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### High Temperature Liquid Chromatography on a Poly(Styrene-Divinybenzene) Stationary Phase

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## High Temperature Liquid Chromatography on a Poly(Styrene-Divinylbenzene) Stationary Phase

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**Abstract:** The performance of poly(styrene-divinylbenzene) stationary phase was evaluated for the separation of test compounds, alkyl aryl ketones, and barbiturates under reversed-phase high performance liquid chromatography (RP-HPLC) at high temperature (100°C–200°C) using water-rich and superheated water as the eluent. Good separations with acceptable column efficiencies were achieved at high column temperatures. Van't Hoff plots for the separations were linear, which suggest that for selected compounds over the temperature range studied, no changes occurred in the retention mechanism. The high temperature RP-HPLC separation technique was successfully applied to the separation of selected barbiturates using 100% pure water as the eluent.

**Keywords:** High temperature HPLC, Poly(styrene-divinylbenzene), Barbiturates, Van't Hoff

### INTRODUCTION

Temperature has recently become an increasingly accepted separation parameter in reversed-phase high performance liquid chromatography (RP-HPLC). High temperature operation in RP-HPLC provides the opportunity to reduce the quantity of organic solvent used in mixed organic-water mobile phase, increases analyte mass transfer rates, and decreases column back pressure and total analysis time significantly.<sup>[1]</sup> High temperature separation has been shown to

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improve analyte resolution by decreasing mobile phase viscosity and by increasing the diffusion rate of the sample species, thus increasing mass transfer of the analyte to the stationary phase and, thereby, decreasing the peak width.<sup>[2]</sup>

High temperature, as an optimization parameter in the separation process of the RP-HPLC system, has been widely studied due to recent findings of alternative stationary phases, which have high thermal stability at high temperatures. Zirconia based columns have received a great deal of attention recently because of their extraordinary stability under extreme thermal and chemical conditions.<sup>[3,4]</sup> Recently, Sanagi and co-workers established a preliminary study on the separation of triazole fungicides using superheated water eluent on a zirconia based stationary phase.<sup>[5,6]</sup> The method developed justified that purified water can be utilized as eluent to completely separate triazole fungicides with excellent detection limits. Poly(styrene-divinylbenzene) (PS-DVB) stationary phase can be regarded as one of the earliest stationary phases introduced, and is able to withstand extended exposure to column temperature as high as 200°C. In contrast to conventional alkyl silane bonded phase, higher temperature will directly accelerate the dissolution of silica in aqueous solution.<sup>[7]</sup> Although PS-DVB has been shown to have high thermal stability characteristics; no comprehensive study has been reported on the performance of PS-DVB stationary phase at high temperature.

In this comprehensive study, we report on the results of high temperature RP-HPLC separation of test compounds, alkyl aryl ketones (AAKs), and barbiturates using water-rich and superheated water on polystyrene-divinylbenzene stationary phase.

## EXPERIMENTAL

### Reagents

HPLC grade acetonitrile (100%) was obtained from J.T. Baker (USA). Deionized water of at least 18 MΩ was purified by a Nano ultra pure water system (Barnstead, USA). Sodium nitrate and alkyl aryl ketones (acetophenone, propiophenone, butyrophenone, and valerophenone) were obtained from Fluka Chemika (Switzerland). Test compounds (benzamide, phenol, benzaldehyde, nitrobenzene, and toluene) were obtained from Riedel-de-Haen (Germany), and barbiturates (allobarbitol, aprobarbitol, buthetal, butal-bital) were obtained from Alltech Assoc. Inc. (Deerfield, USA).

### Chromatographic Conditions

The instrumental set-up was illustrated in a previous report.<sup>[5,6]</sup> In brief, the high temperature HPLC system consisted of a conventional HPLC system coupled with a column oven of a Shimadzu GC-8A Gas Chromatography (Shimadzu Kyoto,

Japan). HPLC separations were carried out using a Waters 515 HPLC pump (Milford, USA) for mobile phase delivery. A Rheodyne 7125 injection valve (Cotati, USA) fitted with a 5  $\mu\text{L}$  loop was used for sample introduction. Analyte peaks were detected using a Shimadzu SPD-6A UV detector (Kyoto, Japan) and were recorded on a Waters 746 Data Module integrator (Milford, USA). A 20 cm  $\times$  0.25 mm I.D. length of stainless-steel tubing was placed in the oven between the injection valve and the column as a pre-heating coil. The column used in this work was packed with PLRP-S 100  $\text{\AA}$  (5  $\mu\text{m}$  PS-DVB) (150 mm  $\times$  2.1 mm I.D.) obtained from Polymer Laboratories (Shropshire, UK). A Jasco 880-81 (Japan) backpressure regulator was connected to the outlet of the detector to maintain a constant backpressure ( $\sim$ 20 bar) in the detector cell.

## Procedure

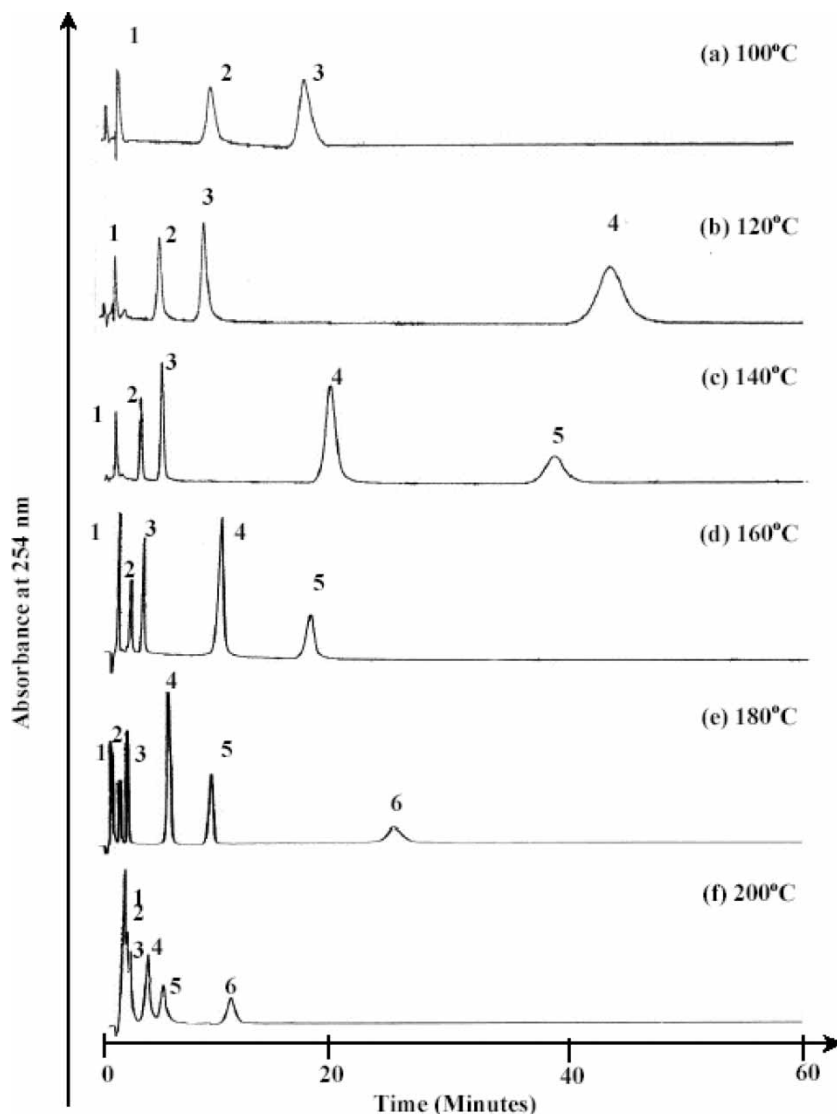
The mobile phase used was prepared by mixing deionized water with acetonitrile and subsequently degassed using a vacuum ultrasonic method. Samples of AAKs, test compounds, and barbiturates dissolved in acetonitrile were injected in triplicate onto the column. Separations of AAKs and test compounds were carried out using acetonitrile-water: 20:80, 10:90, 1:99, and 0:100 (v/v) at high temperatures (100°C–200°C). Separations of barbiturates were performed using 100% pure water as the eluent. The eluent flow rate was 0.5 mL/min and sample injection volume was 1  $\mu\text{L}$ . The sample solvent (acetonitrile) peak was used to determine the void volume of the system. Solute concentrations were between 0.1–2 mg/mL. UV detection at 254 nm (test compounds and AAKs), and 220 nm (barbiturates) were achieved.

## RESULTS AND DISCUSSION

### Performance Study of PLRP-S Column at High Temperature

The separations of test compounds and AAKs, as a function of temperature, with different mobile phase compositions using high flow rates were examined. It was noted that solutes with higher hydrophobicity were retained longer on the stationary phase due to the strong hydrophobic interactions (Fig. 1a and 1b). This is probably due to the retention behaviour on the PS-DVB stationary phase being strongly dependent on the neutral non-polar polystyrene surface, which functions as the active site for reversed-phase separation with aqueous eluent.<sup>[8]</sup>

Table 1 shows the retention factors of the test compounds and AAKs, as a function of temperature, using different percentages of acetonitrile. For each compound studied, there was a marked decrease in retention factors by increasing the temperature from 100°C to 200°C, with 20°C increments, using a high flow rate of 0.5 mL/min. It was observed that the analysis time decreased by



**Figure 1.** (a) Separation of test compounds on PLRP-S column. Chromatographic condition: mobile phase: 100% pure water; flow rate: 0.5 mL/min; temperature: 100°C–200°C; detection: UV absorbance at 254 nm. Peaks: **1** — sodium nitrate; **2** — benzamide, **3** — phenol, **4** — benzaldehyde, **5** — nitrobenzene, **6** — toluene. (b) Separation of alkyl aryl ketones (AAKs) on PLRP-S column. Chromatographic condition: mobile phase: 100% pure water; flow rate: 0.5 mL/min; temperature: 140°C–200°C; detection: UV absorbance at 254 nm. Peaks: **1** — sodium nitrate; **2** — acetophenone, **3** — propiophenone, **4** — butyrophenone, **5** — valerophenone.

(continued)

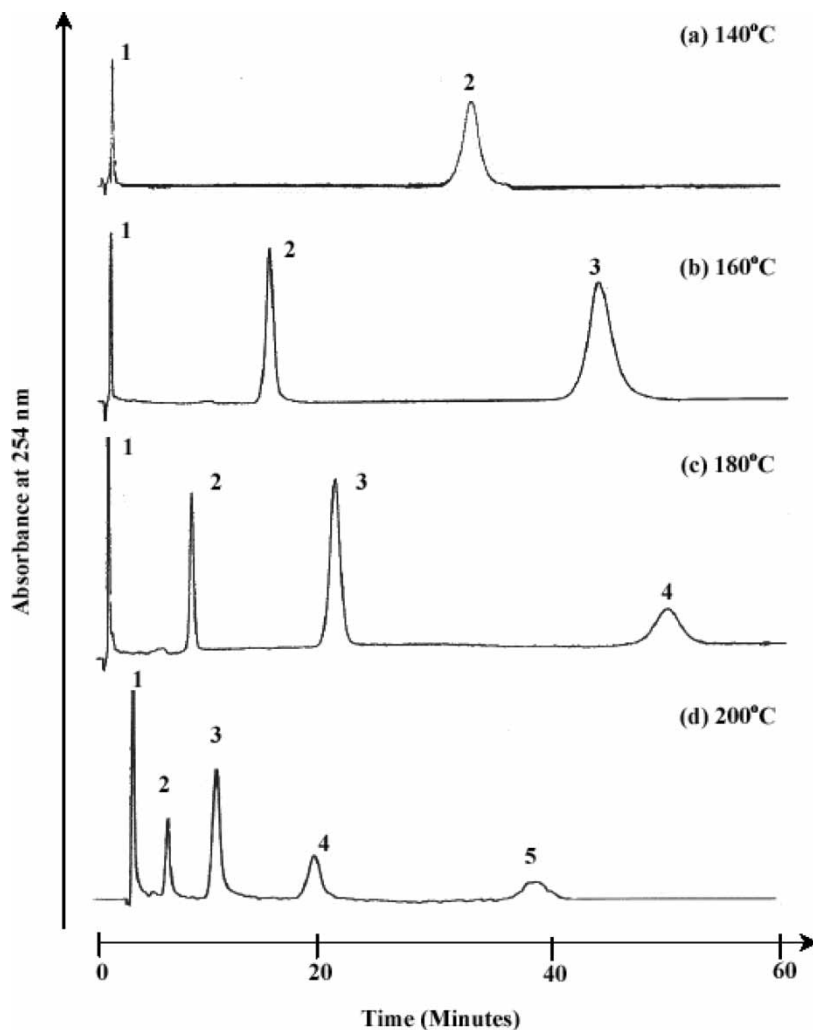


Figure 1. Continued.

about 7-fold when the temperature was increased from 100°C to 200°C, without significant loss in resolution and column efficiency. It was shown that a 1% increase in acetonitrile concentration has the same effect as a 4°C increase in column temperature in controlling solute retention. The result obtained is comparable with our previous report on the separation of triazole fungicides.<sup>[5]</sup> Equivalent separations were obtained at 100°C with 20% acetonitrile, at 140°C with 10% acetonitrile, and at 180°C with 0% acetonitrile. This indicates that retention can be controlled either by the amount of organic solvent in the mobile phase or by column temperature.

**Table 1.** Retention factors of test compounds and AAKs as a function of temperature using different proportions of organic modifier.

Compounds	Mobile phase composition (ACN–water) (v/v)	Retention factor, <i>k</i> (R.S.D. %), column temperature (°C)					
		100	120	140	160	180	200
Acetophenone	<b>20–80</b>	6.88 (0.4)	5.45 (1.6)	3.80 (1.0)	2.57 (0.7)	1.74 (0.7)	1.14 (0.0)
	10–90	28.60 (1.0)	13.55 (0.3)	9.86 (0.7)	5.88 (1.1)	3.26 (0.7)	2.49 (0.0)
	<b>1–99</b>	—	45.34 (0.8)	22.55 (0.5)	12.53 (1.4)	7.10 (0.4)	3.30 (1.3)
	0–100	—	—	25.93 (0.5)	12.44 (0.6)	6.89 (0.7)	3.19 (1.3)
Propiophenone	<b>20–80</b>	17.74 (0.4)	13.37 (1.4)	8.60 (0.8)	5.35 (0.8)	3.36 (0.7)	1.99 (0.0)
	10–90	—	39.72 (0.4)	27.09 (0.7)	14.66 (1.4)	7.31 (0.8)	5.12 (0.4)
	<b>1–99</b>	—	—	—	38.35 (1.3)	19.39 (0.4)	7.71 (1.0)
	0–100	—	—	—	38.16 (0.8)	18.76 (0.5)	7.67 (1.1)
Butyrophenone	<b>20–80</b>	39.28 (0.6)	28.51 (1.5)	17.20 (0.7)	9.95 (0.7)	5.81 (0.9)	3.16 (0.4)
	10–90	—	—	65.99 (0.8)	32.67 (1.6)	14.69 (0.7)	9.57 (0.5)
	<b>1–99</b>	—	—	—	—	47.66 (0.4)	16.39 (1.0)
	0–100	—	—	—	—	46.48 (0.6)	16.75 (1.2)
Valerophenone	<b>20–80</b>	—	61.58 (1.5)	34.71 (0.6)	18.51 (0.7)	10.05 (0.8)	5.02 (0.4)
	10–90	—	—	—	—	29.59 (0.9)	17.94 (0.4)
	<b>1–99</b>	—	—	—	—	—	35.09 (0.9)
	0–100	—	—	—	—	—	37.11 (0.9)

Benzamide	<b>20–80</b>	0.60 (0.0)	0.46 (4.0)	0.41 (0.7)	0.35 (2.1)	0.27 (5.4)	0.23 (0.7)
	10–90	1.85 (1.3)	1.11 (0.6)	0.89 (0.0)	0.60 (0.0)	0.40 (1.5)	0.40 (1.6)
	<b>1–99</b>	4.02 (1.4)	2.37 (1.3)	1.43 (1.1)	0.95 (1.4)	0.62 (0.0)	0.37 (1.6)
	0–100	5.66 (0.3)	2.93 (0.9)	1.58 (0.3)	0.94 (1.1)	0.65 (1.4)	0.29 (2.1)
Phenol	<b>20–80</b>	1.69 (0.4)	1.24 (2.3)	0.96 (0.7)	0.75 (1.0)	0.57 (2.9)	0.44 (0.7)
	10–90	4.79 (1.0)	2.70 (0.4)	1.99 (0.6)	1.31 (0.0)	0.85 (0.5)	0.74 (0.9)
	<b>1–99</b>	8.72 (1.3)	5.01 (1.1)	2.97 (1.0)	1.94 (1.4)	1.28 (0.4)	0.76 (0.8)
	0–100	11.36 (0.4)	5.83 (0.9)	3.15 (0.3)	1.88 (0.7)	1.29 (0.9)	0.66 (0.9)
Benzaldehyde	<b>20–80</b>	5.45 (0.4)	4.10 (1.9)	2.90 (0.6)	2.06 (0.4)	1.43 (1.2)	0.96 (1.3)
	10–90	19.20 (0.8)	9.62 (0.9)	6.89 (0.4)	4.28 (0.2)	2.46 (1.6)	1.93 (0.6)
	<b>1–99</b>	—	25.17 (0.9)	13.33 (0.9)	7.72 (1.1)	4.68 (0.2)	2.38 (0.5)
	0–100	—	32.37 (0.7)	15.06 (0.2)	7.82 (0.3)	4.67 (0.7)	2.24 (0.3)
Nitrobenzene	<b>20–80</b>	11.11 (0.6)	7.76 (2.0)	5.09 (0.5)	3.34 (0.2)	2.16 (1.0)	1.39 (1.1)
	10–90	43.46 (0.8)	19.65 (1.1)	12.98 (0.3)	7.45 (0.2)	4.00 (1.0)	2.92 (0.6)
	<b>1–99</b>	—	—	26.93 (0.7)	14.43 (1.0)	8.17 (0.3)	3.87 (0.3)
	0–100	—	—	30.85 (0.3)	14.75 (0.4)	8.17 (0.7)	3.71 (0.4)
Toluene	<b>20–80</b>	31.33 (0.8)	22.36 (1.7)	14.05 (0.3)	8.73 (0.2)	5.37 (0.6)	3.09 (0.8)
	10–90	—	—	39.31 (0.2)	21.88 (0.3)	10.82 (1.1)	7.57 (0.9)
	<b>1–99</b>	—	—	—	—	23.64 (0.2)	10.16 (0.3)
	0–100	—	—	—	—	23.29 (0.8)	9.71 (0.3)



## Influence of Temperature on a Column Separation Mechanism

The influence of temperature on a column separation mechanism can be calculated from retention data by evaluation of the Van't Hoff plots.<sup>[9]</sup> As a theoretical basis for the Van't Hoff plots, the retention factor is expressed in terms of standard enthalpies and entropies of transfer of a solute from mobile to stationary phase. The relationship between the logarithm of the retention factor ( $\ln k$ ) and enthalpy and entropy equals:

$$\ln k = -\Delta H^\circ/RT + \Delta S^\circ/R + \ln \phi \quad (1)$$

where  $k$  is the measured retention factor value,  $\Delta H^\circ$  the enthalpy,  $\Delta S^\circ$  the entropy,  $T$  the absolute temperature,  $R$  the gas constant, and  $\phi$  the phase ratio of the column.  $\Delta H^\circ$  and  $\Delta S^\circ$  are the standard enthalpy and standard entropy of transfer of a solute from the mobile to the stationary phase.

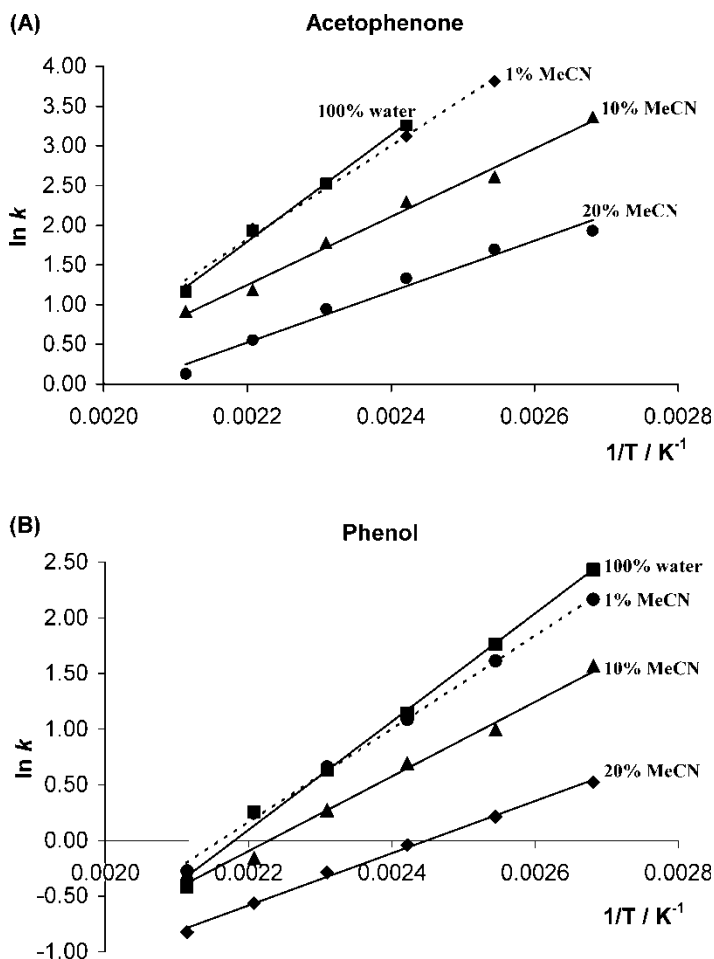
From the results, enthalpy data for acetophenone and phenol at temperatures ranging from 100°C to 200°C, with different mobile phase compositions, were prepared (Table 2). The Van't Hoff plots showed no significant deviation from linearity (Fig. 2). It was, therefore, concluded that over the temperature range no changes in the mixed retention mechanism for compounds occurred. In all cases,  $\Delta H^\circ$  values were negative under the experimental conditions, demonstrating that the retention of compounds studied is an exothermic process. It is energetically more favourable for the compounds to remain in the stationary phase than in the mobile phase. As expected, the  $\Delta H^\circ$  value became more negative with decreasing acetonitrile content in the eluent. This shows that a strong retention interaction between mobile and stationary phase occurs when the percentage of organic modifier in the eluent is decreased.

## Separation of Barbiturates

The high temperature RP-HPLC separation technique was applied to the separation of selected barbiturates using 100% pure water as eluent. Retention

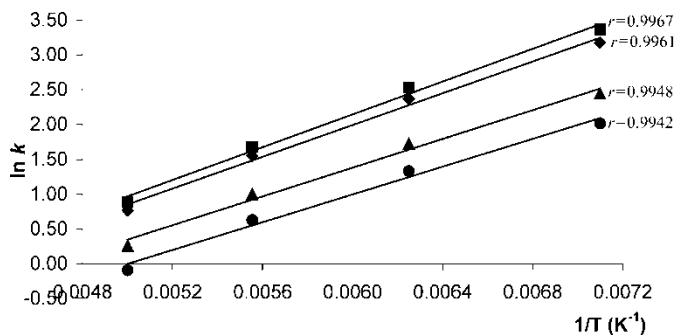
**Table 2.** Enthalpy data for acetophenone and phenol at temperatures ranging from 100°C to 200°C in different mobile phase compositions

Compounds	Mobile Phase Composition: MeCN–water							
	Enthalpy $\Delta H^\circ$ (kJ/mol)				Correlation, $r$			
	20–80	10–90	1–99	0–100	20–80	10–90	1–99	0–100
Acetophenone	–26.63	–35.72	–49.30	–55.76	0.9878	0.9909	0.9930	0.9952
Phenol	–19.49	–27.87	–35.05	–40.28	0.9969	0.9907	0.9974	0.9954

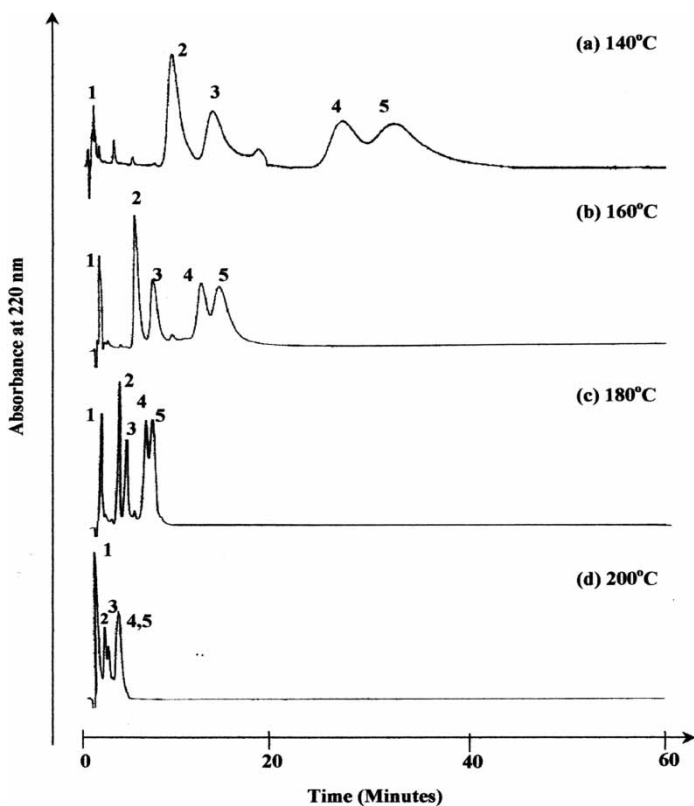


**Figure 2.** Van't Hoff plots for (A) acetophenone and (B) phenol using different proportion of acetonitrile as eluent (acetonitrile/water) at temperature range of 100°C to 200°C.

factors of the barbiturates decreased significantly with increasing column temperature. From the Van't Hoff plots for the barbiturates (Fig. 3), it was obvious that there was no significant deviation from linearity. It was therefore concluded, that over the temperature range there were no changes in the retention mechanism for barbiturates. The elution strength of the 100% pure water mobile phase was strong enough to elute all the barbiturates within a reasonable analysis time at high operating temperatures. The barbiturates were separated completely within 60 minutes, using 100% pure water as the eluent at high temperatures ( $> 140^{\circ}C$ ) (Fig. 4). The analysis time was reduced by about 8-fold when the temperature increased from  $140^{\circ}C$  to



**Figure 3.** Van't Hoff plots for barbiturates using 100% pure water as eluent at temperature range of 140°C to 200°C.



**Figure 4.** Separation of barbiturates on PLRP-S column (150 × 2.1 mm I.D.). Chromatographic condition: mobile phase: 100% pure water; flow rate: 0.5 mL/min; temperature: 140°C–200°C; detection: UV absorbance at 220 nm. Peaks: **1**—solvent; **2**—allobarbital, **3**—aprobarbital, **4**—buthetal, **5**—butalbital.

200°C without significant loss in resolution and column efficiency. It is important to note, that when the column temperature was lower than 140°C, generally, very broad peaks were observed for the barbiturates (results not shown). This was probably due to the poor solubility of the barbiturates in pure water eluent at low temperatures. Water at temperatures of less than 140°C was too polar to solvate most organic compounds.<sup>[10]</sup> Buthetal and butalbital peaks were found to overlap in the separation at 200°C This was probably due to the strong elution strength of the superheated water and similarity of the hydrophobicity value for these two compounds.

## CONCLUSIONS

The performance of the poly(styrene-divinylbenzene) stationary phase using water-rich and superheated water eluent at high temperatures of 100°C to 200°C has been examined. Van't Hoff plots for the separations were linear, suggesting that no changes occurred in the retention mechanism over the temperature range studied. The method developed justified that purified water can be utilized as an eluent to completely separate barbiturates.

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