representative samples (i.e. two 1 mL aliquots for fluid samples or two 1 gram amounts for tissue samples) then diluted using two procedures. The first procedure consists of diluting the sample with additional matrix prior to extraction (sera adding sera), while the second procedure consists of diluting the extract with solvent post-extraction (methanol extract adding additional methanol solvent). If the values are not within 15% of each other additional testing will be required to determine which value is a correct representation of the sample concentration.

Protocol #E00-1311
Amendment 1

AMEND TO READ: Any sample with an area greater than 110% of the highest acceptable standard will be reported as >ULOQ.

REASON: The purpose of this study is to demonstrate the presence of the target analytes in pooled human serum and plasma. It is more important to show that endogenous levels are detectable than to show specifically what those levels are.

5. PROTOCOL READS: SECTION 1.1 The purpose of this study is to quantify perfluorohexanoic acid (C6), perfluoroheptanoic acid (C7), pentadecafluorooctanoic acid (C8), heptadecafluorononanoic acid (C9), nonadecafluorodecanoic acid (C10), perfluorodecanoic acid (C11), perfluorododecanoic acid (C12), tetrahydroperfluorooctane sulfonate (THPFOS), tetrahydroperfluorodecane sulfonate (THPFDS), and perfluorooctanesulfonate (PFOS) in normal pooled human serum and plasma. This study does not have a typical test substance that is dosed onto a specific controlled test system as per a conventional GLP study.

AMEND TO READ: The purpose of this study is to quantify perfluoroheptanoic acid (C7), pentadecafluorooctanoic acid (C8), heptadecafluorononanoic acid (C9), nonadecafluorodecanoic acid (C10), perfluorodecanoic acid (C11), perfluorododecanoic acid (C12), tetrahydroperfluorooctane sulfonate (THPFOS), tetrahydroperfluorodecane sulfonate (THPFDS), and perfluorooctanesulfonate (PFOS) in normal pooled human serum and plasma. This study does not have a typical test substance that is dosed onto a specific controlled test system as per a conventional GLP study.

REASON: The C₆ compound was eliminated from the study.

6. PROTOCOL READS: SECTION 3.1

Test Substances

Test Substance	Formula
Perfluorohexanoic Acid	C ₆ F ₁₁ COOH
Tetradecafluoroheptanoic Acid	C ₈ F ₁₃ COOH
Pentadecafluorooctanoic Acid	C ₇ F ₁₅ COOH
Heptadecafluorononanoic Acid	C ₈ F ₁₇ COOH
Nonadecafluorodecanoic Acid	C ₉ F ₁₉ COOH
Perfluoroundecanoic Acid	C ₁₀ F ₂₁ COOH
Perfluorododecanoic Acid	C ₁₁ F ₂₃ COOH
1H,2H,3H,4H-perfluorooctanesulfonate	C ₈ H ₅ F ₁₃ O ₃ S
1H,2H,3H,4H-perfluorodecane sulfonate	C ₁₀ H ₅ F ₁₇ O ₃ S
Potassium Perfluorooctanesulfonate	C ₈ F ₁₇ SO ₃ ⁻ K ⁺

AMEND TO READ:

Test Substances

Test Substance	Formula
Tetradecafluoroheptanoic Acid	C ₈ F ₁₃ COOH
Pentadecafluorooctanoic Acid	C ₇ F ₁₅ COOH

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Protocol #E00-1311
Amendment 1

Heptadecafluorononanoic Acid	$C_8F_{17}COOH$
Nonadecafluorodecanoic Acid	$C_9F_{19}COOH$
Perfluoroundecanoic Acid	$C_{10}F_{21}COOH$
Perfluorododecanoic Acid	$C_{11}F_{23}COOH$
1H,2H,3H,4H-perfluorooctanesulfonate	$C_8H_5F_{13}O_3S$
1H,2H,3H,4H-perfluorodecane sulfonate	$C_{10}H_5F_{17}O_3S$
Potassium Perfluorooctanesulfonate	$C_8F_{17}SO_3^-K^+$

REASON: The C_8 compound was eliminated from the study.

7. PROTOCOL READS: SECTION 4.0

Reference Substances

Reference Substance	Formula	Traceability #	Source	Physical Description	Purity	Storage Conditions
Perfluorohexanoic Acid	$C_6F_{11}COOH$	TCR-047	SMM* 236-1B-10	Colorless Liquid	TBD**	Frozen
Tetradecafluoro heptanoic Acid	$C_6F_{13}COOH$	TCR-267	Aldrich	Clear Crystals	99.5%	Frozen
Pentadecafluoro octanoic Acid	$C_7F_{15}COOH$	TCR-617	Oakwood Products	White Crystals	> 97%	Ambient Temperature
Heptadecafluoro nonanoic Acid	$C_8F_{17}COOH$	TCR-618	Oakwood Products	White Crystals	> 99%	Ambient Temperature
Nonadecafluoro decanoic Acid	$C_9F_{19}COOH$	TCR-036	Oakwood Products	White Solid	98%	Frozen
Perfluoroundecanoic Acid	$C_{10}F_{21}COOH$	TCR-619	Oakwood Products	White Crystals	> 99%	Ambient Temperature
Perfluorodecanoic Acid	$C_{11}F_{23}COOH$	TCR-037	Oakwood Products	White Powder	96%	Frozen
1H,2H,3H,4H-perfluorooctane sulfonate	$C_8H_5F_{13}O_3S$	TCR-343	SynQuest Labs	White Powder	TBD	Frozen
1H,2H,3H,4H-perfluorodecane sulfonate	$C_{10}H_5F_{17}O_3S$	TCR-627	Pace Analytical	White Crystals	94.7%	Ambient Temperature
Potassium Perfluorooctane sulfonate	$C_8F_{17}SO_3^-K^+$	TCR-018	SMM* 236-1B-10	White Powder	86.9%	Frozen

AMEND TO READ

Reference Substances

Reference Substance	Formula	Traceability #	Source	Physical Description	Purity	Storage Conditions
Tetradecafluoro heptanoic Acid	$C_6F_{13}COOH$	TCR-267	Aldrich	Clear Crystals	99.5%	Frozen
Pentadecafluoro octanoic Acid	$C_7F_{15}COOH$	TCR-617	Oakwood Products	White Crystals	> 97%	Ambient Temperature

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Initial Date

Protocol #E00-1311
Amendment 1

Pentadecafluoro octanoic Acid	C ₇ F ₁₃ COOH	TCR-617	Oakwood Products	White Crystals	> 97%	Ambient Temperature
Heptadecafluoro nonanoic Acid	C ₈ F ₁₇ COOH	TCR-618	Oakwood Products	White Crystals	> 99%	Ambient Temperature
Nonadecafluoro decanoic Acid	C ₉ F ₁₉ COOH	TCR-036	Oakwood Products	White Solid	98%	Frozen
Perfluoroundecanoic Acid	C ₁₀ F ₂₁ COOH	TCR-619	Oakwood Products	White Crystals	> 99%	Ambient Temperature
Perfluorododecanoic Acid	C ₁₁ F ₂₃ COOH	TCR-037	Oakwood Products	White Powder	96%	Frozen
1H,2H,3H,4H-perfluorooctane sulfonate	C ₈ H ₆ F ₁₃ O ₃ S	TCR-343	SynQuest Labs	White Powder	TBD	Frozen
1H,2H,3H,4H-perfluorodecane sulfonate	C ₁₀ H ₆ F ₁₇ O ₃ S	TCR-627	Pace Analytical	White Crystals	94.7%	Ambient Temperature
Potassium Perfluorooctane sulfonate	C ₈ F ₁₇ SO ₃ ⁻ K ⁺	TCR-018	SMM* 236-1B-10	White Powder	86.9%	Frozen

REASON: The C₆ compound was eliminated from the study.

8. PROTOCOL READS: SECTION 6.0

Surrogate Matrix

SURROGATE MATRIX:	SOURCE	TRACEABILITY
Rabbit Serum	Sigma-Aldrich	TCR-686

- 6.4 **Justification of the Surrogate Matrix.** Based on preliminary testing, normal pooled rabbit serum contains very low endogenous levels of the test analytes and is thereby suitable for use as a surrogate matrix.
- 6.5 **Identification of Surrogate Matrix.** Samples shall be identified by the study number, date of initial preparation, test substance or surrogate matrix, sample number, replicate number (if applicable), analyst(s), and project leader.

AMEND TO READ: None

REASON: It was decided not to use rabbit serum curves to generate data for this study.

9. PROTOCOL READS: SECTION 8.3 Limits of Quantitation (LOQ): The lower limit of quantitation (LLOQ) will be determined for each analyte. The level determined as the LLOQ must show a recovery within 75% - 125% for the analytes and must show a response greater than twice that of the blank.

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Initial Date

Protocol #E00-1311
Amendment 1

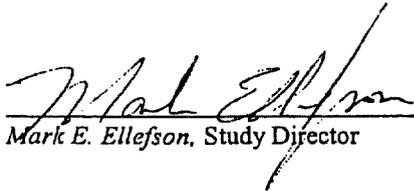
AMEND TO READ: Limits of Quantitation (LOQ): The lower limit of quantitation (LLOQ) will be determined for each analyte. The level determined as the LLOQ must show a recovery within 70% - 130% for the analytes.

REASON: Since this is a screening-type study it was appropriate to relax the acceptable recovery levels in order to avoid re-running sample sets.

Amendment Approval



William K. Reagen, Sponsor Representative 11/8/02
Date



Mark E. Ellefson, Study Director 11/8/02
Date

Exact Copy of Original
CNC 11/11/02
Initial Date

Study Title

Analysis of Endogenous Fluorochemicals in Normal Pooled Human Serum and Plasma

PROTOCOL AMENDMENT NO. 2

Amendment Date:

6 December, 2002

Performing Laboratory

3M Environmental Technology & Safety Services
3M Environmental Laboratory
935 Bush Avenue
St. Paul, MN 55106

Laboratory Project Identification

ET&SS E02-1039

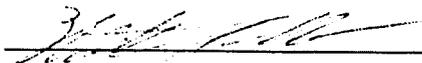
This amendment modifies the following portion(s) of the protocol:

PAGE 3, SECTION 1.1, PROTOCOL READS: The purpose of this study is to quantify, perfluoroheptanoic acid (C7), pentadecafluorooctanoic acid (C8), heptadecafluorononanoic acid (C9), nonadecafluorodecanoic acid (C10), perfluorodecanoic acid (C11), perfluorododecanoic acid (C12), tetrahydroperfluorooctane sulfonate (THPFOS), tetrahydroperfluorodecane sulfonate (THPFDS), and perfluorooctanesulfonate (PFOS) in normal pooled human serum and plasma. This study does not have a typical test substance that is dosed onto a specific controlled test system as per a conventional GLP study.

AMEND TO READ: The purpose of this study is to quantify perfluoroheptanoic acid (C7), pentadecafluorooctanoic acid (C8), heptadecafluorononanoic acid (C9), nonadecafluorodecanoic acid (C10), perfluorodecanoic acid (C11), perfluorododecanoic acid (C12), and perfluorooctanesulfonate (PFOS) in normal pooled human serum and plasma. This study does not have a typical test substance that is dosed onto a specific controlled test system as per a conventional GLP study.

REASON: THPFOS and THPFDS were removed from the list of analytes in the revised report because the data was intended as quantitative and only screening estimates could be obtained. In addition, THPFOS and THPFDS were removed from the list of test substances, section 3.0, page 3 and the list of reference substances, section 4.0, page 4.

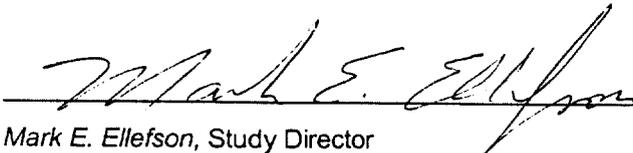
Amendment Approval



William K. Reagen, Sponsor Representative

12/09/02

Date



Mark E. Ellefson, Study Director

12/09/02

Date

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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

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

PROJECT

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

Total Pages: 111

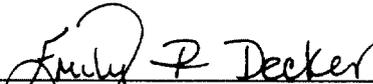
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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

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. § 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. § 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. § 5.2 (3): The date of receipt of for the calf serum sample ID 0204718 was not documented.
4. § 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
3M Environmental

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.

<u>Phase</u>	<u>Date Inspected</u>	<u>Date Reported to Study Director</u>	<u>Date Reported to Exygen Management</u>	<u>Date Reported to Sponsor</u>
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

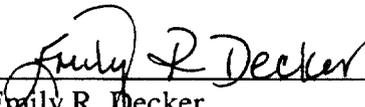
 Date

CERTIFICATION OF AUTHENTICITY

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

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

Study Director, Exygen



Emily R. Decker
Scientist
Exygen Research

10/30/02

Date

Exygen Research Facility Management:



John M. Flaherty
Vice President
Exygen Research

10/30/02

Date

Sponsor Study Monitor, 3M:



William K. Reagan, Ph.D.
3M Environmental

10/31/02

Date

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:

<u>Name</u>	<u>Title</u>
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. Southfield, 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.

Exygen 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 ($\leq -10^{\circ}\text{C}$) 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.

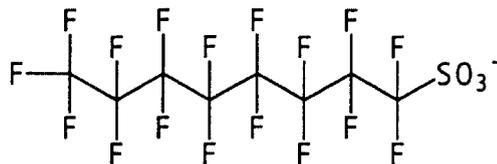
<u>Compound</u>	<u>Exygen Inventory No.</u>	<u>Lot No.</u>	<u>Purity (%)</u>	<u>Expiration Date</u>
PHAA (C6)	SP0002086	NB 117735-32	TBD	01/01/10
TDHA (C7)	SP0002091	PU/07219EU	99.5	01/01/05
PFOA (C8)	SP0002087	210002	>97	07/19/07
PFNA (C9)	SP0002085	H7568	>99	07/19/07
C10	SP0002090	R11K	98	12/01/10
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

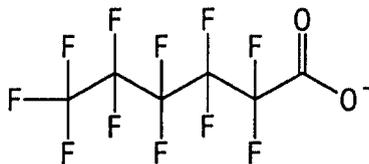
Molecular Weight: 499, as shown



Name: C6

Chemical Name: Perfluorohexanoate

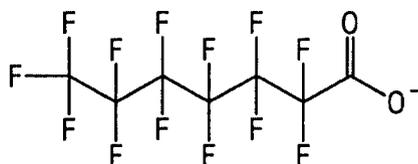
Molecular Weight: 313, as shown



Name: C7

Chemical Name: Perfluoroheptanoate

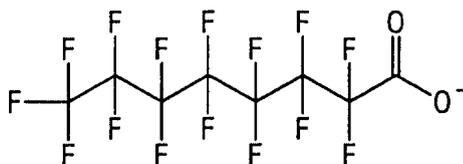
Molecular Weight: 363, as shown



Name: C8

Chemical Name: Pentadecafluorooctanoate

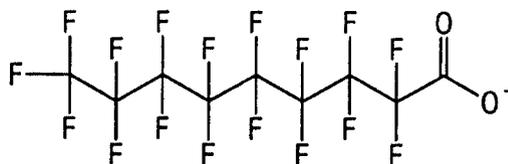
Molecular Weight: 413, as shown



Name: C9

Chemical Name: Heptadecafluorononanoate

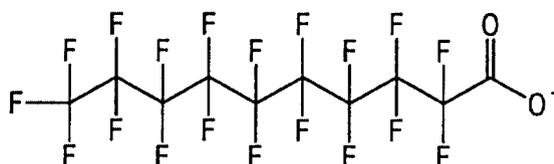
Molecular Weight: 463, as shown



Name: C10

Chemical Name: Nonadecafluorodecanoate

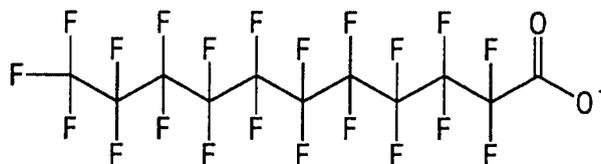
Molecular Weight: 513, as shown



Name: C11

Chemical Name: Perfluoroundecanoate

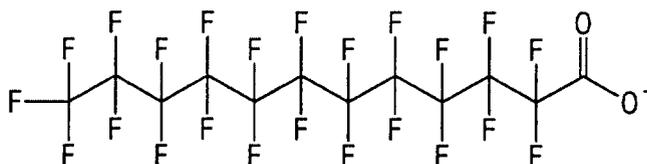
Molecular Weight: 563, as shown



Name: C12

Chemical Name: Perfluorododecanoate

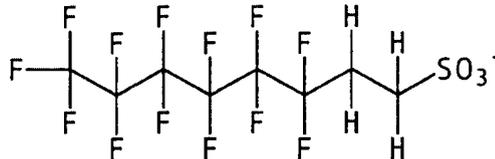
Molecular Weight: 613, as shown



Name: THPFOS

Chemical Name: Tetrahydroperfluorooctane sulfonate

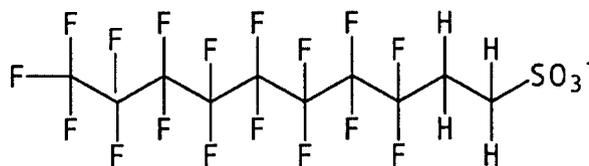
Molecular Weight: 427, as shown



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

- Measure 2 mL of serum sample into a 15 mL disposable centrifuge tube and fortify, if appropriate.
- Add 5 mL of ACN and shake for ~20 minutes on a wrist action shaker.
- Centrifuge tubes at ~3000 rpm for ~ 5 minutes. Carefully decant supernatant into a 50 mL disposable centrifuge tube and add 35 mL of water.
- Load the sample onto a conditioned SPE column. Discard the eluate. Any analyte residues will be trapped on the SPE column at this point.
- 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.
- 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 ($6^{\circ} \pm 2^{\circ}\text{C}$) 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

<u>Time (min)</u>	<u>% A</u>	<u>% B</u>	<u>Flow Rate (mL/min)</u>
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 :

<u>Anion</u>	<u>Parent ion</u>	<u>Daughter ion</u>	<u>Dwell (secs)</u>	<u>Declustering Potential</u>	<u>Collision Energy</u>
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:

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

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

Equation 2:

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

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:

Recovery (%) =

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

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:

$$\begin{aligned} \text{Analyte found (ng/mL)} &= \frac{[54636 - 632.277]}{59435.9} \\ &= 0.9 \text{ ng/mL} \end{aligned}$$

From equation 2:

$$\begin{aligned} \text{Analyte found (ppb)} &= \frac{0.9 \text{ ng/mL} \times 1 \text{ mL}}{2 \text{ mL}} \\ &= 0.45 \text{ ppb (ng/mL)} \end{aligned}$$

From equation 3:

$$\begin{aligned} \% \text{ Recovery} &= \frac{(0.45 \text{ ng/mL} - 0 \text{ ng/mL}) \times 100}{0.5 \text{ ng/mL}} \\ &= 90\% \end{aligned}$$

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

Sample ID	Sponsor ID	Fort Level (ng/mL)	% Recovery										
			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

Sample ID	Sponsor ID	Fort Level (ng/mL)	% Recovery										
			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	C6	C7	C8	C9	C10	C11	C12	PFOS	THPFOS	THPFDS
		(ng/mL)										
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	C6	C7	C8	C9	C10	C11	C12	PFOS	THPFOS	THPFDS
		(ng/mL)										
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)										PFOS	THPFOS	THPFDS
			C6	C7	C8	C9	C10	C11	C12					
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)										PFOS	THPFOS	THPFDS
			C6	C7	C8	C9	C10	C11	C12					
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

*Bio Resource
Sigmund/Aldrich
Golden West*

		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

*Golden West
Chene
Lampson
Immature Research*

		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

*Lampson
Lampson
Lampson*

		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

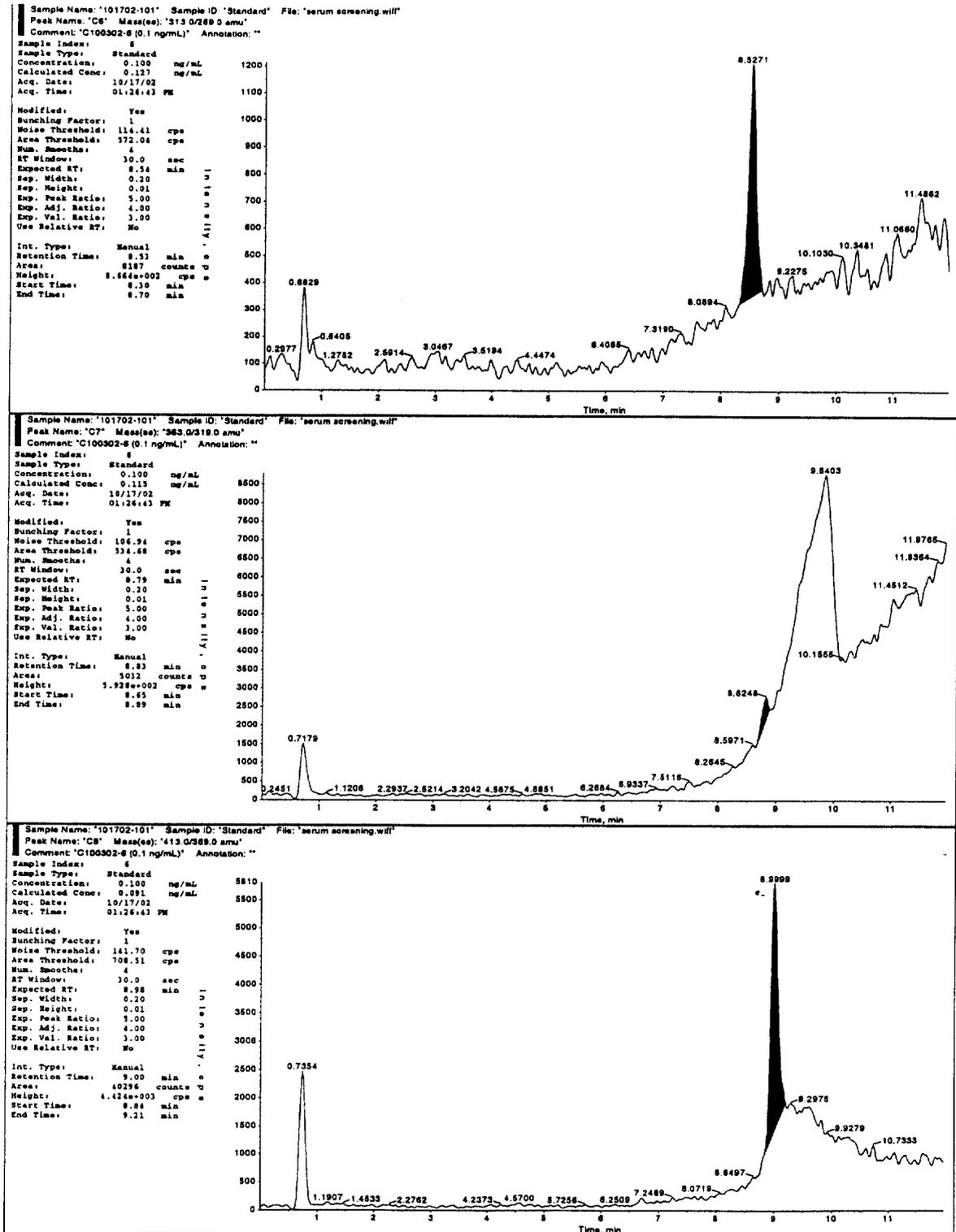


Figure 1 (cont) Chromatogram Representing 0.1 ng/mL Calibration Standard

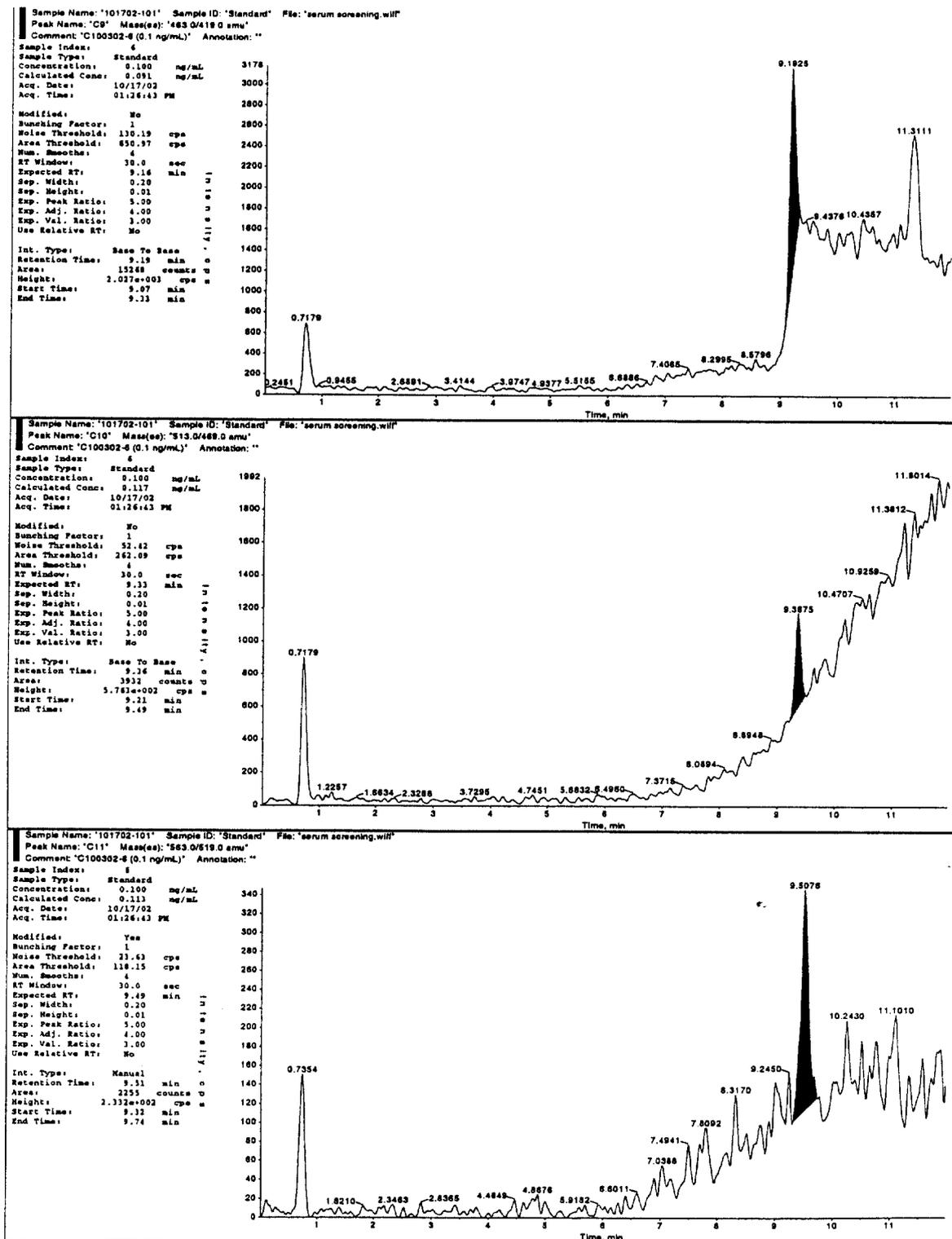


Figure 1 (cont) Chromatogram Representing 0.1 ng/mL Calibration Standard

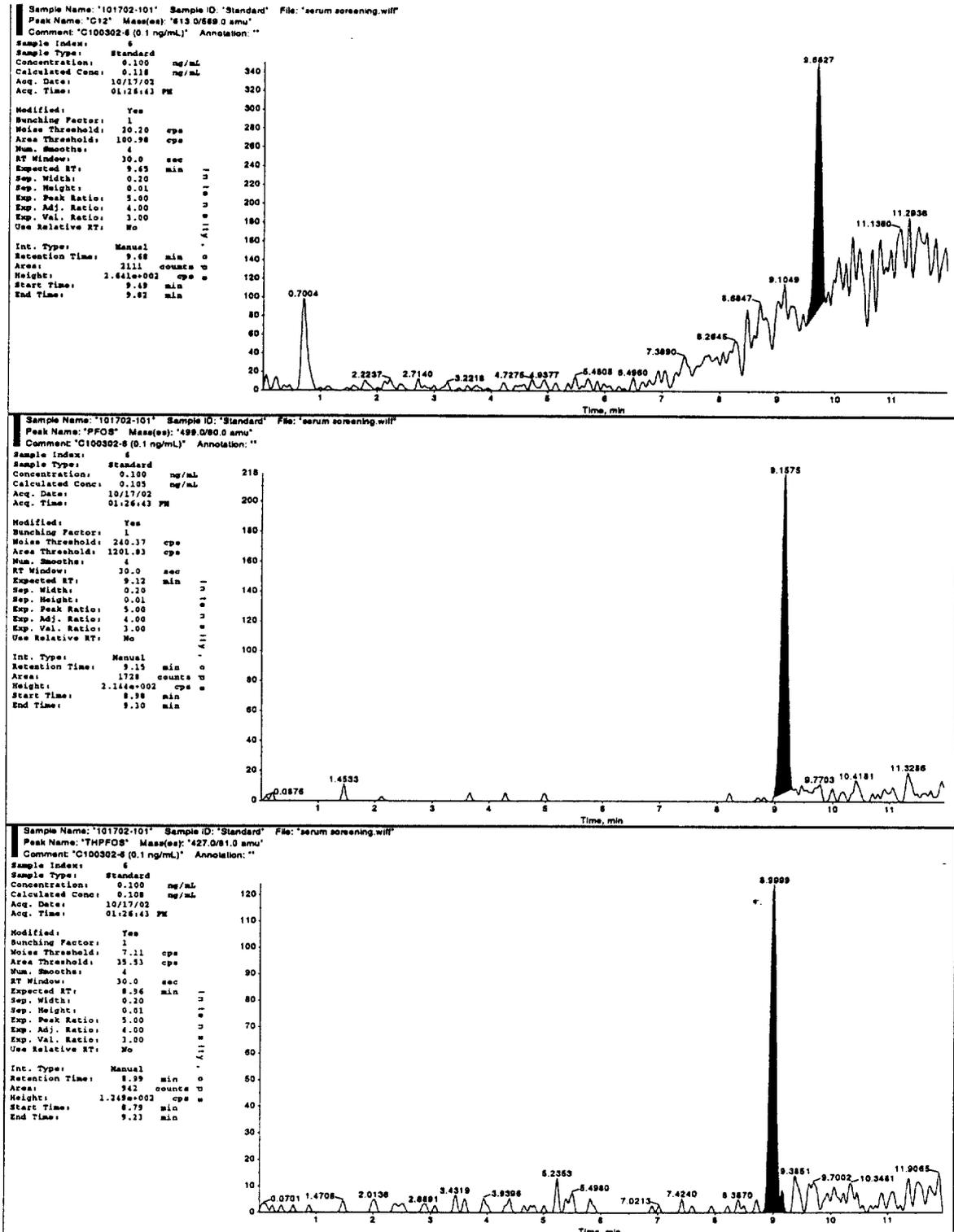


Figure 1 (cont) Chromatogram Representing 0.1 ng/mL Calibration Standard

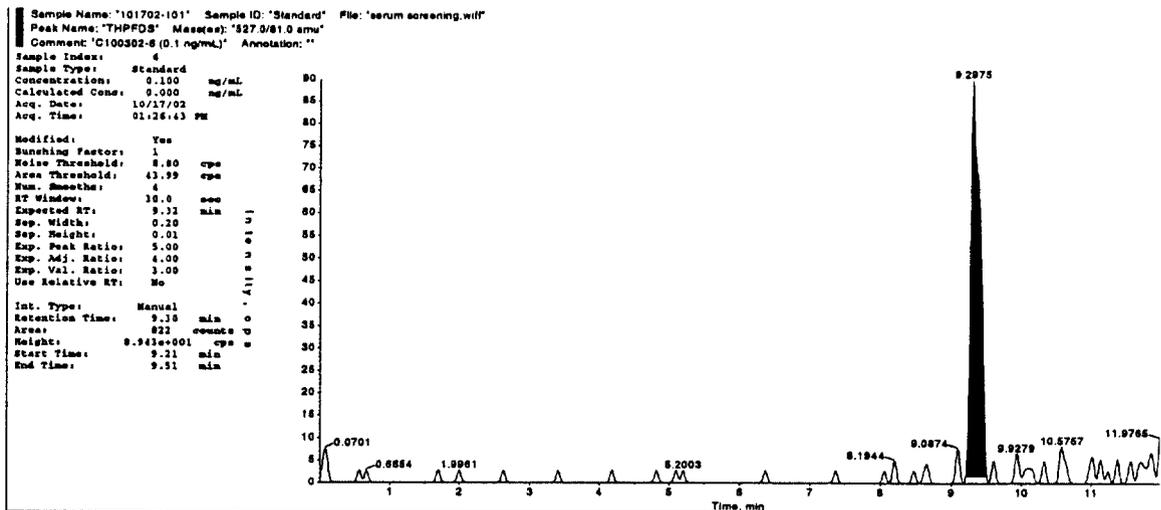


Figure 2 Chromatogram Representing a Fortified Human Plasma Sample at 0.5 ppb (Exygen ID: 0204490 Spk J, Sponsor ID: TCR-674)

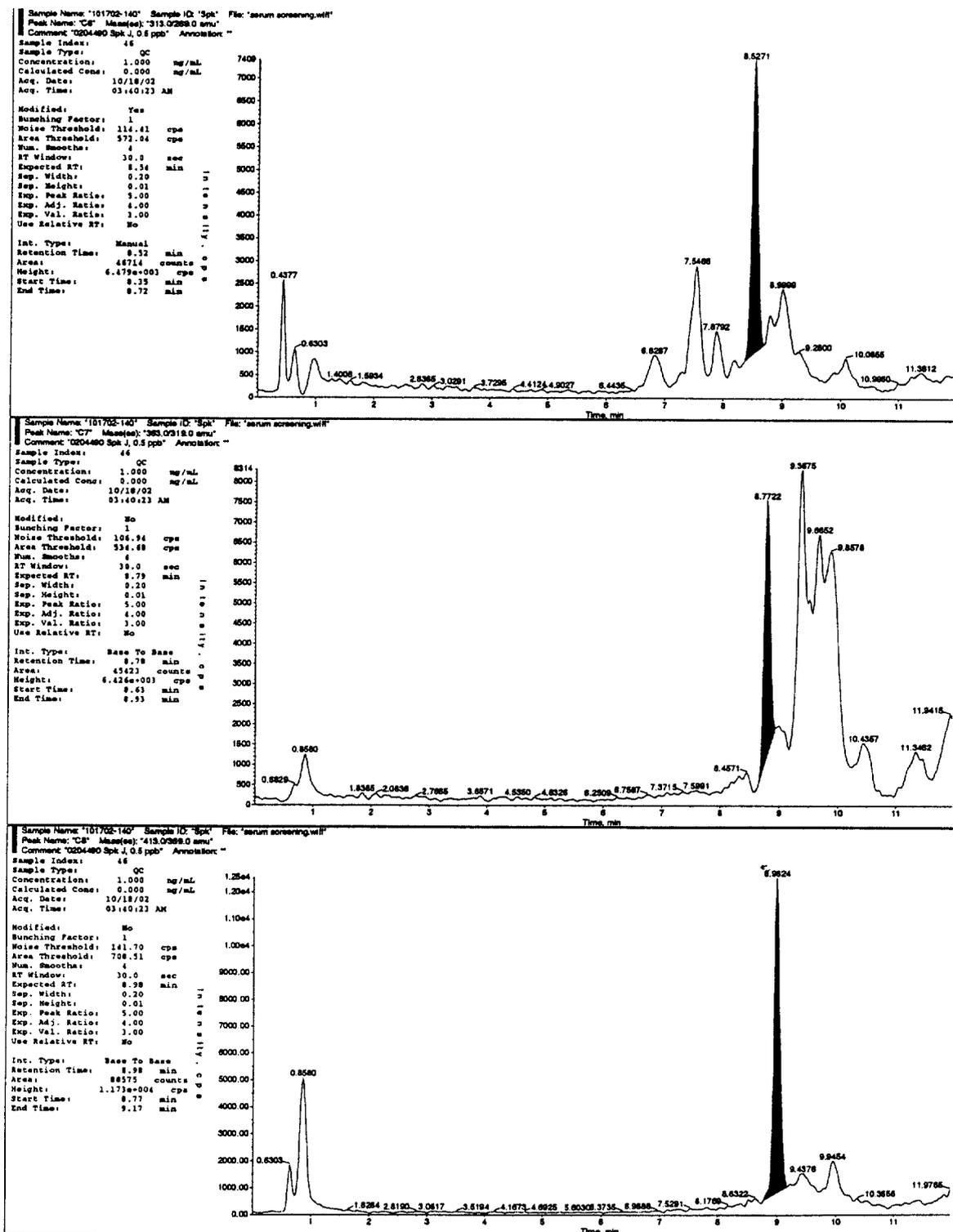


Figure 2 (cont') Chromatogram Representing a Fortified Human Plasma Sample at 0.5 ppb (Oxygen ID: 0204490 Spk J, Sponsor ID: TCR-674)

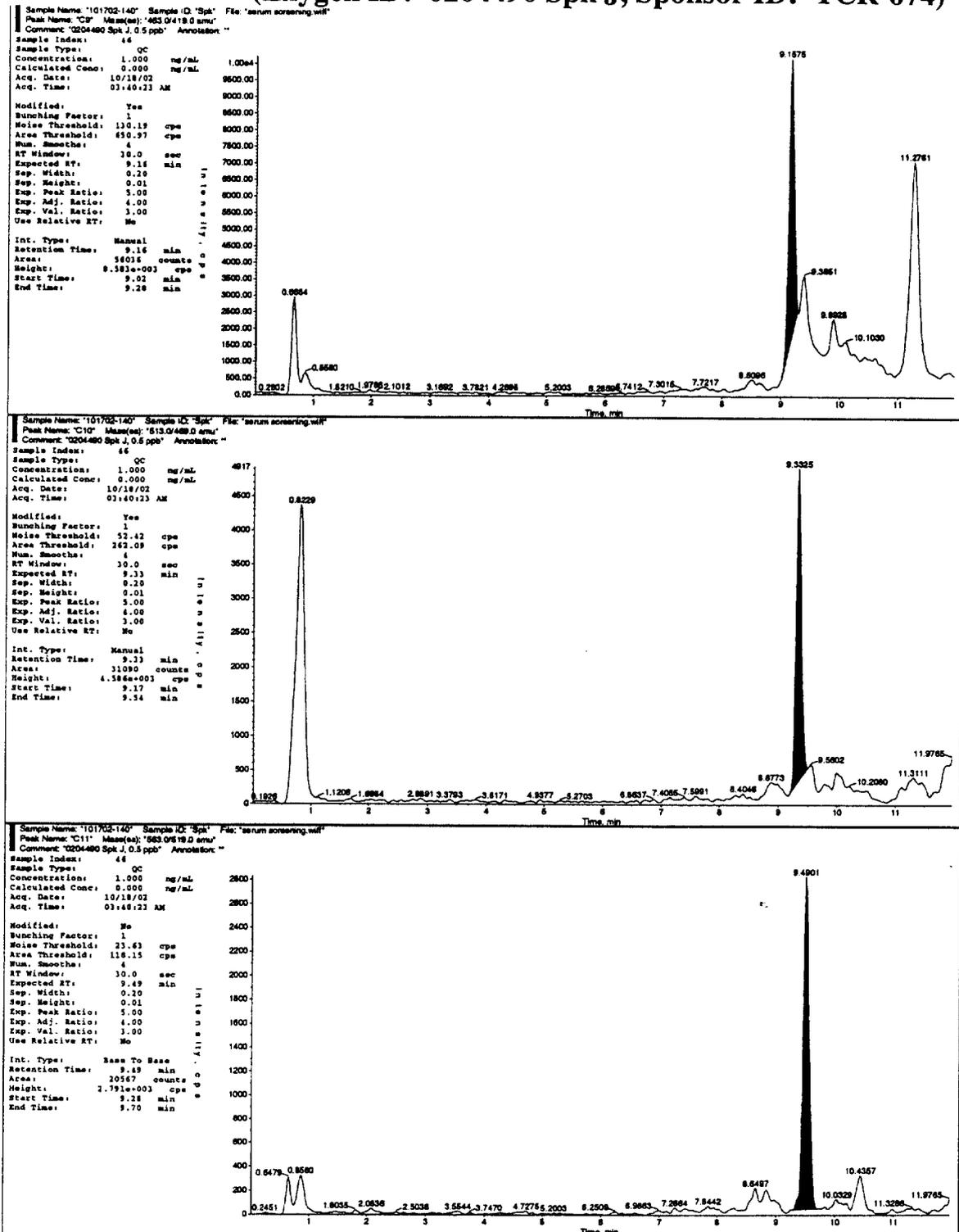


Figure 2 (cont') Chromatogram Representing a Fortified Human Plasma Sample at 0.5 ppb (Oxygen ID: 0204490 Spk J, Sponsor ID: TCR-674)

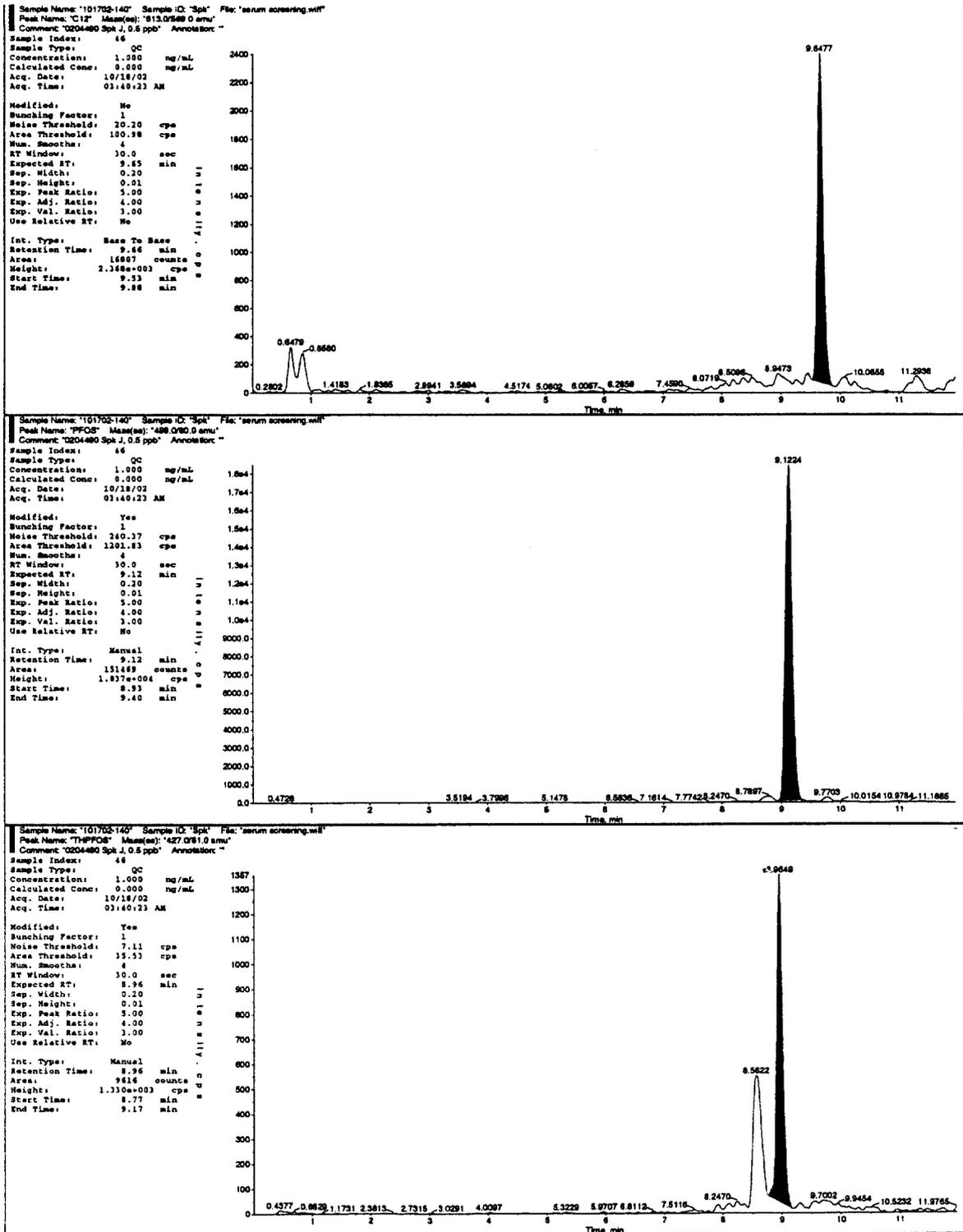


Figure 2 (cont') Chromatogram Representing a Fortified Human Plasma Sample at 0.5 ppb (Oxygen ID: 0204490 Spk J, Sponsor ID: TCR-674)

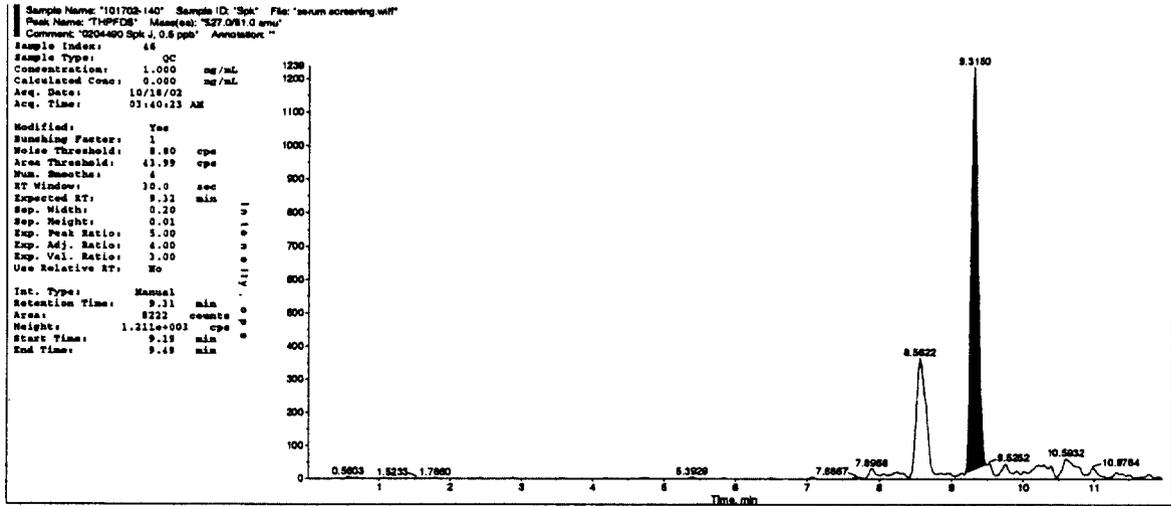


Figure 3

Chromatogram Representing a Human Plasma Sample (Oxygen ID: 0204490, Sponsor ID: TCR-674)

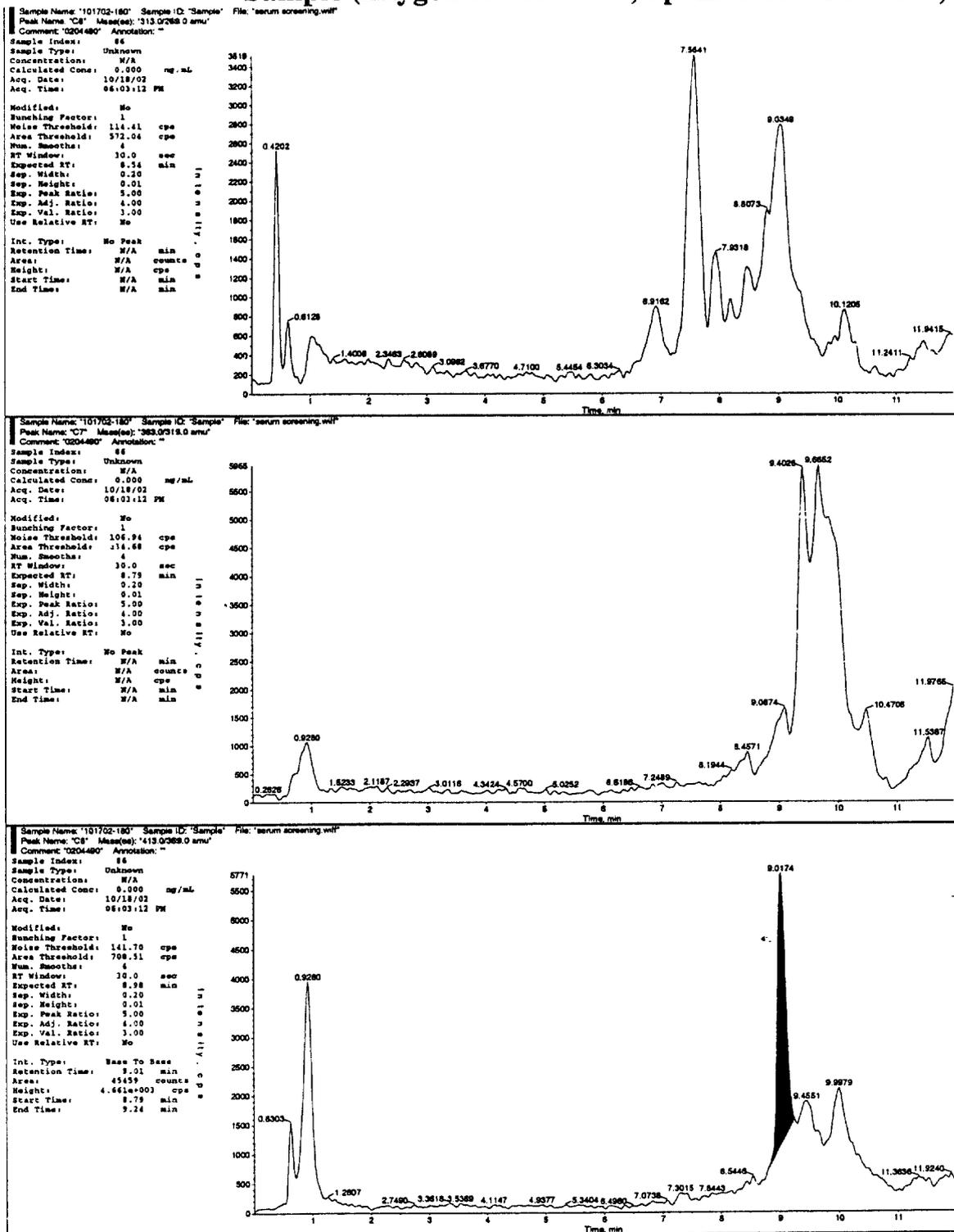


Figure 3 (cont') Chromatogram Representing a Human Plasma Sample (Exygen ID: 0204490, Sponsor ID: TCR-674)

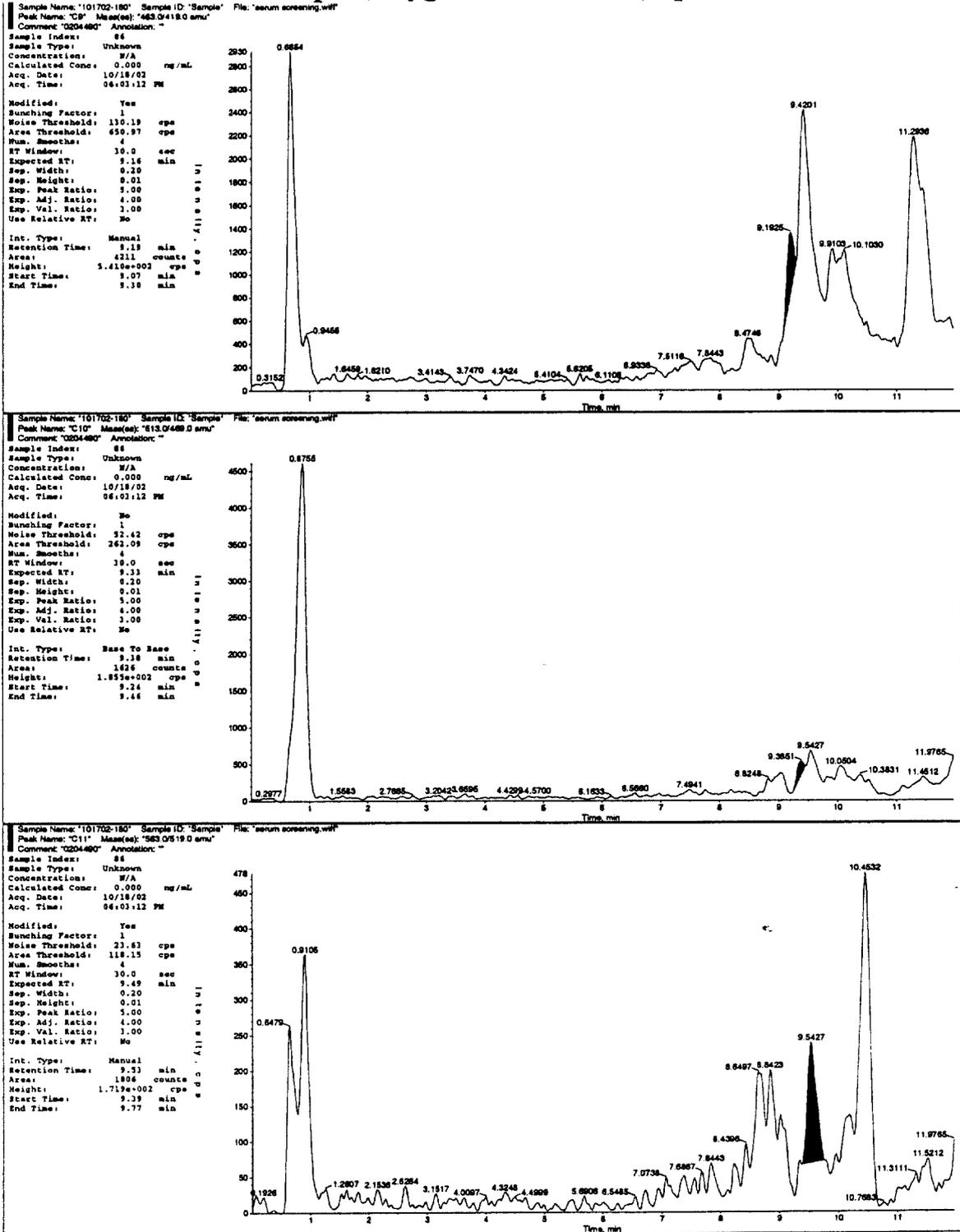


Figure 3 (cont') Chromatogram Representing a Human Plasma Sample (Exygen ID: 0204490, Sponsor ID: TCR-674)

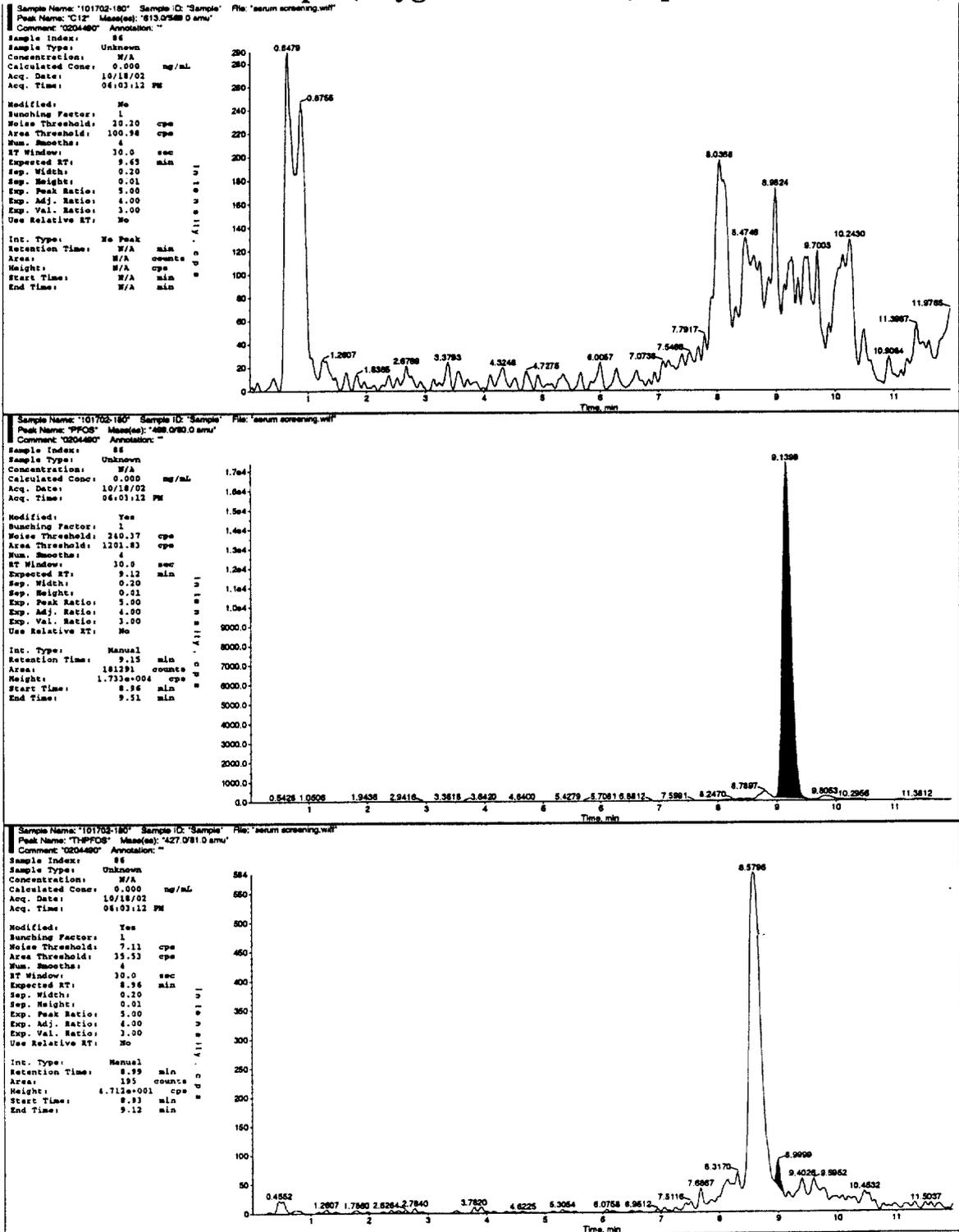


Figure 3 (cont') Chromatogram Representing a Human Plasma Sample (Exygen ID: 0204490, Sponsor ID: TCR-674)

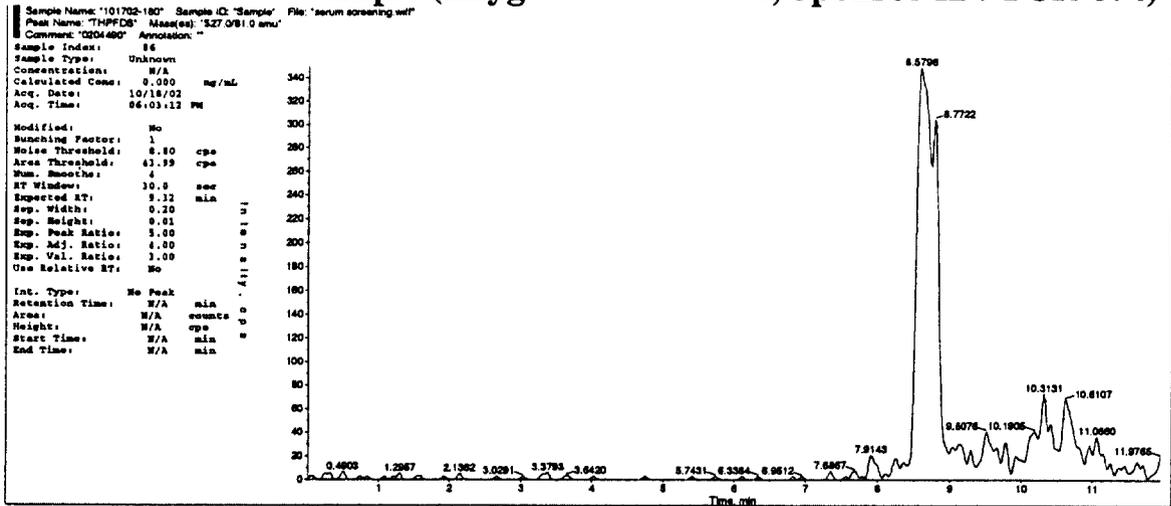
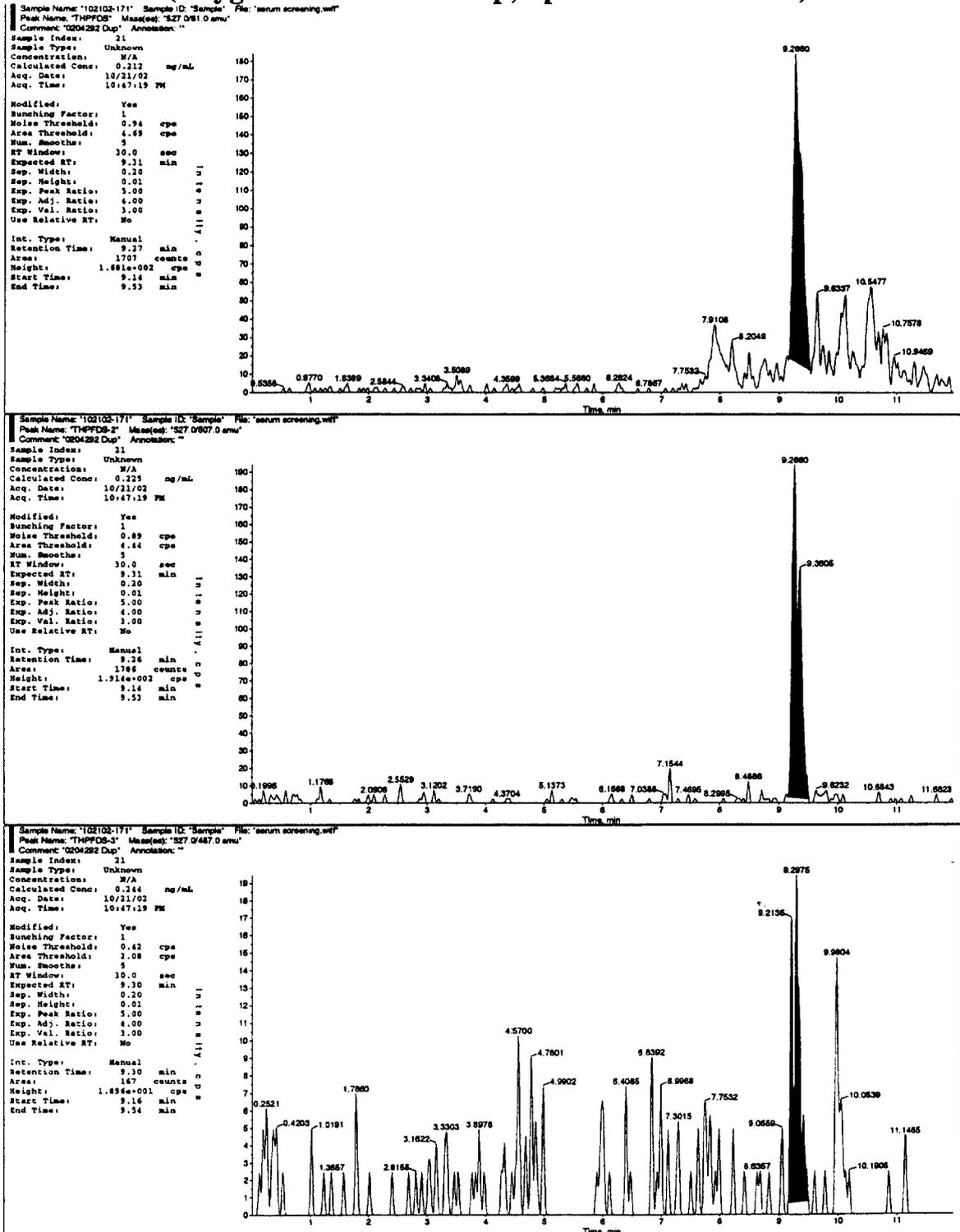


Figure 4 Chromatogram Representing a Sample Analyzed for Three Daughters for THPFDS (Exygen ID: 0204292 Dup, Sponsor ID: X328-A).



APPENDIX A

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

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

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

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

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

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
SM

10/09/02

Date

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

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

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.

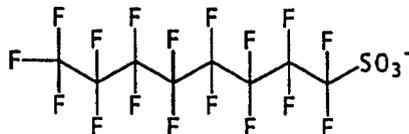
TEST ITEMS

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

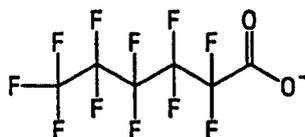


Study Plan: Exp-023-082
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Name: C6

Chemical Name: Perfluorohexanoic acid

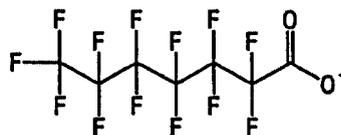
Molecular Weight: 313, as shown



Name: C7

Chemical Name: Perfluoroheptanoic acid

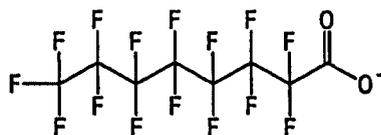
Molecular Weight: 363, as shown



Name: C8

Chemical Name: Pentadecafluorooctanoic acid

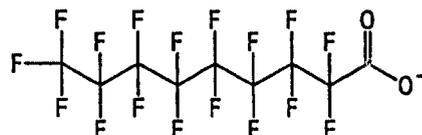
Molecular Weight: 413, as shown



Name: C9

Chemical Name: Heptadecafluorononanoic acid

Molecular Weight: 463, as shown

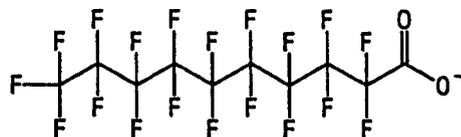


Study Plan: ExP-023-082
 Oxygen Study No.: 023-082

Name: C10

Chemical Name: Nonadecafluorodecanoic acid

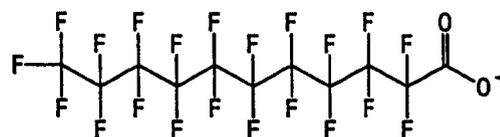
Molecular Weight: 513, as shown



Name: C11

Chemical Name: Perfluoroundecanoic acid

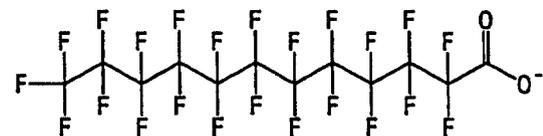
Molecular Weight: 563, as shown



Name: C12

Chemical Name: Perfluorododecanoic acid

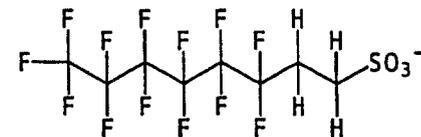
Molecular Weight: 613, as shown



Name: THPFOS

Chemical Name: Tetrahydroperfluorooctane sulfonate

Molecular Weight: 427, as shown

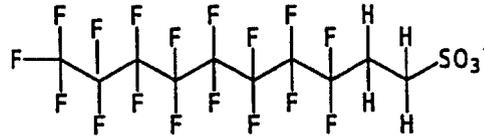


Study Plan: ExP-023-082
Exygen Study No.: 023-082

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."

TESTING FACILITY

Exygen Research
3058 Research Drive
State College, PA 16801
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STUDY DIRECTOR

Emily Decker
Scientist
Exygen Research
Phone: (814) 272-1039
emily.decker@exygen.com

SPONSOR

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

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

SPONSOR STUDY MONITOR

William Reagen
Building 2-3E-09
St. Paul, MN 55133-3331
Phone: (651) 778-6565
wkreagen@mmm.com

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

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Exygen Study No.: 023-082

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 (μ L)	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.

Study Plan: ExP-023-082
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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.

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

- 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.

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 Exygen Study No.: 023-082

- 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.

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

- Analytical standard chromatograms will additionally include the concentration (e.g., $\mu\text{g/mL}$).
- 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.

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

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

John Flaherty, Karen Risha, and Emily Decker

DATE ISSUED

July 30, 2002

SPONSOR

3M Medical Department Corporate Toxicology
3M Center, Building 220-2E-02
St. Paul, MN 55144-1000

PERFORMING LABORATORY

Exygen Research
3058 Research Drive
State College, PA 16801

METHOD NUMBER

ExM-023-071

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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 \pm 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% \pm 9.9% (n=3)	10	108% \pm 4.7% (n=3)
50	98% \pm 3.5% (n=3)	50	111% \pm 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% \pm 8.5% (n=3)	10	88% \pm 9.8% (n=3)	93% \pm 4.7% (n=3)
50	88% \pm 1.5% (n=3)	50	120% \pm 2.1% (n=3)	79% \pm 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% \pm 3.1% (n=3)	10	117% \pm 1.5% (n=3)	89% \pm 2.5% (n=3)
50	94% \pm 2.5% (n=3)	50	111% \pm 4.0% (n=3)	87% \pm 2.1% (n=3)

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

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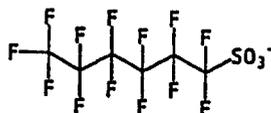
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2. **EXPERIMENTAL COMPOUNDS**

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

PFHS

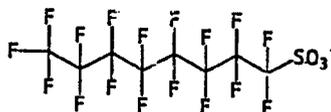
Chemical Name = Perfluorohexanesulfonate
 Molecular weight = 399, as shown



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

PFOS

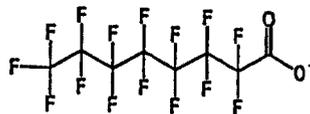
Chemical Name = Perfluorooctanesulfonate
 Molecular weight = 499, as shown



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



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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.

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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.

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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).

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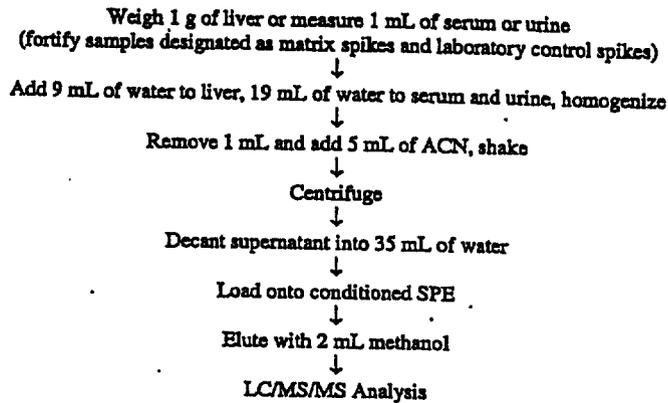
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4. METHOD

4.1. FLOW DIAGRAM

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

Method Flow Diagram



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 CO₂ 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.

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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.

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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 C₈ (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

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from different manufacturers (Keystone Betasil C₁₈ 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 (R² ≥0.985). If calibration results fall outside these limits, then appropriate steps must be taken to adjust instrument operation, and the standards or the relevant set of samples should be reanalyzed.

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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 μ L) 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.

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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 ($R^2 \geq 0.985$). If calibration results fall outside these limits, then appropriate steps must be taken to adjust instrument operation, and the standards or the relevant set of samples should be reanalyzed.
6. Retention times between standards and samples must not drift more than $\pm 4\%$ 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).

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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.

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

Equation 3:

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

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.

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

Exygen Method No: ExM-023-071

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

Study Plan: EXP-023-082
 Oxygen Study No.: 023-082

Oxygen Method No: EXM-023-071

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:		8.5
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

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

Exygen Method No: ExM-023-071

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	118
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	88
0201682 Spk B	10	91
0201682 Spk C	10	89
Average:		89
Standard Deviation:		2.5

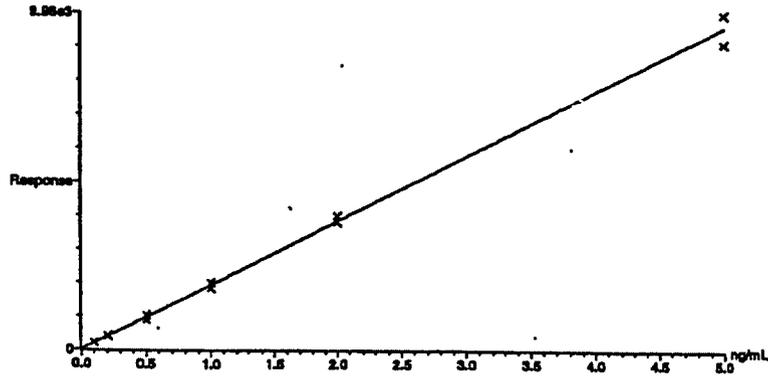
Sample ID	Analyte Added (ng/mL)	Percent Recovery (%)
0201682 Spk D	50	89
0201682 Spk E	50	88
0201682 Spk F	50	85
Average:		87
Standard Deviation:		2.1

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

Oxygen Method No: ExM-023-071

Figure 1. Calibration Curve for PFHS

Compound 1 name: PFHS
Coefficient of Determination: 0.996485
Calibration curve: $1818.28 \cdot x + 3.14154$
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None

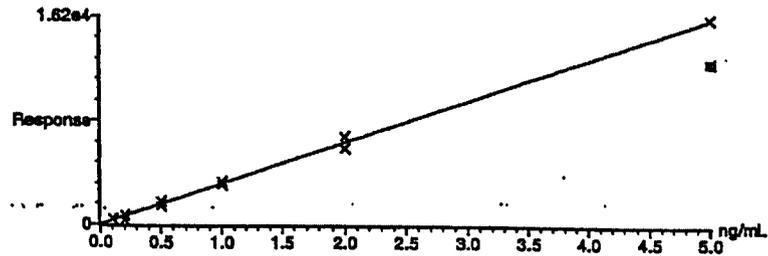


Study Plan: ExP-023-082
Oxygen Study No.: 023-082

Oxygen Method No: ExM-023-071

Figure 2. Calibration Curve for PFOS

Compound 2 name: PFOS
Coefficient of Determination: 0.997009
Calibration curve: $3211.30 \cdot x + 48.9285$
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None

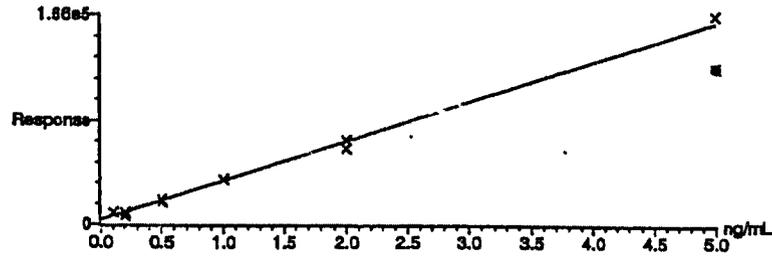


Study Plan: ExP-023-082
Oxygen Study No.: 023-082

Oxygen Method No: ExM-023-071

Figure 3. Calibration Curve for PFOA

Compound 1 name: PFOA
Coefficient of Determination: 0.995945
Calibration curve: $31270.9 * x + 3675.38$
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None



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

Exygen Method No: ExM-023-071

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

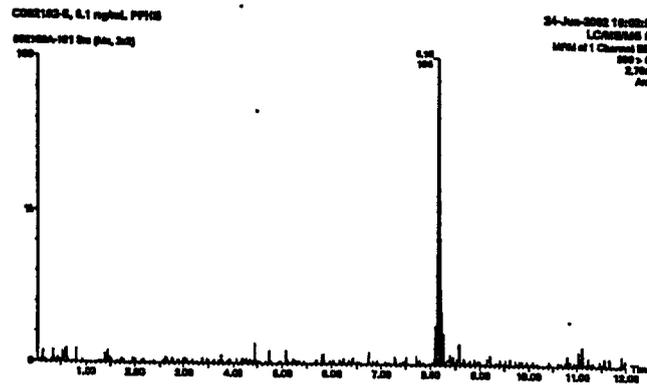
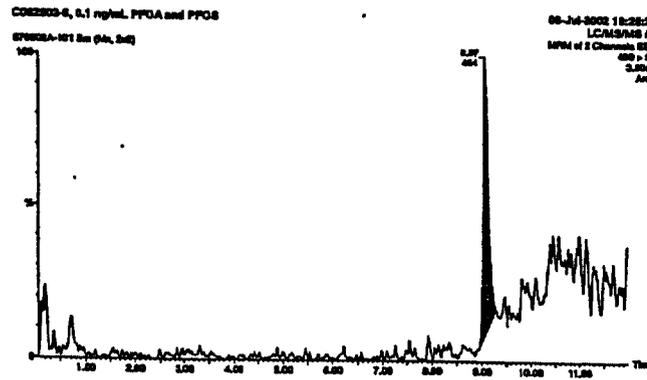


Figure 5. Representative Chromatogram of a 0.1 ng/mL Standard Containing PFOS



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

Exygen Method No: ExM-023-071

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

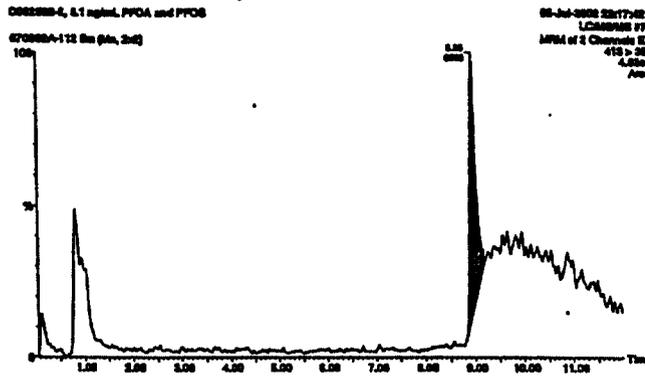
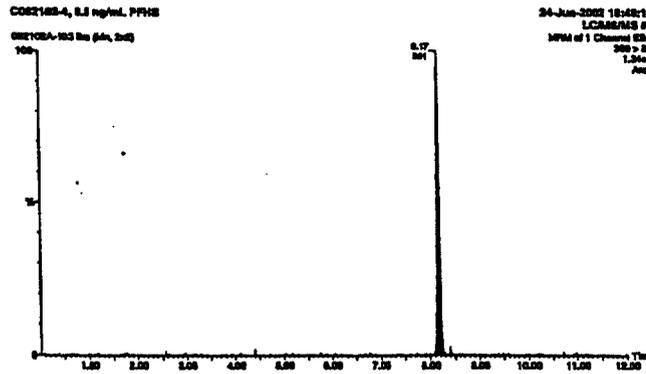


Figure 7. Representative Chromatogram of a 0.5 ng/mL Standard Containing PFHS



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

Exygen Method No: ExM-023-071

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

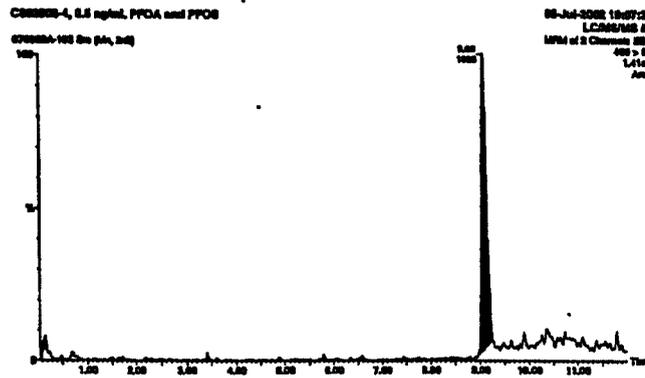
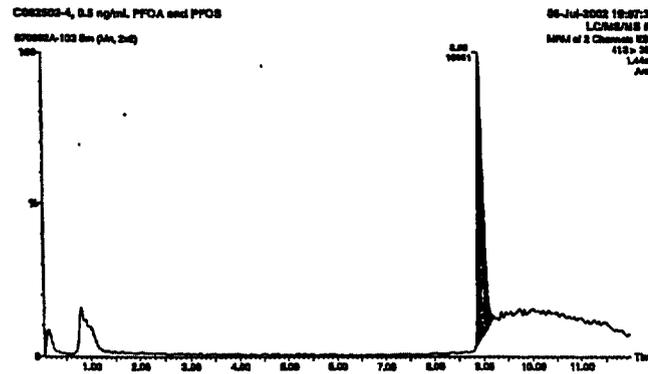


Figure 9. Representative Chromatogram of a 0.5 ng/mL Standard Containing PFOA



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

Oxygen Method No: ExM-023-071

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

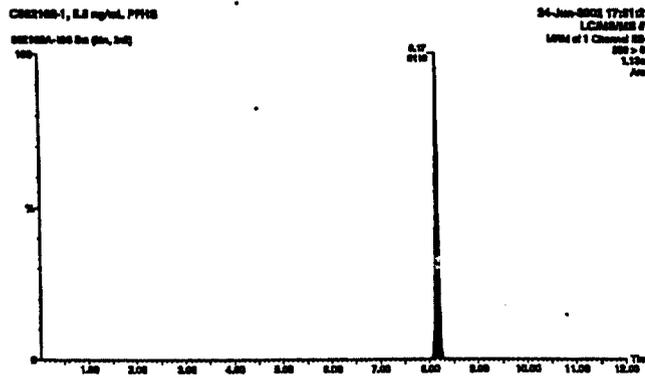
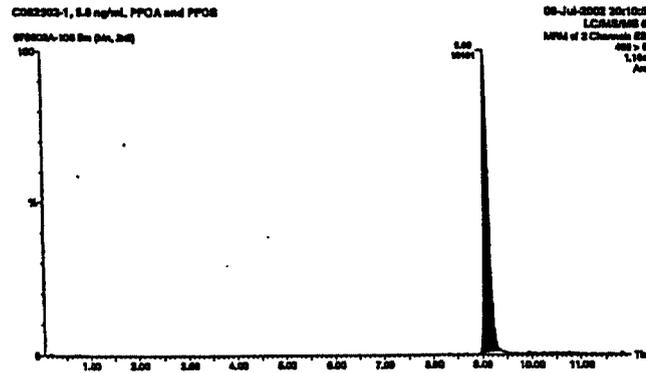


Figure 11. Representative Chromatogram of a 5.0 ng/mL Standard Containing PFOS



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

Exygen Method No: ExM-023-071

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

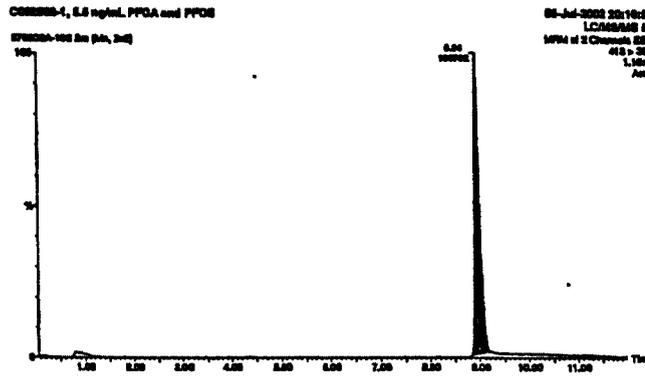
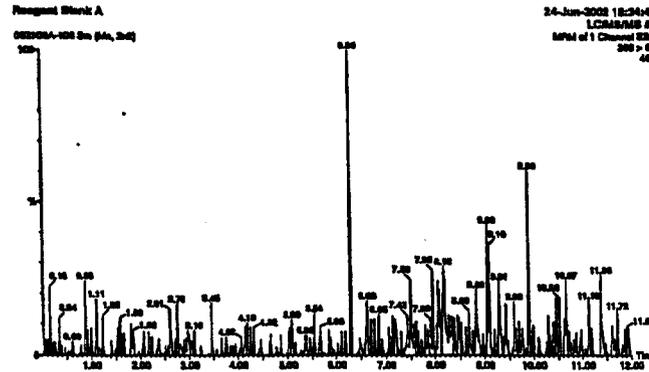


Figure 13. Representative Chromatogram of a Reagent Blank Sample Analyzed for PFHS



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

Oxygen Method No: ExM-023-071

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

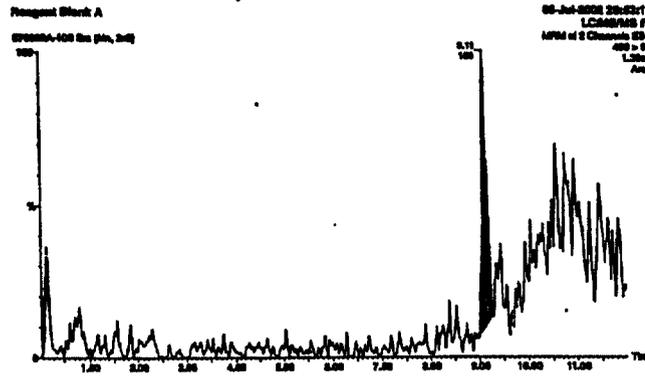
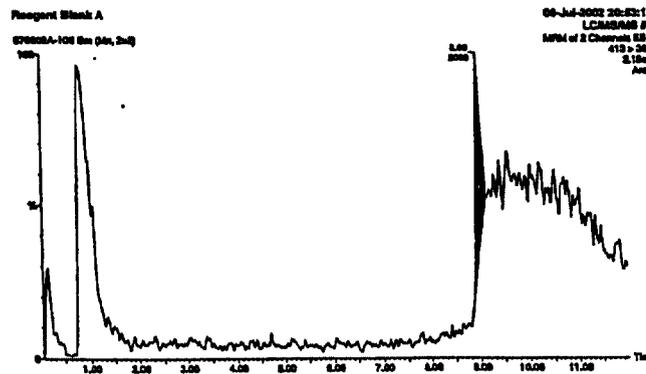


Figure 15. Representative Chromatogram of a Reagent Blank Sample Analyzed for PFOA



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

Oxygen Method No: ExM-023-071

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

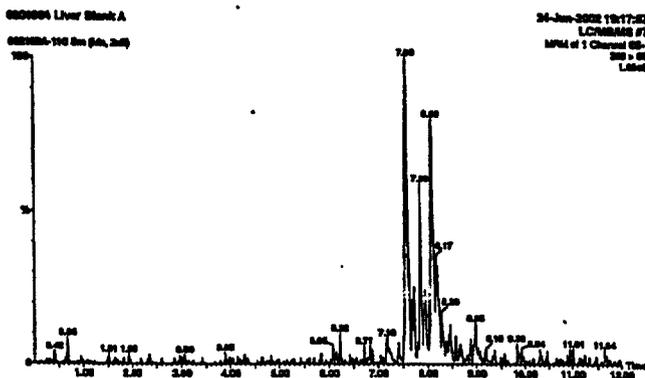
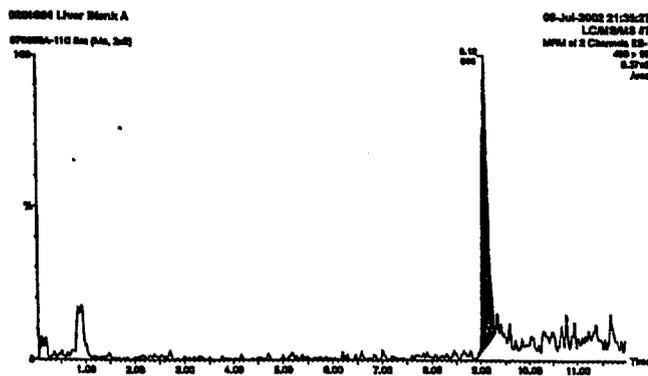


Figure 17. Representative Chromatogram of a Control Liver Sample Analyzed for PFOS



Study Plan: EXP-023-082
Oxygen Study No.: 023-082

Oxygen Method No: ExM-023-071

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

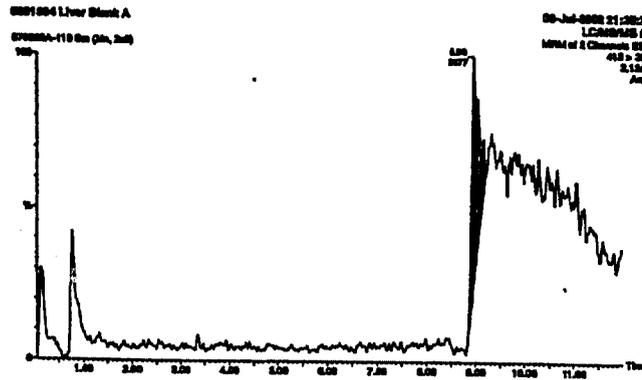
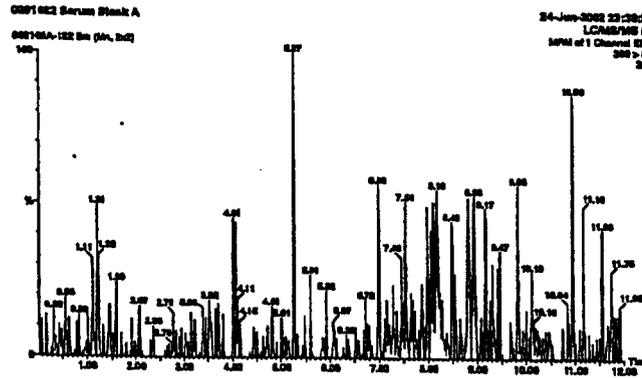


Figure 19. Representative Chromatogram of a Control Serum Sample Analyzed for PFHS



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

Oxygen Method No: ExM-023-071

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

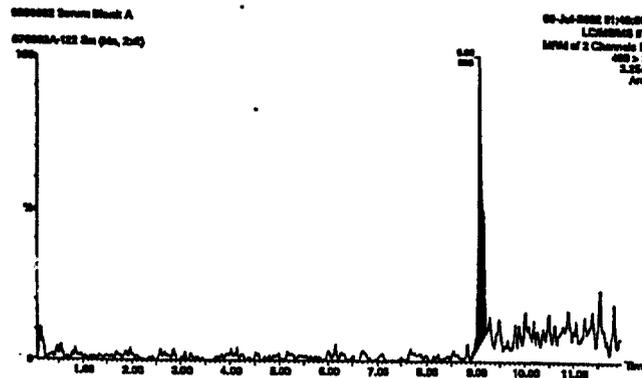
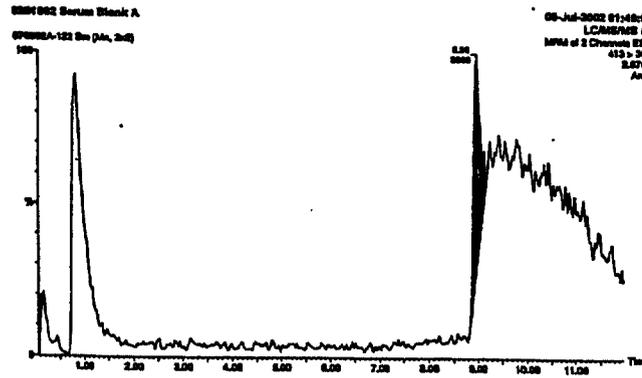


Figure 21. Representative Chromatogram of a Control Serum Sample Analyzed for PFOA



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

Oxygen Method No: ExM-023-071

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

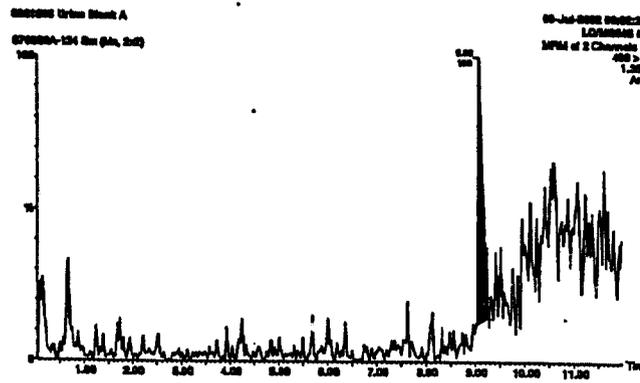
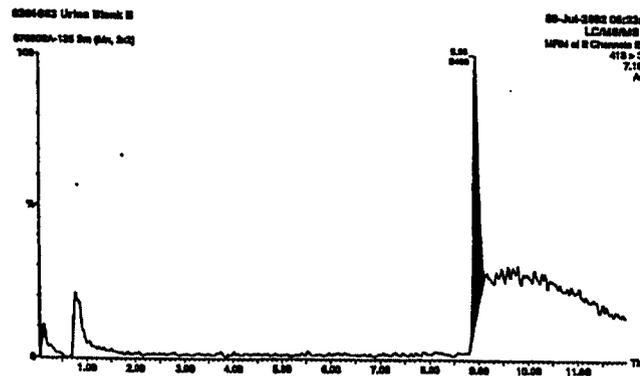


Figure 23. Representative Chromatogram of a Control Urine Sample Analyzed for PFOA



Study Plan: Exp-023-082
Oxygen Study No.: 023-082

Oxygen Method No: ExM-023-071

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

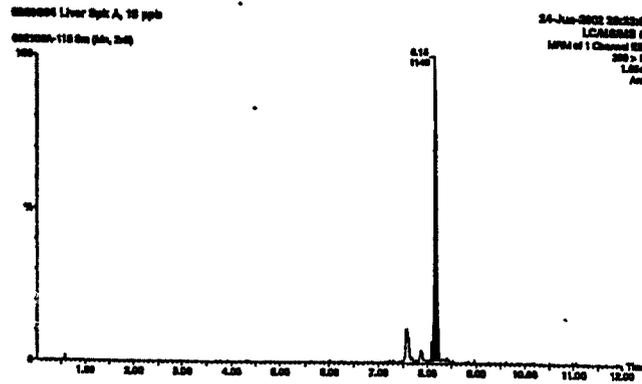
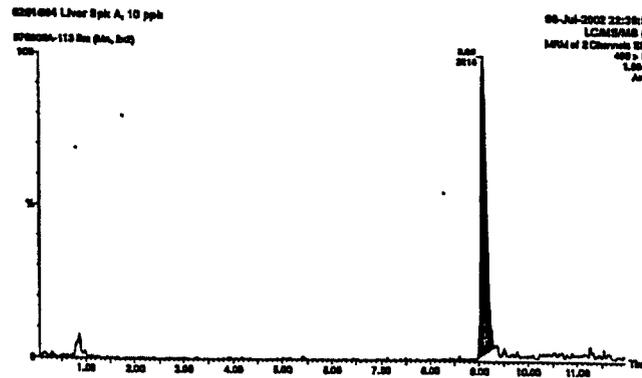


Figure 25. Representative Chromatogram of a Control Liver Sample Fortified at 10 ng/g with PFOS



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

Exygen Method No: ExM-023-071

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

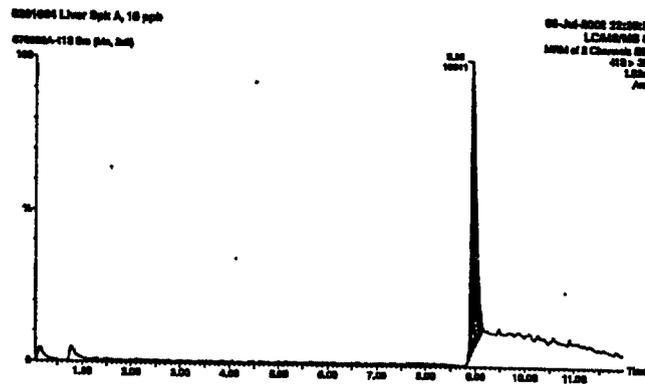
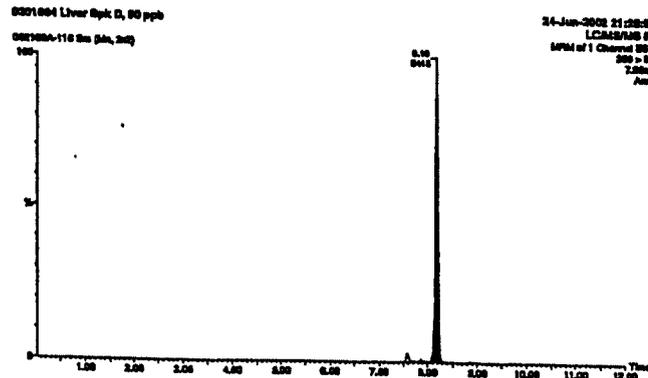


Figure 27. Representative Chromatogram of a Control Liver Sample Fortified at 50 ng/g with PFHS



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

Oxygen Method No: ExM-023-071

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

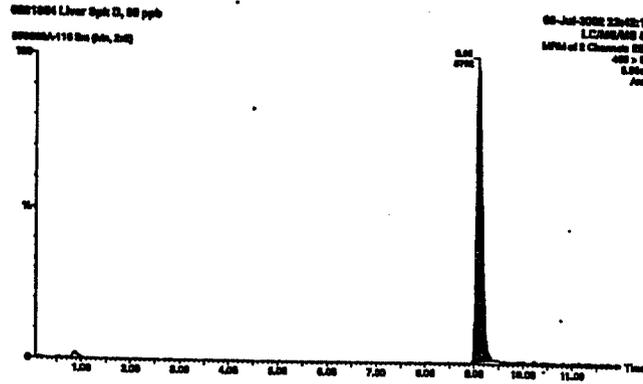
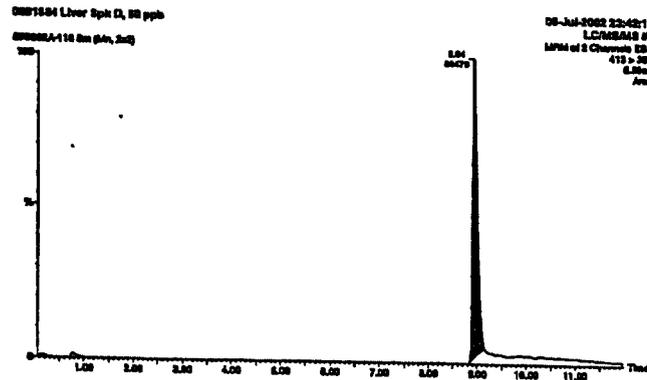


Figure 29. Representative Chromatogram of a Control Liver Sample Fortified at 50 ng/g with PFOA



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

Oxygen Method No: ExM-023-071

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

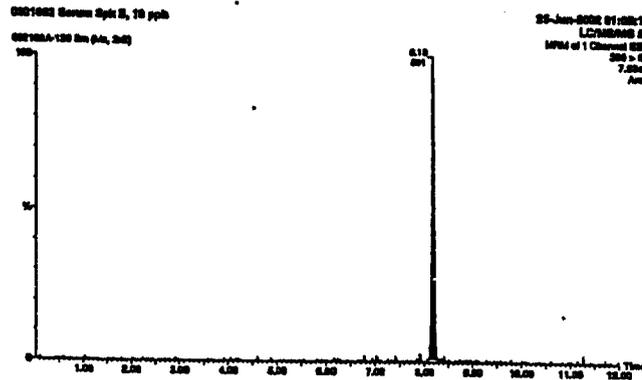
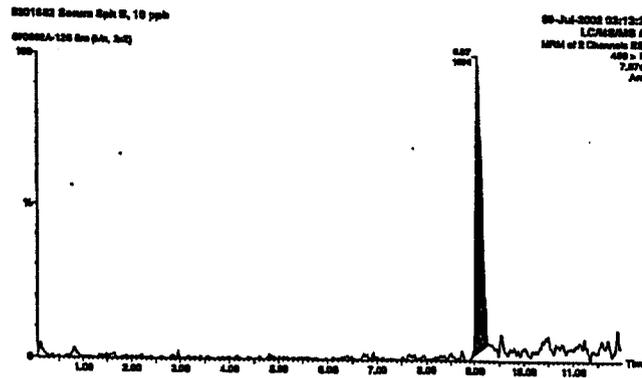


Figure 31. Representative Chromatogram of a Control Serum Sample Fortified at 10 ng/mL with PFOS



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

Exygen Method No: ExM-023-071

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

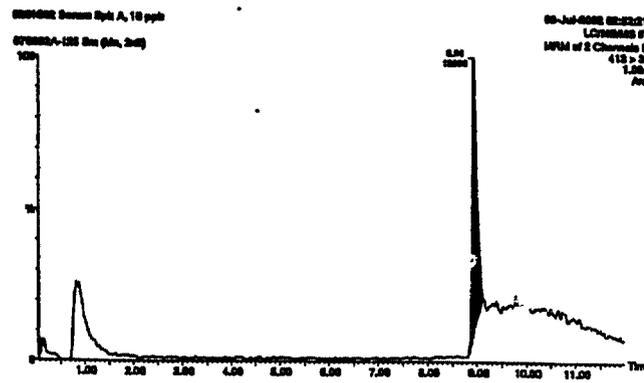
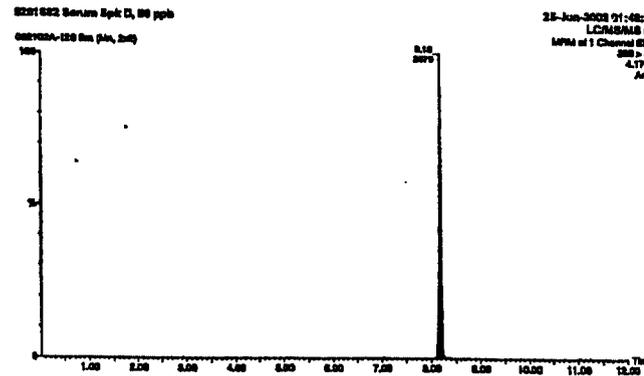


Figure 33. Representative Chromatogram of a Control Serum Sample Fortified at 50 ng/mL with PFHS



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

Exygen Method No: ExM-023-071

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

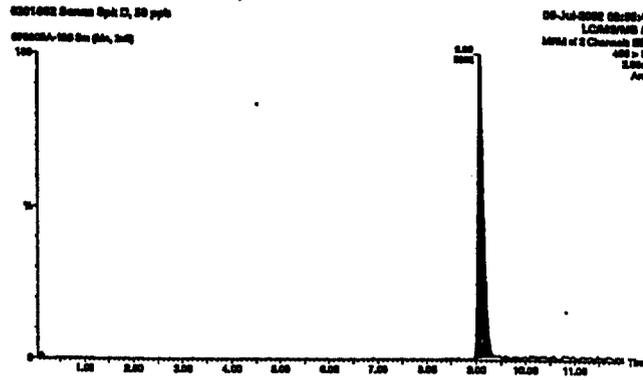
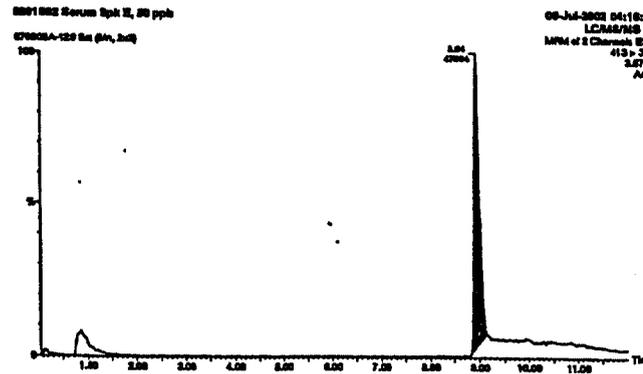


Figure 35. Representative Chromatogram of a Control Serum Sample Fortified at 50 ng/mL with PFOA



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

Oxygen Method No: ExM-023-071

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

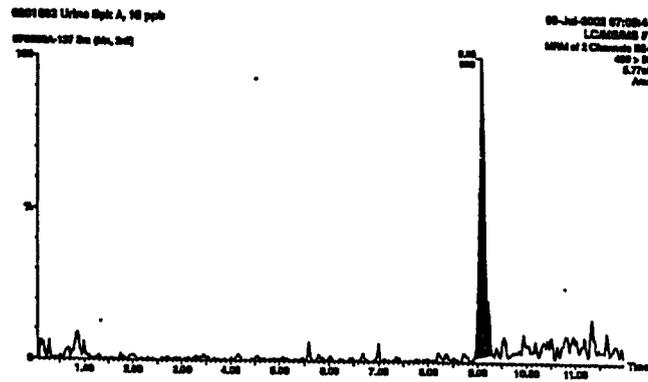
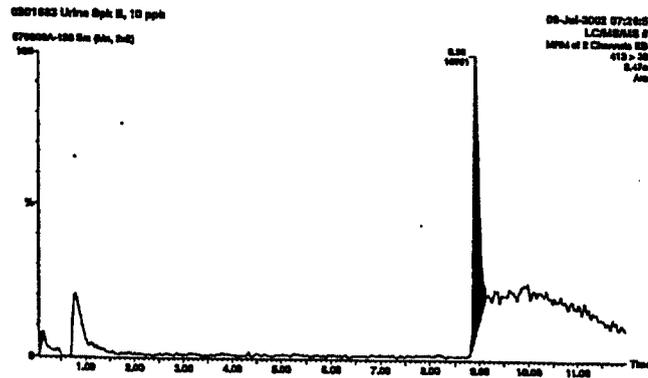


Figure 37. Representative Chromatogram of a Control Urine Sample Fortified at 10 ng/mL with PFOA



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

Exygen Method No: ExM-023-071

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

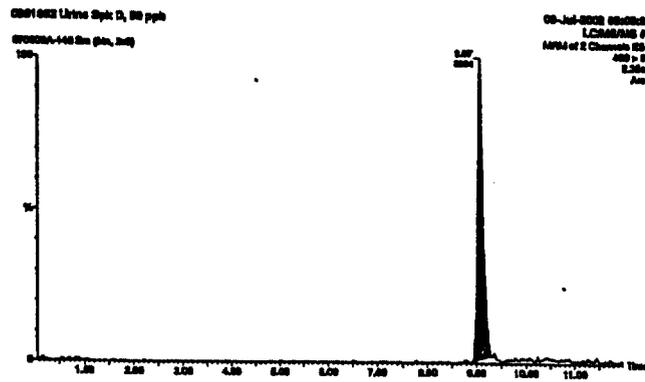
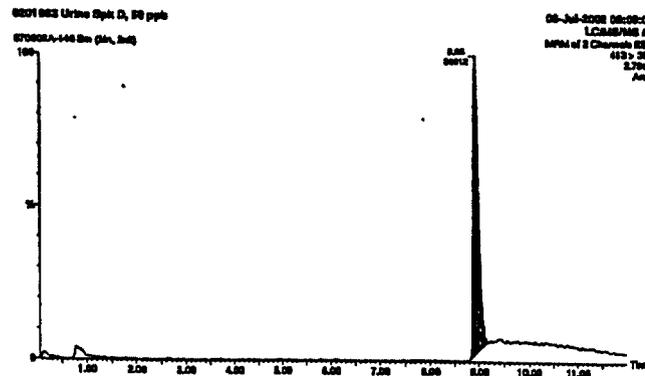


Figure 39. Representative Chromatogram of a Control Urine Sample Fortified at 50 ng/mL with PFOA





PROTOCOL DEVIATION		Page <u>1</u> of <u>1</u>
Deviation Number: <u>1</u>		
Date of Occurrence: <u>10/15/02</u>		
Exygen Study Number: <u>023-082</u>		Protocol Number: <u>ExP-023-082</u>
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		
<small>for example - deviation issued, SOP revision, etc.</small>		
Protocol deviation issued.		
Recorded By: <u>Frederic R. Decker</u>		Date: <u>10/23/02</u>
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.		
Principal Investigator Signature <u>N/A</u>		Date _____
Study Director Signature <u>Frederic R. Decker</u>		Date <u>10/30/02</u>
Management Signature <u>John M. Flaherty</u>		Date <u>10/30/02</u>
(Sponsor) _____		Date _____
Exygen QAU Review _____		Date <u>NCL 10/30/02</u>

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8/25/2002/5

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 State College, PA 16801, USA
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 F: 814.272.1019
 exygen.com



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PROTOCOL DEVIATION	
Deviation Number: <u>2</u>	
Date of Occurrence: <u>10/17/02</u>	
Exygen Study Number: <u>023-082</u>	Protocol Number: <u>EXP-023-082</u>
DESCRIPTION OF DEVIATION	
Analytical Procedure Summary-l. 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 <small>for example - deviation issued, SOP revision, etc.</small>	
Protocol deviation issued.	
Recorded By: <u>Emily R Decker</u>	Date: <u>10/23/02</u>
IMPACT ON STUDY	
No negative impact because final concentration remained the same, but complete elution of the analytes was established.	
Principal Investigator Signature <u>N/A</u>	Date _____
Study Director Signature <u>Emily R Decker</u>	Date <u>10/30/02</u>
Management Signature <u>John M Flaherty</u>	Date <u>10/30/02</u>
(Sponsor)	Date _____
Exygen QAU Review	<u>NCL 10/30/02</u>

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PROTOCOL DEVIATION		Page <u>1</u> of <u>1</u>
Deviation Number: <u>3</u>		
Date of Occurrence: <u>throughout study</u>		
Exygen Study Number: <u>023-082</u>		Protocol Number: <u>ExP-023-082</u>
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 criteria for QC spike recoveries could not always be met.		
ACTIONS TAKEN		
<i>for example - deviation issued, SOP revision, etc.</i>		
1-2. Protocol deviation issued.		
Recorded By: <u>July R Decker</u>		Date: <u>10/30/02</u>
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.		
Principal Investigator Signature <u>N/A</u>		Date _____
Study Director Signature <u>July R Decker</u>		Date <u>10/30/02</u>
Management Signature <u>John M Fleharty</u>		Date <u>11/30/02</u>
(Sponsor) _____		Date _____
Exygen QAU Review		<u>NCC 10/30/02</u>

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PROTOCOL DEVIATION		Page <u>1</u> of <u>1</u>
Deviation Number: <u>4</u>		
Date of Occurrence: <u>throughout study</u>		
Exygen Study Number: <u>023-082</u>		Protocol Number: <u>Exp-023-082</u>
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		
<i>for example - deviation issued, SOP revision, etc.</i>		
1-2. Protocol deviation issued.		
Recorded By: <u>Emily R Decker</u>		Date: <u>10/30/02</u>
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.		
Principal Investigator Signature <u>N/A</u>		Date _____
Study Director Signature <u>Emily R Decker</u>		Date <u>10/30/02</u>
Management Signature <u>John M Flaherty</u>		Date <u>10/30/02</u>
(Sponsor)		Date _____
Exygen QAU Review		<u>NCL 10/30/02</u>

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9/25/2002/5

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Page <u>1</u> of <u>1</u>	
PROTOCOL DEVIATION	
Deviation Number: <u>5</u>	
Date of Occurrence: <u>throughout study</u>	
Exygen Study Number: <u>023-082</u>	Protocol Number: <u>Exp-023-082</u>
DESCRIPTION OF DEVIATION	
1. Analytical Procedure Summary-Method Section 4.5.1---the transition monitored for PFOS was 499 -> 80	
ACTIONS TAKEN <i>for example - deviation issued, SOP revision, etc.</i>	
1. Protocol deviation issued.	
Recorded By: <u>Emily R Decker</u>	Date: <u>10/30/02</u>
IMPACT ON STUDY	
1. No negative impact.	
Principal Investigator Signature <u>N/A</u>	Date _____
Study Director Signature <u>Emily R Decker</u>	Date <u>10/30/02</u>
Management Signature <u>John M. Flaherty</u>	Date <u>10/30/02</u>
(Sponsor)	Date _____
Exygen QAU Review	<u>NCL 10/30/02</u>

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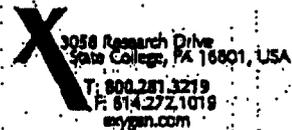
P.02/06



PROTOCOL DEVIATION		Page <u>1</u> of <u>1</u>
Deviation Number: <u>1</u>		
Date of Occurrence: <u>10/15/02</u>		
Exygen Study Number: <u>023-082</u>		Protocol Number: <u>ExR-023-082</u>
DESCRIPTION OF DEVIATION		
Analytical Procedure Summary: <u>Calibration Standards</u>		
Did not prepare extracted standards for human plasma at 1.5 and 2.5 ppb levels.		
ACTIONS TAKEN		
<small>for example: special issue, SOP revision, etc.</small>		
Protocol deviation issued.		
Recorded By: <u>Andy P. Decker</u>		Date: <u>10/23/02</u>
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.		
Principal Investigator Signature: <u>Andy P. Decker</u>	Date: <u>10/30/02</u>	
Study Director Signature: <u>John M. Rakaty</u>	Date: <u>10/30/02</u>	
Management Signature: <u>[Signature]</u>	Date: <u>10/30/02</u>	
(Sponsor) <u>[Signature]</u>	Date: <u>10/30/02</u>	
Exygen QAU Review		<u>Nec 11/20/02</u>

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PROTOCOL DEVIATION		Page <u>1</u> of <u>1</u>
Deviation Number: <u>2</u>		
Date of Occurrence: <u>10/17/02</u>		
Exygen Study Number: <u>023-082</u>		Protocol Number: <u>EXP-023-082</u>
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		
<small>for example - deviation issued SOP revision, etc.</small>		
Protocol Deviation Issued:		
Recorded By: <u>Fred R. Decker</u>	Date: <u>10/23/02</u>	
IMPACT ON STUDY		
No negative impact because final concentration remained the same, but complete elution of the analytes was established.		
Principal Investigator Signature: <u>[Signature]</u>	Date: <u>10/30/02</u>	
Study Director Signature: <u>[Signature]</u>	Date: <u>10/30/02</u>	
Management Signature: <u>[Signature]</u>	Date: <u>10/30/02</u>	
(Sponsor) <u>[Signature]</u>	Date: <u>10/30/02</u>	
Exygen QAU Review: <u>[Signature]</u>		Date: <u>10/30/02</u>

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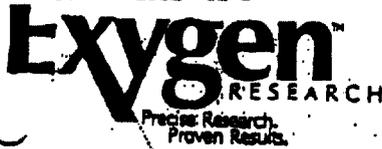
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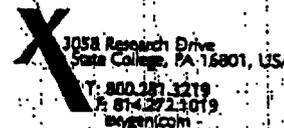
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PROTOCOL DEVIATION		Page <u>1</u> of <u>1</u>
Deviation Number: <u>3</u>		
Date of Occurrence: <u>throughout study</u>		
Exygen Study Number: <u>023-082</u>		Protocol number: <u>DP-023-082</u>
DESCRIPTION OF DEVIATION		
1. Analytical Procedure Summary 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 criteria for QC spike recoveries could not always be met.		
ACTIONS TAKEN		
<i>for example - deviation issued, SOP revision, etc.</i>		
1-2. Protocol deviation issued.		
Recorded By: <u>Andy P. Decker</u>		Date: <u>10/30/02</u>
IMPACTION STUDY		
1. No negative impact because residue was <u>less than 35%</u> 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 THPPDS and THPPDS 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.		
Principal Investigator Signature: <u>N/A</u>		Date: _____
Sivay Director Signature: <u>Andy P. Decker</u>		Date: <u>10/30/02</u>
Management Signature: <u>John M. Hackett</u>		Date: <u>10/30/02</u>
(Sponsor) <u>William Allen</u>		Date: <u>10/30/02</u>
Exygen QAU Review: <u>N/C</u>		Date: <u>10/30/02</u>

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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: corrective action, SOP revision, etc.)

1-2. Protocol deviation issued.

Recorded By: Larry 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.

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

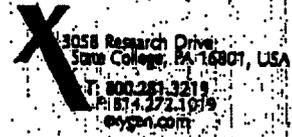
Study Director Signature: [Signature] Date: 10/30/02

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

Exygen QAU Review: NLL 10/30/02

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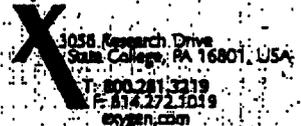
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PROTOCOL DEVIATION		Page 11 of 19
Deviation Number: <u>5</u>		
Date of Occurrence: <u>throughout study</u>		
Exygen Study Number: <u>023-082</u>		Protocol Number: <u>EXP-023-082</u>
DESCRIPTION OF DEVIATION		
1. Analytical Procedure Summary Method Section 4.3.11--the transition monitored for PFO5 was 499 -> 80		
ACTIONS TAKEN		
1. Protocol deviation issued		
Recorded By: <u>[Signature]</u>	Date: <u>10/30/02</u>	
IMPACT ON STUDY		
1. No negative impact.		
Principal Investigator Signature: <u>[Signature]</u>	Date: <u>10/30/02</u>	
Study Director Signature: <u>[Signature]</u>	Date: <u>10/30/02</u>	
Management Signature (Sponsor): <u>[Signature]</u>	Date: <u>10/30/02</u>	
Exygen QAU Review: <u>NCL</u>		Date: <u>10/30/02</u>

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