Temperature Effect on Peak Width and Column Efficiency in Subcritical Water Chromatography

Yu Yang[1,*], Lori J. Lamm[1], Ping He[1], and **Toru Kondo[2]**

[1]Department of Chemistry, East Carolina University, Greenville, NC 27858 and [2]Fuji Silysia Chemical Ltd., Kasugai-Shi, Aichi-Ken, 487-0013, Japan

Abstract

Subcritical water has been recently employed as the mobile phase to eliminate the use of organic solvents in reversed-phase liquid chromatography. Although the influence of temperature on retention in subcritical water chromatography has been reported, the temperature effect on peak width and column efficiency has not yet been quantitatively studied. In this work, several polar and chlorinated compounds are separated using pure subcritical water on Zorbax RX-C8, PRP-1 (polystyrene–divinylbenzene), Hypersil ODS, and ZirChrom-polybutadiene columns. Isothermal separations are performed at temperatures ranging from 60°C to 160°C. The retention time and peak width of analytes are reduced with increasing temperature. However, the column efficiency is either improved or almost unchanged with the increasing temperature in the low-temperature range (lower than the 100°C to 120°C range), but it is decreased when temperature is further raised in the high-temperature range (higher than the 100°C to 120°C range). Therefore, a maximum in column efficiency is obtained at temperatures within the 100°C to 120°C range in most cases.

Introduction

Reversed-phase liquid chromatography (RPLC) is a very popular separation and analysis technique used today. Unfortunately, organic solvents are required to achieve separation in RPLC. An enormous amount of these organic solvents is consumed every day worldwide. These organic solvents are expensive in terms of both purchasing and waste disposal. In addition, they are also potentially harmful to the laboratory environment and the operator. Therefore, searching for nontoxic solvents as the mobile phase for RPLC is of great interest.

Ambient water is too polar to serve as an eluent for reversed-phase separation. Fortunately, the polarity of water decreases with increasing temperature. Therefore, the solubility of organic compounds is dramatically increased in water at elevated temperatures (1–4). For example, the solubility of some pesticides and polycyclic aromatic hydrocarbons is increased several orders of magnitude by raising the water temperature from ambient to 200°C (1–3). Thus, liquid chromatographic (LC) separations can be achieved by using high-temperature (subcritical) water (5–11). With two additional components (an oven and a backpressure regulator or restrictor), a conventional high-performance liquid chromatographic (HPLC)–UV system can be easily modified to a subcritical water separation system. The oven is used to provide the temperature for subcritical water separation, and the backpressure regulator prevents water from boiling when working with temperatures higher than 100°C. The UV detector is placed outside the oven. Depending on the separation temperature and the flow rate of water used, the temperature of the water eluent in the UV flow cell varies, but it is lower than the oven temperature. If a backpressure regulator or a short packed LC column is used to provide the backpressure, they are normally connected to the outlet of the UV flow cell. Thus, the flow cell is under pressure and may be damaged.

Most reports on subcritical water chromatography mainly focus on testing the feasibility of using subcritical water as the mobile phase for reversed-phase separation (5–11). Even though the effect of water temperature on the retention is mentioned in some of these reports (5–11), a quantitative study of the temperature effect on peak width and column efficiency in subcritical water separation has not yet been reported. It should be pointed out that the effects of temperature on retention, viscosity, diffusivity, and the number of plates have been well-investigated in conventional HPLC (12–17). However, the temperature range was generally much narrower and normally went up to 80°C. In addition, organic solvents were involved in the mobile phases of these studies (12–17).

In this work, pure water at elevated temperatures and pressures was used as the eluent to separate several polar analytes and chlorophenols on four commercial columns, which included the Zorbax RX-C8, polymeric PRP-1, Hypersil ODS, and ZirChrom-PBD columns. Separations were performed at temperatures ranging from 60°C to 160°C in an isothermal manner. The peak width was monitored and the number of theoretical plates was calculated to evaluate the temperature effect on column efficiency.

* Author to whom correspondence should be addressed: email yangy@mail.ecu.edu.

Experimental

Separation columns

A polystyrene–divinylbenzene column (PRP-1, 250- × 4.1-mm i.d.) was purchased from Hamilton Company (Reno, NV). A Zorbax RX-C8 column (250- × 4.6-mm i.d.) was obtained from DuPont (Wilmington, DE). A Hypersil ODS column (100- × 4.6-mm i.d.) (Keystone, Bellefonte, PA) was used to separate a phenol mixture. Because the recently developed zirconia-based columns have shown excellent thermal stability and column efficiency (18–20), a ZirChrom-polybutadiene (PBD) column (100- × 2.1-mm i.d.) (ZirChrom Separation Inc., Anoka, MN) was also employed in this study. The particle size was 3 µm for the ZirChrom-PBD column and 5 µm for the other three columns.

Reagents

All analytes used in this study were obtained from Sigma (St. Louis, MO). The stock solutions of the solutes were prepared in methanol (HPLC grade) (Fisher Scientific, Fair Lawn, NJ). The deionized water (18 MΩ) was prepared in our laboratory using a Sybron/Barnstead (Boston, MA) system. All mobile phases were purged using helium gas prior to each use.

Subcritical water separation

A homemade subcritical water chromatography–UV system was employed in this work. A Hewlett-Packard (Avondale, PA) gradient pump Series 1050 was used to deliver the mobile phase. The flow rate was 0.2 mL/min for the ZirChrom-PBD column and 1 mL/min for the other three columns. The outlet of the pump was connected to a Valco injector fitted with a 2-µL sample loop (purchased from Keystone Scientific). The injector was located just outside a Fisher Isotemp oven. A piece of stainless steel tubing (100-cm × 0.005-inch i.d.) (Keystone) was connected between the injector and the separation column as a preheating coil. Both the preheating coil and the separation column were placed inside the oven. The preheating coil acted like a high-temperature water reservoir to ensure that the water eluent reached the desired temperature before entering the separation column. Because water will be vaporized at 1 atm and temperatures at 100°C or higher, backpressure must be applied to the outlet of the column in order to keep water in the liquid state. There are several reasons for avoiding steam in subcritical water chromatography–UV systems. The water mobile phase may stay in liquid near the column inlet, thus causing steam to form inside the separation column near the outlet end if there is not enough backpressure applied. Thus, the mobile phase exists as two separate phases (liquid water and steam) in the separation column. In addition, the UV signal strongly fluctuates if steam exists in the system. This means that the UV detector is not stable when steam passes through the flow cell. In this study, a capillary restrictor (7-cm × 75-µm) (Polymicro Technologies Inc., Phoenix, AZ) was placed outside the oven and between the separation column and the UV flow cell in order to ensure that the water inside the separation column stayed in the liquid state at higher temperatures. Connection unions ($^1/_{16}$ inch to $^1/_{16}$ inch) (Supelco, Bellefonte, PA) were used to connect the restrictor. By $^1/_{16}$-inch stainless steel tubings, the inlet of union 1 and the outlet of union 2 were connected to the column and UV detector, respectively. The fused-silica capillary restrictor was connected with both unions using graphite ferrules (Alltech, Deerfield, IL). We evaluated the influence of the restrictor dimension on the retention time using restrictors having 7 to 30 cm in length and 51 to 103 µm in inner diameter. However, there was no significant effect of the restrictor dimension on the retention time. A restrictor with a length of 7 cm and a 75-µm inner diameter was chosen for all of the experiments reported in this work. An LDC variable wavelength detector (spectro Monitor 3200, Riviera Beach, FL) was used in this separation system. The UV detector was set at a wavelength of 254 nm for the entire work.

After purging the deionized water with helium, the water was continuously pumped through the separation column at either 0.2 or 1 mL/min, depending on the columns used. Then, the oven was turned on and set to a desired temperature. In order to ensure that separations were carried out at the set temperature, the first injection was not made until approximately 20 min after the oven temperature was reached. This allowed the stationary phase in the packed column and the mobile phase to equilibrate to the desired temperature. It should be noted that the temperature of the stationary phase and the mobile phase inside the column lagged behind the oven temperature by approximately 5 to 20 min, depending on the temperature employed. A Hewlett Packard 3396 Series II integrator was used as the data-recording device. The peak width monitored in this work was at half-height, and the number of theoretical plates (N) was computed using the following equation:

$$N = 2\pi(t_R H/A)^2 \qquad \text{Eq. 1}$$

where H and A are the peak height and area, respectively.

Results and Discussion

Zorbax RX-C8 column

The Zorbax RX-C8 column was first used to study the temperature effect on the peak width and column efficiency. The test solutes in this study included pyridine, benzamide, catechol, and guaiacol. The temperature used for the separation of these analytes ranged from 60°C to 100°C because this column was proven to be thermally stable at temperatures up to 100°C for several thousand column volumes (18). In case of coelution, the analytes were injected individually. It is known that the retention time is decreased with increasing temperature. The same trend was observed in this study with all four solutes tested. For example, pyridine was not eluted until approximately 44 min at 60°C (as shown in Figure 1A) (t_0 = ~2.4 min). However, the same analyte was eluted within approximately 16 min at 100°C. It should be noted that the decrease in retention with increasing temperature was in an almost linear fashion (Figure 1A). Figure 1B demonstrates the temperature effect on the peak width for the test analytes. Because the viscosity of water decreased dramatically when the temperature was raised (as shown in Table I) (21,22), the diffusivity was greatly enhanced. Thus, narrower

peaks were obtained at elevated temperatures. Similar to the retention time, the reduction in the peak width with increasing temperature was not dramatic.

The influence of temperature on column efficiency is demonstrated in Figure 1C. Based on the type of curves in Figure 1C, the solutes can be divided into two groups. The first group includes benzamide and pyridine. The peak efficiency of these two solutes was significantly improved with increasing temperature. The number of theoretical plates obtained at 100°C was 53–84% higher than that at 60°C for benzamide and pyridine. This uptrend temperature effect on efficiency was achieved because the reduction in retention was slower than the reduction in peak width when the temperature was raised. This phenomenon can be seen from Figures 1A and 1B. When the temperature was increased from 60°C to 100°C, the reduction in retention time and peak width for benzamide was 49% and 62%, respectively. However, the plate number of catechol and guaiacol was almost unchanged when the temperature was raised from 60°C to 100°C. This means that both the retention time and peak width of catechol and guaiacol were decreased with similar rates when the temperature was increased.

Table I. Temperature Effect on the Viscocity of Water*

Temperature (°C)	Viscocity (cP) At 50 bar	Viscocity (cP) At 100 bar
25	0.8898	0.8889
50	0.5479	0.5487
100	0.2836	0.2849
150	0.1832	0.1844
200	0.1345	0.1357
250	0.1061	0.1075

* Obtained from references 21 and 22.

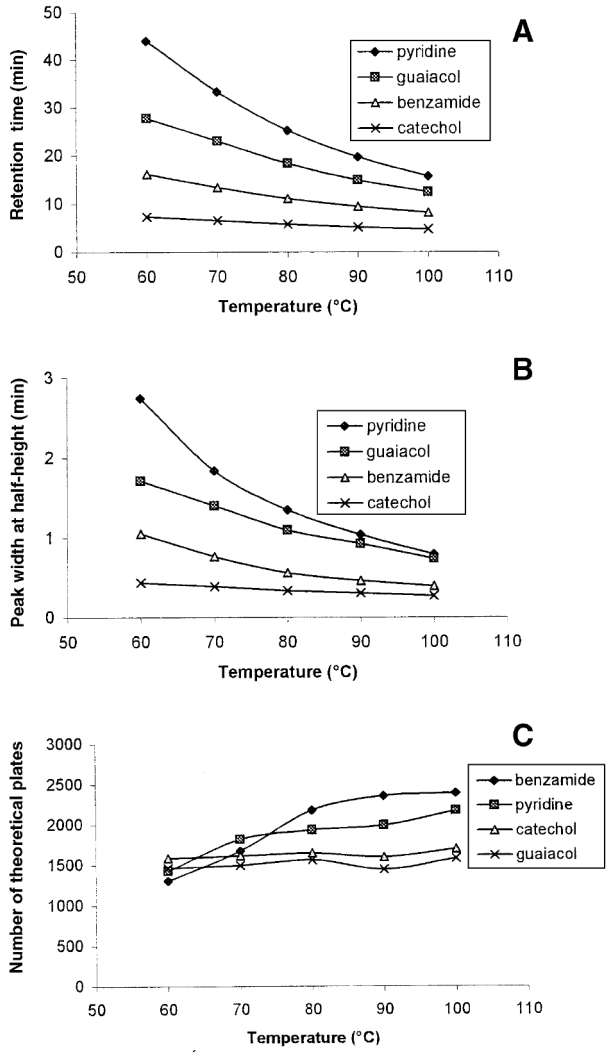

Figure 1. Temperature effect on (A) the retention time, (B) peak width, and (C) number of theoretical plates for separation on the Zorbax RX column.

PRP-1 column

Because the polymeric PRP-1 column is thermally stable at temperatures up to 160°C based on our previous work (18), the temperature range was expanded to 160°C to evaluate the temperature effect on the column efficiency with a greater tempera-

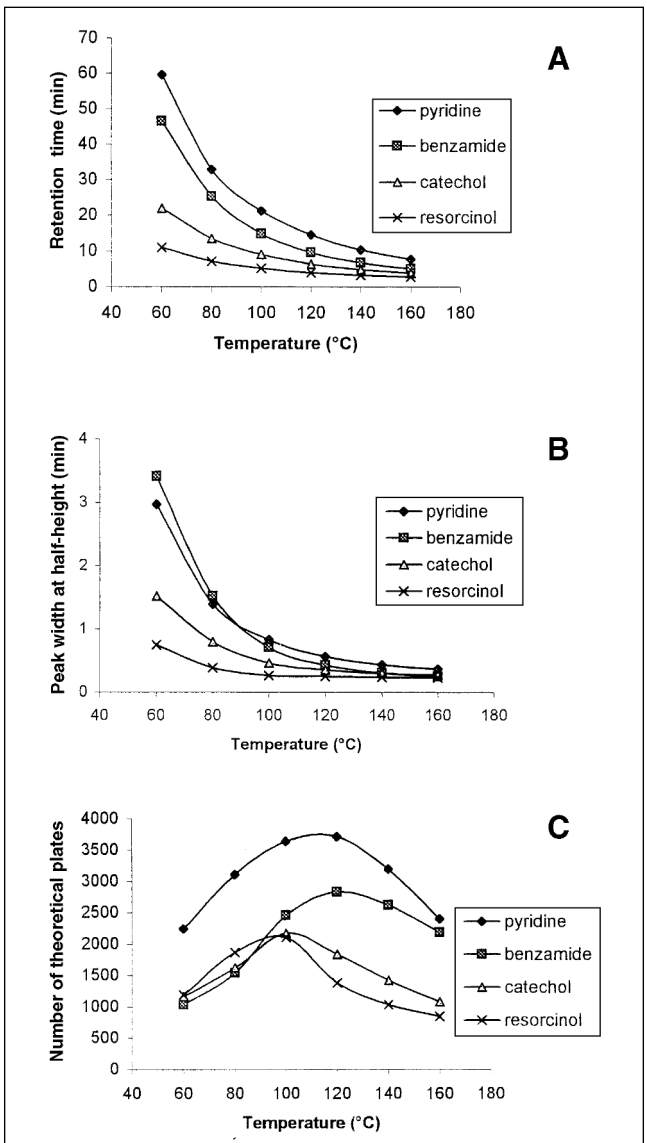

Figure 2. Temperature effect on (A) the retention time, (B) peak width, and (C) number of theoretical plates for separation on the PRP-1 column.

ture range. Therefore, the temperature effect on the peak efficiency of the same or similar solutes (resorcinol, catechol, benzamide, and pyridine) was also investigated by using the PRP-1 column. The separation temperatures ranged from 60°C to 160°C at an interval of 20°C. The analytes were injected individually in case of coelution at higher temperatures. The retention time and peak width were also significantly decreased with increasing temperature as shown in Figure 2 (t_0 = ~2.0 min). The reduction in retention time and peak width was up to 80% for these analytes by raising the separation temperature from 60°C to 160°C. This means that the analysis was 7–9 times faster at 160°C than that at 60°C.

The effect of temperature on peak efficiency is depicted in Figure 2C. Similar to the separation on the Zorbax column, uptrend curves of temperature versus column efficiency were obtained in the temperature range of 60°C to 120°C. However, the number of theoretical plates was decreased when the temperature was further raised to 160°C. Thus, the peak efficiency reached a maximum at temperatures in the 100°C to 120°C range. This maximum of peak efficiency shows that the reduction in retention time was smaller than the reduction in peak width at the low-temperature range, and the decrease in retention time was greater than the decrease in peak width at the high-temperature range. This phenomenon can be clearly seen from Figures 2A and 2B. For example, the retention time of resorcinol was reduced 35% while its peak width experienced a 48% reduction when temperature was raised from 60°C to 80°C (low-temperature range). However, the opposite phenomenon was observed at the higher temperature range. When the temperature was increased from 140°C to 160°C, the decrease in retention time and peak width for catechol was 20% and 8%, respectively.

Hypersil ODS column

In order to further explore the effect of temperature on column efficiency, a mixture of phenol, 2-chlorophenol, and 2,3-dichlorophenol was separated using a Hypersil ODS column. Even though the thermal stability of this column is poorer than the PRP-1 column, we still used a temperature range of 60°C to 140°C. Because the column was exposed to high temperatures only for several hours (the most) in this study, the thermal stability did not get significantly worse within this short period of time based on our previous study (18). Again, both the retention time (t_0 = ~1.0 min) and peak width were decreased with increasing temperature as shown in Figures 3A and 3B. The number of theoretical plates (equivalent to a 25-cm column) was slightly increased for chlorophenols but stayed almost unchanged for phenol when the temperature was increased from 60°C to 100°C. Further raising the temperature from 100°C to 140°C caused a significant decrease in column efficiency (as demonstrated in Figure 3C). This was in agreement with the results obtained by using the PRP-1 column even though different analytes were used.

ZirChrom-PBD column

Based on references 19 and 20, the ZirChrom-PBD column was stable at temperatures up to the range of 150°C to 200°C. Therefore, the phenol mixture was also separated on the ZirChrom-PBD column at temperatures ranging from 60°C to 140°C. Because the inner diameter of the ZirChrom-PBD column was 2.1 mm, a flow rate of 0.2 mL/min was used for this column. Similar to separations on the Hypersil ODS column, the column efficiency was either increased or unchanged when the temperature was raised from 60°C to 100°C (as shown in Figure 4C), but the plate number (equivalent to a 25-cm column) was decreased when the temperature was further increased from 100°C to 140°C. However, the decrease in efficiency was less significant for this zirconia-based column compared with that for the Hypersil column. As can be seen from Figure 4C, increasing the temperature from 60°C to 140°C resulted in either no decrease or a very little decrease (approximately 15%) in efficiency with the ZirChrom-PBD column but a typical 40% decrease with the Hypersil ODS column. This means that the

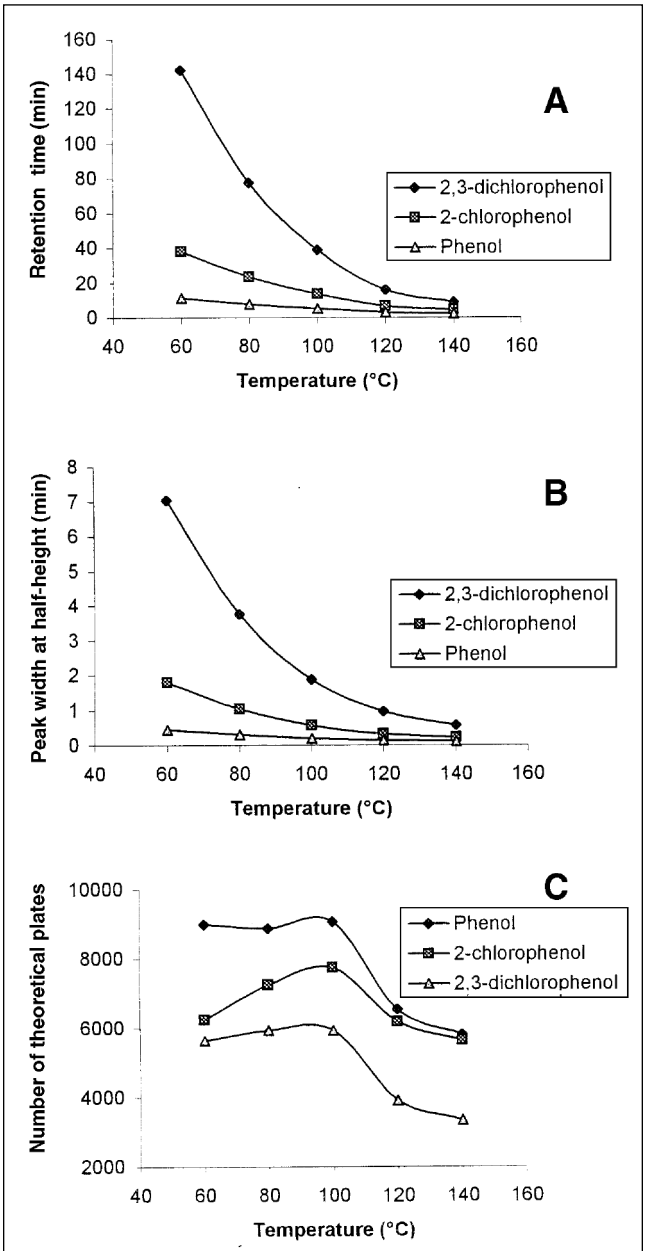

Figure 3. Temperature effect on (A) the retention time, (B) peak width, and (C) number of theoretical plates (equivalent to a 25-cm column) for separation on the Hypersil ODS column.

zirconia-based column is more suitable for separations at higher temperatures. Another benefit associated with the ZirChrom-PBD column is that the analysis time required by this column was much shorter than that required by the Hypersil column. As shown in Figures 3 and 4, separation on the ZirChrom-PBD column was approximately four times faster than that on the Hypersil column, but the column efficiency of the ZirChrom-PBD under the fast analysis conditions was still competitive compared with that obtained by the Hypersil column.

Mechanism for the temperature effect on column efficiency in subcritical water chromatography

To the best of our knowledge, this is the first report that quantitatively describes the effect of water temperature on column efficiency over a wide temperature range in subcritical water chromatography. In many cases, a maximum in column efficiency in subcritical water chromatography was obtained when the water temperature was varied in this work. We believe that this maximal efficiency was caused by two factors that are associated with water temperature. The first one is the mass transfer that improves the efficiency, and the second is the longitudinal diffusion that worsens the column efficiency.

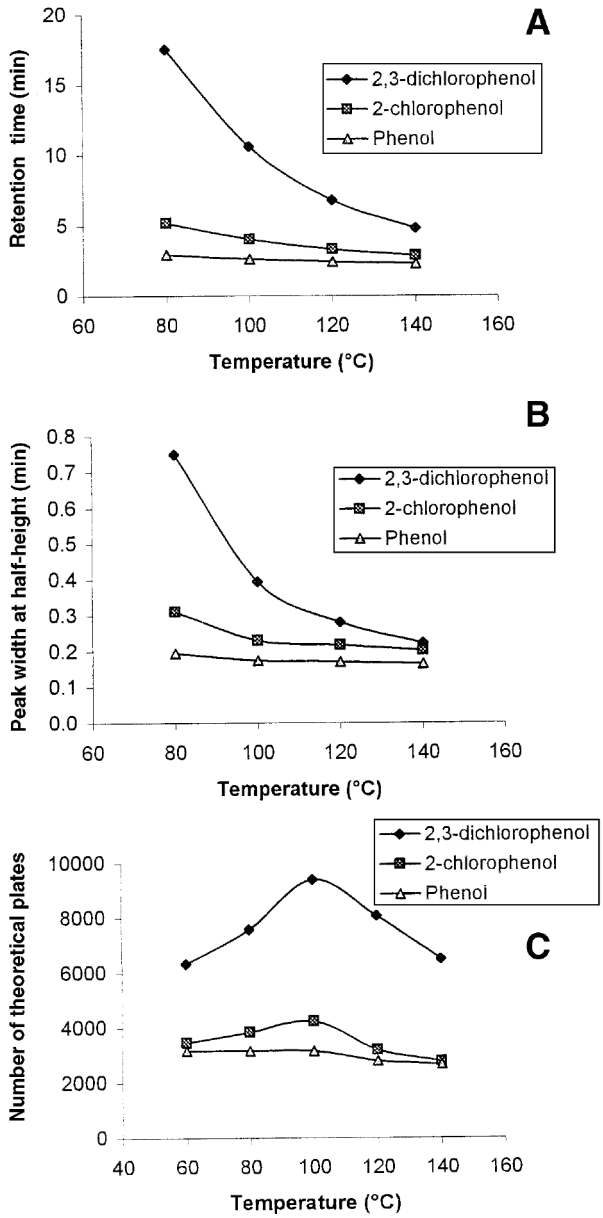

Figure 4. Temperature effect on (A) the retention time, (B) peak width, and (C) number of theoretical plates (equivalent to a 25-cm column) for separation on the ZirChrom-PBD column.

It is well-known that the diffusivity or diffusion coefficient of the mobile phase (D_m) is directly proportional to the absolute temperature and inversely proportional to the viscosity of the mobile phase. Because the viscosity of water is decreased with increasing temperature as demonstrated in reference 22 (Table I), the diffusivity (mass transfer) of water is dramatically increased and the mass transfer resistance (the C_m term in the van Deemter equation) is greatly decreased at elevated temperatures. Thus, narrower bands and higher column efficiency should be expected with increasing temperature. This is why the number of theoretical plates was generally increased when the temperature was raised from 60°C to the 100°C to 120°C range (as illustrated in Figures 1–4). Therefore, we believe that mass transfer may dominate the subcritical water separation process at the lower temperature range (lower than the 100°C to 120°C range).

By increasing the temperature from 100°C to 120°C, the diffusivity is further increased and even better mass transfer results. However, the better mass transfer also causes a greater axial molecular diffusion (longitudinal diffusion, the B term in the van Deemter equation), which makes the column efficiency become poorer. Therefore, the higher the temperature, the greater the longitudinal diffusion (B is directly proportional to D_m in the van Deemter equation) and the lower the column efficiency. This is the reason why the number of plates was decreased when the temperature was raised from the 100°C to 120°C range to the 140°C to 160°C range (Figures 2–4). Therefore, longitudinal diffusion may be the dominating factor that controls the subcritical water separation at the higher temperature range. Carr et al. (20) reported that the column efficiency was decreased for separations using organic solvent–water mixtures at temperatures of 150°C and 200°C, although the authors indicated that this might be caused by the interaction of molecules with the column walls at higher temperatures (20).

Because increasing the separation temperature causes lower mass transfer resistance (the C term in the van Deemter equation decreases) but also greater longitudinal diffusion (the B term in the van Deemter equation increases), a maximal column efficiency may be observed. However, if the decrease in the C term and increase in the B term are similar in the lower temperature range, then they compensate each other. Thus, the efficiency will stay unchanged when the temperature is increased from low temperature to the 100°C to 120°C range. This is evidenced in Figures 1, 3, and 4. However, at a higher temperature range the increase in the B term always exceeds the decrease in the C term. Therefore, the column efficiency was always decreased when the temperature was further raised. This may explain why the number of plates was always decreasing with all of the columns and solutes tested when the temperature was increased from the 100°C to 120°C range to the 140°C to 160°C range (as shown in Figures 2–4).

Conclusion

The elution of several polar analytes has been achieved by using pure water and four different types of commercially available reversed-phase columns at elevated temperatures under moderate pressure to keep the water in the liquid state. A fused-silica capillary restrictor was connected between the separation column and the UV flow cell to provide the backpressure needed to avoid water from boiling at higher temperatures. For all of the analytes studied and columns used, the peak width was decreased with increasing water temperature. For example, the peak width of benzamide obtained on the PRP-1 column was reduced by as much as 93% by raising the temperature from 60°C to 160°C. However, the column efficiency was either improved or remained unchanged initially but then decreased with increasing temperature. Thus, a maximum in efficiency was observed at temperatures in the 100°C to 120°C range in most cases.

Acknowledgments

This research was supported by an award from Research Corporation (CC4607). The authors would also like to thank ZirChrom Separations Inc. for providing the ZirChrom-PBD column. Toru Kondo acknowledges Fuji Silysia Chemical Ltd. and the Department of Chemistry at East Carolina University for providing the opportunity to conduct this research.

References

1. N.D. Sanders. Observation of the solubility of heavy hydrocarbons in near-critical water. *Ind. Eng. Chem. Fundament.* **25:** 171–74 (1986).
2. D.J. Miller and S.B. Hawthorne. Method for determining the solubility of hydrophobic organics in subcritical water. *Anal. Chem.* **70:** 1618–21 (1998).
3. D.J. Miller, S.B. Hawthorne, and A.A. Clifford. Solubility of polycyclic aromatic hydrocarbons in subcritical water from 298 K to 498 K. *J. Chem. Eng. Data* **43:** 1043–46 (1998).
4. Y. Yang, S.B. Hawthorne, and D.J. Miller. Toluene solubility and organic partitioning from gasoline and diesel fuel into water at elevated temperatures and pressures. *J. Chem. Eng. Data* **42:** 908–13 (1997).
5. R.M. Smith and R.J. Burgess. Superheated water—a clean eluent for reversed-phase high-performance liquid chromatography. *Anal. Commun.* **33:** 327–29 (1996).
6. D.J. Miller and S.B. Hawthorne. Subcritical water chromatography with flame ionization detection. *Anal. Chem.* **69:** 623–27 (1997).
7. R.M. Smith and R.J. Burgess. Superheated water as an eluent for reversed-phase high-performance liquid chromatography. *J. Chromatogr. A* **785:** 49–55 (1997).
8. B.A. Ingelse, H.-G. Janssen, and C.A. Cramers. HPLC–FID with superheated water as the eluent: improved methods and instrumentation. *J. High Resolut. Chromatogr. HRC* **21:** 613 (1998).
9. Y. Yang, A.D. Jones, and C.D. Eaton. Retention behavior of phenols, anilines, and alkylbenzenes in liquid chromatographic separations using subcritical water as the mobile phase. *Anal. Chem.* **71:** 3808–13 (1999).
10. O. Chienthavorn and R.M. Smith. Buffered superheated water as an eluent for reversed-phase high-performance liquid chromatography. *Chromatographia* **50:** 485 (1999).
11. T.M. Pwlowski and C.F. Poole. Solvation characteristics of pressurized hot water and its use in chromatography. *Anal. Commun.* **36:** 71–75 (1999).
12. W.R. Melander, B.-K. Chen, and C. Horváth. Mobile phase effects in reversed-phase chromatography I. Concomitant dependence of retention on column temperature and eluent composition. *J. Chromatogr.* **185:** 99–109 (1979).
13. F.V. Warren and B.A. Bidlingmeyer. Influence of temperature on column efficiency in reversed-phase liquid chromatography. *Anal. Chem.* **60:** 2821–24 (1988).
14. T. Welsch, M. Schmid, J. Kutter, and A. Kalman. Temperature of the eluent: a neglected tool in high-performance liquid chromatography? *J. Chromatogr.* **728:** 299–306 (1996).
15. L.C. Sander and S.A. Wise. Subambient temperature modification of selectivity in reversed-phase liquid chromatography. *Anal. Chem.* **61:** 1749–54 (1989).
16. A. Tchapla, S. Heron, H. Colin, and G. Guiochon. Role of temperature in the behavior of homologous series in reversed-phase liquid chromatography. *Anal. Chem.* **60:** 1443–48 (1988).
17. L.A. Cole and J.G. Dorsey. Temperature dependence of retention in reversed-phase liquid chromatography. 1. Stationary-phase considerations. *Anal. Chem.* **64:** 1317–23 (1992).
18. P. He and Y. Yang. "Thermal Stability of Reversed-Phase Columns Under Subcritical Water Conditions". Presented at *Eastern Analytical Symposium*, Atlantic City, NJ, November 2000.
19. J. Li and P.W. Carr. Effect of temperature on the thermodynamic properties, kinetic performance, and stability of polybutadiene-coated zirconia. *Anal. Chem.* **69:** 837–43 (1997).
20. J. Li, Y. Hu, and P.W. Carr. Fast separation at elevated temperatures on polybutadiene-coated zirconia reversed-phase material. *Anal. Chem.* **69:** 3884–88 (1997).
21. Y. Yang, M. Belghazi, A. Lagadec, S.B. Hawthorne, and D.J. Miller. Elution of organic solutes from different polarity sorbents using subcritical water. *J. Chromatogr. A* **810:** 149–59 (1998).
22. L. Haar, J.S. Gallagher, and G.S. Kell. *National Bureau of Standards/National Research Council Steam Tables*. Hemisphere Publishing Corporation, New York, NY, 1984.

Manuscript accepted December 7, 2001.