CHAPTER I

INTRODUCTION

1. MICROWAVE TECHNOLOGY REVIEW

The microwave region of the electromagnetic spectrum lies between infrared radiation and the radio frequency region and it corresponds to wavelengths of 1 cm to 1 m (i.e. frequencies of 30 GHz to 300 MHz respectively). Microwave radiation may interact with matter by three different mechanisms: spectroscopic, ionic conduction, and dielectric polarization. Microwave spectroscopy occurs when molecules absorb microwave radiation and are promoted to higher energy rotational states.[1] Ionic conduction involves the microwave-stimulated migration of ions in an electromagnetic field. It is this mechanism that is responsible for heating of conducting and semi-conducting solids when exposed to microwave radiation.[2] The third mechanism of interaction, dielectric polarization, is primarily responsible for microwave heating of the object. It is this mechanism that is relevant in this research. Dielectric polarization involves the microwave stimulated rotation of molecules that have a dipole moment. It is the electric field component of the microwave radiation that causes the dipoles to respond. As the electric field component increases, molecules possessing dipoles will align with the poles of the electric field. When the field decreases the molecules return to a state of disorder. If a material contains polar molecules, dipoles will generally be in random orientations when no electric field is applied. Polar molecules will align with the poles of the electric field when electric field is applied as shown in Figure 1. Alignment and randomization occurs approximately 4.9×10^9 times per second. Heating occurs as the rotational molecular motion is converted to translational motion via collisions with surrounding molecules.[3]

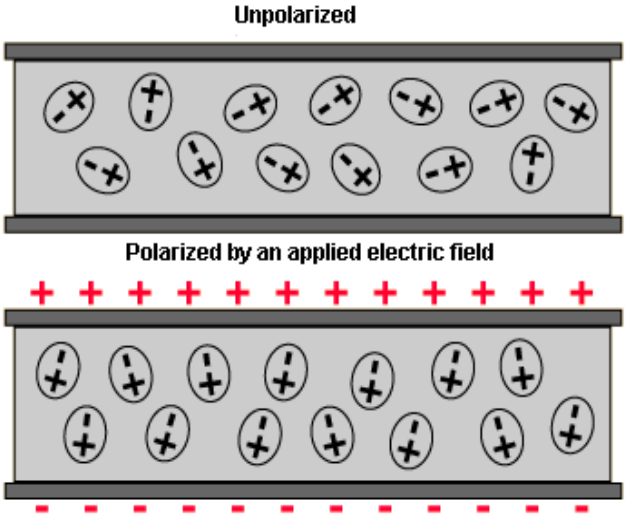

Figure 1. Alignment and randomization of polar molecules resulting from dielectric polarization.

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A major source of microwave dielectric heating lies in the ability of an electric field to polarize charges in a material and the inability of this polarization to follow rapid reversals of the electric field. The total polarization a_t is the sum of several individual components:

$$a_t = a_e + a_a + a_d + a_i$$

where a_e is the electronic polarization arising from the realignment of electrons around certain nuclei; a_a is the atomic polarization which results from the relative displacement of nuclei due to the unequal distribution of charge in the molecule； a_d is the dipolar polarization which results from the orientation of permanent dipoles by the electric field; and a_i is the interfacial polarization (Maxwell-Wagner effect) which happens when there is a build up of charge at interface. [4]

In an oscillating electric field in concert with electromagnetic radiation, the response of a material depends on the time scales of the orientation and disorientation phenomena relative to the frequency of the radiation. The time scales for the polarization and depolarization (a_e and a_a) are much faster than microwave frequencies, so a_e and a_a do not contribute to the dielectric heating effect. The importance of interfacial polarization (a_i) in the microwave region has not been well defined. The time scales for polarization with permanent dipole moments (a_d) are comparable to microwave frequencies, therefore dipolar polarization (a_d) mainly contributes to microwave heating.

Dipolar polarization is due to the dipole moment arising from different electronegativities of atoms in a molecule. If the electric field oscillates slowly, the time taken by the electric field to change direction is longer than the response time of the dipole, therefore the dielectric polarization keeps in phase with the electric field. The electric field is thought to provide the energy to make the molecules rotate into alignment. Some energy is transferred to the random motion every time during this process. A dipole is knocked out of alignment and then realigned. During this process, the temperature hardly rises because the transfer of energy is

so small. If the electric field oscillates rapidly, the dipole moment changes direction faster than the response time of the dipoles. In this case, no energy is absorbed and the material cannot be heated because the dipoles do not rotate.

In the microwave range of frequencies, the time in which the field changes is about the same as the response time of the dipoles. The molecules rotate because of the torques which is the measure of a force's tendency to produce rotation about an axis, but the resulting polarization lags behind the change of electric field. As a result, the material can absorb energy from the changing electric field and is heated.

A reliable device for generating fixed frequency microwaves was first designed by Randall and Booth[3] at the University of Birmingham in England as part of the development of Radar during the Second World War. Domestic and commercial appliances employing microwave energy for heating and cooking began to appear in the United States in the 1950's. The widespread use of microwave ovens in the world came during the 1970s and 1980s as a result of effective Japanese technology. Domestic and industrial microwave heaters are restricted to one or two frequency bands centered at 915MHz and 2450 MHz in order not to interfere with microwaves used for RADAR transmissions and telecommunications.[3] Nowadays microwave heating techniques are widely used in many fields of analytical chemistry. Applications of microwave in this regard include sample digestion for elemental analysis, solvent extraction, sample drying, measurement of moisture, analyte desorption and adsorption, speciation and nebulization of sample solutions, and microwave GC.[4] A brief discussion of several of these applications is given below.

1.1 MICROWAVE-ASSISTED EXTRACTION

Sample preparation is an important part of qualitative and quantitative analysis. In the last decade, analysts have had an increasing demand for new extraction methods which are

amenable to automation, shorten extraction times, and reduce organic solvent consumption. For these purposes some new extraction techniques have arisen, and microwave-assisted extraction (MAE) is one of them. A closed-vessel system for microwave-assisted extraction (MAE) is normally used in analytical scale laboratories. With closed vessels, the solvent can be heated well above its normal boiling point, resulting in enhancing extraction efficiency and speed.[5]

One of the main advantages of MAE is the reduction of extraction time relative to Soxhlet extraction. In conventional heating, a part of the heating time is devoted to heating the vessel before heat is transferred to the solution. Microwaves heat the solution directly, which can accelerate the speed of heating. Additionally, MAE allows for a significant reduction in organic solvent consumption and the possibility of simultaneously running multiple samples. As a result MAE is an attractive alternative to conventional techniques as evidenced by the increasing number of scientific papers published during the last decade.

A commonly used commercial microwave-assisted extraction system is the MES-1000 microwave solvent extraction system, supplied by CEM (Matthews, NC, USA). The system applies 950 W of microwave energy at 100% power and allows for 12 extraction vessels to be irradiated at the same time. One vessel is a reference for controlling heat and pressure. Extraction parameters such as the percent power input, pressure, and temperature in the range of 20-200℃ can be controlled as needed. The samples are placed into lined Teflon PFA (perfluoroalkoxy) vessels. The vessels which may number 12 are located in a carousel, which rotates through 360^{o} during the operation. Magnetic stirring in each extraction vessel is employed in some systems. Efficient stirring can cause fresh solvent contact with the sample surface continuously. If the pressure exceeds the limit, a spring device allows the vessel to open and close quickly, thus releasing the excess pressure from the microwave cavity.[5]

The application of microwave-assisted solvent extraction is widespread in analytical fields, such as highly stable organic pollutants POPs[6,7], pesticides[8-11], phenols[12-14], and antioxidant additives in polymer[15-18]. A major field in analytical chemistry dealing with sample preparation on a routine basis is the pharmaceutical industry, but so far relatively few papers have been published in this area using MAE.

1.2 MICROWAVE LIQUID CHROMATOGRAPHY

High-performance liquid chromatography (HPLC) is the most versatile of the chromatographic techniques because it is applicable to a wide variety of analytes. However, liquid mobile phases have relatively low diffusivities and high viscosities. The diffusivity of the mobile phase is a critical parameter in chromatographic separations. The speed of separation for these major chromatographies increase in the order: HPLC < SFC < GC. The reason is the diffusivities of the corresponding mobile phase increase in the same way. Therefore, HPLC would be a better technique if one can increase the diffusivity of the liquid mobile phase. Previously, diffusivity has been increased by raising the mobile phase temperature.[19-23] However, the disadvantages are that additional hardware is required in order to maintain pressure in the column so that the solvents do not boil at the elevated temperatures, and it was unsuitable for thermal unstable analytes.

Recently, Taylor and Stone[24] employed pulsed microwave radiation to increase the diffusivity of nonpolar mobile phases without heating. Dielectric polarization is usually used for heating, but it is possible to increase the diffusivity of a liquid when the liquid is only subjected to very short “bursts” of microwave radiation. In this way, there was only a minimal effect on the mobile phase temperature. A 25 cm IonPac NS-1 column with a 4 mm i.d. obtained form Dionex (Sunnyvale, CA) was put into a Samsung domestic 600 microwave oven at the standard 2450 MHz frequency. This column was made of polyetheretherketone

instead of metal, so it created no problem in the microwave oven. The peak due to the analyte 2-naphthol was sharpened by using pulsed microwave energy. The increase in plate count with pulsed microwave compared with the slight decrease in plate count when heated in a conventional oven clearly proved that the dielectric polarization phenomenon was the primary cause of the improved efficiency. This study showed it was possible to sharpen peaks in HPLC by applying microwave radiation. This result was encouraging and promising despite the use of a crude microwave system.

2. MICROWAVE GAS CHROMATOGRAPHY

2.1 INTRODUCTION

Gaisford and Walters[25] have developed a microwave GC oven which operates at 919MHz. The microwave GC oven was engineered to generate a uniform microwave field around an open tubular wall coated capillary column which was stated to eliminate cold spots which are more common in a domestic microwave oven. Moreover, the microwave heated only the column because the column contained the only appropriate microwave absorbing material. On the other hand in a conventional GC system the wall of GC oven and the air circulating in the oven are also heated in addition to the column. Selective microwave heating makes column heating more efficient, and faster cooling can be achieved because less material is heated. In the microwave GC, ramp rates up to 6℃/sec, isotherms as high as 450℃, and one minute cool-down times from 350 to 50℃ can be achieved.[25] The oven is small enough to be held in the palm of one's hand. It is anticipated that this instrumentation could result in a productivity increase of 10-20% in a GC lab, which could translate into tens of thousands of dollars per year savings.

2.2 COLUMN AND OVEN

2.2.1 Column for Microwave GC

Capillary columns made of fused-silica and coated with a siloxane polymer are predominantly the columns of choice for GC. Organic molecules and silica do not absorb appreciable microwave energy at the frequencies used by the oven. Therefore, conventional wall coated capillary columns (e.g. fused silica) and any organic compound injected into it or that coats the surface will remain unaffected by the microwaves in the oven. The open tubular capillaries used for the microwave oven are also coated on the outside with a microwave-absorbent material that efficiently converts microwave energy to thermal energy. Thus, the column, stationary phase, and analytes are heated in a uniform, precise, and repeatable manner. The stationary phase of the microwave GC column can be in theory any type used in a conventional GC column. A 100% methyl silicone column was used in this research. The GC column can be heated up to 450℃ thus eliminating the need for special metal-clad columns in high-temperature analyses[25] although this temperature limit will depend on the stationary phase. For a methyl silicone column, the maximum column temperature is 325 ℃.[25] Common capillary gas chromatographic columns have different sizes ranging from 0.1 to 0.53 mm internal diameter and from 4-60 meters length. A column of 30 meters in length with an internal diameter of 0.32 mm was used in this research. The microwave GC column and oven can be installed in a conventional GC oven by connecting the microwave column with the injector and detector of the conventional GC oven as shown in Figure 2.

As shown in Figure 2, the microwave-heatable GC is made of two parts. There is a Antek 3600 microwave controller shown on the right. The host Agilent 6890A conventional GC is on the left wherein the door of the GC oven is removed. The microwave GC oven and column are installed inside the host GC oven. The microwave GC column is connected to the injector and detector of the host GC. A waveguide cable connects the microwave generator in

the microwave controller to the microwave GC oven. A thermocouple inserted into the column is used to measure the column temperature. Air is introduced into the microwave GC oven through a plastic tube to cause faster column cooling.

2.2.2 Microwave Column Oven Versus Conventional Microwave Oven

Conventional microwave ovens are closed metal boxes of at least a cubic foot in volume and the electromagnetic energy is confined. Conventional microwave ovens are operated at 2.45 GHz, so the wavelength of electromagnetic waves is about 122.4 mm. A conventional microwave oven acts as a multi-mode resonant cavity. "A resonant cavity is a structure in which the interference pattern between multiple reflecting electromagnetic waves excited in the cavity resolves itself into a well defined and stable standing wave pattern."[25] A multi-mode resonant cavity is one in which there may be different and unique standing wave patterns. A conventional microwave oven can have many modes because the oven dimensions are many times the wavelength. As a result, there is a complicated pattern of overlapped standing wave patterns from different resonant modes. At some points the electromagnetic field strength is high; at other points the field strength is zero. Objects in such electromagnetic fields are heated in complicated and uneven patterns. The space between adjacent hot spots is typically less than one wavelength. Domestic microwave oven manufacturers attempt to make the temperature of heated objects even by two ways: (1) using metal fans to stir the mode pattern in the oven; (2) rotating platforms to move objects around physically in the oven. Both of the methods can improve heating modestly, but obviously they cannot be used for the microwave GC oven.

In order for a microwave oven to be used for heating a coiled chromatographic column, it must be a single mode oven, so that the electromagnetic field is predictable inside the oven. Non-rectangular oven geometries are preferred because existing chromatographic columns cannot be bent into tight rectangular shapes. As a result, the microwave GC oven used for gas

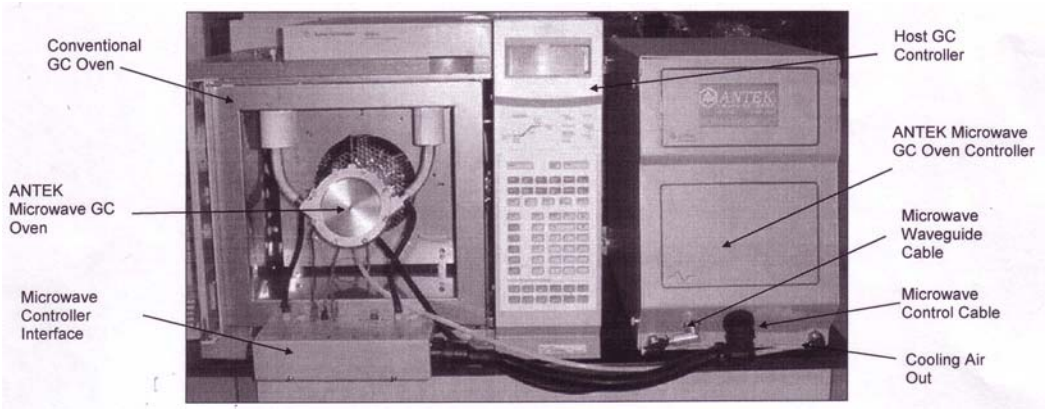

Figure 2. The actual microwave GC instrument in laboratory.

chromatography invented by Gaisford has a single mode resonant cavity which is described as a cylindrical resonant cavity. This cavity can be tuned to deliver microwave power to the column by adjusting the length of the coupling antenna. The microwave wavelength cannot be smaller than the length and/or the diameter of the cavity in order to achieve a single mode resonant cavity. A single mode resonant cavity can ensure that there is no uncertainty in the electromagnetic field distribution.

The Microwave GC Oven has been designed to generate a uniform microwave field around the column and thus eliminate cold spots, therefore the size of the microwave GC oven is designed precisely for it. Microwaves heat the materials that can absorb microwave energy. In a microwave GC oven, only the column is heated because the column contains the only appropriate microwave absorbing material. Selective microwave heating makes more efficient heating possible because energy is transferred directly to the heated object. Moreover, faster cooling can also be achieved because less material is heated. Column cooling can be achieved by introducing air into the oven and reducing microwave energy supply. [25]

2.3 PREVIOUS WORK CONCERNING MICROWAVE GC

There is only one publication related to the applications of microwave GC. Sacks and Brenna have done some work on the comparison of microwave and conventionally heated columns for GC of fatty acid methyl esters.[26] They employed a polyethylene glycol-based column (30 m x 0.25 mm). During the temperature ramp, the microwave oven was heated at a rate of 360 ^{o}C/min starting at 60 ^{o}C. The GC head pressure was 18 psi, resulting in a column mobile phase flow of 1.6 mL/min at 60 ^{o}C. The microwave GC decreased the time for analysis of FAMEs by 20% compared to a conventionally GC. The prospects of microwave

GC were stated to be promising where fast GC is used considering that its rapid heating potential can shorten analysis time greatly.

Negative temperature programming might be a useful tool for analysts to gain separation of co-eluted components without special columns or more expensive techniques. Nazem[27] previous tested the feasibility of negative temperature programming by using microwave GC. Negative temperature programming can cause the column to cool rapidly after the column has been heated up to a relatively high temperature because the thermal mass of the microwave GC oven is small. Air is introduced into the microwave oven by a plastic tube from an air tank when cooling down the column. A difference between actual and set point temperature of the column when the column was cooled was noted. There would be a temperature deviation between actual column temperature and setting column temperature if the cooling was too fast. The effect of different air pressures and different tubing diameters on cooling was studied in Nazem's work.[27] Cooling air pressure was tested from 3 to 50 psi, and 12 psi was shown to be the optimized cooling air pressure. Different tubing sizes from 1/8 to 1/4 inch were tested, and 1/4 inch tubing was the optimum.

My research focused on the study of negative temperature programming and its application to critical peaks which are difficult to separate. Employing negative temperature programming properly may make the peak separation time longer between two peaks which otherwise eluted close to each other.

3. ALTERNATIVE APPROACHES FOR IMPROVING RESOLUTION

Resolution is an important parameter in chromatographic separation. The classic equation for resolution is shown in equation (1). Improving a separation can be accomplished by effecting changes in any of the three parameters, plate number (N), retention factor (k) or

selectivity (α)[28]. R_s=1.0 is sufficient for qualitative analysis, and R_s =1.5 is sufficient for quantitative analysis.

$$R_s = (\alpha - 1/\alpha)(k/k+1)N^{1/2}/4 \quad (1)$$

R_s - resolution
$k = (k_1+k_2)/2$
$k_1 = (W_1)_S/(W_1)_M$
$k_2 = (W_2)_S/(W_2)_M$
α - selectivity
k - average retention factor
k_1 – retention factor of solute 1
k_2 – retention factor of solute 2
N - plate number
$(W_1)_S$, $(W_2)_S$ – the amount of solute in the stationary phase
$(W_1)_M$, $(W_2)_M$ – the amount of solute in the mobile phase

3.1 MAXIMIZE THE PLATE NUMBER OF THE CHROMATOGRAPHIC SYSTEM.

3.1.1 Column length. One simple way to achieve better resolution is to increase column length (L), which increases the number of theoretical plates (N) directly as shown in equation (2). The increase in R_s is less efficient because L is proportional to the square root of R_s. Long columns of 30 m or 60 m are typically used for complex mixture in order to get good resolution.

$$H = L/N \quad (2)$$

H – plate height
L – column length
N – plate number

3.1.2 Linear velocity. The van Deemter equation for open tubular column is shown as equation (3). When H vs. u is plotted, the so-called van Deemter plot takes the shape of a nonsymmetrical hyperbola. As one would expect from the van Deemter equation in which one term is multiplied by velocity while another is divided by it, there is a minimum in the curve (i.e. an optimum velocity which provides the highest efficiency and smallest plate height[28]). The minimum H is desired in the gas chromatography. It is logical to assume that chromatography would be carried out at the optimum linear velocity in order to get the best

resolution. However, linear velocity is inversely proportional to retention time $t_{R,}$ so increasing linear velocity can decrease analysis time. An increasing in linear velocity over the optimum value causes the theoretical plate height (H) to exceed the minimum H (H_{min}). According to theory, operating at $u= 2u_{opt}$ causes only a 25% loss in separation efficiency and a 12% loss in R_s.[29]

3.1.3 Internal diameter. In open tubular columns the speed and resolution are related to the column internal diameter.[30,31] By reducing the internal diameter, a higher efficiency per length (L) is produced. A decrease in column diameter results in a proportionately decreased value of minimum plate height (H_{min}). When the internal diameter is reduced, optimal average linear velocity (u_{opt}) will also be faster. Both results lead to a shorter void time (t_M) and a proportionally shorter analysis time. Since peak capacity is proportional to $d_c^{3,}$ the penalties are much lower sample capacity and much higher carrier gas pressures required.

$$H = B/\bar{u} + C\bar{u} \qquad (3)$$

H – plate height
B – longitudinal molecular diffusion
C – mass transfer in the stationary liquid phase
$\bar{u}$ – average linear velocity

3.1.4 Film thickness. Column film thickness is another factor that affects resolution. To minimize the contribution of mass transfer C term in the van Deemter equation, the film thickness should be small. Rapid diffusion through thin films allows the solute molecules to stay closer together[28], which results in smaller peak width and better resolution. Commercial columns are available with films of 0.1 μm although 0.25 μm films are more common. While thin films give high efficiencies and are good for high-boiling compounds, it should be remembered that they can accommodate only very small sample sizes.

3.2 MAXIMIZE THE SELECTIVITY OF THE CHROMATOGRAPHIC SYSTEM

Chromatographic selectivity is the ability to distinguish between compounds. This can be realized by separation or by detection once the method for sample preparation has been selected. Analysts should select a column which gives the maximum selectivity for the pairs of compounds which basically co-elute.

3.2.1 Comprehensive 2D GC

Comprehensive two-dimensional GC opened a new future for GC.[32,33] The very high separation power offered by comprehensive two-dimensional gas chromatography (GC x GC) allows one, even in case of very complex matrices, to separate the target compounds from the interferences. Two columns of different selectivity can be combined together with or without an intermediate trap.[34] GC X GC is a multidimensional separation technique in which the resolving power of two columns is applied to some or all of the components in a sample. This powerful technique results in very high peak capacity and has been applied to very complex mixtures. 2D-GC is used in the field of trace analysis for the potential of enhanced sensitivity as a result of analytes which are zone-compressed by modulation at the end of a first-dimension column and using a very fast elution in the second-dimension column. 2D-GC is often used in a normal temperature program experiment, but it can be considered faster GC considering the number of resolved peaks achieved in a short given time. "Comprehensive 2D GC means the separation power of each column applies fully to each analyte independently to eliminate the dependencies of the second column's separation on the relative position of peaks from the first column."[35]

Comprehensive GC separations can be accomplished many ways. The most popular method in use today includes thermal modulation at the juncture of columns 1 and 2. Figure 3[35] shows the main principles of GC X GC with a thermal modulator. The typical method is

to blow cooled nitrogen or air over a section of the entrance to column 2 intermittently. When the cooling flow is turned off, the cooled column section rapidly reheats via the circulation of hot oven air. When cooling is applied, peaks that are eluted from column 1 are trapped at the beginning of column 2. When heated, the peaks are released, and they begin to move again in a narrow band to start the secondary separation. During the heating stage, however, any new peak material that exits column 1 will not be trapped, and thus will enter column 2 with the already trapped material. This might produce some unexpected peak overlapping. The second thermal modulator solves this problem. The two modulators are heated and cooled out of phase with each other. Peaks are trapped at the first modulator when it is cooled down. The second modulator is also cooled down and then the first modulator is heated. The trapped peaks move to the cooled second modulator when the first modulator is hot. Then, when the first modulator is cooled down again, the two trapping zones are effectively isolated from each other. The second modulator is heated, and the peaks trapped are released into column 2. Nothing new coming from column 1 enters into column 2 because the first modulator is still cooled at that time. This method effectively isolates the two columns from each other for the purposes of GC X GC.

Typically in 2D GC the first column has ordinary speed and the second column has pretty high speed of separation. The analysis time of column 2 is 0.1 min or less with very short (1-2 m) narrow-bore (100-180 um i.d.) dimensions and linear velocities higher than optimum.

2D GC has been applied to a number of complex and challenging samples. Shellie and Marriott[36] applied 2D GC with quadrupole MS detection to the essential oil of *Pelargonium graveolens*. The area in which 2D GC x GC excels is the elucidation of petroleum samples. Lewis and Hamilton[37] reported the excellent ability of 2D GC with TOF/MS to analyze gasoline at high isomeric complexity. Undoubtedly, 2D GC is a promising technique of GC.

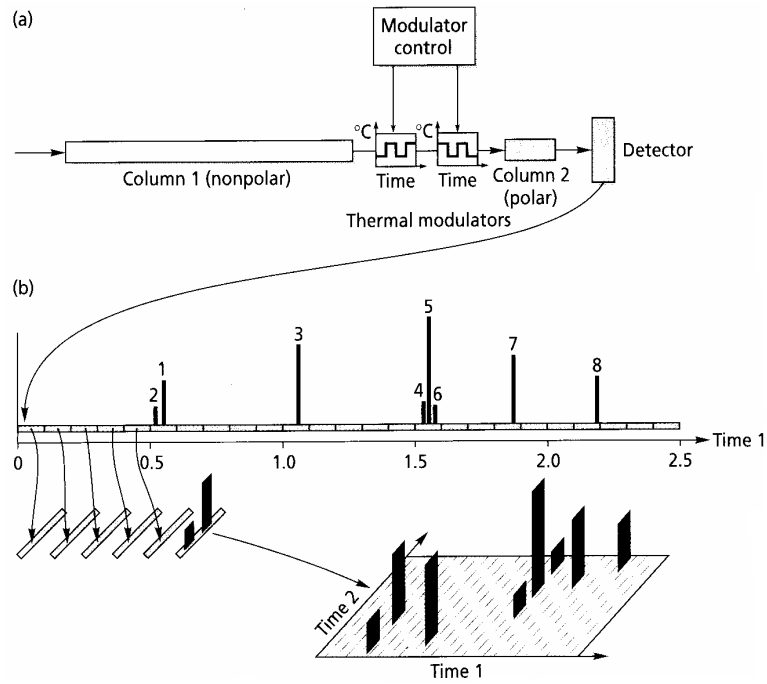

Figure 3.[35] GC X GC system.

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3.2.2 Selective detection

Selective detection can be applied to the compounds which are needed to be separated from each other. Selective detection makes the sample preparation step easier. For example, the electron-capture detector (ECD) is typical detector for halogenated compounds.

MS is another powerful selective detector. Fast GC-MS has the potential to be a powerful tool in routine analytical laboratories by increasing sample throughput and improving laboratory efficiency. GC-MS can rapidly and automatically detect and resolve overlapping peaks for compounds with different mass. "The critical feature of GC-MS that is not observed with fast GC-MS using element selective detectors is that MS gives another adjustable degree of control in sensitivity and selectivity (element selective detectors can be very discriminating between analyte and matrix, but this is not the case from analyte to analyte)."[38] Overlapping control of sensitivity and selectivity allows one instrumental component (GC or MS) to compensate for worse performance with other components, as indicated by the compensation arrows in Figure. 4[38].

Mass spectral deconvolution software is an effective tool to resolve co-eluting peaks in GC-MS. Deconvolution programs are powerful because they automatically do perfect background subtraction of distinct MS spectra to identify individual components within a mixture that has been minimally separated by chromatography. The automated mass spectral deconvolution and identification system from NIST[39] can distinguish between compounds with different mass spectra separated in time by half a scan apart, which is available for free on the Internet (http://chemdata.nist.gov/mass-spc/amdis/).

Speed and/or selectivity can be gained by using deconvolution in GC-MS. A practical measure of selectivity in a separation is peak capacity, which is the number of peaks that could be accommodated in a separation. As stated previously, MS can provide an additional

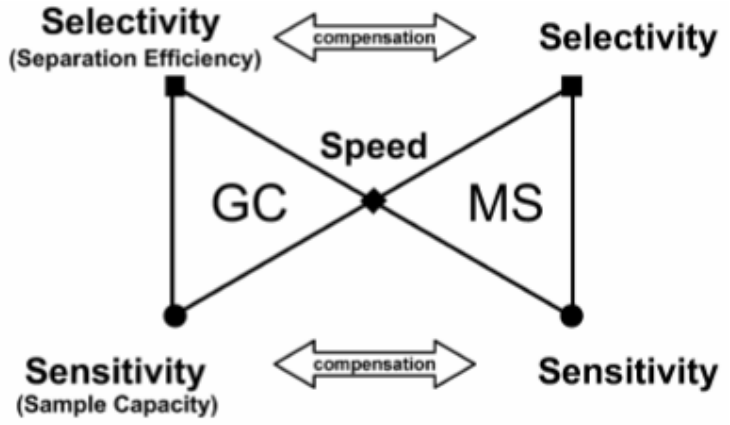

Figure 4.[38] The combination of GC and MS for optimization of speed. The sensitivity and selectivity of each approach can be used to compensate for losses in the other to provide a faster analysis of potentially the same quality.

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degree of selectivity to compensate for losses in GC separation power. For example, a spectral collection rate of 5 Hz for a width of 1 s provides the ability to resolve 10 times more compounds by their distinct mass spectra if the deconvolution program can distinguish peaks separated by half a scan width. Therefore, a 10-fold faster separation could be conducted to achieve the same effective peak capacity as GC with non-selective detection. Actual peak width is immaterial in this calculation, and the effective peak capacity in GC-MS is a factor of 2 greater than the number of spectra acquired across the GC peak. Therefore, 10 points per peak yields a 20-fold higher effective selectivity in GC-MS than GC without MS.[38]

3.3 MAXIMIZE THE RETENTION FACTOR OF THE CHROMATOGRAPHIC SYSTEM

3.3.1 Negative temperature programming

Negative temperature programming has been used in supercritical fluid chromatography (SFC) because a decrease in temperature results in greater solvating power of the mobile phase. Supercritical fluids were first used as chromatographic mobile phases more than four decades ago.[40,41] It has been generally accepted that density changes of the fluids generate important advantages in SFC.[42,43] The largest effects on density from changes of pressure are found near the critical temperature for the fluid. To apply this advantage in SFC, research and applications have focused on pressure programming and temperature programming. Advanced SFC density programming could use both pressure and negative temperature programming to achieve optimum performance. Inverse temperature programming has been reported to be a useful technique to adjust the density of supercritical fluid.[44,45]

Negative temperature programming in SFC has been applied to many compounds. Technical PCB mixtures have been analyzed by packed column supercritical fluid chromatography using negative temperature programming.[46] When using negative

temperature programming the chromatographic resolution was considerably better than under isothermal conditions as the last eluted peaks showed no broadening. The separation of pyrethrins and pyrethroids in SFC was optimized by negative temperature programming.[47] An isobaric (16.2 MPa) negative temperature gradient of -1.2℃/min from 130 to 80℃ holding for 10 min was used. Negative temperature programming is thus an appropriate and highly reproducible substitute for pressure programming in SFC.

Negative temperature programming in GC might be a useful tool for analysts to cause co-eluted components to separate without special columns or more expensive techniques. The selectivity (α) and plate number (N) are constants for a fixed stationary phase and analytes. Therefore, an increase in average retention factor is the only way to improve resolution according to equation (1). The temperature of the column decreases for a short time in negative temperature program. During the cooling ramp the solute prefers to stay in the stationary phase rather than in the mobile phase. Thus, the average retention factor (k) increases at low column temperature, and the value of k/k+1 also increases. As a result R_s is improved using negative temperature programming.

Mass transfer of the solute in negative temperature program is shown in Figure 5. At equilibrium with isothermal conditions, the solute achieves partitions SP and MP in Figure 5a. An instant later the mobile phase moves the analyte downstream. The solute molecules in the MP have moved, therefore, ahead of those in the SP thus broadening the overall zone of molecules in Figure 5b. The solute molecules in the SP and MP refocus when the column temperature decreases during the cooling ramp of a negative temperature program in Figure 5c. Therefore, sharper peaks are expected resulting from the smaller peak width because of low diffusibility at lower temperature during the cooling ramp. Smaller peak width can help to result in better resolution.

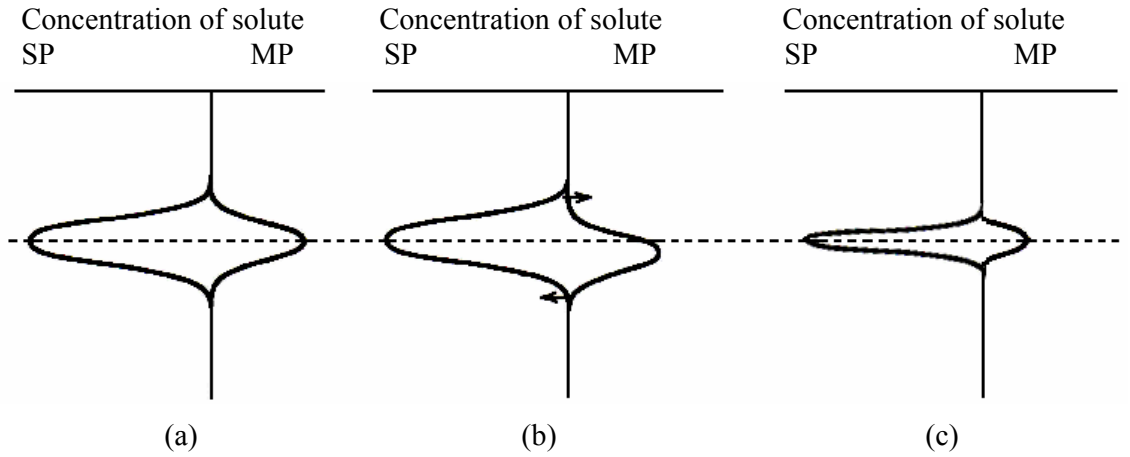

Figure 5. Mass transfer of the solute in negative temperature programming.

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4. POLYCYCLIC AROMATIC HYDROCARBONS

4.1 PROPERTIES, NOMENCLATURE, AND TOXICITY

Polycyclic aromatic hydrocarbons (PAHs) may well be the most widely studied class of environmental pollutants. In the mid-1930s the discovery of carcinogenicity of benzo[a]pyrene, dibenzo[a,h]anthracene[48] and other PAHs provided the landmark for PAHs identification and estimation in the environment. Carcinogenic, mutagenic and the toxic nature of polycyclic aromatic hydrocarbons (PAHs)[49,50] as well as their widespread presence and persistence have provided the impetus for the continuing study of these compounds for environmental monitoring. The importance of PAHs is indicated by the substantial interest given them by analytical chemists today, and it is not uncommon for a novel or improved analytical technique to be evaluated using PAHs as test compounds.

PAHs are formed and released into the environment through natural and man-made sources. Natural sources include volcanoes and forest fire, while the man-made sources include burning of wood, auto mobile exhaust, industrial power generators, incinerators, production of coal tar, coke, asphalt and petroleum, incomplete combustion of coal, oil, gas, garbage, tobacco and charbroiled meat. From these sources, a significant proportion of the PAH emissions enters the atmosphere directly and/or is adsorbed on airborne particulate matter. The dangers posed by the presence of PAHs in the atmosphere are obvious because of their biological properties. Their existence in the surrounding air has a direct impact on the human population. However, most of the experimental evidence for carcinogenicity of PAHs in humans is based on studies carried out on occupational workers exposed to these compounds in the workplace. These studies have associated lung and skin cancer observed in human subjects with exposure to PAHs via inhalation or dermal contact. Studies involving oral administration of PAHs have been performed on animals.

Polycyclic aromatic hydrocarbons can be classified as alternant and nonalternant. Alternant PAHs contain only six-membered rings while nonalternant PAHs posses both six- and odd-numbered rings (e.g. five- or seven-membered rings, etc.). PAHs can also be named how the rings are fused together such as if the benzenoid rings are fused together in a linear fashion, the PAH is an acene (e.g. anthracene, naphthacene, tetracene, pentacene, etc.). When the benzenoid rings are fused together in an angular arrangement, their name ends with phene (e.g. pentaphene).[51] A few examples of environmentally important PAHs are given in Figure 6.

4.2 PAH ANALYSIS

An extensive overview on the methods of PAH anlysis can be found in the reviews of Lee[52], Law[53] and Furton[54]. Gas chromatography is still the most widely used method in analytical chemistry. The fundamental characteristic for a compound to be analyzed by GC is its volatility within the temperature range used. PAHs containing up to 24 carbon atoms may be anlyzed by GC.[55] The general amenability of PAHs to GC analysis has caused this technique to be the principle analytical technique for these compounds in environmental samples for more than forty year.

The PAH[56-59] and nitro-PAH[60-62] content in diesel vehicle exhaust received special attention in the 1990s since diesel fueled private automobiles appeared to be making a comeback, especially in Europe. The US National Institute of Standards and Technology had also developed diesel particulate extracts as standard reference materials.[63]

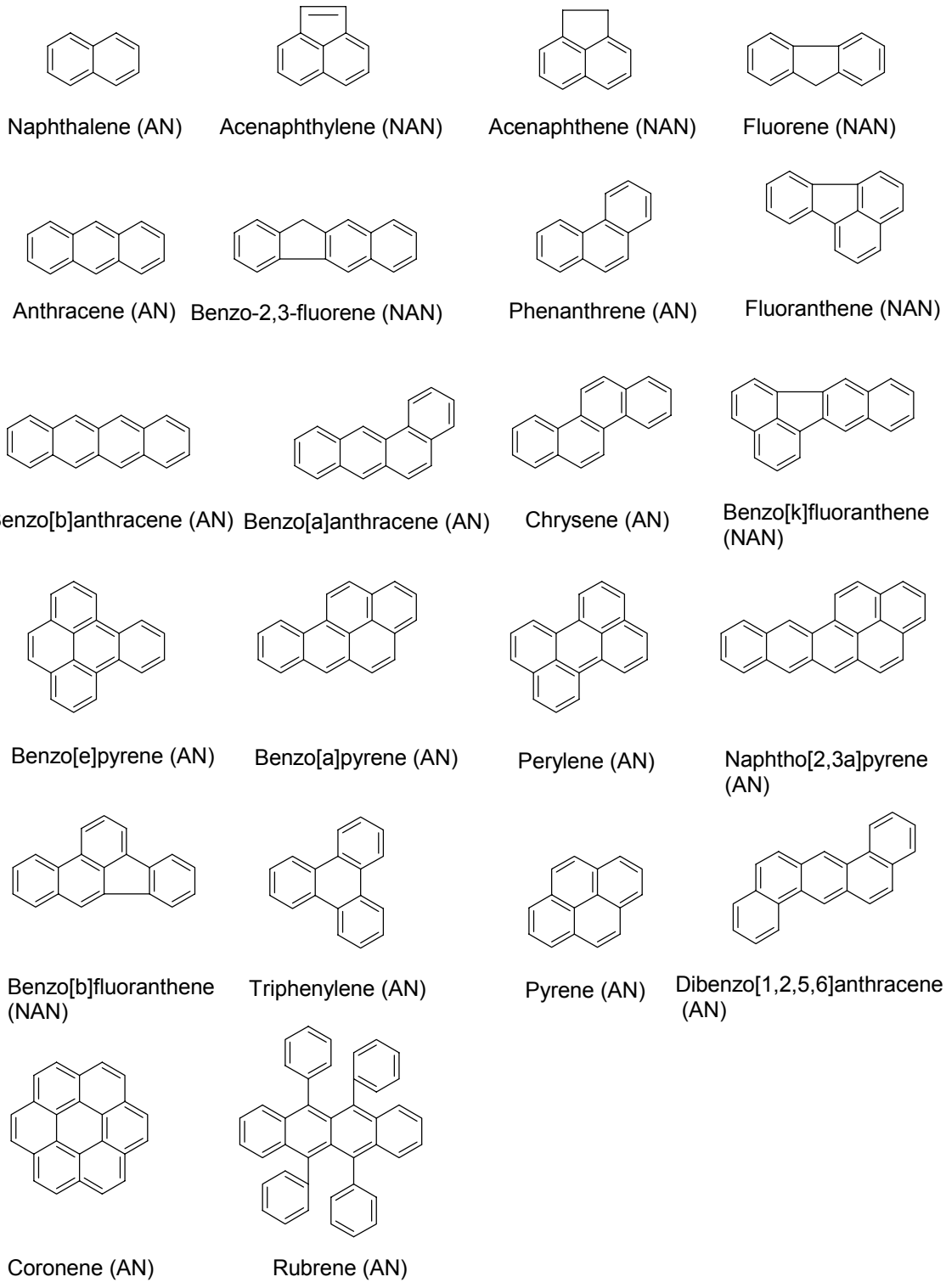


Figure 6. Structure and classification of selected PAHs. ‘‘AN’’ stands for alternant PAHs and ‘‘NAN’’ stands for nonalternant PAHs.

Indoor air has an important impact on a person's well-being. Rogge[64] studied the emissions from natural-gas home appliances, including a space heater and a water heater which were both evaluated to be in good condition. The authors found that mutagens of PAHs formed 22% of the particulate mass emitted, which suggested that exhausts from natural-gas combustion should be given more attention than before.

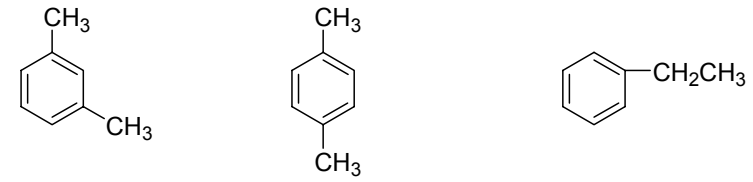
PAHs in urban air has been studied involving the use of GC-MS as the primary technique. Rogge[65] investigated the contribution of biogenic sources of trace amounts of PAHs to urban atmospheres; Fernandez[66] identified and quantified 15 PAHs in urban and semi-urban atmospheres; Roussel[67] used both HPLC and GC-MS to establish the point source of atmospheric PAHs emissions.

In recent years, further improvements in PAH anlysis by GC have seen to the development of more conventional stationary phases specifically for these compounds. For example, Klasson-Wehler [68] and Sinkkoken [69] described the use of improved stationary phases for gas chromatography of PAHs; while Bemgard [70] compared several high-temperature stationary phases for their separation.

The majority of PAH compounds, including the sixteen listed by the US EPA, can be conveniently analyzed by GC. Biologically active PAHs are usually also the more volatile ones, so GC is intuitively, the appropriate method for their analysis. Conversely, the larger or less volatile compounds must be separated by another technique. HPLC is the most popular of these alternative techniques. HPLC is more acceptable for thermally-unstable PAHs and can be used for a much wider range of PAHs with a large number of rings. For instance, Jinno[71] reported the reversed-phase HPLC of a ten-ring system, ovalene. Moreover, the US EPA specifies HPLC as the technique to use for PAHs present in aqueous effluents[72]. Reversed-phase HPLC has tended to be more popular for PAH separation than normal-phase HPLC[73].

However, this does not always mean that it is easier to develop a method for HPLC since more parameters that may be exploited in order to get a satisfactory separation can also be a disadvantage. The general amenability of PAHs to GC analysis and the advantages of GC for resolving power and the capability to detect low concentrations have always made GC/GC-MS to be the principal analytical technique for PAHs analysis.

In our study, efforts have been made to obtain the baseline separation of four critical pairs using negative temperature programming in the microwave GC. In the first part of the study, two non-volatile critical pairs benzo(a)anthracene-chrysene[58,74-76] and phenanthrene-anthracene[74-77] were studied. The standard PAHs mixture was studied to investigate the influence of negative temperature programming versus positive temperature programming for the resolution. The second part of our study, two volatile critical pairs ethylbenzene-m-xylene and m-xylene-p-xylene were studied using negative temperature programming. Ethylbenzene and m-xylene[78] have much lower boiling points than PAHs. In GC m-xylene and p-xylene which typically appear as a single peak in GC were studied to test the limit of negative temperature programming. m-Xylene and p-xylene have a boiling temperature difference of 0.77℃, but they are not separated on a PDMS column[78].



m-Xylene p-Xylene Ethylbenzene

Figure 7. Structure of xylene and ethylbenzene.

CHAPTER II

QUANTITATIVE ANALYSIS AND APPLICATION OF NEGATIVE TEMPERATURE PROGRAMMING

1. INTRODUCTION

Negative temperature programming was employed to analyze and quantitate four different critical pairs of volatile and non-volatile compounds. The influence of holding time in the cooling ramp and reheating rate in negative temperature programs for the resolution were investigated. Optimized conditions were employed to quantitatively analyzed the critical pairs of compounds. Positive temperature programming and negative temperature programming were conducted on the same sample. The results obtained from negative temperature programming were compared to those from positive temperature programming. The main objective of this study was to investigate the feasibility of negative temperature programming for qualitative and quantitative analysis of critical pairs of compounds.

2. EXPERIMENTAL

2.1 APPARATUS

An Antek Instruments (Houston, TX) 3600 microwave controller equipped with microwave GC oven and column installed in A host GC was used for all parts of the study. The host GC system was an Agilent 6890A (Palo Alto, CA). Ultra high purity of helium as carrier gas was obtained from Airgas Inc. (Radnor, PA). A Supelco microwave methyl silicone column (Bellefonte, PA), 30 m x 0.32 mm with 1 μm film thickness was used for the separations. The microwave GC oven control system shown in Figure 8 gives more details of the control system. A thermocouple inserted into the column was used to measure the column temperature. Pressurized air was introduced into the microwave GC oven from an air tank

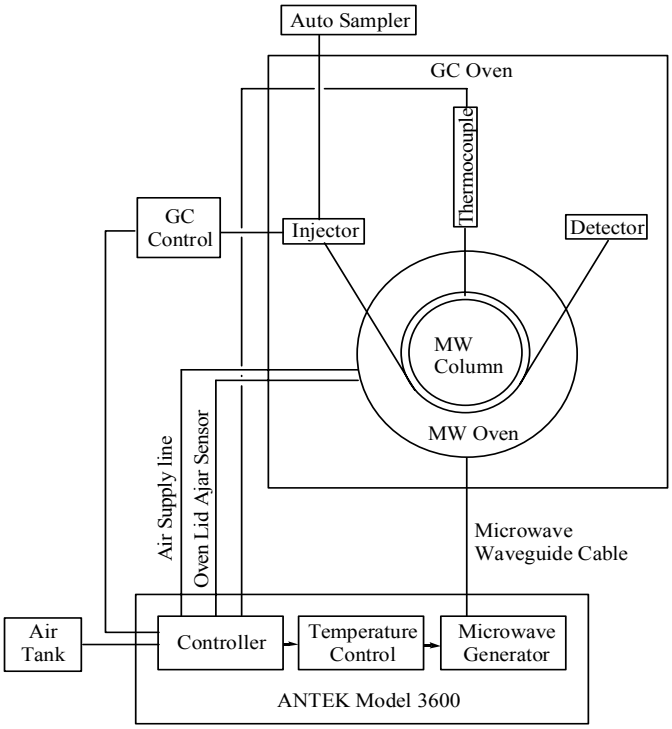


Figure 8. Microwave GC oven control system.

to cause faster column cooling. A waveguide cable connected the microwave generator to the microwave GC oven. The Antek model 3600 microwave controller is a microwave generator and controller designed to produce microwave energy according to operator-selected temperature settings and then transmit the energy to the microwave oven to heat the microwave GC column to the preset temperature levels.

2.2 REAGENTS

HPLC grade methanol and dichloromethane (Burdick & Jackson, Muskegon, MI) were used. Naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, ethylbenzene, m-xylene, and p-xylene were purchased from Aldrich Chemical Co. (Milwaukee, WI). The chemical name, chemical structure, and physical properties were given in Figure 6, Figure 7, Table 1. The standard mixtures at various concentrations were prepared using methanol (MeOH) and methylene chloride (MeOH:CH_2Cl_2 = 1:1) as solvents. A standard mixture at the concentration of 1000 ppm for each component standard was first made and successively diluted to 20 – 100 ppm.

2.3 GC SEPARATION

The inlet temperature was set to 280℃, and the FID detector temperature was set to 290 ℃. Split injection mode with a split ratio of 1:10 was used. The make-up flow rate of helium was 10 mL/min. Cooling air pressure for the microwave GC oven was set at 12 psi.[27] If not noted, the injection volume was kept at 1 μL. A constant inlet pressure mode was used and the optimized inlet pressure was 15 psi.

Compound	Name	Boiling Point (℃)
1	Naphthalene	217.7
2	Acenaphthylene	280
3	Acenaphthene	279
4	Fluorene	298
5	Phenanthrene	340
6	Anthracene	340
7	Fluoranthene	384
8	Pyrene	393
9	Benzo(a)anthracene	437.8
10	Chrysene	448

a. Physical properties of ten PAHs.

Name	Boiling Point (℃)
m-Xylene	139.12
p-Xylene	138.35
Ethylbenzene	136.19

b. Physical properties of xylene and ethylbenzene.

Table 1. Physical properties of analytes.

2.3.1 Determination of the optimum inlet pressure

Since the Antek 3600 microwave controller could not communicate with the electric flow-meter of the host Agilent 6890A GC in this microwave GC system, the constant inlet pressure mode was used instead of the constant flow rate mode. A van Deemter curve ($H = B/\bar{u} + C\bar{u}$) was constructed in order to ascertain the optimum inlet pressure. Typically a solute is chosen and run isothermally at a variety of flow rates in order to obtain the van Deemter curve.

Naphthalene and acenaphthene were chosen because of their low boiling points. Two isothermal temperatures (160℃ and 280℃) were chosen for study. Naphthalene was chosen and run isothermally at 160℃ in the inlet pressure range of 5 - 20 psi. Naphthalene eluted from the column too fast to obtain the reasonable retention time at 280℃ since the boiling point of naphthalene is 217.7℃. Therefore, acenaphthene with a higher boiling point was chosen and run isothermally at 280℃ in the inlet pressure range of 5 – 20 psi. Plate number (N) was obtained by retention time (t_R) and peak width at half height (w_h) according equation (4). Plate height (H) was obtained by column length (L) and plate number (N) using equation (2) (H = L/N). Since van Deemter curve was plotted by plate height (H) versus average linear velocity($\bar{u}$), the average linear velocity was calculated from the retention time (t_M) for a nonretained methane peak (see equation (5)).

$$N = 5.54(t_R/w_h) \quad (4)$$

$$\bar{u} = L/t_M \quad (5)$$

N – plate number
t_R – retention time
W_h – peak width at half height
H – plate height
L – column length
$\bar{u}$ – average linear velocity
t_M – void time

Van Deemter curves at 160℃ are shown in Figures 9a (H vs. inlet pressure) and 9b (H vs. average linear velocity). An optimum inlet pressure was observed at approximately 15 psi which corresponded to an optimum linear velocity of 35 cm/s. A flow rate of 1.7 mL/min at 15 psi could be calculated from the linear velocity and column size. Van Deemter curves at 280℃ are shown in Figures 10a and 10b. Similarly, a 15 psi inlet pressure corresponded to an optimum linear velocity of 30 cm/s and a flow rate of 1.5 mL/min at 280℃. Gas flow rate decreases with increasing column temperature because the viscosity is larger at higher temperature. Therefore, 15 psi was concluded to be the optimum inlet pressure since it gave the lowest plate height (H) at both temperatures.

2.3.2 Temperature programs

Various positive and negative temperature programs were used in the research. Basically the positive temperature program was applied to the sample first to see whether every peak was well separated. Negative temperature programs were applied to improve the separation if the critical pairs of compounds could not achieve baseline separation.

Cooling ramp was deemed to be an important part of negative temperature programming. The rate of cooling ramp was kept at 120℃/min in this research since 120℃/min was the optimized cooling rate for negative temperature programming according to Nezem's work.[27] In microwave GC cooling was faster at higher temperature than at lower temperature. There would be a temperature deviation between actual column temperature and setting column temperature if the cooling was too fast. For example, the actual temperature might be 105℃ when the setting temperature displayed 100℃ on the control panel if the cooling rate is 200 ℃/min was. Each time the column temperature was decreased to 70℃ because it was the lowest temperature that could be achieved rapidly since room temperature air was used for

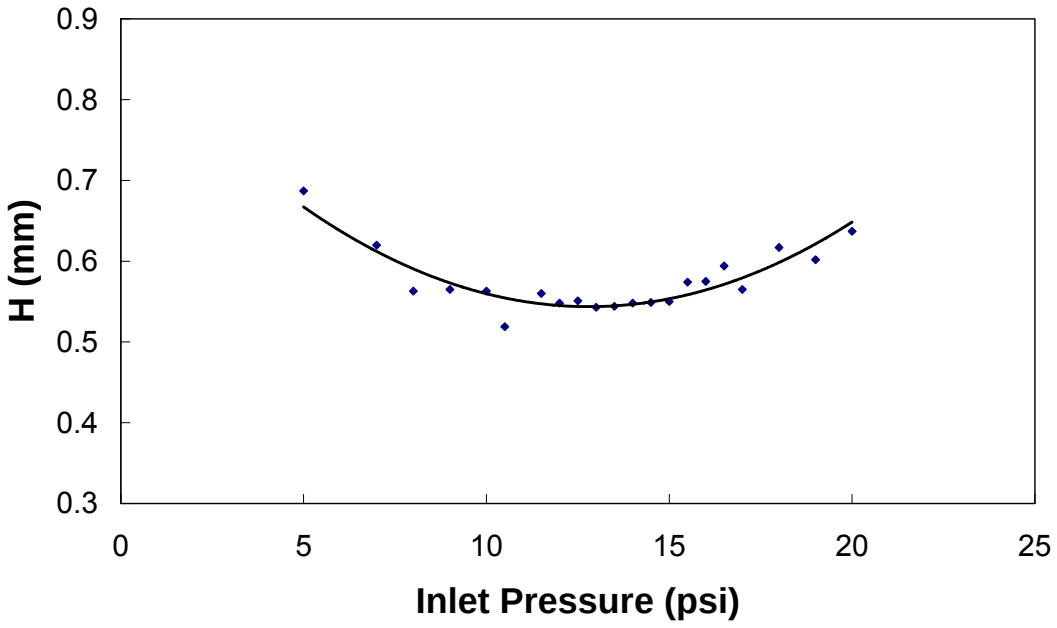


Figure 9a. Van Deemter curve (plate height vs. inlet pressure) of microwave methyl silicone column at 160℃ using naphthalene.

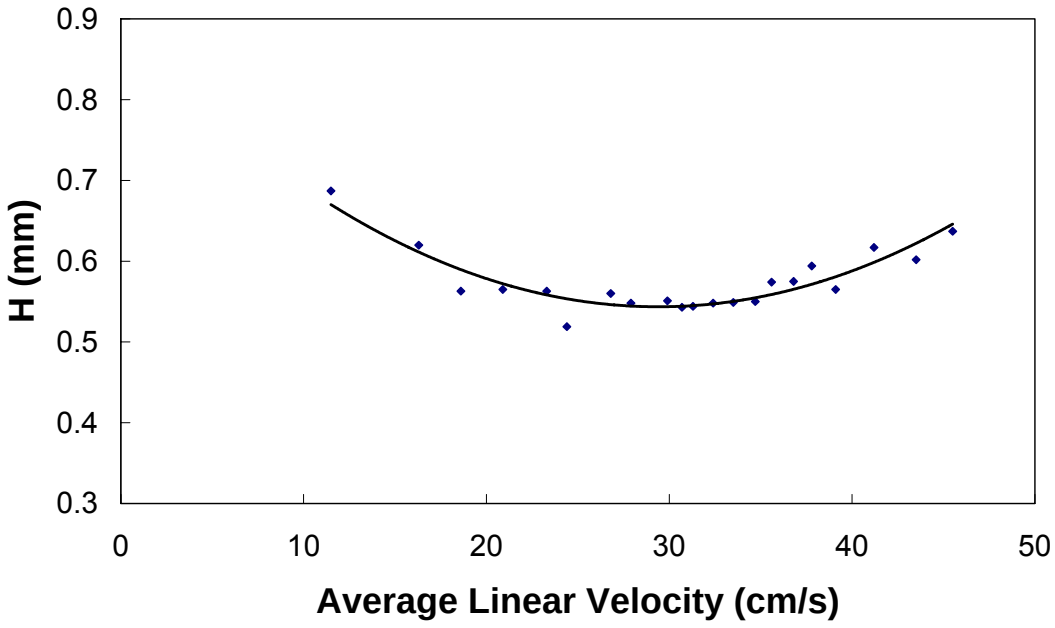


Figure 9b. Van Deemter curve (plate height vs. average linear velocity) of microwave methyl silicone column at 160℃ using naphthalene.

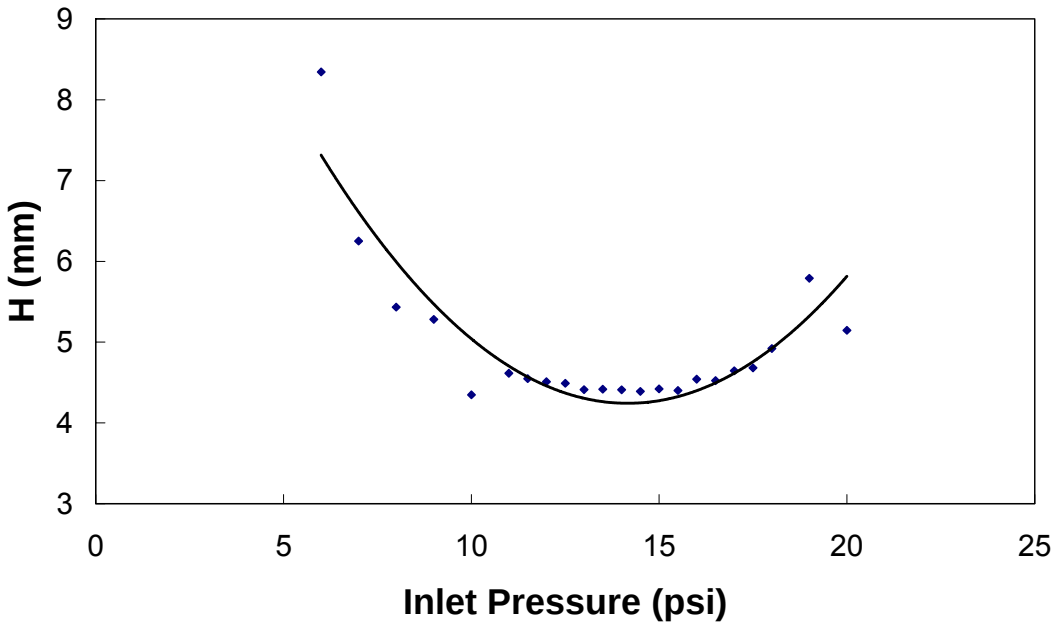


Figure 10a. Van Deemter curve (plate height vs. inlet pressure) of microwave methyl silicone column at 280℃ using acenaphthene.

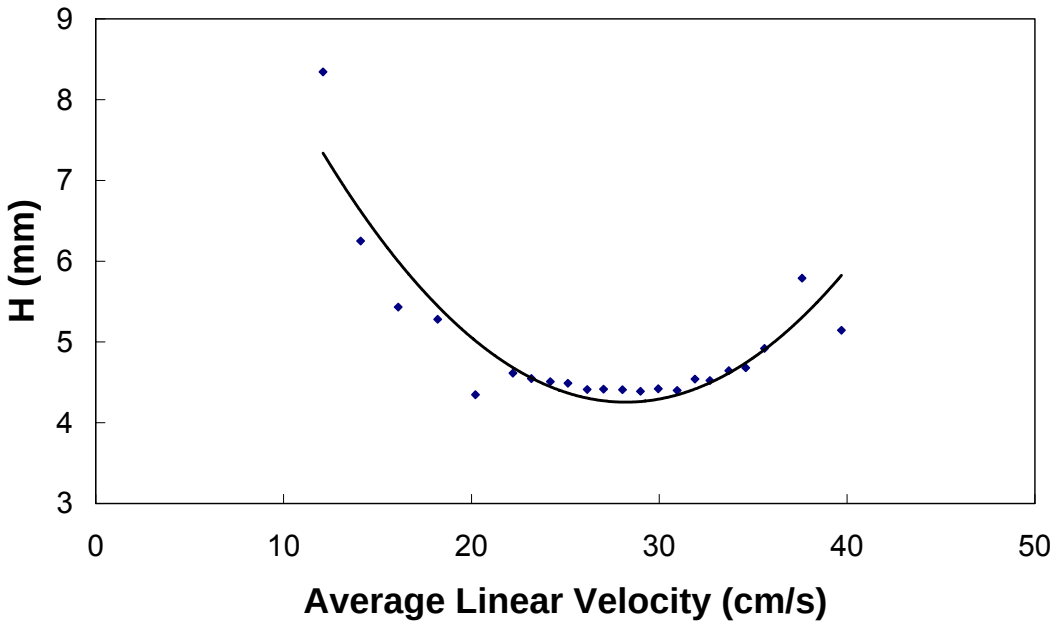


Figure 10b. Van Deemter curve (plate height vs. average linear velocity) of microwave methyl silicone column at 280℃ using acenaphthene.

cooling. Cooling from 300℃ to 290℃ only took one second; while cooling from 70℃ to 60 ℃ took fifteen seconds.

Timing for applying the cooling ramp, holding time at the low temperature, and the reheating rate were investigated in negative temperature programming.

3. RESEARCH AND DISCUSSION

An advantage of microwave-heating GC is negative temperature programming. Short cool-down time make it possible to employ negative temperature programming during the process. In conventional GC, only positive temperature programming is used because the oven cools down slowly due to its great thermal mass. Negative temperature programming might have the potential to be used for separation because the rapid cooling in microwave GC makes it possible. In negative temperature programming initially the column is heated to a certain temperature before the negative cooling ramp. After cooling the column is reheated again.

3.1 COMPARISON OF REPRODUCIBILITY VIA POSITIVE AND NEGATIVE TEMPERATURE PROGRAMMING

Reproducibility was studied for comparison of peak area and retention time via positive and via negative temperature programming. A standard solution of pyrene and benzo(a)anthracene was made at a concentration of 50 μg/mL for pyrene and 60 μg/mL for benzo(a)anthracene. Positive temperature program 1 and two negative temperature programs 3 and 4 were employed for the comparison.

Temperature program 1 (positive): 50℃ for 2 min, increased to 280℃ at 8℃/min and held for 10 min.

Table 2. Reproducibility data for positive temperature program 1.

GC conditions: 50℃ for 2 min, increased to 280℃ at 8℃/min and held for 10 min.

	Run 1	Run 2	Run 3	Average	SD	RSD (%)
Pyrene area	85.2	86.1	84.2	85.17	0.95	1.1
Benzo(a)anthracene area	94.5	96.4	94.4	95.1	1.13	1.2
Pyrene RT (min)	30.35	30.35	30.34	30.35	0.01	0.02
Benzo(a)anthracene RT (min)	35.86	35.85	35.83	35.85	0.01	0.04
Pyrene peak asymmetry	0.99	1.00	1.02	1.00	0.02	1.80
Benzo(a)anthracene peak asymmetry	1.01	1.04	1.06	1.04	0.02	2.11

Table 3. Reproducibility data of negative temperature programs.

a. Program 3.
GC conditions: 50℃ for 2 min, increased to 280℃ at 8℃/min and held for 1.6 min, decreased to 70℃ at 120℃/min, held for 2.0 min, then 30℃/min to 280℃ held for 10 min.

	Run 1	Run 2	Run 3	Average	SD	RSD (%)
Pyrene area	87.4	88.3	89.0	88.2	0.80	0.91
Benzo(a)anthracene area	93.9	98.4	99.1	97.1	2.82	2.90
Pyrene RT (min)	30.32	30.29	30.27	30.29	0.03	0.10
Benzo(a)anthracene RT (min)	44.94	44.93	44.91	44.93	0.02	0.04
Pyrene peak asymmetry	0.99	0.97	0.99	0.98	0.01	1.02
Benzo(a)anthracene peak asymmetry	1.03	0.99	0.97	1.00	0.03	3.00

b. Program 4.
GC conditions: 50℃ for 2 min, increased to 280℃ at 8℃/min and held for 1.6 min, decreased to 70℃ at 120℃/min, held for 2.0 min, then 120℃/min to 280℃ held for 10 min.

	Run 1	Run 2	Run 3	Average	SD	RSD (%)
Pyrene area	87.2	88.9	88.3	88.1	0.86	0.98
Benzo(a)anthracene area	93.1	99.0	97.7	96.6	3.10	3.21
Pyrene RT (min)	30.40	30.38	30.39	30.39	0.01	0.03
Benzo(a)anthracene RT (min)	40.80	40.79	40.79	40.79	0.01	0.02
Pyrene peak asymmetry	0.98	0.94	0.98	0.97	0.02	2.06
Benzo(a)anthracene peak asymmetry	1.02	0.96	0.98	0.99	0.03	3.03

Temperature program 2 (negative): 50℃ for 2 min, increased to 280℃ at 8℃/min and held for 1.6 min, decreased to 70℃ at 120℃/min, held for 2.0 min, then 8℃/min to 280℃ held for 10 min.

Temperature program 3 (negative): 50℃ for 2 min, increased to 280℃ at 8℃/min and held for 1.6 min, decreased to 70℃ at 120℃/min, held for 2.0 min, then 30℃/min to 280℃ held for 10 min.

Temperature program 4 (negative): 50℃ for 2 min, increased to 280℃ at 8℃/min and held for 1.6 min, decreased to 70℃ at 120℃/min, held for 2.0 min, then 120℃/min to 280℃ held for 10 min.

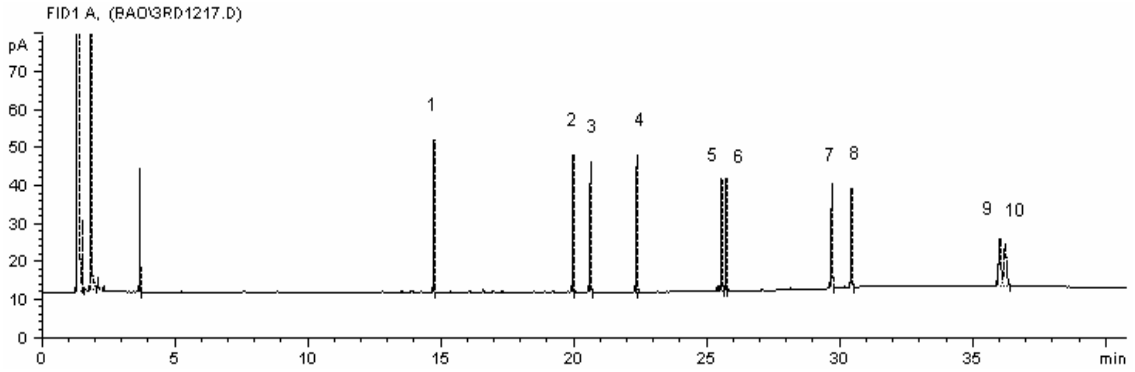
The reproducibility data are listed in Tables 2 and 3. There was no significant difference for peak area RSD, retention time RSD, and peak asymmetry RSD between positive and negative temperature program as shown in Figure 10. Peak area RSD and retention time RSD were acceptable considering manual injection used in the research.

3.2 APPLICATION OF NEGATIVE TEMPERATURE PROGRAMMING FOR NON-VOLATILE COMPOUNDS

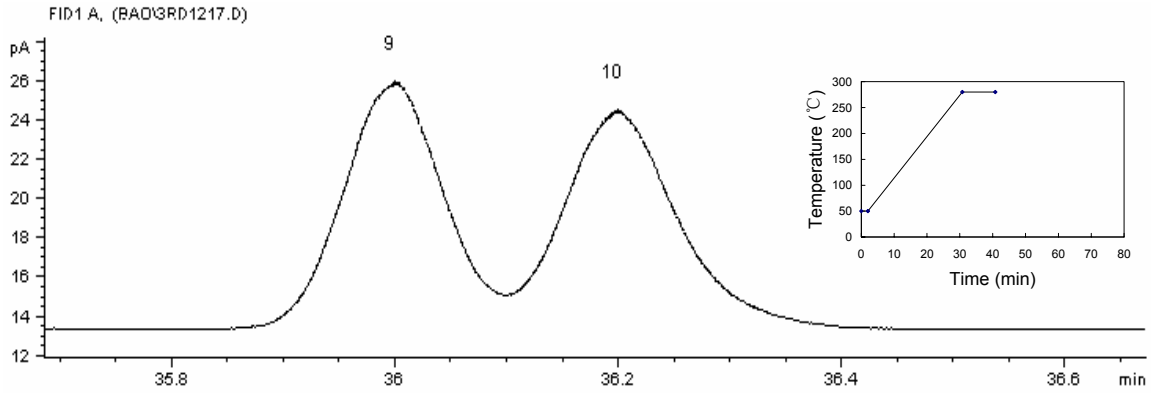
3.2.1 Qualitative analysis for the separation of benzo(a)anthracene and chrysene

The goal of this portion of the work was to study the influence of negative temperature programming on the resolution of benzo(a)anthracene and chrysene. Benzo(a)anthracene and chrysene have high boiling points and were difficult to separate using a methyl silicone column with positive temperature programming. For example, a standard solution of ten PAHs was made in methanol/dichloromethane (50:50). The concentration was 50 ppm for each component. The sample solution (1 μL) was injected into the microwave GC column using positive temperature program 1. The resulting chromatogram is shown in Figure 11. Compounds 1-8 were separated with good peak shapes and resolution. However, compounds 9 and 10 were not baseline separated.

Negative temperature programming was employed in hopes of more effectively resolving compounds 9 and 10. A series of experiments were thus undertaken to ensure that optimal



a. Chromatogram of 10 PAHs mixture using positive temperature program 1. (1) naphthalene, (2) acenaphthylene, (3) acenaphthene, (4) fluorene, (5) phenanthrene, (6) anthracene, (7) fluoranthene, (8) pyrene, (9) benzo(a)anthracene, and (10) chrysene. A methyl silicone capillary column (30 m×0.32 mm×1μm) was used for separation. The temperature program was 50℃ for 2 min, increased to 280℃ at 8℃/min and held for 10 min. The concentration was 50 μg/mL for each compound.



b. Expanded chromatogram of the separation of component 9 and 10 using positive temperature program 1. (9) benzo(a)anthracene, and (10) chrysene. R_s = 1.2.

Figure 11. Chromatogram of ten PAHs using positive temperature program 1.

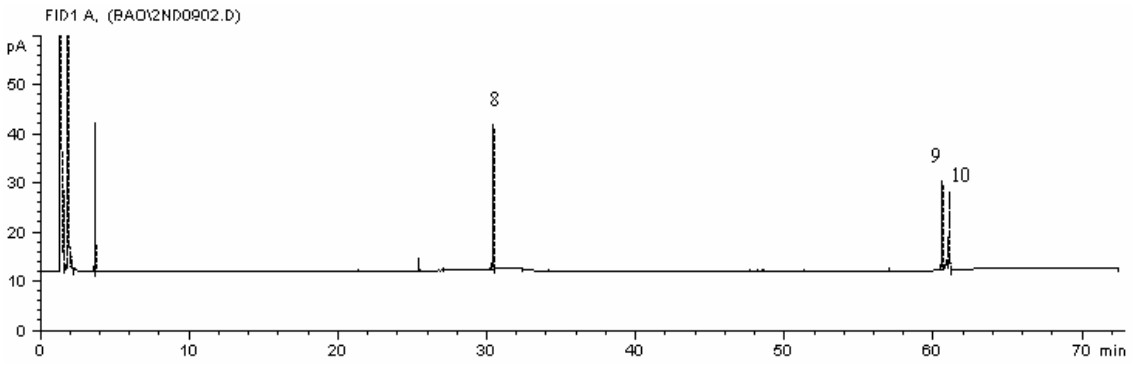
conditions were being used. Three negative temperature programs were applied to test the feasibility for baseline separation of compounds 9 and 10. Only compounds 8, 9, and 10 were injected. Pyrene (compound 8) was used as the internal standard. The resolution of compounds 9 and 10 was successfully improved to R_s = 4.1 using negative temperature program 2 (Figure 12).

Unfortunately the analysis time of negative temperature program 2 was over 60 minutes because of a slow reheating ramp. Thus, the reheating rate was increased from 8℃/min to 30 ℃/min (i.e. program 3). The resolution of compounds 9 and 10 was slightly reduced to 4.0 (Figure 13); while the analysis time was decreased by 15 minutes. The re-heating rate was increased even more to 120℃/min (program 4) in order to shorten the analysis time further. The resolution of compounds 9 and 10 was 3.9 in Figure 14. At the same time, peak width decreased with negative temperature programming because the compounds refocused on the column during the cooling ramp. Peak width decreased even more with an increase in reheating rate as shown in Table 4 since the compounds spent less time on the column.

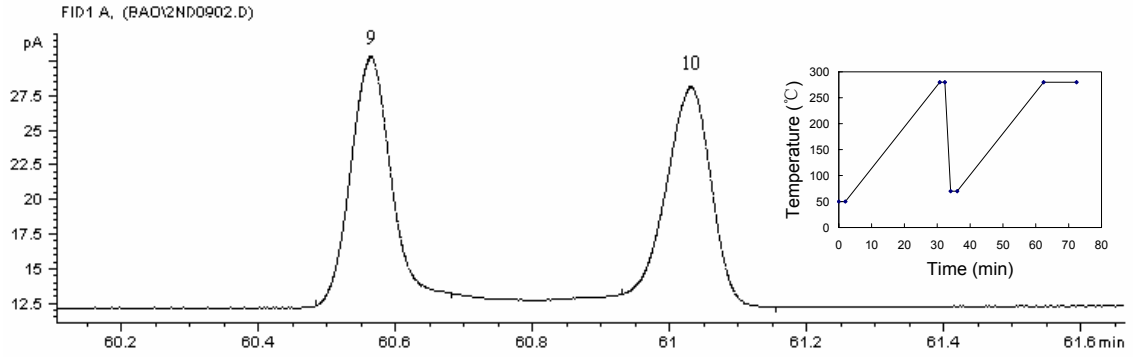
The sample solution of 10 PAHs was run using temperature program 3 in order to study the influence of negative temperature programming for a complex mixture. Compounds 9 and 10 continued to achieve baseline separation in the complex mixture as shown in Figure 15.

3.3.2 Quantitative analysis of negative temperature program

The goal of this portion of the work was to study the feasibility of negative temperature programming for quantitative analysis using program 3. Traditionally, there are several standardization techniques employed in the practice of chromatographic analyses, such as external standards, internal standards, and standard addition. The internal standard method does not require exact or consistent sample volumes or response factors since they are built into the method, so it is good for manual injections used in this research. The internal

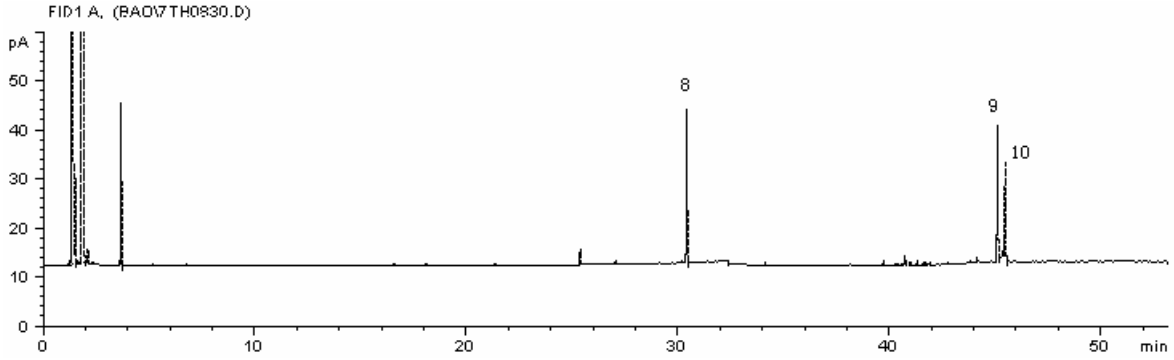


a. Chromatogram of compounds 8, 9 and 10 using negative temperature program 2. (8) pyrene, (9) benzo(a)anthracene, and (10) chrysene. A methyl silicone capillary column (30 m×0.32 mm×1μm) was used for separation. The temperature program was 50℃ for 2 min, increased to 280℃ at 8℃/min and held there for 1.6 min, decreased to 70℃ at 120℃/min, held there for 2.0 min, then 8℃/min to 280℃ held there for 10 min. The concentration was 50 μg/mL for each compound.

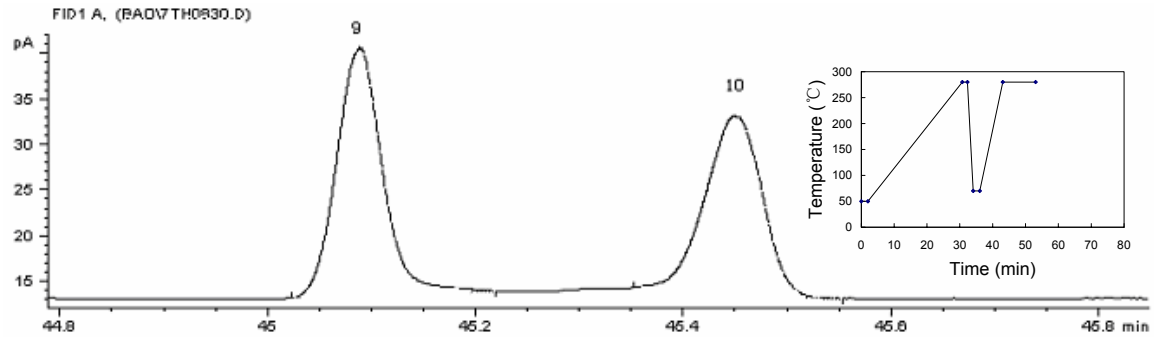


b. Expanded chromatogram of the separation of component 9 and 10 using negative temperature program 2. (9) benzo(a)anthracene, and (10) chrysene. $R_s = 4.1$.

Figure 12. Chromatogram of three PAHs using negative temperature program 2.

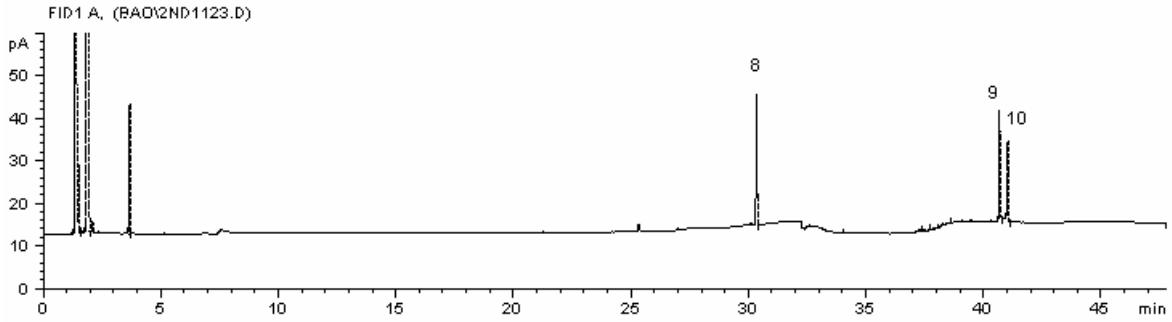


a. Chromatogram of compounds 8, 9 and 10 using negative temperature program 3. (8) pyrene, (9) benzo(a)anthracene, and (10) chrysene. A methyl silicone capillary column (30 m×0.32 mm×1μm) was used for separation. The temperature program was 50℃ for 2 min, increased to 280℃ at 8℃/min and held there for 1.6 min, decreased to 70℃ at 120℃/min, held there for 2.0 min, then 30℃/min to 280℃ held there for 10 min. The concentration was 50 μg/mL for each compound.

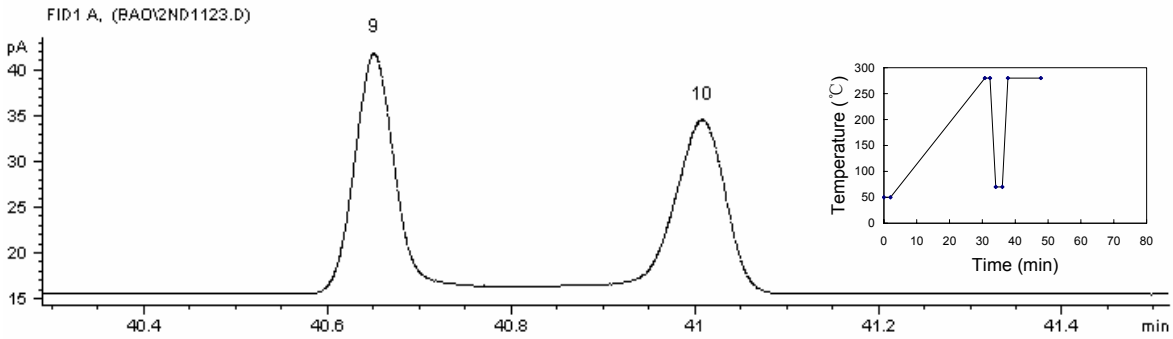


b. Expanded chromatogram of the separation of component 9 and 10 using negative temperature program 2. (9) benzo(a)anthracene, and (10) chrysene. $R_s = 4.0$.

Figure 13. Chromatogram of three PAHs using negative temperature program 3



a. Chromatogram of compounds 8, 9 and 10 using negative temperature program 4. (8) pyrene, (9) benzo(a)anthracene, and (10) chrysene. A methyl silicone capillary column (30 m×0.32 mm×1μm) was used for separation. The temperature program was 50℃ for 2 min, increased to 280℃ at 8℃/min and held there for 1.6 min, decreased to 70℃ at 120℃/min, held there for 2.0 min, then 120℃/min to 280℃ held there for 10 min. The concentration was 50 μg/mL for each compound.



b. Expanded chromatogram of the separation of component 9 and 10 using negative temperature program 2. (9) benzo(a)anthracene, and (10) chrysene. R_s = 3.9.

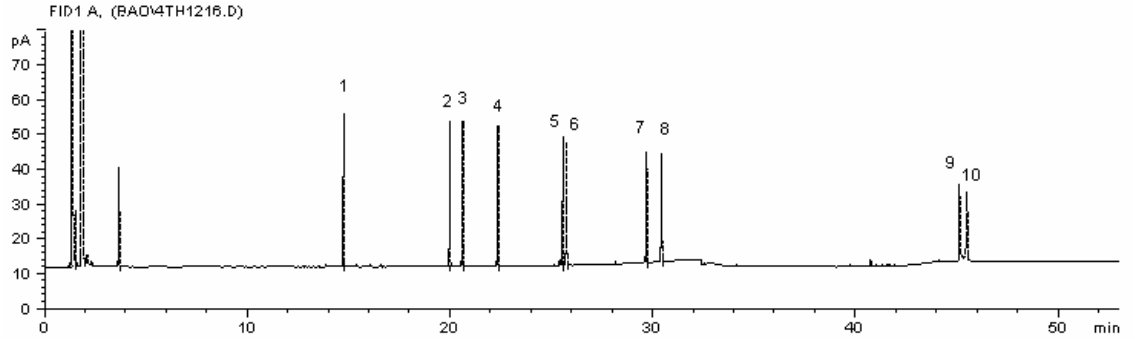
Figure 14. Chromatogram of three PAHs using negative temperature program 4.

Table 4. Comparison of chromatographic parameters via a positive temperature program versus a negative temperature program.

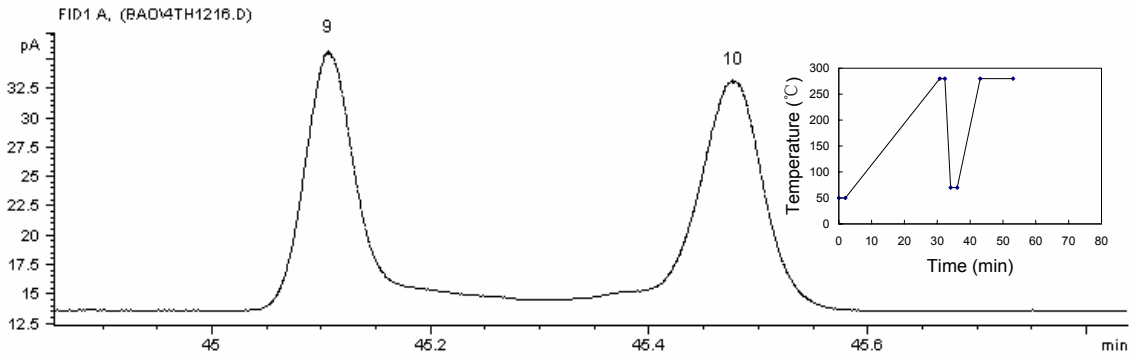
Temperature Program	Reheating rate (℃/min)	9 RT (min)	10 RT (min)	Compd. 9 $W_{1/2h}$ (min)	Compd. 10 $W_{1/2h}$ (min)	R_s
1 (positive)	N/A	36.00 (0.17)*	36.20 (0.17)	0.089 (1.3)	0.093 (2.8)	1.2 (0.82)
2 (negative)	8	60.57 (0.18)	61.03 (0.18)	0.063 (8.9)	0.071 (7.4)	4.1 (7.4)
3 (negative)	30	45.08 (0.05)	45.44 (0.05)	0.047 (3.6)	0.062 (5.4)	4.0 (1.4)
4 (negative)	120	40.65 (0.01)	41.01 (0.01)	0.046 (1.0)	0.060 (2.8)	3.9 (1.5)

RT: retention time.
$W_{1/2}$: peak width at half height.
R_s: resolution.
N/A: not applicable.

* Numbers in parenthesis are % relative standard deviation (n= 3)



a. Chromatogram of 10 PAHs mixture using negative temperature program 3. (1) naphthalene, (2) acenaphthylene, (3) acenaphthene, (4) fluorene, (5) phenanthrene, (6) anthracene, (7) fluoranthene, (8) pyrene, (9) benzo(a)anthracene, and (10) chrysene. A methyl silicone capillary column (30 m×0.32 mm×1μm) was used for separation. The temperature program was The temperature program was 50℃ for 2 min, increased to 280℃ at 8℃/min and held there for 1.6 min, decreased to 70℃ at 120℃/min, held there for 2.0 min, then 30℃/min to 280℃ held there for 10 min. The concentration was 50 μg/mL for each compound.



b. Expanded chromatogram of the separation of component 9 and 10 using negative temperature program 3. (9) benzo(a)anthracene, and (10) chrysene.

Figure 15. Chromatogram of ten PAHs mixture using negative temperature program 3.

standard chosen for this method should be chemically similar to the analytes of interest and be well resolved from them. The internal standard should also elute near the peaks of interest and must be available in high purity. Compound 8 (pyrene) which fullfilled all these requirements was used as internal standard in the research. Five point calibration curves of PAH solutions were established in the range of 20 – 100 μg/mL of each PAH component while the concentration of internal standard was kept at 50 μg/mL. Peak area ratio (area of interest peak/area of internal standard) versus the concentration of the PAH standards was plotted to provide a calibration curve. The calibration curves of compound 9 and 10 as shown in Figure 16 were made by using the data in Table 5. Both calibration curves showed good linearity since the correlation coefficients were greater than 0.999.

The standard solution of compound 8 (pyrene), 9 (benzo(a)anthracene), and 10 (chrysene) with the concentration of 50 ug/mL for each component was injected. The reproducibility data of three runs were shown as below. Numbers in parenthesis are % relative standard deviation (n= 3).

Average area of 8 (50ppm)	Average area of 9 (50ppm)	Average area ratio 9/8	Average area of 10 (50ppm)	Average area ratio 10/8
85.1 (0.47)	76.0 (1.7)	0.894 (1.2)	72.2 (0.88)	0.848 (1.3)

Linear equation for calibration curve of compound 9: $y = 0.0184x - 0.0025$
Average area ratio of 9/8 = 0.894 → x = 48.70ppm
48.70ppm/50ppm x 100% = 97.4%

Linear equation for calibration curve of compound 10: $y = 0.0176x + 0.0133$
Average area ratio of 10/8 = 0.848 → x = 47.45ppm
47.45ppm/50ppm x 100% = 95.0%

The peak area ratios were obtained and plugged into the equations of calibration curves, thus the percentages of recovery were obtained. The percentages of recovery were 97.4% and 95.0% which were both above 95%, so quantitative analysis was achieved using negative temperature program 3.

Table 5. Peak area ratio for calibration curves.

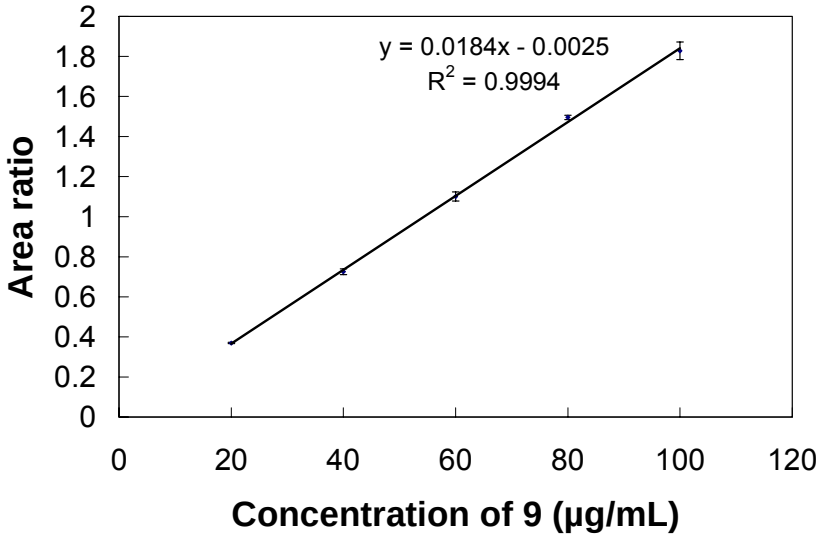
a. Results of area ratio of 9/8 in five solutions of compound 8 and 9 mixture. The concentration of pyrene was 50 µg/mL for pyrene in all five solutions. The concentration of compound 9 (benzo(a)anthracene) was 20 µg/mL, 40 µg/mL, 60 µg/mL, 80 µg/mL, and 100 µg/mL individually in five solutions.

Conc. of compd. 9	Area ratio of compd. 9/8
20 µg/mL	0.37 (0.95)*
40 µg/mL	0.73 (2.0)
60 µg/mL	1.10 (2.1)
80 µg/mL	1.50 (0.73)
100 µg/mL	1.83 (2.4)

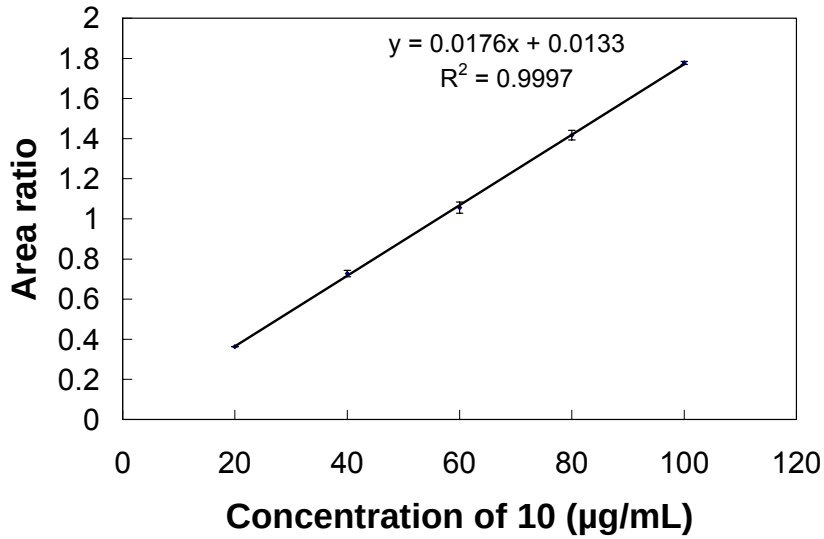
b. Results of area ratio of 10/8 in five solutions of compound 8 and 10 mixture. The concentration of compound 8 (pyrene) was 50 µg/mL for pyrene in all five solutions. The concentration of compound 10 (chrysene) was 20 µg/mL, 40 µg/mL, 60 µg/mL, 80 µg/mL, and 100 µg/mL individually in five solutions.

Conc. of compd. 10	Area ratio of compd. 10/8
20 µg/mL	0.36 (0.17)
40 µg/mL	0.73 (2.2)
60 µg/mL	1.06 (2.6)
80 µg/mL	1.42 (1.7)
100 µg/mL	1.78 (0.42)

* Numbers in parenthesis are % relative standard deviation (n= 3)



a. Calibration curve of compound 9 using negative temperature program 3.



b. Calibration curve of compound 10 using negative temperature program 3.

Figure 16. Calibration curves using negative temperature program 3.

3.2.3 Qualitative analysis of negative temperature program for the separation of phenanthrene and anthracene

The successful application of negative temperature programming for the resolution of benzo(a)anthracene and chrysene caused us to apply it to other critical pairs. Phenanthrene and anthracene are another critical pair in GC since their boiling points are both 340℃ which are lower than those of benzo(a)anthracene and chrysene. The boiling points of benzo(a)anthracene and chrysene are 437.8℃ and 448℃. The temperature programs used in this part of the study were:

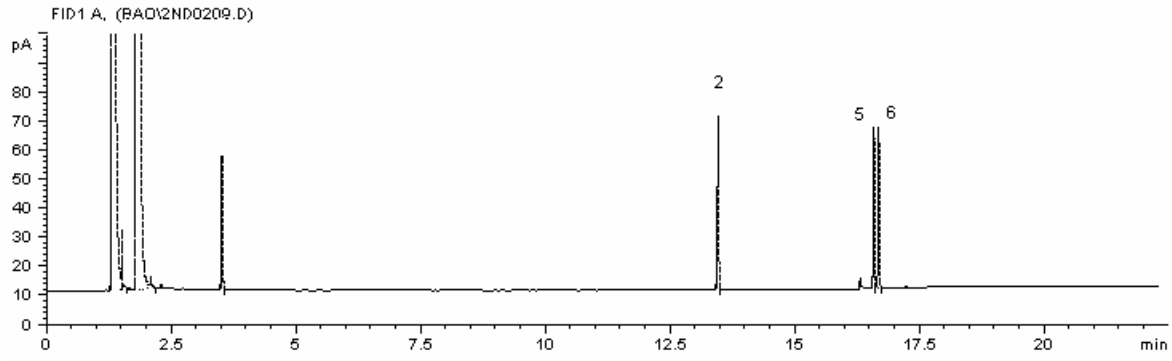
Temperature program 5 (positive): 50℃ for 2 min, increased to 280℃ at 15℃/min and held there for 5.0 min.

Temperature program 6 (negative): 50℃ for 2 min, increased to 250℃ at 15℃/min and held there for 0 min, decreased to 70℃ at 120℃/min, held there for 2.0 min, then 15℃/min to 280℃ held there for 3.0 min.

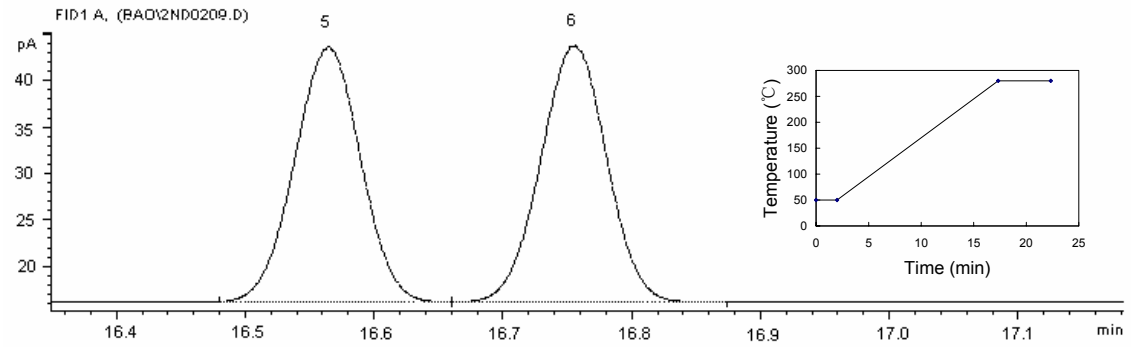
The positive temperature program 5 was employed first. The resolution of compound 5 (phenanthrene) and 6 (anthracene) was 2.0 as shown in Figure 17. The resolution was improved, however, to 5.6 when negative temperature program 6 was employed as shown in Figure 18. The conclusion reached here was that negative temperature programming could be applied to improve the resolution of non-volatile critical pairs, and the application of negative temperature programming was not confined to only one particular critical pair of compounds.

3.3 APPLICATION OF NEGATIVE TEMPERATURE PROGRAMMING FOR VOLATILE COMPOUNDS

The application of negative temperature programming to volatile compounds was next studied in this part. Ethylbenzene and m-xylene are structural isomers for each other. The boiling point of ethylbenzene is 136.19℃ and the boiling point of m-xylene is 139.12℃. Therefore, they were a critical pair in GC separation[78]. A positive temperature program was

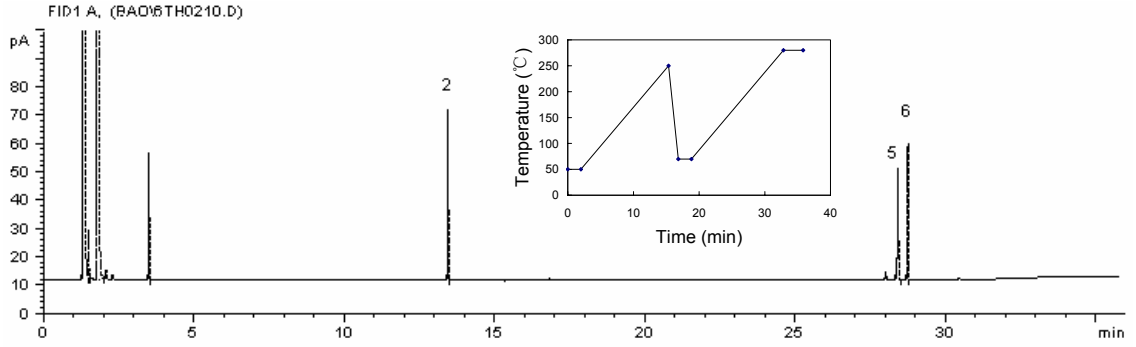


a. Chromatogram of compounds 2, 5, and 6 using positive temperature program. (2) acenaphthylene, (5) phenanthrene, and (6) anthracene. A methyl silicone capillary column (30 m×0.32 mm×1μm) was used for separation. The temperature program was 50℃ for 2 min, increased to 280℃ at 15℃/min and held there for 5.0 min. The concentration was 50 μg/mL for each compound. Acenaphthylene was used as internal standard.

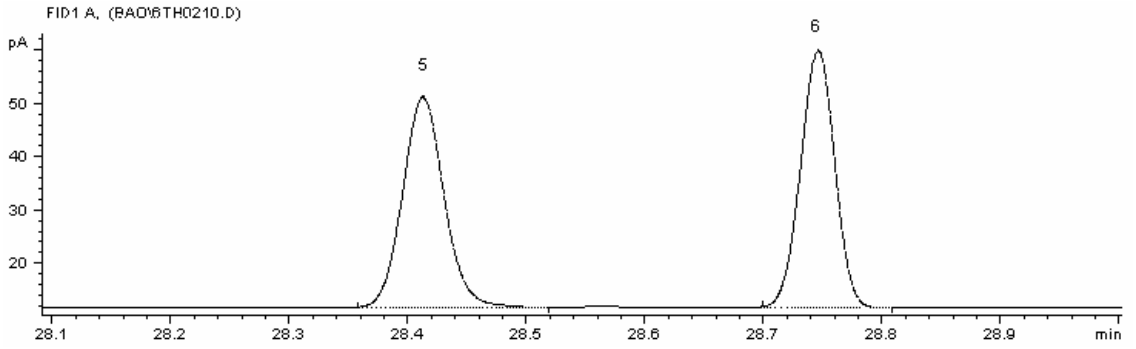


b. Expanded chromatogram of the separation of component 5 and 6 using positive temperature program. (5)phenanthrene, and (6) anthracene. $R_s = 2.0$.

Figure 17. Chromatogram of three PAHs using positive temperature program 5.



a. Chromatogram of compounds 2, 5, and 6 using negative temperature program 6. (2) acenaphthylene, (5) phenanthrene, and (6) anthracene. A methyl silicone capillary column (30 m×0.32 mm×1μm) was used for separation. The temperature program was 50℃ for 2 min, increased to 250℃ at 15℃/min and held there for 0 min, decreased to 70℃ at 120℃ /min, held there for 2.0 min, then 15℃/min to 280℃ held there for 3.0 min. The concentration was 50 μg/mL for each compound. Acenaphthylene was used as internal standard.



b. Expanded chromatogram of the separation of component 5 and 6 using positive temperature program. (5)phenanthrene, and (6) anthracene. R_s = 5.6.

Figure 18. Chromatogram of three PAHs using negative temperature program 6.

employed first. The resolution of ethylbenzene and m-xylene was only 1.4 employing positive temperature program 7 as shown in Figure 19. Baseline separation of ethylbenzene and m-xylene was, however, obtained using negative temperature program 8 with a resolution of 2.0 as shown in Figure 20.

The temperature programs used in this part are listed as below:

Temperature program 7 (positive): 90℃ for 1.5 min, increased to 230℃ at 40℃/min and held for 0 min

Temperature program 8 (negative): 90℃ for 1.5 min, increased to 100℃ at 40℃/min and held for 0 min, decreased to 70℃ at 120℃/min, held for 0 min, then 40℃/min to 230℃ held for 0 min.

Temperature program 9 (negative): 90℃ for 1.5 min, increased to 100℃ at 40℃/min and held for 0 min, decreased to 70℃ at 120℃/min, held for 1 min, then 40℃/min to 230℃ held for 0 min.

Temperature program 10 (negative): 90℃ for 1.5 min, increased to 100℃ at 40℃/min and held for 0 min, decreased to 70℃ at 120℃/min, held for 2 min, then 40℃/min to 230℃ held for 0 min.

Negative temperature programs 8, 9, and 10 as shown in Figure 21 were employed to study the influence of different negative temperature programs for the resolution and peak width. These three negative temperature programs were similar, but the holding time at low column temperature was different. The influence of different holding time for the resolution and peak width was studied. Resolution increased with increasing holding time at 70℃. Peak width increased with increasing holding time in negative temperature programs although it was still smaller than the one in positive temperature program as shown in Table 6. Peak width increased with increasing holding time because the band width would expand when the compounds spent more time in the column.

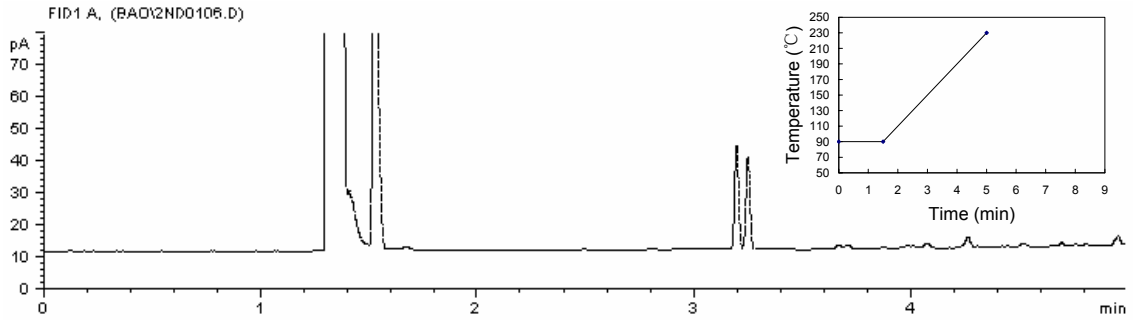
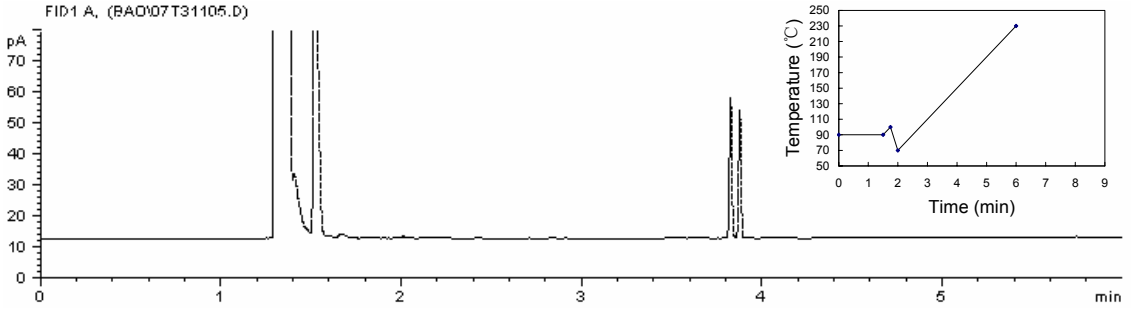
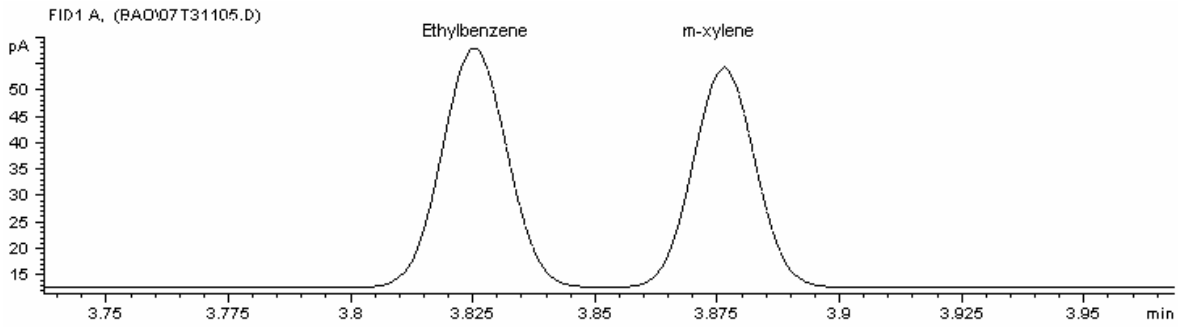


Figure 19. Chromatogram of ethylbenzene and m-xylene using positive temperature program 7. A methyl silicone capillary column (30 m×0.32 mm×1μm) was used for separation. The temperature program was 90℃ for 1.5 min, increased to 230℃ at 40℃/min and held there for 0 min. R_s = 1.4. The concentration was 50 μg/mL for each compound.

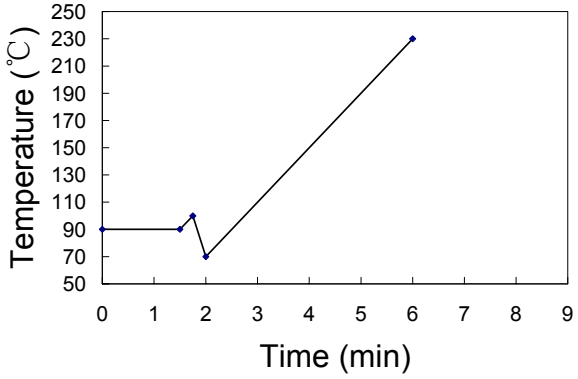


a. Chromatogram of ethylbenzene and m-xylene using negative temperature program 8. A methyl silicone capillary column (30 m×0.32 mm×1μm) was used for separation. The temperature program was 90℃ for 1.5 min, increased to 100℃ at 40℃/min and held there for 0 min, decreased to 70℃ at 120℃/min, held there for 0 min, then 40℃/min to 230℃ held there for 0 min. The concentration was 50 μg/mL for each compound.

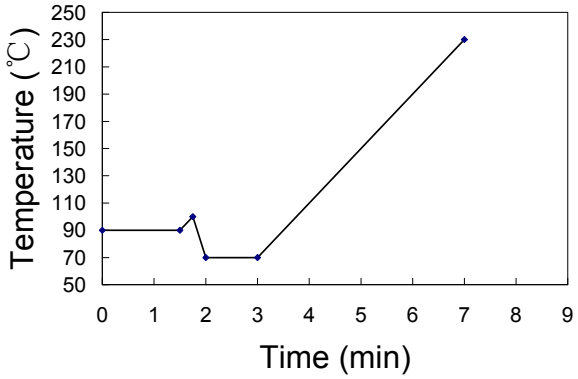


b. Expanded chromatogram of the separation of ethylbenzene and m-xylene using negative temperature program 6. $R_s = 2.0$.

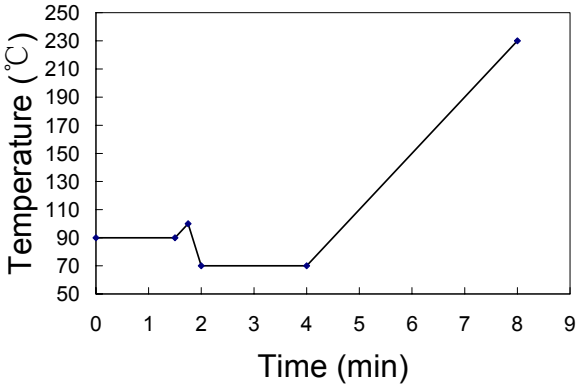
Figure 20. Chromatogram of ethylbenzene and m-xylene using negative temperature program 8.



Program 8



Program 9



Program 10

Figure 21. Graphs of three negative temperature programs.

Table 6. Comparison of chromatographic parameters via a positive temperature program versus three negative temperature programs.

Temp. Program	Hold Time (min)	Ethylbenzene RT (min)	m-Xylene RT (min)	Ethylbenzene $W_{1/2h}$ (min)	m-Xylene $W_{1/2h}$ (min)	R_s
7 (positive)	N/A	3.20 (0.17)*	3.25 (0.17)	0.021 (1.3)	0.023 (2.8)	1.4 (0.82)
8 (negative)	0	3.82 (0.06)	3.88 (0.06)	0.016 (3.9)	0.015 (0.68)	2.0 (2.4)
9 (negative)	1	4.50 (0.12)	4.56 (0.10)	0.017 (6.9)	0.016 (1.1)	2.4 (2.8)
10 (negative)	2	5.13 (0.03)	5.22 (0.03)	0.020 (1.9)	0.018 (0.63)	2.9 (4.2)

* Numbers in parenthesis are % relative standard deviation (n= 3)

3.3.2 Application of negative temperature programming for the separation of m-xylene and p-xylene

Xylene has three substitutional isomers which are m-xylene, p-xylene, and o-xylene. The critical pair of m-xylene and p-xylene are so difficult to separate from each other[78] that they appeared as a single peak in GC chromatogram in positive temperature program 11 as shown in Figure 22. Various negative temperature programs were tried to achieve baseline separation.

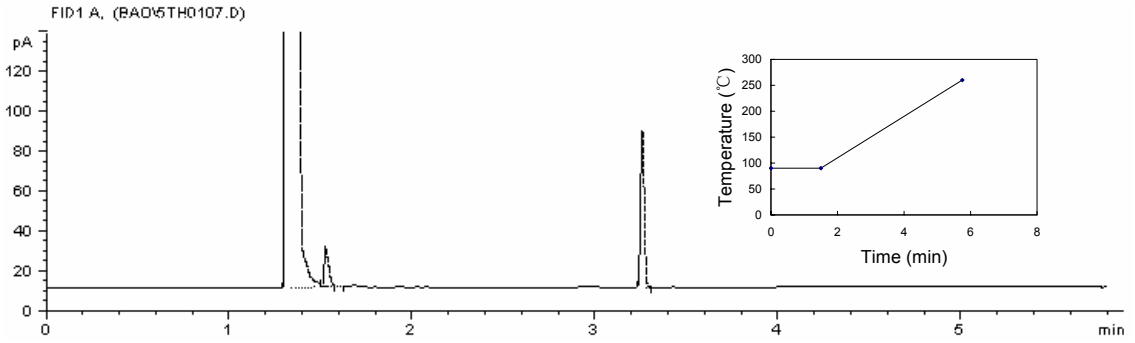
The temperature programs used in this part are listed as below:

Temperature program 11 (positive):90℃ for 1.5 min, increased to 260℃ at 40℃/min and held for 0 min.

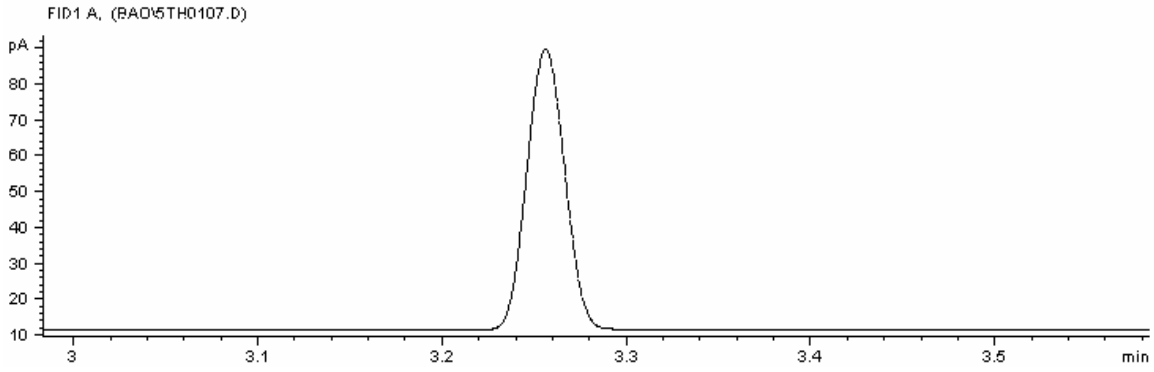
Temperature program 12 (negative): 90℃ for 1.5 min, increased to 120℃ at 40℃/min and held for 0 min, decreased to 70℃ at 120℃/min, held for 0 min, then 40℃/min to 260℃ held for 5 min.

Temperature program 13 (negative): 90℃ for 1.5 min, increased to 120℃ at 40℃/min and held for 0 min, decreased to 70℃ at 120℃/min, held for 1 min, then 40℃/min to 260℃ held for 5 min.

A series of experiments were undertaken to ensure optimal conditions were being used. Baseline separation could not be obtained using neither negative temperature program 12 or 13, although the resolution was improved relative to positive temperature programming as shown in Figure 23. Negative temperature programming was not effective in resolving this critical pair.

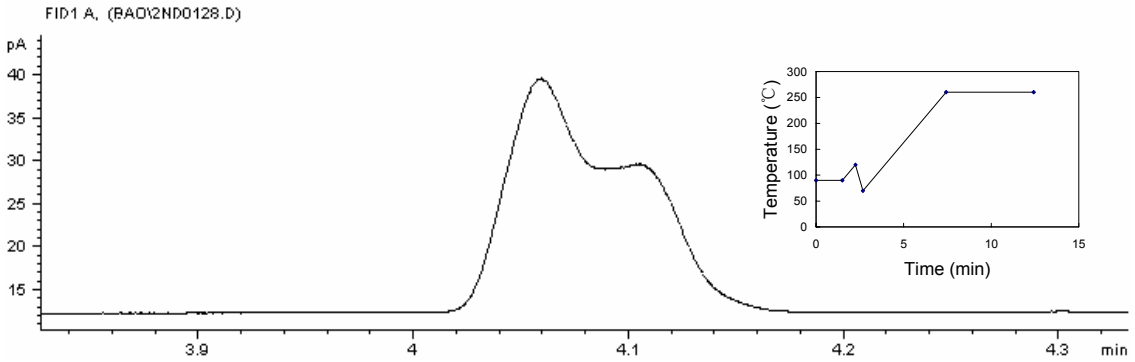


a. Chromatogram of xylene mixture using positive temperature program. A methyl silicone capillary column (30 m×0.32 mm×1μm) was used for separation. The temperature program was 90℃ for 1.5 min, increased to 260℃ at 40℃/min and held for 0 min. The concentration was 50 μg/mL for each compound.

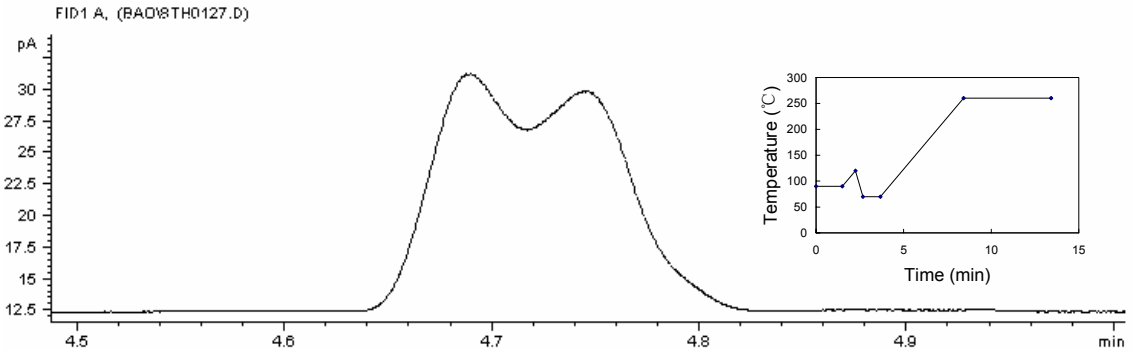


b. Expanded chromatogram of the separation of xylene using positive temperature program . $R_s < 0.5$.

Figure 22. Chromatogram of xylene mixture using positive temperature program 11.



a. Chromatogram of xylene mixture. A methyl silicone capillary column (30 m×0.32 mm ×1μm) was used for separation The temperature program was 90℃ for 1.5 min, increased to 120℃ at 40℃/min and held for 0 min, decreased to 70℃ at 120℃/min, held for 0 min, then 40℃/min to 260℃ held for 5 min. The concentration was 50 μg/mL for each compound.



b. Chromatogram of xylene mixture. A methyl silicone capillary column (30 m×0.32 mm× 1μm) was used for separation The temperature program was 90℃ for 1.5 min, increased to 120℃ at 40℃/min and held for 0 min, decreased to 70℃ at 120℃/min, held for 1 min, then 40℃/min to 260℃ held for 5 min. The concentration was 50 μg/mL for each compound.

Figure 23. Chromatogram of xylene mixture using negative temperature programs.

4. CONCLUSION

Negative temperature programming was used to improve the resolution of the critical pairs of compounds successfully compared to positive temperature programming in qualitative analysis and quantitative analysis. In the research we studied two critical pairs of non-volatile compounds (benzo(a)anthracene-chrysene and phenanthrene-anthracene) and two critical pairs of volatile compounds (ethylbenzene-m-xylene and m-xylene-p-xylene) using negative temperature programming. Qualitative analysis was achieved for three critical pairs among them except the pair of m-xylene-p-xylene. Quantitative analysis was achieved using negative temperature program since the percentages of recovery were above 95% for the critical pair of benzo(a)anthracene and chrysene. Calibration curves of standard solutions using negative temperature program were obtained with good linearities for quantitation. We found that negative temperature program could increase the resolution of overlapped peaks effectively for greater retention factor (k) at low temperature in the cooling ramp. Negative temperature program could decrease the peak width for solute refocusing in the cooling ramp. The influence of the reheating rate in the reheating ramp and the holding time in the cooling ramp in negative temperature programs were studied. The resolution decreased with the increasing reheating rate, and the peak width decreased with the increasing reheating rate and decreasing holding time in the cooling ramp. Negative temperature programming was not effective in resolving the critical pair of m-xylene-p-xylene. The influence of an increase of retention factor and solute refocusing in the cooling ramp was not enough to achieve baseline separation since the properties of these two substitutional isomers were very similar. Negative temperature programming is reliable and robust method for application in improving the resolution of critical peaks.

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VITA

Jianxiong Bao was born on January 5, 1979 in Shanghai, P. R. China. She was awarded her Bachelor of Science degree in Medicinal Chemistry from Shenyang Pharmaceutical University in July, 2000. After graduation she worked in Shanghai Institute of Materia Medica, Chinese Academy of Science in China. She entered Virginia Tech to pursue a Master of Science degree under the guidance of Dr. Larry Taylor in January, 2003.

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