



Studies on the long-term thermal stability of stationary phases in subcritical water chromatography

Ping He, Yu Yang*

Department of Chemistry, College of Arts and Sciences, East Carolina University, Greenville, NC 27858-4353, USA

Abstract

The long-term thermal stability of five commercially available reversed-phase columns has been evaluated under subcritical water conditions (100 and 150 °C). The five columns included Zorbax RX-C₈, Nucleosil C₁₈ AB, Hypersil BDS C₁₈, PRP-1 (poly(styrene–divinylbenzene)), and ZirChrom-PS (polystyrene) columns. Retention factors, plate numbers, and peak areas were monitored over a prolonged period of time. Comparing the three silica-based columns, the Zorbax RX-C₈ column was the most stable followed by the Nucleosil C₁₈ AB column. The Hypersil BDS C₁₈ column was the least stable under subcritical water conditions. The ZirChrom-PS column was stable at 100 °C for at least 7600 column volumes. Of all five columns, the polymeric PRP-1 column was the most stable under subcritical water conditions.

© 2002 Elsevier Science B.V. All rights reserved.

Keywords: Stationary phases, LC; Stability studies; Temperature effects; Subcritical water chromatography

1. Introduction

Since water is very polar at ambient conditions, it is mixed with organic solvents so that it can serve as the mobile phase in reversed-phase liquid chromatography. Conventional reversed-phase separations are normally performed at ambient temperature, even though some separations have been done at elevated temperature which rarely exceeded 100 °C [1–8]. Recently, pure water has been used as the eluent for reversed-phase separation at elevated temperatures [9–23]. The reason behind this new separation technique is that the polarity of water is dramatically decreased when heated under pressure. At 200–250 °C, the dielectric constant of water is similar to that of methanol or acetonitrile [24].

The reported subcritical water chromatography research has been focused on the following areas: separation of polar and non-polar analytes; separation of model drugs; subcritical water separation with different detection techniques including UV, flame ionization detection, and NMR; and studies of retention behavior in subcritical water chromatography [9–23]. Since subcritical water separation requires elevated temperature, an associated concern is whether the stationary phase is thermally stable. This question is especially critical when combined with the fact that most commercially available reversed-phase packing materials are designed for low temperature application. Even though the short-term stability of separation columns under subcritical water conditions has been mentioned in some reports, the long-term thermal stability has been investigated only for polymeric and zirconia-based columns using organic solvent and water mixtures as the eluent.

*Corresponding author. Tel.: +1-252-328-1647; fax: +1-252-328-6210.

E-mail address: yangy@mail.ecu.edu (Y. Yang).

In this study, we evaluated the thermal stability of three silica-based, one zirconia-based, and one polymeric column. The temperatures used were 100 or 150 °C depending on the packing materials. Each column was evaluated for 6000–12 500 column volumes at each elevated temperature. Retention factors and plate numbers were calculated for each chromatogram at a given column volume to monitor any degradation of the stationary phase under subcritical water conditions.

2. Experimental

2.1. Chemicals and reagents

Deionized water (18 MΩ cm) was the only mobile phase used for all experiments in this study. It was obtained from a Nano pure-A-4-holder system in the Chemistry Department at East Carolina University. Fresh water was purged with helium before each use. Benzene, toluene, ethylbenzene, phenol and *m*-xylene were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Uracil, 4-chlorophenol, 2,3-dichlorophenol, 2,3,6-trichlorophenol, aniline, methylbenzoate, *p*-acetophenidide and naphthalene were obtained from Sigma (St. Louis, MO, USA). Caffeine was purchased from Eastman Kodak (Rochester, NY, USA). All test solutions were prepared in HPLC-grade methanol, which was obtained from Fisher Scientific.

2.2. Subcritical water separation

All separations in this study were made with a

Consta Metric 3200 solvent delivery system (LDC Analytical, Riviera Beach, FL, USA), An isotemp programmable oven (Fisher Scientific, forced-air convection), UV detection at 254 nm with a variable-wavelength detector (Knauer), and an HP3396 series II integrator (Hewlett-Packard, Avondale, PA, USA) were used. Sample injection was performed using a Valco manual injector fitted with a 2-μl sample loop (Keystone Scientific, Bellefonte, PA, USA). A piece of fused-silica capillary tubing with dimensions 12 cm×50 μm (Polymicro Technologies, Phoenix, AZ, USA) was used as the restrictor to prevent water from boiling at temperatures above 100 °C.

2.3. Columns evaluated

A total of five different columns have been evaluated in this study including three silica-based, one zirconia-based, and one polymeric column. A Zorbax RX-C₈ column was obtained from DuPont (Wilmington, DE, USA). A Nucleosil C₁₈ AB column and a Hypersil BDS C₁₈ column were purchased from Keystone Scientific. Since the recently developed zirconia-based columns have shown excellent thermal stability and column efficiency [1,2], a ZirChrom-PS (polystyrene) column (ZirChrom Separation, Anoka, MN, USA) was also employed in this study. A poly(styrene–divinylbenzene) column (PRP-1) was provided by Hamilton (Reno, NV, USA). Descriptions of these five columns are given in Table 1.

2.4. Column temperature monitoring

The column temperature was monitored using a

Table 1
Descriptions of the evaluated columns

Description	Column length (cm)	Column I.D. (mm)	Particle size (μm)	Pore size (Å)
Zorbax RX-C ₈ ^a	15	4.6	5	80
Nucleosil C ₁₈ AB ^a	15	2.0	5	100
Hypersil BDS C ₁₈ ^a	15	4.6	5	120
ZirChrom-PS ^b	10	2.1	3	N/A
Hamilton PRP-1 ^c	15	2.1	10	100

^a Silica-based column.

^b Zirconia-based column.

^c Polymeric column.

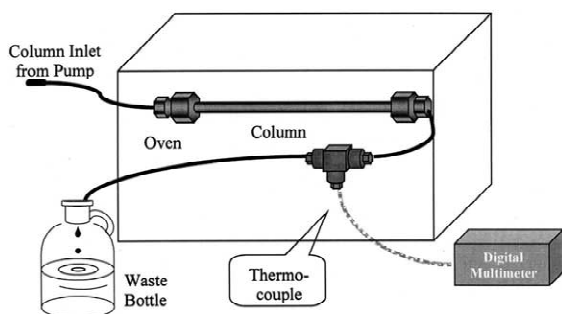


Fig. 1. Schematic diagram of temperature monitoring system.

system as shown in Fig. 1. A stainless steel T-union housed a thermocouple. The T-union was connected to the analytical column and the outlet of the union was directed to the waste bottle. The tubing between the column and the T-union was ~ 3 cm long. The T-union and the tubing were wrapped with insulating material. The Hamilton PRP-1 column was used in this experiment. At the beginning of the temperature-monitoring experiment, the oven was set at a low temperature, such as 25 °C. As soon as the oven was reset to a higher temperature, such as 200 °C, manual recording of the rise of two temperature readings was immediately started. One temperature reading was obtained from the digital display on the oven and the other was obtained from the thermocouple. The temperature readings were recorded every 30 s. The change in temperature as a function of time was then plotted.

2.5. Evaluation of the thermal stability of columns

The water (18 M Ω cm) was purged with helium before each use. Then the pump, oven, and detector were turned on. Approximately 1 h after the desired temperature was reached, the first injection of the day was made. The injection time was recorded and the data were collected. Then the same mixture was injected periodically at the same separation temperature to monitor the long-term change in retention time, plate number, and peak areas to evaluate the thermal stability of the columns. The flow-rates and temperatures used for each column are given in Table 2.

Table 2
Separation conditions for each column

Column brand name	Flow rate (ml/min)	Evaluation temperature (°C)
Zorbax RX-C ₈	1.00	100
Nucleosil C ₁₈ AB	0.40	100
Hypersil BDS C ₁₈	1.00	100
ZirChrom-PS	0.40	100
Hamilton PRP-1	0.40	100, 150

3. Results and discussion

3.1. Column temperature delay

The diameters of the columns evaluated in this study are much greater compared with GC columns. Thus there must be a temperature delay inside the LC columns compared to the oven temperature. As shown in Fig. 2 (top), it took ~ 16 min for the oven to reach 200 °C when heated from 25 °C. However, it required ~ 35 min for the column temperature to reach 200 °C. The temperature delay for 200 °C was 19 min. The delays were shorter for lower tempera-

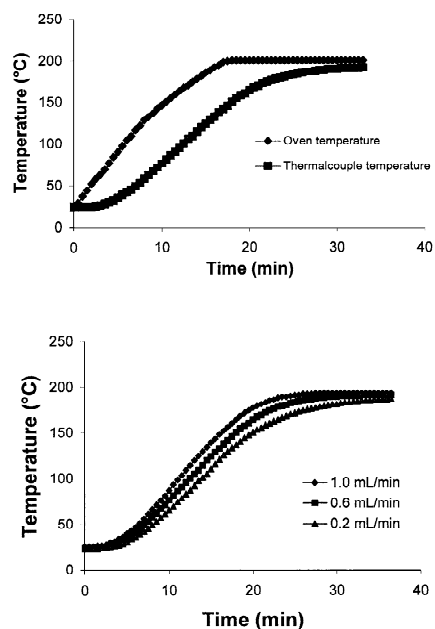


Fig. 2. Comparison of oven temperature and column temperature (top) and comparison of the column temperature at different flow-rates (bottom).

tures. For example, the delays were 6.5, 6.0, and 5.0 min for 150, 100, and 50 °C, respectively. Fig. 2 (bottom) demonstrates the column temperatures obtained at 200 °C by using three different flow-rates of water. We can easily see that the temperature delay was shorter at higher flow-rates but longer at lower flow-rates. This could be attributed to the high heat capacity of water. At higher flow-rate water conducts heat more efficiently in the column than at lower temperature.

3.2. Column thermal stability

3.2.1. Zorbax RX-C₈

Since the column dimensions are 15 cm in length and 0.46 cm in inner diameter, one column volume is equivalent to 2.49 ml ($V = \pi r^2 l = 3.14(0.46/2)^2 \cdot 15 = 2.49 \text{ cm}^3 = 2.49 \text{ ml}$). Thus at a flow-rate of 1 ml/min, 1 min is equivalent to 0.40 column volumes.

A mixture of caffeine, methyl benzoate, and benzene was used in this evaluation. As shown in Fig. 3 (top), the retention factors of analytes did not change very much after the column was exposed to subcritical water (100 °C water) for more than 6000 column volumes. A plot of effective plate number versus column volume is shown in Fig. 3 (bottom). Even though the reproducibility was poor for benzene and methyl benzoate, the efficiency did not worsen after heating for over 6000 column volumes, which indicates that this XDB-C₈ column was stable at 100 °C for at least 6000 column volumes (15 000 min or 250 h).

Fig. 4 contrasts the chromatograms obtained using the Zorbax RX-C₈ column at two different column volumes, 1565 and 6180 column volumes, respectively. It can be seen that the two chromatograms appear very similar, again indicating the good thermal stability of this column at the temperature evaluated.

3.2.2. Nucleosil C₁₈ AB

This is a narrow-bore column with 2-mm internal diameter. The column volume is 0.47 ml. This column was evaluated using a mixture of benzene, toluene, and methyl benzoate at 100 °C for 12 500 column volumes. Based on the values of retention factor and effective plate number, the column was

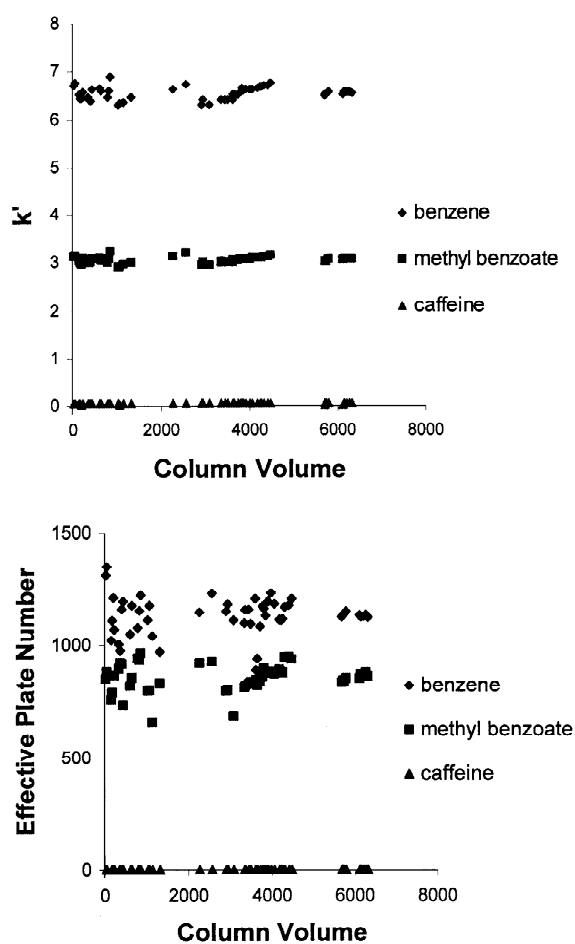


Fig. 3. Retention factor (top) and effective plate number (bottom) versus column volume for Zorbax RX-C₈ column at 100 °C.

stable for up to 8000 column volumes as shown in Fig. 5. However, both retention and column efficiency were decreased for benzene and toluene after 8000 column volumes, indicating that the thermal stability became poorer due to prolonged heating under the subcritical water condition. It has to be pointed out that the retention factor and effective plate number of methyl benzoate were slightly increased with increasing column volume during the entire evaluation period. This phenomenon is evidenced by the migration of the methyl benzoate peak toward longer retention time as shown in Fig. 6. A possible reason for the increased retention for methyl benzoate and decreased retention for benzene and toluene is that the bonded C₁₈

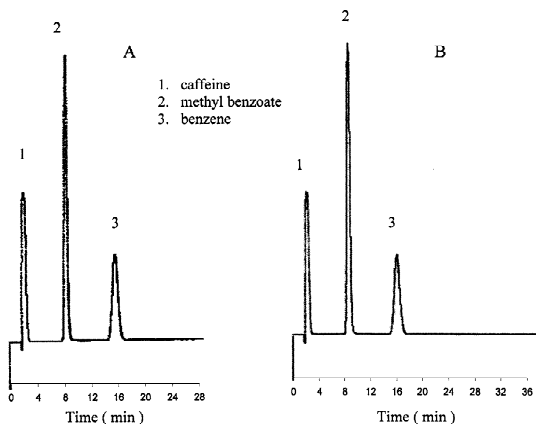


Fig. 4. Chromatograms obtained from Zorbax RX-C₈ column after (A) 1565 and (B) 6180 column volumes at 100 °C.

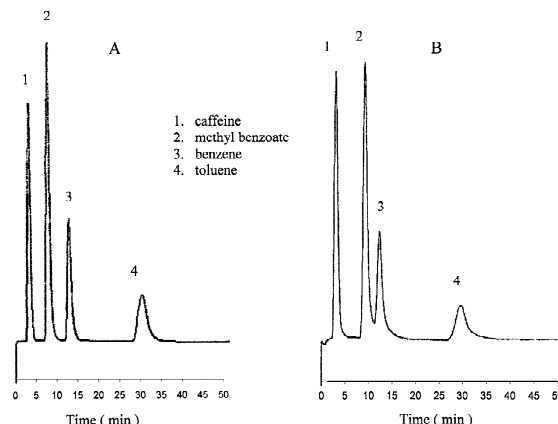


Fig. 6. Chromatograms obtained from Nucleosil C₁₈ AB column after (A) 2960 and (B) 11 901 column volumes at 100 °C.

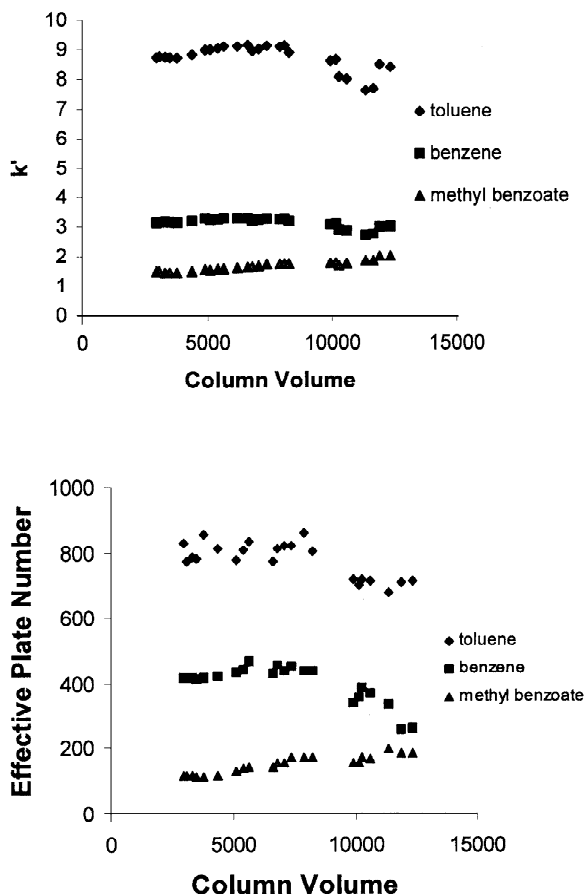


Fig. 5. Retention factor (top) and effective plate number (bottom) versus column volume for Nucleosil C₁₈ AB column at 100 °C.

non-polar phase was gradually stripped from the polar silica surface, resulting a stronger interaction with more polar solutes and weaker interaction with non-polar solutes.

3.2.3. Hypersil BDS C₁₈

This column has the same dimension as the Zorbax RX-C₈ column, and therefore the column volume is 2.49 ml. A mixture of chlorophenols was used to evaluate the thermal stability of this Hypersil BDS C₁₈ column. As shown in Fig. 7, there was a slight increase in retention factor for all three chlorophenols when the column was evaluated at 100 °C for up to 1000 column volumes. On the other hand, there was a significant decline in effective plate number for both the di- and trichlorophenols as depicted in Fig. 7 (bottom). This phenomenon suggests that the stationary phase experienced significant degradation during the course of evaluation. As shown in Fig. 8, the trichlorophenol peak shows considerable broadening after just 746 column volumes of subcritical water applied.

3.2.4. ZirChrom-PS

This is a shorter column with dimensions of 10 cm×2.1 mm. The column volume is 0.35 ml. Since the flow-rate is 0.4 ml/min, 1 min is equivalent to 1.14 column volumes. The three analytes chosen to evaluate the ZirChrom-PS column were naphthalene, *m*-xylene, and toluene. It can be seen from Fig. 9 that although there was a slight decrease

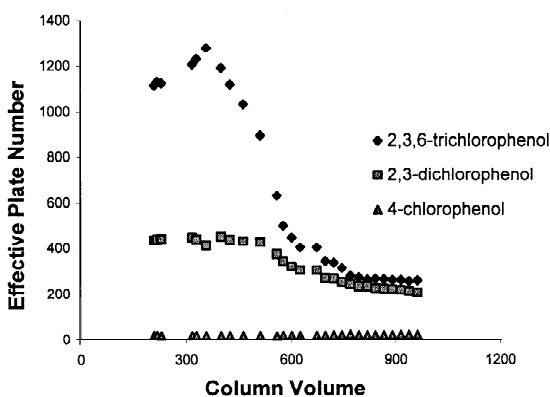
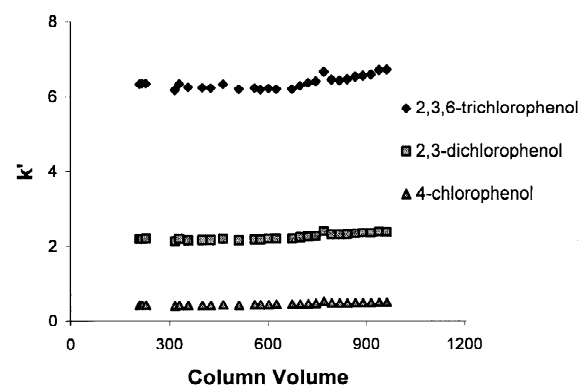


Fig. 7. Retention factor (top) and effective plate number (bottom) versus column volume for Hypersil BDS C₁₈ column at 100 °C.

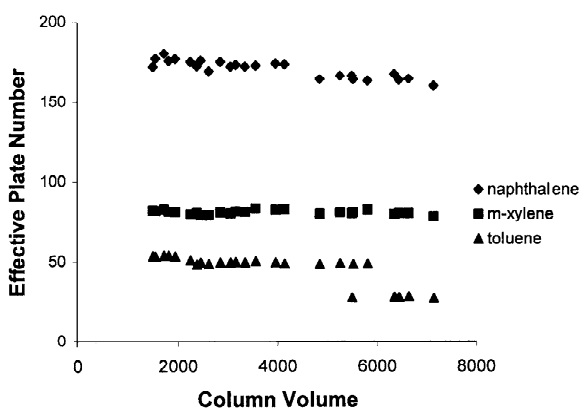
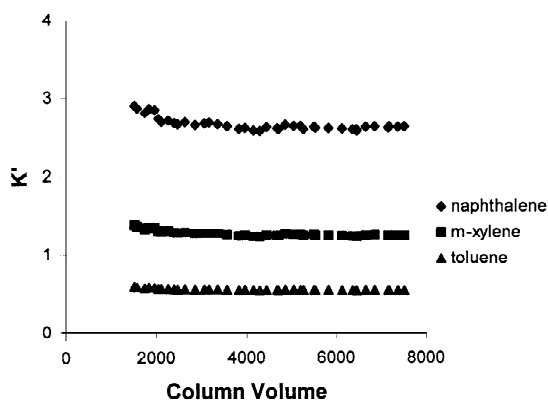


Fig. 9. Retention factor (top) and effective plate number (bottom) versus column volume for ZirChrom-PS column at 100 °C.

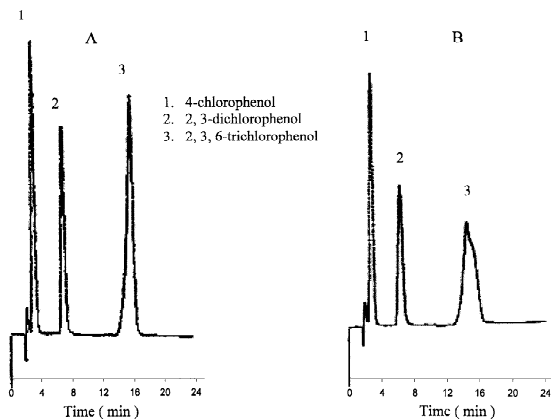


Fig. 8. Chromatograms obtained from Hypersil BDS C₁₈ column after (A) 229 and (B) 746 column volumes at 100 °C.

in retention factor at the beginning of the evaluation, K' then stayed stable for up to 7600 column volumes at 100 °C. Based on the plot of the effective plate number versus column volume (Fig. 9, bottom), there was virtually no change in plate number for *m*-xylene. However, there was a slight decline in plate number for the other two analytes. Fig. 10 shows the chromatograms for the three components obtained at 1733 and 7502 column volumes. Both chromatograms appear very similar. All these results indicated that the ZirChrom PS column was relatively stable at 100 °C.

3.2.5. Hamilton PRP-1

Since this column has dimensions of 15 cm × 2.1 mm, the column volume is 0.52 ml. The analytes used in this evaluation included caffeine, *m*-cresol,

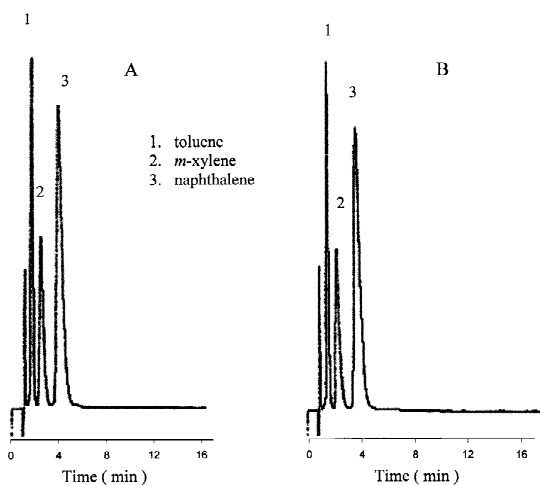


Fig. 10. Chromatograms obtained from ZirChrom PS column after (A) 1733 and (B) 7502 column volumes at 100 °C.

and *p*-acetophenidide. This column was initially evaluated at 100 °C. As shown in Fig. 11, there was no significant change in retention factor while the plate number slightly decreased with increasing column volumes for the three analytes. Further increasing the temperature to 150 °C, there was still no significant change in retention factor (Fig. 12). Interestingly, the plate number of *p*-acetophenidide increased and then stabilized after the first 2500 column volumes while that of the other two analytes changed very little with increase of column volumes as shown in Fig. 12 (bottom). Although we do not fully understand why the plate number of *p*-acetophenidide increased, the data indicated that there was no degradation of the stationary phase at this temperature. Fig. 13 demonstrates the chromatograms obtained at two different column volumes at 150 °C. These observations clearly demonstrated that the PRP-1 stationary phase was rather stable. These results may suggest that high-temperature water is more compatible with the PRP-1 stationary phase. The better thermal stability of the PRP-1 column was expected since it has been widely used in gel permeation chromatography at higher temperatures.

3.3. Reproducibility of peak areas

As previously discussed, the Hamilton PRP-1 and the Zorbax RX-C₈ columns were stable under sub-

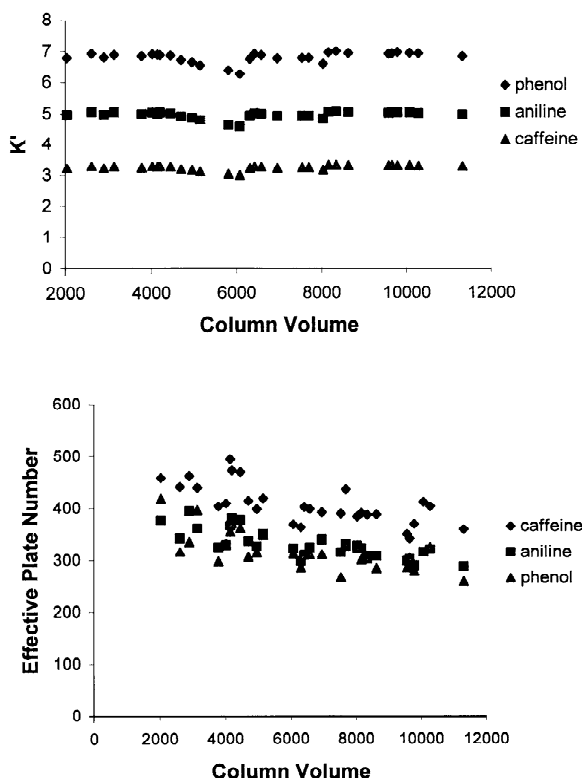


Fig. 11. Retention factor (top) and effective plate number (bottom) versus column volume for Hamilton PRP-1 column at 100 °C.

critical water conditions. Therefore, the reproducibility as measured by the relative standard deviation (RSD) of peak area is summarized in Tables 3 and 4. It can be seen that for a given analyte, caffeine, the Hamilton PRP-1 column yielded a better reproducibility (smaller spread of RSD) than that of the Zorbax RX-C₈ column. This means that the performance of the polymer-based Hamilton PRP-1 column is better than that of the silica-based Zorbax RX-C₈ column under subcritical water conditions.

4. Conclusions

Even though people are normally skeptical about the thermal stability of reversed-phase packing materials at elevated temperatures, our results obtained in this study are quite positive. Based on retention factor, all five columns were stable after being

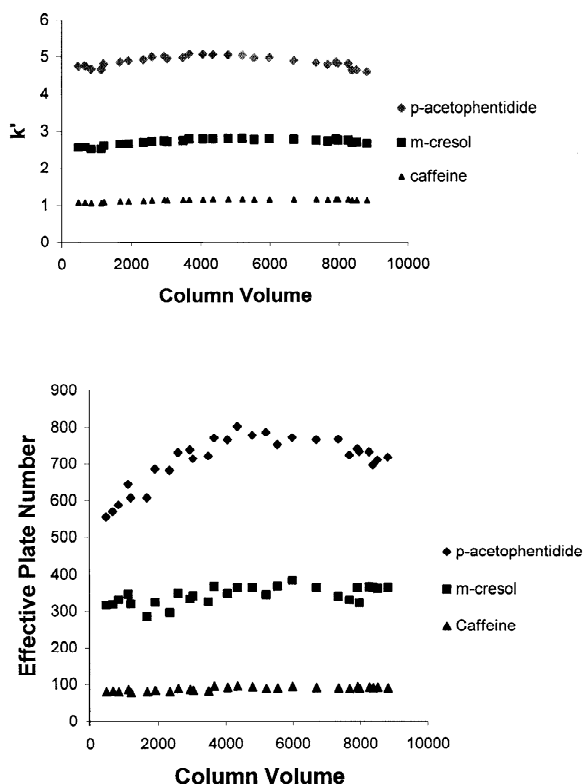


Fig. 12. Retention factor (top) and effective plate number (bottom) versus column volume for Hamilton PRP-1 column at 150 °C.

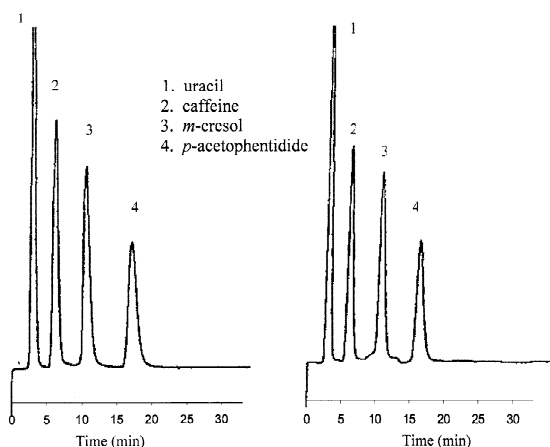


Fig. 13. Chromatograms for Hamilton PRP-1 column after (A) 482 and (B) 8821 column volumes at 150 °C.

Table 3
Reproducibility of peak area for Hamilton PRP-1 column

Temperature (°C)	Analytes	RSD (% , $n > 30$)
100	Uracil	2.6
	Caffeine	3.9
	Aniline	3.8
	Phenol	6.6
150	Uracil	3.5
	Caffeine	2.5
	<i>m</i> -Cresol	4.6
	<i>p</i> -Acetophentidide	3.0

Table 4
Reproducibility of peak area for Zorbax RX-C₈ column

Temperature (°C)	Analytes	RSD (% , $n > 40$)
100	Caffeine	7.2
	Methyl benzoate	10.4
	Benzene	5.9

exposed to subcritical water conditions for at least 6000 column volumes. While the column efficiency of the Zorbax RX-C₈, ZirChrom-PS, and PRP-1 columns did not significantly change for several thousands of column volumes, the peak efficiency obtained by the Nucleosil C₁₈ AB and Hypersil BDS C₁₈ columns worsened after heating for prolonged time. Comparing the three silica-based columns, the Zorbax RX-C₈ column was the most stable followed by the Nucleosil C₁₈ AB column. The Hypersil BDS C₁₈ column was the least stable under subcritical water conditions. The ZirChrom-PS column was stable at 100 °C for at least 7600 column volumes. Of the five columns, the polymer-based Hamilton PRP-1 column was the most stable under subcritical water conditions.

Acknowledgements

This research was supported by an award from Research Corporation and by the 1999 Starter Grant Award from the Society for Analytical Chemists of Pittsburgh. Research/Creative Activity grants from East Carolina University are also appreciated. The authors thank ZirChrom Separation, Inc. for providing the ZirChrom-PS column.

References

- [1] J. Li, P.W. Carr, *Anal. Chem.* 69 (1997) 837.
- [2] J. Li, Y. Hu, P.W. Carr, *Anal. Chem.* 69 (1997) 3884.
- [3] H. Chen, Cs. Horvath, *J. Chromatogr. A* 705 (1995) 3.
- [4] K. Kalghatgi, Cs. Horvath, *J. Chromatogr.* 443 (1988) 343.
- [5] L.C. Sander, L.R. Field, *Anal. Chem.* 52 (1980) 2009.
- [6] H.S. Tchaplal, H. Colin, G. Guiochon, *Anal. Chem.* 60 (1988) 1443.
- [7] L.A. Cole, J.G. Dorsey, *Anal. Chem.* 64 (1992) 1317.
- [8] L.A. Cole, J.G. Dorsey, *Anal. Chem.* 64 (1992) 1324.
- [9] R.M. Smith, R.J. Burgess, *Anal. Commun.* 33 (1996) 327.
- [10] R.M. Smith, R.J. Burgess, *J. Chromatogr. A* 785 (1997) 49.
- [11] O. Chienthavorn, R.M. Smith, *Chromatographia* 50 (1999) 485.
- [12] R.M. Smith, R.J. Burgess, J.R. Bone, *LC·GC: Mag. Sep. Sci.* 17 (1999) 938.
- [13] R.M. Smith, O. Chienthavorn, S.D. Taylor, *Anal. Chem.* 71 (1999) 4493.
- [14] Y. Yang, A.D. Jones, C.D. Eaton, *Anal. Chem.* 71 (1999) 3808.
- [15] T.M. Pwlowski, C.F. Poole, *Anal. Commun.* 36 (1999) 71.
- [16] I.D. Wilson, *Chromatographia* 52 (2000) S28.
- [17] D.J. Miller, S.B. Hawthorne, *Anal. Chem.* 69 (1997) 623.
- [18] B.A. Ingelse, H.-G. Janssen, C.A. Cramers, *J. High Resolut. Chromatogr.* 21 (1998) 613.
- [19] E.W.J. Hooijschuur, C.E. Kientz, U.A.Th. Brinkman, *J. High Resolut. Chromatogr.* 23 (2000) 309.
- [20] Y. Yang, A.D. Jones, J.A. Mathis, M.A. Francis, *J. Chromatogr. A* 942 (2001) 231.
- [21] Y. Yang, L.J. Lamm, P. He, T. Kondo, *J. Chromatogr. Sci.* 40 (2002) 107.
- [22] T. Kondo, Y. Yang, L.J. Lamm, *Anal. Chim. Acta* 460 (2002) 185.
- [23] N. Wu, Q. Tang, J.A. Lippert, M.L. Lee, *J. Microcol. Sep.* 13 (2001) 41.
- [24] Y. Yang, M. Belghazi, A. Lagadek, S.B. Hawthorne, D.J. Miller, *J. Chromatogr. A* 810 (1998) 149.