Investigations into the Extraction and the Determination of Polycyclic Aromatic Hydrocarbons (PAHs) from Water by Solid-Phase Extraction and Capillary Gas Chromatography / Mass Spectrometry

By

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Abstract

Factors affecting the determination of PAHs by capillary GC/MS were studied. The effect of the initial column temperature and the injection solvent on the peak areas and heights of sixteen PAHs, considered as priority pollutants, using crosslinked methyl silicone (DB1) and 5% diphenyl, 94% dimethyl, 1% vinyl polysiloxane (DB5) columns was examined. The possibility of using high boiling point alcohols especially butanol, pentanol, cyclopentanol, and hexanol as injection solvents was investigated.

Studies were carried out to optimize the initial column temperature for each of the alcohols. It was found that the optimum initial column temperature is dependent on the solvent employed. The peak areas and heights of the PAHs are enhanced when the initial column temperature is 10-20 c above the boiling point of the solvent using DB5 column, and the same or 10 C above the boiling point of the solvent using DB1 column. Comparing the peak signals of the PAHs using the alcohols, p-xylene, n-octane, and nonane as injection solvents, hexanol gave the greatest peak areas and heights of the PAHs particularly the late-eluted peaks. The detection limits were at low pg levels, ranging from 6.0 pg for fluorene to 83.6 pg for benzo(a)pyrene.

The effect of the initial column temperature on the peak shape and the separation efficiency of the PAHs was also studied using DB1 and DB5 columns. Fronting or splitting of the peaks was observed at very low initial column temperature. When high initial column temperature was used, tailing of the peaks appeared. Great difference between DB1 and DB5 columns in the range of the initial column temperature in which symmetrical peaks of PAHs can be obtained

is observed. Wider ranges were shown using DB5 column. Resolution of the closely-eluted PAHs was also affected by the initial column temperature depending on the stationary phase employed. In the case of DB5, only the early-eluted PAHs were affected; whereas, with DB1, all PAHs were affected.

An analytical procedure utilizing solid phase extraction with bonded phase silica (C8) cartridges combined with GC/MS was developed to analyze PAHs in water as an alternative method to those based on the extraction with organic solvent. This simple procedure involved passing a 50 ml of spiked water sample through C8 bonded phase silica cartridges at 10 ml/min, dried by passing a gentle flow of nitrogen at 20 ml/min for 30 sec, and eluting the trapped PAHs with 500 µl of p-xylene at 0.3 ml/min. The recoveries of PAHs were greater than 80%, with less than 10% relative standard deviations of nine determinations. No major contaminants were present that could interfere with the recognition of PAHs. It was also found that these bonded phase silica cartridges can be re-used for the extraction of PAHs from water.

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This work is specially dedicated to my wife, son , and daughter for their inspiration.

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Introduction

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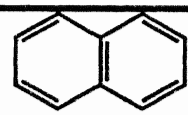
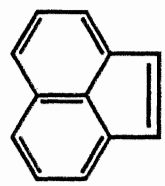
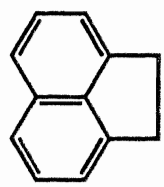
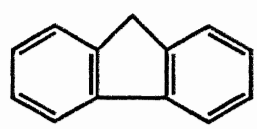
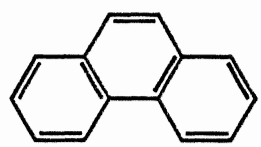
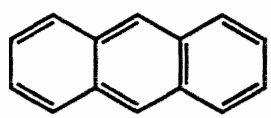
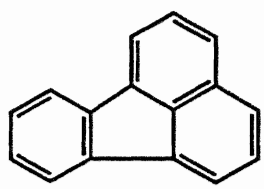
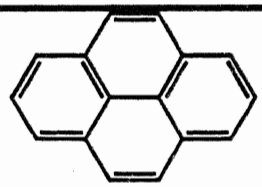
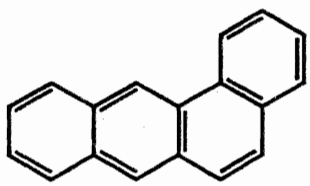
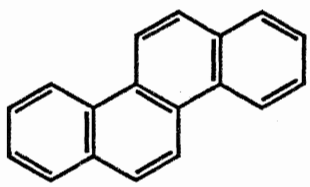
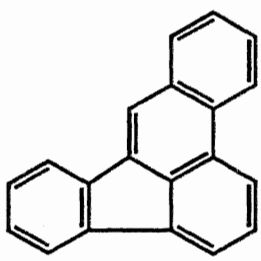
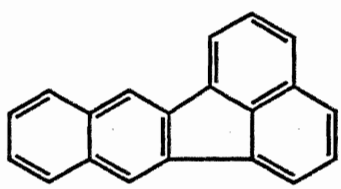
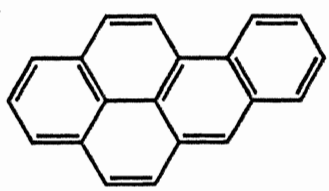
Polycyclic aromatic hydrocarbons (PAHs), polynuclear aromatic hydrocarbons (PNAs) , or polyarenes are composed of two or more fused aromatic (benzene) rings (1-2). They form a large class of chemicals which are found in the atmosphere, soil, and water. Since many of these compounds are known to be carcinogens, their identification and quantification in soil and water are of continuing concern in connection with human exposure through contaminated food and drinking water supplies.

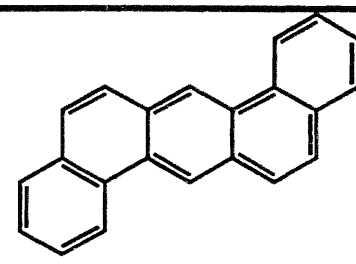
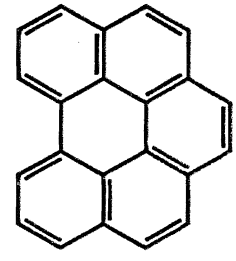
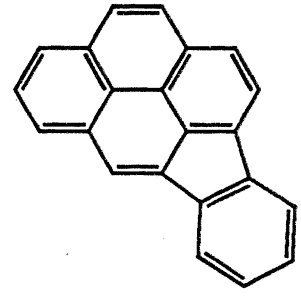
Prior to the introduction of the IUPAC (International Union of Pure and Applied Chemistry) system, various systems of nomenclature have been used to describe PAH structure. Many of these compounds have been named unsystematically, some names reflect the initial isolation of the compound (e.g. naphthalene, pyrene), some reflect their color (fluoranthene), and some reflect the shape of their molecules (coronene) (3).

In 1979, the United States Environmental Protection Agency (EPA) included sixteen PAHs in its list of priority pollutants whose presence should be monitored and limited in effluent waters (4). Table 1 lists these sixteen PAHs along with their structures, molecular weights, and boiling points. They represent the most commonly measured PAHs in the aqueous environment.

Table 1. Structure, Boiling Points, and Molecular Weight of 16 PAHs

Peak #	Cmpd. Name	M.W.	B.P.	Structure
1.	Naphthalene	128	218	
2.	Acenaphthylene	152	276	
3.	Acenaphthene	154	274	
4.	Fluorene	166	294	
5.	Phenanthrene	178	338	
6.	Anthracene	178	340	
7.	Fluoranthene	202	383	

Peak #	Cmpd. Name	M.W.	B.P.	Structure
8.	Pyrene	202	393	
9.	Benz(a)-anthracene	228	431	
10.	Chrysene	228	414	
11.	Benzo(b)-fluoranthene	252	481	
12.	Benzo(k)-fluoranthene	252	481	
13.	Benzo(a)-pyrene	252	496	

Peak #	Cmpd. Name	M.W.	B.P.	Structure
14.	Dibenz(a,h)-anthracene	278	—	
15.	Benzo(g,h,i)-perylene	276	—	
16.	Indeno(1,2,3,cd)-pyrene	276	—	

Polycyclic aromatic hydrocarbons are usually colored, crystalline solids having high melting and boiling points. The physical properties of PAHs are characterized by the conjugated π-electron system, which also accounts for their chemical stability. The boiling points of PAHs are higher than those of the n-alkanes of the same number of carbon atoms (5). As might be anticipated from their nonpolar hydrophobic nature and high molecular weight, PAHs have low solubility in water. However, they are very soluble in organic solvents. The

presence of organic solvents in water can increase the solubilities of PAHs (6). There is a considerable variation in the values for solubility of PAHs in the literature (7,8), which could be due to the differences in the methods of preparation and measurement of the saturated solutions. Nevertheless, solubilities tends to decrease as the molecular weight increases. Naphthalene has a solubility of about 32 ppm while four-ring PAH have solubilities in the 2-20 ppb range (8). Angular PAH isomers are more soluble than the linear isomers. For example phenanthrene is approximately 20 times more water soluble than anthracene. The presence of co-solute in solution and temperature has a great effect on the solubilities of PAH (9). Solubility of phenanthrene increased from 423 to 1277 ppb between 8.5 and 29.9 C. The presence of one or two PAH in solution effected the solubility of an additional PAH in that solution. Naphthalene enhanced the solubility of biphenyl whereas the presence of both naphthalene and phenanthrene increased the solubility of acenaphthene (9). Thus, it is clear that solute-solute interaction have great influence on the solubility of PAHs.

Sources and distribution of PAHs in the environment

Polycyclic aromatic hydrocarbons can be formed from both natural and anthropogenic sources (3). Natural sources may include volcanic activity, natural combustion (forest fires), and biological material. Anthropogenic sources which are predominant and contribute more to environmental pollution, are the result of industrial processes, residential heating, and transportation. In the United States, motor vehicles are thought to be the major source of atmospheric PAHs accounting for approximately 35% of the yearly total (10). Aluminum production, residential heating (wood, coal, oil, and gas), coke production, power generation, and incineration contribute 17%, 12%, 11%, 7%, and 3% respectively (10).

PAHs are the products of incomplete combustion of organic materials, which is considered as the primary source of these compounds in the environment. It is believed that at high temperatures, organic compounds are broken into smaller, unstable molecules (pyrolysis) which then recombine to form larger, relatively more stable aromatic hydrocarbons (pyrosynthesis) (3,11).

Polycyclic aromatic hydrocarbons are found at various levels of concentration throughout the environment. Their concentrations and distribution generally depend on many factors such as geographical location, seasonal variation, and source-point. Several papers have been published on the occurrence and distribution of PAHs in the environment (12-16). PAHs may enter the water with the discharge of sewage, industrial waste, oil spillage, and through rain containing atmospheric contaminants from soources, such as automobile exhausts and forest fires. In most cases there is a direct relationship between the concentrations of PAHs in water and the degree of industrialization

and other human activities. Analysis of ground water has shown a concentration range of 0.001-0.10 μg/L(ppb) of carcinogenic PAH, while untreated river water have approximately ten times higher levels of PAH than ground water (0.01-0.025) μg/L (13). It was assumed that PAH in ground water were leached from surface water through soils containing PAH, while the higher concentration in uncontaminated fresh water lakes originated from aquatic biota in sediment and soil. PAH entering the water from various sources become adsorbed to particulate matter that deposit on the sediment due to their low solubility and hydrophobic nature; thus, the concentration of PAH in sediments are usually higher than those in water. Leaching from the sediments may return some fractions of PAH to the water. In a study of New England River basin (14), the more water-soluble PAH were found in water only. Those of intermediate solubilities, anthracene and phenanthrene, where found in both water and sediment; and those of low solubility, with molecular weights above 228, were found only in sediment.

Toxicity

Not all polycyclic aromatic hydrocarbons are carcinogenic, and even among those that have been shown to cause cancer, carcinogenicity varies widely (1). The carcinogenic activity of PAHs is dependent on the shape, size, and steric factors of the molecule. Tri-, tetra-, penta-, and hexacyclic compounds are more carcinogenic than either smaller or larger compounds. Highly angular configurations are more carcinogenic than either linear or highly condensed compounds (3). The biological activity of PAHs is isomer specific. The relationship between the structures of methylated PAH and their carcinogenicity has been studied (17-20). Methylbenz(a)anthracenes (MBA) and methyl chrysenes (MC) are among the most biologically active alkylated aromatic series. There are six monomethyl chrysene isomers, and of these, only 5-(MC) is an effective tumor initiator and complete carcinogen, when tested on mouse skin. 2-, 3-, 4-, and 6-(MC) are moderately active. The tumor-initiating and carcinogenic activities of 5-(MC) are significantly greater than those of chrysene itself (17). The carcinogenic activity of 12 monomethyl benz(a)anthracenes were compared (18). 7-(MBA) was most active, 6-, 8-, and 12-(MBA) isomers were slightly active, and the remaining isomers were inactive. The presence of a heteroatom in the ring can also alter the carcinogenic properties. For instance, although phenanthrene is inactive, phenanthridine is a carcinogen (21). Nitrogen PAHs show greater carcinogenicity than those methylated PAH with similar structure.

Determination of PAHs in water

Samples of water of various origins can be subjected to chromatographic analysis in two basic procedures, either by direct injection of the water sample or by concentration of the analytes in the water prior to the analysis (22). Environmental water samples can not be analyzed effectively by any chromatographic method without pretreatment of the sample. This is because most samples are either too complex, too dilute, or incompatible with the chromatographic system. In most instances, it is the trace concentrations of compounds that must be determined, thus, it is essential to preconcentrate the analytes prior to the analysis. Regardless of the size and the nature of the composition of the sample, the objective is to obtain a sub-fraction of the original sample enriched in all substances which ensure effective determination

The determination of PAHs in environmental samples has been the subject of many papers in the literature. The concentrations of PAHs in water samples are usually very low, and generally found in the presence of other organic material. Their concentration in water systems ranges from the 1ppt (1ng/L) level in pure ground water to 1ppm (1mg/L) level in heavily contaminated water. PAHs are usually present in real samples as complex mixtures containing components of widely ranging concentrations. The major analytical problem in the analysis of PAHs is the separation and identification of individual components in the presence of other isomers and alkyl derivatives. For example, there are 12 possible structures for the five-ring compounds of molecular weight 278, and if a methyl group is added to the ring, the number of possible isomers increases to 117 (3).

Many analytical techniques usually involving various types of chromatography have been employed for their analysis, such techniques range from relatively simple and rapid qualitative methods to very elaborate and expensive methods for quantitative analysis.

The World Health Organization (WHO) has recommended that the concentration of six specific PAH compounds, fluoranthene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(ghi)perylene, and indeno-(1,2,3-cd)pyrene not exceed 200 ng/L in domestic water (23). It was also recommended that raw water containing 0.1-1.0μg/L of PAH should be adequately treated to reduce their levels to the lowest possible concentration level and that raw waters containing over 1.0μg/L PAH are unsafe for drinking purposes even after treatment (24). Thus, these compounds must be monitored at very low level in water samples. In order to achieve sufficient concentrations for detection, efficient extraction, and separation from other extractable compounds, a concentration step must often precedes the analysis. Extraction with a liquid or a sorbent are commonly used techniques prior to the analysis.

Liquid-Liquid Extraction :

The analysis of organic pollutants in water traditionally relies on liquid-liquid extraction methods. It is based on the partition of solute between an aqueous and an immiscible organic phase, which can be represented by the following equilibrium (25) :

$$[A]_{aq} \Leftrightarrow [A]_{org} \qquad (1)$$

where $[A]_{aq}$ and $[A]_{org}$ are the concentration of the analyte in the aqueous and the organic phase, respectively. The ratio of the analyte A in both phases will be constant regardless of the quantity of A. This ratio is usually referred to as the partition coefficient or distribution coefficient K.

$$K = \frac{[A]_{org}}{[A]_{aq}} \qquad (2)$$

The efficiency of the extraction is dependent mainly on the affinity of the solute for the solvent as measured by the partition coefficient K. The number of extraction steps and the phase ratio (volume of extraction solvent / volume of sample) can also effect the efficiency of extraction. It is well established that more efficient extraction can be achieved by extracting with several small portions of solvent than extracting with a single large one (25). For simple extraction using separating funnels, the partition coefficient should be large since the number of extraction steps performed and the phase volume of the solvent are limited.

The determination of PAHs present in water at the microgram per litre level has usually been carried out by the removal of PAHs from water using liquid-liquid extraction (26-27). Acheson et al. (26) investigated the effect of the initial concentration on the efficiency of extraction of PAHs. It was found that at higher concentrations efficiencies were in the region of 80%; however, at lower concentrations efficiencies may drop below 40%. The lower extraction efficiencies were thought to be due to adsorption of PAHs onto the glass of the mixing vessel, or degradation within the vessel. Accordingly, it was recommended that the extraction should be carried out as soon after sample collection as possible and that it is esseential to sample water directly into the extraction vessel. Better recoveries for low molecular weight PAHs at low concentrations were reported when hexane was used as the extracting solvent (28). The extractions were made by manual shaking for 10 minutes because the experiments made with mechanical shaker did not give reproducible results. Grob et al. (27) also found improved reproducibility using vigorous shaking by hand over various mixing techniques including mechanical shaking, stirring, and ultrasonic treatment.

The partition coefficient may be made favorable by adjusting the pH of the water sample (29). Extraction of water samples by manipulating pH is widely used to fractionate samples into neutral, basic, weakly acidic and strongly acidic fractions. Novotny et al. (30) developed a liquid-liquid partitioning method to isolate PAHs in complex mixtures. They separated the mixtures into acidic, basic, and neutral components. In this method, after pH adjustment with aqueous H_2SO_4 or KOH , the water sample is extracted with an appropriate solvent in a separatory funnel. Then the pH is readjusted and the water sample is reextracted.

Continuous liquid-liquid extraction (CLLE) techniques are used when the sample volume is large and the partition coefficient is small. It allows for continuous extraction of a sample automatically. A number of continuous liquid-liquid extractors have been described (31-33). Godefroot et al. (33) reported a continuous liquid-liquid extraction apparatus that avoids the evaporation of the solvent. The extracts (2ml) were directly analyzed by GC. However, the efficiency of this method for the sub-ppm level were rather low. Although CLLE) equipment is readily available, the time required for extraction (18-24 hr) is usually quoted as a reason for not using this method(31).

There are a number of disadvantages associated with the use of liquid-liquid extraction which makes the technique undesirable. The main disadvantages of the method are the time necessary for the extraction, the co-removal of interfering substances which requires clean-up of the extract, and the need for large volumes of high-purity solvents which are expensive and generate a significant amount of toxic solvent waster. Another disadvantage of liquid-liquid extraction is the evaporation of the solvent to a small volume. This step is the reason for the loss of PAHs. Smith and co-workers (34) have evaluated the performance of several volume reduction methods including rotary evaporation, distillation, and nitrogen evaporation. It was found that all methods gave similar recoveries for high molecular weight PAHs. However, a significant losses of low molecular weight PAHs in methylene chloride were observed by the nitrogen evaporation method. The time required for evaporation was 2-7 hr using these techniques.

An alternative technique to solvent extraction is the use of sorbents to extract organic compounds from aqueous samples. The use of sorbents for preconcentration purposes has been developed very rapidly in the past twenty years due to a number of reasons such as the decrease in the analysis time, cost,

labor, and solvent consumption, relative to the traditional liquid-liquid extraction. Middleton and Rosen (35) were the first successfully to characterize organic components in water. They extracted chlorinated insecticides from several thousands gallons of water using carbon columns and chloroform as the extracting solvent, which is usually referred to as the carbon-chloroform extraction (CCE) method. Even though the recoveries of some pesticides were low, because they were not stable on the activated carbon, the CCE method led to the extensive use of carbon for both analytical and water purification purposes (36-37).

Solid Phase Extraction

The extraction of organic compounds from water by adsorption on solid material followed by elution with an organic solvent has become a very popular technique in the recent years. It is based on the following equilibrium (38)

$$[A]_l \Leftrightarrow [A]_s \qquad (3)$$

where $[A]_l$ and $[A]_s$ are the concentration of the analyte in the liquid and the solid phases, respectively. The partition coefficient K can be given as:

$$K = \frac{[A]_S}{[A]_l} \qquad (4)$$

The principle of solid phase extraction is analogous to that of liquid extraction, the differences are in the extraction medium used and in the resulting effect. By careful selection of a solid phase and the solvent, it is possible to achieve total retention of the analyte by driving the equilibrium toward the solid phase so that K (in equation 4) approaches infinity or total elution of the analyte by forcing the equilibrium to the liquid phase so that K approaches zero(38). There are several factors that makes the use a sorbent advantageous over a liquid for the extraction of organic compounds (22):

1. The partition coefficient can be shifted even more toward the sorbent than in liquid extraction if the sorbent is selected correctly.
2. Adsorption of water on the sorbent is minimal.
3. The " wettability " of the sorbent with water allows for satisfactory contact of the solute and the sorbent surface.
4. The sorbent surface is chemically inert.

In solid phase extraction, two mechanisms are believed to be involved, interaction of solutes with water and adsorption / desorption of solutes from the surface of the sorbent. Interaction of solutes with water depends on the type of organic solute. For hydrophobic solutes, it has been suggested (39) that a non-polar organic molecule is solubilized in water, because of the orientation of many layers of water molecules around the organic molecule. Thus, the water becomes more ordered, and the entropy of water decreases. However, when the organic molecules are adsorbed on the sorbent, the ordered water molecules are dispersed, so that the order of the water system decreases and its entropy increases. This makes the entire process favorable since it usually leads to

negative changes in the free energy. The mechanism of surface adsorption is dependent on the type of interactions between the solute and the sorbent. Hydrophobic solutes are believed to undergo non-polar interactions which occur between the carbon-hydrogen bonds of the sorbent functional groups and the carbon-hydrogen bonds of the solute. These forces are commonly known as Van der Waals or dispersion forces. There are other secondary interactions that might occur during the extraction of the analyte of interest including the interaction between the analyte and other components in the sample, the interaction between the water molecules and the sorbent, and the interaction between the components of the sample and the sorbent. In order to achieve high efficiency of adsorption, the interaction between the analyte of interest and the sorbent must be strong while all other interactions must be weak. The choice of the sorbent plays a major role in achieving high efficiency of adsorption of particular analyte. Generally, hydrophobic molecules are attracted from water by the hydrophobic surface of the sorbent, and hydrophilic molecules are attracted by the hydrophilic surface of the adsorbent. Thus, hydrophobic non-polar sorbents can be employed to extract and concentrate non-polar analytes from aqueous solutions. PAHs are usually extracted from water samples using non-polar sorbents, and are eluted from the sorbent by applying a more hydrophobic solvent than the sorbent. Thus, the " like adsorbs like " principle can often be used successfully

A typical solid phase extraction procedure with reversed phase chromatography includes the following steps:

1. Activation of the sorbent: The role of this step is to ensure maximum contact of the solutes with the pores of the sorbent by wetting the sorbent with an organic water-miscible solvent, usually methanol. This causes opening of the chains in the stationary phase, thus increasing its surface area. A decrease in

the adsorption efficiency may result upon omitting the activation of the sorbent. It was observed that the efficiency of adsorption was decreased when this step was replaced by using a large volume of water(40). Hence, it is always recommended to activate the sorbent before passing the sample. Any of the reversed-phase chromatography solvents, such as acetonitrile, isopropanol, or acetone can be used as an activating solvent; however, methanol is the most commonly used solvent due to its relatively low cost, low toxicity, and ready availability in most laboratories at high purity(41).

2. Conditioning with water: This step removes the activation solvent to enable a proper contact of the sample with the sorbent. Excessive washing with water could reduce the recovery(40)
3. Loading of the sample:
4. Removal of interferences by washing the column with an appropriate eluent, usually water.
5. Removal of water by passing either a stream of air or nitrogen through the column or simply by storing the column in a dessicator. Prolonged drying time sometimes led to decrease in recovery. It was found that leaving the vacuum on for 2 minutes resulted in reduction of recovery of pesticides (41).
6. Desorption of the analyte either by an appropriate solvent using a vacuum, a pump, or a syringe or by heating.

Types of Sorbents

The most commonly employed sorbents for the extraction of polycyclic aromatic hydrocarbons from water are carbon, macroreticular polymeric resins, and bonded-phase silanized silicas.

Carbon Sorbents:

Carbon sorbent was the first medium used for the extraction of organic compounds from water as previously mentioned(35). The most common form of carbon used for trace enrichment of organic compounds is granular activated carbons with large surface areas (300-2000 $m^2 g^{-1}$), a wide pore diameter distribution and a heterogeneous surface with active functional groups(42). The use of activated carbon for concentrating trace compounds in water has been elaborated over the years to various methods such as the carbon chloroform extract method (CCE) and the carbon alcohol extract method (CAE). The main advantage of activated carbon was high adsorption capacity, which allows for the use of a small amount of sorbent to extract a large volume of water, and its high thermal stability, up to 700 C (22). However due to the heterogeneous nature of activated carbons, some problems were encountered. Many organic compounds in water did not adsorb completely on carbon and some compounds adsorbed so strongly that complete elution of substances was impossible. Thus, recoveries were generally low and inconsistent (43-44). Another disadvantage is the high level of backgrounds (blanks). Cleaning the carbon prior to use is not recommended practice. Results showed that there is no differences in the adsorptive power between stock carbon and carbon that was pre-extracted with chloroform and dried (36).

Due to the drawbacks mentioned, improvement of the method resulted in developing more homogeneous structures of carbon such as the graphitized carbon black (GCB). They showed high recovery for low molecular weight polar analytes, chlorinated and organophosphorus pesticides (45). The analysis of PAHs in water was investigated using graphitized carbon black (37, 46). Carbopack B, the commercial name for a non-specific graphitized carbon back with a surface area of 90 m^2/g, was used to extract PAHs (46). 50 mg of Carbopack B were extracted in soxhlet apparatus for 6 hr, one liter of water spiked with PAHs was passed through the cartridge. A mixture of (1:1) light petroleum and toluene was used as the eluting solvent. High recoveries for low molecular weight PAHs were obtained, however low recoveries for anthracene and pyrene, 57% and 48% respectively, were observed. Similar results were observed by Lagana et al. (37). The adsorbed PAHs were eluted by passing toluene-benzene-acetonitrile (5:2:3). Low recoveries of benzo(g,h,i)perylene and indeno(1,2,3-c,d)pyrene were obtained, 58% and 53% respectively. Thus, graphitized carbon black was found unsuitable for the preconcentration of PAHs particularly the high molecular ones.

Polymer Sorbents

Considering the problems associated with the use of activated carbon, synthetic polymers are reported as an alternative for the trace enrichment of organic compounds from water. In 1969, Riley and Taylor (47) were the first to report the use of a styrene-divinylbenzene polymer to accumulate a number of dissolved organic compounds from sea water. These hydrophobic polymers have found extensive use as adsorbents due to their homogeneous structures. The most common used type of polymers for the concentration of PAHs are Styrene-Divinylbenzene copolymers (XAD), Tenax-GC, and Polyurethane polymers.

Styrene-Divinylbenzene copolymers

Poly(styrene-divinylbenzene) and polyacrylate (XAD) resins which manufactured by Rohm and Haas have received the most attention for trace enrichment purposes. The interest of these polymers was developed after the extensive test results and the detailed methodology was presented by Junk and co-workers in 1974 (48). They recommended the following procedure for the treatment of the sorbent. The impurities in XAD-2 and XAD-4 are removed by slurrying in methanol and decanting. The remaining resin beds were purified by sequential solvent extractions with methanol, acetonitrile, and diethyl ether in a Soxhlet extractor for 8hr per solvent. The average recovery of all organics was claimed to be 78%, while the recoveries of the PAHs studied, naphthalene, fluorene, anthracene, and acenaphthene, were 98, 84, 83, 92% respectively.

Both XAD-2 and XAD-4 resins (polystyrene-divinylbenzene) are aromatic in character and very hydrophobic. They are chemically identical, the only difference is in their surface area and average pore diameter. The surface area and the average pore diameter for XAD-2 are 300 m^2/g and 90 $\mathring{A}$, whereas it is 784 m^2/g and 50 $\mathring{A}$ for XAD-4 (22). Results showed no significant differences between these two sorbents for concentrating organic compounds since they have similar adsorption properties. When both resins were compared to carbon, the overall recovery of organic substances for the macroreticular resins was often better (42).

Junk et al.(48) examined the effect of different operating conditions on using XAD-2. No significant change in the recovery was observed when the initial concentration of the sample was changed from tens of ppb to tens of ppm, or

when the flow rate through the column was altered. However, in another study (49) a decrease in the recovery of naphthalene was obtained upon increasing the flow rate of the sample through the column from 3 to 7 ml/min. Changing the pH of the sample was found to have no effect on the extraction efficiency of non-polar compounds such as alkyl benzene and naphthalene (49).

XAD-2 resin has a lower concentration efficiency for low molecular weight aliphatic compounds than for aromatic compounds (50). The retention efficiency also increases with the increasing of the molecular weight in homologous series, and decreases as the polarity of the analytes increases. Better recoveries of phenol were obtained when the polar XAD-7 (methylacrylate polymer) was used in place of XAD-2 (49). The increase in the extraction efficiency of phenol was explained by the attraction of phenol to the more polar XAD-7 rather than the non-polar XAD-2, and that the increase in the surface area of XAD-7 (450 m^2/g) over XAD-2 (300 m^2/g) is insufficient to account for the increase in efficiency. The analysis of marine water for contamination with pesticides and hydrocarbons has also been described (51). The recoveries of pesticides were about 80%, however low recoveries were obtained for hydrocarbons such as phenanthrene, 62%

XAD-7 and XAD-8 (methyl acrylate polymers) have a moderate hydrophilic structure Because of the ester cross-linkage, they have a more polar structure than XAD-2 and, hence, have a higher affinity for polar compounds. The specific surface area and the mean pore diameter for XAD-7 are 450 m^2/g and 80 $\mathring{A}$ respectively, and that of XAD-8 are 140 m^2/g and 250 $\mathring{A}$ respectively (49). XAD-8 was found to favor aliphatic over aromatic compounds (52). The resin seemed to follow an inverse solubility trend. That is, in general, aliphatic compounds are less soluble than aromatic compounds. Likewise, the following functional groups were preferred $-CH_3 > -CO_2H > -CHO > -OH > NH_2$. (50).

The principal disadvantage of the XAD resins is the slow and careful treatment required prior to the use in trace analysis to produce an acceptable blank levels. Both XAD-2 and XAD-4 resin contain significant quantities of a variety of material, including alkylated derivatives of benzene, styrene, naphthalene, and biphenyl (53). The nature of these impurities suggests that they are either residual from the resin manufacturing process (e.g., starting material or secondary byproducts) or artifacts from the degradation of the polymer itself. The contaminant concentrations are much higher for XAD-4 than XAD-2 resin, which attributed to the higher surface area of XAD-4 (53).

Due to the nature and magnitude of the residues, any clean-up procedure can only minimize the extractable material, thus, a significant amount of impurities can be introduced into the eluate even though the resins are carefully cleaned prior to use (54). It was originally assumed that poor blanks were obtained due to insufficient removal of the impurities in the clean-up procedure, however, various clean-up procedures were found to be ineffective in eliminating the impurities in the blank. Picer et al.(55) purified XAD-2 and XAD-4 by extraction for 24hr with acetonitrile in a Soxhlet extractor, 12hr with diethyl ether, and 24hr with methanol. These purified resins gave a large difference in the recoveries for some chlorinated hydrocarbons. An alternative clean-up procedure (56) using thermal desorption followed by washing with methanol gave similar unsatisfactory blanks. However a satisfactory blank was obtained when no water was used in the procedure, hence, it became evident that the presence of impurities was associated with the passage of water sample through the column (56). These findings were in agreement with the results given by Jahangir and co-workers (57). They found that the ability of the styrene-divinylbenzene resins to retain aromatic compounds from aqueous solution decreased with increasing

time of contact with water. These drawbacks seem to preclude the use of XAD-2 resin for the detection of very low levels of organics in water.

Tenax-GC:

Tenax is poly-(2,6-diphenyl-phenylene oxide) with a specific surface area of 19-30 m^2/g and an average pore radius of 720 $\mathring{A}$ (58).Tenax is commonly used for purge-and-trap methods due to its high temperature stability (up to 320 C) which makes it also a suitable sorbent to use for adsorption / thermal procedures for extracting organic compounds directly from water(59). The use of Tenax became popular after the early report by Leoni et al. (60). They employed Tenax for the extraction of pesticides and polycyclic aromatic hydrocarbons. The recovery of the PAHs used in this study (anthracene, perylene, and indeno(1,2,3-c,d)pyrene) at concentration of 0.1 ppb was in the range of 86-96%, and that of pesticides was found to be about 80%. PAHs were extracted with 30 ml of acetone, and the extract was concentrated to 0.2 ml. Conditioning of the sorbent is usually done by solvent extracting twice for 3hr with refluxing ether and then thermally desorbing for 3hr at 280 C with helium gas or by the passage of 0.5L of 50:50 hexane / acetone through the cartridge followed by thermal desorption (61). Beside the lengthy conditioning procedure require prior to the extraction, another drawback of tenax is its low capacity, which makes the use of tenax restricted only to those samples with low analyte concentration.

Polyurethanes:

Two types of polyurethane polymers, open-pore polyurethane (opp) and porous polyurethane foam, are the most widely used polymers of this type for preconcentrating organic compounds from water. Open-pore polyurethane consists of agglomerated spherical particles (1-10μm in diameter) bonded to each other in a rigid, highly permeable structure (62). It is usually prepared by step

growth polymerization of polyisocyanates and polyols in glass columns (63). Opp is compatible with organic solvents, dilute acids and water, however, bases hydrolyse the polymer / glass bonds easily. Also, their use for preconcentrating PAHs appeared to have a limited life. Passing a large volumes of water (12-24L) broke the opp-column bond (63). Navratil et al. (63) evaluated the use of opp columns for the concentration of PAHs from water. Prior to use, the column was rinsed with 100 ml of heptane, 20 ml of methanol, 10 ml of distilled water. One liter of water containing six PAHs (benzo(a)pyrene, pyrene, biphenyl, fluoranthene, naphthalene, and phenanthrene) was passed through the column, and the adsorbed PAHs were eluted by 10 ml methanol. The recovery of PAHs studied was affected by the flow-rate of the sample through the column and by the initial concentration of the analytes in the sample. An increase in the flow-rate decreases the adsorption. A significant change in the recovery occurred when the concentration was increased from 1ppb to 5ppb. For example, the recovery of phenanthrene was 92 and 58 % for 1ppb and 5ppb concentrations respectively. When opp was compared with XAD-2 for concentrating pyrene from water sample, the recoveries were higher using opp-column than XAD-2 resin (63).

Porous polyurethane foam was also studied as a material for the concentration of PAHs from water samples (64-65). The recovery of benzo(a)-pyrene from spiked distilled and tap water was 91 and 65 % respectively (64). The lower efficiency was suggested to be linked to the presence of suspended particles since the tap water after filtration gave similar recoveries to distilled water. It was also recommended that the water should be heated to 60-65 C to desorb benzo(a)pyrene from the suspended particles. Basu et al. (65) investigated the use of polyurethane foam as a replacement for carbon for concentrating PAHs. Several impurities from the foam were leached during the elution process. It was suggested that these impurities could eliminated by precleaning the foam

plugs which involved extracting in a Soxhlet extractor for 24hr with acetone. In this study, 60L of water was passed through the precleaned column. PAHs were eluted with 30ml of acetone, 125 ml of cyclohexane, and the extracts were combined and concentrated. The recovery of the six PAHs was over 90 %, however, for naphthalene, it was found that polyurethane foams are not as effective as XAD resin sorption.

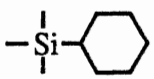
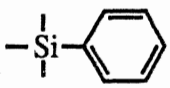
Bonded Silicas :

Advances in sample preparation have been made mainly through the application of the bonded silica chemistry transferred from HPLC column technology to sample preparation (40). Bonded silicas are the most popular supports in reversed-phase liquid chromatography. The development of reversed-phase sorbents for use in solid phase extraction made possible the removal of hydrophobic compounds from aqueous solution. May et al. (66) first reported the use of the reversed-phase liquid chromatographic support for the extraction of organic compounds. C18 bonded phase was used to extract aromatic hydrocarbons from water. In spite of the low recovery of some PAHs studied (e.g.. Benzo(a)pyrene 58%), the advantages of the C18 reversed-phase were numerous. The technique was capable of isolating non-polar hydrocarbons from polar compounds interferences with extreme simple operating conditions so that the time involved in the work-up of the sample was reduced from days to hours.

Many types of bonded silica sorbents are available with different selectivity from numerous suppliers. Table (2) lists the various bonded silica phases available for the extraction of organic compounds. They are grouped according to functionality, non-polar and polar sorbents (67). Each bonded phase is specifically used to isolate a particular chemical functional groups. Selection of the appropriate sorbent depends on the type of the analyte of interest and the

sample matrix. Generally a stationary phase of similar polarity to the compound of interest is used with the sample dissolved in a solvent of opposite polarity. Non-polar sorbents are used extract hydrophobic compounds from polar solvents such as water.

Table 2: Bonded silica phases available for solid phase extraction

	Extraction column	Structure	Analyte Functional Groups	Matrix
Non-polar Extraction	C18 Octadecyl	$-\overset{\vert}{\underset{\vert}{Si}}-C_{18}H_{37}$	*Hydrophobic Groups :*	*Aqueous :*
	C8 Octyl	$-\overset{\vert}{\underset{\vert}{Si}}-C_{8}H_{17}$	-Aromatic rings -Alkyl chains	-Water -Buffers -Biological Fluids
	C2 Ethyl	$-\overset{\vert}{\underset{\vert}{Si}}-C_{2}H_{5}$		
	CH Cyclohexyl			
	PH Phenyl			
Polar Extraction	CN Cyano-propyl	$-\overset{\vert}{\underset{\vert}{Si}}-(CH_2)_3CN$	*Hydrophilic Groups :*	*Non-polar :*
	2OH Diol	$-\overset{\vert}{\underset{\vert}{Si}}-(CH_2)_3OCH_2\underset{OH}{\underset{\vert}{CH}}-\underset{OH}{\underset{\vert}{CH_2}}$	Hydroxyls Amines Heteroatoms (S, O, N)	Hexane Oils Chloroform Lipids
	SI Silica	$-\overset{\vert}{\underset{\vert}{Si}}-OH$		
	NH_2 Amino-propyl	$-\overset{\vert}{\underset{\vert}{Si}}-CH_2CH_2CH_2NH_2$		

Octadecyl (C18) bonded porous silica is the most common sorbent used for the solid phase extraction of organic compounds from aqueous solutions. All the methods described for the liquid-liquid extraction of an analyte from an aqueous phase into an organic solvent are also applicable to solid phase extraction with C18 bonded silica (38). Rostad and co-workers (68) used bonded silica as an alternative to standard liquid-liquid extraction methods. The procedure involved passing a 50-100 ml of the water sample through a bonded-phase extraction column, eluting the adsorbed analytes from the column with 2-4 ml of solvent (1ml of acetonitrile and 2ml of dichloromethane), and evaporating the extract to 0.1 ml with a stream of dry nitrogen, after which the sample was ready for analysis. Even though the recoveries of both methods were comparable, the solid phase extraction method was much simpler than the liquid-liquid partition technique. Also, C18 bonded silica was compared with the standard EPA method 625 for extracting organic compounds from water (69). Method 625 is one of the analytical methods recommended by the Environmental Protection Agency for monitoring of priority pollutants (70). It requires the use of liquid-liquid extraction procedure. 1 to 2 liters of water sample are extracted with methylene chloride using a separatory funnel techniques. The combined extract is then dried and concentrated by evaporation using Kuderna-Danish apparatus. When the EPA method was compared to the C18 bonded silica, the cartridge extraction (C18) method gave recoveries which were equal or better than the recoveries of EPA method (625). The recoveries of naphthalene, acenaphthalene, phenanthrene, pyrene, and chrysene using EPA method (625) were 48, 59, 73, 52, and 49 respectively, whereas recoveries using C18 bonded silica were 83, 70, 67, 52, and 54 respectively. It was found that the cartridge sorbent extraction method has several advantages over the liquid-liquid extraction.

(A) The sampling of water can be undertaken in the field.
(B) The evaporation step is eliminated.
(C) The labor, facilities, and equipment required for extraction is reduced.
(D) Elution procedures yield a fewer interferences than liquid-liquid extraction.

C18 bonded silica found extensive use during the past few years due to the numerous advantages mentioned and to its availability in a small disposable cartridges from various suppliers. The packing materials of solid phase extraction have particle sizes from 30 to 60μm packed into plastic cartridges formed from highly purified polypropylene (to eliminate the leaching of plasticizers) and sandwiched between two porous frits with pore diameter of ca. 20μm. The bottom end of the cartridge is usually terminated in a Luer fitting so that it can be easily connected to a sampling manifold or a syringe needle (42). A wide range of sample size, flow rate through the column, cartridge size, and volume of eluting solvent has been reported for bonded silica extraction (71). Water sample from 20-2000 ml, flow rates from 2-200 ml/min., cartridges sizes from 100-1200 mg, and volume of eluting solvents from 0.1-5 ml have been used.

Applications of bonded silica to environmental waters have included extraction of pesticides (72-76), herbicides (77-80), priority pollutants (81-82), polychlorinated biphenyls (PCBs) (83-84), and polycyclic aromatic hydrocarbons (PAHs) (68-71, 85-86).

Octadecyl (C18) bonded silica was evaluated for the extraction of organochloine pesticides such as aldrin, endrin, HCB, heptachlor, lindane from water (76). The method gave results with spiked tap water similar to those obtained employing solvent extraction. However, the extraction of pesticides with C18 sorbent required less solvent, faster and easier to perform than that solvent

extraction method. The effect of the eluting solvent on the recovery of pesticides was investigated (87). Ethyl acetate was found to yield the best recoveries compared to hexane and light petroleum. The use of larger amounts of Octadecyl-silica and / or larger volume of eluent did not result in improved pesticide recoveries (75). Also herbicides including Simazine, Atrazine, Propazine, and Cyanazine are extracted from water with C18 bonded silica (77). The recoveries of the herbicides investigated were over 90%, and they were extractable over a broad concentration rang from 50 to 5000 ng/l. When compared to XAD-2 resin, the C18 boned silica gave better recoveries.

The efficiency of recovery of polychlorinated biphenyl (PCBs) from water samples using disposable cartridges of C18 bonded silica was investigated in the concentration range from 0.01 to 10 ppm (84). The mean recovery percentage was found to be 95.2%. Hexane was used as an eluting solvent of PCBs from the cartridge. The effect of the initial concentration on the recovery of PCBs was also examined. At low PCBs concentration, ranging from 0.01 to 1 ppb, high and reproducible recovery percentages were obtained. Thus, the adsorption of PCBs onto C18 cartridges is efficient enough to retain a significant amount of PCBs present in the analysed water. However, at higher PCBs concentrations, ranging from 2 to 10 ppb, the extraction efficiency decreased to 50%. The drop of efficiency was explained by the solubility limits of PCBs in water. On-line capillary gas chromatography extraction of PCBs employing C18 column was studied (83). The retained PCBs were eluted by hexane directly into the gas chromatograph after a brief period of N_2 flushing to remove the residual water from the precolumn.

The use of bonded silica mainly C18 cartridges for the extraction of PAHs from aqueous samples has been the subject of many papers (68-71). High

recoveries of the low molecular weight PAHs are usually obtained. When 100 ml of spiked water was forced through a cartridge containing 100 mg C18 bonded silica at a flow rate of about 25 ml/min., and eluted with 0.1 ml of benzene, recoveries of naphthalene, acenaphthylene, acenaphthene, phenanthrene, and fluoranthene were 83, 88, 89, 97, 90% respectively (71). The recovery of the low molecular weight PAHs using C18 cartridge was compared to the recovery employing solvent extraction (71). Comparative results were obtained using both methods as shown in table 3

Table 3: Comparison of PAH recovery using solvent and solid phase extraction, (10 ppb concentrations)

PAHs	Solvent Extraction	C18 Bonded Silica
Naphthalene	23	22
Acenaphthylene	60	69
Acenaphthene	391	376

The effect of several factors on the use of bonded silica for the extraction of PAHs from water samples have been examined. When longer drying time of the cartridge to remove the residual water were used, the recoveries decreased indicating that losses were occurring due to evaporation (86). However, partial drying of the cartridges for only 30 s and equilibration of the ethyl acetate for several minutes before collecting the elute enhanced the recoveries. The flow rate of the sample through the column showed no significant effect on the efficiency of extraction of PAHs (86). This was also observed by other investigators (88-89). Thus, the flow rate does not have to be closely controlled to achieve efficient adsorption. The effect of the initial concentration of the sample on the recovery

was also studied (68). The recoveries of fluorene, phenanthrene, and anthracene were less at higher concentration (200 ppb) than at lower concentration (50-100 ppb). The use of different types of non-polar sorbents for the preconcentration of PAHs was evaluated. The octyl-, octadecyl-, cyclohexyl-, and phenyl-bonded phases showed similar recoveries for PAHs (68). For example, the recoveries of fluorene using octyl-, octadecyl-, cyclohexyl-, and phenyl-bonded silica were 67, 68, 68, 64% respectively. However, Chladek et al. (69) showed that use of C8 sorbent gave better recoveries than C2, C4, and C18 sorbents

Reproducibility of the bonded silica sorbents is the major drawback of the procedure. It usually reduces the confidence of the technique. Variation in separation factor from lot-to-lot were measured for 24 different manufacturer's lots (90). It was found that anthracene / phenanthrene showed substantial lot-to-lot variation in separation factors. Puyear et al. (81) showed that the reproducibility between individual C18 cartridges of the same lot number average ±5%. The reproducibility observed on four columns prepared from the same lot number suggested that reliable results could obtained. However, when two different lots of C18 bonded silica were compared, the average recovery efficiencies of hydrocarbon standards studied were 78% and 63%. Over the past few years manufacturers have improved batch-to-batch reproducibility (91).

Gas Chromatography Determination of PAHs

The choice of any chromatographic method depends mainly on the type of compounds to be determined. Gas chromatography is usually the technique of choice for the separation of thermally stable and volatile compounds. The required properties of gas chromatography (GC) for the separation of PAHs are selectivity toward individual compounds and thermal stability of the stationary phase over the range of temperature necessary to elute all PAHs. The main reasons for the wide application of GC for the separation of PAHs, particularly when it is coupled with mass spectrometry, are high sensitivity and its high separation power. The importance of isomer-specific identification of PAHs , as discussed earlier, required the use of capillary GC/MS for the analysis of complex PAH mixtures (92-93).

The flame ionization detector (FID) and photoionization detector (PID) are commonly coupled with GC for the analysis of organic compounds in water. FID responds with high sensitivity to essentially all organics. For the determination of PAHs, FID is sensitive to low levels. However, it is not selective toward PAHs since it responds proportionately to the number of $-CH_2-$ groups introduced into the flame (94). Acheson et al. (26) were able to detect 10 ng amounts of most PAHs including benzo(a)pyrene and chrysene with FID. The application of a GC-PID system to the analysis of PAHs has been reported (95). It was found that PID system is 10 to 40 times more sensitive to PAHs than the flame ionization detector.

The use of other chromatographic techniques such as High Performance Liquid Chromatography (HPLC) for the analysis of PAHs has been investigated (96-97). The peak capacity of HPLC columns are considerably inferior to capillary GC (97). The capillary GC column possesses a much greater resolving power, in terms of plate number, than the HPLC column so that many more compounds can be separated and detected when capillary GC is employed. Only about 20 PAH peaks can be resolved by HPLC without overlap (96). The complete analysis of environmental samples with as many as 200 PAHs requires the use of capillary GC (98).

Chapter 2:

Experimental Section

Instrumentation

A Hewlett-Packard Model 5890 gas chromatograph coupled with a quadrupole mass spectrometer (Model 5970) with fused silica capillary columns, with (SUPELCO SPB-5, 5% diphenyl, 94% dimethyl, 1% vinyl polysiloxane), and (Hewlett-Packard Ultra 1, cross-linked methyl silicone) as stationary phases was used in the study of PAH determination. Both columns had an approximate length of 25 m, inner diameter of 0.2 mm, and film thickness of 0.33 μm (25m x 0.2mm x 0.33μm). The gas chromatograph was equipped with Model 7673A autosampler, split / splitless injector, and Model 300 computer for data handling. The mass spectrometer was used in the selective ion monitoring (SIM) mode except for the studies of interferences in which the SCAN mode was employed. Helium (supplier: Linde "Zero gas") was used as the carrier gas, the flow rate was set at 0.8 ml / min.

The optimized temperature program used in the determination of PAHs is shown in Table 4, with variations in the initial column temperature depending on the solvent used for injection.

Table 4: Temperature Program

Initial Temp.	Initial Time	Rate (°C/min)	Final Temp.	Final Time
Variable	4.00	20.0	170	0.00
		5.0	190	0.00
		30.0	220	0.00
		20.0	260	25.00

Injection Port Temp.: 260°C.
Transfer Line Temp.: 260°C.

Reagents:

All solvents and standards were obtained from commercial sources and used without purification. Solvents used in this study were p-xylene (b.p. 138 C), o-xylene (b.p. 144 C), toluene (b.p. 110.6 C), methanol (b.p. 65 C), and dichloromethane (b.p. 40 C) (Fisher Scientific, HPLC Grade); butanol (b.p. 117.5 C), pentanol (b.p. 137.3 C), cyclopentanol (b.p. 140.8 C), hexanol (b.p. 158 C), nonane (b.p. 151 C), and n-octane (b.p. 125 C) (Aldrich, HPLC Grade); benzene (b.p. 80.1 C), and ethyl acetate (b.p. 118.5 C) (BDH, Analytical Grade).

PAHs: Three commercially available solutions of PAHs (1ml) were obtained from SUPELCO (Oakville, Ontario).

Standard 1: a mixture of 16 PAHs with different concentrations in benzene and dichloromethane (50:50) as listed in Table 5.

Standard 2: a mixture of 16 PAHs (2000 μg/ml of each of PAH in benzene and dichloromethane (50:50)) as listed in Table 5.

Standard 3: a mixture of 13 PAHs (500 μg/ml of each of PAH in dichloromethane) as those listed in Table 5 except naphthalene, acenaphthene, and fluoranthene.

Each of standard 1 and 2 were dissolved in methanol to give stock solutions of 20 μg/ml of the PAHs. Standard 3 was dissolved in dichloromethane to give a stock solution of 5 μg/ml of each PAH. These stock solutions were used to prepare solutions with various concentrations in different solvents.

Table 5: Concentrations of 16 PAHs in Standard 1

Component:	Concentration (µg/ml)
Naphthalene	1000
Acenaphthylene	2000
Acenaphthene	1000
Fluorene	200
Phenanthrene	100
Anthracene	100
Fluoranthene	200
Pyrene	100
Benzo(a)anthracene	100
Chrysene	100
Benzo(b)fluoranthene	200
Benzo(k)fluoranthene	100
Benzo(a)pyrene	100
Dibenzo(a,h)anthracene	200
Benzo(ghi)perylene	200
Indeno(1,2,3-cd)pyrene	100

Solid-Phase extraction

Water Samples:

Standard water samples were prepared by adding 1ml of 2 μg/ml of each of the PAHs in either methanol or dichloromethane to 100 ml of distilled water from the laboratory.

SPE Cartridges:

Three types of cartridges obtained from (Waters Assoc., Millford, MA) were used in this study. Each cartridges was made from polyethylene and was filled with a silica support having either octa-, or octadecyl-, or tri-octadecyl-silane chemically bonded to the surface. These cartridges were designed to fit onto the end of a syringe. The physical properties of these cartridges are summarized in Table 6.

Table 6: Physical Characteristics of Sep-Pak Packing Material

Packing Material	Mean Weight (mg)	Pore Size (A)	Particle Size (μm)
C8	145	125	37-55
C18	130	125	55-105
tC18	145	125	37-55

SPE Procedure:

The extraction procedure was utilized as follows. The cartridges were first conditioned by the addition of 10 ml of methanol, then flushed with 10 ml of distilled water. The water sample (50 ml) was passed through the appropriate cartridge using a Hamilton syringe at a flow rate of 3 -12 ml / min. After the preconcentration step, the cartridge was dried by passing a gentle flow of nitrogen, approximately 20 ml / min., for 15-120 seconds by using N-Evap (Organomation) evaporator. The cartridge was then eluted with 0.1-1.0 ml of an appropriate elution solvent at flow rates of about 0.3-1 ml / min An aliquot of 3 µl of the eluted solution was injected into the GC/MS for quantification. The flow rate of loading and elution was regulated with the aid of SAGE Model 355 Syringe Pump shown in Figure 1.

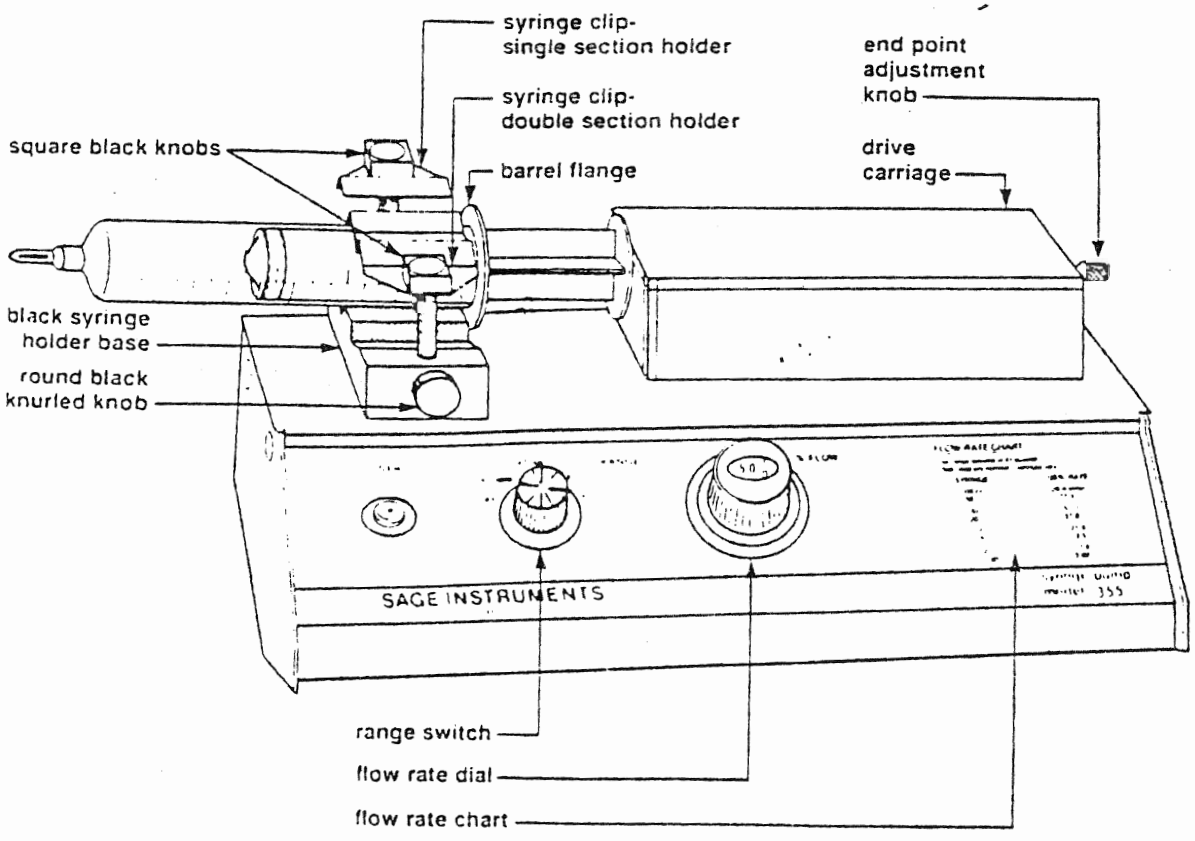


Figure 1: Syringe Pump (Sage Model 355)

Chapter 3:
Results and Discussion

Part A: Investigations into the Factors Affecting the Performance of Capillary GC/MS in the Determination of Polycyclic Aromatic Hydrocarbons (PAHs)

The effect of solvent

Solvents play an important role in governing the performance of chromatography of components on the capillary GC in the determination of PAHs It has been shown that the choice of the solvent employed has great effect on the sensitivity of PAHs particularly the late-eluting ones.(99) Higher boiling point solvents, such as xylenes and toluene, gave enhanced signals that were one to 100 times greater than those in lower boiling point solvents such as dichloromethane and hexane. Similar findings have also been reported by Lee et al. (100). PAHs had higher detector responses in high boiling point solvents (isooctane and toluene) than in low boiling point solvents (cyclohexane). Comparisons of the response factors between solvents with similar polarities such as isooctane and cyclohexane showed a great difference in the PAH signal which was due to their volatility differences. It was seen that the greater the difference in boiling point between the solvents, the more significant the difference in their PAH response. Thus, it was concluded that higher boiling point solvents are more efficient in transferring PAHs from the injection port to the column.

In this study, the possibility of employing high boiling point alcohols as an injecting solvents was examined. Several alcohols such as butanol (117.5 C), cyclopentanol (140.8 C), pentanol (137.3 C), and hexanol (158 C) were used to investigate the effect of these solvents on the performance of capillary GC.

The effect of initial column temperature:

Temperature programming has a great effect on the sensitivity and resolution of PAHs. It has been shown that the effect of initial column temperature is more significant than the effect of temperature during later stages.(99) Thus, studies were carried out by varying the initial column temperature while keeping the rest of the temperature program constant. The temperature program mentioned in the experimental section was used for the study of the effect of the initial column temperature on the solvents.

Each of the alcohols was used separately to prepare a standard solution containing 2μg/ml (2ppm) of the sixteen PAHs. An aliquot of 3μl of each of these solutions was injected into a column of 5% diphenyl, 94% dimethyl, vinyl polysiloxane (DB5) in triplicates using the splitless injection technique. The initial column temperature was varied, 20-30 C above and below the boiling point of each solvent. The mean value of both the peak area and the peak height of each of the sixteen PAHs was determined from the total ion chromatogram (TIC) with selected ion monitoring mode. The peak area and the peak height of each of the solvents were normalized relative to the optimum initial column temperature of that particular solvent. The optimum initial temperature for the late-eluting PAHs depends on the solvent employed. Table (7) and (8) summarize the relative peak area and height for the four alcohols used.

Table 7: Relative peak area of the PAHs in different solvents (%), using DB5 column

	butanol			cyclopentanol			pentanol			hexanol		
b.p. (C)	117			141			137			158		
	Initial Column Temperature											
	127	137	147	151	161	171	147	157	167	158	168	178
Peak#												
2	96	100	92	88	100	95	117	100	91	100	100	96
3	95	100	108	92	100	102	96	100	108	98	100	116
4	95	100	100	90	100	92	104	100	95	98	100	97
5	96	100	93	93	100	81	116	100	84	74	100	96
6	85	100	105	83	100	104	95	100	105	91	100	96
7	87	100	99	85	100	92	102	100	99	93	100	96
8	86	100	97	85	100	92	101	100	98	94	100	97
9	82	100	89	78	100	87	97	100	97	87	100	90
10	82	100	89	78	100	86	98	100	98	87	100	91
11	78	100	80	75	100	83	92	100	97	81	100	86
12	79	100	78	71	100	78	91	100	95	83	100	88
13	76	100	76	73	100	80	88	100	98	80	100	85
14	69	100	69	67	100	73	78	100	93	74	100	77
15	67	100	67	67	100	71	74	100	92	72	100	76
16	72	100	71	67	100	73	78	100	91	73	100	78

Table 8: Relative peak height of the PAHs in different solvents (%), using DB5 column

	butanol			cyclopentanol			pentanol			hexanol		
b.p. (C)	117			141			137			158		
	Initial Column Temperature											
	127	137	147	151	161	171	147	157	167	158	168	178
Peak#												
2	169	100	62	176	100	67	339	100	87	249	100	86
3	160	100	75	218	100	60	298	100	69	259	100	89
4	166	100	61	151	100	62	204	100	66	203	100	72
5	122	100	75	122	100	66	150	100	63	78	100	63
6	98	100	68	98	100	77	126	100	71	159	100	75
7	105	100	90	104	100	83	129	100	78	121	100	90
8	93	100	101	98	100	82	124	100	81	117	100	84
9	83	100	87	77	100	77	98	100	93	93	100	90
10	82	100	86	79	100	79	99	100	91	89	100	89
11	79	100	79	75	100	83	92	100	97	84	100	88
12	77	100	78	75	100	80	91	100	97	81	100	87
13	76	100	76	73	100	80	89	100	94	81	100	83
14	70	100	70	68	100	73	79	100	93	74	100	78
15	67	100	67	67	100	73	77	100	92	72	100	76
16	73	100	70	69	100	74	82	100	94	75	100	76

As we can see from Table (7), that the peak area of the PAHs in all the alcohols are enhanced when the initial column temperature is 10-20 C above the boiling point of the solvent, particularly the late-eluting PAHs. Table (8) also shows that the peak heights of the PAHs are affected by the initial column temperature in a similar manner. However, the peak height of the early-eluting PAHs are higher when the initial column temperature is below or the same as the boiling point of the solvent than when the initial column temperature is above the boiling point of the solvent. This would indicate that sharp peaks of the low molecular weight PAHs (up to 202) are obtained. Figure 2(a), illustrates the effect of the initial column temperature on fluorene (M.W. 166) in hexanol. As the initial column temperature increases, the peak height decreases and the peak broadens. However, the peak height of the late-eluting PAHs reaches its maximum at 20 C above the boiling point of butanol, cyclopentanol, and pentanol, and at 10 C above the boiling point of hexanol. Figures 2 (b-d) show the effect of the initial column temperature on the high molecular weight PAHs (M.W.228-278) in hexanol in which 168 C was found to be the optimum initial column temperature for injection.

The reason for the enhancement of the peak height of the low molecular weight PAHs, especially acenaphthylene, acenaphthene, and fluorene could be due to the reconcentration effect by the solvent. A prerequisite for achieving reconcentration by the solvent effect is recondensation of solvent in the column. Thus, the initial column temperature must be lower than the boiling point of the solvent used during the time period of splitless injection.(101) The result of the solvent effect is the concentration of the sample at the head of the column which r prevents peaks from being broadened. The solvent is retained by the stationary

phase and forms a solvent barrier preventing the migration of the front edge of the plug of solutes until the less volatile components enter the column. The solvent barrier reconcentrates the sample components, causing the original broad band to improve in shape.(102-103) This might explain the high peak height observed for the early-eluting PAHs, since they are more volatile than the late-eluting PAHs. Hence, they would be concentrated by the solvent for a longer period of time.

The cold trapping effect could be the reason for the enhancement of the peak height and area for the high molecular weight PAHs when the initial column temperature is higher than the boiling point of the solvent. The basis of cold trapping is that the column temperature is kept at least 150 C below the boiling point of the components,(103-104) so that the migration speed of the volatile components is reduced until the less volatile components catch up. This results in a condensed sample components band, which evaporates upon the increase of the temperature.

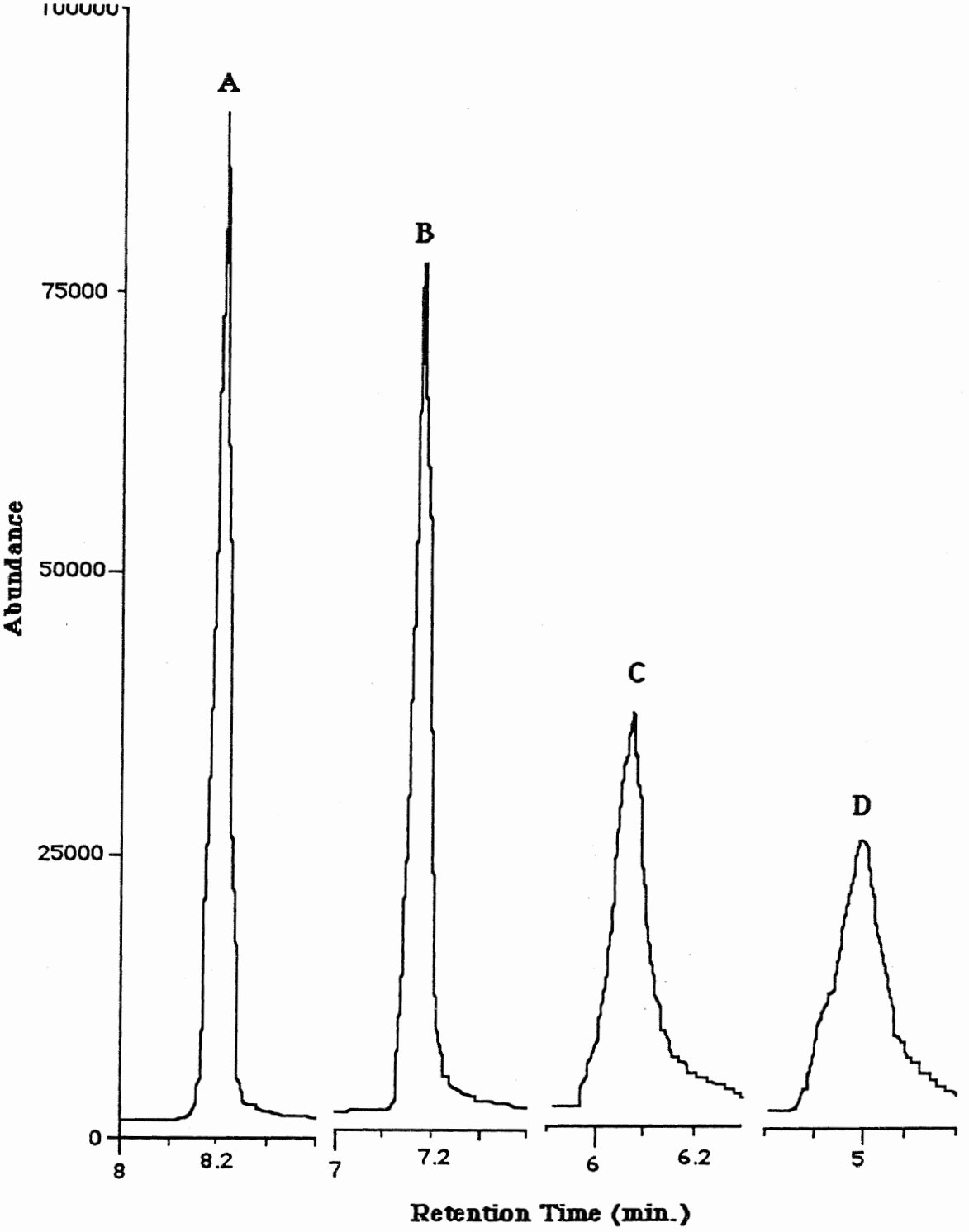


Figure 2(a): The effect of initial column temperature on the peak height of Fluorene in hexanol
Column initial temperature: (A)148 C, (B)158 C, (C)168 C, (D)178 C

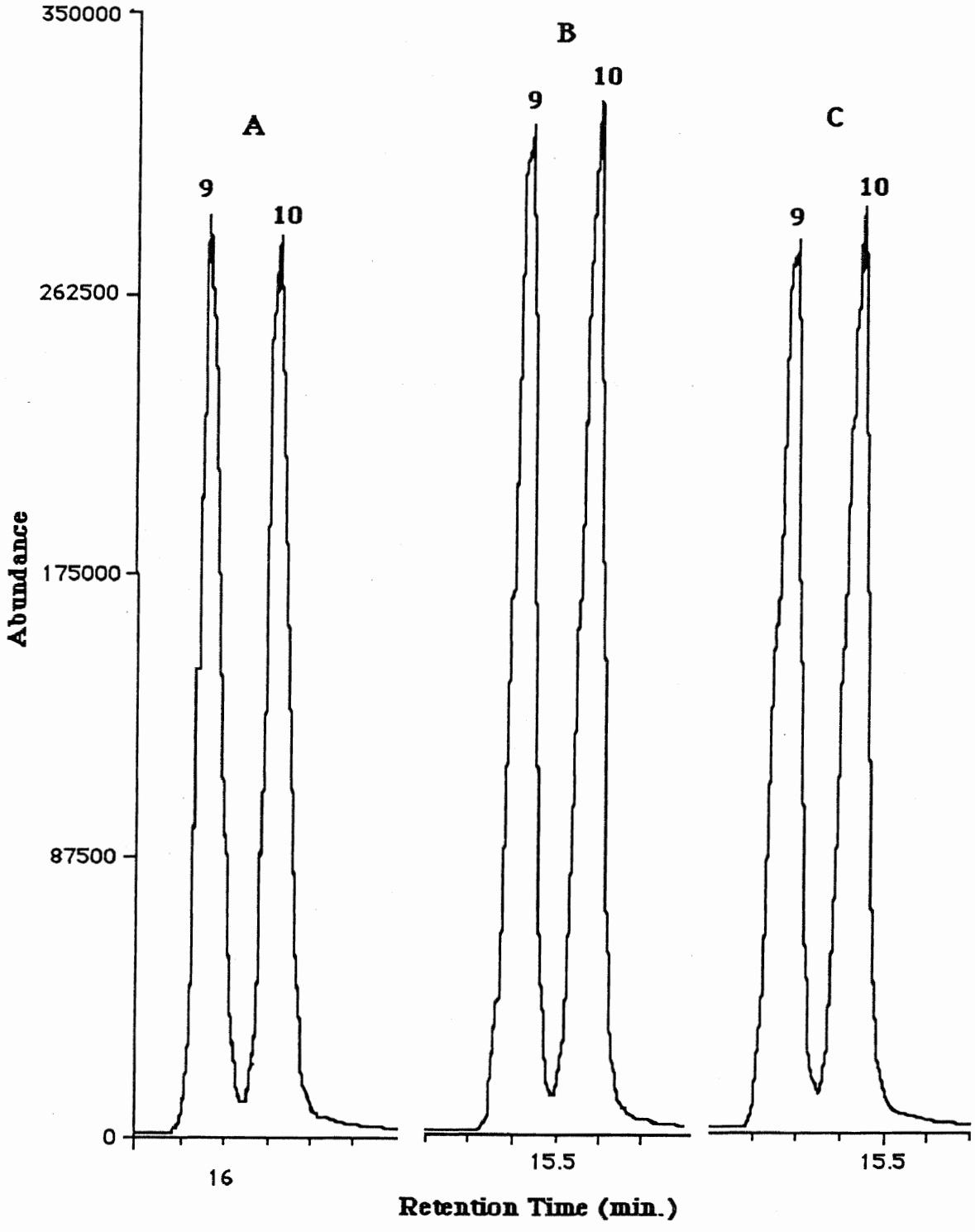


Figure 2(b): The effect of the initial column temperature on the peak height of Benz(a)-anthracene (9) and Chrysene (10) in hexanol

Column initial temperature: (A) 158 C, (B) 168 C, (C) 178 C

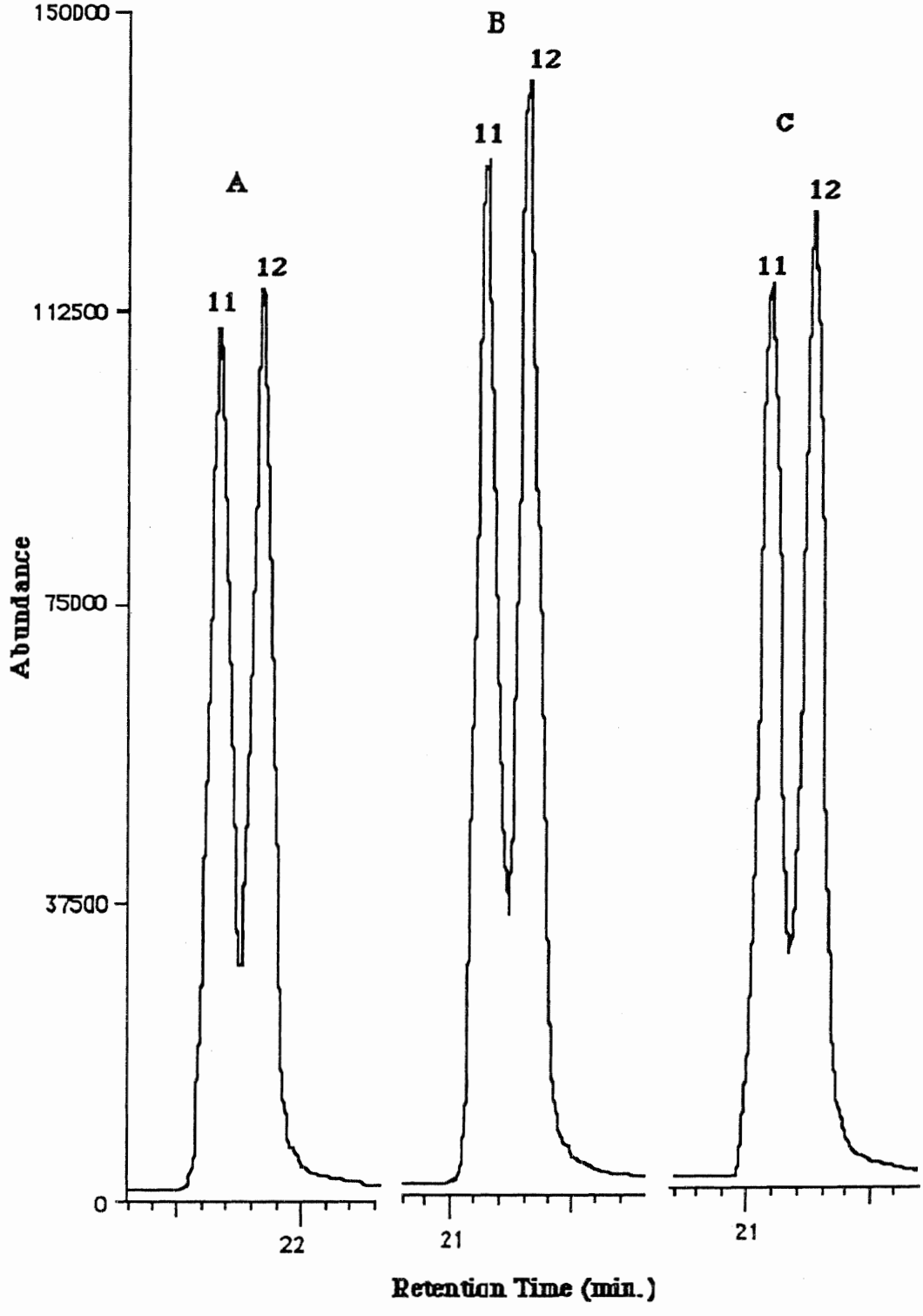


Figure 2(c): The effect of initial column temperature on the peak height of Benzo(b)-fluoranthene (11) and Benzo(k)-Fluoranthene in hexanol

Column initial temperature: (A) 158 C, (B) 168 C, (C) 178 C

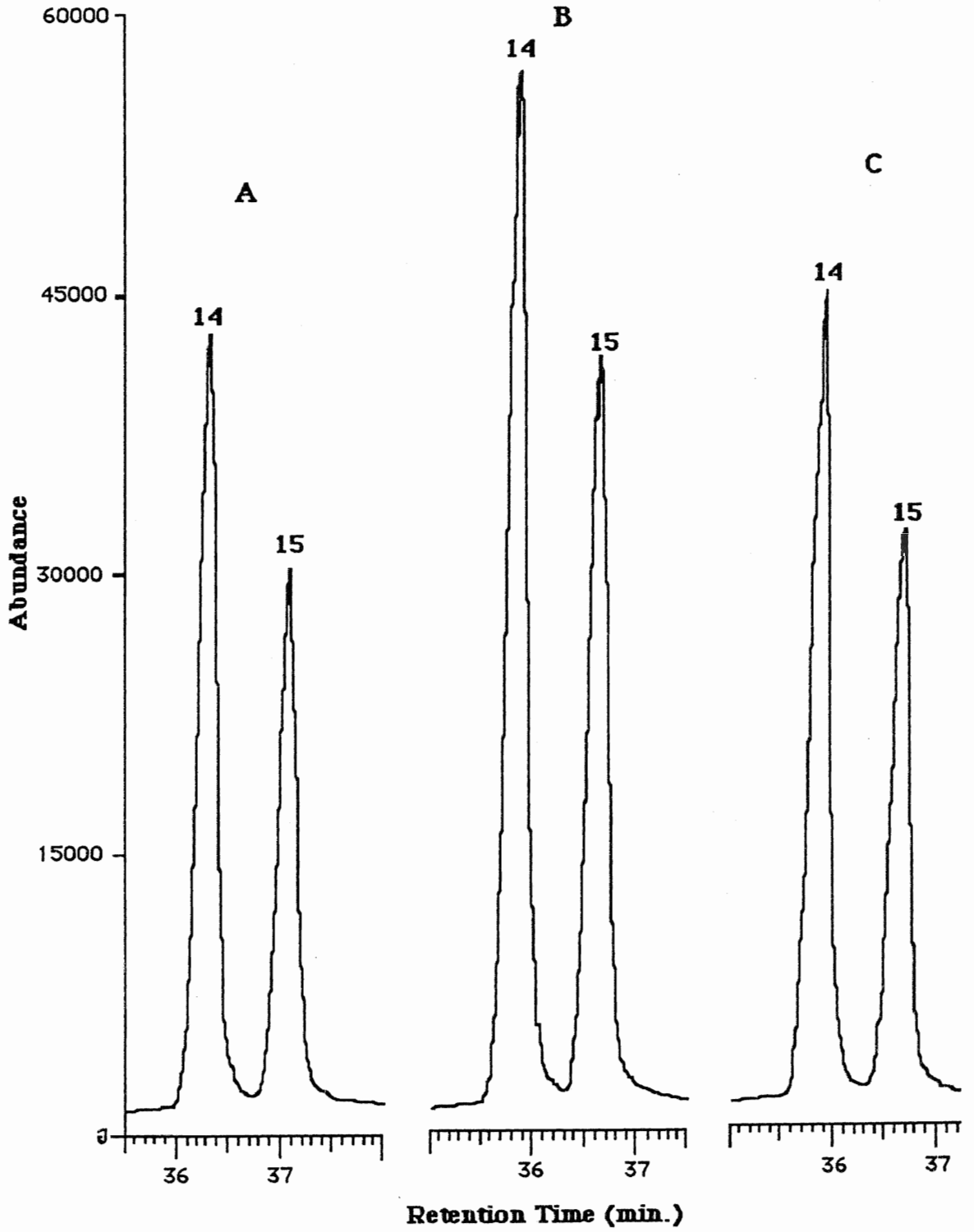


Figure 2(d): The effect of initial column temperature on the peak height of Dibenzo(a,h)-anthracene (14) and Benzo(g,h,i)-perylene in hexanol

Column initial temperature: (A) 158 C, (B) 168 C, (C) 178 C

The effect of the initial column temperature on the peak shape of the sixteen PAHs is more pronounced when the column temperature is very low or very high. It was found that the initial column temperature affects the early-eluting more than the late-eluting peaks.This is clearly demonstrated in Figure 3(a-c) which shows the effect of the initial column temperature on benzo(a)anthracene and chrysene in hexanol, when the initial temperature is 30 C lower or higher than the boiling point of hexanol. As we can see, when the initial temperature is 128 C, fronting or splitting of the peak appears,Figure 3(a). As the initial temperature is increased from 128 C to 158 C, fronting of the peak disappears and a symmetrical peak is obtained (Figure 3(b)). However, with a further increase of the initial temperature from 158 C to 188 C, tailing of the peak appears as shown in Figure 3(c).

The previous observations can be explained as follows. At an initial column temperature lower than the boiling point of the solvent, both the solvent and solutes are condensed in the column inlet. They are moved through the column by the carrier gas, which results in the separation of the solutes into two portion. One retained on the stationary phase and the other moving with the solvent. As the initial column temperature increases, the solvent evaporates and the two portions elute, resulting in splitting or fronting of the peak. When the initial column temperature is the same as the boiling point of the solvent or higher, the solvent is not condensed. Only one portion of the solute is retained by the stationary phase, so that a symmetrical peak would be eluted from the column. If the initial column temperature is too high, the solutes would not condense in the column which could result in tailing of the peak, since cold trapping would be less effective in concentrating the solutes. This could be supported be the fact that the peak shape of the early-eluted PAHs are more affected by the increase of the initial column temperature. Similar results were also obtained when butanol, cyclopentanol, and pentanol were used.

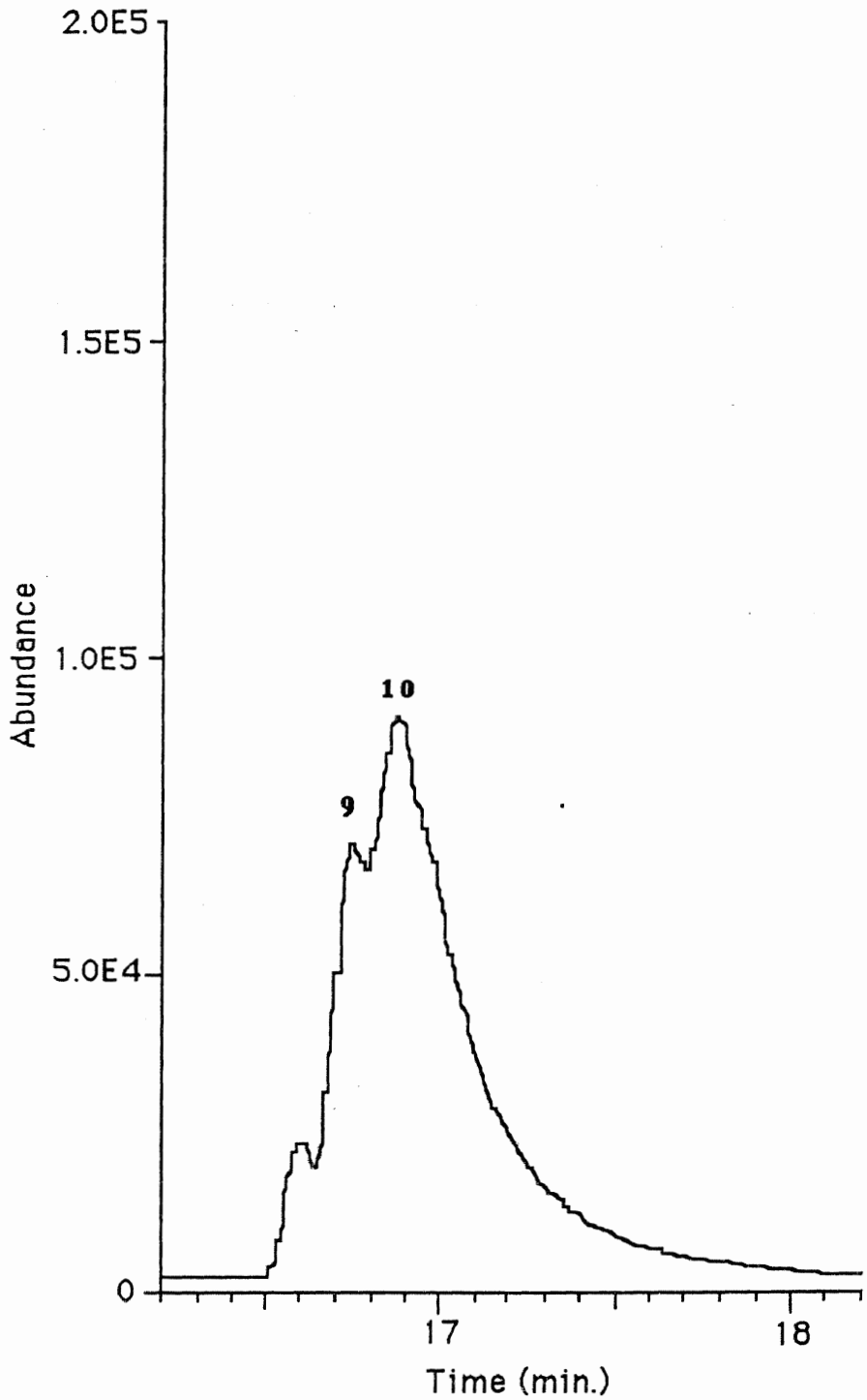


Figure 3(a): Chromatogram of Benz(a) anthracene (9) and chrysene (10) in hexanol at an initial column temperature of 128 C

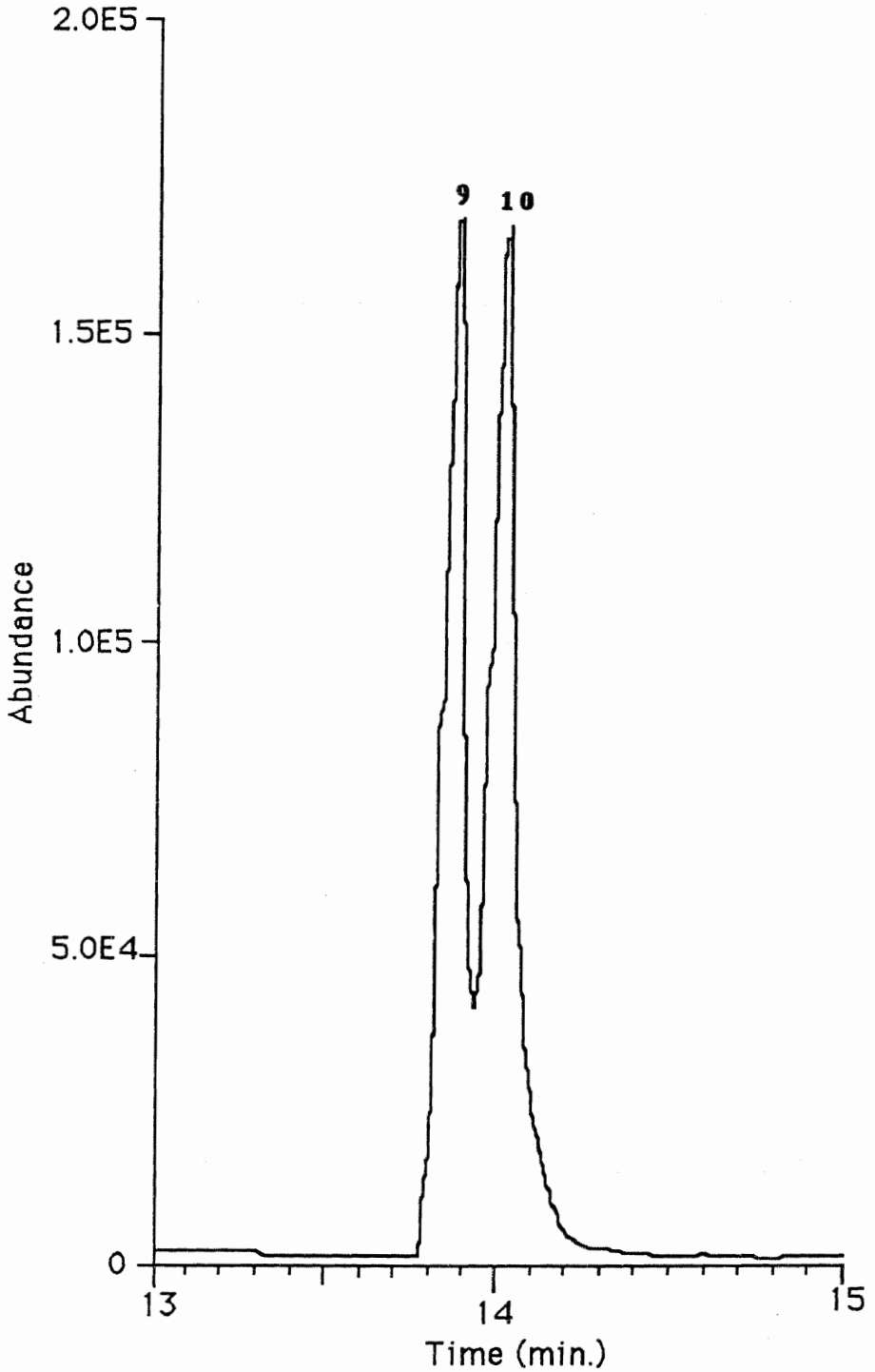


Figure 3(b): Chromatogram of benz(a)anthracene (9) and chrysene (10) in hexanol at an initial column temperature of 158 C

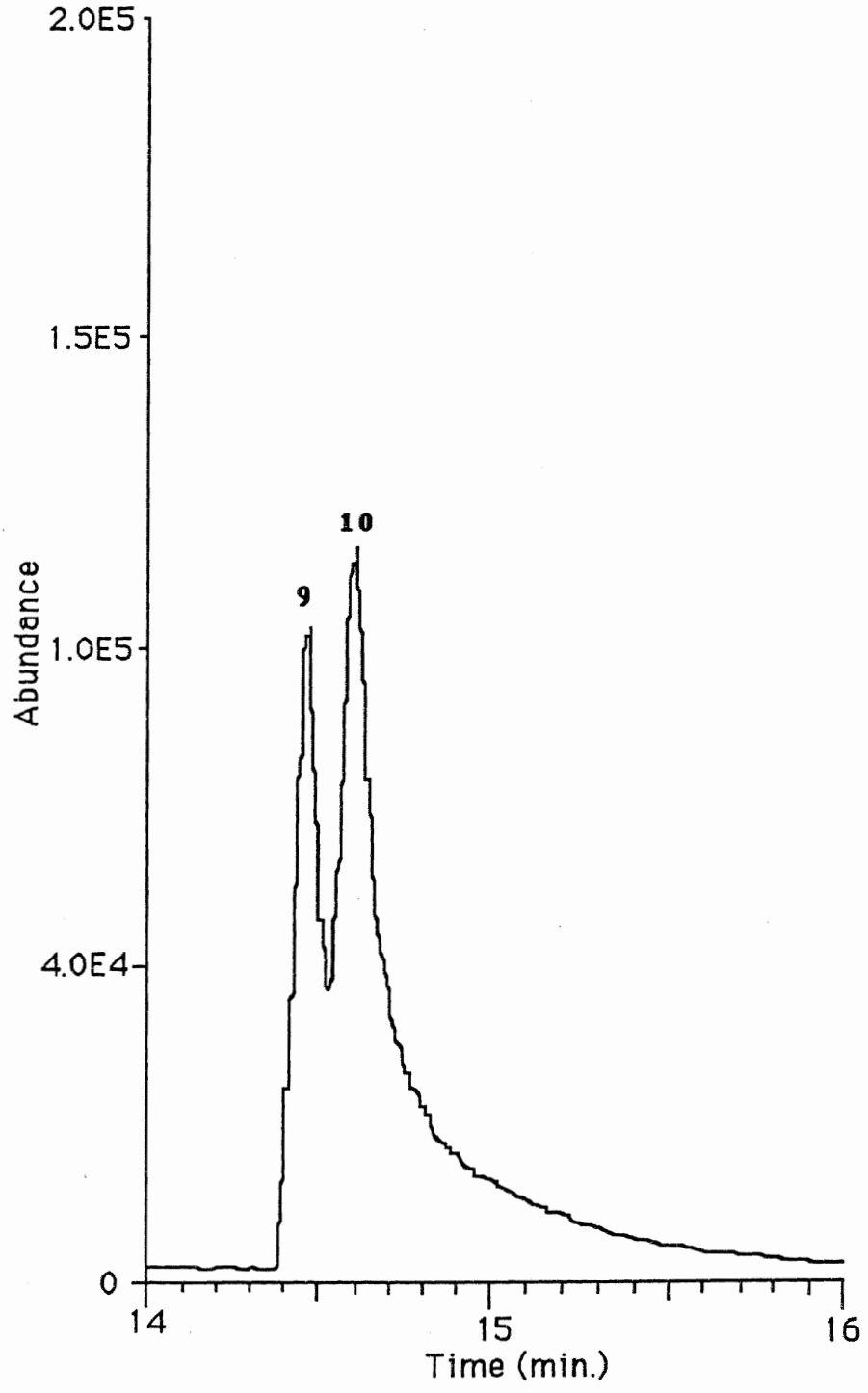


Figure 3(c): Chromatogram of benz(a)anthracene (9) and chrysene (10) in hexanol at an initial column temperature of 188 C

The effect of initial column temperature on the separation efficiency of the PAHs followed a trend similar to the effect on the peak shape, in which the early-eluted PAHs are more affected by the initial column temperature than are the late-eluted PAHs. Figure 4 shows the effect of the initial column temperature on the resolution of peak 5 and 6, 9 and 10, 11 and 12, 14 and 15 in hexanol. These pairs of peaks were chosen because they represent the closest pairs of peaks out of the sixteen PAHs. Resolution was calculated based on the following equation:

$$R_S = 2 (t_{rb} - t_{ra}) / (W_a + W_b)$$

where R_S is the resolution between peaks a and b, t_{ra} and t_{rb} are retention times of peaks a and b respectively, W_a and W_b are widths of peaks a and b respectively.

The effect of the initial column temperature on the resolution can be clearly seen in Figure 4. The effect is more pronounced in the early-eluted peaks than in the late-eluted peaks. The separation efficiency between phenanthrene and anthracene, peaks 5 and 6, increases as the initial column temperature increases up to the boiling point of hexanol, and decreases gradually with the increase of the initial column temperature above the boiling point of hexanol. The decrease in resolution when the initial column temperature is lower than the boiling point of the solvent could be due to an excessive condensation of the solvent which has been shown to cause broadening of the early-eluted peaks. (101) However, the gradual decline in the separation efficiency as the initial column temperature increases is due to the broading of the peak. At an initial column temperature above the boiling point of hexanol, 158 C, phenanthrene and anthracene with boiling points of 338 C and 340 C respectively are not likely to be cold trapped; thus the resultant peaks would be broadened. The resolution of late-eluted PAHs, peaks 11/12 and 14/15, are not significantly affected by the initial column temperature as can be seen in Figure 4. Similar results were also observed with the other solvents.

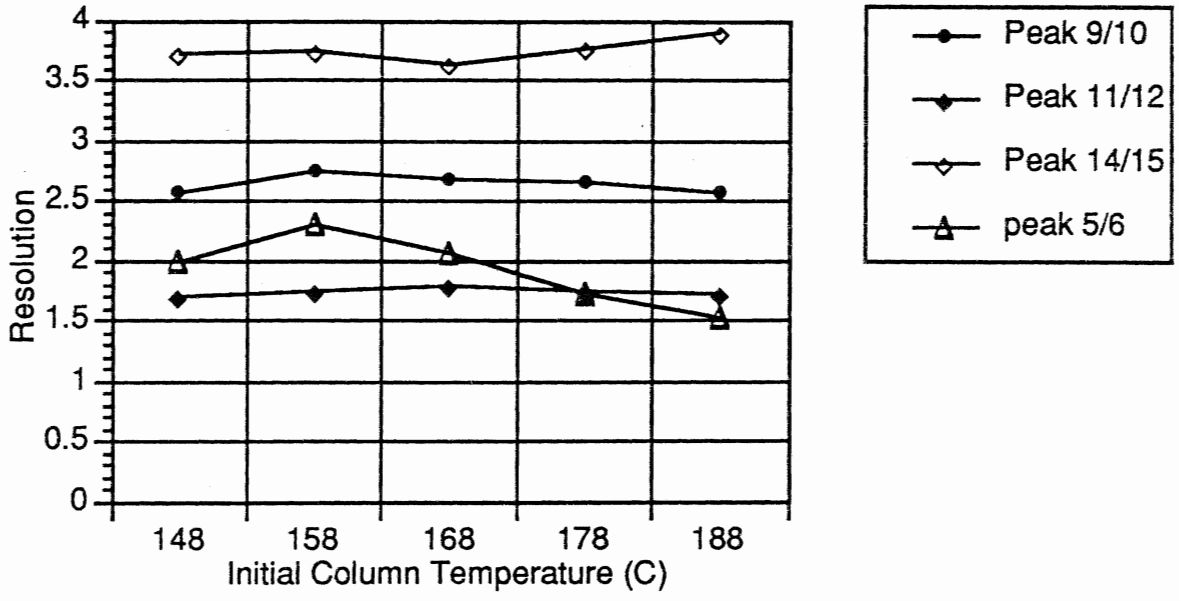


Figure 4: The effect of the initial colomn temperature on resolution (injection solvent: hexanol)

Choice of solvent:

It has been suggested that high boiling point solvents, especially p-xylene, are the most efficient for transferring PAHs from the injector onto the column.(100) Studies were carried out to compare the peak signal of the sixteen PAHs in p-xylene to those in butanol, cyclopentanol, pentanol, and hexanol. The optimum initial column temperature of p-xylene (boiling point 138 C) was found to be 158 C, which is in agreement with the previous findings of the effect of the initial column temperature on alcohols. Standard solutions of 2μg / ml of the sixteen PAHs in the five solvents were prepared, and seven replicate injections of 3μl of each the solutions were introduced onto the column by splitless injection. The mean values of the peak area and peak height of the PAHs in each of the solvents were calculated from the total ion current chromatogram, and normalized relative to 100% peak height and peak area of PAHs in hexanol. The relative peak area and peak height are summarized in Tables 9 and 10 respectively.

The relative peak area of the early-eluted peaks, from acenaphthylene to pyrene, are not significantly different in all solvents except those in butanol where the similarity in the peak area is only up to phenanthrene as shown in Table 9. However, differences in the peak area among the solvents are observed for the late-eluted peaks. Hexanol seems to give larger peak areas than the other solvents. Similar results are also observed by comparing the peak height of other solvents except that butanol and p-xylene gave greater peak heights than hexanol for the early-eluted PAHs, acenaphthylene, acenaphthene, fluorene, phenanthrene, and anthracene, as shown in Table 10. The reason for the great enhancement of the chromatographic signal for the early-eluted PAHs in butanol could be due to the initial column temperature used. At an initial column temperature of 137 C,

the optimum initial column temperature for butanol, the early-eluted PAHs are most likely to be cold trapped in the column. The peak area of these early-eluted PAHs in butanol, Table 9, seems to be in agreement with the other solvents, and only their peak height is being enhanced which could be due to reconcentration effect. It is unlikely to be the solvent effect since the initial column temperature is higher than the boiling point of butanol. However, cold trapping might be the reason for the peak height observed, since these early-eluted PAHs with boiling point between 276 C to 340 C, and retention time less than 10 minutes could be cold trapped at 137 C according to the temperature program mentioned in the experimental section.

The great enhancement of the peak height of the early-eluted PAHs in p-xylene might be due to the high affinity of the solvent to the stationary phase used. The introduction of 5% diphenyl to the stationary phase could increase the wettability of p-xylene to the stationary phase which slows the migration of the solvent. Thus, the more volatile PAHs could be concentrated.

The effect of the solvent on the separation efficiency of the closely-eluted PAHs was investigated as shown in Figure 5. As we can see, all the solvents studied show similar resolution for the late-eluted PAHs. Thus, only the peak response of the PAHs is effected by the type of solvent used. Figure 6 and 7 show the chromatograms of the fifteen PAHs in p-xylene and hexanol. Naphthalene is not shown in the figure because of the acquisition program used in which the starting time was at 3 minutes. It can be seen that the enhanced chromatographic signals are greater in the case of hexanol than that of p-xylene particularly for the late-eluting peaks. Hence, hexanol seems to be more efficient solvent to use for the injection of PAHs.

Table 9: Relative peak area of 15 PAHs (%)
(using DB5 column)

peak#	butanol	cyclopentanol	pentanol	p-xylene	hexanol
2	97	107	93	107	100
3	89	87	95	111	100
4	91	86	97	104	100
5	95	100	101	125	100
6	78	83	90	103	100
7	72	85	88	102	100
8	72	86	92	102	100
9	55	85	86	87	100
10	54	86	87	87	100
11	42	87	82	72	100
12	41	87	81	71	100
13	37	88	79	60	100
14	26	95	75	42	100
15	22	93	73	39	100
16	26	95	75	42	100

Table 10: Relative peak height of 15 PAHs (%)
(using DB5 column)

peak#	butanol	cyclopentanol	pentanol	p-xylene	hexanol
2	155	78	69	202	100
3	152	93	82	248	100
4	158	97	85	208	100
5	123	110	97	153	100
6	120	123	106	178	100
7	87	91	96	111	100
8	81	97	101	112	100
9	55	85	88	85	100
10	55	87	86	88	100
11	43	87	82	75	100
12	40	87	81	70	100
13	38	89	80	61	100
14	27	95	76	44	100
15	22	92	74	40	100
16	27	95	77	44	100

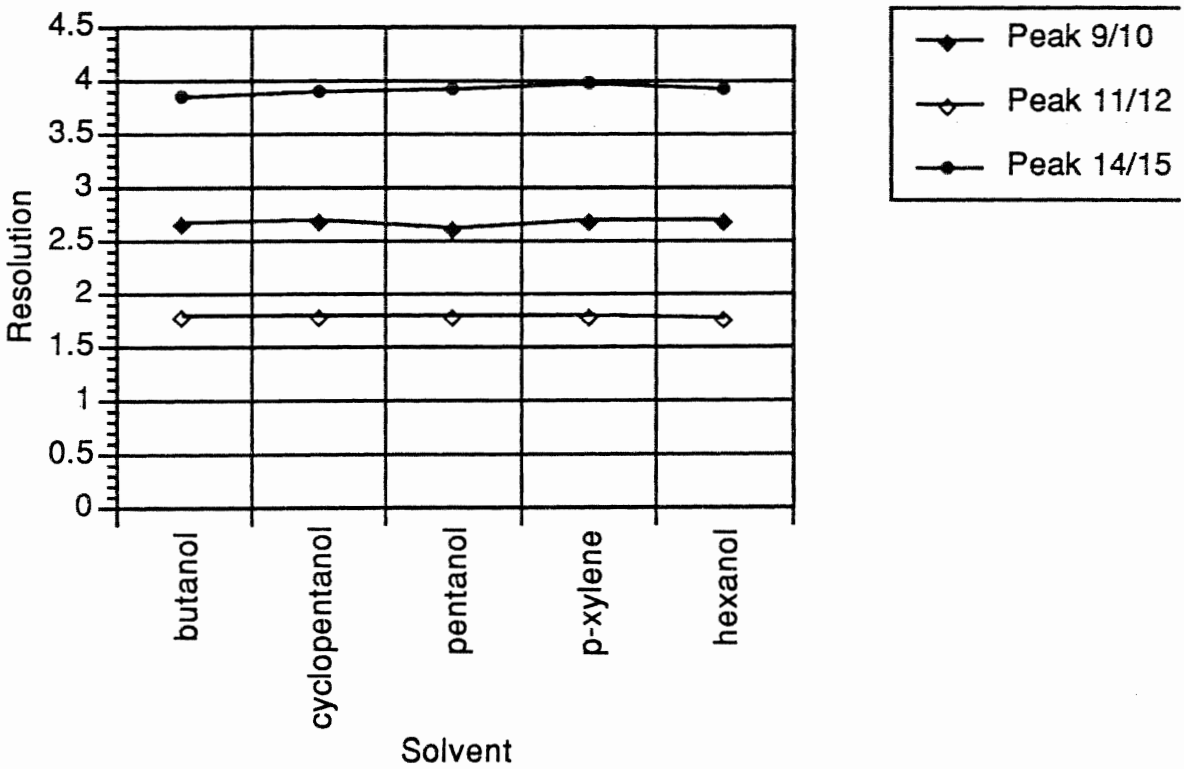


Figure 5: Effect of solvent on the resolution
(DB5 column)

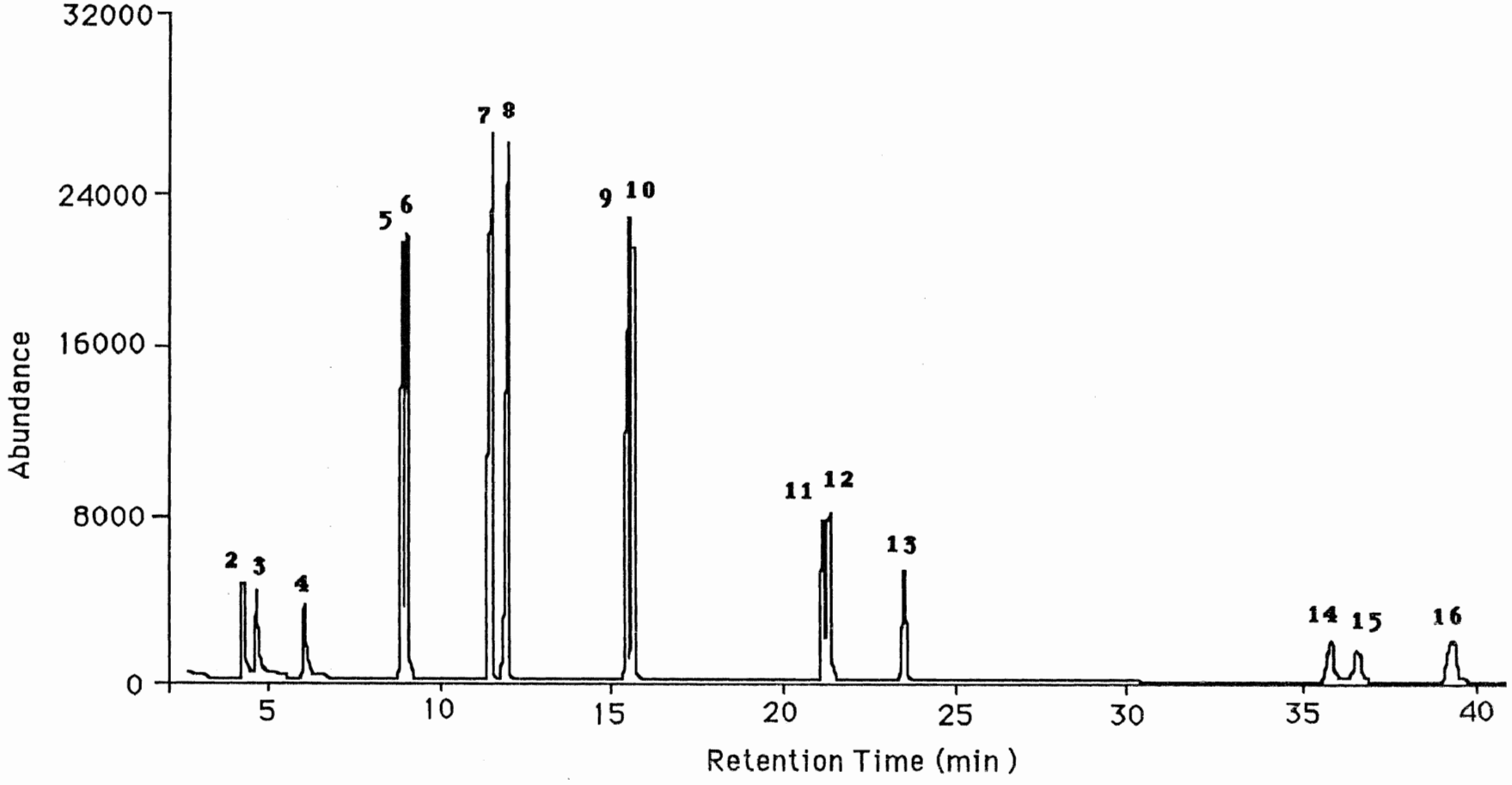


Figure 6: Chromatogram of 15 PAHs (3 µl of 2ppm) **in p-xylene**

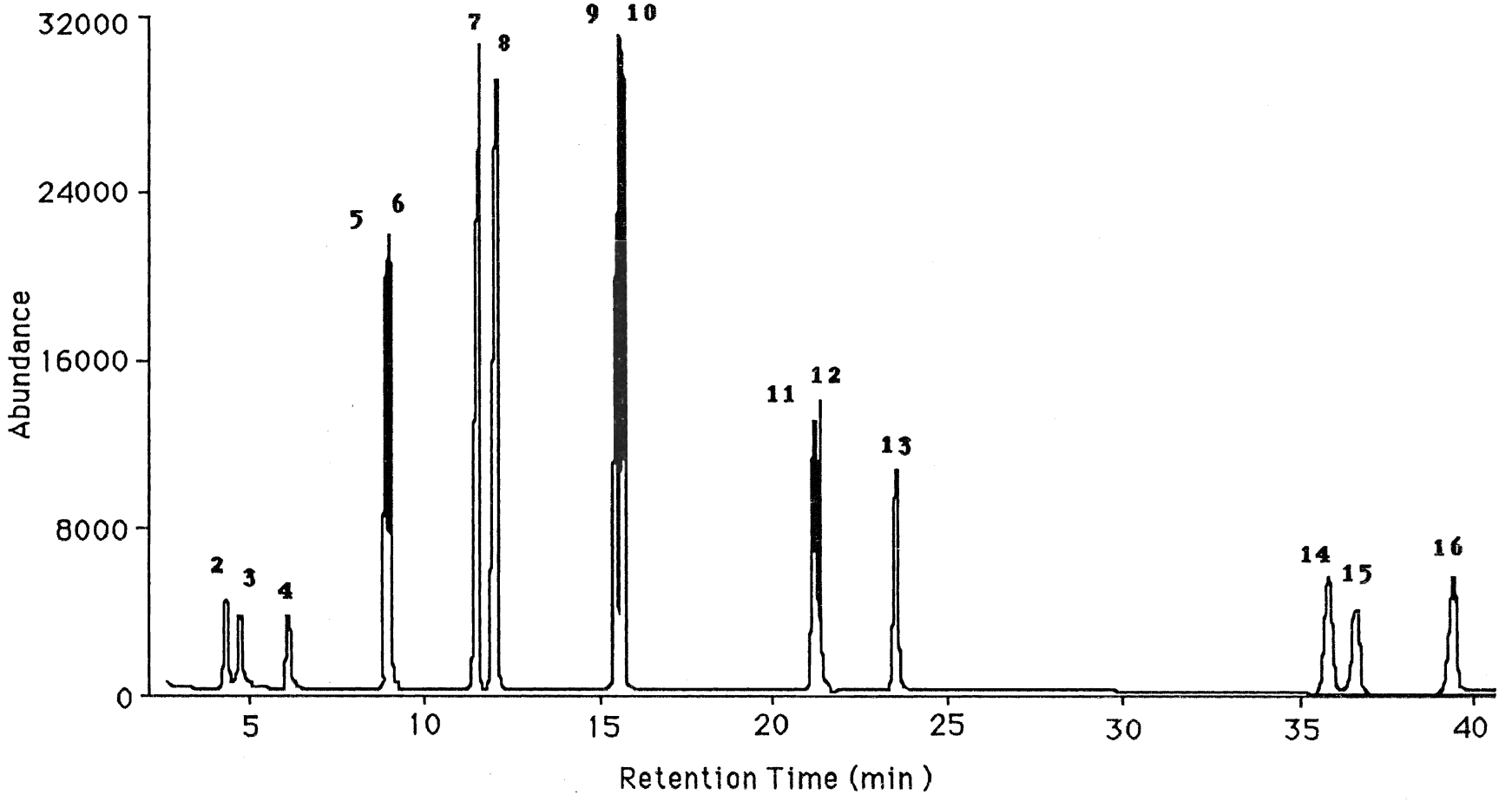


Figure 7: Chromatogram of 15 PAHs (3 µl of 2ppm) **in hexanol**

Calibration of the 13 PAHs , standard 3 in the experimental section, in a concentration range 10 ng / ml to 60 ng / ml were carried out. The correlation coefficient (r) of both the peak area and the peak height calibration curves of each of the PAHs were calculated based on five replicate injections of 3 µl of each of the standard solutions in hexanol using an initial column temperature of 168 C. The correlation coefficients of the 13 PAHs are tabulated in Table 11. The lowest correlation coefficient, 0.978, is obtained for the peak height of dibenz(a,h)-anthracene; otherwise, the results of all the different standards seem to be in good correlation. There are no significant differences in the correlation coefficients of both the peak heights and the peak areas of the PAHs studied. Table 11 also lists the detection limits (S/N=3) of all the 13 PAHs in hexanol. The detection limits were at low pg levels, ranging from 6.0 pg for fluorene to 83.6 pg for benzo(a)pyrene.

In order to determine the precision, the relative standard deviations (RSD) of seven replicate injections of 3 µl of 2 µg / ml of the 15 PAHs in p-xylene and the alcohols were calculated as shown in Table 12. The relative standard deviations of all the PAHs in the various solvents studied based on the peak area are less than 10 % except for indeno(1,2,3,-cd)pyrene, dibenz(a,h)anthracene, and benzo(g,h,i)-perylene in butanol which gave a relative standard deviations of 15%. The standard deviation of the retention times of the 15 PAHs in butanol, cyclopentanol, pentanol, and hexanol were determined based on seven replicate injection as shown in Table 13. The standard deviations are in the range of 0.01 to 0.04 except for those in butanol in which the range of the standard deviations are 0.1 to 0.8. The high standard deviations of the retention time of the late-eluted peaks in butanol is most likely to be due to the low chromatographic signals of those PAHs.

Table 11: Correlation coefficient and the detection limits of 13 PAHs in hexanol.

(calibration range: 10 ng / ml to 50 ng / ml)

peak #	Component	Correlation coefficient (r)		Detection Limit (3σ)
		by peak area	by peak height	(pg)
1	Acenaphthylene	0.998	0.993	6.6
2	Fluorene	0.997	0.996	6.0
3	Phenanthrene	0.999	0.994	9.5
4	Anthracene	0.997	0.991	11.8
5	Fluoranthene	0.992	0.989	16.6
6	Benz(a)-anthracene	0.996	0.998	24.1
7	Chrysene	0.994	0.996	21.0
8	Benzo(b)fluoranthene	0.993	0.994	18.4
9	Benzo(k)fluoranthene	0.997	0.994	19.3
10	Benzo(a)pyrene	0.991	0.997	83.6
11	Indeno(1,2,3,-cd)pyrene	0.989	1.009	57.4
12	Dibenz(a,h)anthracene	0.978	0.987	61.8
13	Benzo(g,h,i,)perylene	0.991	0.983	52.3

Table 12: Relative standard deviations based on peak area (DB5 column)

Peak #	butanol	cyclopentanol	pentanol	p-xylene	hexanol
2	2.7	3.0	2.2	2.7	3.0
3	2.6	3.2	1.0	3.3	3.0
4	3.2	2.5	2.8	2.4	2.8
5	3.4	2.5	2.0	2.9	1.7
6	3.0	3.4	0.8	3.2	2.3
7	3.7	2.7	2.5	3.3	1.9
8	3.8	2.4	2.8	3.0	1.7
9	6.8	1.9	4.8	3.4	2.1
10	6.7	1.7	4.9	3.1	2.0
11	8.6	1.9	5.8	4.0	2.8
12	7.8	2.1	5.7	3.8	2.9
13	9.9	2.8	4.9	3.6	2.6
14	15.8	4.6	2.9	7.4	5.8
15	15.6	5.4	3.3	8.4	7.1
16	15.9	5.4	2.4	7.3	6.5

Table 13: Standard deviations of the retention time of the alcohols (DB5 column)

Peak#	butanol	cyclopentanol	pentanol	hexanol
2	0.09	0.01	0.03	0.01
3	0.17	0.01	0.03	0.01
4	0.07	0.01	0.02	0.01
5	0.10	0.01	0.01	0.01
6	0.16	0.01	0.01	0.01
7	0.08	0.01	0.01	0.01
8	0.12	0.01	0.01	0.01
9	0.22	0.02	0.02	0.01
10	0.22	0.02	0.02	0.01
11	0.44	0.02	0.02	0.02
12	0.35	0.02	0.02	0.02
13	0.59	0.03	0.02	0.02
14	0.70	0.04	0.02	0.03
15	0.84	0.04	0.02	0.03
16	0.83	0.04	0.02	0.04

The effect of the solvent on the determination of PAHs was further examined by comparing the response of PAHs in hexanol to high boiling point aliphatic solvents such as n-octane and nonane. First, the effect of the initial column temperature on the performance of capillary GC in the determination of PAHs using these aliphatic solvents was investigated. Standard solutions of 2μg/ml of the sixteen PAHs in n-octane and nonane were prepared separately. An aliquot of 3μl of each of these solutions was injected into a 5% diphenyl, 94% dimethyl, vinyl polysiloxane with various initial column temperature The mean value of triplicate determinations of the peak area and the peak area of each of the PAHs was calculated from the total ion current chromatogram. Tables (14) and (15) summarize the relative peak area and peak height of each of the solvents to the optimum initial column temperature of that particular solvent. Naphthalene was not detected in nonane because the starting time of aquisition was 3 minutes according to acquisition program used, by which time both naphthalene and the solvent have eluted.

The optimum initial column temperature for injecting PAHs in n-octane and nonane was found to be similar to those observed for alcohols. The optimum initial column temperature for n-octane is the same as the boiling point of the solvent (125 C); whereas, it is 10 C higher than the boiling point of nonane (151 C). The peak height of the sixteen PAHs seem to be more affected by the initial column temperature than the peak area particularly for the late-eluted peaks. At an initial column temperature lower than the optimum, the peak height of the early-eluted peaks are similar or higher than those obtained at the optimum initial temperature, as shown in Figure 8. This could be due to the reconcentration effect of the solvent suggested previously for alcohols.

Table 14: The relative peak area of 16 PAHs (%) in n-octane and nonane (using DB5 column)

	octane				nonane			
b.p. (C)	125				151			
	Initial Column Temperature (C)							
	115	125	135	145	141	151	161	171
Peak#								
1	107	100	97	94	n.d.	n.d.	n.d.	n.d.
2	102	100	94	88	96	102	100	98
3	102	100	93	84	92	95	100	115
4	87	100	89	77	100	102	100	92
5	119	100	94	76	109	106	100	82
6	99	100	87	75	96	98	100	98
7	103	100	86	71	99	100	100	94
8	104	100	87	71	98	100	100	94
9	102	100	78	60	104	101	100	92
10	101	100	88	69	90	96	100	90
11	98	100	83	66	111	103	100	93
12	92	100	93	76	87	89	100	99
13	100	100	83	66	102	95	100	93
14	98	100	83	60	92	85	100	85
15	97	100	82	57	97	85	100	85
16	94	100	73	67	97	84	100	83

n.d. (not detected)

Table 15: The relative peak height of 16 PAHs (%) in n-octane and nonane (using DB5 column)

	octane				nonane			
b.p. (C)	125				151			
	Initial Column Temperature (C)							
	115	125	135	145	141	151	161	171
Peak#								
1	126	100	107	77	n.d.	n.d.	n.d.	n.d.
2	104	100	90	64	109	103	100	63
3	108	100	86	62	115	104	100	59
4	109	100	89	74	131	120	100	50
5	141	100	117	82	113	125	100	60
6	121	100	100	72	114	107	100	63
7	102	100	93	84	88	90	100	92
8	103	100	95	80	90	94	100	87
9	100	100	82	65	82	86	100	90
10	98	100	84	72	77	86	100	88
11	89	100	77	62	86	90	100	92
12	90	100	77	61	81	86	100	91
13	65	100	72	54	87	88	100	90
14	94	100	61	40	84	79	100	76
15	92	100	59	39	88	79	100	77
16	88	100	65	47	90	82	100	79

n.d. (not detected)

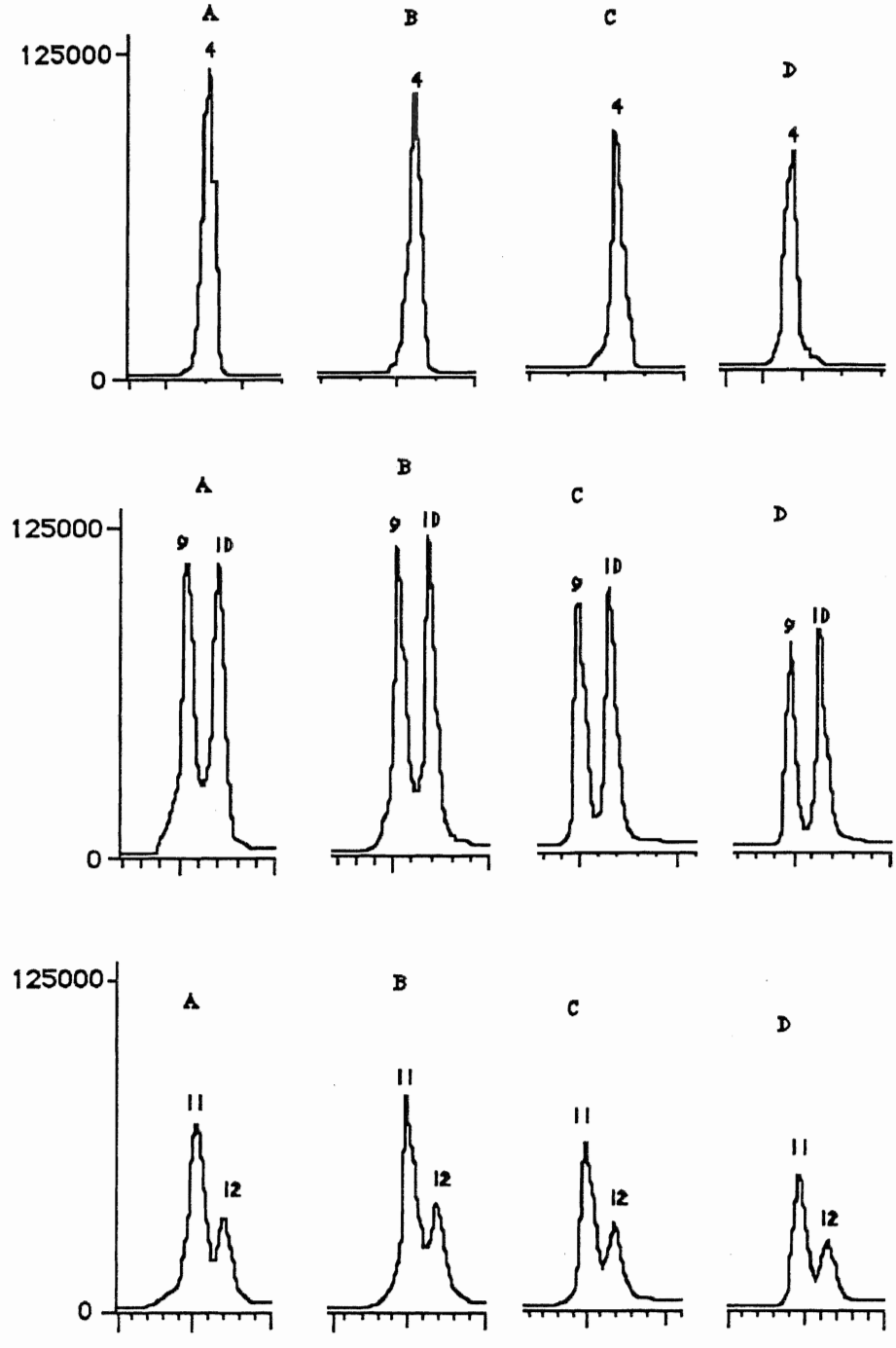


Figure 8: The effect of the initial column temperature on the peak height of Fluorene (4), Benz(a)-anthracene (9), Chrysene (10), Benzo(b)fluoranthene (11), and Benzo(k)fluoranthene (12) in n-octane

Column initial temperature: (A) 115 C, (B) 125 C, (C) 135 C, (D) 145 C

The effect of the initial column temperature on the resolution of the closely-eluted PAHs in n-octane and nonane was examined. Figure 9 illustrates the effect of the initial column temperature on the separation efficiency of peak 5 and 6, 9 and 10, 11 and 12 in n-octane. The resolution between phenanthrene and anthracene, peak 5 and 6, decreases as the initial column temperature increases over the boiling point. The decrease in the separation efficiency might be due to the broadening of the peak in which cold trapping becomes less effective as previously discussed in the case of alcohols. However, the resolution of the late-eluted peaks increases as the initial column temperature increases. This could be the result of the decrease in the peak height as can be clearly seen in Figure 8 in which better separation between peak 9, and 10 is reached as the initial temperature increases.

The relative standard deviations of seven replicate determination of the sixteen PAHs in n-octane and nonane were carried out as shown in Table 16. The precision of the results seems to be similar for both solvents. The relative standard deviations for all PAHs is less than 10% except for the last three peaks in n-octane.

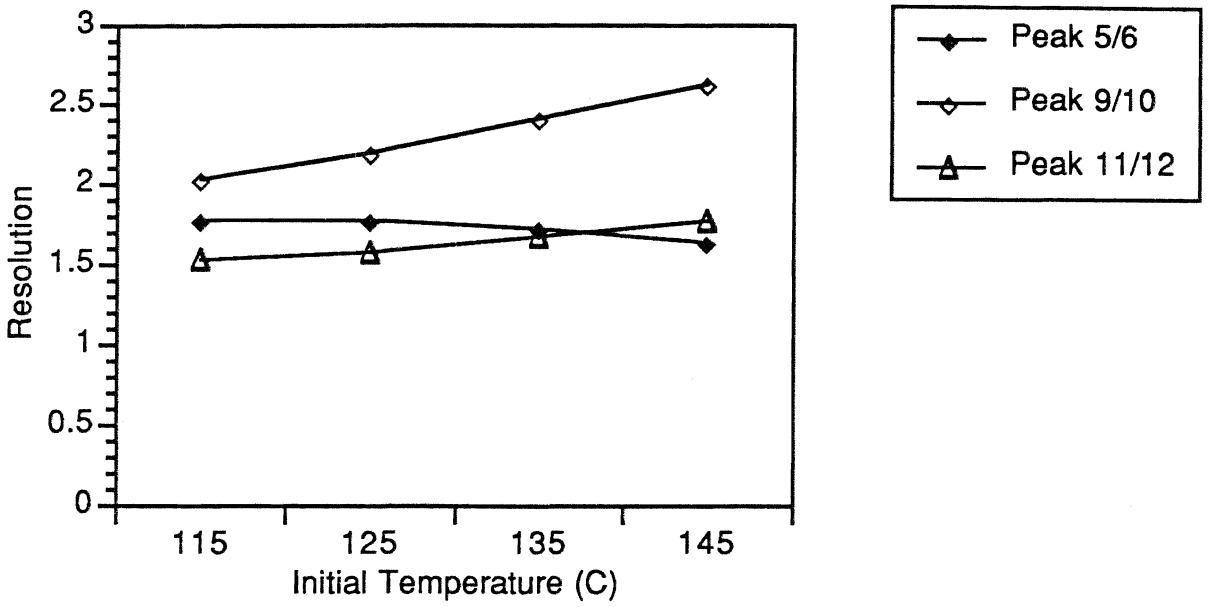


Figure 9: The effect of the initial column temperature on resolution

Solvent: n-octane

Table 16: Relative standard deviation (%)
(Solvent: n-octane, and nonane)
(Column: DB5)

Peak#	Octane (n=7)		Nonane (n=7)	
	Peak height	Peak area	Peak height	Peak area
1	2.5	9.0	2.8	2.5
2	0.7	6.1	2.2	3.2
3	2.1	6.1	4.6	2.3
4	2.2	6.8	7.2	3.2
5	3.8	5.5	4.5	3.7
6	3.3	6.6	2.9	4.0
7	2.5	6.8	4.9	4.4
8	2.0	7.2	2.6	4.5
9	4.2	5.3	3.0	5.7
10	6.0	4.2	4.1	4.3
11	8.8	6.8	2.9	5.9
12	8.9	5.9	2.8	5.1
13	9.1	11.7	3.7	3.5
14	12.0	5.1	8.0	9.5
15	13.1	10.3	8.1	8.4
16	11.3	8.6	8.8	9.8

Having optimized the initial column temperature for the determination of PAHs in n-octane and nonane, the peak responses of the sixteen PAHs in these solvents were compared to those in hexanol. Both the peak area and the peak height of the PAHs in n-octane, nonane, and hexanol were calculated from the total ion current chromatogram, and normalized relative to 100% peak response in hexanol. Table 17 summarizes the relative peak area and height of the these PAHs. As expected, the differences in the peak response correlates with the differences of the boiling point of the solvents especially for the late-eluted peaks Hence, hexanol with the highest boiling point among the solvents show better peak response for the late-eluting peaks. The peak height and the peak area of the early-eluting peaks seem to follow a reverse trend in which n-octane with the lowest boiling point gave the highest peak response followed by nonane and hexanol. This could be explained by the cold trapping effect mentioned earlier. When the initial column temperature is 115 C, the optimum temperature for injecting PAHs in n-octane, the early-eluted PAHs might be cold trapped more efficiently than with a higher initial column temperature.

The effect of n-octane and nonane on the resolution of the late-eluted peaks was compared with that in hexanol as shown in Figure 10. Hexanol gave the highest resolution for peaks 9 and 10,and 11 and 12.

Table 17: The relative peak area and height of n-octane and nonane to hexanol (DB5 column)

	Peak Area			Peak Height		
	hexanol	nonane	octane	hexanol	nonane	octane
Peak#						
2	100	118	130	100	126	132
3	100	101	112	100	138	141
4	100	130	142	100	137	104
5	100	132	144	100	117	133
6	100	96	95	100	123	114
7	100	110	110	100	93	116
8	100	109	111	100	85	102
9	100	118	101	100	85	94
10	100	104	94	100	83	92
11	100	122	95	100	92	91
12	100	90	82	100	88	90
13	100	90	87	100	93	93
14	100	92	83	100	88	84
15	100	92	76	100	84	74
16	100	97	89	100	91	85

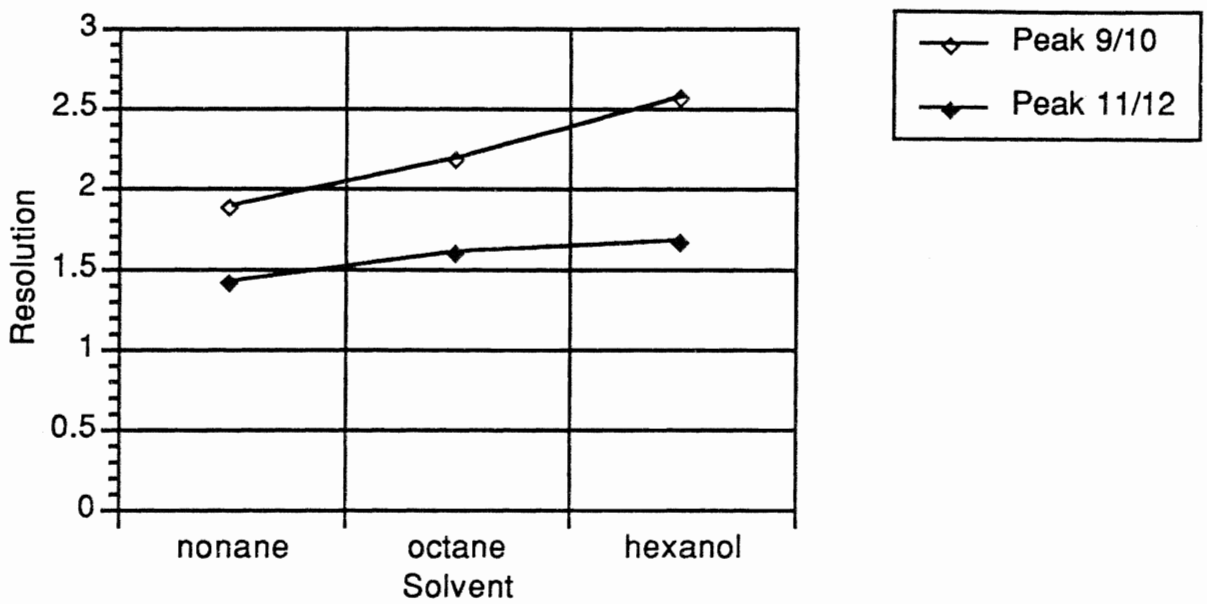


Figure 10 : The effect of n-octane, nonane, and hexanol on resolution (using DB5 column)

Effect of stationary phase:

Further investigations into the effect of the initial column temperature and the solvent on the peak response of the PAHs in alcohols were carried out using a column coated with a crosslinked methyl silicone stationary phase (DB1) instead of the 5% diphenyl, 94% dimethyl, 1% vinyl polysiloxane (DB5) which was employed for all the previous studies. The same conditions as those used with DB5 were carried out. Standard solutions containing 2 μg/ml of the sixteen PAHs in butanol, cyclopentanol, pentanol, and hexanol were prepared separately, and an aliquot of 3 μl of each of these solutions were injected onto the DB1 column. The effect of the initial column temperature was investigated by varying the initial temperature 20 C above and below the boiling point of the solvent.

Both the peak area and the peak height of the PAHs were normalized relative to those obtained for the optimum initial column temperature as shown in Tables 18 and 19 respectively. As seen with DB5, the optimum initial column temperature is dependent on the solvent used. However, the striking observation is that the optimum initial column temperature is the same or 10 C above the boiling point of the solvent used. Thus, the optimum initial column temperature for the determination of PAHs using DB1 column is at least 10 C lower than that of DB5. This could be explained by the presence of 5% diphenyl in the DB5 column. Grob (101) suggested that the introduction of some phenyl groups to the stationary phase increases the wettability of the stationary phase surface by the sample solvent. This seems to be the reason for the higher optimum initial column temperature when DB5 is used, in which the solvent adsorbs strongly to

the stationary phase so that the migration of the solutes is slowed down. This is known as phase soaking phenomenon.(105-106) The most important factor in phase soaking is the wettability of the solvent on the stationary phase; thus, proper solvent and stationary phase is a prerequisite to achieve a phase soaking effect that would eliminate the distortion or broadening of the peaks by reducing the migration speed of the solutes.

The retention time of the PAHs using DB1 was found to be lower than that using DB5. This is usually the result of the increased wettability of the stationary phase by the solvent.(101) Hence, the higher initial column temperature and the higher retention time observed in the case of DB5 compared to DB1 might be due to the phase soaking phenomenon.

Table 18: Relative peak area of the PAHs in alcohols (%), using DB1 column

	butanol				cyclopentanol				pentanol				hexanol			
b.p. (C)	117				140				138				158			
	Initial Column Temperature (C)															
	107	117	127	137	130	140	150	160	118	128	138	148	148	158	168	178
Peak#																
2	99	116	100	89	88	100	57	n.d.	114	109	100	88	73	100	n.d.	n.d.
3	94	102	100	99	75	100	89	83	101	101	100	92	n.d.	100	n.d.	n.d.
4	99	98	100	90	54	100	97	76	100	102	100	89	n.d.	100	85	88
5	79	94	100	85	88	100	98	75	99	101	100	88	127	100	69	38
6	86	90	100	93	50	100	120	95	55	102	100	99	118	100	86	55
7	74	108	100	88	71	100	105	82	58	99	100	99	28	100	82	72
8	95	102	100	87	73	100	108	81	57	100	100	100	116	100	83	72
9	69	93	100	104	78	100	93	71	71	120	100	90	82	100	83	71
10	42	65	100	103	84	100	105	97	47	82	100	77	38	100	89	82
11	70	80	100	91	86	100	76	76	70	115	100	91	118	100	83	73
12	68	92	100	88	79	100	87	73	61	94	100	90	69	100	95	82
13	61	89	100	89	78	100	93	73	64	102	100	91	74	100	93	78
14	60	81	100	98	79	100	92	65	59	95	100	89	80	100	92	83
15	50	70	100	85	78	100	93	66	51	94	100	89	68	100	92	85
16	59	83	100	97	79	100	92	67	59	98	100	91	71	100	92	83

n.d. (not detected)

Table 19: Relative peak height of the PAHs in alcohols (%), usin DB1 column

	butanol				cyclopentanol				pentanol				hexanol			
b.p. (C)	117				140				138				158			
	Initial Column Temperature (C)															
	107	117	127	137	130	140	150	160	118	128	138	148	148	158	168	178
Peak#																
2	171	163	100	59	101	100	4	n.d.	171	124	100	86	96	100	n.d.	n.d.
3	151	147	100	63	69	100	45	25	144	136	100	89	n.d.	100	n.d.	n.d.
4	123	134	100	61	54	100	72	39	103	119	100	72	n.d.	100	60	49
5	59	86	100	73	80	100	85	51	60	81	100	63	82	100	46	23
6	66	91	100	76	48	100	97	50	58	94	100	75	86	100	58	27
7	58	102	100	87	51	100	85	56	53	80	100	90	24	100	72	45
8	54	73	100	78	69	100	105	74	45	75	100	84	82	100	93	62
9	58	83	100	83	81	100	99	71	57	90	100	90	69	100	88	78
10	47	59	100	137	76	100	96	79	47	73	100	89	35	100	97	83
11	61	81	100	88	84	100	91	77	63	93	100	88	85	100	92	78
12	60	87	100	104	81	100	93	75	55	86	100	87	68	100	95	82
13	54	81	100	101	83	100	97	74	55	89	100	83	67	100	89	80
14	54	82	100	115	82	100	95	67	52	90	100	78	74	100	87	83
15	48	70	100	87	82	100	95	68	46	89	100	76	68	100	88	83
16	54	80	100	99	83	100	94	70	54	95	100	76	70	100	85	83

n.d. (not detected)

The effect of solvent on the peak response of the sixteen PAHs was investigated using DB1. The peak area and the peak height of the PAHs in alcohols were compared to those of p-xylene. The optimum initial column temperature for p-xylene was found to be the same as the boiling point of the solvent (138 C), which is in agreement with the previous results obtained with alcohols. The mean values of seven determinations of the peak area and height of the PAHs in each of the solvents were calculated, and normalized relative to 100% in hexanol. Tables 20 and 21 summarize the relative peak area and peak height of the PAHs. The first three peaks were not detected in hexanol because they were eluted prior to the starting time of acquisition. The response of both the peak area and the peak height of the PAHs relate directly to the boiling point of the solvent used. As was the case with DB5, hexanol, with the highest boiling point, gave the greatest peak area and peak height compared to the other solvents used.

The relative standard deviations of the seven replicate injections of each of the solvents are given in Table 22. The relative standard deviations were less than 10% for the early-eluted peaks; however, it was a little higher for the last three peaks.

Table 20: Relative peak area of PAHs (%), using DB1 column

peak#	butanol	cyclopentanol	pentanol	p-xylene	hexanol
4	118	101	97	104	100
5	101	75	92	90	100
6	106	91	85	89	100
7	88	86	77	83	100
8	91	87	80	84	100
9	61	83	68	71	100
10	77	81	77	77	100
11	49	82	60	61	100
12	56	81	65	67	100
13	47	79	57	50	100
14	22	65	42	36	100
15	19	66	44	38	100
16	28	64	45	38	100

Table 21: Relative peak height of the PAHs (%), using DB1 column

peak#	butanol	cyclopentanol	pentanol	p-xylene	hexanol
4	92	77	85	94	100
5	82	52	72	80	100
6	72	82	66	77	100
7	69	95	58	86	100
8	80	69	65	73	100
9	50	69	48	62	100
10	54	72	51	60	100
11	41	65	48	54	100
12	39	70	48	56	100
13	33	67	45	49	100
14	18	59	33	36	100
15	12	52	29	32	100
16	23	61	35	36	100

Table 22: Relative standard deviations of the solvents (n=7)
(Column: DB1)

Peak#	butanol	cyclopentanol	pentanol	p-xylene	hexanol
2	9.8	2.1	2.2	1.4	n.d.
3	9.5	0.7	2.1	2.7	n.d.
4	9.2	5.0	5.3	6.2	7.5
5	8.3	6.9	4.0	4.4	6.0
6	6.4	6.9	6.3	5.7	5.9
7	8.8	7.7	4.6	5.8	3.2
8	7.8	7.5	6.8	5.2	1.8
9	9.4	6.7	8.8	6.5	4.9
10	5.0	3.8	6.8	7.0	3.2
11	9.2	8.1	8.4	7.2	6.5
12	5.6	7.2	9.8	7.1	4.2
13	13.8	8.5	10.2	7.2	6.3
14	8.0	10.8	12.9	8.3	14.4
15	10.5	14.8	15.3	6.3	17.0
16	6.2	7.4	11.6	8.1	14.2

The effect of the initial column temperature on the resolution of the late-eluting PAHs in hexanol was examined as shown in Figure 11. Similar observations for peaks 9 and 10, 11 and 12, 14 and 15 were obtained. Unlike the case of DB5 (Figure 5), where the resolution of the late-eluted peaks were not affected by the change of the initial column temperature, with DB1, the resolution of the late-eluted peaks increases as the initial column temperature increases to 10 C above the boiling point of the solvent. This was the case for the early-eluted peaks with DB5 in which excessive condensation of the solvent was thought to be the reason. This might apply to the late-eluted peaks with DB1, since the late-eluted peaks with DB1 are eluted earlier than with DB5.

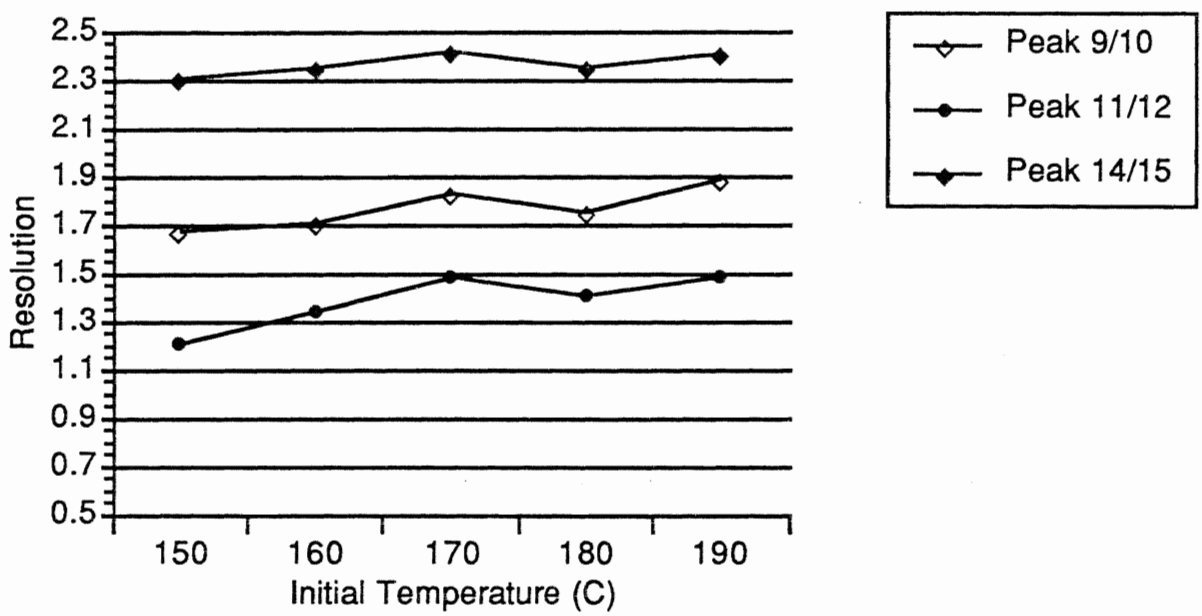


Figure 11: The effect of the initial column temperature on resolution, using DB1 column

The effect of solvent on the resolution of the late-eluted peaks using DB1 was also investigated, as shown in Figure 12. All solvents were injected at their optimum initial column temperature. The separation efficiency of the late-eluted peaks was found to relate directly to the boiling point of the solvent used. The higher the boiling point, the better the separation between the late-eluted peaks. By comparing the effect of the initial column temperature on the resolution of the late-eluted peaks in each solvent, it was found that the resolution increases as the initial temperature increases up to 170 C regardless of the solvent used. Thus, the solvent had no significant effect on the separation efficiency of the late-eluted peaks.

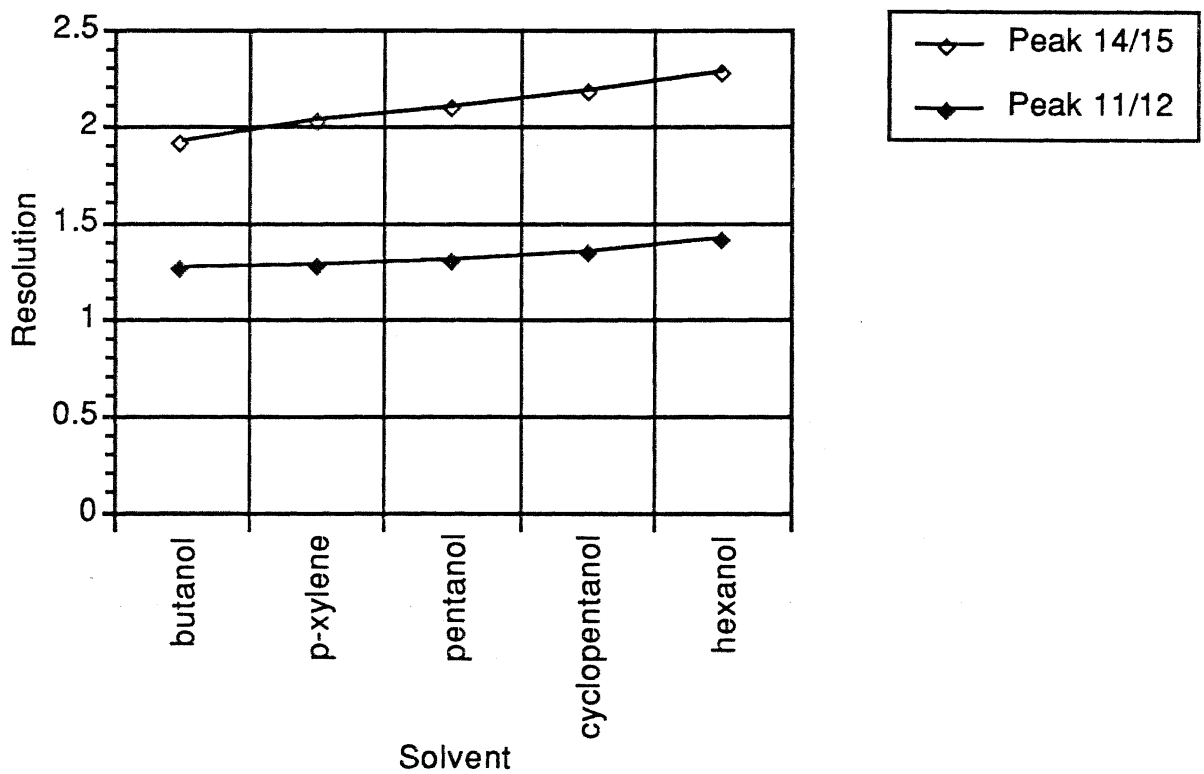


Figure 12: Effect of solvent on resolution, using DB1 column

The effect of the stationary phase on the resolution of the closely-eluted PAHs was studied. Better separation efficiency was obtained with the DB5 column compared to the DB1. Figure 13 compares the separation efficiency between benzo(b)fluoranthene and benzo(k)fluoranthene using DB1 and DB5 columns. The resolution of these peaks is greater when the DB5 column was used. Similar results were also observed by comparing the separation efficiency between indeno(1,2,3,-cd)pyrene and dibenz(a,h)anthracene as shown in Figure 14. The better separation efficiency obtained using DB5 could be due to the 5% diphenyl present in the phase which increases the wettability of the solvent on the stationary phase so that the speed of migration of solutes is slowed down resulting in better separation.

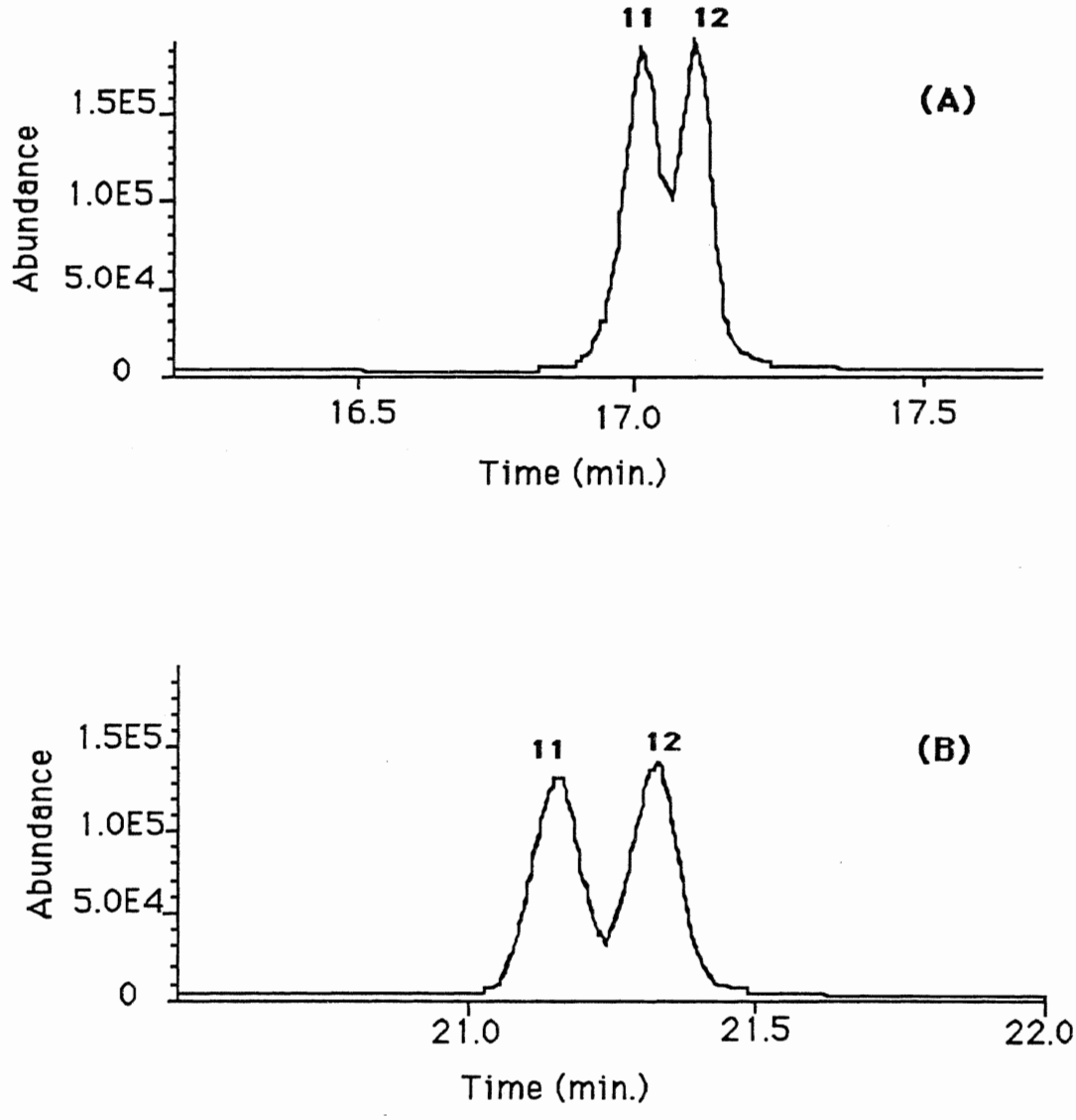


Figure 13: Comparison of separation efficiency of benzo(b)fluoranthene (11) and benzo(k)fluoranthene (12) in hexanol

(A) methyl silicone column

(B) 5% diphenyl, 94% dimethyl, 1%vinyl polysiloxane column

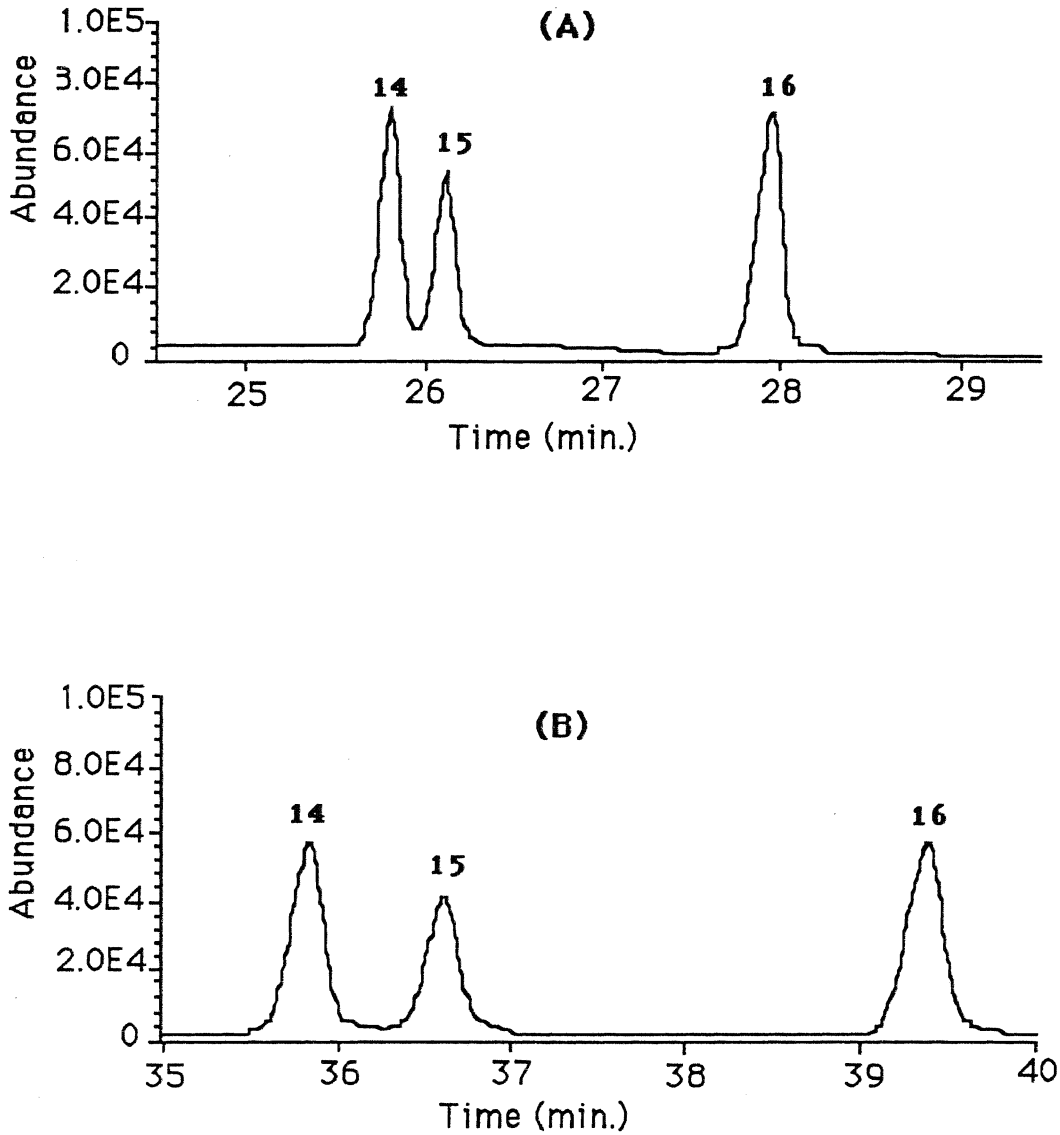


Figure 14: Comparison of separation efficiency of indeno(1,2,3,-cd)pyrene, dibenz(a,h)anthracene, and benzo(g,h,i)perylene in hexanol

(A) methyl silicone column

(B) 5% diphenyl, 94% dimethyl, 1% vinyl polysiloxane column

The effect of the initial column temperature on the peak shape of the sixteen PAHs using the DB1 column was found to be similar to that using the DB5 column. Figure 15 shows the effect of the initial column temperature on the peak shape of the early-eluted PAHs in pentanol. Similar results were also observed with the other solvents. The peak shape of the early-eluted peaks were affected by an initial column temperature as low as 20 C above and below the optimum initial column temperature. At an initial column temperature 20 C lower than the boiling point of the solvent, fronting of the peak was observed. As the initial temperature was increased, the fronting disappeared and a symmetrical peak was obtained. Further increase of the initial temperature resulted in tailing of the peak. The reason for these observations was explained earlier in the case of DB5 column.

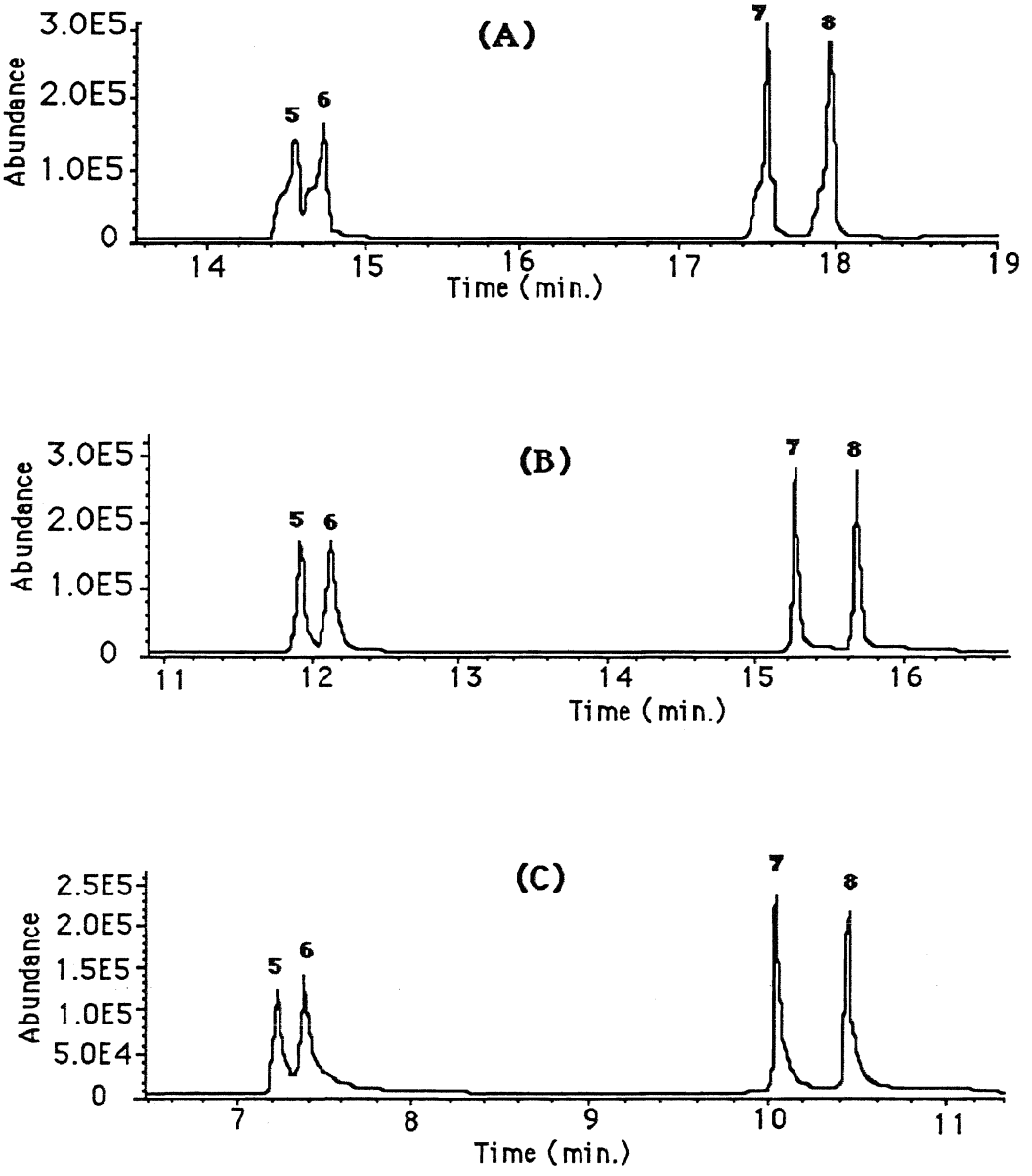


Figure 15: Comparison of peak shape of PAHs in hexanol using DB1 column
Column initial temperature: (A) 118 C (B) 138 C (C) 158 C
Peak: (5) phenanthrene, (6) anthracene, (7) fluoranthene, (8) pyrene

Comparing the effect of the initial column temperature on the peak shape of the sixteen PAHs using DB1 and DB5 columns showed a significant difference between the two phases in the range of the initial column temperature in which symmetrical peaks of PAHs can be obtained. Peak shapes of each of the sixteen PAHs in different solvents were recorded at initial temperatures ranging from 50 C to 260 C depending on the solvent employed. Figures 16 and 17 show the effect of the initial temperature on the peak shape of the sixteen PAHs in hexanol using DB1 and DB5 respectively. Similar figures are also given for different solvents in Appendix 1.

The upper curve in Figures 16-17 shows the maximum initial temperature, above which the peaks starts to tail; whereas the lower curve shows the minimum initial temperature, below which fronting of the peaks is obtained. The region between the two curves represent the initial temperature at which symmetrical peaks are obtained. In general, the region of the symmetrical peaks for the early-eluted peaks is smaller than that of the late-eluted peaks. Also, the ranges of initial temperatures for symmetrical peaks of each of the PAHs were found to be significantly different for the two stationary phases. As can be seen from Figures 16-17 and Appendix 1, the lower curve in DB5 is lower than that in DB1; however the upper curve is not significantly changed in the two columns.

The wider range of the initial temperature with DB5 might be due to the introduction of 5% of diphenyl groups into the stationary phase which is believed to increase the wettability of the solvent on the surface of the stationary phase. (101) Thus, the solvent is strongly adsorbed on the stationary phase, so that the speed of the migration of the PAHs is reduced. This in turn eliminates the splitting or fronting of the peaks.

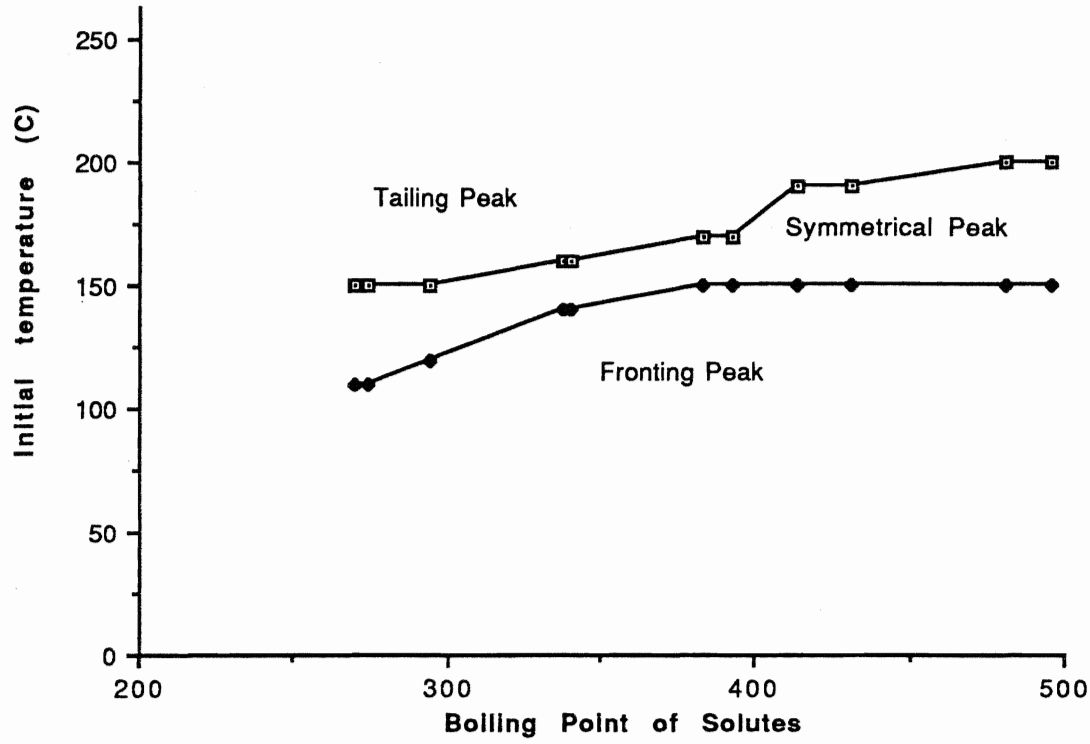


Figure 16: Effect of initial temperature on peak shape of 16 PAHs in hexanol with DB1 column

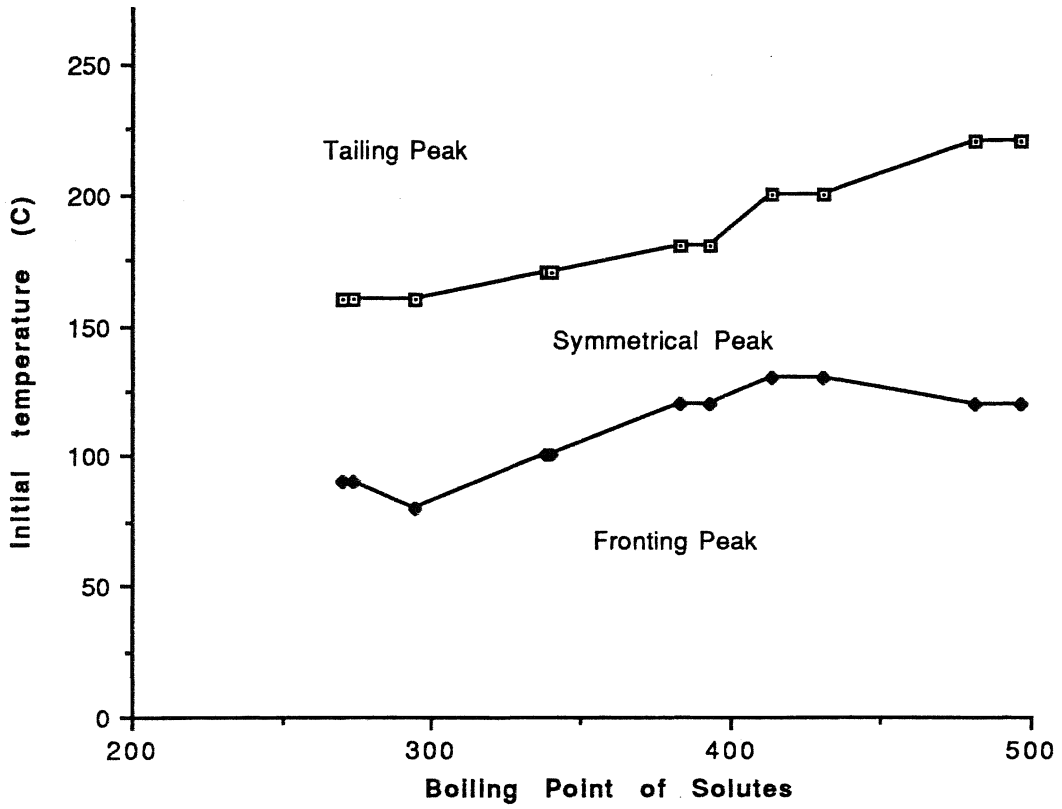


Figure 17: Effect of initial temperature on peak shape of 16 PAHs in hexanol with DB5 column

Effect of Injection Volume

The amount of the volume of samples injected onto the GC column is another parameter that needed to be optimized to determine the optimum conditions for the determinations of PAHs. The effect of injection volume was studied experimentally by varying the injection volume from 1 to 5 µl while keeping the initial column temperature constant. A standard solution containing 2 µg/ml of each of the PAHs in hexanol was introduced into both a methyl silicone (DB1) and a 5% diphenyl, 94% methyl, 1% vinyl polysiloxane (DB5) columns

Table 23 summarizes the effect of the volume injected on the peak area of the sixteen PAHs in hexanol using DB5 column. As can be seen, the higher boiling point PAHs are more affected by the increase of the volume injected than the low boiling point PAHs. This is also illustrated clearly in Figure 18. By comparing the peak height of pyrene, benz(a)-anthracene, and indeno(1,2,3,-cd)-pyrene with different injection volume, the peak area of the late-eluted PAHs increased significantly, as the injected volume increased from 1 µl to 4 µl. The early-eluted peaks remain unchanged. Further increase in the injection volume from 4 µl to 5 µl, seems to reduce the peak area of the late-eluted PAHs, particularly the last six peaks. Similar results were also obtained by using DB1 column as shown in Table 24.

Table 23: Effect of injection volume on peak area of 15 PAHs in hexanol, normalized relative to 1 µl injection

(5% diphenyl, 94% dimethyl, 1% vinyl polysiloxane column)

Peak#	1µL	2µL	3µL	4µL	5µL
2	1.0	0.9	0.8	0.9	0.8
3	1.0	0.9	0.8	0.6	0.8
4	1.0	0.8	0.7	0.8	0.8
5	1.0	0.9	0.9	0.9	0.9
6	1.0	1.0	1.0	1.0	1.0
7	1.0	1.0	0.9	1.0	1.0
8	1.0	1.0	1.0	1.1	1.0
9	1.0	1.1	1.1	1.3	1.3
10	1.0	1.1	1.0	1.2	1.2
11	1.0	1.2	1.2	1.6	1.5
12	1.0	1.2	1.2	1.6	1.5
13	1.0	1.3	1.3	1.9	1.8
14	1.0	1.7	1.8	3.2	3.1
15	1.0	1.9	2.2	4.0	3.9
16	1.0	1.6	1.8	3.1	3.0

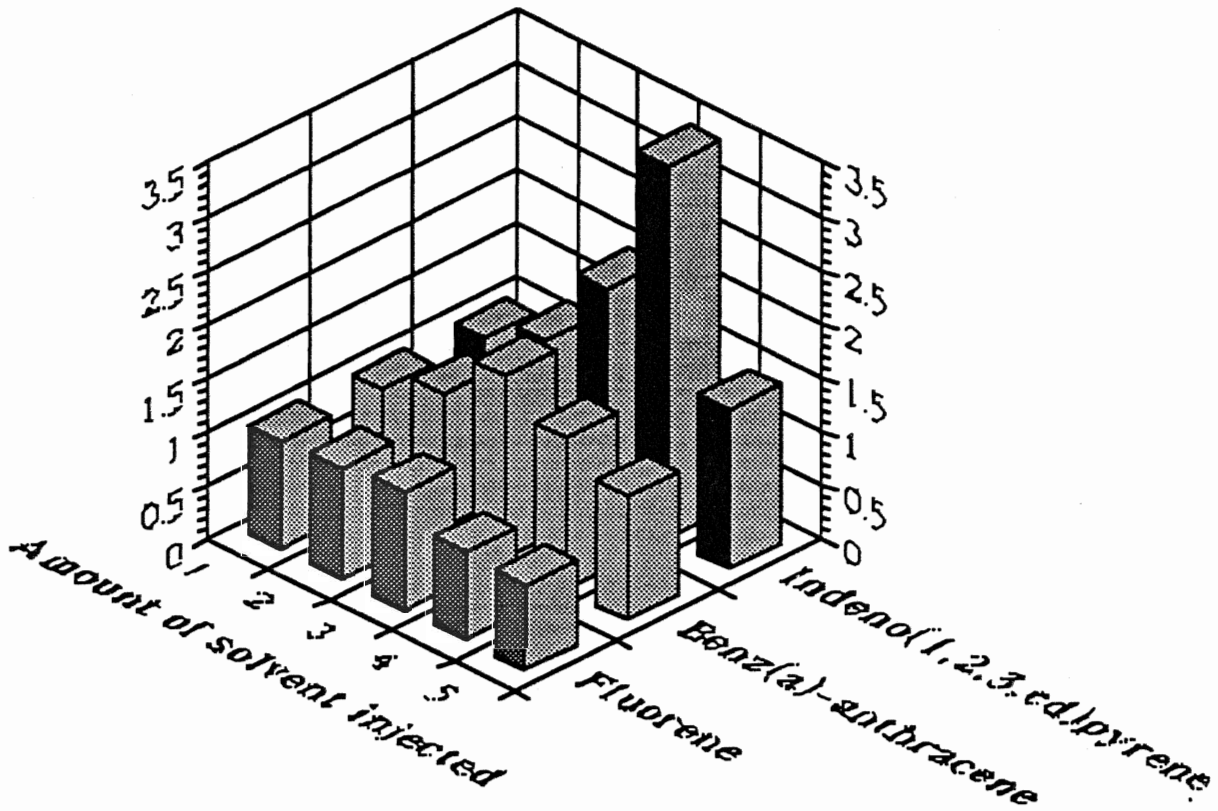


Figure 18: Effect of volume injected on peak area of pyrene, benzo(a)-anthracene, and indeno(1,2,3,-cd)pyrene in hexanol (DB5 column)

Table 24: Effect of injection volume on peak response of 13 PAHs in hexanol (methyl silicone column)

	Peak Area					Peak Height				
	1 µL	2 µL	3 µL	4 µL	5 µL	1 µL	2 µL	3 µL	4 µL	5 µL
Peak										
4	1.0	1.0	1.1	0.8	0.8	1.0	1.3	1.9	1.7	1.9
5	1.0	0.9	1.3	1.3	1.1	1.0	1.5	1.5	1.5	1.4
6	1.0	1.0	1.1	0.9	0.8	1.0	1.3	1.7	1.6	1.5
7	1.0	1.1	1.3	1.0	0.9	1.0	1.1	1.3	1.0	0.9
8	1.0	1.0	1.2	1.0	0.9	1.0	0.9	1.1	1.1	0.9
9	1.0	1.2	1.7	1.4	1.1	1.0	1.3	1.8	1.5	1.2
10	1.0	1.2	1.6	1.3	1.1	1.0	1.1	1.5	1.2	1.0
11	1.0	1.4	2.2	2.0	1.4	1.0	1.4	2.1	1.8	1.3
12	1.0	1.3	2.1	1.9	1.3	1.0	1.4	2.3	2.0	1.4
13	1.0	1.4	2.3	2.4	1.5	1.0	1.5	2.6	2.7	1.7
14	1.0	1.2	2.0	3.4	1.4	1.0	1.3	2.3	3.3	1.5
15	1.0	1.2	1.7	3.9	1.5	1.0	1.3	2.3	3.8	1.7
16	1.0	1.3	2.0	3.5	1.5	1.0	1.4	2.3	3.5	1.7

Similar findings were also reported by Grob et al. (107). They found that small amount of sample cannot be transferred accurately because of the discrimination of high-boiling components. This might be the result of incomplete evaporation of the high-boiling solutes in the vaporization chamber. Another possibility is that the high-boiling components are lost as they are pulled out of the injector when the syringe is removed. The decrease in the peak response when the injection volume was over 4 µl could arise from back diffusion (108-109), in which the pressure wave pushes the excess of vapors backward out of the top of the vaporization chamber upon the dilution with the carrier gas. Vapors may also pressed backward into the carrier gas supply line. Volatile components readily return into the injector, but the high-boiling material may remain there and return after the splittless period or during a subsequent run. Thus, in order to account for the discrimination , several internal standards distributed over the whole molecular range should be added to the sample.

The effect of the injection volume on the resolution of the closely-eluted peaks was also investigated as shown in Figure 19. As anticipated, there is no significant change in the separation efficiency all the four pairs of peaks upon the increase of the injection volume. The relative standard deviation of five replicate injections of each of the volumes was determined using DB5 column. Table 25 shows the low relative standard deviation obtained which indicates the reproducibility of the analysis.

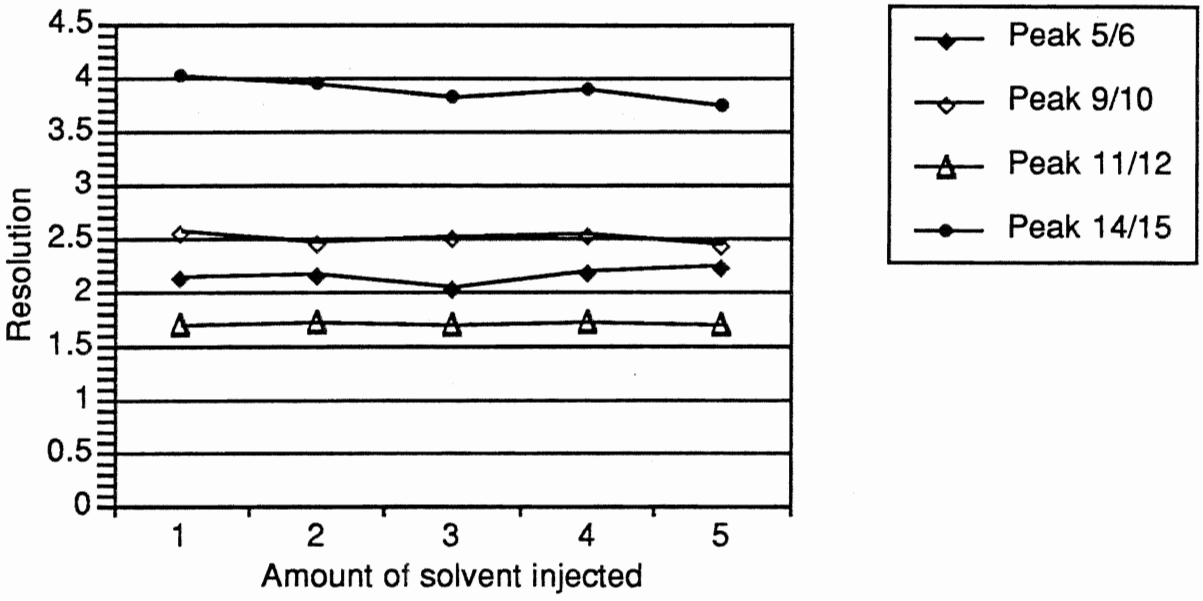


Figure 19: Effect of injection volume of hexanol on resolution
(5% diphenyl, 94% dimethyl, 1% vinyl polysiloxane column)

Table 25: Relative standard deviation (%RSD) of the injection volume of hexanol (n=5)
(DB5 column)

Peak#	1 µL	2µL	3µL	4µL	5µL
2	2.0	1.5	2.9	3.7	1.4
3	1.7	2.4	1.7	1.6	4.5
4	1.2	1.7	4.2	2.2	1.9
5	2.8	1.4	2.9	1.7	1.1
6	1.4	1.6	3.3	1.3	1.5
7	1.5	1.5	2.7	1.6	1.8
8	1.5	1.3	2.7	2.4	2.3
9	1.6	1.7	3.7	1.7	2.9
10	2.0	1.5	3.6	1.6	2.4
11	2.2	3.5	4.9	2.9	4.1
12	2.0	3.8	5.1	2.7	4.0
13	2.9	4.6	5.5	3.5	4.2
14	7.8	10.3	6.5	5.8	5.7
15	3.3	10.7	11.1	6.1	6.0
16	8.9	9.2	5.9	6.8	5.4

Part B: Investigations into the factors affecting the extraction efficiency of PAHs in water using solid phase extraction (SPE)

Although solid-phase extraction is widely used for preconcentration of samples prior to chromatographic analysis, developing methods based on solid-phase extraction is still something of an art.(110) One of the major causes of the poor recoveries of solutes is the incomplete elution. This poor recoveries could be due to the choice of the elution solvent. Based on the likelihood that the solvent is crucial in the elution of analytes, the first phase of this study involved the optimization of the solvent used for the elution of PAHs.

Effect of elution solvent:

In order to establish the optimum conditions for the extraction of PAHs, several elution solvents were evaluated. Non polar solvents such as benzene, p-xylene, and toluene with dielectric constants 2.29, 2.27, and 2.38 respectively were examined based on the idea that non polar solvent have a much greater affinity for hydrophobic solutes such as PAHs. Benzene has been recommended by several investigators to be used as an elution solvent for extracting PAHs from water.(71,86) Alcohols such as butanol, pentanol, cyclopentanol, and hexanol were also examined due to their high solubility in water. It was thought that the poor recoveries of PAHs might be due to the incomplete removal of water during drying of the cartridge so that alcohols,which might dissolve retained water, would be ideal elution solvents

The effect of the elution solvent on the extraction efficiency of PAHs in water using several non polar bonded phase silica sorbents especially octyl (C8), octadecyl (C18), and tri-octadecyl (tC18) cartridges was evaluated. Recovery studies were run on 100 ml spiked water samples using a 50 ml syringe to force the water through the bonded silica cartridges at flow rates of about 10 ml/min. Spiking was performed by adding 1 ml of 2 mg/l (ppm) of the sixteen PAHs in methanol to water volume of 100 ml. One ml of an appropriate solvent was passed through the cartridges to elute the trapped PAHs of which 3 µl was injected into the GC/MS. The data presented in Tables 26 and 27 represent the average recoveries of three determinations of the sixteen PAHs by several elution solvents relative to p-xylene, based on the peak height and area, using C8 cartridges. Figure 20 shows similar total recoveries for the elution solvents with respect to the peak height and area. As can be seen from Tables 26 and 27, comparable results were obtained in the case of p-xylene and benzene for the low molecular weight PAHs; however, p-xylene gave by far the highest recoveries for the high molecular weight PAHs. Low recoveries were observed by using alcohols as elution solvents which could be due to their high polarity which results in low affinity to trap the adsorbed PAHs on the bonded silica phase. Similar results were also obtained using C18 and tC18 cartridges as shown in Appendix 2 and Figure 21. Hence, it was concluded that p-xylene is the solvent of choice for eluting PAHs.

Table 26: Relative recoveries of PAHs by different elution solvents to p-xylene based on peak area using C8 cartridges

	p-xylene	toluene	benzene	butanol	cyclopentanol	pentanol	hexanol
ε	2.27	2.38	2.29	17.1		13.9	13.3
Peak							
2	100	94	84	67	76	72	73
3	100	81	86	65	59	48	67
4	100	99	93	62	57	63	65
5	100	86	67	43	46	50	52
6	100	88	85	47	46	51	53
7	100	82	82	33	34	40	40
8	100	79	85	34	32	38	40
9	100	60	98	37	52	57	34
10	100	77	107	61	71	78	44
11	100	47	80	31	50	54	30
12	100	56	88	48	61	66	36
13	100	46	75	36	52	55	27
14	100	25	16	27	36	34	17
15	100	21	14	7	36	32	15
16	100	30	33	23	36	35	16

Table 27: Relative recoveries of PAHs by different elution solvents to p-xylene based on peak height using C8 cartridges

	p-xylene	toluene	benzene	butanol	cyclopentanol	pentanol	hexanol
ε	2.27	2.38	2.29	17.1		13.9	13.3
Peak							
2	100	94	86	68	85	72	93
3	100	80	73	62	68	65	84
4	100	89	67	54	61	61	69
5	100	82	58	41	47	51	62
6	100	87	65	35	43	46	47
7	100	78	79	23	32	38	40
8	100	74	71	20	28	33	37
9	100	54	100	32	45	53	30
10	100	62	107	45	57	66	34
11	100	44	78	28	45	52	27
12	100	47	76	36	49	57	26
13	100	37	63	27	38	46	19
14	100	23	31	17	26	28	13
15	100	19	41	19	28	24	11
16	100	26	40	19	32	30	13

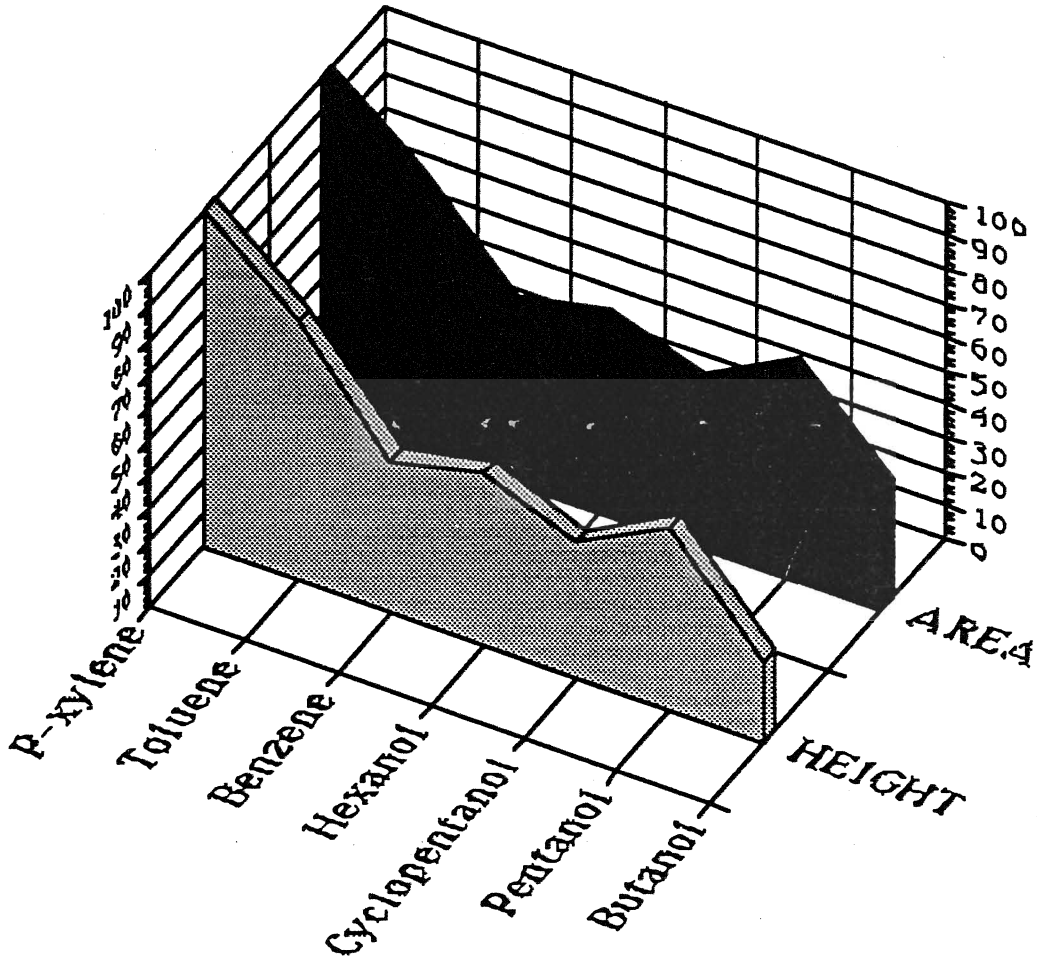


Figure 20: Comparison of the total recoveries of PAHs by various elution solvents in term of peak area and height using C8 cartridges

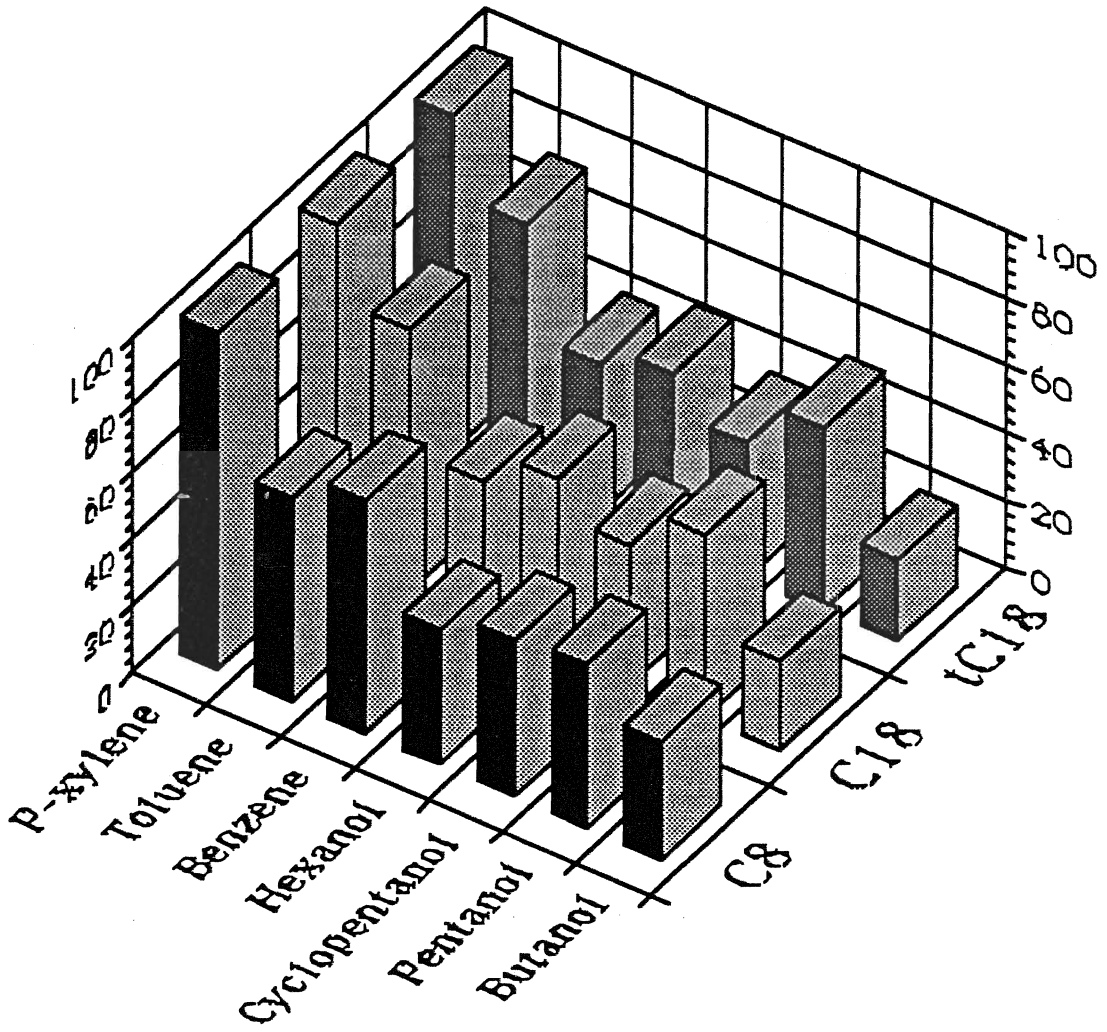


Figure 21: Comparison of the total recoveries of PAHs using different bonded silica phases

Further investigations to optimize the elution solvents were carried out by comparing the recoveries using ethyl acetate to those of p-xylene and o-xylene. Ethyl acetate was used since it has been recommended by Chladek et al. (69) to be used as an elution solvent for PAHs. In their report, only the recoveries of the low molecular weight PAHs, up to chrysene, were presented. The same procedure as the one mentioned earlier was used to carry out the study except that the standard solution contains thirteen PAHs in dichloromethane. Table 28 gives the average recoveries of p-xylene relative to o-xylene and ethyl acetate as eluents for the thirteen PAHs using C8 cartridges. The recoveries reported are means of five analyses. Similar recoveries were obtained for both p-xylene and o-xylene; however, lower recoveries for the low molecular weight PAHs were observed for ethyl acetate. Also, ethyl acetate did not elute the last three PAHs. The use of C18 and tC18 showed similar results to those obtained with C8, as summarized in Appendix 2.

The relative standard deviations of five replicate determination of the thirteen PAHs by p-xylene, o-xylene, and ethyl acetate as elution solvent using C8 cartridge are given in Table 29. The low relative standard deviation indicates the reproducibility of the analysis.

Table 28: Relative recoveries of thirteen PAHs by o-xylene and ethyl acetate to p-xylene using C8 cartridges

	AREA			HEIGHT		
	p-xylene	o-xylene	ethyl acetate	p-xylene	o-xylene	ethyl acetate
ε	2.27	2.57	6.02	2.27	2.57	6.02
Peak#						
1	100	108	101	100	123	110
2	100	77	82	100	91	68
3	100	90	79	100	105	58
4	100	144	89	100	123	77
5	100	146	87	100	142	79
6	100	109	52	100	116	45
7	100	122	n.d.	100	114	n.d.
8	100	115	48	100	112	48
9	100	117	65	100	98	59
10	100	108	32	100	102	44
11	100	96	n.d.	100	90	n.d.
12	100	21	n.d.	100	35	n.d.
13	100	103	n.d.	100	84	n.d.

n.d. (not detected)

Table 29: Relative standard deviations (RSD%) of the average recoveries of the thirteen PAHs by p-xylene, o-xylene, and ethyl acetate as elution solvents using C8 cartridges for 5 replicate determinations

	AREA			HEIGHT		
	p-xylene	o-xylene	ethyl acetate	p-xylene	o-xylene	ethyl acetate
Peak#						
1	3.5	6.8	7.9	4.6	6.8	6.3
2	4.3	3.0	8.0	5.1	3.2	5.2
3	5.0	3.7	8.3	7.5	1.7	5.9
4	4.9	3.4	5.6	5.9	7.1	5.4
5	5.1	3.9	4.3	8.6	4.0	4.0
6	9.9	6.1	8.5	12	7.4	6.2
7	7.6	8.5	2.6	11	5.8	4.8
8	13	4.5	7.3	12	5.1	4.7
9	9.4	7.9	14	12	5.0	9.8
10	14	5.0	34	15	7.2	8.9
11	18	5.0	n.d.	14	6.1	n.d.
12	7.8	10	n.d.	11	4.5	n.d.
13	16	3.2	n.d.	14	4.0	n.d.

n.d. (not detected)

The effect of the elution solvents on the efficiency of extracting of PAHs from water was further examined by comparing the average recoveries of thirteen PAHs eluted by nonane to those eluted by p-xylene using non polar bonded phase silica especially C8, C18, and tC18 cartridges. Nonane was selected because of its lower dielectric constant, 1.97, compared to p-xylene, 2.27. Hence, it was thought to be a potential eluting solvent capable of effectively eluting the trapped PAHs on the bonded silica phase. Tables 30 and 31 represent the average recoveries of six determinations of the thirteen PAHs obtained by using p-xylene and nonane as elution solvents. The relative average recoveries was calculated based on the peak area and the height of each of the PAHs as shown in Table 30 and 31 respectively. The octyl-, octadecyl-, and tri-octadecyl- bonded phases showed similar recoveries for the low molecular weight PAHs by using either p-xylene or nonane as elution solvents. These results are in agreement with those observed by Rostad et al.(68) in which the recoveries of fluorene from water samples were similar when octyl-, octadecyl-, cyclohexyl-, and phenyl- bonded phases were used. However, The octyl- (C8) cartridges with p-xylene as an elution solvent demonstrated better recoveries for the high molecular weight PAHs compared to the other phases. The high recoveries observed for dibenz(a,h)anthracene for phases other than C8 with p-xylene, as seen in Tables 30 and 31, could be due to an experimental error in the elution of the analyte by p-xylene. Thus, it was concluded based on the best overall performance that the C8 bonded phase silica cartridges are sorbents of choice trapping PAHs from water and p-xylene as the elution solvent.

The relative standard deviations of the recoveries using the different bonded phases with p-xylene and nonane as elution solvents are summarized in Table 32. The relative standard deviations are within 10% of the mean except for benzo(g,h,i)perylene.

Table 30: Relative average recoveries in term of the peak area of thirteen PAHs with p-xylene and nonane as elution solvents using different bonded phases

ε	p-xylene 2.27			nonane 1.97		
	C8	**C18**	**tC18**	**C8**	**C18**	**tC18**
Peak#						
1	100	92	91	207	167	55
2	100	101	91	86	105	93
3	100	96	91	94	96	96
4	100	98	93	114	115	122
5	100	95	89	105	98	99
6	100	95	97	89	136	128
7	100	79	83	65	96	91
8	100	82	109	133	111	105
9	100	73	84	20	94	85
10	100	74	78	24	107	97
11	100	30	90	4	31	37
12	100	129	100	53	68	47
13	100	63	79	7	72	71

Table 31: Relative average recoveries in term of the peak height of thirteen PAHs with p-xylene and nonane as elution solvents using different bonded phases

ε	p-xylene 2.27			nonane 1.97		
	C8	**C18**	**tC18**	**C8**	**C18**	**tC18**
Peak#						
1	100	92	82	165	166	39
2	100	97	84	84	94	96
3	100	102	95	92	96	99
4	100	87	92	89	94	101
5	100	96	90	88	75	77
6	100	97	96	62	102	91
7	100	81	89	57	97	84
8	100	78	102	55	89	83
9	100	70	82	12	87	80
10	100	68	75	24	71	62
11	100	58	84	17	50	47
12	100	168	145	104	184	106
13	100	61	80	20	70	60

Table 32: The relative standard deviations of the recoveries of thirteen PAHs by p-xylene and nonane as elution solvents using different bonded phases (n=6)

	p-xylene						nonane					
	Height			Area			Height			Area		
	C8	C18	t18	C8	C18	t18	C8	C18	t18	C8	C18	t18
Peak#												
1	4.5	6.2	11	4.5	2.0	4.8	8.1	13	27	1.6	15	9.2
2	6.8	8.8	7.2	4.6	4.2	3.7	3.1	4.1	5.7	3.0	1.9	1.6
3	1.8	3.9	8.6	3.3	2.5	3.1	3.4	5.3	2.2	1.9	1.9	1.3
4	7.1	5.7	4.4	5.3	1.9	2.5	1.6	5.4	1.9	1.7	1.7	1.0
5	7.1	4.4	3.7	5.1	2.3	1.3	2.9	3.5	4.7	2.2	2.1	1.5
6	11	4.9	4.5	8.4	4.3	4.6	2.1	3.4	1.8	5.6	4.6	4.4
7	7.2	2.8	3.9	6.1	3.3	3.4	4.9	5.7	6.0	7.1	3.9	6.6
8	8.3	3.9	8.6	8.4	5.6	10	8.2	4.5	4.0	6.9	4.2	2.9
9	7.5	3.7	9.4	8.0	2.5	4.6	11	3.6	6.4	35	7.7	4.1
10	9.6	6.2	8.2	7.7	3.1	7.6	12	7.0	8.1	31	7.7	5.2
11	11	13	11	12	40	4.5	11	14	19	26	12	19
12	27	9.5	34	41	36	41	7.1	16	21	22	11	3.3
13	9.8	9.7	13	11	10	13	12	10	13	13	14	35

Volume of the elution solvent:

Determinations of the least amount of solvent required for complete elution of the trapped PAHs on the bonded phase were carried out by passing small volumes of p-xylene through the cartridges. A sequence of 100 µl of p-xylene portions were loaded into the cartridges to determine the volume necessary to elute the PAHs. As low as 200 µl of p-xylene was needed to effectively elute the high molecular weight PAHs trapped on the octyl-bonded phase as shown in Figure 22. However, it was found that at least 500 µl of p-xylene are necessary to completely elute all the sixteen PAHs. Similar results were also observed using C18 and tC18 bonded silica phases as can be seen in Figures 23 and 24, respectively.

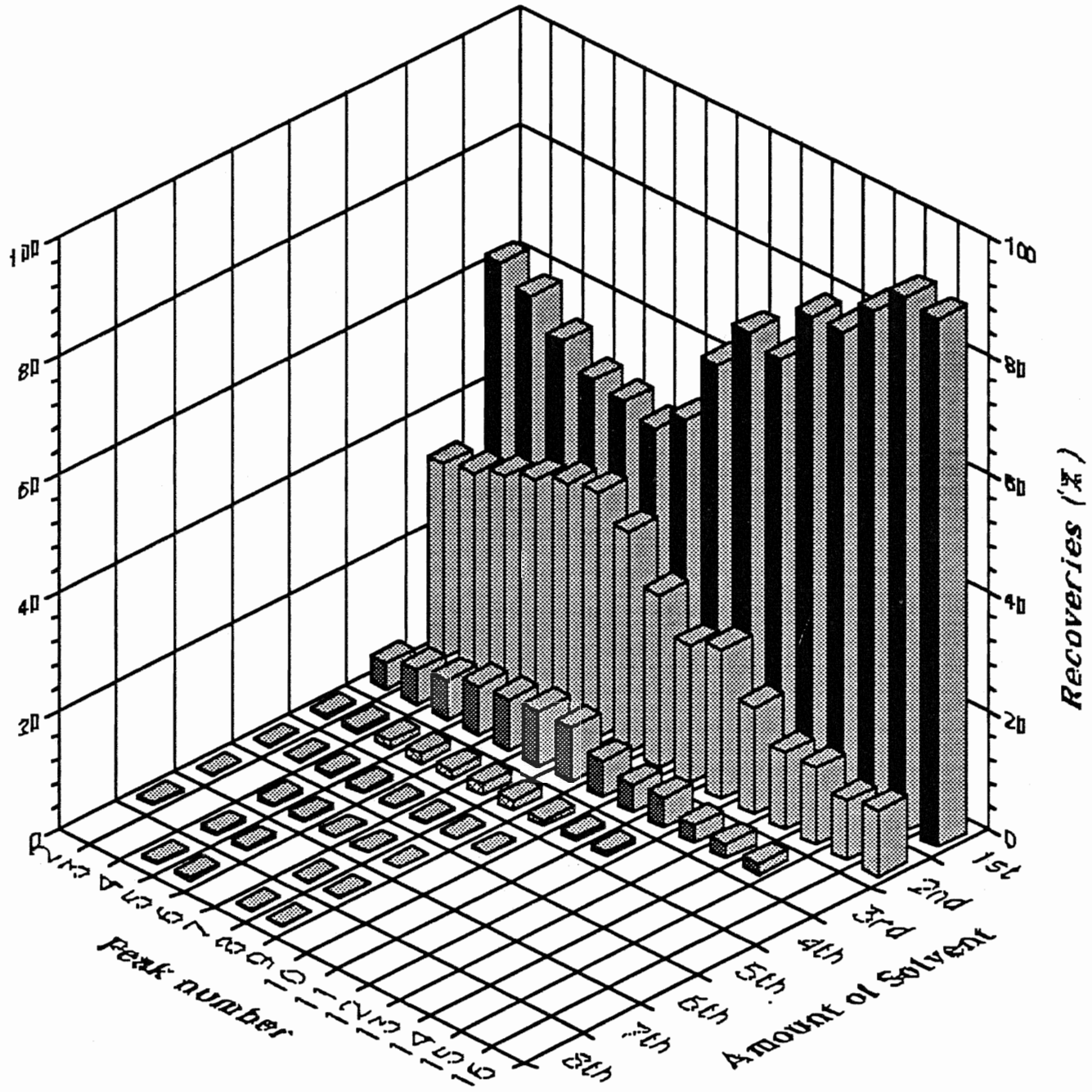


Figure 22: Volume of p-xylene required to elute the PAHs using C8 cartridges

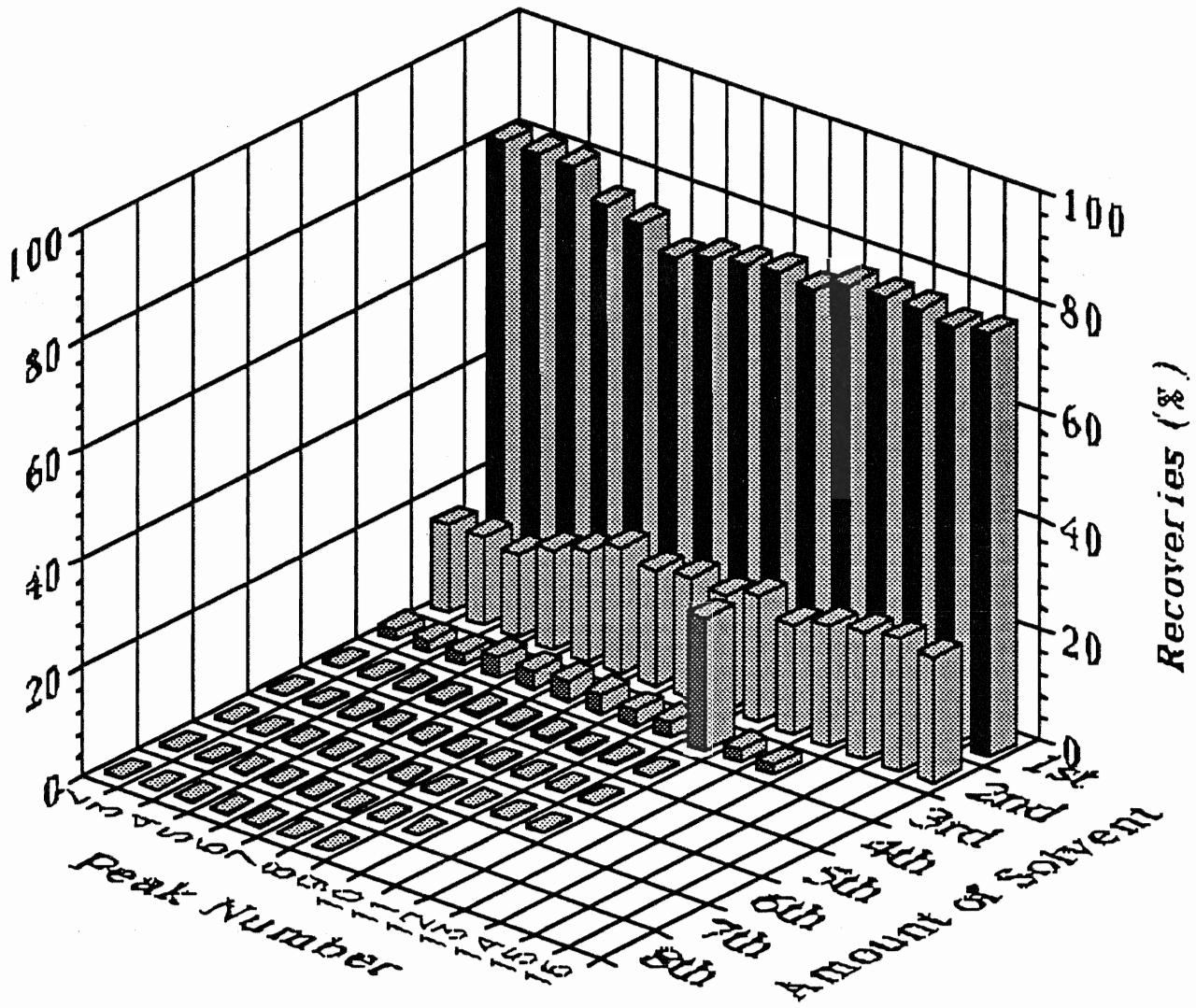


Figure 23: Volume of p-xylene required to elute the PAHs using C18 cartridges

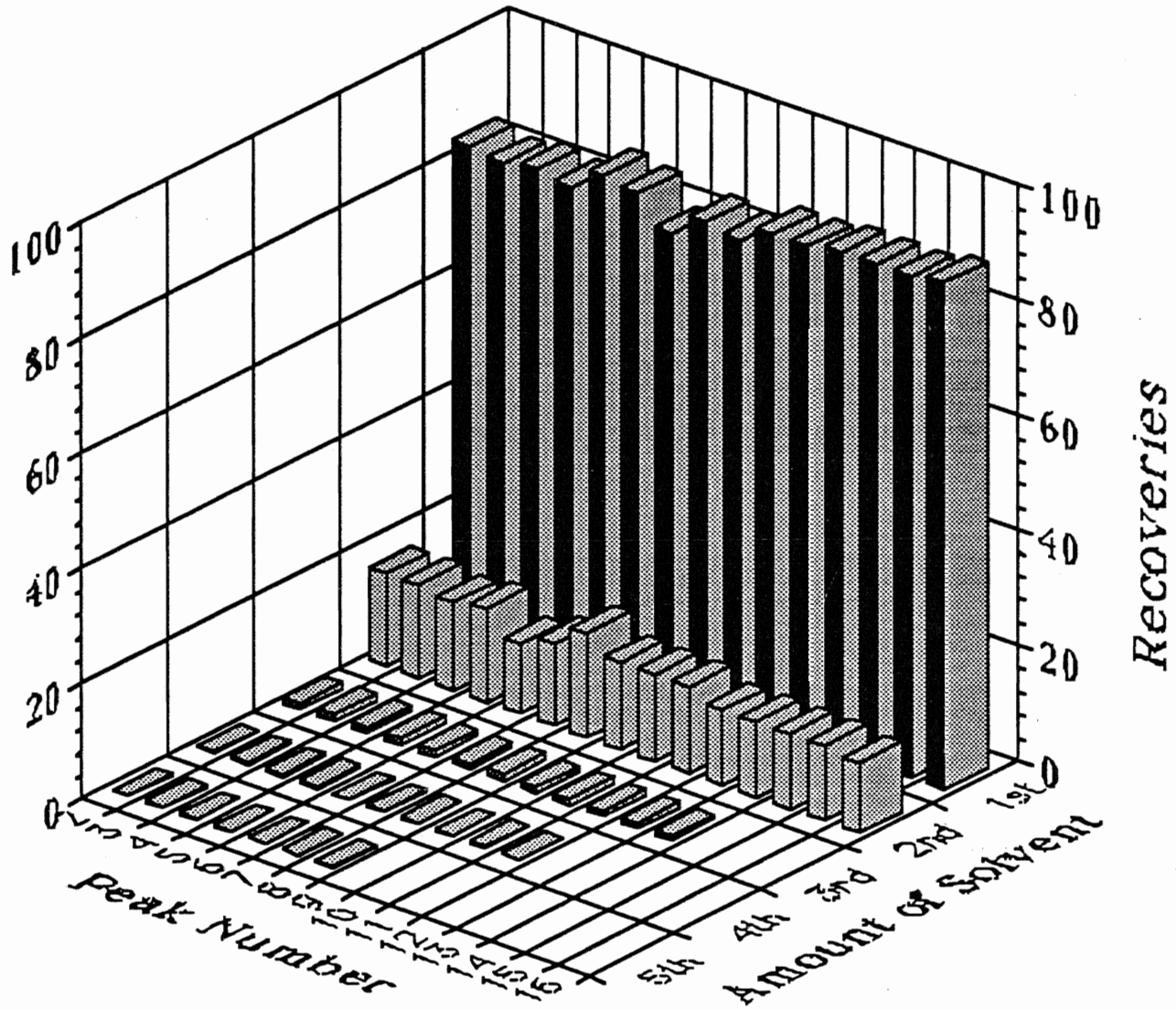


Figure 24: Volume of p-xylene required to elute the PAHs using tC18 cartridges

The effect of the flow rate:

The effect of flow rate of loading the spiked water sample through the C8 cartridges was investigated. The flow rate was varied between 3 ml / min to 12 ml / min. Peak response measurements of the resultant eluants showed no significant difference at these flow rates as shown in Figure 25. Our results and those from other investigators (88-89, 111) indicate that the flow rate does not have to be closely monitored. The peak responses of phenanthrene and anthracene at 5 ml / min appear to be lower than those at the other flow rates. In an independent experiment, the peak responses of phenanthrene and anthracene at flow rates of 3 ml/min to 6 ml/min were found to be similar.

The effect of the flow rate used to elute the trapped PAHs on the C8 bonded phase was also examined. The flow rate of elution solvent appeared to have some effect on the extraction efficiency of PAHs, particularly the low molecular weight PAHs, as shown in Figure 26. As the flow rate increased from 0.3 ml / min 0.5 ml / min, a decline in the peak response was observed. This could be due to a stronger affinity between the low molecular weight PAHs and the sorbent so that longer contact time between the sorbent and the analyte is required for complete elution. Further increase of the flow rate to 1 ml / min resulted in a decrease of the peak response of all PAHs. Thus, the optimum flow rate of eluting the PAHs was chosen to be 0.3 ml / min.

Figure 25: The effect of the flow rate of loading the sample through the C8 cartridges

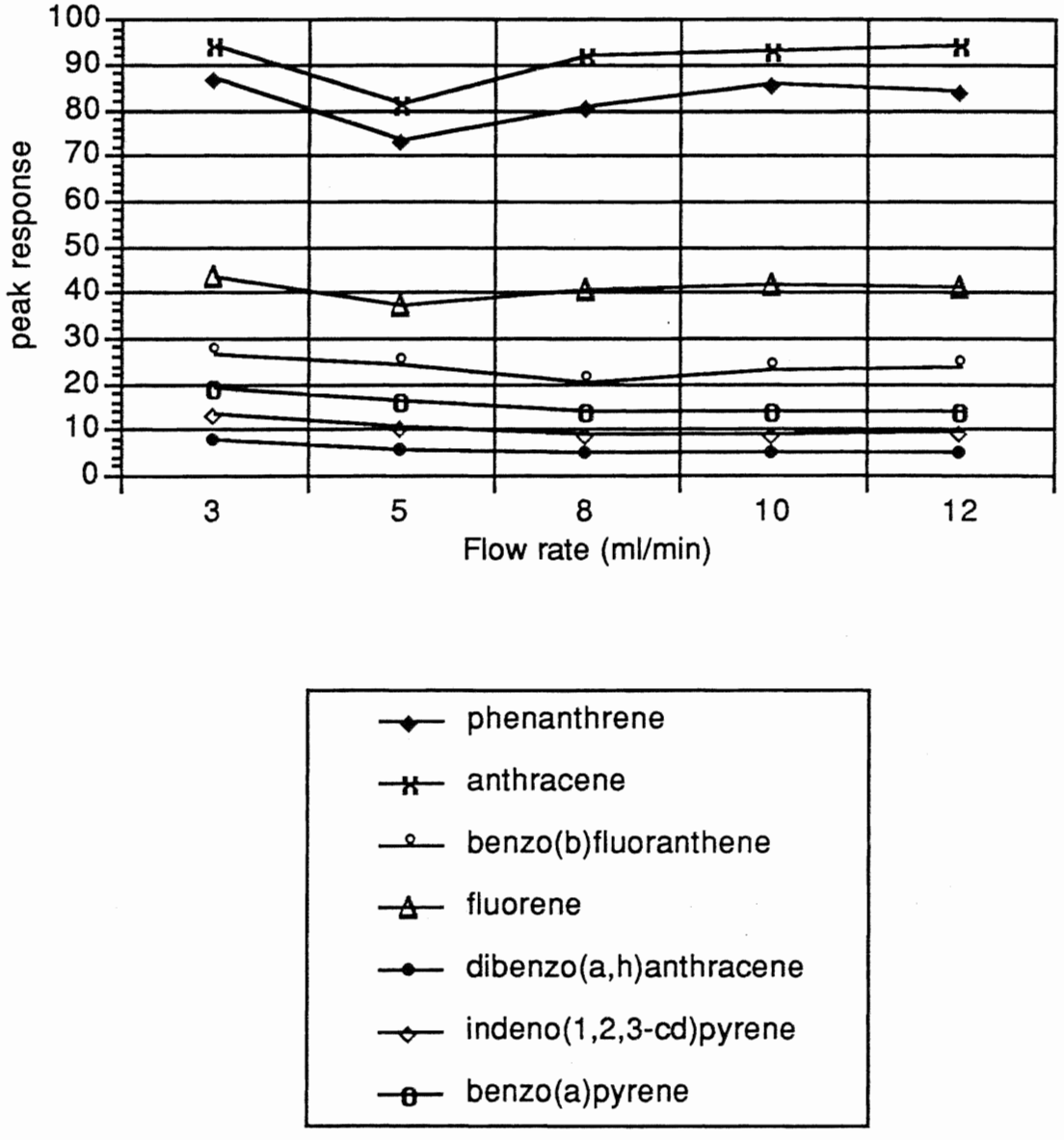
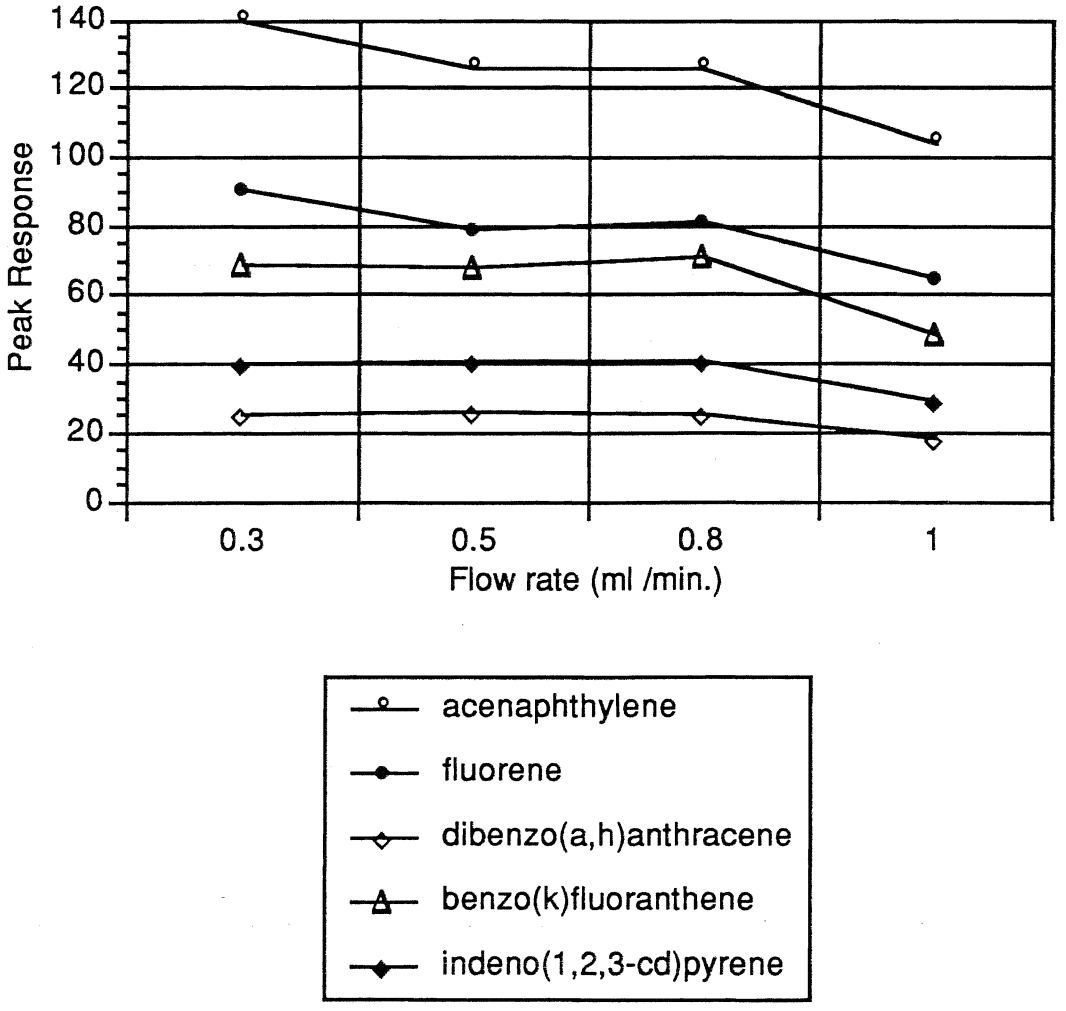


Figure 26: The effect of the flow rate of elution of PAHs by p-xylene



The effect of drying time:

After the water sample is passed through the bonded phase, drying of the cartridge to remove the remaining of the water is the next step. In our study, drying was carried out by blowing a gentle stream of nitrogen, about 20 ml/min, through the cartridge. The effect of the drying time on the recovery of PAHs was investigated. Table 33 shows the data obtained for drying time between 15 and 120 seconds. It was found that the drying times employed at 20 ml/min have no significant effects on the low molecular weight PAHs. This might be due to the strong affinity of the low molecular weight PAHs to the sorbent. However, the high molecular weight PAHs seem to be effected when the drying time is below or above 30 seconds. Losses in recoveries when the drying time is below 30 seconds could be the result of the incomplete removal of the water which might interfere with the solvent and the sorbent interaction.

Table 33: The effect of drying time (seconds) on the recoveries of PAHs by p-xylene

Peak#	Component	15	30	60	90	120
2	Acenaphthylene	105	100	101	110	110
3	Acenaphthene	104	100	100	110	108
4	Fluorene	105	100	100	110	111
5	Phenanthrene	109	100	101	112	114
6	Anthracene	109	100	101	114	115
7	Fluoranthene	113	100	99	114	114
8	Pyrene	113	100	98	114	113
9	Benz(a)anthracene	100	100	90	95	91
10	Chrysene	82	100	87	88	84
11	Benzo(b)fluoranthene	91	100	83	79	77
12	Benzo(k)fluoranthene	61	100	77	77	75
13	Benzo(a)pyrene	67	100	80	77	73
14	Indeno(1,2,3-cd)pyrene	49	100	74	61	60
15	Dibenzo(a,h)anthracene	52	100	63	81	76
16	Benzo(g,h,i)perylene	39	100	65	73	62

Absolute Recoveries of PAHs:

Having optimized the conditions of the extraction of PAHs from water samples, the absolute recoveries of the PAHs were determined. Recoveries were calculated as the amount of PAHs in the solution after completing the concentration procedure compared with the amount present in standard solution. Both the eluted PAHs in p-xylene and the standard solution were injected onto a DB5 column, from which chromatograms of the PAHs were obtained. Recoveries were calculated based on the peak height and the peak area of each of the analytes. The data presented in Table 34 represent the average recoveries of fifteen PAHs achieved when 50 ml of spiked water samples were passed through C8 cartridges at flow rate of 10 ml/min, dried in a gentle flow of nitrogen for 30 sec, and eluted with 500 µl of p-xylene at 0.3 ml/min. The PAH concentrations in water was 20 ng / ml (ppb). The same conditions, as discussed in part A, for the analysis of PAHs in p-xylene using GC/MS were employed. The recoveries reported in Table 34 are the means of nine determinations. It can be seen that the average recoveries are all greater than 80%, particularly significant is the recoveries of the high molecular weight PAHs. The relative standard deviations of these recoveries are summarized in Table 35, in which low relative standard deviations were obtained indicating the good reproducibility of the procedure.

Table 34: Absolute recoveries of the PAHs in p-xylene using C8 cartridges (PAH concentrations in water is 20 ppb)

Peak#	Component	Height	Area
2	Acenaphthylene	133	100
3	Acenaphthene	116	84
4	Fluorene	118	98
5	Phenanthrene	119	104
6	Anthracene	102	90
7	Fluoranthene	109	101
8	Pyrene	105	101
9	Benz(a)anthracene	89	92
10	Chrysene	81	80
11	Benzo(b)fluoranthene	99	100
12	Benzo(k)fluoranthene	84	86
13	Benzo(a)pyrene	89	92
14	Indeno(1,2,3-cd)pyrene	93	94
15	Dibenzo(a,h)anthracene	87	87
16	Benzo(g,h,i)perlene	85	86

Table 35: The relative standard deviations of the recoveries of PAHs in p-xylene using C8 cartridges.
(PAH concentration in water 20ppb)

Peak#	Component	Height	Area
2	Acenaphthylene	9.9	4.8
3	Acenaphthene	12	4.3
4	Fluorene	11	2.2
5	Phenanthrene	10	9.4
6	Anthracene	15	5.7
7	Fluoranthene	10	8.5
8	Pyrene	9.4	9.0
9	Benz(a)anthracene	9.7	7.0
10	Chrysene	5.3	4.2
11	Benzo(b)fluoranthene	9.0	9.1
12	Benzo(k)fluoranthene	6.3	5.8
13	Benzo(a)pyrene	8.4	7.8
14	Indeno(1,2,3-cd)pyrene	9.0	9.2
15	Dibenzo(a,h)anthracene	8.7	9.3
16	Benzo(g,h,i)perlene	9.3	8.8

The effect of the concentrations of PAHs in spiked water samples on the recoveries of PAHs was also examined. The same procedure as mentioned previously was used except that the PAH concentration in water was 2 ng / ml. Similar recoveries to those with 20 ng / ml PAH concentration were obtained except for the last three PAHs where, the PAH concentration was 2 ppb, the recoveries were about 10% higher than those when the PAH concentration was 20 ppb. The results are shown in Figure 27.

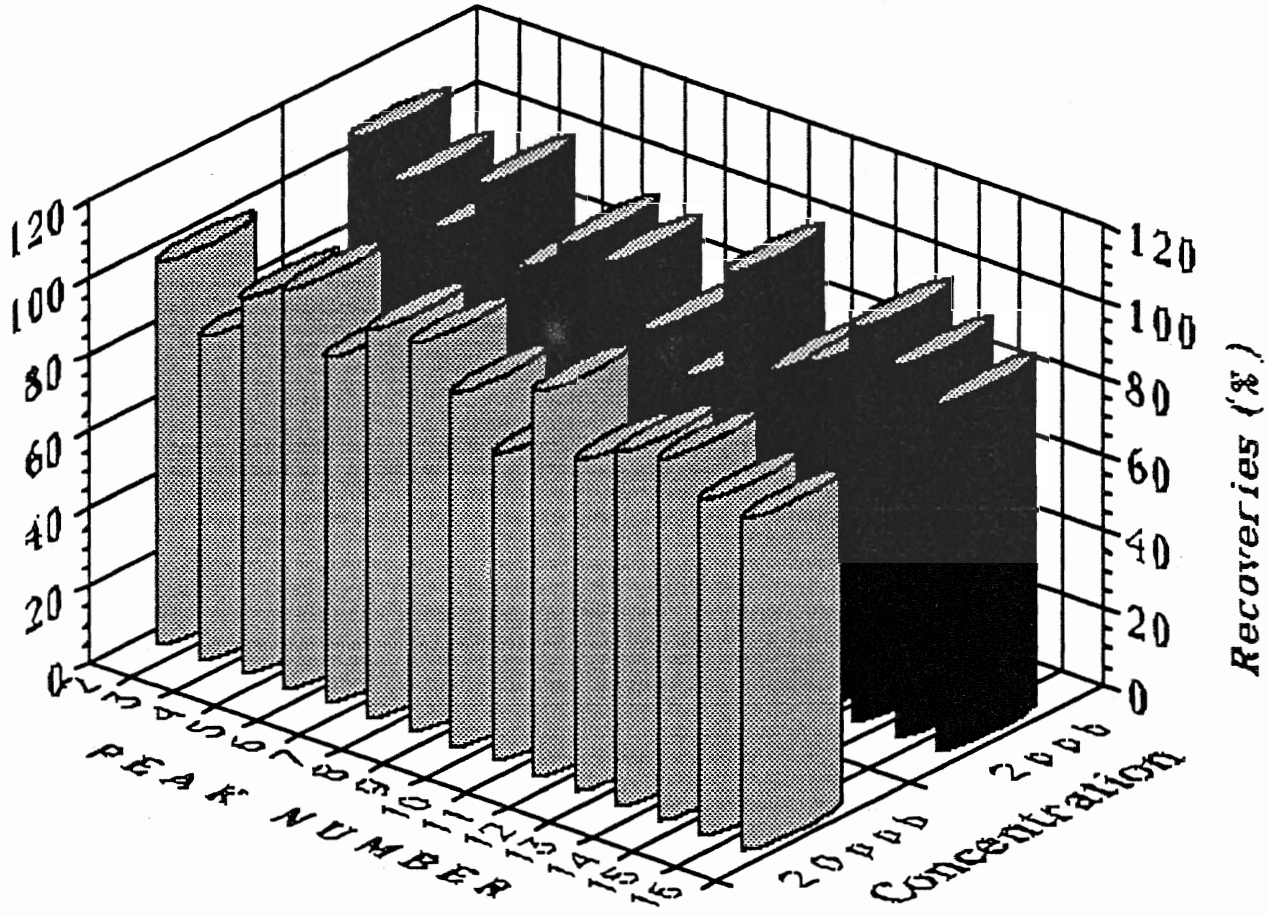
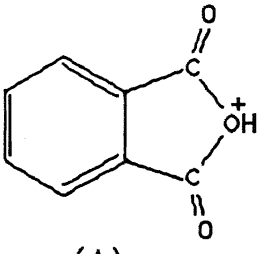


Figure 27: The effect of the PAH concentration in water on the percent recoveries

Studies on possible interferences on the extraction of PAHs from water were carried out by carrying out a GC/MS run using a scan of 55-500 to identify the eluents from the three bonded phase silica, C8, C18, and tC18. Similar chromatograms were obtained for these sorbents as shown in Appendix 3. The same procedure as mentioned earlier was followed for each of the phases. The major contaminants present at low level are primarily residual phthalate esters. These contaminants can be distinguished and they do not interfere with the recognition of PAHs. Phthalate esters can be easily recognized from their electron impact (EI) mass spectra; they all give a very characteristic ion at m/e 149, which is considered to be the protonated anhydride species (A).



(A)

Also, present at very low level were aliphatic hydrocarbons. All chromatograms in Appendix 3 showed peaks corresponding to straight-chain alkanes in which weak molecular ions and typical series of $C_n H_{2n+1}^+$ ions are present. However, all these low level contaminants do not interfere with the recognition of PAHs.

Re-usability of solid-phase extraction columns

It is recommended by the supplier that each cartridge is to be used for only one single analysis. Although the SPE cartridges are relatively inexpensive, the quantity required for the analysis of large numbers of samples raises the question of their reusability. The effect of reusing the bonded phase silica cartridges on the recoveries of PAHs extracted from water samples was examined. After the elution of the trapped PAHs on the sorbent by p-xylene, the cartridge was reconditioned in the same manner as the original conditioning, and used again for the extraction of PAHs from water. Table 36 and 37 present the recoveries of PAHs obtained, based on the peak area and height, respectively, when the C8 cartridges were re-used up to nine times. Similar responses by the peak area and the peak height calculations were obtained, as shown in Figure 28. The results obtained indicate that the C8 cartridges can be re-used for the extraction of PAHs from water samples. The efficiency of C8 cartridges of trapping PAHs from water was decreased by about 20% after they are used once. Similar results were also observed for the C18 cartridges when they were re-used up to five times for the extraction of PAHs from water samples as shown in Table 38. The re-usability of C8, C18, and tC18 during the extraction of PAHs was compared in Figure 29, in which similar results were obtained for all the three sorbents. The relative standard deviations for the recoveries of PAHs by re-using C8 cartridges up to nine times are summarized in Table 39. The relative standard deviations for three determinations were lower than 10% except for the last six PAHs in the ninth use of the cartridges.

Table 36: The relative recoveries of the reusability of C8 cartridges to the first use of the extraction of PAHs from water
(recoveries are based on the peak area of the PAHs)

	1st	2nd	3rd	4th	5th	6th	7th	8th	9th
Peak#									
2	100	94	95	86	81	86	83	76	85
3	100	97	102	93	85	90	90	81	86
4	100	92	91	87	79	86	83	77	82
5	100	90	89	80	83	90	87	79	84
6	100	92	90	81	78	83	83	75	82
7	100	90	88	73	81	82	86	77	85
8	100	89	87	70	81	79	85	74	83
9	100	87	80	76	84	86	94	93	88
10	100	91	84	90	86	99	100	109	104
11	100	83	74	77	86	92	104	99	92
12	100	91	79	87	86	100	108	114	107
13	100	87	75	79	85	94	105	110	99
14	100	87	74	83	80	91	103	105	97
15	100	89	73	86	70	86	90	103	104
16	100	91	74	88	74	88	90	102	99

Table 37: The relative recoveries of the reusability of C8 cartridges to the first use of the extraction of PAHs from water
(recoveries are based on the peak height of the PAHs)

Peak#	1st	2nd	3rd	4th	5th	6th	7th	8th	9th
2	100	86	84	73	61	69	64	56	74
3	100	89	99	87	61	75	66	62	71
4	100	87	88	75	66	77	70	62	75
5	100	94	97	87	85	99	90	84	92
6	100	87	88	73	78	82	79	66	72
7	100	92	89	76	89	89	91	85	94
8	100	88	77	59	72	69	83	78	84
9	100	89	81	75	83	86	98	95	87
10	100	90	85	89	85	100	103	106	100
11	100	85	76	80	89	95	107	102	94
12	100	92	81	89	88	102	111	118	109
13	100	87	77	80	85	95	109	112	91
14	100	90	76	85	81	93	104	107	99
15	100	90	75	89	73	88	93	105	106
16	100	91	75	89	75	89	92	103	101

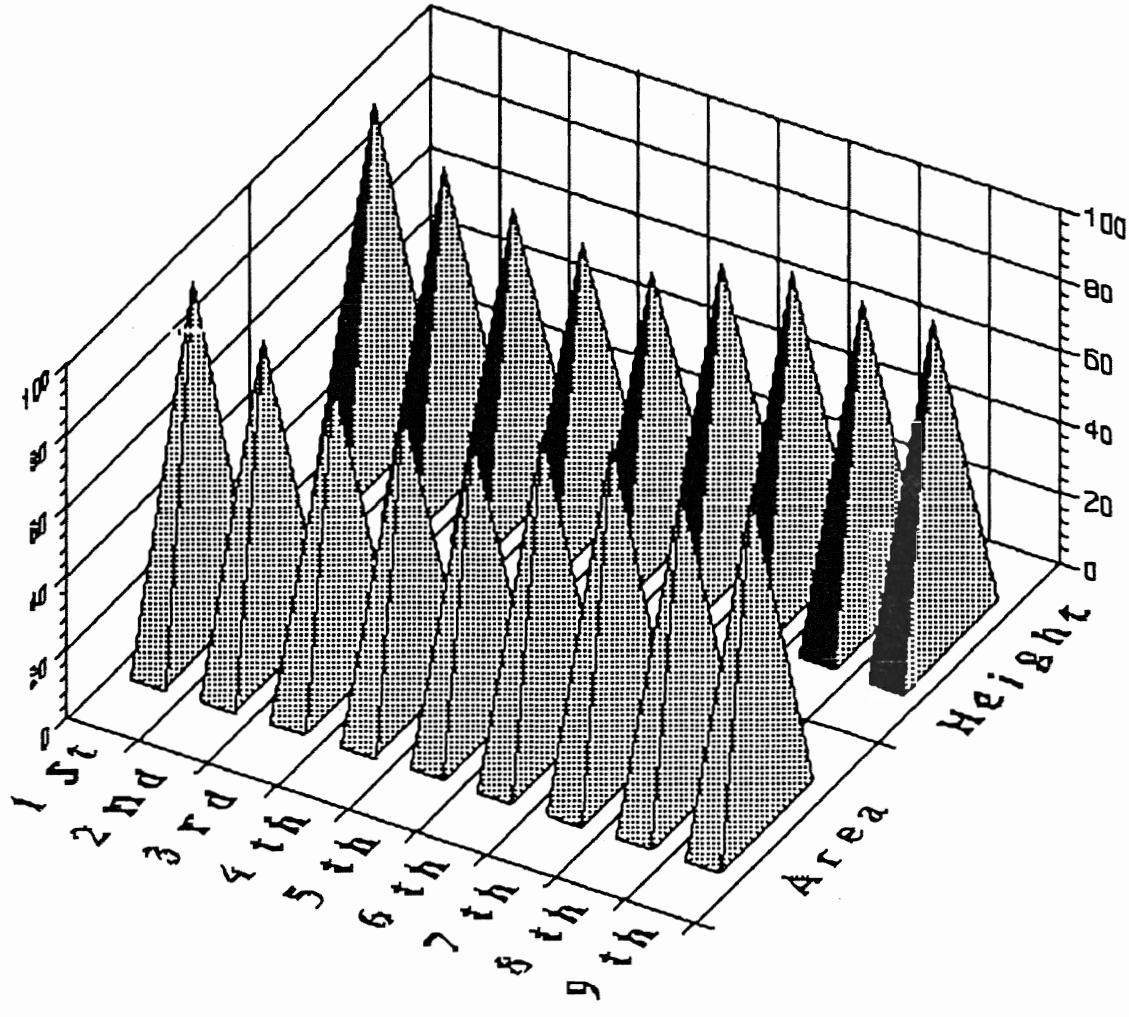


Figure 28: Comparison of the recoveries of PAHs during the re-usability of C8 cartridges based on the peak area and the peak height.

Table 38: The relative recoveries of the reusability of C18 cartridges to the first use of the extraction of PAHs from water

	Area					Height				
	1st	2nd	3rd	4th	5th	1st	2nd	3rd	4th	5th
Peak#										
2	100	92	80	95	87	100	104	98	116	110
3	100	92	82	101	98	100	105	106	128	118
4	100	82	68	94	77	100	100	95	117	106
5	100	90	74	95	85	100	99	84	101	96
6	100	89	72	94	84	100	85	73	103	82
7	100	88	68	91	79	100	96	79	105	85
8	100	87	67	89	77	100	90	65	79	80
9	100	87	73	83	75	100	88	72	83	74
10	100	101	97	98	99	100	97	95	97	97
11	100	71	67	69	66	100	71	66	68	65
12	100	96	97	98	102	100	95	95	96	100
13	100	90	83	86	83	100	90	82	85	84
14	100	95	92	88	92	100	96	93	89	95
15	100	83	82	68	77	100	84	83	71	79
16	100	104	106	92	101	100	104	105	91	102

Table 39: The relative standard deviations of the recoveries of PAHs during the re-usability of C8 cartridges

Peak#	1st	2nd	3rd	4th	5th	6th	7th	8th	9th
2	3.7	1.8	1.5	2.1	2.8	3.5	8.9	9.4	1.9
3	4.3	1.8	0.52	2.1	3.6	3.2	9.9	5.8	2.0
4	3.1	1.9	1.9	6.9	2.6	3.1	7.7	4.6	3.0
5	3.1	2.9	2.5	2.2	3.2	2.5	7.7	5.2	4.3
6	3.6	2.2	1.0	1.9	3.6	2.1	6.9	6.6	0.67
7	3.1	2.4	2.4	2.3	3.4	1.4	6.6	4.4	4.5
8	3.0	2.4	2.6	2.6	3.2	1.9	6.9	4.3	4.3
9	2.4	4.0	3.6	3.3	3.9	2.9	5.4	3.7	8.4
10	1.7	3.8	3.8	3.0	3.8	2.4	5.2	3.8	8.3
11	2.5	5.2	4.5	3.6	4.0	4.7	4.0	7.6	12
12	2.3	6.6	2.9	3.2	3.7	4.6	5.8	8.0	11
13	3.1	5.2	5.1	4.4	3.6	5.3	4.6	6.9	12
14	3.2	7.3	2.3	4.3	4.4	9.4	4.8	7.2	15
15	3.6	7.7	1.7	4.8	4.6	8.7	6.1	7.8	15
16	0.12	7.6	1.0	5.9	6.1	10	5.3	6.6	14

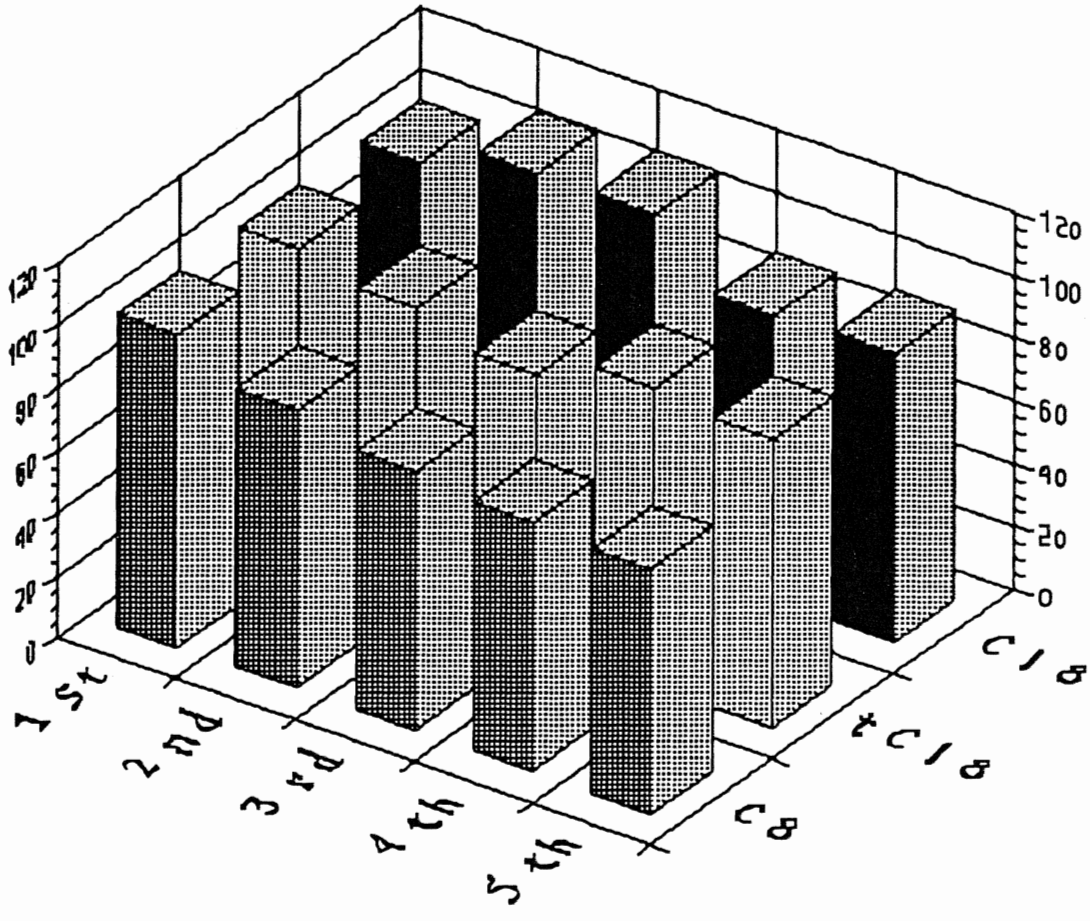


Figure 29: Comparison of the recoveries of PAHs during the re-usability of C8, C18, tC18 cartridges

Summary and Conclusion

High boiling point alcohols were studied as potential injection solvents for the determination of PAHs by capillary Gas Chromatography / Mass Spectrometry. The influence of the initial column temperature on the peak response of the PAHs, injected in butanol (b.p. 117.5 C), pentanol (b.p. 137.3 C), cyclopentanol (b.p. 140.8 C), and hexanol (b.p. 158 C) was investigated. The optimum initial column temperature was shown to be dependent on the injection solvent and the stationary phase employed, particularly for the late-eluted PAHs. The highest enhancement of the PAH peaks was achieved when the initial column temperature was 10-20 C above the boiling point of the solvent if injected onto DB5 column, and the same or 10 C higher than the solvent when DB1 column is used. The peak shape of the PAHs was found to be significantly affected by the initial column temperature regardless of the stationary phase. Fronting or splitting of the peaks was observed at low initial column temperature; however, the fronting disappeared upon the increase on the initial column temperature in which symmetrical peaks were obtained. High initial column temperature caused tailing of the PAH peaks. The range of the initial column temperature in which symmetrical PAH peaks can be obtained was wider when DB5 column is used compared to DB1 column. The separation efficiency of the closely-eluted PAHs was also shown to be affected by the stationary phase. Better resolution of the PAH peaks was obtained with DB5 column compared to DB1 column.

Among the alcohols studied, hexanol gave the greatest peak areas and heights of the late-eluted PAHs. Comparing the peak responses of the PAHs in hexanol to other high boiling point solvents such as p-xylene, nonane, and n-octane, it was concluded that hexanol should be the solvent of choice for the injection of PAHs onto the DB5 column.

Solid-phase extraction:

Preliminary experiments were carried out to examine the use of the high boiling point alcohols as elution solvents in the solid phase extraction of PAHs from water samples. Alcohols were used in attempt to increase the recoveries of the PAHs. Low recoveries of PAHs were thought to be caused by the incomplete elution of the trapped analytes on the bonded phase which might be due to interferences from the retained water. Hence, alcohols with their high solubility in water were examined in this study to overcome the problem of the low recoveries. Butanol, pentanol, cyclopentanol , and hexanol were evaluated as elution solvents for the extraction of PAHs from water. Unfortunately, the recoveries of spiked PAHs were low. The use of less polar solvents than alcohols as elution solvents was investigated. P-xylene gave by far the highest recoveries for the high molecular weight PAHs compared to toluene, butanol, pentanol, cyclopentanol, and hexanol. Further studies were carried out to compare the recoveries obtained when p-xylene is used as an elution solvent to other solvents such as benzene and ethyl acetate which have been recommended by many investigators to be as elution solvents for the extraction of PAHs from water samples. Again, p-xylene was shown to be the most proper

elution solvent especially for the late-eluted PAHs which were not eluted by ethyl acetate. From this work, it was clear that p-xylene is the elution solvent of choice for the extraction of PAHs from water samples. The influence of the volume of p-xylene, the type of the sorbent, the flow rate, and the drying time of the cartridges on the recoveries of PAHs was investigated. By comparing the recoveries using non polar bonded phase silica sorbents such as octyl (C8), octadecyl (C18), and tri-octadecyl (tC18) cartridges, similar recoveries were obtained for the low molecular weight PAHs; however, C8 demonstrated better recoveries for high molecular weight PAHs. The volume of p-xylene needed to elute the PAHs was found to depend on the molecular weight of the PAH. As low as 200 µl of p-xylene was required to elute the high molecular weight PAHs. For complete elution of the PAHs, 500 µl of p-xylene was chosen as the optimum volume. The flow rate of passing the water sample through the C8 cartridge was shown to have no affect of the recoveries of PAHs. However, high flow rate to elute the trapped PAHs gave lower recoveries of PAHs. Passing a gentle flow of nitrogen to dry the cartridges for 30 sec was found to give the best recoveries of PAHs. The absolute recoveries of PAHs from water, based on the optimum conditions were greater than 80 % regardless of the PAH concentration in the water sample. The relative standard deviations of nine replicate determination of the sixteen PAHs at 20 ng/ml were lower than 10%. In terms of interferences, it was found that no major contaminants were present that would interfere with the recognition of PAHs by using GC / MS. An added advantage to using the bonded phase silica for the extraction of PAHs from water is that these cartridges were

found to be reusable for more than once. Thus, the number of the cartridges needed for routine analysis of the PAHs would be reduced.

The use of this simple solid phase extraction procedure provides a rapid, efficient, and reproducible method for the determination of PAHs. The one-step extraction and concentration procedure minimizes analyte losses and the possible addition of contaminants. Very little solvent is needed for extraction; moreover, the method is faster and easier to perform than the conventional solvent extraction method. In addition to these advantages, cartridge sampling can be undertaken in the field, which eliminate the need for shipping and storing large volumes of water.

Appendix 1

The effect of the initial column temperature on the peak shape of the sixteen PAHs

Standard solutions of 2μg / ml of the sixteen PAHs in various solvents were injected onto two separate columns coated with different stationary phases. The effect of the initial column temperature on the peak shape of the sixteen PAHs using the two stationary phases was investigated as shown in the following pages.

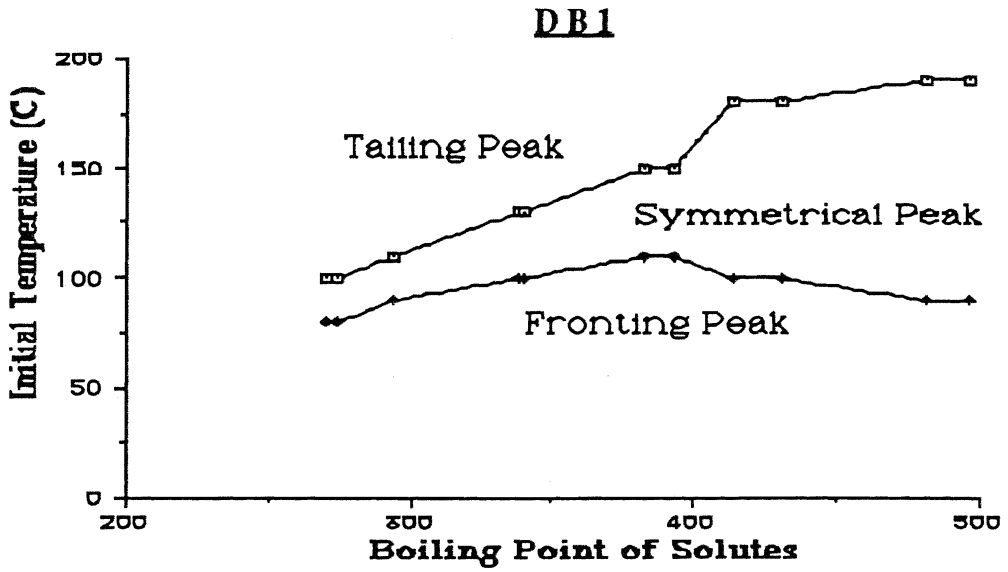
The stationary phases are:

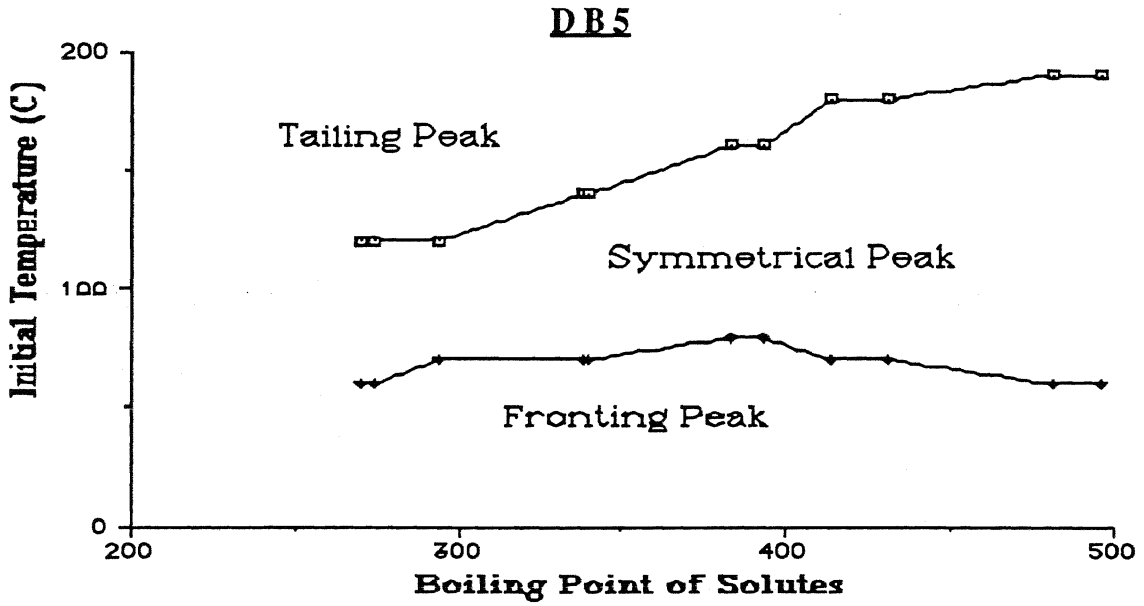
1. Crosslinked methyl silicone (DB1)
2. 5% diphenyl, 94% dimethyl, 1% vinyl polysiloxane (DB5)

The solvents used in this study are: (the order of the solvents is the same as they are shown in the following pages)

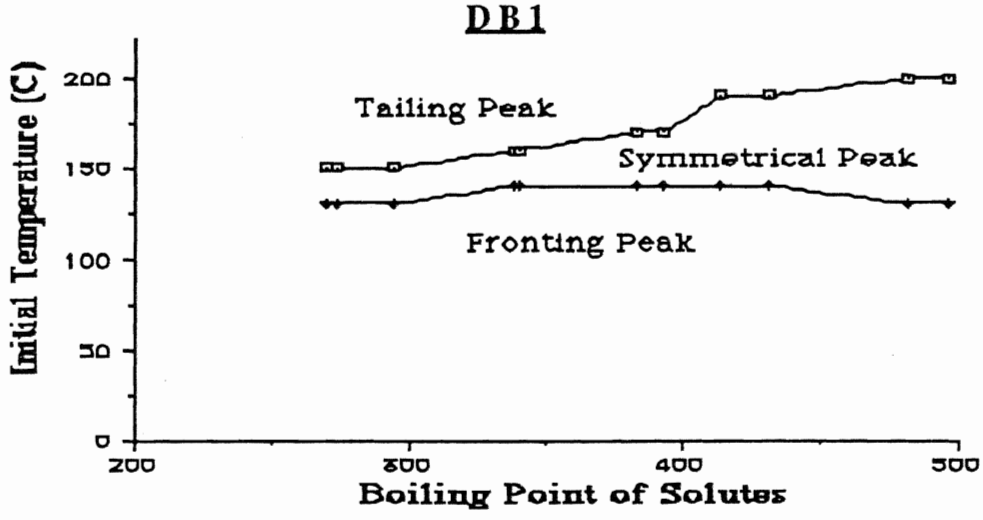
1. butanol
2. cyclopentanol
3. pentanol
4. p-xylene
5. n-octane
6. nonane

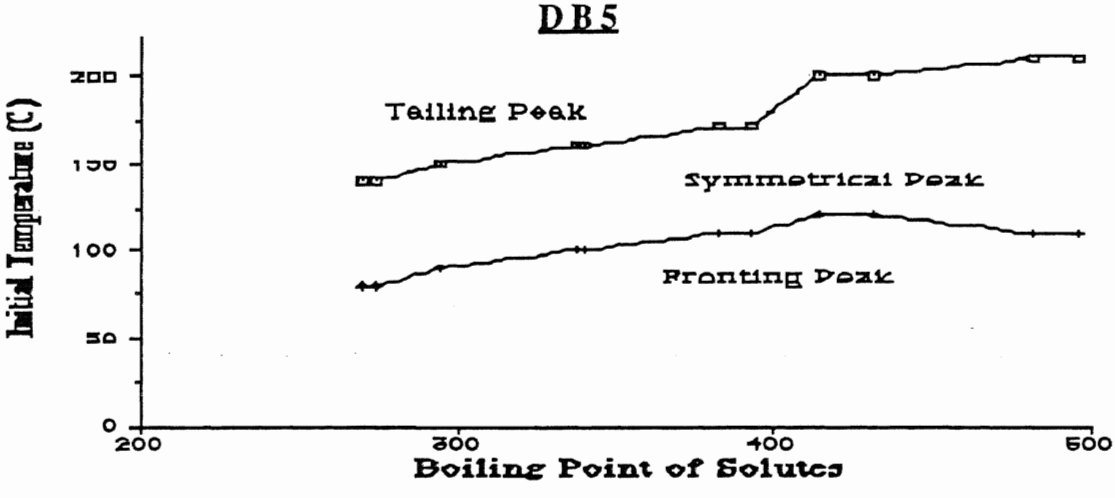
Butanol


DB1
Initial Temperature (C)
200
150
100
50
0
Tailing Peak
Symmetrical Peak
Fronting Peak
200
300
400
500
Boiling Point of Solutes

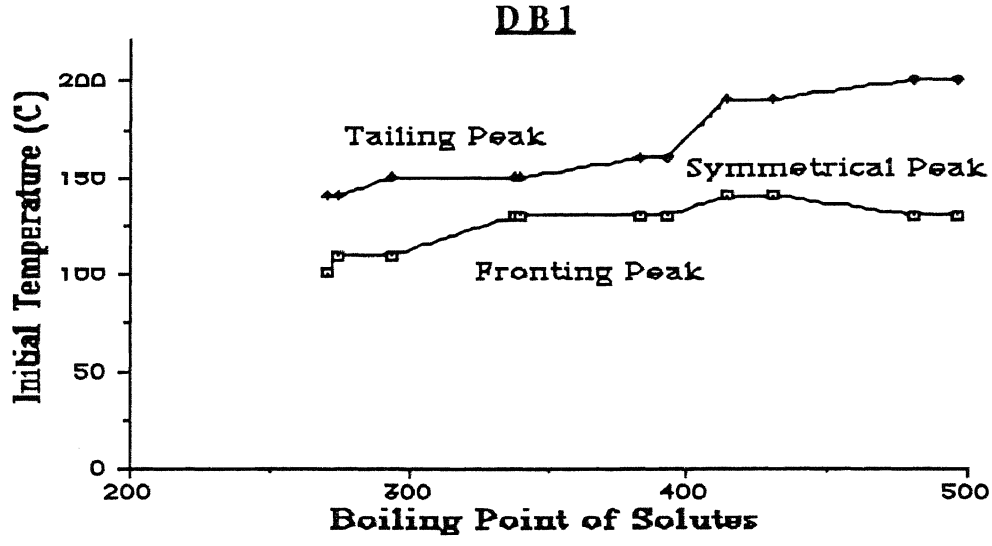

DB5
Initial Temperature (C)
200
100
0
Tailing Peak
Symmetrical Peak
Fronting Peak
200
300
400
500
Boiling Point of Solutes

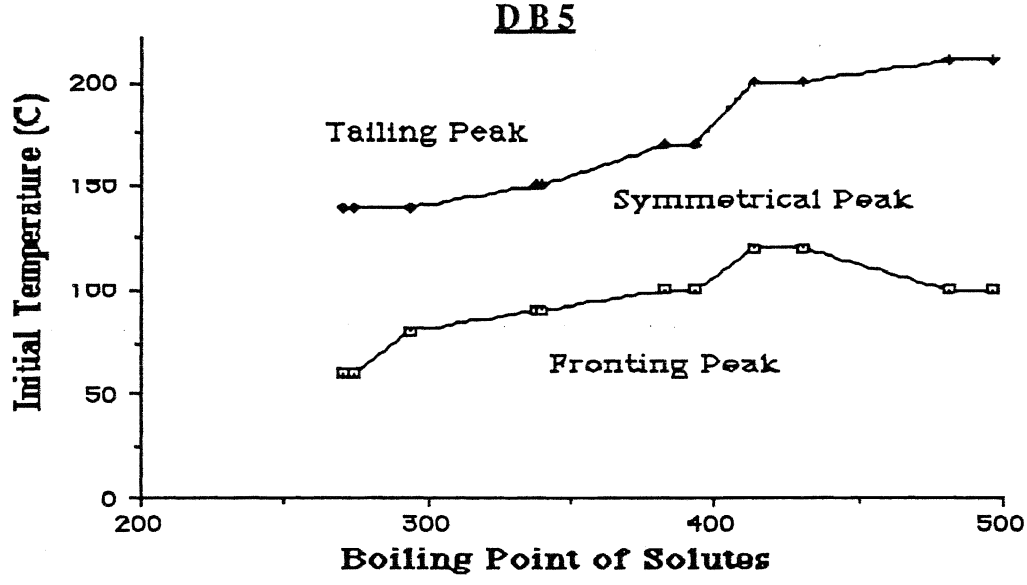
Cyclopentanol





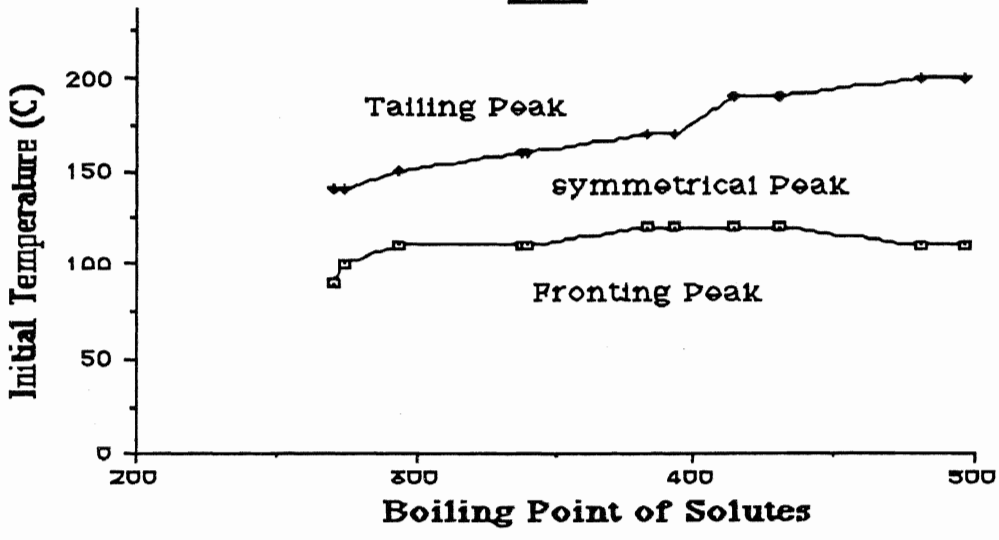
Pentanol


DB1
Initial Temperature (C)
200
150
100
50
0
Tailing Peak
Symmetrical Peak
Fronting Peak
200
300
400
500
Boiling Point of Solutes

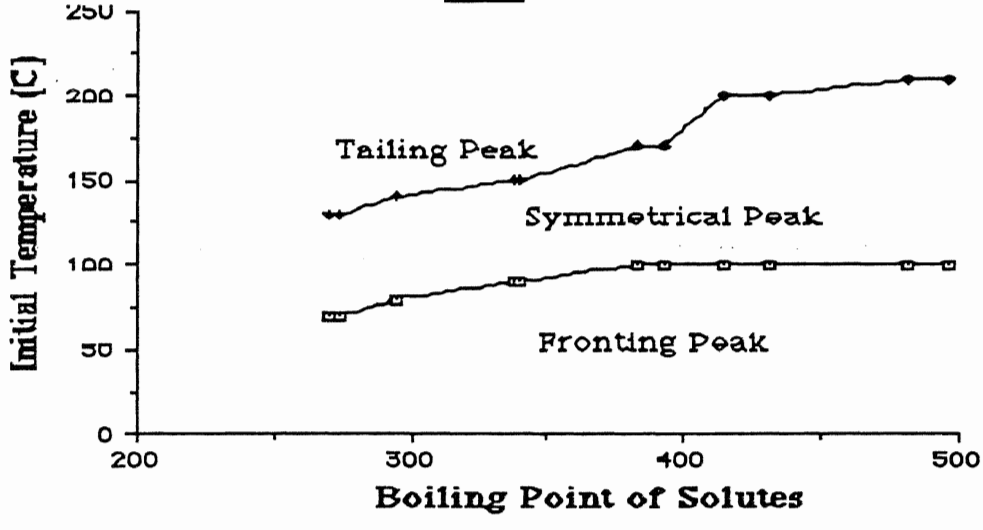

DB5
Initial Temperature (C)
200
150
100
50
0
Tailing Peak
Symmetrical Peak
Fronting Peak
200
300
400
500
Boiling Point of Solutes

P-xylene

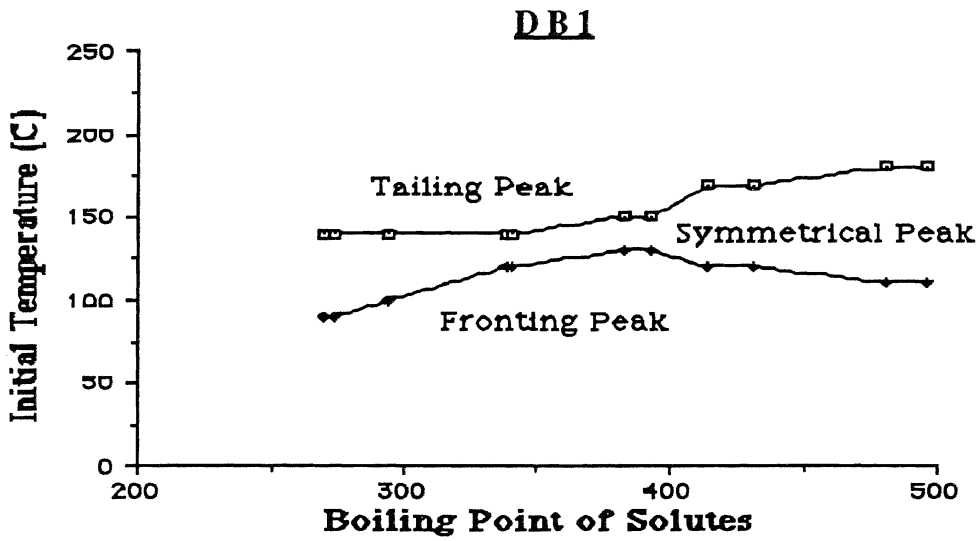
DB1

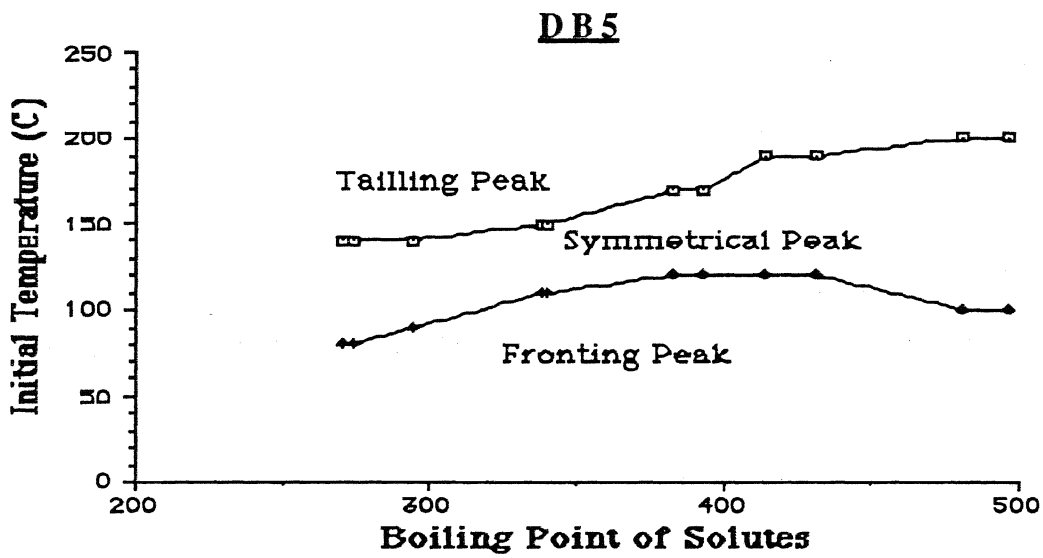


DB5

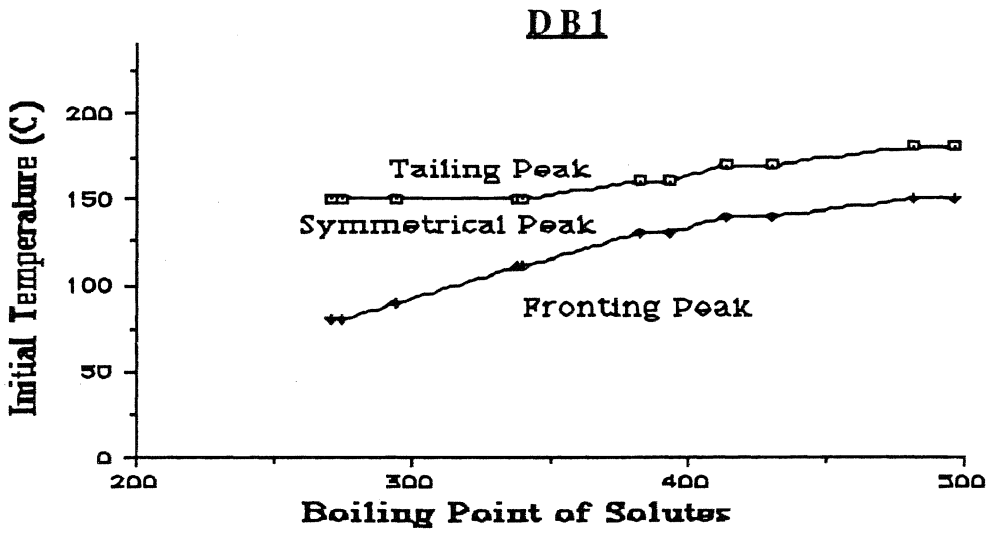


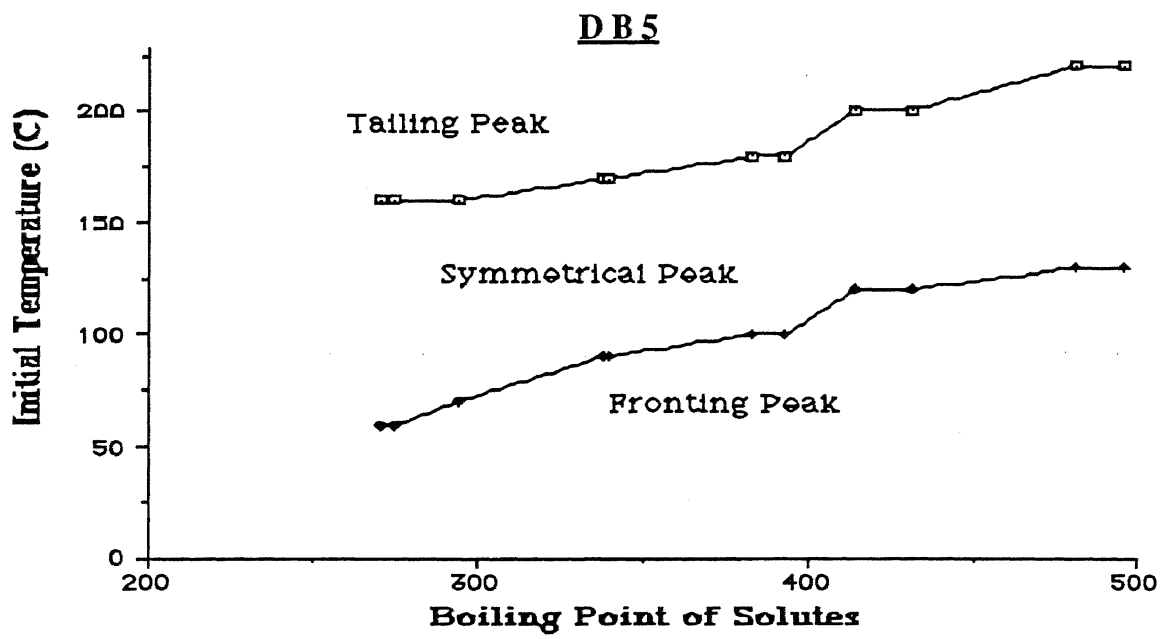
n-Octane


DB1
Initial Temperature (C)
250
200
150
100
50
0
200
300
400
500
Boiling Point of Solutes
Tailing Peak
Symmetrical Peak
Fronting Peak


DB5
Initial Temperature (C)
250
200
150
100
50
0
200
300
400
500
Boiling Point of Solutes
Tailling Peak
Symmetrical Peak
Fronting Peak

Nonane


DB1
Initial Temperature (C)
200
150
100
50
0
Tailing Peak
Symmetrical Peak
Fronting Peak
200
300
400
500
Boiling Point of Solutes


DB5
Initial Temperature (C)
200
150
100
50
0
Tailing Peak
Symmetrical Peak
Fronting Peak
200
300
400
500
Boiling Point of Solutes

Appendix 2

The effect of the elution solvent on the extraction efficiency of PAHs in water using octadecyl (C18) and tri-octadecyl (tC18) cartridges

The average recoveries of three determinations for spiked water samples based on the peak height and peak area of sixteen and thirteen PAHs using octadecyl (C18) and tri-octadecyl (tC18) cartridges are summerized in the following tables.

Relative recoveries of sixteen PAHs with different elution solvents to p-xylene based on the peak height using C18 cartridges

ε	p-xylene 2.27	toluene 2.38	benzene 2.29	butanol 17.1	cyclopentanol	pentanol 13.9	hexanol 13.3
Peak							
1	100	96	75	81	65	85	n.d.
2	100	100	80	70	70	91	74
3	100	101	72	67	69	66	60
4	100	91	56	59	55	63	59
5	100	93	46	43	48	43	61
6	100	82	47	42	46	38	60
7	100	84	50	23	42	31	45
8	100	83	47	26	40	30	44
9	100	84	30	14	37	45	45
10	100	106	39	25	47	70	59
11	100	76	20	12	35	56	43
12	100	87	23	0.5	39	70	56
13	100	64	50	14	33	55	44
14	100	38	21	n.d.	22	45	37
15	100	39	41	n.d.	14	55	43
16	100	40	14	n.d.	28	57	46

n.d. (not detected)

Relative recoveries of sixteen PAHs with different elution solvents to p-xylene based on the peak area using C18 cartridges

ε	p-xylene 2.27	toluene 2.38	benzene 2.29	butanol 17.1	cyclopentanol	pentanol 13.9	hexanol 13.3
Peak							
1	100	98	79	66	67	72	n.d.
2	100	122	99	88	92	120	97
3	100	102	75	64	65	67	71
4	100	91	73	59	60	60	63
5	100	90	57	46	52	49	57
6	100	96	69	49	54	48	56
7	100	89	59	40	42	36	45
8	100	91	58	37	41	36	43
9	100	92	36	19	41	48	46
10	100	64	51	38	61	77	67
11	100	74	23	12	36	57	44
12	100	87	27	38	45	75	73
13	100	76	30	10	40	60	55
14	100	41	9	n.d.	23	50	42
15	100	18	66	n.d.	6	73	54
16	100	29	3	n.d.	31	60	49

n.d. (not detected)

Relative recoveries of sixteen PAHs with different elution solvents to p-xylene based on the peak height using tC18 cartridges

ε	p-xylene 2.27	toluene 2.38	benzene 2.29	butanol 17.1	cyclopentanol	pentanol 13.9	hexanol 13.3
Peak							
1	100	123	81	79	75	105	n.d.
2	100	113	71	63	83	108	78
3	100	106	81	57	63	71	68
4	100	114	74	57	69	65	69
5	100	112	61	46	56	69	59
6	100	83	47	32	41	50	48
7	100	108	67	26	43	51	51
8	100	91	69	24	37	44	46
9	100	69	36	10	30	42	47
10	100	76	43	17	33	51	54
11	100	59	29	8	30	48	53
12	100	63	32	10	30	50	50
13	100	52	23	4	25	42	42
14	100	36	11	n.d.	17	35	34
15	100	34	n.d.	8	14	50	29
16	100	41	25	3	25	41	37

n.d. (not detected)

Relative recoveries of sixteen PAHs with different elution solvents to p-xylene based on the peak area using tC18 cartridges

	p-xylene	toluene	benzene	butanol	cyclopentanol	pentanol	hexanol
ε	2.27	2.38	2.29	17.1		13.9	13.3
Peak							
1	100	98	81	69	74	75	n.d.
2	100	106	86	75	88	100	75
3	100	103	83	67	76	74	68
4	100	95	60	59	65	69	59
5	100	99	61	46	53	59	55
6	100	99	80	46	54	58	56
7	100	96	71	39	41	49	48
8	100	99	74	34	39	48	48
9	100	79	42	11	33	47	52
10	100	89	56	33	42	61	62
11	100	61	31	8	25	47	51
12	100	72	46	4	35	55	64
13	100	62	23	3	28	47	49
14	100	51	4	n.d.	26	52	52
15	100	15	n.d.	1	11	42	32
16	100	44	14	1	24	43	41

n.d. (not detected)

Relative recoveries of thirteen PAHs by o-xylene and ethyl acetate to p-xylene using C18 cartridges

	AREA			HEIGHT		
	p-xylene	o-xylene	ethyl acetate	p-xylene	o-xylene	ethyl acetate
ε	2.27	2.57	6.02	2.27	2.57	6.02
Peak#						
1	100	76	86	100	46	75
2	100	68	81	100	51	72
3	100	74	75	100	60	61
4	100	48	62	100	37	48
5	100	76	70	100	69	65
6	100	54	49	100	52	32
7	100	96	n.d.	100	91	n.d.
8	100	55	43	100	56	39
9	100	119	74	100	98	69
10	100	77	13	100	67	40
11	100	95	n.d.	100	91	n.d.
12	100	95	n.d.	100	67	n.d.
13	100	100	n.d.	100	125	n.d.

n.d. (not detected)

Relative standard deviations (%RSD) of average recoveries of the thirteen PAHs by p-xylene, o-xylene, and ethyl acetate using C18 cartridges

	AREA			HEIGHT		
	p-xylene	o-xylene	ethyl acetate	p-xylene	o-xylene	ethyl acetate
Peak#						
1	2.7	3.6	4.3	10	3.6	6.3
2	1.6	2.0	2.5	8.7	6.9	4.6
3	1.0	3.9	3.8	4.6	1.0	4.6
4	1.0	3.3	5.0	6.6	6.2	7.6
5	0.1	2.7	6.7	1.9	2.5	4.9
6	3.1	4.2	8.9	4.8	4.6	9.3
7	3.8	2.6	6.0	2.4	2.4	4.5
8	2.1	1.7	13	2.3	3.3	10
9	0.8	1.6	8.1	1.3	3.0	8.3
10	5.0	3.5	4.3	5.9	3.9	15
11	3.6	3.7	n.d.	6.6	4.1	n.d.
12	11	9.4	n.d.	8.6	5.2	n.d.
13	3.0	5.3	n.d.	3.0	4.3	n.d.

n.d. (not detected)

Relative recoveries of thirteen PAHs by o-xylene and ethyl acetate to p-xylene using tC18 cartridges

	AREA			HEIGHT		
	p-xylene	o-xylene	ethyl acetate	p-xylene	o-xylene	ethyl acetate
ε	2.27	2.57	6.02	2.27	2.57	6.02
Peak#						
1	100	80	95	100	52	89
2	100	203	75	100	90	78
3	100	74	61	100	62	50
4	100	87	92	100	63	69
5	100	76	63	100	65	45
6	100	53	22	100	47	16
7	100	67	n.d.	100	50	n.d.
8	100	48	22	100	44	17
9	100	53	10	100	45	14
10	100	45	32	100	40	26
11	100	37	n.d.	100	33	n.d.
12	100	31	n.d.	100	26	n.d.
13	100	37	n.d.	100	33	n.d.

n.d. (not detected)

Relative standard deviations (%RSD) of average recoveries of the thirteen PAHs by p-xylene, o-xylene, and ethyl acetate using tC18 cartridges

	AREA			HEIGHT		
	p-xylene	o-xylene	ethyl acetate	p-xylene	o-xylene	ethyl acetate
Peak#						
1	2.6	5.1	0.7	5.6	4.8	1.2
2	2.3	5.6	1.4	5.4	6.8	0.8
3	1.5	4.0	2.2	2.5	5.0	3.0
4	1.1	3.6	2.1	2.8	6.3	2.8
5	1.2	2.3	3.6	0.6	4.9	2.8
6	1.8	7.0	4.1	2.0	7.8	5.4
7	1.3	6.1	1.1	1.5	5.7	1.2
8	3.5	6.7	11	3.6	6.7	2.7
9	3.3	6.0	1.2	2.9	7.4	3.7
10	1.1	6.0	8.8	1.3	6.9	2.8
11	1.7	3.2	n.d.	3.2	5.4	n.d.
12	8.5	12	n.d.	2.4	1.5	n.d.
13	2.5	1.9	n.d.	1.4	3.1	n.d.

n.d. (not detected)

Appendix 3

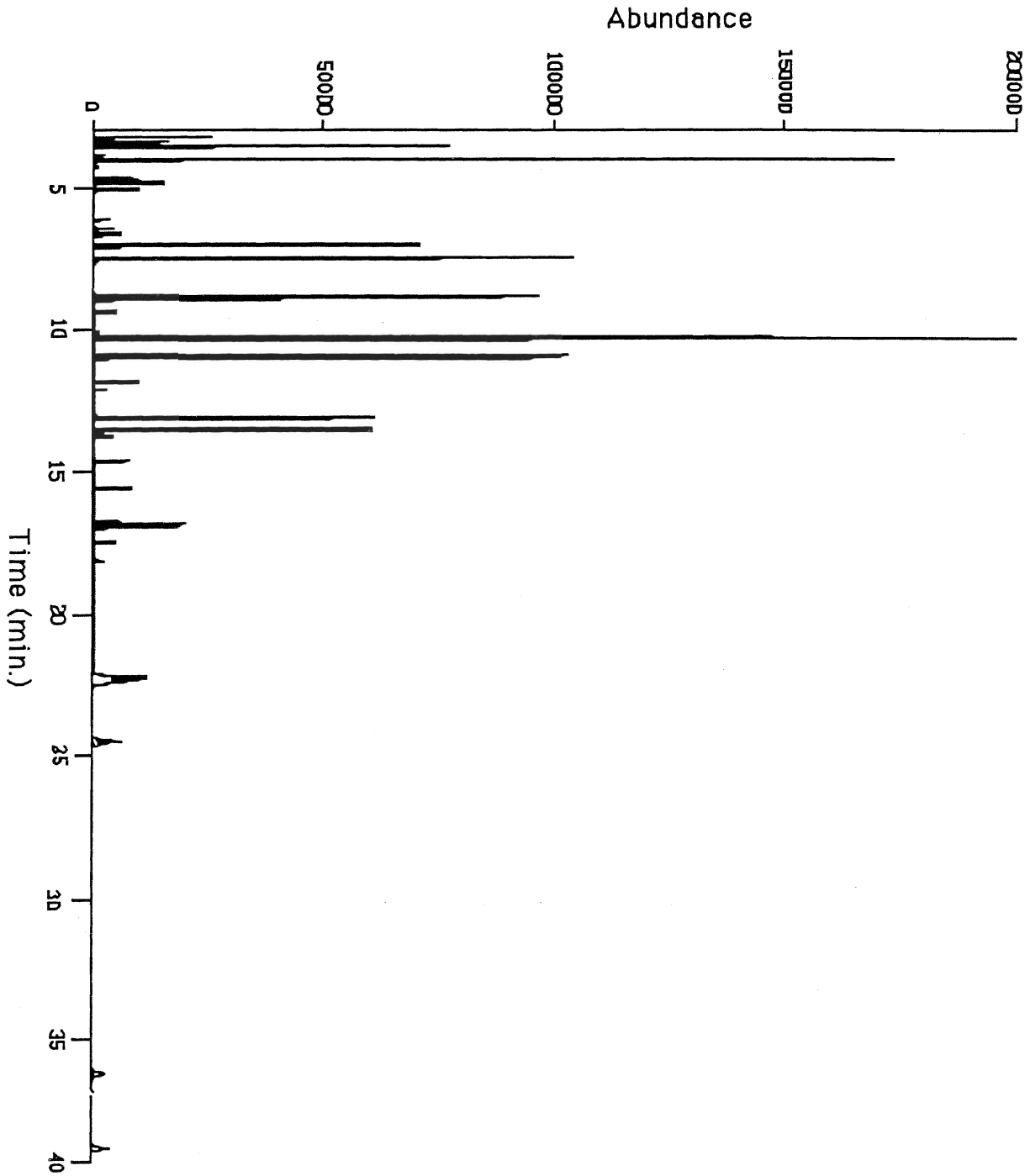
Comparison of chromatograms of sixteen PAHs extracted from water by C8, C18, and tC18 cartridges for possible interferences.

Determinations of possible interferences were carried out by scanning the solutions eluted by p-xylene through C8, C18, tC18 bonded phase silica.

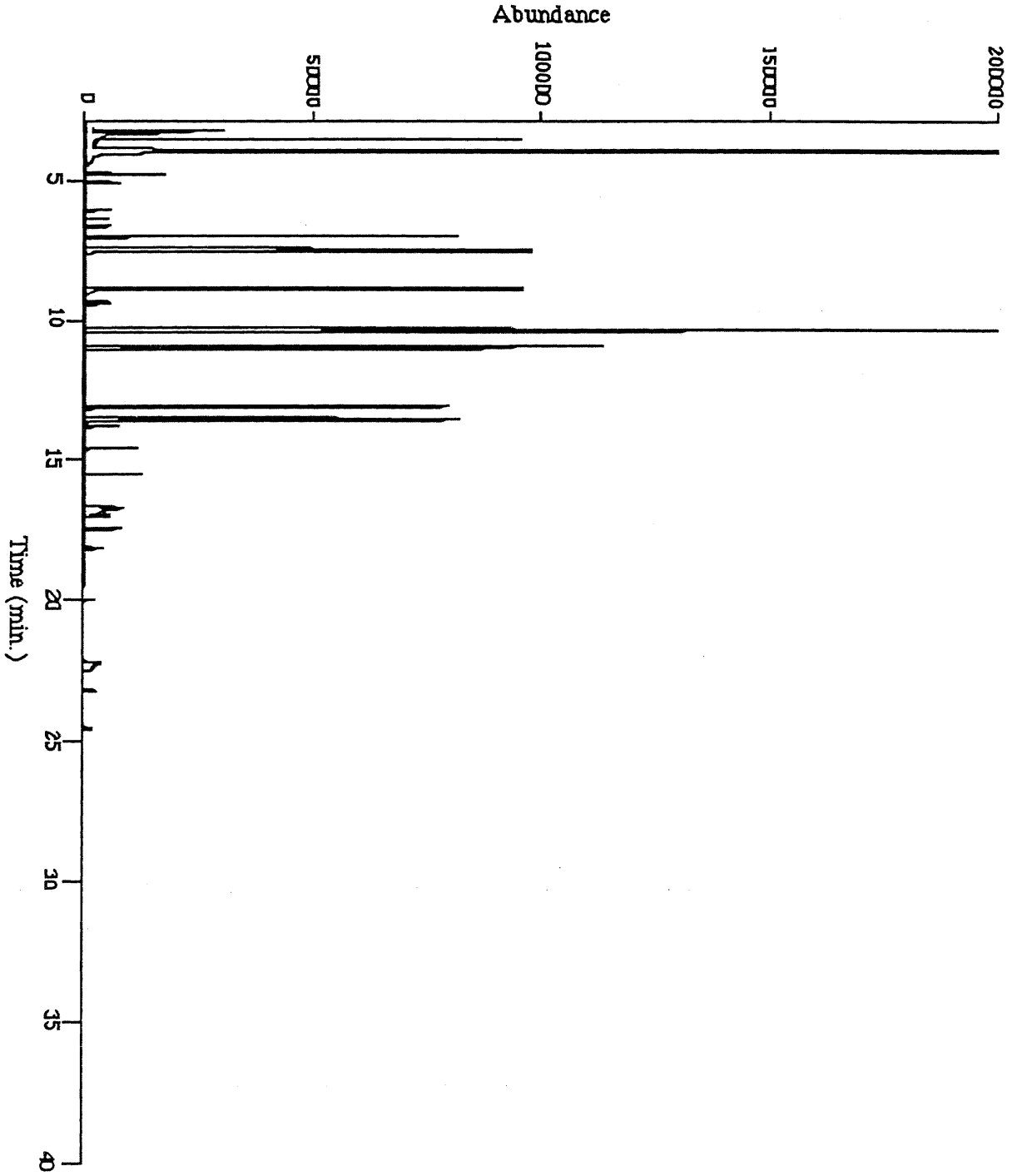
The order of chromatograms:

1. C8
2. C18
3. tC18

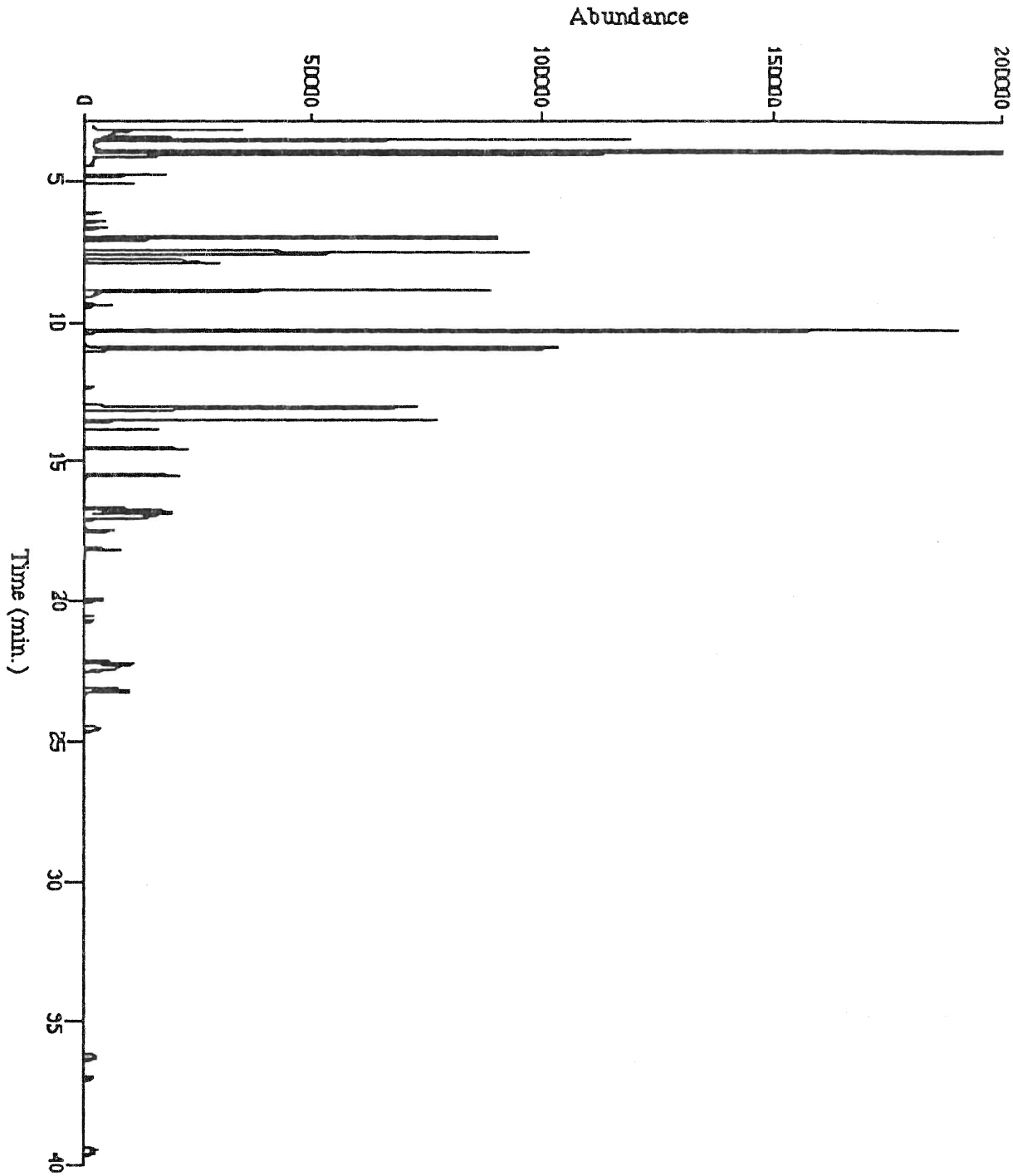
(1) Chromatogram of 16 PAHs eluted by p-xylene through C8 cartridge
(SCAN mode)



(2) Chromatogram of 16 PAHs eluted by p-xylene through C18 cartridge
(SCAN mode)



(3) Chromatogram of 16 PAHs eluted by p-xylene through tC18 cartridge
(SCAN mode)



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