226-1151

mf#64776

3M

Study Title

ANALYSIS OF ENDOGENOUS FLUOROCHEMICALS IN NORMAL POOLED HUMAN SERUM AND PLASMA

Data Requirement

40 CFR Part 792

Author

Mark E. Ellefson

Study Completion Date

13 November, 2002

Revised Report

9 December, 2002

Performing Laboratory

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

Project Identification

3M Environmental Laboratory Study Number E02-1039

Total Number of Pages

225

CONTAIN NO CBI

This page has been reserved for specific country requirements.

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 — Date 12/9/02

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

000095

QUALITY ASSURANCE STATEMENT

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

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

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

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

Tonya K Rude — 12/6/02

QAU Representative — *Date*

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

STUDY INFORMATION

Sponsor

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

Study Director

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

Study Location

Testing Facility

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

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

Study Dates

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

Location of Archives

All original raw data and the report have been archived at the 3M Environmental Laboratory according to 3M Standard Operating Procedures.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

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. ^{1}Value < 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. ^{1}Value < 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

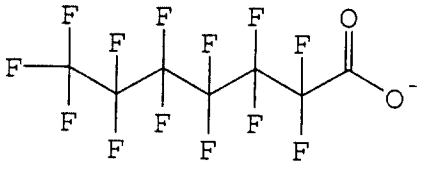
TEST SUBSTANCE	FORMULA
Tridecafluoroheptanoate (C7)	$C_6F_{13}COO^-$
Pentadecafluorooctanoate (C8)	$C_7F_{15}COO^-$
Heptadecafluorononanoate (C9)	$C_8F_{17}COO^-$
Nonadecafluorodecanoate (C10)	$C_9F_{19}COO^-$
Perfluoroundecanoate C11)	$C_{10}F_{21}COO^-$
Perfluorododecanoate (C12)	$C_{11}F_{23}COO^-$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

The molecular structures are given below.

Name: C7

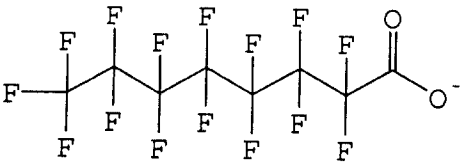
Chemical Name: Perfluoroheptanoate

Molecular Weight: 363, as shown



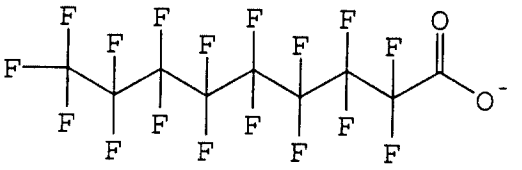
Name: C8

Chemical Name: Pentadecafluorooctanoate
Molecular Weight: 413, as shown



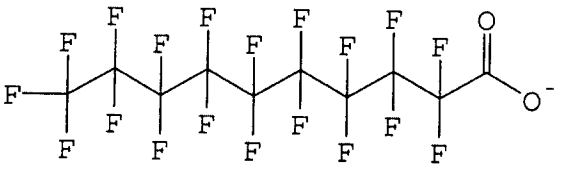
Name: C9

Chemical Name: Heptadecafluorononanoate
Molecular Weight: 463, as shown



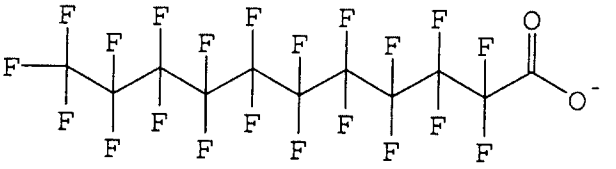
Name: C10

Chemical Name: Nonadecafluorodecanoate
Molecular Weight: 513, as shown



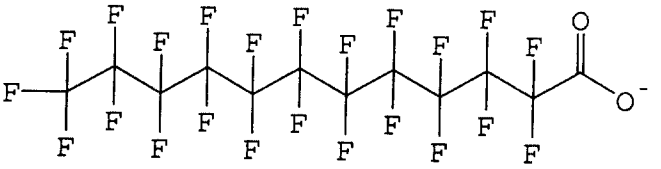
Name: C11

Chemical Name: Perfluoroundecanoate
Molecular Weight: 563, as shown



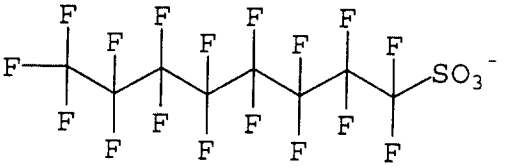
Name: C12

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 heptanioc 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	$C_8F_{17}SO_3^-K^+$	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., Southield, 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 Matricies." 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 C_{18} 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 Matricies." 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 C_{18} 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™ C_{18} 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:

Time (min.)	% B
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:

(C_7)

Channel	Parent Ion (m/z)	Daughter Ion (m/z)	Collision Energy (eV)	Cone (V)
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

(C_8)

Channel	Parent Ion (m/z)	Daughter Ion (m/z)	Collision Energy (eV)	Cone (V)
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

(C_9)

Channel	Parent Ion (m/z)	Daughter Ion (m/z)	Collision Energy (eV)	Cone (V)
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

(C_{10})

Channel	Parent Ion (m/z)	Daughter Ion (m/z)	Collision Energy (eV)	Cone (V)
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

(C_{11})

Channel	Parent Ion (m/z)	Daughter Ion (m/z)	Collision Energy (eV)	Cone (V)
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

(C_{12})

Channel	Parent Ion (m/z)	Daughter Ion (m/z)	Collision Energy (eV)	Cone (V)
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

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

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

ANALYTICAL RESULTS

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

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

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

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

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

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

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

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. ^{1}Value < 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. ^{1}Value < 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	$C_7O_2F_{13}$
C8	412.9637	412.9659	-5.4	$C_8O_2F_{15}$
C9	462.9613	463.9627	-3.0	$C_9O_2F_{17}$
PFOS	498.9280	498.9297	-3.3	$C_8O_3F_{17}S$
C10	512.9615	512.9595	3.9	$C_{10}O_2F_{19}$
C11	562.9542	562.9563	-3.8	$C_{11}O_2F_{21}$

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

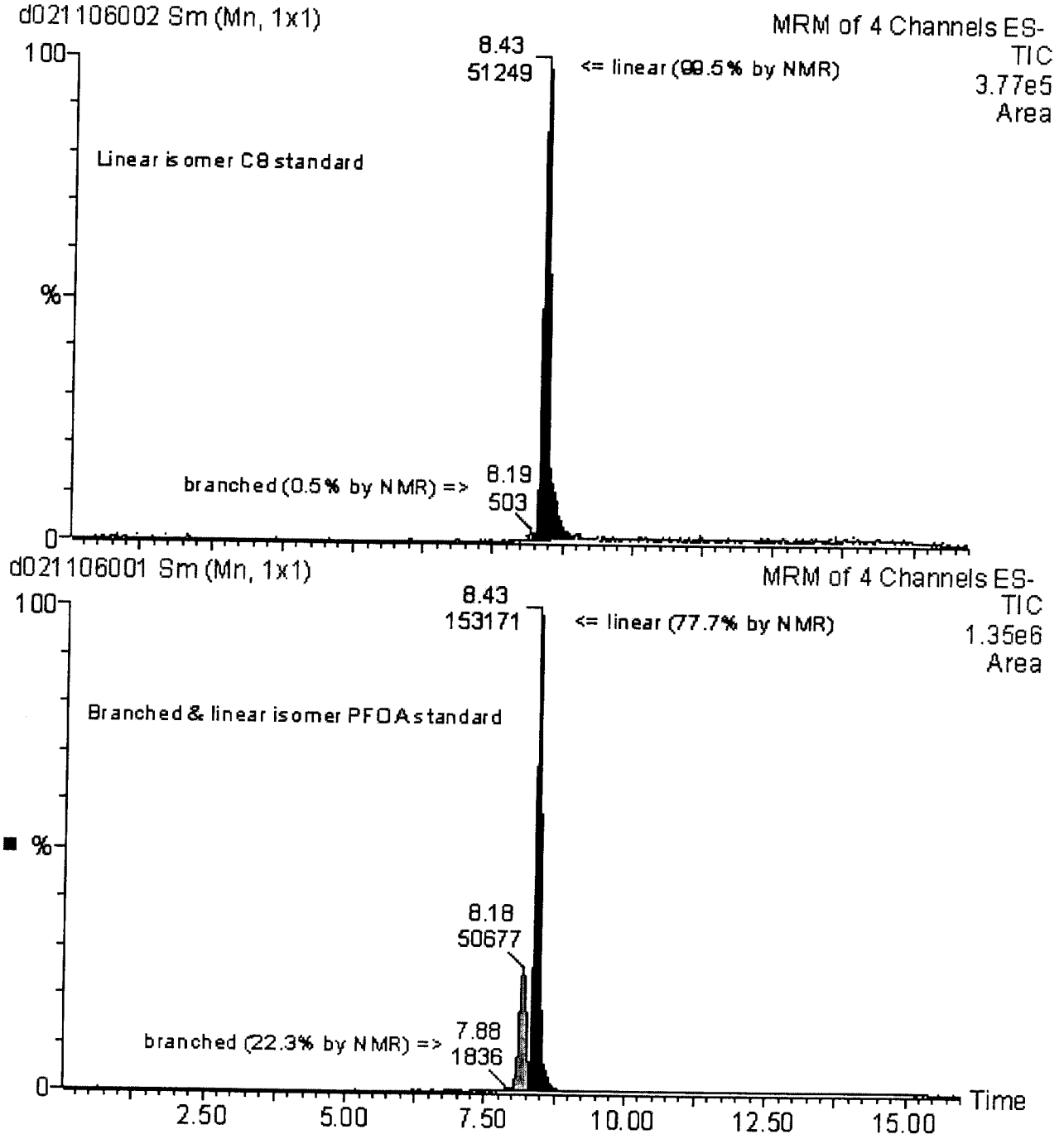
Test Substance	Parent Ion (m/z)	Daughter Ions (m/z)
C_7	363	319, 119
C_8	413	369, 219, 169, 119
C_9	463	419, 269, 219, 169, 119
C_{10}	513	469, 319, 269, 219, 169
C_{11}	563	519, 319, 219, 169, 119
C_{12}	613	569, 419, 319, 269, 219, 169, 119
PFOS	499	130, 99, 80

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.

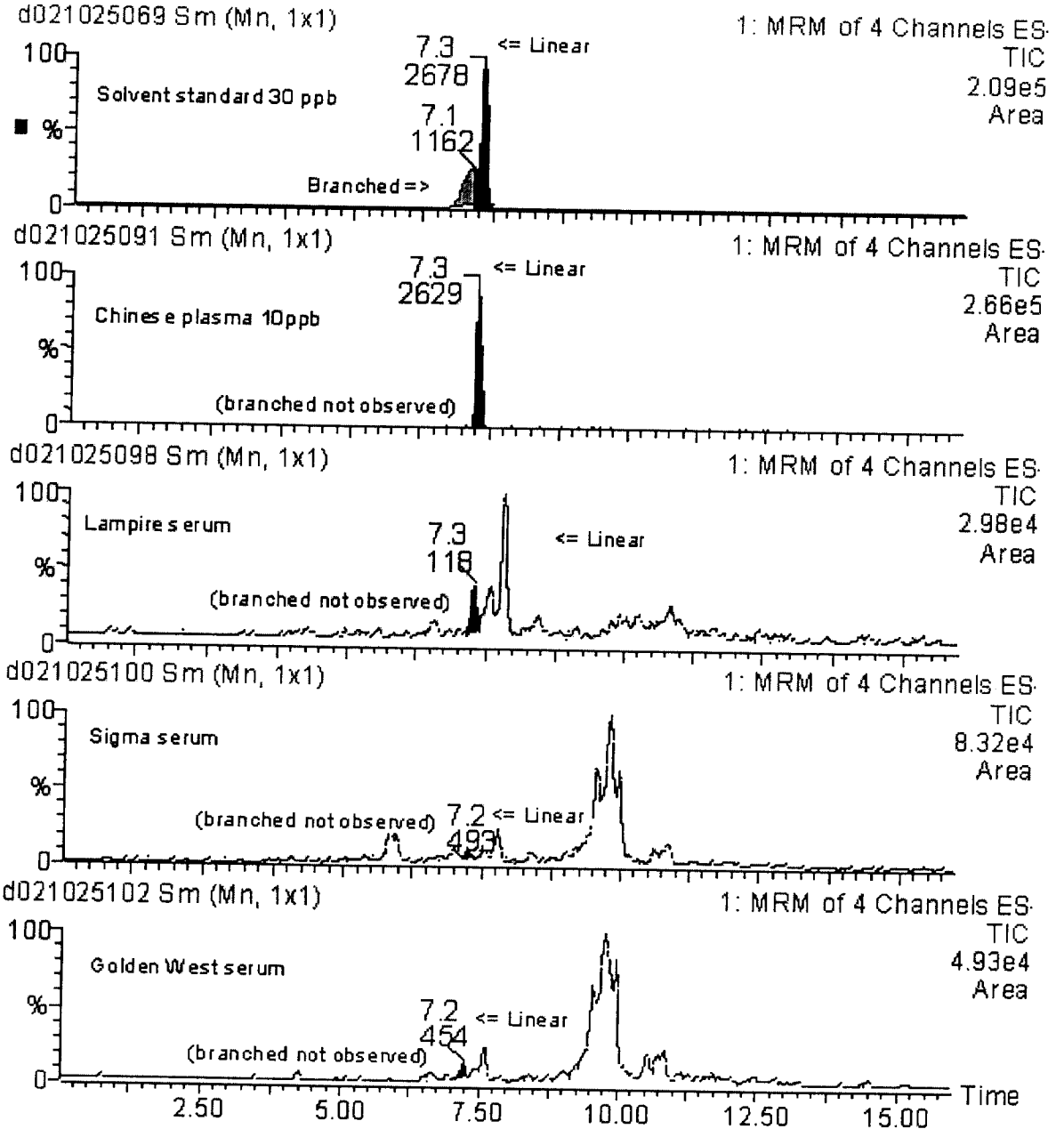
Figure 1: NMR Certified 99.5% Linear Isomer PFOA and Mixed Branched and Linear Isomer PFOA Standards



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.

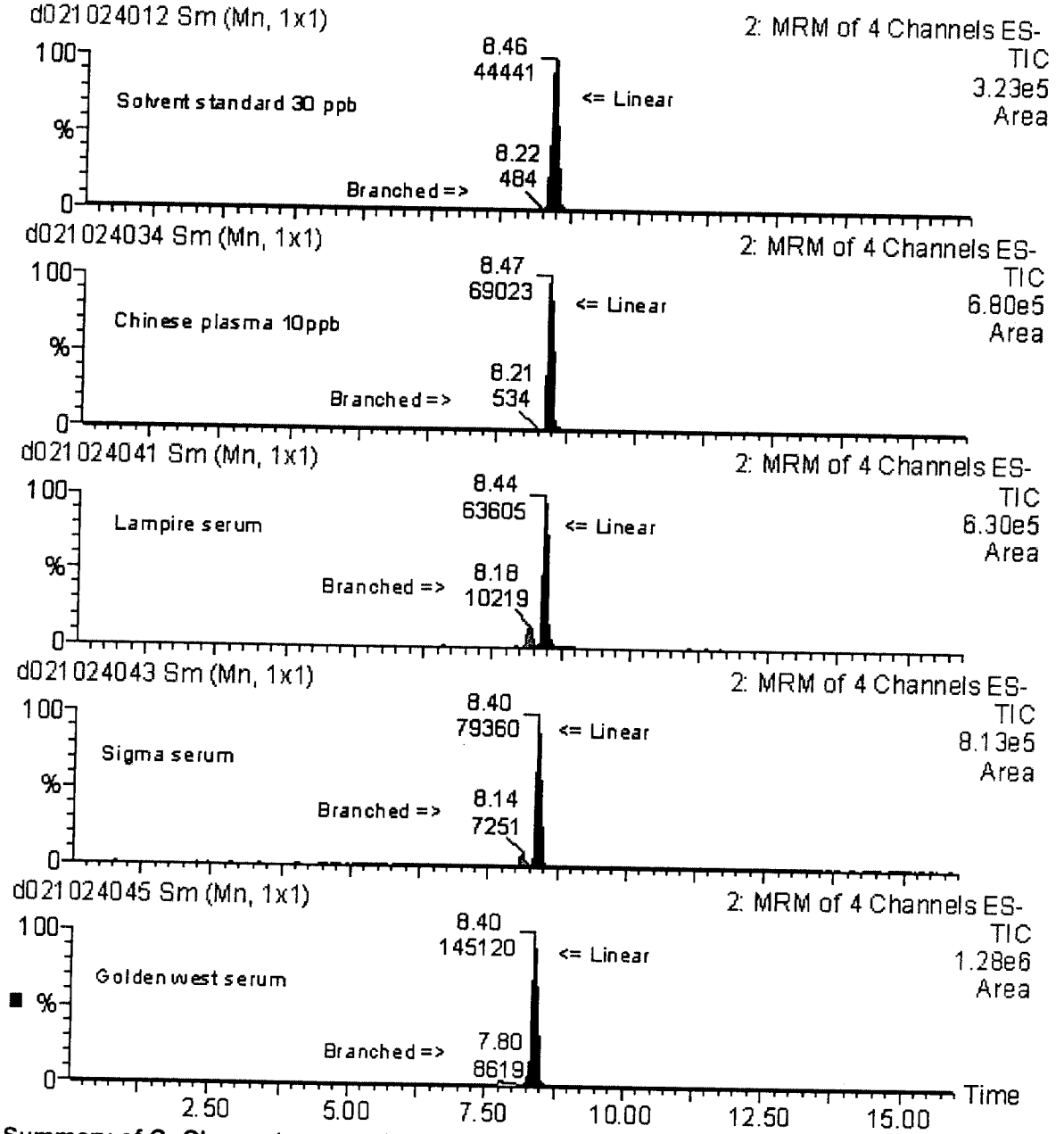
000115

Figure 2: C_7 Isomer Distribution



Summary of C_7 Acid Chromatograms: Low to non-detect levels of the branched isomers of C_7 acid were observed in these lots of commercial pooled human serum.

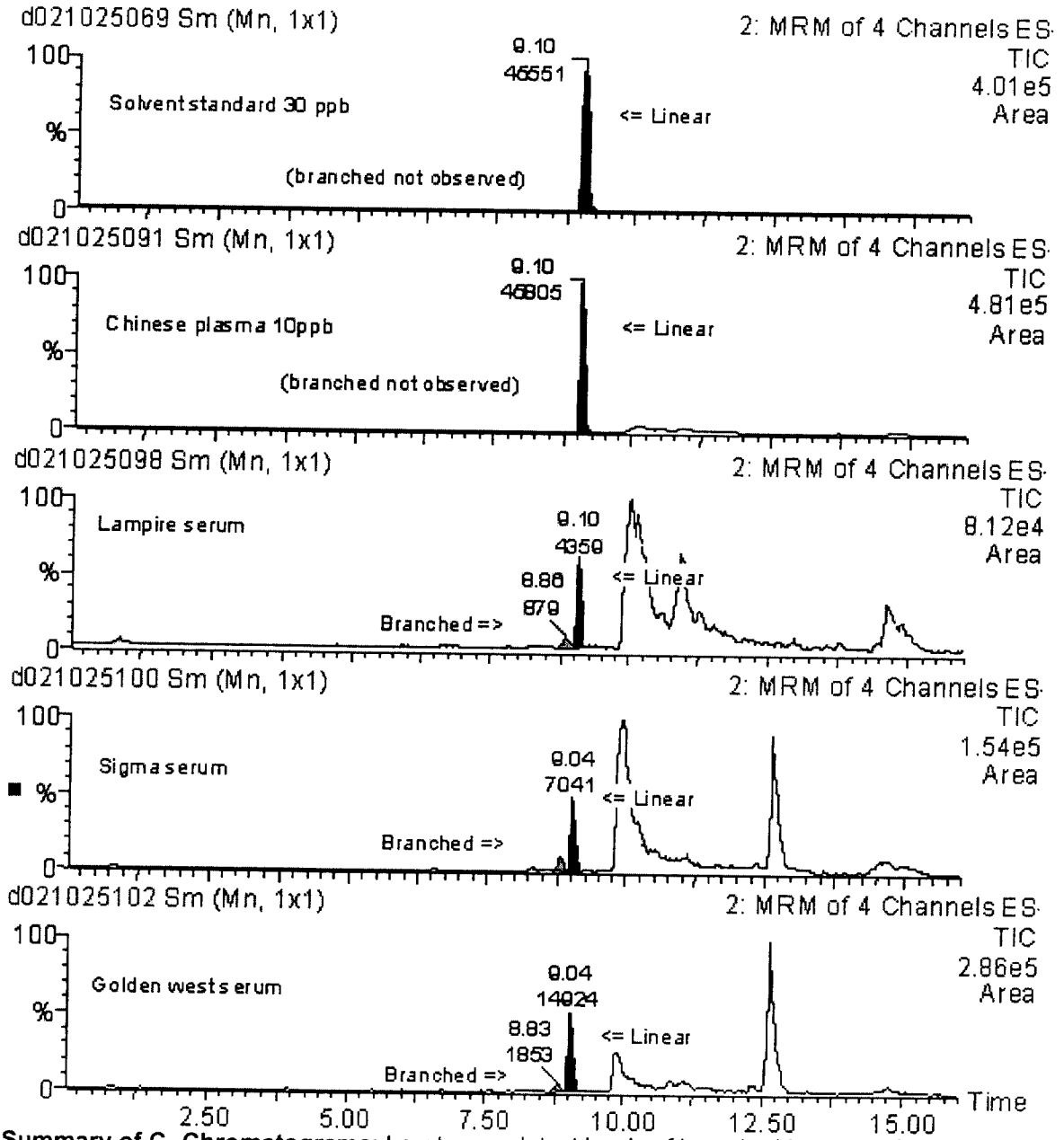
Figure 3: C_8 Isomer Distribution



Summary of C_8 Chromatograms: Low to non-detect levels of branched isomers of C_8 were observed in these lots of commercial pooled human serum.

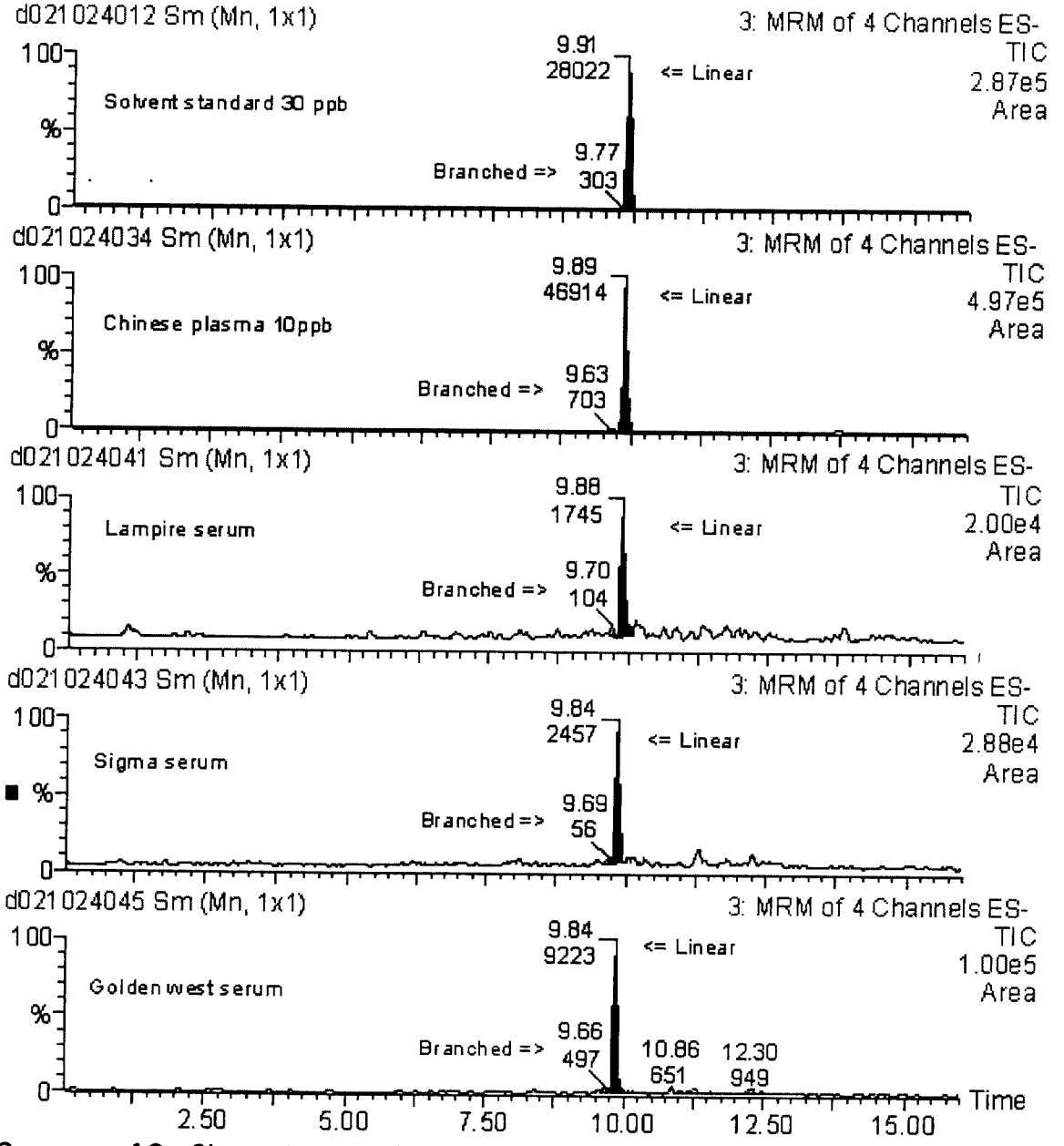
000117

Figure 4: C_9 Isomer Distribution



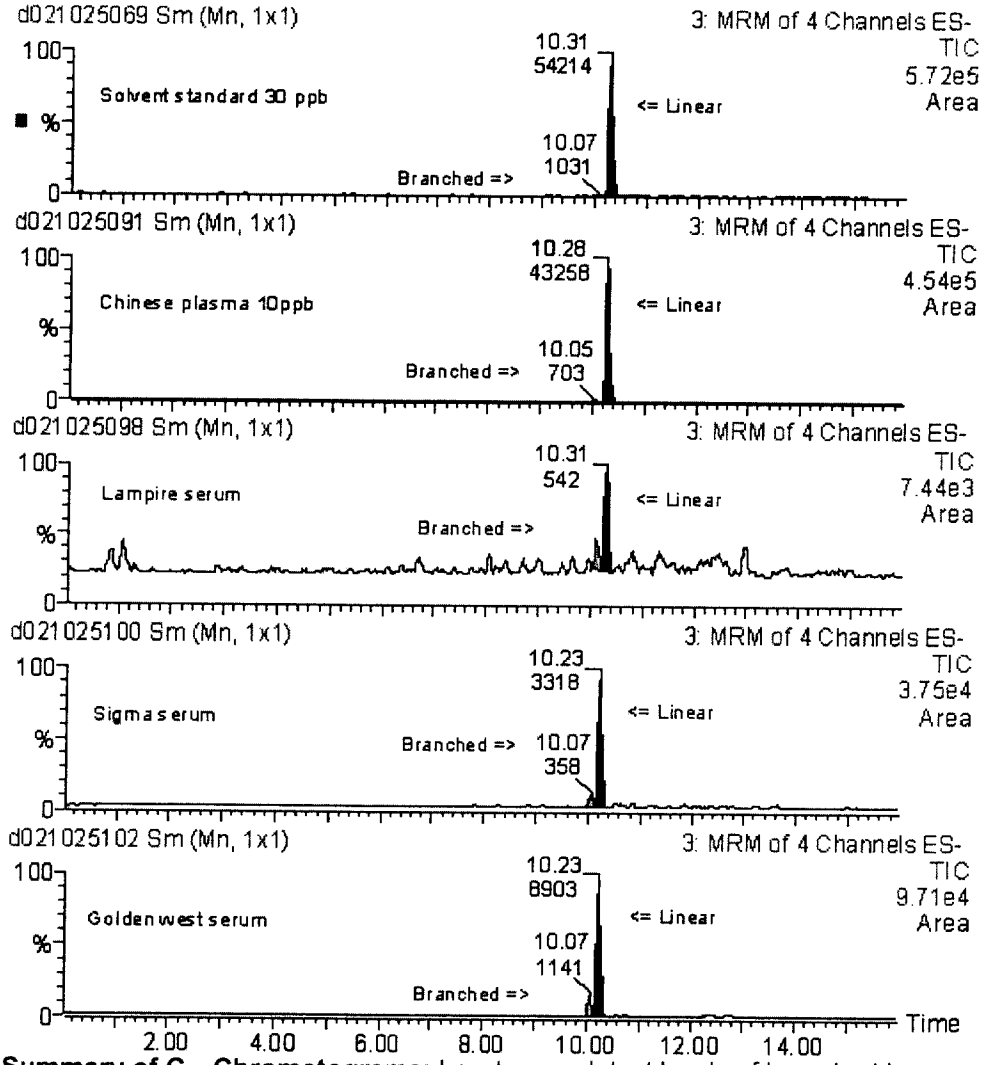
Summary of C_9 Chromatograms: Low to non-detect levels of branched isomers of C_9 were observed in these lots of commercial pooled human serum.

Figure 5: C_{10} Isomer Distribution



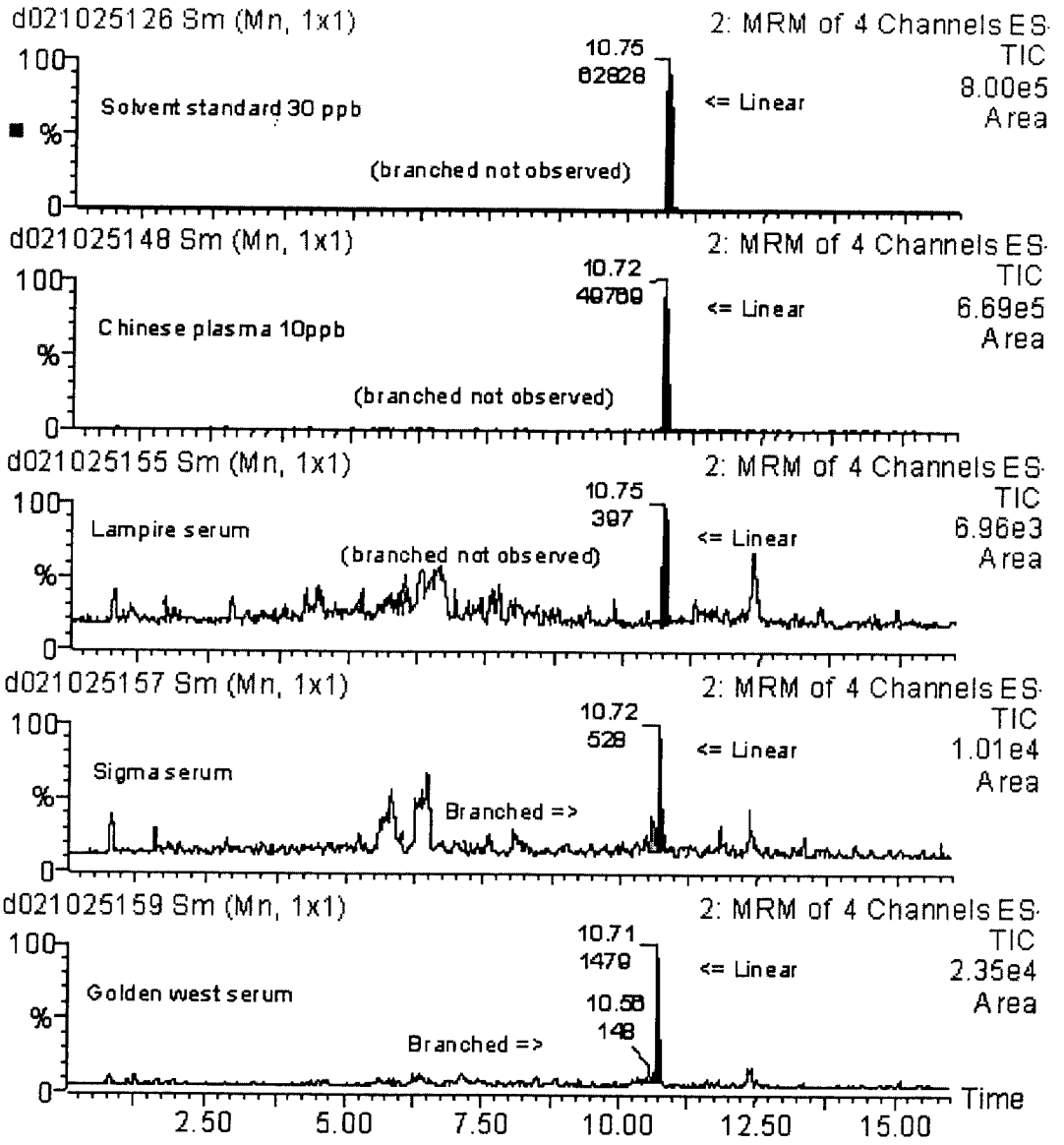
Summary of C_{10} Chromatograms: Low to non-detect levels of branched isomers of C_{10} were observed in these lots of commercial pooled human serum.

Figure 6: C_{11} Isomer Distribution



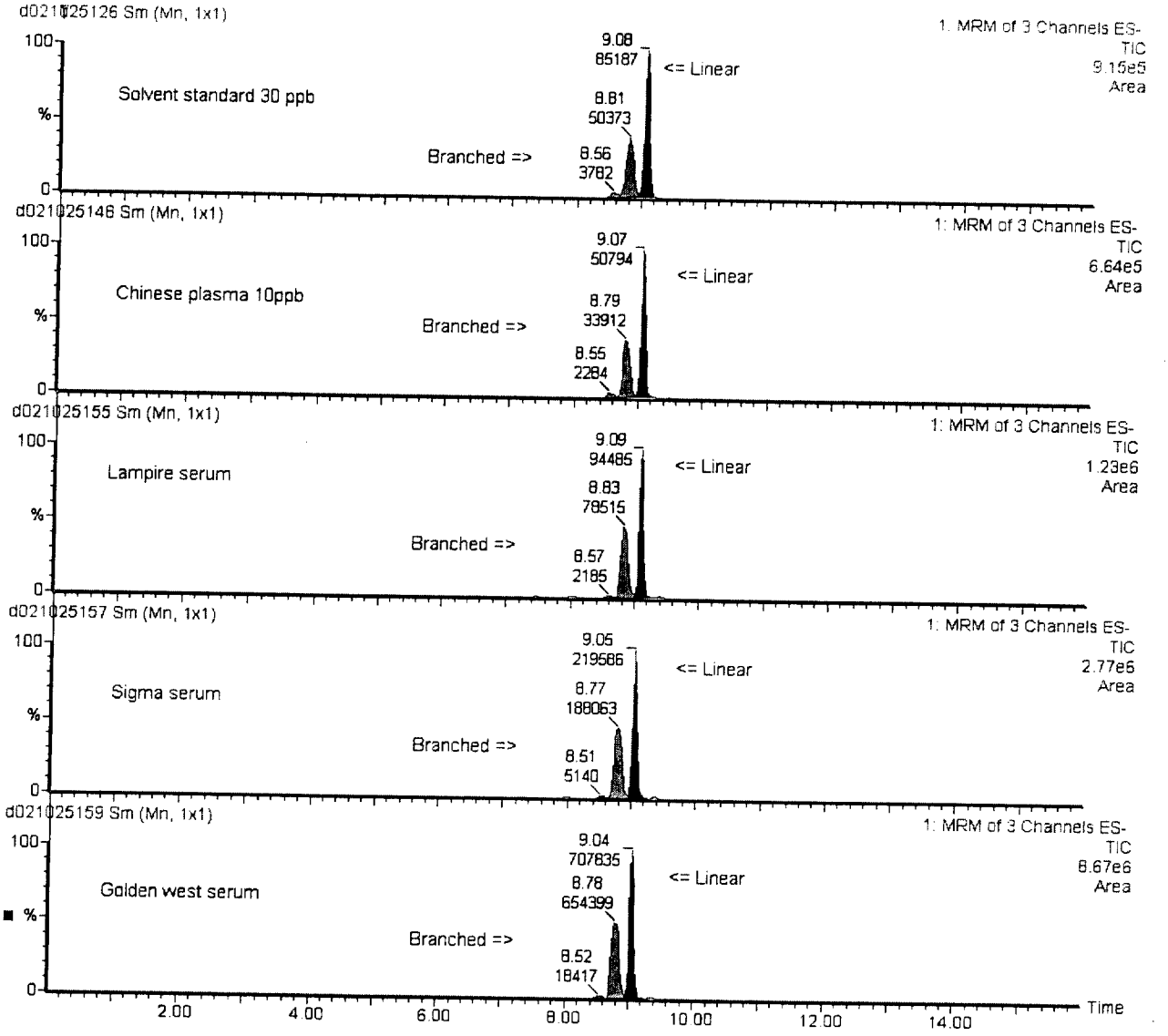
Summary of C_{11} Chromatograms: Low to non-detect levels of branched isomers of C_{11} were observed in these lots of commercial pooled human serum.

Figure 7: C_{12} Isomer Distribution



Summary of C_{12} Chromatograms: Low to non-detect levels of branched isomers of C_{12} 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:

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

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:

Mark E. Ellefson 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: ☐ SOP ☒ Method ☐ Equipment Procedure
(Check one) ☐ 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 ±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: Cindy Carlson Date: 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: 11/1/02

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

3M Environmental Laboratory

Deviation No. 1
(assigned by Study Director or Project Lead at the end of study or project)

000128

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:

02/18/02

Willliam K. Reagen
Laboratory Manager

Date

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

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 C_{18} 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.

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 sampule 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 C_{18} (Waters)

75 mL sample reservoir (or other appropriate size)

Crimp cap glass autovials and caps

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

Crimpers

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

7 Reagents and Standards

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

Acetonitrile, HPLC grade or equivalent

Methanol, HPLC grade or equivalent

Ammonium acetate, reagent grade or equivalent

Biological fluids or tissues, frozen from supplier

7.1 Reagents preparation

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

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

7.2 Target analyte standard preparation

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

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

Bring to volume with methanol for a stock standard of approximately 1000 ppm (µg/mL).

Dilute the stock solution with methanol for a working standard 1 solution of approximately 50 ppm. Example calculation: 1000 µg/mL x 5 mL/100 mL = 50 µg/mL.

Dilute working standard 1 with methanol to produce a working standard 2 solution of approx. 5.0 ppm. Example calculation: 50 µg/mL x 10 mL/100mL = 5.0 µg/mL.

Dilute working standard 1 with methanol to produce a working standard 3 solution of approx. 0.50 ppm. Example calculation: 50 µg/mL x 1.0 mL/100 mL = 0.5 µg/mL.

7.3 Surrogate standard preparation

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

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

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

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

7.4 Internal standard preparation

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

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

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

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.

Exact Copy of Original
Initial Date

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

Exact Copy of Original
Initial 10/3/16 Date

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.

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 ug/mL	1.5	5.00 ng/g or ng/mL	0.375 ng/mL
0.500 ug/mL	3.0	10.0 ng/g or ng/mL	0.750 ng/mL
0.500 ug/mL	8.0	25.0 ng/g or ng/mL	2.00 ng/mL
0.500 ug/mL	16	50.0 ng/g or ng/mL	4.00 ng/mL
0.500 ug/mL	32	100 ng/g or ng/mL	8.00 ng/mL
5.00 ug/mL	5.6	175 ng/g or ng/mL	14.0 ng/mL
5.00 ug/mL	8.0	250 ng/g or ng/mL	20.0 ng/mL
5.00 ug/mL	16	500 ng/g or ng/mL	40.0 ng/mL
5.00 ug/mL	24	750 ng/g or ng/mL	60.0 ng/mL
5.00 ug/mL	32	1000 ng/g or ng/mL	80.0 ng/mL
5.00 ug/mL	40	1250 ng/g or ng/mL	100 ng/mL
50.0 ug/mL	5.0	1500 ng/g or ng/mL	125 ng/mL
50.0 ug/mL	6.0	1750 ng/g or ng/mL	150 ng/mL
Surrogate Std 100 ug/mL	10	6500 ng/g or ng/mL	500 ng/mL

11 Procedures

11.1 Tissue Sample Preparation

Obtain frozen tissue samples

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

Weigh the tissue directly into a tared plastic sampule 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 sampule vial, or other volume as determined by the analyst and documented in a note to file.

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

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

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

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

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 sampule 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.

Exact Copy of Original
LMK 12/31/07
Initial Date

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.

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 µL injection

Inject/sample = 1

Cycle time = 10.0 minutes

Flow rate = 300 µL/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 10–500uL/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

Exact Copy of Original
Initial 10/31/01 Date

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:

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

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

IV = Initial volume

DV = Diluent volume (reagent grade water)

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

AR = Analytical result from MassLynx summary

DF = Dilution factor

FV = Final volume

MA = Matrix amount

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

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

Calculate spike percent recoveries using the following equation:

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

Exact Copy of Original
Initial Date

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):

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

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

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

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%.

Exact Copy of Original
LJM 10/31/08
Initial Date

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

Exact Copy of Original
Initial Date 10/31/02

19 Revisions

Revision Number	Revision Description	Revision Date
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

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

Attachment A – Extraction Worksheet

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

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

Sample Number	Sample Number or description	Volume of diluted sample removed	Amount and spike mix used	Amount and surrogate spike mix used	Type of column used and lot	Elution solvent and volume	Comments

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 polypropelene 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 _____ uL 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.

Exact Copy of Original
CMK 10/31/07
Initial Date

Attachment B - Sample Weight/Volume Worksheet

Prep Date(s):
Analyst(s):
Sample Matrix:
Method/Revision:

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

Sample ID	Initial Wt./Vol. g/mL/L	Water Volume added (mL)	Volume Removed (mL)		Comments

Form Completion Verified By:_____

Exact Copy of Original
Initial Date

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	

Exact Copy of Original
CMC 10/31/01
Initial Date

Attachment D: Dilutions Summary Worksheet

Study:
Dilution Date/Analyst:
Box Number:

Solvent/TN Number:
Extraction Date/Analyst:
Matrix/Timepoint:

Sample Number or Description	Dilutions								
	1/	1/	1/	1/	1/	1/	1/	Comments	Verified By:

Notes:
1/10 dilution = _____ of sample + _____ of solvent

Exact Copy of Original
Initial 10/31/02 Date

Form Completion Verified By: _____

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

Chineese Plasma, Serum Data Cal Curves

Analyte	Calibration Range, ng/mL	R^2	System Suitability Samples, %RSD	CCV #1 recovery, %	CCV #2 recovery, %	CCV #3 recovery, %	Matrix Blanks
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

Chineese Plasma, Plasma Data Cal Curves

Analyte	Calibration Range, ng/mL	R^2	System Suitability Samples, %RSD	CCV #1 recovery, %	CCV #2 recovery, %	Matrix Blanks
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

Chineese 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_2O w/ 2mM $NH_4CH_3CO_2$	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			

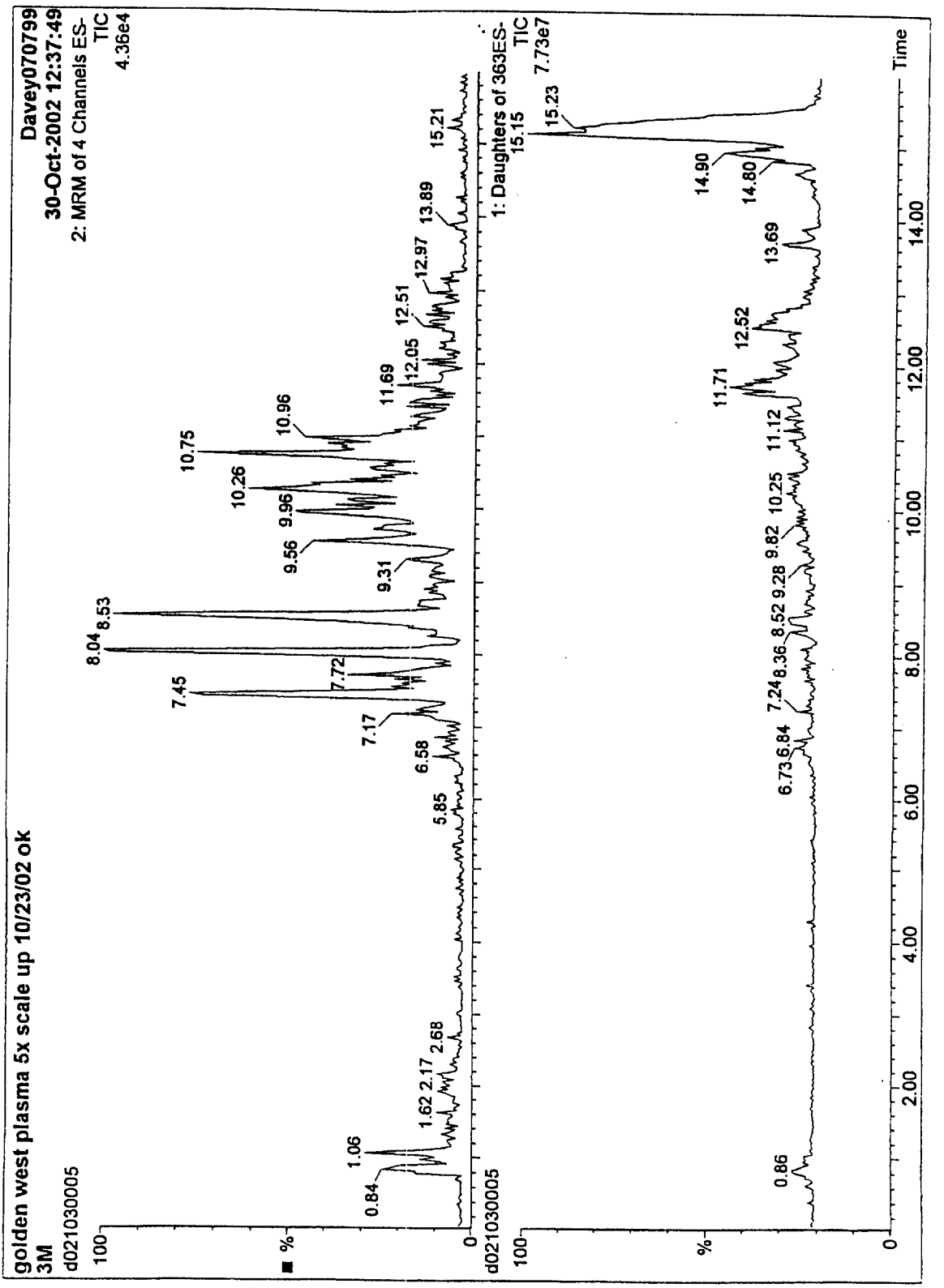
Accurate mass measurements and elemental compositions obtained in this analysis are summarized in Table 2:

Table 2

Retention Time	Accurate Mass	Theoretical Mass	Mass Deviation (ppm)	Probable Formula
5.8 min	362.9739	362.9691	13.3	$C_7O_2F_{13}$
6.3 min	412.9637	412.9659	-5.4	$C_8O_2F_{15}$
6.5 min	526.9693	526.9610	15.8	$C_{10}H_4O_3F_{17}S$
6.69 min	462.9613	463.9627	-3.0	$C_9O_2F_{17}$
6.71 min	498.9280	498.9297	-3.3	$C_8O_3F_{17}S$
6.72 min	512.9615	512.9595	3.9	$C_{10}O_2F_{19}$
6.8 min	426.9734	426.9674	14.1	$C_8H_4O_3F_{13}S$
7.1 min	562.9542	562.9563	-3.8	$C_{11}O_2F_{21}$

ATTACHMENT C: CHROMATOGRAMS

C7 ACID

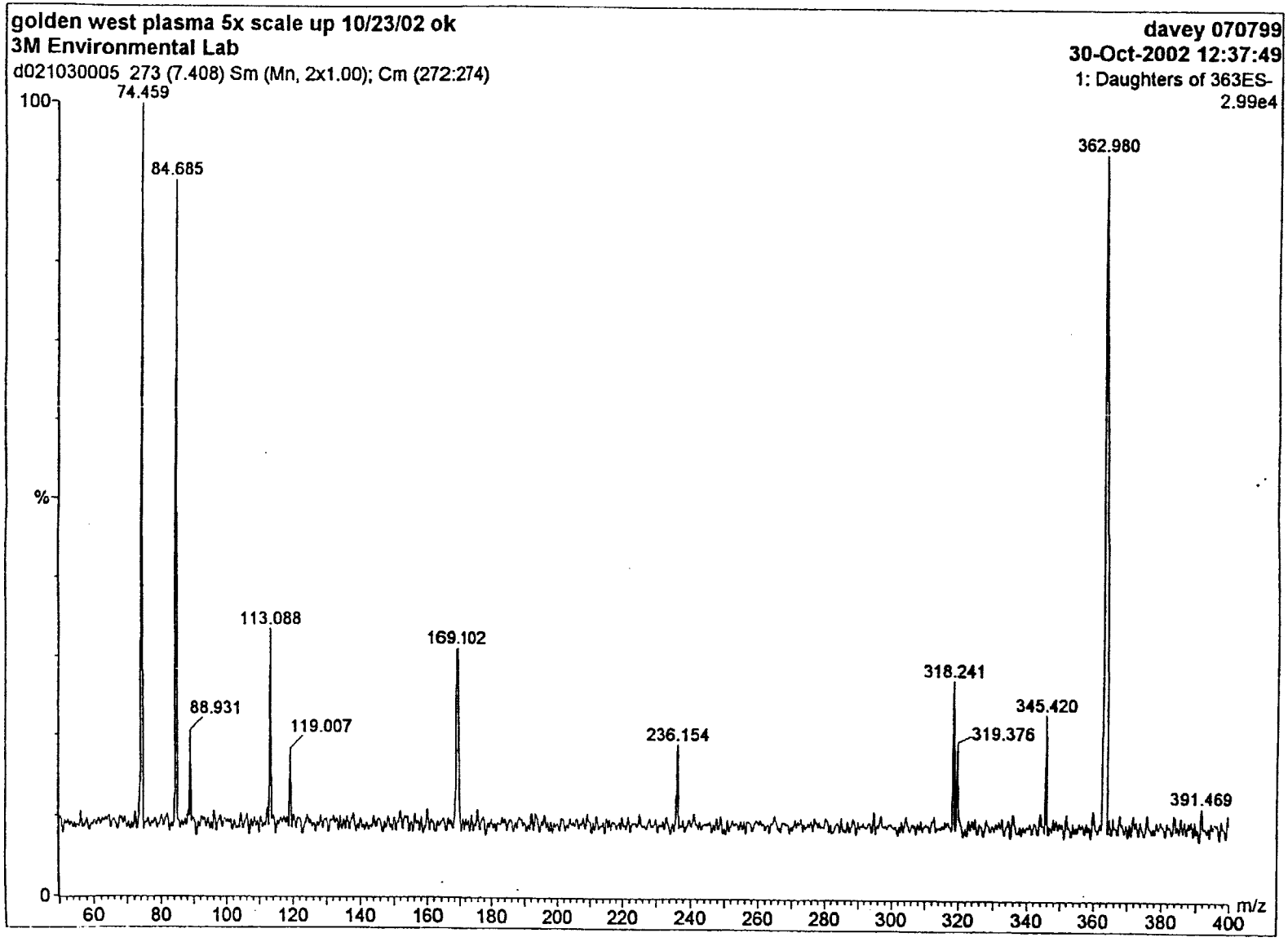


Exact Copy of Original

Initial Date

000156

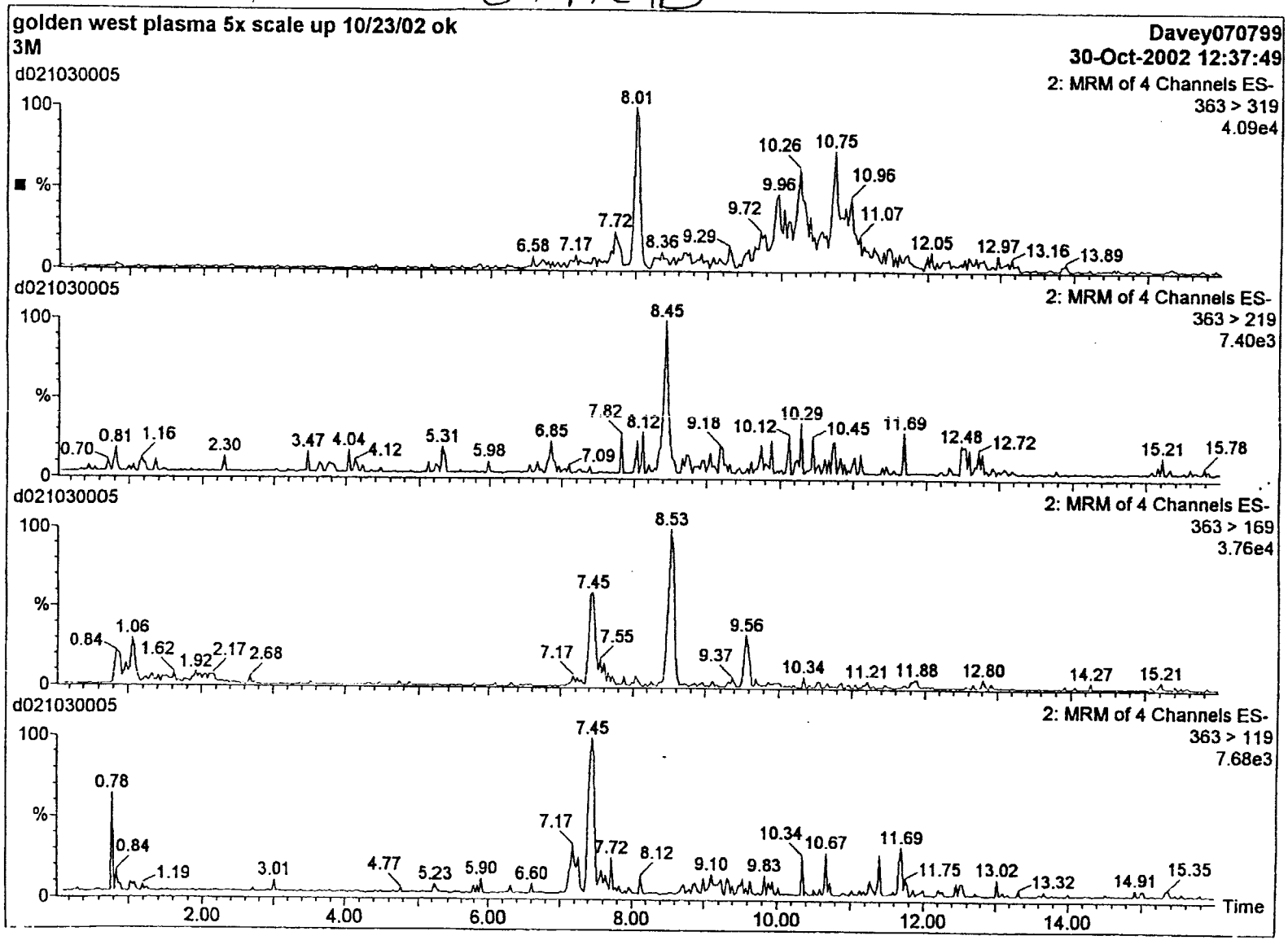
C7 ACID



Exact Copy of Original

Cmc 10/31/02
Initial Date

C7 AC ID

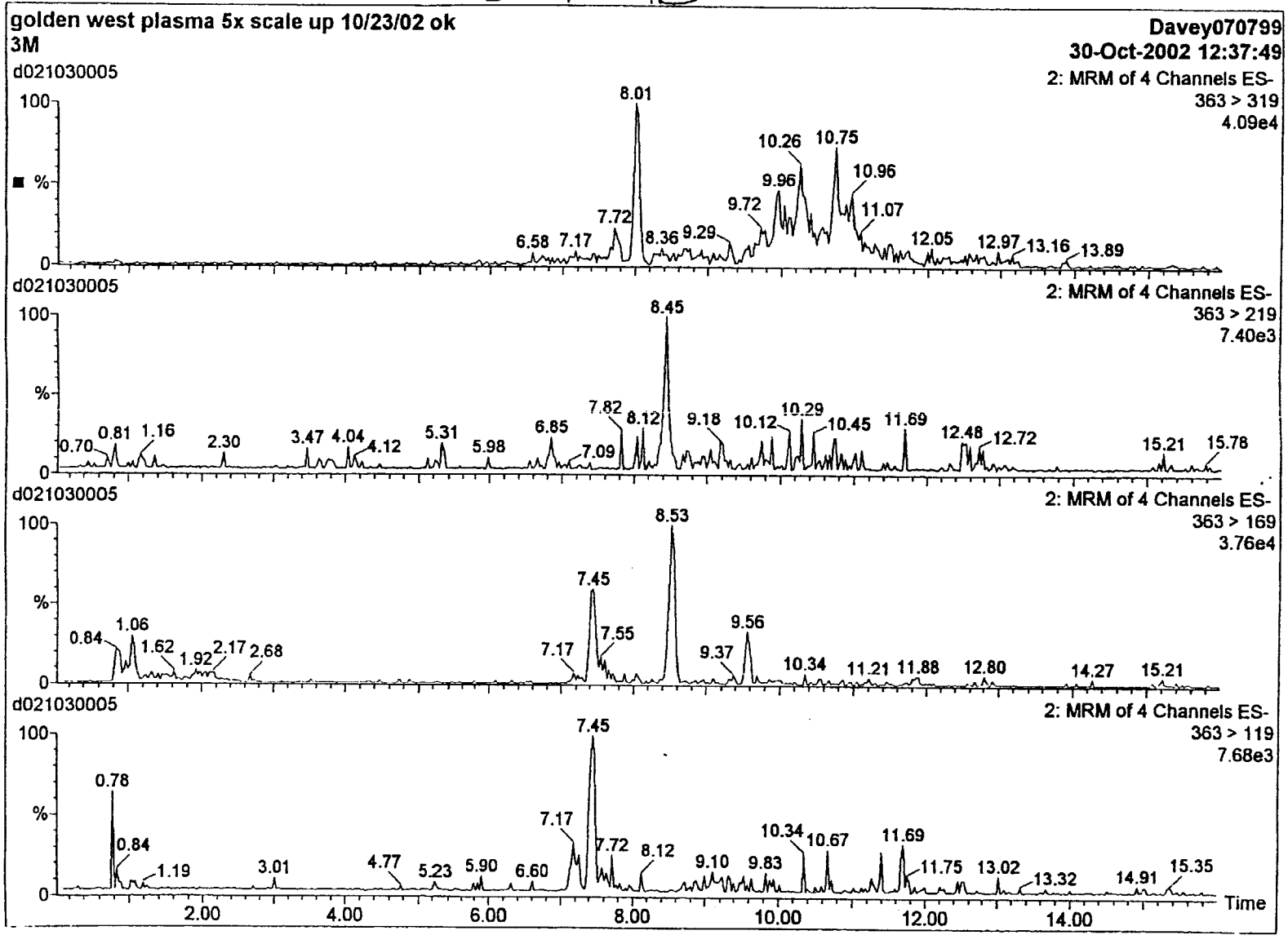


Exact Copy of Original

CMC 10/31/02
Initial Date

000158

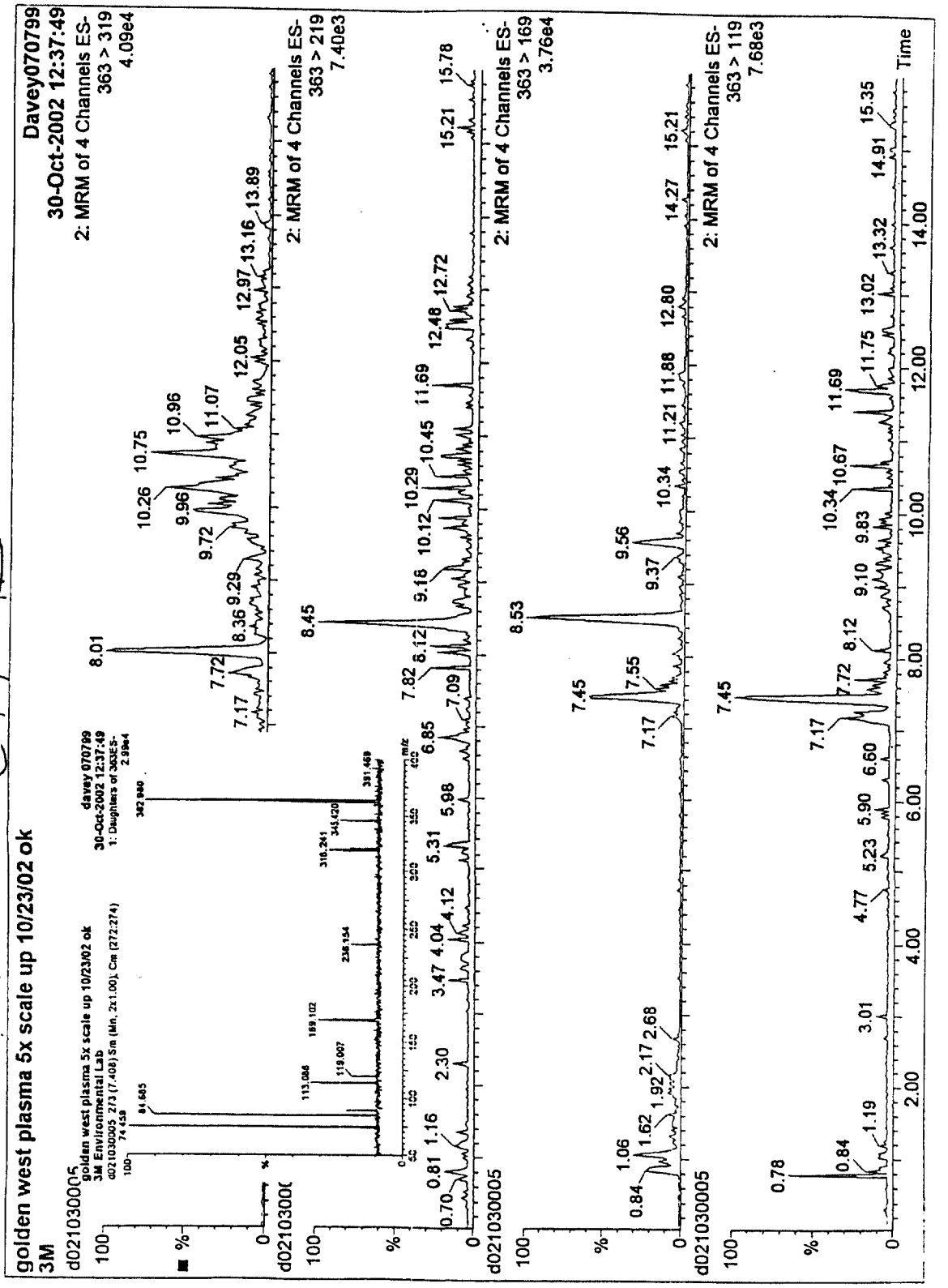
C7 AC ID



Exact Copy of Original

CMC 10/31/02
Initial Date

000159

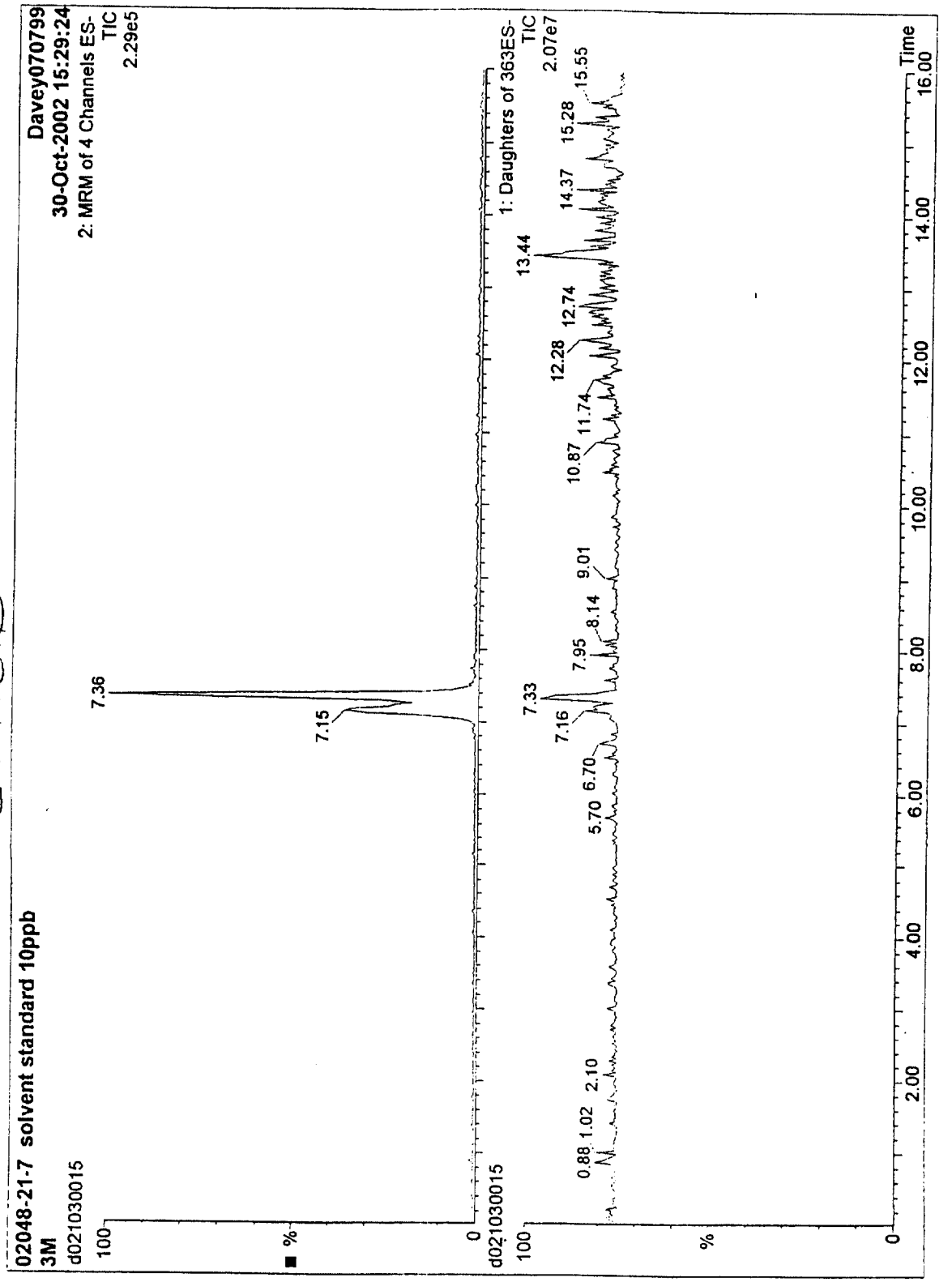


Exact Copy of Original

10/31/02
Initial Date

000160

C7 ACID

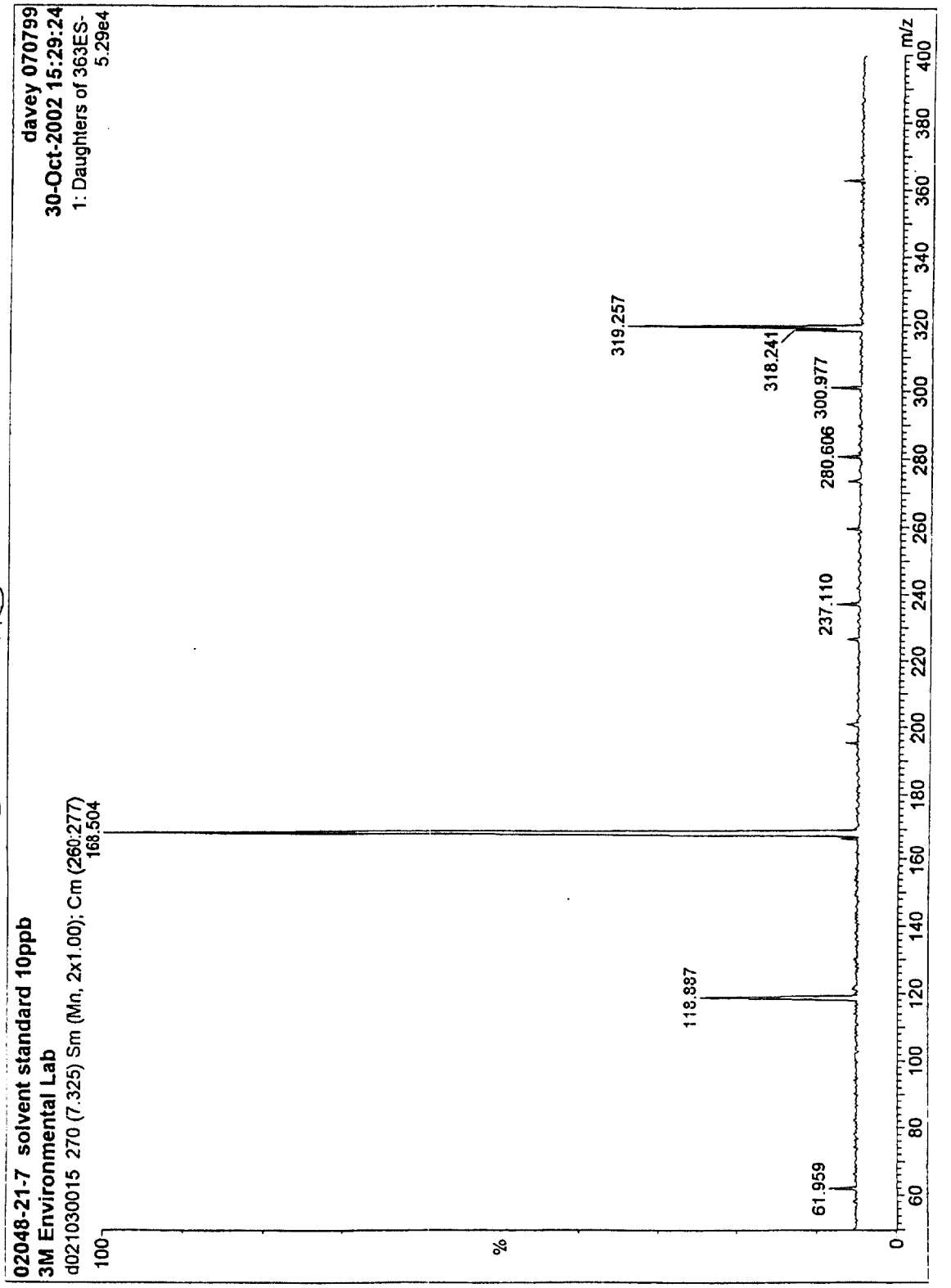


Exact Copy of Original

Chris 11/12/02
Initial Date

000161

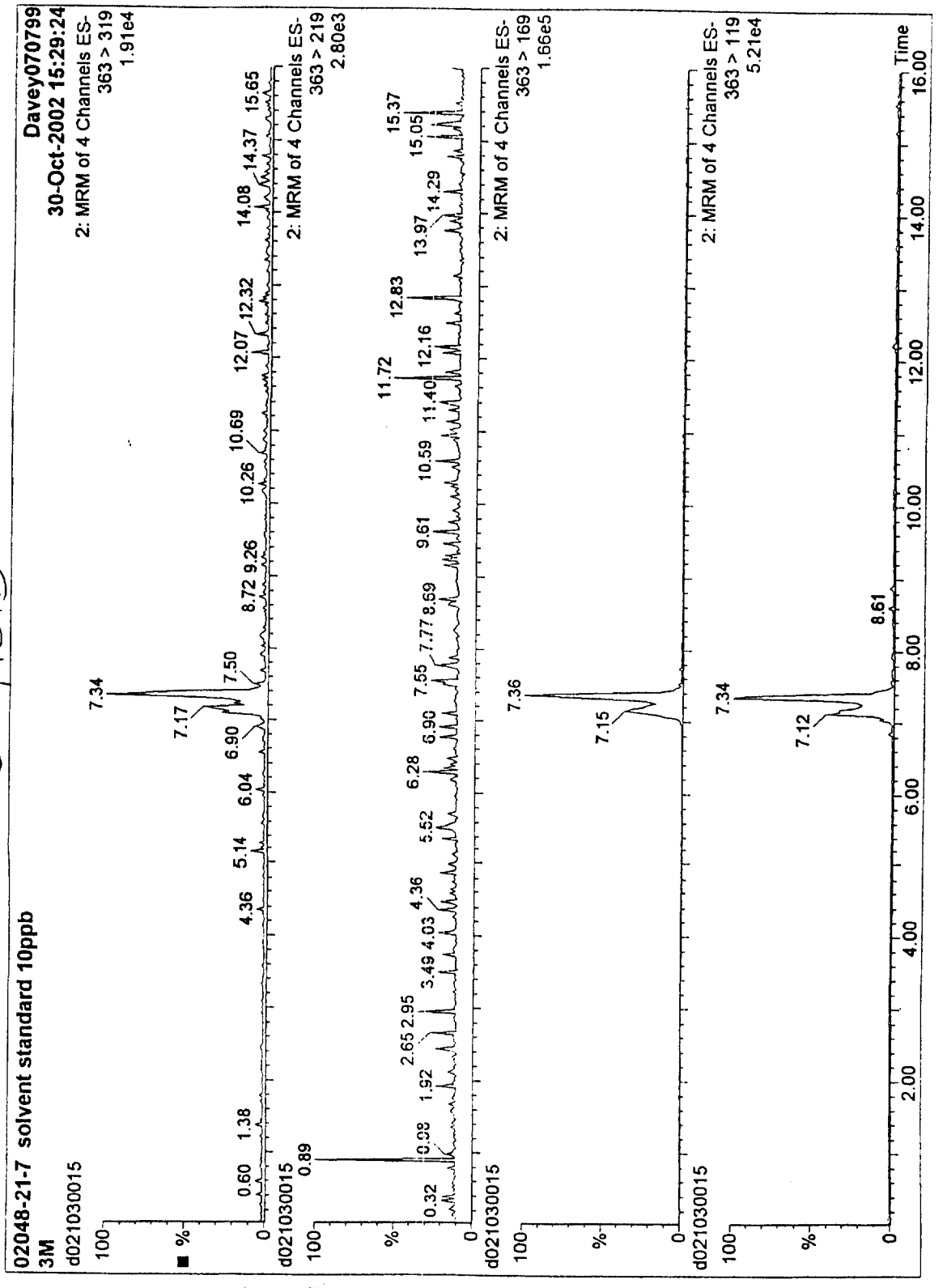
C7 ACID



Exact Copy of Original

CHK 11/7/02
Initial Date

000162

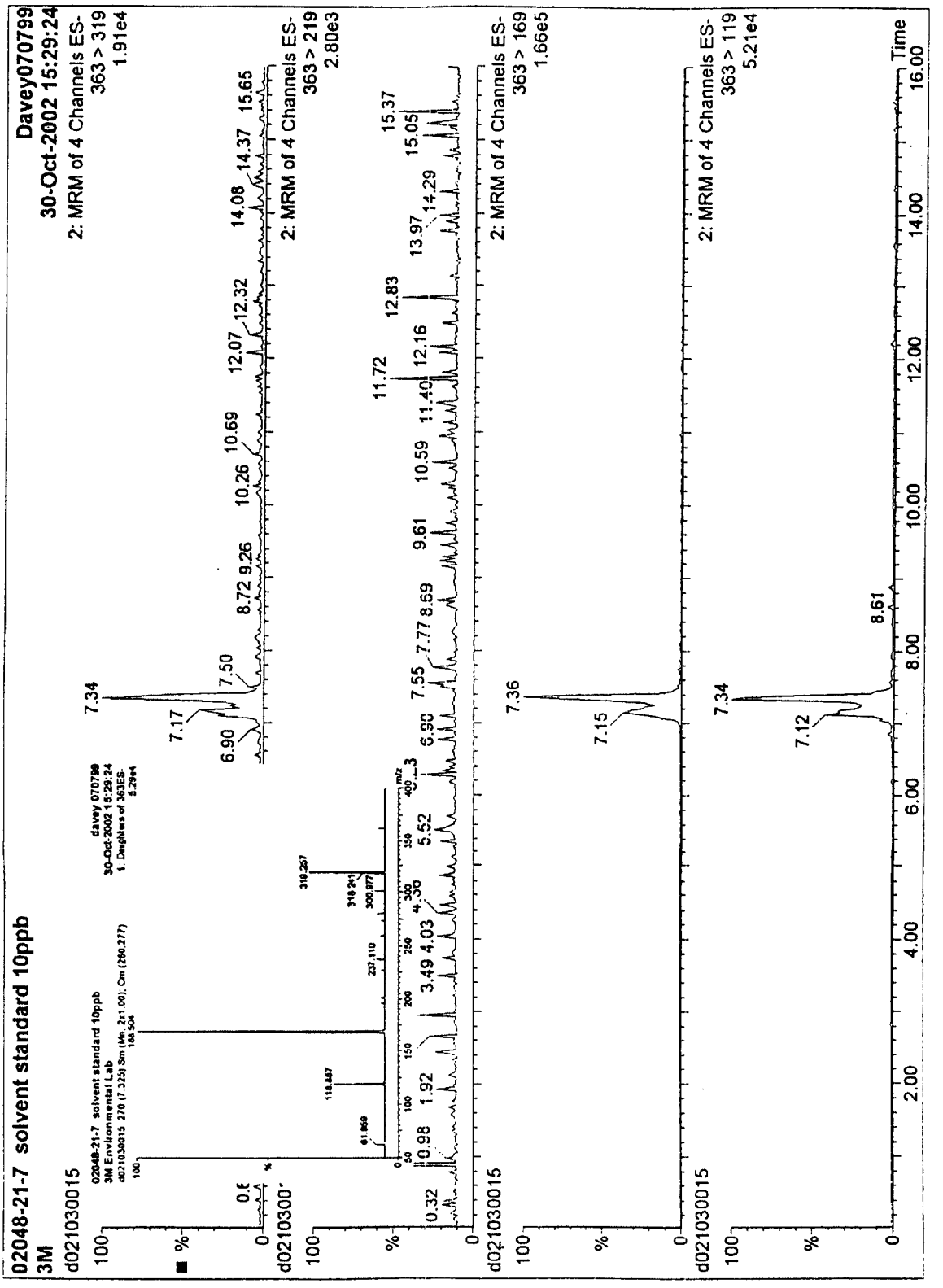


Exact Copy of Original

LMK 11/7/02
Initial Date

000163

C7 AcID



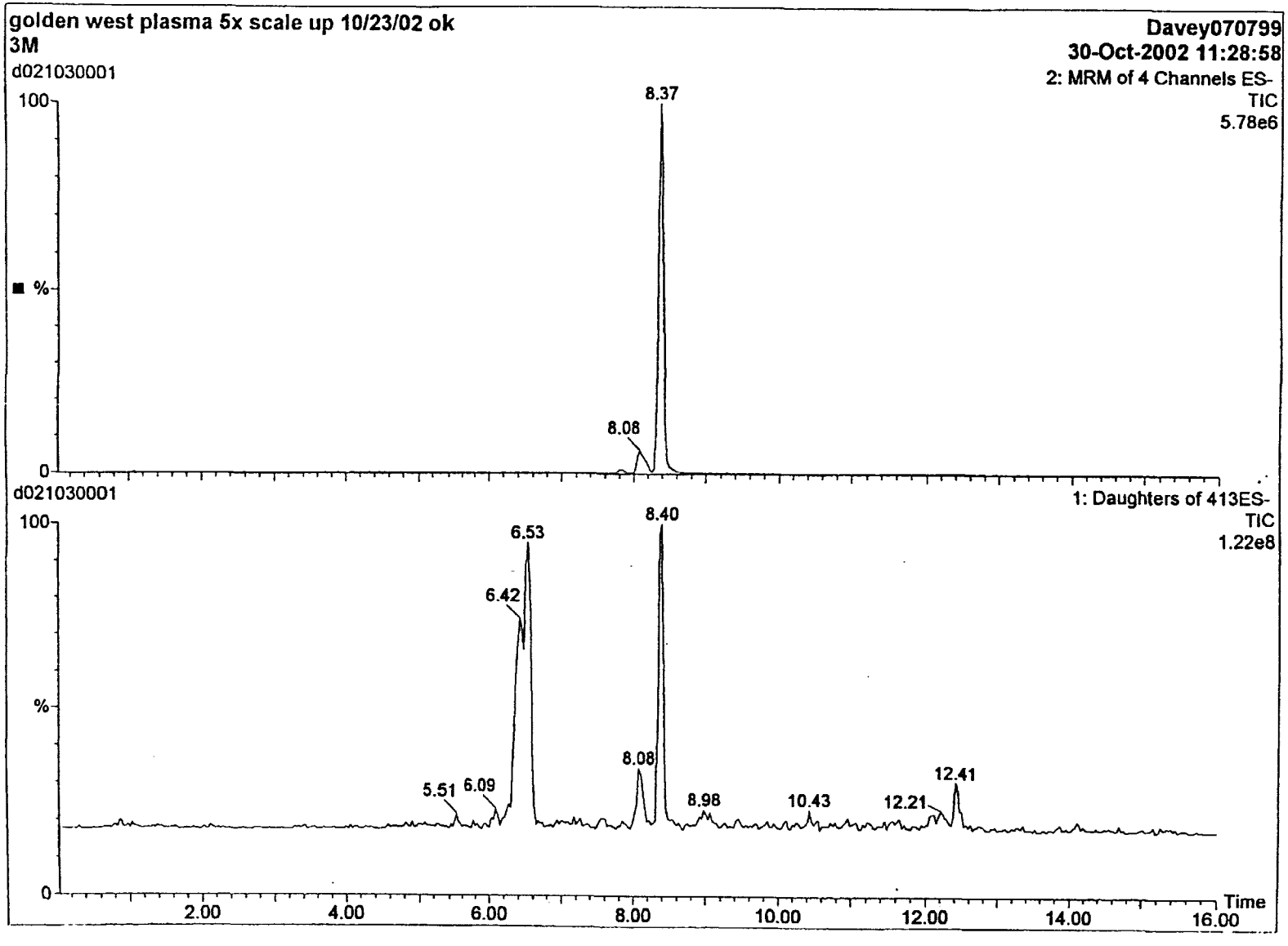
Exact Copy of Original

cmx 4/1/02

Initial Date

000164

C8 ACID

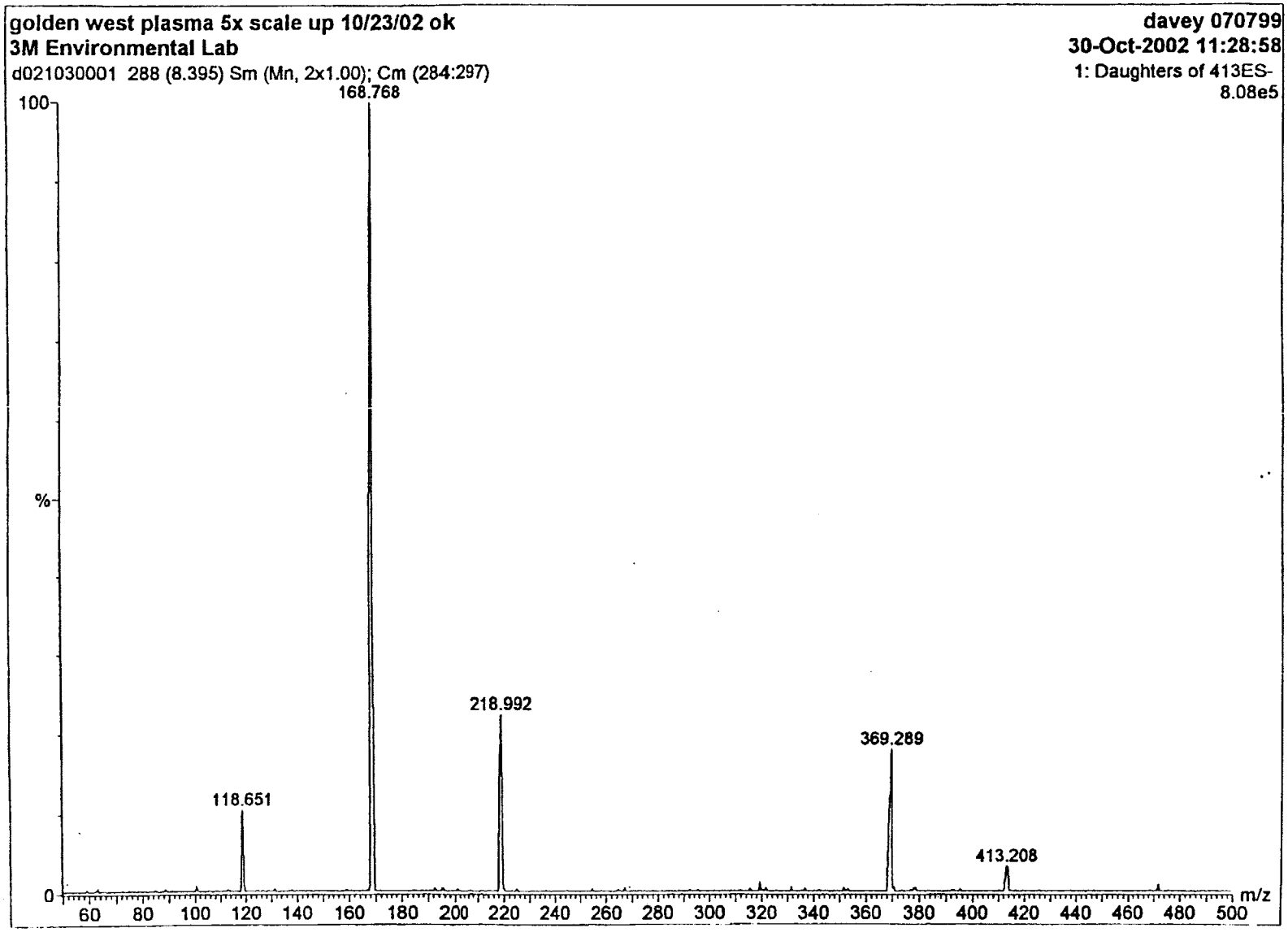


Exact Copy of Original

CMC 10/31/02
Initial Date

000165

C8 ACID



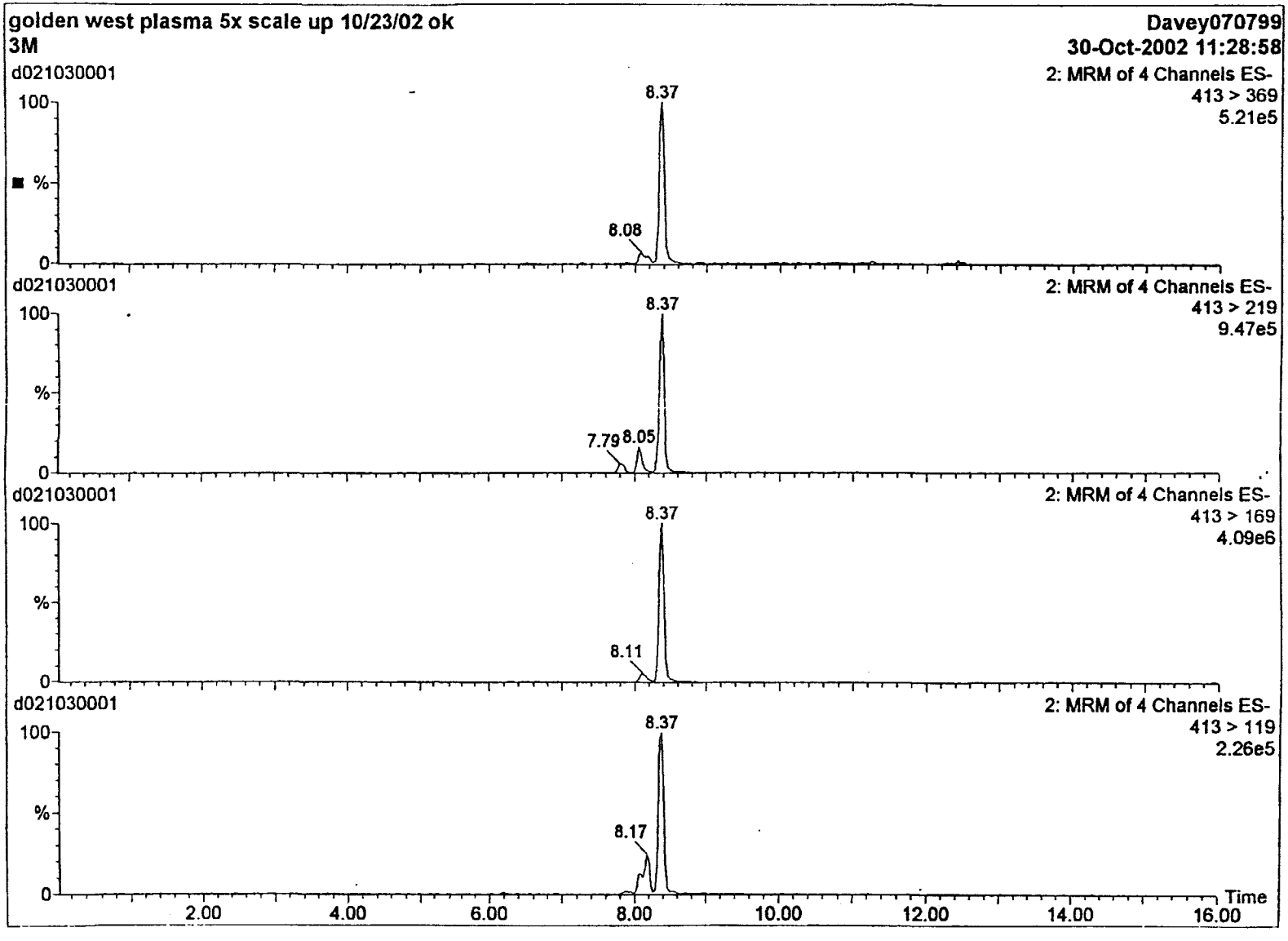
Exact Copy of Original

CMC 10/31/02

Initial Date

000166

C8 ACID

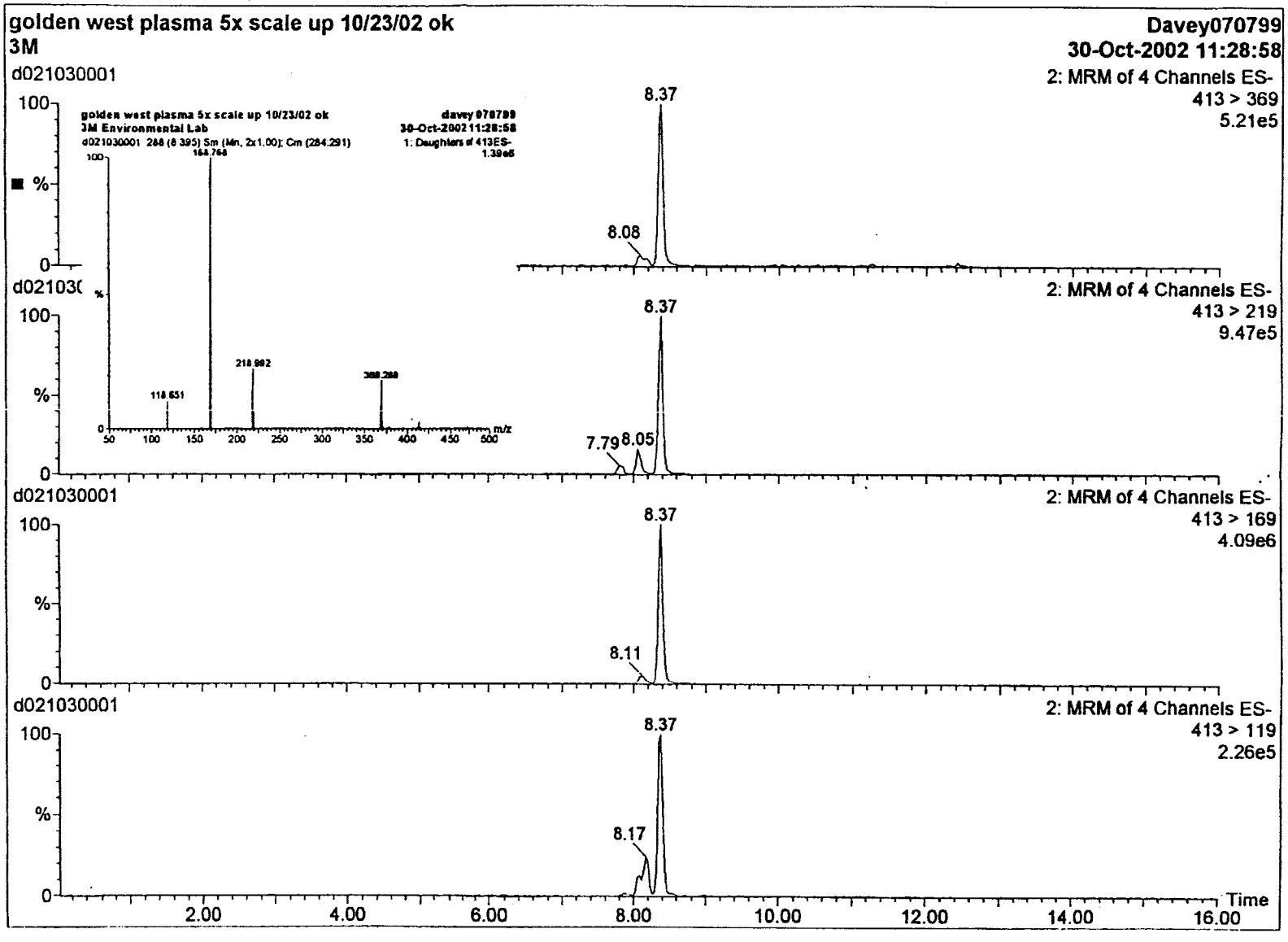


Exact Copy of Original

CMC 10/31/02
Initial Date

000167

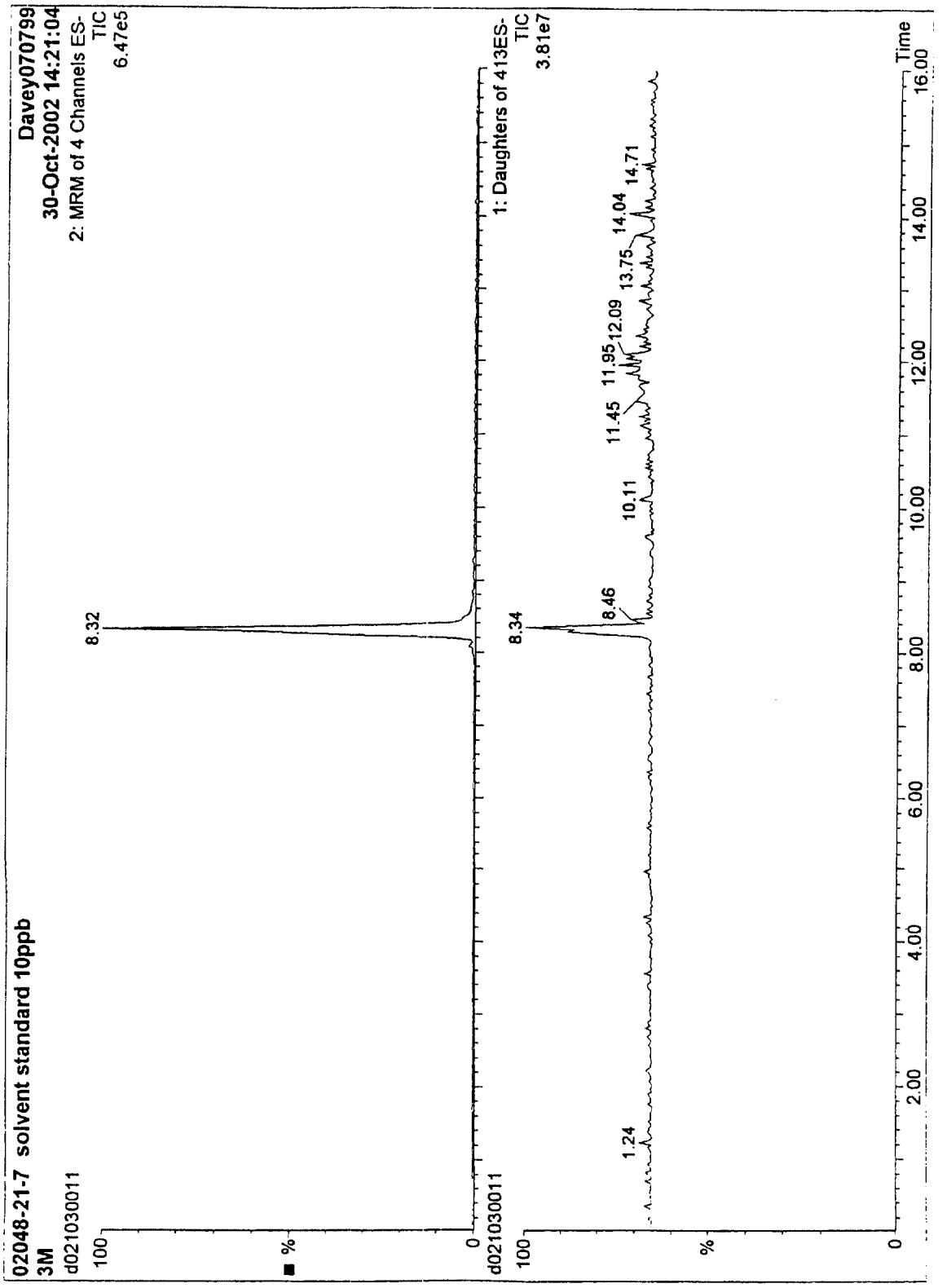
C8 PFOA ID



Exact Copy of Original

CMC 10/31/02
Initial Date

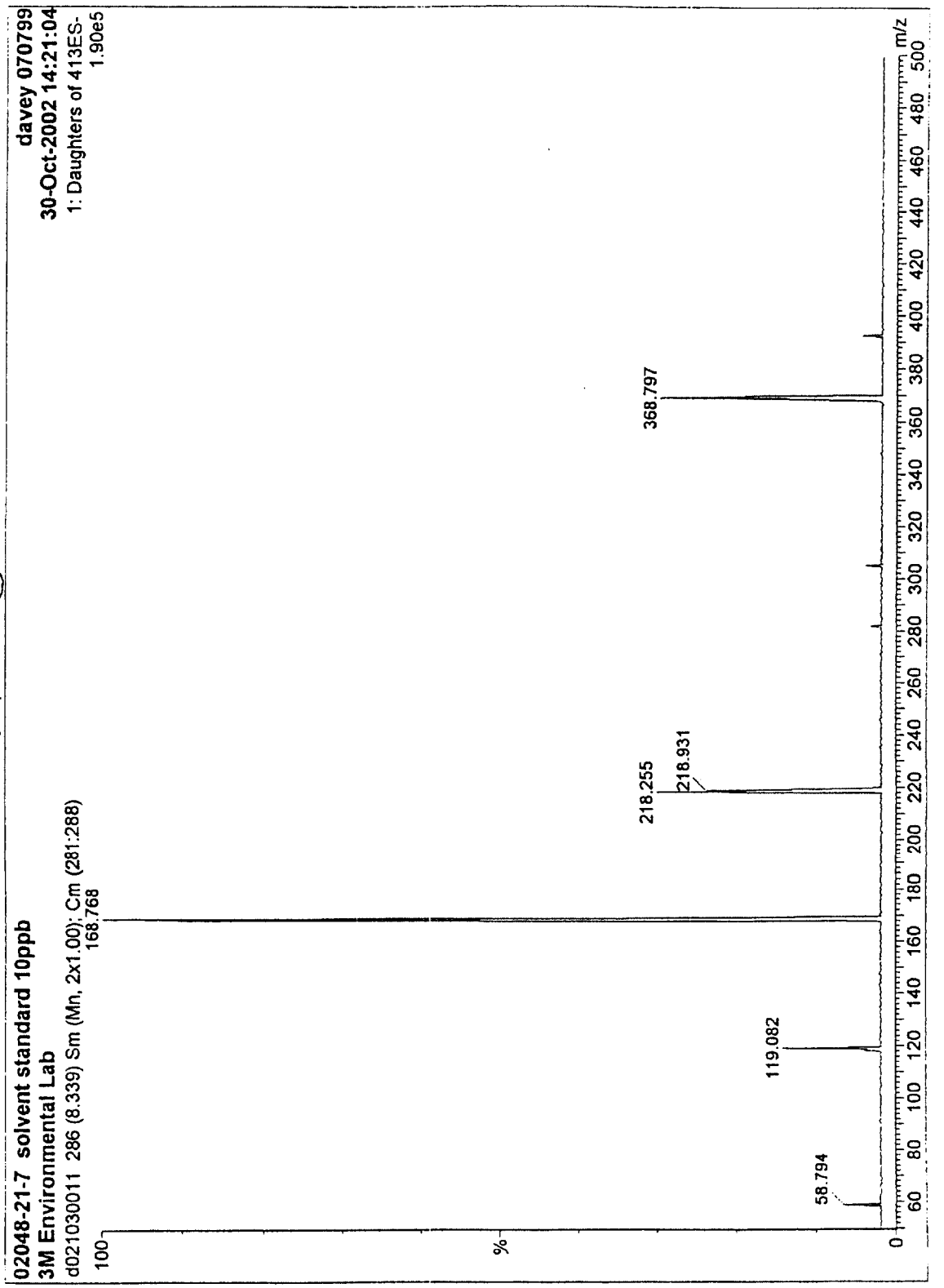
000168



Exact Copy of Original

Initial Date

000169

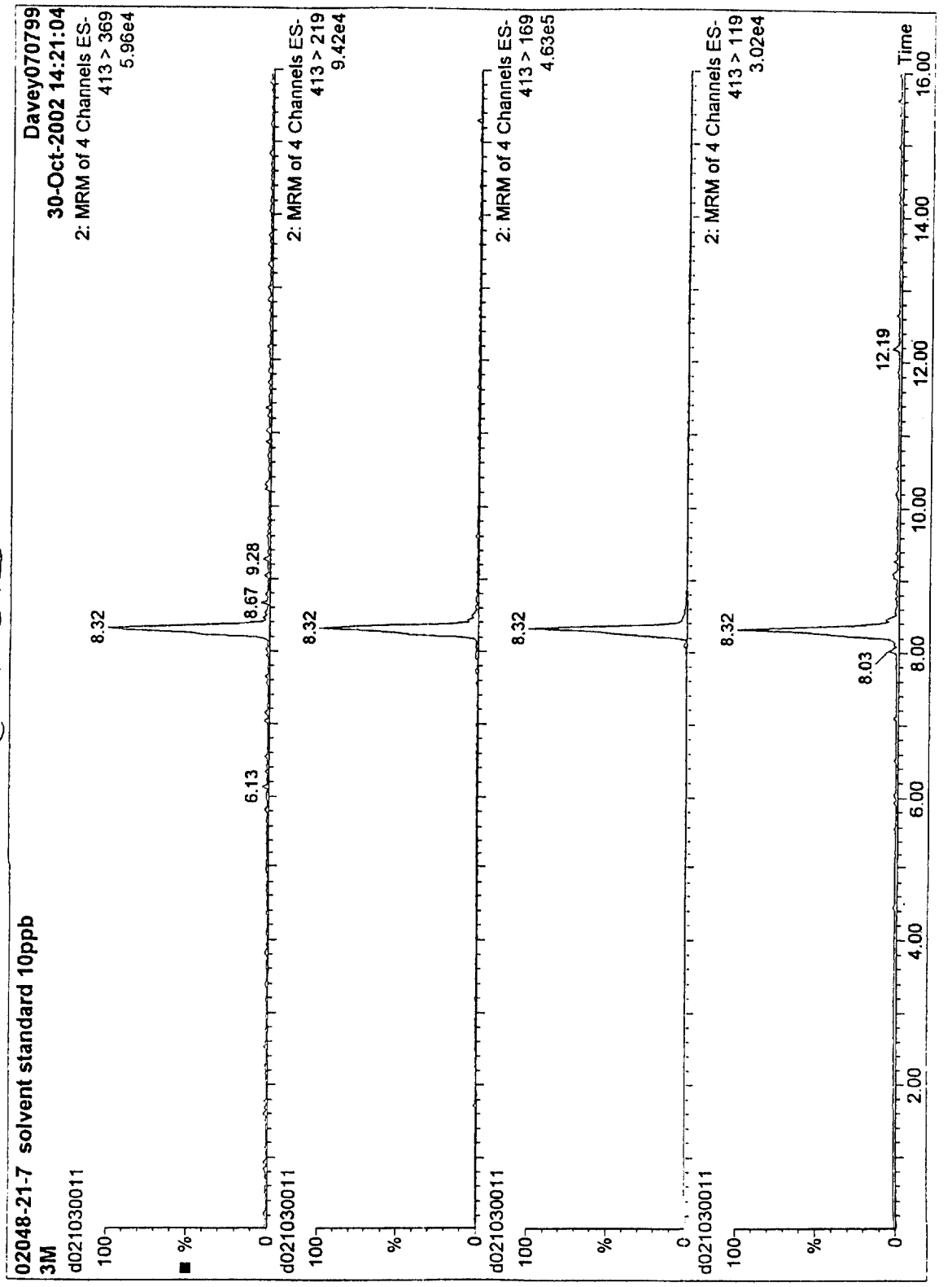


C8 PCID

Exact Copy of Original

CMS 11/1/02
Initial Date

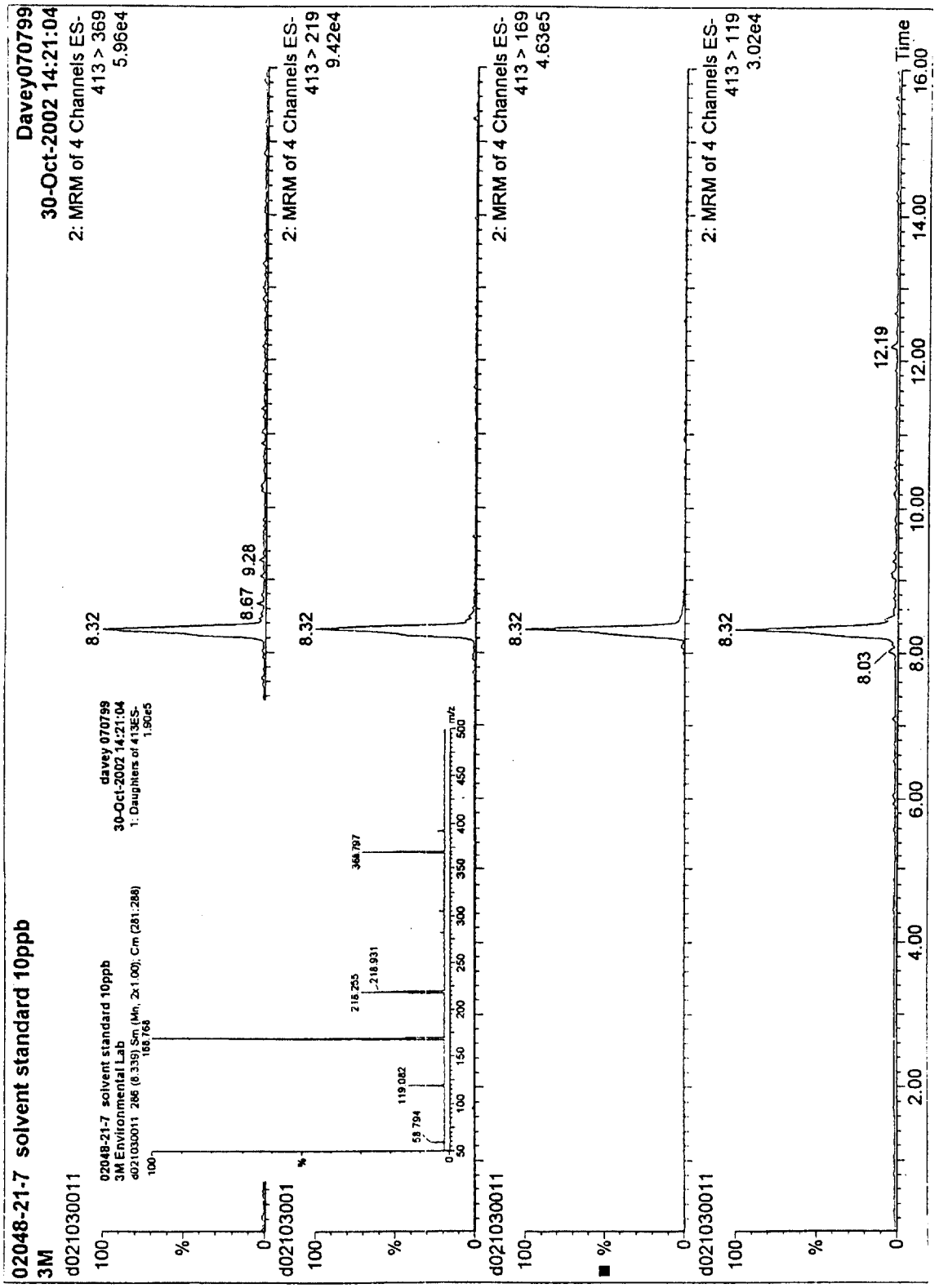
0001170



Exact Copy of Original

Initial Date

000171



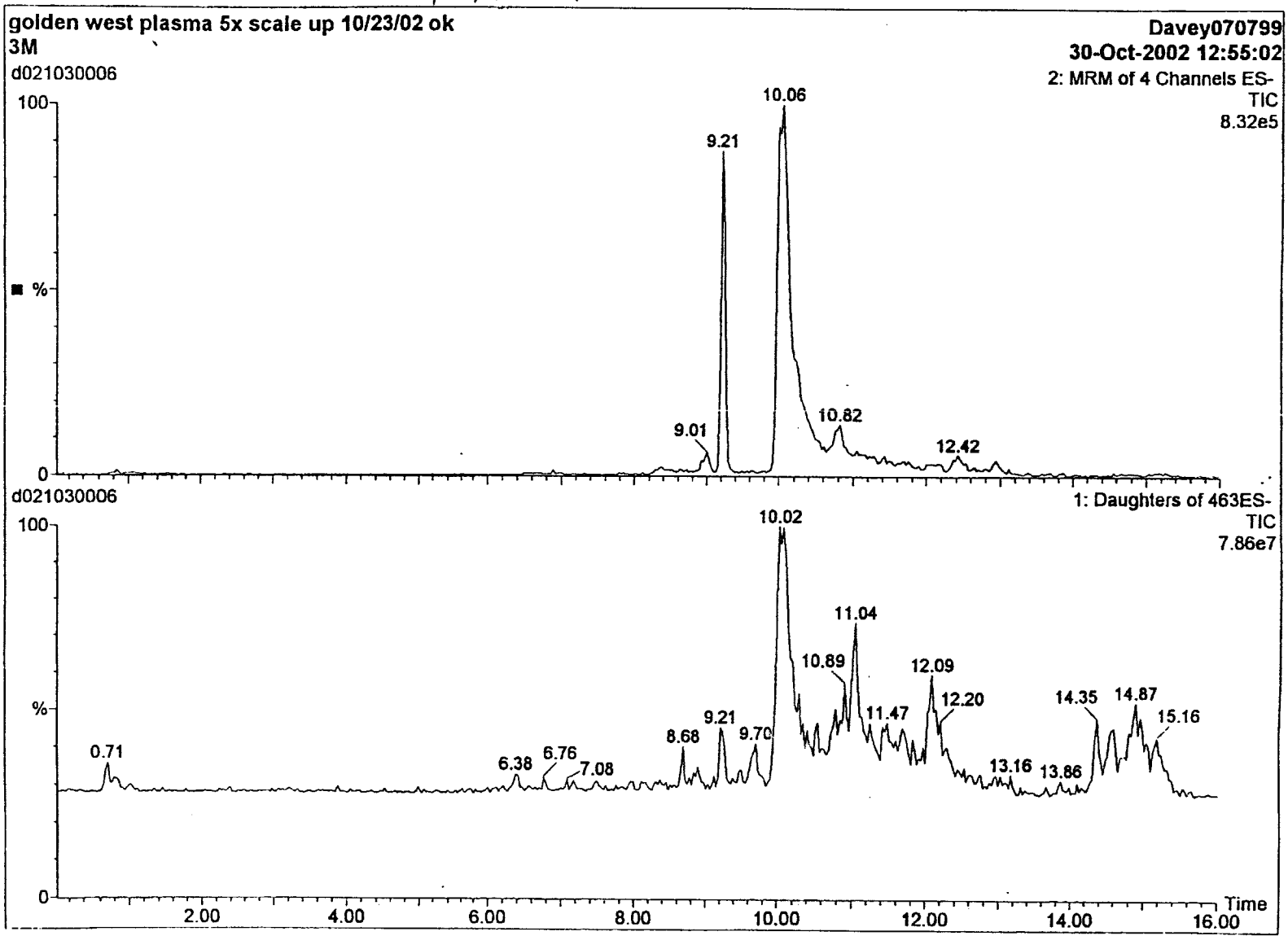
C8 HCID

Exact Copy of Original

Initial 11/7/02 Date

000172

C9 ACID



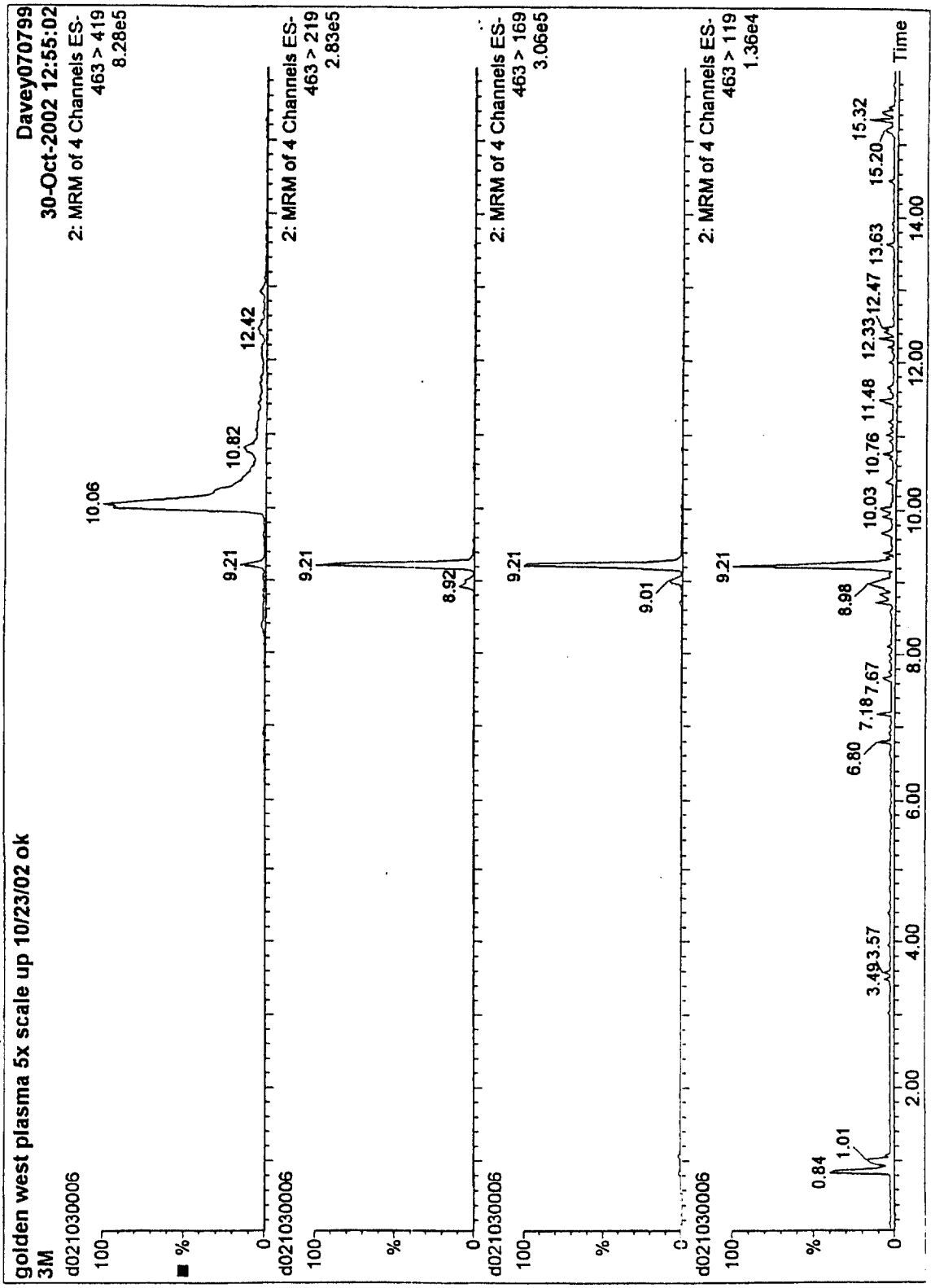
Exact Copy of Original

CMC 10/31/02

Initial Date

000173

Q19 ACID

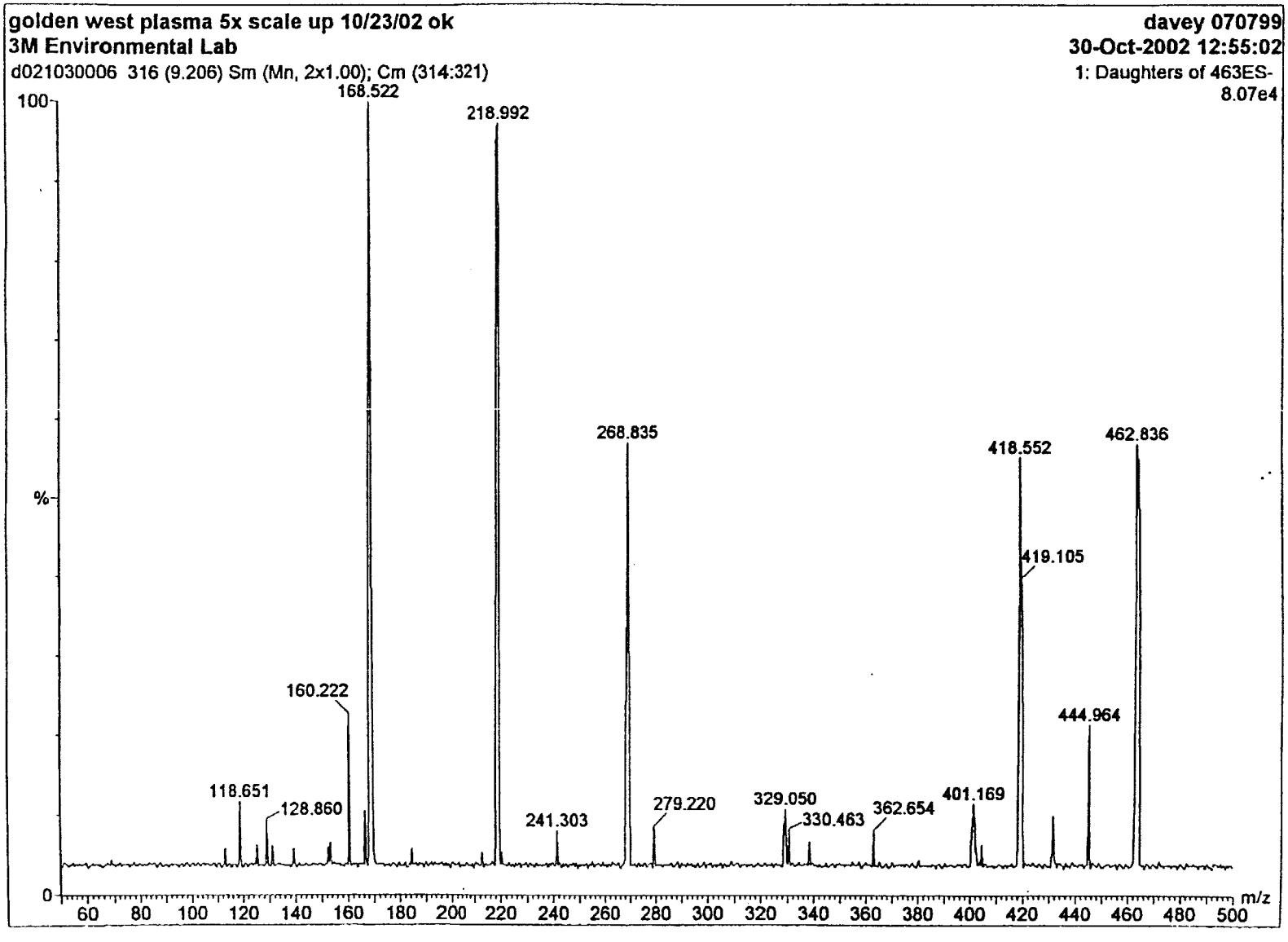


Exact Copy of Original

Initial Date 10/31/02

000174

C9 PFUnD

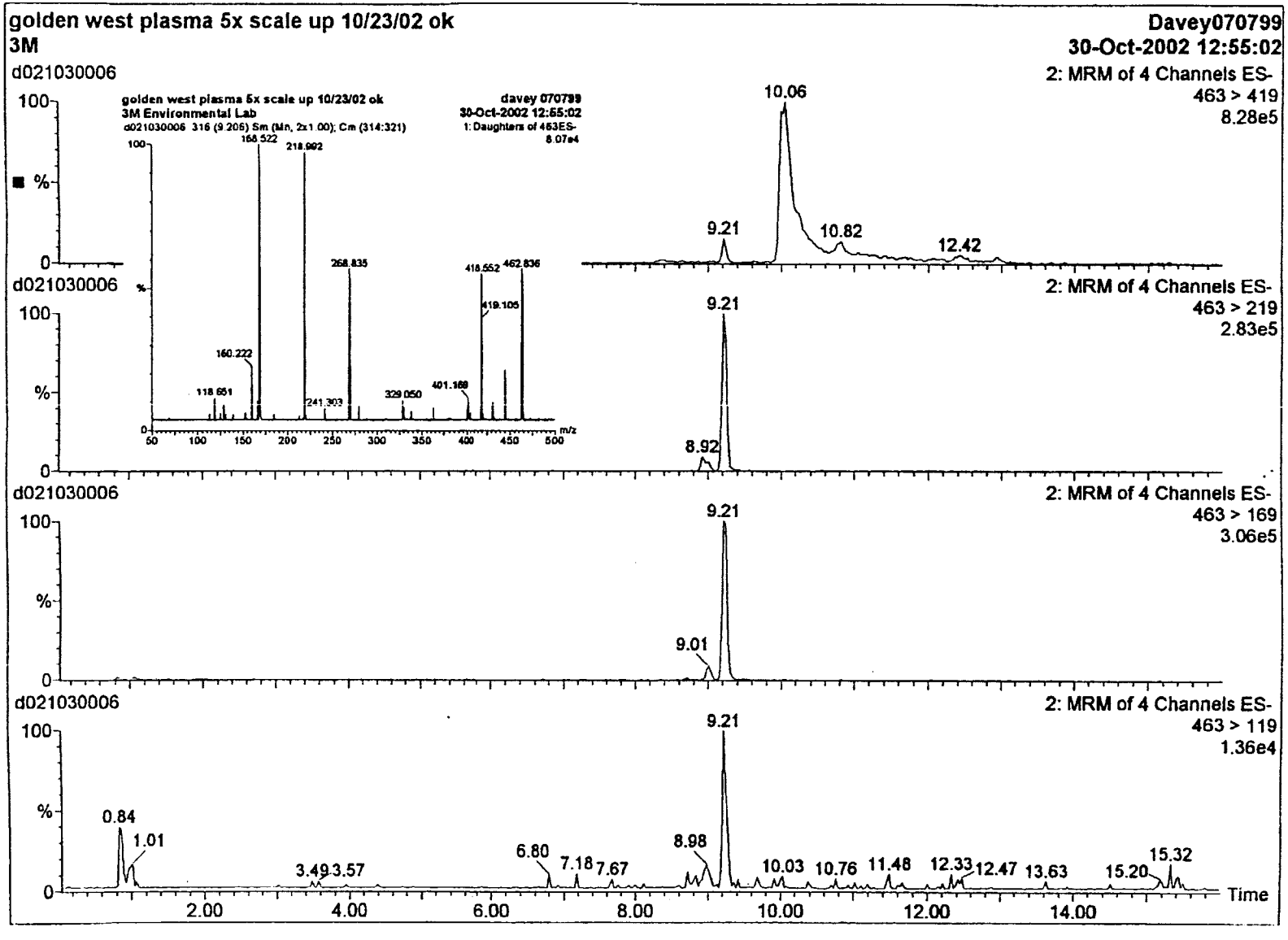


Exact Copy of Original

CMC 10/31/02
Initial Date

000175

C9 ACID

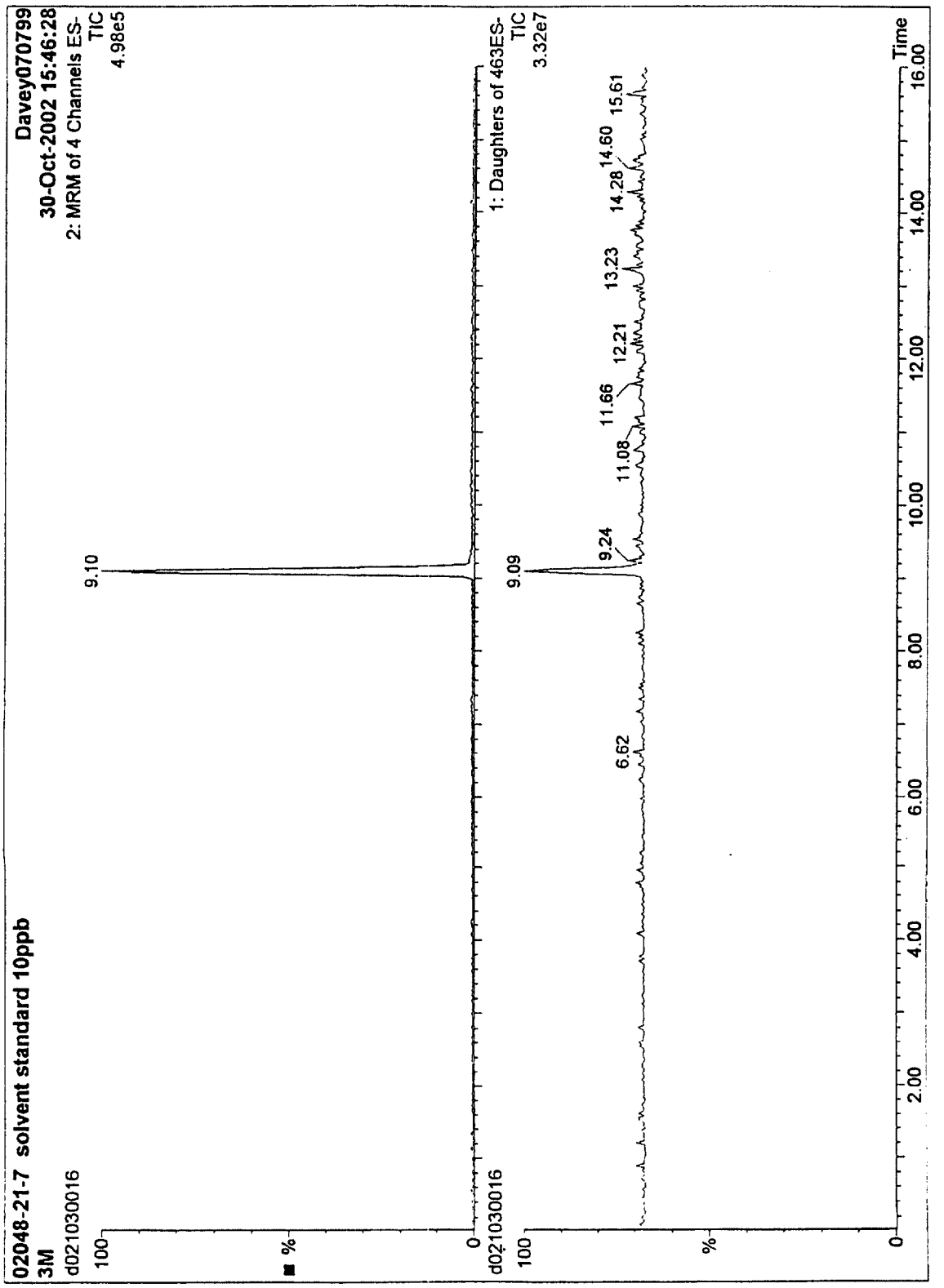


Exact Copy of Original

CMC 10/31/02
Initial Date

000176

C9 ACID



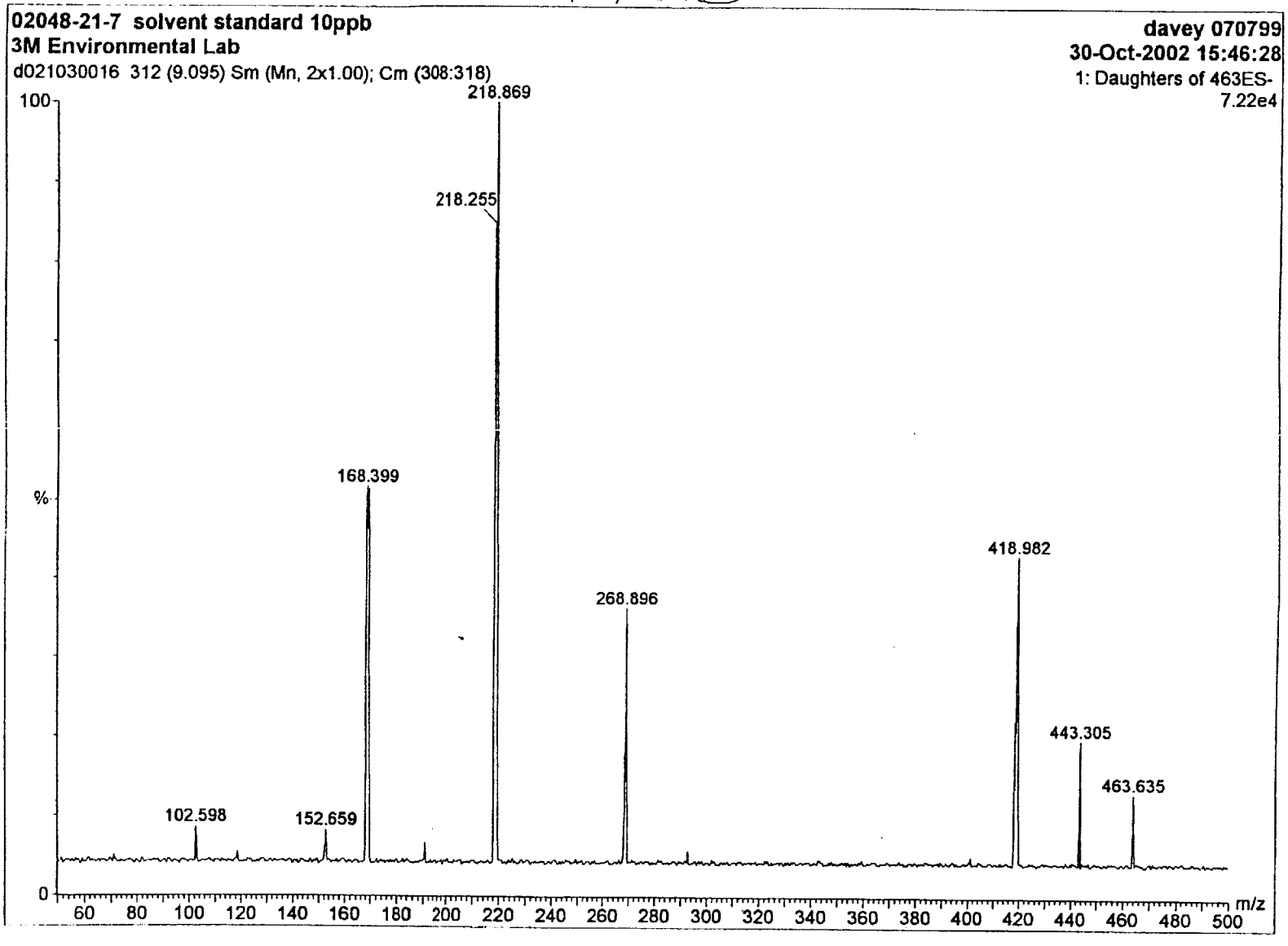
Exact Copy of Original

GMC 11/7/02

Initial Date

000177

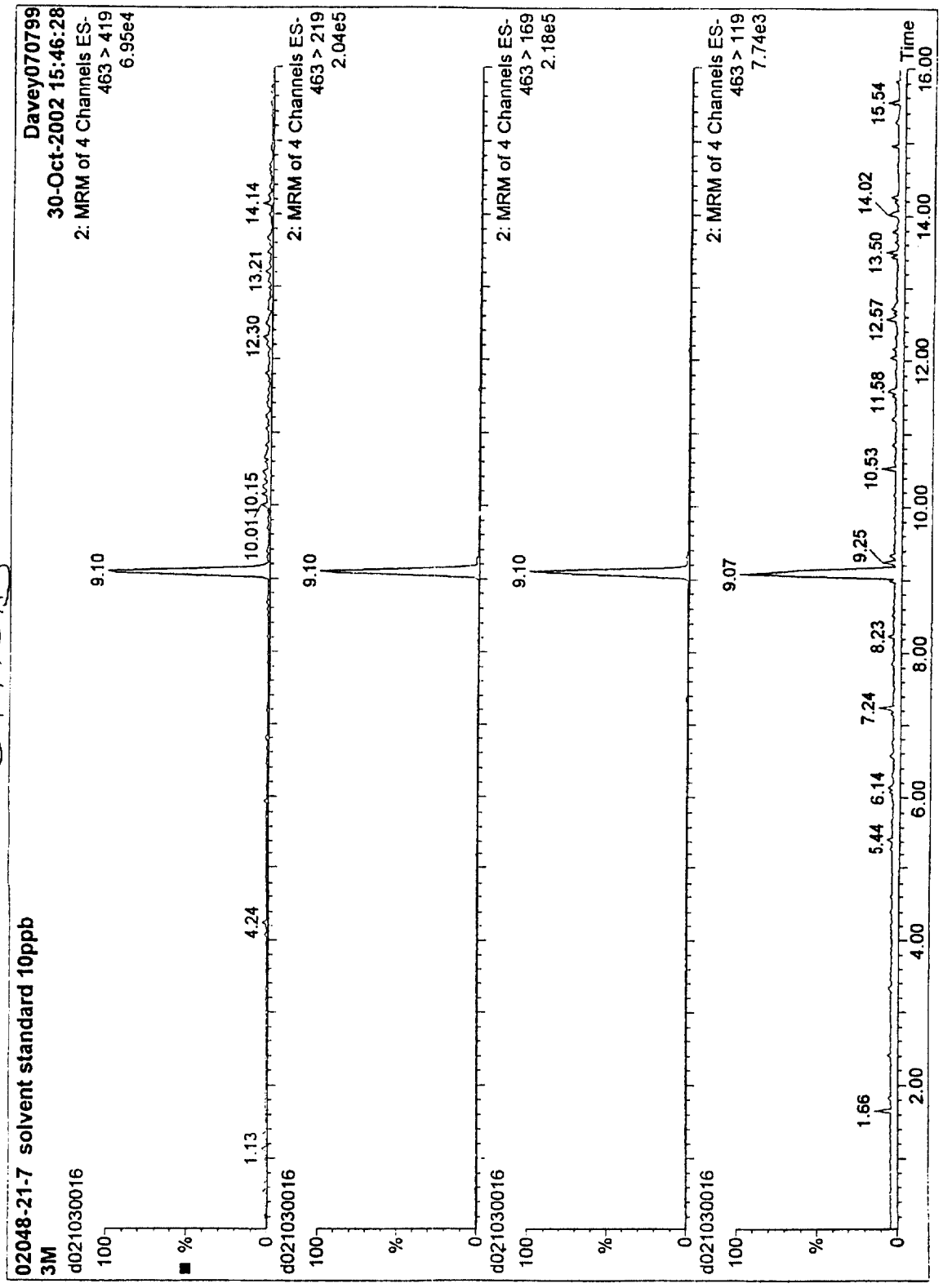
C9 ACID



Exact Copy of Original

CMC 11/1/02
Initial Date

000178

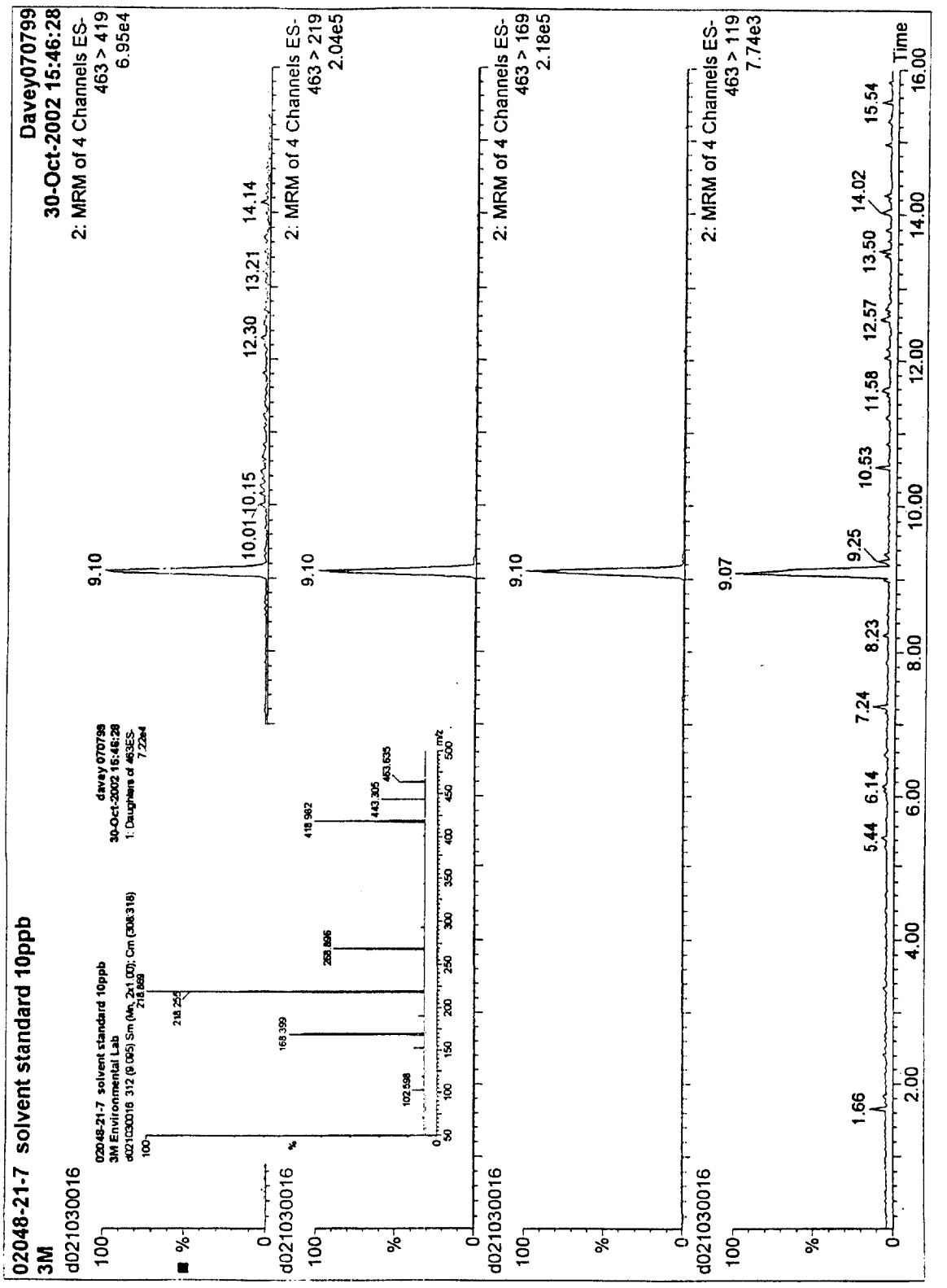


CQ PCID

Exact Copy of Original

Initial Date

000179



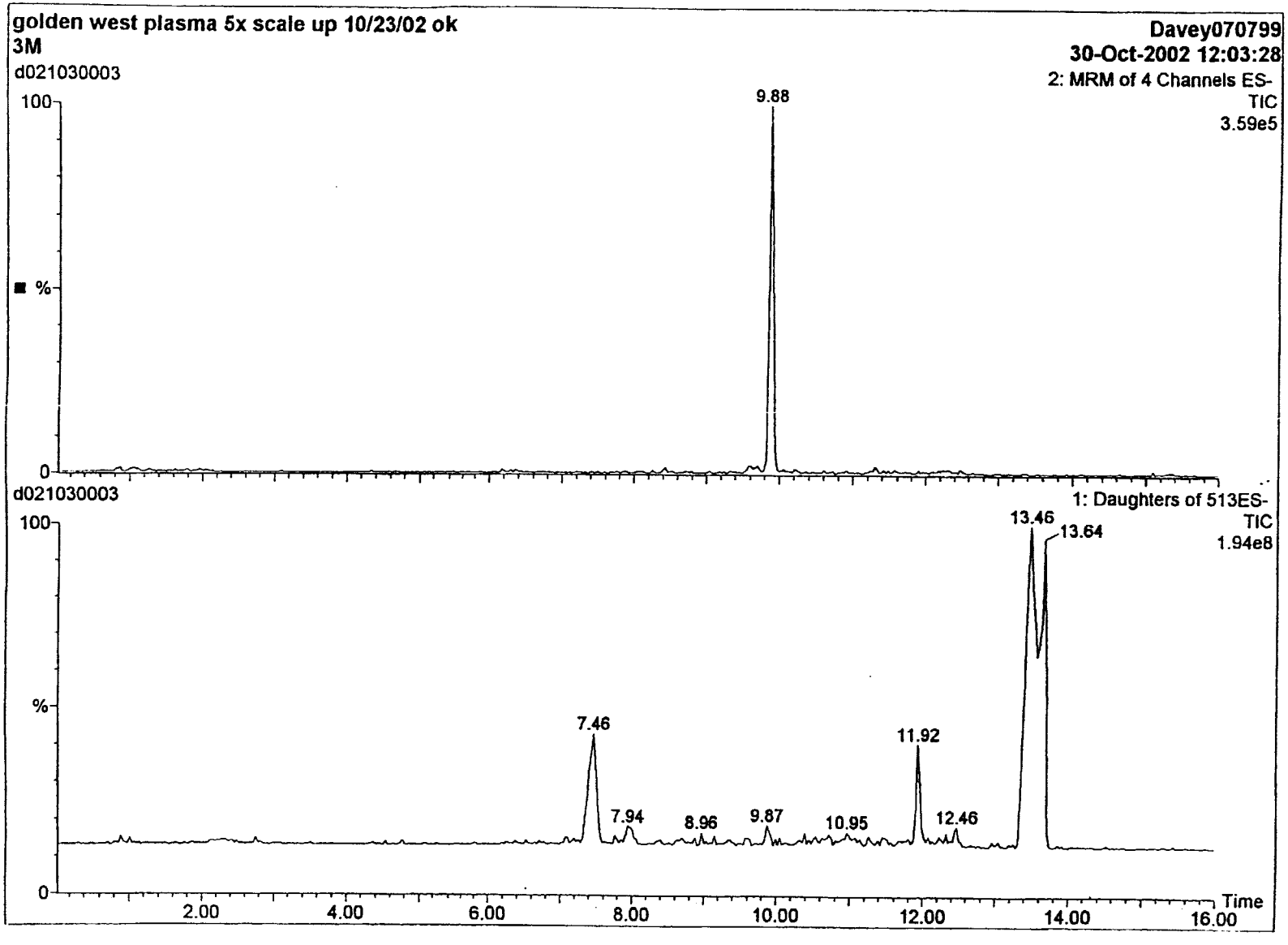
CG AAD

Exact Copy of Original

Initial Date 11/1/02

000180

C10 ACID

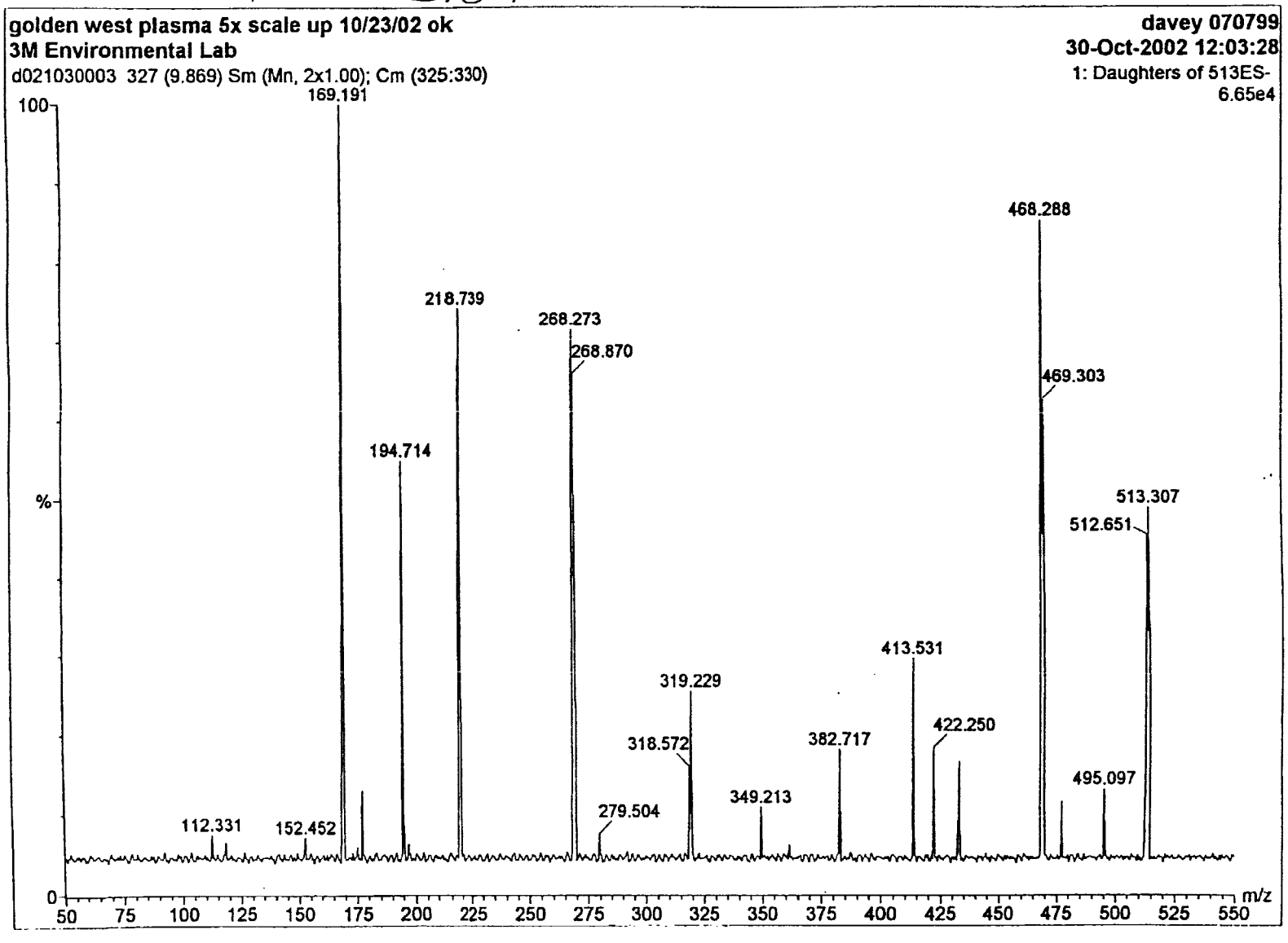


Exact Copy of Original

CMC 10/31/02
Initial Date

000181

5' C10 ACID

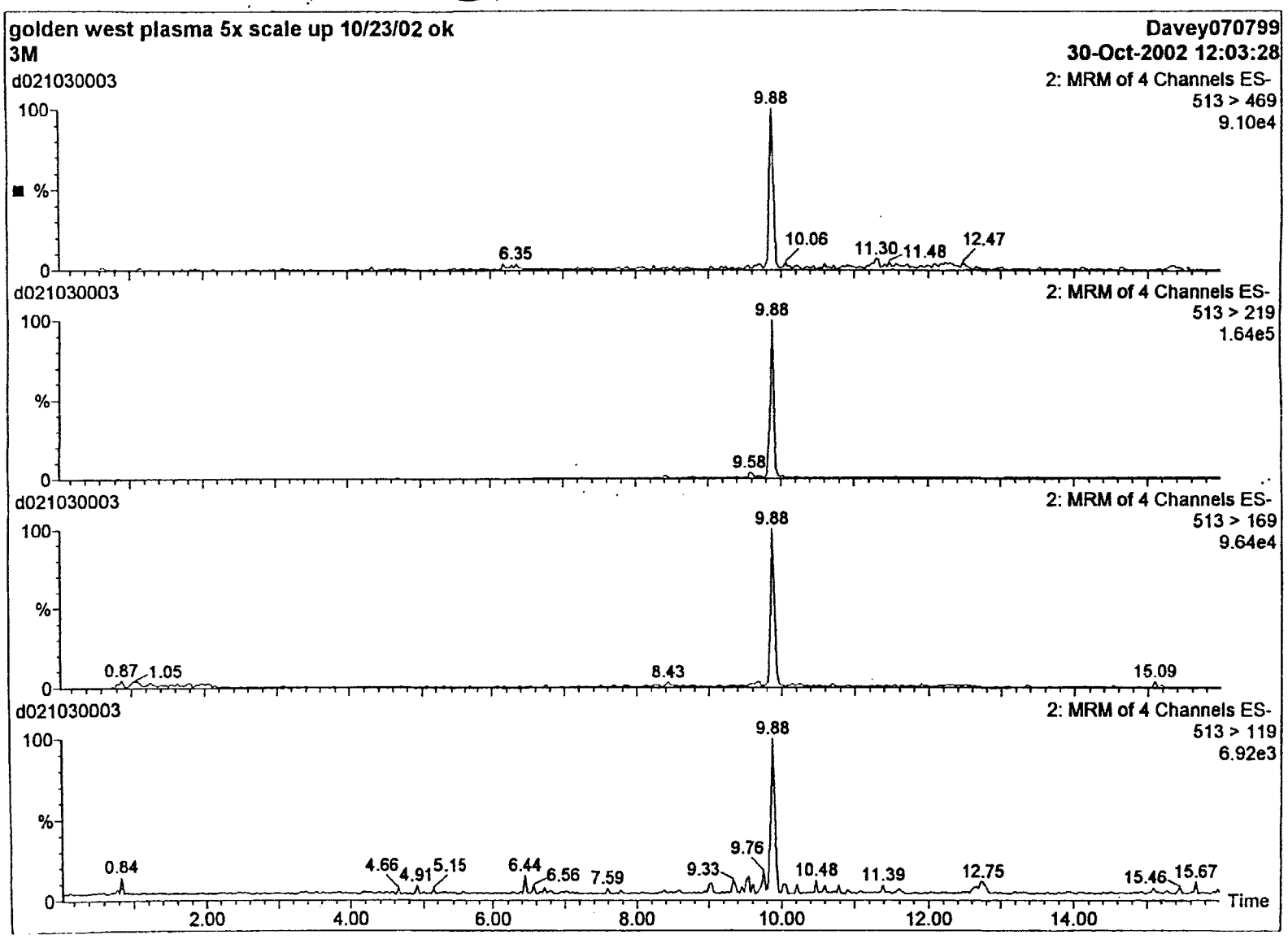


Exact Copy of Original

CMC 10/31/02
Initial Date

000182

C10 ACID



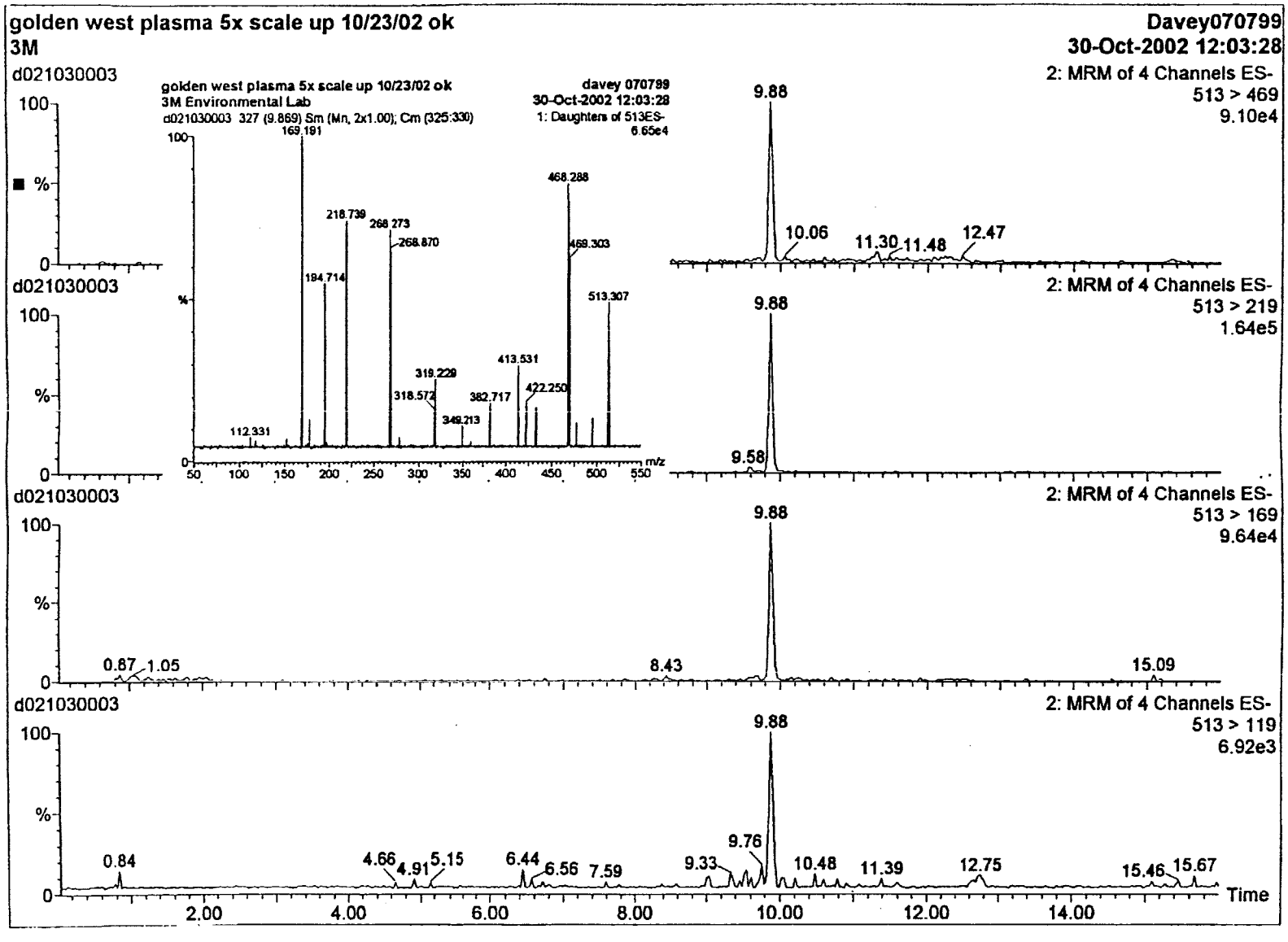
Exact Copy of Original

CMC 10/31/02

Initial Date

000183

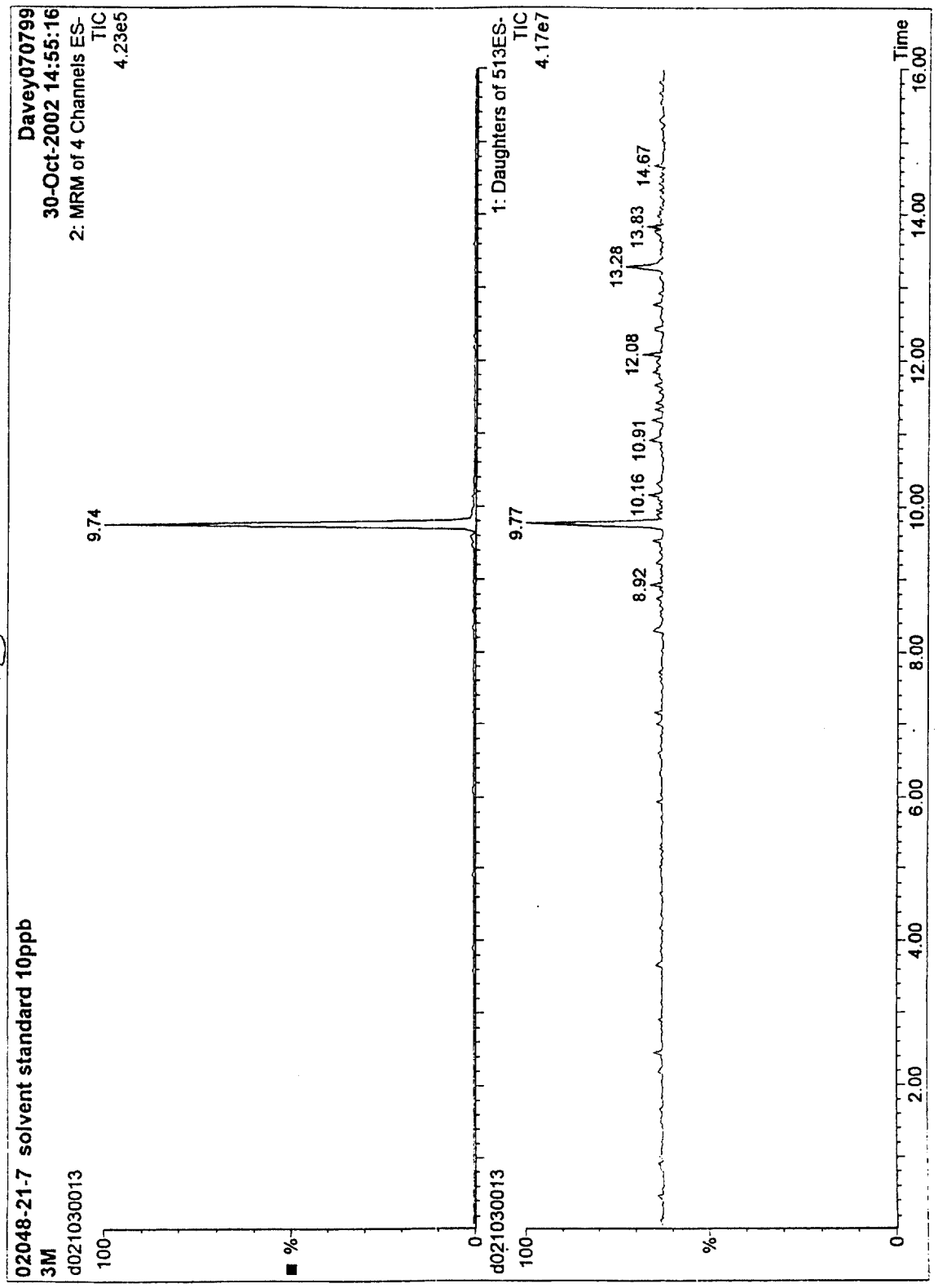
C10 ACID



Exact Copy of Original

CMC 10/31/02
Initial Date

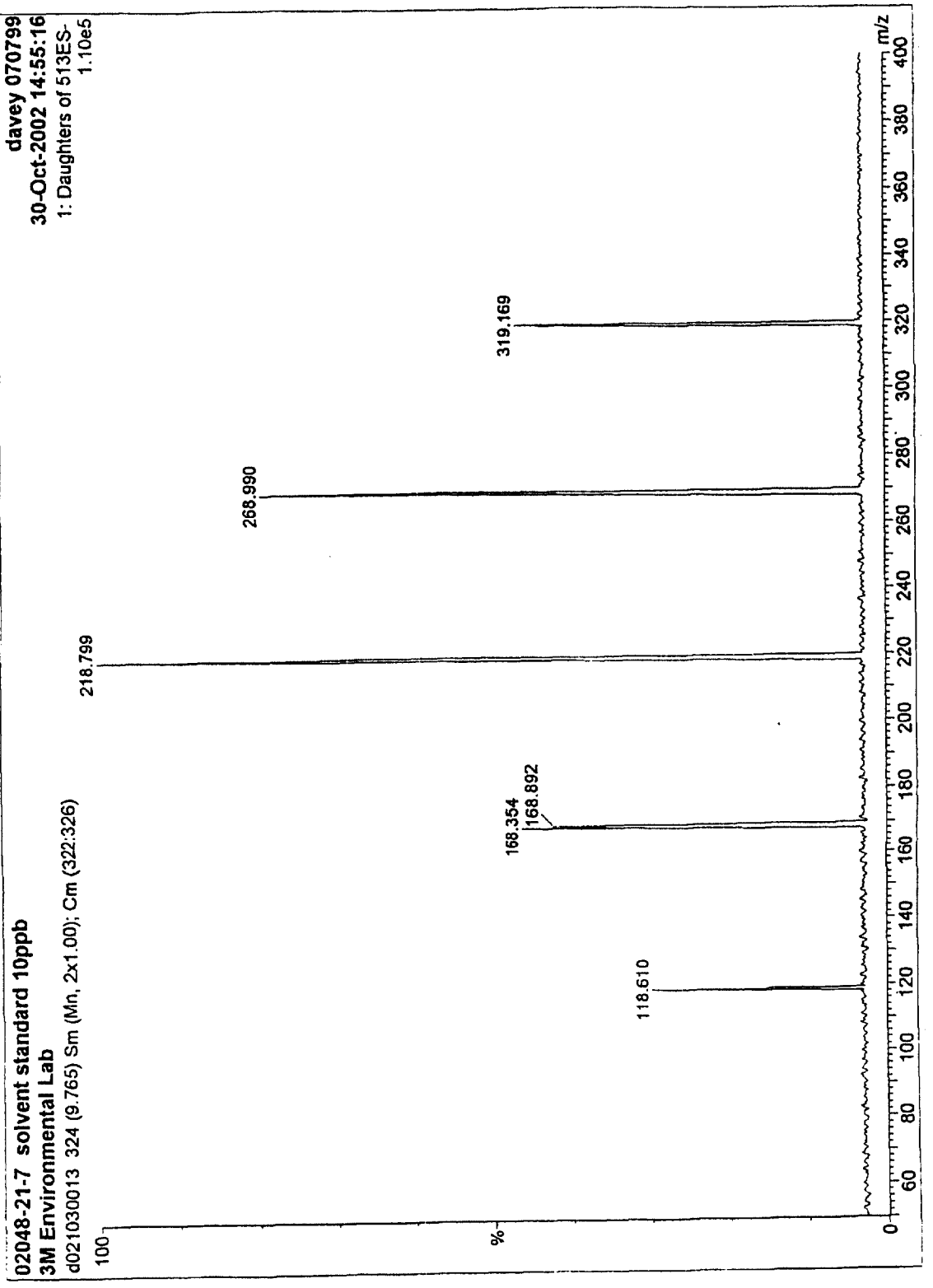
000184



Exact Copy of Original

Initial Date

000185



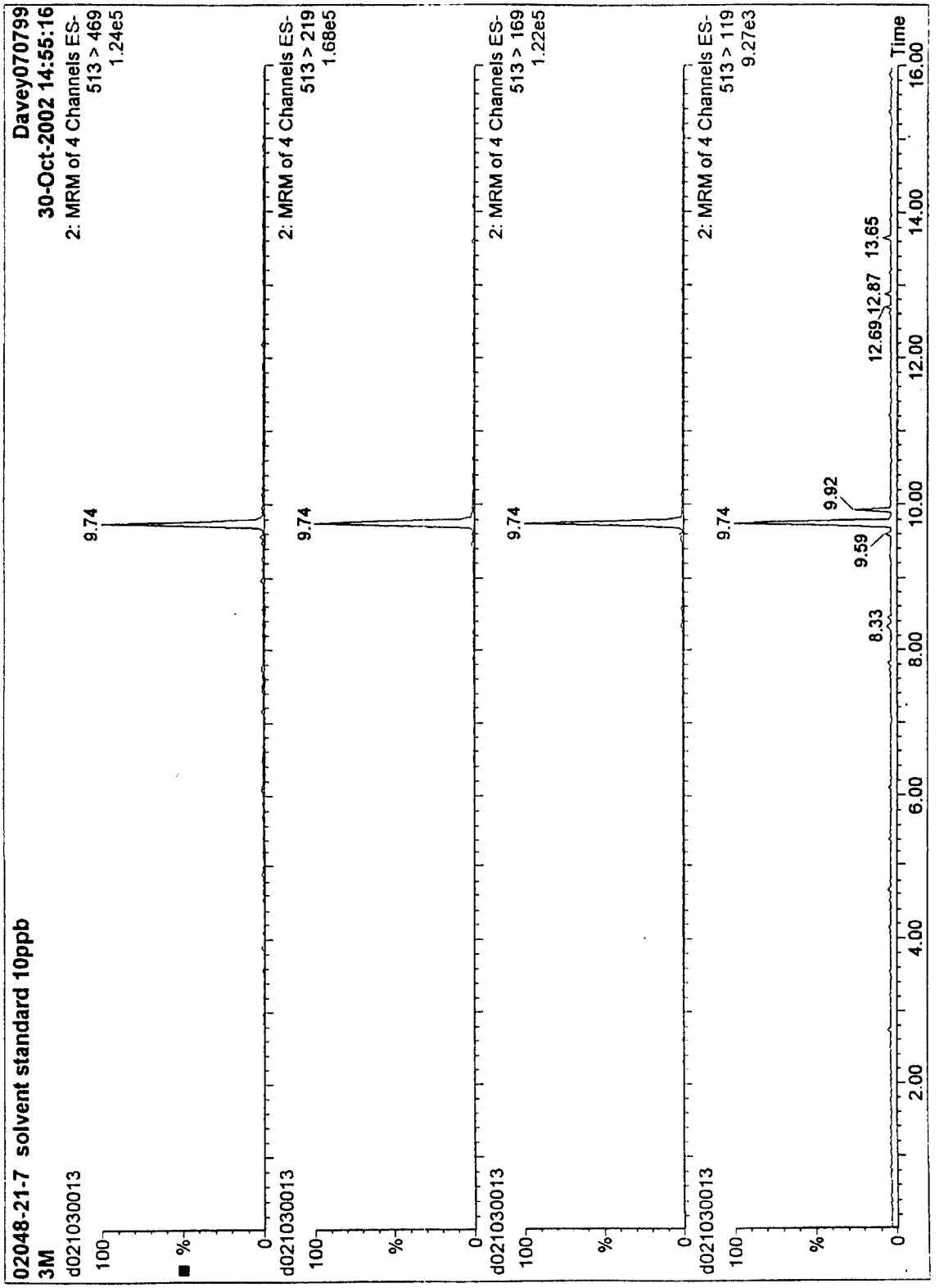
Exact Copy of Original

Initial Date

11/21/02

000186

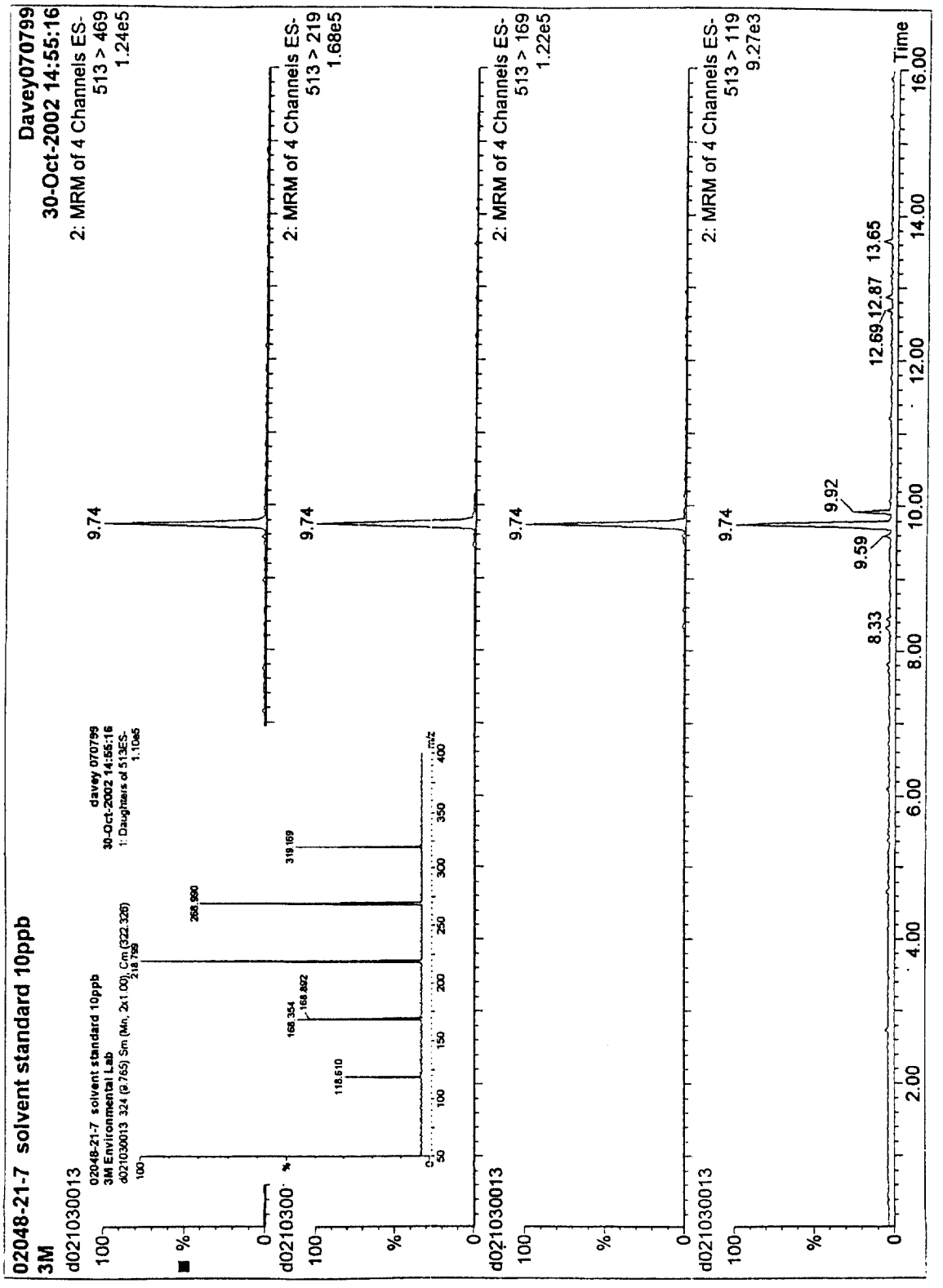
C1D 2&1D



Exact Copy of Original

Initial Date

000187



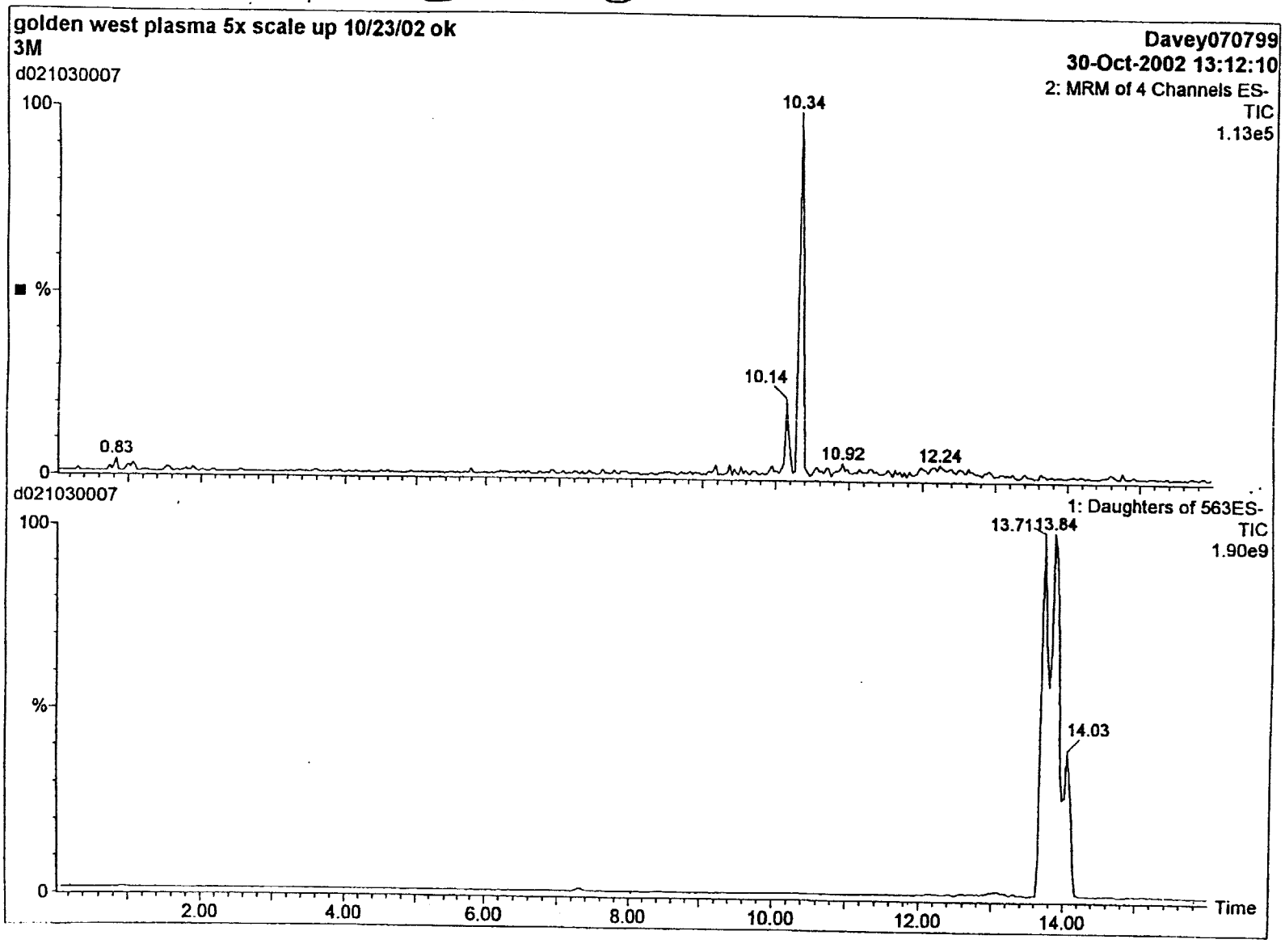
CID ACID

Exact Copy of Original

Initial Date

000188

C11 ACID



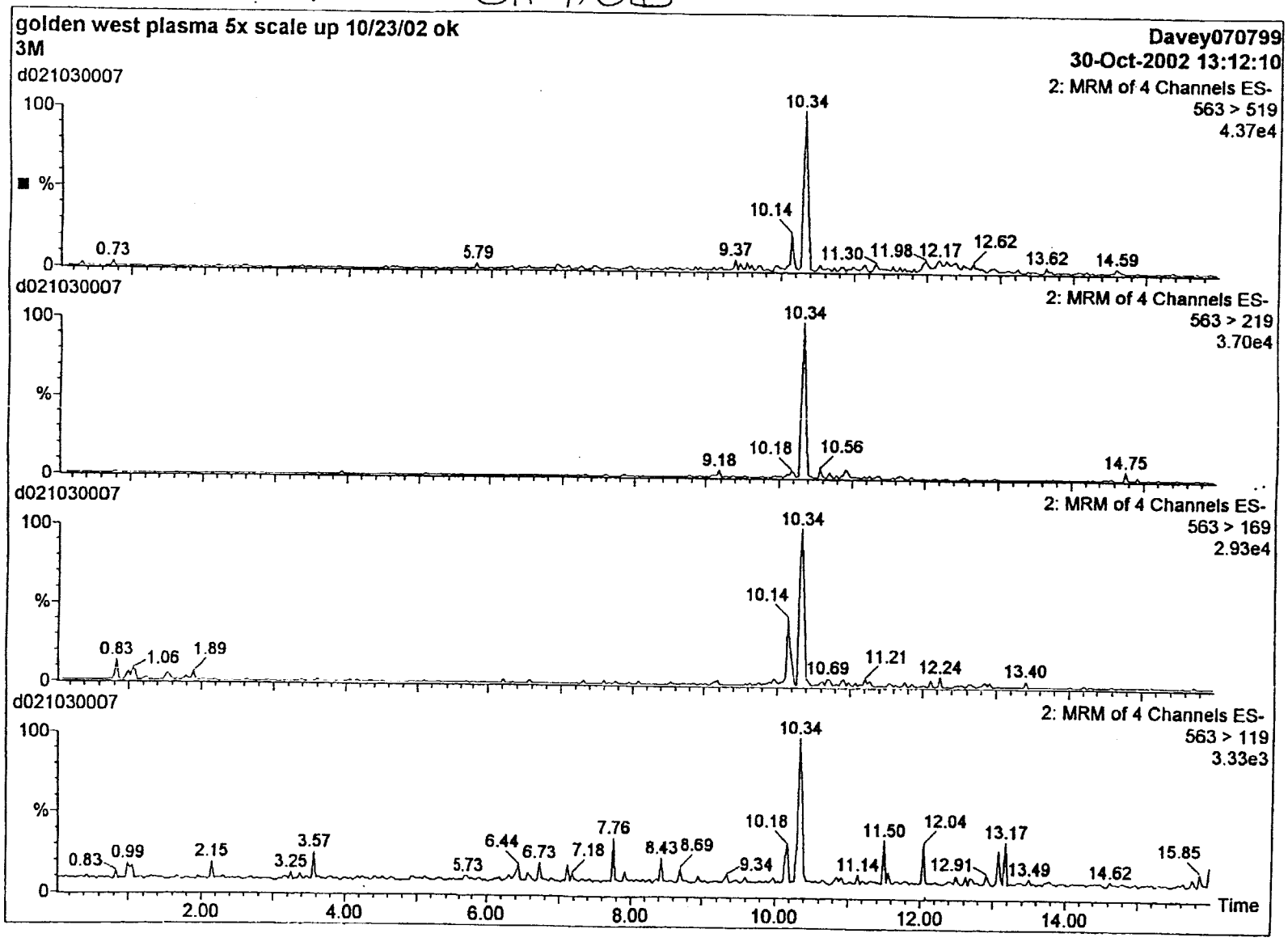
Exact Copy of Original

CMC 10/31/02

Initial Date

000189

C11 ACID

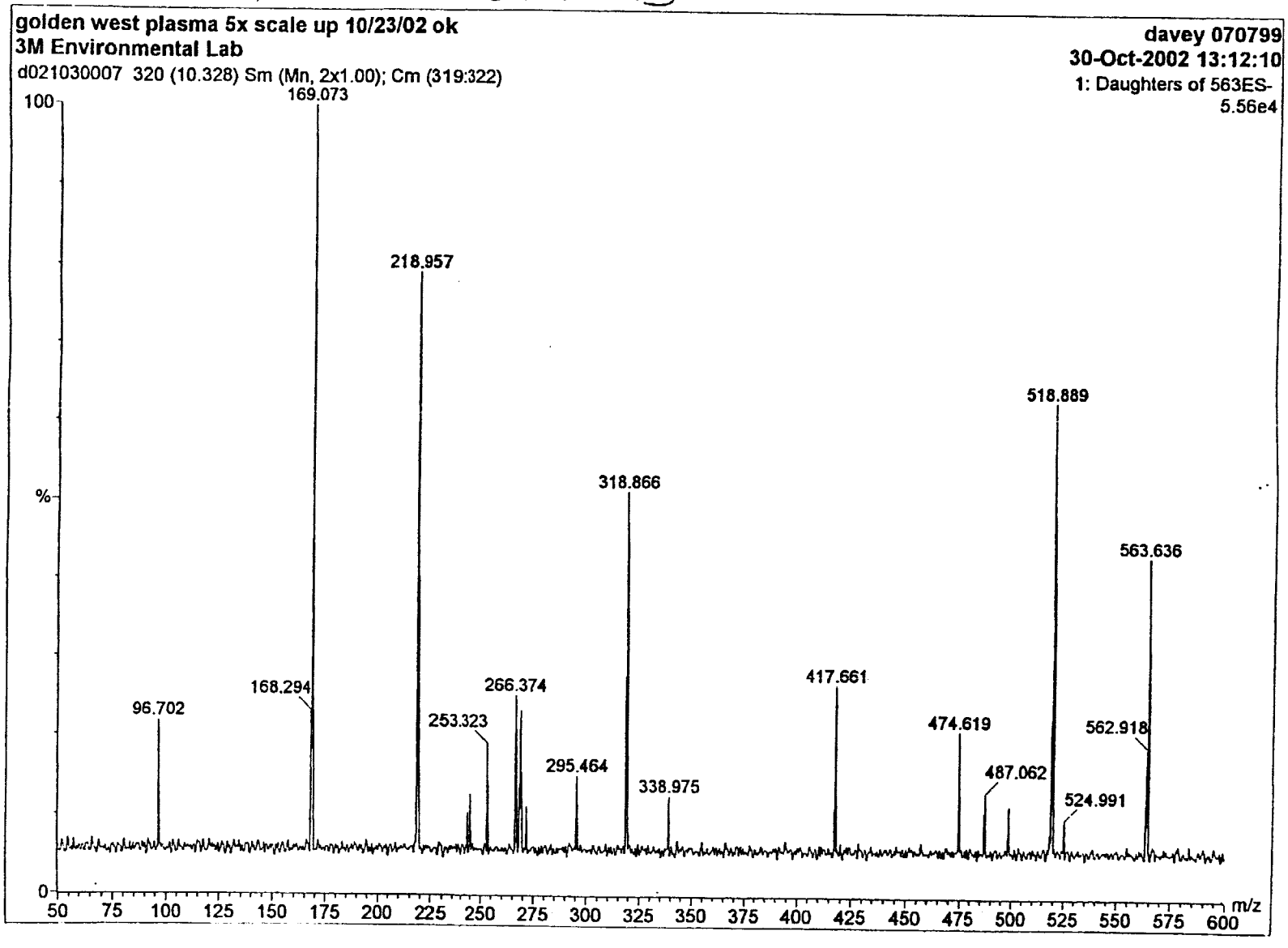


Exact Copy of Original

CMC 10/31/02
Initial Date

000190

C11 ACID

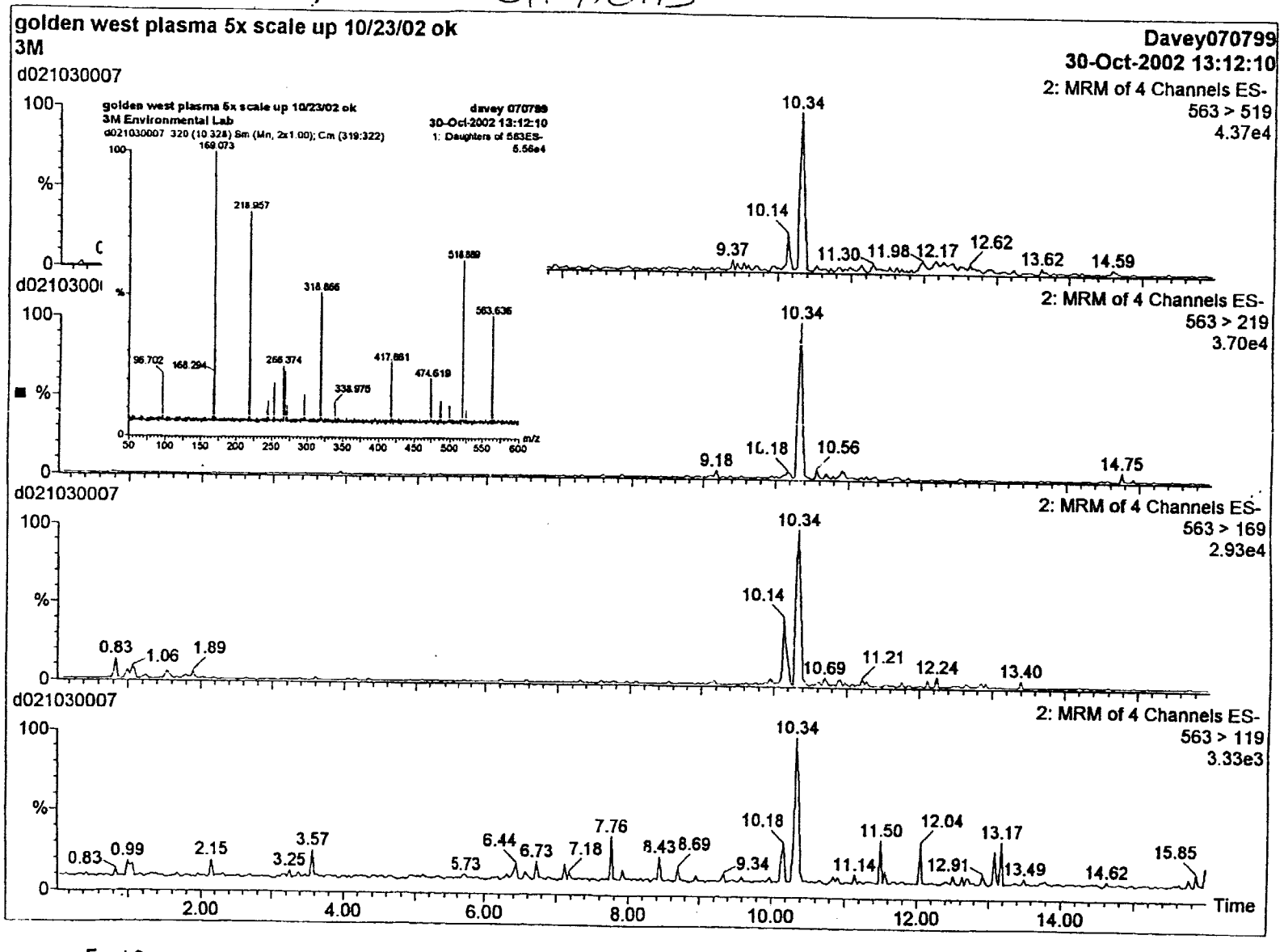


Exact Copy of Original

CMC 10/31/02
Initial Date

000191

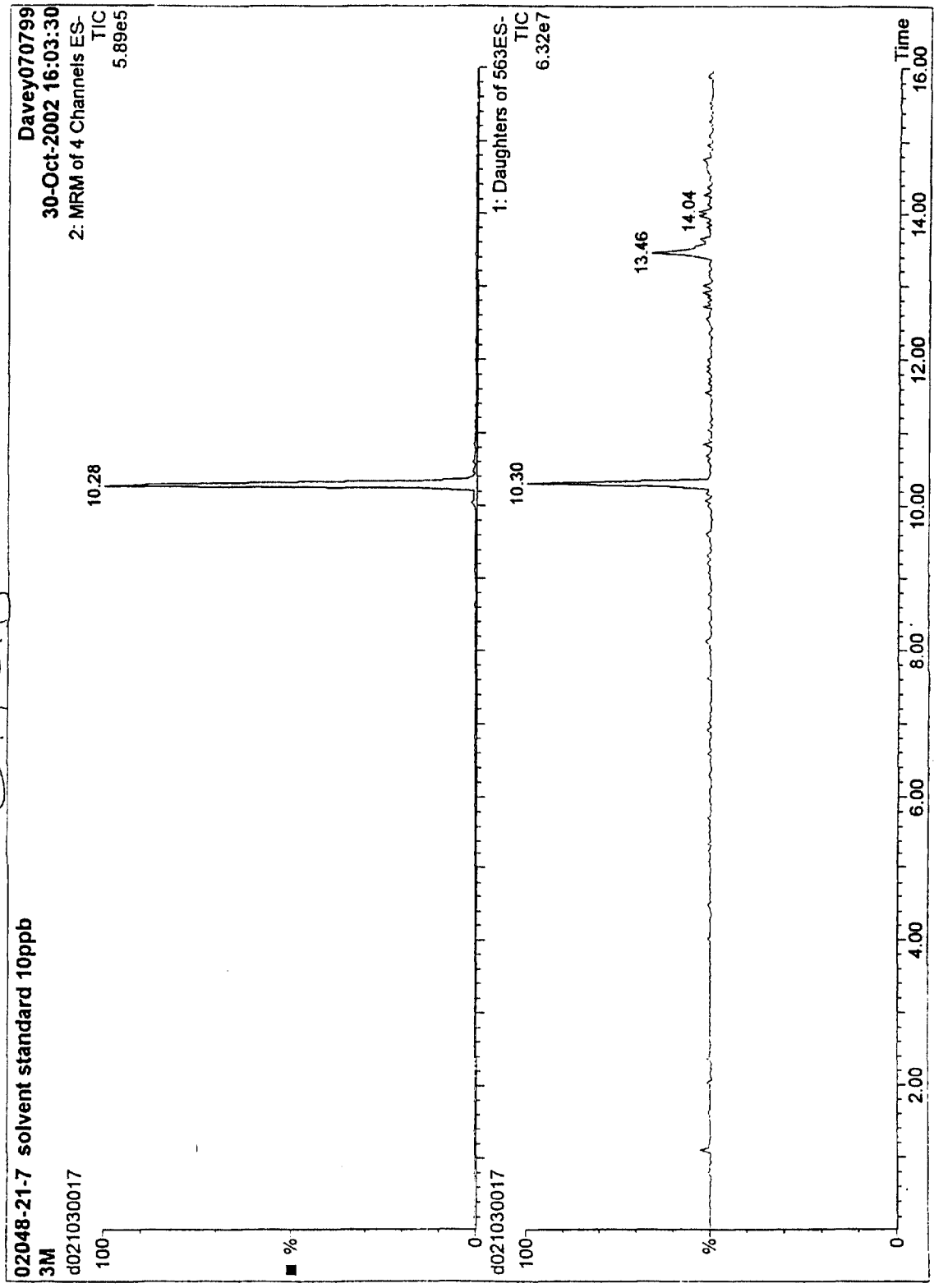
C11 ACID



Exact Copy of Original

CMC 10/31/02
Initial Date

000192

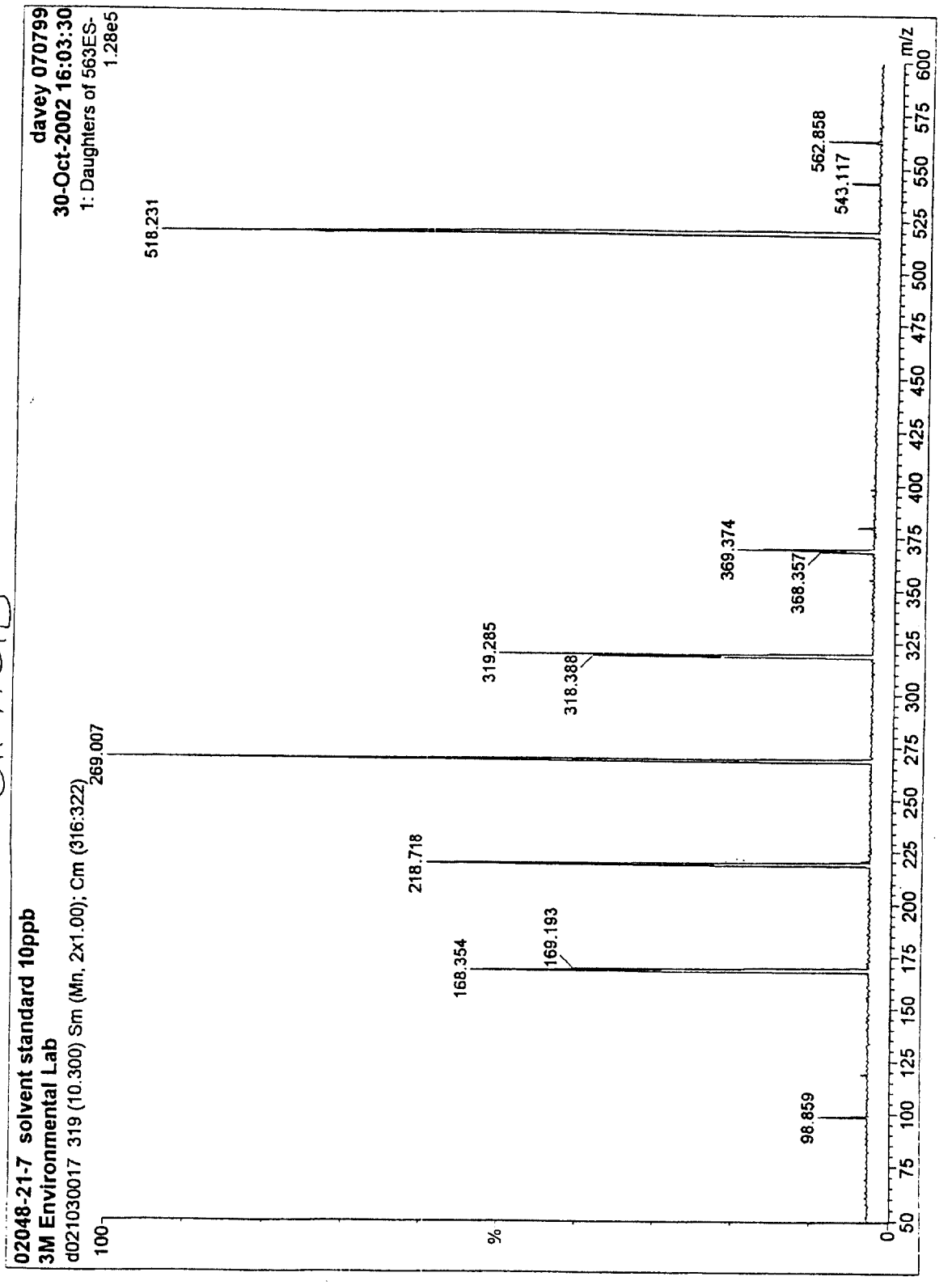


C11 ACID

Exact Copy of Original

Initial Date

000193



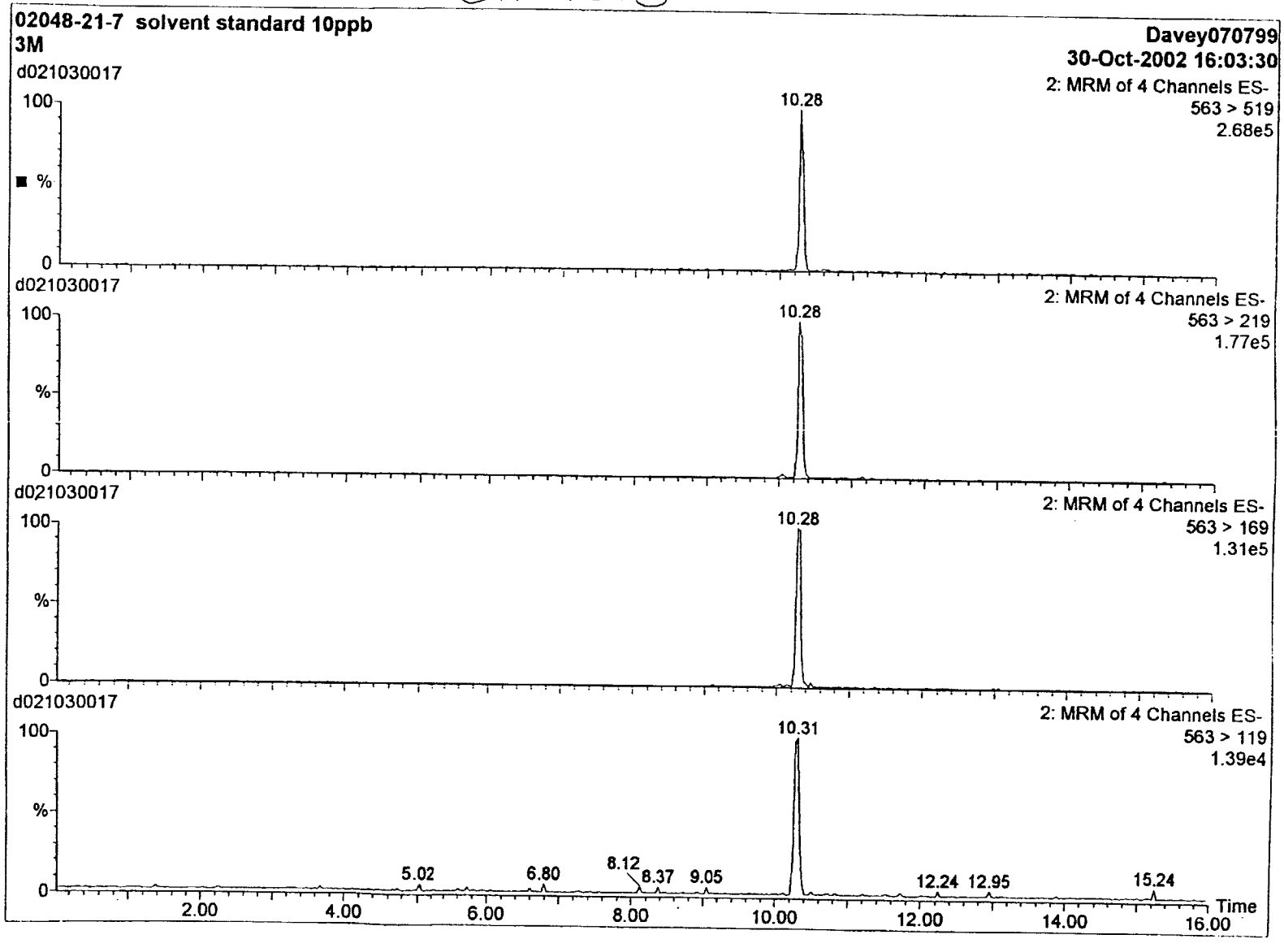
C11 ACID

Exact Copy of Original

CMC 11/4/02
Initial Date

000194

C11 ACID

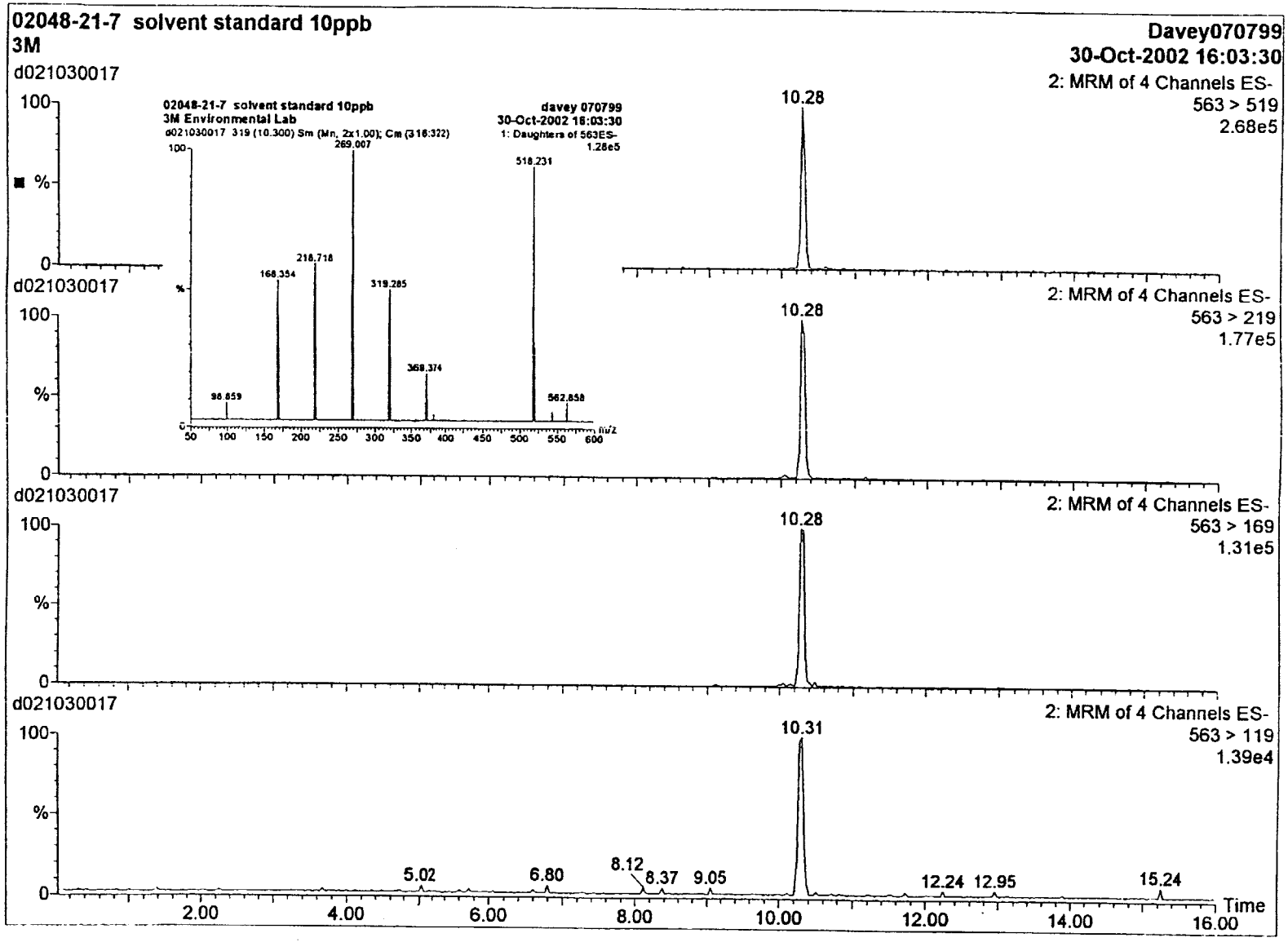


Exact Copy of Original

CMC 11/1/02
Initial Date

000195

C11 ACID

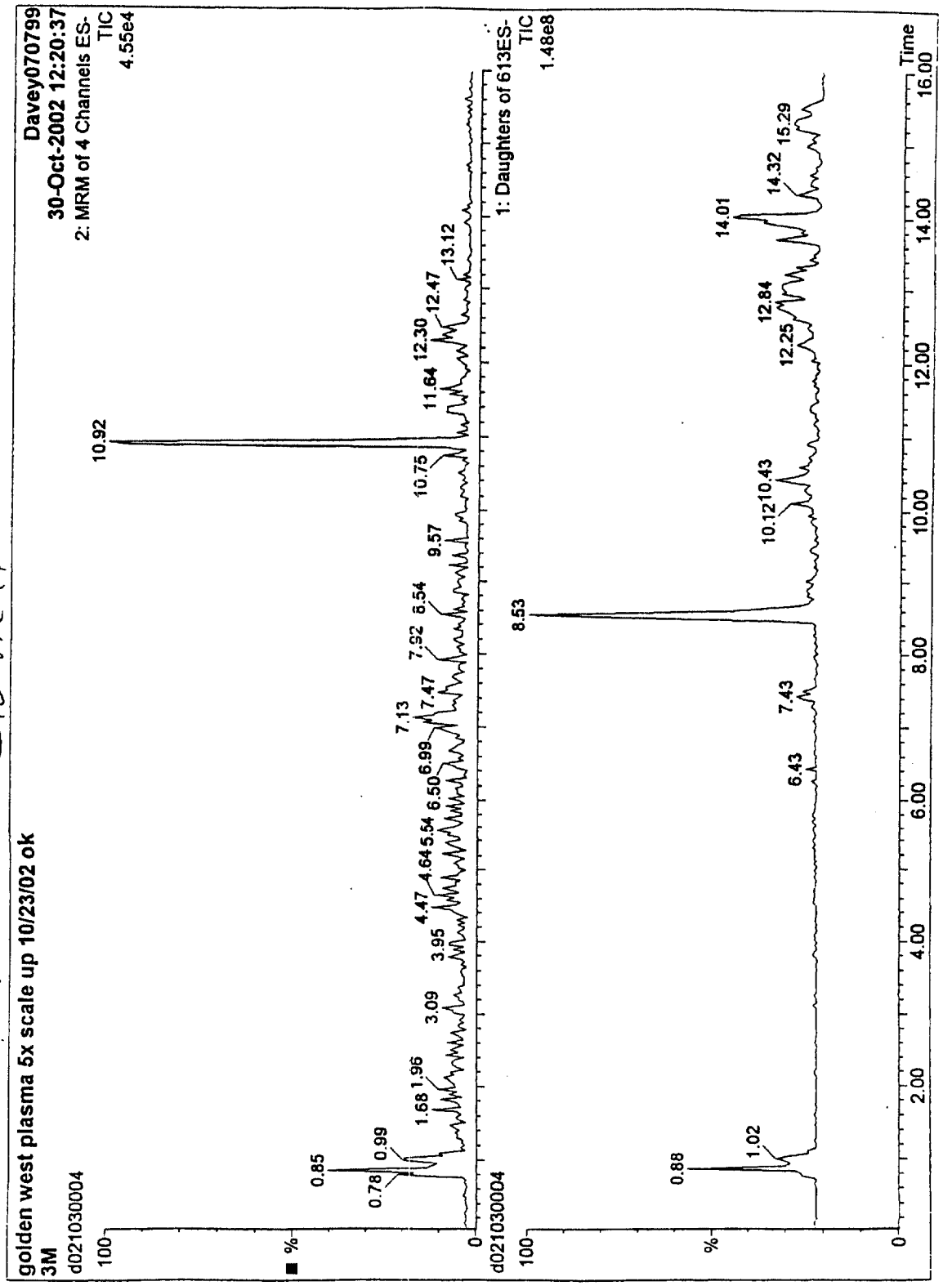


Exact Copy of Original

CMC 11/1/02
Initial Date

000196

C12 H2 (P

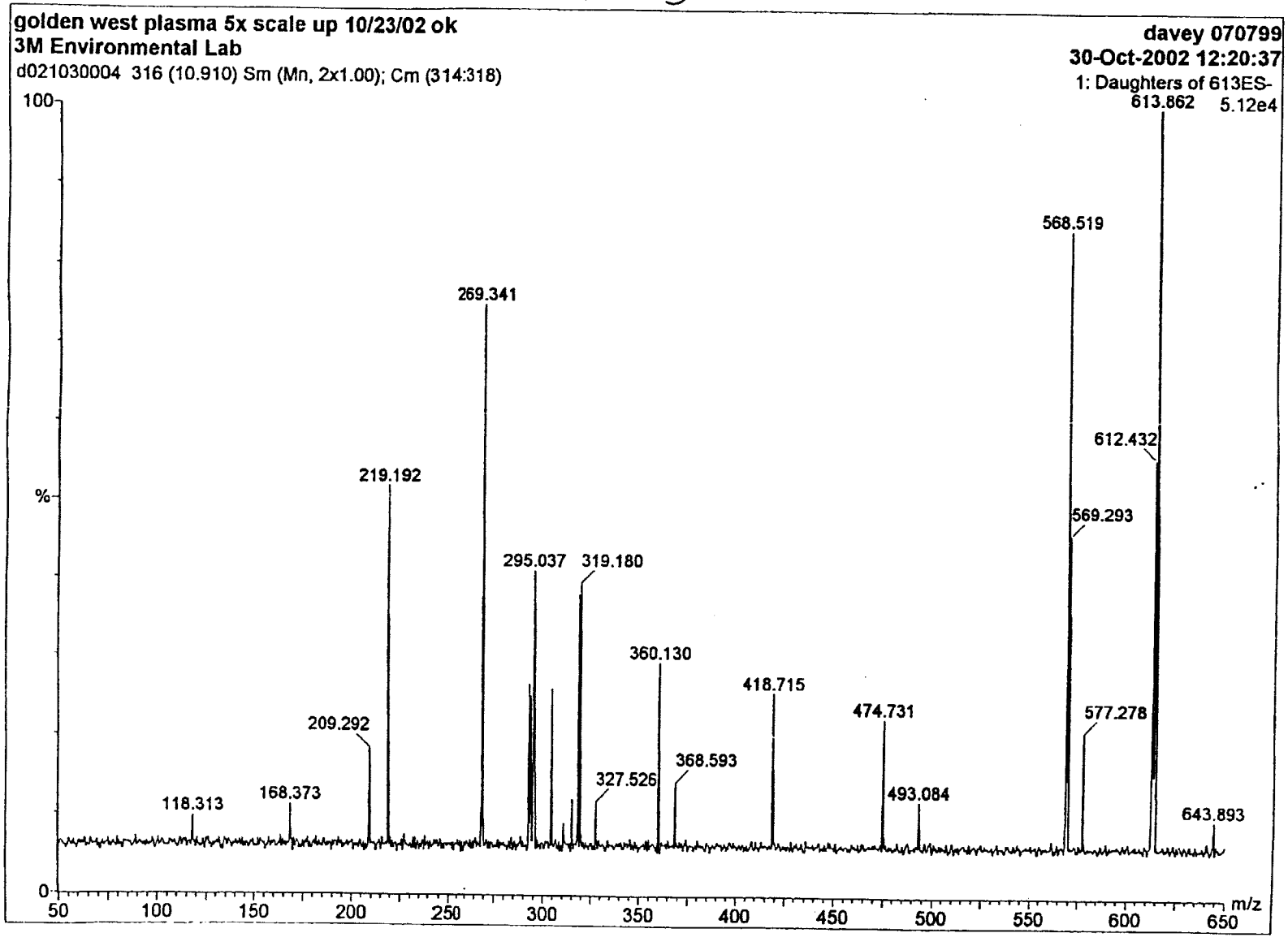


Exact Copy of Original

LDC 10/31/02
Initial Date

000197

C12 ACID

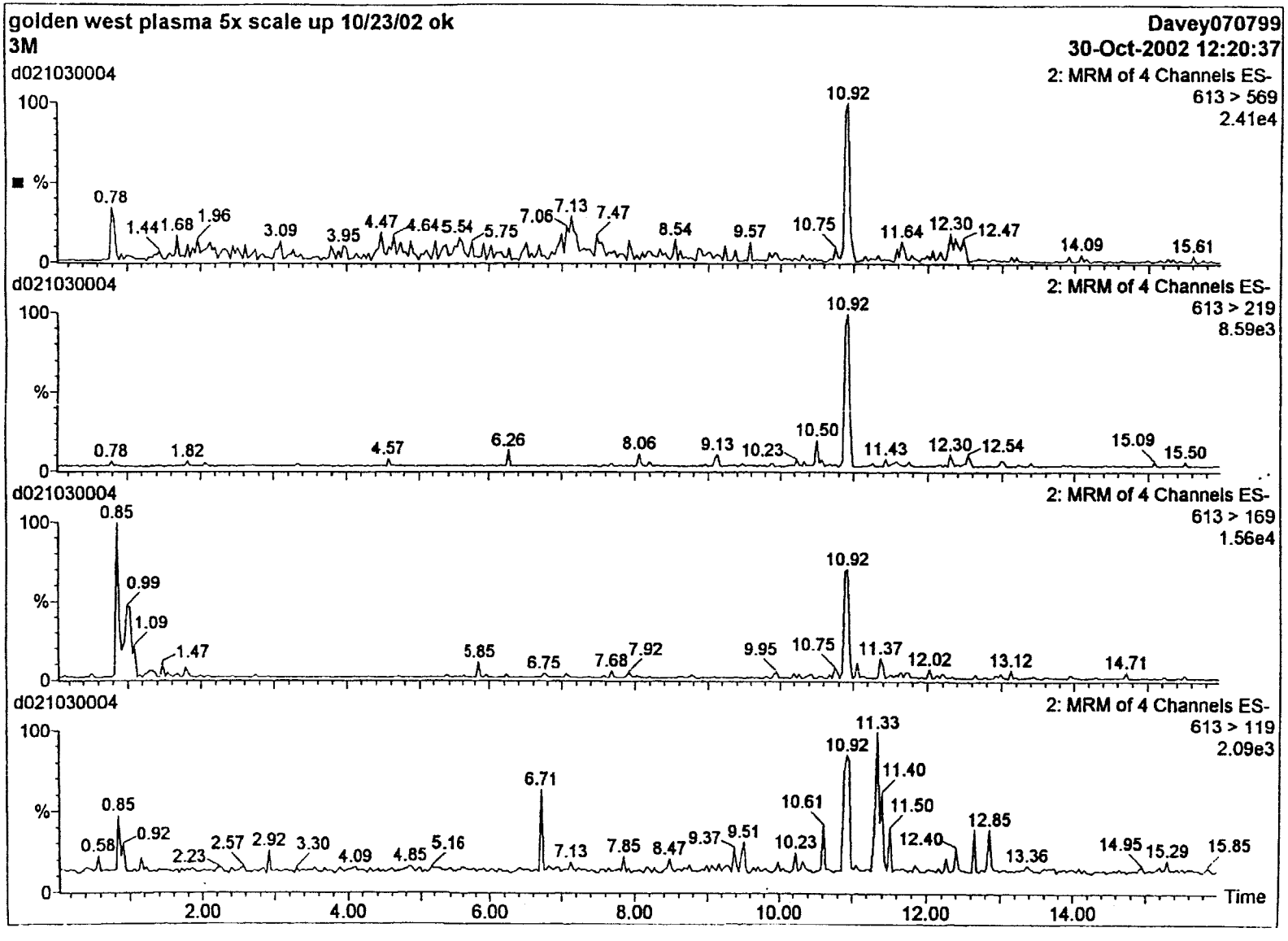


Exact Copy of Original

CMC 10/31/02
Initial Date

000198

C12 ACID

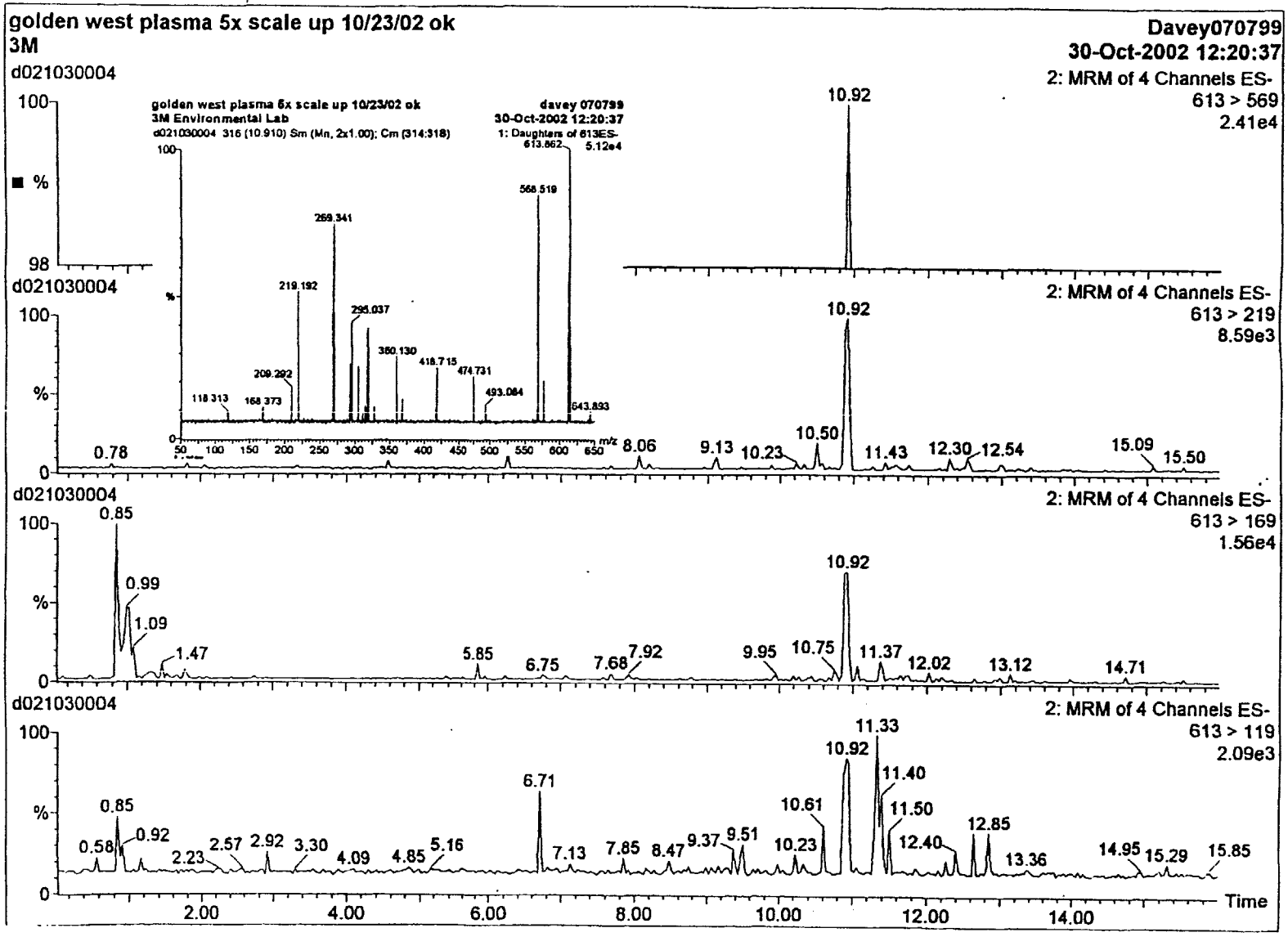


Exact Copy of Original

CMC 10/31/02
Initial Date

000199

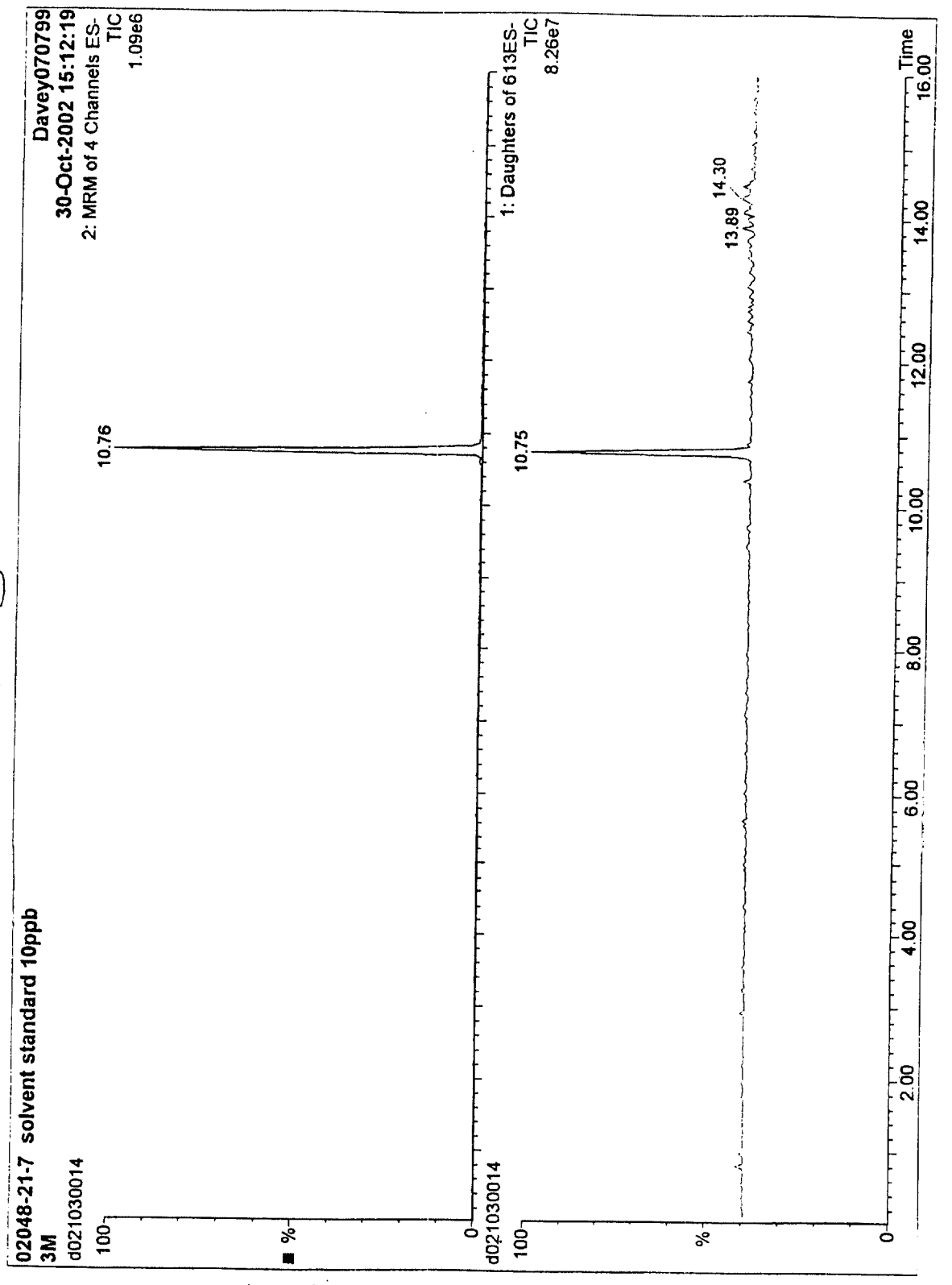
C12 ACID



Exact Copy of Original

CMC 10/31/02
Initial Date

000200

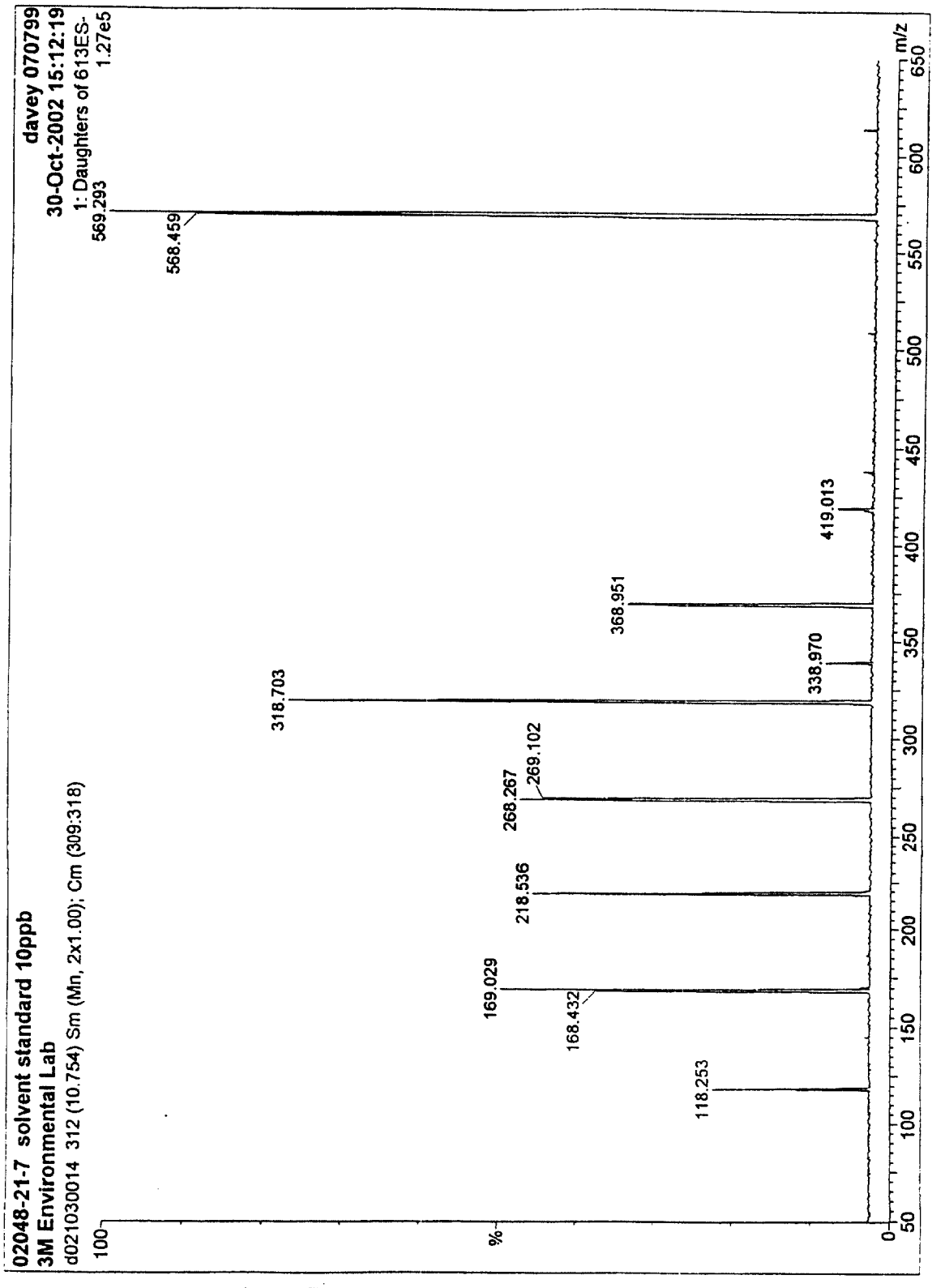


Exact Copy of Original

CMC 11/1/02
Initial Date

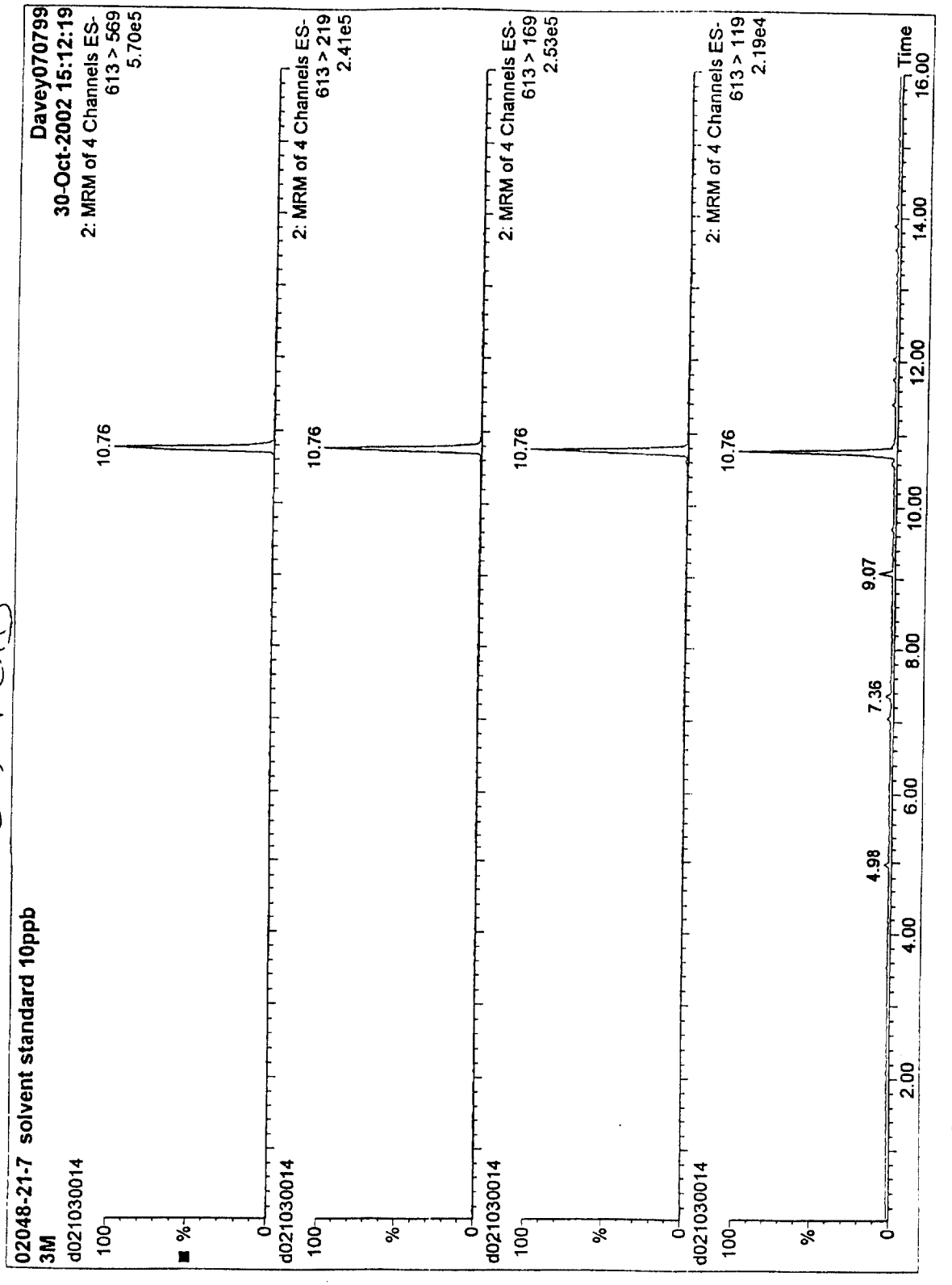
000201

C12 PCID



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

000202

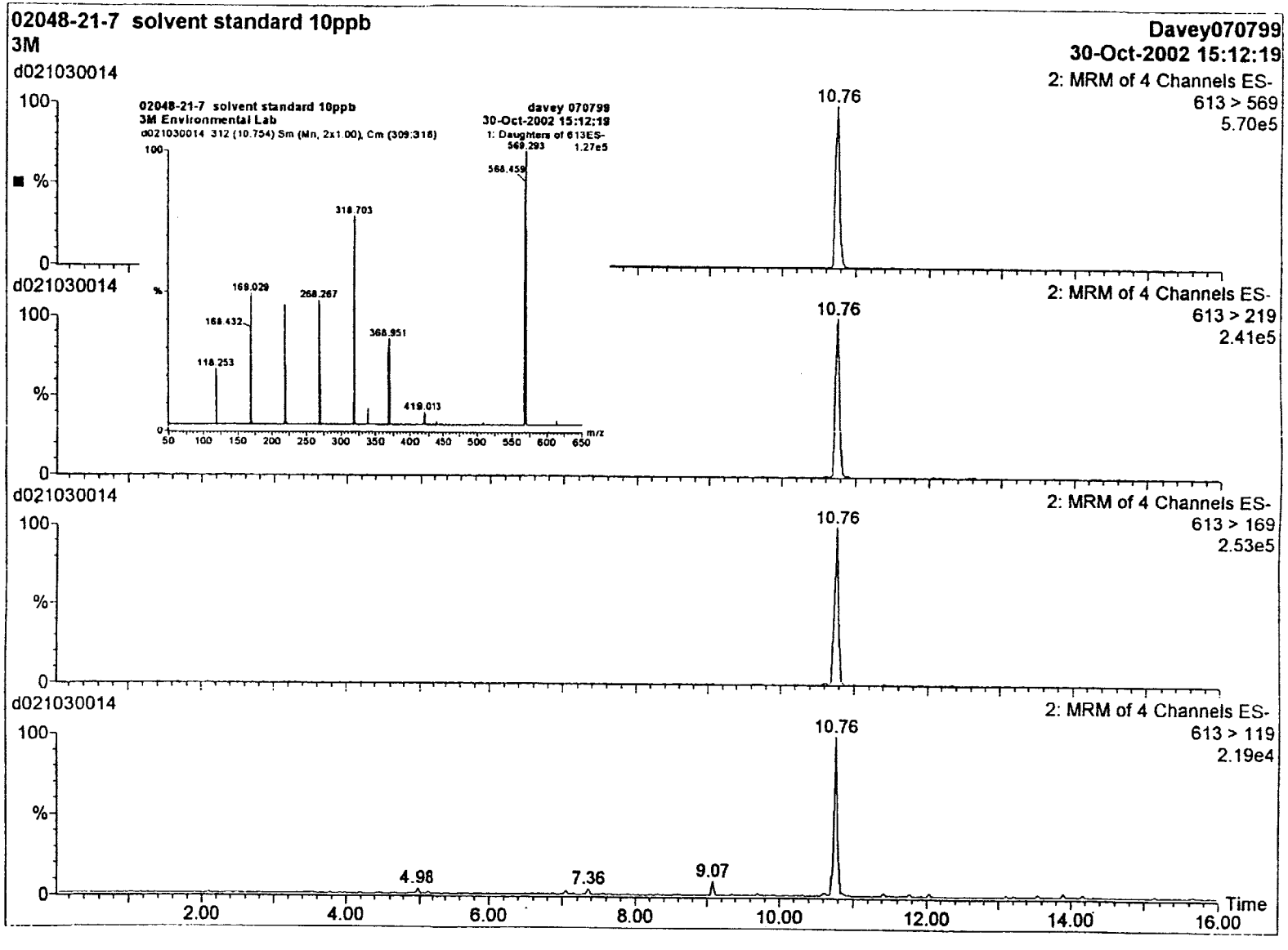


Exact Copy of Original

CMC 11/1/02
Initial Date

000203

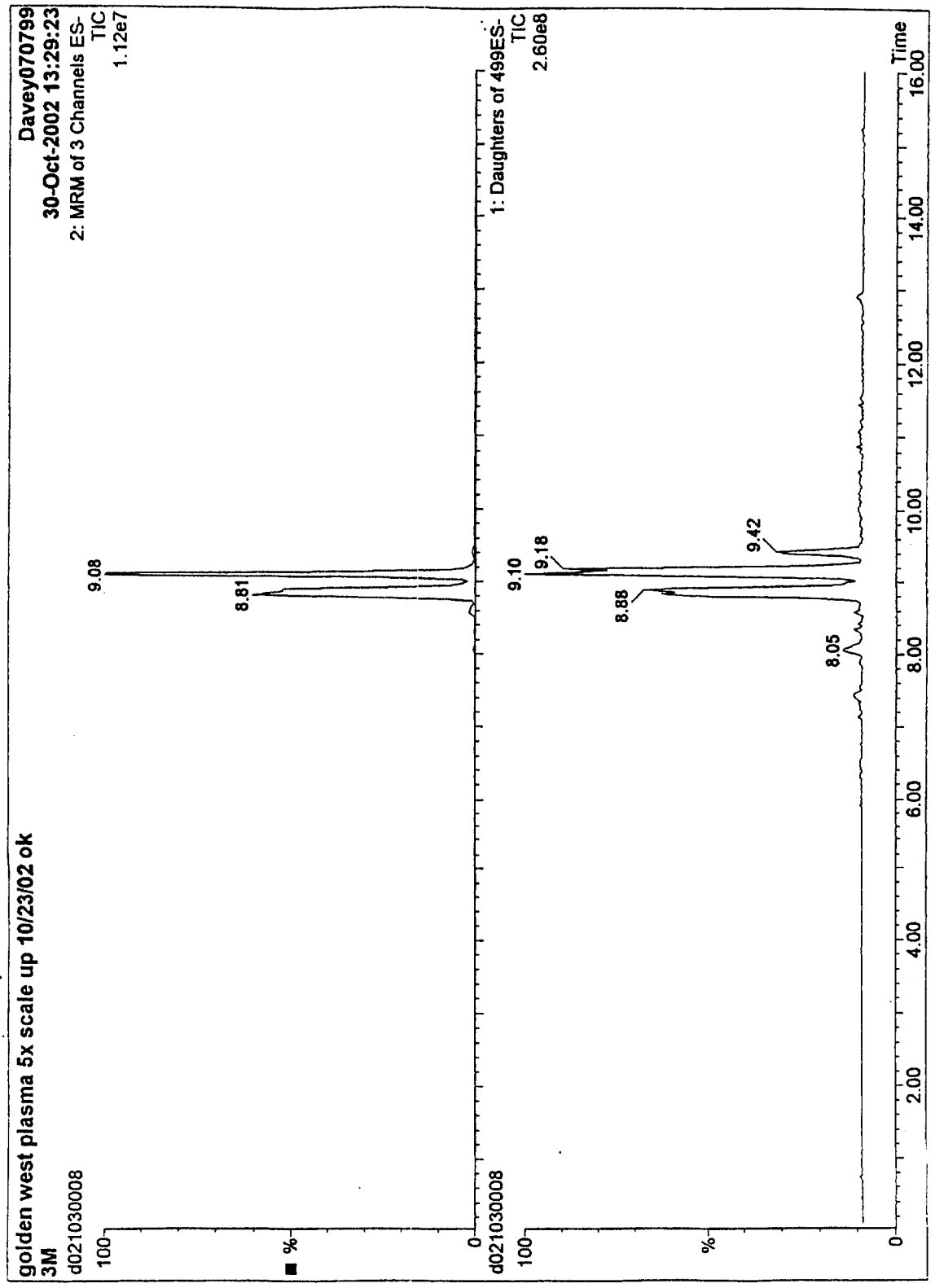
C12 ACID



Exact Copy of Original

CMC 11/1/02
Initial Date

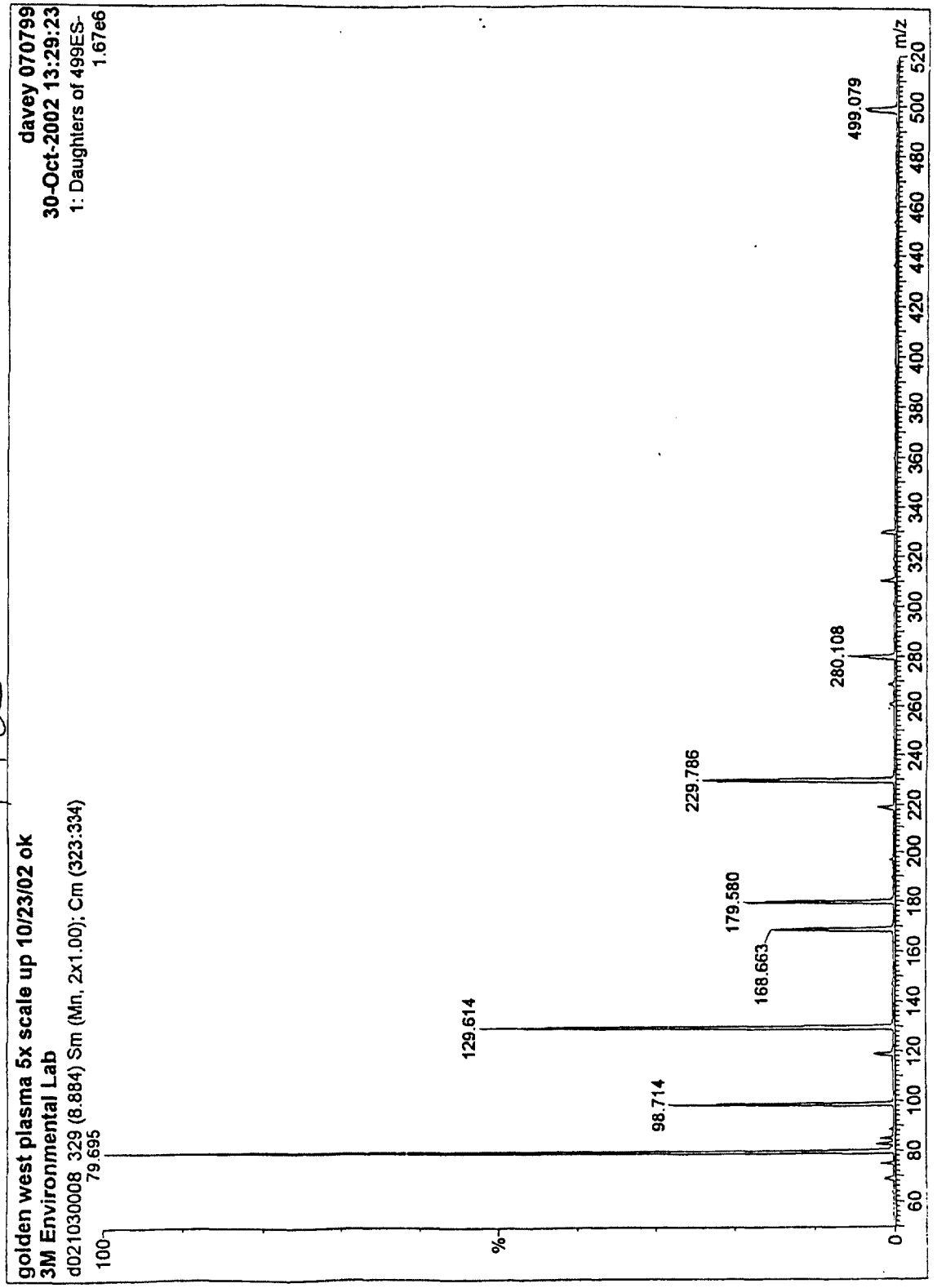
000204



Exact Copy of Original

CMC 10/31/02
Initial Date

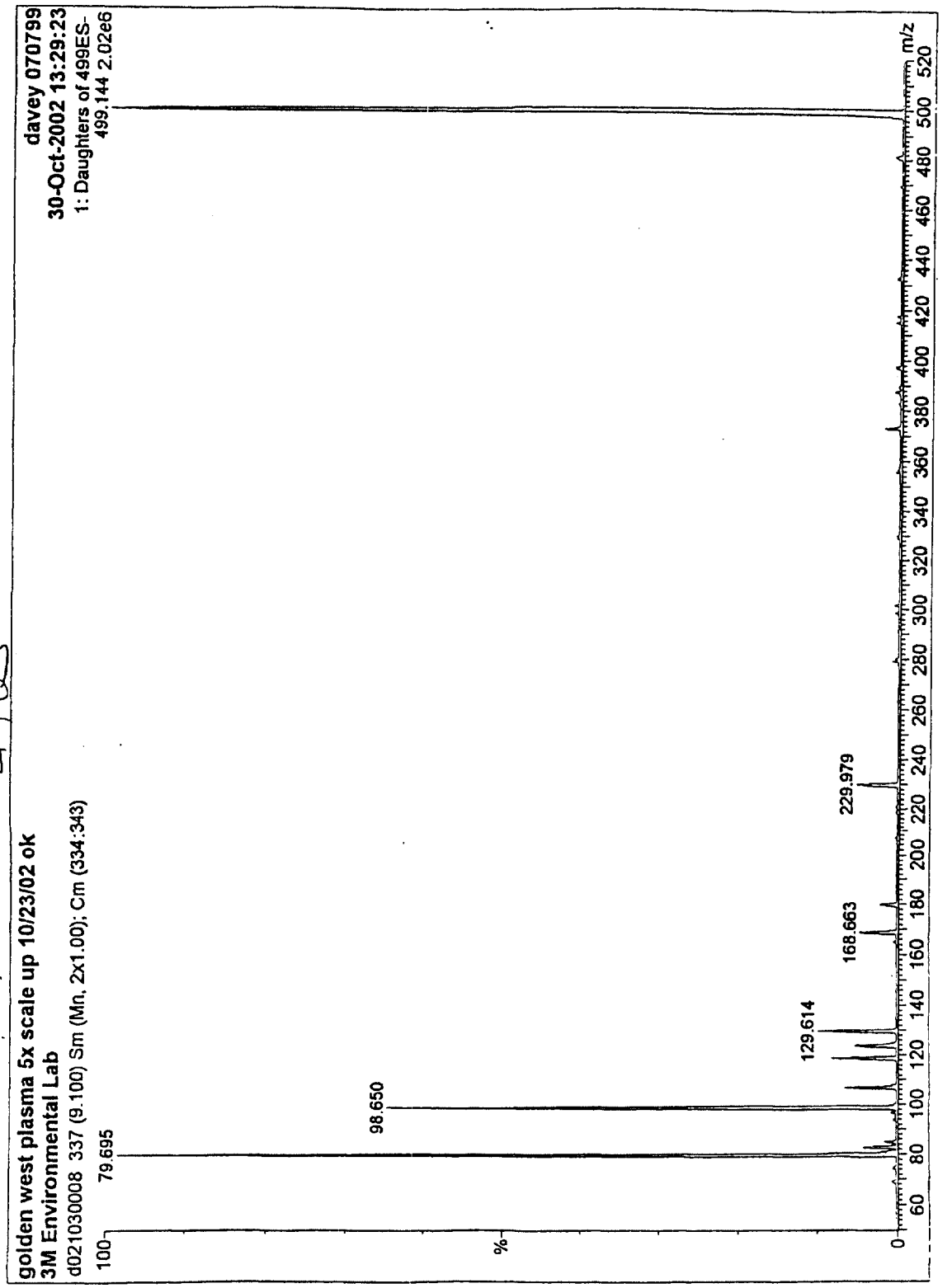
000205



Exact Copy of Original

Initial Date

000206



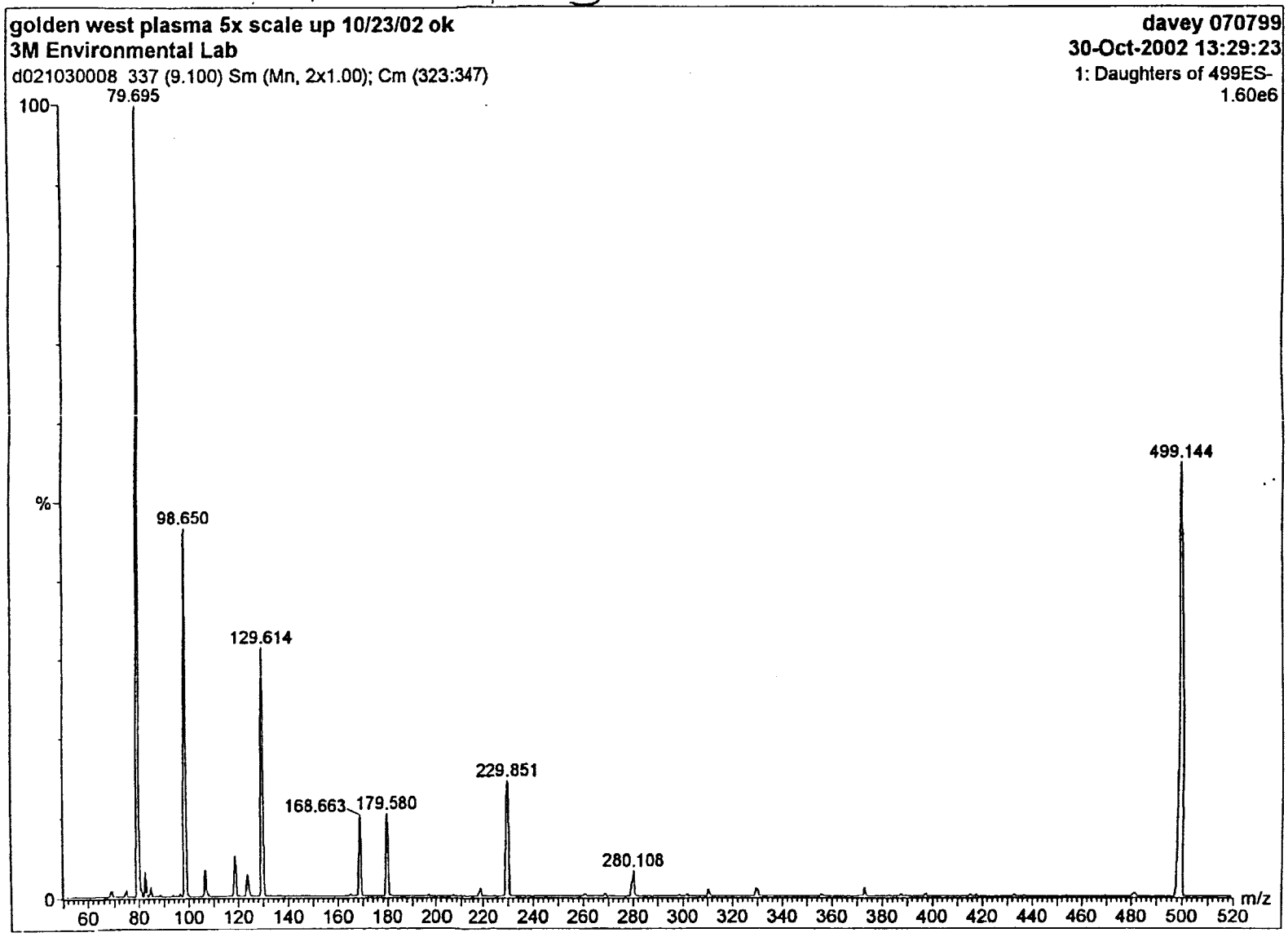
PFOS

Exact Copy of Original

CMC 10/31/02
Initial Date

000207

PFOS

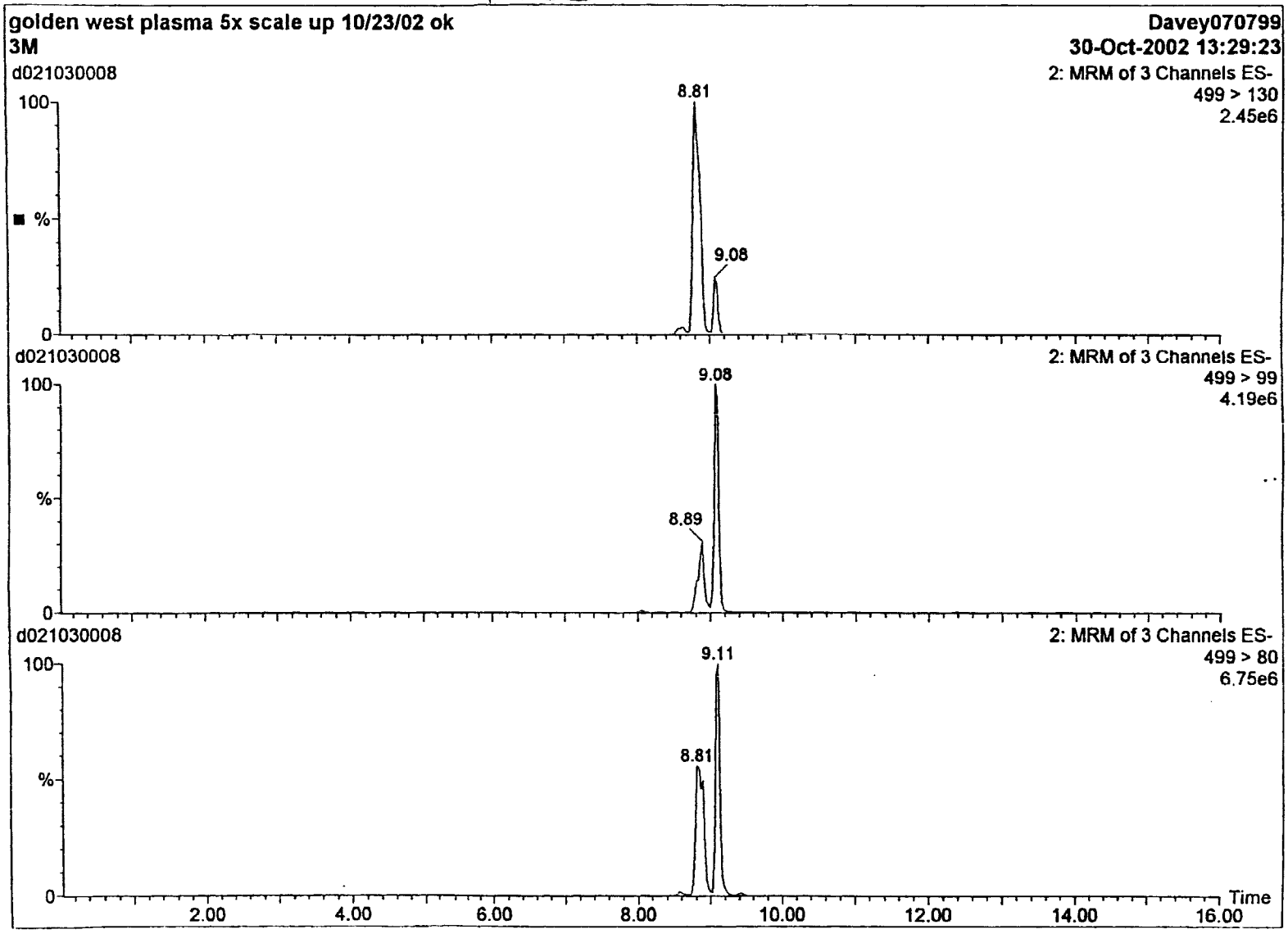


Exact Copy of Original

CML 10/31/02
Initial Date

000208

PFOS

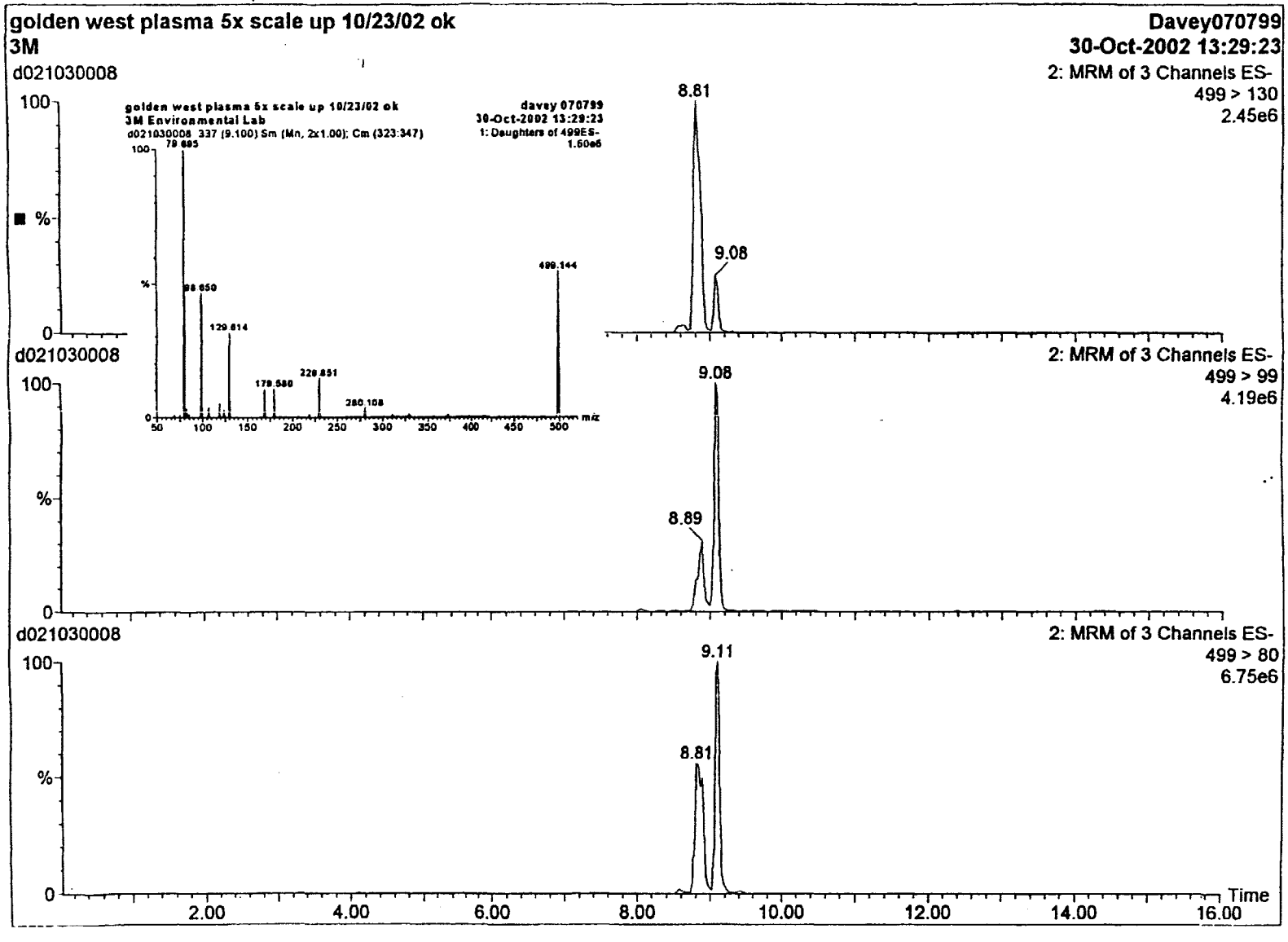


Exact Copy of Original

CMK 10/31/02
Initial Date

000209

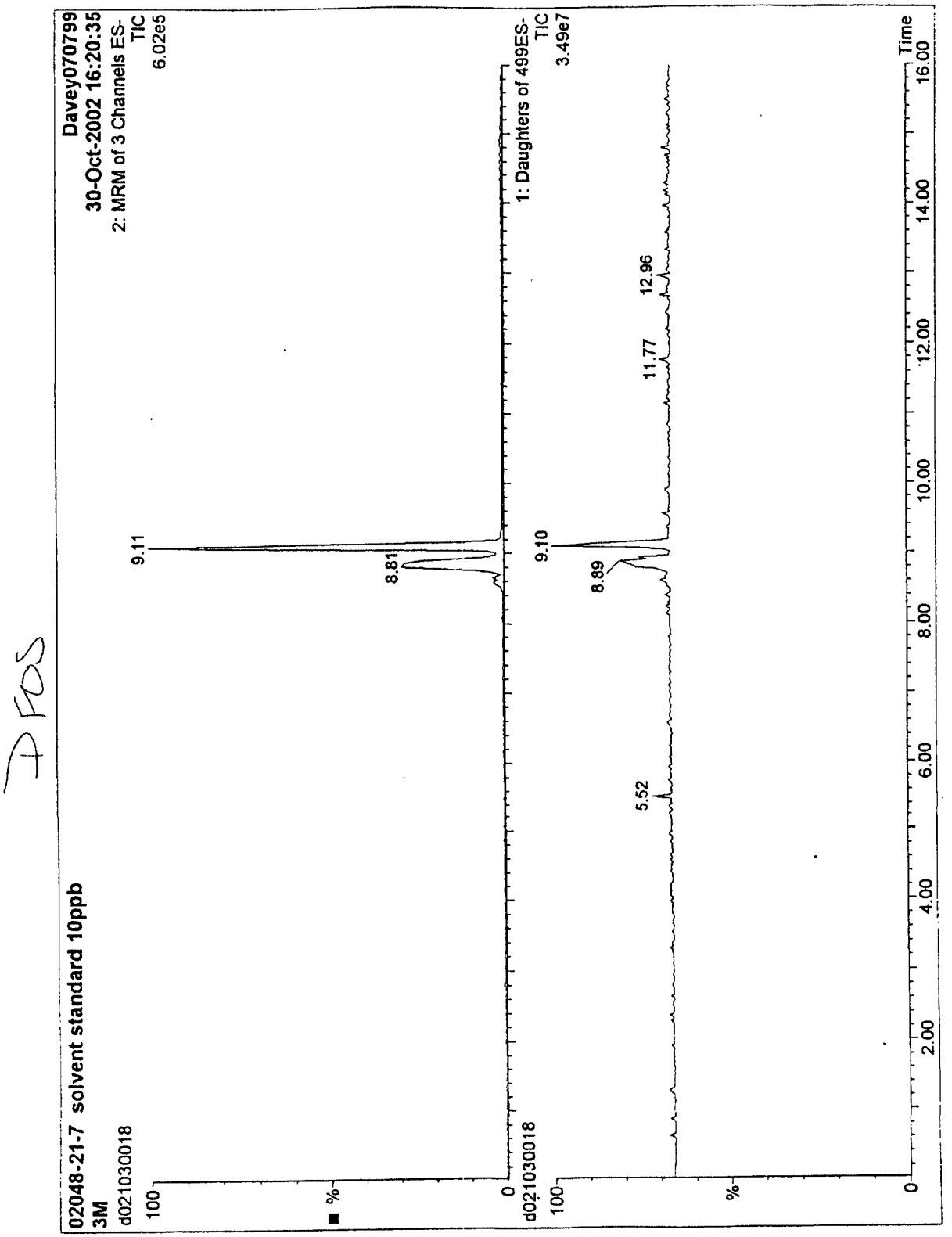
PFOS



Exact Copy of Original

CMC 10/31/02
Initial Date

000210



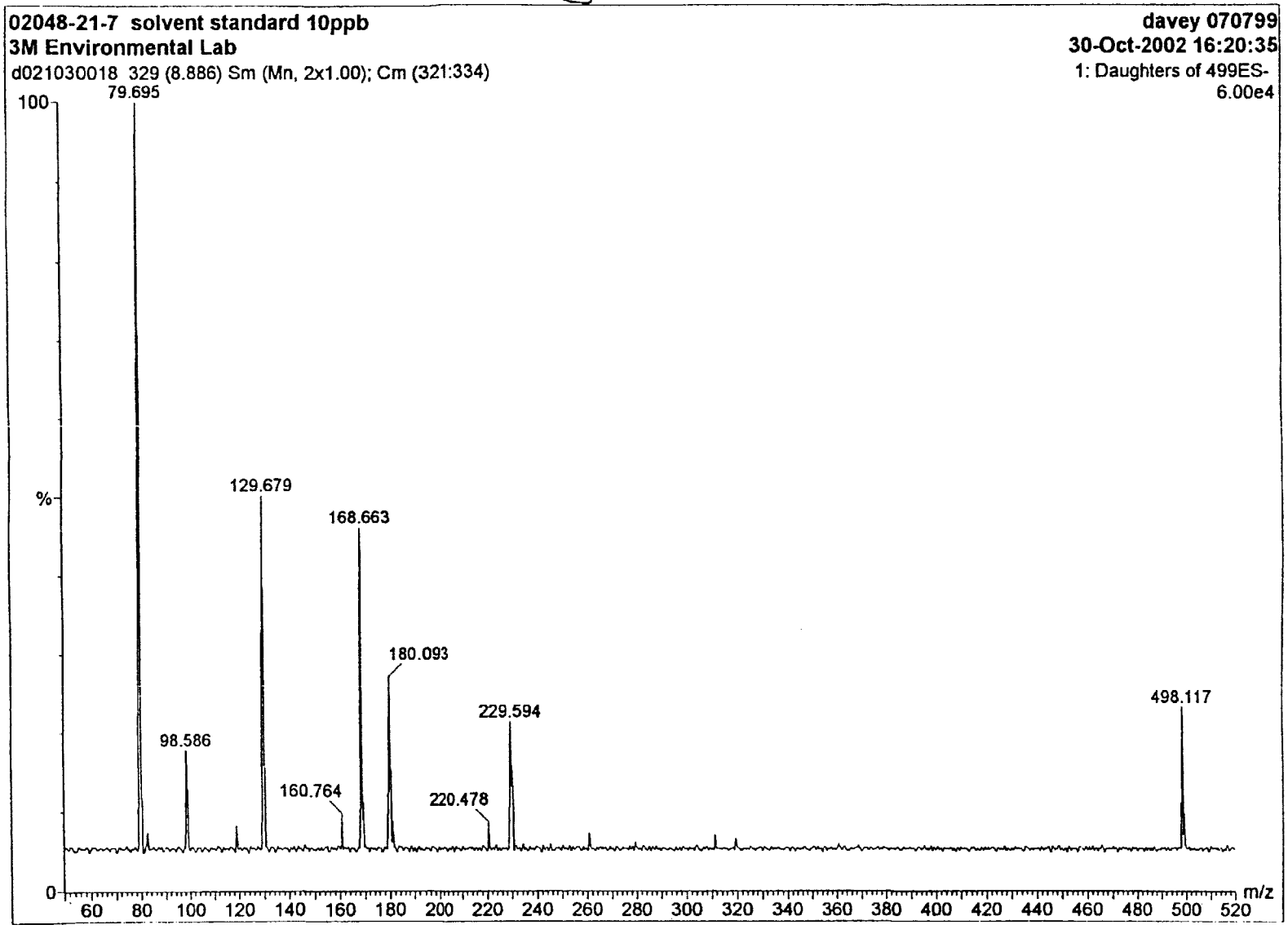
PFOS

Exact Copy of Original

Initial Date

000211

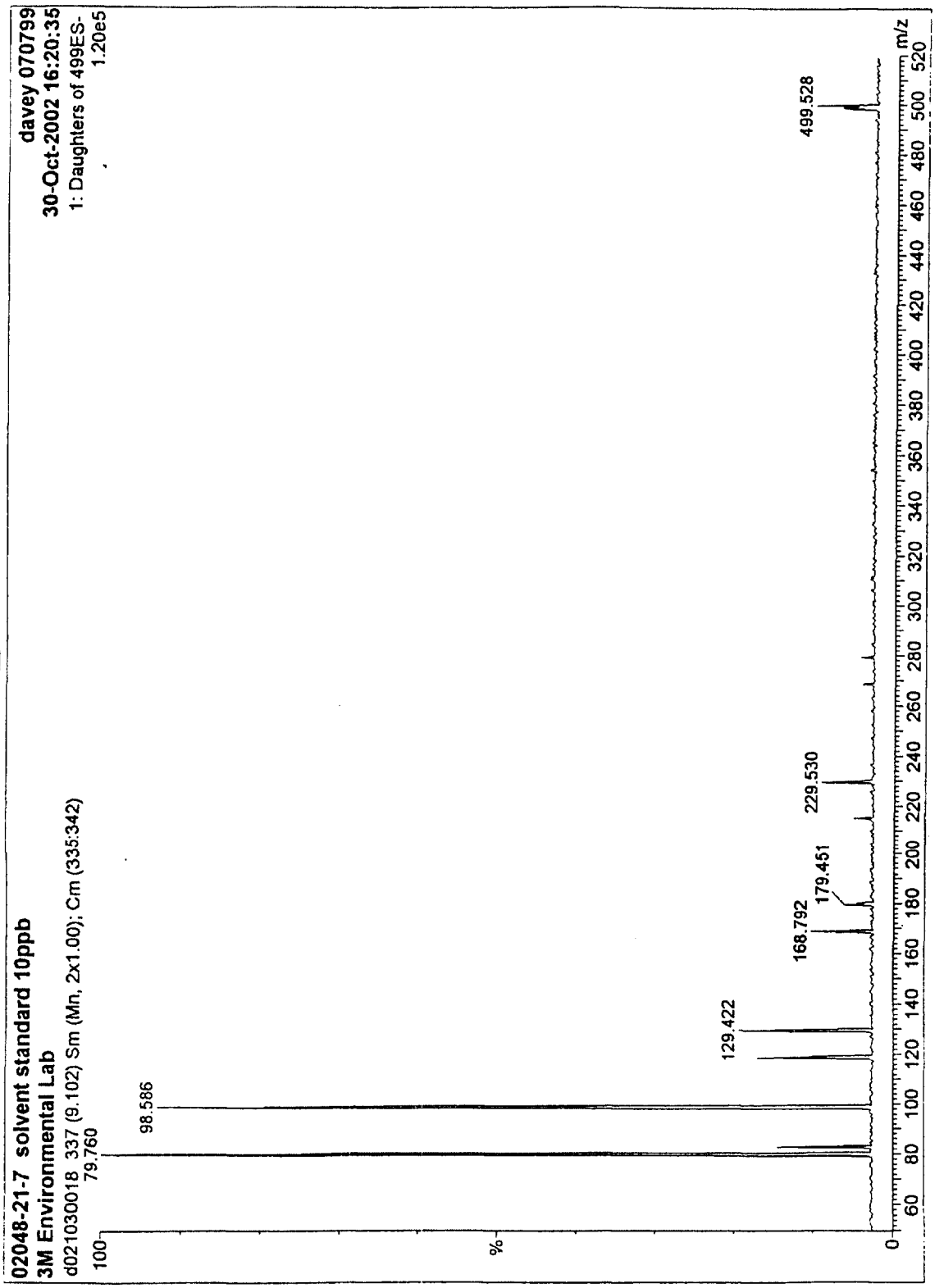
PFOS



Exact Copy of Original

CM 11/1/02
Initial Date

000212

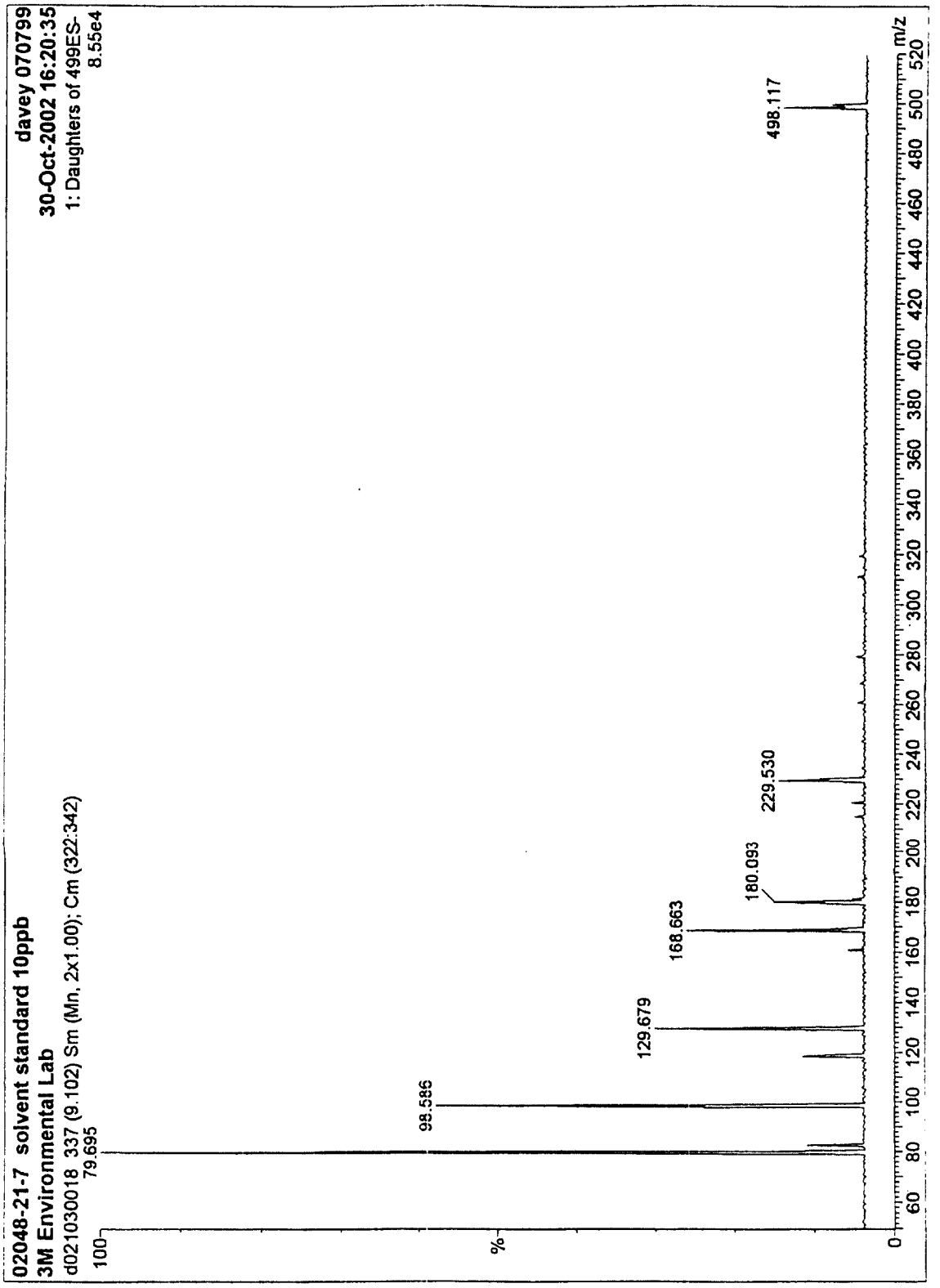


PFOS

Exact Copy of Original

Initial Date

000213

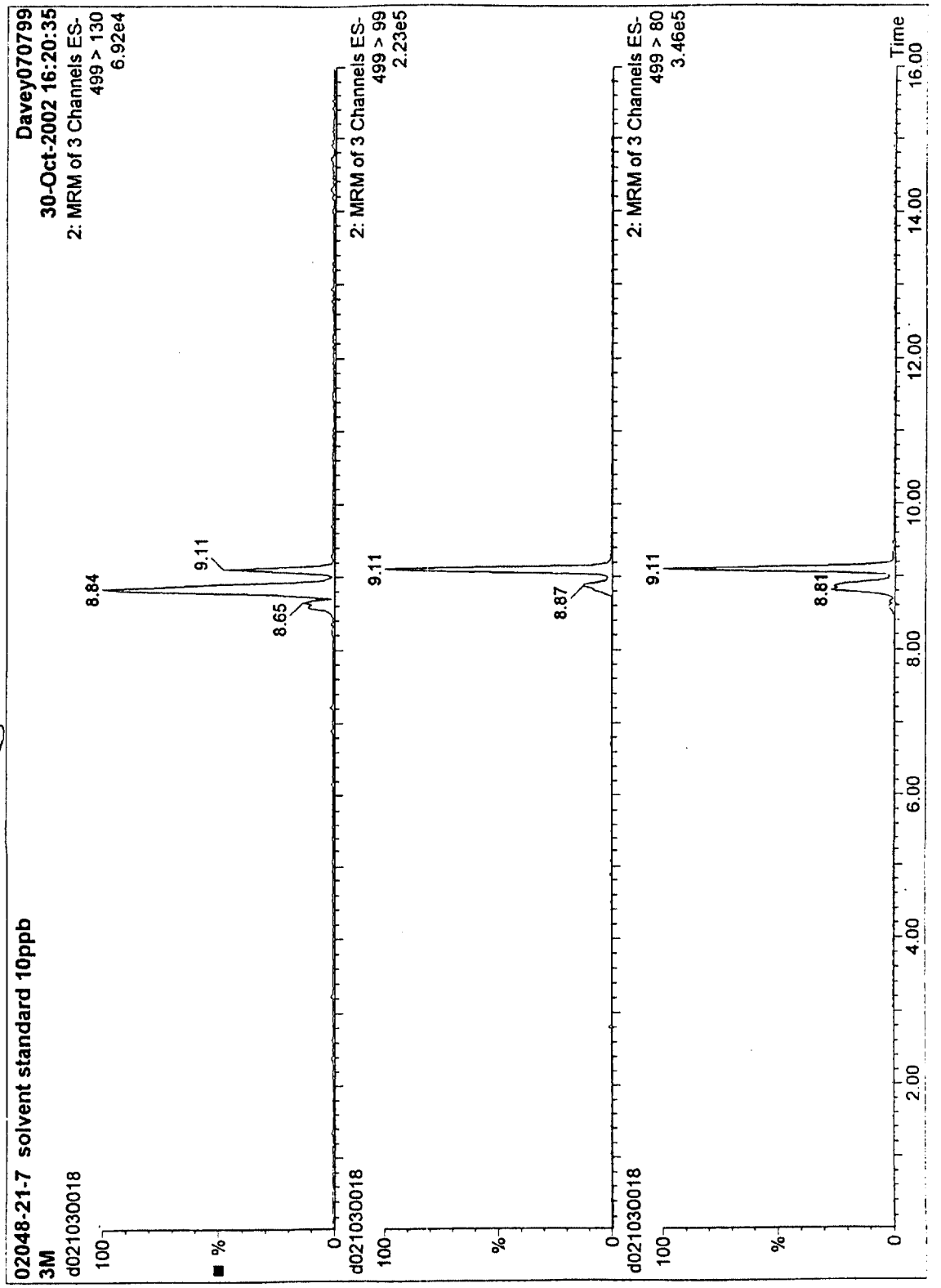


P&S

Exact Copy of Original

Initial Date

000214

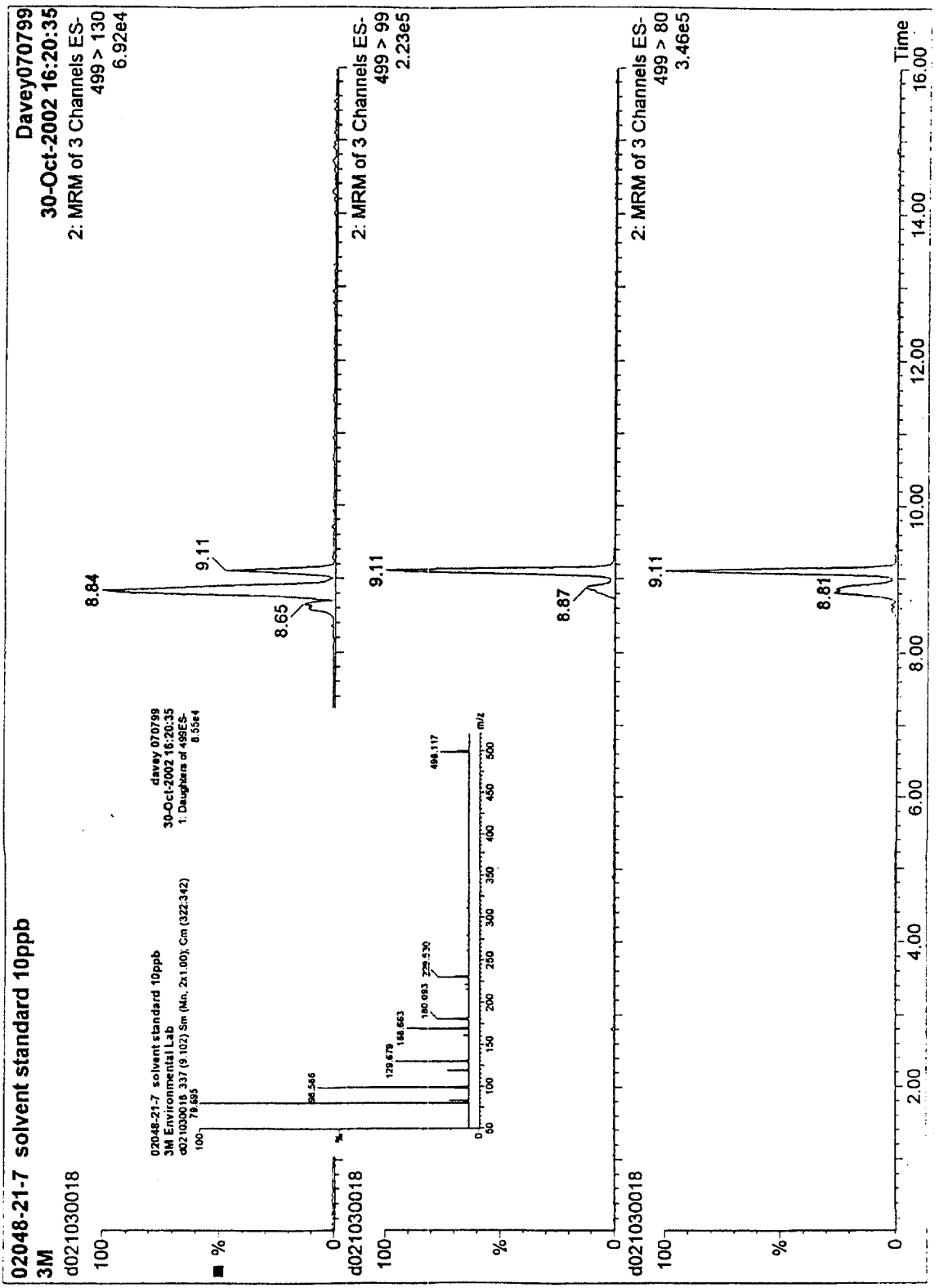


PFOS

Copy of Original

Initial Date

000215



Exact Copy of Original

Initial Date

000216

ATTACHMENT D: PREP SHEETS AND TRACEABILITY INFORMATION

SPE Columns Extraction Worksheet

Prep Date: 10/23/2002 10230 2
Analysts initials: OK OK

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

Sample Number or description	Volume of sample filtered (ml)	Type of column used	Amount and spike mix used	Elution solvent and volume	Comments
Blank milli-q	2000 ml	Waters, 1g, 6ml	NA	2ml of MeOH TN-A-8313	
Lampire Serum	2000 ml	Waters, 1g, 6ml	NA	2ml of MeOH TN-A-8313	TCR-688
Sigma Serum	2000 ml	Waters, 1g, 6ml	NA	2ml of MeOH TN-A-8313	TCR-689
Golden West Serum	2000 ml	Waters, 1g, 6ml	NA	2ml of MeOH TN-A-8313	TCR-690
Bioresource Serum	2000 ml	Waters, 1g, 6ml	NA	2ml of MeOH TN-A-8313	TCR-687
Lampire Plasma	2000 ml	Waters, 1g, 6ml	NA	2ml of MeOH TN-A-8313	TCR-685
Golden West Plasma	2000 ml	Waters, 1g, 6ml	NA	2ml of MeOH TN-A-8313	TCR-684
Innovative Resources Plasma	2000 ml	Waters, 1g, 6ml	NA	2ml of MeOH TN-A-8313	TCR-683

OK 102302

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;
ACN TN-A-8 OK 102302 430T ; LOT OF COLUMN: W 21981

Additional comments:

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

Samples were transferred onto colum using Tygon tubing. OK 1023-02

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

~~Non~~ GLP SPE Columns Extraction Worksheet

ETS 223111.0102

Prep Date: CMC 10/24/02 10/11/2002 10.11.02
Analysts initials: OK OK

Method Revision: ~~ETS-231~~ (05)
Study Number: E 02-1039

Sample Number or description	Volume of sample filtered (ml)	Type of column used	Amount and spike mix used	Elution solvent and volume	Comments
Blank milli-q-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-686
rabbit serum curve-0.1ppb	400 ml	Waters, 1g, 6ml	2ul of 02050-14	2 ml of MeOH TN-A-6310	TCR-686
rabbit serum curve-0.25ppb	400 ml	Waters, 1g, 6ml	5ul of 02050-14	2 ml of MeOH TN-A-6310	TCR-686
rabbit serum curve-0.5ppb	400 ml	Waters, 1g, 6ml	10ul of 02050-14	2 ml of MeOH TN-A-6310	TCR-686
rabbit serum curve-0.75ppb	400 ml	Waters, 1g, 6ml	15ul of 02050-14	2 ml of MeOH TN-A-6310	TCR-686
rabbit serum curve-1ppb	400 ml	Waters, 1g, 6ml	20ul of 02050-14	2 ml of MeOH TN-A-6310	TCR-686
rabbit serum curve-2.5ppb	400 ml	Waters, 1g, 6ml	50ul of 02050-14	2 ml of MeOH TN-A-6310	TCR-686
rabbit serum curve-5ppb	400 ml	Waters, 1g, 6ml	100ul of 02050-14	2 ml of MeOH TN-A-6310	TCR-686
rabbit serum curve-10ppb	400 ml	Waters, 1g, 6ml	200ul of 02050-14	2 ml of MeOH TN-A-6310	TCR-686
rabbit serum curve-30ppb	400 ml	Waters, 1g, 6ml	600ul of 02050-14	2 ml of MeOH TN-A-6310	TCR-686
Lampire serum blank-1	400 ml	Waters, 1g, 6ml	NA	2 ml of MeOH TN-A-6310	TCR-688
Lampire serum blank-2	400 ml	Waters, 1g, 6ml	NA	2 ml of MeOH TN-A-6310	TCR-688
Lampire serum MS-0.5ppb	400 ml	Waters, 1g, 6ml	10ul of 02050-14	2 ml of MeOH TN-A-6310	TCR-688
Lampire serum MS-5ppb	400 ml	Waters, 1g, 6ml	100ul of 02050-14	2 ml of MeOH TN-A-6310	TCR-688
Sigma serum blank-1	400 ml	Waters, 1g, 6ml	NA	2 ml of MeOH TN-A-6310	TCR-689
Sigma serum blank-2	400 ml	Waters, 1g, 6ml	NA	2 ml of MeOH TN-A-6310	TCR-689
Sigma serum MS-0.5ppb	400 ml	Waters, 1g, 6ml	10ul 02050-14	2 ml of MeOH TN-A-6310	TCR-689
Sigma serum MS-5ppb	400 ml	Waters, 1g, 6ml	100ul 02050-14	2 ml of MeOH TN-A-6310	TCR-689
Golden West serum blank-1	400 ml	Waters, 1g, 6ml	NA	2 ml of MeOH TN-A-6310	TCR-690
Golden West serum blank-2	400 ml	Waters, 1g, 6ml	NA	2 ml of MeOH TN-A-6310	TCR-690
Golden West serum MS-0.5ppb	400 ml	Waters, 1g, 6ml	10ul of 02050-14	2 ml of MeOH TN-A-6310	TCR-690
Golden West serum MS-5ppb	400 ml	Waters, 1g, 6ml	100ul of 02050-14	2 ml of MeOH TN-A-6310	TCR-690
Bioreseource research serum blank-1	400 ml	Waters, 1g, 6ml	NA	2 ml of MeOH TN-A-6310	TCR-687
Bioreseource research serum blank-2	400 ml	Waters, 1g, 6ml	NA	2 ml of MeOH TN-A-6310	TCR-687
Bioreseource research serum MS-0.5ppb	400 ml	Waters, 1g, 6ml	10ul of 02050-14	2 ml of MeOH TN-A-6310	TCR-687
Bioreseource research serum MS-5ppb	400 ml	Waters, 1g, 6ml	100ul of 02050-14	2 ml of MeOH TN-A-6310	TCR-687

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
CMC 10/31/02
Initial Date

Additional comments: ACN TN-A-6194 OK 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: VWR #6916074 CENTRIFUGE: HH # EQUIP# 02118.51

~~Non~~ GLP SPE Columns Extraction Worksheet

Prep Date: MEC 10/24/02 10/10/2002 10/10 02
Analysts initials: OK CK

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

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

Extraction Steps (initial when/if done):

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

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

Additional comments:
CENTRIFUGE NMS # C21PP55 ; shaken: VWR SC SER# 04IC94

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.

Exact Copy of Original
Initial 11/1/02 Date

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

Form ETS-4-15.0

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)	

Exact Copy of Original

CMC 11/1/02
Initial Date

USE LOG
Human plasma from China **Human plasma** **TCR-674**

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

Exact Copy of Original

CMC 11/1/02
Initial Date

Test Control and Reference Substance Log

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

CMC 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 11/1/07
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

CMC 11/1/02
Initial Date

Test Control and Reference Substance Log

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

○ **Archived/Substance Not Available**

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

Exact Copy of Original

CMC 11/1/02
Initial Date

000230

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

000233

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

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

Exact Copy of Original

CMC 11/1/07
Initial Date

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

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

Exact Copy of Original

CMC 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 assinged TN-A-6286 10/11/02 ok**
Attachment(s)	

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
-	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

CMS 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

CMC 11/1/02
Initial Date

000239

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

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

Exact Copy of Original

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

( ) **Archived/Substance Not Available**

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

Exact Copy of Original

CMC Initial 11/1/02 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

000244

Standard curve tracking sheet

Prep date: 10/7/2002 10702 CKJ
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	10.36	10.24	10.02	10.06
02048-21-8	750	15.00	15.32	15.50	15.22	15.15	15.46	15.54	15.37	15.03	15.09
02048-21-9	1000	20.01	20.43	20.66	20.29	20.20	20.61	20.72	20.49	20.04	20.12
02048-21-10	1500	30.01	30.64	31.00	30.43	30.30	30.92	31.08	30.73	30.06	30.18

Signature: Urijeka Kuglizi

Verified by: Cindy Carlson 10/31/02

Exact Copy of Original

CMK 10/31/02
Initial Date

000245

Standard mix tracking sheet

Sera Super mix for SPE 10X MDV

Prep date: 10/7/2002 Exp. 11/13/02
Analyst: OK OK 10.7.02
Type of the mix: sera super mix
Target analytes: C6, C7, C8, C9, C10, C11, C12, THPFOS, THPFDS, PFOS

Study Number: Sera SPE validation
Final Solvent&TN-A #: MeOH/6308
Final Volume of the mix: 25

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: Vujadka Knežjan Verified by: Cindy Cardona 10/31/02

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

SINGLE COMPONENT PREPARATION LOG

Date: 10.10.02

Book No. 02 050
Page No. 14

Analyst: OKJ

Description: 100ppb SUPER MIX FOR SPE

Stock Number: 02002-63

Weight or Volume Used: 5 ml

Balance ID: NA

Concentration or Purity: 500ppb

Other Correction Factors: NA

Corrected Weight: NA

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

Final Volume: 25 ml

Final Concentration: 100ppb

Storage Location: 2nd fl.

Expiration Date: 11.13.02

Exact Copy of Original

LMC 10/31/02
Initial Date

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

000247

SINGLE COMPONENT PREPARATION LOG

Date: 10/10/02

Book No. 02 050
Page No. 17

Analyst: OK

Description: 0.75ppb SUPERMIX

Stock Number: M2cc02-63

Weight or Volume Used: 37.5 µl

Balance ID: NA

Concentration or Purity: 5cppb

Other Correction Factors: NA

Corrected Weight: NA

Solvent and TN-A Number: MeOH / TNA6310

Final Volume: 25ml

Final Concentration: 0.75ppb

Storage Location: 2nd fl.

Expiration Date: 11/3/02

Exact Copy of Original

CMC 10/31/02
Initial Date

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

SINGLE COMPONENT PREPARATION LOG

Date: 10-10-02

Book No. 02 050
Page No. 16

Analyst: CKJ

Description: 0.25 ppb SUPERMIX

Stock Number: 02-02-63

Weight or Volume Used: 12.5 µl

Balance ID: NA

Concentration or Purity: (R) OK 10-10-02 N 50ppb

Other Correction Factors: NA

Corrected Weight: NA

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

Final Volume: 25 mL

Final Concentration: 0.25ppb

Storage Location: 2nd fl.

Expiration Date: 11-13-02

Exact Copy of Original

CMK 10/31/02
Initial Date

Reviewed by: _____ 10/1/02
Signature Date

000249

MULTI-LEVEL PREPARATION LOG

Book No. 02 048
Page No. 21

Description: CURVE FOR SERA SPE - SUPERMIX
Date Prepared: ~~10-6-02~~ Revised 10-7-02 Storage Location: 2nd fl.
Prepared By: CK Expiration Date: 11-13-02

Solvent: MEOH
3M Trace #: IN-A-G=202

Standard ID #	02 048-21-1	02 048-21-2	02 048-21-3	02 048-21-4	02 048-21-5	02 048-21-6	02 048-21-7	02 048-21-8
Description ppb	0.1	0.5	1	2.5	5	7.5	10	15

Component Standards				Amount of Component Standard Added µl							
Description	ID #	Conc	Expiration Date	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7	Std 8
SUPERMIX FOR SERA SPE	02a02-63	200ppb	11-13-02	5	25	50	125	250	375	500	750
						OK 10-7-02					
Final Volume µl				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 ppb	20	30				OK 10-7-02		

Component Standards				Amount of Component Standard Added µl							
Description	ID #	Conc	Expiration Date	Std 9	Std 10	Std 11	Std 12	Std 13	Std 14	Std 15	Std 16
SUPERMIX FOR SERA SPE	02a02-63	200ppb	11-13-02	1000	1600						
		OK 10-7-02							OK 10-7-02		
Final Volume µl				25	→						
Final Concentration ppb				20	30				OK 10-7-02		

Reviewed by: [signature] 10/11/02

Exact Copy of Original
Initial [signature] Date 10/21/02

3M ENVIRONMENTAL LABORATORY E02-10398

000250

MULTI-COMPONENT PREPARATION LOG

Book No. 02 002
Page No. 63

Description: SUPERMIX FOR 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

Standard ID #	02 002-63

Component Standard						Corrected Weight	Final Concentration ppb
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 TPM	2.26.03	12.5 µl			501
PFOS	~~02040~~ (use) 02022-56	1048 ppm	11.15.02	12 µl			503
	Final Volume	1 ml		25	Approximate Concentration of the mix		500 ppb

OK
10 6 02

Reviewed by: _____ Signature, 10/11/02 Date

Exact Copy of Original
10/31/02 Date

000251

Test Control and Reference Substance Log

Substance trade name or reference #	TDHA	TCR Substance #	TCR-99131-025
Substance/chemical name:	Tridecafluoroheptanoic Acid	TCR #	TCR-267
Lot/batch #:	PU/07219EU	Received from:	Aldrich
3M #	NA		
Expiration date:	01/01/2005	Amount received (wt. or vol):	25 g, 37.1357 g gross wt.
Initials:	SRP	Date:	12/21/1999
Number/size of containers:	1/ 30 mL plastic container	Shipper:	Courier
Condition:	clear crystals	MSDS (y/n)	● 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

Exact Copy of Original

CMC 12/31/02
Initial Date

000252

E02-1039
TCR-99131-25
TCR-99131-26

CERTIFICATE OF ANALYSIS

3M ENVIRONMENTAL
SCOTT POST
651 778 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 7988 OF EDITION I, VOLUME 1 OF "THE ALDRICH LIBRARY OF FT-IR SPECTRA".
FLUORINE NMR	CONFORMS TO STRUCTURE.
GAS LIQUID CHROMATOGRAPHY	99.5 %
QUALITY CONTROL ACCEPTANCE DATE	JUNE 1999

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

ALDRICH CHEMICAL COMPANY
DAVID SWESSEL
JANUARY 26, 2000

** TOTAL PAGE.04 **

000253

USE LOG

TDHA
Tridecafluoroheptanoic Acid
TCR-99131-025

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

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

SINGLE COMPONENT PREPARATION LOG

Date: 8/29/02

Book No. 02 040
Page No. 58

Analyst: RWW

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 @ RWW-10/1/02

Storage Location: 2nd Floor - Rm Temp.

Expiration Date: 2/28/03

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

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

000255

SINGLE COMPONENT PREPARATION LOG

Date: 8/29/02

Book No. 02 040
Page No. 58

Analyst: RWW

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 RWW-10/1/02

Storage Location: 2nd Floor - Rm Temp.

Expiration Date: 2/28/03

Exact Copy of Original

CMC 11/1/02
Initial Date

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

000256

3M SPECIALTY MATERIALS MANUFACTURING DIVISION ANALYTICAL LABORATORY

Request # **GID:71638**

To: Lisa Stevenson - (8-5568) – ET&SS – 2-3E-09

From: Tom Kestner - (3-5633) - SMMD Analytical Lab - 236-2B-11

Subject: **Characterization of TCR-99131-025 by ^{1}H-NMR and ^{19}F-NMR Spectroscopy**

Date: October 30, 2002

SAMPLE DESCRIPTION:

- TCR-99131-025, lot PU/07219EU from the Telomer project.
  Nominal product = $CF_3(CF_2)_n$-CO_2H, where average n ≈ 5 (white solid).

Sample	Spectra #'s	Experiment Descriptions
TCR-99131-025	H71638.GID.401	400 MHz 1D ^{1}H-NMR in a $CFCl_3$/acetone-d_6 solvent mixture + p-HFX cross integration/internal std.
TCR-99131-025	F71638.GID.401	376 MHz 1D ^{19}F-NMR in a $CFCl_3$/acetone-d_6 solvent mixture + p-HFX cross integration/internal std.
TCR-99131-025	F71638.GID.COSY.502	470 MHz 2D ^{19}F-NMR COSY (correlated spectroscopy) experiment in a $CFCl_3$/acetone-d_6 solvent mixture.

OBJECTIVE:

This sample was subjected to a combination of ^{1}H-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(trifluoro-methyl)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 ^{1}H-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}H/^{19}F-NMR internal standard to allow the calculation of the absolute weight percent concentrations of specific components, or 2) a ^{1}H/^{19}F-NMR cross integration standard to permit the cross correlation of the relative ^{1}H and ^{19}F signal intensities for evaluation of the overall sample composition.

RESULTS:

The ^{1}H-NMR and ^{19}F-NMR spectral data indicated this sample was a relatively high purity form of the nominal product, $CF_3(CF_2)_n$-CO_2H, where the average value of n = 5.05. Small amounts of a few impurity components, including probable isomers, were also assigned. A ^{1}H/^{19}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}H/^{19}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

TMK 10/31/02
Initial Date

October 30, 2002

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

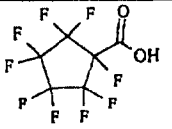
Copies of the NMR spectra are attached with the paper copy of this report for your reference. If you have any questions about these results, please let me know.

Tom Kestner

c: Mark Ellefson
Rick Payfer
Ron Purcell
William Reagen

File Reference: ts71638.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 $^{1}H/^{19}F$-NMR Cross Integration Analysis

Component Structures [1]	$^{1}H/^{19}F$-NMR Relative **Weight%** Concentrations (single trial analysis)
$CF_3(CF_2)_n$-CO_2H (where average n = 5.05)	98.24%
Probable **internal branched isomers** $CF_3(CF_2)_x$-$CF(CF_3)$-$(CF_2)_y$-CO_2H (where x≠0 and assume x+y = 3.05 for calculation purposes)	0.87%
Probable **terminal isopropyl branched isomers** $(CF_3)_2$-CF-$(CF_2)_n$-CO_2H (assume n=3.05 for calculation purposes)	0.52%
A probable ether/acid as possible O-[CF_2CF_2-$CO_2H]_2$	0.13%
Probable 	0.11%
C_nH_{2n+2} saturated aliphatic hydrocarbons and functional aliphatic components, C_nH_{2n+1}-X, where –X can be possible ether and ester functional groups.	≈0.11%
Possible $CF_3(CF_2)_n$-CO_2CH_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.

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

000258

Certificate of Analysis

Nominal Product: $CF_3(CF_2)_n$-CO_2H, where average n ≈ 5

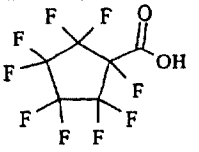
Tridecafluoroheptanoic acid

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 ^{1}H-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}F/^{1}H-NMR Spectral Analyses

Component Structures [1]	^{1}H/^{19}F-NMR Relative **Weight%** Concentrations (single trial analysis)
$CF_3(CF_2)_n$-CO_2H (where average n = 5.05)	≤98.2% **Purity**
Probable **internal branched isomers** $CF_3(CF_2)_x$-$CF(CF_3)$-$(CF_2)_y$-CO_2H (where x≠0 and assume x+y = 3.05 for calculation purposes)	0.87%
Probable **terminal isopropyl branched isomers** $(CF_3)_2$-CF-$(CF_2)_n$-CO_2H (assume n=3.05 for calculation purposes)	0.52%
A probable ether/acid as possible O-$[CF_2CF_2$-$CO_2H]_2$	0.13%
 Probable	0.11%
C_nH_{2n+2} saturated aliphatic hydrocarbons and functional aliphatic components, C_nH_{2n+1}-X, where –X can be possible ether and ester functional groups.	≈0.11%
Possible $CF_3(CF_2)_n$-CO_2CH_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

File Reference: CofA_TCR-99131-025_Lot PU-07219EU.doc

Test Control and Reference Substance Log

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

○ Archived/Substance Not Available

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

Exact Copy of Original

CMC 10/31/02
Initial Date

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
CMC Initial 10/31/02 Date

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

Exact Copy of Original

CMC 10/31/02
Initial Date

000262

SINGLE COMPONENT PREPARATION LOG

Date: 8/15/02

Book No. 02 040
Page No. 45

Analyst: RWW

Description: Pentadecafluorooctanoic Acid Std. K C7F15COOH

Stock Number: TCR-017

Weight or Volume Used: 0.0615g

Balance ID: 914

Concentration or Purity: NA

Other Correction Factors: NA

Corrected Weight: 0.0615g

Solvent and TN-A Number: MeOH/TN-A-0243

Final Volume: 50ml

Final Concentration: 1230ppm

Storage Location: 2nd Floor - Room Temp

Expiration Date: 2/15/03

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

Reviewed by: Tim N. Stevenson 08/15/02
Signature, Date

000263

3M SPECIALTY MATERIALS MANUFACTURING DIVISION ANALYTICAL LABORATORY

Request # **GID:71638**

To: Lisa Stevenson - (8-5568) – ET&SS – 2-3E-09

From: Tom Kestner - (3-5633) - SMMD Analytical Lab - 236-2B-11

Subject: **Characterization of TCR-617 by ^{1}H-NMR, ^{19}F-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 = $CF_3(CF_2)_n$-CO_2H, 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 reported 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 $(C_6F_{13}O)$-CO_2H ether acid.

OBJECTIVE:

This sample was subjected to a combination of ^{1}H-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. 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(trifluoro-methyl)benzene (p-HFX), and then totally dissolved in deuterated acetone (acetone-d_6) for subsequent analysis by NMR. A 500 MHz ^{1}H-NMR spectrum and a 470 MHz ^{19}F-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 ^{1}H/^{19}F-NMR internal standard to allow the calculation of the absolute weight percent concentrations of specific components, or 2) a ^{1}H/^{19}F-NMR cross integration standard to permit the cross correlation of the relative ^{1}H and ^{19}F signal intensities for evaluation of the overall sample composition.

RESULTS:

The combined ^{1}H-NMR, ^{19}F-NMR, and LC/MS analyses indicated this sample was a high purity form of the nominal product, $CF_3(CF_2)_n$-CO_2H, where the average value of n = 6.02. Small amounts of a few impurity components, including probable isomers, were also assigned. A ^{1}H/^{19}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 combined single trial ^{1}H/^{19}F-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.

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

October 28, 2002

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

RESULTS (cont.):

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

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

Tom Kestner

c: Joel Miller
Rick Payfer
Ron Purcell
William Reagen

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

Component Structures [1]	NMR Relative **Weight%** Concentrations (single trial measurement)
$CF_3(CF_2)_n$-CO_2H where average n = 6.02 by ^{19}F-NMR. LC/MS showed n=6 (major), n=5, n=4 (minors).	99.52%
Probable $(CF_3)_2$-CF-$(CF_2)_n$-CO_2H assume n=4 for calculation purposes	0.39%
Probable $(C_6F_{13}O)$-CO_2H acyclic ether acid as possible CF_3CF_2-O-$CF(CF_3)$-CF_2CF_2-CO_2H	0.057%
Possible $CF_3(CF_2)_x$-$CF(CF_3)$-$(CF_2)_y$-CO_2H where x≠0, y≠0 and assume x+y = 4 for calculation purposes	0.019%
Possible $(CF_3)_3$-C-$(CF_2)_n$-CO_2H assume n=3 for calculation purposes	0.013%
Possible C_nH_{2n+2} saturated aliphatic hydrocarbons	0.0079%

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

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

Certificate of Analysis

Nominal Product: $CF_3(CF_2)_n$-CO_2H, where average n ≈ 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, ^{1}H-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}F/^{1}H-NMR and LC/MS Analyses

Component Structures [1]	^{1}H/^{19}F-NMR Relative **Weight%** Concentrations (single trial analysis)
$CF_3(CF_2)_n$-CO_2H where average n = 6.02 by ^{19}F-NMR. LC/MS showed n=6 (major), n=5, n=4 (minors).	≤99.51% **Purity**
Probable $(CF_3)_2$-CF-$(CF_2)_n$-CO_2H assume n=4 for calculation purposes	0.39%
Probable $(C_6F_{13}O)$-CO_2H acyclic ether acid as possible CF_3CF_2-O-$CF(CF_3)$-CF_2CF_2-CO_2H	0.057%
Possible $CF_3(CF_2)_x$-$CF(CF_3)$-$(CF_2)_y$-CO_2H where x≠0, y≠0 and assume x+y = 4 for calculation purposes	0.019%
Possible $(CF_3)_3$-C-$(CF_2)_n$-CO_2H assume n=3 for calculation purposes	0.013%
Possible C_nH_{2n+2} saturated aliphatic hydrocarbons	0.0079%

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

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

File Reference: CofA_TCR-617_Lot 210002.doc

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:	**C9HF17O2**
Comments	**Shipping comments: Corrosive solid, No 3M ID# - must ship ground small quantity exception (30 mL/g or less per vial). LAS 10/02/02**
Attachment(s)	TCR-618.pdf

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

000267

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

CERTIFICATE OF ANALYSIS

Date: 27-Nov-01

Material: Heptadecafluorononanoic acid

Cat.No.: 2263

Cas No.: [375-95-1]

Lot No.: H7568

Assay: 99+% by NaOH titration

Appearance: Off-white solid

Melting Point: 57-62°C

Exact Copy of Original
CMC 10/3/02
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
Page No. 46

Analyst: RWN

Description: Heptadeca Fluorooctanoic Acid Stock (C8F17COOH

Stock Number: TCR-C18

Weight or Volume Used: 0.0634g

Balance ID: 914

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: Lisa A. Stevenson 08/15/02
Signature, Date

000270

3M SPECIALTY MATERIALS MANUFACTURING DIVISION ANALYTICAL LABORATORY

Request # **GID:71638**

To: Lisa Stevenson - (8-5568) – ET&SS – 2-3E-09

From: Tom Kestner - (3-5633) - SMMD Analytical Lab - 236-2B-11

Subject: **Characterization of TCR-618 by ^{1}H-NMR and ^{19}F-NMR Spectroscopy**

Date: October 28, 2002

SAMPLE DESCRIPTION:

- TCR-618, lot H7568 from the Telomer project.
  Nominal product = $CF_3(CF_2)_n$-CO_2H, where average n ≈ 7 (white solid).

Sample	Spectra #'s	Experiment Descriptions
TCR-618	H71638.GID.402	400 MHz ^{1}H-NMR in acetone-d_6 solvent + p-HFX cross integration/internal std.
TCR-618	F71638.GID.402/.403	376 MHz ^{19}F-NMR in acetone-d_6 solvent + p-HFX cross integration/internal std.

OBJECTIVE:

This sample was subjected to a combination of ^{1}H-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(trifluoro-methyl)benzene (p-HFX), and then totally dissolved in deuterated acetone (acetone-d_6) for subsequent analysis by NMR. A 400 MHz ^{1}H-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}H/^{19}F-NMR internal standard to allow the calculation of the absolute weight percent concentrations of specific components, or 2) a ^{1}H/^{19}F-NMR cross integration standard to permit the cross correlation of the relative ^{1}H and ^{19}F signal intensities for evaluation of the overall sample composition.

RESULTS:

The ^{1}H-NMR and ^{19}F-NMR spectral data indicated this sample was a high purity form of the nominal product, $CF_3(CF_2)_n$-CO_2H, where the average value of n = 6.955. Small amounts of a few impurity components were also assigned. A ^{1}H/^{19}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}H/^{19}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

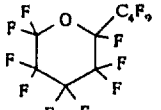
Tom Kestner

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 $^{1}H/^{19}F$-NMR Cross Integration Analysis

Component Structures [1]	NMR Relative **Weight%** Concentrations (single trial measurement)
$CF_3(CF_2)_n$-CO_2H where average n = 6.955	98.02%
Probable H-$(CF_2)_n$-CO_2H assume n = 8 for calculation purposes	1.10%
Probable $CF_3(CF_2)_n$-CO_2CH_3 where average n = 6.955	0.65%
A probable cyclic ether  as possible or similar	0.20%
Total inorganic fluoride (at least 3-types), including some possible HF	0.021%
Possible C_nH_{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: $CF_3(CF_2)_n$-CO_2H, where average n ≈ 7

Heptadecafluorononanoic acid

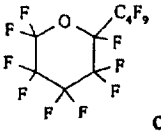
Product Code: TCR-618, Lot H7568

October 28, 2002

Tom Kestner

The sample of **TCR-618, lot H7568** was analyzed using a combination of ^{19}F-NMR and ^{1}H-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}F/^{1}H-NMR Spectral Analyses

Component Structures [1]	^{1}H/^{19}F-NMR Relative **Weight%** Concentrations (single trial analysis)
$CF_3(CF_2)_n$-CO_2H where average n = 6.955	≤98.02% **Purity**
Probable H-$(CF_2)_n$-CO_2H assume n = 8 for calculation purposes	1.10%
Probable $CF_3(CF_2)_n$-CO_2CH_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 C_nH_{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

File Reference: CofA_TCR-618_Lot H7568.doc

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

CMK 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 ^{1}H-NMR and ^{19}F-NMR Spectroscopy**

Date: October 28, 2002

SAMPLE DESCRIPTION:

- SD036, lot R11K from the Telomer project.
  Nominal product = $CF_3(CF_2)_n$-CO_2H, 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 ^{1}H-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 ^{1}H-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}H/^{19}F-NMR internal standard to allow the calculation of the absolute weight percent concentrations of specific components, or 2) a ^{1}H/^{19}F-NMR cross integration standard to permit the cross correlation of the relative ^{1}H and ^{19}F signal intensities for evaluation of the overall sample composition.

RESULTS:

The ^{1}H-NMR and ^{19}F-NMR spectral data indicated this sample was a relatively high purity form of the nominal product, $CF_3(CF_2)_n$-CO_2H, where the average value of n = 7.85. Small amounts of a few impurity components, including probable isomers, were also assigned. A ^{1}H/^{19}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}H/^{19}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 **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

CJYY 10/31/02
Initial Date

October 28, 2002

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

Tom Kestner

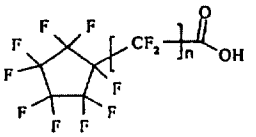
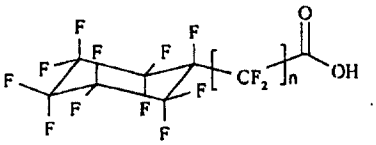
c: Rick Payfer
Ron Purcell
William Reagen

File Reference: ls71638.GID.SD036_Lot R11K_Telomer Project.DOC/101

TABLE-1

Sample: SD036, Lot R11K from the Telomer project.

Overall Compositional Results by $^{1}H/^{19}F$-NMR Cross Integration Analysis

Component Structures [1]	NMR Relative **Weight%** Concentrations (single trial measurement)
$CF_3(CF_2)_n$-CO_2H where average n = 7.85	98.01%
Probable $CF_3(CF_2)_x$-$CF(CF_3)$-$(CF_2)_y$-CO_2H where x≠0 and assume x+y = 5.85 for calculation purposes	1.41%
Probable $(CF_3)_2$-CF-$(CF_2)_n$-CO_2H 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 C_nH_{2n+2} saturated aliphatic hydrocarbons	0.026%
Total inorganic fluoride	≥0.0095%

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

Exact Copy of Original

LMK 10/31/02
Initial Date

000276

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

Appearance: White solid

Melting Point: 83-85°C

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

Yumei Yang
Yumei Yang
Quality Control

USE LOG
Nonadecafluorodecanoic acid Nonadecafluorodecanoic acid SD036

Gross Wt./Vol. Before withdrawal Balance ID 1	Amnt. withdrawn (mass or vol) Balance ID 2	Gross wt/vol after withdrawal Balance ID 1	Purpose (enter standard number or reason for removal)	Balance ID 1	Balance ID 2	Initials	Date
33.9944g	0.1196g	33.8784g	standard 02022-90	914	-	RWW	07/02/2002

Exact Copy of Original

CMC 10/31/02
Initial Date

SINGLE COMPONENT PREPARATION LOG

Date: 7/2/02

Book No. 02 022
Page No. 90

Analyst: RWW

Description: PFDA Stock

Stock Number: SD036

Weight or Volume Used: 103.1 mg

Balance ID: 914

Concentration or Purity: 98%

Other Correction Factors: NA

Corrected Weight: 101.0 mg

Solvent and TN-A Number: Methanol - TN-A-0205

Final Volume: 100 ml

Final Concentration: 1010 ppm

Storage Location: 2nd Floor

Expiration Date: 1/2/03

Exact Copy of Original

CMC Initial

10/31/02 Date

Reviewed by: Cindy Carlson 7/17/02
Signature, Date

279

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:	C11HF21O2
Comments	Shipping comments: Harmful solid, No 3M ID# - must ship ground small quantity exception (30 mL/g or less per vial). LAS 10/02/02
Attachment(s)	TCR-619.pdf

Exact Copy of Original

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 ^{1}H-NMR and ^{19}F-NMR Spectroscopy**

Date: October 29, 2002

SAMPLE DESCRIPTION:

- TCR-619, lot U11N from the Telomer project.
  Nominal product = $CF_3(CF_2)_n$-CO_2H, where average n ≈ 9 (white powder).

Sample	Spectra #'s	Experiment Descriptions
TCR-619	H71638.GID.404	400 MHz ^{1}H-NMR in acetone-d_6 solvent + p-HFX cross integration/internal std.
TCR-619	F71638.GID.405	376 MHz ^{19}F-NMR in acetone-d_6 solvent + p-HFX cross integration/internal std.

OBJECTIVE:

This sample was subjected to a combination of ^{1}H-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 ^{1}H-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}H/^{19}F-NMR internal standard to allow the calculation of the absolute weight percent concentrations of specific components, or 2) a ^{1}H/^{19}F-NMR cross integration standard to permit the cross correlation of the relative ^{1}H and ^{19}F signal intensities for evaluation of the overall sample composition.

RESULTS:

The ^{1}H-NMR and ^{19}F-NMR spectral data indicated this sample was a relatively high purity form of the nominal product, $CF_3(CF_2)_n$-CO_2H, where the average value of n = 8.63. Small amounts of a few impurity components, including probable isomers, were also assigned. A ^{1}H/^{19}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}H/^{19}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

CMY 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: ls71638.GID.TCR-619_Lot U11N_Telomer Project.DOC/101

TABLE-1

Sample: TCR-619, Lot U11N from the Telomer project.
Overall Compositional Results by $^{1}H/^{19}F$-NMR Cross Integration Analysis

Component Structures [1]	NMR Relative **Weight%** Concentrations (single trial measurement)
$CF_3(CF_2)_n$-CO_2H (where average n = 8.63)	96.4%
Probable **internal branched isomers** $CF_3(CF_2)_x$-$CF(CF_3)$-$(CF_2)_y$-CO_2H (where x≠0 and assume x+y = 6.63 for calculation purposes)	2.6%
Probable **trans-olefins** of general form: trans-$CF_3(CF_2)_x$-CF=CF-$(CF_2)_y$-CO_2H (assume x+y = 6.63 for calculation purposes)	0.89%
Possible **terminal isopropyl branched isomers** $(CF_3)_2$-CF-$(CF_2)_n$-CO_2H (assume n=6.63 for calculation purposes)	0.12%
Probable C_nH_{2n+2} saturated aliphatic hydrocarbons	0.032%
Total **inorganic fluoride**	≥0.0017%

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

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

282

Certificate of Analysis

Nominal Product: $CF_3(CF_2)_n$-CO_2H, where average n ≈ 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 ^{1}H-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}F/^{1}H-NMR Spectral Analyses

Component Structures [1]	^{1}H/^{19}F-NMR Relative **Weight%** Concentrations (single trial analysis)
$CF_3(CF_2)_n$-CO_2H (where average n = 8.63)	≤96.4% **Purity**
Probable **internal branched isomers** $CF_3(CF_2)_x$-$CF(CF_3)$-$(CF_2)_y$-CO_2H (where x≠0 and assume x+y = 6.63 for calculation purposes)	2.6%
Probable **trans-olefins** of general form: trans-$CF_3(CF_2)_x$-CF=CF-$(CF_2)_y$-CO_2H (assume x+y = 6.63 for calculation purposes)	0.89%
Possible **terminal isopropyl branched isomers** $(CF_3)_2$-CF-$(CF_2)_n$-CO_2H (assume n=6.63 for calculation purposes)	0.12%
Probable C_nH_{2n+2} saturated aliphatic hydrocarbons	0.032%
Total **inorganic fluoride**	≥0.0017%

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

Tom Kestner

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

File Reference: CofA_TCR-619_Lot U11N.doc

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

CERTIFICATE OF ANALYSIS

Date: 15-Jul-02

Material: Perfluoroundecanoic acid

Cat.No.: 2265

Cas No.: [4234-23-5]

Lot No.: U11N

Assay: 99+% by NaOH titration

Appearance: White solid

Melting Point: 92-98°C

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

Yumei Yang
QC Manager

USE LOG

Perfluoroundecanoic acid
Perfluoroundecanoic acid
TCR-619

Gross Wt./Vol. Before withdrawal Balance ID 1	Amnt. withdrawn (mass or vol) Balance ID 2	Gross wt/vol after withdrawal Balance ID 1	Purpose (enter standard number or reason for removal)	Balance ID 1	Balance ID 2	Initials	Date
15.5264g	0.0691g	15.4573g	standard 02040-65	914	-	RWW	08/29/2002

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

000285

SINGLE COMPONENT PREPARATION LOG

Date: 8/29/02

Book No. 02 040
Page No. 65

Analyst: KWW

Description: Perfluoroundecanoic Acid

Stock Number: TCR-619

Weight or Volume Used: 0.0678g

Balance ID: 914

Concentration or Purity: NA

Other Correction Factors: NA

Corrected Weight: 0.0678g

Solvent and TN-A Number: MeOH/TN-A-644

Final Volume: 50ml

Final Concentration: 1356 ppm

Storage Location: 2nd Floor - Rm Temp

Expiration Date: 2/28/03

Exact Copy of Original

CMC 11/1/02
Initial Date

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

000286

Test Control and Reference Substance Log

Substance trade name or reference #	**Perfluorododecanoic acid**	TCR Substance #	**SD037**
Substance/chemical name:	**Perfluorododecanoic acid**	TCR #	**TCR-37**
Lot/batch #:	**R24K**	Received from:	**Oakwood Products Inc 4/2/99**
3M #	**ID#2266**		
Expiration date:	**12/01/2010**	Amount received (wt. or vol):	**25g, 43.4548 g gross wt.**
Initials:	**JCP**	Date:	**04/27/1999**
Number/size of containers:	**1/120 mL plastic bottle**	Shipper:	**N/A**
Condition:	**white powder MCH 07/22/99**	MSDS (y/n)	● 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

Initial 10/31/02 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 ^{1}H-NMR and ^{19}F-NMR Spectroscopy**

Date: October 28, 2002

SAMPLE DESCRIPTION:

- SD-037, lot R24K from the Telomer project.
  Nominal product = $CF_3(CF_2)_n$-CO_2H, where average n ≈ 10 (white powder).

Samples	Spectra #'s	Experiment Descriptions
SD037	H71638.GID.405	400 MHz ^{1}H-NMR in acetone-d_6 solvent + p-HFX cross integration/internal std.
SD037	F71638.GID.406	376 MHz ^{19}F-NMR in acetone-d_6 solvent + p-HFX cross integration/internal std.

OBJECTIVE:

This sample was subjected to a combination of ^{1}H-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 ^{1}H-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}H/^{19}F-NMR internal standard to allow the calculation of the absolute weight percent concentrations of specific components, or 2) a ^{1}H/^{19}F-NMR cross integration standard to permit the cross correlation of the relative ^{1}H and ^{19}F signal intensities for evaluation of the overall sample composition.

RESULTS:

The ^{1}H-NMR and ^{19}F-NMR spectral data indicated this sample was a high purity form of the nominal product, $CF_3(CF_2)_n$-CO_2H, where the average value of n = 10.042. Small amounts of a few impurity components, including probable isomers, were also assigned. A ^{1}H/^{19}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}H/^{19}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
SD037, Lot R24K: Telomer Project

Tom Kestner

c: Rick Payfer
Ron Purcell
William Reagen

File Reference: ls71638.GID.SD037_Lot R24K_Telomer Project.DOC/101

TABLE-1

Sample: SD037, Lot R24K from the Telomer project.
Overall Compositional Results by $^{1}H/^{19}F$-NMR Cross Integration Analysis

Component Structures [1]	NMR Relative **Weight%** Concentrations (single trial measurement)
$CF_3(CF_2)_n$-CO_2H where average n = 10.02	99.65%
Probable $(CF_3)_2$-CF-$(CF_2)_n$-CO_2H assume n=8 for calculation purposes	0.13%
Possible $CF_3(CF_2)_x$-$CF(CF_3)$-$(CF_2)_y$-CO_2H where x≠0, y≠0 and assume x+y = 8 for calculation purposes	0.088%
Probable chlorinated impurity, Cl-CF_2CF_2-R_f, possibly as Cl-$(CF_2)_{11}$-CO_2H	0.049%
Possible methyl ester impurity as $CF_3(CF_2)_n$-CO_2CH_3 where average n = 10.02	0.048%
Probable C_nH_{2n+2} saturated aliphatic hydrocarbons	0.016%
Toluene	0.015%

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: $CF_3(CF_2)_n$-CO_2H, where average n ≈ 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 ^{1}H-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 $^{19}F/^{1}H$-NMR Spectral Analyses

Component Structures [1]	$^{1}H/^{19}F$-NMR Relative **Weight%** Concentrations (single trial analysis)
$CF_3(CF_2)_n$-CO_2H where average n = 10.02	≤99.65% **Purity**
Probable $(CF_3)_2$-CF-$(CF_2)_n$-CO_2H assume n=8 for calculation purposes	0.13%
Possible $CF_3(CF_2)_x$-$CF(CF_3)$-$(CF_2)_y$-CO_2H where x≠0, y≠0 and assume x+y = 8 for calculation purposes	0.088%
Probable chlorinated impurity, Cl-CF_2CF_2-R_f, possibly as Cl-$(CF_2)_{11}$-CO_2H	0.049%
Possible methyl ester impurity as $CF_3(CF_2)_n$-CO_2CH_3 where average n = 10.02	0.048%
Probable C_nH_{2n+2} saturated aliphatic hydrocarbons	0.016%
Toluene	0.015%

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

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

File Reference: CofA_SD037_Lot R24K.doc

E02-1039
SD-057

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%
Perfluorododecanic 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	914	-	RWW	08/29/2002

Exact Copy of Original

CWS 6/3/02
Initial Date

SINGLE COMPONENT PREPARATION LOG

Date: 8/29/02

Book No. 02 040
Page No. 55

Analyst: RWW

Description: Perfluorododecanoic Acid - $C_{11}F_{23}COOH$

Stock Number: SD037

Weight or Volume Used: 0.0563g

Balance ID: 914

Concentration or Purity: NA

Other Correction Factors: NA

Corrected Weight: 0.0563g

Solvent and TN-A Number: MeOH-TN-A-4244

Final Volume: 50ml

Final Concentration: 1126 ppm

Storage Location: 2nd Floor - Rm Temp

Expiration Date: 2/29/03

Exact Copy of Original

CMC 11/1/02
Initial Date

Reviewed by: Lisa A. Stevenson 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 Traceablitity number was TN-A-2130 PMR 04/07/99 Moved to cold storage (< 0C) on 05/16/00 LAC 05/16/00 Standard has been moved to Freezer 19 in room 347 KJD 06/06/00 Standard was stored at room temperature prior to 05/16/00. LAC 12/19/00 Shipping Codes: FC-S000-0185-6(<30 g on dry ice) LAC 06/14/01 Shipping Comments: Dangerous goods In excepted quantity of Class 6, UN2811. REGULATED-TOXIC 98-0211-0888-5 (>30 g (1 oz sample)) Not on dry ice. LAC 01/08/02
Attachment(s)	 sd018msds.pdf  sd018nmrreq53030.pdf  sd018nmrreq61517.pc  E00-1682-COA-SD018-PFOS-Rev3

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

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

INTERIM CERTIFICATE OF ANALYSIS

Revision 3

Centre Analytical Laboratories COA Reference #: 023-018A
3M Product: PFOS, Lot 217
Reference #: SD-018
Purity: 86.9%

Test Name	Specifications	Result
Purity[1]		**86.9%**
Appearance	White Crystalline Powder	Conforms
Identification NMR		Positive
Metals (ICP/MS) 1. Calcium 2. Magnesium 3. Sodium 4. Potassium[2] 5. Nickel 6. Iron 7. Manganese		 1. 0.005 wt./wt.% 2. 0.001 wt./wt.% 3. 1.439 wt./wt.% 4. 6.849 wt./wt.% 5. <0.001 wt./wt.% 6. 0.005 wt./wt.% 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 2. Fluoride 3. Bromide 4. Nitrate 5. Nitrite 6. Phosphate 7. Sulfate[4]		 1. <0.015 wt./wt.% 2. 0.59 wt./wt.% 3. <0.040 wt./wt.% 4. <0.009 wt./wt.% 5. <0.006 wt./wt.% 6. <0.007 wt./wt.% 7. 8.76 wt./wt.%
Organic Acids [5] (IC) 1. TFA 2. PFPA 3. HFBA 4. NFPA		 1. <0.1 wt./wt.% 2. <0.1 wt./wt.% 3. 0.10 wt./wt.% 4. 0.28 wt./wt.%
Elemental Analysis[6]: 1. Carbon 2. Hydrogen 3. Nitrogen 4. Sulfur 5. Fluorine	 1. Theoretical Value = 17.8% 2. Theoretical Value = 0% 3. Theoretical Value = 0% 4. Theoretical Value = 5.95% 5. Theoretical Value = 60%	 1. 12.48 wt./wt.% 2. 0.244 wt./wt.% 3. 1.74 wt./wt.% 4. 8.84 wt./wt.% 5. 54.1 wt./wt.%

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

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

INTERIM CERTIFICATE OF ANALYSIS

Revision 3

Centre Analytical Laboratories COA Reference #: 023-018A

Date of Last Analysis: 08/31/00

Expiration Date: 08/31/06

Storage Conditions: Frozen ≤-10°C

Re-assessment Date: 08/31/06

[1]Purity = 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%

[2]Potassium is expected in this salt form and is therefore not considered an impurity.

[3]Purity by DSC is generally not applicable to materials of low purity. No endotherm was observed for this sample.

[4]Sulfur 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

[6]Theoretical value calculations based on the empirical formula, $C_8F_{17}SO_3^-K^+$ (MW=538)

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

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

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

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: Chas Simons 10/11/01
Charles Simons Date
Scientist, Centre Analytical Laboratories

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

Reviewed By: John M Flaherty 10/11/01
John Flaherty Date
Laboratory Manager, Centre Analytical Laboratories

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**

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

SINGLE COMPONENT PREPARATION LOG

Date: 5/13/02

Book No. 02 022
Page No. 56

Analyst: RWN

Description: PFOS 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: MeOH/TH-A-06034

Final Volume: 50ml

Final Concentration: 1048ppm

Storage Location: R16

Expiration Date: 11/13/02

Exact Copy of Original

CMC 10/31/02
Initial Date

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

ATTACHMENT E: PROTOCOL AND AMENDMENT

3M

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

Exact Copy of Original
CMC 10/31/08
Initial Date

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	10 October 2002
Experimental Termination Date	01 November 2002

Exact Copy of Original

CMC 10/31/02
Initial Date

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	$C_5F_{11}COOH$
Tetradecafluoroheptanoic Acid	$C_6F_{13}COOH$
Pentadecafluorooctanoic Acid	$C_7F_{15}COOH$
Heptadecafluorononanoic Acid	$C_8F_{17}COOH$
Nonadecafluorodecanoic Acid	$C_9F_{19}COOH$
Perfluoroundecanoic Acid	$C_{10}F_{21}COOH$
Perfluorododecanoic Acid	$C_{11}F_{23}COOH$
1H,2H,3H,4H-perfluorooctanesulfonate	$C_8H_5F_{13}O_3S$
1H,2H,3H,4H-perfluorodecane sulfonate	$C_{10}H_5F_{17}O_3S$
Potassium Perfluorooctanesulfonate	$C_8F_{17}SO_3^-K^+$

Exact Copy of Original

CMK 10/31/05
Initial Date

4.0 Reference Substances

Reference Substances

Reference Substance	Formula	Traceability #	Source	Physical Description	Purity	Storage Conditions
Perfluorohexanoic Acid	$C_5F_{11}COOH$	TCR-047	SMM* 236-1B-10	Colorless Liquid	TBD**	Frozen
Tetradecafluoro heptanioc Acid	$C_6F_{13}COOH$	TCR-267	Aldrich	Clear Crystals	99.5%	Frozen
Pentadecafluoro octanoic Acid	$C_7F_{15}COOH$	TCR-617	Oakwood Products	White Crystals	> 97%	Ambient Temperature
Heptadecafluoro nonanoic Acid	$C_8F_{17}COOH$	TCR-618	Oakwood Products	White Crystals	> 99%	Ambient Temperature
Nonadecafluoro decanoic Acid	$C_9F_{19}COOH$	TCR-036	Oakwood Products	White Solid	98%	Frozen
Perfluoroun decanoic Acid	$C_{10}F_{21}COOH$	TCR-619	Oakwood Products	White Crystals	> 99%	Ambient Temperature
Perfluorodo decanoic Acid	$C_{11}F_{23}COOH$	TCR-037	Oakwood Products	White Powder	96%	Frozen
1H,2H,3H,4H-perfluorooctane sulfonate	$C_8H_5F_{13}O_3S$	TCR-343	SynQuest Labs	White Powder	TBD	Frozen
1H,2H,3H,4H-perfluorodecane sulfonate	$C_{10}H_5F_{17}O_3S$	TCR-627	Pace Analytical	White Crystals	94.7%	Ambient Temperature
Potassium Perfluorooctane sulfonate	$C_8F_{17}SO_3^-K^+$	TCR-018	SMM* 236-1B-10	White Powder	86.9%	Frozen

*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

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

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., Southield, 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".

Exact Copy of Original

CMK 10/31/02
Initial Date

8.0 Data Quality Objectives

8.1 Absolute Recovery:

8.1.1 The absolute recovery of the method will be evaluated separately in human serum and plasma and rat serum. For each matrix, the samples will be fortified at two levels of (500ppt and 5ppb). All samples will be extracted through the method, and analyzed by comparison with external calibration of non-extracted standards. The non-extracted standard calibration curves will be prepared in methanol, and will consist of a minimum of nine (9) levels, including a methanol blank. The best appropriate regression will be used to describe this curve (for best accuracy at all levels of the standards).

8.1.2 The accuracy (% recovery) and precision (%CV of the recoveries) will be determined at each level, and for all levels combined. There is no control limit for accuracy; precision must be better than 15% at each level.

8.2 Calibration: Calibration curves will be prepared from extracted matrix standards, in Chinese plasma and rabbit serum. The curves will consist of a minimum of nine (9) levels and a matrix blank. The equation will be determined by regression analysis using the peak areas of the analyte. The accuracy of each level will be verified. Any level outside 75% - 125% of nominal must be deactivated, and regression re-calculated, except the LLOQ which must be within 30% of nominal. All levels must show a response greater than twice that of the blank. A maximum of four (4) levels may be deactivated in any one set, or the set will be re-analyzed.

8.3 Limits of Quantitation (LOQ): The lower limit of quantitation (LLOQ) will be determined for each analyte. The level determined as the LLOQ must show a recovery within 75% - 125% for the analytes and must show a response greater than twice that of the blank. These limits will be determined during the course of the study and documented in the raw data. Should the LLOQ level calibration be de-activated in a particular set, the practical limit of quantitation for this set will be raised to the next acceptable level. Samples below the practical LOQ of that set will be reanalyzed until quantitated in a set including the validated LLOQ.

The upper limit of quantitation (ULOQ) will be determined in serum and plasma. The level determined as the ULOQ must show a recovery within 75% - 125% for the analytes. These limits will be determined during the course of the study and documented in the raw data. Should the ULOQ level calibration standard be de-activated in a particular set, the practical limit of quantitation for this set will be lowered to the next highest acceptable level.

Any sample with an area greater than 110% of the highest acceptable standard will need to be diluted into the range of the calibration curve. If samples are diluted into the range of the curve during analyses and enough sample remains, a post-run dilution validation will be performed to verify sample values. To

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

Exact Copy of Original
CMC 10/31/07
Initial Date

contain updated methods incorporating any changes or improvements.

10.1.6 A description of the test system.

10.1.7 A description of any circumstances that may have affected the quality or the integrity of the data.

10.1.8 The name of the Study Director and the names of other scientists, professionals, and supervisory personnel involved in the study.

10.1.9 A description of the transformations, calculations, or operations performed on the data, a summary and analysis of the analytical chemistry data, and a statement of the conclusions drawn from the analyses.

10.1.10 Statistical methods used to evaluate the data, if applicable.

10.1.11 The signed and dated reports of each of the individual scientists or other professionals involved in the study, if applicable.

10.1.12 The location where raw data and the final report are to be stored.

10.1.13 A statement prepared by the quality assurance unit listing the dates that study inspections and audits were made and the dates of any findings reported to the Study Director and Management.

10.2 If it is necessary to make corrections or additions to a final report after it has been accepted, the changes will be made in the form of an amendment issued by the Study Director. The amendment will clearly identify the part of the final report that is being amended, provide the reasons for the amendment, and will be signed by the Study Director.

11.0 Quality Assurance

11.1 The 3M Environmental Laboratory Quality Assurance Unit will review the protocol and audit study conduct, data, and the final report to determine compliance with Good Laboratory Practice Standards and with 3M Environmental Laboratory Standard Operating Procedures. The Quality Assurance Unit will report all findings to the Sponsor Representative and the Study Director.

12.0 Location of Raw Data, Records, and Final Report

12.1 Original data or copies thereof, will be available at 3M Environmental Laboratory. When the final report is completed all original paper data, including those items listed below, will be retained in the archives of 3M Environmental Laboratory following signing of the final report.

12.2 The following raw data and records will be retained in the study folder in the archives according to 3M Environmental Laboratory SOPs.

12.2.1 Approved protocol and amendments

Exact Copy of Original
CMC 10/31/08
Initial Date

12.2.2 Study correspondence

12.2.3 Shipping records

12.2.4 Raw data

12.2.5 Approved final report (original signed copy)

12.2.6 Electronic copies of data

12.3 The following supporting records will be retained separately from the study folder in the archives according to 3M Environmental Laboratory SOPs:

12.3.1 Training records

12.3.2 Calibration records

12.3.3 Instrument maintenance logs

12.3.4 Standard operating procedures, equipment procedures, and methods

13.0 Sample Retention

13.1 A portion of the reference substances used in the study will be retained in the laboratory for a period of not less than 2 years after a report is issued.

13.2 Sample extracts will be retained and refrigerated for a period of not less than three months from the time of analysis.

14.0 Protocol Amendments and Deviations

14.1 Amendments and deviations to the protocol will be in the form of written amendments signed by the Study Director and the Sponsor Representative. Amendments will be considered as part of the protocol and will be attached to the final protocol. All changes to the protocol will be indicated in the final report. Any other changes will be in the form of written deviations, signed by the Study Director and filed with the raw data.

15.0 Attachments

None

Protocol Signature

Study Director:

Mark E. Ellefson 10/10/02

Mark E. Ellefson Date:
3M Environmental Laboratory

Protocol Approval

Sponsor:

10/10/02

William K. Reagen, Ph.D. Date:
3M Environmental Laboratory

Exact Copy of Original

Cmc 10/31/02
Initial Date

Study Title

Analysis of Endogenous Fluorochemicals in Normal Pooled Human Serum and Plasma

PROTOCOL AMENDMENT NO. 1

Amendment Date:

October 31, 2002

Performing Laboratory

3M Environmental Laboratory
Building 2-3E-09
935 Bush Avenue
St. Paul, MN 55144-1000

Laboratory Project Identification

3M Environmental Laboratory Study LIMS #E02-1039

Exact Copy of Original

CMC Initial 11/11/02 Date

Protocol #E00-1311
Amendment 1

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

1. PROTOCOL READS: SECTION 8.1.1: The absolute recovery of the method will be evaluated separately in human serum and plasma and rat serum. For each matrix, the samples will be fortified at two levels of (500ppt and 5ppb). All samples will be extracted through the method, and analyzed by comparison with external calibration of non-extracted standards. The non-extracted standard calibration curves will be prepared in methanol, and will consist of a minimum of nine (9) levels, including a methanol blank. The best appropriate regression will be used to describe this curve (for best accuracy at all levels of the standards).

AMEND TO READ: The absolute recovery of the method will not be evaluated as the matrix effects render this type of comparison limited in its usefulness.

REASON: A planned change in the study's direction.

2. PROTOCOL READS: SECTION 8.2: Calibration: Calibration curves will be prepared from extracted matrix standards, in Chinese plasma and rabbit serum. The curves will consist of a minimum of nine (9) levels and a matrix blank.
AMEND TO READ: Calibration: Calibration curves will be prepared from extracted matrix standards in Chinese plasma. The curves will consist of a minimum of nine (9) levels. Reasons for not using one or more of these standards in the construction of a calibration curve are up to the discretion of the analyst and will be documented in the raw data.

REASON: The samples were run against 9 extracted standards without a blank, but not all of these standards were used to construct the curve. Additionally rabbit curves were not included in the data for this study.

3. PROTOCOL READS: SECTION 7.1: Analysis of the test and reference substances will be conducted in the 3M Environmental Laboratory. The analyses will be conducted as described by 3M Environmental Laboratory Method ETS-8-231, "Solid Phase Extraction and Analysis of Fluorochemical Compounds from Biological matrices".
AMEND TO READ: Analysis of the test and reference substances will be conducted in the 3M Environmental Laboratory. The analyses will be conducted as described by 3M Environmental Laboratory Method ETS-8-231, "Solid Phase Extraction and Analysis of Fluorochemical Compounds from Biological matrices". Selected ion monitoring will be used to detect the presence of the target analytes. The masses scanned will be documented in the raw data and in the final report.
REASON: Add clarity.

4. PROTOCOL READS: SECTION 8.3 Any sample with an area greater than 110% of the highest acceptable standard will need to be diluted into the range of the calibration curve. If samples are diluted into the range of the curve during analyses and enough sample remains, a post-run dilution validation will be performed to verify sample values. To perform the dilution validation, one sample will be separated into two representative samples (i.e. two 1 mL aliquots for fluid samples or two 1 gram amounts for tissue samples) then diluted using two procedures. The first procedure consists of diluting the sample with additional matrix prior to extraction (sera adding sera), while the second procedure consists of diluting the extract with solvent post-extraction (methanol extract adding additional methanol solvent).
If the values are not within 15% of each other additional testing will be required to determine which value is a correct representation of the sample concentration.

Exact Copy of Original

EMC 11/11/02
Initial Date

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 C_6 compound was eliminated from the study.

6. PROTOCOL READS: SECTION 3.1

Test Substances

Test Substance	Formula
Perfluorohexanoic Acid	$C_5F_{11}COOH$
Tetradecafluoroheptanioc Acid	$C_6F_{13}COOH$
Pentadecafluorooctanoic Acid	$C_7F_{15}COOH$
Heptadecafluorononanoic Acid	$C_8F_{17}COOH$
Nonadecafluorodecanoic Acid	$C_9F_{19}COOH$
Perfluoroundecanoic Acid	$C_{10}F_{21}COOH$
Perfluorododecanoic Acid	$C_{11}F_{23}COOH$
1H,2H,3H,4H-perfluorooctanesulfonate	$C_8H_5F_{13}O_3S$
1H,2H,3H,4H-perfluorodecane sulfonate	$C_{10}H_5F_{17}O_3S$
Potassium Perfluorooctanesulfonate	$C_8F_{17}SO_3^-K^+$

AMEND TO READ:

Test Substances

Test Substance	Formula
Tetradecafluoroheptanioc Acid	$C_6F_{13}COOH$
Pentadecafluorooctanoic Acid	$C_7F_{15}COOH$

Exact Copy of Original
CHC 11/11/02
Initial Date

Heptadecafluorononanoic Acid	$C_8F_{17}COOH$
Nonadecafluorodecanoic Acid	$C_9F_{19}COOH$
Perfluoroundecanoic Acid	$C_{10}F_{21}COOH$
Perfluorododecanoic Acid	$C_{11}F_{23}COOH$
1H,2H,3H,4H-perfluorooctanesulfonate	$C_8H_5F_{13}O_3S$
1H,2H,3H,4H-perfluorodecane sulfonate	$C_{10}H_5F_{17}O_3S$
Potassium Perfluorooctanesulfonate	$C_8F_{17}SO_3^-K^+$

REASON: The C_6 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	$C_5F_{11}COOH$	TCR-047	SMM* 236-1B-10	Colorless Liquid	TBD**	Frozen
Tetradecafluoro heptanoic Acid	$C_6F_{13}COOH$	TCR-267	Aldrich	Clear Crystals	99.5%	Frozen
Pentadecafluoro octanoic Acid	$C_7F_{15}COOH$	TCR-617	Oakwood Products	White Crystals	> 97%	Ambient Temperature
Heptadecafluoro nonanoic Acid	$C_8F_{17}COOH$	TCR-618	Oakwood Products	White Crystals	> 99%	Ambient Temperature
Nonadecafluoro decanoic Acid	$C_9F_{19}COOH$	TCR-036	Oakwood Products	White Solid	98%	Frozen
Perfluoroun decanoic Acid	$C_{10}F_{21}COOH$	TCR-619	Oakwood Products	White Crystals	> 99%	Ambient Temperature
Perfluorodo decanoic Acid	$C_{11}F_{23}COOH$	TCR-037	Oakwood Products	White Powder	96%	Frozen
1H,2H,3H,4H-perfluorooctane sulfonate	$C_8H_5F_{13}O_3S$	TCR-343	SynQuest Labs	White Powder	TBD	Frozen
1H,2H,3H,4H-perfluorodecane sulfonate	$C_{10}H_5F_{17}O_3S$	TCR-627	Pace Analytical	White Crystals	94.7%	Ambient Temperature
Potassium Perfluorooctane sulfonate	$C_8F_{17}SO_3^-K^+$	TCR-018	SMM* 236-1B-10	White Powder	86.9%	Frozen

AMEND TO READ

Reference Substances

Reference Substance	Formula	Traceability #	Source	Physical Description	Purity	Storage Conditions
Tetradecafluoro heptanoic Acid	$C_6F_{13}COOH$	TCR-267	Aldrich	Clear Crystals	99.5%	Frozen
Pentadecafluoro octanoic Acid	$C_7F_{15}COOH$	TCR-617	Oakwood Products	White Crystals	> 97%	Ambient Temperature

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

Pentadecafluoro octanoic Acid	$C_7F_{15}COOH$	TCR-617	Oakwood Products	White Crystals	> 97%	Ambient Temperature
Heptadecafluoro nonanoic Acid	$C_8F_{17}COOH$	TCR-618	Oakwood Products	White Crystals	> 99%	Ambient Temperature
Nonadecafluoro decanoic Acid	$C_9F_{19}COOH$	TCR-036	Oakwood Products	White Solid	98%	Frozen
Perfluoroun decanoic Acid	$C_{10}F_{21}COOH$	TCR-619	Oakwood Products	White Crystals	> 99%	Ambient Temperature
Perfluorodo decanoic Acid	$C_{11}F_{23}COOH$	TCR-037	Oakwood Products	White Powder	96%	Frozen
1H,2H,3H,4H-perfluorooctane sulfonate	$C_8H_6F_{13}O_3S$	TCR-343	SynQuest Labs	White Powder	TBD	Frozen
1H,2H,3H,4H-perfluorodecane sulfonate	$C_{10}H_5F_{17}O_3S$	TCR-627	Pace Analytical	White Crystals	94.7%	Ambient Temperature
Potassium Perfluorooctane sulfonate	$C_8F_{17}SO_3^-K^+$	TCR-018	SMM* 236-1B-10	White Powder	86.9%	Frozen

REASON: The C_6 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.

Exact Copy of Original

CMC 11/11/02
Initial Date

Protocol #E00-1311
Amendment 1

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

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

Amendment Approval

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

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

Exact Copy of Original
CMC 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

12/09/02

William K. Reagen, Sponsor Representative — Date

Mark E. Ellefson 12/09/02

Mark E. Ellefson, Study Director — Date