Investigations into the Determination of Polycyclic Aromatic Hydrocarbons (PAHs) and Polychlorinated Biphenyls (PCBs) by Capillary Gas Chromatography

by

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Abstract

Factors involved in the determination of PAHs (16 priority PAHs as an example) and PCBs (10 PCB congeners, representing 10 isomeric groups) by capillary gas chromatography coupled with mass spectrometry (GC/MS, for PAHs) and electron capture detection (GC/ECD, for PCBs) were studied, with emphasis on the effect of solvent. Having various volatilities and different polarities, solvent studied included dichloromethane, acetonitrile, hexane, cyclohexane, isooctane, octane, nonane, dodecane, benzene, toluene, p-xylene, o-xylene, and mesitylene. Temperatures of the capillary column, the injection port, the GC/MS interface, the flow rates of carrier gas and make-up gas, and the injection volume were optimized by one factor at a time method or simplex optimization method.

Under the optimized conditions, both peak height and peak area of 16 PAHs, especially the late-eluting PAHs, were significantly enhanced (1 to 500 times) by using relatively higher boiling point solvents such as p-xylene and nonane, compared with commonly used solvents like benzene and isooctane. With the improved sensitivity, detection limits of between 4.4 pg for naphthalene and 30.8 pg for benzo[g,h,i]perylene were obtained when p-xylene was used as an injection solvent.

Effect of solvent on peak shape and peak intensity were found to be greatly dependent on temperature parameters, especially the initial temperature of the capillary column. The relationship between initial temperature and shape of peaks from 16 PAHs and 10 PCBs were studied and compared when toluene, p-xylene, isooctane, and nonane were used as injection solvents. If a too low initial temperature was used, fronting or

split of peaks was observed. On the other hand, peak tailing occurred at a too high initial column temperature. The optimum initial temperature, at which both peak fronting and tailing were avoided and symmetrical peaks were obtained, depended on both solvents and the stationary phase of the column used. On a methyl silicone column, the alkane solvents provided wider optimum ranges of initial temperature than aromatic solvents did, for achieving well-shaped symmetrical GC peaks. On a 5% diphenyl: 1% vinyl: 94% dimethyl polysiloxane column, when the aromatic solvents were used, the optimum initial temperature ranges for solutes to form symmetrical peaks were improved to a similar degree as those when the alkanes were used as injection solvents. A mechanism, based on the properties of and possible interactions among the analyte, the injection solvent, and the stationary phase of the capillary column, was proposed to explain these observations.

The effect of initial temperature on peak height and peak area of the 16 PAHs and the 10 PCBs was also studied. The optimum initial temperature was found to be dependent on the physical properties of the solvent used and the amount of the solvent injected. Generally, from the boiling point of the solvent to 10 °C above its boiling point was an optimum range of initial temperature at which the highest peak height and peak area were obtained.

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Literature Review and Introduction

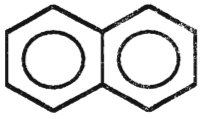
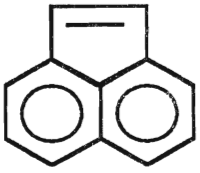
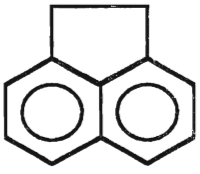
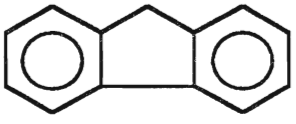
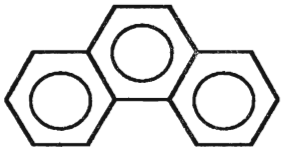
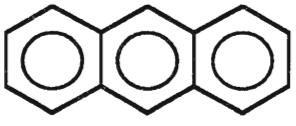
I. General Description of Polycyclic Aromatic Hydrocarbons (PAHs)

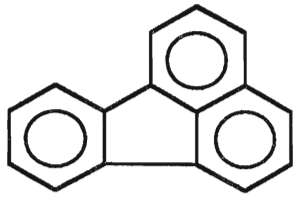
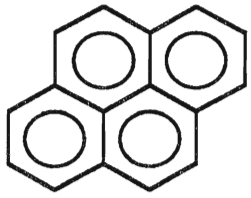
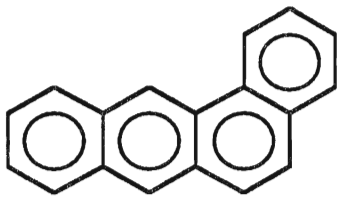
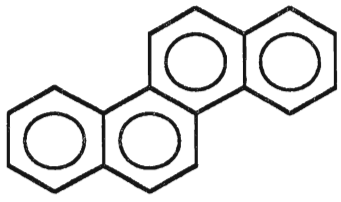
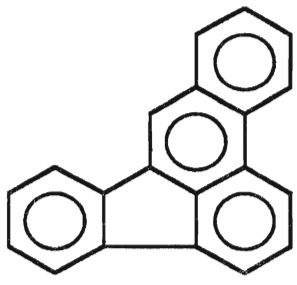
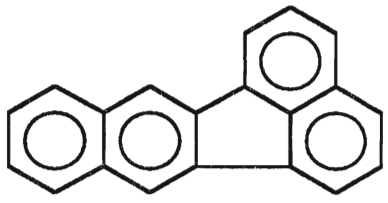
Polycyclic (or polynuclear) aromatic hydrocarbons (PAHs) (1, 2) or polycyclic aromatic compounds (PACs) (3) are aromatic hydrocarbons with two or more six-membered or six- and five-membered rings. The interlinked rings have at least two atoms in common. The nomenclature of PAHs has varied and has included common names and International Union of Pure and Applied Chemistry (IUPAC) names. Common names of PAHs are derived from the origin of the PAH, spectral property (color), or the shape of their molecules. Although IUPAC introduced its systematic nomenclature for PAHs in 1971 (4), some common names are still being used in practice since they have passed into general use for long time. Table 1 lists sixteen representative PAHs, along with their structures, molecular weights and boiling points. These 16 PAHs are also well known as the priority toxic compounds listed by the Environmental Protection Agency (EPA), USA.

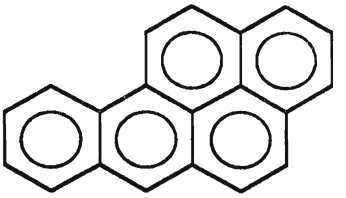
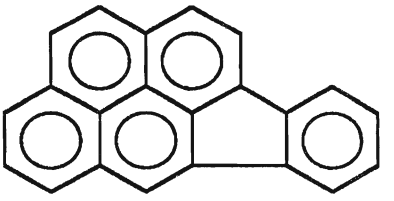
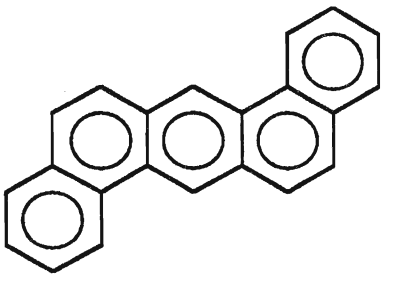
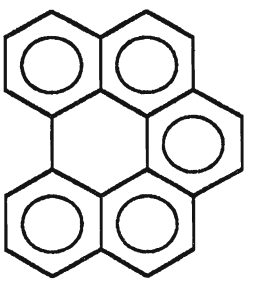
The physical and chemical properties of PAHs have been summarized by Zander (5). The most important feature of PAHs is their conjugated π-electron (ring) systems. The conjugation results in the chemical stability and distinct physical and spectroscopic properties of PAHs. Characteristic absorption and fluorescence spectra of different PAHs can be obtained due to absorption of ultraviolet (UV) or visible radiation by

the transition of an electron from the π- to π^*-orbital. This spectroscopic property has been applied to the determination of PAHs.

Table 1. Structures, boiling points and molecular weights of 16 PAHs

Peak#	Component Name	MW	b.p. (°C)	Structure
1	Naphthalene	128	218	
2	Acenaphthylene	152	270	
3	Acenaphthene	154	274	
4	Fluorene	166	294	
5	Phenanthrene	178	338	
6	Anthracene	178	340	

7	Fluoranthene	202	383	
8	Pyrene	202	393	
9	Benz(a)-anthracene	228	431	
10	Chrysene	228	414	
11	Benzo(b)fluoranthene	228	481	
12	Benzo(k)fluoranthene	252	481	

13	benzo(a)pyrene	252	496	
14	Indeno(1,2,3,-cd)pyrene	276	-	
15	Dibenz(a,h)anthracene	278	-	
16	Benzo(g,h,i)perylene	276	-	

PAHs are hydrocarbons of low volatility and have much higher boiling points than the n-alkanes of the same carbon number. Except for some hydrogenated derivatives, almost all PAHs are solids at ambient temperature. They are highly soluble in aromatic solvents and in relatively polar non-aqueous solvents such as dichloromethane. The solubilities of PAHs are very low in water, unless they contain a polar substituent group. Thus, the accumulation of PAHs in the environment

occurs mostly by adsorbing on particles and sediments rather than by dissolving in water (6). But the solubilities of PAHs in water polluted with organic solvents can be dramatically increased (7, 8).

PAHs have been known as chemically stable and inert compounds (9). When PAHs react, they tend to retain their conjugated ring systems. Reactions normally occur by electrophilic substitution to give derivatives, rather than by addition (10). Oxidation of PAHs and photochemical transformation (photooxidation, photolysis) have also been reported (11-15).

Toxicity

Since cancer among chimney sweeps in Britain were first reported in 1775 (16), the harmful effects of soot, tar, and pitch attracted great attention, leading to the identification of PAHs (in pitch) as carcinogenic constituents in the 1930's (17). Since then, the awareness and studies of toxicity of PAHs have grown continuously. By 1976, more than 30 PAHs and several hundred PAH derivatives were reported to cause carcinogenic effects (18). Polycyclic aromatic hydrocarbons are now known as the largest group of chemical carcinogens among the chemicals of environmental concern (19).

Toxicity and metabolism related to PAHs have been reviewed in a number of books (20-22). It is likely that no single mechanism of carcinogenity can be proposed, and that the associations of carcinogens with different biologically important molecules can all be of importance. The alterations of the structures of RNA and DNA through their reactions with oxygenated PAH derivatives are likely to affect their biological

functions, to cause mutations, and to cause chromosomal damage. Reactions of PAHs with proteins have been proposed (23-24).

Formation and Emission Sources of PAHs

PAHs can be formed by thermal decomposition of any organic material containing carbon and hydrogen. Formation may be based on two major mechanisms, pyrolysis, or incomplete combustion, and pyrosynthesis or carbonization. At high temperatures, organic compounds are partially decomposed to smaller, unstable molecules. This process is known as pyrolysis. The cracked fragments, mostly radicals, recombine to yield larger, relatively stable aromatic hydrocarbons. The latter process is called pyrosynthesis (25-26). Therefore, the incomplete combustion of organic material would lead to the formation of PAHs.

Sources of PAHs found in the environment can be divided into natural sources and anthropogenic sources. The natural sources of PAHs (27-29) include volcanic activity, biosynthesis by algae, plants or bacteria, and natural combustion such as forest and prairie fires.

Compared with natural sources, the anthropogenic sources are predominant and more important to environment pollution (30, 31). Due to human activities, the anthropogenic sources of PAHs mainly include industrial sources, power and heat generation, residential heating, incineration and open fires, and automobiles. Among them, residential and industrial combustions of fuels are the major sources. Amount of PAHs released depends on the raw materials and the combustion technology. For example, the emission of PAHs by burning wood can be typically 40 mg PAHs/kg dry wood (31). Coal burning can release as much as 60 mg PAHs

per kg coal. The sources and formation of PAHs have been reviewed in a number of books (1, 3, 31, 32)

Determination of PAHs

Widespread concern of environmental pollution by PAHs has emphasized the need for measurements of the presence and concentration of PAHs in a variety of samples. A great number of analytical techniques have been developed for the determination of PAHs (3). Lee *et al.* (3) have reviewed the methods for the determination of PAHs. These methods include chromatography, mass chromatography, ultraviolet (UV) absorption and luminescence spectroscopy, nuclear magnetic resonance (NMR) and infrared spectroscopy (IR). Because of high compositional complexity of PAH mixtures in many environmental samples, appropriate separation techniques are often needed for identification and quantitation of PAHs. Thus, chromatography with different detectors has played a very important role in PAH analysis. In the following sections, high performance liquid chromatography (HPLC) and gas chromatography (GC) will be discussed, with emphasis on GC.

II. Determination of PAHs by gas chromatography

The history of chromatography can be traced back to the mid-19th century, when Runge used a paper chromatograph for the separation of dyes. Column chromatography was then developed and the term "chromatography" was first used in 1906 by Tswett (33). In 1941, Martin

and Synge (34) first proposed that a gas could be used as a mobile phase in chromatography. However, because the facilities for controlling gas are much more complicated than those for a liquid phase, the idea of gas chromatography (GC) was not applied in the practice until 1954, when Ray (35) obtained the first gas chromatogram. The first commercial GC instrument was introduced in 1955. Since then, the development of GC has been very dramatic (36-39). Thus, GC has been widely applied to the analysis of many different organic samples. The development and application of GC for the determination of PAHs has been extensively studied as summarized in a number of reviews (40-44). Accurate identification and quantitation of trace amounts of PAHs require a technique with high separation efficiency and good sensitivity. GC has shown the potential of fulfilling these requirements.

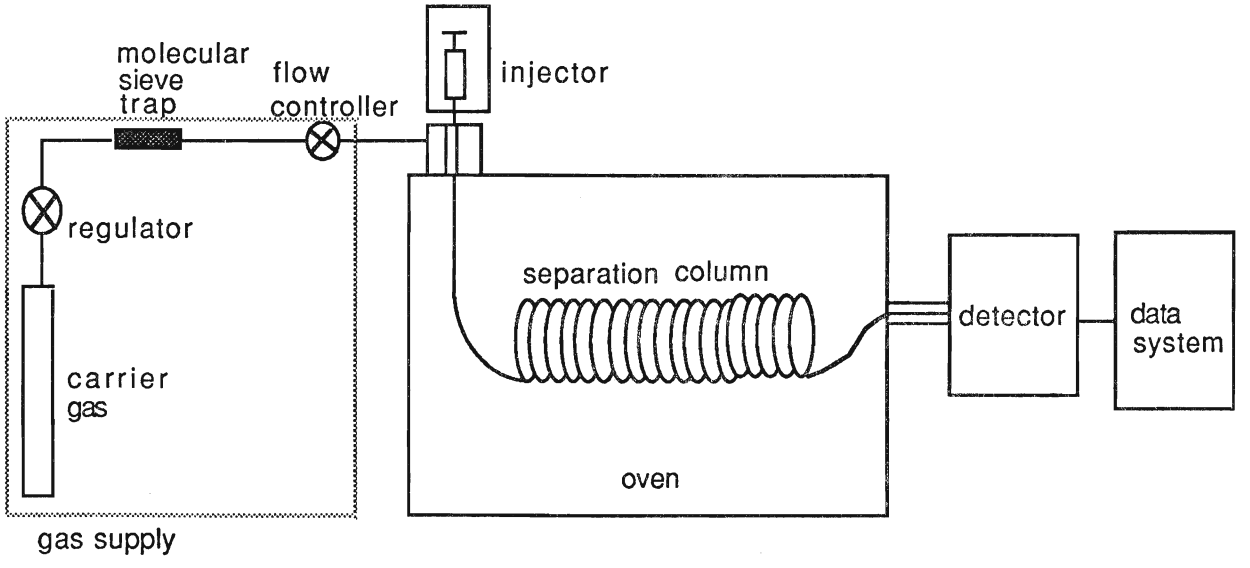


Figure 1. Schematic diagram of a gas chromatograph

A GC instrument, as shown schematically in Figure 1, basically comprises a supply of carrier gas, a sample injection system, a separation column at a controlled temperature, and a detector and data system. A GC column is attached to the injection port and sample is introduced into the carrier gas stream at a temperature sufficient to ensure vaporization of all sample components. The vaporized samples are then transferred from the injection port into the column, and undergo the chromatographic separation process. A detector, attached directly to the column exit, monitors individual sample components as they are eluted from the column. A recording of responses of sample components with time forms a chromatogram. The details of each major GC components will be discussed in the following sections, with the concerns of their applications to the analysis of PAHs.

Carrier Gas

Carrier gas flow must be carefully controlled and remain constant in order to obtain high efficient sample transfer and reproducible retention behaviour. This control is usually achieved by using pressure regulators and flow meters. The carrier gas itself must be thermally stable and chemically inert toward the samples analyzed and the liquid stationary phase of the column. Among other inert gases, helium and hydrogen have been most commonly used as GC carrier gases, because they can be used at high flow rate without substantial loss of separation efficiency.

Column

Basically there are two types of GC columns, packed column and capillary column. In the 1950s and 1960s, packed columns were mainly used for organic analysis by GC. The packed column is a metal or glass tube packed with solid support particles of uniform size (80-120 mesh). The support particles are coated with the liquid stationary phase. The support material should have high surface area and should be chemically inert so that it can not interact with either the samples or the stationary phase. Diatomites, which are skeletons of a single cell algae, have been found to fulfil the requirements, and have been commonly used as support materials. The choice of liquid stationary phase, on the other hand, is generally based on the type of sample to be analyzed. A number of papers(45-46) give useful information for selecting stationary phase for specific types of solute. Various kinds of PAH samples were analyzed by using packed column GC (47-50). However, packed columns did not provide sufficient separation in most cases. Although the increase of packed column length resulted in a higher separation efficiency, a number of drawbacks, such as long retention times, high column temperatures, and considerable back pressure were also encountered (51).

The great demands for high efficiency of separation in trace organic analysis encouraged the analysts to develop capillary columns. The concept of the capillary column was first described by Golay (52) in 1958. The extraordinary progress of capillary column was made possible by the invention of the glass capillary drawing machine in 1960 (53), because it made the practical use of high inertness of glass possible.

Capillary GC has been now developed to the point where it is easy to used as a routine analytical tool.

Capillary GC has become the dominant method for the determination of PAHs. Three major advantages of capillary columns over packed columns have been identified in the literature (3, 37, 42, 54-55) and are summarized below:

(1). High separation efficiency. Although the resolution per unit length of a capillary column may be not significantly different from that of a packed column, the length of a capillary column can be easily as long as 30 m or even longer, whereas a packed column is normally 3 m or shorter because of the difficulties of coiling and installation into the oven. Thus, the total number of theoretical plates of a capillary column (normally 600,000) is much higher than that of packed columns (less than 10,000) (56). The separation of a large number of PAHs and PAH isomers becomes possible using capillary columns. Even isomers with only minor structural differences, can be easily distinguished by capillary GC. This has been demonstrated by practical examples of the determination of PAHs in complex sample matrices (37, 42, 57-58).

(2). Fast separation. Although a capillary column may be ten times longer than a packed column, the total time required for the analysis is actually shorter, since hundreds of components may be separated in a single run. Also, fast separation can be achieved with a short capillary column as clearly demonstrated by Wright and Lee (59) in the determination of PAHs in coal tar. PAHs ranged from naphthalene to coronene were successfully separated on a 4-m long capillary column (SE-52, 0.3 mm i.d., 0.25 μm) in only 25 minutes.

(3). Capillary columns make the combination of GC with other techniques, such as mass spectrometry and infrared spectrometry, much easier. In terms of the analysis of PAHs, the combined ancillary techniques give more positive and complete results in identification. For instance, the recently available GC/IR/MS instrument (60) can provide retention data, mass spectra and IR spectra at the same time. Both the mass spectrum and IR spectrum contain very useful information which can lead to the elucidation of the structures of components of interest. Among the ancillary techniques, GC/MS is the most well established technique applied to the qualitative and quantitative analysis for PAHs as well as other organic compounds. The identification of PAHs with different molecular weights is easily obtained with their characteristic mass spectra. Although the mass spectra of many PAH isomers are nearly identical, the positive identification of isomers are also made with the additional information on their chromatographic behaviours.

The first capillary column was made from a Tygon tube in 1958 (52). During the early age of the capillary column, copper, nickel, and stainless steel, were used as column materials. A few application of capillary metal columns in the analysis of PAHs were also reported (61-64). However, chromatographers soon lost interest in capillary metal columns in practice due to the difficulties in coating stationary phase on the surface of metal tubes and to the lack of inertness of the surface. Glass was found to be desirable compared with plastics and metals, because glass provides very low catalytic activity (i.e. high inertness). This feature became even more important in the analysis of labile components in complex matrices. However, the poor wettability of glass

surface with organic liquids was a severe problem, which required for the use of skilful technology in deactivation and coating (43, 65).

The development of a greater understanding of the surface chemistry of glass improved the technology of capillary column considerably. The practical solutions used were surface corrosion with HCl or HF (66, 67) and deposition of solid particles (67, 68) prior to the coating of the stationary phase. The purpose of these treatments was to increase roughness of the glass surface so that it would be easier to coat a liquid thin layer of organic film. The use of flexible fused silica in 1979 began a new generation of capillary columns (69).These columns are made of pure SiO_2 and are extremely rugged when an external coating of polymide polymer is applied.

The quality of a capillary column is controlled by film thickness, which should be uniform along the entire column length (70). Thus, the coating procedure plays an important role in producing an efficient and durable column. Detailed studies on factors affecting the film thickness and homogeneity during coating were reported in several papers (70-74) and reviews (55, 75).

Deactivation of the inner surface of the column strongly affects the separation efficiency. If the deactivation is not complete, many problems, such as peak tailing, loss of sample in the column, even decomposition, may be encountered. Therefore, the procedures of deactivation are extremely important for satisfactory analysis. Among other coating materials, methylcyclosiloxanes has been used to deactivate fused silica as well as glass (76, 77). High temperature treatment has also proved to help complete the deactivation (76).

The nature of the stationary phase not only affects the selectivity of column, but also the stability and entire performance of the capillary column in the analysis. As a result of developments in column techniques, various kinds of capillary columns with different stationary phases have been manufactured. These have been discussed in a number of reviews (41, 42, 46, 78). Practical examples of the selection of stationary phases for the determination of PAHs have been extensively summarized (41, 42, 44). The conventional gum phases are most commonly used. They include methylpolysiloxanes (columns: SE-30, OV-1), 5% phenyl methylsiloxane (SE-52), and 5% phenyl and 1% vinyl methylpolysiloxanes (SE-54) (41-43).

Since the early 1980s, cross-linked silicone polymers have become the most favoured (79-83). The cross-linked phases involve two kinds of structure. One is formed by thermally condensing hydroxyl and alkoxyl groups to split out water, alcohols and ethers. In this kind of phase, Si-O-Si bonds are formed (84, 85). Capillary columns prepared in this way can be used at temperatures up to 320°C routinely in the analysis of PAHs (86). However, the drawback of this kind of cross-linked column is that it is less efficient and more active than conventional phases. This problem resulted in another development of free-radical cross-linked polysiloxane stationary phases (87). The methyl groups form carbon-carbon bonds attached to silicon atoms (i.e. Si-C-C-Si). By preparing suitable cross-linked phases, the selectivity of the column for separating PAHs is improved (88). The advantages of capillary columns with cross-linked stationary phases are high thermal stability and non-extractable column coatings (89-90).

Detectors

In the application to PAH analysis, the detectors coupled with GC can be divided into two general kinds, non-selective and selective detectors. Non-selective detectors respond to almost all the effluents from the GC; whereas selective detectors only selectively detect the specific type of component upon their setting conditions. Flame ionization detector (FID) is the most common non-selective detector for GC in the determination of PAHs. Selective detectors include electron capture detector (ECD), gas-phase spectroscopic detectors, mass spectrometry with selective ion monitoring, and specific heteroatom detectors.

1. Flame ionization detector (FID)

As a most common conventional detector for the determination of PAHs (91-92), FID provides a number of important advantages: wide linear dynamic range, good sensitivity and reliability in routine analysis, and simple maintenance. The typical detection limits for PAHs are at the 1 ng level. Since FID provides stable responses within a wide linear range, there is usually no need for multi-point calibrations. Quantitation based on internal standards in adequate. The advantages of FID have been widely applied to determine PAHs in various kinds of samples (41-42). However, the shortcoming of FID is that the sample matrix severely interferes with the determinations due to the non-selective response of FID. Therefore, samples undergo extensive clean-up before they are analyzed by GC/FID.

2. Electron capture detector (ECD)

ECD is the most sensitive detector for the compounds containing electronegative substituents, such as chlorine, sulfur and oxygen. The relatively less sensitive responses of the ECD to PAHs make the application of ECD in the determination of PAHs unpopular. Nevertheless, there are some published studies on the analysis of PAHs by ECD. One of those involved in the study of the carcinogenesis (93-94), since the electron affinity of PAHs was found to be related to the carcinogenic properties of PAHs, and ECD was used to differentiate the affinity among PAHs. Another application of ECD, combined with FID, was to identify different PAH isomers. The information of different ratios of PAHs from ECD and FID can be used as additional confirmation for the identification of PAH mixtures (49, 95-96). However, this also creates the need to determine response factors of each PAHs. The narrow linear range and the baseline drift with the temperature program are also the disadvantages of ECD.

3. Flame photometric detector (FPD)

FPD, which was originally developed for selective determination of sulfur- and phosphorous-containing pesticides, was found useful in the determination of PAHs containing these heteroatoms (97-98). Another heteroatom detector is the nitrogen sensitive detector. It was used to improve fingerprinting of PAHs containing nitrogen heterocycles (58, 99-100). However, one of the serious problems of such detector is response quenching if there are non-sulfur or non-nitrogen compounds present.

4. Spectroscopic detectors

Gas-phase spectroscopic detectors include the gas-phase ultraviolet spectrometric detector (UV) and the fluorescence detector (FD). UV was explored in the use of GC in the early 1960s (101-102). Because UV detection is sensitive to aromatics, it was coupled with GC for the analysis of PAHs (101). However, there were many problems involved in coupling UV with GC, such as dead volume and design and installation of sample cell. This technique is still under development, improvements have been made by Novotny and co-workers (103).

Gas phase fluorescence detector has also been extensively studied (104). Oxygen quenching response and carrier gas dilution were found to be problematic for the analysis of PAHs as well as for other organic analysis. Some techniques such as fast scan, spectral subtraction, and enrichment by removing carrier gas before the effluent goes in the detector, were developed to solve these problems (104-107). Compared with gas phase UV detectors, FD offers higher sensitivity and greater selectivity in the determination of PAHs because of the high fluorescence of PAHs. In addition to gas phase FD, liquid phase FD was also applied to analyze PAH effluents from GC (108). The principle is that GC effluent is adsorbed or dissolved in a proper solution before the measurement of fluorescence, which is similar to the FD for liquid chromatography. A limitation of both FD and UV detectors is that they are not able to analyze all PAHs in a single run.

5. Mass spectrometric detector (MSD)

Each of the above detectors has shown certain advantages. However, none of these detectors can provide complete information for identification and quantitation at the same time. This problem was largely overcome with the successful combination of GC with MS. The application of GC to PAH analysis has been greatly advanced by this achievement. GC/MS provides not only good sensitivity, but also structural characteristics of PAHs. Along with retention data, both identification and quantitation for a wide range of PAHs are easily accomplished by GC/MS in a single run. Thus GC/MS has now become a widely accepted technique for the routine analysis of PAHs (40-41).

The success of GC/MS combination has been attributed to three major developments (109-110). The development of high resolution capillary column is one of the most important aspects. The small amount of effluent from the capillary GC does not cause problem to the vacuum system so that the requirement of high vacuum in the ion source can be fulfilled. Therefore, the extremely narrow capillary column (usually 0.2-0.3 mm i.d.) is able to be directly interfaced with the ion source of a MS. Secondly, the increasing availability of computer and data handling systems makes it possible to record complete spectra from the mass spectrometer even with fast scans. Finally, different kinds of ionization methods and detection modes of mass spectrometry improve further the sensitivity and selectivity.

A modern mass spectrometer which is coupled with the GC generally consists of four elements: ion source, mass analyzer, detector, and vacuum system. An ion source is used to generate a beam of ions from the sample components eluted from a capillary column. The ion source is

followed by a mass analyzer, which separate ions of different masses. The separated ions finally reach the detector where the abundance of the ions is measured. At the same time, the mass spectra of components in the sample are digitized and recorded by a computer. A vacuum system is required to ensure that ions travel to the detector without colliding with residual gas molecules. Comprehensive discussions of these aspects have been provided in two books (109-110).

A. Ion source

In GC/MS instruments, the ionizer basically incorporates lens plates to focus the ion beam as well as to extract and accelerate the ions into the mass analyzer. The ions are produced by many ionization techniques (111-112), such as electron impact (EI), chemical ionization (CI), field ionization (FI), field diffusion (FD), and fast atom bombardment (FAB).

Electron impact ionization is the most common method employed in GC/MS. Electrons are produced by a heated filament and accelerated across an ionization chamber. When the effluents from GC pass through this ionization chamber, electrons in the chamber transfer their energy to the sample molecules. The molecules become excited and form molecular ions. If the excess of energy is transferred from electrons to molecules, further cleavage takes place and fragments are produced, resulting in rich spectra. The spectra with fragments is one of the advantages of EI because they provide detail information on the structure of compound of interest. On the other hand, EI is not satisfactory for the analysis of thermally unstable components because of the decomposition in ion source. Fortunately, most PAHs are stable enough to be ionized by EI. The

EI spectra of PAHs mostly contain high intensity molecular ion (M^+) and relatively low intensity ions resulted from the losses of hydrogen atoms ($(M-nH)^{n+}$). The alkyl substituted PAHs also produce $(M-15)^+$ and $(M-29)^+$ due to the loss of CH_3 and C_2H_5 groups, respectively. When PAHs contain heteroatoms, such as oxygen, sulfur, and nitrogen, the spectral characteristics of these atoms are also observed in the mass spectra. The detailed characteristics of EI spectra of PAHs have been summarized by Lee *et al.* (3).

The availability of standard EI spectra in on-line GC/MS instrument libraries and in the literature has been well realized as an advantage. However, the EI spectra may not be sufficient for the identification of PAH isomers, because the EI spectra of PAH isomers are very similar to one another. The use of a high resolution capillary column to separate PAH isomers before they are eluted into the MS has been the method to deal with this problem. The reliable and characteristic retentions of specific PAH isomers can additionally confirm the identification (3).

In addition to EI, chemical ionization (CI) is also utilized in GC/MS instruments. The process of CI is rather different from that of EI. In the CI method, a reagent gas is introduced into the ion source. Molecules of reagent gas are bombarded with high energy electrons to produce reactant ions. For example, methane, the most common reagent gas, is bombarded with electrons to form CH_5^+, CH_4^+, CH_3^+, CH_2^+, etc. When analyte molecules, eluted from the GC, enter the ion chamber, the reagent gas ions react with them to become uncharged methane and to produce analyte ions. This process continuously repeats. Since sample molecules do not directly connect with high energy electrons during CI, the ionization of sample components is much "softer" than EI ionization. Thus, the CI

spectrum of a molecule usually contains intense $(M+H)^+$ ion. Other fragmentations are very weak and may not be detected. This characteristic of CI has been widely applied to obtain the molecular weight of unknown analytes.

In CI ionization, both positive and negative ionization modes have been used. But the majority of studies and applications of CI has involved positive ions. Methane is most commonly used as reagent gas in positive chemical ionization. The use of CI for the identification of PAHs has been reported (90, 113-115). However, CI is not as sensitive as EI for the quantitation of PAHs. The CI spectrum alone is often not sufficient for the positive identification due to the lack of fragment information. In modern GC/MS, both EI and CI are often installed so that they can be complementary.

Other ionization methods, such as FI and FD, have also been used in GC/MS (109-110). But for the identification and quantitation of PAHs by GC/MS, EI and CI are still preferred.

B. Mass analyzer

Once ionized, the sample molecules and their fragments can be separated on the basis of their different mass-to-charge ratios. To accomplish this, many kinds of mass analyzers have been used. Among them, quadrupole and magnetic sector are the most commonly used in GC/MS instruments. A quadrupole mass analyzer consists of four rods. The opposite rod pairs are electrically connected, one of which carries a positive voltage, the other carries a negative voltage. The voltages applied to the rods consist of a direct current (DC) and a radio frequency (RF) voltage. The mass resolution is determined by the ratio of the DC to

RF voltage. Normally unit mass resolution is obtained during an ion passing through the rods to the detector. To obtain a mass scan, the DC and RF voltages are varied with a constant ratio. The change of DC and RF voltages can be very fast. Therefore, the quadrupole mass spectrometer has the main advantage of fast scan, which is specially desired in the GC/MS instrument since components are continuously eluted out of column during GC run. With the computer system, a modern quadrupole GC/MS easily handle four mass spectra per second.

The problems of a quadrupole mass analyzer are low resolution and low sensitivity at high mass. The use of high resolution capillary columns can easily result in complete resolution of components over a very short time so that the quadrupole analyzer is still the most widely applied technique of MS in analytical usage (116). New scan methods, which will be discussed later, dramatically improve the sensitivity of a mass spectrometer. The recent improvement and applications of quadrupole mass analyzers in analytical chemistry have been demonstrated in a recent review (117).

The magnetic sector mass spectrometer is another type which is commonly used in GC/MS systems. Ions with a unit charge are separated by magnetic field according to the equation $m/z=H^2R^2/2V$,
where m is the the mass of an ion, z is its charge, H is the strength of the magnetic field, R is the radius of curvature of the path for the ion transferring from ion source to a detector, V is the accelerating voltage. A mass scan can be obtained by varying H or V. In practice, H is most commonly varied at constant V.

The magnetic mass analyzer may consist of single focus, where only a magnet is used. When both electrostatic field and magnetic field are

applied, the mass spectrometer is called double focusing type. Usually the electrostatic field is used prior to the magnet sector.

A magnetic analyzer usually provides high resolution. However, the scan rate of a magnetic sector mass spectrometer is slow and limited because of magnetic field hysteresis. In the application of GC/MS, the fast scan is preferred to high resolution. Thus, a quadrupole mass spectrometer is more often used.

In the application of a mass spectrometer to the analysis, both scan mode and selective ion monitoring (SIM) are applied. With scan mode, a desired range of mass is sequentially scanned. This method may be often used to identify unknown components in complex sample matrices. However, SIM mode is preferred when quantitation of a known component is needed, because of its superior detection limit. SIM mode includes single ion monitoring (118) and multiple ion monitoring (119). With the single ion monitoring, only one m/z value of compound of the interest is focused. The time of each cycle is significantly shortened and more time is spent on the monitoring of the selective ion. Also, the matrix eluted at the same retention time is avoided by selectively monitoring the ion of interest. Therefore, the signal-to-noise ratio (S/N) and ultimately the sensitivity and selectivity of GC/ MS are improved. With multiple ion monitor (119), a number of ions were selectively detected and, in most cases, molecular ions of interest are chosen as the characteristic mass (120). The sensitivity with this mode is also better than with scan. Nowadays, almost all kinds of GC/MS instruments from different manufacturers contain both SCAN and SIM modes. They are also widely and successfully used in the determination of PAHs (121).

Sample introduction methods for capillary gas chromatography

Since capillary columns provide much smaller sample capacity compared with conventional packed columns, the introduction of sample into the capillary column becomes very important in order to provide sufficient amount of sample for GC detectors, to avoid overload on the column inlet, and to prolong the effective column lifetime. Great efforts have been made on the development of ideal injection methods for capillary gas chromatography for specific purposes. Although there is still no single method suitable for all kinds of sample analyses, three techniques, split, splitless, and on-column injections, have been commonly used for different applications.

To avoid the overloading of sample on the low capacity capillary column, the split injection method was developed (122-123). With this technique, the vapor of sample in the injector is split into two streams, one of which is transferred into the capillary column by carrier gas, the other is vented. The split ratio of the two streams can be regulated by changing the flow rates of the carrier gas and purge gas. Split injection has been the conventional method for organic analysis (122-123). However, the main shortcoming of this method is that a very small portion of injected sample, typically less than 1% (124), is transferred onto the column and detected. This limits its application mainly to the analysis of samples containing high concentration of analytes. For trace analysis, which is the case for most environmental samples, an injection method with higher sample transfer efficiency is required.

The technique of splitless injection was developed to avoid the split without allowing the separation efficiency to suffer. The basic principle of splitless injection was described in 1965 (125). At this early-stage of the development of splitless injection, samples were vaporized in an injector at relatively high temperature. The vaporized samples were introduced into a cold precolumn or a cold column over a long period of time and then quickly heated to start separation (126-129). However, the wide applicability of splitless injection was not realized until 1969, when Grob (130) described the basic operation of the technique and demonstrated its application to steroid analysis. The basic mechanism introduced was cold trapping (130-131). But later investigations confirmed that the "solvent effect" (132-133) was involved in the splitless injection. Since Harris (134) utilized a "solvent effect" to improve performance by splitless injection, Grob (132-133) has developed splitless injection to a very high degree. We will further discuss, in the next section, the details of solvent effect, cold trapping, and other effects related to the splitless injection.

For comparison, the advantages of splitless injection over the split injection are summarized as below.

(1) Diluted sample can be injected for analysis with no need of preconcentration. This eliminates possible errors from the preconcentration steps, which not only concentrate the analytes of interest, but also accumulate the matrices that may interfere with the determination.

(2) Splitless injection is useful for a full range of substances with various volatilities, except for the compounds eluted before the

solvent; whereas non-volatile or low volatility substances are discriminated with split injection.

(3) No additional equipment, such as splitter, is needed.

However, because the splitless injection is very much dependent on the solvent effect, the operational parameters are more critical and have to be carefully optimized (135-136).

Springer *et al.* (137) have compared split with splitless injection for PAH analysis by capillary GC. PAHs with 2 to 4 rings obtained by both injection methods gave similar linear responses. However, splitless injection is subject to over-all lower error in quantitation due to decreased molecular weight discrimination in the splitless injection mode. Splitless injection has been the most common technique of sample introduction for PAH analysis with capillary GC.

Another very attractive method of sample introduction is on-column injection (138, 139). Many aspects of on-column injection are similar to those of splitless injection. The main difference between them is that with on-column injection, it does not require the process of vaporization. Sample is directly injected onto a capillary column. Soon after on-column injection was introduced, it was a great relief to many analysts because of getting rid of some problems which occurred in splitless injection. For example, one of the important parameters for both split and splitless injection methods, vaporizer temperature, is no longer required. The problem of efficiency of transferring sample vapor from injector to the column does not exist in the case of on-column injection. No septum is used so that there are no impurities deposited on the column from the septum. Diluting solvents do not interfere the peak width and retention, which otherwise are often seen in splitless injection.

In regard to the development and application of on-column injection for capillary GC, Grob has given detailed discussion in his recent book (139). Along with the development, solvent effect was observed in on-column injection, similar to that in splitless injection.

The further understanding of on-column injection becomes the basis for developing more sophisticated techniques. For example, the combination of HPLC with capillary GC was successful because it used on-column injection and retention gaps (139).

The major shortcoming of on-column injection is that the accumulation of non-volatile components shortens the lifetime of the column, especially the initial part of the column. This may be the reason that on-column injection is not common for the analysis of PAHs, especially for high molecular PAHs.

III. Solvent effect in capillary GC with splitless injection

As discussed above, multiple steps are involved in the process of splitless injection of components into capillary column. The solvent participates in all the steps: the evaporation of diluted sample in injection port, the transfer of evaporated sample into column inlet, and chromatographic performance between the column inlet and the column exit. During these processes, solvent plays an important role in controlling the performance of chromatography of components on the capillary column, which strongly affect both quantitative and qualitative analyses. Solvent can provide positive effect but can also be nuisance, causing distortion of chromatographic peaks. In order to achieve the best

solvent effect and to avoid the distortion caused by the solvent, it is very important to understand the mechanism of the solvent effect. The following discussion will be focussed on the different situations resulted from the solvent when splitless injection is used.

In splitless injection, one of the main disadvantages is the slow sample introduction into column. This causes all solute bands to be broadened. This phenomenon is called band broadening in time (140). Grob (123) has concluded three important characteristics of band broadening in time. (1) In isothermal runs, all peaks of solutes are broadened equally. (2) The peaks are distorted reproducibly. (3) The broadening effect diminishes with the increase of temperature and disappears about 80 °C to 100 °C above the injection temperature.

A few methods were used to prevent band broadening in time. One of them is the solvent effect. Solvent effect was first applied to reconcentrate the initially broadened bands in splitless injection (133). The process of reconcentration by solvent effect is as follows. A sample injected into a hot vaporization chamber (i.e. injection port) is vaporized. The vaporized sample is transferred into the column inlet and condensed, because the column is generally held at a temperature of at least 30 °C below the boiling point of the solvent used. The recondensed solvent in the column inlet is retained by the stationary phase to form a "hill" of solvent. The solvent "hill" acts as a barrier, retarding or even stopping the migration of the front edge of plug of solutes before the less volatile sample material enters the column. The rear of plug of solute migrates faster than the front edge since there is less solvent at the rear edge. Therefore, the rear of the plug of solutes is able to catch up the front

edge resulting in narrow bands. Solvent is vaporized again upon the increase of the column temperature.

By using a visible column, Grob and Grob Jr. (133) first discovered the above solvent effect when isomers of nonane were used as solutes. Prior to this work, Deans (141) discussed the similar effect based on mathematic calculations. According to Grob's results, Jennings *et al.* (142) theoretically described such solvent effect in terms of the three fundamental parameters describing the partitioning process in the column.

In order to achieve solvent effect of reconcentrating broadened bands of solutes, the conditions used are extremely important. Column temperature is one of the most important parameters. The proper temperature is dependent on the volatility of solvent used. It is usually found that column temperature must be at least 20 to 30 °C below the boiling point of the solvent used during the time period of splitless injection (123). For example, when the column temperature was kept at 45 °C, the peaks of the isomeric alkanes C_9 and C_{10} in pentane were distorted and the separation of them was ruined, because pentane (b.p. 36 °C) did not recondense at temperature of 45 °C. However, sharp peaks of these solutes were successfully achieved with n-heptane (b.p. 98 °C) as solvent, while other parameters were kept unchanged. Another example is the comparison of peaks of alkane C_7 to C_9 when n-hexane (b.p. 68 °C), 2,3-dimethylbutane (b.p. 58 °C), n-pentane (b.p. 36 °C), and isopentane (b.p. 27 °C) were used as solvent, respectively (123). At a column temperature of 25 °C, sharp peaks were obtained with n-hexane and 2,3-dimethylbutane as solvents whose boiling points were 42 and 52 °C, respectively, above the column temperature used, but not with the other

two low boiling point solvents. Other factors such as the amount of solvent injected and injection speed were also found to affect the reconcentration effect (132,133).

A number of studies (132, 133, 136, 138, 142) have demonstrated that the solvent effect can dramatically improve the shape of peaks so that the best separation can be achieved. However, many problems are also encountered. One of the major problems is called "partial solvent trapping" (143). When the solvent effect is used to reconcentrate the broadened bands, the solvent must be condensed. The condensed solvent forms a layer on the surface of the column, functioning as a temporary stationary phase. Therefore, such a layer of solvent may influence the chromatographic behaviour of certain sample components. Three situations were observed (143).

First, solutes are completely retained by the solvent layer, which is called full solvent trapping effect. When full solvent trapping is obtained, the bands of solutes are reconcentrated, resulting in well-shaped chromatographic peaks. In order to achieve full solvent trapping, the solvent must recondense in the column inlet and remain there at least until the sample transfer from the injector to the column is complete. Therefore, the polarity of solvent and solutes must be very similar in order completely to retain solutes in the solvent.

Secondly, when the solutes greatly differ from solvent in polarity and also are very volatile, the solutes are not retained at all, resulting in the deformation or broadening of peaks of solutes. For example, when hexane was used as injection solvent, the peak of ethanol was broadened,

whereas sharp peaks of alkanes such as C_{10} and C_{11} were obtained in the same run (143).

The most often observed situation is that components are only partially retained by the solvent, which is between full solvent trapping and none solvent trapping. For example, in hexane solvent, the aromatic compounds such as benzene, toluene, and ethyl benzene are eluted in the shape of fronting, like a chair shape (143). The degree of fronting is decreased from benzene, to toluene, and then ethyl benzene, due to the increase of trapping efficiency of hexane to these solutes. Partial solvent trapping was also observed with split injection and on-column injection, as long as the solvent is condensed in the column inlet (143).

The solvent trapping effect was further confirmed by Grob Jr. (144) with two-step chromatography. From his results, Grob Jr. claimed that the stationary phase was not important in the column inlet where solvent trapping effects occurred (143, 144), because a relatively thick layer of solvent in column inlet served as a temporary stationary phase.

The peak distortion due to partial solvent trapping shows the same characteristics as that caused by band broadening in time, as demonstrated in a number of papers (140, 143-145). The initial band broadening caused by partial solvent trapping is due to the slow evaporation of the condensed solvent (143-145). The peak width of partial trapped components are usually determined by the evaporation time of the solvent, which may last from 5 seconds to several minutes (143-145).

The phenomena of peak broadening and distortion were also ascribed to the "band broadening in space" (140, 145). The band broadening in space is due to spreading of the sample components through a flow of the liquid

of sample in column inlet (140, 145-148). When the vapor of sample is transferred into the column inlet, sample is recondensed on column inlet as a plug. However, this plug of the liquid sample (i.e. condensed sample) flows further into the column and loses some liquid from its rear, because the liquid sample coats on the wall of the column. This section of column coated with recondensed sample is called "flooded zone". Both solvent trapping and band broadening in space (149-151) are related to this flooded zone. However, band broadening in space and solvent trapping are occasionally addressed to different components (150). The highly volatile components are more subject to partial solvent trapping effects, because parts of vapor of volatile components are not trapped on the column inlet. The components with high boiling point are mostly influenced by band broadening in space due to the flow of condensed components along with the solvent. The flooding solvent can spread the components over several decimetres of the column inlet. The band width of these components corresponds to the length of the flooded zone.

In addition to the flow of liquid sample into column inlet, the bottom part of the injector is also found to cause the band broadening in space (145). During splitless injection, the injector is kept at high temperature to evaporate sample, while the column is kept at a temperature low enough to recondense the solvent and analytes. A temperature gradient may exist between the bottom part of the injector, including the split exit, the fitting and screw of the column attachment, and the column. When the sample vapor is transferred from the injector into the column, the high boiling components may be partially retained at the column entrance or bottom part of the injector, whereas the volatile components may pass through the whole connection and further flow into

the column in the oven. Grob Jr. (145) demonstrated this effect visually by injection of a fluorescent PAH, perylene in dichloromethane, into a transparent column kept at ambient temperature, while the injector temperature was at 270 °C. Perylene was found to mostly retained in the warm part of column between the hot injector and the cool oven. However, a small part of perylene was spread out in a poorly reproducible patten in the column. Thus, a distorted peak was observed. The bottom part of injector may also cause band broadening in space when the temperature program rises rapidly. Because the attachment may cause the delay of temperature increasing (behind the oven temperature), the components retained in the initial section of the column at the bottom part of the injector may delay the chromatographic migration, resulting in the broadening band of the solutes (151).

Cold trapping is the most common method to solve the problem of peak broadening or distortion. When cold trapping is applied to reconcentrate the broadened band due to the slow transfer of sample from injector to column inlet (i.e. band broadening in time), the purpose is to reduce the migration speed of the advanced sample so that the rear sample materials can have a chance to catch up. Therefore, the column is kept at a low temperature during injection, and then raised for the chromatographic separation. In order to obtain a cold trapping effect, Grob (123) recommended a minimum temperature difference of 80 °C between the initial column temperature and the approximate elution temperature of the analyte. Hence, if a component is eluted at 250 °C, the initial column temperature during injection should be kept at 170 °C or lower. In general, it is believed that compounds with boiling points of 150 °C higher than the column temperature will be cold trapped (142).

The reconcentration power of cold trapping is dominated by the ratio of migration speeds of solutes at the temperature of injection and of elution. The "15 °C rule" has been used empirically to estimate the migration speeds in relation to the temperature. The so-called "15 °C rule" (123) means that migration speed is increased by a factor of 2^n when the column temperature is increased by (15Xn)°C. In other words, the reconcentration factor is 2, 4, 8, 16..., when the difference of column temperature between the injection and the elution is 15, 30, 45, 60 °C..., respectively.

Cold trapping has also been used as an essential step in many injection techniques, such as falling needle method (152), injection onto precolumn (153), and intermediate trapping in multi-column analysis (154, 155). Cold trapping with different injection methods has also been used to the analysis of PAHs (156, 157), while the details on cold trapping in general have been discussed (123, 158).

Phase soaking (150, 159, 160), a specific kind of solvent effect, has also been found useful to prevent peaks from broadening or distortion. Phase soaking occurs in capillary column beyond the flooded inlet section where solvent trapping takes place, which may otherwise result in band broadening in space. The most important factor in phase soaking is the wettability of the solvent on the stationary phase. When solvent has polarity similar to the stationary phase, the flooding effect may be eliminated by phase soaking. In the presence of phase soaking effect, the solvent-saturated stationary phase may reduce the migration speed of advanced sample to let rear sample catch up. Also when the solute band is crossed by the rear edge of the solvent band, different migration speed within the solute band may be produced. The front of the solute band

moves slower than the rear of the solute band due to the existence of the solvent at the front of the solute band. Therefore, the condensed sample is reconcentrated to a narrow band. For instance, when the solvent effect is applied to reconcentrate the band of n-octane in n-heptane, the initial band of octane is broadened with band width about 2 min when the band starts migration in the analytical part of column. After the band of octane migrated through the analytical column with 2 to 5 meters, the band was reconcentrated in band width of less than 1 second (159) due to the phase soaking effect.

Proper stationary phase and solvent should be chosen to obtain a phase soaking effect for the analysis. In addition, column length in 25 m or longer may be necessary to achieve the successful reconcentration effect by phase soaking (151).

To avoid band broadening by flooding effect, retention gap was introduced (161, 162). Basically, a retention gap consists of an uncoated section of column inlet. The length of such uncoated column inlet is dependent on the length of the flooded zone. Usually it is at least as long as flooded zone. the mechanism of retention gap for reconcentrating flooded band is that when front edge of liquid sample in column inlet reaches the coated section of column, the speed of migration of the liquid sample is retarded; whereas the liquid sample at the rear edge flows relatively faster in uncoated section and tends to reach the front part. This process provides the function of reconcentration of band, which is rather similar to the process of reconcentration by classical solvent effect.

In addition to uncoated retention gap, the coated retention gap may be also useful (163). But the film of stationary phase in retention gap should be thinner than that in the section of separating column.

In order to achieve sufficient reconcentration effect by retention gap, the volume of sample and bore size of retention gap should be utilized properly. A large volume of sample easily creates a long flooded zone which makes the band broadening in space very severe. Thus, sample volume is usually limited to a few microliters under normal conditions (163). Very long retention gaps may also be needed (161).

As implied by its reconcentration mechanism, a retention gap should provide retention power as low as possible in order for the liquid sample to move fast in flooded zone. On the other hand, retention gap requires retention power in order to minimize the length of flooded zone (164, 165). Therefore, the property and condition of a retention gap should be optimized. Grob *et al.* (165-167) have investigated the length of flooded zone of different solvents on different retention gaps. The factors, including inertness of the surface, depth and length of the retention gap, and the length of the flooded zone, were considered for evaluation. Their results suggested that silylation is the preferred method of deactivation and that roughening of the internal wall lead to a drastic shortening of the flooded zone due to the increase in retention power. The DPTMDS deactivated fused silica capillary was found to be an outstanding retention gap. But the carbowax deactivated glass or fused silica capillary is advantageous for methanol and water solutions because of excellent wettability of this surface. Detailed studies have be discussed by Grob in his recent book (139).

Retention gaps are not only applied to reconcentrate broadened bands, but are also utilized to analyze dirty samples. It is particularly attractive for the analysis of samples with a high content of high boiling or non-volatile by-products (168, 169). Also, retention gaps have been recently developed as an important technique for combining LC with capillary GC by using on column injection (139,170). The automated on-line HPLC-HRGC has been established and applied to the analysis of various samples (139).

IV. Some recent development related to GC

LC/GC

Although capillary GC has proved very efficient, it is not always powerful enough to resolve every component in very complex mixtures. In practice, pre-column separation (off-line) has proved to be very useful. Therefore, on-line liquid chromatograph/gas chromatography (LC/GC) coupling has been developed for sequential and automated analysis, i.e., specific-component isolation or chemical-class fractionation by LC prior to chromatographic analysis by GC.

Direct coupling of LC to GC generally involves the isolation of the LC fraction of interest, the transfer of the specific LC fraction into the GC column, and the volatilization of the transferred LC solvent and solutes into the gas mobile phase (171). One major difficulty to couple LC to GC lies in the fact that the two chromatographic systems operate with different mobile phases. The introduction of relatively large volumes (10 µl-10 ml) of liquid LC effluent into a GC column at inlet temperatures

below that of LC effluent boiling point can cause flooding (or overloading) of the GC column (5). This leads to distortion, broadening or spitting of GC peaks. However, this problem was overcome with a retention gap, a 30-cm to 5-m length of uncoated, deactivated capillary prior to the GC separation column.

The LC/GC interfaces developed include three major types. They are the autoinjector (172-175), on-column (176), and gas transfer (or loop-type) interfaces (177-179). In most studies on LC/GC described in the literature, normal-phase LC with low-boiling, non-polar solvents has been used; whereas only about 15% of LC/GC applications featured reversed-phase LC due to the mobile phase difficulties (171).

Supercritical Fluid Chromatography (SFC)

A supercritical fluid (SF) is defined as a substance existing at temperature and pressure above its supercritical points. First reported by Klesper *et al.* (180) in 1962, supercritical fluid chromatography (SFC) has undergone dramatic developments (181), especially with the application of fused silica capillary columns to SFC (182, 183).

One of the important advantages of chromatography using supercritical mobile phases is that the increased diffusion coefficients of SFs compared with liquids can result in faster separation or greater resolution. Also, compared with gases, SFs can solubilize thermally labile and non-volatile solutes. Upon the decompression of the solution, the solute is introduced into the vapor phase for detection. Therefore, SFC is suitable for the analysis of non-volatile and thermally labile compounds.

The column technique used for SFC has been basically adopted from GC (for the capillary column format) and from LC (for the packed column format). As temperature programming is used to change retention behaviour for GC and solvent programming is used for LC, pressure gradient is most widely used in SFC to alter solvent strength and therefore solute retention (184). Gradients in temperature or mobile phase composition can also be used.

Non-polar or low-polarity solvents, such as N_2O, CO_2, ethane, propane, pentane, xenon, SF_6, and freons, have been studied for SFC. Carbon dioxide has been found to be the most appropriate fluid in many SFC applications due to its low critical temperature (31 °C), non-toxicity, and lack of interference with most detection methods. However, for highly polar and high molecular weight solutes, there is still a lack of proper fluid system. Although polar fluids, such as NH_3, exhibit useful properties, the complications resulting from its reactivity have limited its application.

Almost all detectors applied to GC and HPLC have been investigated for SFC. The current most universal detectors for SFC are FID and UV absorption. The combination of SFC and MS, based on decompression of the fluid directly into the mass spectrometer ion source, has made a significant progress (185, 186). But the current SFC/MS interfacing methods are limited by the reliance on analyte volatility for both transport through the SFC pressure restrictor and subsequent gas-phase ionization processes. FT-IR has also been reported as a detection method for SFC (187).

A SFC/GC application to the analysis of PAH includes the isolation of PAHs from a complex liquid hydrocarbon samples (188). Selective

extractions of PAHs from a solid matrix (189) and from diesel exhaust particulates (190) have also been reported by using supercritical fluid extraction (SFE) (171).

GC/FT-IR/MS

Despite its undoubted usefulness, GC/MS often can not differentiate structural isomers. However, if both Fourier transform infrared (FT-IR) and MS spectral data are collected simultaneously, complementary spectral data are beneficial for positive identification. While MS can provide molecular weights, halogen isotopic clusters, and characteristic fragmentation information, FT-IR can distinguish isomers, provide group frequency data and absorption coefficients. Therefore, the hyphenation of GC with FT-IR and MS has been studied (191).

The first direct-linked GC/FT-IR/MS system was developed in 1980, as described in a paper published in 1981 (192). Either parallel or serial linkage of the three components is possible. If the serial arrangement is used, the dead volume between the GC and the MS is encountered, resulted in a potential degrading of chromatographic resolution (193). Thus for GC/FT-IR/MS systems, parallel interface has been often applied. A light pipe (194-197), or internally gold-coated glass tube, served as a parallel split interface between the GC and the two spectrometers. Most of the effluent (95% or more) is splitted to the IR spectrometer, and the rest to the mass spectrometer. This arrangement maximizes the amount of sample reaching the less sensitive IR spectrometer. Hence, the full value of GC/FT-IR/MS is obtained, as both types of spectra are obtained for all components separated by the GC.

Environmental applications of directly linked GC/FT-IR/MS systems were reported (198-200) soon after the successful interfacing of the instrument. Because of the complementary nature of IR and mass spectral data, more GC peaks can be identified when GC/FT-IR/MS is used than when either GC/FT-IR or GC/MS is applied separately (201-206).

V. Determination of PAHs by HPLC

A liquid chromatography instrument generally consists of: a solvent reservoir for the mobile phase, a solvent pump (or pumps) to force the mobile phase through the chromatographic system, a precolumn to resaturate the mobile phase with the stationary phase and a guard column to prevent contamination of the separation column, a pressure gauge inserted close to the column to measure column inlet pressure, a sampling or injection device to introduce the sample into the column, the separation column, and a detector with data acquisition/handling device.

Modern HPLC has been developed as a competitive method for the determination of PAHs. There are two main reasons related to the progress of application of HPLC in the analysis of PAHs. The first is the development of chemically bonded stationary phases (207). Various kinds of chemically bonded phases provide not only high efficiency of separation, but also better selectivity. Therefore, different isomeric groups of PAHs can be separated by choosing proper stationary phases. The second reason is the successful application of fluorescence detectors (FD) in HPLC (208). Almost all PAHs are capable of fluorescing, whereas many matrices do not have fluorescence. Thus, the determination of PAHs can be achieved without interference from many other matrix materials,

which are incapable of fluorescence. HPLC can be generally classified into two categories according to the principles of separation. They are normal phase and reversed phase LC. The application of both normal phase and reversed phase LC to the determination of PAHs will be briefly discussed, along with the recent progress.

Normal phase LC

In normal phase liquid chromatography, it usually involves polar chemically bonded stationary phase and non-polar mobile phase. The elution order of the chromatographic process is from non-polar to polar components, i.e., from saturated hydrocarbons, to olefinic and aromatics, and then compounds with increasing polarities. Stationary phases studied (209-212) consist of a variety of polar groups. They are amine (NH_2), diamine ($R(NH_2)_2$), nitrile (CN), diol ($R(OH)_2$), ether (ROR), and nitrophenyl (NO_2). The normal phase packing materials are produced by bonding these polar groups to the silica particles. The stationary phase with NO_2 group bonded to silica gel provided better selectivity (209), compared with NH_2 stationary phase.

The elution of PAHs on a number of stationary phases were according to the number of aromatic rings of PAHs (210, 211, 213). PAHs containing less aromatic rings elute first. The effect of alkyl-side chains on the retention of PAHs were found relatively less significant (213-215). Many applications of normal phase LC to the determination of PAHs have been reported (216-218). However, a limitation of normal phase LC for the determination of PAHs is that it is often insufficient enough for the separation of PAH isomers.

Reversed phase LC

The concept of reversed phase LC was first reported in 1950 (219). A polar mobile phase was used to separate fatty acids on the modified siliceous stationary phase, which was a hydrophobic partitioning layer. The later studies in the 1960's extended the knowledge of various surface reaction. The application of reversed phase LC to the separation of PAHs was then first succeeded by Schmit *et al.* (220) in 1971. A chemically bonded octadecylsilane (C_{18}) stationary was used in their work. Since then, reversed C_{18} stationary phase LC has been the most popular method in the separation of PAHs. The most important advantages of the reversed phase LC are its high efficiency and a variety of stationary phases available, which provide unique selectivity for the separation of PAH isomers. Because the variation of carcinogenic toxicity among the PAH isomers, the necessity of complete separation of PAH isomers is demanded.

Silica is also the most common support material in reversed phase LC. The chemical bondings between the support material and stationary phase usually include four types. They are ester (Si-OR), amino (Si-NR), carbon (Si-CR_3), and siloxane (Si-O-Si-CR_3). Stationary phases are normally alkyl and the surface coverage with bonded phase is an important factor to affect the column efficiency. A wide range of researches demonstrated that the coverage of surface on substrate strongly affect the selectivity of reversed phase column for the separation of PAHs (221-224). The systematic evaluation of the effect of surface coverage on the selectivity of a number of stationary phases for

the separation of PAHs were also described (225, 226). Polymeric phases provided the better selectivity than monomeric phases for the separation of PAHs, and the separation efficiency was improved by increasing the surface coverage of polymeric phases (225, 226).

Particle size also influences the chromatographic performance of PAHs. A number of studies (227-231) demonstrated that the retention of PAHs on both polymeric and monomeric stationary phases decreases with the increase of pore diameter. The heavily loaded polymeric phases on wide pore substrates were found to provide better separation of PAHs. This was ascribed to the high coverage of surface.

The combination of different kinds of stationary phases has been found to improve selectivity and separation efficiency (232, 233). With a combination of different columns or with a mixed stationary phase column, good separation of PAHs were reported (232, 233). Column selectivity of a column with mixed stationary phases and a column by coupling a few short columns with different stationary phases showed the same (233).

In addition to the above aspects of columns, the structural properties of PAHs are also important, since the components directly interact with the stationary and mobile phases. The relationship between PAHs and their chromatographic retentions has been known well dependent on the number of aromatic nuclei (234, 235). The relationship between the shape of PAHs and the retention on reversed phase LC has also been studied. The shape of PAHs was described in terms of the length-to-breadth (L/B) ratios (218). Generally, the LC retention increases with the increase of L/B ratio of the PAH. The property of planarity or nonplanarity of PAHs is considered as an extension of the

molecular description of L/B ratio to include parameters of thickness of molecular structure of the PAH (218, 236-238). PAHs with planar and linear structures are generally retained longer than non-planar and non-linear PAHs.

Detection

The most common detectors for HPLC are UV absorption and fluorescence. These detectors are sensitive and selective to PAH analysis. UV detector includes fixed wavelength and multi-wavelength modes. Fixed wavelength UV detector was found to provide higher sensitivity than multi-wavelength UV detector (239). The optimum wavelength in the determination of PAHs can provide both good selectivity and sensitivity (240). For example, the maximum absorbance of benzo[a]pyrene was obtained at 290 nm, where only a little intensity was from perylene. The selective determination of fluoranthene and pyrene was also accomplished by using wavelengths 340 nm and 360 nm, respectively. A number of papers have also described the use of multiple wavelength UV detector coupled with LC in the determination of PAHs (241-243). It appeared more useful for identification.

Fluorescence detection is ideally suited for the determination of PAH components separated by HPLC. Almost all of PAHs have intensity fluorescence with individual spectral characteristics (244). The characteristic fluorescence spectra can be used for possible identification of specific PAH in complex PAH mixtures. By choosing proper excitation and emission wavelengths, a higher degree of specificity can be obtained, resulting in less interference from sample

matrix. Besides PAHs, only a limited number of compounds are able to provide fluorescence. Thus PAHs can be analyzed in the presence of many other classes of compounds with little or no sample clean-up. The detection limits can be obtained as low as sub-picogram levels(245, 246). Therefore, HPLC with fluorescence detection has been applied to determine qualitatively and quantitatively PAHs in many kinds of samples, such as air particulates (247), diesel exhaust (248), cigarette smoke (249), petroleum products (250), water (251), and many other environmental samples (218, 252).

However, the major problem of fluorescence detector is fluorescence quenching of PAHs. Oxygen and other compounds, such as nitromethane, were found to quench the fluorescence of some PAHs (253-255). In some cases, this was used as advantage to determine certain PAHs without interference from others (256).

Recent Advances in HPLC

One of the recent advances in HPLC is the development of microcolumns (capillary and mirobore column) (257-259). The application of microcolumn LC to the analysis of PAH containing samples has been reported (260). Some of the advantages of using microcolumn in LC include higher separation efficiencies, improved detection performance, reduced solvent flow rate and amount, and the ability to work with smaller amounts of samples, which benefit to trace analysis (257). This technique is still in the process of development (257, 261).

The combination of liquid chromatography with mass spectrometry (MS) has been another major advance in HPLC (262). With MS as a detector

for LC, identification information is enriched by mass spectra of effluents in addition to retention data. Mass spectrometry, with a variety of available ionization techniques, also provides high sensitivity. The difficulty in combining LC with MS, however, is developing an appropriate interface (263). Significant progress has been made toward interfacing LC with MS (262). Moving belt interface, direct liquid introduction, thermospray, heated pneumatic nebulizer, atmospheric pressure ionization, and electrospray have been developed to couple LC with MS, as discussed in a few reviews (262-266). The application of LC/MS in the determination of PAHs have also been reported (267, 268). The recent development and application of HPLC were also summarized in an A-page paper (269) and a biennial fundamental review paper (270) in *Analytical Chemistry*.

VI. Brief Introduction on Determination of PCBs

Polychlorinated biphenyls (PCBs) was first synthesized by Schmidt and Schultz (271) in 1881. Due to their unique characteristics of both thermal and chemical stability, mixtures of PCBs have been widely used since 1930, as non-flammable oils, especially in connection with electrical transformers, condensers, and paint.

Despite their use in industry, PCBs are toxic (272-274). In humans, chronic ingestion of small amounts (10 mg kg^{-1}) for more than 50 days causes chloracne. Prolonged, continuous exposure to even low doses may also cause serious human health problems. Widespread application of PCBs has resulted in a persistent and ubiquitous environmental problem. Swedish scientist Jensen (275) first found PCBs in fish and birds in

1966. Then in 1968, a very serious food poison accident happened in Japan. Poisoned victims consumed the rice oil which was contaminated by PCBs leaked from a heat exchanger. This so-called "Yusho" poisoning incident (276) drew great attention on problem of PCB contamination of foods. The increased cancer of PCB contamination is also derived from the fact that by late 1971, the environmental pollution had resulted in global contamination of wildlife (277).

An important step in controlling environmental pollution by PCBs is the determination of PCBs. In theory, the total number of possible PCB products resulting from chlorination of biphenyl can be as many as 209. This creates difficulties in qualitative and quantitative analyses of PCBs in environmental samples. Analytical problems and methods for the determination of PCBs have been reviewed (278, 279). Due to the complicated nature of PCB mixtures, chromatographic methods (280), especially gas chromatography, have been often applied (281-284).

VII. Research Goal

In the determination of trace amounts of PAHs by a capillary GC, both good sensitivity and separation efficiency are desirable. Grob(132, 133) and Jennings(142) have described solvent effect and cold trapping as the two principal functions that lead to the reconcentration of analyte vapors in the capillary column. As a result of these effects, sharp and narrow GC peaks were achieved. However, the use of these effects is still not widely applied. This is probably due to the lack of full understanding of the mechanism of the effects and also to the fact that the instrumental conditions needed for achieving good separation, symmetrical GC peak shape, and desired sensitivity appeared to be critical. A number of papers (285-287) have reported that the responses of solutes, such as PAHs (285), and organophosporus pesticides(286, 287), varied with the injection solvents. The authors suggested that there should be consistency in the solvent used for the standards and samples in capillary GC to deal with the problem caused by the use of different solvents. No further studies were performed on this matter. Some very important factors, such as the relationship between the boiling point of the solvent and the GC conditions, were not investigated.

To achieve the best separation and the highest sensitivity in the determination of PAHs, especially the late-eluting PAHs, which often give poor sensitivity by capillary GC(288), it is necessary to study the effect of solvent, the relationship between the boiling point of solvent and the optimum initial temperature of the column, and other factors that might affect the performance in the determination of PAHs by GC/MS. This study should lead to an understanding of the mechanism of the

solvent effect and the process of sample transfer from the injector to column. The findings on the determination of PAHs we expect to be applicable to the determination of PCBs by capillary GC with electron capture detection.

Chapter 2. Experimental Section

Instrumentation

A Hewlett-Packard Model 5890 gas chromatography, equipped with a Model 5970 quadrupole mass spectrometer (GC/MS) was used for the determination of PAHs. Another Hewlett-Packard Model 5890 gas chromatography coupled with electron capture detector (GC/ECD) was used to determine PCBs. Both sets of instrument were equipped with Model 7673A autosamplers, split/splitless injection modes, and Model 300 computer controlling and data acquisition systems. A fused silica capillary column coated with crosslinked methyl silicone gum as the stationary phase (Hewlett-Packard Ultra 1, crosslinked methyl silicone column) was used in the determination to PCBs. This column 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). In the studies of PAH determination, columns Hewlett- Packard Ultra 1 and SUPELCO SPB-5 (30m x 0.25mm x 0.25μm) were used. The stationary phase of the latter column was 5% diphenyl: 94% dimethyl: 1% vinyl polysiloxane.

Carrier gas

Helium (Linde "Zero gas") was used as carrier gas for the GC/MS system. The flow rate of helium, measured at the mass spectrometer pump exhaust, was approximately 0.8 ml min^{-1} at column temperature of 120 °C and room temperature of 25 °C. On the GC/ECD system, hydrogen

was used as carrier gas and nitrogen as make-up gas. The flow rate of carrier gas (H_2) was 0.8 ml min^{-1}. The make-up gas (N_2) pressure was kept at 40 psi.

Reagents

PAHs: A commercially available solution (10 ml) of a mixture of 16 PAHs (2000 μg ml^{-1} of each PAH in dichloromethane and benzene (50: 50)), as listed in Table 1, was obtained from SUPELCO (Oakville, Ontario, Canada). This standard was dissolved in benzene to give a stock solution containing 40 μg ml^{-1} of each PAH. The solutions containing 0.02-6.0 μg ml^{-1} of PAHs in different solvents were made by dilution of appropriate volumes of this stock solution.

PCBs: A mixture of 10 representative PCBs, as shown in Table 2, was obtained from SUPELCO (Bellefonte, PA, USA). Each of these PCB congeners represents one isomeric group. Dissolved in hexane, this PCB mixture was used as stock solution, with their concentrations listed in Table 2. Working solutions were prepared by diluting appropriate amounts of this stock solution in appropriate solvents.

All stock solutions and standard solutions of PAHs and PCBs were kept in a refrigerator at approximately 4 °C. They were taken out and allowed to reach room temperature prior to use.

Table 2. Name and Concentration of 10 PCBs

Peak #	Name of Component	Concentration ($\mu g\ ml^{-1}$)	
		Stock solution	Working solution
1	2-chlorobiphenyl	100	10
2	3,3'-dichlorobiphenyl	100	10
3	2,4,5-trichlorobiphenyl	10	1
4	2,2',4,4'-tetrachlorobiphenyl	10	1
5	2,3',4,5',6-pentachlorobiphenyl	10	1
6	2,2',3,3',6,6'-hexachlorobiphenyl	10	1
7	2,2',3,4,5,5',6-heptachlorobiphenyl	5	0.5
8	2,2',3,3',4,4',5,5'-octachlorobiphenyl	5	0.5
9	2,2',3,3',4,4',5,5',6-nonachlorobiphenyl	5	0.5
10	2,2',3,3',4,4',5,5',6,6'-decachlorobiphenyl	5	0.5

Solvents used in the study included dichloromethane (b.p. 40 °C), hexane (b.p. 68 °C), toluene (b.p. 110.6 °C), p-xylene (b.p. 138 °C), o-xylene (b.p. 144 °C), and acetonitrile (b.p. 81.6 °C) (Fisher Scientific, HPLC Grade); cyclohexane (b.p. 80.7 °C) and isooctane (b.p. 99.2 °C) (Caledon Laboratories, HPLC Grade); octane (b.p. 125 °C), nonane (b.p. 151 °C), and dodecane (b.p. 215 °C) (Aldrich, Analytical Grade); benzene (b.p. 80.1 °C) (BDH, Analytical Grade); and mesitylene (b.p. 162 °C) (Estman Kodak, Analytical Grade).

Table 3. Temperature programs used in the determination of PAHs.

Level	Initial Temp.(°C)	Initial Time(Min)	Rate (°C Min^{-1})	Final Temp.(°C)	Final Time (Min)
			Program One		
1	100	1	20.0	150	1.5
2			30.0	200	4.0
3			30.0	220	4.0
4			30.0	250	0.2
5			1.0	263	2.0
6			50.0	270	0.0
7			1.0	280	0.0
			Program Two		
1	variable	4	20.0	150	0.0
2			5.0	187	0.0
3			30.0	220	0.0
4			20.0	260	25

Purge-off time: 0.6 min.

Injection port temp.: 250 °C

Transfer-line temp.: 250 °C (program one), 260 °C (program two)

Temperature Program

Temperature programs used in the determination of PAHs are shown in Table 3. Program one in Table 3 was initially used for some experiments. Results shown in Figures 2(a) and 2(b) and Tables 4(a) and 4(b) in the later "Results and Discussion" section were obtained under the temperature program one. With further optimization and the following consideration, the temperature program two was chosen for the rest of studies on the determination of PAHs.

Optimization of the temperature program was studied in an attempt to separate all the 16 PAH peaks, especially the closest peak pairs, i.e., peaks 5 and 6, 9 and 10, 11 and 12, and 14 and 15, without losing sensitivity. The methyl silicone column was used as separation column and toluene was used as injection solvent. The first four peaks were easily separated and eluted during the first temperature period. Temperature rates, 3, 5, 8, 10, and 15 $^{o}C\ min^{-1}$, for level 2 were studied. The complete separation of peaks 5 and 6 was obtained only when this temperature rate was lower than 8 $^{o}C\ min^{-1}$. With other solvents, such as nonane, p-xylene, and isooctane, the complete separation of peaks 5 and 6 was also achieved at the temperature rate below 8 $^{o}C\ min^{-1}$. Therefore, a temperature rate of 5 $^{o}C\ min^{-1}$ was applied during the separation of peaks 5 and 6. During the time period corresponding to level 3 in the temperature program, only peaks 7 and 8 were eluted and they were easily separated from each other. Therefore, a fast increase of temperature from 187 ^{o}C to 220 ^{o}C was chosen in order to give higher signals of peaks 7 and 8. The rest of peaks came out during the last temperature period. With different temperature rates, 5, 10, 15, 20, 25,

and 30 °C, there was no significant change on resolution of these peaks. But the peak heights were increased and elution time was shorter if this rate was higher than 20 °C min^{-1}. We also found that the complete separation of peaks 14 and 15 can only be achieved when the oven temperature was lower than 260 °C at the time when these peaks were eluted. If the temperature was too low (for example 220 °C), however, the peak heights of the late-eluting peaks were reduced significantly due to the widening of these peaks. Under the consideration of separation and sensitivity, temperature program two shown in Table 3 was found to be suitable and was used for the determination of PAHs unless stated. It should be noted that the initial temperature was dependent on the solvent used. If the solvent boiling point was higher than 130 °C, level 1 was replaced by a level with only the initial temperature and an initial time of 4 min.

In the determination of PCBs, a 1 µl aliquot of sample solution was injected onto the crosslinked silicone column at an initial temperature which depended on the solvent used. The oven was kept at the initial temperature for 2 minutes. The temperature was then increased to 250 °C at a rate of 8 °C min^{-1} and was held for 15 minutes. Temperatures of both injection port and detector were 250 °C. A purge-off time of 30 seconds was applied to allow splitless injection of samples.

Chapter 3 Results and Discussion

I. An Investigation into Factors Affecting Performance in the Determination of PAHs by Capillary GC/MS

The Effect of Solvent

The sixteen PAHs available commercially represented a reasonably wide range of compounds from the list of priority pollutants. They are listed in Table 1 with their boiling points. The order of the list in Table 1 also corresponds to the order in which the PAHs are eluted from the capillary GC column.

Several solvents with different boiling points and different polarities were used to investigate the effect of solvent on the determination of PAHs by GC/MS. In an initial trial, the seven solvents with the lowest boiling points, listed in Table 4, were chosen as solvents for the injection of the PAHs. Each of these solvents was used separately to prepare a standard solution containing 2 $\mu g\ ml^{-1}$ of each of the sixteen PAHs. An aliquot of 3 μl of this solution was then introduced into the capillary GC column via splitless injection. Both peak area and peak height of each PAH were determined from the total ion current chromatogram (TIC) with selected ion monitoring. For comparison, the peak areas and peak heights of all PAH peaks, with all seven solvents, were normalized, based on the results obtained with toluene as the solvent. The relative peak area and peak height are summarized in Tables 4(A) and 4(B), respectively. As we can see, the peak areas generally increase with the boiling point of the solvent, except for the first five

peaks in isooctane. The striking observation is that the late eluting peaks from the toluene solution are much larger than from all the other solvents. This suggests that toluene is much more efficient in transferring the higher molecular weight PAHs onto the column. The peaks from fluorene, phenanthrene, anthracene, fluoranthene and pyrene in low boiling solvents are more comparable in height with similar peaks from the toluene solutions.

In order further to confirm the importance of solvent boiling point on PAH determination by GC/MS, benzene, toluene, p-xylene, o-xylene, were used as solvents and their effects were investigated. These solvents have similar polarities, with dielectric constants (ε) of 2.284, 2.379, 2.270, and 2.568, respectively, but with different boiling points 80.1, 110.6, 138, and 144 °C, respectively. Under similar conditions, as discussed above, except that the initial temperature was increased from 100 °C to 120 °C, total ion current chromatograms of PAHs in these four solvents were obtained. The peak height of each PAH, relative to the peak height of the same PAH in toluene as 100%, are shown in Table 5. Despite their similar polarity, these solvents caused significant differences in signals from PAHs, due probably to their difference in volatility. Again, the greater the difference of boiling point between these solvents is, the more significant difference in relative peak height of late-eluting peaks is observed. Similar observations were reported by Lee *et al.* (285), where solvents with similar polarity, such as acetonitrile and methanol, gave significantly different responses. This also suggests that this difference in behaviour might be due to the difference in the boiling point between these two solvents.

Table 4 a . Relative peak area of 16 PAHs in differenct solvents (%)

Component	bp (°C)	peak #	solvent	dichloromethane	hexane	benzene	cyclohexane	acetonitrile	isooctane	toluene
			ε	9.080	1.890	2.284	2.023	38.8	1.940	2.379
			bp (°C)	40	68	80.1	80.7	81.6	99.2	110.6
						Relative Peak Area				
Naphthalene	218	1		84	89	79	81	64	118	100
Acenaphthylene	270	2		77	84	75	78	63	118	100
Acenaphthene	274	3		77	83	75	76	63	115	100
Fluorene	294	4		69	77	71	72	61	111	100
Phenanthrene	338	5		55	67	62	63	53	112	100
Anthracene	340	6		61	78	73	72	61	99	100
Fluoranthene	383	7		44	63	59	63	54	97	100
Pyrene	393	8		42	61	57	60	53	94	100
Benzo[a]anthracene	431	9		15	30	33	35	33	60	100
Chrysene	414	10		24	65	50	52	49	69	100
Benzo[b]fluoranthene	481	11		6	17	20	21	25	37	100
Benzo[k]fluoranthene	481	12		11	26	31	32	36	47	100
Benzo[a]pyrene	496	13		5	17	21	21	27	35	100
Indeno[1,2,3-c d]pyrene	-	14		21	6	9	7	14	16	100
Dibenz[ah]anthracene	-	15		0(nd)	6	11	8	14	16	100
Benzo[ghi] perylene	-	16		1	8	12	9	17	17	100

nd - not detected

Table 4b . Relative peak height of 16 PAHs in different solvents. (%)

peak #	solvent	dichloro-methane	hexane	benzene	cyclohexane	acetonitrile	isooctane	toluene
	ε	9.080	1.89	2.284	2.023	38.8	1.940	2.379
	bp (°C)	40	68	80.1	80.7	81.6	99.2	110.6
					Relative Peak Height			
1		18	23	37	35	19	103	100
2		56	75	79	79	54	84	100
3		65	82	86	87	61	87	100
4		85	106	108	107	84	107	100
5		92	115	107	110	92	119	100
6		85	107	109	108	91	113	100
7		65	95	89	96	82	111	100
8		61	91	85	87	78	106	100
9		20	42	45	48	45	69	100
10		23	50	52	55	50	73	100
11		7	23	26	27	33	45	100
12		8	23	27	29	32	44	100
13		5	17	27	21	27	37	100
14		1	5	9	6	14	16	100
15		0	4	8	6	11	13	100
16		1	7	11	8	16	17	100

Table 5. Relative Peak Height of TIC of 16 PAHS(%)

(3 μl of 2 μg ml-1)

	Solvent	Benzene	Toluene	p-xylene	o-xylene
	bp (°C)	80.1	110.6	138	144
	ε	2.284	2.379	2.270	2.568
peak #			Relative Peak Height		
1		37	100	94	109
2		60	100	142	167
3		40	100	138	157
4		45	100	92	61
5		38	100	52	65
6		54	100	50	45
7		55	100	49	49
8		55	100	59	53
9		45	100	91	92
10		46	100	71	70
11		70	100	117	109
12		66	100	99	96
13		41	100	112	107
14		24	100	147	120
15		21	100	162	134
16		27	100	143	118

The heights of peaks 4 to 10 with xylenes as solvent are lower than those in toluene. This is likely caused by the band broadening resulted from the solvent recondensation. At the initial column temperature of 120 °C, toluene is not likely recondensed, but (p-, o-) xylenes, with boiling points of 138 and 144 °C, may be recondensed at this temperature.

The determinations of the same amount of PAHs in benzene and toluene, as solvents, gave the chromatograms shown in Figure 2 with temperature program one in Table 2. Comparing Figure 2(a) with Figure 2(b), one can clearly see the enhancement of chromatographic signals when the solvent is toluene, especially the dramatic enhancement of the late eluting peaks. The enhancement effect of the higher boiling point solvent on the TIC signal may be due to two reasons. First, the higher responses of PAHs in toluene may be caused by the reconcentration effect of the solvent. In Grob's model of the solvent effect (133), the vaporized material is transferred onto the column essentially as a mixture. In the first stage of separation on the column, the solvent shifts away from the sample components and the relatively wider band of solvent blocks the expansion of the vapours of the solutes so that the solutes are reconcentrated in a narrow band. The condensation of solvent is likely to occur at column temperatures below 100 °C when toluene is used as solvent. This is indicated by the changes of resolution of peak 5 and 6, 9 and 10, 11 and 12 as shown in the Figure 3, which is obtained at the column initial temperature 100 °C. Resolution was calculated based on the following equation:

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

where R_S is 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 resolution of Peaks 5 and 6, 9 and 10 became worse with isooctane and toluene as solvents. The loss of separation efficiency is possibly due to the recondensation of solvent resulting in the band broadening in space(140, 145). Although recondensation of solvent reduces the separation efficiency, it may help to complete the transfer of PAHs from injector into column. Thus the responses of the solutes are greater from toluene solutions than from benzene solutions. Secondly, the low responses of PAHs in benzene may be caused by the loss of sample vapor due to the expansion backwards (289).

The solvent itself would not be expected to change the ionization process in the MS detector since the solutes and solvent will be separated in time. This was confirmed by the observations that the ratio of $(M+1)^+/M^+$ of each PAH was independent of solvent, which is demonstrated in Table 6. If the solvent participated in the process of ionization of the PAH, the abundance of $(M+1)^+$ would be changed due to chemical ionization in addition to electron impact ionization.

Since different solvents vary in volatility, the column temperature used for a specific solvent should be dependent on its boiling point. In order to apply the enhacement of signals of PAHs owing to toluene as discussed above in practice, it is certainly important to find the best column temperature which also provides the best separation.

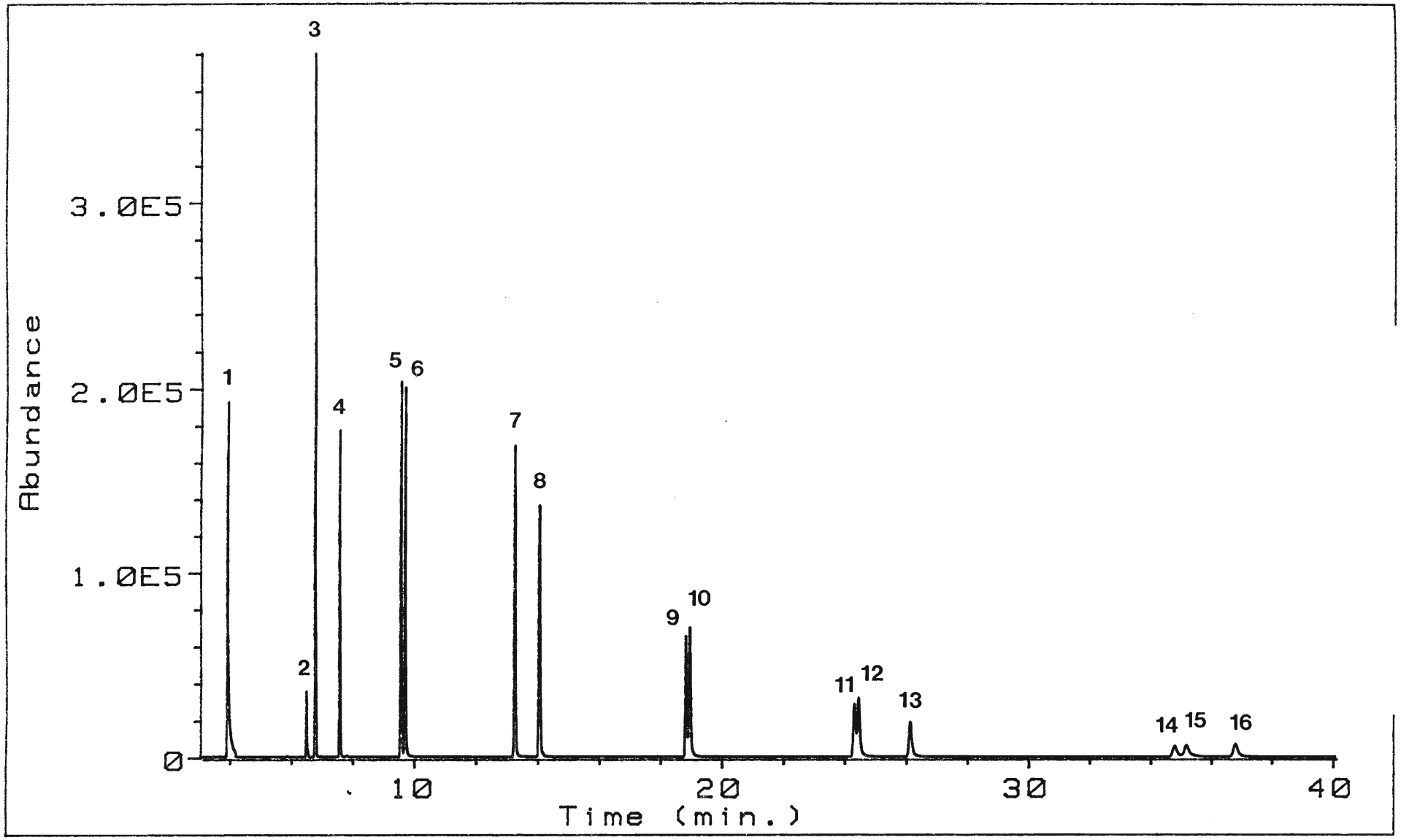


Figure 2(a). Chromatogram of 16 PAHs (3 µl of 2 µg ml^{-1}) in benzene.

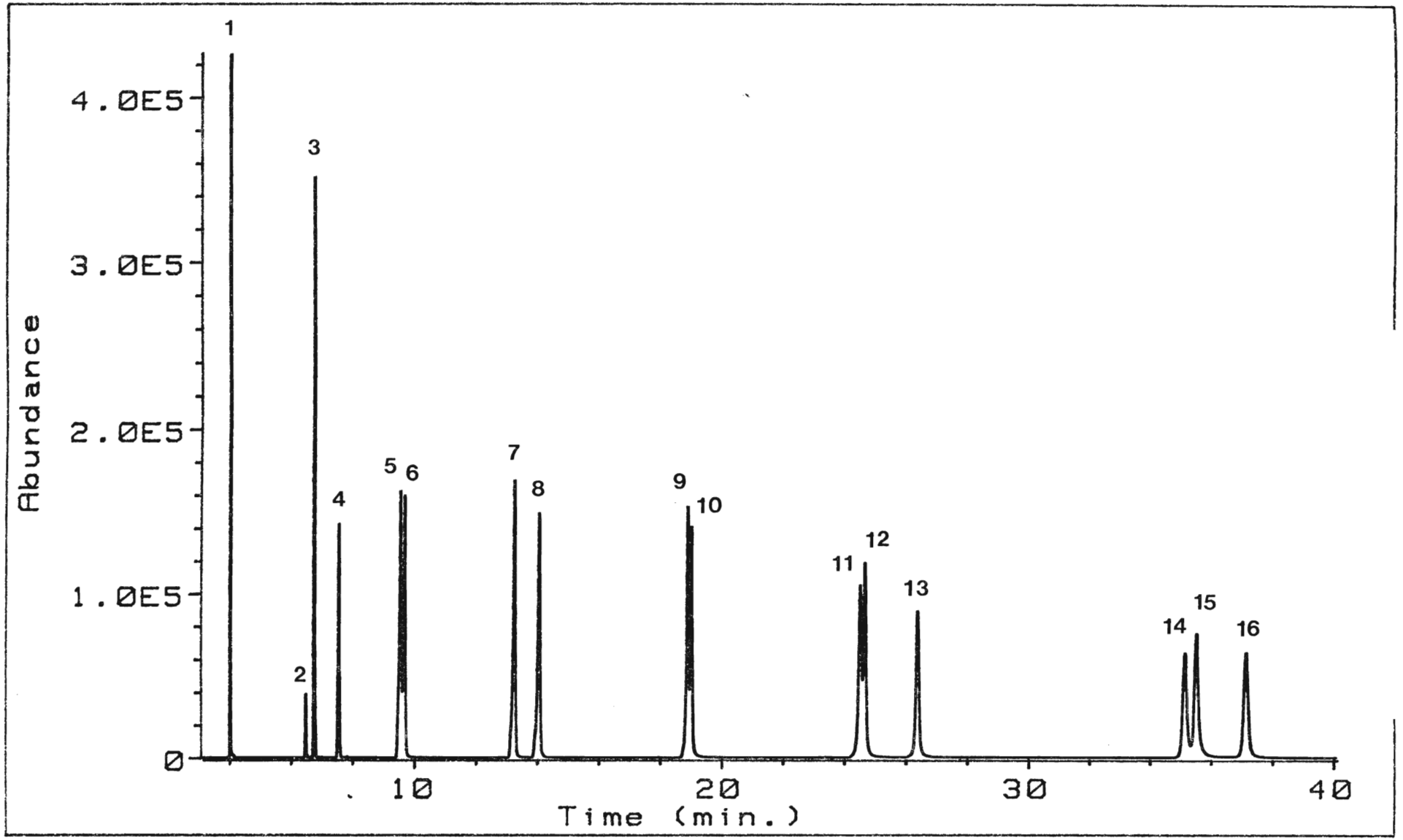


Figure 2(b). Chromatogram of 16 PAHs (3 µl of 2 µg ml^{-1}) in toluene.

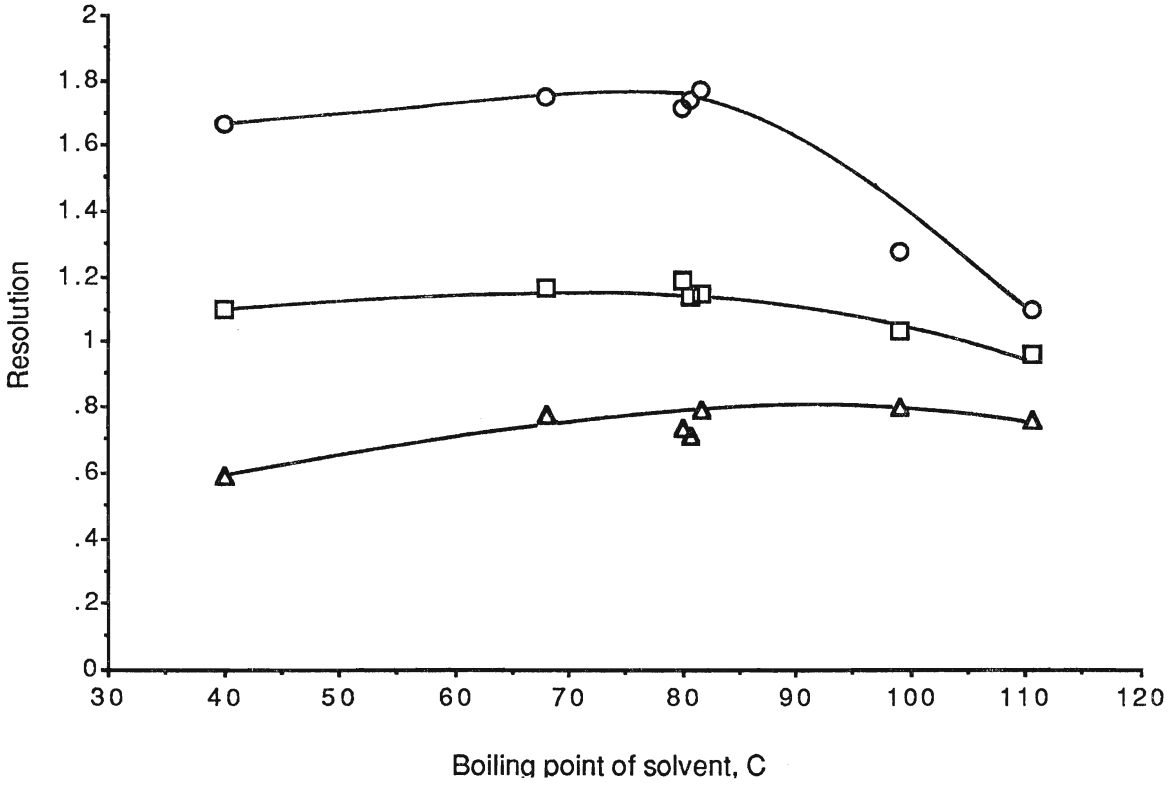


Figure 3. Effect of solvent on the resolution

○ Peak 5/6 □ Peak 9/10 △ Peak 11/12

Solvent	DCM	Hexane	Benzene	Cyclohexane	Isooctane	Toluene
b.p. (°C)	40	68	80.1	80.7	81.6	110.6

Table 6. The ratio of $(M + 1)^+/ M^+$ of 16 PAHs in different solvent

peak #	M^+	solvent	$(M+1)^+/M^+$ benzene	toluene	p-xylene	o-xylene
1	128		0.10	0.11	0.10	0.10
2	152		0.12	0.12	0.12	0.14
3	154		0.16	0.12	0.13	0.12
4	166		0.13	0.14	0.16	0.13
5	178		0.15	0.15	0.12	0.15
6	178		0.16	0.15	0.17	0.17
7	202		0.17	0.17	0.18	0.17
8	202		0.19	0.18	0.17	0.18
9	228		0.17	0.18	0.20	0.22
10	228		0.18	0.19	0.19	0.21
11	252		0.21	0.22	0.22	0.23
12	252		0.25	0.22	0.22	0.22
13	252		0.20	0.22	0.23	0.21
14	276		0.22	0.25	0.22	0.22
15	278		0.29	0.24	0.27	0.21
16	276		0.26	0.22	0.23	0.20

The Effect of Initial Temperature

Column temperature and temperature programming were found to affect the resolution and sensitivity of the mixture of PAHs. The temperature program mentioned in the experimental part was found to be optimum in the determination of PAHs in toluene solution, with regard to both resolution and sensitivity. This temperature program was also

appropriate when PAHs were dissolved in other solvents, except that the initial temperature was changed. The effect of initial temperature on the determination of PAHs was found to be more significant than temperatures during later stages. Therefore, studies on the effect of initial temperature were carried out while the rest of temperature program was kept unchanged.

The effect of initial temperature on peak shape is clearly demonstrated in Figure 4. Figure 4 compares chromatograms of phenanthrene and anthracene in toluene under the initial temperatures of (a)100 °C, (b) 110 °C, (c) 120 °C, (d) 130 °C, and (e) 140 °C. At the lower initial temperature, fronting of the peak appeared as shown in Figure 4(a). With the increase of initial temperature from 100 to 120 °C, the fronting of peaks gradually disappeared, and a symmetrical peak was obtained when the initial temperature was 120 °C (Fig. 4c). With a further increase of initial temperature, from 120 to 140 °C, tailing of the peaks appeared, leaving asymmetric peaks, shown in Figure 4e. Similar results were also obtained using other solvents. Fronting appeared at initial temperatures lower than the boiling point of the solvent; tailing appeared at initial temperatures higher than 20 °C above the boiling point of the solvent. The effect of initial temperature on peak shape is more significant for the eight early-eluting peaks than for the late-eluting peaks.

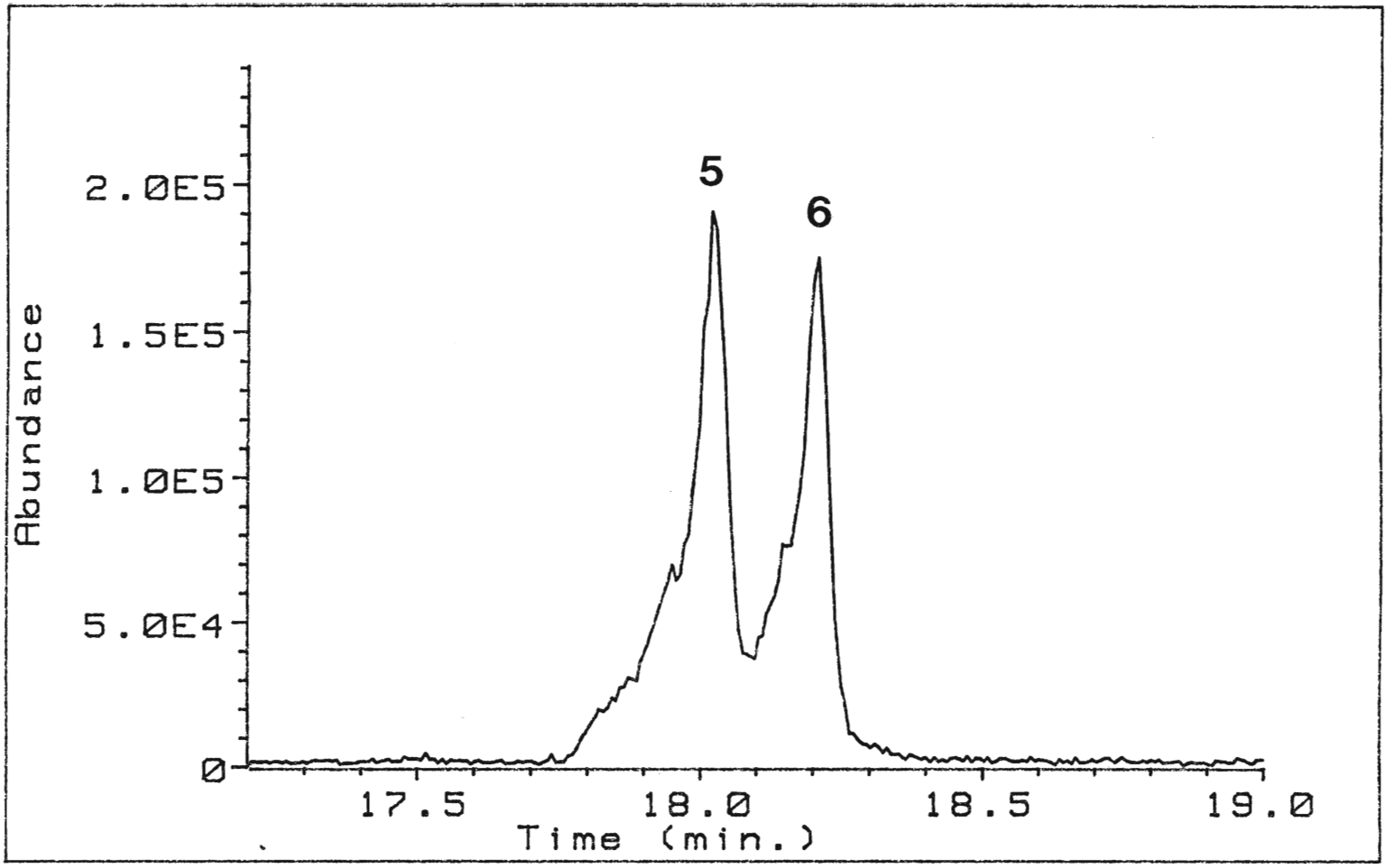


Figure 4(a). Chromatogram of phenanthrene and anthracene in toluene at an initial column temperature of 100 °C.

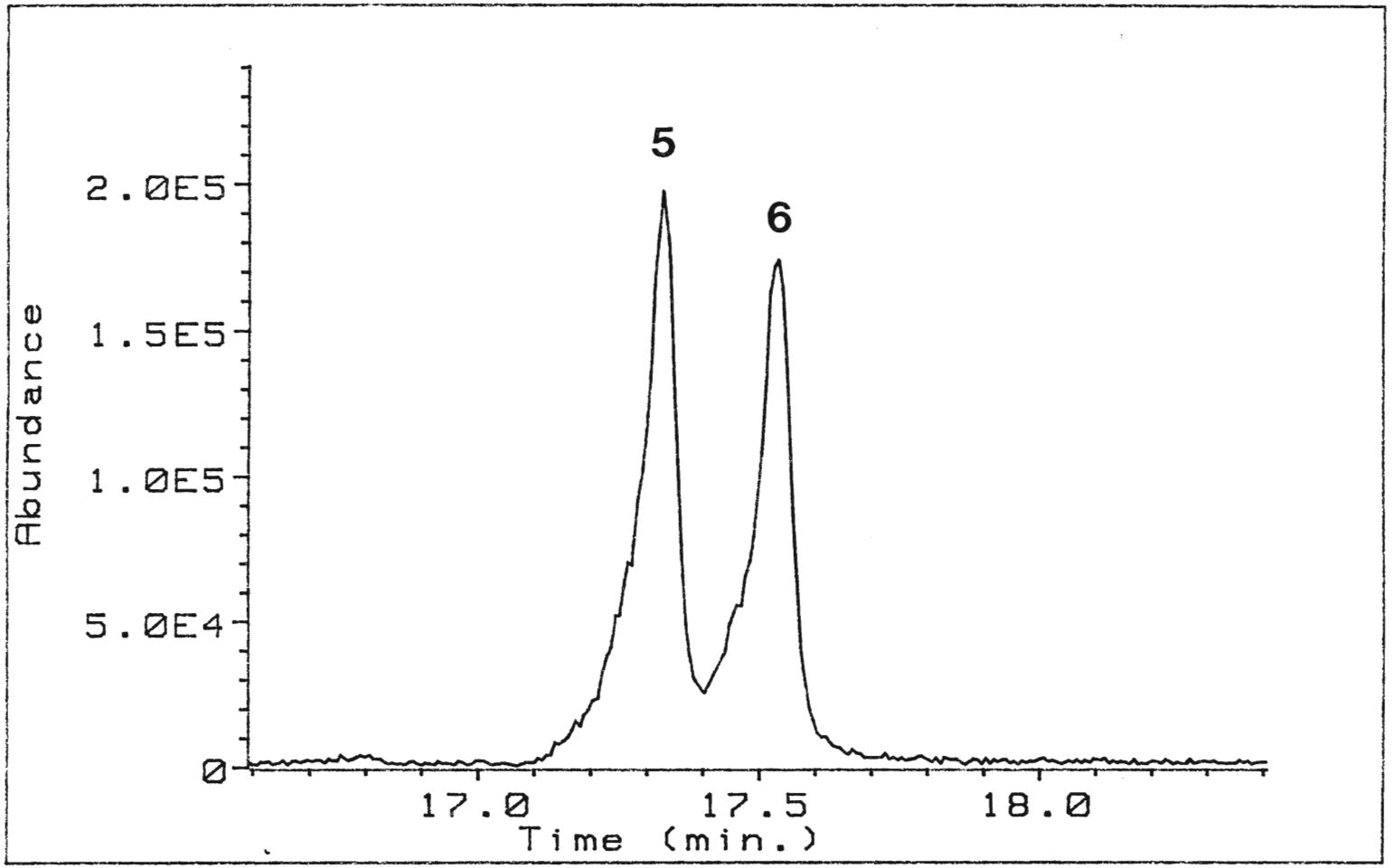


Figure 4(b). Chromatogram of phenanthrene and anthracene in toluene at an initial column temperature of 110 °C.

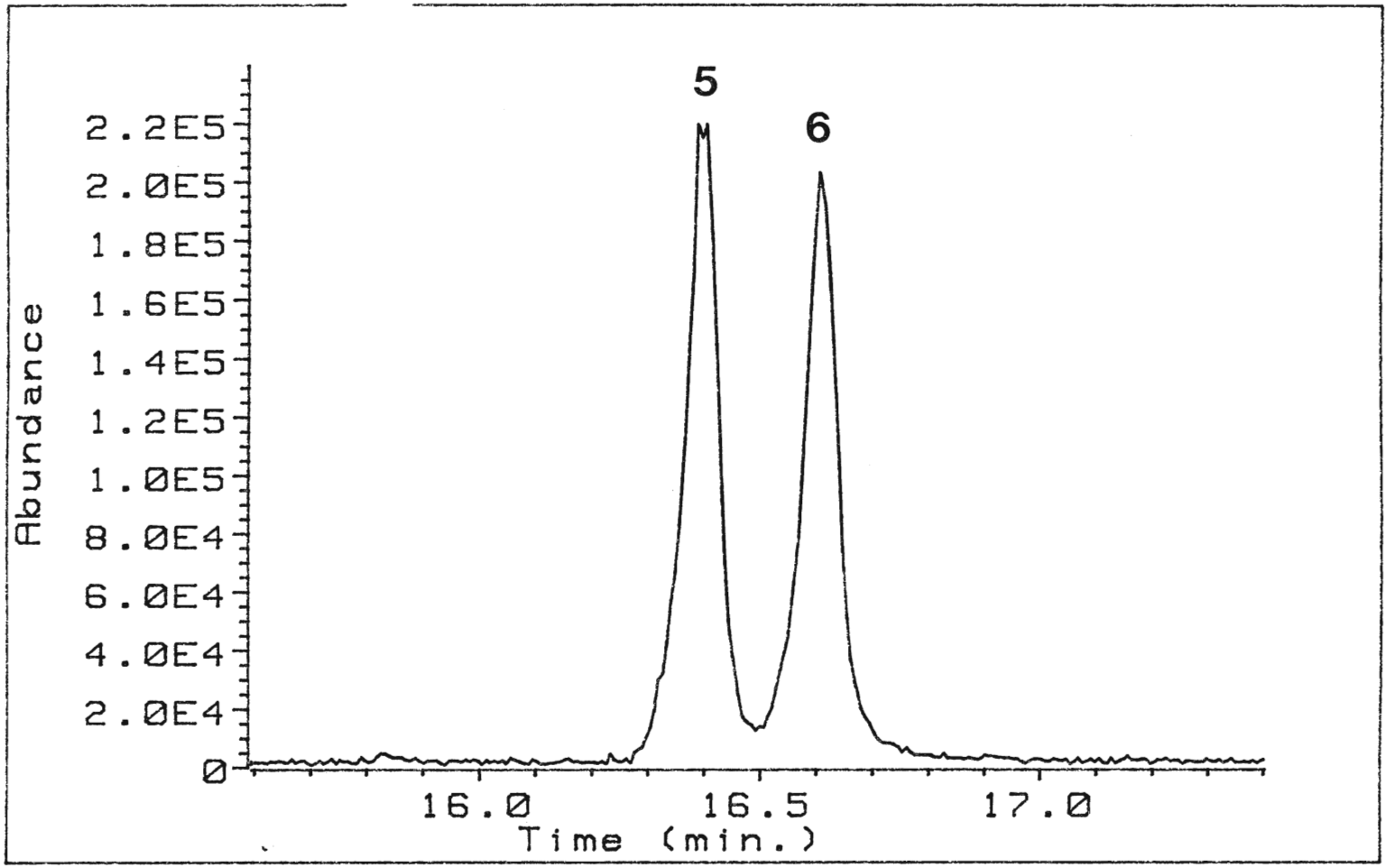


Figure 4(c). Chromatogram of phenanthrene and anthracene in toluene at an initial column temperature of 120 °C.

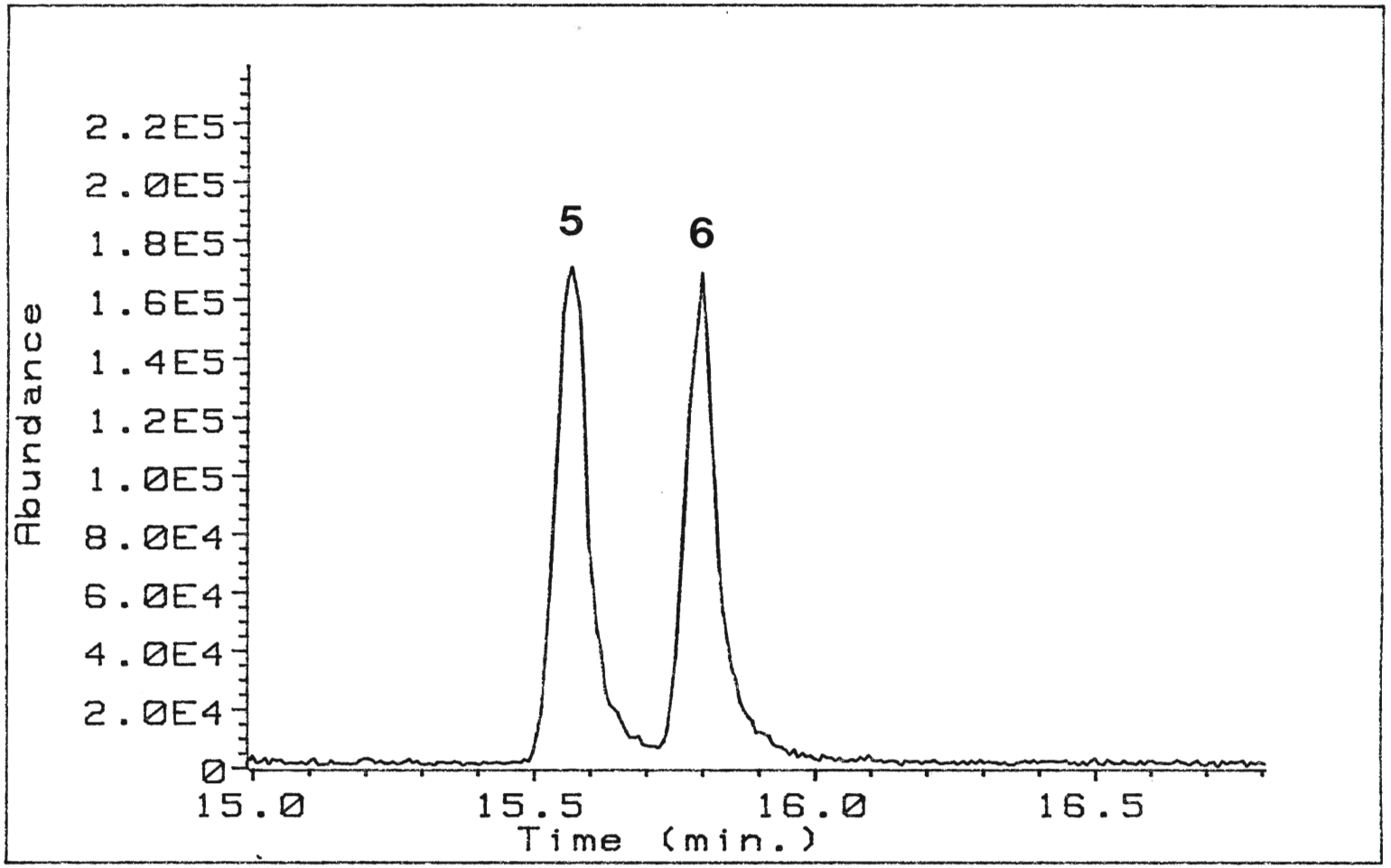


Figure 4(d). Chromatogram of phenanthrene and anthracene in toluene at an initial column temperature of 130 °C.

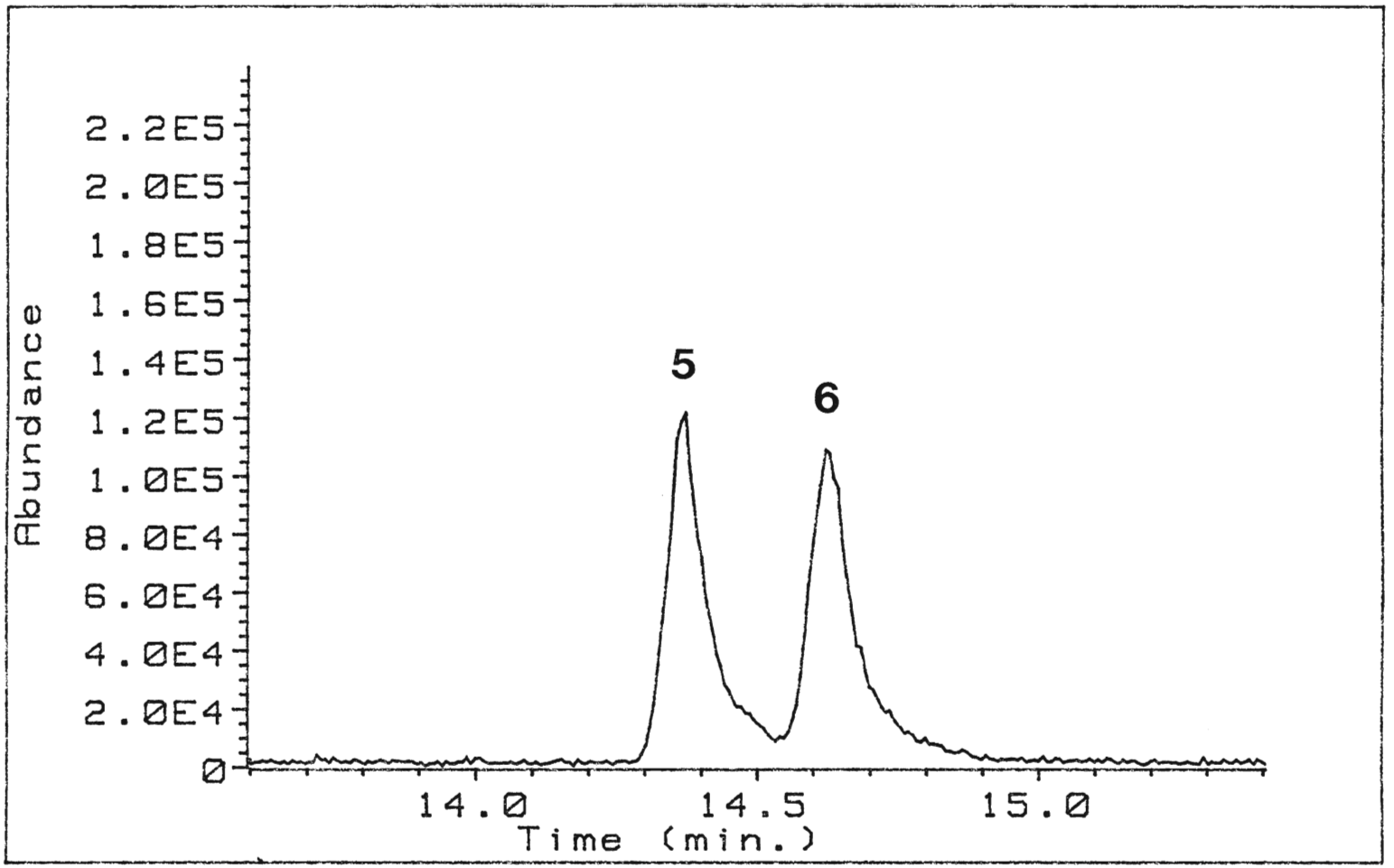


Figure 4(e). Chromatogram of phenanthrene and anthracene in toluene at an initial column temperature of 140 °C.

The changes of peak shapes also reflect the change of separation efficiency as shown in Figure 5. The resolutions between peaks 5 and 6, 9 and 10, and 11 and 12, which were the closest pairs of peaks, were obtained at initial temperature from 100 to 130 °C. As we can see from Figure 5, the resolution of peak 5 and 6 increases with increasing initial column temperature from 100 to 120 °C and remains constant over the range of temperature of 120 to 130 °C. However, the changes of resolutions of peak 9 and 10, 11 and 12 are not significant. This also suggests that the condensation of solvent with relatively high boiling point, such as toluene, occurs at column temperatures lower than their boiling points. The recondensed solvent may cause the broadening solute bands, especially the early-eluting components. Thus the resolution of early-eluting peaks, such as peaks 5 and 6, becomes worse when the initial column temperature is below the boiling point of the solvent.

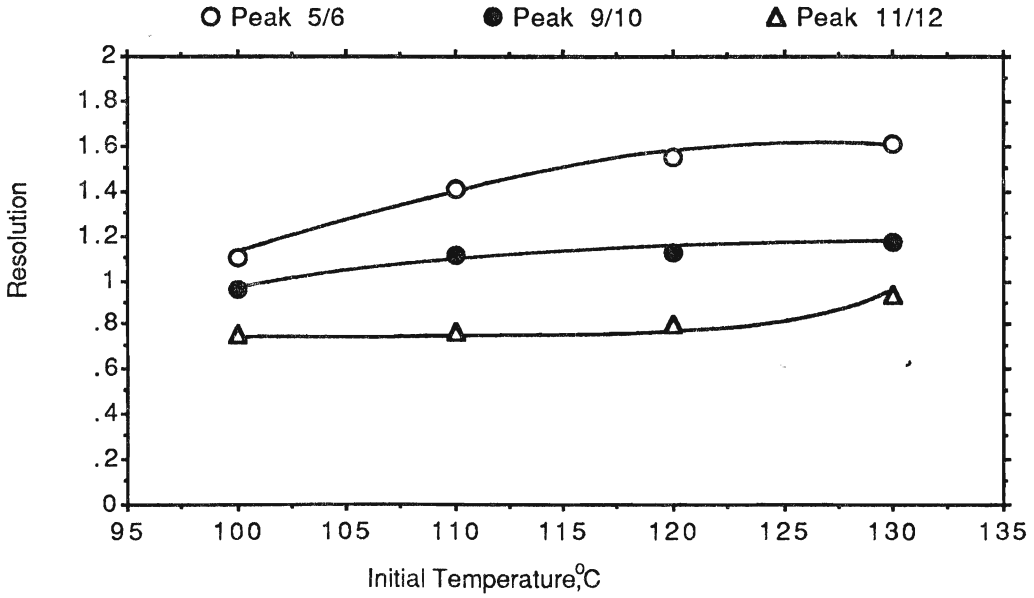


Figure 5. Effect of initial temperature on the resolution

In order to determine the effect of initial temperature on peak height and area, aliquots of 3 μl of 2 μg ml^{-1} PAHs, made separately in benzene, toluene, o-xylene, p-xylene, respectively, were injected sequentially. At different initial temperatures, varying within 10-20 °C above and below the boiling point of the solvent, TICs of 16 PAHs were obtained. Peak heights and peak areas were determined, and relative peak height and the peak area of each PAH were then calculated compared with those determined for 10 °C above the boiling point of the solvent. The results are summarized in Table 7. As we can see, the optimum initial temperature depends on the solvent used. At an initial temperature between the boiling point of the solvent and 10-20 °C higher than the boiling point, the highest response, measured by peak area and peak height, was achieved. Results in Table 7 indicate that the solvent with the lower boiling point (e.g. benzene at 80 °C) showed a smaller range of optimum initial temperature. If a higher boiling solvent (e.g. xylene) was used, the changes in response of the PAHs were small within a relatively wider initial temperature range Thus, the most symmetrical peaks with the highest responses were achieved by using an initial temperature 10-20 °C above the boiling point of the solvent used. The data listed in Table 8 are based on these conditions. Initial temperatures of 90 °C and 120 °C are optimum for benzene and toluene as solvents, respectively. The peak area and peak height of PAHs in Table 8 show that toluene gives higher sensitivity than benzene.

Table 7 Relative peak area and height of 16 PAHs in aromatic solvents at different initial temperature

bp. (°C)	benzene 80			toluene 110.6				p-xylene 138				o-xylene 144			benzene			toluene					p-xylene				o-xylene		
	Initial Column Temperature (°C)																												
	80	90	100	100	110	120	130	128	138	148	158	144	154	164	80	90	100	100	110	120	130	140	128	138	148	158	144	154	164
Peak #	Relative Peak Area														Relative Peak Height														
1	167	100	54	92	113	100	88	-	-	-	-	-	-	-	114	100	68	64	88	100	48	15	-	-	-	-	-	-	
2	142	100	72	81	108	100	89	113	120	100	89	18	100	-	106	100	76	98	109	100	50	32	86	98	100	68	77	100	-
3	147	100	73	83	114	100	114	106	112	100	85	113	100	-	108	100	80	94	98	100	71	33	113	90	100	73	86	100	-
4	135	100	78	88	111	100	121	108	111	100	85	116	100	87	104	100	74	90	95	100	80	42	138	125	100	83	101	100	99
5	138	100	71	90	111	100	113	104	113	100	97	109	100	80	109	100	67	89	93	100	79	56	67	88	100	67	161	100	98
6	134	100	74	78	106	100	108	105	111	100	94	108	100	78	106	100	68	90	91	100	85	56	67	88	100	67	113	100	100
7	136	100	74	85	106	100	114	107	108	100	98	104	100	86	108	100	75	81	96	100	83	59	73	84	100	82	136	100	111
8	126	100	72	87	107	100	112	102	105	100	96	102	100	85	110	100	78	76	90	100	79	63	64	76	100	82	129	100	114
9	127	100	70	104	112	100	113	120	115	100	140	98	100	77	113	100	71	87	104	100	79	70	96	95	100	99	115	100	96
10	121	100	70	85	103	100	109	91	96	100	101	123	100	96	116	100	71	79	97	100	82	71	85	87	100	100	156	100	100
11	126	100	65	100	104	100	115	110	112	100	97	106	100	78	121	100	70	95	103	100	88	83	102	101	100	100	114	100	86
12	120	100	70	95	101	100	101	96	94	100	100	125	100	93	118	100	66	86	96	100	88	79	91	100	100	97	128	100	94
13	111	100	71	100	112	100	111	101	101	100	99	112	100	84	113	100	60	91	109	100	81	75	99	95	100	95	122	100	92
14	118	100	71	94	101	100	120	104	105	100	108	118	100	75	117	100	51	100	108	100	74	66	101	102	100	107	126	100	88
15	122	100	67	92	102	100	120	101	109	100	106	118	100	78	115	100	54	88	100	100	74	66	100	103	100	100	116	100	77
16	114	100	50	111	126	100	120	98	105	100	102	125	100	84	116	100	60	94	104	100	80	96	98	97	100	100	78	100	90

Table 8. Relative peak area and height

(under conditions of temperature program two and injection volume: 3 µl)

	Solvent		Solvent	
initial temperature (°C)	Benzene 90	Toluene 120	Benzene 90	Toluene 120
peak #	Relative Area		Relative Height	
1	76	100	59	100
2	66	100	107	100
3	67	100	120	100
4	65	100	125	100
5	67	100	111	100
6	63	100	99	100
7	61	100	71	100
8	60	100	73	100
9	45	100	48	100
10	49	100	51	100
11	37	100	39	100
12	37	100	37	100
13	33	100	34	100
14	24	100	25	100
15	22	100	22	100
16	24	100	24	100

While other parameters were kept constant and the initial time was varied from 2 to 10 minutes, a series of determinations of the PAHs in toluene and p-xylene were carried out. The effect of the length of time at the initial column temperature is not as significant as initial temperature. Resolutions of peaks 5 and 6, 9 and 10, 11 and 12, and 14 and 15, at different initial times, were not changed significantly. The relatively better resolution between the closest pairs of peaks and sensitivity of the sixteen PAHs were achieved with an initial time of 8 minutes for toluene and 4 minutes for p-xylene. When a solvent of higher boiling point was used, different initial temperature was necessary for optimum sensitivity and resolution. Thus, for p-xylene the initial temperature was 148 °C; the initial time was kept at 4 minutes.

Temperature of Injection Port

The injection port temperature directly affects the efficiency of sample vaporization, therefore it also affects the sensitivity of determination. As the injection port temperature was increased from 200 °C to 250 °C, the peak areas of most of the 16 PAHs gradually increased. Areas of PAH peaks reached a maximum at the injection port temperature of 250 °C and remained similar between 250 °C and 260 °C. When the injection port temperature was increased further to 270 °C and 280 °C, a slight decrease of peak areas was observed. A similar effect on peak heights was also obtained.

Table 9. Simplex Optimization

Experiment Number (Vertex)	Simplex Formed by Vertex	Temperature of Injector (°C)	Initial Temperature (°C)	Mean Abundance of 16 Peaks (x 10^5)
1		250	120	0.83
2		250	130	0.80
3		260	120	1.4*
4	1, 3, 2	260	110	1.1
5	3, 4, 1	270	110	0.74
6	3, 5, 4	270	120	0.90
7	3, 6, 5	260	130	1.4
8	3, 7, 6	250	130	0.80
9	confirm 3	260	120	1.4*

* mean of three trials

To obtain optimum conditions for both injection port temperature and initial temperature in the determination of PAHs, a simplex optimization method was applied to optimize these two factors. The mean value of peak heights of all 16 individual PAH peaks of the TIC was used as the response since all 16 peaks changed in a similar fashion. Toluene was used to prepare standard solutions. Both factors were varied at the same time, regulated by the simplex optimization method. A full simplex optimization was accomplished with nine experimental units. The results, summarized in Table 9, indicate that the highest response for the 16 PAHs was obtained at the optimum condition of injection port temperature of 260 °C and initial temperature of 120 °C demonstrated in Table 9.

temperature of 260 °C and initial temperature of 120 °C demonstrated in Table 9.

Temperature of Transfer-line

The temperature of the interface (i.e., transfer-line) between the GC column and the MS detector is also a factor that could influence sensitivity. It was found that relatively a high temperature of the interface is needed in order to reach the highest sensitivity. As shown in Figure 6, the peak area of three representive peaks 3, 7, and 10 increases with the increase of transfer-line temperature from 250 to 280 °C. An interface temperature of 260 °C was chosen since it gave sufficient sensitivity and was the highest temperature in the temperature program and reasonably below the limiting temperature (300 °C as suggested by the manufacturer). At the above range of transfer-line temperature, the resolutions of peaks 5 and 6, 9 and 10, and 11 and 12 were also examined. The resolutions of these peaks indicated in Figure 7 were not affected appreciably by this factor within the range of temperatures used. Also, the length of the interface (20 cm) is very short, compared with the 25 meters of the capillary column, and would not be expected to have a significant effect upon resolution.

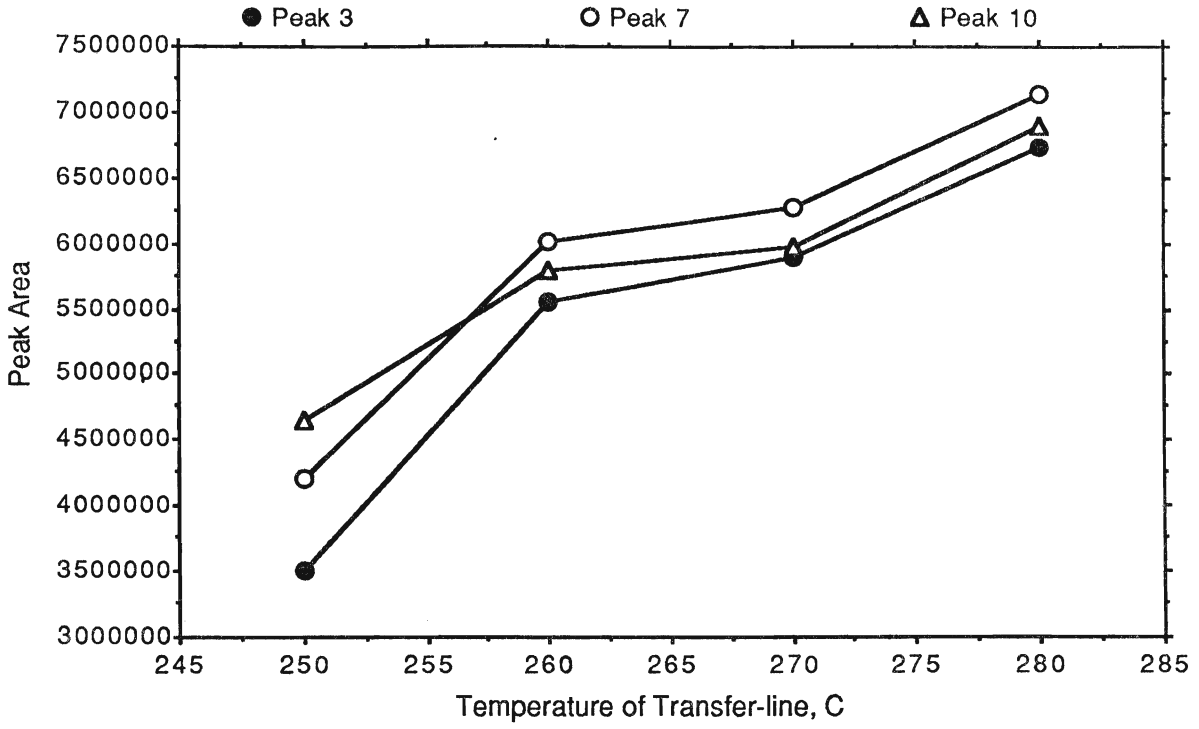


Figure 6. Effect of Temperature of Transfer-line on Peak Area

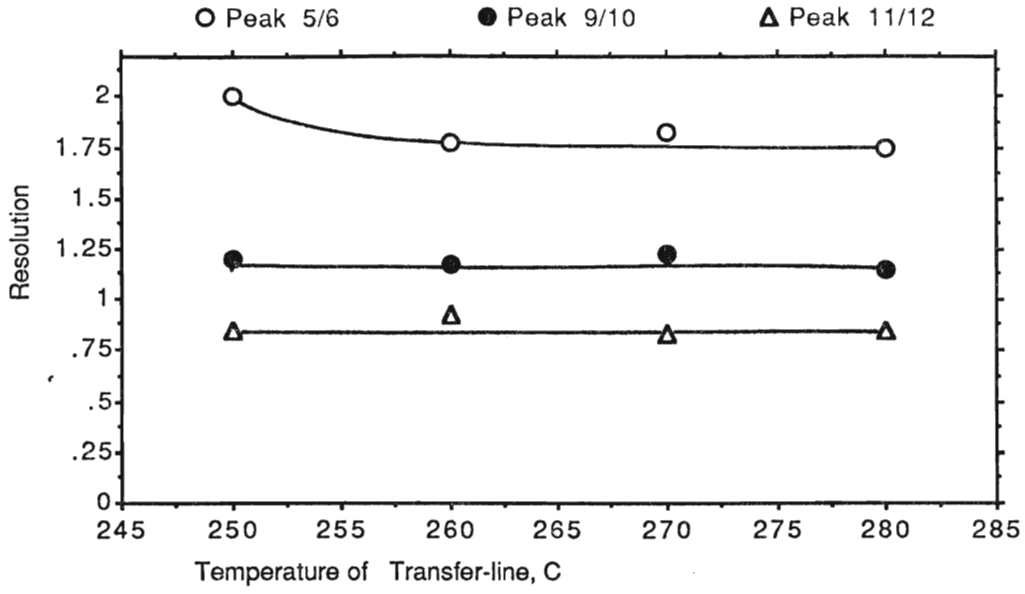


Figure 7. Effect of Transfer-line Temperature on Resolution

Column Head Pressure

The carrier gas must be regulated to provide constant pressure as well as a continuous flow. Thus the flow controller in the instrument requires a 10-15 psi differential between input from the cylinder to output to injection port (i.e., column head) as recommended by the manufacturer. A pressure of 60 psi on the gas cylinder pressure gauge was also suggested. In order to observe the effect of column head pressure on the response of PAHs, TICs of 16 PAHs in toluene were obtained while varying column head pressure between 10 and 15 psi. Peak area and peak height of representative PAHs were then integrated. While the column head pressure was increased from 11 psi to 12.5 psi, sensitivity of PAHs gradually increased. As the column head pressure continuously increasing from 12.5 psi to 14.5 psi, the peak areas of PAHs

showed a small decrease. Within this range, there was no change in the resolution of PAHs. Therefore, a column head pressure of 12.5 psi was chosen with the consideration of better sensitivity. At this pressure, the corresponding carrier gas (He) flow rate, measured at the pump exhaust, was approximately 0.8 ml $min.^{-1}$ at a column temperature 120 ^{o}C.

Analytical Figures

Calibrations of 16 PAHs in toluene were carried out under the optimum conditions, as discussed above. The range of concentrations of PAHs for these experiments was from 0.02 μg ml^{-1} to 6 μg ml^{-1}. Both peak area and peak height were used as response. Correlation coefficients of the calibration curves of the sixteen PAHs are summarized in Table 10. They were better than 0.97 for all 16 PAHs determined, with concentration ranging from 0.20 μg ml^{-1} to 6.0 μg ml^{-1}. There was no significant difference of correlation coefficients of calibration curves of PAHs by peak area from those by peak height.

Table 10. Correlation coefficients of calibration curves of 16 PAHs (calibration range: 0.2 μg ml^{-1} - 6.0 μg ml^{-1})

Peak #	Component name	Correlation coefficient (r) by peak area	by peak height
1	Naphthalene	1.00	0.997
2	Acenaphahylene	1.00	0.997
3	Acenaphthene	1.00	0.992
4	Fluorene	1.00	0.998
5	Phenanthrene	0.999	0.996
6	Anthracene	0.999	0.998
7	Fluoranthene	0.995	0.993
8	Pyrene	0.995	0.996
9	Benzo[a]anthracene	0.994	0.977
10	Chrysene	0.995	0.993
11	Benzo[b]fluoranthene	0.992	0.989
12	Benzo[k]fluoranthene	0.992	0.991
13	Benzo[a]pyrene	0.989	0.990
14	Indeno[1,2,3,-c,d]pyrene	0.987	0.986
15	Dibenzo[a,h]anthracene	0.970	0.976
16	Benzo[g,h,i]perylene	0.984	0.983

Table 11. Relative standard deviations (RSD)

Peak #	RSD (%) Peak Area (n=7)	Peak Height (n=7)	Retention Time (min.) (n=9)
1	4.3	3.9	0.45
2	2.2	5.4	0.09
3	2.4	6.0	0.09
4	2.2	6.6	0.08
5	2.5	5.3	0.05
6	2.3	6.2	0.05
7	3.4	4.3	0.04
8	3.0	6.0	0.04
9	3.6	6.6	0.05
10	6.4	5.1	0.05
11	6.0	5.5	0.08
12	6.6	7.0	0.05
13	6.2	7.7	0.05
14	8.9	8.3	0.07
15	10	11	0.07
16	8.7	10	0.08

Seven replicate determinations of 3 ng (3 μl of 1 μg ml^{-1}) of each of 16 PAH in toluene were carried out under the optimum conditions in order to determine the precision. As shown in Table 11, a relative standard deviations (RSD) in the range of 3.9-11.0% based on peak height, and 2.2-10.5% based on peak area were obtained for the 16 PAHs determined. Nine replicate determinations of 3 ng of 16 PAHs gave the RSD values of retention time of 0.45% for naphthalene and less than 0.1% for the remaining 15 PAHs determined.

The detection limits (S/N=3) for the determination of 16 PAHs by GC/MS were at low pg levels, as shown in Table 12, ranging from 2.4 pg for naphthalene to 94.7 pg for benzo[a]pyrene, when determinations were made in toluene, and 4.4 pg for naphthalene to 30.8 pg for benzo[a]pyrene for determinations in p-xylene.

Evaluation of the Use of Toluene as Solvent

The results discussed above show the usefulness of toluene as solvent for the determination of PAHs by capillary GC/MS. One way to apply toluene to the PAH determinations is to use toluene to extract PAHs from samples and also to use toluene as solvent for sample introduction into the GC. With this approach, toluene is used throughout the whole procedure of PAH analysis. Another approach to apply toluene to improve analytical property by GC is to use toluene as a make-up solvent to prepare an sanalyte solution just before the sample injection procedure. In this case, PAHs may be extracted from samples by using other solvents in addition to toluene. After solvent is carefully evaporated, the analytes are made in toluene followed by the determination by GC. In order to

examine the possibility of the latter approach, the following experiments were performed. Standard solutions of 0.40 μg ml^{-1} of each of 16 PAHs were first made in benzene and cyclohexane (25 ml), respectively. Each solution was then gently evaporated to dryness at room temperature by using a stream of nitrogen gas. The residue was then dissolved in toluene and made up to 5.0 ml with toluene. An aliquot of 3 μl of this solution was injected into the capillary GC for the determination of PAHs, and the recoveries of each PAH were calculated by comparing with standard PAHs in toluene without evaporation procedure. The recovery results are shown in Table 13. Through this evaporation process, most PAHs were quantitatively recovered with recoveries of (100 ± 10) %, whereas the four earlier eluting PAHs were not completely recovered. Because of their relatively higher volatility, the four earlier eluting PAHs might be lost during the evaporation process. However, the evaporation process can be used in the determination of higher molecular weight PAHs, which have lower volatility. In the case of determination of higher molecular weight PAHs by GC-MS, toluene proved superior in enhancing sensitivities over the commonly used solvents, for example, benzene.

Table 12. Detection Limits of PAHs in Toluene and P-xylene (under conditions of temperature program two and injection volume 3μl)

Peak#	Component	Detection Limit (3σ) (pg) Solvent Toluene Initial Temp. 120 (°C)	p-xylene 148 (°C)
1	Naphthalene	2.4	4.4
2	Acenaphthylene	10.5	7.5
3	Acenaphthene	8.7	8.0
4	Fluorene	24.6	8.8
5	Phenanthrene	27.0	3.0
6	Anthracene	49.2	3.0
7	Fluoranthene	16.8	2.7
8	Pyrene	13.5	2.8
9	Benz[a]anthracene	34.5	4.4
10	Chrysene	17.4	4.1
11	Benzo[b]fluoranthene	33.3	11.7
12	Benzo[k]fluoranthene	28.2	10.7
13	Benzo[a]pyrene	94.7	17.1
14	Indeno[1,2,3-cd]pyrene	69.0	43.6
15	Dibenz[a,h]anthracene	85.8	43.6
16	Benzo[g,h,i]perylene	62.1	30.8

Table 13. Recoveries of 16 PAHs evaporated from cyclohexane and benzene

Peak #	Recovery (%) by peak area		by peak height	
	Benzene	Cyclohexane	Benzene	Cyclohexane
1	18	17	21	54
2	50	67	18	66
3	57	70	55	72
4	71	80	70	82
5	81	99	81	92
6	86	100	85	99
7	92	105	91	100
8	91	104	85	108
9	102	108	95	104
10	105	110	95	102
11	98	103	95	103
12	100	102	97	102
13	95	102	94	102
14	95	97	95	100
15	96	97	96	95
16	98	95	97	94

II. Study of Effect of Solvent and Stationary Phase on the Chromatographic Behaviour of PAHs

The preliminary observations discussed in the previous section showed that peak shapes of PAHs on capillary GC/MS were influenced by the initial temperature used (292). A number of early-eluting peaks of 16 priority PAHs in toluene were fronting when the initial temperature was lower than 110 °C. When the initial temperature was higher than 130 °C, peak tailing occurred. To give more detailed information, the effect of initial temperature on peak shape was further investigated and proved to be more severe when initial temperature is very low. Since the gas chromatographic separation of analytes is dependent on the physical interactons among the analytes, gas phase, and stationary phase, it is necessary to study the gas chromatographic behaviour of PAHs influenced by the properties of solvent used for injection and by the stationary phase. In order to obtain the best separation and sensitivity, studies of inter-relations among various factors such as initial temperature, injection volume, solvent and stationary phase were also felt to be important to be performed. Since the previous results suggested that better sensitivities of PAHs were obtained with high boiling point solvent, a wide range of solvents with boiling points from 40 °C of dichloromethane to 215 °C of dodecane were studied.

Effect of initial temperature of what on peak shape

A series of experiments were performed with the initial temperature varied from 88 °C to 258 °C. GC/MS peak profiles were obtained with the crosslinked methyl silicone column, when a 2 μl solution of 2 μg ml^{-1} of each PAH in p-xylene was injected at an initial temperature of (a) 88 °C, (b) 128 °C, and (c) 138 °C, respectively. The representative peaks 5 to 8 were chosen to demonstrate the effect of initial temperature on peak shape. As we can see from Figure 8, peak shape varies significantly with the initial column temperature. When the initial temperature is 138 °C, a chromatogram with symmetrical GC peaks is obtained as shown in Figure 8 (c). With the decrease of the initial temperature, however, the effect of fronting and splitting of PAHs peaks became greater. Fronting of the GC peaks appeared at an initial column temperature of 128 °C (Fig. 8b) and each peak was split into two when the initial temperature was 88 °C (Fig. 8a). Similar effects were observed when PAHs were made in other aromatic solvents, such as benzene and toluene. This splitting phenomenon was mainly observed for the first 10 peaks of the 16 PAHs.

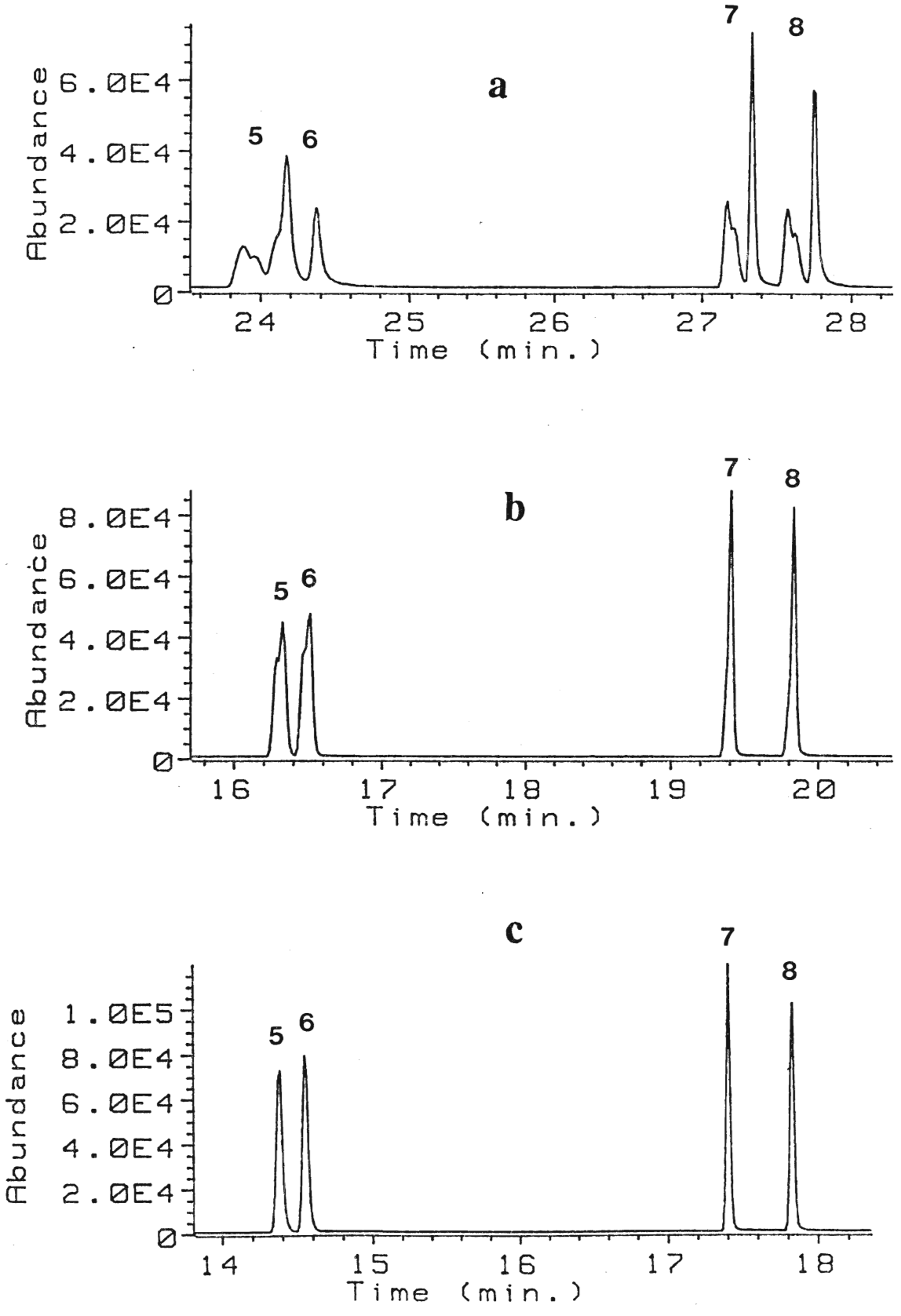


Figure 8. GC peak profiles of PAHs (2µl of 2 µg ml^{-1}) in p-xylene.
Column initial temperature: (a)-88 °C, (b)-128 °C, and (c)-138 °C.
Peak: 5- phenanthrene, 6- anthracene, 7- fluoranthene, and 8- pyrene.

In order further to study the dependence of peak profiles on initial temperature, peak shapes of each of 16 PAHs in different solvents were recorded at different initial temperatures, while other parameters were kept constant. The relation between peak shape of each PAH and initial temperature is illustrated in Figures 9-12. Figures 9, 10, 11, and 12 were obtained when toluene, p-xylene, isooctane and nonane, respectively, were used as solvents. The boiling points on the X axis correspond to each PAH determined. Peaks 13, 15 and 16 are not presented in these figures, because the latter three behaved similarly to peak 14 and their peak shapes were not significantly changed with the change of initial temperature. The upper curve in Figures 9-12 shows the maximum initial temperatures, above which peaks start tailing. The lower curve shows the minimum initial temperature, below which fronting of peaks is observed. These two curves divide the space into three regions. Within the bottom region, under the minimum initial temperature curve, GC peaks began to exhibit fronting and the fronting or splitting of peaks worsens as the temperature decreases. Similarly tailing peaks were observed in the top region above the maximum initial temperature curve. In the middle region, between the two curves, symmetrical peaks were obtained, indicating the optimum initial temperature with regard to peak shape. As shown in Figures 9-12, the optimum initial temperature varies with different PAHs. This optimum range generally increases with the increase of boiling points of PAHs. Thus the early eluting PAH peaks have narrow critical ranges, whereas the late eluting PAHs give much wider optimum range.

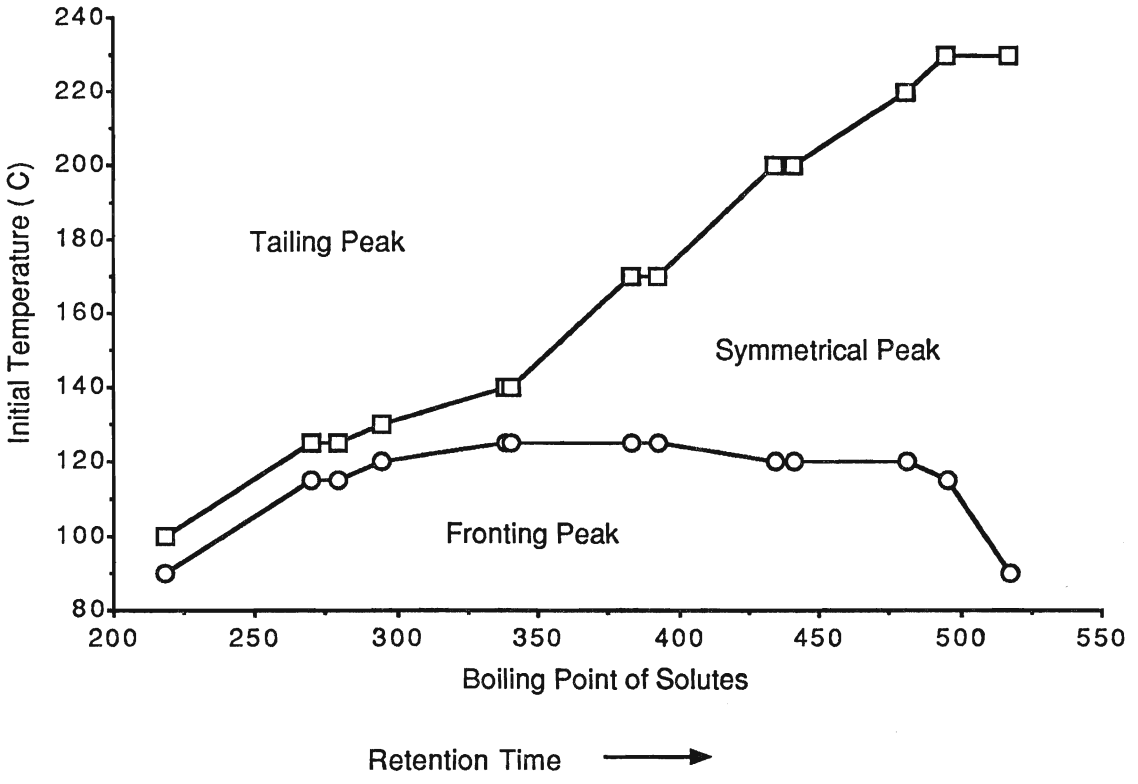


Figure 9. Effect of Initial Temperature on Peak Shape of 16 PAHs (2 µl of 2 µg ml^{-1}) in Toluene

O Initial Temperature of Low Limit □ Initial Temperature of High Limit

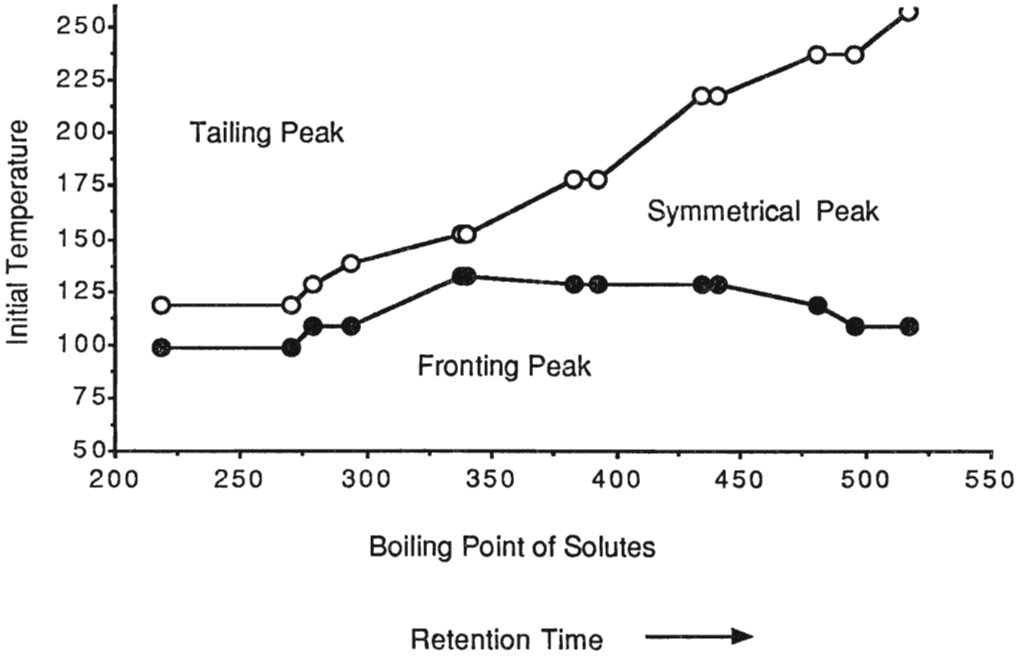


Figure 10. Effect of Initial Temperature on Peak Shape of 16 PAHs (2 µl of 2 µg ml^{-1}) in p-xylene

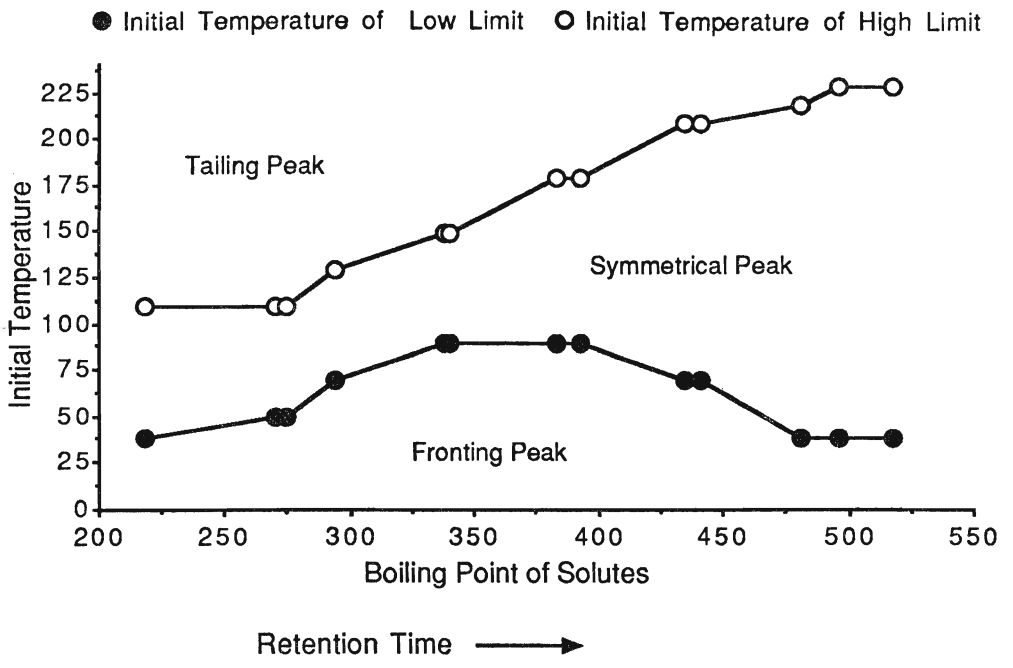


Figure 11. Effect of Initial Temperature on Peak Shape of 16 PAHs (2 µl of 2 µg ml^{-1}) in Isooctane

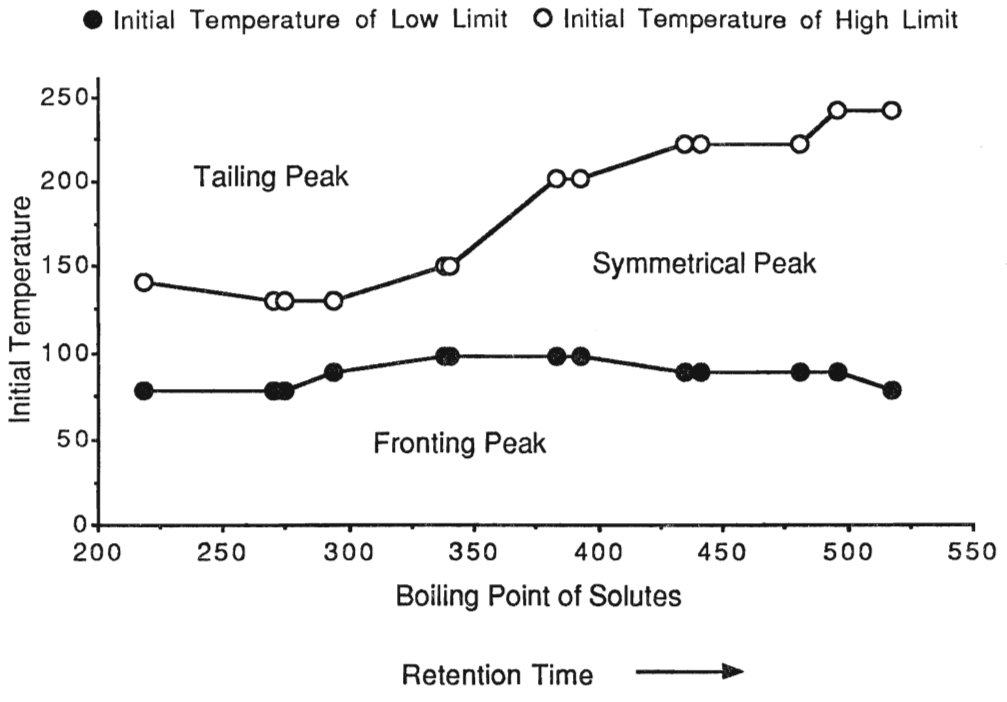


Figure 12. Effect of Initial Temperature on Peak Shape of 16 PAHs in Nonane

The optimum initial temperature range also depends on the solvent used for the injection of PAHs. Comparing Figures 9, 10, 11, and 12, we can see that the ranges of initial temperature for symmetrical peaks are larger in alkane solvents such as isooctane and nonane than that in aromatic solvents like toluene and p-xylene. The optimum ranges of initial temperature for symmetrical peaks 2 and 3, for example, are approximately 110 to 120 °C in toluene, 108 to 138 °C in p-xylene, 49 to 109 °C in isooctane, and 81 to 141 °C in nonane.

From the above results, it is possible to provide a description which explains the effect of initial temperature on peak shape. At the first stage of separation on the column, solutes and solvent are carried from the injector into the column inlet and condensed on the stationary phase, when the initial temperature is low enough. The condensed solutes and solvent are carried along the column by the carrier gas. Thus a process resembling liquid chromatographic separation occurs during this initial low temperature period. This results in the separation of the solute into two portions, one being in the front with solvent and another being retained on the stationary phase. As the column temperature increases, the solvent evaporates and gas chromatogaphic separation gives peaks of PAHs from both portions, resulting in two peaks for each early-eluting PAH.

If the initial temperature used is up to or 10 °C higher than the boiling point of the solvent used, there may be no major condensation of solvent on the column, liquid-chromatographic-like separation between condensed solvent and PAHs does not occur. Solutes remain in one portion only. Therefore, no peak split can be observed and symmetrical peaks are formed at an initial temperature of 10 °C above the boiling point of the

solvent used. Although the classic solvent effect (133) may not occur under this condition, cold trapping (123, 142) plays an important role in condensing the solutes and giving symmetric chromatographic peaks. Generally, a solute can be cold trapped under a temperature of more than 150 °C below its boiling point (142). Thus narrow and symmetrical peaks of PAHs are obtained as a result of the cold trapping effect, since the optimum initial column temperatures shown in Figures 9 to 12 are well below 150 °C lower than the boiling points of PAHs except the first one, naphthalene (bp. 218 °C). If the initial temperature is too high, solvent has shifted away quickly, and PAHs are relatively strongly adsorbed on the stationary phase. This adsorption results in the tailing of peaks at high initial column temperature.

Different effects of initial temperature on the peak shape from different kinds of solvents are probably derived from the properties of solubility of PAHs in the solvents and the wettability of the solvents on the surface of capillary column (i.e. the affinity between the solvent and the stationary phase of the column). When aromatic solvents such as toluene and xylene are used at a low initial temperature, the condensed solvent tends to spread over the column inlet due to their poor wettability on the methyl silicone column (i.e. low affinity between the aromatic solvent and methyl silicone stationary phase). As a result of this process, while a part of PAHs is retained on the column inlet, the condensed aromatic solvent easily retains a portion of PAHs to migrate along the column since PAHs are soluble in these solvents. Therefore, the split peaks are readily observed.

Even at initial temperatures 10 °C below the boiling points of the aromatic solvents, the fronting or splitting of PAH peaks were obtained,

as shown in Figures 9 and 10. When alkane solvents, such as isooctane and nonane, were used for the injection of PAHs, however, peak shape of PAHs changed much less significantly with the variation of initial temperature. At low initial temperature, for example, 30 °C under the boiling point of the solvent used, the solvent and PAHs were also condensed. But unlike the previous situation where aromatic solvents were used, the migration of solvent layer should be slower than that of aromatic solvent because alkane solvents are more strongly adsorbed by the methyl stationary phase due to their higher affinity (or better wettability). Therefore, even if a portion of solutes migrates with the alkane solvent, the speed of this process is controlled by the migration of alkane solvent on the column stationary phase, which is low. After components pass through the entire column length, the small separation during the column inlet may be eliminated. In addition, due to the low distribution coefficients of PAHs in alkane solvents, the amount of PAHs carried away by the alkane solvent is also very small. Instead, PAHs are more likely condensed by the cold trapping effect to stay in one portion on the stationary phase at this low temperature. Thus, even when the initial temperature was 60 °C below the boiling point of isooctane and nonane, no peak split, but only the fronting was observed.

This explanation can be further demonstrated when benzene and cyclohexane were used as solvents, which have similar boiling points but different affinities to PAHs and to the methyl silicone stationary phase. At an initial temperature of 50 °C, which are 30 °C lower than the boiling points of these two solvents, the different chromatographic behaviours of the 16 PAHs in the two solvents were observed and are shown in Figure 13, with representative peaks 2 to 8. When benzene was used as solvent,

split peaks were observed as illustrated in Figure 13 (a). Symmetrical peaks of PAHs were obtained when cyclohexane is used as solvent [Figure 13 (b)]. Since these solvents have similar boiling points, the effect of their volatilities is negligible. Therefore, the different chromatographic behaviours of PAHs in these solvents may be caused by two factors, the affinity between the solvent and the stationary phase (methyl silicone) and the destribution coefficient of PAHs in the solvent. The affinity between benzene and methyl silicone stationary phase is lower than that between cyclohexane and methyl stationary phase. Therefore, benzene carrying PAHs moves faster than cyclohexane on the stationary phase of the column inlet. More PAHs may also be carried out by benzene than by cyclohexane, because of much higher solubility of PAHs with benzene but not with cyclohexane. Thus, the split phenomenon is observed from benzene, not from cyclohexane.

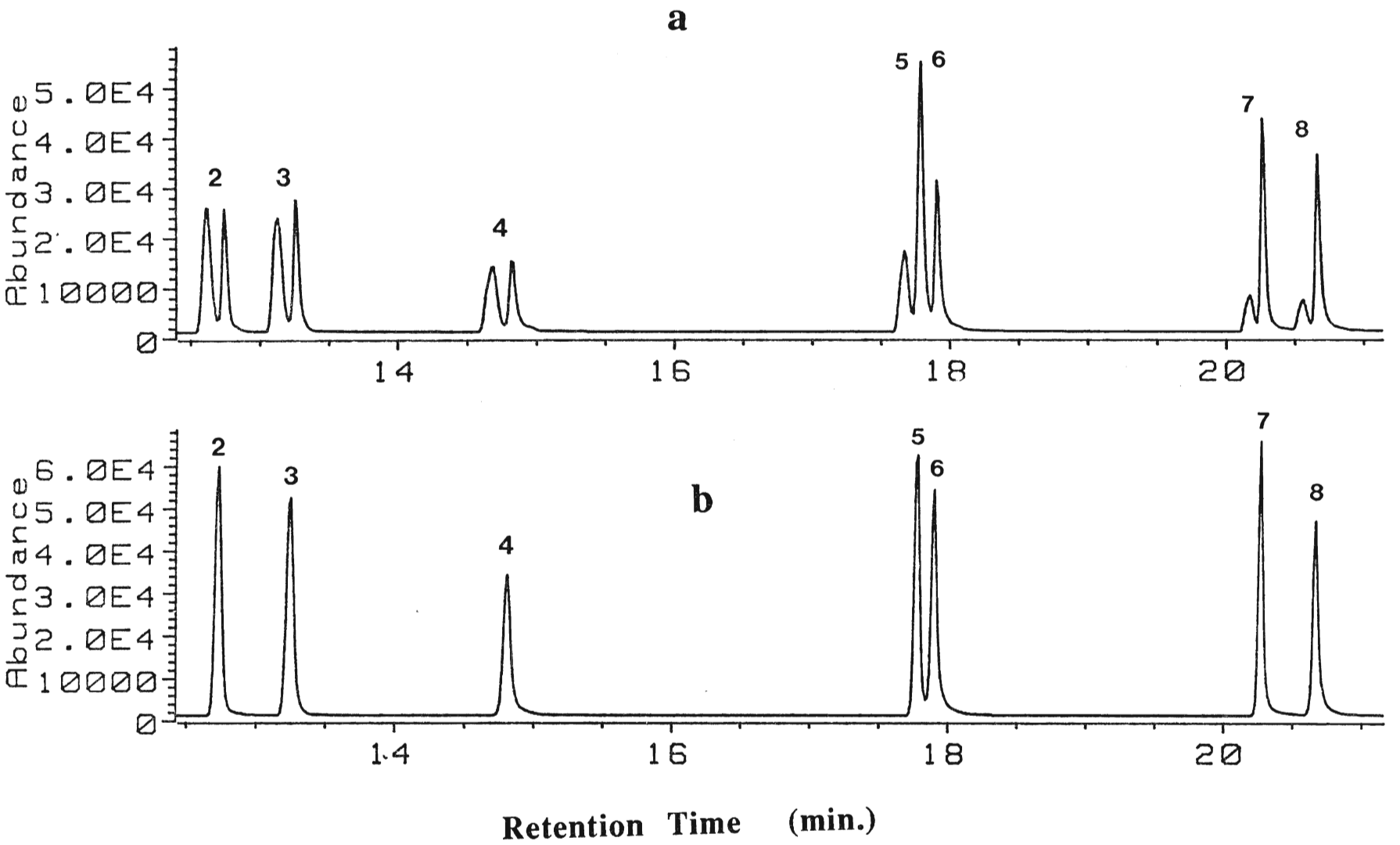


Figure 13. GC peak profiles of seven PAHs (peaks 2-8) in (a) - benzene; and (b) - cyclohexane (Initial column temperature: 50 °C)

Effect of stationary phase on the peak shape

In order further to investigate the effect of the affinity between solvent and stationary phase as well as the effect of initial column temperature on peak shape, both cross-linked methyl silicone column and 5% diphenyl: 94% dimethyl: 1% vinyl polysiloxane column were used to separate 2 μl of 2 μg ml^{-1} PAHs in p-xylene and in nonane. When p-xylene was used as solvent, the initial column temperature was varied from 58 to 258 °C. The ranges of initial temperatures for symmetrical peaks of each PAHs were found to be significantly different on these two columns with different stationary phases. Table 14 summarizes the characteristic temperatures of the 16 PAHs in p-xylene, separated on the two columns. T_1 is the minimum of initial temperature below which the fronting of PAH peak occurs. T_2 is the maximum of initial temperature above which the tailing of peak appears. ΔT (T_2 - T_1) is the difference between the tailing temperature (T_2) and the fronting temperature (T_1). It shows how wide the range of initial column temperature for obtaining the symmetrical peak of each PAHs is. As shown in Table 14, the lower limits of initial temperatures (T_1) are much lower on the 5% diphenyl: 94% dimethyl: 1% vinyl polysiloxane column than on the methyl silicone column. The upper limits (T_2), however, appear very little different, probably within the experimental error, between the two columns used. Thus the ranges of initial temperature (ΔT) for symmetrical peaks are increased when 5% diphenyl: 94% dimethyl: 1% vinyl polysiloxane bonded phase is used instead of crosslinked methyl silicone phase. The increase of symmetrical peak range may be because the introduction of 5% diphenyl in the stationary phase greatly improves the wettability of p-

xylene on the stationary phase (i.e. higher affinity between p-xylene and 5% diphenyl on the stationary phase). Thus, the spread of PAHs in condensed p-xylene on the column inlet is forced to slow down. As a result, no split peaks are observed within a wider initial temperature range. This is consistent with the observation that alkane solvents provide wide ranges of initial temperatures for symmetrical peaks, when methyl silicone column is used. The results in Table 14 also describe that the increase of ΔT as stated above is greater for the early-eluting eight peaks than for the late-eluting peaks, when the methyl silicone column is replaced by 5% diphenyl: 94% dimethyl: 1% vinyl polysiloxane column. This is because solvent remains in contact with the early-eluting components for a longer time as it gradually moves away from the solutes.

When nonane is used as solvent, the comparison of optimum temperatures on the two columns are shown in Table 15. The minimum initial temperatures (T_1) are lower on 5% diphenyl: 94% dimethyl: 1% vinyl polysiloxane stationary phase than on methyl stationary phase. The high limits (T_2) of the first ten peaks are also decreased on 5% diphenyl: 94% dimethyl: 1% vinyl polysiloxane stationary phase. Hence, the ranges of initial temperatures (ΔT) for symmetrical peaks of PAHs in nonane are not significantly changed when the stationary phase is changed from methyl silicone to 5% diphenyl: 94% dimethyl: 1% vinyl polysiloxane. This is because the reduction of 6% methyl group on the entire stationary phase is negligible compared with the remaining 94% methyl group. The affinity between 5% diphenyl: 94% dimethyl: 1% vinyl polysiloxane stationary phase and nonane does not significantly differ from that between methyl silicone stationary phase and nonane.

Table 14. Effect of stationary phase on peak shape of 16 PAHs (2 µl of 2 µg ml^{-1}) in p-xylene

stationary phase	crosslinked methyl silicone			5% diphenyl: 94% dimethyl: 1% vinyl polysiloxane		
peak#	T_1	T_2	ΔT (T_2-T_1)	T_1	T_2	ΔT (T_2-T_1)
1	98	118	20	58*	138	80
2	98	118	20	68	128	60
3	108	128	20	68	128	60
4	108	138	30	68	138	70
5	138	158	20	98	148	50
6	138	158	20	98	148	50
7	128	178	50	98	188	90
8	128	178	50	98	188	90
9	128	218	90	108	198	90
10	128	218	90	108	198	90
11	118	238	120	78	228	150
12	118	238	120	78	228	150
13	108	238	130	78	228	150
14	108	258	150	78	258	180
15	98	258	160	78	258	180
16	98	258	160	78	258	180

* At which the peak is still not fronting.

T_1- below which the peak starts fronting.

T_2- above which the peak starts tailing.

Table 15. Effect of stationary phase on peak shape of 16 PAHs (2 µl of 2 µg ml^{-1}) in nonane

stationary phase	crosslinked methyl silicone			5% diphenyl :94% dimethyl : 1% vinyl polysiloxane		
peak#	T_1	T_2	ΔT (T_2-T_1)	T_1	T_2	ΔT (T_2-T_1)
1	81	151	70	51*	131	80
2	81	141	60	61	121	60
3	81	141	60	61	121	60
4	91	141	50	71	121	50
5	101	151	50	91	141	50
6	101	151	50	91	141	50
7	101	201	100	81	181	100
8	101	201	100	81	181	100
9	91	221	130	121	201	80
10	91	221	130	121	201	80
11	91	221	130	91	221	130
12	91	221	130	91	221	130
13	91	231	140	91	231	140
14	81*	251	170	51*	251	200
15	81*	251	170	51*	251	200
16	81*	251	170	51*	251	200

* at which the peak is not fronting.

T_1- below which the peak starts fronting.

T_2- above which the peak starts tailing.

With the introduction of diphenyl into methyl silicone stationary phase, it not only provides wider optimum initial temperature range, but also improves the separation efficiency. This is indicated by comparing the chromatograms of PAHs in p-xylene, obtained on both methyl silicone and 5% diphenyl: 94% dimethyl: 1% vinyl polysiloxane columns at an initial column temperature of 138 °C. The peaks 9-15 are shown in Figure 14 because the separations between peaks 9 and 10, 11 and 12, and 14 and 15 have proved to be the most difficult pairs among the 16 PAHs. As shown in Figure 14, the resolutions of peaks 9 and 10, 11 and 12 are only about 1.0 and 0.7, respectively, on the column with methyl silicone stationary phase. However, when 5% diphenyl: 94% dimethyl: 1% vinyl polysiloxane column is used without change of any other parameters, the completed separation of peaks 9 and 10, and 14 and 15 are obtained. The resolution of peaks 11 and 12 is improved from 0.7 to 1.0. This improvement in the separation efficiency was also achieved with 5% diphenyl: 94% dimethyl: 1% vinyl polysiloxane column when nonane was used as solvent and an optimum initial temperature was applied. Therefore, 5% diphenyl: 94% dimethyl: 1% vinyl polysiloxane bonded stationary phase column is preferred in the separation of the 16 PAHs.

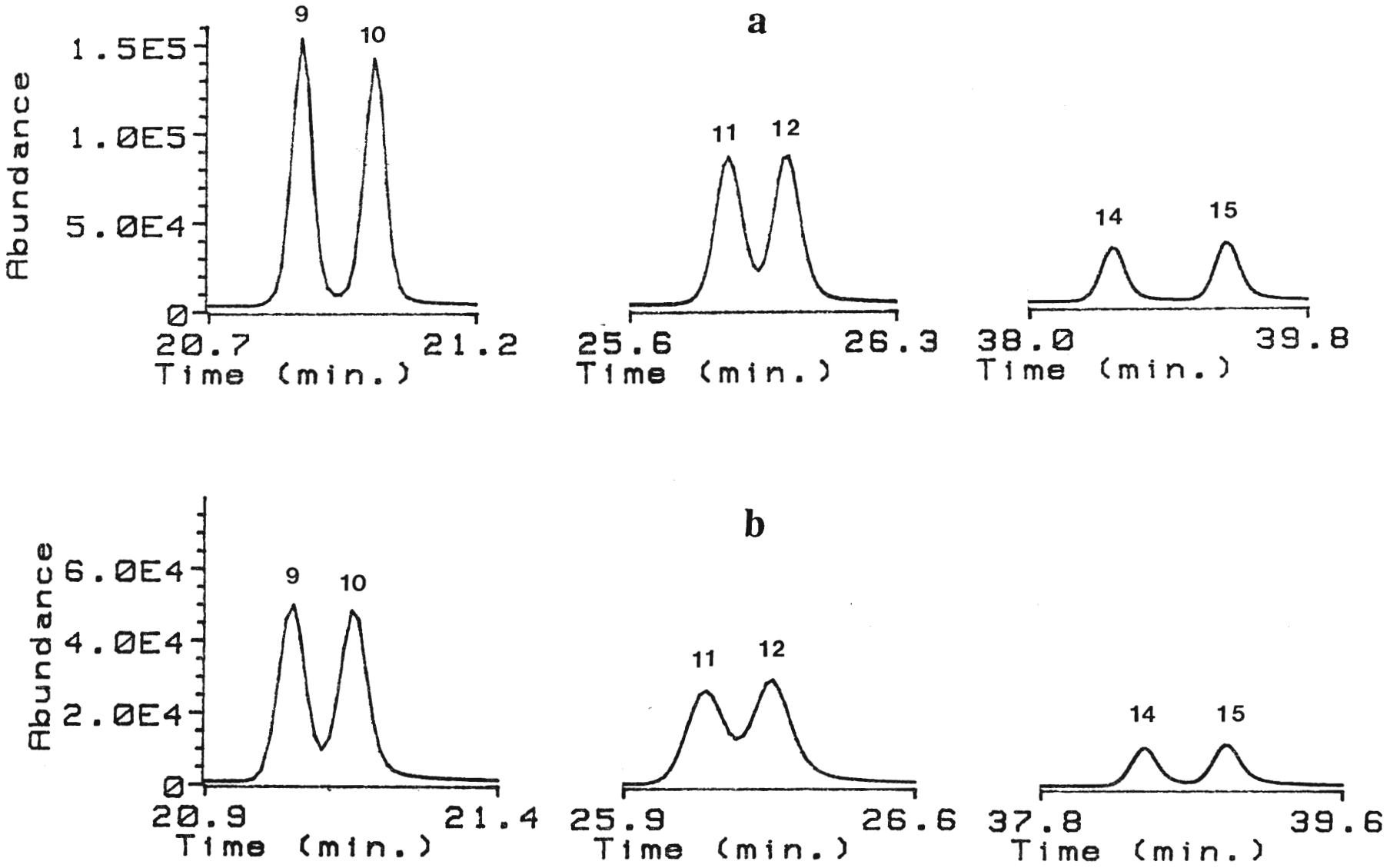


Figure 14. Comparison of separation efficiency of some PAHs (peaks 9-15) in p-xylene on (a) 5% diphenyl: 94% dimethyl: 1% vinyl polysiloxane column; and (b) methyl silicone column.

Effect of Injection Volume

It has been suggested that the splitting of GC peaks with on-column injection can be avoided by reducing the injection volume (290). Therefore, the effect of injection volume was studied in an attempt to eliminate peak distortion in the determination of PAHs by GC/MS. When the initial temperature was 88 °C, chromatograms of PAHs in p-xylene were obtained from the methyl silicone column, while varying the injection volume from 1 to 3 μl. Peak shape of each chromatogram was then examined. Results showed that with this improper initial temperature (88 °C), peak shapes of the 16 PAHs were not improved when the injection volume was reduced from 3 μl to 1 μl. Split peaks were obtained with injection of 1-3 μl sample. On the other hand, if the initial temperature is increased to 138 °C, which is in the optimum range, symmetric peaks were consistently obtained as the injection volume of PAHs in p-xylene was varied from 1 to 3 μl.

Further study on the effect of injection volume on the peak shape of PAHs was carried out with 5% diphenyl: 94% dimethyl: 1% vinyl polysiloxane column. Aliquots of 1 μl and 2 μl of a solution containing 2 μg ml^{-1} of 16 PAHs in p-xylene were injected at the initial temperatures ranging from 58 to 258 °C. The shape of each peak in the chromatograms was examined and the relation between initial temperature and peak shape was obtained. As shown in Table 16, when the injection volume was 1 μl, the lower limits (T_1) of initial temperatures are generally lower than those obtained with 2 μl, except for the first four peaks where they give similar results between 1 and 2 μl injection. There is no change of upper limits (T_2) observed between 1 and 2 μl injection volume. When the

column temperature is below 108 °C, i.e. 30 °C lower than the boiling point of p-xylene, p-xylene is expected to recondense on column inlet. The more solvent is recondensed, the more energy is required to re-evaporate the condensed solvent in the short time during which the distortion of band does not occur. Hence, the higher initial temperature is required when 2 µl of solution is injected compared with 1 µl solution. If the recondensation of solvent does not occur, the injection volume does not affect the peak shape significantly. This is why the upper limits of initial temperatures are almost constant with the change of injection volume from 1 to 2 µl. Similar observations are also recorded with nonane as solvent, as the ranges of the initial temperatures for forming symmetric peaks are summarized in Table 17.

Table 16. Effect of injection volume on peak shape of 16 PAHs (2 µl of 2 µg ml^{-1}) in p-xylene

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

injection volume	1 µl			2 µl		
peak#	T_1	T_2	ΔT (T_2-T_1)	T_1	T_2	ΔT (T_2-T_1)
1	58*	128	>70	58*	138	>80
2	68	128	60	68	128	60
3	68	128	60	68	128	60
4	68	138	70	68	138	70
5	78	148	70	98	148	50
6	78	148	70	98	148	50
7	68	188	120	98	188	90
8	68	188	120	98	188	90
9	68	198	130	108	198	90
10	68	198	130	108	198	90
11	68	228	160	78	228	150
12	68	228	160	78	228	150
13	68	228	160	78	228	150
14	58	258	200	78	258	180
15	58	258	200	78	258	180
16	58	258	200	78	258	180

* At which the peak is still not fronting.

T_1- below which the peak starts fronting.

T_2- above which the peak starts tailing.

Table 17. Effect of injection volume on peak shape of 16 PAHs (2 μl of 2 μg ml^{-1})in nonane

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

injection volume	1 μl			2 μl		
peak#	T_1	T_2	ΔT (T_2-T_1)	T_1	T_2	ΔT (T_2-T_1)
1	51*	121	>70	51*	131	>80
2	51*	111	>60	61	121	60
3	51*	111	>60	61	121	60
4	51*	121	>70	71	121	50
5	71	141	70	91	141	50
6	71	141	70	91	141	50
7	61	181	120	81	181	100
8	61	181	120	81	181	100
9	61	201	140	121	201	80
10	61	201	140	121	201	80
11	51*	>221	180	91	221	130
12	51*	>221	180	91	221	130
13	51*	>231	190	91	231	140
14	51*	>251	200	51*	251	>200
15	51*	>251	200	51*	251	>200
16	51*	>251	200	51*	251	>200

* at which the peak is not fronting.
T_1- below which the peak starts fronting.
T_2- above which the peak starts tailing.

With the change of injection volume, the responses of PAHs are found to be changed, as one might expect. Tables 18-20 summarize the responses, measured as both peak height and peak area, of PAHs with respect to injection volumes of 1, 2, and 3 μl. When toluene was used as solvent (Table 18), peak area and peak height generally increase with the

increase of injection volume from 1 μl to 3 μl. When p-xylene (Table 19) and nonane (Table 20) were used, the results (Table 19-20) indicate that the responses are non-linear versus the injection volume in the range of 1 to 3 μl. When injection volume is increased from 1 μl to 2 μl, the peak heights and peak areas of PAHs in p-xylene or nonane increase twice or more than twice. If injection volume is further increased from 2 μl to 3 μl, the changes of responses are not significant. This may be mainly ascribed to the loss of analyte during the sample injection and transfer processes. When a large amount of sample is injected, the evaporation of solutes in the injector may not be complete at the injection port temperature used (260 °C). Thus, the transfer of sample from the injector to the column may be inefficient. Also, the volume of sample vapour in the injector may be larger than the volume of the injector. This leads the loss of sample vapour through septum purge system. In addition, when injection volume is changed, the conditions for giving the optimum response may also be changed accordingly. For example, conditions for obtaining results in Table 19 are optimized for 1-2 μl of PAHs in p-xylene. These conditions may not be appropriate with an injection volume of 3 μl. These results suggest that the calibration of sample with standards must be performed with the same injection volume. Internal standard calibration can be used to avoid the above problem.

Table 18. Effect of injection volume on sensitivity of 16 PAHs in toluene

(Crosslinked methyl silicone column; temperature program two with an initial temperature 120 °C and an initial time of 8 min.)

Injection volume	1	2	3	1	2	3
Peak #	relative peak area			relative peak height		
1	1.0	2.1	2.9	1.0	2.2	3.0
2	1.0	2.1	2.9	1.0	2.1	2.8
3	1.0	2.1	2.9	1.0	1.9	2.6
4	1.0	2.2	3.0	1.0	2.0	2.8
5	1.0	2.2	3.1	1.0	2.1	3.0
6	1.0	2.3	3.2	1.0	2.2	3.2
7	1.0	2.2	3.3	1.0	2.2	3.2
8	1.0	2.2	3.3	1.0	2.3	3.5
9	1.0	2.5	4.0	1.0	2.4	4.0
10	1.0	2.3	3.6	1.0	2.5	4.1
11	1.0	2.7	4.6	1.0	2.7	4.5
12	1.0	2.4	4.0	1.0	2.8	4.7
13	1.0	2.9	5.2	1.0	3.2	5.9
14	1.0	2.9	6.1	1.0	3.1	6.5
15	1.0	3.1	6.6	1.0	3.2	7.2
16	1.0	2.7	5.4	1.0	2.9	5.9

Table 19. Effect of injection volume on sensitivity of 16 PAHs in p-xylene

(Crosslinked methyl silicone column; temperature program two with an initial temperature of 148 °C and an initial time 2 min.)

Injection volume	1	2	3	1	2	3
Peak #	relative peak area			relative peak height		
1	1.0	2.0	2.5	1.0	2.6	3.4
2	1.0	1.9	2.4	1.0	2.4	3.1
3	1.0	2.0	2.5	1.0	2.5	2.8
4	1.0	2.0	2.3	1.0	2.1	2.8
5	1.0	2.0	2.3	1.0	2.1	2.2
6	1.0	2.0	2.3	1.0	2.1	2.3
7	1.0	2.0	2.3	1.0	1.8	2.1
8	1.0	2.0	2.3	1.0	2.0	2.0
9	1.0	2.2	2.4	1.0	2.1	2.4
10	1.0	2.1	2.2	1.0	2.2	2.3
11	1.0	2.3	2.5	1.0	2.3	2.2
12	1.0	2.2	2.1	1.0	2.4	2.3
13	1.0	2.4	2.4	1.0	2.5	2.6
14	1.0	2.6	2.6	1.0	2.6	2.6
15	1.0	2.5	2.4	1.0	2.7	2.6
16	1.0	2.5	2.4	1.0	2.4	2.3

Table 20. Effect of injection volume on sensitivity of 16 PAHs in nonane

(Crosslinked methyl silicone column; temperature program: 121 °C, hold for 4 min. 8 °C/min. → 280 °C, 3 mim. 4 °C/min. → 300 °C. Transferline temp. 280 °C, injector temp. 260 °C.)

Injection volume	1	2	3	1	2	3
Peak #	relative peak area			relative peak height		
1	1.0	1.5	1.7	1.0	1.7	1.8
2	1.0	1.7	2.0	1.0	1.8	2.1
3	1.0	1.7	2.0	1.0	1.8	2.0
4	1.0	1.7	2.1	1.0	1.7	2.1
5	1.0	1.8	2.2	1.0	1.7	2.0
6	1.0	1.8	2.2	1.0	1.6	1.9
7	1.0	1.8	2.3	1.0	1.5	1.9
8	1.0	1.9	2.3	1.0	1.5	1.8
9	1.0	2.0	2.6	1.0	1.6	2.2
10	1.0	1.8	2.3	1.0	1.6	2.0
11	1.0	2.1	2.9	1.0	1.9	2.6
12	1.0	1.9	2.5	1.0	1.8	2.5
13	1.0	2.1	2.9	1.0	2.0	2.8
14	1.0	2.2	3.4	1.0	2.0	2.8
15	1.0	2.2	3.3	1.0	2.3	3.6
16	1.0	2.1	3.2	1.0	2.1	3.2

Inter-relation among initial temperature, peak shape and sensitivity

Since the significant change of peak shape with the variation of initial temperature was observed, the relation between the sensitivity and peak shape was investigated. When 2 μl of 2 μg ml^{-1} of each PAH in isooctane was injected under the initial temperature ranging from 59 to 129 °C, the various peak shapes were recorded and the chromatograms were integrated to measure peak area and peak height. For the convenience of comparison, peak area and peak height were normalized based on those obtained at the initial temperature of 99 °C (i. e. the boiling point of isooctane). The relative peak area and height of the 16 PAHs are summarized in Table 21. The results of the last four peaks might not be accurate owing to their low intensities. As we can see from Table 21, the changes in relative area are basically not significant when the initial temperature is in the range of 79 to 119 °C. Although the shapes of peaks 4-10 were distorted at the initial temperature 59 °C, their peak areas increased slightly. This may be because solvent condensation improves the transfer efficiency of sample from the injector to the column. This increase can also be derived from the integration error due to the distortion of peaks. The change of peak height, on the other hand, was different from that of peak area. When the initial temperature is below 79 °C, which is out of the optimum range, the heights of peaks 2 to 6 are decreased. This is the temperature range where the fronting or splitting of peaks occurs, resulting in the decrease of peak height. Similar responses in peak height were obtained at initial temperatures between 79 and 119 °C, where symmetrical peaks were

achieved as shown in Figure 11. There are generally similar peak heights for the late-eluting peaks 9 to 13 even when the initial temperature is in the range of 59 to 119 °C. These results indicate that the changes of peak heights follow the same trend as the variations of peak shapes. Peak heights are reduced when asymmetric peaks are obtained. The further increase of initial temperature influences both peak area and height. For example, when the initial temperature was 129 °C, which was 30 °C above the boiling point of isooctane, both peak area and peak height of the PAHs were reduced. This effect could be due to the volatility of solvent as explained previously. In the previous study, we have also found that when aromatic solvents, such as benzene, toluene, and p-xylene, were used as injection solvent, the reduction of responses of 16 PAHs was observed when the initial temperature was 20 °C above the boiling points of solvents respectively. However, if the solvent has a higher boiling point, such as xylenes, the decrease of response, caused by increasing the initial temperature above the boiling points of the solvents, was not as significant as with the low boiling point solvents.

Table 21. Effect of initial temperature on peak area and height of PAHs (2 μl of 2 μg ml^{-1}) in isooctane

(Crosslinked methyl silicone column)

Initial Temp.(ºC)	59	79	89	99	109	119	129
Peak#			Relative peak area				
1	109	101	102	100	128	89	83
2	112	101	101	100	96	90	83
3	115	103	102	100	96	90	83
4	116	103	101	100	96	90	83
5	123	103	99	100	94	89	82
6	100	99	99	100	94	86	81
7	114	98	98	100	91	87	78
8	113	97	97	100	92	86	79
9	113	100	93	100	80	81	67
10	96	90	88	100	88	90	77
11	112	108	94	100	78	83	65
12	102	99	88	100	81	93	70
13	91	94	96	100	74	89	58
14	115	126	95	100	73	81	47
15	112	147	120	100	87	93	45
16	115	102	92	100	79	93	56
Peak#			Relative peak height				
1	145	132	118	100	97	52	17
2	74	84	95	100	96	83	45
3	78	89	100	100	105	88	51
4	76	84	92	100	108	94	59
5	73	78	87	100	101	97	78
6	69	78	87	100	102	98	78
7	85	80	90	100	101	110	90
8	87	80	94	100	103	104	90
9	98	90	87	100	86	92	76
10	95	90	86	100	91	93	80
11	108	100	89	100	82	86	66
12	104	100	88	100	83	91	69
13	94	94	82	100	76	88	60
14	123	124	95	100	77	88	52
15	124	136	104	100	86	93	51
16	118	112	88	100	81	91	51

When nonane was used as the solvent, both peak area and height were also obtained under the different initial temperatures varying from 111 to 181 °C. The variation of responses with initial temperature is demonstrated in Table 22. Again, the change of peak height is more significant than that of peak area. With an initial temperature between 111 and 161 °C, peak area is not significantly changed. When the initial temperature is increased up to 181 °C, the peak areas of early-eluting peaks are reduced whereas the late-eluting peaks are still not affected. The reduction in peak areas of early-eluting peaks is likely caused by band broadening at high initial temperature. This band broadening may be sufficiently reconcentrated by cold trapping for high boiling point PAHs, resulting in no loss of response. When the initial temperature is lowered from 151 to 111 °C, the peak heights of the first four peaks were significantly increased. The last six peaks are essentially identical. Peaks 5-8 had lower peak heights at 111 °C, probably due to the fronting of these peaks. When the initial temperature was above 161 °C, a dramatic decrease of peak heights of early-eluting peaks was observed. These changes, again, correspond to the change of peak shape, as shown in Figure 12.

A similar phenomenon was observed when p-xylene was used, as shown in Table 23. The results show that the change of peak shape affects peak response. Therefore, the proper initial temperature chosen for GC is extremely important in order to obtain better resolution as well as sensitivity.

Table 22. Effect of initial temperature on peak area and peak height of 16 PAHs (2 µl of 2 µg ml^{-1}) in nonane

(Crosslinked methyl silicone column)

Initial Temp. (ºC)	111	121	131	141	151	161	181
Peak#			Relative peak area				
1	99	95	90	97	100	94	-
2	99	102	95	99	100	90	-
3	99	102	97	98	100	95	77
4	99	100	97	98	100	93	84
5	102	104	99	99	100	92	70
6	101	100	95	96	100	101	105
7	108	105	100	100	100	94	99
8	107	105	99	99	100	93	102
9	125	113	102	99	100	89	100
10	91	101	99	90	100	90	105
11	124	112	104	101	100	91	99
12	100	106	100	97	100	94	102
13	112	107	107	104	100	93	100
14	113	112	107	103	100	95	103
15	112	108	106	105	100	95	99
16	115	113	109	103	100	98	101
Peak#			Relative peak height				
1	160	136	143	137	100	32	-
2	164	177	163	139	100	63	-
3	162	166	151	131	100	65	27
4	134	138	140	125	100	71	32
5	78	94	102	111	100	82	52
6	79	95	106	109	100	90	58
7	75	86	97	106	100	86	65
8	68	88	86	93	100	84	79
9	86	95	97	99	100	88	94
10	82	93	97	100	100	90	93
11	105	107	103	100	100	90	99
12	96	105	102	101	100	93	98
13	99	106	106	101	100	90	101
14	112	111	107	102	100	94	103
15	112	110	106	102	100	94	105
16	111	110	106	100	100	95	101

Table 23. Effect of initial temperature on peak area and height of PAHs (2 µl of 2 µg ml^{-1}) in p-xylene

(Crosslinked methyl silicone column)

Initial temp. (ºC)	108	128	138	148	158
Peak#		Relative peak area			
1	-	-	100	-	-
2	150.	100	100	101	87
3	151	101	100	106	90
4	140	101	100	106	87
5	split	102	100	109	87
6	split	101	100	108	91
7	110	96	100	108	88
8	110	96	100	109	88
9	116	97	100	107	94
10	76	98	100	104	92
11	77	99	100	111	103
12	76	98	100	104	95
13	80	101	100	110	92
14	71	101	100	105	97
15	68	101	100	102	95
16	73	101	100	102	97
Peak#		Relative peak height			
1	-	-	100	-	-
2	149	101	100	91	56
3	125	94	100	94	59
4	80	67*	100	92	60
5	split	61*	100	103	75
6	split	58*	100	100	74
7	62	73*	100	98	76
8	65	81	100	110	80
9	77	96	100	107	96
10	75	99	100	107	95
11	77	99	100	111	101
12	75	99	100	109	103
13	75	99	100	112	93.8
14	68	103	100	105	98
15	68	101	100	107	102
16	73	101	100	102	99

*Fronting peak. - not detected due to solvent delay time too long.

Precision is another important analytical factor. One might expect a higher standard deviation if peaks are distorted irregularly. When symmetric peaks are achieved, good precision of retention time and peak response are also expected. This is indeed the case in the determination of PAHs by GC/MS. Table 24 lists the relative standard deviations (RSD) of chromatographic retention time and responses, measured by peak height and peak area, of the 16 PAHs in nonane solvent. The cross-linked methyl silicone column was used. At initial temperatures of 121 and 141 °C, both well in the optimum range, reasonably low and comparable relative standard deviations of seven replicate determinations are obtained, as shown in Table 24. Except for the first peak, very low RSD values of retention time are obtained. The relatively higher deviation of the first peak in retention time is likely caused by the solvent close to it. These results are consistent with the fact that the symmetric peaks are obtained under the given conditions.

Table 24. RSDs of peak area, height and retention time of 16 PAHs (2 μl of 2 μg ml $^{-1}$) in nonane

(Crosslinked methyl silicone column)

	RSD (%)					
	peak area		peak height		retention time	
initial temp.(°C)	121	141	121	141	121	141
peak #						
1	5.1	4.1	7.8	6.2	0.4	0.3
2	4.8	5.0	6.2	7.4	0.04	0.06
3	5.2	6.3	6.3	8.1	0.02	0.05
4	5.5	6.1	5.5	6.7	0.04	0.02
5	5.4	5.3	4.7	4.8	0.02	0.01
6	5.4	5.3	3.1	6.2	0.01	0.01
7	6.2	5.5	4.1	5.7	0.01	0.02
8	6.1	5.3	3.2	4.3	0.00	0.02
9	7.2	3.1	5.5	2.7	0.01	0.02
10	5.8	7.7	4.8	3.0	0.01	0.03
11	6.6	2.6	5.9	2.8	0.02	0.03
12	6.4	6.3	5.1	3.6	0.01	0.03
13	5.5	3.9	6.0	3.1	0.02	0.03
14	5.2	3.0	4.8	3.5	0.04	0.04
15	6.2	3.4	5.7	2.6	0.03	0.04
16	4.4	3.8	5.2	3.0	0.04	0.04

Selecting Solvent

The results discussed above indicate that appropriate solvent should be chosen in order to improve the GC performance in the determination of PAHs. The priority criteria for selecting solvent are such that good separation efficiency and sufficient sensitivity are achieved under the optimum instrumental conditions. For good separation among PAH peaks, it is necessary to consider peak shape and to prevent GC peaks from broadening, fronting, tailing or splitting. In order to obtain symmetric peaks of PAHs over a wide range of column temperatures, the solvent with polarity similar to the stationary phase of the column is preferred to prepare the PAH solution for injection. For example, an alkane solvent such as nonane is preferred when methyl silicone column is used. If an aromatic solvent such as p-xylene is used, better chromatographic performance of PAHs is obtained on 5% diphenyl: 94% dimethyl: 1% vinyl polysiloxane stationary phase than on methyl silicone stationary phase.

Sensitivity is another factor to be concerned when good peak shape is achieved. We have found that better responses of 16 PAHs from GC/MS were obtained when injection solvents with relatively higher boiling points were used, such as xylenes and toluene compared with other common solvents such as hexane, isooctane, and benzene. Based on the above facts, we further studied some other solvents with even higher boiling point, such as mesitylene, nonane and dodecane.

For comparison, when the injection solvents were hexane, benzene, isooctane, toluene, p-xylene, o-xylene, mesitylene, nonane, and dodecane under the initial temperatures of 10 °C above the boiling points of these

solvents, the relative peak area and peak height are summarized in Tables 25 and 26. As shown in the Tables, both peak area and peak height of the late-eluting 10 PAHs are approximately 1 to 3.5 times higher in p-xylene, o-xylene, and nonane than in toluene. Responses of PAHs in other solvents with low boiling points are much lower than that in toluene, as reported previously. Although the results show that better responses of the last eight peaks were obtained with dodecane and mesitylene as solvent than with toluene, the first four peaks were eluted with solvent so that no results were obtained. Tables 25 and 26 also show that the better responses of the first eight peaks are obtained with isooctane as solvent. Therefore, it is not necessary to use high boiling point solvent if only the first four peaks are concerned. For the last eight peaks, solvents with relatively high boiling points such as xylenes and nonane give better responses.

Since xylenes and nonane were promising to be the best solvents, five injections of each solution were performed when solvents were p-xylene, o-xylene, nonane, and dodecane. The results were normalized according to the peak height and area of 16 PAHs, when p-xylene was used as solvent. The average of relative peak height and area of five determinations are listed in Table 27. Table 27 suggests that nonane gives 40% to 100% higher sensitivity than p-xylene. o-Xylene had a similar response as p-xylene. When dodecane is used, the responses are not better than those in p-xylene. This indicates that there is a proper range of boiling point of solvent providing better sensitivity. This range is dependent on the boiling points of solutes.

Table 25. Relative peak area of PAHs (2 µl of 2 µg ml^{-1}) in different solvents (Crosslinked methyl silicone)

	temp. program one					temp. program two					
solvent	benzene	CH_3CN	hexane	isooctane	toluene	p-xylene	o-xylene	mesitylene	octane	nonane	dodecane
	initial temperature (^oC)										
peak#	90	90	78	109	120	148	154	165	136	161	225
1	73	51	90	102	100	72	57	-	85	84	-
2	70	50	89	99	100	82	65	-	92	96	-
3	68	49	86	97	100	81	64	-	89	98	-
4	66	47	83	94	100	83	69	137	88	101	-
5	73	51	87	100	100	99	82	133	97	116	-
6	71	47	85	99	100	101	84	155	96	131	171
7	63	45	78	93	100	106	88	146	90	135	77
8	66	45	79	95	100	111	93	160	91	142	80
9	54	33	63	79	100	157	132	201	83	193	66
10	47	29	50	69	100	133	116	178	75	169	108
11	44	30	47	69	100	200	175	212	80	235	84
12	39	25	37	61	100	180	154	207	70	217	117
13	39	27	39	64	100	215	188	399	79	269	112
14	26	23	22	54	100	295	263	319	74	354	129
15	23	19	20	53	100	355	316	382	78	434	151
16	24	25	23	54	100	266	238	270	71	314	119

Table 26. Relative peak height of PAHs (2 µl of 2 µg ml^{-1}) in different solvents (Crosslinked methyl silicone)

	temp. program one					temp. program two					
solvent	benzene	CH_3CN	hexane	isooctane	toluene	p-xylene	o-xylene	mesitylene	octane	nonane	dodecane
	initial temperature (°C)										
peak#	90	90	78	109	120	148	154	165	136	161	225
1	122	102	204	182	100	101	60	-	171	67	-
2	107	89	120	142	100	81	65	-	114	74	-
3	102	86	110	130	100	79	63	-	107	73	-
4	95	74	93	120	100	79	66	187	97	75	-
5	94	66	84	113	100	82	68	96	90	85	-
6	79	55	75	105	100	79	68	103	82	86	32
7	65	47	69	90	100	112	87	142	83	117	18
8	66	47	72	94	100	108	89	115	93	125	23
9	55	34	55	81	100	156	139	307	82	193	45
10	51	32	50	71	100	150	125	187	77	179	54
11	43	29	44	69	100	198	172	329	77	229	72
12	42	27	39	64	100	201	177	211	75	234	85
13	41	28	39	67	100	232	204	341	81	284	82
14	27	23	22	54	100	301	274	406	75	363	114
15	25	19	20	53	100	358	323	350	76	424	130
16	26	24	22	53	100	262	247	228	72	310	107

Table 27. Effect of solvent on PAH responses

(Crosslinked methyl silicone column)

solvent	p-xylene		o-xylene		nonane		dodecane	
initial temp.(oC)	138		144		141		215	
peak#	area	height	area	height	area	height	area	height
1	100	100	99	105	137	200	-	-
2	100	100	105	104	142	171	-	-
3	100	100	100	99	136	157	-	-
4	100	100	100	102	139	166	-	-
5	100	100	100	102	140	148	134	222
6	100	100	103	103	142	152	142	225
7	100	100	102	106	143	137	124	134
8	100	100	103	106	146	129	123	139
9	100	100	106	103	156	142	95	79
10	100	100	105	103	147	140	112	88
11	100	100	109	110	158	156	99	74
12	100	100	114	111	156	158	93	72
13	100	100	118	120	182	187	101	76
14	100	100	119	118	176	174	74	62
15	100	100	114	117	177	172	75	60
16	100	100	123	122	177	174	74	58

According to the peak shape and sensitivity, nonane is one of the best solvents for the determination of the 16 PAHs, when a methyl silicone column is used for the separation. A disadvantage of the use of nonane for the preparation of standard solution of PAHs is that it has low solubility of PAHs. However, nonane can be used as dilution solvent, whatever solvent is used to prepare PAH stock solution. In addition, nonane is not as toxic as aromatic solvents. If 5% diphenyl: 94% dimethyl: 1% vinyl polysiloxane column is used, xylenes are also suitable solvents.

On this column, the xylenes also give wide range of initial temperatures to produce symmetrical peak shape and provide good sensitivity.

III. Determination of PCBs by Capillary Gas Chromatography with Electron Capture Detection and Splitless Injection

As discussed above, we have obtained very different peak responses by using different solvents for the injection. Solvents with higher boiling points, such as xylenes and nonane, significantly improved the sensitivities of PAHs, especially higher boiling point PAHs. Since, in many respects, PCBs have similar physical properties to PAHs, we expect this same effect should apply to the determination of PCBs by capillary gas chromatography. By studying effects of various factors on the chromatographic performance of PCBs on capillary GC/ECD, the results will be valuable to confirm previous observations.

In order to obtain a method for the quantitation of PCBs, optimization was performed by the one factor at a time method. Since ECD is the most sensitive detector for electron-rich components like PCBs, ECD was used in this work. When identification is required, the optimum conditions for GC/ECD can be simply used to GC/MSD. The responses of PCBs from ECD generally increase with the number of chlorines on the biphenyl, whereas high chlorinated PCBs give low sensitivity when MSD is used as a detector.

Effect of solvent on responses of PCBs

A preliminary study was carried out to observe the effect of solvents on the responses of PCBs. A standard containing 10 PCBs, as listed in Table 2 in the "Experimental Section", was used as representative PCBs. Each of these 10 PCBs represents one PCB isomeric group.

Eight solvents with a wide range of boiling points, from 40 °C of dichloromethane to 215 °C of dodecane, as listed in Table 28, were used to study their effect on PCB responses. An aliquot of 1 μl of the 10 PCB mixture in each of eight solvents was independently injected. A chromatogram of 10 PCBs was obtained on the GC/ECD under the preliminary conditions stated in Table 28. Each peak in the chromatogram was integrated to give peak area and peak height. For convenience, the peak area and peak height were normalized based on the results from PCBs in nonane solution. The relative peak area and peak height are summarized in Table 28. Both relative peak area and peak height in Table 28 show that in higher boiling solvents such as p-xylene and nonane, PCBs give higher responses on GC/ECD. Although peak areas of PCBs are comparable when dodecane, nonane and p-xylene are used as solvent, peak heights of 10 PCBs from the dodecane solution are lower than those in nonane and p-xylene. This is probably because, with this high boiling point solvent (215 °C), the cold trapping effect may not be sufficiently achieved, resulting in wide solute bands. Based on these results, p-xylene and nonane were chosen as representative examples of aromatic and alkane solvents, for further investigations into other factors affecting the chromatographic behaviour of PCBs.

Table 28. Responses of PCBs in different solvents

Conditions: crosslinked methyl silicone column; injector temperature 250 °C; detector temp. 250 °C;temp. program: bp. (°C), 1 min. → 8 °C/min. → 250 °, 15min..
purge off 30 sec.; injection volume 1µl; makeup gas 40 psi; carrier gas flow rate about 0.8 ml $min.^{-1}$.

Solvent	DCM	Hexane	Benzene	Isooctane	Toluene	p-Xylene	Nonane	Dodecane
bp.(°C)	40	68	80	99	110	138	151	215
Peak#				Relative Peak Area				
1	151	68	83	71	92	110	100	98
2	71	57	73	61	83	102	100	95
3	80	59	77	64	84	100	100	88
4	80	59	77	64	84	100	100	104
5	72	54	71	60	82	101	100	102
6	77	50	67	58	78	101	100	106
7	51	43	60	52	74	103	100	98
8	36	35	48	45	65	103	100	96
9	33	32	45	42	62	103	100	96
10	34	33	45	42	62	103	100	97
Peak#				Relative Peak Height				
1	132	67	81	65	88	119	100	84
2	64	54	69	59	78	105	100	86
3	54	48	59	54	69	95	100	84
4	58	52	65	59	77	101	100	86
5	57	50	68	59	79	104	100	86
6	58	48	65	58	75	102	100	81
7	44	26	55	50	71	101	100	75
8	32	34	46	43	61	105	100	83
9	31	32	43	42	61	101	100	85
10	33	32	44	43	62	103	100	88

Effect of temperatures on the sensitivity of PCBs

An appropriate temperature of the injector often determines the efficiency of evaporation of injected solutes especially for those solutes, such as PCBs, which have low volatility. Therefore, this temperature affects the efficiency of transfer of solutes from the injector to the GC column and eventually affects the sensitivity. As shown in Figures 15 and 16, when the injector temperature is varied from 220 °C to 280 °C, different values of peak area and peak height are obtained from 1 µl of 10 PCBs in p-xylene with an initial temperature of 128 °C. Within the range of injector temperature from 220 °C to 260 °C, there is no significant change in peak area and peak height of all ten PCB peaks. When the injector temperature is increased from 260 to 280 °C, both peak area and peak height decrease, especially for the late-eluting peaks. This phenomenon was also found in the determination of PAHs. A possible explanation is that sufficient evaporation of the 10 PCBs in p-xylene is obtained during the injector temperature 220 and 260 °C. If the injector temperature is too high, the volume of vapour created from 1 µl 10 PCBs in p-xylene may be larger than that of the injector. Thus, a portion of sample vapour may be lost during the processes of evaporation and transfer. The similar results and the same phenomenon from 1 µl of 10 PCBs in p-xylene solution were also obtained when the initial temperature was decreased from 128 to 118 °C. Also, if p-xylene was replaced by nonane, as a solvent, the optimum range of the injector temperature was found to be the same.

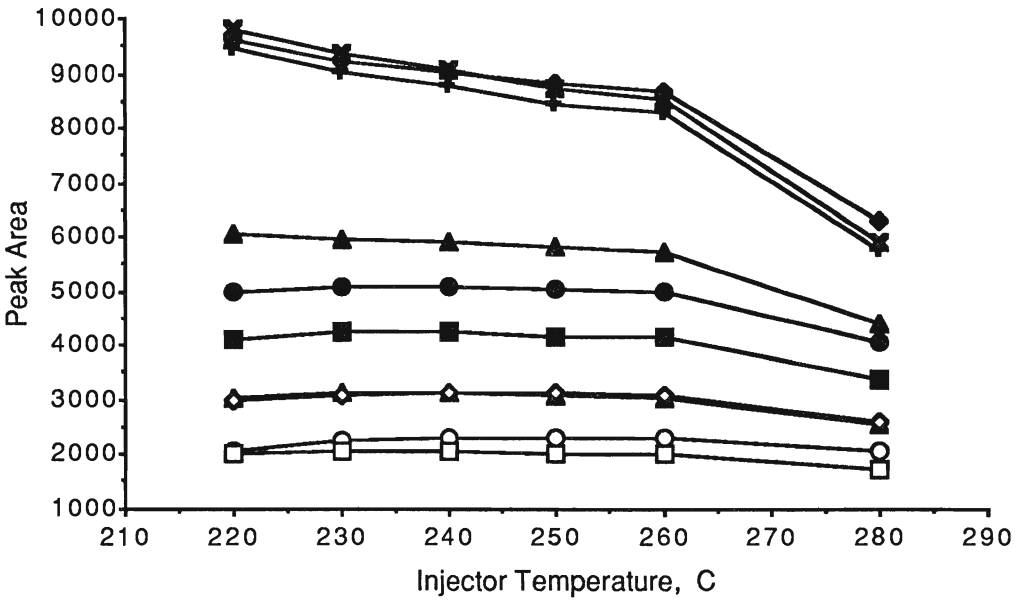


Figure 15. Effect of injector temperature on peak area of 10 PCBs (1 µl) in p-xylene

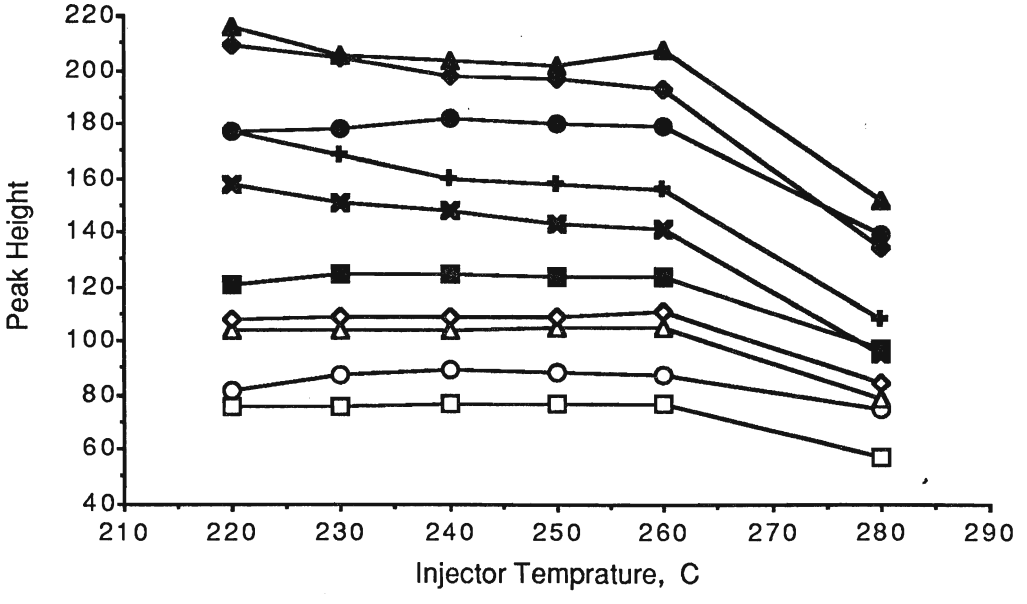


Figure 16. Effect of injector temperature on peak height of 10 PCBs (1 µl) in p-xylene

○ Peak 1 □ Peak 2 △ Peak 3 ◇ Peak 4 ● Peak 5
■ Peak 6 ▲ Peak 7 ◆ Peak 8 ✚ Peak 9 ✖ Peak 10

(Initial temperature: 128 °C) .

After sample vapour is transferred into the column inlet, the temperature of the column, during the initial period of time, significantly affects the chromatographic behaviour of the components in the sample injected. This was clearly demonstrated in the determination of PAHs, discussed in previous sections. Therefore, it was necessary to perform a series of experiments to study the effect of initial temperature on the chromatographic behaviour and responses of PCBs. To start with, peak shape with relation to the initial temperature was studied. Similar to PAHs, the peak shape of 10 PCBs depends upon the initial column temperature, as illustrated in Figure 17. p-Xylene was used as a solvent for the injection of PCBs. As described in the figure, when the initial temperature is between 88 and 98 °C, peaks of PCBs exhibit fronting. If the initial temperature is further reduced to below 88 °C, peaks are split. If the initial temperature is too high, peaks start tailing. The temperature at which tailing starts varies with different PCBs. Peak 1 (i.e. 2-chlorobiphenyl) begins tailing at an initial temperature above 128 °C. When the initial temperature is greater than 148 °C, tailing of peaks 2, 3, and 4, (3,3'- dichlorobiphenyl, 2, 4, 5- trichlorobiphenyl, and 2, 2', 4, 4'- tetrachlorobiphenyl, respectively) occur. The rest of the peaks start tailing at an initial temperature above 188 °C. Between these critical initial temperatures, symmetrical peaks of PCBs are obtained. As we can see from Figure 17, the optimum initial temperature ranges for achieving symmetrical peaks are 88 - 128 °C for peak 1, 98-148 °C for peaks 2-4, and 98-188 °C for the last six PCB peaks.

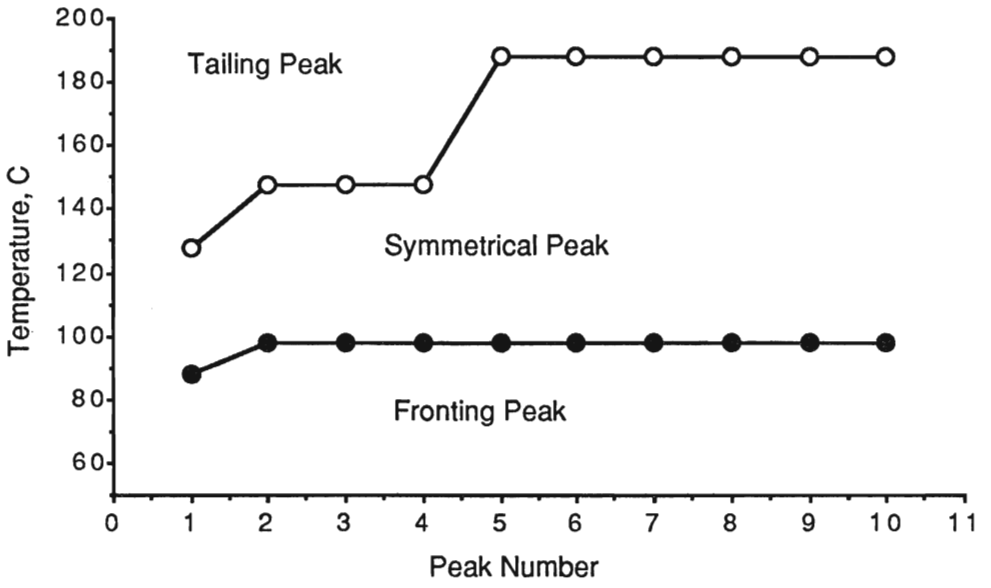


Figure 17. Effect of initial temperature on peak shape of 10 PCBs

Each peak number corresponds to one of the 10 PCBs. The initial temperature is varied from 88 to 188 °C.

Peak area and peak height of PCBs were also investigated with various initial temperatures in the range between 88 and 188 °C. As demonstrated in Figure 18, the areas of peaks 1 to 4 are not significantly changed with the variation of the initial temperature. The areas of the last six peaks gradually decrease as the initial temperature increases from 88 to 158 °C. This decrease is less than 20 percent. The change of peak height, on the other hand, depends more on the peak shape. Figure 19 shows that the highest values of peak height are generally obtained at initial temperatures between 98 and 138 °C. A significant decrease in peak height of the first peak is clearly shown in Figure 19 when the initial temperature is higher than 158 °C. This is likely because, at this initial temperature, the peak shows severe tailing, as shown in Figure 17.

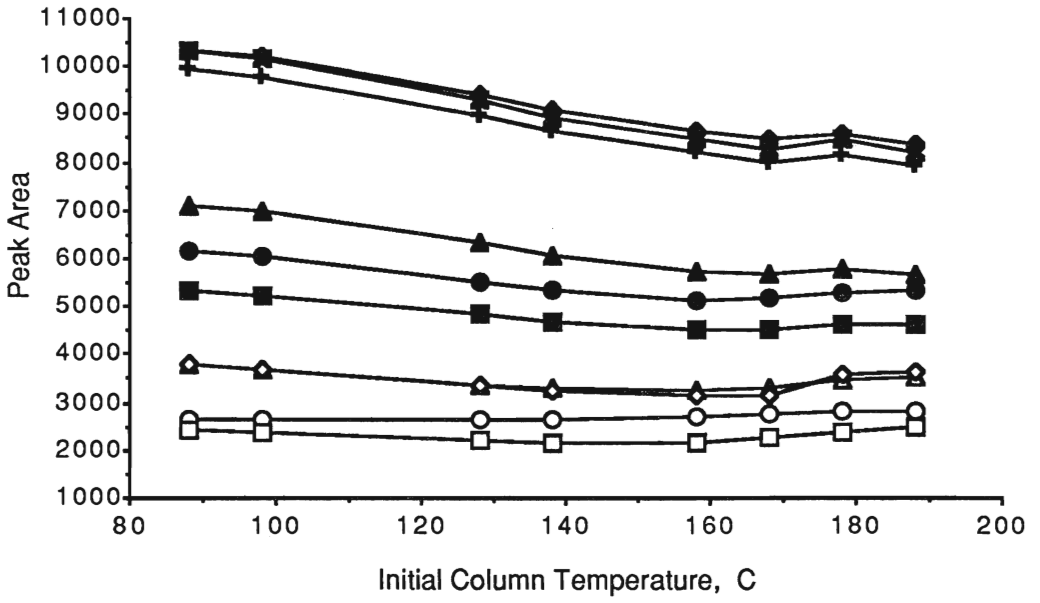


Figure 18. Effect of initial column temperature on peak area of 10 PCBs in p-xylene

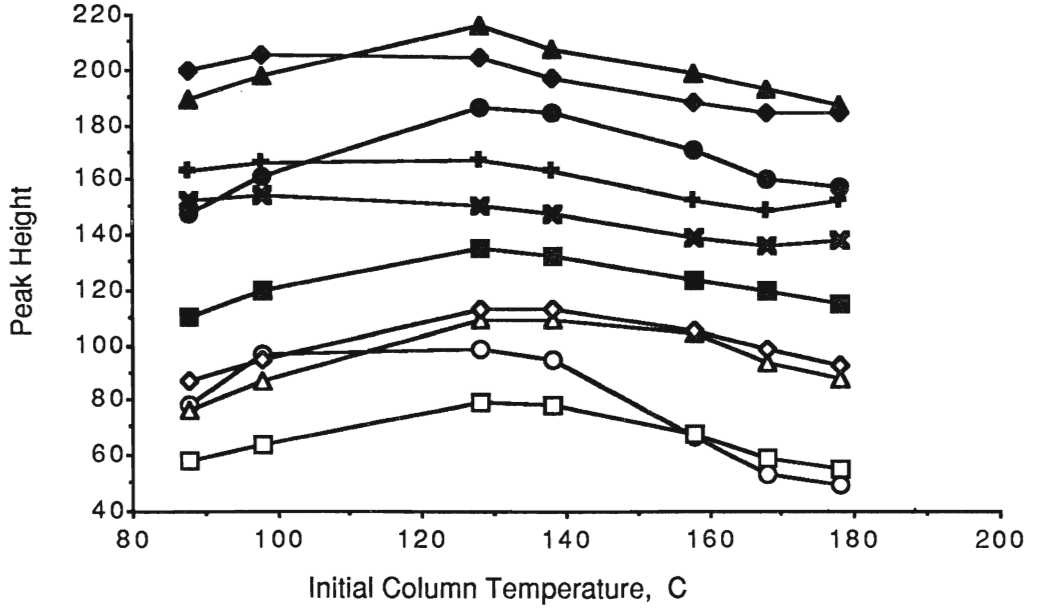


Figure 19. Effect of initial column temperature on peak height of 10 PCBs in p-xylene

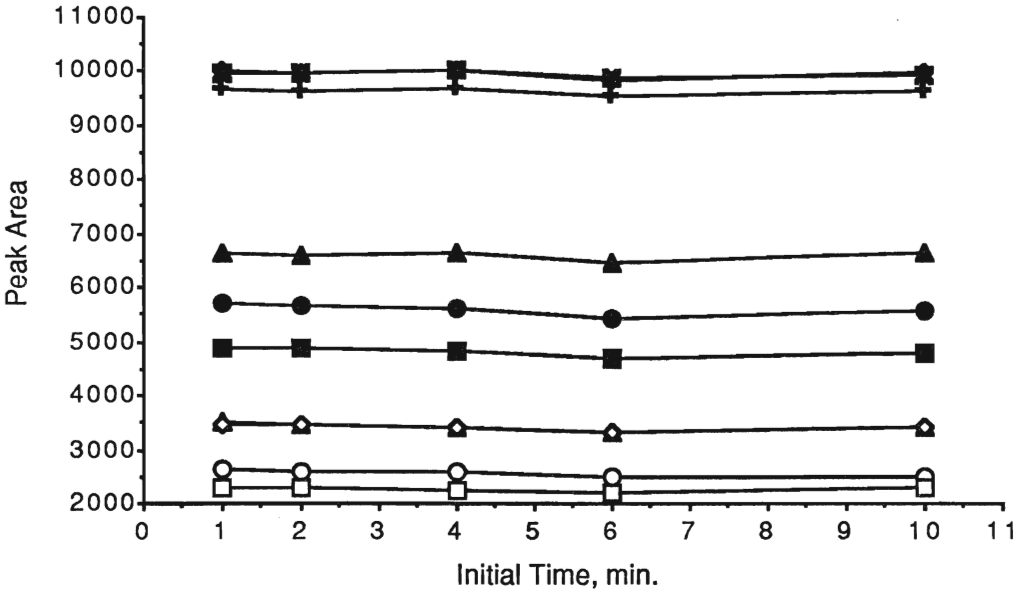


Figure 20. Effect of initial time on peak area of 10 PCBs in p-xylene

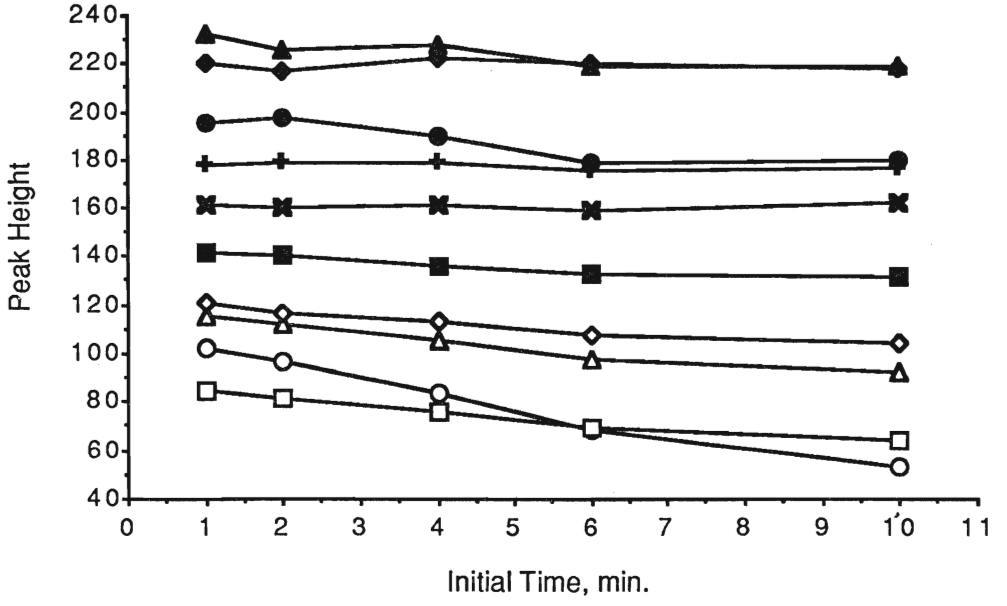


Figure 21. Effect of initial time on peak height of 10 PCBs in p-xylene temperature program: 128 °C → initial time (min.) → 8 °C/min. → 250 °C, held for 15 min.; 1 µl of injection volume.

○ Peak 1 □ Peak 2 △ Peak 3 ◇ Peak 4 ● Peak 5
■ Peak 6 ▲ Peak 7 ◆ Peak 8 ✚ Peak 9 ✖ Peak 10

When 1 μl of PCBs in p-xylene is injected under the temperature program stated in Figure 20, the period of time during which the initial temperature is held was found not to affect responses significantly. As shown in Figure 20, a constant peak area is obtained within a range of the initial time studied from 1 to 10 min. If the response is measured as peak height, its relation to the initial temperature is shown in Figure 21. Relatively higher peaks are obtained with the initial time in the range of 1 to 4 min., while a slight decrease in peak height of 10 PCBs is observed if the initial time is over 4 min. Therefore, an initial temperature of 2 min. was chosen.

After being held at the initial temperature for an initial 2 min., the column is heated to a temperature of 250 °C, to perform the separation. The rate of increase of the column temperature was studied with regard to peak shape and response. This change in temperature rate produced little change in peak shape. At an optimum initial temperature (128 °C) and an optimum initial time (2 min..), symmetrical peaks from 1 μl of 10 PCBs in p-xylene were obtained with the temperature rates of 4, 6, 8, 10, and 12 °C/min.. Also, only slight enhancement of peak area with the increase of temperature rate were observed, as shown in Figure 22. The changes of peak area from ten peaks show the same trends. Peak heights, however, are more subject to the change of the temperature rate. This is demonstrated in Figure 23. The heights of peaks 1 to 6 are significantly increased with the increase of temperature rate. The remaining 4 peaks give similar peak heights within the range of temperature rates of 6 and 12 °C/min. If the temperature is below 6 °C/min., peak heights of ten PCBs are all decreased. For the 10 PCBs studied, they are all well separated from one another. However, for the complex PCB mixtures, such

as Arochlor 1260, complete separation of many PCB congeners may not be easily achieved. As shown in Figure 24, PCBs elute faster with a higher temperature rate, at which the separation of PCB mixtures is not complete. Therefore, high temperature rate is not recommended for the analysis of complex PCB mixtures by GC, even though a higher peak height is obtained. A temperature rate of 8 °C/min is used in the rest of this work which give good separation and reasonably high sensitivity.

With the above one factor at a time optimization method, the temperature factors were optimized. The optimum conditions are injector temperature 250 °C; initial temperature 10 °C below the boiling point of the solvent used; initial time 2 min.; temperature rate 8 °C/min. At these optimum conditions, the effect of solvent on responses was studied again to ensure that nonane and p-xylene are the best choice of the solvent. The relative peak area and peak height obtained under optimum conditions are summarized in Table 29. These results demonstrate that the optimum responses of PCBs studied are obtained when either nonane or p-xylene are used as solvents for injection. Solvents such as hexane and benzene, with low boiling points, and dodecane, with high boiling point, gave low responses for both the 10 PCBs and the 16 PAHs.

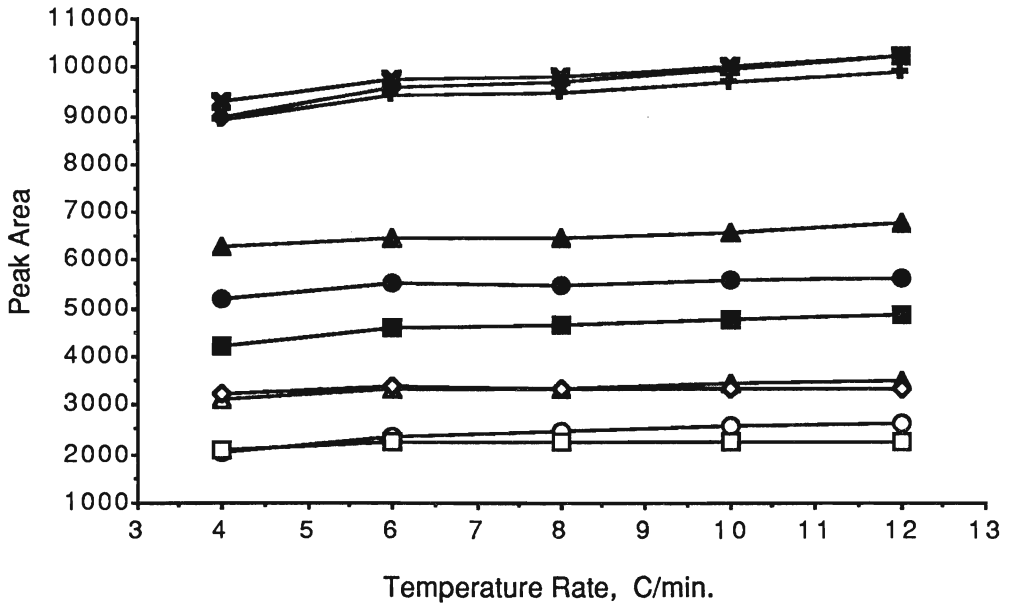


Figure 22. Effect of temperature rate on peak area of 10 PCBs

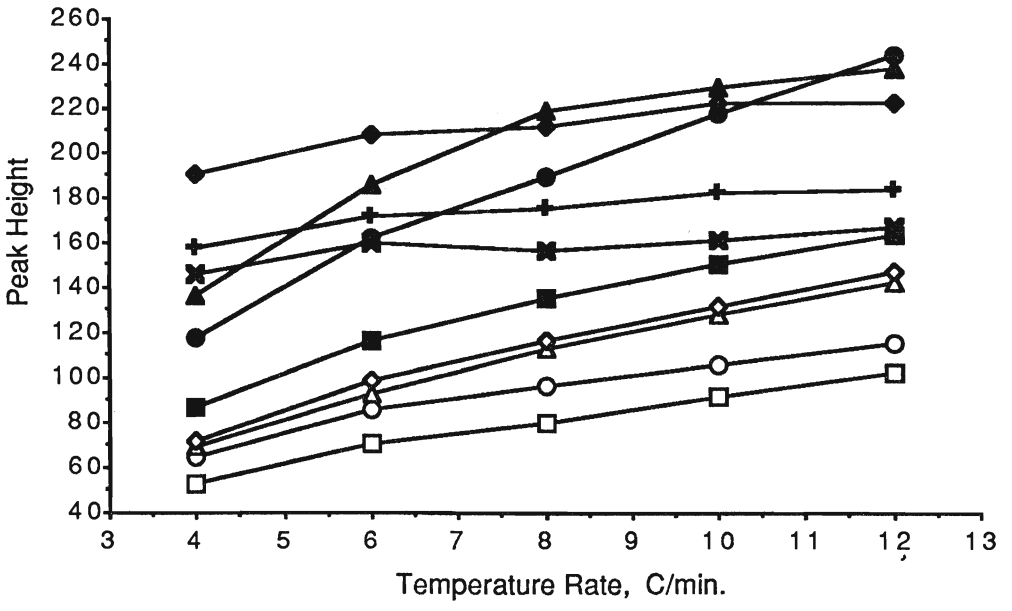


Figure 23. Effect of temperature rate on peak height of 10 PCBs

○ Peak 1 □ Peak 2 △ Peak 3 ◇ Peak 4 ● Peak 5
■ Peak 6 ▲ Peak 7 ◆ Peak 8 ✚ Peak 9 ✖ Peak 10

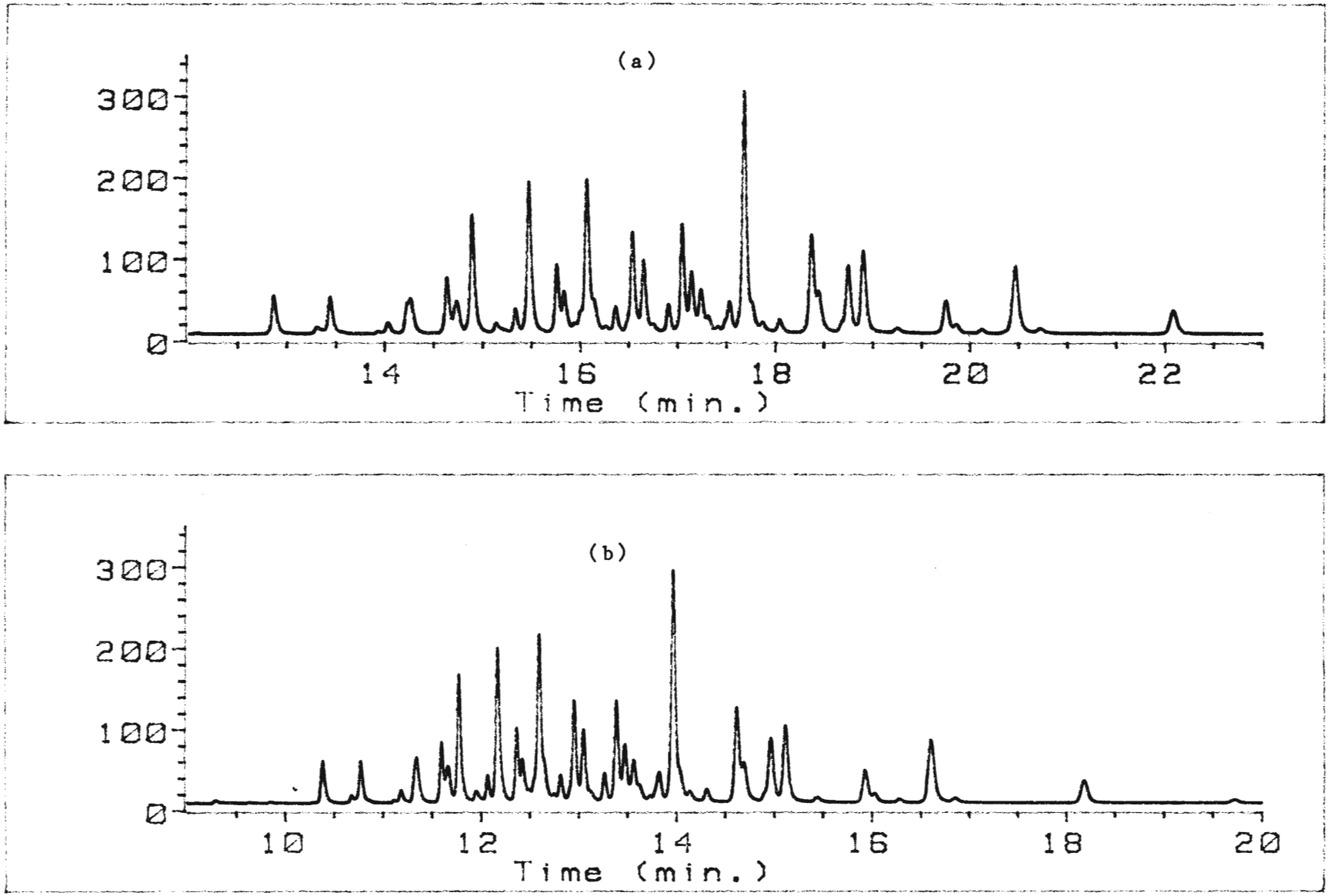


Figure 24. Chromatograms from 1 µl of 10 µg ml^{-1} of a PCB mixture (Arochlor 1260) in nonane. Temperature rate: **(a)** 8 °C min^{-1} ; and **(b)** 12 °C min^{-1}.

Table 29. Effect of solvent on response

solvent	DCM	Hexane	Benzene	isooctane	Toluene	p-Xylene	nonane
initial temp. (°C)	30	58	70	89	100	128	141
Peak #			Relative peak area				
1	84	63	68	54	79	98	100
2	55	52	61	50	78	98	100
3	63	55	64	53	78	96	100
4	66	57	66	55	80	97	100
5	59	53	61	51	77	97	100
6	59	52	60	50	74	95	100
7	46	44	53	46	71	97	100
8	32	36	31	39	63	98	100
9	30	34	42	37	62	99	100
10	30	35	42	38	62	98	100
Peak#			Relative peak height				
1	61	53	54	45	69	97	100
2	45	45	51	43	68	97	100
3	41	40	43	38	56	86	100
4	47	46	52	44	66	95	100
5	46	46	51	45	67	97	100
6	46	48	53	49	70	94	100
7	36	39	45	40	65	95	100
8	27	32	38	36	57	94	100
9	26	32	37	34	54	97	100
10	29	34	40	37	60	98	100

The pressure of make-up gas and the flow rate of carrier gas

Unlike the MSD, which requires high vacuum, ECD needs certain amount of make-up gas to achieve the optimum gas flow (carrier gas flow + make-up gas flow) required for its giving the optimum sensitivity. The influence of make-up gas (N_2) pressure on the responses of the 10 PCBs is illustrated in Figure 25 and Figure 26. As these figures show, when the pressure of make-up gas is below 30 psi, both peak area and peak height are reduced significantly. Within the range of the make-up gas pressure from 30 to 40 psi, the highest peak area and peak height were obtained. When too much make-up gas is introduced into the ECD, the sensitivity of PCBs is reduced again. It is possible that the excess make-up gas significantly increases the diffusion of the solute vapour in detector cell.

Since the make-up gas (N_2) is introduced into the ECD a few millimetres away from the column exit, it should not interfere with the retention time. As Table 30 demonstrates, the differences of retention times obtained at make-up gas pressure 20, 30, 35, 40, 45, and 50 psi are within 0.02 min..

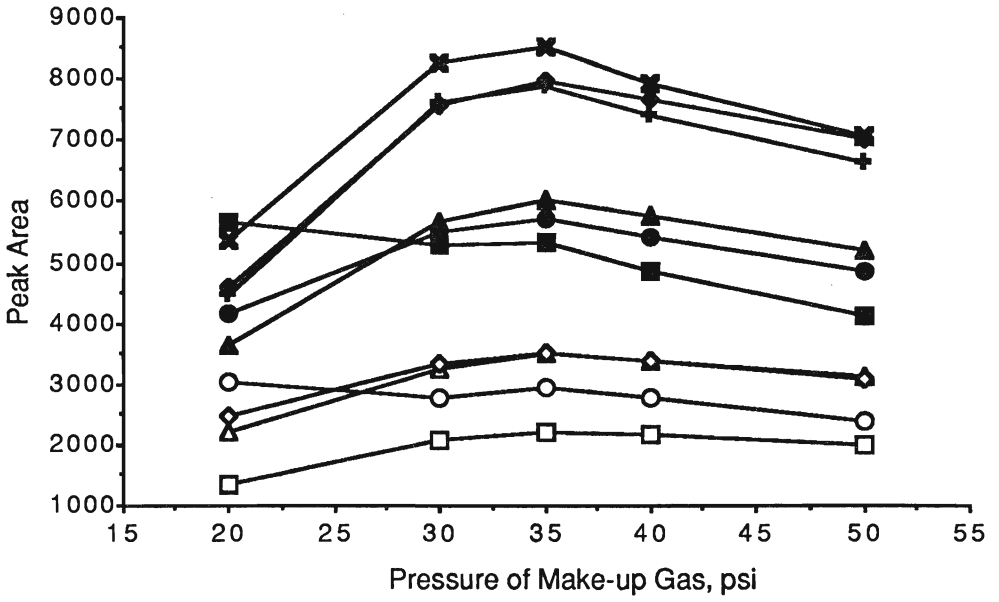


Figure 25. Effect of make-up gas (N_2) pressure on peak area of 10 PCBs

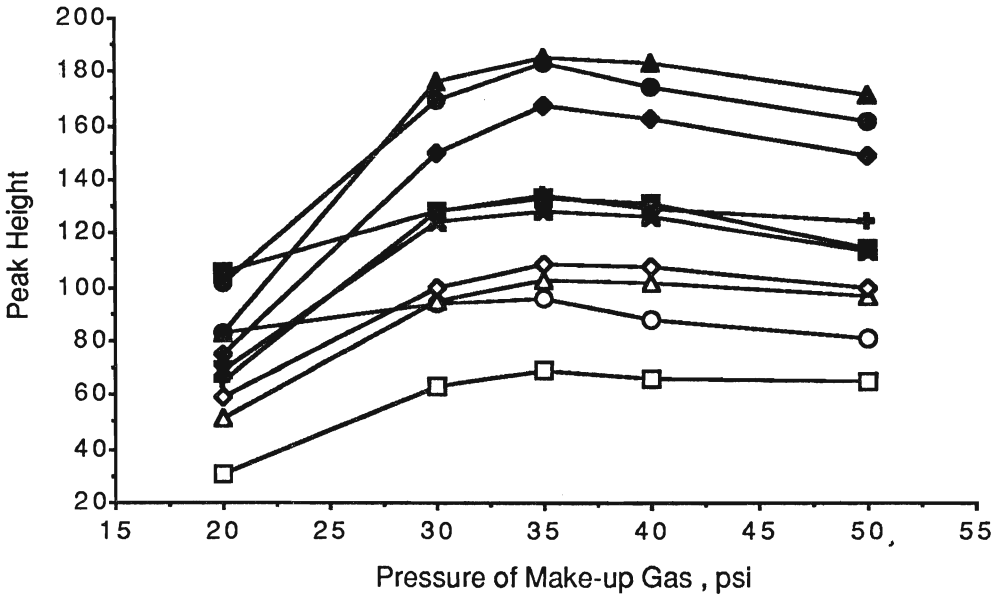


Figure 26. Effect of make-up gas (N_2) pressure on peak height of 10 PCBs

○ Peak 1 □ Peak 2 △ Peak 3 ◇ Peak 4 ● Peak 5
■ Peak 6 ▲ Peak 7 ◆ Peak 8 ✚ Peak 9 ✖ Peak 10

Table 30. Effect of make-up gas pressure on retention time (1μl of 10 PCBs in nonane)

	Makeup gas(N_2) pressure (psi)					
	20	30	35	40	45	50
Peak #	Retention time (min.)					
1	4.54	4.54	4.54	4.53	4.53	4.54
2	7.72	7.72	7.72	7.72	7.71	7.72
3	8.80	8.80	8.80	8.80	8.79	8.80
4	10.14	10.14	10.14	10.13	10.13	10.13
5	11.66	11.66	11.65	11.65	11.65	11.65
6	12.90	12.89	12.89	12.89	12.89	12.89
7	15.58	15.57	15.57	15.56	15.56	15.56
8	19.31	19.31	19.30	19.30	19.30	19.30
9	21.03	21.03	21.03	21.02	21.03	21.03
10	22.70	22.70	22.69	22.69	22.70	22.70

The carrier gas flow rate dramatically affects retention time of a chromatographic peak. The higher carrier gas flow rate is used, the shorter retention time is obtained. Therefore, the carrier gas flow rate should be controlled very well and kept constant in order to achieve precise retention data for identification.

Carrier gas flow not only affects the retention time, but also strongly influences the band width of solute, that will result in the different sensitivity, particularly in peak height. When the make-up gas

pressure is kept at 38 psi constantly and optimum temperatures are used, the flow rate of carrier gas was increased from 0.6 to 1.6 ml $min.^{-1}$, corresponding to column head pressure 10 and 20 psi. Over this range of flow rate of carrier gas, no significant change of peak area was observed, as shown in Figure 27. However, when the flow rate is increased from 0.6 to 0.8 ml $min.^{-1}$ (i.e. column head pressure 13 psi), Figure 28 shows that the peak heights are significantly increased. When the flow rate is further increased from 0.8 to 1.6 ml $min.^{-1}$, peak heights of the first seven peaks show very little change, whereas the peak heights of peaks 8, 9, and 10 continue to increase with the increase of carrier gas flow rate. Two possible factors may be contributed to this phenomenon. First, the improved peak heights of PCBs at higher carrier gas flow rate are probably due to the increase of speed of transferring solutes from the injector to the column. The transfer of late-eluting PCBs with higher boiling points are normally slower than the early-eluting PCBs. The band widths of late-eluting PCBs are widened by the slow transfer process. When the carrier gas flow rate is increased, the process of transfer is shortened. Therefore, the band widths of these PCBs are narrowed, resulting in the improvement of peak height. Secondly, according to Van Deemter curve, the highest theoretical plate numbers n (i.e. the shortest height of each theoretical plate) are obtained at certain carrier gas flow rate, at the bottom of Van Deemter curve. When the flow rate is around the bottom of Van Deemter curve, the greatest peak height is achieved and also the change of peak sharpness is small, because there is only small change of theoretical plate numbers. Therefore, it is possible when the flow rate is between 0.8 and 1.6 ml $min.^{-1}$, the optimum separation efficiency is achieved for peaks 1 to 7, whereas the last three PCBs may

still need higher flow rate to reach the highest number of theoretical plates.

Effect of injection volume on optimum conditions

The optimum conditions discussed above were obtained when injection volume was 1 μl. If the volume of injected sample is changed, the optimum conditions may not be the same. Further studies, therefore, were carried out to examine the optimum conditions when the injection volume is varied.

Since the initial column temperature has proved to a major factor to affect the GC peak shape, it was studied with respect to different injection volume. An aliquot of 1 and 2 μl of 10 PCBs in nonane was independently injected with the variation of initial temperature. Table 31 compares the fronting and tailing temperatures of each of the ten peaks obtained with 1 and 2 μl of injection volume. As we can see from Table 31, while the upper limits of initial temperatures are similar, the minimum initial temperatures for symmetric peaks are higher with 2 μl than with 1 μl of injection volume. Thus, with the injection of 2 μl of PCBs, the optimum initial temperature range of each 10 PCBs for obtaining symmetric peaks is approximately 40 °C narrower compared with that from 1 μl of PCB solution.

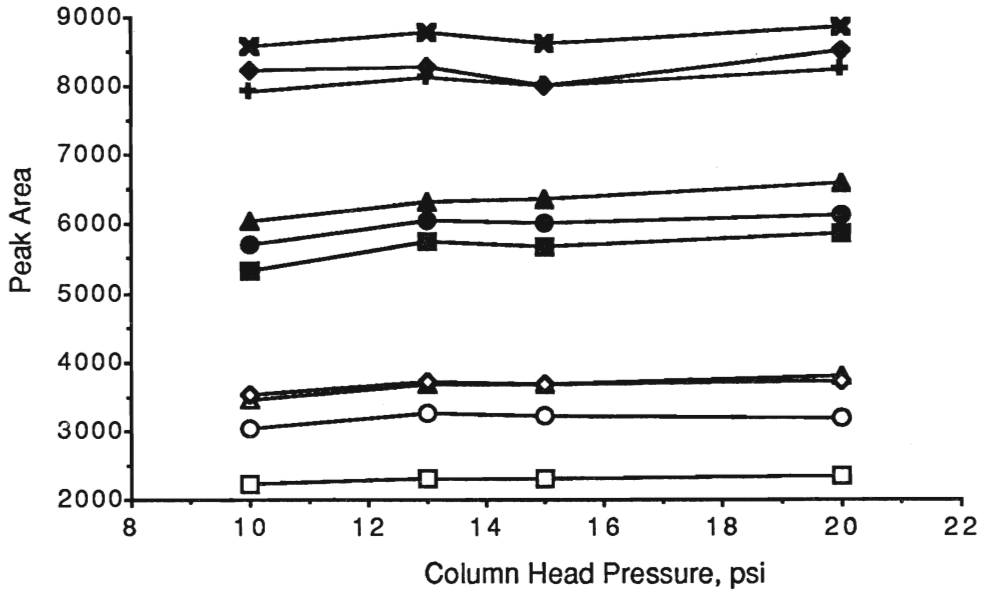


Fig 27. Effect of carrier gas flow on peak area of 10 PCBs

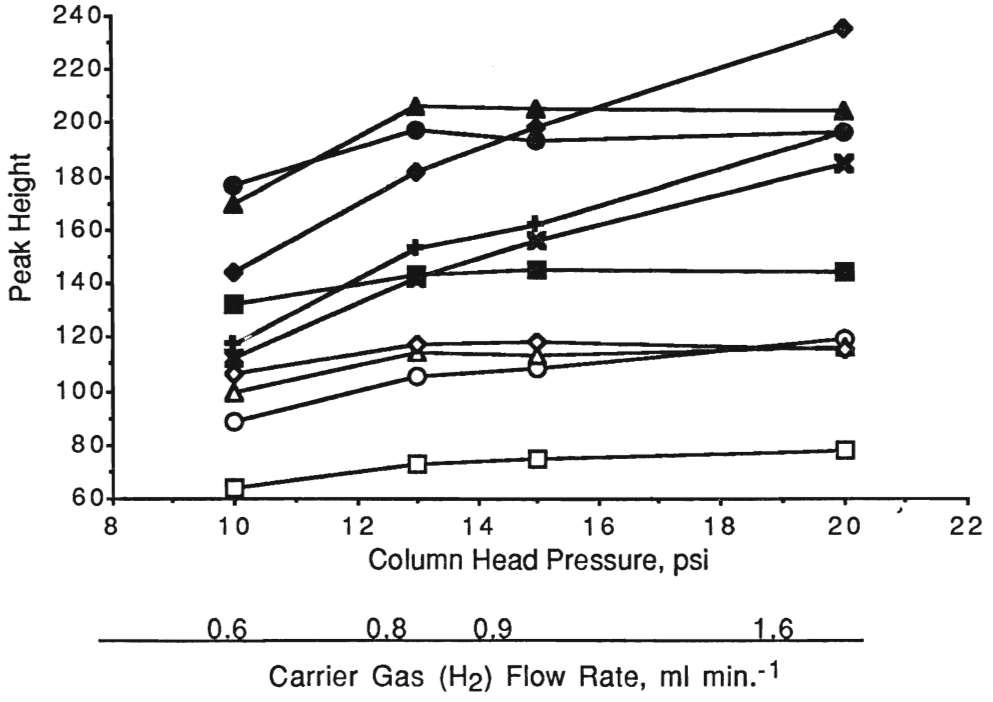


Figure 28. Effect of carrier gas flow on peak height of 10 PCBs

○ Peak 1 □ Peak 2 △ Peak 3 ◇ Peak 4 ● Peak 5
■ Peak 6 ▲ Peak 7 ◆ Peak 8 ✚ Peak 9 ✖ Peak 10

Table 31. Effect of injection volume on the optimum initial temperature range for symmetrical peaks
(1μl of 10 PCBs in nonane)

inject. vol.	1 μl			2 μl		
Peak#	Low limits (T_1)	High limits (T_2)	ΔT (T_2-T_1)	Low limits (T_1)	High limits (T_2)	ΔT (T_2-T_1)
1	51	141	90	81	141	60
2	81	161	80	121	161	40
3	81	161	80	121	161	40
4	81	161	80	121	161	40
5	91	191	100	131	191	60
6	91	191	100	131	191	60
7	91	221	130	131	221	90
8	101	221	120	141	221	80
9	101	221	120	141	221	80
10	101	221	120	151	221	70

The optimum initial temperature for obtaining the best peak response is also changed with the increase of injection volume. Figures 29 and 30 show peak height of 10 PCBs versus initial temperature, with an injection volume of 1 μl (Figure 29) and 2 μl (Figure 30). As demonstrated in Figure 29, the optimum range of initial temperature is between 111 and 151 °C with an injection volume of 1 μl. However, when 2 μl solution is injected, the optimum initial temperature is between 151 and 161 °C, as shown in Figure 30. The variation of peak height obtained at different initial temperatures is much more dramatic with 2 μl injection volume. The effect of initial temperature on the peak area is less significant than peak height. This is demonstrated in Figures 31 and 32.

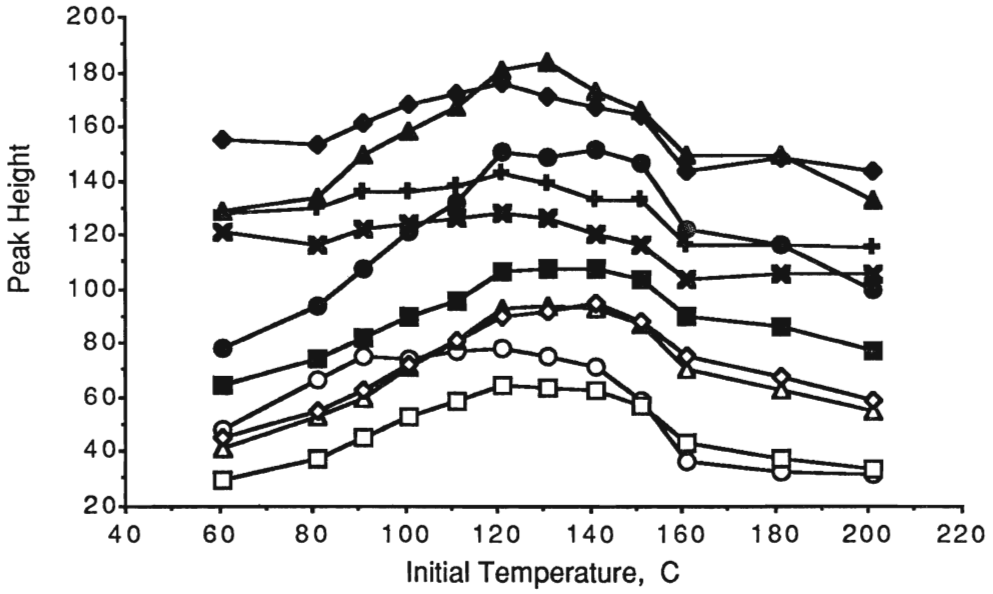


Figure 29. Effect of initial temperature on peak height from 1 μl of 10 PCBs in nonane

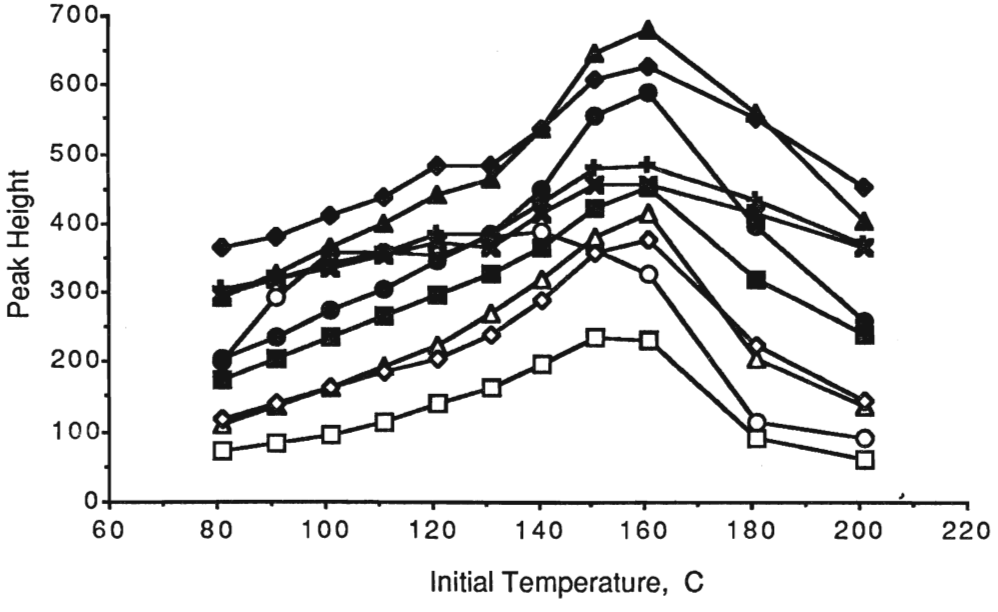


Figure 30. Effect of initial temperature on peak height from 2 μl of 10 PCBs in nonane

○ Peak 1 □ Peak 2 △ Peak 3 ◇ Peak 4 ● Peak 5
■ Peak 6 ▲ Peak 7 ◆ Peak 8 ✚ Peak 9 ✖ Peak 10

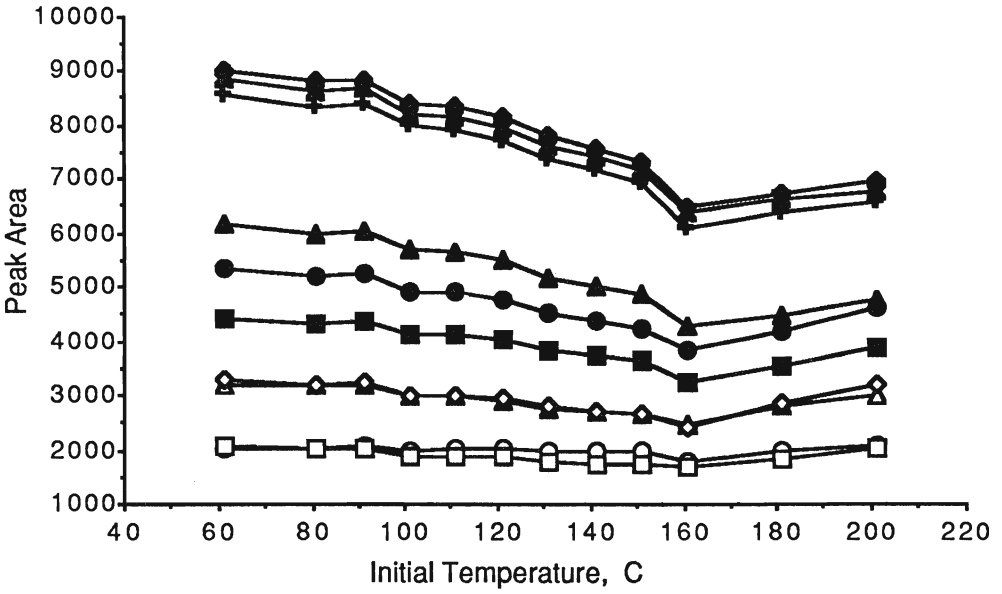


Figure 31. Effect of initial temperature on peak area from 1 µl of 10 PCBs in nonane

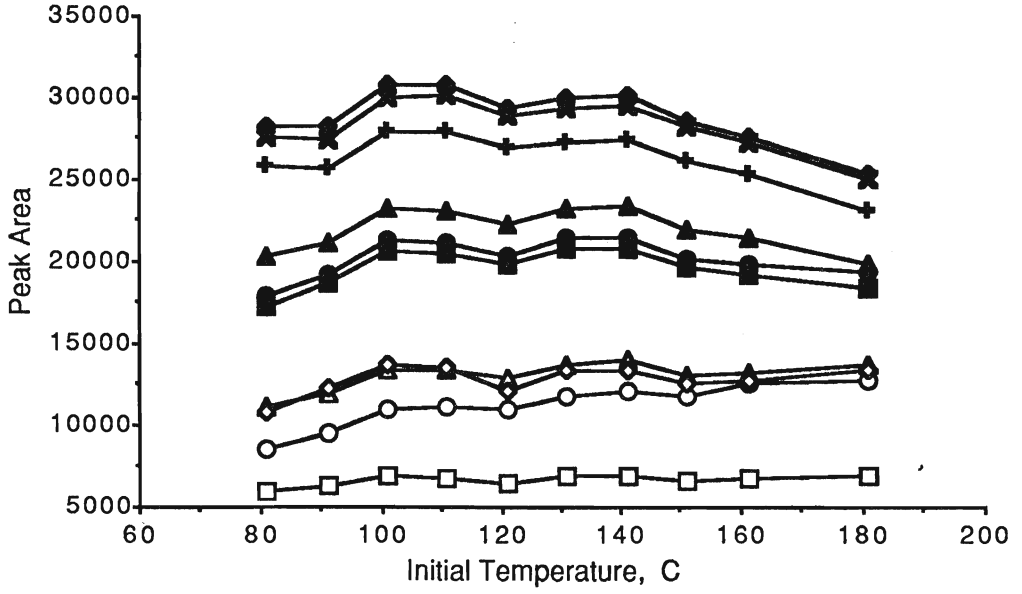


Figure 32. Effect of initial temperature on peak area from 2 µl of 10 PCBs in nonane

○ Peak 1 □ Peak 2 △ Peak 3 ◇ Peak 4 ● Peak 5
■ Peak 6 ▲ Peak 7 ◆ Peak 8 ✚ Peak 9 ✖ Peak 10

When more sample is injected, higher energy may be required to complete the evaporation of sample, as suggested in previous section on PAHs. This was confirmed in the determination of PCBs. When 1 µl of 10 PCBs with nonane as a solvent was injected, the optimum peak area was obtained over the range of injector temperature of 220 and 260 °C as shown in Figure 33. With this range of injector temperatures, the optimum peak heights of the ten PCBs are also obtained. If the injector temperature is further increased above 260 °C, the loss of sensitivity occurs. When the volume of sample injected is increased up to 2 µl, the optimum injector temperature is also increased. Figure 34 suggests that the injector temperature should be higher than 260 °C in order to obtain the optimum peak area. With an injector temperature below 260 °C, the responses are reduced, which is likely due to insufficient evaporation.

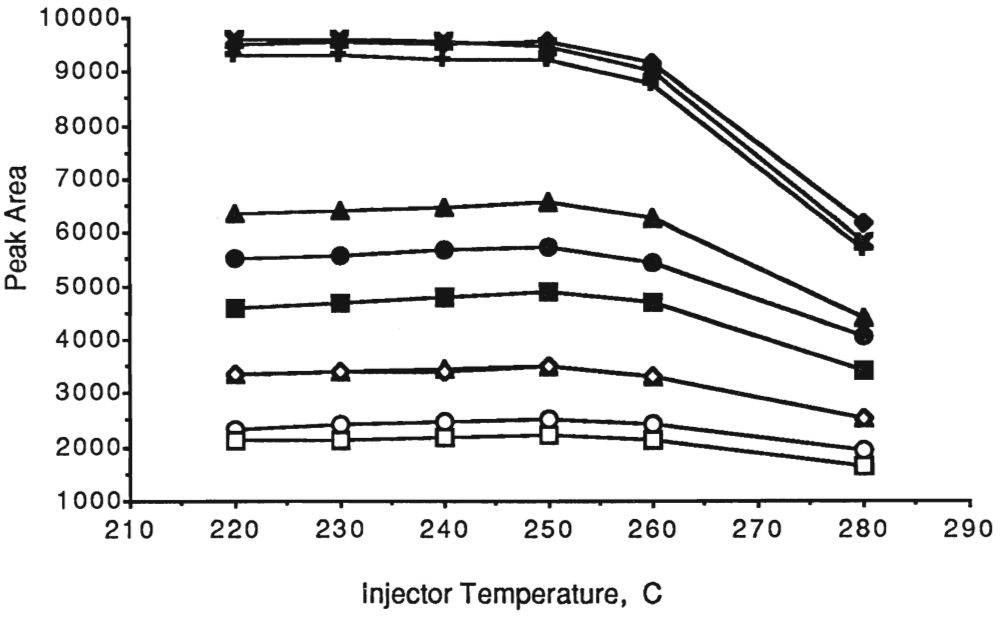


Figure 33. Effect of injector temperature on peak area from 1 µl of 10 PCBs in nonane

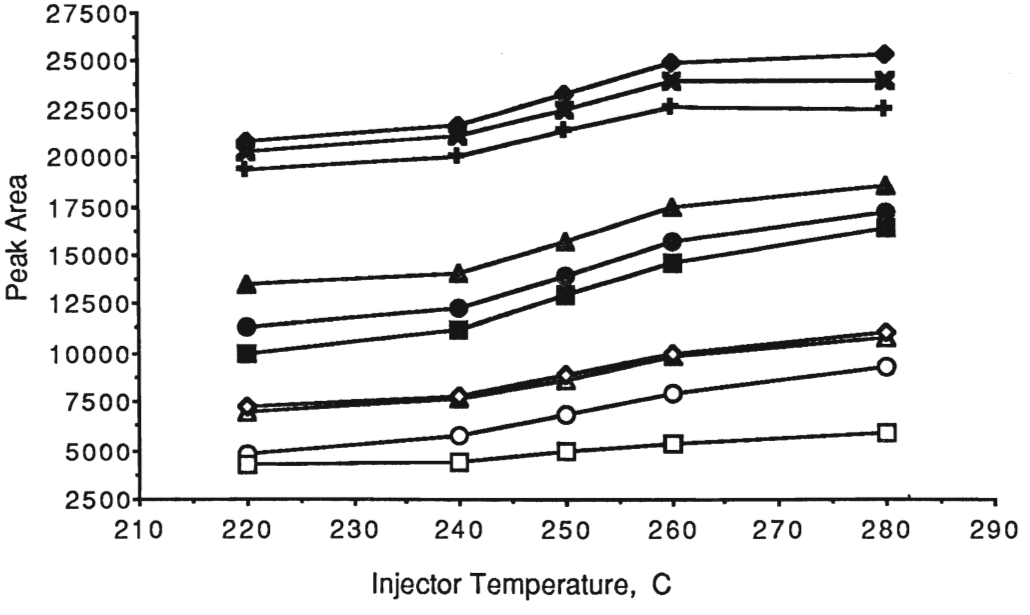


Figure 34. Effect of injector temperature on peak area from 2 µl of 10 PCBs in nonane

○ Peak 1 □ Peak 2 △ Peak 3 ◇ Peak 4 ● Peak 5
■ Peak 6 ▲ Peak 7 ◆ Peak 8 ✚ Peak 9 ✖ Peak 10

The function of injection volume on sensitivity

With regard to the quantity of solute, one might expect that the response of the solute increases linearly with the increase of injection volume. However, this was not found to be true in this work when peak height is used as response factor. As discussed above, the optimum peak height and area of 1 μl and 2 μl of 10 PCBs in nonane were obtained, respectively, at the initial temperature 118 and 151 °C. Therefore, these two initial temperatures were used here. When the injection volume is increased from 1 to 3 μl at an initial temperature 118 °C, which is 33 °C lower than the boiling point of nonane, the peak area linearly increases with the increase of injection volume. The relative peak area and linear regression correlation coefficient of each ten PCBs are listed in Table 32. When the initial temperature is increased to 151 °C (b.p. of nonane), the peak area of each peak is also found to be linear with the injection volumes. In both cases, the linear regression correlation coefficient of each equation is within 0.994 and 1.00, as Table 32 shows.

Table 32. The relation between peak area and injection volume

Initial. temp.	118 °C				151 °C			
inject. vol (µl)	1	2	3		1	2	3	
Peak #	Relative peak area			r^2	Relative peak area			r^2
1	1.0	1.9	2.6	0.999	1.0	2.1	3.3	0.999
2	1.0	1.9	2.8	0.999	1.0	1.9	2.9	0.997
3	1.0	2.0	2.9	1.00	1.0	2.1	3.5	0.994
4	1.0	1.9	2.8	0.999	1.0	2.1	3.4	0.997
5	1.0	2.0	2.9	1.00	1.0	2.1	3.3	0.997
6	1.0	1.9	2.8	1.00	1.0	2.1	3.3	0.999
7	1.0	2.0	3.0	1.00	1.0	2.0	3.2	0.998
8	1.0	2.0	2.9	1.00	1.0	2.0	3.0	1.00
9	1.0	2.0	2.9	1.00	1.0	1.9	2.9	1.00
10	1.0	2.0	3.0	1.00	1.0	1.9	2.9	1.00

The peak heights corresponding to injection volumes vary with the initial temperatures. The curve of peak height verses injection volume was not linear. Table 33 shows that peak heights are only less than 50% increased when injection volume is doubled at the initial temperature 118 °C. With further increase of injection volume from 2 to 3 µl, no increase of peak heights of PCBs is found except for the first one. There may be several reasons responsible for the loss of peak heights with relatively high injection volume. The most important factor is improper

initial temperature. With an initial column temperature of 118 °C, chromatograms from 1, 2, and 3 μl of 10 PCBs in nonane were obtained and shown in Figure 35. As shown in Figure 35 (a), peaks from 1 μl of PCBs are symmetrical. The fronting of peaks appear from 2 μl of PCBs (Fig. 35b). Fronting of peaks becomes more severe and even split when 3 μl of PCBs is injected [Figure 35 (c)].

Table 33. The relation between peak height and injection volume

Initial. temp.	118 °C				151 °C			
inject. vol.(μl)	1	2	3		1	2	3	
Peak #	Relative peak height			r^2	Relative peak height			r^2
1	1.0	1.9	2.7	0.998	1.0	3.6	5.6	0.994
2	1.0	1.3	1.2	0.623	1.0	2.4	2.5	0.809
3	1.0	1.3	1.2	0.497	1.0	2.3	2.3	0.790
4	1.0	1.2	1.2	0.493	1.0	2.2	2.2	0.759
5	1.0	1.2	1.1	0.267	1.0	2.0	2.1	0.822
6	1.0	1.3	1.3	0.736	1.0	2.0	2.2	0.873
7	1.0	1.3	1.3	0.614	1.0	1.9	2.1	0.861
8	1.0	1.5	1.5	0.755	1.0	1.9	2.2	0.916
9	1.0	1.5	1.5	0.817	1.0	1.9	2.2	0.934
10	1.0	1.5	1.6	0.849	1.0	2.0	2.3	0.923

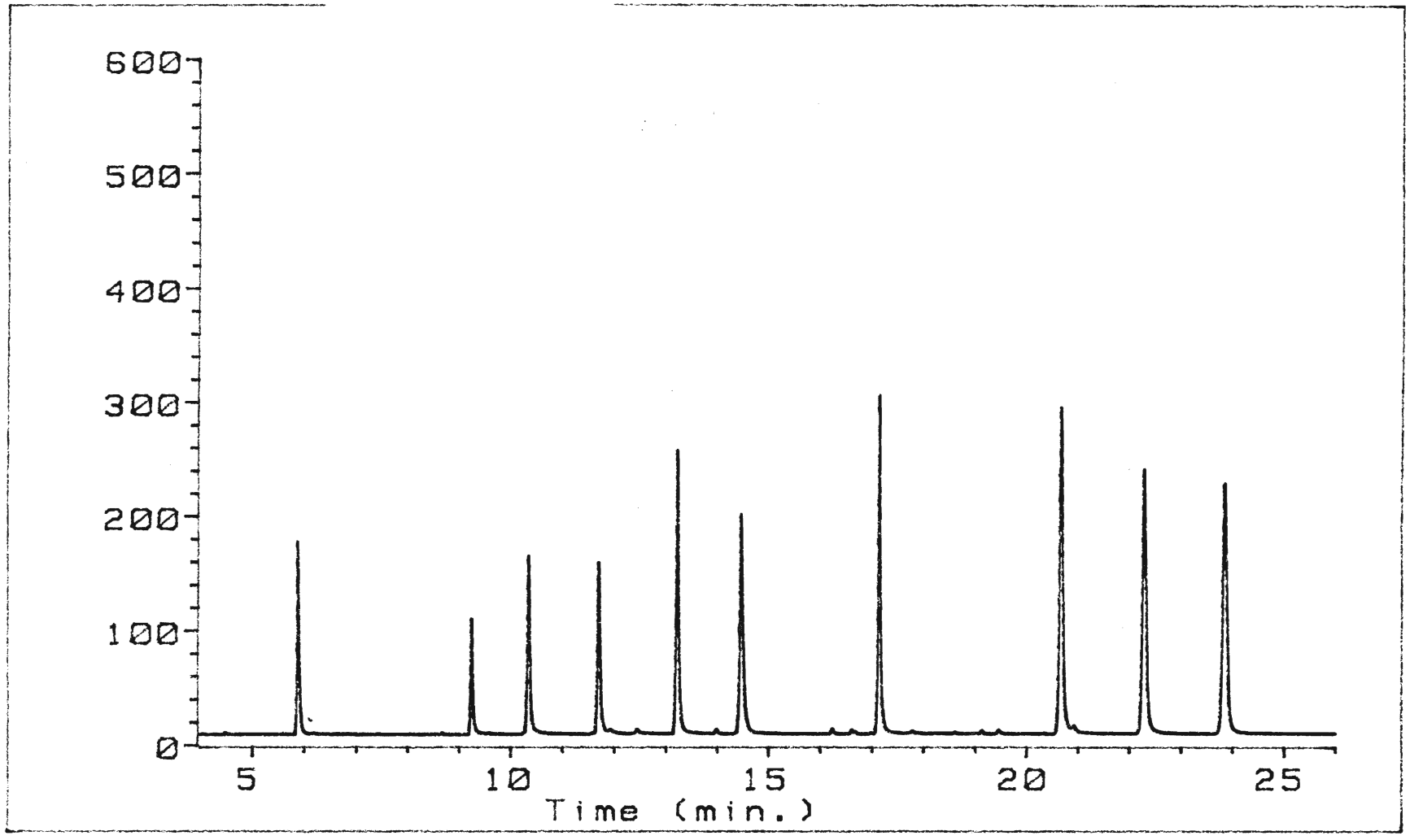


Figure 35 a. Chromatogram from 1 µl of 10 PCBs in nonane at an initial temperature of 118 °C.

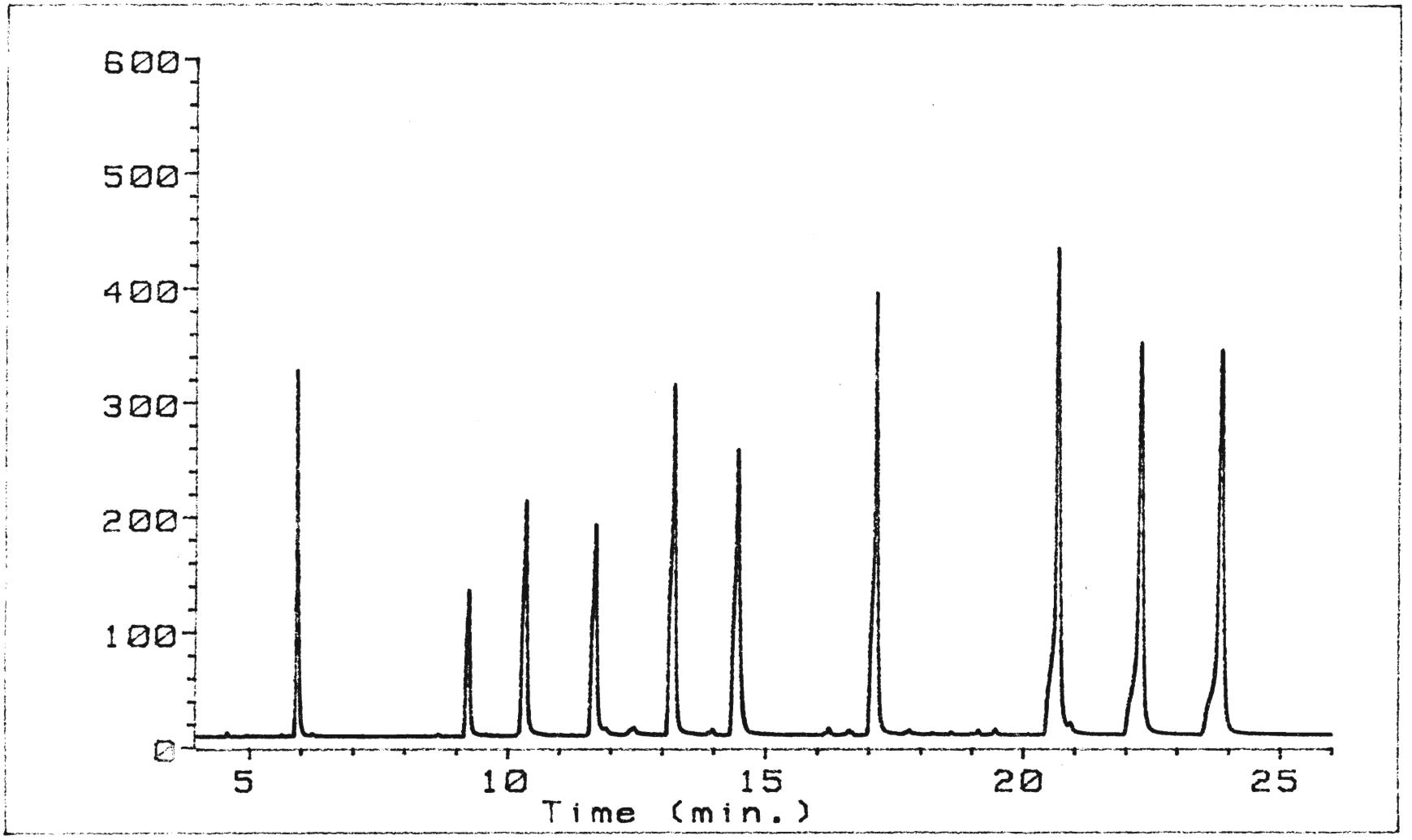


Figure 35 **b**. Chromatogram from 2 µl of 10 PCBs in nonane at an initial temperature of 118 °C.

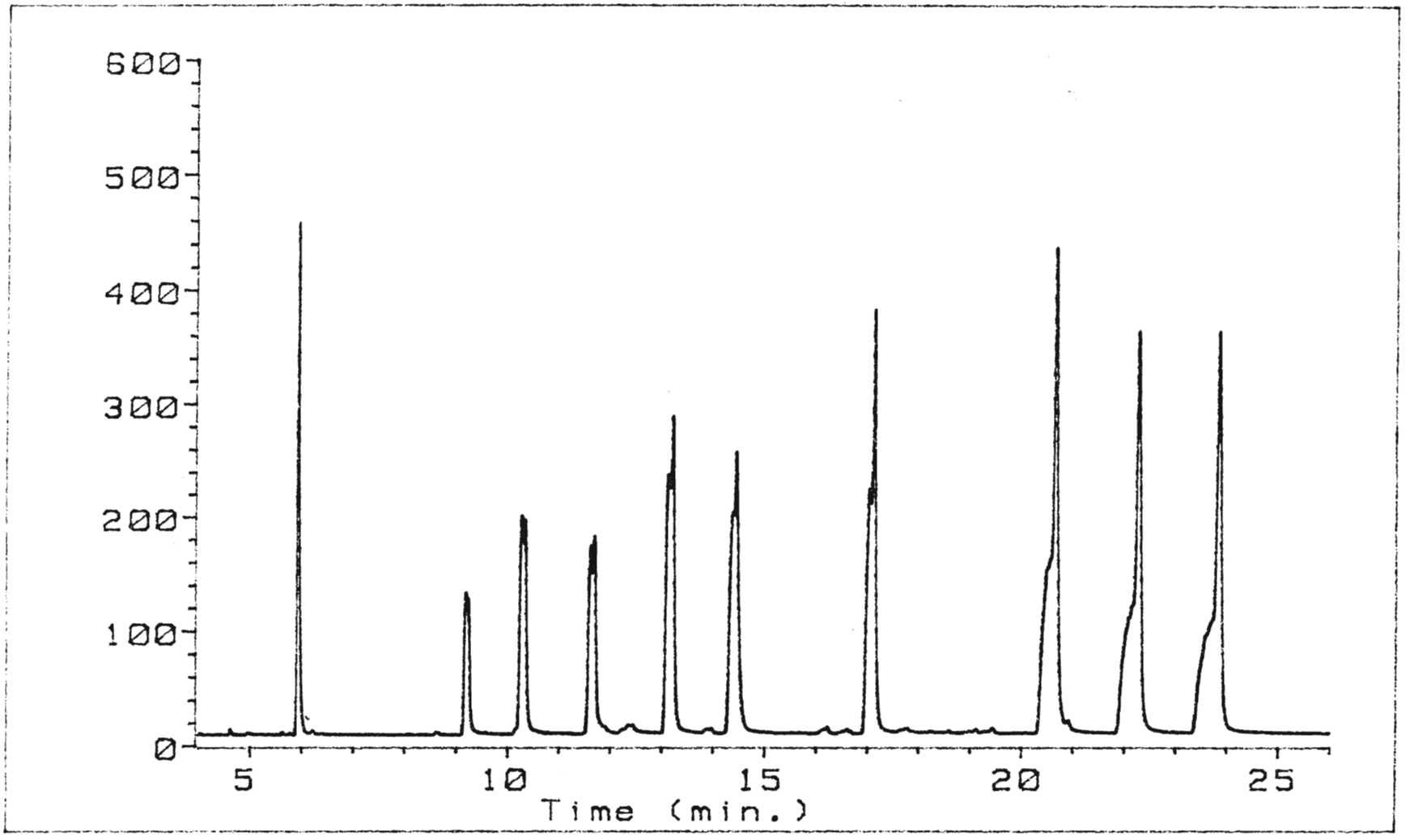


Figure 35 c. Chromatogram from 3 µl of 10 PCBs in nonane at an initial temperature of 118 °C.

In order to improve the peak shape when high injection volume is used, a higher initial temperature (151 °C) was used to obtain the responses from PCBs. Figure 36 shows chromatograms from 1, 2 and 3 µl of 10 PCBs in nonane at an initial temperature of 151 °C. All symmetrical peaks are obtained with injection volume of 1 µl (Figure 36a) and 2 µl (Figure 36b). Only slight fronting of peaks is observed at an injection volume of 3 µl (Figure 36c). This improvement of peak shape results in the increase of peak heights from 2 µl of PCB sample, described in Table 33. Table 33 shows that the peak heights are increased with the increase of injection volume. The peak heights of 2 µl of PCBs are generally twice high as those of 1 µl sample. However, the increase of peak heights from the 3 µl injection, compared with 2 µl of injection is small.

The above results suggest that, when the initial temperature is low enough to obtain the solvent effect, the injection volume is limited to 1 µl for the most symmetrical peak and best sensitivity. When the initial temperature is 151 °C, with nonane as solvent, the solvent effect is not likely to be achieved. However, 2 µl of injection volume can be used successfully and better sensitivity is also obtained. Although the solvent effect is not able to be used to improve the sensitivity at this initial temperature, the cold trapping effect occurs and it improves the sensitivity. The loss of peak heights at injection volume 3 µl is possibly due to overloading of the column. The bands of PCBs are broadened and hence have lower peak height. Even when the column flow rate is increased to 1.6 ml $min.^{-1}$ (i.e. column head pressure 20 psi), the peak heights of 3 µl of PCBs in nonane are still lower than three times of peak heights from 1 µl of 10 PCBs.

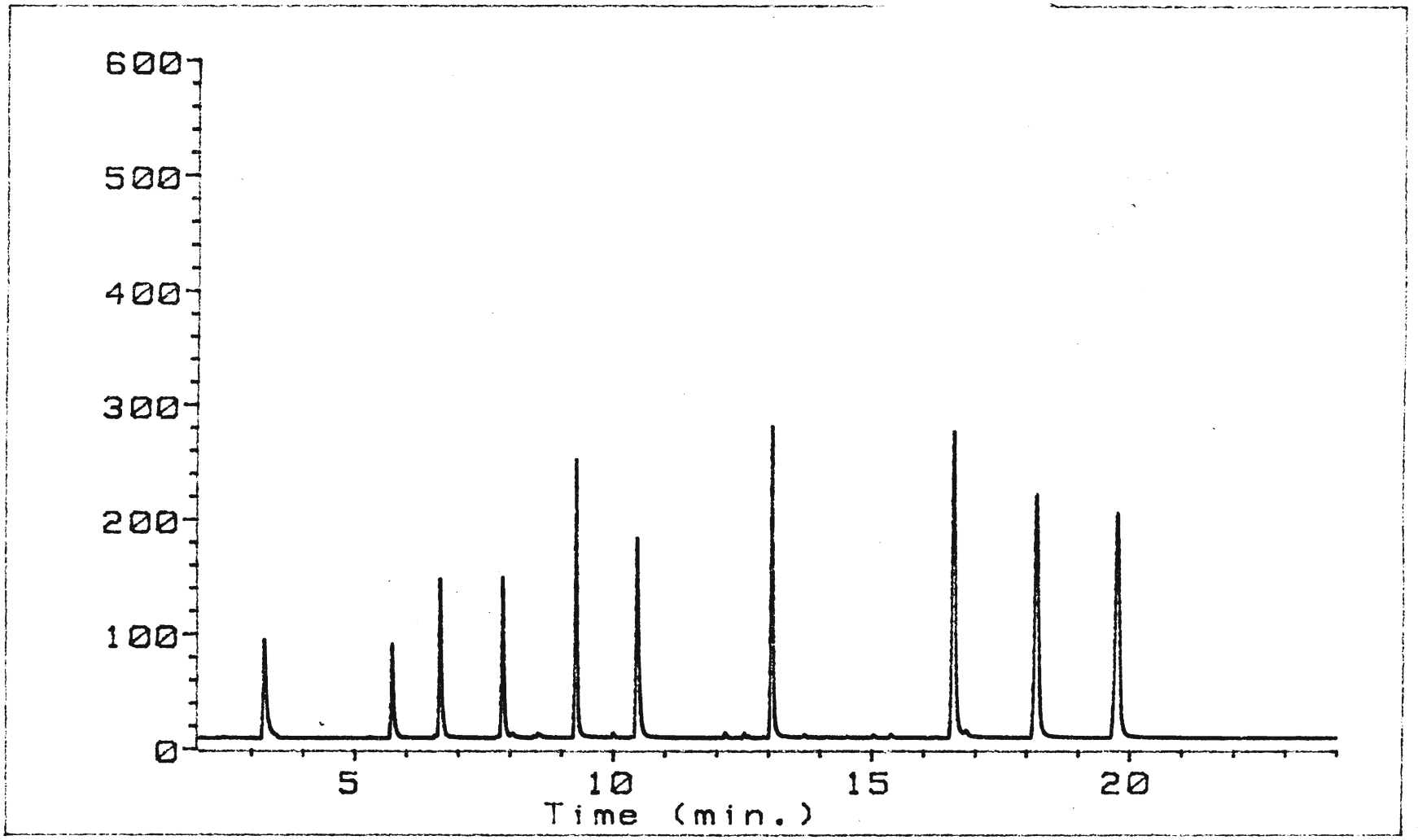


Figure 36 a. Chromatogram from 1 µl of 10 PCBs in nonane at an initial temperature of 151 °C.

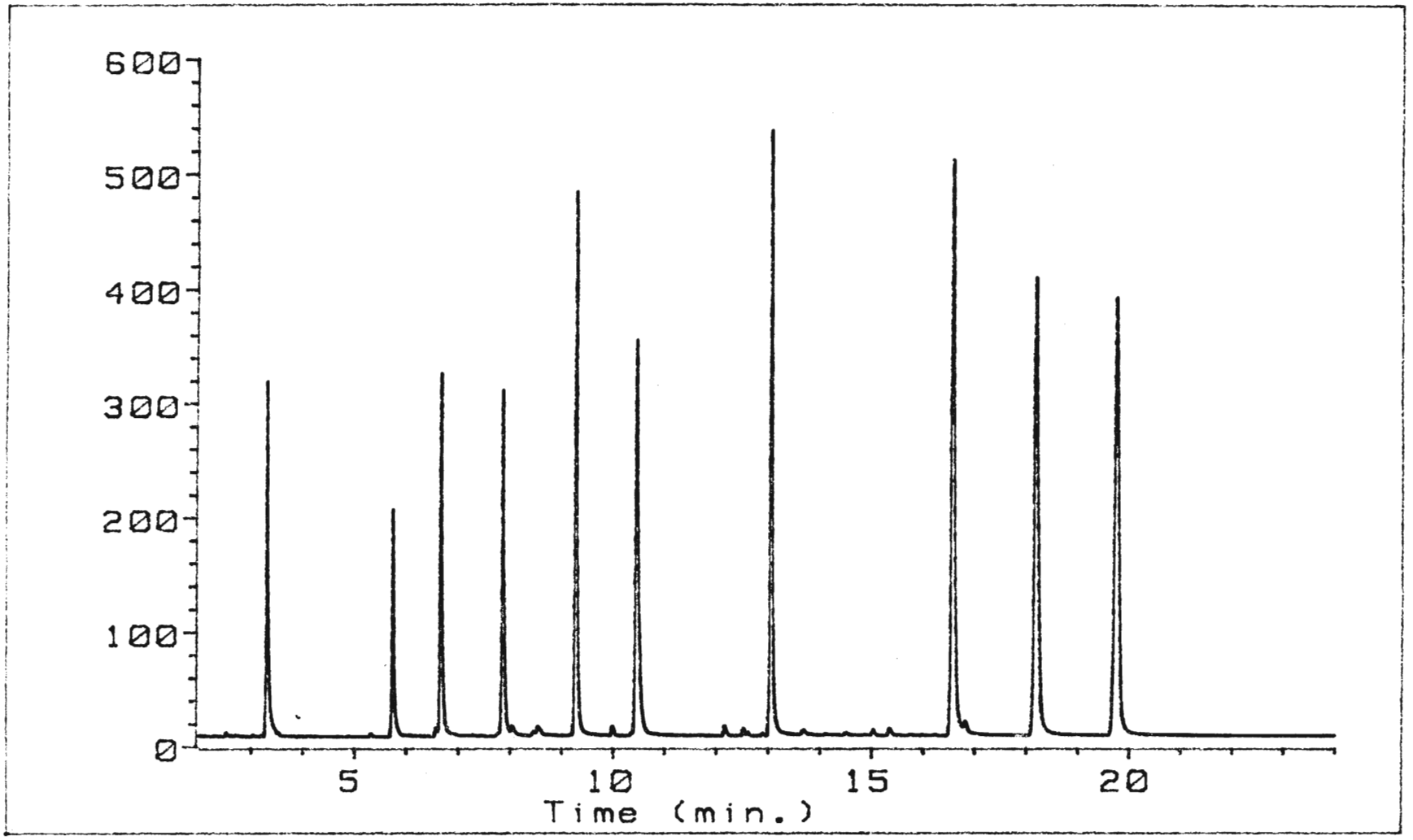


Figure 36 **b**. Chromatogram from 2 µl of 10 PCBs in nonane at an initial temperature of 151 °C.

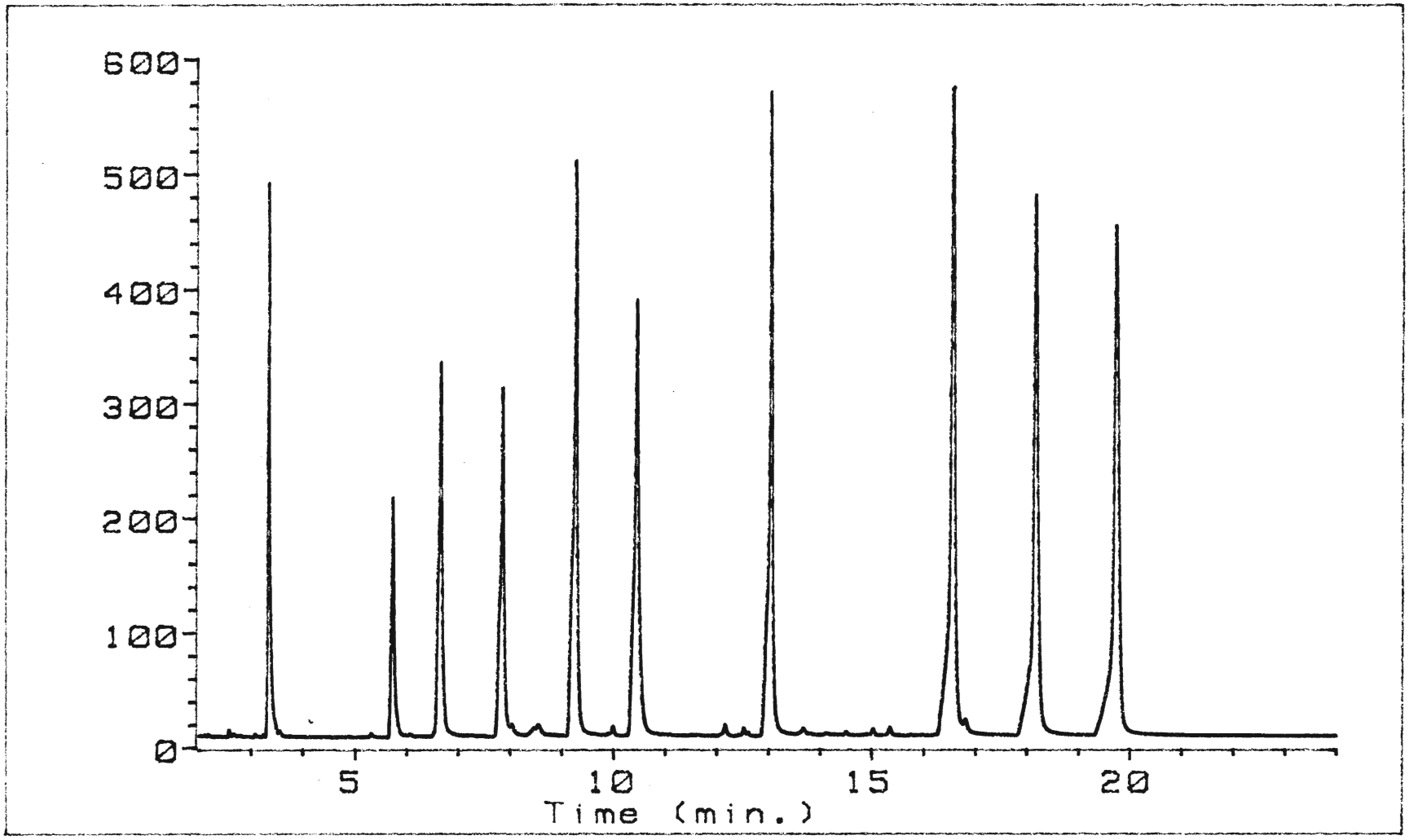


Figure 36 c. Chromatogram from 3 µl of 10 PCBs in nonane at an initial temperature of 151 °C.

The effect of structure of solvent

In the previous sections, the effect of affinity between solvent and stationary phase was found to influence the peak shapes of PAHs, especially at initial temperatures low enough to produce the solvent effect. The dissimilarity of stationary phase and solvent causes fronting or splitting of peaks. This makes it difficult to apply the solvent effect to enhance GC signals. However, the previous study showed that the distortion of peak can be eliminate by using proper solvent and initial temperature. It is of concern that the distortion of peaks caused by the solvent may also exist in the determination of PCBs. In fact, the above results of initial temperature affecting the peak shape and response indicate that the condensed solvent may be the reason for peak fronting. Further experiments were performed to observe more details of effect of solvent on the chromatographic behaviour of PCBs.

With a crosslinked methyl silicone column, an aliquot of 1 μl of 10 PCBs solutions in benzene, toluene, p-xylene, hexane, and isooctane were independently injected at initial temperatures of 40 °C below the boiling points of solvents used. At this level of initial temperature, no fronting of any of the ten PCBs occurs with hexane or isooctane as solvents. However, when the aromatic solvents were used for injections, interesting phenomena were observed. From benzene (Figure 37a) to toluene (Figure 37b), the fronting of PCB peaks was reduced, and no fronting of PCB peaks was observed when p-xylene was used as solvent (Figure 37c). At initial temperatures of 50 °C below the boiling points of these solvents, peaks from PCBs in p-xylene are only slightly fronting, as shown in Figure 38a. However, the peaks of PCBs in toluene (Figure 38b) and benzene (Figure 38c), are severely split.

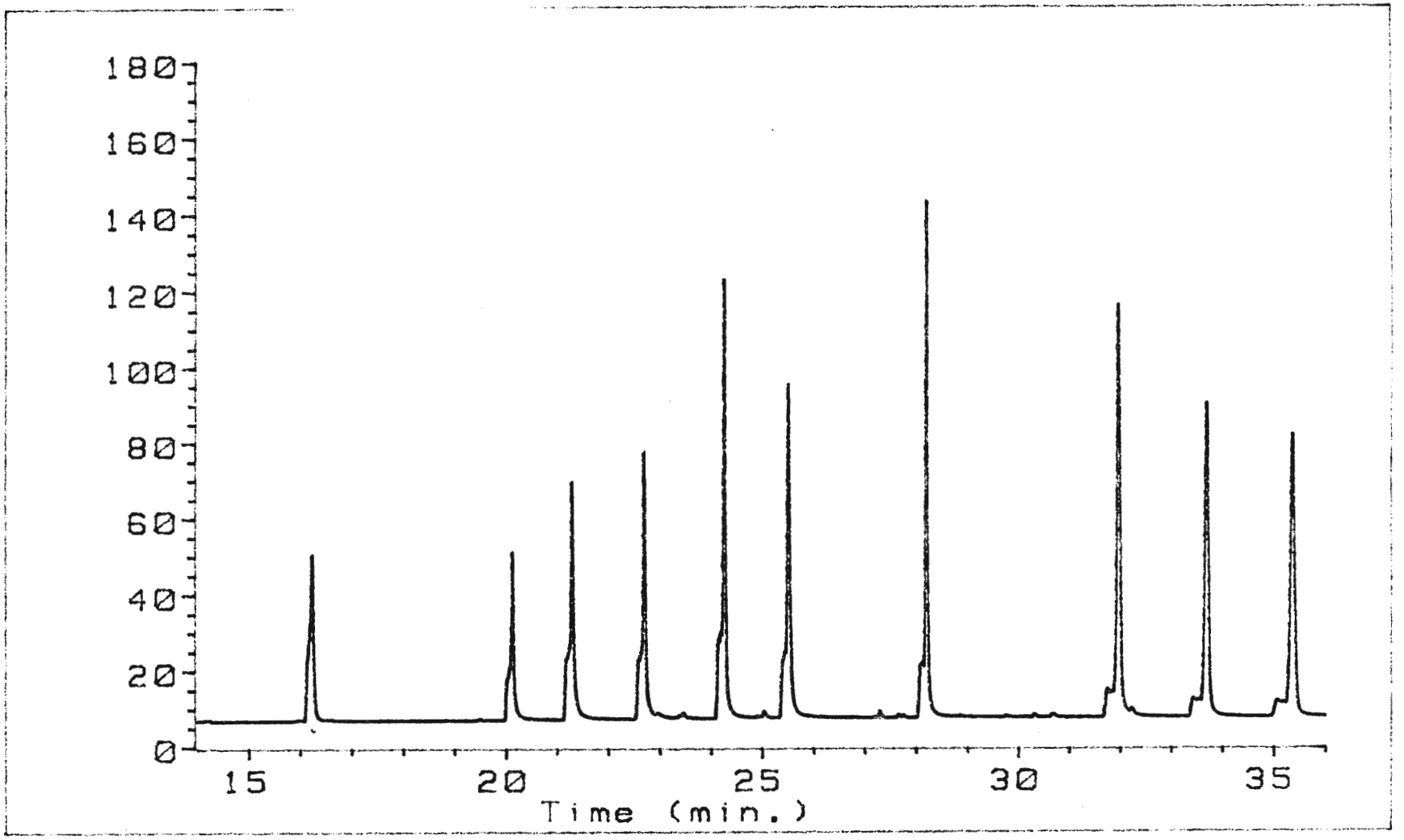


Figure 37 a. Chromatogram of 10 PCBs in benzene at an initial temperature of 40 °C (b.p.-40).

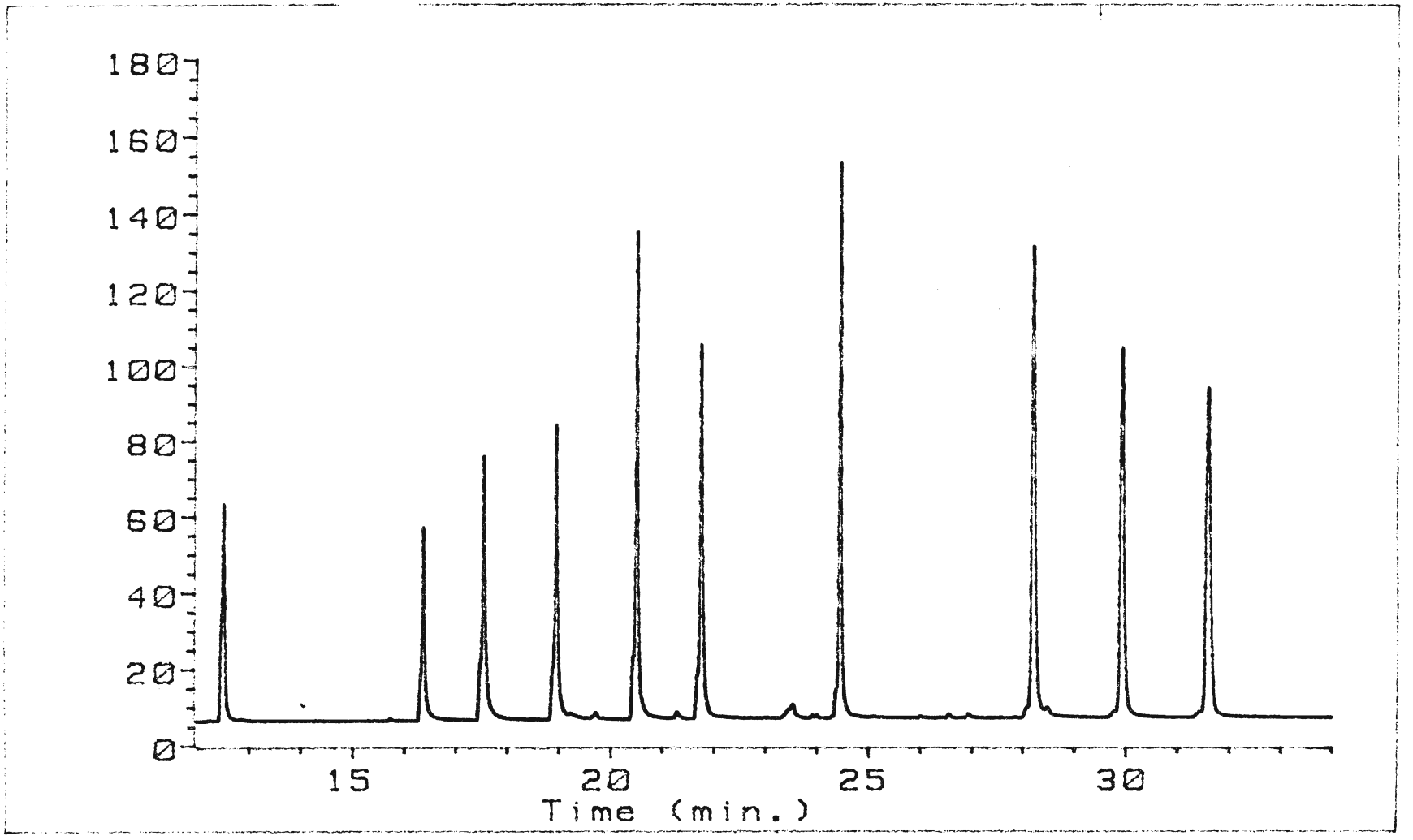


Figure 37 **b**. Chromatogram of 10 PCBs in toluene at an initial temperature of 70 °C (b.p.-40).

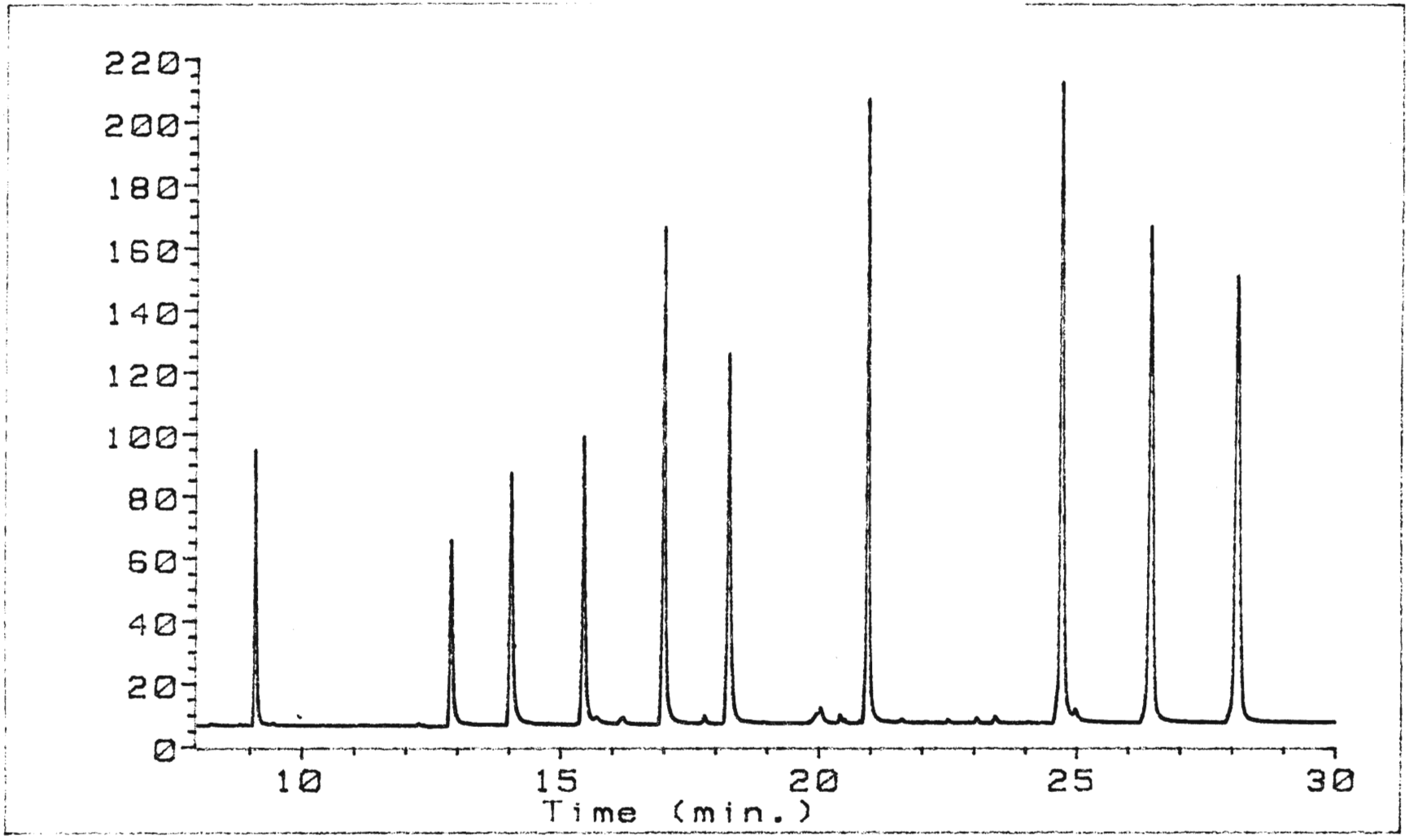


Figure 37 c. Chromatogram of 10 PCBs in p-xylene at an initial temperature of 98 °C (b.p.-40).

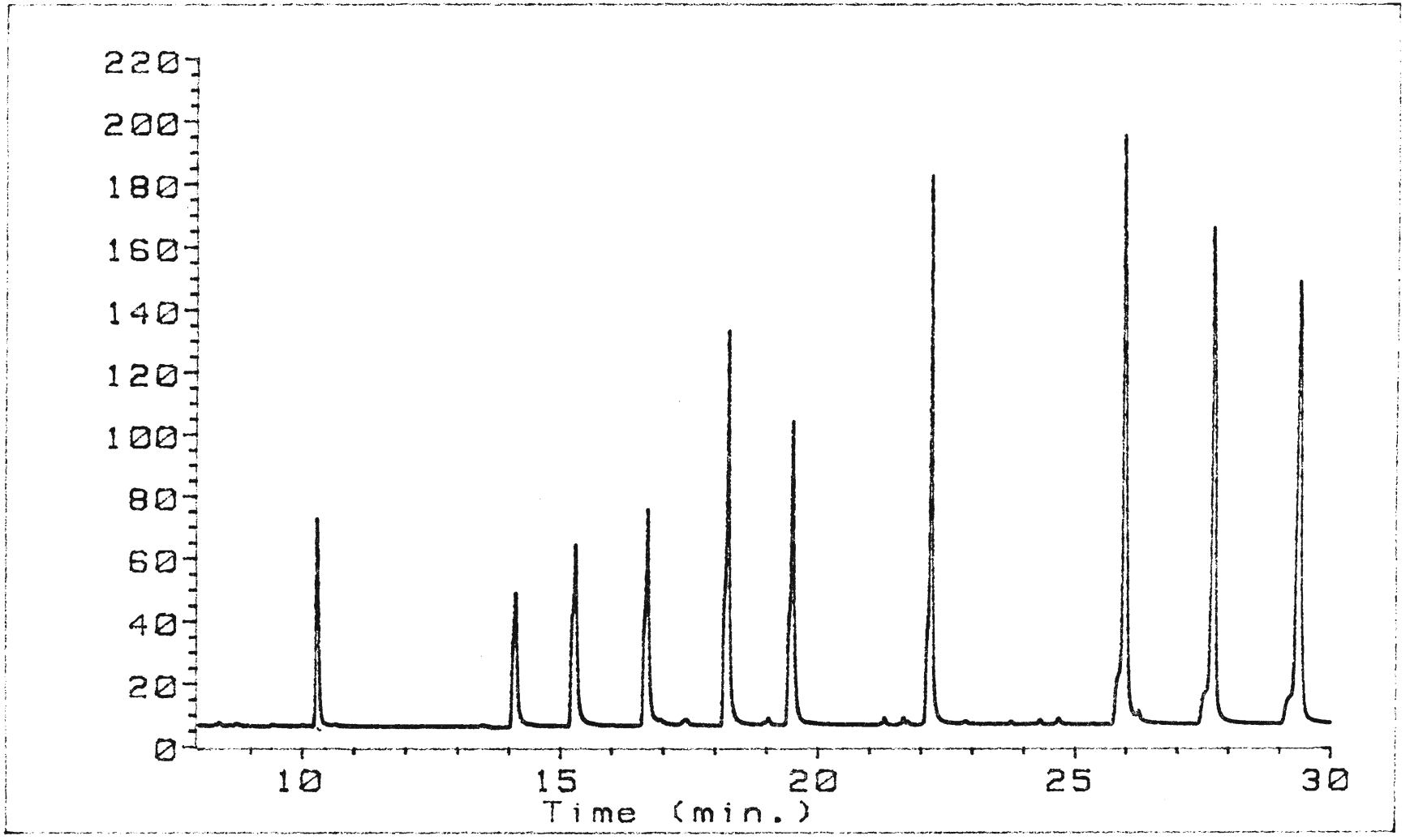


Figure 38 a. Chromatogram of 10 PCBs in p-xylene at an initial temperature of 88 °C (b.p.-50).

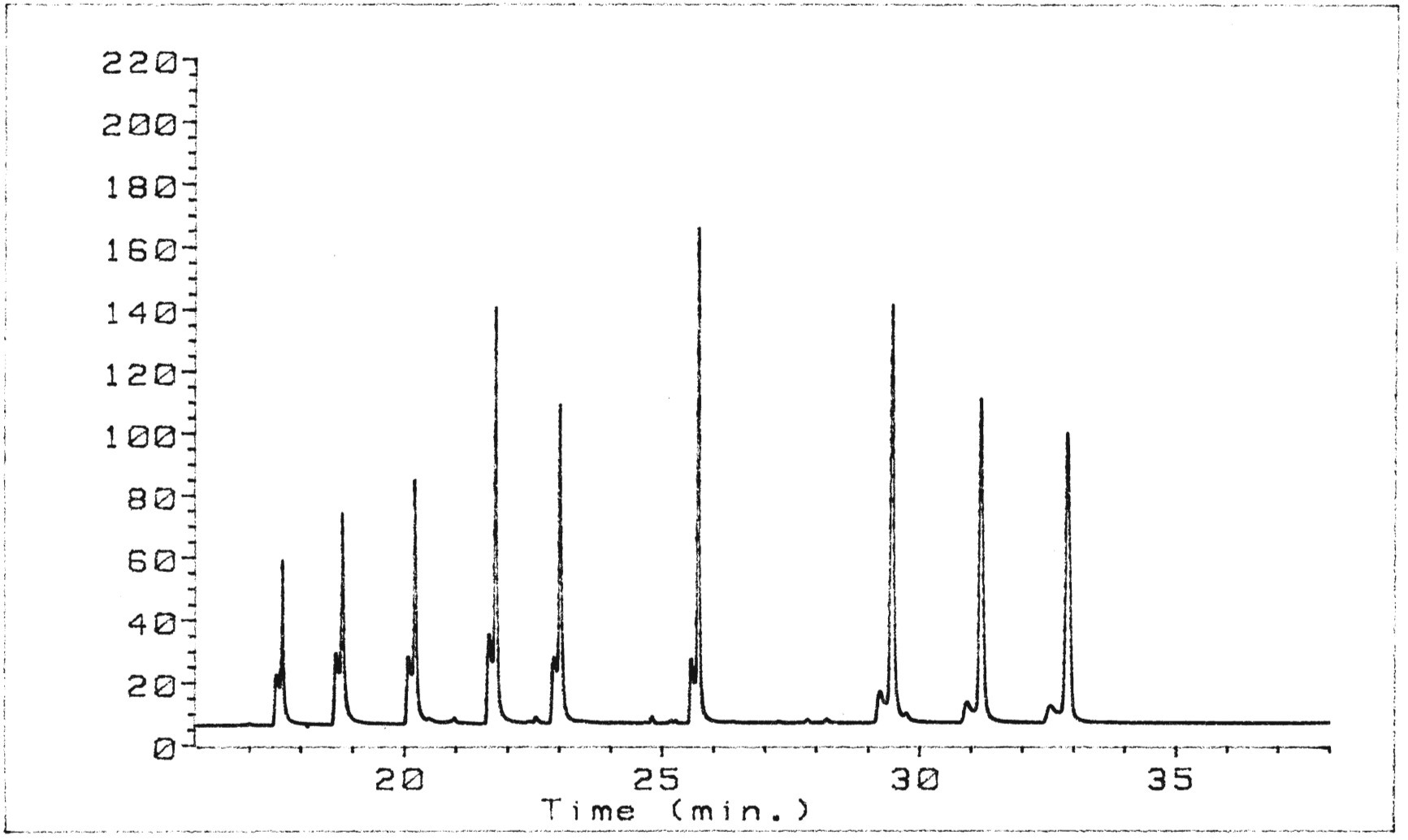


Figure 38 **b**. Chromatogram of 10 PCBs in toluene at an initial temperature of 60 °C (b.p.-50).

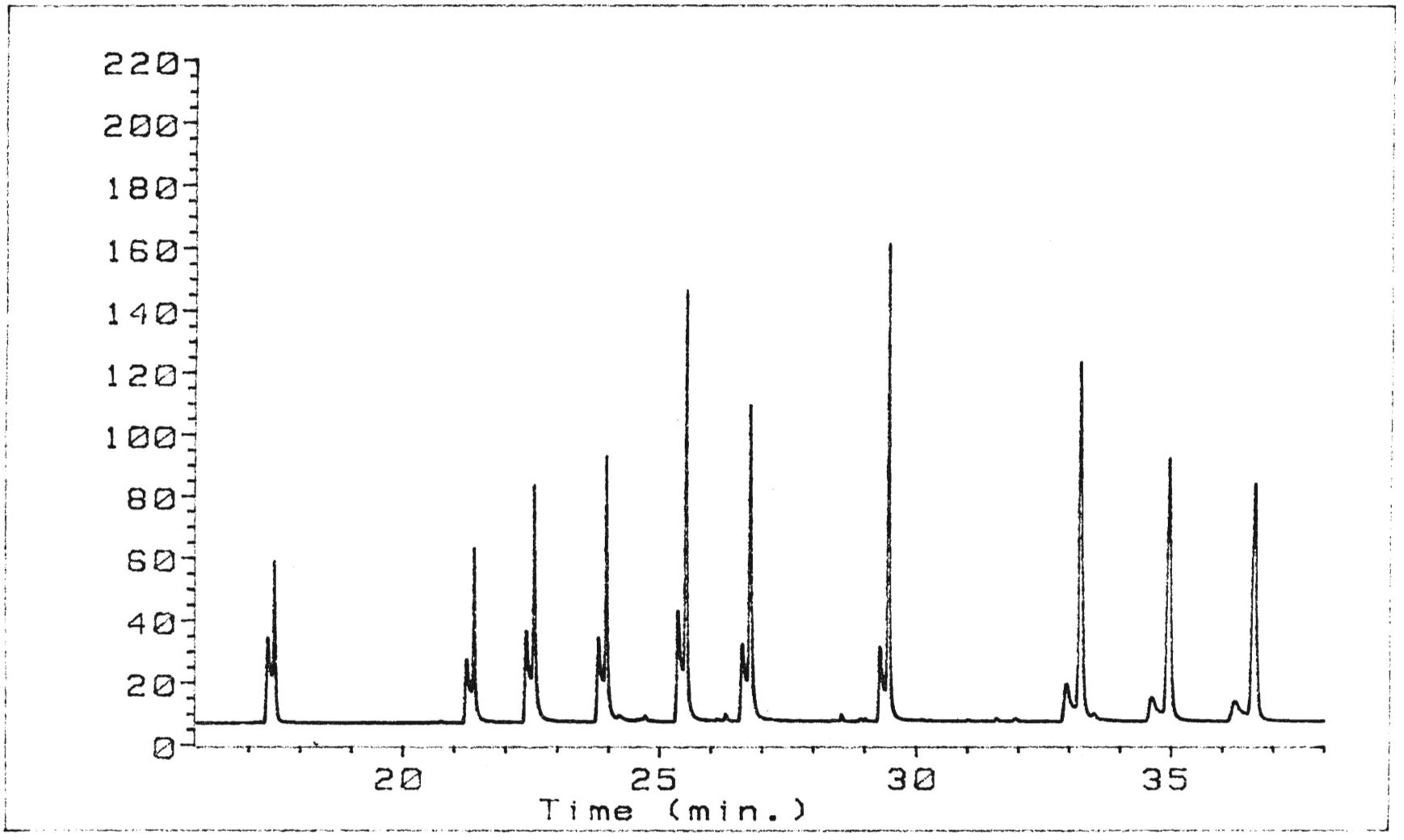


Figure 38 c. Chromatogram of 10 PCBs in benzene at an initial temperature of 30 °C (b.p.-50).

At the initial column temperatures 40 °C below the boiling points of these aromatic solvents, the solvents should be recondensed on the column inlet. If the condensed solvent provides a reconcentration effect, the peak will be narrowed to enhance the sensitivity. This seems to explain why symmetrical peaks are formed with p-xylene as solvent. However, this does not fit with the severe fronting observed with benzene and toluene. Among these three solvents, one would not expect to see significant differences in solubility of PCBs. The different volatility of the solvents should not cause above different behaviours, because the least volatile solvent, p-xylene, should be more easily condensed and should therefore produce longer bands of condensed solvent. Possibly, two kinds of movement exist during the process on column inlet. One is that the solutes spread along with the movement of solvent, which causes fronting or splitting of analyte peaks. On the other hand, the adsorption of solvent on the stationary phase and the cold trapping effect reduce the speed of solvent migration and the spread of the solutes by solvent. The cold trapping effect and solvent recondensation occurred in all three cases where benzene, toluene, and p-xylene were used as solvents. However, the adsorption capacity of the stationary phase for the solvents may not be the same because of their different structures. The two methyl groups on the structures of p-xylene molecules could improve the affinity between p-xylene and methyl silicone stationary phase. The increased affinity and wettability of p-xylene, compared with those of benzene and toluene, on the stationary phase reduced the speed of solvent migration and the spread of the solutes by the solvent. Therefore, fronting of PCB peaks is reduced with the change of injection solvent from benzene to toluene and is eliminated in p-xylene at the above conditions.

Conclusion and Proposal

Twelve solvents with a wide range of boiling points from 40 °C of dichloromethane to 215 °C of dodecane were studied in the determination of PAHs and PCBs by capillary GC with splitless injection. The great improvement of sensitivities of the 16 PAHs, considered as the priority pollutants, were achieved by using high boiling solvents. Particularly for the late-eluting PAHs, the higher boiling point solvents such as toluene, xylenes, and nonane, gave greatly enhanced signals that were between 1 and 500 times greater than the signals from PAHs in injection solvents such as dichloromethane, hexane, benzene, and isooctane. The various results suggested that the highest enhancement can be achieved by choosing a proper solvent for a specific range of PAHs. In this study, nonane was found to give the best sensitivity for these 16 PAHs.

In order to obtain symmetrical peaks and the best sensitivity of PAHs in any kind of solvent, initial column temperature was found to be the most important factor. When the initial column temperature was below the boiling point of the solvent used for injection, splitting or fronting of peaks was observed, whereas the symmetrical peaks were generally obtained at the initial temperatures at or 10 to 20 °C higher than the boiling point of the solvent used. If the initial temperature was too high, tailing of peaks were produced. During the range of initial temperature from boiling point to 20 °C above the boiling point of solvent, the solvent effect was not obtained. The cold trapping effect, however, played an important role to reconcentrate analyte in the column, resulted in symmetrical peaks with high sensitivity.

Peak height was related to peak shape and hence the greatest peak height value was obtained only when a symmetrical peak was achieved. The dependence of peak area on peak shape was reflected on the fact that a lower accuracy in integrating peak area was encountered when peak fronting or tailing occurred. Therefore, the criteria for the selection of solvents and experimental conditions should include the consideration of good peak shape and then high sensitivity.

The optimum ranges of initial column temperatures were found to be strongly dependent on the properties of solvent and stationary phase, the amount of solvent injected, and the boiling points of PAHs. When the methyl silicone stationary phase column was used to separate PAHs, alkane solvents gave the wide range of initial temperatures for forming symmetrical peaks, whereas the wide range of initial temperatures of symmetrical peaks were obtained with the injection of both aromatic solvent and alkane solvent on the 5% diphenyl: 1% vinyl: 94% dimethyl polysiloxane bonded stationary phase column. The optimum ranges of initial temperatures increased with the decrease of injection volume from 2 μl to 1 μl. Also, the higher boiling PAHs have wider ranges of initial temperatures than the lower boiling PAHs.

A mechanism was proposed to explain the effect of solvent on peak shape with the consideration of interactions among solvent, stationary phase of the column, and the analyte. This mechanism may help to understand the effect of solvent and therefore to choose appropriate solvent and stationary phase.

With optimized conditions and p-xylene as an injection solvent, the method has been adopted by our colleagues to the determination of PAHs

in river sediment and fish samples. This method has proved to be promising for the environmental analysis.

By using GC/MS, detection limits (S/N=3) obtained range from 2.4 pg for naphthalene to 86 pg for benzo[g,h,i]-perylene in toluene, and from 4.4 pg for naphthalene to 30.8 pg benzo[g,h,i]perylene in p-xylene. Relative standard deviations (RSD) of seven replicate determination of 3 ng of PAHs in toluene, ranged from 2.2 to 10.5%, when measured by peak area, and 3.9-11.0%, when measured by peak height. RSDs of 5 injections of 2 μl of 2 μg ml^{-1} 16 PAHs in nonane were 2.6 to 7.7% by peak area and 2.6 to 8.1% by peak height.

Analytical parameters in the determinations of PCBs by capillary GC/ECD were optimized by one-factor-at-a-time method. As in the study of PAH determination, effect of solvents on sensitivity and peak shape of PCBs were examined. Signal enhancement was achieved by choosing appropriate solvent for injection. Nonane, as an injection solvent, gave the highest sensitivity and wide initial temperature range for symmetrical peak in the determination of 10 representative PCBs. Studies of chromatographic behavior with relation to the property of solvent further confirmed the mechanism proposed.

Since the sensitivity is strongly dependent on the evaporation and transfer efficiencies in injector and the reconcentration effect on the column inlet, the injection of co-solvent or mixture solvent, consisting of a low boiling solvent such as dichloromethane and hexane and a high boiling solvent may provide advantages. With a mixture of two solvents for sample injection, the low boiling point solvent provides sufficient evaporation of samples and the high boiling point solvent may give appropriate reconcentration effects (cold trapping effect and solvent

effect). Thus GC peaks with enhanced sensitivity and good separation efficiency may be achieved by using the co-solvent.

As we discovered, the highest sensitivity in the determination of both PAHs and PCBs were obtained when the xylenes and nonane were used as injection solvents. If the solvent for the injection and for the extraction is the same, it may simplify analytical procedures. However, the common problem in using nonane and the xylenes for the extraction of analyte from samples is derived from their low volatilities. The difficulty in evaporating these high boiling point solvents for preconcentration made them improper for the Soxhlet extraction and ultra-sonic extraction. It may be possible, however, to overcome this problem by using mixtures of nonane or the xylenes with more volatile solvents, such as dichloromethane and hexane.

We have done a preliminary experiment in the determination of PCBs in surface water by GC/ECD coupled with preconcentration by using solid phase extraction. Unfortunately, the recoveries of spiked PCBs were very low probably due to the improper conditions for the the solid phase extraction. With further study and optimization, this method may be useful for identification and quantitation of trace levels of PCBs in environmental water samples.

Reaction between PAHs and trinitrobenzene (TNB) to form molecular complex has been known for many decades (291). Based on the literature(291), PAH-TNB complexes were synthesized by reacting equimolecular quantities of PAH and TNB in hot solvent such as alcohol, benzene, and glacial acetic acid. The complexes were needle-shaped crystals after hot solvent was evaporated. Compared with PAHs, their molecular complexes with TNB give stronger UV and fluorescence spectra. This may be applied

to improve sensitivity and selectivity in the determination of PAHs by Chromatograph with UV or fluorescence detection. A HPLC (Perkin Elmer Series 300) with UV/visible detector was used. In an initial experiment, 0.5 ml of 100 μg ml^{-1} of 16 PAHs and approximately 20 mg TNB were dissolved and mixed in 20 ml alcohol. A UV absorption spectrum was taken for this mixture on a DMS-100 UV/visible spectrophotometer (Varian) with wavelength scan mode. This spectrum was compared with those of PAHs and TNB and it showed no UV absorption wavelength shift nor signal enhancement, indicating no evidence of the formation of molecular complexes between PAHs and TNB. A single PAH, benzo[g,h,i]perlyene, and TNB were then mixed in hot alcohol. Upon the evaporation of the solvent (alcohol), we did obtain the needle-crystals in orange-red color. The orange-red color of the product was deeper than its starting materials. However, no extra peak besides those of TNB and benzo[g,h,i]perlyene was found when this product was dissolved in acetonitrile and determined by HPLC/UV. The reason for not obtaining expected more sensitive peak of molecular complex of the PAH is still unknown. It may be because the PAH-TNB complexes are unstable in the solution or because the formation of the complex requires more critical conditions. Nevertheless, it may be useful to perform more experiment on this idea.

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