Malaysian Journal of Chemistry, 2004, Vol. 6, No. 1, 055 - 066

High Temperature High Performance Liquid Chromatography of Triazole Fungicides on Carbon-Clad Zirconia Stationary Phase

Mohd Marsin Sanagi,* See Hong Heng, Wan Aini W. Ibrahim, Ahmedy Abu Naim
Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia,
81310 Skudai, Johor, Malaysia

Abstract : Temperature is an important separation parameter in reversed-phase high performance liquid chromatography. In this work, high-temperature high performance liquid chromatography on a carbon-clad zirconia column using water-rich eluent is evaluated as a new approach for the separation of selected triazole fungicides. The selectivity and retention patterns of triazole fungicides, hexaconazole, tebuconazole, propiconazole, and difenoconazole were investigated at temperatures of 100 °C to 150 °C. Excellent separations were achieved for propiconazole, tebuconazole and difenoconazole under all conditions studied. Despite insufficient separations between hexaconazole and propiconazole, slight separations were observed for the *cis* and *trans* propiconazole isomers. Van't Hoff plots for the separations were linear suggesting that no changes occurred in the retention mechanism for the selected fungicides over the temperature range studied.

Abstrak : Suhu merupakan parameter penting dalam kromatografi cecair prestasi tinggi fasa terbalik. Dalam kajian ini, kromatografi cecair prestasi tinggi bersuhu tinggi dengan turus zirkonia tersalut karbon menggunakan pengelusi kaya-air telah dikaji sebagai suatu pendekatan baru bagi pemisahan racun rumpai triazola. Kepilihan dan pola penahanan rumpai triazola heksakonazola, tebukonazola, propikonazola, and difenokonazola telah dikaji pada suhu 100 °C hingga 150 °C. Pemisahan yang sangat baik diperoleh bagi propikonazola, tebukonazola, dan difenokonazola pada semua keadaan kajian. Sementara tiada pemisahan yang lengkap diperoleh bagi heksakonazola dan propikonazola, sedikit pemisahan diperoleh bagi pasangan *cis* dan *trans* bagi propikonazola. Lakaran Van't Hoff bagi pemisahan tersebut adalah linear yang menandakan tiada perubahan berlaku pada mekanisme penahanan bagi rancun rumpai terpilih berkenaan pada julat suhu kajian.

Received : 10.11.03; accepted : 28.05.04

Introduction

Control of column temperature as an optimization parameter in the separation process of the reversed-phase high performance liquid chromatography (RP-HPLC) has been widely investigated. 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 [1]. Elevated column temperature operation in RP-HPLC can be used as a tool to overcome the flow rate problem associated with high back pressure, allowing the use of higher flow rates that otherwise could not be applied. The pressure reduction is due to a decrease in eluent viscosity with increasing temperature. The lower viscosity decreases the pressure drop across the column and allows higher linear velocities as the limit of pump pressure is approached [2]. An increase in temperature also increases the diffusion coefficients of the mobile phase and the analytes. According to the Stokes–Einstein relationship, the diffusion coefficient is directly proportional to the absolute temperature and inversely proportional to the viscosity. The high-temperature separation has been shown to improve analyte resolution by decreasing mobile phase viscosity and by increasing the diffusion rate of the analytes, thus increasing mass transfer of the analyte to the stationary phase and thereby decreasing the peak width [3].

High temperature as an optimisation parameter in the separation process of the RP-HPLC system has been widely studied due to the recent findings of alternative stationary phases that have high thermal stability at high temperatures. Polystyrene-divinylbenzene (PS-DVB) stationary phase can be regarded as one of the earliest stationary phases introduced which is able to withstand exposure to mobile phase at extreme pHs (1-14) and column temperature as high as 200 ^{o}C [4]. Sanagi and See [5] in their paper described a comprehensive study on PS-DVB column using water-rich and superheated water eluent at high column temperatures up to 200 ^{o}C. Recently, mixtures of barbiturates have been separated on PS-DVB column using pure water eluent [6]. Zirconia based column have received a great deal of attention recently because of their extraordinary stability under extreme thermal and chemical conditions. Zirconia based stationary phase can be regarded as one of the latest high thermal stability stationary phases introduced and is able to

withstand extended exposure to column temperature as high as 150 ^{o}C. Zirconia by itself has very rich surface chemistry and able to operate at a wider pH range (1-14). In contrast, for conventional alkyl silane bonded phase, higher temperature will directly accelerate the dissolution of silica in aqueous solution [7]. Zirconia column coated with polybutadiene (PBD) have been widely used as a reversed-phase (RP) stationary phase because it is a much more durable substrate compared with silica, while not imparting the high retentive characteristics of the aromatic polymer-based column [8-10]. Carbon-clad zirconia columns with unique selectivity have been used as stationary phase in liquid chromatography. Carbon based supports have been found to have different selectivities compared to traditional bonded phase reversed phase LC media. These phases have properties somewhat in between those of reversed-phase and normal-phase sorbents [11]. The carbon support is generally more retentive towards polar compounds and is often more selective for the separation of structural isomers and homologues than the conventional reversed-phase supports [12]. Jackson *et al.* [13] reported the separation of diastereomers formed by derivatizing enantiomers with Mosher's reagent on carbon-coated zirconia column. A variety of structural isomers have been separated on carbon-clad zirconia and ODS-bonded silica columns by Weber and Carr [12]. The carbon column resolves the isomer mixtures to a greater extent and in a shorter time despite its lower efficiency than did the ODS column.

Fungicides have been developed since the discovery of the antifungal activity of N1-substituted azoles in the late 1960s [14]. According to the chemical classification and timing of application, fungicides can be categorized into three groups. Protectant fungicides are applied to the plant surface before infection occurs. Curative fungicides are applied after initial infection before it produces visible symptoms. Eradicant fungicides are applied when an infection has already become visible and for preventing further sporulation and spread of the disease [15]. Triazole fungicides are well known as curative fungicides, thanks to their excellent protective and a relatively low resistance risk compared with other fungicides such as benzimidazoles and dicarboximides. The common structural moiety for triazole fungicides is the 1,2,4-triazole ring, which is connected to the hydrophobic backbone through position 1 [16]. Currently, triazoles comprise about 25 commercial agrochemicals and represent the latest group of modern agricultural fungicides. Most of the triazole fungicides are used against rusts, powdery mildews, and scabs [14].

Capillary gas chromatography (GC) in conjunction with various selective detectors is still the most popular separation technique in fungicide residue analysis because of its high sensitivity and selectivity [17,18]. However, the required derivatization and poor GC properties limit the use of GC in favor of liquid chromatography (LC), which does not required derivatization. The increasing use of LC is chiefly the result of its suitability for labile and polar fungicides that require derivatization prior to GC analysis [19,20]. Fungicide analysis has also been carried out by capillary electrophoresis (CE) [21,22]. Little has been reported on the separation of triazole series fungicides by HPLC. Recently, the separation of 14 triazole fungicides was demonstrated by Wu and co-workers [16] using CE separation technique. However, the efficiency of the separation was not completely reported in the paper. In order to establish a high efficiency separation technique on triazole fungicides, it is thus of interest to develop high-temperature high performance liquid chromatography (HT-HPLC) separation technique using carbon-clad zirconia as stationary phase, which has not been reported in any research work.

The aim of the present study is to develop a novel high temperature high performance liquid chromatography (HT-HPLC) separation method on selected triazole fungicides (hexaconazole, tebuconazole, propiconazole, and difenoconazole) (Table 1) using mobile phase with low proportions of organic modifier. The performance of carbon-clad zirconia column (ZirChrom-CARB) for the separation of triazole fungicides is also investigated.

Experimental

Reagents

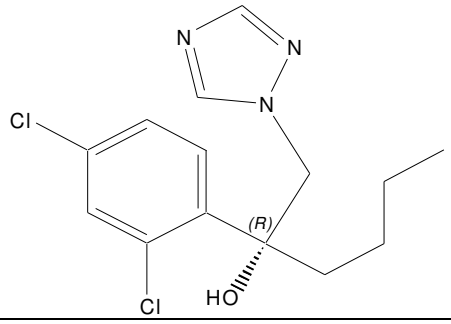
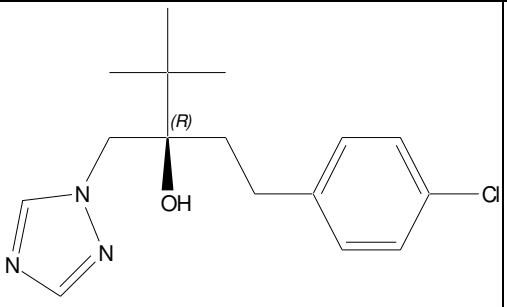
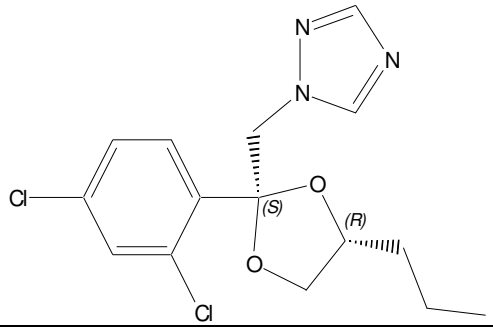
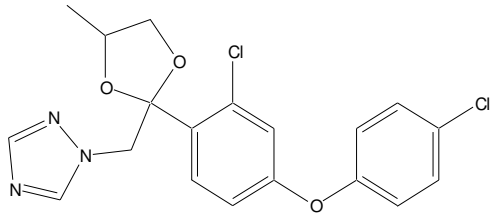
HPLC grade acetonitrile was obtained from Caledon Laboratories Ltd. (Canada). Double-distilled deionised water of at least 18 MΩ was purified by Nano ultra pure water system (Barnstead, USA). Triazole fungicides (hexaconazole, tebuconazole, propiconazole, and difenoconazole) were obtained from Dr. Ehrenstorfer (Augsburg, Germany).

Chromatographic conditions

The instrumental set-up is shown in Fig. 1. The high temperature HPLC systems consisted of a conventional HPLC system coupled with an oven of a Perkin Elmer Autosystem Gas Chromatography (USA). HPLC separations were carried out using a Waters 515 HPLC pump (Milford, USA) for mobile phase delivery. Samples were injected into the system using a 25 μL syringe (Hamilton, Australia). A Rheodyne 7125 injection valve (Cotati, USA) fitted with a 20 μL loop was used for sample introduction. Analyte peaks were detected using a Shimadzu SPD-6A UV detector (Kyoto, Japan) and were recorded on a Hewlett Packard HP 3396 Series II integrator (USA). A length of stainless-steel tubing (30 cm × 0.5 mm I.D.) was placed in the oven

between the injection valve and column as a mobile phase pre-heating coil. Separations were carried out on a column (100 mm × 2.1 mm I.D.) packed with 3 µm ZirChrom-CARB 300 Å (ZirChrom Separations, Anoka, MN, USA). The column and the pre-heating coils were placed together in the oven. A JASCO 880-81 (Japan) backpressure regulator was attached at the outlet of the detector to maintain a constant backpressure of about 20 bars in the detector cell.

Table 1: Properties of four triazole fungicides

Fungicides	Structure	Molecular Weight	Log P*
Hexaconazole (*RS*)-2-(2,4-dichlorophenyl)-1-(1*H*-1,2,4-triazol-1-yl)hexan-2-ol $C_{14}H_{17}Cl_2N_3O$		314.21	3.77
Tebuconazole (*RS*)-1-*p*-chlorophenyl-4,4-dimethyl-3-(1*H*-1,2,4-triazol-1-ylmethyl)pentan-3-ol $C_{16}H_{22}ClN_3O$		307.82	4.01
Propiconazole *cis-trans*-1-[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-ylmethyl]-1*H*-1,2,4-triazole $C_{15}H_{17}Cl_2N_3O_2$		342.22	4.16
Difenoconazole *cis-trans*-3-chloro-4-[4-methyl-2-(1*H*-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-2-yl]phenyl 4-chlorophenyl ether $C_{19}H_{17}Cl_2N_3O_3$		406.26	4.79

* : Prediction of logarithm of Partition Coefficient (*n*-Octanol/Water) (log P) according to mechanic molecular modeling methods by using CS ChemOffice® Chem3D Pro version 5.0 and CS ChemDraw® Ultra version 6.0 computer software.

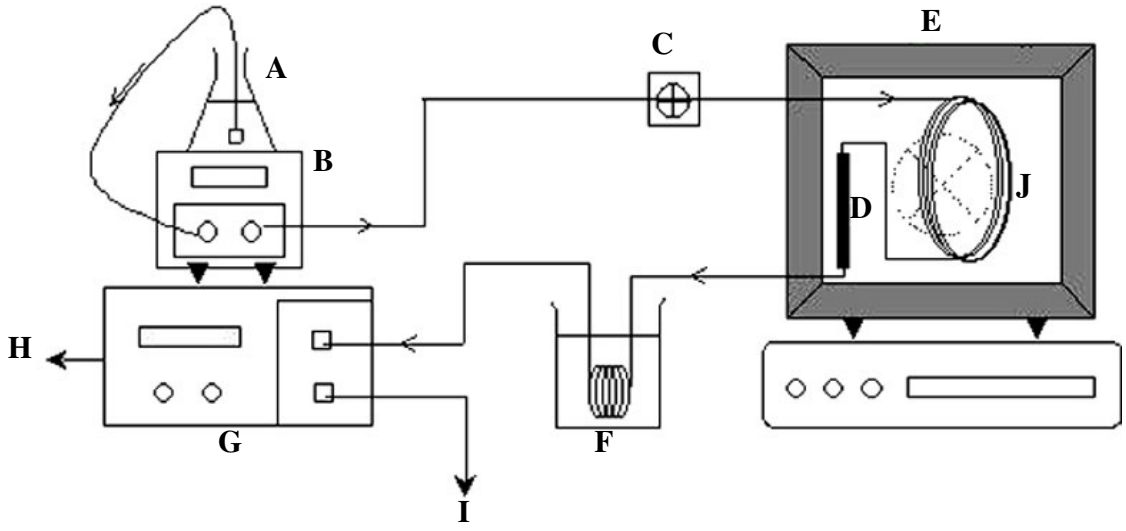


Figure 1 : Experimental set-up: A, mobile phase reservoir; B, HPLC pump; C, injection valve; D, column; E, oven; F, cooling system (ice water); G, UV detector; H, integrator; I, backpressure regulator; J, mobile phase pre-heating coil.

Procedure

The mobile phase used was prepared by mixing double distilled deionised water with acetonitrile and the mixture was subsequently degassed using vacuum-ultrasonic method. Samples of triazole fungicides dissolved in acetonitrile were injected in triplicate onto the column. Separation was carried out on ZirChrom-CARB column using acetonitrile-water: 10:90, 5:95, and 1:99 (v/v) at high temperatures (100 °C-150 °C). The eluent flow rate was 0.5 mL/min and sample injection volume was 1 µL. Solute concentrations were 0.05-0.1 mg/mL. UV detection of analytes for the comparison study was at 220 nm.

Results and discussion

Elution behaviour of triazole fungicides on a carbon-clad zirconia column

Reversed-phase HPLC separations of four triazole fungicides on a ZirChrom-CARB column at various temperatures ranging from 100 °C to 150 °C with different mobile phase compositions are illustrated in Fig.2a-c. In this work, an elevated flow rate of 0.5 mL/min was used to take advantage of the increased analyte mass transfer at high column temperatures. The elution order of the fungicides was hexaconazole, propiconazole, tebuconazole, and difenoconazole. It was noticed that the elution sequence showed a poor relationship with the triazole molecular weight and log P value. Carbon phases have radically different selectivities compared to traditional bonded phase reversed-phase liquid chromatographic media. Conventional reversed-phases typically separate on the basis of polarity, shape, and size of the solute, while carbon phases separate on the basis of these properties plus π-π and dipole-induced dipole interactions, which are most evident in the extraordinarily high retention exhibited by multiplanar aromatic ring compounds [23,24]. Weber and Carr [12] carried out a study on the comparison of carbon-clad zirconia and conventional RP stationary phase. The results demonstrate that an increase in the dipolarity of the test solute enhances retention on carbon supports, but on conventional ODS phases an increase in dipolarity always decreases retention. Besides, carbon support has much more greater selectivity for the separation of both non-polar and polar isomers and retention mechanism is estimated exclusively an adsorption process on the rigid carbon surface.

Based on the results gathered in this study, the elution behaviour of triazole fungicides was strongly dependent on the dipolarity and contribution of the planar aromatic on the solutes. Hexaconazole was first eluted across the column followed by propiconazole, tebuconazole, and difenoconazole. All the triazole fungicides have a common structural moiety, which is the 1,2,4-triazole ring. Propiconazole and difenoconazole are two types of triazoles fungicides that exist in their *cis*- and *trans*-isomers. Hexaconazole and propiconazole have very similar chemical structures, hydrogen backbone with

a substituted phenyl group at one end, and an alkyl group at the other end. The only difference is that propiconazole has a substituted dioxolan group instead of normal alkyl group bonded with the hydrogen backbone. This results in the dipolarity for propiconazole being much higher than hexaconazole. Tebuconazole with a hydroxyl group bonded with the chiral carbon centre and only single chloro-substituted with the benzene ring showed increased solute retention on the rigid planar carbon surface. Difenoconazole's hydrogen backbone has two substituted phenyl groups at both ends that make it highly retentive on the rigid carbon surface.

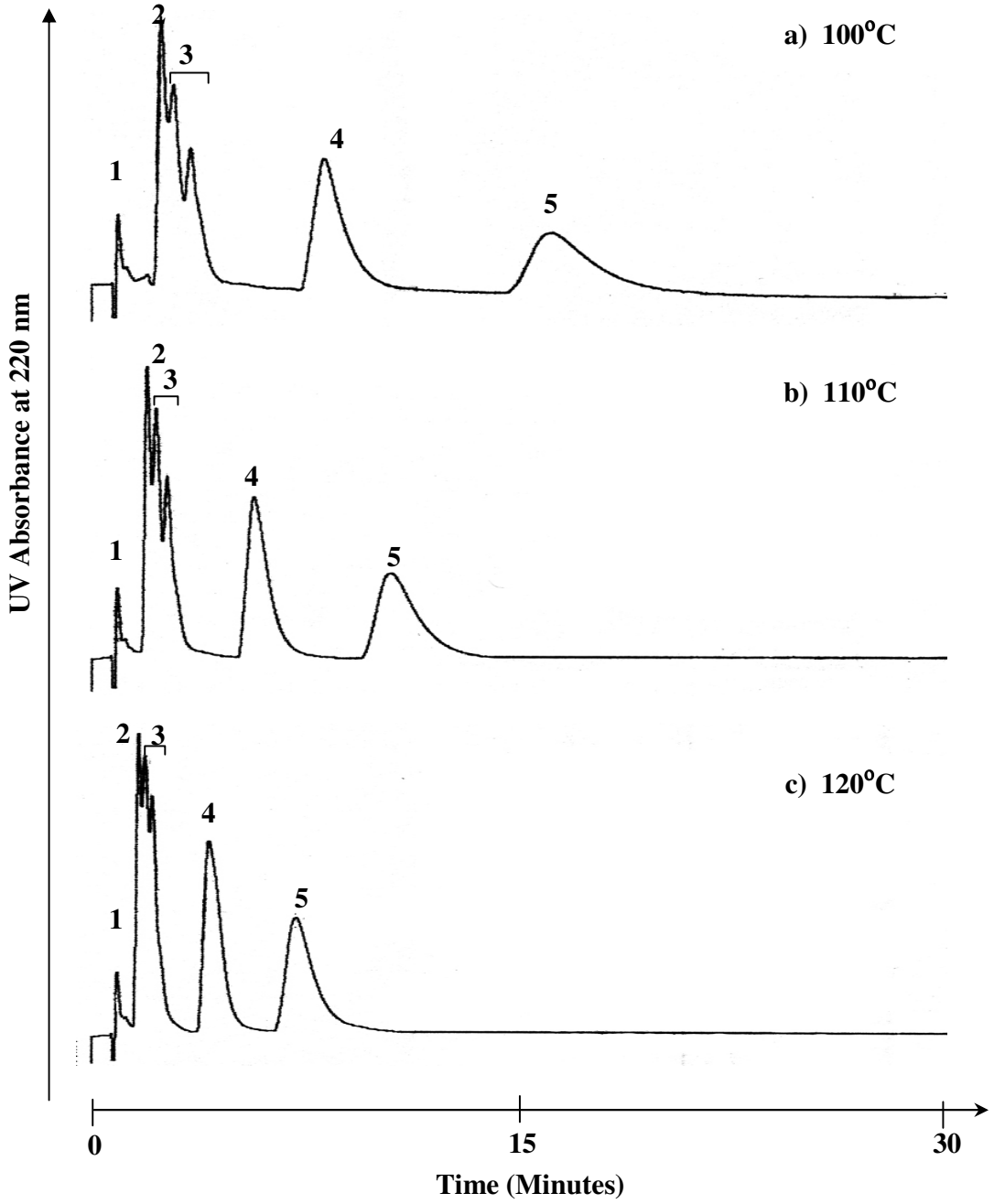


Figure 2a : Separation of four triazole fungicides on ZirChrom-CARB column (100 × 2.1 mm I.D.). Chromatographic condition: mobile phase: acetonitrile-water 10:90 (v/v); flow rate: 0.5 mL/min; temperature: 100 °C-120 °C; detection: UV absorbance at 220 nm; injection volume: 1 µL; solute concentration: 0.1 mg/mL. Peaks: **1** – solvent; **2** – hexaconazole, **3** – propiconazole, **4** – tebuconazole, **5** – difenoconazole.

Selectivity and resolution on carbon-clad zirconia column

The high temperature RP-HPLC separations (Fig.2a-c) show that there were excellent separations between propiconazole (peak 3), tebuconazole (peak 4) and difenoconazole (peak 5). Slight separations were observed for the *cis* and *trans* isomers of propiconazole. However, hexaconazole (peak 2) and propiconazole were not sufficiently separated under all conditions studied. Although several published

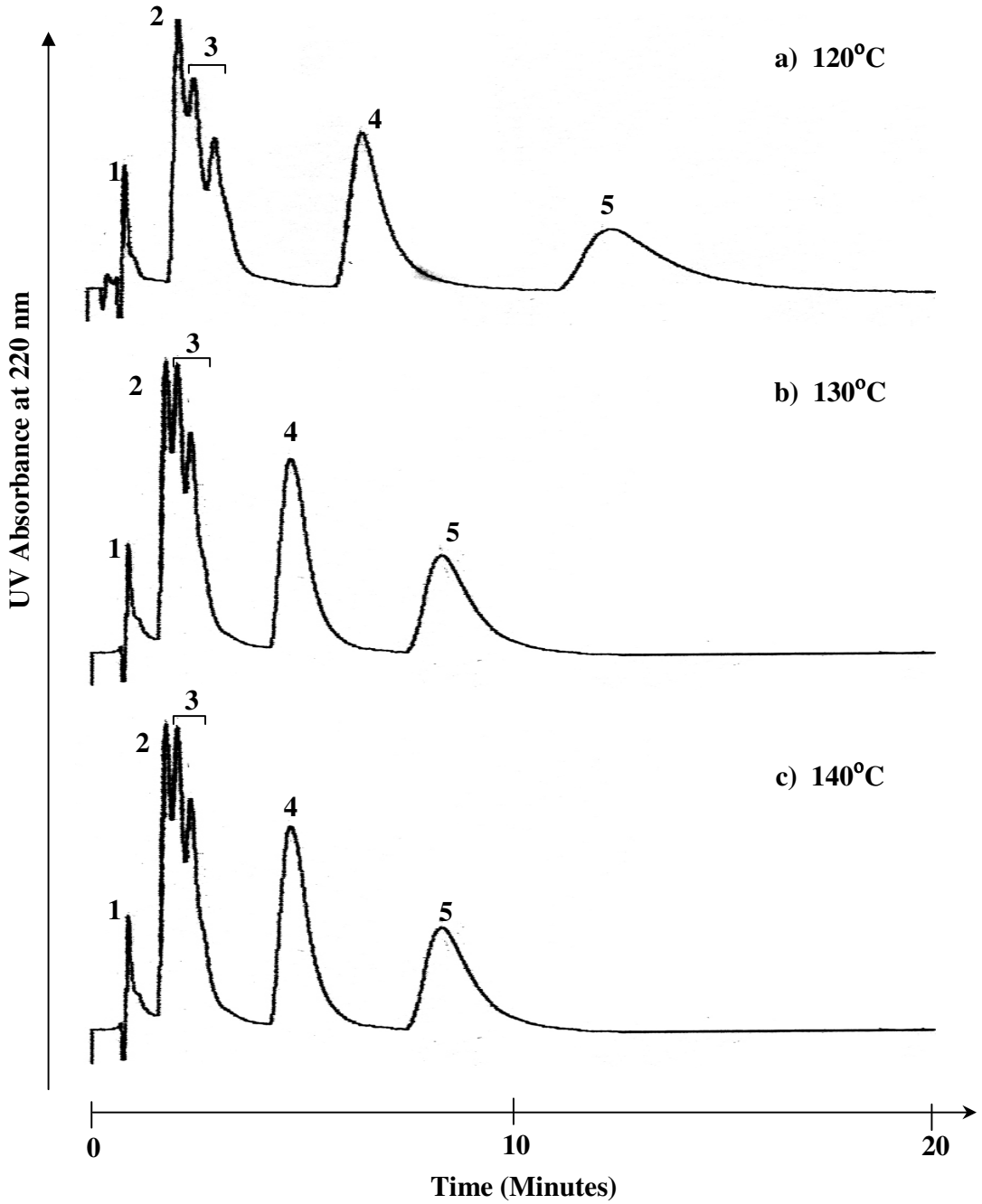


Figure 2b : Separation of four triazole fungicides on ZirChrom-CARB column (100 × 2.1 mm I.D.). Chromatographic condition: mobile phase: acetonitrile-water 5:95 (v/v); flow rate: 0.5 mL/min; temperature: 120 °C-140 °C; detection: UV absorbance at 220 nm; injection volume: 1 µL; solute concentration: 0.1 mg/mL. Peaks: **1** – solvent; **2** – hexaconazole, **3** – propiconazole, **4** – tebuconazole, **5** – difenoconazole.

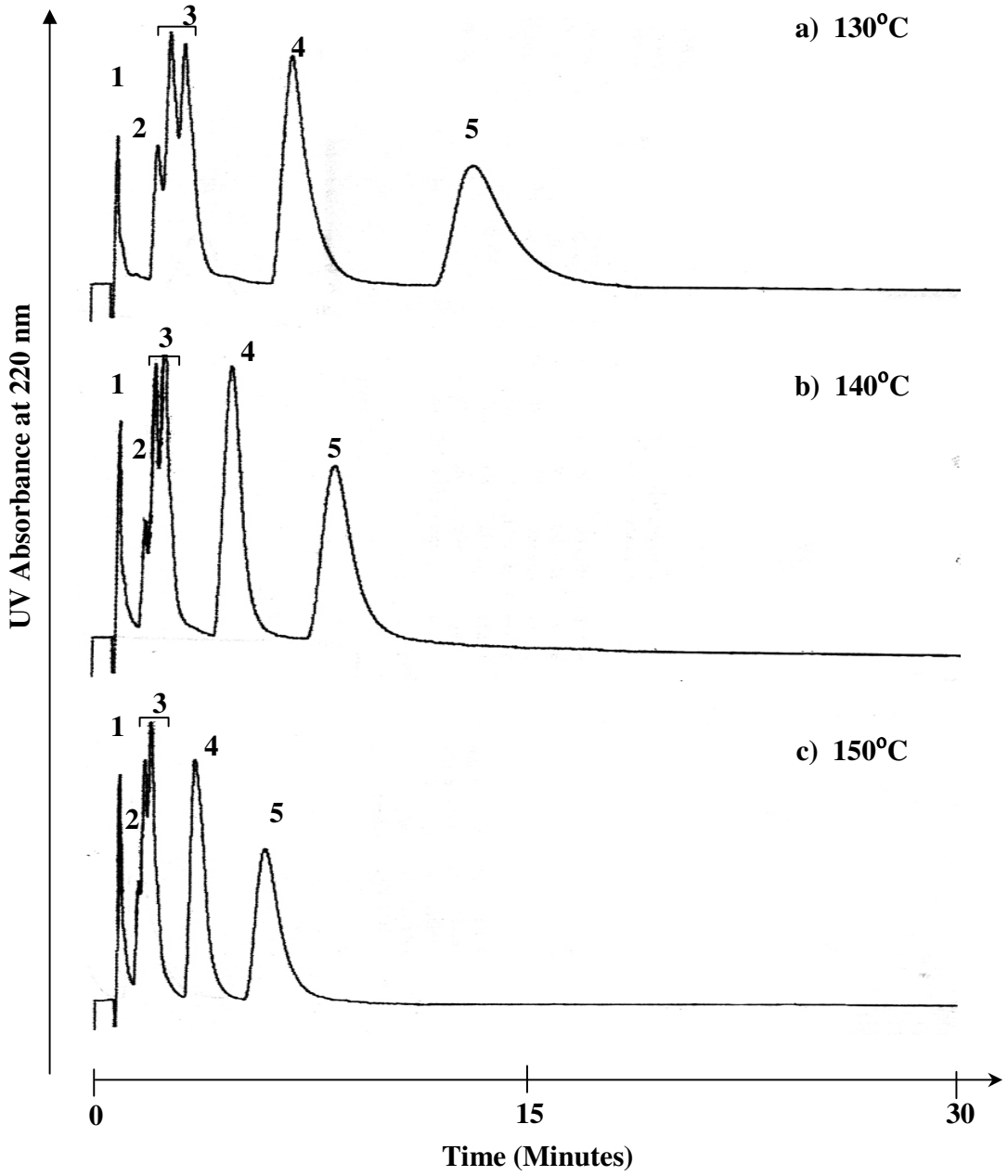


Figure 2c : Separation of four triazole fungicides on ZirChrom-CARB column (100 × 2.1 mm I.D.). Chromatographic condition: mobile phase: acetonitrile-water 1:99 (v/v); flow rate: 0.5 mL/min; temperature: 120 °C-150 °C; detection: UV absorbance at 220 nm; injection volume: 1 µL; solute concentration: 0.05-0.1 mg/mL. Peaks: **1** – solvent; **2** – tebuconazole, **3** – hexaconazole, **4** – propiconazole, **5** – difenoconazole.

papers already clearly demonstrated that carbon-clad zirconia column has a unique selectivities for the separation of isomer and homologues, difenoconazole, a structural and optical isomer, was not successfully separated. This was probably caused by the use of water-rich mobile phase in all cases studied. There might be a possibility that water-rich eluent was unable to wet the carbon-clad zirconia particles leading to insufficient mass transfer, hence poor resolution and selectivity on the stationary phase. This phenomenon was also observed on a PBD-coated zirconia stationary phase [25]. The use of high column temperature might also be another reason leading to this phenomenon. Sander *et al*. [26]

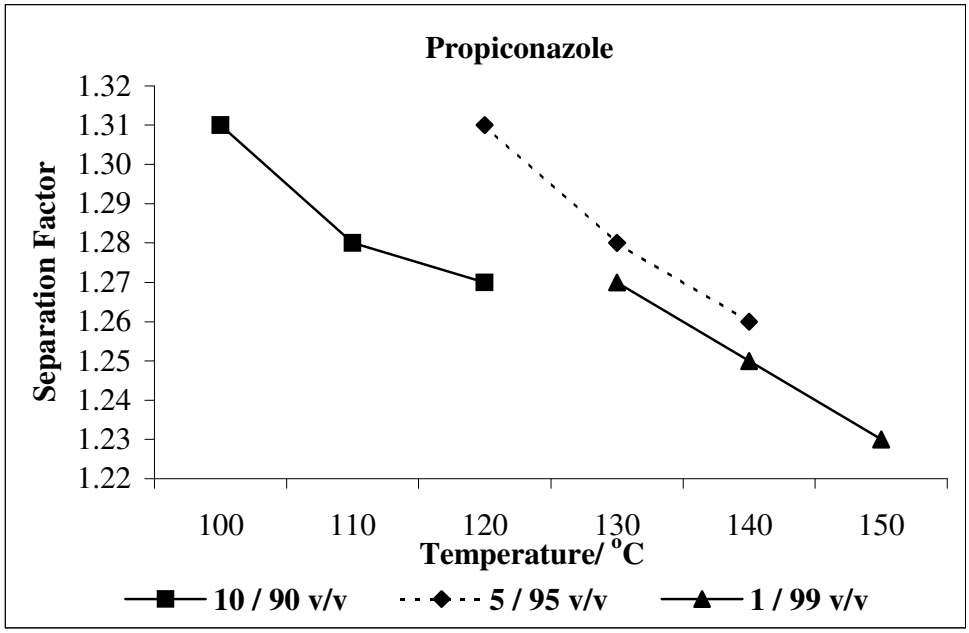


Figure 3 : Relationship of separation factor as a function of mobile phase composition at different column temperature on Carbon-clad zirconia column. Solutes: propiconazole.

in their paper described that for entropy-dominated retention processes, isomer with rigid, well-defined structures can be separated on the basis of differences in molecular shape and shape recognition is usually increased at reduced temperature.

The variation of separation factor for the propiconazole isomers at high temperatures and different organic compositions in the mobile phase was further investigated by plotting two-dimensional graphs (Fig. 3). It was found that at a given column temperature, the separation factor values were inversely proportional to the percentage of organic modifier used in mobile phase. In addition, in contrast to that for PBD-coated zirconia columns [**8, 9**], high proportion of organic modifier in the eluent generally resulted higher separation factor values on carbon-clad zirconia stationary phase. This is probably due to the differences on retention mechanism between these two columns. A zirconia-PBD phase is more on partitioning retention mechanism relative to carbon phases, which is exclusively an adsorption retention mechanism.

Influence of temperature on retention factor

For each compound studied, there was a marked decrease in retention factors with increasing temperature from 100 ^{o}C to 150 ^{o}C with 10 ^{o}C increments using elevated flow rate at different proportions of acetonitrile (Table 2). It was observed that a 5 % change in concentration of acetonitrile in the mobile phase produced a 1.5-fold decrease in retention factor at each column temperature, respectively, without significant loss in resolution and column efficiency. Based on the retention factor value gathered in this study, we noticed that a 1 % increase in acetonitrile concentration has the same effect as a 3 ^{o}C increase in column temperature in controlling solute retention. Several studies have directly compared the effects of changing the solvent composition and column temperature. Bowermaster and McNair [27] observed that a 1 % increase in methanol concentration had approximately the same effect as a temperature increase of 4 ^{o}C. Chen and Horvath [28] found a similar relationship after examining a series of homologous *n*-alkylbenzenes in acetonitrile/water (1 % : 5 ^{o}C). As shown in Fig. 2a-c, equivalent separations were obtained at 110 ^{o}C with 10 % acetonitrile, at 130 ^{o}C with 5 % acetonitrile, and at 140 ^{o}C with 1 % acetonitrile. This indicates that retention can be controlled either by the amount of organic solvent in the mobile phase or by column temperature.

Influence of temperature on column efficiency

Hexaconazole was used as solute to examine the effect of high temperature on column efficiency. The results and variations of column efficiency as a function of temperature using low compositions of organic modifier are as shown in Fig. 4. For each mobile phase composition investigated, the overall trend for column efficiency (*N*/m) was directly proportional to the column temperature.

Table 2 : Retention factors of four triazole fungicides on carbon-clad zirconia column as a function of temperature using different proportions of organic modifier

Compounds	Mobile Phase	Retention factor, *k* (R.S.D. %)					
	MeCN~water	Column temperature (^{o}C)					
	(v/v)	100	110	120	130	140	150
Hexaconazole	10~90	1.74 (1.2)	1.20 (0.8)	0.87 (0.5)	–	–	–
	5~95	–	–	1.46 (0.3)	1.04 (0.0)	0.74 (0.0)	–
	1~99	–	–	–	1.68 (0.0)	1.15 (0.4)	0.84 (0.6)
Propiconazole		2.24 (1.3)	1.56 (1.6)	1.12 (0.8)	–	–	–
	10~90	2.93 (1.3)	1.99 (0.6)	1.43 (0.7)			
		–	–	1.89 (0.5)	1.35 (0.6)	0.96 (0.5)	–
	5~95			2.48 (0.4)	1.73 (0.5)	1.21 (0.4)	
		–	–	–	2.20 (0.2)	1.48 (0.0)	1.08 (0.0)
	1~99				2.80 (0.5)	1.86 (0.0)	1.33 (1.5)
Tebuconazole	10~90	8.39 (1.3)	5.51 (0.3)	3.77 (0.5)	–	–	–
	5~95	–	–	6.56 (0.2)	4.50 (0.3)	3.02 (1.0)	–
	1~99	–	–	–	7.29 (0.9)	4.70 (0.4)	3.27 (0.6)
Difenoconazole	10~90	17.51 (1.8)	11.01 (0.2)	7.34 (0.7)	–	–	–
	5~95	–	–	13.45 (0.6)	8.80 (0.5)	5.91 (1.0)	–
	1~99	–	–	–	14.85 (0.9)	9.13 (0.4)	6.29 (0.4)

* R.S.D. % was based on triplicate injection

– Not studied

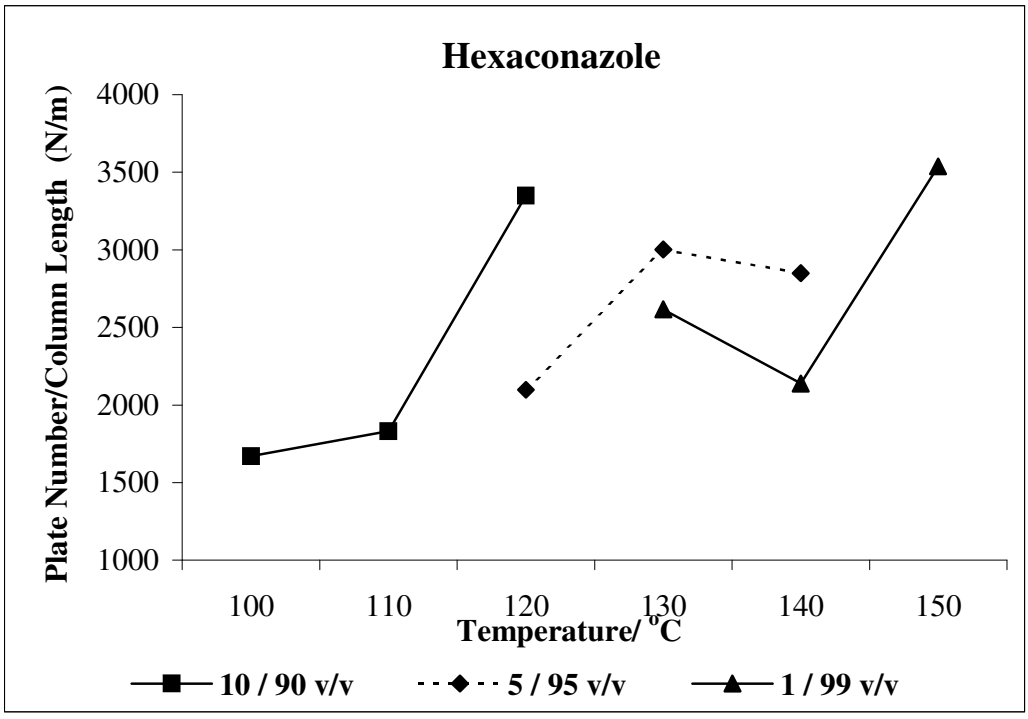


Figure 4 : Variation of column efficiency as a function of temperature using low proportion of organic modifier on carbon-clad zirconia column. (Solute: Hexaconazole)

However, the results showed that the optimum column efficiency for each mobile phase composition examined were located at different temperature range. According to Smith [29], very narrow peaks are also more prone to errors in the measurement of the peak width. Hence, small differences in efficiencies should not be regarded as significant. Besides, the overall column efficiency values for hexaconazole on carbon-clad zirconia column were not satisfactory. In this study, it was observed that the performance of the carbon phase column after heated at high temperature using water-rich eluent for a few days usually showed a significant decrease in column efficiency. The column required reconditioning by using 100 % THF to recover the column efficiency. Thus, it is strongly recommended that for prolonged operations, the carbon phase column should be used only with mobile phase containing at least 5-10 % of organic modifier. However, low efficiency on hexaconazole could be affected more significantly by extra-column band-broadening factors due to the rapid elution process on the carbon phase column. Carr and Li [30] reported that low column efficiency at high temperature could probably be due to the longitudinal molecular diffusion that is more pronounced at high temperatures. However, by using high linear velocity region, the longitudinal molecular diffusion is no longer significant.

Influence of temperature on separation mechanism

The influence of temperature on column separation mechanism can be calculated from retention data by evaluation of the Van't Hoff plots. As a theoretical basis for the Van't Hof plots the retention factor is expressed in terms of standard enthalpies and entropies of transfer from mobile to stationary phase. The relation between the logarithm of the retention factor (ln k) and enthalpies and entropies equals [31]:

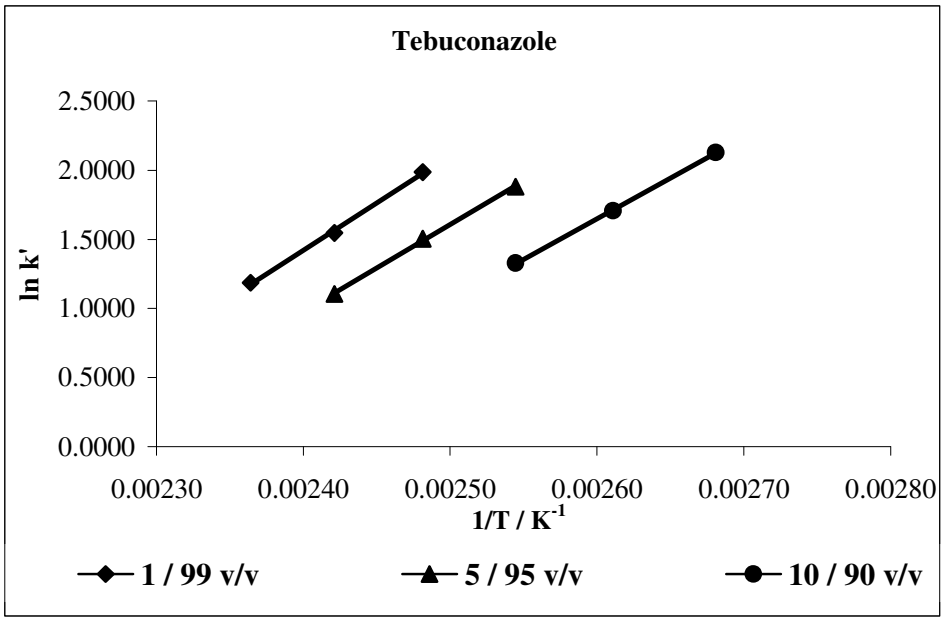
$$\ln k = -\Delta H^o/RT + \Delta S^o/R + \ln \phi \qquad (1)$$

where k is the measured retention value, ΔH^o the enthalpy, ΔS^o the entropy, T the absolute temperature, R the gas constant and ϕ the phase ratio of the column. ΔH^o and ΔS^o are the standard enthalpy and standard entropy of transfer of a solute from the mobile phase to the stationary phase.

From the Van't Hoff plots for the tebuconazole and hexaconazole illustrated in Figure 5, it was obvious that there was no significant deviation from linearity. A rectilinear plot indicates that the same separation mechanism prevails across the entire temperature range of interest [32]. In all cases ΔH^o values were negative under the experimental conditions, demonstrating that retention of compounds studied is an exothermic process (Table 3). It is energetically more favourable for the compounds to remain in the stationary phase than in the mobile phase. As expected, the value of ΔH^o became more negative with decreasing acetonitrile content in the eluent. This showed that a strong retention interaction between mobile and stationary phase occurred when the percentage of organic modifier in eluent decreased. Recently, Dasgupta and Kephart [32] reported that rectilinear Van't Hoff plots were obtained for all solutes on both polybutadiene and carbon-coated zirconia column over temperature range studied. They described that the nature of carbon phase remains unaltered throughout the temperature range studied.

Table 3 : Enthalpy data for the hexaconazole and tebuconazole at high column temperatures on Carbon-clad zirconia column.

Compounds	Temperature range: 100 °C to 150 °C					
	Enthapy ΔH^o(kJ/mol)			Correlation, r		
	Mobile phase composition: MeCN~water			Mobile phase composition: MeCN~water		
	10~90	5~95	1~99	10~90	5~95	1~99
Hexaconazole	–42.26	–45.84	–49.15	0.9993	0.9998	0.9984
Tebuconazole	–48.76	–52.32	–56.85	0.9998	0.9991	0.9983



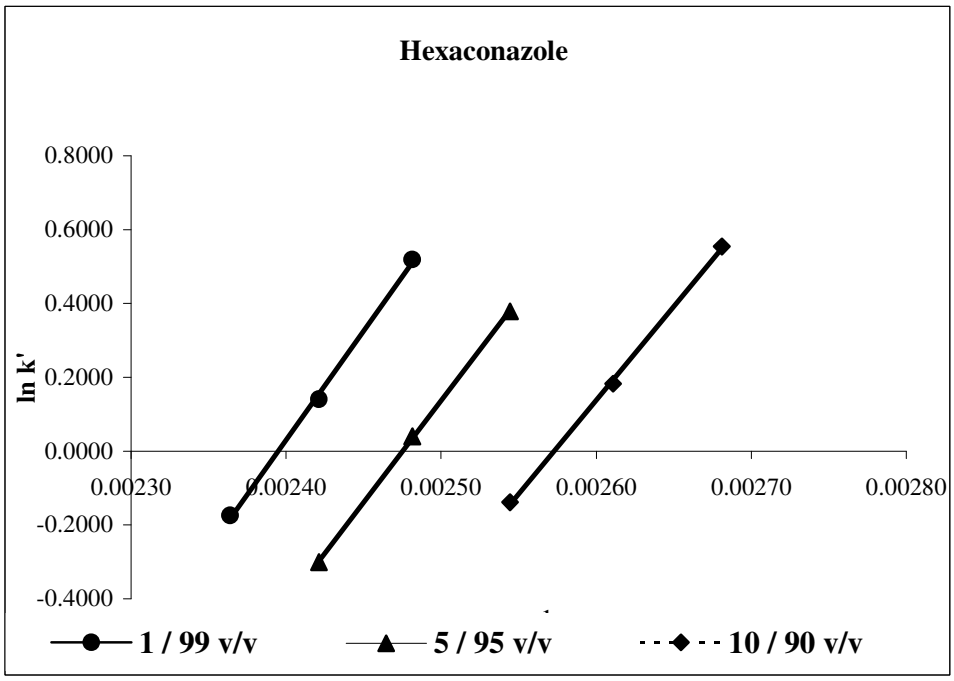


Figure 5: Van't Hoff plots for tebuconazole (above) and hexaconazole (below) using different proportions of acetonitrile in the eluent at high column temperature on Carbon-clad zirconia column.

Acknowledgement

The authors would like to thank Universiti Teknologi Malaysia and the Ministry of Science, Technology and Innovation, Malaysia, for financial support through the IRPA programme Vote Number 74091 and a studentship for H. H. See.

References:

1. Djordjevic, N. M., Fowler, P. W. J., and Houdiere, F. (1999) *J. Micro. Sep.*, **11**, 403-413.
2. Carr, P. W. and Thompson, J. D. (2002) *Anal. Chem.,* **74**, 1017-1023.
3. Dasgupta, P. K. and Kephart, T. S. (2000) *Analytica Chimica Acta,* **414**, 71-78.

4. Lee D. P. (1982) *J. Chromatogr. Sci.,* **20**, 203-208.
5. Sanagi, M. M. and See, H. H. (2003) Proceedings of the International Conference on Advancement In Science and Technology (iCAST), Kuala Lumpur, Malaysia, **105-107**.
6. See H. H., Sanagi M. M., Naim A. A., and Hussain A. (2003) in "Analytical Chemistry: Application and Current Issues" Z.B Assim, F.B. Ahmad, I.B. Jusoh, S. Lau, and M. Mohamed (Eds.), Monograph of the 16th Malaysian National Symposium on Analytical Chemistry, Sarawak, Malaysia, **31-37**.
7. McNeff C., Zigan L., Johnson K., Carr P. W., Wang A. and Weber-Main A. M. (2000) *LCGC*, **18 (5)**, 514-529.
8. Carr P. W., Hu Y., and Li J. (1997) *Anal. Chem.*, **69**, 3884-3888.
9. Sun J. and Carr P. W. (1995) *Anal. Chem.*, **67**, 3717-3721.
10. Rigney M. P., Funkenbush E. F. and Carr P. W. (1990) *J. Chromatogr*, **499**,291-304.
11. Jackson P. T. and Carr P. W. (2002) *J. Chromatogr. A.,* **958**, 121-123.
12. Weber T. P. and Carr P. W. (1990) *Anal. Chem.,* **62**, 2620-2625.
13. Jackson P. T., Kim T. Y., and Carr P. W. (1997) *Anal. Chem.,* **69**, 5011-5017.
14. Stehmann C. and De Waard M. A. (1996) *Crop Protection*, **15**, 39-47.
15. Whitehead R. (1995) "The UK Pesticide Guide" CAB International & BCPC.
16. Wu Y. S., Lee H. K. and Li S. F. Y. (2001) *J. Chromatogr. A.,* **912**, 171-179.
17. Paoli M. D., Tacchedo M., Damiato V., Fabbro D., and Bruno R. (1997) *J. Chromatogr. A., **765***, 127-131.
18. Columé A., Cardenas S., Gallego M. and Valcarcel M. (2000) *J. Chromatogr. A.,* **882**, 193-203.
19. Blasco C., Pico Y., Manes J. and Font G. (2002) *J. Chromatogr. A.,* **947**, 227-235.
20. Blasco C., Pico Y., and Font G. (2002) *J. AOAC Int.,* **3**, 704-710.
21. Rodriguez R., Pico Y., Font G. and Manes J. (2001) *J. Chromatogr. A., **879***, 253-254.
22. Rodriguez R., Manes J. and Pico Y. (2003) *Anal. Chem. **75***, 452-459.
23. Jackson P. T. and Carr P. W. (1998) *CHEMTECH*, **28**, 29.
24. Jackson P. T., Schure M. R. and Carr P. W. (1997) *Anal. Chem.,* **69**, 416.
25. Li J. and Carr P. W. (1996) *Anal. Chem.,* **68**, 2857-2868.
26. Sander L. C., Pursch M. and Wise S. A. (1999) *Anal. Chem.,* **71**, 4821.
27. Bowermaster J. and McNair H. (1983) *J. Chromatogr.*, **279**, 431.
28. Chen M., and Horvath C. (1997) *J. Chromatogr. A.*, **788**, 51.
29. Smith R. M. (1988) "Gas and liquid chromatography in analytical chemistry" *John Wiley & Sons, Chichester*
30. Carr P. W. and Li J. (1997) *Anal. Chem.*, **69**, 2202-2206.
31. Vervoort R. J. M., Ruyter E., Debets A. J. J., Claessens H. A., Cramers C. A. and Jong G. J. (2002) *J. Chromatogr. A.*, **964**, 67-76.
32. Dasgupta P. K. and Kephart T. S. (2000) *Talanta*, **56**, 977-987.