Technical Report No. 320
September 1994

Organic Carbon Characterization of Advanced Treated Wastewater at Water Factory 21, Orange County

by

Martin Reinhard, Wang-Hsien Ding and Yoshiko Fujita

Department of Civil Engineering
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Sponsored by

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and

National Water Research Institute
10500 Ellis Avenue
Post Office Box 20865
Fountain Valley, California 92728-0865
(WQ-92-03)

Project Officer: Mike Wehner, Orange County Water District

Department of CIVIL ENGINEERING
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ACKNOWLEDGMENTS

Mr. Brian Okey at the Water Quality Laboratory at OCWD collected all samples described in this report. Dr. Jerry Leenheer at the U. S. Geological Survey (Arvada, CO) provided us with invaluable advice and assistance in developing the analytical methods for this project. At Stanford University, Dr. Lois Durham and Ms. Haroula Dris in the Department of Chemistry provided assistance for the NMR analyses. Finally, we thank Ms. Eva Orwin in the Department of Civil Engineering for assistance in sample preparation.

In addition to the support provided by the Orange County Water District and the National Water Research Institute (WQ-92-03), financial assistance was received through a Tyng Fellowship administered by Williams College, Williamstown, Massachusetts. The GC/TSQ MS was obtained through an equipment grant from the U. S. Department of Energy (DE-FG05-87ER75377).

EXECUTIVE SUMMARY

A methodology was developed to characterize the dissolved nonvolatile organic carbon content (DOC) of Water Factory 21 (WF21) effluents (Q8, Q9 and Q22B) and three different groundwaters. The well waters were from: the Talbert seawater intrusion barrier monitoring well M21 which is recharged by WF21 blend; Yorba Linda Water District Well 11 (YLWD11) which is recharged by Santa Ana River water; and deep well 1 (DW1) which is not impacted by water of wastewater origin. DW1 water is used for blending with WF21 effluents prior to injection. For each sample, the DOC was characterized in the aggregate or "bulk" form and a subfraction was characterized as a complex mixture of individual trace organics using gas chromatography and mass spectrometry (GC/MS).

The DOC fraction was separated from the total organic carbon (TOC) content by filtration, removing particles and bacteria. Volatiles were removed during concentration by evaporation. The bulk DOC was separated into three operationally defined fractions: humic acid, fulvic acid and hydrophilic. A mass balance was obtained relying on elemental analyses of the fulvic and humic acid fractions, and DOC measurements of the hydrophilic fractions. In addition, the fulvic acid fractions were characterized by ^{1}H NMR spectroscopy. Results were compared with DOC characterizations reported for natural and polluted waters. Individual trace compounds were converted into volatile alkyl derivatives and characterized by GC/MS.

The bulk DOC characteristics of the WF21 waters differed from those observed for deep groundwater and from those typically reported for natural waters. For all three waters from Water Factory 21 (Q8, Q9 and Q22B) and the Yorba Linda Water District Well #11 (YLWD11) the DOC

consisted only of fulvic acids and hydrophilics. Humic acids were not detected. DW1 and M21 groundwater contained ten and 6.5 percent humic acid, respectively. It appears reasonable to propose that the humic acid in M21 originates from the DW1 water. Although the removal processes of RO and activated carbon treatment are different, the DOC in the effluents of the activated carbon columns (Q8) and the RO-plant (Q22B) was similarly proportioned between hydrophilic and fulvic acid fractions: approximately 50% hydrophilic, and 33% - 44% fulvic acid.

The DOC in waters of wastewater origin exhibited elemental ratios that were different from those reported for natural waters. Fulvic acid fractions from the WF21 waters and the two recharged groundwaters, M21 and YLWD11, all exhibited high (> 1.5) H/C atomic ratios, indicating that saturated aliphatic or alicyclic structures predominate over aromatic structures in the complex mixtures. Only the DW1 fulvic and humic acids exhibited H/C ratios (1.3 and 1.2, respectively) considered within the range representative of natural humic substances. The H/C ratio for the M21 humic acid was also slightly high (1.4), perhaps indicating the impact of organics of wastewater origin. Consistent with this observation are the N/C atomic ratios for the fulvic and humic acid fractions from the WF21 waters and the M21 and YLWD11 groundwaters: all were high relative to reference natural aquatic humic substances.

The fulvic acid ^{1}H NMR spectra showed the broad unresolved humps characteristic of complex mixtures, with the two groundwaters DW1 and YLWD11 showing the fewest distinct signals indicative of specific structures. The ^{1}H NMR fingerprints of the three WF21 waters and M21 groundwater were very similar to each other, indicating a correlation between origin and spectral appearance.

The analytical method used for specific trace organics characterization entailed concentration by evaporation, esterification, and analysis with GC/EI-MS, GC/CI-MS and GC/TSQ-MS techniques. The lower limit of detection was below one microgram per liter.

Specific compounds that were detected included compounds of anthropogenic origin, microbial transformation products of anthropogenic substances and metabolites of microbial processes. Anthropogenic compounds included EDTA and NTA and alkylphenol polyethoxylates (APEs). Known microbial transformation products of anthropogenic precursors that were detected included partially oxidized APEs and linear alcohol ethoxylates (LAEs). Compounds that appeared to be microbial metabolites included glyoxal, methyl glyoxal, alkanedioic acids and several multi-functional aldehydes and acids that were not further identified.

GC/MS analyses revealed significant differences and similarities among the different water samples. In WF21 effluent, the presence of several classes of anthropogenic compounds was detected including EDTA, NTA, and LAEs and APEs and their transformation products such as dicarboxylated LAEs and APEs, and dicarboxylated polyethylene glycols. Other compound classes that were detected include aldehydes, dicarboxylic acids, and fatty acids. In Q8 and Q9, EDTA was by far the most prominent individual compound with a concentration of 290 µg/L. Most other contaminants were present at concentrations less than 10 µg/L. Chlorination of Q8 converted APEs into brominated APEs but left many of the other compounds unchanged. In the RO treated effluent, a similar but much weaker profile of anthropogenic compounds was observed. EDTA was detected at 4 µg/L and most other compounds at levels below 1 µg/L. The total concentration of trace organics detected in Q22B was 13 µg/L. Most trace organic contaminants appear to be attenuated or transformed during transport to M21. The total concentration of detectable trace organics in M21 was 110 µg/L. EDTA, which was detected at 82 µg/L, was the most prominent compound. Other compounds were present at the low microgram per liter range. The apparent lack of attenuation of EDTA suggests that this compound is refractory under these conditions and may possibly serve as a tracer for water of wastewater origin. Further study is needed, however, to test this proposition.

Interestingly, the organics profile observed in WF21 was not evident in YLWD11 although its water originates from the Santa Ana River. In Orange County, the Santa Ana River contains a high proportion of treated effluents from upstream municipal wastewater treatment plants. In YLWD11, the compounds detected included compounds classified as microbial metabolites such as glyoxal (140 µg/L) but not EDTA. The total concentration of detectable trace organics was approximately 450 µg/L but for many compounds the structure and source remain to be identified. In the deep well water used for blending (DW1), no anthropogenic compounds were detected, as expected. The total concentration of detectable trace organics was only 2.4 µg/L

The database that has been developed is based on a very limited sample set and only tentative conclusions are possible. The public health significance of these data and their implications for the water industry need to be addressed in more detailed studies.

SECTION 1

INTRODUCTION

1.0 Background

In many regions of the United States, growing populations are straining conventional fresh water resources and local groundwater aquifers are being depleted at a rapid pace. Saltwater intrusion has become a problem in some coastal areas, as seawater follows the artificially induced gradients caused by dropping water tables. Recognition of the need for effective water resource management resulted in the creation of the Orange County Water District (OCWD) in 1933, which from its inception has been charged with the responsibility of protecting and maintaining Orange County's groundwater basin.

The OCWD's comprehensive groundwater management plan includes several projects to increase basin water supplies, through recharge of both imported waters and local waters. The main sources of imported water are the State Water Project and the Colorado River. Two of the principal local sources of water for recharge are the Santa Ana River and reclaimed wastewater produced at Water Factory 21 (WF21). The Santa Ana River enters Orange County from neighboring Riverside County, carrying a high proportion of effluents discharged by municipal wastewater treatment plants upstream. In Orange County, the river water is diverted into off-channel percolation basins and introduced into the forebay groundwater basin by infiltration.

WF21 is an advanced wastewater treatment plant which provides reclaimed water for direct injection into the groundwater basin, specifically for OCWD's Talbert Gap seawater intrusion barrier project. Secondary effluent is received from the Orange County Sanitation District's treatment plant in Fountain Valley and subjected to advanced treatment via one of two

different treatment trains : the advanced wastewater treatment (AWT) train that includes chemical clarification, recarbonation, filtration and activated carbon adsorption, or the reverse osmosis (RO) treatment train that replaces the carbon contact with reverse osmosis. The resulting effluents are of very high quality, as measured by the parameter of total organic carbon (TOC). The AWT effluent has averaged 5.4 mg/l TOC and the RO effluent 0.8 mg/l TOC. These values compare favorably to the 4.5 mg/l historical average of the local deep groundwater (Rigby 1990).

The OCWD has developed an extensive water quality monitoring program to ensure the safety and quality of its groundwater supply. Production wells, monitoring wells in the Santa Ana River recharge zones, and test wells throughout the basin are regularly analyzed for a suite of primary and secondary water quality parameters, as are samples taken from several points along the WF21 treatment system. WF21 in particular was the subject of intensive investigations by Stanford University from 1978-1982, in which samples taken at different stages of treatment were analyzed for EPA priority pollutants (McCarty *et al.* 1978; McCarty *et al.* 1980; McCarty *et al.* 1982). In the influent, 25 of the 100 priority pollutants were found routinely. In the effluent, most priority pollutants were effectively removed and either non-detectable or present at concentrations below 1 µg/l. Exceptions were the trihalomethanes which were formed during chlorination and were present at levels above 1 µg/l. In addition to priority pollutants the occurrence of some specific non-priority pollutants was detected. The most prominent group was comprised of the halogenated and nonhalogenated residues of alkylphenol polyethoxylate surfactants.

1.1 Motivation for Project

The OCWD has been operating WF21 since the early 1970's, and since the beginning has been required to blend the WF21 effluents with local deep well (DW) water prior to injection. The historical average blend has been 1:1:1 AWT:RO:DW, resulting in a blend that has averaged 3.6 mg/l TOC (Rigby 1990). In June of 1990, OCWD proposed to change the blend to eliminate the deep well water component, relying instead solely on WF21 water. The proposed injection water would consist of two-thirds RO water, and one-third non-RO water, with a projected average 2.3 mg/l TOC. Also this year, however, the Office of Drinking Water (ODW) in the California Department of Health Services proposed new criteria for recharge of groundwater using reclaimed municipal wastewater. Among the proposed criteria was a maximum TOC level of 1 mg/l for direct injection. The ODW based this figure on a 1987 Report of the Scientific Advisory Panel (SAP) which noted that treated wastewater with a TOC content of less than 1 mg/l can be achieved, and that at this level, "essentially all identifiable trace organic compounds should be absent in detectable concentrations" (Groundwater Recharge Committee Report 1990). Using the 1 mg/l cutoff, neither the 1:1:1 blend nor the proposed 1:2:0 blend (AWT:RO:DW) would be acceptable. Under the current operating conditions only 100% RO treatment satisfies the proposed standard.

The OCWD believes that a simple TOC cutoff for reclaimed wastewaters used for direct injection is inappropriate. Because the TOC of a natural water or a wastewater can and usually does incorporate a wide spectrum of organic compounds, the parameter generally does not correlate well with relevant biological, toxicological or chemical properties. The OCWD bases this contention on the earlier (1978-1982) studies performed by Stanford University which found no priority pollutants in the WF21 effluents at levels above drinking water standards. The OCWD also points out that historical groundwater monitoring data from wells down-gradient of the injection locations show that the TOC decreases as the injected water travels through the

aquifer. For example, at Huntington Beach well #98 the chloride concentration is equal to that of the injection water, indicating complete breakthrough of the injection water, but the TOC concentration has remained stable at 1.0 mg/l for approximately 10 years. Travel time to the well has been estimated to be 2.7 - 3 years (Wesner and Herndon 1990). Apparently during that time some of the TOC is removed from the water. Removal of organic carbon during groundwater transport by biological and chemical processes is a well recognized phenomenon (Roberts *et al.* 1982) and it appears to be occurring in the Orange County groundwater basin as well.

However, although removal of TOC is "well recognized," it is not yet "well understood." A large part of the difficulty lies in the fact that little is known about the actual composition of TOC in most natural waters and wastewaters. The TOC in natural waters is an extremely complex mixture of trace organics, many of which are not amenable to traditional methods of molecular identification. The TOC of reclaimed wastewaters has similar characteristics. The previous work performed by Stanford University on the WF21 effluents focused on the volatile organics that make up the majority of the priority pollutant lists. However, most of the TOC in the WF21 effluents is in fact non-volatile, and although a large number (> 100) of specific compounds were analyzed in 1978-1982, because virtually all were at trace (ppb) levels the fraction of the TOC characterized was no more than a few percent. In the work reported here, we have aimed to characterize much of the TOC which remained unexamined in the earlier studies. In addition to specific compound identification, we have also characterized the bulk organic composition of the waters in a manner amenable to comparison with published data on natural waters. With a more comprehensive picture of the organic composition of the WF21 effluents, it will be possible to better understand the TOC removal which occurs in the aquifer, and to optimize pretreatment for wastewater to be used for groundwater recharge.

1.2 Study Objectives and Overall Approach

The objective of this study was to provide data that would assist in:

- setting standards for groundwater recharge with treated wastewater;
- optimizing treatment objectives of advanced wastewater treatment plants with respect to groundwater recharge;
- evaluating the quality of groundwater that was recharged with water of wastewater origin; and
- planning more detailed studies that would give insight into attenuation during transport.

The overall experimental approach was to:

- develop an analytical protocol that would reveal unique characteristics of the TOC and which could be used for investigating transformation processes during groundwater recharge; and
- test and apply the standard analytical protocol to characterize and compare the TOC of WF21 effluents and groundwaters with differing levels of wastewater impact.

Unlike the earlier characterization work which focused on the organic priority pollutants (McCarty *et al.* 1978; McCarty *et al.* 1980; McCarty *et al.* 1982), the work reported here was concerned with the polar and high molecular weight organics, which in fact constitute the majority of the TOC in the OCWD waters. We limited the study to the dissolved organic carbon fraction, because the particulate fraction appeared to be very small. Samples were filtered prior to analysis. Therefore in mass balance calculations, the non-volatile and non-particulate organic carbon, referred to here as dissolved organic carbon (DOC), is assumed to be 100 percent. To emphasize this we will hereafter always use the term DOC in this document. DOC analyses

indicate the non-specific bulk organic carbon which is present at milligram per liter concentrations, as opposed to the specific trace organic compounds that are present at micro- or nanogram per liter levels.

We designed our experimental approach assuming that certain compound classes warranted special attention, based on previous analyses and experience with wastewater treatment processes. These classes were expected to comprise the major portions of the injected DOC and/or are of greatest health concern. The materials include detergent-derived surfactant residues, humic substances (natural biopolymers) and halogenated products formed during chlorination. We also sought to identify specifically measurable compounds which may serve as tracers for the movement of water and indicator compounds for transformation processes. Surfactant residues are commonly found in secondary and tertiary treated wastewaters; in fact the nonionic alkylphenol polyethoxylates were detected in the WF21 effluents during the earlier studies (McCarty *et al.* 1982; Reinhard *et al.* 1982). Surfactant residues have also been detected in groundwater; Field *et al.* reported the occurrence of anionic and nonionic surfactant residues in a groundwater plume of secondary treated wastewater (Field *et al.* 1992). Specifically, Field *et al.* found carboxylated residues of linear alkylbenzene sulfonates and alkylphenol polyethoxylates in groundwater 500 m from the infiltration site, indicating that these compounds are both poorly biodegradable and mobile in the groundwater environment.

Humic substances are ubiquitous in natural and "non-natural" waters, accounting for half or more of the DOC in most natural waters (Thurman 1985). These high molecular weight heterogeneous materials also have been reported as major components of treated wastewaters (Amy *et al.* 1987), indicating incomplete removal during treatment. Moreover, a recent study at the University of Karlsruhe determined that humic materials actually appear to be produced during biological treatment of wastewaters (Link *et al.* 1989). Humic substances, in addition to their role as a major constituent of DOC, are of interest because they have been implicated as a

source of halogenated disinfection byproducts (Christman *et al.* 1990), the other specific category of compounds which we designed our approach to address. These byproducts demand attention primarily because of potential health concerns.

The analytical protocol that we developed for characterization consists of two parts: a bulk DOC characterization component and a specific compound identification component. The approach for the bulk DOC characterization was developed from approaches commonly applied to the study of DOC in natural waters. The DOC was fractionated into operationally defined humic acid, fulvic acid, and hydrophilic fractions, and the mass balance of carbon between the three fractions was determined. In addition, elemental analyses and proton nuclear magnetic resonance (^{1}H NMR) spectra were obtained for the fulvic acid fractions. The results were compared with published results for natural waters, with the aim being to determine which characteristics were similar between natural and wastewaters, and which differed. The specific compound identification component relied on gas chromatography with mass spectrometric detection (GC/MS); MS analysis is crucial to idenfication of unknown compounds within a complex matrix. For most of the compounds, reference standards are not commercially available. Detailed information about the methods developed for the bulk DOC characterization and the specific compound identification are presented in Sections 2 and 3, respectively.

This report includes data obtained for six different waters obtained from Orange County, hereafter referred to as samples Q8, Q9, Q22B, M21, DW1, and YLWD11. The first three waters are from WF21 and are described in the following subsection. M21 is groundwater from the Talbert aquifer (the uppermost aquifer in the groundwater basin) drawn from monitoring well 21, located approximately 50 feet south of injection well I-5 in the Talbert injection barrier. Travel time to this well is on the order of 1 - 2 months, and the water is assumed to be 100% injection derived at this point. DW1 is groundwater from the deepest (800+ ft from the surface) aquifer in the basin, drawn from deep well 1, located approximately 100 yards east of M21.

Water from this well is assumed to be unaffected by the recharged wastewater, and DW1 water is used for blending with the WF21 effluents prior to injection. YLWD11 is groundwater from Yorba Linda Water District well 11, located near Warner Basin in northeastern Orange County. This well is in the recharge zone of the Santa Ana River recharge basins, and is perforated in six intervals, ranging from a depth of 115 ft. to 514 ft.

1.3 Water Factory 21

WF21 has been in operation since 1975, providing a reliable source of injection water for the Talbert Gap seawater intrusion barrier. Over the years upgrades and improvements have maintained the plant as a state of the art wastewater reclamation facility. As noted earlier, WF21 has two treatment trains: the advanced wastewater treatment (AWT) system that includes chemical clarification, recarbonation, multimedia filtration and activated carbon adsorption, and the reverse osmosis (RO) treatment train that replaces the carbon contact with reverse osmosis. The RO system is especially effective at removing DOC. The ratios of wastewater apportioned to the two treatment trains can be adjusted by the plant operators. Currently the AWT system can treat up to 15 mgd of secondary effluent, while the RO system can process 5 mgd.

A schematic of the treatment processes and sampling locations at WF21 is presented in Figure 1.1. The samples which we have analyzed for this report are Q8, Q9, and Q22B. As shown in Figure 1.1, Q8 is the effluent from the granular activated carbon (GAC) contactors, prior to chlorination, and Q9 is the GAC effluent after chlorination. Q22B is the RO effluent, treated identically as Q8 up to and through the multimedia filtration, and then diverted to the RO system. (The valve shown in Figure 1.1 between the GAC and the RO systems is normally closed.)

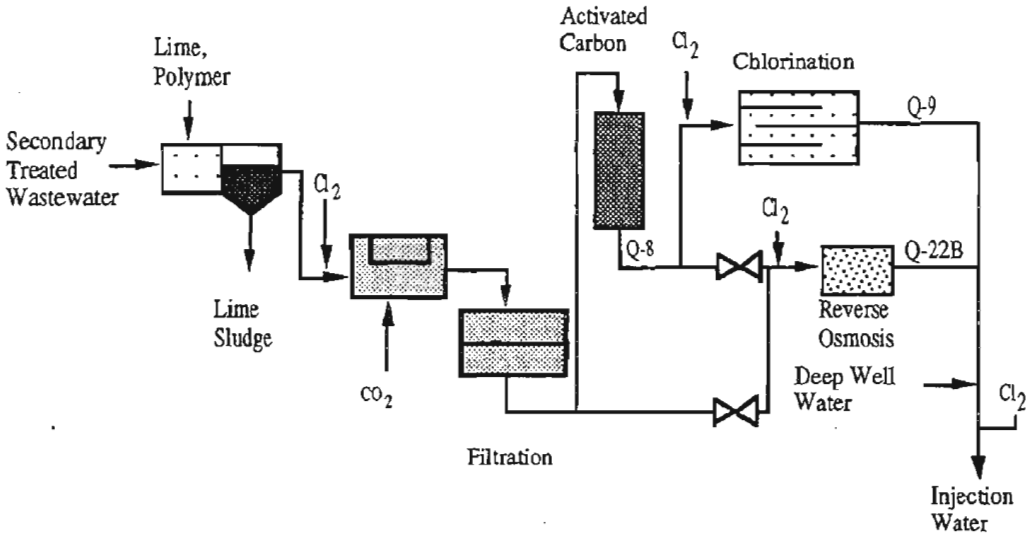


Figure 1.1 Schematic of Water Factory 21

1.4 Organization of this Report

This report is organized into six sections plus appendices. The procedures and findings of the bulk DOC characterization study are described in Section 2. Mass balances of carbon, elemental analysis results, and ^1H NMR spectra are presented. Section 3 contains the methods and results of the specific compound identification effort, including details about some of the standard reference work, and estimated concentrations for positively and tentatively identified compounds. Section 4 presents conclusions from the previous two sections, and Section 5 includes recommendations for future work. The bibliography for the entire document is contained in Section 6. Appendices A through F contain electron-impact ionization (EI) mass spectra for Q8, Q9, Q22B, M21, DW1 and YLWD11, respectively.

SECTION 2

BULK DOC CHARACTERIZATION

2.0 Approach

The approach taken for the aggregate DOC characterization in the WF21 effluents and groundwater is derived from approaches commonly applied to the study of "natural organic matter," in particular humic substances, in natural waters. Humic substances are natural polymers that result from the decomposition, transformation, and polymerization of plant and animal residues, and are found in both soil and water. Often the brown or yellowish color of natural waters is attributed to high humic content. Secondary and tertiary treated wastewaters too have been found to contain humic substances (Manka and Rebhun 1982; Amy *et al.* 1987), due to both their presence in the original natural water and possible formation during biological treatment (Link *et al.* 1989). Despite their ubiquitous appearance in the environment, and the fact that they have been studied since their first reported extraction from peat in the late 18th century (Achard 1786), a comprehensive characterization of humic substances has thus far eluded environmental scientists. The difficulty lies in the fact that there are no specific molecular structures for humic substances, unlike other chemical entities. Humic substances have been defined as "A general category of naturally occurring, biogenic, heterogeneous organic substances that can be generally characterized as being yellow to black in color, of high molecular weight, and refractory" (Aiken *et al.* 1985). This vague description makes no reference to function, as humic substances have evaded definition in terms of this attribute as well. For practical purposes then humic substances are generally defined operationally (i.e., by isolation technique) and are characterized in their aggregate form.

Methods for isolating and concentrating aquatic humic substances are not standardized, but many of the current practitioners rely on column sorption techniques, especially with the Amberlite XAD resins. These are nonionic macroporous copolymers that rely on the "hydrophobic effect" as the main driving force for sorption (Aiken 1985). XAD-8 resin was chosen as the sorbent for this work because of the many reported applications of this particular resin in humic substance isolation (Aiken *et al.* 1979; Leenheer 1981; Thurman and Malcolm 1981; Kukkonen *et al.* 1990). Dr. Jerry Leenheer of the U. S. Geological Survey in Arvada, CO developed and taught us a procedure which results in the isolation of three fractions: humic acid, fulvic acid, and a hydrophilic fraction. The humic acid is defined here as the fraction of the DOC that becomes insoluble at pH $\leq$ 2, the fulvic acid is the fraction that remains soluble at pH $\leq$ 2 and is sorbed (reversibly) onto the XAD-8 resin, and the hydrophilic fraction is that fraction of DOC which does not sorb to the XAD-8 column at pH 2. During 1992 the fractionation method was tested for 10 and 7 liter volumes of Q8 and M21, and although complete DOC mass balance data were not available, comparison of ^{1}H NMR spectra of the fulvic acid fractions suggested that the method was reproducible.

In this report, results obtained during 1993 and 1994 for pre-chlorination GAC effluent (Q8), chlorinated GAC effluent (Q9), and reverse osmosis effluent (Q22B) from Water Factory 21, and groundwaters from wells M21, DW1, and YLWD11, are presented. A method blank using HPLC grade water was also run. For each sample a mass balance of organic carbon divided among the three fractions was determined. In addition, the fulvic and humic acid isolates were freeze-dried and when quantities permitted, subjected to elemental analysis and ^{1}H NMR analysis. Elemental analysis has been commonly applied to isolated humic substances (Thurman and Malcolm 1981; Huffman and Stuber 1985; Peschel and Wildt 1988), as has ^{1}H NMR (Wershaw 1985; Thorn 1987; Grasso *et al.* 1990), and therefore comparison with published data for "typical" natural waters is possible.

Experimental details are given below in Section 2.1. Mass balance results for the DOC fractionation are discussed in Section 2.2, and elemental analysis results are presented in Section 2.3. The ^{1}H NMR spectra are discussed in Section 2.4.

2.1 Experimental Section

Chemicals. Reagent water was obtained from a Milli-Q laboratory water purification system (Millipore Corp., Bedford, MA). For the method blank, HPLC reagent water was purchased from J.T. Baker, Inc. (Phillipsburg, NJ). Acetonitrile was HPLC grade and purchased from Aldrich Chemical Co. Inc. (Milwaukee, WI) or J.T. Baker, Inc. Reagent grade hydrochloric acid was purchased from Mallinckrodt Specialty Chemicals Co. (Paris, KY) and reagent grade sodium hydroxide pellets from J.T. Baker, Inc. Deuterium oxide (99.9%) was purchased from Aldrich or Isotec, Inc. (Miamisburg, OH).

Sample Collection and Storage. Approximately twenty liter grab samples were collected at Water Factory 21 or from the various well sites in Orange County by OCWD personnel, and shipped in coolers packed with ice via overnight delivery to the Stanford Water Quality Lab. The samples were contained within teflon bags inside plastic carboys. Upon arrival at Stanford, all samples were stored at 4 °C. Prior to concentration, all samples were filtered through 0.3 μm glass fiber filters (Gelman A/E type).

Sample Concentration and Isolation of the Humic Acid Fraction. Seventeen to eighteen liters of filtered water were concentrated by low temperature rotary evaporation (<50 °C, 27 in. Hg vacuum) to volumes of less than 500 ml. The concentrates were acidified to pH 2 with concentrated HCl and the volumes adjusted to approximately 500 ml using Milli-Q water. The acidified samples were then allowed to sit overnight at 4 °C. The following day the concentrate

was filtered through an 0.3 μm glass fiber filter, followed by a rinse with approximately 30 ml of Milli-Q water at pH 2 (acidified with HCl). The filtrate was reserved for fractionation on the XAD-8 into hydrophilic and fulvic acid fractions.

Precipitated humic acids deposited on the filter were redissolved and rinsed through the filter with approximately 15 ml of 0.5N NaOH, into a test tube. The basic filtrate was then acidified to approximately pH 1 with concentrated HCl in order to re-precipitate the humic acids, and these were centrifuged for 20 minutes at 2850G. If brown solids were visible, the supernatant was decanted, and the humics resuspended in a few ml of Milli-Q water and centrifuged again. This procedure was repeated once or twice more, until the supernatant reached approximately pH 5. At this point all of the supernatant was pipetted off. The dark brown residue was redissolved in 1 to 2 ml of Milli-Q water at neutral pH, and freeze-dried.

Isolation of the Hydrophilic and Fulvic Acid Fractions on XAD-8 Resin. Following removal of 10 ml of filtered concentrate for DOC analysis, the remainder of the concentrate was applied to a 100 ml bed volume column of Amberlite XAD-8 resin, initially cleaned according to the procedure of Leenheer and Huffman (Leenheer and Huffman 1979), and cleaned between uses by rinses with 200 ml of 0.5N NaOH, 200 ml of Milli-Q water, 200 ml of 75% acetonitrile/25% water at pH 2 (HCl), and 200 ml of Milli-Q water. The column was stored between uses in 75% acetonitrile/25% water at neutral pH, and just prior to use was rinsed with another 4 liters of Milli-Q water.

The portion of the concentrate that did not sorb to the column was the hydrophilic fraction; this was collected along with an additional 300 ml of dilute acid (Milli-Q water acidified to pH 2 with hydrochloric acid) used to rinse the XAD-8. The fulvic acid fraction was eluted from the column with 200 ml of 75% acetonitrile/25% water at pH 2 (HCl) and collected separately.

The acetonitrile and most of the water was removed from the fulvic acid fraction by rotary evaporation. Most of the HCl was removed by distillation with the acetonitrile as an azeotrope. Additional portions of acetonitrile and water at neutral pH were added to the flask and distilled off until the concentrate was at neutral pH. The aqueous solution of fulvic acids was then freeze-dried in a Labconco Dry Ice Benchtop Freeze Dry System (Labconco, Kansas City, Missouri). Figure 2.1 is a schematic of the bulk DOC fractionation procedure.

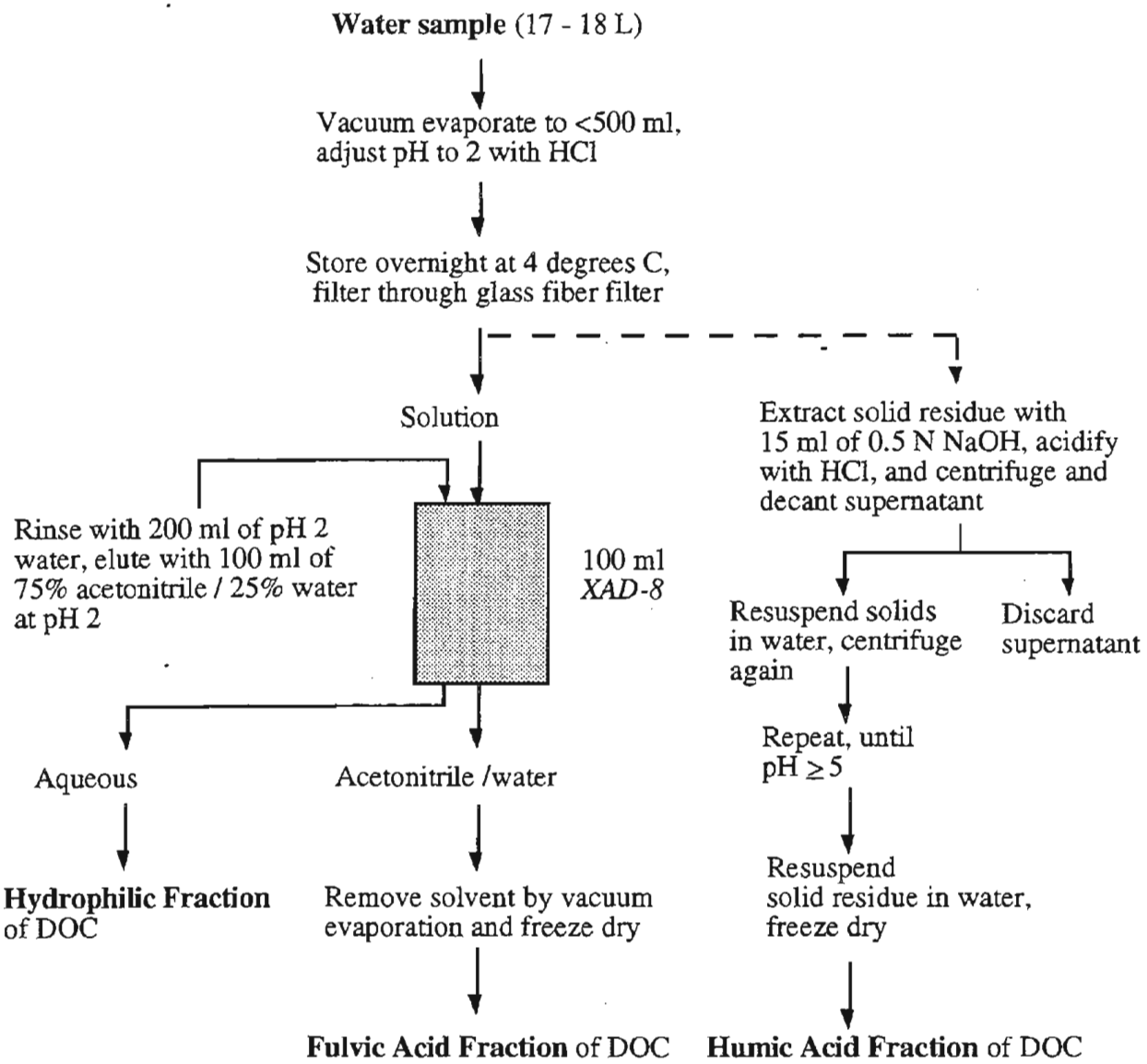


Figure 2.1 Bulk DOC Fractionation Procedure

DOC Measurement. Most of the DOC measurements reported here were made on a Dohrmann DC-80 TOC Analyzer (Rosemount Analytical/Dohrmann, Santa Clara, CA). Calibration standards were prepared with potassium hydrogen phthalate and Milli-Q water with phosphoric acid. All samples were filtered (0.2 μm or 0.3 μm) and acidified to pH 2 (with phosporic acid) prior to measurement. Later measurements were made on an OI-700 TOC analyzer (O. I. Analytical, College Station, TX) in the Orange County Water District Organics Laboratory.

Proton Nuclear Magnetic Resonance (^{1}H NMR) Spectroscopy. A Varian XL-400 NMR spectrometer (Varian Analytical Instruments, San Fernando, CA) was used to measure the ^{1}H NMR spectra. Dr. Lois Durham of the Chemistry Department performed the majority of the analyses. The spectra were obtained at 400 MHz and generated by a pulse delay of 4 s. Freeze-dried fulvic acids (approx. 10-20 mg) were dissolved in 1-2 ml of D_2O and filtered through glass wool. The peak for HOD was used as reference and set to 4.8 ppm.

Elemental Analysis. The freeze-dried fulvic and humic acids were sent to Huffman Laboratories, Inc. (Golden, CO 80403) for elemental analysis. C and H were always measured, and when quantities permitted, N, ash, Cl, Br, O and S were measured as well.

Ion Chromatography. In order to correct the measurement of chlorine in the Q8 and Q9 fulvic acid fractions for the presence of inorganic chloride, 3.1 mg and 7.7 mg portions of the Q8 and Q9 fulvic acids, respectively, were redissolved in separate 1 ml Milli-Q water aliquots for analysis by ion chromatography (IC). A Dionex Series 4000i instrument with a HPIC-AS4A column (Dionex Corporation, Sunnyvale, CA), an anion micro membrane suppressor, and a conductivity detector was used. Analyses were isocratic, with a 50/50 mixture of 0.75 mM sodium bicarbonate-2.2 mM sodium carbonate and 5 mM sodium borate as eluent, at a rate of 2 ml/min. Chloride was quantified by comparing retention time and peak area with external standards. After back calculating the total amount of inorganic chloride present in the original

isolated fulvic acid, the percentage of chlorine reported by Huffman Laboratories was adjusted downward to exclude the fraction due to inorganic chloride. Sufficient quantities of Q22B and M21 fulvic acid (the only other fulvic acids for which elemental chlorine analysis results were obtained) were not available for chloride analysis by IC. However, 0.9 mg of the method blank fulvic acid fraction was redissolved in 1 ml of Milli-Q water and analyzed by IC; the chlorine concentrations of the Q22B and M21 fulvic acids were then corrected using the calculated total amount of chloride in the method blank fulvic acid.

2.2 DOC Fractionation Results

The samples received from OCWD for fractionation on XAD-8 are listed in Table 2.1, along with the average DOC values before concentration. The DOC for the method blank is also included. The amounts of organic carbon remaining after concentration (and filtration of the concentrate to remove precipitated humic acids) are given in Table 2.2, along with the DOC fractionation results. The organic carbon in the fulvic and humic acid fractions was estimated from the percent carbon determined by elemental analysis (see Section 2.3).

Inspection of Table 2.2 reveals that losses during the concentration step (reducing 17-18 liters to 500 milliliters) varied significantly among the samples. The recovery of DOC after the concentration step was very high for Q8 (102%); this was not unexpected, given that following activated carbon treatment most of the remaining DOC components would be relatively polar, and thus not easily removed by evaporation. DOC recovery after concentration of the Q22B was 87%. Unfortunately, direct measurements of the DOC in the Q9 and M21 sample concentrates were precluded by problems with the organic carbon analyzer, but the sums of the fractions isolated totaled 85% of the original DOC for Q9 and 74% for M21; these values represent minimum percent recoveries for the concentration step. The bulk DOC analyses of both the DW1 and YLWD11 groundwaters were marked by losses of 30% and 35%, respectively, of

Table 2.1 OCWD Samples for Bulk DOC Characterization

Sample	Date Collected	Volume (l)	DOC[a] (mg/l)	Total C (mg)
Q8	3/8/93	17.0	4.2	71
Q9	6/14/93	17.7	6.0	106
Q22B	9/20/93	18.0	0.92	17
M21	7/20/93	18.0	1.1	20
DW1	10/26/93	17.0	2.6	43.9
YLWD11	11/30/93	18.0	1.5	27.4
Method Blank	-	18.0	0.11	2.0

[a] Average of three measurements for Q8, Q9, M21 and DW1; two measurements for YLWD11, one measurement for Q22B.

Table 2.2 Organic Carbon Fractionation

Sample	DOC after conc.[a]		Hydrophilic		Fulvic Acid		Humic Acid	
	mg C	%C[b]	mg C	%C[b]	mg C	%C[b]	mg C	%C[b]
Q8	72.5	102	34.3	48	31.3	44	0	0
Q9	n.a.	-	55.2	52	35.2	33	0	0
Q22B	14.8	87.1	9.2	54	5.8	34	0	0
M21	n.a.	-	3.1	16	10.1	51	1.3	6.5
DW1	26.0	59.2	5.6	12.8	3.3	7.5	4.5	10.3
YLWD11	17.9	65.3	5.8	21.2	11.5	31.9	0	0
Method Blank	1.6	70.0	0.89	44.5	0.51	25.5	0	0

[a] Measured after filtration to remove precipitated humic acid; therefore does not include DOC attributed to humic acid fraction.
[b] Percentage of total DOC in original water.
n.a. Not available.

DOC during concentration (after correction for the humic acid in DW1), indicating that relative to the Q8 and Q22B, more of the DOC in the DW1 and YLWD11 samples was distillable under the rotoevaporator conditions used. Finally, the method blank HPLC water also apparently contained a significant fraction of distillable DOC; 30% was lost during concentration.

For the DOC that was recovered, results shown in Table 2.2 indicate that the three WF21 effluents, Q8, Q9 and Q22B, are similar in terms of the distribution of DOC between the three fractions, despite the wide range in DOC concentrations (from < 1 mg/l in Q22B to 6 mg/l in Q9). Approximately half of the DOC was classified as hydrophilic. Amy *et al.* also fractionated GAC-treated secondary wastewaters on XAD-8, and obtained similar results: 55-58% of the non-purgeable organic carbon was isolated in the hydrophilic fraction, operationally defined in the same manner as in our method (Amy *et al.* 1987). Humic acid was not recovered from any of the three reclaimed waters.

The M21 groundwater fractionation results appear quite different from those for the WF21 effluents, even though the M21 and Q22B samples were quite similar in terms of nominal DOC content. Much less of the M21 DOC (16%) was measured in the hydrophilic fraction, and over 50 percent was included in the fulvic acid fraction. The decrease in hydrophilic DOC may signify the occurrence of biological activity in the aquifer; hydrophilic compounds are generally more available to microorganisms and therefore more likely to be transformed. Fulvic acid is by definition refractory, and thus an increase in its relative contribution to total DOC following biological removal of hydrophilic compounds is expected. Humic acid was also recovered from the M21 groundwater, unlike the WF21 effluents. The humic acid may derive from the non-wastewater portion of the injection blend (the concentration of humic acid in M21 in fact corresponds to the 3-fold dilution of DW1 humic acid that would result from a 1:1:1 blend of Q9, Q22B and DW1) and/or transformation of the WF21 effluents' DOC during subsurface travel.

As noted earlier, both the DW1 and YLWD11 waters exhibited large losses of volatile DOC during concentration. However, outside of that common attribute, the two groundwaters yielded very different fractionation results. DW1, like M21, contained some humic acid, while YLWD11, like Q8, Q9 and Q22B, yielded no humic acid. Much of the DOC (66%) in the filtered concentrate of DW1 was not recovered, a possible indication of irreversible (at least by elution with the 75/25 acetonitrile/water blend at pH 2) sorption on the XAD-8 resin. Almost two thirds of the DOC that was recovered from the resin column was isolated in the hydrophilic fraction. According to Thurman, in general the DOC in groundwater is more hydrophilic than the DOC in surface water; in the groundwaters he studied the fraction of hydrophobic DOC was less than 35%, while in surface waters the corresponding figure was usually 50-60% (Thurman 1985). Thurman postulates that the difference between surface waters and groundwaters with respect to hydrophilic/hydrophobic balance is due to the long residence time of the organic substances in the groundwater, which results in either adsorption of hydrophobic components onto the aquifer solids or degradation of those hydrophobic compounds into simpler organic acids by native microorganisms. The hydrophilic/hydrophobic balance for DW1, a deep groundwater with a long storage time, appears to support his contention. In contrast, the M21 groundwater, with a short storage time and recent impact from reclaimed effluent, yielded quite different results.

In contrast, theYLWD11 behaved quite differently. Only a very small portion (3.4%) of the filtered DOC of the YLWD11 concentrate was not recovered, and of the recovered DOC two-thirds was in the fulvic acid fraction (which would be included in the "hydrophobic" fraction under Thurman's definition). In this respect the YLWD11 was similar to the M21 groundwater, for which 77% of the recovered non-humic acid DOC was classified as fulvic acid. However, as noted previously, no humic acid DOC was isolated from the YLWD11 water, while a small fraction (6.5%) was obtained from the M21 groundwater. As the YLWD11 well water is derived from percolated river water containing a high proportion of secondary treated effluent, this result

could signify insufficient residence time of the wastewater DOC in the aquifer for formation of humic acids.

2.3 Elemental Analysis of Fulvic and Humic Acid Fractions

Table 2.3 gives the elemental analysis results for the fulvic acids from the six samples and the method blank. Included in Table 2.3 for comparison are "typical" values for fulvic acids from groundwater, river water, and lake water, as suggested in a literature review by E. M. Thurman (Thurman 1985). Table 2.4 contains C, H, and N results for the only two humic acid fractions isolated, from M21 and DW1 groundwater; insufficient quantities precluded measurement of other elements. "Typical" values for ground and river water humic acids are included (Thurman 1985).

In general, the percent carbon values for the fulvic and humic acid fractions appear to be low relative to the reported "typical" values. This may reflect in part the high (again, relative to the reported "typical" values) concentrations of ash, which could be attributable in large part to residues from sample preparation and cleanup. Unfortunately, the quantity of the fulvic acid fraction isolated from the method blank was not sufficient to permit ash analysis. Because of the possible error introduced by the high ash content, atomic ratios are a more meaningful parameter of comparison. In particular, H/C, O/C, and N/C ratios are frequently reported in the literature, and are therefore amenable to comparison. The calculated atomic ratios for the fulvic acids and humic acids are presented in Table 2.5 and Table 2.6, respectively. Table 2.6 does not include values for O/C, as limited quantities did not permit measurement of oxygen in the humic acid samples.

Table 2.3 Elemental Composition of Fulvic Acids

Sample	% C	% H	% O	% N	% S	% Cl	% Br	% Ash
Q8	42.0	5.4	23.1	4.1	4.1	0.8[b]	2.2	15.6
Q9	46.8	6.0	34.1	3.1	n.a.	1.5[b]	1.1	7.5
Q22B	25.5	4.8	n.a.	4.0	n.a.	10[c]	n.a.	n.a.
M21	32.5	4.4	28.0	3.3	n.a.	9.7[c]	n.a.	8.5
DW1	42.0	4.5	n.a.	1.7	n.a.	n.a.	n.a.	25.0
YLWD11	32.0	4.5	n.a.	1.8	n.a.	n.a.	n.a.	24.0
Method Blank	19.6	4.5	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
"Typical groundwater"[a]	59.7	5.9	31.6	0.9	n.a.	n.a.	n.a.	1.2
"Typical river water"[a]	51.9	5.0	40.3	1.1	n.a.	n.a.	n.a.	1.5
"Typical lake water"[a]	52.0	5.2	39.0	1.3	n.a.	n.a.	n.a.	5.0

[a] From Thurman 1985, p. 288.
[b] Corrected for inorganic chlorine residue using I.C. measurement of chloride in sample fulvic acid.
[c] Corrected for inorganic chlorine residue using I.C. measurement of chloride in method blank fulvic acid.
n.a. Not available, because insufficient material available for analysis.

Table 2.4 Elemental Composition of Humic Acids

Sample	% C	% H	% N
M21	37.3	4.4	2.4
DW1	58.7	5.9	2.2
"Typical groundwater"[a]	62.1	4.9	3.2
"Typical river water"[a]	50.5	4.7	2.0

[a] From Thurman 1985, p. 288.

Table 2.5 Atomic Ratios for Fulvic Acids

Sample	H/C	O/C	N/C
Q8	1.5	0.41	.083
Q9	1.5	0.55	.057
Q22B	2.3	n.a.	.14
M21	1.6	0.65	.087
DW1	1.3	n.a.	.035
YLWD11	1.7	n.a.	.048
Method Blank	2.7	n.a.	n.a.
"Typical groundwater"[a]	1.2	0.40	.013
"Typical river water"[a]	1.2	0.58	.018
"Typical lake water"[a]	1.2	0.56	.021

[a] Calculated from data reported by Thurman 1985, p. 288.
n.a. Not available, because insufficient material available for O or N analysis.

Table 2.6 Atomic Ratios for Humic Acids

Sample	H/C	N/C
M21	1.4	.055
DW1	1.2	.032
"Typical groundwater"[a]	0.95	.044
"Typical river water"[a]	1.1	.034

[a] Calculated from data reported by Thurman 1985, p. 288.

The H/C ratio indicates the degree of unsaturation in the carbon skeleton: a low H/C ratio reflects greater unsaturated and aromatic character. The OCWD fulvic and humic acid samples all exhibit high H/C ratios, indicating that saturated aliphatic or alicyclic structures predominate over aromatic structures in the complex mixtures. Steelink notes that "H/C ratios are clustered around 1.0 for most soil and aquatic humates and fulvates...Ratios above 1.3 indicate that the material may be a nonhumic substance." (Steelink 1985) It is interesting to note that according to this criterion, only the DW1 fulvic and humic acid fractions appear to contain "natural" humic substances. The DW1 groundwater is in fact the one water analyzed that was purported to be pristine--i.e., untainted by anthropogenic pollutants.

The O/C ratio reflects the degree of oxidation of the organic compounds. The OCWD samples for which data are available (the Q8, Q9 and M21 fulvic acids) do not appear remarkable in comparison to the reference natural fulvic acids. It is interesting to note however the increase in O/C between Q8 and Q9 (see Table 2.5); this is likely due to the oxidation of organic compounds by the active chlorine species.

The N/C ratios for the OCWD fulvic and humic acids are generally high relative to the reference values, perhaps indicating the municipal wastewater origin of the waters. "Humic materials" isolated on XAD-2 resin by Peschel and Wildt from both secondary wastewater and Ruhr River water (presumably impacted by municipal and industrial waste water effluents) exhibited similar N/C ratios (Peschel and Wildt 1988). Measured by this parameter, once again the DW1 groundwater, of all of the analyzed samples, appears to be the most like a "typical natural water," for both fulvics and humics.

Elemental analysis results other than C, H, O, N and ash are infrequently reported for humic substances. Huffman and Stuber did however report chlorine analysis results for an aquatic fulvic acid isolated from Coal Creek in Colorado (Huffman and Stuber 1985). The fulvic

acid was analyzed by four different labs, and the measured values for chlorine ranged from 0.097 to 0.23% (Huffman and Stuber 1985). These are significantly lower than the chlorine values measured for the Q8, Q9, Q22B and M21 fulvic acids, which may reflect the formation of organochlorine compounds during water and wastewater disinfection. The chlorine percentages for Q22B and M21 in particular appear to be high (10% and 9.7%, respectively), but it is important to remember that these values were "corrected" for inorganic chloride by assuming the same amount of chloride as was measured in the method blank, because there was not enough of the original sample fulvic acids to measure chloride directly. It may be that the sample fulvic acids contained more inorganic chloride than the method blank "fulvic acid," thereby reducing the estimated organochlorine content. If this is the case, then the back-calculated concentrations of chlorine in the fulvic fractions of the original waters--32 µg/l and 54 µg/l, for Q22B and M21, respectively--may be conservative (high) estimates. The corresponding values for Q8 and Q9 are 15 µg/l and 30 µg/l. These values are less than the total organic halogen content (150 µg/l) reported for a chlorinated secondary wastewater used for infiltration in Phoenix, AZ (Bouwer *et al.* 1982), but they do not account for organochlorine in the hydrophilic fraction (nor in the humic acid fraction, in the case of M21). Christman *et al.* in fact found that the majority of the identified byproducts of the chlorination of an aquatic humic acid were acids (Christman *et al.* 1990), which would be recovered in the hydrophilic fraction. Some of those byproducts may be brominated as well; the formation of brominated products during water chlorination has been well documented (Reinhard *et al.* 1982; Voudrias and Reinhard 1988). The bromine measured in the Q8 and Q9 fulvic acids (2.2% and 1.1%, respectively) may be due to this mechanism. The measured values are comparable to the 3% bromine concentration reported by Malcolm *et al.* for humic material isolated from a chlorinated reverse osmosis effluent near Yuma, AZ (Malcolm *et al.* 1981).

Huffman and Stuber also reported elemental analysis results for sulfur in the same aquatic fulvic acid analyzed for chlorine; percentages ranged from 0.28 to 0.98% (Huffman and Stuber

1985). Once again, these are significantly lower than the one measurement of sulfur available from the OCWD samples, 4.1% in the Q8 fulvic acid. Because data from the other OCWD waters and the method blank are not available, it is difficult to comment on the significance of this figure.

2.4 ^{1}H NMR Spectra of Fulvic Acid Fractions

The ^{1}H NMR spectra of the fulvic acid fractions isolated from the six OCWD samples are presented in Figure 2.2. The NMR spectrum of the fulvic acid fraction isolated from the method blank is shown in Figure 2.3, along with the spectrum of a hexaethylene glycol standard. In each fulvic acid spectrum, the sharp signal at 4.8 ppm represents the hydrogen of HOD (either a contaminant or produced by hydrogen exchange with the D_2O solvent), and the signal at approximately 1.95 ppm indicates hydrogen of acetonitrile, incompletely removed during the fulvic acid cleanup. Because the NMR samples were not all prepared at the same concentration due to sample quantity limitations, relative intensities of peaks should not be compared between samples.

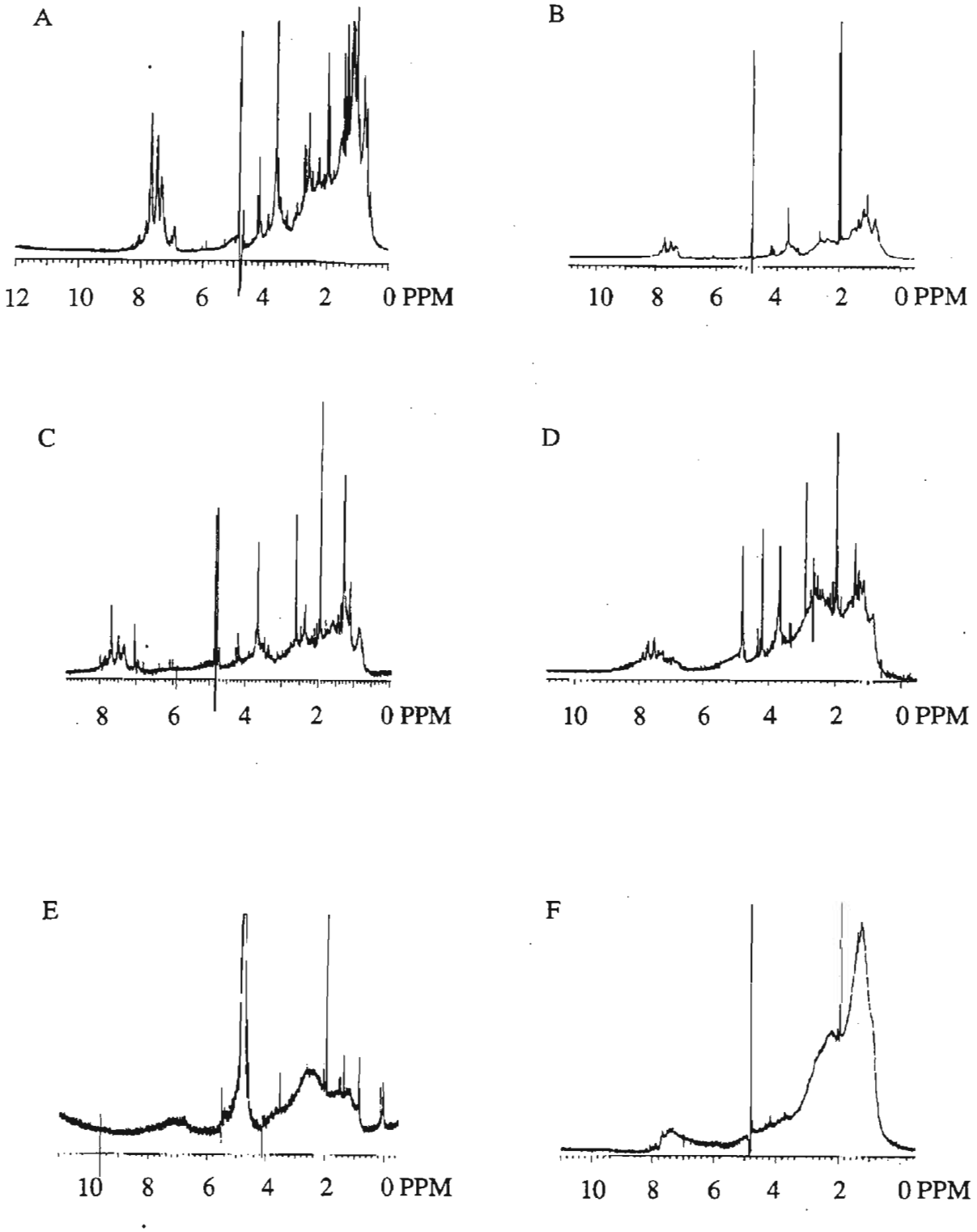


Figure 2.2 ^{1}H NMR Spectra of Fulvic Acids Isolated from (A) Q8, (B) Q9, (C) Q22B, (D) M21, (E) DW1 and (F) YLWD11

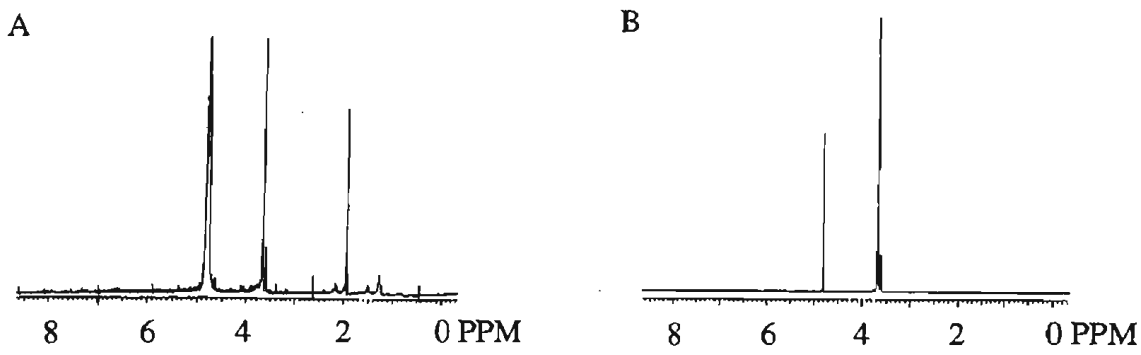


Figure 2.3 ^1H NMR Spectra of (A) Method Blank Fulvic Acids and (B) Hexaethylene glycol standard in D_2O.

In contrast to the method blank (Figure 2.3A), which has very few features other than the water and acetonitrile signals, the spectra of all of the OCWD samples (Figure 2.2) are marked by broad, unresolved humps characteristic of complex mixtures, with some sharp signals indicative of specific structures. Spectra of this type are most useful as "fingerprints" for fulvic acids from different sources, rather than as diagnostics for specific compounds. In this case, the fulvic acids from the three WF21 waters (Q8, Q9, and Q22B) and the WF21 effluent-recharged groundwater, M21, have similar fingerprints, indicating their common origin. Each of the four spectra exhibit the "four major broad resonances characteristic of humic substances" described by Thorn (Thorn 1987), in the regions from 0.5 - 2 ppm, 2 - 3 ppm, 3 - 5 ppm, and 6.5 - 8 ppm. The first region (0.5 - 2 ppm) may be attributed primarily to aliphatic hydrogens, and the farthest downfield region (6.5 - 8 ppm) to aromatic protons. In the region between 2 and 3 ppm, the signals may indicate protons on carbons alpha to carbonyl, carboxylic acid, ester, or amino groups, or benzylic protons. GC/MS data indicate the presence of all of these structural classes in the WF21 effluents and M21 groundwater (see Section 3.3). The signals between 3 and 5 ppm are generally attributed to protons on methyl, methylene, and methyne carbons directly bonded to oxygen and nitrogen (Thorn 1987). The strong signal between 3.5 and 4 ppm may be associated with protons of polyalkyloxylate chains. Figure 2.3B, the spectrum of the hexaethylene glycol

standard, indicates that the primary signals for polyethoxy hydrogens occur around 3.6 ppm. This correlates well with the findings of Leenheer *et al.*, who analyzed dissolved organics in Mississippi River water, using a similar isolation method relying on XAD-8 adsorption (Leenheer *et al.* 1991); a reproduction of their spectrum of the Mississippi River dissolved organic solutes is shown in Figure 2.4. Also using standards, they assigned the chemical shift at 3.6 ppm to protons attached to poly(ethylene) glycol and poly(propylene) glycol units, and attributed these to residues of nonionic surfactants. Compounds of this type are prominent features in GC/MS chromatograms of WF21 effluent extracts (see Section 3.3). The NMR spectrum of the M21 fulvic acid (Figure 2.2D) also exhibits a peak at 3.6 ppm, and the GC/MS analyses provide further proof of the persistence of these poly(ethylene) and poly(propylene) compounds during groundwater transport.

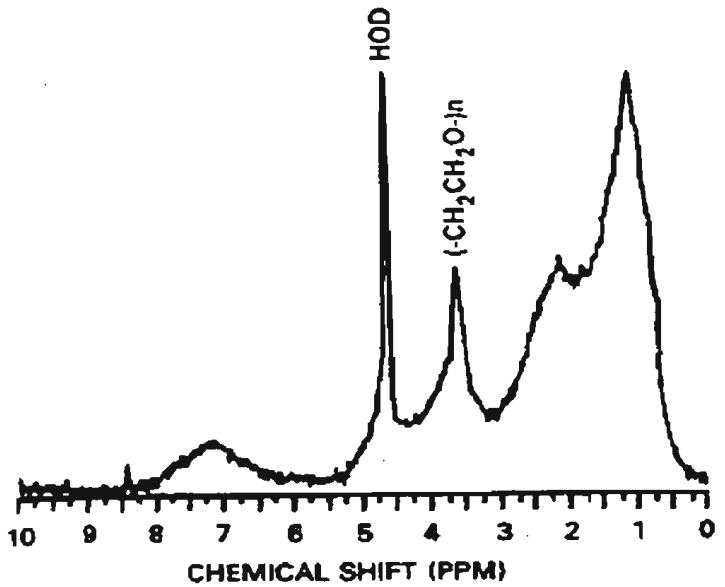


Figure 2.4 ^{1}H NMR Spectrum of Organic Solutes Isolated From Samples Collected From the Mississippi River, reprinted with permission from (Leenheer *et al.* 1991) Copyright 1991 American Chemical Society.

The appearance in the method blank fulvic acid (Figure 2.3A) of the peaks in the 3.6 ppm region, indicative of polyalkyloxy hydrogens, was surprising; the residues may derive from either the HPLC water used for the blank, or from the Milli-Q water used in sample preparation. It is important to note however that, unlike the case for WF21 samples and the M21 ground water, the area of the 3.6 ppm signal relative to the water peak at 4.8 ppm is very small. Integration of the peak areas (not shown) in the method blank indicated a water/polyethoxy hydrogen ratio of 31.

In general, the NMR spectra of the DW1 and YLWD11 groundwaters show a lack of fine structure, in comparison to the WF21 samples and the M21 groundwater. The spectrum of the DW1 fulvic fraction (Figure 2.2E) is marked by the dominant HOD peak at 4.8 ppm; for some unknown reason related to the sample chemistry it was difficult to decouple that peak in order to allow better visualization of the other signals. In general though, the DW1 fulvic fraction has much weaker signals in the region from 0.5 to 2 ppm than the WF21 and M21 samples, for which this region dominates. The aromatic region (6.5 - 8 ppm) is much less prominent, with no distinct peaks, and there is just one small spike around 3.6 ppm, as opposed to the significant peak in the WF21 and M21 samples. GC/MS analyses did not detect the presence of any confirmed poly(ethylene) glycol and poly(propylene) glycol structures in DW1.

The ^{1}H NMR spectrum of the YLWD11 fulvic acid (Figure 2.2F) bears a striking resemblance to the spectrum acquired by Leenheer *et al.* for the dissolved organics in the Mississippi River (Figure 2.4), except for the absence of the poly(ethylene)/poly(propylene) glycol peak at 3.6 ppm. There are no sharp signals (other than the HOD at 4.8 ppm and the acetonitrile at 1.95 ppm), and the major feature is the broad peak in the aliphatic hydrogen region (0.5 - 2 ppm), with the shoulder between 2 and 3 ppm. The spectrum seems to reflect increasing humification of the organic components of the Santa Ana River water (which is composed

primarily of secondary effluent from treatment plants upstream of Orange County), and/or selective removal of specific compounds during percolation into the groundwater.

SECTION 3

IDENTIFICATION OF SPECIFIC ORGANIC RESIDUES USING GC/EI-MS AND GC/CI-MS TECHNIQUES

3.0 Approach

The approach for specific compound identification relies on gas chromatography with mass spectrometric detection (GC/MS). MS detection is crucial to the elucidation of unknown compounds within a complex matrix. Standard reference compounds are often unavailable for trace organic residues, and therefore compounds with identical structure must be prepared. GC provides an efficient and reliable pre-separation method for MS detection. MS/MS techniques were used to analyze unknown mass spectra, and electron impact (EI) and chemical ionization (CI) techniques were used in combination for structural elucidation. Because many of the DOC components in the WF21 effluents and groundwaters are nonvolatile, derivatization is necessary to increase volatility. The compounds are then detected by MS as their derivatives. The methods developed here for the specific compound identification involve concentration of samples by rotary evaporation, derivatization by propanol/formic acid/acetyl chloride, and identification and semi-quantitation by capillary GC/EI-MS, GC/CI-MS and GC/TSQ-MS analysis. Groups of nonionic surfactants and their acidic metabolites were used as standard compounds to evaluate the methods and generate a reference mass spectral data base.

3.1 Methods

3.1.1 Materials and Reagents

Unless noted otherwise all solvents and chemicals were "Baker Resi-Analyzed" grade from J.T. Baker (J.T. Baker, Inc., Phillipsburg, NJ, USA) and were used without further

purfication. Formic acid and acetyl chloride were purchased from Aldrich (Aldrich Chem. Co., Milwaukee, WI, USA). Sodium sulfate (J.T. Baker) was used after baking overnight at 105 °C, after cooling, it was stored in a dessicator. The lauryl alcohol (C_{12}), myristyl alcohol (C_{14}) and palmityl alcohol (C_{16}) polyethoxy carboxylates mixture was a gift from Dr. Francesc Ventura (Sociedad General de Aguas de Barcelona, Barcelona, Spain) and was used as received. Diglycolic acid, tetraethylene glycol (P*4*EG), pentaethylene glycol (P*5*EG) and polypropylene glycol mixture were purchased from Aldrich Chemical Co. (Milwaukee, WI, USA).

3.1.2 Samples

Samples (1 liter) were collected and shipped to the Stanford Water Quality Laboratory in ice-cooled containers. Upon arrival, the samples were immediately filtered through 0.2 µm nylon filter (Nylon 66, Alltech, Deerfield, IL USA) and then stored at 4 °C until analyzed.

3.1.3 Reference Compounds Preparation

Oxidation. The mixed P*4*EG and P*5*EG standards or polypropylene glycol mixture (100 mg each) were converted from the alcohol form to the corresponding dicarboxylates by oxidation with Jones reagent (Reinhard et al. 1982). Saturated magnesium sulfate solution was added to the acid dichromate reaction mixture to facilitate extraction of the products. The mixture was extracted with 60 ml methylene chloride three times, dried with sodium sulfate, filtered through a filter paper and concentrated down to 10 ml by rotary evaporation under vacuum.

Derivatization of Standards. The derivatization procedure was performed according to the method reported by Schaffner and Giger (Schaffner and Giger 1984), with minor modifications. A portion of the mixture was transferred to a 25-ml pear-shaped flask and dried by a gentle stream of nitrogen. The residue was redissolved in 2 ml of 50 % formic acid and evaporated to

complete dryness by rotary evaporation under vacuum. For propylation, 1.5 ml of n-propanol/acetyl chloride reagent (9/1, v/v) was added to the flask (or methanol/acetyl chloride for methylation). Reaction at 85 ^{o}C for one hour converted the carboxylic acids to propyl esters (or methyl esters). After cooling, chloroform 1 ml was added to the reaction mixture. Then 5 ml of 2 % potassium bicarbonate solution was added, and after venting the flask, the mixture was shaken vigorously using a vortex mixer. The top aqueous phase was removed and discarded. This extraction step was repeated. The extract was dried by sodium sulfate (about 0.5 g), and concentrated to approximately 0.1 ml by a stream of nitrogen. The extract was then transferred to an 0.1 ml glass insert and evaporated to dryness. Finally the residue was redissolved in toluene to obtain a concentration of 0.1-0.2 mg/ml.

Sample Preparation. Each subsample 400 ml was concentrated by rotary evaporation to approximately 10 ml, and then transferred to a 25 ml pointed tip flask and evaporated to dryness under vacuum. Then the concentrate was derivatized in the same manner as described above. If an emulsion formed, the organic layer was drained into a centrifuge tube which was capped and centrifuged for 3 minutes or until the emulsion was broken. The top aqueous phase was discarded. This extraction step was repeated. The final residue was taken up in 40 µl toluene containing 20 ng/µl chrysene-d_{12} (Cambridge Isotope Lab. Inc., Andover, MA, USA) as an internal standard. The method blank was prepared from 400 ml purified Milli-Q water (Milli-Q system, Millipore Corp., MA, USA) with the same concentration and derivatization procedures as described above.

3.1.4 Analytical Instruments and Procedures

Electron ionization (EI) mass spectra were obtained using a Hewlett-Packard 5890 gas chromatograph (Hewlett-Packard, Palo Alto, CA, USA) directly connected to a Hewlett-Packard 5970 MSD mass spectrometer ion source with a DB-5 capillary column (30 m x 0.32 mm id.,

0.25 μm film; J&W Scientific, Folsom, CA, USA). Spectra were compared with the references in computerized NBS and EPA-NIH mass spectra library data bases. The electron multiplier voltage was set at 1800 V and the scan range was 45 to 500 amu. An 1 μl sample was injected by splitless technique. The GC temperature program was as follows: 100 °C (held for 5 min) followed by a temperature ramp at 5 °C/min to 300 °C (held for 5 min). The injector temperature was maintained at 280 °C.

Chemical ionization (CI) mass spectra were obtained using a Finnigan Triple-Stage-Quadrupole Mass Spectrometer (TSQ-70; Finnigan MAT, San Jose, CA, USA) with CI ion volume in Q1MS mode. A Varian Model 3400 gas chromatograph (Varian Corp., Sunnyvale, CA, USA) was directly connected to the TSQ-70 with a 30 meter DB-5 capillary column (same as in the H-P system). The Q1MS was tuned using perfluorotributylamine (PFTBA) on *m/z* 219 and *m/z* 414 with methane reagent gas on. Helium was used as the carrier gas at a head pressure of 8 psi. A 2 μl sample was injected by splitless technique. The GC temperature program was the same as in the H-P system. The injector temperature was maintained at 300 °C. Methane or *iso*-butane was used as CI reagent gas. The CI conditions were: ion source pressure 2 Torr (read from "Ion Source" pressure gauge); ionization energy 70 eV; electron multiplier voltage 1500 V; emission current 200 μA; ionization temperature 150 °C; mass range *m/z* 100 - 600; scan time 1 second.

The product-ion mass spectra from GC/TSQ-MS were generated with argon. The collision gas pressure was 1.6 - 1.7 mTorr and the collision energy was -4.0 V. The Q3 scan range was from *m/z* 30 to 40 or 50 units above the parent ions transmitted from the Q1, and the scan time was 1 second.

3.1.5 Identification and Quantitation

Identification. Evidence for compound identification by GC/MS was obtained via the following steps: (a) acquire the EI mass spectrum; (b) acquire the CI mass spectrum with methane and *i*-butane as the reagent gases ; (c) identify the molecular ion from CI data; (d) propose structural assignments and fragmentation mechanisms from the EI fragmentation and the CI molecular ion data; (e) confirm the structural assignment by comparing with reference EI mass spectra from the NBS and EPA-NIH libraries or with the EI mass spectrum of a standard compound; (f) if necessary, acquire the MS/MS product-ion mass spectrum to confirm the fragmentation mechanism. A compound was considered positively identified if two or more of the following criteria were satisfied: (1) the EI spectrum matched with the reported spectrum of a reference compound, (2) the expected molecular ion was confirmed with CI-MS, and (3) the product-ion spectrum of characteristic ions matched the expected fragmentation pattern. In several cases, reference compounds were synthesized for studying MS fragmentation.

Quantitation. Concentrations of most individual compounds were determined semi-quantitatively by comparing the EI base ion (obtained using the HP system) with the *m/z* 240 ion of chrysene-d_{12}. Thus, the concentrations given were based on the assumption that the response factors for the internal standard and the analyte were the same. The concentration was calculated as follows:

$$C_s = \frac{A_s * C_{is}}{A_{is} * DF}$$

C_S : Concentration of compound (µg/L);

C_{IS} : Concentration of internal standard (20 µg/ml);

A_S : Base ion peak area of compound;

A_{IS} : Base ion peak area of internal standard;

DF : Dilution factor: x10,000 for samples Q22B, M21 and DW1;

x1,000 for sampled Q8, Q9 and YLWD11.

The concentrations of EDTA and NTA in the sample were estimated based on an external standard calibration with deuterated EDTA and NTA.

3.2 Mass Spectra of Reference Compounds

3.2.1 Alkylphenol Polyethoxylates and their Carboxylates

Alkylphenol polyethoxylates (APEO) are non-ionic surfactants commonly used for industrial and agricultural applications. APEO compounds consist of branched (tertiary) dodecyl, nonyl (NPEO) or octyl (OPEO) phenol attached to a hydrophilic polyethylene glycol chain having 1 to 30 ethoxy (EO) units. It has been well documented that APEO compounds may be transformed in biological treatment systems by: (a) biodegradation (shortening) of the polyethoxy chain and/or (b) carboxylation of some of the terminal EO units, resulting in the formation of alkylphenol polyethoxy carboxylates (APEC).

In this study, octylphenol polyethoxylates are denoted as OP*n*EO; the *n* indicates the number of EO units attached to the octylphenoxy group. In OP*n*EC (the carboxylated form of OP*n*EO), the *n* indicates the number of unaltered ethoxy units plus the terminal CH_2COOH (or in derivatized form, $CH_2COOC_3H_7$) group.

Representative EI mass spectra of the octylphenol mono- and diethoxylates are shown in Figure 3.1 (a) and (b), respectively. The most significant ions are produced by benzylic cleavage $[M-71]^+$, and are very common for most EI mass spectra of OP*n*EO. The ion at *m/z* 135 was attributed to a loss of the polyethoxy chain with H transfers from the $[M-71]^+$ ion. Figures 3.2 (a), (b) and (c) show the methylated octylphenol mono-, di- and triethoxy carboxylates, respectively. The benzylic cleavage $[M-71]^+$ is also very common for most OP*n*EC compounds. The relatively strong ion at *m/z* 117 was attributed to the cleavage of the

ethereal O-C bond in δ position to the carboxyl group. The OP*I*EC does not have an equivalent structure in the δ position and the ion at *m/z* 117 is very weak.

The CI (methane) mass spectra of OP*n*EO and OP*n*EC are shown in Figures 3.3 and 3.4, respectively. The molecular ions indicated by the CI spectra agreed with those of the EI spectra. Although benzylic cleavage $[MH-72]^+$ was observed in the CI spectra, the more important fragmentation was olefin (C_8H_{16}) displacement producing the ion $[MH-112]^+$. These ions indicated the length of the EO side chain. The ion at *m/z* 113 $[C_8H_{17}]^+$ resulted from the alkyl ion displacement from the phenoxy ring. More detailed studies of the EI and CI mass spectra of halogenated and non-halogenated OPEO and OPEC has been reported by Stephanou *et al.* (Stephanou *et al.* 1988).

3.2.2 Linear Alcohol Polyethoxylates and their Carboxylates

Linear alcohol polyethoxylates (LAEO) are one of the fastest growing nonionic surfactant classes on the market. The growth rate for these compounds has been forecasted as high as 5 to 7 precent per year (Ainsworth 1994). The biodegradation pathway of LAEO has been proposed from laboratory studies (Larson and Games 1985; Steber and Wierich 1985). These compounds may be transformed by three primary biodegradation processes: (a) stepwise oxidative cleavage of ethoxy units, resulting in the formation of linear alcohol polyethoxy carboxylates (LAEC), (b) scission of the oxyethylene chain from the alkyl chain followed by oxidation of both products, the alkane and polyethylene glycol chain (PEG), and (c) oxidation of the alkyl chain to produce the carboxy-alkyl-PEG [$HOOC-(CH_2)_{m-1}$-PEG].

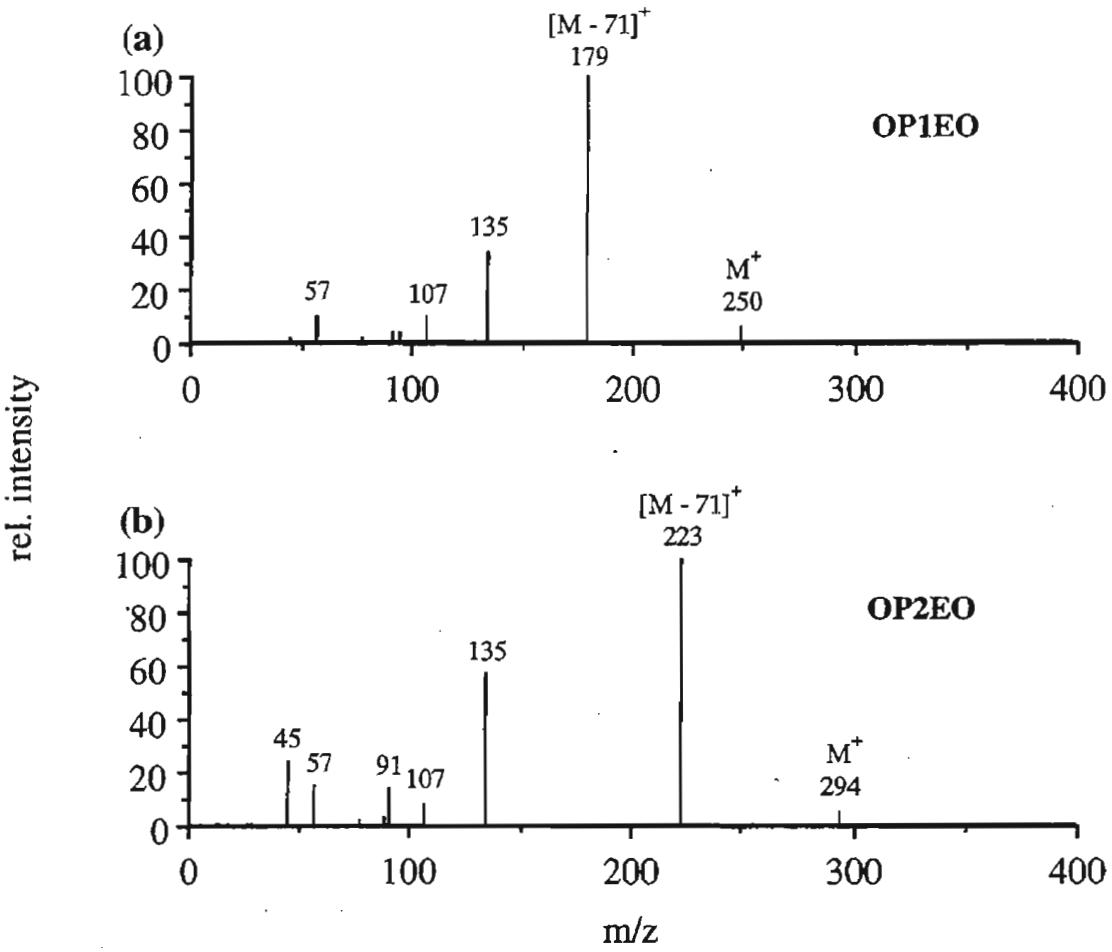


Figure 3.1 EI mass spectra of (a) octylphenol monoethoxylate and (b) octylphenol diethoxylate.

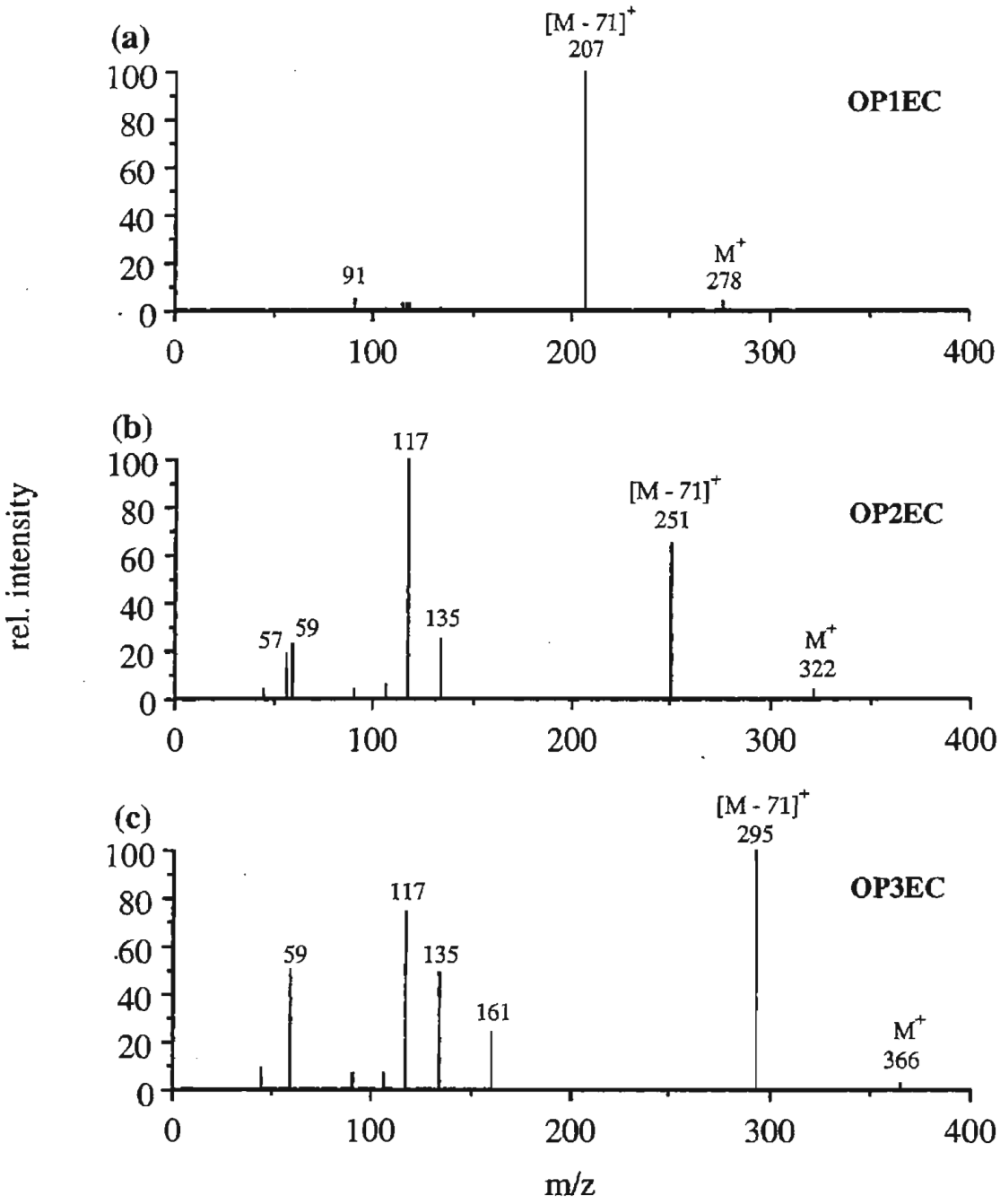


Figure 3.2 EI mass spectra of (a) octylphenol acetic acid-, (b) octylphenol ethoxy acetic acid- and (c) octylphenol diethoxy acetic acid-methyl esters.

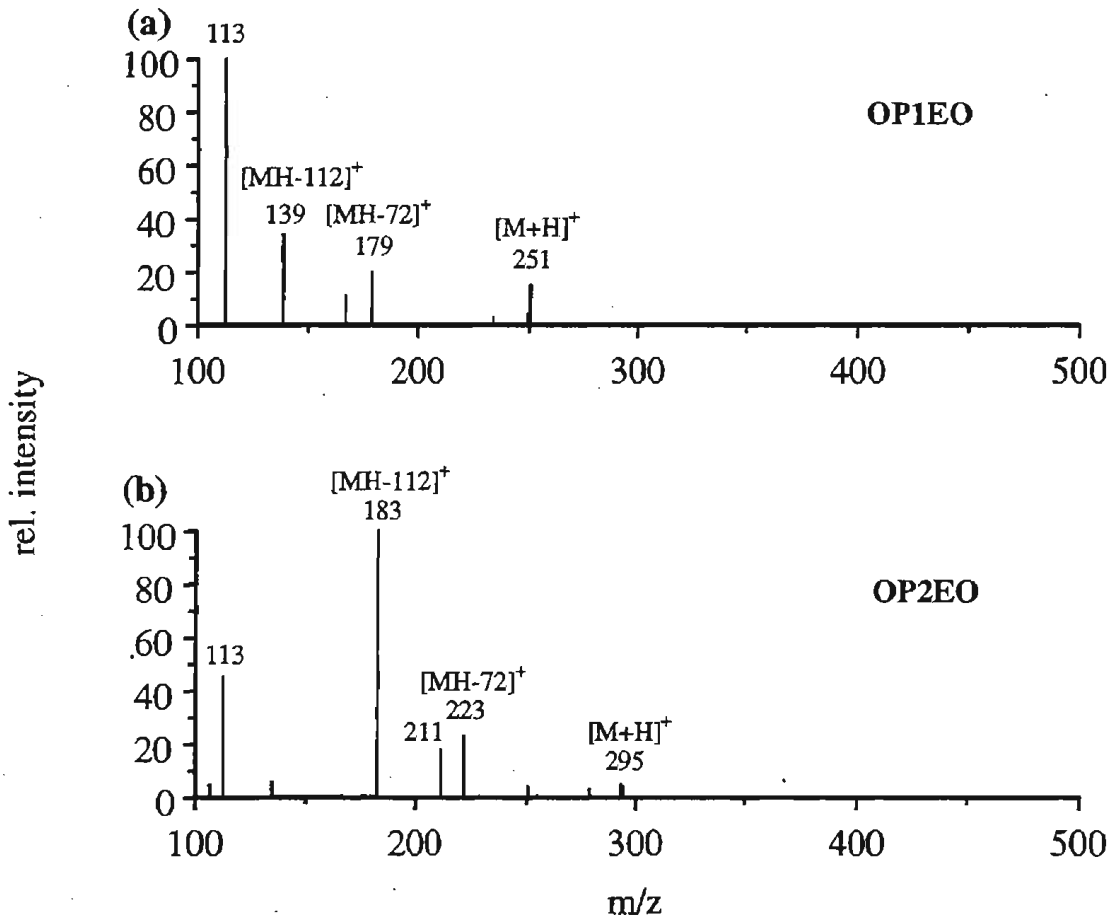


Figure 3.3 CI (methane) mass spectra of (a) octylphenol monoethoxylate and (b) octylphenol diethoxylate.

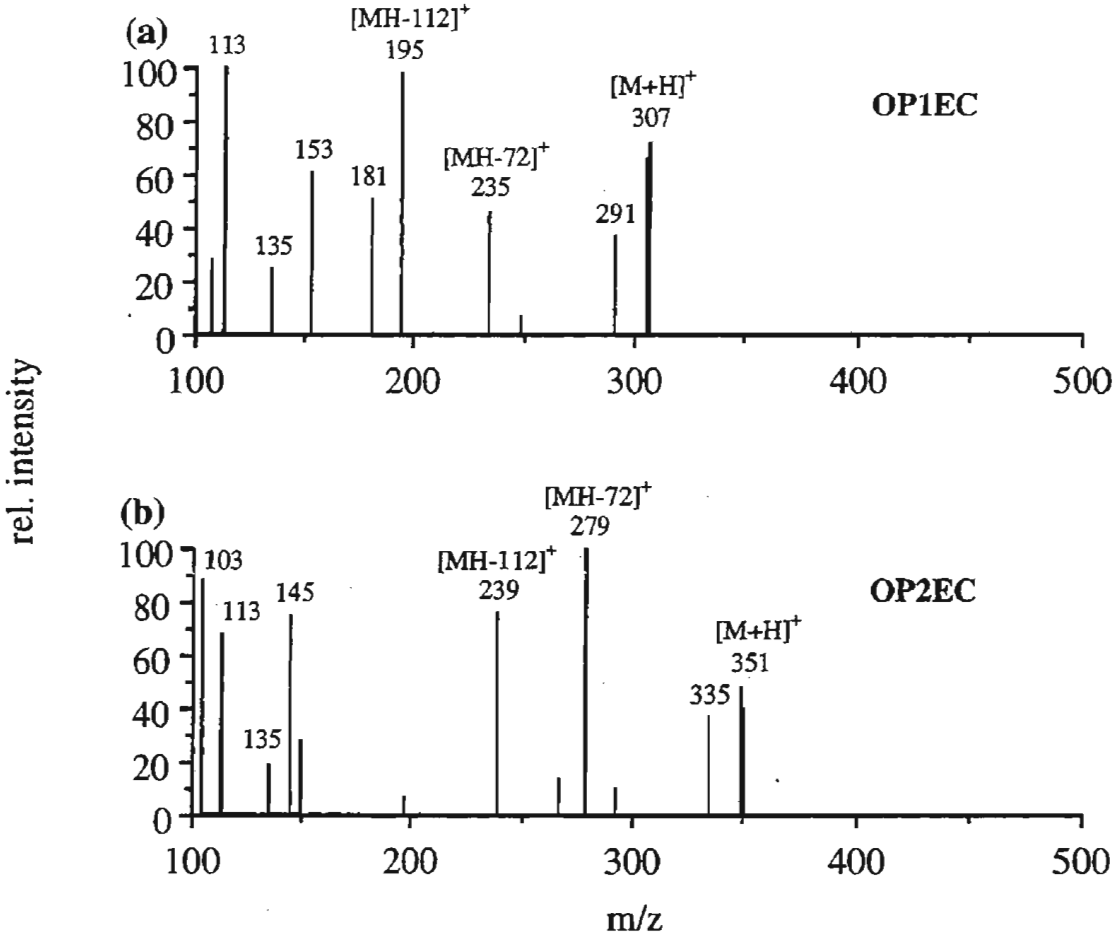


Figure 3.4 CI (methane) mass spectra of (a) octylphenol acetic acid- and (b) octylphenol ethoxy acetic acid-propyl esters.

In this study, lauryl alcohol polyethoxylates are denoted as $C_{12}nEO$, where *n* indicates the number of EO units attached to the lauryl alcohol. $C_{12}nEO$ denotes their acidic metabolites, the *n* indicates the number of unaltered ethoxy units plus the terminal CH_2COOH (or in derivatized form, $CH_2COOC_3H_7$) group.

Representative EI mass spectra of the lauryl alcohol mono- and hexaethoxylates are shown in Figures 3.5 (a) and (b), respectively. They only show uncharacteristic ion series at the low mass end and do not allow us to determine molecular weight and structure. However, the CI

technique provides both reliable molecular weight (from $[M+H]^+$ and $[M+29]^+$ adducts) and some structural characteristic information. Figures 3.6 (a) - (d) show the CI (methane) mass spectra of lauryl alcohol mono- to tetraethoxylates, respectively. A relatively strong $[M-H]^+$ ion was observed in the $C_{12}nEO$ mass spectra and was attributed to initial hydride abstraction from the alkyl chain (Munson and Field 1966; Tsang and Harrison 1975). The protonated molecular ions $[M+H]^+$ were base ions in all cases. These ions may have been formed by protonation of the ethoxy oxygens by CH_5^+ and $C_2H_5^+$. A detailed study of the CI (methane) mass spectra of LAEO and their fragmentation mechanisms has been reported by Stephanou (Stephanou 1984).

The CI (methane) mass spectra of propylated $C_{12}nEC$ with one to four ethoxy units are summarized in Table 3.1. The numbers in parentheses indicate the relative intensities of the ions. The corresponding CI (methane) mass spectra of propylated $C_{12}nEC$ are shown in Figure 3.7. A relatively strong $[M-H]^+$ ion was observed in the $C_{12}nEC$ mass spectra and was attributed to initial hydride abstraction from the alkyl chain (Munson and Field 1966; Tsang and Harrison 1975). The protonated molecular ions $[M+H]^+$ were base ions in all cases and may have been formed by protonation of the ethoxy or carbonyl oxygens by CH_5^+ and $C_2H_5^+$. When there is a long ethoxy chain the oxygen of the ethoxy group is expected to be preferentially protonated over the oxygen of the carbonyl group. The ions $[MH-168]^+$ are diagnostically important because these fragments indicate both the length of the ethoxy chain in $C_{12}nEC$ and the length of the alkyl group. These ions were formed by displacing the alkyl group as an olefin via a hydrogen transfer to the protonated ether oxygen and cleavage of the alkyl-oxygen bond (Scheme 3.1) (Stephanou 1984; Ventura *et al.* 1991). The ion at *m/z* 233 was attributed to loss of H_2O from $[MH-168]^+$ ion, and the ions at *m/z* 189 and 145 were attributed to the loss of ethylene oxides (C_2H_4O) from ion $[MH-168-H_2O]^+$. The ions $[MH-88]^+$ were attributed to the neutral loss of propyl formate ($HCOOC_3H_7$). The ions $[MH-42]^+$ and $[MC_2H_5-42]^+$ may be produced by proton rearrangement and loss a propene ($CH_2=CH-CH_3$) from ions $[M+H]^+$

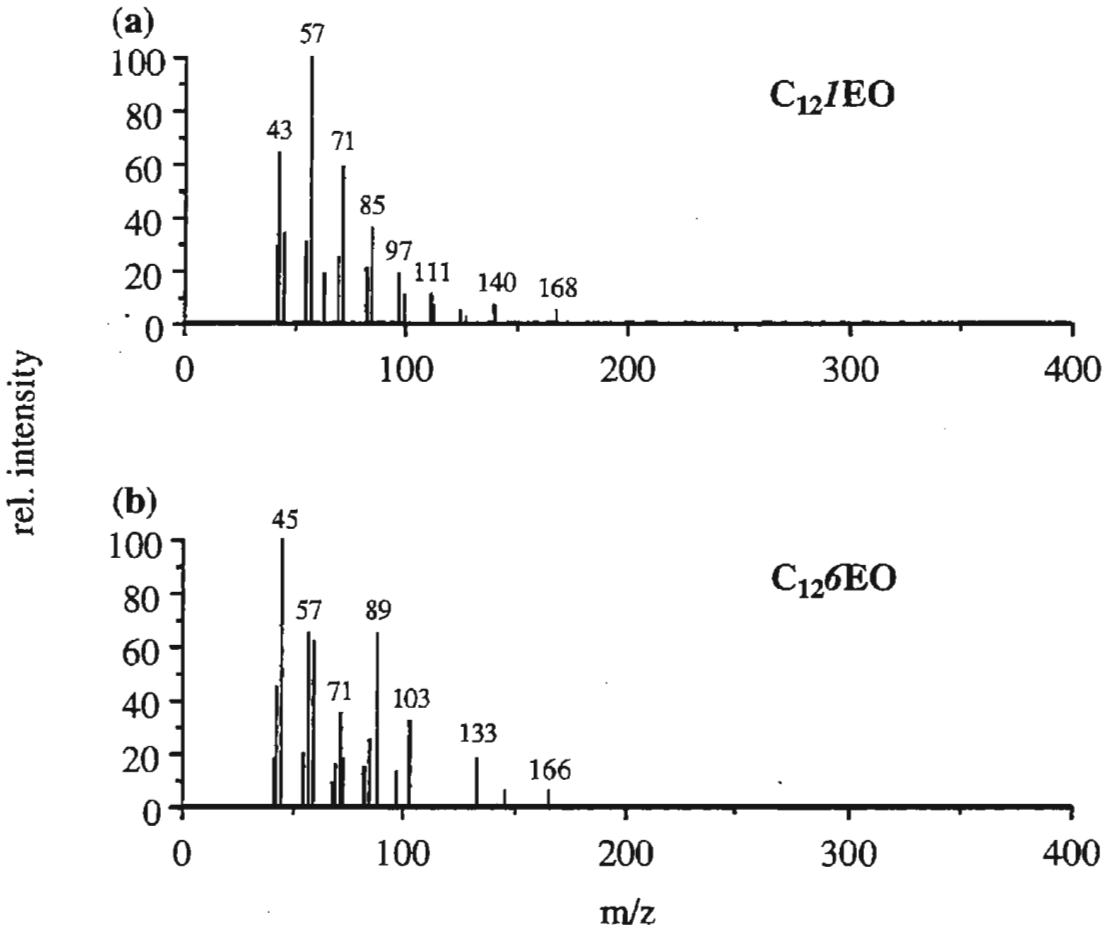


Figure 3.5 EI mass spectra of (a) lauryl alcohol monoethoxylate and (b) lauryl alcohol hexaethoxylate.

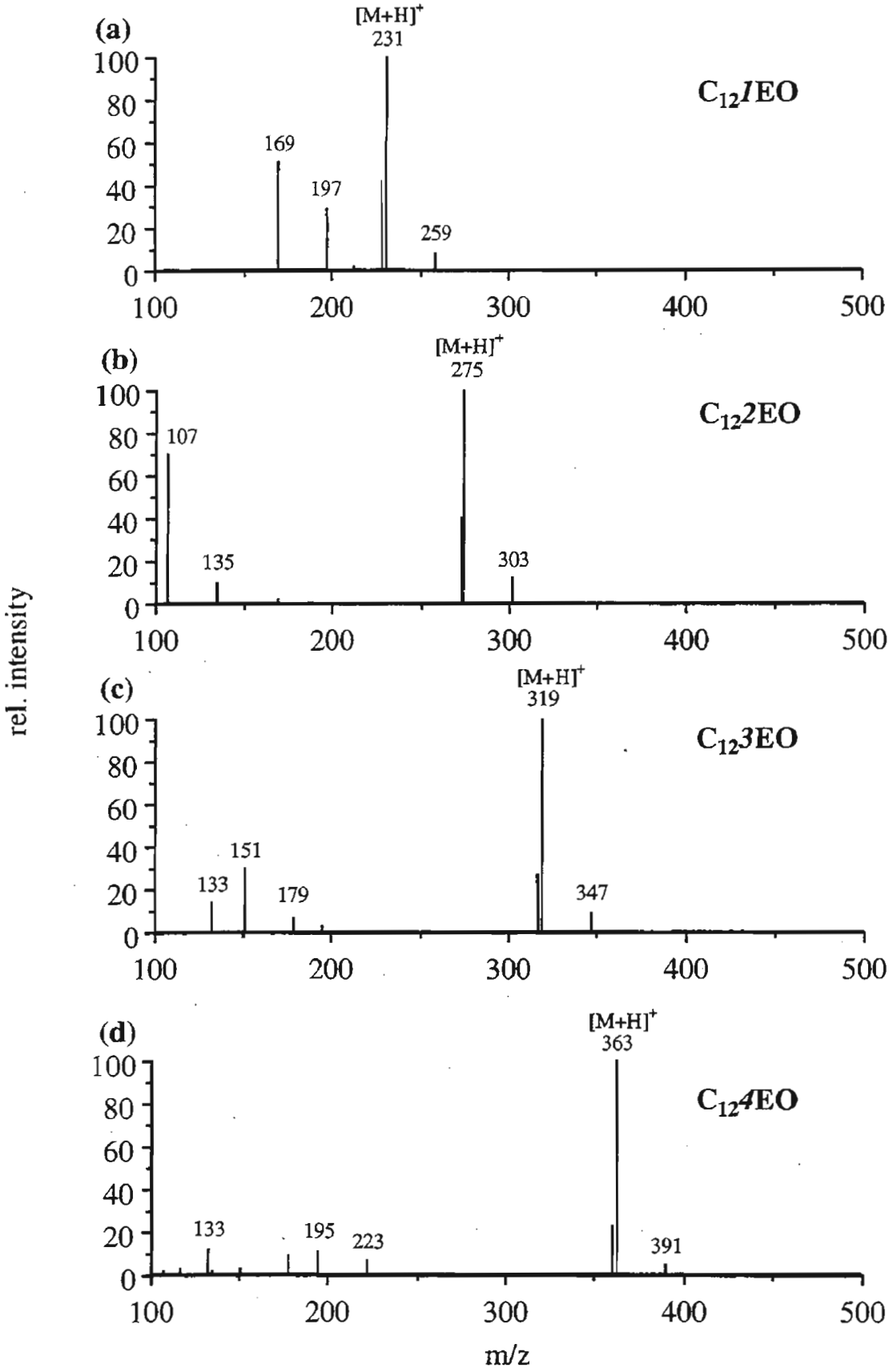


Figure 3.6 The CI (methane) mass spectra of lauryl alcohol (a) mono-, (b) di-, (c) tri- and (d) tetraethoxylates.

Table 3.1 Methane CI Mass Spectra of Propylated C_{12}*n*EC: $[C_{12}H_{25}\text{-O-}(CH_2CH_2O)_{n-1}\text{-}CH_2COOC_3H_7,\ n = 1 - 4]$

Ions	Neutral Loss	n = 1[a] (C_{12}*1*EC)	n = 2[a] (C_{12}2EC)	n = 3[a] (C_{12}*3*EC)	n = 4[a,b] (C_{12}*4*EC)
$[M-H]^+$		285(40)	329(62)	373(75)	417(56)
$[M]^+$		286(14)	330(22)	374(24)	418(19)
$[M+H]^+$		287(100)	331(100)	375(100)	419(100)
$[MH-168]^+$	$C_{12}H_{24}$	119(85)	163(69)	207(88)	251(85)
$[MH-88]^+$	$HCOOC_3H_7$	199(24)	243(17)	287(18)	331(11)
$[MH-42]^+$	CH_2=CH-CH_3	245(9)	289(6)	333(6)	377(6)
$[M+C_2H_5]^+$.		315(6)	359(1)	n.d.	n.d.
$[MC_2H_5-42]^+$	CH_2=CH-CH_3	273(13)	317(25)	361(11)	405(6)
$[MC_2H_5-168]^+$	$C_{12}H_{24}$	147(10)	191(11)	235(25)	279(15)
$[C_{12}H_{25}]^+$ (*m/z* 169)		(32)	(9)	(11)	(17)
Fragmentations (Scheme 3.1)[c]					
m/z 233					(15)
m/z 189				(18)	(19)
m/z 145			(51)	(63)	(73)
m/z 103			(27)	(35)	(37)

[a] The number of *n* indicates the number of unaltered ethoxy units plus the terminal $CH_2COOC_3H_7$ group. In the structure, n-1 is used to explain this alternation.
[b] Also *m/z* 257(41), *m/z* 163 (14).
[c] For C_{12}*3*EC and C_{12}2EC, the fragmentation starts at $[MH-168]^+$, the first loss is H_2O, and for C_{12}*3*EC the second loss is ethylene oxide.
n.d.: not detected.

and $[M+C_2H_5]^+$ (Scheme 3.2) (Munson and Field 1966). The homologs of propylated myristyl alcohol and palmityl alcohol polyethoxy carboxylates (C_{14}*n*EC and C_{16}*n*EC, respectively) have been studied under the same GC/CI-MS conditions. The fragmentation mechanisms and many of the resulting ions were similar to those in propylated C_{12}*n*EC. Figure 3.8 shows the CI mass spectra of propylated C_{14}*4*EC (a) and C_{16}*4*EC (b). The fragment ions $[MH-196]^+$ and $[MH-224]^+$, which were attributed to the olefin displacement of the respective alkyl chains, were identical to those observed for C_{12}*n*EC. The ions of *m/z* 233, 189 and 145 were attributed to the losses of H_2O and ethylene oxides from ions $[MH-196]^+$ and $[MH-224]^+$, respectively,

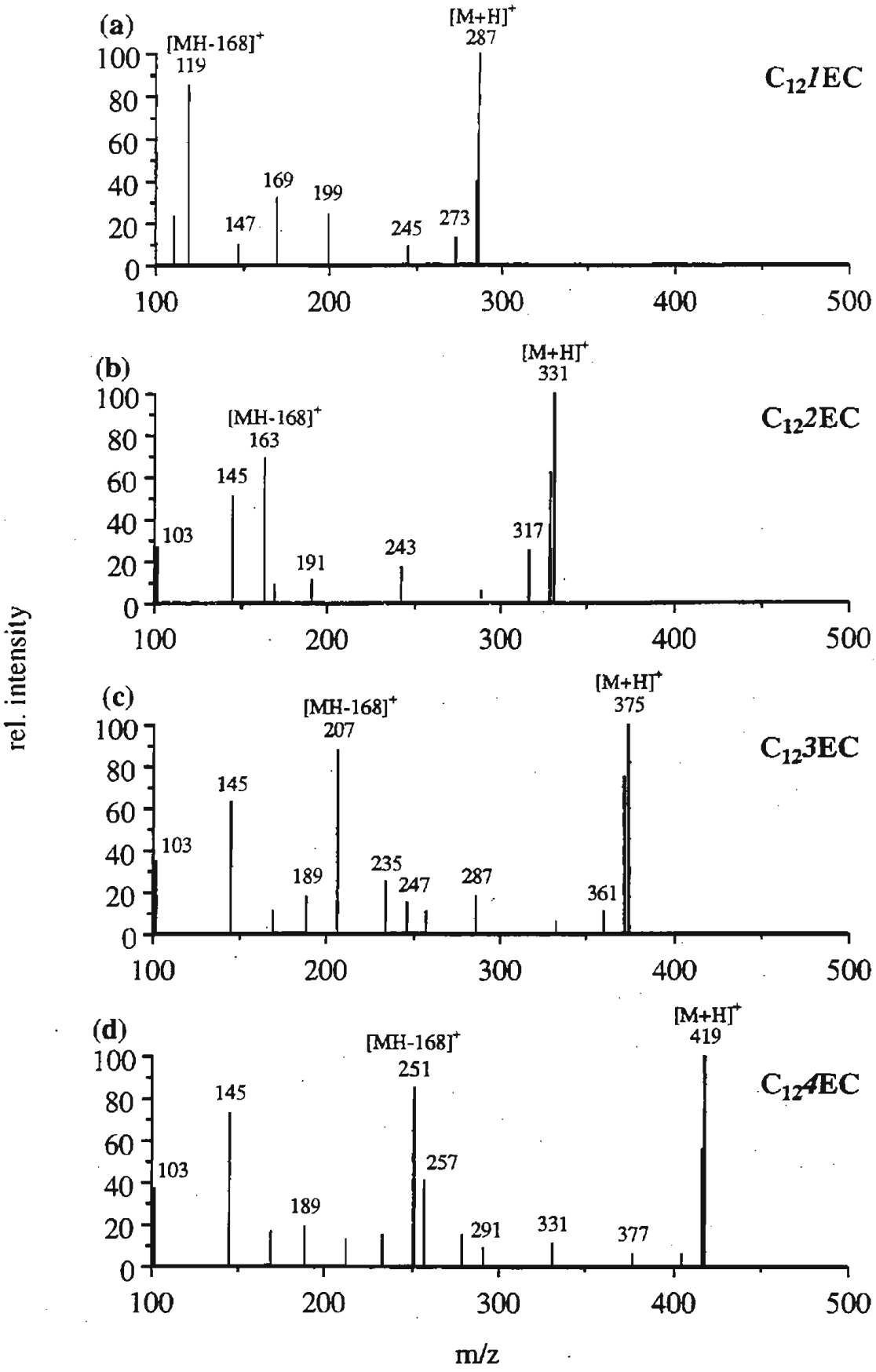
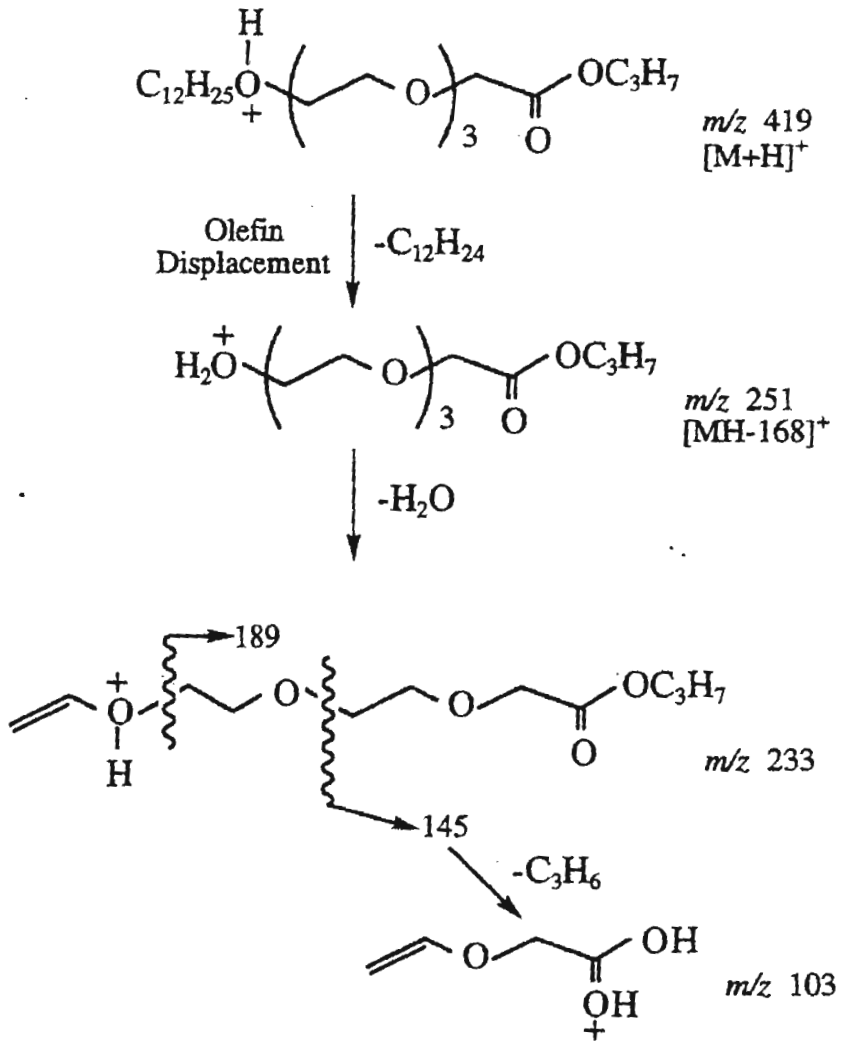
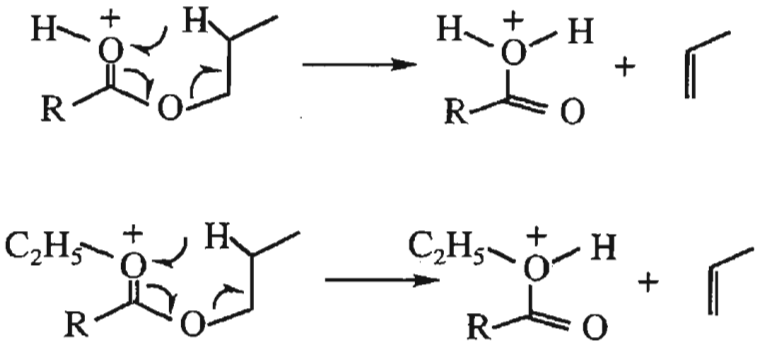


Figure 3.7 The CI (methane) mass spectra of lauryl alcohol (a) acetic acid-, (b) ethoxy acetic acid-, (c) diethoxy acetic acid- and (d) triethoxy acetic acid-propyl esters.


m/z 419
[M+H]+
Olefin
Displacement
-C12H24
m/z 251
[MH-168]+
-H2O
189
m/z 233
145
-C3H6
m/z 103

Scheme 3.1

as described in Scheme 3.1. Neutral loss of propyl formate $[MH-88]^+$ was also observed. A detailed study of the CI (methane) mass spectra of propylated LAEC has been reported by Ding *et al.* (Ding *et al.* 1994A).



Scheme 3.2

3.2.3. Polyethylene Glycols and their Dicarboxylates

Polyethylene glycols (PEGs) are synthetic polymers with many applications and a significant fraction is discharged to the environment. In addition, PEGs provide the hydrophilic head group of LAEO and their biodegradation pathways are described as in Section 3.2.2. In this study, polyethylene glycols are denoted as P*n*EG, and their dicarboxylate metabolites are denoted as P*n*EGDC. The *n* indicates the number of unaltered ethoxy units plus the terminal CH_2COOH (or in derivatized form, $CH_2COOC_3H_7$) groups.

Figure 3.9 shows the (a) EI and (b) CI mass spectra of hexaethylene glycol. The CI mass spectrum yields both molecular weight and fragmentation information. The CI (methane) mass spectra of propylated P*n*EGDC with two, four and five ethoxy units are summarized in Table 3.2. The numbers in parentheses indicate the relative intensities of the ions. The CI mass spectra of

propylated P*n*EGDC are shown in Figure 3.10. The protonated molecular ions $[M+H]^+$ were base ions in all cases, except in P5EGDC. The ions $[MH-88]^+$, attributed to the neutral loss of

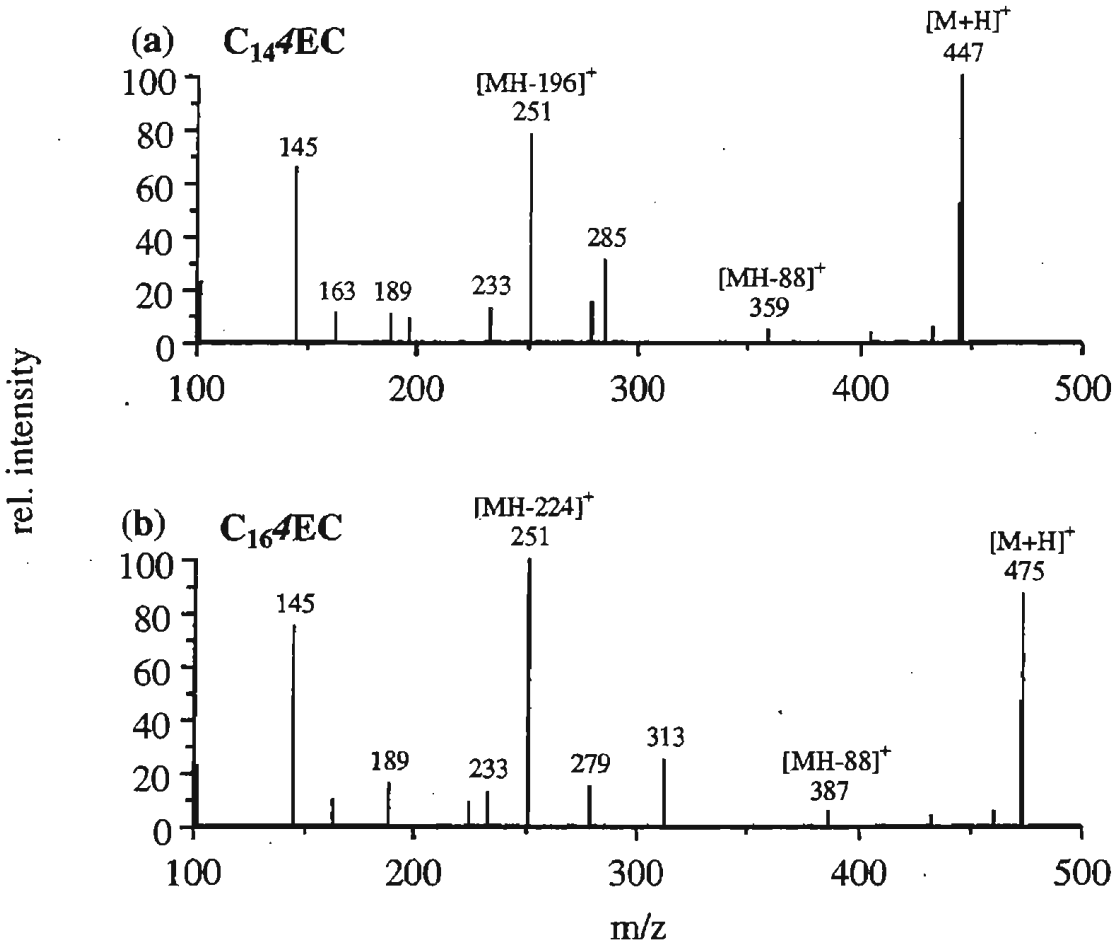


Figure 3.8 The CI (methane) mass spectra of (a) myristyl alcohol triethoxy acetic acid- and (b) palmityl alcohol triethoxy acetic acid-propyl esters.

propyl formate ($HCOOC_3H_7$), were observed in all P*n*EGDC. The CI mass spectra of P*n*EGDC shows similar fragmentation as in C_{12}*n*EC after cleavage of one terminal $HCOOC_3H_7$ from the MH^+ ion. For P5EGDC, the ion at *m/z* 233 was obtained from cleavage at the first ether linkage leading to loss of $O{=}CH_2$ from $[MH-88]^+$ ion, and the ions at *m/z* 189 and 145 were attributed to the losses of ethylene oxides (Scheme 3.3). The relatively intense ion *m/z* 159 $[MH-60]^+$ was observed in P2EGDC and was tentatively attributed to the loss of propanol from the propylated

diglycolate ester. This fragmentation may be explained as the result of a bifunctional interaction as described by Weinkam (Scheme 3.4) (Weinkam and Gal 1976). A detailed study of the CI mass spectra of propylated PnEGDC has been reported by Ding *et al.* (Ding *et al.* 1994A).

Table 3.2. Methane CI Mass Spectra of Propylated P*n*EGDC
$[C_3H_7OOCCH_2\text{-}O\text{-}(CH_2CH_2O)_{n\text{-}2}\text{-}CH_2COOC_3H_7,\ n = 2, 4, 5]$

Ions	Neutral Loss	n = 2[a] P2EGDC	n = 4[a] P4EGDC	n = 5[a] P5EGDC
$[M]^+$		218(7)	306(11)	350(7)
$[M+H]^+$		219(100)	307(100)	351(90)
$[MH-88]^+$	$HCOOC_3H_7$	131(54)	219(46)	263(100)
$[MH-42]^+$	$CH_2=CH-CH_3$	177(60)	265(38)	309(45)
$[MC_2H_5-42]^+$	$CH_2=CH-CH_3$	205(26)	293(19)	337(15)
$[MH-60]^+$	HOC_3H_7	159(39)	n.d.	n.d.
Fragmentations (Scheme 3.3)[b]				
m/z 233				(14)
m/z 189			(6)	(33)
m/z 145			(58)	(60)
m/z 103			(9)	(14)

[a] The number of *n* indicates the number of unaltered ethoxy units plus two terminal $CH_2COOC_3H_7$ groups. In the structure, n-2 is used to explain this alternation.
[b] The fragmentation starts at $[MH-88]^+$, the first loss is $O=CH_2$, followed by the loss of ethylene oxides.
n.d.: not detected.

3.2.4 Polypropylene Glycols and Their Carboxylates

The CI (methane) mass spectra of propylated propylene glycol carboxylates (P*n*PGC) with two to five propoxy groups were studied for comparison with the mass spectra of PnEGDC. The carboxylate group was obtained from the oxidation of poly(*iso*-propylene glycols) at one end, and the ketone was produced from the oxidation of secondary alcohol at the other end.

The CI (methane) mass spectra obtained from propylated P*n*PGC with two to five propoxy units are summarized in Table 3.3. The CI mass spectra of propylated P*n*PGC are shown in Figure 3.11. The protonated molecular ions, $[M+H]^+$, were observed in all cases.

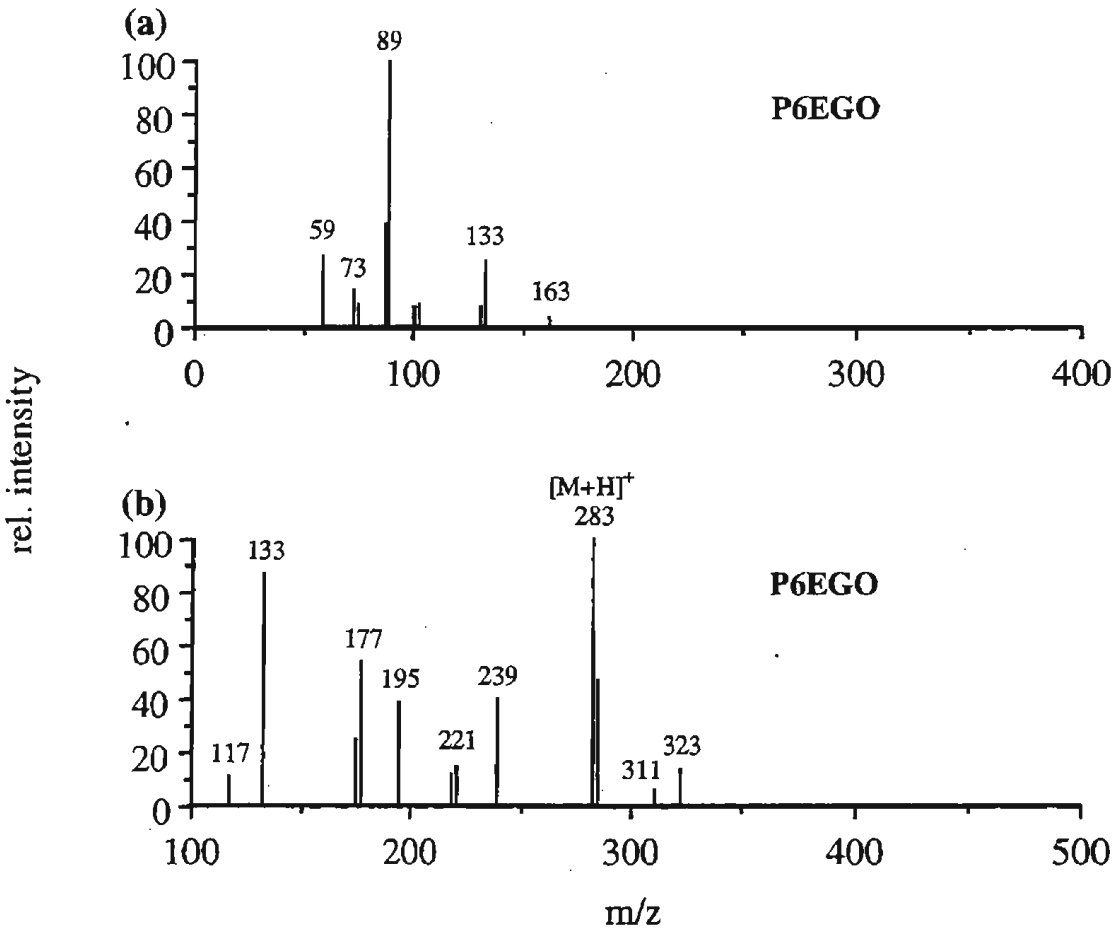


Figure 3.9 (a) EI and (b) CI mass spectra of hexaethylene glycol.

The fragmentation of propylated P5PGC is illustrated in Scheme 3.5. The ions $[MH-74]^+$, attributed to the loss of $CH_3C(O)CH_2$-OH, are important characteristic fragments. The ions at *m/z* 231, 173 and 115 were attributed to the losses of propylene oxides (C_3H_6O) from ion $[MH-74]^+$. The ion *m/z* 131 may be produced by proton rearrangement and loss of a propene (CH_2=CH-CH_3) from ion *m/z* 173 (Scheme 3.2). The ions $[MH-88]^+$, attributed to the neutral

loss of propyl formate ($HCOOC_3H_7$), were observed in all cases. The fragmentation starting at the ester side produced the relatively weak ions *m/z* 217, 159 and 101 (Scheme 3.6).

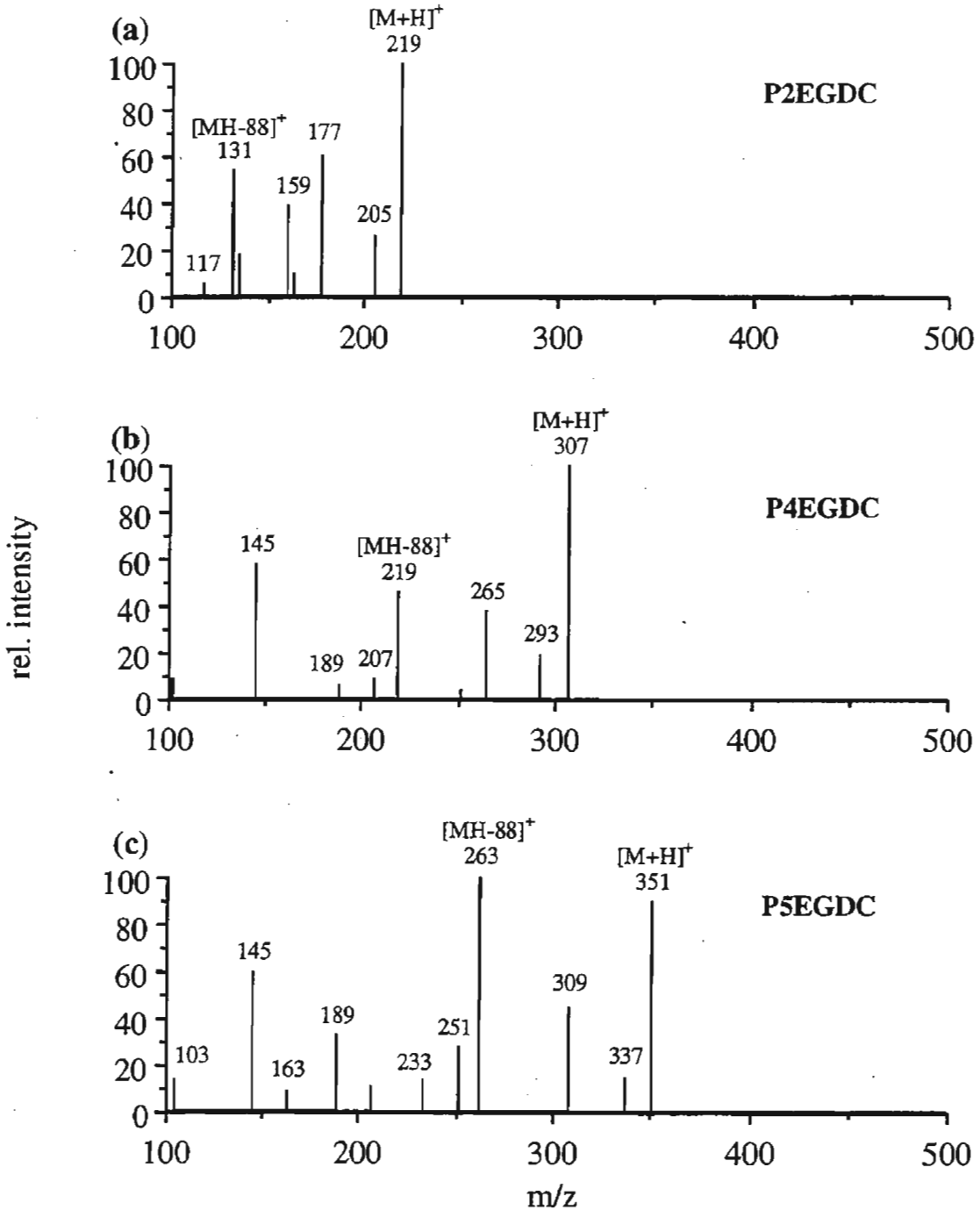
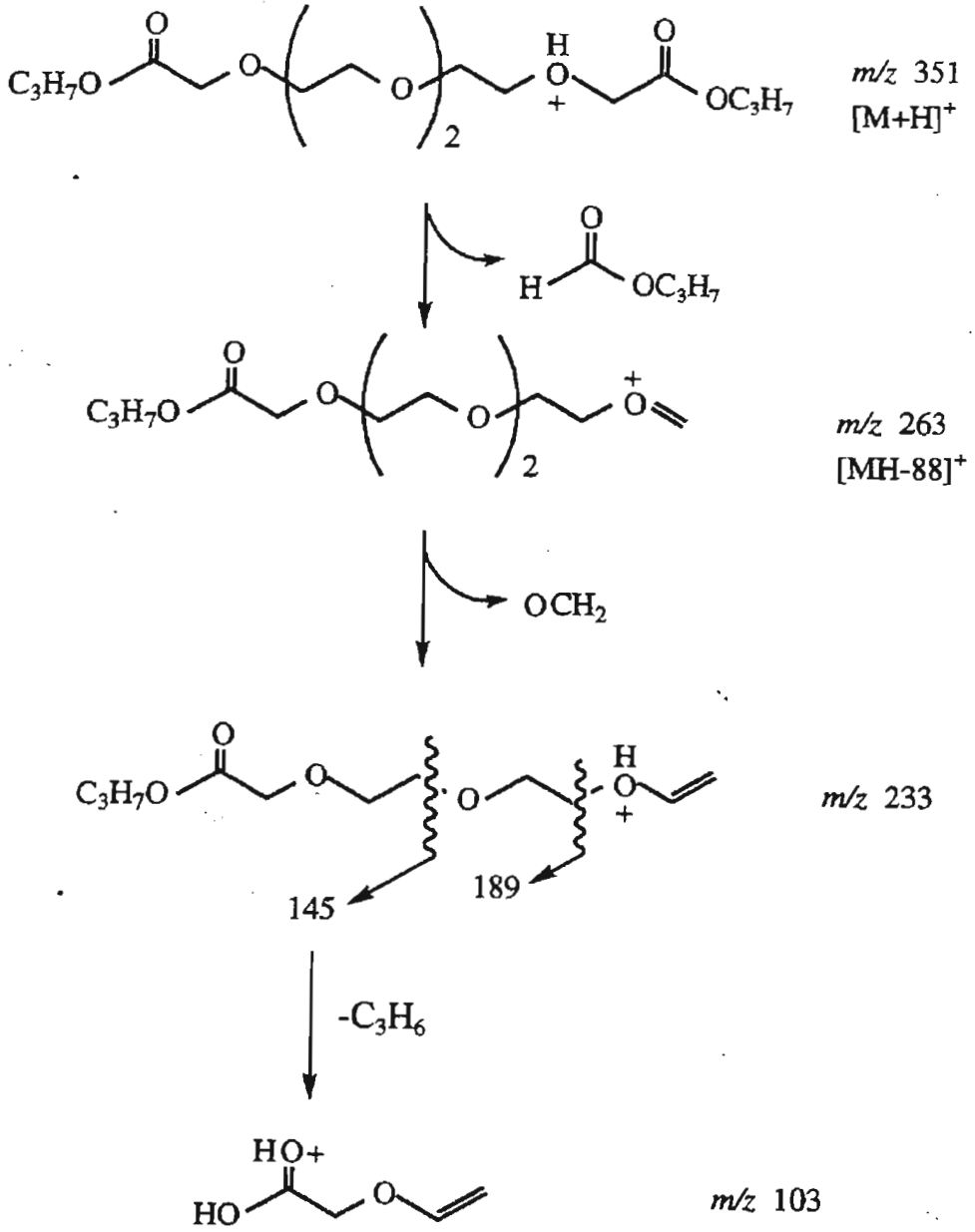
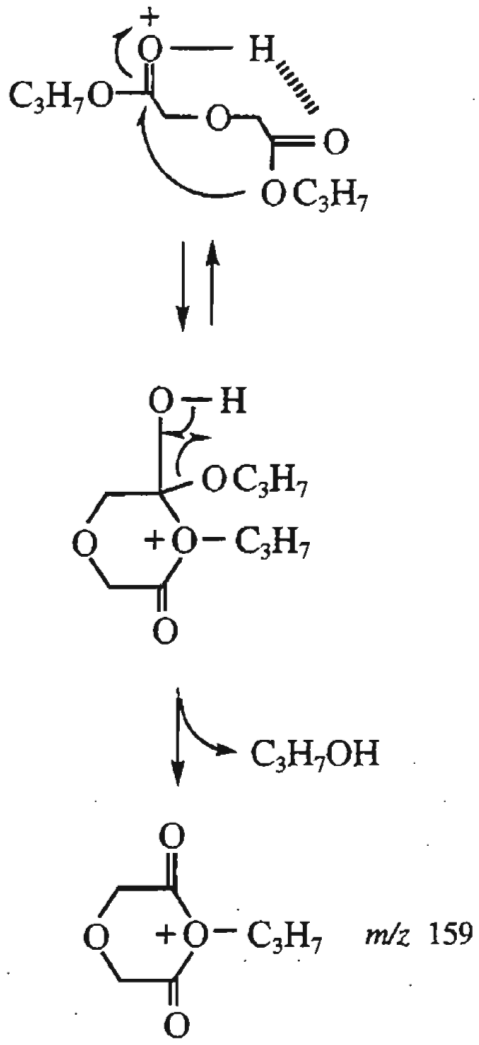


Figure 3.10 The CI (methane) mass spectra of propylated (a) diethylene glycol-, (b) tetraethylene glycol- and (c) pentaethylene glycol-dicarboxylates.



Scheme 3.3



Scheme 3.4

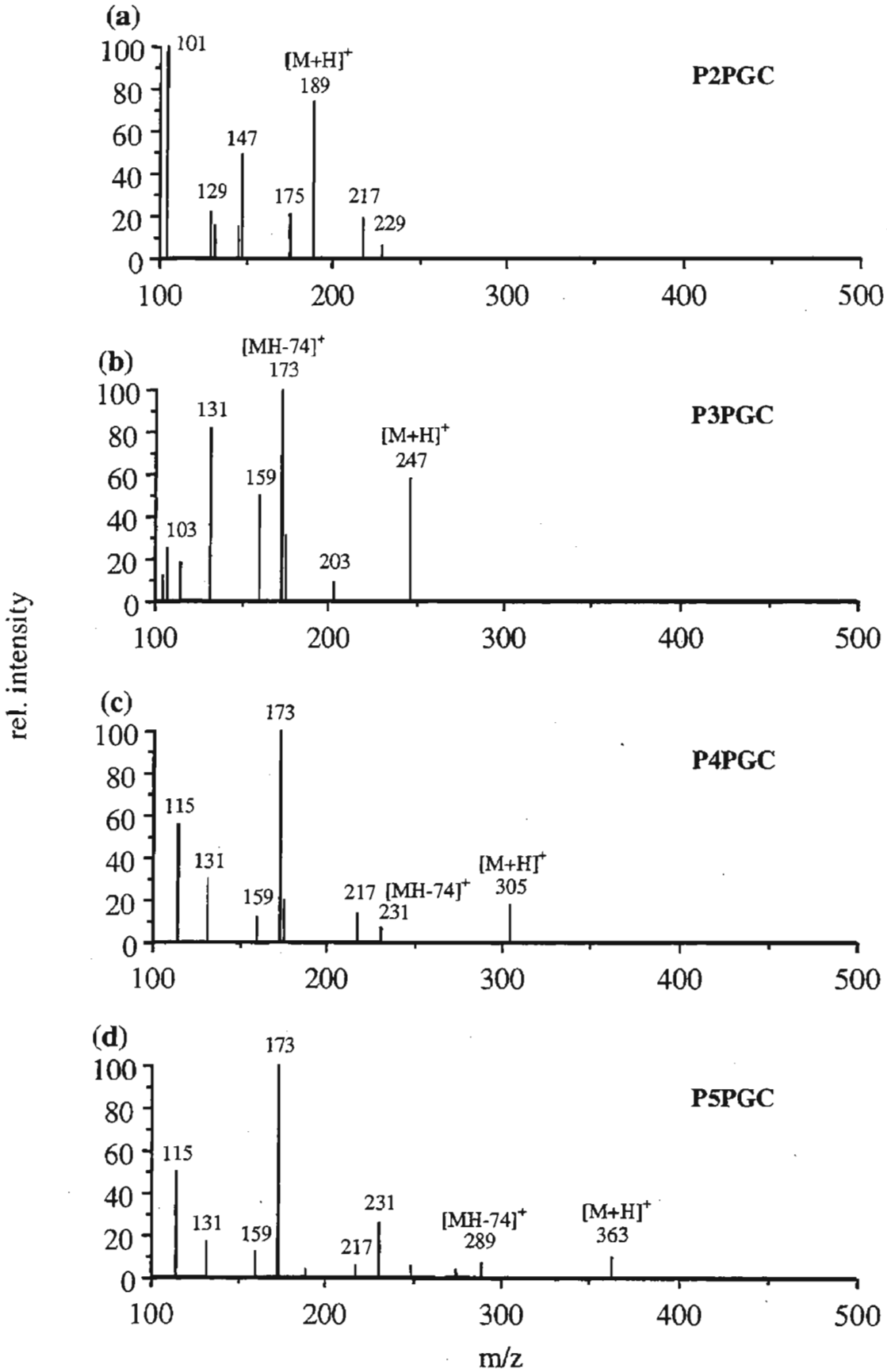
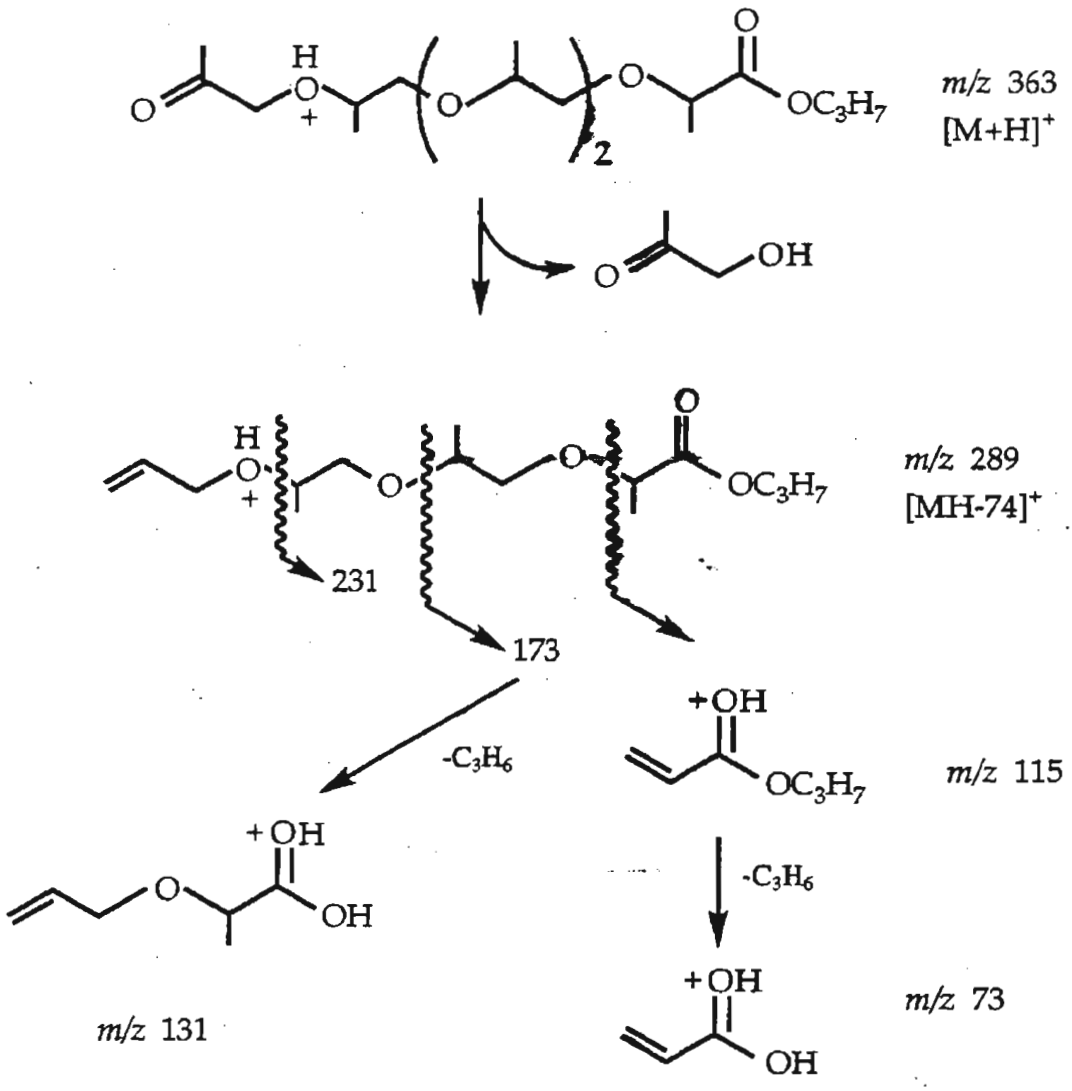
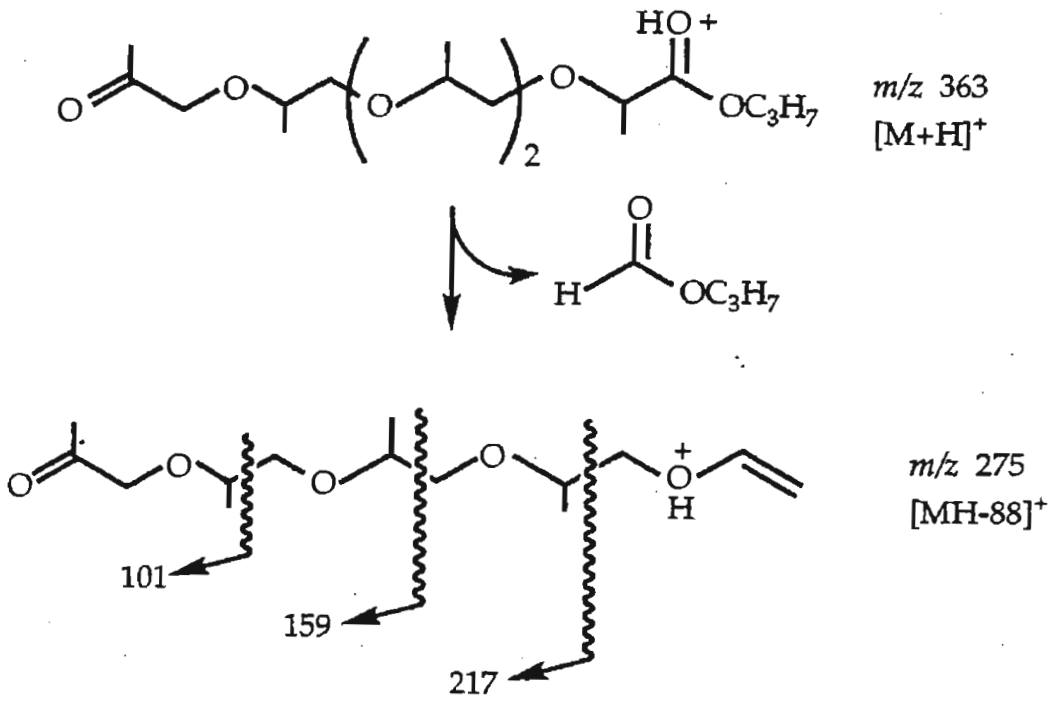


Figure 3.11 The CI (methane) mass Spectra of propylated (a) dipropylene glycol-, (b) tripropylene glycol-, (c) tetrapropylene glycol- and (d) pentapropylene glycol-carboxylates.



Scheme 3.5



Scheme 3.6

Table 3.3. CH_4 CI Mass Spectra of Propylated P*n*PGC:
$[O{=}C(CH_3)CH_2\text{-}O\text{-}(CH(CH_3)CH_2O)_{n\text{-}2}\text{-}CH_2COOC_3H_7,\ n = 2 - 5]$

Ions	n = 2 @ [a]	n = 3 [a]	n = 4 [a]	n = 5 [a]
$[M]^+$	188(16)	246(4)	304(2)	--
$[M+H]^+$	189(74)	247(58)	305(18)	363(10)
Fragmentation I (Scheme 3.5)				
$[MH-74]^+$	115(4)	173(100)	231(7)	289(7)
m/z 231	--	--	--	(26)
m/z 173	--	--	(100)	(100)
m/z 131	--	(82)	(30)	(17)
m/z 115	--	(18)	(56)	(50)
Fragmentation II (Scheme 3.6)				
$[MH-88]^+$	101(100)	159(50)	217(14)	275(4)
m/z 217	--	--	--	(6)
m/z 159	--	--	(12)	(12)
m/z 101	--	(12)	(4)	(8)

@ *m/z* 147(49), m/z 129(22), m/z 175(21) and m/z 217(19) were observed.

[a] The number of *n* indicates the number of unaltered ethoxy units plus two terminal $CH_2COOC_3H_7$ groups. In the structure, n-2 is used to explain this alternation.

3.2.5 NTA and EDTA

Ethylenediamine tetraacetic acid (EDTA) is a common chelating agent and phosphate substitute used as a stabilizer in detergent formulations. Nitrilotriacetic acid (NTA), a structurally related compound, is increasingly substituted for EDTA because of its greater biodegradability. Figure 3.12 shows the EI mass spectra of propylated (a) NDA (nitrilodiacetic acid, a degradation product of NTA), (b) NTA and (c) EDTA, respectively. The major ion in EI spectrum, *m/z* 130 for NDA and *m/z* 230 for NTA, corresponds to homolytic cleavage of a propyl formate ion ($[COOC_3H_7]^+$, *m/z* 87) at the α-position. The ion *m/z* 230 of EDTA may result from the homolytic cleavage of the center of carbon-carbon bond

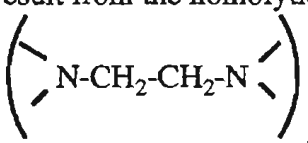


Figure 3.13 shows the corresponding CI mass spectra. The CI spectra show the protonated molecular ions $[M+1]^+$ and the ions $[MH-88]^+$, produced by the neutral loss of propyl formate ($HCOOC_3H_7$). The relatively simple CI mass spectrum of EDTA was observed when *i*-butane, a more softer ionization gas, was used.

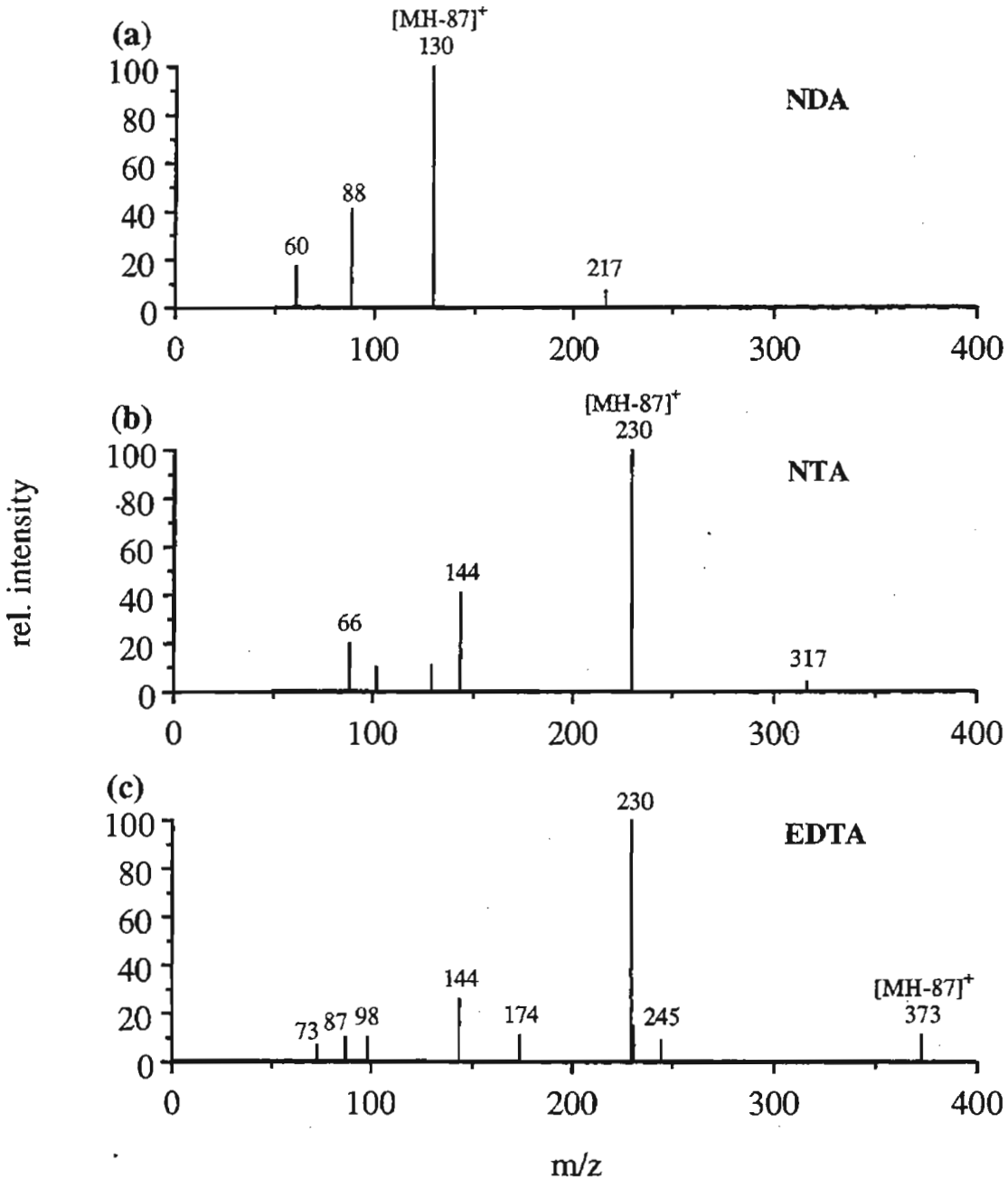


Figure 3.12 The EI mass spectra of propylated (a) NDA, (b) NTA and (c) EDTA.

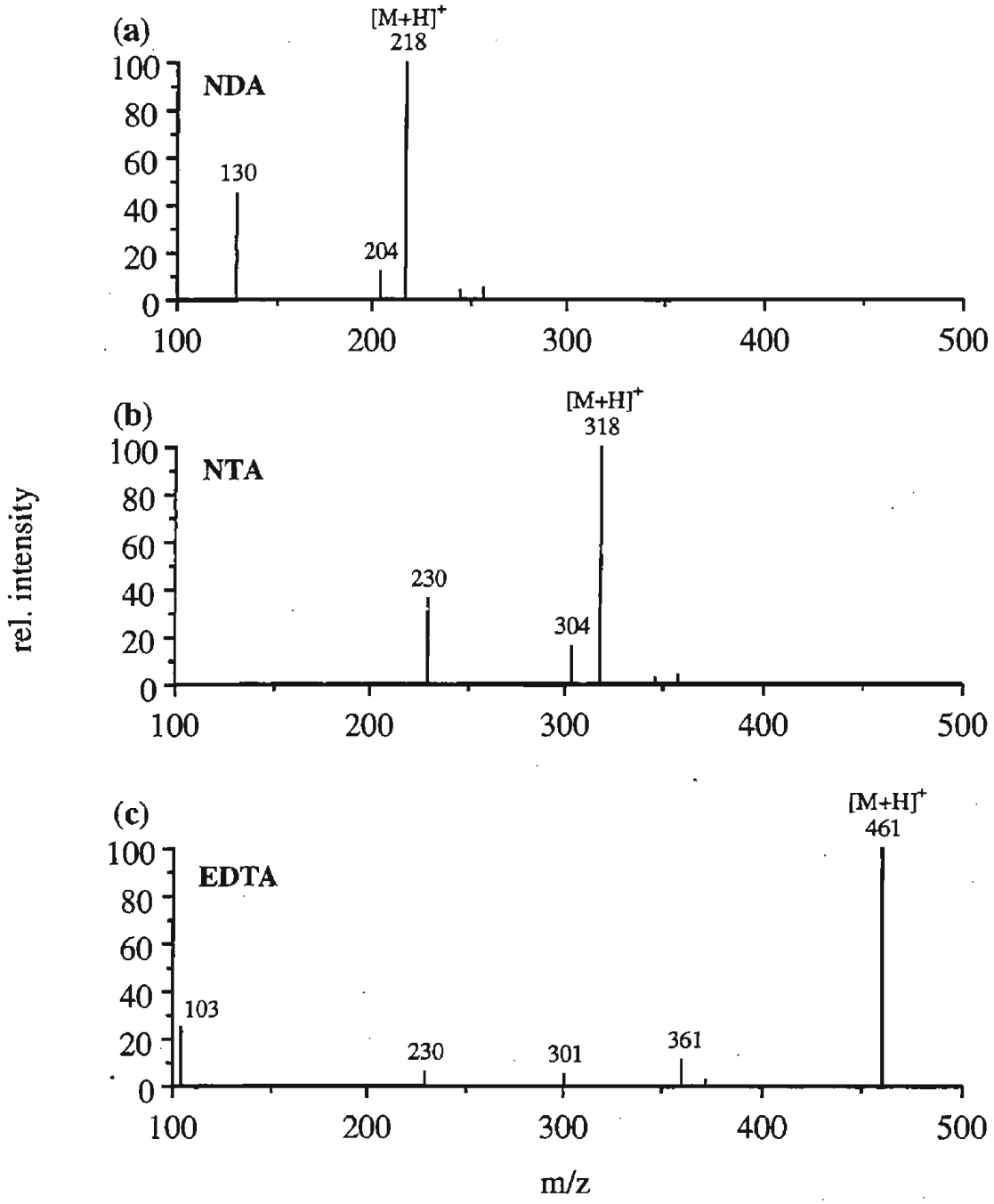


Figure 3.13 The CI (methane) mass spectra of propylated (a) NDA, (b) NTA and (c) EDTA (*i*-butane as reagent gas)

3.3 Analyses of Water Samples

3.3.1 Samples Q8 and Q9

Figures 3.14 and 3.15 show the total ion chromatograms obtained by GC/EI-MS analysis of the propylated Q8 and Q9 effluents collected on December 9, 1993, respectively. Tables 3.4 and 3.5 list the compounds that were positively or tentatively identified along with the molecular formulas, postulated molecular weights and estimated concentrations (μg/L) in Q8 and Q9, respectively. The individual EI mass spectra of the Q8 and Q9 effluents are shown in Appendices A and B, respectively.

Carboxylic acids and carbonyl compounds dominate the positively and tentatively identified compounds in these two effluents. Ethylenediamine tetraacetic acid (EDTA), a common chelating agent and phosphate substitute used as a stabilizer in detergent formulations, is by far the most prominent compound detected in the samples. Nitrilotriacetic acid (NTA), a structurally related compound that is increasingly substituted for EDTA because of its greater biodegradability, was also present in the samples along with its degradation product nitrilodiacetic acid (NDA). The concentrations of these two compounds were significantly lower than the EDTA concentrations. Their EI and CI mass spectra are shown in Figures 3.12 and 3.13, respectively. A detailed mass spectral interpretation has been described in Section 3.2.5.

A group of alkylcarboxylated residues of alkylphenoxy ethoxy carboxylates (APEC), referred to here as carboxyalkylphenoxy ethoxy carboxylates (CAPEC), were detected in sample Q8 (peak numbers 32, 33, 35-40 and 42, 43 in Table 3.4). Figure 3.16 shows representative EI and CI (*i*-butane) mass spectra of dipropylated carboxyalkylphenoxy acetic acid [(a) and (b)]. Figure 3.17 shows representative EI and CI (*i*-butane) mass spectra of dipropylated carboxyalkyl-phenoxy ethoxy acetic acid [(a) and (b)]. The corresponding brominated CAPEC residues were detected in Q9 effluent (peak numbers 36, 39, 40, and 44 in Table 3.5). These

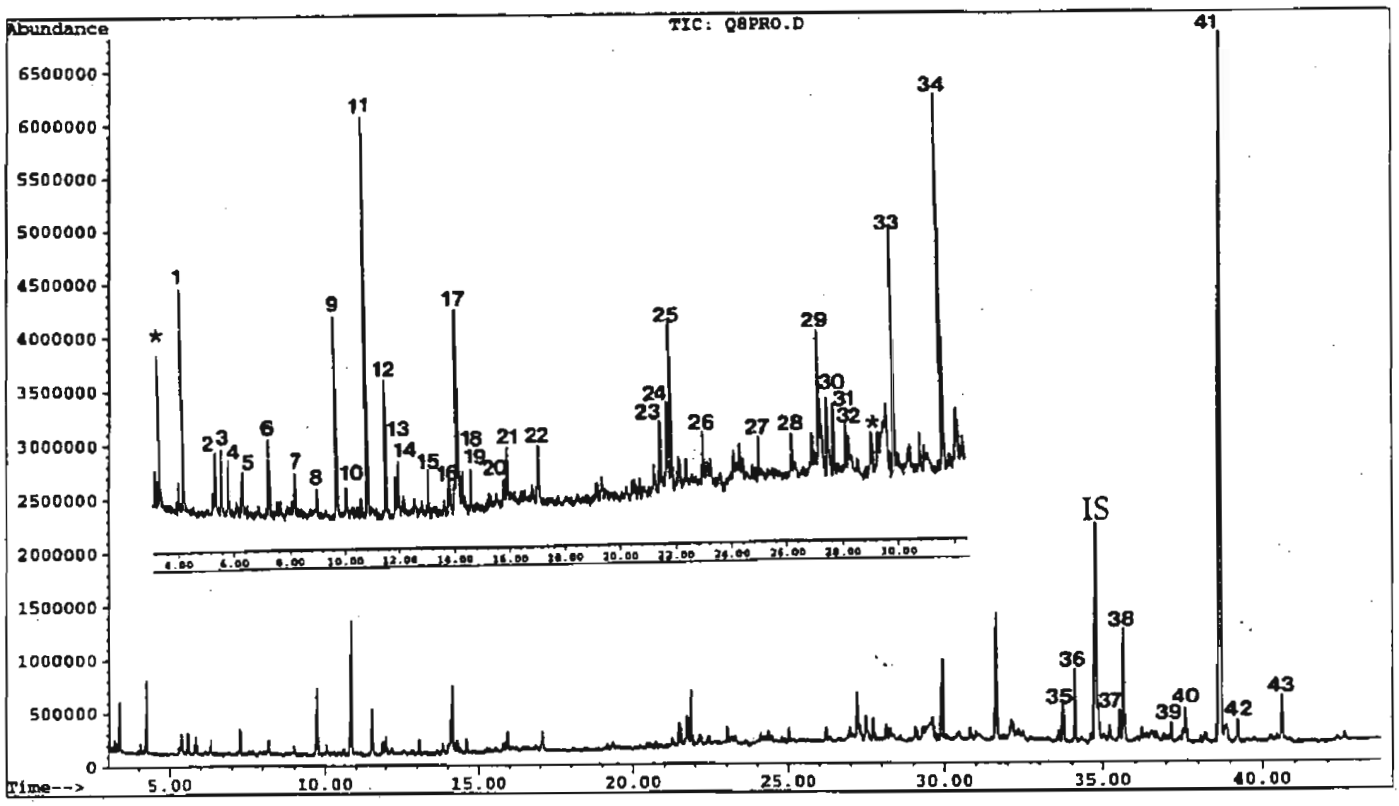


Figure 3.14 GC/EI-MS total ion chromatogram from the propylated Q8 effluent (sample collected on 12/9/93). Peak numbers refer to Table 3.4. Peaks that were found in the method blank are labeled with asterisks. "IS" indicates the internal standard.

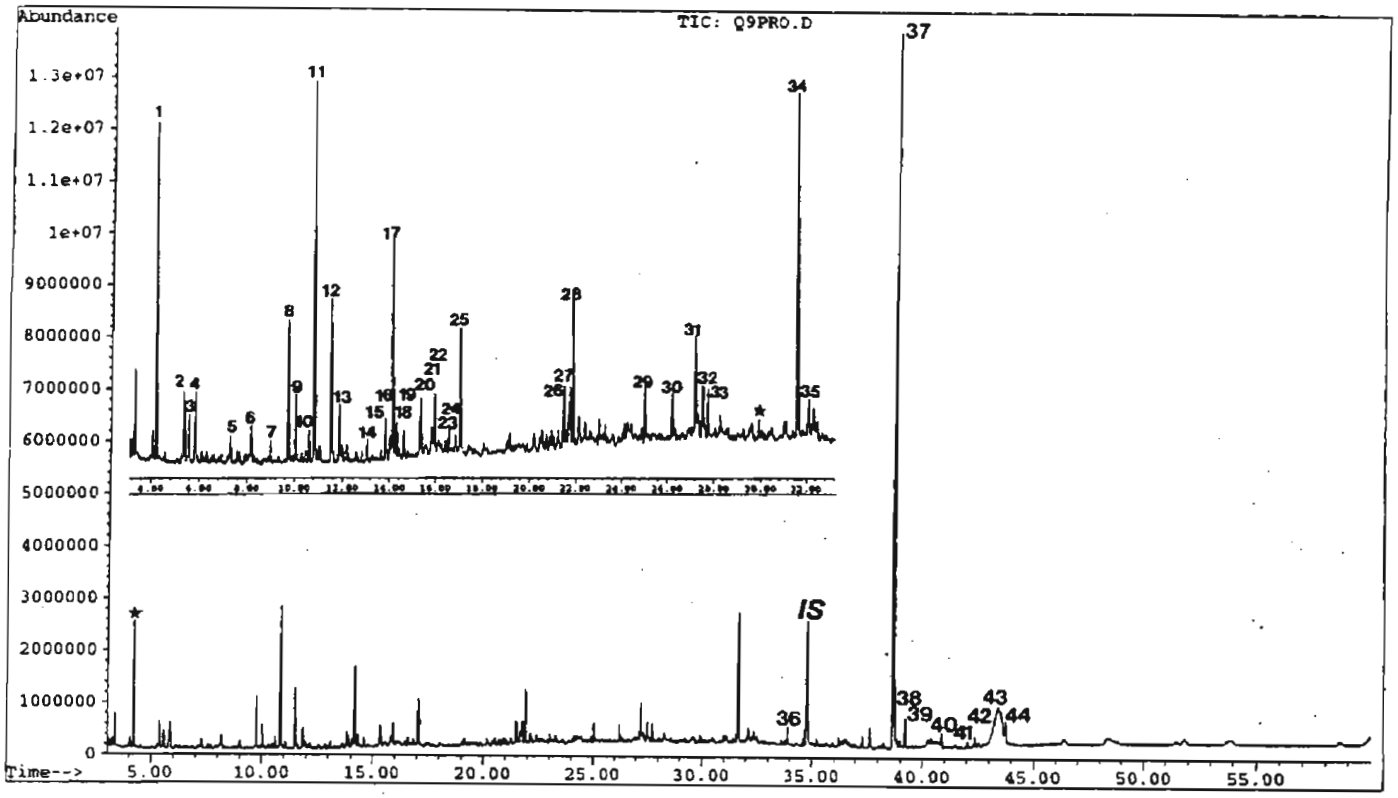


Figure 3.15 GC/EI-MS total ion chromatogram from the propylated Q9 effluent (sample collected on 12/9/93). Peak numbers refer to Table 3.5. Peaks that were found in the method blank are labeled with asterisks. "IS" indicates the internal standard.

Table 3.4 Specific Compounds in Q8 Effluent

Peak	Retention Time (min)	Major ions (m/z)[a]	MW[b]	Estimated Conc. (µg/L)	Positive and Tentative Identifications
1	4.22	89, 131	174	2.8	CH_3-CO-CH(OC_3H_7)$_2$, (Methyl glyoxal)
2	5.36	89, 93,131, 135	N	0.7	Aldehyde
3	5.57	74, 99, 143	N	1.4	
4	5.82	59, 89, 133	174	0.6	
5	6.33	91,119,134	135	0.9	N,N,3-Trimethylbenzeneamine[d]
6	7.27	89,131,135/137,179/181	N	0.8	Aldehyde
7	8.18	87, 105, 129	188	0.8	
8	9.00	168	N	0.5	
9	9.72	88, 89, 130, 131	173	4.8	
10	10.05	109, 139, 168, 203	168	0.5	CH_3-(C_4H_2O)-$COOC_3H_7$, (Methyl furylic acid)
11	10.84	89, 131	218	7.7	$C_2H_5OCH_2CH_2CH_2$-CH(OC_3H_7)$_2$, Ethoxy-butyraldehyde
12	11.51	101, 143	202	5.1	Butanedioic acid, dipropyl ester[d]
13	11.88	117, 141, 159	200	0.7	
14	12.00	99,141,183	N	1.6	
15	13.06	89, 131, 173	172	1.1	C_3H_5-CH(OC_3H_7)$_2$
16	13.84	89, 131, 159	218	0.8	
17	14.13	89, 131	262	4.4	(C_3H_7O)$_2$CH-CH(OC_3H_7)$_2$, (Glyoxal)
18	14.30	115, 157	N	0.7	
19	14.60	89, 131	N	0.8	(C_3H_7O)$_2$CH-$CH_2CH_2COOC_3H_7$, (4-oxobutanoic acid)
20	15.81	59,88, 130	217	0.7	Nitrilodiacetic acid (NDA), dipropyl ester[d]
21	15.94	85, 89, 103, 131, 145, 187	274	0.8	Aldehyde-ethoxycarboxylic acid
22	17.06	111,129, 171	230	0.6	Hexanedioic acid, dipropyl ester[d]
23	21.46	103, 117, 145, 159, 203	276	1.6	Diethoxypropoxy dicarboxylic acid, dipropyl ester
24	21.73	103, 145, 159, 203, 246	276	0.9	Diethoxypropoxy dicarboxylic acid, dipropyl ester
25	21.86	103, 145, 159, 189	276	3.4	C_3H_7OOC-CH_2O-C_3H_6O-CH_2-$COOC_3H_7$
26	23.00	141,183,225	N	0.5	
27	25.03	129, 171, 231	N	0.6	
28	26.21	144, 230, 317	317	0.9	Nitrilotriacetic acid (NTA), tripropyl ester[d]
29	27.20	103, 117, 145, 159, 203, 247	334	1.5	C_3H_7OOC-CH_2O-(C_3H_6O)$_2$-CH_2-$COOC_3H_7$
30	27.49	103,117, 159, 203	334	1.2	Diethoxydipropoxy dicarboxylic acid, dipropyl ester
31	27.71	103,117, 159, 203	334	1.0	Diethoxydipropoxy dicarboxylic acid, dipropyl ester
32	28.14	193,235	322	1.4	C_3H_7OOC-(C_3H_6)-C_6H_4-OCH_2-$COOC_3H_7$
33	29.92	193,235	322	8.3	C_3H_7OOC-(C_3H_6)-C_6H_4-OCH_2-$COOC_3H_7$
34	31.61	185, 213, 300	300	7.8	Naphthalene dicarboxylic acid, dipropyl ester[d]
35	33.73	115,147,195,219 249,321,350	350	1.6	C_3H_7OOC-CH_2-(C_4H_8)-C_6H_4-OCH_2-$COOC_3H_7$

36	34.11	193,235,305	364	6.7	C_3H_7OOC-(C_3H_6)-(C_3H_6)-C_6H_4-OCH_2-$COOC_3H_7$
37	35.53	103,145,279	366	1.7	C_3H_7OOC-(C_3H_6)-C_6H_4-OC_2H_4-OCH_2-$COOC_3H_7$
38	35.66	193,235	364	11	C_3H_7OOC-(C_3H_6)-(C_3H_6)-C_6H_4-OCH_2-$COOC_3H_7$
39	37.13	115,147,195,219 249,321,350	392	1.0	C_3H_7OOC-(C_4H_8)-(C_4H_8)-C_6H_4-OCH_2-$COOC_3H_7$
40	37.60	115,147,195,219 249,321,350	392	1.5	C_3H_7OOC-(C_4H_8)-(C_4H_8)-C_6H_4-OCH_2-$COOC_3H_7$
41	38.721	144, 230, 373	460	290*c*	Ethylenediamine tetraacetic acid (EDTA), tetrapropyl ester*d*
42	39.22	103, 145, 249,279	408	0.9	C_3H_7OOC-(C_3H_6)-(C_3H_6)-C_6H_4-OC_2H_4-OCH_2-$COOC_3H_7$
43	40.61	103,145,279	408	2.0	C_3H_7OOC-(C_3H_6)-(C_3H_6)-C_6H_4-OC_2H_4-OCH_2-$COOC_3H_7$

a Base ion is underlined.
b Molecular weight calculated for the propylated derivative, obtained from *i*-butane CI mass spectrum.
c Quantified using external standard calibration.
d Compound was positively identified.
N: Not identified.

Table 3.5. Specific Compounds in Q9 Effluent

Peak	Retention Time (min)	Major ions (m/z)[a]	MW[b]	Estimated Conc. (µg/L)	Positive and Tentative Identifications
1	4.24	89, 131	174	9.6	CH_3-CO-$CH(OC_3H_7)_2$, (Methyl glyoxal)
2	5.38	89, 93,131, 135	N	2.0	Aldehyde
3	5.59	74, 99, 143	N	2.0	
4	5.87	59, 83, 133	174	1.9	
5	7.27	89, 131	N	0.1	Aldehyde
6	8.18	87, 105, 129	188	1.4	
7	9.00	168	N	0.8	
8	9.75	88, 89, 130, 131	173	7.0	
9	10.05	109, 139, 168, 203	168	2.9	CH_3-(C_4H_2O)-$COOC_3H_7$, (Methyl furylic acid)
10	10.60	76, 117	N	3.0	
11	10.84	89, 131	218	18	$C_2H_5OCH_2CH_2CH_2$-$CH(OC_3H_7)_2$, Ethoxy-butyraldehyde
12	11.51	101, 143	202	13	Butanedioic acid, dipropyl ester[d]
13	11.88	117, 141, 159	200	2.0	
14	13.07	89, 131, 173	172	1.0	C_3H_5-$CH(OC_3H_7)_2$
15	13.84	89, 131, 159	218	2.4	
16	14.03	73, 117, 135, 159, 177	246	0.6	
17	14.15	89, 131	262	11	$(C_3H_7O)_2CH$-$CH(OC_3H_7)_2$, (Glyoxal)

18	14.31	115, 157	216	1.5	
19	14.61	89, 131	246	1.4	$(C_3H_7O)_2CH\text{-}CH_2CH_2COOC_3H_7$, (4-oxobutanoic acid)
20	15.33	91, 105, 118, 133, 163, 180	N	1.0	Benzylic compound
21	15.81	88, 130	217	1.8	Nitrilodiacetic acid (NDA), dipropyl ester[d]
22	15.94	85, 89, 103, 131, 145, 187	274	1.3	Aldehyde-ethoxycarboxylic acid
23	16.57	101, 143, 171	N	2.4	Dicarboxylic acid, dipropyl ester
24	16.83	85, 131, 145, 173	N	1.0	Aldehyde-ethoxycarboxylic acid
25	17.07	111,129, 171	230	2.9	Hexanedioic acid, dipropyl ester[d]
26	21.48	103, 117, 145, 159, 203	276	2.7	Diethoxypropoxy dicarboxylic acid, dipropyl ester
27	21.74	103, 145, 159, 203, 246	276	1.4	Diethoxypropoxy dicarboxylic acid, dipropyl ester
28	21.89	103, 145, 159, 189	276	5.9	$C_3H_7OOC\text{-}CH_2O\text{-}C_3H_6O\text{-}CH_2\text{-}COOC_3H_7$
29	25.02	129, 171, 231	N	2.3	
30	26.22	144, 230, 317	317	2.0	Nitrilotriacetic acid (NTA), tripropyl ester[d]
31	27.22	103, 117, 145, 159, 203, 247	334	2.7	$C_3H_7OOC\text{-}CH_2O\text{-}(C_3H_6O)_2\text{-}CH_2\text{-}COOC_3H_7$
32	27.50	117, 159, 203	334	1.8	Diethoxydipropoxy dicarboxylic acid, dipropyl ester
33	27.73	117, 159, 203	334	1.5	Diethoxydipropoxy dicarboxylic acid, dipropyl ester
34	31.64	185, 213, 300	300	16	Naphthalene dicarboxylic acid, dipropyl ester[d]
35	32.12	117, 145, 159, 203	N	1.6	Poly(ethoxy)poly(propoxy) dicarboxylic acid
36	33.87	213, 215, 271, 273, 313, 315	400	0.3	$C_3H_7OOC\text{-}(C_3H_6)\text{-}C_6H_3Br\text{-}OCH_2\text{-}COOC_3H_7$
37	38.72	144, 230, 373	460	390[c]	Ethylenediamine tetraacetic acid (EDTA), tetrapropyl acid[d]
38	38.99	103, 145, 215	N	0.9	Ethoxycarboxylic acid
39	39.22	213, 215, 313, 315	422	2.0	$C_3H_7OOC\text{-}(C_3H_6)\text{-}(C_3H_6)\text{-}C_6H_3Br\text{-}OCH_2\text{-}COOC_3H_7$
40	40.86	103, 145, 327, 329	422	0.8	$C_3H_7OOC\text{-}(C_3H_6)\text{-}(C_3H_6)\text{-}C_6H_3Br\text{-}OCH_2\text{-}COOC_3H_7$
41	42.03	103, 145	N	0.6	Ethoxycarboxylic acid
42	42.34	103, 145, 213	N	0.9	Ethoxycarboxylic acid
43	43.39	103, 145, 189	N	10	Ethoxycarboxylic acid
44	43.74	103, 145, 213, 215, 357, 359	486	2.0	$C_3H_7OOC\text{-}(C_3H_6)\text{-}(C_3H_6)\text{-}C_6H_3Br\text{-}OC_2H_4\text{-}OCH_2\text{-}COOC_3H_7$

[a] Base ion is underlined.

[b] Molecular weight calculated for the propylated derivative, obtained from *i*-butane CI mass spectrum.

[c] Quantified using external standard calibration.

[d] Compound was positively identified.

N: Not identified.

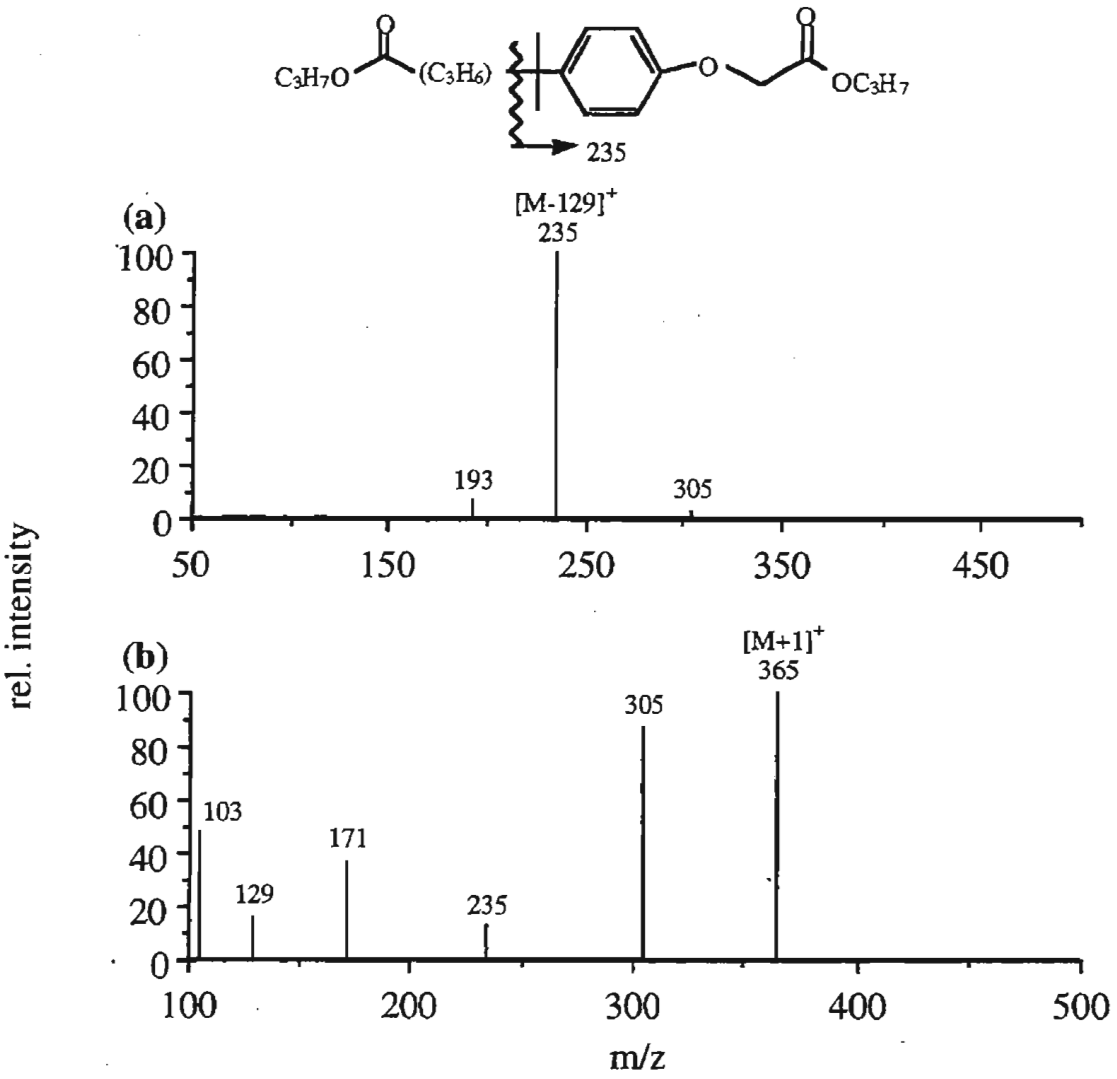


Figure 3.16 (a) EI and (b) CI (*i*-butane) mass spectra of dipropylated carboxyalkyl-phenoxy acetic acid from Q8 effluent.

were probably formed during chlorination of Q8 at WF21. The preferential formation of brominated over chlorinated byproducts during water chlorination has been well documented (Reinhard and Goodman 1982; Stephanou 1985; Voudrias and Reinhard 1988). The selected ion mass chromatograms (in the EI mode) of brominated CAPEC's with characteristic ion pairs *m/z* 313/315, 327/329 and 357/359 from propylated Q9 effluent are shown in Figure 3.18. Typical

EI and CI (*i*-butane) mass spectra of dipropylated brominated carboxyalkylphenoxy acetic acid from Q9 effluent are shown in Figure 3.19.

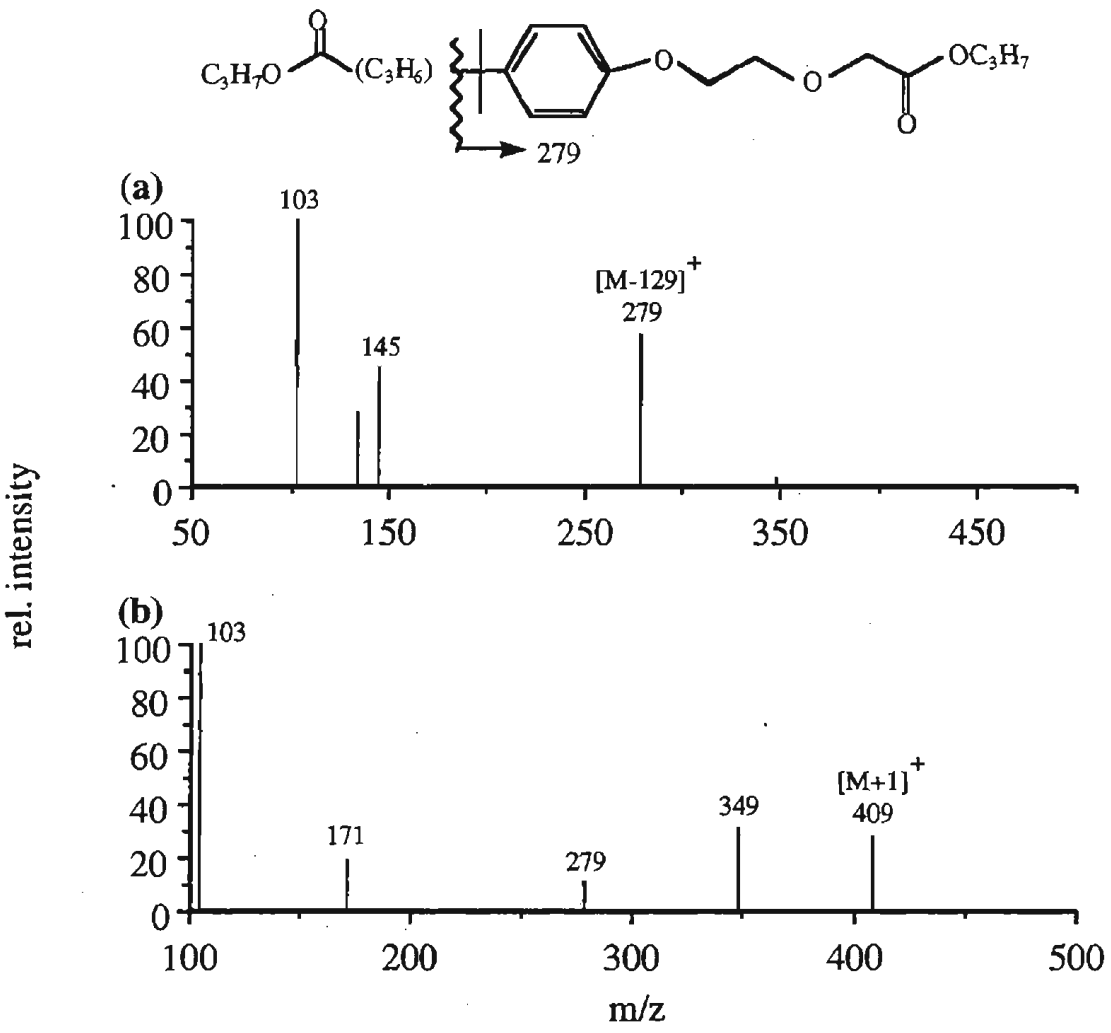


Figure 3.17. (a) EI and (b) CI (*i*-butane) mass spectra of dipropylated carboxyalkylphenoxy ethoxy acetic acid from Q8 effluent.

The most significant ions in EI spectra are produced by benzylic cleavage $[M-129]^+$. This characteristic cleavage has been observed for most EI mass spectra of APEC and halogenated APEC compounds (Reinhard and Goodman 1982; Stephanou 1985; Stephanou *et al.* 1988). The CI spectra show strong protonated molecular ions $[M+H]^+$ and the ions $[MH-60]^+$,

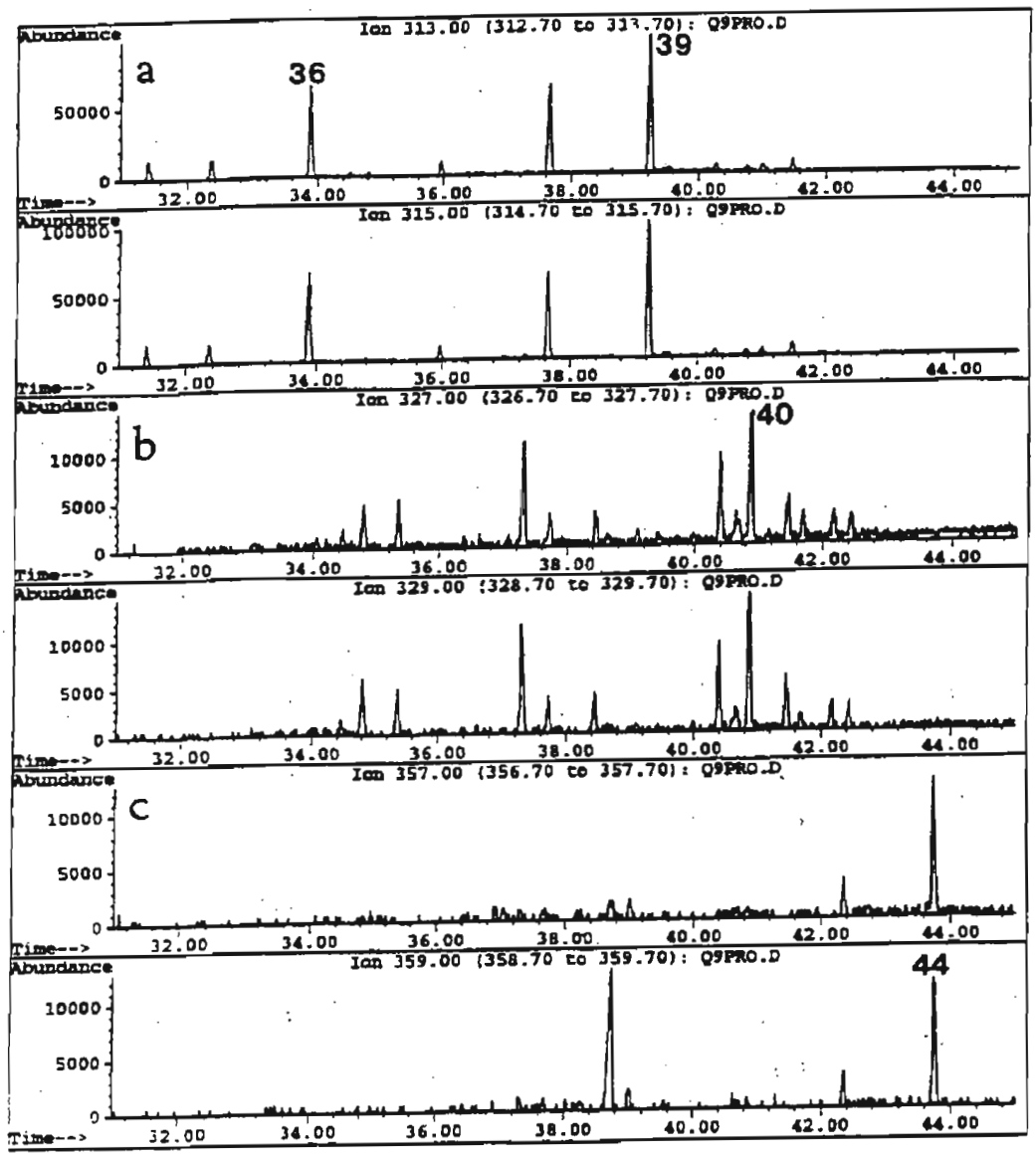


Figure 3.18 Selected ion mass chromatograms (in the EI mode) of propylated brominated-CAPEC's with characteristic ion pairs (a) *m/z* 313/315, (b) *m/z* 327/329 and (c) *m/z* 357/359 from Q9 effluent (sample collected on 12/9/93). Peaks numbers refer to Table 3.5.

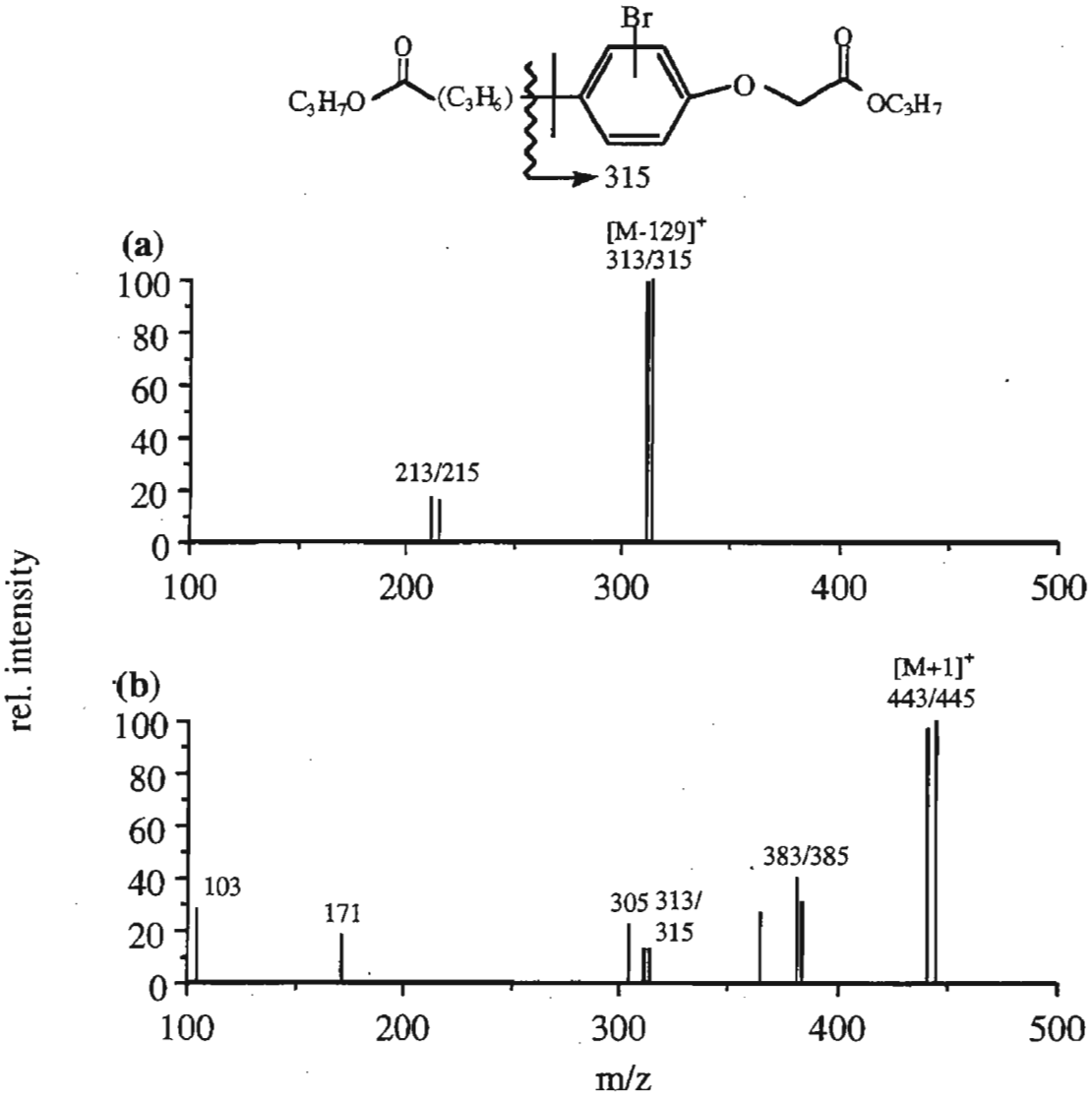


Figure 3.19. (a) EI and (b) CI (*i*-butane) mass spectra of dipropylated brominated carboxyalkylphenoxy acetic acid from Q9 effluent.

attributed to the loss of propanol (HOC_3H_7). The benzylic cleavage was also observed in all of the CI spectra of the CAPEC's, but at lower intensity than in the EI spectra. The ions at *m/z* 171 (as shown in Figures 3.16, 3.17, and 3.19), were tentatively assigned to $[C_3H_7COO\text{-}C_3H_6\text{-}C_3H_6]^+$, representing the carboxyalkyl ion displacement from the phenoxy ring. Cleavage at the same point was observed in the CI mass spectra of OPEC and halogenated OPEC.

Carboxylation of the alkyl side chain of APEC was determined by comparison of methylated and propylated extracts of the same samples under the same analytical conditions (Ding, *et al.* 1994B). Figure 3.20 shows the CI (*i*-butane) mass spectra of the dimethyl/dipropyl

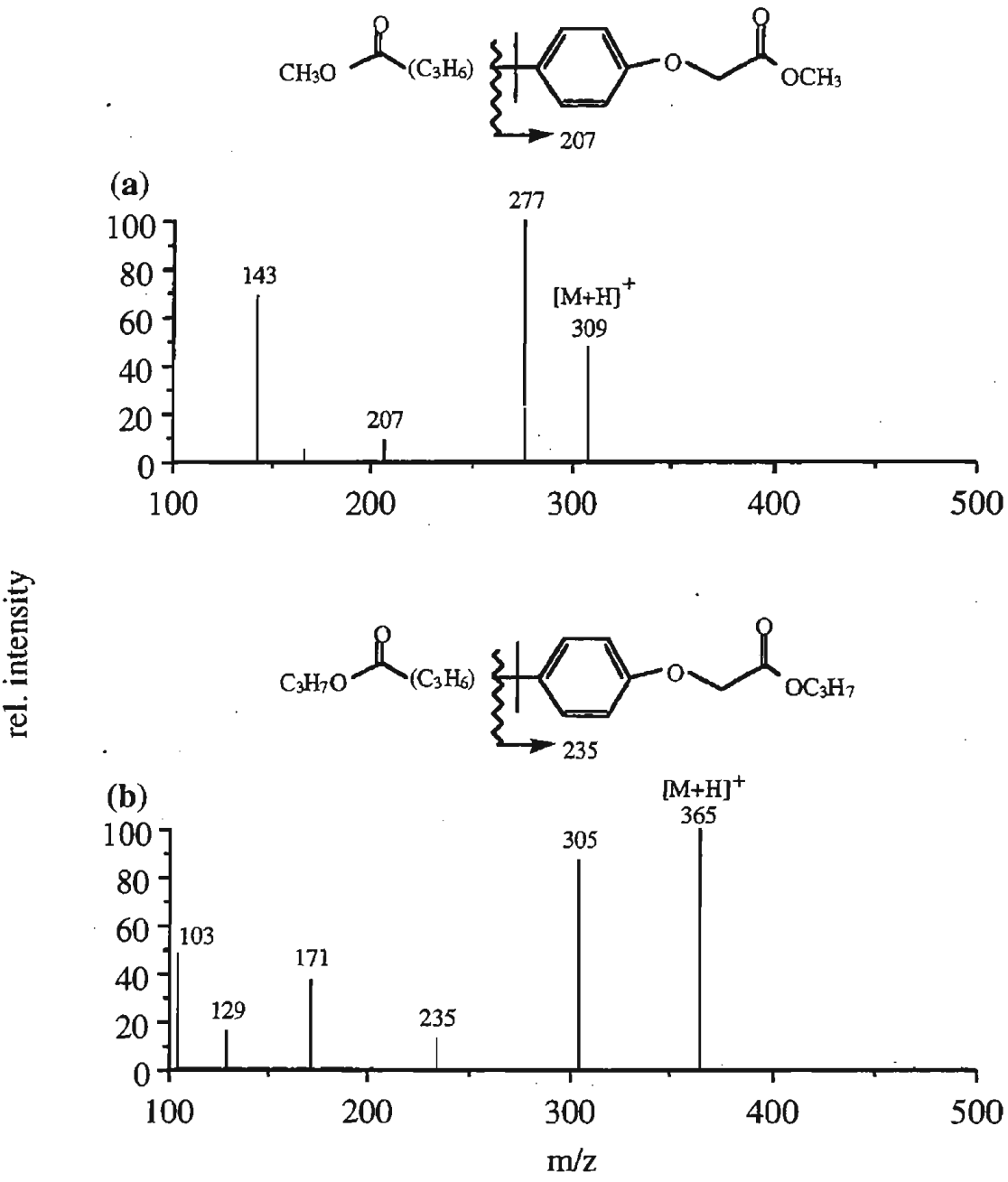


Figure 3.20. CI (i-butane) mass spectra of carboxyalkylphenoxy acetic acid (a) dimethyl- and (b) dipropyl-esters.

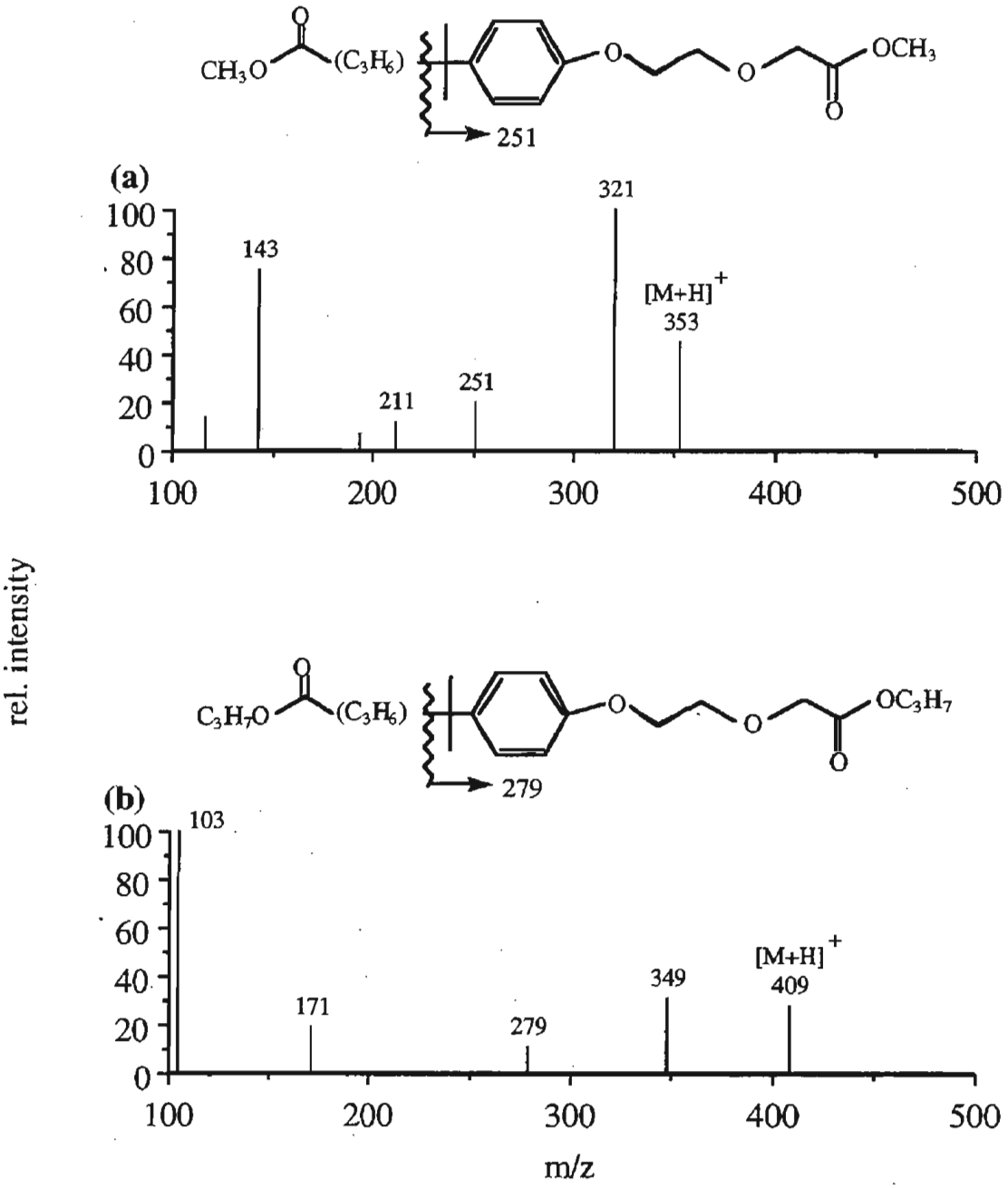


Figure 3.21. CI (i-butane) mass spectra of carboxyalkylphenoxy ethoxy acetic acid (a) dimethyl- and (b) dipropyl-esters.

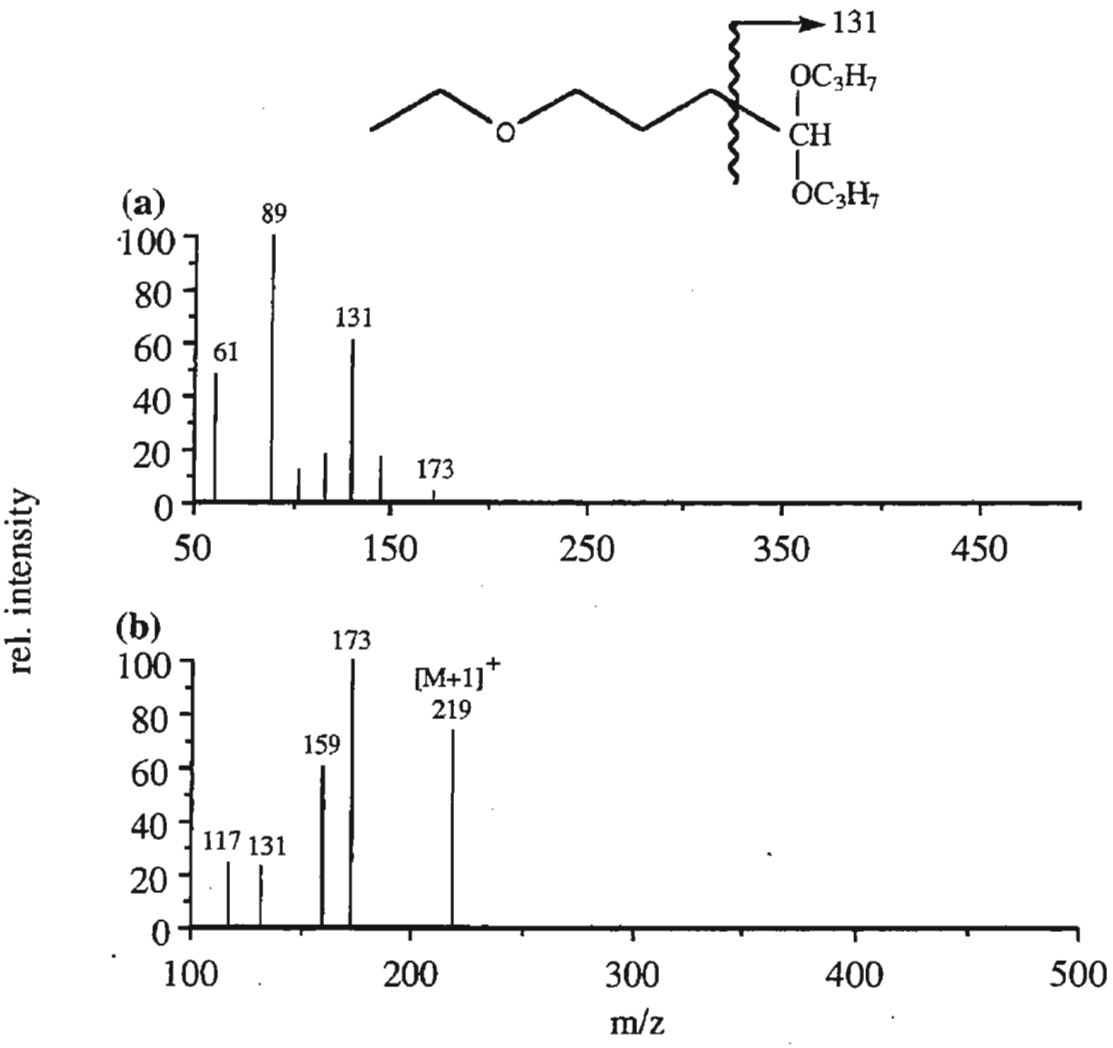


Figure 3.22. (a) EI and (b) CI (*i*-butane) mass spectra of ethoxy butyraldehyde dipropyl acetal from propylated Q8 effluent.

esters of carboxyalkylphenoxy acetic acid. Figure 3.21 shows the CI (i-butane) mass spectra of dimethyl/dipropyl esters of carboxyalkylphenoxy ethoxy acetic acid. The mass difference of the molecular ions in the CI mass spectra was 56 amu, which represents the difference between dipropylated (two $-COOC_3H_7$) and dimethylated (two $-COOCH_3$) CAPEC's. However, the difference between the methylated and propylated base ions in the EI mass spectra was 28 amu,

which represents the difference between the two benzylic cleavage products $[C_3H_6\text{-}C_6H_4\text{-}OCH_2COOC_3H_7]^+$ and $[C_3H_6\text{-}C_6H_4\text{-}OCH_2COOCH_3]^+$. Therefore we concluded that the

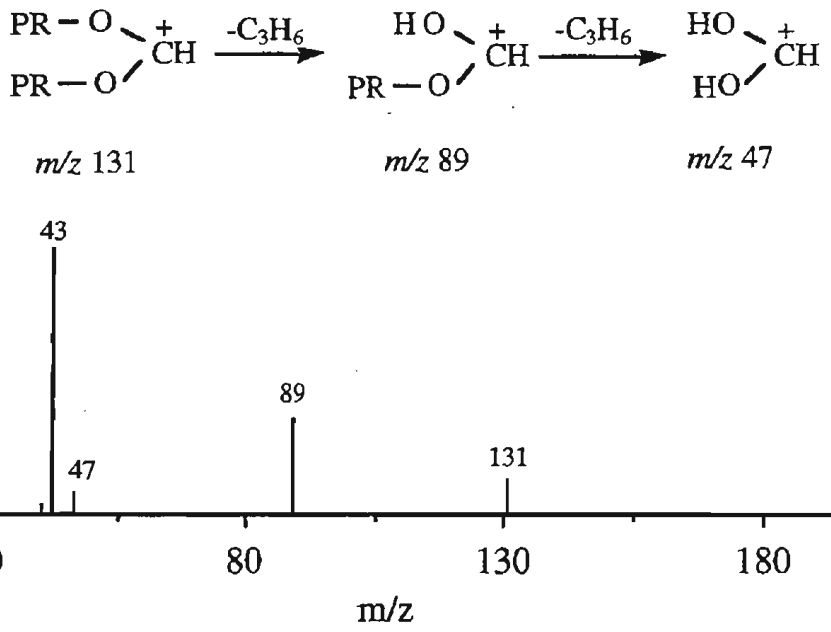


Figure 3.23. The product-ion mass spectrum of *m/z* 131 from ethoxy butyraldehyde dipropyl acetal in propylated Q8 effluent.

second methyl/propyl ester group must be attached to the alkyl chain side of the molecular. The carboxylation of the alkyl chains of APEO was postulated by Schöberl based on elemental analysis (Schöberl *et al.* 1981). Complete structural assignments of the carboxylated alkyl group could not be made because of the complex isomeric structures of the original nonyl side chains.

Many of the detected compounds in Q8 and Q9 effluents appear to be residues of non-aromatic poly(ethoxy), poly(propoxy) and poly(ethoxy)(propoxy) type compounds. These types of residues were also found in surface water by ^1H and ^{13}C NMR spectrometry analysis (Leenheer *et al.* 1991). They may derive from biodegradation of linear alcohol polyethoxylates (Steber and Wierich 1985), or block copolymers of polyethylene glycol and polypropylene

glycol, both of which are, like the APE, common nonionic surfactant classes. Some may also be derived from ether type solvents. Small aliphatic dicarboxylic acids are also present in Q8 and Q9. Oxidation (Liao *et al.* 1982) or chlorination (de Leer *et al.* 1985) of aquatic humic material is a possible origin for some of these compounds.

A group of aldehydes were detected as dipropylated acetal derivatives. The EI and CI (*i*-butane) mass spectra of tentatively identified ethoxy butyraldehyde dipropyl acetal (peak number 11 in Table 3.4) from sample Q8 are shown in Figure 3.22. The ion at *m/z* 173, as a base ion in CI mass spectrum (Figure 3.22(b)), was attributed to the neutral loss of ethanol from the protonated molecular ion. For confirmation of the proposed fragmentation, the product-ion mass spectrum from GC/TSQ-MS (in the EI mode) was studied. Figure 3.23 depicts the product-ion mass spectrum of *m/z* 131 from ethoxy butyraldehyde dipropyl acteal. The *m/z* 131 ion probably represents the dipropoxy acetal portion remaining after loss of the ethoxy propyl group through homolytic α-cleavage at the carbon-carbon bond . The presence of two propoxy groups on the same carbon provides a powerful reaction-initiating site (McLafferty 1980). Stepwise losses of propene (C_3H_6, 42 amu) provide fragment ions of *m/z* 89 and 47 as shown in Figure 3.23. The strong fragment ion *m/z* 43 may originate from propyl ion ($[C_3H_7]^+$). The carbonyl group identification for the aldehydes can be accomplished by monitoring the characteristic ions, such as *m/z* 131, 89 and 47 ions. The aldehydes may derive from industrial wastewater (Le Lacheur *et al.* 1993) or wet precipitation (Kawamura 1993).

3.3.2 Sample Q22B

Figure 3.24 shows the total ion chromatogram obtained by GC/EI-MS analysis of the propylated Q22B effluent collected on December 9, 1993. Table 3.6 lists the compounds that were positively or tentatively identified along with the molecular formulas, postulated molecular weights and estimated concentrations (µg/L) in Q22B. The individual EI mass spectra are shown in Appendix C.

Table 3.6 Specific Compounds in Q22B Effluent

Peak	Retention Time (min)	Major ions (m/z)[a]	MW[b]	Estimated Conc. (µg/L)	Positive and Tentative Identifications
1	9.71	88, 130	173	0.2	
2	11.51	101, 143	202	0.1	Butanedioic acid,dipropyl ester[d]
3	13.82	89, 131	218	0.2	
4	14.13	89, 131	262	0.1	$(C_3H_7O)_2CH\text{-}CH(OC_3H_7)_2$, (Glyoxal)
5	14.59	89, 131	246	0.1	$(C_3H_7O)_2CH\text{-}CH_2CH_2COOC_3H_7$, (4-oxobutanoic acid)
6	15.45	191, 206	206	0.1	2,4-bis(1,1-dimethylethyl)phenol[d]
7	15.94	85, 103, 131, 145, 187	274	0.2	Aldehyde-ethoxycarboxylic acide
8	16.14	104, 133, 151, 175, 193		0.3	
9	17.06	111, 129, 171	230	0.4	Hexanedioic acid, dipropyl ester[d]
10	18.44	89, 131, 171, 201		0.5	Aldehyde
11	19.75	91, 119, 161, 205, 248		0.1	
12	20.52	89, 113, 131, 173, 215	266	0.1	Aldehyde
13	20.60	89, 131, 163, 165, 223, 225, 265, 267		0.1	Brominated aromatic/aldehyde
14	20.88	61, 89, 131,185, 224	272	0.2	Aldehyde
15	21.99	77, 141, 170	213	0.2	N-butylbenzenesulfonamide[d]
16	23.11	89, 131, 181, 199, 233, 258		0.5	Aldehyde
17	24.44	101, 143, 171, 203, 245, 273	272	0.3	
18	25.02	87, 111, 129, 171, 231	272	0.9	
19	26.22	144, 230, 264, 317	317	0.2	Nitrilotriacetic acid (NTA) tripropyl ester[d]
20	27.29	89, 131, 183, 209, 227	312	2.2	Aldehyde
21	28.51	89, 131, 156, 197		0.2	Aldehyde
22	31.93	61, 102, 115, 171, 267, 326	326	0.1	Octadecanoic acid, dipropyl ester[d]

23	32.15	129, 171, 200, 202, 311, 313		0.4	
24	34.10	193, 235, 364	364	0.2	C_3H_7OOC-(C_3H_6)-(C_3H_6)-C_6H_4-OCH_2-$COOC_3H_7$
25	35.48	149, 191		0.3	Phthalate
26	35.66	193, 235	364	0.2	C_3H_7OOC-(C_3H_6)-(C_3H_6)-C_6H_4-OCH_2-$COOC_3H_7$
27	37.64	103, 145, 235, 313, 315, 357, 359		0.1	Brominated alkylphenoxyacetic acid
28	38.64	230, 373, 461	460	4.3[c]	Ethylenediamine tetraacetic acid (EDTA) tetrapropyl acid[d]

[a] Base ion is underlined.
[b] Molecular weight calculated for the propylated derivative, obtained from *i*-butane CI mass spectrum.
[c] Quantified using external standard calibration.
[d] Compound was positively identified.
N: Not identified.

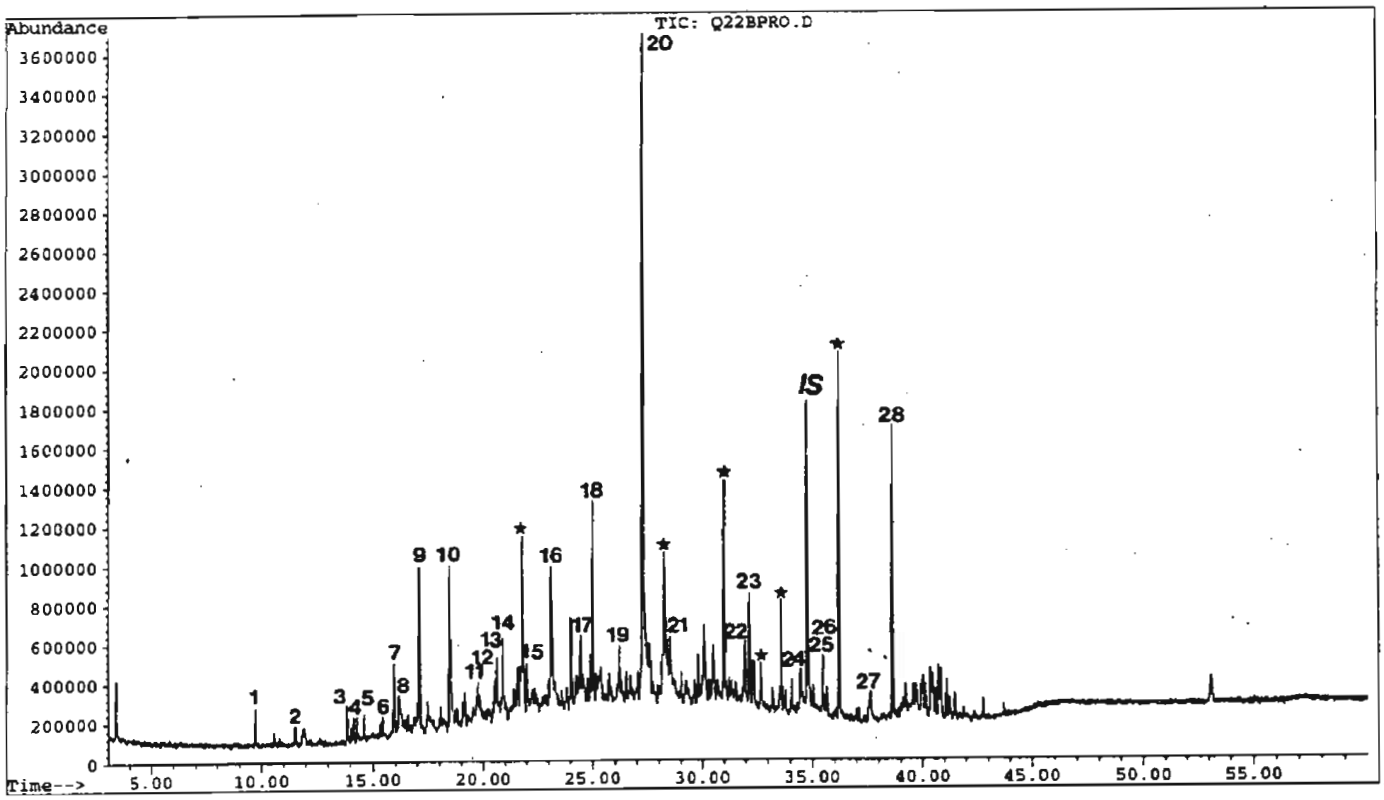


Figure 3.24 GC/EI-MS total ion chromatogram from the propylated Q22B effluent (sample collected on 12/9/93). Peak numbers refer to Table 3.6. Peaks that were found in the method blank are labeled with asterisks. "IS" indicates the internal standard.

3.3.3 Sample M21

Figure 3.25 shows the total ion chromatogram obtained by GC/EI-MS analysis of the propylated M21 water collected on December 13, 1993. Table 3.7 lists the compounds that were positively or tentatively identified along with the molecular formulas, postulated molecular weights and estimated concentrations (μg/L) in M21. The individual EI mass spectra are shown in Appendix D.

Table 3.7 Specific Compounds in M21 Groundwater

Peak	Retention Time (min)	Major ions (m/z)[a]	MW[b]	Estimated Conc. (μg/L)	Positive and Tentative Identifications
1	10.78	89, 131, 173	218	0.3	$C_2H_5OCH_2CH_2CH_2$-$CH(OC_3H_7)_2$, Ethoxy-butyraldehyde
2	11.50	101, 143	202	0.3	Butanedioic acid, dipropyl ester[d]
3	13.80	89, 131, 159	218	0.5	
4	14.11	89, 131, 161	262	0.3	$(C_3H_7O)_2CH$-$CH(OC_3H_7)_2$, (Glyoxal)
5	15.42	57, 191		0.3	2,4-bis(1,1-dimethylethyl)phenol
6	15.79	60, 88, 130, 217	217	0.3	Nitrilodiacetic acid (NDA) dipropyl ester[d]
7	15.90	85, 89, 131, 187	187	0.2	Aldehyde
8	16.53	101, 143, 171	230	0.5	Dicarboxylic acid , dipropyl ester
9	17.05	111, 129, 171	230	3.0	Hexanedioic acid, dipropyl ester[d]
10	17.92	84, 144, 172		0.2	
11	18.42	89, 99, 131, 159, 201, 2256	200	0.2	Aldehyde
12	20.77	89, 113, 131, 173, 215	215	0.4	Aldehyde
13	21.44	103, 145, 159, 203	276	0.8	Diethoxypropoxy dicarboxylic acid, dipropyl ester
14	21.71	103, 145, 149, 159, 203	276	1.0	Diethoxypropoxy dicarboxylic acid, dipropyl ester
15	21.84	103, 145, 159, 199, 234	276	0.6	Ethoxycarboxylic acid
16	23.99	152, 171, 213	272	0.2	
17	24.99	129, 171, 231	242	0.9	
18	26.19	147, 163, 230, 264	317	0.1	Nitrilotriacetic acid (NTA) tripropyl ester[d]
19	26.40	83, 129, 171, 237, 279	292	0.2	
20	26.92	193, 235	278	3.1	Alkylphenoxyacetic acid
21	27.18	103, 145, 159	334	0.3	C_3H_7OOC-CH_2O-$(C_3H_6O)_2$-CH_2-$COOC_3H_7$
22	27.46	59, 117, 159, 203	334	0.3	Diethoxydipropoxy dicarboxylic acid, dipropyl ester
23	27.70	59, 117, 159, 203	334	0.3	Diethoxydipropoxy dicarboxylic acid, dipropyl ester
24	28.12	193, 235	306	0.7	Alkylphenoxyacetic acid
25	28.85	161, 249	298	0.1	Alkylphenoxyacetic acid
26	29.01	193, 235, 292	298	0.1	Alkylphenoxyacetic acid

27	30.78	147, 161, 193, 249, 277, 306	343	0.5	Alkylphenoxyacetic acid
28	31.60	213, 300	338	3.5	Naphthalene dicarboxylic acid
29	31.90	61, 102, 115	326	0.1	Octadecanoic acid, propyl ester[d]
30	32.12	193, 235, 336	336	0.7	Alkylphenoxyacetic acid
31	33.02	103, 145, 279	322	0.8	Alkylphenoxyethoxyacetic acid
32	33.70	219, 249, 321	350	0.5	$C_3H_7OOC\text{-}CH_2\text{-}(C_4H_8)\text{-}C_6H_4\text{-}OCH_2\text{-}COOC_3H_7$
33	34.00	103, 145, 279, 350		0.1	Alkylphenoxyethoxyacetic acid
34	34.31	103, 145, 249, 305	350	0.1	$C_3H_7OOC\text{-}CH_2\text{-}(C_4H_8)\text{-}C_6H_4\text{-}OCH_2\text{-}COOC_3H_7$
35	34.60	131, 173, 219, 261	392	1.5	Aromatic/aldehyde
36	36.88	103, 145, 235	332	0.1	Alkylphenoxyacetic acid
37	37.48	103, 145, 279	380	0.2	Alkylphenoxyethoxyacetic acid
38	38.70	230, 373, 460	460	82[c]	Ethylenediamine tetraacetic acid (EDTA) tetrapropyl ester[d]
39	38.83	103, 145, 173	436	2.0	Ethoxycarboxylic acid
40	42.48	103, 145, 217	480	1.9	Ethoxycarboxylic acid

[a] Base ion is underlined.
[b] Molecular weight calculated for the propylated derivative, obtained from *i*-butane CI mass spectrum.
[c] Quantified using external standard calibration.
[d] Compound was positively identified.
N: Not identified.

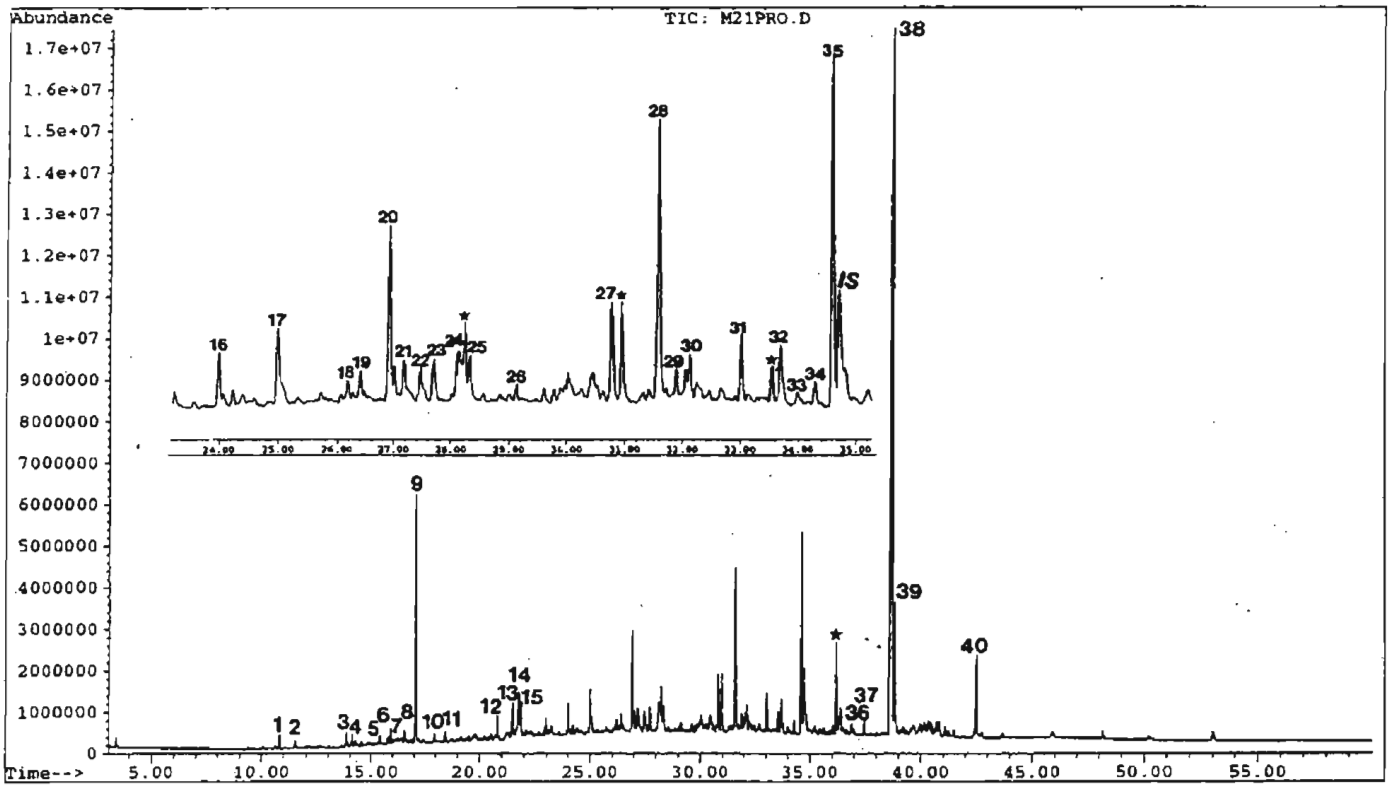


Figure 3.25 GC/EI-MS total ion chromatogram from the propylated M21 groundwater (sample collected on 12/13/93). Peak numbers refer to Table 3.7. Peaks that were found in the method blank are labeled with asterisks. "IS" indicates the internal standard.

3.3.4 Sample DW1

Figure 3.26 shows the total ion chromatogram obtained by GC/EI-MS analysis of the propylated DW1 water collected on December 13, 1993. Table 3.8 lists the compounds that were positively or tentatively identified with the molecular formulas, postulated molecular weights and estimated concentrations (μg/L) in DW1. The individual EI mass spectra are shown in Appendix E.

Table 3.8 Specific Compounds in DW1 Groundwater

Peak	Retention Time (min)	Major ions (m/z)[a]	MW[b]	Estimated Conc. (μg/L)	Positive and Tentative Identifications
1	6.31	134	135	0.2	Benzeneamine[d]
2	6.78	89, 131, 143	142	0.1	
3	8.96	168	N	0.2	
4	10.78	89, 131	218	0.2	$C_2H_5OCH_2CH_2CH_2$-CH(OC_3H_7)$_2$, Ethoxy-butyraldehyde
5	11.50	101, 143	202	0.6	Butanedioic acid, dipropyl ester[d]
6	15.43	191, 206	206	0.2	bis-(1,1 or 2,4)dimethylethyl phenol[d]
7	15.91	85, 131, 187	274	0.1	Aldehyde-ethoxycarboxylic acid
8	17.05	111, 129, 171	230	0.3	Hexanedioic acid, dipropyl ester[d]
9	24.02	141, 159, 201, 213	302	0.1	Naphthalene type compound
10	25.73	149		0.3	Phthalate
11	31.90	61, 102, 326	326	0.1	Octadecanoic acid, propyl ester[d]

[a] Base ion is underlined.
[b] Molecular weight calculated for the propylated derivative, obtained from *i*-butane CI mass spectrum.
[d] Compound was positively identified.
N: Not identified.

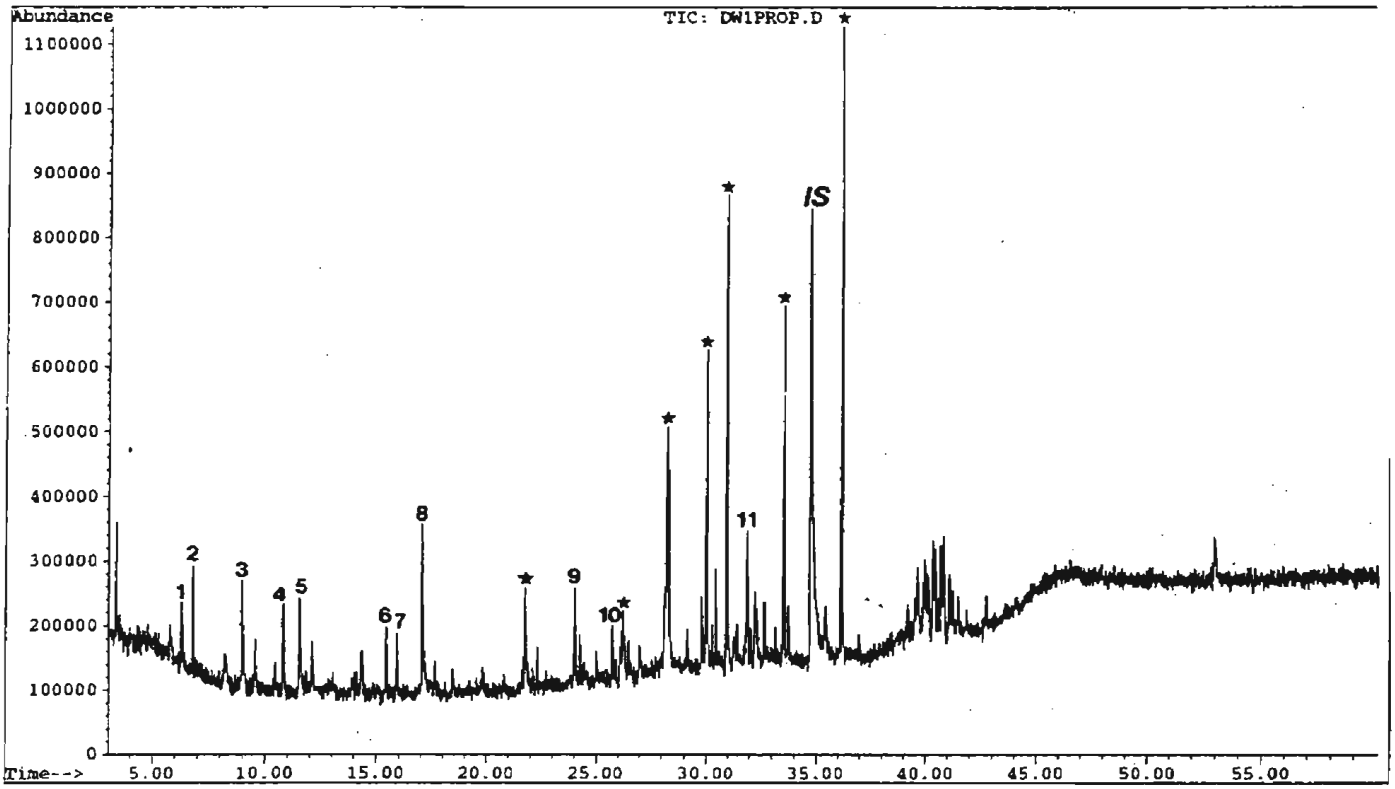


Figure 3.26 GC/EI-MS total ion chromatogram from the propylated DW1 groundwater (sample collected on 12/13/93). Peak numbers refer to Table 3.8. Peaks that were found in the method blank are labeled with asterisks. "IS" indicates the internal standard.

3.3.5 Sample YLWD11

Sample YLWD11 water came from the recharge zone of Santa Ana River water basins. Figure 3.27 shows the total ion chromatogram obtained by GC/EI-MS analysis of the propylated YLWD11 water collected on November 30, 1993. Table 3.9 lists the compounds that were positively or tentatively identified along with the molecular formulas, postulated molecular weights and estimated concentrations (µg/L) in YLWD11. The individual EI mass spectra are shown in Appendix F.

Table 3.9 Specific Compounds in YLWD11 Groundwater

Peak	Retention Time (min)	Major ions (m/z)[a]	MW[b]	Estimated Conc. (µg/L)	Positive and Tentative Identifications
1	3.29	47, 89, 131	N	1.5	
2	4.19	47, 89, 115, 131	174	7.2	CH_3-CO-CH(OC_3H_7)2, (Methyl glyoxal)
3	5.33	89, 93,95, 131, 135, 137	174	3.2	
4	7.25	89, 131, 137, 139, 179, 181	N	2.1	
5	8.29	61, 89, 114, 132	191	1.6	
6	9.45	58, 75, 89, 104, 131, 146	290	120	
7	9.74	88, 130	N	31	
8	10.80	61, 89, 115, 131	218	33	$C_2H_5OCH_2CH_2CH_2$-CH(OC_3H_7)2, Ethoxy-butyraldehyde
9	14.13	47, 61, 89, 119, 131, 161	262	140	(C_3H_7O)2CH-CH(OC_3H_7)2, (Glyoxal)
10	14.56	47, 89, 131	246	12	(C_3H_7O)2CH-$CH_2CH_2COOC_3H_7$, (4-oxobutanoic acid)
11	16.61	47, 89, 117, 131, 159	230	1.7	$C_6H_{11}O$-CH(OC_3H_7)2
12	17.53	61, 89, 103, 137, 149, 179, 209	250	2.4	
13	18.04	47, 73, 89, 115, 131, 146, 157	263	2.7	
14	18.32	73, 89, 108, 115, 131, 171	240	0.7	C_7H_9O-CH(OC_3H_7)2
15	18.42	47, 73, 89, 115, 131	240	1.1	C_7H_9O-CH(OC_3H_7)2
16	19.13	47, 89, 131	230	42	
17	20.13	47, 89, 131, 169	268	1.7	
18	20.30	47, 89, 115, 131	268	1.7	
19	20.51	47, 89, 100, 131, 191	254	3.1	
20	20.93	47, 89, 92, 131	268	1.2	
21	21.03	47, 73, 89, 115, 131	268	1.2	

22	21.33	47, 89, 131	288	1.3	$(C_3H_7O)_2CH\text{-}C_5H_{10}\text{-}COOC_3H_7$
23	22.00	89, 117, 131, 159, 261	332	1.8	
24	27.00	47, 89, 131, 169	286	9.7	
25	29.94	47, 73, 89, 115, 131, 199, 241	344	13	$(C_3H_7O)_2CH\text{-}C_9H_{18}\text{-}COOC_3H_7$
26	30.12-	47, 89, 115, 131, 199, 241	344	12	$(C_3H_7O)_2CH\text{-}C_9H_{18}\text{-}COOC_3H_7$

a Base ion is underlined.

b Molecular weight calculated for the propylated derivative, obtained from *i*-butane CI mass spectrum.

N: Not identified.

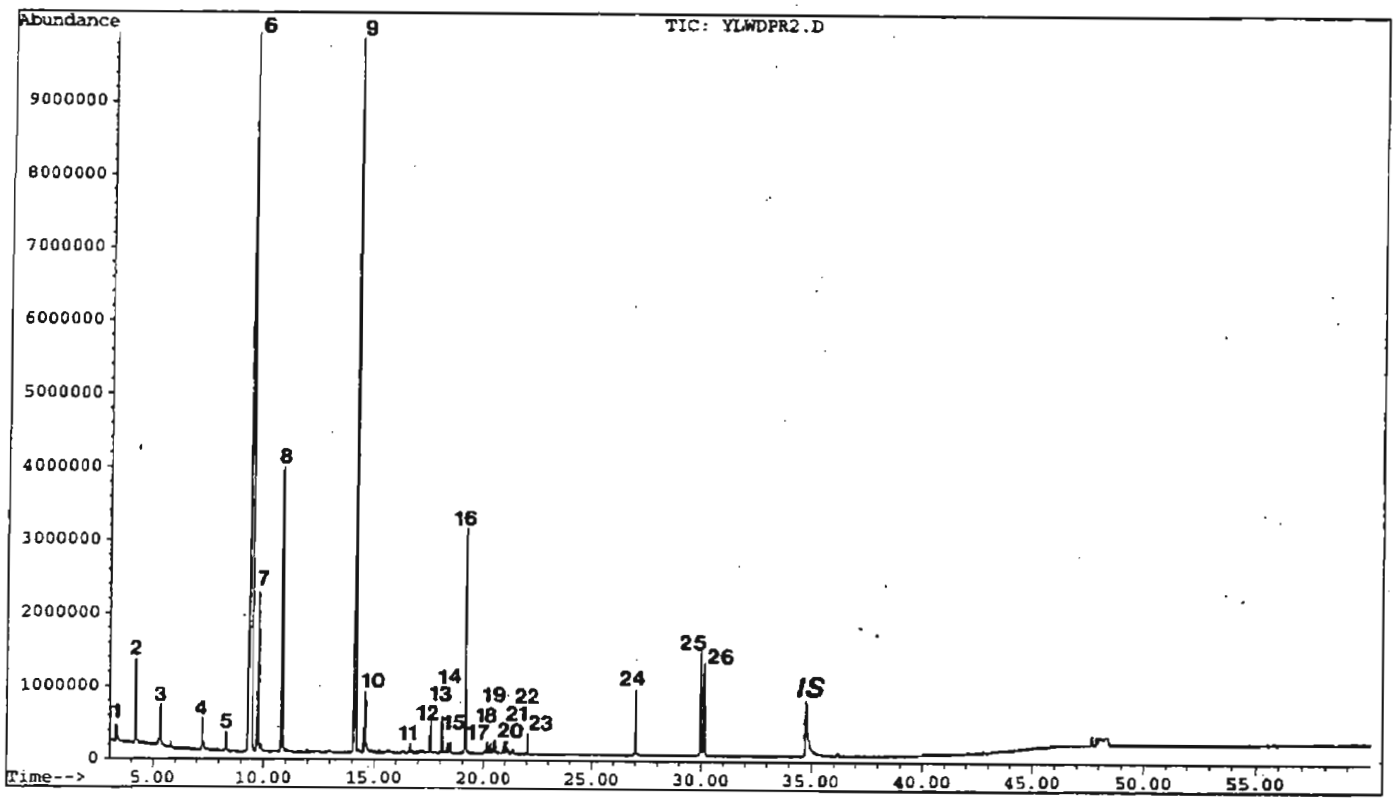


Figure 3.27 GC/EI-MS total ion chromatogram from the propylated YLWD# 11 water (sample collected on 12/13/93). Peak numbers refer to Table 3.9. "IS" indicates the internal standard.

SECTION 4
CONCLUSIONS

4.1 Bulk DOC Characterization

(1) Despite the wide disparity in DOC concentrations (from < 1 mg/l in Q22B to 6 mg/l in Q9), the DOC in all three of the WF21 waters (Q8, Q9 and Q22B) was similarly proportioned between hydrophilic and fulvic acid fractions: approximately 50% hydrophilic, and 33% - 44% fulvic acid.

(2) No humic acid was recovered from any of the WF21 waters, nor from the YLWD11 groundwater. Ten percent of the DOC in the DW1 groundwater was classified as humic acid, and 6.5 % in the M21 groundwater.

(3) The fulvic acid fractions from the WF21 waters and the two recharged groundwaters, M21 and YLWD11, all exhibited high (> 1.5) H/C atomic ratios, indicating that saturated aliphatic or alicyclic structures predominate over aromatic structures in the complex mixtures. Only the DW1 fulvic acid exhibited an H/C ratio (1.3) considered within the range representative of natural humic substances.

(4) The H/C ratio for the M21 humic acid was also slightly high (1.4); the DW1 humic acid however exhibited an H/C ratio (1.2) considered within the range of natural humic substances.

(5) The O/C atomic ratio in the fulvic acid fraction increased following chlorination of Q8 (to produce Q9); this is likely due to the oxidation of organic compounds by the active chlorine species.

(6) N/C atomic ratios for the fulvic and humic acid fractions from the WF21 waters and the M21 and YLWD11 groundwaters were all high relative to reference natural aquatic humic substances, a possible indicator of the samples' wastewater origins. More work is needed to confirm this proposition, however.

(7) Chlorine contents in the WF21 fulvic acids were significantly higher than the value reported for a creek fulvic acid, probably due to chlorine disinfection during water and wastewater treatment.

(8) All of the fulvic acid ^{1}H NMR spectra showed the broad unresolved humps characteristic of complex mixtures, with the two groundwaters DW1 and YLWD11 showing the fewest distinct signals indicative of specific structures. The ^{1}H NMR fingerprints of the three WF21 waters and M21 groundwater were very similar to each other, indicating a correlation between origin and spectral appearance.

(9) Strong signals at 3.6 ppm, indicative of polyethoxy and polypropoxy type structures, were exhibited in all of the ^{1}H NMR spectra of the WF21 and M21 fulvic acids. These signals did not appear in the DW1 and YLWD11 groundwaters.

4.2 Identification of Specific Organic Residues

(1) An analytical approach was developed that combines concentration by evaporation, derivatization, and analysis with GC/EI-MS, GC/CI-MS and GC/TSQ-MS techniques. It was used for the characterization and identification of trace organic residues present in treated wastewater effluents and groundwaters at nanogram per liter levels. Specific compounds that were detected included compounds of anthropogenic origin, microbial transformation products of anthropogenic substances and metabolites of microbial processes. Anthropogenic compounds included EDTA and NTA and alkylphenol polyethoxylates. Known microbial transformation products of anthropogenic precursors that were detected included partially oxidized APEs and LAE. Compounds that appeared to be microbial metabolites included glyoxal, methyl glyoxal, alkanedioic acids and several aldehydes that were not specifically identified.

(2) The public health significance of the detected organic residues with regard to water quality remains to be evaluated. The compounds positively or tentatively identified here are not regulated by the U. S. Environmental Protection Agency.

(3) EDTA was by far the most prominent compound detected in samples Q8, Q9 and M21. EDTA was also the most prominent compound in Q22B, but at a much reduced concentration relative to the other WF21 effluents, indicating effective removal during reverse osmosis. NTA, a structually related compound, was also detected in samples Q8, Q9, Q22B and M21, but at much lower concentrations.

(4) During chlorination of Q8 a significant fraction of the CAPEC compounds was converted into brominated CAPEC's. The N,N,3-trimethylbenzeneamine was removed during chlorination.

(5) In samples Q8 and Q9, approximately 50 synthetic and natural organic compounds were detected ranging in concentration from 0.5 μg/L to 140 μg/L. The total was 300 to 400 μg/L (approximately 5% of the TOC).

(6) In sample Q22B, approximately 80% of the total chromatographable products were positively or tentatively identified at concentrations ranging from 0.1 μg/L to 4.3 μg/L. The total amount of the detected compounds was 13 μg/L.

(7) In sample M21, approximately 90% of the total chromatographable products were positively or tentatively identified at concentrations ranging from 0.1 μg/L to 82 μg/L. The total amount of the detected compounds was 110 μg/L.

(8) In sample DW1, approximately 9 trace organics were detected at concentrations ranging from 0.1 μg/L to 0.6 μg/L.

(9) In sample YLWD11, approximately 40 % of the total chromatographable products were tentatively identified at concentrations ranging from 0.7 μg/L to 140 μg/L. A homologous series of ω-oxocarboxylic acids and alkoxy aldehydes were tentatively identified. The total amount of the detected compounds was approximately 450 μg/L.

SECTION 5

RECOMMENDATIONS

Methods routinely used to characterize the chemical and biological properties of DOC lack the specificity required to accurately predict water quality changes during groundwater recharge, and to specify minimum groundwater residence times, travel distances, and water quality standards. Adequate biological and chemical degradation models that apply to aquifer environments characterized by complex mixtures of organic compounds and low concentrations do not exist. Although many cases of groundwater recharge have been studied, results of different investigations are difficult to compare, because the organic carbon was characterized with nonspecific and non standardized methods. Field studies generally lack adequate controls and have not been supplemented with detailed laboratory studies. Based on these considerations and the results of this study, the following specific recommendations can be made:

(1) Analytical methodology development for the characterization of natural water and wastewater DOC should continue. Special emphasis should be given to characterization of fraction using group specific analyses, desalting procedures for organic concentrates, and to the testing and development of sample preparation and derivatization procedures which make analytes amenable to GC/MS or HPLC analysis. Advanced MS techniques such as Fast Atomic Bombardment (FAB) and thermospray LC/MS should be employed.

(2) Field studies should be conducted to guide laboratory studies and the analytical characterization effort. DOC characterization and specific GC/MS methods should be applied to the study of fate and transport processes during groundwater recharge. Questions that should be addressed include: What fractions are biodegradable under field

conditions? What processes affect carbon turnover in aquifers? What factors affect the maximum observed removals, i.e., the minimum residual concentrations?

(3) Model system studies in which the natural conditions are reproduced under controlled conditions are necessary. Such model systems should consist of columns that are packed with biologically active aquifer or model solids and fed with synthetic wastewaters. Isotopically labeled substrates should be used if possible. Questions that should be addressed include: How do mixtures of substrates support biological growth? What fraction of the organic carbon is mineralized, immobilized or converted into humic substances? Which biodegradation models apply to the low carbon, complex mixture environment found in aquifers?

(4) Other recharge operations should be studied using the same analytical and experimental approach to extend the data base and to demonstrate the general applicability of the concepts developed here. The organic carbon compositions of the different recharge waters should be well-characterized, and compared in order to determine whether uniform standards can be applied nationwide to similar wastewater recharge operations.

SECTION 6

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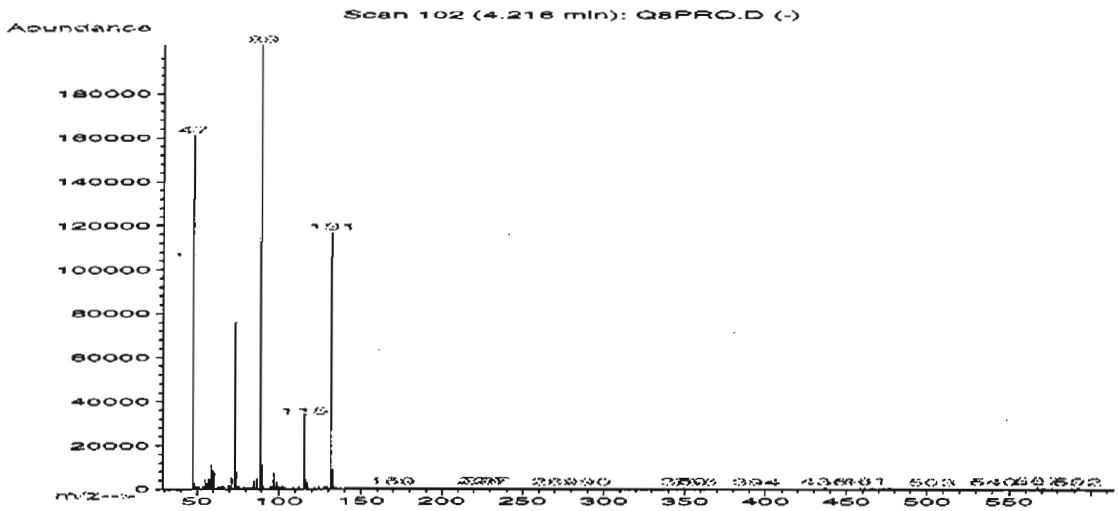
APPENDICES

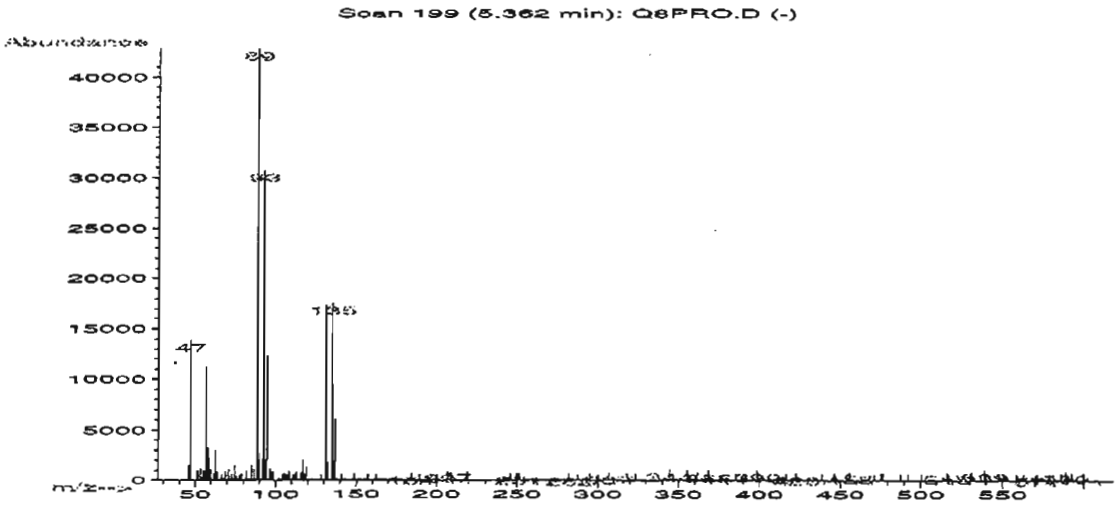
APPENDIX A

Spectra from propylated extract of Q8 (collected 12/9/93), run on EDTA2.M. Numbered as in Table 3.4.

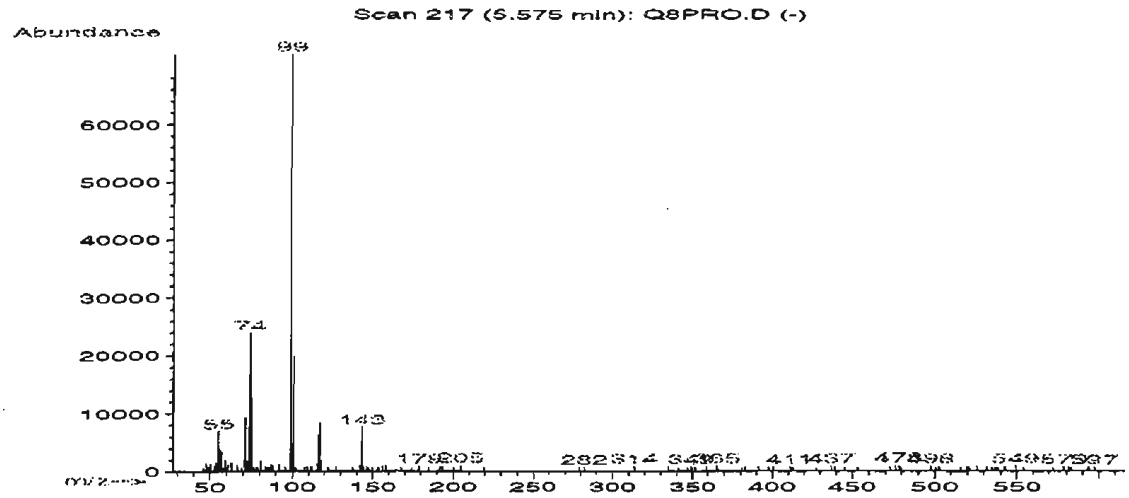
1



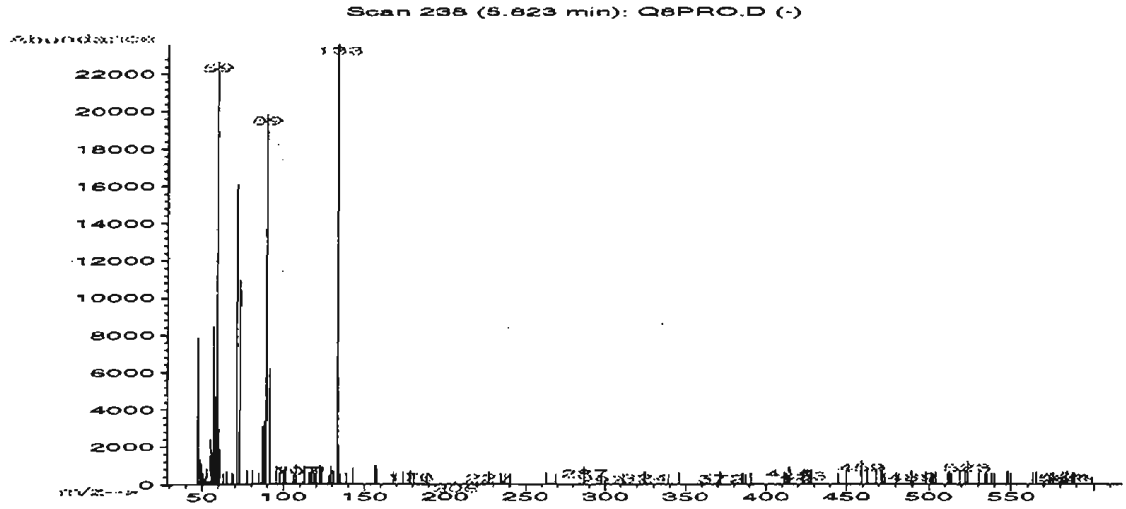
2



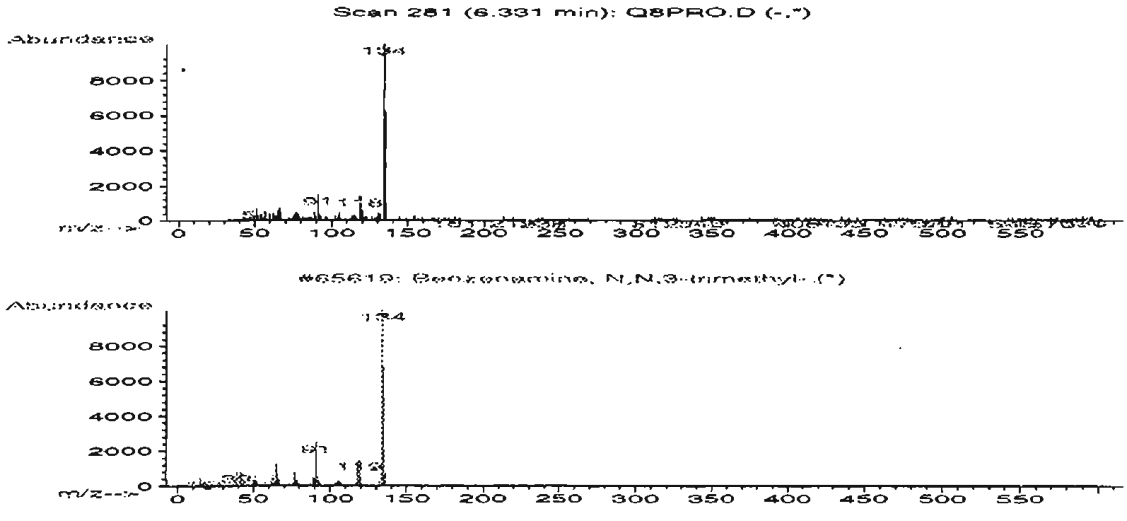
3


Scan 217 (5.575 min): Q8PRO.D (-)
Abundance
99
74
55
143
m/z-->

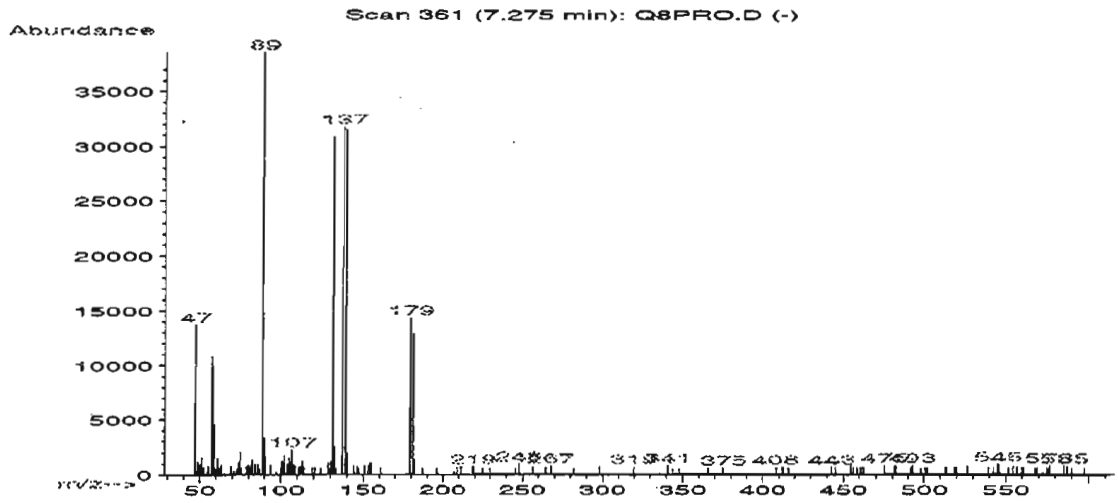
4


Scan 238 (6.623 min): Q8PRO.D (-)
Abundance
m/z-->

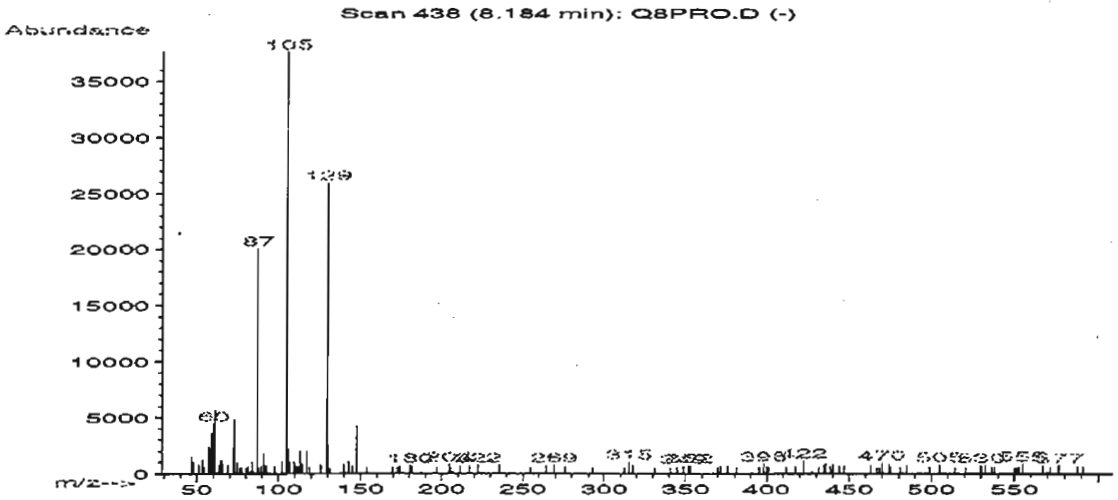
5


Scan 281 (6.331 min): Q8PRO.D (-,*)
Abundance
134
#65619: Benzenamine, N,N,3-trimethyl- (*)
Abundance
134
m/z-->

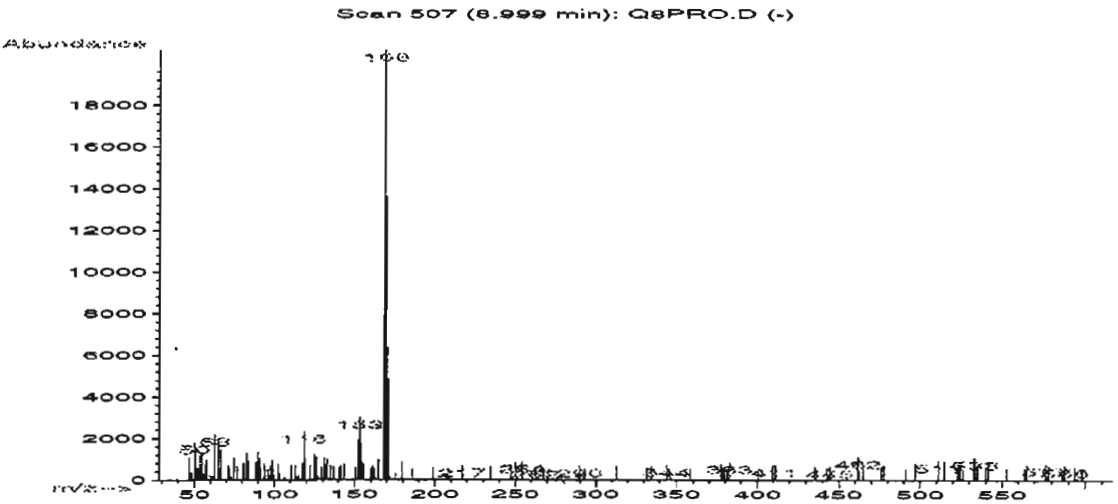
6


Scan 361 (7.275 min): Q8PRO.D (-)
Abundance
89
137
47
179
107
m/z-->

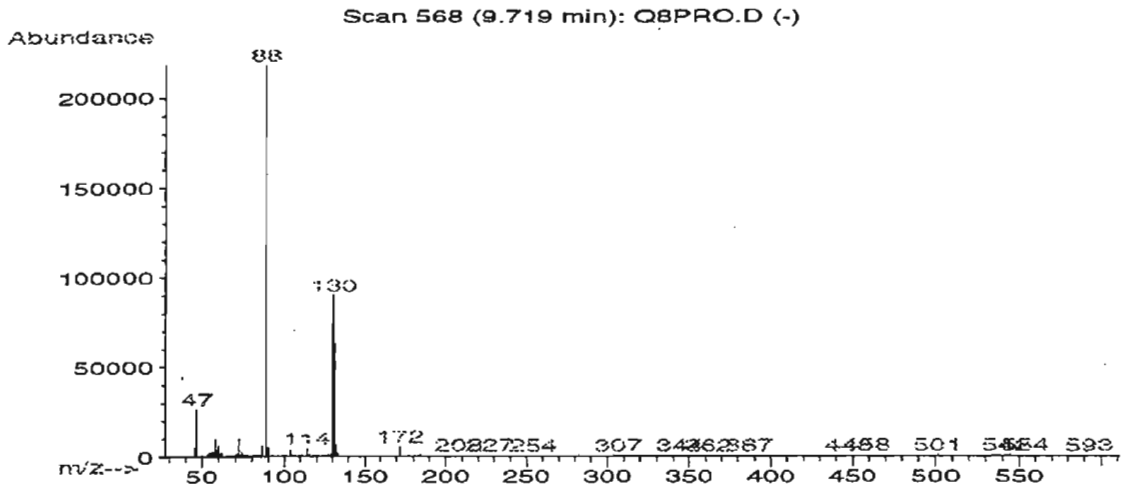
7


Scan 438 (8.184 min): Q8PRO.D (-)
Abundance
105
129
87
69
m/z-->

8


Scan 507 (8.999 min): Q8PRO.D (-)
Abundance
100
149
113
m/z-->

9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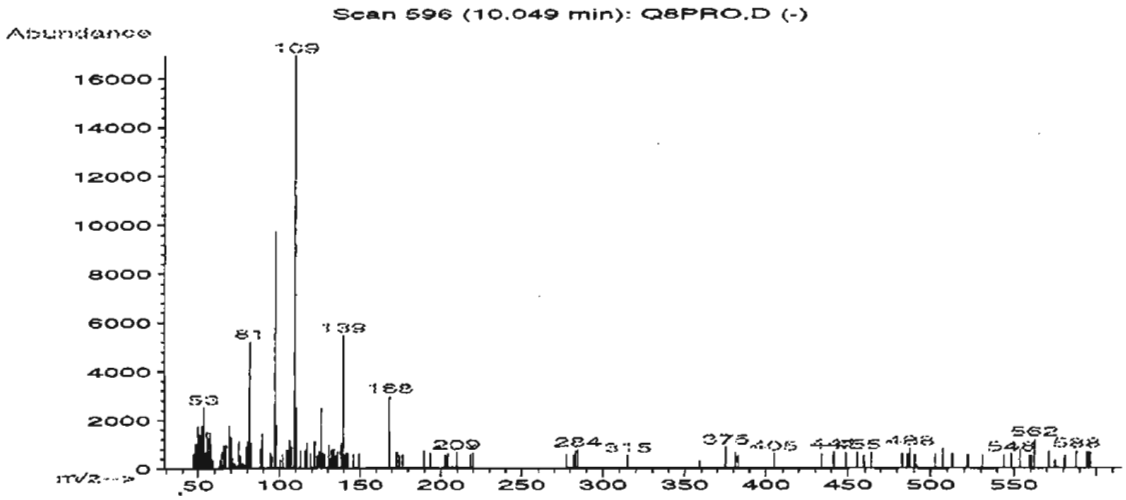
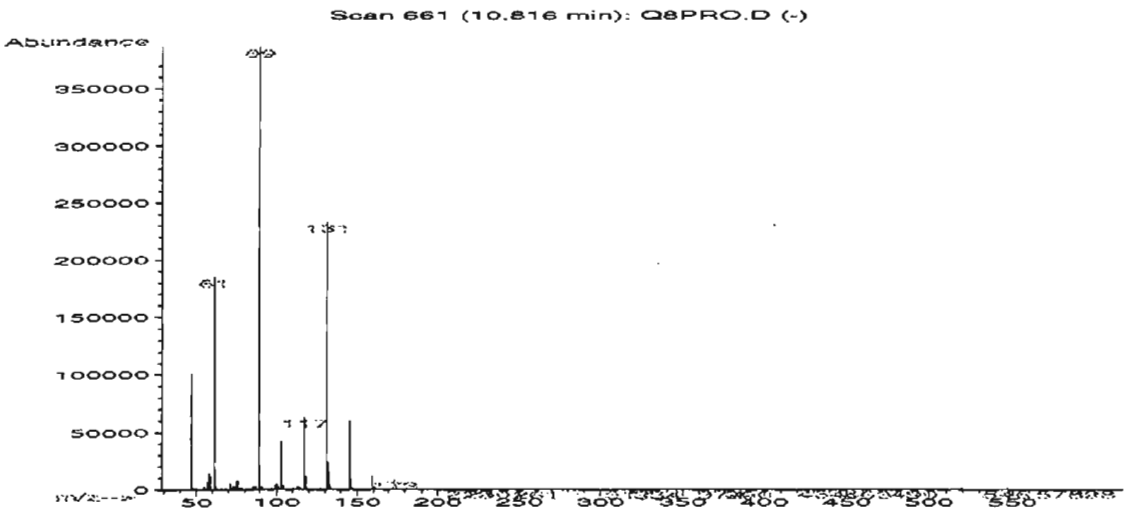
Abundance
88
130
47
172
m/z-->

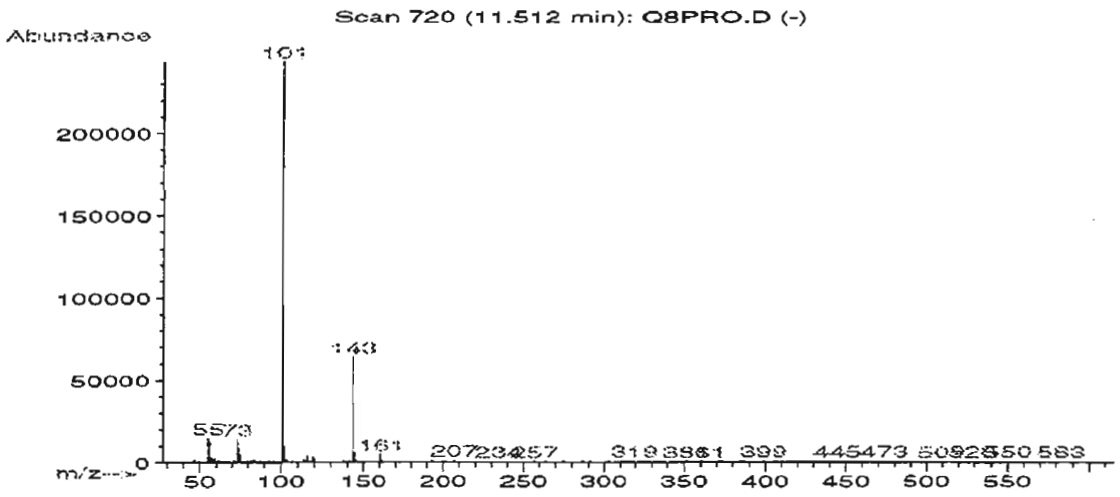
10


Scan 596 (10.049 min): Q8PRO.D (-)
Abundance
169
81
139
53
188
209
284
315
375
405
562
588
m/z-->

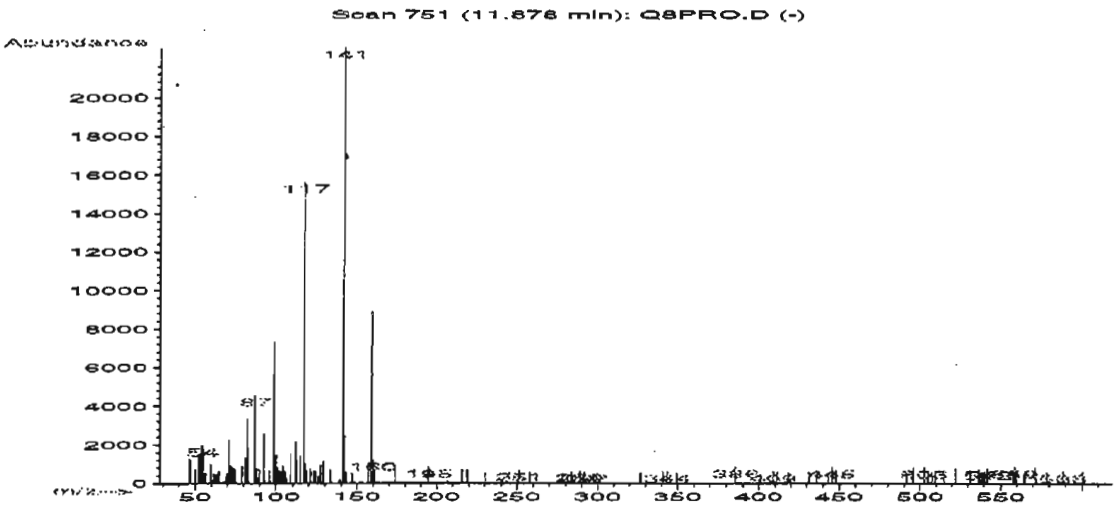
11


Scan 661 (10.816 min): Q8PRO.D (-)
Abundance
99
131
81
117
m/z-->

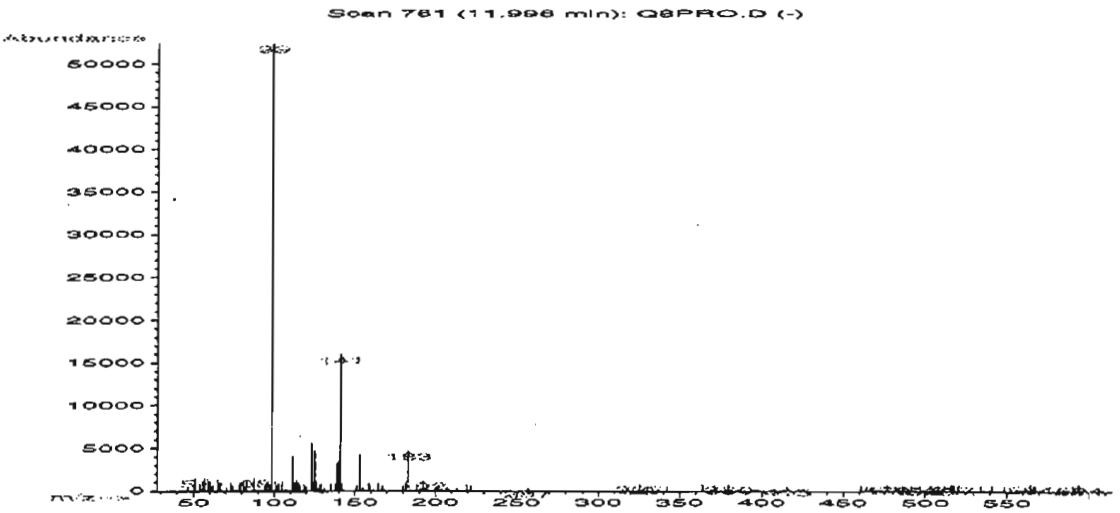
12


Scan 720 (11.512 min): Q8PRO.D (-)
Abundance
101
143
161
m/z-->

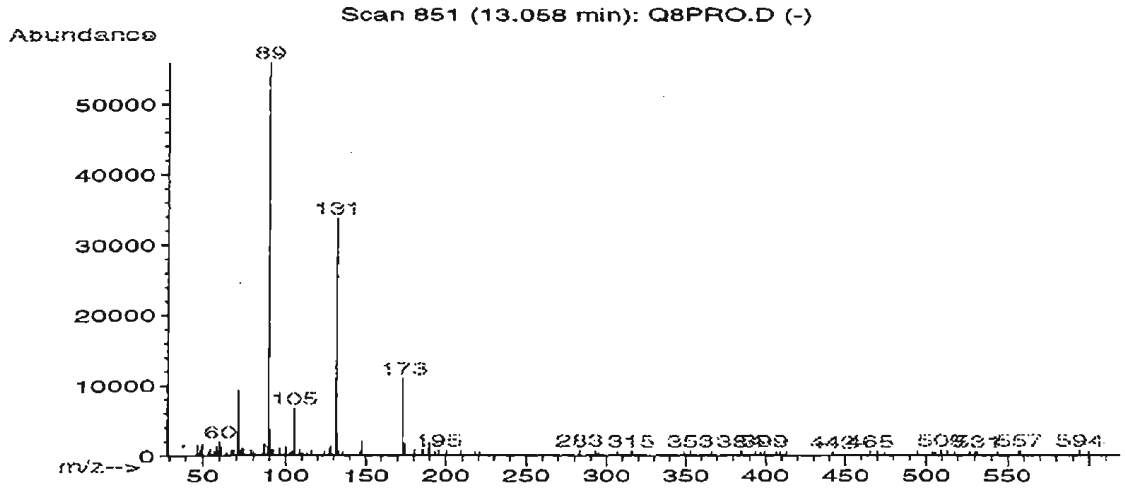
13


Scan 751 (11.878 min): Q8PRO.D (-)
Abundance
141
117
m/z-->

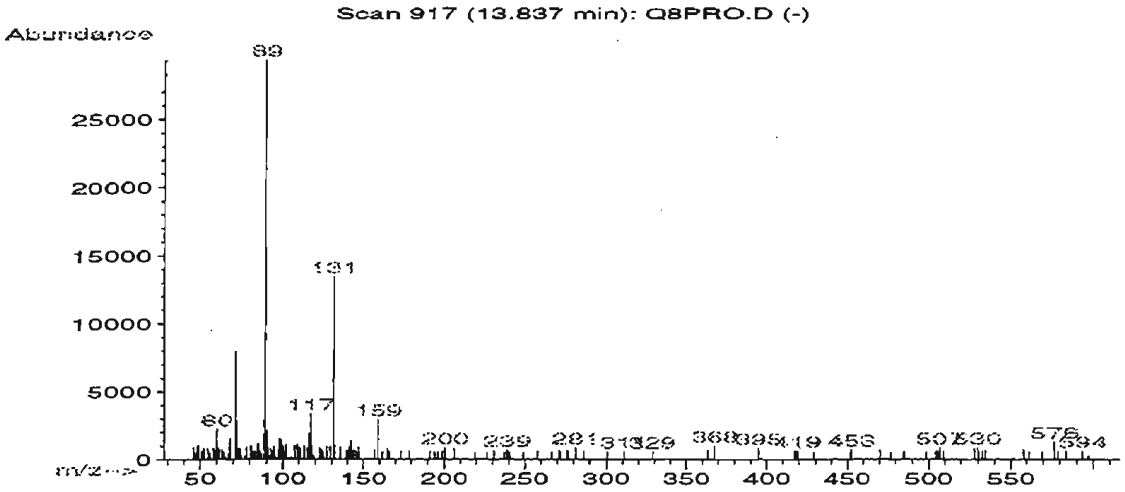
14


Scan 761 (11.998 min): Q8PRO.D (-)
Abundance
m/z-->

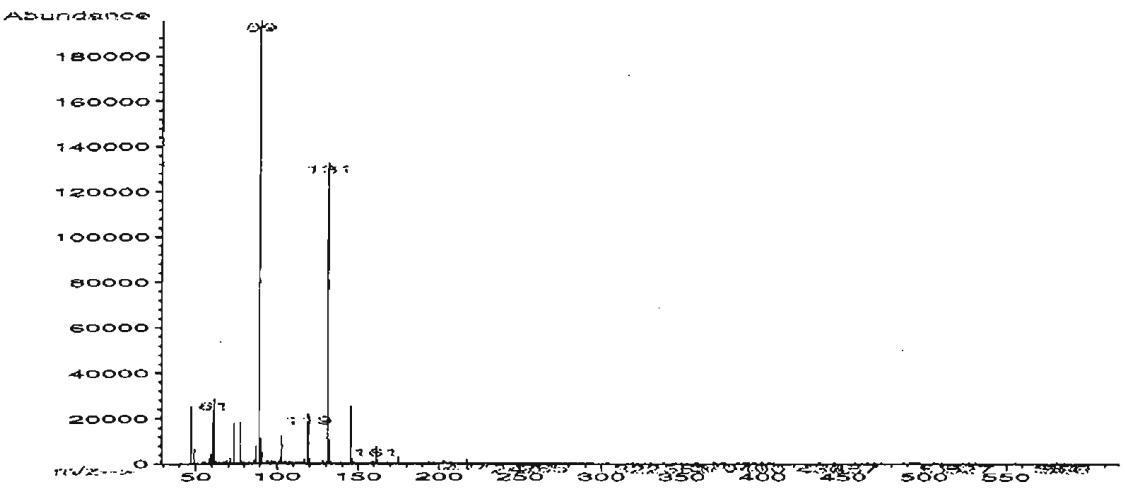
15


Scan 851 (13.058 min): Q8PRO.D (-)
Abundance
89
131
173
105
60
195
m/z-->
50000
40000
30000
20000
10000
0
50 100 150 200 250 300 350 400 450 500 550

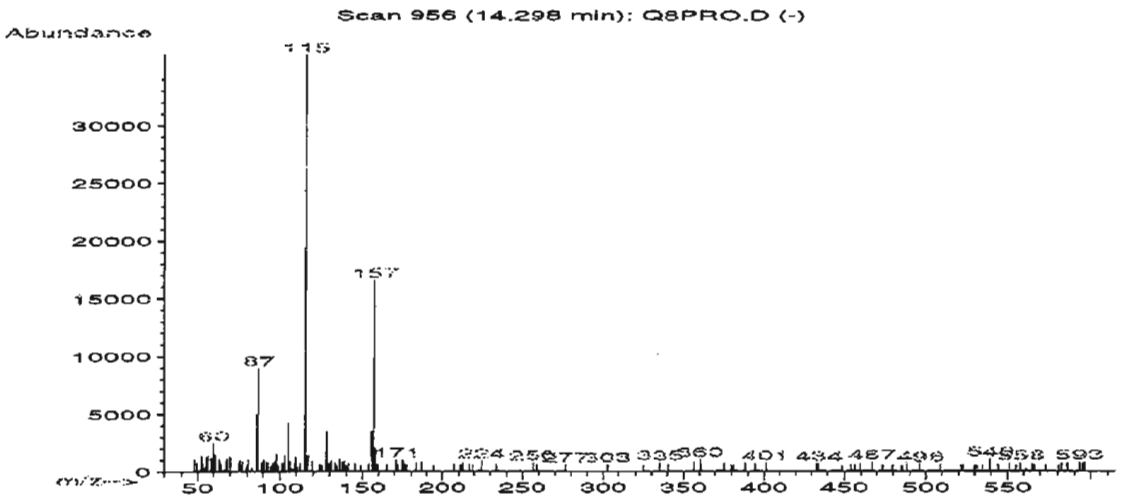
16


Scan 917 (13.837 min): Q8PRO.D (-)
Abundance
89
131
117
159
80
200
m/z-->
25000
20000
15000
10000
5000
0
50 100 150 200 250 300 350 400 450 500 550

17


Scan 942 (14.132 min): Q8PRO.D (-)
Abundance
89
131
m/z-->
180000
160000
140000
120000
100000
80000
60000
40000
20000
0
50 100 150 200 250 300 350 400 450 500 550

18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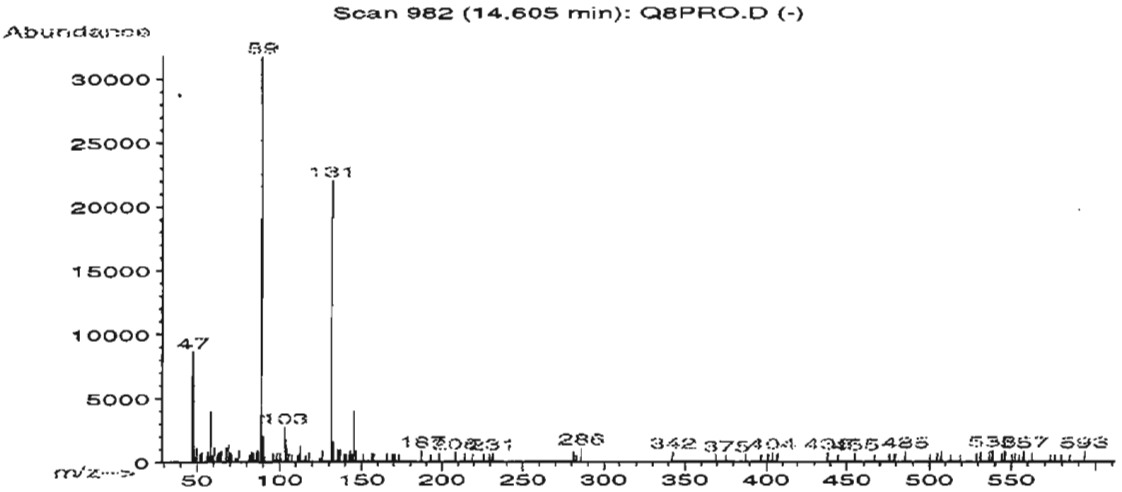
Abundance
115
157
87
60
171
m/z-->

19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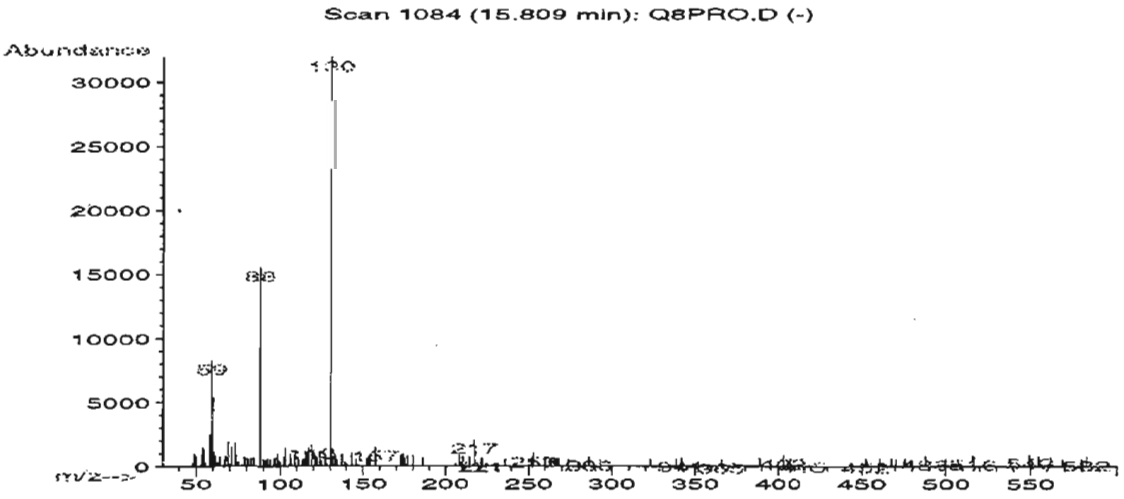
Abundance
59
131
47
103
m/z-->

20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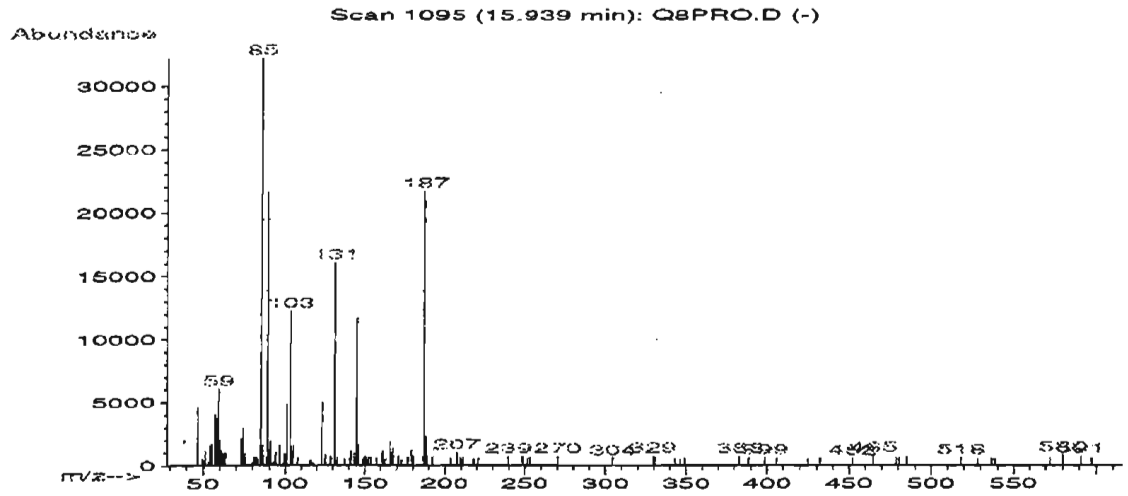
Abundance
115
87
59
217
m/z-->

21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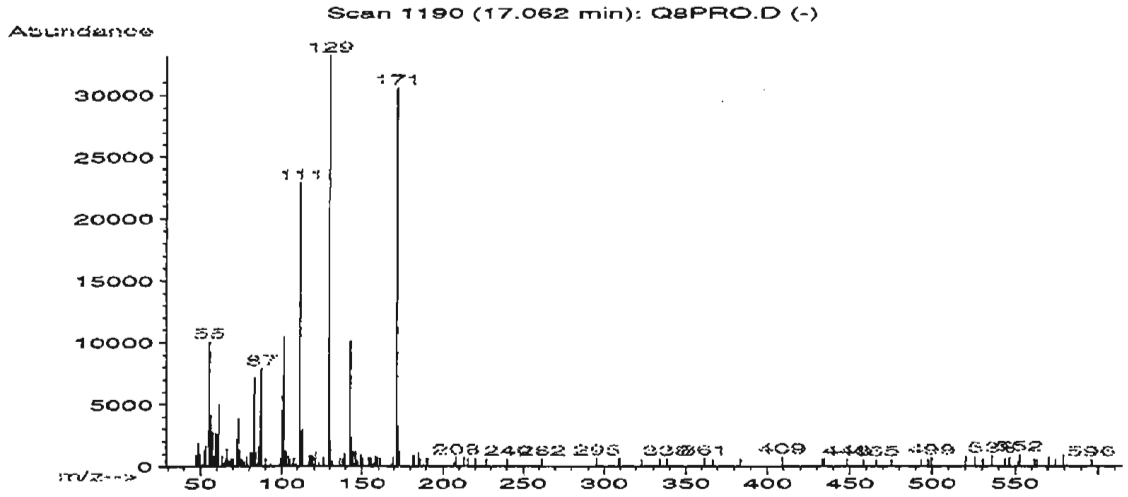
Abundance
m/z-->

22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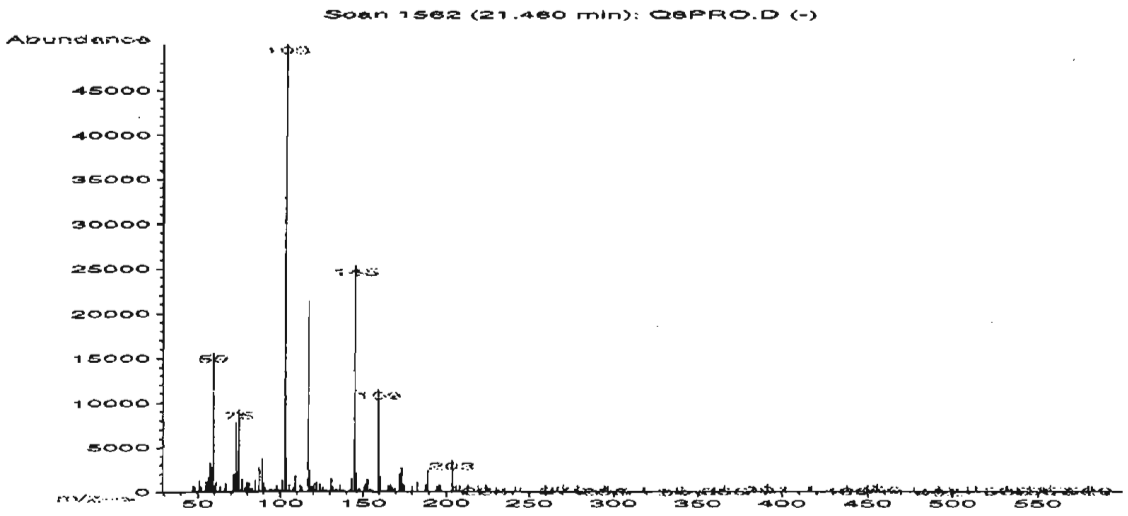
Abundance
m/z-->

23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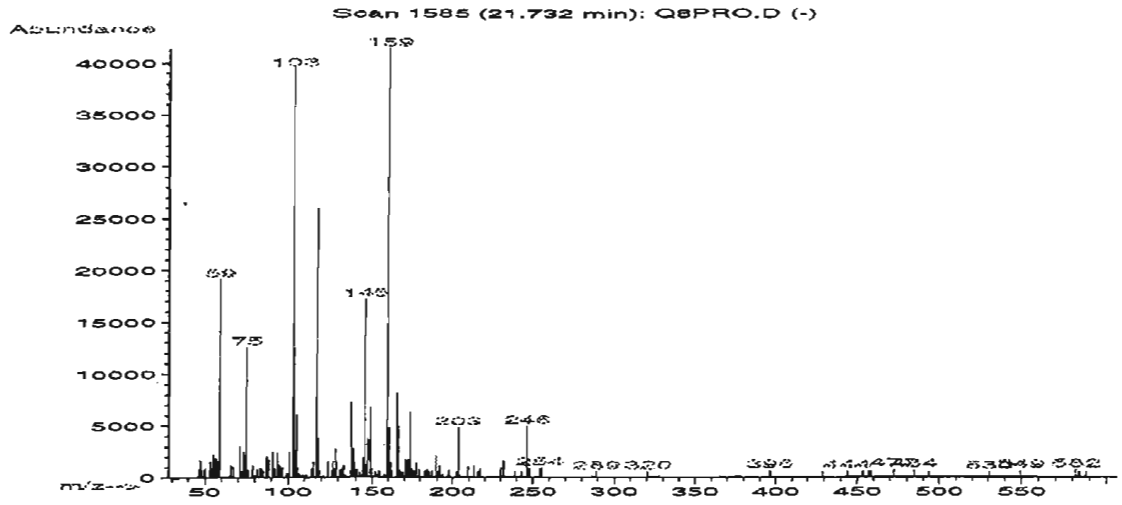
Abundance
m/z-->

24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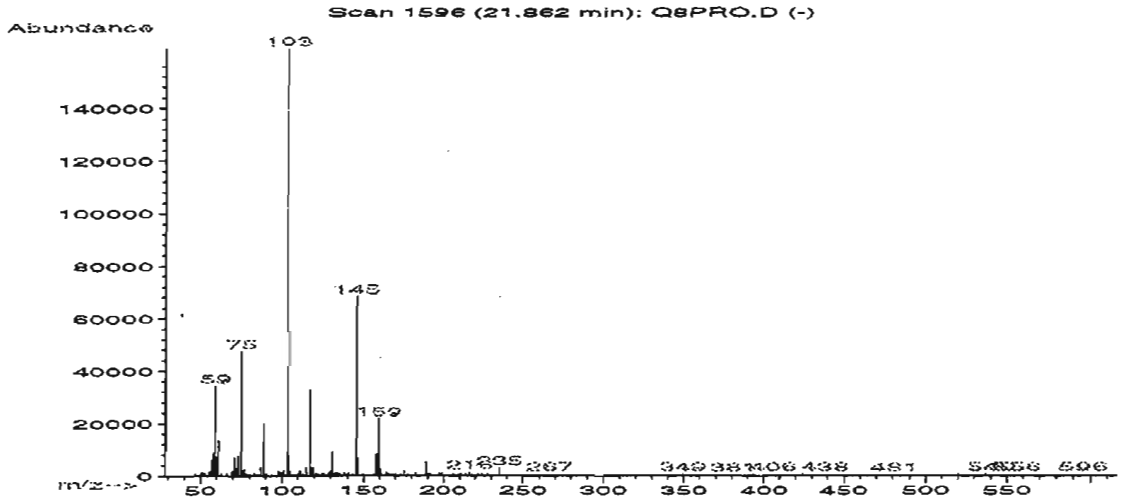
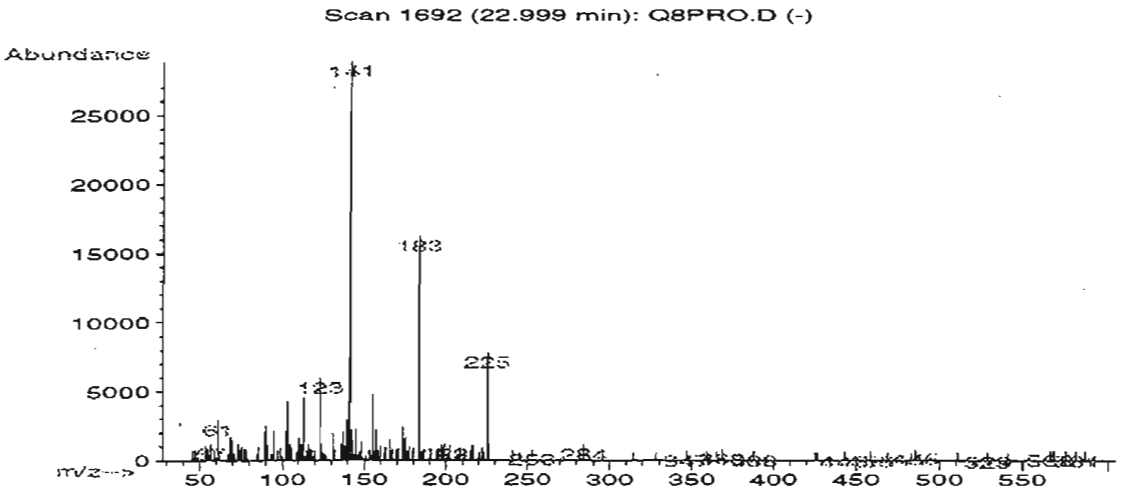
Abundance
159
103
59
148
75
203
246
m/z-->

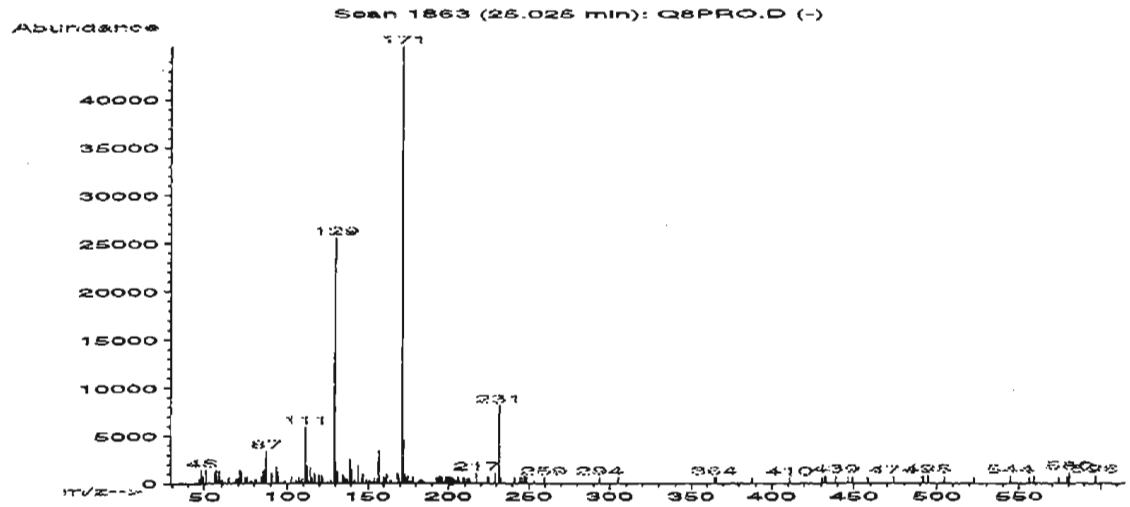
25


Scan 1596 (21.862 min): Q8PRO.D (-)
Abundance
103
145
75
59
159
m/z-->

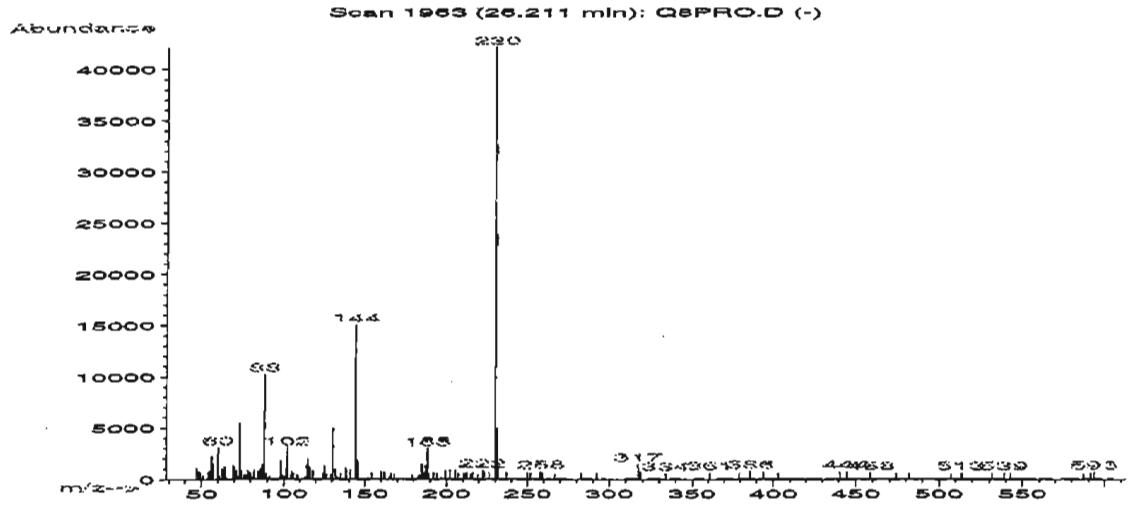
26


Scan 1692 (22.999 min): Q8PRO.D (-)
Abundance
141
183
225
123
m/z-->

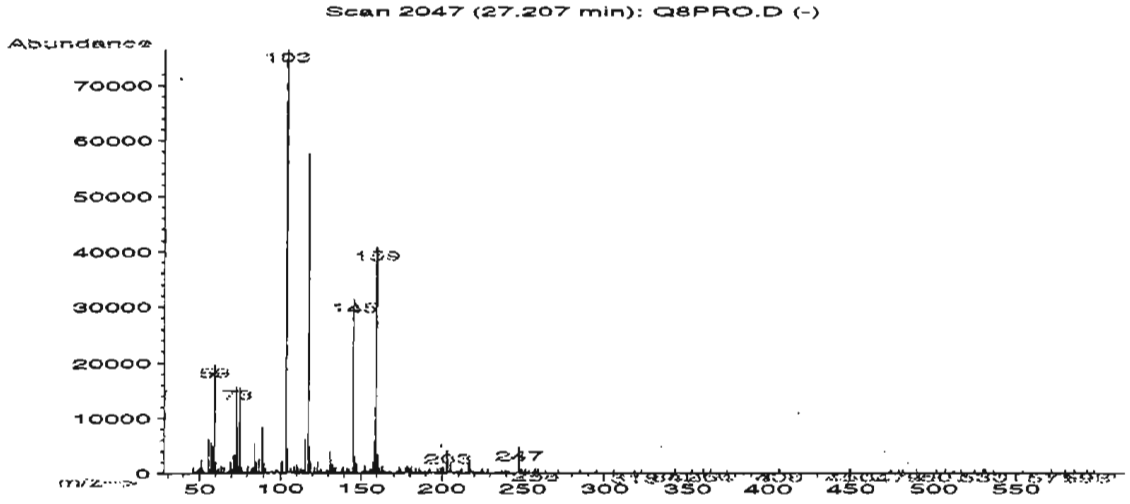
27


Scan 1863 (26.026 min): Q8PRO.D (-)
Abundance
171
129
231
111
97
217
m/z-->

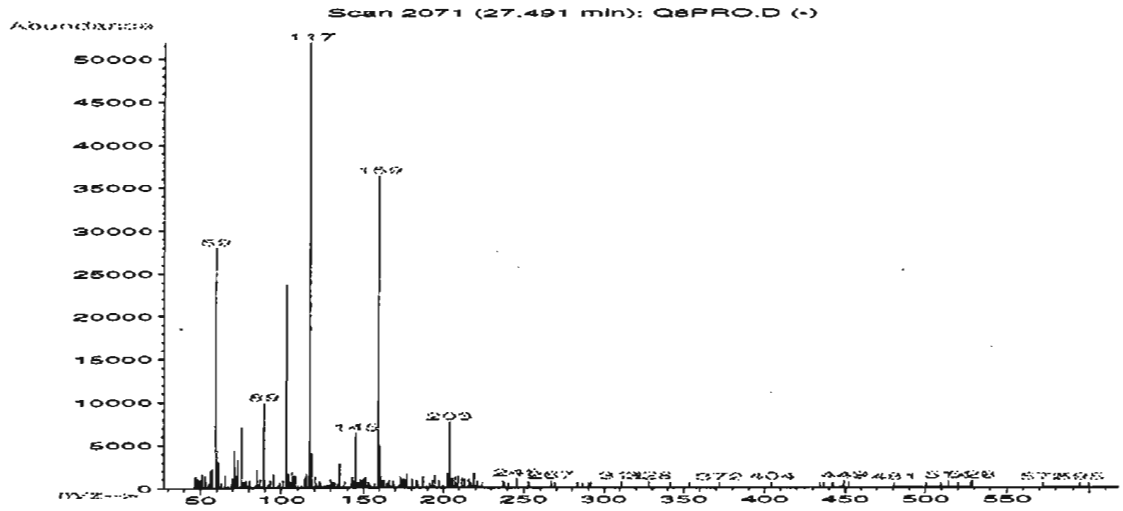
28


Scan 1963 (26.211 min): Q8PRO.D (-)
Abundance
220
144
83
m/z-->

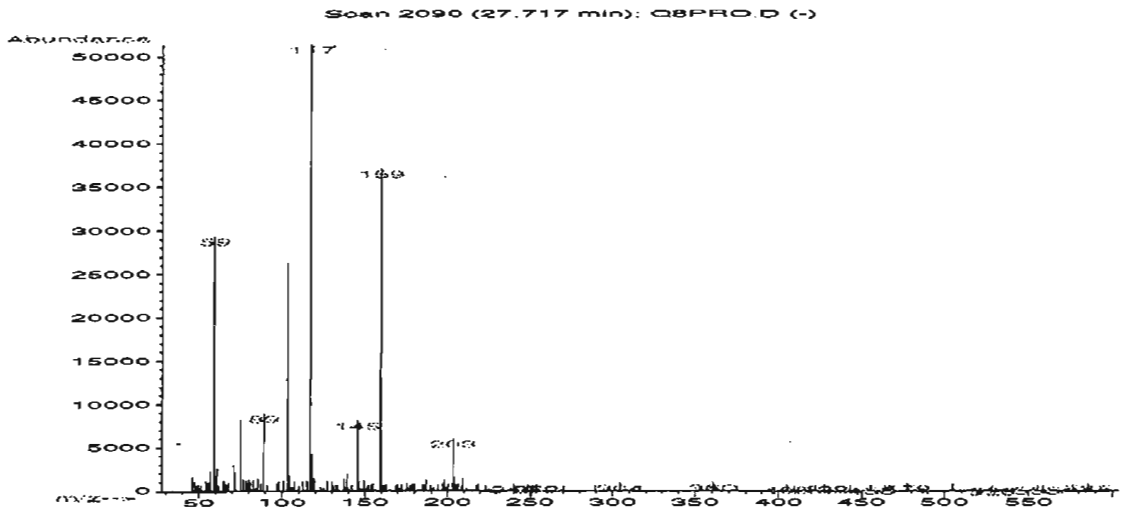
29


Scan 2047 (27.207 min): Q8PRO.D (-)
Abundance
103
159
145
247
m/z-->

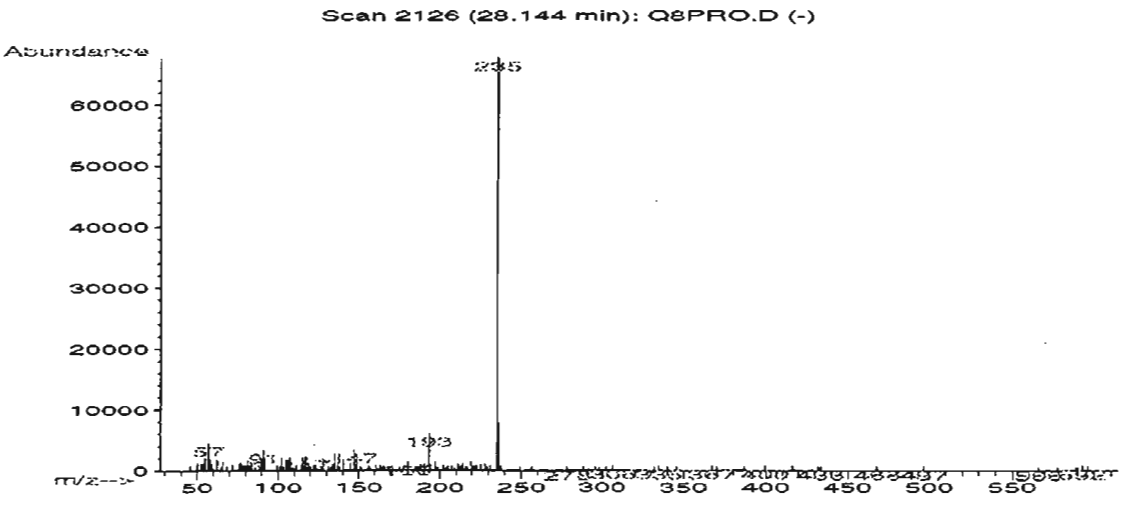
30


Scan 2071 (27.491 min): Q8PRO.D (-)
Abundance
m/z-->

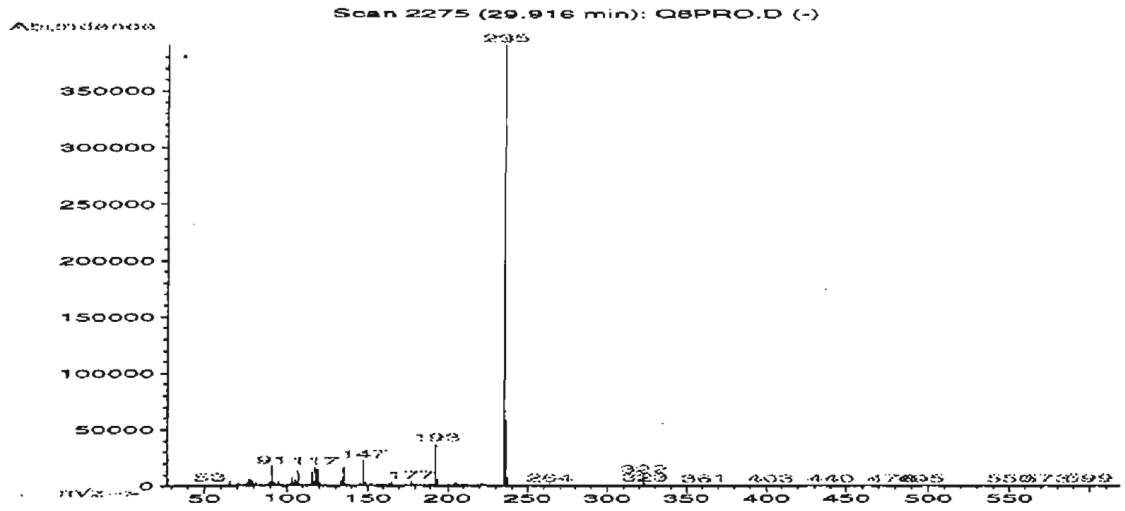
31


Scan 2090 (27.717 min): Q8PRO.D (-)
Abundance
m/z-->

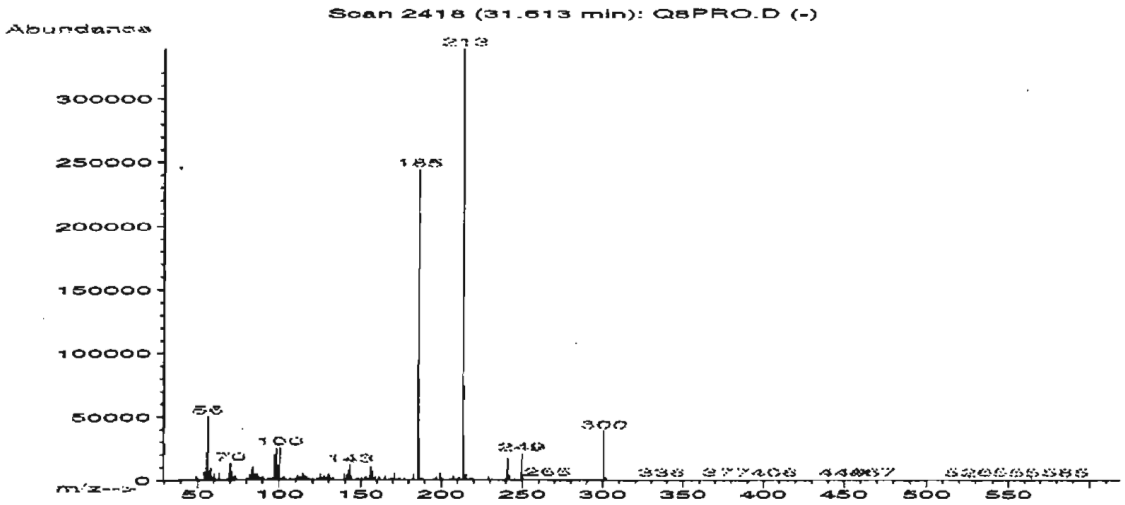
32


Scan 2126 (28.144 min): Q8PRO.D (-)
Abundance
m/z-->

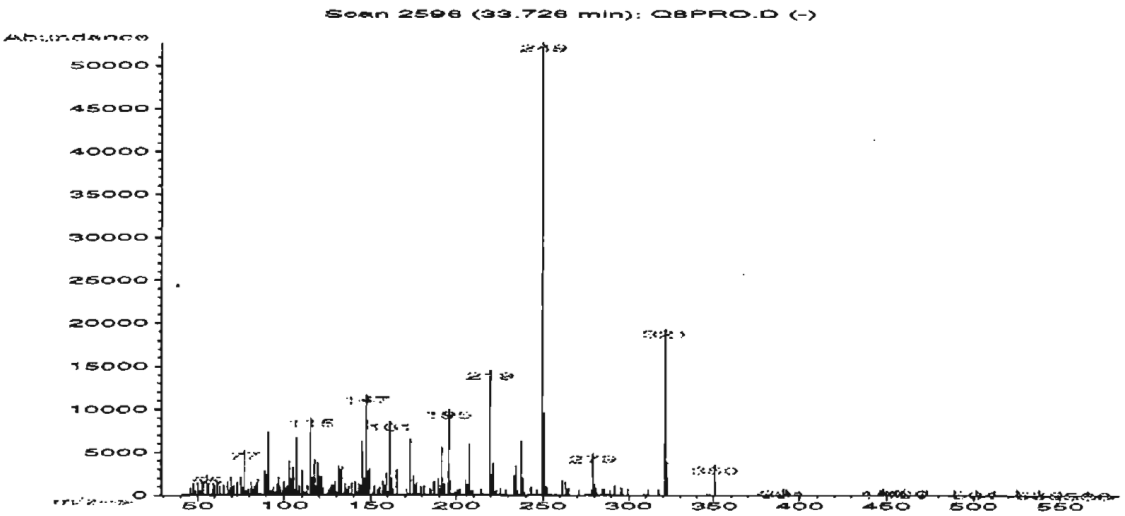
33


Scan 2275 (29.916 min): Q8PRO.D (-)
Abundance
m/z-->

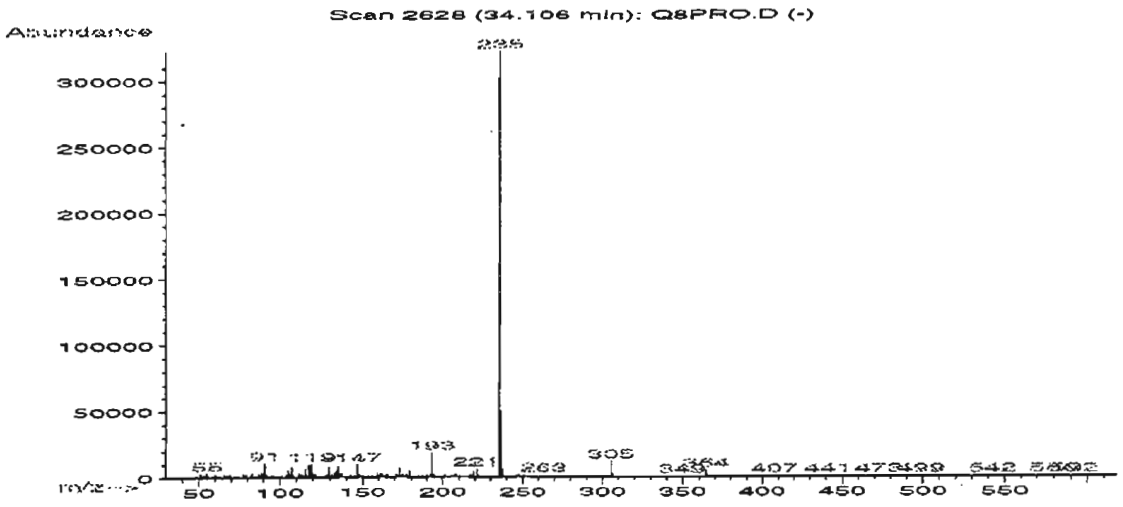
34


Scan 2418 (31.613 min): Q8PRO.D (-)
Abundance
m/z-->

35

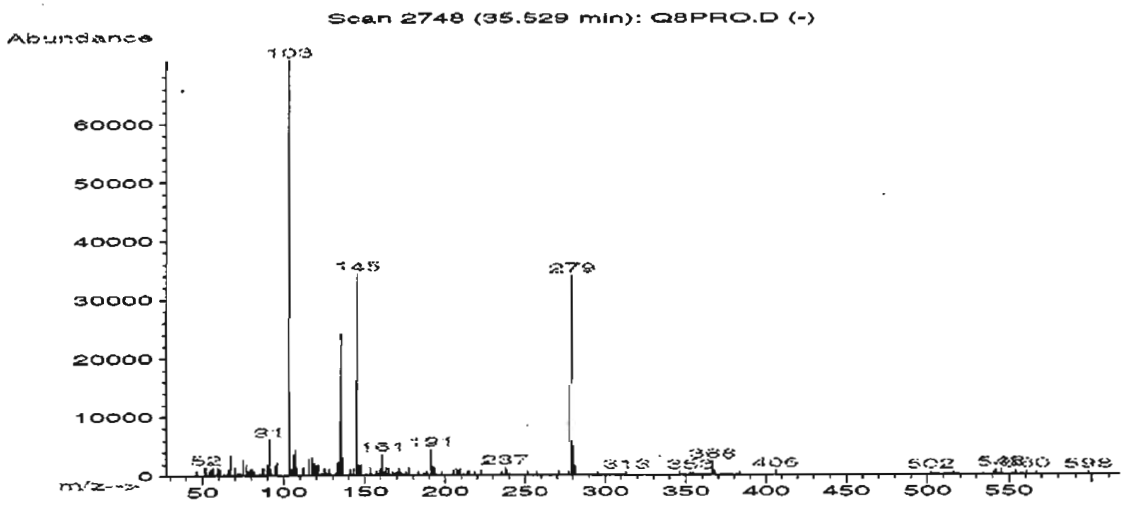

Scan 2598 (33.726 min): Q8PRO.D (-)
Abundance
m/z-->

36

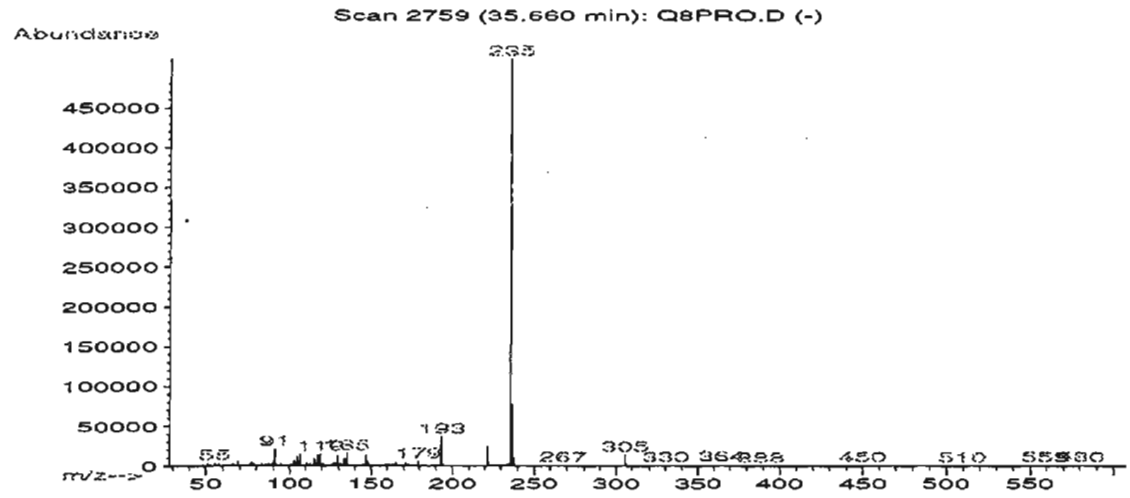


Internal standard at 34.758 minutes.

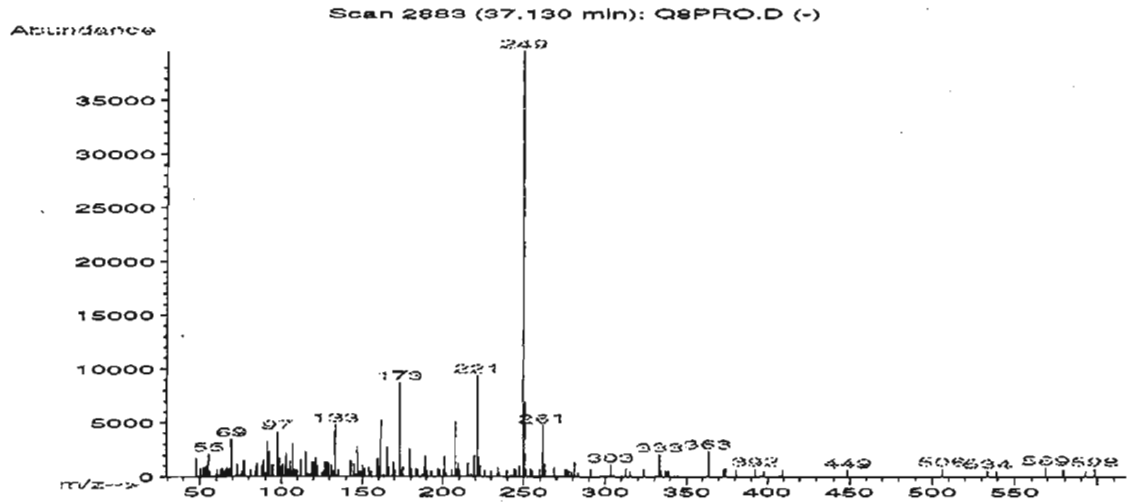
37



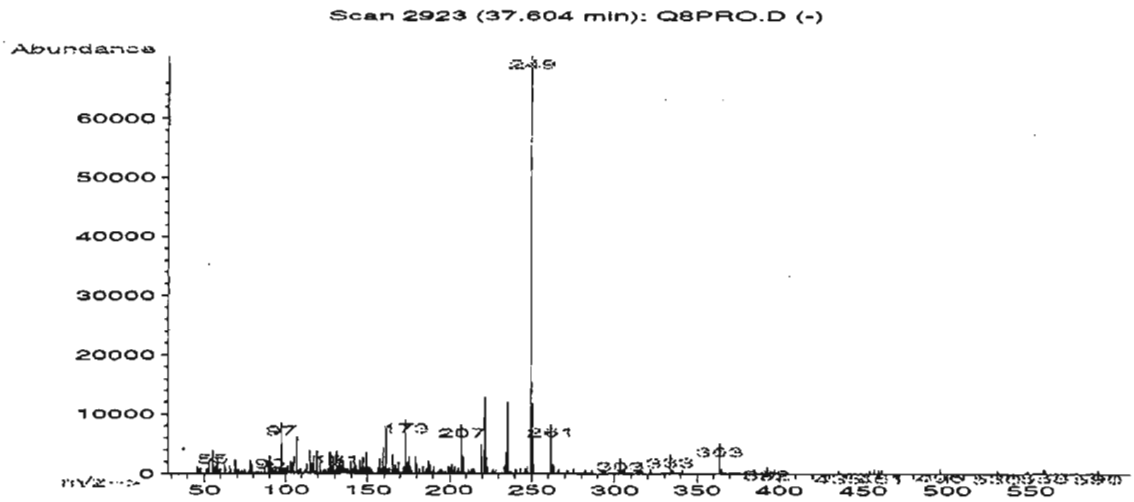
38


Scan 2759 (35.660 min): Q8PRO.D (-)
Abundance
m/z-->

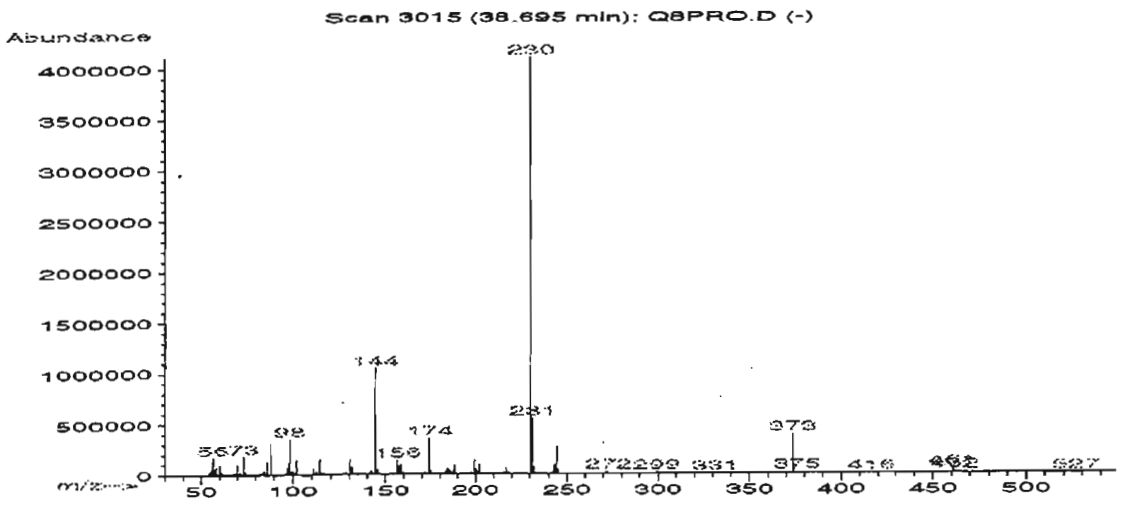
39


Scan 2883 (37.130 min): Q8PRO.D (-)
Abundance
m/z-->

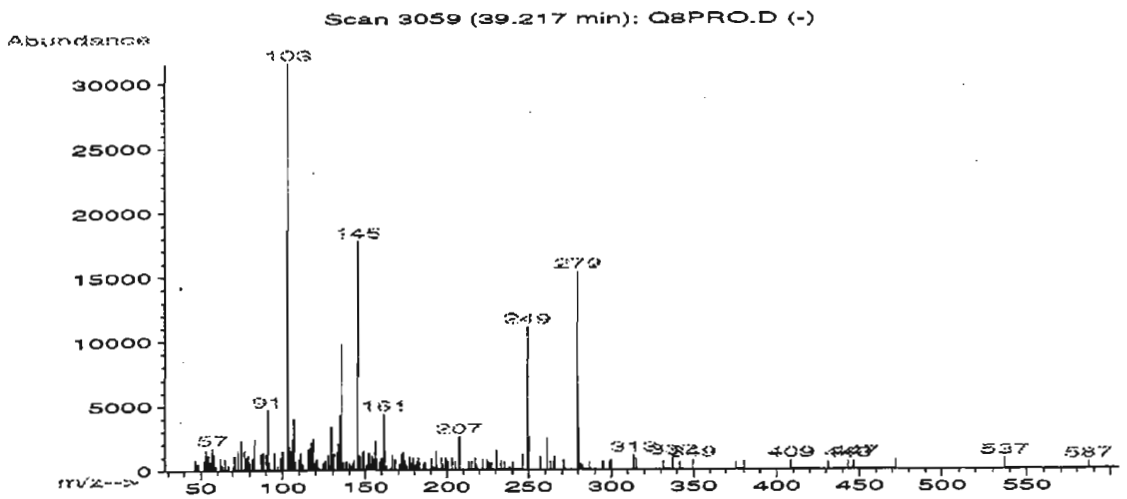
40


Scan 2923 (37.604 min): Q8PRO.D (-)
Abundance
m/z-->

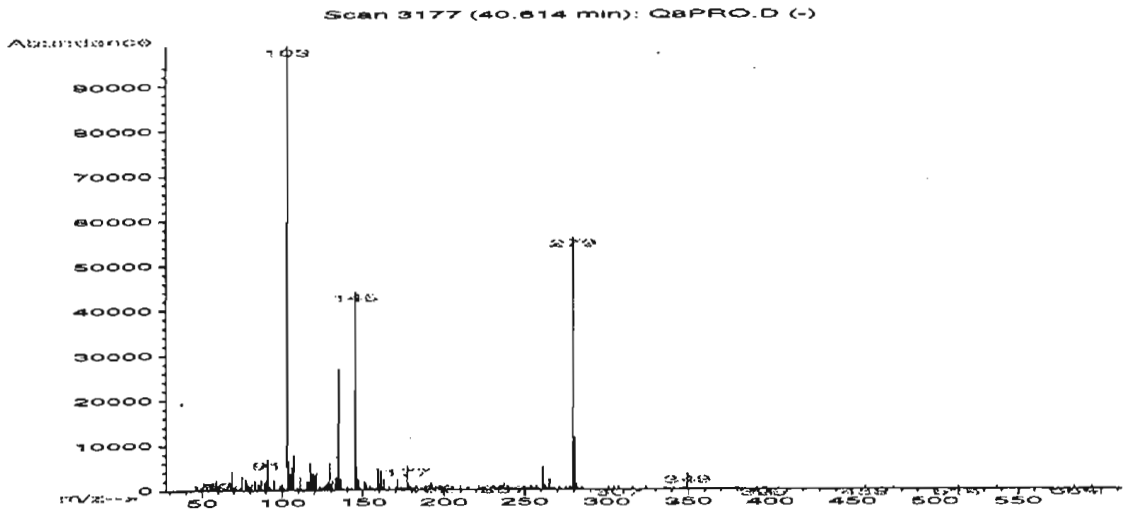
41


Scan 3015 (38.695 min): Q8PRO.D (-)
Abundance
m/z-->

42


Scan 3059 (39.217 min): Q8PRO.D (-)
Abundance
m/z-->

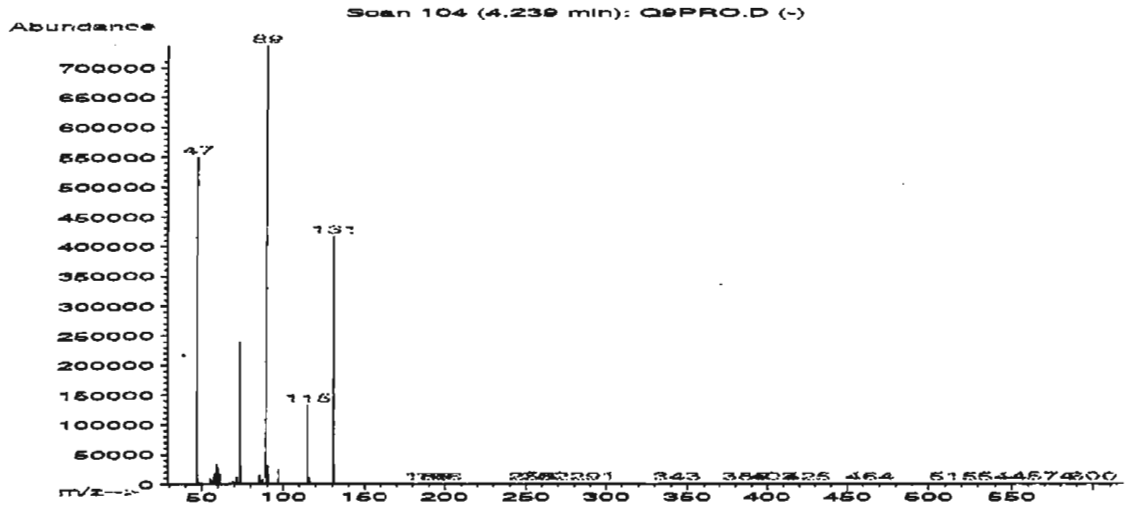
43


Scan 3177 (40.614 min): Q8PRO.D (-)
Abundance
m/z-->

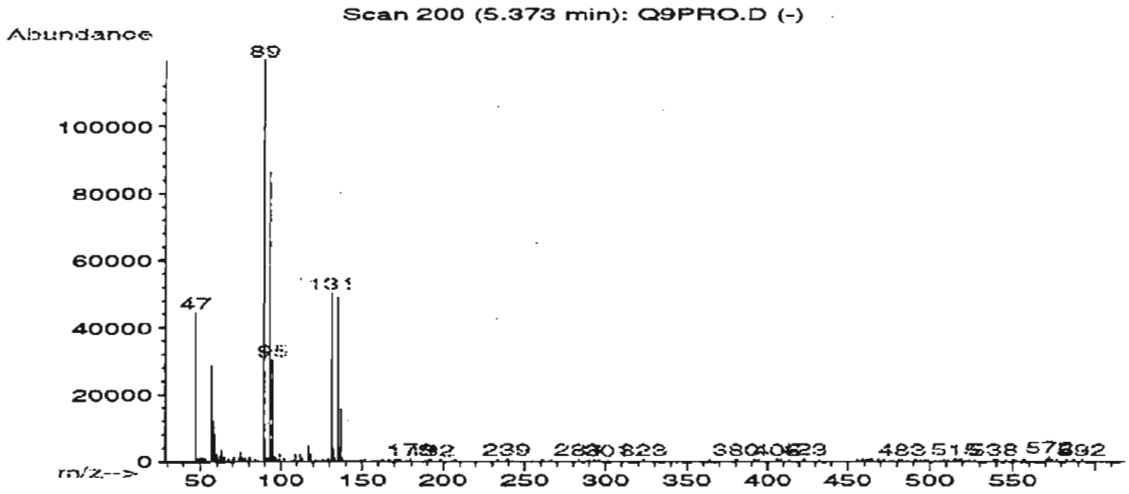
APPENDIX B

Spectra from propylated extract of Q9 (collected 12/9/93), run on EDTA2.M. Numbered as in Table 3.5.

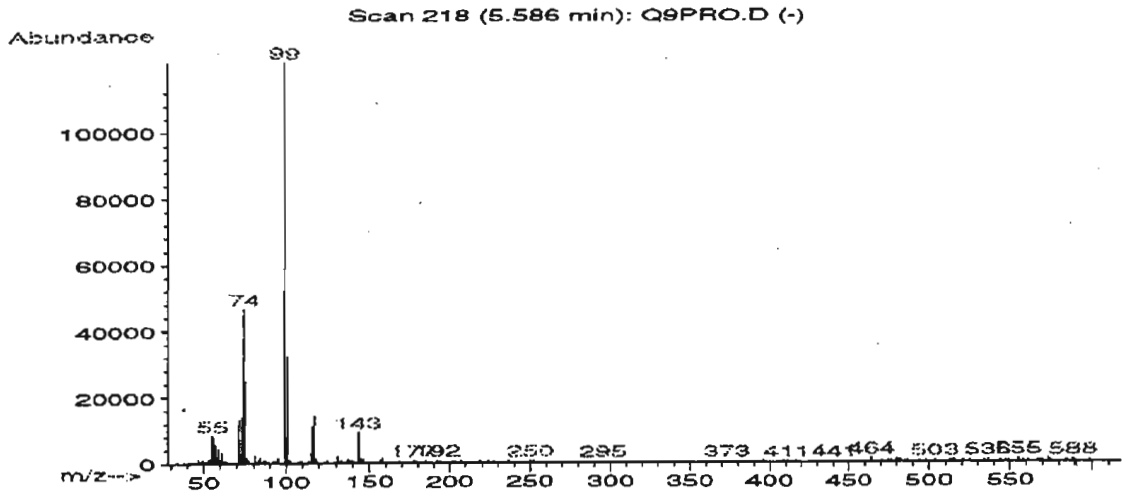
1



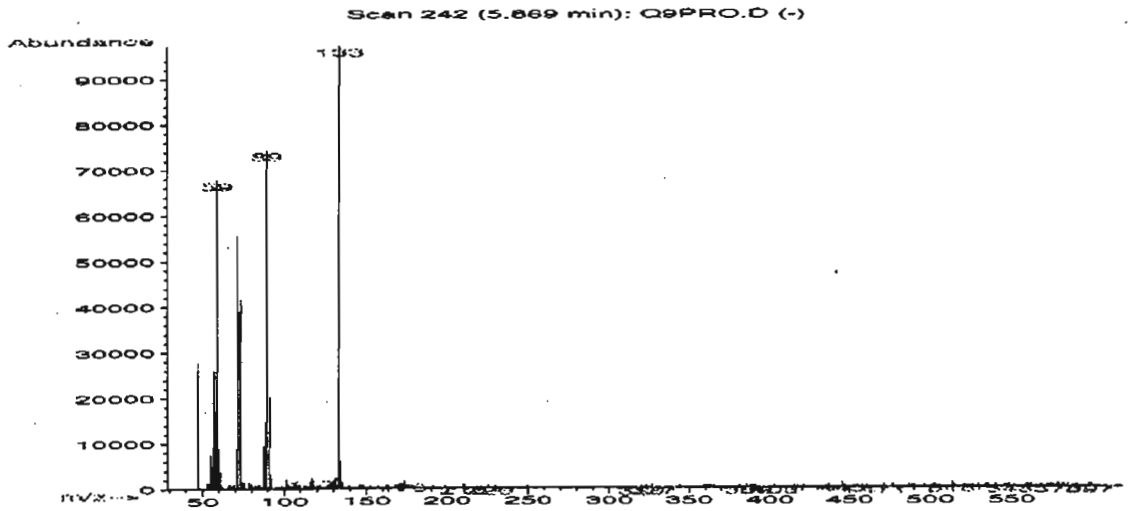
2



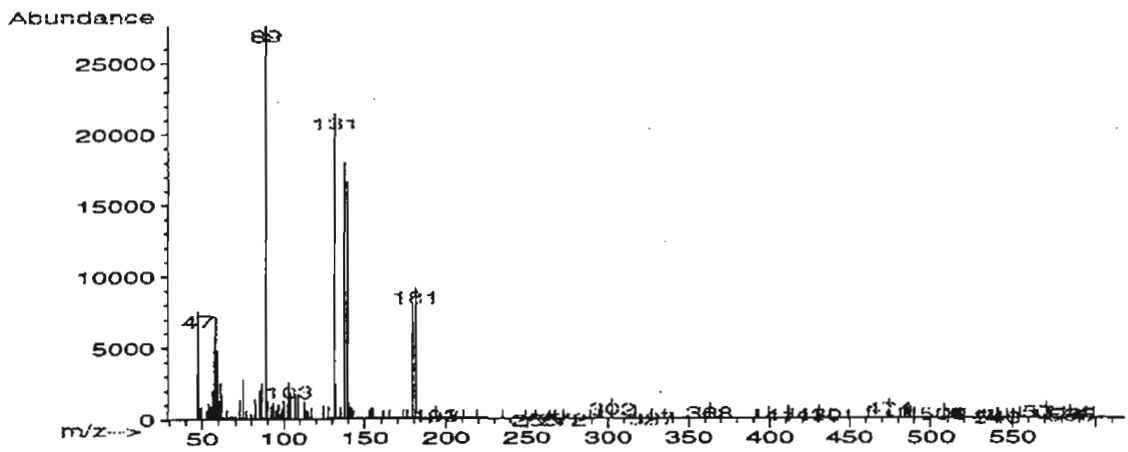
3


Scan 218 (5.586 min): Q9PRO.D (-)
Abundance
99
74
56
143
m/z--->
100000
80000
60000
40000
20000
0
50 100 150 200 250 300 350 400 450 500 550

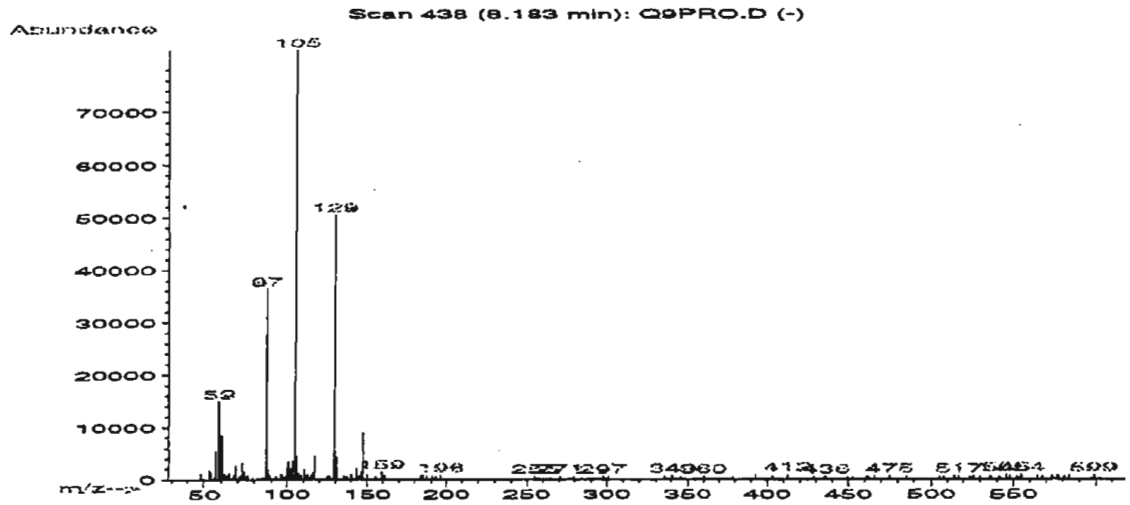
4


Scan 242 (5.869 min): Q9PRO.D (-)
Abundance
193
99
74
m/z--->
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
50 100 150 200 250 300 350 400 450 500 550

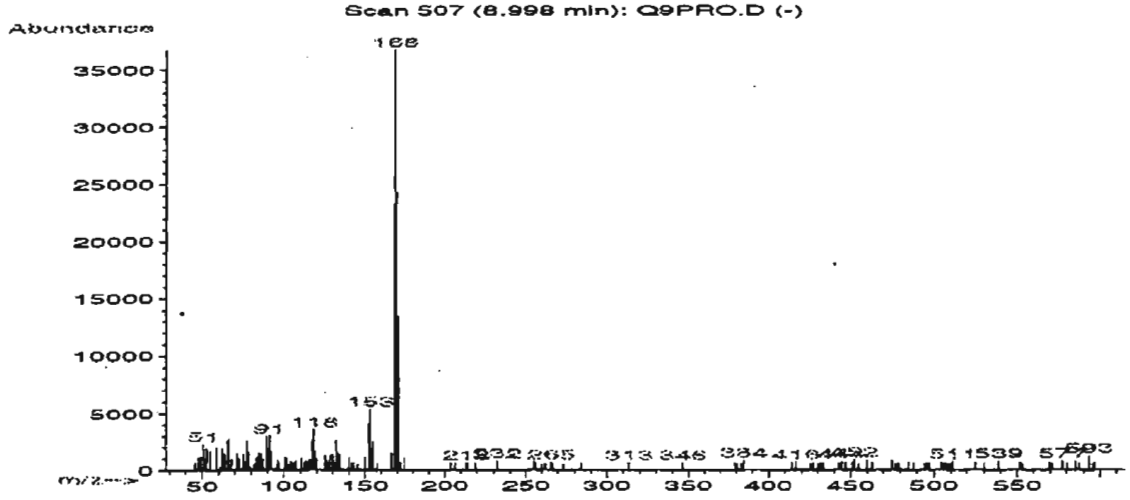
5


Scan 361 (7.274 min): Q9PRO.D (-)
Abundance
85
137
181
47
m/z--->
25000
20000
15000
10000
5000
0
50 100 150 200 250 300 350 400 450 500 550

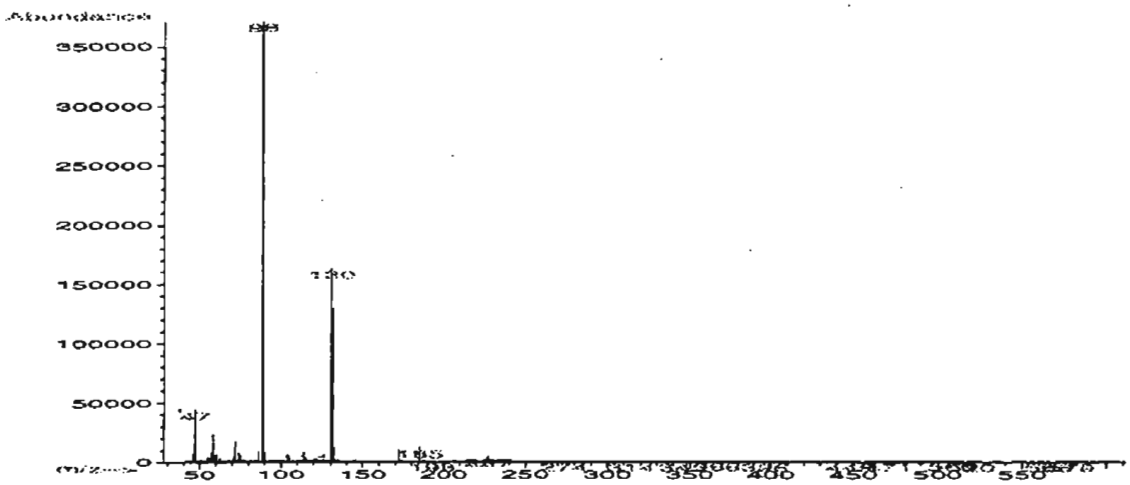
6


Scan 438 (8.183 min): Q9PRO.D (-)
Abundance
105
129
97
52
159
m/z-->

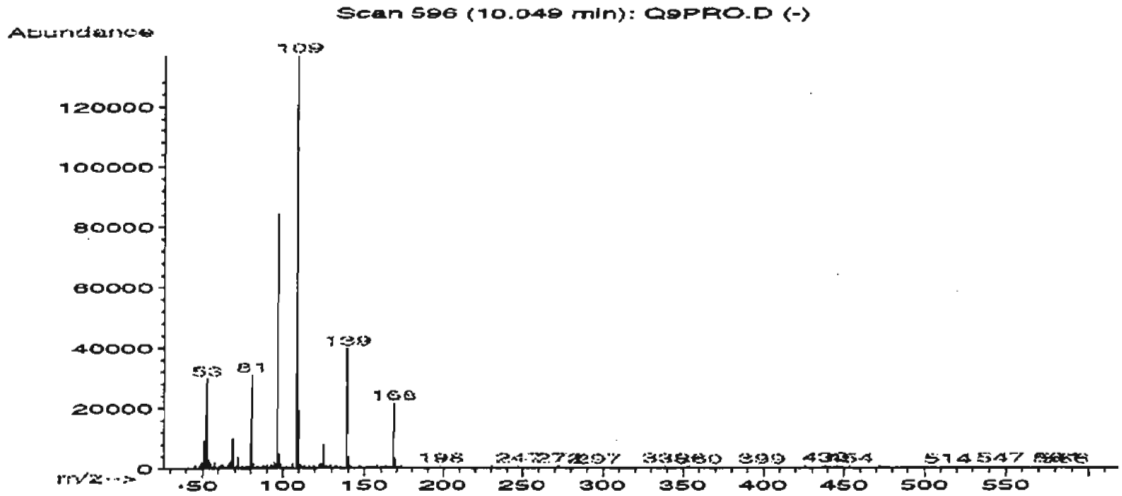
7


Scan 507 (8.998 min): Q9PRO.D (-)
Abundance
168
153
91
116
51
m/z-->

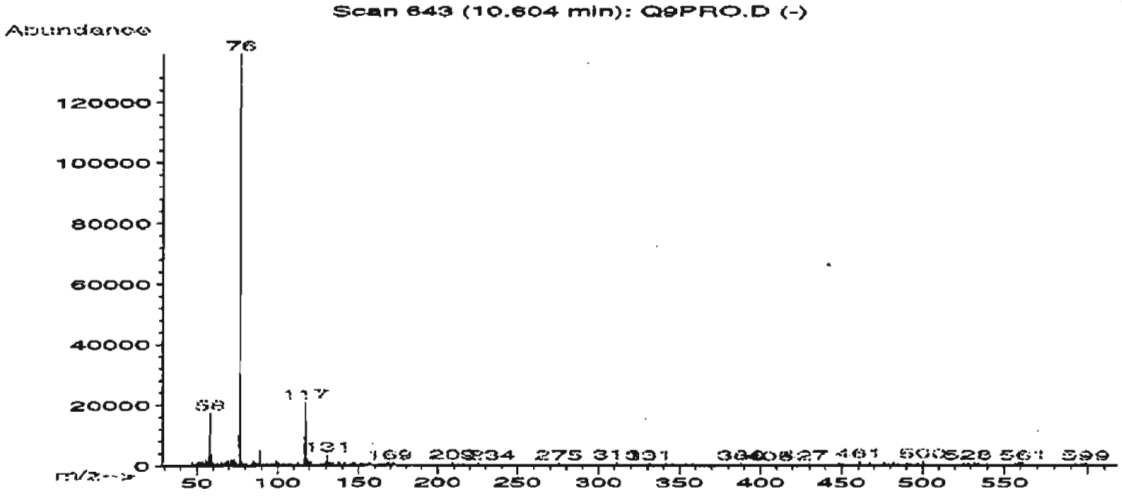
8


Scan 571 (9.754 min): Q9PRO.D (-)
Abundance
m/z-->

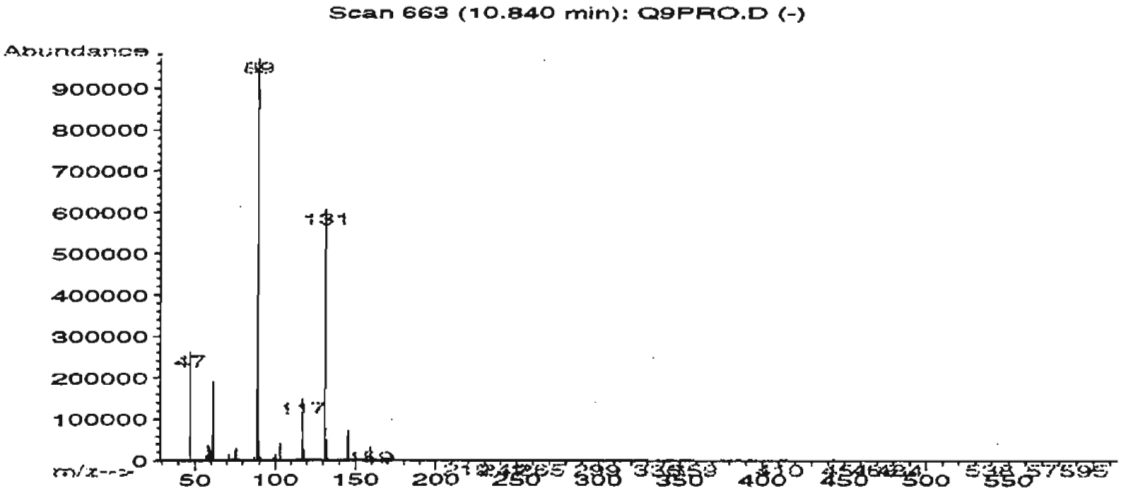
9


Scan 596 (10.049 min): Q9PRO.D (-)
Abundance
109
120000
100000
80000
60000
40000
20000
0
53
81
139
166
m/z-->
50
100
150
200
250
300
350
400
450
500
550

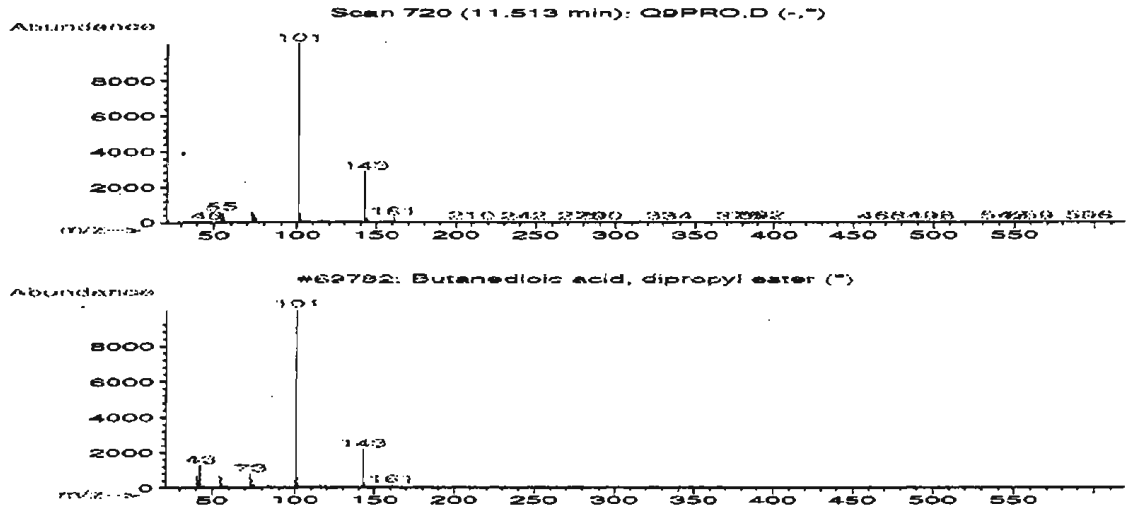
10


Scan 643 (10.604 min): Q9PRO.D (-)
Abundance
76
120000
100000
80000
60000
40000
20000
0
68
117
131
m/z-->
50
100
150
200
250
300
350
400
450
500
550

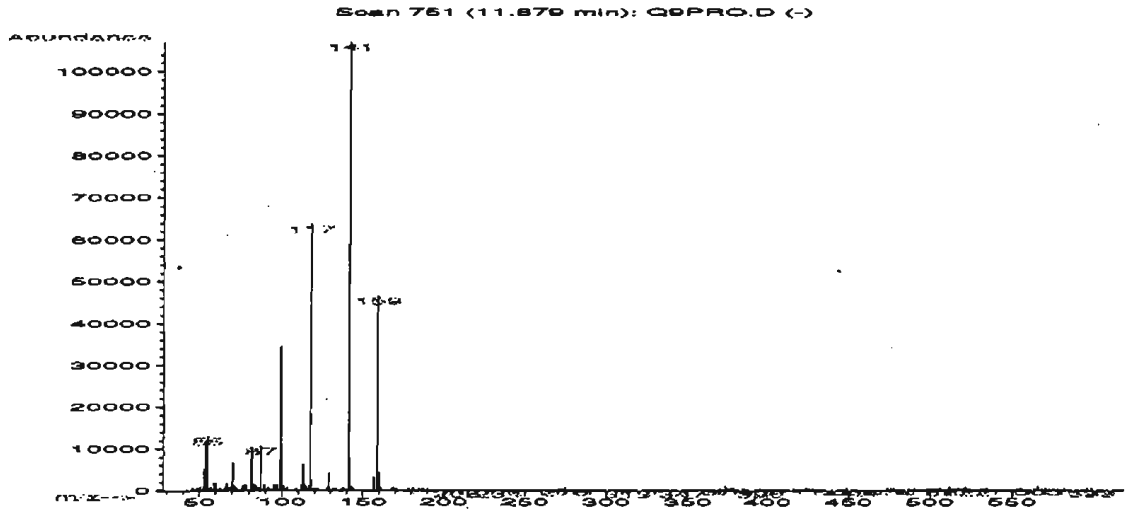
11


Scan 663 (10.840 min): Q9PRO.D (-)
Abundance
69
900000
800000
700000
600000
500000
400000
300000
200000
100000
0
131
47
117
m/z-->
50
100
150
200
250
300
350
400
450
500
550

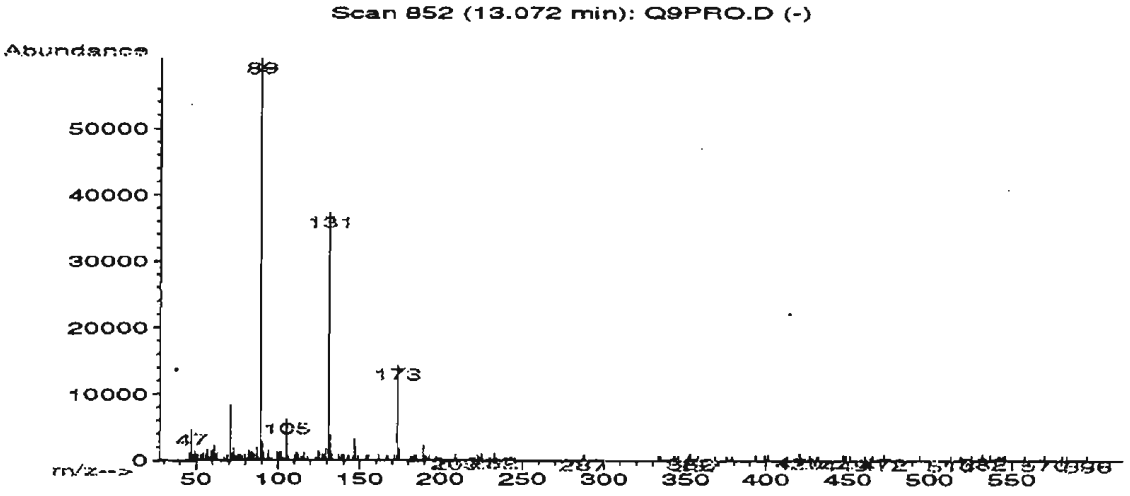
12


Scan 720 (11.513 min): Q9PRO.D (-,*)
Abundance
m/z--->
#62782: Butanedioic acid, dipropyl ester (*)
Abundance
m/z-->

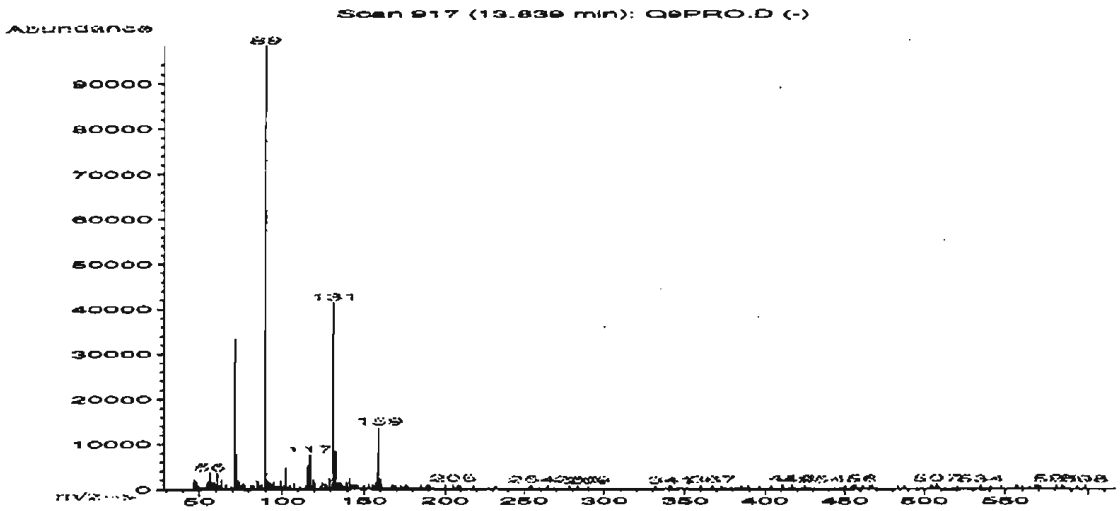
13


Scan 761 (11.879 min): Q9PRO.D (-)
Abundance
m/z-->

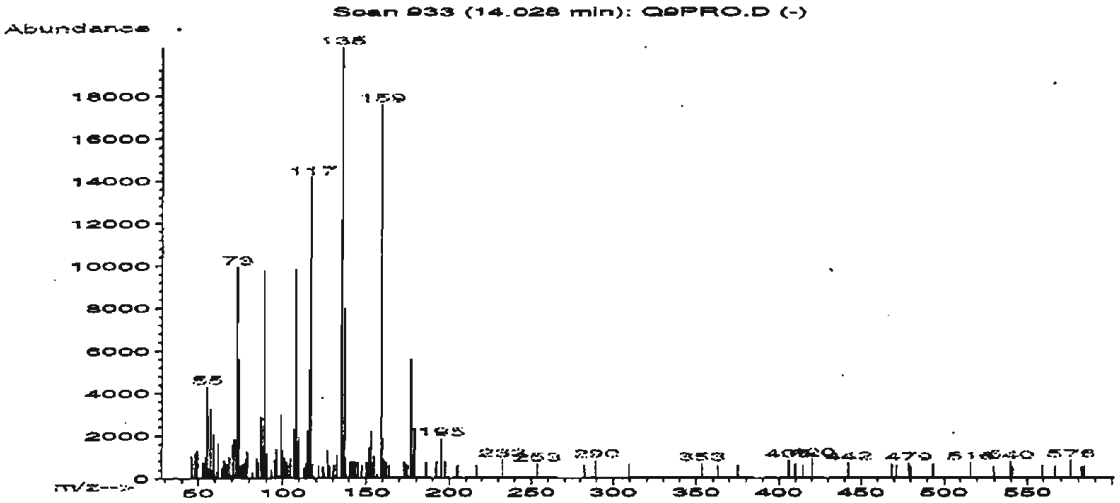
14


Scan 852 (13.072 min): Q9PRO.D (-)
Abundance
m/z-->

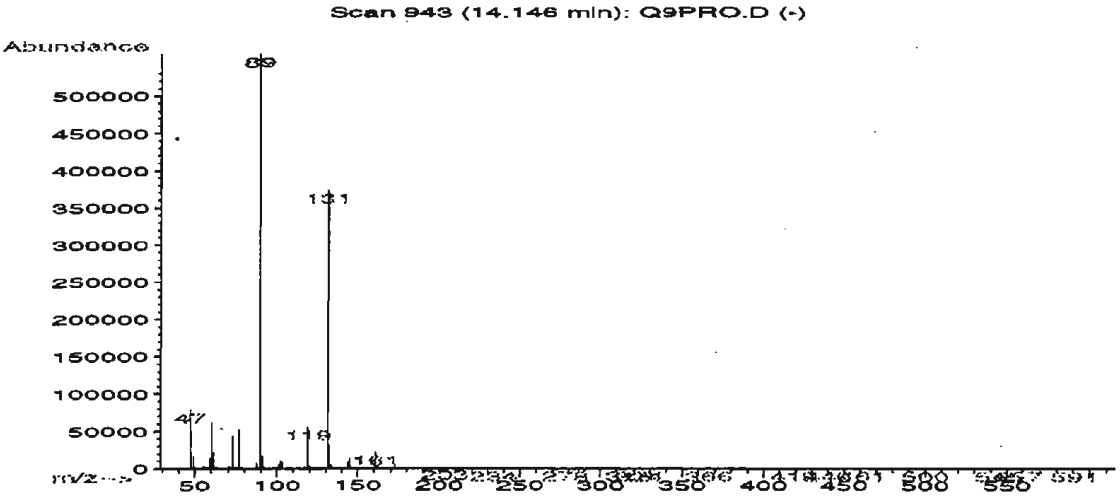
15


Scan 917 (13.839 min): Q9PRO.D (-)
Abundance
89
131
159
117
m/z-->

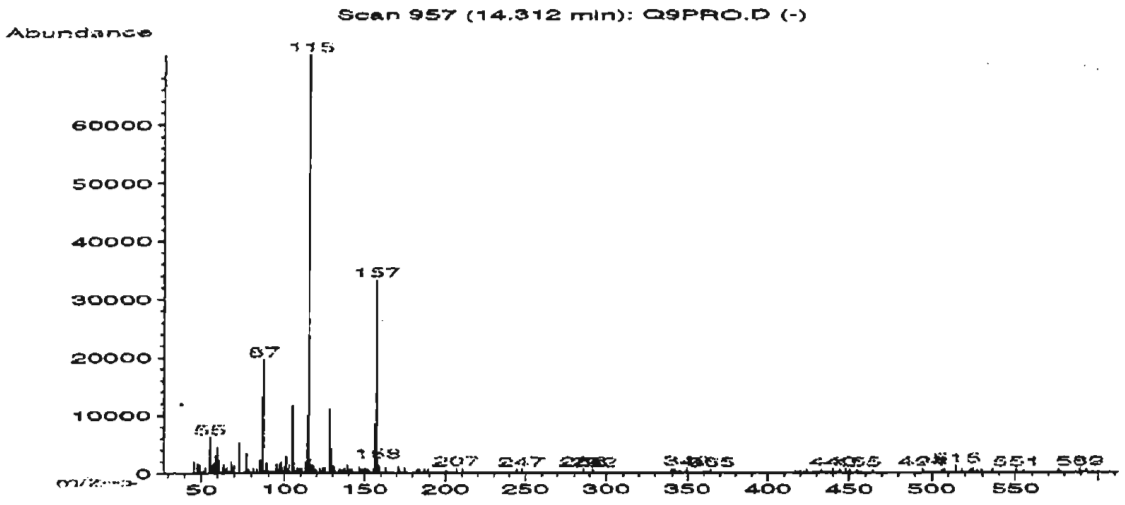
16


Scan 933 (14.028 min): Q9PRO.D (-)
Abundance
135
159
117
73
55
195
m/z-->

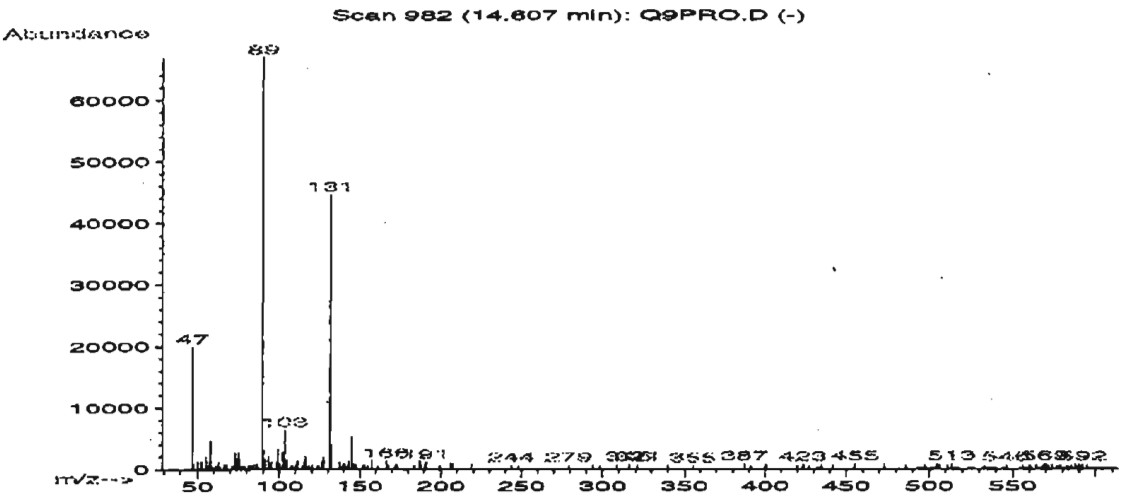
17


Scan 943 (14.146 min): Q9PRO.D (-)
Abundance
89
131
47
116
m/z-->

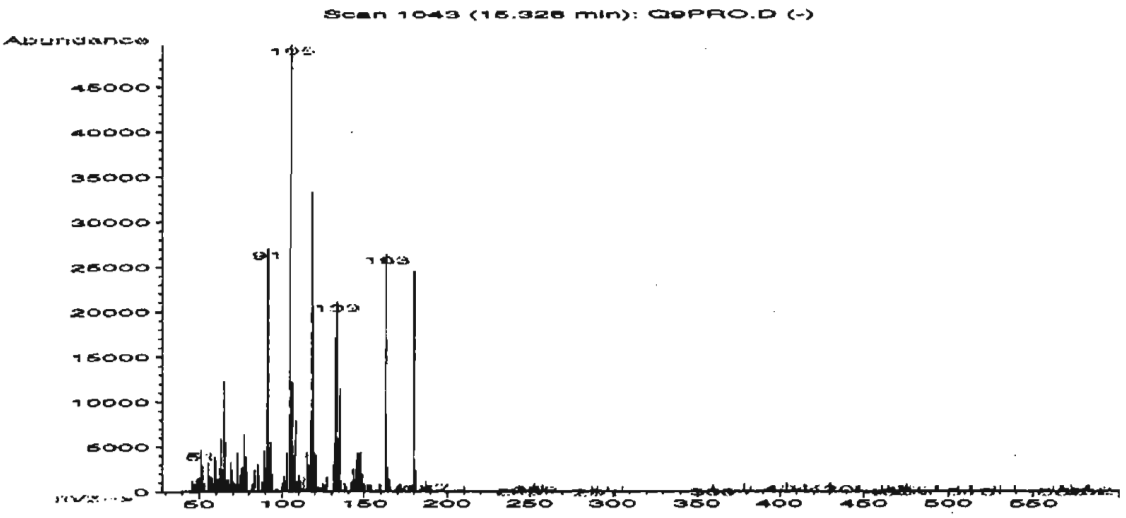
18


Scan 957 (14.312 min): Q9PRO.D (-)
Abundance
115
157
67
55
m/z-->

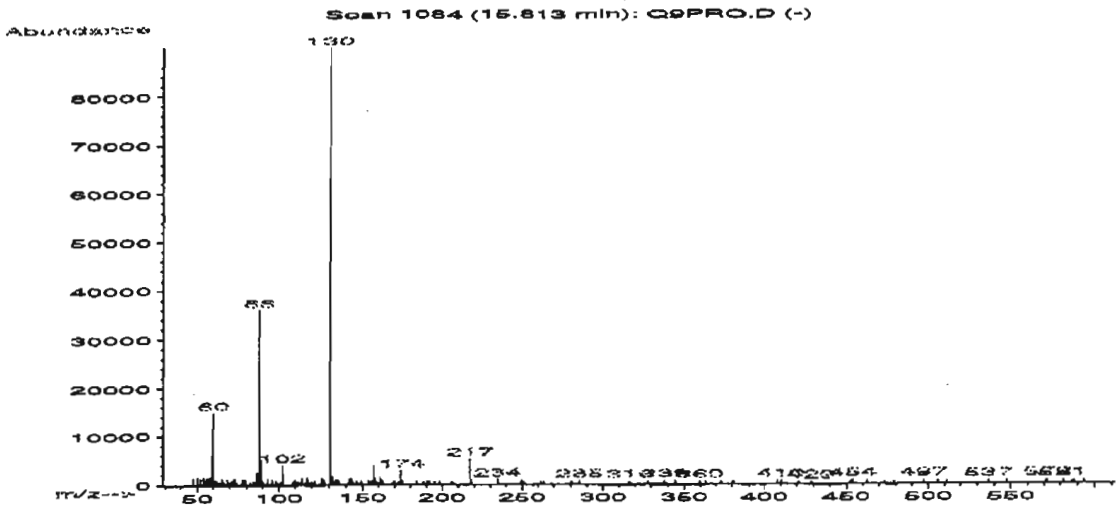
19


Scan 982 (14.607 min): Q9PRO.D (-)
Abundance
89
131
47
m/z-->

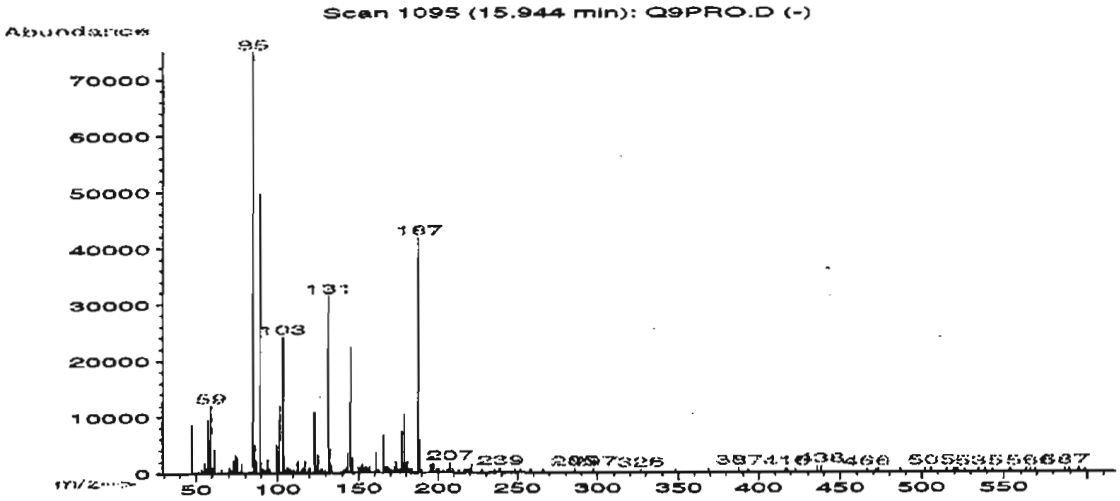
20


Scan 1043 (15.326 min): Q9PRO.D (-)
Abundance
m/z-->

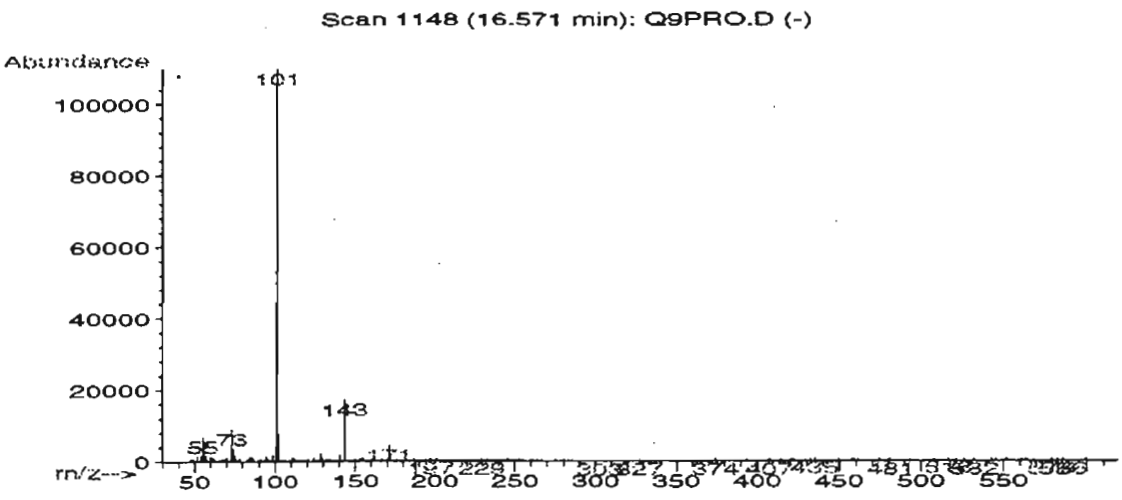
21


Scan 1084 (15.813 min): Q9PRO.D (-)
Abundance
130
80000
70000
60000
50000
40000
30000
20000
10000
0
55
69
102
174
217
234
m/z-->
50
100
150
200
250
300
350
400
450
500
550

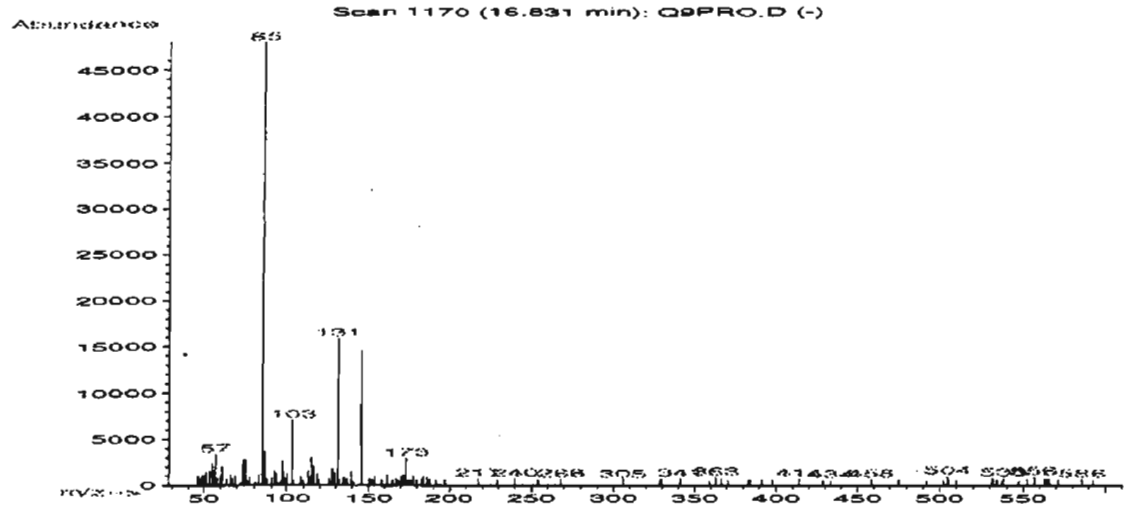
22


Scan 1095 (15.944 min): Q9PRO.D (-)
Abundance
95
70000
60000
50000
40000
30000
20000
10000
0
167
131
103
69
207
239
m/z-->
50
100
150
200
250
300
350
400
450
500
550

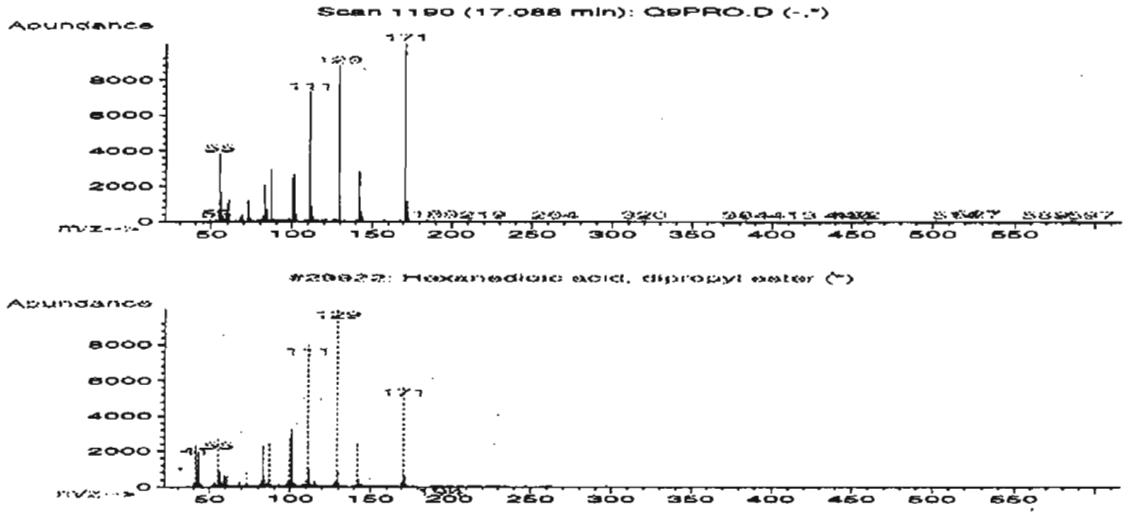
23


Scan 1148 (16.571 min): Q9PRO.D (-)
Abundance
101
100000
80000
60000
40000
20000
0
143
73
55
111
m/z-->
50
100
150
200
250
300
350
400
450
500
550

24

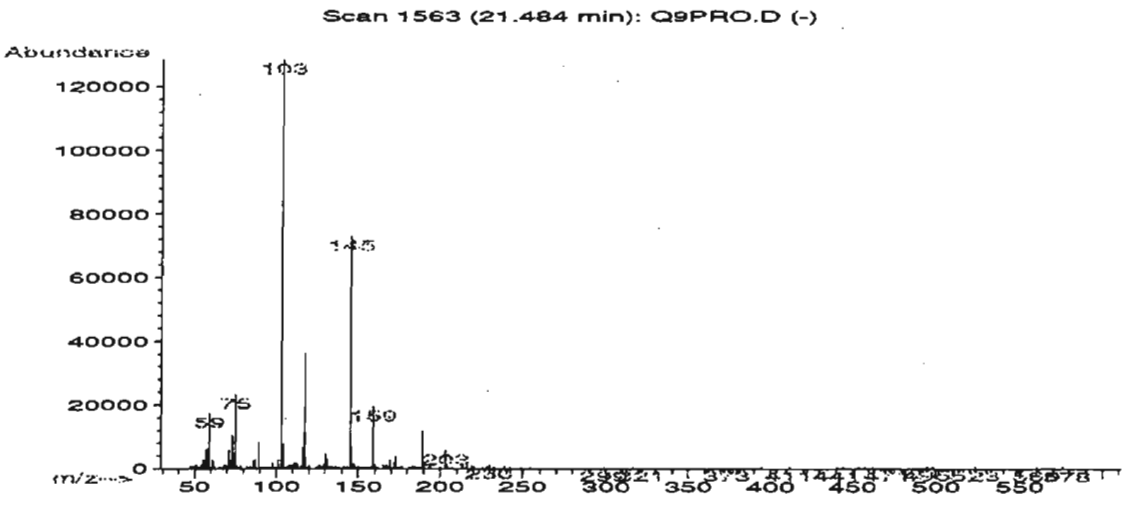

Scan 1170 (16.831 min): Q9PRO.D (-)
Abundance
m/z-->

25

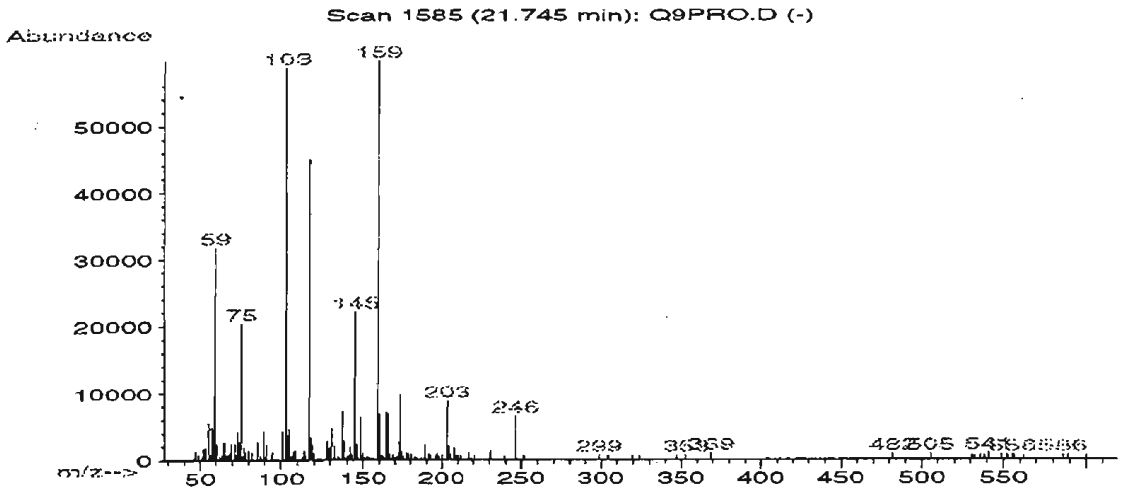

Scan 1190 (17.088 min): Q9PRO.D (-,*)
Abundance
m/z-->

#20923: Hexanedioic acid, dipropyl ester (*)
Abundance
m/z-->

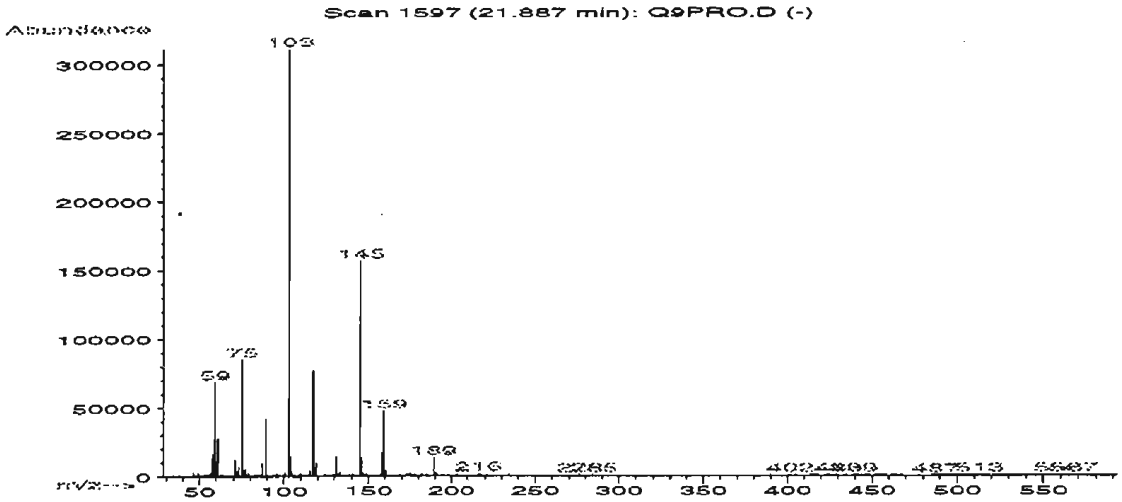
26


Scan 1563 (21.484 min): Q9PRO.D (-)
Abundance
m/z-->

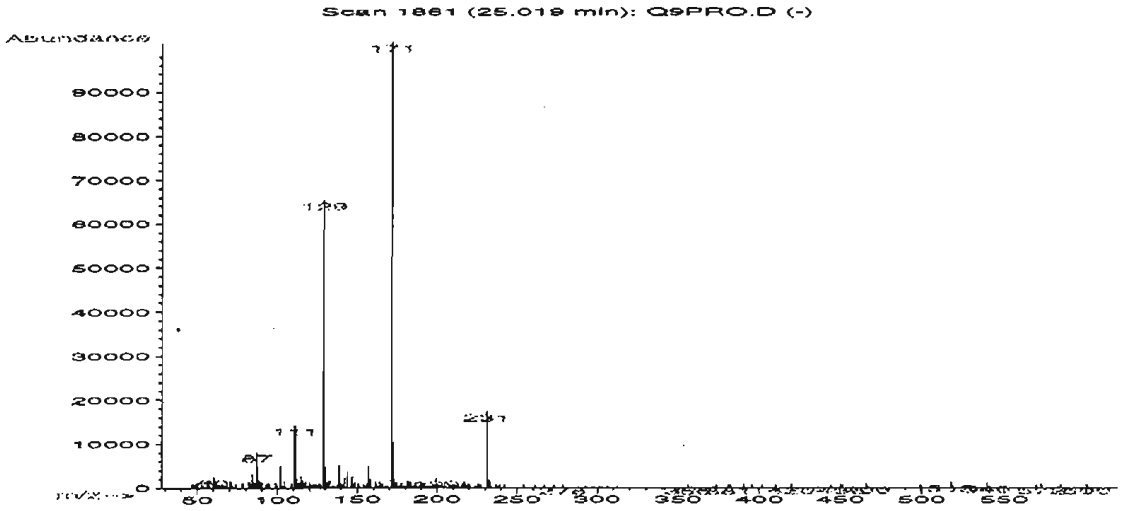
27


Scan 1585 (21.745 min): Q9PRO.D (-)
Abundance
103
159
59
75
145
203
246
m/z-->

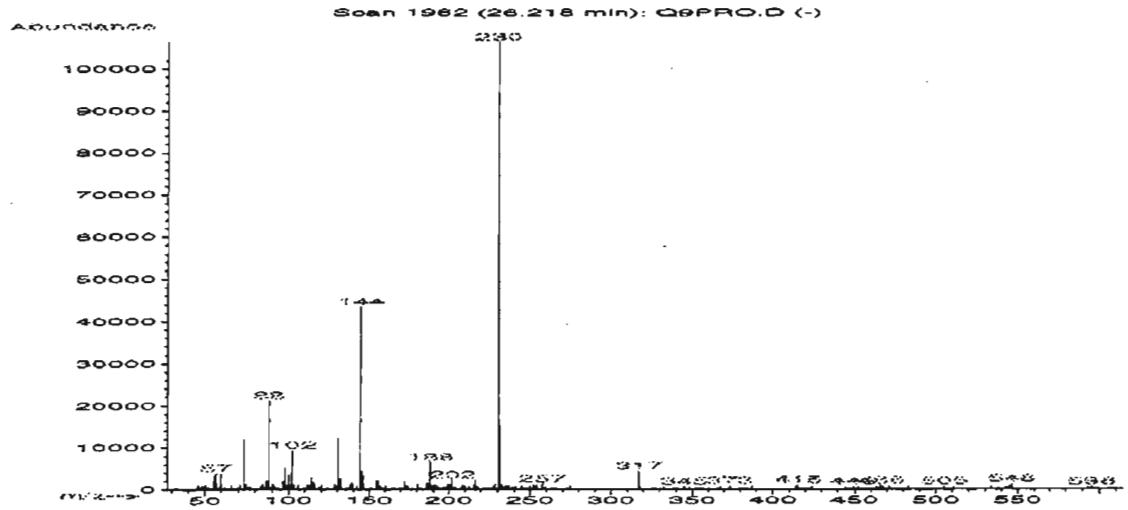
28


Scan 1597 (21.887 min): Q9PRO.D (-)
Abundance
103
145
75
59
159
189
m/z-->

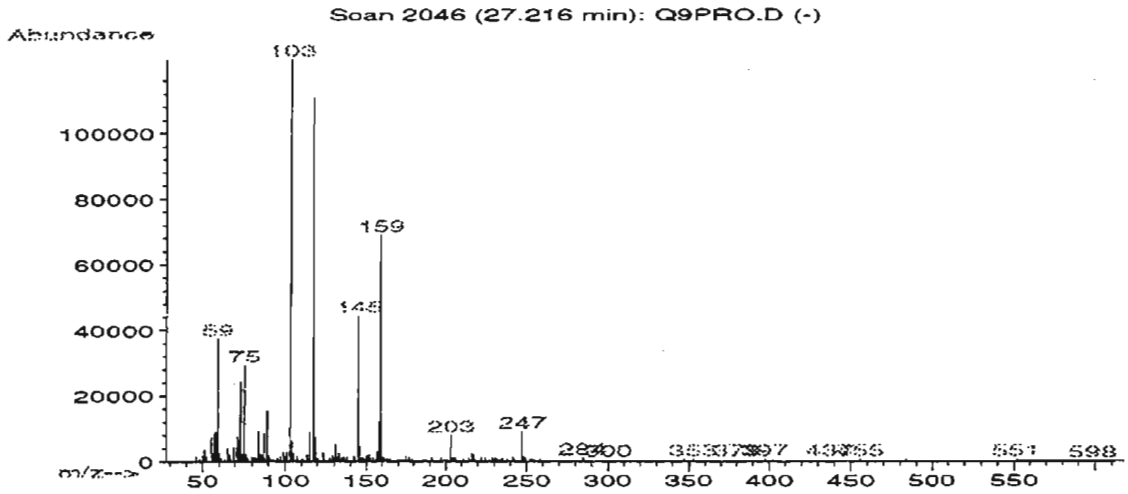
29


Scan 1861 (25.019 min): Q9PRO.D (-)
Abundance
111
129
111
97
m/z-->

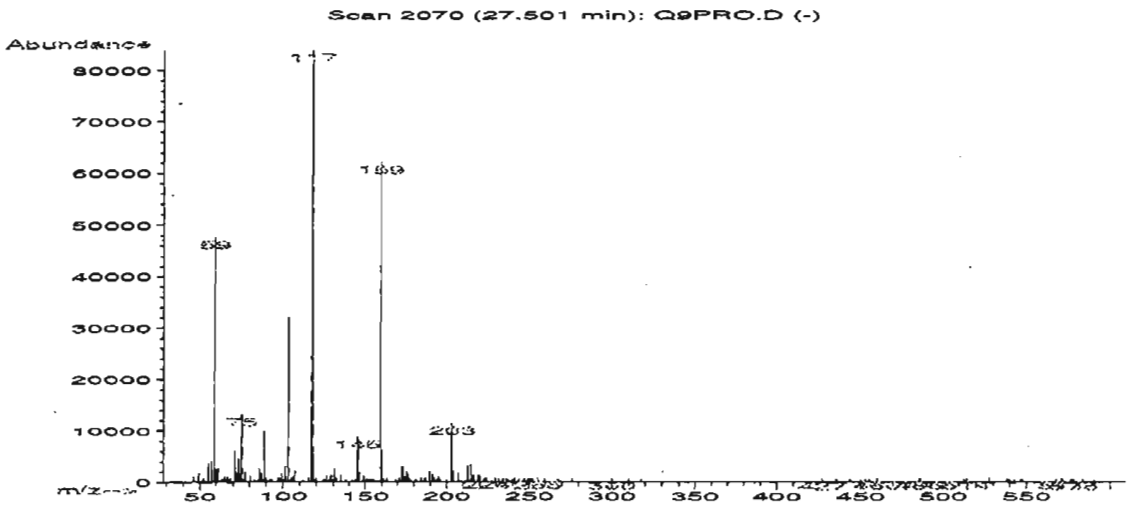
30


Scan 1962 (26.218 min): Q9PRO.D (-)
Abundance
m/z-->

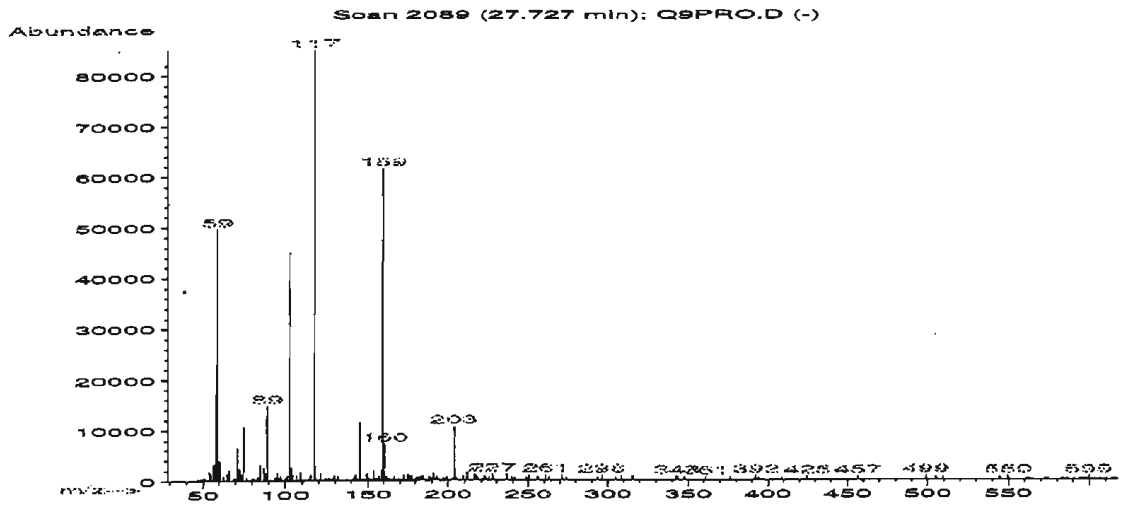
31


Scan 2046 (27.216 min): Q9PRO.D (-)
Abundance
103
159
145
59
75
203
247
m/z-->

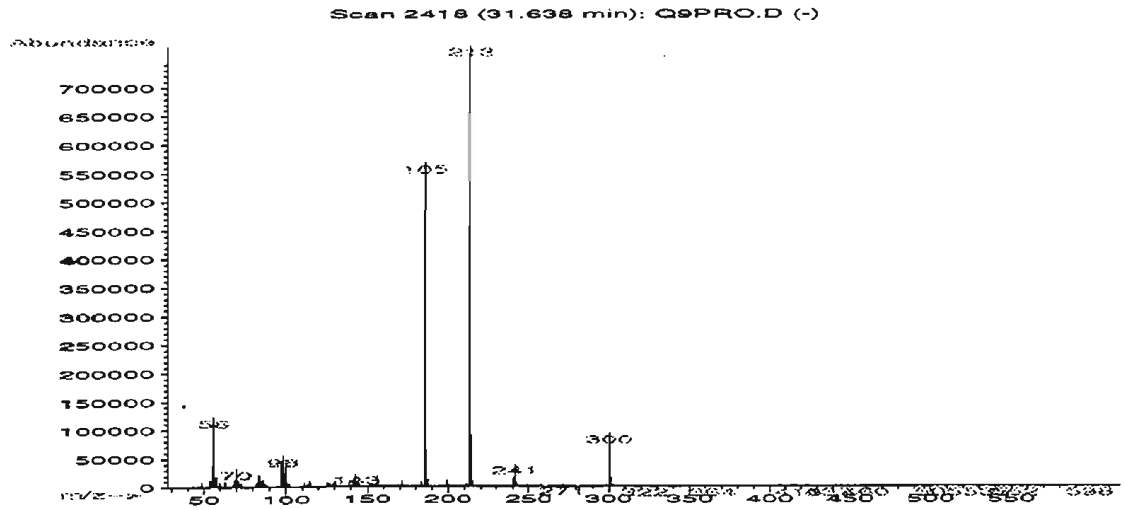
32


Scan 2070 (27.501 min): Q9PRO.D (-)
Abundance
117
159
59
75
145
203
m/z-->

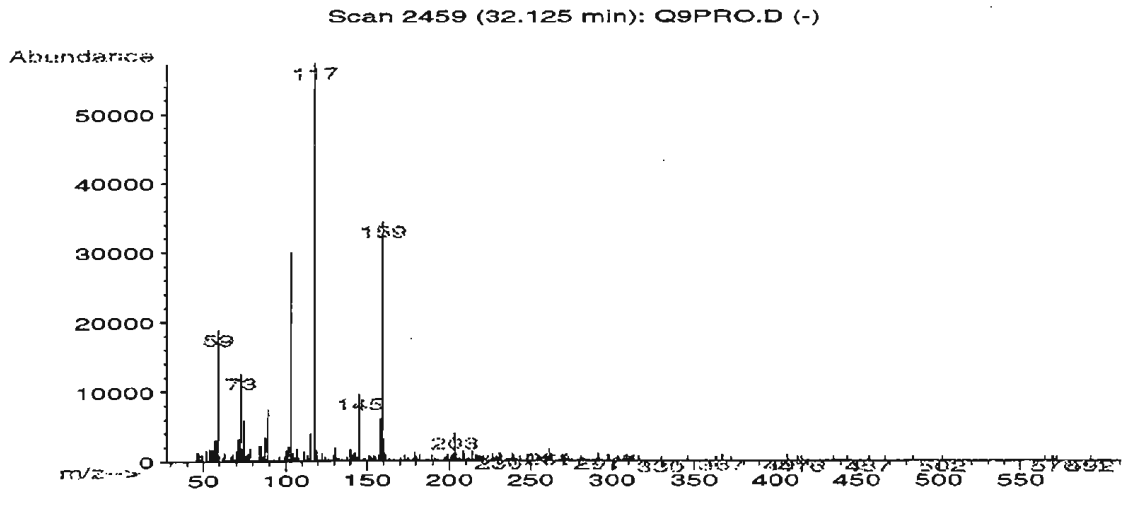
33


Scan 2089 (27.727 min): Q9PRO.D (-)
Abundance
m/z-->

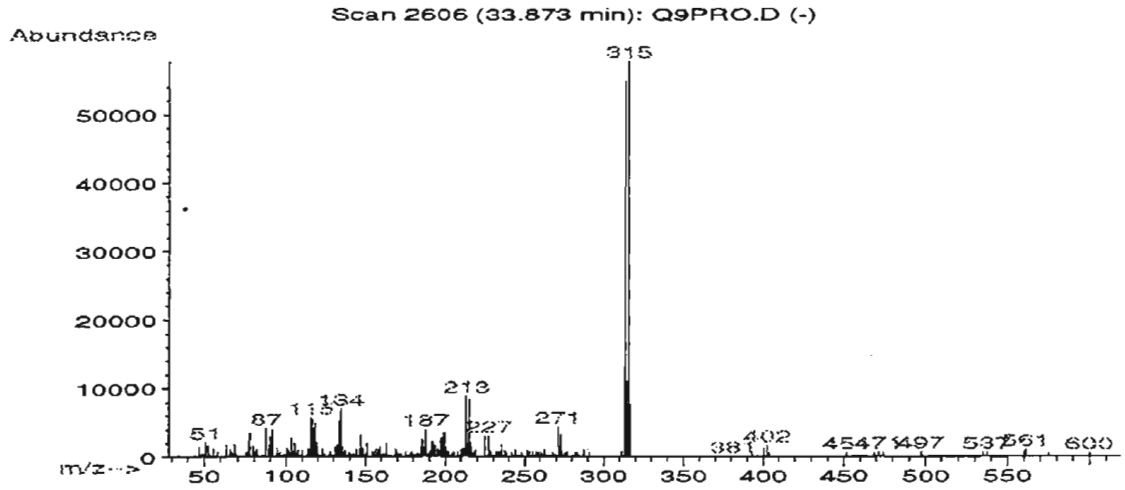
34


Scan 2418 (31.638 min): Q9PRO.D (-)
Abundance
m/z-->

35

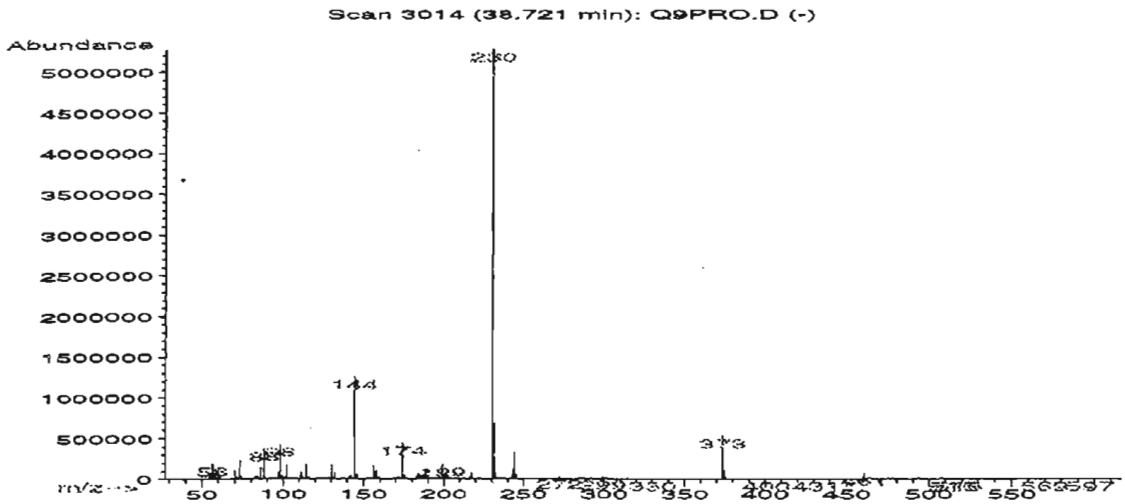

Scan 2459 (32.125 min): Q9PRO.D (-)
Abundance
m/z-->

36

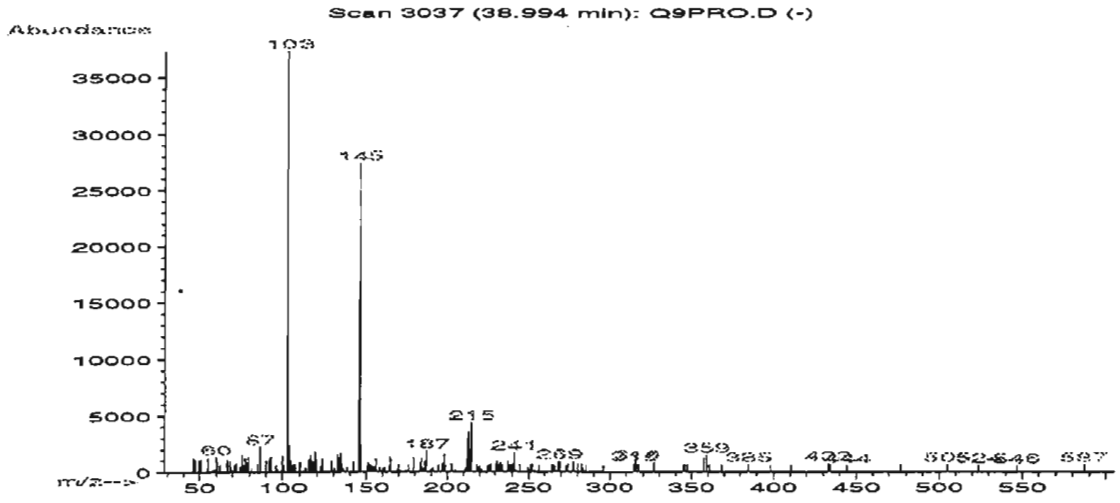


Internal standard at 34.789 minutes.

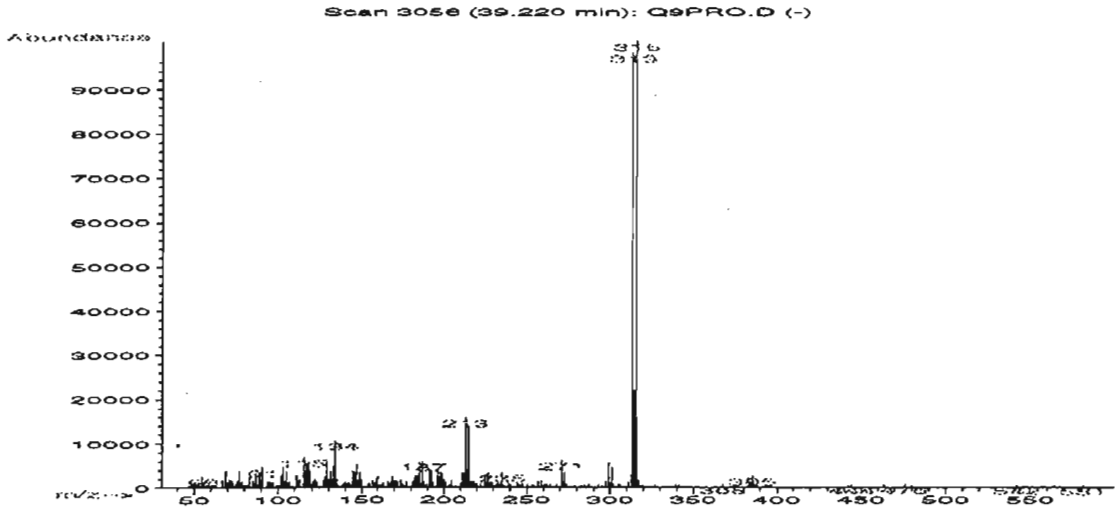
37



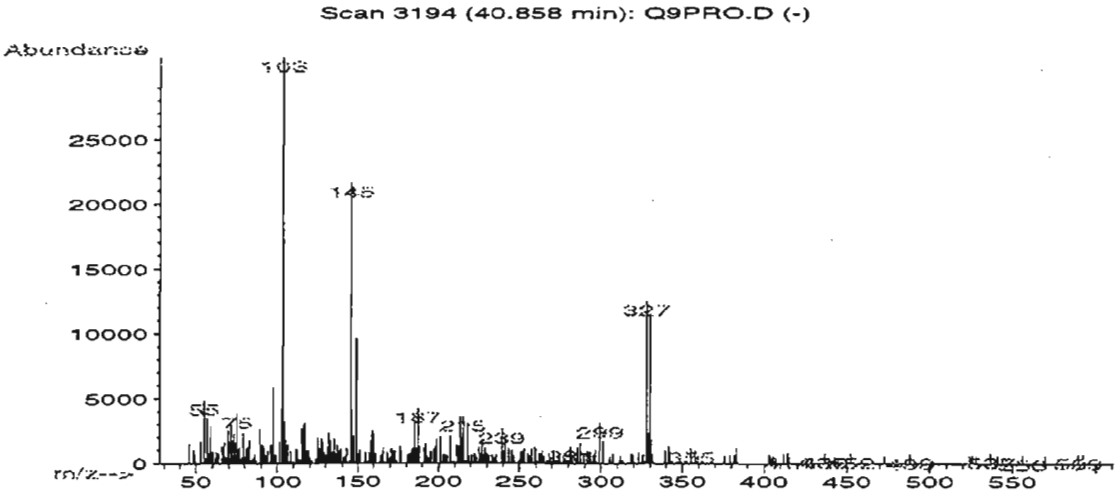
38


Scan 3037 (38.994 min): Q9PRO.D (-)
Abundance
103
145
215
187
241
359
m/z-->

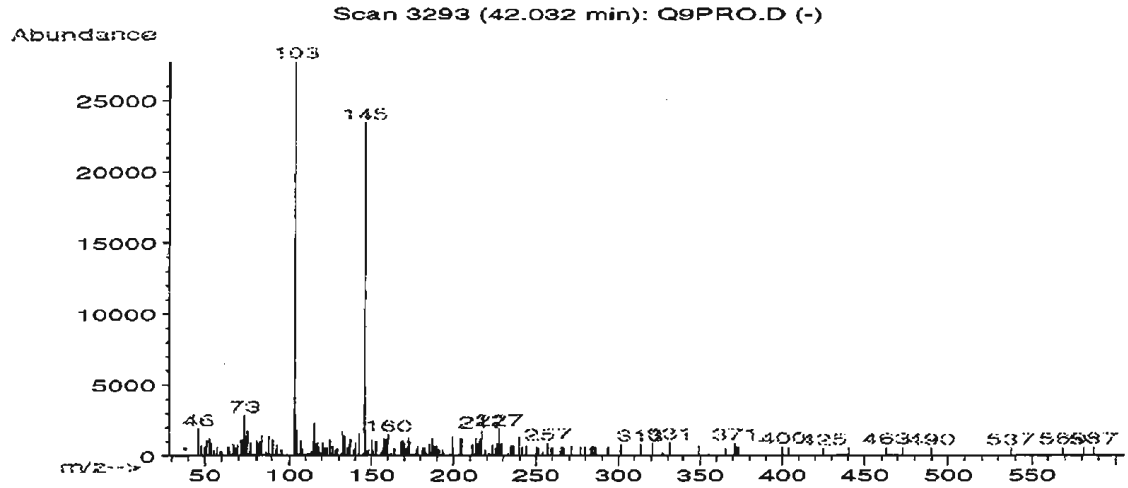
39


Scan 3056 (39.220 min): Q9PRO.D (-)
Abundance
213
m/z-->

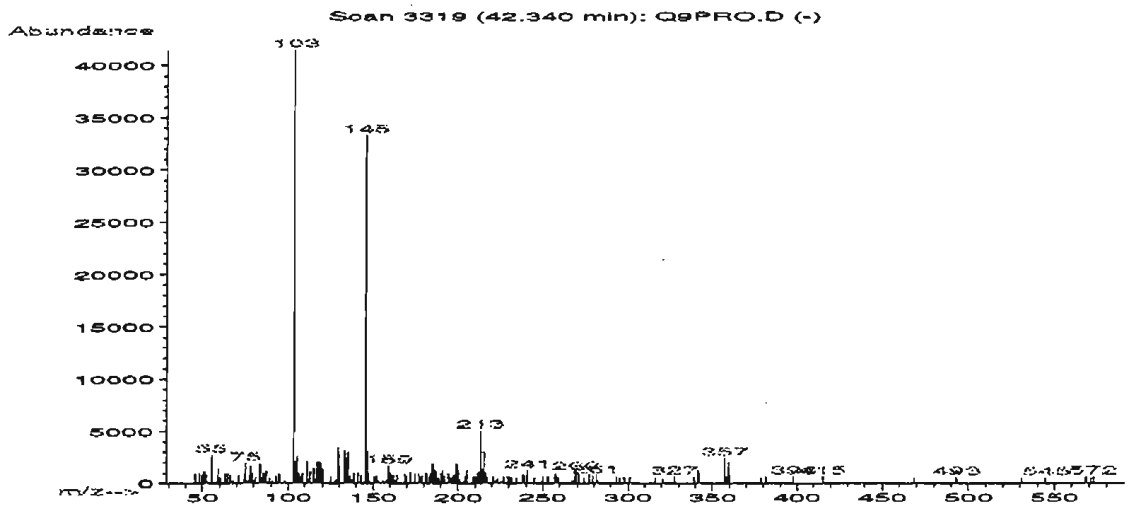
40


Scan 3194 (40.858 min): Q9PRO.D (-)
Abundance
103
145
327
187
215
239
299
m/z-->

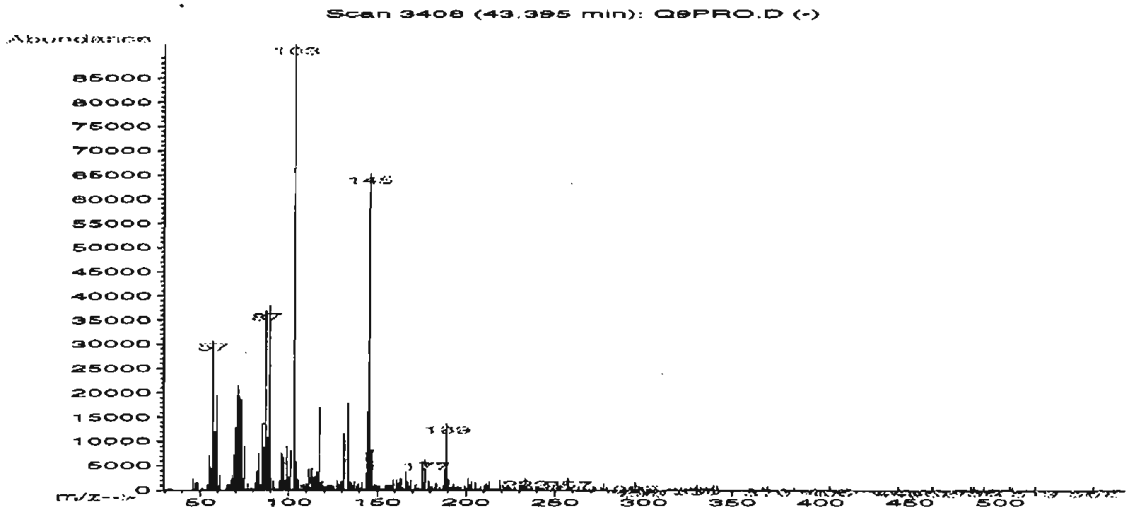
41


Scan 3293 (42.032 min): Q9PRO.D (-)
Abundance
m/z-->

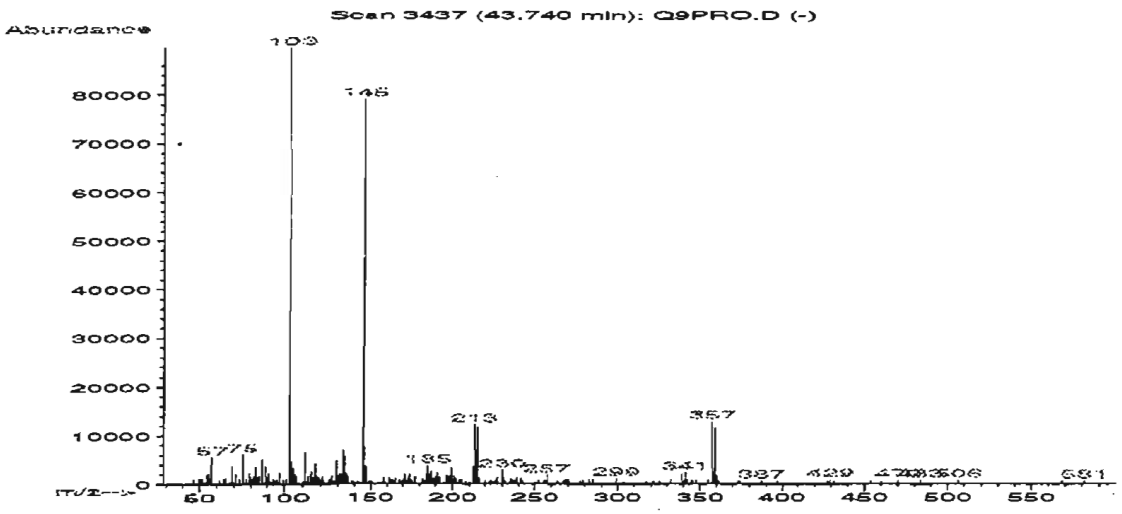
42


Scan 3319 (42.340 min): Q9PRO.D (-)
Abundance
m/z-->

43


Scan 3408 (43.395 min): Q9PRO.D (-)
Abundance
m/z-->

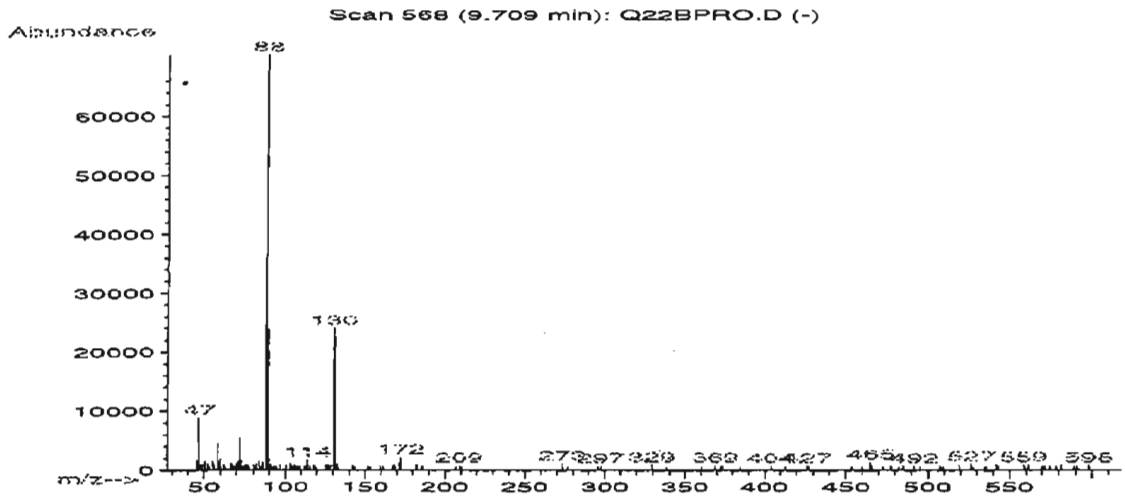
44


Scan 3437 (43.740 min): Q9PRO.D (-)
Abundance
103
145
213
357
5775
155
230
257
299
341
337
m/z
80000
70000
60000
50000
40000
30000
20000
10000
0
60
100
150
200
250
300
350
400
450
500
550

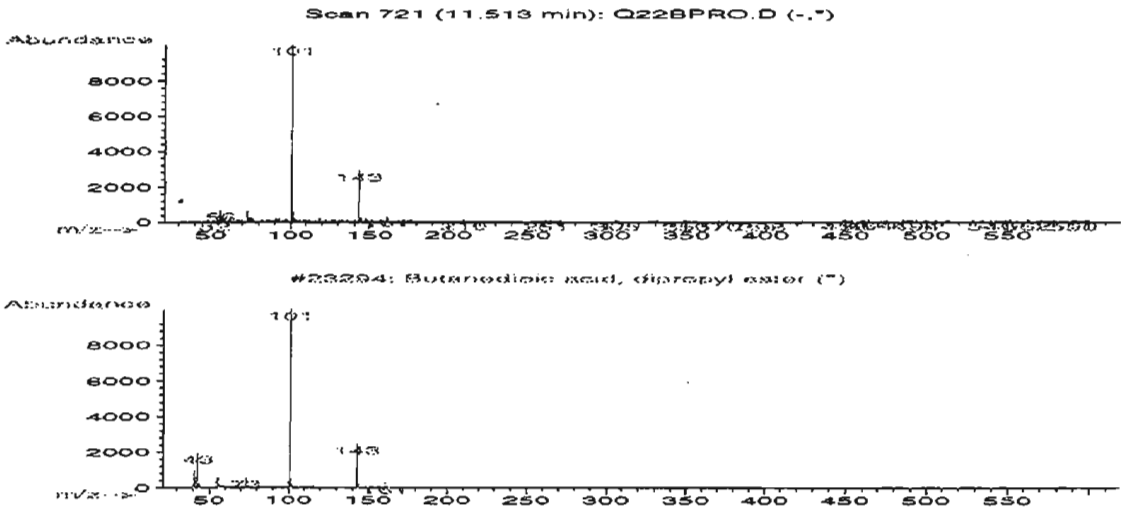
APPENDIX C

Spectra from propylated extract of Q22B (collected 12/9/93), run on EDTA2.M. Numbered as in Table 3.6.

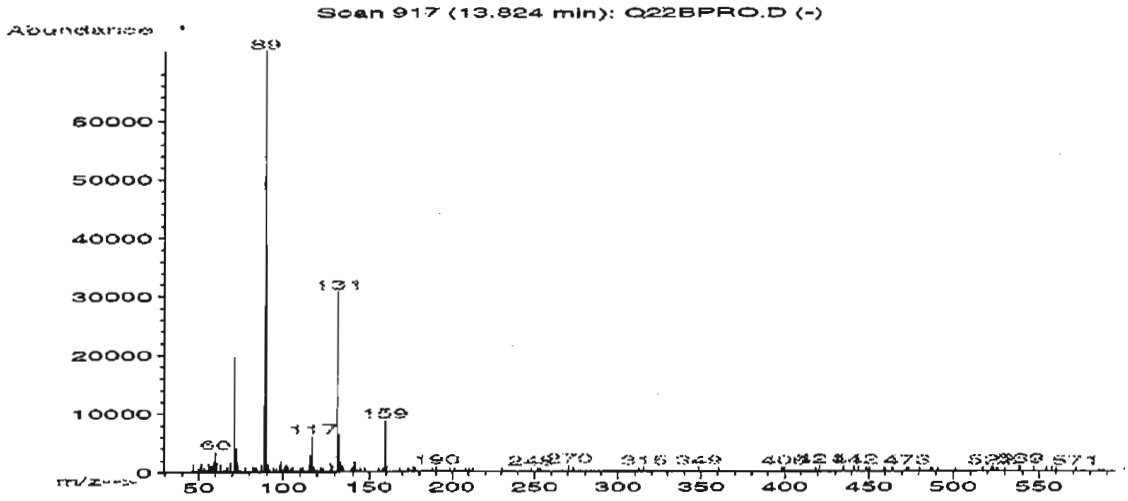
1



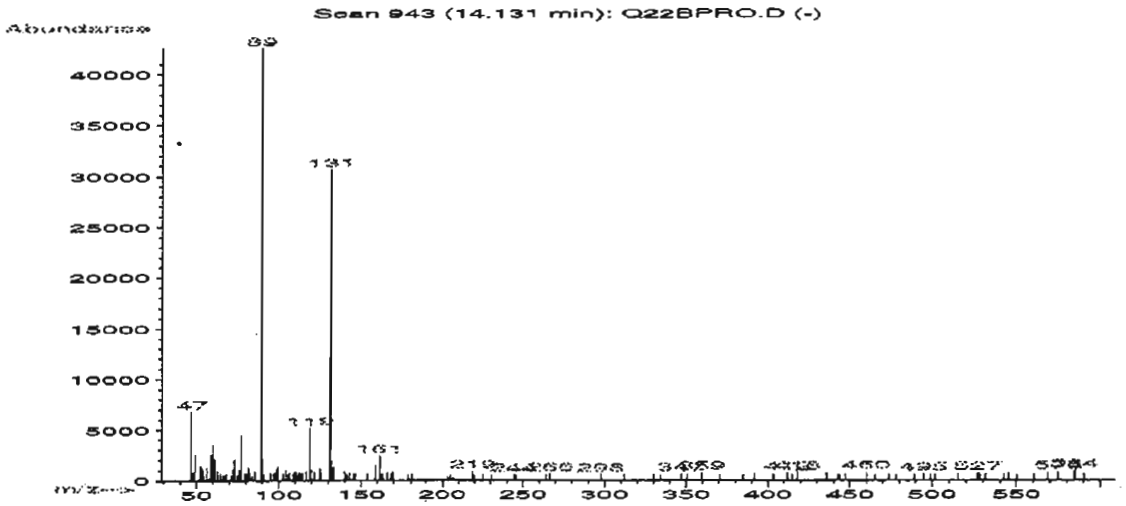
2



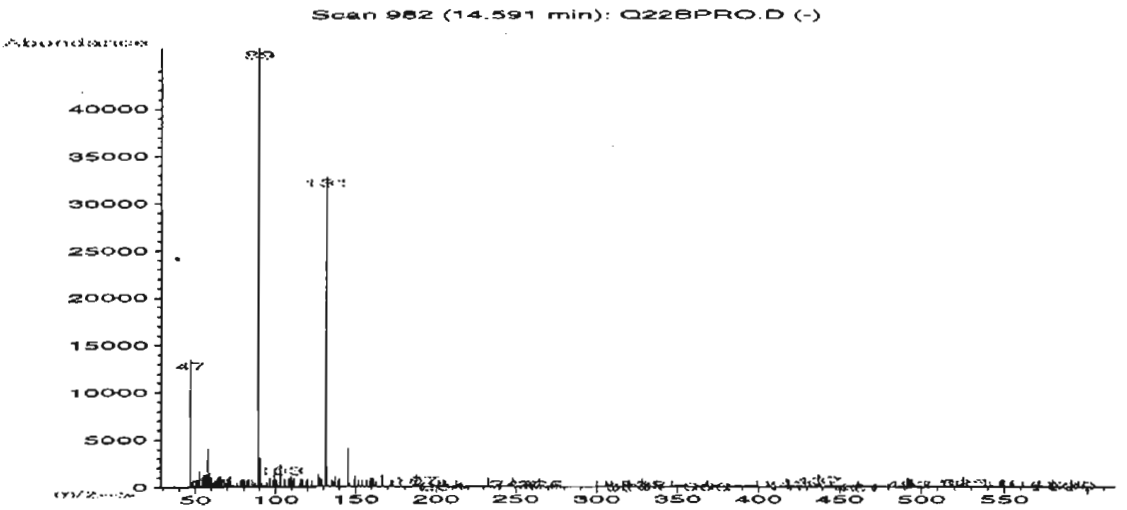
3


Scan 917 (13.824 min): Q22BPRO.D (-)
Abundance
89
131
159
117
60
m/z-->

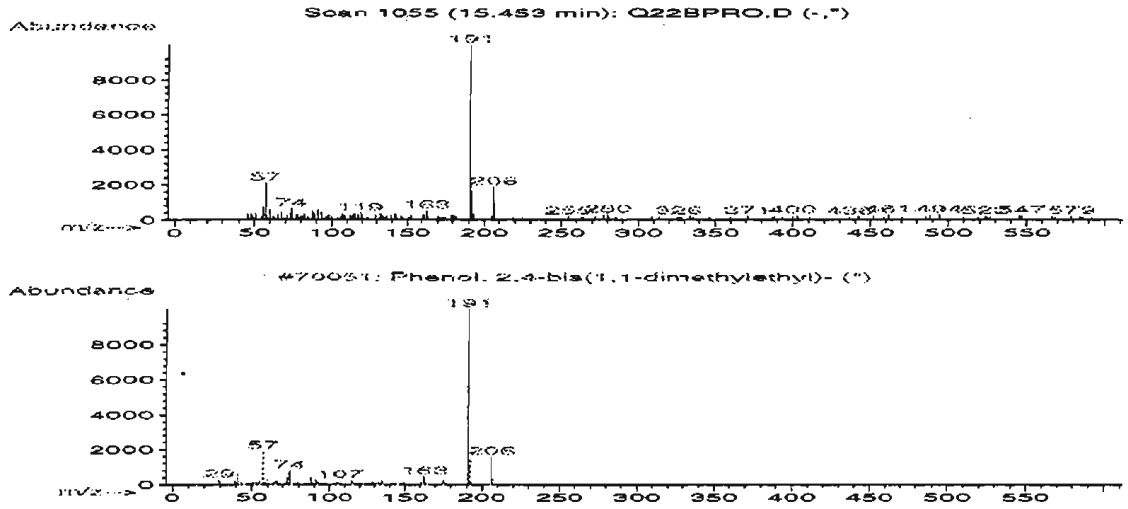
4


Scan 943 (14.131 min): Q22BPRO.D (-)
Abundance
89
131
47
115
161
m/z-->

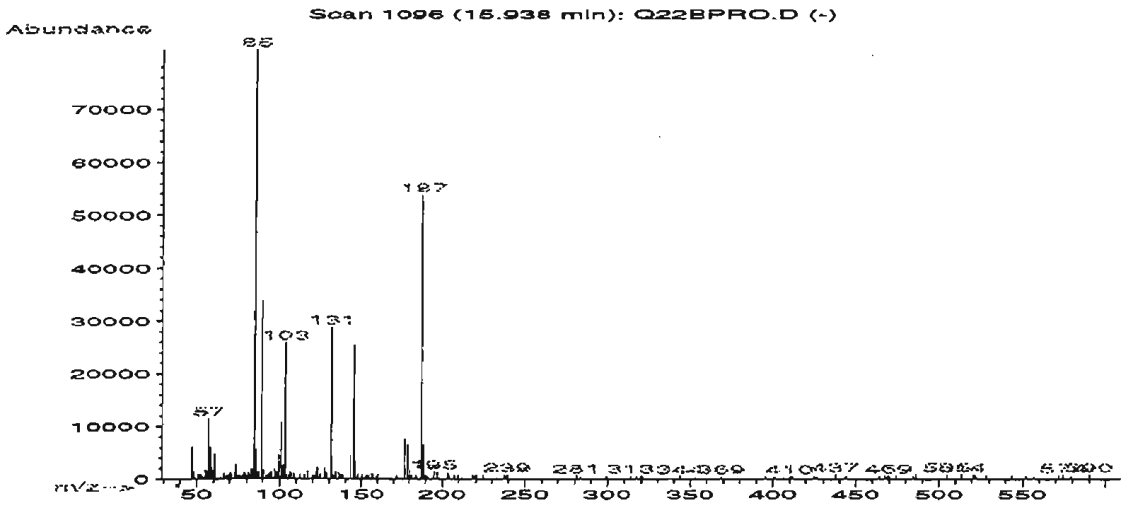
5


Scan 982 (14.591 min): Q22BPRO.D (-)
Abundance
89
131
47
m/z-->

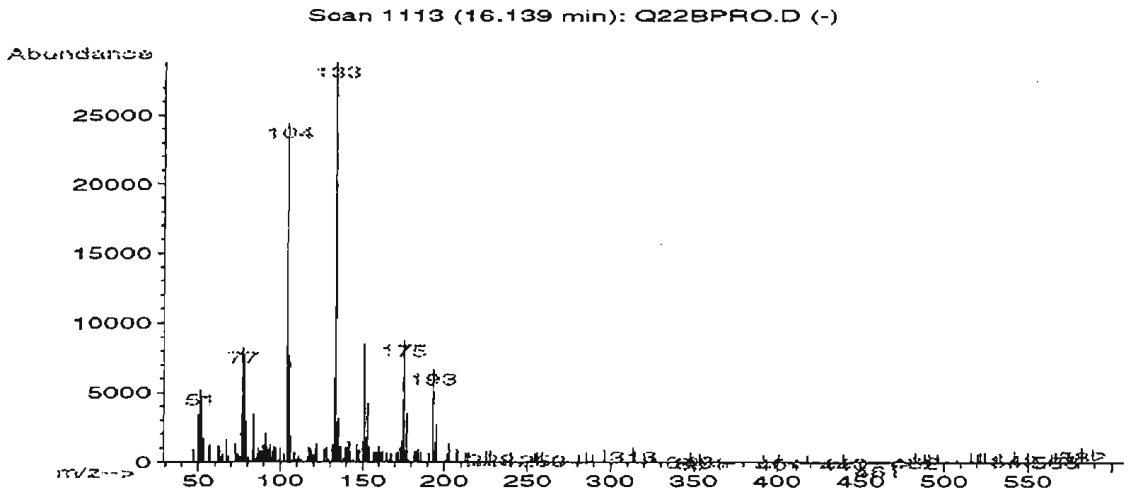
6


Scan 1055 (15.453 min): Q22BPRO.D (-,*)
Abundance
191
8000
6000
4000
2000
0
57
74
119
163
206
m/z-->
0 50 100 150 200 250 300 350 400 450 500 550
#70051: Phenol, 2,4-bis(1,1-dimethylethyl)- (*)
Abundance
191
8000
6000
4000
2000
0
57
74
107
163
206
m/z-->
0 50 100 150 200 250 300 350 400 450 500 550

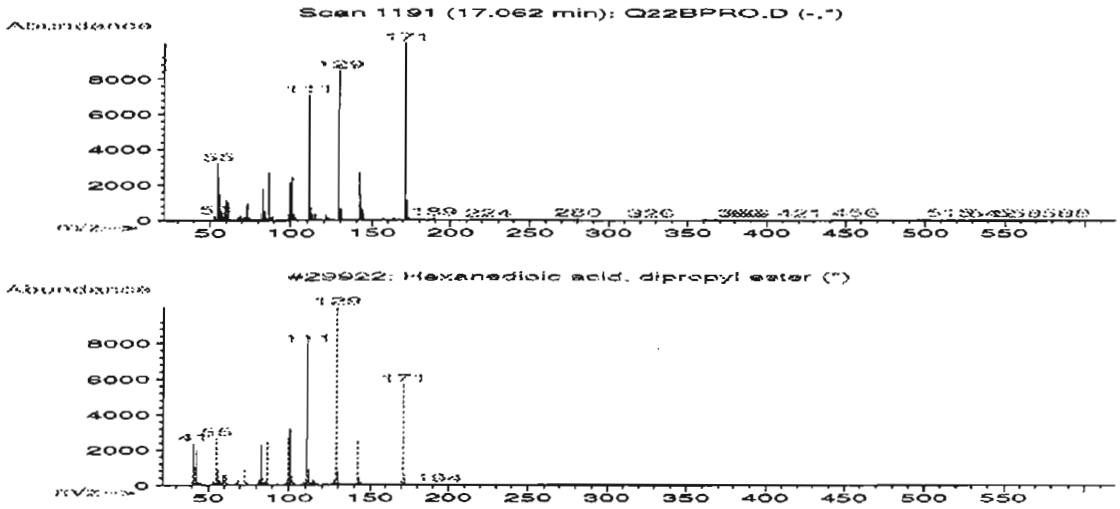
7


Scan 1096 (16.938 min): Q22BPRO.D (-)
Abundance
85
70000
60000
50000
40000
30000
20000
10000
0
197
103
131
57
m/z-->
50 100 150 200 250 300 350 400 450 500 550

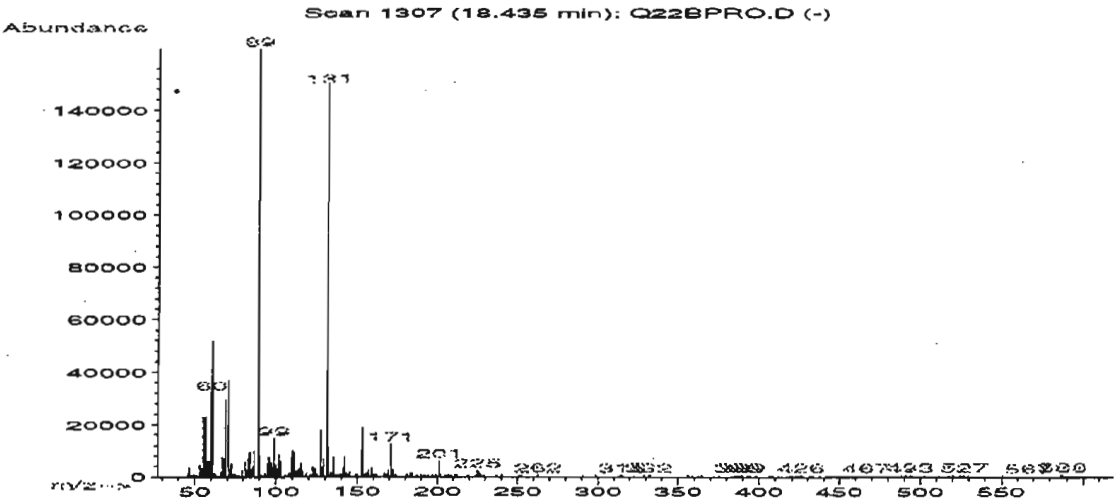
8


Scan 1113 (16.139 min): Q22BPRO.D (-)
Abundance
133
25000
104
20000
15000
10000
77
175
193
5000
0
54
m/z-->
50 100 150 200 250 300 350 400 450 500 550

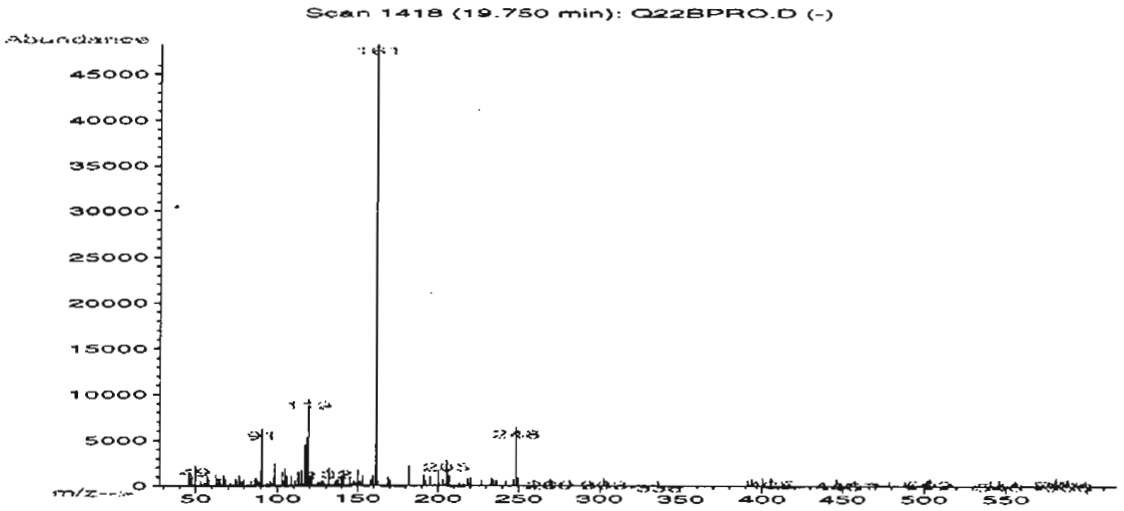
9


Scan 1191 (17.062 min): Q22BPRO.D (-.*)
Abundance
m/z--->
#29922: Hexanedioic acid, dipropyl ester (*)

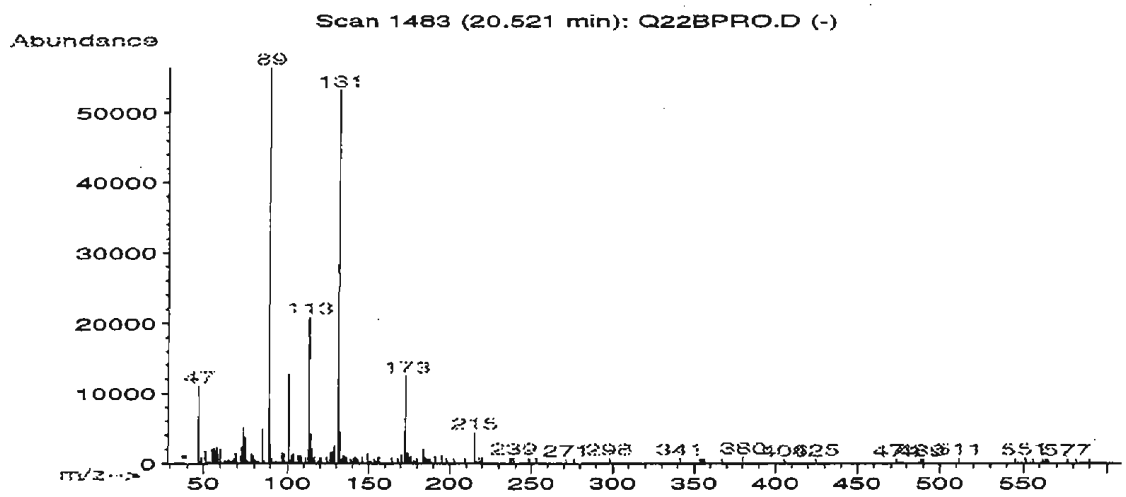
10


Scan 1307 (18.435 min): Q22BPRO.D (-)
Abundance
m/z-->

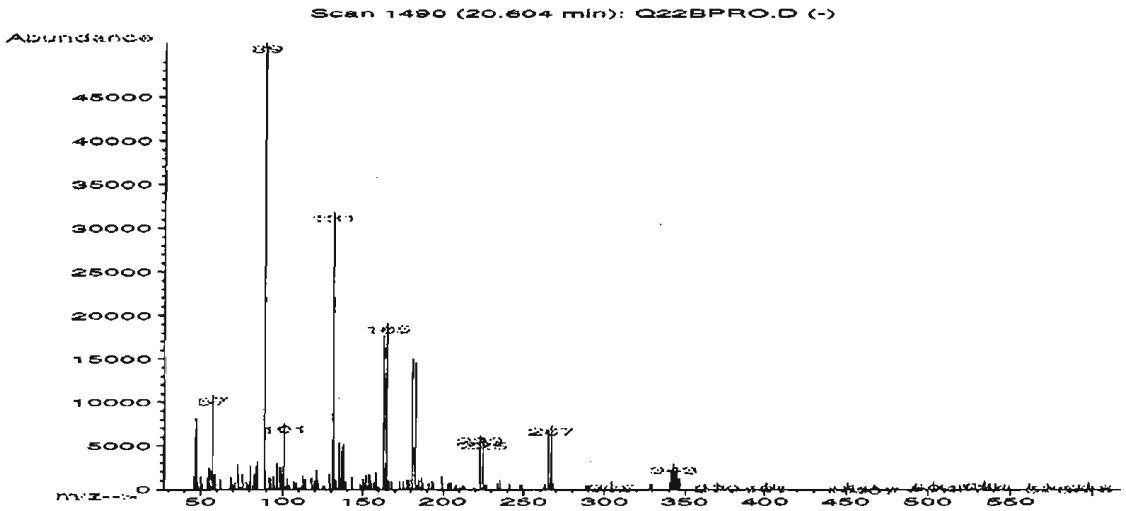
11


Scan 1418 (19.750 min): Q22BPRO.D (-)
Abundance
m/z-->

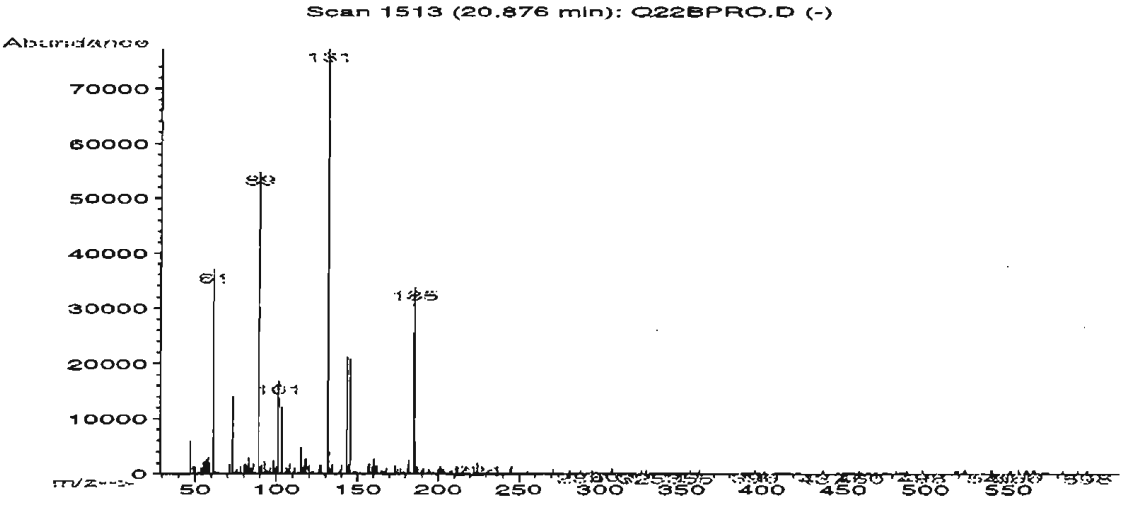
12


Scan 1483 (20.521 min): Q22BPRO.D (-)
Abundance
89
131
113
173
47
215
m/z-->

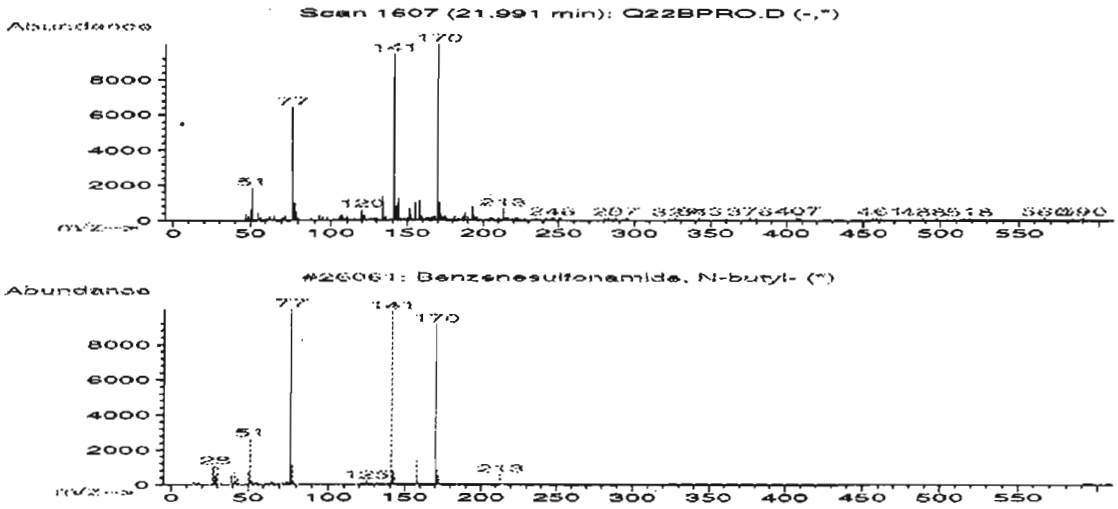
13


Scan 1490 (20.604 min): Q22BPRO.D (-)
Abundance
89
131
173
47
101
217
m/z-->

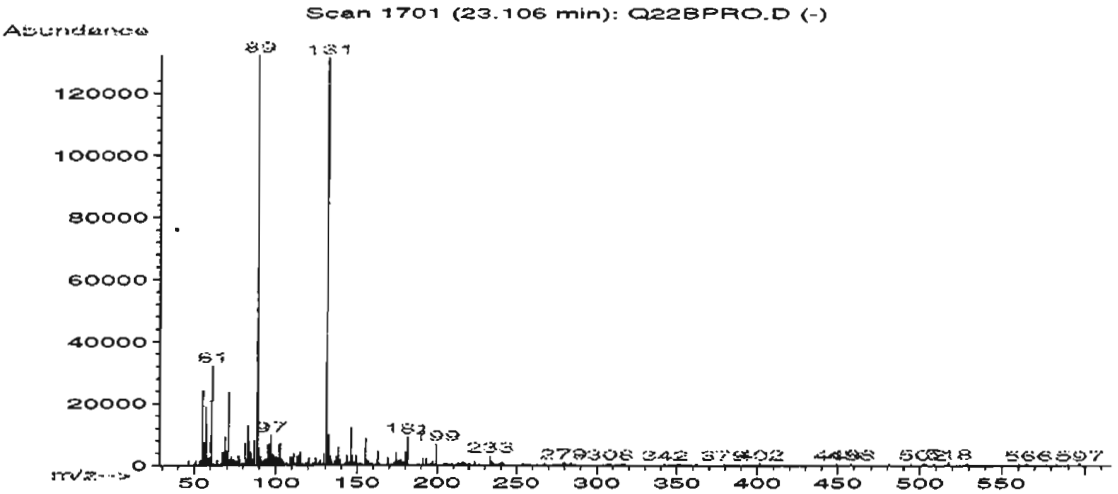
14


Scan 1513 (20.876 min): Q22BPRO.D (-)
Abundance
131
89
61
175
101
m/z-->

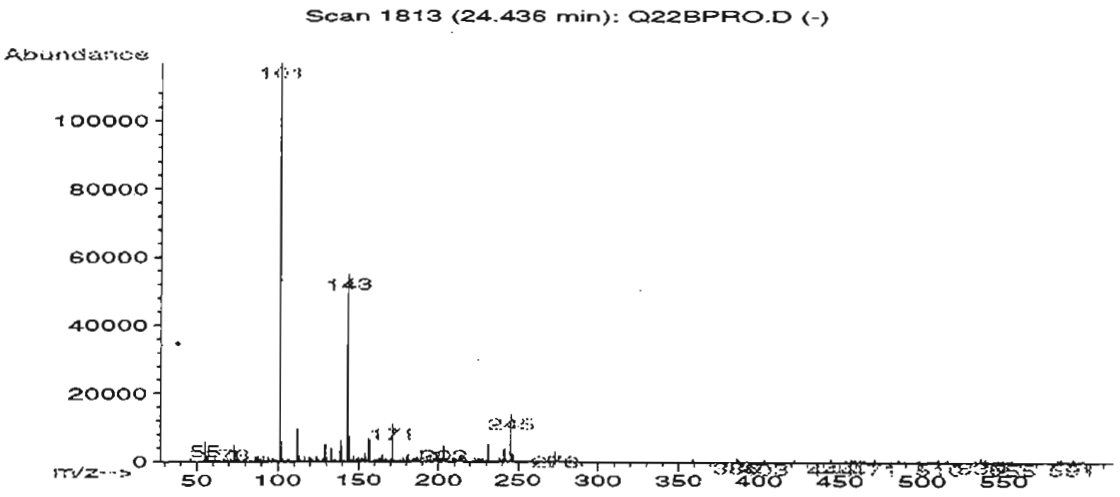
15


Scan 1607 (21.991 min): Q22BPRO.D (-,*)
Abundance
m/z--->
#26061: Benzenesulfonamide, N-butyl- (*)
Abundance
m/z--->

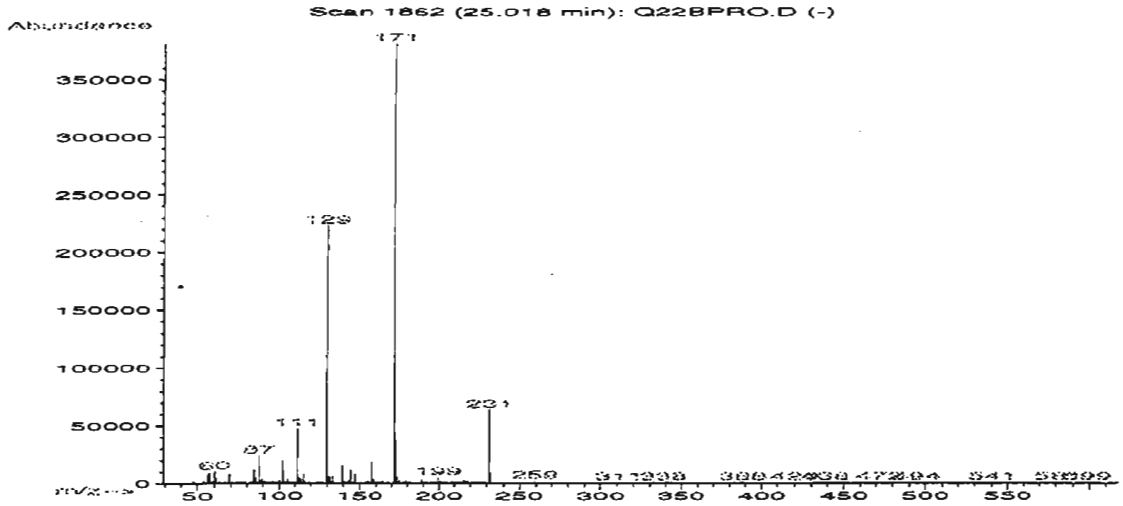
16


Scan 1701 (23.106 min): Q22BPRO.D (-)
Abundance
m/z--->

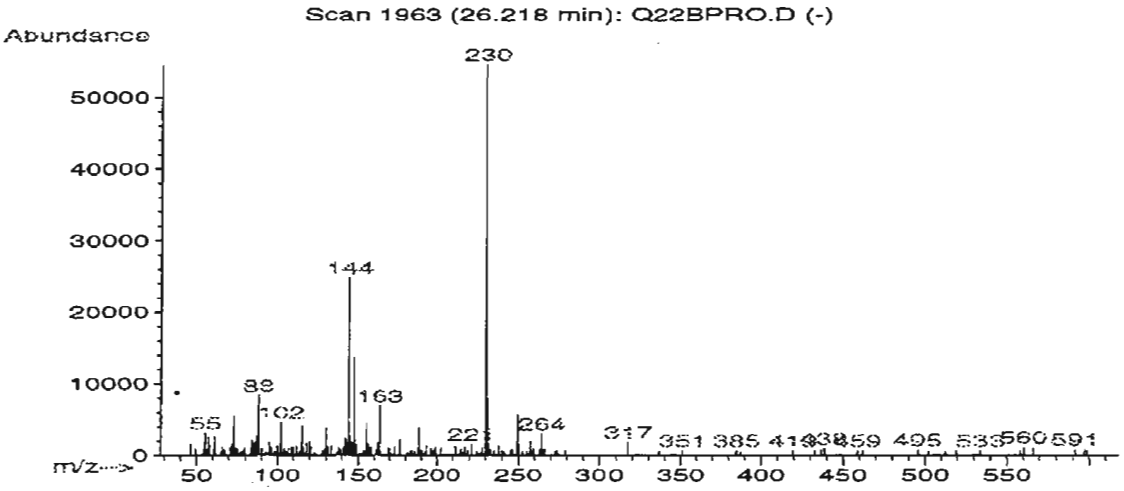
17


Scan 1813 (24.436 min): Q22BPRO.D (-)
Abundance
m/z--->

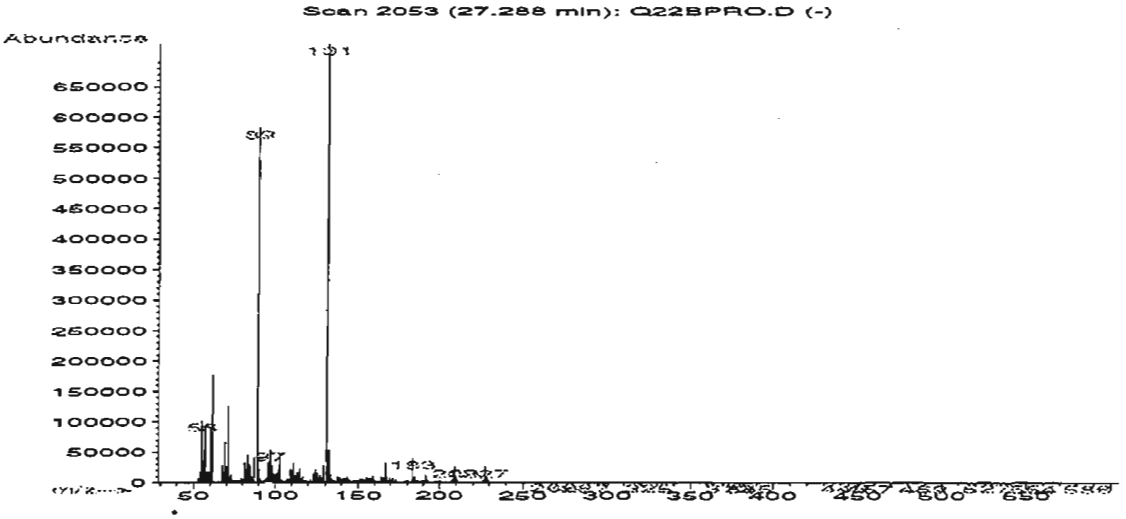
18


Scan 1862 (25.018 min): Q22BPRO.D (-)
Abundance
171
129
231
111
97
69
m/z
350000
300000
250000
200000
150000
100000
50000
0
50
100
150
200
250
300
350
400
450
500
550

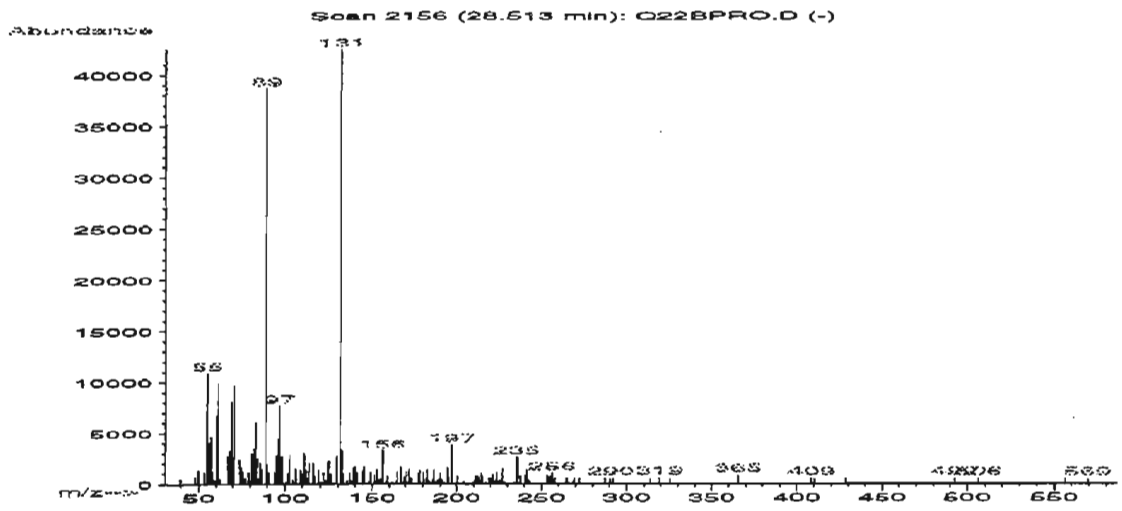
19


Scan 1963 (26.218 min): Q22BPRO.D (-)
Abundance
230
144
88
163
102
55
264
317
m/z
50000
40000
30000
20000
10000
0
50
100
150
200
250
300
350
400
450
500
550

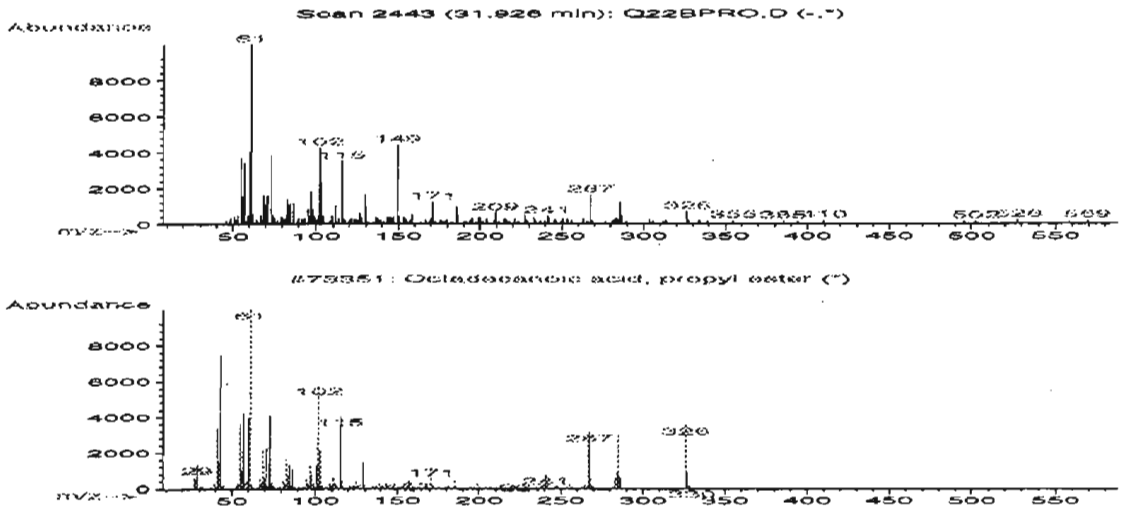
20


Scan 2053 (27.288 min): Q22BPRO.D (-)
Abundance
151
69
m/z
650000
600000
550000
500000
450000
400000
350000
300000
250000
200000
150000
100000
50000
0
50
100
150
200
250
300
350
400
450
600
650

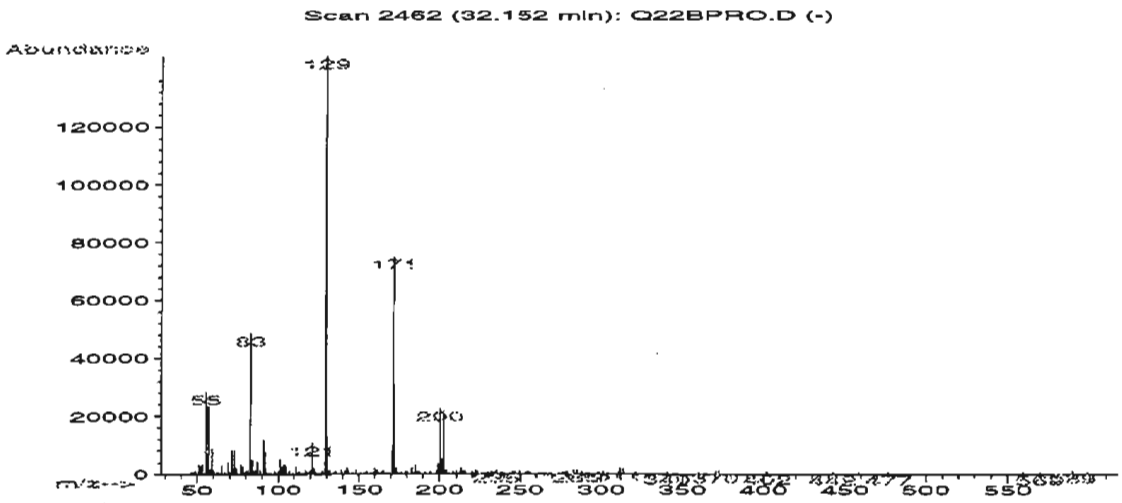
21


Scan 2156 (28.613 min): Q22BPRO.D (-)
Abundance
m/z-->

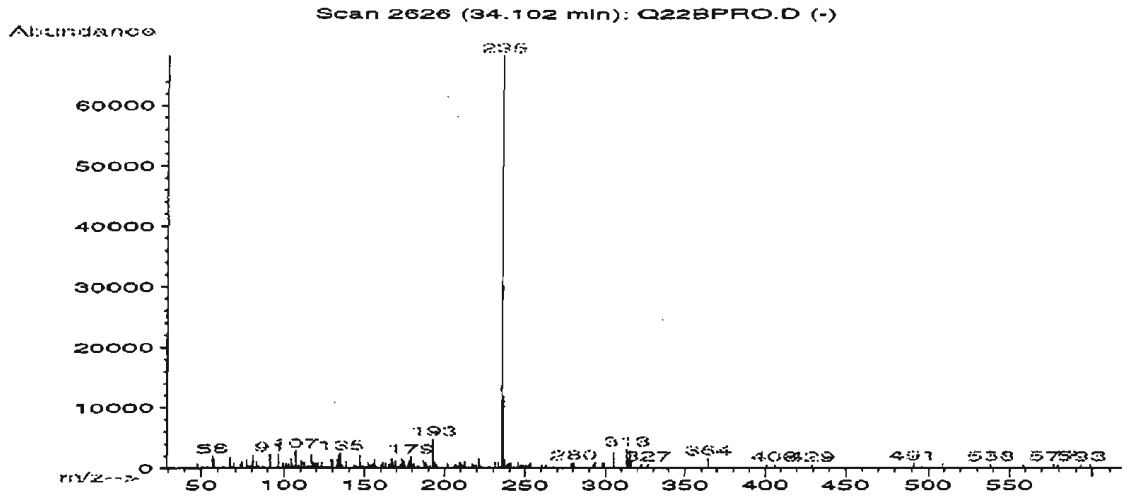
22


Scan 2443 (31.926 min): Q22BPRO.D (-.*)
Abundance
#75351 : Octadecanoic acid, propyl ester (*)
Abundance
m/z-->

23

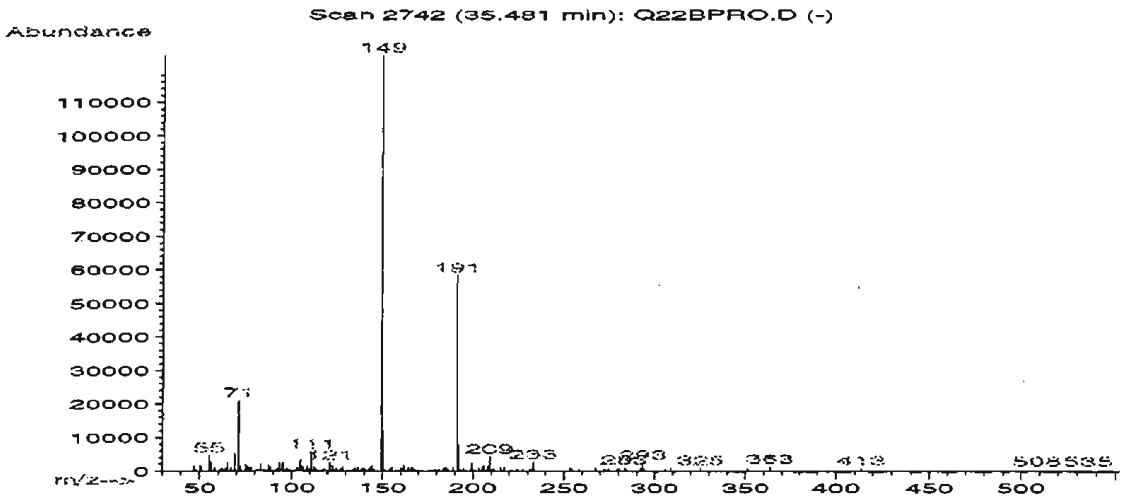

Scan 2462 (32.152 min): Q22BPRO.D (-)
Abundance
m/z-->

24

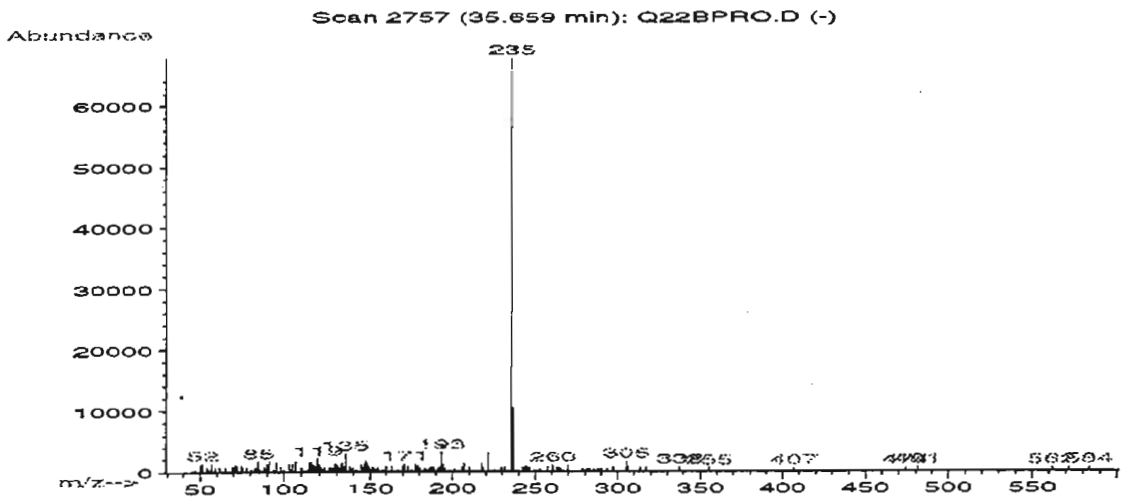


Internal standard at 34.756 minutes.

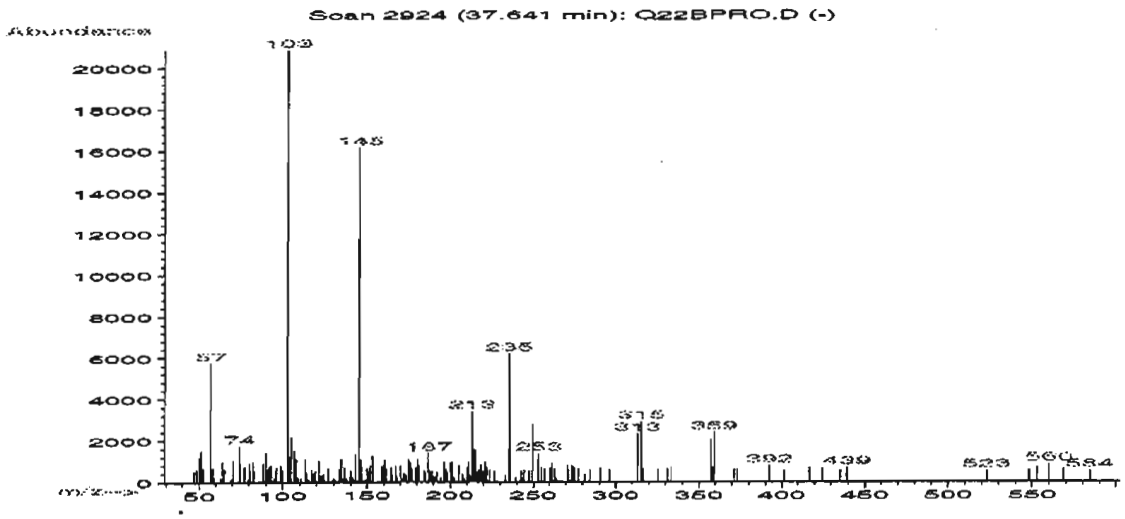
25



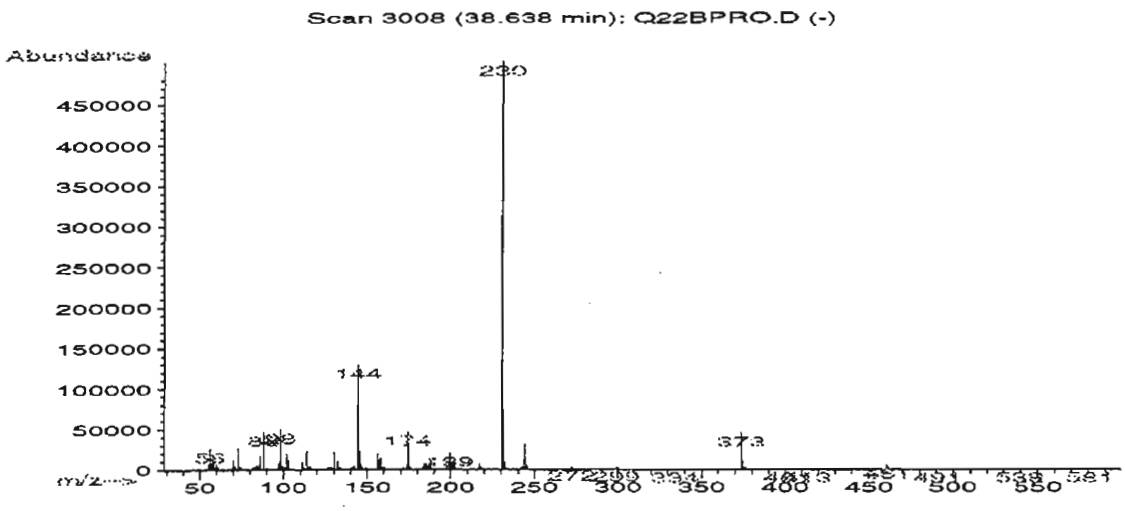
26


Scan 2757 (35.659 min): Q22BPRO.D (-)
Abundance
235
m/z-->

27


Scan 2924 (37.641 min): Q22BPRO.D (-)
Abundance
103
145
57
74
197
213
236
252
315
313
359
392
439
523
560
584
m/z-->

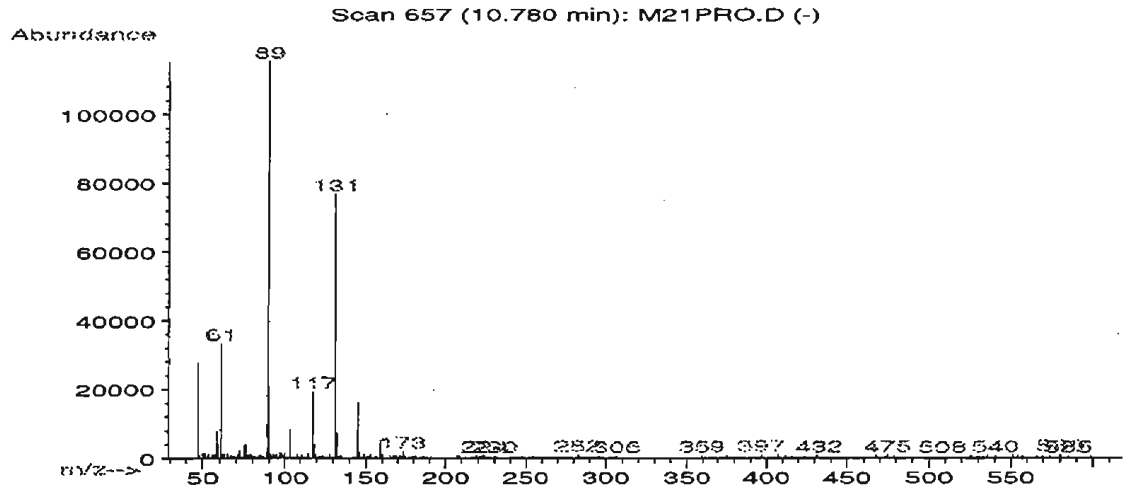
28


Scan 3008 (38.638 min): Q22BPRO.D (-)
Abundance
230
144
174
373
m/z-->

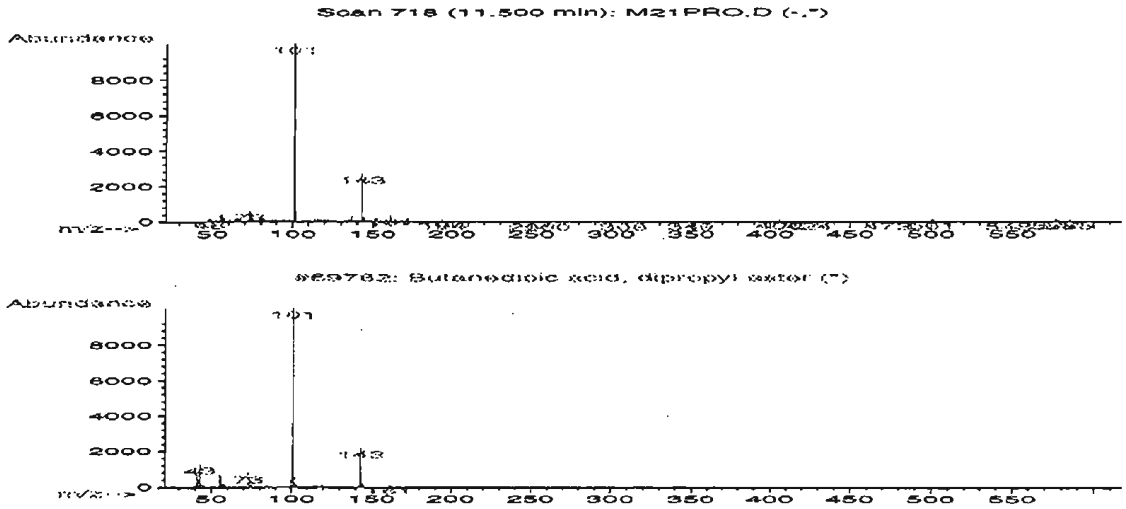
APPENDIX D

Spectra from propylated extract of M21 (collected 12/13/93), run on EDTA2.M. Numbered as in Table 3.7.

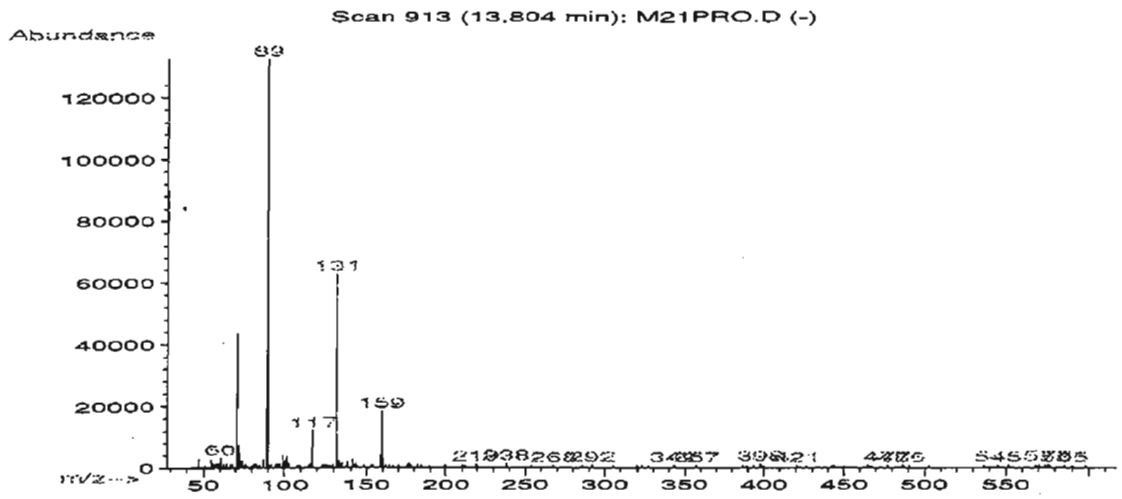
1



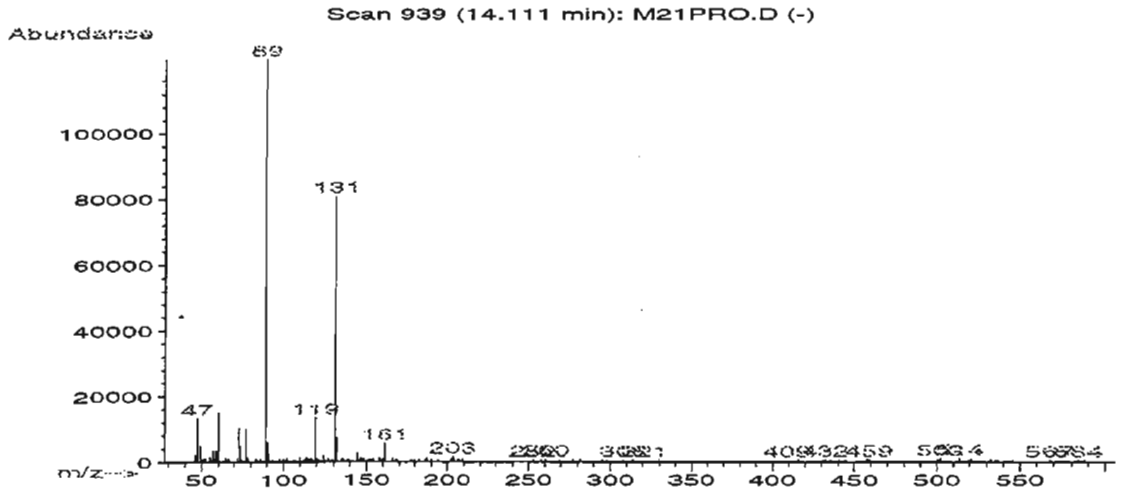
2



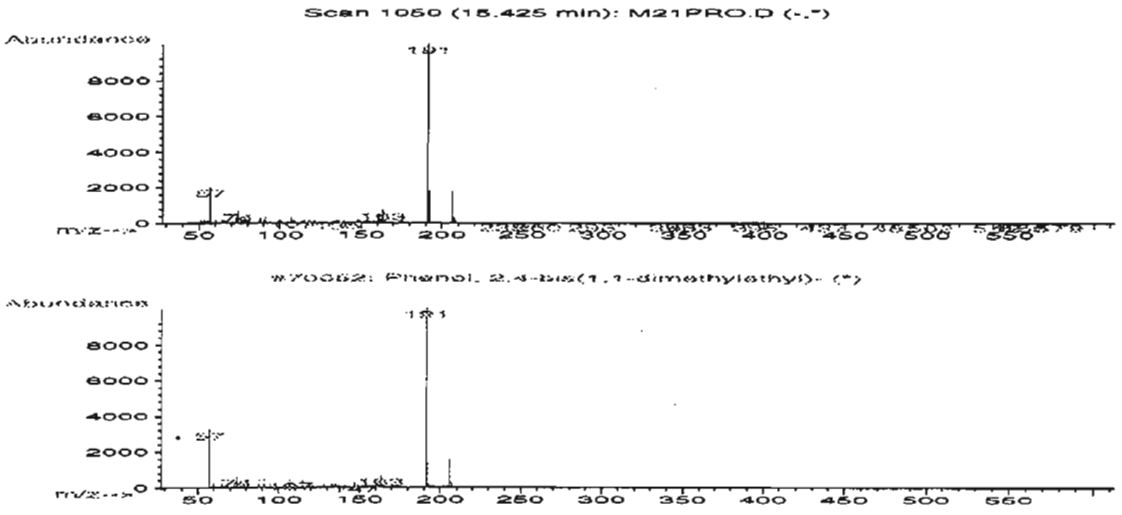
3


Scan 913 (13.804 min): M21PRO.D (-)
Abundance
69
131
159
117
60
m/z-->

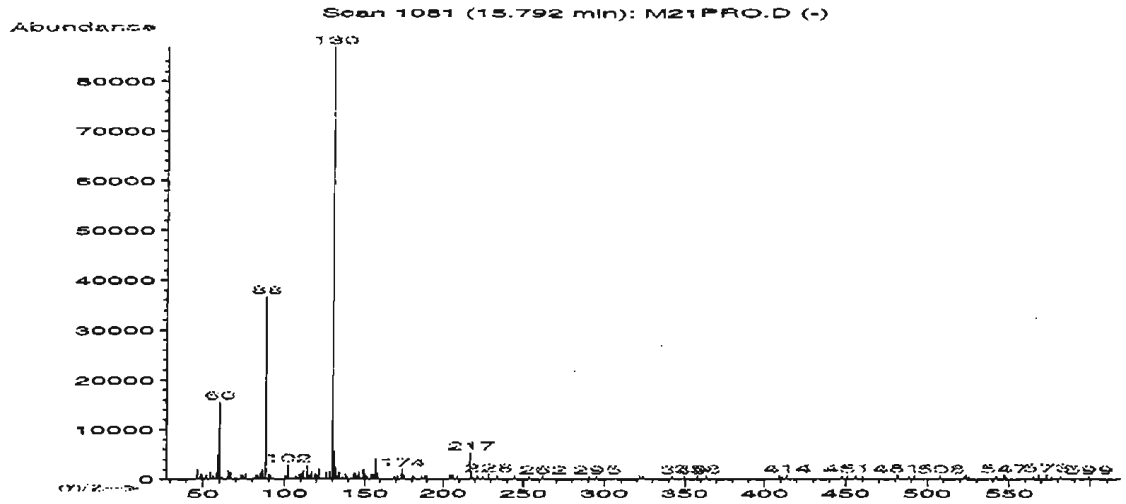
4


Scan 939 (14.111 min): M21PRO.D (-)
Abundance
69
131
47
119
161
203
m/z-->

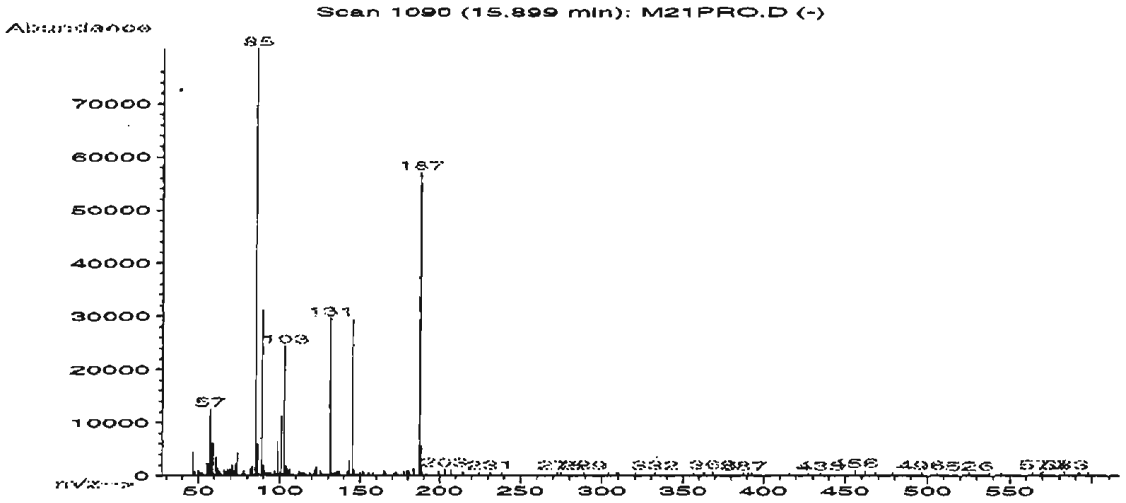
5


Scan 1050 (15.425 min): M21PRO.D (-.*)
Abundance
191
m/z-->

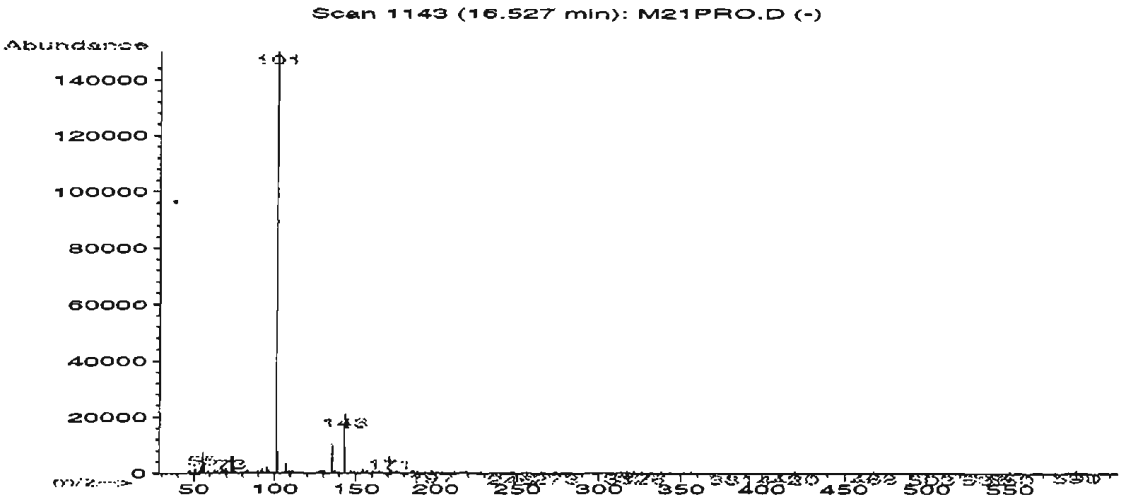
6


Scan 1081 (15.792 min): M21PRO.D (-)
Abundance
130
80
66
217
m/z

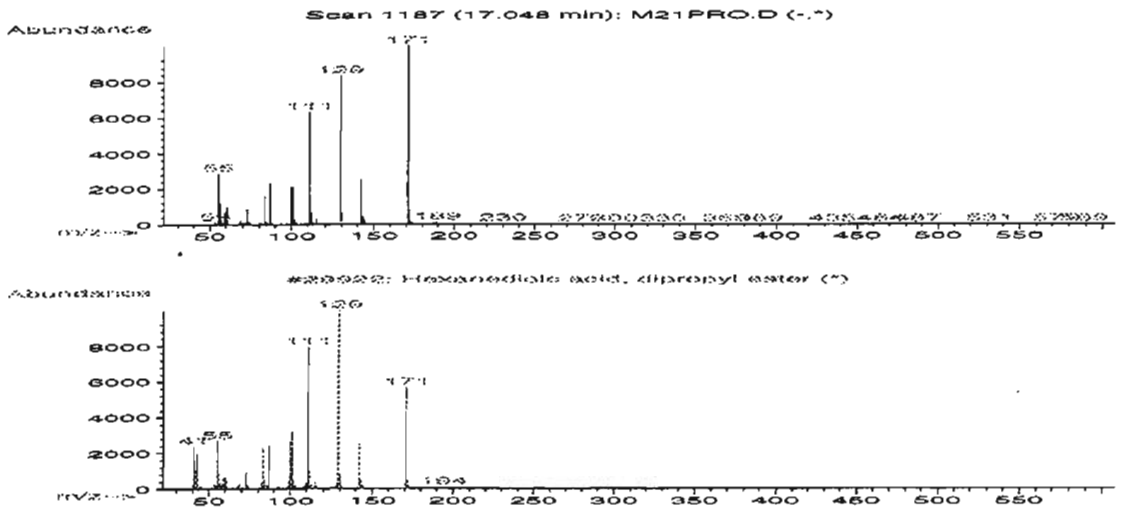
7


Scan 1090 (15.899 min): M21PRO.D (-)
Abundance
95
187
131
103
67
m/z

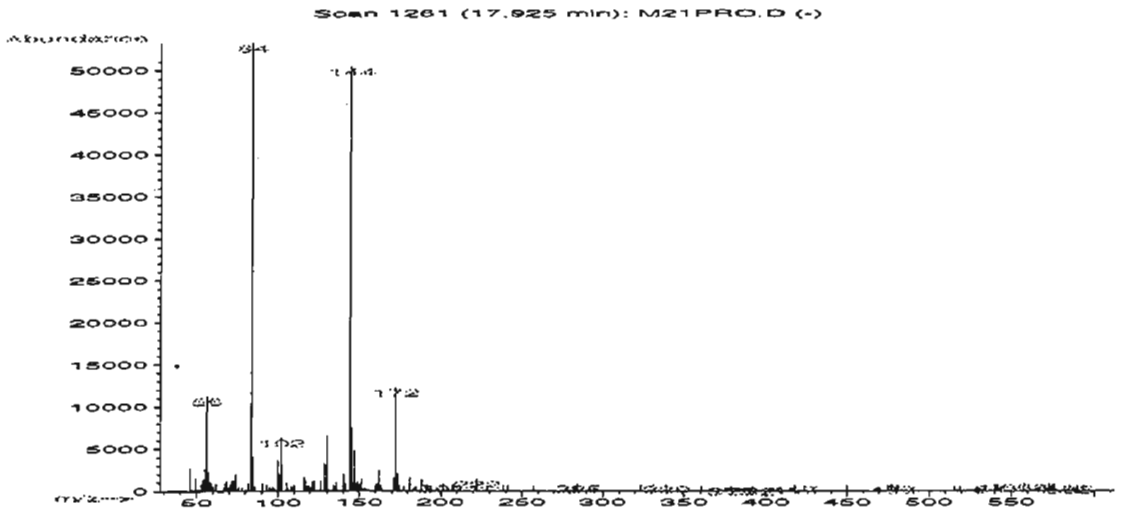
8


Scan 1143 (16.527 min): M21PRO.D (-)
Abundance
141
143
m/z

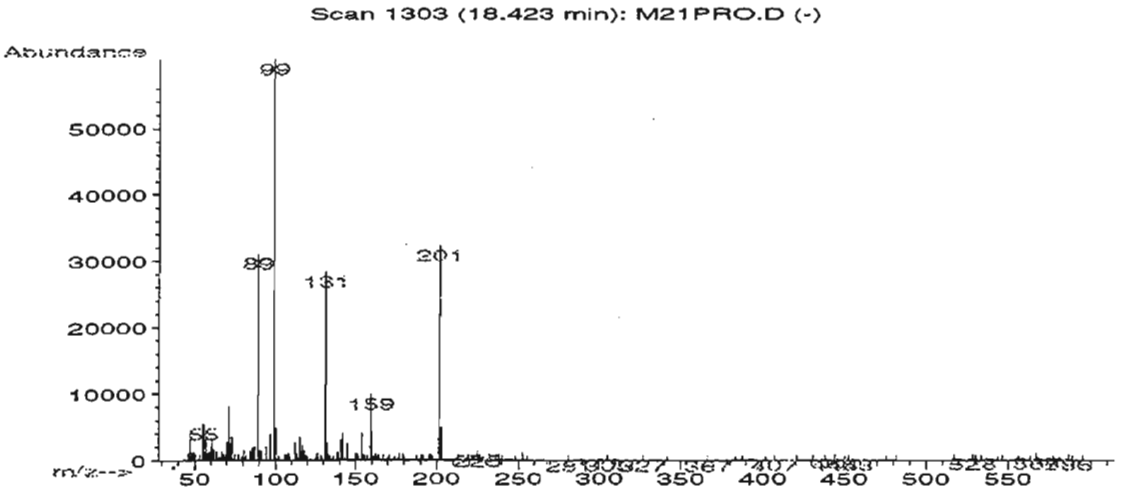
9


Scan 1187 (17.048 min): M21PRO.D (-,*)
Abundance
m/z-->

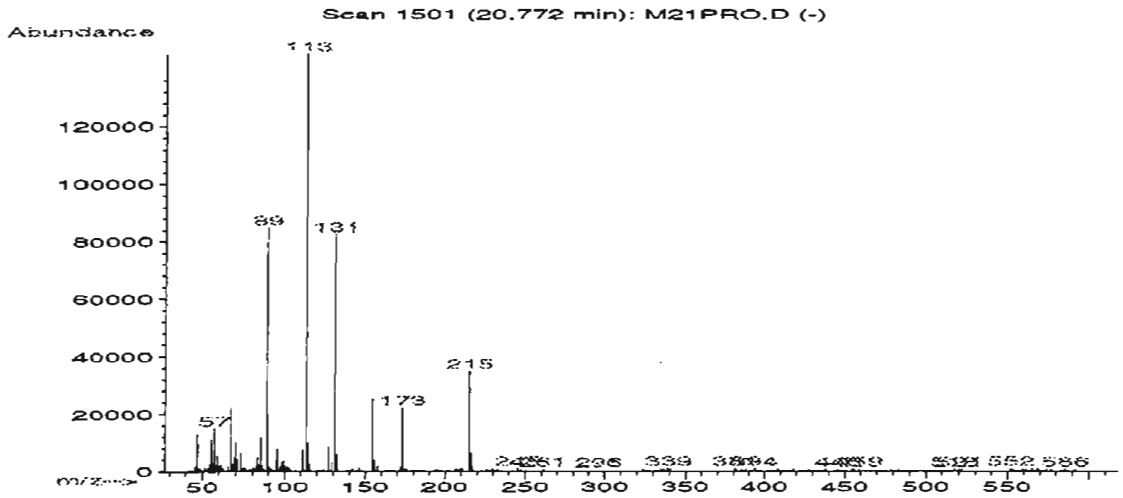
10


Scan 1261 (17.925 min): M21PRO.D (-)
Abundance
m/z-->

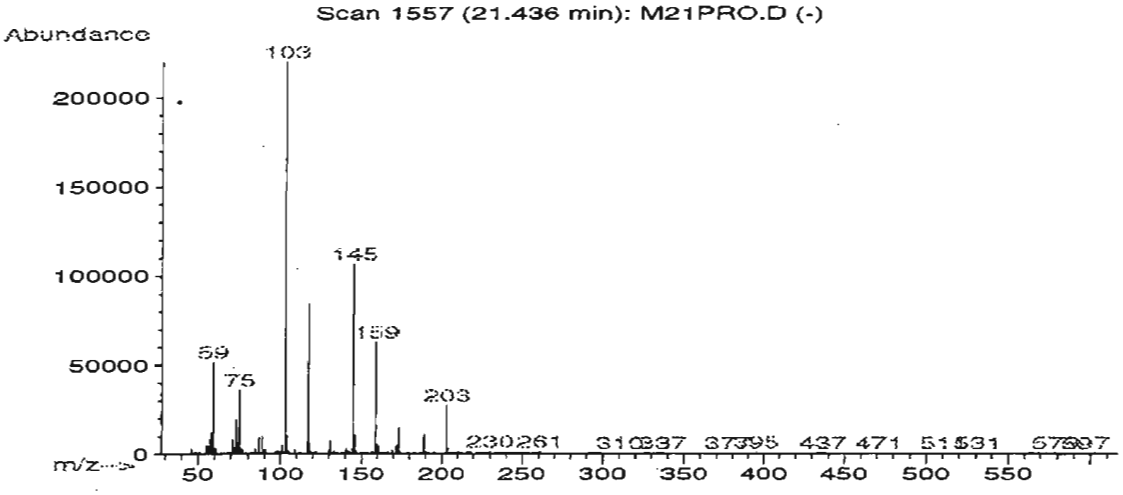
11


Scan 1303 (18.423 min): M21PRO.D (-)
Abundance
m/z-->

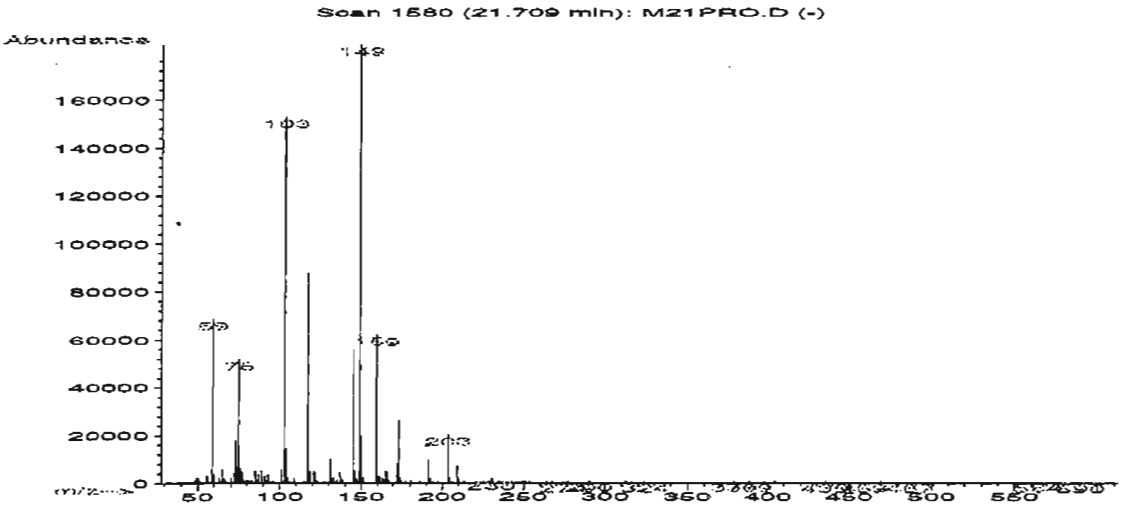
12


Scan 1501 (20.772 min): M21PRO.D (-)
Abundance
113
89
131
215
173
57
m/z--->
120000
100000
80000
60000
40000
20000
0
50 100 150 200 250 300 350 400 450 500 550

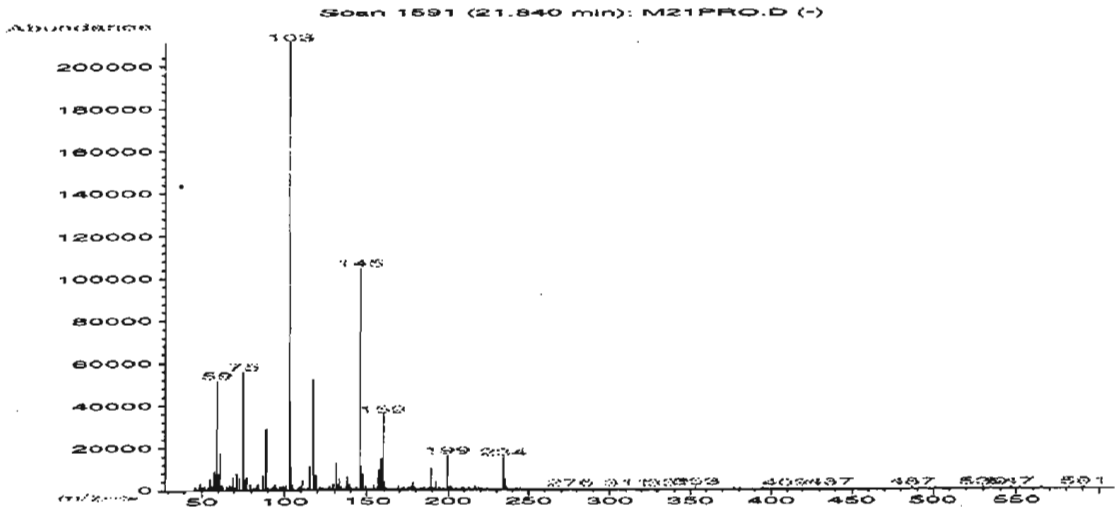
13


Scan 1557 (21.436 min): M21PRO.D (-)
Abundance
103
145
159
59
75
203
m/z--->
200000
150000
100000
50000
0
50 100 150 200 250 300 350 400 450 500 550

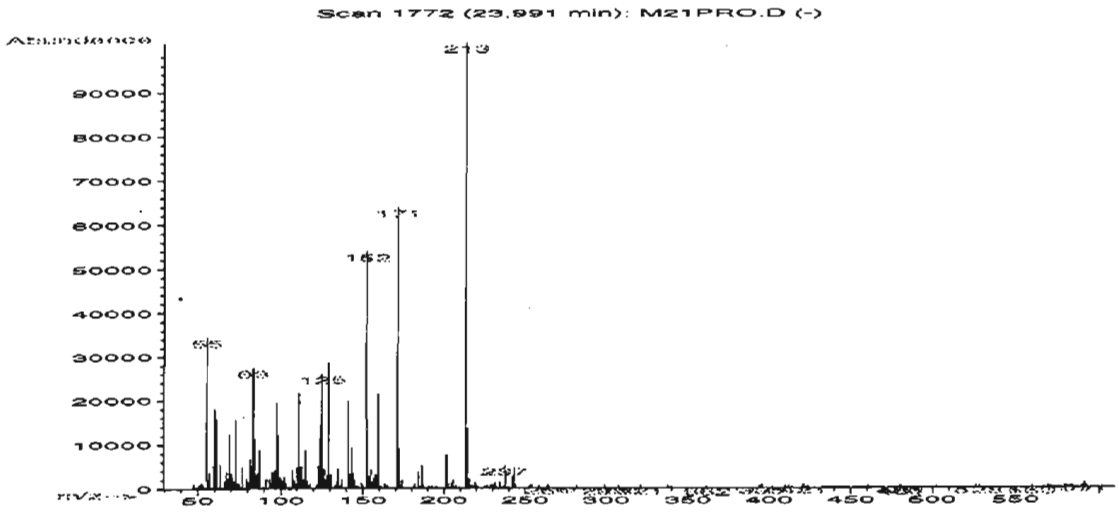
14


Scan 1580 (21.709 min): M21PRO.D (-)
Abundance
145
103
59
75
159
203
m/z--->
160000
140000
120000
100000
80000
60000
40000
20000
0
50 100 150 200 250 300 350 400 450 500 550

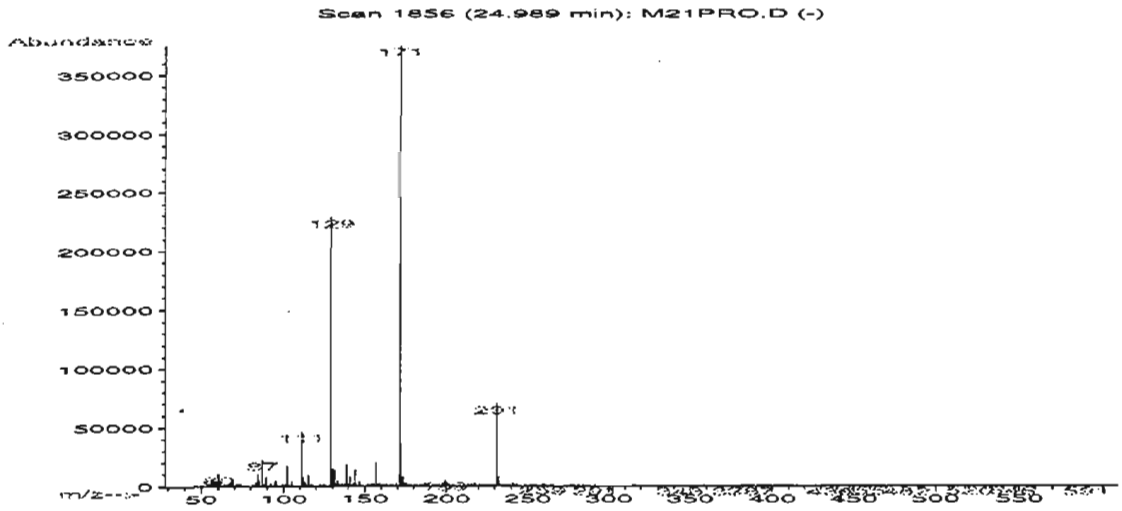
15


Scan 1591 (21.840 min): M21PRO.D (-)
Abundance
m/z-->

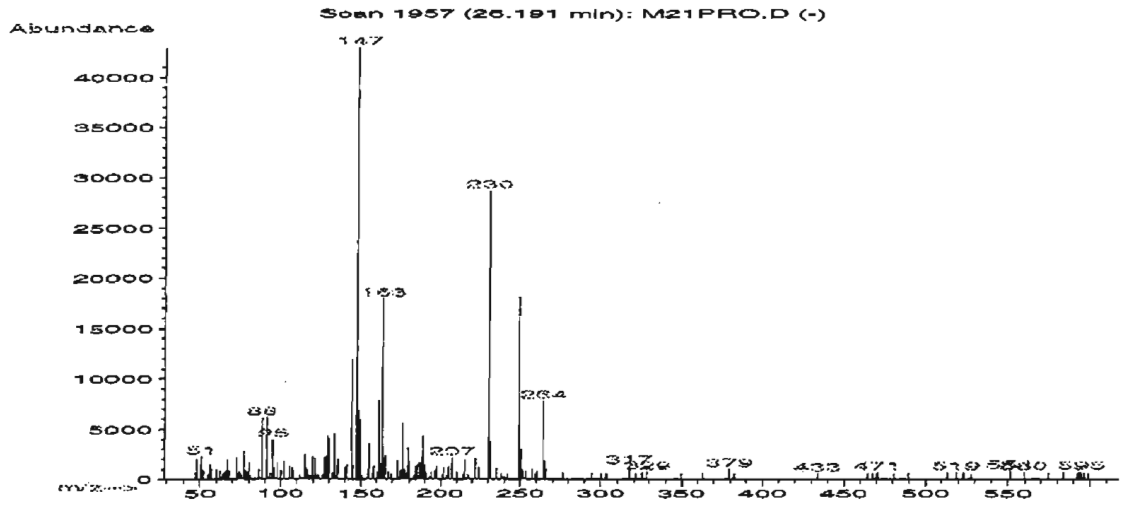
16


Scan 1772 (23.991 min): M21PRO.D (-)
Abundance
m/z-->

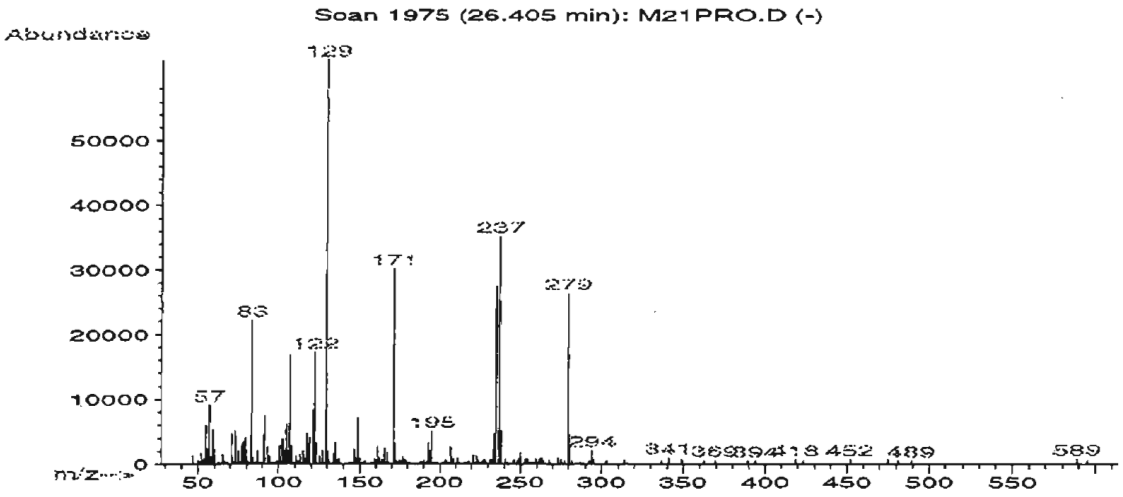
17


Scan 1856 (24.989 min): M21PRO.D (-)
Abundance
m/z-->

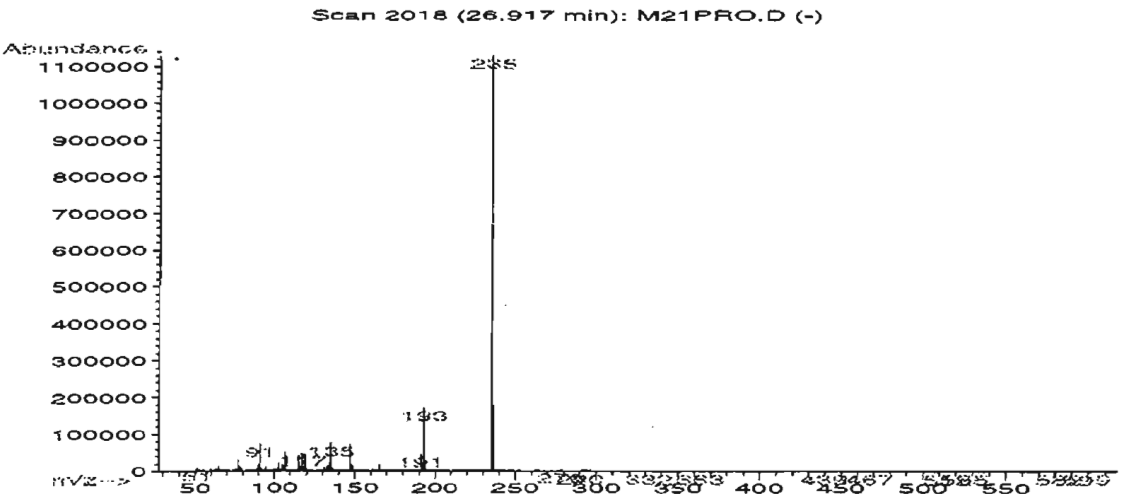
18


Scan 1957 (26.191 min): M21PRO.D (-)
Abundance
147
230
183
264
89
95
51
207
317
379
433
471
519
m/z-->

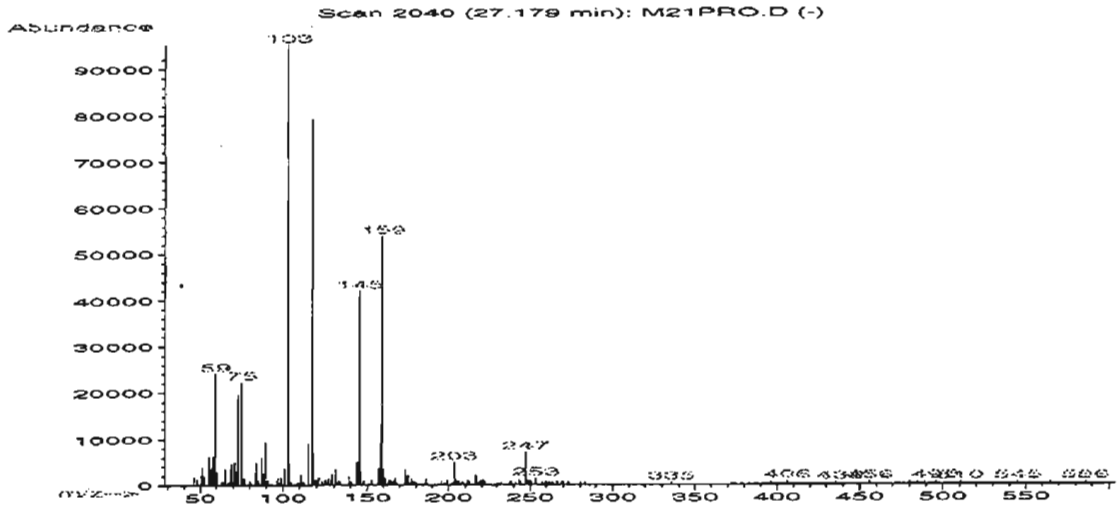
19


Scan 1975 (26.405 min): M21PRO.D (-)
Abundance
129
237
171
279
83
122
57
195
294
341
369
394
418
452
489
589
m/z-->

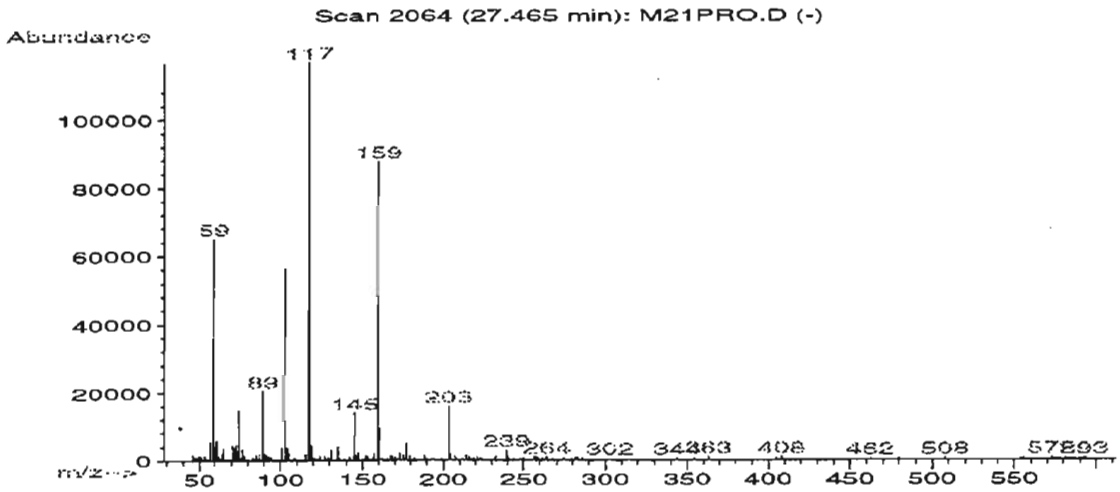
20


Scan 2018 (26.917 min): M21PRO.D (-)
Abundance
255
193
91
139
151
m/z-->

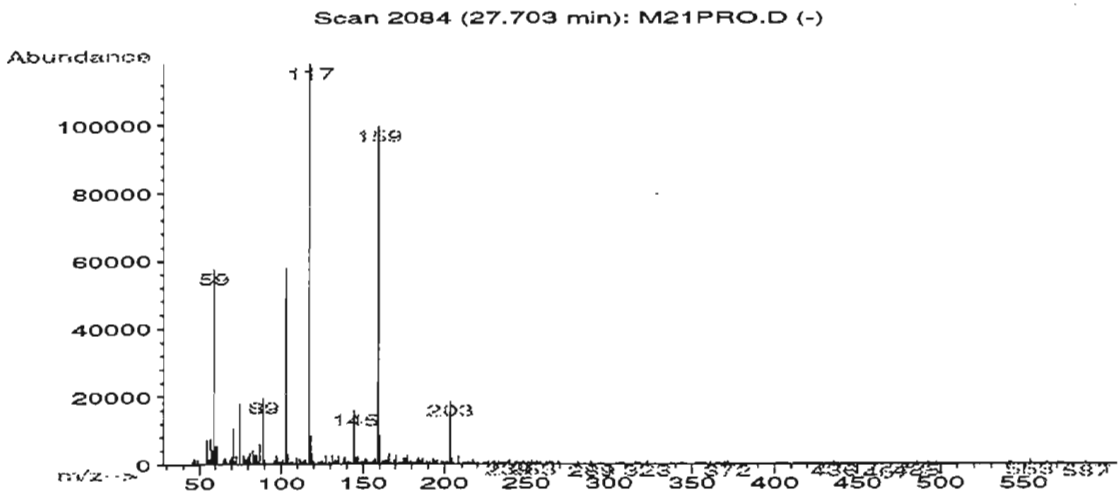
21


Scan 2040 (27.179 min): M21PRO.D (-)
Abundance
m/z-->

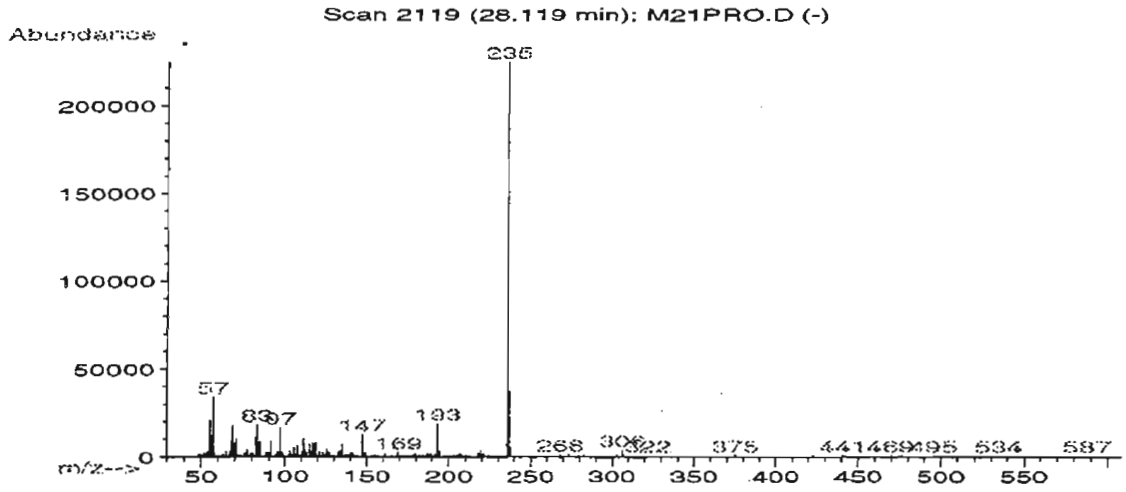
22


Scan 2064 (27.465 min): M21PRO.D (-)
Abundance
m/z-->

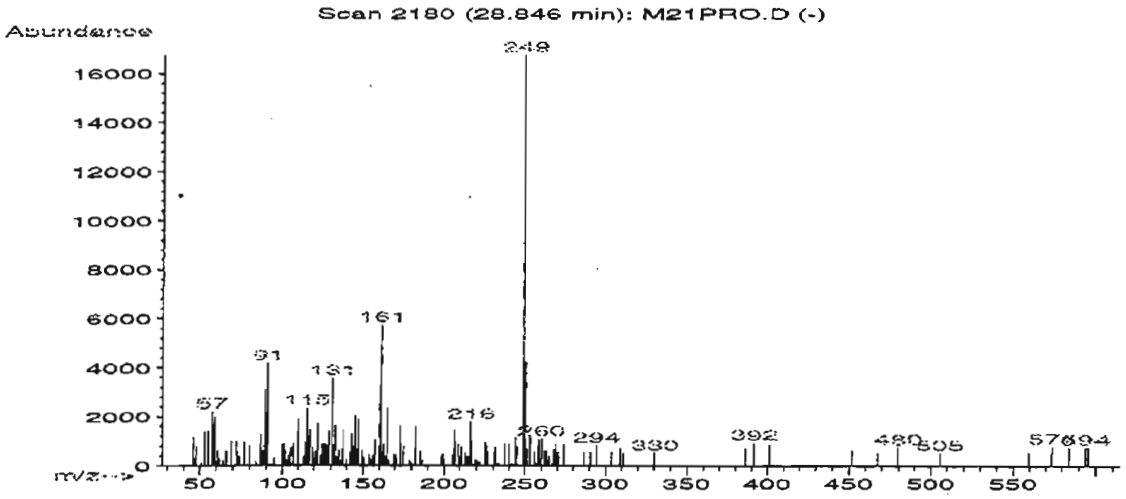
23


Scan 2084 (27.703 min): M21PRO.D (-)
Abundance
m/z-->

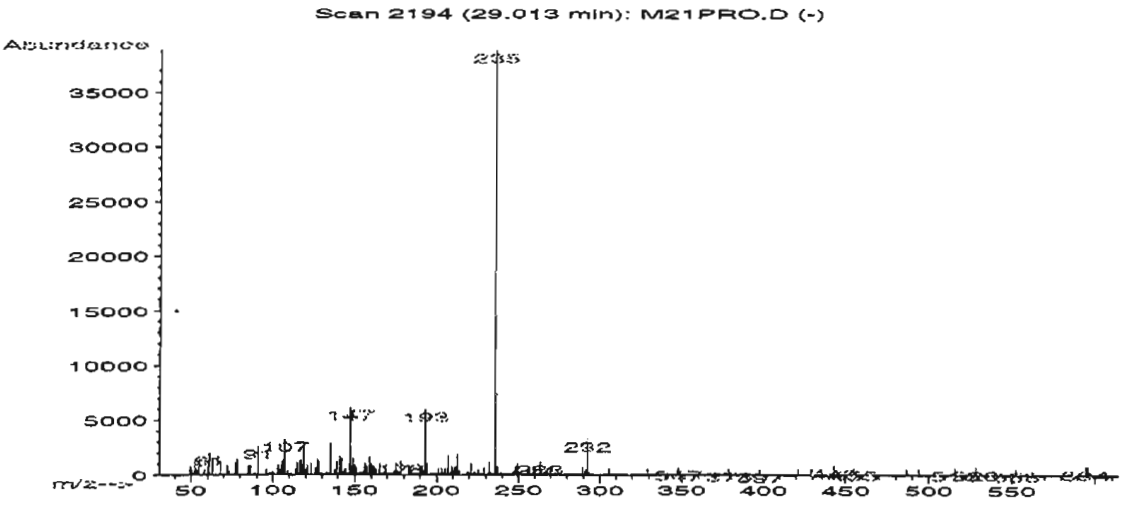
24


Scan 2119 (28.119 min): M21PRO.D (-)
Abundance
235
200000
150000
100000
50000
57
83
97
147
169
193
268
306
322
375
441
469
495
534
587
m/z-->
0
50
100
150
200
250
300
350
400
450
500
550

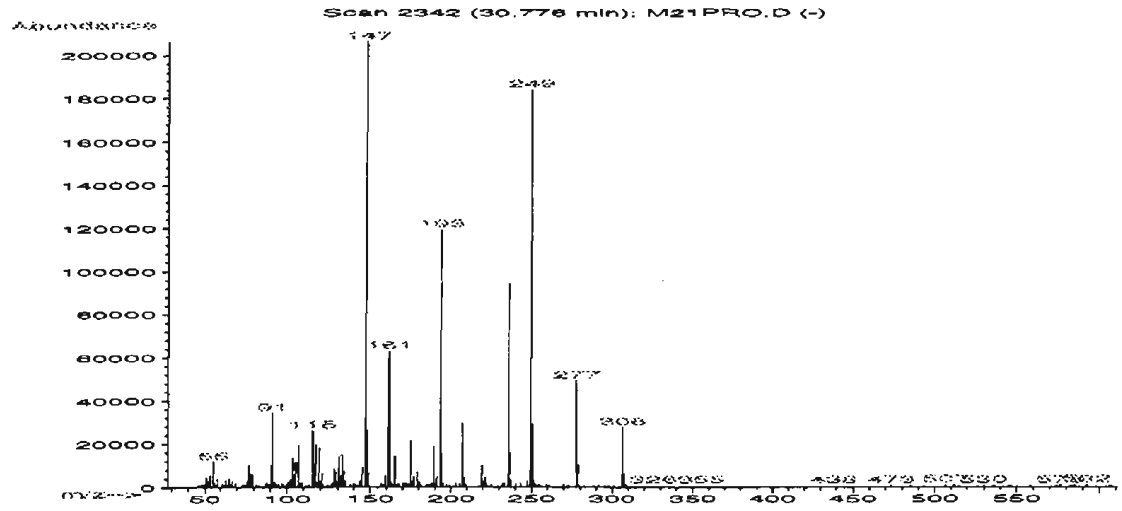
25


Scan 2180 (28.846 min): M21PRO.D (-)
Abundance
249
16000
14000
12000
10000
8000
6000
4000
2000
161
91
181
115
57
216
260
294
330
392
480
505
576
594
m/z-->
0
50
100
150
200
250
300
350
400
450
500
550

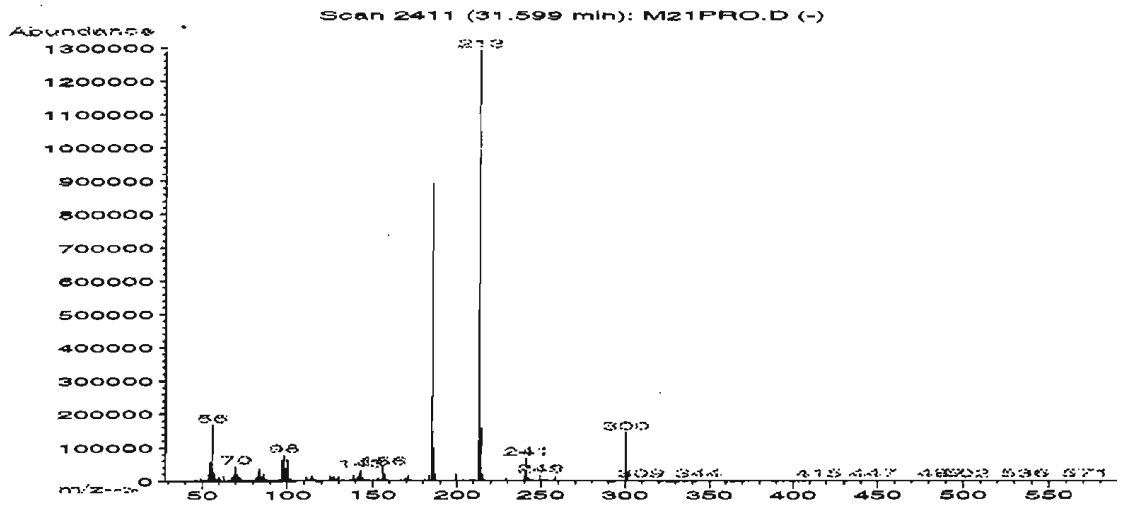
26


Scan 2194 (29.013 min): M21PRO.D (-)
Abundance
235
35000
30000
25000
20000
15000
10000
5000
147
193
97
232
m/z-->
0
50
100
150
200
250
300
350
400
450
500
550

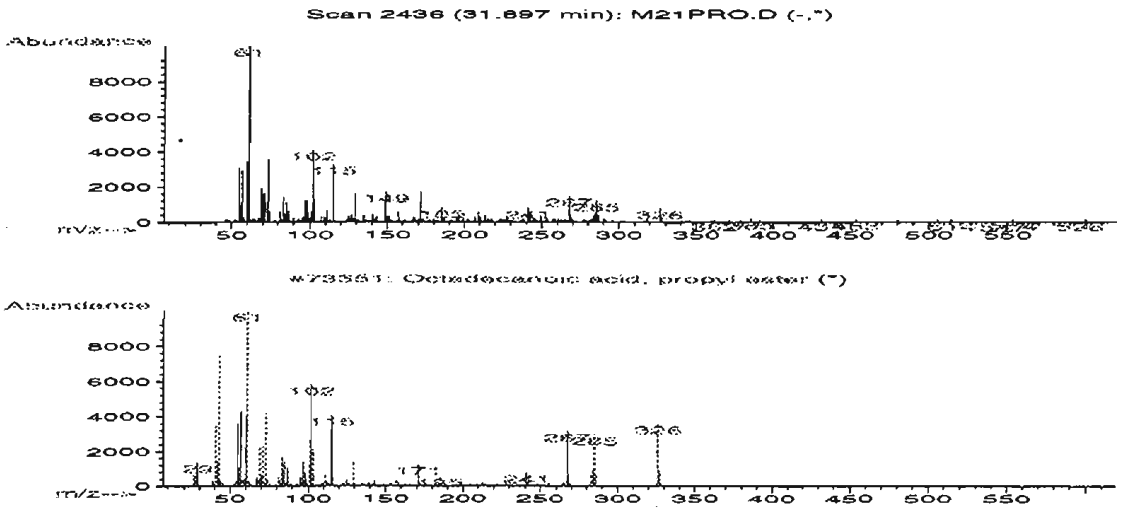
27


Scan 2342 (30.778 min): M21PRO.D (-)
Abundance
147
249
199
121
277
91
308
115
66
m/z-->

28

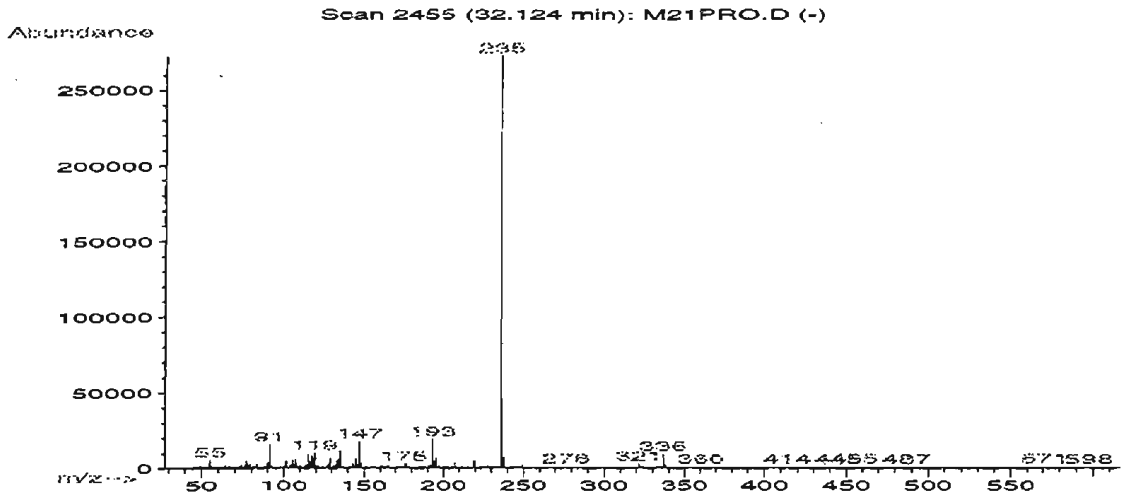

Scan 2411 (31.599 min): M21PRO.D (-)
Abundance
213
56
300
70
98
241
m/z-->

29

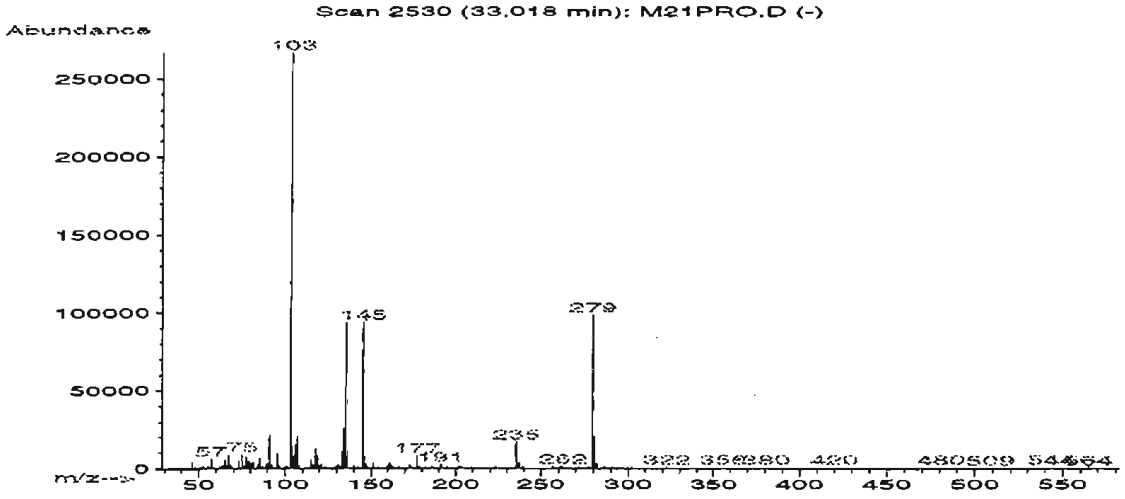

Scan 2436 (31.897 min): M21PRO.D (-,*)
Abundance
69
102
115
346
m/z-->

#73561: Octadecanoic acid, propyl ester (*)
Abundance
69
102
115
346
m/z-->

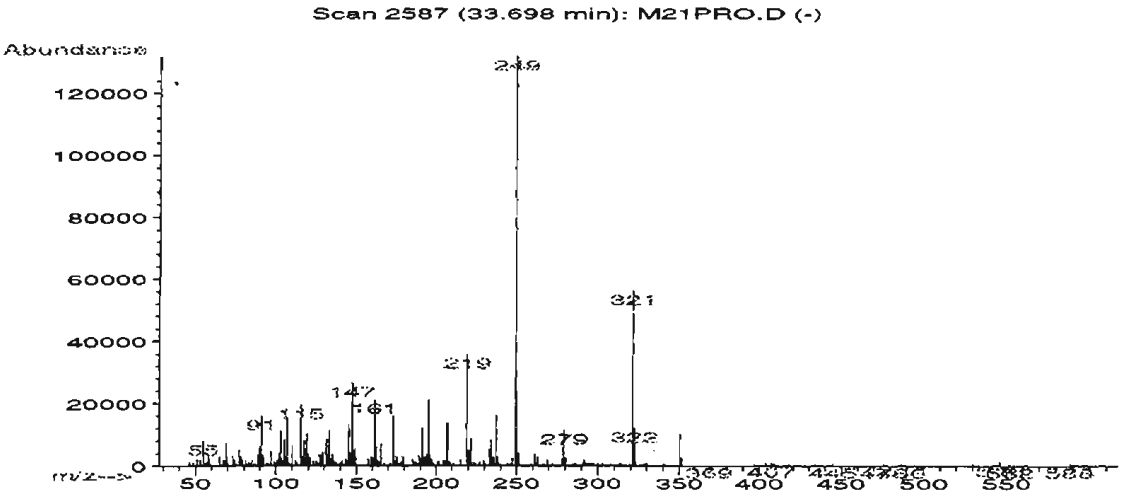
30


Scan 2455 (32.124 min): M21PRO.D (-)
Abundance
m/z-->

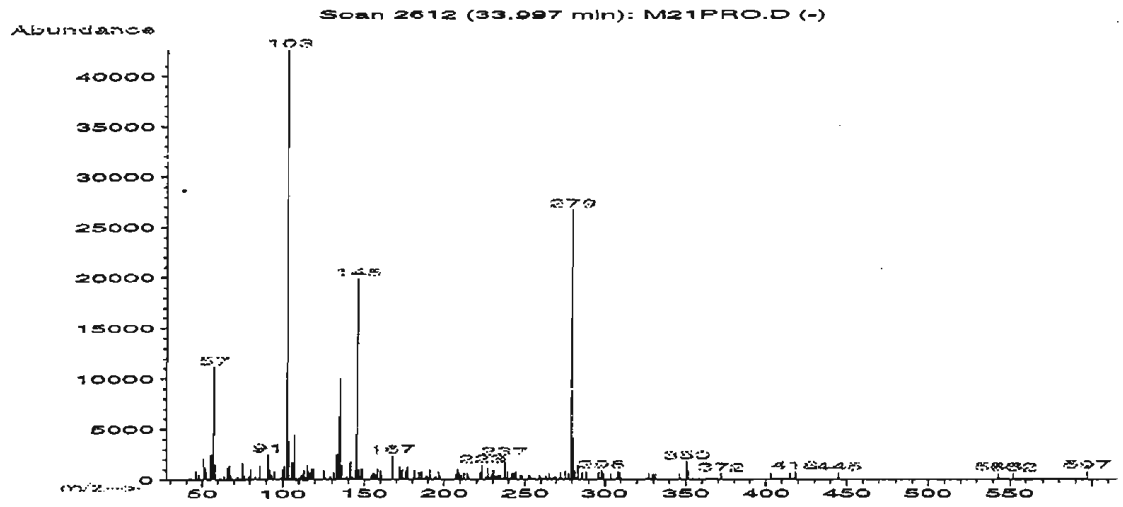
31


Scan 2530 (33.018 min): M21PRO.D (-)
Abundance
m/z-->

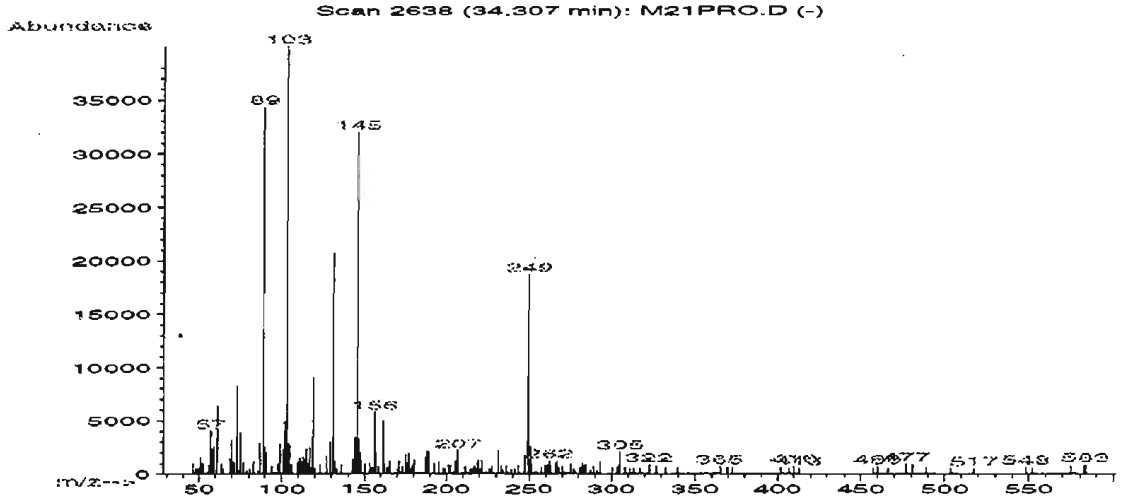
32


Scan 2587 (33.698 min): M21PRO.D (-)
Abundance
m/z-->

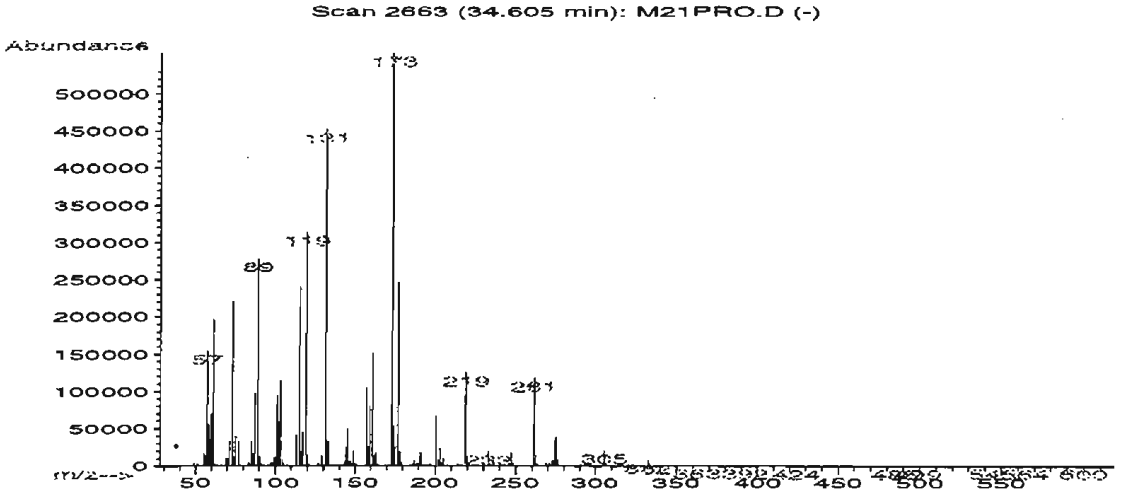
33


Scan 2612 (33.997 min): M21PRO.D (-)
Abundance
m/z-->

34

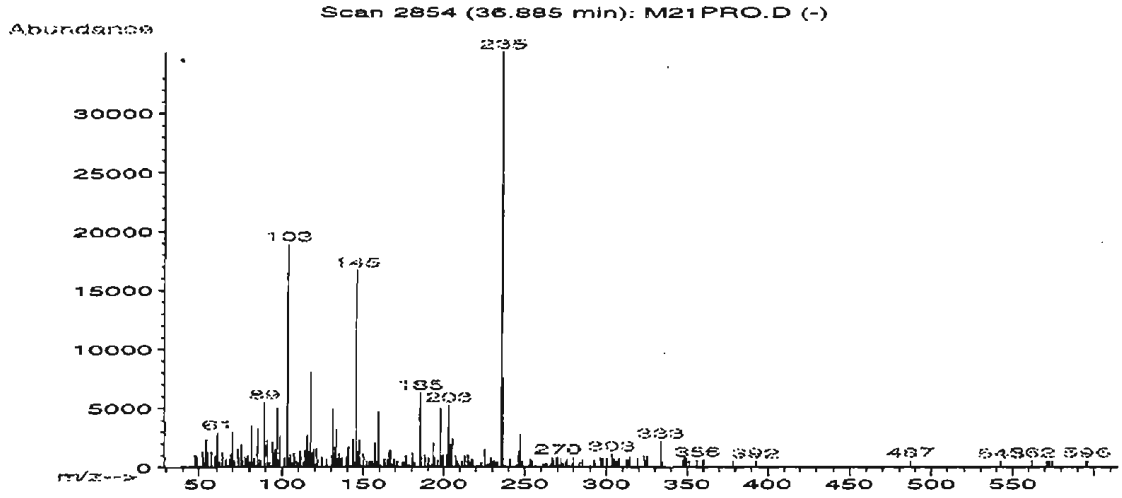

Scan 2638 (34.307 min): M21PRO.D (-)
Abundance
m/z-->

35

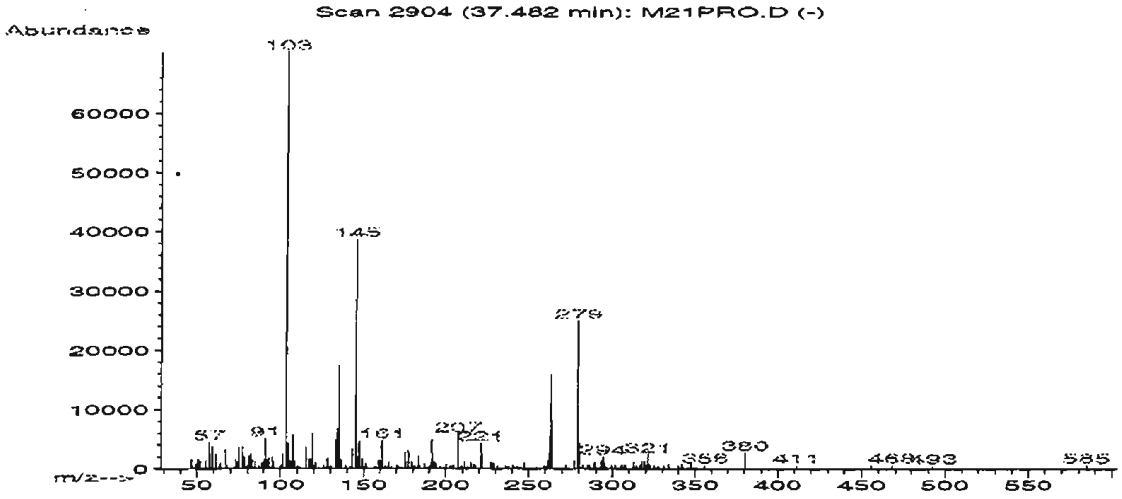

Scan 2663 (34.605 min): M21PRO.D (-)
Abundance
m/z-->

Internal standard at 34.712 minutes.

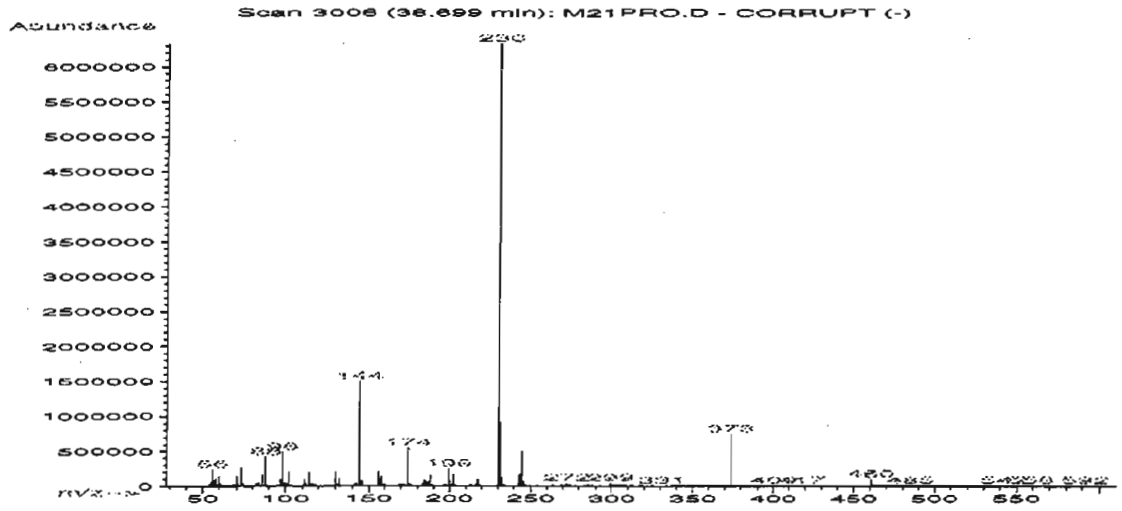
36



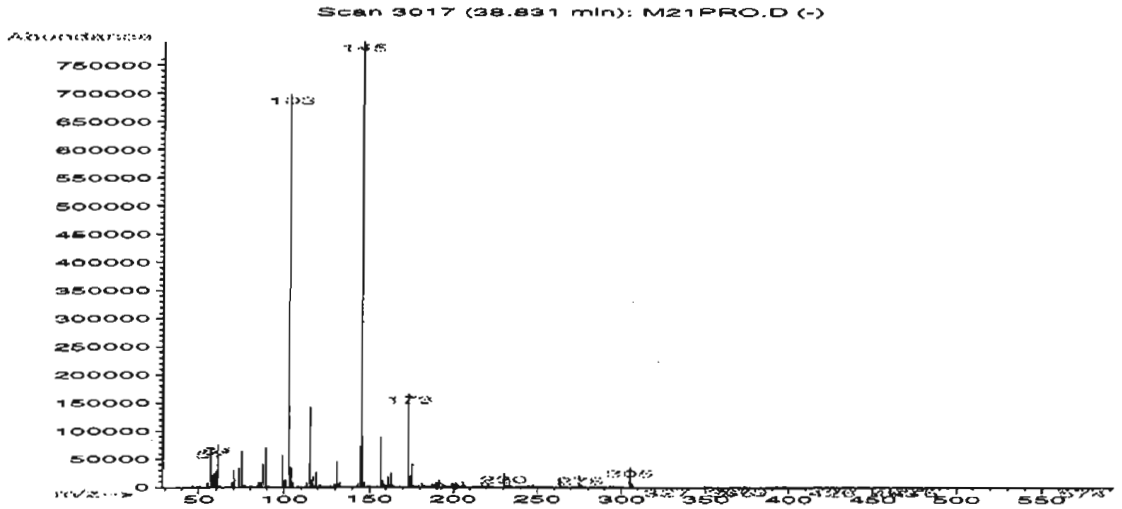
37



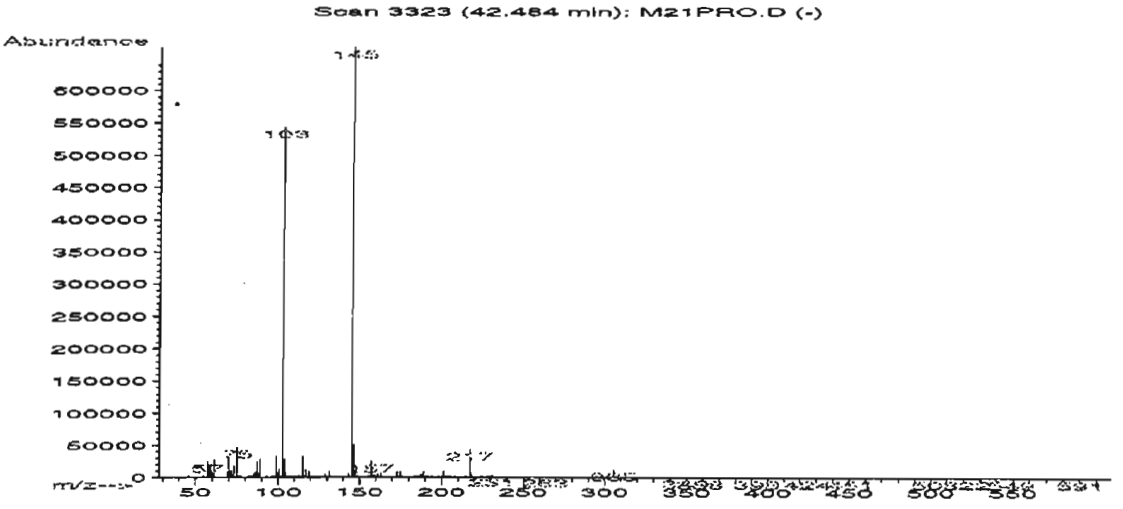
38


Scan 3008 (38.699 min): M21PRO.D - CORRUPT (-)
Abundance
m/z-->

39


Scan 3017 (38.831 min): M21PRO.D (-)
Abundance
m/z-->

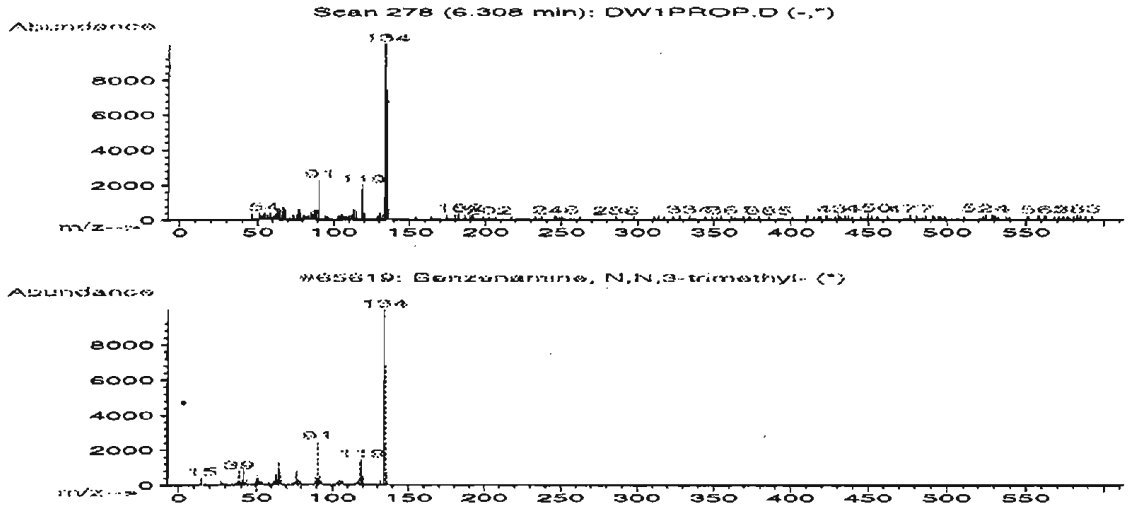
40


Scan 3323 (42.484 min): M21PRO.D (-)
Abundance
m/z-->

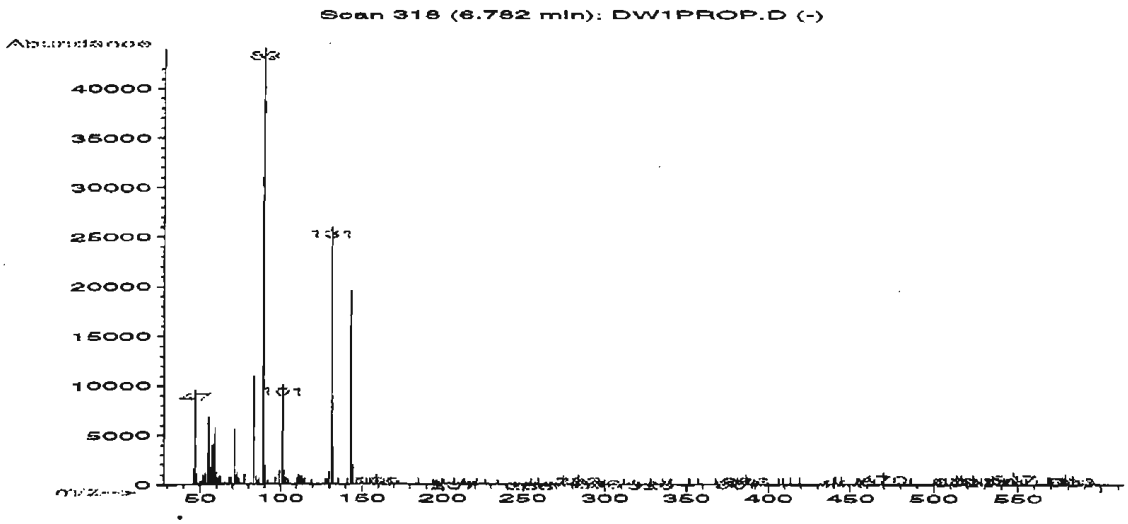
APPENDIX E

Spectra from propylated extract of DW1 (collected 12/13/93), run on EDTA2.M. Numbered as in Table 3.8.

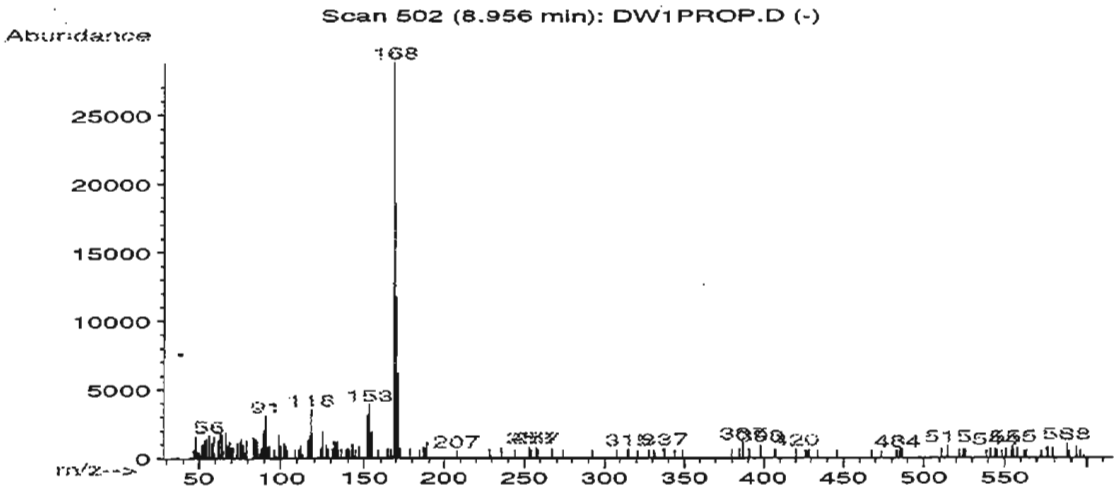
1



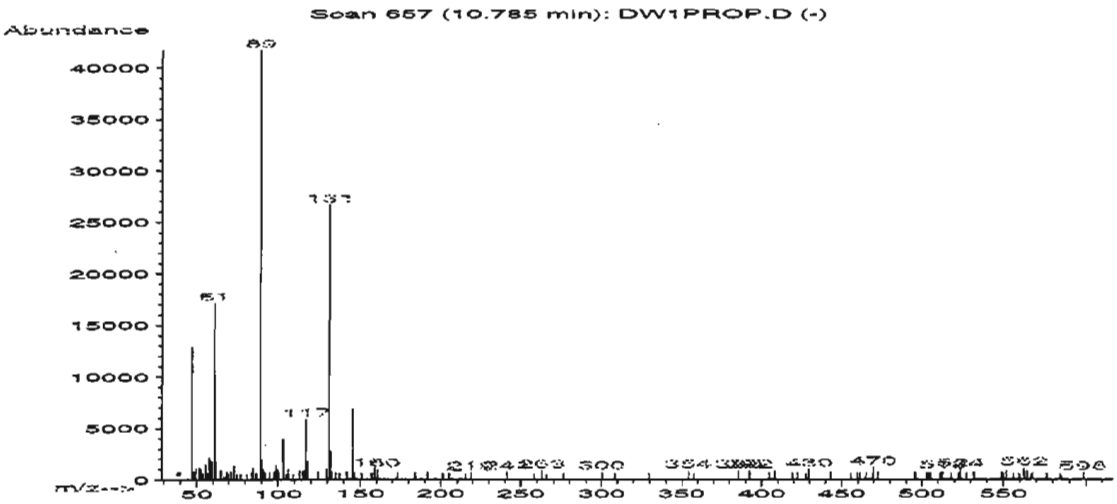
2



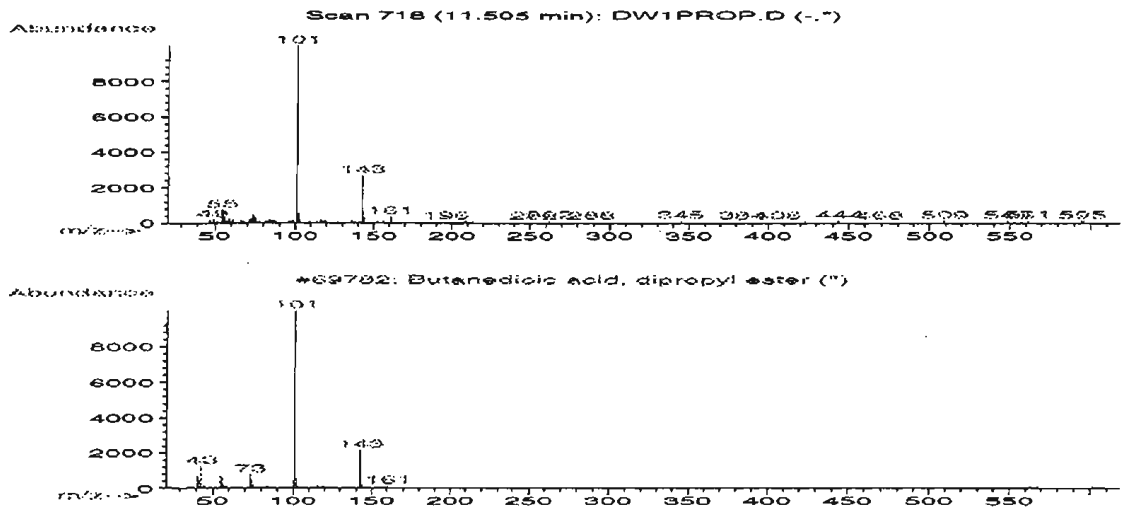
3


Scan 502 (8.956 min): DW1PROP.D (-)
Abundance
168
25000
20000
15000
10000
5000
0
56
91
116
153
207
m/z-->
50
100
150
200
250
300
350
400
450
500
550

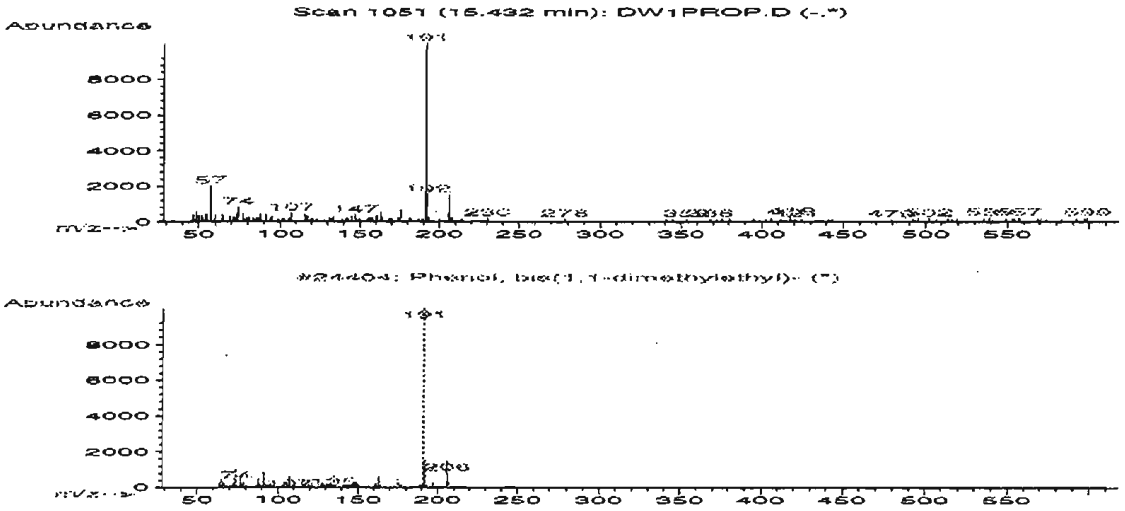
4


Scan 657 (10.785 min): DW1PROP.D (-)
Abundance
69
40000
35000
30000
25000
20000
15000
10000
5000
0
51
117
133
150
m/z-->
50
100
150
200
250
300
350
400
450
500
550

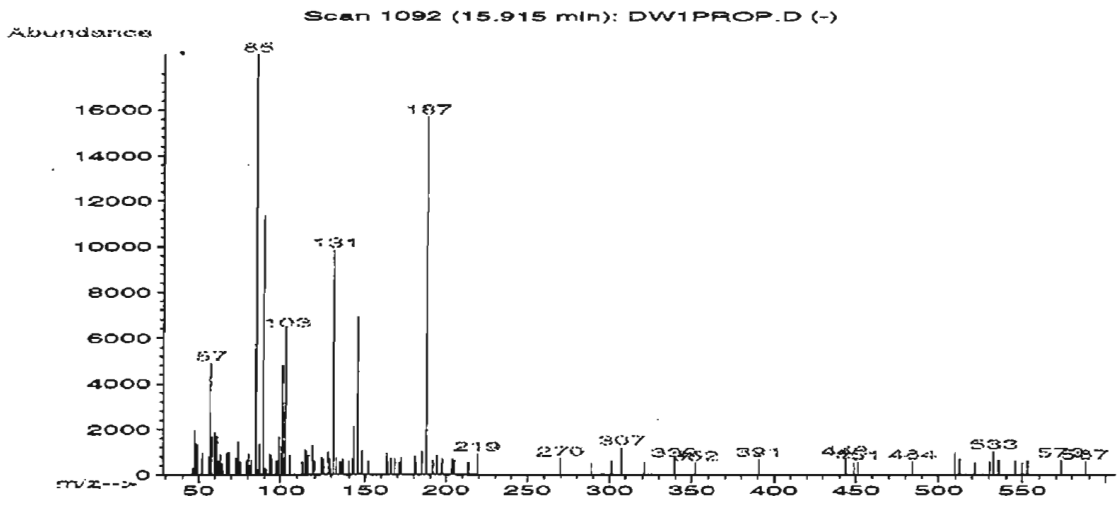
5


Scan 718 (11.505 min): DW1PROP.D (-.*)
Abundance
101
143
#69702: Butanedioic acid, dipropyl ester (*)
Abundance
101
143
161

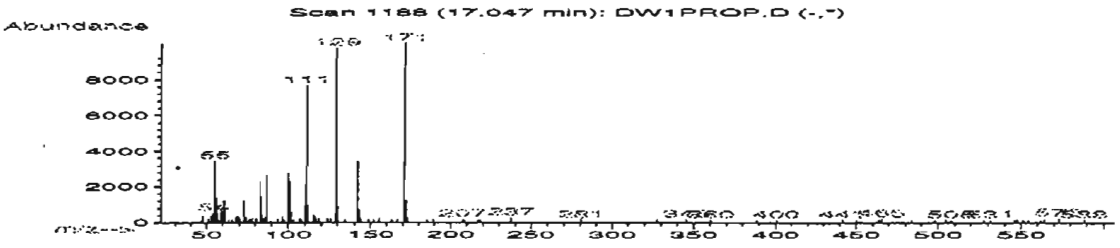
6

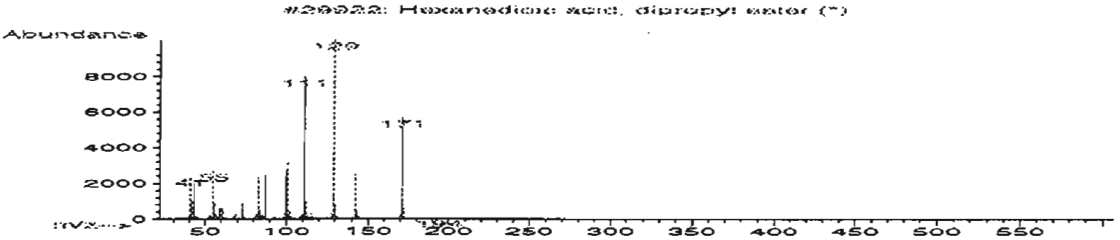

Scan 1051 (15.432 min): DW1PROP.D (-.*)
Abundance
101
57
#24404: Phenol, bis(1,1-dimethylethyl)- (*)
Abundance
101

7

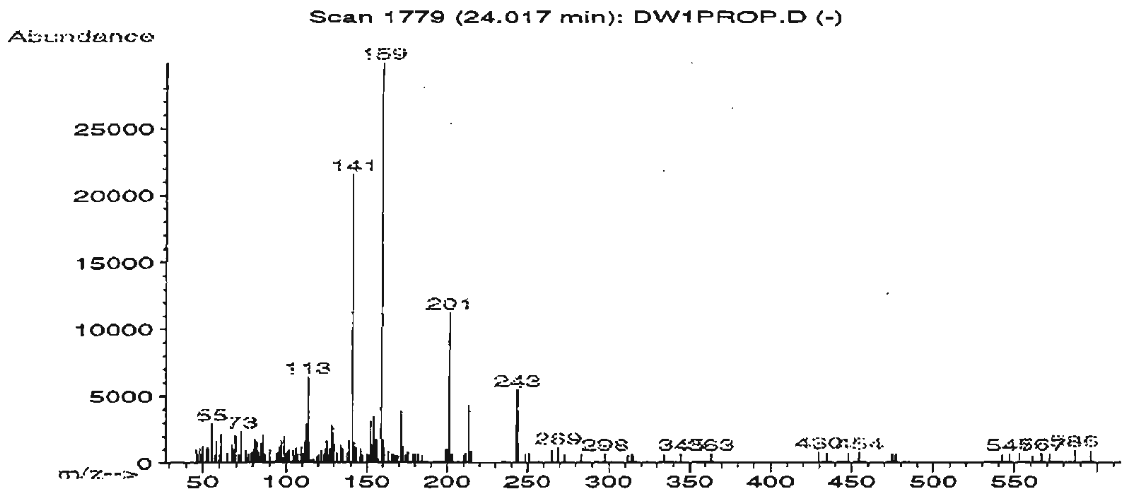

Scan 1092 (15.915 min): DW1PROP.D (-)
Abundance
85
187
131
103
57
219
270
307
331
533
m/z-->

8

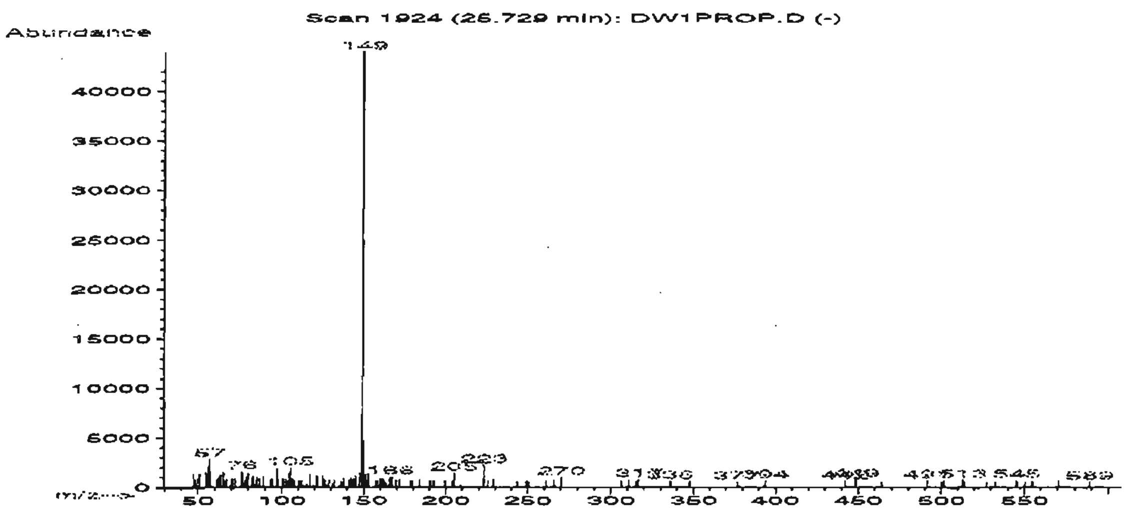

Scan 1188 (17.047 min): DW1PROP.D (-,*)
Abundance
129
171
111
55
m/z-->


#29922: Hexanedioic acid, dipropyl ester (*)
Abundance
129
111
171
m/z-->

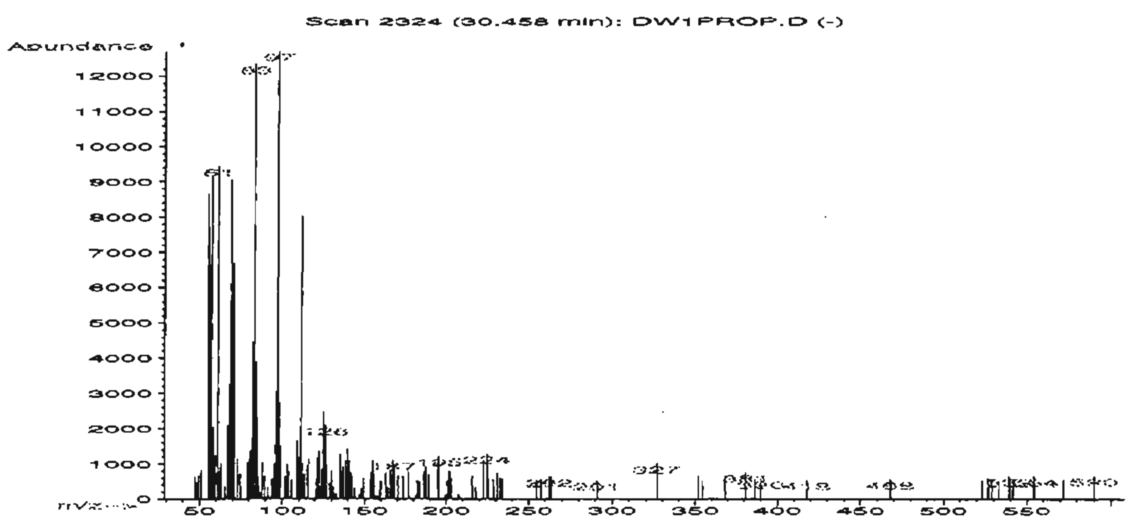
9


Scan 1779 (24.017 min): DW1PROP.D (-)
Abundance
m/z-->
159
141
201
113
243
55
73
269
298

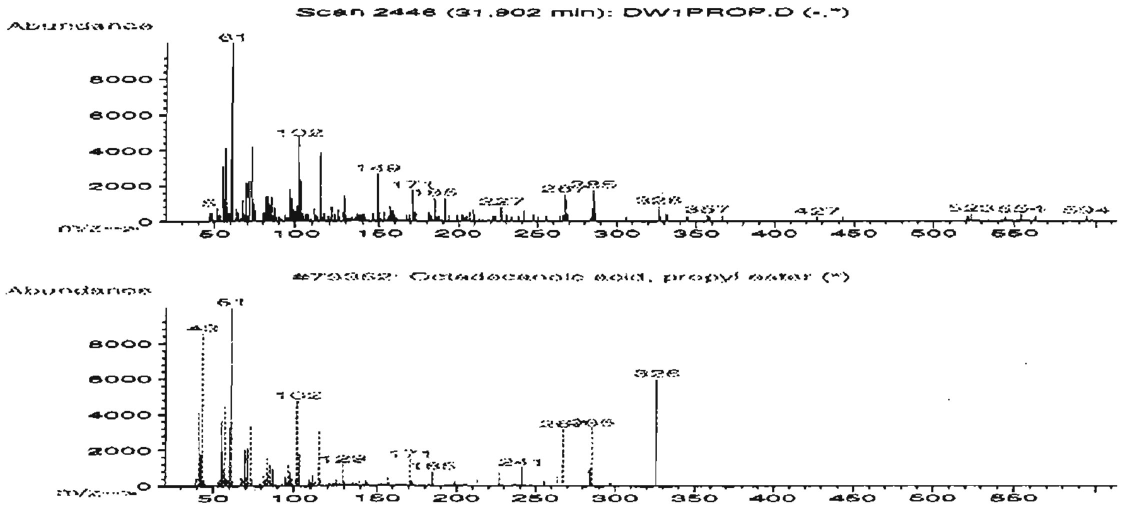
10


Scan 1924 (26.729 min): DW1PROP.D (-)
Abundance
m/z-->
149
57
76
105
205
223
270

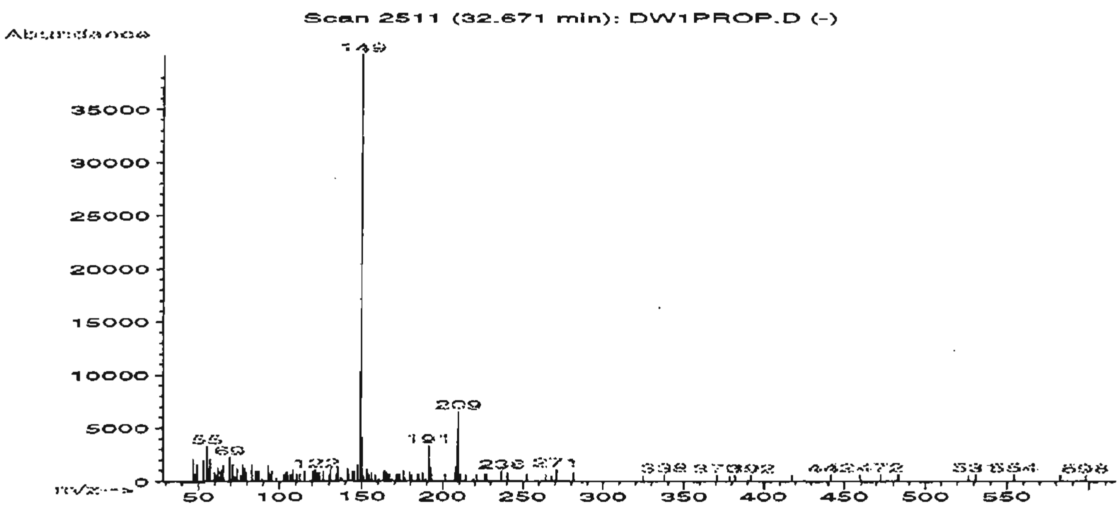
11


Scan 2324 (30.458 min): DW1PROP.D (-)
Abundance
m/z-->
126
327

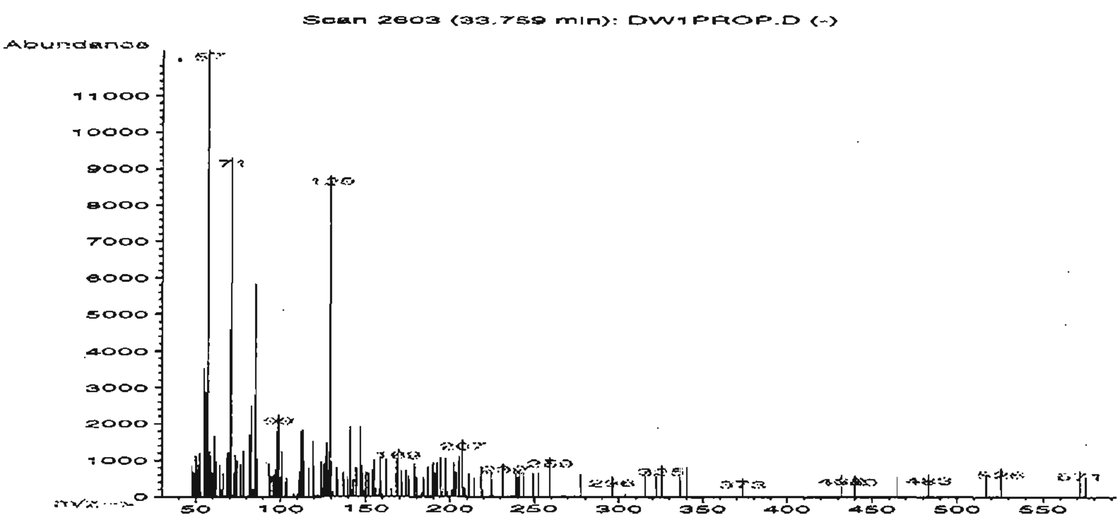
12


Scan 2446 (31.902 min): DW1PROP.D (-,*)
Abundance
m/z-->
Octadecanoic acid, propyl ester (*)
Abundance
m/z-->

13


Scan 2511 (32.671 min): DW1PROP.D (-)
Abundance
m/z-->

14

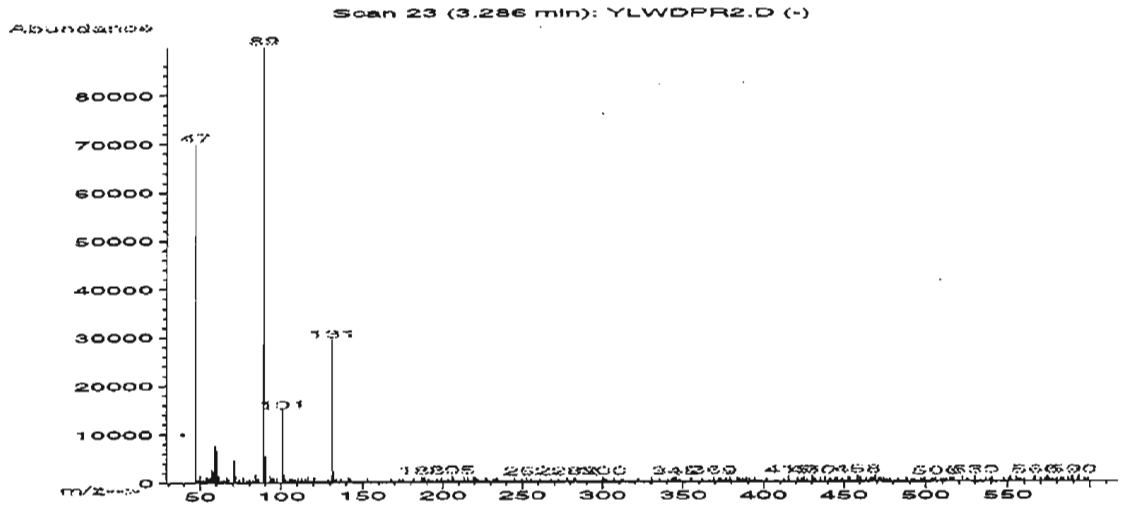

Scan 2603 (33.759 min): DW1PROP.D (-)
Abundance
m/z-->

Internal standard at 34.749 minutes.

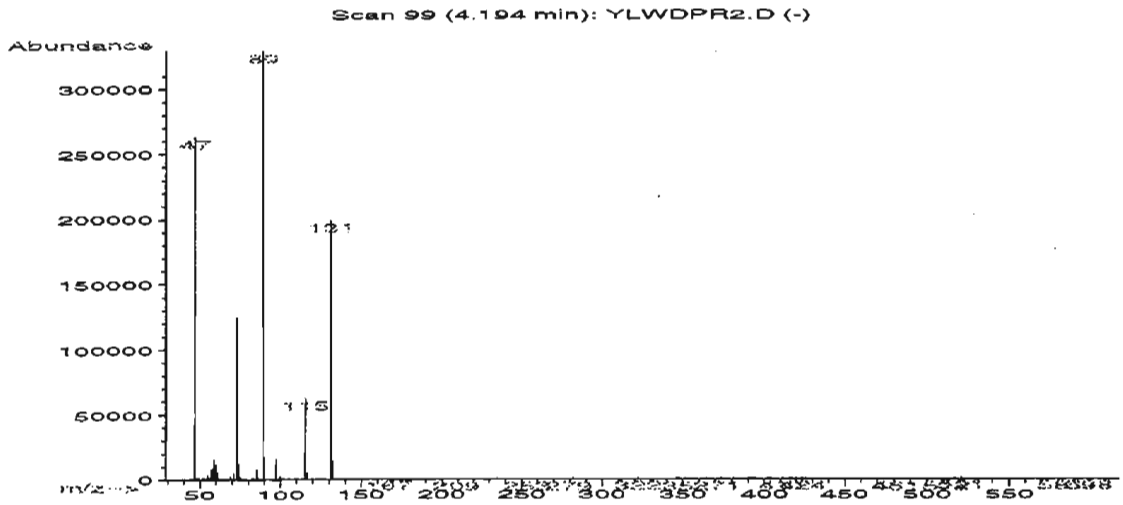
APPENDIX F

Spectra from propylated extract of YLWD11 (collected 11/30/93), run on EDTA2.M. Numbered as in Table 3.9.

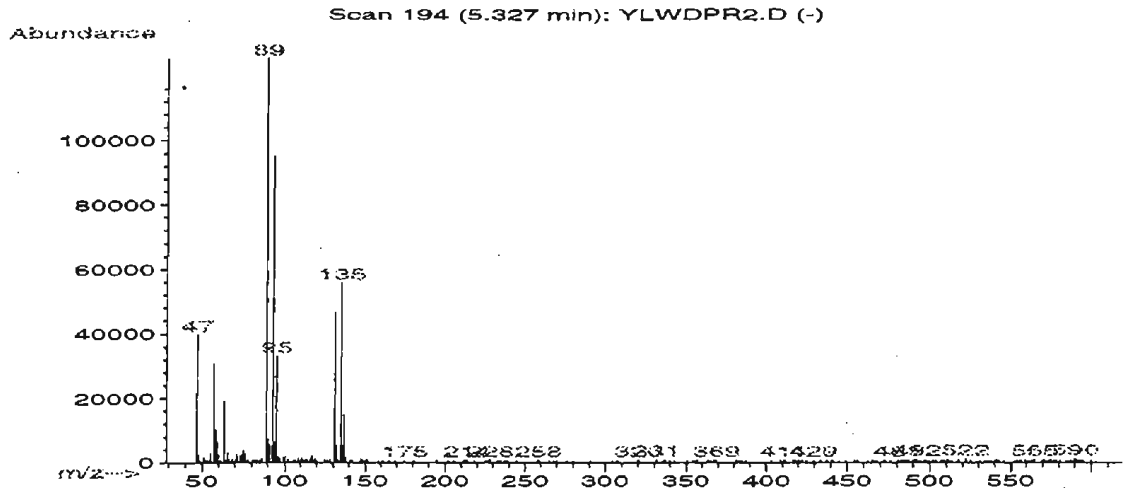
1



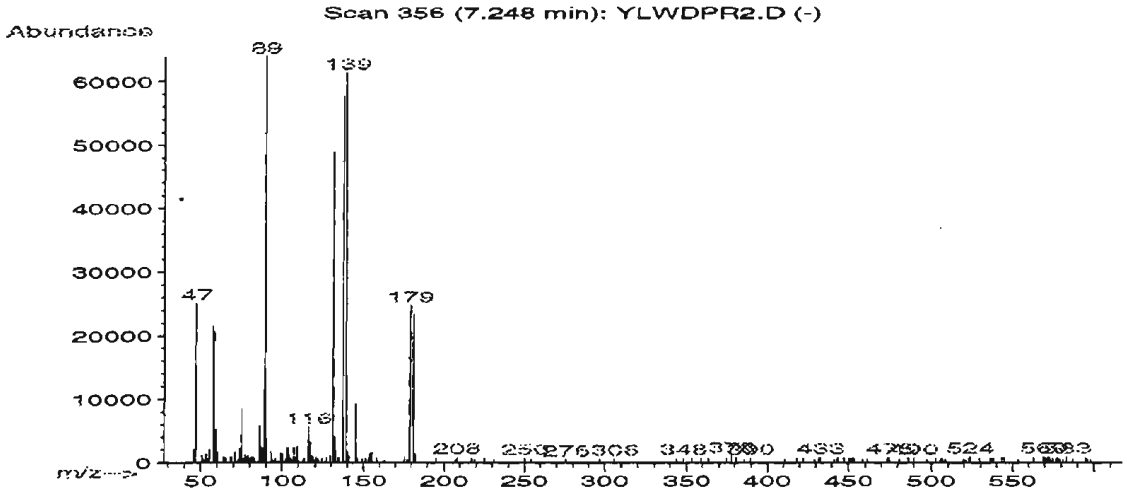
2



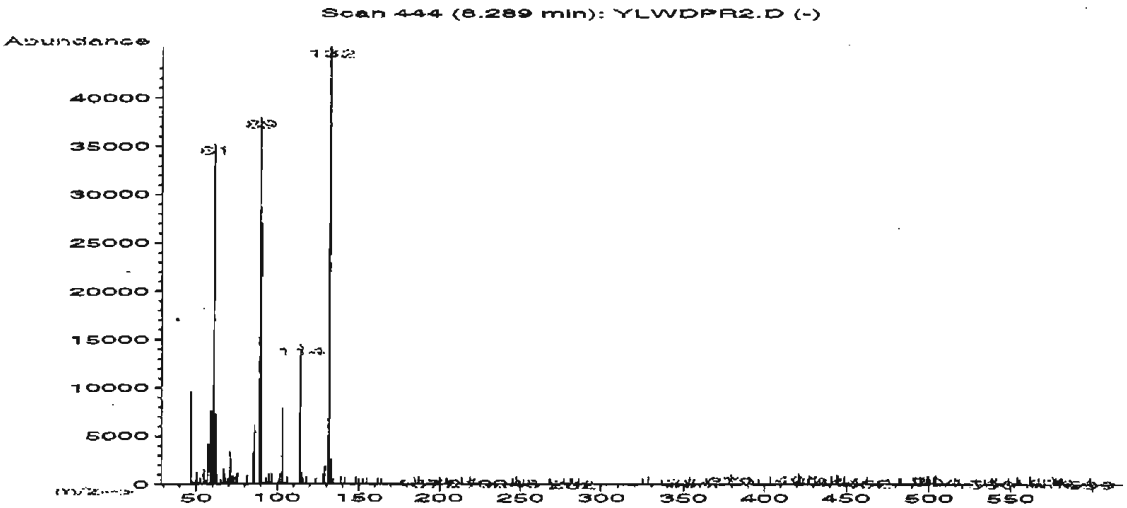
3


Scan 194 (5.327 min): YLWDPR2.D (-)
Abundance
89
135
47
85
m/z-->

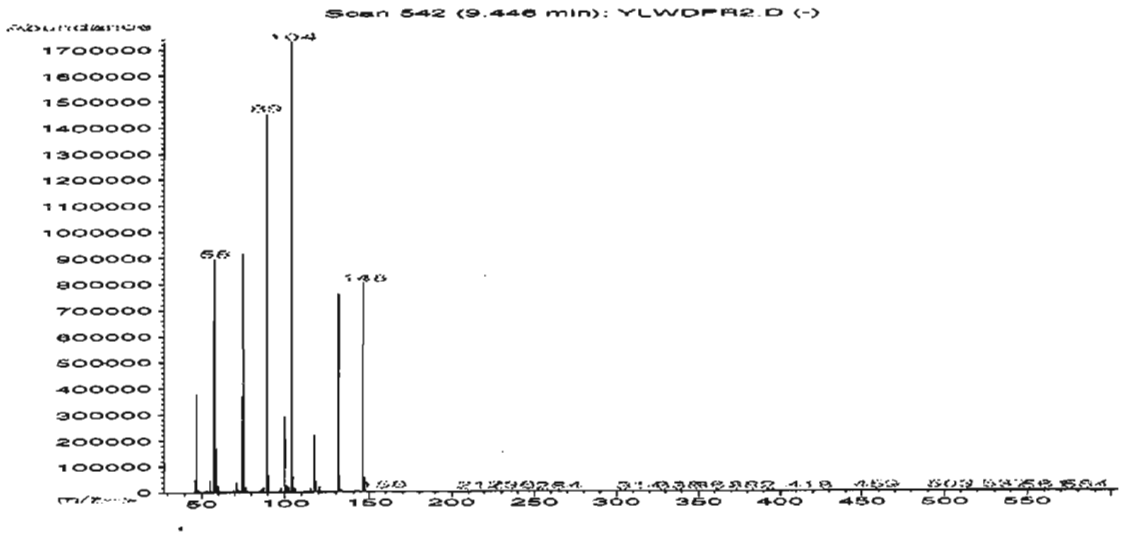
4


Scan 356 (7.248 min): YLWDPR2.D (-)
Abundance
89
139
47
179
116
m/z-->

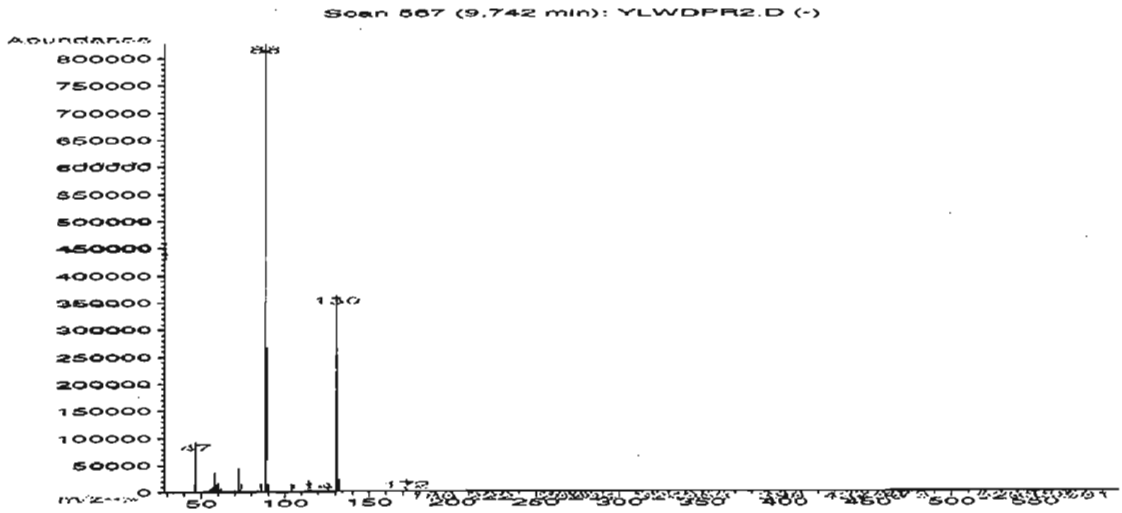
5


Scan 444 (8.289 min): YLWDPR2.D (-)
Abundance
132
91
m/z-->

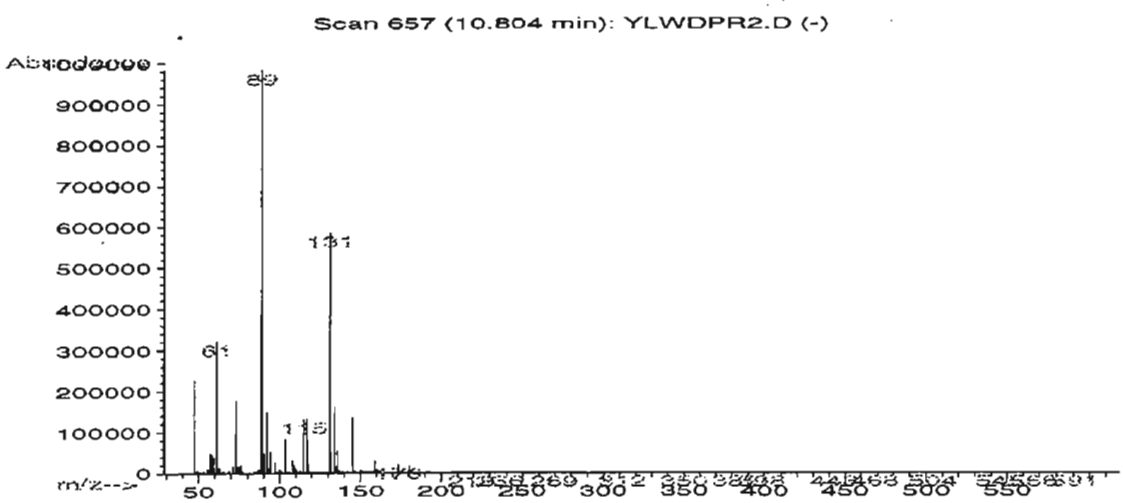
6


Scan 542 (9.446 min): YLWDPR2.D (-)
Abundance
m/z-->

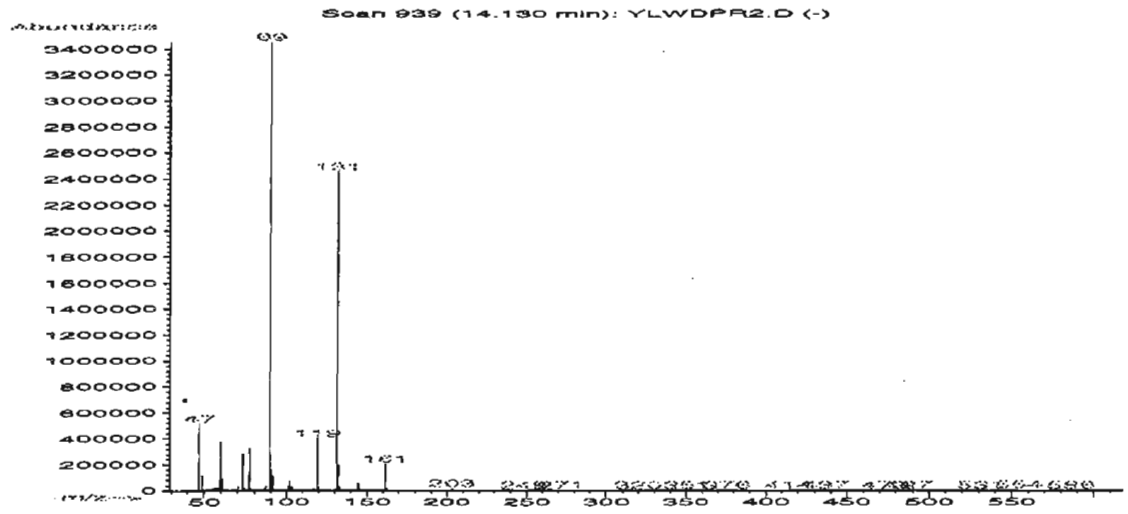
7


Scan 567 (9.742 min): YLWDPR2.D (-)
Abundance
m/z-->

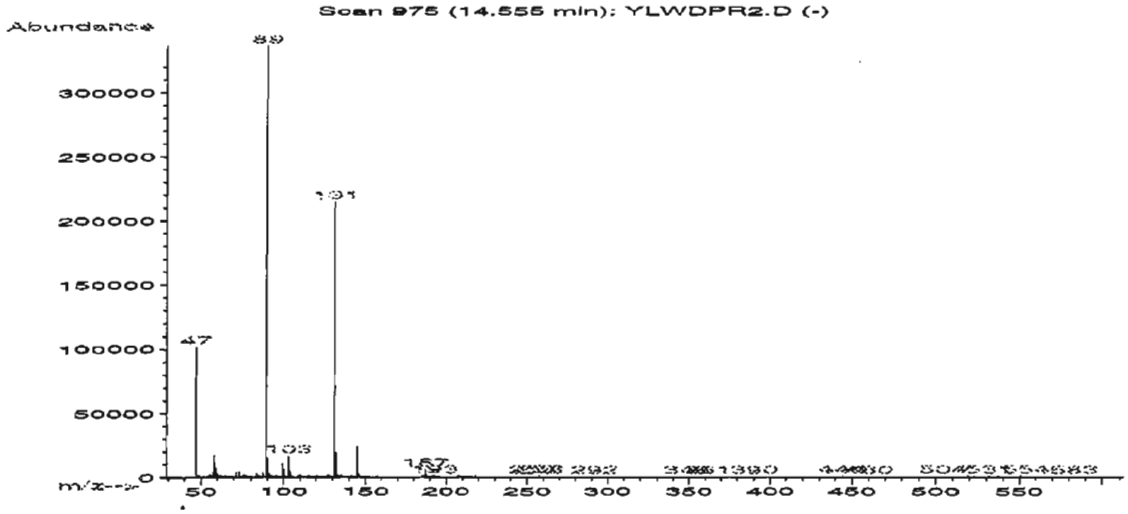
8


Scan 657 (10.804 min): YLWDPR2.D (-)
Abundance
m/z-->

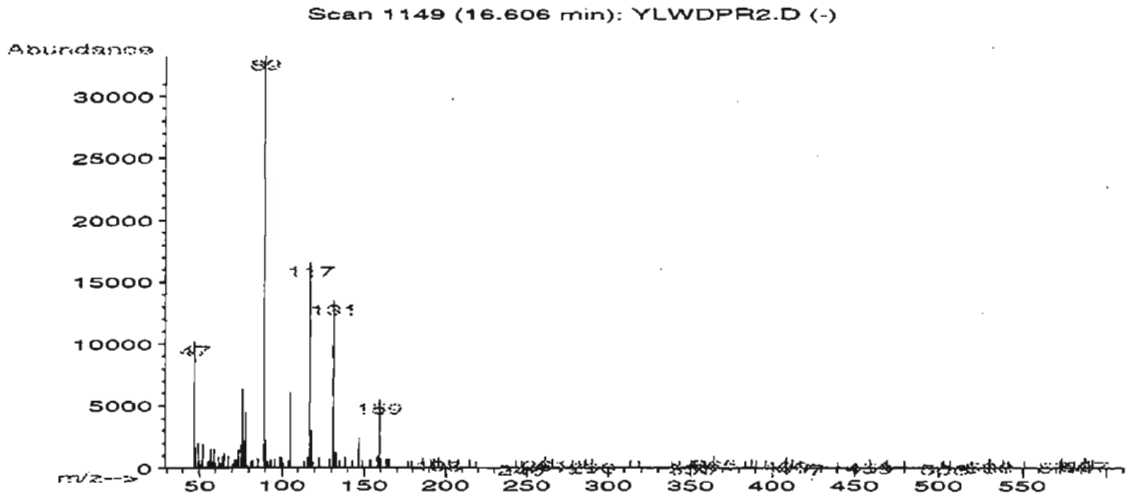
9


Scan 939 (14.190 min): YLWDPR2.D (-)
Abundance
m/z-->

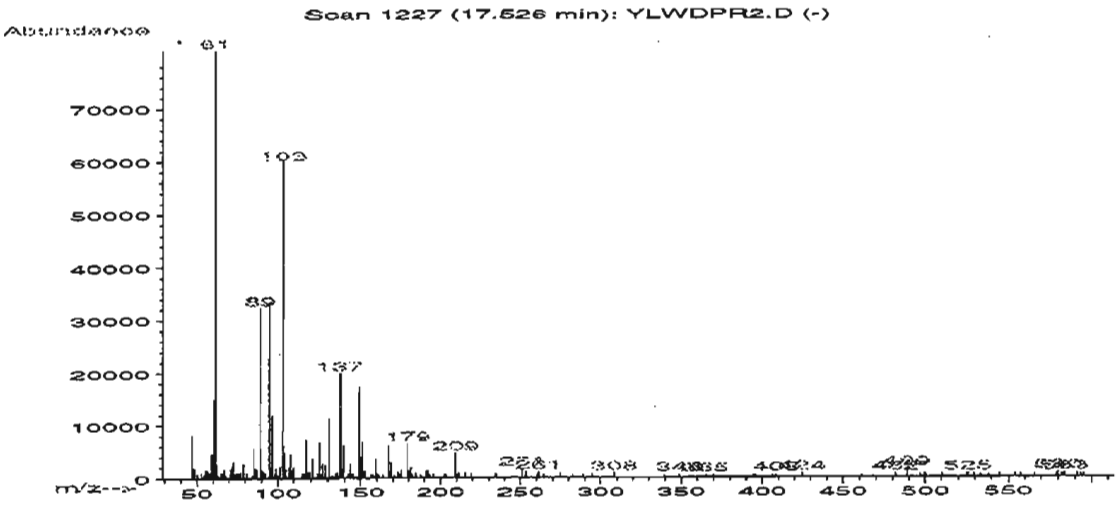
10


Scan 975 (14.555 min): YLWDPR2.D (-)
Abundance
m/z-->

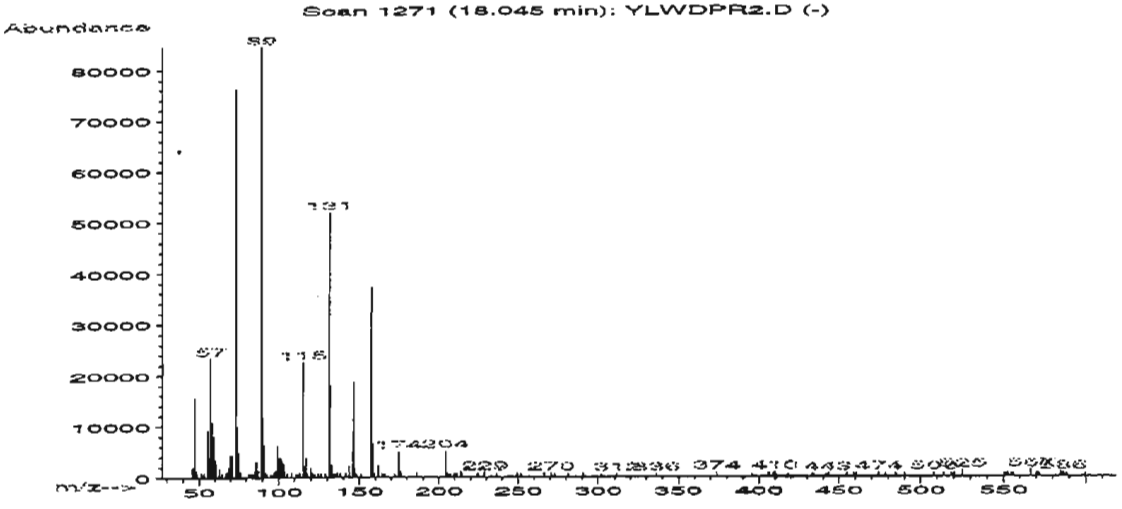
11


Scan 1149 (16.606 min): YLWDPR2.D (-)
Abundance
m/z-->

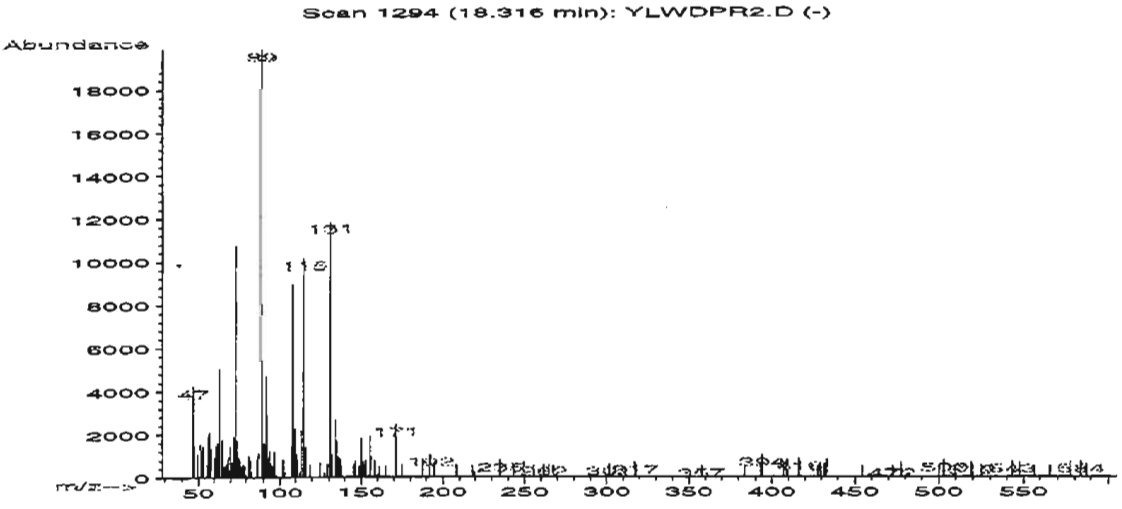
12


Scan 1227 (17.526 min): YLWDPR2.D (-)
Abundance
m/z-->

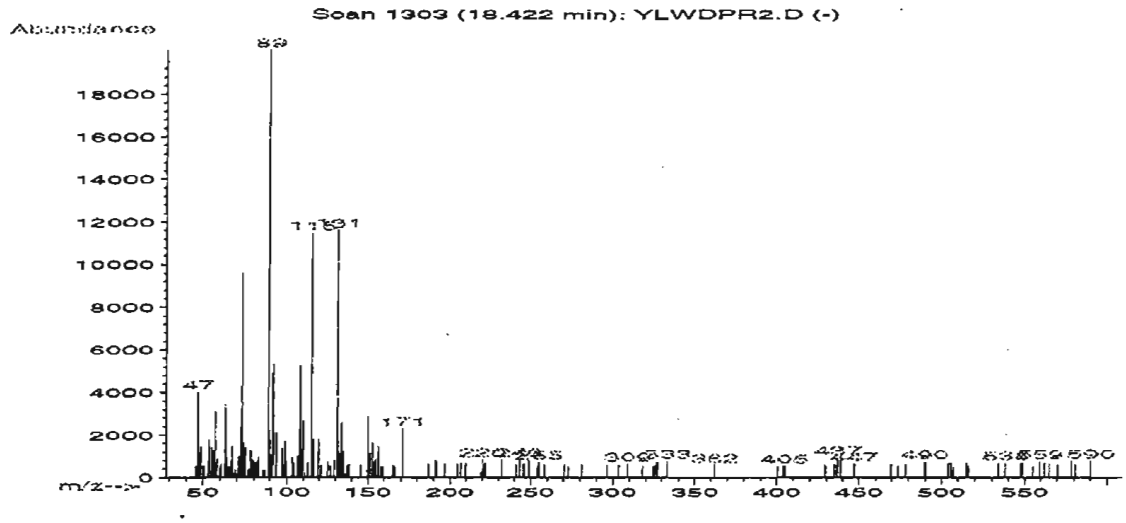
13


Scan 1271 (18.045 min): YLWDPR2.D (-)
Abundance
m/z-->

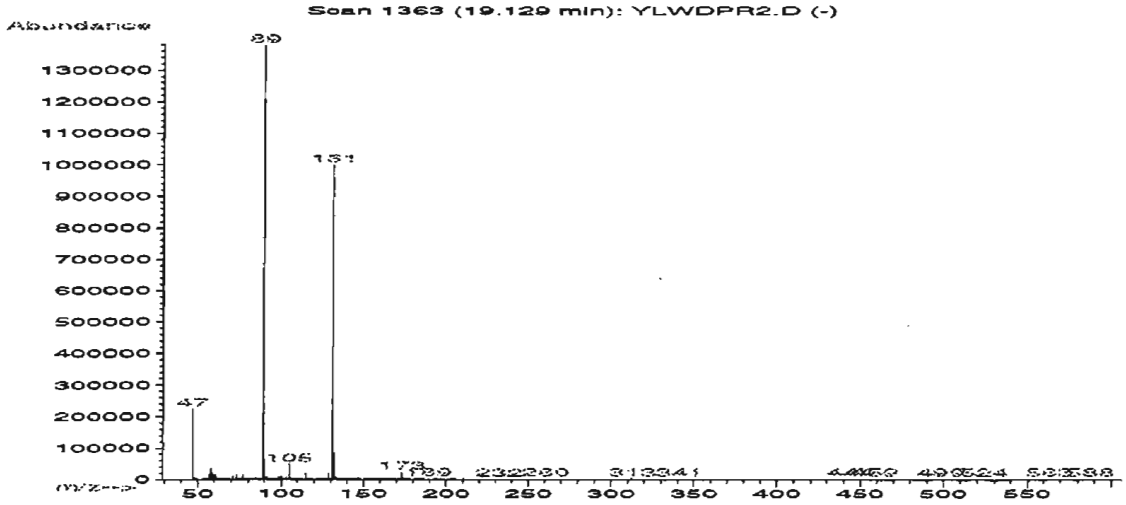
14


Scan 1294 (18.316 min): YLWDPR2.D (-)
Abundance
m/z-->

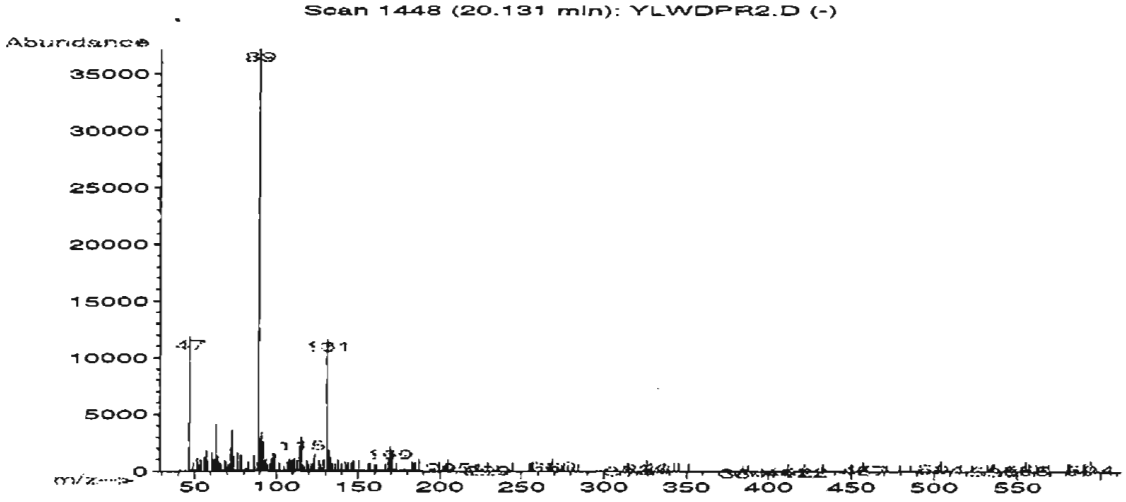
15


Scan 1303 (18.422 min): YLWDPR2.D (-)
Abundance
m/z-->

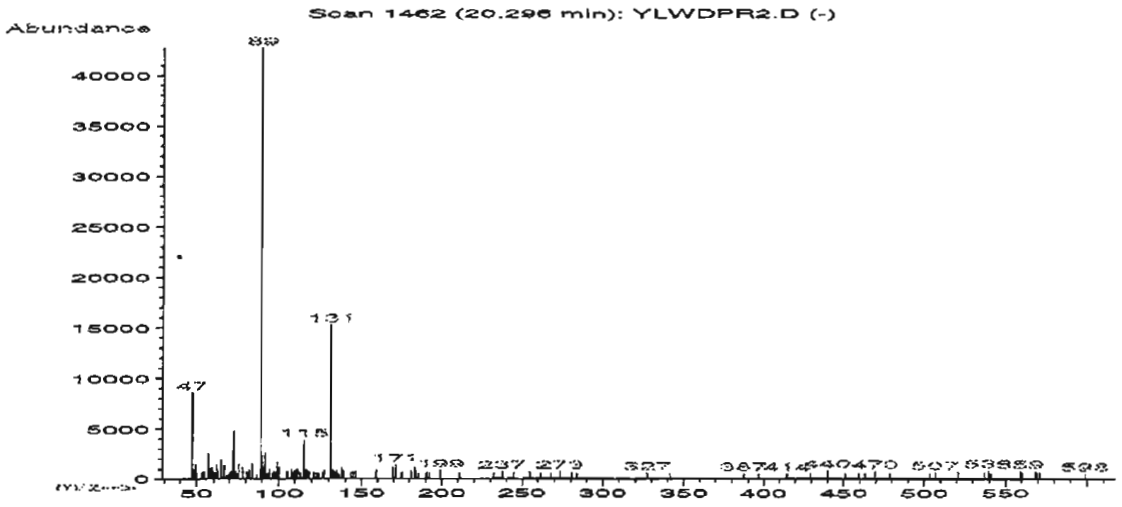
16


Scan 1363 (19.129 min): YLWDPR2.D (-)
Abundance
m/z-->

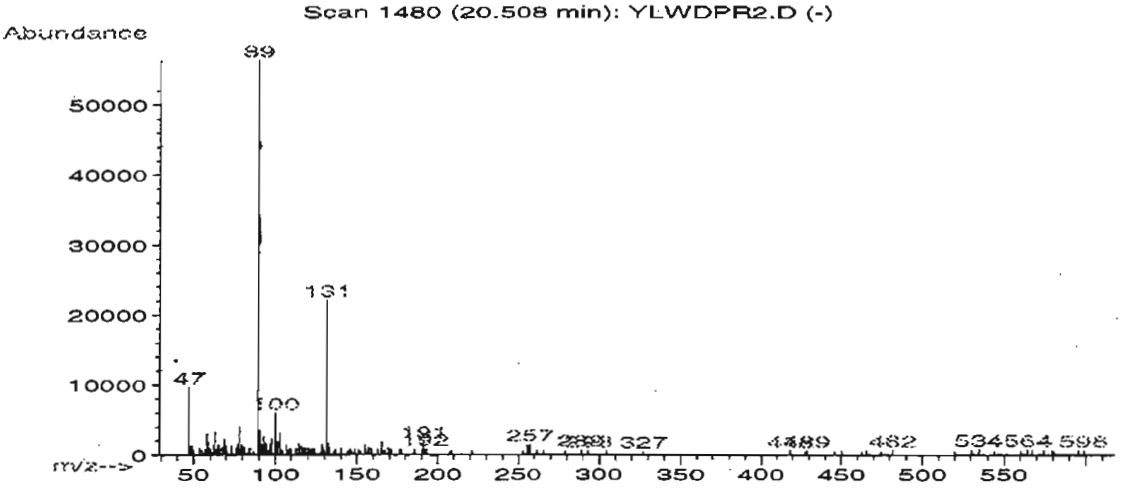
17


Scan 1448 (20.131 min): YLWDPR2.D (-)
Abundance
m/z-->

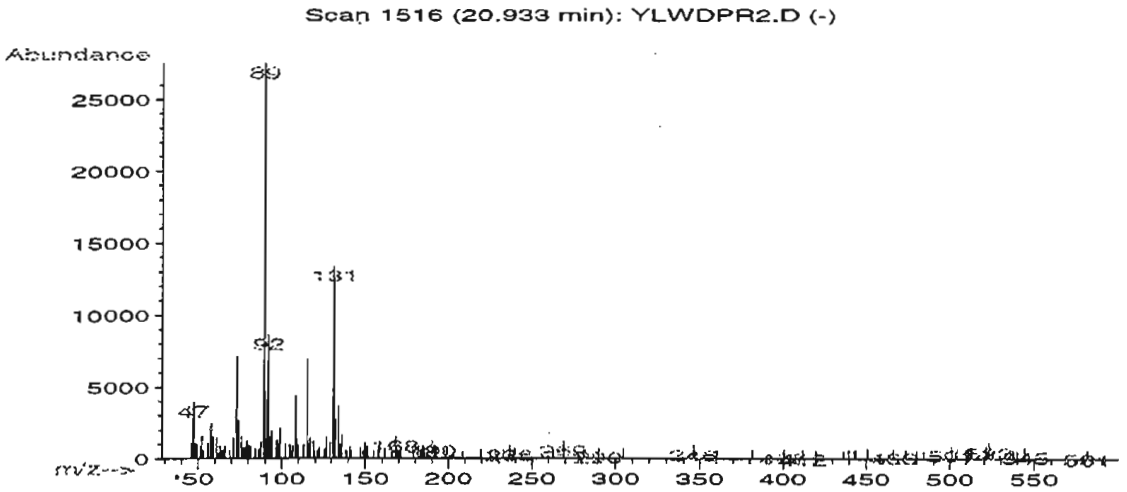
18


Scan 1462 (20.296 min): YLWDPR2.D (-)
Abundance
m/z-->

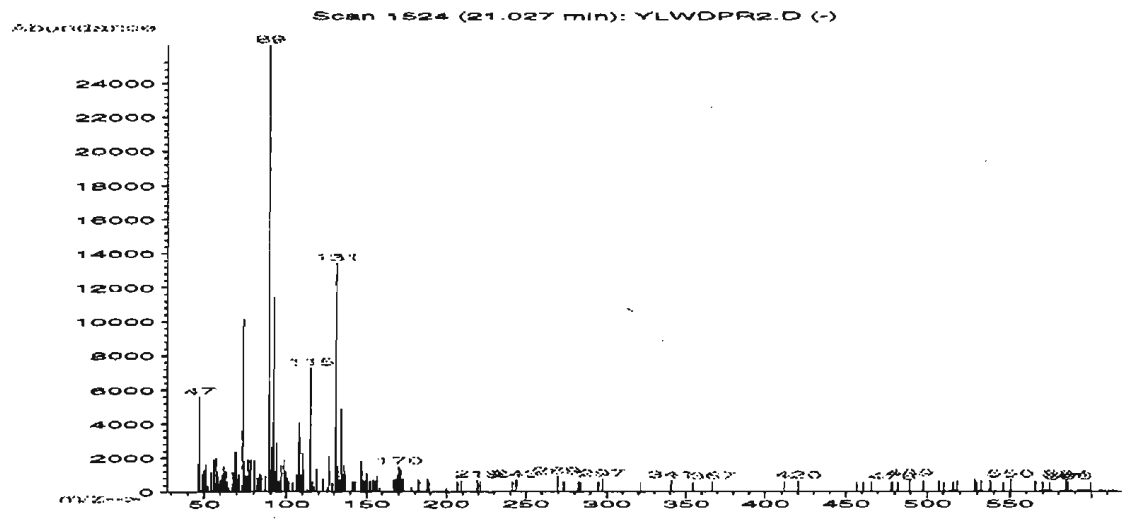
19


Scan 1480 (20.508 min): YLWDPR2.D (-)
Abundance
m/z-->

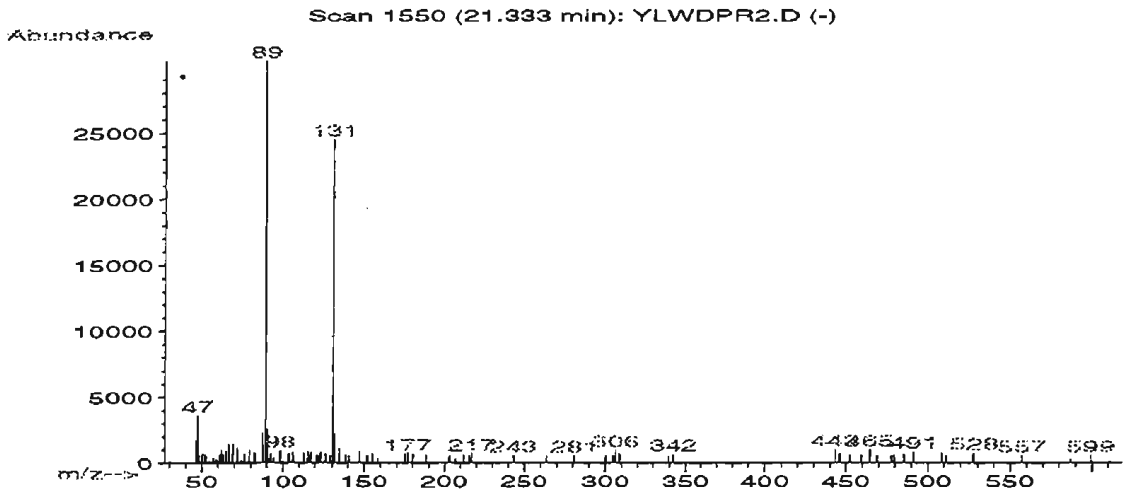
20


Scan 1516 (20.933 min): YLWDPR2.D (-)
Abundance
m/z-->

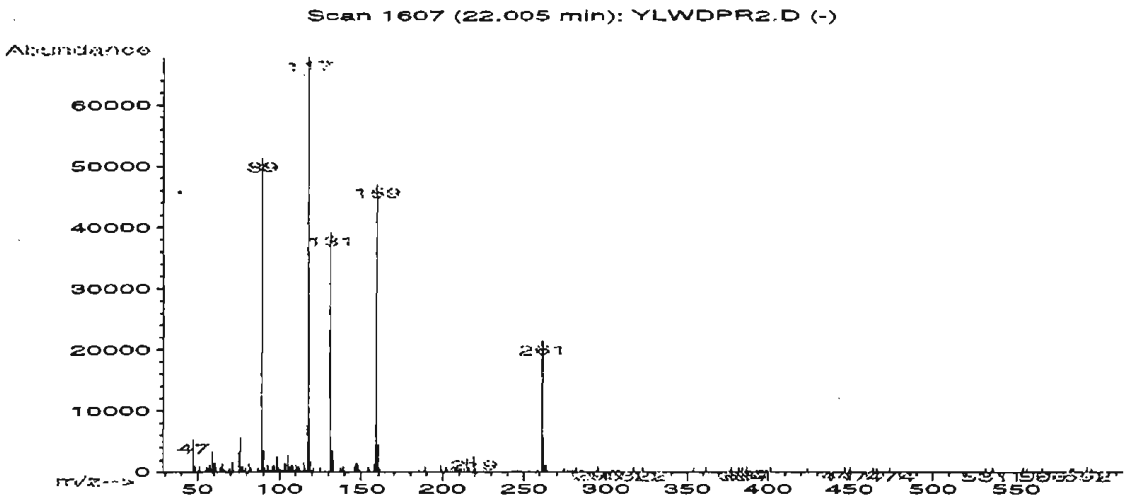
21


Scan 1524 (21.027 min): YLWDPR2.D (-)
Abundance
89
131
135
47
170
m/z-->

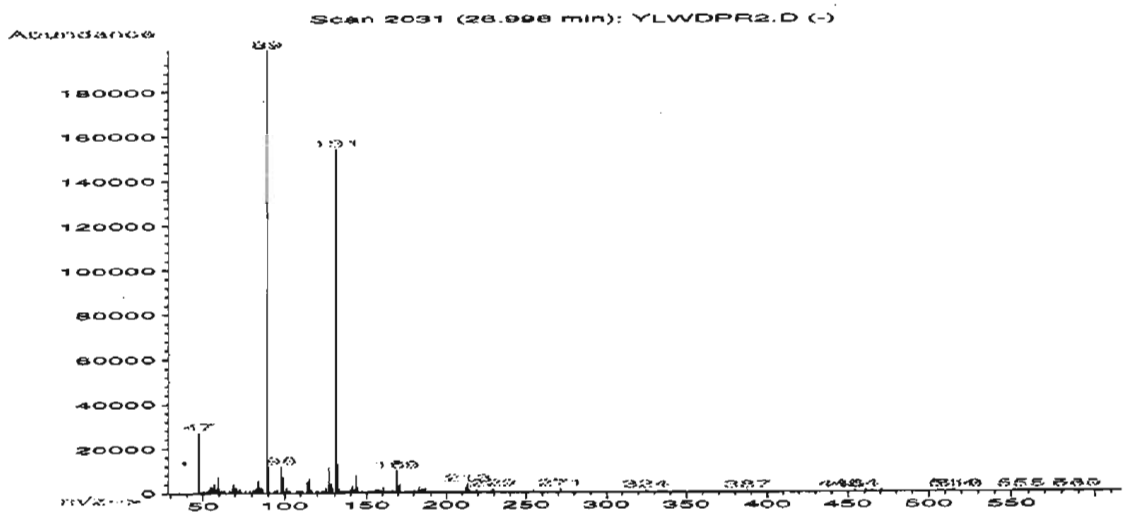
22


Scan 1550 (21.333 min): YLWDPR2.D (-)
Abundance
89
131
47
98
177
342
m/z-->

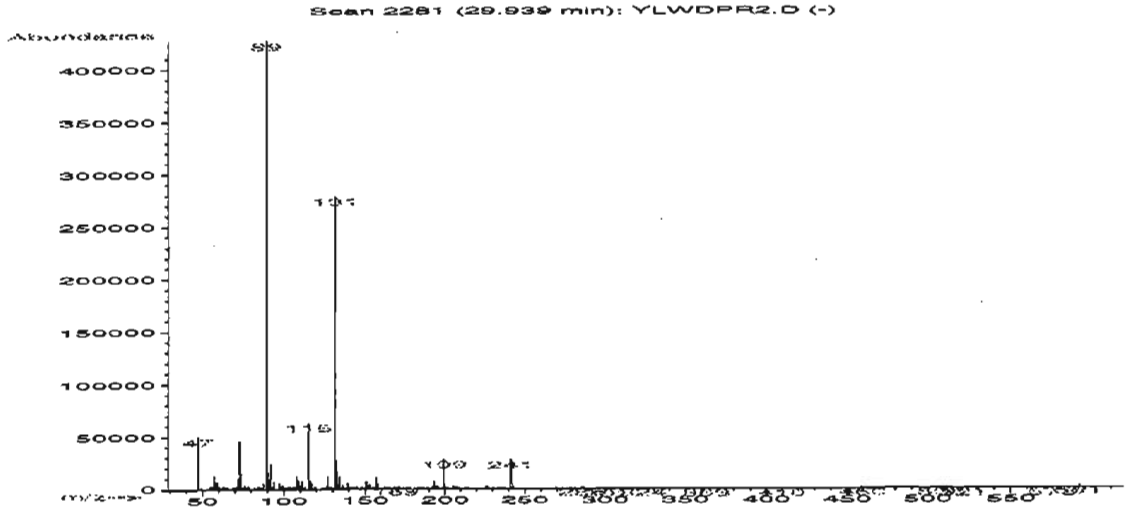
23


Scan 1607 (22.005 min): YLWDPR2.D (-)
Abundance
89
131
47
m/z-->

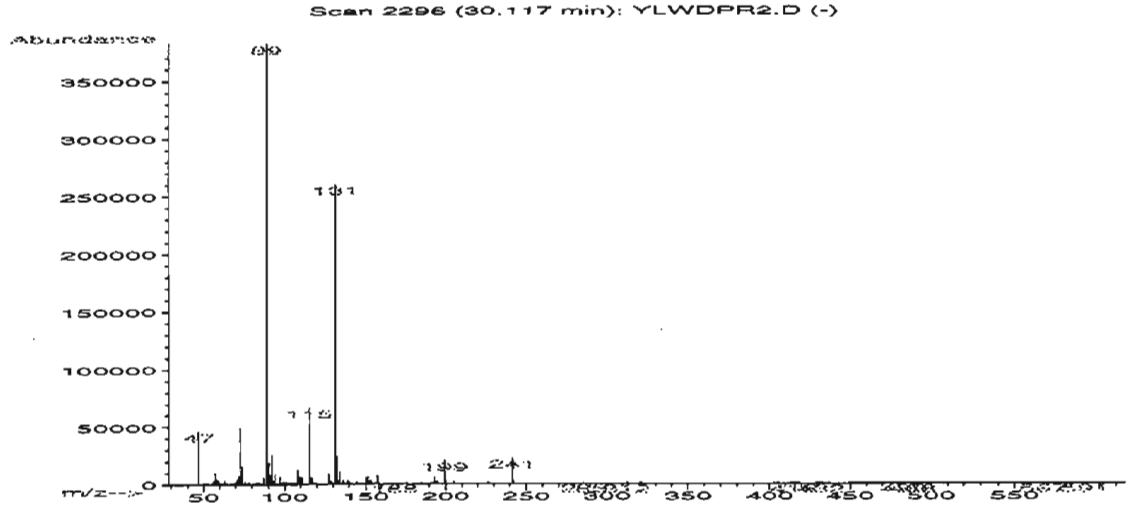
24


Scan 2031 (28.998 min): YLWDPR2.D (-)
Abundance
m/z-->

25


Scan 2281 (29.939 min): YLWDPR2.D (-)
Abundance
m/z-->

26


Scan 2296 (30.117 min): YLWDPR2.D (-)
Abundance
m/z-->

Internal standard at 34.742 minutes.