Negative Temperature Programming Using Microwave Open Tubular Gas Chromatography

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Abstract

A microwave gas chromatography (GC) column oven is engineered to generate a uniform microwave field around an open tubular column with the elimination of cold spots, which are common in a domestic microwave oven. Short cool-down time in microwave heating makes it possible to employ negative temperature programming for the enhanced separation of compounds during the process. The feasibility of negative temperature programming in microwave GC is investigated for the analysis and quantitation of four different pairs of nonvolatile and volatile compounds. The influence of intermediate column cooling rate, holding time in the cooling ramp, and reheating rate after the cooling ramp for enhanced resolution are investigated. The results obtained from negative temperature programming are compared with those from positive temperature programming. Negative temperature programming affords greater resolution of some critical pairs of analytes.

Introduction

Currently, microwave heating techniques are widely used in many fields of analytical chemistry. Applications of microwaves in this regard include sample digestion, solvent extraction, sample drying, moisture determination, analyte desorption, nebulization of sample solutions, and chromatography. The applications to chromatography are probably the least obvious, and they constitute the focus of this manuscript.

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. Previously, diffusivity has been increased by raising the mobile phase temperature (1–5). However, additional hardware is required in order to maintain pressure in the column so that the solvents do not boil at the elevated temperatures, and high-temperature HPLC is unsuitable for thermally unstable analytes.

Recently, Taylor and Stone (6) employed pulsed microwave radiation to increase the diffusivity of polar analytes in a nonpolar mobile phase without significant heating. A 25-cm IonPac NS-1 Dionex column (Sunnyvale, CA) with a 4-mm i.d. was put into a domestic 600-W microwave oven at the standard 2450 MHz frequency. The column was made of polyetheretherketone instead of metal, so it created no problem in the microwave oven. The peak caused by the analyte (2-naphthol) was sharpened by using pulsed microwave energy. The increase in plate count with pulsed microwaves, compared with the slight decrease in plate count when the column was heated in a conventional oven, clearly proved that the dielectric polarization phenomenon was the primary cause of the improved efficiency. Further work is needed to establish the feasibility and usefulness of this system.

Gaisford and Walters (7) have developed a microwave gas chromatography (GC) oven, which operates at 919 MHz. 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 that are common in a domestic microwave oven. The microwave energy heated only the column because the column contained the only appropriate microwave absorbing material. In a conventional GC system, the walls of the GC oven and the air circulating in the oven are also heated in addition to the column. Selective microwave heating can potentially make column heating more efficient, and faster cooling of the column can be achieved because less material is heated. In the microwave GC, ramp rates up to 6°C/s, isotherms as high as 450°C, and 1-min cool-down times from 350°C to 70°C can be achieved (7). Conventional wall-coated capillary columns (e.g., fused silica) and most any organic compound injected into it or that coats the surface (such as polyimide) will remain essentially unaffected by the microwaves in the oven. The open tubular capillaries used for the microwave oven are unique in that they are coated on the outside with a microwave-absorbent material that efficiently converts microwave energy into thermal energy. 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 microwave GC column oven can be installed in a conventional GC oven by connecting the microwave column to the injector and detector of a conventional GC oven. 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

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plastic tube to facilitate faster column cooling.

The microwave GC oven used for GC has a single mode 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 or diameter (or both) of the cavity in order to achieve a single mode resonant cavity.

Sacks and Brenna have compared microwave and conventionally heated columns for GC of fatty acid methyl esters (8). They employed a polyethylene glycol-based column (30 m × 0.25 mm). The microwave oven was heated at a rate of 360°C/min starting at 60°C. The GC head pressure was 18 psi, resulting in a carrier gas flow of 1.6 mL/min at 60°C. The microwave GC decreased the time of analysis of fatty acid methyl esters by 20% compared with a conventional GC. The prospects of microwave GC are promising when fast GC is used, considering that its rapid heating potential can shorten analysis time greatly.

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. The largest effects on density from changes of temperature are found near the critical temperature for the fluid. Negative temperature programming in SFC has been applied to many compounds, such as mixtures of polychlorinated biphenyls (9). 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 also optimized by negative temperature programming (10). An isobaric (16.2 MPa) negative temperature gradient of –1.2°C/min from 130°C to 80°C with a hold tune of 10 min was used.

Negative temperature programming in GC might be a useful tool for the separation of coeluted components without special columns or more expensive techniques. The selectivity (a) and plate number (N) are constants for a fixed stationary phase and analytes. Therefore, an increase in average retention factor (k) is the only way to improve resolution according to the master resolution equation listed later. Negative temperature programming may be an efficient way to directly influence the k and re-focus analytes during the separation. During the cooling ramp, the solute resides more in the stationary phase than in the mobile phase. Thus, the average k increases at low column temperature, and the value of $k/k + 1$ is predicted to increase.

$$R_s = (a - 1/a)(k/k + 1)N^{1/2}/4 \qquad \text{Eq. 1}$$

where, R_s is resolution, a is the selectivity, and N is the plate number. Mass transfer of the solute during negative temperature programming is shown schematically in Figure 1A. At equilibrium and isothermal conditions, the solute partitions between the stationary phase (SP) and mobile phase (MP). An instant later, the MP 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 (Figure 1B). The solute molecules in the SP and MP refocus when the column temperature decreases during the cooling ramp (Figure 1C). Therefore, sharper peaks are expected, resulting from the smaller peak width because of low diffusibility at the lower temperature during the cooling ramp.

Experimental

Apparatus

An Antek Instruments (Houston, TX) model 3600 microwave controller with a cavity resonance of 2.445 GHz, equipped with microwave GC oven/column installed in a host GC oven, was used. The host GC system was an Agilent 6890A (Palo Alto, CA). Ultrahigh purity helium was obtained from Airgas Inc. (Radnor, PA). A Supelco (Bellefonte, PA) methyl silicone column (30 m × 0.32 mm, 1-µm film thickness), appropriately coated with microwave-sensitive material, was used for the separations. The microwave GC oven column control system shown in Figure 2 gives more details of the control system. A thermocouple (Antek) inserted beside the column was used to measure the column temperature. Pressured air was used to rapidly cool the oven column. A waveguide cable (Antek) connected the microwave generator to the microwave GC oven/column.

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.

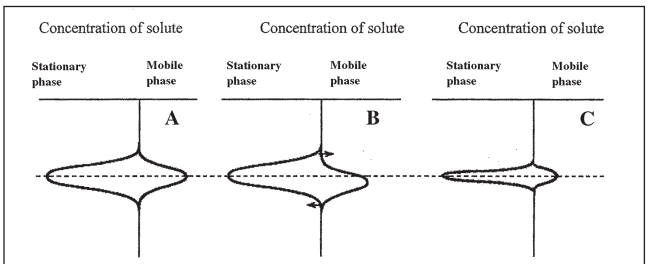

Figure 1. Proposed distribution of solute between stationary and mobile phases: (time zero) before negative temperature programming (A); (time zero + Δ) before negative temperature programming (B); and (time zero + 2Δ) after negative temperature programming (C).

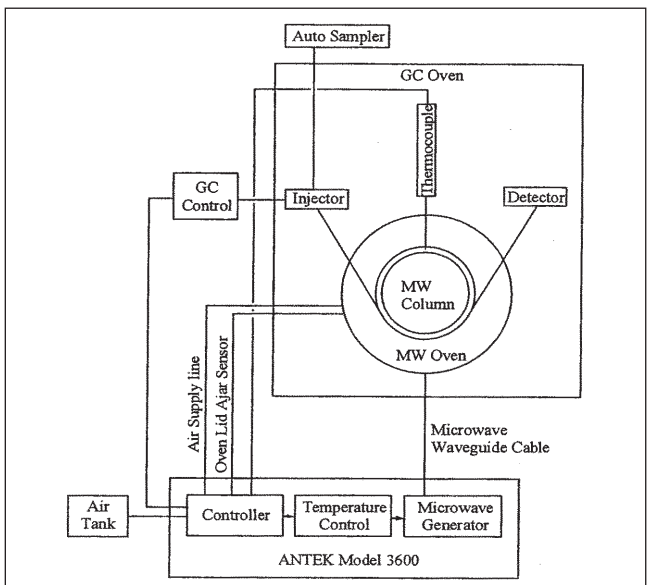

Figure 2. Schematic of microwave GC oven control system.

(Milwaukee, WI). Standard mixtures at various concentrations were prepared using methanol–methylene chloride (1:1, v/v).

GC separation

The inlet temperature was 280°C, and the flame ionization detector temperature was set to 290°C. The make-up flow rate of helium was 10 mL/min. Cooling air for the microwave GC oven column was set at 12 psi. The injection volume was 1 µL, split 1:10. The column was operated in the constant inlet pressure mode. The optimized inlet pressure was 15 psi.

Results and Discussion

Negative temperature programming might have potential in GC because of the rapid heating/cooling afforded by exclusive microwave stimulation of the column coating. Initially, the column was heated to a certain intermediate temperature; then a rapid negative cooling ramp was instituted, after which the column was heated to its final temperature. This manuscript describes, for the first time, efforts to improve resolution of pairs of analytes using negative temperature programming via microwave GC. Two nonvolatile critical pairs, benzo(a)anthracene–chrysene and phenanthrene–anthracene, were studied along with a more volatile pair, ethylbenzene–*m*-xylene.

Separation of benzo(a)anthracene and chrysene

A standard solution of the two polycyclic aromatic hydrocarbons (PAHs) was made in methanol–dichloromethane (1:1). The concentration was 50 ppm for each component. The sample solution (1 µL) was injected into the microwave GC oven/column using positive temperature program 1. The resulting chromatogram is shown in Figure 3A. As can be seen, the two compounds were not baseline separated, resolution (R_s) = 1.2. Negative temperature programming was employed in hopes of increasing resolution. Three negative temperature programs were applied to test the feasibility of enhancing baseline separation. In each case, resolution of the two compounds was successfully improved to approximately R_s = 4.1. Unfortunately, the analysis time was over 60 min because of the slow reheating ramp in program 2. When the reheating rate after the temperature drop was increased from 8°C/min to 30°C/min, the resolution of the compounds was still near 4.0, and the analysis time was decreased by 15 min. The reheating rate was increased even more to 120°C/min (program 4) in order to shorten the analysis time further. The resolution was 3.9 (Figure 3B). At the same time, peak width decreased with negative temperature programming as shown in Table I. A sample solution of 10 PAHs was run using negative temperature program 3 in order to study the influence of negative temperature programming for a complex mixture. The two analytes of interest continued to achieve baseline separation in the complex mixture.

The feasibility of negative temperature programming for quantitative analysis was next studied. The internal standard chosen for this method was pyrene which was pure and eluted near the analytes of interest. Five point calibration curves were established in the range of 20–100 µg/mL of each PAH component. Peak-area ratio (area of interest peak/area of internal standard) versus the concentration of the PAH standard was plotted to provide a calibration curve. Calibration curves for both analytes showed good linearity with correlation coefficients greater than 0.999. A stan-

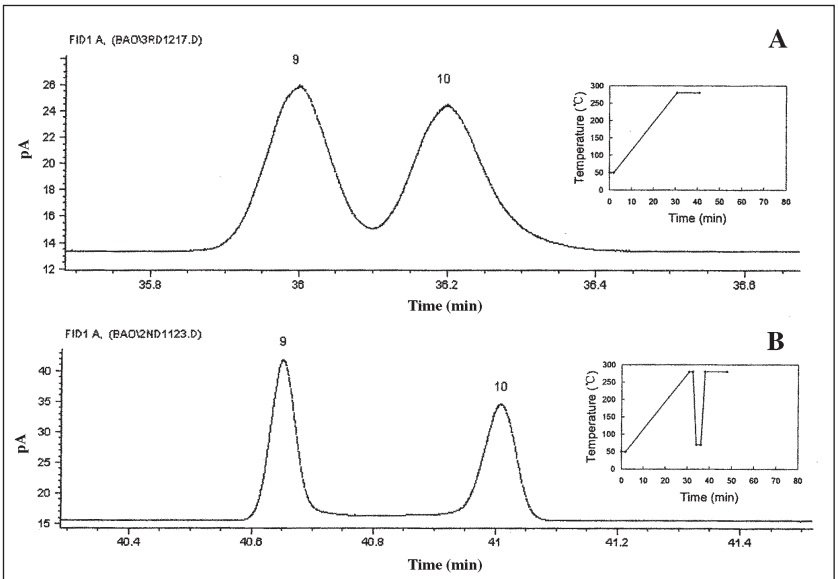

Figure 3. Expanded chromatogram of benzo(a)anthracene and chrysene using: positive temperature program 1 (50°C for 2 min, increased to 280°C at 8°C/min, and held for 10 min, R_s = 1.2) (A) and negative temperature program 4 (50°C for 2 min, increased to 280°C at 8°C/min and held for 1.6 min, decreased to 70°C at 120°C/min, held for 2.0 min, then 120°C/min to 280°C and held for 10 min, R_s = 3.9) (B).

Table I. Comparison of Chromatographic Parameters for a Positive and Three Negative Temperature Programs*

Temperature program	Reheating rate (°C/min)	t_R (min)	t_R (min)	Benzanthracene W_h (min)	Chrysene W_h (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)

* Abbreviations: t_R = retention time, W_h = peak width at half height, R_s = resolution, and N/A = not applicable.
† Numbers in parenthesis are percent relative standard deviation (n = 3).

dard solution of pyrene, benzo(a)anthracene, and chrysene with a concentration of 50 µg/mL for each component was then injected into the microwave oven/column. The peak area ratios were obtained and plugged into the equation for each calibration curve. The percentages of recovery were 97.4% and 95.0%, which indicated that quantitative analysis was achieved using negative temperature programming.

Separation of phenanthrene–anthracene

Phenanthrene and anthracene is another critical pair in GC because their boiling points are both 340°C, which are lower than those of either benzo(a)anthracene or chrysene. A positive temperature program was employed first. The resolution of phenanthrene and anthracene was calculated to be 2.0 (Figure 4A). The resolution improved, however, to 5.6 when negative temperature program 6 was employed (Figure 4B). The conclusion reached here was that negative temperature programming can be applied to improve the resolution of nonvolatile pairs, and the application is not confined to only one particular pair of compounds.

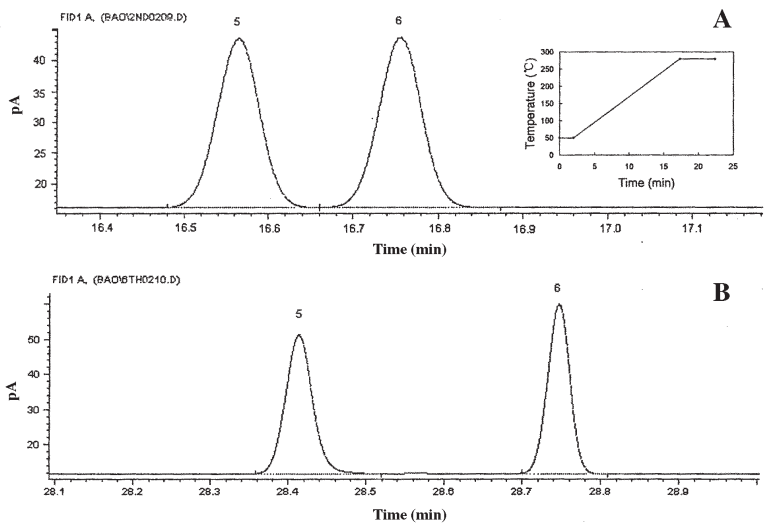

Figure 4. Expanded chromatogram of phenanthrene and anthracene using positive temperature program 5 (50°C for 2 min, increased to 280°C at 15°C/min and held for 5.0 min, R_s = 2.0) (A); and negative temperature program 6 (50°C for 2 min, increased to 250°C at 15°C/min and held for 0 min, decreased to 70°C at 120°C/min, held for 2.0 min, then 15°C/min to 280°C and held for 3.0 min R_s = 5.6) (B).

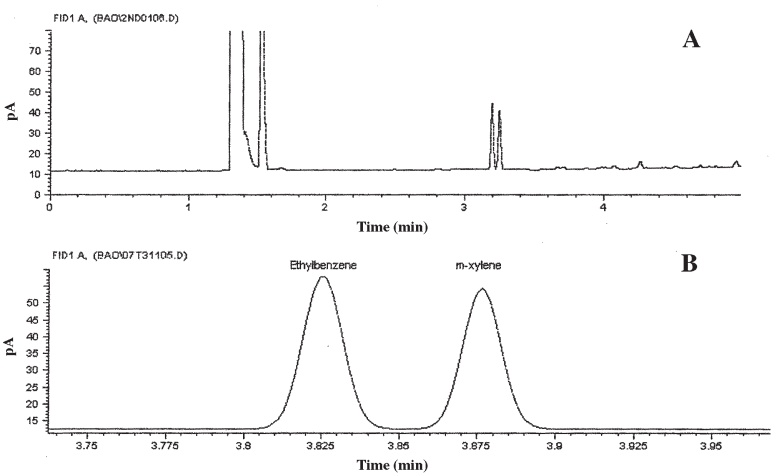

Figure 5. Chromatograms of ethylbenzene and *m*-xylene using positive temperature program 7. A methyl silicone capillary column (30 m × 0.32 mm × 1 µm) was used for separation. The temperature program was 90°C for 1.5 min, increased to 230°C at 40°C/min and held for 0 min, R_s = 1.4 (A); and the temperature program was 90°C for 1.5 min, increased to 100°C at 40°C/min and held for 0 min, decreased to 70°C at 120°C/min and held for 0 min, then 40°C/min to 230°C and held for 0 min, R_s = 2.0 (B).

Separation of volatile pairs

The application of negative temperature programming to volatile compounds was next studied. Ethylbenzene and *m*-xylene are structural isomers with boiling points of 136.18°C and 139.12°C, respectively. The resolution of ethylbenzene and *m*-xylene was only 1.4 for the positive temperature program chosen (Figure 5A). Baseline separation of ethylbenzene and *m*-xylene was, however, obtained using negative temperature programming with a resolution of 2.0 (Figure 5B). The reheating ramp rate was identical to the positive program used.

Three negative temperature programs were employed to study the influence of holding time at low column temperature on the resolution of ethylebenzene and *p*-xylene (Table II). All parameters in the three negative temperature programs were the same except for the holding time in the cooling ramp. Under these conditions, resolution increased with increasing holding time at 70°C. Peak width, however, increased with increased holding time, although it was smaller than that achieved with positive temperature programming.

Our attention turned next to the attempted separation via negative temperature programming of *m*-xylene and *p*-xylene. This critical pair is so difficult to separate (7,8) that only a single peak is observed in the GC chromatogram with positive temperature programming (Figure 6). Various negative temperature programs were attempted, unsuccessfully, to achieve baseline separation (Figure 7).

Conclusion

Negative temperature programming was used to improve the resolution of the critical pairs of compounds. Negative temperature programming was found to greatly improve the resolution of critical pairs compared with positive temperature programming in qualitative analysis. Calibration curves of standard solution using a negative temperature program were obtained with good linearities for quantitation. Quantitative analysis was achieved using a negative temperature program because the percentages of recovery were above 95% for the

Table II. Comparison of Chromatographic Parameters for a Positive Versus Three Negative Temperature Programs*

Temperature program	Hold time (min)	Ethylbenzene t_R (min)	*m*-Xylene t_R (min)	Ethylbenzene W_h (min)	*m*-Xylene W_h (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)

* Abbreviations: t_R = retention time, W_h = peak width at half height, R_s = resolution, and N/A = not applicable.
† Numbers in parenthesis are percent relative standard deviation (n = 3).

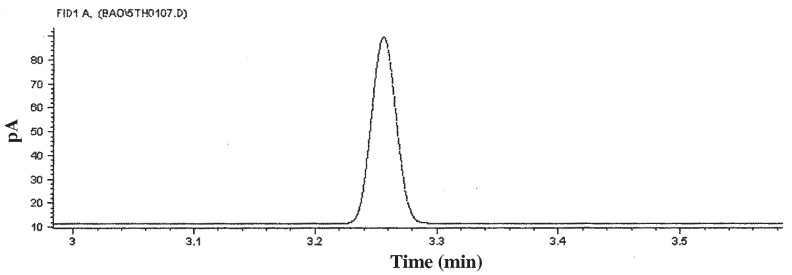

Figure 6. Expanded chromatogram of the xylene mixture using positive temperature program 11 (90°C for 1.5 min, increased to 260°C at 40°C/min and held for 0 min), R_s < 0.5.

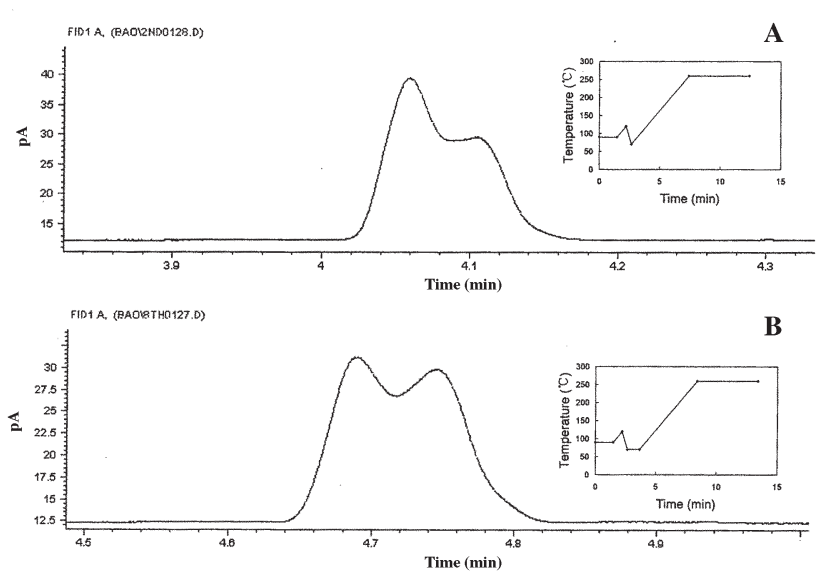

Figure 7. Chromatogram of the xylene mixture. A methyl silicone capillary column (30 m × 0.32 mm × 1 µm) was used for separation. The temperature program was 90°C for 1.5 min, increased to 120°C at 40°C/min and held for 0 min, decreased to 70°C at 120°C/min, held for 0 min, then 40°C/min to 260°C and held for 5 min (A). The temperature program was 90°C for 1.5 min, increased to 120°C at 40°C/min and held for 0 min, decreased to 70°C at 120°C/min and held for 1 min, then 40°C/min to 260°C and held for 5 min.

critical pair of benzo(a)anthracene and chrysene. The negative temperature program could increase the resolution of overlapped peaks effectively for greater k at a low temperature in the cooling ramp. The 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 is a reliable and robust method for application in improving the resolution of critical peaks.

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