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Final Report
on
Methods Development for Total Organic Carbon Accountability

by

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Contents

Introduction

Experimental

Results

Summary

Appendix A - Biuret Active Substances

Appendix B - GC/FID FAME Analysis

Appendix C - Analysis of B and A Standard Mix By EPA-625
Methodology

Appendix D - Acidic Liquid Extraction Results

Appendix E - Basic Liquid Extraction Results

Appendix F - Diethyl Ether Extraction Results

Appendix G - Solid Phase Extraction Results

Appendix H - LC and LC/MS Analysis Results

Appendix I - Procedures and Instrumental Parameters

Appendix J - Solid Phase Extraction Recovery Study

Introduction

This report describes the efforts completed during the contract period beginning November 1, 1990 and ending April 30, 1991. Samples of product hygiene and potable water from WRT 3A were supplied by NASA/MSFC prior to contract award on July 24, 1990. Humidity condensate samples were supplied on August 3, 1990. During the course of this contract chemical analyses were performed on these samples to qualitatively determine specific components comprising the measured organic carbon concentration. In addition, these samples and known standard solutions were used to identify and develop methodology useful to future comprehensive characterization of similar samples.

Standard analyses including pH, conductivity and total organic carbon (TOC) were conducted. Colorimetric and enzyme linked assays for total protein, bile acids, B-hydroxybutyric acid, methylene blue active substances (MBAS), urea nitrogen, ammonia and glucose were also performed. Gas chromatographic procedures for non-volatile fatty acids and EPA priority pollutants were also performed. Lastly, liquid chromatography was used to screen for non-volatile, water soluble compounds not amenable to GC techniques.

Methods development efforts were initiated to separate and quantitate certain chemical classes not classically analyzed in water and wastewater samples. These included carbohydrates, organic acids, and amino acids. Finally, efforts were initiated to identify useful concentration techniques to enhance detection limits and recovery of non-volatile, water soluble compounds.

Experimental Methods

Samples

Ten separate samples were supplied collected from five separate locations. Duplicate samples, one preserved (H_2SO_4 to $\text{pH} \leq 2$) and one unpreserved at our request, were obtained from each location.

Upon collection, samples were immediately transported on ice to the UAH/CSLS Environmental Laboratory. The acid preserved samples were immediately refrigerated at $4\text{ }^\circ\text{C}$. Following removal of aliquots for pH and conductivity measurements, the unpreserved samples were frozen in acid leached high density polypropylene bottles along with a reagent water "storage blank".

Humidity condensate samples provided by MSFC on August 3, 1990, were collected during exercise periods after the conclusion of WRT 3A. It is unclear as to what equipment and level of exercise was obtained and how this testing period differed from those conducted during WRT 3A. As before, duplicate samples were provided with one preserved using H_2SO_4 and one unpreserved. Upon collection, samples were immediately transported on ice to the UAH/CSLS Environmental Laboratory. The preserved samples were refrigerated at $4\text{ }^\circ\text{C}$. The unpreserved samples were immediately tested for pH and conductivity, then frozen in acid leached high density polypropylene bottles.

Due to delays in contract procurement the samples had exceeded accepted holding times by as much as 60 days upon contract award. It should be noted that any presentation of findings using these samples should be labeled "suspect" and are therefore

qualitatively and quantitatively inconclusive. However, the primary purpose of the initial analysis was to verify laboratory performance using EPA 625 and EPA 525 methodology and to determine principal components accounting for the measured TOC. For these reasons and the much higher level of measured TOC (3-30 times higher than condensate and potable samples, respectively) our primary analyses focused on samples collected from the hygiene tanks. Since these samples had the highest TOC it was felt that they would possess the greatest diversity of organics as well as possibly maintain greater sample stability.

Routine checks to determine TOC were used to verify sample integrity. Results of the TOC monitoring indicated changes in measured TOC concentration with some samples over time. In addition, there is no assurance that the compounds comprising the initial TOC have remained constant over time. The data presented in this and all previous reports should therefore be viewed with caution. Representations made regarding compound identity may not necessarily reflect compounds which were present in the original sample at the time of collection. Due to the limitations of sample volume during WRT testing and the requirement to obtain analytical data necessary to characterize the process stream it was difficult to obtain adequate water for a purely methods development effort such as this. However, in this regard, the use of standard solutions of known composition and concentration seemed a more prudent choice to meet the stated objectives of this effort.

Standards

Standards were purchased or prepared for EPA base neutral acid (BNA) extractable compounds, volatile fatty acids and organic alcohols. The prepared standard solutions were then used either as "neat" calibrating solutions or as representative samples to determine recovery percentages of various procedures.

Conductivity, pH and TOC

Conductivity and pH measurements were performed using an YSI model 35 conductance meter with a K=1 cm cell and an Orion model EA92 ion analyzer, respectively. TOC analysis was performed on preserved samples using an Astro 2001 Total Organic Carbon Analyzer. TOC was determined by a Total carbon minus Total inorganic carbon method in an effort to account for volatile purgable compounds which may be lost in sequential TIC - TOC methods where the TOC reported is more accurately a non-purgable organic carbon (NPOC). Total Carbon in the samples was determined by acidic UV/persulfate oxidation of an entire sample followed by a total inorganic carbon measurement of a duplicate sample. This TC - TIC method more accurately measures volatile, purgable organics which may be lost in a TIC first determination.

Methylene Blue Active Substances (MBAS)

Standard Method 5540C (MBAS) for anionic surfactant was performed on hygiene sample WRT-3A-23-0-G*M*-4-HT3, using a Perkin-Elmer Lambda 4B UV/Vis spectrophotometer to assess the contribution of these compounds to hygiene TOC. Anionic sulfates and possibly sulfonates are present in cleansing agents used in the hygiene shower, dishwasher, and clotheswasher.

Total Protein

A modified Biuret method using Bicinchoninic acid as an absorbing complexing reagent for Cu^{+1} produced in the Biuret reaction was used in this determination. Solutions of Bovine Serum Albumin in reagent water were used as standards.

Bile Acids

Bile acids were determined using an enzymatic procedure (Sigma 450). As described, bile acids are oxidized to 3-oxo-bile acids with a subsequent reduction of NAD to NADH. Nitro-blue tetrazolium salt is reduced to formazan in a coupled enzymatic procedure which is measured at 530 nm. The results are calculated and reported as total bile acids as cholate.

B-Hydroxybutyric acid

B-hydroxybutyric acid (B-HBA) was determined colorimetrically as described in Sigma 310. In this procedure B-HBA is oxidized to acetoacetate by B-HBA dehydrogenase subsequently reducing NAD to NADH. The increase in NADH is followed spectrophotometrically at 340 nm and is directly proportional to the concentration of B-HBA in the sample.

GC/FID Fatty Acid Analysis

Free fatty acids were determined using liquid-liquid extraction of derivitized methyl esters. A 20 mL sample was lyophilized (freeze dried) overnight and reconstituted in 1 mL reagent water. 3 mL of 3.75 N NaOH was added and the sample heated at 100 °C for 30 minutes to saponify any complexed acids (tri-acyl glycerides) present. Methyl esters were prepared using alcoholic HCl at 80 °C for 10 minutes. Fatty acid methyl esters (FAMES)

were extracted using hexane-t-butyl ether. Analysis of FAMES were conducted by gas chromatography using a Flame Ionization Detector (FID). FAME identification were tentatively made based on retention time and response of known standards by comparison to an established peak naming table containing over 130 known fatty acid methyl esters. Quantitation was calculated based on the obtained area counts using an experimentally determined response factor.

GC/MS Methods

GC/MS analyses were performed using a Hewlett Packard 5890 GC equipped with an HP 5988 mass spectrometer. A 25 meter HP Ultra 2 crosslinked phenyl/methyl polysiloxane column was used for all analyses.

General Liquid/Liquid Extractions

Methylene Chloride Extraction

A slightly modified EPA 625 method for semi-volatile priority pollutants was performed on a preserved one liter aliquot of WRT-3A-23-0-G*M*-4-H-HT3. The sample was spiked with Environmental Resources Base/Neutrals standard lot #91108. Run concurrently with each unknown sample were a one liter reagent water method blank and one liter of the ERA BAN standard prepared per instructions. PFTBA was used for instrument tuning. Samples were extracted manually in two liter separatory funnels according to the method and concentrated to 1mL in K-D apparatus. Deuterated surrogate standards were not available, so quantitation is an approximation based on the ERA standard "method blank" and neat standard as external standards.

Diethyl Ether Extraction

A liquid/liquid extraction using diethyl ether patterned after EPA 625 was performed on a one liter aliquot of WRT-3A-23-0-G*M*-1-H-HT2 in an effort to extract more polar compounds than with the methylene chloride. Extraction was manual using 2L separatory funnels and concentration to 1 mL with K-D apparatus and N₂ stream. PFTBA was used for tuning. The 1 ppm butylated hydroxytoluene (BHT) peroxidation inhibitor served as internal standard.

Solid Phase Extraction

A C₁₈ solid phase extraction patterned after EPA 525 drinking water method was performed on a 500 ml aliquot of WRT-3A-23-0-G*M*-4-H-HT3 hygiene sample. The ERA BNA standard lot #91108 was again used as an analytical control check as an EPA 525 sample was not available. Spiked hygiene water, an ERA standard "method blank" and a water blank were extracted using J T Baker C₁₈ SPE cartridges (500 mg) and manifold according to EPA 525. Throughout the course of this contract other solid phase absorbants were used. These included C₈, phenyl and diol (J. T. Baker Company). Each of these columns were used per manufacturers instructions unless otherwise noted.

LC and LC/MS Analysis

Conventional LC with UV and refractive index detection as well as LC/MS have been performed on HT3 samples with limited success. 100 mL aliquots were diluted to 500 mL with acetonitrile, then extracted using J T Baker diol SPE columns (500 mg). The columns were conditioned with 10 mL 50/50 methanol

and water. Samples were eluted with two 500 uL aliquots of mobile phase.

For conventional LC a Shimatzu SLC-6A system with auto sampler and column oven was used. The mobile phase was water at 0.7 mL/min on a Bio-Rad HPX-87C organic acid/alcohol column at 50 °C. Detection was by refractive index and UV at 215 nm.

Results

Conductivity, pH and TOC results are presented in Table 1. The TOC values presented in Table 1 are the means plus or minus standard deviation (n=3). The results of these samples are consistent with other samples collected and reported by the Boeing Laboratory during WRT 3A.

MBAS analysis using sodium dodecylsulfate as a standard, gave 0.5 ppm surfactants as methylene blue active substances. Since MBAS may overestimate surfactant concentrations in complex waste streams, this suggests that unremoved surfactants do not contribute more than 0.6 % of the 80.5 ppm TOC present in this sample.

Results of the protein determinations are presented in Table 2. Supplemental data is included as Appendix A. A number of reducing compounds including carbohydrates produce positive Biuret results. For this reason, we have chosen to refer to these compounds as "total biuret reactive substances". Subsequent analysis for glucose (Sigma 16 UV) failed to detect this specific carbohydrate. This result is not surprising considering the age of the sample as any carbohydrate would have been rapidly

assimilated by contaminating microorganisms. Colorimetric assay of biuret reactive substances for both Hygiene tank and Humidity condensate samples were in the low ppm range. This represents approximately 5% of the measured TOC.

Determination of bile acids revealed approximately 1.3 mg/L total bile acids calculated as cholate. Specific potential interfering compounds are unknown to the authors at present. B-hydroxybutyric acid was also determined by a colorimetric procedure. Typical sample analysis revealed BHBA concentrations to be on the order of approximately 10-15 mg/L in the hygiene water samples assayed. This represents a relative high percentage of TOC and may certainly be considered as a potential major component. Furthermore, B-HBA was detected in all samples analyzed. Potential interfering compounds are unknown to the authors at this time.

Several fatty acids were detected from samples collected from the hygiene tank #2. Quantitative results for eight fatty acids detected from this sample are presented in Table 3. Concentrations were calculated using an area count response factor of 2.6 pg obtained from MIDI, manufacturer of the FAME standards used for calibration. Raw data is included as Appendix B. Again, since samples have exceeded holding times these quantitative results should be interpreted carefully as they may represent only an "order of magnitude" type assessment.

TABLE 1
Initial Sample Data

MSFC SAMPLE ID	TOC (ppm)	pH	conductivity (uS)
WRT-3A-23-0-G*M*-7-H-PT3	5.9 ± 0.6	3.86	26.8
WRT-3A-23-0-G*M*-6-H-PT2	3.4 ± 0.3	4.05	24.3
WRT-3A-23-0-G*M*-8-H-PT4	7.5 ± 0.5	3.87	77.1
WRT-3A-23-0-G*M*-4-H-HT3	80.5 ± 2.9	4.67	10.0
WRT-3A-23-0-G*M*-1-H-HT2	62.3 ± 1.6	4.98	12.1
WRT-3A-23-0-G*M*-24-H-TNK	18.9 ± 0.5	5.70	17.5

Table 2
Colorimetric Determination of Biuret Reactive Substances

Sample	Abs. @ 562nm	Conc. (ppm)
WRT-3A-23-0-G*M*-1-H-HT2	0.254	3.4
WRT-3A-23-0-G*M*-4-H-HT3	0.214	2.8
WRT-3A-23-0-G*M*-24-H-TNK	0.116	1.8

Table 3
Fatty Acid Methyl Esters Quantitative Results

Compound	Retention Time	ECL	Conc. (ppb)
Ukn 15.549	9.623	15.549	67
16:0	10.378	16.0	107
18:0	13.860	18.0	53
22:0	20.731	22.039	93
26:0	26.895	25.946	1053
Ukn 26.336	27.551	26.336	381
Ukn 27.765	29.962	27.765	104
29:0	32.924	29.004	220

Results of EPA 625 liquid-liquid extraction indicated good recovery of standard compounds. Compounds present and recoveries from the sample matrix are presented in Table 4a. Library matches for standard compounds were also acceptable (Appendix C). Six chromatographic peaks were present in the acid preserved hygiene sample which were not present in the blanks. Chromatograms and selected spectra are included in Appendix D.

Five relatively low molecular weight semi-volatiles were present in the methylene chloride extract of HT3 not present in the blank. Several of these semi-volatiles contained a rather unusual ion fragment M/Z 127 probably indicating iodine in these compounds. The library identifications included in Appendix D are presented as a general guide as to compound class. In several cases, they are not the actual compound present. Based on the area counts obtained and a standard response factor it is felt that semivolatile compounds make up on a small fraction of total, as these compounds were most likely present in sub parts per million levels.

The liquid/liquid extraction of the unpreserved (frozen) sample from HT2, produced 12 tentatively identified compounds not present in the blank. Comparison with ppb level standards suggests that all the identified compounds are present in low ppb levels. Recovery data for a commercial semi-volatiles standard diluted in reagent water and extracted as previously described are presented in Table 4b (Appendix C). Recoveries were not as good as in the acid extraction of a preserved sample from HT3. The base/neutral standard available for these determinations

Table 4a**Recoveries of ERA BNA Standard from sample matrix**

Compound	Retention Time (minutes)	Std Conc. (ppb)	%Recovery
Acenaphthylene	19.34	144.0	99
Benzyl Alcohol	11.02	159.0	53
bis(2-chloroethyl) ether	9.89	34.0	58
2-Chloronaphthalene	17.99	24.9	86
Di-n-Butylphthalate	26.72	36.3	118
Hexachloroethane	11.91	68.2	95
Naphthalene	14.31	13.1	73
Phenanthrene	24.55	90.0	98

Table 4b**EPA 625 Methylene Chloride Liquid/Liquid Extraction****ERA BNA Std. Diluted per Instructions**

Peak#	Compound Name	Std. Concentration	%Recovery
1	Bis 2-Chloroethyl Ether	34 ug/L	121%
2	Benzyl Alcohol	159 ug/L	23%
3	Hexachloroethane	68.2 ug/L	112%
4	Naphthalene	13.1 ug/L	98%
5	2-Chloronaphthalene	24.9 ug/L	70%
6	Acenaphthylene	144 ug/L	70%
7	Phenanthrene	90 ug/L	48%
8	Dibutyl Phthalate	36.3 ug/L	42%

should have been extracted more efficiently from basic solution. This result is thus far unexplained, but may simply be a result of the extraction precision of the method (\pm 20-50% for most 625 compounds) perhaps compounded by small errors due to manual injections and external standards. Specific compounds tentatively identified are presented in Table 5. Individual chromatograms and spectra are included as Appendix E. Toluene and xylene contaminants present in the methylene chloride, used for this extraction, are noted and were not reported as compounds identified in Table 5.

Likewise, using ether extraction nine chromatographic peaks were present in the HT2 sample not present in the method blank. Several of these compounds were not detected in the methylene chloride extract of HT3. Chromatograms and selected spectra are included in Appendix F. Iodopentane and iodoform are positively identified. Only two of these compounds, RT 11.6 and RT 12.3, do not contain iodine as is illustrated in the spectra presented and the extracted ion chromatogram for ion 127. The Butylated hydroxytoluene, RT 20.2, appears on the extracted ion chromatogram, since its total ion abundance was several million counts. This was orders of magnitude greater than any of the isolated compounds. The semi-volatiles present in this extract are also estimated to be in sub part per million levels.

Recoveries obtain using EPA 525 methodology are included as Table 6. Bis (2-chloroethyl) ether and benzyl alcohol were recovered in only trace amounts. Since deuterated internal surrogate standards were not available, the "method blank" and neat standard served as external standards.

Table 5

EPA Base/Neutral Extract of Unpreserved Hygiene Tank #2

Tentative Peak Identifications

Peak #	Retention Time	Identification
1	5.4 min.	Iodobutane
2	8.4 min.	Cyclohexenone
3	9.2 min.	Benzaldehyde
4	11.5 min.	Diethyl Benzene
5	21.6 min.	Diethyl Phthalate
6	21.9 min.	substituted Benzeneethanol
7	22.9 min.	long-chain hydrocarbon
8	26.8 min.	Isobutyl Phthalate
9	27.0 min.	substituted Cyclohexane
10	31.5 min.	Benzyl Butyl Phthalate
11	31.8 min.	Hexanedioic Acid, dioctyl ester
12	33.8 min.	Bis(2-ethylhexyl) Phthalate

Table 6

C₁₈ Solid Phase Extract

Compound	Retention Time (minutes)	Std Conc. (ppb)	%Recovery
Acenaphthylene	19.34	144.0	93
Benzyl Alcohol	11.02	159.0	trace
bis(2-chloroethyl) ether	9.89	34.0	trace
2-Chloronaphthalene	17.89	24.9	78
Di-n-Butylphthalate	26.72	36.3	trace
Hexachloroethane	11.74	68.2	74
Naphthalene	14.19	13.1	108
Phenanthrene	24.55	90.0	49

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 No significant chromatographic peaks are present for the hygiene sample which did not occur in the method blank or water blank (Appendix G). This further indicates that semivolatile compounds do not account for a significant portion of the TOC.

Good resolution of eight chromatographic peaks using LC with refractive index and UV detection is illustrated in Appendix H. These represent the principal components since these are relatively unconcentrated samples. Retention times obtained correlate closely to short chain primary alcohols and carboxylic acids, unfortunately LC retention time alone is not enough to identify unknown compounds. Multiple wavelength methods required more time than available during the initial analysis. In addi-

tion, numerous alcohol and acid standards must be run to obtain adequate matches for all peaks. Finally, recovery problems in the diol solid phase extraction have been noted for these classes of compounds because of their high affinity to water. The reagent water mobile phase required for this column did not produce sufficient ionization for thermospray LC/MS application.

An alternative LC procedure was attempted for LC/MS using 0.1 molar ammonium acetate buffer as mobile phase on a Hamilton PRP-X300 column. The acetate buffer provides better thermospray ionization. Chromatogram and selected spectra are included in Appendix H. Thermospray mass spectra for several reference standard carboxylic acids obtained under our experimental conditions are included as reference.

Three compounds were barely detected from a diol extracted HT3 sample, and more work on establishing detection limits and optimizing extraction conditions are obviously necessary.

Method Development

Method development efforts initiated during this reporting period include a liquid chromatography procedure for carbohydrates and organic acids, a liquid chromatography procedure for amino acids and a gas chromatography procedure for alcohols and organic acids. Method development activities have resulted in the determination of instrumental parameters required for the separation, identification and quantification of target compounds. A summary of the instrumental parameters and tables of retention times obtained for representative standard compounds are included as Appendix I.

Sample Concentration

Lyophilized Samples

Several 25 mL aliquotes of HT2 and HT3 were lyophilized (freeze dried) and reconstituted in 1 ml of various solvents including water, methanol, and hexane. Analysis for these fractions is not complete at this time, as the hexane and methanol fractions contain some compounds which cause excessive column bleed on the siloxane column used. The water fractions are awaiting LC analysis as time permits.

Solid Phase Extraction (SPE)

Solid phase extraction technology was evaluated as a potential method to concentrate analytes for subsequent liquid/gas chromatographic analysis. The results of the preliminary evaluation are summarized in Table 7. The actual procedures and mass spectra are included as Appendix J.

Table 7
EXTRACTION PHASE

Analyte	Octadecyl C18	Octyl C8	Phenyl C6	Diol (-OH) _n	Recovery
Polynuclear Aromatics	60-95%	N/O ¹	N/A ²	N/A	
Miscible Alcohols	N/A	N/A	N/O	20-70%	
Volatile Fatty Acids	N/A	N/O	55-72%	28-35%	
Pesticides	N/A	48-101%	N/O	N/A	

² N/A - Not Adequate - Unsuitable Phase

¹ N/O - Not Optimized - Some Recovery - More Development Required

Summary

Liquid/liquid extractions for semi-volatiles account for only a small fraction of TOC present in hygiene water samples. Sample preservation and extraction pH affect the number and type of compounds recovered. Approximately 27 compounds at low ppb levels have been tentatively identified in hygiene samples using liquid/liquid techniques to date. These are summarized in Table 8.

In addition, several principle components, which may account for as much as 30% of the TOC have been tentatively identified. These include non-volatile fatty acids, bile acids, B-hydroxybutyric acid and proteins. Preliminary results indicate that simple colorimetric tests, like the modified Biuret determination of protein, may have some utility for determining the concentrations of various chemical groups contributing to TOC. Additional work on standard solutions will be necessary to assess the effect of interfering compounds. In addition, other colorimetric assays will be used to identify and detect principal components.

Gas chromatography analysis for fatty acid methyl esters have produced quantitative data for eight fatty acid or related compounds. These compounds are present in hygiene samples at or below low ppm levels.

Apparently the principle components present in hygiene water samples are relatively low molecular weight alcohols, aldehydes, carboxylic acids, and fatty acids. As indicated by initial hypothesis standard EPA and APHA methods will not achieve TOC accountability.

Table 8

Compounds Detected in WRT Samples to Date			
Number	Tentative ID	Method	Sample
1	3-hydrox-2-butanone	EPA 625/acid	HT3
2	Toluene	EPA 625/acid	HT3
3	iodobutane	EPA 625/acid	HT3
		EPA 625 base-neutral & ether liquid/liquid	HT2
4	iodo-isobutane	EPA 625/acid	HT3
5	1-Iodo-3-methyl-butane	EPA 625/acid	HT3
		& ether liquid/liquid	HT2
6	Iodopropane	ether liquid/liquid	HT2
7	Iodoform	ether liquid/liquid	HT2
8	substituted benzene B.P. 120	ether liquid/liquid	HT2
9	substituted benzene B.P. 136	ether liquid/liquid	HT2
10	2-cyclohexene- 1 -one	EPA 625/base-neu.	HT2
11	Benzaldehyde	EPA 625/base-neu.	HT2
12	Diethylbenzene	EPA 625/base-neu.	HT2
13	Diethyl Phthalate	EPA 625/base-neu.	HT2
14	substituted benzyl alcohol B.P. 107	EPA 625/base-neu.	HT2
15	long-chain hydrocarbon B.P. 55	EPA 625/base-neu.	HT2
16	Butyl methyl phthalate	EPA 625/base-neu.	HT2
17	substituted Cyclohexane	EPA 625/base-neu.	HT2
18	Hexanedioc acid ester	EPA 615/base-neu.	HT2
19	Bis(2-ethylhexyl) phthalate	EPA 625/base-neu.	HT2
20	Ukn fatty acid ECL 15.549	FAME	HT2
21	Fatty Acid 16:0	FAME	HT2
22	Fatty Acid 18:0	FAME	HT2
23	Fatty Acid 22:0	FAME	HT2
24	Fatty Acid 26:0	FAME	HT2
25	Ukn Fatty Acid ECL 26.336	FAME	HT2
26	Ukn Fatty Acid ECL 27.765	FAME	HT2
27	Fatty Acid 29:0	FAME	HT2

Chemical Class Test	Sample	Concentration
Methylene Blue Active Substances (surfactants)	HT2	<1 mg/L
Biuret Active Substances (proteins)	HT2	3.4 mg/L
Bile Acids	HT3	2.8 mg/L
B-hydroxybutyric acid	HT	3.1 mg/L
	HT	10.0 mg/L

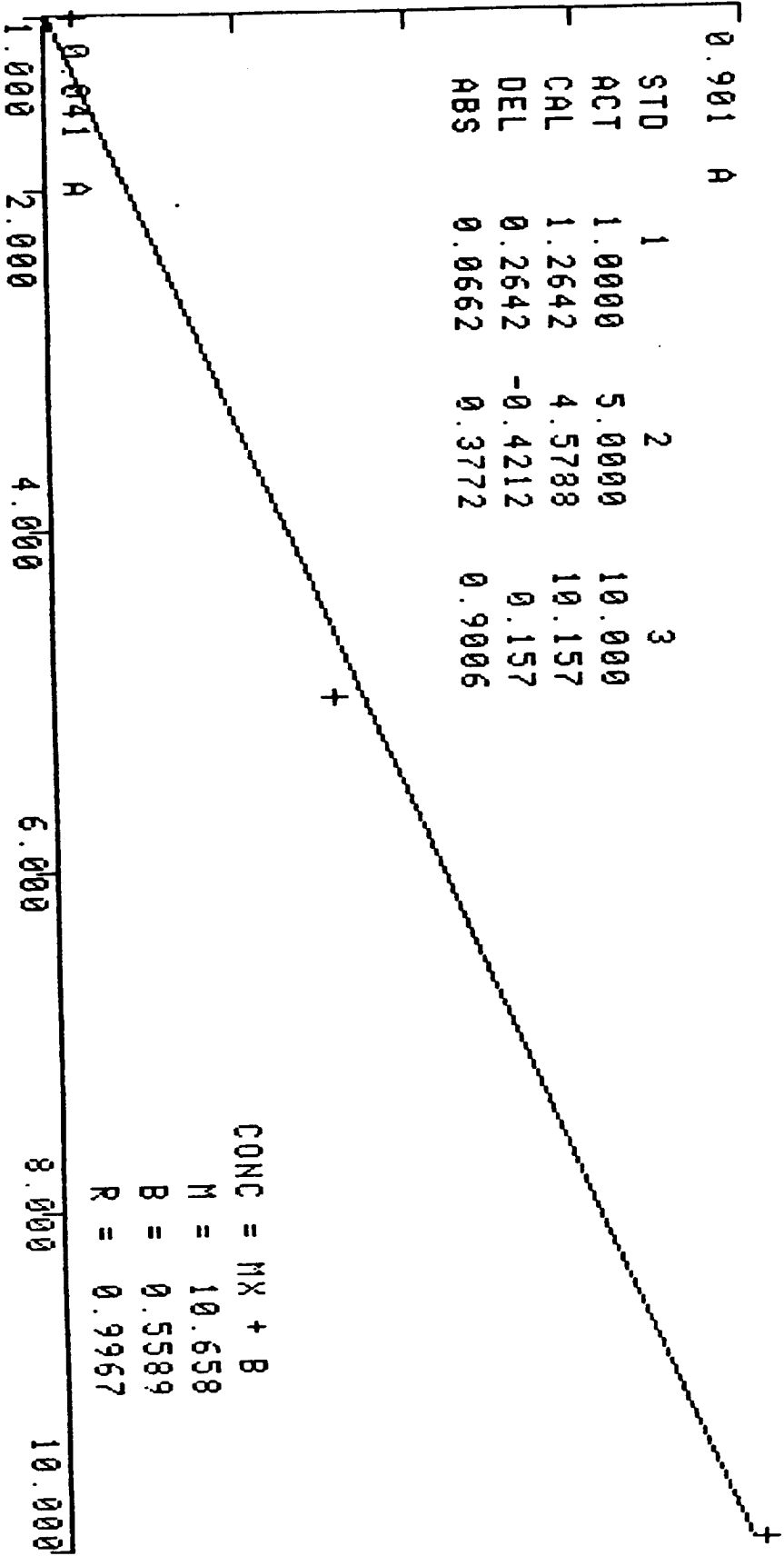
LC/MS work on unconcentrated samples has not been as successful as anticipated due to the relatively insensitive nature of LC. There is simply not enough analyte present to detect by LC/MS without preconcentration. Additional work toward development of a efficient preconcentration step for these analytes is obviously required.

Appendix A

Biuret Active Substances

0.901 A

STD	1	2	3
ACT	1.00000	5.00000	10.0000
CAL	1.2642	4.5788	10.157
DEL	0.2642	-0.4212	0.157
ABS	0.0662	0.3772	0.9006



Appendix B
GC/FID FAME Analysis

THE ANALYSIS OF LIPIDS

Hewlett-Packard has developed a microbial identification system using a gas chromatograph which identifies microbes based on the amount and types of fatty acids found in the cells. This system was used to separate, identify and quantitate the fatty acids found in the water reclamation samples. The sample preparation procedure and GC parameters can be found in the Hewlett-Packard HP 5898A Microbial Identification System Operating Manual, and below. The only modifications to the procedure are a substitution of 10 ml of liquid sample for analysis as opposed to approximately 40 mg live wet cells, and the samples were shaken by hand as opposed to rotated during the extraction phase.

Additionally, a table of known fatty acids and their relative retention times is included below.

GC parameters:

Injection volume:	2 μ l
Split ratio:	1:50
Initial Oven Temp:	170°C
Initial Time:	0 min
Rate A:	5°/min
Rate B:	30°/min
Final Temp A:	270°C
Final Temp B:	300°C
Final Time A:	0 min
Final Time B:	1 min
Inj Temp:	250°C
Det Temp:	300°C
Equil Time:	3 min
Carrier Gas:	Hydrogen
Carrier Flow:	30 ml/min
Make-up Gas:	Helium
Make-up Gas Flow:	30 ml/min
Air Flow:	400 ml/min
Range:	2
Attenuation:	0

PEAK NAME & QUANTIZING LIBRARY

Peak Definitions			Qualitative Calib.					Quantitative Calib.					
Indxl	Peak Name	Nominal RI	Nominal Window	Nominal RT	Cal. Mix	RefI	Fa Plot	CalI	ChkI	QntI	Nom.	Min.	Max.
INbr.					12345678	PK.	IGrpl	FaMIxI	MixI	Grpl	Amount	Amount	Amount
1	9:0	9.000	0.015	2.401 CII.....	T	0	0	1	1	1	5.556	4.79	5.36
2	8:0 3OH	9.385	0.010	2.584	3	6	0	0	1	0.000	0.00	0.00
3	unknown 9.521	9.521	0.010	2.648	-1	0	0	0	1	0.000	0.00	0.00
4	10:0 ISO	9.605	0.015	2.680 .I.....	T	3	1	0	0	1	0.000	0.00	0.00
5	10:0	10.000	0.015	2.876 CIII....	T	0	0	1	1	1	11.111	9.59	10.68
6	9:0 3OH	10.400	0.010	3.145	3	6	0	0	1	0.000	0.00	0.00
7	11:0 ISO	10.605	0.015	3.275 .I.....	T	3	1	0	0	1	0.000	0.00	0.00
8	11:0 ANTEISO	10.693	0.015	3.333 .I.....	T	3	2	0	0	1	0.000	0.00	0.00
9	unknown 10.928	10.928	0.010	3.488	-1	0	0	0	1	0.000	0.00	0.00
10	11:0	11.000	0.015	3.535 CIII....	T	0	0	1	1	1	5.556	4.79	5.36
11	unknown 11.097	11.097	0.010	3.620	-1	0	0	0	1	0.000	0.00	0.00
12	10:0 2OH	11.157	0.010	3.672 I.....	.	3	5	0	1	1	0.000	1.89	2.51
13	10:0 3OH	11.423	0.010	3.905 I.....	.	3	6	0	1	1	0.000	0.90	1.20
14	unknown 11.541	11.541	0.010	4.000	-1	0	0	0	1	0.000	0.00	0.00
15	12:0 ISO	11.608	0.015	4.067 .I.....	T	3	1	0	0	1	0.000	0.00	0.00
16	12:0 ANTEISO	11.699	0.015	4.147	T	3	2	0	0	1	0.000	0.00	0.00
17	unknown 11.798	11.798	0.010	4.233	-1	0	0	0	1	0.000	0.00	0.00
18	12:1 AT 11-12	11.925	0.015	4.344 ...I....	.	3	7	0	0	1	0.000	0.00	0.00
19	12:0	12.000	0.015	4.410 C.II....	T	0	0	1	1	1	11.111	9.59	10.68
20	11:0 ISO 3OH	12.090	0.010	4.589 .I.....	.	3	3	0	0	1	0.000	0.00	0.00
21	unknown 12.112	12.112	0.010	4.534	-1	0	0	0	1	0.000	0.00	0.00
22	11:0 2OH	12.158	0.010	4.584	3	5	0	0	1	0.000	0.00	0.00
23	11:0 3OH	12.441	0.010	4.897	3	6	0	0	1	0.000	0.00	0.00
24	unknown 12.486	12.486	0.010	4.947	-1	0	0	0	1	0.000	0.00	0.00
25	13:0 ISO	12.612	0.015	5.086 .I.....	T	3	1	0	0	1	0.000	0.00	0.00
26	13:0 ANTEISO	12.701	0.015	5.184 .I.....	T	3	2	0	0	1	0.000	0.00	0.00
27	13:1 AT 12-13	12.931	0.010	5.438 ...I....	.	3	7	0	0	1	0.000	0.00	0.00
28	13:0	13.000	0.015	5.514 CIII....	T	0	0	1	1	1	5.556	4.79	5.36
29	12:0 ISO 3OH	13.898	0.010	5.643 .I.....	.	3	3	0	0	1	0.000	0.00	0.00
30	12:0 2OH	13.178	0.010	5.748 ...I....	.	3	5	0	0	1	0.000	0.00	0.00
31	12:1 3OH	13.289	0.010	5.895 ...I....	.	1	0	0	0	1	0.000	0.00	0.00
32	14:1 ISO E	13.380	0.010	6.025	10	1	0	0	1	0.000	0.00	0.00
33	12:0 3OH	13.455	0.010	6.113 .I.....	.	3	6	0	0	1	0.000	0.00	0.00
34	unknown 13.566	13.566	0.010	6.259	-1	0	0	0	1	0.000	0.00	0.00
35	14:0 ISO	13.618	0.015	6.328 .I.....	T	3	1	0	0	1	0.000	0.00	0.00
36	14:0 ANTEISO	13.707	0.015	6.445	T	3	2	0	0	1	0.000	0.00	0.00
37	13:0 ISO 2OH	13.814	0.010	6.586 .I.....	.	3	4	0	0	1	0.000	0.00	0.00
-1	14:1 TRANS 9/CIS 9	13.894	0.010	6.691 ...I....	.	3	9	0	0	1	0.000	0.00	0.00
-1	14:1 CIS 9/TRANS 9	13.903	0.010	6.703 ...I....	.	3	8	0	0	1	0.000	0.00	0.00
38	unknown 13.961	13.961	0.010	6.780	-1	0	0	0	1	0.000	0.00	0.00
39	14:0	14.000	0.015	6.831 CIII....	T	0	0	1	1	1	11.111	9.59	10.68
40	13:0 ISO 3OH	14.110	0.010	6.995 .I.....	.	3	3	0	0	1	0.000	0.00	0.00
41	13:0 2OH	14.191	0.010	7.117	3	5	0	0	1	0.000	0.00	0.00
42	unknown 14.258	14.258	0.010	7.217 .I.....	.	1	0	0	0	1	0.000	0.00	0.00
43	15:1 ISO E	14.387	0.010	7.410	10	1	0	0	1	0.000	0.00	0.00
44	15:1 ISO F	14.414	0.010	7.458 .I.....	.	10	2	0	0	1	0.000	0.00	0.00
45	15:1 ISO G	14.441	0.010	7.490	10	3	0	0	1	0.000	0.00	0.00
-2	15:1 ISO H/13:0 3OH	14.460	0.010	7.519	10	4	0	0	1	0.000	0.00	0.00

Peak Library File: IDUOL:PEAK_LIB Library Version: 1.3 04-APR-86
 Number of Peaks: 142 Number of features: 130 Number Calib. Mixes: 4

PEAK NAME & QUANTIZING LIBRARY

Peak Definitions				Qualitative Calib.				Quantitative Calib.						
Indx	Nominal Name	Nominal RI	Nominal Window	Cal. Mix	Ref	Fm Plot	Cal	Chk	Qnt	Nom.	Min.	Max.		
Nbr.	Peak Name	RT		12345678	Plk.	IGrplFam	Mix	Mix	Grpl	Amount	Amount	Amount		
-2	13:0 3OH/15:1 I/H	14.470	0.010	7.534	3	6	0	0	1	0.000	0.00	0.00
-2	15:1 ISO I/13:0 3OH	14.477	0.010	7.544	..I.....	.	10	5	0	0	1	0.000	0.00	0.00
46	unknown 14.503	14.503	0.010	7.583	..I.....	.	1	0	0	0	1	0.000	0.00	0.00
47	15:1 ANTEISO A	14.526	0.010	7.617	-1	1	0	0	1	0.000	0.00	0.00
48	15:0 ISO	14.621	0.015	7.759	..I.....	T	3	1	0	0	1	0.000	0.00	0.00
49	15:0 ANTEISO	14.711	0.015	7.894	..I.I....	T	3	2	0	0	1	0.000	0.00	0.00
50	15:1 A	14.792	0.010	8.015	-1	0	0	0	1	0.000	0.00	0.00
51	15:1 B	14.856	0.010	8.111	-1	0	0	0	1	0.000	0.00	0.00
52	15:1 CIS 10	14.904	0.010	8.182	...I....	.	3	10	0	0	1	0.000	0.00	0.00
53	unknown 14.966	14.966	0.010	8.275	-1	0	0	0	1	0.000	0.00	0.00
54	15:0	15.000	0.015	8.326	CIII....	T	0	0	1	1	1	5.556	4.79	5.36
55	14:0 ISO 3OH	15.117	0.010	8.515	3	3	0	0	1	0.000	0.00	0.00
56	14:0 2OH	15.285	0.010	8.658	..I.....	.	3	5	0	1	1	0.000	-1.89	2.51
57	16:1 ISO E	15.386	0.010	8.951	..I.....	.	10	1	0	0	1	0.000	0.00	0.00
58	16:1 ISO F	15.415	0.010	8.997	10	2	0	0	1	0.000	0.00	0.00
59	16:1 ISO G	15.442	0.010	9.041	10	3	0	0	1	0.000	0.00	0.00
60	16:1 ISO H	15.461	0.010	9.072	..I.....	.	10	4	0	0	1	0.000	0.00	0.00
-3	16:1 ISO I/14:0 3OH	15.482	0.010	9.106	10	5	0	0	1	0.000	0.00	0.00
-3	14:0 3OH/16:1 ISO I	15.490	0.010	9.119	..I.....	.	3	6	0	1	1	0.000	0.90	1.20
61	unknown 15.549	15.549	0.010	9.214	..I.....	.	-1	0	0	0	1	0.000	0.00	0.00
62	16:0 ISO	15.626	0.015	9.339	..I.I....	T	3	1	0	0	1	0.000	0.00	0.00
63	unknown 15.665	15.665	0.010	9.482	-1	0	0	0	1	0.000	0.00	0.00
64	16:0 ANTEISO	15.717	0.015	9.486	T	3	2	0	0	1	0.000	0.00	0.00
65	16:1 A	15.757	0.010	9.551	-1	0	0	0	1	0.000	0.00	0.00
66	16:1 B	15.774	0.010	9.578	..I.....	.	1	0	0	0	1	0.000	0.00	0.00
67	16:1 CIS 9	15.817	0.010	9.648	..III....	.	3	8	0	0	1	0.000	0.00	0.00
-4	15:0 ISO 2OH/16:1t9	15.843	0.010	9.690	3	4	0	0	1	0.000	0.00	0.00
-4	16:1 TRANS 9/15:2OH	15.856	0.010	9.711	..I.....	.	3	9	0	0	1	0.000	0.00	0.00
68	16:1 C	15.908	0.010	9.795	-1	0	0	0	1	0.000	0.00	0.00
69	16:0	16.000	0.015	9.944	CIII....	T	0	0	1	1	1	11.111	9.59	10.68
70	15:0 ISO 3OH	16.135	0.010	10.172	...I....	.	3	3	0	0	1	0.000	0.00	0.00
71	15:0 2OH	16.217	0.010	10.311	...I....	.	3	5	0	0	1	0.000	0.00	0.00
72	17:1 ISO E	16.387	0.010	10.599	10	1	0	0	1	0.000	0.00	0.00
73	17:1 ISO F	16.416	0.010	10.648	..I.I....	.	10	2	0	0	1	0.000	0.00	0.00
74	17:1 ISO G	16.433	0.010	10.677	..I.....	.	10	3	0	0	1	0.000	0.00	0.00
75	17:1 ISO H	16.461	0.010	10.724	10	4	0	0	1	0.000	0.00	0.00
-5	17:1 ISO I/ANTEI B	16.476	0.010	10.749	10	5	0	0	1	0.000	0.00	0.00
-5	17:1 ANTEISO B/i I	16.486	0.010	10.766	...I....	.	1	0	0	0	1	0.000	0.00	0.00
76	15:0 3OH	16.504	0.010	10.797	3	6	0	0	1	0.000	0.00	0.00
77	17:1 ANTEISO C	16.525	0.010	10.832	-1	0	0	0	1	0.000	0.00	0.00
78	17:1 ANTEISO A	16.541	0.010	10.859	-1	1	0	0	1	0.000	0.00	0.00
79	unknown 16.580	16.580	0.010	10.925	-1	0	0	0	1	0.000	0.00	0.00
80	17:0 ISO	16.629	0.015	11.008	..I.....	T	3	1	0	0	1	0.000	0.00	0.00
81	17:0 ANTEISO	16.722	0.015	11.166	..I.....	T	3	2	0	0	1	0.000	0.00	0.00
82	17:1 A	16.773	0.010	11.252	-1	0	0	0	1	0.000	0.00	0.00
83	17:1 B	16.792	0.010	11.284	..I.....	.	1	0	0	0	1	0.000	0.00	0.00
84	17:1 CIS 10	16.818	0.010	11.328	...I....	.	3	10	0	0	1	0.000	0.00	0.00
85	17:1 C	16.862	0.010	11.403	-1	0	0	0	1	0.000	0.00	0.00
86	17:0 CYCLO	16.888	0.015	11.446	..I.I....	T	1	0	0	0	1	0.000	0.00	0.00

Peak Library File: IDUOL:PEAK_LIB Library Version: 1.3 84-APR-86
 Number of Peaks: 142 Number of features: 130 Number Calib. Mixes: 4

PEAK NAME & QUANTIZING LIBRARY

Peak Definitions			Qualitative Calib.					Quantitative Calib.						
Indx		Nominel	Name	Nominel	Cal. Mix	Ref	Fm Plot	Call	Chk	Qnt	Nom.	Min.	Max.	
(Nbr.)	Peak Name	RT	Window	RT	12345678	Ph.	Grp	Fam	Mix	Mix	Grp	Amount	Amount	Amount
87	unknown 16.918	16.918	0.010	11.497		-1	0	0	0	1	0.008	0.00	0.00
88	17:0	17.000	0.010	11.636	Cl.I....	T	0	0	1	1	1	5.556	4.79	5.36
89	16:1 2OH	17.047	0.010	11.717		3	12	0	0	1	0.000	0.00	0.00
90	16:0 ISO 3OH	17.145	0.010	11.886		3	3	0	0	1	0.000	0.00	0.00
91	16:0 2OH	17.235	0.010	12.041	I.....		3	5	0	1	1	0.000	1.89	2.51
92	18:1 ISO F	17.418	0.010	12.342	..I.....		10	2	0	0	1	0.000	0.00	0.00
93	18:1 ISO G	17.440	0.010	12.394		10	3	0	0	1	0.000	0.00	0.00
94	18:1 ISO H	17.460	0.010	12.420		10	4	0	0	1	0.000	0.00	0.00
95	16:0 3OH	17.520	0.010	12.531	..II....		3	6	0	0	1	0.000	0.00	0.00
96	18:3 CIS 6,12,14	17.577	0.010	12.630	T	-1	0	0	0	1	0.000	0.00	0.00
97	18:0 ISO	17.632	0.015	12.724		3	1	0	0	1	0.000	0.00	0.00
-6	18:2 CIS 9,12/18:0a	17.720	0.010	12.876		-1	0	0	0	1	0.000	0.00	0.00
-6	18:0 ANTEISO/18:2 c	17.727	0.010	12.888		3	2	0	0	1	0.000	0.00	0.00
98	18:1 CIS 9	17.769	0.010	12.960	..I.....		3	8	0	0	1	0.000	0.00	0.00
-7	18:1 CIS 11/t 9/t 6	17.822	0.010	13.051		1	0	0	0	1	0.000	0.00	0.00
-7	18:1 TRANS 9/t6/c11	17.825	0.010	13.057		3	9	0	0	1	0.000	0.00	0.00
-7	18:1 TRANS 6/t9/c11	17.826	0.010	13.058		-1	0	0	0	1	0.000	0.00	0.00
99	18:1 TRANS 11	17.850	0.010	13.113		-1	0	0	0	1	0.000	0.00	0.00
100	18:1 B	17.919	0.010	13.219		1	0	0	0	1	0.000	0.00	0.00
101	18:0	18.000	0.015	13.350	CIII....	T	0	0	1	1	1	11.111	9.59	10.68
102	17:0 ISO 3OH	18.164	0.010	13.640	..I.....		3	3	0	0	1	0.000	0.00	0.00
103	17:0 2OH	18.249	0.010	13.787		3	5	0	0	1	0.000	0.00	0.00
104	TBSA	18.392	0.010	14.033	..I.....		1	0	0	0	1	0.000	0.00	0.00
105	19:1 ISO I	18.473	0.010	14.172		-1	0	0	0	1	0.000	0.00	0.00
106	17:0 3OH	18.535	0.010	14.279		3	6	0	0	1	0.000	0.00	0.00
107	19:0 ISO	18.633	0.015	14.447	T	3	1	0	0	1	0.000	0.00	0.00
108	19:0 ANTEISO	18.729	0.010	14.613		3	2	0	0	1	0.000	0.00	0.00
-8	unknown 18.756/19:1	18.756	0.010	14.659		-1	0	0	0	1	0.000	0.00	0.00
-8	19:1 CIS 10/18.576	18.768	0.010	14.680	..I.....		3	10	0	0	1	0.000	0.00	0.00
109	19:1 TRANS 7	18.823	0.010	14.774	..I.....		1	0	0	0	1	0.000	0.00	0.00
-9	un 18.846/18.850	18.846	0.010	14.814		-1	0	0	0	1	0.000	0.00	0.00
-9	un 18.850/.846/19cy	18.850	0.010	14.835		-1	0	0	0	1	0.000	0.00	0.00
-9	19:0 CYCLO C9-10/un	18.867	0.010	14.850		-1	0	0	0	1	0.000	0.00	0.00
110	19:0 CYCLO C11-12	18.980	0.015	14.907	T	-1	0	0	0	1	0.000	0.00	0.00
111	19:0	19.000	0.015	15.079	CIII....	T	0	0	1	1	1	5.556	4.79	5.36
112	18:1 2OH	19.088	0.010	15.220	..I.....		3	12	0	0	1	0.000	0.00	0.00
113	18:0 2OH	19.264	0.010	15.527	..I.....		3	5	0	0	1	0.000	0.00	0.00
114	unknown 19.368	19.368	0.010	15.703		-1	0	0	0	1	0.000	0.00	0.00
115	20:4 CIS 5,8,11,14	19.395	0.010	15.749		-1	0	0	0	1	0.000	0.00	0.00
116	18:0 3OH	19.551	0.010	16.014		3	1	0	0	1	0.000	0.00	0.00
117	20:0 ISO	19.635	0.010	16.157		-1	0	0	0	1	0.000	0.00	0.00
118	unknown 19.735	19.735	0.010	16.326		1	0	0	0	1	0.000	0.00	0.00
119	20:1 CIS 11	19.770	0.010	16.386	..I.....		-1	0	0	0	1	0.000	0.00	0.00
120	20:1 TRANS 11	19.833	0.010	16.493		-1	0	0	0	1	0.000	0.00	0.00
121	20:0	20.000	0.015	16.776	CII.....	T	0	0	1	1	1	11.111	9.59	10.68

IO: 1 CALIBRATION MIX
 Bottle: 1 CALIBRATION [EUKARY]

RT	Area	Ar/Ht	Respon	ECL	Name	%	Comment 1	Comment 2
1.586	33067000	0.066	. . .	7.044	SOLVENT PEAK		< min rt	
1.908	968	0.024	. . .	7.689		< min rt	
2.562	45892	0.027	1.298	9.000	9:0	3.34		
3.061	99177	0.028	1.181	10.000	10:0	6.57	Peak match -0.0008	
3.691	842	0.029	. . .	10.913			
3.751	52423	0.031	1.089	11.000	11:0	3.20	Peak match -0.0053	
3.889	21798	0.032	1.077	11.151	10:0 20H	1.32	Peak match 0.0090	
4.132	10340	0.033	1.057	11.417	10:0 30H	0.61	Peak match 0.0024	
4.665	109960	0.034	1.020	12.000	12:0	6.29	Peak match -0.0023	
5.813	57216	0.038	0.972	13.000	13:0	3.12	Peak match -0.0004	
7.176	116470	0.042	0.941	14.000	14:0	6.15	Peak match 0.0002	
8.715	60339	0.044	0.926	15.000	15:0	3.14	Peak match -0.0043	
9.046	25576	0.045	0.925	15.199	14:0 20H	1.33	Peak match 0.0072	
9.520	11457	0.047	0.924	15.484	Sun In Feature 3	0.59	Peak match 0.0026	14:0 30H/16:1 ISO I
10.378	121550	0.047	0.924	16.000	16:0	6.30	Peak match -0.0024	
12.106	60507	0.050	0.933	17.000	17:0	3.17	Peak match -0.0031	
12.511	27523	0.051	0.936	17.230	16:0 20H	1.45	Peak match 0.0046	
13.864	125340	0.050	0.950	18.000	18:0	6.68	Peak match -0.0013	
14.899	1359	0.052	. . .	18.591			
15.614	63508	0.051	0.972	19.000	19:0	3.46	Peak match -0.0004	
17.339	123130	0.053	0.998	20.000	20:0	6.90	Peak match 0.0001	
18.331	1000	0.040	. . .	20.588			
19.026	63003	0.053	1.025	21.000	21:0	3.62	Peak match 0.0005	
20.671	129580	0.054	1.049	22.000	22:0	7.63	Peak match -0.0005	
22.260	64677	0.055	1.070	23.000	23:0	3.88	Peak match 0.0000	
23.806	63705	0.055	1.084	24.000	24:0	3.87	Peak match 0.0005	
25.303	62663	0.054	1.089	25.000	25:0	3.83	Peak match -0.0002	
25.504	1133	0.059	. . .	25.119			
26.785	26752	0.582	. . .	25.880		> max ar/ht	
27.546	35270	0.475	. . .	26.332		> max ar/ht	
29.961	2455	0.085	. . .	27.765			
30.356	123860	0.083	1.024	28.000	28:0	7.12	Peak match -0.0003	
32.598	3321	0.120	. . .	28.910		> max ar/ht	
32.929	2059	0.111	. . .	29.045			
34.978	2487	0.112	. . .	29.877			
35.282	128660	0.115	0.891	30.000	30:0	6.43		
*****	11457	SUMMED FEATURE 3	0.59	12:0 ALDE ?	unknown 10.928
*****	16:1 ISO I/14:0 30H	14:0 30H/16:1 ISO I

Solvent Ar Total Area Named Area % Named Total Amt Mbr Ref ECL Deviation Ref ECL Shift

33067000 1845032 1768354 95.84 1782454 0

GOOD PEAK MATCHING: PEAK POSITION MATCHING ERROR (RMS) IS 0.0033.

LONGOS (Rev 1.0) * NO MATCH *

ID: 8319006 FATTY ACIDS HT-2 (20 ML)
 Bottle: 2 SAMPLE (EUKARY)

RT	Area	Ar/Ht	Respon	ECL	Name	%	Comment 1	Comment 2
1.589	28105000	0.056	. . .	7.051	SOLVENT PEAK	< min rt	
9.623	1030	0.075	0.924	15.545	unknown 15.549	29.34	ECL deviates -0.004	
10.378	1641	0.049	0.924	16.000	16:0	46.78	ECL deviates 0.000	Reference 0.000
13.860	815	0.057	0.950	18.000	18:0	23.88	ECL deviates 0.000	Reference -0.002
20.731	1437	0.102	. . .	22.039		
26.895	16201	0.750	. . .	25.946	> max ar/ht	
27.551	5866	0.252	. . .	26.336	> max ar/ht	
29.962	1603	0.073	. . .	27.767		
32.924	3391	0.147	. . .	29.044	> max ar/ht	
36.649	2450	0.143	. . .	30.556	> max rt	

(ng/ml) pp6

67
 107
 53
 93
 1053
 381
 104
 220
 159

Solvent Ar	Total Area	Named Area	% Named	Total Amt	Nbr Ref	ECL Deviation	Ref ECL Shift
28105000	31984	3486	10.90	3243	2	0.002	0.002

* QUESTION ANALYSIS: TOTAL AREA LESS THAN 5000. CONCENTRATE AND RE-RUN.

2237

*** ANALYSIS NOT GOOD ENOUGH FOR LIBRARY SEARCH ***

Response Factor = 2.6 pg

Inj volume = 2 µl

Appendix C

Analysis of B and A Standard Mix By EPA-625 Methodology



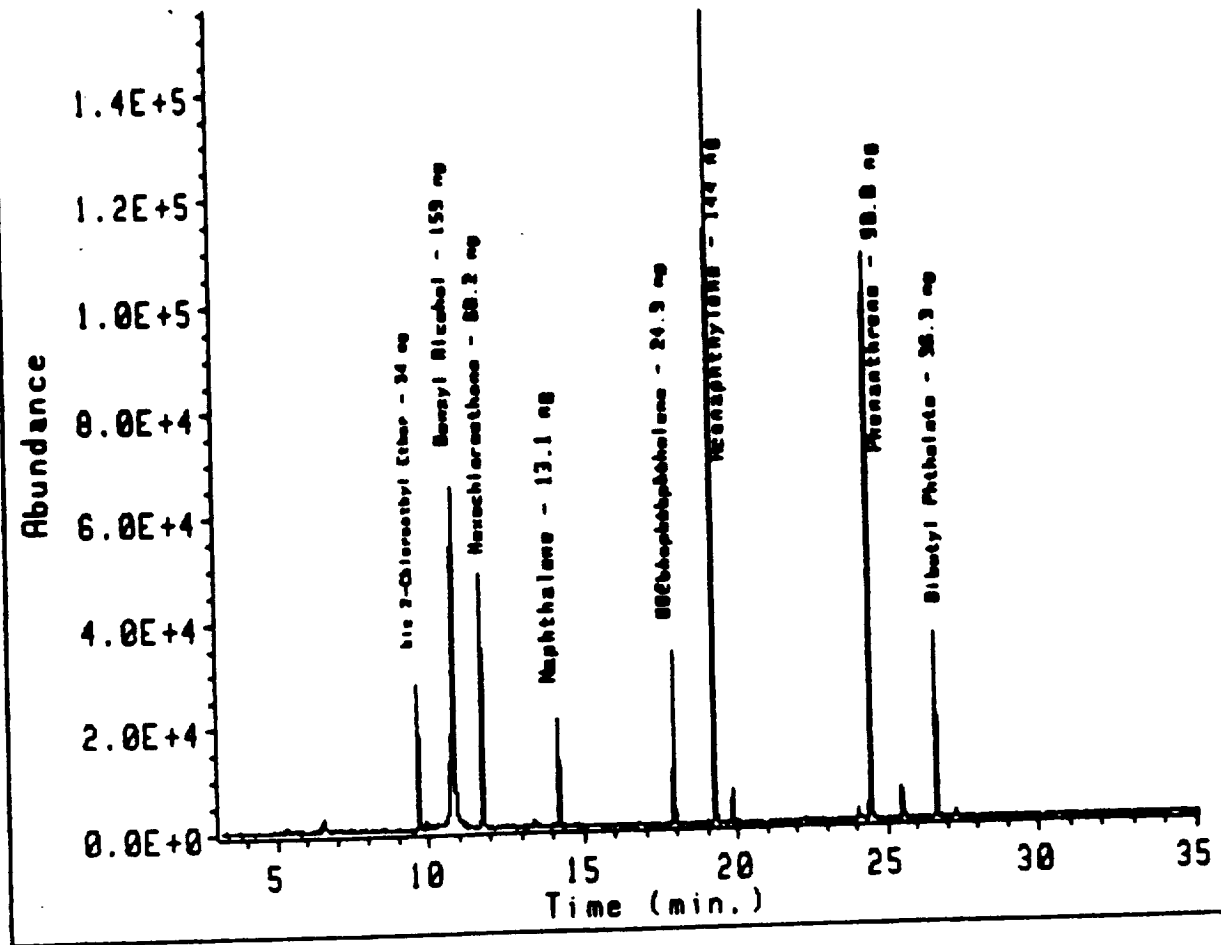
Certification

Priority Pollutant™/CLP Quality Control Standards

Parameter	LOT NO. 91108 Certified Value µg/l	Advisory Range µg/l
BASE/NEUTRALS		
Acenaphthylene	144	48-209
Benzyl Alcohol	159	24-287
bis(2-Chloroethyl) ether	34.0	4.1-53
2-Chloronaphthalene	24.9	15-29
Di-n-butylphthalate	36.3	5.4-43
Hexachloroethane	68.2	27-77
Naphthalene	13.1	5.0-17
Phenanthrene	90.0	49-108

ORIGINAL PAGE IS
OF POOR QUALITY

ERA BASE/NEUTRALS STD LOT 491100



ORIGINAL PAGE IS
OF POOR QUALITY

Appendix D

Acidic Liquid Extraction Results

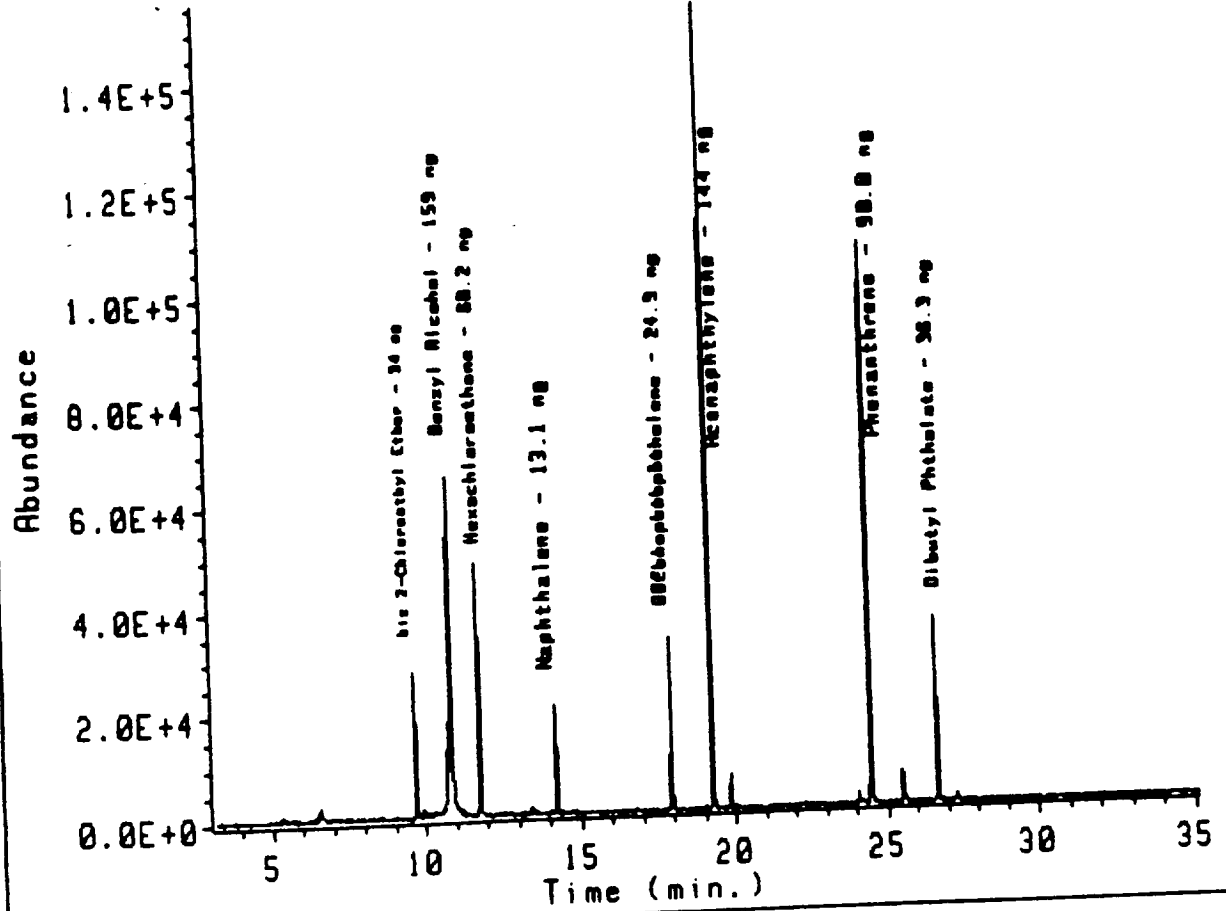


Certification

PriorityPollutnT™/CLP Quality Control Standards

Parameter	LOT NO. 91108 era Certified Value µg/l	Advisory Range µg/l
BASE/NEUTRALS		48-209
Acenaphthylene	144	24-287
Benzyl Alcohol	159	4.1-53
bis(2-Chloroethyl)ether	34.0	15-29
2-Chloronaphthalene	24.9	5.4-43
Di-n-butylphthalate	36.3	27-77
Hexachloroethane	68.2	5.0-17
Naphthalene	13.1	49-108
Phenanthrene	90.0	

ERA BASE/NEUTRALS STD LOT 491108

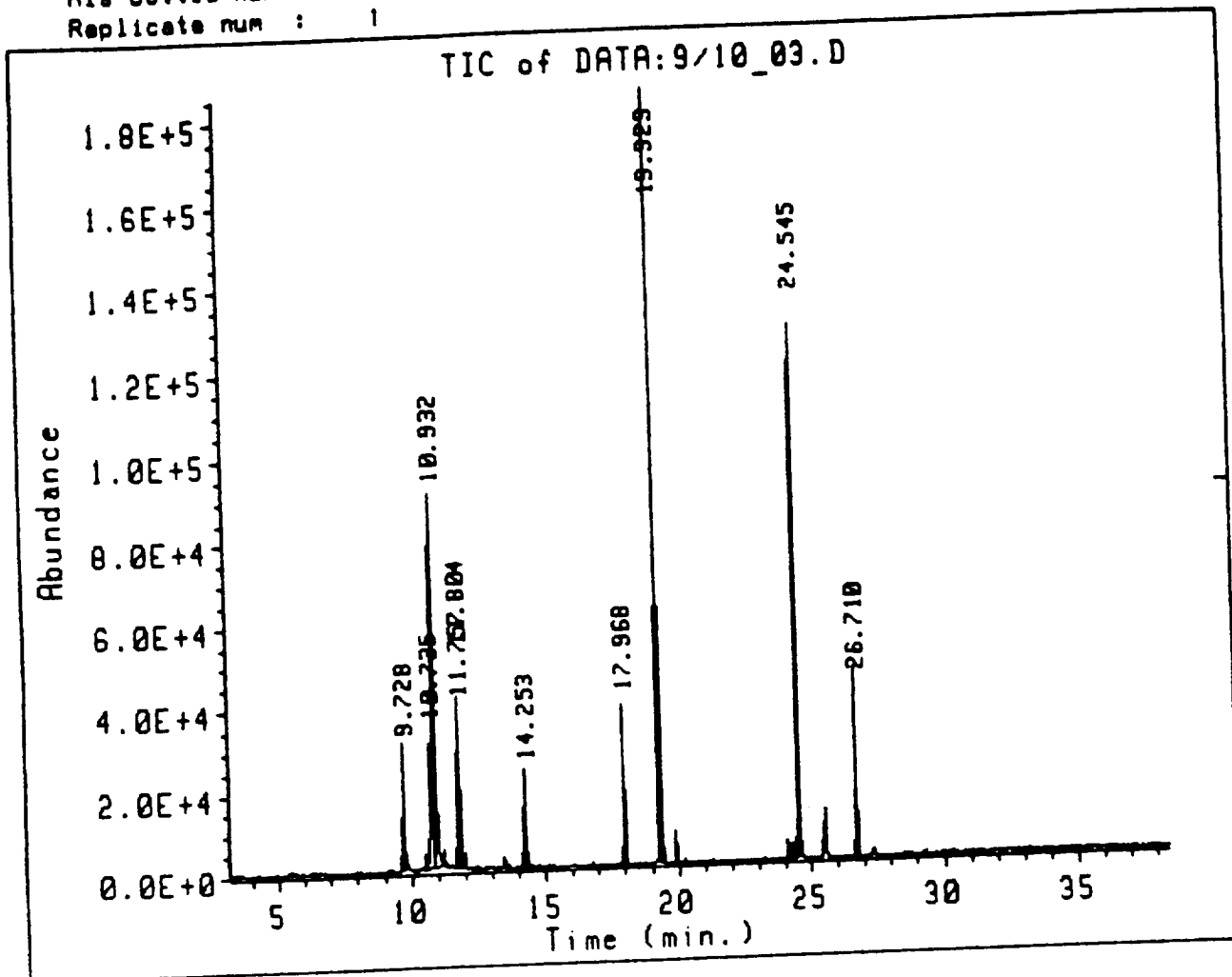


Data file: DATA:9/10_03.D
File type: GC / MS DATA FILE

Sample Name: ERA STANDARD LOT 891108 - NEAT
Misc Info: 2.0 uL INJ - 11 psig - Undiluted Std. for Quantit.
Operator : B BENSON

Instrument: MS_5988
Inlet : GC

Sequence index : 0
Als bottle num : 0
Replicate num : 1



TIC of DATA:9/10_03.D 10 integration peaks found.

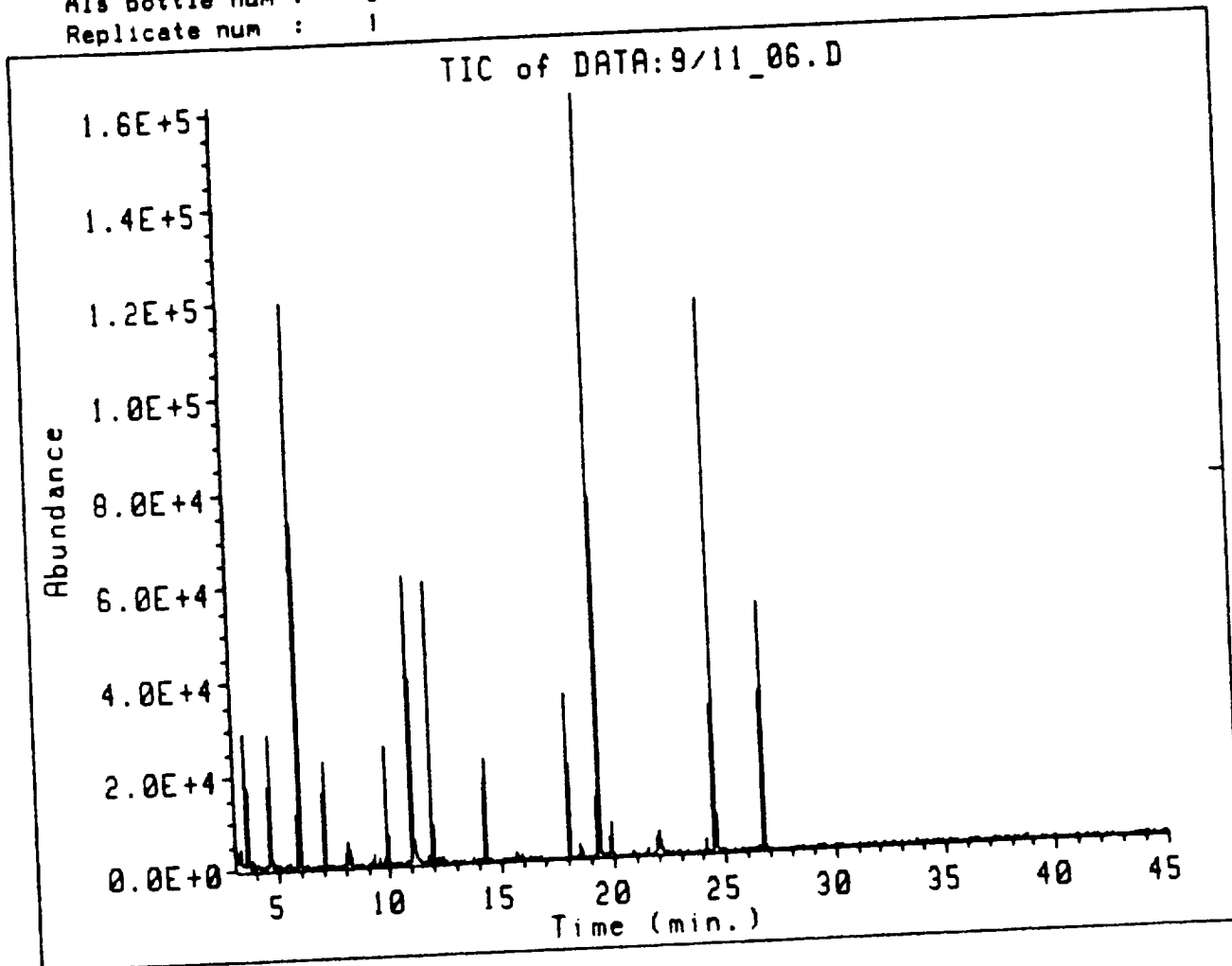
Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	9.728	BV	0.059	955326	9.605	9.839
2	10.735	VV	0.089	1712477	10.510	10.841
3	10.932	VV	0.076	4208404	10.841	11.265
4	11.757	BV	0.050	869829	11.666	11.776
5	11.804	V8	0.055	1336331	11.776	11.990
6	14.253	88	0.049	717485	14.118	14.363
7	17.968	88	0.048	1134114	17.907	18.057
8	19.323	88	0.051	5818141	19.234	19.457
9	24.545	BV	0.070	4205998	24.304	24.617
10	26.710	88	0.065	1110176	26.634	26.800

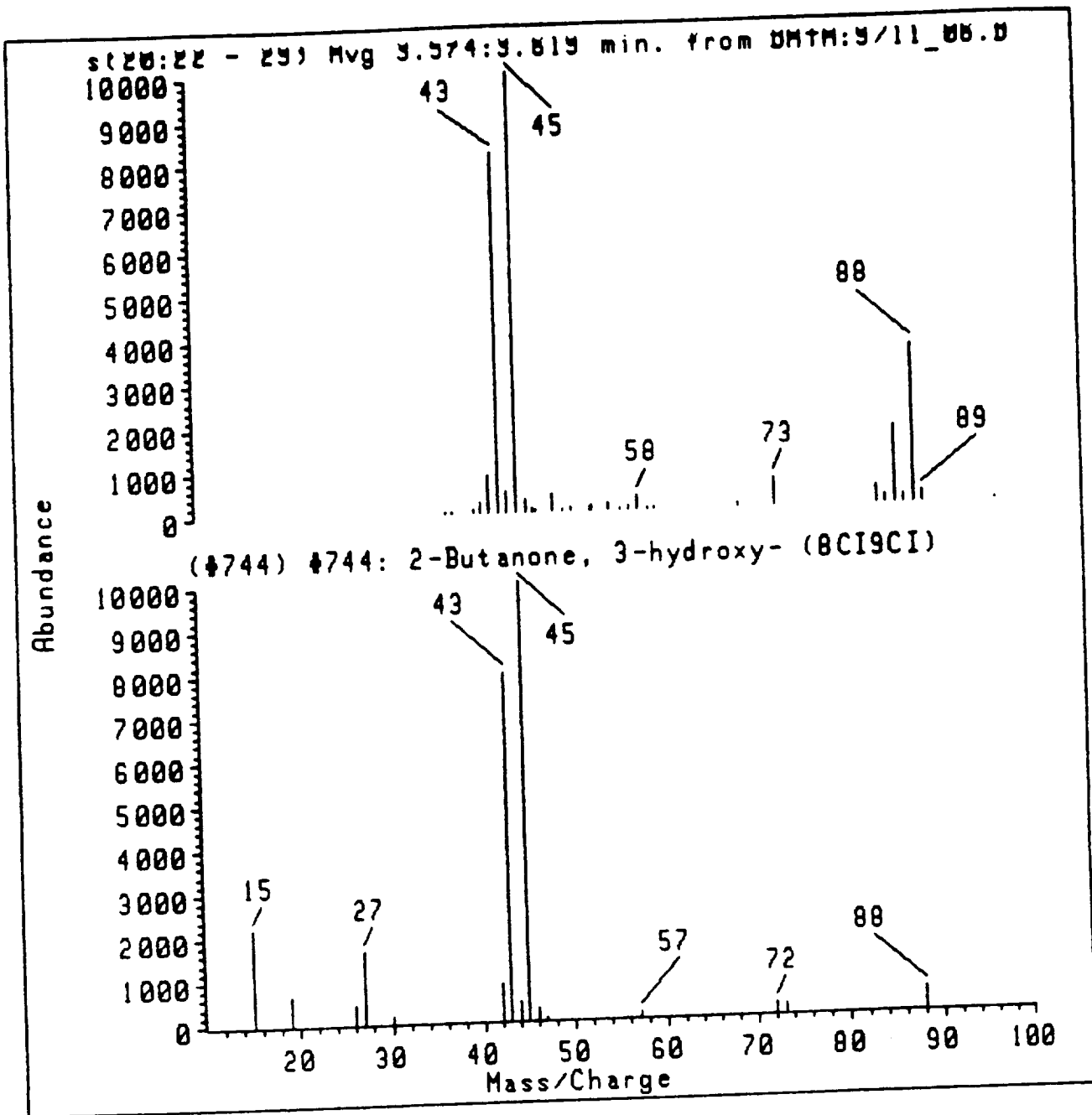
Data file: DATA:9/11_06.D
File type: GC / MS DATA FILE

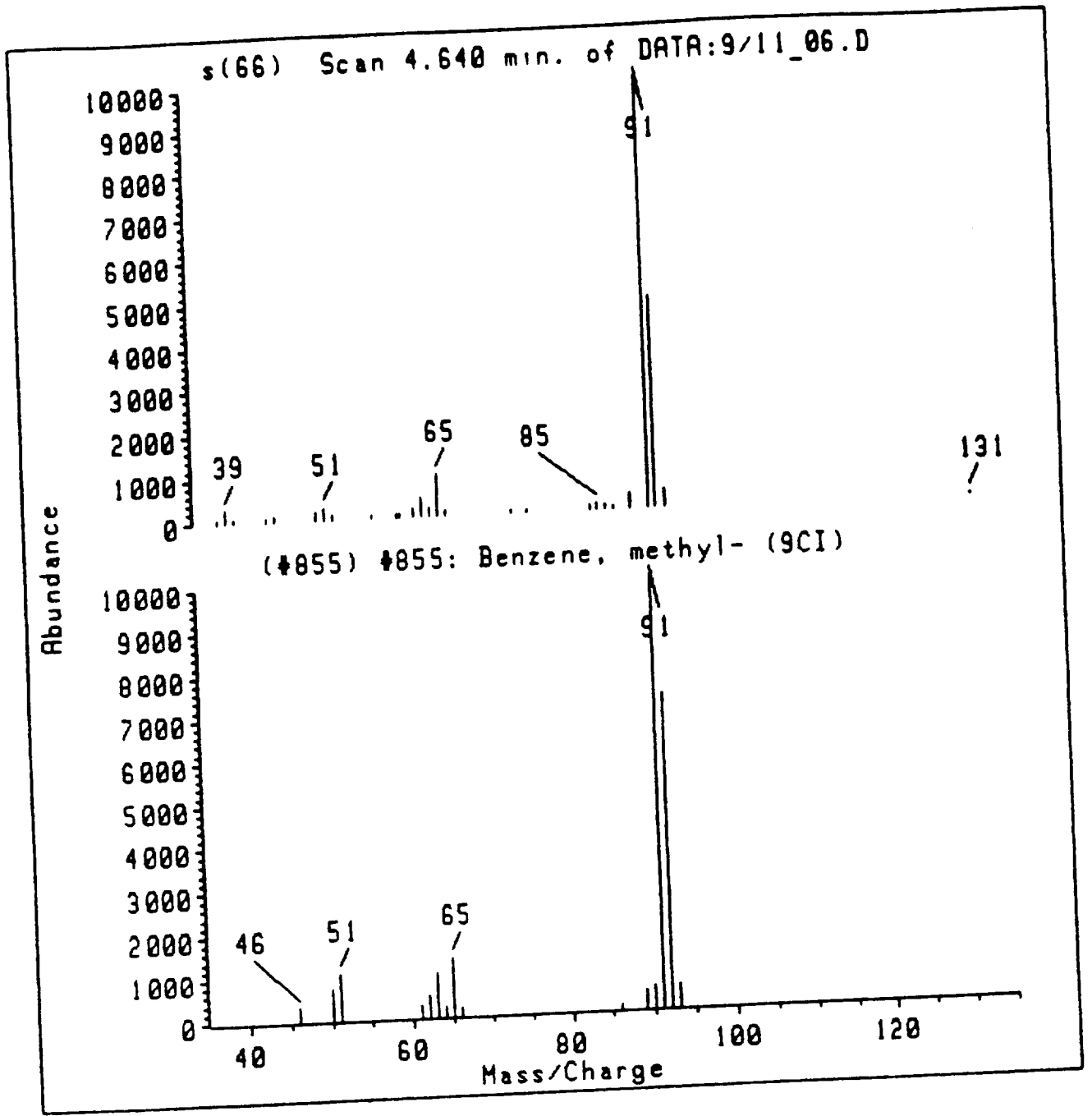
Sample Name: BENSON SAMPLE NUMBER 3
Misc Info: 2.0 uL INJ - 11 psig
Operator : MUK

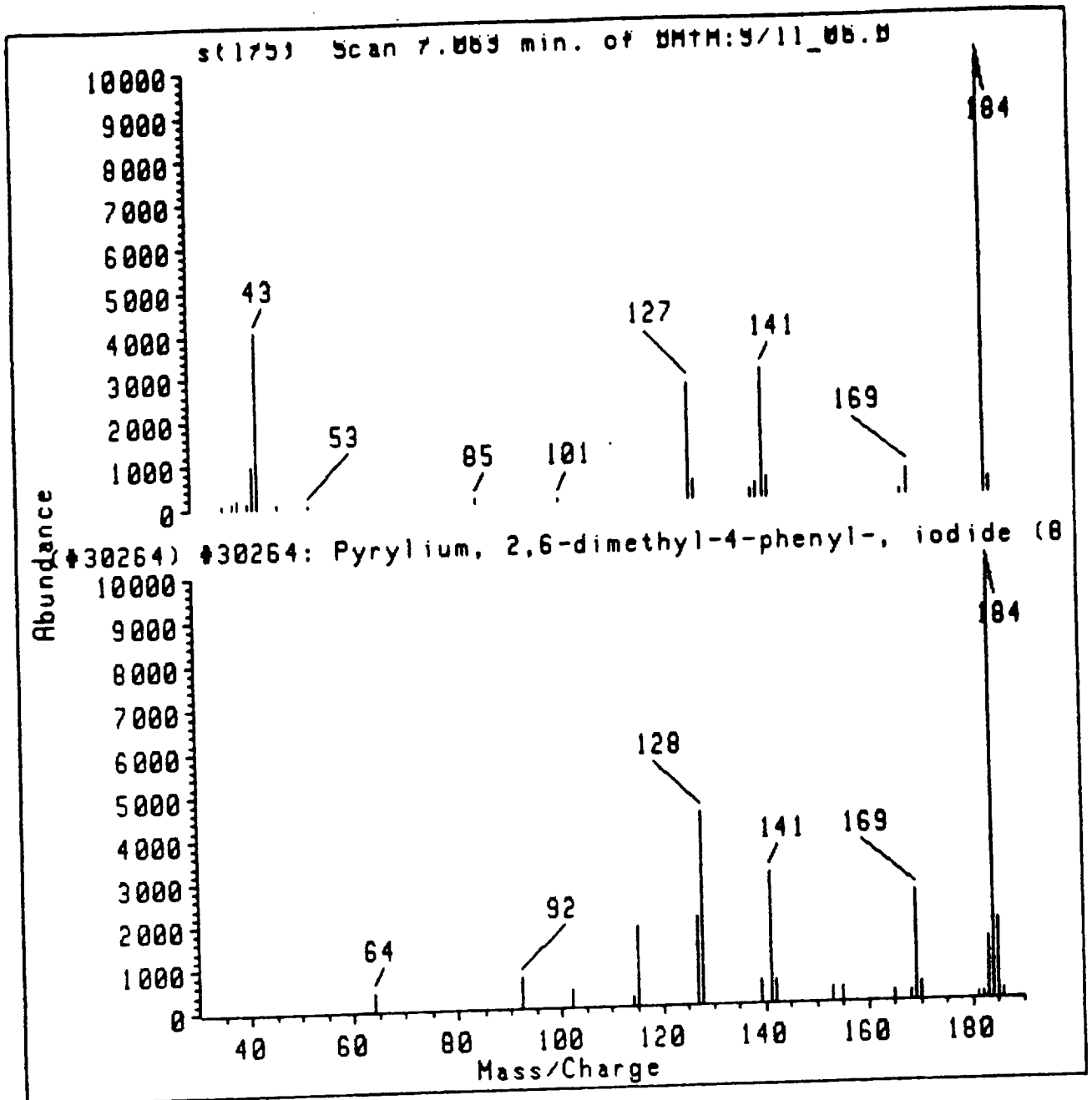
Instrument: MS_5988
Inlet : GC

Sequence index : 0
Als bottle num : 0
Replicate num : 1



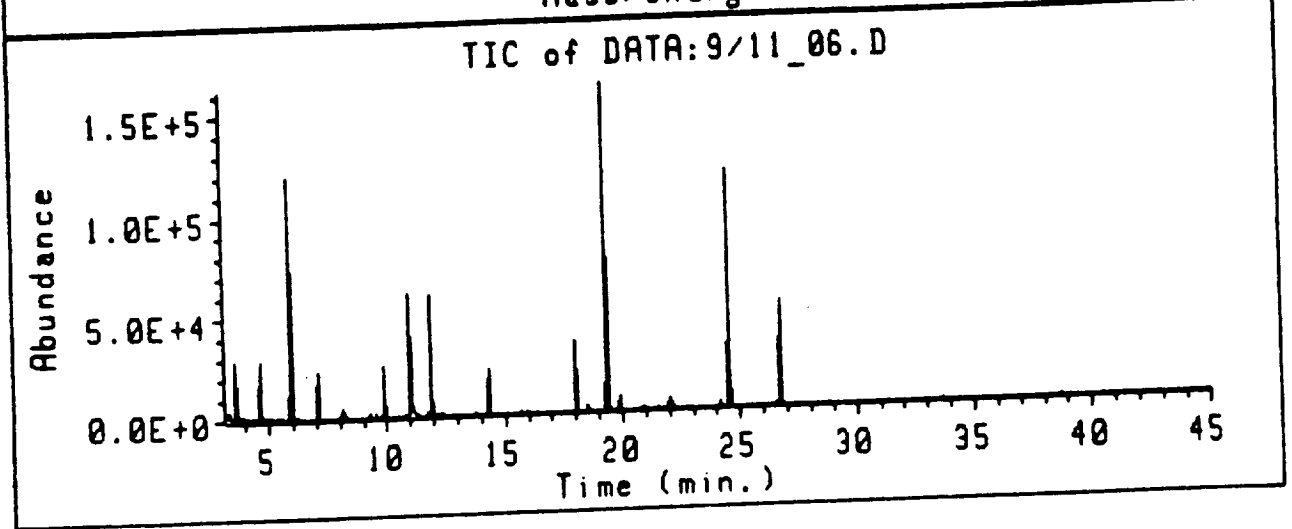
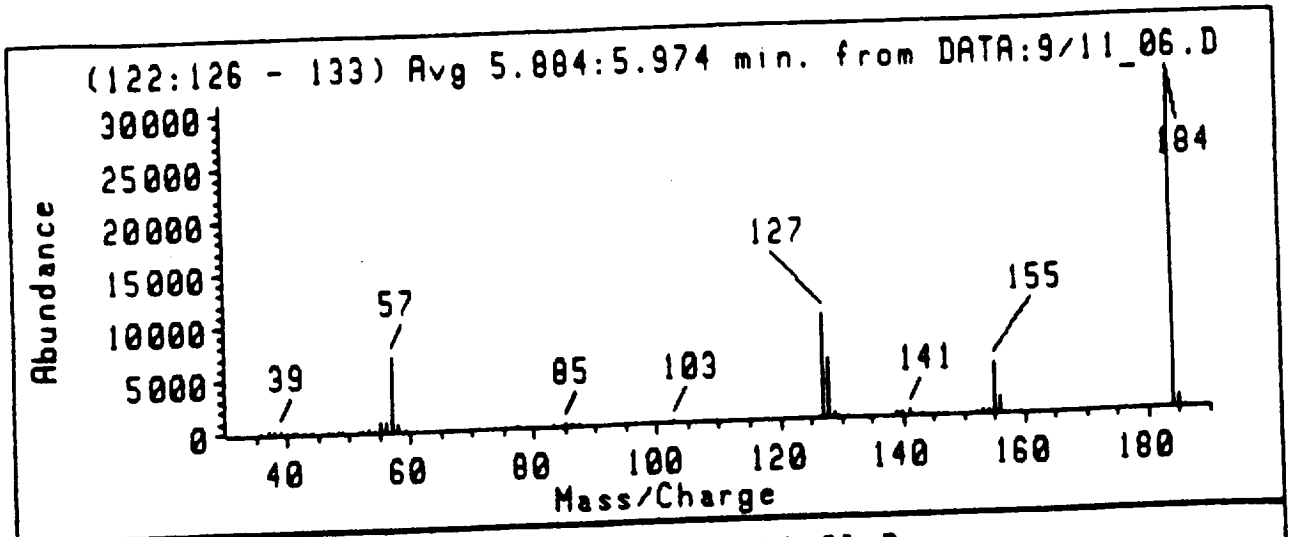






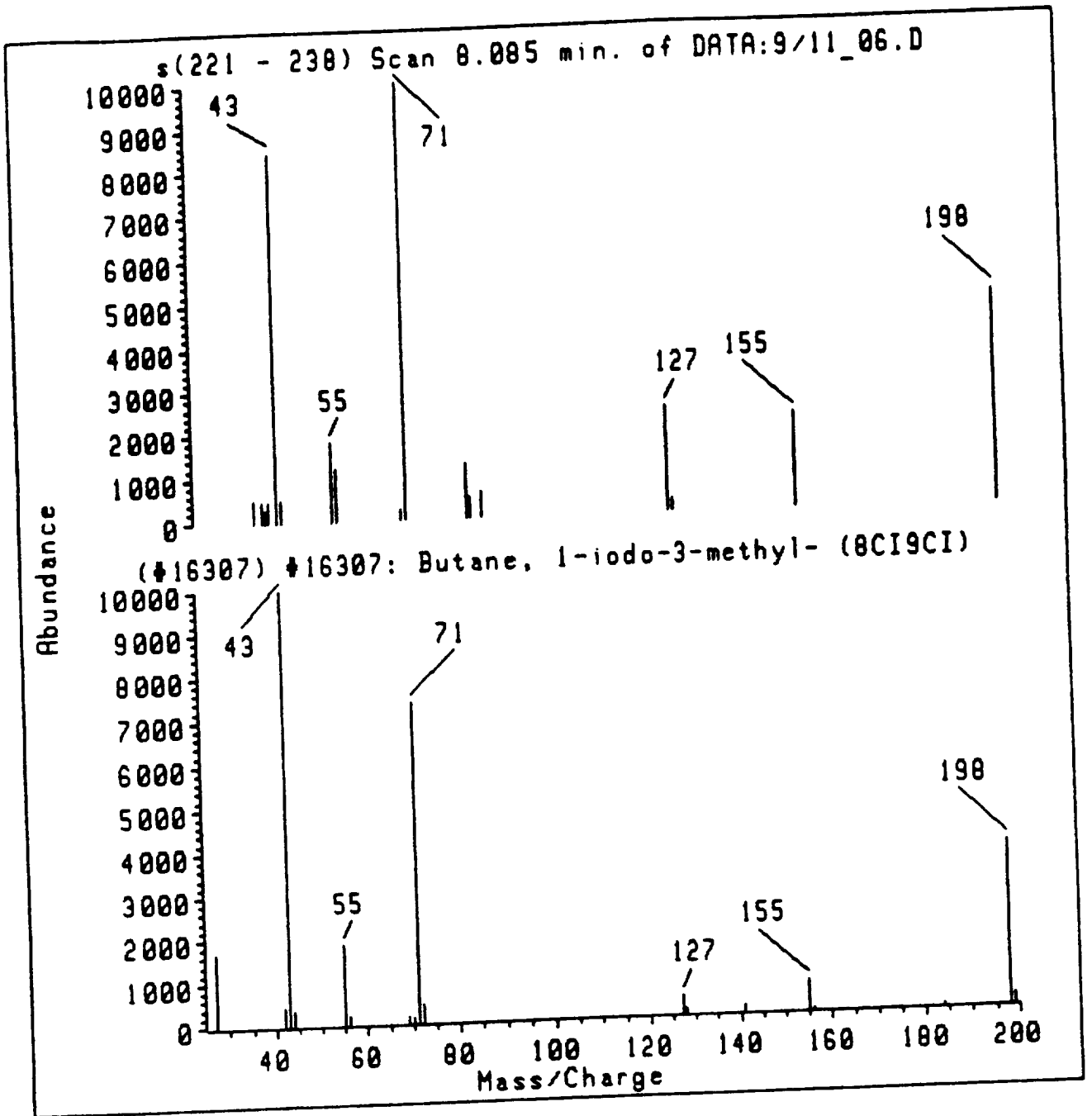
Scan 7.063 min. of DATA:9/11_06.D

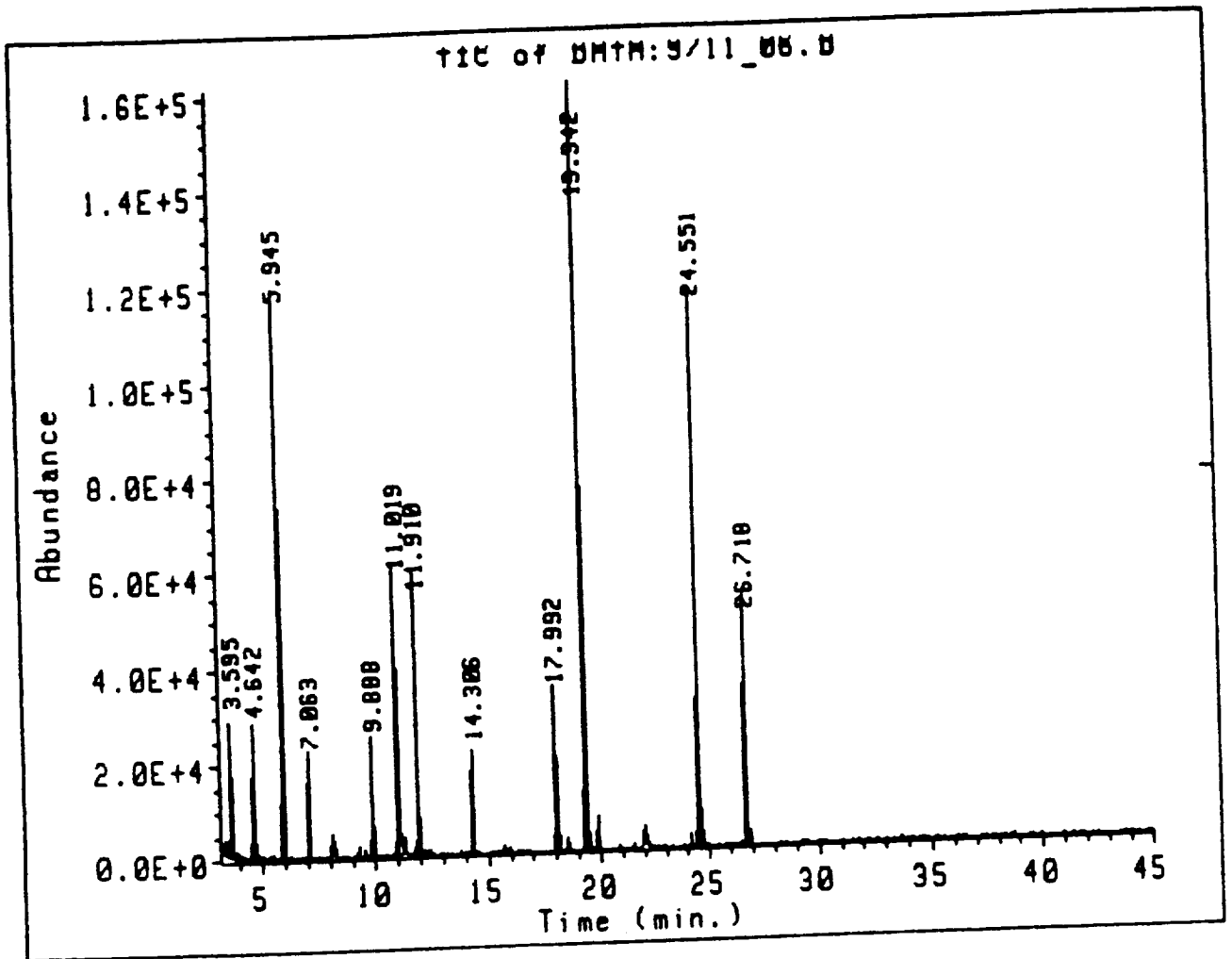
AMU.	Abundance	AMU.	Abundance	AMU.	Abundance
35.95	47.00	52.75	30.00	141.05	2871.00
37.95	109.00	85.00	82.00	141.95	439.00
39.05	180.00	101.00	57.00	167.95	94.00
41.05	108.00	126.90	2596.00	168.95	561.00
41.95	936.00	127.90	399.00	183.95	9689.00
42.95	3920.00	138.90	157.00	184.95	318.00
46.70	46.00	139.90	307.00		



Avg 5.884:5.974 min. from DATA:9/11_06.D

AMU.	Abundance	AMU.	Abundance	AMU.	Abundance
35.80	25.40	54.00	17.40	129.65	11.40
36.95	144.20	55.00	733.20	131.05	23.60
37.95	165.20	56.00	653.00	138.90	145.40
38.95	239.80	57.00	6959.80	139.15	12.80
40.00	41.80	58.00	503.20	139.95	188.00
40.95	25.00	59.15	26.60	141.00	317.80
41.70	10.60	77.25	7.80	142.90	17.80
43.00	34.60	82.95	60.40	152.00	54.60
44.05	39.80	85.00	83.60	152.95	80.40
46.80	9.20	85.95	24.00	154.00	230.00
48.40	7.20	86.25	7.20	154.95	4624.00
48.95	24.20	87.20	18.40	156.00	1336.00
51.40	9.20	102.90	10.00	183.95	30994.40
51.65	8.60	126.90	9630.80	185.00	967.60
52.25	13.80	127.90	5409.40	186.00	39.40
53.05	133.80	128.90	301.20		





Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	3.595	BB	0.052	788240	3.519	3.686
2	4.642	BM	0.064	1208298	4.499	4.714
3	5.945	BB	0.063	4339767	5.813	6.035
4	7.063	BB	0.044	561023	6.993	7.138
5	9.888	BB	0.039	550939	9.832	9.954
6	11.019	BB	0.078	2222233	10.912	11.335
7	11.910	BM	0.039	1273857	11.825	11.964
8	14.306	BB	0.042	526831	14.230	14.386
9	17.992	BB	0.047	970455	17.915	18.115
10	19.342	BB	0.057	5766990	19.207	19.507
11	24.551	BB	0.057	4107705	24.417	24.707
12	26.718	BB	0.067	1315329	26.644	26.867

Recovery of Base/Neutrals with respect to neat standard

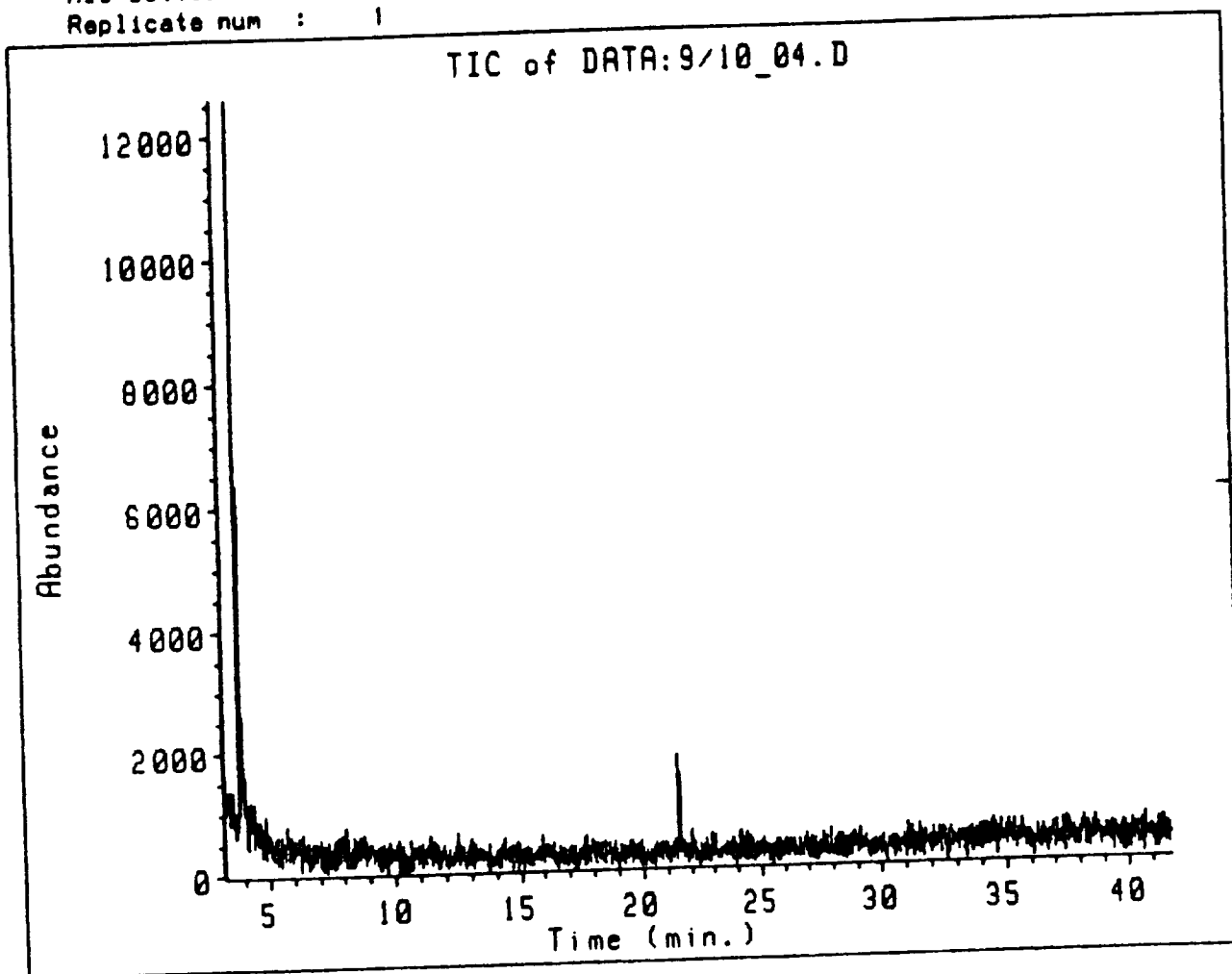
Peak #	Rt.	% Recovery
5	9.89	$550939 / 955326 \times 100 = 57.67\%$
6	11.02	$2222233 / 4268464 \times 100 = 52.80\%$
7	11.91	$1273857 / 1336331 \times 100 = 95.32\%$
8	14.31	$526831 / 717485 \times 100 = 73.43\%$
9	17.99	$970455 / 1134114 \times 100 = 85.57\%$
10	19.34	$5766990 / 5818141 \times 100 = 99.12\%$
11	24.55	$4107705 / 4205998 \times 100 = 97.66\%$
12	26.72	$1315329 / 1110174 \times 100 = 118.48\%$

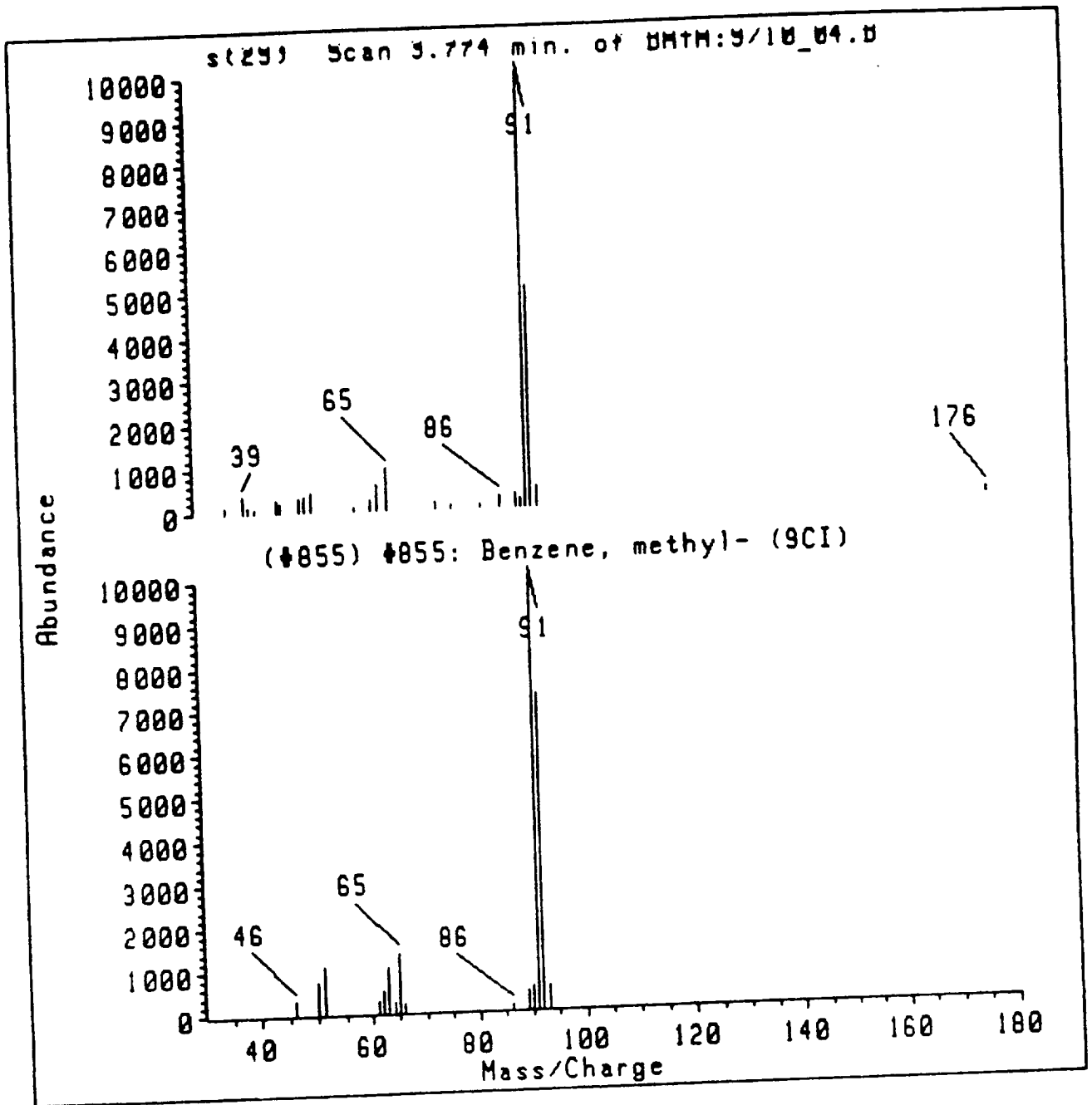
Data file: DATA:9/10_04.D
File type: GC / MS DATA FILE

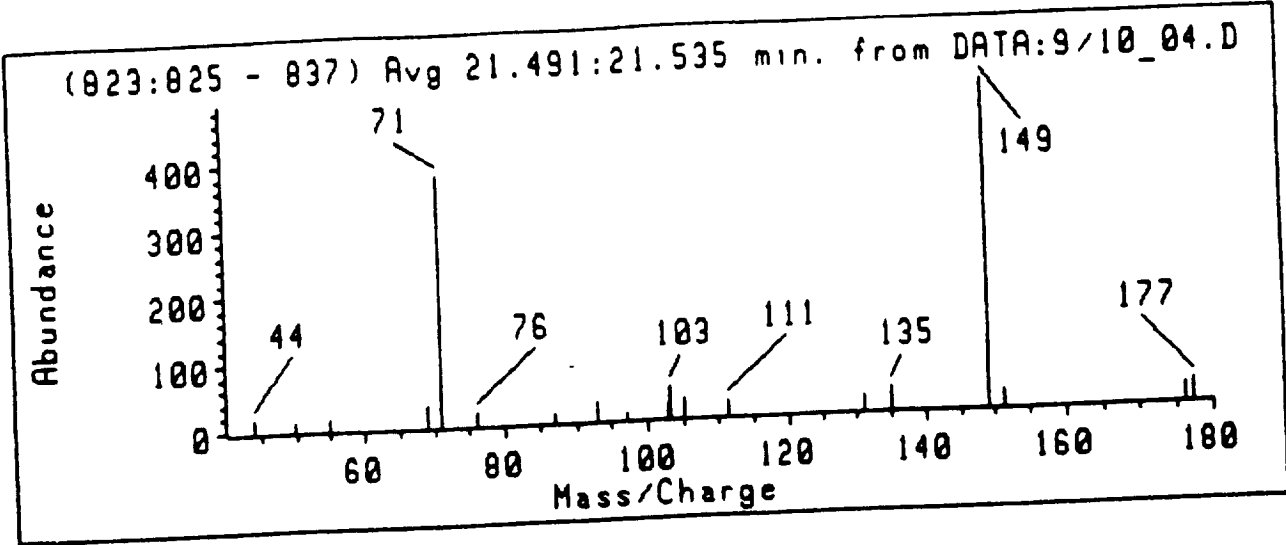
Sample Name: WATER BLANK - EPA 625 EXTRACTION
Misc Info: 2.0 uL INJ - 11 psiq - METHOD BLANK
Operator : B BENSON

Instrument: MS_5988
Inlet : GC

Sequence index : 0
Als bottle num : 0
Replicate num : 1

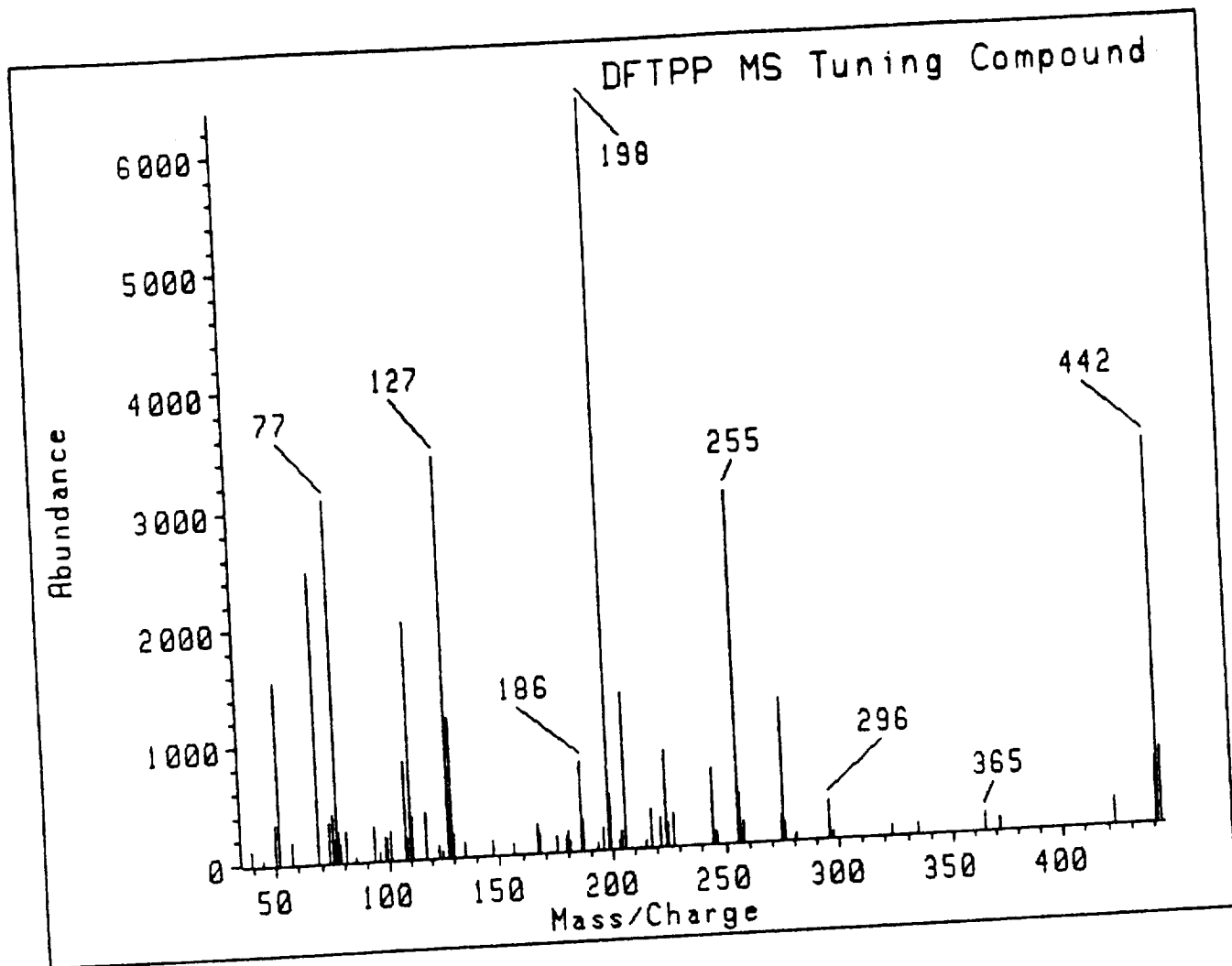






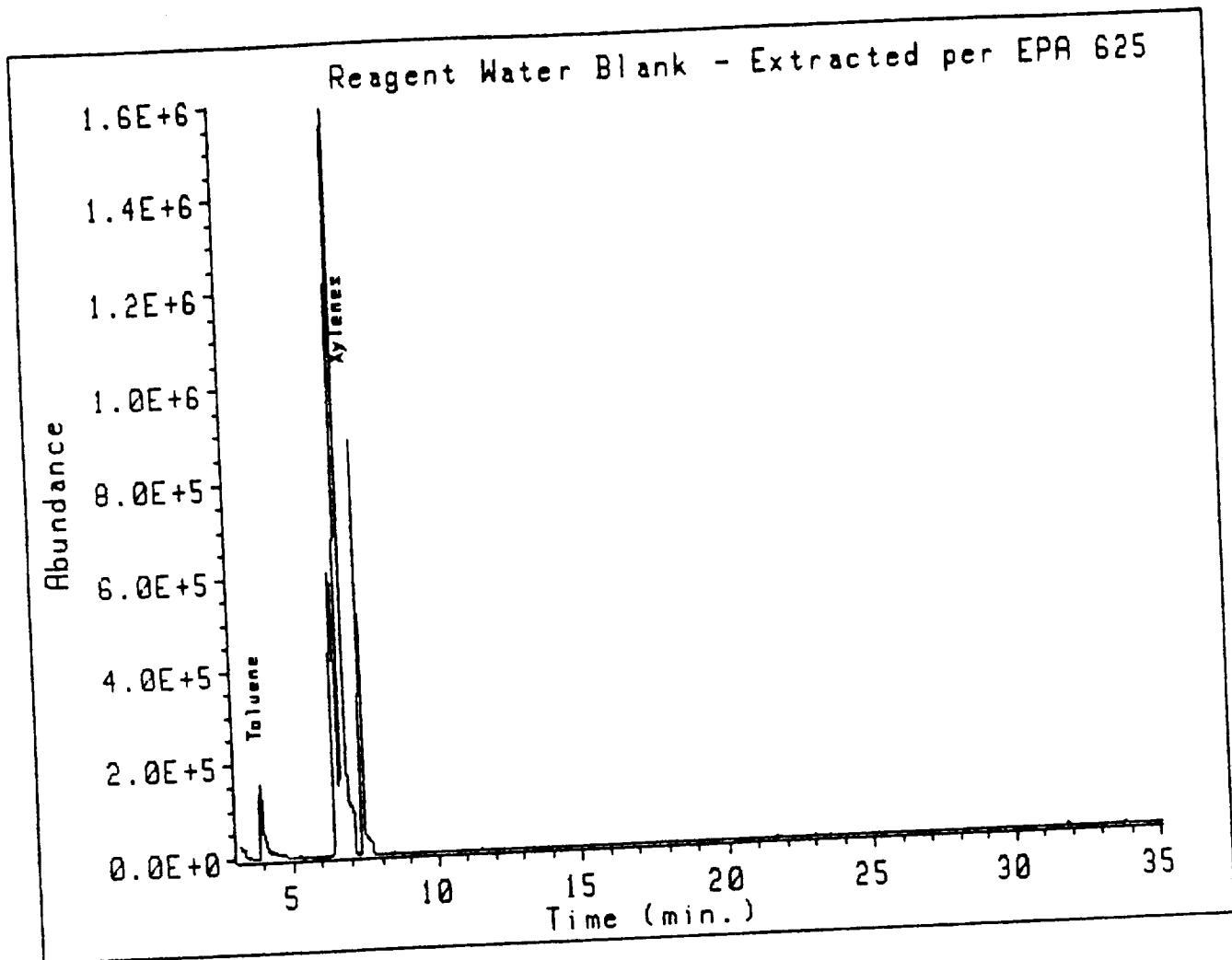
Appendix E

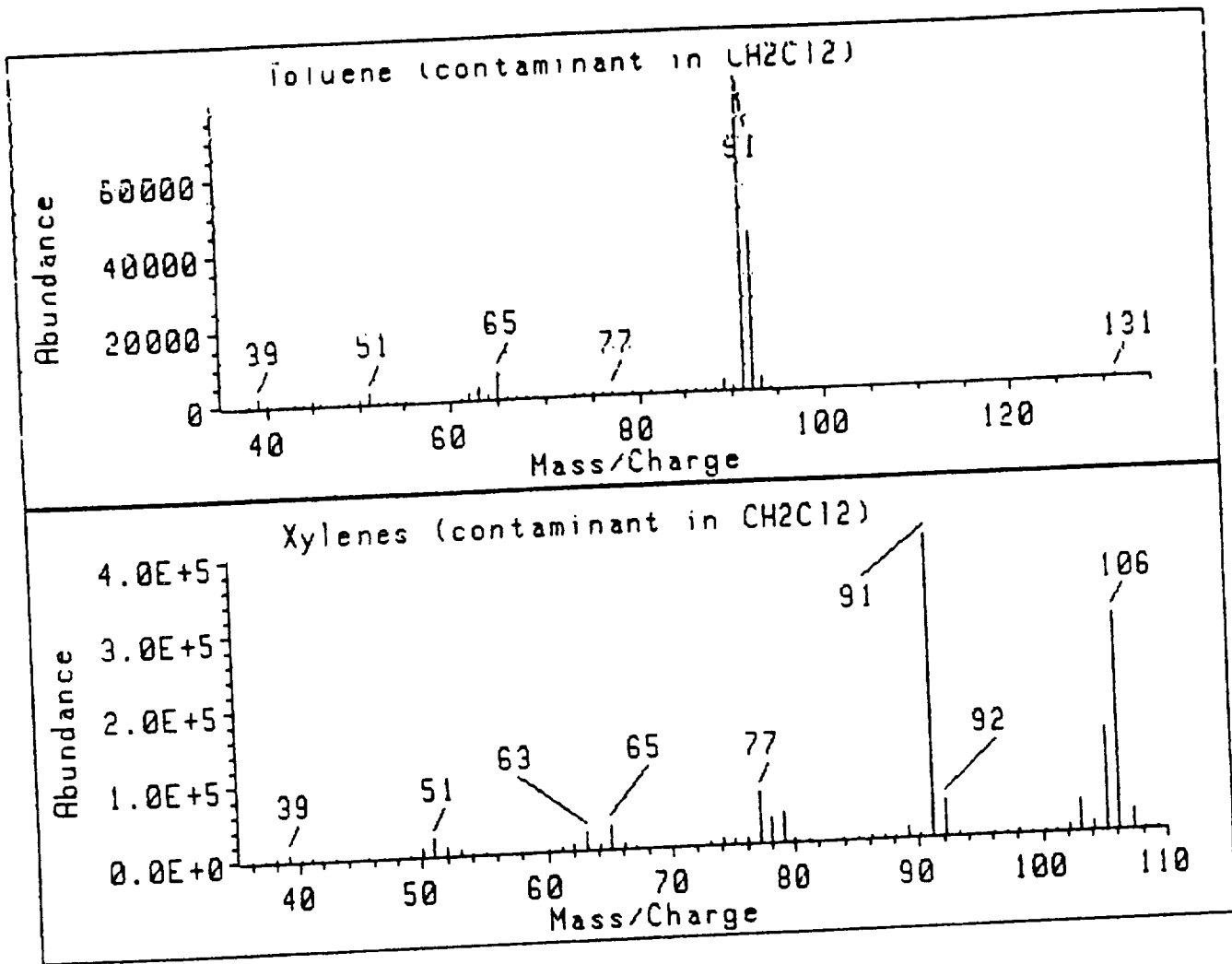
Basic Liquid Extraction Results



Scan 20.376 min. of DATA:1/16_02.D

AMU.	Abundance	AMU.	Abundance	AMU.	Abundance
39.05	104.00	127.15	3407.00	224.15	809.00
44.05	29.00	128.15	205.00	225.15	188.00
50.05	339.00	129.15	1166.00	227.15	271.00
51.05	1540.00	130.15	194.00	244.15	627.00
57.05	172.00	135.15	105.00	245.15	100.00
68.95	2470.00	147.15	104.00	246.00	91.00
74.05	327.00	156.15	83.00	255.00	2999.00
75.05	398.00	156.40	73.00	256.00	407.00
76.05	167.00	167.15	238.00	257.20	42.00
77.05	3064.00	168.15	159.00	258.05	167.00
78.05	260.00	175.25	134.00	274.05	212.00
78.95	150.00	179.15	108.00	275.05	1209.00
81.05	246.00	180.00	155.00	276.05	135.00
85.05	27.00	181.15	74.00	281.20	47.00
93.15	282.00	186.15	745.00	296.05	310.00
96.25	56.00	187.15	254.00	297.20	40.00
98.15	198.00	193.00	52.00	323.20	82.00
99.15	171.00	196.15	181.00	334.20	82.00
101.00	244.00	198.00	6380.00	365.05	148.00
107.15	821.00	199.15	453.00	372.05	88.00
108.15	172.00	203.15	60.00	423.05	215.00
110.15	2003.00	204.15	147.00	441.15	544.00
111.15	354.00	206.15	1310.00	442.15	3282.00
117.15	373.00	215.00	45.00	443.15	630.00
123.00	103.00	217.00	321.00		
125.15	50.00	221.15	242.00		



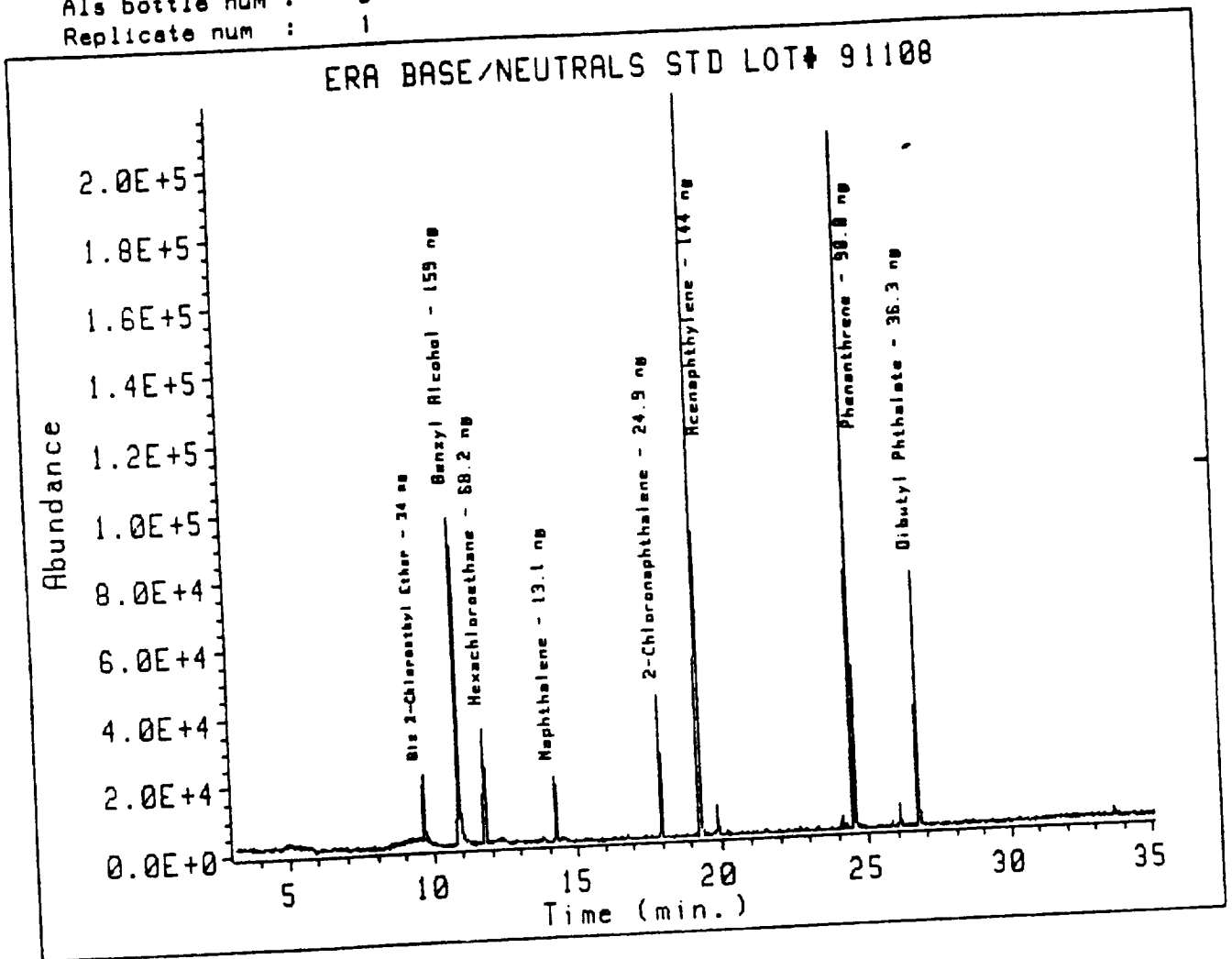


Data file: DATA:1/16_01.D
File type: GC / MS DATA FILE

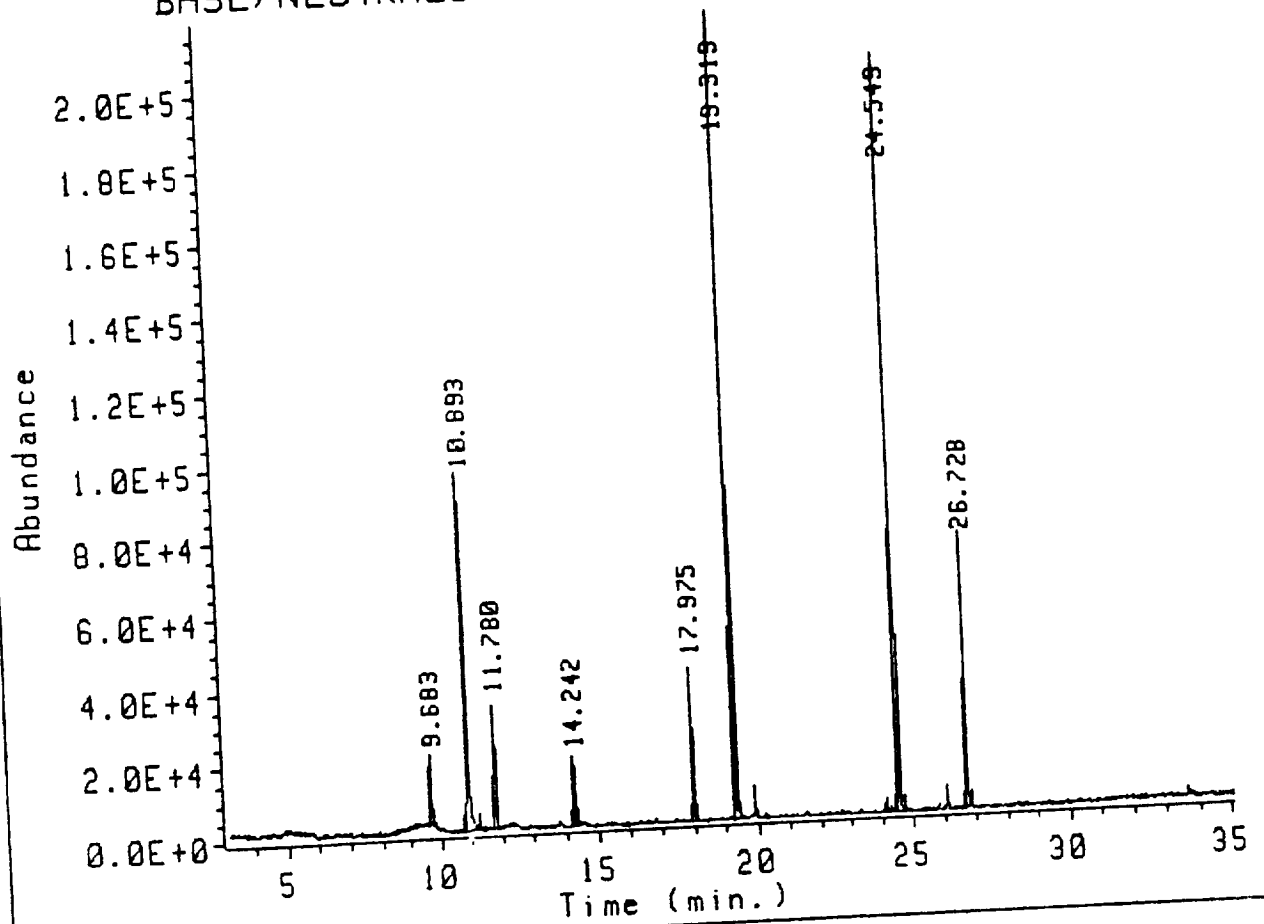
Sample Name: NEAT STANDARD - ERA BNA Std #91108
Misc Info: 1.0 ul inj - 8 psig
Operator : B BENSON

Date : 16 Jan 91 11:45 am
Instrument: MS_5988
Inlet : GC

Sequence index : 0
Als bottle num : 0
Replicate num : 1



BASE/NEUTRALS STD #91108 Integration Results



TIC of DATA:1/16_01.D 9 integration peaks found.

Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	9.683	BB	0.053	591022	9.614	9.804
2	10.893	BB	0.071	3984233	10.709	11.257
3	11.780	BV	0.049	1007761	11.693	11.861
4	14.242	BB	0.047	508422	14.164	14.332
5	17.975	BB	0.044	1106799	17.909	18.088
6	19.319	BV	0.051	6844013	19.228	19.454
7	24.549	BV	0.050	6195442	24.438	24.707
8	26.728	BB	0.047	2051028	26.607	26.842

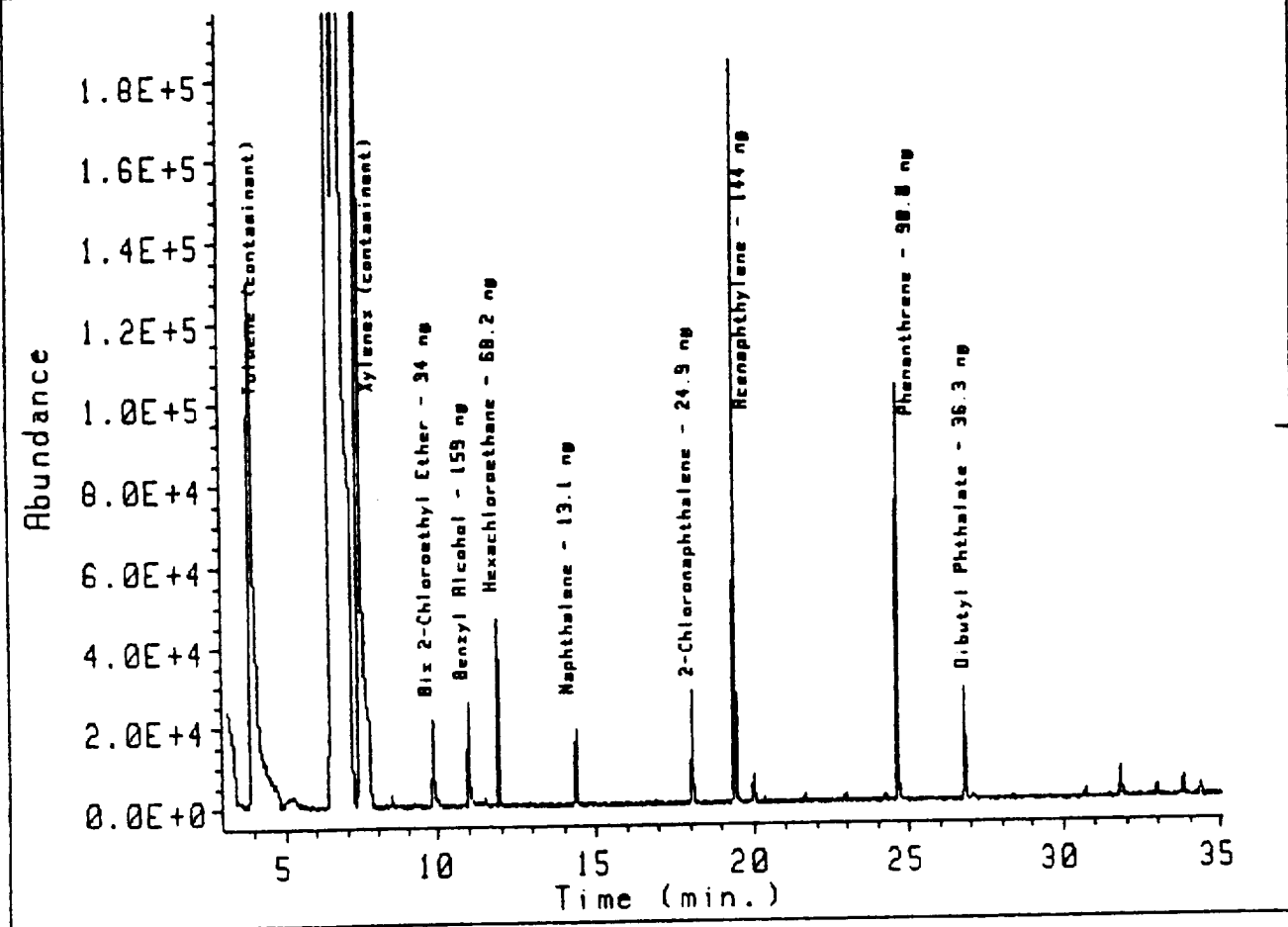
Data file: DATA:1/16_04.D
File type: GC / MS DATA FILE

Sample Name: ERA BNA Std - CH2CL2 EXTRACTED STANDARD #91108
Misc. Info: 1.0 ul inj - .8 psig
Operator : 9 BENSON

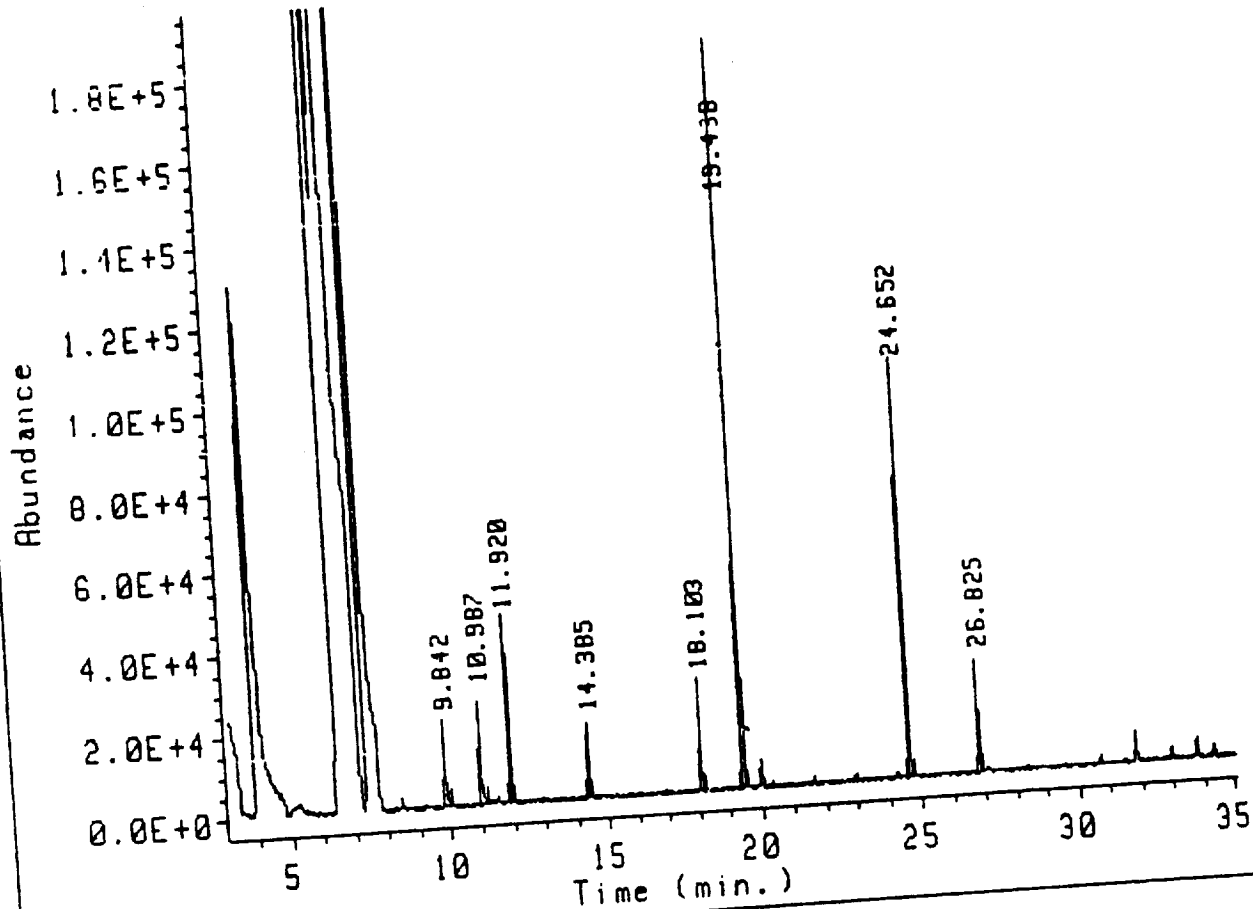
Date : 16 Jan 91 2:32 pm
Instrument: MS_5988
Inlet : GC

Sequence index : 0
Als bottle num : 0
Replicate num : 1

EPA 625 EXTRACT of ERA Base/Neutral Std # 91108 in H



TIC of DATA: 1/16_04.D



TIC of DATA:1/16_04.D 8 integration peaks found.

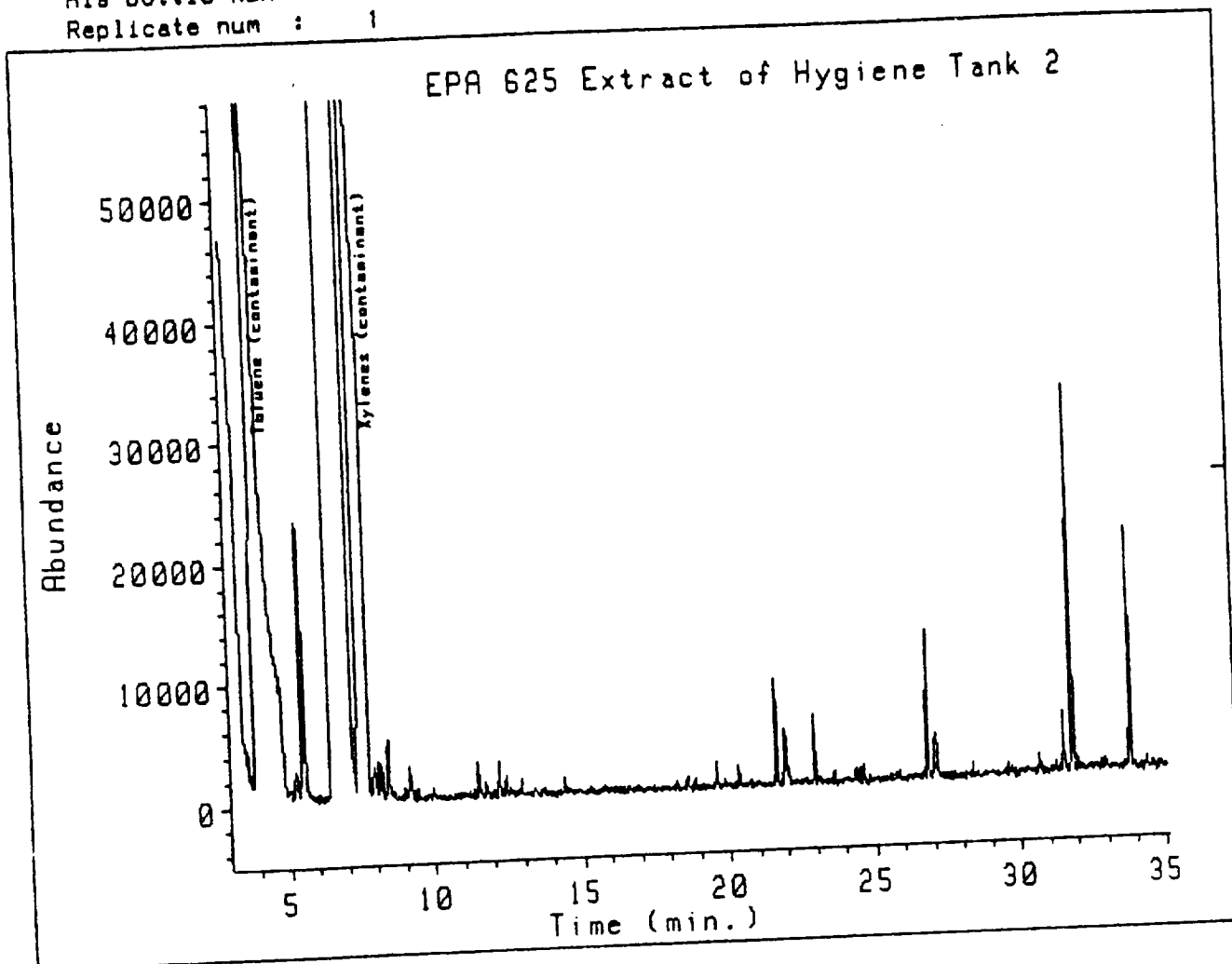
Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	9.842	BB	0.054	717214	9.753	10.044
2	10.987	BV	0.053	918446	10.915	11.183
3	11.920	BB	0.043	1127141	11.854	12.054
4	14.385	BV	0.072	499439	14.310	14.467
5	18.103	BB	0.067	772676	18.007	18.231
6	19.438	BV	0.046	4787863	19.348	19.552
7	24.652	BB	0.070	2986620	24.519	24.810
8	26.825	BB	0.049	861116	26.731	26.922

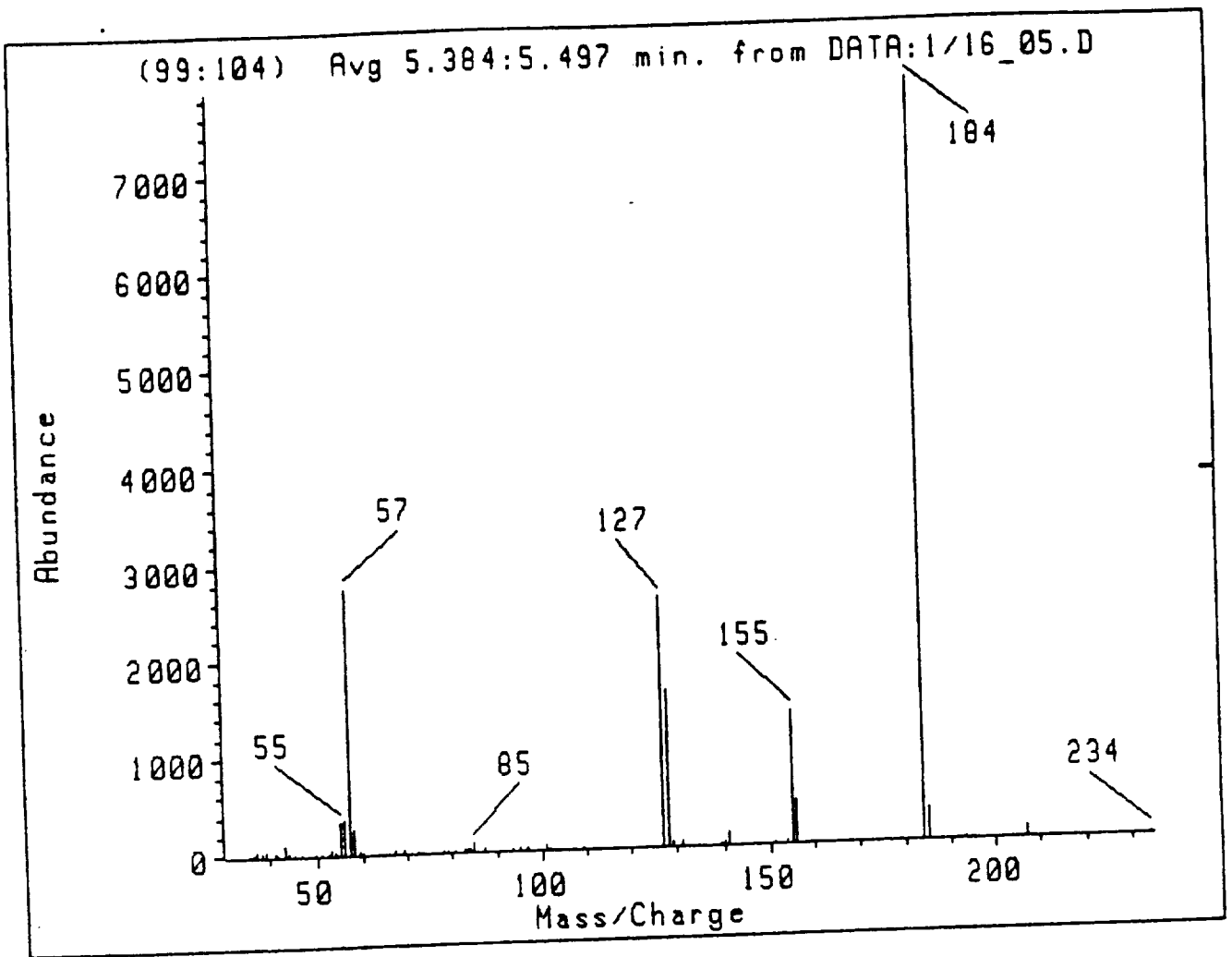
Data file: DATA:1/16_05.D
File type: GC / MS DATA FILE

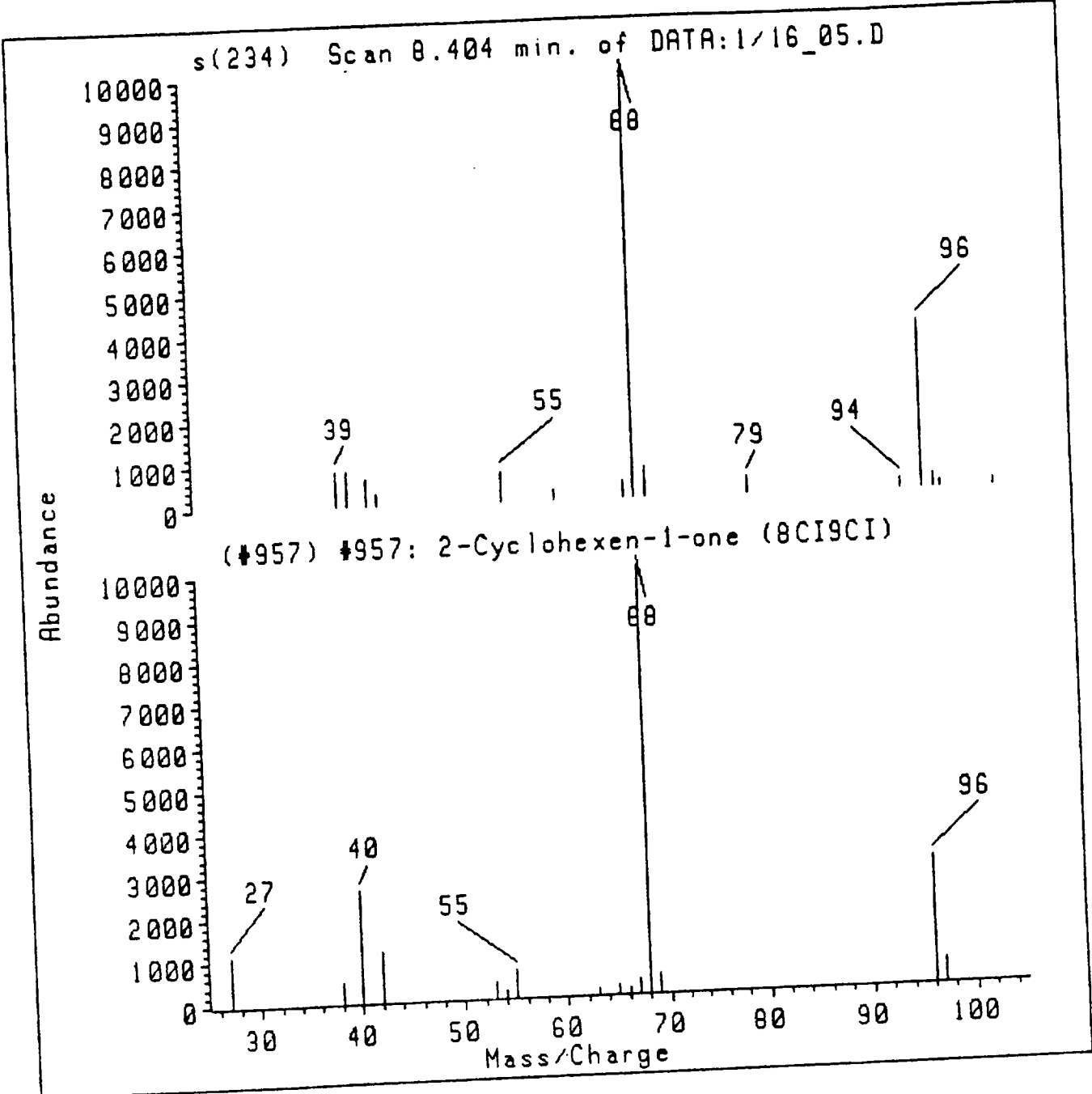
Sample Name: Hygiene Tank 2 MeCL EXTRACTED per EPA 625
Misc Info: 2.0 ul inj - 8 psig
Operator : B BENSON

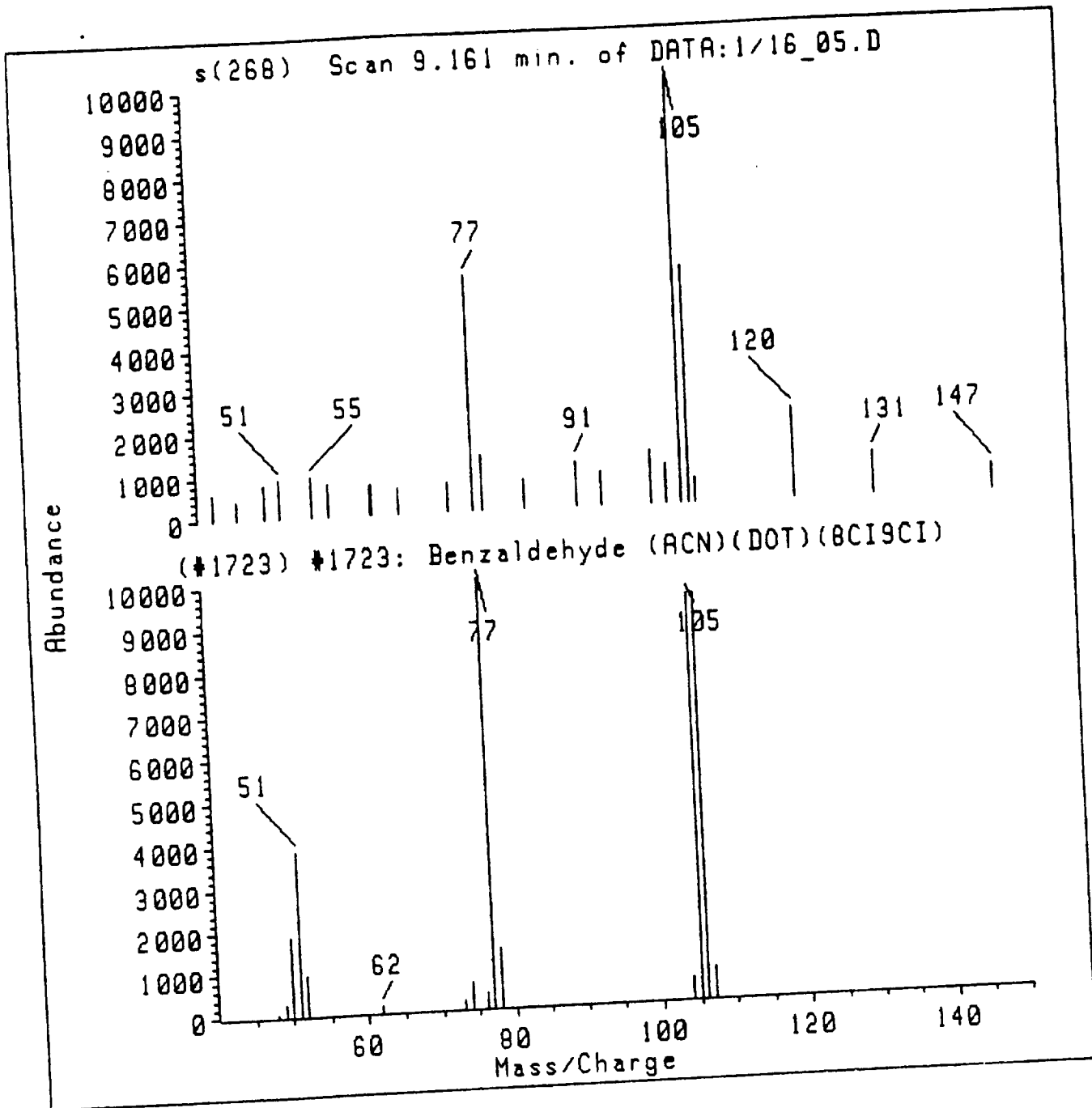
Date : 16 Jan 91 3:29 pm
Instrument: MS_5988
Inlet : GC

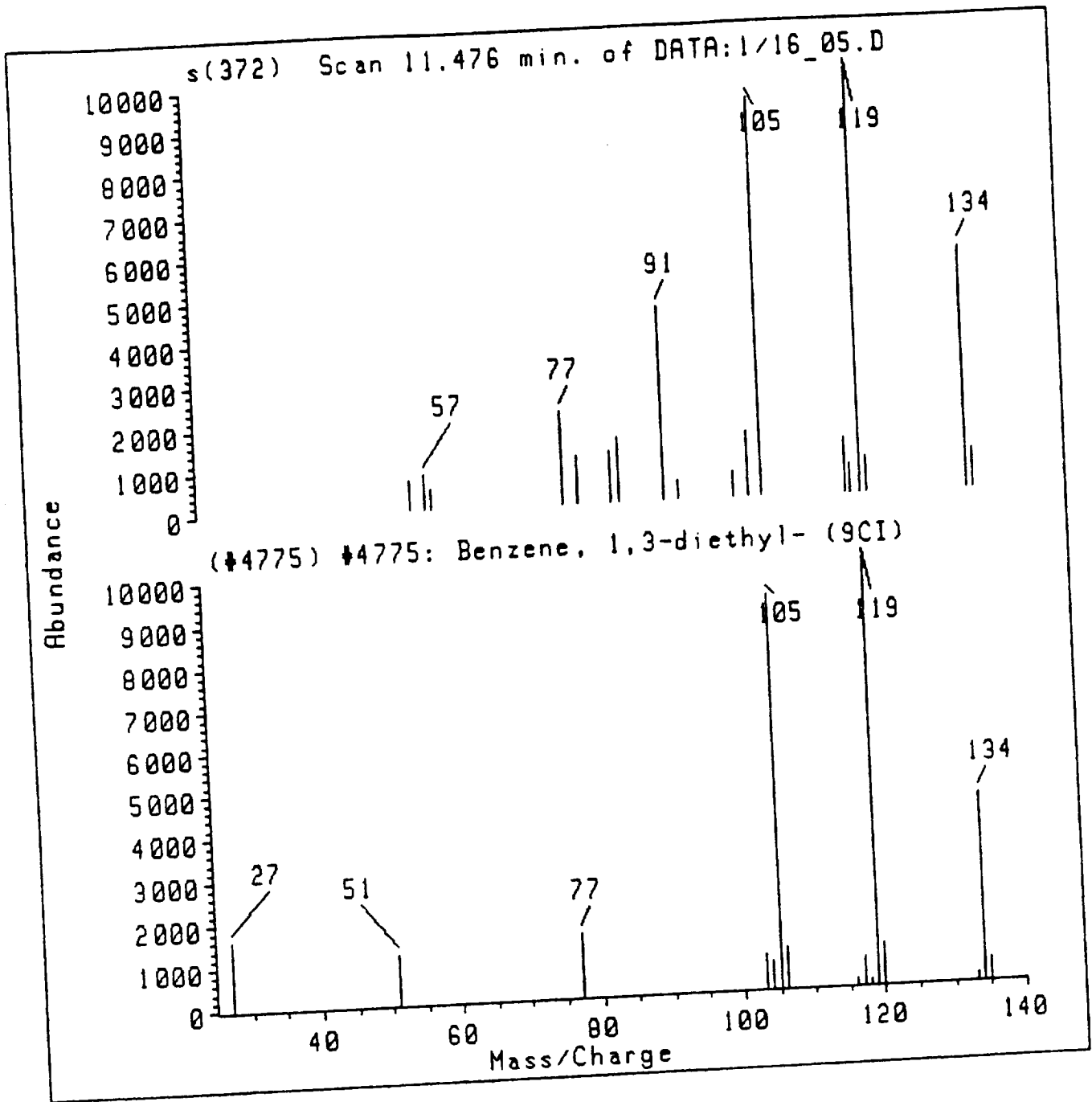
Sequence index : 0
Als bottle num : 0
Replicate num : 1

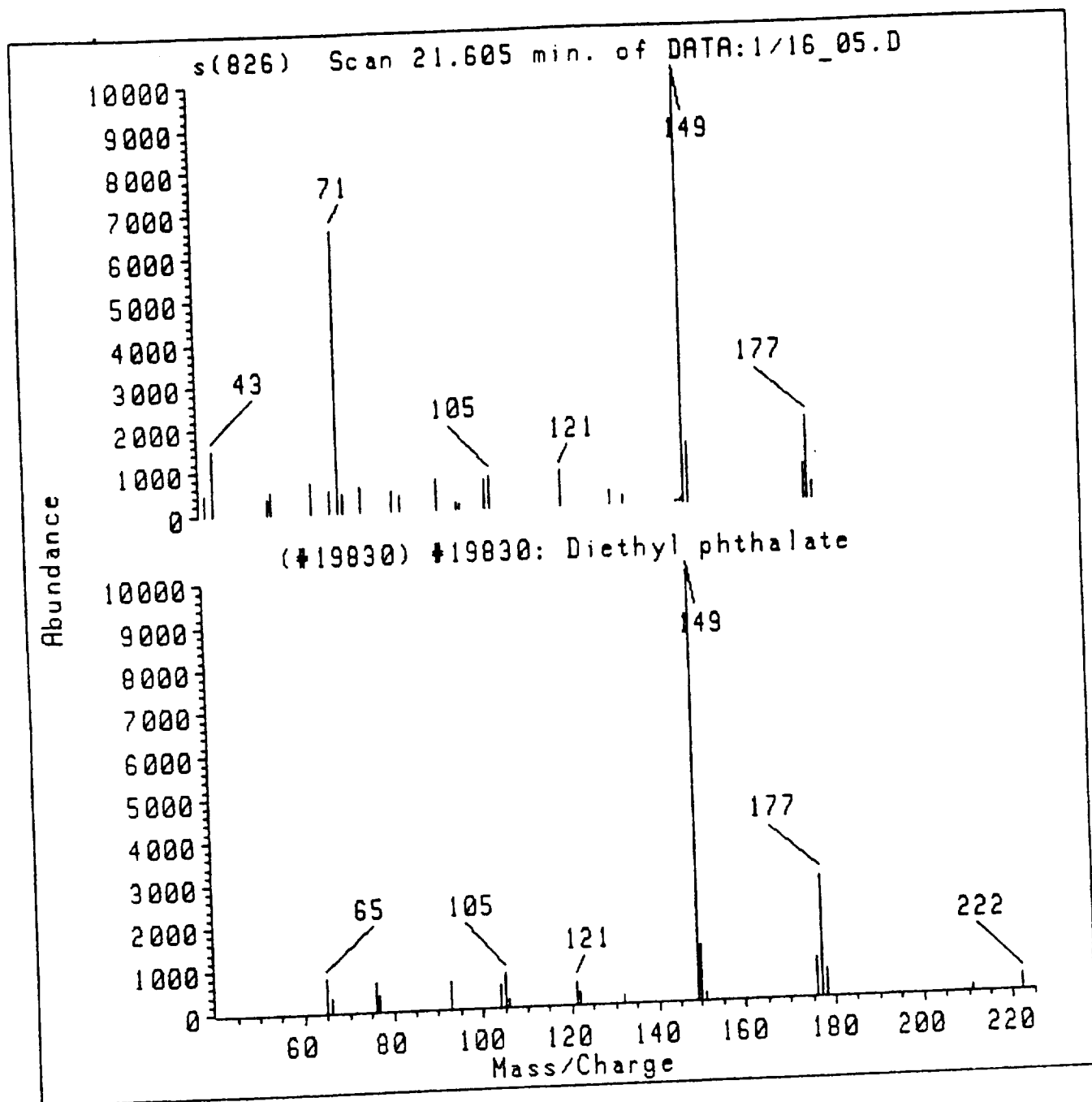


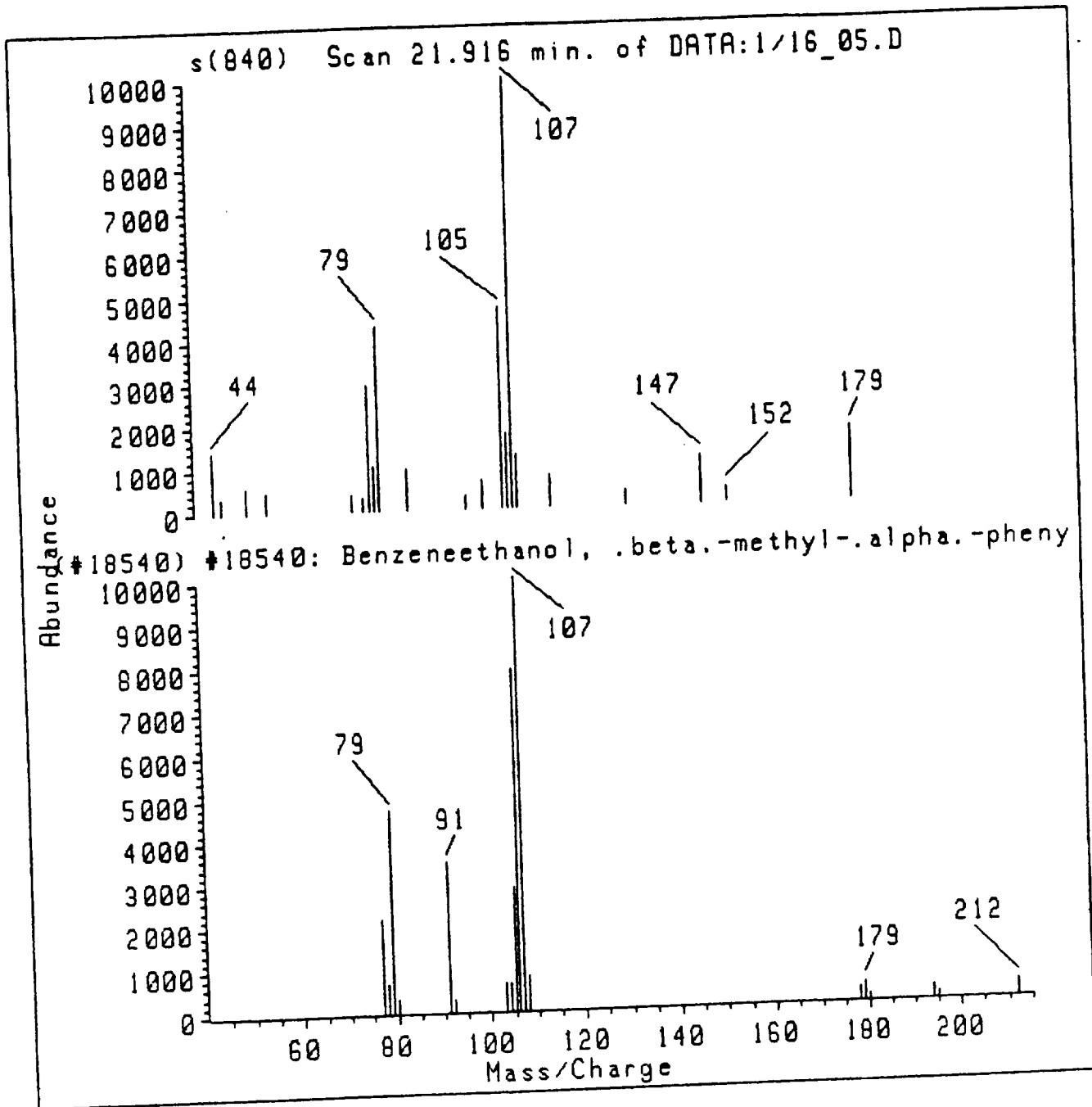


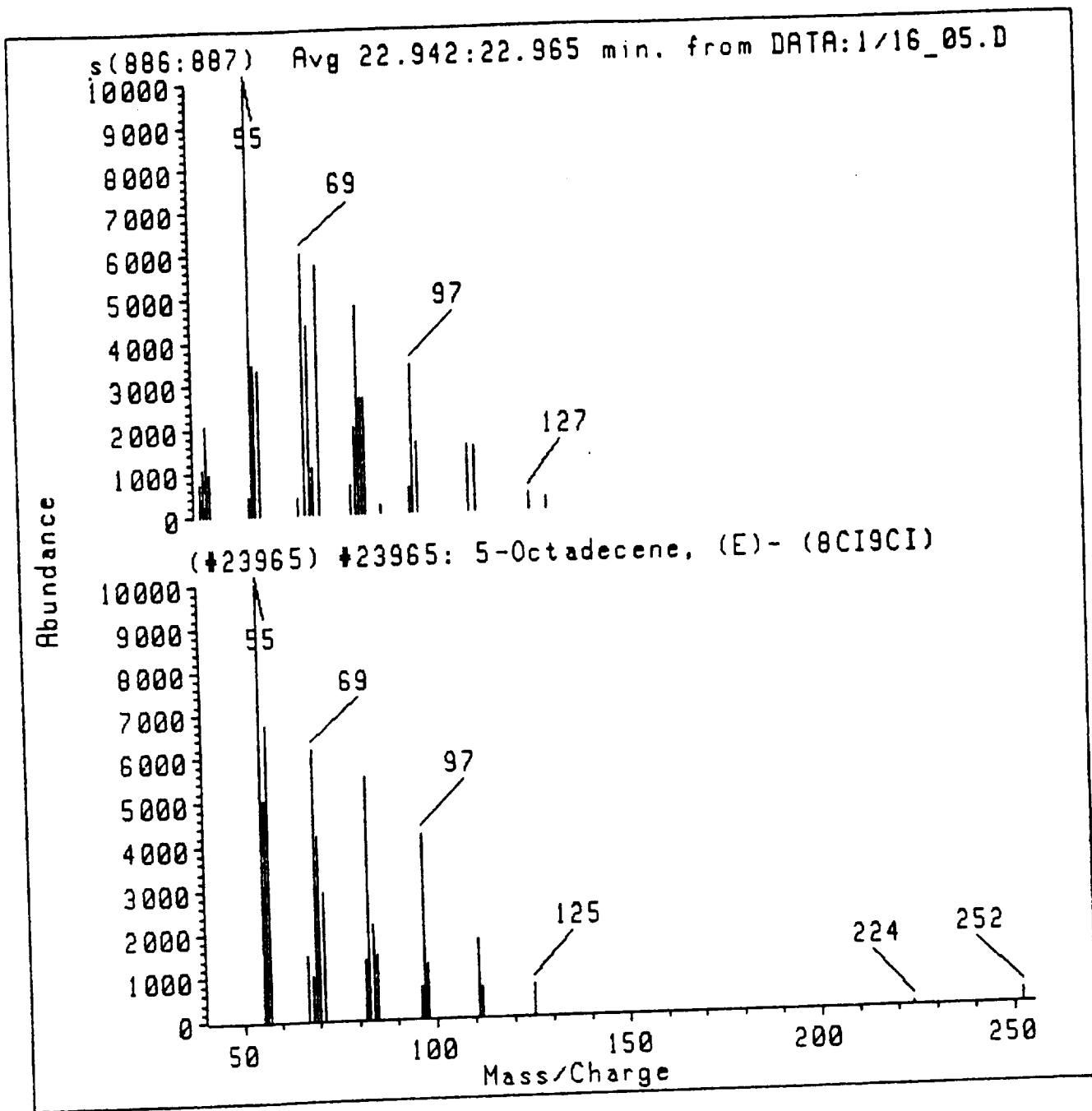


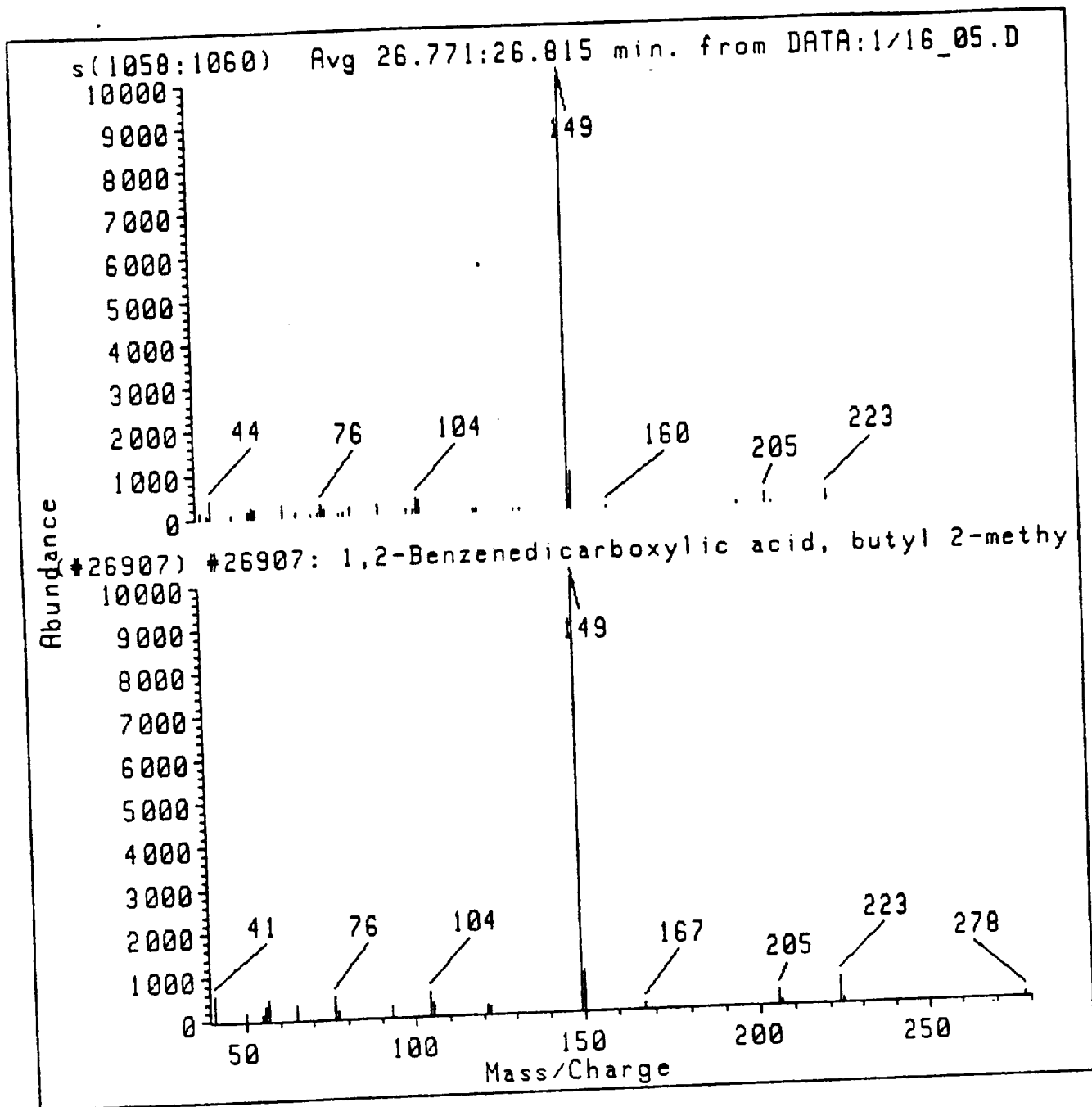




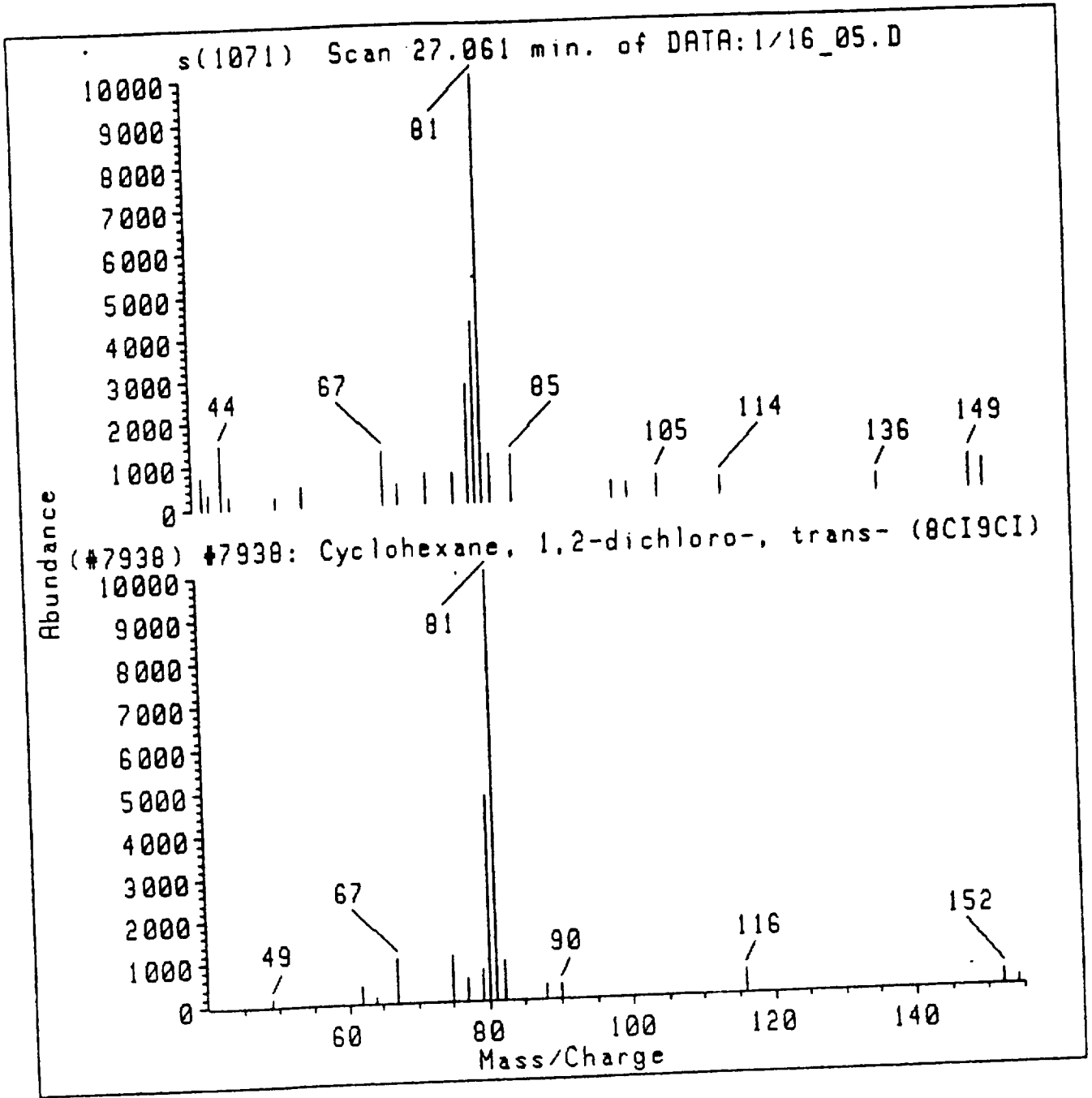


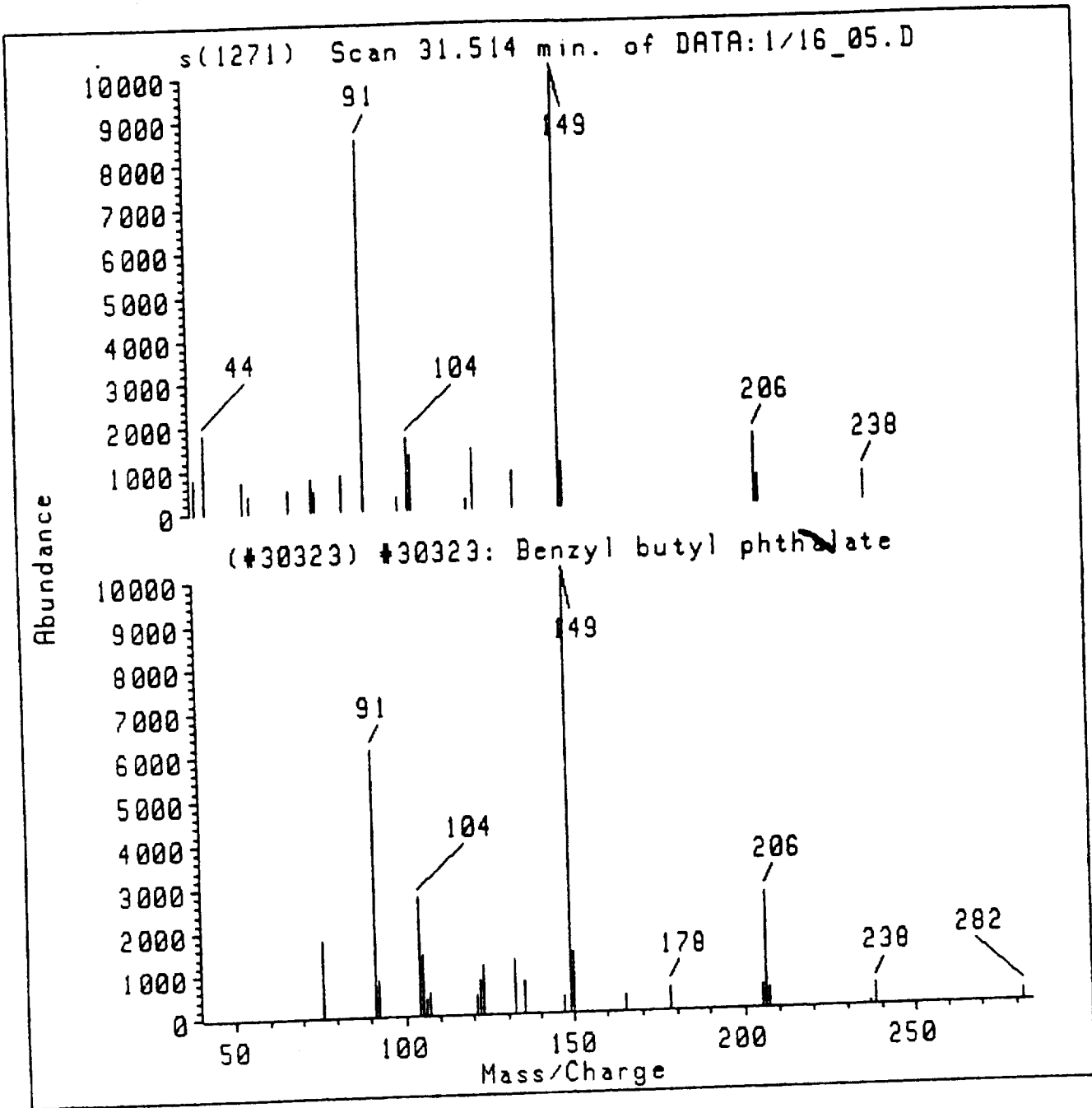


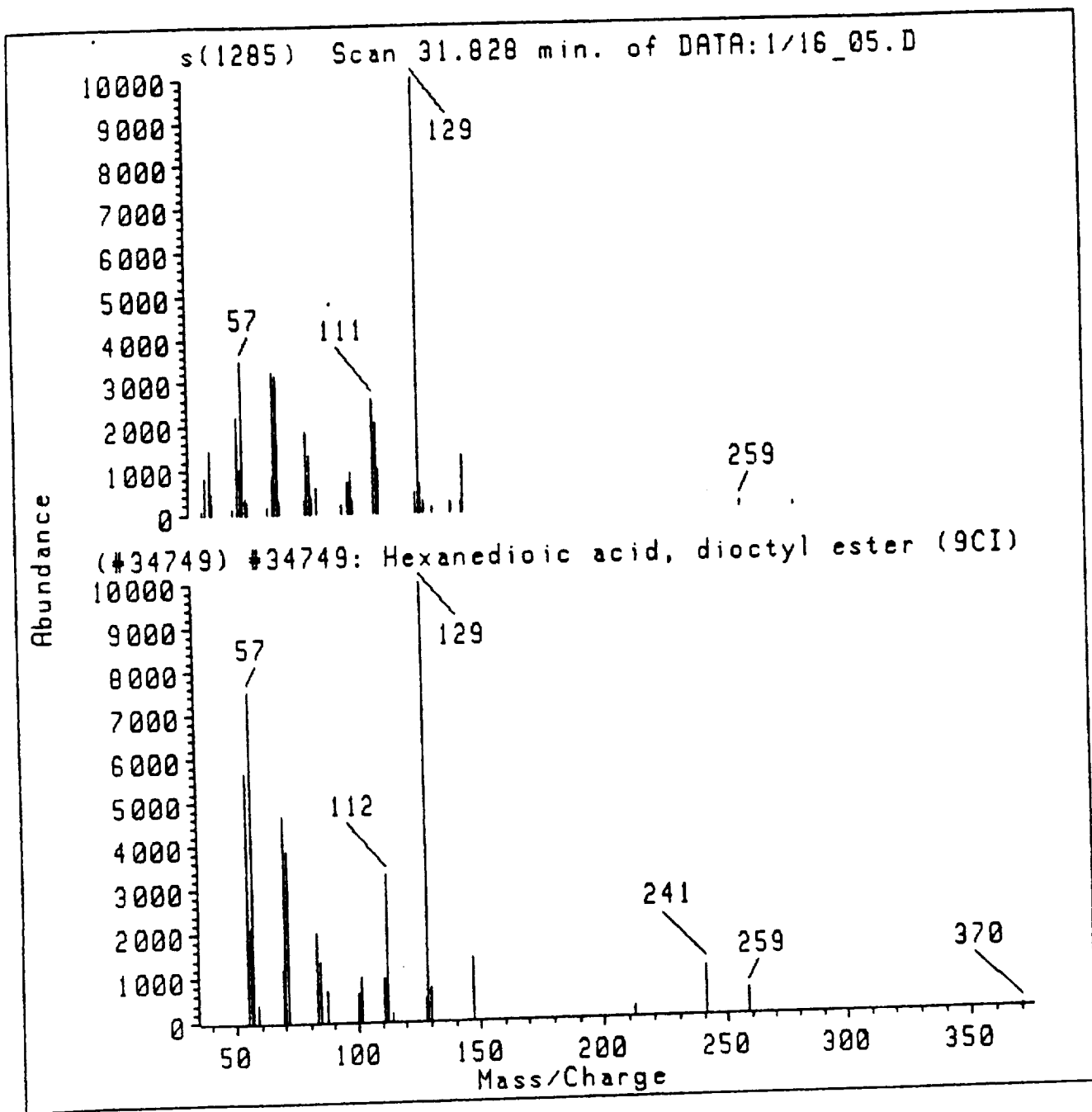




- read.







Appendix F

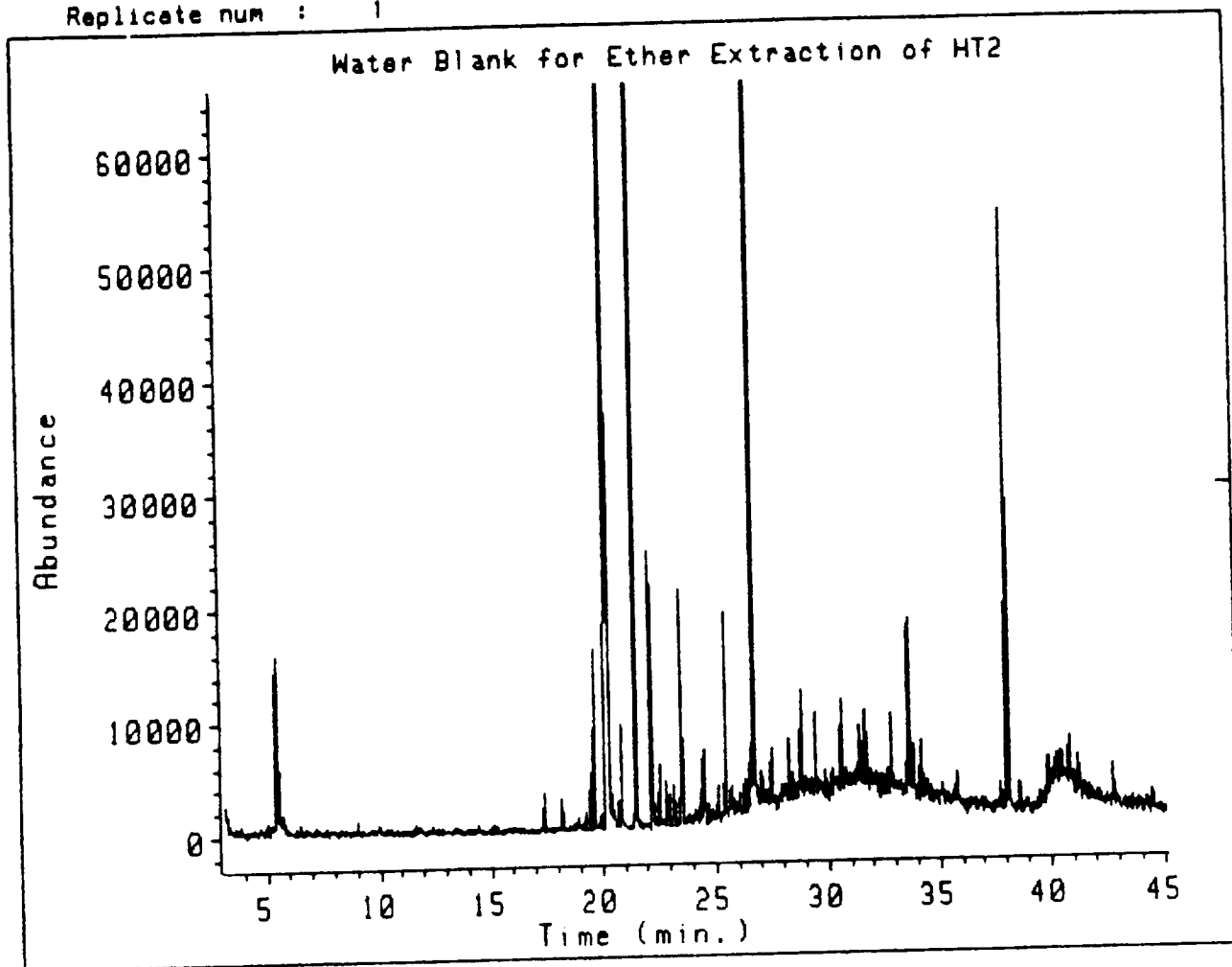
Diethyl Ether Extraction Results

Data file: DATA:10/11_01.D
File type: GC / MS DATA FILE

Sample Name: WATER BLANK for Ether Extraction of HT-2
Misc Info: 3.0 uL inj. - 11 psig SAMPLE 10/10_01
Operator : B BENSON

Instrument: MS_5988
Inlet : GC

Sequence index : 0
Als bottle num : 0
Replicate num : 1

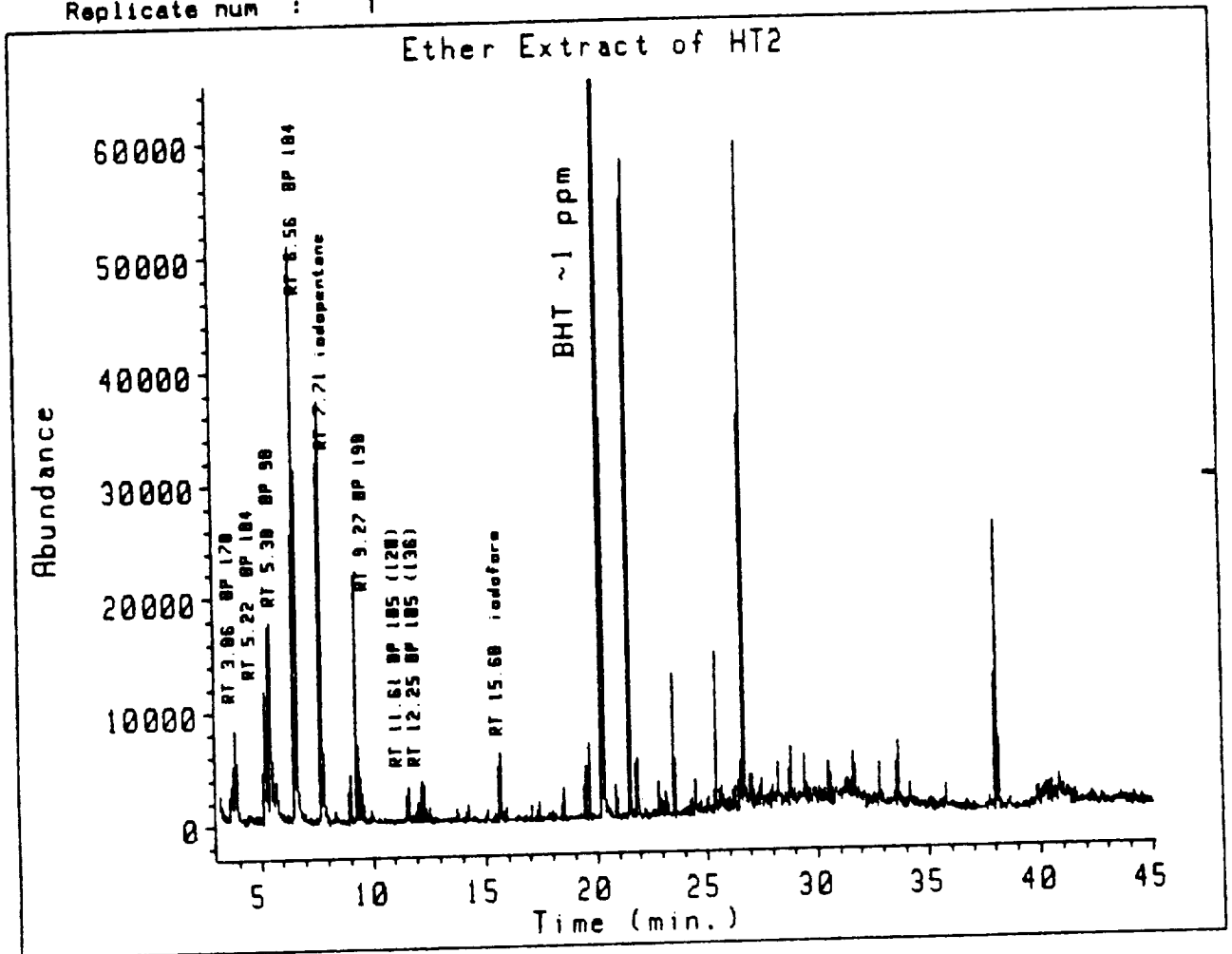


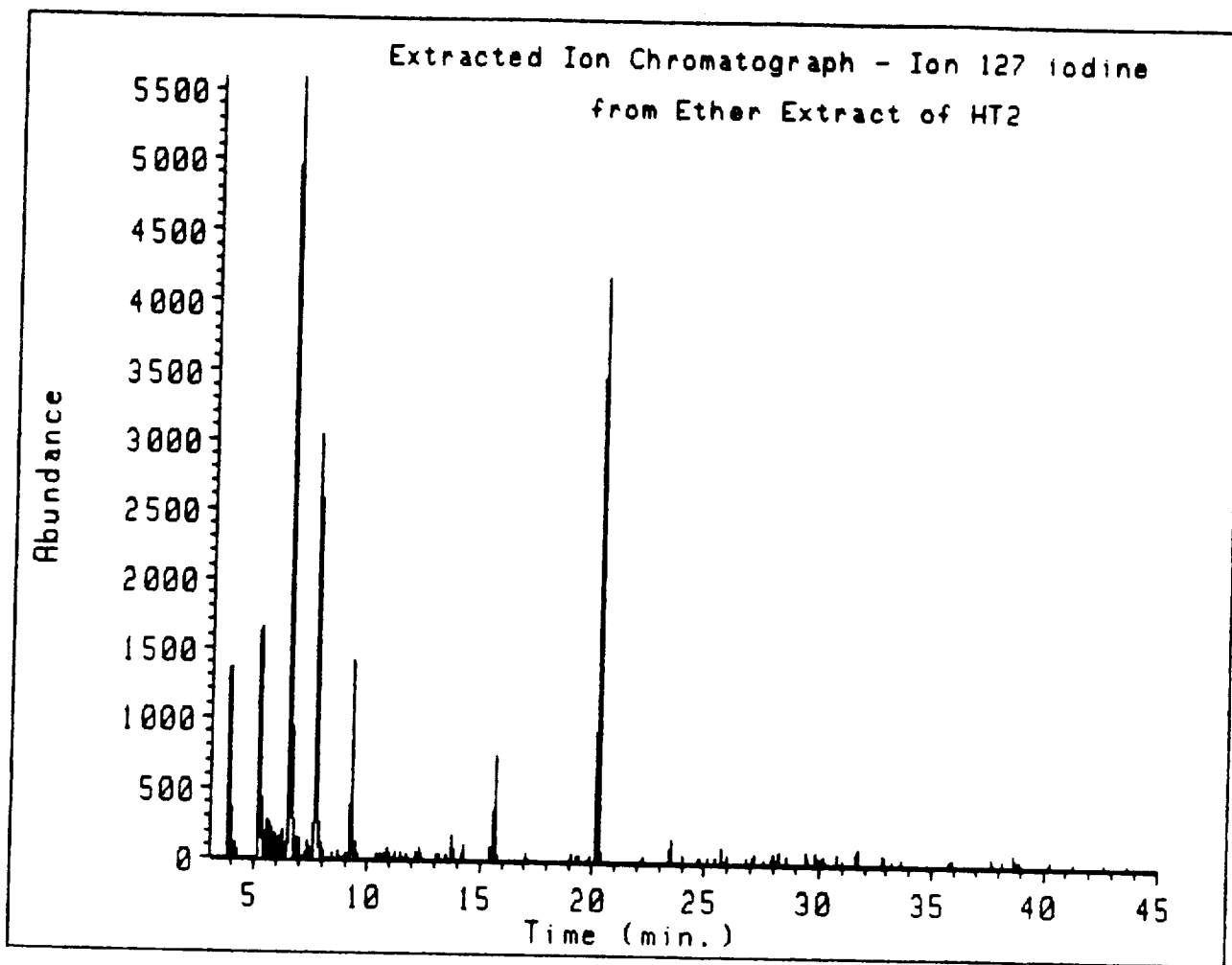
Data file: DATA:10/11_02.D
File type: GC / MS DATA FILE

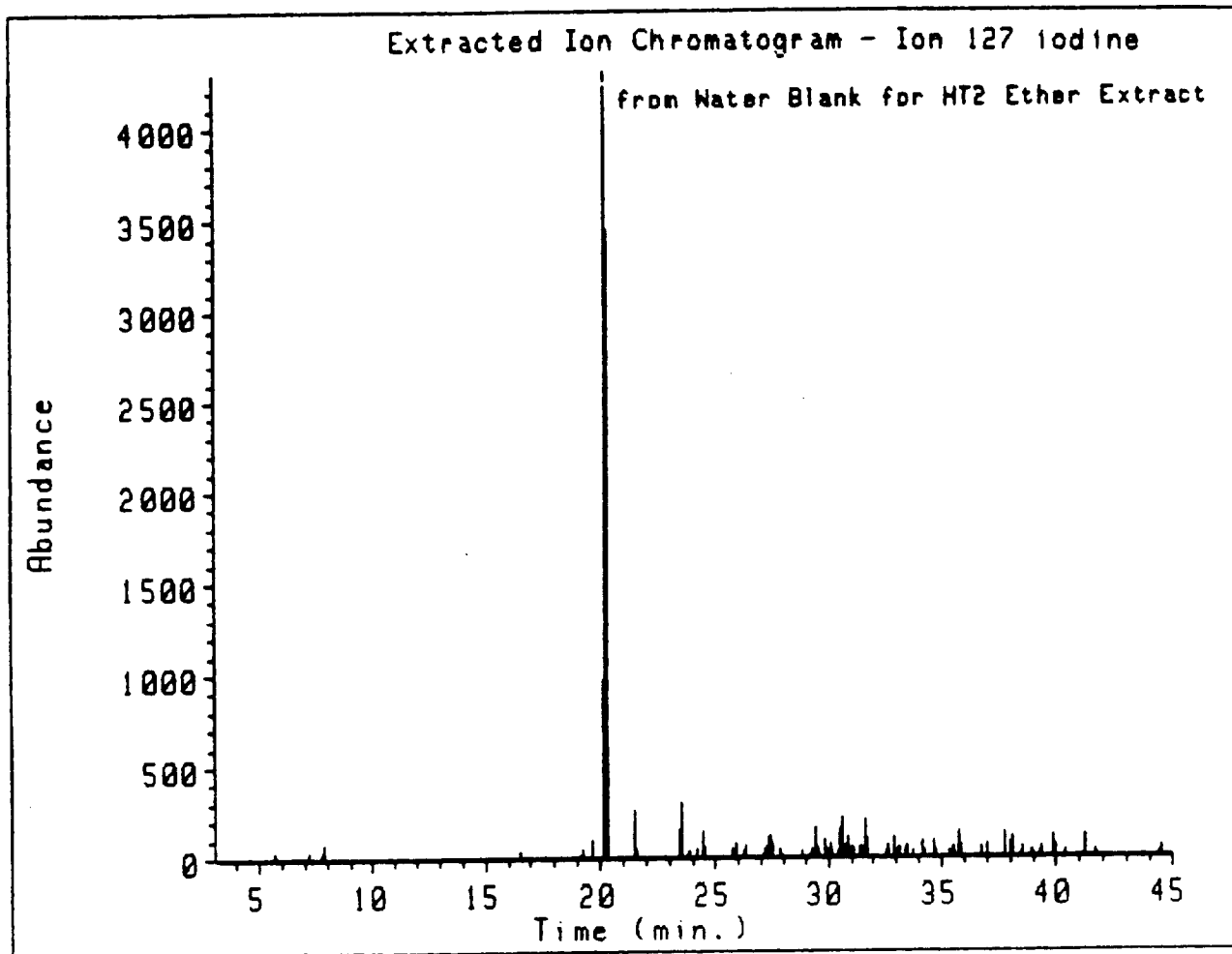
Sample Name: HYGIENE WATER - Ether Extraction of HT-2 - WRT 3A
Misc Info: 3.0 uL inj. - 11 psig SAMPLE 10/10_02
Operator : B BENSON

Instrument: MS_5988
Inlet : GC

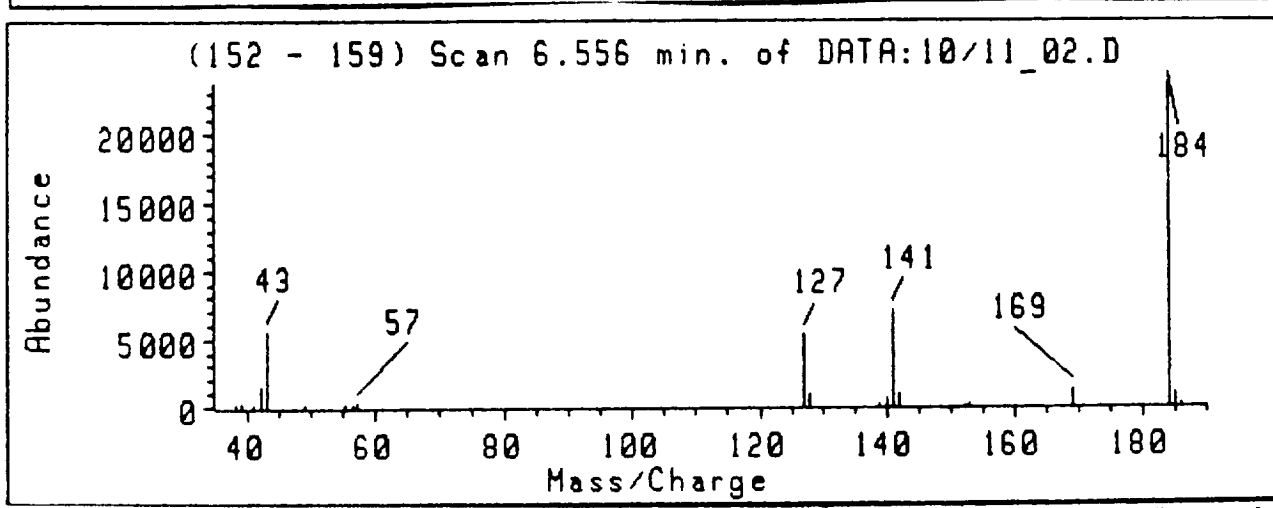
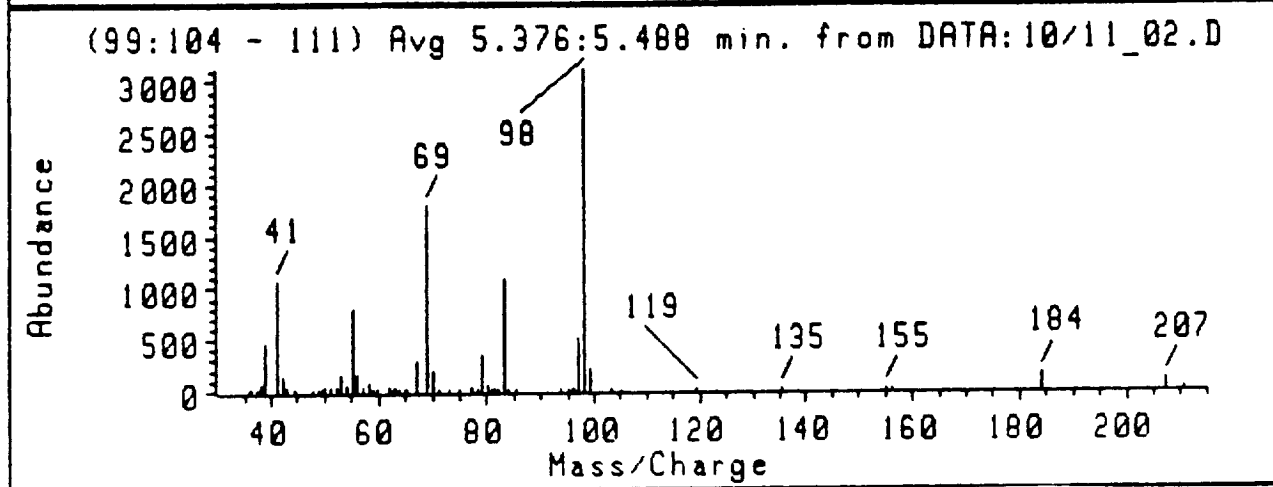
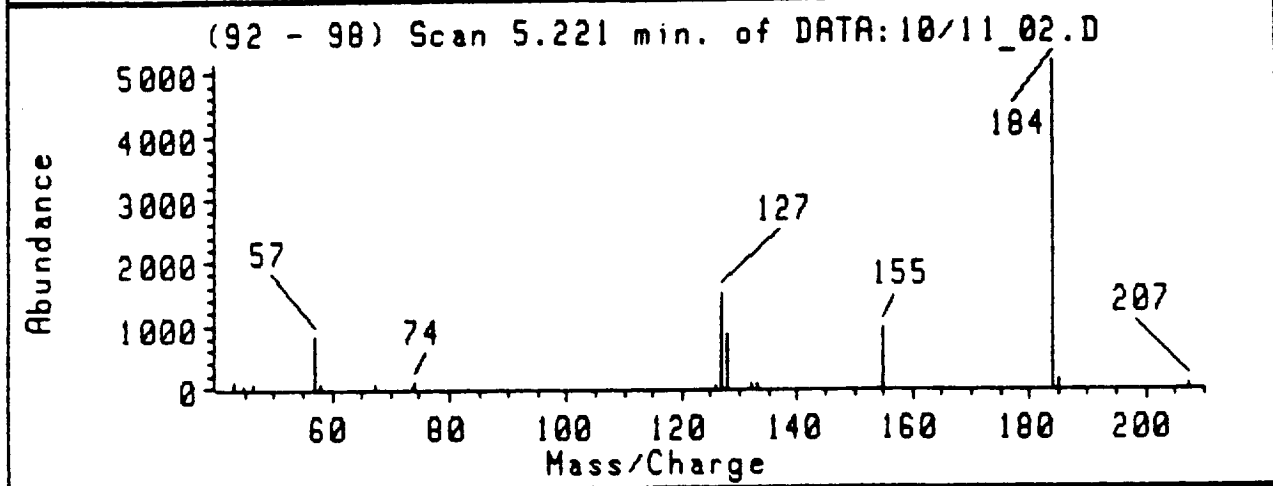
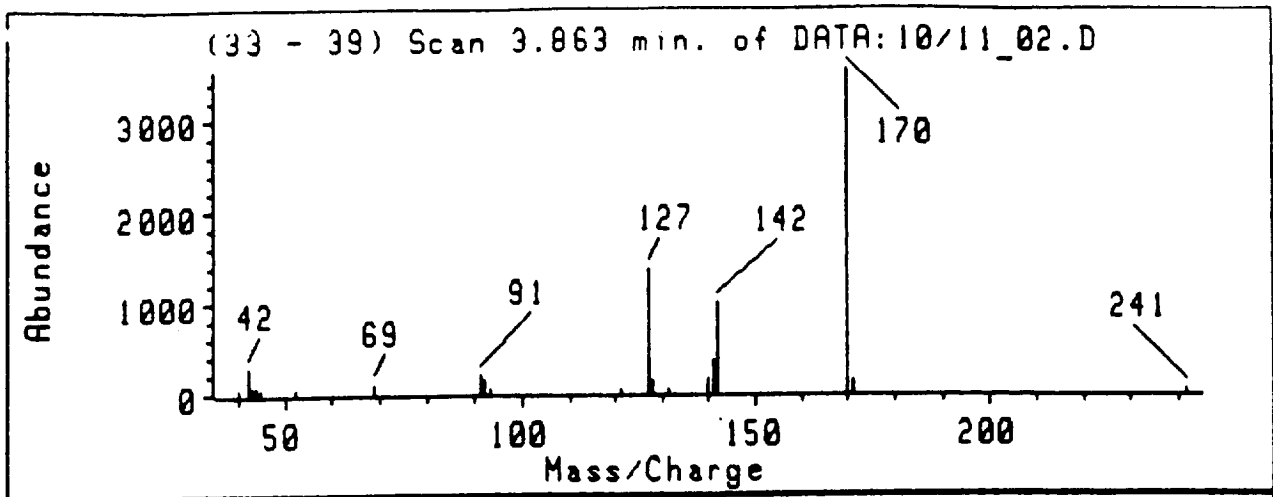
Sequence index : 0
Als bottle num : 0
Replicate num : 1

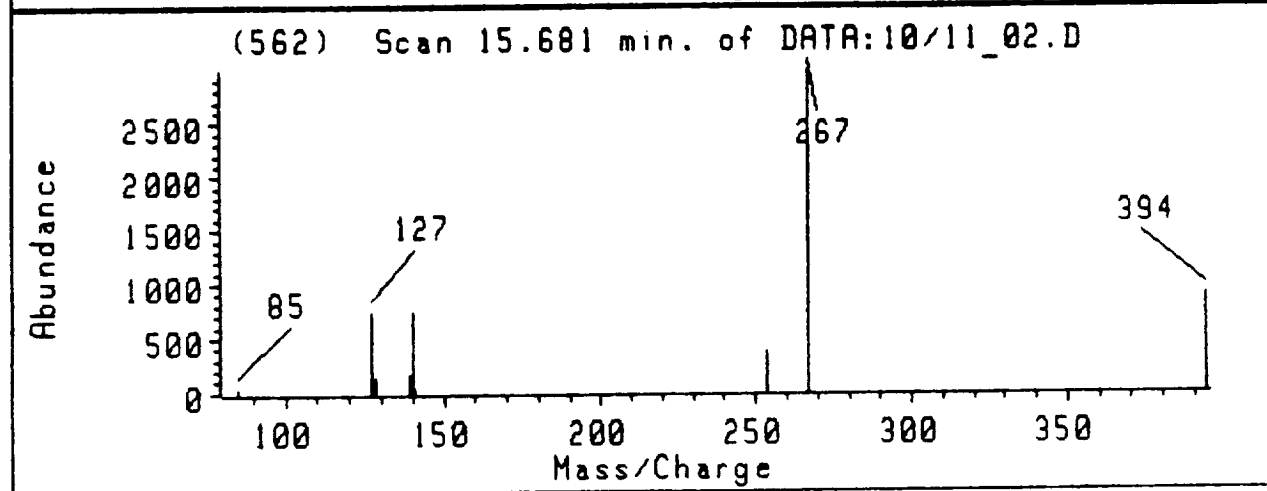
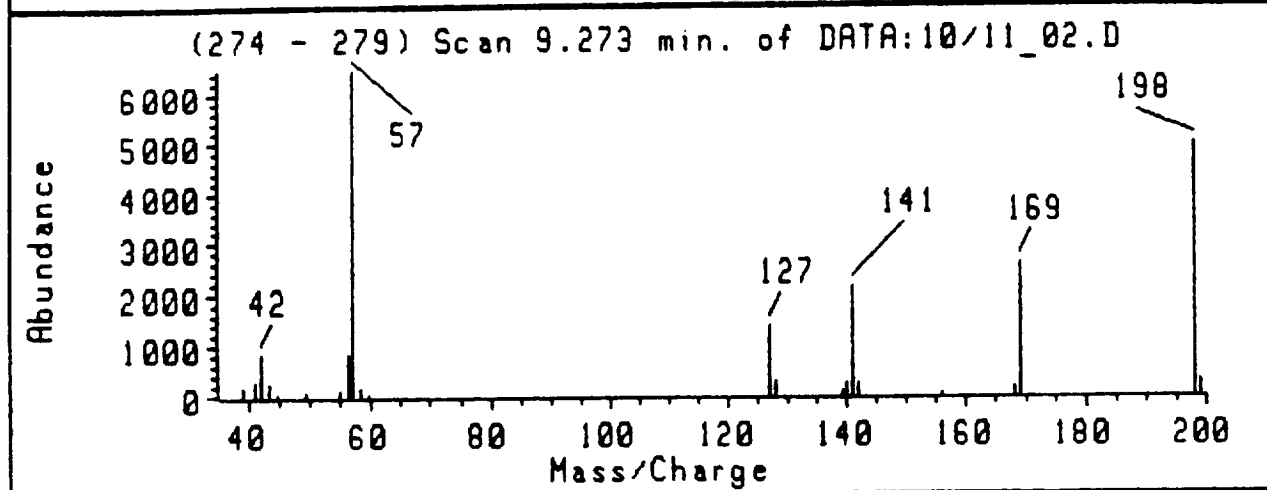
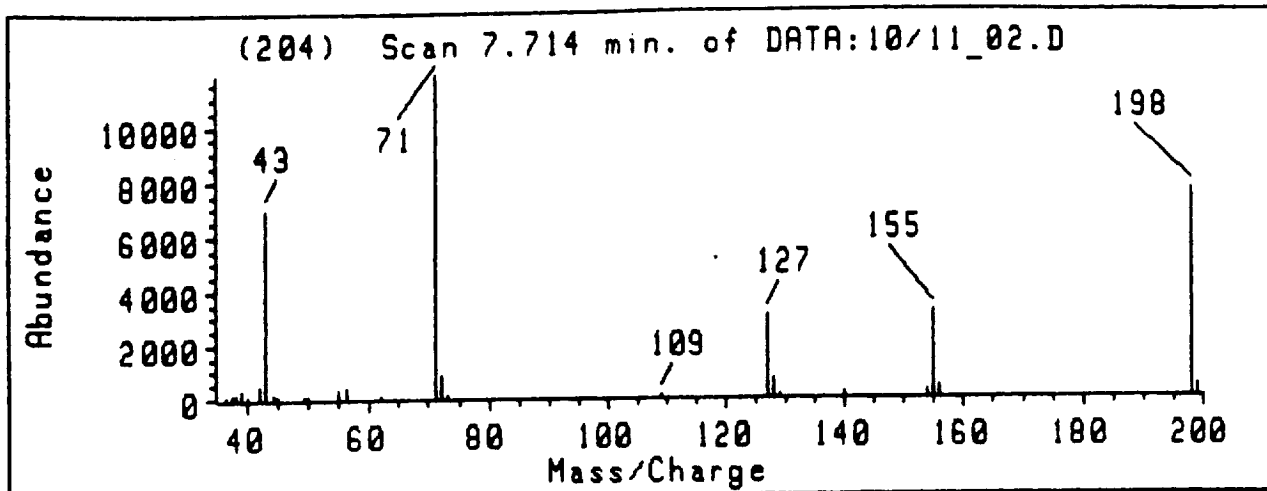


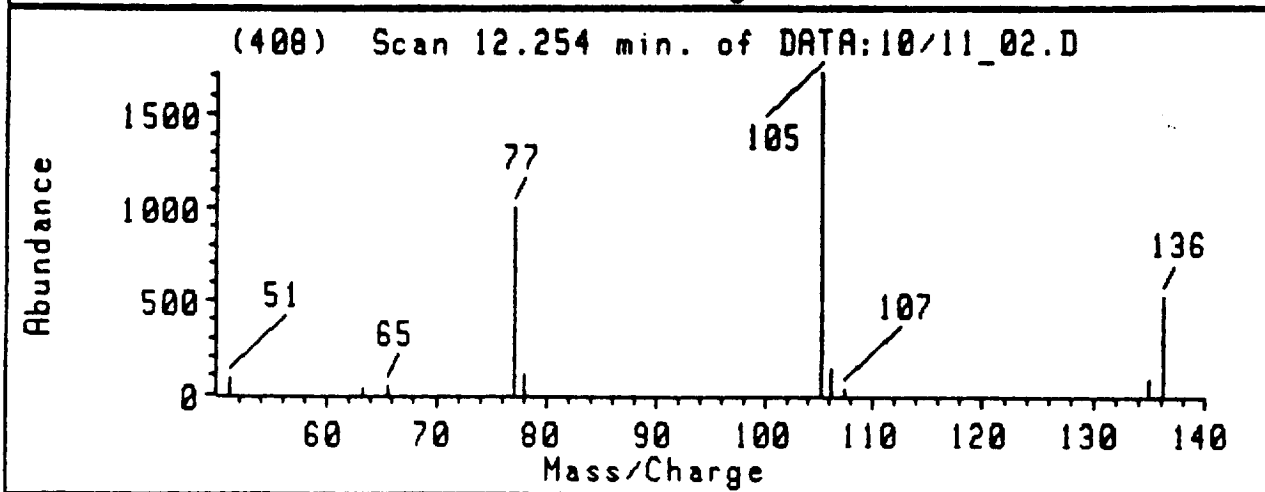
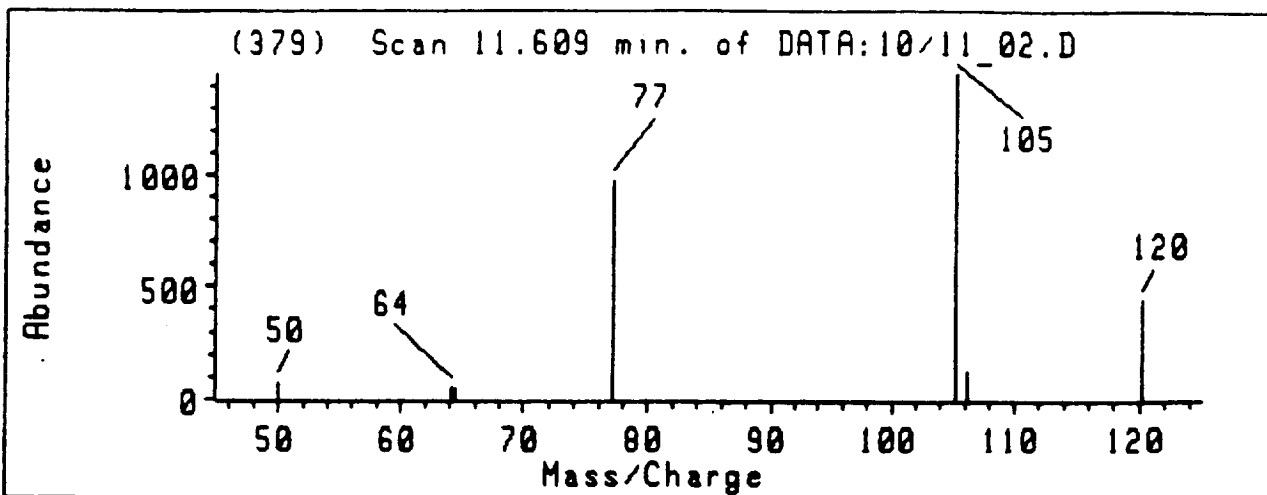




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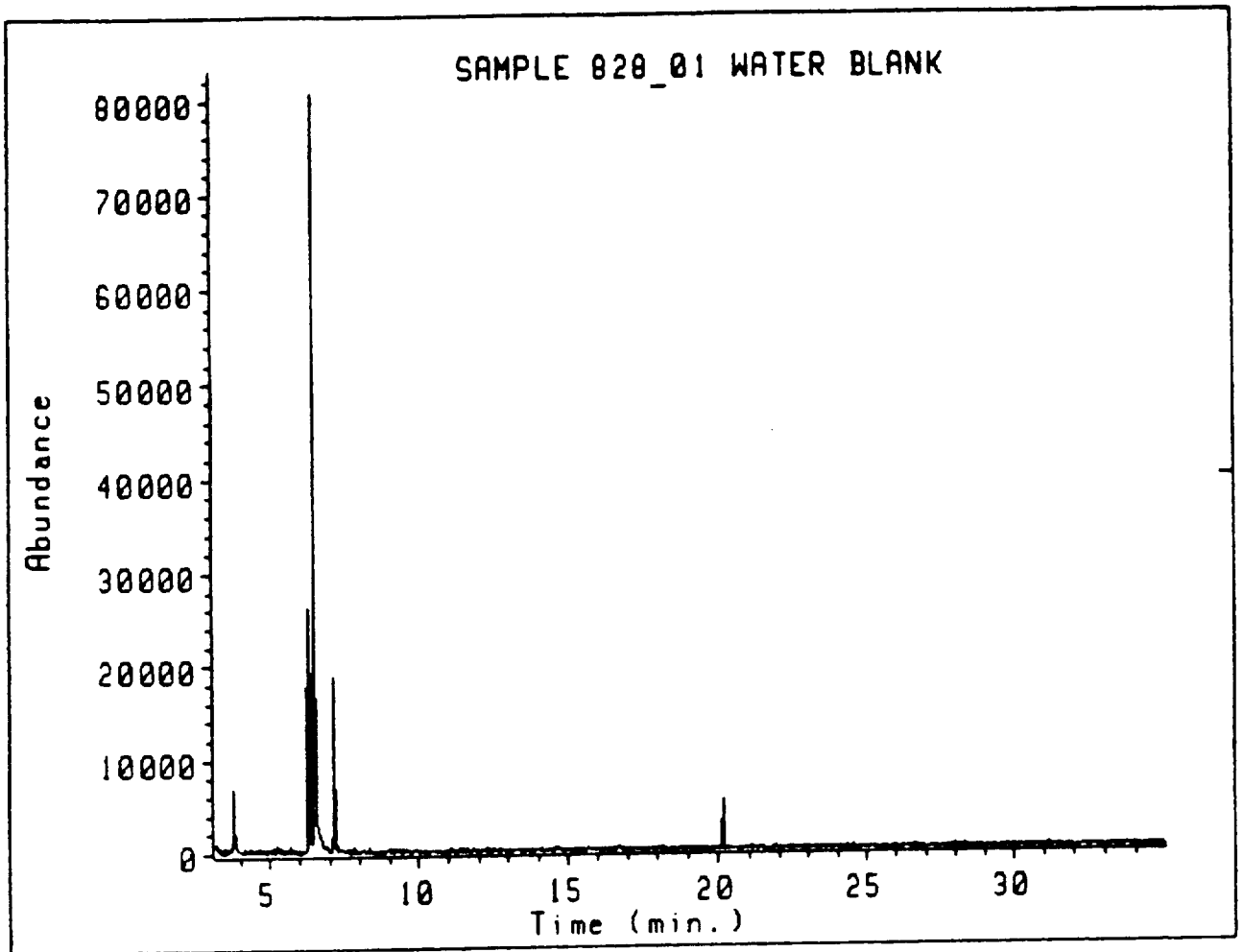


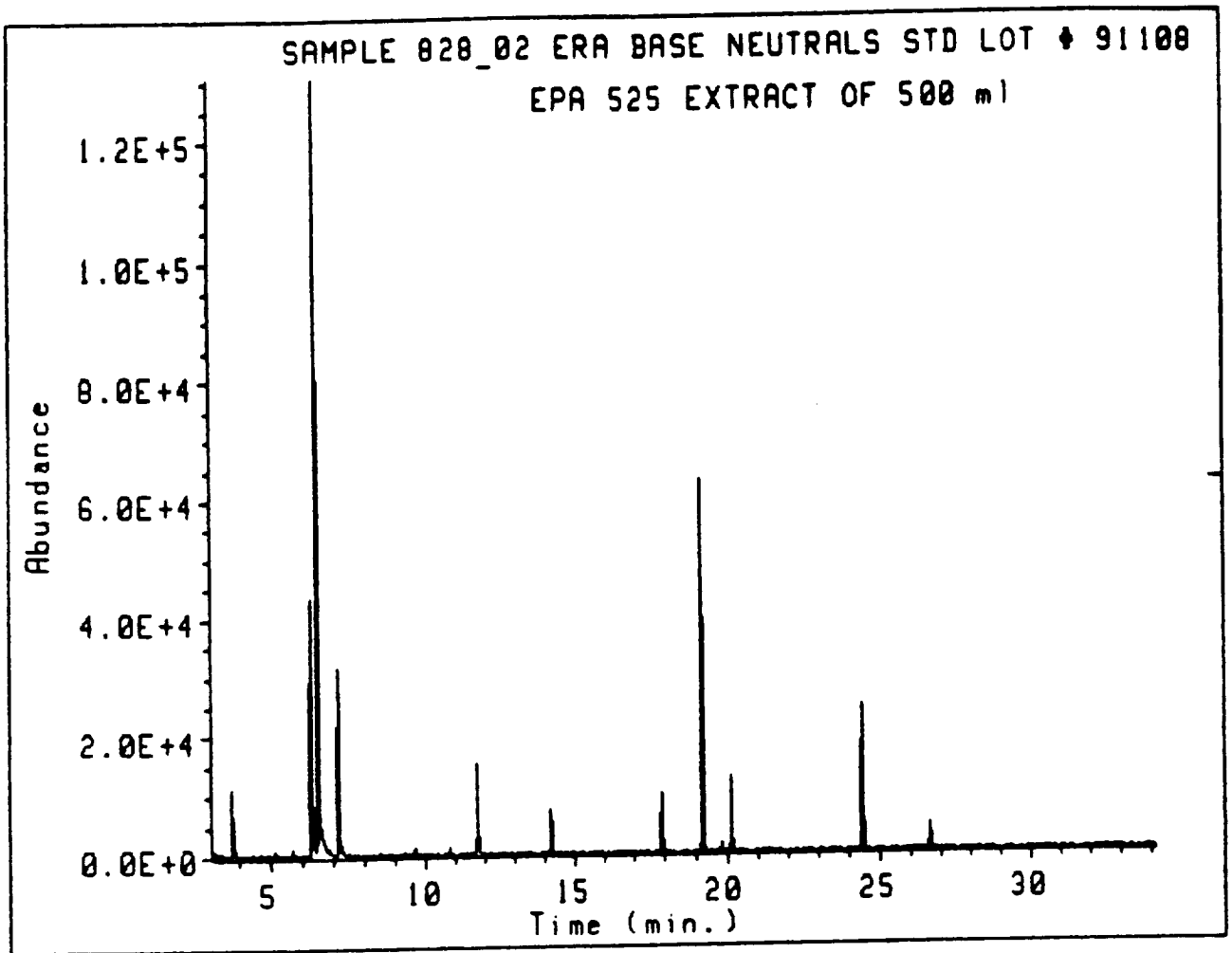


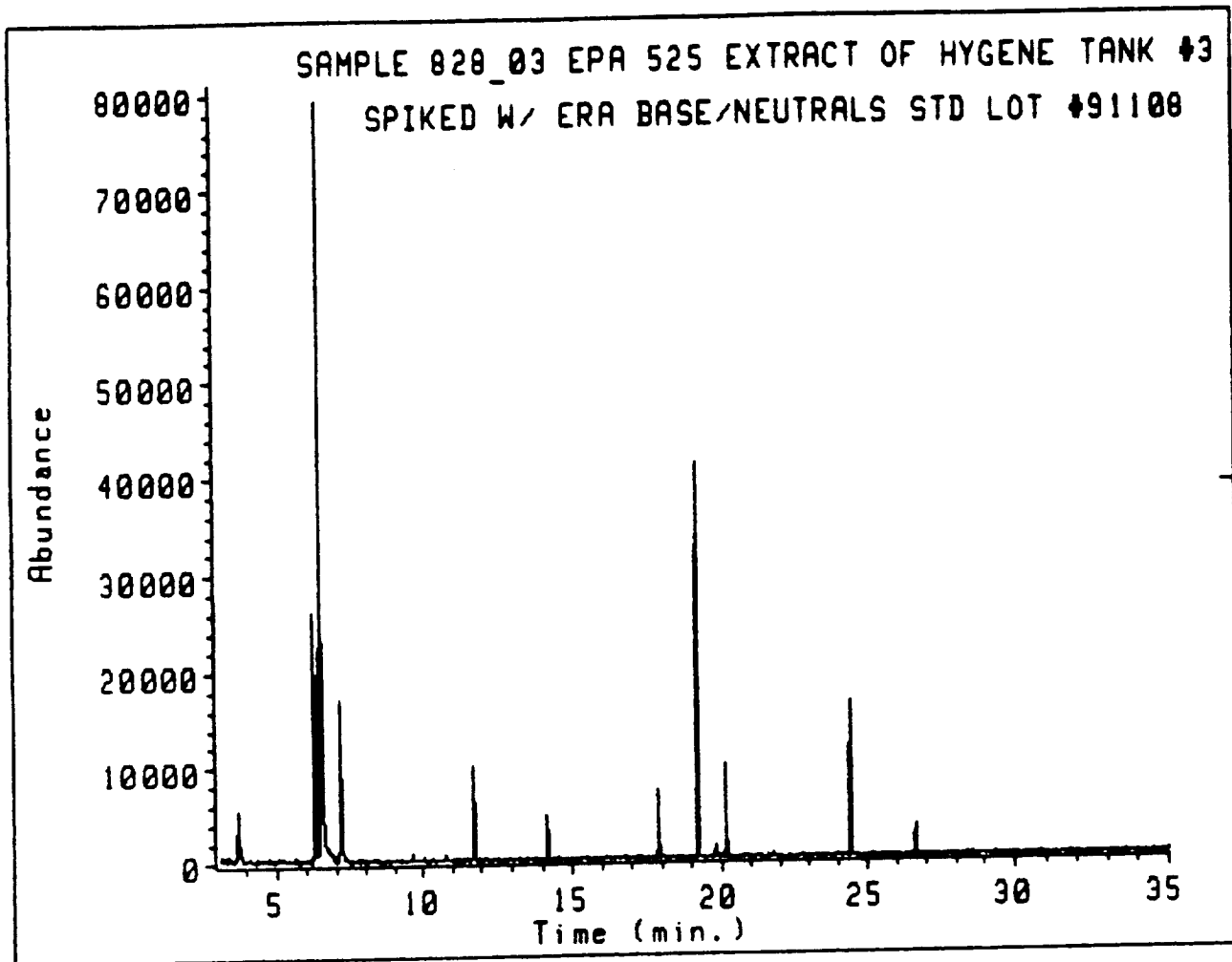


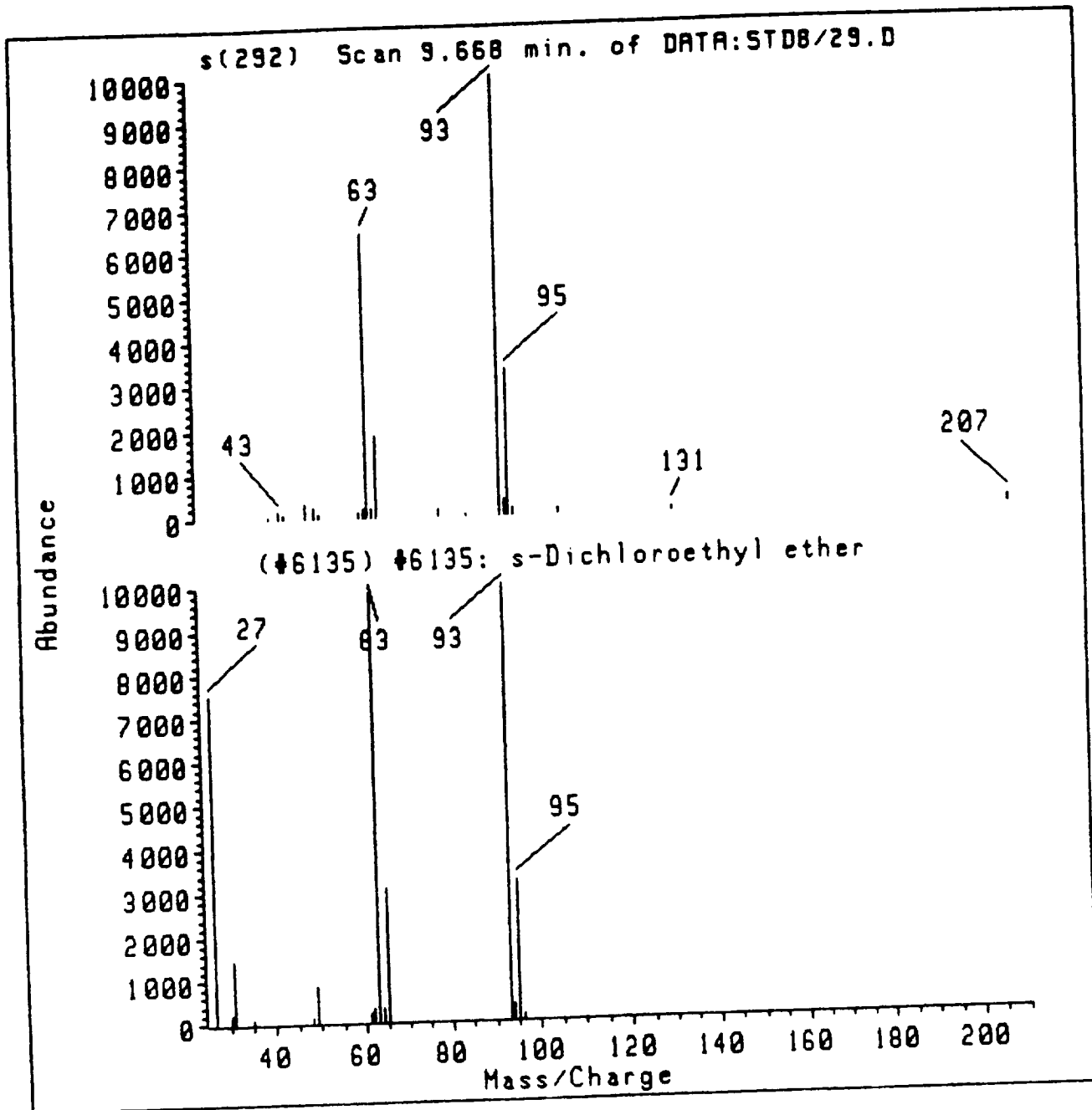
Appendix G

Solid Phase Extraction Results

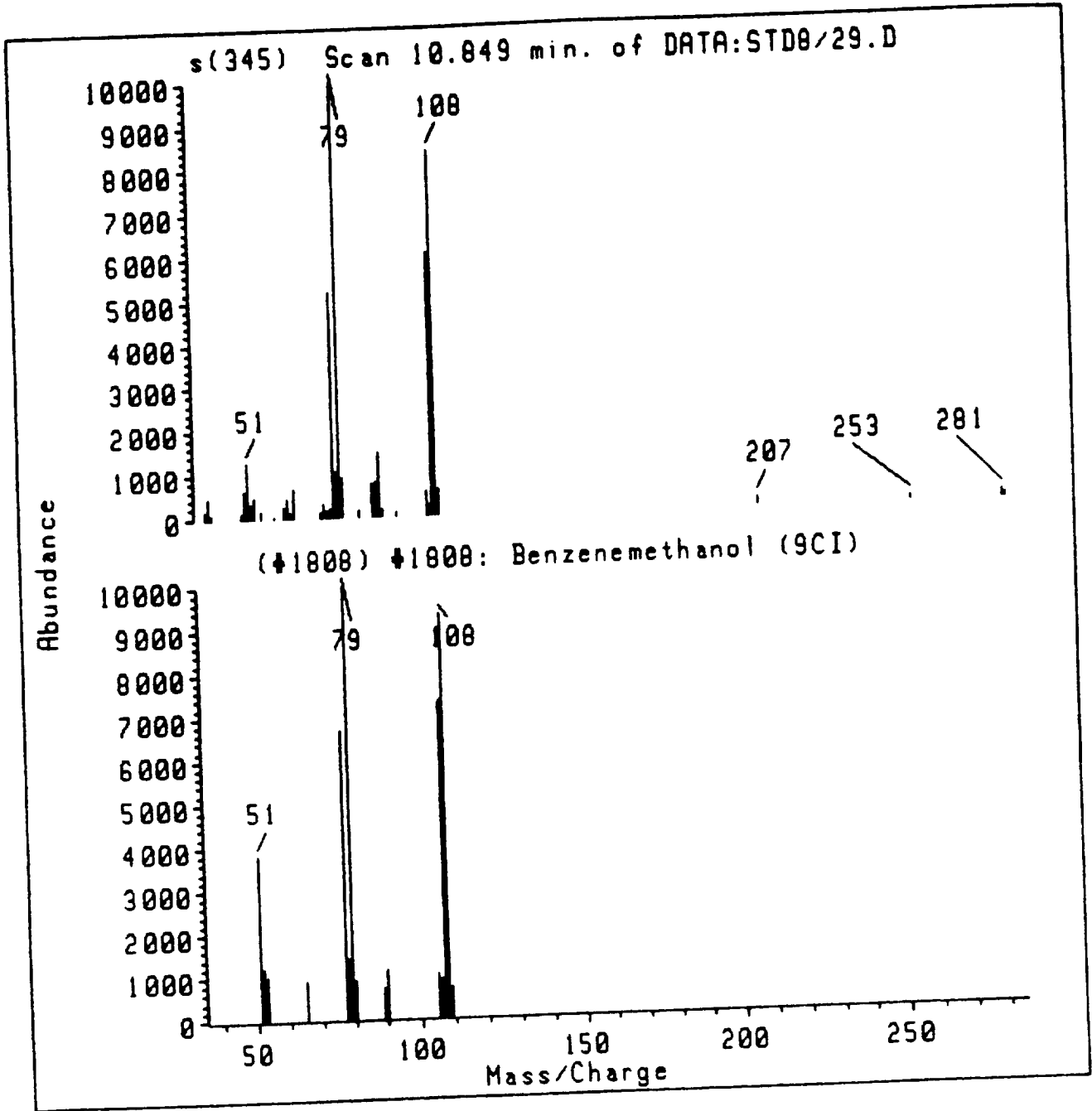


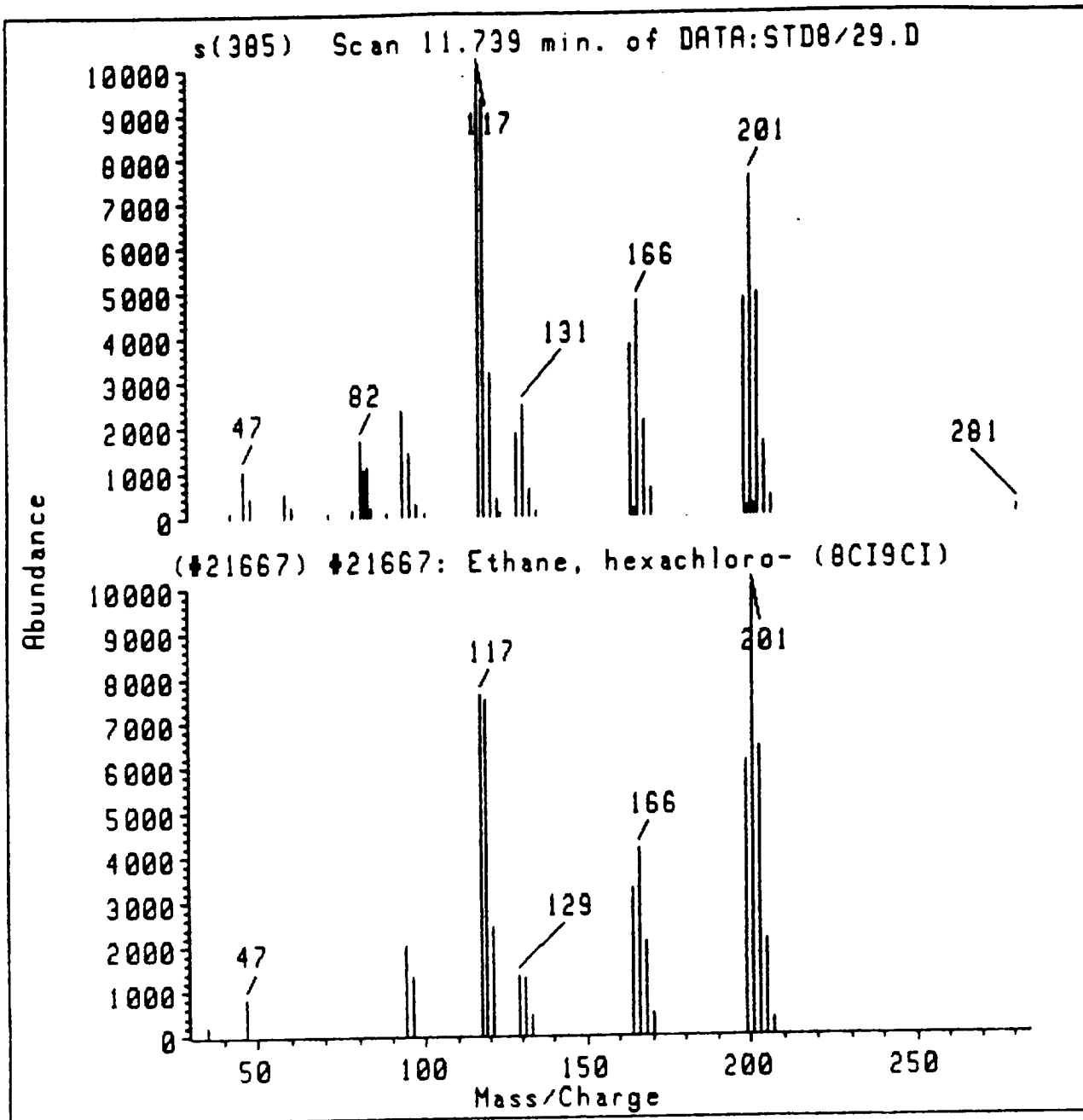


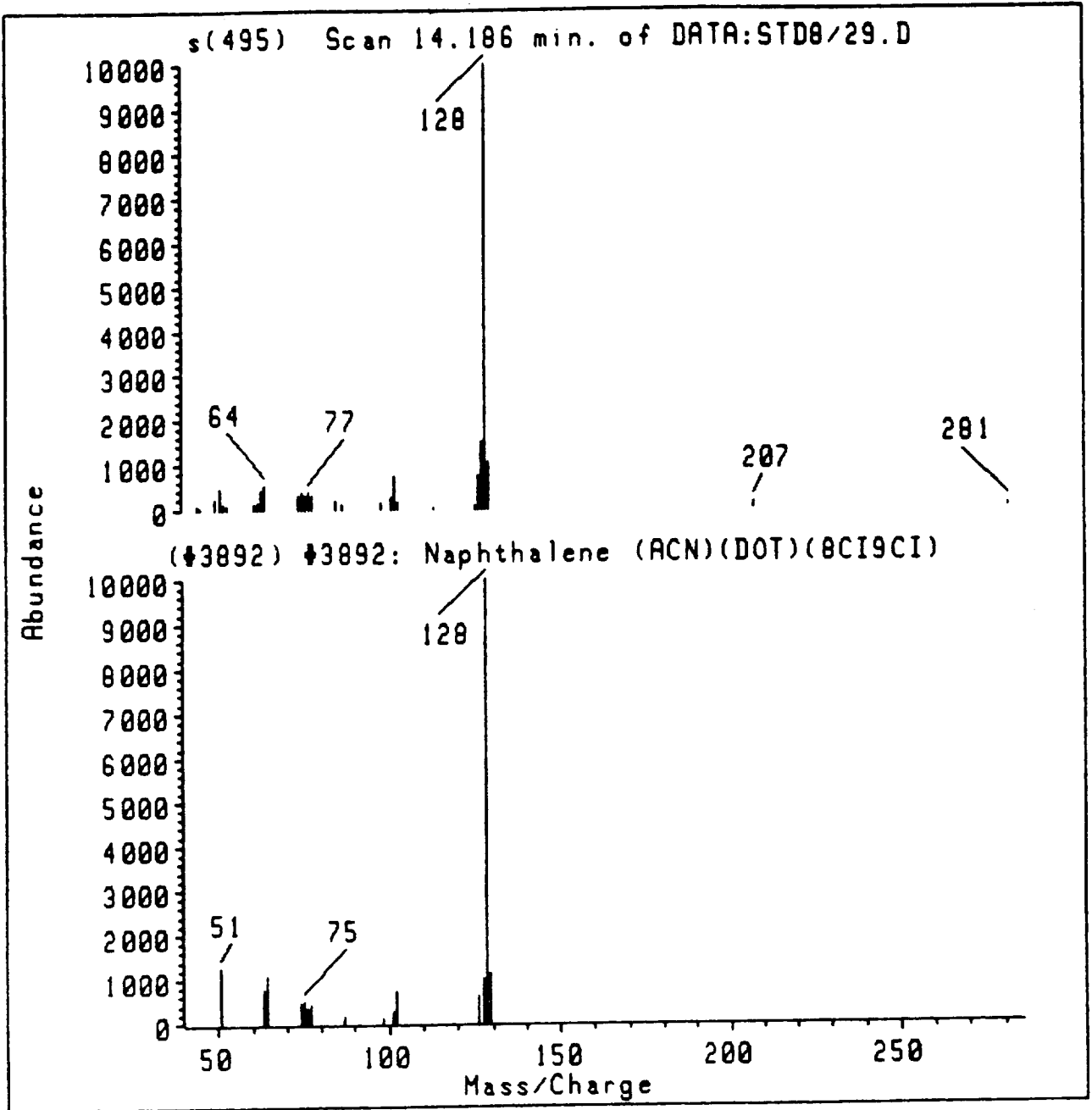


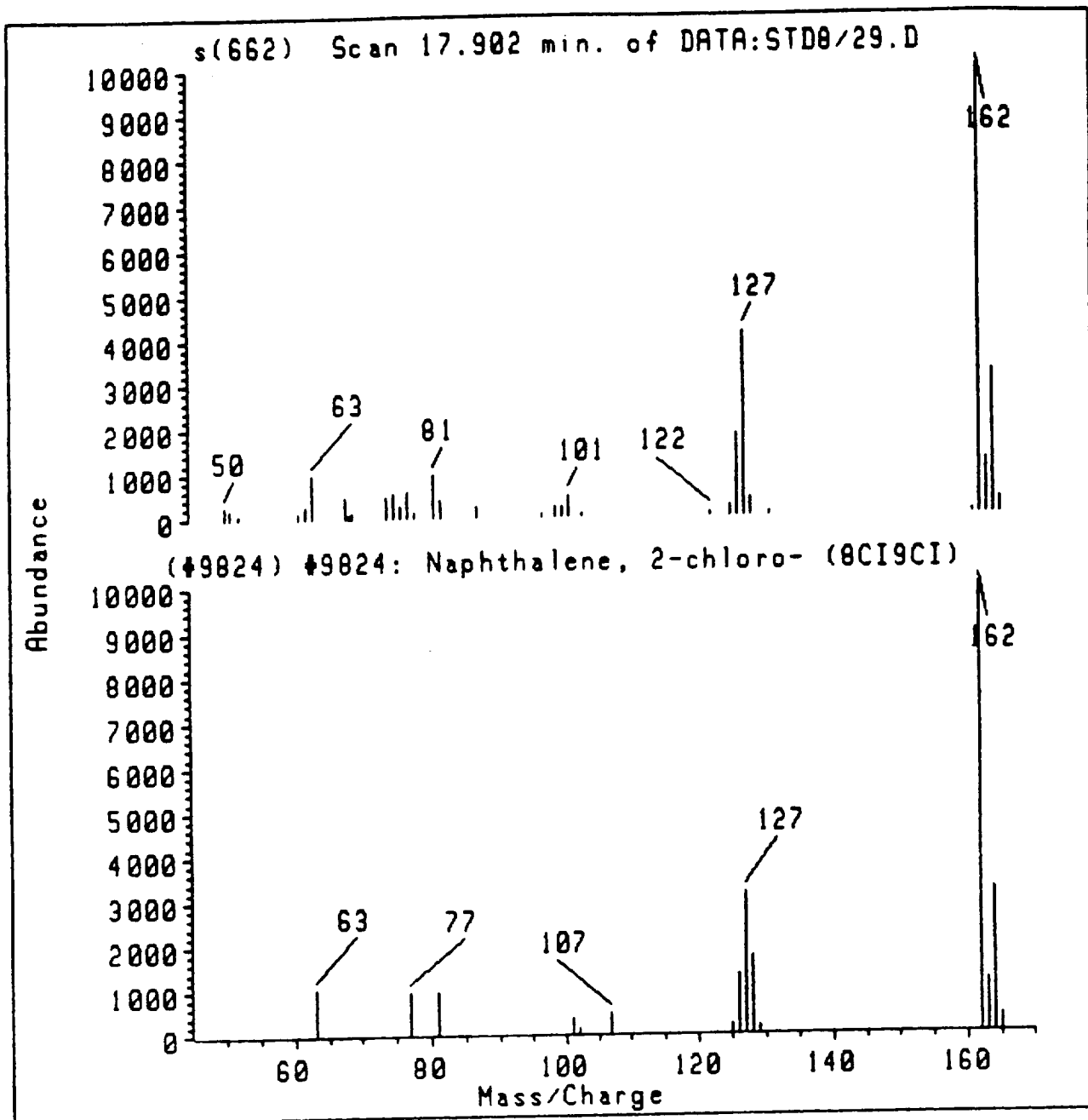


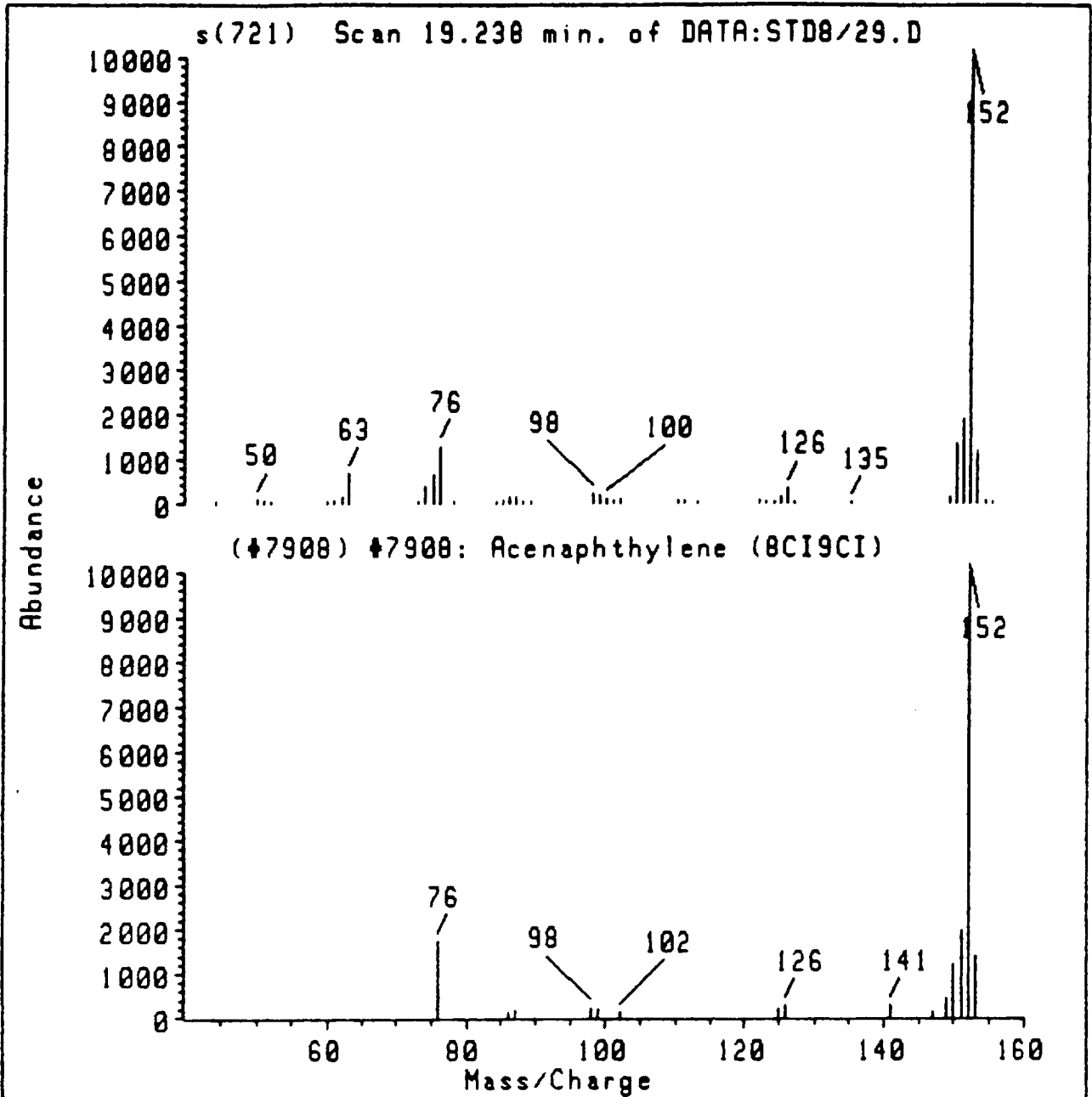
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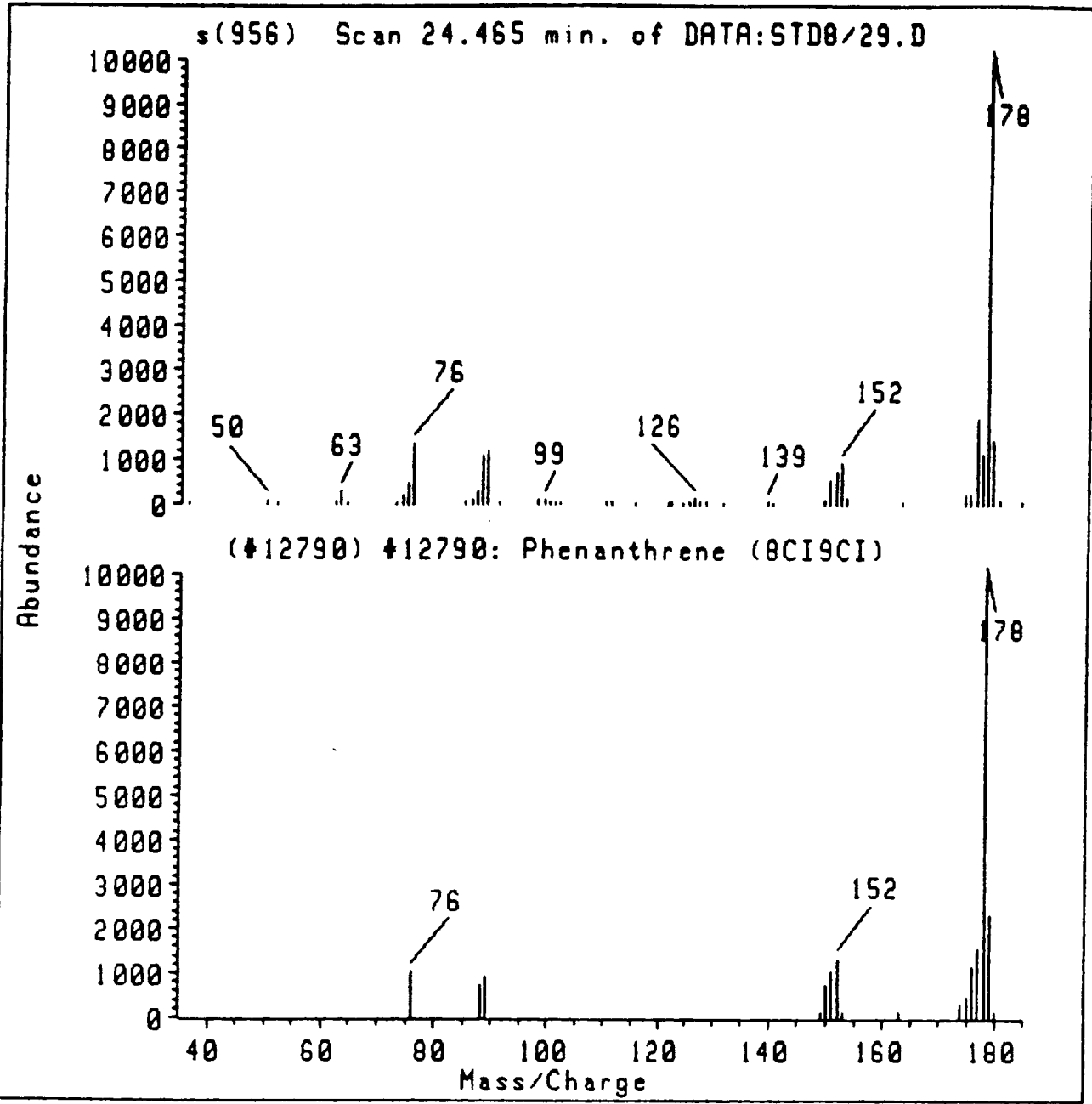


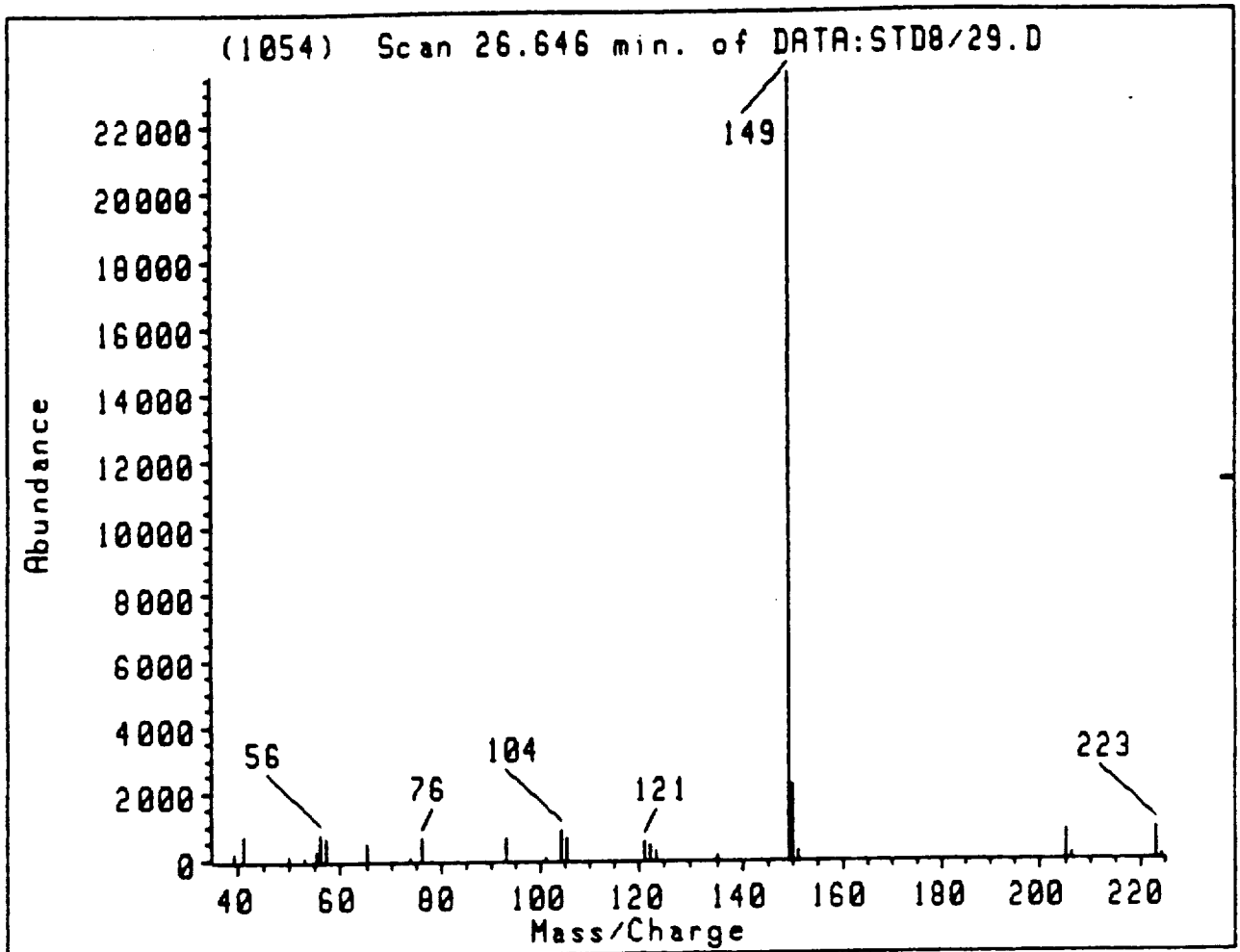


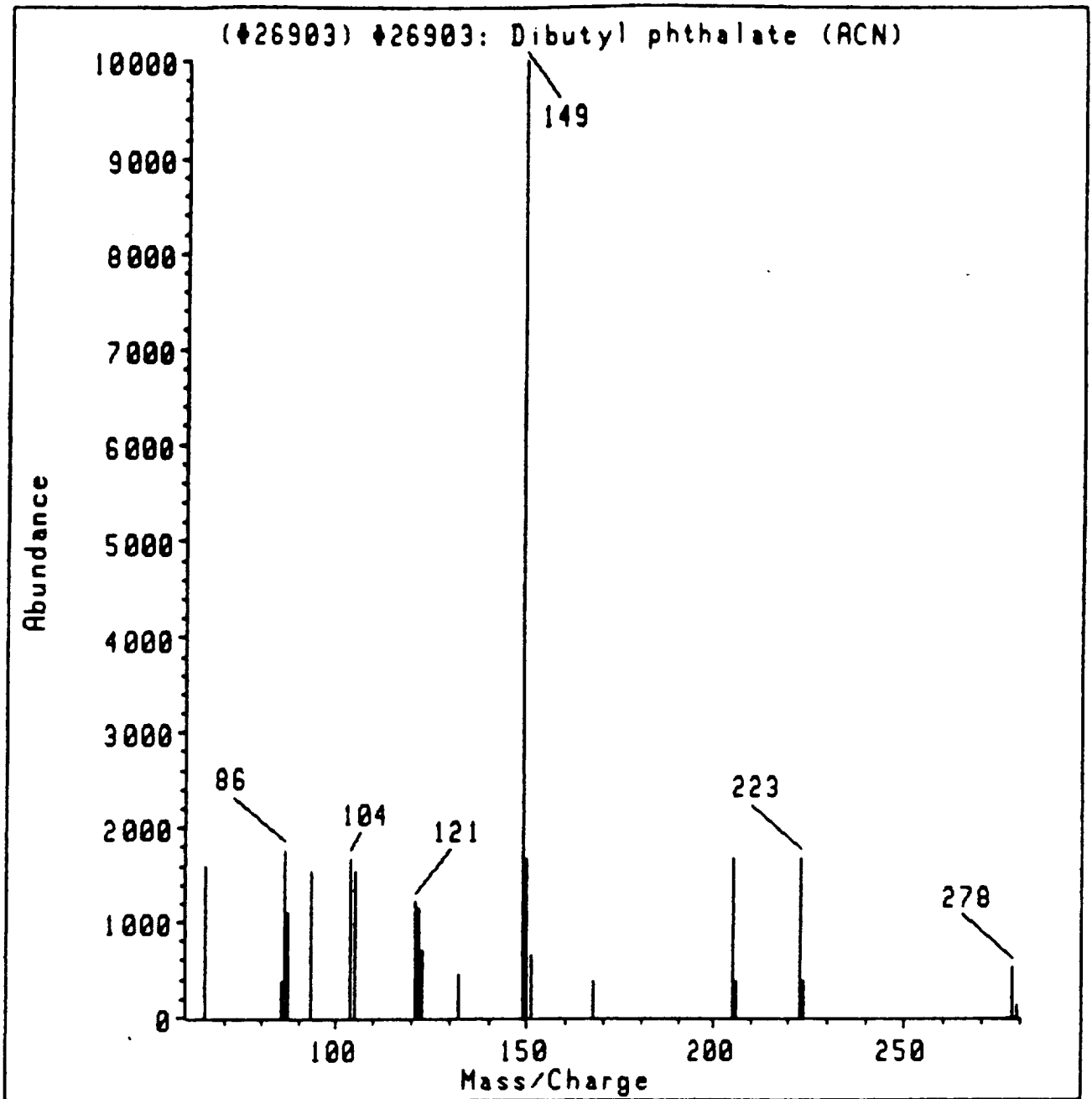


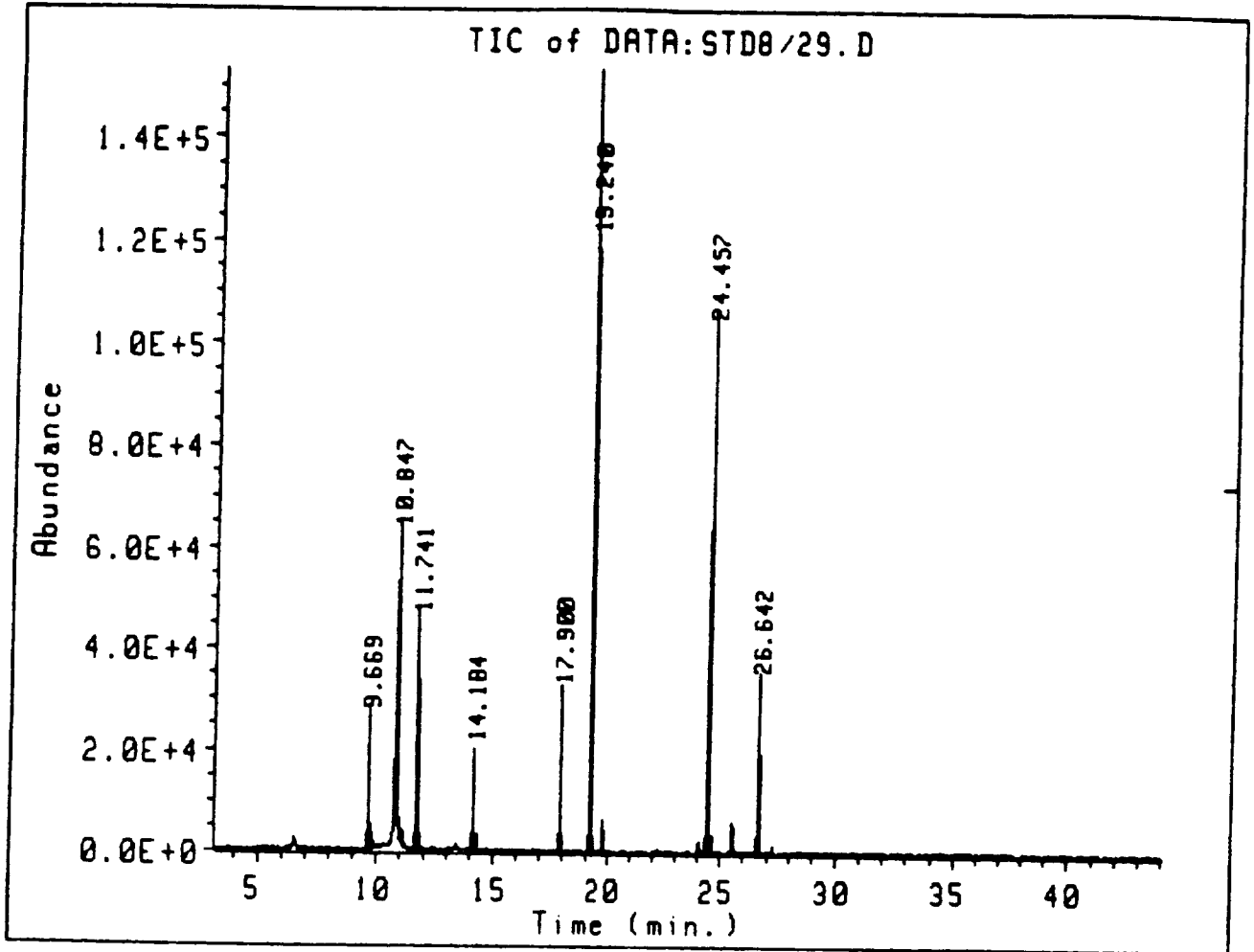










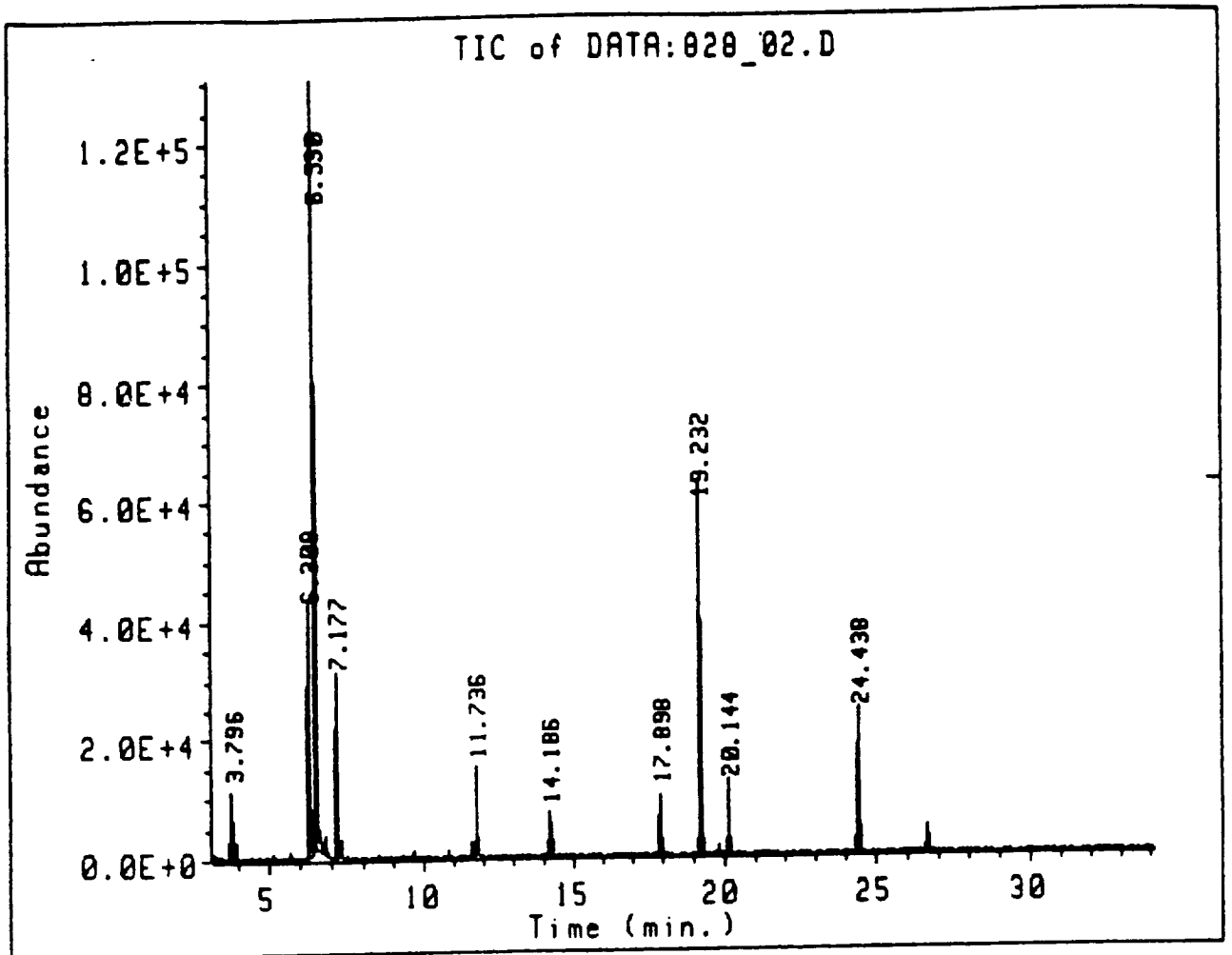


TIC of DATA:STD08/29.D 8 integration peaks found.

Page 1

Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	9.669	BB	0.056	619795	9.559	9.820
2	10.847	BB	0.069	1307732	10.785	11.141
3	11.741	BB	0.072	<u>1192887</u>	11.654	11.899
4	14.184	BB	0.039	<u>445618</u>	14.117	14.317
5	17.900	BH	0.057	<u>778998</u>	17.816	17.955
6	19.240	BB	0.049	<u>4396432</u>	19.131	19.374
7	24.457	BB	0.053	<u>3443289</u>	24.335	24.636
8	26.642	BB	0.043	841402	26.530	26.730

extract of ...
conc. to 4 ml to 3 μ l injected
so conc. should be 3/8 of next standard
on previous page

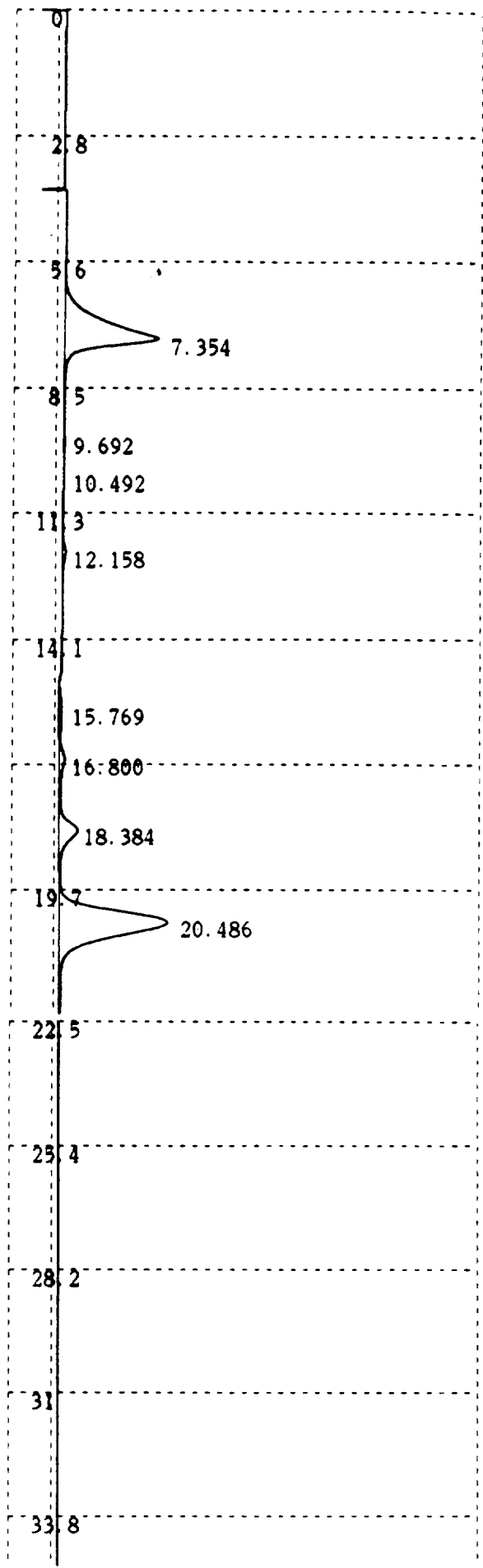
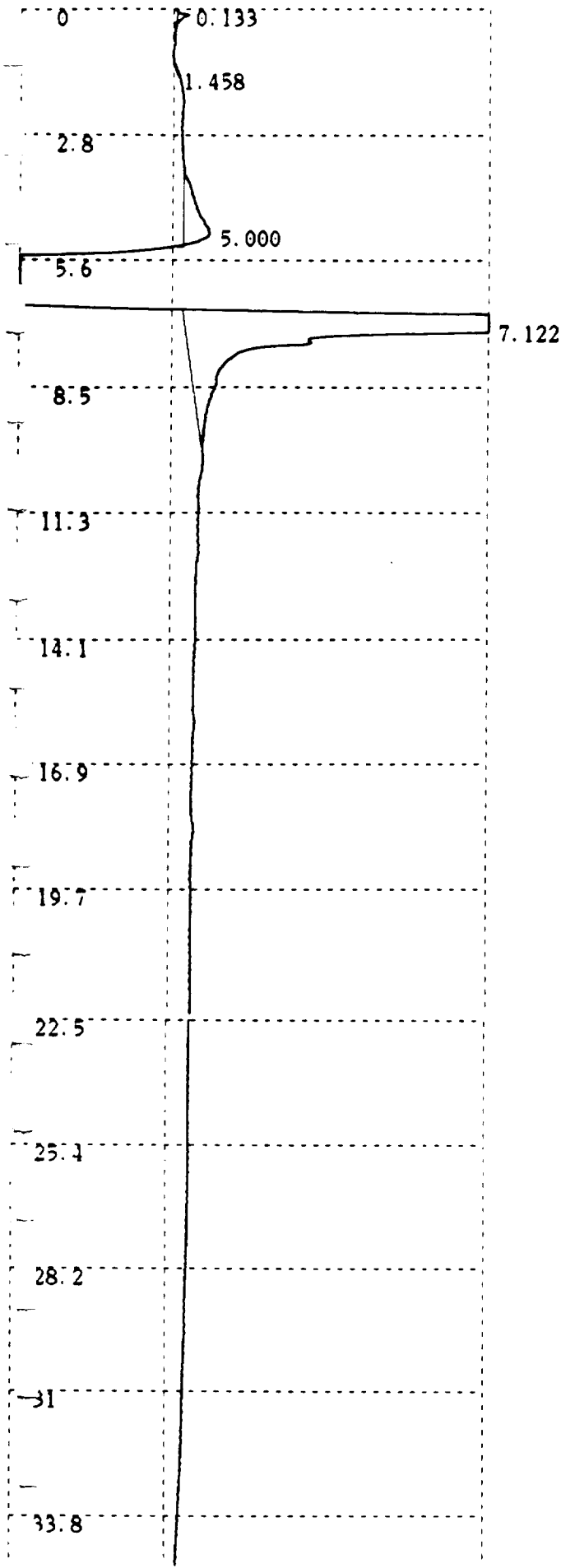


Peak	Ret Time	Type	Width	Area	Start Time	End Time
1	3.796	BB	0.051	265162	3.732	3.932
2	6.308	BB	0.042	1028211	6.240	6.462
3	6.530	BB	0.041	3053986	6.474	6.819
4	7.177	BB	0.040	700886	7.120	7.290
5	11.736	BB	0.039	<u>330625</u>	11.567	11.805
6	<u>14.186</u>	BB	0.058	<u>180480</u>	14.120	14.299
7	<u>17.898</u>	BB	0.038	<u>227913</u>	17.843	17.968
8	<u>19.232</u>	BB	0.059	<u>1535996</u>	19.158	19.370
9	20.144	BB	0.038	249498	20.061	20.217
10	24.438	BB	0.074	<u>636514</u>	24.352	24.531

Rt	Name	% Recovered
11.74	Hexachloroethane	$(330625 \div 447332) \times 100 = 74\%$
14.19	Naphthalene	$(180480 \div 167106) \times 100 = 108\%$
17.89	2-chloronaphthalene	$(227913 \div 292124) \times 100 = 78\%$
19.23	Acenaphthalene	$(1535996 \div 1648662) \times 100 = 93\%$
24.44	Phenanthrene	$(636514 \div 1291233) \times 100 = 49\%$

Appendix H

LC and LC/MS Analysis Results



H-1

Amadru
8/30/90 Diol column extraction of Hygiene tank #3
Note Book - 001 Page 92

061

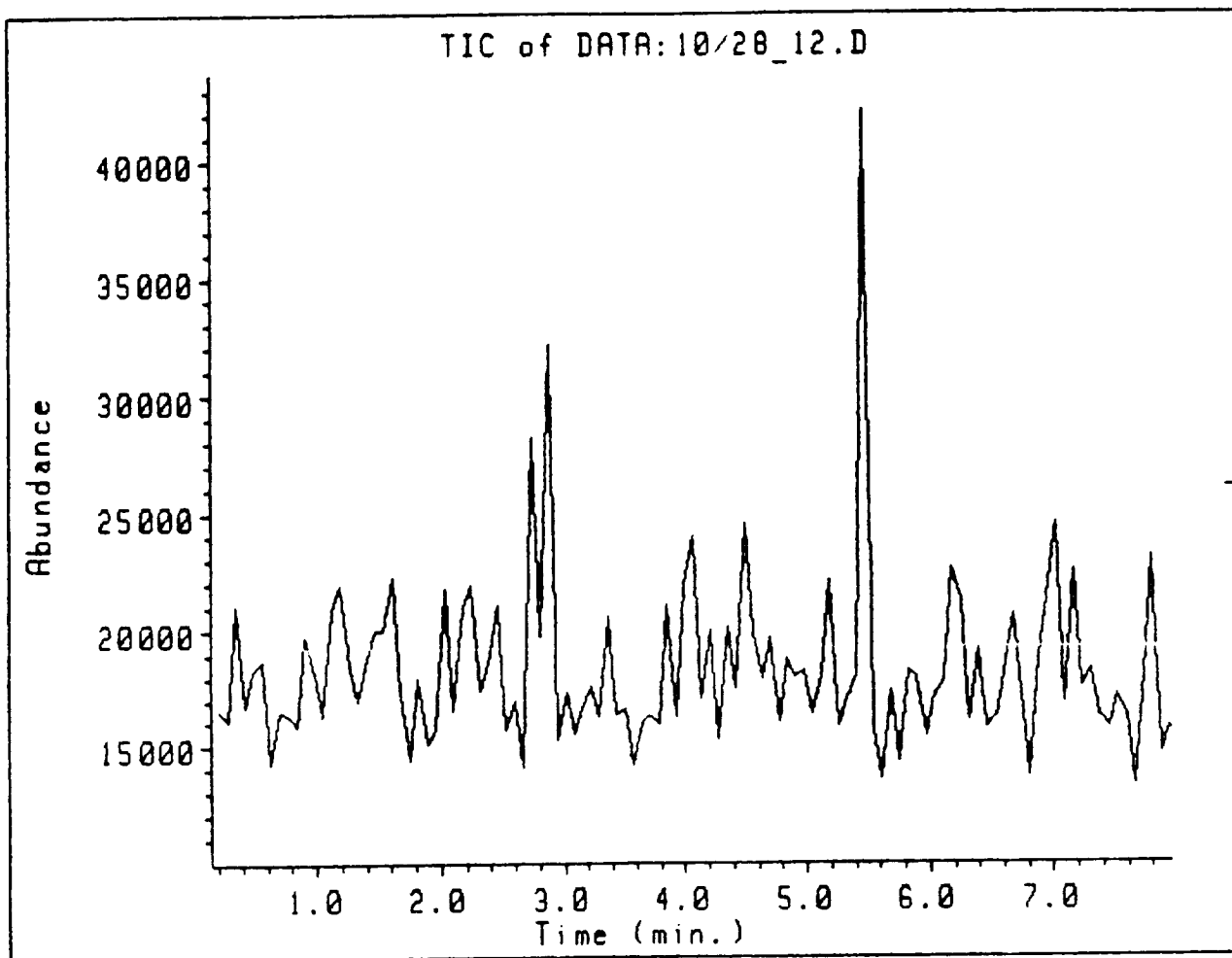
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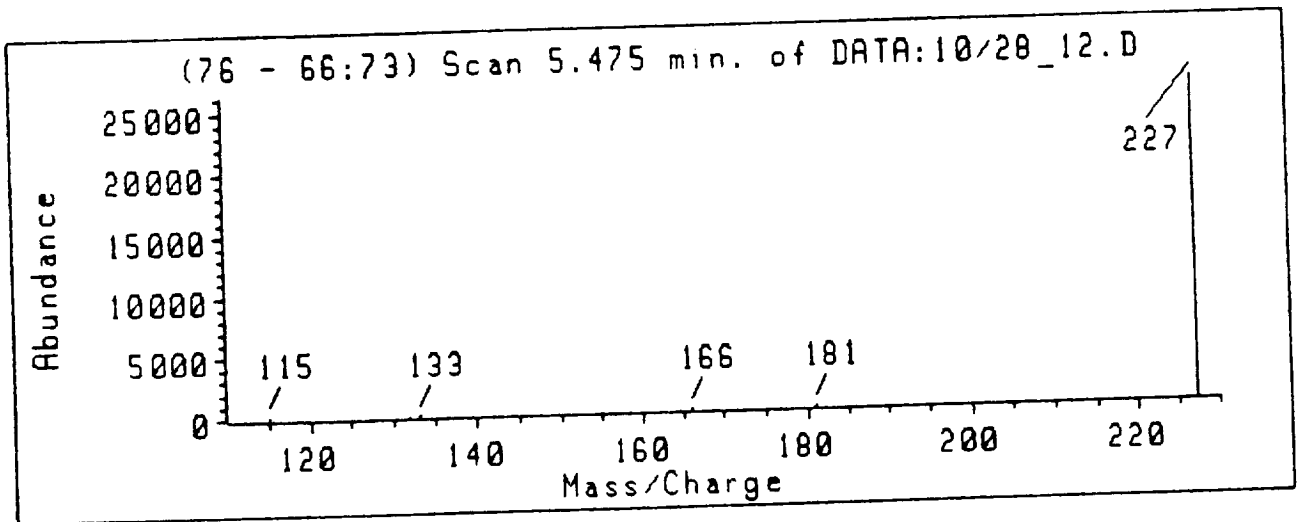
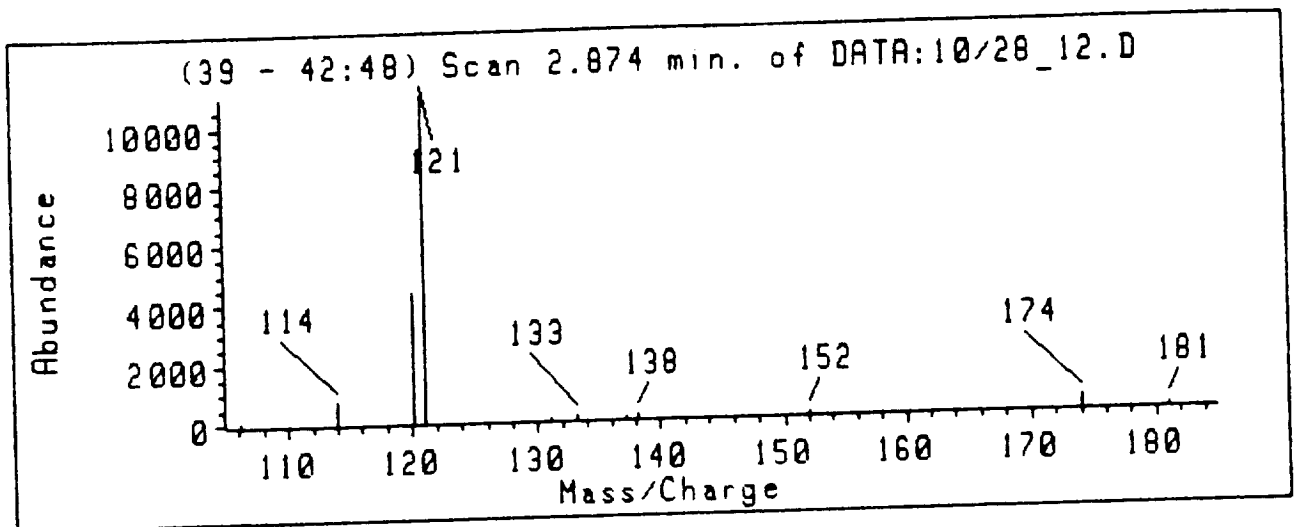
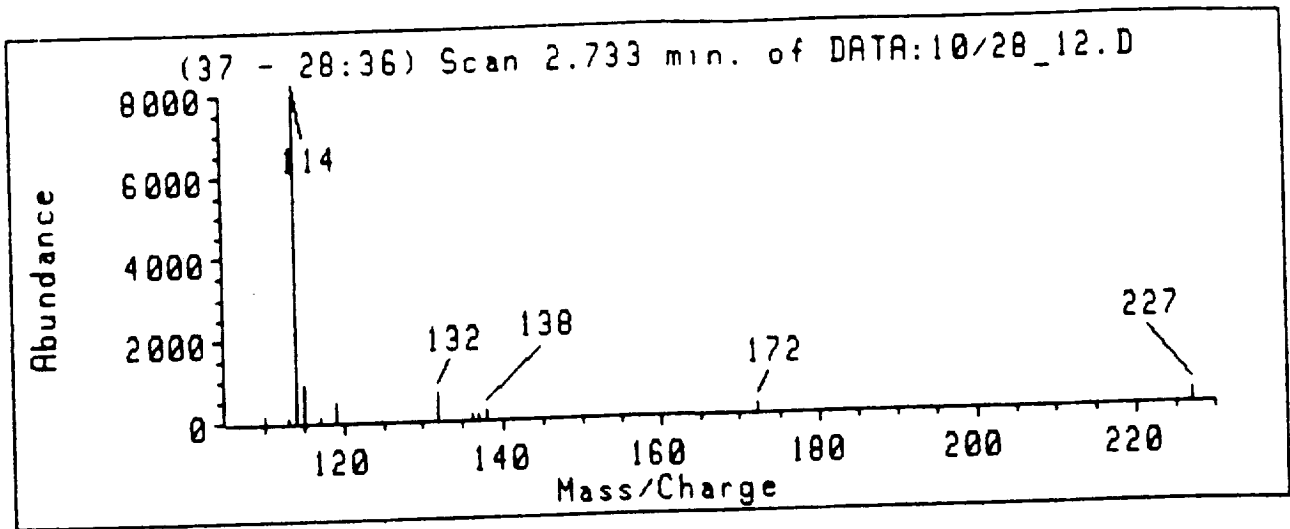
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File type: LC / MS DATA FILE

Sample Name: Hygiene Water HT#3 - Bio-Rad Ion-Ex Filtered
Misc Info: 25 uLinj - PRP-X300 @ 1.0 ml/min
Operator : B BENSON

Instrument: MS_5988
Inlet : TS

Sequence index : 0
Als bottle num : 0
Replicate num : 1



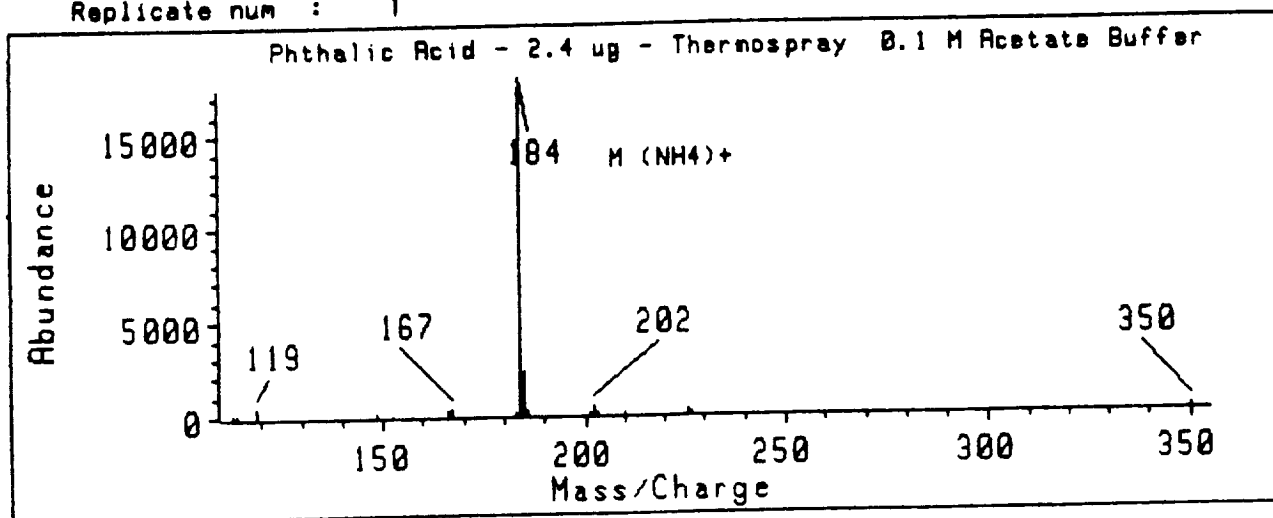


Date file: DATA:10/28_01.D
File type: LC / MS DATA FILE

Sample Name: THALLIC ACID - 1.2 mg/10 ml
Misc Info: 25 uL INJ
Operator : B BENSON

Date : 29 Oct 90 1:59 pm
Instrument: MS_5988
Inlet : TS

Sequence index : 0
Als bottle num : 0
Replicate num : 1

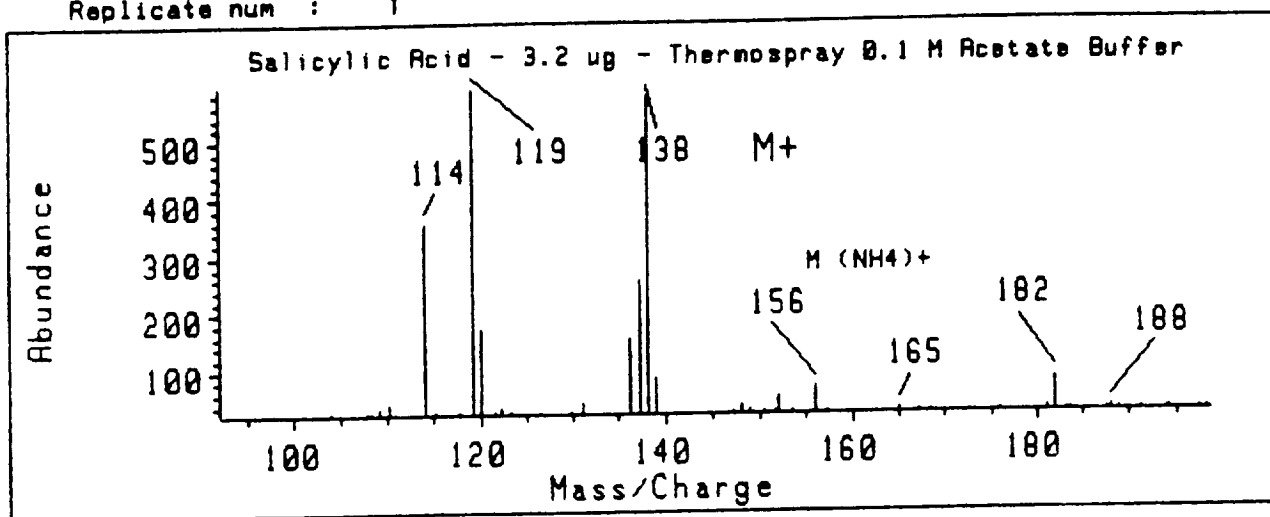


Data file: DATA:10/28_02.D
File type: LC / MS DATA FILE

Sample Name: SALICILIC ACID - 1.6 mg/10 ml
Misc Info: 25 uL INJ
Operator : B BENSON

Date : 29 Oct 90 2:07 pm
Instrument: MS_5988
Inlet : TS

Sequence index : 0
Als bottle num : 0
Replicate num : 1

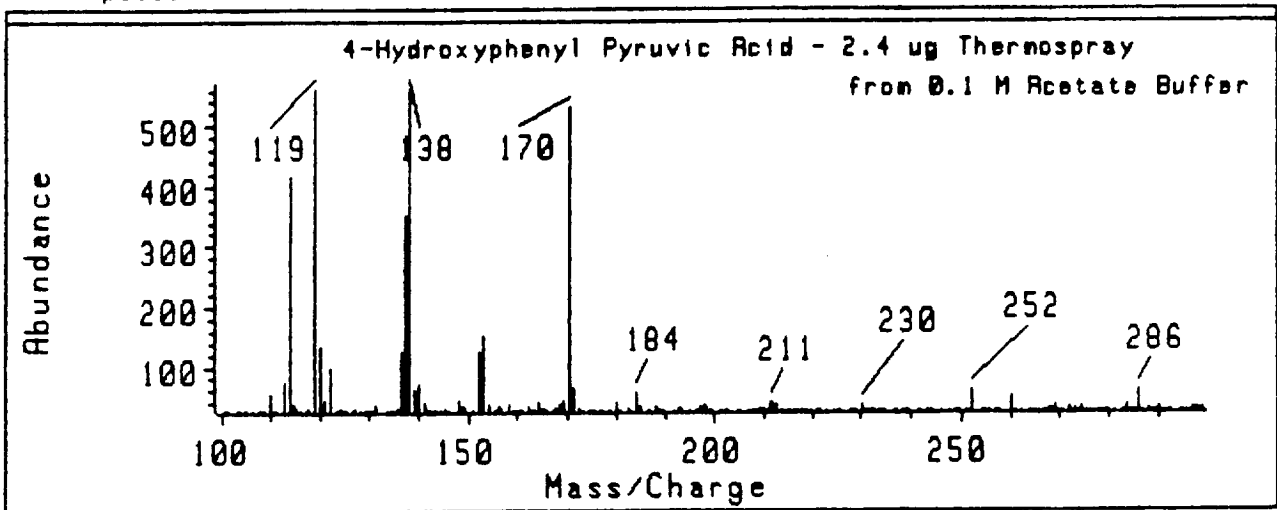


Data file: DATA:10/28_03.D
File type: LC / MS DATA FILE

Sample Name: 4-HYDROXYPHENYL PYRUVIC ACID - 1.2 mg/10ml
Misc Info: 25 uL INJ
Operator : B BENSON

Date : 29 Oct 90 2:16 pm
Instrument: MS_5988
Inlet : TS

Sequence index : 0
Als bottle num : 0
Replicate num : 1

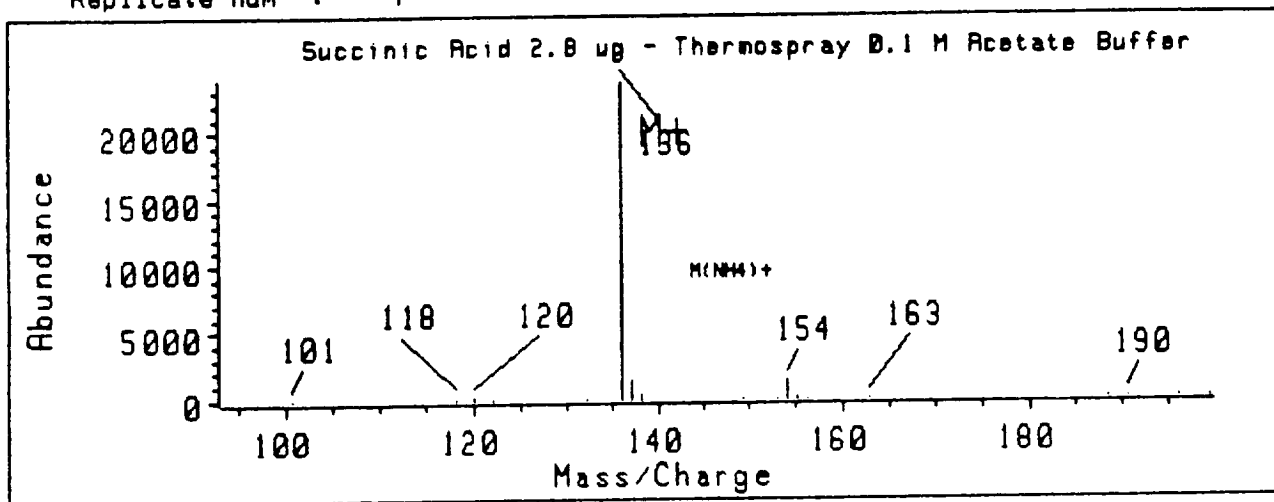


Data file: DATA:10/28_05.D
File type: LC / MS DATA FILE

Sample Name: SUCCINIC ACID - 1.4 mg/10ml
Misc Info: 25 uL INJ
Operator : B BENSON

Date : 29 Oct 90 2:30 pm
Instrument: MS_5988
Inlet : TS

Sequence index : 0
Als bottle num : 0
Replicate num : 1

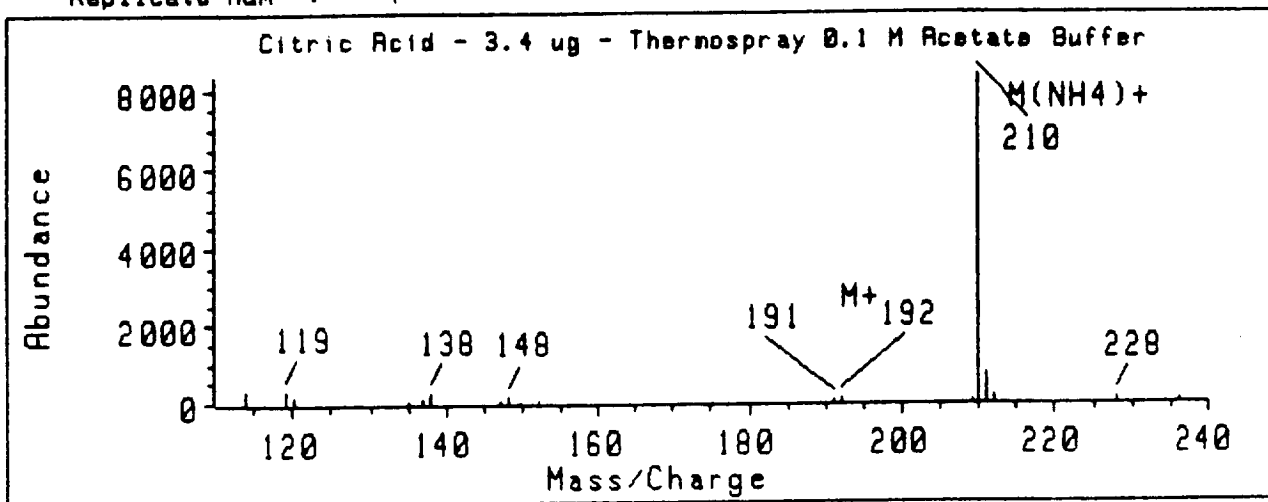


Data file: DATA:10/28_06.D
File type: LC / MS DATA FILE

Sample Name: CITRIC ACID - 1.7 mg/10ml
Misc Info: 25 uL INJ
Operator : B BENSON

Date : 29 Oct 90 2:34 pm
Instrument: MS_5988
Inlet : TS

Sequence index : 0
Als bottle num : 0
Replicate num : 1



Appendix I

Procedures and Instrumental Parameters

TABLE 1. pH indicators for culture media

Name	pK'	pH range and colors*	Concn usually employed in media (g/liter) ^b	AMBI (1944) or 0.01 N NaOH needed to dissolve 0.1 g
Phenol red	7.8	6.9(Y)-8.5(R)	0.010-0.030	28.2
Bromothymol blue	7.1	6.1(Y)-7.7(B)	0.010-0.032	16.0
Bromocresol purple	6.2	5.4(Y)-7.0(P)	0.010-0.032	18.5
Chlorophenol red	6.0	5.1(Y)-6.7(R)	0.015	23.6
Bromocresol green	4.7	3.8(Y)-5.4(B)	0.020	14.3

* Symbols: Y, yellow; B, blue; P, purple; R, red.

^b For addition to culture media, dissolve in alcohol or prepare an aqueous solution using 0.01 N NaOH.

lect the granules by centrifugation. Suspend the granules in water and dialyze against running tap water until free from chloride (use AgNO₃ on a small portion; there should be no precipitate of AgCl formed). Dialyze further against distilled water. In a series of centrifugations, wash the granules several times with acetone. Place the granules in a Soxhlet apparatus and continuously extract them with acetone-ether (2:1, vol/vol) for 3 days to remove non-PHB lipids. Further extract the granules with hot diethyl ether, pulverize them, and dry them under a vacuum. Determine the dry weight

After drying, disperse the phosphate buffer by means and sonic oscillated suspension autoclaving, and calculate how much one needs to add a final concentration overlay describe

MISSING

isocaproic Acid →

20.4.16. Potass

Prepare 0.2 M KH₂PO₄. Mix the solutions in Table 2 to obtain the appropriate pH value. The mixture (0.2 M phosphate buffer) can be further diluted to give the desired molarity. Also see part 6.1.2.

20.4.17. Phosphate-E

10x Stock solution
Na₂HPO₄·anh
NaH₂PO₄·H₂O
NaCl, reagent

Dissolve ingredients in volume of 1,000 ml

Working solution
Stock solution
Distilled water

MISSING

isobutanol →

TABLE 2. Number of parts of K₂HPO₄ solution and KH₂PO₄ solution required to obtain potassium phosphate buffer of various pH levels

K ₂ HPO ₄	KH ₂ PO ₄	pH
49	51	6.8
55	45	6.9
61	39	7.0
67	33	7.1
72	28	7.2
77	23	7.3

20.4.18. Standard Solutions for Gas Chromatography (7)

Standard mixture of volatile fatty acids (ca. 1 meq/100 ml)

Formic acid	0.087 ml ✓
Acetic acid, glacial	0.057 ml ✓
Propionic acid	0.075 ml ✓
Isobutyric acid	0.092 ml ✓
Butyric acid	0.091 ml ✓
Isovaleric acid	0.109 ml ✓
Valeric acid	0.109 ml ✓
Isocaproic acid	0.126 ml ✓
Caproic acid	0.126 ml ✓
Heptanoic acid	0.142 ml ✓
Distilled water	100 ml

When using the standard mixture, acidify and extract 1 ml of the solution with ether for gas chromatography.

Standard mixture of alcohols. The following list shows the amount of each alcohol to be added to 100 ml of water to obtain the final concentration in parentheses.

Ethanol	0.1 ml	(1.7 mM) ✓
Propanol	0.036 ml	(0.5 mM) ✓
Isobutanol	0.005 ml	(0.05 mM) ✓
Butanol	0.01 ml	(0.1 mM) ✓
Isopentanol	0.006 ml	(0.05 mM) ✓
Pentanol	0.008 ml	(0.05 mM) ✓

Extract 1 ml of the mixture with ether for gas chromatography.

Procedure for the analysis of sugars and organic acids using HPLC

Simple sugars and low molecular weight organic acids can be monitored simultaneously with HPLC using a BioRad organic acid column and a Refractive Index detector. The detection limit was determined to be 0.5 ppm for both sugars and acids. With this procedure, however, both water and soap component peaks are visible.

MATERIALS

HP1090 Mainframe with autosampler
HP85B Data Station
HP Refractive Index Detector
HP 3392A Integrator
BioRad HPX-87H Organic Acid Analysis Column (with guard column)
HP autosampler vials
0.01N H₂SO₄

METHOD

Sample preparation:
Shake samples before filtration.
Filter each sample through a Gelman LC-13 or Acrodisc filter (0.45/μm) using a 10cc luer-loc glass syringe.
Collect the sample eluent in an autosampler vial and cap using Hewlett-Packard's vial crimper.
Place in autosampler carriage and inject.

HPLC parameters:
Solvent: 0.01N H₂SO₄ (purged with Helium)
Flow rate: 0.8 ml/min
Column: BioRad Organic Acid Analysis Column with guard column
Column Oven temperature: 65°C
Detector temperature: 40°C
Chart Speed: 0.5 cm/min
Attenuation: 2 or 3 depending on concentration of sample components
Peak width: 0.04
Threshold: 3
Area reject: 0
Injection volume: varied as listed in data table, depending on concentration of sample components

Sugars And Organic Acids Standard

Results from a 2 μ l injection

<u>Component</u>	<u>Retention time(min)</u>	<u>Area</u>	<u>Concentration (ppm)</u>
Maltose	5.76		
Lactose	5.76		
Citric Acid	6.00		
Glucose	6.89	71746	96
Pyruvic Acid	7.18	52893	102
Fructose	7.42	82615	92
Arabinose	8.02	82233	101
Lactic Acid	9.69	48472	148
Formic Acid	10.48	70258	257
Acetic Acid	11.45	67953	150
Propionic Acid	13.52	28677	68
Butyric Acid	16.55	Not integrated	accurately
Valeric Acid	22.61	55968	95
Hexanoic Acid	32.98	48988	93

Other standards not included above:

<u>Component</u>	<u>Retention time (min)</u>
Mannose	7.31
Galactose	7.34
Xylose	7.40
Pyruvic Acid	7.16
Acetone	16.84
Methanol	14.89
2-Propanol	19.27
2-Ketobutyric	8.01
Inositol	7.16
MgCl ₂	4.60
Heptanoic Acid	51
3-Hydroxybutyric	10.4

The Analysis Of Amino Acids

The Bio-Sil ODS-5S column is an excellent column for the separation and quantitation of dansylated amino acids. Dansylation adds a strong UV chromophore to the amino acid, which greatly increases the sensitivity of the analysis when using a UV detector. The HPLC system used for sample evaluation was the HP1090 Mainframe with autosampler and diode array detector, the HP85B Data Station, and the HP 2225-A Printer.

MATERIALS

Dansyl Chloride by Sigma
0.2M NaHCO₃ (adjusted to a pH of 9.5 to 10 with NaOH)
0.03M NaH₂PO₄ (adjusted to a pH of 6.5 with NaOH)
Acetonitrile
Amino Acid Standards by Sigma and Supelco

PROCEDURE

Preparation of Amino Acid Standard:

In a 500 ml volumetric flask, add the following standards and dilute with deionized water:

TABLE 1

Standard	mg/500ml	Concentration (ppm)	Retention Time (min)	Detection Limits (ppm)
Aspartic	13.75	27.5	9.6	3.2
Glutamic	13.14	26.28	10	4.1
Asparagine	14.72	29.44	13.0	1.7
Citrulline	15.74	31.48	13.7	2.2
Threonine	14.63	29.26	14.9	1.5
Glycine	16.24	32.48	15.0	0.8
Alanine	14.30	28.6	15.6	1.2
Arginine	13.67	27.34	16.0	6.7
Proline	16.15	32.3	16.7	0.8
Valine	14.42	28.84	17.9	1.6
Leucine	13.84	27.68	19.7	1.8
Tryptophan	12.23	24.46	20.2	2.7
Cystine	13.55	27.1	20.3	1.7
Ornithine	14.27	28.54	25.9	1.3
Lysine HCl	14.36	28.72	26.2	1.4
Histidine	14.95	29.9	26.7	1.1
Tyrosine	16.52	33.04	29.4	2.1

(Note: Phenylalanine has the same retention time as Cystine. Cystine was included in the Ersatz model and Phenylalanine was not; therefore, Cystine was added to the standard mix.)

0.2M NaHCO₃ buffer plus Internal Standards:

8.4g of NaHCO₃ were added to less than 500 ml of deionized water and brought to a pH of 9.5 with NaOH. This solution was transferred to a 500 ml volumetric flask to which 60.02mg of Serine and 64.03 mg of Methionine were added. The buffer was then brought to volume with deionized water and thoroughly mixed.

Serine (120.04ppm) and Methionine (128.06ppm) had retention times of 14.1 and 18.4 minutes, respectively when analyzed with the same solvent system as the above Amino Acid Standard.

Preparation of samples:

Shake each sample vigorously.

Pipet (Using an Eppendorf 1ml pipetter) 2ml of sample into an amber vial.

Add 0.5 ml of 0.2M NaHCO₃ buffer plus internal standards to the vial, mix. (Use an Eppendorf 0.5 ml pipetter)

Add 2 ml of Dansyl chloride (5mg/ml in Acetonitrile).

Cap, shake, and allow to react for 1 hour.

Then add 1 ml of 2% Methylamine HCl (in water) to stop the reaction.

Transfer to autosampler vials, cap with HP's crimper, and place in autosampler carriage for injection.

Run duplicates per every 10 samples.

Use the above procedure to prepare a fresh standard for each analysis.

Place this standard at the beginning and at the end of the sample set for analysis.

HPLC parameters:

Column: Bio-Sil ODS 5-9

Solvent: A gradient system as follows:

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1* METHOD SEPARATION

SOS-CONFIG A=1 , B=1 , C=1

FLOW = 1
% B= 5 , C= 0

MAXPRESS = 250

MINPRESS = 0

OVENTEMP = 36

INJVOLUM = 15

SLOWDOWN = 2

STOPTIME = 45

POSTTIME = 3

COLUMNSW = 0

E1=0 E2=0 E3=0 E4=0

AT 0.1 FLOW - 1

AT 0.1 % B= 5 , C= 0

AT 30 % B= 50 , C= 0

AT 35 % B= 95 , C= 0

AT 40 % B= 95 , C= 0

AT 45 % B= 5 , C= 0

1*END-OF-LIST

METHOD1040

1* DAD Signals

A S-250,10 R-550,100 M=1 I=1 P

B S-260,10 R-550,100 M=1 I=1

C S-0,0 R-0,0 M=0 I=1

1* DAD Intset

A TH = 5 AR = 5

B TH = 5 AR = 5

C TH = 5 AR = 5

1* DAD Plotset

A RANGE = 50 ZEROX = 10

B RANGE = 50 ZEROX = 10

C RANGE = 50 ZEROX = 10

1* DAD

PEAKWIDTH = 0.05 ; min

CHARTSPEED = 1 ; cm/min

STOPTIME = 0 ;

1* DAD Spectra

FROM = 210; nm

TO = 400; nm

STEP = 4 ; nm

MEM = 0 ; none

1* DAD Integrator

REPORT TYPE = 1 ; area%

RATIO SIGNALS =

PLOT MODE = 2 ; offline

ANNOTATION = 3 ; markers

1*Signal (min) (min)

DO FROM -0 TO -0

1* DAD Expert

LAMPCURR = 1 ; low

TIMEBASE = 0 ; auto

MONITOR = P

AUTO-OFF = 1440 ; min

POSTTIME = 0.1 ; min

SIGNAL MATCH = 0.125

1* DAD Calibration

WINDOW = 5 %

REF WINDOW = 5 %

UNITS =

DEFAULT PEAK# = 0 ; uncal peaks

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Appendix J

Solid Phase Extraction Recovery Study

PROCEDURES

C18 Solid Phase Extraction

Column: BakerBond™ 500mg C18 Phase

Sample: Neutralize and filter 10.0 ml water sample

Column Conditioning: 10 ml Methylene Chloride then air dry 1 minute, followed by 10 ml Methanol and 10 ml reagent water without letting phase go dry.

Sample Addition: With reservoir attached add 10 ml of prepared sample and aspirate at 5 in. Hg. vacuum. Air dry 10 minutes.

Sample Elution: Elute with two 500 ml aliquots of Methylene Chloride.

C8 Solid Phase Extraction

Column: BakerBond™ 500mg C8 Phase

Sample: Neutralize and filter 10.0 ml of water sample

Column Conditioning: Condition with two 3 ml aliquots of Methanol followed by 2 ml reagent water without letting phase go dry.

Sample Addition: With reservoir attached add 10 ml of prepared sample and aspirate at 5 in. Hg. vacuum. Wash with 1 ml reagent water then air dry 5 minutes.

Sample Elution: Elute with two 500 ml aliquots of 50:50 Hexane/Diethyl ether.

Diol Solid Phase Extraction

Column: BakerBond™ 500 mg Diol Phase

Sample: Filter and adjust pH with HCL or NaOH to ionize analytes

of interest, dilute 6:1 with 50:50 Methanol/Diethyl Ether.

Column Conditioning: Condition with two 2 ml volumes of Methylene Chloride followed by 5 ml reagent water without letting solid phase go dry.

Sample Addition: With reservoir attached aspirate 10 ml of prepared sample at 5 in. Hg. vacuum. Wash in 2 ml Methylene Chloride.

Elution: Elute analytes with three 500 ml portions of 100:1 isopropanol/Glacial acetic acid.

Phenyl Solid Phase Extraction

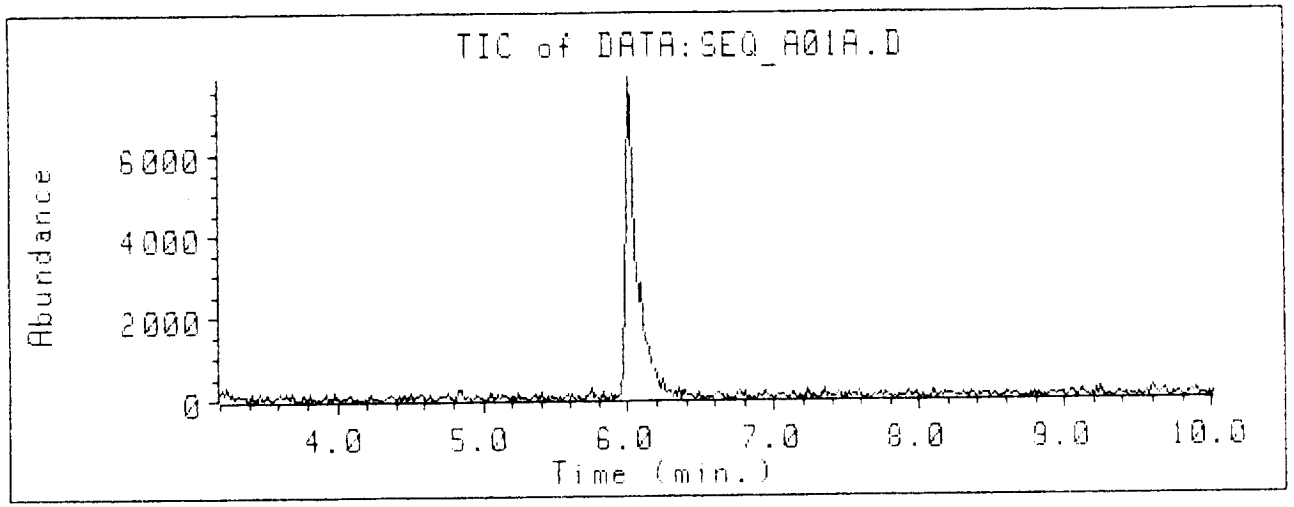
Column: BakerBondTM 500 mg Phenyl (C6) Phase

Sample: Neutralize and filter 10 ml of water sample

Column Conditioning: Condition with two 3 ml volumes of Methanol followed by two 3 ml volumes of water. Do not let solid phase go dry.

Sample Addition: With reservoir attached aspirate 10 ml of prepared sample at 5 in. Hg. vacuum. Wash with 3 ml reagent water.

Elution: Elute with two 500 ml aliquots of acetonitrile

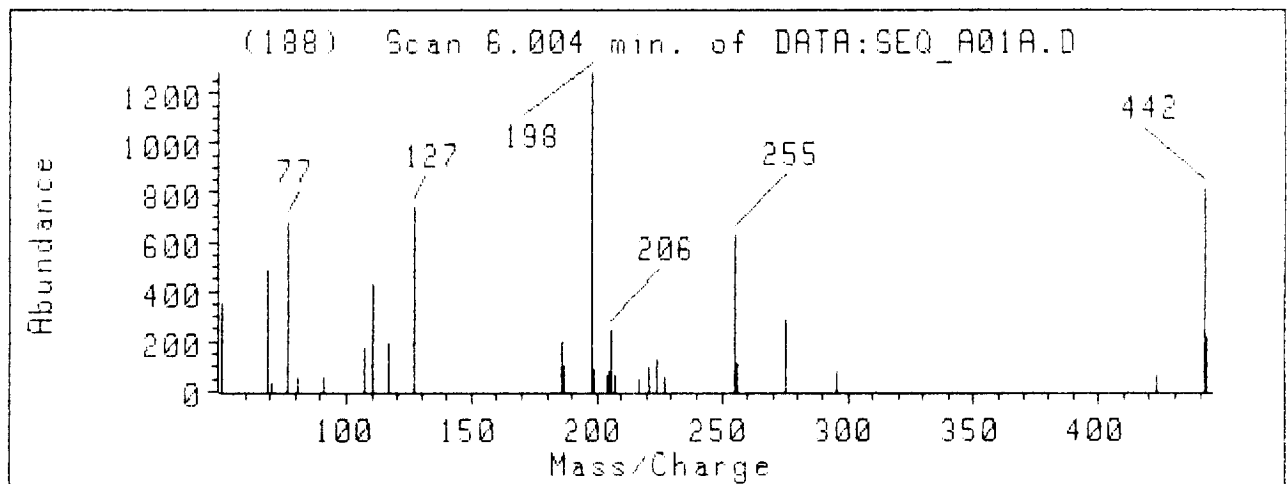
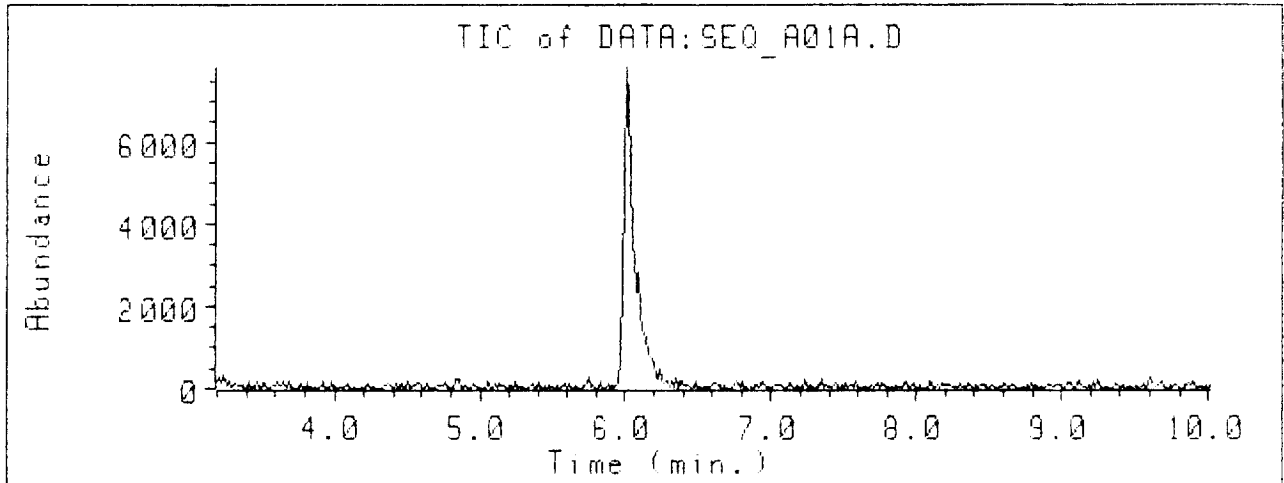


Data file: DATA:SEQ_A01A.D
File type: GC / MS DATA FILE

Sample Name: DFTPP MS TUNING CPD 25 ng/ul
Misc Info:
Operator :

Date : 14 Mar 91 4:43 pm
Instrument: MS_5988
Inlet : GC

Sequence index : 1
Als bottle num : 1
Replicate num : 1

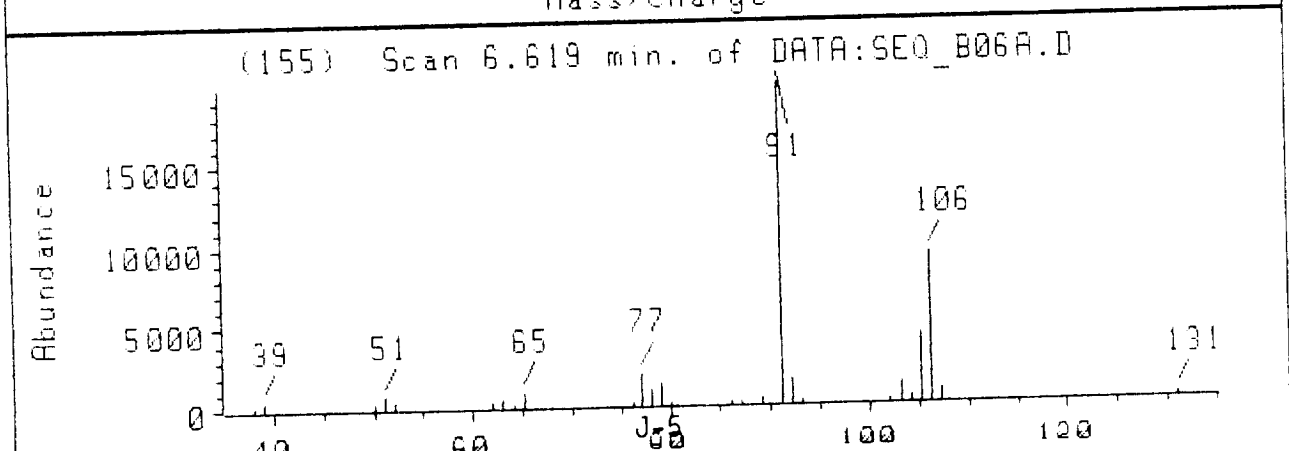
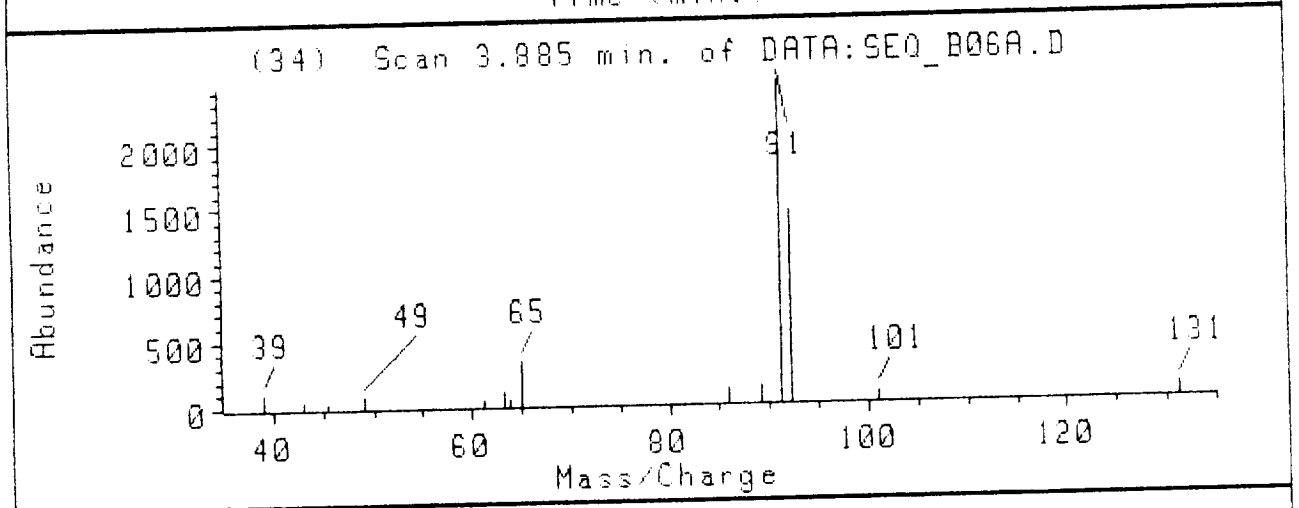
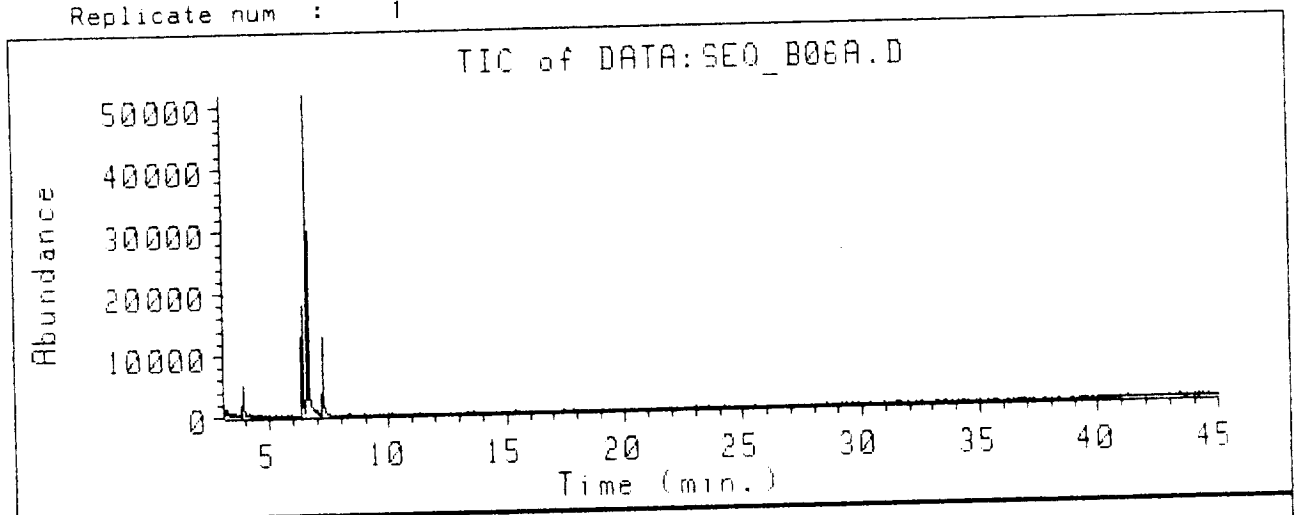


Data file: DATA:SEQ_B06A.D
File type: GC / MS DATA FILE

Sample Name: 3/14_04 C18 SPE H2O BLANK
Misc Info:
Operator :

Date : 14 Mar 91 8:27 pm
Instrument: MS_5988
Inlet : GC

Sequence index : 2
Als bottle num : 6
Replicate num : 1

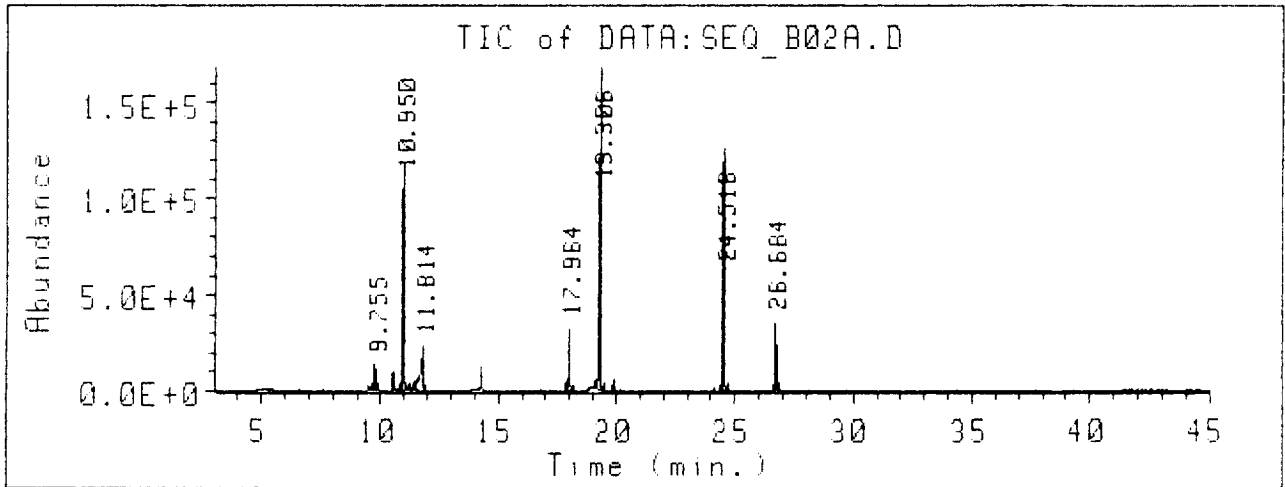


Data file: DATA:SEQ_B02A.D
File type: GC / MS DATA FILE

Sample Name: ERA BASE/NEUTRALS STD LOT #91108 NEAT- not extracted
Misc Info:
Operator :

Date : 14 Mar 91 4:59 pm
Instrument: MS_5988
Inlet : GC

Sequence index : 2
Als bottle num : 2
Replicate num : 1



TIC of DATA:SEQ_B02A.D 7 integration peaks found.

Page 1

Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	9.755	BB	0.059	358734	9.632	9.899
2	10.950	BB	0.058	4060975	10.836	11.215
3	11.814	BB	0.116	2077215	11.360	11.907
4	17.964	BB	0.040	943236	17.807	18.186
5	19.306	BB	0.056	6126915	19.089	19.524
6	24.518	BB	0.055	4410530	24.409	24.710
7	26.684	BB	0.044	952720	26.573	26.841

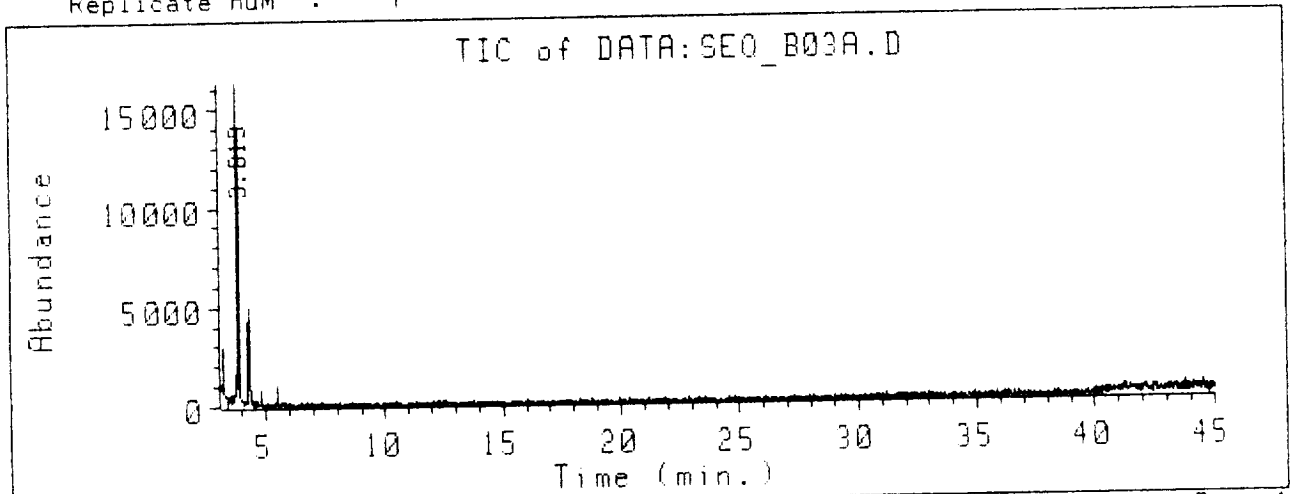
ORIGINAL SOURCE
OF POOR QUALITY

Data file: DATA:SEQ_B03A.D
File type: GC / MS DATA FILE

Sample Name: 3/14_01 CBH6- SPE of Al. Std.
Misc Info:
Operator :

Date : 14 Mar 91 5:51 pm
Instrument: MS_5988
Inlet : GC

Sequence index : 2
Als bottle num : 3
Replicate num : 1



TIC of DATA:SEQ_B03A.D 1 integration peaks found.

Page 1

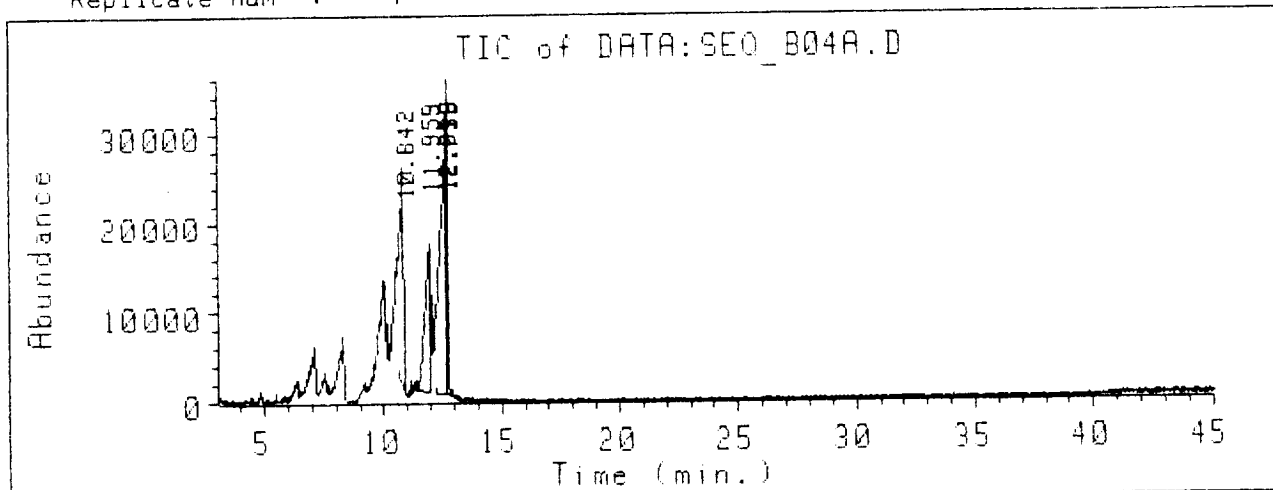
Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	3.819	BV	0.027	212313	3.732	3.846

Data file: DATA:SEQ_B04A.D
File type: GC / MS DATA FILE

Sample Name: 3/14_02 C6H6 SPE of F.A. Std.
Misc Info:
Operator :

Date : 14 Mar 91 6:43 pm
Instrument: MS_5988
Inlet : GC

Sequence index : 2
Als bottle num : 4
Replicate num : 1



TIC of DATA:SEQ_B04A.D 4 integration peaks found.

Page 1

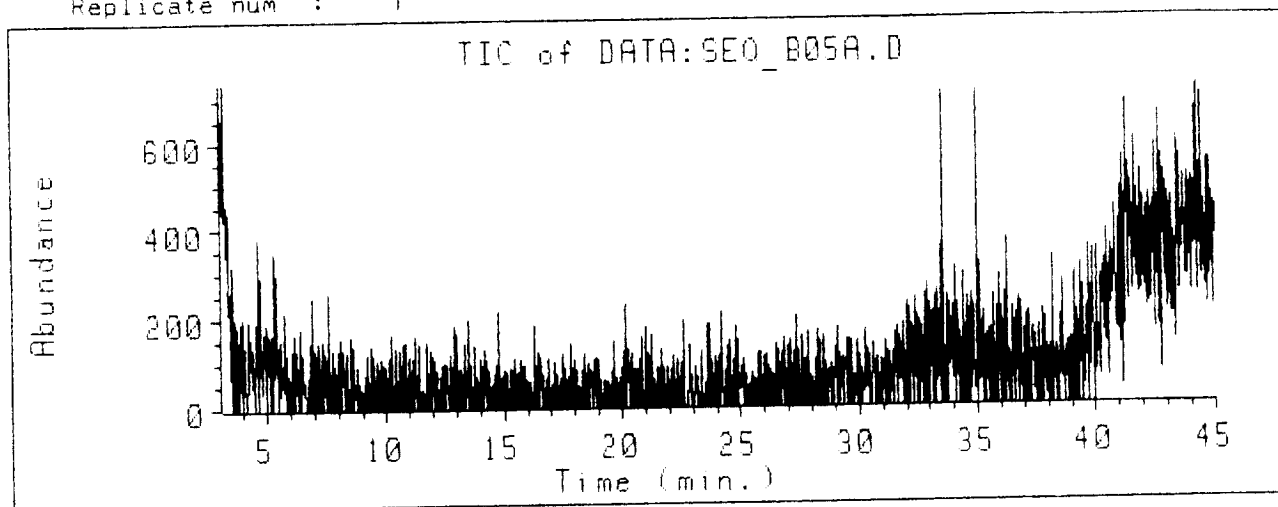
Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	10.842	VB	0.119	2362780	10.666	10.942
2	11.959	PV	0.186	2390949	11.450	12.000
3	12.612	VV	0.192	4048585	12.231	12.630
4	12.739	VB	0.099	2414825	12.630	12.877

Data file: DATA:SEQ_B05A.D
File type: GC / MS DATA FILE

Sample Name: 3/14_03 06H6 SPE H2O BLANK
Misc Info:
Operator :

Date : 14 Mar 91 7:35 pm
Instrument: MS_5988
Inlet : GC

Sequence index : 2
Als bottle num : 5
Replicate num : 1



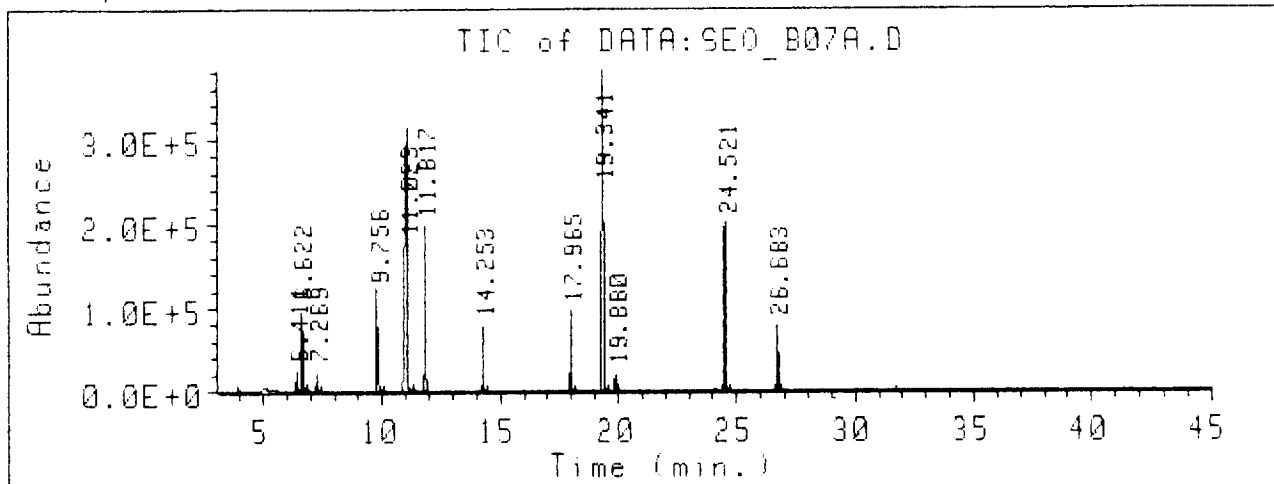
Data file: DATA:SEQ_B07A.D
File type: GC / MS DATA FILE

Sample Name: 3/14_05 C18 SPE of ERA BN Std. 91108

Misc Info:
Operator :

Date : 14 Mar 91 9:19 pm
Instrument: MS_5988
Inlet : GC

Sequence index : 2
Als bottle num : 7
Replicate num : 1



TIC of DATA:SEQ_B07A.D 12 integration peaks found.

Page 1

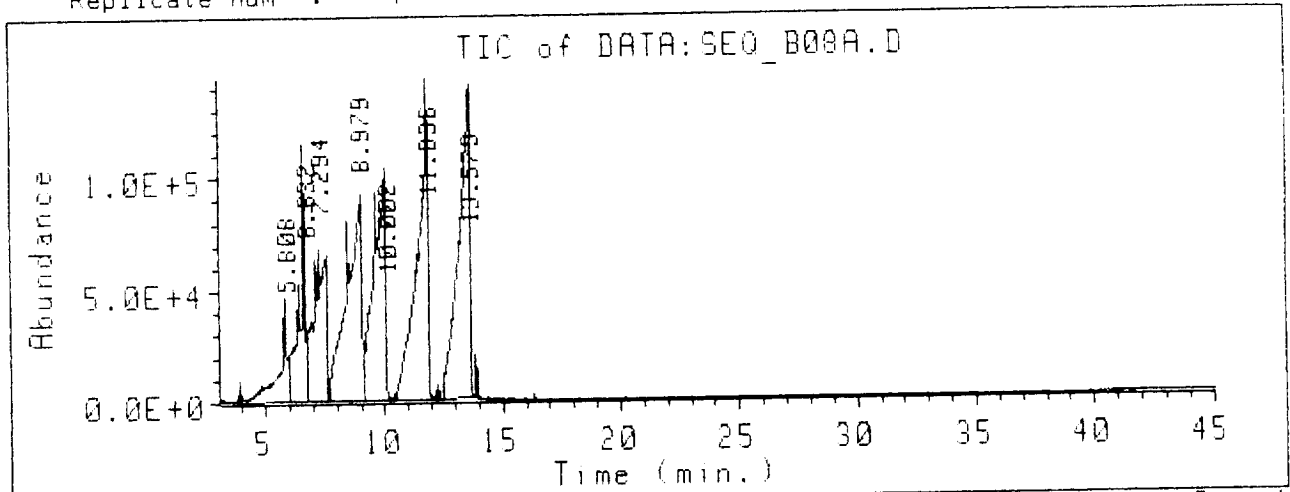
Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	6.411	BV	0.053	812085	6.342	6.565
2	6.622	VB	0.044	2519367	6.565	6.844
3	7.269	BB	0.040	551295	7.212	7.413
4	9.756	BB	0.050	3535131	9.688	10.067
5	11.023	BB	0.098	21299163	10.837	11.283
6	11.817	BV	0.051	6237204	11.729	11.922
7	14.253	BB	0.046	2085436	14.183	14.428
8	17.965	BB	0.047	2588474	17.897	18.153
9	19.341	BB	0.060	16388301	19.213	19.614
10	19.880	BB	0.068	540879	19.804	20.038
11	24.521	BB	0.059	7544732	24.388	24.756
12	26.683	BB	0.048	2259973	26.596	26.853

Data file: DATA:SEQ_B08A.D
File type: GC / MS DATA FILE

Sample Name: 3/14_06 C18 SPE of F.A. Std.
Misc Info:
Operator :

Date : 14 Mar 91 10:11 pm
Instrument: MS_5988
Inlet : GC

Sequence index : 2
Als bottle num : 8
Replicate num : 1



TIC of DATA:SEQ_B08A.D 7 integration peaks found.

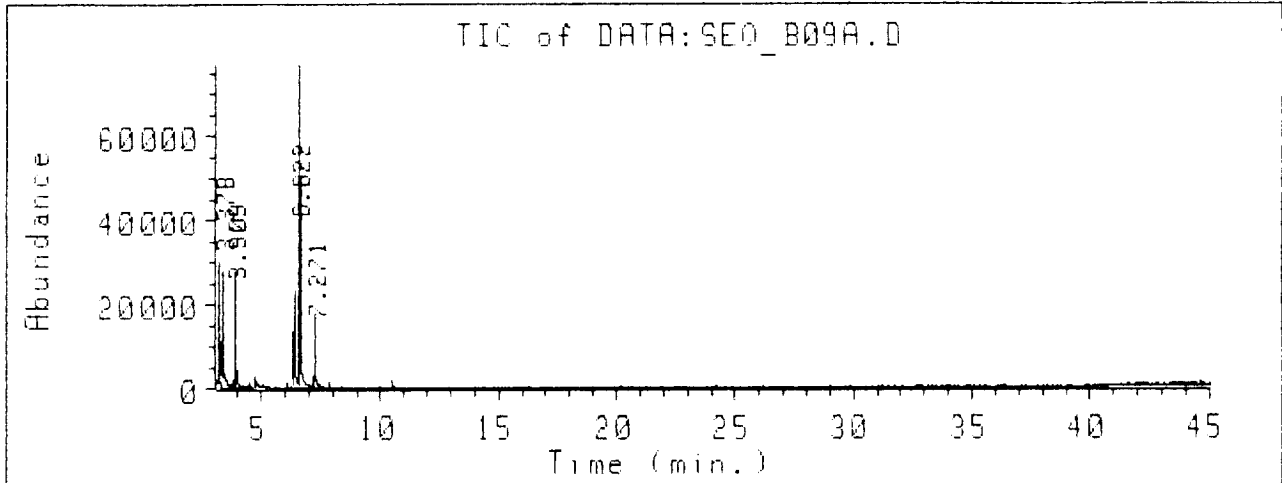
Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	5.808	BV	0.431	10131726	3.783	5.971
2	6.632	VV	0.277	16304195	5.971	6.775
3	7.294	VV	0.536	22881921	6.775	7.668
4	8.979	VV	0.577	39189283	7.668	9.142
5	10.002	VV	0.458	35194190	9.142	10.415
6	11.836	PV	0.395	43284800	10.415	12.201
7	13.579	PV	0.431	43136331	12.201	13.809

Data file: DATA:SEQ_B09A.D
File type: GC / MS DATA FILE

Sample Name: 3/14_07 C18 SPE of Al. Std.
Misc Info:
Operator :

Date : 14 Mar 91 11:03 pm
Instrument: MS_5988
Inlet : GC

Sequence index : 2
Als bottle num : 9
Replicate num : 1



TIC of DATA:SEQ_B09A.D 4 integration peaks found.

Page 1

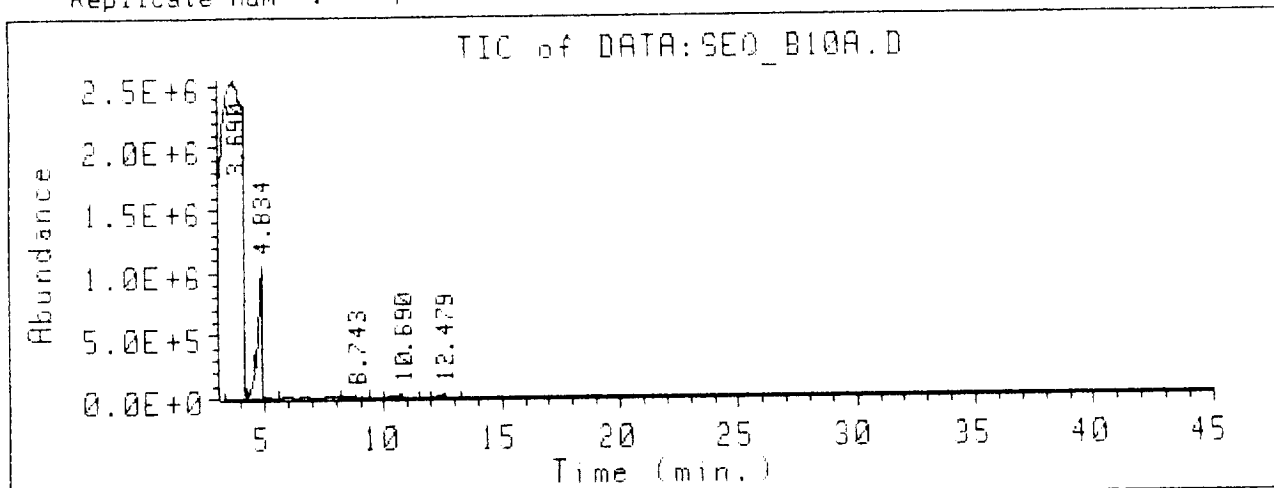
Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	3.378	PH	0.153	1228697	3.269	3.849
2	3.909	VH	0.163	836691	3.849	4.451
3	6.622	BV	0.167	3134758	6.123	7.172
4	7.271	PB	0.155	586245	7.172	7.841

Data file: DATA:SEQ_B10A.D
File type: GC / MS DATA FILE

Sample Name: 3/14_08 DIOL SPE H2O BLANK
Misc Info:
Operator :

Date : 14 Mar 91 11:55 pm
Instrument: MS_5988
Inlet : GC

Sequence index : 2
Als bottle num : 10
Replicate num : 1



TIC of DATA:SEQ_B10A.D 5 integration peaks found.

Page 1

Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	3.690	PH	0.828	1259965125	3.269	4.245
2	4.834	VV	0.236	120356250	4.245	5.564
3	8.743	VB	0.379	4070408	8.161	9.411
4	10.690	BB	0.374	5826570	9.992	11.511
5	12.479	BB	0.325	4072895	11.958	13.253

Data file: DATA:SEQ_B11A.D
File type: GC / MS DATA FILE

Sample Name: 3/14_09 DIOL SPE of F.A. Std.

Misc Info:

Operator :

Date : 15 Mar 91 12:47 am

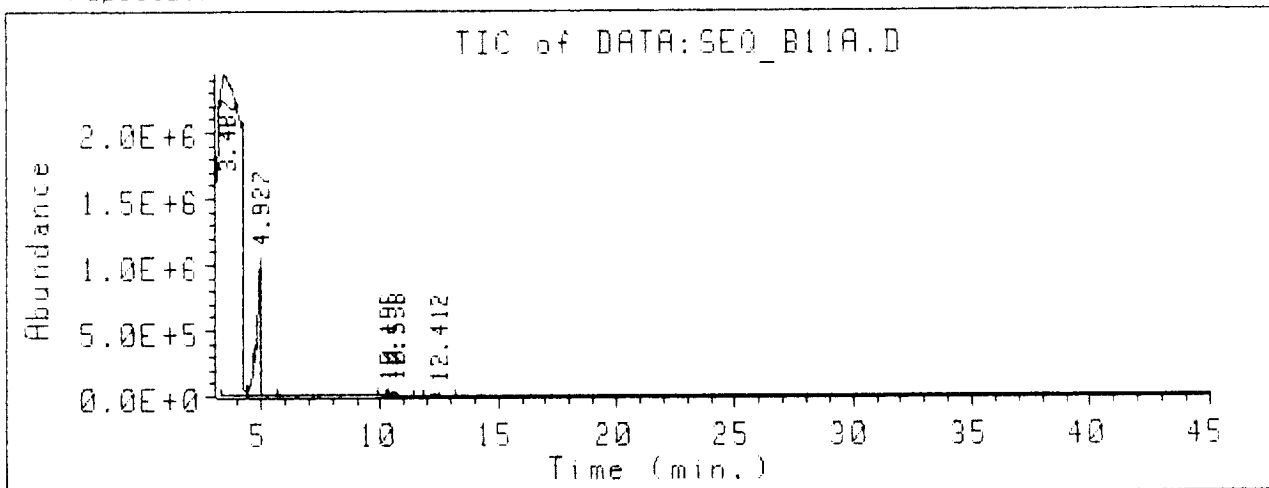
Instrument: MS_5988

Inlet : GC

Sequence index : 2

Als bottle num : 11

Replicate num : 1

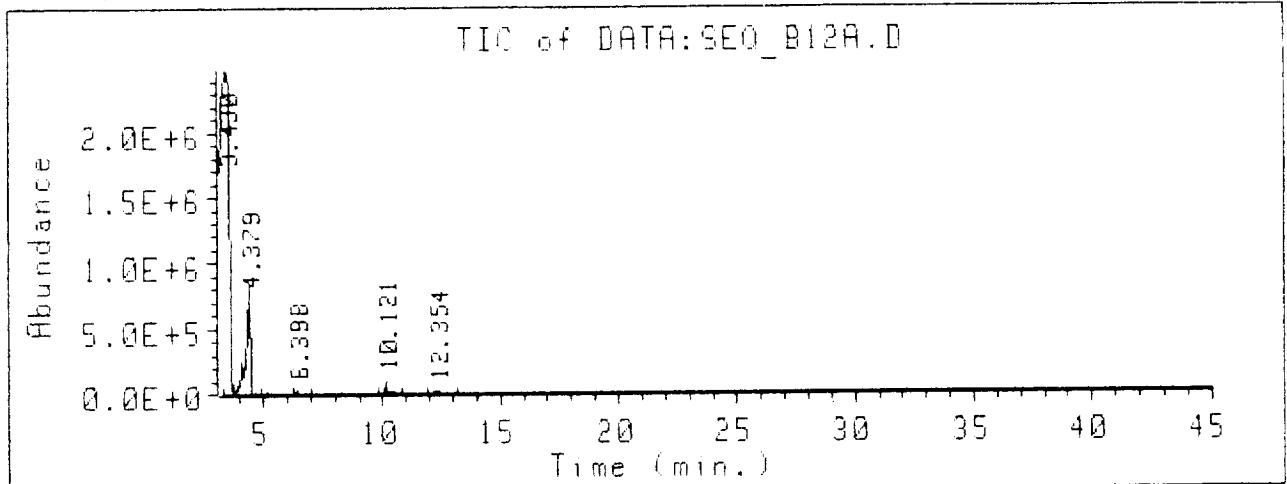


Data file: DATA:SEQ_B12A.D
File type: GC / MS DATA FILE

Sample Name: 3/14_10 DIOL SPE of Al. Std.
Misc Info:
Operator :

Date : 15 Mar 91 1:39 am
Instrument: MS_5988
Inlet : GC

Sequence index : 2
Als bottle num : 12
Replicate num : 1



TIC of DATA:SEQ_B12A.D 5 integration peaks found.

Page 1

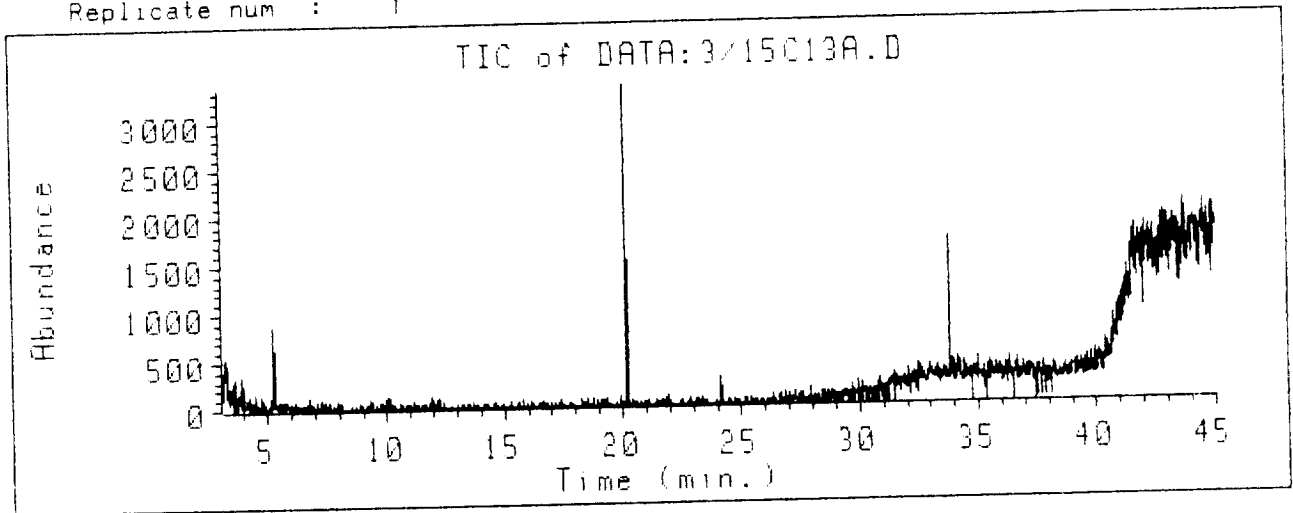
Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	3.430	PH	0.336	499874979	3.269	3.754
2	4.379	VV	0.237	96031805	3.754	4.921
3	6.398	VV	0.121	1492269	6.250	7.020
4	10.121	BB	0.213	5361856	9.788	10.771
5	12.354	BB	0.282	3306727	11.887	13.137

Data file: DATA:3/15C13A.D
File type: GC / MS DATA FILE

Sample Name: 3/14_11 C8 SPE H2O BLANK
Misc Info:
Operator :

Date : 15 Mar 91 5:35 pm
Instrument: MS_5988
Inlet : GC

Sequence index : 3
Als bottle num : 13
Replicate num : 1

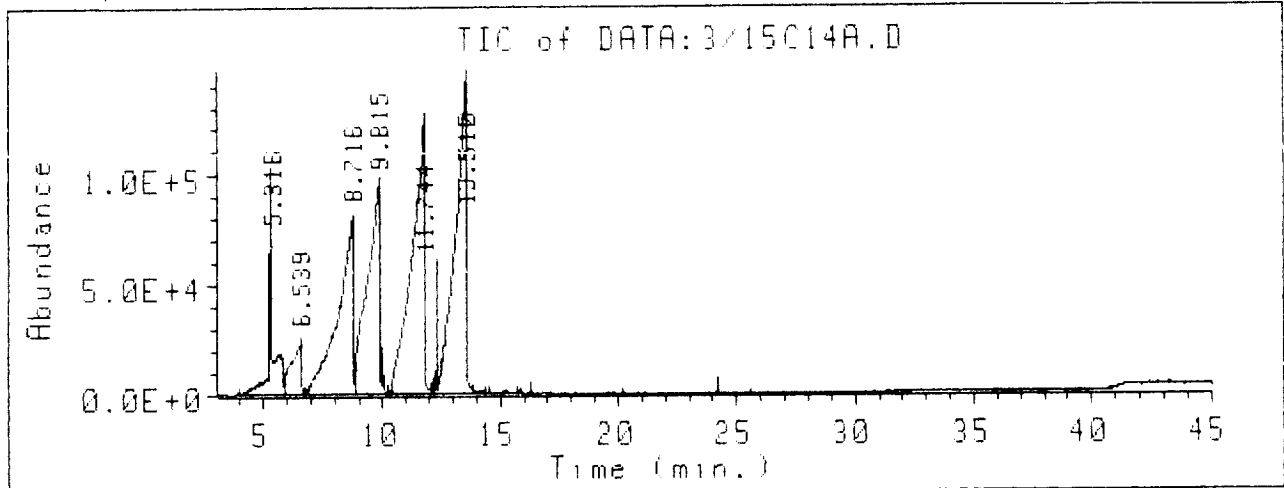


Data file: DATA:3/15014A.D
File type: GC / MS DATA FILE

Sample Name: 3/14_12 08 SPE of F.A. Std.
Misc Info:
Operator :

Date : 15 Mar 91 6:27 pm
Instrument: MS_5988
Inlet : GC

Sequence index : 3
Als bottle num : 14
Replicate num : 1



TIC of DATA:3/15014A.D 6 integration peaks found.

Page 1

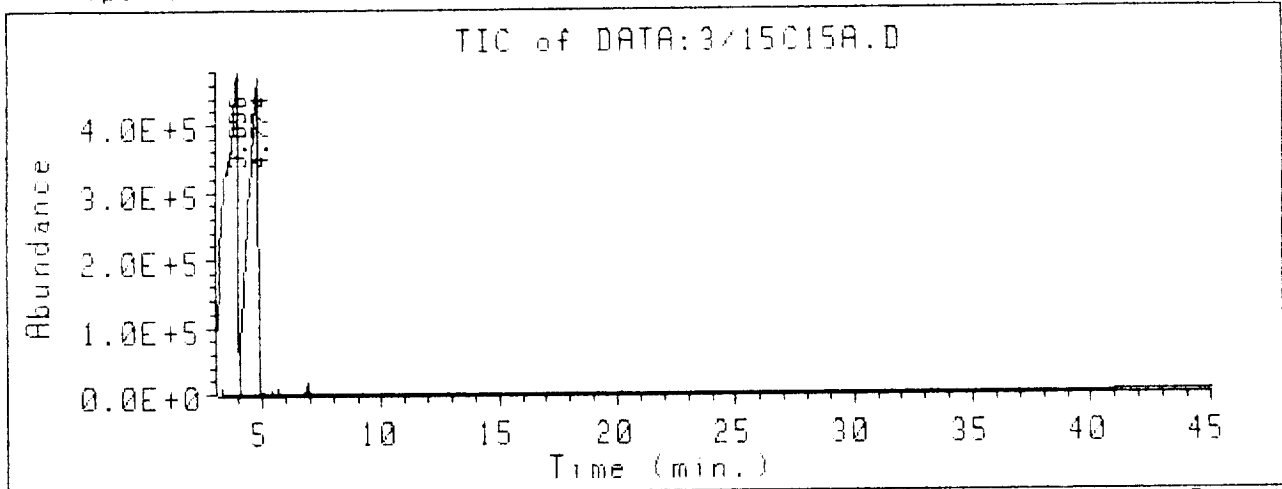
Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	5.316	BV	0.337	9848150	4.006	5.905
2	6.539	VV	0.438	6790321	5.905	6.776
3	8.716	PV	0.579	35182302	6.776	8.876
4	9.815	VV	0.495	35146986	8.876	10.261
5	11.744	VV	0.545	46643933	10.261	12.071
6	13.510	VB	0.465	48450903	12.071	14.483

Data file: DATA:3/15015A.D
File type: GC / MS DATA FILE

Sample Name: 3/14_13 C8 SPE of Al. Std.
Misc Info:
Operator :

Date : 15 Mar 91 7:19 pm
Instrument: MS_5988
Inlet : GC

Sequence index : 3
Als bottle num : 15
Replicate num : 1



TIC of DATA:3/15015A.D 2 integration peaks found.

Page 1

Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	3.896	PH	0.469	158164437	3.269	4.072
2	4.774	VH	0.396	135306240	4.072	5.656

Data file: DATA:3/15016A.D
File type: GC / MS DATA FILE

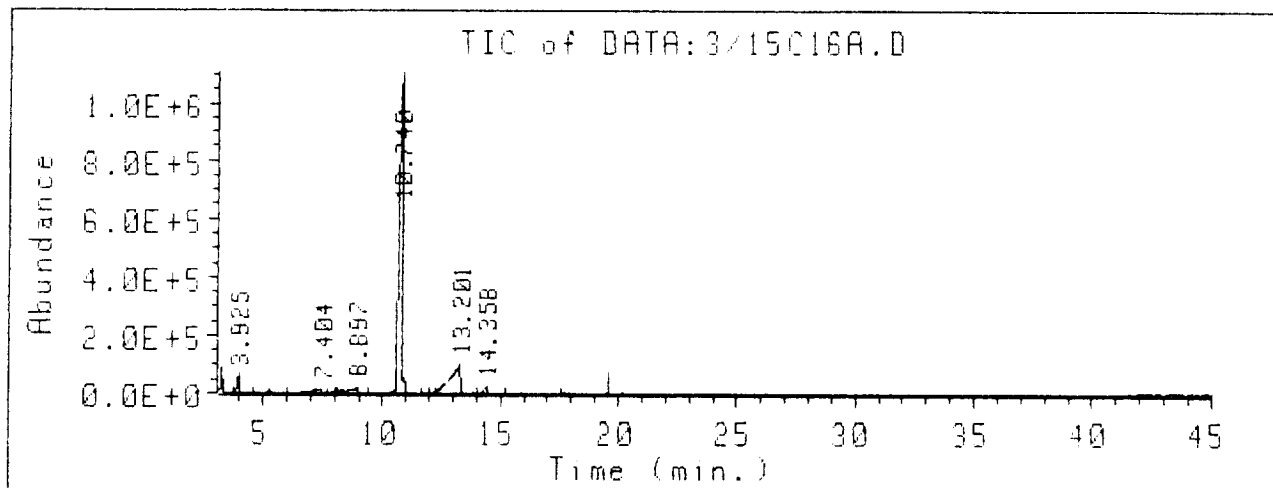
Sample Name: 3/14_14 C8 SPE of Organic Acids Std.

Misc Info:

Operator :

Date : 15 Mar 91 8:12 pm
Instrument: MS_5988
Inlet : GC

Sequence index : 3
Als bottle num : 16
Replicate num : 1



TIC of DATA:3/15016A.D 6 integration peaks found.

Page 1

Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	3.925	BV	0.196	4001542	3.715	4.541
2	7.404	BV	0.447	5503382	5.993	7.667
3	8.897	VV	0.776	7747099	7.667	9.408
4	10.740	PB	0.237	124548708	9.408	11.641
5	13.201	BV	0.483	32038884	11.998	14.052
6	14.358	VB	0.292	2924514	14.052	15.169

Data file: DATA:3/15017A.D
File type: GC / MS DATA FILE

Sample Name: 3/14_15 08 SPE of Al. Std. (Melvin's)
Misc Info:
Operator :

Date : 15 Mar 91 9:04 pm
Instrument: MS_5988
Inlet : GC

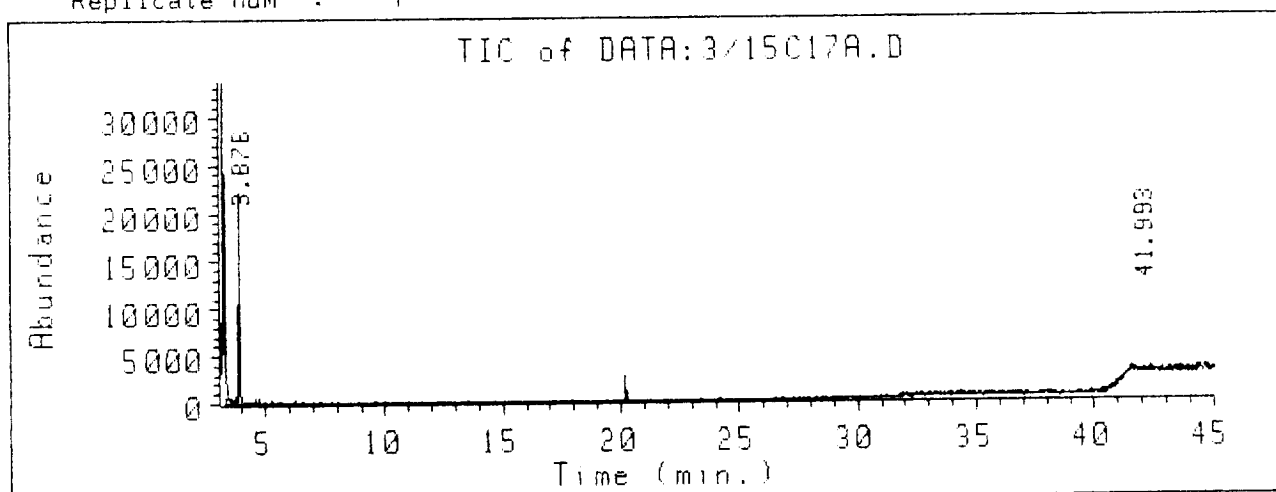
Sequence index : 3
Als bottle num : 17
Replicate num : 1

Data file: DATA:3/15017A.D
File type: GC / MS DATA FILE

Sample Name: 3/14_15 08 SPE of Al. Std.
Misc Info:
Operator :

Date : 15 Mar 91 9:04 pm
Instrument: MS_5988
Inlet : GC

Sequence index : 3
Als bottle num : 17
Replicate num : 1



TIC of DATA:3/15017A.D 2 integration peaks found.

Page 1

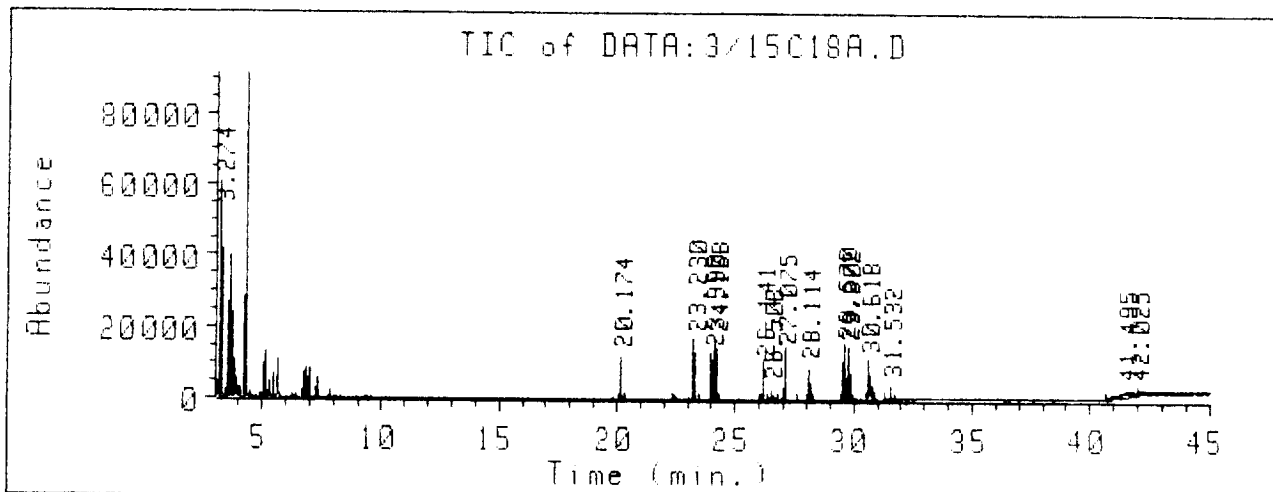
Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	41.993	PH	0.179	788404	3.269	3.715
2	3.876	VH	0.111	761591	3.715	4.562

Data file: DATA:3/15C18A.D
File type: GC / MS DATA FILE

Sample Name: 3/14_16 C8 SPE of Altech Pesticide Std. 7241
Misc Info:
Operator :

Date : 15 Mar 91 9:56 pm
Instrument: MS_5988
Inlet : GC

Sequence index : 3
Als bottle num : 18
Replicate num : 1



TIC of DATA:3/15C18A.D 15 integration peaks found.

Page 1

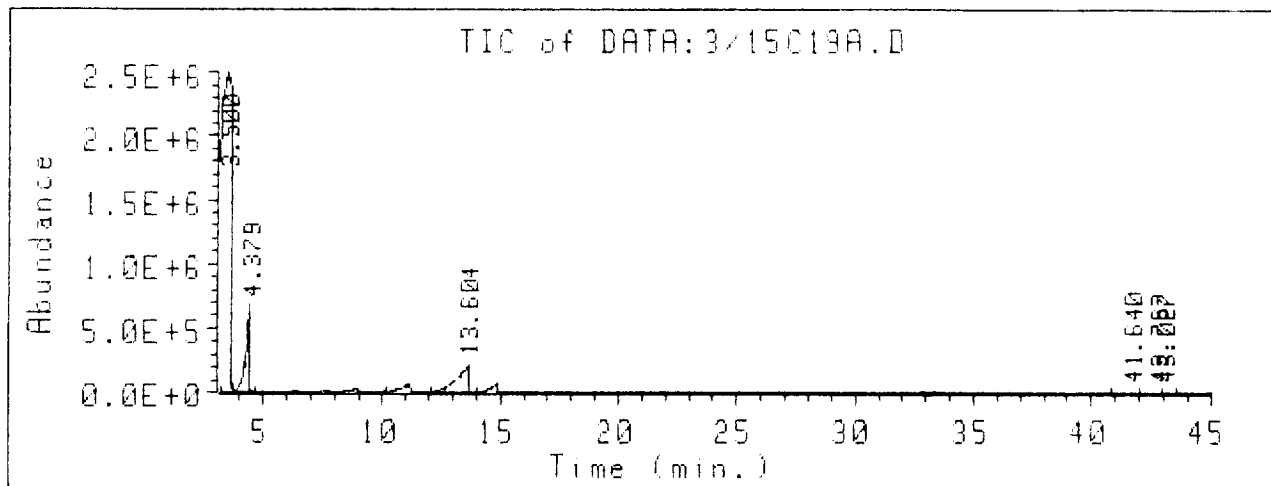
Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	3.274	PH	0.096	1671936	3.224	3.492
2	20.174	BB	0.045	305453	20.088	20.355
3	23.230	BV	0.088	499784	22.363	23.433
4	23.990	PH	0.054	384396	23.433	24.080
5	24.168	PH	0.084	481800	24.080	24.326
6	26.141	BV	0.096	295359	25.954	26.282
7	26.508	PV	0.117	173772	26.282	26.779
8	27.075	VB	0.050	380293	26.992	27.560
9	28.114	BV	0.045	222562	28.028	28.272
10	29.570	BH	0.078	418744	29.411	29.648
11	29.802	PV	0.111	615717	29.648	29.925
12	30.618	BV	0.093	520970	30.504	30.906
13	31.532	BV	0.081	113196	31.263	31.694
14	41.495	BV	0.569	478215	40.653	41.970
15	42.025	VV	0.114	52526	41.970	42.125

Data file: DATA:3/15019A.D
File type: GC / MS DATA FILE

Sample Name:
Misc Info:
Operator :

Date : 15 Mar 91 10:48 pm
Instrument: MS_5988
Inlet : GC

Sequence index : 3
Als bottle num : 19
Replicate num : 1



TIC of DATA:3/15019A.D 6 integration peaks found.

Page 1

Peak#	Ret Time	Type	Width	Area	Start Time	End Time
1	3.500	PH	0.363	620618969	3.224	3.771
2	4.379	VH	0.202	72793304	3.771	4.654
3	13.604	BB	0.505	73488176	12.094	14.038
4	41.640	VV	0.653	748671	40.803	42.038
5	42.767	VV	0.632	469676	42.038	42.925
6	43.027	VB	0.265	193365	42.925	43.551