HIGH PERFORMANCE LIQUID CHROMATOGRAPHY COUPLED WITH MASS SPECTROMETRY METHOD DEVELOPMENT AND APPLICATIONS

THESIS

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Kai Zhou

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CHAPTER ONE

INTRODUCTION TO API/MS

1.1 Mass Spectrometry

Mass Spectrometry is an analytical technique which may be used to 1) identify unknown mass/charge ratios, 2) quantify unknown compounds and 3) elucidate the structure and chemical properties of molecules.

A mass spectrometer is an instrument that measures the masses of individual molecules that have been converted into ions. Each mass spectrometer consists of an ionization source, a mass analyzer, an ion detector and a data system. The general working procedure for a mass spectrometer is: the liquid, solid or vapor sample is introduced through an inlet, gas phase ions are produced, then sorted in the mass analyzer according to their mass-to-charge (m/z) ratio, then collected by a detector. In the detector the ion flux is converted to a proportional electric current. The data system records the magnitude of these electrical signals as a function of m/z and converts this information into a mass spectrum [1]. There are many kinds of ionization sources including: electron ionization (EI), chemical ionization (CI), fast atom/ion bombardment (FAB), secondary ion mass spectrometry (SIMS), thermospray (TSP), electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI). ESI and APCI ionize ions at atmospheric pressure and then transfer the ions into the mass spectrometer.

1.2 Electrospray Ionization (ESI)

The first experiment using electrospray was carried out by Chapman in the late 1930s [2]. In the late 1960s, Dole et al used this technique for sample ionization for mass spectrometry [3]. But the most important discovery was attributed to Fenn and Mann *et al.* who developed the modern-day technique of electrospray ionization-mass spectrometry (ESI-MS) [4]. The electrospray is produced by applying a strong electric field, under atmospheric pressure, to a liquid passing through a capillary tube. The electric field is obtained by applying a potential difference of 3-6 kV between this capillary and the counter electrode separated by 0.3-2 cm, producing electric fields on the order of 10^6 V m^{-1}. This field induces a charge accumulation at the liquid surface located at the end of the capillary, which will break to form highly charged droplets. A gas injected coaxially at a low flow rate allows dispersion of the spray to be limited in space. While the solvent is evaporating, the droplets keep shrinking. When their repelling coulombic forces are about the same as their cohesion forces, the division of the droplet occurs. These droplets proceed via a cascade of ruptures, producing smaller and smaller droplets. When the electric field on the surface of the droplet becomes large enough, a desorption of ions from the surface occurs. The higher the compound concentration at the surface of the droplet, the higher the sensitivity that can be obtained. "ESI is very incredible, because it is able to analyze samples with a molecular weight of over 130,000 with just a simple quadrupole!" [5].

ESI can analyze small molecules, tryptic digests (for mass mapping), oligonucleotides (including some non-covalent binding interactions), large and small

peptides (even those containing metal ions), carbohydrates and even C_{60} fullerenes [6-8]. Polar compounds such as amines, peptides, and proteins are best ionized by ESI.

1.3 Atmospheric Pressure Chemical Ionization (APCI)

APCI is an ionization technique that exploits gas-phase ion-molecule reactions at atmospheric pressure. In this method, the primary ions are produced by a corona discharge. APCI is a soft ionization method. It is mainly applied to non-polar compounds such as hydrocarbons and steroids with moderate molecular weight up to about 1500 D and generally gives monocharged ions [1]. The analyte in solution from a direct inlet probe or a liquid chromatograph elutes at a flow rate between 0.2 and 2 ml min^{-1} and is introduced directly into a pneumatic nebulizer where it is converted into a thin fog. Droplets then are displaced by the gas flow through a heated quartz tube called a desolvation/vaporization chamber. The heat transferred to the spray droplets allows vaporization of the mobile phase and of the sample in the gas flow. The temperature of this chamber is controlled, which makes the vaporization condition independent of the flow, the nature of the mobile phase, and the sample in the gas phase. The hot gas and the compounds leave this tube. After desolvation, they are carried to a corona discharge needle where ionization occurs.

1.4 The Quadrupole Ion Trap

A quadrupole ion trap consists of four parallel poles or rods, which are bent to form a closed loop. The outer rods made up the circular electrode and the top and bottom rods make up the caps. The overlapping of a direct potential with an alternative one gives

a "three dimensional quadrupole" in which ions of all masses are trapped in a three-dimensional trajectory. Ions with different masses are present together inside the trap. As the ions repel each other in the trap, their trajectories expand as a function of time. To avoid ion losses by this expansion, care has to be taken to reduce the trajectory. This is accomplished by maintaining a pressure of helium gas in the trap that removes excess energy from the ions by collision. The ion trap may be used as a mass spectrometer by applying a resonant frequency along the *z* axis to expel ions of a given mass.

1.5 Tandem Mass Spectrometry in the Ion Trap

Tandem mass spectrometry, also called MS/MS is a method that is widely used to identify the chemical structure of analytes. The general procedure is: 1, Select ions of one mass-to-charge ratio by expelling all the others from the ion trap. This can be performed either by selecting the precursor ion at the apex of the stability diagram or by resonant expulsion of all ions except for the selected precursor. 2, Let these ions fragment. Energy is provided by collisions with the helium gas, which is always present. This fragmentation can be improved by excitation of the selected ions by irradiation at their secular frequency. 3, Analyze the ions by one of the described scanning methods: stability limit or resonant ejection. 4, Alternatively, select a fragment in the trap and let it fragment further. This step can be repeated to obtain MS^n spectra.

Coupling two stages of mass analysis can be very useful in identifying compounds in complex mixtures and in determining structures of unknown substances. In product ion scanning, the most frequently used MS/MS mode, product ion spectra of ions of any chosen m/z value represented in the conventional mass spectrum are generated. From a mixture of ions in the source region or collected in an ion trap, ions of a particular m/z

value are selected in the first stage of mass analysis. These "parent" or "precursor" ions are fragmented and then the product ions resulting from the fragmentation are analyzed in a second stage of mass analysis. If the sample is a pure compound and fragment-forming ionization is used, the product spectra obtained from the fragment ions in the normal mass spectrum can provide much additional information for structural analysis. If the sample is a mixture and soft ionization is used to produce, for example, predominantly $[M+H]^+$ ions, the second stage of MS can be used to obtain an identifying mass spectrum for each component in the mixture. MS/MS can also be useful in eliminating interferences in single ion monitoring experiments when an ion signal at the m/z of interest is produced. For sector, quadrupole and time-of-flight instruments, each stage of mass analysis requires a separate mass analyzer. For quadrupole ion trap or ICR mass spectrometers, the MS/MS experiment can be conducted sequentially in time within a single mass analyzer [1].

1.6 Liquid Chromatography Coupled with Mass Spectrometry (LC/MS)

LC-MS is considered an important and mature analytical technique. It is applied widely in environmental, pharmaceutical, and natural product analysis. Combining LC and MS offers the possibility of taking advantage of both LC as a powerful separation technique, and MS as a sensitive detection and identification technique. A complex mixture thus can be separated by LC first and then introduced into the mass spectrometer. This can occur in two ways: the eluted compounds are collected and analyzed off-line; or the LC is connected directly to the MS and the mass spectra are acquired while the compounds of the mixture are eluted from the analytical column. The latter method operates on-line and is most commonly used. In order to couple LC and MS together to

conduct on-line analysis, interfaces have been developed to be placed between the LC column outlet and the mass analyzer in order to solve the incompatibility between the LC column outlet and the MS high vacuum. Over the past 25 years, researchers have built various LC-MS interfaces such as particle-beam, thermospray, continuous-flow FAB, electrospray and atmospheric chemical ionization (APCI). Currently, API based LC-MS interfaces, *i.e.*, electrospray and APCI are the most widely used approaches, while other interfaces like particle-beam, thermospray and continuous-flow FAB are also used to a more limited extent [9].

CHAPTER TWO

IDENTIFICATION OF ORGANOSULFUR COMPOUNDS IN ARABIAN CRUDE OIL

2.1 Introduction

Crude oil is a naturally occurring, flammable liquid whose chemical composition varies depending on the source. Crude oil and refinery products primarily consist of hydrocarbons (50%-90%) with the remainder composed of compounds containing N, S, O, and trace amounts of organometallics. The sulfur containing compounds include thiols, sulfides, disulfides and thiophene type compounds as well as inorganic sulfur. The sulfur compounds (0.025% - 11% by weight) in petroleum products create significant problems: they poison catalysts used in processing, and several sulfur heterocycles are suspected mutagens or carcinogens. Upon combustion of fuels rich in sulfur, sulfur dioxides, a major component of acid rain, are liberated [10]. Inorganic sulfur can be successfully removed from crude oil by a variety of physical separation methods, but organosulfur compounds are much more difficult to eliminate [11]. In order to convert crude oil into a more valuable fuel, obtaining the compositional analysis of different crude oils is desirable.

Rudzinski *et al* developed an ESI-MS method to effectively ionize polyaromatic organosulfur compounds by addition of Pd(II) into the sample solution thus enabling PASH compounds to be detected by the mass spectrometer [12]. It was reported that the ionization of the polyaromatic organosulfur compounds was due to electron transfer to

the Pd(II) cation from the PASH compounds [13]. The goal of this research was to apply this method, which was developed based on five standard PASH compounds, to an Arabian crude oil, and to identify and characterize organosulfur compounds in the crude oil. To achieve an ideal separation and characterization, a series of classical analytical methods were used. The overall procedure involved fractionation of the Arabian crude oil by using distillation and open column chromatography, followed by high performance liquid chromatography (HPLC), and last ESI-MS to identify the compounds.

2.2 Experimental

2.2.1 Reagents and Chemicals

HPLC grade Hexane, dichloromethane, methanol, toluene, acetonitrile, chloroform, diethyl ether, diethyl amine, palladium chloride, aluminum oxide (Neutral, Brockman activity I, 80/200 mesh), dibenzothiophene (DBT), benzonaphthothiophene (BNTP), 4,6-dimethyldibenzothiophene (4,6-DBT) and 2-methyldibenzothiophene (2-DBT) and thianthrene were obtained from Sigma-Aldrich (Milwaukee, WI, U.S.A). Silica gel (100/200) was obtained from Fisher Scientific (Pittsburgh, PA).

2.2.2 Fractionation Procedure for the Arabian Crude Oil

2.2.2.1 Distillation

Arabian crude oil was separated into light (<200°C at 760 torr.), middle (200-310°C at 760 torr), heavy (<260°C at 20 torr) and residue fractions by a series of distillations based on the maximum boiling points.

2.2.2.2 SARA Separation

Alumina was activated at 220°C for 96 hours. 10g activated alumina was pre-wet with hexane and poured into a glass column (1.1 x 30cm). The heavy fraction collected from distillation was separated into saturate, aromatic and resin fraction by the SARA method [14-16]. Heavy distillate 0.325g was dissolved in 5ml hexane and loaded at the top of the column. 40ml hexane was used to elute the saturates, 80ml toluene was used to elute the aromatic hydrocarbons, and 20ml toluene-methanol (80:20) was used to elute the resin fraction[17].

2.2.2.3 Ligand Exchange Chromatography

Ligand exchange chromatography (LEC) was applied to further separate the aromatic hydrocarbons into polyaromatic hydrocarbons (PAH), polyaromatic sulfur heterocycle (PASH), and polar sulfur compound (PSC) fractions [18]. 100ml distilled water was added to $PdCl_2$(1g) and silica gel (20g), then stirred. The wet gel was dried overnight at 95°C and activated at 200°C for 24 hours. The activated gel was pre-wet by hexane-chloroform (50:50) and packed into a column (1.1x30cm). 31.3mg aromatic hydrocarbon was loaded on top of the column. 30ml hexane-chloroform (50:50) was used to elute the PAH fraction, an additional 50ml hexane-chloroform (50:50) was used to elute the PASH fraction. 100ml chloroform-diethyl ether (90:10) was used to elute the PSC fraction. Fractions were dried by evaporating the solvents.

2.2.3 Instrumentation

2.2.3.1 HPLC Separation

The liquid chromatography system consisted of two Beckman Model 110B solvent delivery systems attached to a Beckman Model 406 Analog Interface Module connected to an ASA Systems computer with GOLD software. A Rheodyne injector was attached to a SUPELCUSIL LC-NH_2 column (25cm x 30mm) connected to a Beckman Model 166 variable wavelength detector set at 254 nm. 4 x 10^{-4}g PASH sample was dissolved in 2ml dichloromethane-hexane (10:90), and 20µl sample was injected via the injector. 100% hexane was used as the mobile phase and the flow-rate was set at 0.6ml/mın.

2.2.3.2 Mass Spectrometry

Mass spectra were obtained on a Finnigan LCQ ion trap MS equipped with an electrospray ionization (ESI) source. MS and MS^2 full scan mode were used for the identification of organosulfur compounds. A Gateway 2000 computer with Xcalibur 1.2 software was used for data collection and analysis.

Sample preparation followed a protocol previously developed for the identification of standard PASH compounds [12]. PASH sample 2 x 10^{-4}g was dissolved in 1ml CH_3OH-CH_3CN (50:50), then $PdCl_2$ was added to the sample to a final concentration of 1mM.. All of the samples were injected by direct infusion at a flow rate of 3µl/min using the dual syringe pump of the Finnegan LCQ instrument. The nitrogen sheath gas flow-rate was 60 arbitrary units; ion mode positıve; ionspray voltage 3.5kV; capillary temperature 200°C; capillary voltage 60V; tube lens offset voltage 40V, and the source fragmentation energy was optimized for each compound. Each MS scan was the

average of 10 microscans and data for the mass spectra were based on 2-125 scans. MS was performed in full scan mode over the m/z range 100-500.

MS/MS experiments were performed to further identify the PASH compounds. From the PASH fraction's MS full scan spectrum, peaks with the same m/z ratio as the standard organosulfur compounds' m/z ratios were picked to conduct tandem mass spectrometry. The collision energy was optimized for each compound in order to obtain spectra showing both the parent ion peak and stable fragments.

2.3 Results and Discussion

2.3.1 Distillation

Table 2.1 shows the results of Arabian crude oil distillation. As the boiling points of the compounds increased, their densities increased, and the fractions become darker and denser.

Fraction	Boiling Point	Volume	Density
Arabian Crude Oil	N.A	230mL	0.93g/mL
Light Distillate	<200°C@760torr	55mL	0.79g/mL
Middle Distillate	200-310°C@760torr	65mL	0.98g/mL
Heavy Distillate	<260°C@20torr	26mL	1.13g/mL
Residue	>260°C@20torr	59mL	1.26g/mL

Table 2.1 Results of the Arabian Crude Oil distillation

2.3.2 SARA Separation

Figure 2.1 is the flow chart for the SARA separation. The separation was conducted on the heavy distillate of Arabian crude oil.

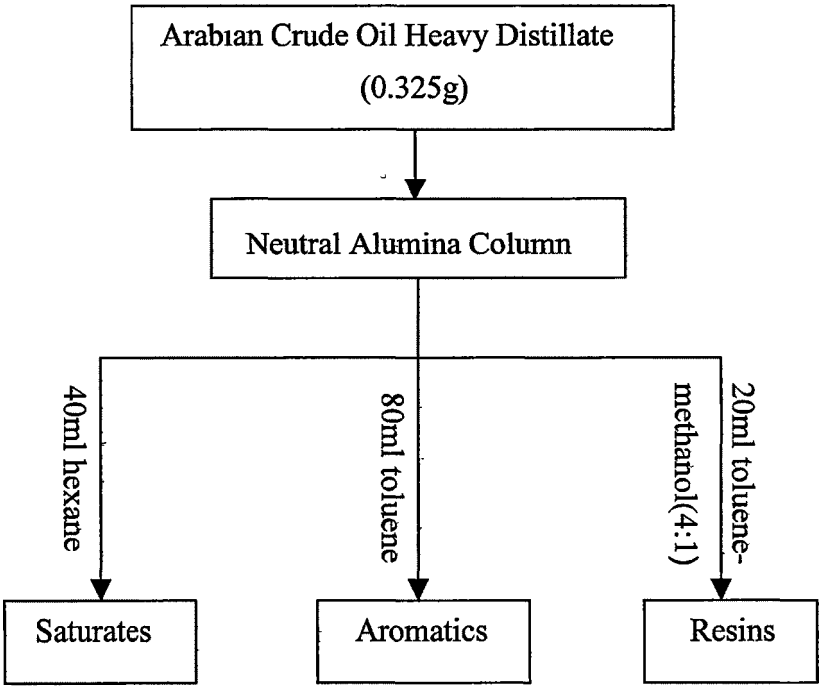


Figure 2.1 SARA Separation Scheme

2.3.3 Ligand Exchange Chromatography Separation

Figure 2.2 shows the flow chart for the ligand exchange chromatographic separation. The separation of aromatic hydrocarbons by ligand exchange chromatography is through a metal-π binding interaction.

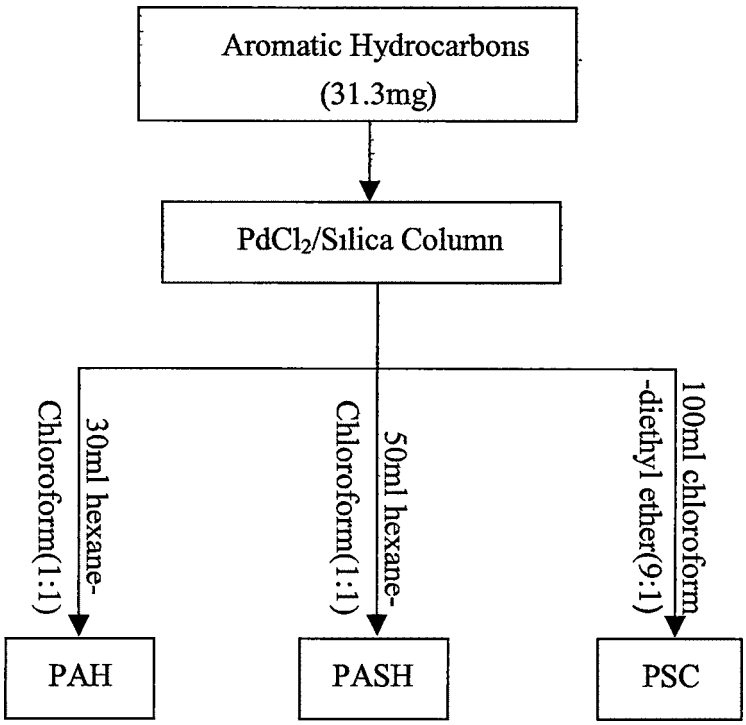


Figure 2.2 Ligand Exchange Chromatography Scheme

2.3.4 HPLC Separation

The separation of the PASH fraction on an amino bonded phase column with 100% hexane mobile phase is based on the number of aromatic rings in the compound. Compounds containing more aromatic rings are more polarizable so they elute later. Figure 2.3 is the UV spectrum for the HPLC separation of the PASH fraction. Three major peak envelopes were detected by the UV detector. By comparing the retention time of the peaks with those of standard PASH compounds, it was determined that the second

unresolved peak corresponds to 3-ring compounds, and the third peak corresponds to 4-ring compounds. Based on the absorbance values, the second peak was the strongest peak, indicating that the PASH fraction of the heavy distillate from the Arabian crude oil contains mostly 3-ring aromatic sulfur hydrocarbons.

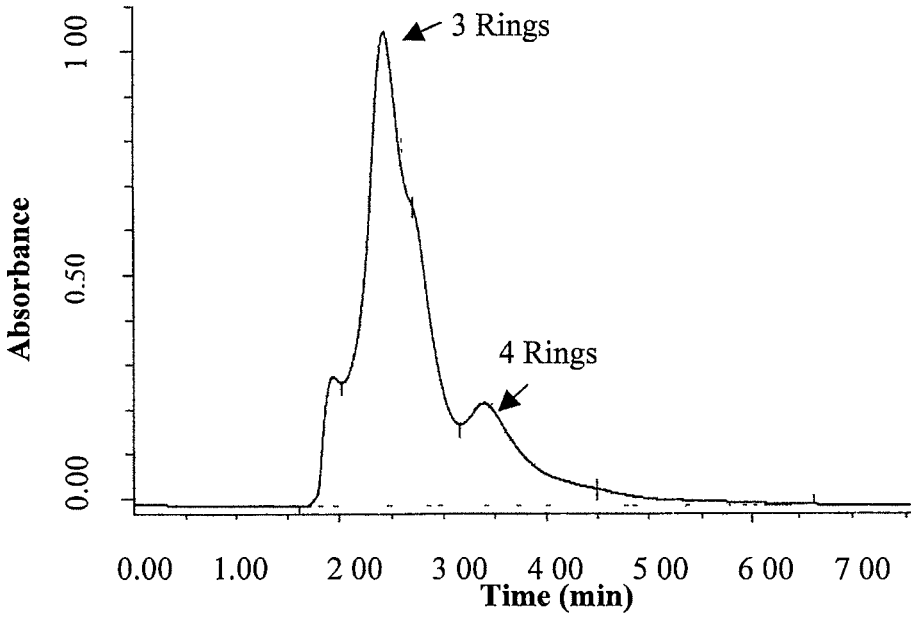


Figure 2.3 HPLC separation of Arabian Crude Oil PASH fraction LC-NH_2 column, UV detection at 340nm, hexane as mobile phase

2.3.5 Mass Spectrometry

Pd^{2+} can abstract one electron from the sulfur atom of the PASH compounds to form the Pd^{+} ion, PASH compounds lose one electron to form a molecular cation, and both PASH radical cations and Pd^{+}-solvent complexes can be detected in positive ion mode ESI/MS [12]. $PdCl_2$ was added into the PASH fraction to help the PASH compounds ionize, and thus be detected by mass spectrometry. Figure 2.4 is the MS full

scan of the PASH fraction. The spectrum indicates that the major part of this fraction is composed of compounds with molecular weights ranging from 150 to 450D. Figure 2.5 is the enlarged m/z range 140-250 of Figure 2.4. On the enlarged spectrum, peaks with m/z 184, 198, 212 and 216 correspond to the molecular weights of compounds DBT, 2-DBT, 4,6-DBT and BNTP using their MS full scan standard spectrum generated from a previous study [12]. The pattern (184+14n) corresponds to a DBT homologous series and indicates that these organosulfur compounds are probably present in Arabian crude oil. Final proof requires tandem mass spectrometry. If these compounds' tandem mass spectra match the corresponding standard organosulfur compounds' tandem mass spectra, then a positive identification is assumed.

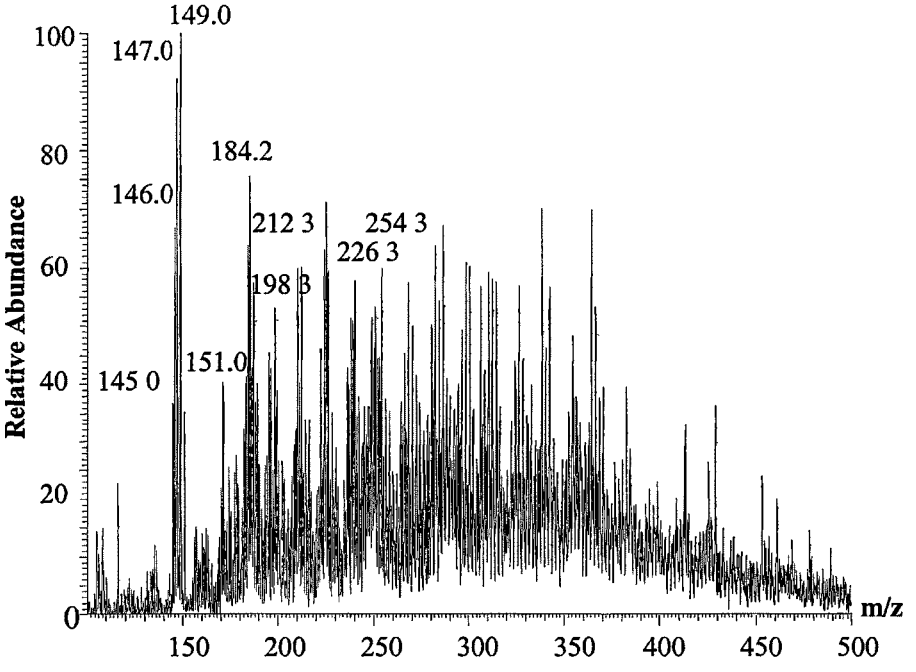


Figure 2.4 MS full scan of the PASH fraction introduced by direct infusion. Positive ion mode, $2x10^{-4}$ M sample with 1mM $PdCl_2$ in CH_3OH-CH_3CN (50:50).

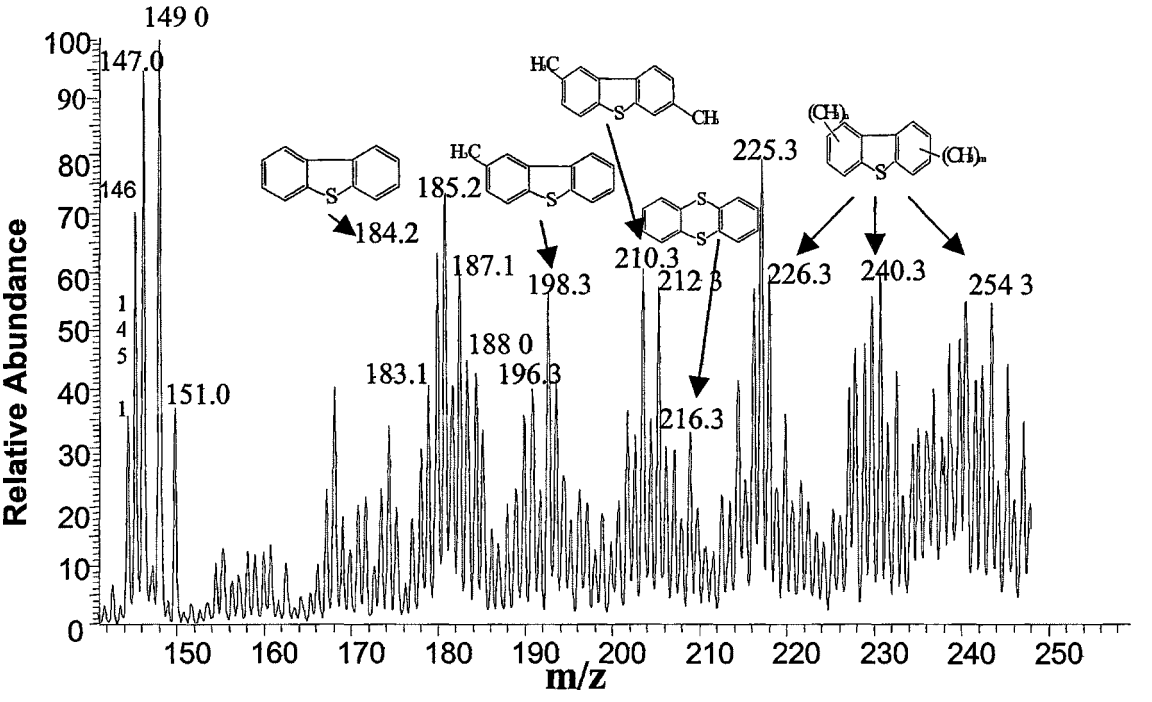


Figure 2.5 Enlarged full-scan MS of Arabian crude oil PASH fraction in CH_3OH-CH_2Cl_2 (50:50) The concentration of Pd(II) was 1mM. Ionspray voltage 3 5kV, capillary temperature 200°C, capillary voltage 60V, tube lens offset voltage 40V.

2.3.6 Tandem Mass Spectrometry

Figures 2.6-2.8 show the ESI/MS/MS spectra of m/z 184, 198 and 212 from the Arabian Crude Oil. Standard ESI/MS/MS spectra of DBT, 2-DBT and 4,6-DBT generated from a previous study [12] were used to confirm the identity of the unknown compounds. In Figure 2.6, the presence of a molecular ion peak at 184 and the loss of 32 strongly suggest that the m/z 184 peak is DBT. In figure 2.7, the presence of a molecular ion peak at 198, the M-15 peak at 183, and the M-1-32 peak at 165 provide evidence for 2-DBT in the PASH fraction. In figure 2.8, the presence of a molecular ion peak at 212, the M-1 peak at 211, the M-15 peak at 197 and the M-1-32 peak at 179 suggest the presence of 4,6-DBT in the Arabian crude oil PASH fraction. As for the other unidentified peaks in each of the three spectra, these peaks may be attributed to other

compounds with the same molecular weight as the three identified organosulfur compounds.

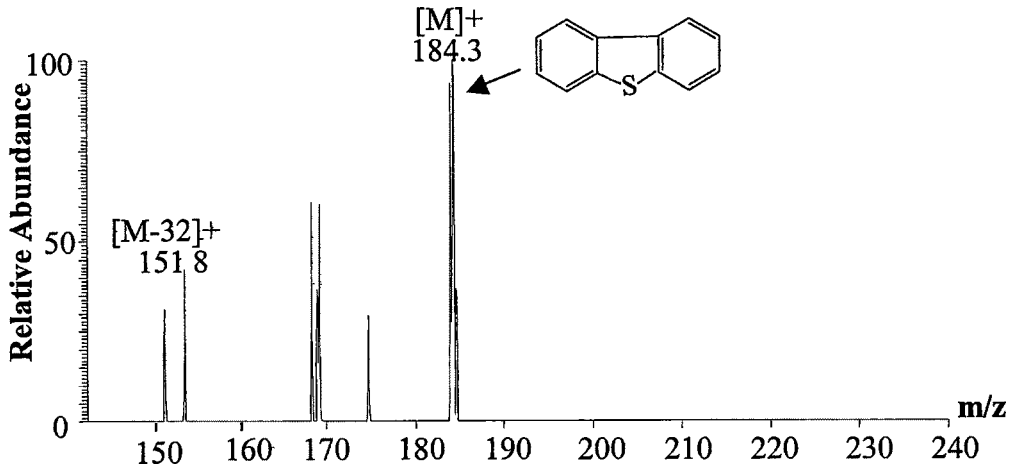


Figure 2.6 MS/MS Data for M=184, Collision Energy: 40%

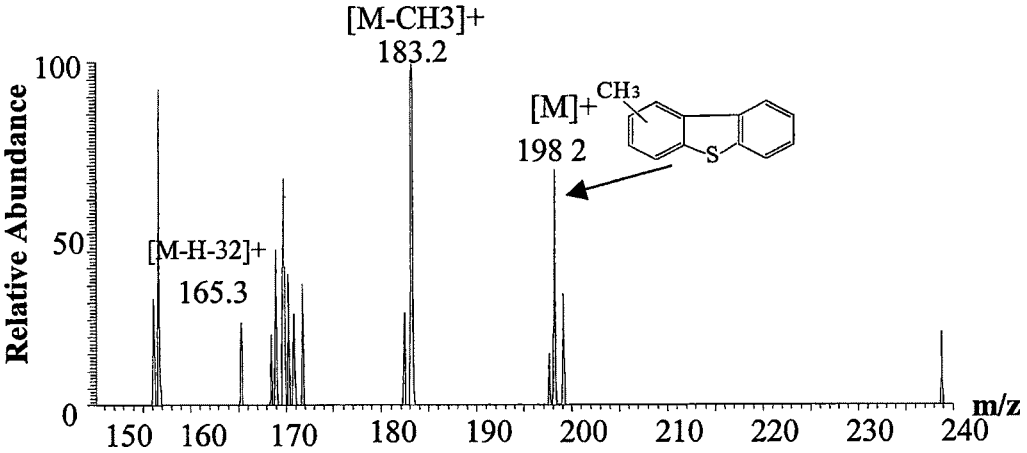


Figure 2.7 MS/MS Data for M=198, collision energy 41%.

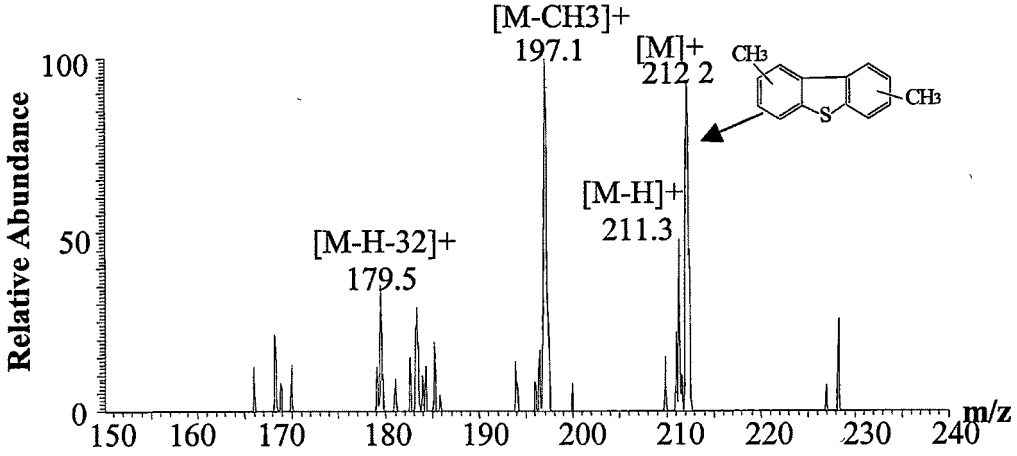


Figure 2.8 MS/MS Data for M=212, Collision Energy: 40%

2.4 Conclusions

The identification of polyaromatic sulfur hydrocarbon compounds by ESI-MS and ESI/MS/MS in the presence of Palladium(II) is very effective. The Pd(II) acts as an ionization enhancing reagent. It reacts with the sulfur atom of the organosulfur compound and abstracts an electron from the sulfur atom to the Pd(II). The organosulfur compounds form a radical cation which can then be detected by mass spectrometry. The successful application of this method to an Arabian Crude Oil proves the practical significance of a method previously developed [12].

CHAPTER THREE
DISINFECTION BY-PRODUCTS OF OZONATED INDOOR AIR ANALYSIS

3.1 Introduction

While indoor air issues have plagued humans since prehistoric times (openings in caves were made to exhaust smoke from fires), there is a growing recognition of the importance of indoor air quality on the collective health and economy of the United States. The average American spends more than 21 hours each day in indoor environments. Although there is a general expectation that homes, schools and workplaces provide a safe and healthy environment for their occupants, there is increasing evidence that, for most Americans, exposure to toxic chemicals is dominated by routine daily activities that occur indoors. The levels of most air pollutants in indoor air exceed those observed outdoors, even in the nation's most polluted cities [19].

It was recently estimated that lost productivity due to illnesses generated by poor indoor air quality results in up to a 170 billion dollar annual loss to the U.S. economy. This figure does not reflect the magnitude of individual suffering that can occur due to such illnesses. There is also a growing concern about a nearly 50% increase in the number of Americans who suffer from asthma over the past 20 years. Many experts now believe that poor indoor air quality plays a major role in this trend. Sources of indoor pollution are numerous and often poorly understood. Outdoor air entering buildings

through natural or forced ventilation, generally contains only a small percentage of the toxic chemicals observed in indoor air. Many toxic chemicals are released to indoor environments by consumer products, including deodorizers, cleaning solutions, moth cakes, dry-cleaned clothes, paints, and candles. Even activities as seemingly harmless as cooking have been observed to lead to elevated levels of pollutants [20].

There are other types of indoor air pollutants called disinfection by-products (DBPs) which are of concern to scientists. DBP studies were started 30 years ago. The first discovered DBPs were chloroform and other trihalomethanes in drinking water [21]. Because of that, initial DBP studies focused on drinking water. Scientists found that during the water disinfection process, while disinfectants such as chlorine and ozone killed microorganisms, they also reacted with natural organic matter (NOM) that exists in almost all sorts of water, and generated so called disinfection by-products including trihalomethanes, aldehydes and ketones. The primary motivation for detecting DBPs has been concern over DBPs' health risks ranging from cancer to birth defects. As more and more DBPs in drinking water are identified, people's growing desire for a better living environment demands that research be expanded to a wider area. Indoor air DBP studies are the consequence.

Recent indoor DBP analyses mainly focused on ozone generated DBPs. Ozone is one of the most popular disinfectants which is effective against resistant microorganisms. It becomes more and more widely used for treating drinking water and for environmental disinfection. When the indoor environment is contaminated by pathogenic microorganisms, disinfection is needed to kill the microorganisms and prevent people and animals from being infected. While a large amount of ozone was injected into

contaminated buildings to kill microorganisms, many compounds in indoor space readily react with ozone to form a variety of harmful or irritating by-products [22-25]. These chemicals are called volatile organic compounds (VOC), and are stored and later released from carpet, wall paper, adhesives, and other indoor materials. In the disinfection process, the reaction between ozone and indoor materials produces a variety of aldehydes [23]. In addition, ozone may also increase the indoor air concentration of formic acid [25], both of which can irritate the lungs if produced in sufficient quantities. Some of the potential by-products produced by ozone's reactions with other chemicals are themselves very reactive and capable of producing irritating and corrosive by-products [24, 26-27]. Therefore, there is an immediate need for developing analytical methods to isolate and identify these harmful DBPs.

LC-MS has been used to discover DBPs in drinking water and air samples for several years, ever since people found that GC-MS can not detect highly polar or nonvolatile DBPs. ESI and APCI are currently the most effective ionization techniques being used with LC-MS, permitting the lowest detection limits. But low molecular weight (<200Da) DBPs are difficult to distinguish from the high chemical background inherent in the low molecular weight region of the LC-MS mass spectrum. The chemical background is due to solvents (*e.g.* water, acetonitrile, methanol) used in LC-MS. The chemical background is made up of protonated molecular ions of these solvents, together with a number of dimers, trimers and adducts of these solvents with sodium and other ions. Because the peaks in this low mass region do not generally rise above the baseline of the chemical background, they are not readily distinguished. As a result, the most

successful studies to date involve the identification of unknown DBPs by LC-MS after derivatization.

The original derivatization work was performed on carboxylic acid DBP for analysis by GC-MS. Once methylated the esters produced, sharp and well defined GC-MS peaks allowing for better detection and good separation [28]. Later on, a new derivatization method was applied to identify polar carbonyl compounds by GC-MS. A popular derivatizing agent called pentafluorobenzylhydroxylamine (PFBHA) was used to react with polar carbonyl compounds such as formaldehyde, cyanoformaldeyde, propanal and glyoxal. This reaction rendered the polar compounds non-polar, allowing them to be extracted from water and analyzed by GC-MS. The PFBHA derivatives also exhibited a base peak in their mass spectra at m/z 181, which enabled all of the derivatives to be quickly distinguished from surrounding, non-derivatized chemicals in the complex mixture. In some cases, so-called double derivatization which means PFBHA derivatization followed by methylation was used to detect aldo- and keto-acids (*e.g.* glyoxylic acid, pyruvic acid) by GC-MS [29].

Ultimately the derivatization idea was transferred to LC-MS, where it was used to increase the effectiveness of LC/MS by increasing the molecular weight to a value which would put it in a region above the chemical background and by imparting a readily ionizable group to the molecule thus increasing the sensitivity for MS [28]. Some derivatization compounds have been successfully used to realize the detection of low molecular weight DBPs. These compounds include dinitrophenylhydrazine (DNPH) and 4-dimethylamine-6-(4-methoxyl-1-naphthyl)-1,3,5-triazine-2-hydrazine (DMNTH). DNPH is used to derivatize highly polar carbonyl DBPs. This derivatization allows

highly polar DBPs from an ozonated sample to be preconcentrated, it imparts significant molecular weight to the molecule to boost the molecular weight above 200Da, and gives the nearly un-ionizable aldehydes and ketones an ionizable group that permits sensitive detection by negative ion LC-MS. The DMNTH derivatization method like the DNPH method, was initially developed for the analysis of carbonyl compounds in air. It has been recently shown to be effective for detecting and identifying highly polar ozone DBP, such as pyruvic acid, glyoxylic acid and 5-ketohexanal.

DNPH derivatization followed by LC-MS has been the most common analytical method used to analyze aldehydes and ketones in air [30-32]. It has been reported that ozone DBPs include carbonyl containing compounds such as aldehydes and ketones [33-45], and their molecular weights range from as low as 30 up to hundreds of Daltons. When DNPH reacts with these carbonyl containing DBPs, the amino group of DNPH reacts with the carbonyl group of the compounds to generate carbonyl DNPH derivatives. It was also reported that, the ESI positive and negative ion mode showed insufficient detection limits due to strong fragmentation, and the same was valid for APCI positive ion mode [32,46]. APCI negative ion mode generated only $[M-H]^-$ ion peaks for the mass spectra, and therefore was considered as the best mode for the detection of carbonyl-DNPH derivatives.

The purpose of this research was to develop an LC/MS analytical method based on standard carbonyl-DNPH derivatives which are believed present in ozonated indoor air. The standard compounds' HPLC retention times and MS/MS spectra were obtained. The method was then applied to ozonated indoor air samples in order to identify as many aldehyde and ketone DBPs as possible.

3.2 Experimental

3.2.1 Reagents and Chemicals

HPLC grade acetonitrile was obtained from EM Science (Gibbstown, NJ, U.S.A). Carbonyl-DNPH Mix was purchased from SUPELCO (Bellefonte, PA, U.S.A) (Catalog Nos. 47285-U and 4M7285-U). The carbonyl-DNPH Mix contained 15 aldehyde or ketone DNPH derivatives: Formaldehyde-2,4-DNPH, Acetaldehyde-2,4-DNPH, Acrolein-2,4-DNPH, Acetone-2,4-DNPH, Propionaldehyde-2,4-DNPH, Crotonaldehyde-2,4-DNPH, Butyraldehyde-2,4-DNPH, Isovaleradehyde-2,4-DNPH, Valeraldehyde-2,4-DNPH, Hexaldehyde-2,4-DNPH, Benzaldehyde-2,4-DNPH, *m*-Tolualdehyde-2,4-DNPH, *o*-Tolualdehyde-2,4-DNPH, *p*-Tolualdehyde-2,4-DNPH, 2,5-Dimethylbenzaldehyde-2,4-DNPH, at a concentration of 15ug/mL of each compound (as carbonyl) in acetonitrile. In the experiments, the mixture was diluted by 50% acetonitrile/water to a final concentration of 0.15μg/mL, 0.015μg/mL or 0.0015μg/mL as needed.

Thirty-one DNPH derivatized air samples in 100% acetonitrile were obtained from Dr. Richard Corsi's research group at the University of Texas at Austin and used without any pre-treatment. The results from thirty-one samples will be reported in this thesis.

3.2.2 HPLC Separation

The high performance liquid chromatography system consisted of two Gilson module 306 pumps, connected to a Gilson 805 Manometric module and a 811C dynamic mixer. A SSI 100uL loop injector was attached to a polaris 3u C18-A 4.6x50mm reverse phase column (MetaChem Technologies Inc., Torrance, CA, U.S.A), connected to a Gilson module 118 UV/VIS detector set at a wavelength of 360nm. 10~25μL samples

were injected each time. The flow rate was set at 400uL/min. Separation was carried out at 25°C using the following mobile phase gradients: 0~5min, 50% acetonitrile/water, 5~25min, from 50% acetontrile/water to 100% acetontrile.

3.2.3 Mass Spectrometry by Direct Infusion of Standard Carbonyl-DNPH Derivative Mix

Direct infusion experiments were performed on a standard carbonyl-DNPH mix to obtain the optimized mass spectrometry conditions. Mass spectra were obtained on a Finnigan LCQ quadrupole ion trap mass spectrometer equipped with an atmospheric pressure chemical ionization (APCI) source in negative ion mode. MS full scan mode was used for the direct infusion experiments. Sample injection was through an electronically controlled, integrated, dual syringe pump. A Gateway 2000 computer with Xcalibur 1.2 software was used for date collection and analysis. Other parameters were set as follows: nitrogen sheath gas flow-rate, 60 arbitrary units; vaporization temperature, 450°C; capillary temperature, 150°C; capillary voltage, 20-40V; tube lens off set, 10-30V; discharge current, 5-10μA. Each MS scan was based on the average of 5 microscans and data for the mass spectra were based on 4-100 scans. The sample direct injection flow rate was set at 15μL/min.

3.2.4 LC/MS Analysis for the Standard Carbonyl-DNPH Derivative Mix

The HPLC/MS experiments were conducted by connecting the Gilson module 118 UV/VIS detector outlet to the sample inlet of the Finnigan LCQ ion trap mass spectrometer APCI probe. For the LC separation, 10-25μL sample was injected each time. The flow rate was set at 400uL/min. Separation was carried out at 25°C using the

following mobile phase gradients: 0~5min, 50% acetonitrile/water, 5~25min, from 50% acetontrile/water to 100% acetontrile.

Mass spectrometry parameters were set as follows: nitrogen sheath gas flow-rate, 65 arbitrary units; vaporizor temperature, 450 °C; capillary temperature, 150 °C; capillary voltage, 20-40V; tube lens off set, 10-30V; discharge current, 5-10μA and the auxiliary gas flow rate , 15 arbitrary units. The APCI high flow program was used for LC/MS experiments.

In order to determine the detection limit, the 0.15μg/mL, 0.015μg/mL and 0.0015μg/mL carbonyl-DNPH compound mixes were tested. Single ion monitoring (SIM) mode was applied by setting the mass spectrometer scan at the appropriate m/z for each standard compound.

3.2.5 LC/MS/MS for Standard Carbonyl-DNPH Compounds

LC/MS/MS experiments were conducted by switching the mass spectrometer between MS mode and MS/MS mode manually while the LC was running. When a peak started showing up on the UV spectrum, the compound's m/z value was detected by the mass spectrometer under MS full scan mode. The mass spectrometer was then switched to MS/MS mode very quickly using the compound's m/z value as the parent ion. LC/MS/MS was performed to 1) obtain each carbonyl-DNPH compound's standard fragmentation pattern, 2) identify those DNPH-derivatives which were co-eluted from the column and 3) distinguish compounds having the same molecular weight. The collision energy of MS/MS was set between 20% and 28% on the fly for each compound in order to generate enough fragmentation information.

3.2.6 Application to Ozonated Indoor Air Samples

The optimized LC/MS method generated by running the carbonyl-DNPH compound mix was used to identify the DBPs from ozonated indoor air samples. MS full scan mode over the m/z range 200 to 450D was used instead of SIM mode since the compounds present in each sample were unknown. Compound identifications were based on their m/z values and HPLC retention times. If the unknown compounds' m/z value and chromatographic retention time matched the data generated from running standard DNPH derivatives, the unknown compound was identified.

3.3 Results and Discussion

3.3.1 HPLC Separation

Figure 3.1 shows the HPLC separation of the 15 carbonyl-DNPH compound standard mixture. Of the 15 compounds, 12 major peaks were obtained with retention times which varied between 5.22 and 17.80 min. Peaks 3 and 10 show more than one compound co-eluting from the column. The elution order on the reverse phase column was based on the hydrophobicity of the compounds. Compounds with shorter aliphatic chains were eluted earlier than compounds with longer chains, branched chain compounds were eluted earlier than straight chain compounds. Among the 15 compounds, acetone and acrolein have very similar structure and polarity, *m*-tolualdehyde, *o*-tolualdehyde and *p*-tolualdehyde have almost all the same characteristics except for the methyl group's position. Using the standard carbonyl-DNPH derivative mix's HPLC-UV spectrum supplied by the company as a reference, based on the grouping, spacing, relative intensity and the order of elution of the peaks, peak 1

corresponds to formaldehyde, peak 2 to acetaldehyde, peak 3 to acetone and acrolein coeluting from the column, peak 4 to propionaldehyde, peak 5 to crotonaldehyde, peak 6 to butyraldehyde, peak 7 to benzaldehyde, peak 8 to isovaleraldehyde, peak 9 to valeraldehyde, peak 10 to *m*-tolualdehyde, *o*-tolualdehyde and *p*-tolualdehyde co-eluting from the column, peak 11 to hexaldehyde, and peak 12 to 2,5-dimethylbenzaldehyde. To further separate these compounds, a longer or smaller diameter particle column is need.

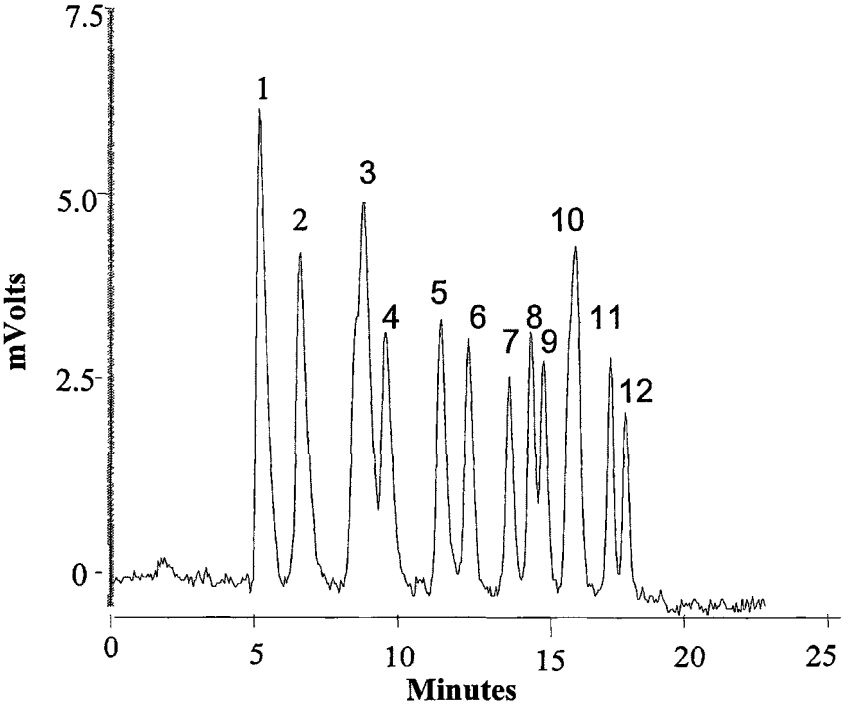


Figure 3.1 HPLC separation of 0.15μg/mL carbonyl-DNPH compound mix. C18 reverse phase column (3u, 4.6x50mm), UV detection at 310 mn.

3.3.2 Mass Spectrometry by Direct Infusion of Standard Carbonyl-DNPH Derivative Mix

Figure 3.2 shows the direct infusion mass spectrum of the carbonyl-DNPH mix. Eleven $[M-H]^-$ peaks with extremely low backgrounds were obtained. Because within the 15 compounds, there are only 11 different molecular weights, all of the possible compounds were detected. As for the lowest detection limit, the 0.0015μg/ml standard DNPH-derivative mix generated a spectrum with a S/N equal to 3:1. The APCI(-) was a very effective and sensitive ionization mode for the analysis of carbonyl-DNPH compounds. Because APCI is a soft ionization source, it doesn't generate many fragments. Moreover, the derivatized carbonyl-DNPH is easily deprotonated to generate $[M-H]^-$, which enables the use of negative ion mode APCI. By using negative ion mode, the S/N ratio could be dramatically increased compared to positive ion mode. The lowest detection limit for MS direct infusion reached 10pg for tolualdehyde-DNPH derivative and 2,5-dimethylbenzaldehyde-DNPH derivative.

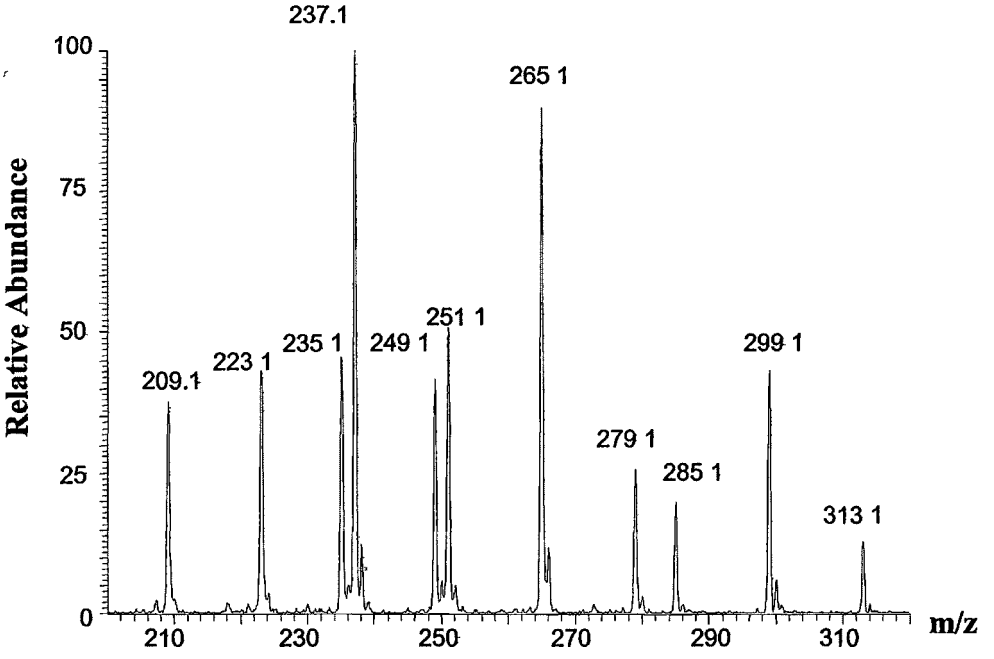


Figure 3.2 Direct infusion MS full scan of 0.15μg/mL carbonyl-DNPH mix. Each m/z value corresponds to the DNPH derivative of formaldehyde at 209.1; acetaldehyde at 223.1; acrolein at 235.1; acetone and propionaldehyde at 237.1; crotonaldehyde at 249.1, butyraldehyde at 251.1; isovaleraldehyde and valeraldehyde at 265.1; hexaldehyde at 279.1; benzaldehyde at 285.1, *m, o, p*-toluadehyde at 299.1; 2,5-dimethylbenzaldehyde at 313.1.

3.3.3 LC/MS analysis for standard Carbonyl-DNPH Derivative Mix

Figure 3.3 shows the 0.15μg/mL sample's chromatograms obtained from the UV detector and the mass spectrometer in SIM (single ion monitoring) mode. As compounds were separated by the C18 reverse phase column according to their different hydrophobicities, they were sent to the mass spectrometer one by one and detected under the SIM mode. Each compound was identified by both its HPLC retention time and m/z value of its $[M-H]^-$ peak. Figure 3.4 shows the SIM chromatograms for 0.15μg/mL, 0.015μg/mL and 0.0015μg/mL of the carbonyl-DNPH standard mixture. All the compounds were detected at the 0.15μg/mL and 0.015μg/mL concentration levels. At the

0.0015μg/mL concentration level, most of the compounds were not detected, except for the tolualdehyde-DNPH isomers (*m,o,p*-tolualdehyde) and 2,5-dimethylbenzaldehyde-DNPH. The detection limit for LC/MS reached the 150pg level for all compounds, and 15pg for the tolualdehyde-DNPH isomers (*m,o,p*-tolualdehydes) and 2,5-dimethylbenzaldehyde-DNPH.

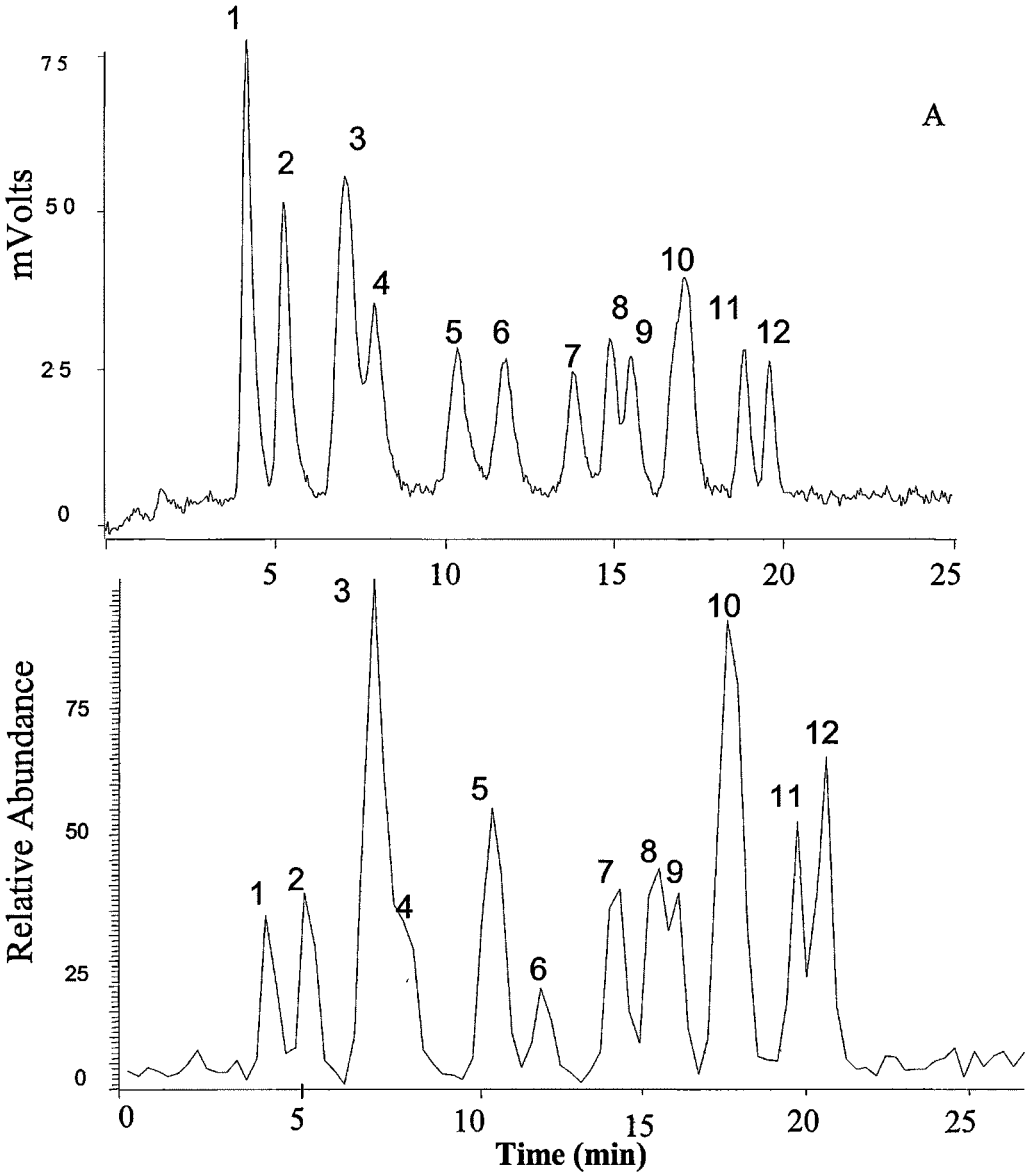


Figure 3.3A) UV chromatogram for 0.15μg/mL carbonyl-DNPH standard mixture B) TIC (total ion chromatogram) for carbonyl-DNPH standard mixture using SIM mode The numbers correspond to the DNPH derivatives of (1) formaldehyde, (2) acetaldehyde, (3) acetone, acrolein, (4) propionaldehyde (5) crotonaldehyde, (6) butyraldehyde, (7) benzaldehyde, (8) isovaleraldehyde, (9) valeraldehyde, (10) m-tolualdehyde, o-tolualdehyde, p-tolualdehyde, (11) hexaldehyde, (12) 2,5-Dimethylbenzaldehyde.

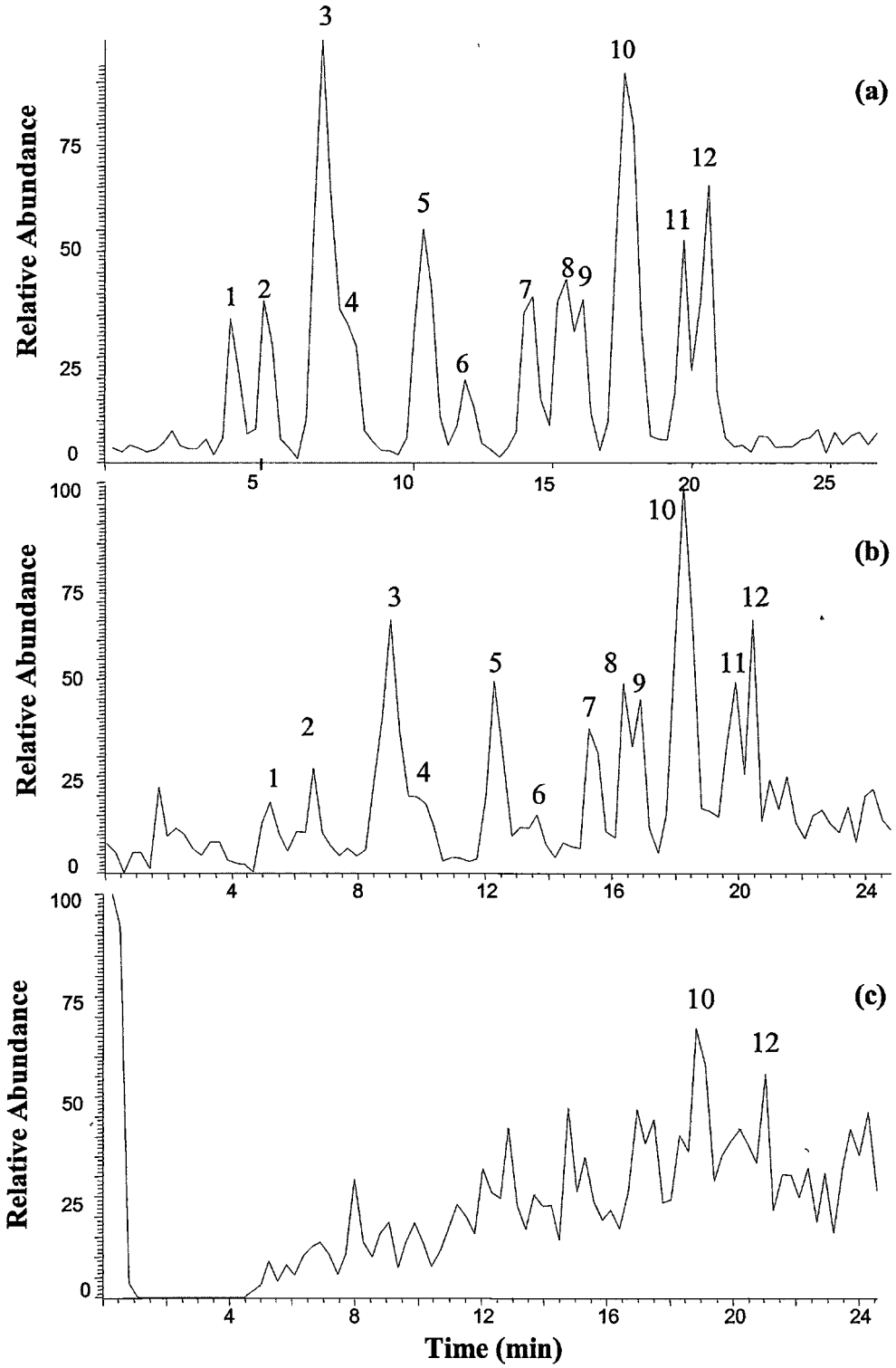


Figure 3.4 Total ion chromatogram (SIM mode) of (a) 0.15µg/mL DNPH-carbonyl standard mix. (b) 0.015µg/mL DNPH-carbonyl standard mix. (c) 0.0015µg/mL DNPH-carbonyl standard mix. DNPH derivative of 1) formaldehyde, 2) acetaldehyde, 3) acetone, acrolein, 4) propionaldehyde, 5) crotonaldehyde, 6) butyraldehyde, 7) benzaldehyde, 8) isovaleraldehyde, 9) valeraldehyde, 10) m-tolualdehyde, o-tolualdehyde, p-tolualdehyde, 11) hexaldehyde, 12) 2,5-Dimethylbenzaldehyde.

3.3.4 LC/MS/MS for Standard Carbonyl-DNPH Compounds

LC/MS/MS experiments were performed on precursor ion peaks at 209, 223, 235, 237, 249, 251, 265, 279, 285, 299, 313D. Table 3.1 lists each compound's parent ion peak and the major fragments in their MS/MS spectra. Figure 3.5 shows the MS/MS spectrum for each compound. Fragment ions at m/z 120, 122, 152 and 179 were attributed to the 2,4-dinitrophenylhydrazone moiety. These fragment intensities were relatively low. A strong m/z 163 fragment ion indicates aldehyde-DNPH derivatives. α-unsaturated aldehydes showed a neutral loss of 47 [HNO_2] from the $[M-H]^-$ ion. α-saturated aldehydes showed neutral losses of m/z 30 [NO], 45 [$-NO_2+H$], 46 [NO_2] from $[M-H]^-$ ion. Aromatic aldehydes showed a strong $[M-H-164]^-$ fragment ion peak.

Compounds	Precursor [M-H]	Fragments
formaldehyde	209	163, 120, 179, 151, 123, 153
acetaldehyde	223	179, 163, 152, 178, 120, 193
acrolein	235	167, 163, 179, 152, 158, 200, 204, 188, 120
acetone	237	179, 151, 207, 152, 191
propionaldehyde	237	179, 163, 150, 122, 120, 158, 152, 192, 219, 191
crotonaldehyde	249	152, 192, 172, 179, 232, 163, 202, 214
butyraldehyde	251	163, 179, 152, 221, 120, 189, 206, 126, 191
isovaleraldehyde	265	163, 179, 151, 126, 220, 120, 176
valeraldehyde	265	163, 152, 192, 235, 191, 203, 205
hexaldehyde	279	163, 152, 179, 234, 249, 120, 151, 261, 191
benzaldehyde	285	238, 163, 121, 179, 120, 192
m-,*o*-,*p*-tolualdehtde	299	252, 163, 179, 135, 151, 120, 206
2,5-demethyl benzaldehyde	313	266, 163, 149, 179, 285, 120

Table 3.1 MS/MS data for carbonyl-DNPH standard compounds.

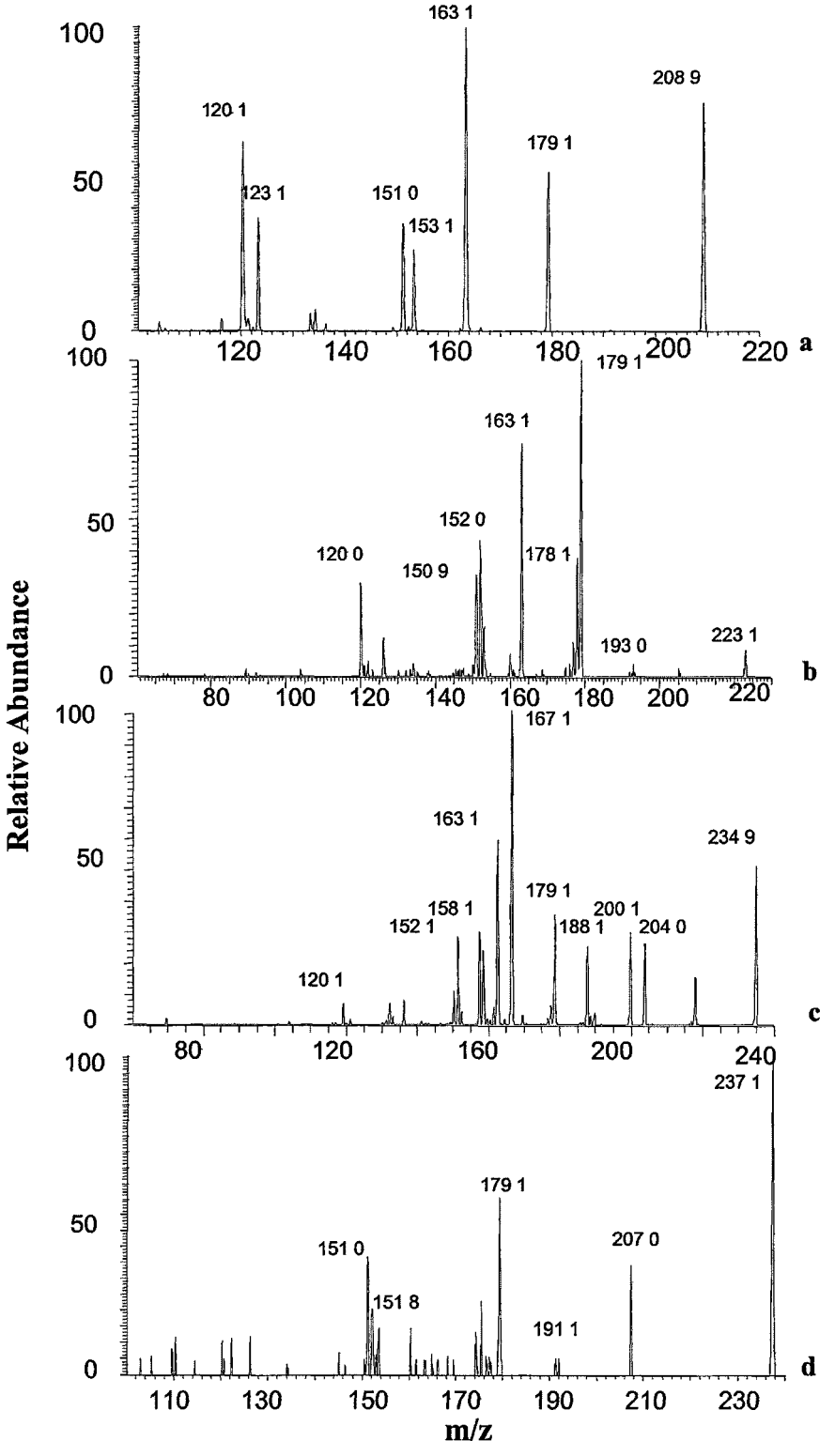
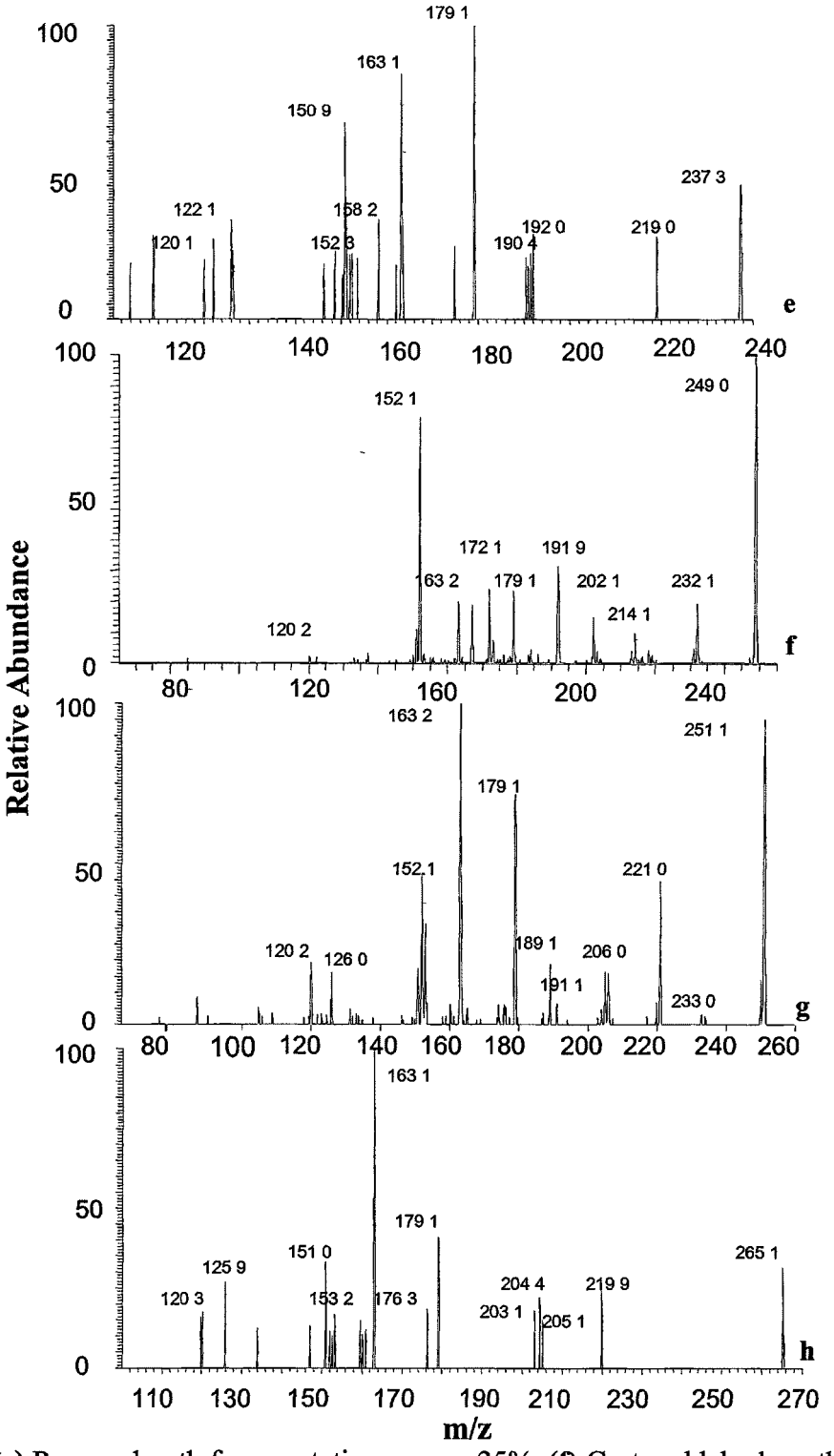
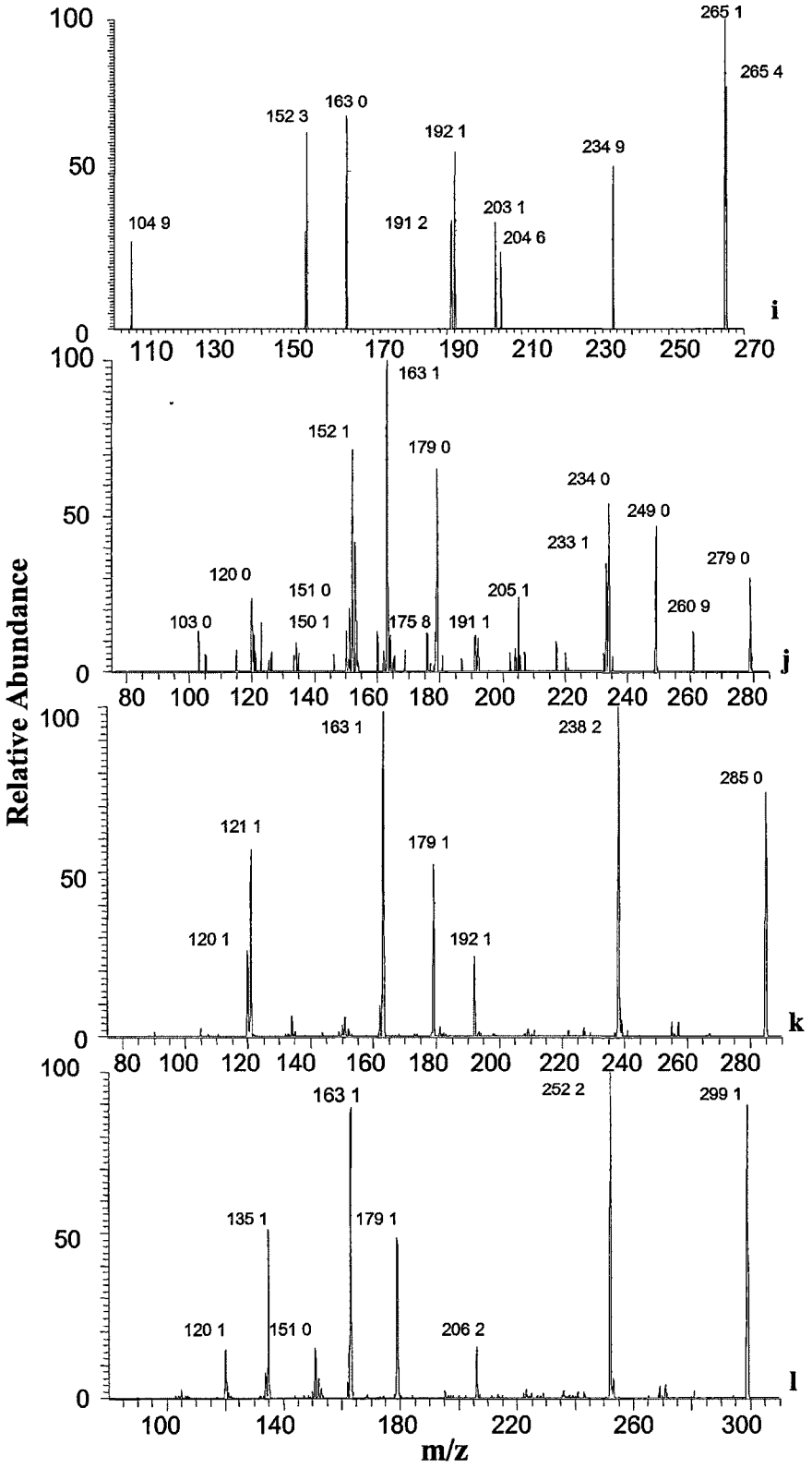


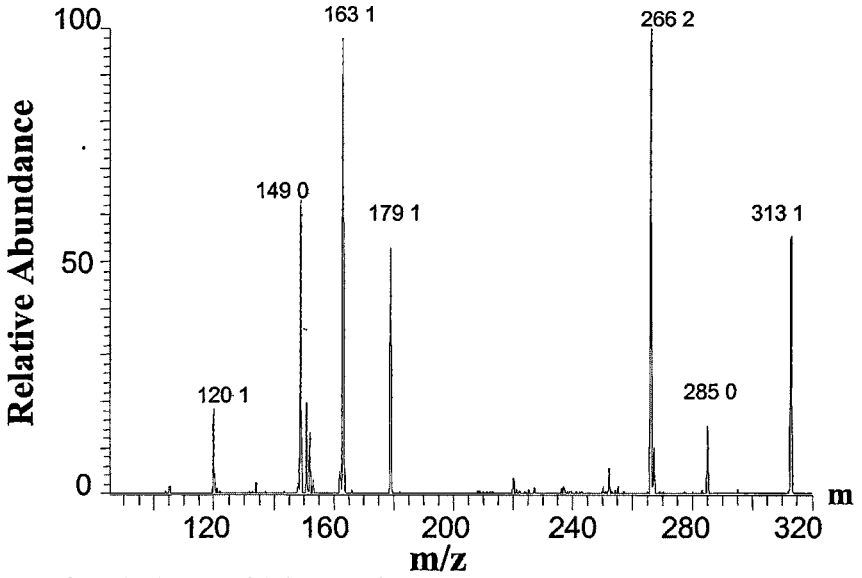
Figure 3.5, MS/MS spectrum of DNPH derivative of (a) Formaldehyde with fragmentation energy 20%, **(b)** Acetaldehyde with fragmentation energy 25%, **(c)** Acrolein with fragmentation energy 25%, **(d)** Acetone with fragmentation energy 25%.



(e) Propanal with fragmentation energy 25%, **(f)** Crotonaldehyde with fragmentation energy 25%, **(g)** Butanal with fragmentation energy 25%, **(h)** 3-Methylbutanal with fragmentation energy 25%



(i) Pentanal with fragmentation energy 25%, **(j)** Hexanal with fragmentation energy 26%, **(k)** Benzaldehyde with fragmentation energy 26%, **(l)** *m,o,p*-Tolualdehyde with fragmentation energy 26%.



(m) 2,5-dimethyl benzaldehyde with fragmentation energy 28%.

3.3.5 Application to ozonated indoor air sample

Since the standard carbonyl-DNPH derivatives' LC/MS total ion chromatogram data was obtained, by comparing the LC/MS data of the unknown air samples to the standard compounds, the DNPH derivatives contained in the air samples could be identified and the corresponding aldehydes or ketones could be determined. Thirty one different ozone treated indoor air samples were analyzed. Figures 3.6 to 3.14 show the UV spectra and the LC/MS total ion chromatograms of nine ozonated air samples. Figures 3.15 to 3.36 show the LC/MS total ion chromatograms of 22 ozonated air samples. Tables 3.2 to 3.33 list the detailed LC/MS results for these thirty-one air samples including the LC retention time, compound m/z value, ion intensity and compound identity.

3.4 Conclusions

APCI(-) is very suitable for DNPH-carbonyl derivatives identification. Their molecular ion peaks are easily observed. The MS/MS experiments can help to distinguish aldehydes and ketones, α-saturated aldehydes and α-unsaturated aldehydes, aromatic aldehydes and unsaturated aldehydes. DNPH derivatization coupled with LC/MS to identify aldehydes and ketones formed during the indoor air disinfection process is a very effective method to detect and determine the disinfection by-products in air samples. The easy sample preparation, the accuracy and the high efficiency allow people to conduct qualitative analysis of ozone treated air samples. This study was only focusing on the ozone disinfection byproducts: aldehydes and ketones. By applying this method, thirty-one different indoor air samples obtained from the outgassing of various indoor materials such as wallpaper, ceiling tile, nylon, concrete, carpet, polished linoleum, particle board, etc. were successfully identified.

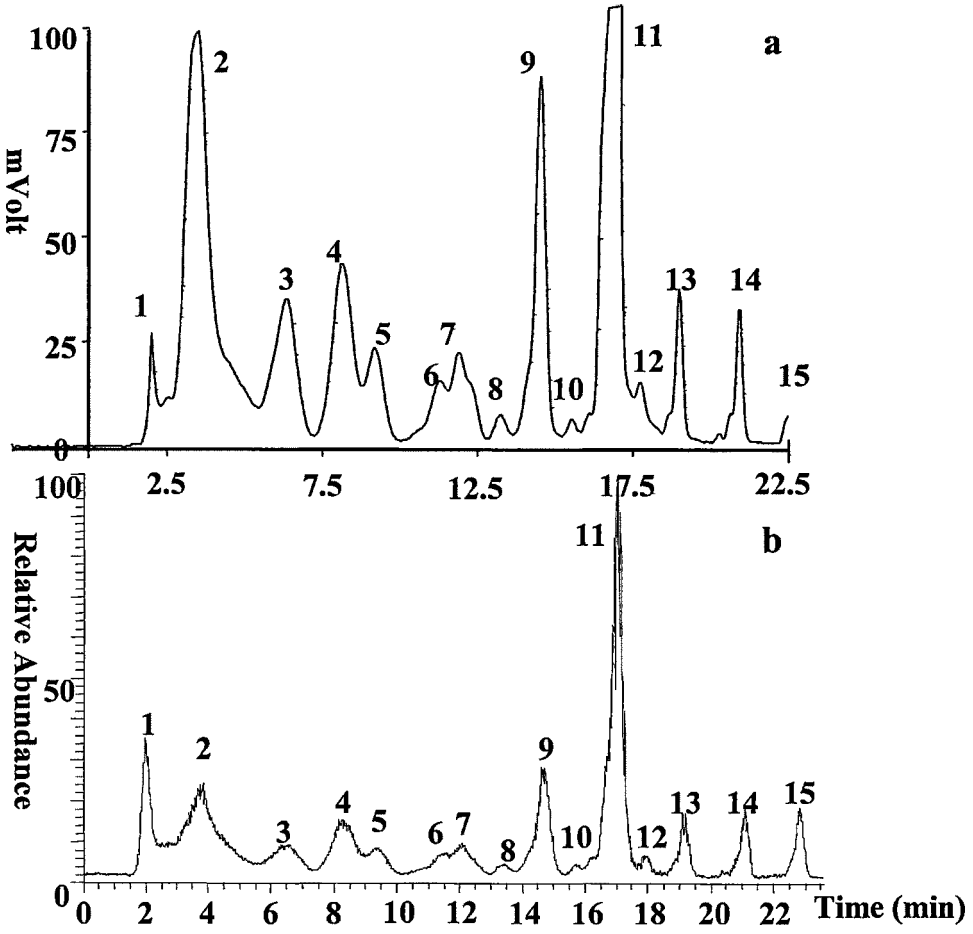


Figure 3.6 a) UV chromatogram for sample 366957
b) LC/MS total ion chromatogram for sample 366957

Peak No.	Retention Time	m/z	Ion Intensity	Compound
1	1 98mn	293, 225	2.67E6, 3.76E5	?, *Formic Acid*
2	3 87min	218	1 76E+05	?
3	6.52min	223	6 65E+05	Acetaldehyde
4	8 71min	295, 237	7 75E5, 6.19E5	?, Acetone
5	9 27min	237	7 20E+05	Propioaldehyde
6	12 07mn	251	7 80E+05	Butayraldehyde
7	13.87min	285	1.18E+05	Benzaldehyde
8	14 19min	265	8 25E+05	Isovaleraldehyde
9	14.63min	265	2 94E+06	Valeraldehyde
10	15.25min	337	1.71E+05	C9 carboxyl acid
11	17.04min	279	6 15E+06	Hexaldehyde
12	18 00mn	293	3 75E+05	C7 aldehyde
13	19.18min	293	1 49E+06	C7 aldehyde
14	21.08mn	307	1.43E+06	C8 aldehyde
15	22 85mn	321	1.52E+06	C9 aldehyde

Table 3.2 LC/MS data for sample 366957

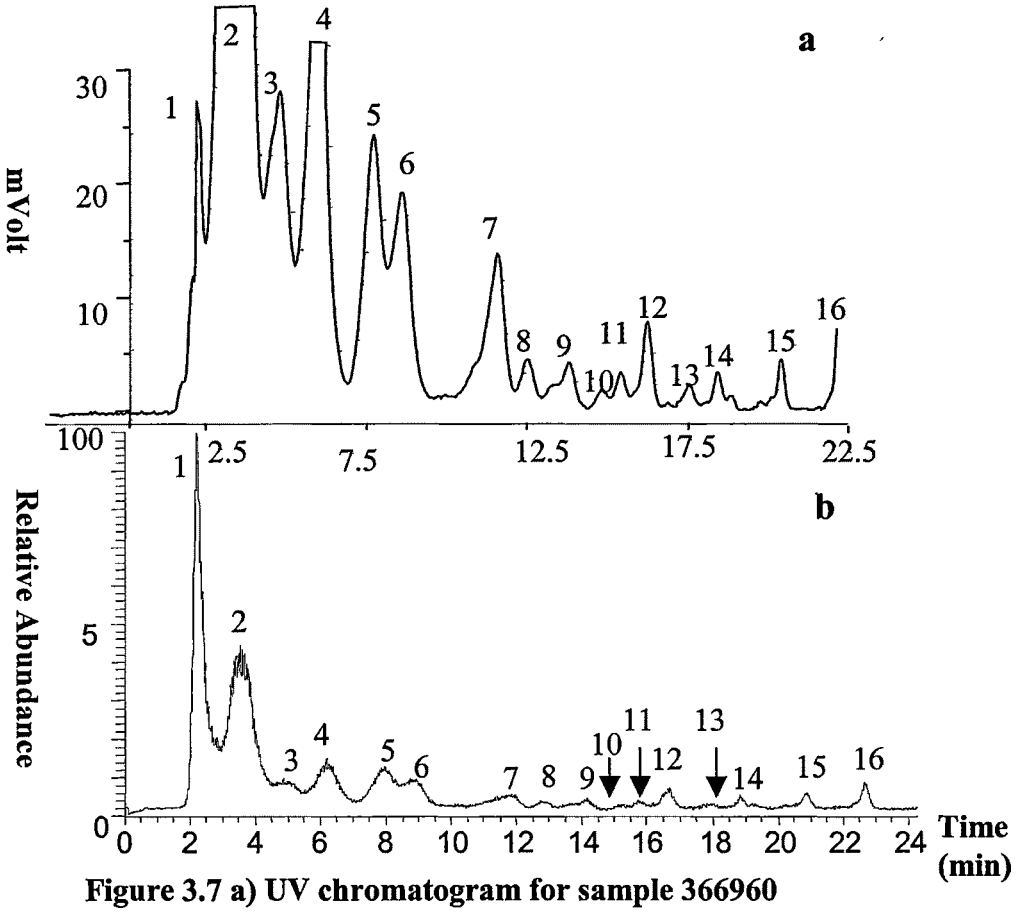


Figure 3.7 a) UV chromatogram for sample 366960
b) LC/MS total ion chromatogram for sample 366960

Peak No	Retention Time	m/z	Ion Intensity	Compound
1	2 22mn	293, 225	7.99E6, 4 22E5	?, *Formic Acid*
2	3.52min	233, 218	1.55E6, 1 38E6	?, ?
3	4.97min	234	4.23E+05	?
4	6.18min	223	9.46E+05	Acetaldehyde
5	7 97min	237	9 93E+05	Acetone
6	8.80min	237	6.59E+05	Propionaldehyde
7	11 41mn	251	1 97E+05	Butyraldehyde
8	11 80mn	285	1.39E+05	Benzaldehyde
9	14.15mn	265	1.87E+05	C5 aldehyde
10	15.30min	299	1.43E+04	Tolualdehyde
11	15.75mn	337,431	4.87E4, 1 21E5	?, ?
12	16.71mn	279	4 01E+05	Hexaldehyde
13	18.01min	445	5.64E+04	?
14	18.84mn	293	1.71E+05	C7 aldehyde
15	20 86min	307	2.40E+05	C8 aldehyde
16	22.68min	321	4 86E+05	C9 aldehyde

Table 3.3 LC/MS data for sample 366960

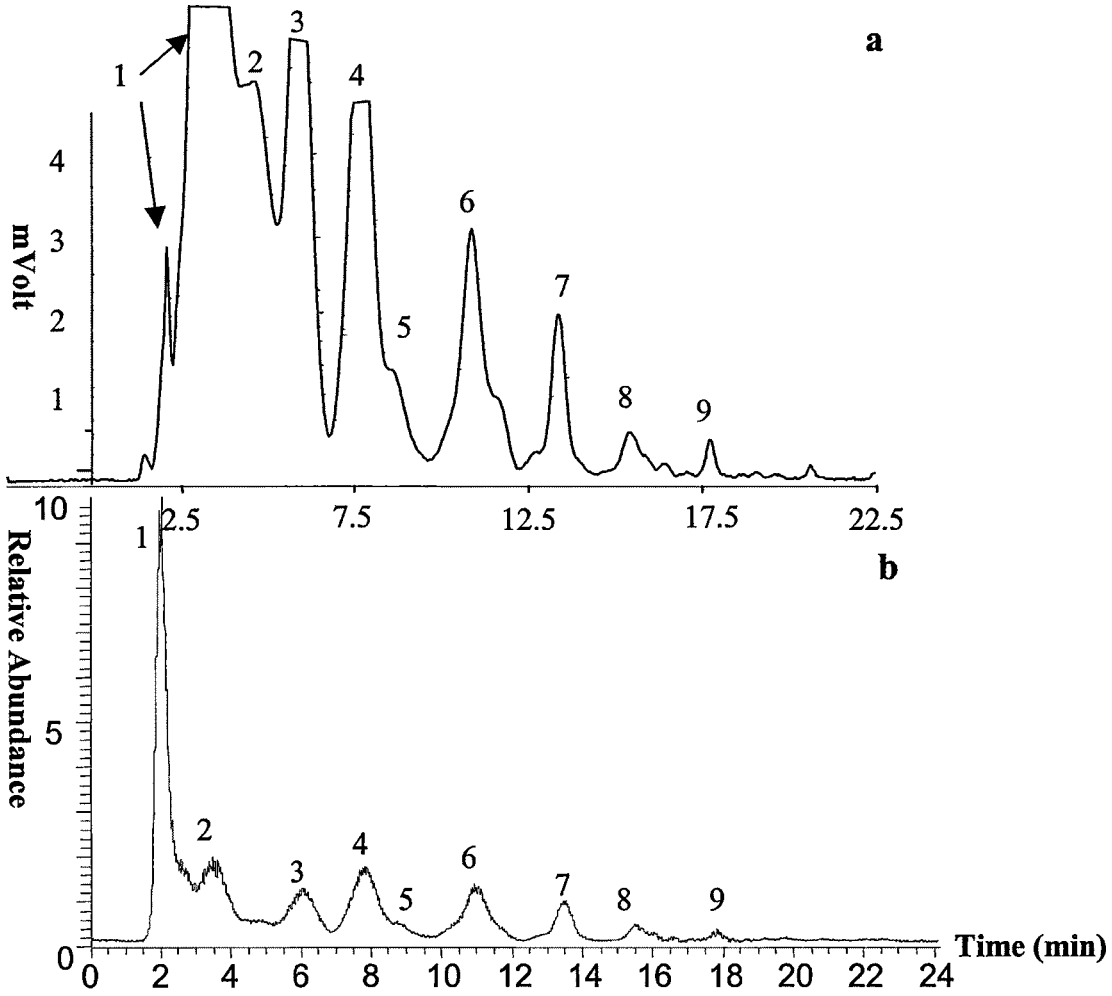


Figure 3.8 a) UV chromatogram for sample 366967
b) LC/MS total ion chromatogram for sample 366967

Peak No.	Retention Time	m/z	Ion Intensity	Compound
1	2.03min	293, 225	1.32E7, 9.51E5	?, *Formic acid*
2	3.49min	233, 218	6.80E5, 1.14E6	?, ?
3	6.10min	223	1.74E+06	acetaldehyde
4	7.83mm	237	3.90E+06	acetone
5	8.83mm	237	4.51E+05	propionaldehyde
6	10.95min	251	1.88E+06	butyraldehyde
7	13.52min	265	1.33E+06	C5 aldehyde
8	15.51min	279	5.54E+05	hexaldehyde
9	17.83min	293, 445	1.16E5, 1.78E5	C7 aldehyde, ?

Table 3.4 LC/MS data for sample 366967

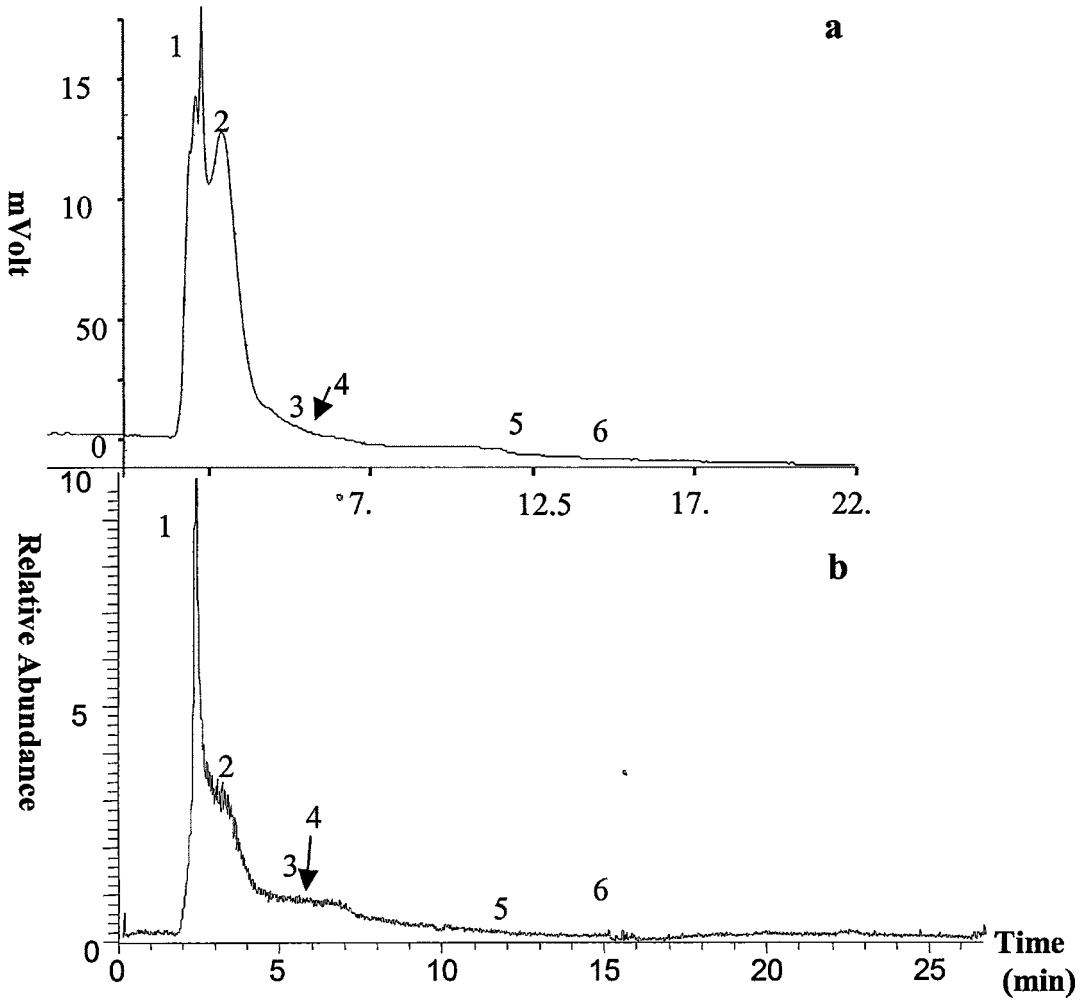


Figure3.9 a) UV chromatogram for sample 366970
b) LC/MS total ion chromatogram for sample 366970

1	2.31min	293	5.57E+06	?
2	3.08min	218	8.31E+05	?
3	6.49min	363	1.28E+05	?
4	6.62min	237	6.72E+04	acetone
5	12.00mn	265	2.35E+03	C5 aldehyde
6	15.01mn	279	1.76E+03	hexaldehyde

Table 3.5 LC/MS data for sample 366970.

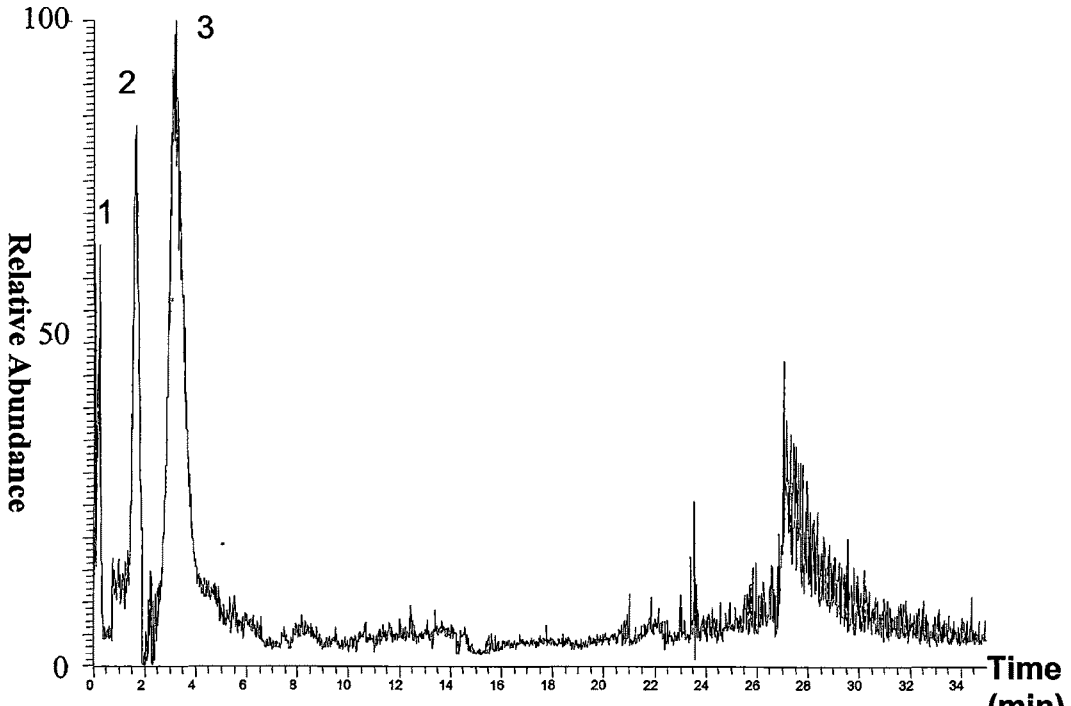


Figure 3.10 LC/MS total ion chromatogram for sample 432546

Peak No	Retention Time	m/z	Ion Intensity	Compound
1	0 08min	227	1 16E+06	?
2	1 66min	293, 211, 225	1 50E6, 7 50E5, 6 00E5	?, ?, *Formic acid*
3	3 24min	233, 218	2 40E6, 1 20E6	?, ?

Table 3.6 LC/MS data for sample 432546

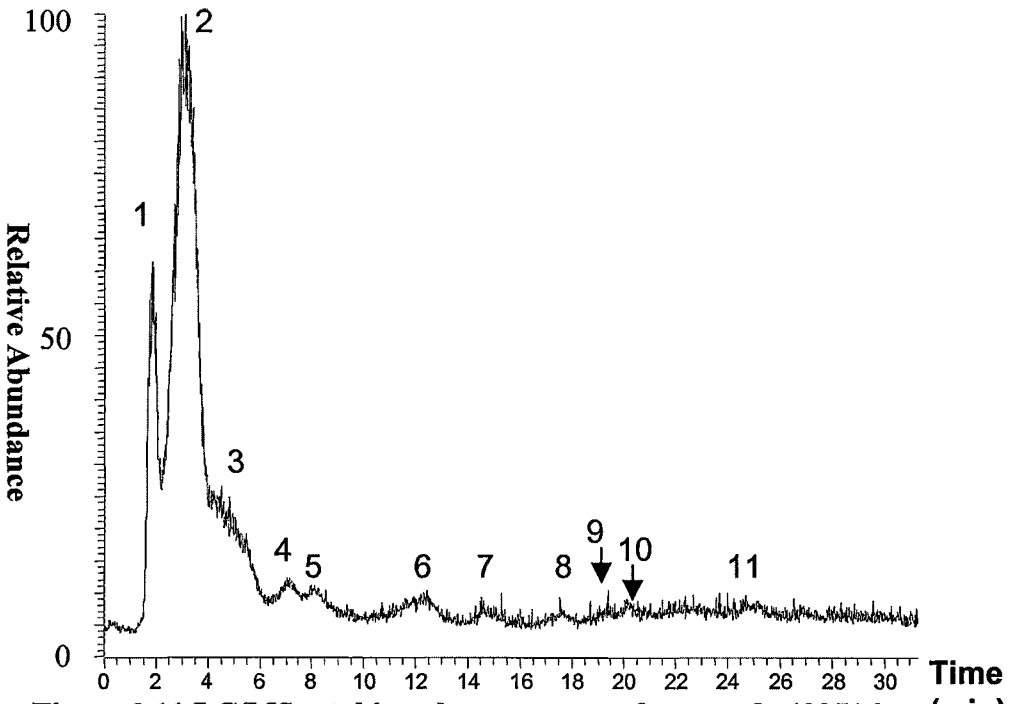


Figure 3.11 LC/MS total ion chromatogram for sample 432516

Peak No	Retention Time	m/z	Ion Intensity	Compound
1	1 87min	293, 225	1 36E6, 9.38E5	?, *Formic Acid*
2	3 14min	218, 223, 348	1.32E6, 9 24E5, 5 20E5	?, Acetaldehyde, ?
3	4 83min	294	2 71E+05	?
4	7 03min	237	1 70E+05	Acetone
5	8 12min	237	6 05E+04	Propionaldehyde
6	12 45min	218, 251	1 17E5, 6 69E4	?, Butyraldehyde
7	14 52min	265	2 39E+04	C5 aldehyde
8	17.53min	431,279,227	1 36E4, 1 09E4, 1 09E4	?, Hexaldehyde, ?
9	19 39min	293	1 53E+04	C7 aldehyde
10	20 13min	445,407,263	2 23E4, 1 54E4, 1 54E4	?,?,?
11	>24 68min	339, 369	2 03E4, 1 85E4	?, ?

Table 3.7 LC/MS data for sample 432516

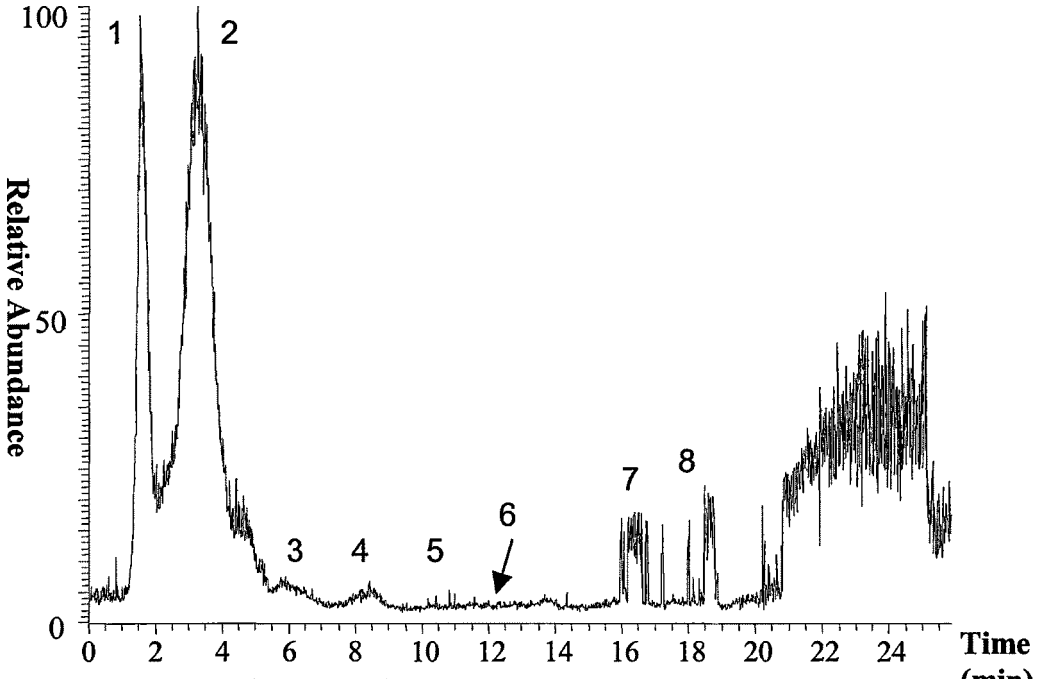


Figure 3.12 LC/MS total ion chromatogram for sample 432531

Peak No	Retention Time	m/z	Ion Intensity	Compound
1	1 57min	293	2.46E+06	?
2	3 28min	233, 218	1 56E6, 1 40E6	?, ?
3	5 89min	294, 223	8 51E4, 5.11E4	?, Acetaldehyde
4	8.41min	237	1 06E+05	Acetone/Propionaldehyde
5	10 38min	227	7 91E+03	?
6	12 42min	251	9 81E+03	Butyraldehyde
7	16 40min	417, 211, 265	1 00E4, 8 26E3, 5.90E3	?, ?, C5 aldehyde
8	18.00min	227, 279	8 65E3, 7 78E3, 5.19E3	?, Hexaldehyde,

Table 3.8 LC/MS data for sample 432531

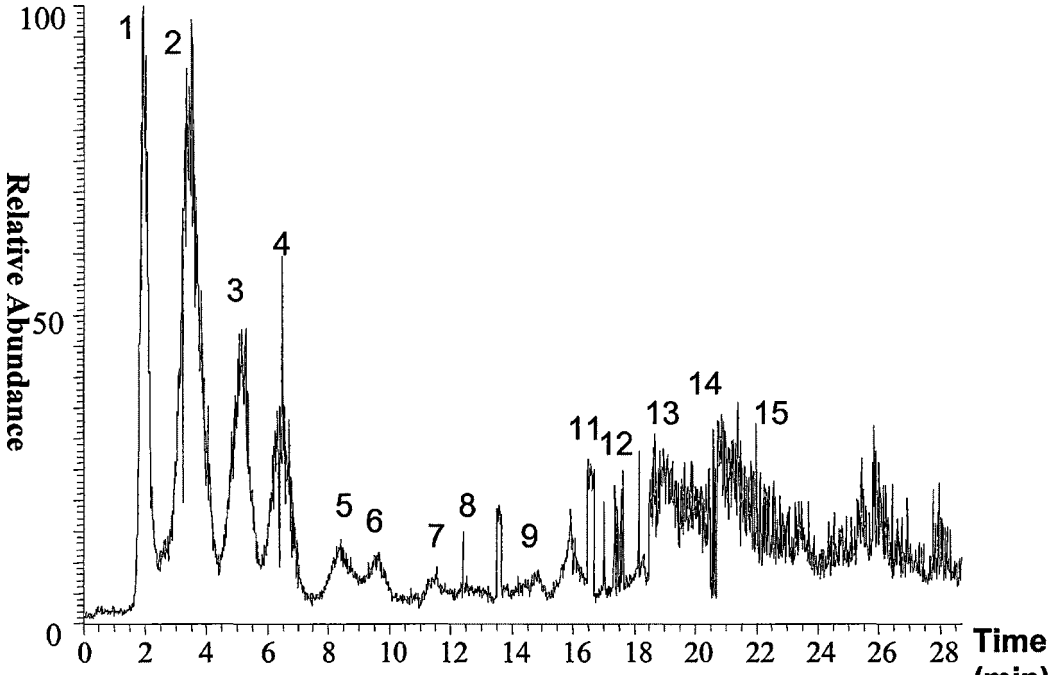


Figure 3.13 LC/MS total ion chromatogram for sample 432545

Peak No.	Retention Time	m/z	Ion Intensity	Compound
1	1 97min	293	2 86E+06	?
2	3 52min	233	1 37E6, 5 48E5	?
3	5.29min	209, 218	8 17E5, 6 94E5	Formaldehyde, ?
4	6 47min	223	7 53E+05	Acetaldehyde
5	8 40min	237	2 41E+05	Acetone
6	8 51min	237	1 49E+05	Propionaldehyde
7	11 55min	251, 433, 249	2 86E4, 1 43E4, 1 43E4	Butyraldehyde, ?, ?
8	12 41min	251	5 65E+04	Butyraldehyde
9	14 91min	285	8 04E+04	Benzaldehyde
10	15 93min	265	7.69E+04	Isovaleraldehyde
11	16 49min	265	2 46E+04	Valeraldehyde
12	17 67min	299	1 05E+04	Tolualdehyde
13	18 96min	279	5 96E+04	Hexaldehyde
14	20 86min	293	2 19E+04	C7 aldehyde
15	22 00min	307, 339, 369	3 15E+04	C8 aldehyde, ?, ?

Table 3.9 LC/MS data for sample 432545

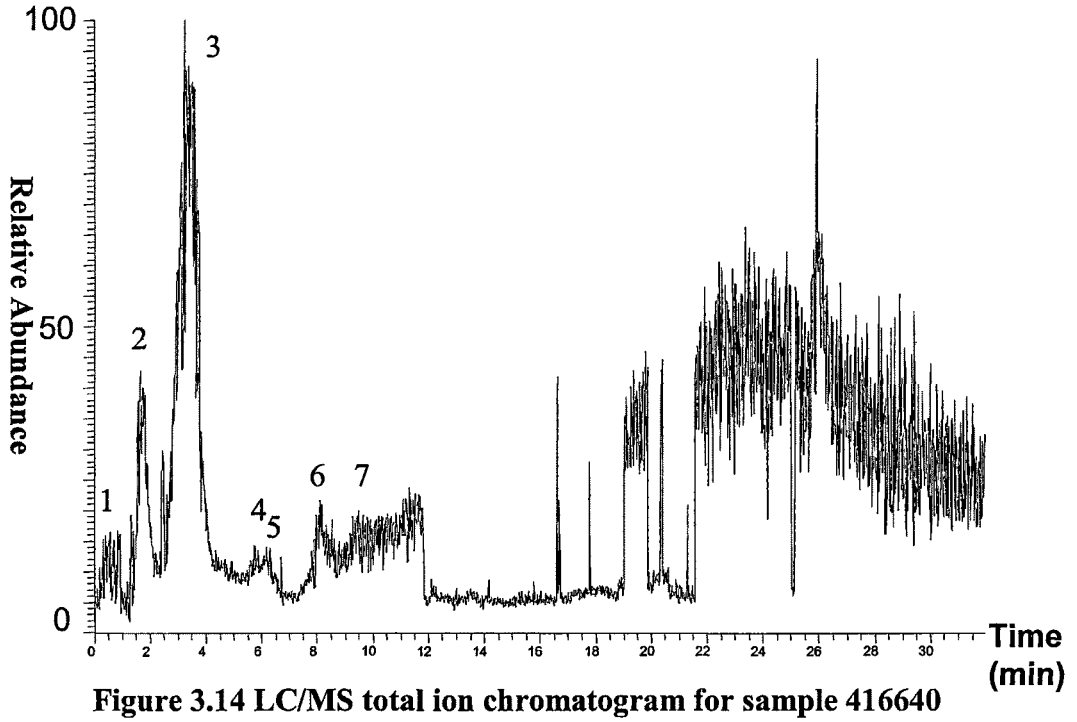


Figure 3.14 LC/MS total ion chromatogram for sample 416640

Peak No	Retention Time	m/z	Ion Intensity	Compound
1	0 86min	227	1 36E6, 9 38E5	?
2	1 65min	293, 225, 211	1 32E6, 9 24E5, 5 20E5	?, *formic Acid*, ?
3	3 27min	233, 218	2.71E+05	?, ?
4	5.75min	294	1 70E+05	?
5	6 28min	223	6 05E+04	Acetaldehyde
6	8 11min	237	1 17E5, 6 69E4	Propionaldehyde
7	9 28min	227	2 39E+04	?

Table 3.10 LC/MS data for sample 416640

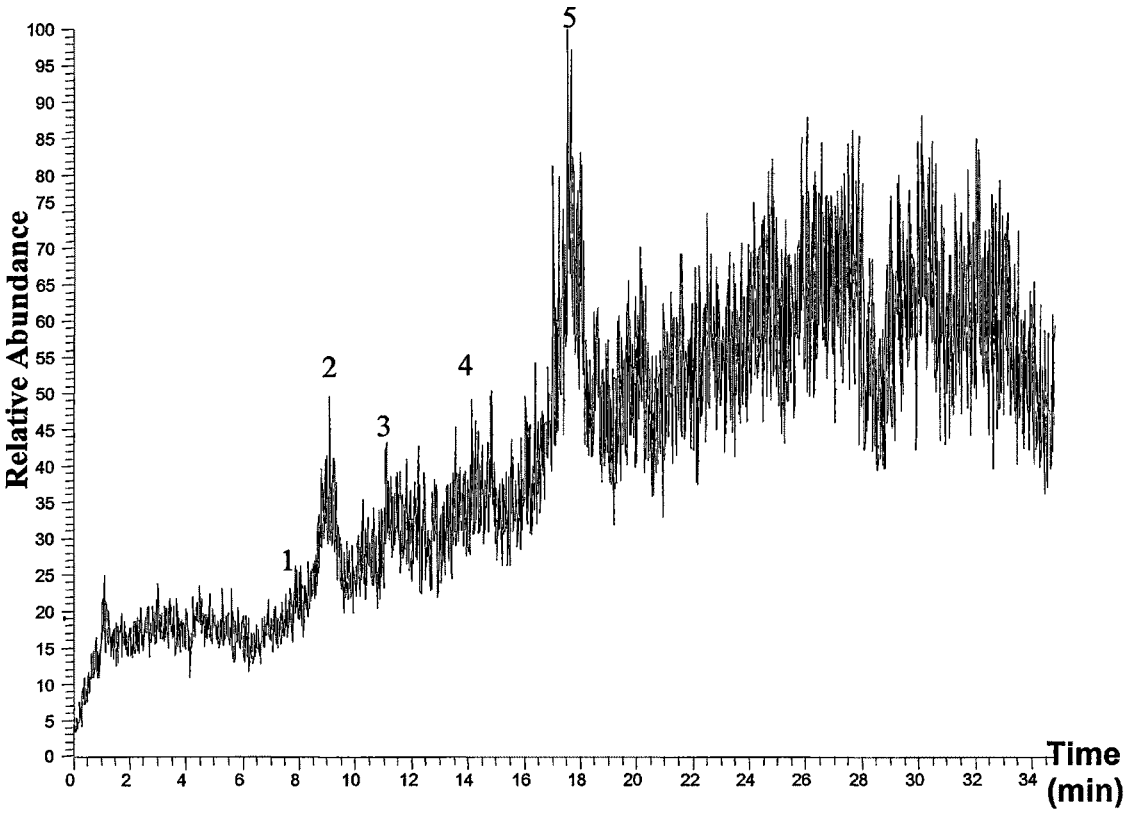


Figure 3.15 LC/MS total ion chromatogram for sample 466690.

Peak No	Retention Time	m/z	Ion Intensity	Compound
1	8 06min	223	1 27E+04	Acetaldehyde
2	9 11min	237	5 91E+04	Acetone/Propionaldehyde
3	11 51min	251	6 28E+03	Butyraldehyde
4	14 14min	265	4 46E+04	C5 aldehyde
5	17 55min	279	1 47E+05	Hexaldehyde

Table 3.11 LC/MS data for sample 466690.

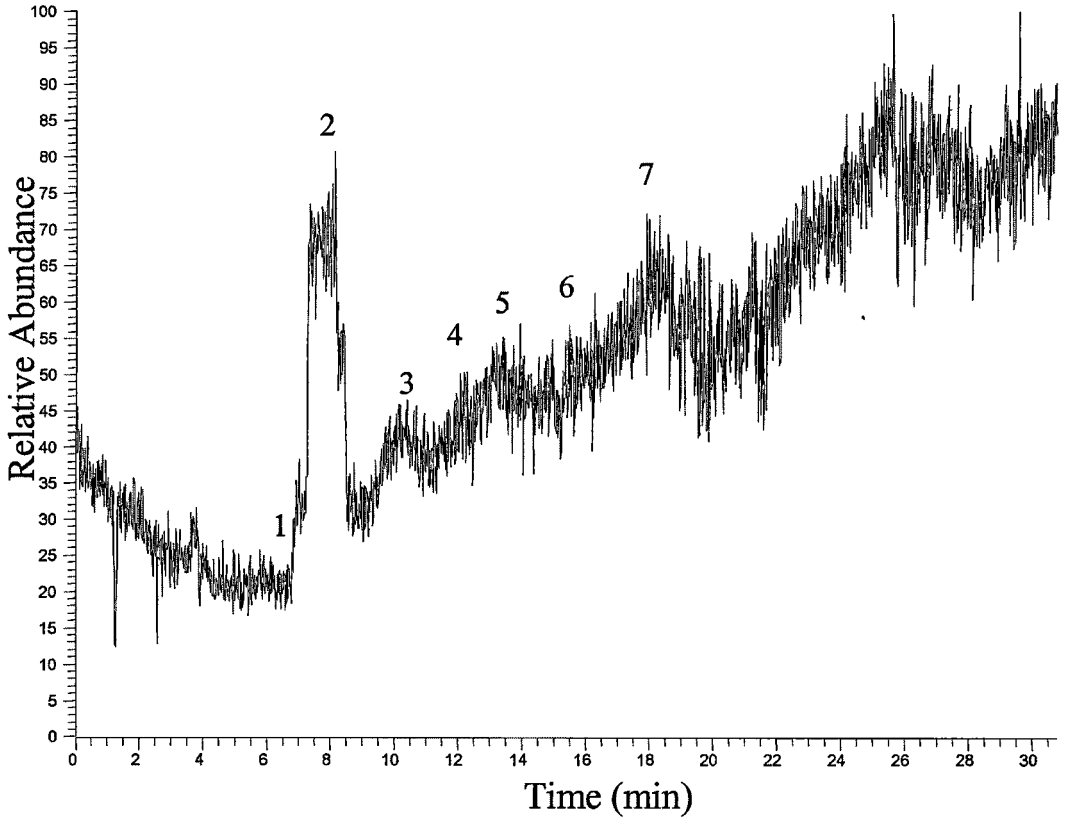


Figure 3.16 LC/MS total ion chromatogram for sample 421429.

Peak No	Retention Time	m/z	Ion Intensity	Compound
1	6.77min	223	2.04E+03	Acetaldehyde
2	8.15min	217, 241	4.99E4, 2.45E4	?, ?
3	9.86min	237	4.64E+04	Acetone/Propionaldehyde
4	12.09min	249	3.30E+03	Crotonaldehyde
5	17.55min	251	5.97E+03	Butyraldehyde
6	15.89min	265	2.27E+03	C5 aldehyde
7	17.91min	217, 249, 279	6.28E4, 2.51E4, 3.14E3	?, ?, Hexaldehyde

Table 3.12 LC/MS data for sample 421429.

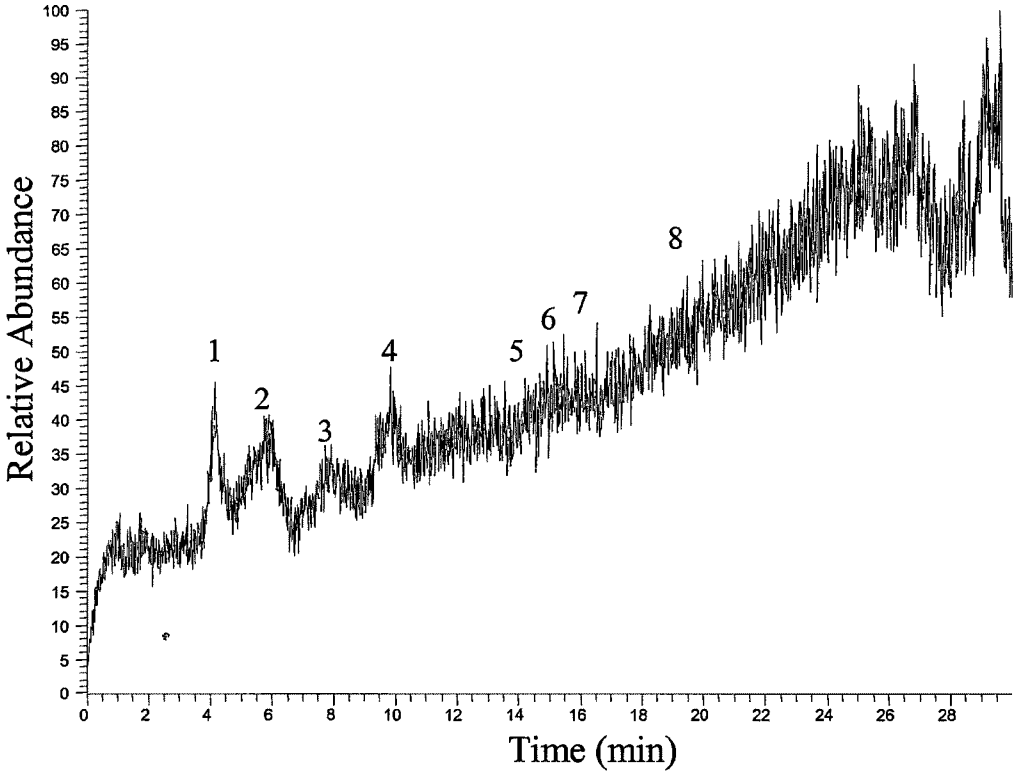


Figure 3.17 LC/MS total ion chromatogram for sample 421405.

Peak No	Retention Time	m/z	Ion Intensity	Compound
1	4.15mm	428, 429	2.46E+04	?, ?
2	5.91mm	348	4.16E+04	?
3	7.93mm	223	7.90E+03	Acetaldehyde
4	9.83mm	237	4.46E+04	Acetone/Propionaldehyde
5	13.18mm	251	3.03E+04	Butyraldehyde
6	14.90mm	285	3.35E+03	Benzaldehyde
7	15.65mm	265	3.96E+03	C5 aldehyde
8	19.80mm	279	3.01E+03	Hexaldehyde

Table 3.13 LC/MS data for sample 421405.

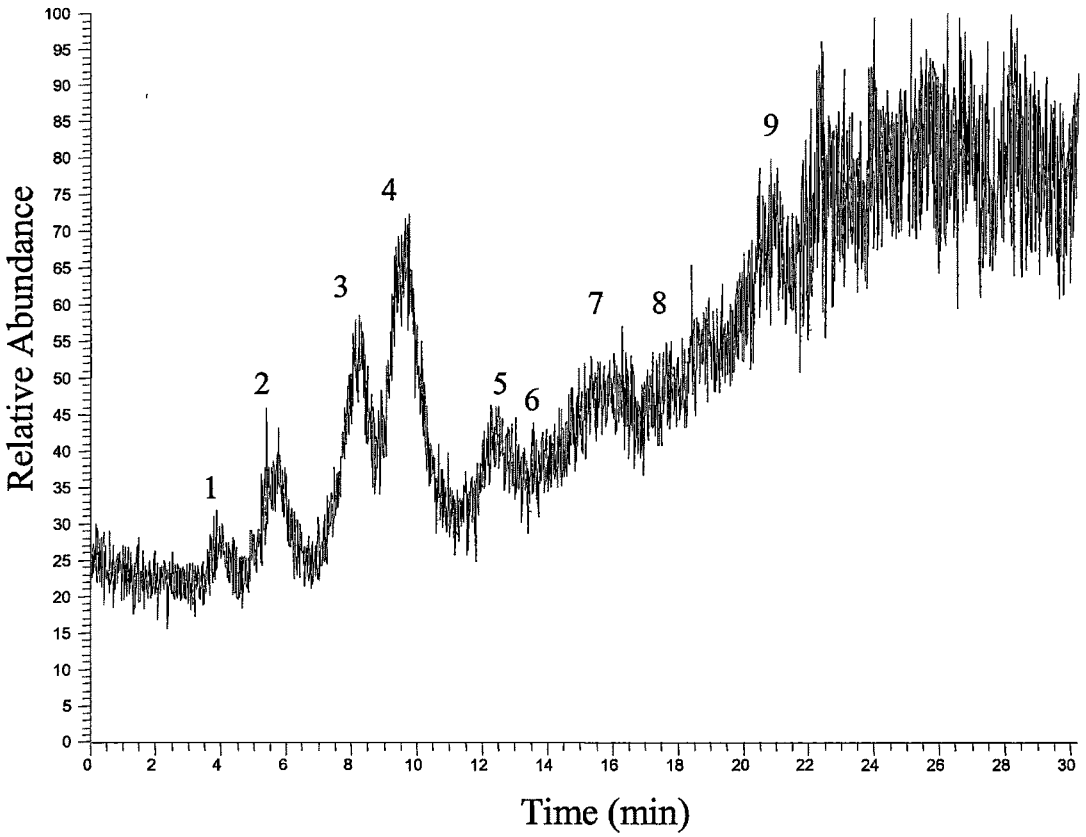


Figure 3.18 LC/MS total ion chromatogram for sample 421722.

Peak No	Retention Time	m/z	Ion Intensity	Compound
1	3 85mm	241, 217,293, 377	6 83E+03	?, ?, ?, ?
2	5 73mm	348	5 45E+04	?
3	8.24mm	223	1 45E+05	Acetaldehyde
4	9 74mm	237	1.85E+05	Acetone/Propioaldehyde
5	12 26mm	251	3 95E+04	Butyraldehyde
6	13 53mm	285	2 75E+04	Benzaldehyde
7	16 02mm	265	4 03E+04	C5 aldehyde
8	17 83mm	241, 217	4 70E4, 1 88E4	?, ?
9	20 46mm	279	5 62E+04	Hexaldehyde

Table 3.14 LC/MS data for sample 421722.

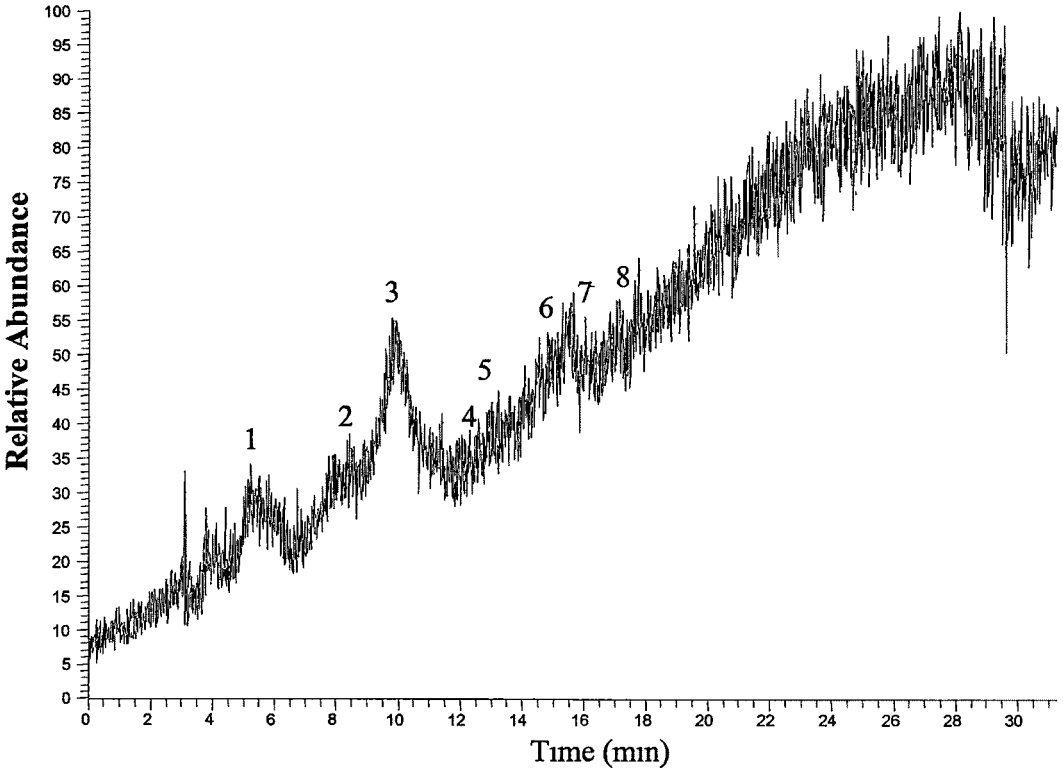


Figure 3.19 LC/MS total ion chromatogram for sample 466320.

Peak No	Retention Time	m/z	Ion Intensity	Compound
1	5 25min	348		?
2	8 43min	223	2 78E+03	Acetaldehyde
3	9 82min	237	6 59E+04	Acetone/Propionaldehyde
4	12 33min	249	1 71E+03	Crotonaldehyde
5	12 97min	251	3 84E+03	Butyraldehyde
6	14 68min	285	1 53E+03	Benzaldehyde
7	16 13min	265	3 26E+03	C5 aldehyde
8	17.43min	279	2 03E+03	Hexaldehyde

Table 3.15 LC/MS data for sample 466320.

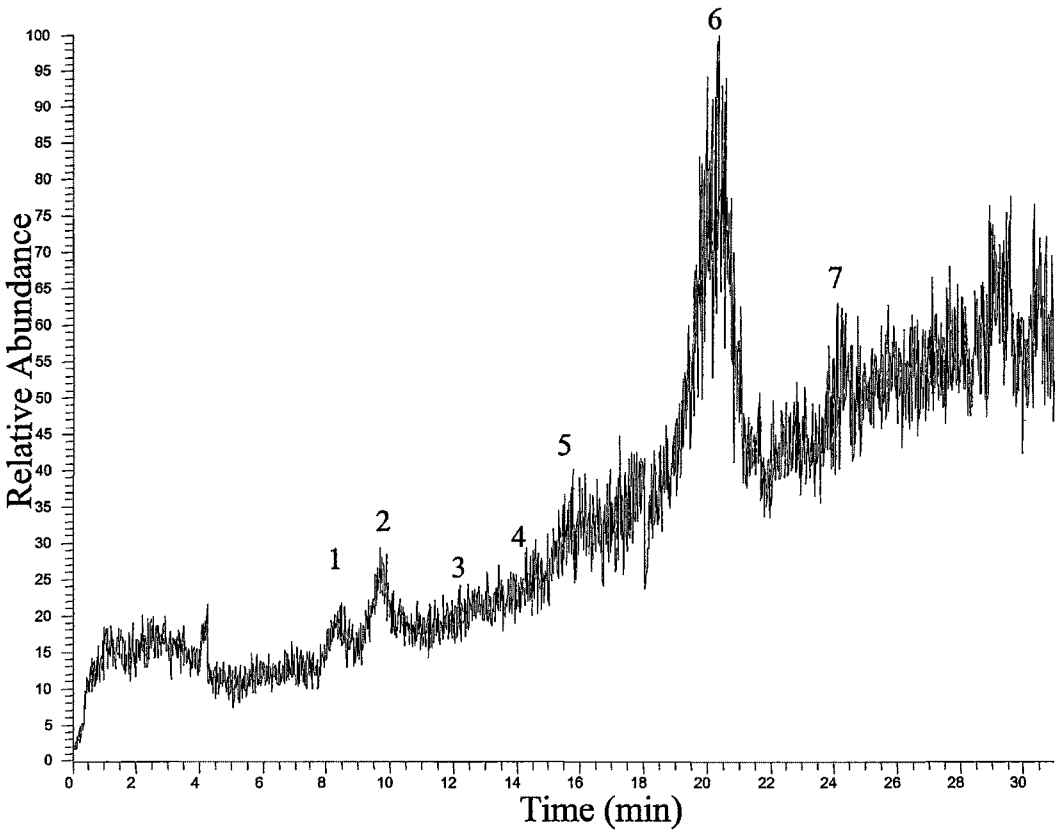


Figure 3.20 LC/MS total ion chromatogram for sample 466687.

Peak No	Retention Time	m/z	Ion Intensity	Compound
1	8 37min	223	2 60E+04	Acetaldehyde
2	9 75min	237	5 23E+04	Acetone/Propionaldehyde
3	12 64min	251	3 48E+03	Butyraldehyde
4	14 29min	285	1 25E+03	Benzaldehyde
5	15 97min	265	4 09E+04	C5 aldehyde
6	20 39min	279	2 48E+05	Hexaldehyde
7	24 11min	241	8 25E+04	?

Table 3.16 LC/MS data for sample 466687.

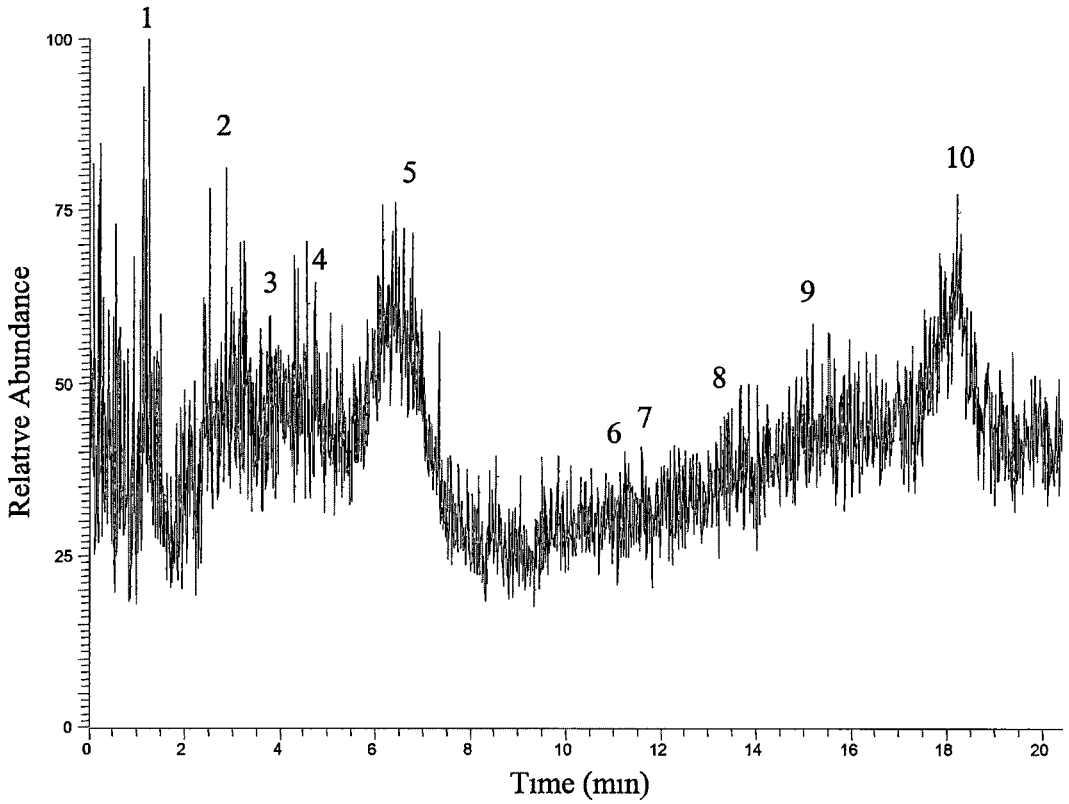


Figure 3.21 LC/MS total ion chromatogram for sample 466252.

Peak No.	Retention Time	m/z	Ion Intensity	Compound
1	1.27min	233	2.37E+04	?
2	2.87min	233,218	2.14E5,1.71E5	?,?
3	3.93min	209	1.50E+05	Formaldehyde
4	4.81min	223	2.30E+05	Acetaldehyde
5	6.61min	237	4.94E+05	Acetone/Propionaldehyde
6	11.40min	251	2.61E+04	Butyraldehyde
7	12.17min	265	2.86E+04	?
8	13.56min	285	5.54E+04	Benzaldehyde
9	15.09min	265	6.94E+04	C5 aldehyde
10	18.20min	279	3.68E+05	Hexaldehyde

Table 3.17 LC/MS data for sample 466252.

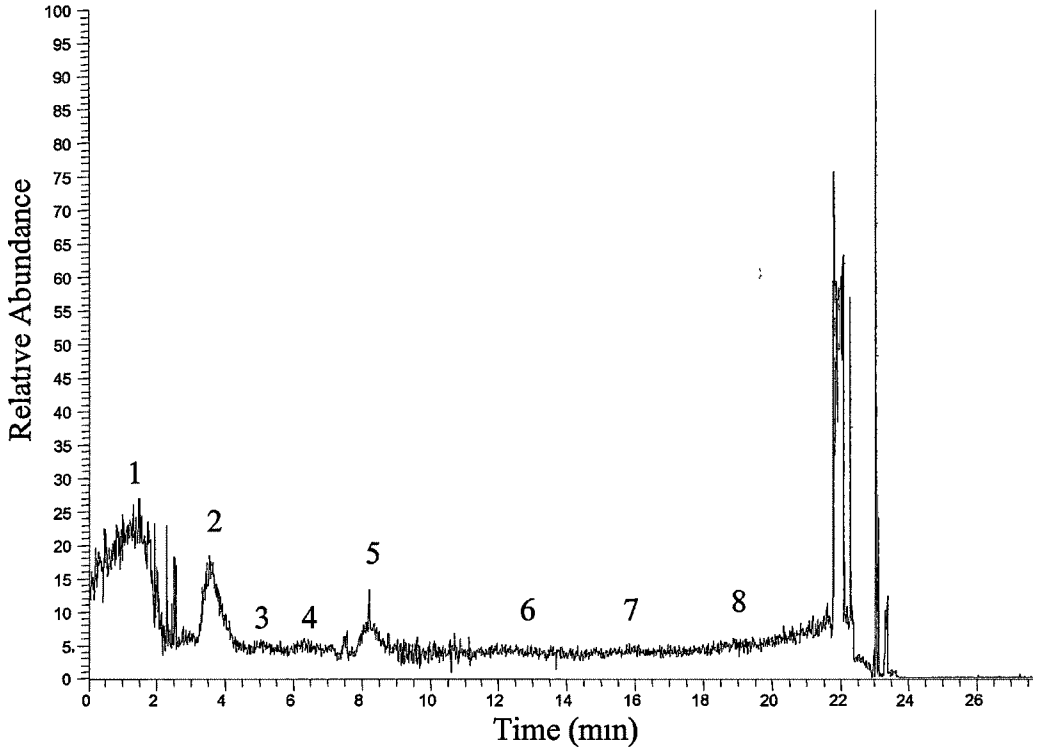


Figure 3.22 LC/MS total ion chromatogram for sample 421743.

Peak No	Retention Time	m/z	Ion Intensity	Compound
1	1 46mm	233	1 79E+04	?
2	3 58mm	233,218	1 27E+05	?,?
3	5 05mm	209	9 76E+03	Formaldehyde
4	6 28mm	223	2 22E+04	Acetaldehyde
5	8 21mm	237	7 13E+04	Acetone/Propionaldehyde
6	12 76mm	251	4 80E+03	Butyraldehyde
7	16 10mm	265	3 04E+03	C5 aldehyde
8	18.99mm	279	2 19E+03	Hexaldehyde

Table 3.18 LC/MS data for sample 421743.

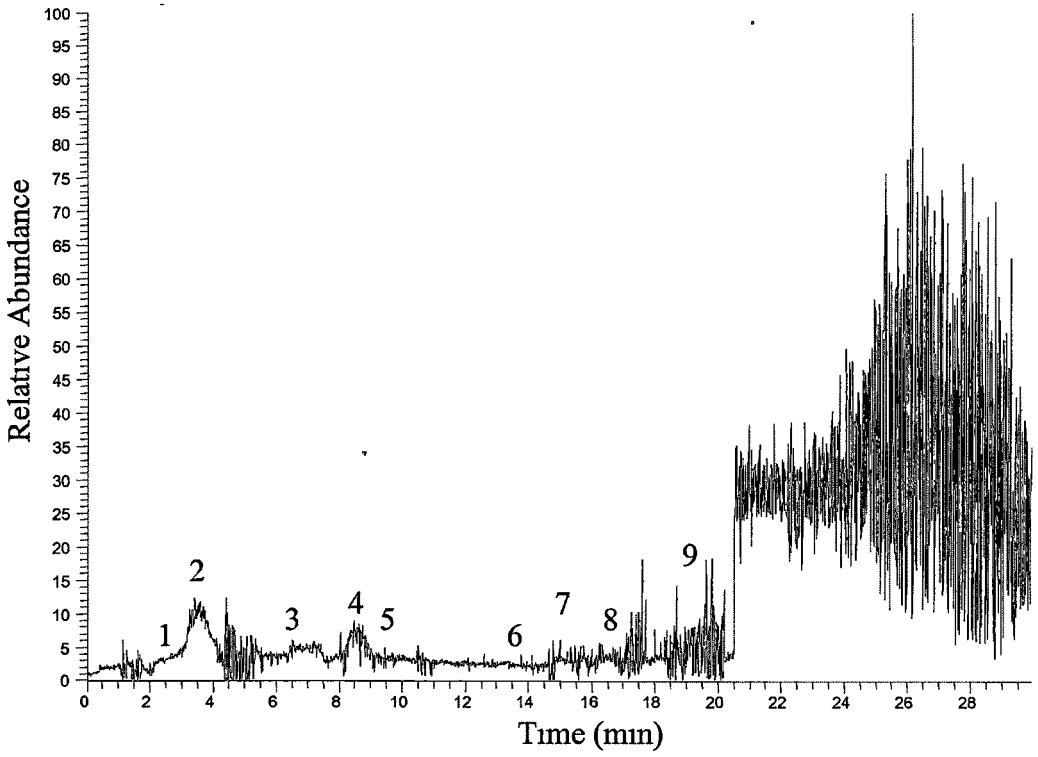


Figure 3.23 LC/MS total ion chromatogram for sample 466322.

Peak No	Retention Time	m/z	Ion Intensity	Compound
1	2 49mm	225	2 42E+04	?
2	3 39mm	233	1 22E+05	?
3	6 41mm	223	3 02E+04	Formaldehyde
4	8 57mm	237	1 70E+05	Acetaldehyde
5	9 63mm	237	1 71E+04	Propioaldehyde
6	13 20mm	251	1 80E+04	Butyraldehyde
7	15 05mm	285	3 85E+04	Benzaldehyde
8	16 47mm	265	4 16E+04	C5 aldehyde
9	19 20mm	279	4 97E+04	Hexaldehyde

Table 3.19 LC/MS data for sample 466322.

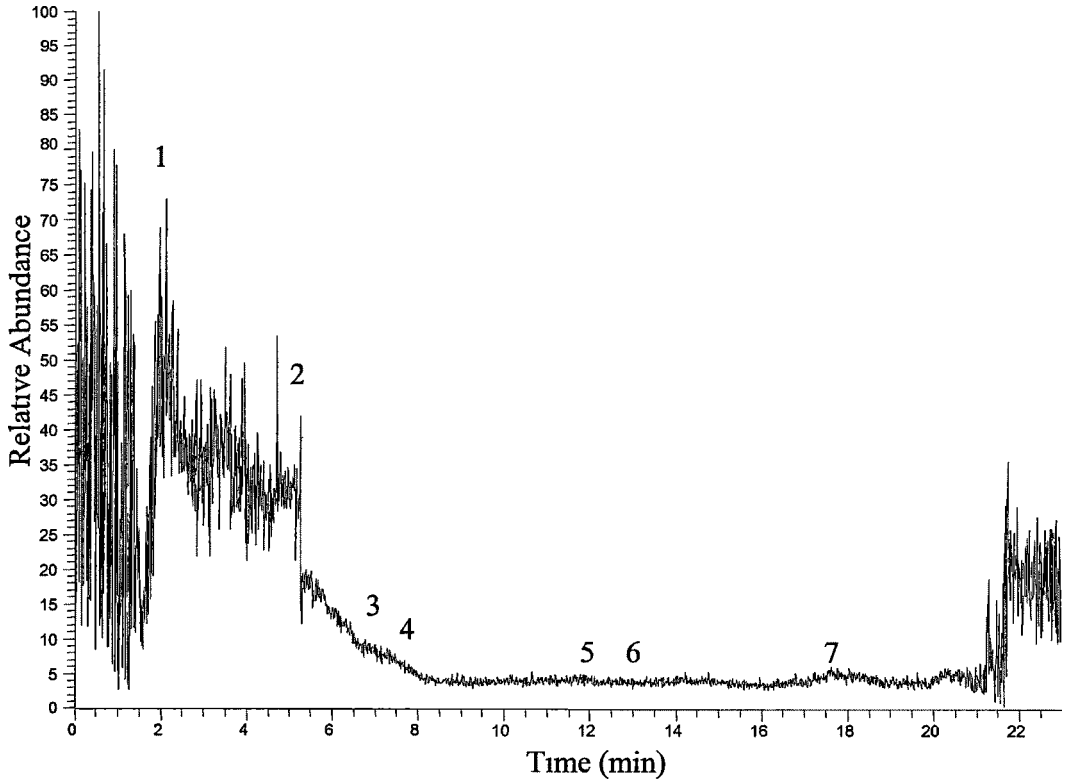


Figure 3.24 LC/MS total ion chromatogram for sample 466321.

Peak No	Retention Time	m/z	Ion Intensity	Compound
1	2 13min	225	4 10E+04	?
2	5 24min	233	2 93E5,1 46E5	?,?
3	6 91min	209	3 58E+04	Formaldehyde
4	7 90min	223	5 89E+04	Acetaldehyde
5	12 04min	237	3 61E+05	Acetone/Propionaldehyde
6	14 96min	249	3 42E+03	Crotonaldehyde
7	17 75min	251	2 09E+04	Butyraldehyde

Table 3.20 LC/MS data for sample 466321.

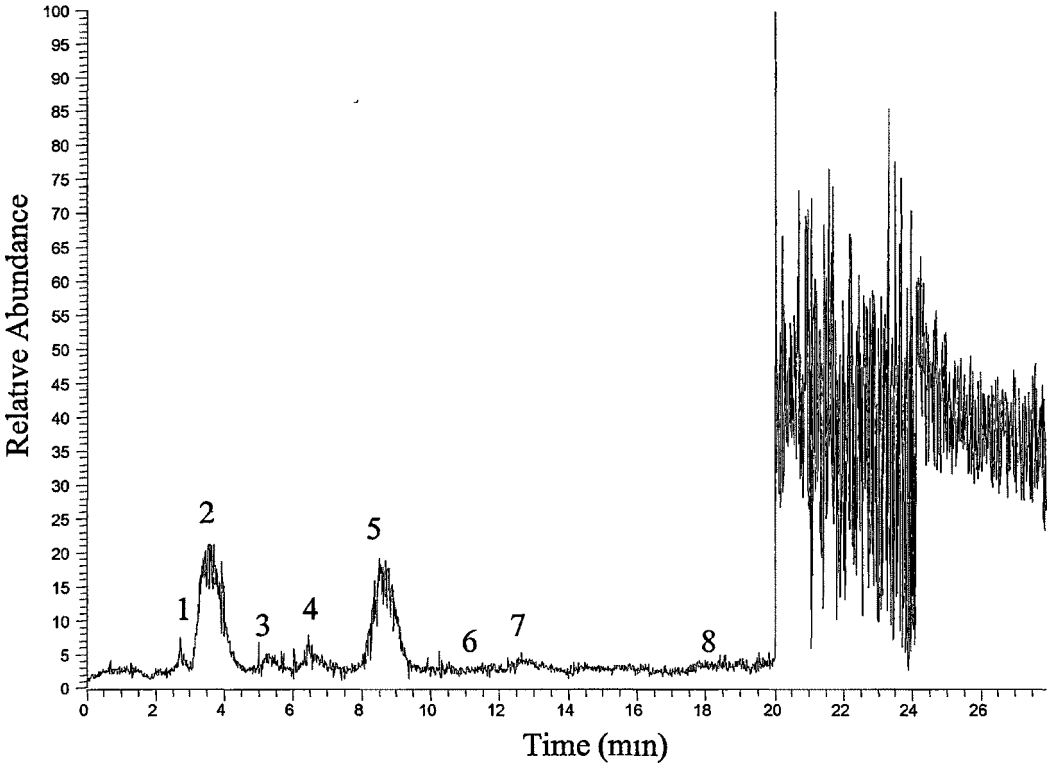


Figure 3.25 LC/MS total ion chromatogram for sample 421443.

Peak No	Retention Time	m/z	Ion Intensity	Compound
1	2 73mm	225	4.10E+04	?
2	3.56mm	233	1 46E+05	?
3	5 25mm	209	3 58E+04	Formaldehyde
4	6 46mm	223	5 89E+04	Acetaldehyde
5	8 50mm	237	3 61E+05	Acetone/Propionaldehyde
6	11 83mm	249	3.42E+03	Crotonaldehyde
7	12 64mm	251	2.09E+04	Butyraldehyde
8	18.33mm	279	8 87E+03	Hexaldehyde

Table 3.21 LC/MS data for sample 421443.

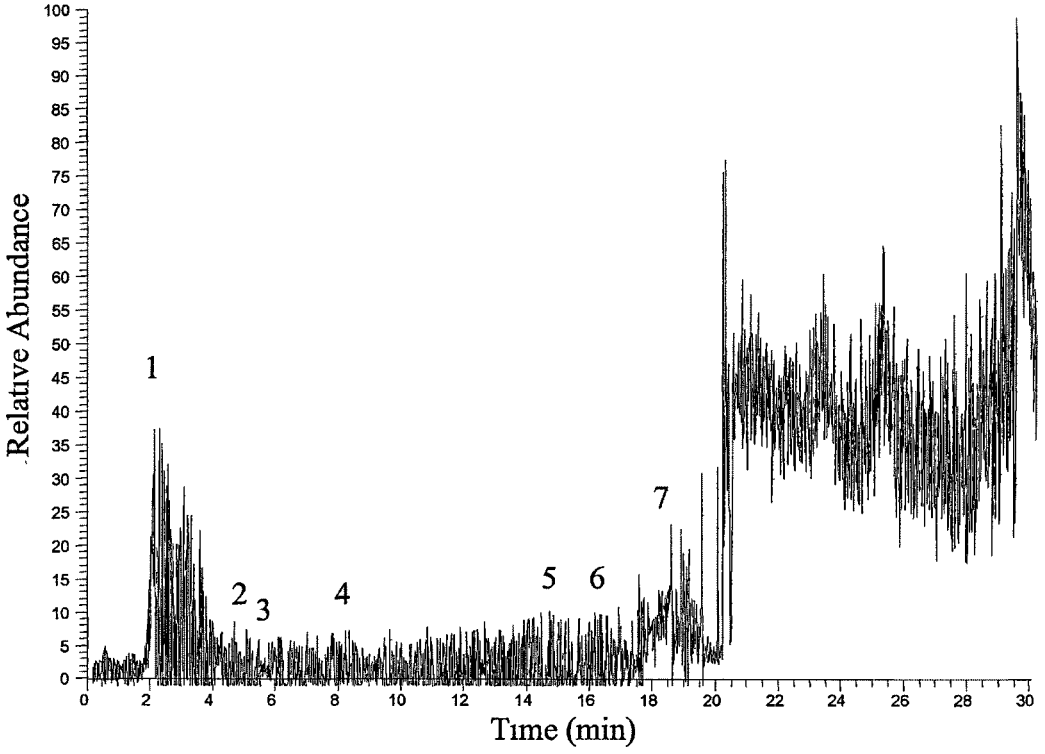


Figure 3.26 LC/MS total ion chromatogram for sample 466689.

Peak No	Retention Time	m/z	Ion Intensity	Compound
1	2 21min	233,218	1 49E5,5 96E4	?,?
2	4 95min	209	6 80E+03	Formaldehyde
3	5 68min	223	5 62E+03	Acetaldehyde
4	8 18min	237	1 35E+04	Acetone/Propionaldehyde
5	14 97min	285	9 95E+03	Benzaldehyde
6	16 13min	265	1.17E+04	C5 aldehyde
7	18.42min	279	1.31E+05	Hexaldehyde

Table 3.22 LC/MS data for sample 466689.

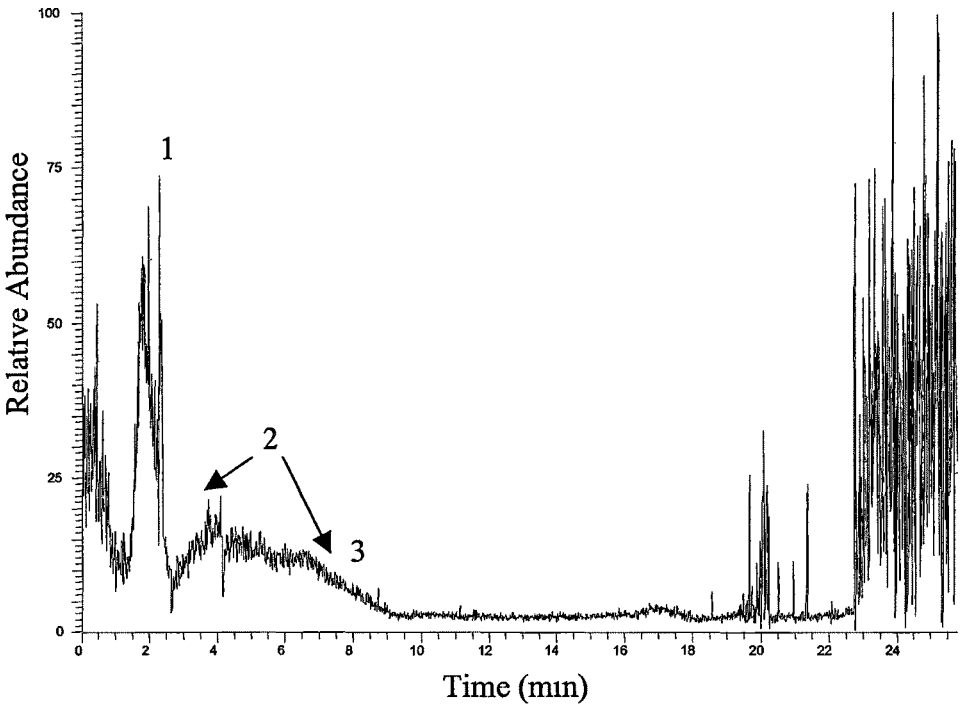


Figure 3.27 LC/MS total ion chromatogram for sample 412961.

Peak No	Retention Time	m/z	Ion Intensity	Compound
1	2 27mn	212,366,349	1 74E+05	?,?,?
2	3 35-7.55mn	362,289,349	6 04E+04	?,?,?
3	8 09mn	346	8 09E+03	?

Table 3.23 LC/MS data for sample 412961.

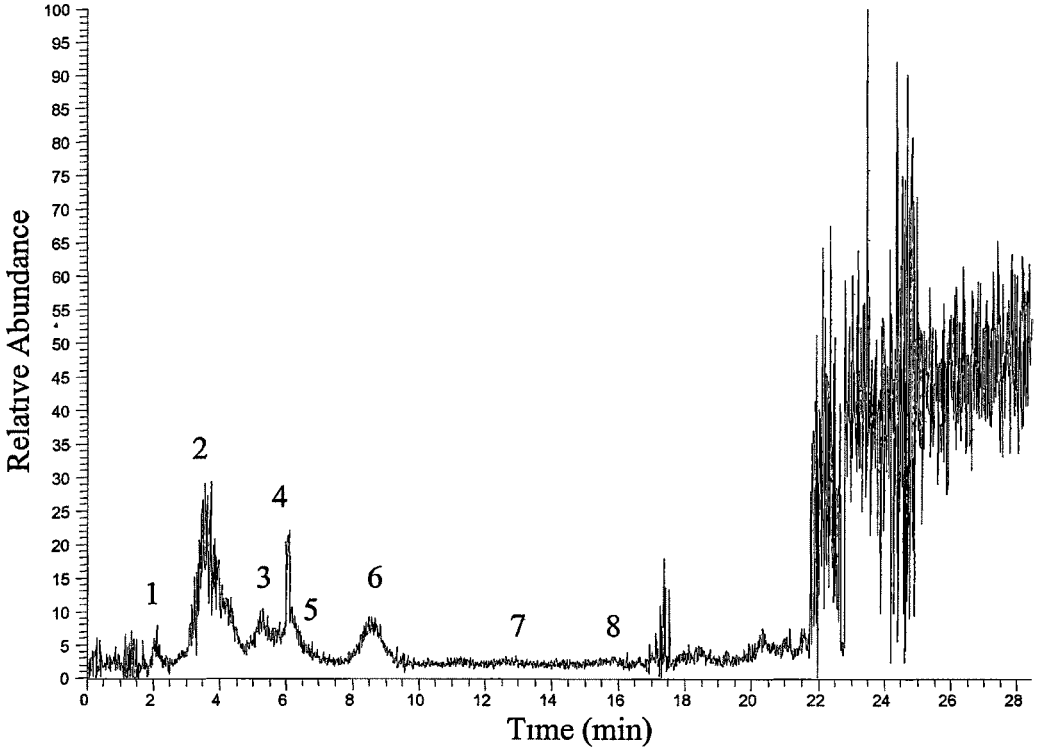


Figure 3.28 LC/MS total ion chromatogram for sample 416600.

Peak No	Retention Time	m/z	Ion Intensity	Compound
1	2 11mn	225	4 84E+04	?
2	3 74mn	233,218	2 05E51 64E5	?,?
3	5 30mn	235	9.28E+04	?
4	6 09mn	294	1 86E+05	?
5	6 62mn	223	2 31E+04	Acetaldehyde
6	8 58mn	237	8 60E+04	Acetone/Propionaldehyde
7	13.06mn	251	5 89E+03	Butyraldehyde
8	15 91mn	265	3.16E+03	C5 aldehyde

Table 3.24 LC/MS data for sample 416600.

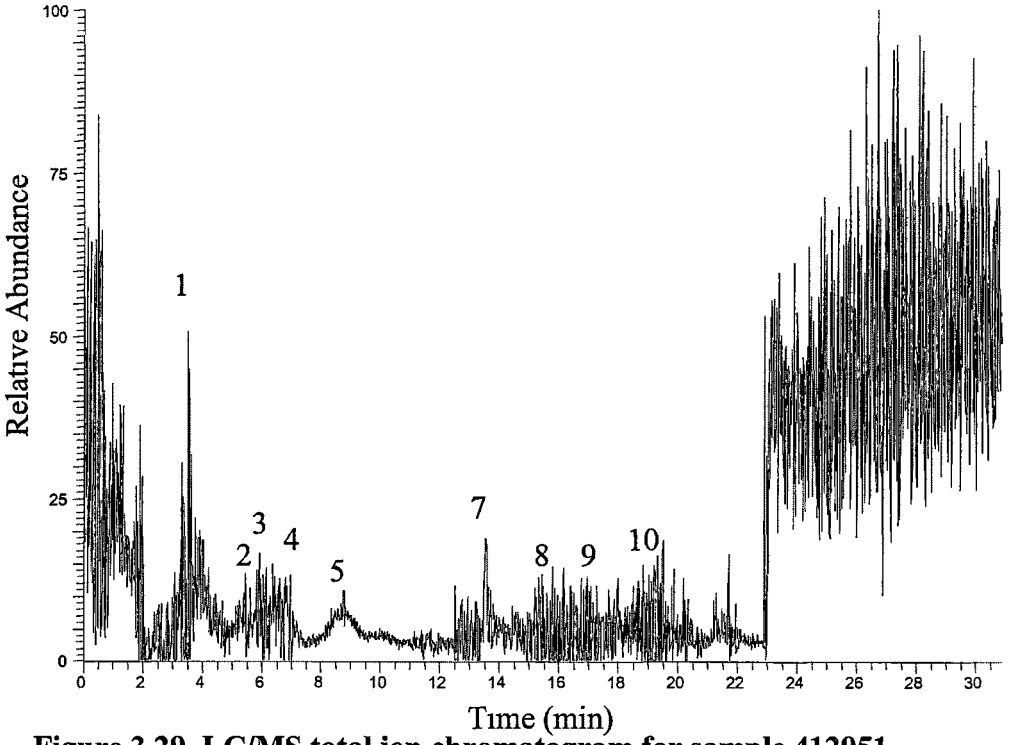


Figure 3.29 LC/MS total ion chromatogram for sample 412951.

Peak No	Retention Time	m/z	Ion Intensity	Compound
1	3 52min	233,218	9 69E4,8 72E4	?,?
2	5 39min	235	3 03E+04	?
3	5 90min	294	3 33E+04	?
4	6 74min	223	7 00E+04	Acetaldehyde
5	8 76min	237	5 71E+04	Acetone
6	9 99min	237	2.22E+04	Propionaldehyde
7	13 53min	251	1 97E+04	Butyraldehyde
8	15 29min	285	7 53E+03	Benzaldehyde
9	16 55min	265	5 63E+03	C5 aldehyde
10	19 27min	279	8 77E+03	Hexaldehyde

Table 3.25 LC/MS data for sample 412951.

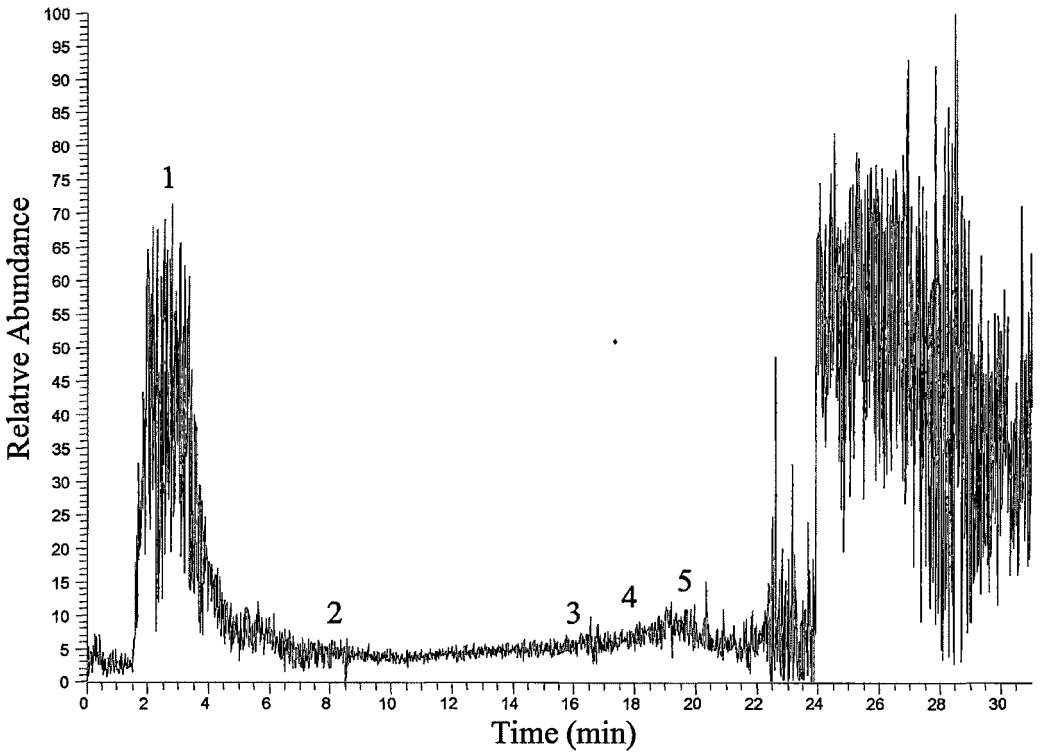


Figure 3.30 LC/MS total ion chromatogram for sample 410450.

Peak No	Retention Time	m/z	Ion Intensity	Compound
1	2 78mm	218,233	2 18E5,1 74E5	?, ?
2	8 08mm	237	6 58E+03	Acetaldehyde
3	16 01mm	265	2 83E+03	C5 aldehyde
4	17.71mm	299	2 11E+03	Tolualdehyde
5	19 54mm	279	2 66E+03	Hexaldehyde

Table 3.26 LC/MS data for sample 410450.

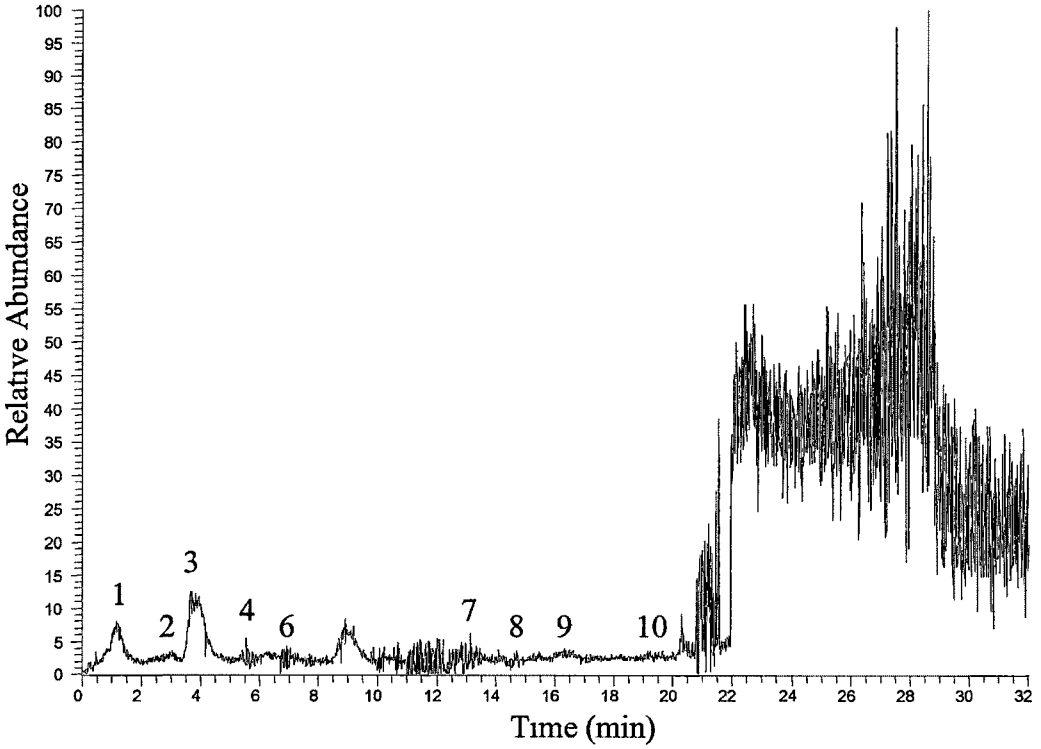


Figure 3.31 LC/MS total ion chromatogram for sample 412952.

Peak No	Retention Time	m/z	Ion Intensity	Compound
1	1.19min	267,218	2 28E4,1 37E4	?, ?
2	3 07min	225	2·84E+04	?
3	3 85min	218,233	1 35E+05	?,?
4	5 54min	218,209	7.65E3,4 09E3	?,Formaldehyde
5	6 93min	223	1 86E+04	Acetaldehyde
6	8 89min	237	1 12E+05	Acetone/Propioaldehyde
7	13 29min	233,251	7 43E3,5 18E3	?, Butyraldehyde
8	14 94min	285	1.11E+03	Benzaldehyde
9	16 37min	265	4 85E+03	C5 aldehyde
10	19 66min	279	5 00E+03	Hexaldehyde

Table 3.27 LC/MS data for sample 412952.

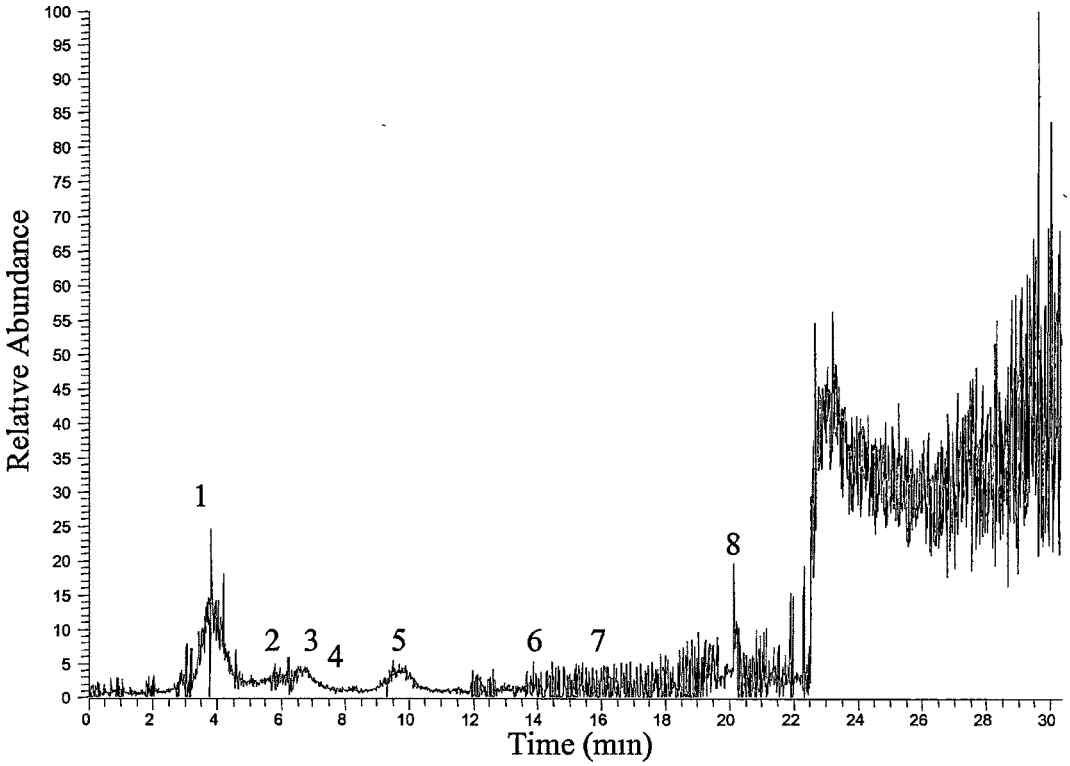


Figure 3.32 LC/MS total ion chromatogram for sample

Peak No	Retention Time	m/z	Ion Intensity	Compound
1	3 79min	218,233	1 85E5, 1 76E5	?, ?
2	5 92min	235,209	4 00E4,7 32E3	?, formaldehyde
3	6 50min	294	1 12E+05	?
4	7 69min	223	3 86E+03	Acetaldehyde
5	9.69min	237	9 32E+04	Acetone/Propionaldehyde
6	14 05min	251,301	4 70E3,4 23E3	Butyraldehyde,?
7	15.96min	265	3.35E+03	C5 aldehyde
8	20 13min	279,395	1 23E4,8 61E3	Hexaldehyde,?

Table 3.28 LC/MS data for sample 416591.

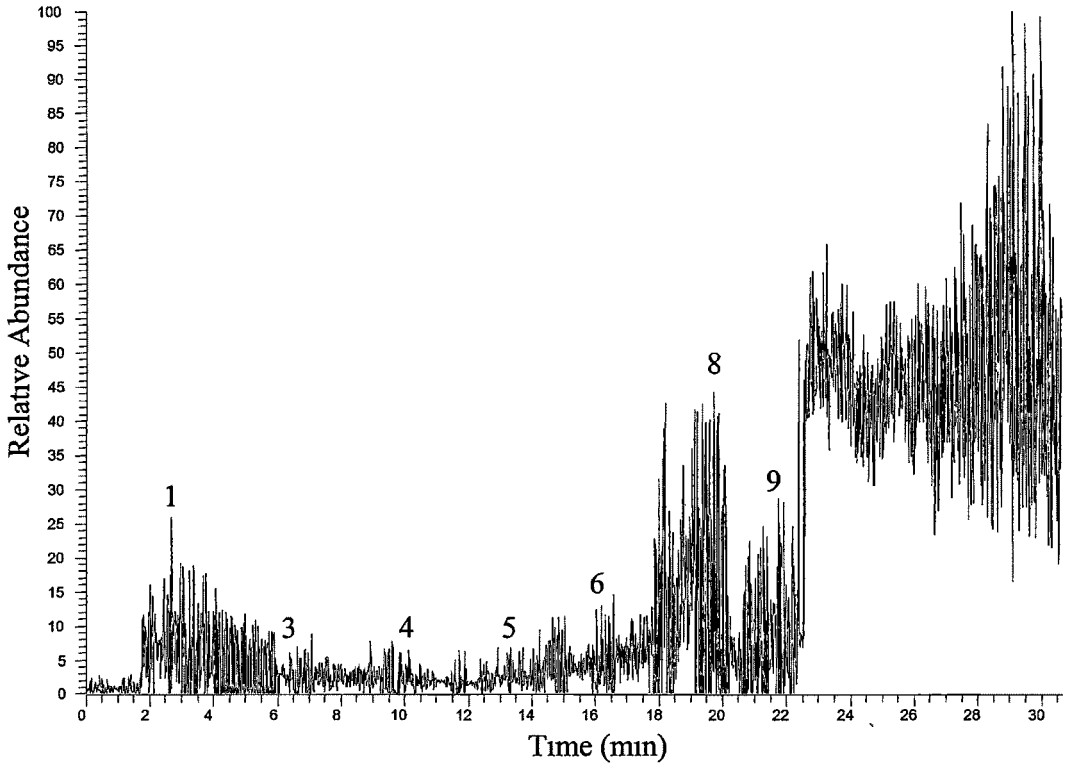


Figure 3.33 LC/MS total ion chromatogram for sample 416585.

Peak No	Retention Time	m/z	Ion Intensity	Compound
1	2 70min	217,225, 233	3 63E+04	?, ?, ?
2	5 97min	209	2 52E+03	Formaldehyde
3	6 30min	223	1 10E+04	Acetaldehyde
4	10 12min	237	1 82E+04	Acetone/Propionaldehyde
5	13 34min	251	1 27E+04	Butyraldehyde
6	16 17min	265	5 17E+04	?
7	16 58min	265	8 91E+04	C5 aldehyde
8	18 48min	279	2 50E+05	Hexaldehyde
9	21 76min	293	6.62E+04	?

Table 3.29 LC/MS data for sample 416585.

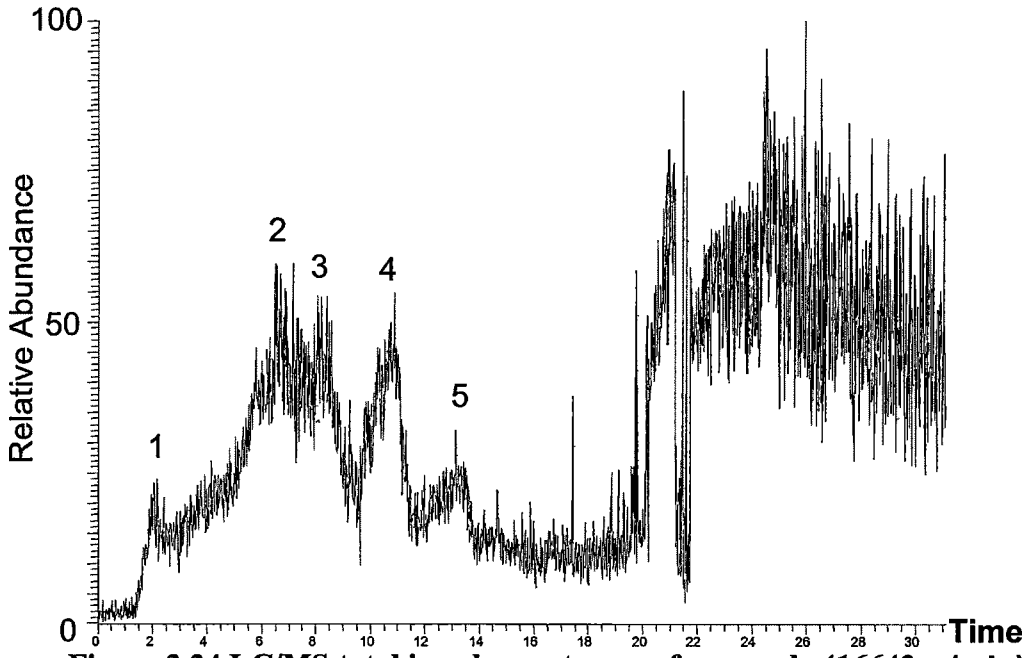


Figure 3.34 LC/MS total ion chromatogram for sample 416643.

Peak No	Retention Time	m/z	Ion Intensity	Compound
1	2 16min	273, 257	2 25E+04	?, ?
2	6 50min	258, 228, 288	4 42E4, 2 65E4, 2 65E4	?, ?, ?
3	8 40min	286	4.35E+04	?
4	10 89min	300	2.39E+04	?
5	13 15min	301, 348	1 28E4, 5 12E4	?, ?

Table 3.30 LC/MS data for sample 416643.

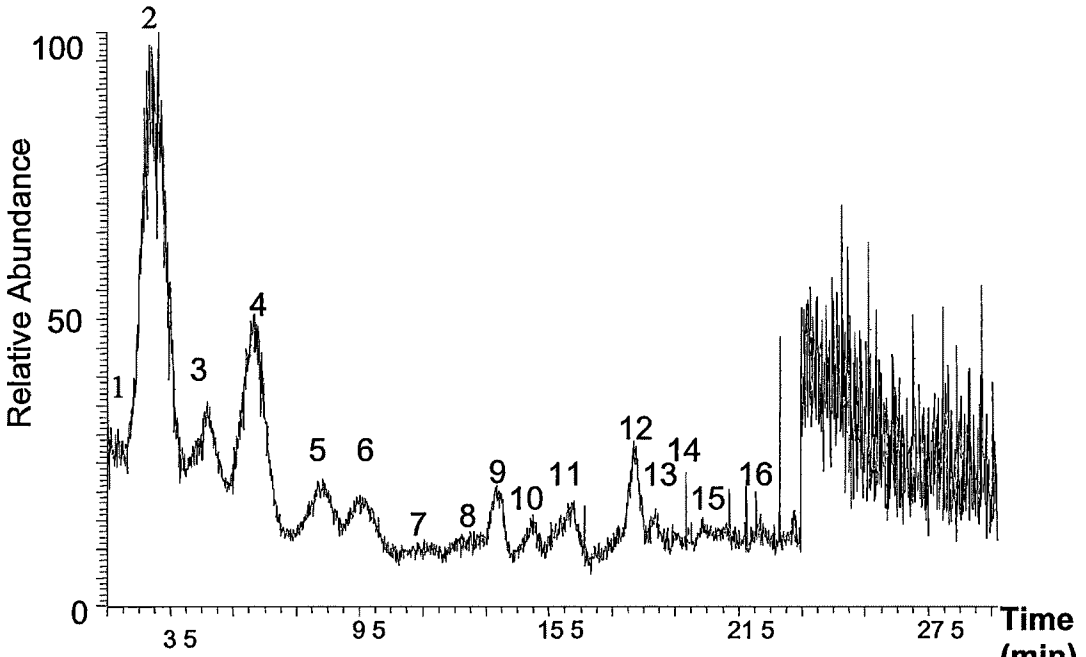


Figure 3.35 LC/MS total ion chromatogram for sample 416629.

Peak No	Retention Time	m/z	Ion Intensity	Compound
1	1 80mm	211, 225	1 60E5, 1 60E5	?, *Formic Acid*
2	3 17mm	218, 223	1 23E6, 9 25E5	?, ?
3	4 75mm	218, 209	2 42E5, 1 45E5	?, Formaldehyde
4	6 18mm	223, 218, 259	3 46E5, 2 42E5, 2 42E5	Acetaldehyde, ?, ?
5	8 36mm	237, 273, 227	6 85E4, 3.42E4, 2 74E4	Propionaldehyde, ?, ?
6	9 52mm	218, 237	1 07E5, 1 02E5	?, Acetone
7	11 46mm	227	1 83E+04	?
8	12 47mm	251	4 23E+04	Butyraldehyde
9	13 83mm	218	2 50E+05	?
10	14 98mm	285	1 00E+05	Benzaldehyde
11	16 28mm	265, 301, 445	4 68E4, 2 80E4, 2 34E4	C5 aldehyde, ?, ?
12	18 19mm	279, 431	3 05E4, 1 14E5	C6 aldehyde, ?
13	18 87mm	279,315	6 71E4, 3 36E5	Hexaldehyde, ?
14	19 85mm	293, 289	1 51E4, 1 06E4	C7 aldehyde, ?
15	20 83mm	293, 445	2 63E4, 7 9E3	C7 aldehyde, ?
16	22 21mm	307, 369	2 25E4, 1 35E4	C8 aldehyde, ?

Table 3.31 LC/MS data for sample 416629.

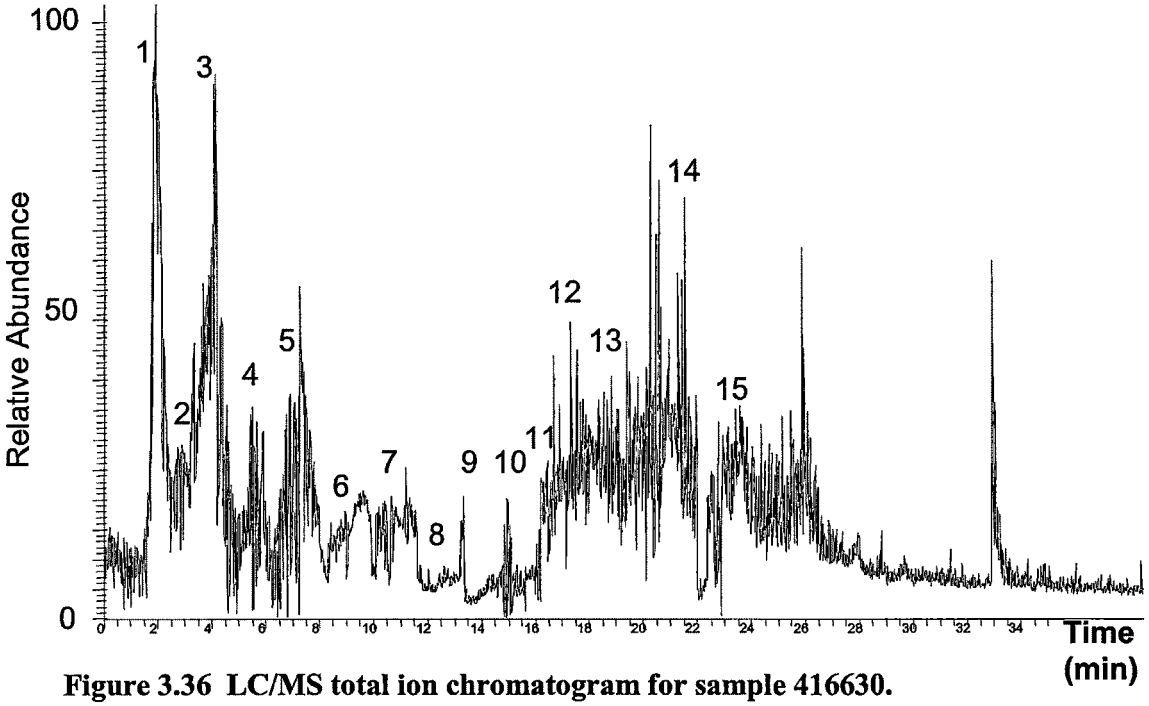


Figure 3.36 LC/MS total ion chromatogram for sample 416630.

Peak No	Retention Time	m/z	Ion Intensity	Compound
1	1 78min	293	1 99E+06	?
2	2 53min	225	2 68E+05	Formic Acid
3	3 79min	218, 233	8 71E5, 5 22E5	?, ?
4	5 17min	218, 209	2 52E5, 1 51E5	?, Formaldehyde
5	6 66min	223, 259, 218	4 70E5, 1 88E5, 1 88E5	Acetaldehyde, ?, ?
6	8 83min	237	3 81E+05	Acetone
7	10 23min	237, 273	1 93E5, 9.65E4	Propionaldehyde, ?
8	12 22min	309, 227, 251	2.26E4, 2.15E4, 1 92E4	?, ?, Butyraldehyde
9	13 70min	287	1 03E+05	?
10	15.28min	285	1 35E+05	Benzaldehyde
11	15 83min	265	1 31E+05	Valeraldehyde
12	17 02min	299	2.94E+04	Tolualdehyde
13	18 55min	279, 431	1 31E5, 7 44E4	Hexaldehyde, ?
14	21 58min	293	3 62E+04	C7 aldehyde
15	23 67min	307, 339, 321	3 41E4, 2 73E4, 1.36E4	C8 aldehyde, ?, C9 aldehyde

Table 3.32 LC/MS data for sample 416630.

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VITA

Kai Zhou was born in Chengdu, Sichuan, China, on August 29, 1977, the daughter of Mingxian Zhou and Lejia Yao. After completing her study at Shude High School, Chengdu, Sichuan, in 1996, she entered Sichuan University, Chengdu, Sichuan. She received the degree of Bachelor of Science from Sichuan University in July 2000. From January 2001 to June 2002, she studied at Texas Christian University graduate college. She transferred to Texas State University-San Marcos graduate college in August 2002. During the following two years, she was employed as teaching assistant and research assistant with Chemistry & Biochemistry department at Texas State University-San Marcos.

Permanent Address: 2501 Lake Austin Blvd. N106
Austin, Texas 78703

This thesis was typed by Kai Zhou.