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Organic Compounds in Cyanea capillata and Chrysaora quinquecirrha

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Abstract

Cyanea capillata and *Chrysaora quinquecirrha* are two species of jellyfish which emit an "iodoform-like" odor. Attempts to identify the compound(s) which produce this odor have been made using chromatography techniques. Thus far, results obtained from column chromatography and GC-MS analysis have been inconclusive. However, these data suggest an hypothesis that may explain the origin of the odor. A low molecular-weight, highly volatile compound, such as ethylene, is a likely candidate for this elusive, odor-causing chemical.

Introduction

Interest in this project stemmed from literature reports of various toxins found in marine organisms and the detection of an odorous compound in jellyfish. Toxins in marine animals include a wide range of molecules classified as acids, polyethers, and proteins (Yasumoto & Murata, 1993). Some of the most odorous poisons are the halogenated compounds. For example, 2,6-dibromophenol and 3-chloroindole have been identified in marine hemichordates (Ashworth & Comier, 1997; and Higa & Scheuer, 1975). Halogenated phenols, pyrroles, and benzylalcohols have also been identified in other benthic organisms such as annelids (e.g. Woodin et al., 1987). Halogenated compounds from these annelids are known to have antibiotic and antifungal activity (Woodin et al., 1987).

The initial interest in these compounds was prompted by their "iodoform-like" odor, and the detection of a similar scent has been detected in the jellyfish, *Cyanea capillata* and *Chrysaora quinquecirrha* (Balser, unpubl.). Although bioactive compounds have been reported from other cnidarians, odorous compounds in jellyfish have not yet been identified (Pawlik, 1993). We, therefore, employed techniques previously used to isolate and identify unknown compounds in biological tissues in an attempt to isolate the odor-causing compounds in *Cyanea capillata* and *Chrysaora quinquecirrha* . Specifically, we used gas chromatography with a mass spectral detector (GC-MS) and column chromatography in an attempt to identify the chemical structure of the odorous compounds in these two

species of jellyfish.

Cyanea capillata, more commonly known as Lion's mane jellyfish, and *Chrysaora quinquecirrha* are, more commonly known as the stinging nettle, in the class scyphozoa, order Semaeostomeae. In general, scyphozoans are found abundantly throughout the world from cold to tropical waters. They have a life cycle which is dominated by the medusa or free swimming stage, more commonly known as the jellyfish phase. Scyphomedusae are known to protect themselves with specialized cells called nematocytes which house organelles called nematocysts. The nematocysts contain various types of toxins including neurotoxic (affecting the nervous system), myotoxic (affecting the muscle tissue), hemolytic (affecting the blood cells), and necrotic poisons (causing death of tissue). Additionally, jellyfish, are carnivorous and use nematocysts to catch a wide range of prey including zooplankton and small fish. Furthermore, the toxins found in the nematocysts are proteinaceous and do not emit an odor.

The odor emitted by *Cyanea capillata* and *Chrysaora quinquecirrha* resembles ozone and smells slightly chlorinated. Similarities in smell to halogenated compounds in other invertebrate animals (specifically, the hemichordate, *Saccoglossus kowalevskii*) suggested that the compound in these jellyfish was perhaps a halogenated phenol or pyrrole. The general hypothesis of this study was that the odorous compounds present in these species of jellyfish are separate from the nematocyst toxins and possibly function as anti-predatory or anti-bacterial agents. Possible functions of odorous, halogenated compounds isolated from other marine animals include anti-microbial and toxic properties (Higa & Sakemi, 1982). The work presented here specifically attempts to identify the odor-causing compounds in these jellyfish and to determine if halogenated compounds are present.

Materials and Methods

Instrumentation and Standards

Two species of jellyfish, *Cyanea capillata* and *Chrysaora quinquecirrha,* were extracted in an attempt to isolate odorous compounds. In addition to these tissues, a bacterial sample and a series of standards were also analyzed. All of the samples during the experiment were analyzed using GC-MS or thin layer chromatography ,(TLC). The GC-MS data were obtained from a HP 6890 GC equipped with a column of HP-5MS crossed linked with 5% PHME siloxane. The column was 30m long, 0.25mm in diameter, and had a film thickness of 0.25um. The GC was equipped with a mass detector. In addition to

GC-MS analysis, TLC was performed using four different solvents (diethyl ether, petroleum ether, acetone, and a 1:1 mixture of petroleum ether and ethyl ether). Analysis via TLC demonstrates the presence of UV or IR active compounds. The standard solutions were prepared by dissolving 0.1g of known compounds in 40 ml of methanol. The standards tested were 2,4,6-tribromophenol, 2,6-dibromophenol, bromohydroquinone, and a mixture of all three. These known solutions were analyzed using GC-MS (spectra 1-4) and TLC (appendix 2) analysis.

Tissue Preparation

Samples of live *Chrysaora quinquecirrha* were delivered from Gulf Specimens, Panacea, Florida to the laboratory at Illinois Wesleyan University (IWU) on 9 September, 1997 and were frozen 2 days later using liquid nitrogen, sealed in a plastic bag, and stored in an ultracold, -80 freezer. The specimens were observed to have the iodoform-like odor.

On 8 October , 1997, 19.6g of frozen, *Chrysaora quinquecirrha* tissue were thawed to room temperature in 50ml of HPLC-grade methanol. Interestingly, the odor was no longer present. The tissue was homogenized in a 40ml Kontes glass homogenizer for approximately 7 minutes. The suspension was gravity filtered, and the residue washed with 50 ml of fresh methanol. Next, the filtrate was centrifuged in a Marathon 22 KBR at 7,500 rpm for 5 minutes. Following centrifugation, 50ml of nanopure water and 75ml of ethyl acetate were added, and the solution was allowed to partition. The organic layer was removed and examined via GC-MS analysis. Extractions series are summarized in Appendix 1 by trial #.

Approximately 2 weeks later, a second *Chrysaora quinquecirrha* extraction was performed. However, a mortor and pestle using dichromix washed sea sand was used to homogenize the tissue in response to the observation that the Kontes glass homogenizer did not disrupt the tissue effectively. The remainder of the extraction was similar to the aforementioned extraction (appendix 1, trial 2). Again, the sample was examined via GC-MS analysis.

Next, specimens of *Cyanea capillata* were collected from waters around San Juan Islands, Washington, frozen in liquid nitrogen, and shipped on dry ice to the laboratory at IWU. Upon arrival, the animals were observed to have a strong iodoform-like odor prior to storage in the ultracold, -80 freezer. Two weeks later, approximately 50g of this tissue were removed from the freezer and extracted using the previous method. The previously detected odor was not present. The sample was again tested via GC-

MS analysis (appendix 1, trial 3). Additionally, TLC was performed using a 1:1 mixture of petroleum ether and ethyl ether.

Cyanea capillata tissue was extracted a second time using a new technique. Approximately 373g of tissue were treated with 350ml of acetone during homogenation in a mortor using a pestle with dichromixed sea sand. The suspension was gravity filtered, and the filtrate was partitioned twice, once with 200ml of pet. ether and once with 200ml of ethyl ether. Both organic layers were evaporated by rotary evaporation. The petroleum ether layer yielded a small amount of a yellowish-orange oil, and the ethyl ether layer yielded a cloudy oil and a white solid. All three samples were tested via TLC analysis (appendix 2).

A third *Cyanea capillata* extraction was then performed. This time however, a blender was used instead of the mortor and pestle. This alteration in the method was used with hopes of disrupting the tissue more than possible with the mortor and pestle. The rest of the extraction was similar to the previous extraction with *C. capillata* (appendix 1, trials 6 & 7). The three samples obtained again were analyzed by GC-MS and TLC. The petroleum ether oil was also analyzed via column chromatography. The column was prepared by packing 10g of silica with 3mm of sand on top. Approximately 0.2g of the oil was added with 50ml of petroleum ether. A decreasing ratio (9:1 to 1:1) of petroleum ether to ethyl ether was added in 40ml aliquots. Seventy fractions were collected and tested via TLC (see appendix 2).

A trial to check the effectiveness of the extractions was performed on a 0.2g, thawed sample of the acorn worm, *Saccoglossus kowalewski*. The specimen was homogenized with a mortor and pestle with dichromixed sea sand in the presence of acetone. The aqueous solution was extracted with 5ml of pet ether and analyzed via GC-MS. The data conclusively showed halogenated compounds (spectrum 37). Therefore, the extraction methods used here are believed to be effective in isolating these halogenated compounds.

Live *Chrysaora quinquecirrha* were ordered from Marine Biological Laboratory (MBL), Woods Hole, MA. and arrived on January 22, 1998 at the IWU laboratory. Interestingly, these specimens produced slime, or mucus when removed from the water. Additionally, a faint odor was noted to be present. The tentacles were removed from the bell, and each section was extracted using the previous method. This extraction was repeated for the next two days on two more jellyfish (appendix 1, trials 8, 9, &10). All the samples were analyzed via GC-MS analysis.

On 25 March, 1998, 10 small *Cyanea capillata* were received from MBL. A faint, barely detectable,

odor was present in some specimens. Three separate extractions using the previous method were done (appendix 1, trial 11, 12, &13). Again, all samples were analyzed via GC-MS. In a concurrent experiment conducted by Andy Boyden, pieces of these jellyfish were removed and plated with bacterial cultures. His results show the jellyfish tissue that possessed the iodoform-like odor produced zones of inhibition in plate cultures and was associated with growth of an as yet unidentified rod-shaped bacterium.

Consequently, the unique bacteria was cultured and scraped into a vial. Two extractions were done, one using 1ml of chloroform and the other using1ml of petroleum ether. The samples were then filtered through a FH filter with a pore size of 0.5um and analyzed via GC-MS analysis.

Additional *Cyanea capillata* from MBL arrived at the IWU laboratory on April 8, 1998. The iodoform-like odor was present. Three specimens were combined and blended for approximately 2 minutes. The resulting solution was split into 4 equal parts and extracted with 4 different solvents (chloroform, ethyl acetate, petroleum ether, and 1:1 of chloroform and methanol). All samples were analyzed via GC-MS. In addition to the extraction a headspace analysis was done. Two live specimens were placed into a flask with an airtight septum over the opening. A gas sample was removed and injected into the GC-MS.

The final attempt to identify the elusive compound was done on approximately 70g, wet weight, of *Cyanea capillata*. The tissue was blended for 2 minutes. Fifteen ml of deuterated (d)-chloroform were added and allowed to stir for 24 hours with nitrogen bubbling. The chloroform layer was removed, dried, and analyzed via GC-MS and proton and 13C NMR. The remaining aqueous layer was extracted with petroleum ether and analyzed via GC-MS analysis. Finally, the remaining aqueous solution was distilled to remove the salt and then analyzed via GC-MS analysis.

Results

Chrysaora quinquecirrha

Analysis of three specimens of *C.quinquecirrha* with GC-MS indicates an absence of brominated and iodinated compounds in ethyl acetate and petroleum ether extracted samples (spectra 5-13). The characteristic double peaks at 79 and 81 (m/z) indicative of bromine were absent as was the typical iodine peak at 127.

Large peaks near the end of each chromatograph are thought to represent cholesterol or similar compounds. Although these peaks were not identified accurately by the MS, because of the original MS

settings, comparison of spectra shown in spectra 7 & 33, suggests that these peaks represent cholesterol.

Spectra of GC-MS show a consistent peak at a retention time of 35 minutes (spectra 9 & 11). This peak is identified by the MS as a long-chain carbon with a structure similar to fatty acids, typical of fats.

One of the largest GC peaks in each of these samples came off the column around 39 minutes and could be identified by the MS with 90% accuracy. Mass spectrometry indicates that this peak results from benzenedicarboxylic acid, more commonly known as phthlate, with a molecular weight of 279. Throughout the spectra, the side chains on this molecule identified by MS differ, from isooctyl esters to n-octyl esters (spectra 6 & 8).

Cyanea capillata

Analysis of 6 specimens of *C.capillata* with GC-MS indicates an absence of brominated and iodinated compounds in samples extracted with ethyl acetate, petroleum ether, and ethyl ether extracted samples (spectra 15-28). The characteristic double peaks at 79 and 81 (m/z) indicative of bromine were absent as was the typical iodine peak at 127.

As seen in the *C.quinquecirrha,* large peaks at the end of the chromatographs are thought to represent cholesterol or similar compounds (spectrum 33). The MS has identified the compounds with 99% assurance. These results are expected since cholesterol is a major component of biological membranes. Additionally, the long-chain carbon structures have been identified in *C. capillata* (spectra 17, 21&27). These molecules were found in petroleum ether and ethyl ether extractions. Again, these fatty acid molecules are typical of fats, and are found in biological membranes.

As seen in the *C. quinquecirrha* tissue, the phthalate compounds also are present in the *C. capillata* extractions with petroleum ether and ethyl ether (spectra 16, 20, 23, and 25). The possible side chains on phthalate in this tissue ranged from methylpropyl to n-octyl groups.

The set of extractions done in March (appendix 1, trials 12 and 13) yielded inconclusive results, probably due to insufficient amounts of tissue during the extractions. The spectra obtained showed no signs of halogenated compounds, phthlate, or the long-chain acid molecules (spectra 26-28).

Similar results were obtained from the April extractions (appendix 1, trials 15-19). However, the compounds that were identified from these samples were cholesterol (spectrum 33) and air (spectra 32,

34, & 36). The air peaks are present in all samples in April because of a leaky septum which rendered the data unusable. This is unfortunate because of all samples analyzed, these specimens emitted the strong odor during the time of extraction.

Saccoglossus kowalewski and Bacteria

Results from *S. kowalewski* show the presence of bromination (spectrum 37). The 79 and 81 (m/z) peaks indicative of bromine are present as are the two peaks at 224 and 226 which represent the mass of the molecule without either isotope of bromine as a side group.

The bacterial results are inconclusive because of contamination from the column. The compounds identified by MS in these extractions are siloxane compounds (spectra 38&39). The column used is cross-linked with 5% PHME siloxane, indicating that the contamination was coming from the column.

Discussion

The original hypothesis of this study was that the odorous compounds observed in *Cyanea capillata* and *Chrysaora quinquecirrha* are halogenated and probably function as anti-predatory or anti-bacterial agents. Therefore, the extraction techniques used were aimed at recovering halogenated compounds similar to those found in other invertebrate animals.

Known halogenated compounds found in other marine organisms were used as standards. The data obtained from these standard chemicals from GC-MS and TLC analysis provided a basis to examine the unknown compounds in jellyfish. In the mass spectral data, halogenated compounds have unique signatures. For example, since bromine occurs as equal isotopes with mass to charge ratios (m/z) of 79 and 81, these peaks indicate the presence of bromine. Additionally, iodine gives unique peaks at 127 and (mass - 127), which represents the molecule after iodine is knocked off. Furthermore, the TLC analysis on the knowns was useful in choosing a solvent which effectively separates halogenated types of compounds. Additionally, these data gave Rf values which could be compared to the Rf values of the unknowns.

The first extraction on *Chrysaora quinquecirrha* (appendix 1, trial 1) utilized a technique that isolated and identified brominated compounds in an acorn worm, *Saccoglossus kowalewski* (Woodin et al,

1987). However, none of the GC data showed any evidence of the presence of halogenated compounds (spectrum 5). In fact, the only peak which could be identified with confidence was phthalate, found at a retention time of 50 minutes. This compound was found in tissues of both species tested during this project.

Observations during the first extraction using a glass Kontes homogenizer revealed that the tissue was not being homogenized enough to expose internal compounds to the solvent. Therefore, a mortor and pestle with sea sand was used in the second extraction. Once again however, none of the data showed any sign of halogenated compounds.

The next set of extractions used *Cyanea capillata* tissue. Again, prior to being in the ultracold freezer the specimens were observed to contain the iodoform-like odor. However, during the time of the extractions the odor was no longer present. In spite of this, the tissue was extracted as before. The results from the GC-MS analysis were again inconclusive in showing the presence of halogenated compounds. However, products were obtained from evaporation of the solvents. The smearing of the spots indicated little if any separation had occurred. The oils and solid obtained were tested via TLC, and the results were inconclusive. The oil and solid from the evaporation of ethyl ether did not spot indicating no UV or IR active compounds were present.

A larger extraction was done on *Cyanea capillata* tissue. In an attempt to recover more product, acetone was used in conjunction with a blender to further disrupt the tissue. In addition to acetone, petroleum ether and ethyl ether were used as the extracting solvents. This time a greater yield of the two oils and solid was obtained, and samples were tested via GC-MS and TLC analysis. The GC-MS data was again inconclusive, as was the TLC data because of spot smearing in all used solvents. Smearing indicates that the samples were not separate on the chromatography plates. Therefore, column chromatography was utilized in an attempt to separate the oil from the petroleum ether extraction which spotted on TLC. The fractions obtained from the column smeared again during TLC analysis. From this, it was concluded that the separation was not clean enough to use NMR analysis.

Live animals were ordered in an attempt to test samples which actually contained the odor. During the next five extractions, the data again showed no signs of halogenation. Therefore, a new hypothesis was made. The data obtained thus far, supports the hypothesis that the odor-causing compound is a highly-volatile, low molecular-weight gas, like ethylene. The hypothesis is supported by the disappearance of the compound at -80 C in the ultracold freezer. Additionally, if ethylene were

present, the GC-MS would not be able to conclusively identify it because the MS would mistake it for nitrogen in the air.

In accord with the hypothesis that the compound might be ethylene, data obtained in a study by Andy Boyden demonstrated the growth of an unusual bacterium on plates smeared with the jellyfish tissue that contained the odor. Therefore, it was deduced that possibly the bacteria are producing or assisting in the production of the elusive compound(s). Some bacteria are known to produce toxic compounds and ethylene (Taiz & Zeiger, 1991). In line with these reports, the bacteria were extracted with petroleum ether in an attempt to locate a compound similar to what is seen in the jellyfish data. However, thus far a leaky septum in the GC has rendered the data unusable.

A final attempt to isolate the compound in jellyfish was performed on the basis that ethylene might be the product. The extraction with d-chloroform was allowed to stir overnight with nitrogen bubbling over it in order to concentrate the sample. The d-chloroform was then tested via NMR and GC-MS analysis. However, the NMR spectrum was inconclusive and the GC-MS data has yet to be analyzed.

Future work on this project will examine the hypothesis that the compound might be a highly-volatile, low-molecular weight compound. The GC-MS data from the last sample of chloroform will be analyzed and a new , longer NMR analysis will be performed on the d-chloroform sample. Tests which can be done in an attempt to locate a compound of this nature include using a GC equipped with a flame-ionization detector. Additionally, oven conditions on the GC will be altered to lower temperatures with headspace analysis. Furthermore, attempts to dissolve the compound will include bubbling the headspace through petroleum ether and then concentrating the solution.

Overall, the project has not yielded any conclusive data to show the identity of the elusive compound. However, the data do support the new hypothesis put forth and the research will be continued in that direction.

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Appendix 1
extractions

Trial #	Date	Specimen	Weight	Homogenizer	Solvent	Analysis
1	10/8/97	*Chrysaora quinquecirrha*	19.6g	Kontes homogenizer	75ml of ethyl acetate	GC-MS
2	10/22/97	*Chrysaora quinquecirrha*	30.3g	mortor & pestle	45ml of ethyl acetate	GC-MS
3	10/26/97	*Cyanea capillata*	50.3g	mortor & pestle	40ml of ethyl acetate	GC-MS
4	11/5/97	*Cyanea capillata*	375g	mortor & pestle	350ml acetone & 200ml of pet ether	TLC
5	11/5/97	*Cyanea capillata*	375g	mortor & pestle	350ml of acetone & 200ml of ethyl ether	TLC
6	11/11/97	*Cyanea capillata*	1kg	blender	300ml of acetone & 200ml of pet ether	GC-MS & TLC
7	11/11/97	*Cyanea capillata*	1kg	blender	300ml of acetone & 150ml of ethyl ether	GC-MS & TLC
8	1/22/98	*Chrysaora quinquecirrha*	33.7g	blender	25ml of acetone & 25ml of pet ether	GC-MS
9	1/23/98	*Chrysaora quinquecirrha*	53.1g	blender	15ml of pet ether	GC-MS
10	1/24/98	*Chrysaora quinquecirrha*	21.6g	blender	15ml of pet ether	GC-MS
11	3/25/98	*Cyanea capillata*	18.5g	blender	20ml of pet ether	GC-MS
12	3/25/98	*Cyanea capillata*	8g	blender	20ml of pet ether	GC-MS
13	3/25/98	*Cyanea capillata*	7g	blender	20ml of pet ether	GC-MS
14	4/7/98	Bacteria	0.1g	blender	1ml of pet ether &1ml of chloroform	GC-MS
15	4/8/98	*Cyanea capillata*	170g	blender	15ml of chloroform	GC-MS
16	4/8/98	*Cyanea capillata*	170g	blender	15ml of ethyl acetate	GC-MS
17	4/8/98	*Cyanea capillata*	170g	blender	15ml of pet ether	GC-MS
18	4/8/98	*Cyanea capillata*	170g	blender	15ml of 1:1 (chloro & methanol)	GC-MS
19	4/11/98	*Cyanea capillata*	69g	blender	15ml of d-chloroform	GC-MS &NMR

Appendix 2
TLC data

standards

compound	solvent	Rf
2,6-dibromophenol	pet ether	0.100
2,4,6-tribromophenol	pet ether	0
hydroquinone	pet ether	0
mixture of all three	pet ether	0

compound	solvent	Rf
2,6-dibromophenol	acetone	0.708
2,4,6-tribromophenol	acetone	0.741
hydroquinone	acetone	0.667
mixture of all three	acetone	0.743

compound	solvent	Rf
2,6-dibromophenol	diethyl ether	0.588
2,4,6-tribromophenol	diethyl ether	0.896
hydroquinone	diethyl ether	0.406
mixture of all three	diethyl ether	0.692, 0.754, 0.846

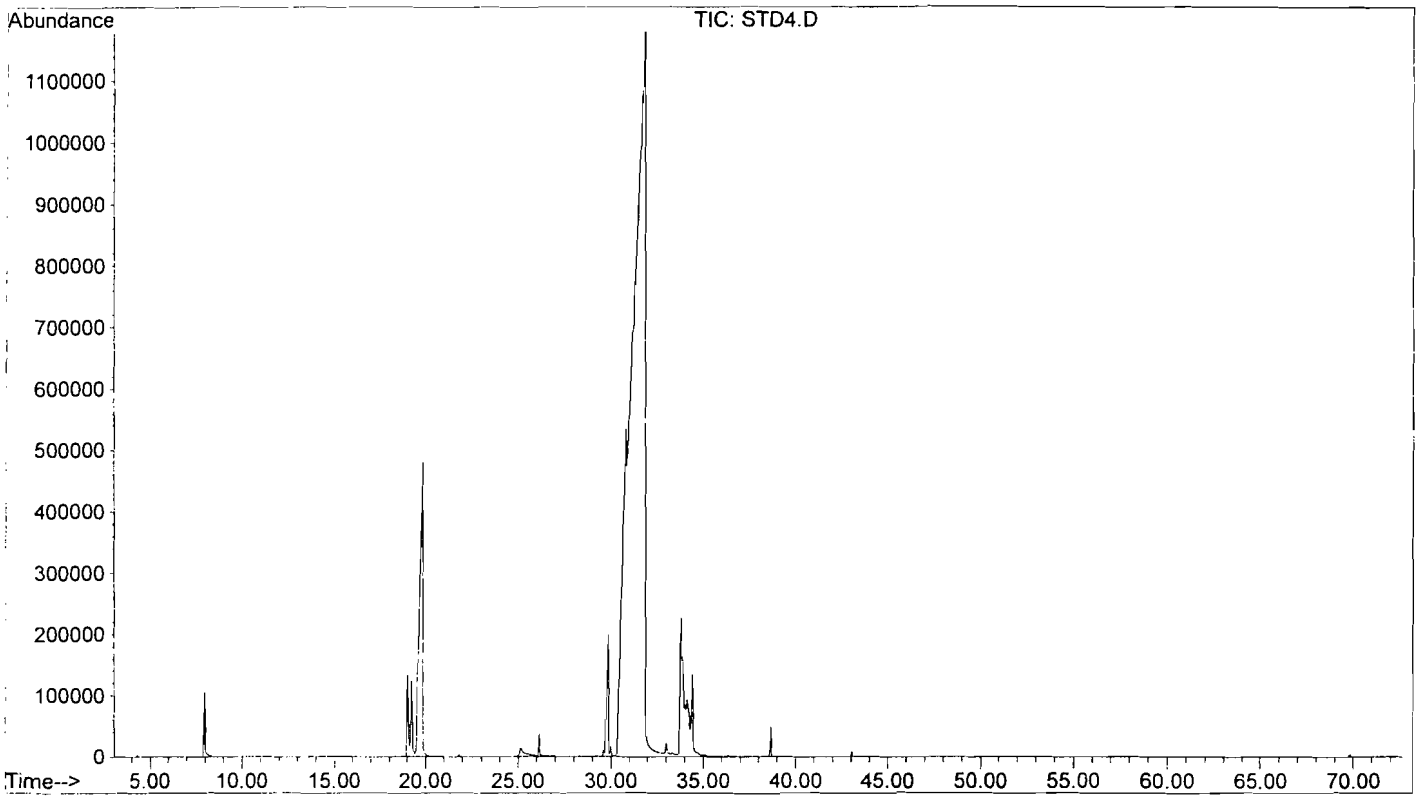
compound	solvent	Rf
2,6-dibromophenol	1:1	0.707
2,4,6-tribromophenol	1:1	0.159
hydroquinone	1:1	0.345
mixture of all three	1:1	0.672, 0.344, 0.000

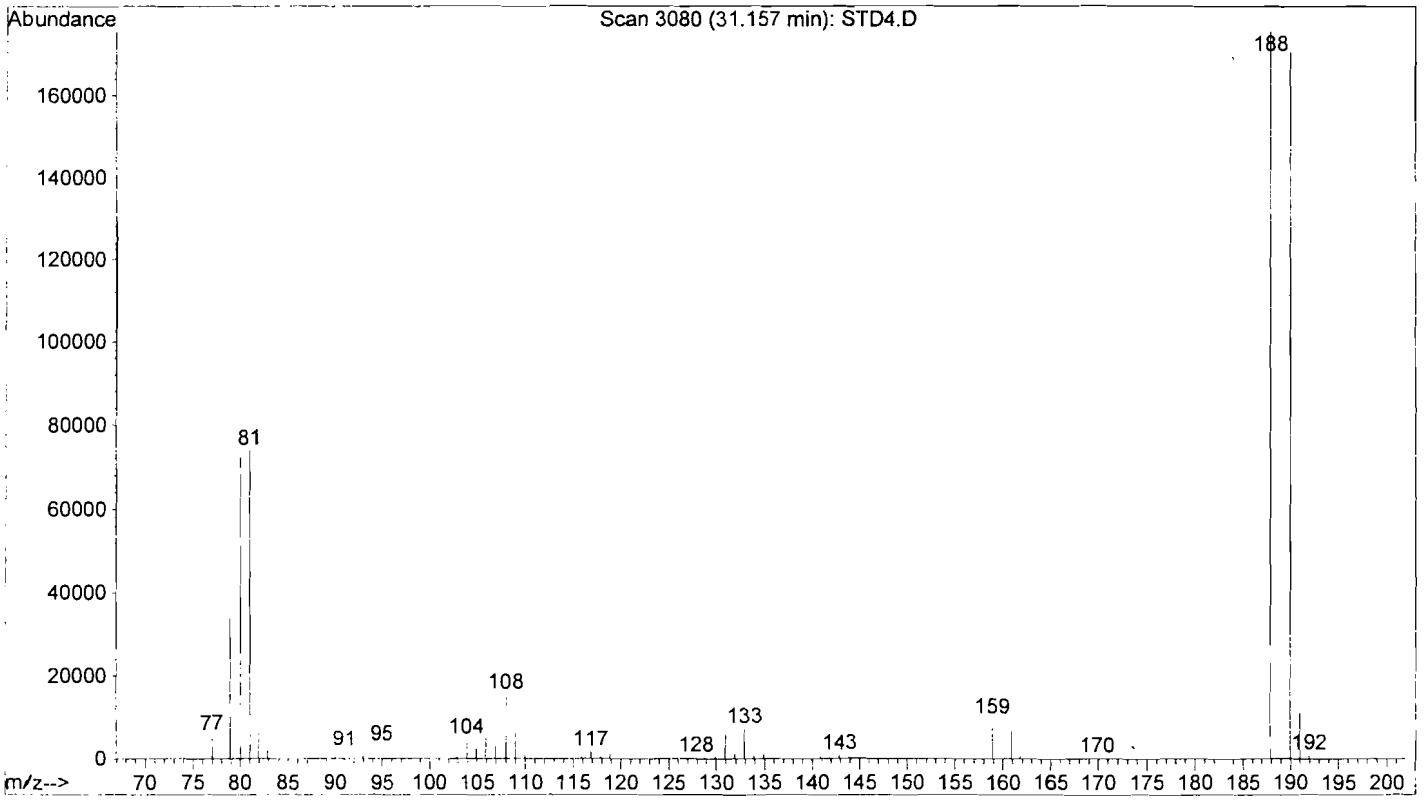
column chromatography data
(solvent 1:1 of pet ether and diethyl ether)
(appendix 1, trial 6&7)

fraction	Rf
1-14	no spot
15-16	0.733
17-20	0.683 & 0.708
21-32	no spot
33-38	0.561
39-40	no spot
41-43	0.593
44-50	no spot
51-53	0.231, 0.481, & 0.615
54-57	0.400
58-70	no spot

Spectrum 1

File : C:\HPCHEM\1\DATA\STD4.D
Operator : Kris Mitchell
Acquired : 6 Oct 97 1:22 pm using AcqMethod INVERT
Instrument : GC/MS Ins
Sample Name: bromohydroquinone
Misc Info :
Vial Number: 1





Spectrum 2

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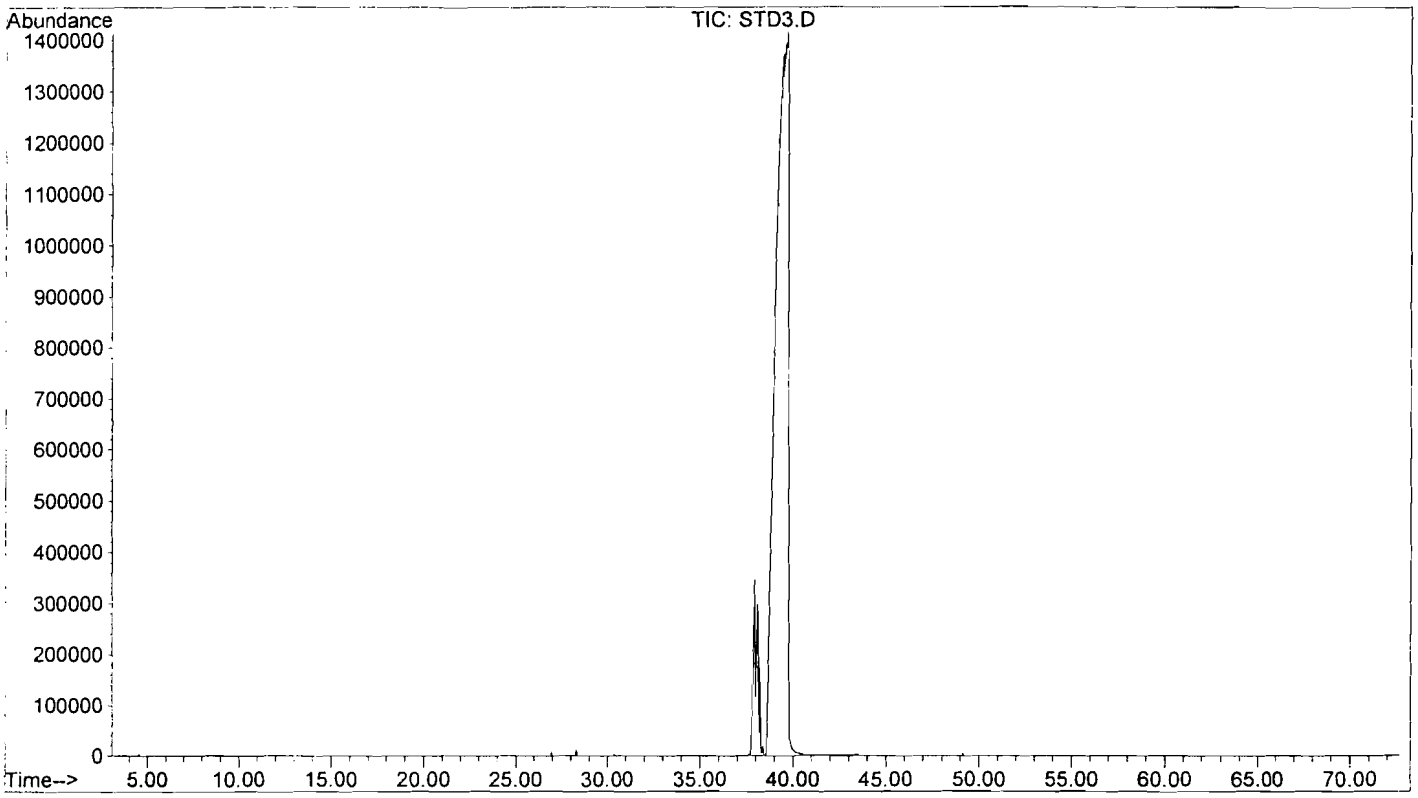
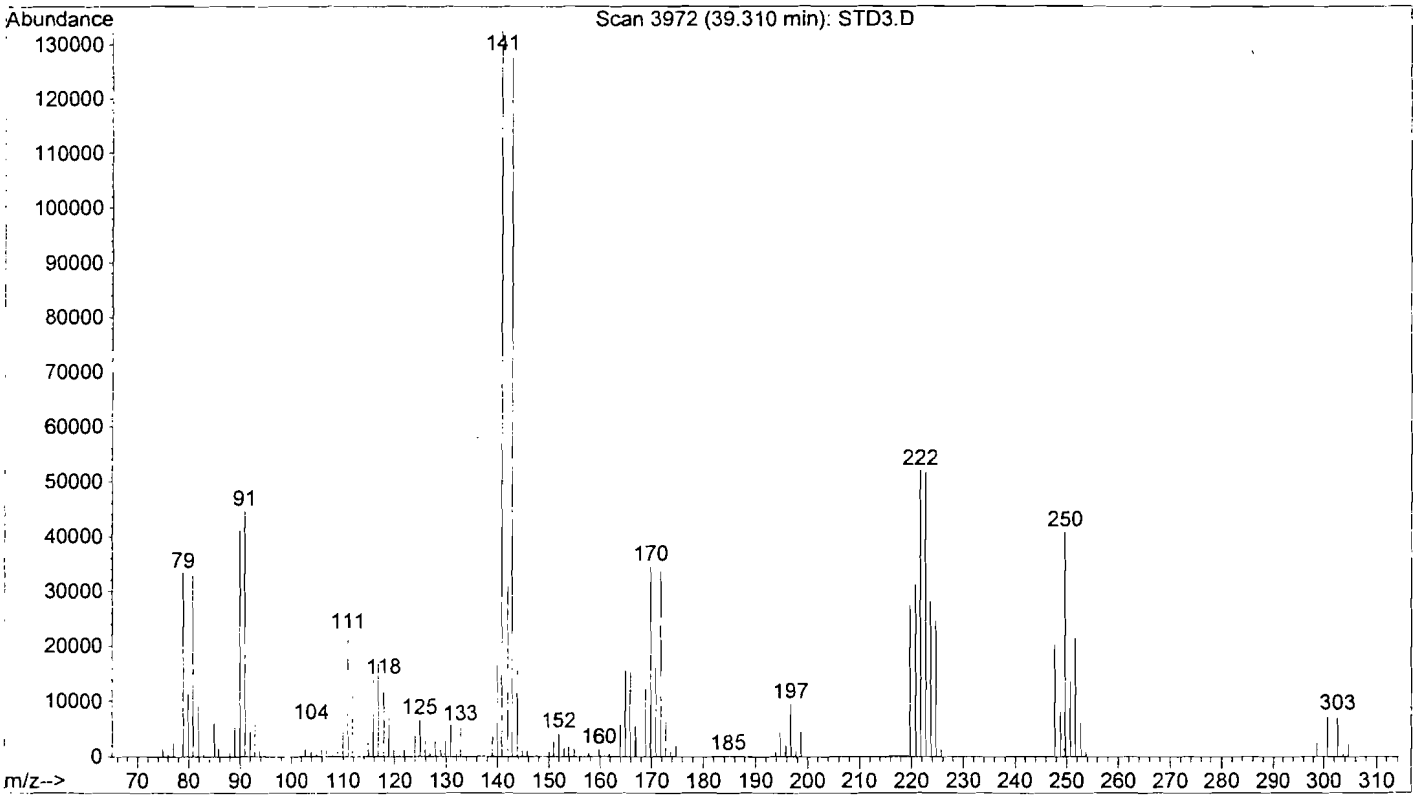
Acquired : 6 Oct 97 11:08 am using AcqMethod INVERT
Instrument : GC/MS Ins
Sample Name: 2,4,6 tribromo phenol
Misc Info : .1 g of each in 40 mLof methanol
Vial Number: 1





Spectrum 3

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Operator : Kris Mitchell
Acquired : 3 Oct 97 1:25 pm using AcqMethod INVERT
Instrument : GC/MS Ins
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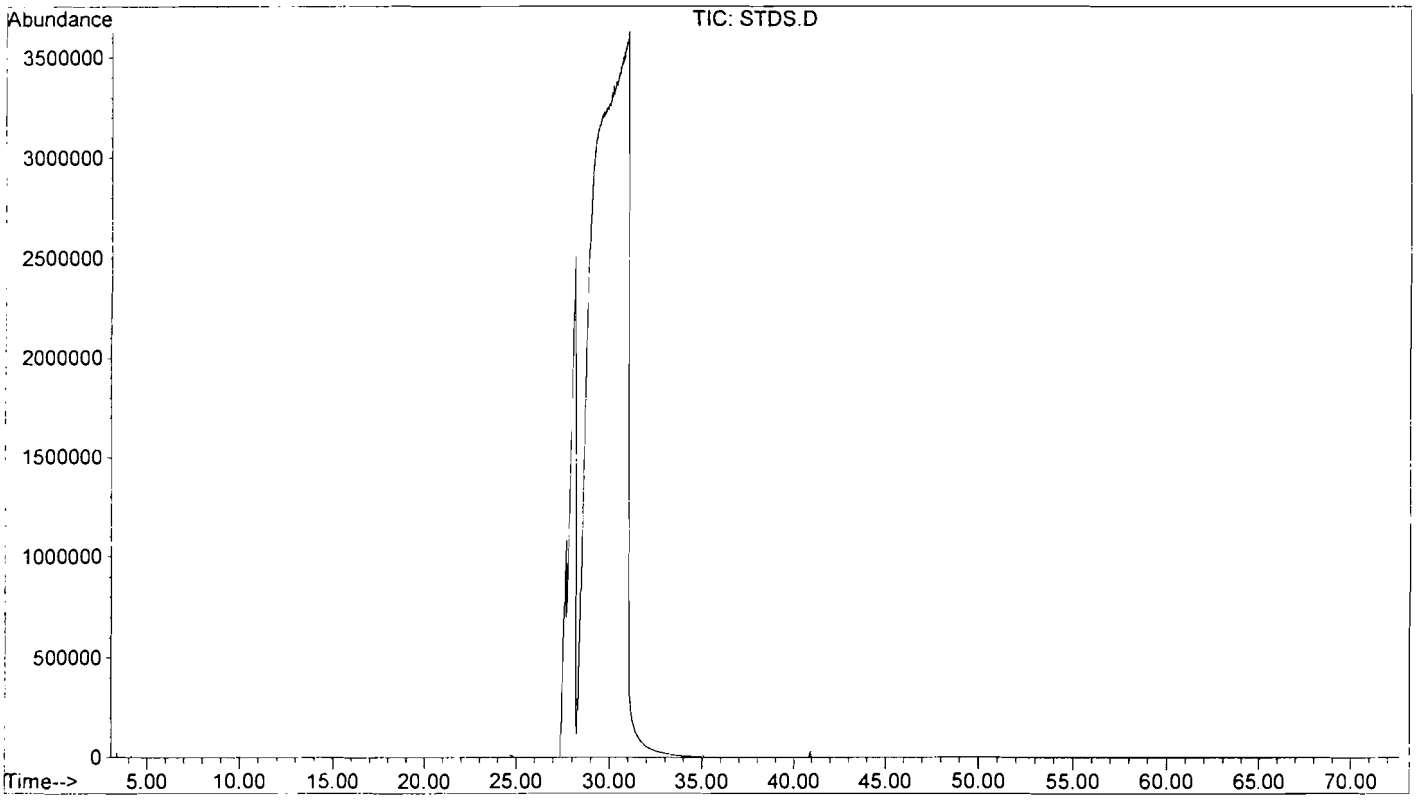
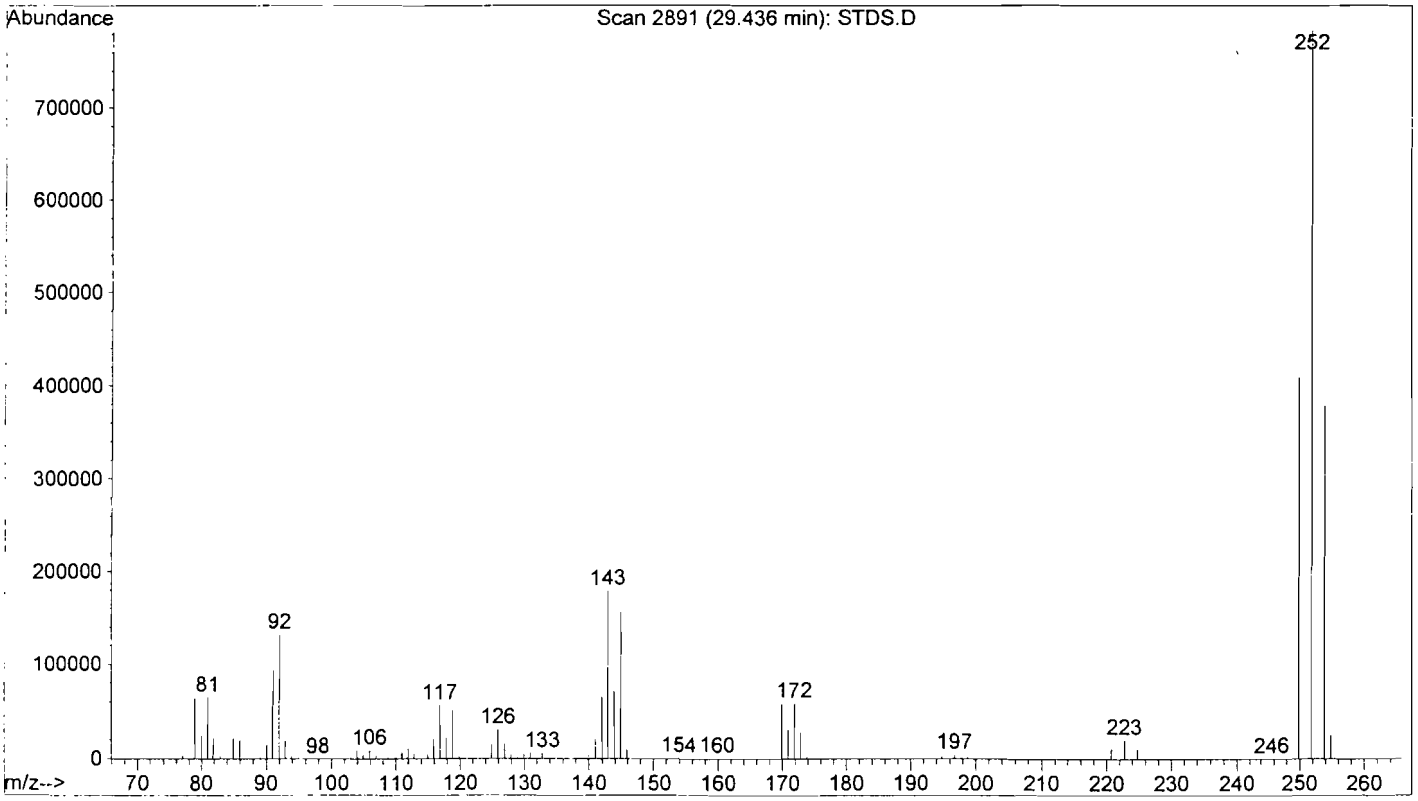
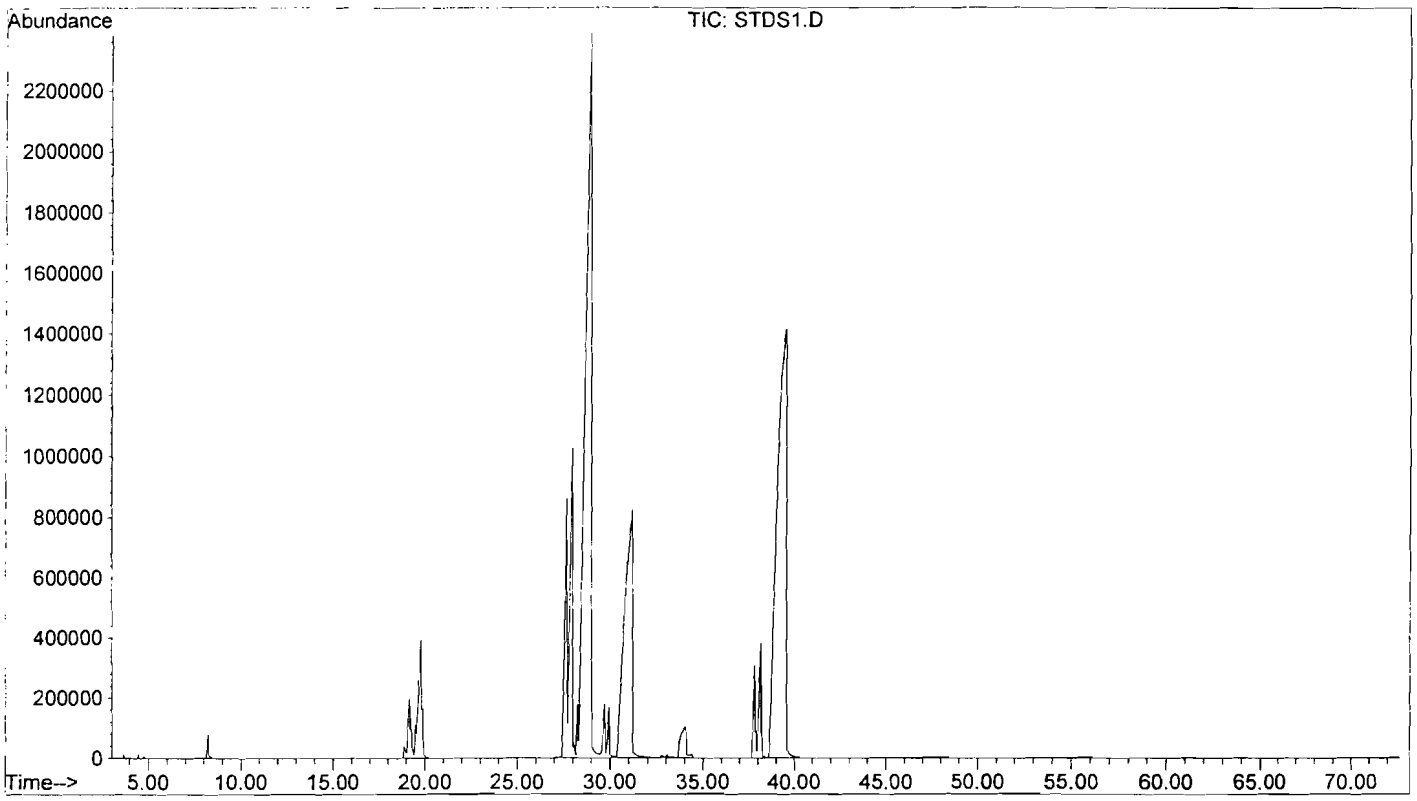
Misc Info : .2 g in 5 mL MeOH
Vial Number: 1

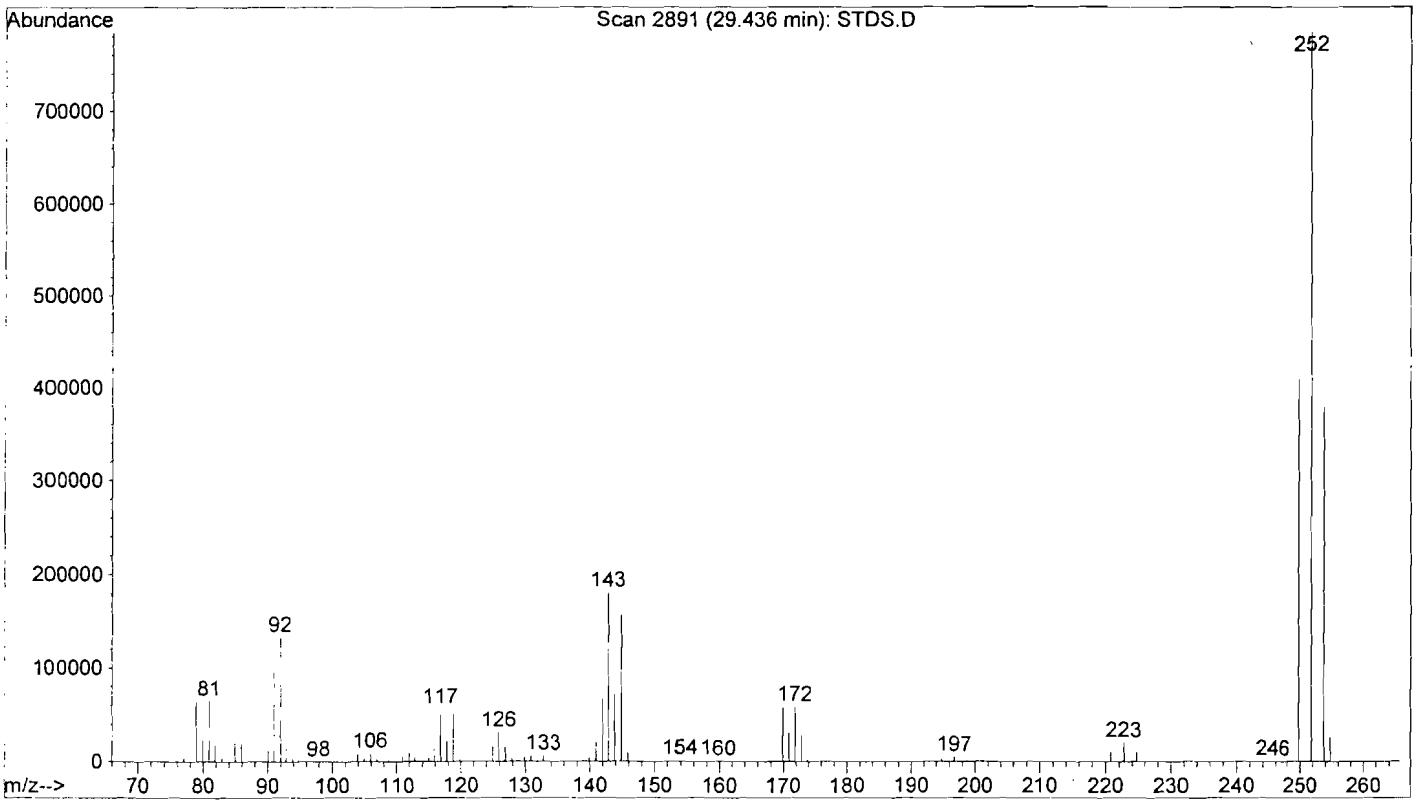




Spectrum 4

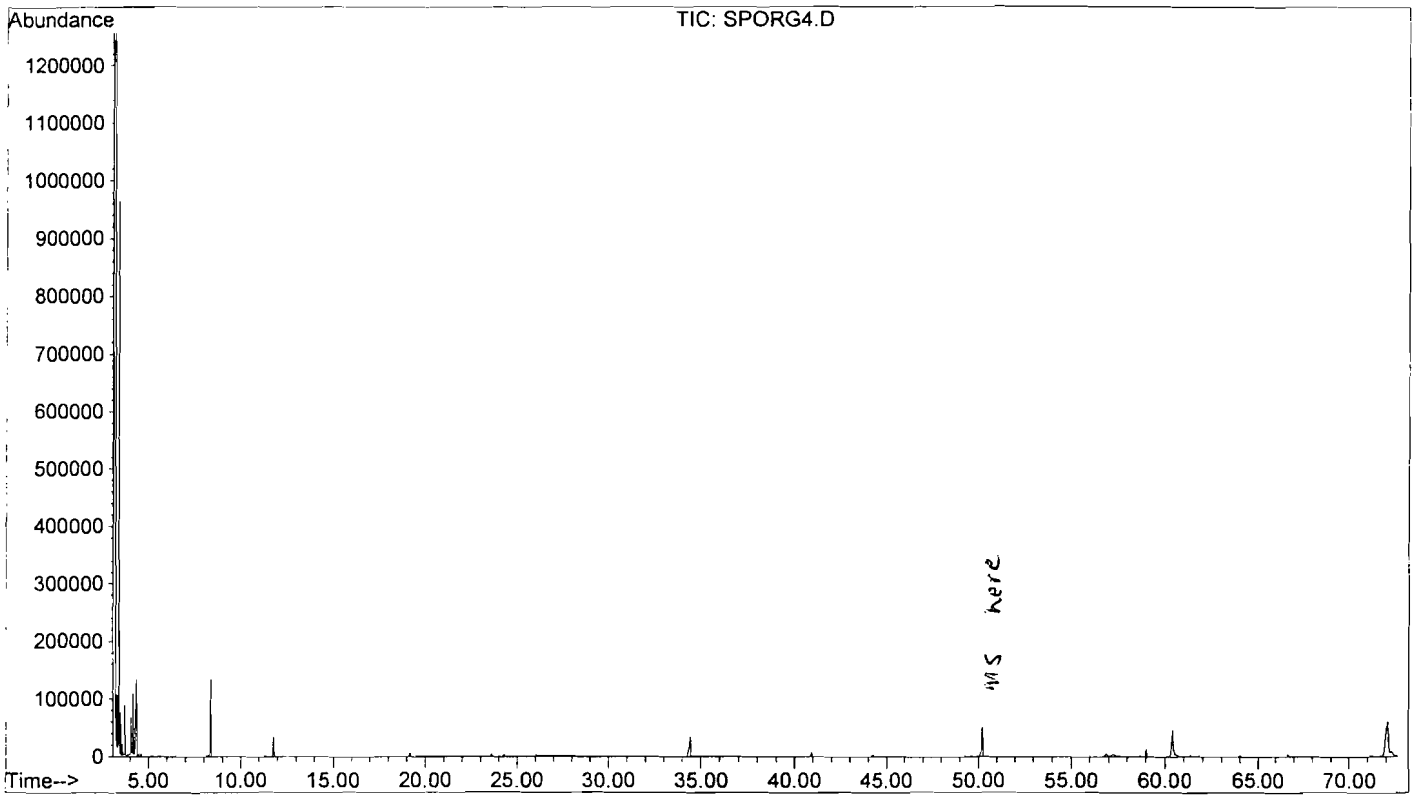
File : C:\HPCHEM\1\DATA\STDS1.D
Operator : Kris Mitchell
Acquired : 3 Oct 97 2:46 pm using AcqMethod INVERT
Instrument : GC/MS Ins
Sample Name: Standard Mixture
Misc Info : .1 g of each in 40 mLof methanol
Vial Number: 1

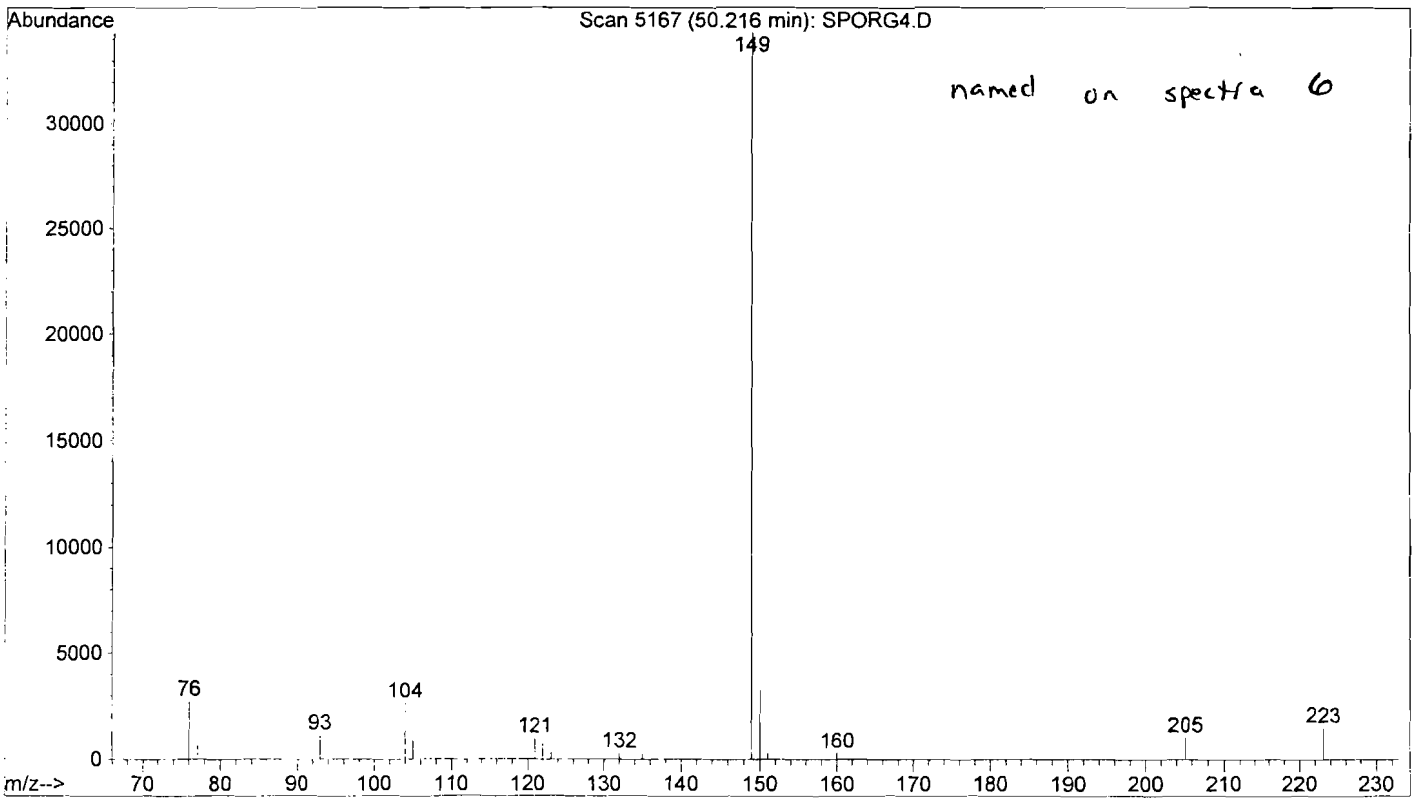




Spectrum 5

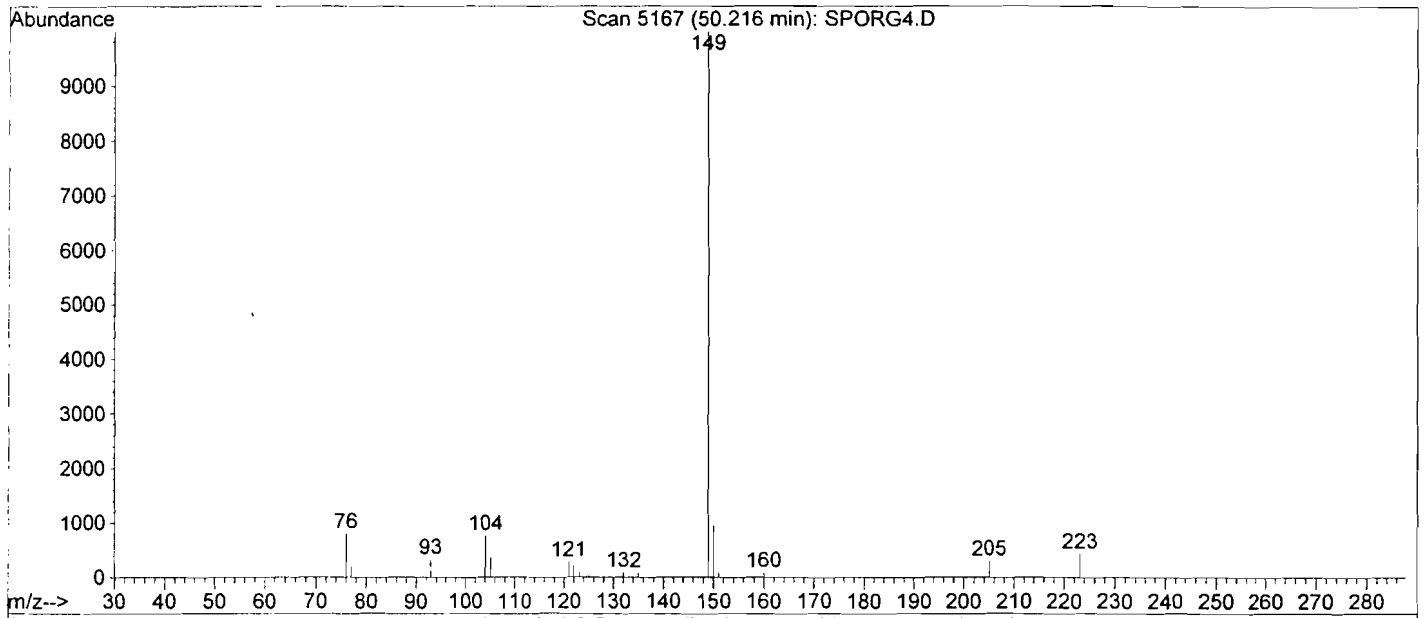
File : C:\HPCHEM\1\DATA\SPORG4.D
Operator : Kris Mitchell
Acquired : 14 Oct 97 8:39 am using AcqMethod INVERT
Instrument : GC/MS Ins
Sample Name: sample in organic layer concentrated with N2
Misc Info :
Vial Number: 1

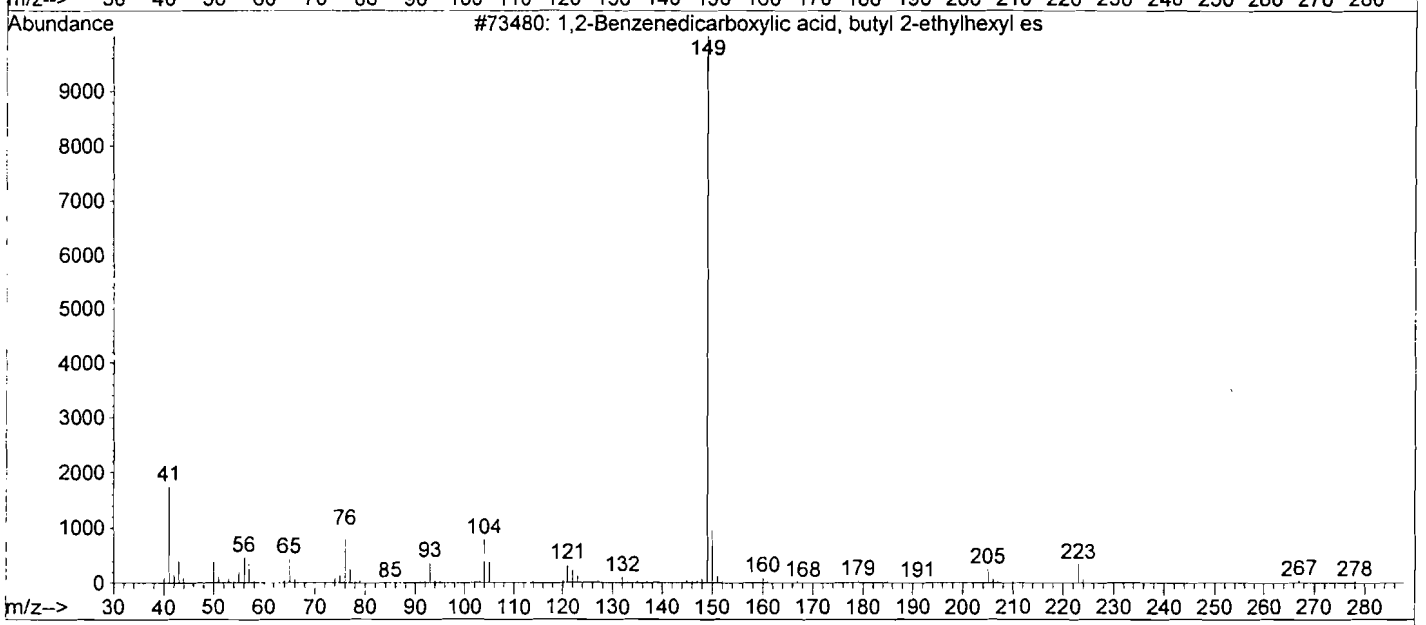


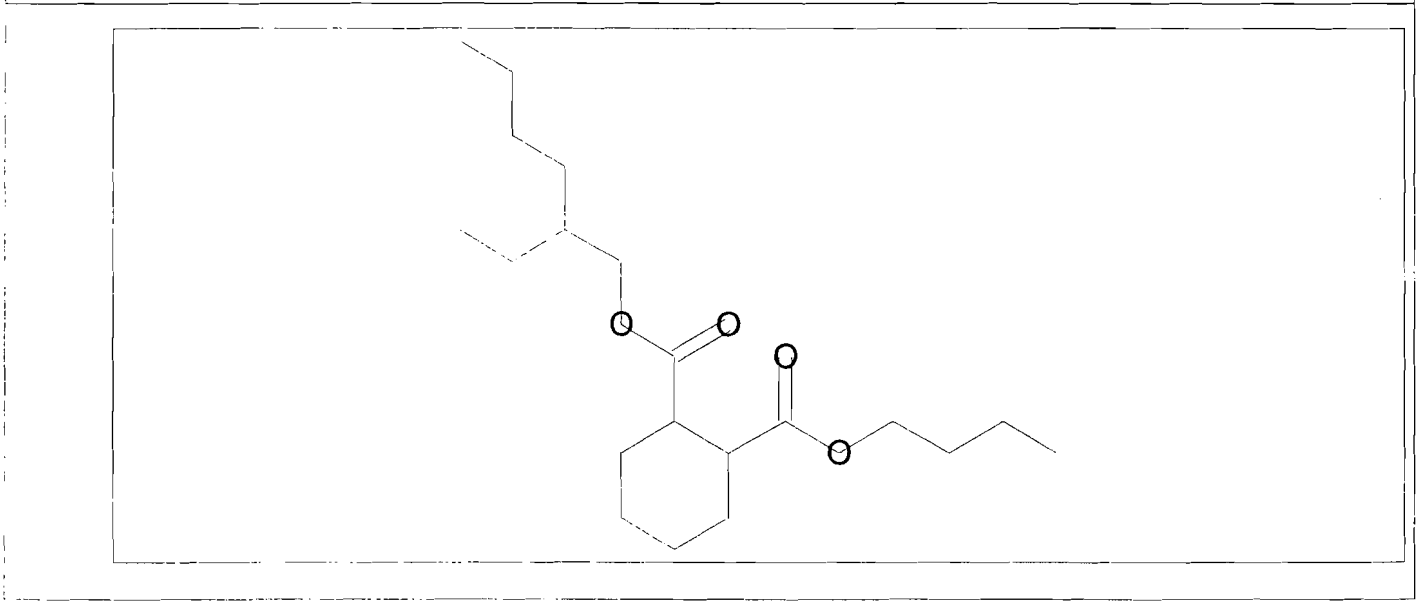


Spectrum 6

Library Searched : C:\DATABASE\NBS75K.L
Quality : 90
ID : 1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl este

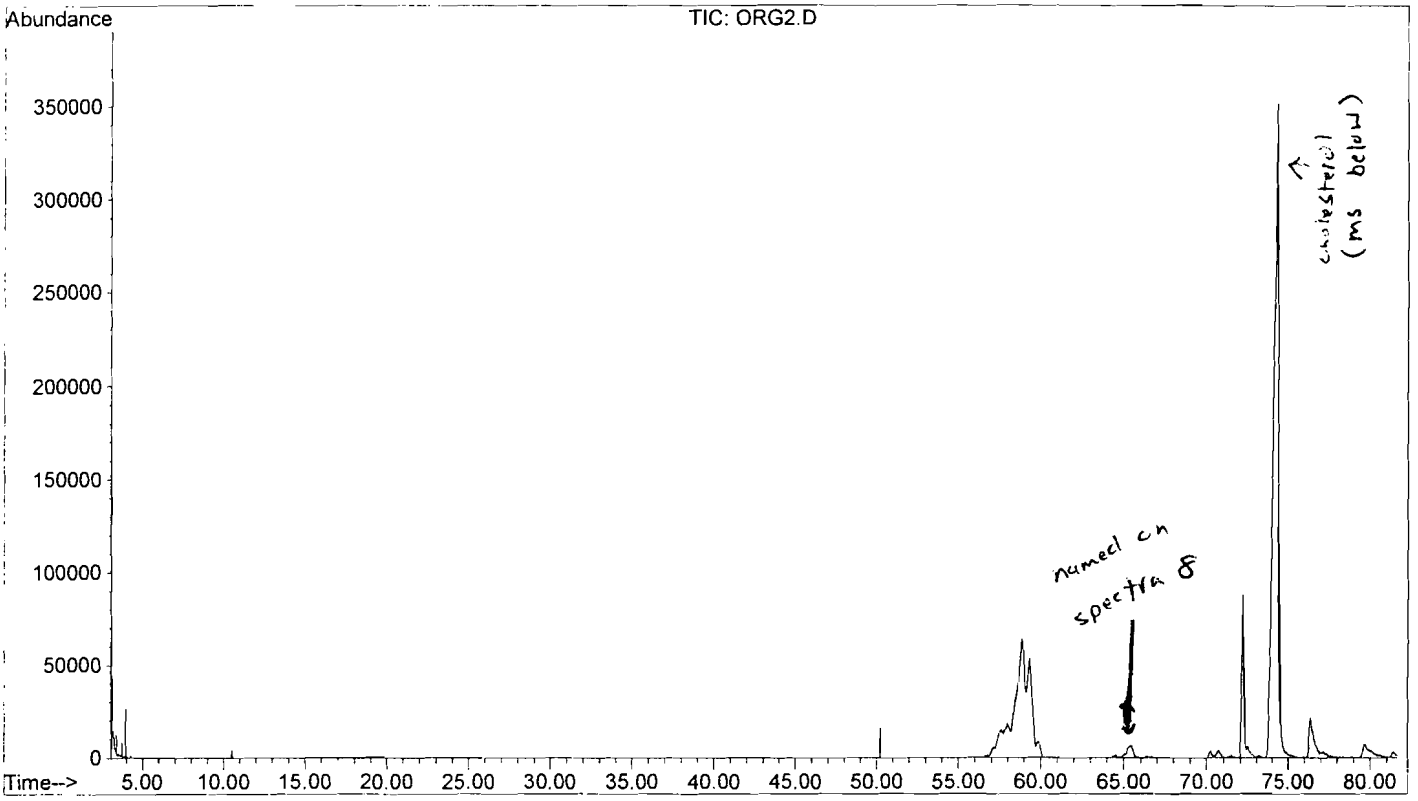


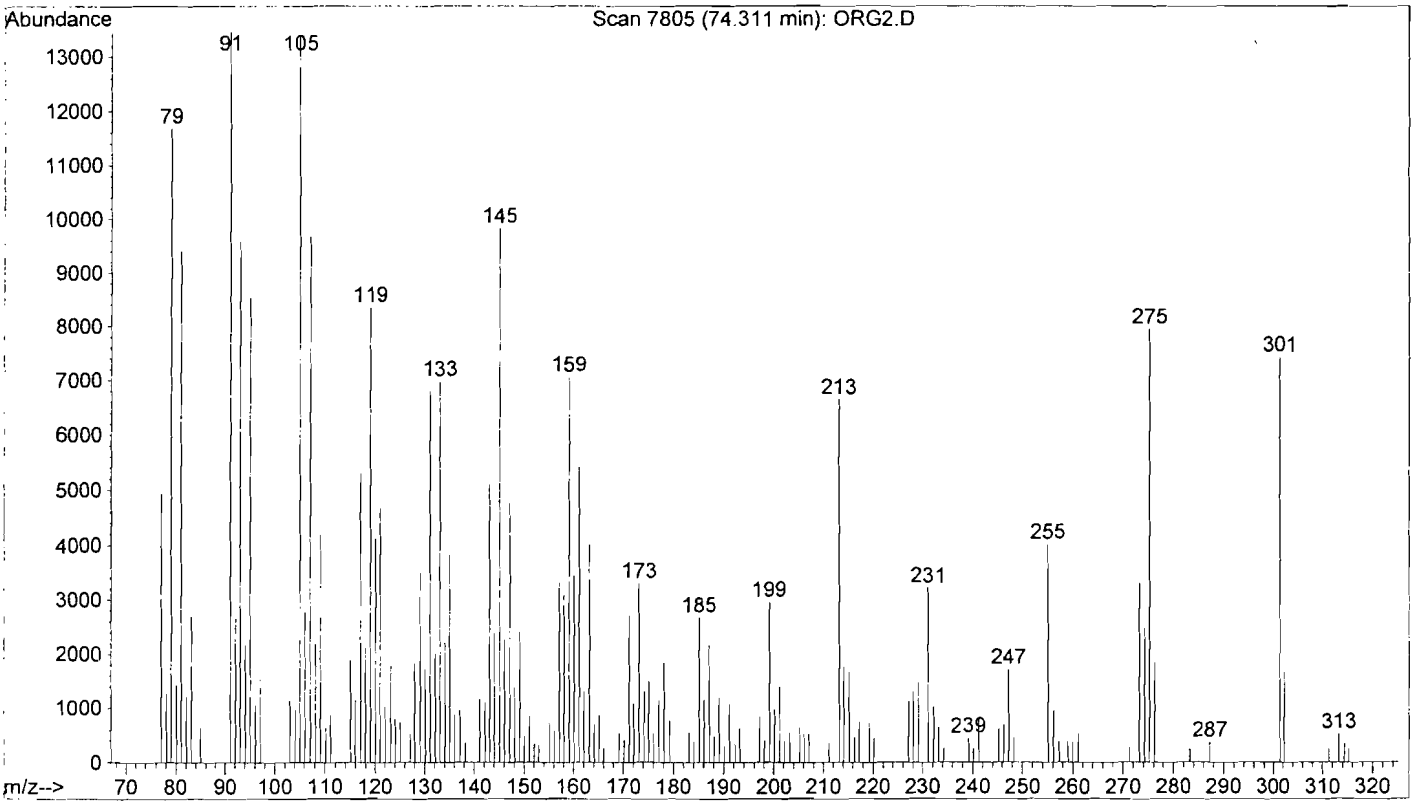




Spectrum 7

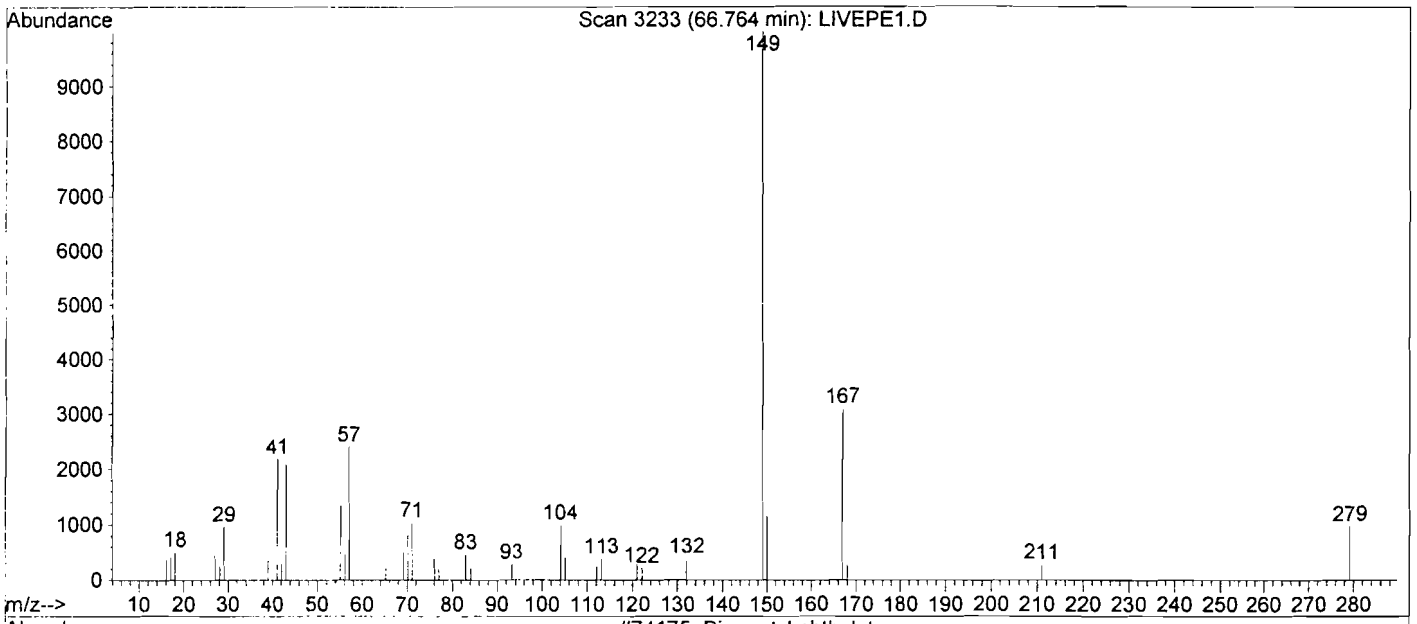
File : C:\HPCHEM\1\DATA\ORG2.D
Operator : Kris Mitchell
Acquired : 22 Oct 97 10:26 am using AcqMethod INVERT
Instrument : GC/MS Ins
Sample Name: second extraction trial 1 of org
Misc Info : in ethyl acetate
Vial Number: 1

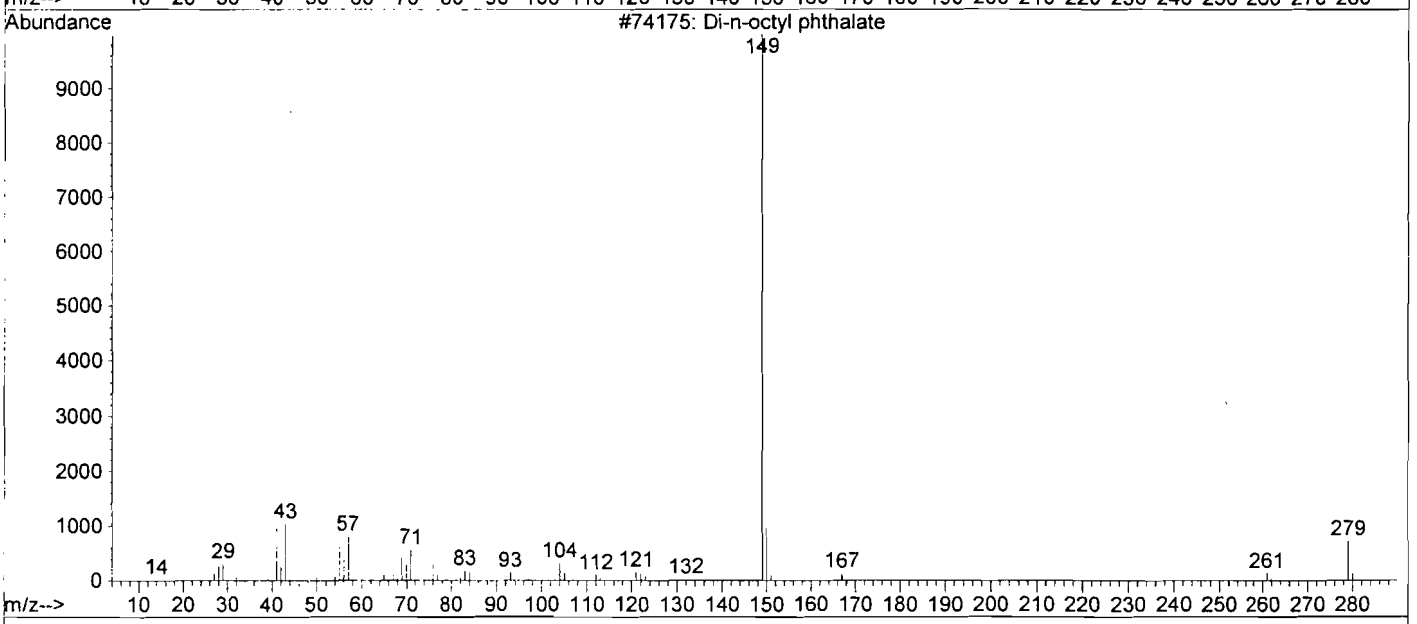


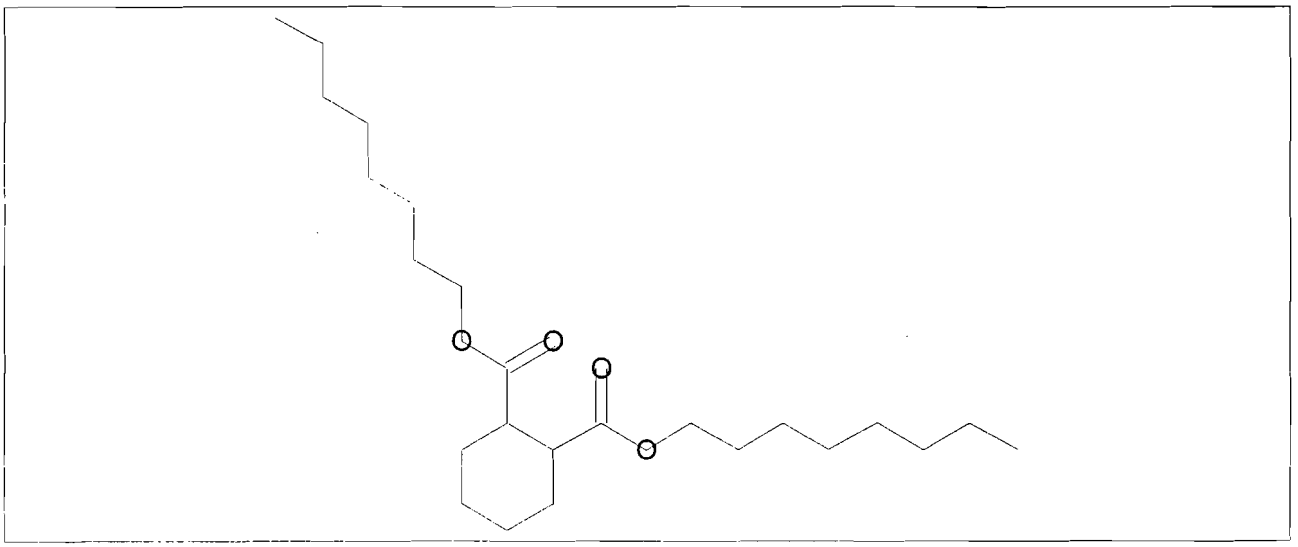


Library Searched : C:\DATABASE\NBS75K.L
Quality : 59
ID : Di-n-octyl phthalate

Spectrum 8

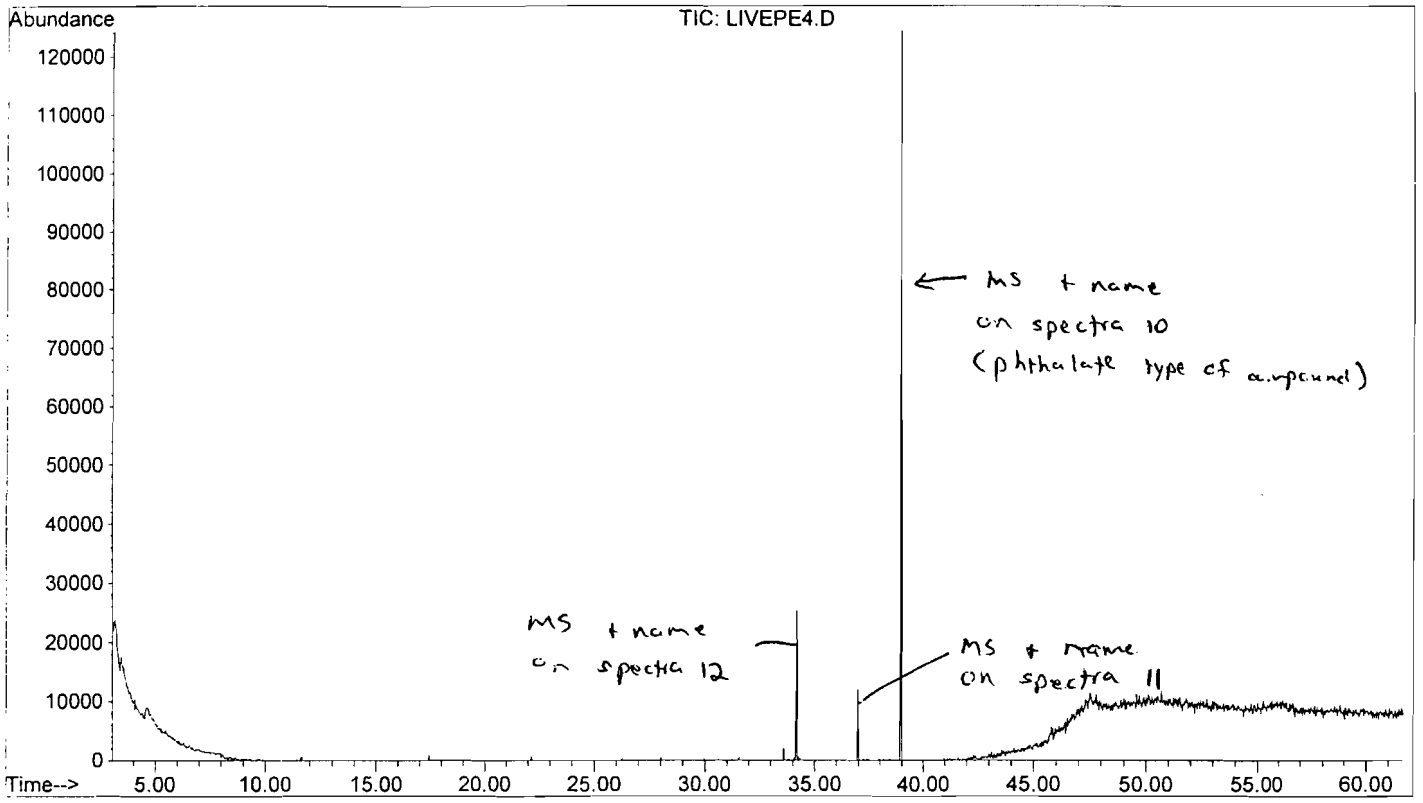


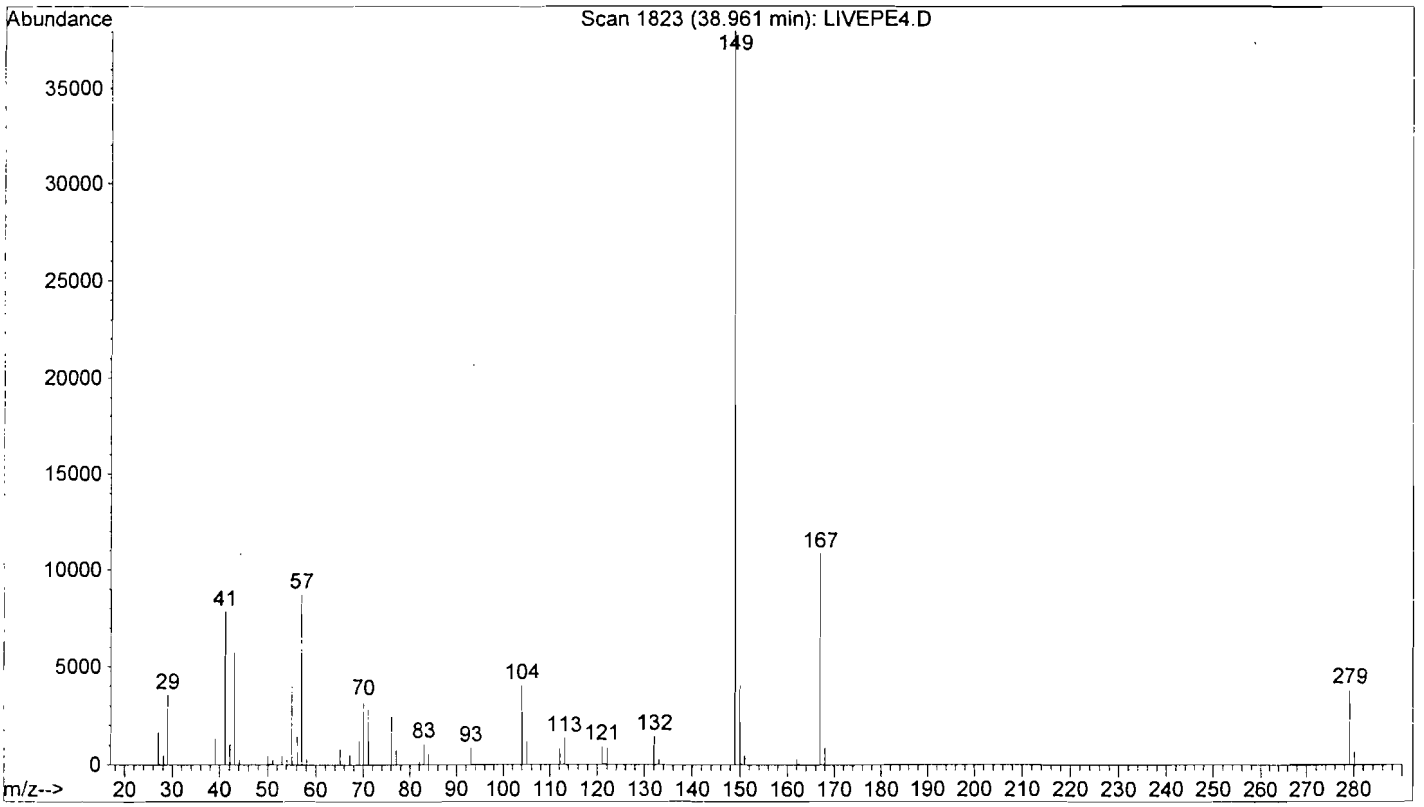




Spectrum 9

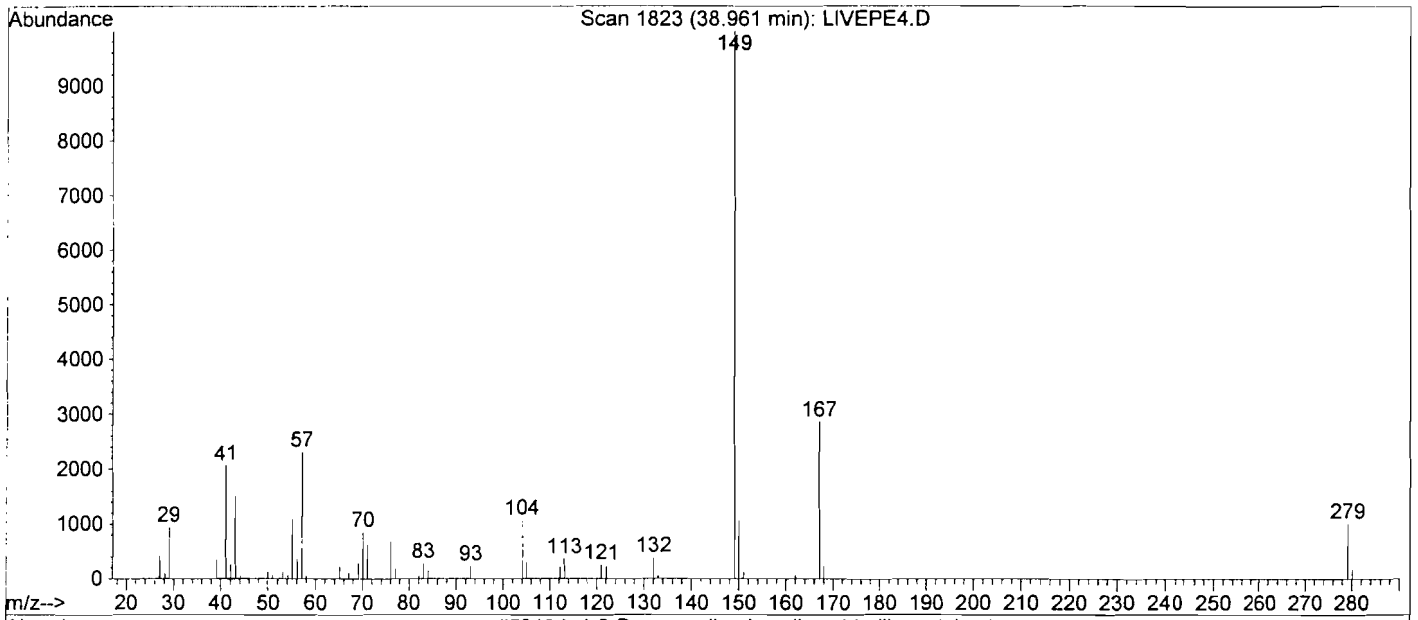
File : C:\HPCHEM\1\DATA\LIVEPE4.D
Operator : Kris Mitchell
Acquired : 24 Jan 98 10:32 am using AcqMethod INVERT
Instrument : GC/MS Ins
Sample Name: livechrpete4
Misc Info : chrysaora-live bell-in15mlpe1-24-98
Vial Number: 1

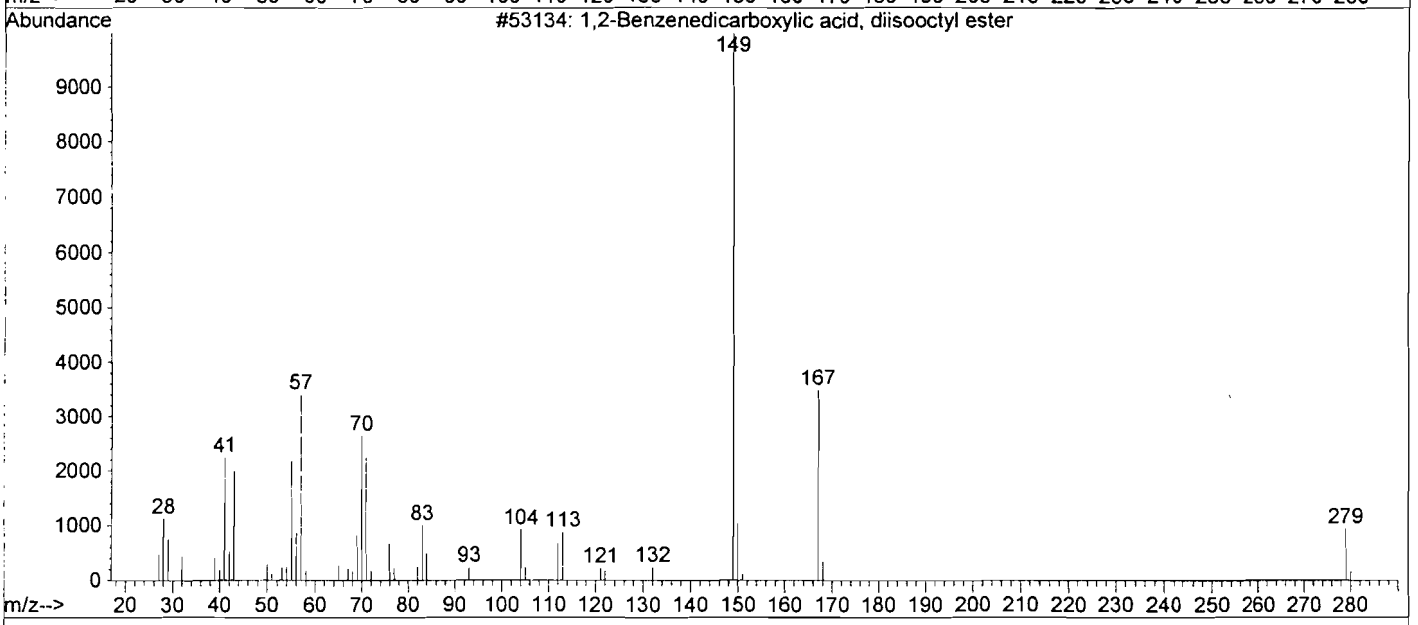


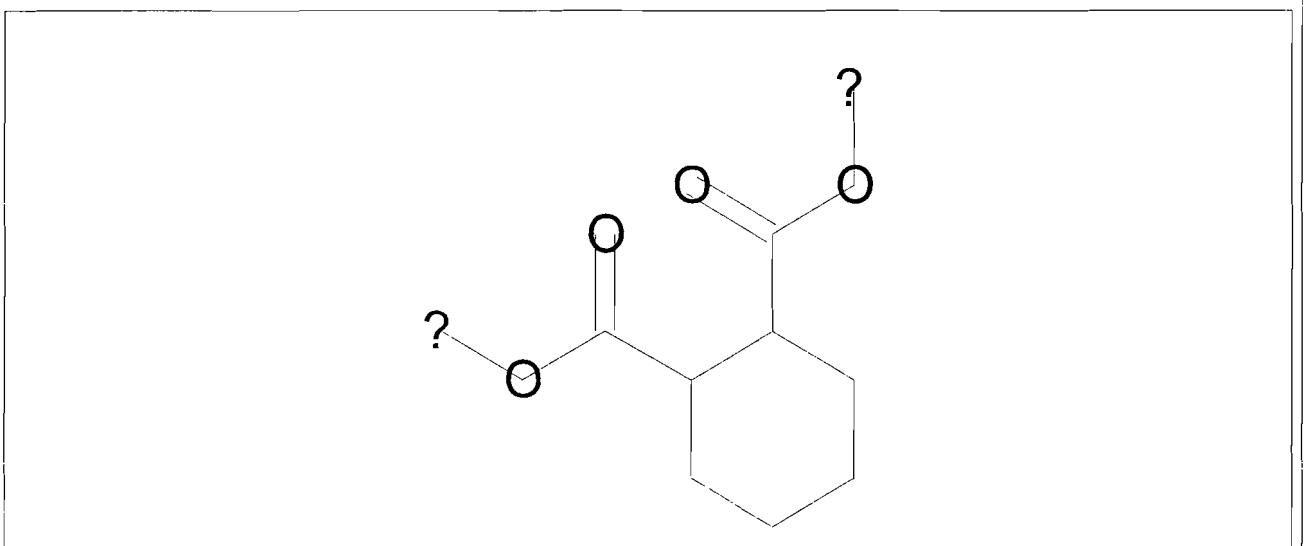


Library Searched : C:\DATABASE\NBS75K.L
Quality : 74
ID : 1,2-Benzenedicarboxylic acid, diisooctyl ester

Spectrum 10

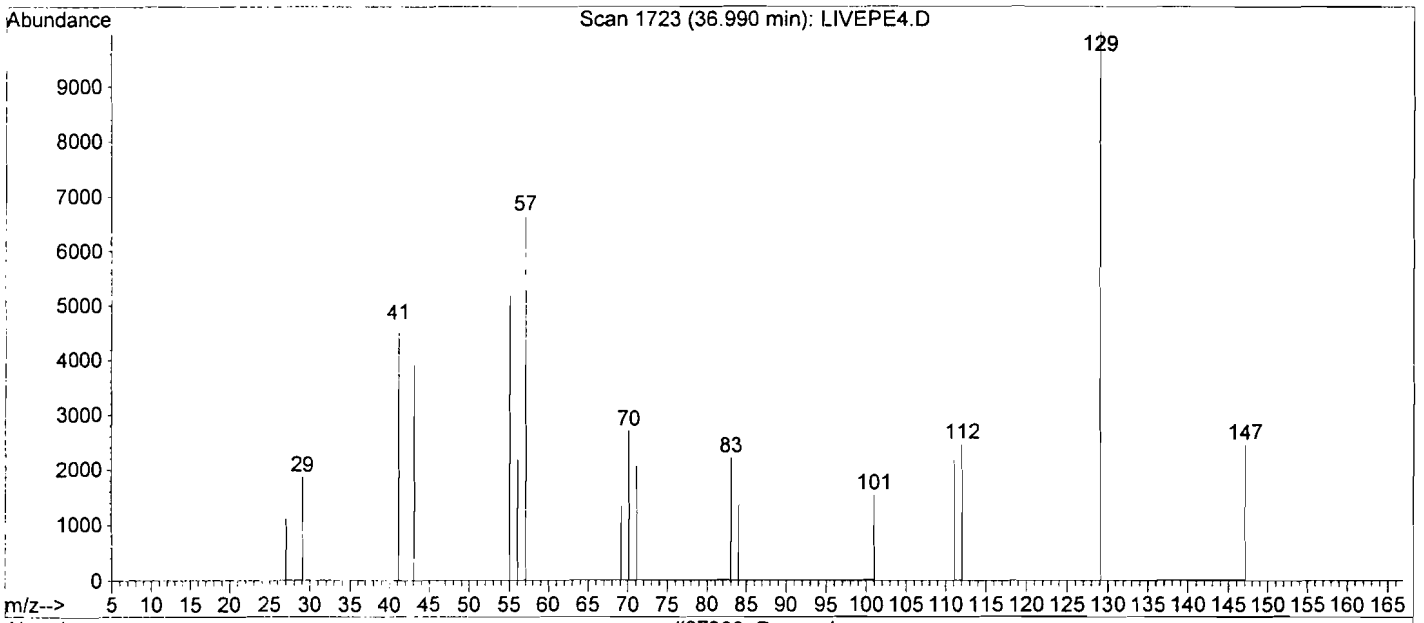


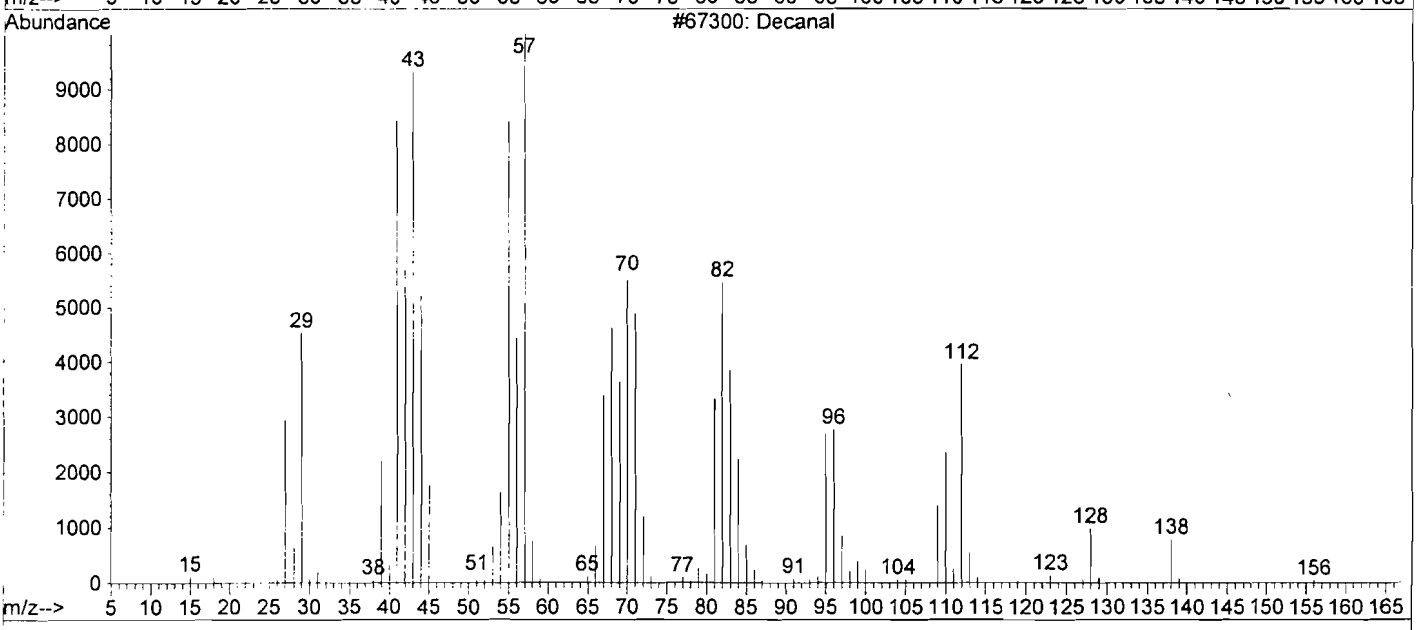


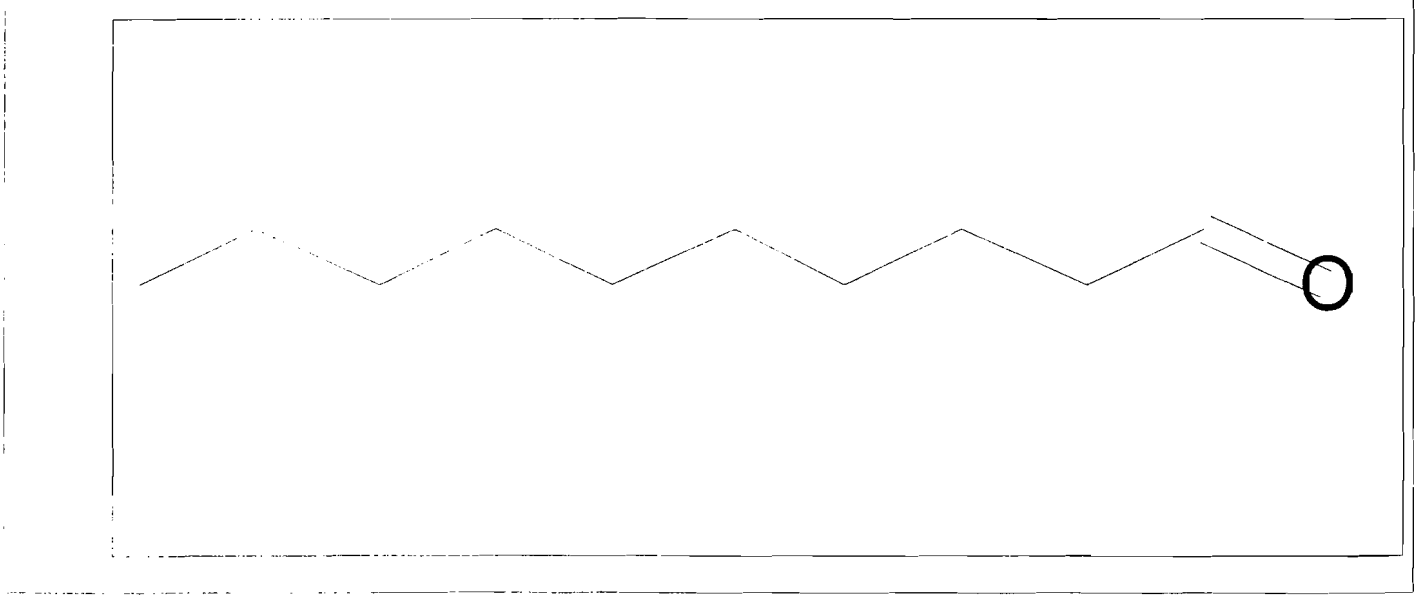


Library Searched : C:\DATABASE\NBS75K.L
Quality : 35
ID : Decanal

Spectrum 11

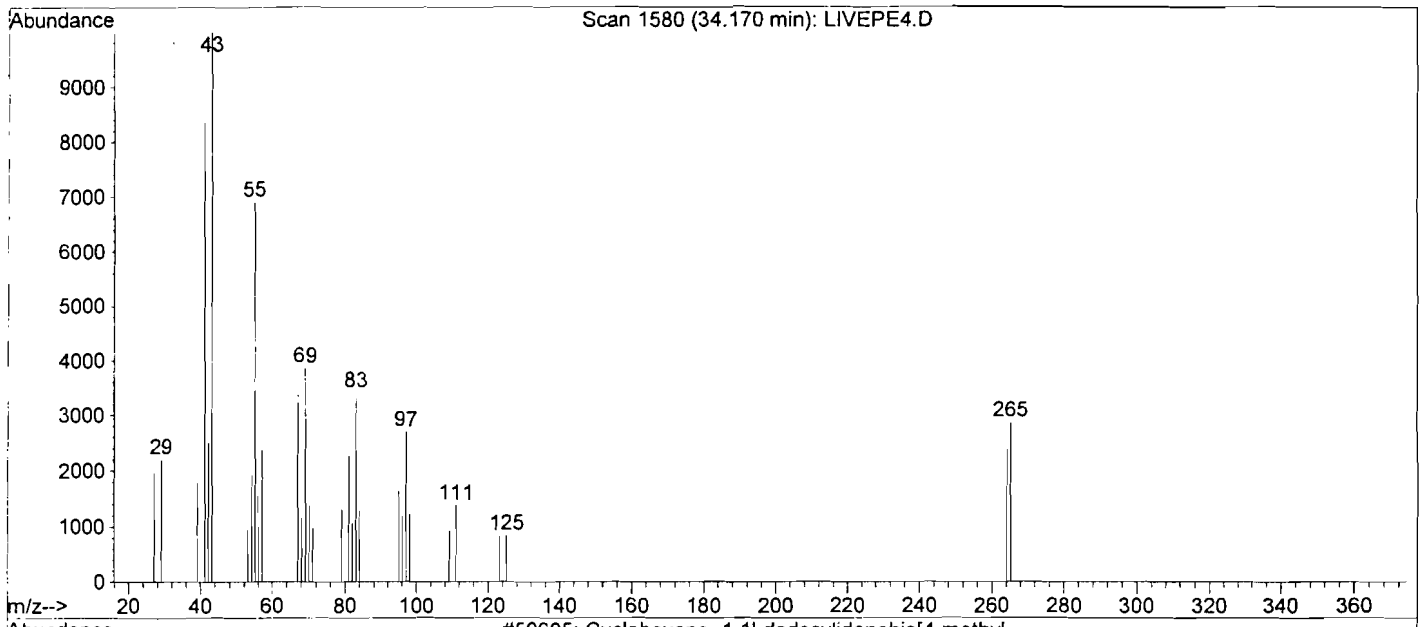


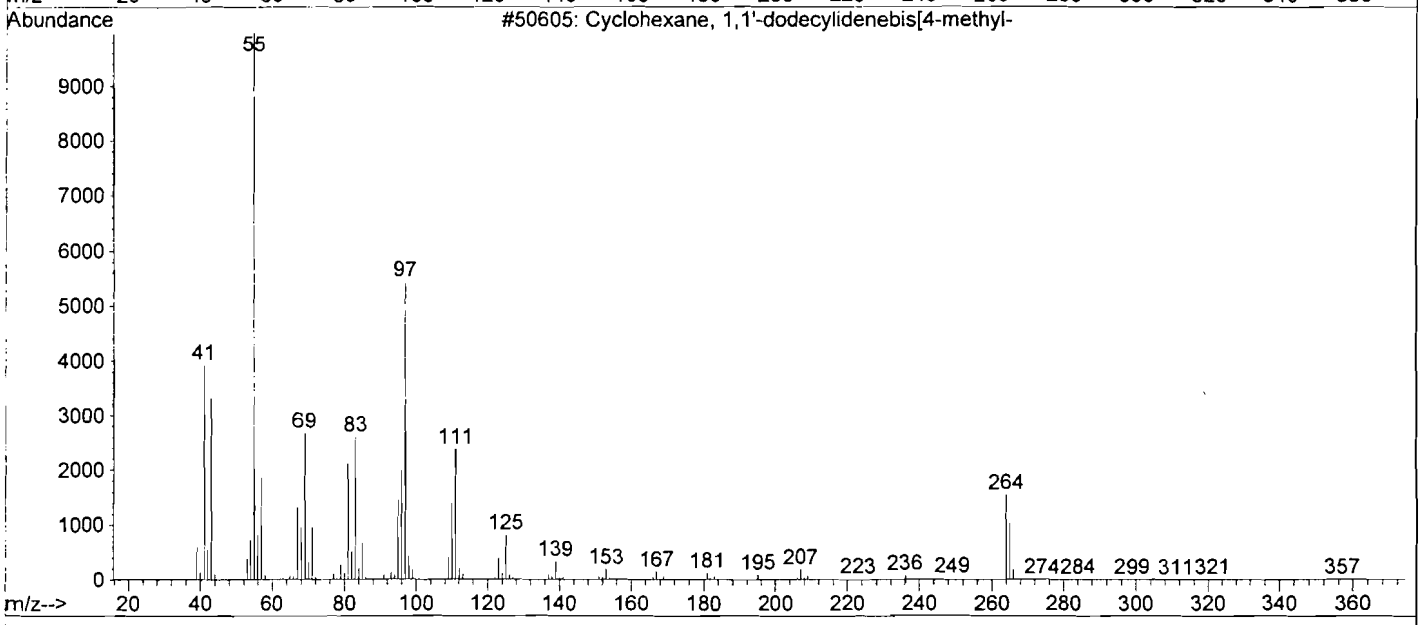


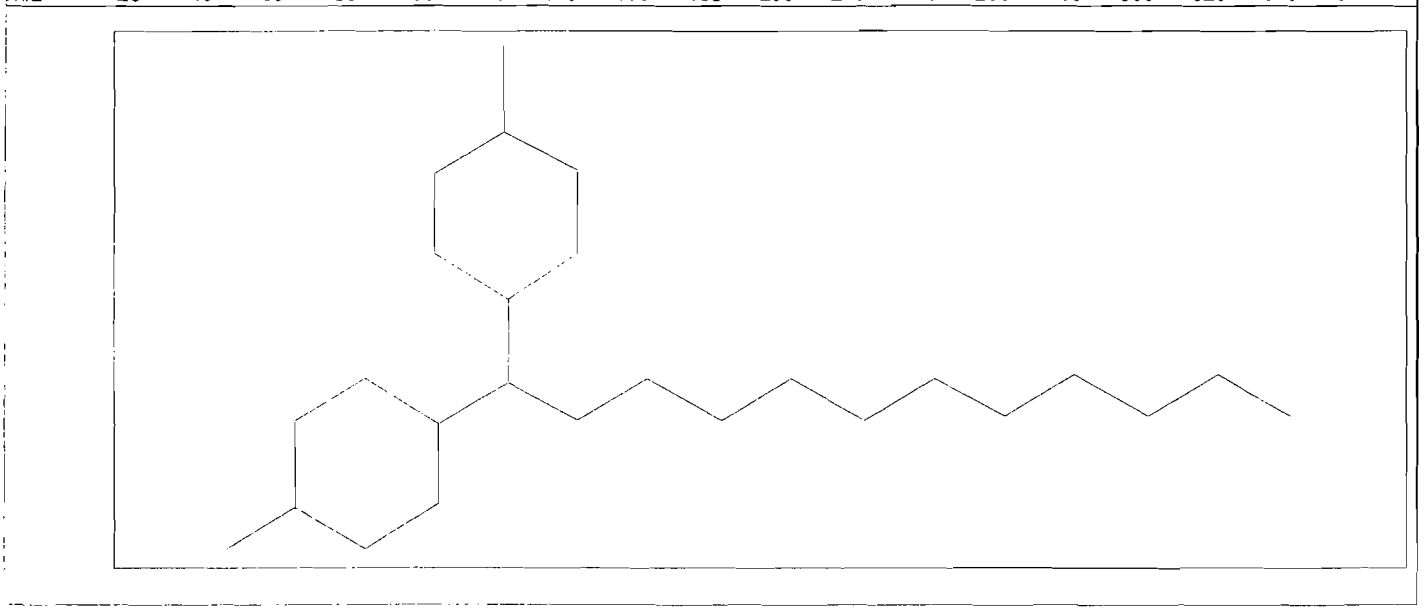


Library Searched : C:\DATABASE\NBS75K.L
Quality : 50
ID : Cyclohexane, 1,1'-dodecylidenebis[4-methyl-

Spectrum 12

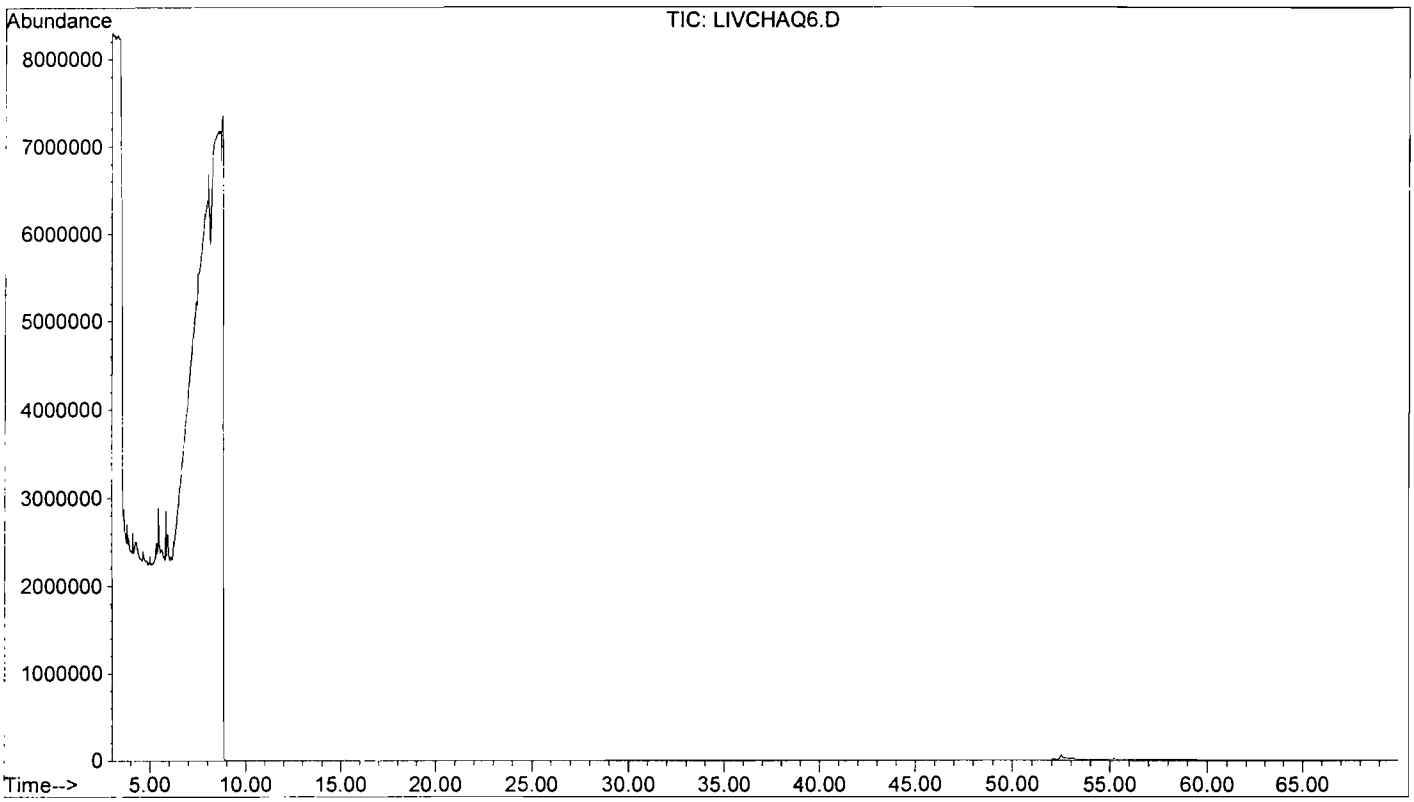


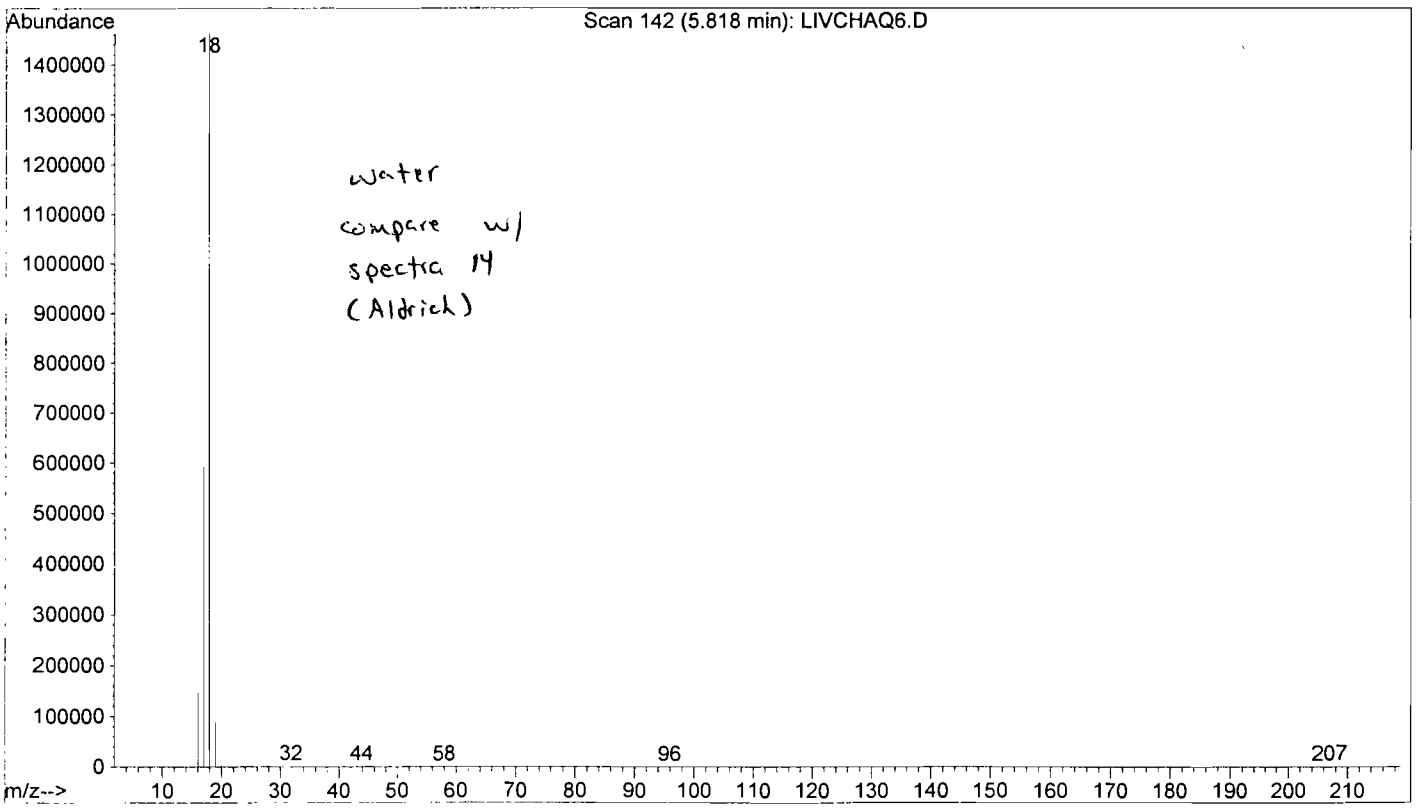




spectrum 13

File : C:\HPCHEM\1\DATA\LIVCHAQ6.D
Operator : Kris Mitchell
Acquired : 26 Jan 98 11:53 am using AcqMethod INVERT
Instrument : GC/MS Ins
Sample Name: livechaq6
Misc Info : livechtentaq-1-26-98
Vial Number: 1

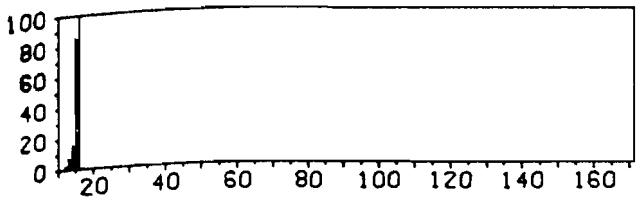




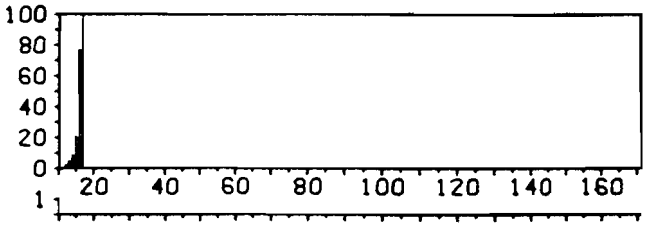
water
compare w/
spectra 14
(Aldrich)

Spectrum 14

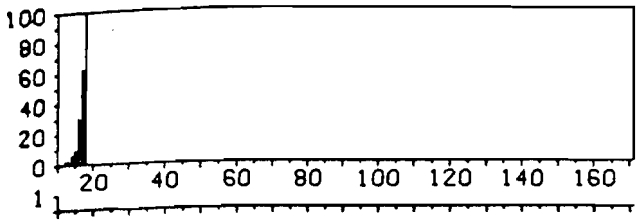
METHANE
16.03130 C H4 AA-1-3



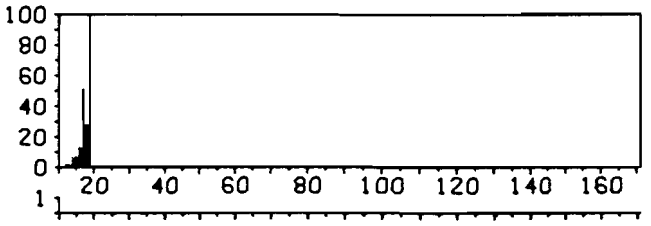
MONODEUTEROMETHANE
17.03758 C H3 D AA-1-6



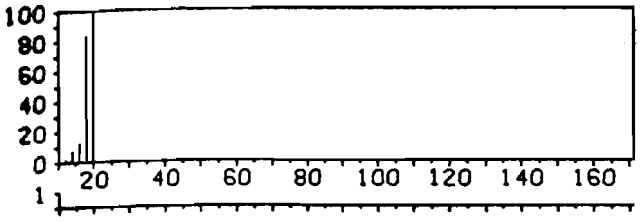
DIDEUTEROMETHANE
18.04385 C H2 D2 AA-2-1



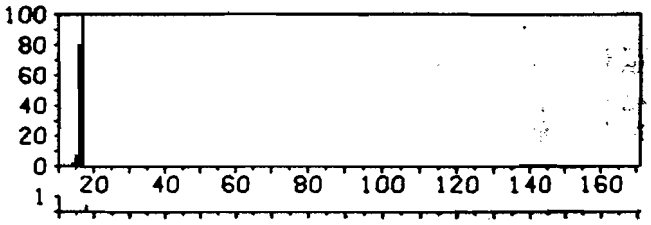
TRIDEUTEROMETHANE
19.05013 C H D3 AA-2-2



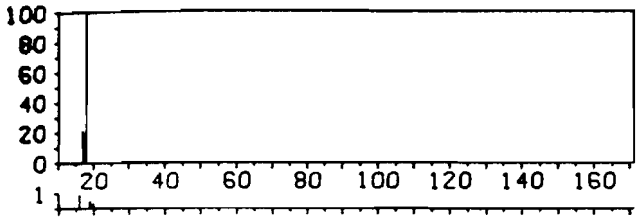
TETRADEUTEROMETHANE
20.05641 C D4 AA-2-3



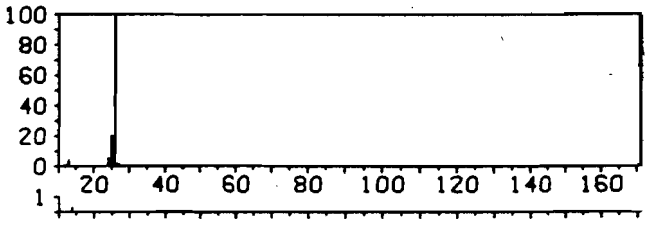
AMMONIA
17.02655 H3 N AA-1-5



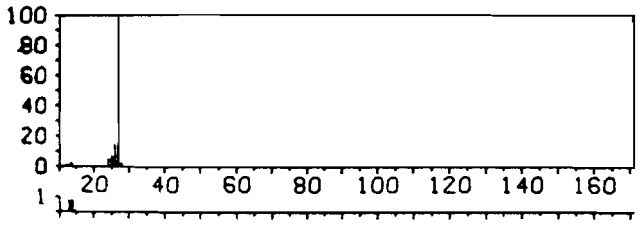
WATER
18.01056 H2 O AA-1-7



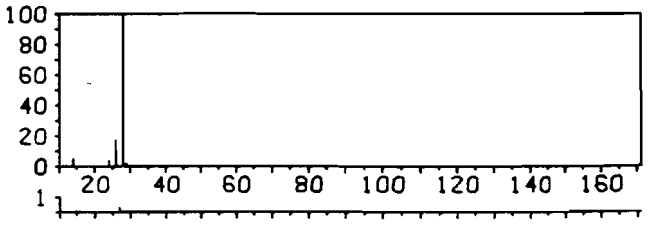
ACETYLENE
26.01565 C2 H2 AD-3413



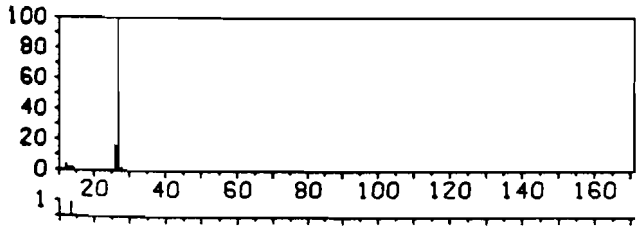
MONODEUTEROACETYLENE
27.02193 C2 H D AD-3411



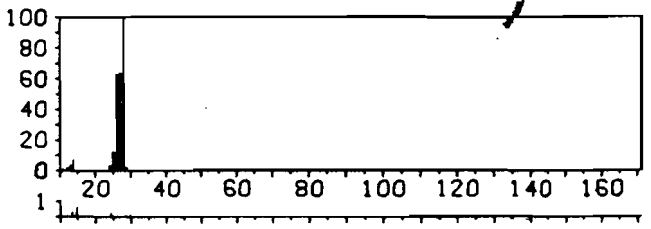
DIDEUTEROACETYLENE
28.02820 C2 D2 AD-3412



HYDROGEN CYANIDE
27.01090 C H N AA-2-6

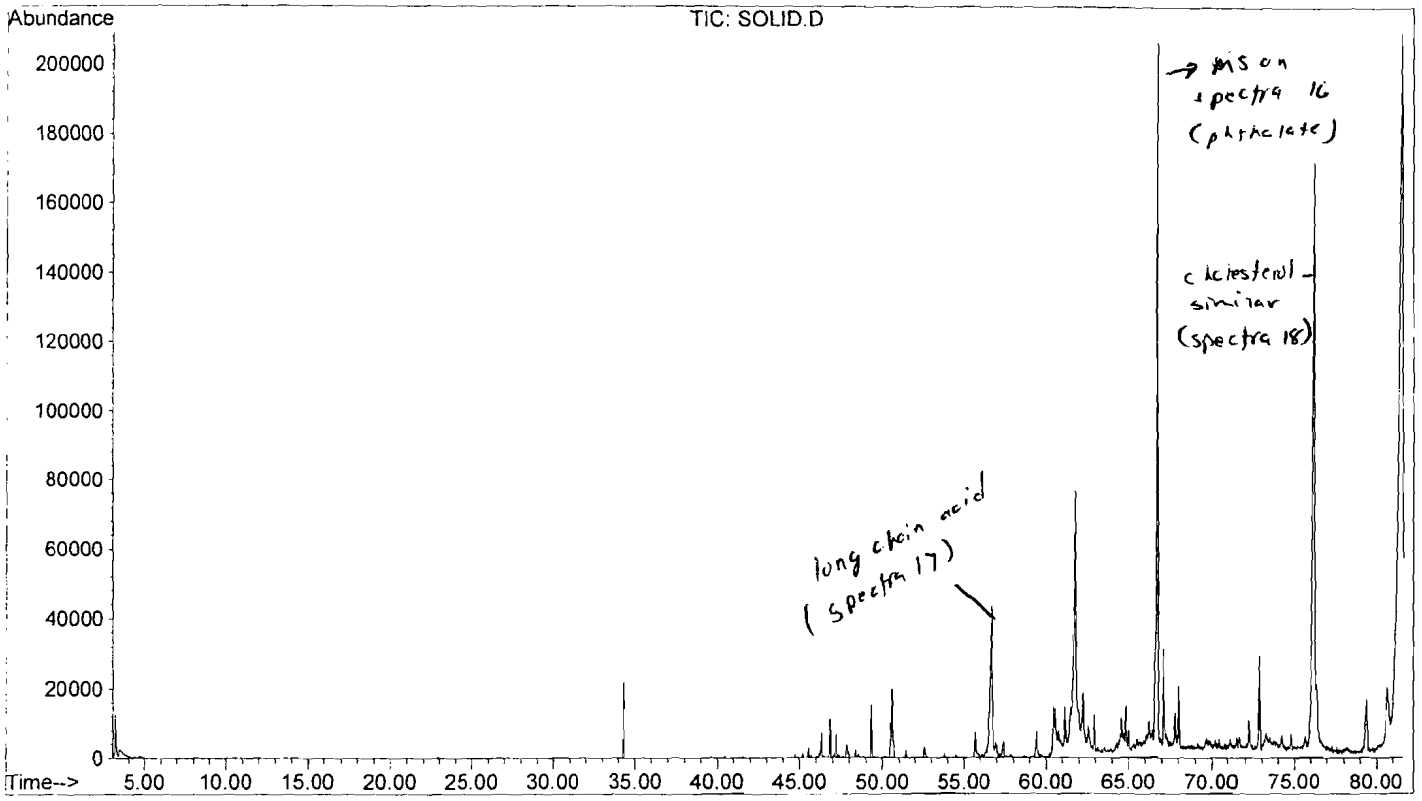


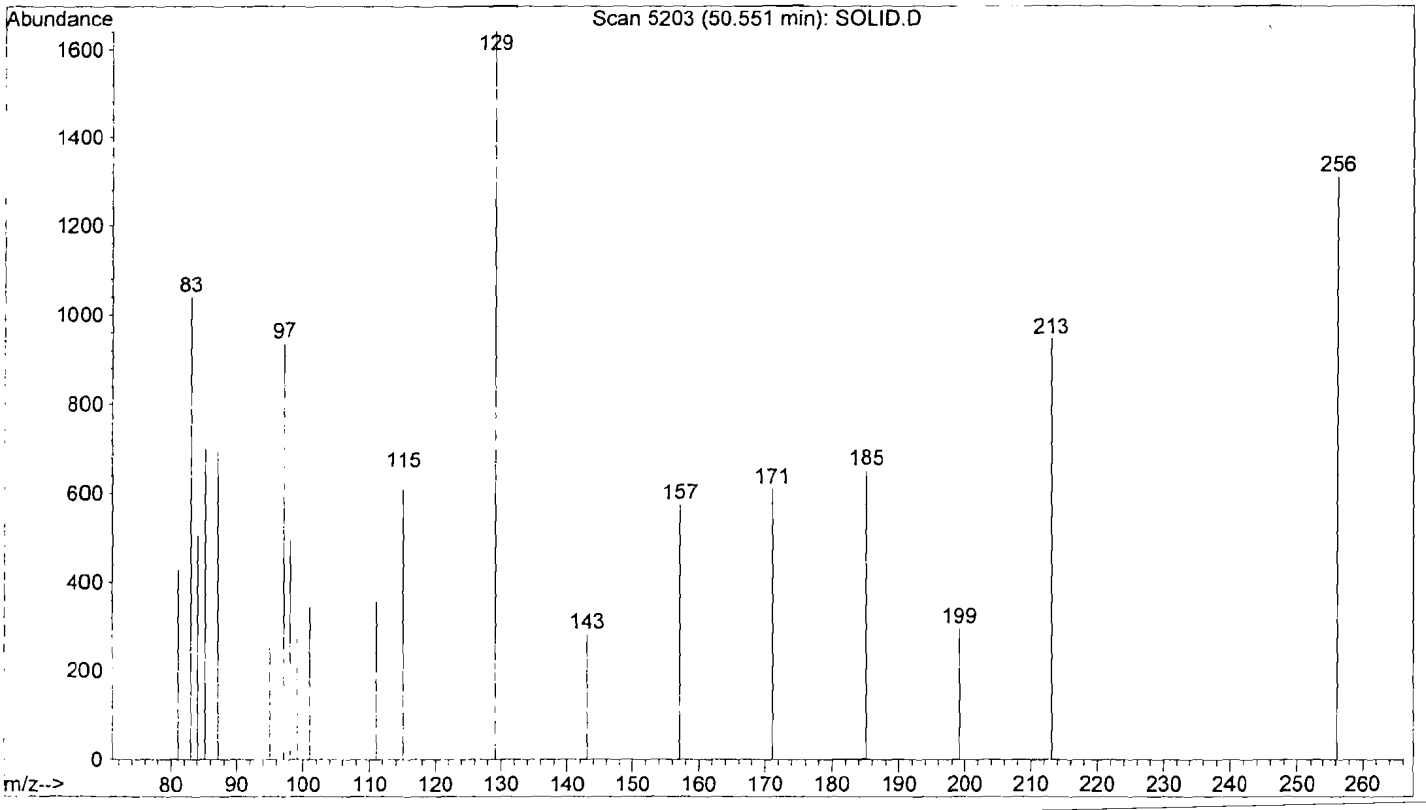
ETHENE(ETHYLENE)
28.03130 C2 H4 AA-3-5



Spectrum 15

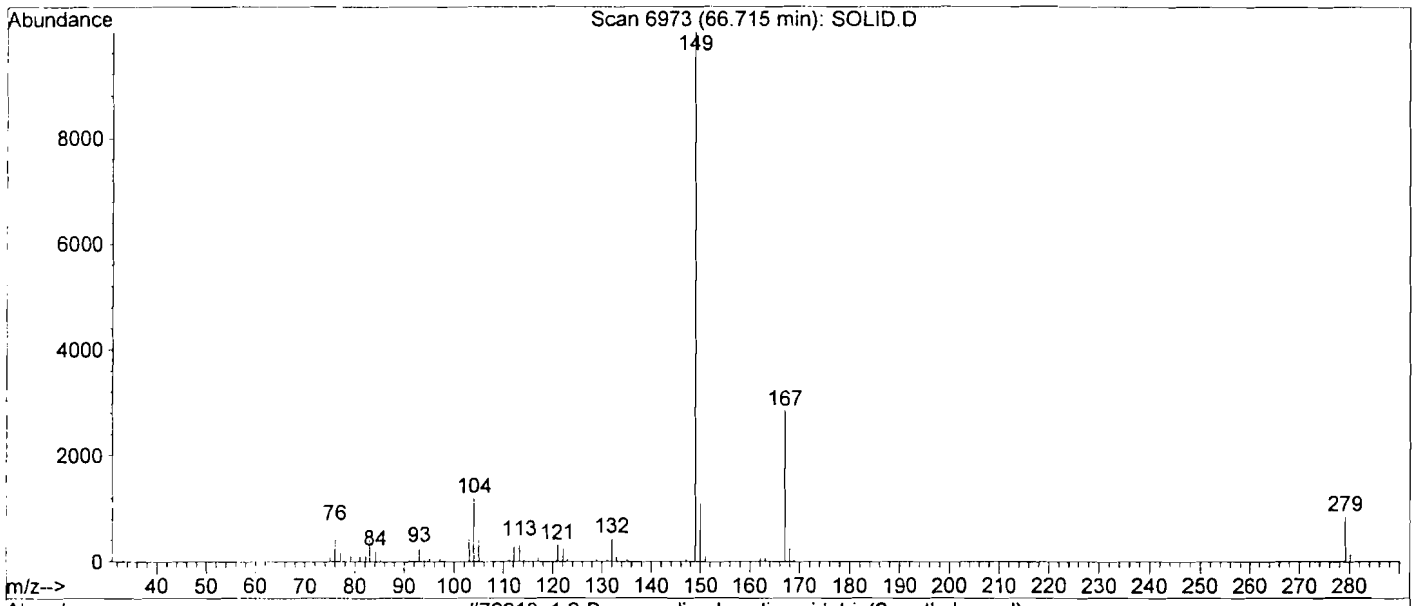
File : C:\HPCHEM\1\DATA\SOLID.D
Operator : Kris Mitchell
Acquired : 19 Nov 97 9:13 am using AcqMethod INVERT
Instrument : GC/MS Ins
Sample Name: ethyl ether solid
Misc Info : solid from ethyl ether in 2ml
Vial Number: 1

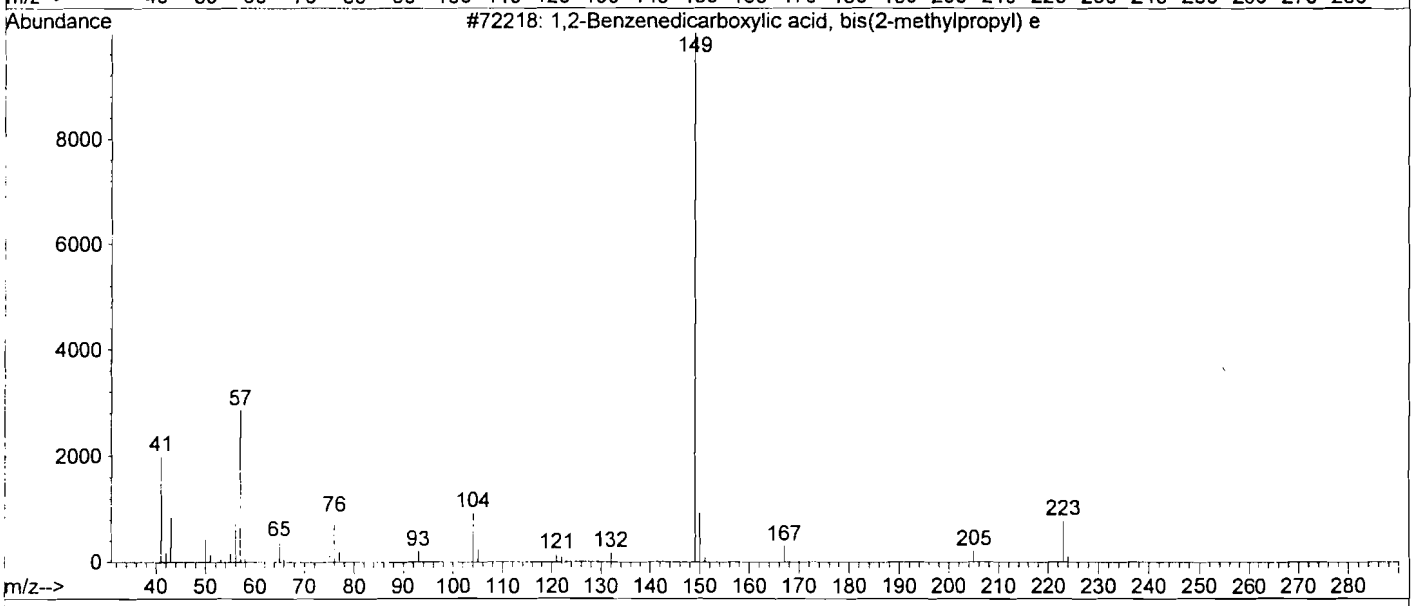


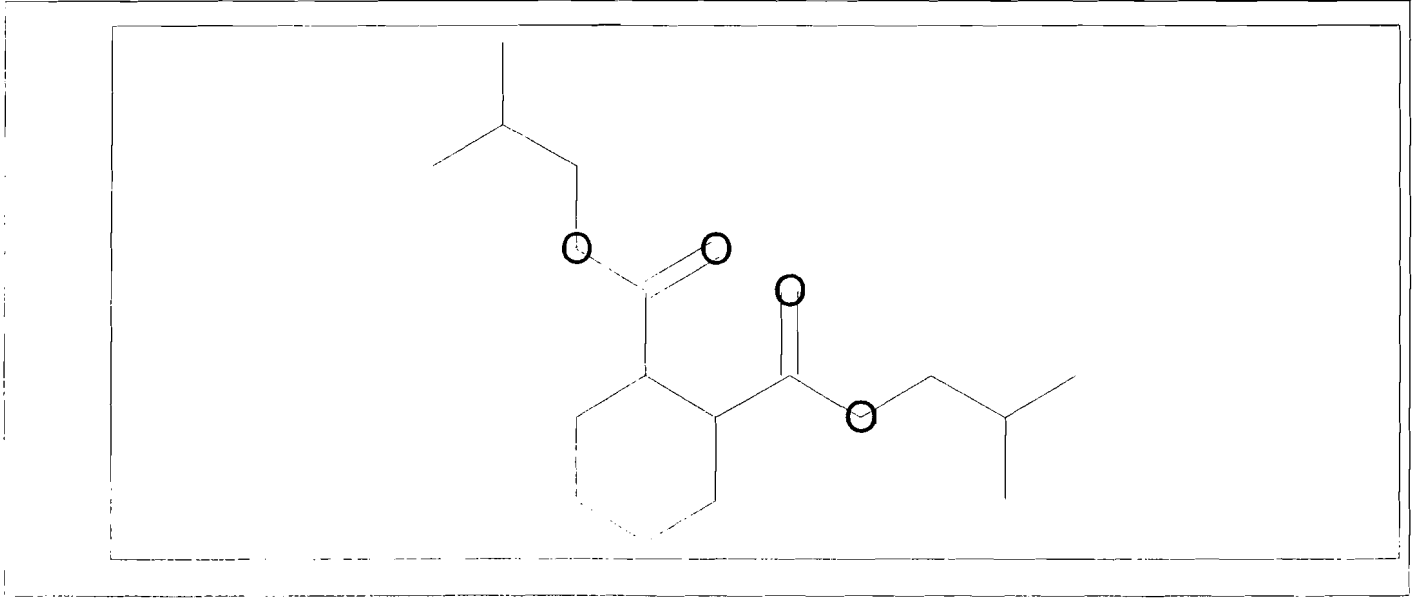


Library Searched : C:\DATABASE\NBS75K.L
Quality : 59
ID : 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) este
r

Spectrum 16

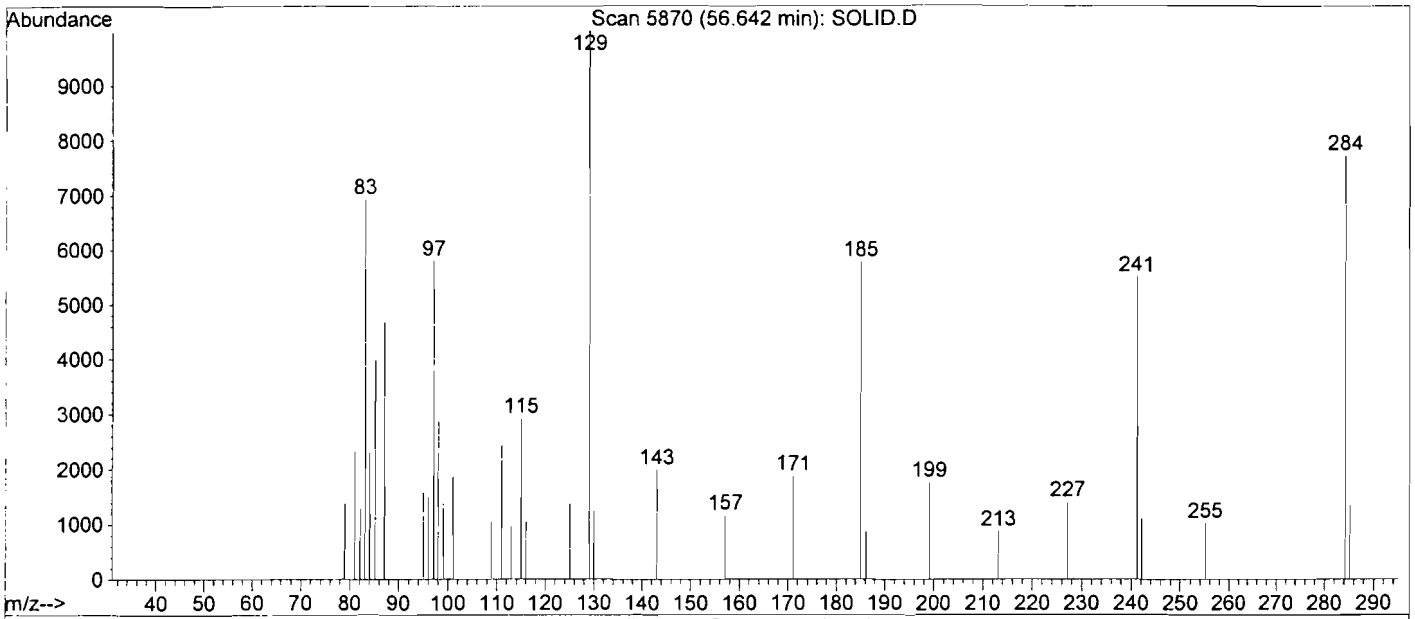


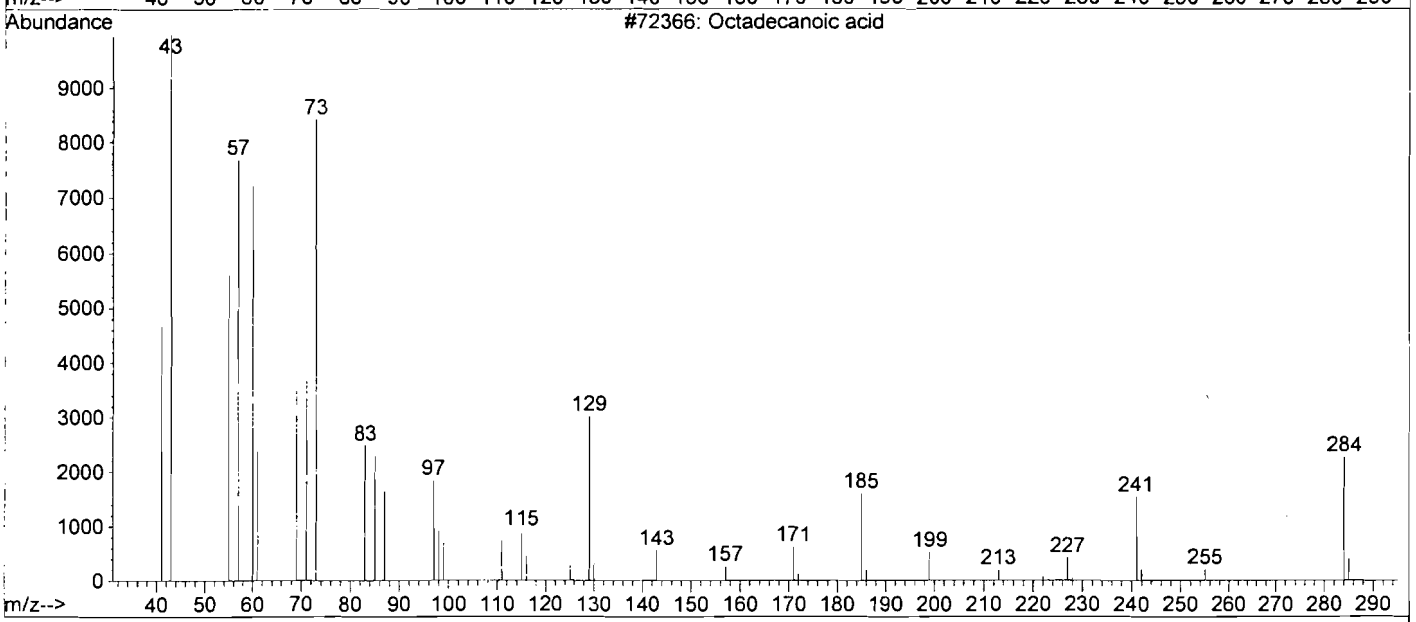




Library Searched : C:\DATABASE\NBS75K.L
Quality : 93
ID : Octadecanoic acid

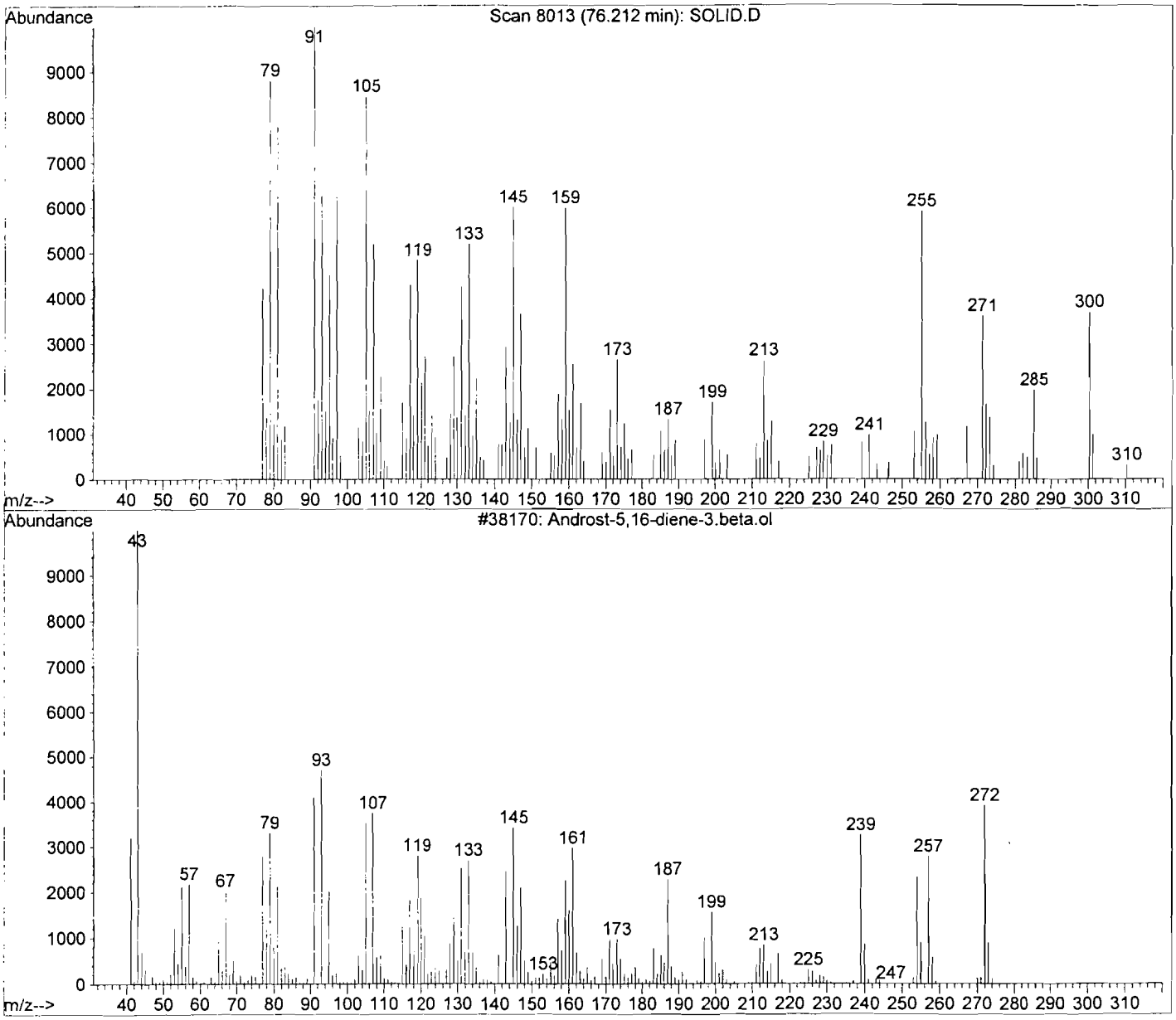
Spectrum 17





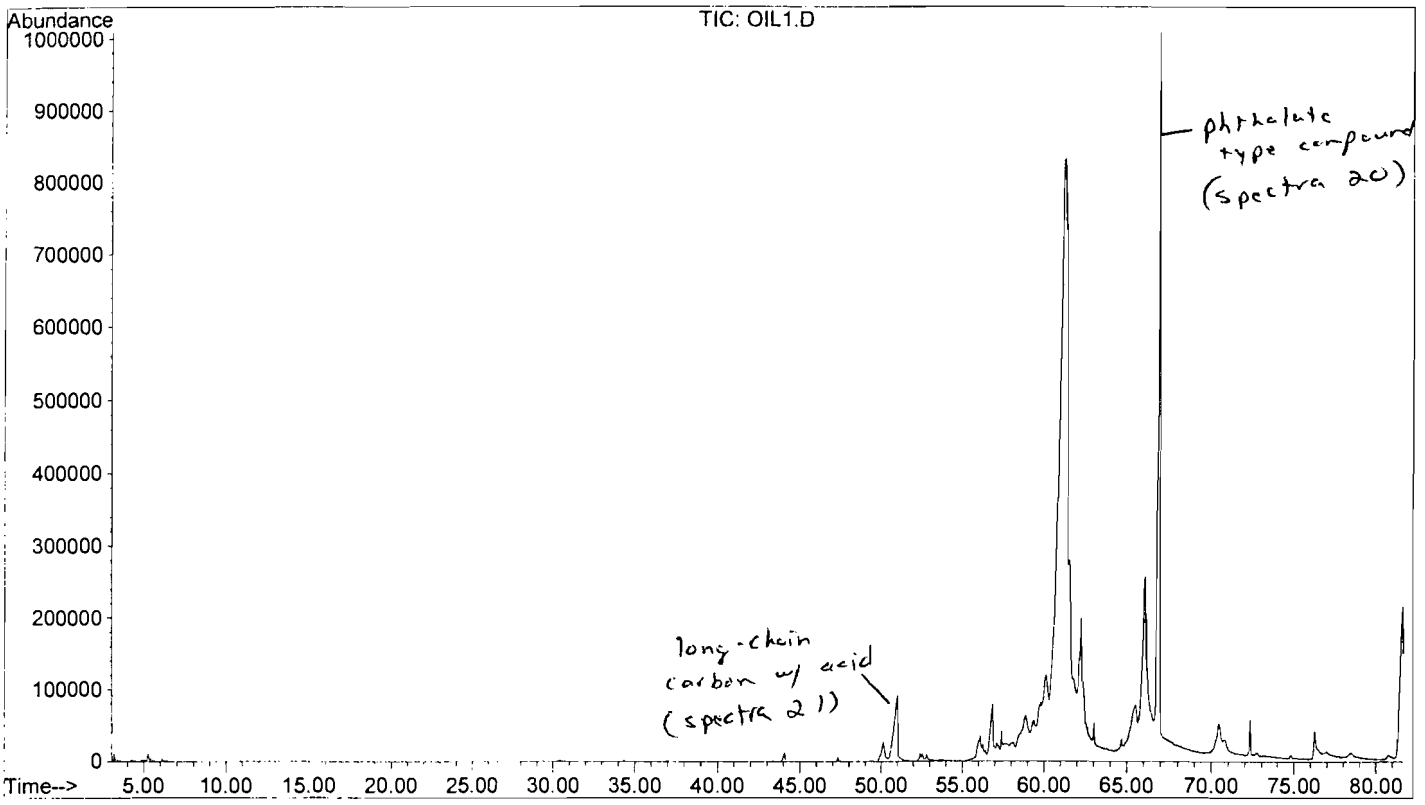
Library Searched : C:\DATABASE\NBS75K.L
Quality : 43
ID : Androst-5,16-diene-3.beta.ol

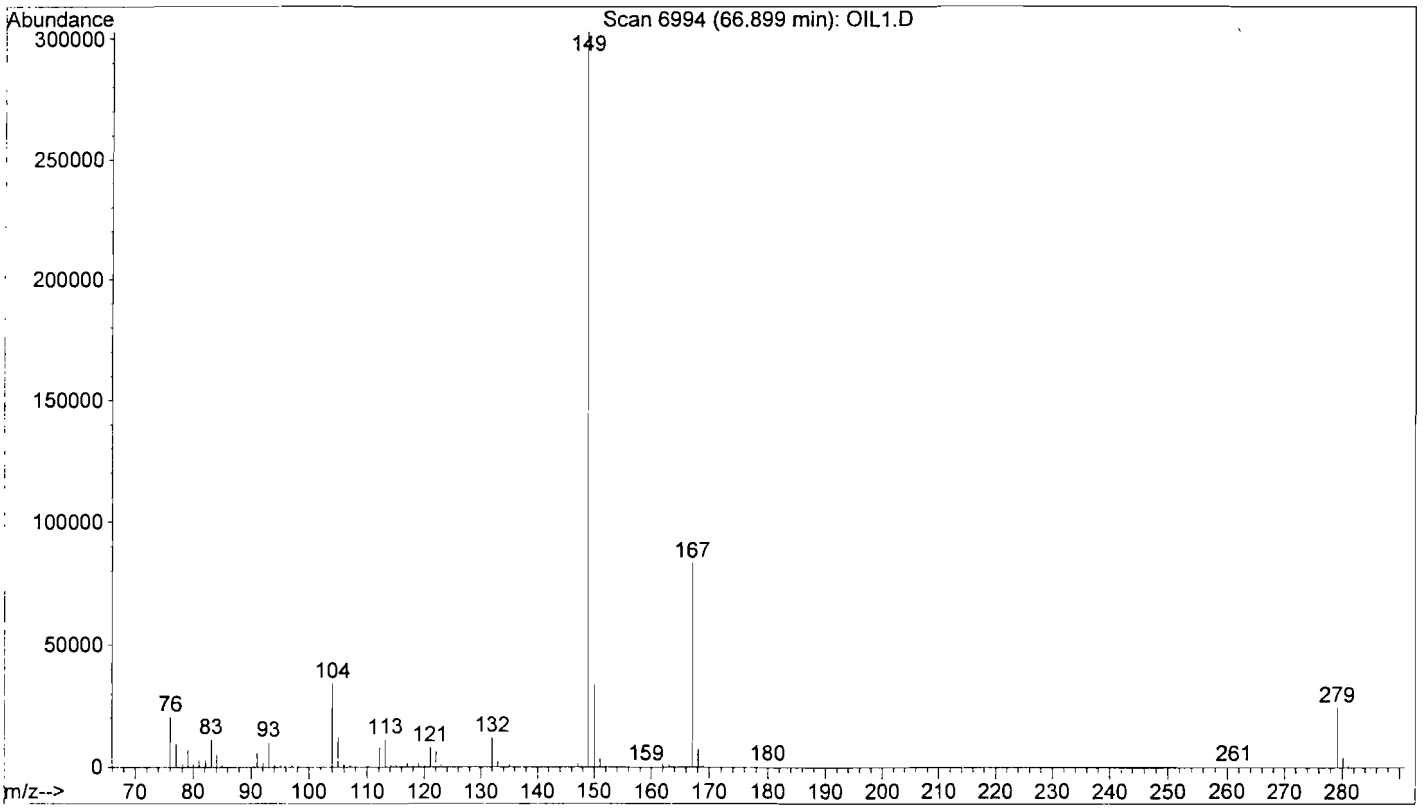
Spectrum 18



Spectrum 19

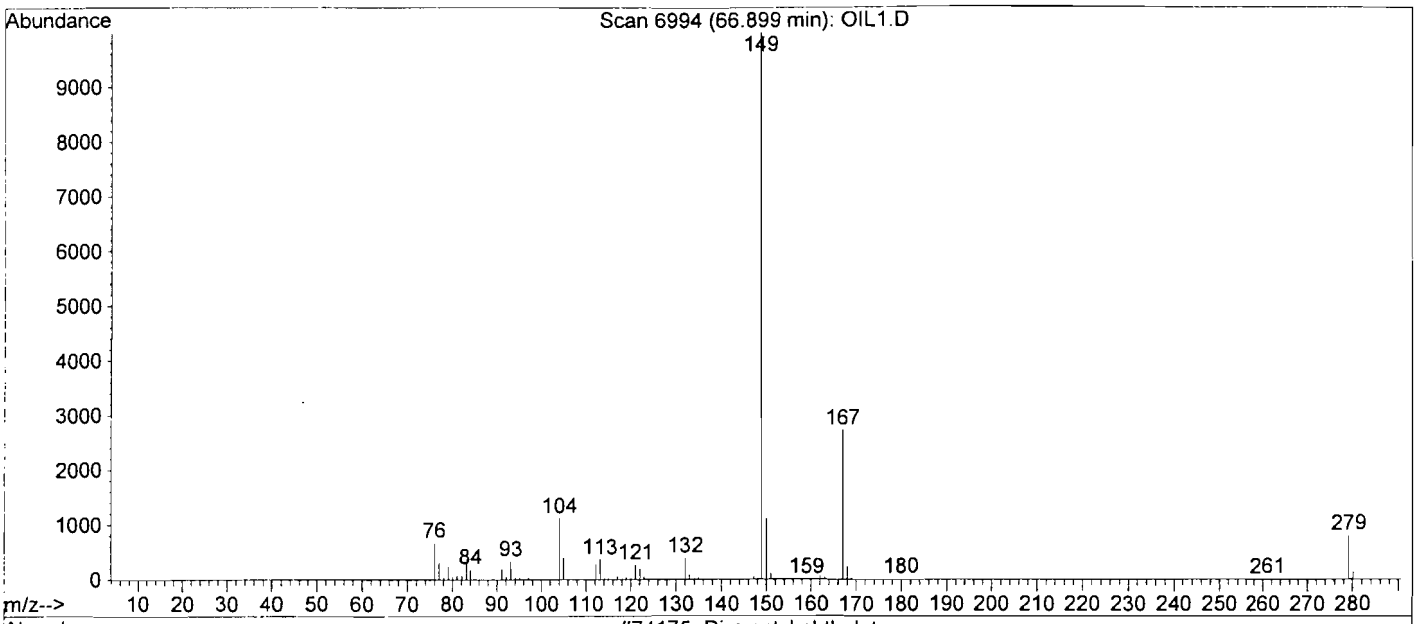
File : C:\HPCHEM\1\DATA\OIL1.D
Operator : Kris Mitchell
Acquired : 19 Nov 97 11:15 am using AcqMethod INVERT
Instrument : GC/MS Ins
Sample Name: pet ether solid
Misc Info : oil from pet ether in 5ml
Vial Number: 1

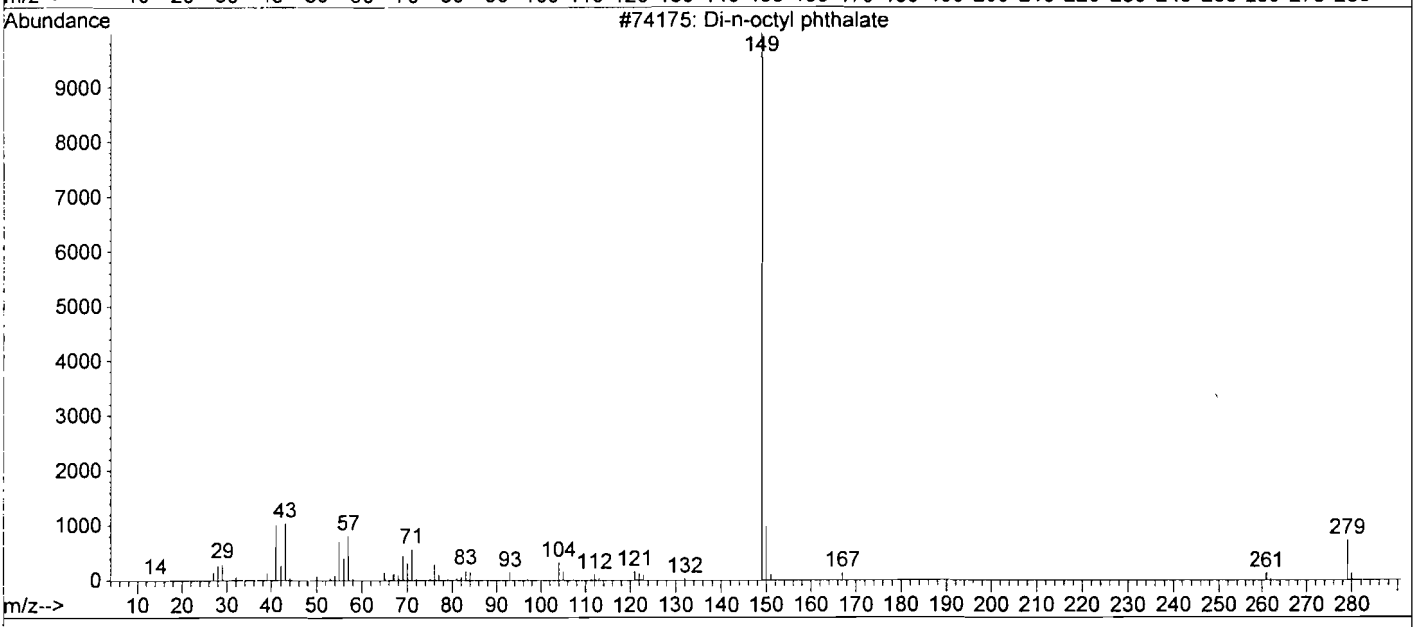


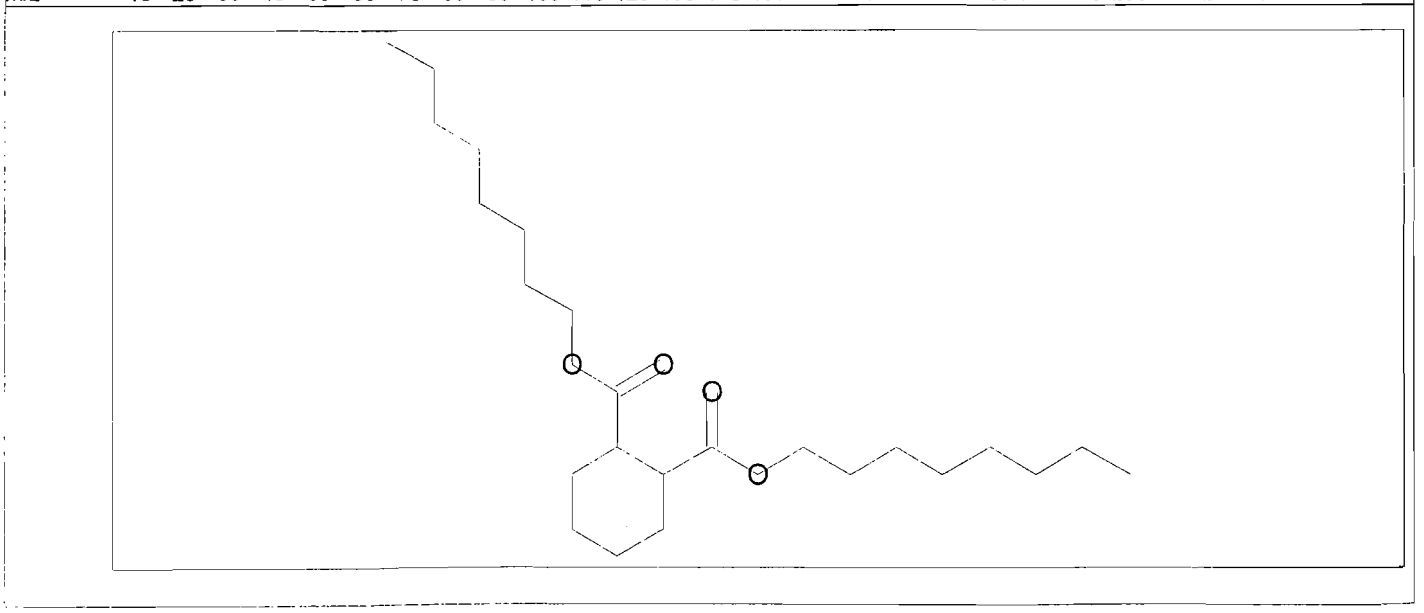


Library Searched : C:\DATABASE\NBS75K.L
Quality : 59
ID : Di-n-octyl phthalate

Spectrum 20

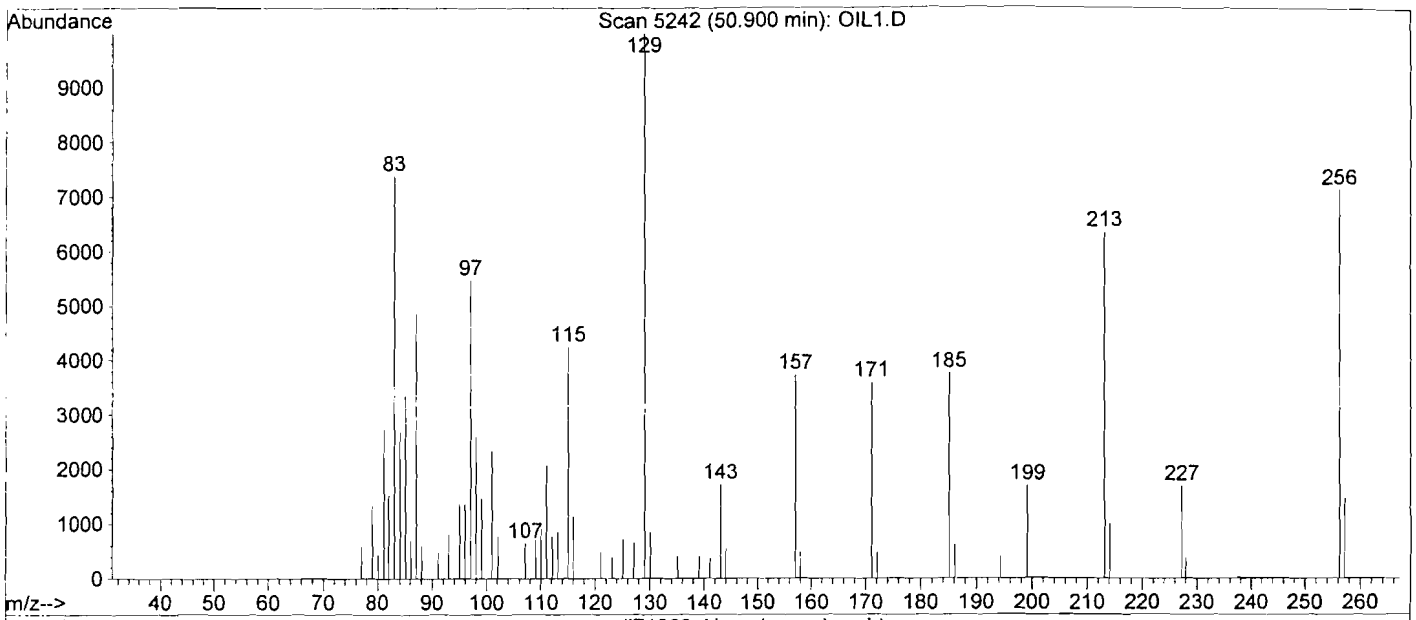


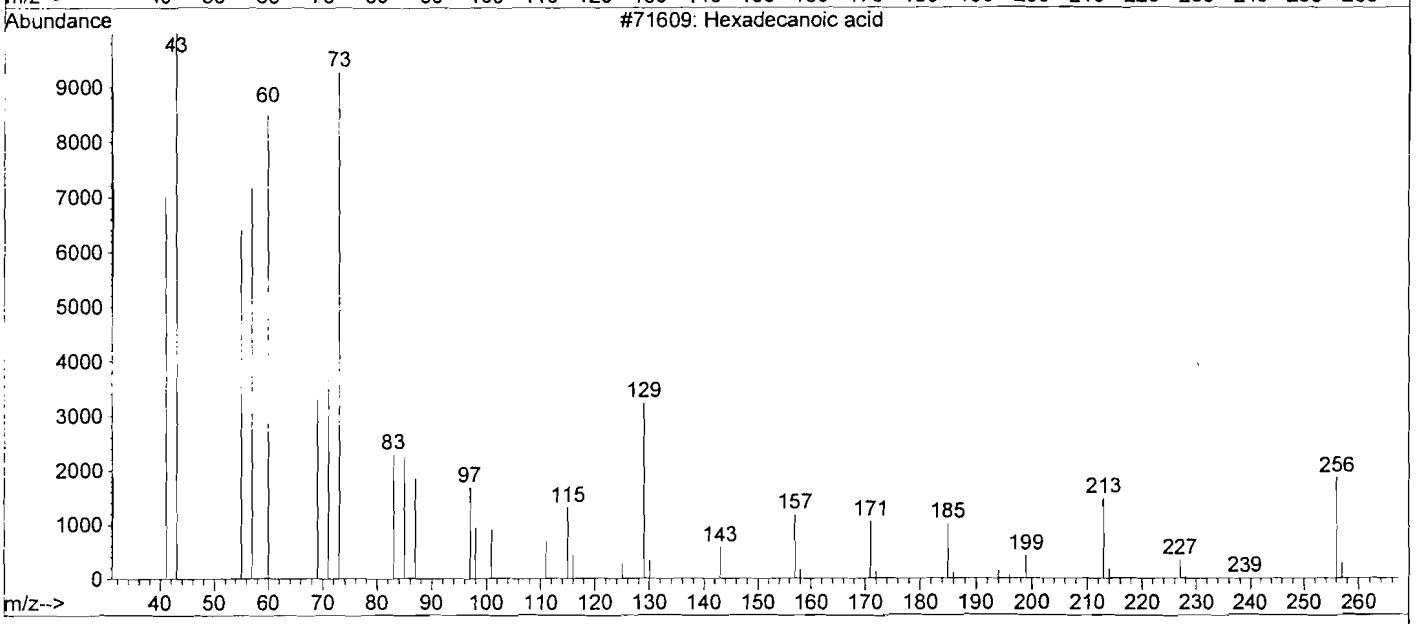


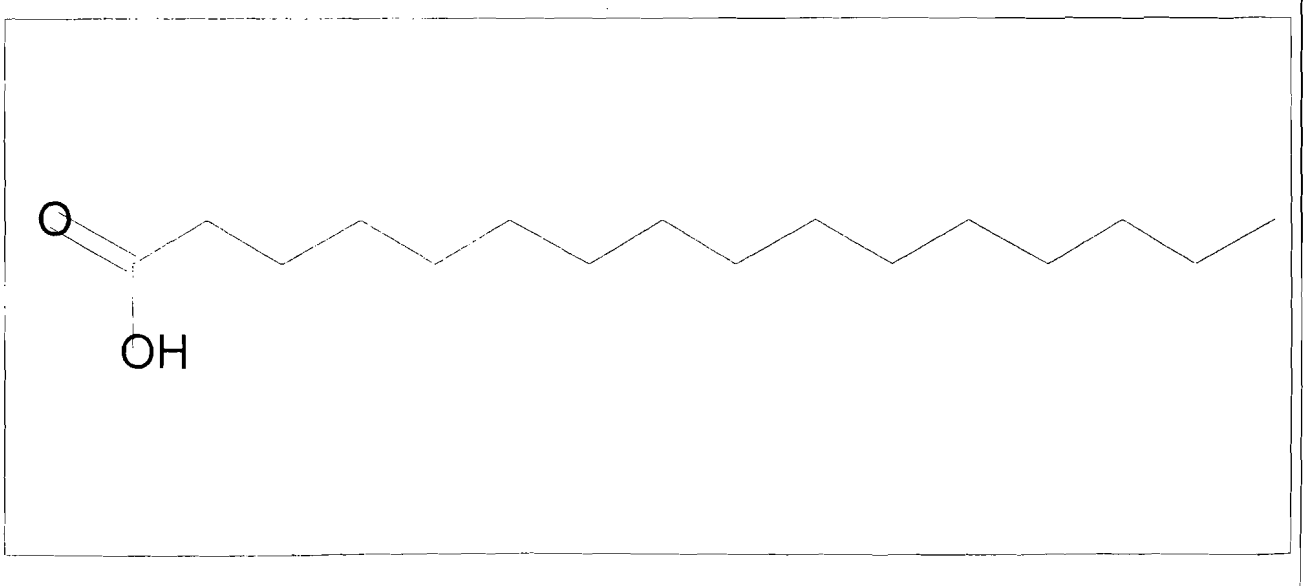


Library Searched : C:\DATABASE\NBS75K.L
Quality : 94
ID : Hexadecanoic acid

Spectrum 21

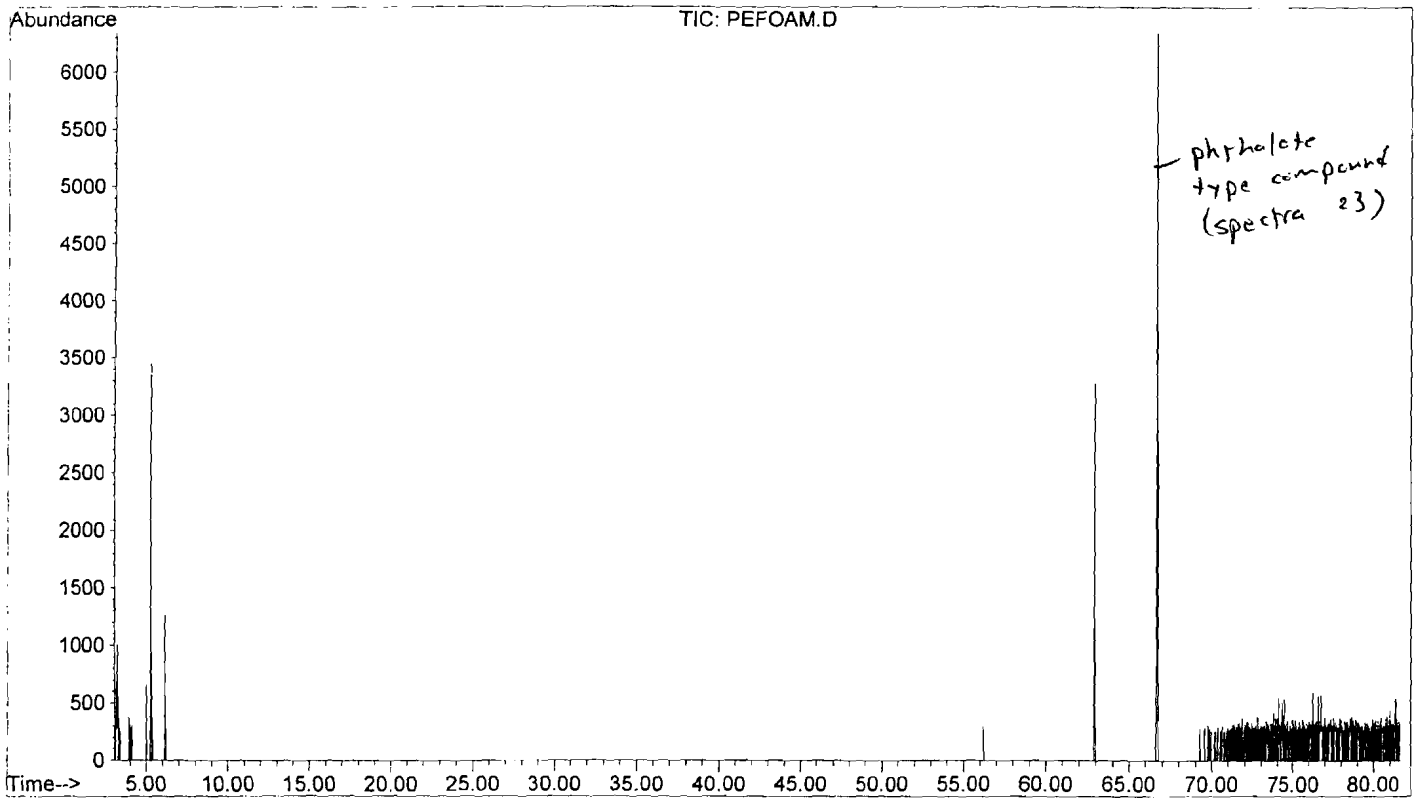


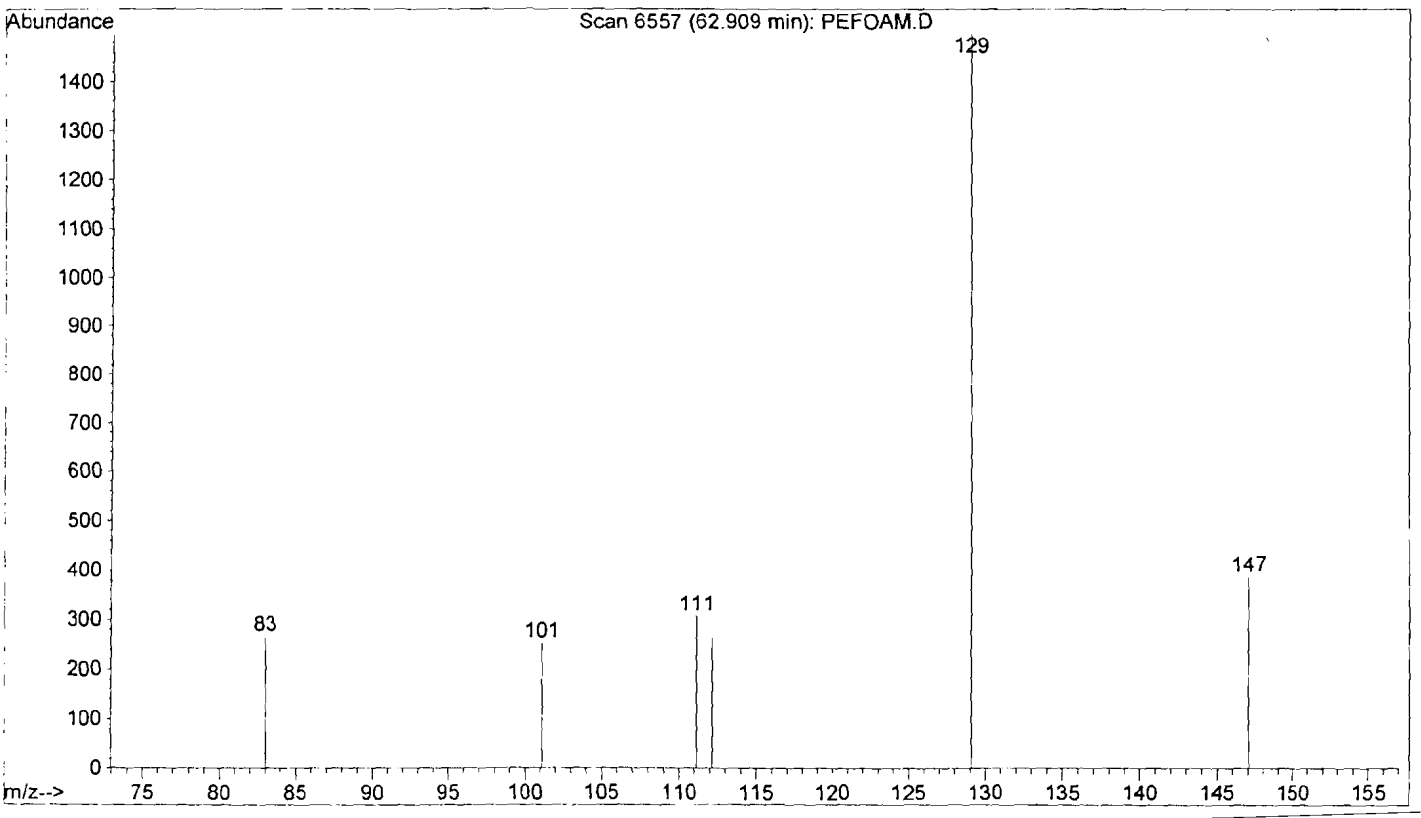




Spectrum 22

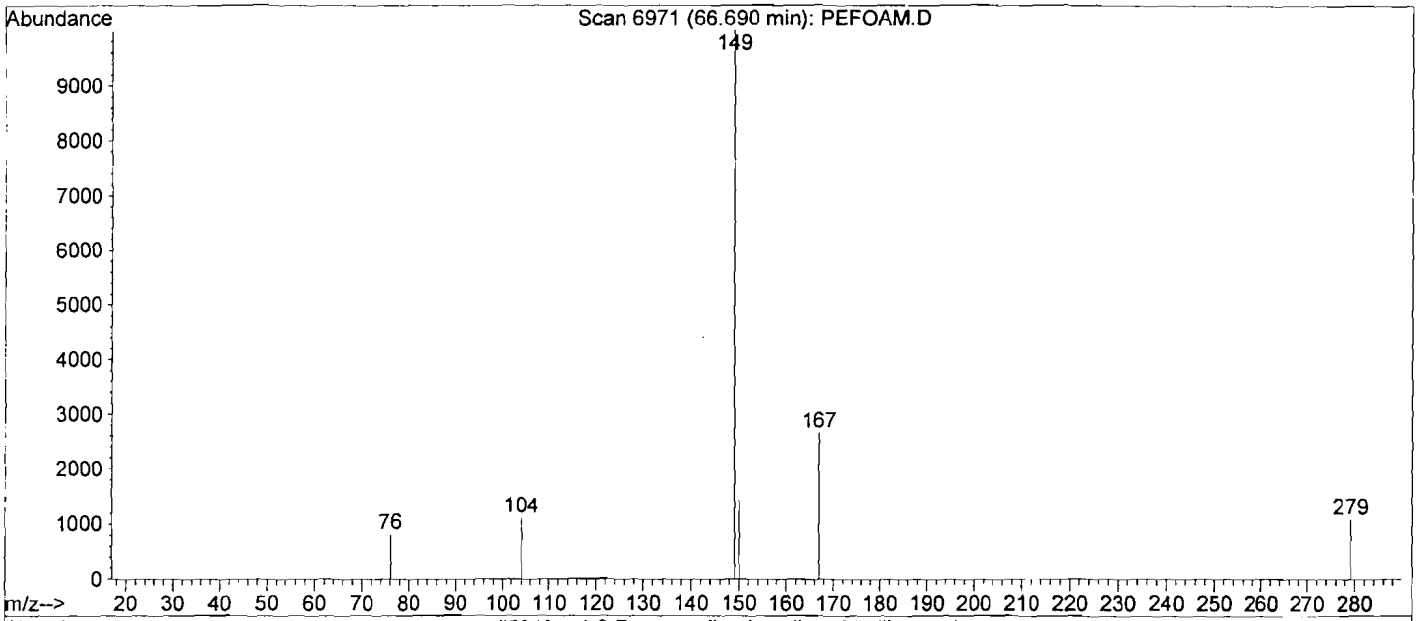
File : C:\HPCHEM\1\DATA\PEFOAM.D
Operator : Kris Mitchell
Acquired : 21 Nov 97 9:17 am using AcqMethod INVERT
Instrument : GC/MS Ins
Sample Name: pet ether foam extract
Misc Info : cyenea foam extract from pe 11/21
Vial Number: 1

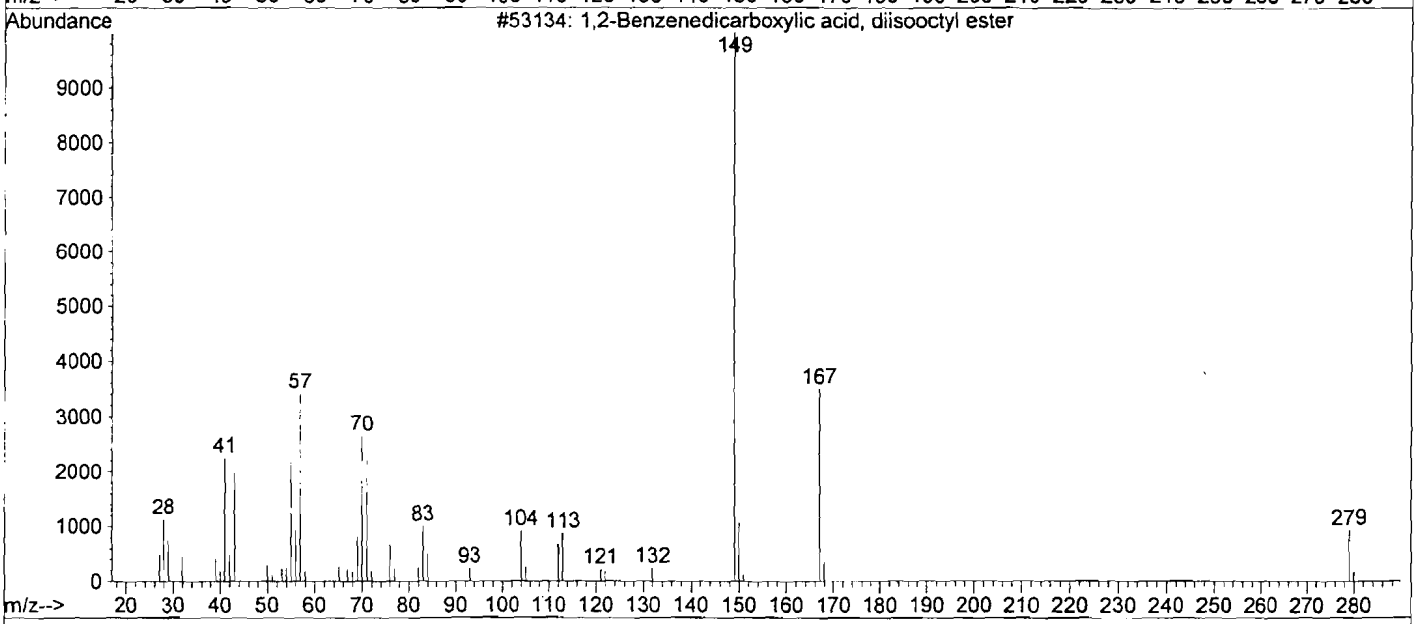


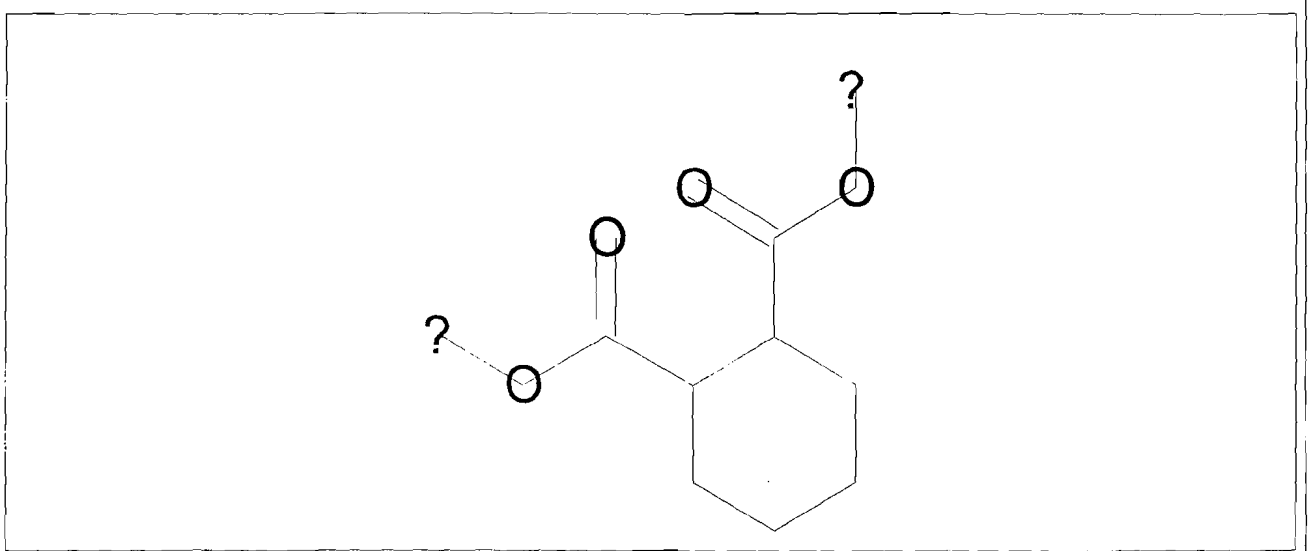


Library Searched : C:\DATABASE\NBS75K.L
Quality : 83
ID : 1,2-Benzenedicarboxylic acid, diisooctyl ester

Spectrum 23

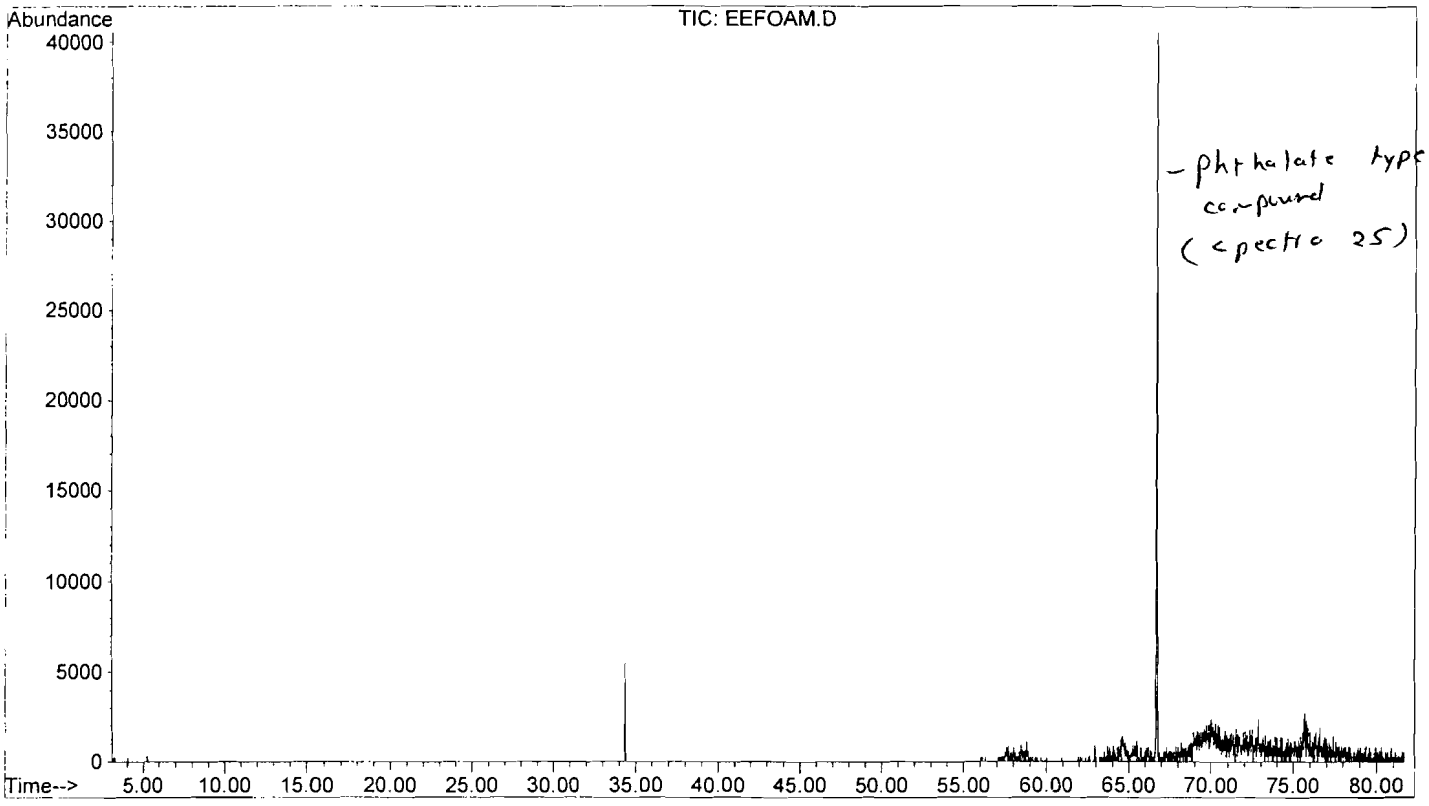




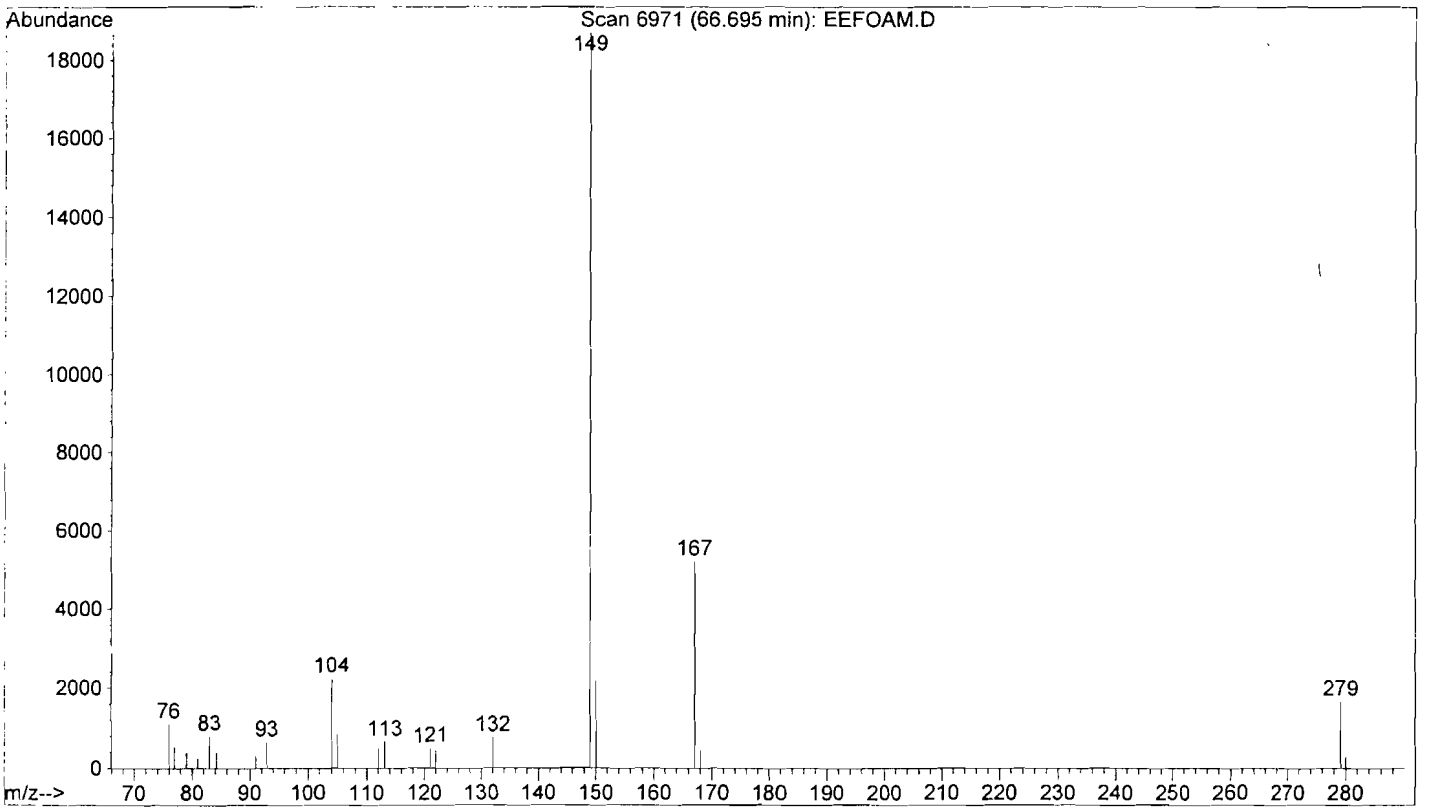


Spectrum 24

File : C:\HPCHEM\1\DATA\EEFOAM.D
Operator : Kris Mitchell
Acquired : 20 Nov 97 9:56 pm using AcqMethod INVERT
Instrument : GC/MS Ins
Sample Name: ethyl ether foam extract
Misc Info : cyenea foam extract from ee 11/20
Vial Number: 1

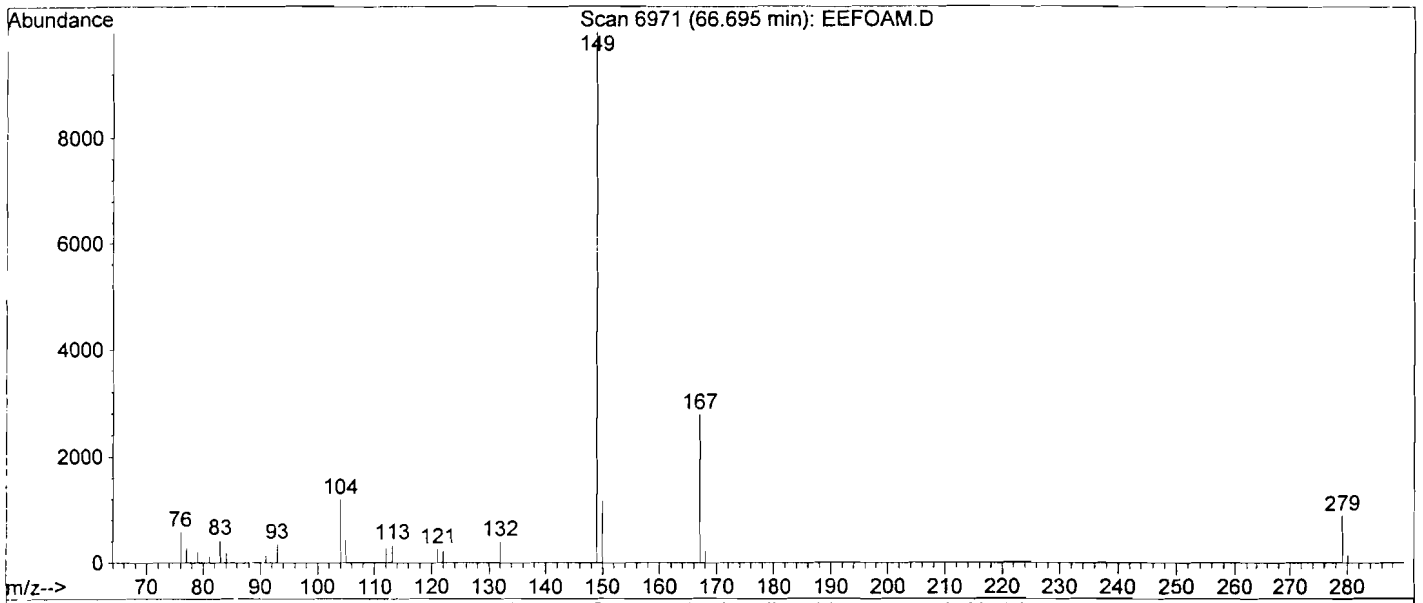


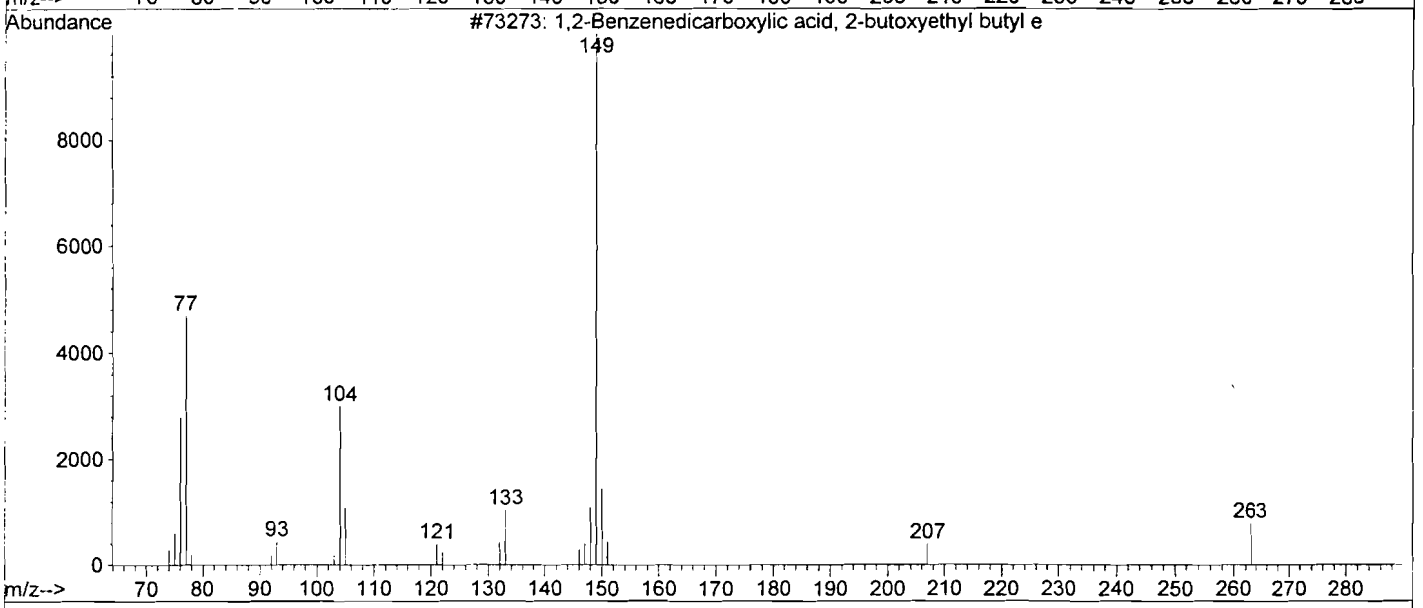
- phthalate type compound (spectra 25)

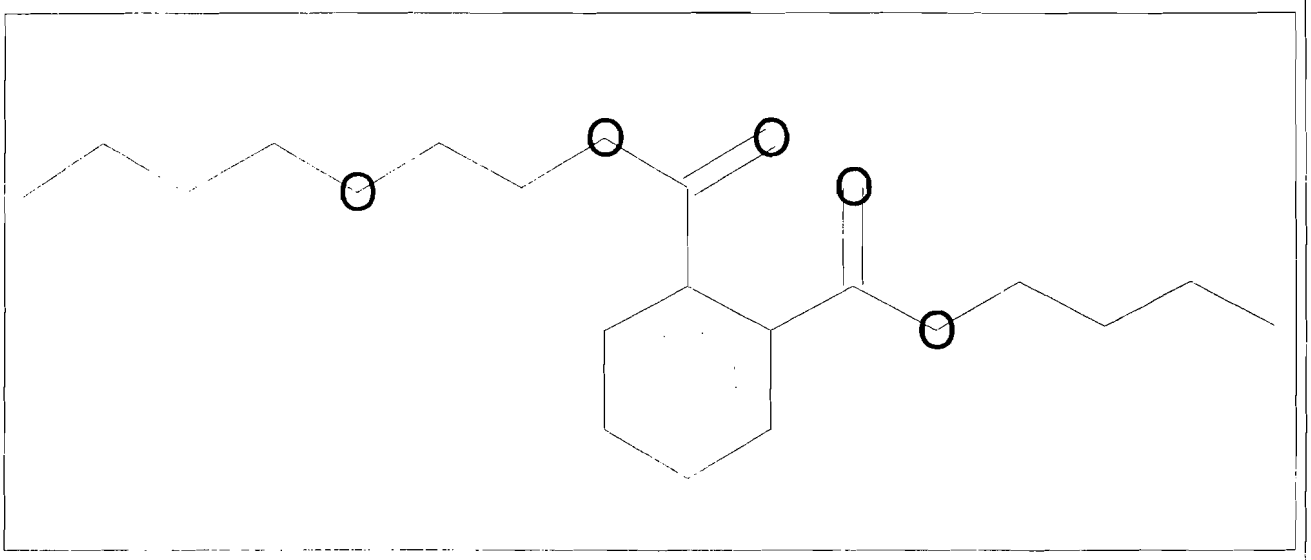


Library Searched : C:\DATABASE\NBS75K.L
Quality : 59
ID : 1,2-Benzenedicarboxylic acid, 2-butoxyethyl butyl este
r

Spectrum 25

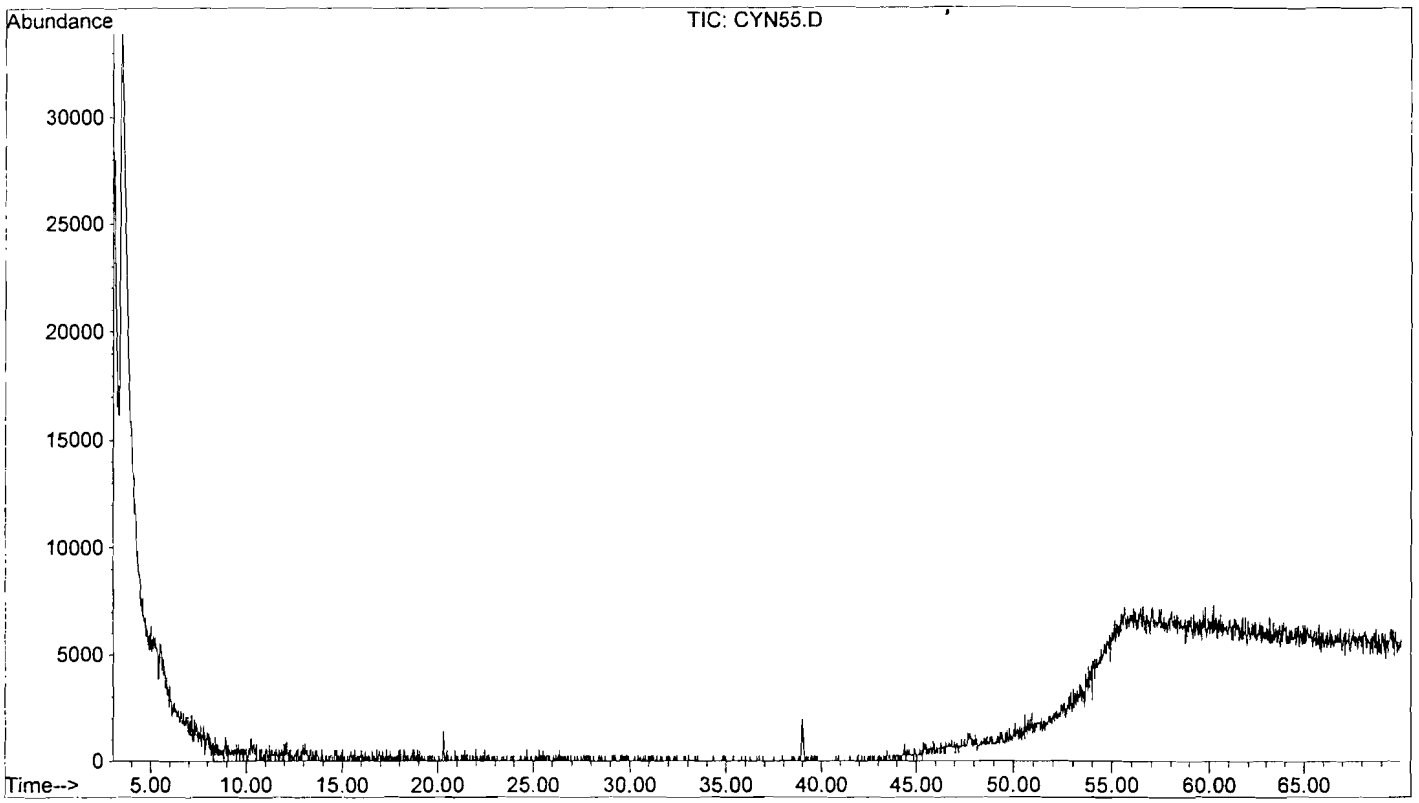


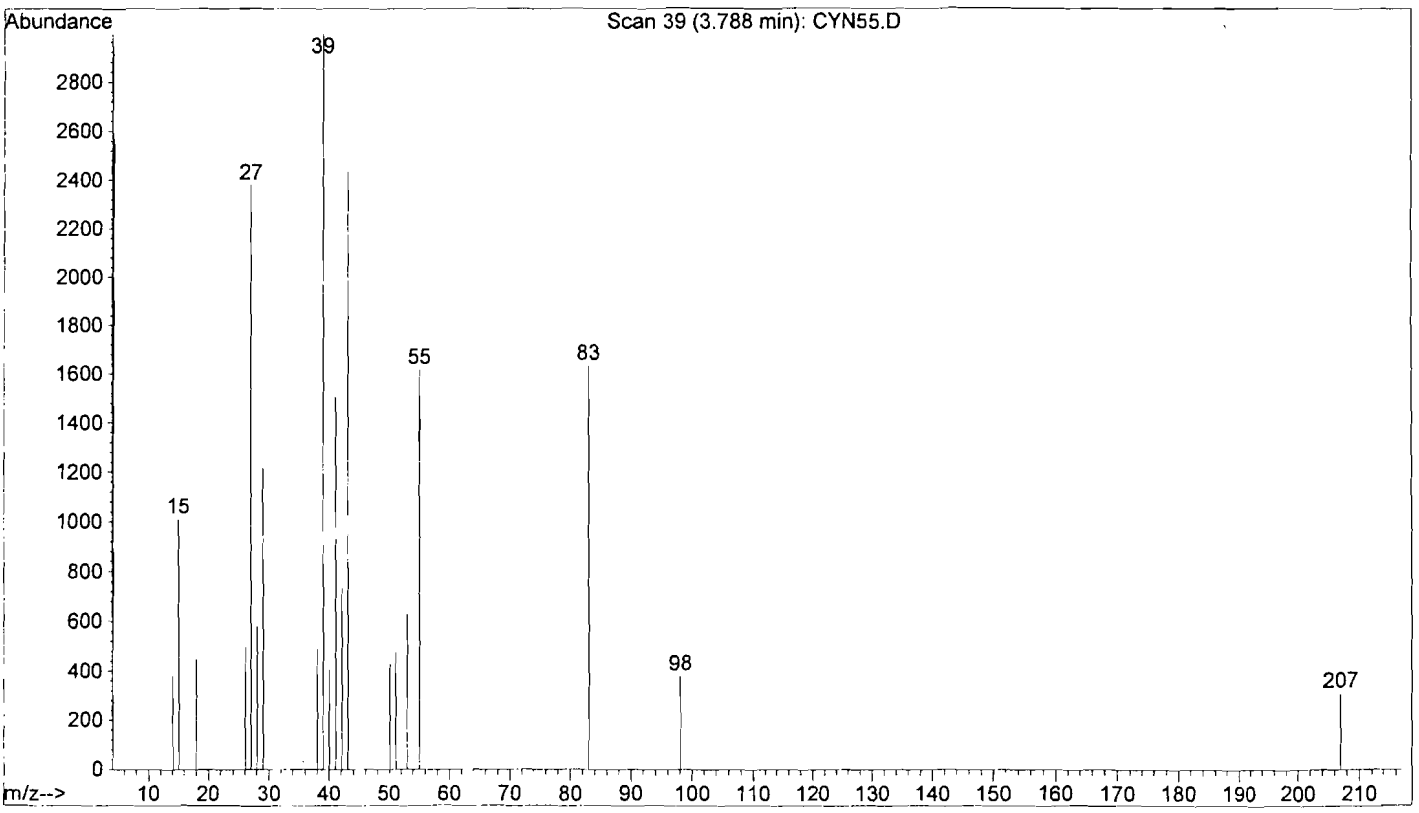




Spectrum 26

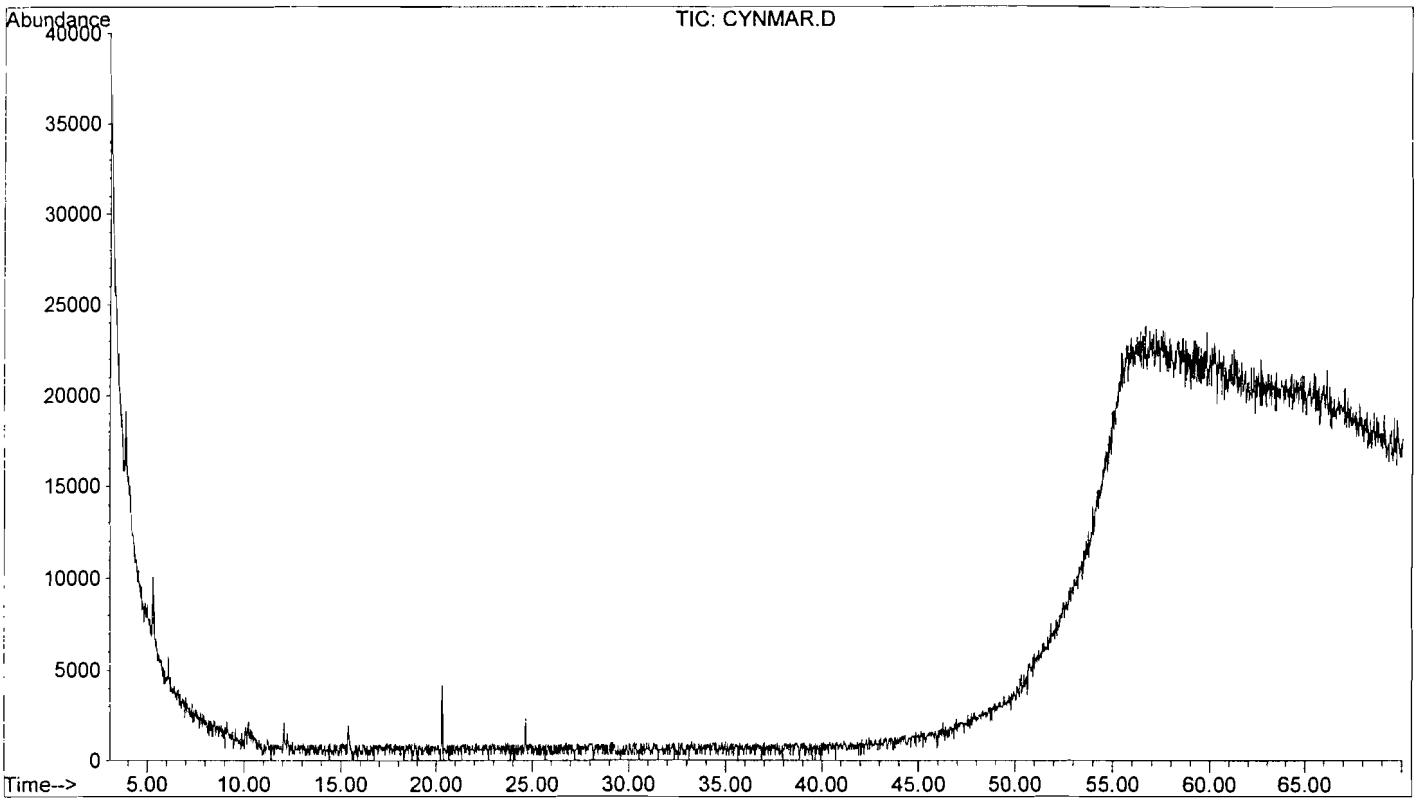
File : C:\HPCHEM\1\DATA\CYN55.D
Operator : Kris Mitchell
Acquired : 25 Mar 98 4:45 pm using AcqMethod INVERT
Instrument : GC/MS Ins
Sample Name: cyn553-25
Misc Info : cyenea in 5pe and ace 3-25-98
Vial Number: 1

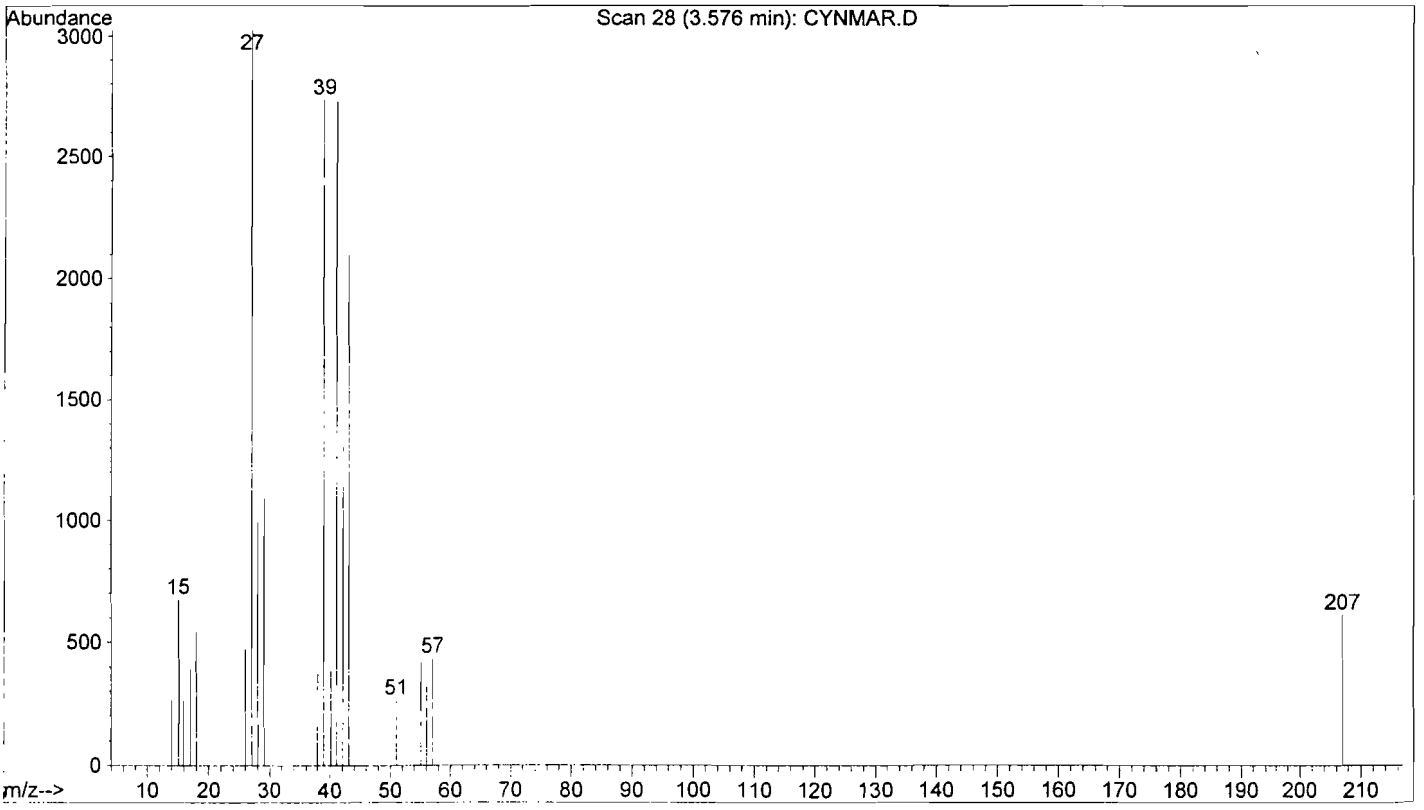




Spectrum 27

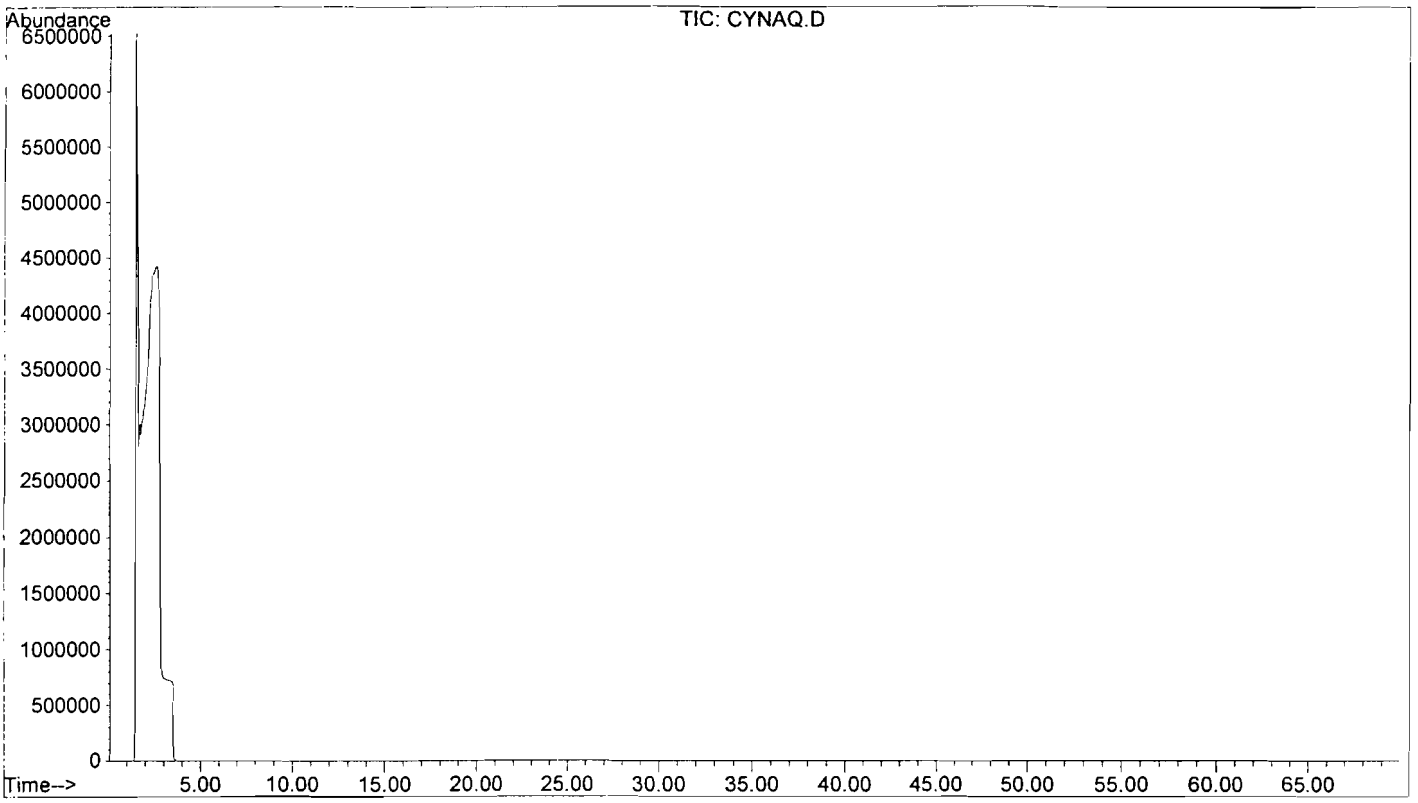
File : C:\HPCHEM\1\DATA\CYNMAR.D
Operator : Kris Mitchell
Acquired : 25 Mar 98 11:20 am using AcqMethod INVERT
Instrument : GC/MS Ins
Sample Name: cyenea 18.5 mar
Misc Info : cyenea 18.5g 3-25-98
Vial Number: 1

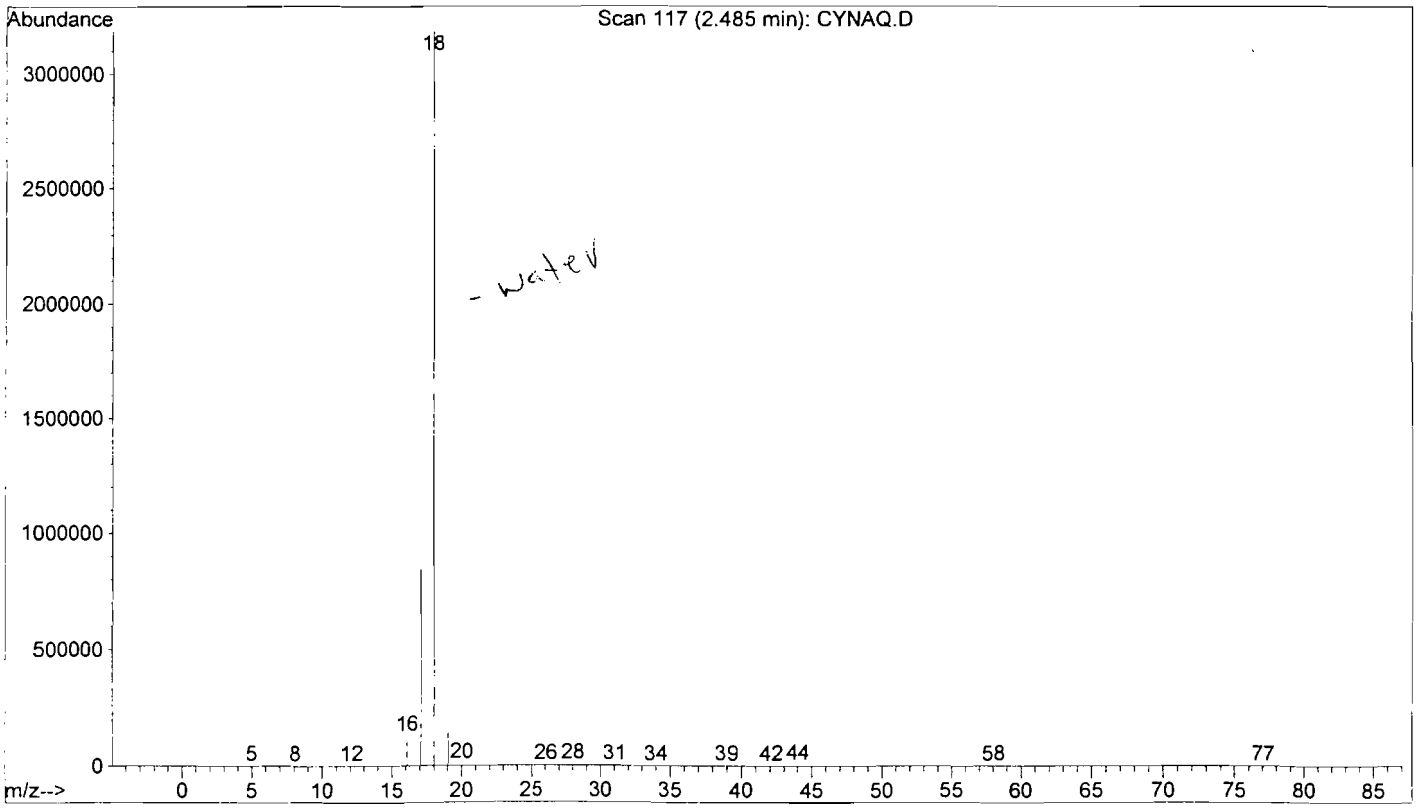




Spectrum 28

File : C:\HPCHEM\1\DATA\CYNAQ.D
Operator : Kris Mitchell
Acquired : 25 Mar 98 10:44 pm using AcqMethod INVERT
Instrument : GC/MS Ins
Sample Name: cynaq3-25
Misc Info : cyenea aqueous 3-25-98
Vial Number: 1

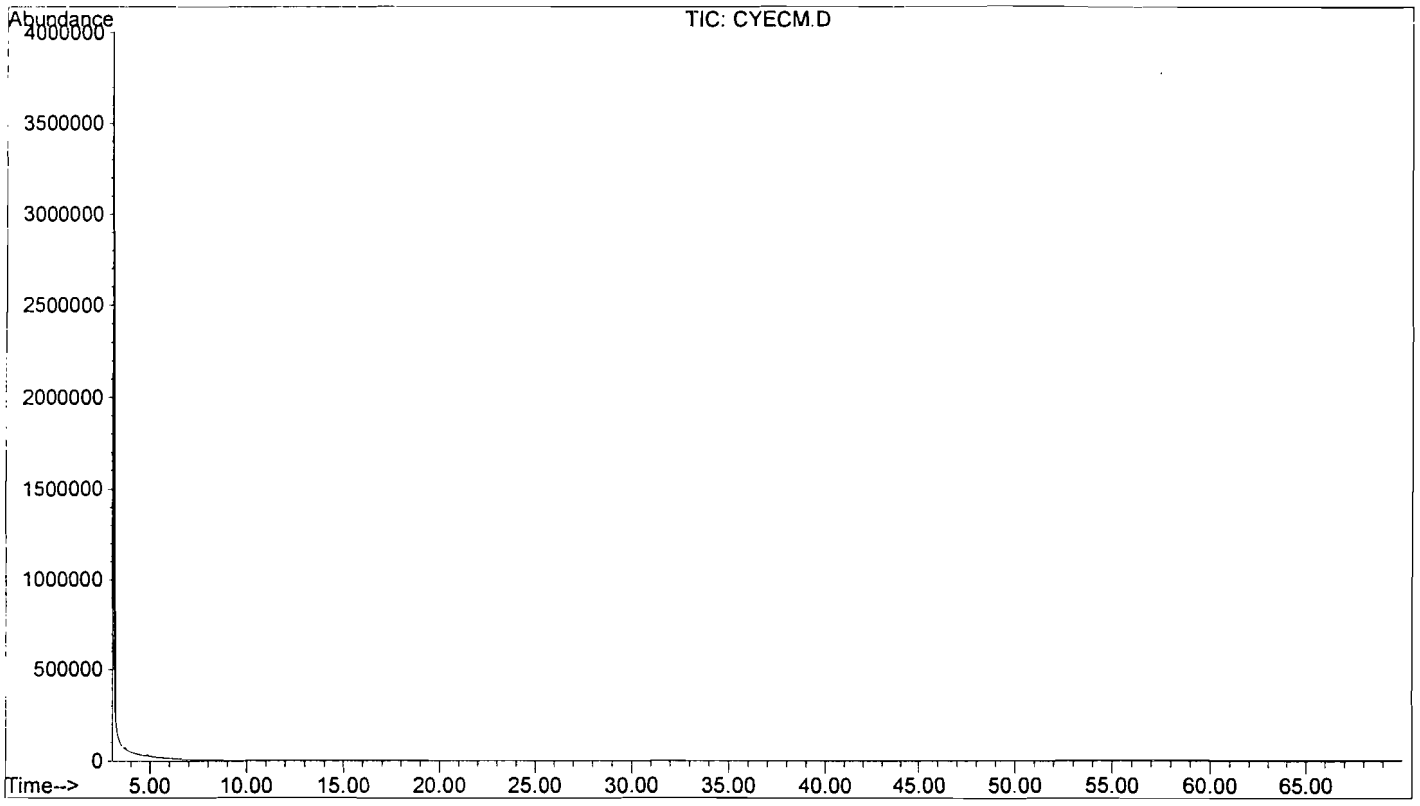


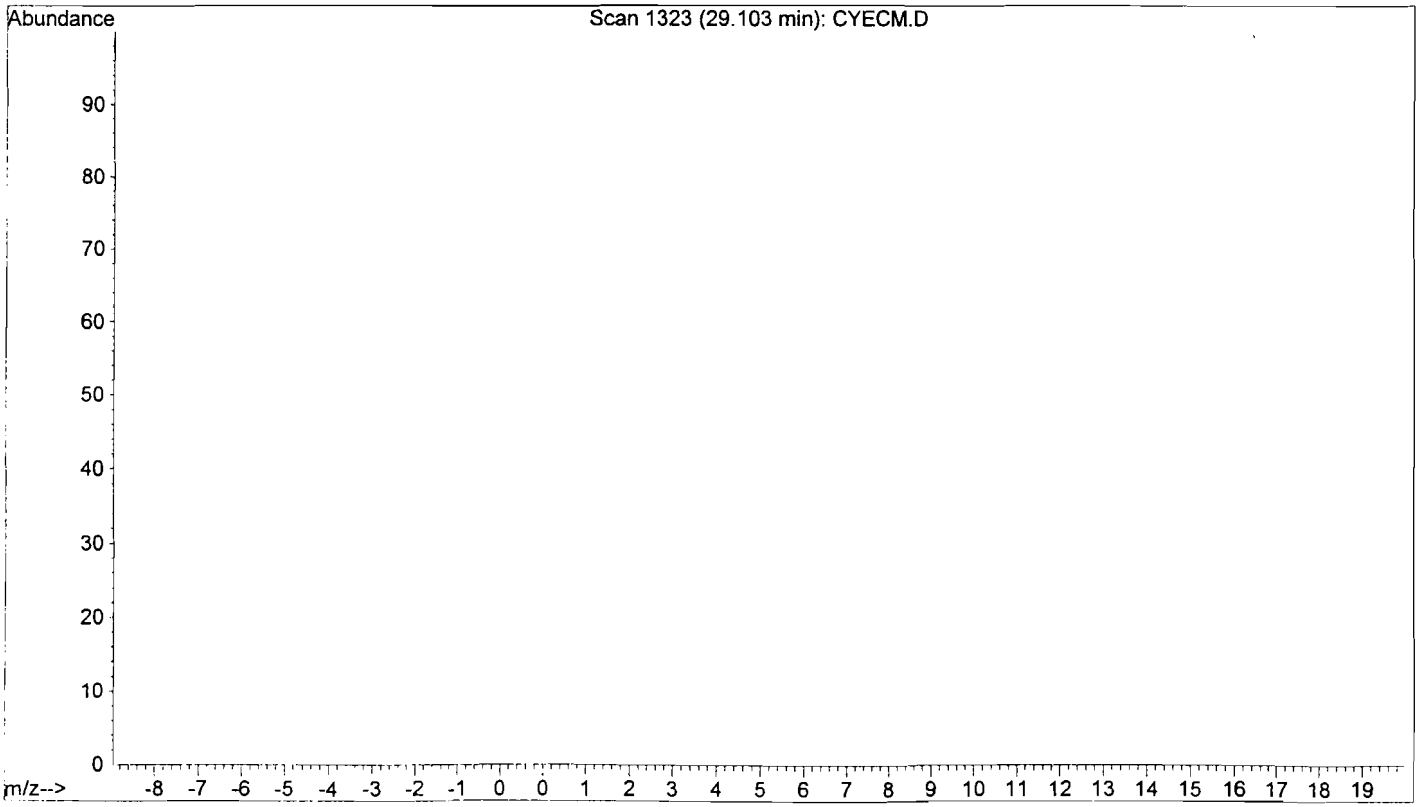


- water

Spectrum 29

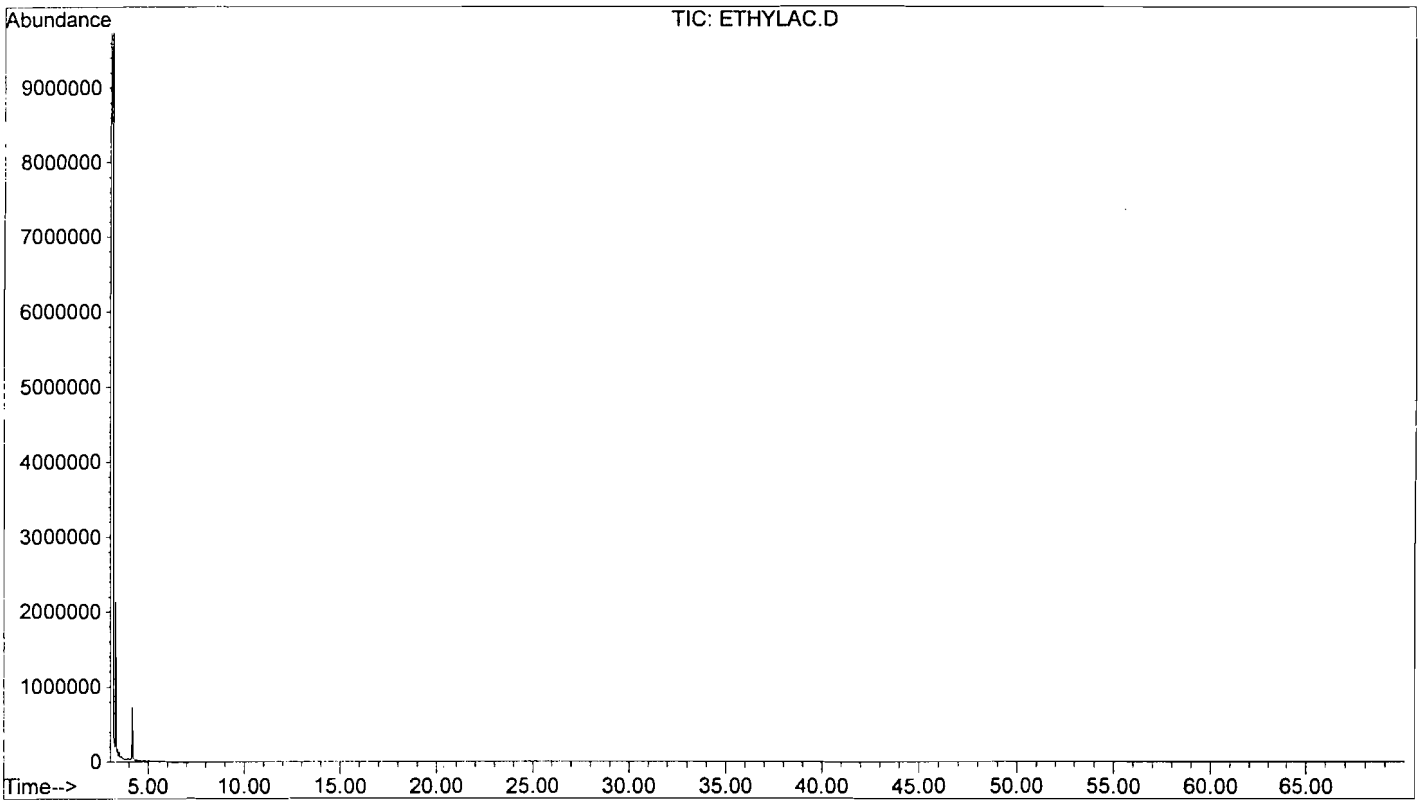
File : C:\HPCHEM\1\DATA\CYECM.D
Operator : Kris Mitchell
Acquired : 9 Apr 98 7:22 pm using AcqMethod INVERT
Instrument : GC/MS Ins
Sample Name: cyenea in 1:1
Misc Info : chloroform:methanol 4-9-98
Vial Number: 1

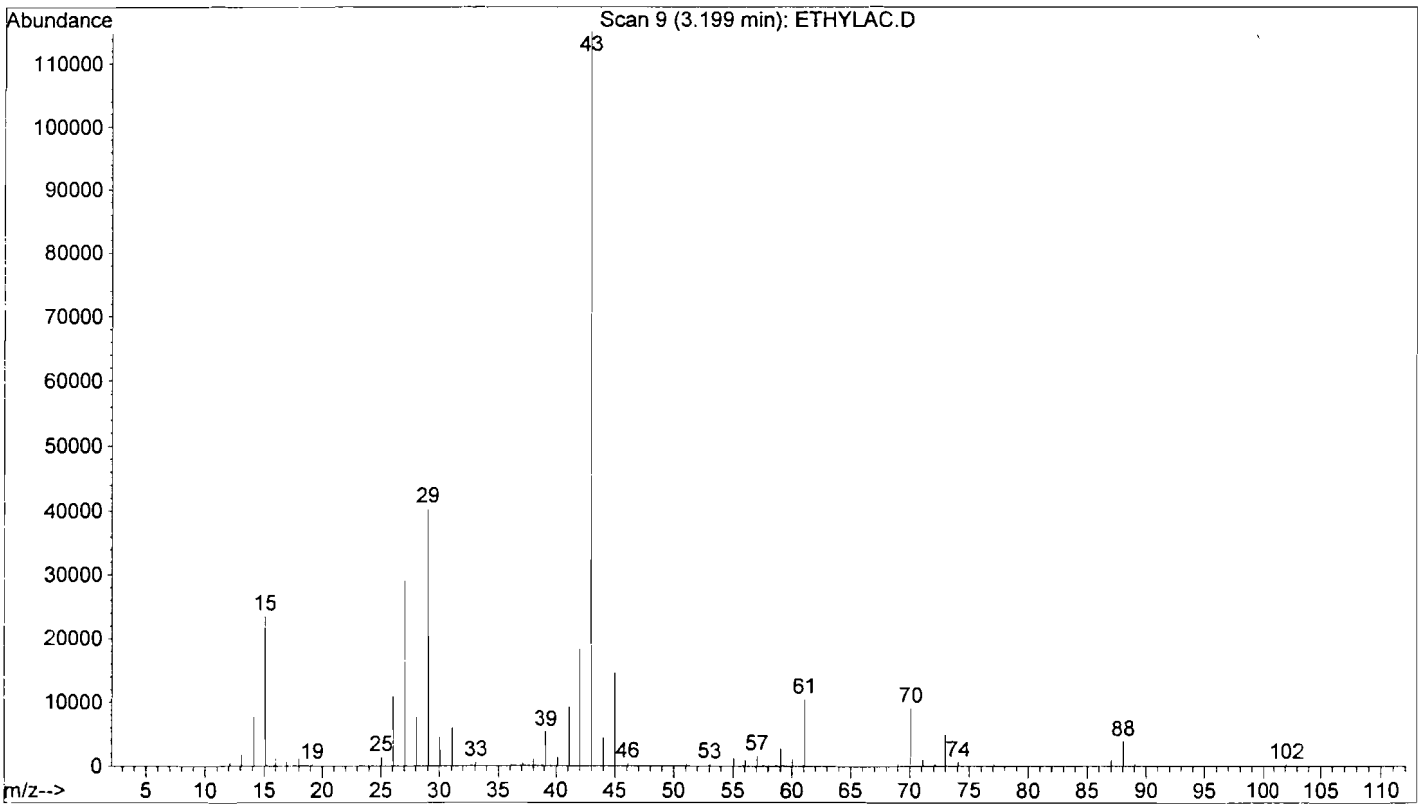




Spectrum 30

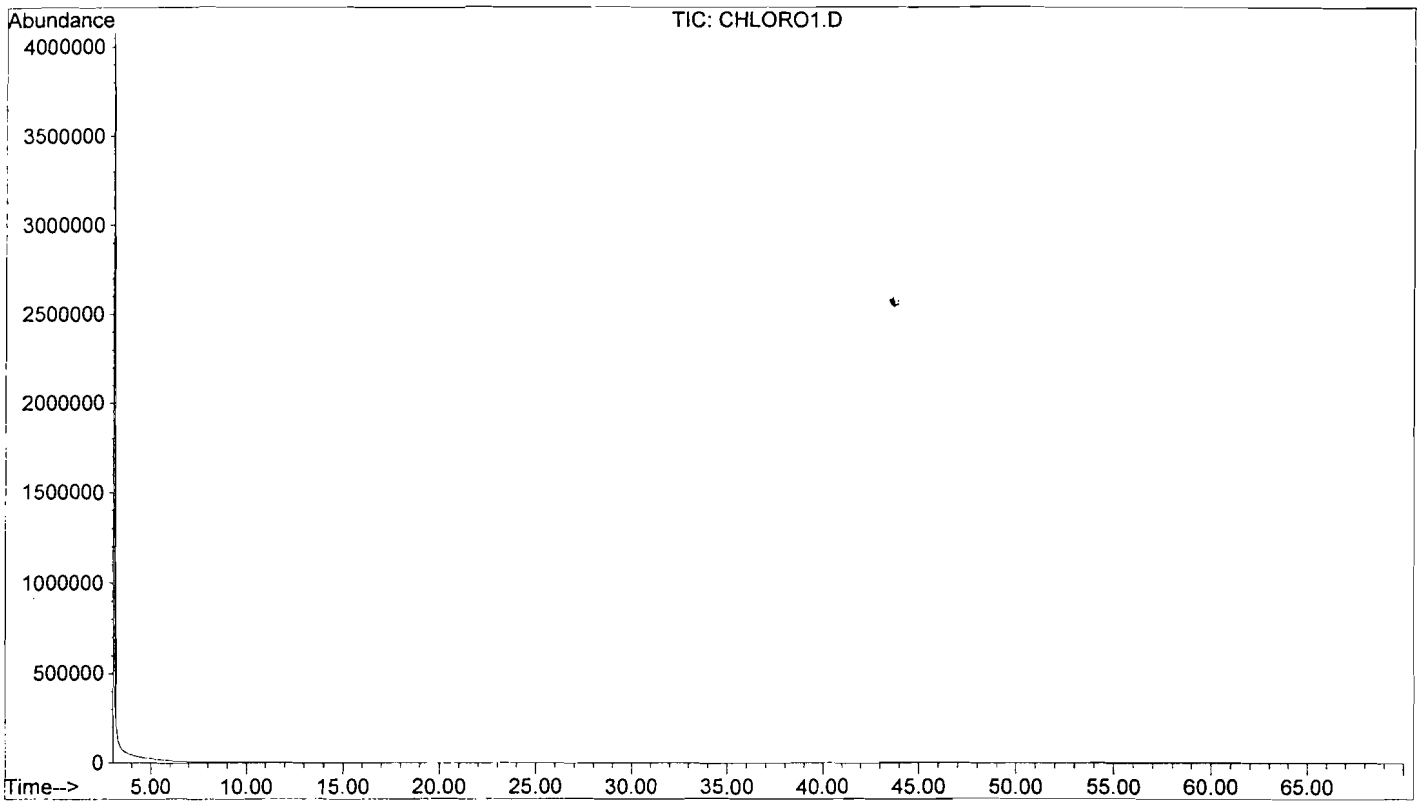
File : C:\HPCHEM\1\DATA\ETHYLAC.D
Operator : Kris Mitchell
Acquired : 10 Apr 98 9:43 am using AcqMethod INVERT
Instrument : GC/MS Ins
Sample Name: ethyl acetate
Misc Info : cyenea in ethyl acetate 4-10-98
Vial Number: 1

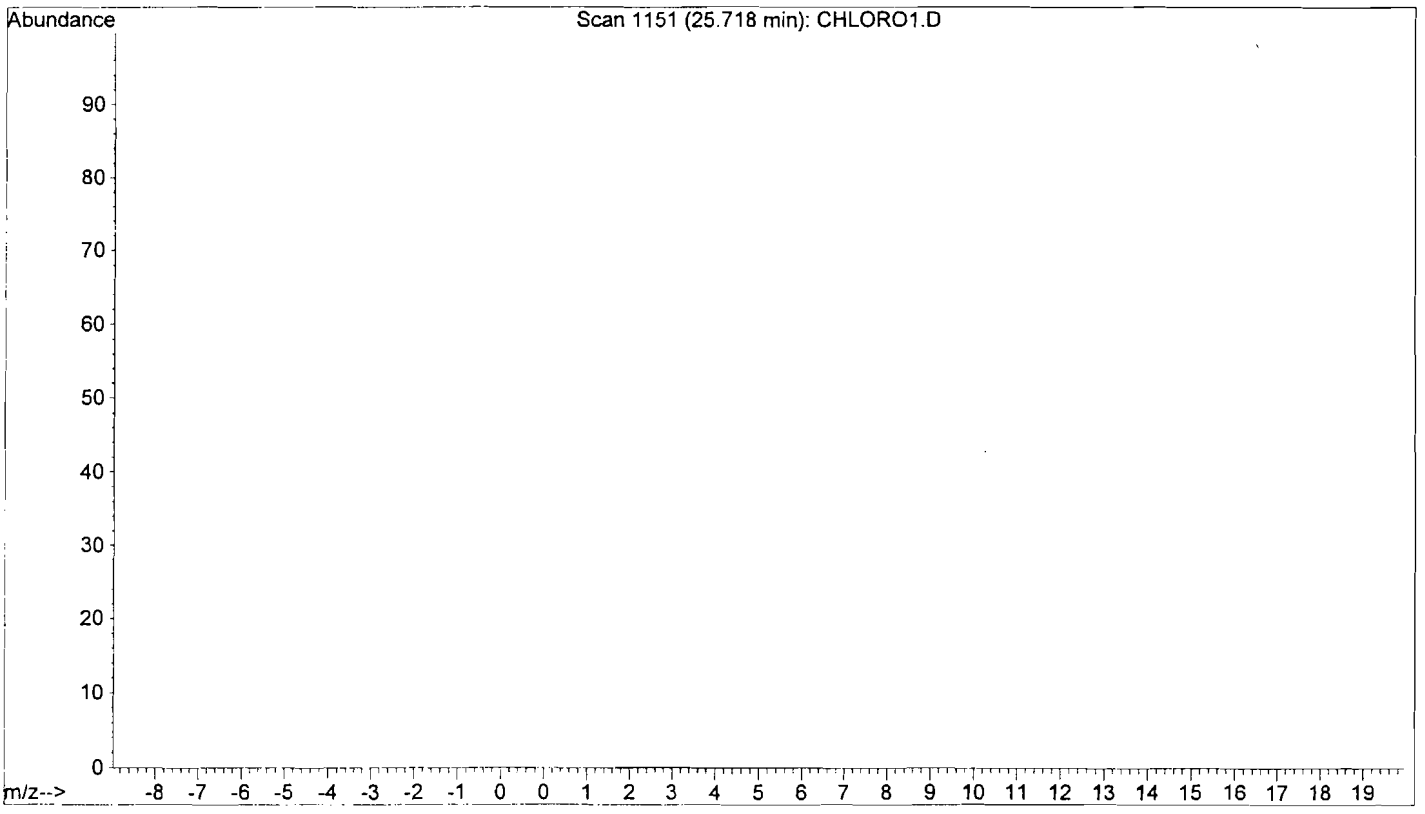




Spectrum 31

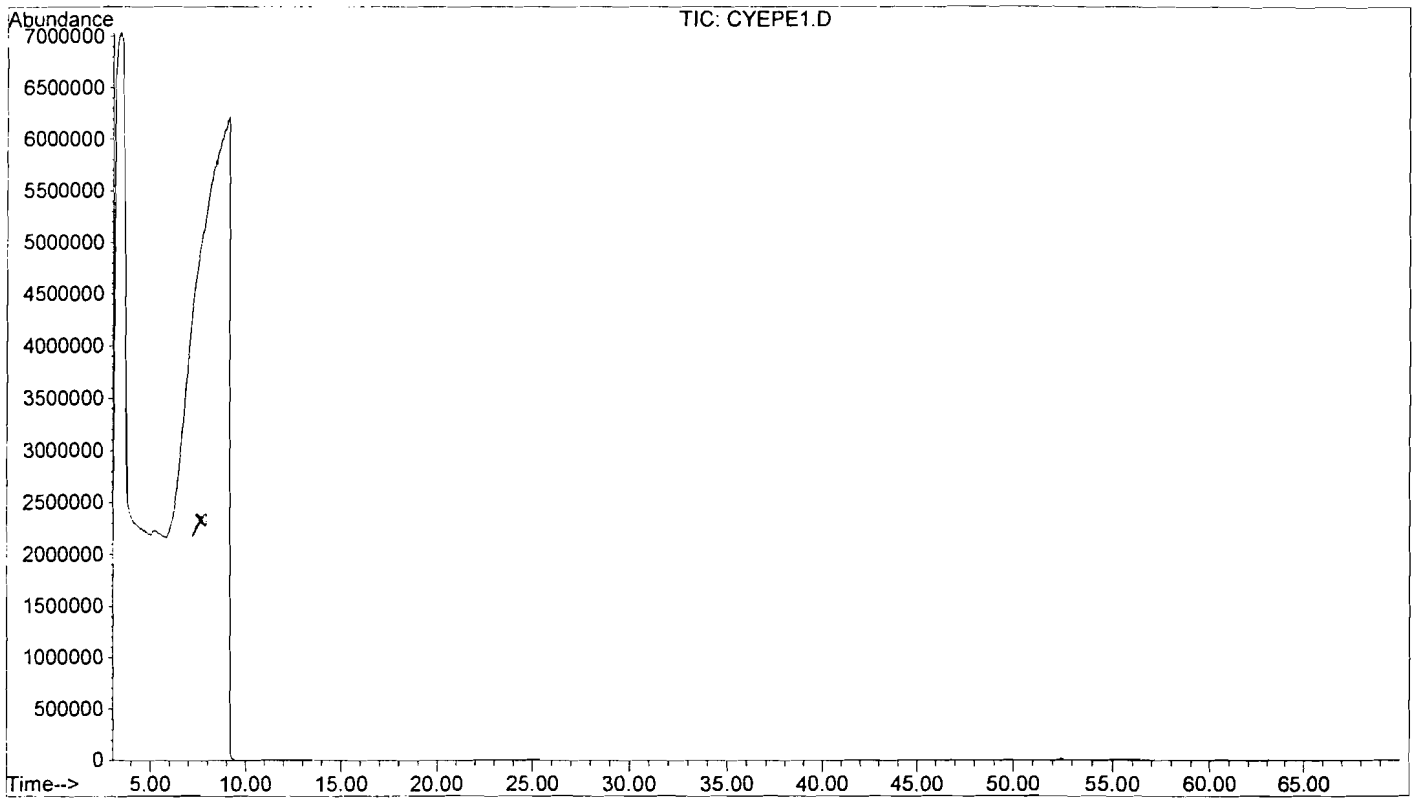
File : C:\HPCHEM\1\DATA\CHLORO1.D
Operator : Kris Mitchell
Acquired : 10 Apr 98 1:25 pm using AcqMethod INVERT
Instrument : GC/MS Ins
Sample Name: chloroform 1
Misc Info : cyenea in chloroform 1 4-10-98
Vial Number: 1

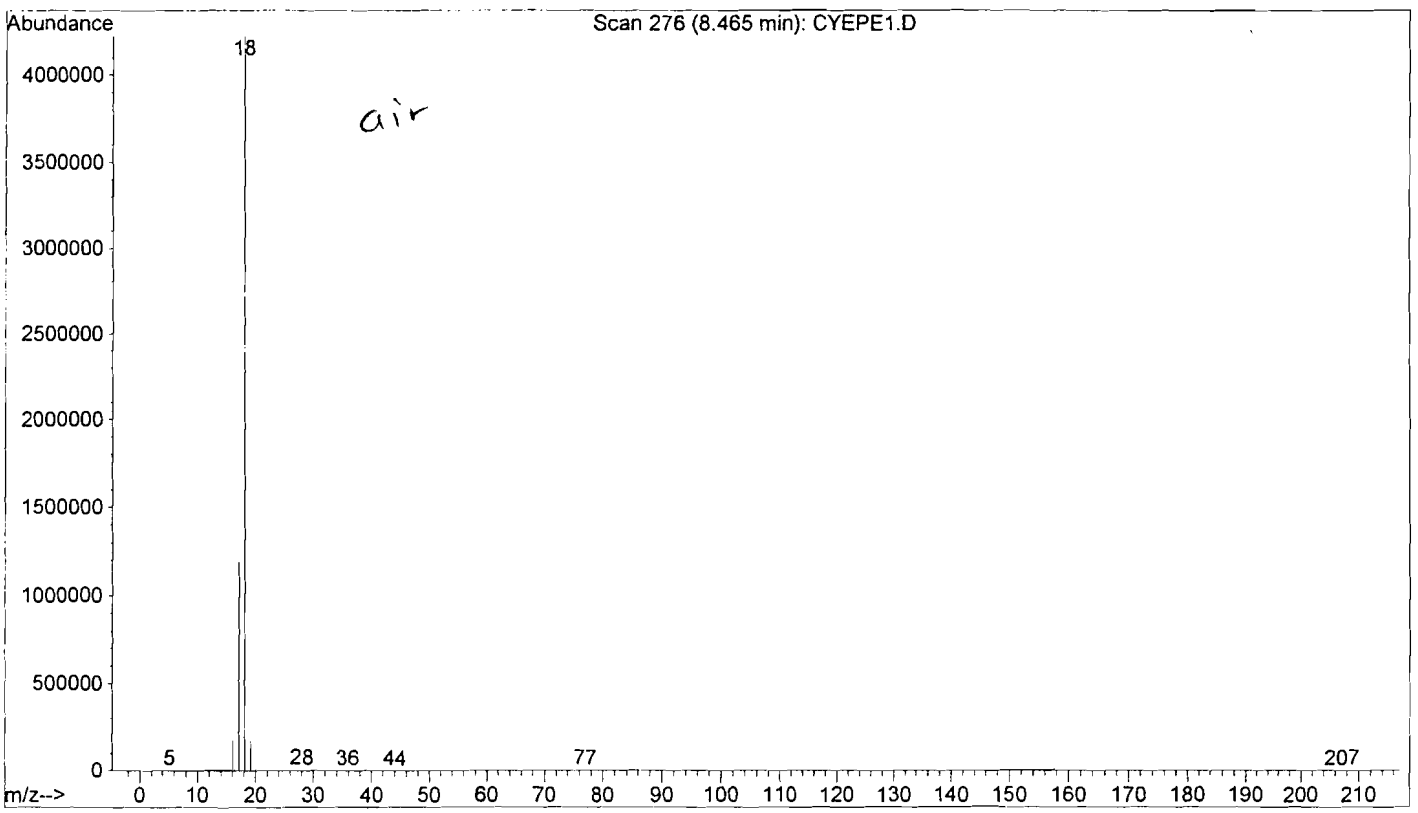




Spectrum 32

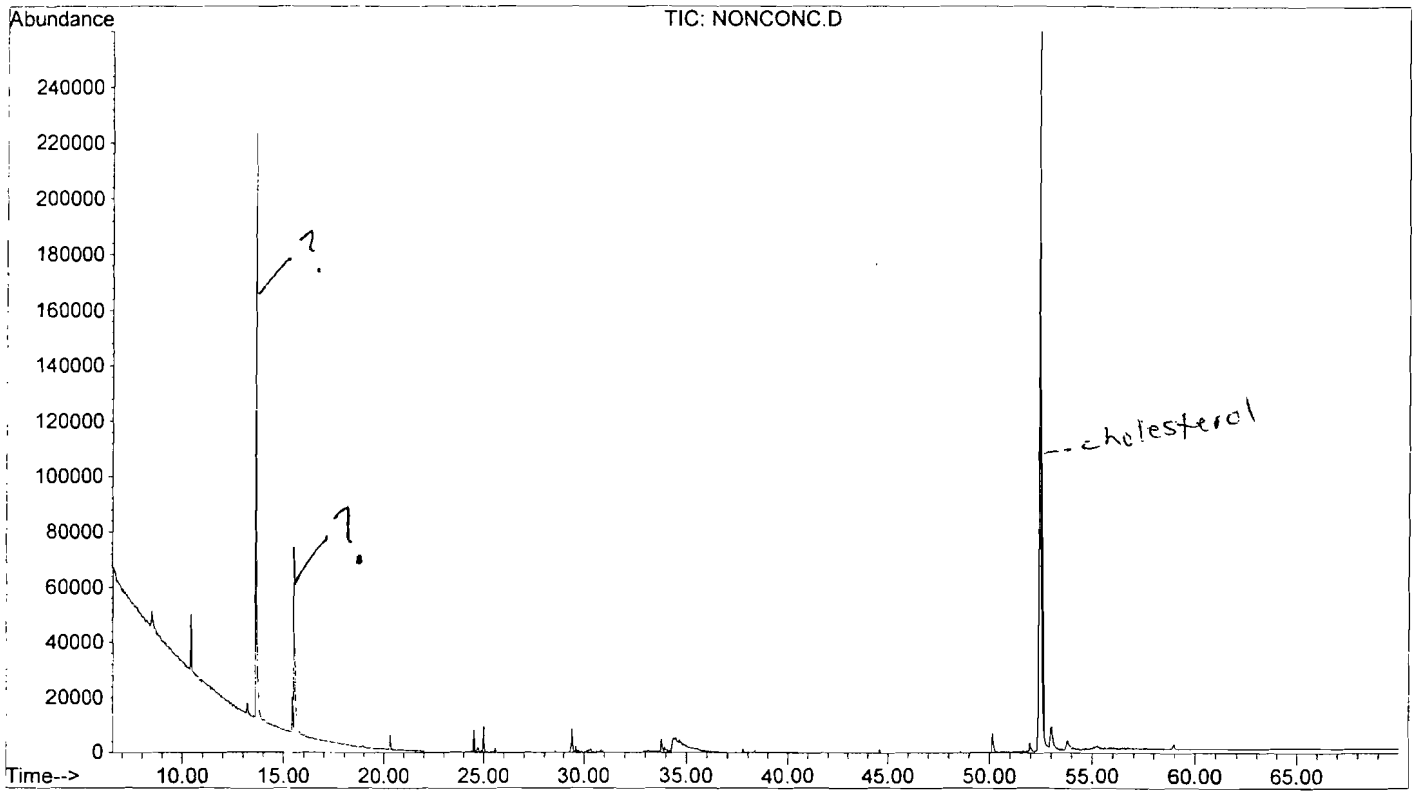
File : C:\HPCHEM\1\DATA\CYEPE1.D
Operator : Kris Mitchell
Acquired : 10 Apr 98 2:42 pm using AcqMethod INVERT
Instrument : GC/MS Ins
Sample Name: petro ether 1
Misc Info : cyenea in pet ether 1 4-10-98
Vial Number: 1

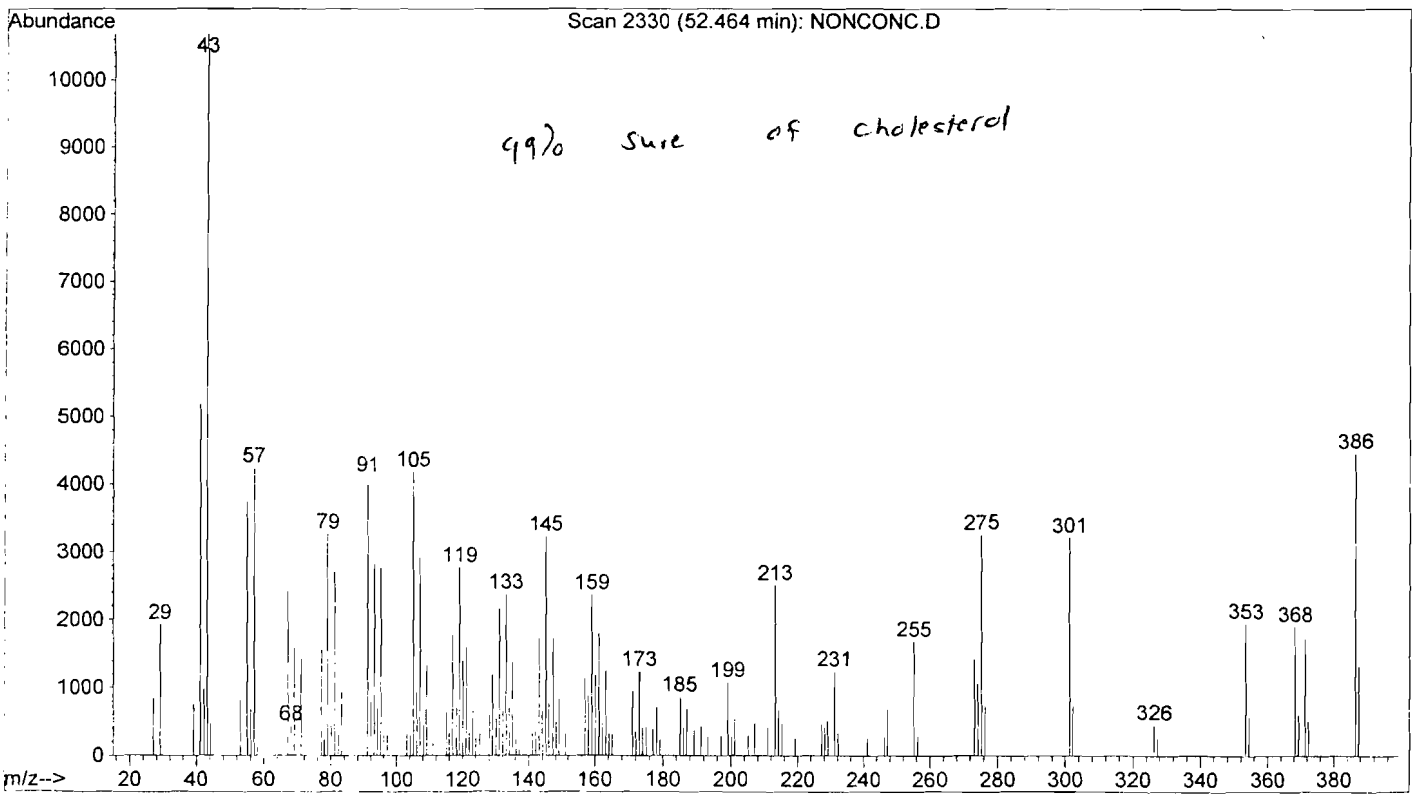




Spectrum 33

File : C:\HPCHEM\1\DATA\NONCONC.D
Operator : Kris Mitchell
Acquired : 12 Apr 98 3:02 pm using AcqMethod INVERT
Instrument : GC/MS Ins
Sample Name: noncontaminated chlo
Misc Info : noncontaminated chloroform 4-12-98
Vial Number: 1

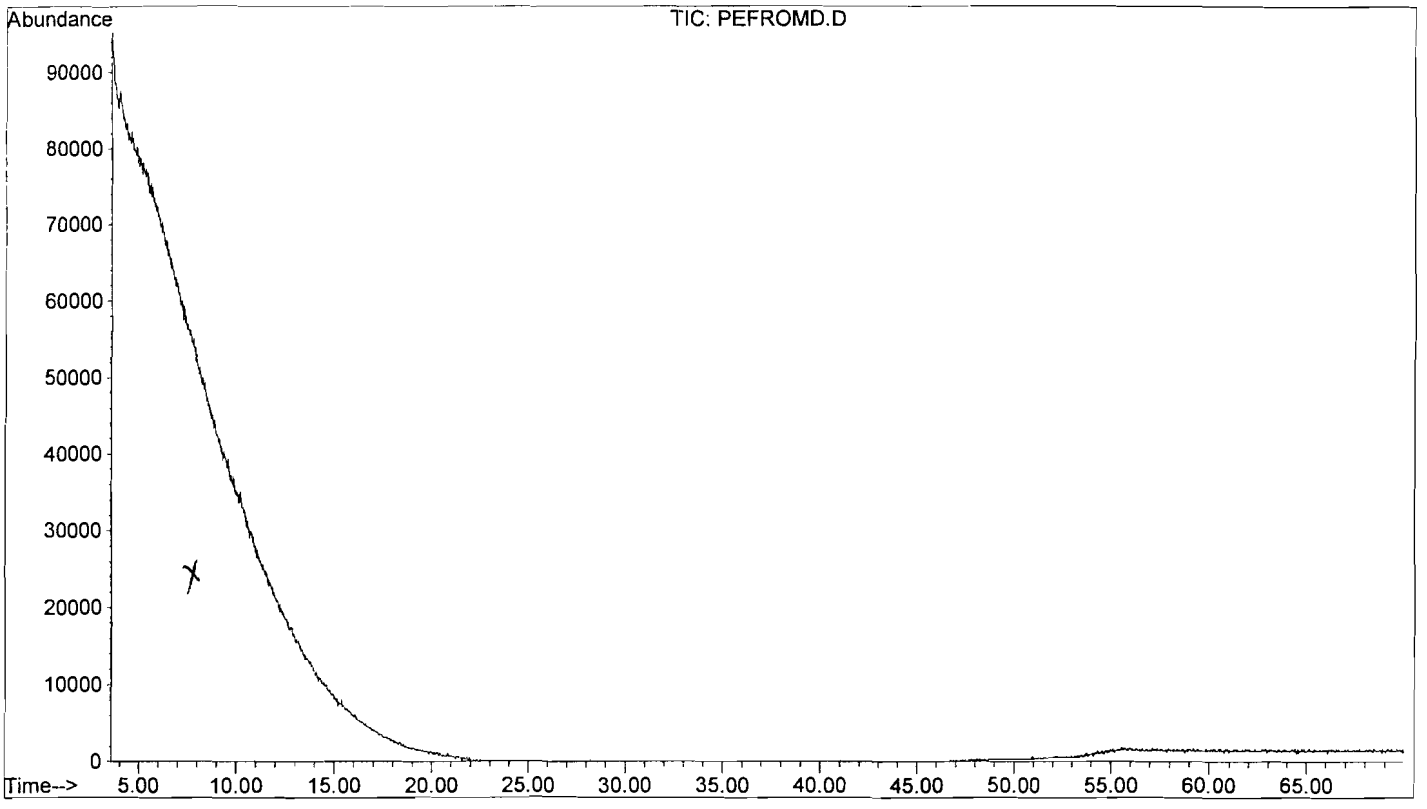


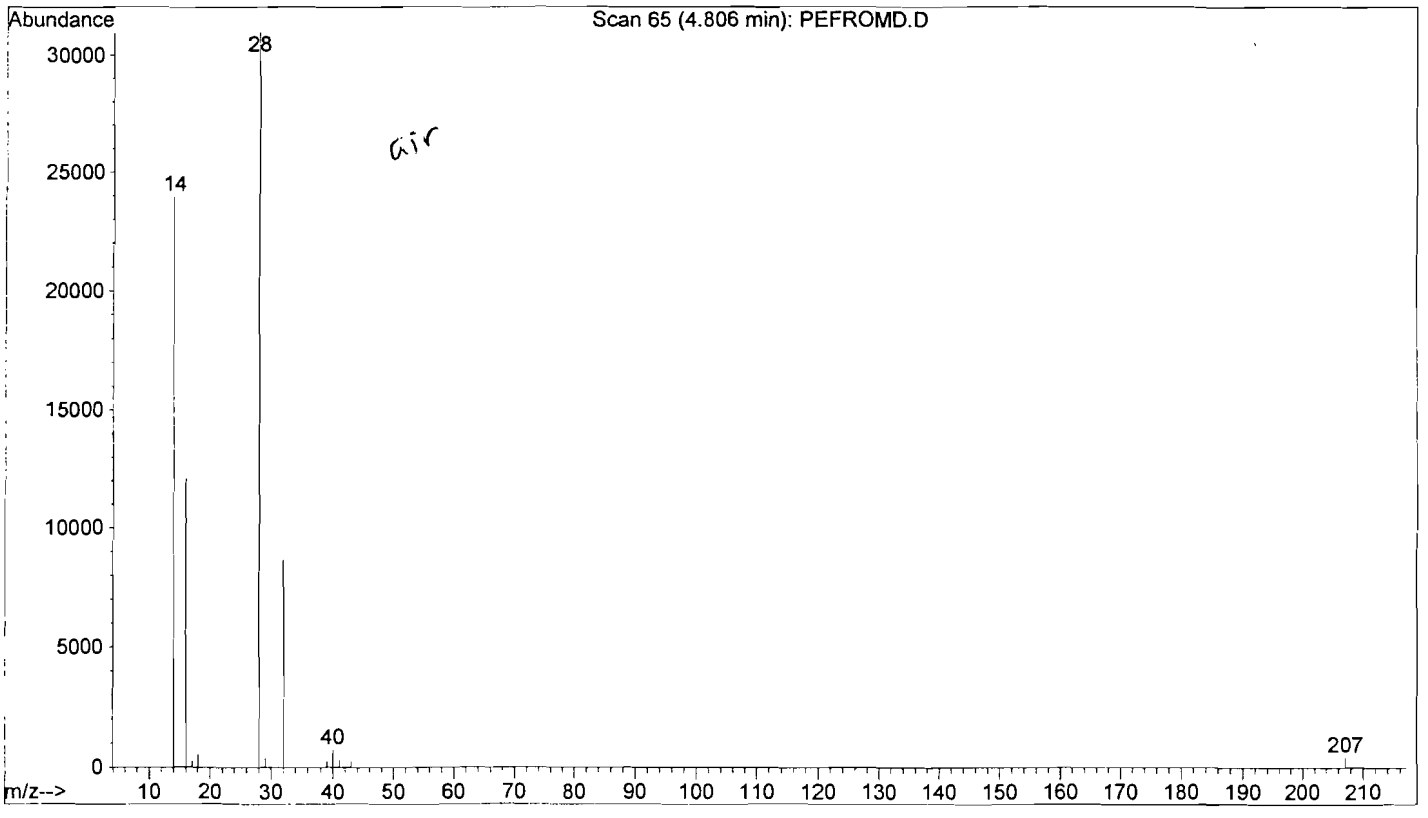


99% sure of cholesterol

Spectrum 34

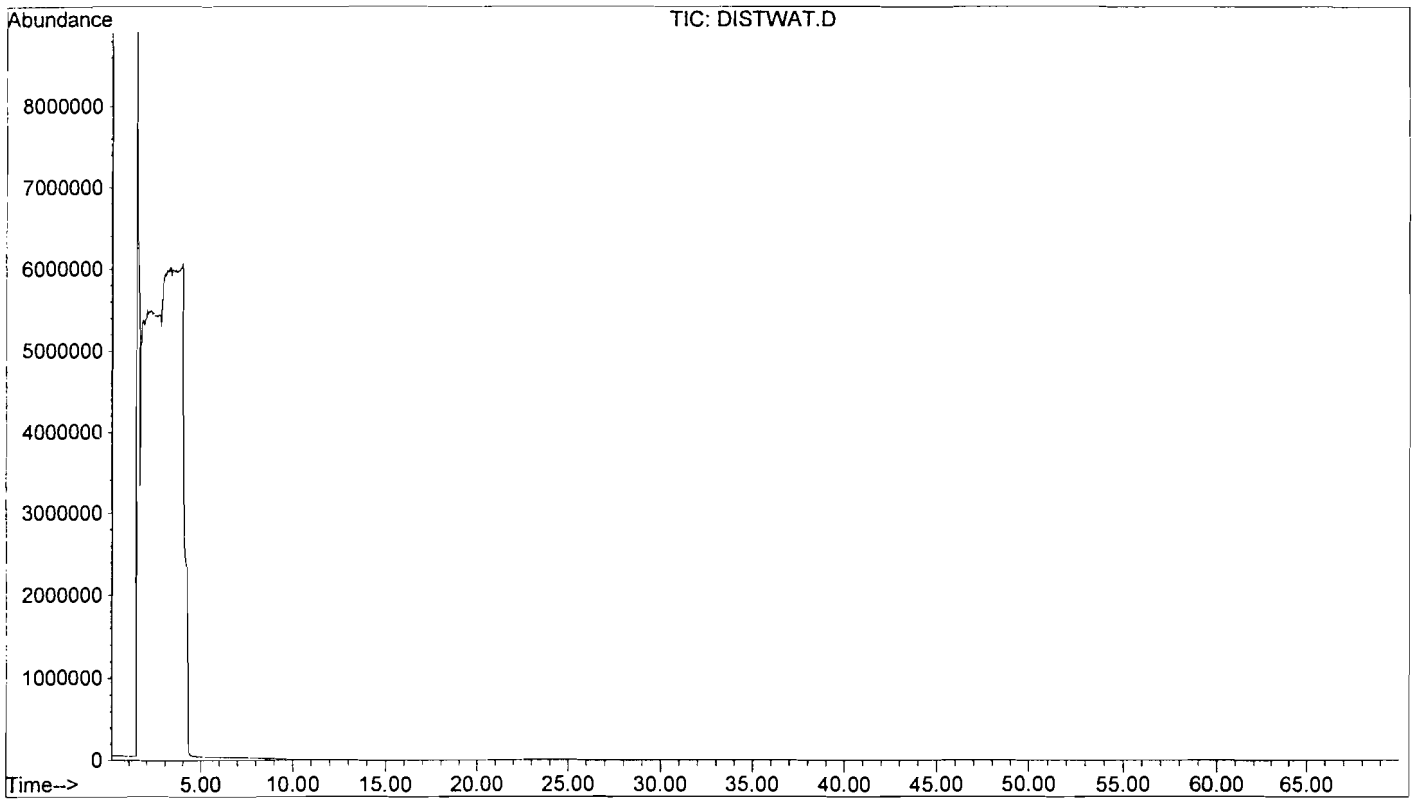
File : C:\HPCHEM\1\DATA\PEFROMD.D
Operator : Kris Mitchell
Acquired : 14 Apr 98 8:58 am using AcqMethod INVERT
Instrument : GC/MS Ins
Sample Name: petether before dist.
Misc Info : pet ether before distillation 4-14-98
Vial Number: 1

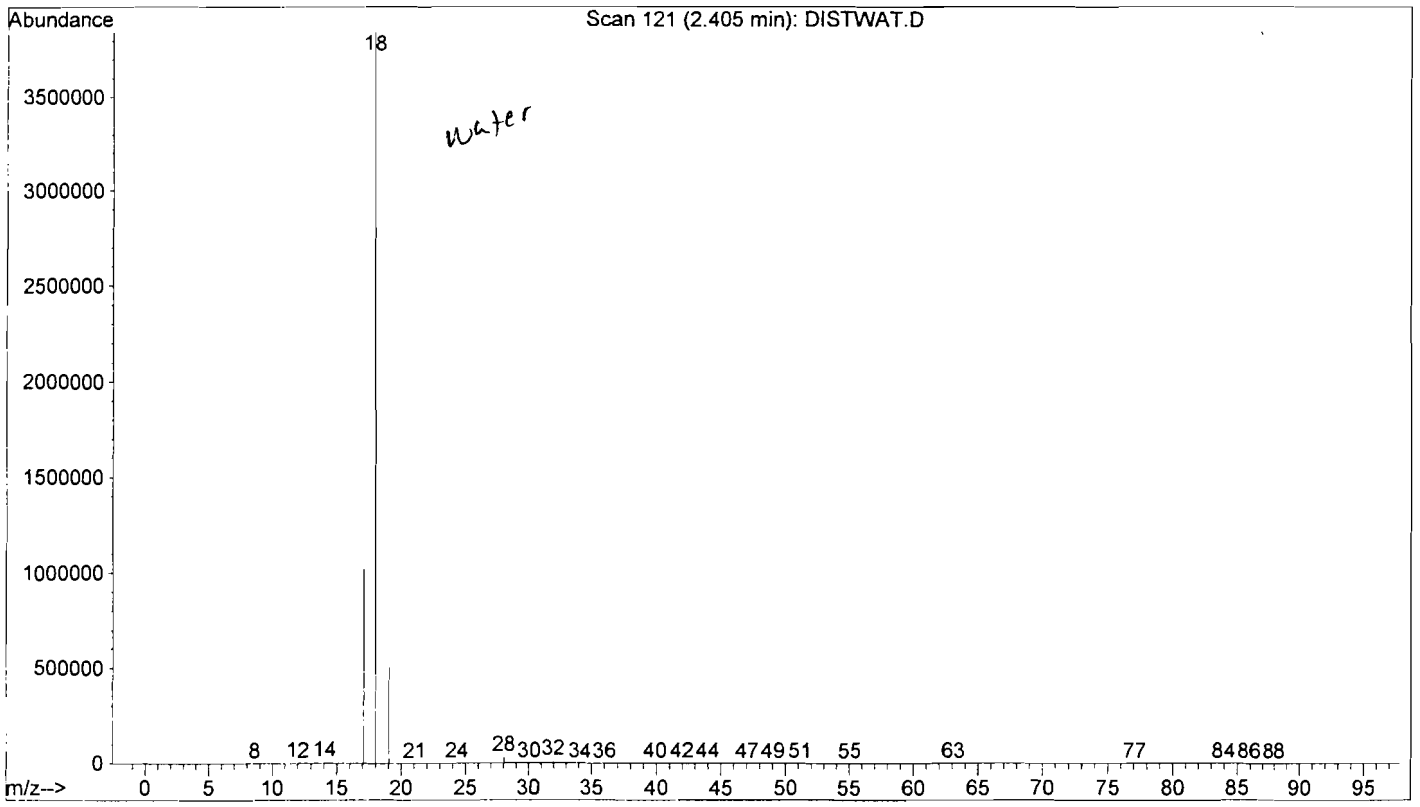




Spectrum 35

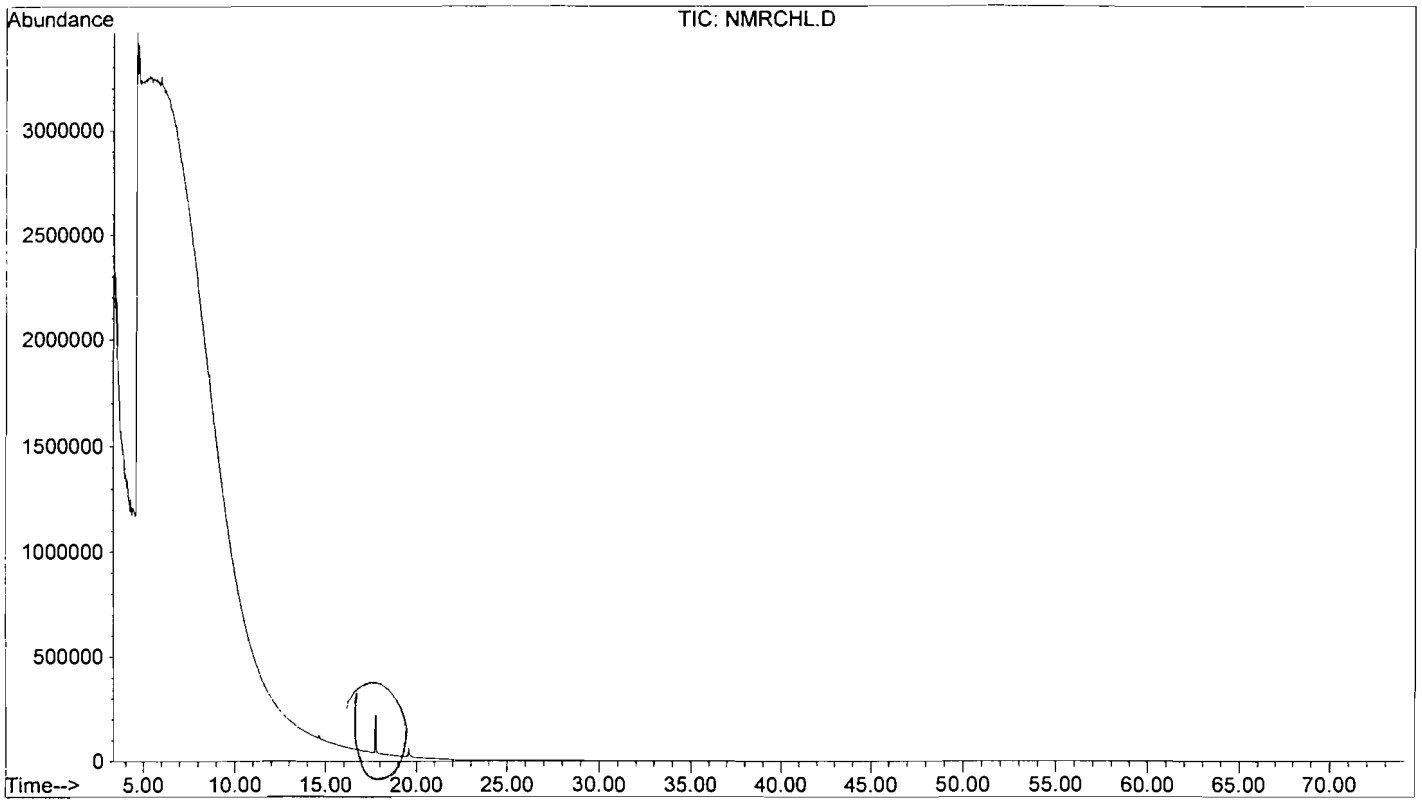
File : C:\HPCHEM\1\DATA\DISTWAT.D
Operator : Kris Mitchell
Acquired : 13 Apr 98 7:05 pm using AcqMethod INVERT
Instrument : GC/MS Ins
Sample Name: distillate
Misc Info : distillate water layer 1 4-13-98
Vial Number: 1

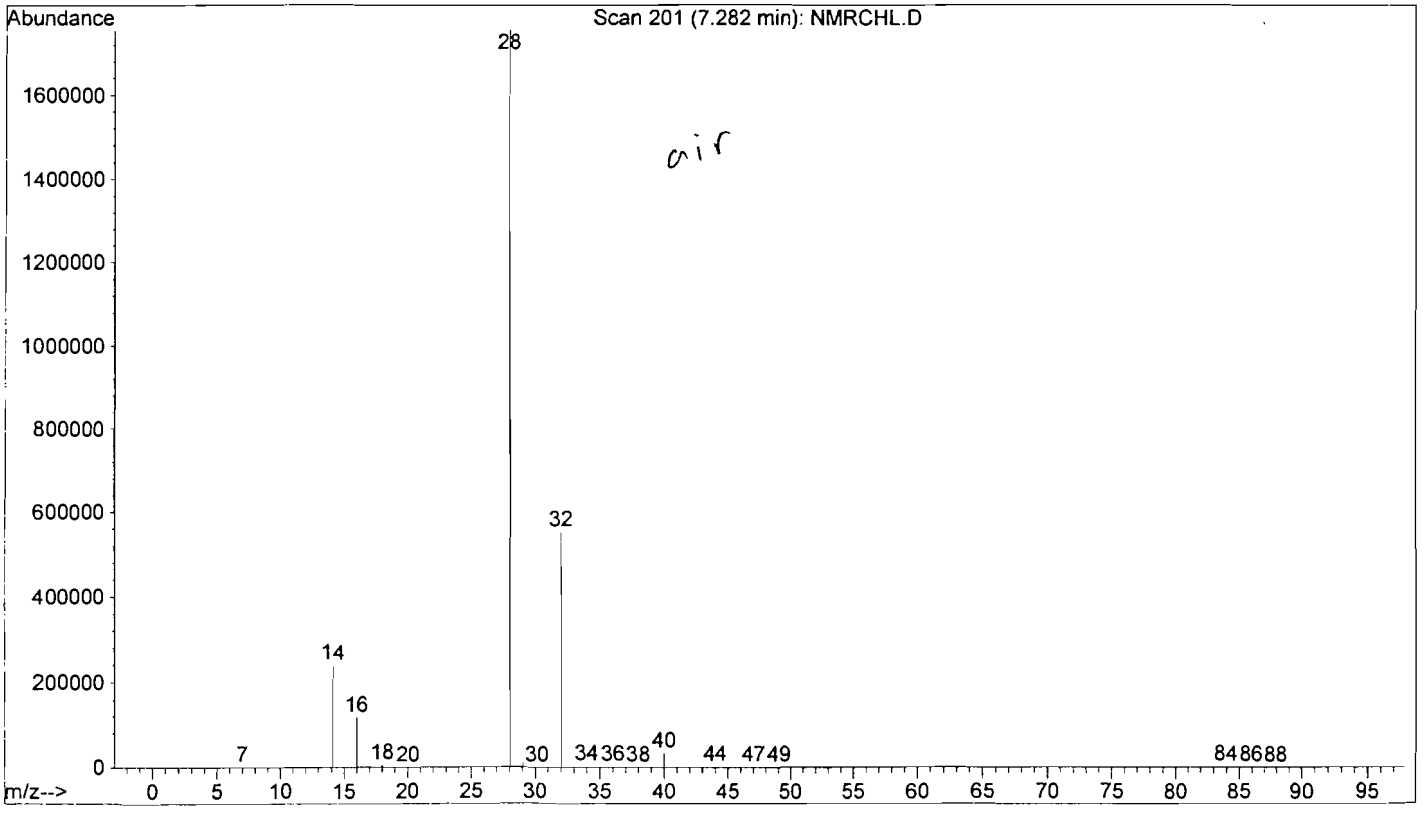




Spectrum 36

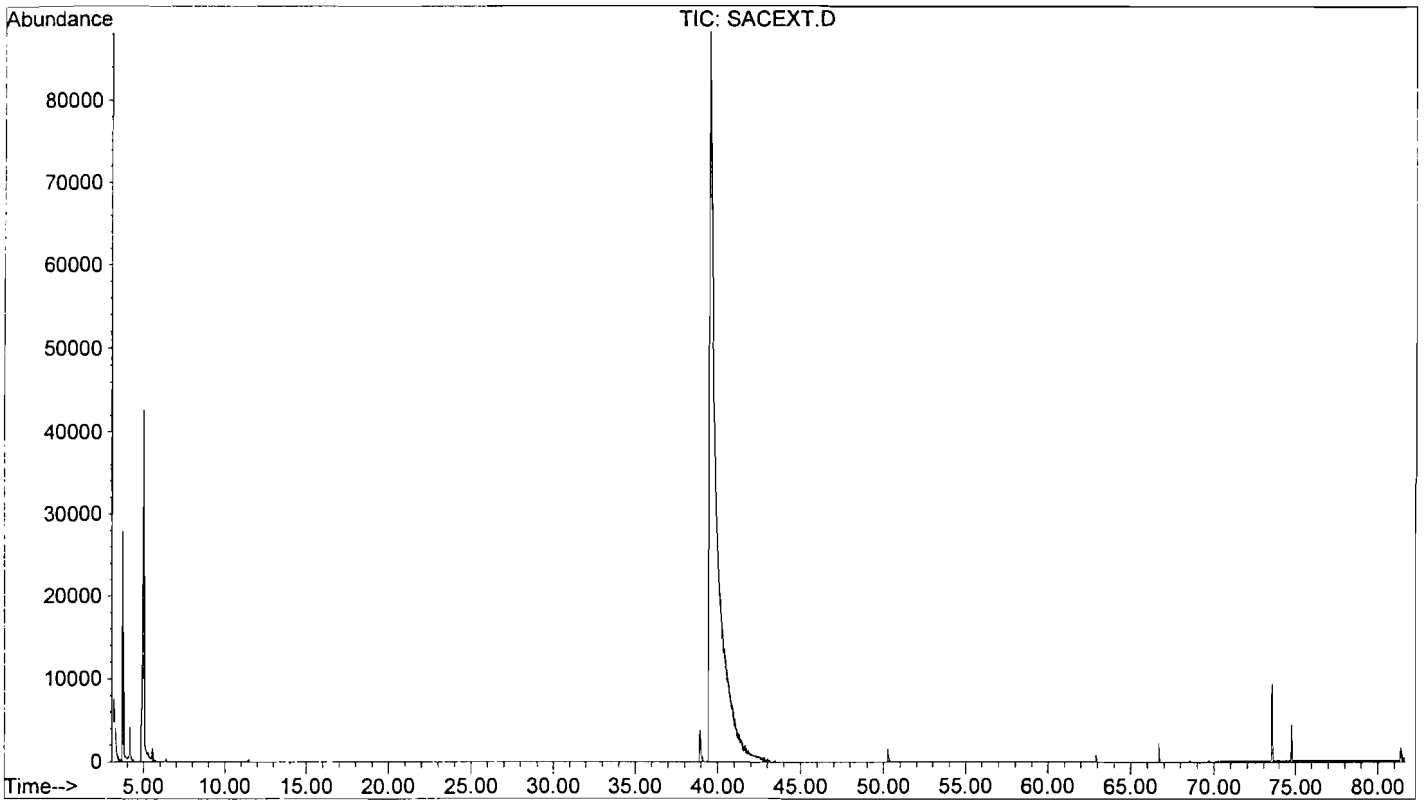
File : C:\HPCHEM\1\DATA\NMRCHL.D
Operator : Kris Mitchell
Acquired : 17 Apr 98 3:06 pm using AcqMethod INVERT
Instrument : GC/MS Ins
Sample Name: Chloro after NMR
Misc Info : Chloroform after NMR 4-17-98
Vial Number: 1

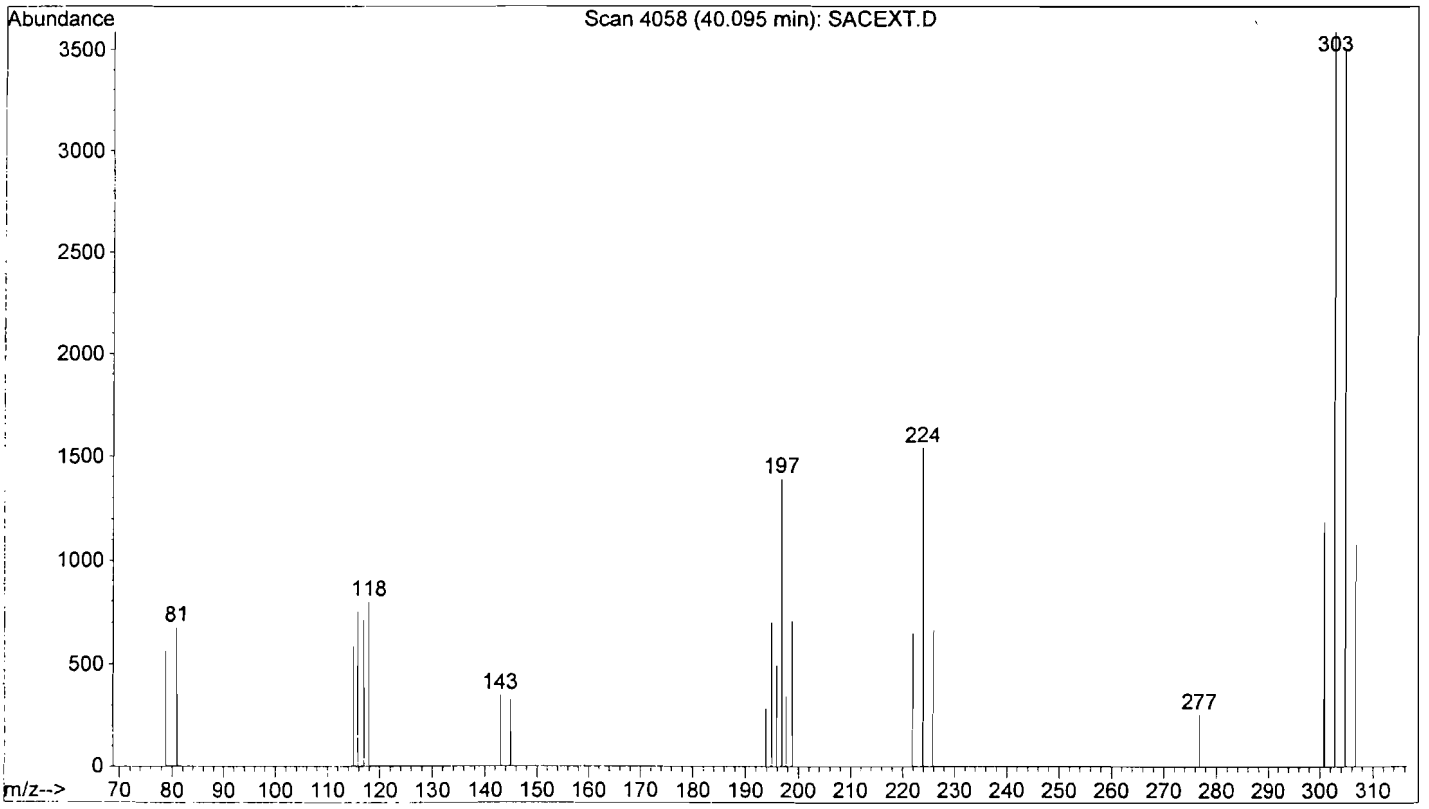




Spectrum 37

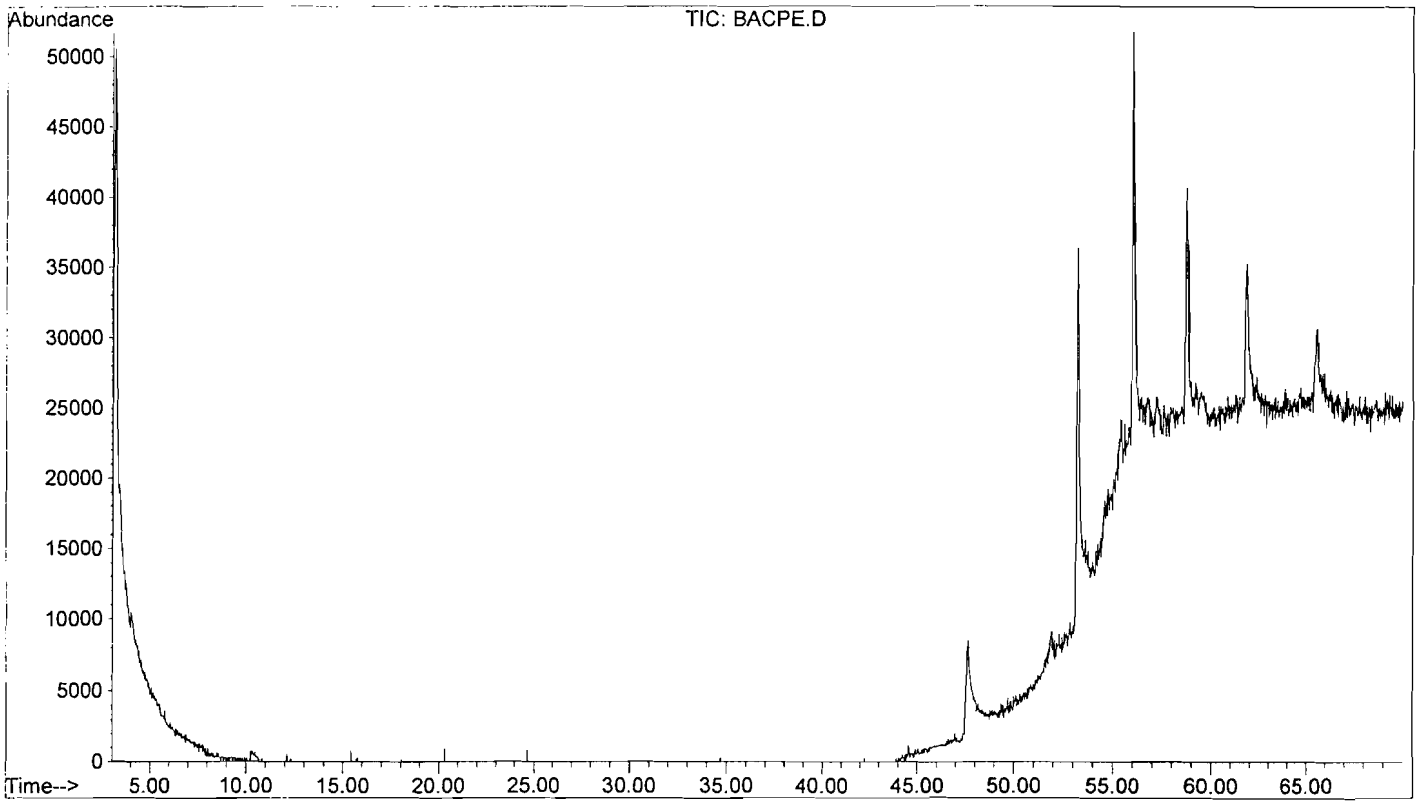
File : C:\HPCHEM\1\DATA\SACEXT.D
Operator : Kris Mitchell
Acquired : 26 Nov 97 11:35 am using AcqMethod INVERT
Instrument : GC/MS Ins
Sample Name: sac. whole extract
Misc Info : sac. in acetone and pet ether 11/26/97
Vial Number: 1

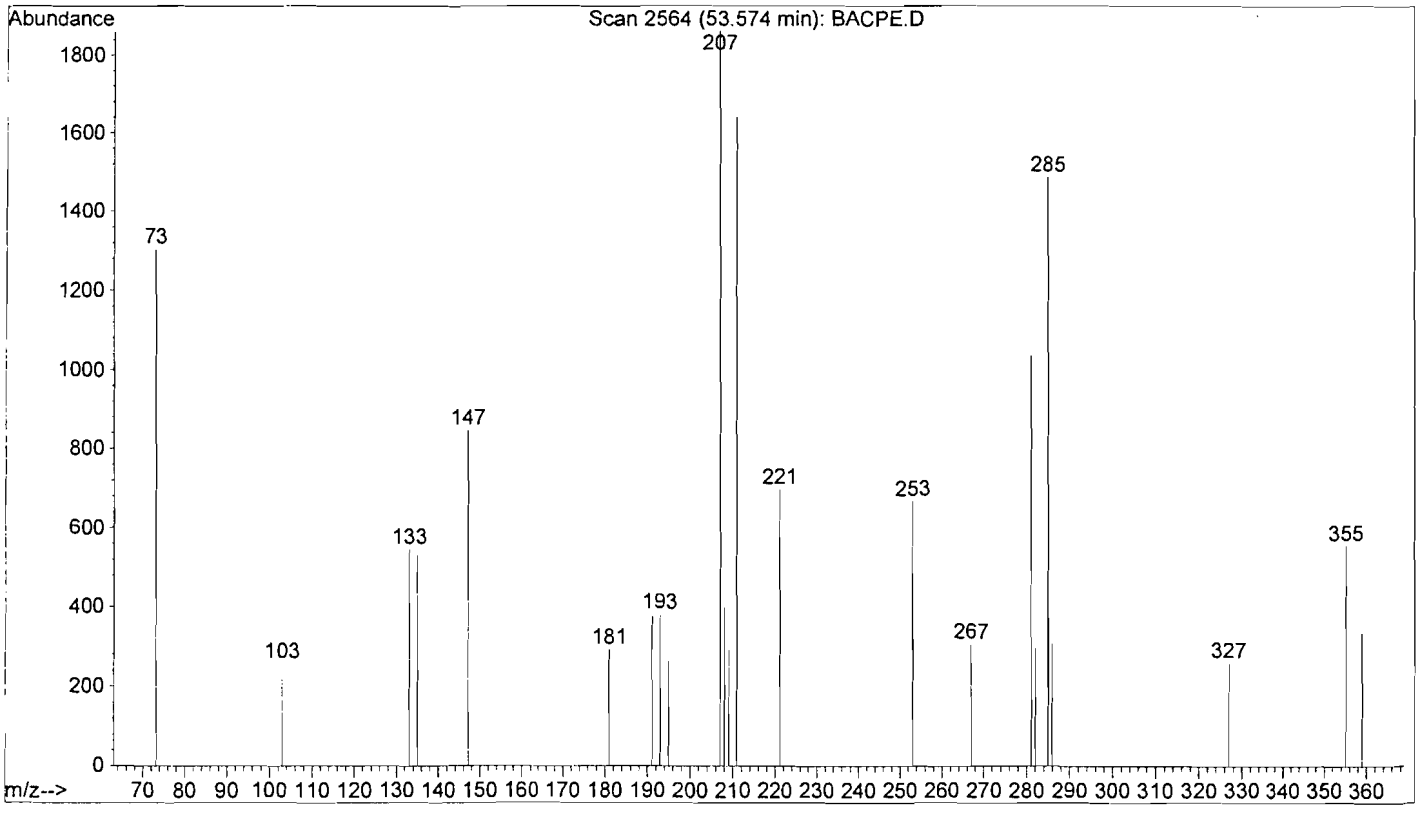




Spectrum 38

File : C:\HPCHEM\1\DATA\BACPE.D
Operator : Kris Mitchell
Acquired : 8 Apr 98 9:11 am using AcqMethod INVERT
Instrument : GC/MS Ins
Sample Name: bac in pe
Misc Info : bacteria in 1ml pe 4-8-98
Vial Number: 1





Spectrum 39

Library Searched : C:\DATABASE\NBS75K.L
Quality : 14
ID : 1,1,1,3,5,7,7,7-Octamethyltetrasiloxane