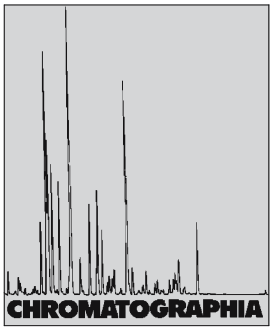
Effect of Temperature on the Chromatographic Behavior of Epirubicin and its Analogues on High Purity Silica Using Reversed-Phase Solvents



2007, *65*, 519–526

Lili Dong[1,2], Junxiong Huang[1,2,✉]

[1] State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, P.O. Box 2871, Beijing 100085, China; E-Mail: junxionghuang@yahoo.com
[2] Graduate School of the Chinese Academy of Sciences (GSCAS), Beijing 100049, China

Received: 13 August 2006 / Revised: 17 October 2006 / Accepted: 10 January 2007
Online publication: 15 March 2007

Abstract

The influence of temperature on the retention behavior of epirubicin and its analogues on high purity silica with reversed-phase solvents has been systematically investigated. It was found that temperature effects on retention are highly dependent on the type and concentration of organic modifier, as well as the pH of the mobile phase. In organic-rich mobile phases, the type of organic modifier plays an important role. With an aprotic solvent as modifier, retention times show anomalous increases with elevated temperature. At the same time, both efficiency and resolution are significantly improved but this is not the situation with a protic solvent as modifier. In addition, temperature shows different effects on retention time and selectivity when the pH is changed, and temperature-dependent selectivity reversal is found at higher pHs. In aqueous-rich mobile phases, regardless of the nature of the organic solvent and pH, retention of solutes drops as temperature is raised. It seems that the effect of temperature on chromatographic behavior of the solutes on bare silica using mobile phases containing various organic modifiers or pHs, results from a number of different retention mechanisms.

Keywords

Column liquid chromatography
High purity silica
Reversed-phase solvents
Temperature effects on retention
Van't Hoff plots

Introduction

In liquid chromatography, the temperature may have an effect on physical parameters such as solubility, vapor pressure, viscosity, and diffusivity and subsequently on retention, column efficiency, selectivity, column back pressure, and on stationary phase properties. Thus, nearly all the parameters of importance in liquid chromatography are more or less influenced by temperature. Therefore, over the past few years, the use of temperature as a variable controlling selectivity and separation speed has become more popular [1, 2]. However, most of the studies on temperature effects have focused on the two classical chromatographic modes—reversed-phase liquid chromatography (RPLC) and normal phase liquid chromatography (NPLC) [3–11]. Work on the role of temperature on bare silica using reversed-phase solvents is beginning to appear.

Basic compounds can be separated on bare silica with aqueous-rich mobile phases [12, 13] or with organic-rich eluents [14, 15]. The effects of temperature on the retention behavior of small compounds on bare silica at certain compositions of aqueous-organic mobile phases has been reported [16, 17]. The purpose of the present work was to study the influence of temperature on the retention of some basic small compounds on high purity silica with reversed-phase eluents including both high organic/low aqueous and the low organic/high aqueous mobile phases.

Experimental

Apparatus and Reagents

The HPLC system from a TSP (Thermo Separation Products, San Jose, CA, USA) consisted of a SpectraSYSTEM P4000 pump, a SpectraSYSTEM AS3000 autosampler with a column oven, and a Spectra FOCUS diode array detector. Chromatographic system control, data acquisition, and chromatographic analysis were with TSP PC1000 Chromatography Manager software (3.0 version).

In the present work, a high purity silica column (Kromasil KR100-5SIL,

DOI: 10.1365/s10337-007-0200-3
0009-5893/07/05

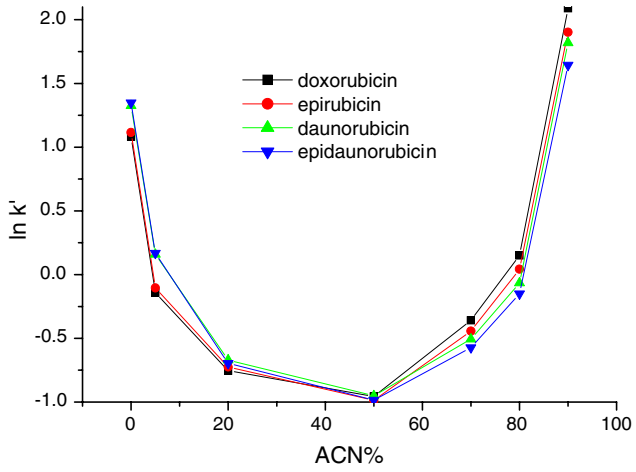


Fig. 1. Effect of ACN concentration on the retention factor of epirubicin and its analogues. Conditions: Kromasil 100-5SIL (5 µm); mobile phase: acetonitrile/sodium formate buffer (30 mM, pH 2.9)

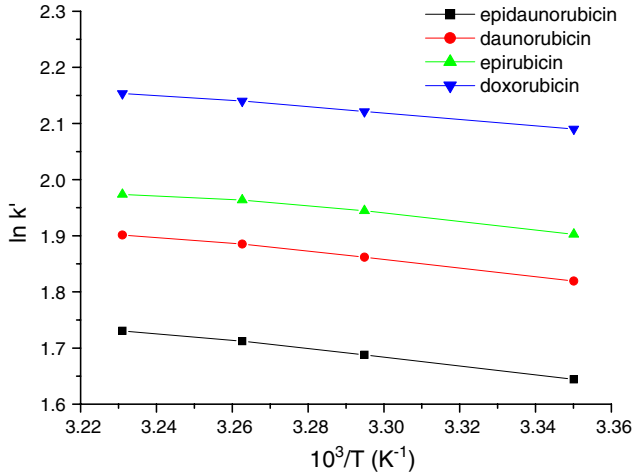


Fig. 2. Effect of temperature upon retention with acetonitrile as modifier. Conditions: Kromasil 100-5SIL (5 µm); mobile phase: 90% (*v*/*v*) acetonitrile in sodium formate buffer (30 mM, pH 2.9) and UV detection 254 nm for epirubicin and its analogue, 80% acetonitrile and UV detection 280 nm for hexapeptide and atenolol, 66% acetonitrile and UV detection 235 nm for thymosin α1; flow-rate: 1.0 mL min^{-1}; injections: 20 µL

Table 1. Temperature effect on the asymmetry factors (*As*) and efficiency (*N*) of epirubicin in 90% acetonitrile or methanol

Temperature (°C)	ACN		MeOH	
	As	*N* (Plates m^{-1})	*As*	*N* (Plates m^{-1})
25	1.11	32,011	1.04	13,318
30	1.10	32,466	1.05	12,228
35	1.09	32,596	1.02	12,554
40	1.09	55,385	1.06	13,434

Other conditions are the same as given in Fig. 2

250 × 4.6 mm I.D., pore size 100 Å, particle size 5 µm, Eka Chemicals AB, Bohus, Sweden). The pK_a of silanols on the silica surface is 4.2. A Kromasil KR100-5C_{18} column (250 × 4.6 mm I.D., 5 µm) was use for comparison. Acetonitrile (ACN) and methanol (MeOH) were of HPLC-grade (Tedia Company, USA). All other solvents and reagents were of analytical-reagent grade. Water was purified with a Milli-Q system (Bedford, Mass USA). Note that buffer concentrations and pH values refer only to the aqueous portion of the eluents. The buffers were prepared from sodium formate, adjusting to the required pH with formic acid (~88%, *v*/*v*) or from sodium acetate with acetic acid. Four anthracycline antibiotics including epirubicin (pK_a 8.08), doxorubicin (pK_a 8.34), daunorubicin (pK_a 8.46) and epidaunorubicin (pK_a 8.2) used as model compounds were obtained as a gift from Hisun Pharmaceutical Inc. (Zhejiang, China).

Chromatographic Conditions

The mobile phases were mixed from formate buffers and organic solvents at specified compositions. The flow rate was 1.0 mL min^{-1} and the injection volume was 20 µL. The column temperature was controlled with an oven mounted in a SpectraSYSTEM AS3000 autosampler, maintaining the temperature within ±0.5 °C of the setpoint.

Results and Discussion

Chromatographic retention on bare silica using aqueous-organic mobile phase involves hydrophilic interaction, ion-exchange, and hydrophobic retention [14, 16, 18]. The relative strength of various adsorption forces depends on the composition of the mobile phase. As shown in Fig. 1, under aqueous-rich environment, the retention of epirubicin and its analogues increased with increasing hydrophobicity of the solutes. Increasing water content in the mobile phase results in longer retention times, which is similar to that experienced in typical RPLC [19]. When an organic solvent was the major component, polar compounds were retained longer than non-polar ones, which was opposite to that obtaining in the former case. In this case, water is the stronger solvent and an increase in water content causes a rapid drop in retention. The above results showed that on the same bare silica column, with the same binary reversed-phase eluents but in different ratios, due to the different controlled mechanisms, various chromatographic behaviors can be observed. Therefore, the effects of temperature on the retention of solutes

were investigated in organic-rich (90% organic modifier) and aqueous-rich (95% aqueous) mobile phases.

Temperature Effect in Organic-Rich Mobile Phase

Temperature Effect on Retention Behavior Using Various Types of Organic Modifiers

ACN—aprotic solvent as organic modifier in mobile phase The effect of column temperature upon retention (ln k) of solutes was investigated using the van't Hoff equation:

$$\ln k = -\frac{\Delta H^o}{RT} + \frac{\Delta S^o}{R} + \ln \phi$$

where $\Delta H°$ is the enthalpy of the sorption process, $\Delta S°$ is the change in entropy and φ is the phase ratio [20].

Figure 2 shows the van't Hoff plots obtained with 90% ACN mobile phase. Negative slopes of van't Hoff curves for four solutes were found and retention time showed a continuous increase with elevated temperature, which is inconsistent with "normal behavior" (decreased retention at elevated temperature). The negative slopes of the van't Hoff curves mean positive or unfavorable $\Delta H°$. On bare silica with aqueous-organic mobile phases, a significant amount of water is associated with the silanols on the silica surface. The hydration shell may act as pseudo stationary phase. Since the values of $\Delta H°$ (the retention enthalpies of four anthracycline antibiotics ranged from 4.5 to 6.1 kJ mol^{-1}) were far below the interaction energy between a surface silanol group and water (about 260.4 kJ mol^{-1}) [21], it is unlikely that the solutes dislodge water molecules associated with the surface of the silica but more likely partition within a hydration shell close to the surface of the silica. The amount of adsorbed solvent may change with temperature, which would make a temperature effect more complex. However, since there is little data available on the thermodynamics of water adsorbed onto silica, it was not possible to rationalize these results further. More detailed studies are needed for a better understanding of the thermodynamics of organic-rich mobile phases.

As presented in Fig. 2, the slopes of four anthracycline antibiotics were parallel, indicating that the relative retentions (α) between them are almost constant and independent of temperature. The results show that the temperature has the same influence on the retention of the different solutes. It also proves that increasing retention at higher temperature is not caused by thermally induced change in the molecular configuration of the solutes, but by a temperature induced change in the retention interactions of the solutes on the surface of silica.

Table 1 shows the temperature effect on the kinetics parameters such as column efficiency and peak asymmetry (As) using epirubicin as an example, (the other solutes exhibited similar behavior). In 90% ACN mobile phase, the peak shape was symmetric within the temperatures range tested (As < 1.2). The most obvious change was the improvement of column efficiency. From 25 to 40 °C, the efficiency increased by about 70%.

Combined the data of retention factor, selectivity and column efficiency discussed above, we can estimate the resolution of the four analogues of epi-

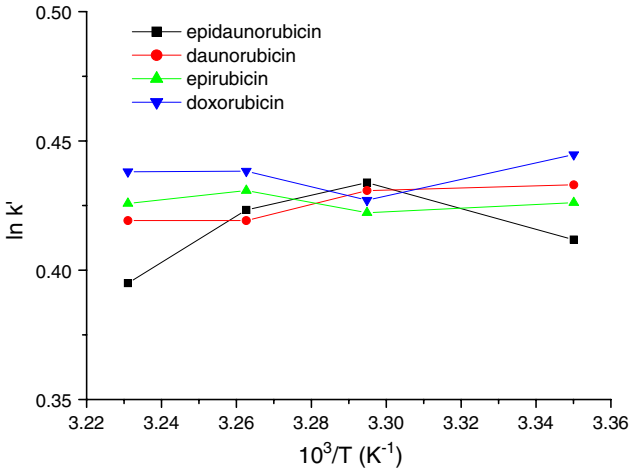


Fig. 3. Effect of temperature upon retention with MeOH as modifier in the mobile phase. Other conditions as given in Fig. 2

Table 2. Temperature effect on the asymmetry factors (As) and efficiency (N) of epirubicin at various pHs

Temperature (°C)	pH = 2.9		pH = 4.2		pH = 5.2	
	As	N (Plates m^{-1})	As	N (Plates m^{-1})	As	N (Plates m^{-1})
25	1.11	32,011	2.29	4,779	2.39	2,978
30	1.10	32,466	2.23	4,820	2.29	3,898
35	1.09	32,596	2.20	5,554	2.31	4,280
40	1.09	55,385	2.23	5,595	2.26	4,515

Other conditions are the same as given in Fig. 2

Table 3. Temperature effect on the asymmetry factors (As) and efficiency (N) of epirubicin in 5% organic modifier mobile phase

Temperature (°C)	MeOH		ACN	
	As	N (Plates m^{-1})	As	N (Plates m^{-1})
25	2.76	578	1.45	11,998
30	2.40	328	1.37	11,720
35	2.15	402	1.40	12,097
40	2.74	454	1.58	11,551

Other conditions as in Figs. 5 or 6

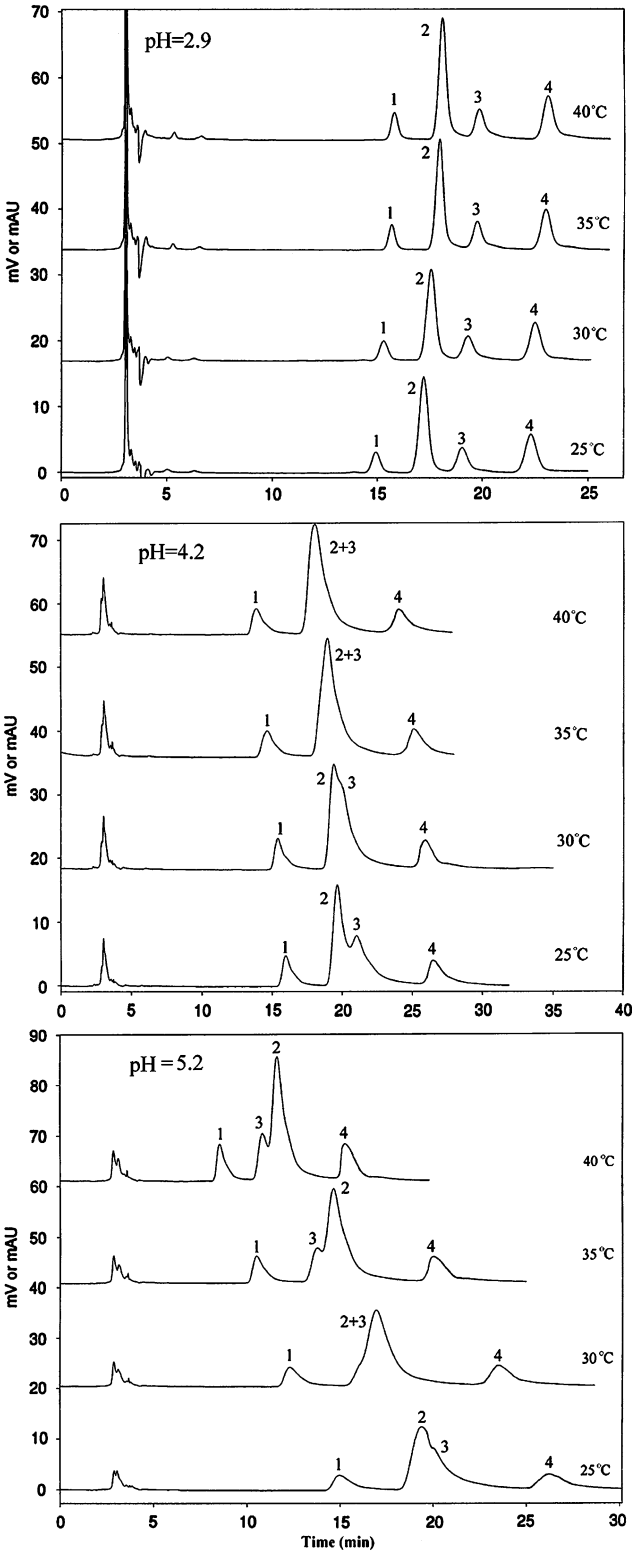


Fig. 4. Temperature effect upon chromatographic retention at different pHs in high organic mobile phase. Peaks **1** epidaunorubicin, **2** daunorubicin, **3** epirubicin, **4** doxorubicin. The data for pH 5.2 were determined in acetate buffer. Other conditions are the same as given in Fig. 2

rubicin at different temperatures according to the Purnell equation [5]:

$$R_s = 1/4\ N^{0.5}[(a-1)/a]\ [k/(1+k)]$$

where R_s is resolution, N is the column plate number, α is the separation factor (k_2/k_1), and k is the retention factor (in this case the average retention factor of two adjacent peaks k_1 and k_2). Among the three parameters relative to R_s, α was almost constant, k increased a little (an average increase in retention time for the four model compounds was only 1.5 min from 25 to 40 °C), but a large increase was found in column plate number. As a result, resolution was improved at higher temperature. Though temperature is not likely to offer dramatic changes in selectivity, the increase of resolution could be useful for a critical peak pair due to the improvement of column efficiency. Moreover, higher temperature reduces system back pressure. Thus, temperature is a parameter as important as others for optimization of chromatographic behavior.

MeOH—protic solvent as organic modifier in the mobile phase When MeOH was employed as organic modifier instead of ACN, the retention of seven solutes exhibited disordered changes with increasing temperature, as shown in Fig. 3. Some of them displayed increasing retention with elevated temperature, others showed the opposite. The different temperature effects are the result of multiple retention mechanisms. In addition to hydrophilic and ion-exchange interactions, solvent-solute and solvent-stationary phase hydrogen bondings are also involved [22]. The combination of these interactions resulted in different temperature effects from that in the ACN mobile phase.

Based on the slopes of plots in Fig. 3, the relative retention α between the solutes as a function of temperature could be deduced. The values of α changed slightly and even reversed elution order was observed with changing temperature, (also different from that with ACN as modifier).

The variation of peak asymmetry and column efficiency with raising temperature seemed not obvious in 90% MeOH, as displayed in Table 1, but improvements of both *As* and *N* were observed in ACN-contained mobile phase. All these results show that temperature plays a more complex role on retention of solutes in MeOH than ACN due to the multiplicity and complexity of the retention mechanisms. For this reason, the following studies were preformed only with eluents containing ACN.

Temperature Effect at Various Buffer pHs

Figure 4 showed the temperature effect upon chromatographic separation at dif-

ferent pHs. With elevated temperature, an increasing trend of retention was observed at pH 2.9; after that the retention values of the solutes slightly dropped at pH 4.2; finally, a significant decrease of retention was achieved at pH 5.2. These results reflect the transition of retention mechanisms with changing pHs. As the buffer pH varies from 2.9 to 5.2, the ionization of the silanols on the silica surface gradually increases and the solutes are kept in a protonated state [23], offering more and more opportunity for cation-exchange. The major mechanism governing the retention of solutes is transferred from hydropilic to ion-exchange interaction. When ion-exchange becomes the dominant mechanism, the pK_a of the analyte plays an important role in affecting retention and selectivity. According to previous work [8, 24], an increase in temperature could cause a reduction of protonated species. Thus, the retention of solutes at higher temperature would decrease as observed in Fig. 4 (pH 4.2 and 5.2) due to the weakened ion-exchange contribution.

In Fig. 4, in addition to the different change in retention, the selectivity between the adjacent peak pair, epirubicin and daunorubicin, was also different at different pHs. At pH 2.9, the value of α remained essentially unchanged regardless of the temperature (discussed above); then, when pH was increased, the elution time of epirubicin became closer and closer to daunorubicin. At pH 4.2, they co-eluted as a single peak at 30 °C. At pH 5.2, with increasing temperature, their elution order was even reversed. It means that the impact of temperature on retention for various solutes is different. As discussed above, at higher pH (pH 4.2 or 5.2), because ion-exchange interaction was greater, the pK_a of the bases becomes an important parameter governing retention. As proposed in previous literature [8, 9, 24], the pK_a of solutes decreases with increasing temperature, and the smaller the pKa of the base is, the more it decreases. Since the pK_a value of epirubicin (pK_a 8.08) is smaller than that of daunorubicin (pK_a 8.46), a greater reduction of the protonated species will be obtained for epirubicin. This implies that the ion-exchange contribution to the retention factor is smaller. Consequently, the retention decreased more rapidly for epirubicin than for daunorubicin, and the two solutes move closer and closer with increasing temperature. When the temperature reach 30 °C, the two analytes co-elute as a single peak at pH 4.2. Furthermore, at pH 5.2, with stronger ion-exchange interaction, the influence of temperature on the shift of pK_a is larger, and a reversal of elution order appears at 35 °C. The results of retention behavior at different pHs show us that column temperature can be used as an additional parameter to manipulate selectivity at higher pH mobile phases.

Table 2 shows that the column efficiencies are influenced by temperature at different pHs (epirubicin as the example).

Table 4. Temperature effect on the asymmetry factors (*As*) and efficiency (*N*) of epirubicin at various pHs

Temperature (°C)	pH = 2.9		pH = 4.2		pH = 5.2	
	As	*N* (Plates m^{-1})	*As*	*N* (Plates m^{-1})	*As*	*N* (Plates m^{-1})
25	1.45	11,998	2.38	4,865	4.28	983
30	1.37	11,720	2.27	4,481	3.85	776
35	1.40	12,097	2.22	3,986	3.60	806
40	1.58	11,551	1.98	3,762	3.32	1,025

Conditions as in Fig. 8

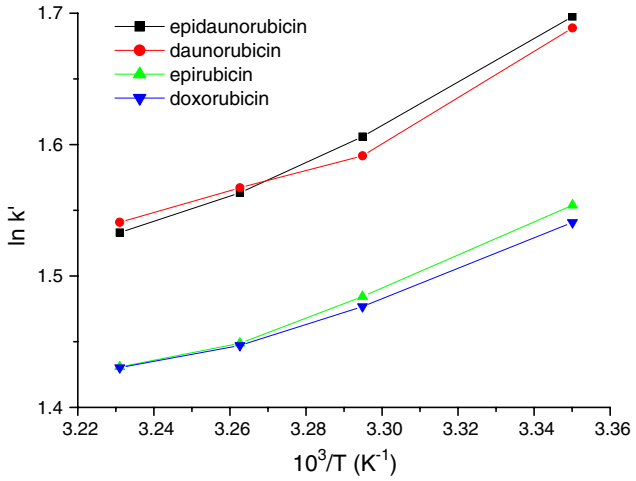


Fig. 5. Effect of temperature upon retention in high aqueous mobile phase with acetonitrile as modifier. mobile phase: 5% (*v*/*v*) acetonitrile in sodium formate buffer (30 mM, pH 2.9) other conditions as in Fig. 2

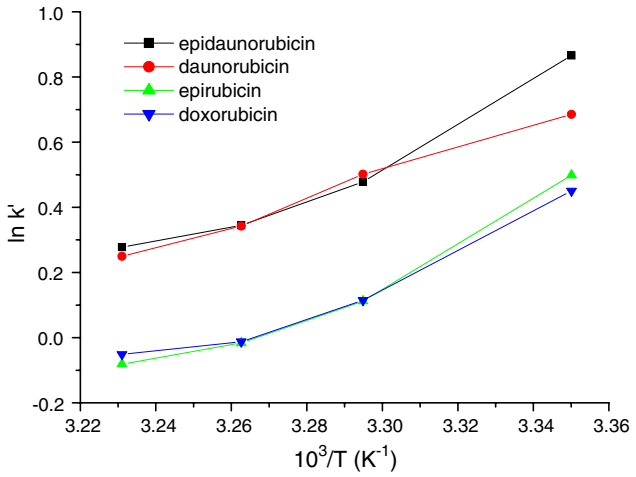


Fig. 6. Effect of temperature upon retention in high aqueous mobile phase with methanol as modifier. Other conditions as in Fig. 5

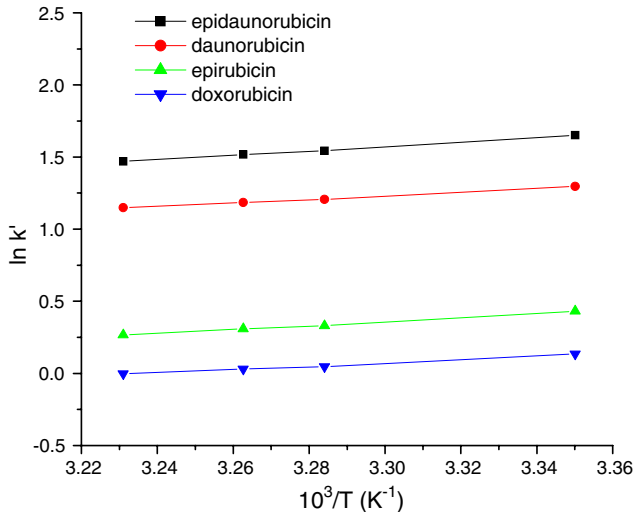


Fig. 7. Effect of temperature upon retention of seven compounds on an RPLC column RPLC. Conditions: Kromasil 100-5C18 (5 μm); mobile phase: 30% (*v*/*v*) acetonitrile in formic acid buffer (pH 2.9). Other conditions as in Fig. 5

It shows that at constant temperature the higher the pH, the lower the efficiency. This behavior can be ascribed to the effect of ion-exchange interaction. As discussed above, ion-exchange interaction is responsible for retention of solutes at higher pHs. However, it is a slow mass transfer step [9], thus leading to lower efficiency. With increasing temperature, improved efficiencies are observed at all the pHs tested. However, compared with the case of lower pH, poor column efficiency at higher pH was disadvantageous to the separation, though a useful change of selectivity could be obtained.

Temperature Effect in Aqueous-Rich Mobile Phase

Temperature Effect on Retention Behavior of the Solutes using Various Types of Organic Modifiers Phase

The influence of temperature on the retention factor of solutes using 5% ACN and MeOH mobile phases are shown in Figs. 5, 6, respectively. Positive slopes of the van't Hoff curves for all the compounds were found, which is in line with what is usually observed in ordinary RPLC (decreased retention time with increasing temperature). The shapes of the van't Hoff plots in Figs. 5, 6 display similar patterns, which are very different from those in organic-rich mobile phases where the patterns of van't Hoff plots were different in aprotic and protic mobile phases. Bidlingmeyer and coworker [16] investigated the effect of temperature on the retention of lipophilic amines on bare silica with MeOH mobile phase. Though the amount of MeOH they used was up to 70%, the retention of analytes did not exhibit hydrophilic behavior, but typical reversed-phase behavior. Moreover, none of the van't Hoff plots were linear. All the results were similar to the results in Figs. 5, 6. They attributed the nonlinear van't Hoff plot to the mixed retention mechanism where electrostatic and adsorptive forces were responsible for the retention of solutes on bare silica in the reversed-phase eluents.

In order to prove the hypothesis, a comparison experiment was carried out on a C18 column, as shown in Fig. 7. It was found that similar to the aqueous-rich case, an increase in temperature caused shorter retention time for the compounds tested, but the van't Hoff plot of each analyte was a straight line with an average value of the regression coefficient r^2 of 0.9950. As discussed above, non-linear van't Hoff curves in water-rich mobile phases on silica results from mixed retention mechanisms and the relative strengths of the different interactions changes with increasing temperature. Whereas in typical RPLC, because of the bonding of silanols with hydrophobic groups, the amount of residue silanols on the stationary phase is less than that on bare silica, thus the role of ion-exchange interaction is less important and hydrophobic interaction is mainly responsible for the retention of the solutes. As a result, a linear plot of ln k against $1/T$ was obtained. The results are consistent with the explanation proposed by Bidlingmeyer [16]. The comparison of the experimental results between bare silica with a high water content mobile phase and classical RPLC shows that the temperature effect shared some degree of agreement in the two cases, i. e. retention of solutes decreased at higher temperature. However, they also presented difference. In our case, in spite of MeOH or ACN as organic modifier, the relationships of ln k versus $1/T$ were linear in RPLC, but non-linear in the case of bare silica with organic-poor eluents.

Under aqueous-rich conditions, bare silica acts as a weak reversed phase material, and the chromatogram (Fig. 8) showing poor efficiency is typical of what was earlier reported [25]. For instance, the separation between doxorubicin and epirubicin, as well as daunorubicin and epidaunorubicin was incomplete, though sufficient resolution was obtained between the former two pairs and the latter two. In addition, peak shapes were wide with tailing accompanying poor column efficiency, as summarized in Table 3 (epirubicin as example). Efficiency appears to change little within the temperature range tested. The results with ACN as organic modifier parallel those of MeOH except for a better efficiency. An overall loss in resolution was obtained at higher temperature in this chromatographic mode, contrary to the situation in organic-rich mobile phases where higher temperature is beneficial for separation process.

Temperature Effect at Various Mobile Phase pHs

The influence of temperature on the retention of compounds was also investigated at various pHs as shown in Fig. 8. It's obvious that within the pH range tested, the ln k values of the model compounds demonstrated a clear trend towards decreasing retention as temperature increased, which was very different from that in high level ACN phases where the changes in retention time with increasing temperature varied with pH. In fact, the main difference between the two chromatographic modes appeares at lower pH. One is shorter retention time at higher temperature, the other is an opposite trend. This behavior can be ascribed to the differences of retention mechanisms between them. In the case with high

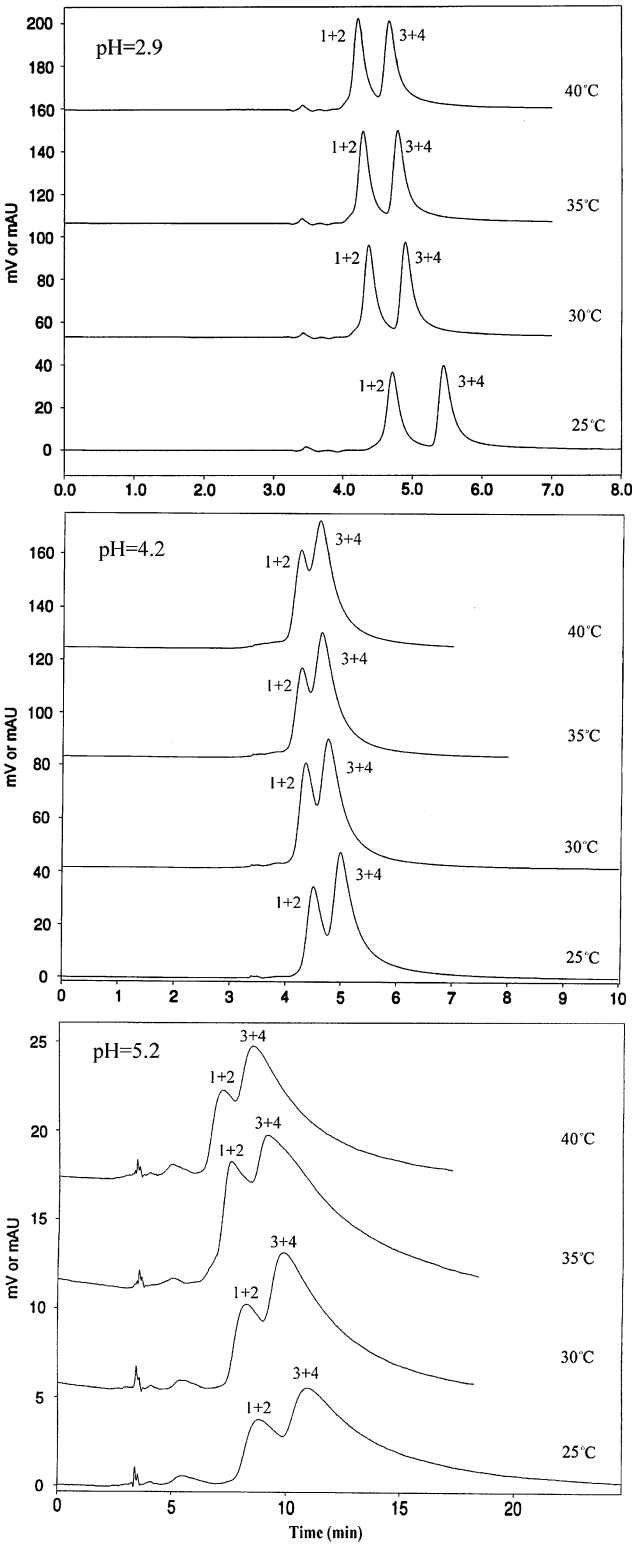


Fig. 8. Temperature effect upon chromatographic retention at different pHs in high aqueous mobile phase. Peaks **1** doxorubicin, **2** epirubicin, **3** daunorubicin, **4** epidaunorubicin. The data of pH 5.2 were determined in acetate buffer. Other conditions are the same as given in Fig. 5, except the buffer pH is varied

aqueous phases, at lower pH, hydrophobic retention is the controlled mechanism, but under the condition of high organic solvent phases hydrophilic interaction is responsible for solute retention. At higher pH, due to decreasing pK_a of solutes at higher temperature [8], the reduction of protonated species weakened its ion-exchange interactions on the surface of silica. As a result, decreasing trends in retention were exhibited in both cases.

Table 4 showed data of asymmetry factors and efficiency at different pHs for epirubicin. A dramatic reduction in efficiency was found with increasing pH. Basically, temperature brought no noticeable beneficial effect on column efficiency or to the asymmetry factor.

Conclusions

It had been found that temperature plays different roles when the type and content of organic modifier or pH in the mobile phase are changed. For the compounds examined in an organic-rich environment, temperature response varied with the type of organic solvent. With ACN as modifier, resolution was improved due to better efficiency at higher temperature. Different changes of retention with temperature were observed at various pHs and dramatic changes of selectivity were obtained at higher pH value owning to temperature-dependent pK_a shift. However, in aqueous-rich mobile phases, the impact of temperature on the retention is not sensitive to the nature of the organic modifier. Decreasing retention factor with increasing temperature and nonlinear van't Hoff plots were observed with both aprotic and protic solvents. Moreover, an increase in temperature led to decreasing retention at all pHs tested. All these effects of temperature on retention, reflect the different retention mechanisms involved.

References

1. Li JW, Carr PW (1997) Anal Chem 69:2193–2201
2. Yang XQ, Ma LJ, Carr PW (2005) J Chromatogr A 1079:213–220
3. Li J (1998) Anal Chim Acta 369:21–37
4. Dolan JW (2002) J Chromatogr A 965:195–205
5. Chester TL, Coym JW (2003) J Chromatogr A 1003:101–111
6. Claessens HA, Straten MA (2004) J Chromatogr A 1060:23–41
7. Castells CB, Gagliard LG, Ráfols C, Rosés M, Bosch E (2004) J Chromatogr A 1042:23–36
8. Buckemaier SMC, McCalley DV, Euerby MR (2004) J Chromatogr A 1060:117–126
9. Gagliardi LG, Castells CB, Ráfols C, Rosés M, Bosch E (2005) J Chromatogr A 1077:159–169
10. McCarthy JP (1994) J Chromatogr A 685:349–355

11. Lee W, Cho D, Chun BO, Chang T, Ree M (2001) J Chromatogr A 910:51–60
12. Crommen J (1979) J Chromatogr 186:705–724
13. Svendsen H, Greibrokk T (1981) J Chromatogr 212:153–166
14. Bidlingmeyer BA, Del Rios JK, Korpi J (1982) Anal Chem 54:442–447
15. Law B (1990) Trends Analyt Chem 9:31–36
16. Bidlingmeyer BA, Henderson J (2004) J Chromatogr A 1060:187–193
17. Guo Y, Gaiki S (2004) J Chromatogr A 1074:71–80
18. Naidong W (2003) J Chromatogr B 796:209–224
19. Qi Y, Huang JX (2001) J Liq Chromatogr Relat Technol 24:2837–2848
20. Melander W, Campbell DE, Horváth C (1978) J Chromatogr 158:215–225
21. Zhuravlev LT (2000) Colloids Surf A Physicochem Eng Asp 173:1–38
22. Li RP, Huang JX (2004) J Chromatogr A 1041:163–169
23. Nawrocki J, Rigney RP, McCormick A, Carr PW (1993) J Chromatogr A 657:229–282
24. Buckemaier SMC, McCalley DV, Euerby MR (2004) J Chromatogr A 1026:251–259
25. Li RP, Huang JX (2005) J Liq Chromatogr Relat Technol 28:2737–2751