



Study Title

ANALYSIS OF ENDOGENOUS FLUOROCEMICALS IN NORMAL POOLED HUMAN SERUM AND
PLASMA

Data Requirement

40 CFR Part 792

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GLP COMPLIANCE STATEMENT

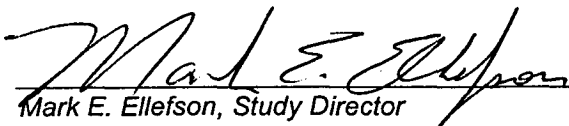
Study Title: Analysis of Endogenous Fluorochemicals in Normal Pooled Human Serum and Plasma

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

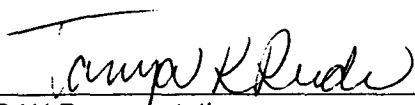
QUALITY ASSURANCE STATEMENT

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


QAU Representative

12/6/02
Date

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STUDY INFORMATION

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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All original raw data and the report have been archived at the 3M Environmental Laboratory according to 3M Standard Operating Procedures.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.

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.

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

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.

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

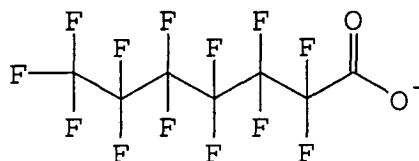
TEST SUBSTANCE	FORMULA
Tridecafluoroheptanoate (C7)	$C_6F_{13}COO^-$
Pentadecafluorooctanoate (C8)	$C_7F_{15}COO^-$
Heptadecafluorononanoate (C9)	$C_8F_{17}COO^-$
Nonadecafluorodecanoate (C10)	$C_9F_{19}COO^-$
Perfluoroundecanoate (C11)	$C_{10}F_{21}COO^-$
Perfluorododecanoate (C12)	$C_{11}F_{23}COO^-$
Perfluorooctane sulfonate (PFOS)	$C_8F_{17}SO_3^-$

The molecular structures are given below.

Name: C7

Chemical Name: Perfluoroheptanoate

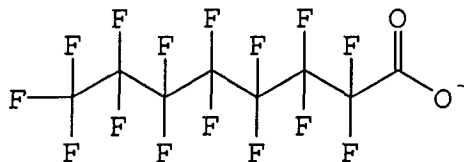
Molecular Weight: 363, as shown



Name: C8

Chemical Name: Pentadecafluorooctanoate

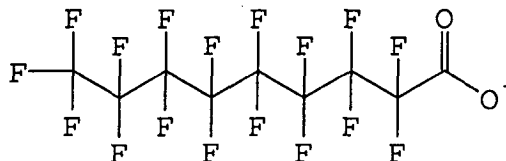
Molecular Weight: 413, as shown



Name: C9

Chemical Name: Heptadecafluorononanoate

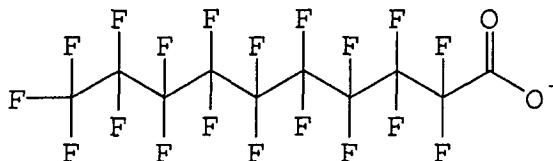
Molecular Weight: 463, as shown



Name: C10

Chemical Name: Nonadecafluorodecanoate

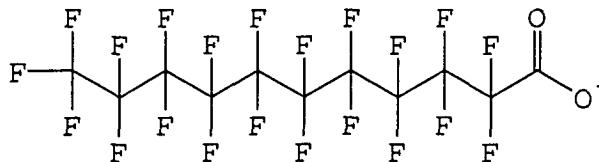
Molecular Weight: 513, as shown



Name: C11

Chemical Name: Perfluoroundecanoate

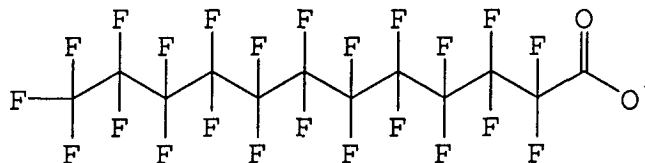
Molecular Weight: 563, as shown



Name: C12

Chemical Name: Perfluorododecanoate

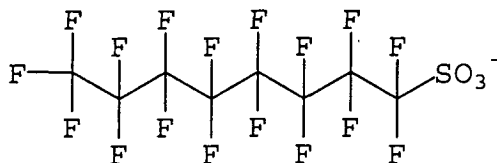
Molecular Weight: 613, as shown



Name: PFOS

Chemical Name: Perfluorooctanesulfonate

Molecular Weight: 499, as shown



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.

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

(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

(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

PFOS

<u>Channel</u>	<u>Parent Ion</u> <u>(m/z)</u>	<u>Daughter Ion</u> <u>(m/z)</u>	<u>Collision</u> <u>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 $\pm 25\%$ 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 $\pm 30\%$. 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.

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.

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.

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

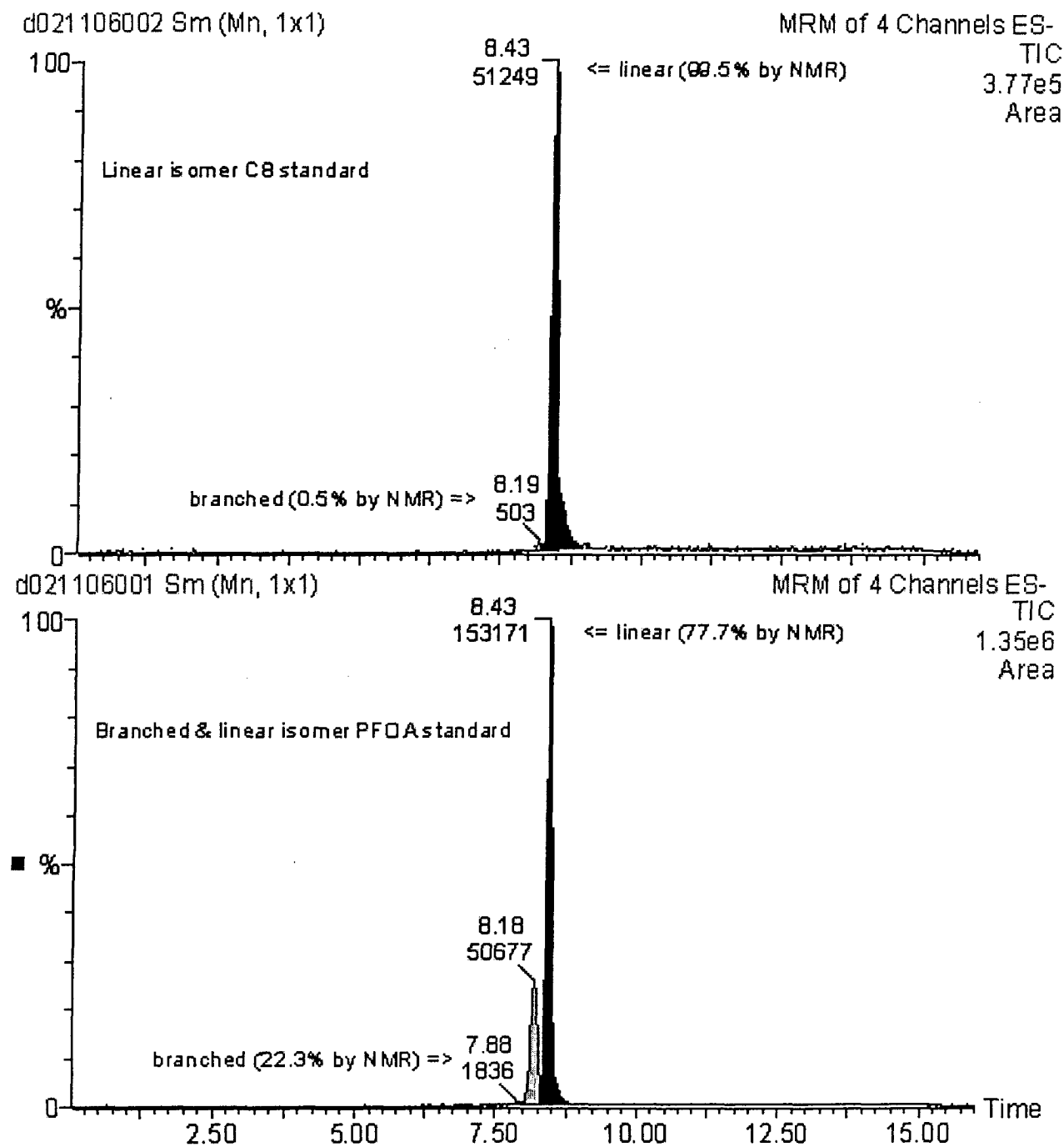
Test Substance	Parent Ion (m/z)	Daughter Ions (m/z)
C ₇	363	319, 119
C ₈	413	369, 219, 169, 119
C ₉	463	419, 269, 219, 169, 119
C ₁₀	513	469, 319, 269, 219, 169
C ₁₁	563	519, 319, 219, 169, 119
C ₁₂	613	569, 419, 319, 269, 219, 169, 119
PFOS	499	130, 99, 80

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

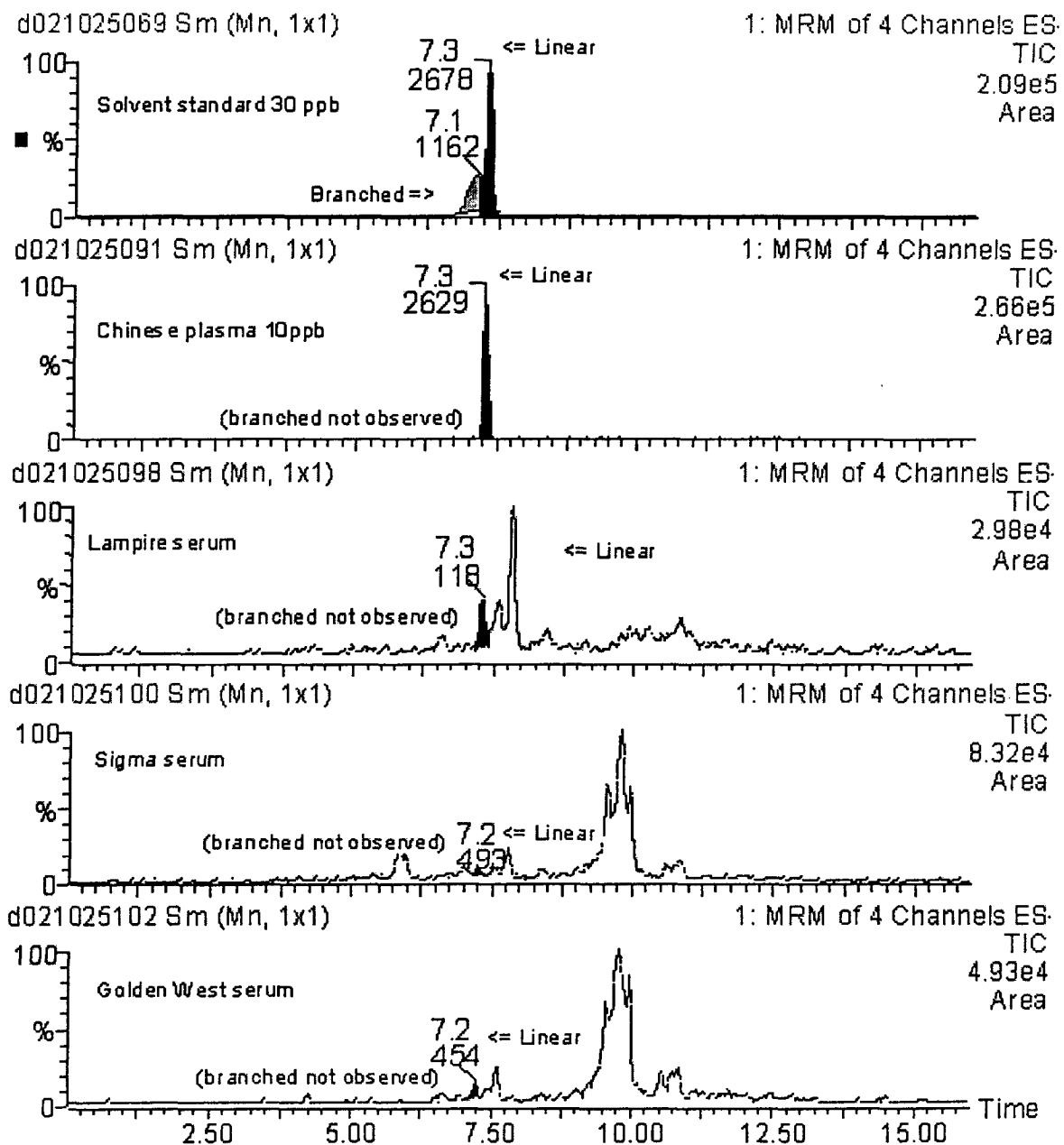
extracts of commercial pooled human serum. Pooled human plasma collected in central China was used as the blank matrix to construct an extracted calibration curve because preliminary screening results indicated it did not contain quantifiable levels of endogenous test substances.

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



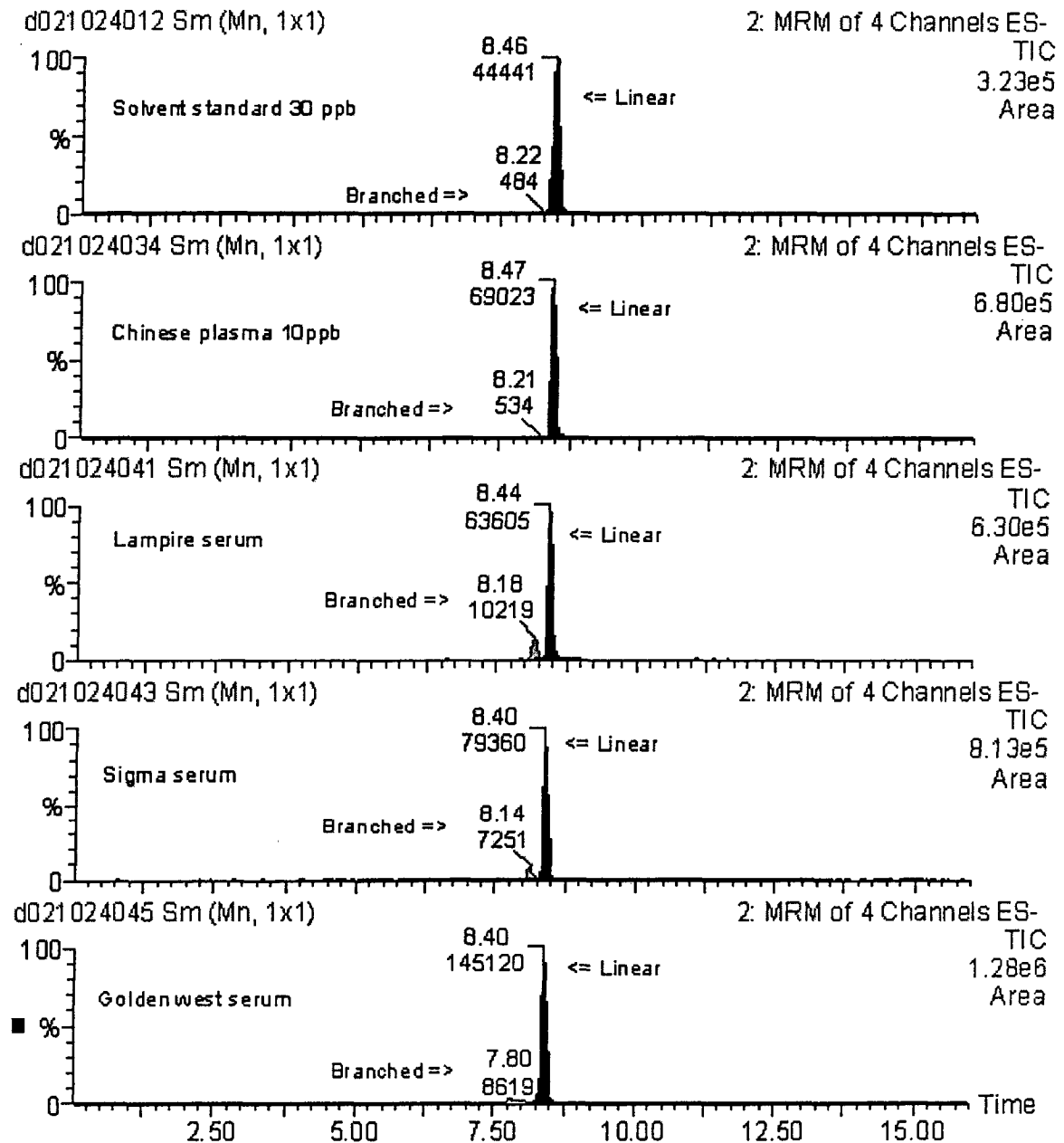
Summary of PFOA Standards: NMR certified standard of the linear isomer PFOA contains 0.5% branched and 99.5% linear isomers. NMR certified standard of mixed branched and linear isomer PFOA contains 22.3% branched and 77.7% linear isomers.

Figure 2: C₇ Isomer Distribution



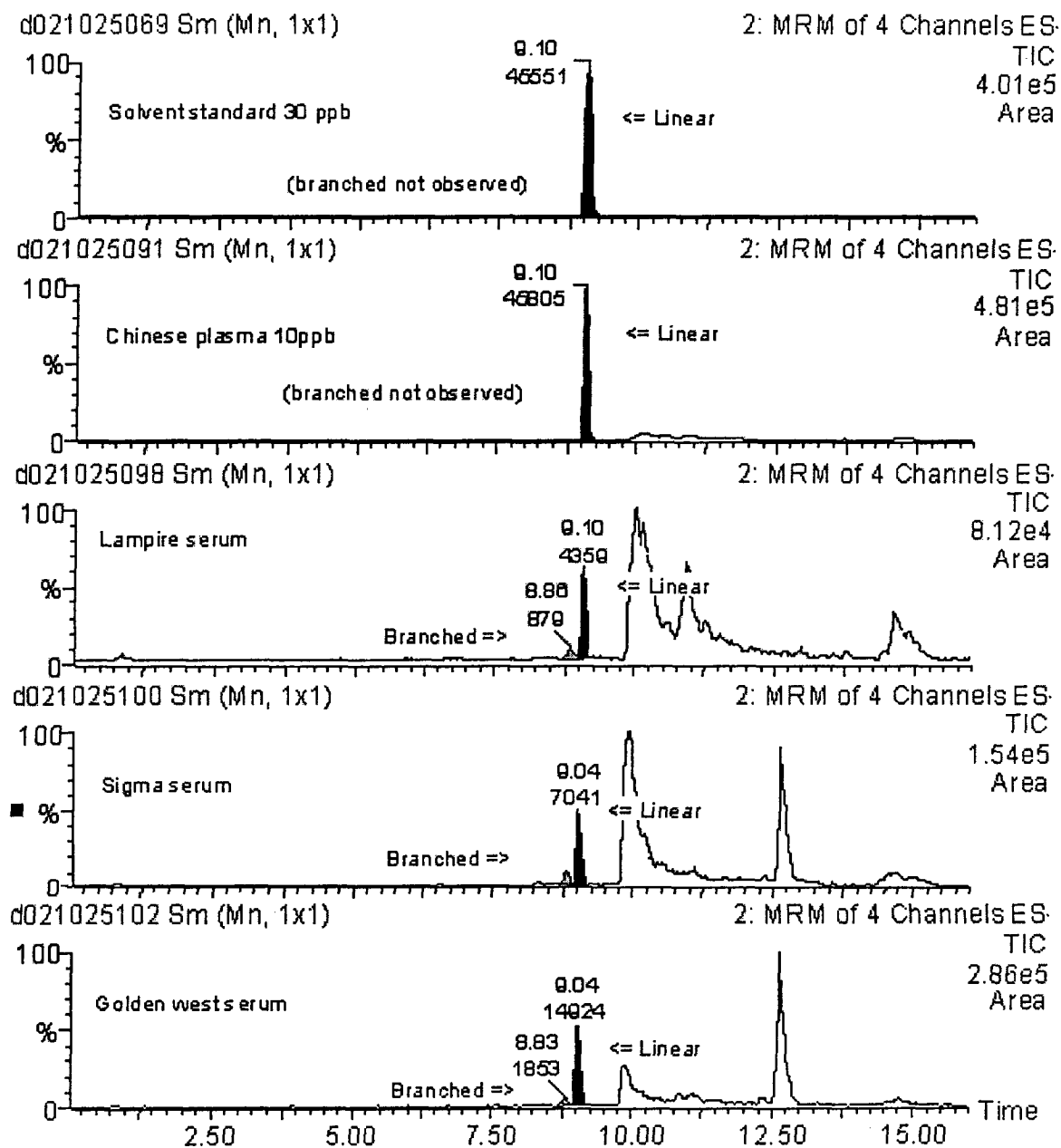
Summary of C₇ Acid Chromatograms: Low to non-detect levels of the branched isomers of C₇ acid were observed in these lots of commercial pooled human serum.

Figure 3: C₈ Isomer Distribution



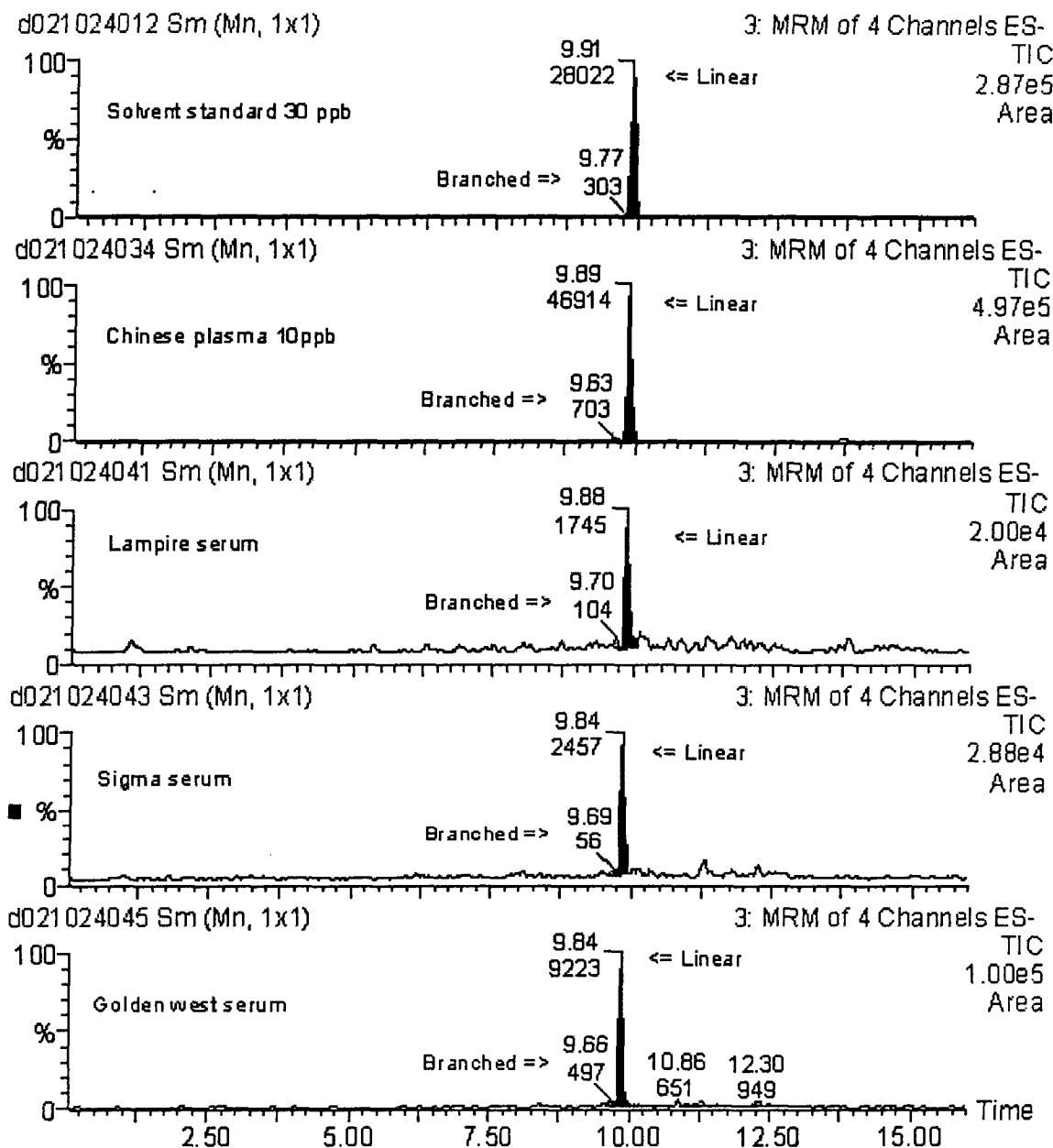
Summary of C₈ Chromatograms: Low to non-detect levels of branched isomers of C₈ were observed in these lots of commercial pooled human serum.

Figure 4: C₉ Isomer Distribution



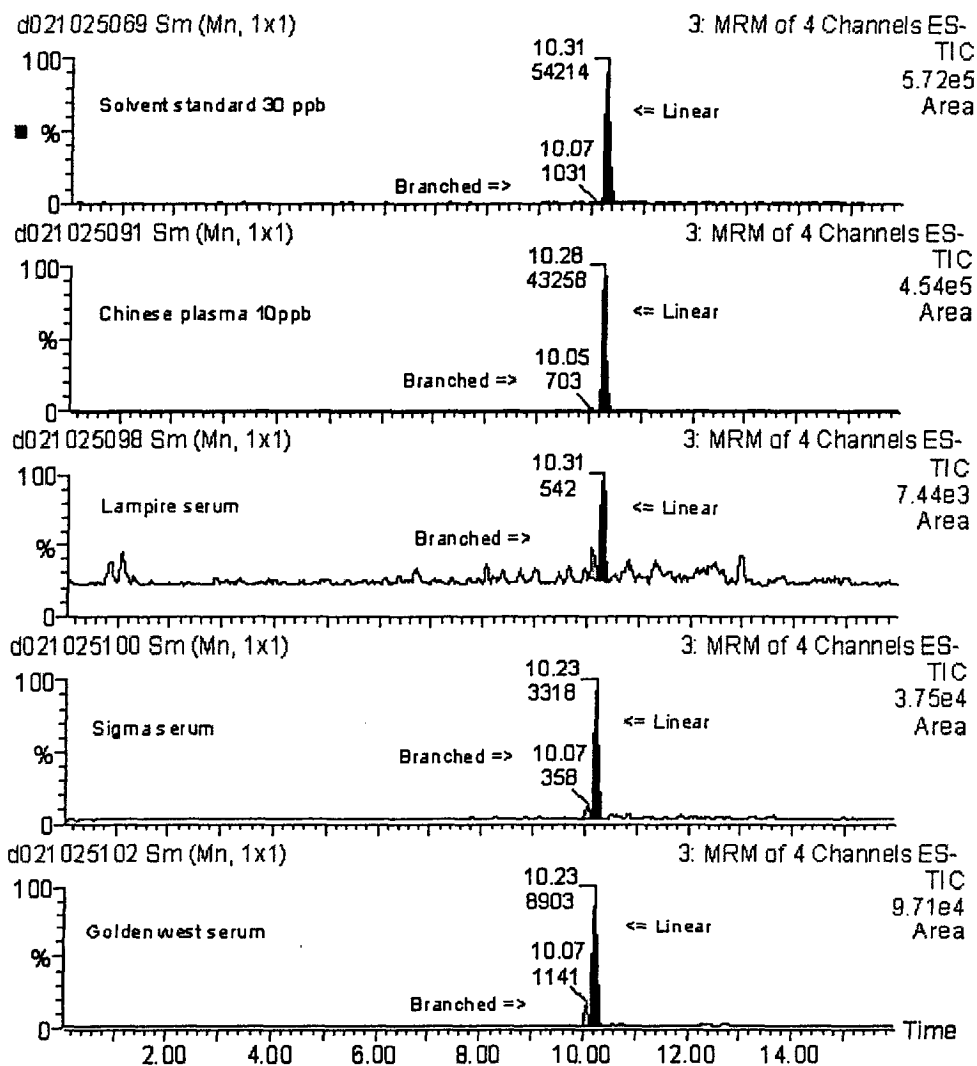
Summary of C₉ Chromatograms: Low to non-detect levels of branched isomers of C₉ were observed in these lots of commercial pooled human serum.

Figure 5: C₁₀ Isomer Distribution



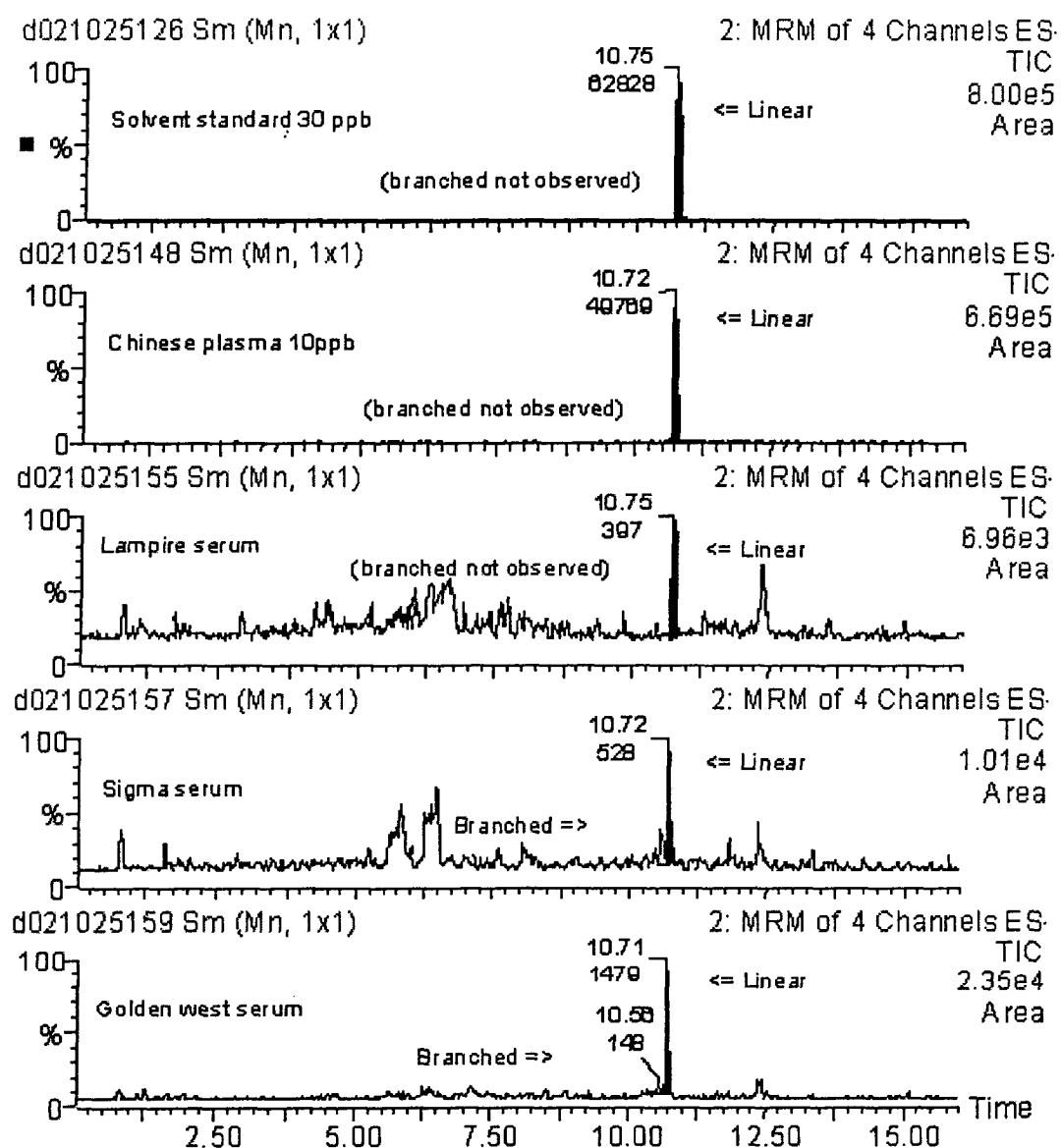
Summary of C₁₀ Chromatograms: Low to non-detect levels of branched isomers of C₁₀ were observed in these lots of commercial pooled human serum.

Figure 6: C₁₁ Isomer Distribution



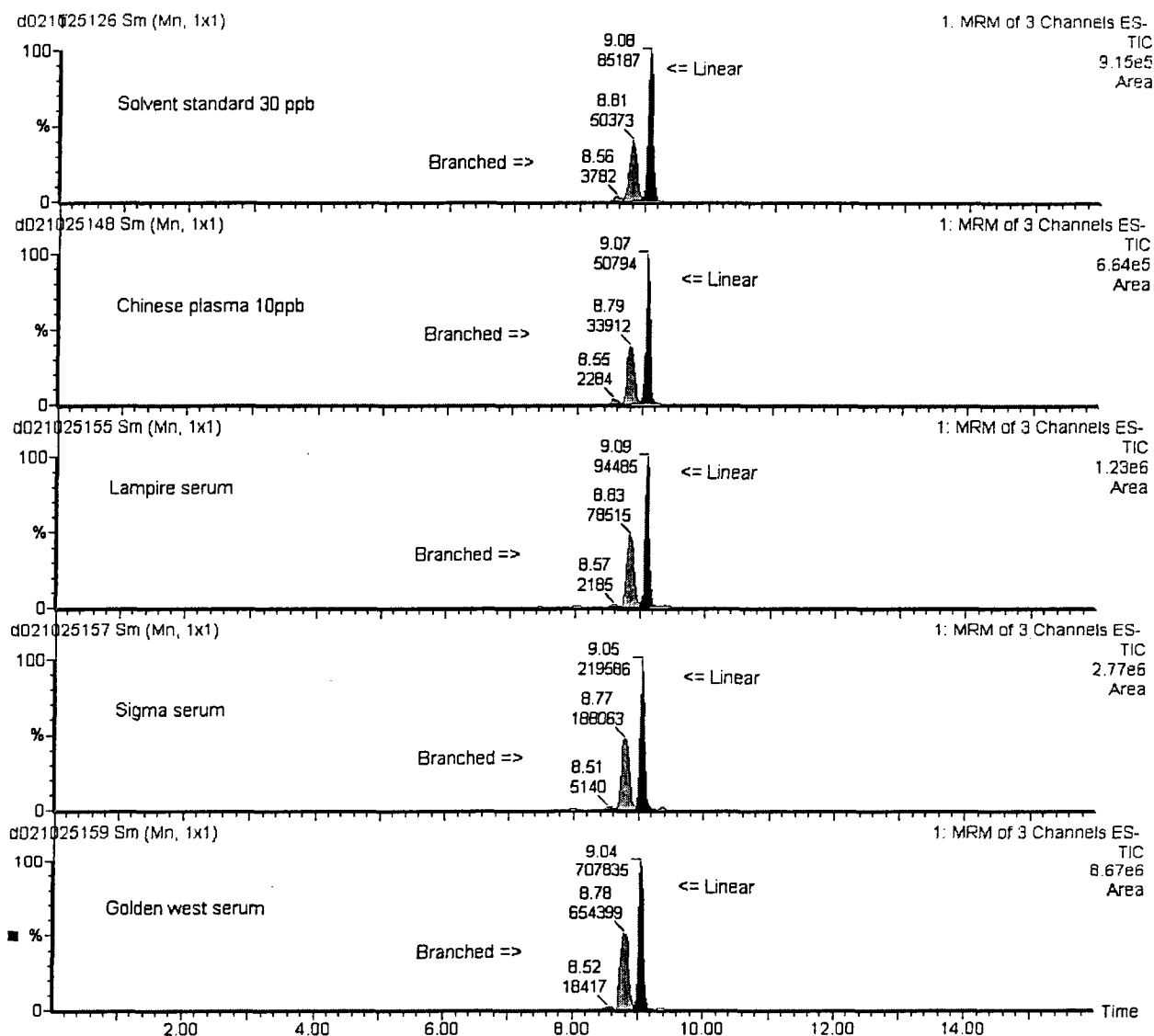
Summary of C₁₁ Chromatograms: Low to non-detect levels of branched isomers of C₁₁ were observed in these lots of commercial pooled human serum.

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.

Figure 8: PFOS Isomer Distribution



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

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.

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.

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

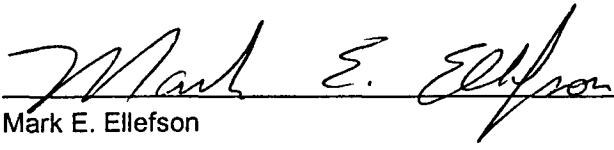
REPORT REVISIONS

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.

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

ATTACHMENT A: METHOD

Record of Deviation

I. Identification	
Study / Project No.	E02-1039
Deviation Type: (Check one)	☐ SOP ☒ Method ☐ Equipment Procedure ☐ Protocol ☐ 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:	
(such as amendment issued, SOP revision, etc.)	
No additional action will be needed.	
Recorded By:	Date:
Cindy Carlson	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: (Study Director / Project Lead)	Date:
[Signature]	11/1/02

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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

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Initial Date

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

Page 1 of 19

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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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.

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 1
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 $\mu\text{g/mL}$	1.5	5.00 ng/g or ng/mL	0.375 ng/mL
0.500 $\mu\text{g/mL}$	3.0	10.0 ng/g or ng/mL	0.750 ng/mL
0.500 $\mu\text{g/mL}$	8.0	25.0 ng/g or ng/mL	2.00 ng/mL
0.500 $\mu\text{g/mL}$	16	50.0 ng/g or ng/mL	4.00 ng/mL
0.500 $\mu\text{g/mL}$	32	100 ng/g or ng/mL	8.00 ng/mL
5.00 $\mu\text{g/mL}$	5.6	175 ng/g or ng/mL	14.0 ng/mL
5.00 $\mu\text{g/mL}$	8.0	250 ng/g or ng/mL	20.0 ng/mL
5.00 $\mu\text{g/mL}$	16	500 ng/g or ng/mL	40.0 ng/mL
5.00 $\mu\text{g/mL}$	24	750 ng/g or ng/mL	60.0 ng/mL
5.00 $\mu\text{g/mL}$	32	1000 ng/g or ng/mL	80.0 ng/mL
5.00 $\mu\text{g/mL}$	40	1250 ng/g or ng/mL	100 ng/mL
50.0 $\mu\text{g/mL}$	5.0	1500 ng/g or ng/mL	125 ng/mL
50.0 $\mu\text{g/mL}$	6.0	1750 ng/g or ng/mL	150 ng/mL
Surrogate Std 100 $\mu\text{g/mL}$	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-Turax 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 µL/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:

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

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 (µg/mL):

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

Calculate actual concentration of analyte in tissue (µg/g):

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

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 < 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 A – Extraction Worksheet

Study Number:
Prep Date:
Analysts initials:
Box#:

Method Revision: ETS-8-231.1
Matrix:
Sample Timepoint:

[illegible]

Blank matrix _____ TN-A-_____; Amount weighed/aliquoted: _____ g/mL

1. Homogenize sample
2. Aliquot 1 mL of diluted matrix into 15 mL polypropylene tube
3. Spike samples accordingly
4. Add ____ mL of ACN (TN-A-_____) to each diluted sample and shake or vortex mix
5. Shake sample for 20 min @ ____ rpm (Shaker _____)
6. Centrifuge sample for 10 min @ ____ rpm (Centrifuge _____)
7. Add 40 mL of _____ water to 50 mL polypropylene centrifuge tube.
8. Decant extract into centrifuge tubes with water
9. Shake sample slightly to ensure proper mixing
10. Attach 6 mL C18 SPE cartridges and 75 mL reservoirs to vacuum manifold
11. Condition column with two washes of ~5 mL MeOH (TN-A-_____) - do not allow column to go to dryness
12. Wash column with two washes of ~5 mL _____ water - do not allow column to go to dryness
13. Filter sample through conditioned column, discarding filtrate
14. Allow column to go to dryness. After dripping stops, draw a high vacuum through column for at least 5 minutes.
15. Elute column with solvent (_____) (TN-A-_____) into appropriate 15 mL centrifuge tube
16. Spike samples with _____ μ L of internal standard # _____, conc. _____
17. Transfer sample into appropriately labeled autovial and cap

Note: In vacuum steps above set the vacuum chamber at approximately 15 kPa - this should give approximately 5-7 mL/min elution flow. Flows may vary through cartridges - kPa may be raised for slow tubes and drying after most have been drawn down and shut off.

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Study Number:
Equipment Number:
Final Solvent & TN Number:

[illegible]

Form Completion Verified By: _____

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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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ATTACHMENT B: DATA TABLES

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	High spike	Low 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	16.78	16.44
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	R ²	System Suitability				Matrix Blanks
			Samples, %RSD	CCV #1 recovery, %	CCV #2 recovery, %	CCV #3 recovery, %	
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

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	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	1.30	4.78	9.89	12.34	13.15	16.19	16.20	20.32	13.56	20.34	15.61	15.55	17.32	21.63
C ₁₂	< LOQ	< LOQ	0.52	5.02	< LOQ	< LOQ	0.48	4.53	< LOQ	< LOQ	0.44	4.80	< LOQ	< LOQ	0.58	5.66
C ₁₁	< LOQ	< LOQ	0.65	5.18	< LOQ	< LOQ	0.46	4.42	< LOQ	0.10	0.52	4.95	0.13	0.14	0.84	6.16
C ₁₀	< LOQ	< LOQ	0.40	4.88	< LOQ	< LOQ	0.49	4.01	0.15	0.17	0.57	5.29	0.19	0.15	0.78	6.08
C ₉	< LOQ	< LOQ	0.59	4.91	0.37	0.50	0.78	4.88	0.42	0.65	0.72	5.11	0.68	0.49	0.84	6.00
C ₈	< LOQ	< LOQ	< LOQ	4.71	2.30	2.91	3.34	7.31	3.46	4.28	3.34	7.94	3.04	3.09	3.86	9.16
C ₇	< LOQ	< LOQ	0.28	5.28	0.20	< LOQ	0.52	5.43	0.45	0.13	0.32	5.34	< LOQ	< LOQ	0.48	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				
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%	78%	< LOQ
C ₁₀	0.10 - 30.3	0.999695	2.76		91%	83%	< LOQ
C ₉	0.25 - 30.4	0.999847	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

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

Quantitated with Chinese Plasma Calibration Curve

All data is in units 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%

**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%

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

ATTACHMENT C: CHROMATOGRAMS

C7 ACID

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

3M

d021030005

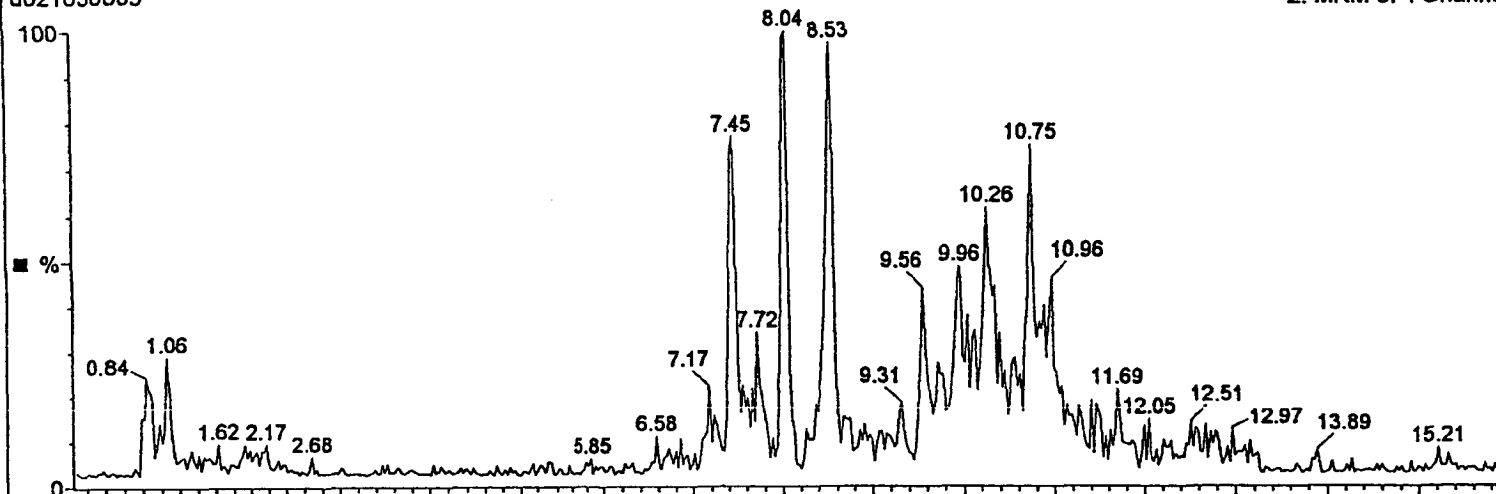
Davey070799

30-Oct-2002 12:37:49

2: MRM of 4 Channels ES-

TIC

4.36e4

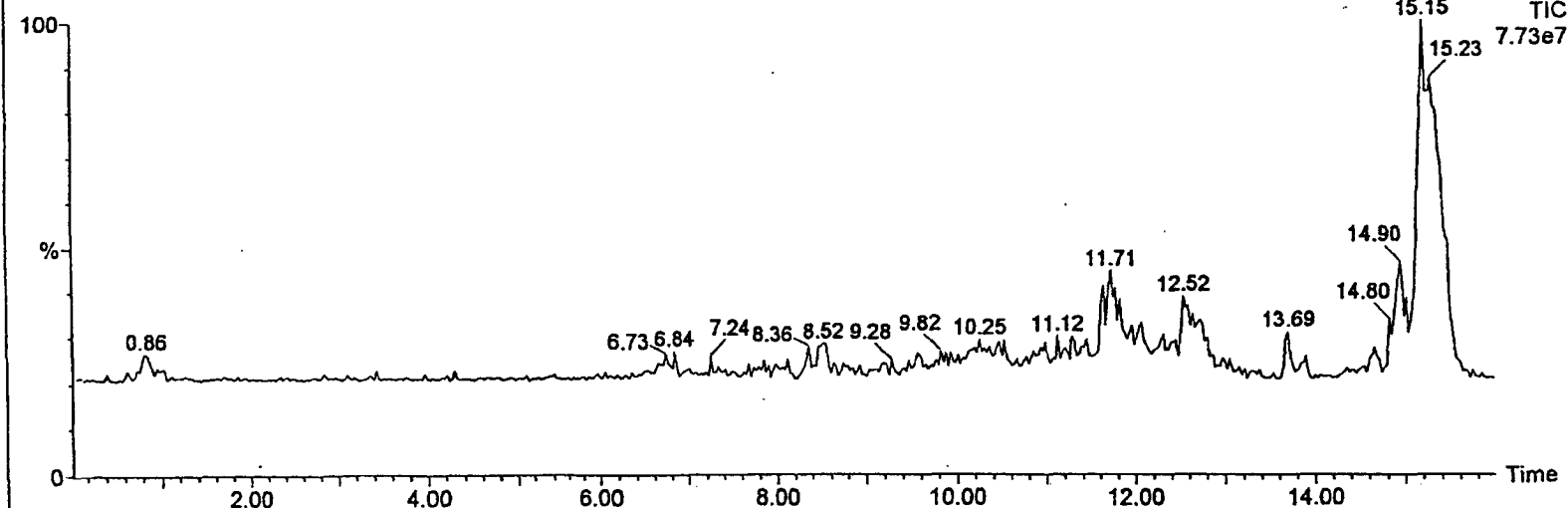


d021030005

1: Daughters of 363ES-

TIC

15.15 15.23 7.73e7



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Initial Date

C7ACID

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

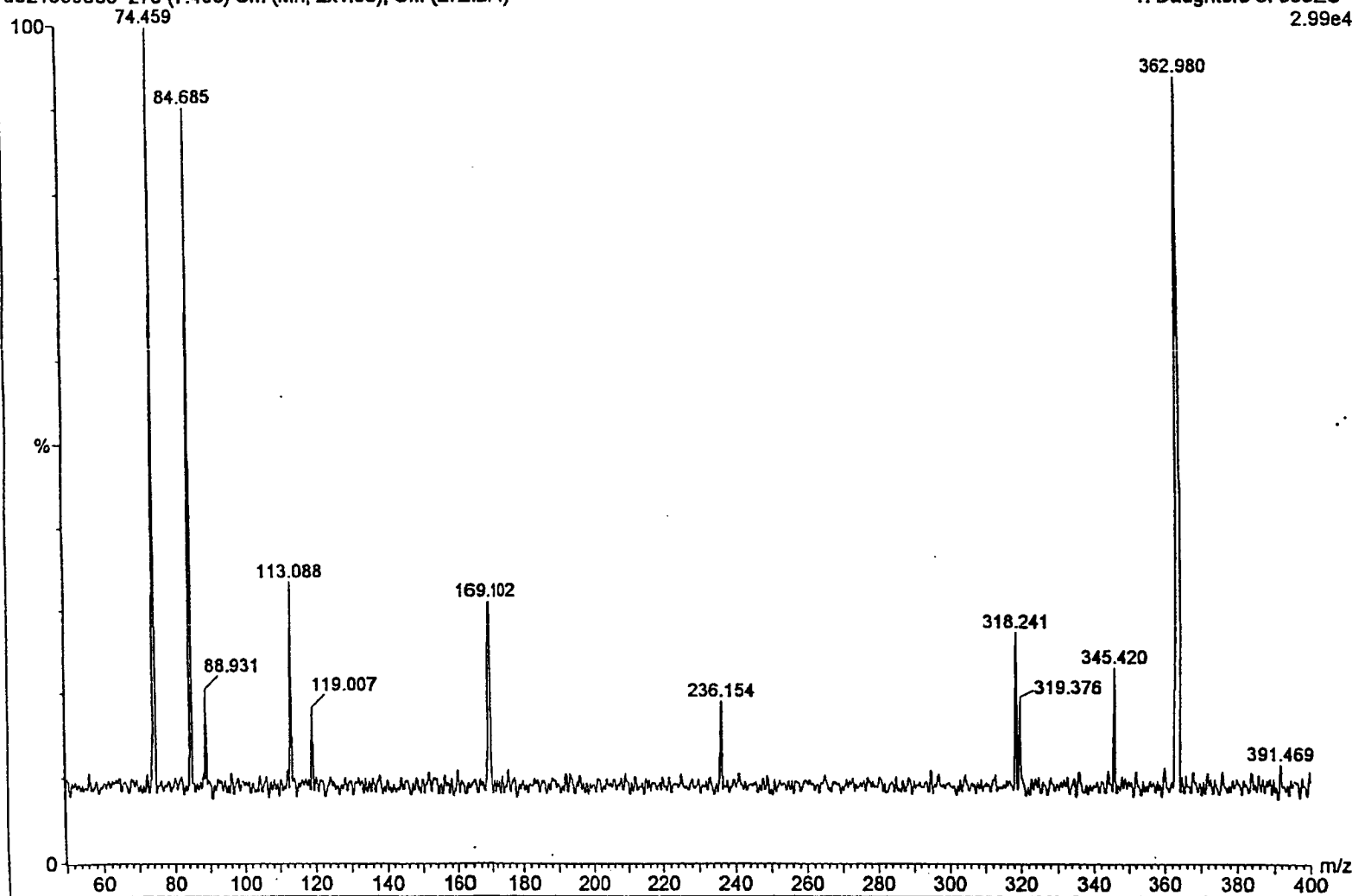
3M Environmental Lab

d021030005 273 (7.408) Sm (Mn, 2x1.00); Cm (272:274)

davey 070799

30-Oct-2002 12:37:49

1: Daughters of 363ES-
2.99e4



Exact Copy of Original

Cinc 10/31/02
Initial Date

C7AC10

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

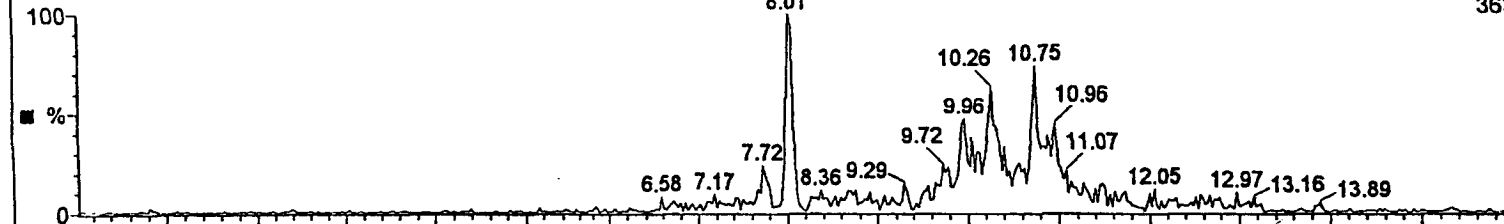
3M

d021030005

Davey070799

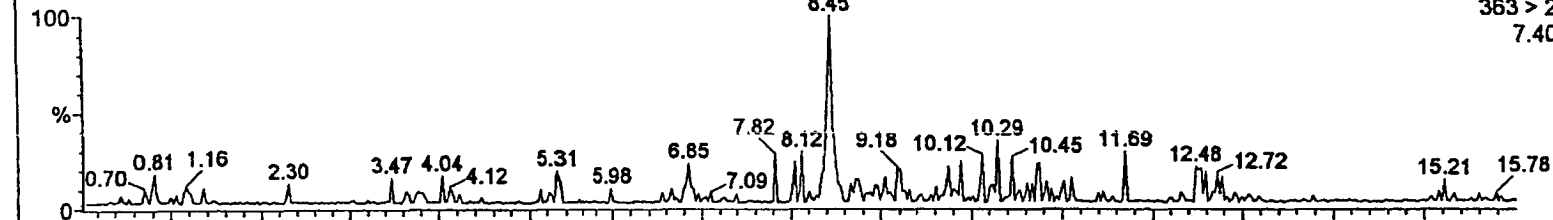
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2: MRM of 4 Channels ES-
363 > 319
4.09e4



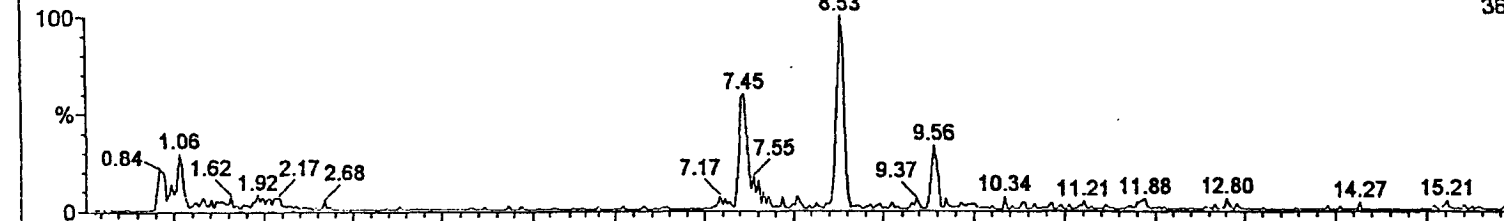
d021030005

2: MRM of 4 Channels ES-
363 > 219
7.40e3



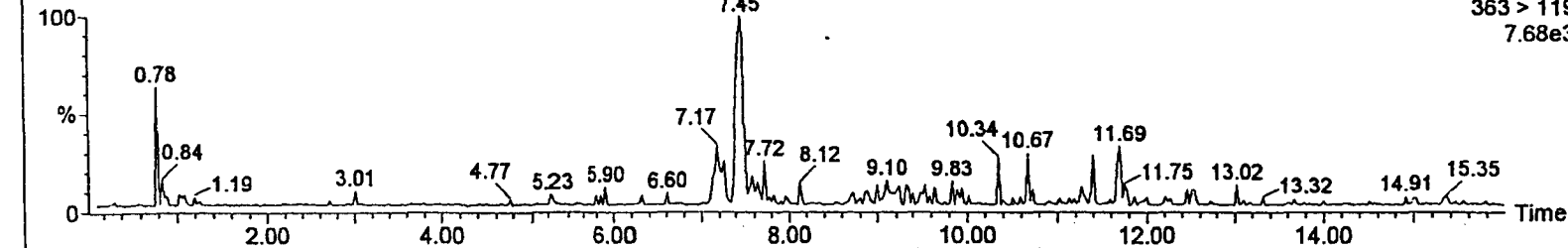
d021030005

2: MRM of 4 Channels ES-
363 > 169
3.76e4



d021030005

2: MRM of 4 Channels ES-
363 > 119
7.68e3



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Cmc 10/31/02
Initial Date

C7AC10

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

3M

d021030005

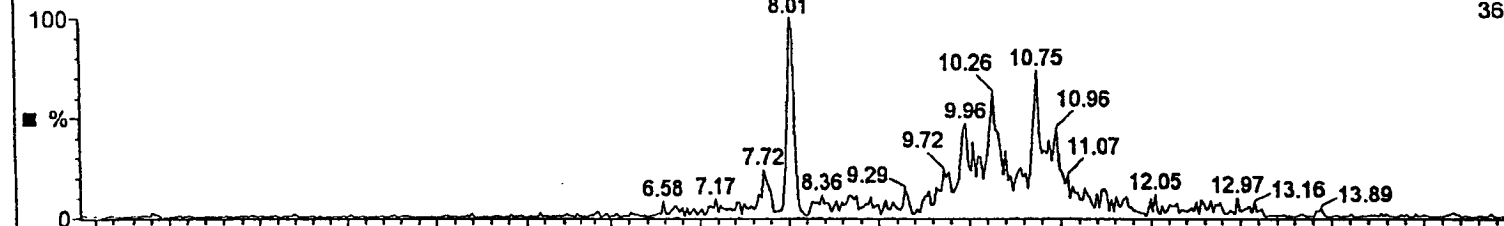
Davey070799

30-Oct-2002 12:37:49

2: MRM of 4 Channels ES-

363 > 319

4.09e4

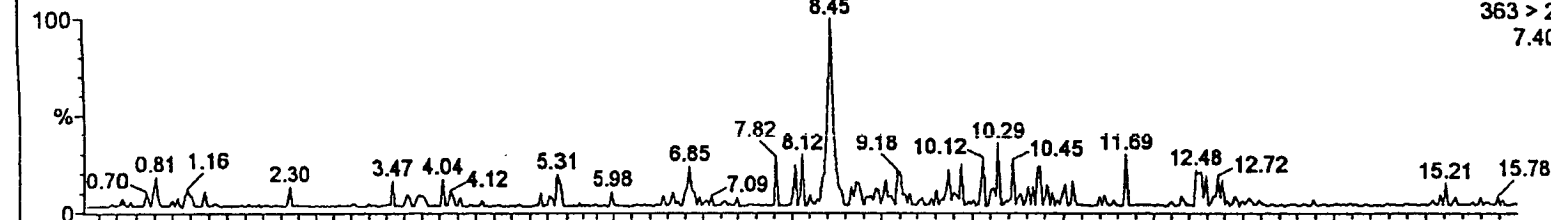


d021030005

2: MRM of 4 Channels ES-

363 > 219

7.40e3

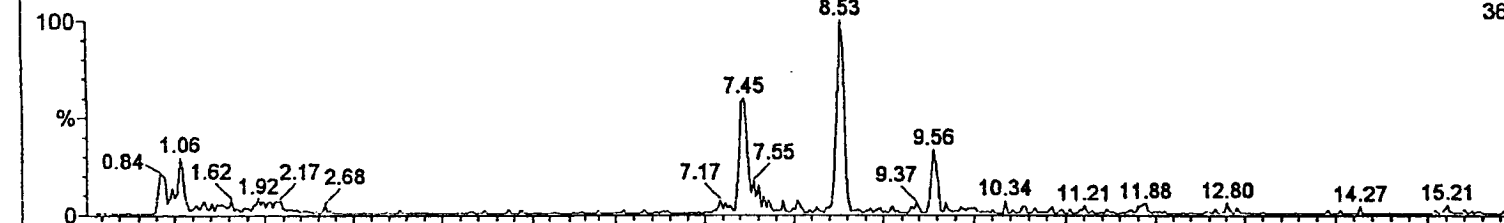


d021030005

2: MRM of 4 Channels ES-

363 > 169

3.76e4

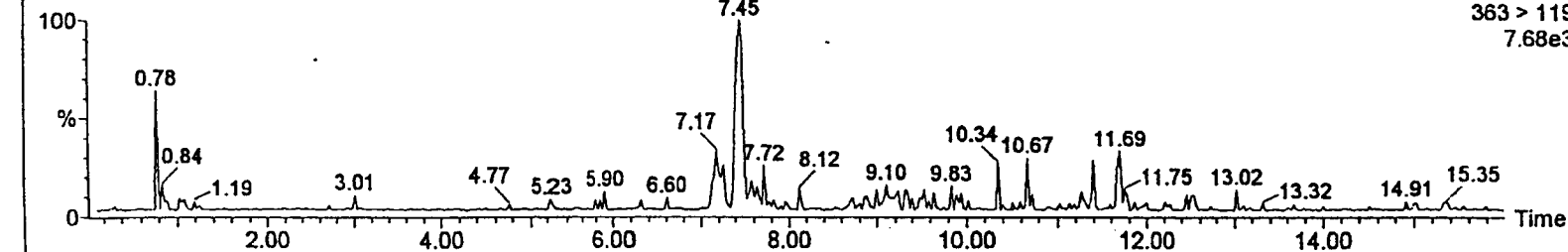


d021030005

2: MRM of 4 Channels ES-

363 > 119

7.68e3



Exact Copy of Original

Cmc 10/31/02
Initial Date

C7 ACID

golden west plasma 5x scale up 10/23/02 ok
3M

Davey070799

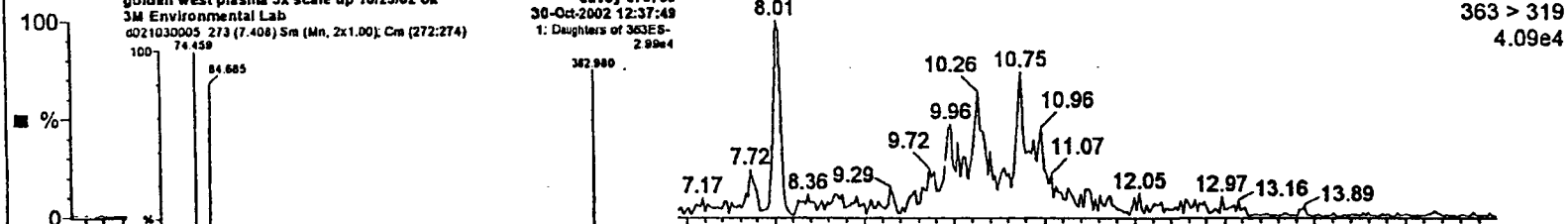
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2: MRM of 4 Channels ES-
363 > 319
4.09e4

d021030005

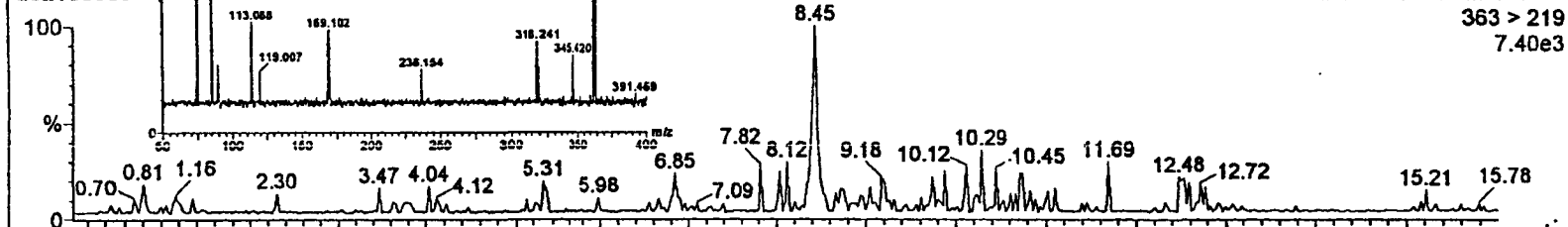
golden west plasma 5x scale up 10/23/02 ok
3M Environmental Lab
d021030005 273 (7.408) Sm (Mn, 2x1.00); Cm (272:274)

davey 070799
30-Oct-2002 12:37:49
1: Daughters of 363ES-
2.99e4



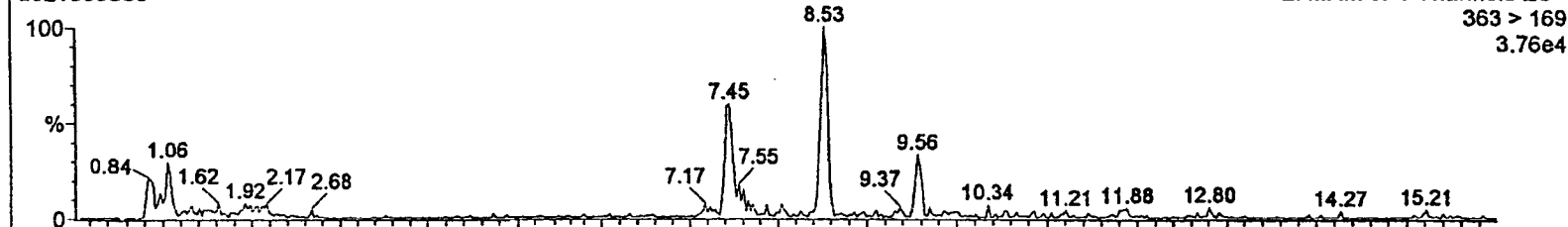
2: MRM of 4 Channels ES-
363 > 219
7.40e3

d021030005



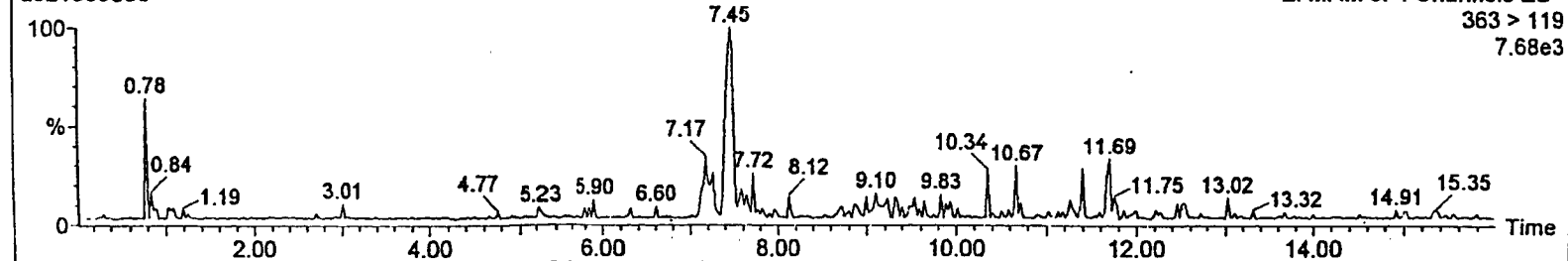
2: MRM of 4 Channels ES-
363 > 169
3.76e4

d021030005



2: MRM of 4 Channels ES-
363 > 119
7.68e3

d021030005



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CMC 10/31/02
Initial Date

C7 ACID

02048-21-7 solvent standard 10ppb

3M

d021030015

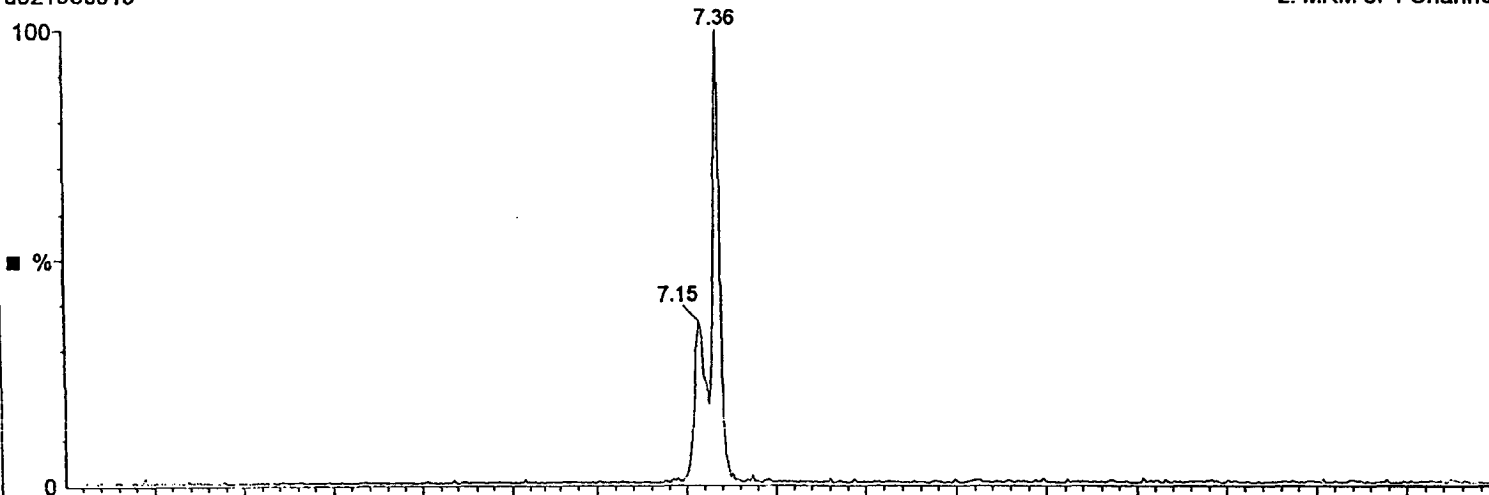
Davey070799

30-Oct-2002 15:29:24

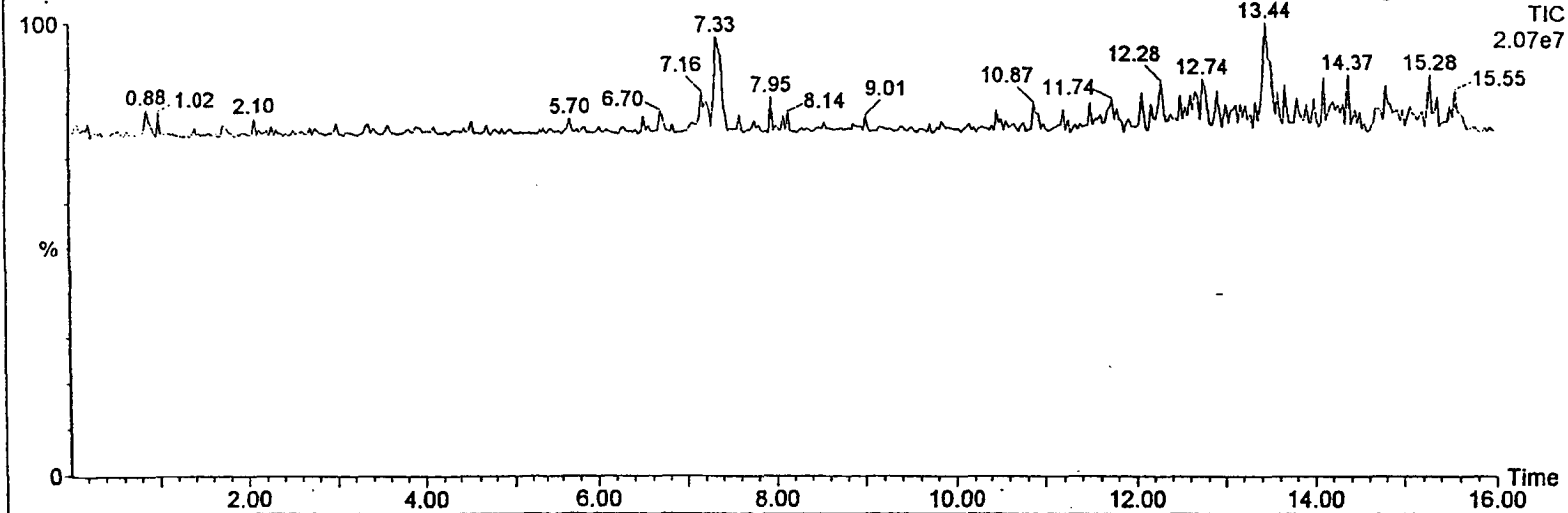
2: MRM of 4 Channels ES-

TIC

2.29e5



d021030015



1: Daughters of 363ES-

TIC

2.07e7

Exact Copy of Original

Initial CME Date 11/1/02

C7 ACID

02048-21-7 solvent standard 10ppb

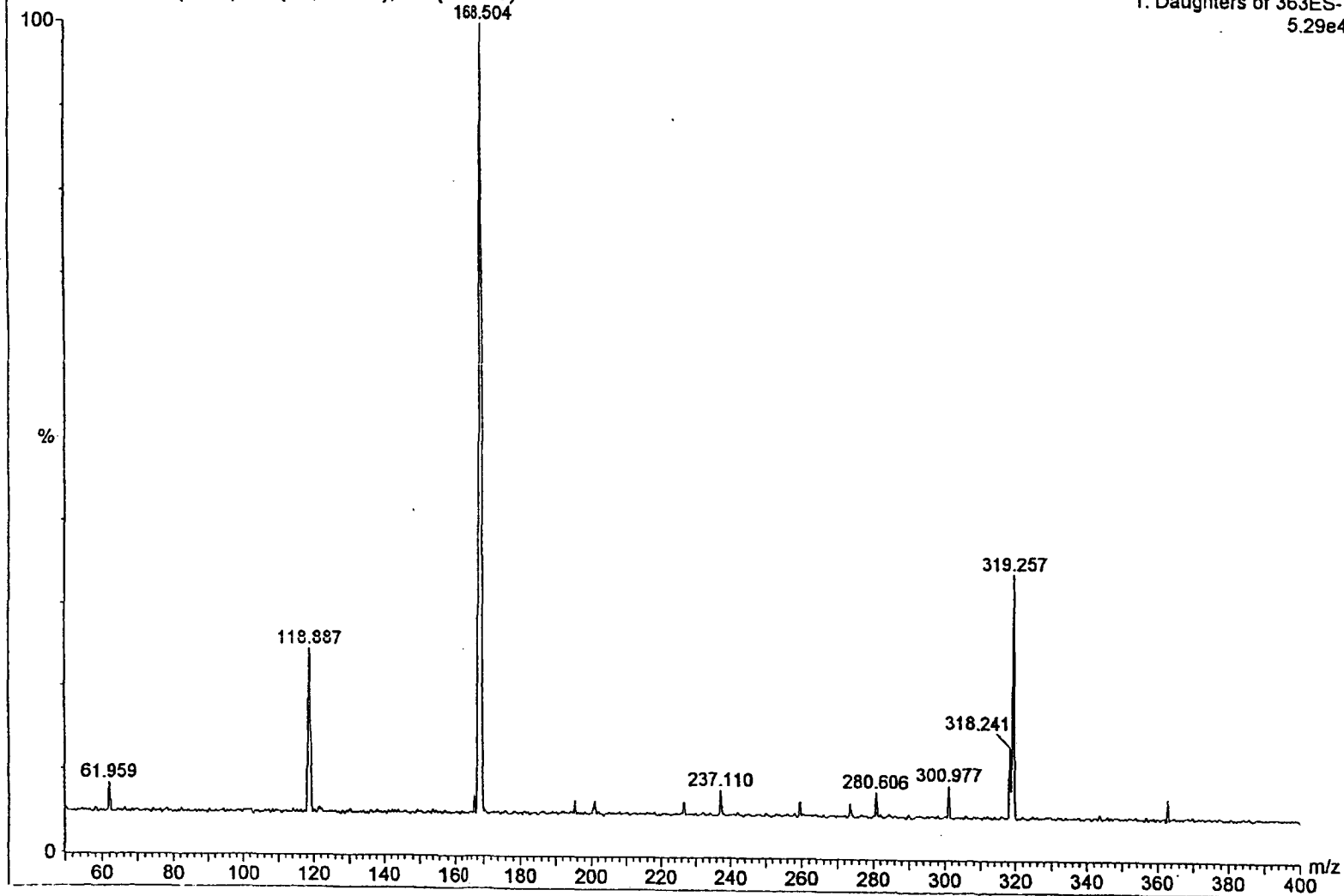
3M Environmental Lab

d021030015 270 (7.325) Sm (Mn, 2x1.00); Cm (260:277)

davey 070799

30-Oct-2002 15:29:24

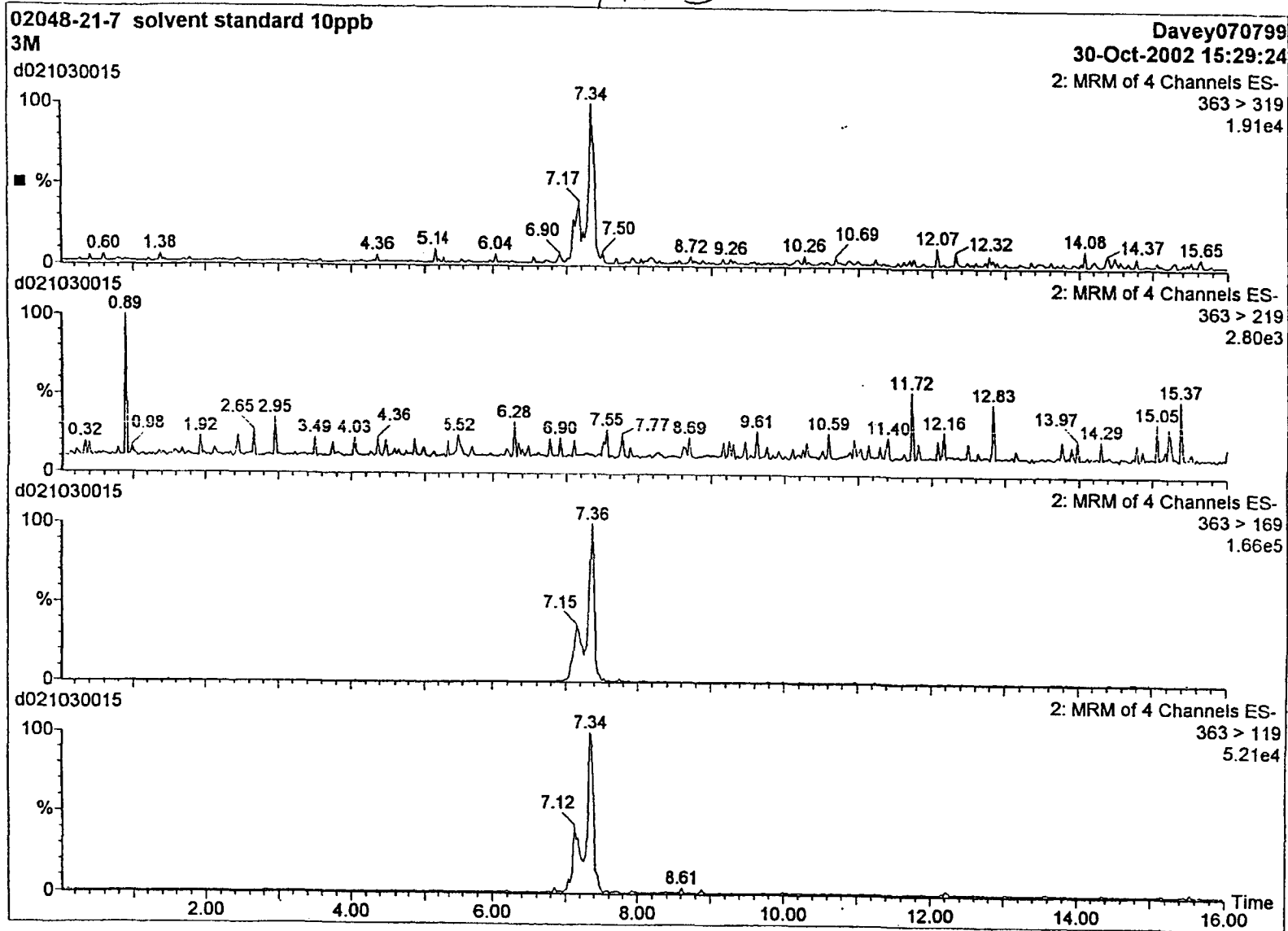
1: Daughters of 363ES-
5.29e4



Exact Copy of Original

CHX 11/1/02
Initial Date

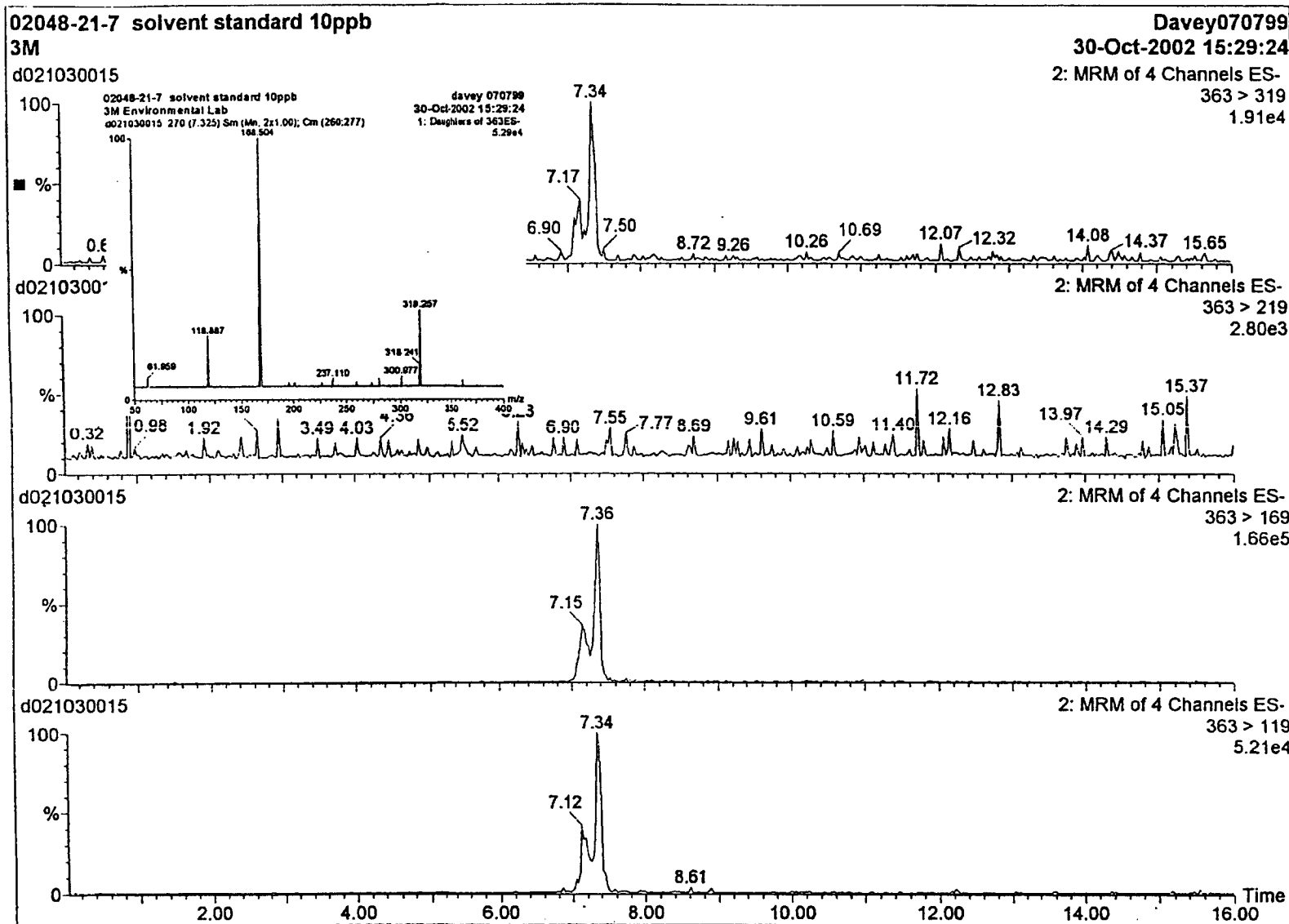
C7 ACID



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CMC 11/1/02
Initial Date

C7 ACID



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E02-1039

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CMX 11/1/02
Initial Date

C8 ACID

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

3M

d021030001

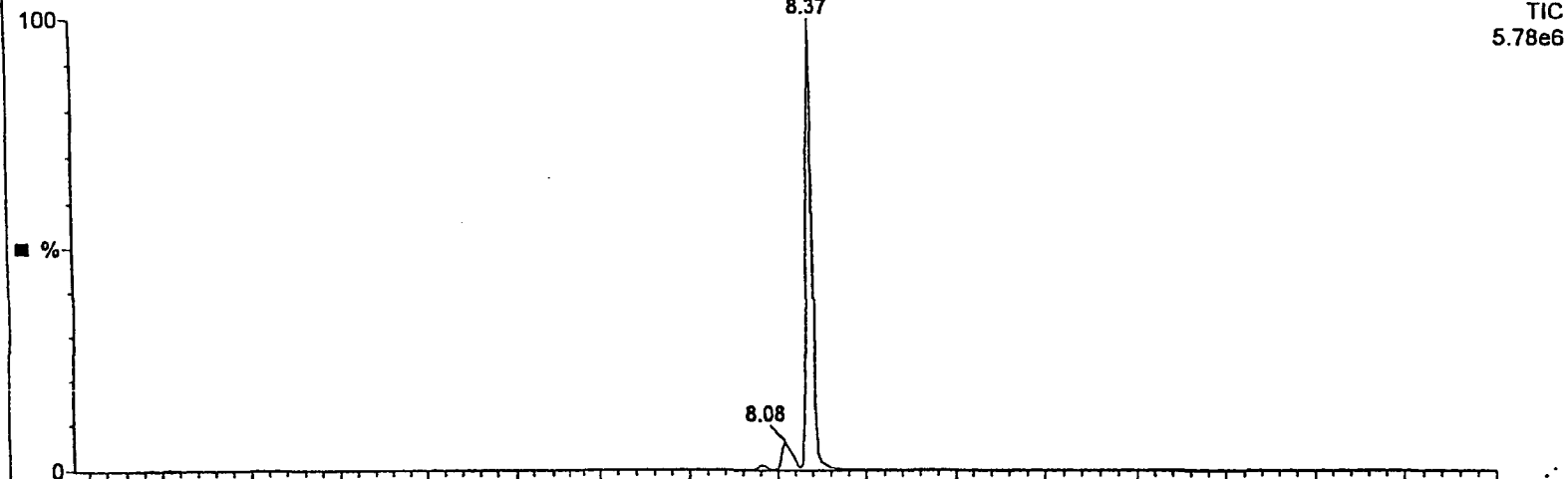
Davey070799

30-Oct-2002 11:28:58

2: MRM of 4 Channels ES-

TIC

5.78e6

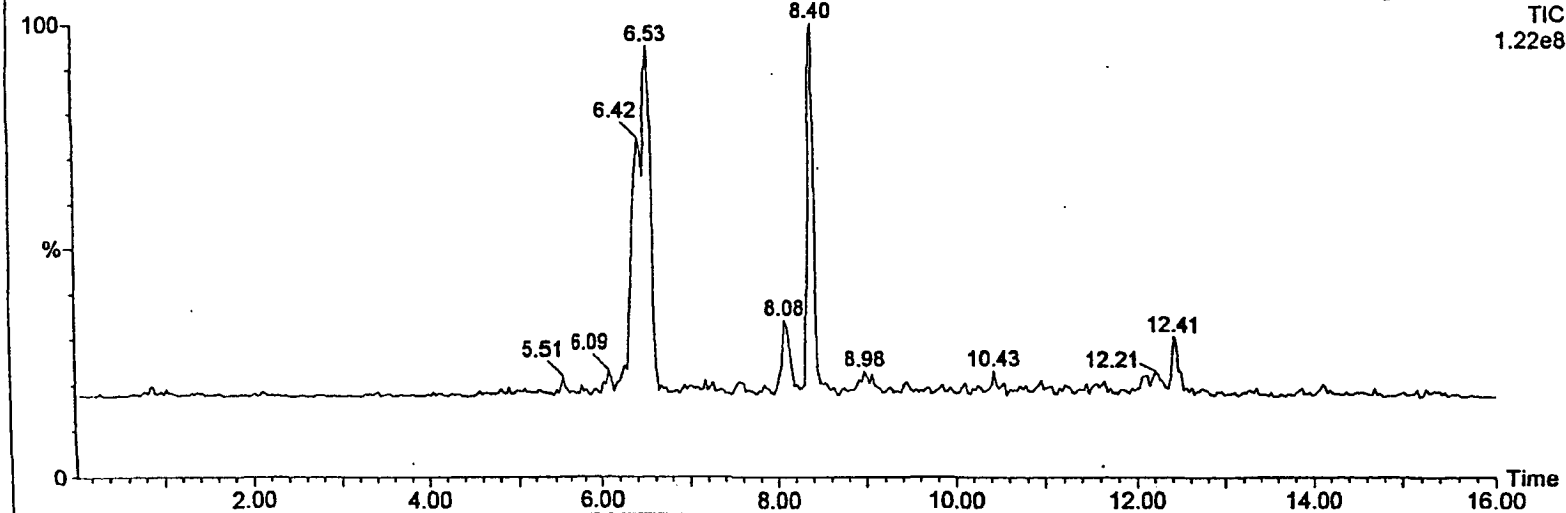


d021030001

1: Daughters of 413ES-

TIC

1.22e8



Exact Copy of Original

CMC 10/31/02
Initial Date

C8 ACID

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

3M Environmental Lab

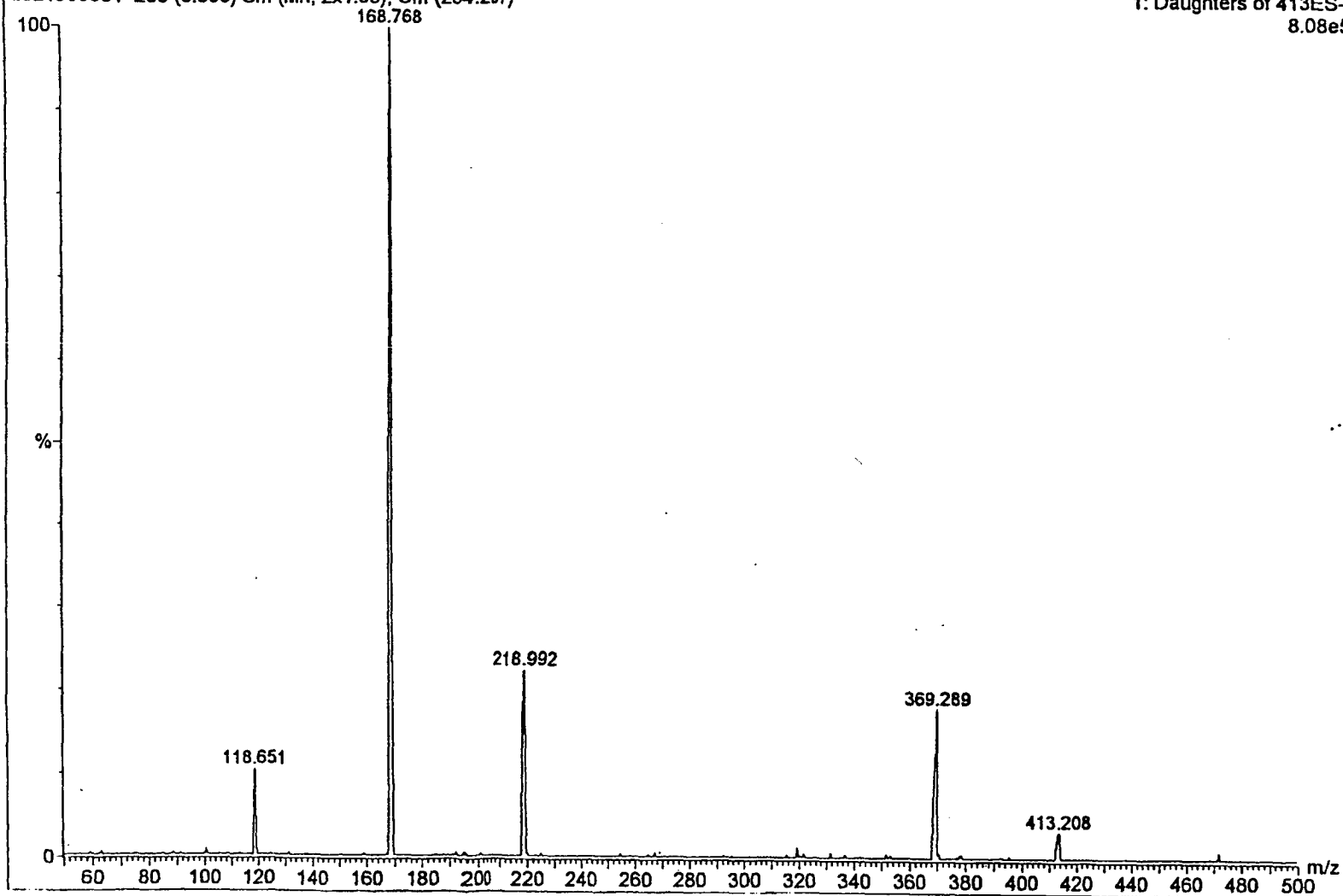
d021030001 288 (8.395) Sm (Mn, 2x1.00); Cm (284:297)

davey 070799

30-Oct-2002 11:28:58

1: Daughters of 413ES-

8.08e5



Exact Copy of Original

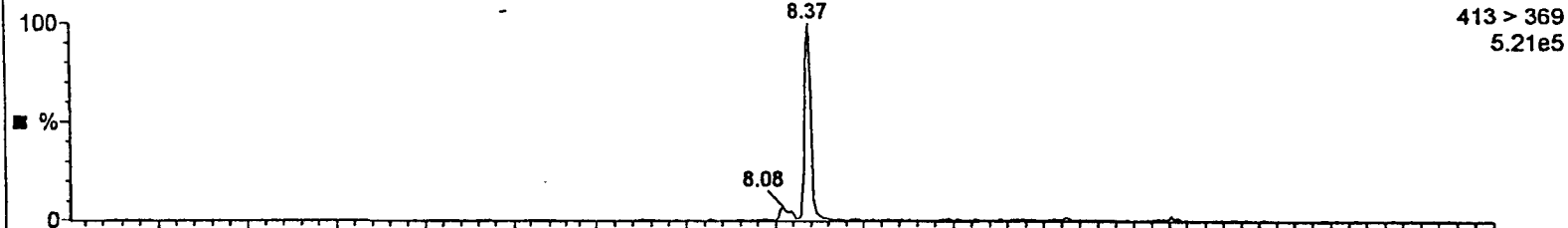
CIM 10/31/02
Initial Date

C8 ACID

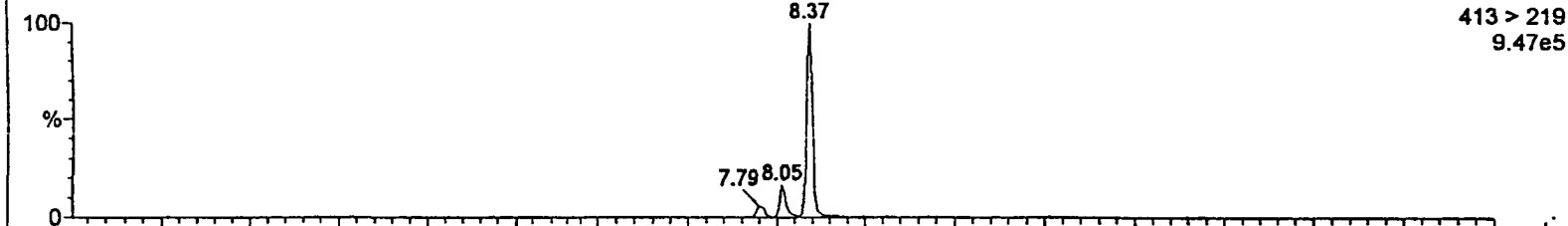
golden west plasma 5x scale up 10/23/02 ok

3M

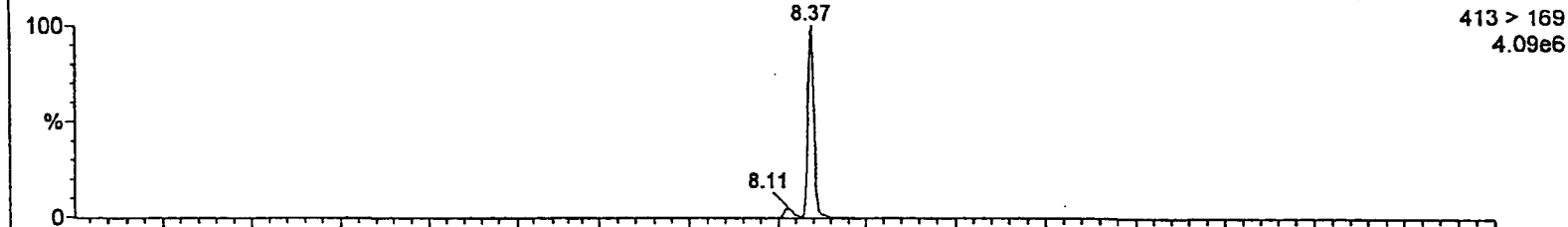
d021030001



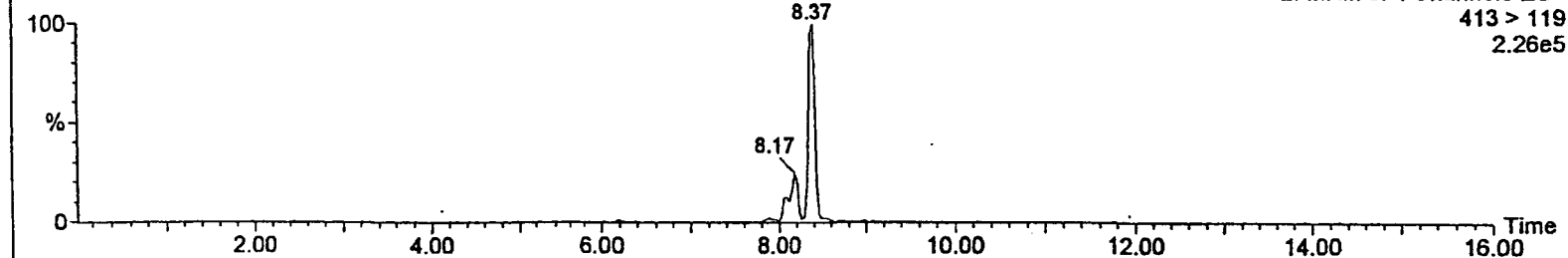
d021030001



d021030001



d021030001



Exact Copy of Original

CMC 10/31/02
Initial Date

CSACID

02048-21-7 solvent standard 10ppb

3M

d021030011

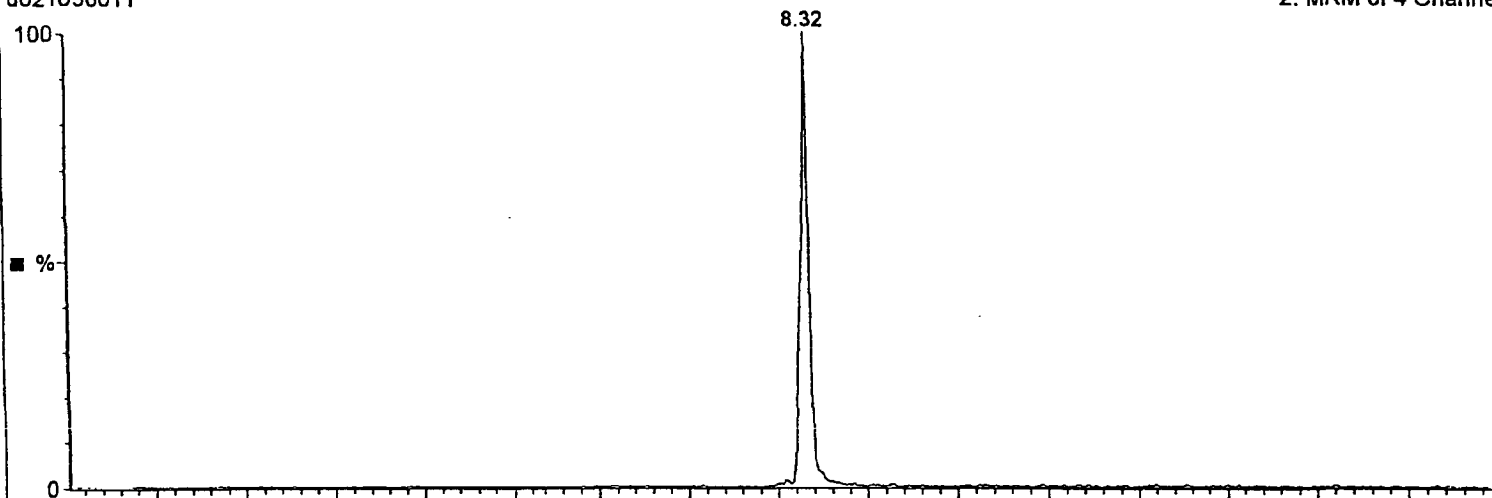
Davey070799

30-Oct-2002 14:21:04

2: MRM of 4 Channels ES-

TIC

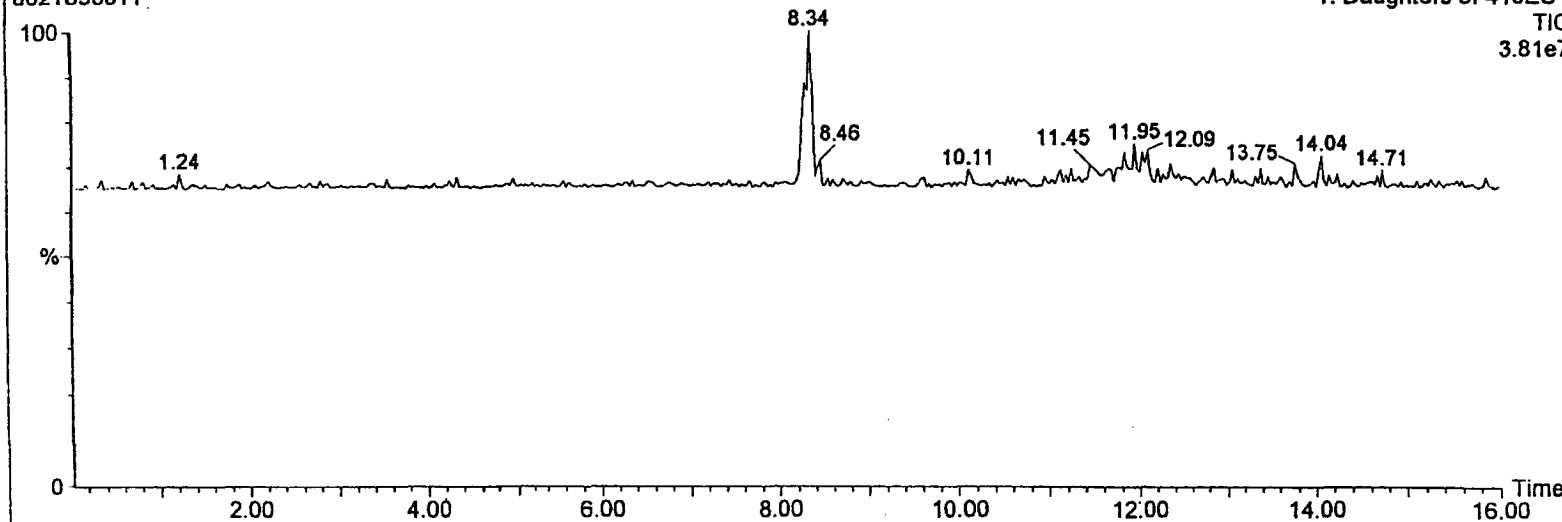
6.47e5



d021030011

1: Daughters of 413ES-

TIC
3.81e7



Exact Copy of Original

CMC 11/1/02
Initial Date

C8 ACID

02048-21-7 solvent standard 10ppb

3M Environmental Lab

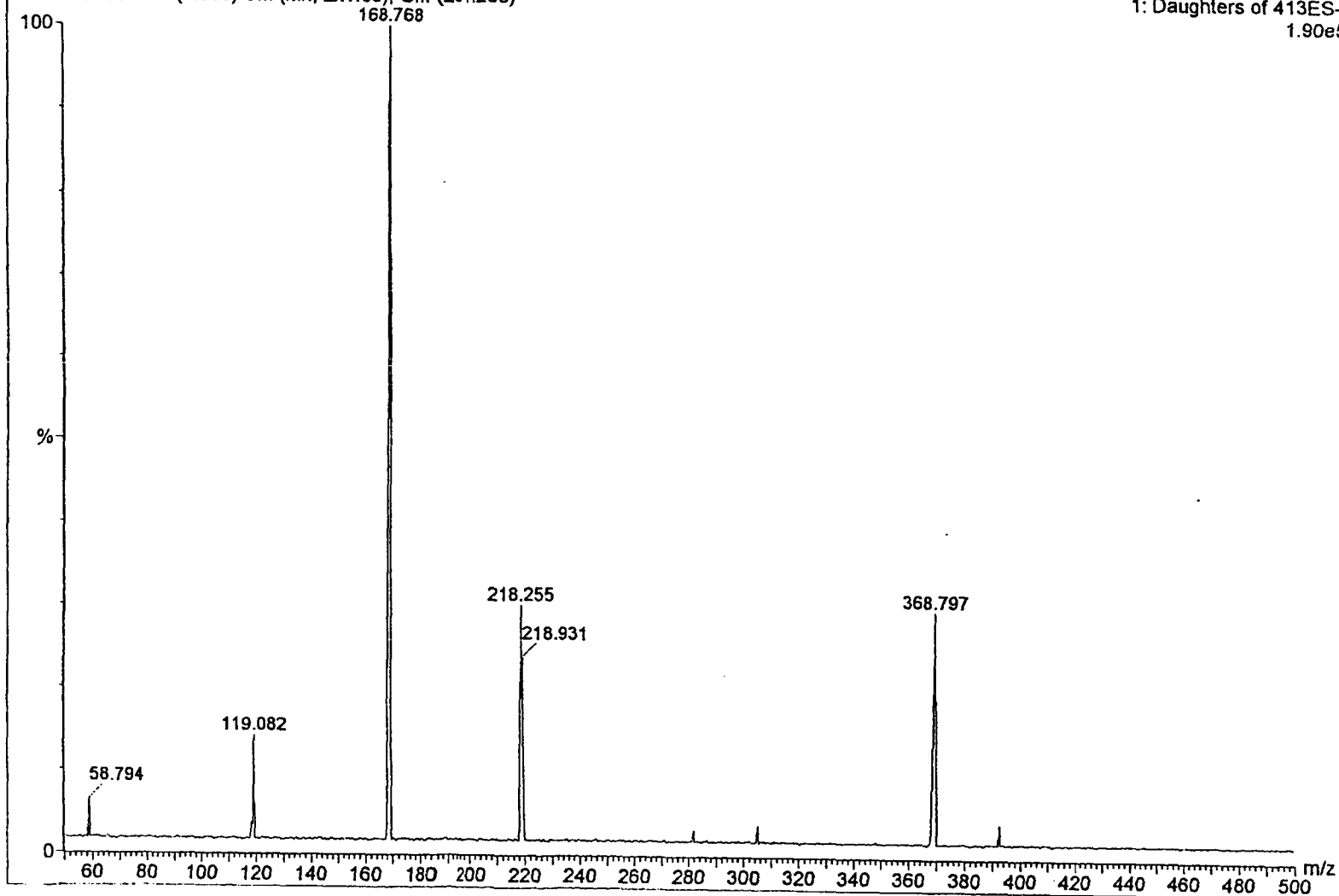
d021030011 286 (8.339) Sm (Mn, 2x1.00); Cm (281:288)

davey 070799

30-Oct-2002 14:21:04

1: Daughters of 413ES-

1.90e5



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Cmc 11/1/02

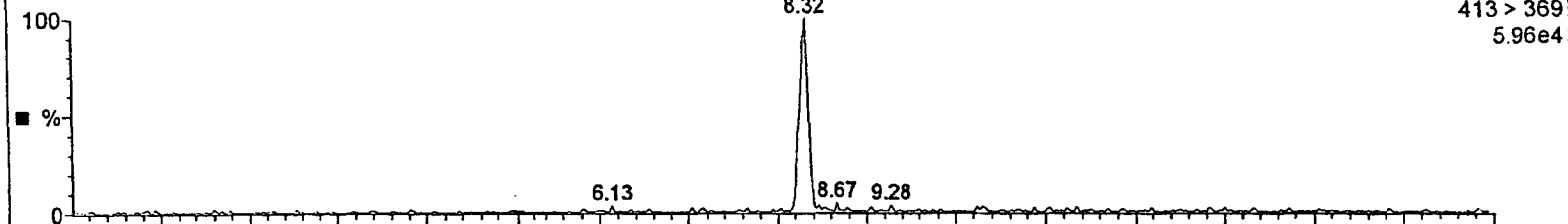
Initial Date

CSACID

02048-21-7 solvent standard 10ppb

3M

d021030011



Davey070799

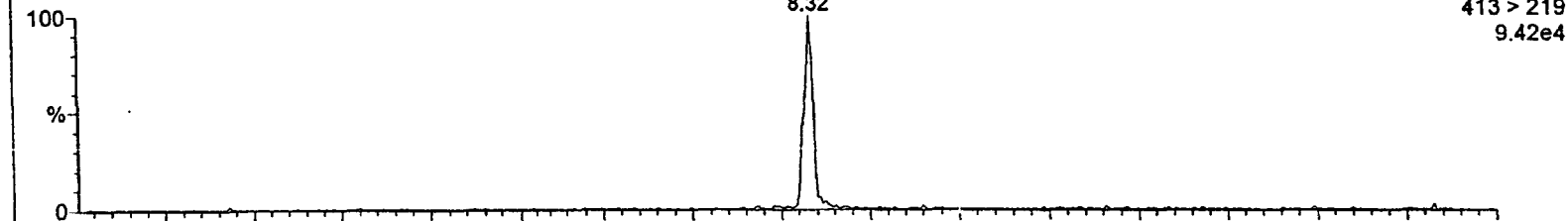
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2: MRM of 4 Channels ES-

413 > 369

5.96e4

d021030011

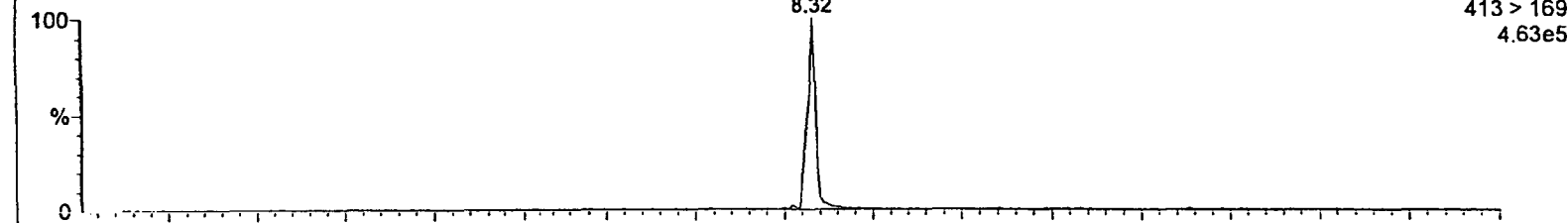


2: MRM of 4 Channels ES-

413 > 219

9.42e4

d021030011

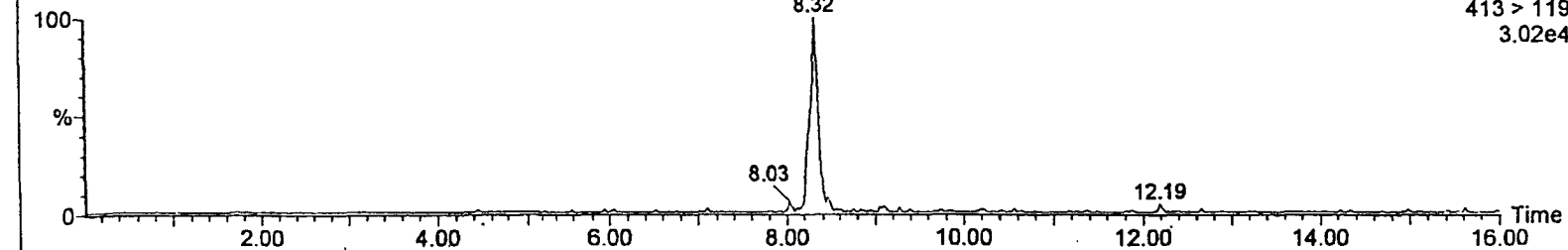


2: MRM of 4 Channels ES-

413 > 169

4.63e5

d021030011



2: MRM of 4 Channels ES-

413 > 119

3.02e4

Exact Copy of Original

cmc 11/1/02
Initial Date

CS ACID

02048-21-7 solvent standard 10ppb

3M

d021030011

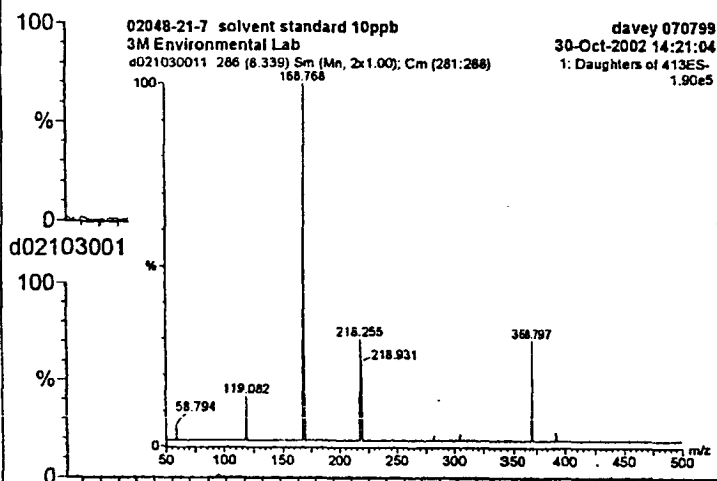
Davey070799

30-Oct-2002 14:21:04

2: MRM of 4 Channels ES-

413 > 369

5.96e4

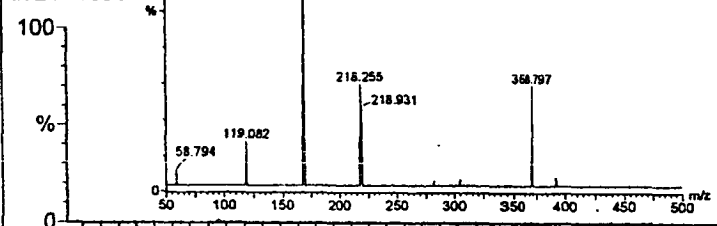


d02103001

2: MRM of 4 Channels ES-

413 > 219

9.42e4

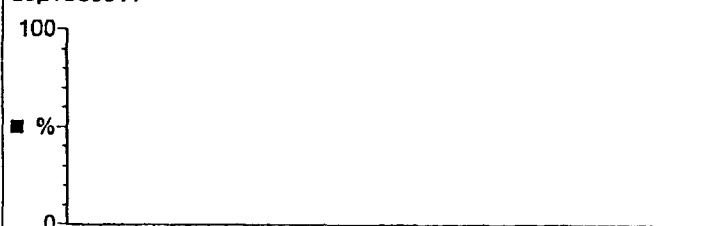


d021030011

2: MRM of 4 Channels ES-

413 > 169

4.63e5

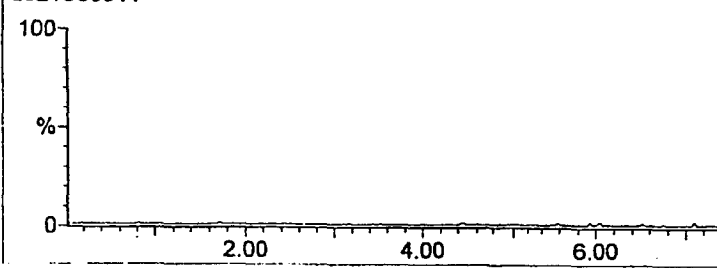


d021030011

2: MRM of 4 Channels ES-

413 > 119

3.02e4



3M ENVIRONMENTAL LABORATORY
E02-1039

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Initial Date

C9 acid

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

3M

d021030006

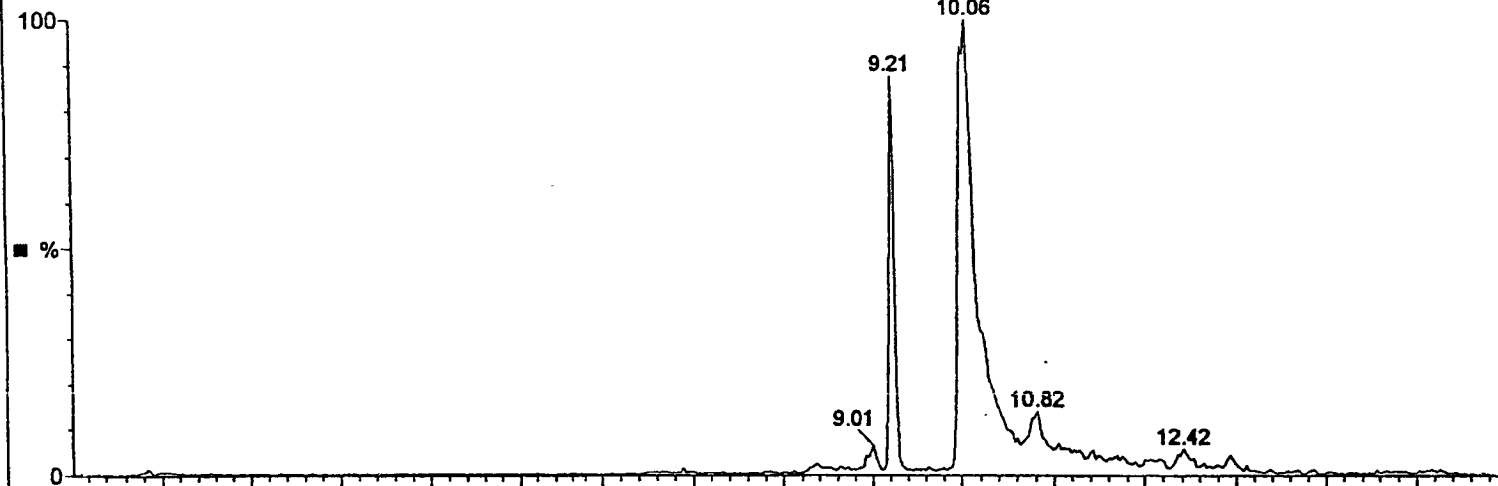
Davey070799

30-Oct-2002 12:55:02

2: MRM of 4 Channels ES-

TIC

8.32e5

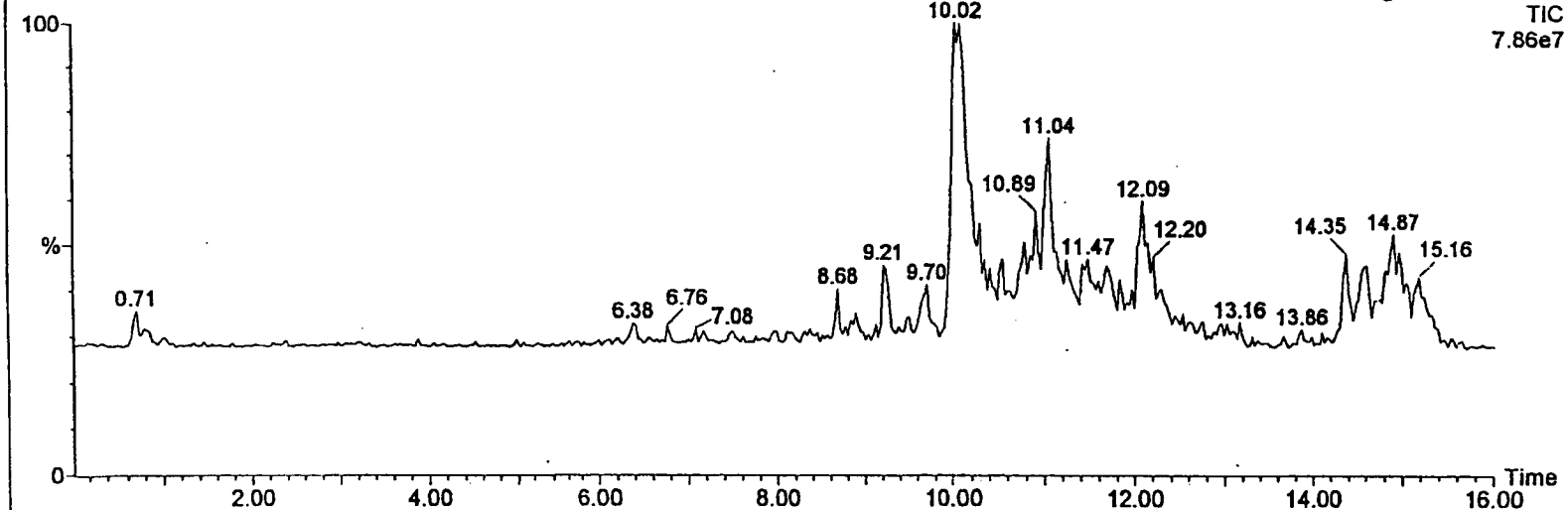


d021030006

1: Daughters of 463ES-

TIC

7.86e7



Exact Copy of Original

cmc 10/31/02
Initial Date

Q9 ACID

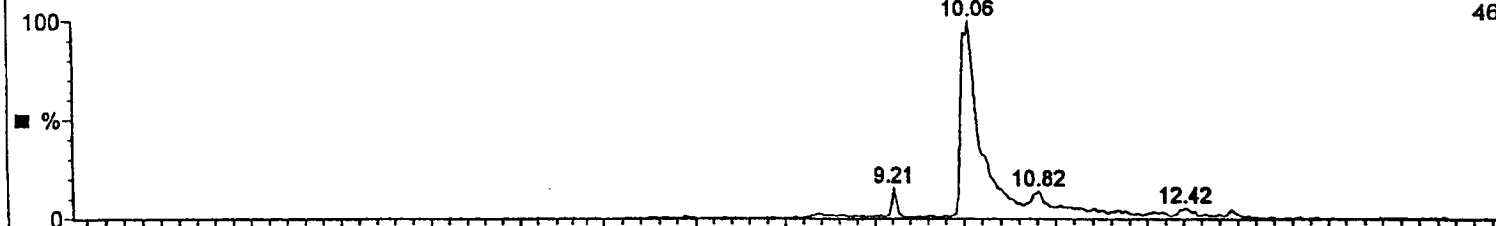
golden west plasma 5x scale up 10/23/02 ok
3M

Davey070799

30-Oct-2002 12:55:02

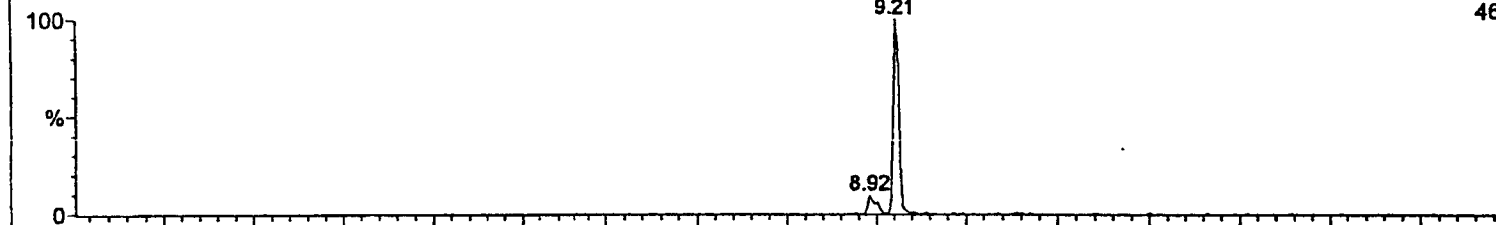
2: MRM of 4 Channels ES-
463 > 419
8.28e5

d021030006



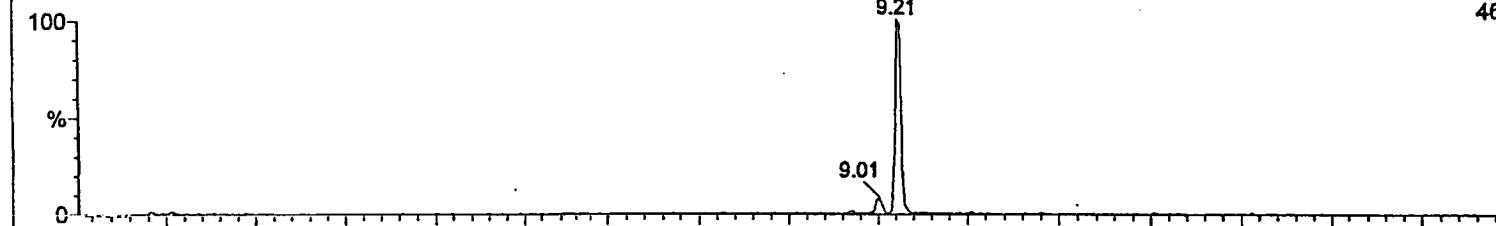
d021030006

2: MRM of 4 Channels ES-
463 > 219
2.83e5



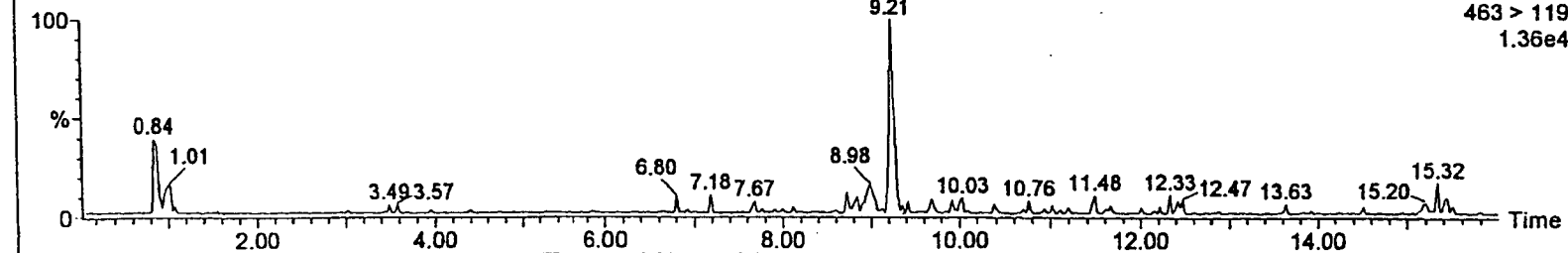
d021030006

2: MRM of 4 Channels ES-
463 > 169
3.06e5



d021030006

2: MRM of 4 Channels ES-
463 > 119
1.36e4



3M ENVIRONMENTAL LABORATORY
E02-1039

Exact Copy of Original

Initial cmc Date 10/31/02

C9 ACID

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

3M Environmental Lab

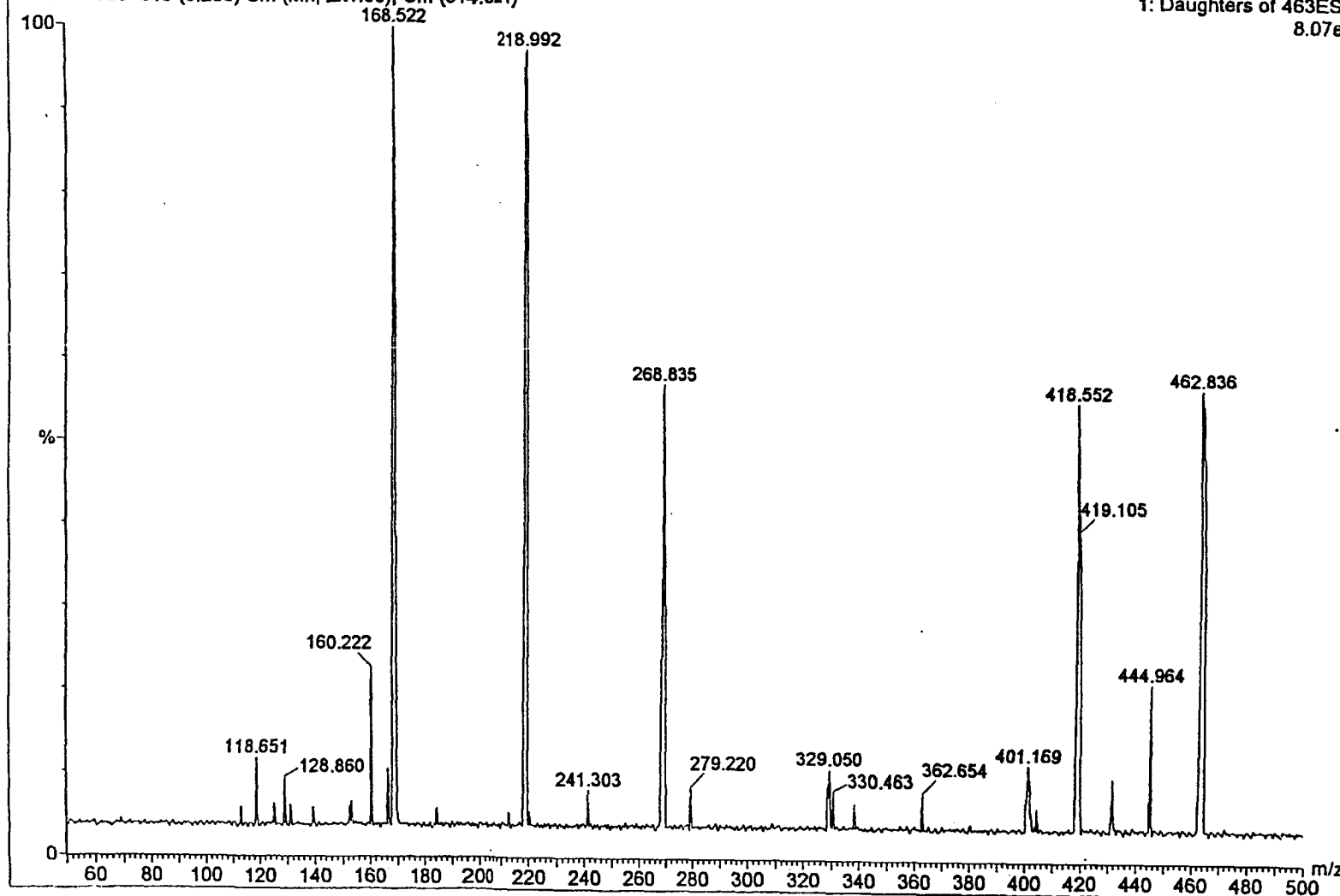
d021030006 316 (9.206) Sm (Mn, 2x1.00); Cm (314:321)

davey 070799

30-Oct-2002 12:55:02

1: Daughters of 463ES-

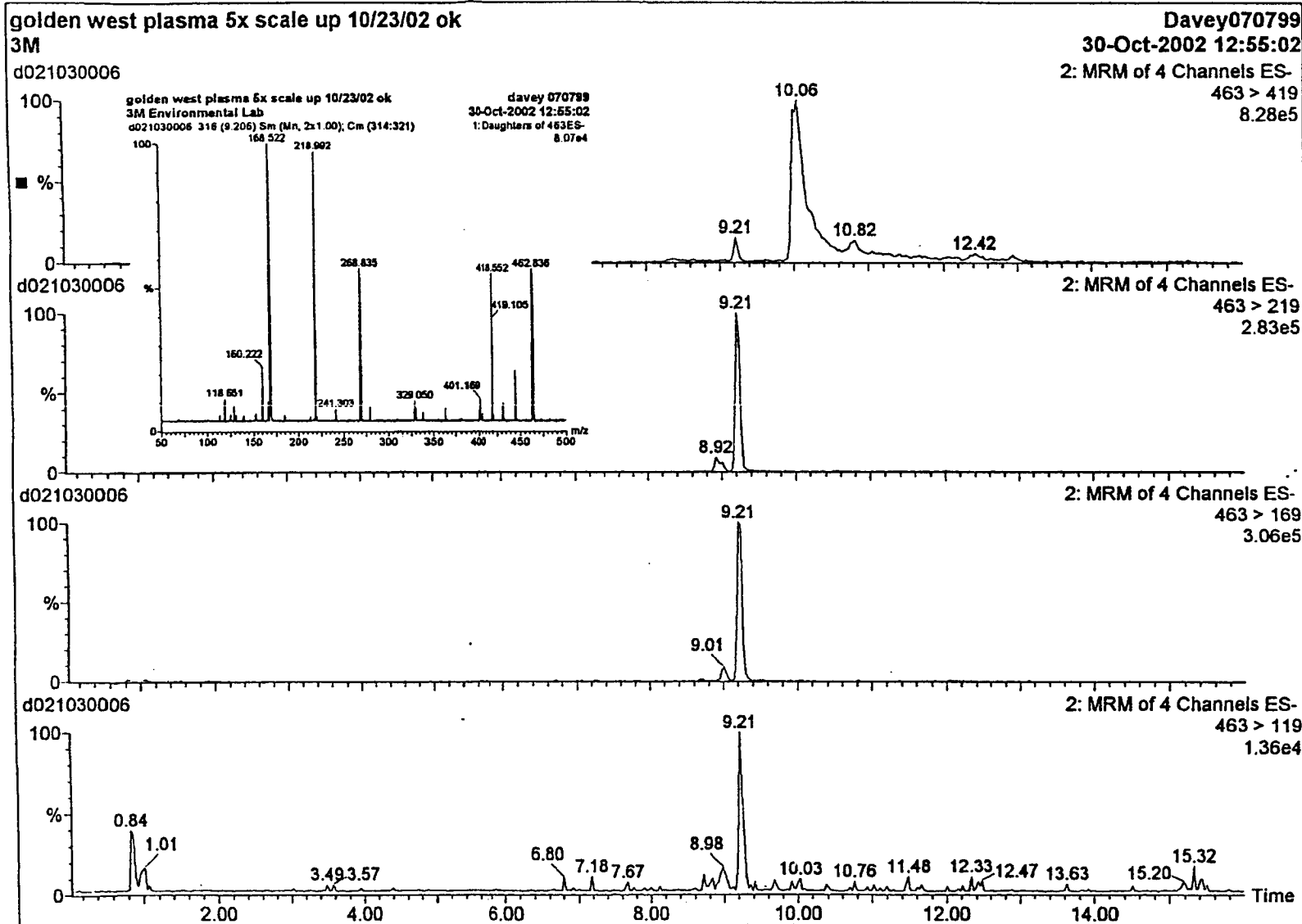
8.07e4



Exact Copy of Original

Cm 10/31/02
Initial Date

C9 ACID



3M ENVIRONMENTAL LABORATORY
E02-1039

Exact Copy of Original

CMC 10/31/02
Initial Date

C9 ACID

02048-21-7 solvent standard 10ppb

3M

d021030016

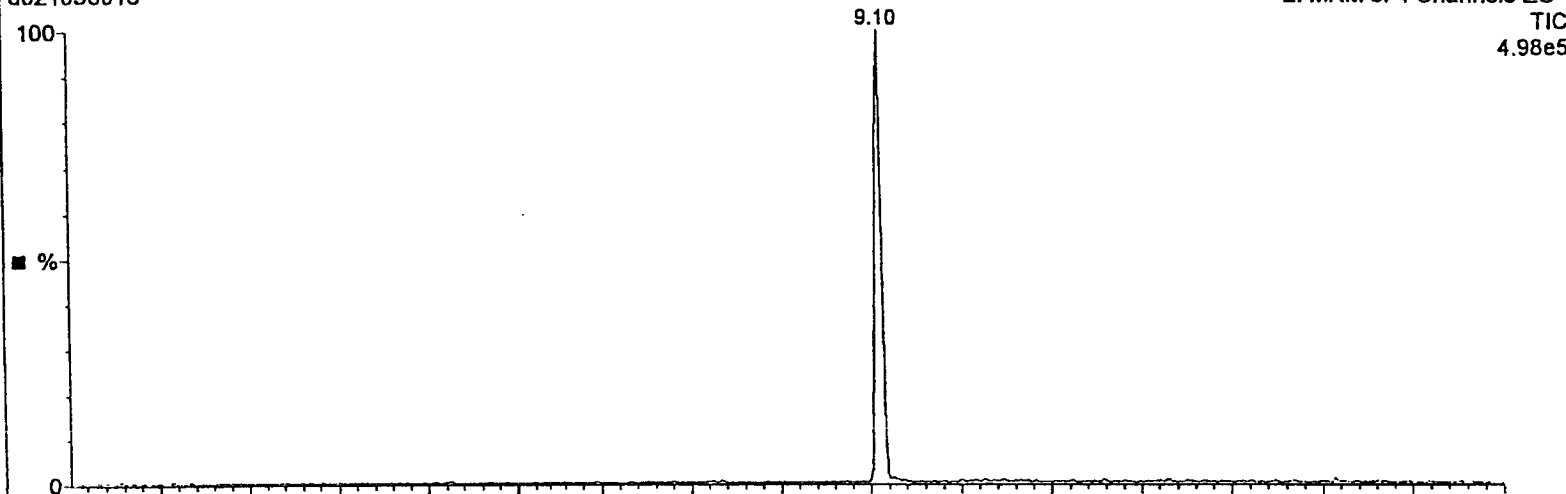
Davey070799

30-Oct-2002 15:46:28

2: MRM of 4 Channels ES-

TIC

4.98e5

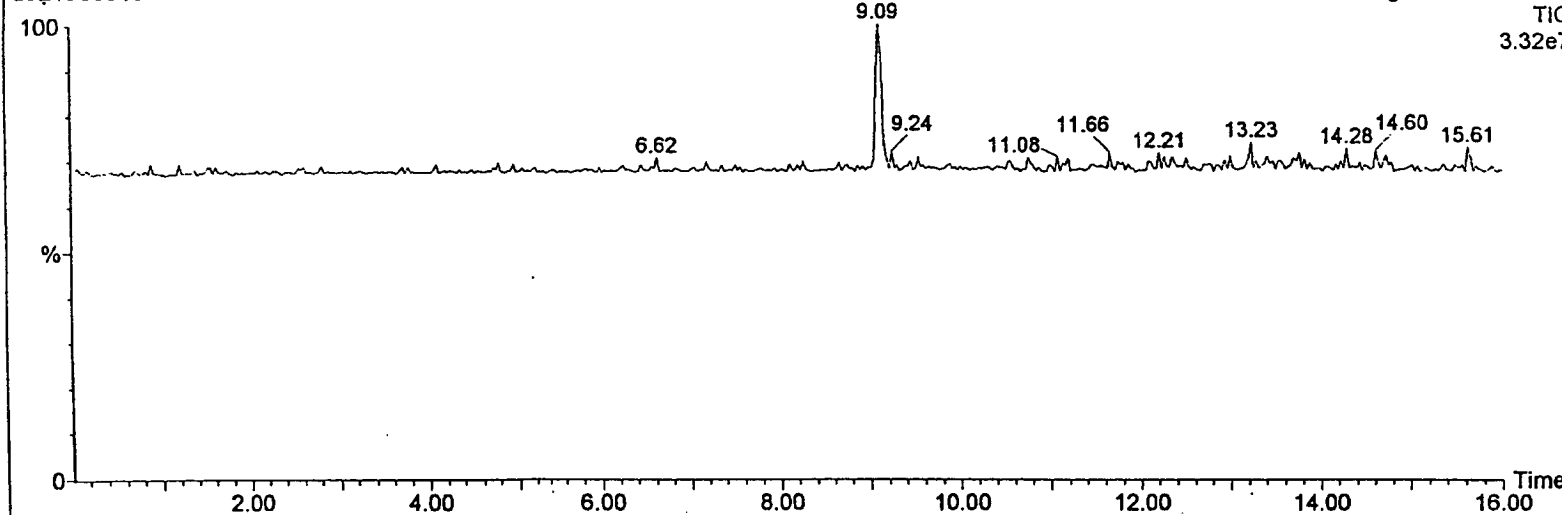


d021030016

1: Daughters of 463ES-

TIC

3.32e7



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CG ACID

02048-21-7 solvent standard 10ppb

3M Environmental Lab

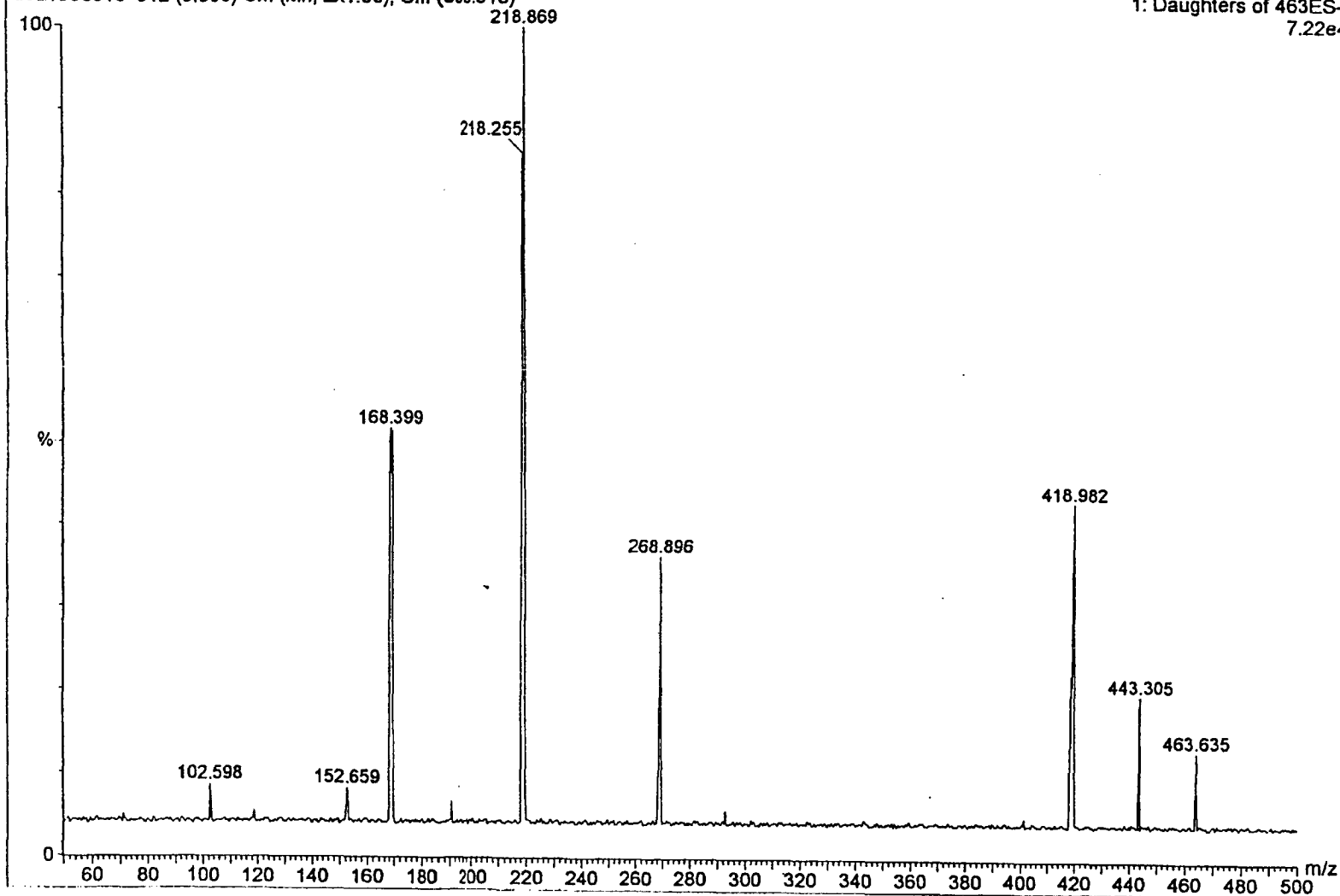
d021030016 312 (9.095) Sm (Mn, 2x1.00); Cm (308:318)

davey 070799

30-Oct-2002 15:46:28

1: Daughters of 463ES-

7.22e4



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Initial Date

C9 ACID

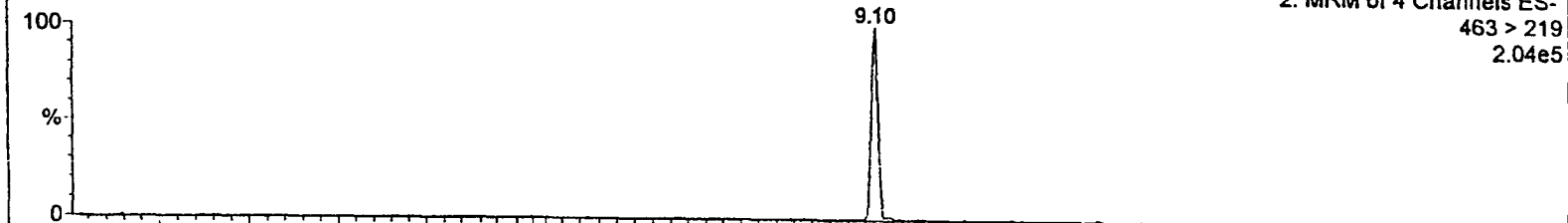
02048-21-7 solvent standard 10ppb

3M

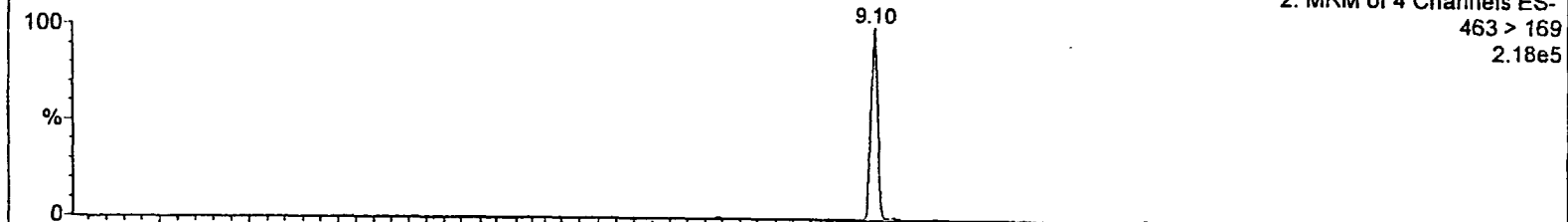
d021030016



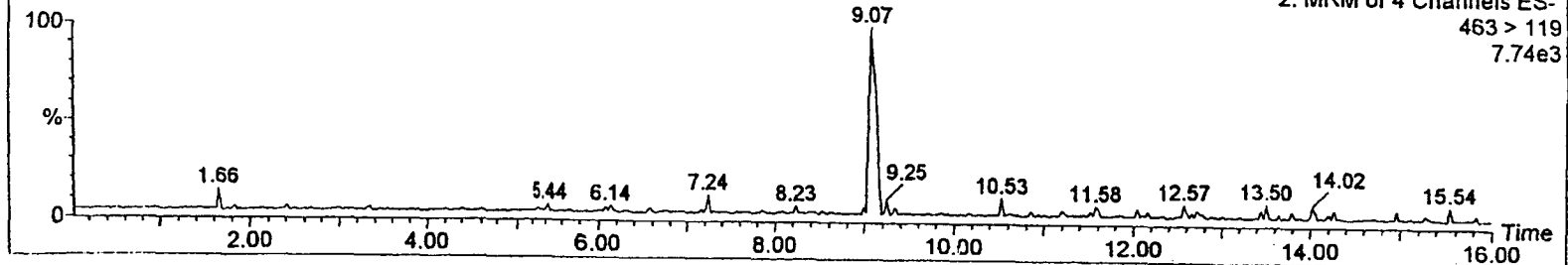
d021030016



d021030016



d021030016



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E02-1039

CG ACID

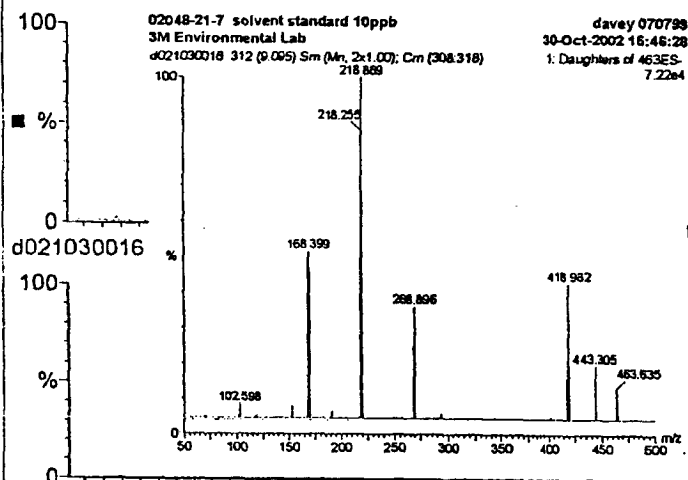
02048-21-7 solvent standard 10ppb
3M

Davey070799

30-Oct-2002 15:46:28

2: MRM of 4 Channels ES-
463 > 419
6.95e4

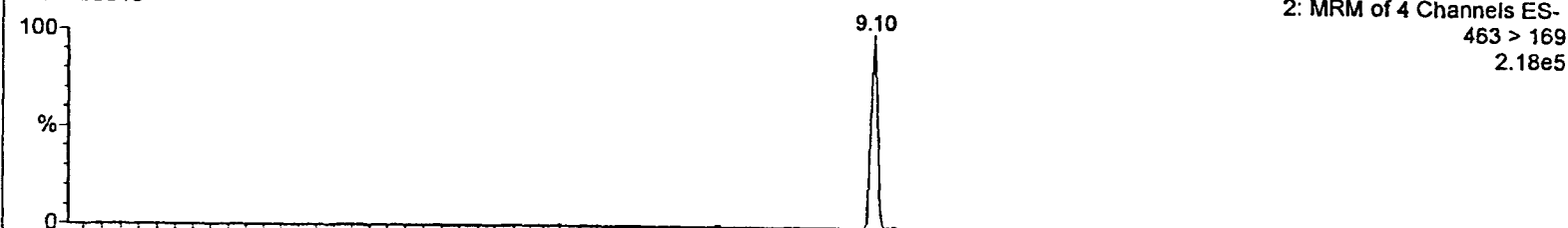
d021030016



d021030016

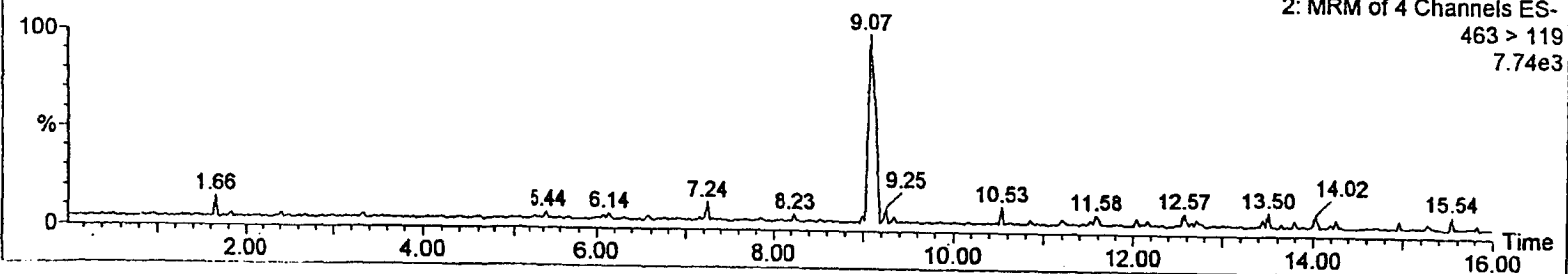
2: MRM of 4 Channels ES-
463 > 219
2.04e5

d021030016



d021030016

2: MRM of 4 Channels ES-
463 > 119
7.74e3



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Initial Date

C10 ACID

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

3M

d021030003

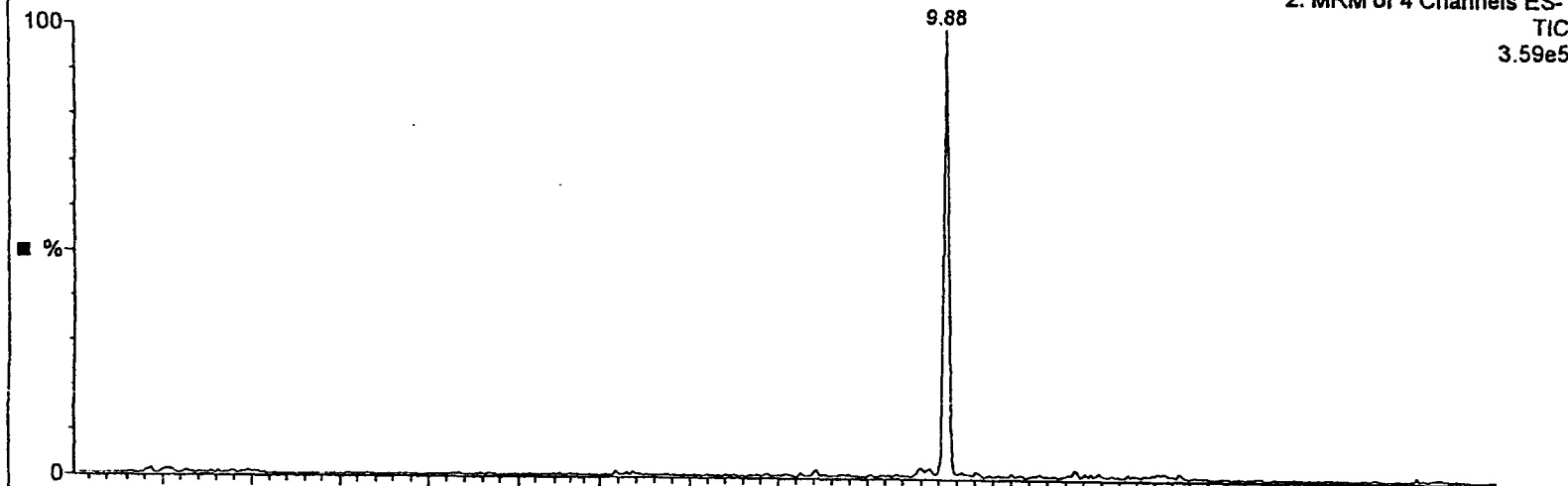
Davey070799

30-Oct-2002 12:03:28

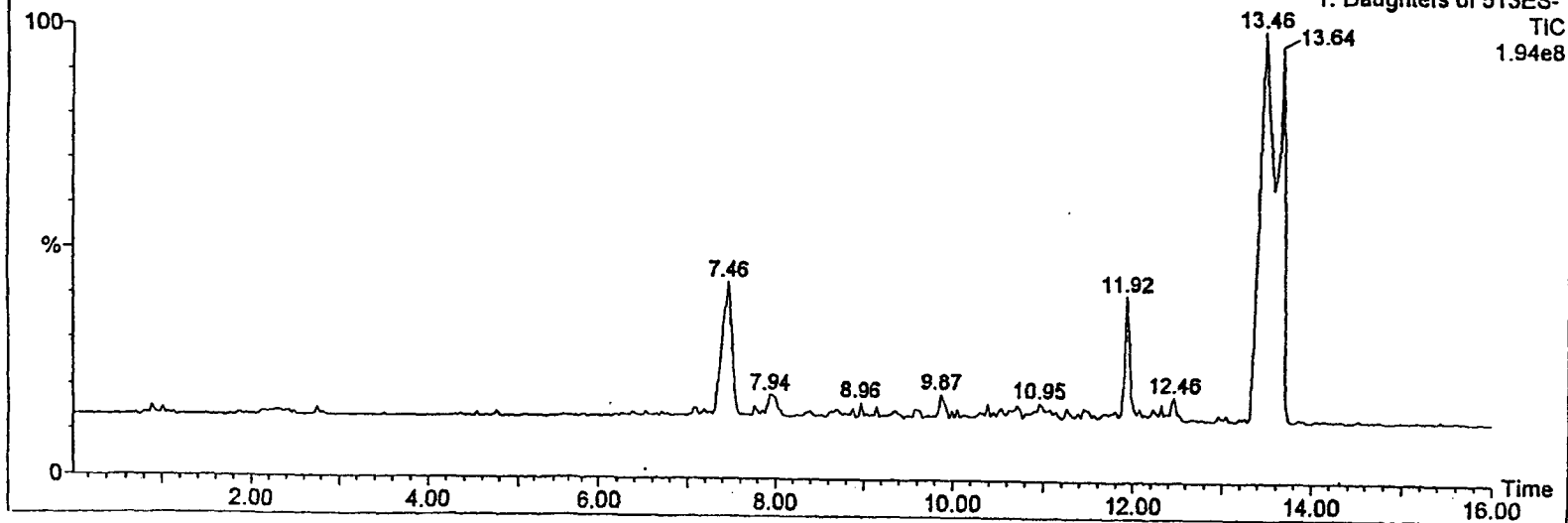
2: MRM of 4 Channels ES-

TIC

3.59e5



d021030003



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CMC 10/31/02
Initial Date

C10 ACID

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

3M Environmental Lab

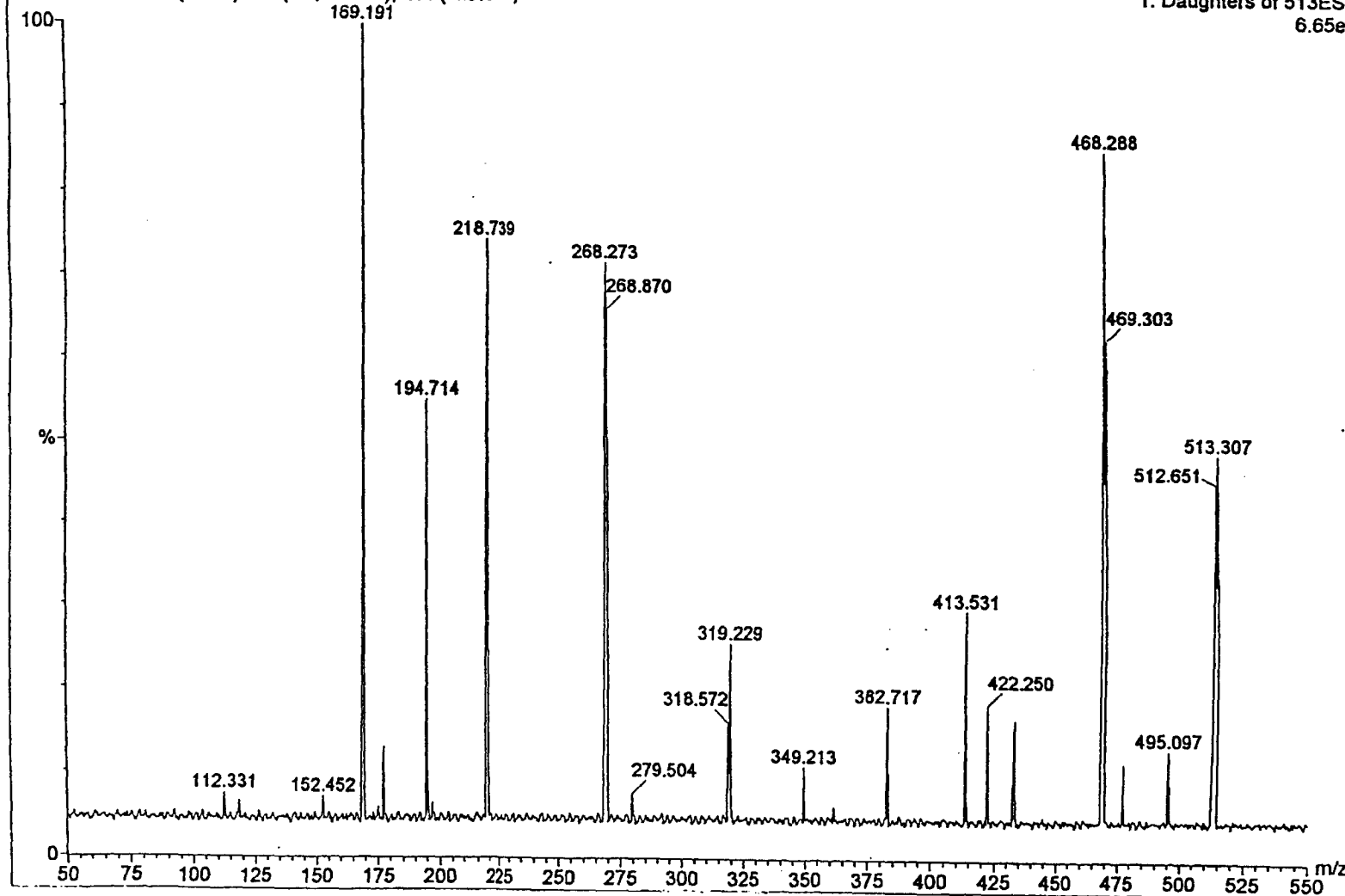
d021030003 327 (9.869) Sm (Mn, 2x1.00); Cm (325:330)

davey 070799

30-Oct-2002 12:03:28

1: Daughters of 513ES-

6.65e4



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Cm 10/31/02
Initial Date

C10 ACID

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

3M

d021030003

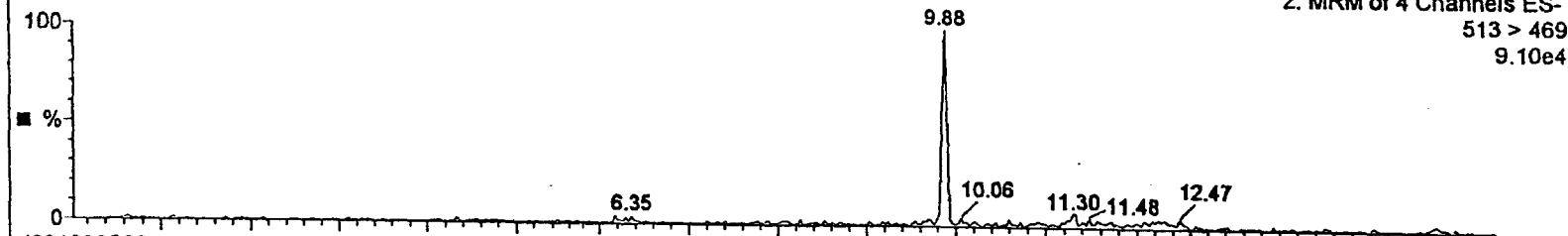
Davey070799

30-Oct-2002 12:03:28

2: MRM of 4 Channels ES-

513 > 469

9.10e4



d021030003

2: MRM of 4 Channels ES-

513 > 219

1.64e5

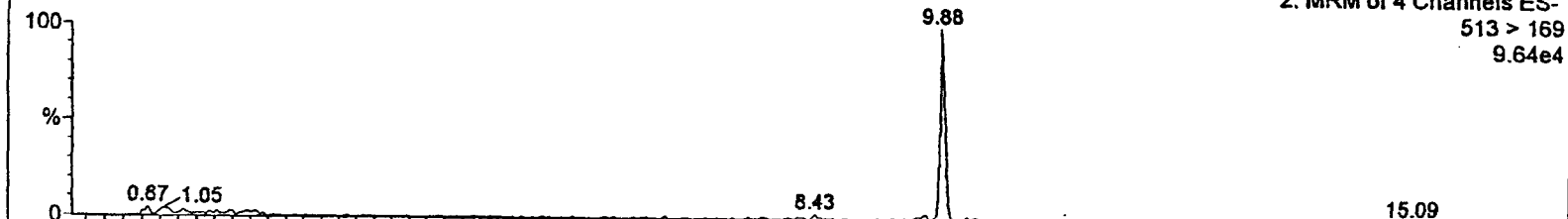


d021030003

2: MRM of 4 Channels ES-

513 > 169

9.64e4

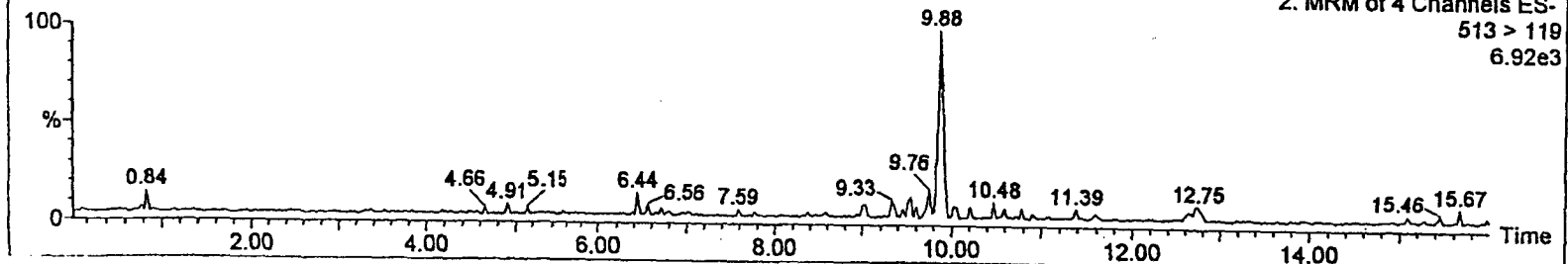


d021030003

2: MRM of 4 Channels ES-

513 > 119

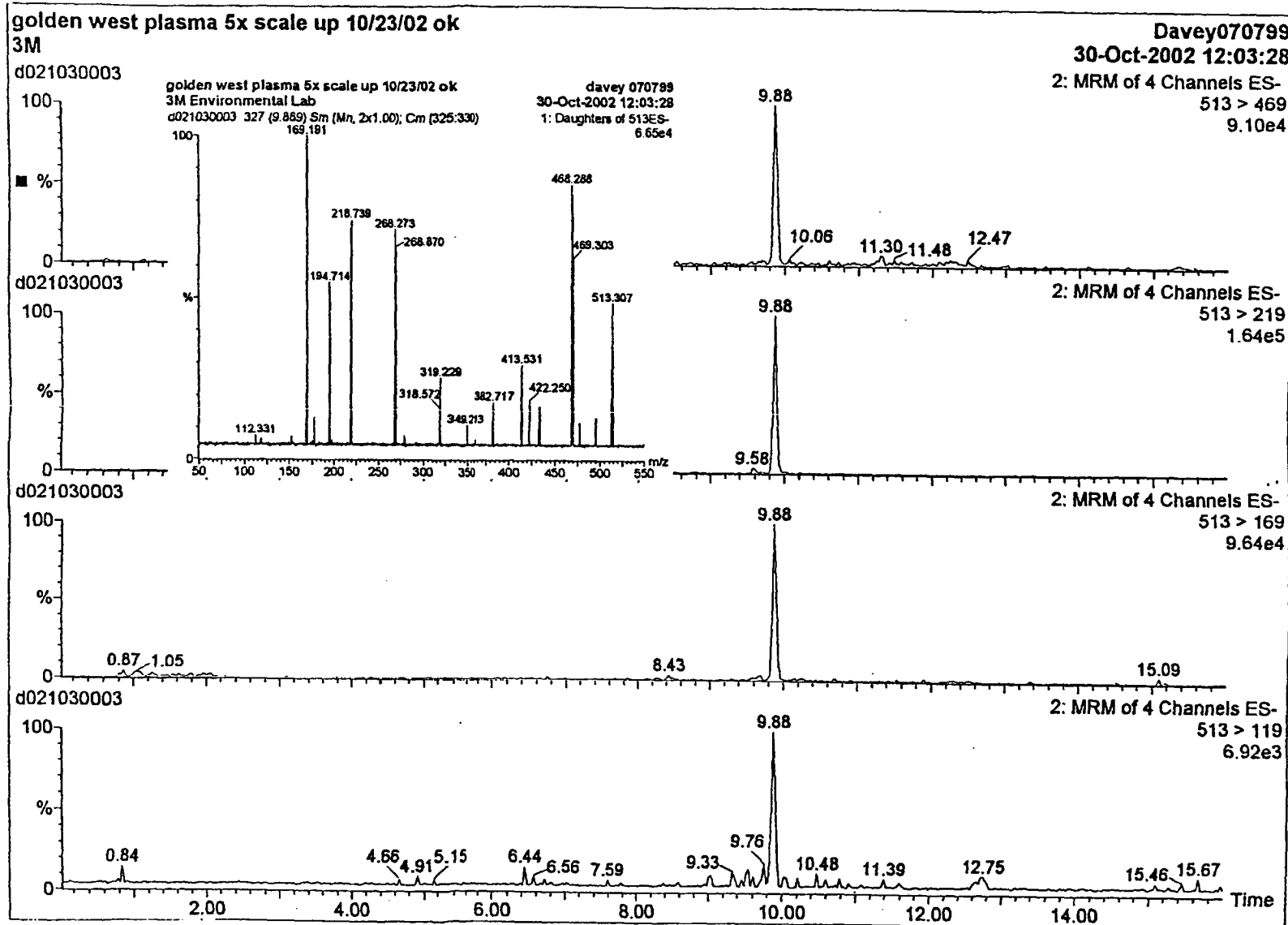
6.92e3



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C10 ACID



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CML 10/31/02
Initial Date

C10 ACID

02048-21-7 solvent standard 10ppb

3M

d021030013

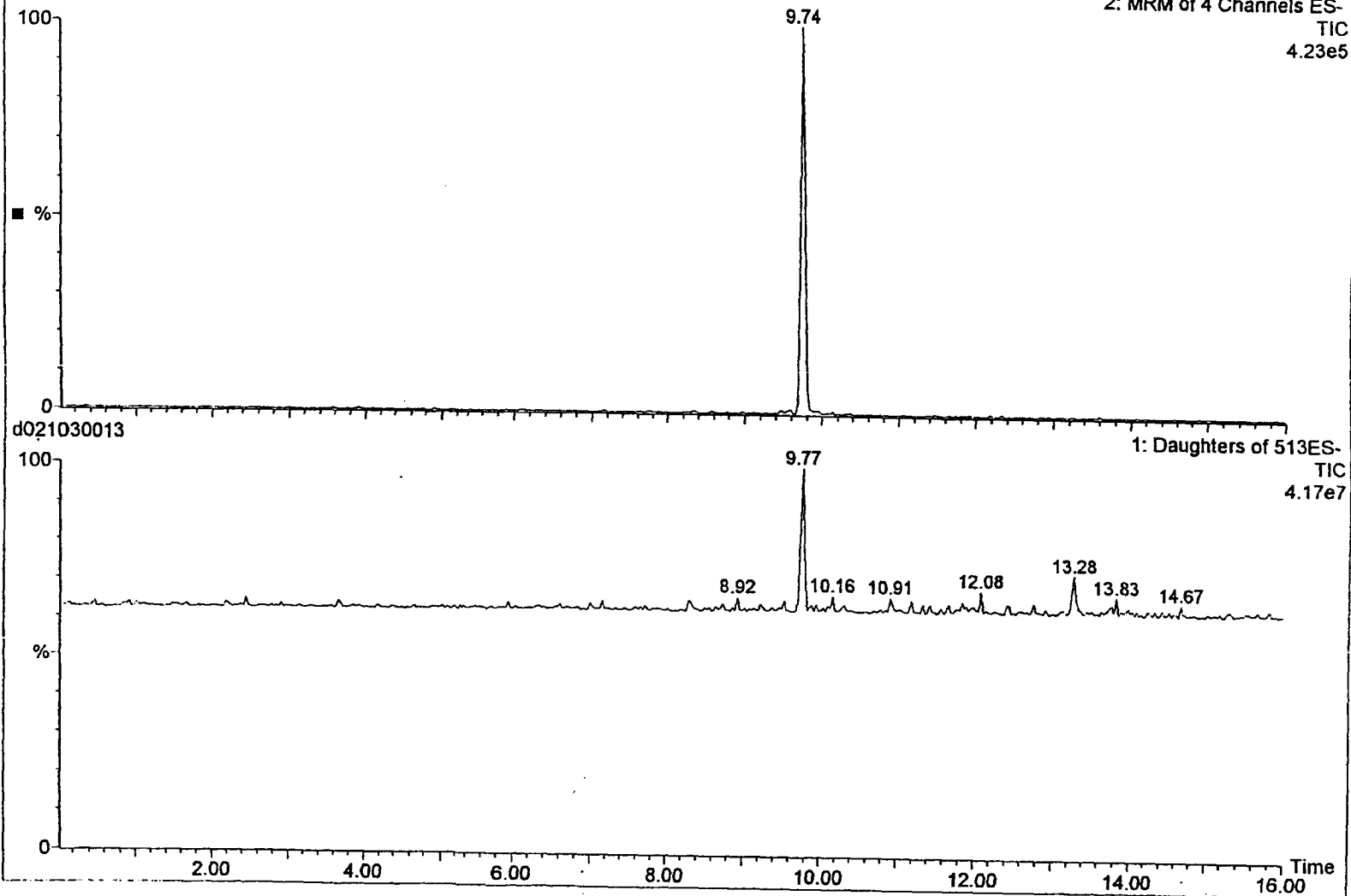
Davey070799

30-Oct-2002 14:55:16

2: MRM of 4 Channels ES-

TIC

4.23e5



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Initial Date

3M ENVIRONMENTAL LABORATORY
E02-1039

CID ACID

02048-21-7 solvent standard 10ppb

3M Environmental Lab

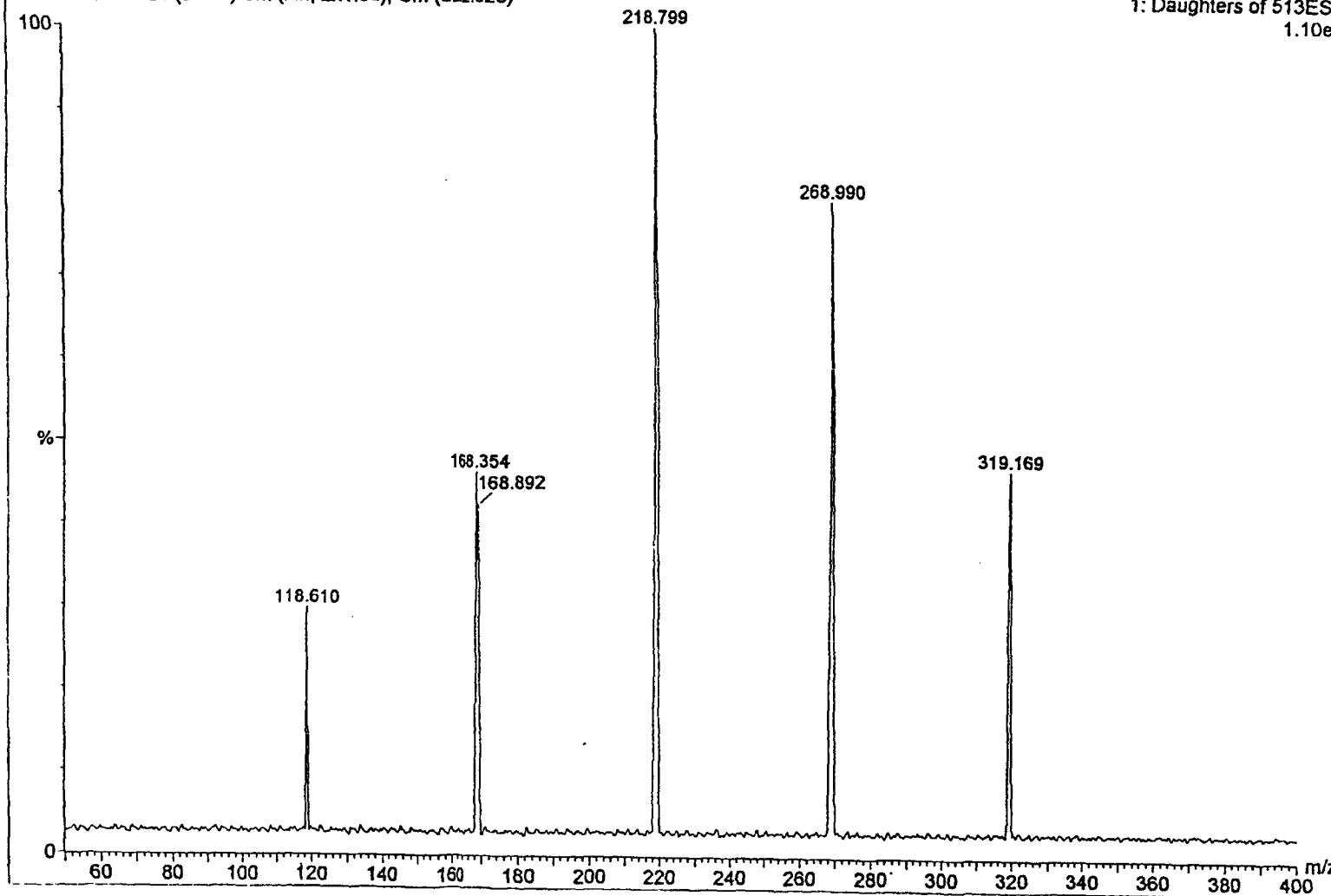
d021030013 324 (9.765) Sm (Mn, 2x1.00); Cm (322:326)

davey 070799

30-Oct-2002 14:55:16

1: Daughters of 513ES-

1.10e5



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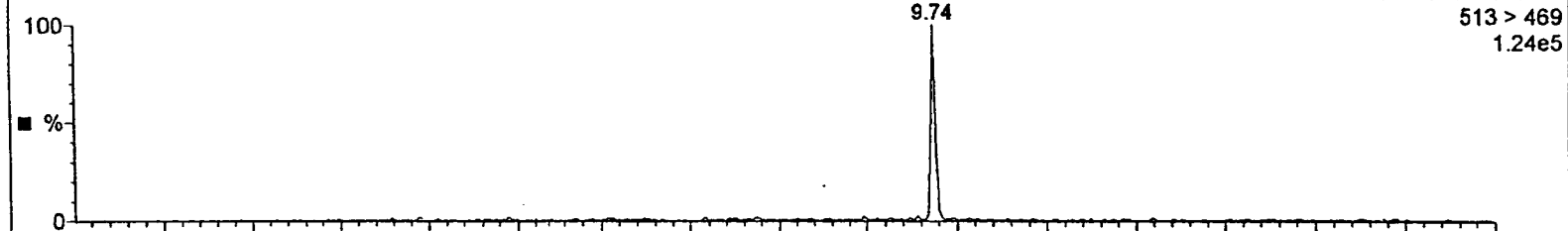
3M ENVIRONMENTAL LABORATORY
E02-1039

C10 ACID

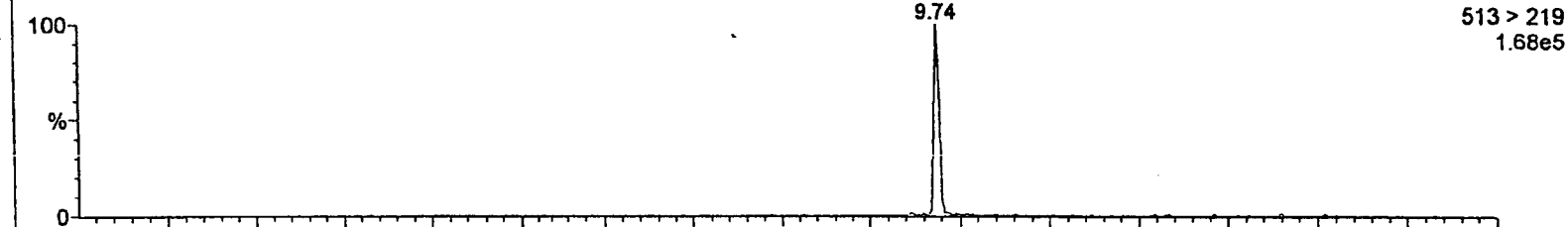
02048-21-7 solvent standard 10ppb

3M

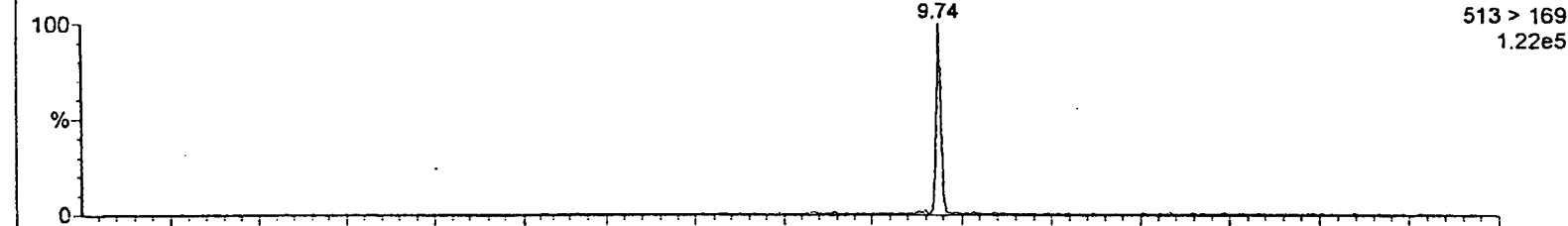
d021030013



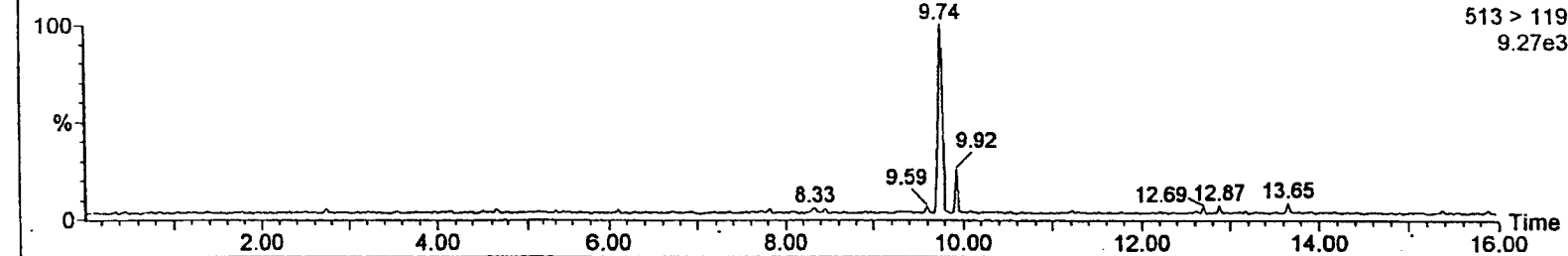
d021030013



d021030013



d021030013



3M ENVIRONMENTAL LABORATORY
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CNC 11/10
Initial Date

CID ACID

02048-21-7 solvent standard 10ppb
3M

Davey070799

30-Oct-2002 14:55:16

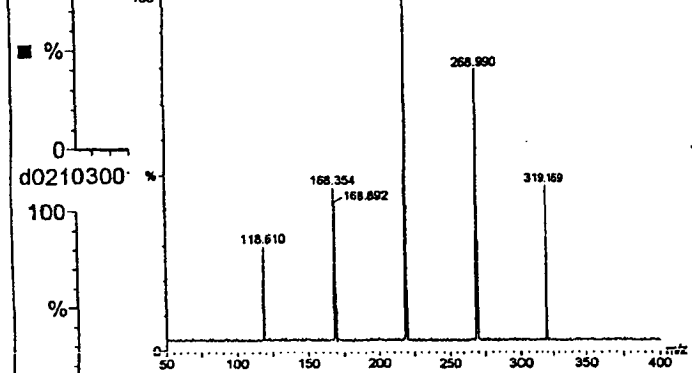
2: MRM of 4 Channels ES-

513 > 469

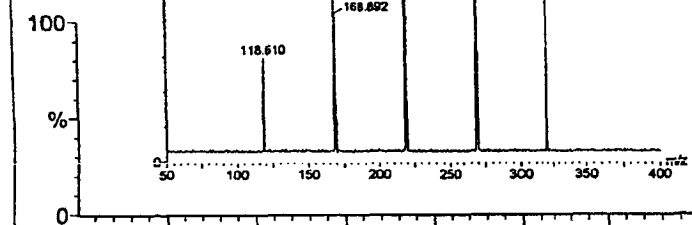
1.24e5

d021030013

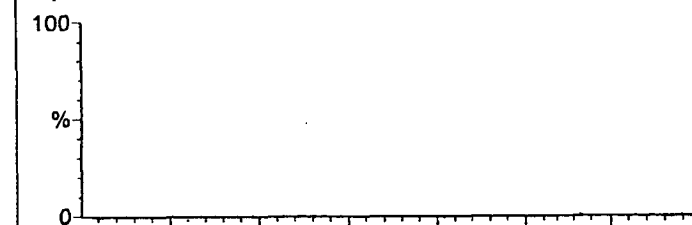
02048-21-7 solvent standard 10ppb
3M Environmental Lab
d021030013 324 (9.765) Sm (Mn, 2x1.00): Cm (322.326)
davey 070799
30-Oct-2002 14:55:16
1: Daughters of 513ES-
1.10e5



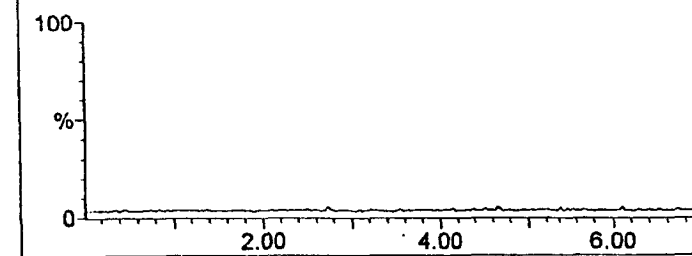
d021030013



d021030013



d021030013



2: MRM of 4 Channels ES-

513 > 219

1.68e5

2: MRM of 4 Channels ES-

513 > 169

1.22e5

2: MRM of 4 Channels ES-

513 > 119

9.27e3

Exact Copy of Original

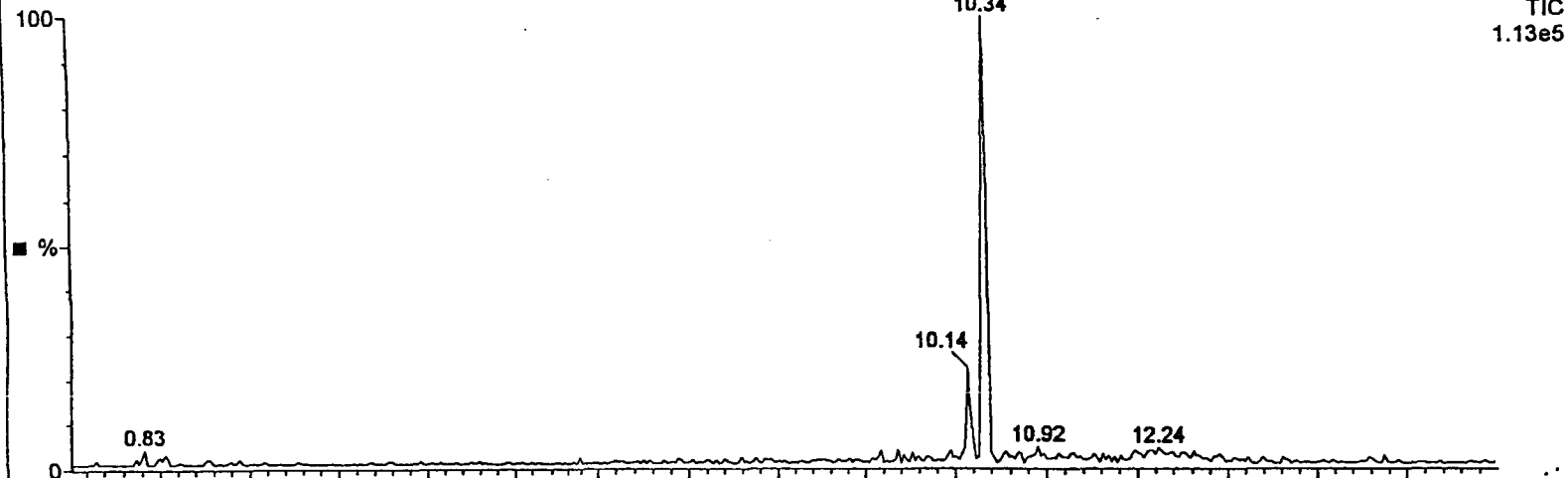
Initial Date

C11 ACID

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

3M

d021030007



Davey070799

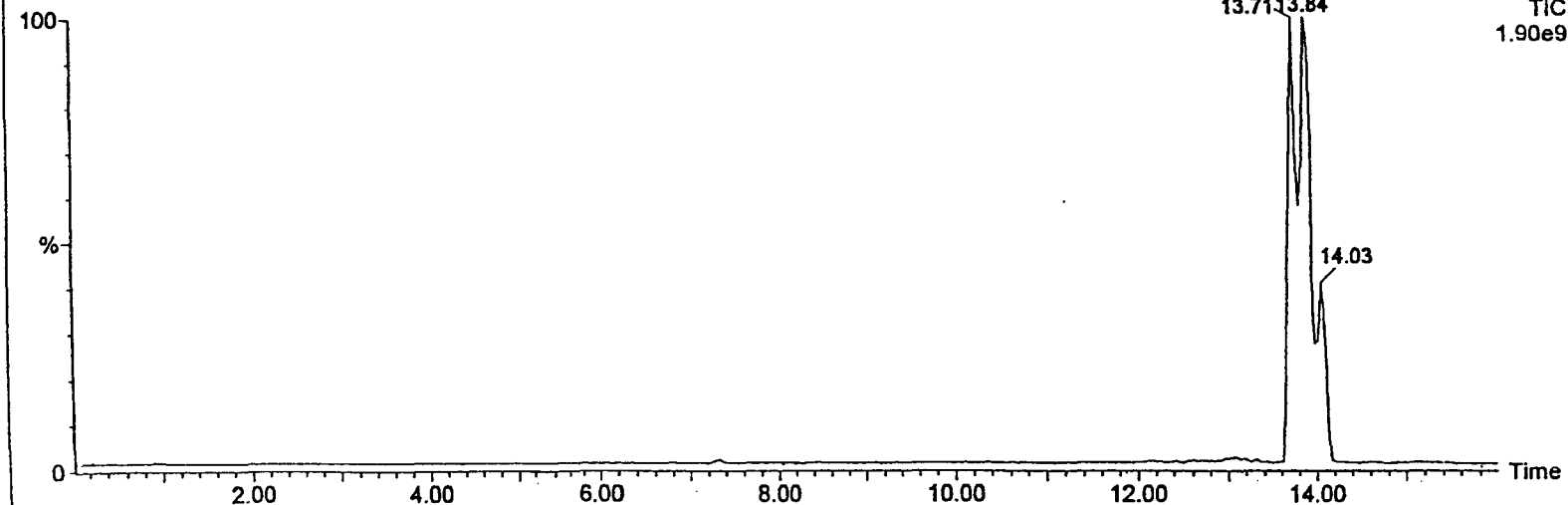
30-Oct-2002 13:12:10

2: MRM of 4 Channels ES-

TIC

1.13e5

d021030007



1: Daughters of 563ES-

TIC

1.90e9

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CMC 10/31/02
Initial Date

C11 ACID

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

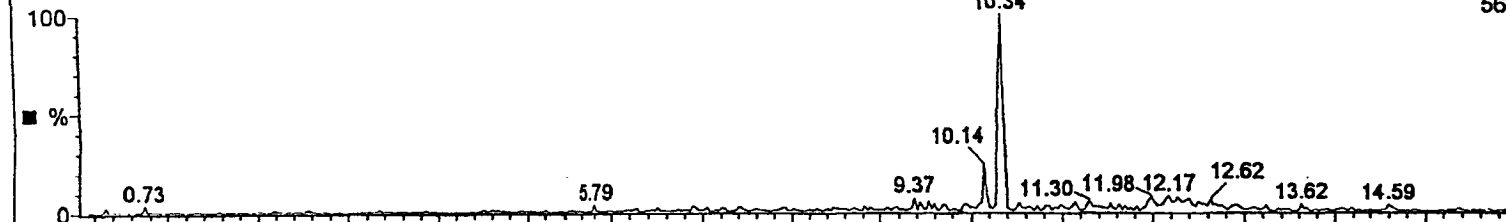
3M

d021030007

Davey070799

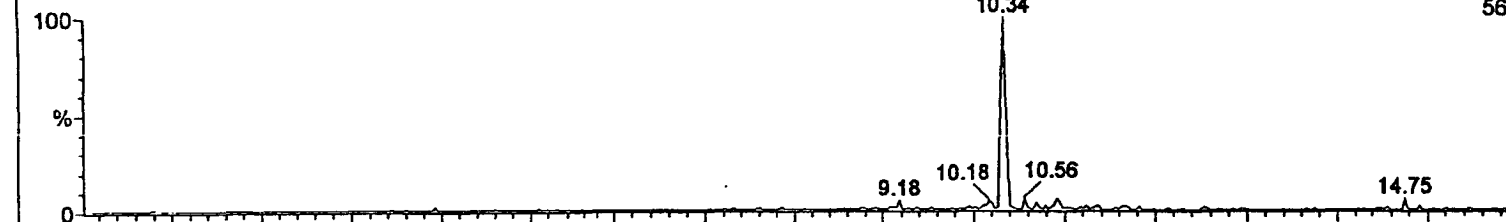
30-Oct-2002 13:12:10

2: MRM of 4 Channels ES-
563 > 519
4.37e4



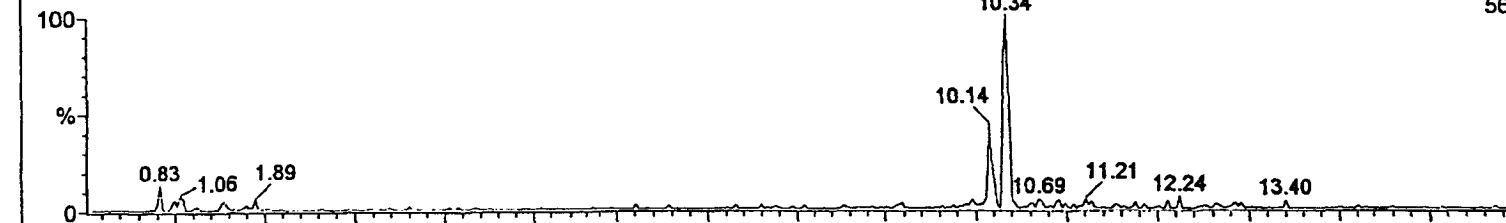
d021030007

2: MRM of 4 Channels ES-
563 > 219
3.70e4



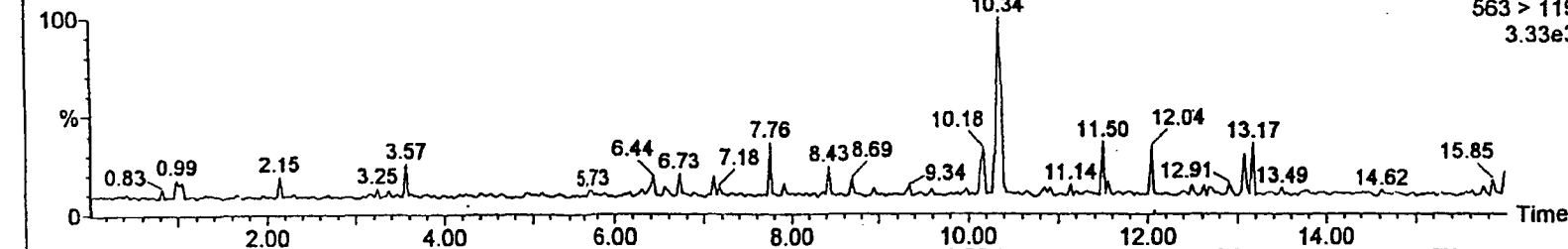
d021030007

2: MRM of 4 Channels ES-
563 > 169
2.93e4



d021030007

2: MRM of 4 Channels ES-
563 > 119
3.33e3



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CIIACID

golden west plasma 5x scale up 10/23/02 ok
3M Environmental Lab

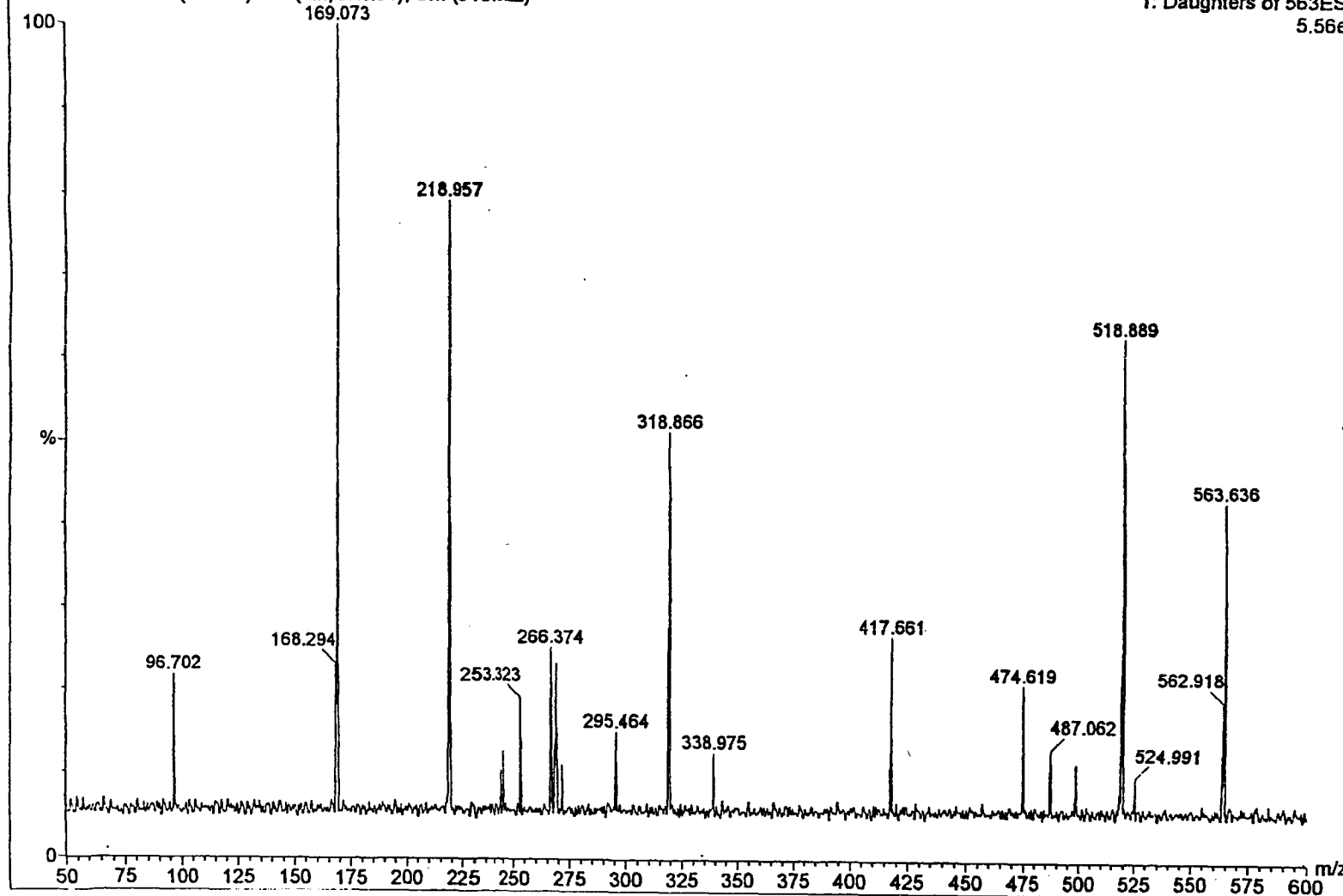
d021030007 320 (10.328) Sm (Mn, 2x1.00); Cm (319:322)

davey 070799

30-Oct-2002 13:12:10

1: Daughters of 563ES-

5.56e4



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Cmc 10/31/02
Initial Date

C11 ACID

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

3M

d021030007

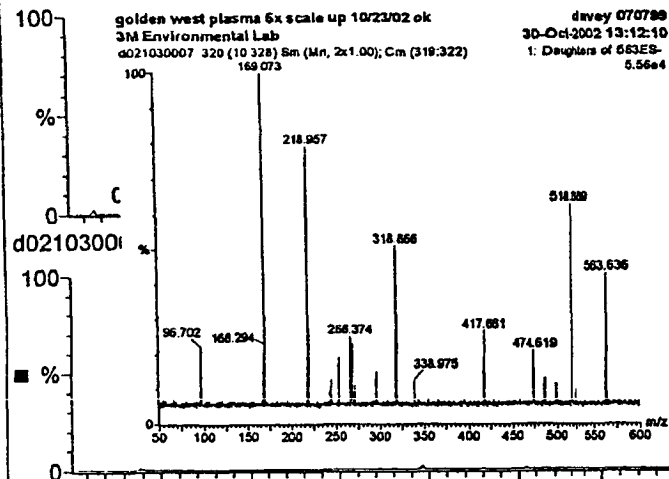
Davey070799

30-Oct-2002 13:12:10

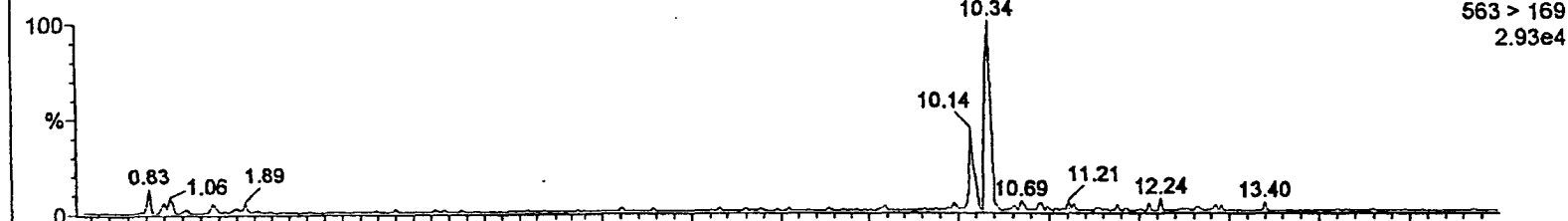
2: MRM of 4 Channels ES-

563 > 519

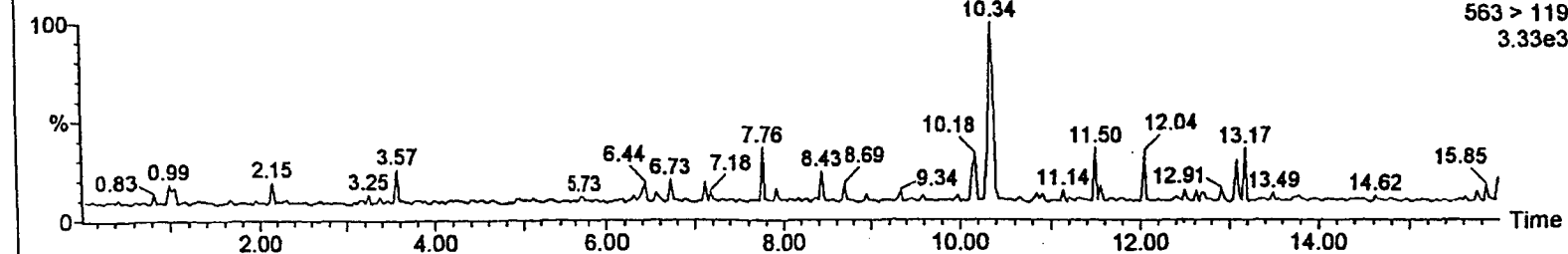
4.37e4



d021030007



d021030007



2: MRM of 4 Channels ES-
563 > 119
3.33e3

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CINC 10/31/02
Initial Date

C11 ACID

02048-21-7 solvent standard 10ppb
3M

d021030017

Davey070799

30-Oct-2002 16:03:30

2: MRM of 4 Channels ES-

TIC

5.89e5

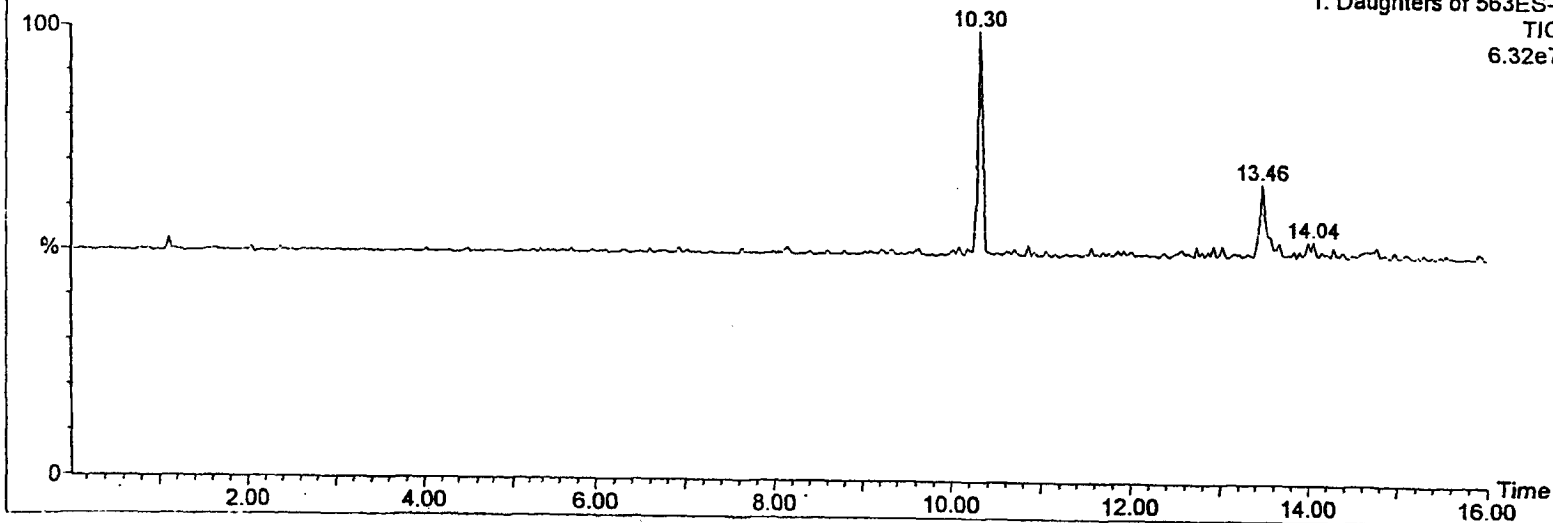


d021030017

1: Daughters of 563ES-

TIC

6.32e7



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3M ENVIRONMENTAL LABORATORY
E02-1039

C11 ACID

02048-21-7 solvent standard 10ppb

3M Environmental Lab

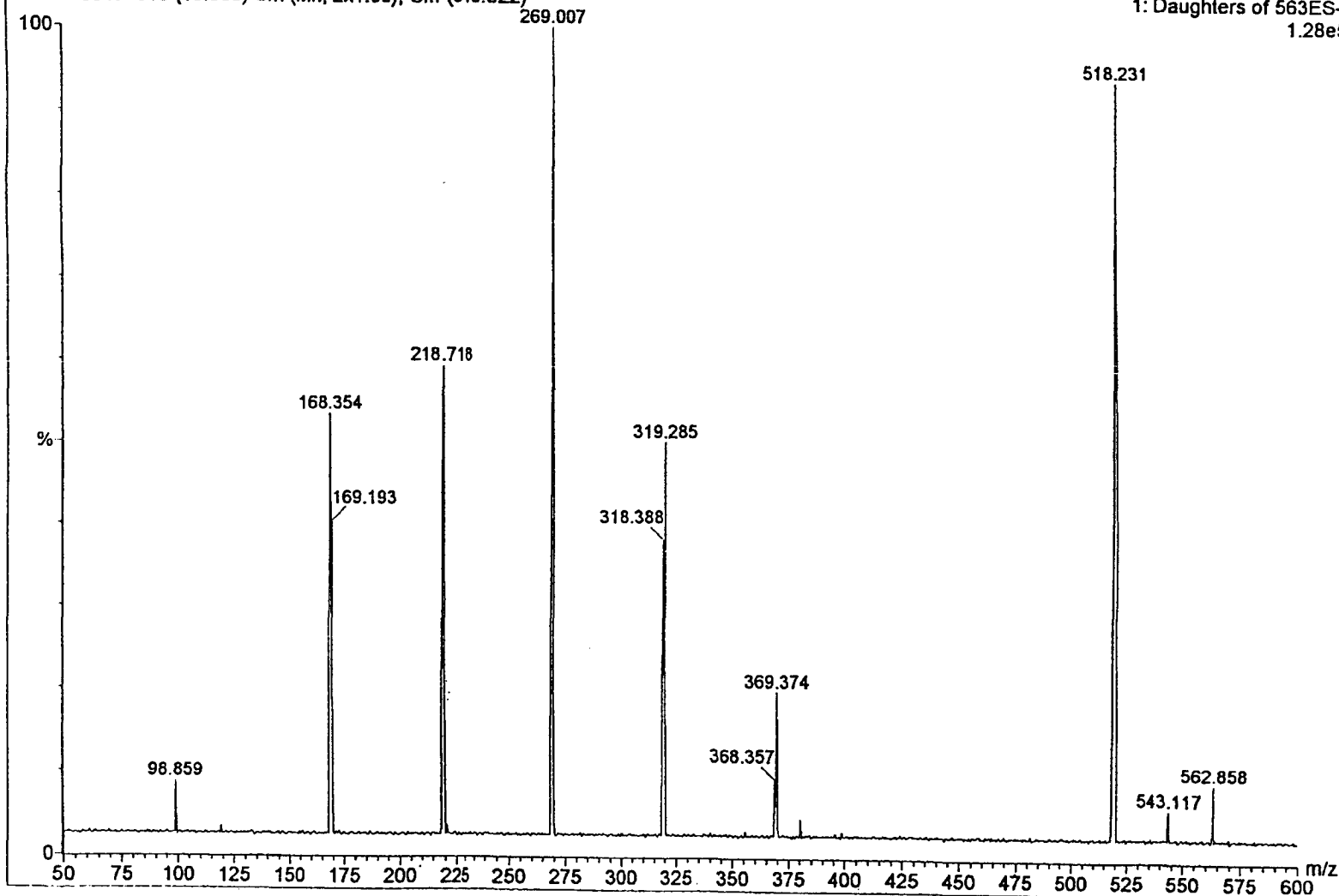
d021030017 319 (10.300) Sm (Mn, 2x1.00); Cm (316:322)

davey 070799

30-Oct-2002 16:03:30

1: Daughters of 563ES-

1.28e5



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3M ENVIRONMENTAL LABORATORY
E02-1039

C11 ACID

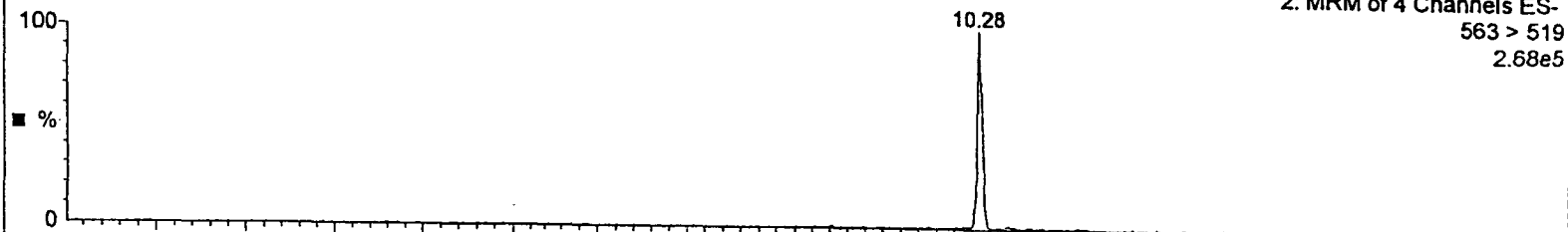
02048-21-7 solvent standard 10ppb
3M

Davey070799

30-Oct-2002 16:03:30

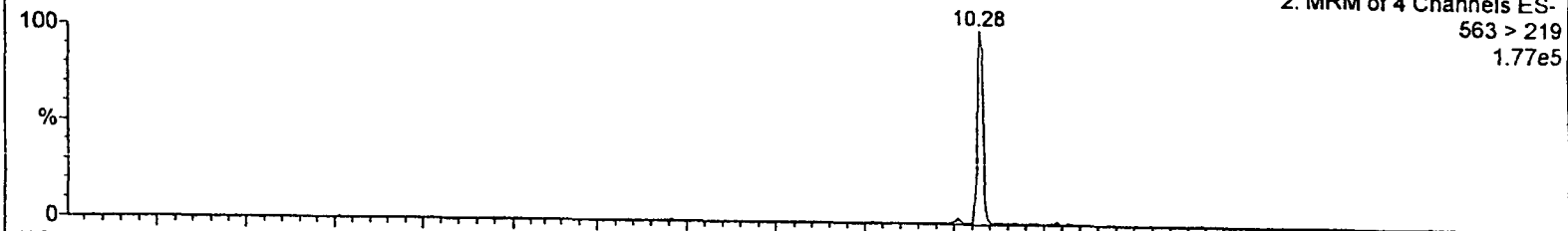
2: MRM of 4 Channels ES-
563 > 519
2.68e5

d021030017



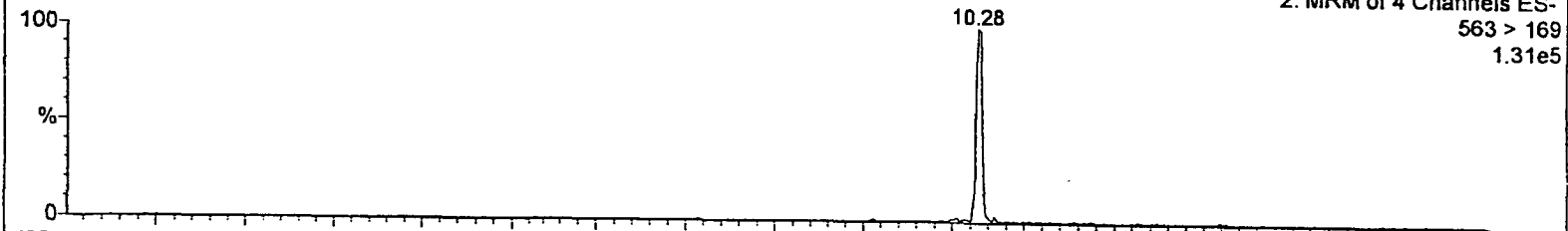
d021030017

2: MRM of 4 Channels ES-
563 > 219
1.77e5



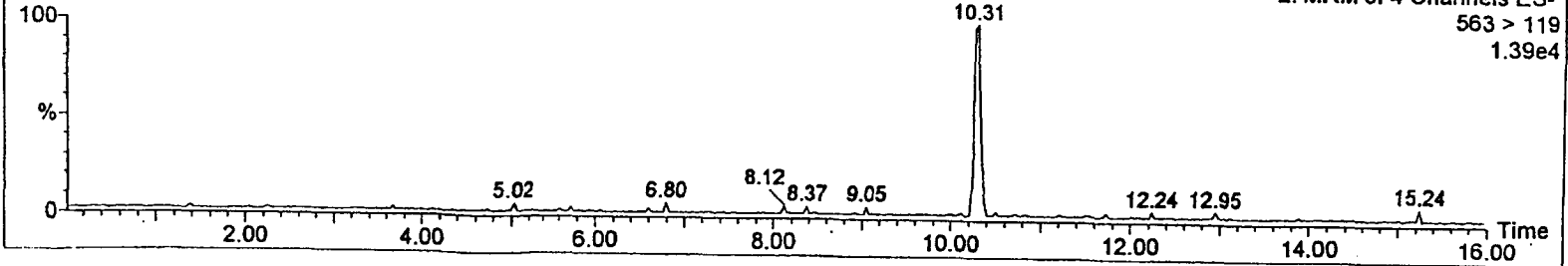
d021030017

2: MRM of 4 Channels ES-
563 > 169
1.31e5



d021030017

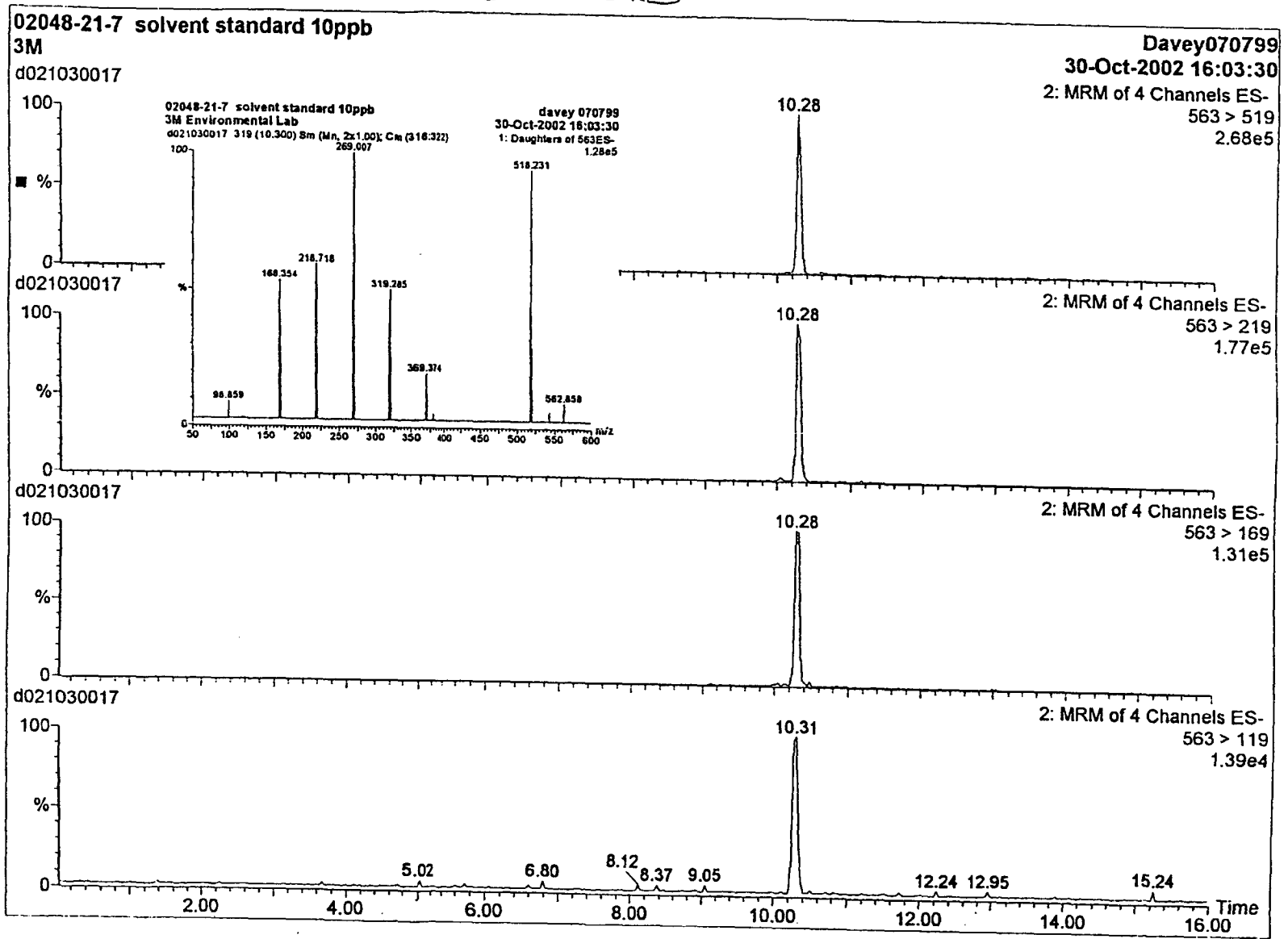
2: MRM of 4 Channels ES-
563 > 119
1.39e4



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C11 ACID



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 E02-1039

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 Initial Date

C12 AC 1D

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

3M

d021030004

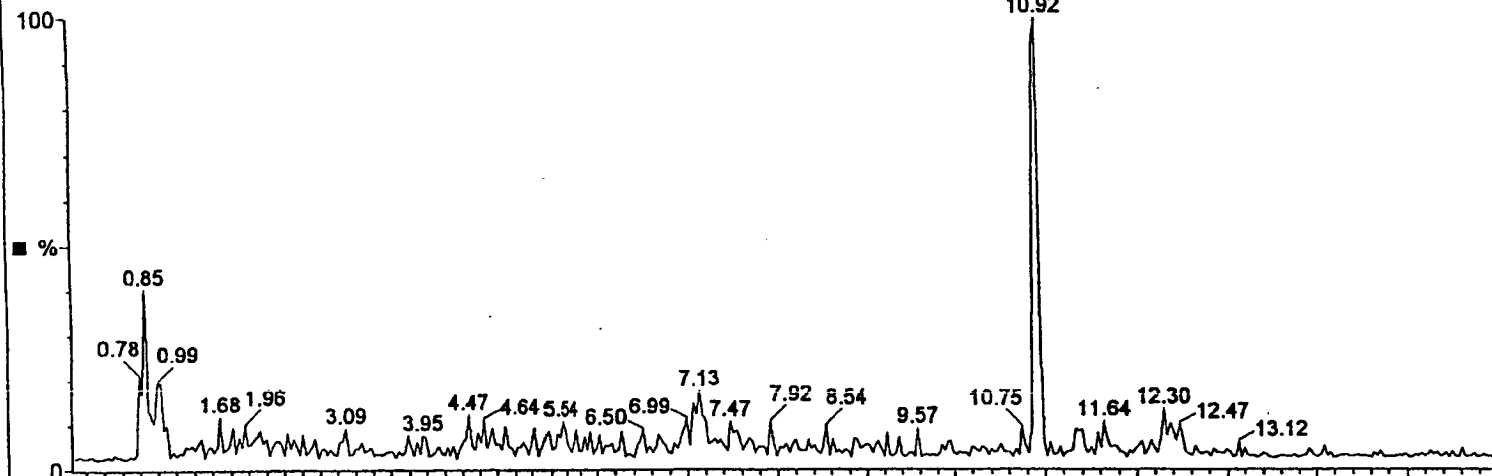
Davey070799

30-Oct-2002 12:20:37

2: MRM of 4 Channels ES-

TIC

4.55e4

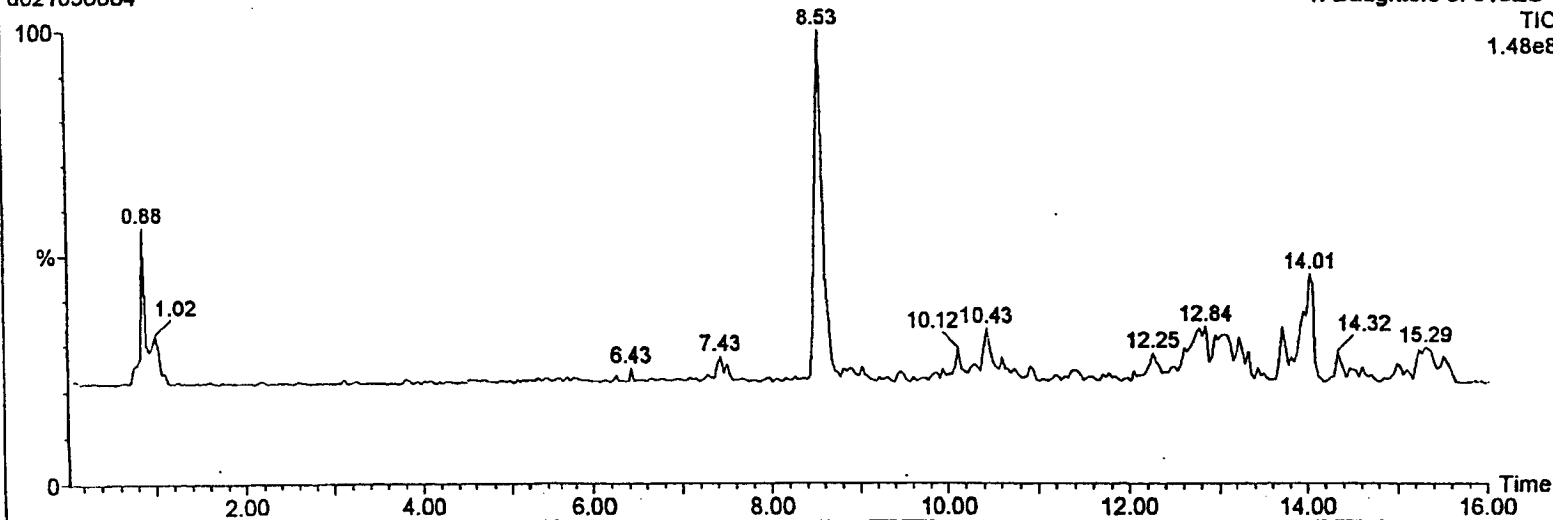


d021030004

1: Daughters of 613ES-

TIC

1.48e8



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CMC 10/31/02
Initial Date

C12 ACID

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

3M Environmental Lab

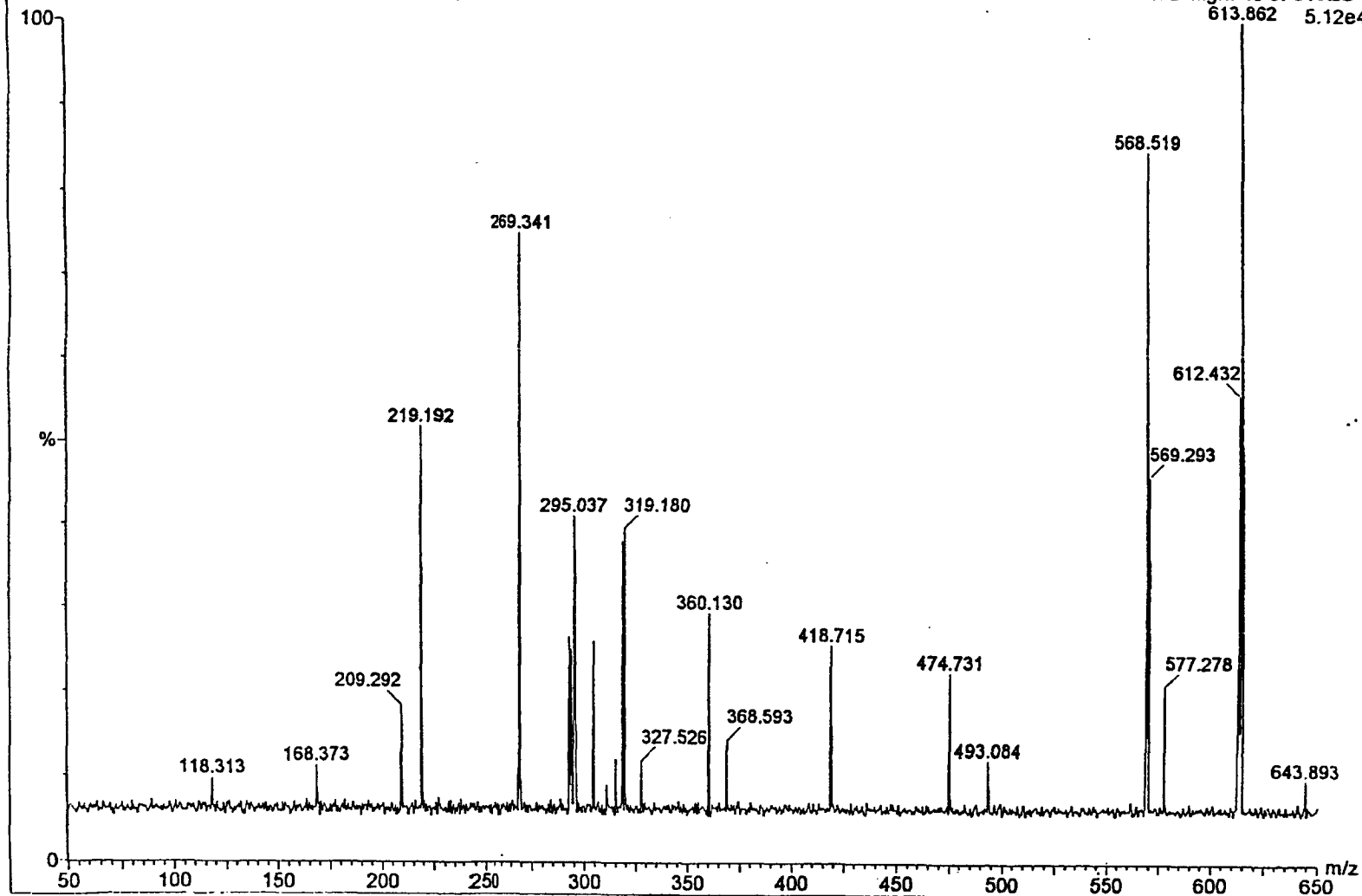
d021030004 316 (10.910) Sm (Mn, 2x1.00); Cm (314:318)

davey 070799

30-Oct-2002 12:20:37

1: Daughters of 613ES-

613.862 5.12e4



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CML 10/31/02
Initial Date

C12 ACID

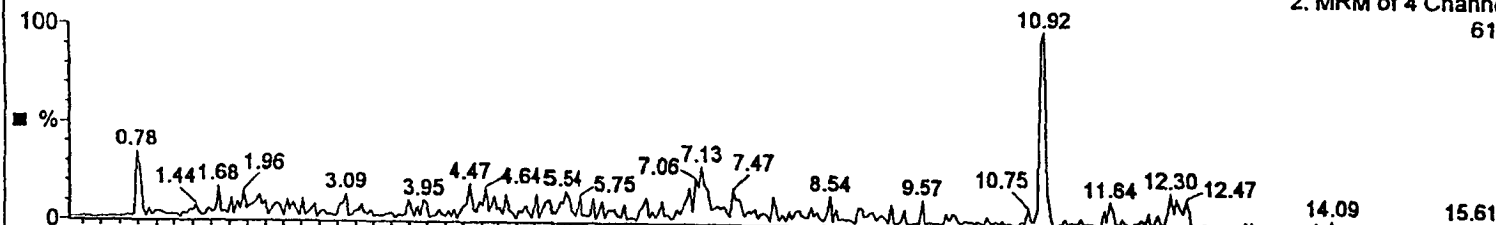
golden west plasma 5x scale up 10/23/02 ok
3M

Davey070799

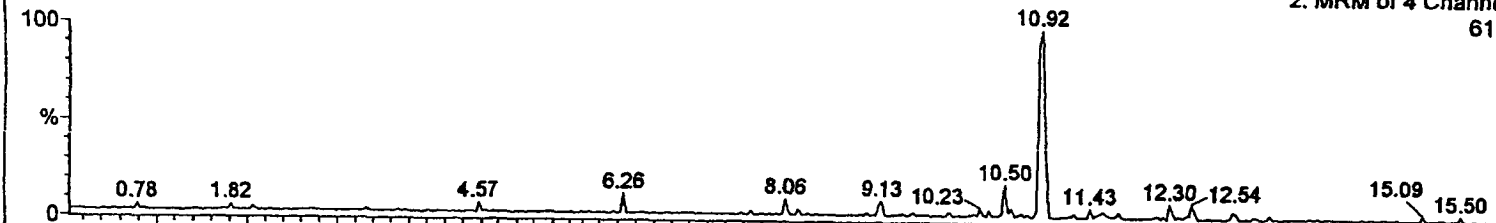
30-Oct-2002 12:20:37

2: MRM of 4 Channels ES-
613 > 569
2.41e4

d021030004

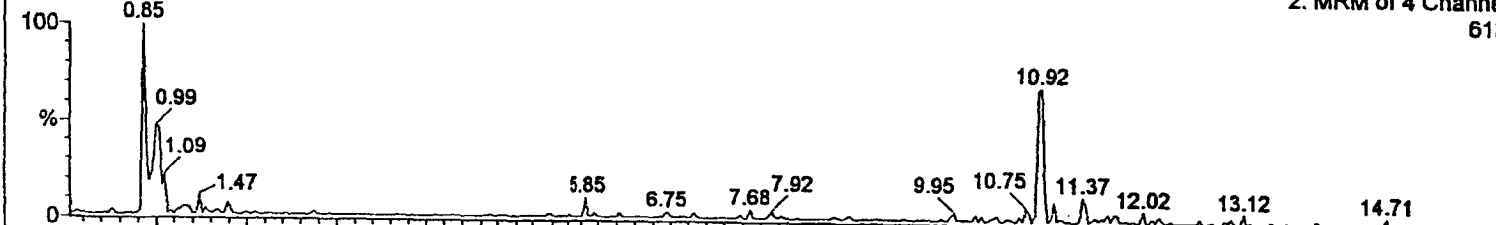


d021030004



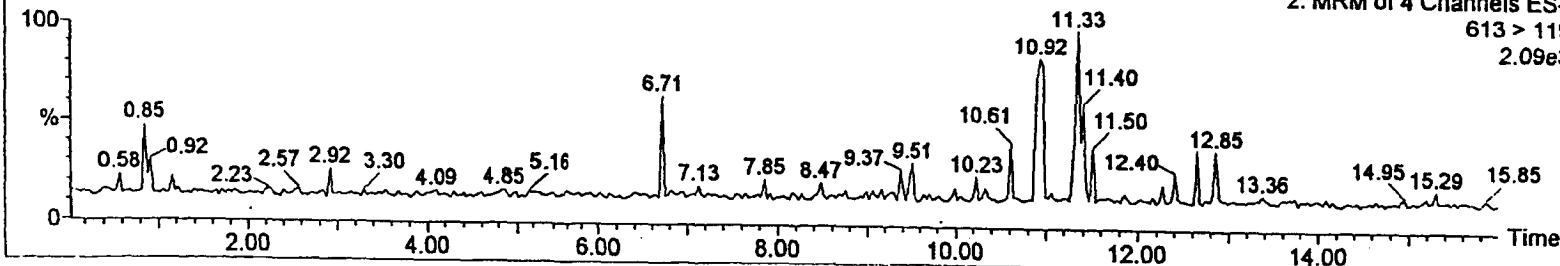
2: MRM of 4 Channels ES-
613 > 219
8.59e3

d021030004



2: MRM of 4 Channels ES-
613 > 169
1.56e4

d021030004



2: MRM of 4 Channels ES-
613 > 119
2.09e3

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Initial CML Date 10/31/02

C12 ACID

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

3M

d021030004

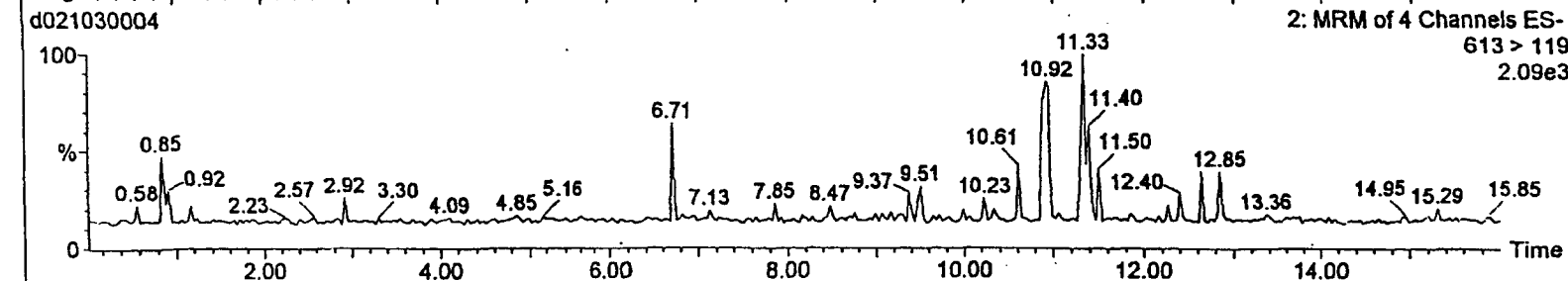
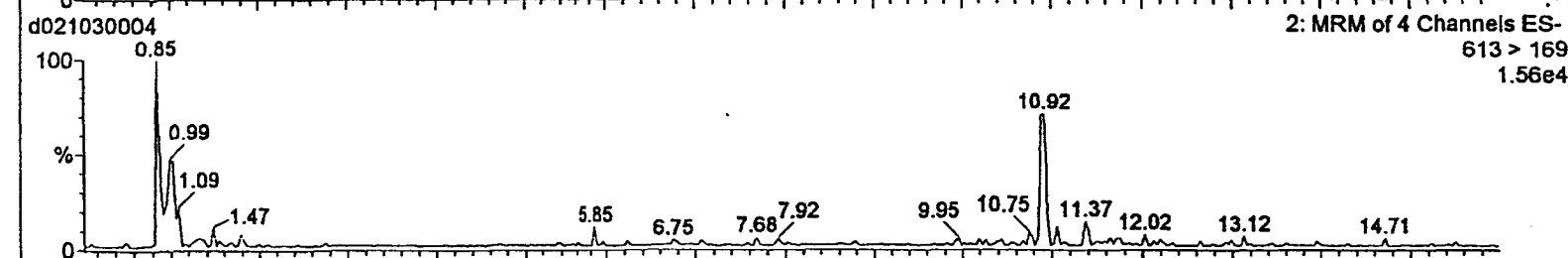
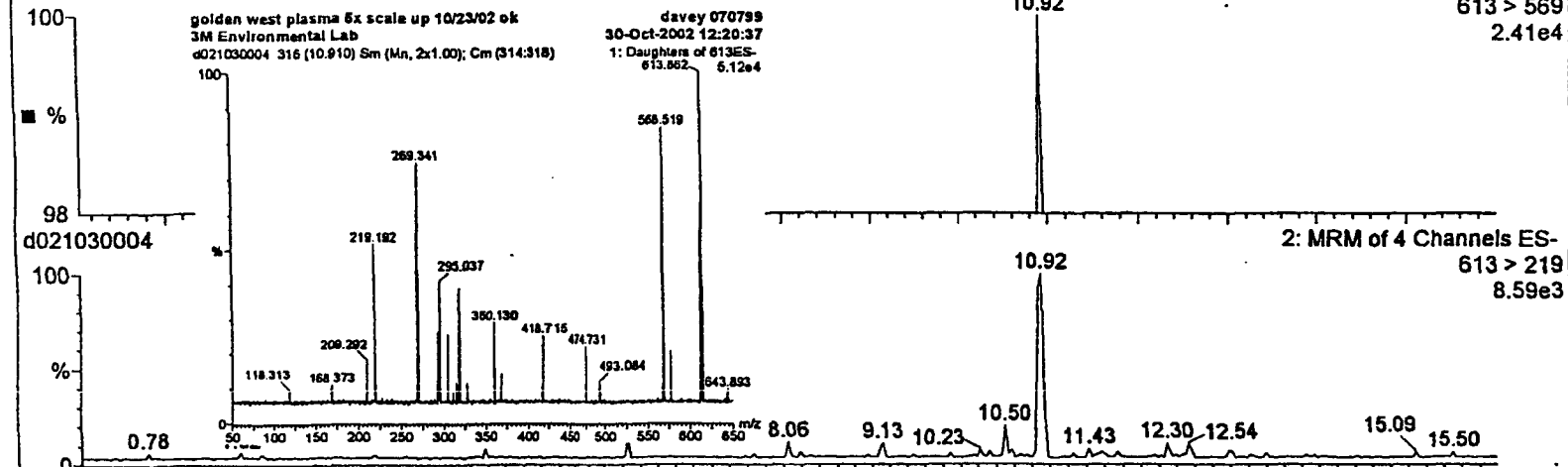
Davey070799

30-Oct-2002 12:20:37

2: MRM of 4 Channels ES-

613 > 569

2.41e4



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CMC 10/31/02
Initial Date

C12 ACID

02048-21-7 solvent standard 10ppb

3M

d021030014

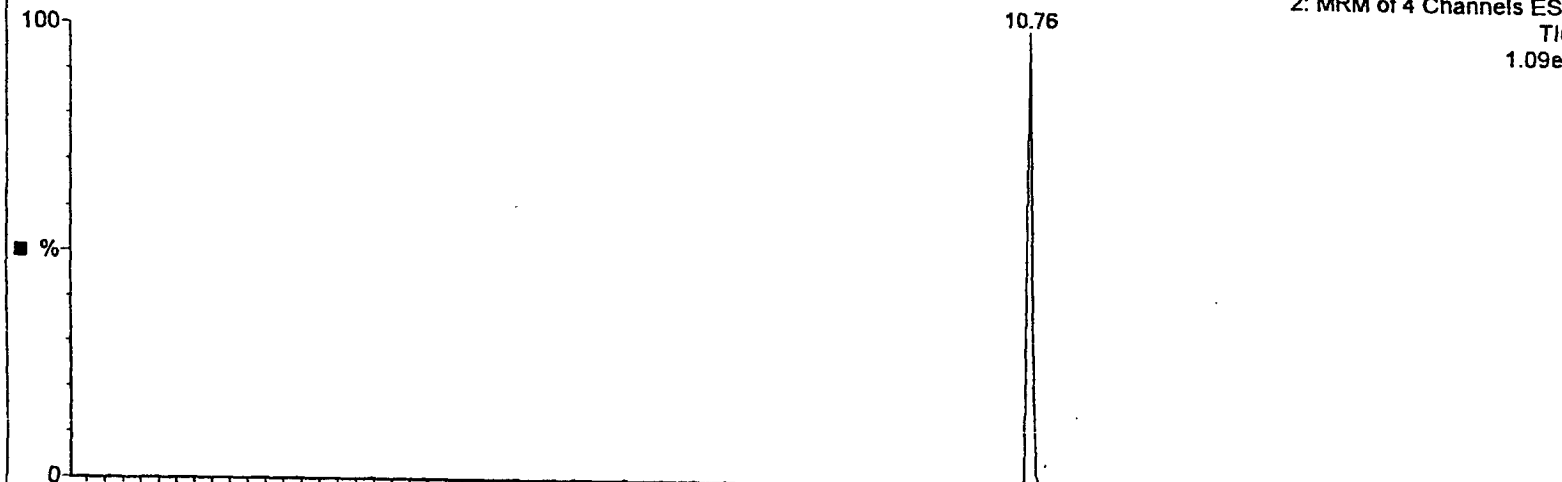
Davey070799

30-Oct-2002 15:12:19

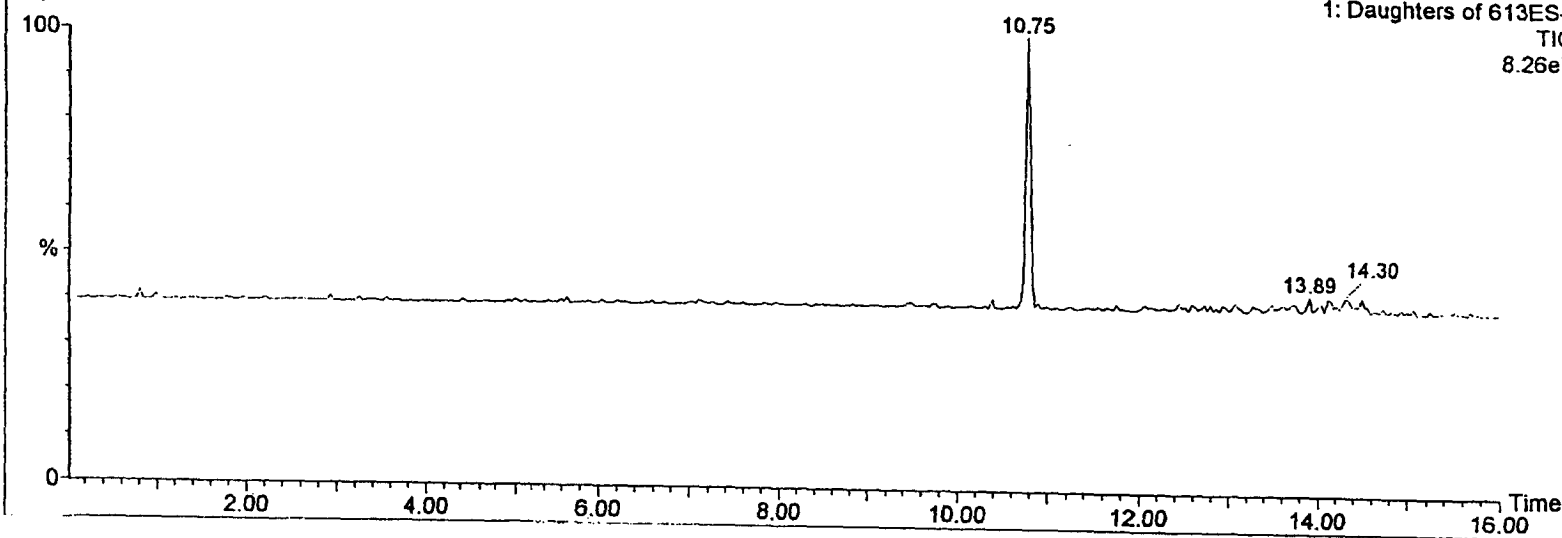
2: MRM of 4 Channels ES-

TIC

1.09e6



d021030014



1: Daughters of 613ES-

TIC

8.26e7

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Initial

Date

C12 ACID

02048-21-7 solvent standard 10ppb

3M Environmental Lab

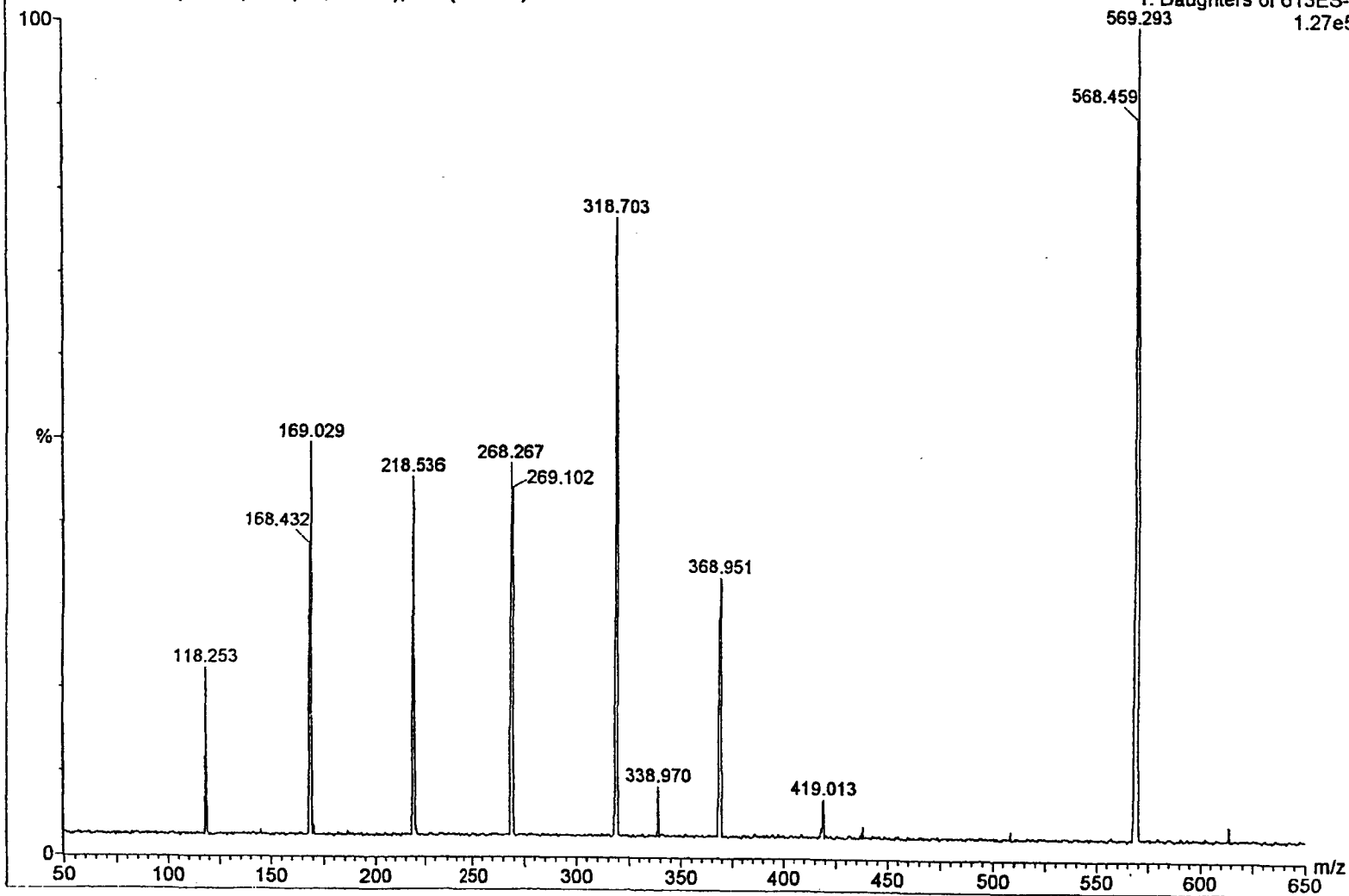
d021030014 312 (10.754) Sm (Mn, 2x1.00); Cm (309:318)

davey 070799

30-Oct-2002 15:12:19

1: Daughters of 613ES-

569.293 1.27e5



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CMC 11/1/02
initial Date

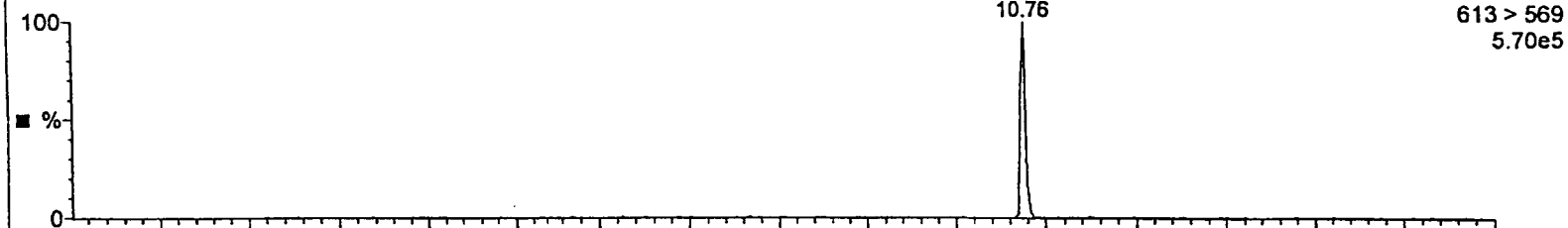
3M ENVIRONMENTAL LABORATORY
E02-1039

C17 ACID

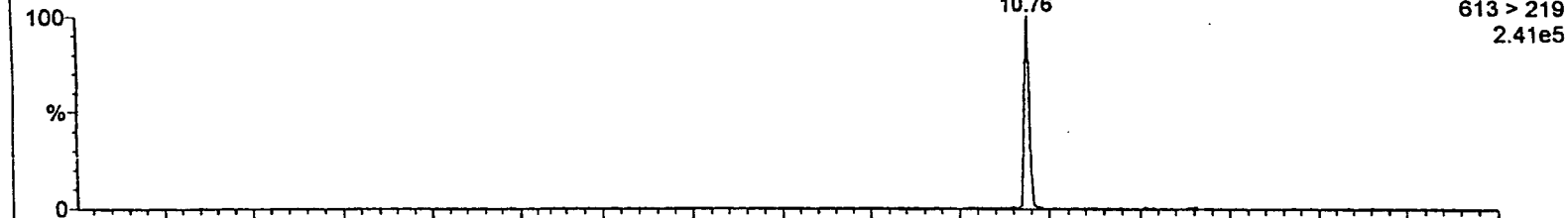
02048-21-7 solvent standard 10ppb

3M

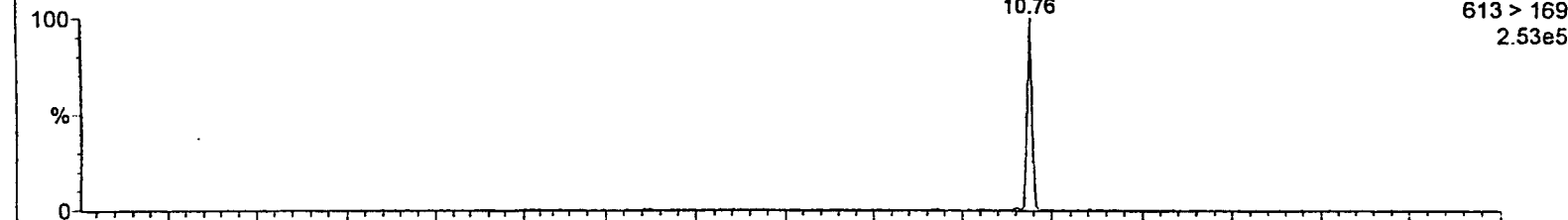
d021030014



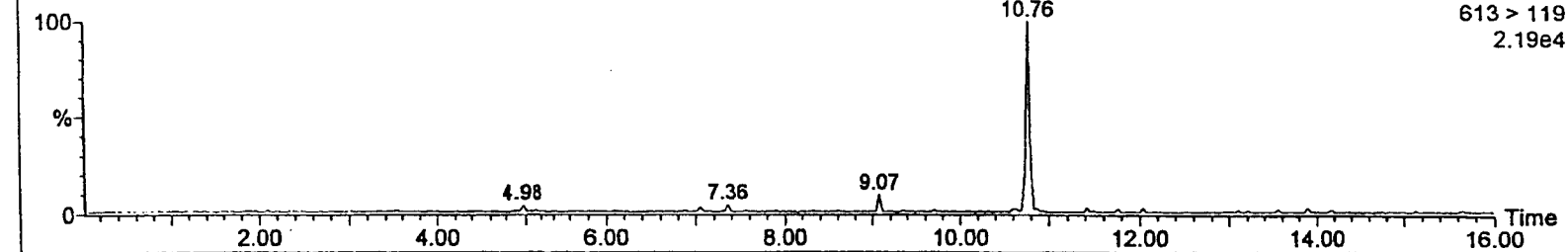
d021030014



d021030014



d021030014



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CMC 11/10
initial Date

C12 ACID

02048-21-7 solvent standard 10ppb

3M

d021030014

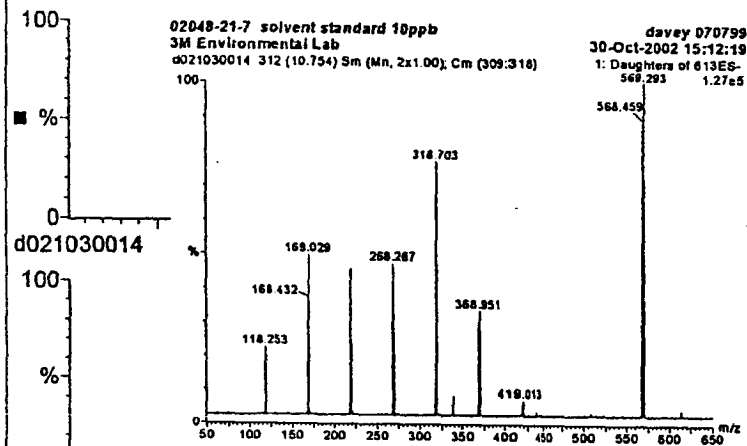
Davey070799

30-Oct-2002 15:12:19

2: MRM of 4 Channels ES-

613 > 569

5.70e5



d021030014

2: MRM of 4 Channels ES-

613 > 219

2.41e5

d021030014

2: MRM of 4 Channels ES-

613 > 169

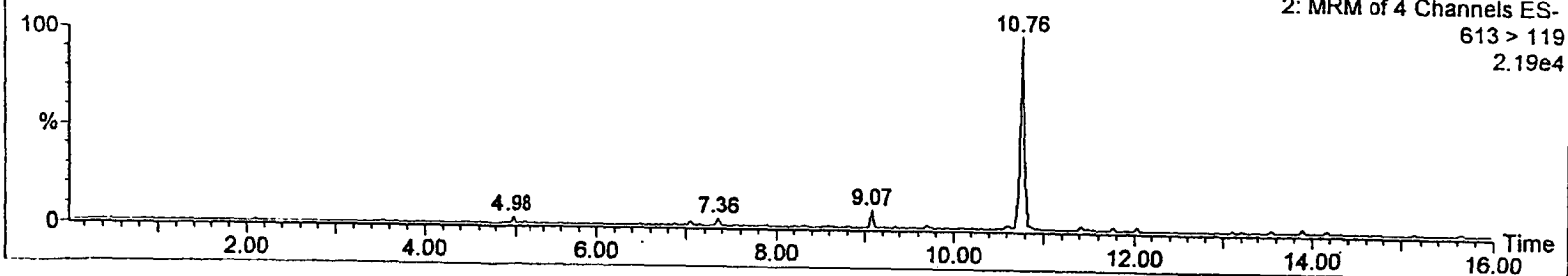
2.53e5

d021030014

2: MRM of 4 Channels ES-

613 > 119

2.19e4



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Initial Date

PFOS

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

3M

d021030008

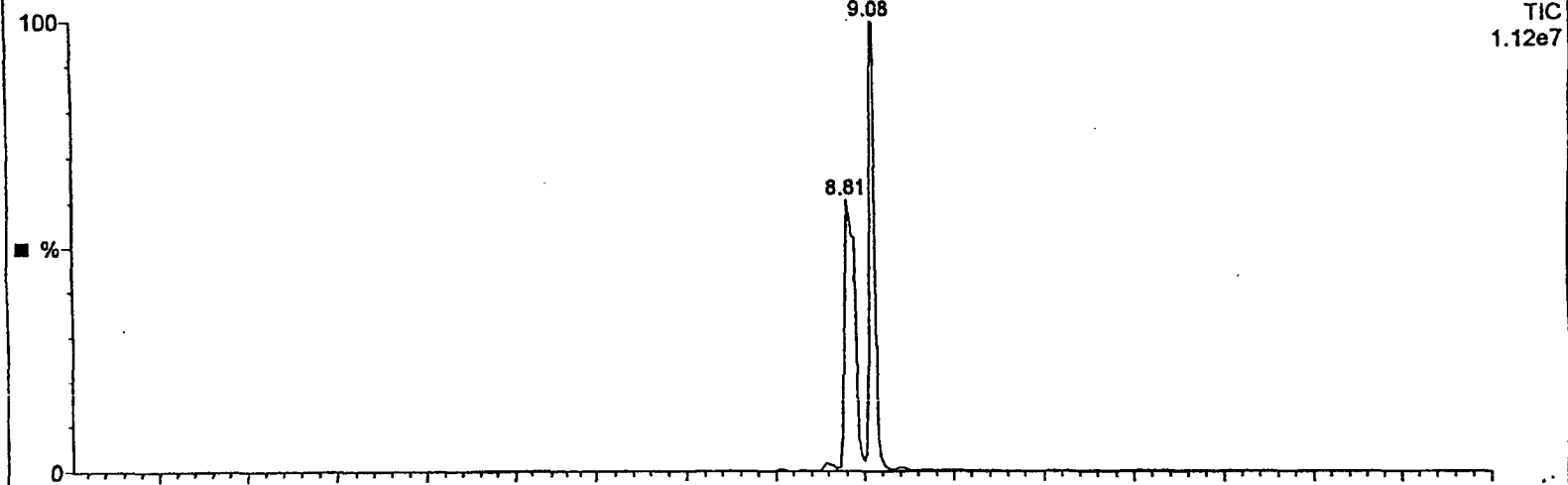
Davey070799

30-Oct-2002 13:29:23

2: MRM of 3 Channels ES-

TIC

1.12e7

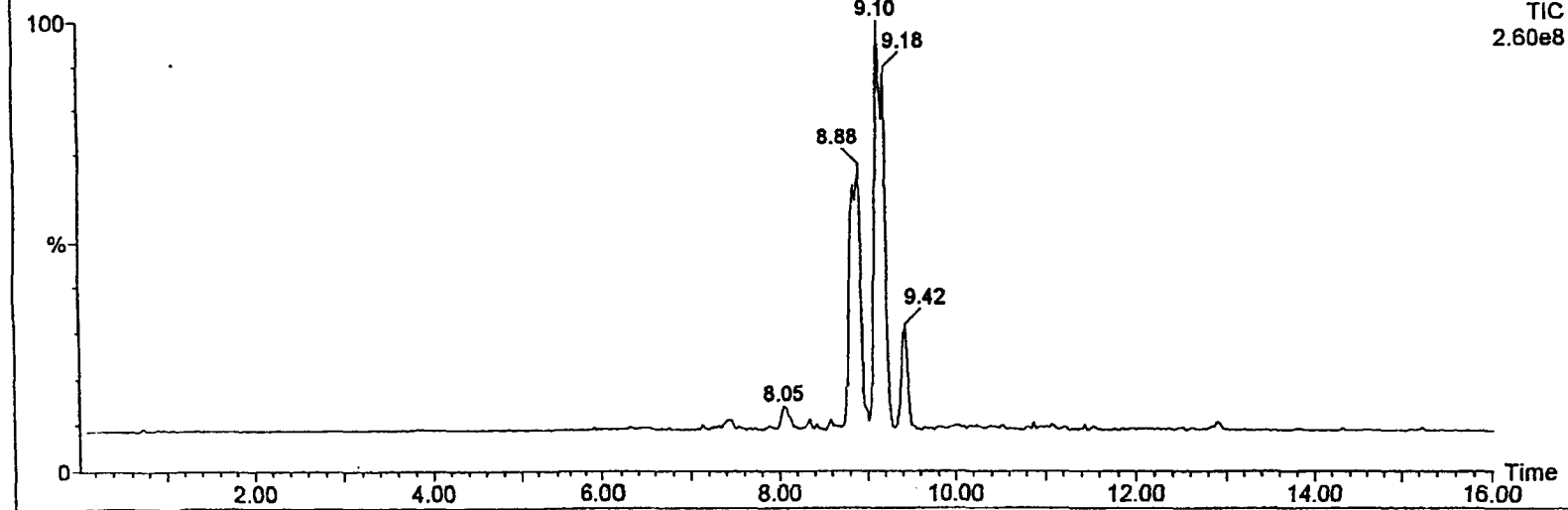


d021030008

1: Daughters of 499ES-

TIC

2.60e8



3M ENVIRONMENTAL LABORATORY
E02-1039

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CMC 10/31/02
Initial Date

PFOS

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

3M Environmental Lab

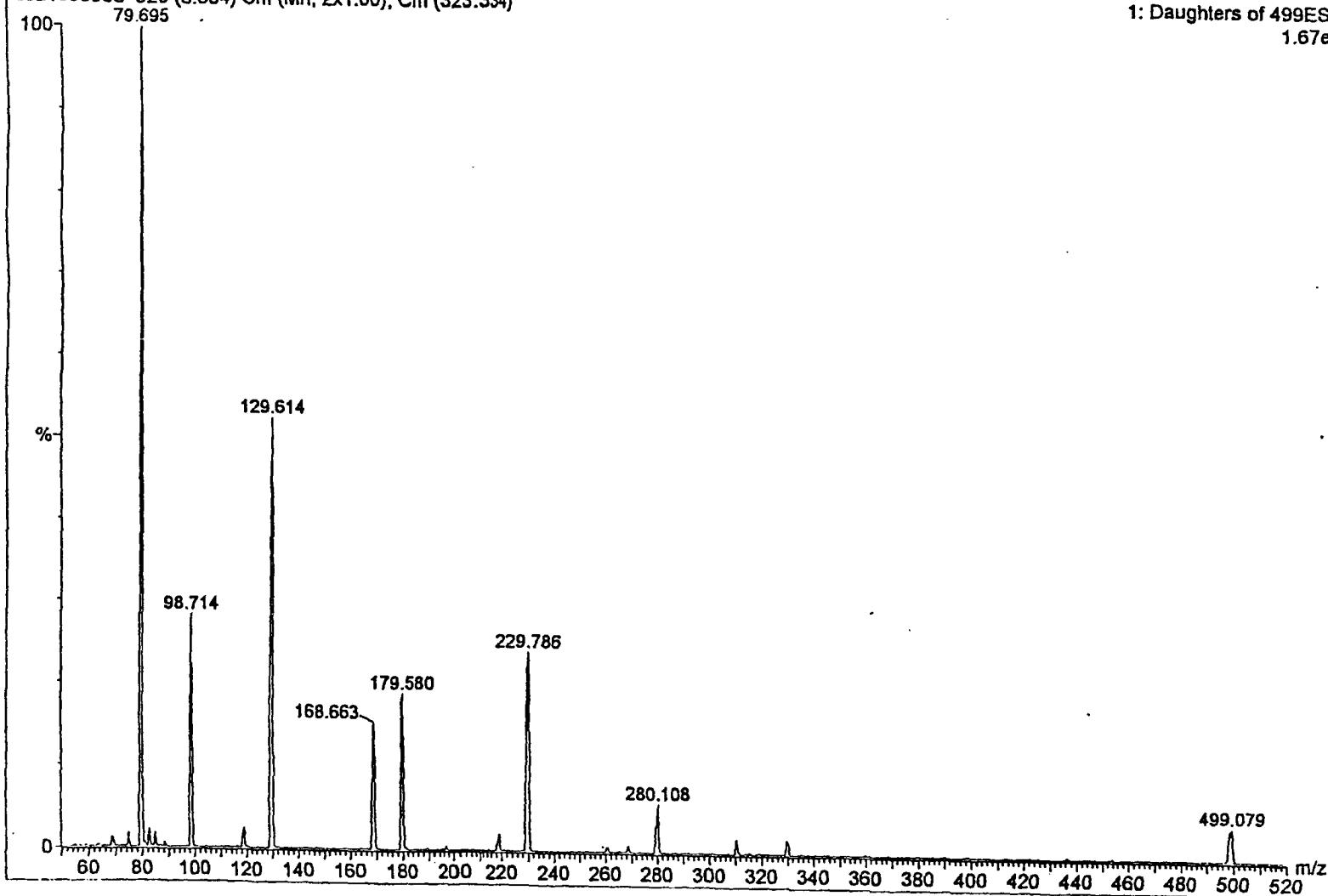
d021030008 329 (8.884) Sm (Mn, 2x1.00); Cm (323:334)

davey 070799

30-Oct-2002 13:29:23

1: Daughters of 499ES-

1.67e6



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Initial CML Date 10/31/02

DFOS

golden west plasma 5x scale up 10/23/02 ok
3M Environmental Lab

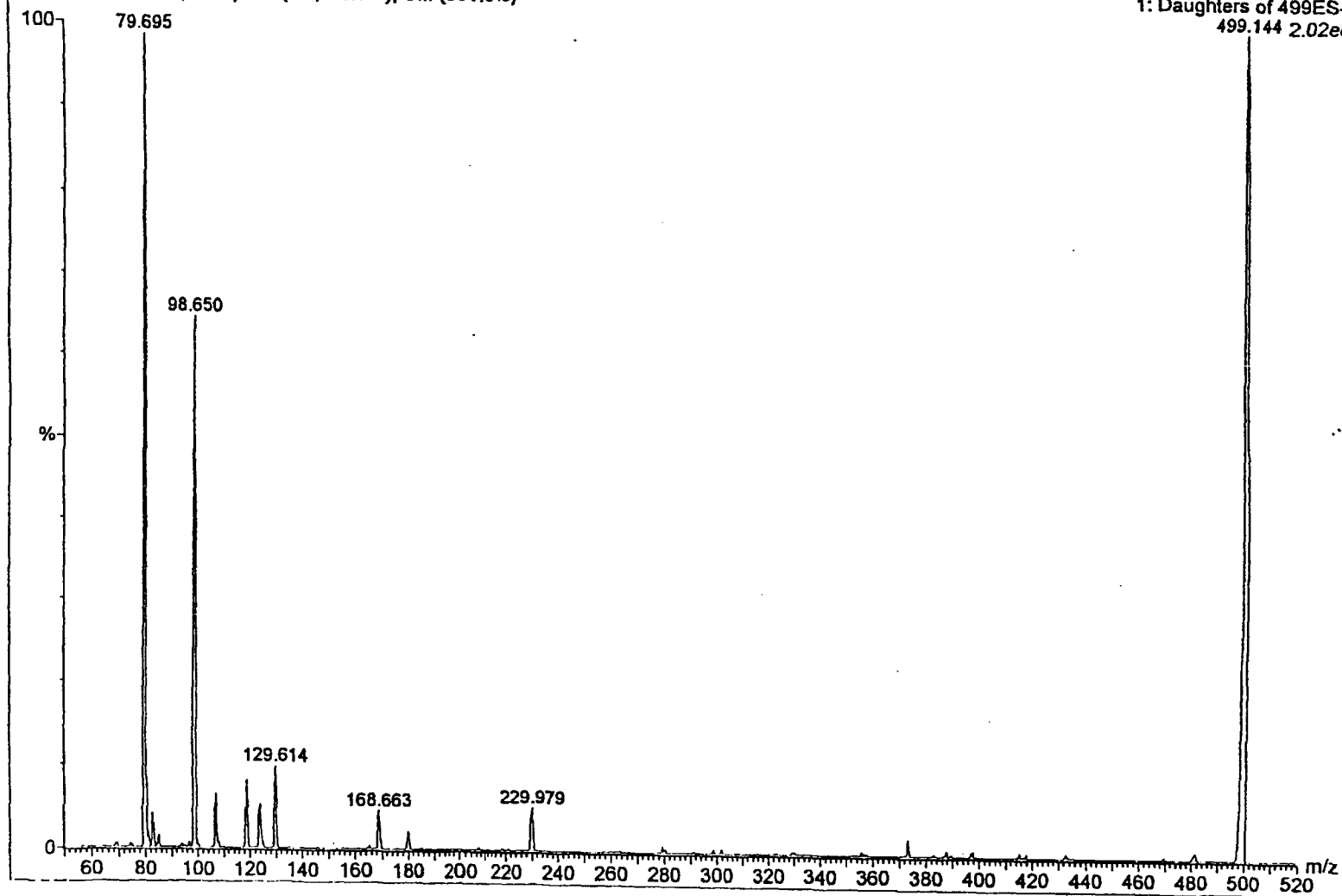
d021030008 337 (9.100) Sm (Mn, 2x1.00); Cm (334:343)

davey 070799

30-Oct-2002 13:29:23

1: Daughters of 499ES-

499.144 2.02e6



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Cmc 10/31/02
Initial Date

PFOS

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

3M Environmental Lab

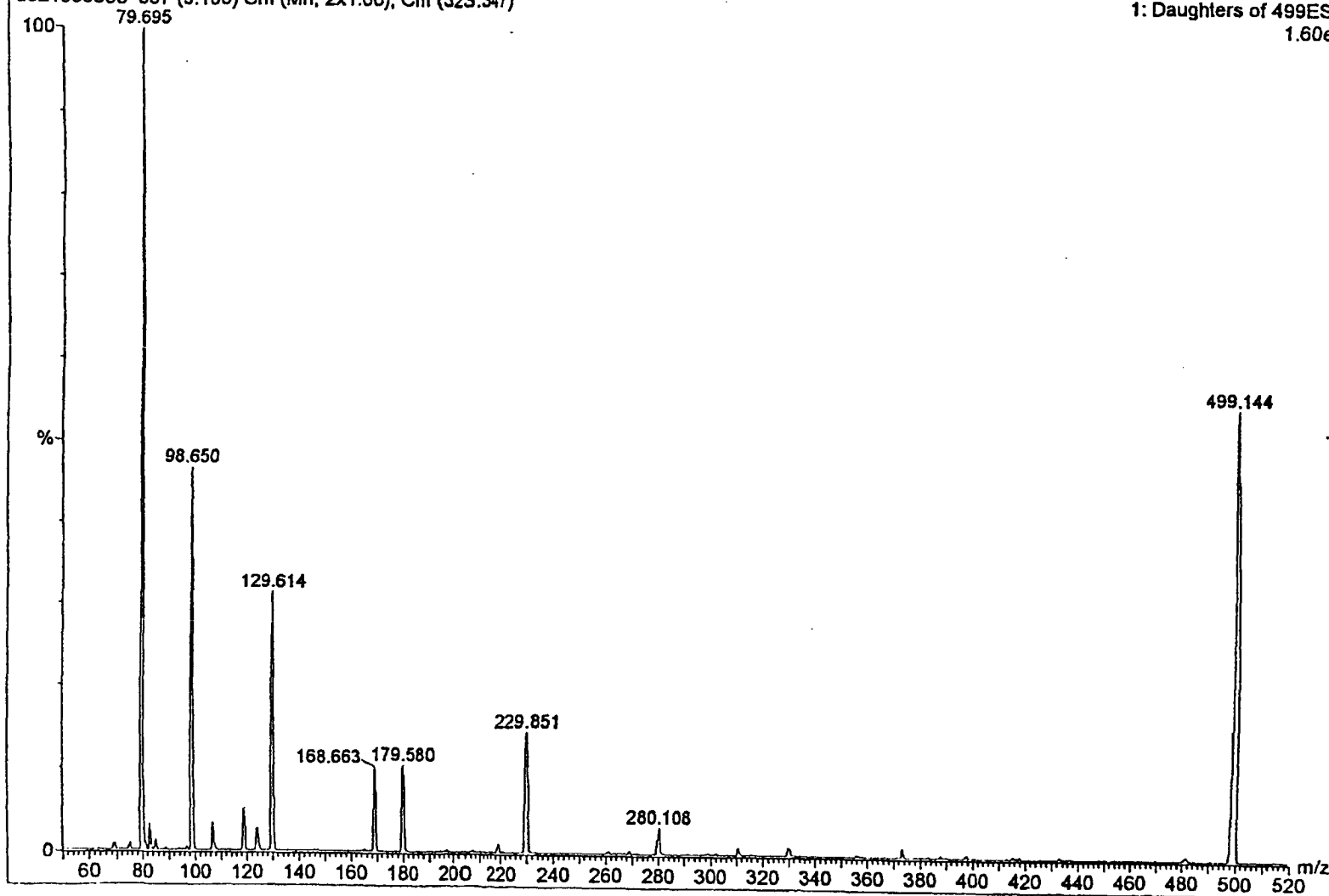
d021030008 337 (9.100) Sm (Mn, 2x1.00); Cm (323:347)

davey 070799

30-Oct-2002 13:29:23

1: Daughters of 499ES-

1.60e6



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CML 10/31/02
Initial Date

DFOS

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

3M

d021030008

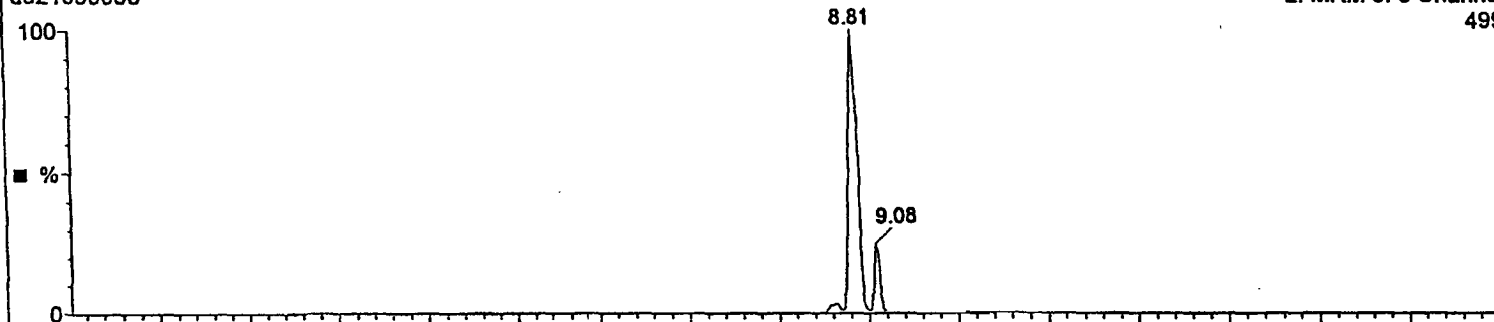
Davey070799

30-Oct-2002 13:29:23

2: MRM of 3 Channels ES-

499 > 130

2.45e6

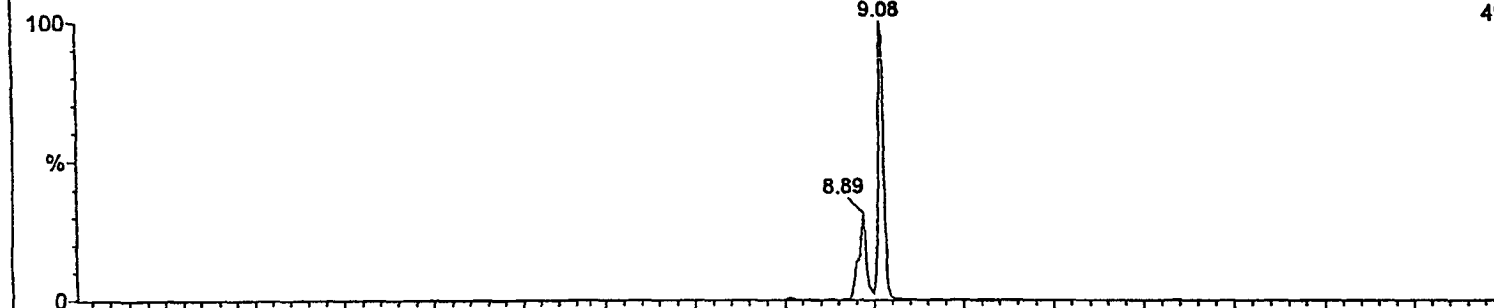


d021030008

2: MRM of 3 Channels ES-

499 > 99

4.19e6

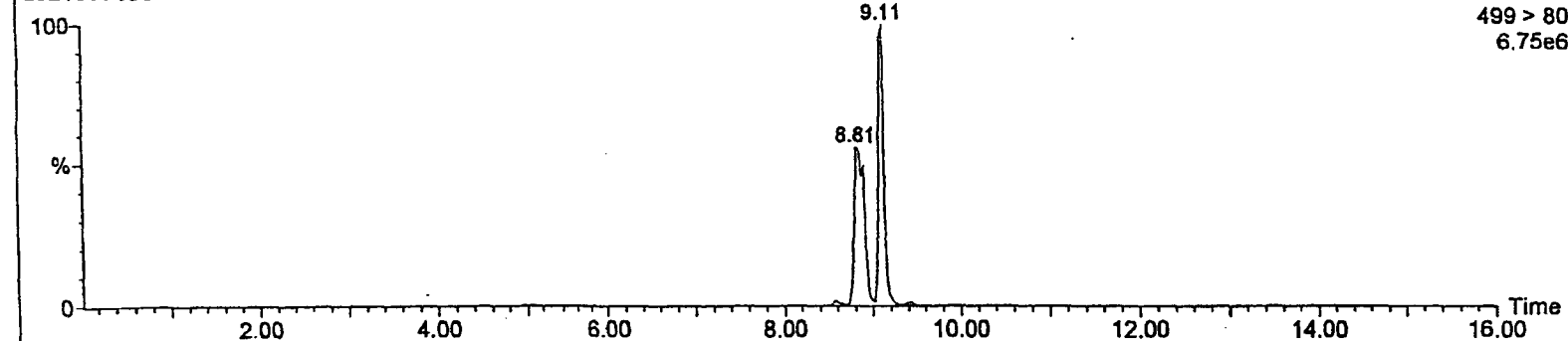


d021030008

2: MRM of 3 Channels ES-

499 > 80

6.75e6



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Initial CNC Date 10/31/02

PFOS

golden west plasma 5x scale up 10/23/02 ok
3M

Davey070799

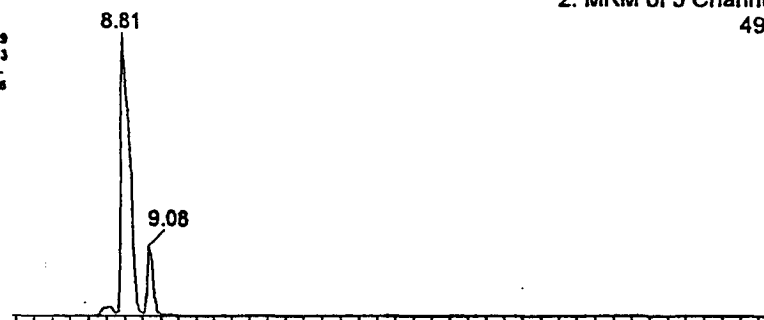
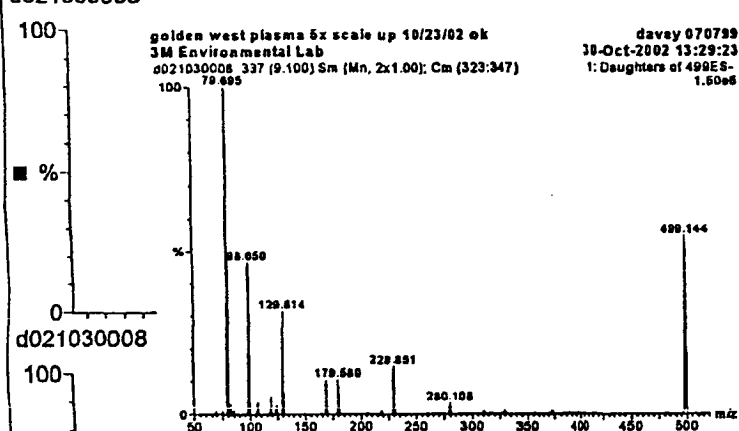
30-Oct-2002 13:29:23

2: MRM of 3 Channels ES-

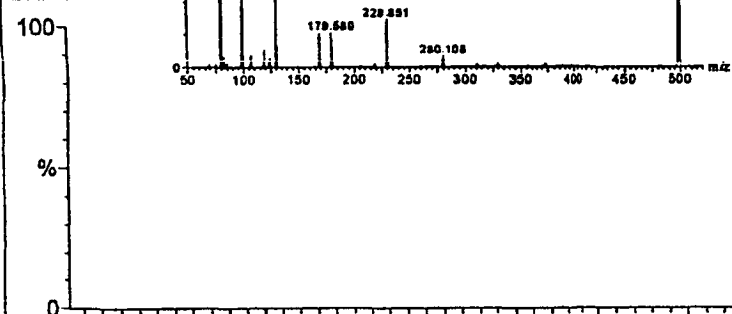
499 > 130

2.45e6

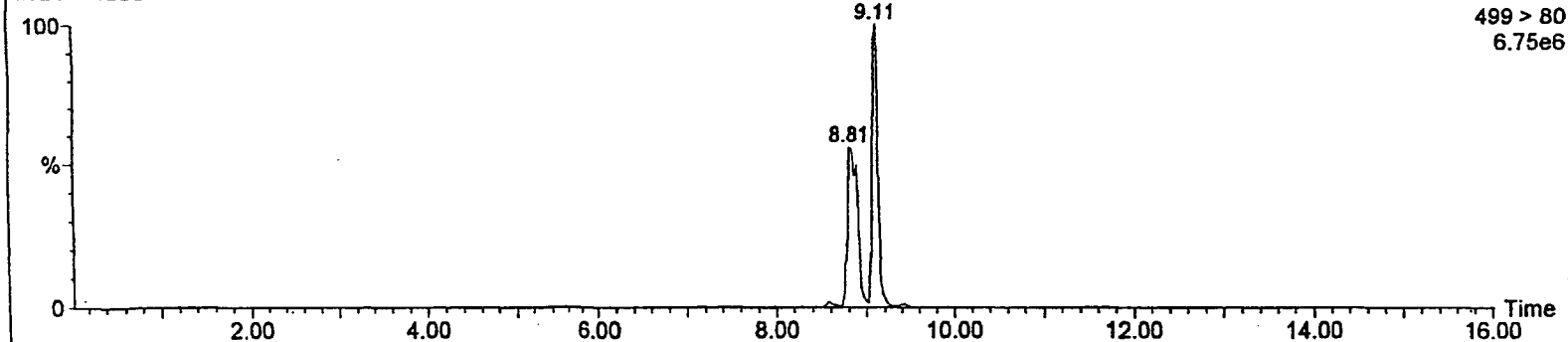
d021030008



d021030008



d021030008



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cmc 10/31/02
Initial Date

D FOS

02048-21-7 solvent standard 10ppb

3M

d021030018

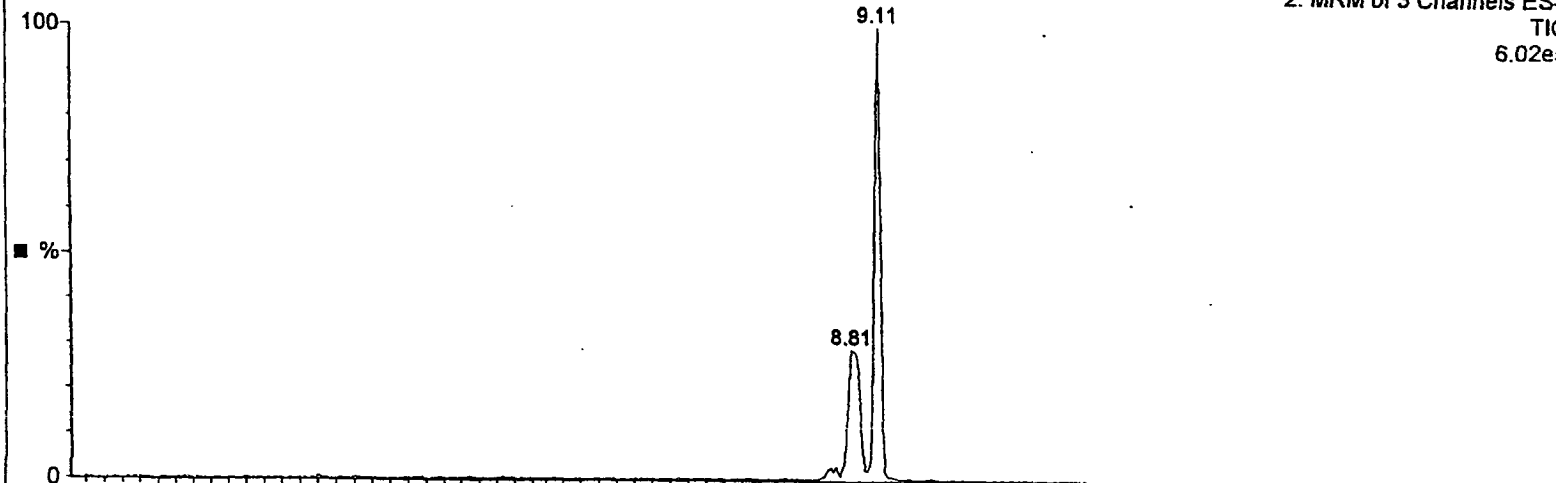
Davey070799

30-Oct-2002 16:20:35

2: MRM of 3 Channels ES-

TIC

6.02e5

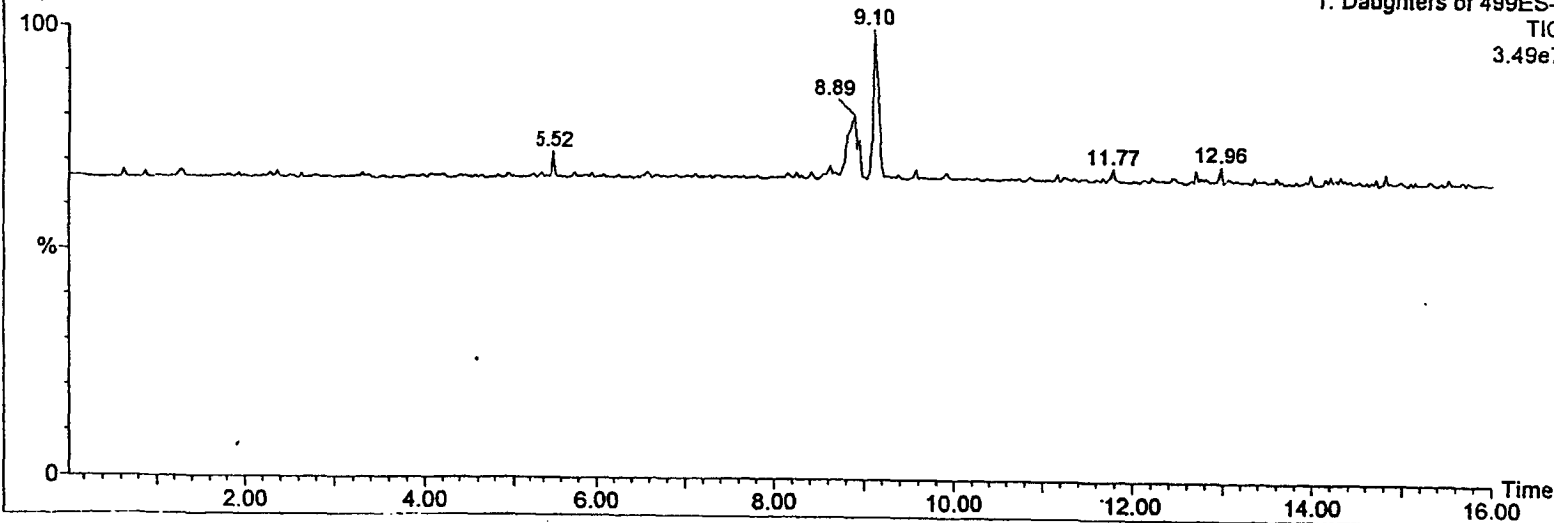


d021030018

1: Daughters of 499ES-

TIC

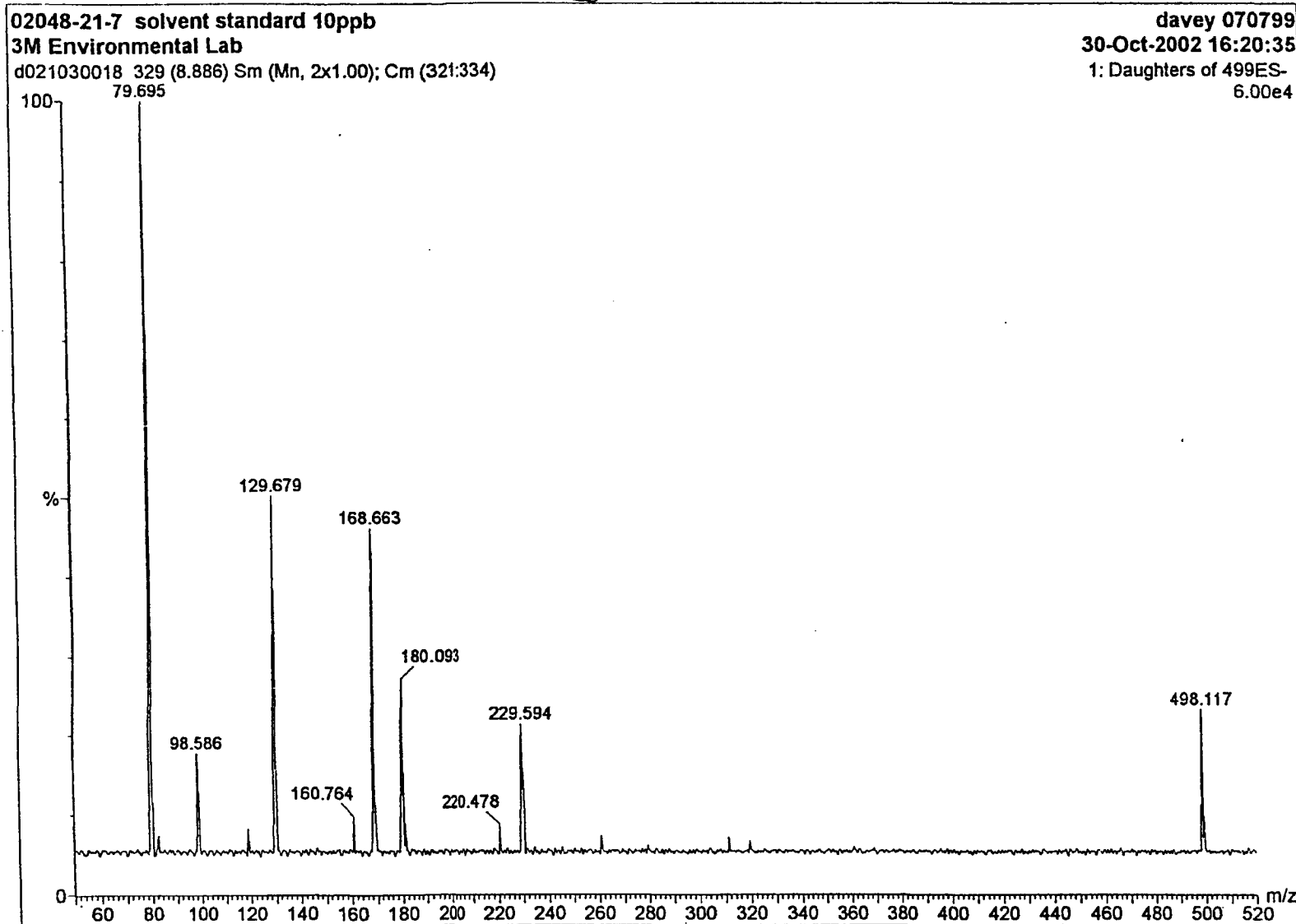
3.49e7



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Initial CMK Date 11/11/02

PFOS



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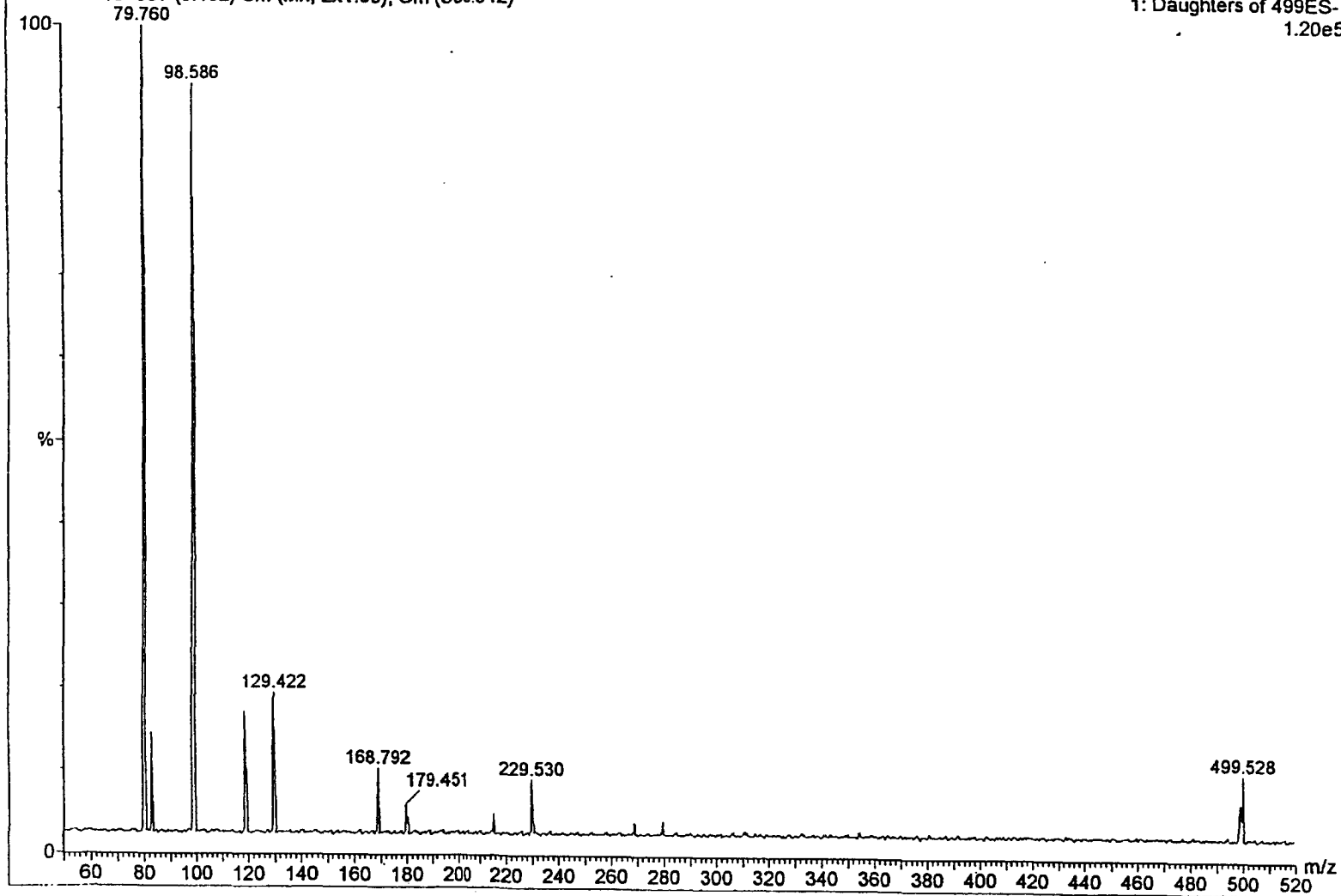
cm 11/1/02
Initial Date

PFOS

02048-21-7 solvent standard 10ppb
3M Environmental Lab

d021030018 337 (9.102) Sm (Mn, 2x1.00); Cm (335:342)

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



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CME 11/1/02
Initial Date

PROS

02048-21-7 solvent standard 10ppb

3M Environmental Lab

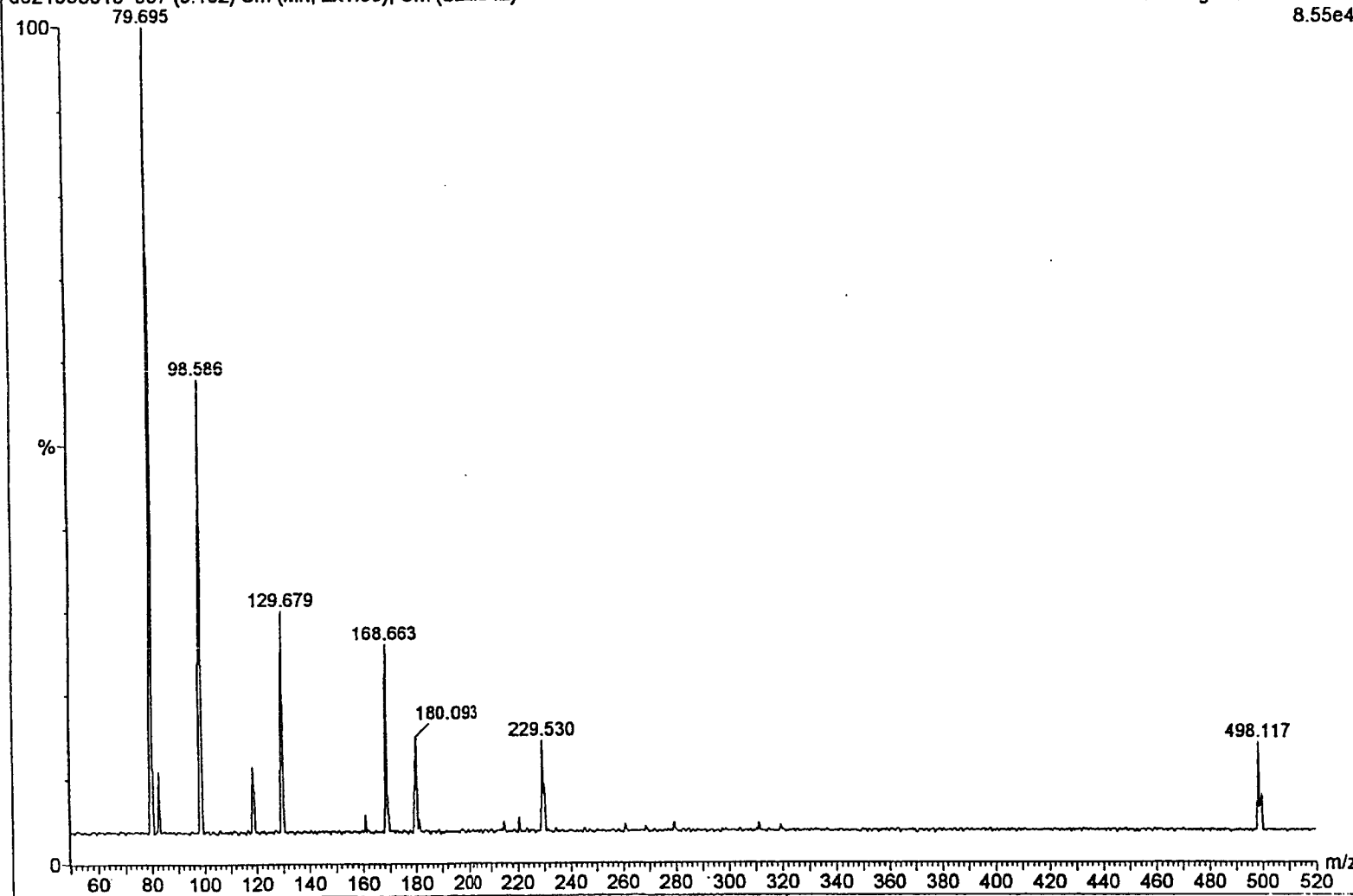
d021030018 337 (9.102) Sm (Mn, 2x1.00); Cm (322:342)

davey 070799

30-Oct-2002 16:20:35

1: Daughters of 499ES-

8.55e4



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CMC 11/1/02
Initial Date

PFOS

02048-21-7 solvent standard 10ppb

3M

d021030018

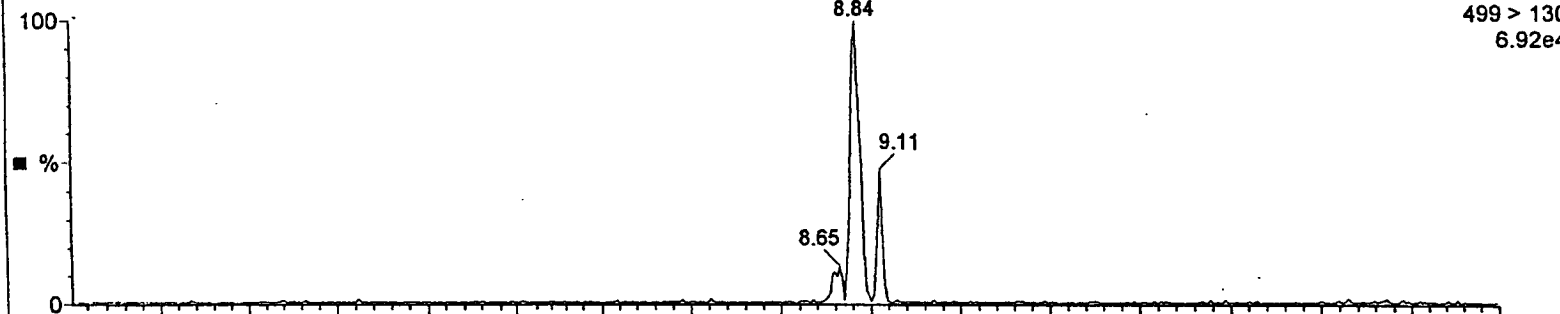
Davey070799

30-Oct-2002 16:20:35

2: MRM of 3 Channels ES-

499 > 130

6.92e4

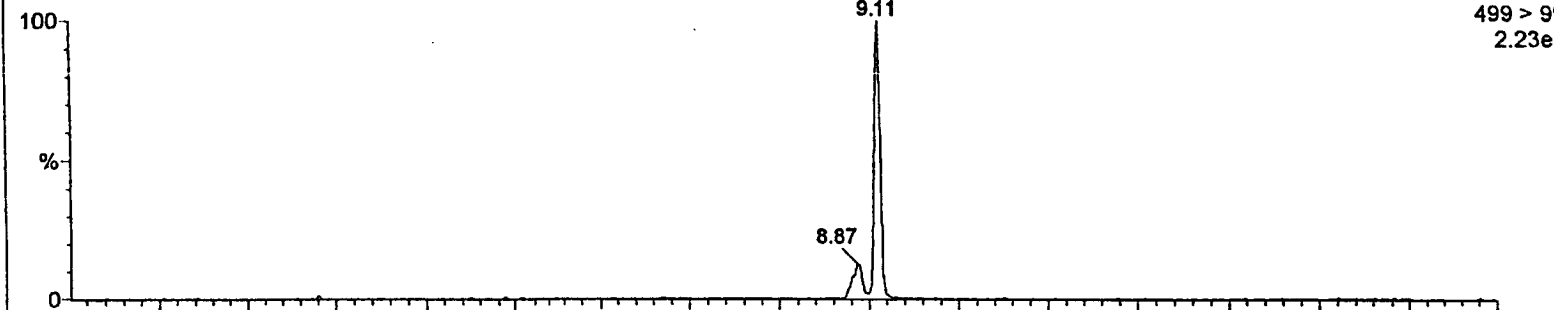


d021030018

2: MRM of 3 Channels ES-

499 > 99

2.23e5

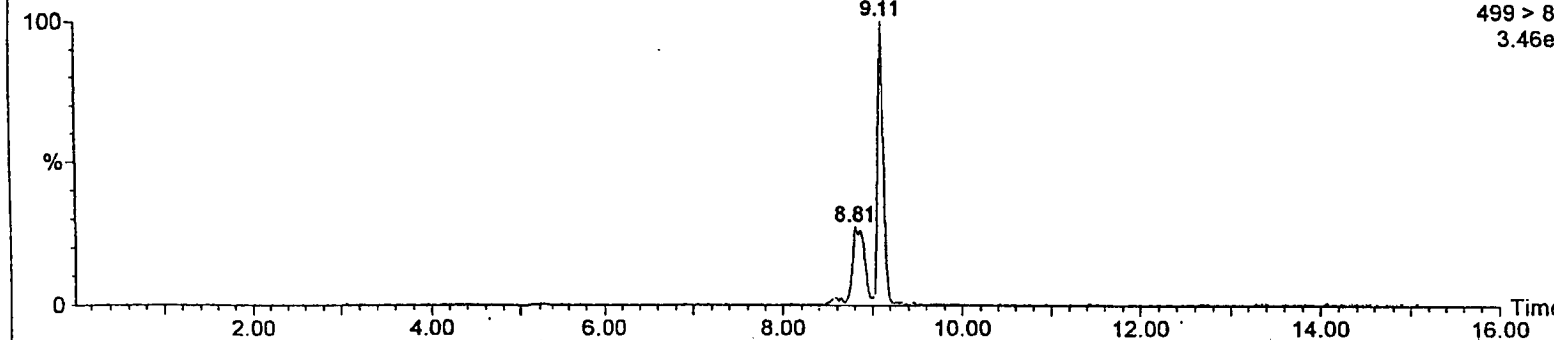


d021030018

2: MRM of 3 Channels ES-

499 > 80

3.46e5



Copy of Original

CMC 11/10
Initial Date

DFCS

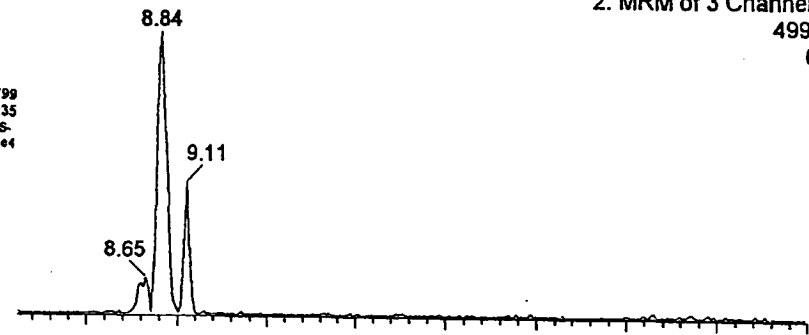
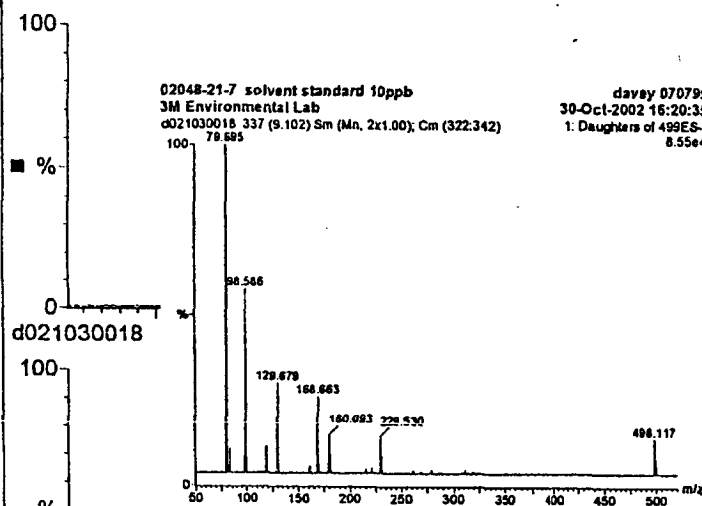
02048-21-7 solvent standard 10ppb
3M

Davey070799

30-Oct-2002 16:20:35

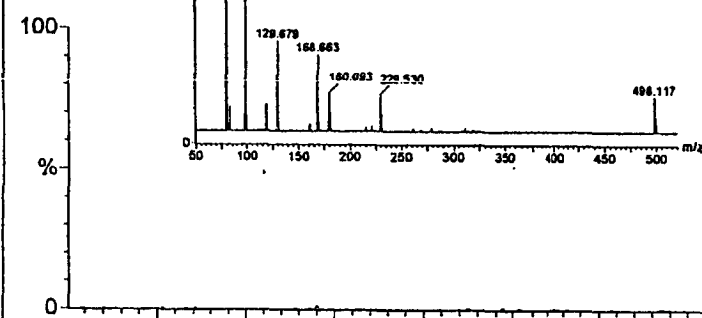
2: MRM of 3 Channels ES-
499 > 130
6.92e4

d021030018



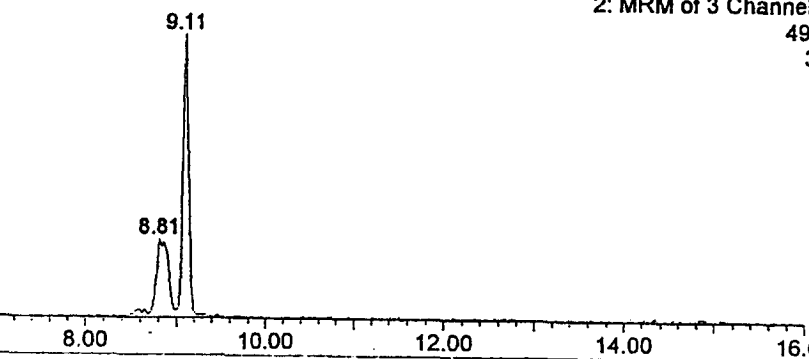
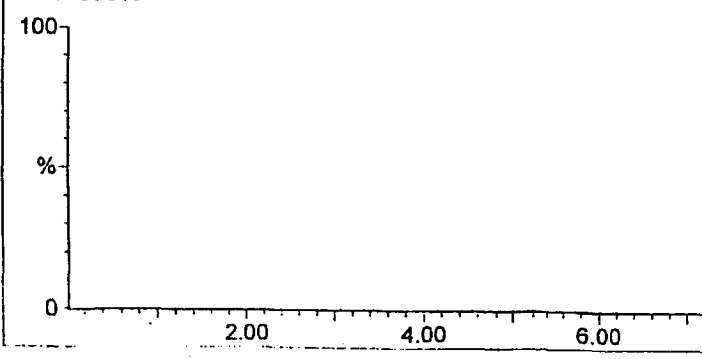
2: MRM of 3 Channels ES-
499 > 99
2.23e5

d021030018



2: MRM of 3 Channels ES-
499 > 80
3.46e5

d021030018



3M ENVIRONMENTAL LABORATORY
E02-1039

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Initial *LMC* Date *11/1/02*

ATTACHMENT D: PREP SHEETS AND TRACEABILITY INFORMATION

Non GLP SPE Columns Extraction Worksheet

Prep Date:
Analysts initials:

10/11/2002
OK

Method Revision: ETS-231
Study Number: E 02-1039

ETS 2231 10/11/02

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 of 02050-14	2 ml of MeOH TN-A-6310	TCR-889
Sigma serum MS-5ppb		400 ml	Waters, 1g, 6ml	100ul 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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CMK 10/31/02
Initial Date

Additional comments: ACN IN A-6194 CK 10/11/02

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 W/RE #041696 CENTRIFUGE NH 3 E600P# 02112355

Non GLP SPE Columns Extraction Worksheet

Prep Date:
Analysts initials:

MEC 10/24/02 or 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-874
Chinese plasma curve-0.25ppb	400 ml	Waters, 1g, 6ml	5ul of 02050-14	2 ml of MeOH TN-A-6310	TCR-874
Chinese plasma curve-0.5ppb	400 ml	Waters, 1g, 6ml	10ul of 02050-14	2 ml of MeOH TN-A-6310	TCR-874
Chinese plasma curve-0.75ppb	400 ml	Waters, 1g, 6ml	15ul of 02050-14	2 ml of MeOH TN-A-6310	TCR-874
Chinese plasma curve-1ppb	400 ml	Waters, 1g, 6ml	20ul of 02050-14	2 ml of MeOH TN-A-6310	TCR-874
Chinese plasma curve-2.5ppb	400 ml	Waters, 1g, 6ml	50ul of 02050-14	2 ml of MeOH TN-A-6310	TCR-874
Chinese plasma curve-5ppb	400 ml	Waters, 1g, 6ml	100ul of 02050-14	2 ml of MeOH TN-A-6310	TCR-874
Chinese plasma curve-10ppb	400 ml	Waters, 1g, 6ml	200ul of 02050-14	2 ml of MeOH TN-A-6310	TCR-874
Chinese plasma curve-30ppb	400 ml	Waters, 1g, 6ml	600ul of 02050-14	2 ml of MeOH TN-A-6310	TCR-874
Chinese plasma blank-1	400 ml	Waters, 1g, 6ml	NA	2 ml of MeOH TN-A-6310	TCR-874
Chinese plasma blank-2	400 ml	Waters, 1g, 6ml	NA	2 ml of MeOH TN-A-6310	TCR-874
Chinese plasma MS-0.5ppb	400 ml	Waters, 1g, 6ml	10ul of 02050-14	2 ml of MeOH TN-A-6310	TCR-874
Chinese plasma MS-5ppb	400 ml	Waters, 1g, 6ml	100ul of 02050-14	2 ml of MeOH TN-A-6310	TCR-874
Lampire Plasma blank-1	400 ml	Waters, 1g, 6ml	NA	2 ml of MeOH TN-A-6310	TCR-885
Lampire Plasma blank-2	400 ml	Waters, 1g, 6ml	NA	2 ml of MeOH TN-A-6310	TCR-885
Lampire plasma MS-0.5ppb	400 ml	Waters, 1g, 6ml	10ul	2 ml of MeOH TN-A-6310	TCR-885
Lampire plasma MS-5ppb	400 ml	Waters, 1g, 6ml	100ul	2 ml of MeOH TN-A-6310	TCR-885
Golden West Plasma blank-1	400 ml	Waters, 1g, 6ml	NA	2 ml of MeOH TN-A-6310	TCR-884
Golden West Plasma blank-2	400 ml	Waters, 1g, 6ml	NA	2 ml of MeOH TN-A-6310	TCR-884
Golden West Plasma MS-0.5ppb	400 ml	Waters, 1g, 6ml	10ul of 02050-14	2 ml of MeOH TN-A-6310	TCR-884
Golden West Plasma MS-5ppb	400 ml	Waters, 1g, 6ml	100ul of 02050-14	2 ml of MeOH TN-A-6310	TCR-884
Innovative Research Plasma blank-1	400 ml	Waters, 1g, 6ml	NA	2 ml of MeOH TN-A-6310	TCR-883
Innovative Research Plasma blank-2	400 ml	Waters, 1g, 6ml	NA	2 ml of MeOH TN-A-6310	TCR-883
Innovative Research Plasma MS-0.5ppb	400 ml	Waters, 1g, 6ml	10ul of 02050-14	2 ml of MeOH TN-A-6310	TCR-883
Innovative Research Plasma MS-5ppb	400 ml	Waters, 1g, 6ml	100ul of 02050-14	2 ml of MeOH TN-A-6310	TCR-883

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;

Exact Copy of Original

Circ 10/31/02
Initial Date

Additional comments:

CHPT. FUGA AND # 02118855: elution: VWS SC: SER# 091694

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

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.

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LME Initial 11/11/02
Date

Recorded By:

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)	☐ Y ☒ 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)	

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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

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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)	Y 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 11/1/02
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
-	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

Cinc 11/1/02
Initial Date

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	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 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	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

CAC 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

Exact Copy of Original
Cmc 11/1/02
 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
-	8ml	-	E02-1039, validation	na	na	OK	10/10/2002

Exact Copy of Original

CMC 11/1/02
Initial Date

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)	☐ Y ☒ 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

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
CNC 11/1/02
 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 11/1/02
Initial Date

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 assigned 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 11/1/02
 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
-	10ml	-	E02-1039	-	-	OK	10/23/2002

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

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 11/1/02
Initial Date

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 11/1/02
Initial Date

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 11/1/02
Initial Date

Test Control and Reference Substance Log

Substance trade name or reference #	Pooled Human Serum from Golden 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

CMLC 11/1/02
Initial Date

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
-	10ml	-	E02-1039	-	-	OK	10/23/2002

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

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

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

Standard curve tracking sheet

Prep date: 10/7/2002
 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: Sera SPE validation
 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	10.38	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

[Handwritten Initials] *[Handwritten Date]*
 Initial Date

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	1268	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 K. Jain

Verified by: Cindy Carlson 10/31/02

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

SINGLE COMPONENT PREPARATION LOG

Date: 10.10.02

Book No. 02 050

Analyst: OKJ

Page No. 14

Description: 100 ppb SUPER MIX FOR SPE

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

LHC 10/31/02
Initial Date

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

SINGLE COMPONENT PREPARATION LOG

Date: 10/10/02
Analyst: OK
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: NA
Solvent and TN-A Number: MeOH / TN-A 6310
Final Volume: 25 ml
Final Concentration: 0.75 ppb
Storage Location: 2nd fl.
Expiration Date: 11/13/02

Exact Copy of Original

cmc 10/31/02
Initial Date

Reviewed by:

Signature

Date

SINGLE COMPONENT PREPARATION LOG

Date: 10/10/02 Book No. 02 050
Analyst: OK 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 10/10/02
50 ppb Other Correction Factors: NA
Corrected Weight: NA 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: 11/13/02

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CMX 10/31/02
Initial Date

Reviewed by:

Signature

Date

MULTI-LEVEL PREPARATION LOG

Description: CURVE FOR SEPA SPE - SUPERMIX
 Date Prepared: 10-6-02 10:02
 Prepared By: OK
 Storage Location: 2nd fl.
 Expiration Date: 11-13-02
 Solvent: MeOH
 3M Trace #: TN-A-G-002

Book No. 02 048
 Page No. 21

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				pplb	0.1	0.5	1	2.5	5	7.5	10	15

Component Standards				Amount of Component Standard Added μ l								
Description	ID #	Conc	Expiration Date	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7	Std 8	
SUPERMIX FOR SEPA SPE	02002-63	100ppb	11-13-02	5	25	50	125	250	375	500	750	
						OK						
						10-7-02						
Final Volume				μ l	25							
Final Concentration				ppb	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				pplb	20	30					
Component Standards				Amount of Component Standard Added μ l							
Description	ID #	Conc	Expiration Date	Std 9	Std 10	Std 11	Std 12	Std 13	Std 14	Std 15	Std 16
SUPERMIX FOR SEPA SPE	02002-63	100ppb	11-13-02	1000	1600						
		OK									
		10-7-02									
Final Volume				25							
Final Concentration				ppb	20	30					

Reviewed by:

Mark E. [Signature] 10/11/02

3M ENVIRONMENTAL LABORATORY
 E02-1039

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 Initial *Line*
 Date 10/31/02

MULTI-COMPONENT PREPARATION LOG

Book No. 02 002

Page No. 63

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

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

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 µl	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 PPM	2-26-03	12.5 µl			501
PFOS	02040-56	1048 PPM	11-15-02	12 µl			503
Final Volume				µl	25	Approximate Concentration of the mix	500 ppm

OK
10602

Reviewed by:

Signature

Date

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

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)	☒ Y ☐ N
Retain	0.3994 g	Date of Retain	02/15/2000
☐ 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:	C6F13COOH		
Comments	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 Shipping comments: Corrosive, No 3M ID# - must ship ground small quantity exception (30 mL/g or less per vial). LAS 10/02/02		
Attachment(s)	  tcr99131-25msds.pdf tcr99131-25cofa.pdf		

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CML 10/31/02
Initial Date

CERTIFICATE OF ANALYSIS

3M ENVIRONMENTAL
SCOTT POST
451 778 5890

PO NBR:

PRODUCT NUMBER: 34204-1

LOT NUMBER: 07219EU

PRODUCT NAME: TRIDECAFLUOROHEPTANOIC ACID, 99%

FORMULA: C₇HF₁₃O₂

FORMULA WEIGHT: 364.06

APPEARANCE

WHITE CRYSTALLINE SOLID

INFRARED SPECTRUM

CONFORMS TO STRUCTURE AND STANDARD AS
ILLUSTRATED ON PAGE 798B OF EDITION 1,
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

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suitability of the product for its particular use. See reverse side of invoice
or packing slip for additional terms and conditions of sale.

015 HKEY-11/02

** TOTAL PAGE.04 **

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

Exact Copy of Original

Cmc 10/31/02
Initial Date

SINGLE COMPONENT PREPARATION LOG

Date: 8/29/02 Book No. 02 040
Analyst: RWJ 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: Chris A. Stevenson 10/01/02
Signature Date

SINGLE COMPONENT PREPARATION LOG

Date: 8/29/02 Book No. 02 040
Analyst: RWJ Page No. 58
Description: Tridecafluoroheptanoic Acid (TDHA)
Stock Number: 99131-25
Weight or Volume Used: 0.0625g 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^{3 @ 100-100/102} 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

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 ^{19}F -NMR Spectroscopy
Date: October 30, 2002

SAMPLE DESCRIPTION:

- TCR-99131-025, lot PU/07219EU from the Telomer project.
Nominal product = $\text{CF}_3(\text{CF}_2)_n\text{-CO}_2\text{H}$, where average $n \approx 5$ (white solid).

Sample	Spectra #'s	Experiment Descriptions
TCR-99131-025	H71638.GID.401	400 MHz 1D ^1H -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 ^{19}F -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 CFCl_3 /deuterated acetone (acetone- d_6) solvent mixture for subsequent analysis by NMR. Initial one-dimensional (1D) 400 MHz ^1H -NMR and 376 MHz ^{19}F -NMR spectra were acquired at room temperature using a Varian UNITYplus 400 FT-NMR spectrometer. A two-dimensional (2D) ^{19}F -NMR correlated spectroscopy (COSY) experiment was also acquired using a Varian UNITY INOVA 500 FT-NMR spectrometer to facilitate assignment of ^{19}F signals associated with various impurity components. This sample preparation method permitted the p-HFX to be used as either 1) a $^1\text{H}/^{19}\text{F}$ -NMR internal standard to allow the calculation of the absolute weight percent concentrations of specific components, or 2) a $^1\text{H}/^{19}\text{F}$ -NMR cross integration standard to permit the cross correlation of the relative ^1H and ^{19}F signal intensities for evaluation of the overall sample composition.

RESULTS:

The ^1H -NMR and ^{19}F -NMR spectral data indicated this sample was a relatively high purity form of the nominal product, $\text{CF}_3(\text{CF}_2)_n\text{-CO}_2\text{H}$, where the average value of $n = 5.05$. Small amounts of a few impurity components, including probable isomers, were also assigned. A $^1\text{H}/^{19}\text{F}$ -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 $^1\text{H}/^{19}\text{F}$ -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.

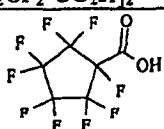
Tom Kestner

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

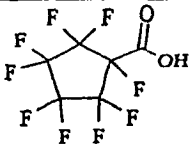
October 30, 2002

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:	C ₈ H ₁₅ O ₂
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

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: 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

Exact Copy of Original

CINC 11/31/02
Initial Date

Yumei Yang
Yumei Yang
QC Manager

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

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: M.OH/TN-A-6243

Final Volume: 50ml

Final Concentration: 1230ppm

Storage Location: 2nd Floor - Room Temp.

Expiration Date: 2/15/03

Exact Copy of Original

CRC 10/31/02
Initial Date

Reviewed by: J. A. Stevenson 08/15/02
Signature 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 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.

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 Reagan

File Reference: ls71638.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 $^1\text{H}/^{19}\text{F}$ -NMR Cross Integration and LC/MS Analyses

Component Structures ¹	NMR Relative Weight% Concentrations (single trial measurement)
$\text{CF}_3(\text{CF}_2)_n\text{-CO}_2\text{H}$ where average $n = 6.02$ by ^{19}F -NMR. LC/MS showed $n=6$ (major), $n=5$, $n=4$ (minors).	99.52%
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 Initial 10/31/02 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


 Initial Date 10/31/02

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)	Y () 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:	C ₉ H ₉ F ₁₇ O ₂
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
Cmc 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: 27-Nov-01

Material: Heptadecafluorononanoic acid

Cat.No.: 2263

Cas No.: [375-95-1]

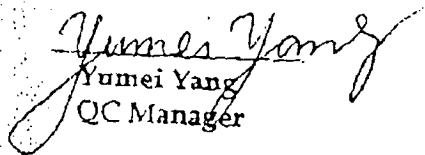
Lot No.: H7568

Assay: 99+⁰% 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

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

SINGLE COMPONENT PREPARATION LOG

Date: 8/15/02

Book No. 02 040

Analyst: RW

Page No. 46

Description: Heptadeca Fluorooctanoic Acid Stock C8 F17COOH

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-6243

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

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 ^{19}F -NMR Spectroscopy
Date: October 28, 2002

SAMPLE DESCRIPTION:

- TCR-618, lot H7568 from the Telomer project.

Nominal product = $\text{CF}_3(\text{CF}_2)_n\text{-CO}_2\text{H}$, where average $n \approx 7$ (white solid).

Sample	Spectra #'s	Experiment Descriptions
TCR-618	H71638.GID.402	400 MHz ^1H -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 ^{19}F -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- d_6) for subsequent analysis by NMR. A 400 MHz ^1H -NMR spectrum and 376 MHz ^{19}F -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 $^1\text{H}/^{19}\text{F}$ -NMR internal standard to allow the calculation of the absolute weight percent concentrations of specific components, or 2) a $^1\text{H}/^{19}\text{F}$ -NMR cross integration standard to permit the cross correlation of the relative ^1H and ^{19}F signal intensities for evaluation of the overall sample composition.

RESULTS:

The ^1H -NMR and ^{19}F -NMR spectral data indicated this sample was a high purity form of the nominal product, $\text{CF}_3(\text{CF}_2)_n\text{-CO}_2\text{H}$, where the average value of $n = 6.955$. Small amounts of a few impurity components were also assigned. A $^1\text{H}/^{19}\text{F}$ -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 $^1\text{H}/^{19}\text{F}$ -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.

CMK 10/31/02
Initial Date

October 28, 2002

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

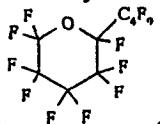
Tom Kestner

c: Rick Payfer
Ron Purcell
William Reagen

File Reference: ls71638.GID.TCR-618_Lot H7568_Telomer Project.DOC/100

TABLE-1

Sample: TCR-618, Lot H7568 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 $n = 6.955$	98.02%
Probable $\text{H-(CF}_2)_n\text{-CO}_2\text{H}$ 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%
Possible $\text{C}_n\text{H}_{2n+2}$ 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

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

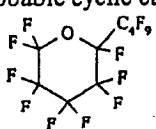
Tom Kestner

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

CME 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 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

CKV 10/31/02
Initial Date

October 28, 2002

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

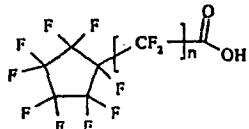
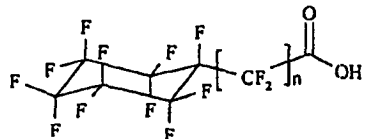
Tom Kestner

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.

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 Initial LMC 10/31/02
 Date

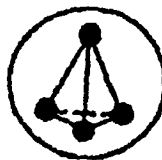
OAKWOOD PRODUCTS

Fax: 803-739-6957

3M ENVIRONMENTAL LABORATORY
Feb 14 2000 1:18

E02-1039

SD-036



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%
Perfluorodecanoic acid 98% min.
Products less volatile than Perfluorodecanoic acid <1%

Appearance: White solid

Melting Point: 83-85°C

Exact Copy of Original

Initial Date
CVR 12/31/02

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

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CNR 10/31/02
Initial Date

SINGLE COMPONENT PREPARATION LOG

Date: 7/2/02 Book No. 02 022
Analyst: RWW Page No. 90
Description: PFDA stock
Stock Number: SD036
Weight or Volume Used: 103.1mg Balance ID: 914
Concentration or Purity: 98.7% Other Correction Factors: NA
Corrected Weight: 101.0mg Solvent and TN-A Number: Methanol-TN-A-06205
Final Volume: 100ml Final Concentration: 1010ppm
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)	Y () 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:	C ₁₁ HF ₂₁ O ₂
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
Cmc 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 TCR-619 by ^1H -NMR and ^{19}F -NMR Spectroscopy
Date: October 29, 2002

SAMPLE DESCRIPTION:

- TCR-619, lot U11N from the Telomer project.
Nominal product = $\text{CF}_3(\text{CF}_2)_n\text{-CO}_2\text{H}$, where average $n \approx 9$ (white powder).

Sample	Spectra #'s	Experiment Descriptions
TCR-619	H71638.GID.404	400 MHz ^1H -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 ^{19}F -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- d_6) for subsequent analysis by NMR. A 400 MHz ^1H -NMR spectrum and a 376 MHz ^{19}F -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 $^1\text{H}/^{19}\text{F}$ -NMR internal standard to allow the calculation of the absolute weight percent concentrations of specific components, or 2) a $^1\text{H}/^{19}\text{F}$ -NMR cross integration standard to permit the cross correlation of the relative ^1H and ^{19}F signal intensities for evaluation of the overall sample composition.

RESULTS:

The ^1H -NMR and ^{19}F -NMR spectral data indicated this sample was a relatively high purity form of the nominal product, $\text{CF}_3(\text{CF}_2)_n\text{-CO}_2\text{H}$, where the average value of $n = 8.63$. Small amounts of a few impurity components, including probable isomers, were also assigned. A $^1\text{H}/^{19}\text{F}$ -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 $^1\text{H}/^{19}\text{F}$ -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 ^{19}F -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

CIX 10/31/02
Initial Date

October 29, 2002

3M SMMD 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 $^1\text{H}/^{19}\text{F}$ -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 $n = 8.63$)	96.4%
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.

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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

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Cmc 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

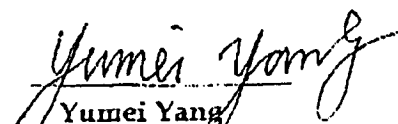
Assay: 99+% by NaOH titration

Appearance: White solid

Melting Point: 92-98°C

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Cmc 10/31/02
Initial Date


Yumei Yang
QC Manager

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

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CWC 10/3/02
Initial Date

SINGLE COMPONENT PREPARATION LOG

Date: 8/29/02 Book No. 02 040
Analyst: RWN Page No. 65
Description: Per 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: THOH/TN-A-LL44
Final Volume: 50mL Final Concentration: 1356 ppm
Storage Location: 2nd Floor - Rm Temp Expiration Date: 2/29/03

Exact Copy of Original

CINC 11/1/02
Initial Date

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

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)	Y 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

JM 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 ^{19}F -NMR Spectroscopy
Date: October 28, 2002

SAMPLE DESCRIPTION:

- SD-037, lot R24K from the Telomer project.
Nominal product = $\text{CF}_3(\text{CF}_2)_n\text{-CO}_2\text{H}$, where average $n \approx 10$ (white powder).

Samples	Spectra #'s	Experiment Descriptions
SD037	H71638.GID.405	400 MHz ^1H -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 ^{19}F -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- d_6) for subsequent analysis by NMR. A 400 MHz ^1H -NMR spectrum and a 376 MHz ^{19}F -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 $^1\text{H}/^{19}\text{F}$ -NMR internal standard to allow the calculation of the absolute weight percent concentrations of specific components, or 2) a $^1\text{H}/^{19}\text{F}$ -NMR cross integration standard to permit the cross correlation of the relative ^1H and ^{19}F signal intensities for evaluation of the overall sample composition.

RESULTS:

The ^1H -NMR and ^{19}F -NMR spectral data indicated this sample was a high purity form of the nominal product, $\text{CF}_3(\text{CF}_2)_n\text{-CO}_2\text{H}$, where the average value of $n = 10.042$. Small amounts of a few impurity components, including probable isomers, were also assigned. A $^1\text{H}/^{19}\text{F}$ -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 $^1\text{H}/^{19}\text{F}$ -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

CM 10/3/02
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: Is71638.GID.SD037_Lot R24K_Telomer Project.DOC/101

TABLE-1

Sample: SD037, Lot R24K 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 $n = 10.02$	99.65%
Probable $(\text{CF}_3)_2\text{-CF-(CF}_2)_n\text{-CO}_2\text{H}$ assume $n=8$ for calculation purposes	0.13%
Possible $\text{CF}_3(\text{CF}_2)_x\text{-CF(CF}_3)_y\text{-CO}_2\text{H}$ where $x \neq 0$, $y \neq 0$ and assume $x+y = 8$ for calculation purposes	0.088%
Probable chlorinated impurity, $\text{Cl-CF}_2\text{CF}_2\text{-R}_i$, 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 Date

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

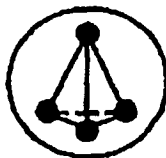
Quantitative Compositional Results by Combined $^{19}\text{F}/^1\text{H}$ -NMR Spectral Analyses

Component Structures ¹	$^1\text{H}/^{19}\text{F}$ -NMR Relative Weight% Concentrations (single trial analysis)
$\text{CF}_3(\text{CF}_2)_n\text{-CO}_2\text{H}$ where average $n = 10.02$	$\leq 99.65\%$ 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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CML 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:** 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

Exact Copy of Original

Cmc 10/31/02
Initial Date

Yumei Yang
Yumei Yang
Quality Control

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	014	-	RWW	08/29/2002

Exact Copy of Original
CME 12/3/02
Initial Date

SINGLE COMPONENT PREPARATION LOG

Date: 8/29/02
Analyst: RWW
Description: Perfluorododecanoic Acid - C₁₂F₂₃COOH
Stock Number: SD037
Weight or Volume Used: 0.0523g Balance ID: 914
Concentration or Purity: NA Other Correction Factors: NA
Corrected Weight: 0.0523g Solvent and TN-A Number: MeOH-TNA-4244
Final Volume: 50ml Final Concentration: 1126 ppm
Storage Location: 2nd Floor - Rm Temp Expiration Date: 2/29/03

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Reviewed by: Jim A. Stenerson 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)	Y 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.px  E00-1682-COA-SD018-PFOS-Rev3

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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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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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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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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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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: 52.39mg 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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Reviewed by: A. Stevenson 06/26/02
Signature Date

ATTACHMENT E: PROTOCOL AND AMENDMENT



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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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 ₁₃ COOH
Tetradecafluoroheptanoic Acid	C ₈ F ₁₅ 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 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 ₃ S	TCR-343	SynQuest Labs	White Powder	TBD	Frozen
1H,2H,3H,4H-perfluorodecane sulfonate	C ₁₀ H ₈ 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

*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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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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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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- 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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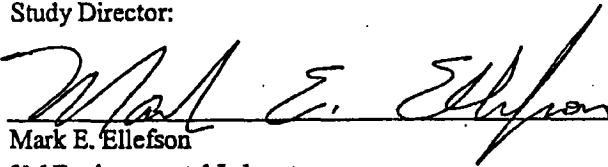
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Analysis of Endogenous Fluorochemicals

E02-1039

Protocol Signature

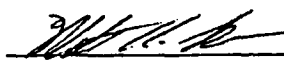
Study Director:


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:

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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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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.

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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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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 ⁺

REASON: The C₆ 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 ₆ F ₁₁ COOH	TCR-047	SMM* 236-1B-10	Colorless Liquid	TBD**	Frozen
Tetradecafluoro heptanoic Acid	C ₈ 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 ₅ F ₁₃ O ₃ S	TCR-343	SynQuest Labs	White Powder	TBD	Frozen
1H,2H,3H,4H-perfluorodecane sulfonate	C ₁₀ H ₅ 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

AMEND TO READ

Reference Substances

Reference Substance	Formula	Traceability #	Source	Physical Description	Purity	Storage Conditions
Tetradecafluoro heptanoic Acid	C ₈ 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

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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
Perfluoroun decanoic 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 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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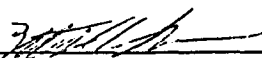
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AMEND TO READ: Limits of Quantitation (LOQ): The lower limit of quantitation (LLOQ) will be determined for each analyte. The level determined as the LLOQ must show a recovery within 70% - 130% for the analytes.

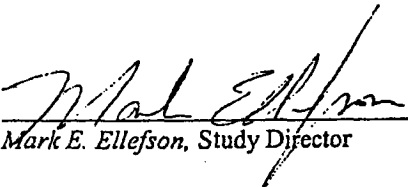
REASON: Since this is a screening-type study it was appropriate to relax the acceptable recovery levels in order to avoid re-running sample sets.

Amendment Approval



William K. Reagen, Sponsor Representative
11/8/02

Date



Mark E. Ellefson, Study Director
11/8/02

Date

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Initial 11/11/02

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

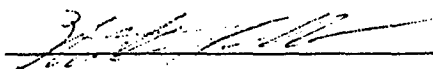
This amendment modifies the following portion(s) of the protocol:

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.

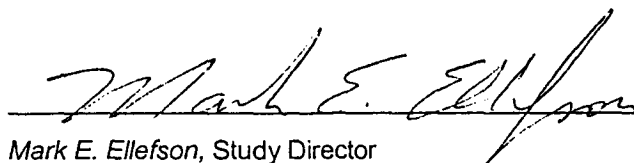
AMEND TO READ: The purpose of this study is to quantify perfluoroheptanoic acid (C7), pentadecafluorooctanoic acid (C8), heptadecafluorononanoic acid (C9), nonadecafluorodecanoic acid (C10), perfluorodecanoic acid (C11), perfluorododecanoic acid (C12), and perfluorooctanesulfonate (PFOS) in normal pooled 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