PERFORMANCE STUDY OF CARBON-CLAD ZIRCONIA STATIONARY PHASE IN HIGH TEMPERATURE REVERSED-PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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Abstract. The performance of carbon-clad zirconia (CCZ) stationary phase was evaluated for the separations of test compounds (benzaldehyde, benzamide, nitrobenzene, and toluene) and alkyl aryl ketones (acetophenone, propiophenone, butyrophenone and valerophenone) at high temperature (100°C-160°C) in reversed-phase high performance liquid chromatography (RP-HPLC) using low compositions of organic modifier and 100% pure water as eluent. Improved separation mechanism and faster analysis time were achieved during high operating temperatures. Van't Hoff plots were linear for the investigated CCZ column, which showed that over the temperature range, no changes occurred in the retention mechanism. The use of alkyl aryl ketone homologs as a retention index scale in high temperature RP-HPLC was investigated. Although the retention factors of the test compounds were greatly affected by increasing the column temperature, there were only slight variations in their respective retention index values.

Keywords: Carbon-clad zirconia, high temperature high performance liquid chromatography

Abstrak. Prestasi turus zirkonia tersalut karbon (CCZ) telah dikaji bagi pemisahan sebatian ujian (benzaldehid, benzamida, nitrobenzena, dan toluena) dan alkil aril keton (asetofenon, propiofenon, butirofenon, dan valerofenon) dalam sistem kromatografi cecair prestasi tinggi fasa terbalik (RP-HPLC) pada suhu tinggi (100°C-160°C) menggunakan fasa bergerak dengan pelarut organik berkomposisi rendah dan 100% air tulen. Mekanisme pemisahan yang lebih baik dan masa analisis yang lebih pendek diperolehi apabila pengoperasian sistem pada suhu tinggi dijalankan. Lakaran keluk Van't Hoff yang linear bagi turus CCZ membuktikan bahawa tiada perubahan mekanisme penahanan terhadap sebatian analit terpilih pada julat suhu yang dikaji. Siri homolog alkil aril keton telah digunakan sebagai skala indeks penahanan dalam pembangunan indeks penahanan dalam sistem RP-HPLC suhu tinggi. Walaupun faktor muatan sebatian ujian itu amat dipengaruhi oleh perubahan suhu turus, namun perubahan nilai indeks penahanan sebatian berkenaan adalah kecil.

Kata kunci: Karbon tersalut zirkonia, kromatografi cecair prestasi tinggi pada suhu tinggi

1.0 INTRODUCTION

High performance liquid chromatography (HPLC), in spite of its slow start, has now become a major analytical technique for analysis of a wide range of compounds that are too involatile or too labile for analysis by gas chromatography. Reports of

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applications in the fields of biochemistry, pharmacy and food chemistry are appearing monthly [1]. The advantages of HPLC over other forms of liquid chromatography are: (i) the HPLC column can be used many times without regeneration; (ii) the resolution achieved on such columns far exceeds that of the older methods; (iii) the technique is less dependent on the operator's skill, and reproducibility is greatly improved; (iv) the instrumentation of HPLC lends itself to automation and quantitation; (v) analysis times are generally much shorter; and (vi) preparative liquid chromatography is possible on a much larger scale.

Zirconia particles coated with a carbon layer developed by Carr *et al.* [2] seem to be substantially different from other carbon supports. The resulting chemical-vapor deposited (CVD) carbon-coated material is useful as a reversed phase support for liquid chromatography. As expected, this material demonstrates much better selectivity for the separation of both non-polar and polar isomers than do conventional reversed phase support. The extreme retentive and selective nature of the carbon support makes it an excellent candidate for consideration when conventional chemically bonded reversed-phase supports fail. Carbon-clad zirconia supports are stable at elevated temperatures and pH. This is comparable to that of unmodified zirconia and polybutadiene-coated zirconia.

A more general acceptance of the importance of temperature as a variable in LC has taken place. This is based on the well-known decrease in viscosity of the mobile phase and the increase in analyte diffusivity at higher temperatures. The consequence of reduced viscosity is lower back pressure over the column, allowing higher speed or longer columns with higher plate numbers. The consequence of increased diffusivity is increased mass transfer, usually resulting in improved column efficiency [3]. This research concentrated on the operating and separation performance of RP-HPLC at high temperature by using mobile phase with low compositions of organic modifier on a ZirChrom CARB, a carbon-clad zirconia column. The use of the alkyl aryl ketones (AAK) retention index scale in high temperature RP-HPLC system using carbon-clad zirconia stationary phase with low composition of organic modifier was also investigated.

2.0   MATERIALS AND METHODS

2.1   Materials

Acetonitrile (HPLC grade) was obtained from Caledon Laboratories Ltd (Canada). Distilled deionized water was prepared in the laboratory using AquaMax Ultrapure Purification System with a distilled water feed. Test compounds comprising of benzaldehyde, benzamide, nitrobenzene and toluene were obtained from MERCK (Schuchardt, Germany). Acetophenone, propiophenone, butyrophenone and valerophenone (alkyl aryl ketones retention index standards) were obtained from Fluka chemika (Switzerland).

2.2 Instrumentation

The high performance liquid chromatography (HPLC) system used in this study was as described in a previous work (Figure 1) [4, 5]. It consisted of a Waters 515 HPLC pump (Milford, USA) for mobile phase delivery, a Shimadzu SPD-6A UV-Spectrophotometric Detector (Kyoto, Japan) for analyte peaks detection and a Shimadzu Gas Chromatograph GC-8A oven (Kyoto, Japan). A Rheodyne 7125 injection valve (Cotati, USA) fitted with a 20 µL loop was used for sample introduction. Data was processed using a HP 3396 Series II Integrator. A 30 cm × 0.25 mm I.D. length of stainless-steel pre-heating coil was placed in the oven between the injection valve and the column. The column used in this research was a carbon-clad zirconia column (Zirchrom-CARB, 100 mm × 2.1 mm I.D., 3 µm particles, (ZirChrom Separations, Anoka, MN, USA). A thermometer (Zecol, England) with temperature range of 20°C-360°C was placed inside the oven to measure the exact oven temperature.
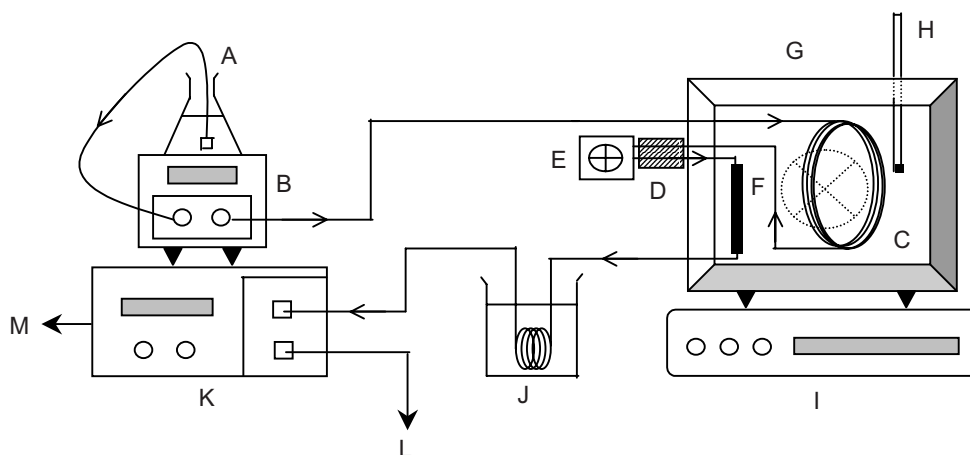

Figure 1   Instrument set-up. Components: A – mobile phase reservoir; B – HPLC pump; C – mobile phase pre-heating coils; D – heat insulator; E – injection port; F – column; G – oven; H – thermometer; I – temperature controller; J –coolant reservoir; K – UV detector; L – waste bottle; M – integrator

2.3 Chromatographic Conditions and Procedures

Mobile phases were prepared by mixing acetonitrile and distilled deionized water. Water-acetonitrile mixture was degassed prior to use. The degassed mobile phase was pumped into the HPLC system for 30 minutes or more to achieve stability. The system was flushed overnight at a flow rate of 0.1 mL/min to ensure the column was equilibrated when a change was made to the mobile phase composition. If the GC oven was used to heat up the column, at least 30 minutes was allowed to stabilize the

column temperature and equilibrium of the whole system. After stabilizing the system, the detector was warmed up for at least 20 minutes. Preliminary analysis was performed on standard samples to determine the retention time and elution order of the analytes.

2.4   Separation at High Temperatures

Experiments were implemented by heating the column at 100°C, 120°C, 140°C and 160°C. Mobile phase consisting of a mixture of acetonitrile-water (20:80 v/v) was eluted at a flow rate of 0.5 mL/min. Test compounds and AAKs mixture (1 µL) were injected into the system consecutively in triplicates. The experiment was repeated for mobile phase compositions of 10:90 and 100% pure distilled deionized water. Detection wavelength was at 254 nm for all runs. The value of the void volume (t_o) was determined using the solvent peak.

The performance of ZirChrom-CARB at various temperatures using low composition organic modifier were examined by analyzing the retention factor, k'; separation factor, α; theoretical number of separation plates per column length, N/m; plate height, H and the resolution, R_s.

3.0   RESULTS AND DISCUSSION

3.1   Elution Behaviour on a Carbon-Clad Zirconia Column

The elution order for the AAKs was directly proportional to the molecular weight of the solute. Acetophenone with the lowest molecular weight was first eluted, followed by propiophenone, butyrophenone and valerophenone (Figure 2). This was probably due to the hydrophobic interactions that occurred on the carbon-clad zirconia stationary phase. The carbon support is not only hydrophobic but it also retains solutes through electronic π-π interaction. Increase of the alkyl portion in the solute structure of the AAKs resulted in the greater contribution of hydrophobic interactions compared to the π-π interaction [6].

The elution order of the test compounds were not directly proportional to the molecular weight. Increase of dipolarity of test solutes increases retention on carbon support [7]. This carbon support is more sensitive to the solute polarizability, dipolarity and shape. Carbon supports are more retentive than conventional supports, especially for the more polar solutes. Nitro and dinitrobenzene are known to be strong π-acceptors [8]. Thus, nitrobenzene was eluted last among the test compounds (Figure 3).
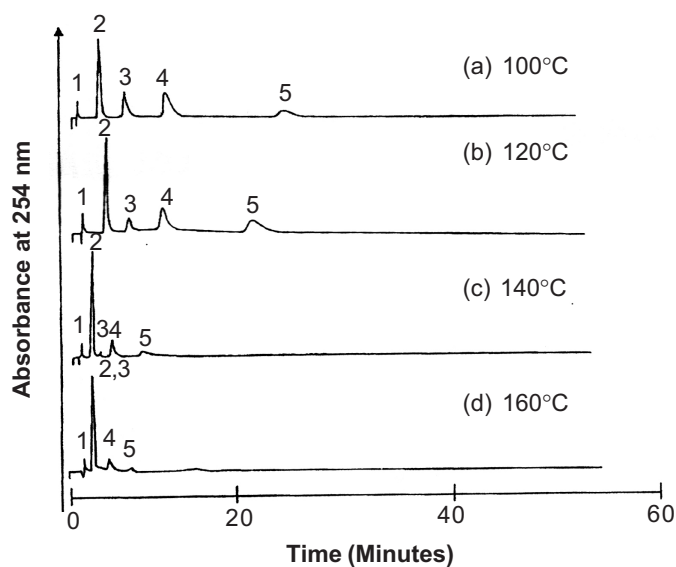
Figure 2 High temperature HPLC separations of AAKs on carbon-clad zirconia column. Chromatographic conditions: Mobile phase:acetonitrile-water 10:90; Flow rate: 0.5 mL/min. Peaks; **1**-solvent, **2**-acetophenone, **3**- propiophenone, **4**-butyrophenone, **5**-valerophenone
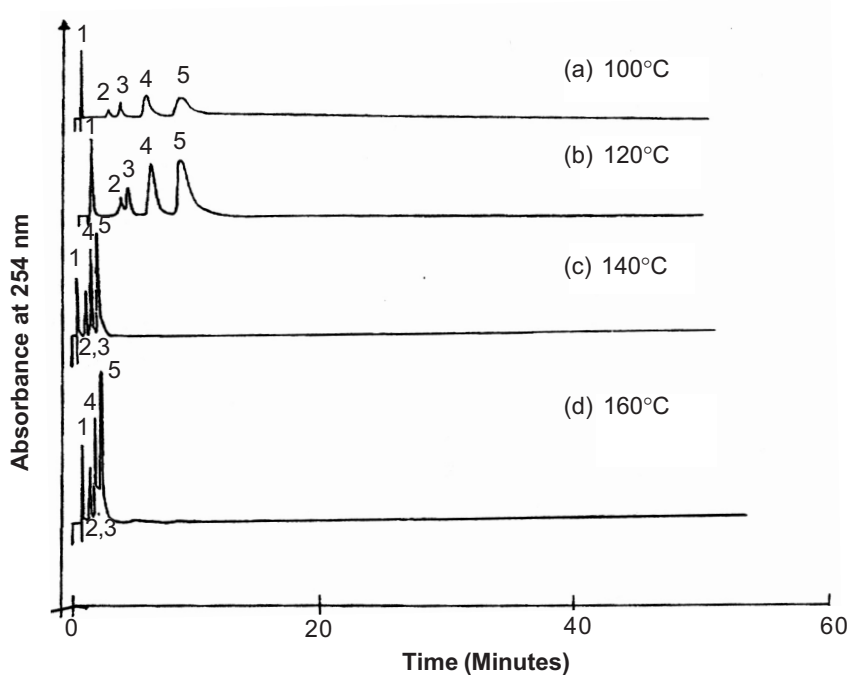

Figure 3 High temperature HPLC separation of test compounds on carbon-clad zirconia column. Chromatographic conditions: Mobile phase: acetonitrile-water 0:100; Flow rate: 0.5 mL/min. Peaks; **1**-solvent, **2**-benzamide, **3**-toluene, **4**-benzaldehyde, **5**-nitrobenzene

3.2   Separation of Test Compounds at High Temperatures

In order to reduce the usage of organic solvents in RP-HPLC system, low compositions of organic modifier were used. A decrease in retention factor with increasing temperature from 100°C to 160°C was observed for both the AAKs and test compounds. In addition, retention factors increased significantly with decreasing percentage of acetonitrile. It was observed that when 100% pure water was used at 160°C, only acetophenone was eluted. Two compounds, acetophenone and toluene
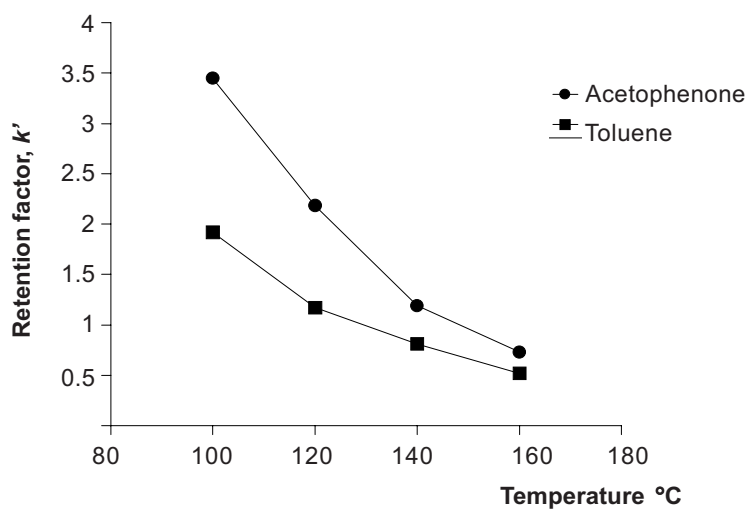
Figure 4   Variation of retention factor as a function of temperature for mobile phase composition acetonitrile-water 10:90 v/v at different column temperature on carbon-clad zirconia column
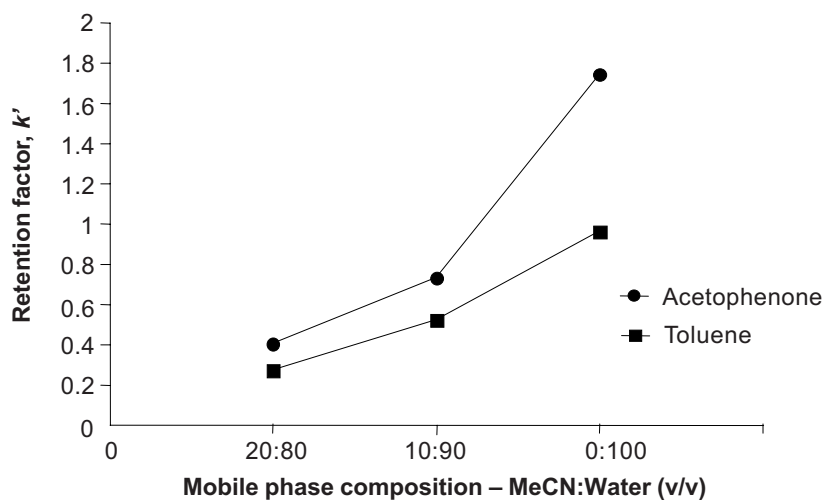
Figure 5   Variation of retention factors as a function of mobile phase composition at temperature 160°C

were chosen to illustrate the variation of retention factor as a function of temperature for mobile phase composition acetonitrile-water 10:90 (v/v) and retention factor as a function of mobile phase composition at 160°C (Figures 4-5).

Acetophenone and benzamide were used as test compounds to examine the effect of high temperature on column efficiency (Table 1). For the AAKs, column efficiency (N/m) markedly decreased when the column temperature was increased. Maximum of peak efficiency across the mobile phase range showed that the reduction in retention time was smaller than the reduction in peak width [9]. Reduced plate height was observed when the percentage of organic modifier was reduced from 20% to 0% for a particular temperature. Illustrations on variation of column efficiency as a function of temperature for acetophenone and benzamide are shown in Figure 6a & 6b.

Table 1 Relationship between theoretical plates number, N, theoretical plate height, H and theoretical plates numbers divided by column length, N/m, as a function of temperature using different mobile phase compositions

| Temperature (°C) | Mobile phase composition (Acetonitrile-Water) | | | | | | | | |
	N			N/m (m^{-1})		(R.S.D %)	Hx10^{-4} (m)		
	20:80	10:90	0:100	20:80	10:90	0:100	20:80	10:90	0:100
AAK standard (Acetophenone)									
100	476	524	-	4760	5240	-	2.1	1.9	-
120	343	454	-	3430	4540	-	2.9	2.2	-
140	219	386	-	2190	3860	-	4.7	2.6	-
160	200	292	330	2000	2920	3300	5.0	3.4	3.0
Test compound (Benzamide)									
100	261	384	-	2610	3840	-	3.8	2.6	-
120	118	369	-	1180	3690	-	8.5	2.7	-
140	153	182	-	1530	1820	-	6.5	5.5	-

(a)

(b)
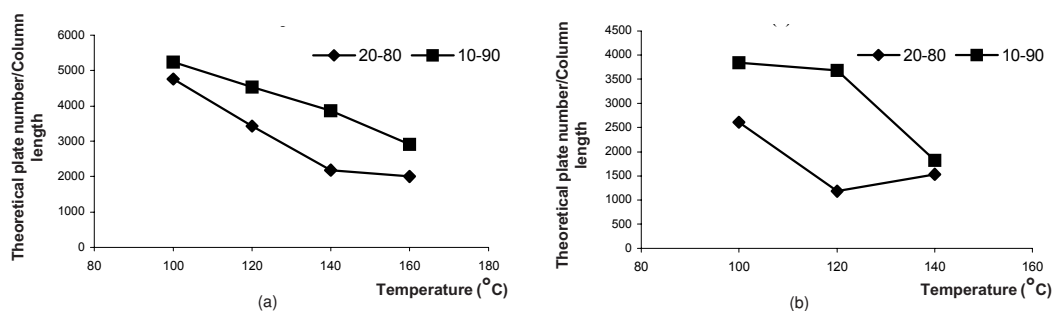
Figure 6 Variation of column efficiency as s function of temperature using low composition of organic modifier (a) Acetophenone and (b) Benzamide

3.3 Effect of Column Temperature on Column Separation Mechanism

Enthalpy data for acetophenone and toluene at temperatures ranging from 100°C to 160°C are shown in Table 2. The van't Hoff plots for both compounds showed no significant deviation from linearity (Figure 7). Van't Hoff plots were linear for the investigated CCZ column, which showed that over the temperature range, no changes occurred in the retention mechanism. In all cases, ΔH^o values were negative under the experimental conditions, indicating that retention of compounds was an exothermic process. The value of ΔH^o reflects the degree of interaction between analytes and the stationary phase and a more negative ΔH^o indicates a higher degree of interaction [10].

Table 2 Enthalpy data for acetophenone and toluene at temperatures ranging from 100°C to 160°C in different mobile phase compositions

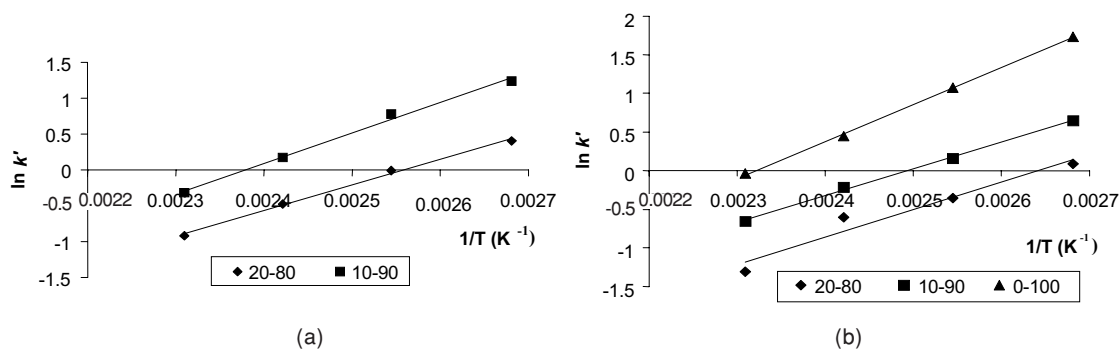| Compounds | Temperature range: 100 °C to 150°C | | | | | |
	Enthalpy ΔH^o(kJ/mol)			Correlation, r		
	Mobile phase composition: MeCN:water			Mobile phase composition: MeCN:water		
	20:80	10:90	0:100	20:80	10:90	0:100
Acetophenone	–29.70	–35.27	–	0.9982	0.9970	–
Toluene	–29.51	–35.07	–40.00	0.9732	0.9988	0.9996

Figure 7 Van't Hoff plots for (a) acetophenone and (b) toluene using different proportion of acetonitrile as eluent at temperature range of 100°C to 160°C

3.4 Retention Index in High Temperature RP-HPLC

The alkyl aryl ketones have been identified as suitable retention index standards for a wide range of analytes, because of their ready detectability, easy availability and similar polarities and hence retentions, to many aromatic analytes [11,12]. The retention indices could form the basis of a retention prediction system, whose conclusions would be more generally applicable than values calculated for one specific retention system.

The retention factors and retention indices of AAKs and test compounds for the mobile phase compositions, acetonitrile-water, 20:80 v/v and 10:90 v/v were calculated and shown in Table 3 and 4 respectively. Retention indices of the test compounds based on AAKs were calculated by plotting $\log k'$ against carbon number X 100 for the standards to a linear correlation using a least squares routine and then interpolating the $\log k'$ values of each of the compounds. The homologues showed a close correlation between the $\log k'$ and the carbon number (C_n X 100) with r values generally exceeding 0.9966 (Table 3-4).

Although the retention factors were greatly affected by increasing the column temperature, there was only a slight variation in the retention index values of the test compounds (Figures 8-9). The AAKs retention index was insensitive to uncertainties in the value of t_0 and to small changes in solvent composition. It is based on the linear relationship between the retention factor, k' and the number of carbon atom (C_n) in the standards [12].

Table 3 Retention factors and retention indices of AAKs and test compounds on a carbon-clad zirconia column at different temperatures in acetonitrile-water (20:80 v/v)

Compound	Retention factor, k'				Retention Index, I				
	Temperature°C				Temperature°C				
	100	120	140	160	100	120	140	160	Nominal
Standard									
Acetophenone	1.5	0.99	0.62	0.4	791	807	807	782	800
Propiophenone	3.05	1.92	1.16	0.7	897	918	920	888	900
Butyrophenone	5.36	3.2	1.84	1.1	981	1003	1003	973	1000
Valerophenone	11.25	6.29	3.43	1.94	1092	1116	1116	1080	1100
Test compounds									
Benzamide	0.52	0.35	0.23	0.27	632	634	627	708	
Toluene	1.09	0.7	0.55	0.27	743	750	785	708	
Benzaldehyde	1.09	0.7	0.55	0.45	743	750	785	804	
Nitrobenzene	2.07	1.27	0.96	0.45	839	849	886	804	
Slope	0.0029	0.0026	0.0024	0.0023					
Intercept	–2.1164	–2.1037	–2.1434	–2.1968					
Correlation, r	0.9975	0.9973	0.9966	0.9981					

Table 4 Retention factors and retention indices of AAKs and test compounds on a carbon-clad zirconia column at different temperatures in acetonitrile-water (10:90 v/v)

Compound	Retention factor, *k'*				Retention Index, *I*				
	Temperature°C				Temperature°C				
	100	**120**	**140**	**160**	**100**	**120**	**140**	**160**	**Nominal**
Standard									
Acetophenone	3.45	2.18	1.19	0.73	795	795	805	832	800
Propiophenone	7.27	4.34	2.28	0.73	907	898	913	832	900
Butyrophenone	13.82	7.75	3.75	2.14	1003	984	996	973	1000
Valerophenone	25.81	16.38	7.52	6.21	1096	1097	1113	1113	1100
Test compounds									
Benzamide	1.27	0.82	0.46	0.52	645	648	646	787	
Toluene	1.92	1.17	0.81	0.52	707	701	740	787	
Benzaldehyde	2.36	1.48	0.81	0.85	738	737	740	852	
Nitrobenzene	4.21	2.55	1.36	0.85	826	818	827	852	
Slope	0.0029	0.0029	0.0026	0.0033					
Intercept	–1.7679	–1.9655	–2.0163	–2.881					
Correlation, *r*	0.9982	0.9978	0.9963	0.8913					
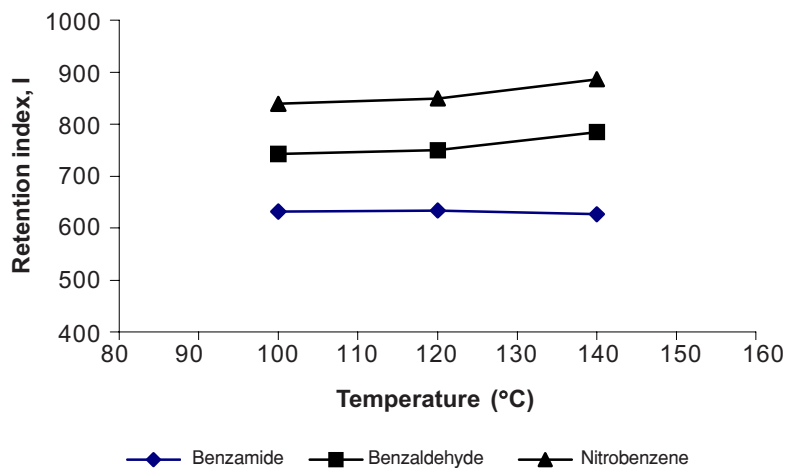
Figure 8 Variation of retention index values of test compounds at different column temperature using mobile phase acetonitrile-water, 20:80 v/v
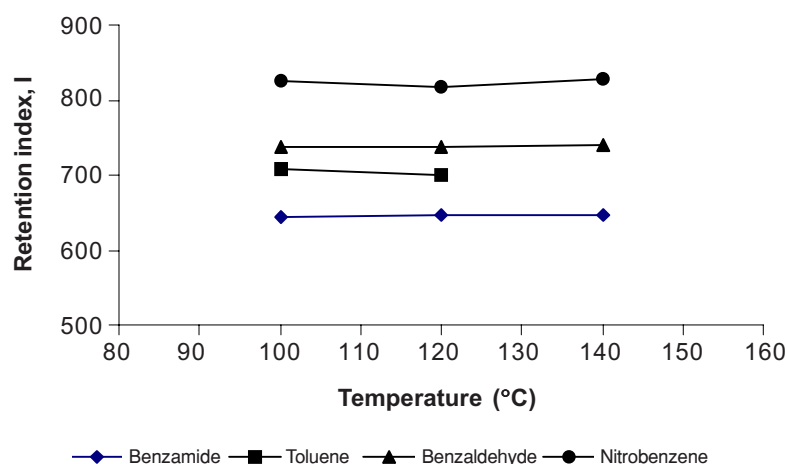
Figure 9   Variation of retention index values of test compounds at different column temperature using mobile phase acetonitrile-water, 10:90 v/v

4.0   CONCLUSIONS

The performance of the carbon-clad zirconia stationary phase using mobile phases with low organic modifier composition at elevated temperatures of 100°C to 160°C has been examined. The stationary phase is not only hydrophobic but also retains solutes through electronic π-π interaction. This carbon support is more sensitive to the solute polarizability, dipolarity and shape. It was observed that a better separation mechanism was achieved under high operating temperatures. The van't Hoff plots for acetophenone and toluene showed no significant deviation from linearity. It can be concluded that, over the studied temperature range, no change in the retention mechanism for the compounds occurred. Alkyl aryl ketone homologs from acetophenone to valerophenone were used as retention index scale in this study. Although the retention factors were greatly affected by increasing the column temperature (100°C to 160°C), there were only slight variations in the retention index values for the test compounds. The retention index values of the test compounds were unaffected by the composition of the mobile phase while operating at high temperatures.

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