SEPARATION, DETECTION AND CHARACTERIZATION OF ORGANOSULFUR COMPOUNDS USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY COUPLED WITH ATMOSPHERIC PRESSURE CHEMICAL IONIZATION MASS SPECTROMETRY

THESIS

Presented to the Graduate Council of
Texas State University-San Marcos
in Partial Fulfillment of the Requirements

For the Degree

Master of SCIENCE

By

Vidya Rai, B. S.

San Marcos, Texas
December 2003

ACKNOWLEDGEMENTS

My deepest and most sincere appreciation to Dr.Walter Rudzinski, my research advisor and professor in the Chemistry and Biochemistry Department, for his constant guidance, trust, support and patience throughout this research. He has been a source of inspiration and every interaction with him has been a great learning experience.

My sincere thanks to my committee members, Dr. Michael Blanda and Dr. Michael Korn, for being on the committee.

I would also like to thank Mr. Dan Millikan and Dr.Xuemei Luo for their help with the mass spectrometer. Special thanks to all the faculty, staff and graduate students in the Chemistry and Biochemistry Department of Texas State University, for their friendship and assistance.

Finally, I would like to thank my family, for their love, encouragement and support throughout my life.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

SEPARATION, DETECTION AND CHARACTERIZATION OF ORGANOSULFUR COMPOUNDS USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY COUPLED WITH ATMOSPHERIC PRESSURE CHEMICAL IONIZATION MASS SPECTROMETRY

by

Vidya Rai, B.S.

Texas State University-San Marcos

December 2003

SUPERVISING PROFESSOR: Dr. Walter Rudzinski

HPLC-APCI-MS/MS using tropylium cation as a sensitizing agent has been applied for the detection of standard PASH compounds: DBT, 2-DBT, 4,6-DBT, TAN and BNTP. The HPLC approach relied on the separation of the PASH compounds according to ring size on an amino bonded phase column. Fractions containing the 3-and 4- ring

compounds were isolated then separated on a PRP (polystyrene divinyl benzene) column. Post-column addition of tropylium after the PRP column, but just before the APCI source of a Finnegan LCQ ion trap mass spectrometer allowed for the detection of organosulfur compounds. Tandem mass spectrometry was performed to study the fragmentation patterns. An Arabian crude oil was fractionated and previously unidentified compounds were detected.

CHAPTER I

INTRODUCTION

Everyday about 10 million metric tons of crude oil are pumped from the Earth. Most (about 90%) of the oil is burned for energy [1]. The problem with crude oil and other fossil fuels is that the combustion products are harmful to the earth. CO_2 emissions have contributed to global warming; whereas, NO_2 and SO_2 emissions produce acid rain, which dissolves buildings, kills forests and poisons lakes [2]. In addition sulfur compounds in petroleum are known to poison catalysts used in processing, and several polycyclic aromatic hydrocarbons (PAH) and polycyclic aromatic sulfur heterocycles (PASH) are ubiquitous environmental pollutants and are potentially carcinogenic, mutagenic and toxic [3].

For the conversion of crude oils into more valuable fuels by pyrolysis and hydrocracking, more information about the chemical composition of the crude is needed. A range of chromatographic techniques like open-column liquid chromatography (OCLC), medium pressure liquid chromatography (MPLC), high performance liquid chromatography (HPLC), gas chromatography (GC), supercritical fluid chromatography (SFC) etc., have been employed for the characterization of petroleum and related products [4]. In the case of gasoline fractions and light distillates these techniques are applied to separate and determine individual compounds. But it was recognized long

ago that the characterization of high boiling petroleum fractions in terms of the individual components is difficult. Even gas chromatography/mass spectrometry (GC/MS) one of the most powerful analytical tools available is limited to relatively low- and medium-boiling fractions. This is because with an increase in the number of aromatic and naphthenic rings in a molecule, the boiling point increases, and the sample is not sufficiently volatile. The problem is further complicated by the presence of heteroatoms involving a variety of functional groups at an immense number of possible locations within a molecule which creates a vast number of isomers [5].

Hydrocarbon type analysis (HTA) by chromatography is popular. Here the goal of chromatographic separation is to prepare chemically meaningful and operationally well-defined "compound class" fractions for further molecular characterization [5]. A classic example of HTA is the Saturate-Aromatic-Resin-Asphaltene (SARA) method [6]. The first step involves extraction of the petroleum sample into a hydrocarbon solvent such as pentane. The Asphaltenes (aggregrates of extended polyaromatics, naphthenic acids, fatty acids, metalloporphyrins, ionic salts and polyhydric phenols) are pentane insoluble and precipitate out of solution. The pentane soluble portion (maltenes) is separated into saturates (alkanes and cycloparaffins) [7], aromatics (mono-, di-, and polycyclic aromatic hydrocarbons with alkyl side chains) [7] and resins (aggregates with a multitude of buiding blocks such as polycyclic aromatic hydrocarbons, sulfoxides, amides, thiophenes, pyridines, quinolines and carbazoles) [7] by elution from an alumina column using solvents of increasing eluotropic strength.

High performance liquid chromatography (HPLC) has been used extensively for the separation of compound-class fractions for further molecular characterization. Matsunaga

applied normal phase liquid chromatography using several commercially available packings including silica, alumina, porous polystyrene gel and chemically bonded phases e.g., NO_2, NH_2, CN and sulfonic acid on Nucleosil. In his study he compared the various packings for the separation of aromatic compounds by ring number, separation of polar compounds (nitrogen- and oxygen- containing compounds) from polynuclear aromatic hydrocarbons, and the resolution between polar compounds with different functional groups [8]. He found that for the separation of aromatic compounds based on ring size, Nucleosil NO_2 gave the best selectivity followed by Nucleosil NH_2. Polystyrene gel exerted a unique adsorption of aromatic compounds with hexane as eluant, probably due to Π-Π bonding interactions. For the separation of aromatics and polar compounds, silica gave the best resolution while overlapping was seen with Nucleosil NO_2, CN and sulfonic acid. Nucleosil NH_2, alumina and silica gave good resolution between phenols and nitrogen compounds and good separation of nitrogen compounds by types was obtained using silica and Nucleosil sulfonic acid.

Wise et al., used reversed phase-liquid chromatography with fluorescence detection for the measurement of PAHs in environmental samples. They compared the separation of 16 PAHs on a C_{18} polymeric phase versus a C_{18} monomeric phase. It was found that C_{18} polymeric phase offered excellent selectivity for the separation of PAH isomers [9].

The LC separations of high boiling or residuum hydrocarbons on the basis of aromatic ring size is a formidable task. A detailed comparison of alumina, amino bonded silica (NH_2-silica) and (2,4- dinitroanilinopropyl) silica (DNAP- silica) for the HPLC separation of aromatic hydrocarbons based on ring size was made with 86 model compounds present in petroleums, coal liquids or shale oils. It was found that DNAP-

silica and alumina were more sensitive to molecular structure but less sensitive to substituent effects than NH_2-silica. On the basis of retention strengths and grouping tendencies, the DNAP- silica was considered superior than the other two for aromatic ring size separation [10].

Winston. K. Robbins developed a fully automated HPLC-2 system for the quantitative measurement of the distribution of both aromatic carbon and mass in heavy distillates. As no single column was completely adequate for separating the entire range of polarity in heavy distillates, and there was no universal quantitative detector for HPLC, the HPLC-2 system made use of 2 columns and 2 detectors. The DNAP column was chosen because it had excellent aromatic ring selectivity. To compensate for its poor resolution of the saturates and monoaromatics it was coupled with a propylaminocyano (PAC) column. The diode array detector (DAD) was used to calculate the aromaticity (%C) and the evaporative mass detector (EMD) was used to calculate the % mass distribution. Unique detector algorithms were applied to quantitate the aromaticity and mass of 6 groups (saturates, 1-4 ring aromatics and polar compounds) separated from oils using solvent gradients on the coupled columns [11].

In yet another HPLC approach the total aromatic content of a diesel fuel was found by precisely determining the mono-, di and polyaromatic species. There was evidence that not all the polyaromatic content in the samples was being determined owing to both poor chromatographic signal amplitude (level in fuel is low) and non elution of some of the higher ring components. To overcome this a backflush operation was incorporated; wherein, the direction of flow of mobile phase is reversed through the column after the elution of the diaromatics. The polyaromatics, owing to a shorter flow path, gave a

narrower peak width and higher amplitude. In addition the higher number components that were previously held on the column were totally eluted and counted in the total aromatic content [12].

Almost all available HPLC detectors have been used for detection and quantification of aromatics in petroleum samples. These include spectroscopic detectors such as UV-visible, fluorescence, diode array detectors and infrared and bulk property detectors such as refractive index, evaporative light scattering and flame ionization. Mass spectrometric detectors have also been used with HPLC. However for hydrocarbon (HC) type determinations, problems arise from the lack of a detection system that provides a uniform response factor for HC types. Hayes and Anderson have overcome the above problem by using a dielectric constant detector for HPLC. The method was able to determine saturates (+olefins), alkyl benzenes and polynuclear aromatics in HC liquids boiling below 600° C. The separation used 3 columns in series, i.e two, 5 μm PAC columns preceding a 10 μm tetranitrofluorenimino (TENF) column using n-butyl chloride as the mobile phase [13].

Though the above mentioned HPLC techniques have been used for the analysis of saturates and aromatics in petroleum samples they can also be used for the analysis of NSO (nitrogen, sulfur, oxygen containing) compounds which are also a mixture of saturates, mono-, di-, tri-, polyaromatic and fused ring systems.

This research focuses on the analysis of polycyclic aromatic sulfur heterocycles (PASH), a specific type of NSO compound present in crude oil. Lately, there has been an increasing interest in PASH due to several reasons e.g., their potential

mutagenic/carcinogenic properties, the difficulty of desulfurizing them for the production of low-sulfur fuels, their photoreactions in the aqueous phase after oil spills, their effects on microbial metabolism and their potential as possible indicators for the maturity of crude oils and source rocks [14]. PASH is a difficult class to analyze as it has a very complex composition, containing widely varying concentrations of numerous isomers. In addition PASH have properties very similar to PAH, so it is very difficult to separate them from each other.

GC coupled with MS is a powerful analytical tool for the characterization of low molecular weight hydrocarbons and heteroatomic molecules having up to 30 or so carbon atoms. Capillary column GC provides excellent resolution between major component peaks permitting both identification and quantification of individual components for light petroleum distillates. For e.g., a list of about 400 identified peaks in whole gasoline samples was provided by Whittemore in 1979 [4]. For high molecular weight, difficult to volatilize compounds like PASH, GC has certain limitations. The high temperature employed for volatilization of compounds ends up causing their decomposition. For e.g., in the analysis of the PASH fraction of a coal liquid by GC about 80- 90% of certain 3 and 4 membered PASH compounds were desulfurized in the column or injection port of the gas chromatograph where the temperature was about 285 ^{0}C [15]. In addition there is degradation of the front end of the capillary column as nonvolatile components from the sample accumulate over time.

Nishioka et al., have developed a rapid method for the isolation of PASH from the aromatic fraction of complex mixtures based on ligand exchange chromatography (LEC) using silica gel impregnated with $PdCl_2$. The approach takes advantage of the selective

interaction of Pd^{2+} with PASH. The method was applied to a coal liquid and petroleum heavy ends, and the PASH fraction was then examined by capillary column gas chromatography with flame ionization and flame photometric detection as well as GC-MS. The results revealed the presence of PASH having 2 to 6 aromatic rings [15].

Online LC-MS is an alternative technique for the molecular characterization of PASH. In order to couple LC with MS, an ion source is needed which transforms the analyte species in solution to free ions in the gas phase on a continuous, as opposed to a batch-wise, basis. Traditional methods of ionization for mass spectrometry such as electron impact, or chemical ionization usually produce atoms or molecules that have lost or gained one or more electrons during a gas phase encounter with an ion or another electron. An implicit requirement of such ionization is that the analyte must be volatile enough to produce a useful concentration of gas molecules as collision partners for the ionizing entities [16]. Thus to extend MS to high boiling molecules other sources have to be considered.

Hsu and Qian have used liquid chromatography/thermospray/mass spectrometry (LC/TSP/MS) to characterize petroleum fractions boiling greater than 1050°F. TSP is mainly a chemical ionization technique using solvent vapor as a reagent gas that selectively ionizes molecules with high proton affinity. Though it utilizes heat to nebulize the LC effluent it does not deposit excess energy into sample molecules resulting in the formation of intact molecular ions with little fragmentation [17].

More recently electrospray ionization (ESI) has been playing an important role in producing ions of large, complex and fragile species that cannot possibly be vaporized for ionization by classical methods. ESI is an example of an atmospheric pressure ionization

(API) process. This technique transfers ions from solution to the gas phase by ionizing the sample at atmospheric pressure and then transferring the ions into a mass spectrometer. An electrospray is produced by applying a strong electric field under atmospheric pressure, to a liquid passing through a capillary tube with a weak flux (1-10 μl/min). The electric field is obtained by applying a potential difference of 3-6 kV between this capillary and the counter-electrode, separated by 0.3-2cms [18]. This field induces a charge accumulation at the liquid surface located at the end of the capillary and thus charged droplets are produced. This is the first major step in the production of gas-phase ions by electrospray from electrolyte ions in solution. When the charged droplets shrink into ultimately very small highly charged droplets by solvent evaporation, the electrical charge density at the surface of the droplets increases. The small droplets produce gas phase ions by either an ion evaporation mechanism or charged residue mechanism [19].

ESI is a soft ionization technique because fragmentation of ions can be avoided. Generally ESI requires a more polar solvent, e.g., water, methanol or acetonitrile and sometimes the addition of an acid or base to promote ionization. Generally ions are formed either through protonation of basic groups or deprotonation of acidic groups depending on the characteristics of the solvent.

Atmospheric pressure chemical ionization (APCI) is another ionization technique which uses gas phase ion-molecule reactions at atmospheric pressure. Here the sample or chromatographic effluent is directly introduced into a pneumatic nebulizer where it is converted into a thin fog by high speed air. Droplets are then displaced by the gas flow through a heated quartz tube called a vaporization chamber. The heat transferred to the

spray droplets allows the vaporization of the mobile phase and of the sample in the gas flow. The hot gas (120 °C) and the compounds leaving this tube and arriving at the reaction area of the source are chemically ionized through proton transfer in positive ion mode and through electron transfer or proton loss in negative ion mode. Generally the evaporated mobile phase acts as the ionizing gas and yields pseudomolecular $(M+ H)^+$ and $(M-H)^-$ ions in the positive and negative ion modes, respectively. The electrons needed for the primary ionization are produced via corona discharge. The ionization of the substrate is very efficient as it occurs at atmospheric pressure and so with a high collision frequency. The rapid desolvation and vaporization of the droplets reduce thermal decomposition considerably and thus preserve the molecular species [18].

From the above description of TSP, ESI and APCI it is apparent that these techniques work well with sample molecules which are ionic in nature and undergo acid/base chemistry. Since PASH are neither ionic nor do they undergo easy protonation and deprotonation analyzing them using the above ionizing sources becomes difficult.

Van Berkel and coworkers in several reports have demonstrated that electron transfer reactions are an efficient means of generating radical cations of aromatic, heteroaromatic and other highly conjugated systems [20]. For e.g., they ionized neutral PAH, a heteroaromatic, a substituted aromatic, and the highly conjugated molecule buckminsterfullerene (C_{60}) in solution via reactions with the chemical electron-transfer reagents: trifluoroacetic acid, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), and antimony pentafluoride and then detected these as their respective radical cations in the gas phase by ESI-MS.

Another approach for the selective determination of difficult to ionize species is

coordination-ion spray. Here positively or negatively charged metal complexes are formed by the addition of a suitable metal ion to an analyte prior to detection by MS. Metals of first and eighth transition groups such as Cu^{+}, Ni^{+2}, Pd^{+2}, Pt and Ag^{+} are employed. These form stable Π- Π or Π allyl complexes with unsaturated compounds [21]. In a specific case Ag^{+} has been used to analyze heavy aromatic hydrocarbons in petroleum fractions by ESI-MS. The aromatic compounds react with the silver ion to form abundant adduct ions such as $[M+Ag]^{+}$ and $[2M+Ag]^{+}$ [22].

In yet another approach Rudzinski et al., used Pd^{2+} to produce radical cations of PASH which were identified by ESI- MS. The process involved an electron transfer between the metal ion and the PASH compound [23]. They further studied the solvent and matrix effects in the above MS analysis. It was found that in a hydrogenated oil matrix PASH compounds in the presence of $PdCl_2$ in (50:50) CH_3OH:CH_2Cl_2 gave an enhanced response when compared to a PASH mixture prepared in (50:50) CH_3OH:CH_3CN. This suggested that CH_2Cl_2 is an important solvent for radical cation stabilization [24].

Recently Airiau and co-workers analyzed PAH using HPLC/ESI-MS/MS after reaction with tropylium cation which produces positive ions [25]. The tropylium cation (TR+) is a strong Π acceptor and it recognizes PAH by Π- Π interactions and almost quantitatively forms the $[PAH- TR]^{+}$ 1:1 cation complex [26]. This complex which is formed in solution can also undergo a charge transfer from [PAH-TR]+ to $[PAH]^{+\cdot}$ in the electrospray interface.

In this research HPLC-APCI-MS/MS using tropylium as the sensitizing agent has been applied for the separation, detection and characterization of standard PASH

compounds as well as those present in an Arabian crude oil. The first step involved using standard compounds such as dibenzothiophene (DBT), 2-methyl- dibenzothiophene (2-DBT), 4,6-dibenzothiophene (4,6-DBT), thianthrene (TAN) and benzonaphthothiophene (BNTP) to establish the method which was then adapted to a crude oil. The Arabian crude oil was distilled and the heavy distillate (<260°C, under vacuum) was subjected to the SARA method of column chromatography [6]. The aromatic fraction thus obtained was treated by LEC using silica impregnated with $PdCl_2$ to concentrate the PASH [15]. This PASH fraction was finally cleaned by a novel solid phase extraction (SPE) approach on a reversed phase LC-NH_2 cartridge. A two dimensional HPLC approach was developed. In the first dimension an LC-NH_2 (amino bonded phase) column separated the PASH based on ring size [8]. These different ring size fractions were then further resolved in a second dimension using a PRP (polystyrene divinyl benzene) column [8]. The eluent from the PRP column was combined with tropylium just before entering the APCI source of a Finnigan LCQ ion trap mass spectrometer. All flow and solvent conditions were optimized. In the presence of tropylium, molecular ions of PASH formed and can be identified [25]. HPLC/MS and tandem HPLC/MS was then done on this purified PASH. Tandem MS was performed to study the fragmentation patterns. Single ion monitoring (SIM) of standards determined the exact retention time in the ion chromatogram. Finally the limit of detection of the standards in the oil matrix was found.

There were two issues that had to be overcome in the above analytical method. The high flow rate of the LC effluent had to be reduced before entering the MS which provides optimal results only at lower flow rates. This was done by running the second LC experiment using the PRP column with a low flow rate (0.3 ml/min) which was

within the working range for the APCI source which could tolerate flows up to 1.0 ml. The second was the compatibility of the LC effluent with the ion source as well as the crude oil. Hexane:dichloromethane (70:30) was optimal for the LC separation. This mixture was not only compatible with the APCI source but also dissolved the crude oil. In addition, the dichloromethane aided the stabilization of the radical cations of PASH [24].

CHAPTER II

EXPERIMENTAL

2.1 Reagents and Chemicals

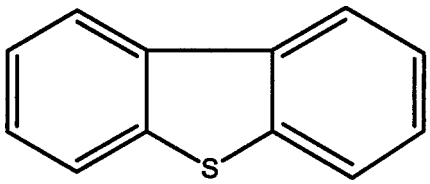
Methanol, acetonitrile (CH_3CN), hexane, chloroform (CH_3Cl), dichloromethane (CH_2Cl_2), toluene and diethyl ether were obtained from EM Science (Gibbstown, NJ). Arabian crude oil was obtained from Motiva Enterprises LLC; alumina (Neutral, Brockmann Activity 1, 150 mesh), silica gel (70/270) and tropylium tetrafluoroborate were obtained from Aldrich (Milwaukee, WI, USA). The organosulfur compounds: dibenzothiophene (DBT, 98 %), thianthrene (TAN, 97 %), 4,6-dimethyl-dibenzothiophene (4,6-DBT, 97 %), benzonaphthothiophene (BNTP, 99 %) were obtained from Aldrich while 2-methyl dibenzothiophene (2-DBT, purity unknown) was obtained from Sigma-Aldrich (Milwaukee, WI, USA). Fig 2.1 shows the structure and formula weight (F.W.) of each of the standards.

2.2 Preparation of Standard Solutions

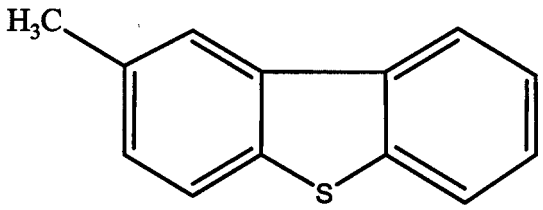
2.2.1 Tropylium Tetrafluoroborate Stock Solution

7.12 mg of tropylium tetrafluoroborate was dissolved in 2 ml of a solution of CH_2Cl_2 : CH_3CN (80:20). After 1 hour the solid was totally dissolved and turned into a clear

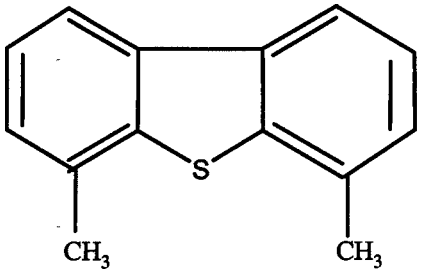
solution with a concentration of 20 mM. 50 μl of the 20 mM solution was taken and diluted to 1 ml in 100% CH_2Cl_2. The resulting solution had a concentration of 1 mM.



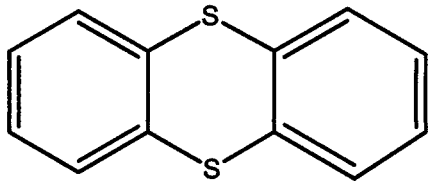
Dibenzothiophene
(DBT) F.W. 184.26



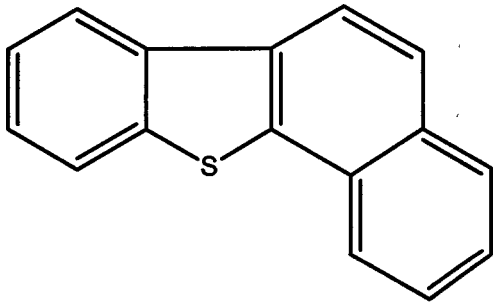
2-methyl-dibenzothiophene
(2-DBT) F.W. 198.28



4,6-dimethyl-dibenzothiophene
(4,6-DBT) F.W. 212.32



Thianthrene
(TAN) F.W. 216.32



Benzonaphthothiophene
(BNTP) F.W. 234.32

Fig 2.1 Structures of standard organosulfur compounds.

2.2.2 Organosulfur Standard Solutions

1.84 mg DBT, 2.13 mg TAN, 2.34 mg BNTP, 1.98 mg 2-DBT and 2.12 mg 4,6-DBT were each dissolved in 10 ml hexane. The concentration of each organosulfur standard solution was 1 mM. These were then diluted in hexane to produce solutions which varied from 10^{-4} to 10^{-6} M. To prepare the standard mixture 100 μl of each of the standard 1mM solutions were mixed together and diluted with hexane to a final volume of 1 ml. Each of the organosulfur standards had a final concentration of 10^{-4} M.

2.3 Fractionation Procedure for the Arabian Crude Oil

2.3.1 Distillation

The Arabian crude oil was distilled. A light distillate boiling up to 200°C was collected under atmospheric pressure. A middle distillate boiling between 200-310°C was collected under atmospheric pressure. A heavy distillate was obtained under reduced pressure (20 torr) keeping the temperature below 260°C. The residue was the remaining non-volatile fraction. See Fig 2.2 for the fractionation scheme.

2.3.2 Saturate–Aromatic-Resin-Asphaltene (SARA) Fractionation Method

The heavy distillate was subjected to the SARA method of column chromatography. The Alumina was activated at 220°C for 96 hours. 10 g activated alumina was pre-wet with hexane and packed into a glass column (1.1x 12.5 cm). 0.325 g heavy distillate was dissolved in 5 ml hexane and loaded at the top of the alumina column. 40 ml hexane was used to elute the saturates, 80 ml toluene was used to elute the aromatic hydrocarbons, 20

ml toluene:methanol (80:20) was used to elute the resin. The asphaltenes remained on the column. The fractions were evaporated to dryness. See Fig 2.2 for the fractionation scheme.

2.3.3 Ligand Exchange Chromatography (LEC) of the Aromatic Fraction

100 ml distilled water was added to 1g $PdCl_2$ and mixed thoroughly. This was then added to 20 g silica gel. The supernatant was discarded and the wet gel dried overnight at 95°C. This was then activated at 200°C for 24 hours. 6 g activated gel was pre-wet by a mixture of hexane:chloroform (50:50) and packed in a column (1.1 x 12.5 cm). 50 mg of the aromatic fraction was dissolved in 5 ml of hexane:chloroform (50:50) and loaded on top of the column. 30 ml hexane:chloroform (50:50) was used to elute the polycyclic aromatic hydrocarbons (PAH) fraction, while an additional 50 ml was used to elute the polycyclic aromatic sulfur heterocycle (PASH) fraction. 100 ml chloroform:diethylether (90:10) was used to elute the sulfur containing polycyclic aromatic compound (SPAC) fraction. Fractions were then evaporated to dryness. See Fig 2.2 for the fractionation scheme.

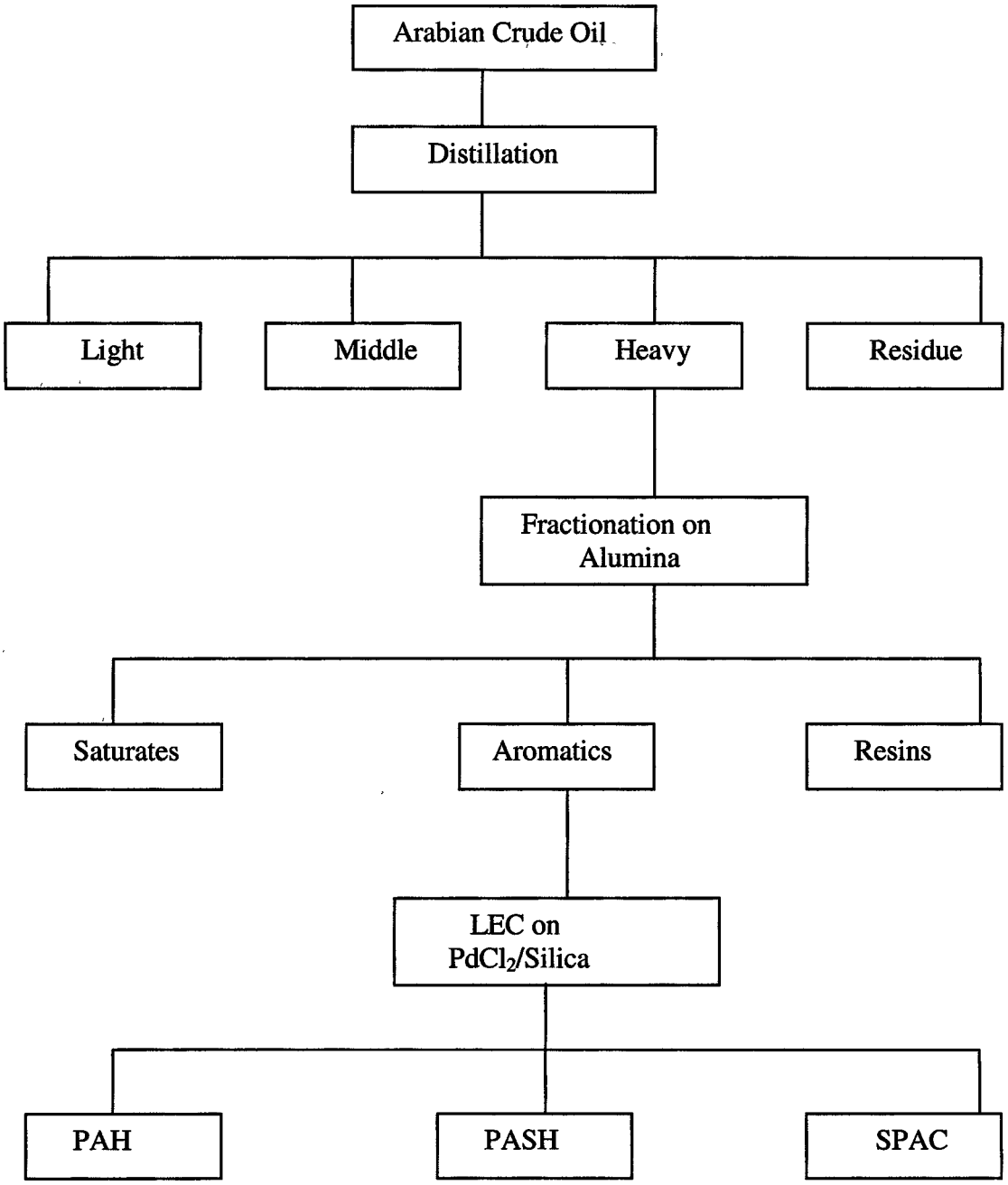


Fig 2.2 Scheme for the fractionation of the Arabian Crude Oil.

2.3.4 Clean up of the Polycyclic Aromatic Sulfur Heterocycles using Solid Phase Extraction (SPE)

Solid phase extraction on a LC -NH_2 cartridge (Supelco LC-NH_2, 3ml) was done to remove excess $PdCl_2$ from the PASH fraction. The tube was pre-wet with 2 ml hexane:chloroform (50:50).1 mg of PASH sample was dissolved in 2 ml hexane:chloroform (50:50) and loaded on top of the extraction tube. 5 ml of solvent mixture was added to clean the sample. 6 ml CH_2Cl_2 was finally added to elute all of the PASH sample. The excess palladium stayed on the cartridge resulting in a PASH sample which was colorless. The sample was evaporated to dryness. 0.7 mg PASH sample was redissolved in 0.7 ml hexane for all further experiments.

2.4 High Performance Liquid Chromatography/Mass Spectrometry

2.4.1 Instrumentation

The liquid chromatography system consisted of two Gilson 306 solvent delivery systems attached to a Gilson 506C Interface Module connected to a HCS (Hometown Computing, San Marcos, Tx) computer with Unipoint version 1.71 software. The above setup was connected to a Rheodyne 7125s 100μl injector and a Gilson 118 Uv-Vis detector set at 254 nm. Both a supelcosil LC-NH_2 column and a polystyrene divinyl benzene (PRP) column were used for the 2-dimensional HPLC separation.

Mass spectra were obtained on a Finnigan LCQ ion trap mass spectrometer equipped with an APCI source. A gateway 2000 computer with Navigator 2.1 software was used for system control as well as collection and analysis of the data.

MS was performed in full scan mode as well as single ion monitoring (SIM) mode. MS^2 was performed and peak fragments monitored over the m/z range 100-300. Each scan was the average of 3 microscans and data for the MS spectra were obtained continuously. Instrumental parameters for the APCI source were as follows: nitrogen sheath gas flow-rate, 70 (arbitrary units); ion mode, positive; corona discharge current, 5 μA; vaporizer temperature, 450 ^{0}C; capillary temperature, 149 ^{0}C; capillary voltage, 17 V; and tube lens offset voltage, 15 V.

2.4.2 Separation of Standards and Oil

All mobile phases were filtered through a 0.45 μm nylon filter prior to use to remove particulates and dissolved gases. All HPLC runs were conducted under isocratic conditions.

50 μl of 10^{-4} M organosulfur standard mixture or 25 μl of a solution containing 1 mg PASH/1 ml hexane was injected into the LC-NH_2 column (250 x 30 mm; dp=5μm) (Supelco, Bellefontaine, PA) and separated using 100% hexane mobile phase at a flow rate of 1 ml/min. Two separate fractions were collected in the case of the standards as well as the oil. These were blown down and reconstituted in 25 μl hexane:CH_2Cl_2 (70:30).

25 μl of each fraction were then sequentially injected into the polymer PRP column (150 x 4.6 mm; dp=10 μm) (Parker) with hexane:CH_2Cl_2 (70:30) as the mobile phase at a flow rate of 0.3 ml/min.

2.4.3 Post Column Derivatization

All LC/MS experiments were performed with the PRP column attached to the MS, using hexane:CH_2Cl_2 (70:30) mix at a flow rate of 0.3 ml/ml. All LC/MS runs involved a post column derivatization using 1 mM tropylium solution in 100% dichloromethane. Before entering the probe of the mass spectrometer the mobile phase from the HPLC system was combined at a standard tee with tropylium solution at a concentration of 1mM added at a flow rate of 15 μl/min.

2.4.4 LC/MS Experiments in Single Ion Monitoring (SIM) Mode

In order to follow the elution of the 5 organosulfur standards individually, single ion monitoring was performed for DBT, TAN, 4,6-DBT, BNTP and 2-DBT on their $[M]^{+\cdot}$ ions at m/z 184.26, 216.32, 212.32, 234.32 and 198.28 respectively.

2.4.5 LC/MS Experiments in Full Scan Mode

LC/MS experiments were performed in the full scan mode on fractions 1 and 2 of the organosulfur standard mixture as well as Fractions 1 and 2 isolated from the crude oil. Mass spectra were obtained at particular elution times corresponding to each of the standards.

2.4.6 LC/MS/MS and MS^3 Experiments in Full Scan Mode

LC/MS/MS experiments were performed on both the standard organosulfur compounds as well as on Fractions 1and 2 of the PASH fraction of the crude oil in order to ascertain characteristic fragmentation patterns. LC/MS^3 experiments were

also performed on some of the more intense peaks present in the PASH fraction. An Isolation width of 1u was employed. The collision energy was gradually increased until both the precursor and product ions could be observed. The collision energy is the voltage introduced in the ion trap which provides kinetic energy to parent ions in order to facilitate their dissociation via colliding with inert gas molecules. The collision energies used are reported with each spectrum in Chapter 3.

2.4.7 Limit of Detection of Organosulfur Standards in the PASH Matrix

A dilute solution of PASH was prepared by mixing 0.6 mg PASH/6 ml of hexane. The Limit of detection (LOD) for each of the organosulfur compounds in this PASH matrix was found by adding the standard solutions in the concentration range of 10^{-6} M to 10^{-4} M.

CHAPTER III

RESULTS AND DISCUSSION

3.1 HPLC of the Organosulfur Standard Mixture Using a LC-NH_2 Column

The amino bonded phase column (LC-NH_2) separates aromatic compounds based on ring size rather than substituents [8]. The organosulfur standard mixture was separated on a LC-NH_2 column according to ring size. (See Fig. 3.1). Through spiking with authentic standards it was found that the first 2 peaks (t_R= 3.28 and 3.52 minutes) correspond to 3 ring compounds and the third peak (t_R= 4.51 min) corresponds to the 4 ring compound (BNTP).

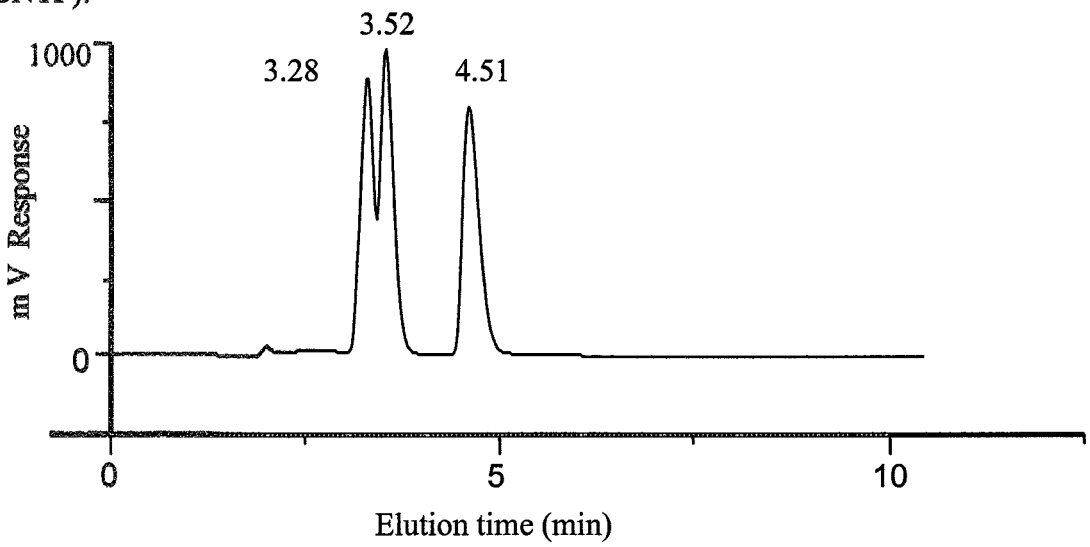


Fig.3.1 HPLC profile for a mixture of organosulfur compounds.

LC-NH_2 column, 250 x 30 mm, dp=5 μm ; Mobile Phase, 100% hexane; Flow Rate, 1ml/min; UV detection at 254 nm.

The first 2 peaks will be designated Fraction 1and the third peak Fraction 2. Fraction 1 was collected between 2.9 and 3.8 minutes and Fraction 2 between 4.1 and 4.8 minutes then blown down. These were reconstituted in hexane:CH_2Cl_2 (70:30) for the next set of HPLC runs.

3.2 HPLC of the Organosulfur Standard Mixture Using a PRP Column

Fraction 1 was sent through a PRP column with hexane:CH_2Cl_2 (70:30) as mobile phase. See Fig. 3.2 below.

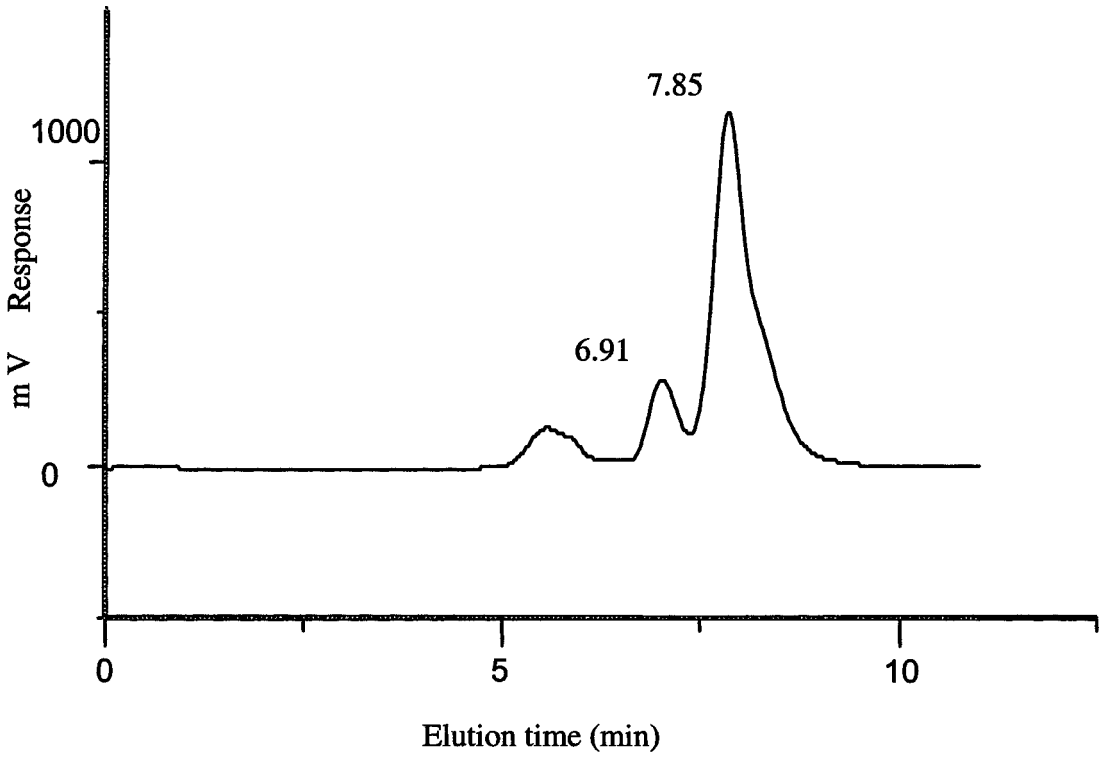


Fig.3.2 HPLC of Fraction 1 collected from the LC-NH_2 column.

PRP column, 150 x 4.6 mm, dp=10 μm; Mobile Phase, hexane:CH_2Cl_2 (70:30); Flow Rate, 0.3ml/min; UV detection at 254 nm.

The second fraction collected from the LC-NH_2 column gave a pronounced peak at 8.91 min on the PRP column which was assumed to be BNTP. See Figure 3.3.

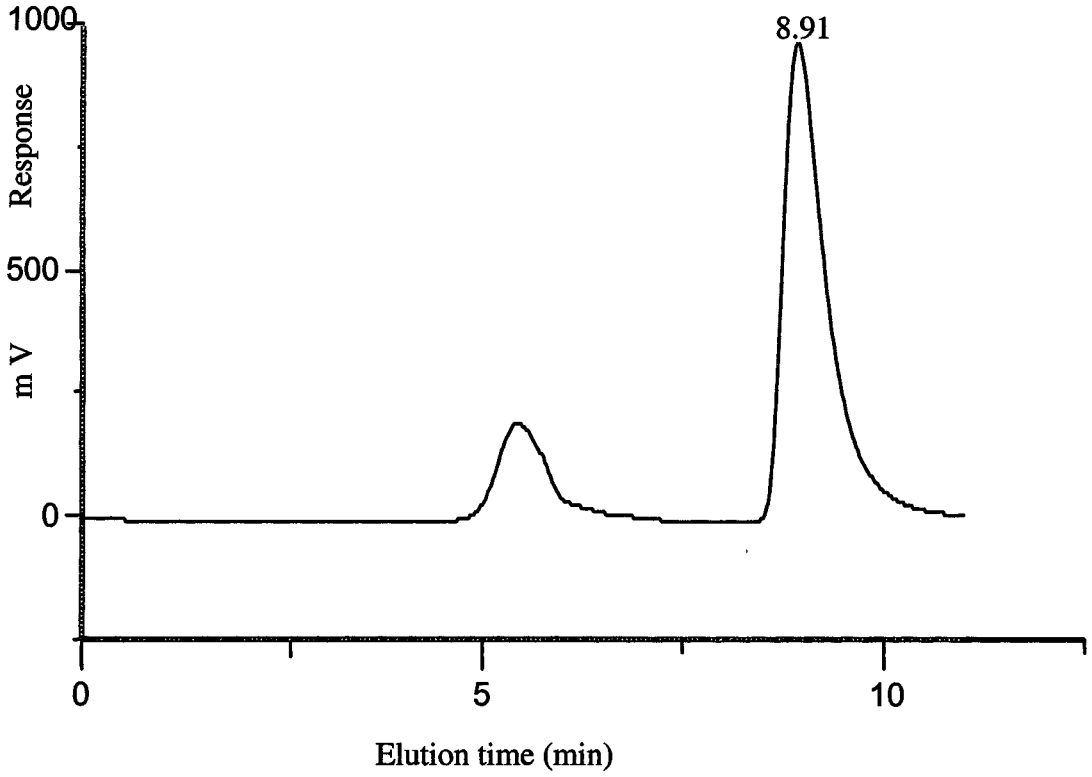


Fig.3.3 HPLC of Fraction 2 collected from the LC-NH_2 column.

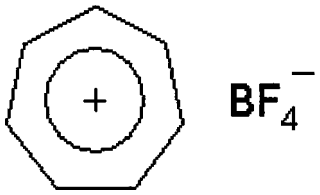
PRP column, 150 x 4.6 mm, dp=10 μm; Mobile Phase, hexane:CH_2Cl_2 (70:30); Flow Rate, 0.3ml/min; UV detection at 254 nm.

The PRP column is made up of a polymer (polystyrene divinyl benzene). The aromatic rings on this packing interact very strongly with the aromatic rings of the organosulfur standard compounds via Π- Π bonding interactions [8]. This allows the use of a strong solvent like dichloromethane for the separation. Dichloromethane is required at this stage, since the LC effluent from the PRP column is sent to the mass spectrometer where radical cations are formed, and dichloromethane plays an important role in the

stabilization of these radical cations. It can also be seen that in Fraction 1, (see Fig 3.2) the three ring standard compounds namely DBT, 2-DBT, 4,6- DBT and TAN are not well resolved. Initial runs with 100% hexane as the mobile phase were also conducted. Though a good separation of the three ring standards was achieved on the PRP column with this mobile phase, it was found that radical cations were not formed in the mass spectrometer and thus this mobile phase composition was unsuitable for the LC/MS experiments.

3.3 HPLC/APCI/MS of 1 mM Tropylium Solution

Fig 3.4 shows the structure of tropylium tetrafluoroborate. Fig 3.5 shows the mass spectrum of 1mM tropylium tetrafluoroborate solution in full scan mode.



F. W. 177.9

Fig 3.4 Structure of Tropylium Tetrafluoroborate.

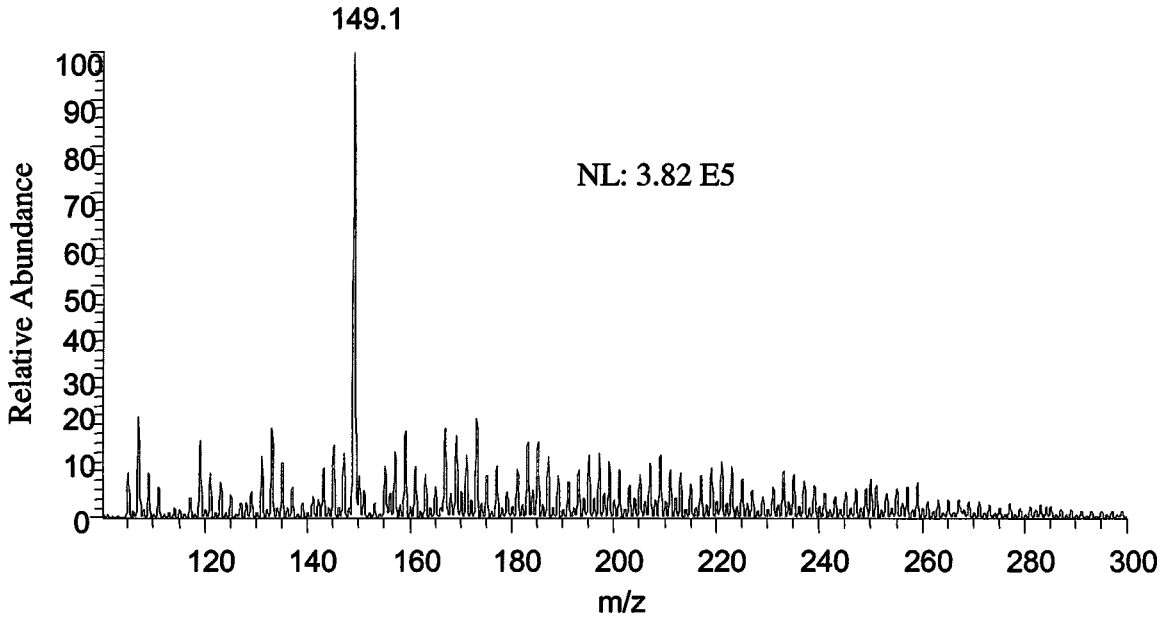


Fig. 3.5 APCI/MS scan of 1 mM tropylium in full scan mode.

PRP column, 150 x 4.6 mm, dp=10 μm ;Mobile Phase, hexane:CH_2Cl_2 (70:30); Flow Rate, 0.3ml/min; 1mM tropylium tetrafluoroborate solution, Flow Rate, 15 μl/min.

HPLC/APCI/MS was done with only the mobile phase from the LC combining with 1 mM tropylium solution at the tee. In Fig 3.5 it can be seen that there is an intense peak at m/z 149 with a signal intensity of 10^5. The identity of the peak is unknown. It could be due to $[Tr+3F+H]^{+\cdot}$.

3.4 HPLC/APCI/MS for Fractions 1& 2 Using Single Ion Monitoring (SIM) Mode

In order to find out the identity as well as the order of elution of the organosulfur standards present in each of the Fractions, experiments were done in SIM mode in the mass spectrometer. Fig 3.6 and 3.7 show the elution profiles for the organosulfur standard compounds present in Fractions 1and 2 respectively.

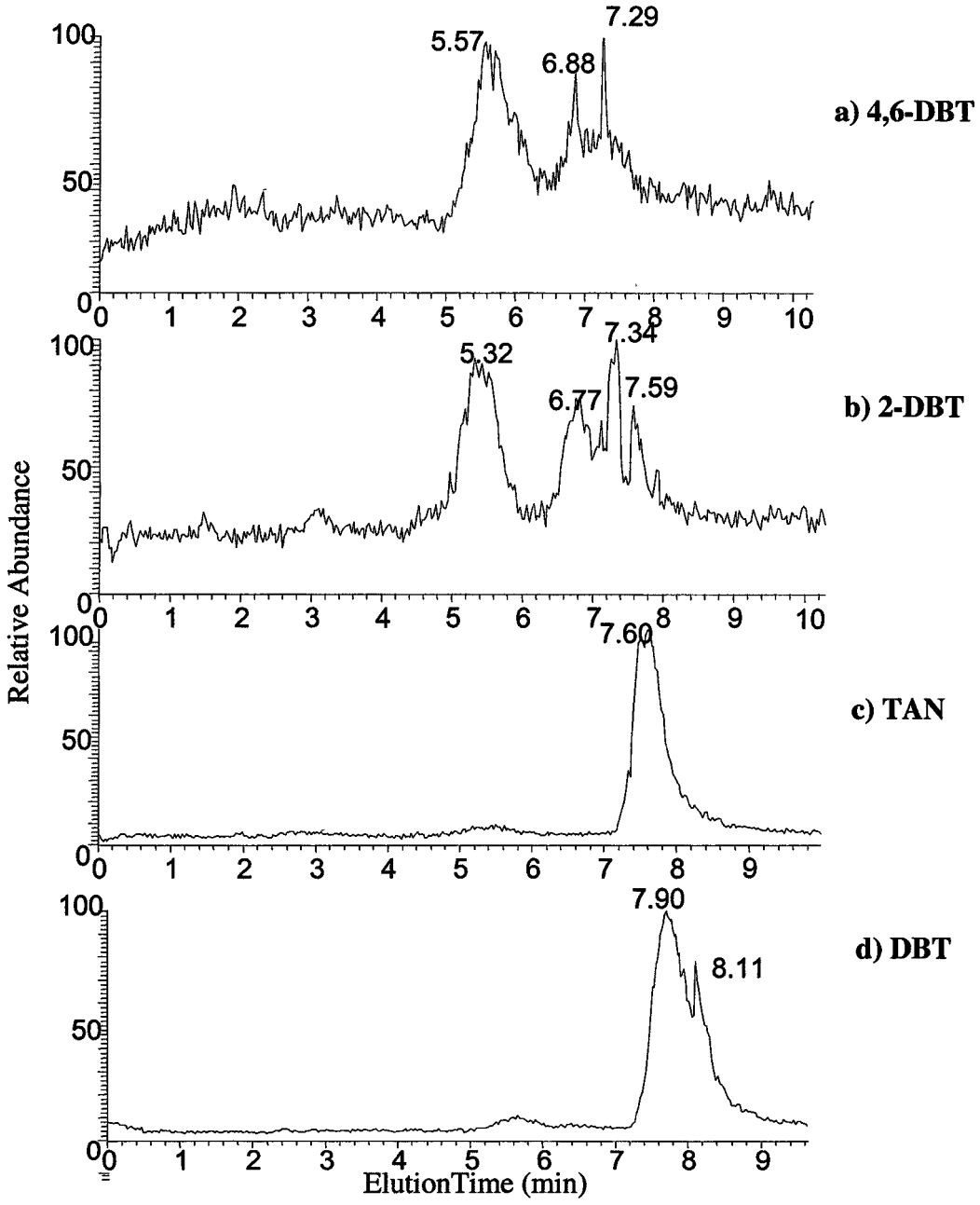


Fig 3.6 Chromatograms of Fraction 1 showing the elution profile for each of the organosulfur standards.

PRP column, 150 x 4.6 mm, dp=10 μm; Mobile Phase, hexane:CH_2Cl_2 (70:30); Flow Rate, 0.3ml/min; post column addition of 1mM tropylium tetrafluoroborate solution, Flow Rate, 15 μl/min, SIM mode, m/z set at a) 212.32 b) 198.28 c) 216.32 d) 184.26

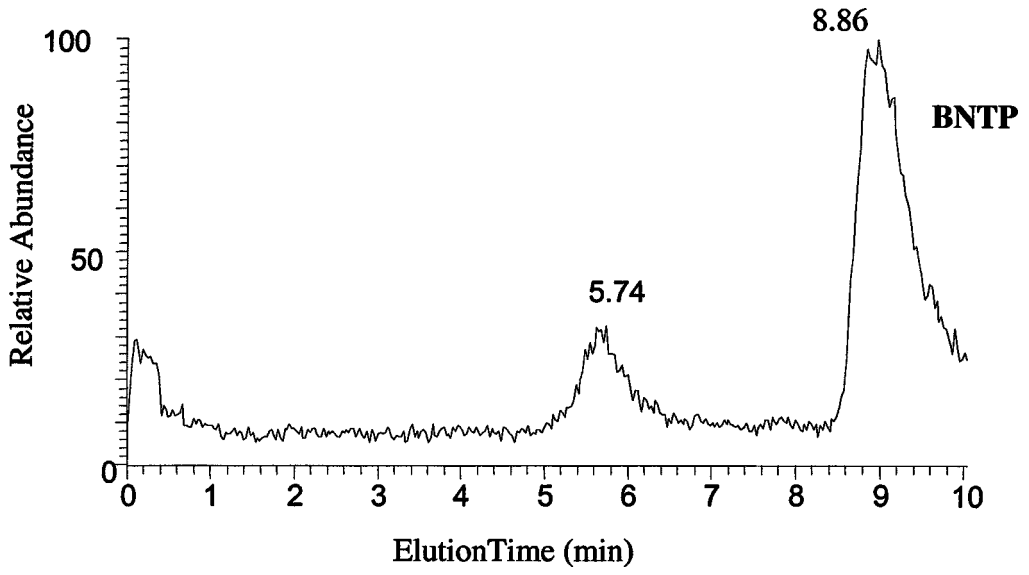


Fig 3.7 Chromatogram of Fraction 2 showing the elution profile for BNTP.

PRP column, 150 x 4.6 mm, dp=10 μm; Mobile Phase, hexane:CH_2Cl_2 (70:30); Flow Rate, 0.3ml/min; post column addition of 1mM tropylium tetrafluoroborate solution, Flow Rate, 15 μl/min, SIM mode, m/z set at 234.32.

From the SIM data it is possible to determine the elution time of TAN (7.6 min) and DBT (about 8.0 min), since there is only a single peak in the SIM chromatogram, but the ion chromatograms of 4,6-DBT, 2-DBTand BNTP are more complicated. There is more than 1 peak for the above standards. In order to get more information about the elution time for these three compounds, HPLC was done wherein the three compounds were added individually to the organosulfur standard mixture. In each case the 10^{-4} M organosulfur standard mixture was mixed with 10^{-4} M organosulfur compound (i.e., 4,6-DBT, 2- DBT and BNTP) in a 1:2 ratio. 20 μl of each of the

above solutions was injected into the PRP column with hexane: CH_2Cl_2 (70:30) as mobile phase at a flow rate of 0.3 ml/min.

3.5 HPLC of 4,6-DBT, 2-DBT and BNTP

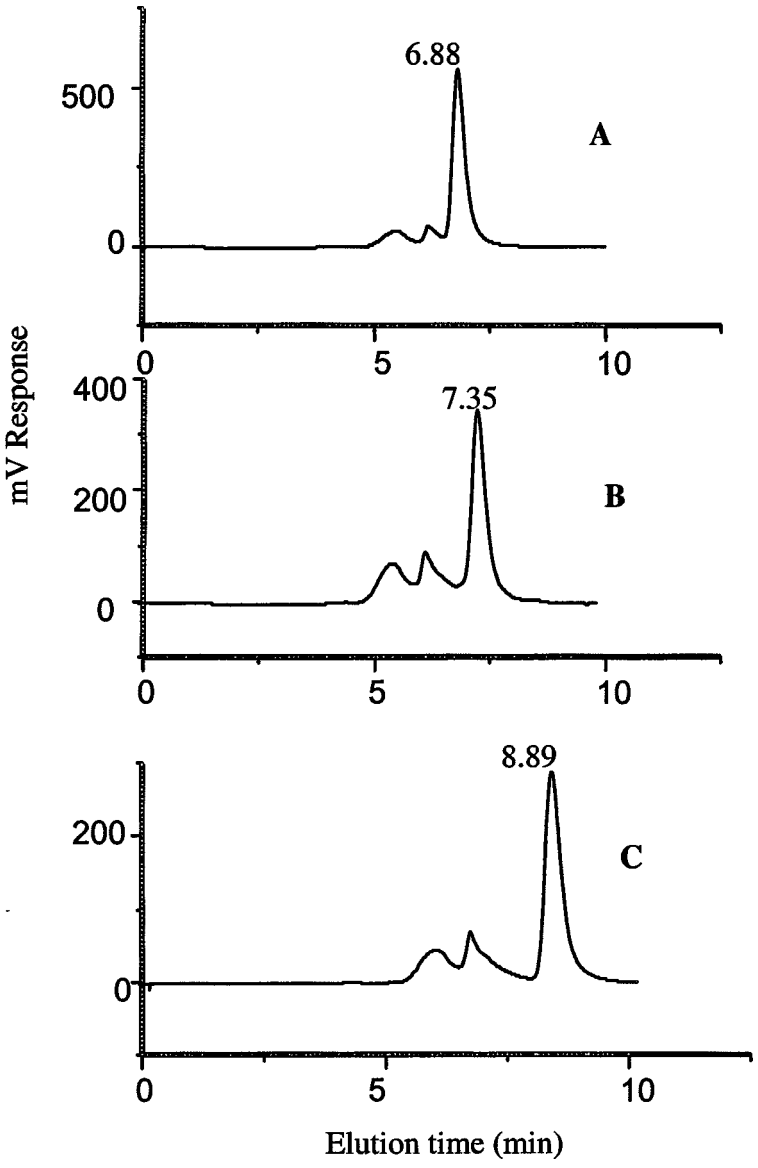


Fig 3.8. Chromatograms for the organosulfur standard mixture after the addition of: A) 4,6-DBT B) 2-DBT and C) BNTP

PRP column, 150 x 4.6 mm, dp=10 μm; Mobile Phase, hexane:CH_2Cl_2 (70:30); Flow Rate, 0.3ml/min; UV detection at 254 nm.

Fig 3.8 shows the chromatograms for the organosulfur standard mixture after the addition of 4,6-DBT, 2-DBT and BNTP. From the above we can infer that 4,6-DBT comes out at 6.88 min followed by 2-DBT at 7.35 min and BNTP at 8.9 min since these show an increase in intensity after the addition of the standard compound.

Summarizing the SIM and the standard addition chromatographic results show that the organosulfur standards elute as follows: 4,6 DBT at 6.88 min, 2-DBT at 7.35 min, TAN at 7.6 min, DBT at 8.0 min and BNTP at 8.9 min. 4,6–DBT comes out first due to the presence of 2 methyl groups in its molecular structure which leads to steric hindrance and therefore a lesser interaction with the PRP column. 2-DBT comes out next as it has one methyl group followed by TAN and DBT with no methyl groups. BNTP has 4 rings and a stronger Π bonding interaction and comes out last.

The PRP column does not appear to sufficiently resolve the three ring compounds to justify the added complexity of using a two dimensional HPLC approach. The amino bonded phase gives 2 peaks for the three ring compounds and the PRP column does not resolve them any further. (Compare Fig 3.1 with Fig 3.2). The PRP column however does have the advantage that it allows the addition of CH_2Cl_2 to the mobile phase which allows better sensitivity in the APCI/MS approach.

3.6 HPLC/APCI/MS for Fractions 1 & 2 in Full Scan Mode

Fig 3.9 shows the MS full scan of the organosulfur standard mixture in the absence of tropylium. It can be seen that in the absence of tropylium there is no appreciable signal intensity for any of the organosulfur standards implying that tropylium is needed for the formation of radical cations of the organosulfur compounds.

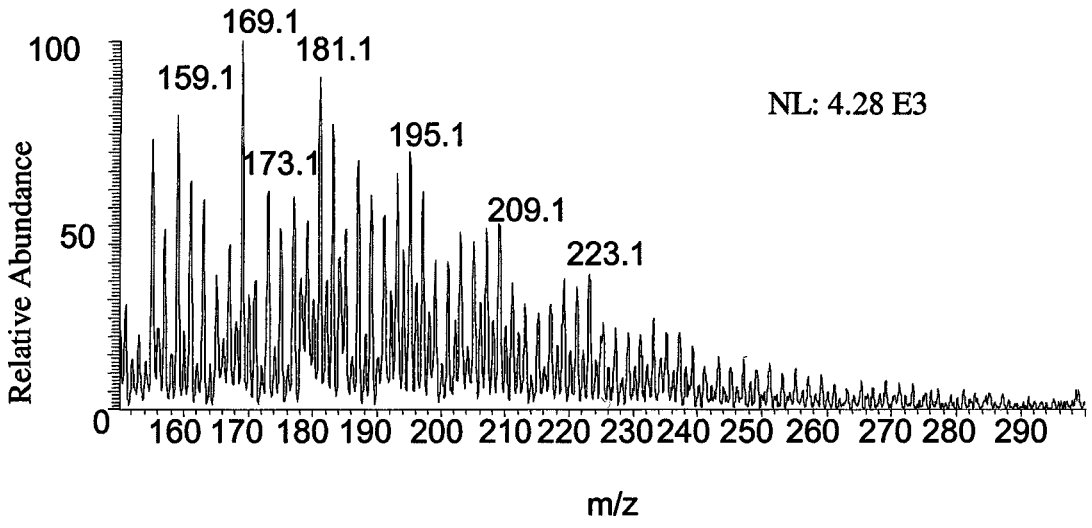


Fig. 3.9 HPLC/APCI/MS scan of organosulfur standard mixture in the absence of tropylium.

PRP column, 150 x 4.6 mm, dp=10 μm; Mobile Phase, hexane:CH_2Cl_2 (70:30); Flow Rate, 0.3ml/min.

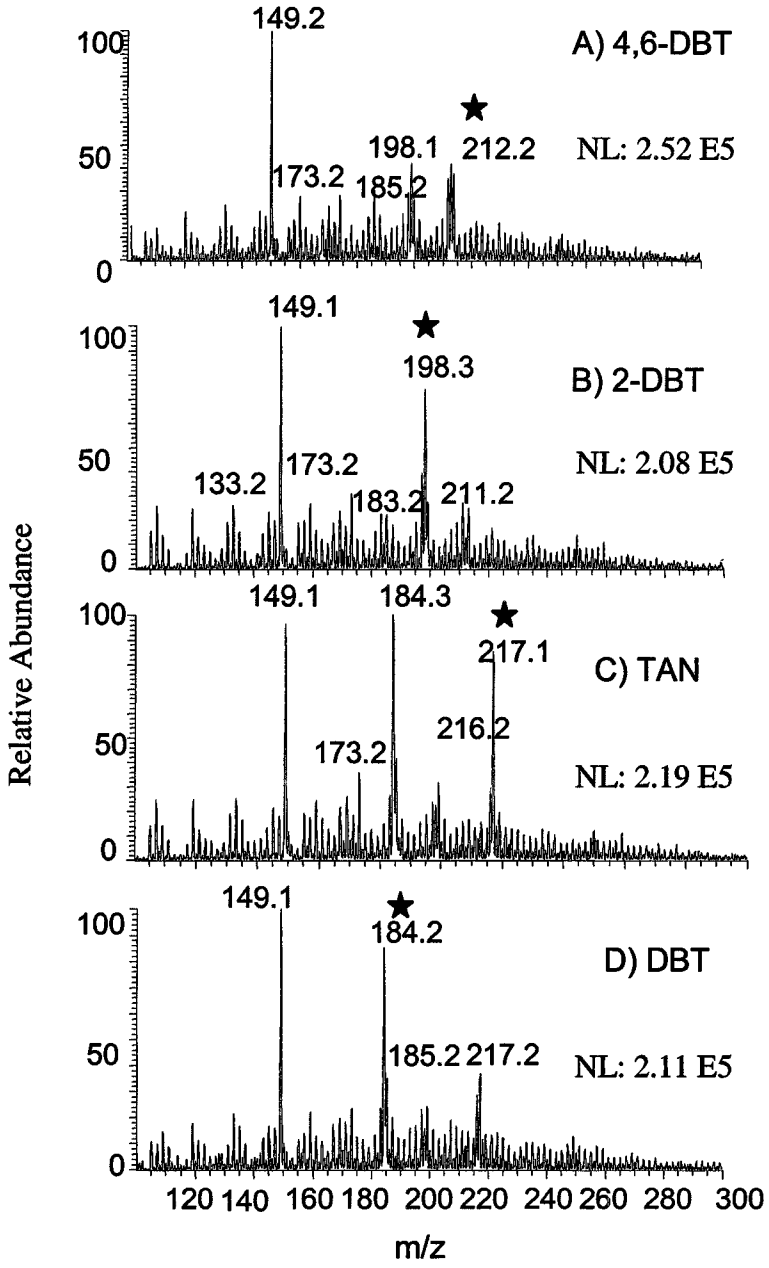


Fig 3.10 HPLC /APCI /MS of Fraction 1.

PRP column, 150 x 4.6 mm, dp=10 μm ; Mobile Phase, hexane:CH_2Cl_2 (70:30); Flow Rate, 0.3ml/min; post column addition of 1mM tropylium tetrafluoroborate solution, FlowRate, 15 μl/min. Full scan mode, A) 6.88 min B) 7.34 min C) 7.60 min D) 8.0 min.

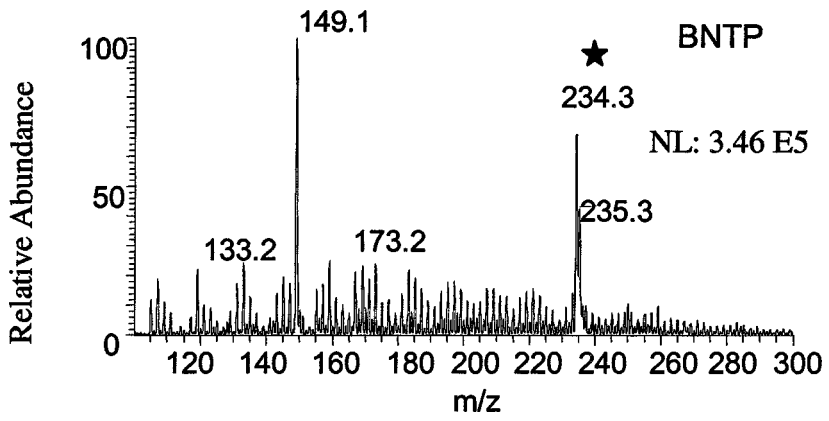


Fig 3.11 HPLC /APCI /MS scan of Fraction 2.

PRP column, 150 x 4.6 mm, dp=10 μm; Mobile Phase, hexane:CH_2Cl_2 (70:30); Flow Rate, 0.3ml/min ; post column addition of 1mM tropylium tetrafluoroborate solution, Flow Rate, 15 μl/min. Full scan mode, 8.9 min.

Fig 3.10 and Fig 3.11 show the MS full scan at 6.88 min, 7.35 min, 7.6 min, 8.0 min and 8.9 min which correspond to the elution times of 4,6 -DBT, 2- DBT, TAN, DBT and BNTP respectively. Each compound showed an intense molecular ion peak at its respective m/z (212.2, 198.3, 216.2, 184.2 and 234.3) with signal intensity at or above 10^5 at a concentration of 10^{-4} in the presence of 1 mM tropylium. A closer look at the spectrum of 4,6 DBT (6.88 min) reveals the presence of a peak at m/z 198.1 and m/z 185.2 which may be the molecular ion peaks of 2-DBT and DBT. Also 2-DBT (7.34 min) shows a peak at m/z 183.2 (which may be the molecular ion peak of DBT) and TAN (7.6 min) shows a peak at m/z 184.3 (which may be the molecular ion peak of DBT). This indicates that the organosulfur compounds may be losing hydrocarbon moieties such as

methyl groups either in the APCI ionization source or in the heated capillary region. In the case of TAN (t_R= 7.60 min) it seems like there is a loss of sulfur resulting in an intense peak at m/z 184.3. Initially it was thought that, since these peaks essentially overlap (see Fig 3.2) that the additional peaks are the result of incomplete separation in the PRP column. To verify the same, an HPLC/APCI/MS experiment was conducted in the full scan mode with an organosulfur standard mixture without 2-DBT. It was found that a molecular ion peak at m/z 198.2 (2-DBT) was still present at 6.88 mins which is the elution time of 4,6-DBT. This confirmed that 4,6-DBT was indeed losing a methyl group to give 2-DBT.

3.7 HPLC/APCI/MS/MS for Organosulfur Standards in Full Scan Mode

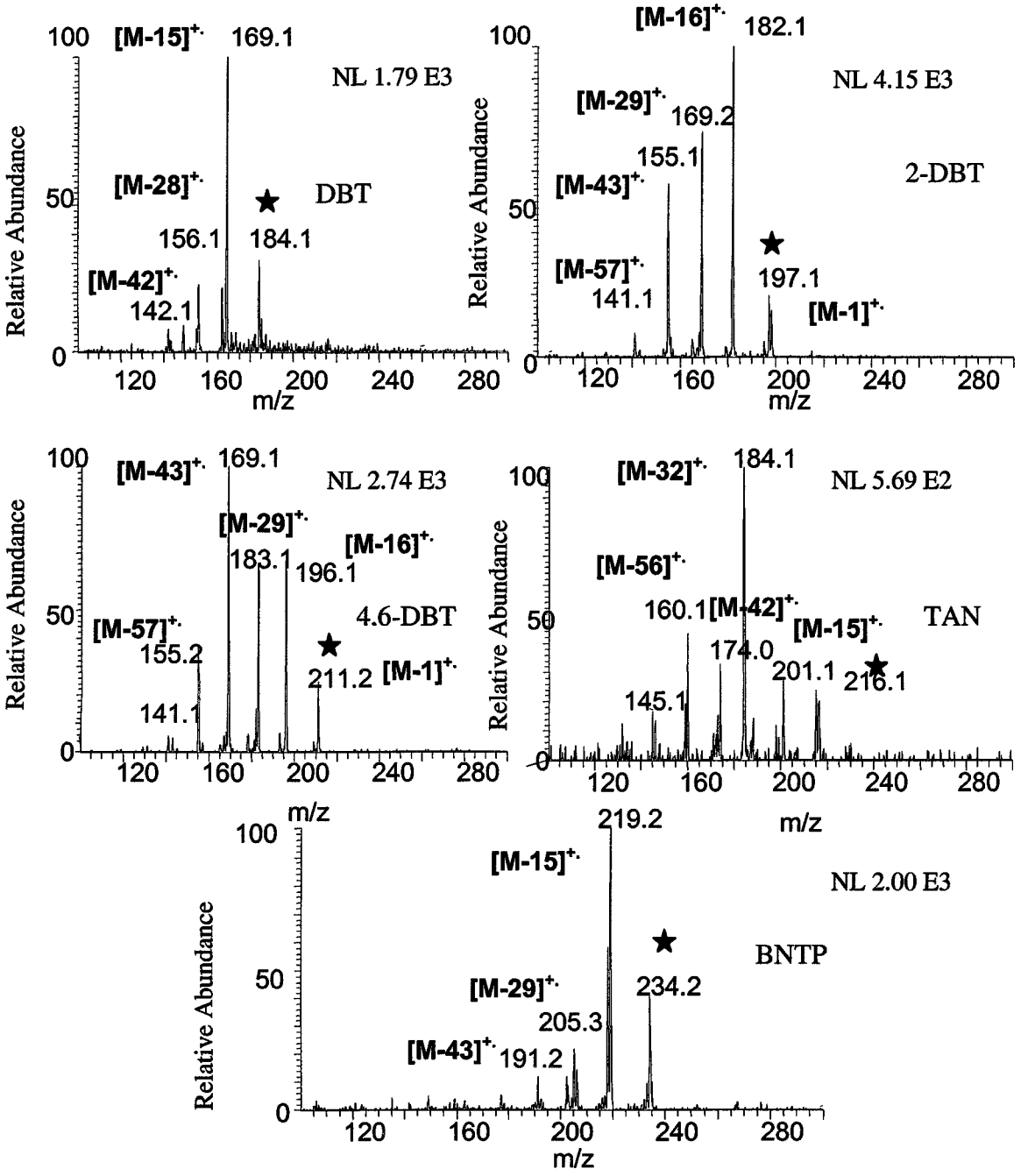


Fig 3.12 HPLC/APCI/MS/MS for organosulfur standards. Mobile Phase, hexane:CH_2Cl_2 (70:30); Flow Rate, 0.3ml/min ; post column addition of 1mM tropylium tetrafluoroborate solution, Flow Rate, 15 μl/min. Collision energies are 38, 41,41, 41, 43% for DBT, 2-DBT, 4,6 DBT, TAN & BNTP respectively.

Fig 3.12 shows the APCI/MS/MS at 6.88 min, 7.35 min, 7.60 min, 8.0 min, 8.9 min which correspond to the elution times of DBT, 2-DBT, 4-6 DBT, TAN, and BNTP. It can be observed from the MS/MS spectra that DBT, TAN and BNTP have parent ions which are stable radical cations, while 2-DBT and 4,6-DBT have parent ions missing one hydrogen. It is suspected that the methyl groups on these two compounds lose one hydrogen during the ionization even before entering the ion trap [27]. 4,6 DBT and 2-DBT have identical losses of 16, 29, 43 and 57 Daltons which could be [M-H-CH_3], [M-CH_2-CH_3], [M-CH_2-CH_2-CH_3] and [M-CH_2-CH_2-CH_2-CH_3] groups. TAN and DBT had identical losses of 15 and 42 which could be[M-CH_3] and [M-H-CH_2-CH_2-CH_3] groups. TAN was the only PASH which had a [M-32] peak which is probably a loss of sulfur atom. BNTP had losses of 15, 29, and 43 which could be [M-CH_3], [M-CH_2-CH_3] and [M-CH_2-CH_2-CH_3] groups. In all cases the signal intensities for the product ions was above 10^3 except for thianthrene which was 10^2. Tandem mass spectrometry studies done on the above organosulfur compounds using an ESI source, CH_3OH:CH_3CN (50:50) and palladium resulted in a neutral loss of 32 Daltons which was a sulfur atom [23]. Whereas in this research which involved HPLC/APCI/MS/MS of organosulfur standards in (70:30) hexane:dichloromethane in the presence of tropylium it was found that removal of a sulfur atom from the organosulfur compound was not possible except in the case of TAN. The ion source or the solvent conditions or both could be important factors for the removal of the sulfur atom.

Thus having established the above HPLC/APCI/MS/MS method which could successfully separate and identify standard organosulfur compounds, experiments were

conducted to see if the above approach might work on the PASH present in an Arabian crude oil.

3.8 Fractionation of the Arabian Crude Oil

3.8.1 Distillation

Table 3.1 shows the results from the distillation of the Arabian Crude Oil:

Table 3.1: Results From the Distillation of Arabian Crude Oil

Fraction	Boiling Point	Recovery (%)
Crude Oil	n.a	99
Light Distillate	< 200 ^{0} C @ 760 torr	27
Middle Distillate	200 - 310 ^{0} C @ 760 torr	27
Heavy Distillate	< 260 ^{0} C @ 20 torr	13
Residue	> 260 ^{0} C @ 20 torr	32

3.8.2 Saturate–Aromatic-Resin–Asphaltene (SARA) Fractionation Method

SARA Fractionation done on the heavy distillate gave the following results. SeeTable 3.2.

Table 3.2: SARA Fractionation of Arabian Crude Oil

	Heavy distillate (%)
Saturates	25
Aromatics	15
Resins	9

The asphaltenes were not recovered.

3.8.3 LEC of the Aromatic Fraction

The results from the LEC of the Aromatic Fraction are shown below in Table 3.3.

Table 3.3: LEC of the Aromatic Fraction

	Aromatic %
PAH	> 100
PASH	14
SPAC	>100

Both the PAH and SPAC fractions obtained after LEC were greater than a 100%. The PAH fraction was the first to be eluted using hexane: chloroform (50:50), so any excess $PdCl_2$ may have come off from the column alongwith it. The SPAC fraction was eluted

last using chloroform:diethylether (90:10), and diethylether being a polar solvent may have resulted in elution of the $PdCl_2$ [15].

3.8.4 SPE of PASH

1 mg of PASH when further cleaned up using SPE yielded 0.7 mg of PASH implying a 30% loss.

Fig 3.13 below shows the fractionation scheme for the Arabian crude oil with the results.

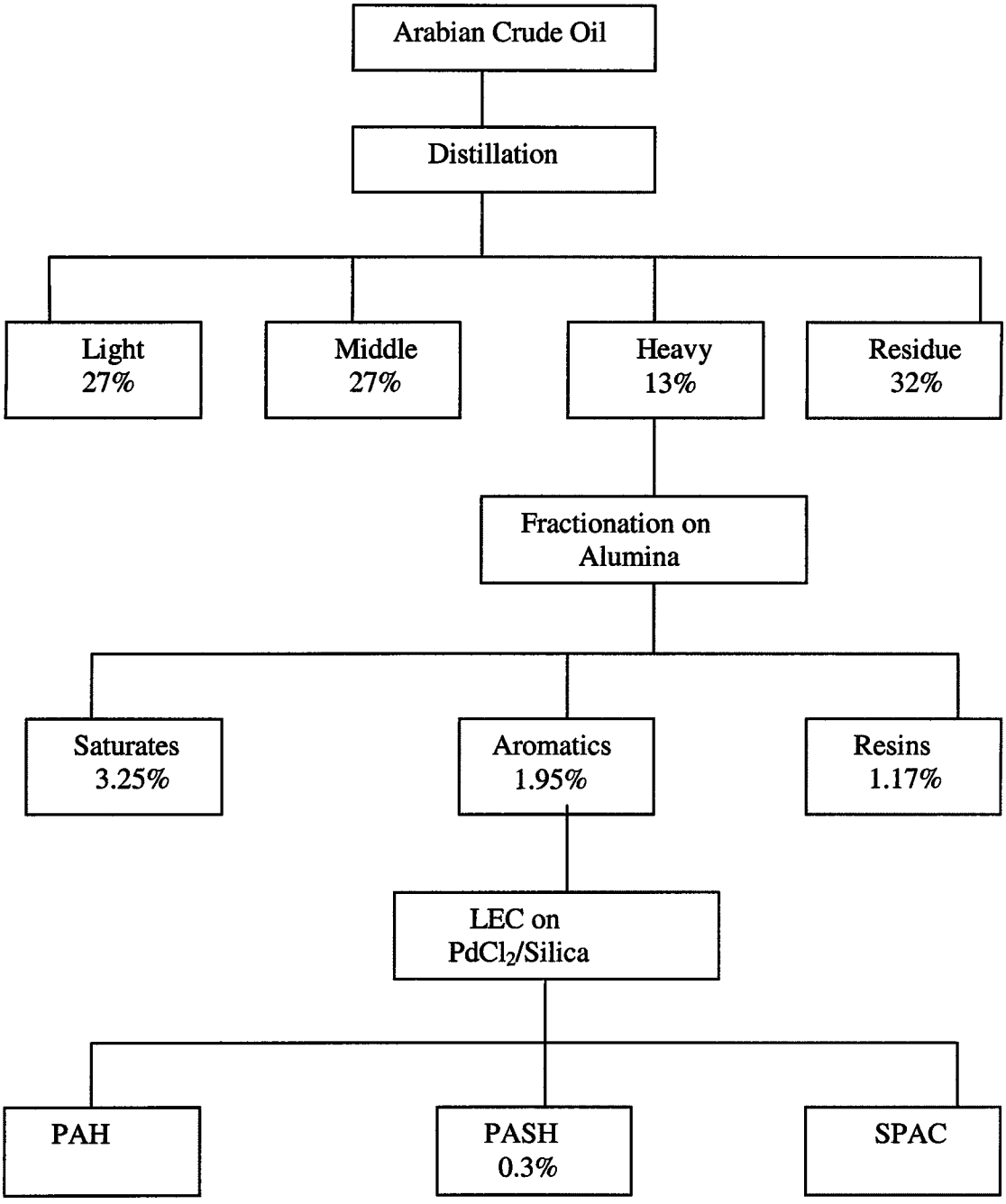


Fig 3.13 Results for the fractionation of the Arabian Crude Oil.

3.9 HPLC of the PASH Fraction of an Arabian Crude Oil Using the LC-NH_2 Column

The PASH fraction isolated from the Arabian Crude Oil (fractionation procedure described in Section 2.3) was separated on the LC-NH_2 column. Fig 3.14 shows the chromatographic separation.

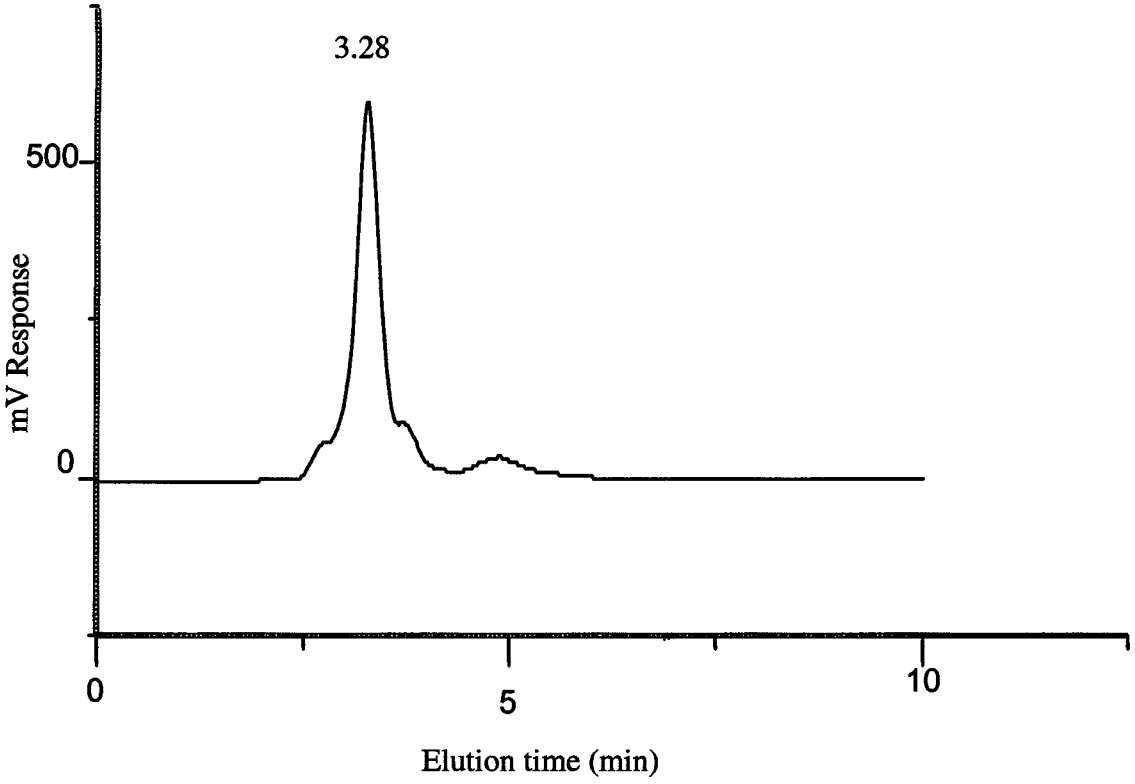


Fig.3.14 HPLC profile for PASH fraction from Arabian Crude Oil.

LC-NH_2 column, 250 x 30 mm, dp=5 μm; Mobile Phase, 100% hexane; Flow Rate, 1ml/min; UV detection at 254 nm.

From the chromatogram it can be observed that the first peak comes out at 3.28 min which is identical to the retention time of the first peak in the LC-NH_2 separation of the

standards (See page 22 , Fig 3.1) and the second peak is around 4.7-4.8 min which is also close to the retention time of the second peak in the standards chromatogram (4.50 min) suggesting that the PASH fraction contains mostly 3 ring but some 4 ring compounds. The percentages of the 3 ring and 4 ring compounds when calculated were found to be 95 % and 5 % respectively.

3.10 HPLC of PASH Fractions 1 and 2 Using Polymer PRP Column

The PASH Fraction 1 collected from the LC-NH_2 column when sent through a PRP column gave the chromatogram shown below.

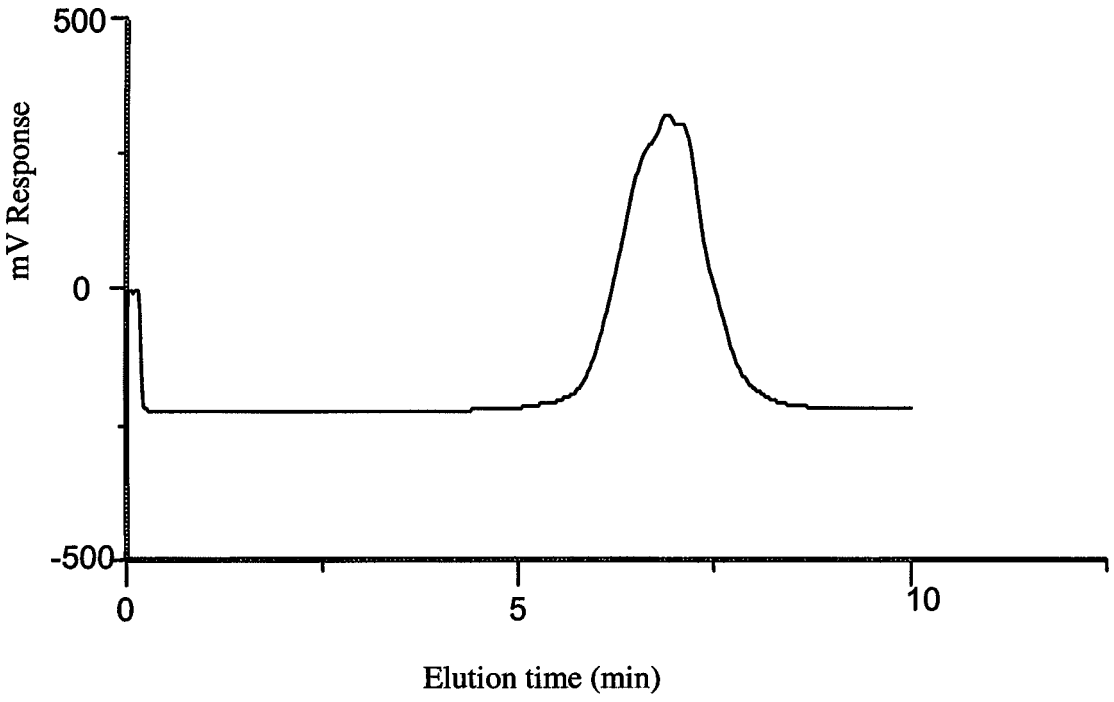


Fig. 3.15 HPLC of PASH Fraction 1 collected from LC-NH_2 column.

PRP column, 150 x 4.6 mm, dp=10 μm ; Mobile Phase, hexane:CH_2Cl_2 (70:30); Flow Rate, 0.3ml/min; UV detection at 254 nm.

The PASH Fraction 2 collected from the LC-NH_2 column when sent through the PRP column gave a peak between 5.1 and 8.6 min. The absorbance is very low which shows a reduced concentration of these higher ring species in this Fraction. See Fig 3.16.

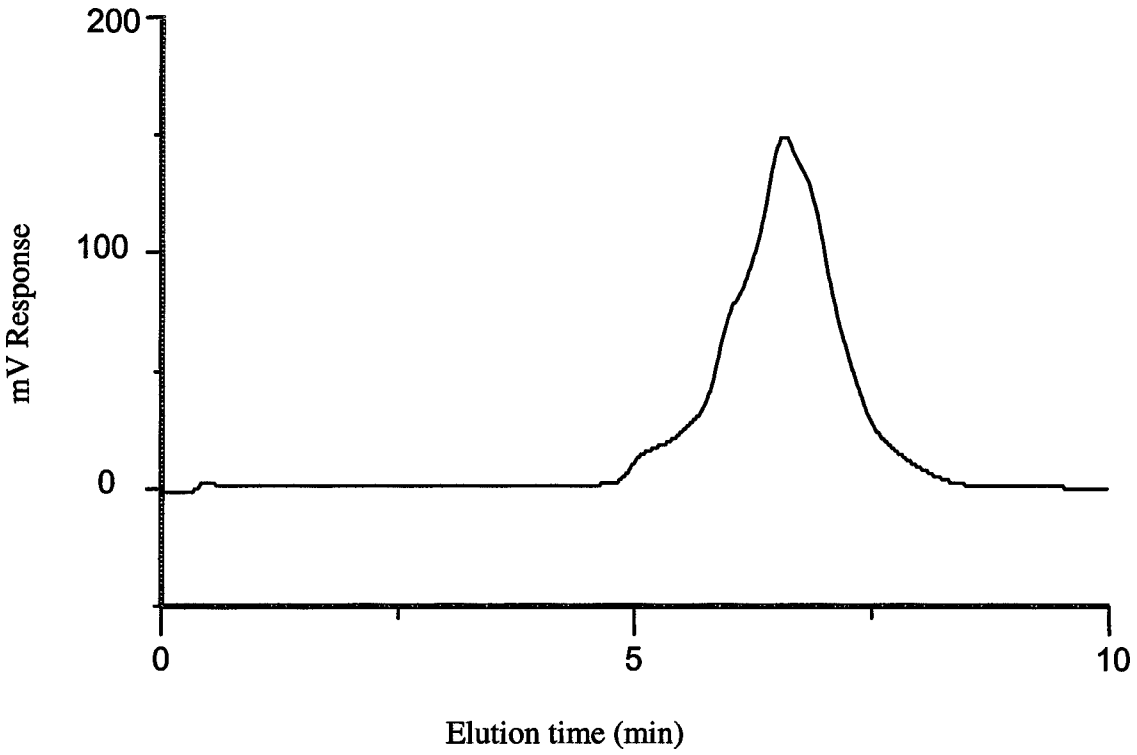


Fig. 3.16 HPLC of PASH Fraction 2 collected from LC-NH_2 column.

PRP column, 150 x 4.6 mm, dp=10 μm; Mobile Phase, hexane:CH_2Cl_2 (70:30); Flow Rate, 0.3ml/min; UV detection at 254 nm.

Fig. 3.15 and 3.16 show a broad envelope between 5.0 and 9.0 mins. This time interval is within the range of retention time for the 3 and 4 ring organosulfur standards. (See page 23 and 24, Fig 3.2 and 3.3). There are no individual peaks. This was expected as the oil is a complex mixture, and under the experimental conditions, individual components cannot be resolved.

3.11 HPLC/APCI/MS for PASH Fraction 1

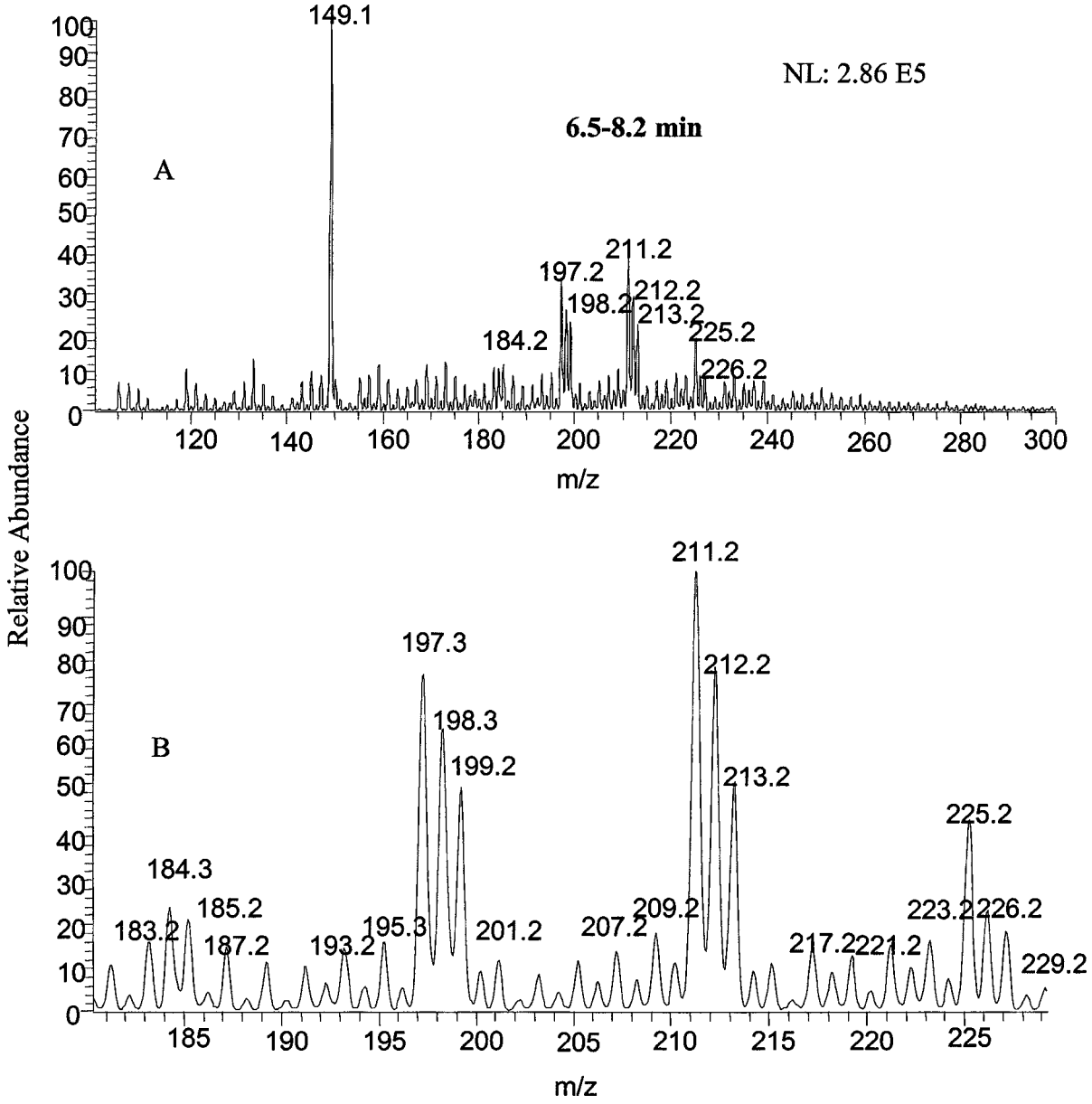


Fig. 3.17 HPLC/APCI/MS scan of PASH Fraction 1 between 6.5 – 8.2 min.

PRP column, 150 x 4.6 mm, dp=10 μm; Mobile Phase, hexane:CH_2Cl_2 (70:30); Flow Rate, 0.3ml/min; post column addition of 1mM tropylium tetrafluoroborate solution, Flow Rate, 15 μl/min. Full scan mode, Mass range: (A) 100-300 Da; (B) 180-230 Da.

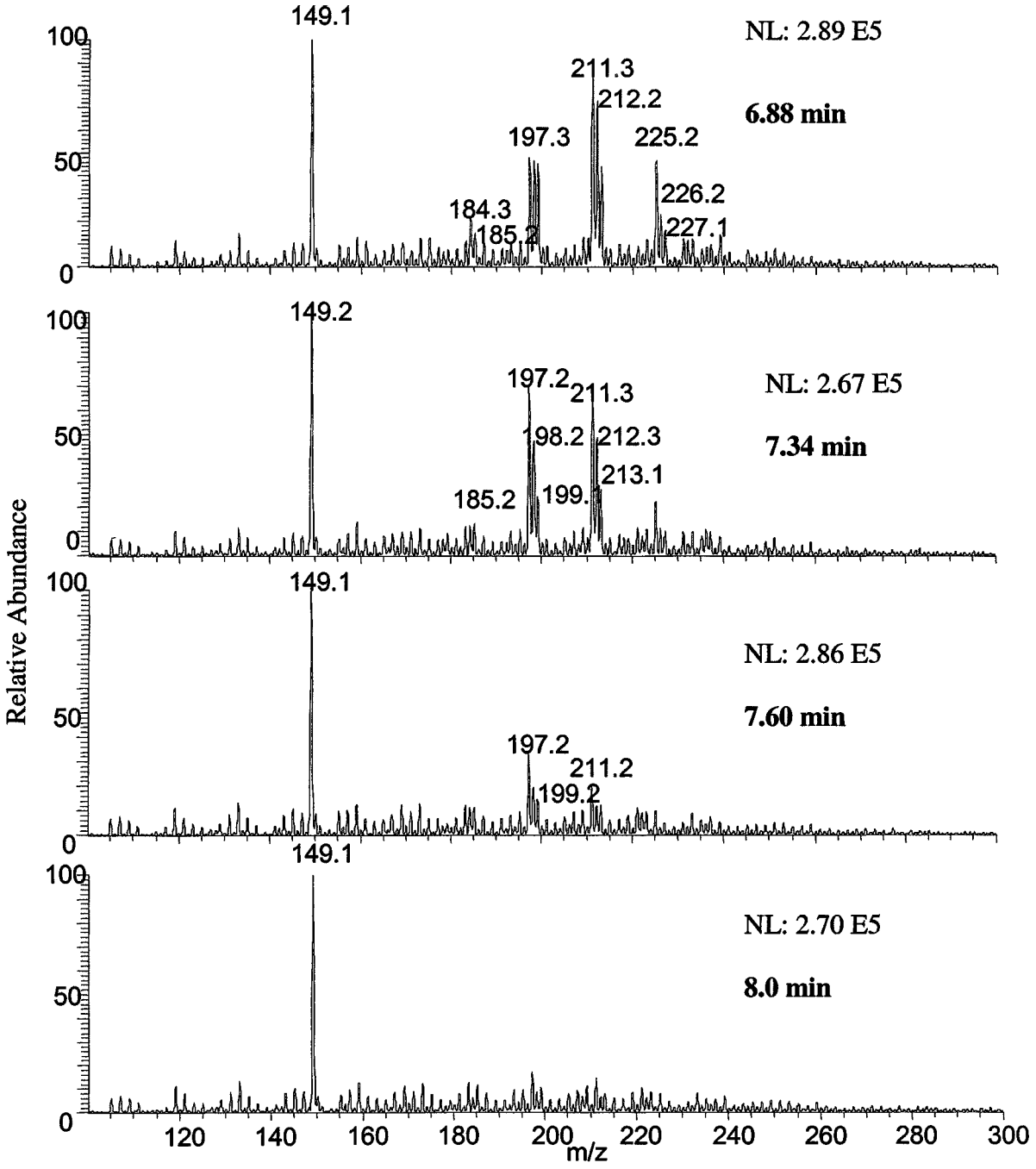


Fig. 3.18 HPLC/APCI/MS scans of PASH Fraction 1.

PRP column, 150 x 4.6 mm, dp=10 μm; Mobile Phase, hexane:CH_2Cl_2 (70:30); Flow Rate, 0.3ml/min; post column addition of 1mM tropylium tetrafluoroborate solution, Flow Rate, 15 μl/min. Full scan mode $\mathbf{t_R}$ (A) 6.88 min (B) 7.34 min (C) 7.60 min (D) 8.0min.

HPLC/APCI/MS of PASH Fraction 1 gave the mass spectra in Fig 3.17 and Fig 3.18. It can be seen in Fig 3.17 (A) that molecular ions at m/z 184.2, 197.2, 211.2, 225.2 are present. The first three molecular ion peaks correspond to DBT, 2-DBT and 4,6- DBT whereas the identity of the peak at 225.2 is not known. A closer look at Fig 3.17 (B) shows that the four molecular ions have a characteristic pattern with $[M-1]^{+\cdot}$, $[M]^{+\cdot}$, $[M+1]^{+\cdot}$ molecular ions formed in each case. In addition, there is a difference of 14 Da between 197.2, 211.2 and 225.2 which may be attributed to a CH_2 group. This means that 225.2 is probably a DBT with 3 methyl groups. The large peak at 149 is tropylium cation associated with another molecule.

Fig 3.18 shows the full-scan MS of PASH Fraction 1 at 6.88, 7.34, 7.60 and 8.0 min respectively. It can be seen that at 6.88 min which is the elution time of 4,6 DBT molecular ions with m/z 211.3, 197.3, 225.2 and 184.3 form with signal intensity of 10^5. 211.3 confirms the presence of 4,6-DBT. 197.3 and 184.3 are the molecular ions of 2-DBT and DBT respectively (maybe due to dissociation of 4,6-DBT) and 225.2 is DBT with 3 methyl groups. At 7.34 min which is the elution time of 2-DBT molecular ions with m/z 211.3 and 197.2 form. 197.2 confirms the presence of 2-DBT. At 7.6 and 8.0 min which are the elution times of TAN and DBT respectively there are no peaks corresponding to the above compounds. This shows the absence of these 2 compounds in this fraction of the oil.

3.12 HPLC/APCI/MS for PASH Fraction 2.

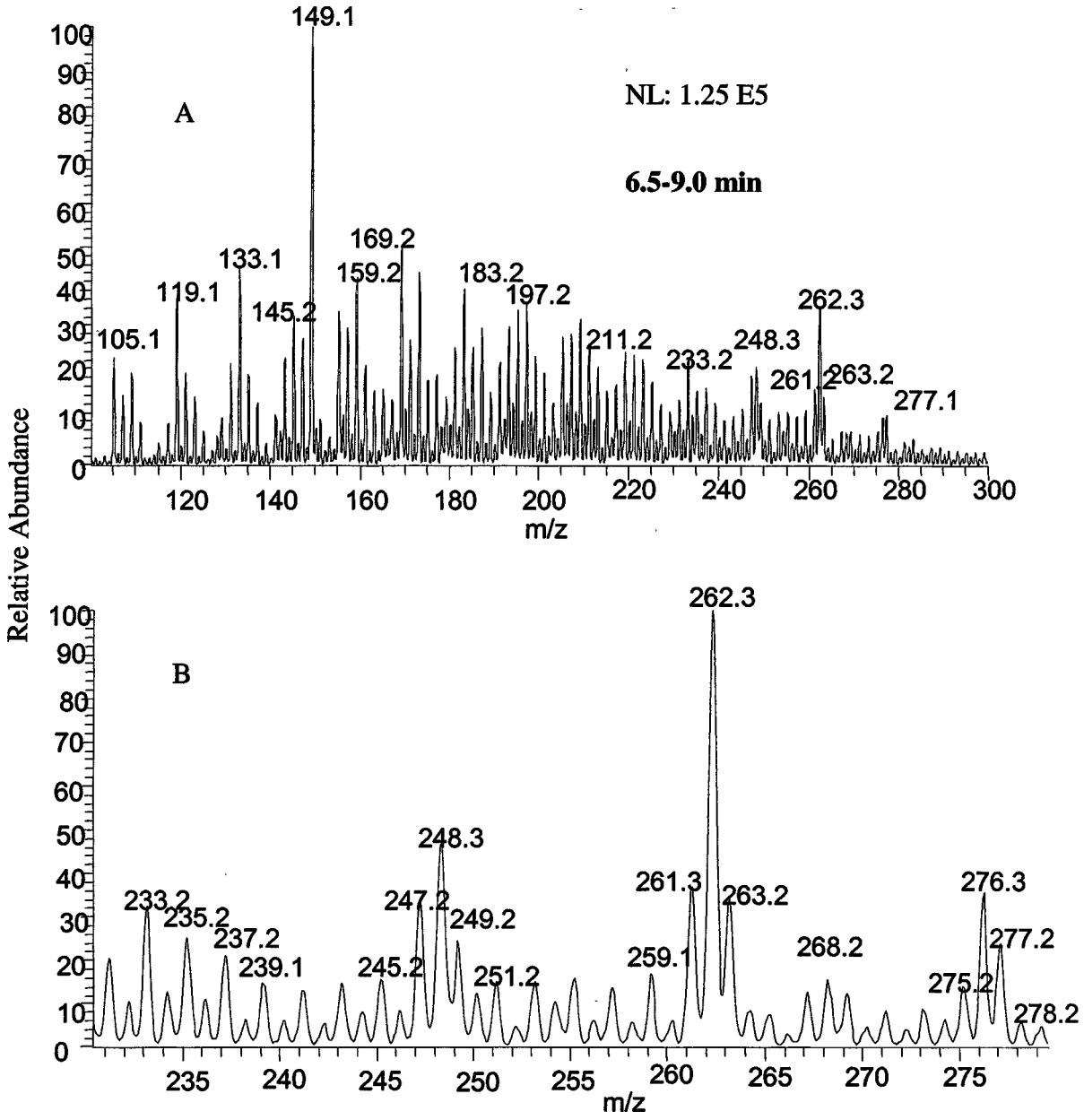


Fig. 3.19 HPLC/APCI/MS scan of PASH Fraction 2 between 6.5–9min.

PRP column, 150 x 4.6 mm, dp=10 μm; Mobile Phase, hexane:CH_2Cl_2 (70:30); Flow Rate, 0.3ml/min; post column addition of 1mM tropylium tetrafluoroborate solution, Flow Rate, 15 μl/min. Full scan mode, Mass range: (A) 100-300 Da; (B) 230-280 Da.

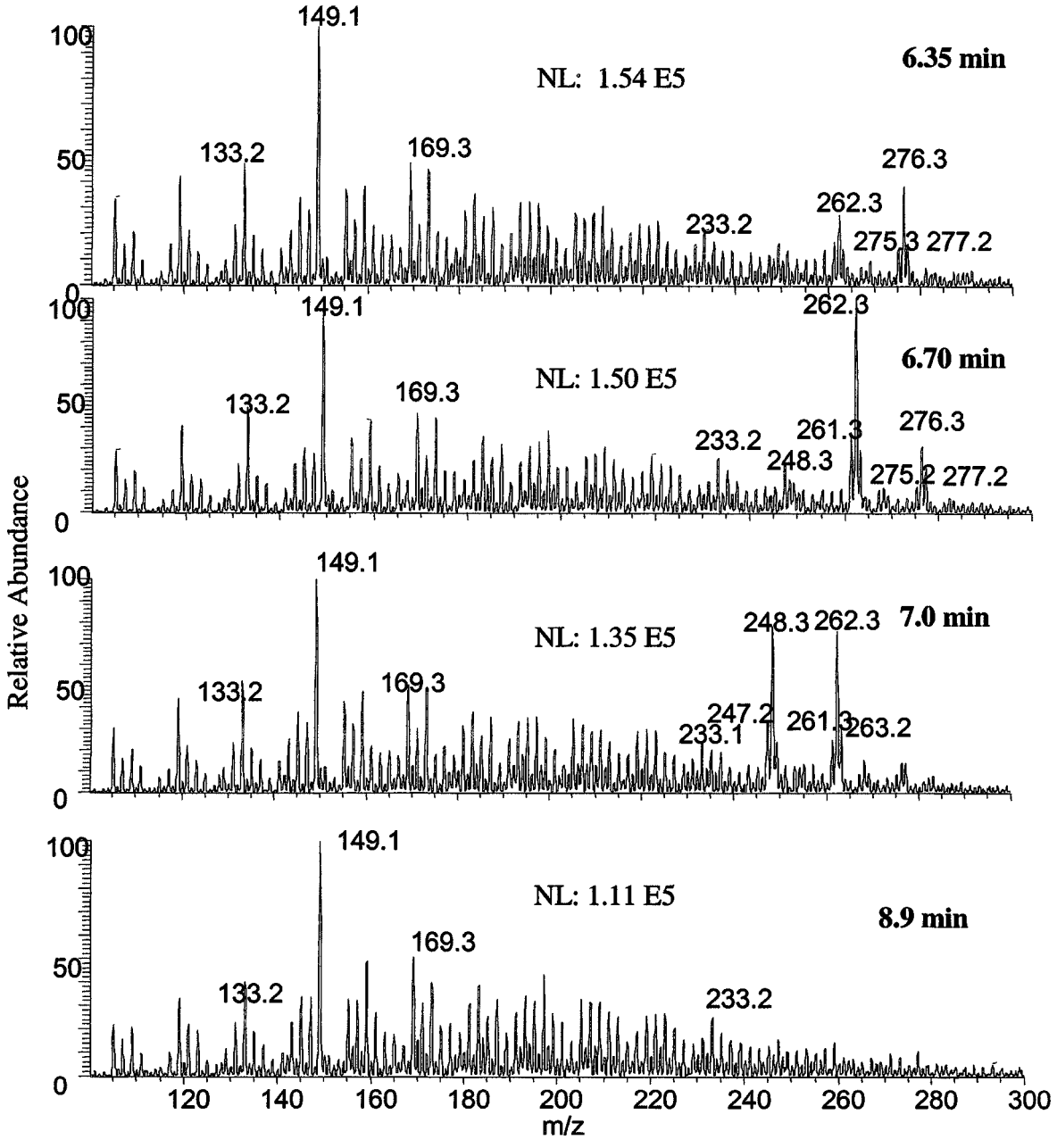


Fig. 3.20 HPLC/APCI/MS scans of PASH Fraction 2.

PRP column, 150 x 4.6 mm, dp=10 μm; Mobile Phase, hexane:CH_2Cl_2 (70:30); Flow Rate, 0.3ml/min; post column addition of 1mM tropylium tetrafluoroborate solution, Flow Rate, 15 μl/min. Full scan mode t_R (A) 6.35 min (B) 6.70 min (C) 7.00 min (D) 8.9 min.

Fig 3.19 (A) shows the mass spectrum of PASH Fraction 2 between 6.5- 9.0 min. It can be seen that there are many peaks in addition to 149 but at low intensity which suggests that the concentration of the 4 ring compounds in this fraction is low as verified in the HPLC separation. A closer look at the spectrum shows peaks separated by 14 mass units. Even though these groupings appear to be peaks, they are not associated with any retention time and hence do not correspond to anything. From Fig 3.19 (B) which is the mass spectrum of PASH Fraction 2 between 230-280 Da it can be seen that Fraction 2 of the oil has molecular ion peaks at m/z 233.2, 248.3, 262.3 and 276.3. The peak at 233.2 corresponds to BNTP. The intensity of the peak is very small and this can also be seen in Fig 3.20 D. At 8.9 min which is the elution time of BNTP the peak for 233.2 is hardly above the noise level. This suggests that BNTP is present at a very low concentration in this fraction of the oil. At 6.35, 6.70 and 7.0 min you have molecular ions with m/z 276.3, 262.3 and 248.3 respectively. They have the same characteristic pattern as Fraction 1 with $[M-1]^{+\cdot}$, $[M]^{+\cdot}$, $[M+1]^{+\cdot}$ ions formed in each case which could mean they are PASH compounds. In fact, they are very likely to be derivatives of BNTP (m/z 234.32) as there is a constant difference of 14 Da between 234.32, 248.3, 262.3 and 276.3 which may be a CH_2 group. Also, the mass spectrum at 6.35 min contains the peak at 276, the mass spectrum at 6.70 min contains the peak at 262.3 and the mass spectrum at 7.0 min contains the peak at 248, these are very likely methylated BNTP compounds, since the more alkylated the PASH, the earlier the elution time.

3.13 HPLC/APCI/MS/MS for PASH in the Full Scan Mode

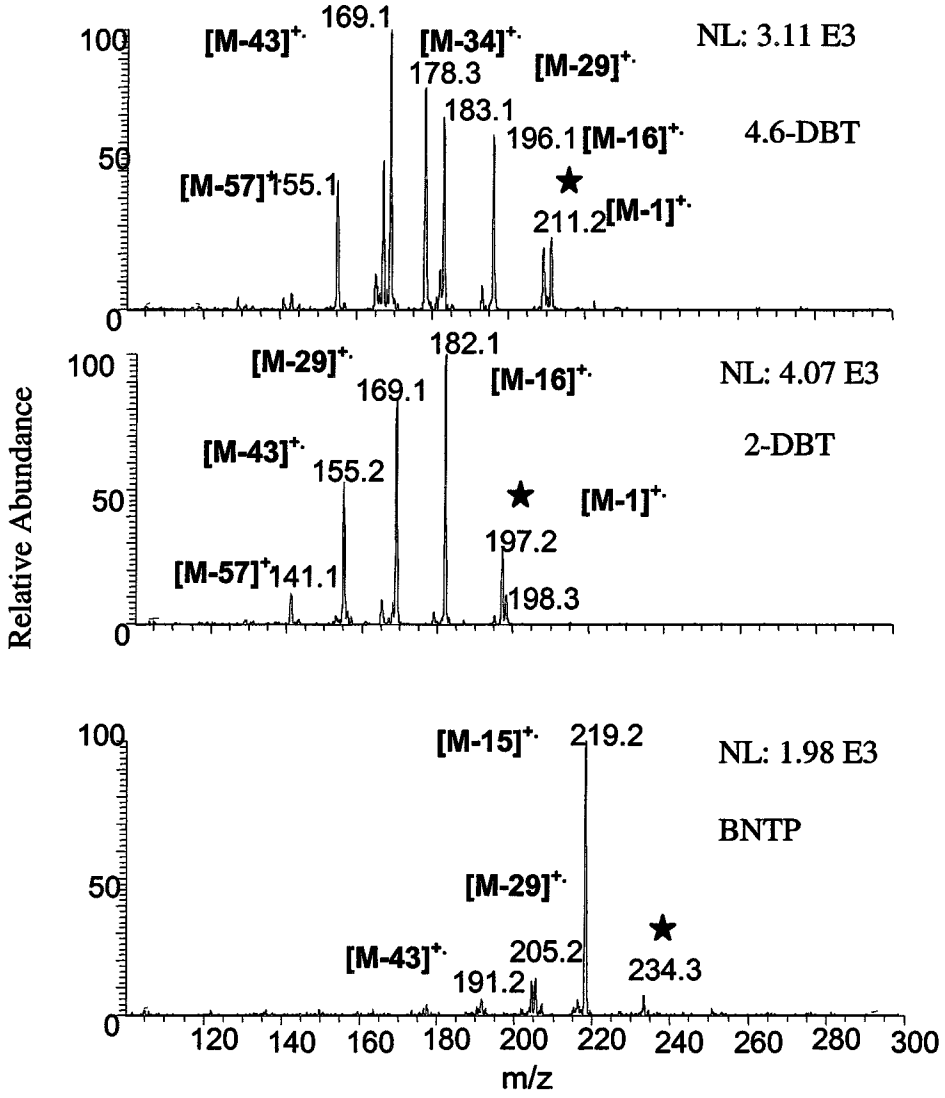


Fig 3.21 HPLC/APCI/MS/MS for PASH Fraction.

Mobile Phase, hexane:CH_2Cl_2 (70:30); Flow Rate, 0.3ml/min; post column addition of 1mM tropylium tetrafluoroborate solution, Flow Rate, 15 μl/min. Collision energies are 41,41,43 % respectively. $\mathbf{t_R}$ (A) 6.88 min (B) 7.34 min (C) 8.9 min.

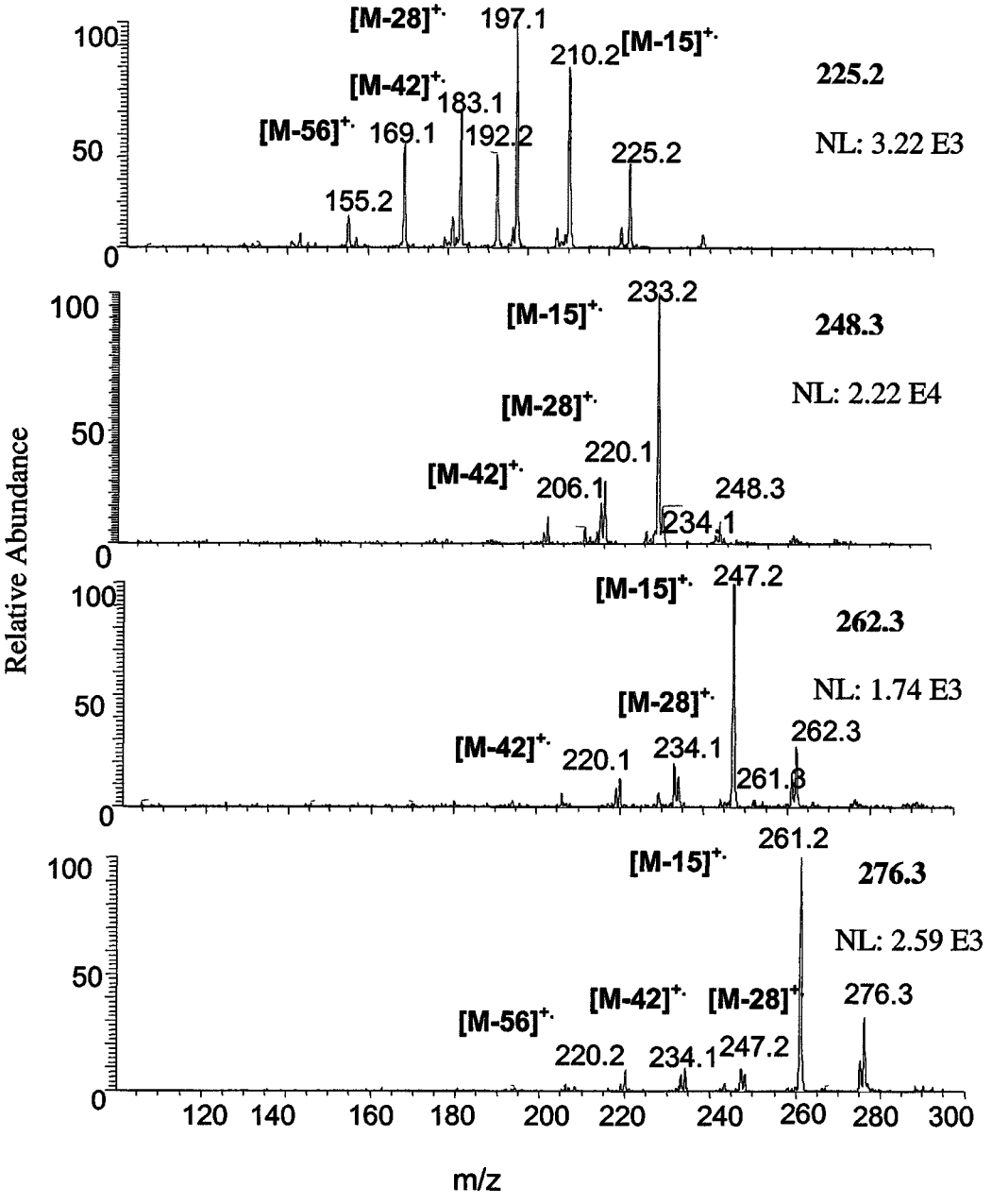


Fig 3.22 HPLC/APCI/MS/MS for PASH Fraction.

Mobile Phase, hexane:CH_2Cl_2 (70:30); Flow Rate, 0.3ml/min ; post column addition of 1mM tropylium tetrafluoroborate solution, Flow Rate, 15 μl/min. Collision energies are 41, 43, 45 and 44 %. m/z (A) 225.2 (B) 248.3 (C) 262.3 (D) 276.3.

Some of the peaks in the PASH fraction were subjected to tandem mass spectrometry experiments. Fig 3.21 shows the fragmentation patterns of three PASH compounds corresponding to the t_R of 4,6-DBT, 2-DBT and BNTP. It can be observed that they are very similar to that of the standards. (See page 36). Thus, we can conclude that the peaks with m/z 211.2, 197.2 and 233.2 are 4,6-DBT, 2-DBT and BNTP respectively.

Fig 3.22 shows the MS/MS scans of the peaks at 225.2, 262.3, 248.3 and 276.3. It can be seen that they have fragmentation patterns identical to that of the organosulfur standards.Thus the above MS/MS data confirm that the molecular ion peaks at 225.2, 262.2, 248.3 and 276.3 are definitely PASH compounds. To strengthen the above deduction MS^3 was conducted on these peaks. Fig 3.23 shows the MS^3 spectra for the peaks at 225.2, 262.2, 248.3 and 276.3.

For MS^3 experiments particular product ions from the MS^2 were chosen and further fragmented. For 225.2, since it was thought to be a derivative of DBT the peak at m/z 183.1 was chosen and fragmented. It can be seen that the fragmentation pattern is identical to that of the standard (see page 36) confirming that 225.2 is a methylated DBT. Similarly for 248.3, 262.3 and 276.3 since they were thought to be derivatives of BNTP the peaks at m/z 233.1 and 234.1 were chosen and fragmented. It can be seen that these also have the same fragmentation pattern as the standard BNTP (see page 36) implying that these are alkylated BNTPs. Thus the MS^3 data further confirms that these newly identified peaks are PASH compounds.

3.14 HPLC/APCI /MS^3 for PASH Fraction

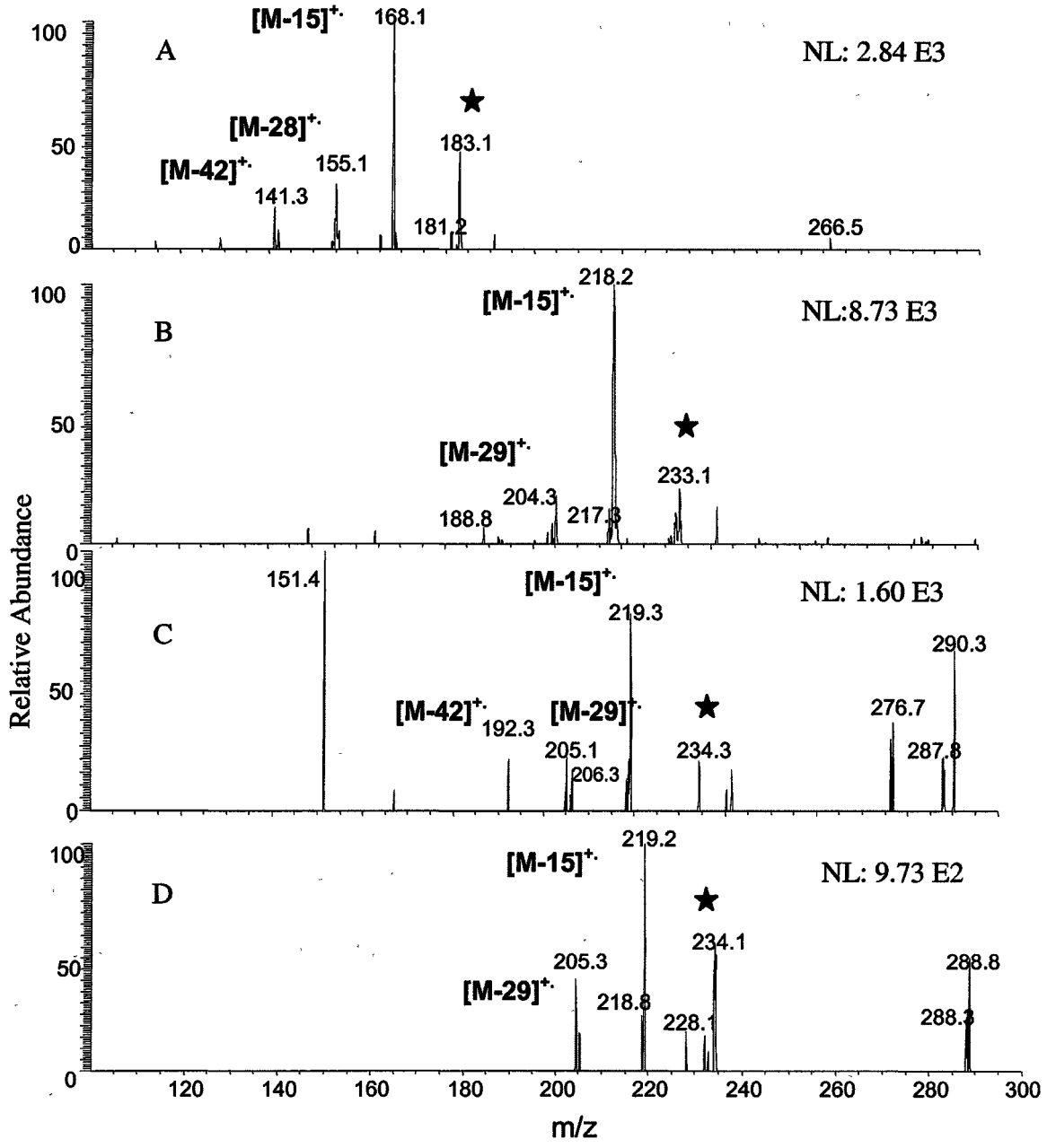


Fig 3.23 HPLC/APCI/MS^3 for PASH Fraction.

Mobile Phase, hexane:CH_2Cl_2 (70:30); Flow Rate, 0.3ml/min ; post column addition of 1mM tropylium tetrafluoroborate solution, Flow Rate, 15 μl/min. Collision energies are 32, 38, 29 and 34% respectively. m/z (A) 225.2 (B) 248.3 (C) 262.3 (D) 276.3.

3.15 Limit of Detection (LOD) of OrganoSulfur Compounds in the PASH Matrix

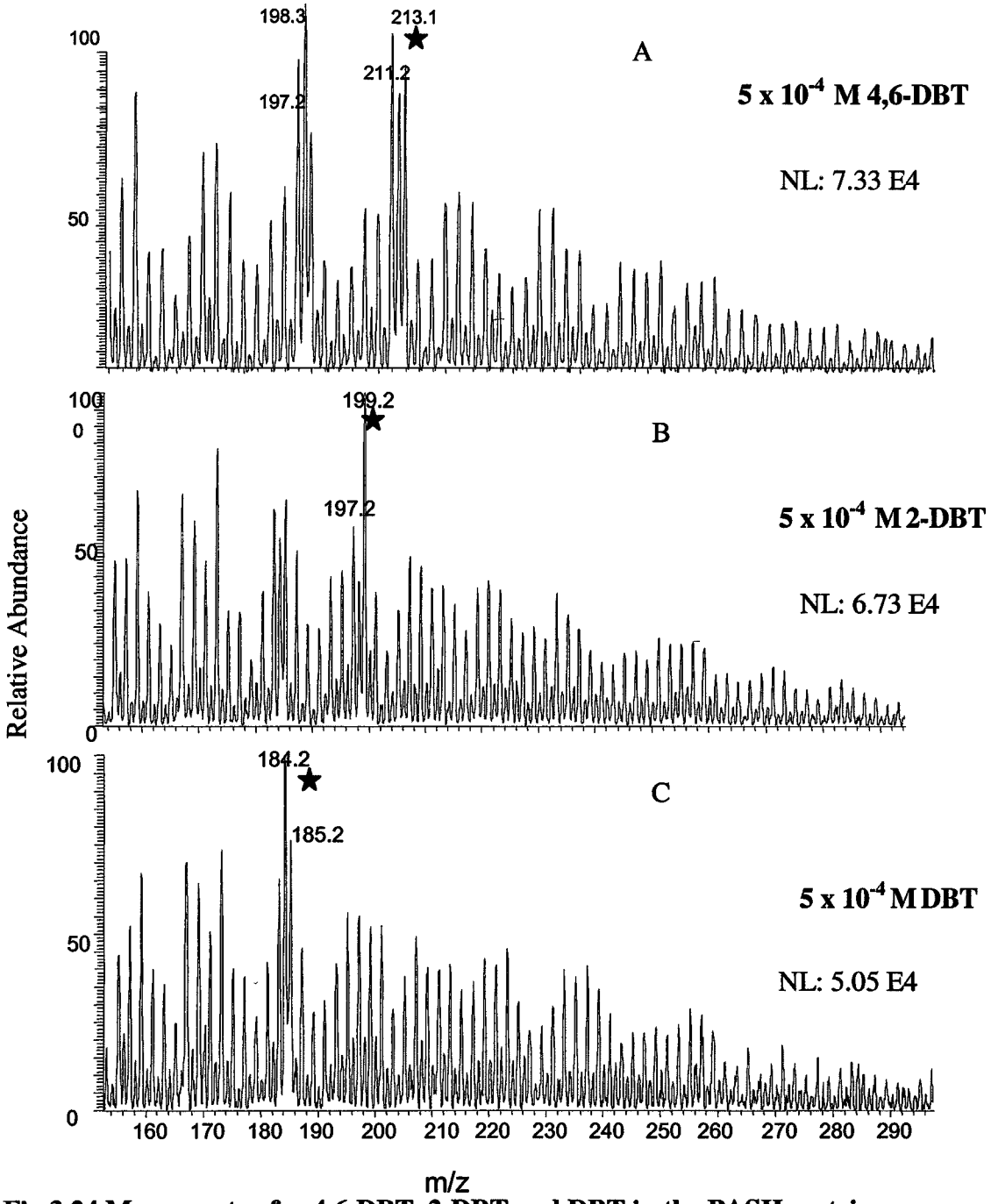


Fig 3.24 Mass spectra for 4,6-DBT, 2-DBT and DBT in the PASH matrix

Matrix: 0.6 mg PASH/6ml hexane, 1mM tropylium tetrafluoroborate solution, Flow Rate, 15 μl/min. (A) 4,6-DBT (B) 2-DBT (C) DBT

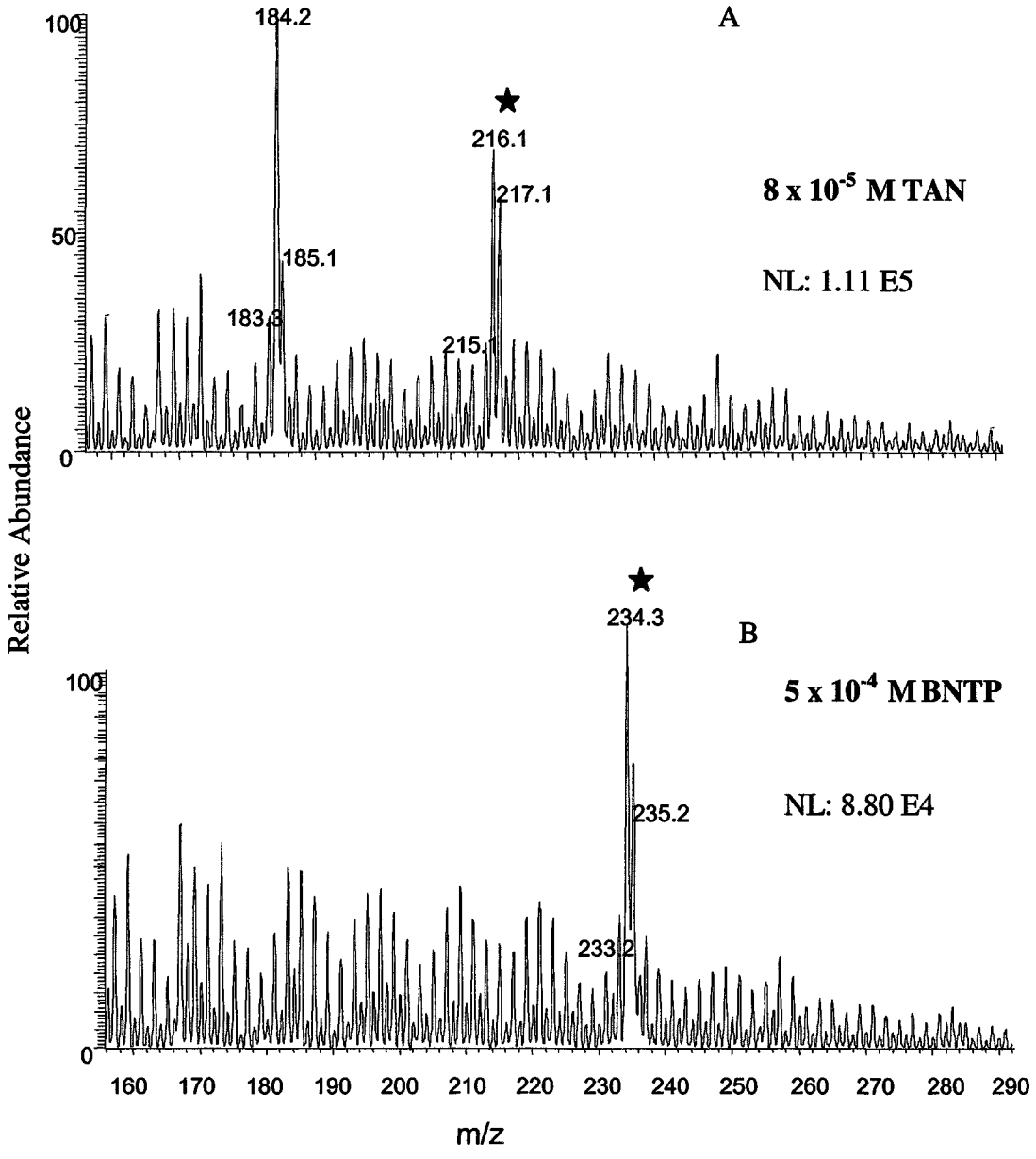


Fig 3.25 Mass spectra for TAN and BNTP in the PASH matrix.

Matrix: 0.6 mg PASH/6ml hexane, 1mM tropylium tetrafluoroborate solution, Flow Rate, 15 μl/min. (A) TAN (B) BNTP

Fig 3.24 and Fig 3.25 show mass spectra used for determining the LOD for the organosulfur standards in the PASH matrix. It was found that for 2-DBT, 4,6-DBT, DBT and BNTP the LOD was 5 x 10^{-4} M. TAN had the lowest detection limit at 8 x 10^{-5} M. TAN has the lowest oxidation potential and therefore the highest sensitivity as previously reported [23]. Table 3.4 lists the LODs for the organosulfur standards in the hydrogenated oil matrix in the presence of $Pd^{2+\cdot}$ as well as the LODs for the organosulfur standards in the PASH matrix in the presence of tropylium tetrafluoroborate .

Table 3.4: LOD of Organosulfur Standards

Compounds	LOD in hydrogenated Oil in the presence of Pd^{2+}	LOD in PASH in the presence of Tr^{+}
TAN	3.0 x 10^{-7} M	8.0 x 10^{-5} M
DBT	1.8 x 10^{-4} M	5.0 x 10^{-4} M
2-DBT	5.0 x 10^{-5} M	5.0 x 10^{-4} M
4,6-DBT	7.5 x 10^{-6} M	5.0 x 10^{-4} M
BNTP	1.8 x 10^{-5} M	5.0 x 10^{-4} M

In the case of palladium studies, the LOD of organosulfur standards were found in a hydrogenated oil matrix (10 mg/ml) in CH_3OH: CH_2Cl_2 (50:50) solvent mixture where as in the case of the tropylium studies, the LOD of organosulfur standards were found in the PASH matrix (0.6 mg/6ml) in 100% hexane. This difference in the nature of the matrix and solvent conditions may be one of the reasons for the better LOD in the palladium (II) studies.

CHAPTER IV

CONCLUSION

HPLC/APCI/MS and HPLC/APCI/MS/MS were performed on organosulfur standard compounds and PASH fractions isolated from an Arabian crude oil. The standard compounds and PASH were separated using high performance liquid chromatography and later identified by a very novel post column addition of tropylium cations prior to APCI/MS detection. Detection of the compounds involved a charge transfer reaction between the sulfur compounds and tropylium. Non alkylated species appear as radical cations in the APCI source where as alkylated species exhibit the loss of hydrogen and alkyl groups.

Tandem MS was performed on the standards as well as the PASH. The PASH had fragmentation patterns identical to the standards thus confirming their presence in the oil. MS/MS showed a constant loss of 16, 29, 43 and 57 Da for the alkylated species. TAN was the only PASH which showed a loss of 32 attributed to sulfur. From all the chromatographic and mass spectrometric data it can be concluded that high performance liquid chromatography coupled with atmospheric pressure chemical ionization mass spectrometry in the presence of tropylium as a sensitizing agent can be used to separate, identify and confirm the presence of polyaromatic sulfur heterocycles in a complex

matrix.

REFERENCES

1. Monticello, D. J. *Curr Opin Microbiol.* **2000**, 11, 540-546.
2. Monticello, D. J. *Chemtech.* **1998**, 28(7), 38-45.
3. Delgado, M. A.; Tena, R. C.; Montelango, F. I. *Chromatographia, Italia.* **1999**, 50, 235 -238.
4. Barman, B. N.; Cebolla,V. L.; Membrado, L. *Crit . Rev. Anal. Chem.* **2000**, 30, 75-120.
5. Boduszynski, M. M. *Energy & Fuels*, **1988,** 2(5), 597-613.
6. Ali, M. F.; Bukhari, A.; Misbah-ul-Hassan. *Fuel Science and Technology Int'l.* **1989**, 7(8), 1179-1208.
7. Rudzinski, W. Grant Application R825503, SouthWest Texas State University, SWT – IEIS.
8. Matsunaga, A. *Anal. Chem.* **1983**, 55, 1375 – 1379.
9. Wise, S. A.; Sandra, L. C.; May, W.E. *Journal of Chromatography*, **1993**, 642, 329 – 349.
10. Thomson, J. S.; Grizzli, P. L. *Analytical Chemistry*, **1982**, 54 (7), 1071-1078.
11. Robbins, W.K. *Journal of Chromatographic Science*, **1998**, 36, 457 – 466.
12. McKerrell, E. H. *Fuel*, **1993,** 72, 1403 – 1409.
13. Hayes, P. C.; Anderson, S. D. *Anal. Chem.* **1985**, 57, 2094-2098.

14. Andersson, J. T.; Schmid, B. *Journal of Chromatography A*, **1995**, 693, 325 – 338.

15. Nishioka, M.; Campbell, R. M.; Lee, M. L.; Castle, R. N. *Fuel*, **1986**, 65, 270 – 273.

16. Zhan, D.; Fenn, J.B. *International Journal of Mass Spectrometry*, 194 **(2000),** 197 – 208.

17. Hsu, C.S.; Qian, K. *Energy & Fuels*, **1993**, 7, 268-272.

18. Hoffmann, E.; Stroobant V. (Eds.) *Mass spectrometry, Principles and Applications* **1999**, John Wiley, Chichester.

19. Cole, R.B. *Electrospray Ionisation Mass spectrometry* **1997**, Wiley, Chichester.

20. Van Berkel, G.J.; Asano, K.G. *Anal. Chem.* **1994**, 66, 2096 –2102.

21. Bayer, E. G.; Frorer, P.; Rental, C. *Angew Chem. Int. Ed.* **1998**, 38, 992-995.

22. Roussis, S. G.; Prouix, R. *Anal. Chem.* **2002**, 74, 1408 – 1414.

23. Rudzinski, W.E.; Zhang, Y.; Luo, X. *J. Mass Spectrom.* **2003**, 38, 167-173.

24. Rudzinski, W.E.; Luo, X. *Fuel Chemistry Division Preprints*, **2003**, 47(1).

25. Airiau, C.Y.; Brereton, R.G.; Crosby, J. *Rapid Commun, Mass Spectrom.* **2001**, 15, 135-140.

26. Morikawi, H.; Imaede, A.; Arakawa, R. *Arial. Commun.* **1999**, 36, 53 – 56.

27. Silverstein, R. M.; Webster, F. X. (Eds.) *Spectrometric Identification of Organic compounds* **1998**, John Wiley and sons, Inc, New York.

VITA

Vidya Rai was born in Karnataka, India, on June 24, 1974, the daughter of Jayanthi Hegde and Shivaram Hegde. After completing her work at Baldwin Girls High School, Karnataka, India, she entered Jyoti Nivas College, Karnataka, in August 1992. She received a Bachelor of Science from Jyoti Nivas College in August 1995. She went on to do her masters in organic chemistry at the Bangalore University and received a Master's in Science in August 1997. She began graduate school during the Fall of 2001 in the Department of Chemistry and Biochemistry at Texas State University.

Permanent Address: 303, 6 Main,
HAL II Stage, Indiranagar
Bangalore-560038
Karnataka, India

This thesis was typed by Vidya Rai