Retention Studies in HPLC

by

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A Master Thesis

Submitted in partial fulfilment of the requirements for the award of

Master of Philosophy

of the Loughborough University of Technology

December 1992

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Acknowledgments

I would like to thank my supervisor Dr. R. M. Smith for all his help and advice throughout the course of this study.

Thanks are due to my friends, colleagues and technical staff for their assistance.

I would also like to thank my husband Yi-Fan for his encouragement and help.

Finally, I wish to thank Polymer Laboratories for their gift of a column and Phase Separation for stationary phase.

Abstract

The effect of increasing temperature on retention time, efficiency and peak symmetry on a PS-DVB column was investigated using two sets of homologous compounds, phenylalkanols (benzyl alcohol to 5-phenylpentanol) and alkyl aryl ketones (benzaldehyde to heptanophenone) as standard compounds. It was found that efficiency and peak symmetry can be improved by an increase of temperature in both RP-HPLC and NP-HPLC. In NP-HPLC, the use of a low level of polar organic modifier can reduce retention times and give improved peak shapes and column efficiencies. A different elution order was obtained in RP-HPLC compared with NP-HPLC.

The study was extended to retention prediction in RP-HPLC. Retention indices can be calculated from the molecular structure of a compound as the sum of the parent index, substituent indices, and interaction indices. In this study the substituent contributions have been determined for 7 aromatic functional groups and 8 aliphatic functional groups over a range of methanol, acetonitrile and THF buffer eluents based on the alkyl aryl ketone retention index scale. The effect of intramolecular hydrogen-bonding in THF has been studied. A general prediction model was used for the retention prediction of disubstituted compounds. It was found that the use of the Hammett constant (σ) had its limitations and was not suitable if THF was the eluent.

Contents

Chapter 1. Fundamental Relationships in Chromatography

1.1 Introduction to HPLC

The initial aim of this study was to investigate the effect of temperature on efficiency and related features of polystyrene-divinylbenzene (PS-DVB) columns in reversed and normal phase high performance liquid chromatography (RP- and NP-HPLC). The study was extended later to the retention prediction in RP-HPLC. In this Chapter the basic principles used in these studies will be described.

Since chromatography was reported by Tswett in 1903 (1), chromatography as a useful technique of analytical chemistry has been rapidly developed. There are many forms of chromatography, including gas chromatography (GC), thin-layer chromatography (TLC), ion-exchange chromatography (IEC), size exclusion chromatography (SEC), high performance liquid chromatography (HPLC) and more recently supercritical fluid chromatography (SFC). Among them, HPLC is one of the most powerful of all the chromatographic techniques. It can often easily achieve separations of analytes that would be difficult or impossible by other forms of chromatography.

There are two phases in HPLC, one is the mobile phase which can be an aqueous organic mixture, a buffer solution or a mixture of organic solvents, depending on the chromatographic method and on the detector that is used, the other is the stationary phase. When a mixture to be separated is introduced at the top of the column, it will be carried through the column by the mobile phase. If a component of the mixture is adsorbed weakly onto the surface of the stationary phase, it will travel down the column faster than other solutes that are more strongly adsorbed. The amounts of time that molecules spend in the column are very dependent on the experimental conditions, such as the nature of the stationary phase, composition of the mobile phase, flow rate of the mobile phase, and the temperature as well as the relative proportions of the stationary and the mobile phase (2).

1.2 Concepts

To estimate whether a separation is good and whether a column is performing well, a number of parameters are usually used as standard measurements. In this section some basic concepts, which were used in this study will be discussed.

1.2.1 Retention Time

The retention (t_R) time of a solute is the time between the instant of sample introduction and when the detector senses the maximum concentration of the retained solute. When solutes pass through the column, the molecules of the solute spend part of the time in the mobile phase and part in the stationary phase. The time that molecules spend in the mobile phase is called the column dead time or void volume (t_O), which is the same for all molecules and represents the time for the mobile phase to pass through the column. The time that molecules spend in the stationary phase is called the adjusted retention time (t_R'). The relationship between dead time (t_O), retention time (t_R) and adjusted retention time (t_R') is given by Figure 1.2.1 and the equation 1.1.

$$t_R = t_R' + t_O. \qquad (1.1)$$

The retention time of the solute is an important parameter for reporting the separation of mixtures, but the reproducibility of the retention time of the same analyte determined in different laboratories or in the same laboratory but on different instruments can be very poor (2). If the methods in the literature are repeated the retention time often differs from the published values. One cause of these difficulties is that the retention time in HPLC is very sensitive and can be

altered by even small changes in the stationary or mobile phase or in the operating conditions (2).

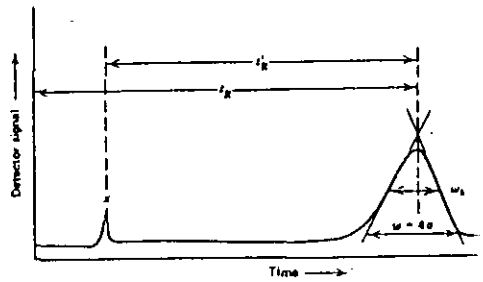

Figure 1.2.1 the relationship between retention time, adjusted retention time and dead time

1.2.2 Capacity Factor

There is another conventional way to measure the retention, which is the capacity factor (k') calculated as the adjusted retention time of the analyte (t_R') divided by the retention time of an unretained compound (t_O), (equation 1.2)

$$k' = t_R'/t_O = (t_R - t_O)/t_O. \qquad (1.2)$$

In theory, the capacity factor should compensate for changes in the eluent flow rate and column dimensions. However, as the value of void volume (t_O) is very small, even minor errors in its measurement can cause a large variation in the capacity factors. In practice, so far there is not an accurate and standard method to obtain very reproducible void volumes in HPLC. The methods that has been reported include the extrapolation of the homologous series to the zero carbon homologue (3,4), using unretained compounds such as acetone (5), uracil (6,7), sodium nitrate (8) and D_2O (9,10), or by the system peak (11). Bidlingmeyer and co-workers found that the use of unretained compounds as void volume markers gave a simpler, rapid and

more reliable procedure rather than extrapolation of the retention times of a series of homologues (12).

1.2.3 Retention Index

Because of the problems with the reproducibility of the retention time and the capacity factor, it is desirable to have another parameter which is more robust than the retention time or the capacity factor to small differences in the experimental conditions. A retention index based on the relative retention of an analyte to a series of standards covering a range of polarities could avoid many of the problems, and could be used to compare the results from the different instruments in the same laboratory or even between laboratories.

In gas-liquid chromatography, the Kovats' retention index value (13) of an analyte based on the retention times of n-alkanes has been widely accepted as a reference scale. It can be expressed as the following equation 1.3.

$$\text{Retention Index} = 100n + 100(\log t_R' - \log t_{Rn}')/(\log t_{Rn+1}' - \log t_{Rn}') \qquad (1.3)$$

where :

t_R' = adjusted retention time of the analyte of interest,

t_{Rn}' = adjusted retention time of the normal alkane with carbon number n emerging before the analyte of interest,

t_{Rn+1}' = adjusted retention time of the normal alkane with carbon number n+1 emerging after the analyte of interest.

As defined in equation (1.3), the retention indices of the n-alkanes are 100 times their carbon number. However the n-alkane scale can not be adopted directly for LC because of the low polarity and thus limited retention range of the alkanes. They also lack a significant chromophere and would be inconvenient or impossible to

detect on most instruments using the widely employed UV detector (14).

It has been recognised in RP-HPLC that there is also a linear relationship (15,16,17) between logarithms of the capacity factors of the members of a homologous series and their carbon number, i.e.

$$\log k' = A \times n_c + B \qquad (1.4)$$

This linear relationship has been verified to be valid for a wide range of homologous series in HPLC. In a review, Smith (18) described many homologous series which has been suggested as suitable retention index scale standards, including n-alkanes, n-alkylbenzenes, alkan-2-ones, alkyl aryl ketones, esters and polycyclic hydrocarbons. Of these the alkan-2-one scale proposed by Baker (19) and the alkyl aryl ketone scale proposed by Smith (14) have been most widely used.

1.2.4 Efficiency

Efficiency is measure of the performance of a column. It can be defined as follows

$$N = 16(t_R/w_b)^2 = 5.54(t_R/w_h)^2, \qquad (1.5)$$

where:

w_b = peak width at the base line,

w_h = peak width at half height of peak.

Column efficiency can also be expressed as either the number of theoretical plates (N), or the height equivalent to a theoretical plate (HETP), which is the column length (L) divided the column plate count

$$H = L/N \qquad (1.6)$$

A good general description of the relationship between the linear flow velocity (u) and the column efficiency expressed as the plate height is given by the Van Deemter equation (20)

$$H = A + B/u + Cu, \qquad (1.7)$$

where A is the eddy diffusion term, B/u is related to longitudinal diffusion term, and Cu is the mass transfer term. From equation (1.7), it can be determined that the highest column efficiency will be obtained at $u = (B/C)^{1/2}$. It can be seen that at low velocity the main contribution to the plate height is from the longitudinal diffusion term, and at high velocity the mass transfer term dominates the equation.

1.2.5 Peak Symmetry

The ideal shape of each of the peaks in a chromatogram is symmetrical and approximately the normal curve or Gaussian shape, but this is not always achieved in practice. Peak asymmetry can be characterised by the equation

$$\text{Peak Asymmetry} = b/a \qquad (1.8)$$

Where a and b are measured at 10% of the peak height as shown in Figure (1.2.2).

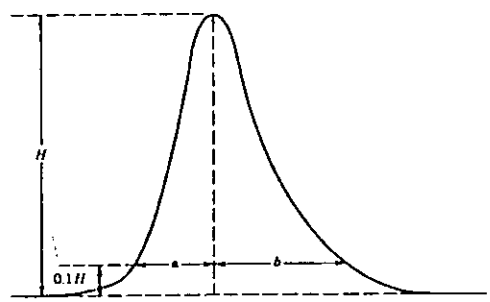

Figure 1.2.2 the measurement of peak asymmetry

In theory, the Gaussian shape is closely approached if the analyte has undergone a sufficiently large number of sorptions and desorptions, as in the case for most peaks with partition ratios of about 1 or greater. In fact, an asymmetrical peak is usually evidence that some undesirable interaction is taking place in the system (21).

1.2.6 Enthalpy - Entropy

Linear relationship is found between the logarithm of the capacity factors measured at an appropriate reference temperature and the corresponding enthalpies for the particular chromatographic process. It is due to enthalpy - entropy compensation, which is manifests itself in a linear dependence of the overall free energy changes on the corresponding enthalpy change for intrinsically similar physical-chemical phenomena (22).

Enthalpy - entropy compensation is conveniently expressed by the relationship

$$\Delta H^{o} = \beta \Delta S^{o} + \Delta G^{o} \quad \text{or} \quad \Delta G^{o} = \Delta H^{o} - \beta \Delta S^{o}, \qquad (1.9)$$

where ΔH^{o} is the Gibbs free energy of a physical-chemical interaction at compensation temperature β, and ΔH^{o} and ΔS^{o} are the corresponding standard enthalpy and entropy respectively. The equation implies that in the vicinity of β changes in ΔH^{o} are offset by changes in ΔS^{o}, so that the free-energy change is practically independent of temperature. When enthalpy-entropy compensation is observed with a family of compounds in a particular chemical transformation the the values of β and ΔG^{o} are invariant (22).

The free energy change for the process is expressed by

$$\Delta G^{o} = -RT\ln K = -RT\ln (k'/\phi) \qquad (1.10)$$

where R is the gas constant, K is related to the thermodynamic equilibrium constant. The capacity factor k' is equal to the phase ratio of the column ϕ times K. Substituting equation (1.10) into (1.9), a relationship can be derived which is called Van't Hoff plot, that is,

$$\ln k' = - \Delta H^{o}/RT + \Delta S^{o}/R + \ln \phi. \qquad (1.11)$$

If the mechanism for the retention process does not change over the temperature range the Van't Hoff plot yields a straight line for ln k' against 1/T and the enthalpy of the reaction is constant (23).

Chapter 2. Introduction to Polystyrene-divinylbenzene Columns

2.1 Development of polystyrene-divinylbenzene columns

The first modern liquid chromatography that utilised polymeric packing materials was the amino acid analyser introduced by Moore and Stein in 1954 (24). They used a glass column containing irregular particles of less than 74 μm diameter of sulphonated polystyrene cross-linked with 4% divinylbenzene. They obtained better resolution than using polystyrene with a higher percentages of cross-linking. In the early 1960's the first spherical polymeric microparticles were introduced. However, most of these early polymers were not rigid and because of a low degree of cross-linking were compressed under the high eluent flow velocities normally employed with LC equipment.

Moore (25) developed a second type of gel structure, which was a macroporous gel. In this gel, the monomers are polymerised in the presence of a diluent that is a solvent for the monomer but not for the polymer. It became possible for a higher degree of cross-linking to be present.

The PS-DVB packings were developed by Hollis (26) as solid absorbents for gas chromatography. It was reported that they could also be used for liquid chromatography (27). Martinu *et al* . (28) used the Porapak packings as absorbents in both thin-layer and column liquid chromatography for the separation of aromatic hydrocarbons.

After Rohm and Haas marketed a series of absorbent resins, the Amberlite XAD series (29), polystyrene-divinylbenzene (PS-DVB) packing materials were researched as ion exchange resins (30) and for partition chromatography (31).

In the late 1960's, with the development of HPLC, Horvath (32) introduced fast liquid chromatography using pellicular column packings which were prepared by coating glass beads with PS-DVB. Kirkland developed a controlled surface porosity support to go with their similar liquid chromatography system (33,34).

Collet *et al* . (35) looked at six polyamides, coated on non-porous spherical glass pellicular beads, and found that the capacity factor could be controlled by varying packing loading and the number of functional groups on the polymer. Fritz and Willis (36) used high speed liquid chromatography with gas pressure to separated mixtures of phenols on macroporous polymethacrylate resin XAD7 which had been sieved through a 325 mesh sieve. Chu and Pietrzyk (37) reported that crushed and sieved XAD2 (75 to 100 μm) could be used as a support for the HPLC separation of organic bases.

Hanson and Sievers (38) found that bonded phases could be produced by the use of an excess of polymer or by the introduction of functional groups through monomers that also contained an active hydrogen.

The early polystyrene based materials were more or less sensitive to solvent changes. However, modern reversed phase columns filled with more rigid porous polystyrene beads have become available. The packing materials seem to be less sensitive to swelling and shrinking than the previously available polystyrenes (39) because of the improvement in the rigidity of porous polystyrene packing materials. There are a range of pore sizes of modern commercially available PS-DVB columns. These rigid copolymer matrices can be utilised without derivatization for reversed phase chromatography (40).

2.2 The characterisation of PS-DVB

The stability of chromatographic columns is very often a problem. It can cause a reduction of efficiency and less of resolution. The reproducibility of retention times may also be affected. For silica gel based packing materials, the instability can arise from the solubility of the silica gel skeleton in aqueous eluents (39). Polymer based packing materials do not suffer from this disadvantage. Unlike the conventional alkyl functionalized silica gels, PS-DVB columns are operable over wider pH range, typically pH(1-13), without the use of guard columns or damage to the packing (41).

It was reported that the PS-DVB columns had a much higher retentive power than Spherisorb-ODS and it was therefore necessary to use a high proportion of organic modifier to give similar retention times (42).

Lee and co-workers (41) described the changes of retention times with pH on a spherical 10μm porous polymeric adsorbent PRP-1 columns. In general, at pH values from 2 to 4, nucleosides and bases are in a positively charged state, due to pK_as less than 4 and their retention times are relatively low. As the pH is increased the positive charge is lost due to deprotonation and the retention times of the neutral species increase to a maximum at around pH 7. Retention times then decrease as the pH is raised further, and the solutes become negatively charged due to pK_a values in the region 8-13.

It has been reported (43,44) that polymeric packings tend to swell significantly in tetrahydrofuran (THF) depending on the methods used to produce the material. The macroporous polymers are synthesised using a "porogen" which solubilizes the monomer but is not soluble in the polymer. The resulting material contains both macroporosity, due to pores between the microspheres and their agglomerates, and microporosity, due to pores within the microspheres (44). The microporosity may be inaccessible to solvent or solute. It is also quite reasonable to presume that the transition from microsphere to pore structure is not discontinuous, but rather exists as a continuum from heavily crosslinked polymer chains to non-crosslinked polymer chains (45).

Bowers and co-workers (46) have reported that peak asymmetry is probably the main limitation of polystyrene-divinylbenzene packing material for reversed phase chromatography, because the region of less heavily crosslinked chains could engage in the multiple interactions with solute molecules that possess π electrons, resulting in the observed tailing. Further, they (47) found a solute with a large π^* (dipolarity or polarizability) is relatively more retained on a polymeric phase than a solute with a low π^*. THF can be adsorbed into a polystyrene resin to a greater extent than

acetonitrile. Methanol and water are also adsorbed on the aromatic resin, but to a lesser extent than either THF or acetonitrile. THF will be the strongest organic co-solvent since the overall hydrophobicity of the solutes will be the major factor in the separation.

Nevejans and Verzele (48) have also reported a swelling of the microporous structure of PRP-1 with THF on the basis of a comparison of surface area and pore volumes measured using two techniques. The differences in chromatographic behaviour observed are presumably the result of specific interactions between the solutes and the stationary phase.

Coppi and co-workers (49) observed the chromatographic behaviour of Chromosorb 101 and pointed out that the mechanism of solute retention is mainly due to non-polar interactions between the solute molecules and the stationary phase. The behaviour of these styrenes copolymers resembles that of a C_{18} silica bonded phase. It was reported that no noticeable peak tailing was obtained because of its hydrophobic surface and non-polar interactions.

Stuurman *et al* . (50) used infrared spectroscopy and chromatographic techniques to study the properties of a number of PS-DVB copolymers, and reported that the column materials were spectroscopically very similar. Their surface are chemically neither homogeneous nor stable during use. The main chromatographic differences between polystyrene and silica gel packings is that the microporosity in polystyrene phases is not constant and can change with the nature of the eluent and the sample (51). Early polystyrene phases with a low degree of cross-linking will collapse in an eluent with low affinity for polystyrene and are less microporous in these eluents than modern polystyrene packings, which are often heavily cross-linked. However, there are no convenient ways to produce a polystyrene phase with both high mechanical strength and a very dense structure without micropores. This is due to the size of the monomeric divinylbenzene units themselves (51).

2.3 The application of PS-DVB column

Because of the shortcomings of ODS columns, such as instability at high pH value and the presence of residual silanol groups, PS-DVB columns are being used more frequently in modern liquid chromatography.

An extensive range of separations have been carried out on PS-DVB column, which include organic acids (29,52), organic bases (37,53), carboxylic acids (54), aromatic acids (55), weak mono and di-protic organic acids and ampholytes (56,57), amino acids, peptides and derivatives (58), wine acids (59), benzenesulphonic acids (60), alkylbenzenesulphonates (61), benzenesulphonates (62), nucleosides and corresponding bases (41), pyrrolizidone alkaloids (63), phenols (64), polyphenols (65), chlorophenols (59,66,67,68), nitrophenols (67), chlorophenoxyacetic acids (68), anilines (64), anti-epileptic drugs (60), sulpha drugs (60,69), tetracyclines (70,71), alkyl benzyl dimethylammonium chloride and alkylpyridium halides (72), aromatic hydrocarbons (73,74,75,76), organic anions (77), giberellins (78), trace concentration of air pollutants (79,80,81), protein (82), penicillins (83) and ammonium (84).

Stuurman *et al.* (50) reported that retained non-polar solutes should be eluted with a mobile phase containing THF, uncharged acid and anions can be chromatographed without difficulty. Cations of amines should be chromatographed only with a mobile phase of low pH and uncharged amino-alcohols can not be chromatographed without the addition of a competitor.

The linear relationship between log k' and the mobile phase composition has been verified by Coppi and co-workers (85) on a PS-DVB column. The selectivity could be enhanced by varying the pH of the mobile phase.

Smith *et al* . (86) investigated the retention of the members of a homologous series on a PS-DVB column using different eluent systems. In general the linear relationship between the logarithm of capacity factors and carbon numbers of members of the series was verified, but the earlier members of each series gave abnormal behaviour, possibly because of the effect of deviation of the void volume. The peak shapes and column efficiencies were improved with the change of the

organic component of the eluent from methanol to acetonitrile or THF. The ternary eluents, for example, adding 1 - 5% THF and 5% acetonitrile to methanol-water (90:10) were used, in each case the efficiency improved marginally and longer retentions still caused a decrease in efficiency. Other researchers (87,88,89,90) have also investigated the relationship between logarithm of capacity factor and the carbon number of homologous on the PS-DVB column.

2.4 Introduction to current work

The studies of polymer columns, especially PS-DVB column, have been carried out by a number of researchers. It has also been examined in this laboratory by Smith *et al.* (86). However, only a few studies have been published on the effects of operational parameters on the efficiency of PS-DVB columns and separations at different temperatures and flow rates of the mobile phase have not been discussed in detail. The aim of this study was to investigate the effect of temperature on efficiency and related features of PS-DVB packing material in both reversed phase and normal phase HPLC. The effect of the flow rate of the mobile phase will be investigated as well.

Chapter 3. Experimental Work on PS-DVB Column

3.1 Chemicals

Mobile phase:

Methanol, acetonitrile, hexane and iso-propanol were HPLC grade (FSA Laboratory Supplies, Loughborough, U.K.).

Standard samples:

Standard compounds were reagent grade. Benzyl alcohol, 3-phenylpropanol, 5-phenylpentanol, hexanophenone and heptanophenone from Aldrich Chemical Company Ltd. (U.K.), acetophenone from BDH Chemicals Ltd. (Poole, U.K.), propiophenone from Hopkin & Williams Ltd. (Essex, U.K.), butyrophenone and valerophenone from Koch-Light laboratories Ltd. (Colnbrook Bucks, U.K.). Sodium nitrate AR grade and iso-octane AR grade were from various sources.

3.2 HPLC equipment

HPLC separations were carried out using a Philips 4015 pump and Philips 4025 UV variable-wavelength detector set at 254 nm. The samples (10 µl) were injected using a 7125 Rheodyne valve fitted with a 20 µl loop. All samples were separated on a PLRP-S (PS-DVB) 5 µm column (150x4.6) from Polymer Labs (U.K.) which was encased in a circulating water jacket connected to a thermostatically controlled water bath. A heater or a cooling unit was used to set the different temperatures. Retention times were recorded using a Hewlett-Packard 3390 integrator. A SE 120 chart recorder was used to measure peak shapes.

3.3 Experimental procedure

3.3.1 Solution Preparation

All solutes were prepared by dissolving sufficient compound to enable detection using the UV detector set at 0.04 aufs. For alkyl aryl ketones (AAK), a solution of six compounds was prepared, from acetophenone to hexanophenone,

3.3.2 Void Volume Solution

Void volume marker solution was prepared by dissolving 0.06g sodium nitrate in 10ml water for RP-HPLC. Pure iso-octane was used as void volume solution in NP-HPLC.

3.3.3 Experimental Method

The column was allowed to equilibrate with mobile phase for at least 1 hour at 1 ml/min flow rate before use. Flow rate was altered between 0.1 to 1.5 ml/min depending on the organic solvents and their composition.

10 µl AAK mixture solution was injected followed by benzaldehyde, phenylalkanols (benzyl alcohol to 5-phenylpentanol) and the void volume marker. This sequence was repeated three times for each different mobile phase. The same column was used through the experiments.

Temperature was varied from 10 ^{o}C to 80 ^{o}C.

3.4 Data calculations

Each solute was injected three times under the same condition. The retention times were calculated based on equation (1.1) as the mean value of the three

retention times measured for a solute and this value was used for all subsequent calculations. The capacity factors were calculated according to the equation (1.2). The efficiency and peak asymmetry of solutes were the average value of three measurements based on equation (1.5) and equation (1.8).

Chapter 4. Results and Discussion for PS-DVB Column

PS-DVB as a reversed phase column material in HPLC has the advantages of wide pH range and stability, compared with ODS silica (59). However, it has been observed that it often gives severe peak tailing and low efficiency especially for longer retained compounds with methanol as eluent. These problem are caused by diffusion of solutes in and out of micropores in the stationary phase (51). It was believed that one way to improve efficiency and peak symmetry would be to increase the temperature (51) and that particular benefit could be obtained when methanol was the eluent. This is because that methanol causes a poor affinity for the stationary phase which cause the shrinking of PS-DVB material, reducing particle size and producing the lower diffusion rate (87). Increasing temperature also reduces capacity factors and improves peak shapes, and then increases the efficiencies. Another way in controlling chromatography performance in RP-HPLC is to change the composition of organic modifier of the eluent. It has been reported that an increase of 3.75°C amounts to a 1% methanol increase (88).

4.1 The effect of temperature in RP-HPLC

In this work two homologous series of compounds, alkyl aryl ketones (AAK, acetophenone to heptanophenone) plus benzaldehyde and phenylalkanols (benzyl alcohol to 5-phenylpentanol) were used as standard samples and sodium nitrate solution was used as a void volume marker. Methanol-water (90:10) and acetonitrile-water (70:30) were used as the mobile phase respectively. The capacity factors, efficiencies and peak symmetry of the two homologous series of compounds at various temperatures are listed in Tables 4.1.1 to 4.1.4.

From the experimental data, the retention increased with increasing the chain length of the homologues, but efficiency and peak symmetry became worse.

Table 4.1.1 The effect of temperature for phenylalkanols on PS-DVB column (methanol-water 90:10)

Temperature	Compounds [a]				
T(°C)	1	2	3	4	5
	Capacity factor				
10	0.61	0.91	1.20	1.75	2.66
20	0.60	0.79	1.06	1.49	2.12
30	0.54	0.72	0.91	1.26	1.80 (before)[b]
30	0.51	0.68	0.89	1.25	1.79 (after)[b]
40	0.48	0.65	0.79	1.08	1.50
50	0.43	0.55	0.68	0.92	1.23
60	0.40	0.51	0.62	0.78	1.03
70	0.38	0.46	0.56	0.69	0.89
80	0.34	0.42	0.49	0.61	0.73
	Efficiency				
10	1162	1112	979	936	837
20	1430	1270	1223	1083	960
30	2242	1840	1822	1795	1985 (before)
30	1329	1308	1196	1105	1086 (after)
40	2312	2170	2031	2059	2102
50	1734	1922	2189	2174	2123
60	1871	1887	2161	2103	2194
70	1817	1817	1974	2015	2035
80	1852	1601	1779	2000	1892
	Asymmetry				
10	2.10	2.20	2.18	2.37	2.39
20	1.80	1.81	2.02	2.12	2.39
30	0.72	1.21	1.19	1.27	1.53 (before)
30	1.24	1.81	1.89	2.23	2.40 (after)
40	1.07	1.16	1.14	1.18	1.31
50	0.93	1.04	1.00	1.11	1.19
60	1.14	1.25	1.11	1.25	1.24
70	1.25	1.24	1.28	1.33	1.37
80	1.33	1.32	1.33	1.38	1.43

[a] 1. benzyl alcohol 2. 2-phenylethanol 3. 3-phenylpropanol 4. 4-phenylbutanol 5. 5-phenylpentanol

[b] sequence of temperatures (°C): 30 (before), 40, 50, 60, 70 and 80 then 10, 20 and 30 (after).

Table 4.1.2 The effect of temperature for AAK on PS-DVBcolumn (methanol-water 90:10)

Temperature	Compounds[a]						
T(°C)	1	2	3	4	5	6	7
	Capacity factor						
10	3.83	4.18	7.43	10.59	15.72	25.07	40.38
20	3.02	3.36	5.66	7.93	11.49	17.85	28.18
30	2.45	2.63	4.30	5.80	8.16	12.23	18.42 (before)[b]
30	2.40	2.59	4.29	5.83	8.27	12.55	18.96 (after)[b]
40	1.99	2.16	3.41	4.48	6.17	8.94	13.09
50	1.60	1.70	2.59	3.35	4.50	6.34	9.00
60	1.30	1.39	2.04	2.59	3.40	4.65	6.43
70	1.12	1.17	1.68	2.09	2.69	3.58	4.80
80	0.95	0.99	1.37	1.67	2.10	2.72	3.56
	Efficiency						
10	550	449	312	275	270	264	337
20	715	619	424	371	387	366	384
30	1247	1158	848	711	694	674	615 (before)
30	745	762	607	457	445	455	463 (after)
40	1146	1522	1186	1033	1027	984	916
50	1170	1682	1516	1377	1350	1370	1247
60	1600	2065	1863	1771	1813	1746	1626
70	1931	2321	2096	2029	2070	2052	2001
80	1951	2021	2000	1862	1917	1956	1840
	Asymmetry						
10	2.24	2.29	2.63	2.06	2.44	2.47	2.01
20	2.02	2.04	2.04	2.06	2.40	2.23	2.26
30	0.92	1.76	1.90	1.89	2.06	1.92	2.43 (before)
30	1.68	2.14	2.15	2.13	2.08	1.89	2.43 (after)
40	0.73	1.51	1.69	1.66	1.85	1.79	2.09
50	0.74	1.25	1.36	1.33	1.48	1.51	1.75
60	0.98	1.10	1.31	1.27	1.29	1.39	1.55
70	1.08	1.07	1.28	1.18	1.25	1.27	1.42
80	1.16	1.15	1.27	1.07	1.23	1.27	1.36

[a] 1. benzaldehyde 2. acetophenone 3. propiophenone 4. butyrophenone 5. valerophenone 6. hexanophenone 7. heptanophenone

[b] as Table 4.1.1.

Table 4.1.3 The effect of temperature for phenylalkanols on PS-DVB column (acetonitrile-water 70:30)

Temperature	Compounds[a]				
T(°C)	1	2	3	4	5
	Capacity factor				
10	0.53	0.61	0.74	0.97	1.26
20	0.54	0.60	0.72	0.95	1.26
30	0.42	0.47	0.60	0.79	1.06
55	0.36	0.43	0.53	0.69	0.90
80	0.33	0.38	0.47	0.61	0.77
	Efficiency				
10	1303	1192	1395	1483	1659
20	1303	1149	1263	1408	1585
30	1645	1649	1948	1825	2071
55	1431	1493	1721	2000	1717
80	1583	1371	1508	1795	2062
	Asymmetry				
10	2.15	1.76	2.11	1.94	2.02
20	2.15	1.81	2.01	2.09	2.17
30	1.63	1.33	1.49	1.54	1.51
55	1.67	1.70	1.50	1.53	1.58
80	1.65	1.64	1.93	1.69	1.80

[a] 1. benzyl alcohol 2. 2-phenylethanol 3. 3-phenylpropanol 4. 4-phenylbutanol 5. 5-phenylpentanol

Table 4.1.4 The effect of temperature for AAK on PS-DVB column (acetonitrile-water 70:30)

Temperature	Compounds[a]						
T(°C)	1	2	3	4	5	6	7
	Capacity factor						
10	1.64	1.50	2.57	3.71	5.33	7.92	11.83
20	1.47	1.40	2.02	3.36	4.80	7.04	15.68
30	1.23	1.19	1.98	2.80	3.96	5.74	8.36
55	0.93	0.92	1.45	2.00	2.75	3.82	5.34
80	0.75	0.77	1.15	1.51	2.01	2.70	3.63
	Efficiency						
10	1582	1478	1338	1196	1199	1201	1084
20	1477	1339	1409	1281	1361	1379	1318
30	2242	2228	2163	2123	2062	2235	2014
55	1961	1723	1954	2138	2172	2236	2414
80	2150	2062	2071	2207	2269	2405	2492
	Asymmetry						
10	2.00	1.94	2.07	2.19	2.09	2.09	1.75
20	2.03	2.02	2.19	1.96	2.04	2.03	1.67
30	1.51	1.61	1.89	1.76	1.75	1.73	1.54
55	1.50	1.66	1.58	1.50	1.60	1.51	1.46
80	1.43	1.51	1.56	1.44	1.54	1.35	1.35

[a] 1. benzaldehyde 2. acetophenone 3. propiophenone 4. butyrophenone 5. valerophenone 6. hexanophenone 7. heptanophenone

4.1.1 Relationship between Logarithm of Capacity Factor and Carbon Number in RP-HPLC

It was reported that there is linear relationship in RP-HPLC between log k' and carbon number of homologues (2,14,52,54,62,73,86). The relationships in the present study have been plotted (Figure 4.1.1 to 4.1.4). The correlations are given in Tables 4.1.5 and 4.1.6. Benzaldehyde was not included within AAK because of the difference between its molecular structure and that of AAK. The correlations of AAK were much better than those of phenylalkanols in acetonitrile, but they were similar in methanol. This was probably because the retention times of the phenylalkanols were shorter than those of AAK in methanol and much shorter in acetonitrile. Therefore, the effect of any deviation of the void volume was less for AAK than for phenylalkanols. The relationship between log k' and the carbon number can also be expressed as the following equation (4.1)

$$\log k' = n_C \log \alpha + \log \beta, \qquad (4.1)$$

where k' is the capacity factor, n_C is the number of methylenes in homologous side chain, α is the selectivity and β is the capacity factor of hypothetical zeroth homologue. Therefore the higher the slope, the higher the selectivity. In general, the selectivity in HPLC decreases with increasing temperature (88). This was confirmed by the experimental data in methanol/water (Table 4.1.5), as slopes decreased with increasing temperature for both homologous series. The slopes of AAK in acetonitrile (Table 4.1.6) also decreased with increasing temperature, but not as much as they did in methanol. For phenylalkanols, all the slopes also showed reductions with temperature in methanol. However, they were very similar in acetonitrile. This meant that the selectivity of solutes in acetonitrile did not change very much especially for short retained ones. The elution strength of acetonitrile is greater than methanol. The solute always shows less retention, poorer selectivity

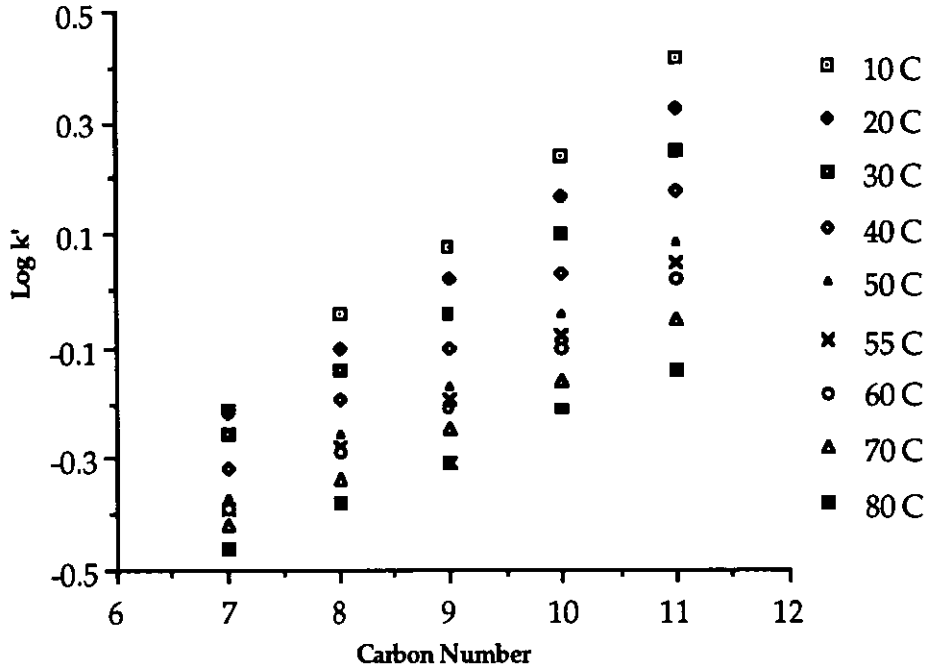

Figure 4.1.1 Log k' against carbon number for phenylalkanols (methanol-water 90:10)

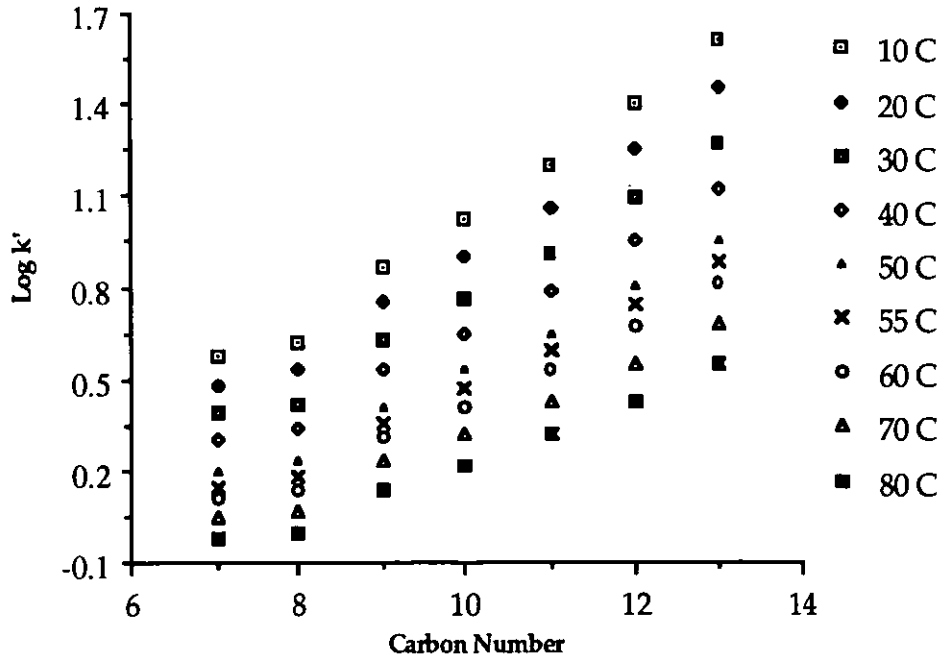

Figure 4.1.2 Log k' against carbon number for AAK (methanol-water 90:10)

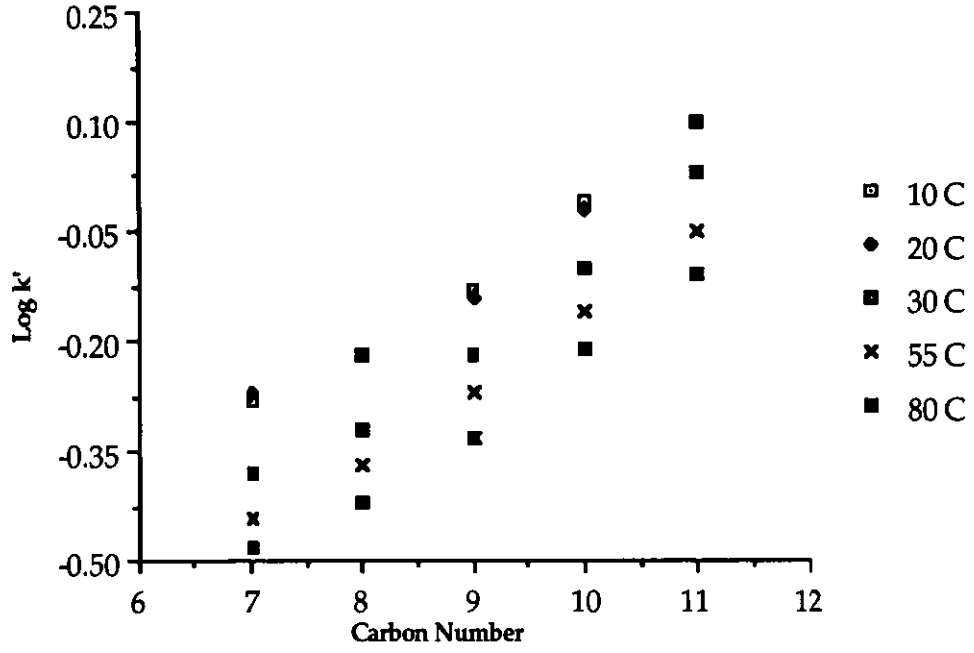

Figure 4.1.3 Log k' against carbon number for phenylalkanols (acetonitrile-water 70:30)

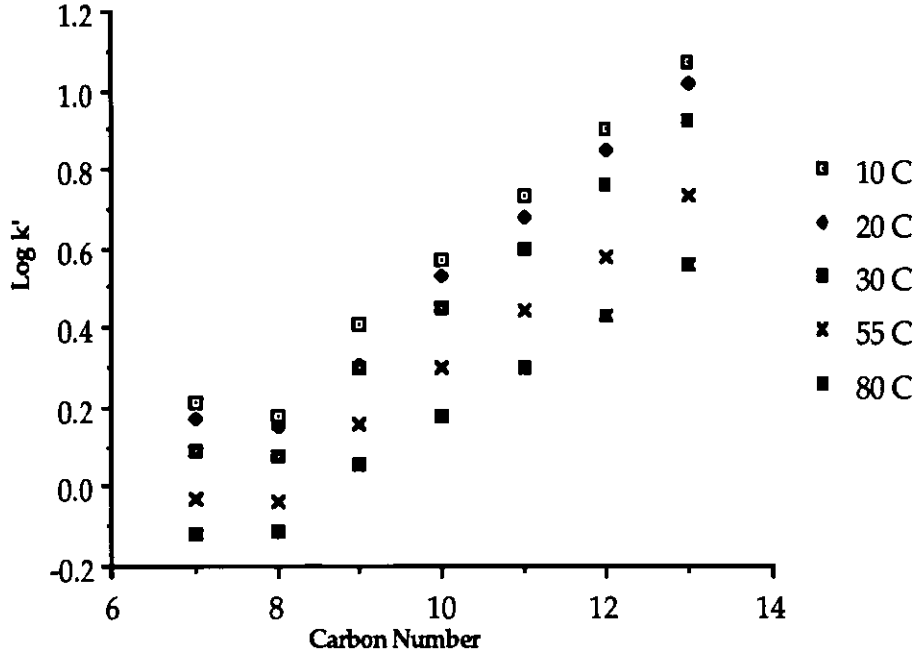

Figure 4.1.4 Log k' against carbon number for AAK (acetonitrile-water 70:30)

Table 4.1.5 The relationship of log k' and carbon number for AAK and phenylalkanols with methanol-water (90:10) at 1 ml/min flow rate on PS-DVB column

Temperature	Phenylalkanols		AAK[a]	
°C	Slope	Correlation	Slope	Correlation
10	0.154	0.996	0.192	0.996
20	0.137	0.995	0.179	0.997
30	0.126	0.994	0.165	0.997
40	0.122	0.994	0.151	0.997
50	0.114	0.995	0.140	0.997
55	0.108	0.996	0.136	0.996
60	0.101	0.995	0.130	0.996
70	0.092	0.997	0.118	0.995
80	0.081	0.997	0.106	0.996

[a] Acetophenone to heptanophenone

Table 4.1.6 The relationship of log k' and carbon number for AAK and phenylalkanols with acetonitrile-water (70:30) at 1 ml/min flow rate on PS-DVB column

Temperature	Phenylalkanols		AAK[a]	
°C	Slope	Correlation	Slope	Correlation
10	0.097	0.985	0.174	0.997
20	0.094	0.973	0.175	0.998
30	0.104	0.983	0.164	0.996
55	0.099	0.993	0.150	0.996
80	0.095	0.987	0.131	0.997

[a] acetophenone to heptanophenone

in acetonitrile than in methanol. Therefore, the change in selectivity with temperature is bigger in methanol than in acetonitrile.

In a paper by Smith and Garside (86), It was reported that the slopes of the correlations should be same for the different series at the same temperature since for the different homologous series the methylene increment should be constant but the slopes of phenylalkanols and alkyl aryl ketones with acetonitrile-water and THF-water were significantly different. Similar marked variations were found for AAK and phenylalkanols homologous series in the present study.

4.1.2 Relationship between Logarithm of Capacity Factor and the Reciprocal of Temperature in RP-HPLC

The relationship between Gibbs free energy, ΔG^o, and logarithm of the capacity factor can be represented as

$$\log k' = -\Delta G^o/RT + \phi, \qquad (4.2)$$

where ϕ is the phase ratio, T is the absolute temperature and R is the gas constant. This equation can be written with the appropriate enthalpy, ΔH^o, and entropy, ΔS^o, as

$$\ln k' = -\Delta H^o/RT + \Delta S^o/R + \phi, \qquad (4.3)$$

where ΔH^o is the standard enthalpy change of solute transfer from the mobile phase to the stationary phase, ΔS^o is the associated change in the standard entropy. The experiments showed that some of the data fitted such a linear relationship of log k' *vs* 1/T well and good correlations were obtained, especially for the case of methanol-water as eluent (Table 4.1.7, and Figures 4.1.5 to 4.1.8).

As reported by Vigh and co-worker (89), the slopes in methanol-water (Table

Table 4.1.7 The relationship between log k' and 1/T for phenylalkanols and AAK with acetonitrile-water (70:30) and methanol-water (90:10) at 1 ml/min flow rate on PS-DV column

Compounds	Eluent			
	Methanol		Acetonitrile	
	Slope	Correlation	Slope	Correlation
benzyl alcohol	3.851	0.978	3.157	0.905
2-phenylethanol	4.926	0.996	3.045	0.926
3-phenylpropanol	5.526	0.998	2.956	0.960
4-phenylbutanol	6.577	0.999	3.010	0.959
5-phenylpentanol	7.887	0.997	3.240	0.972
benzaldehyde	8.770	0.998	4.881	0.980
acetophenone	9.121	0.998	4.367	0.969
propiophenone	10.55	0.999	4.769	0.936
butyrophenone	11.59	0.999	5.745	0.968
valerophenone	12.70	1.000	6.257	0.967
hexanophenone	14.00	1.000	6.184	0.965
heptanophenone	15.37	0.999	7.460	0.965

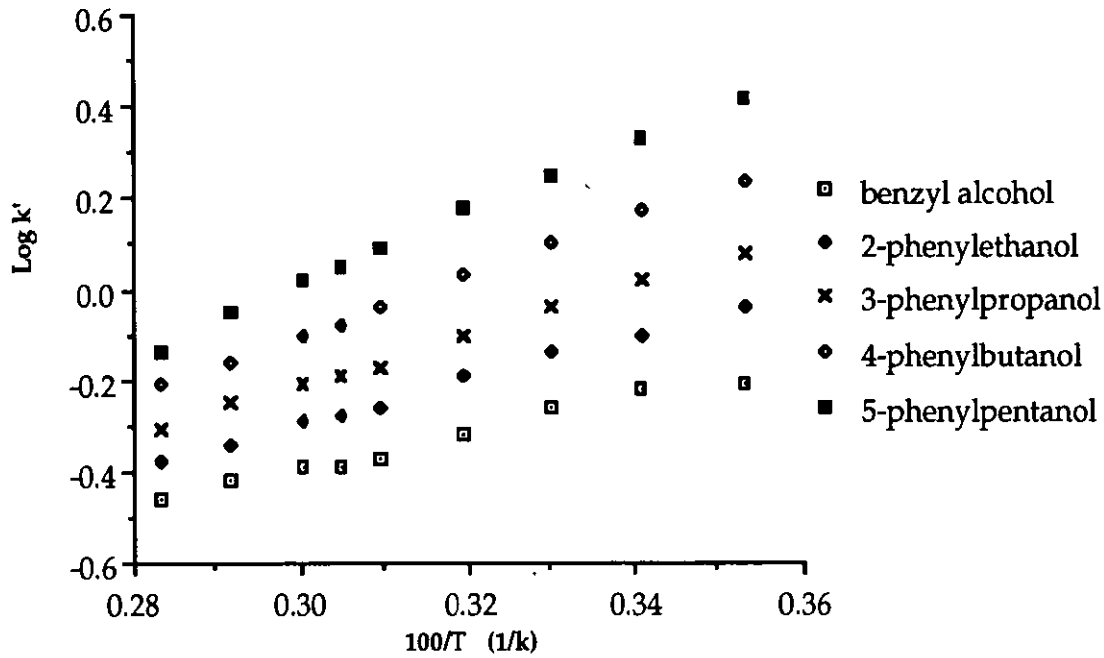

Figure 4.1.5 Log k' against 100/T for phenylalkanols (methanol-water 90:10)

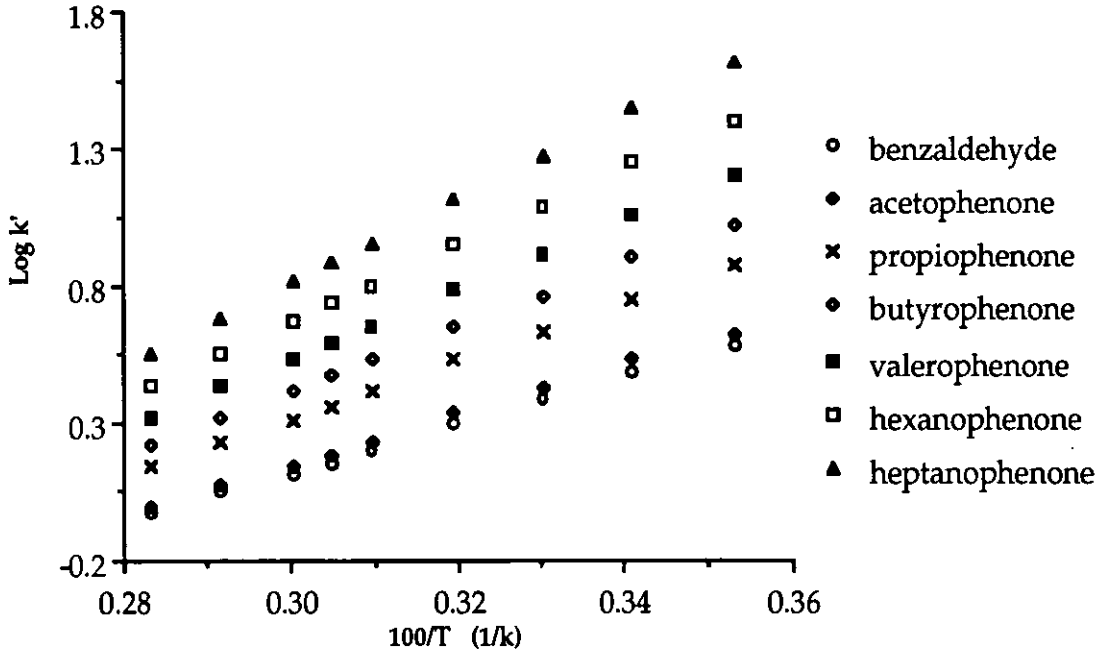

Figure 4.1.6 Log k' against 100/T for AAK (methanol:water 90-10)

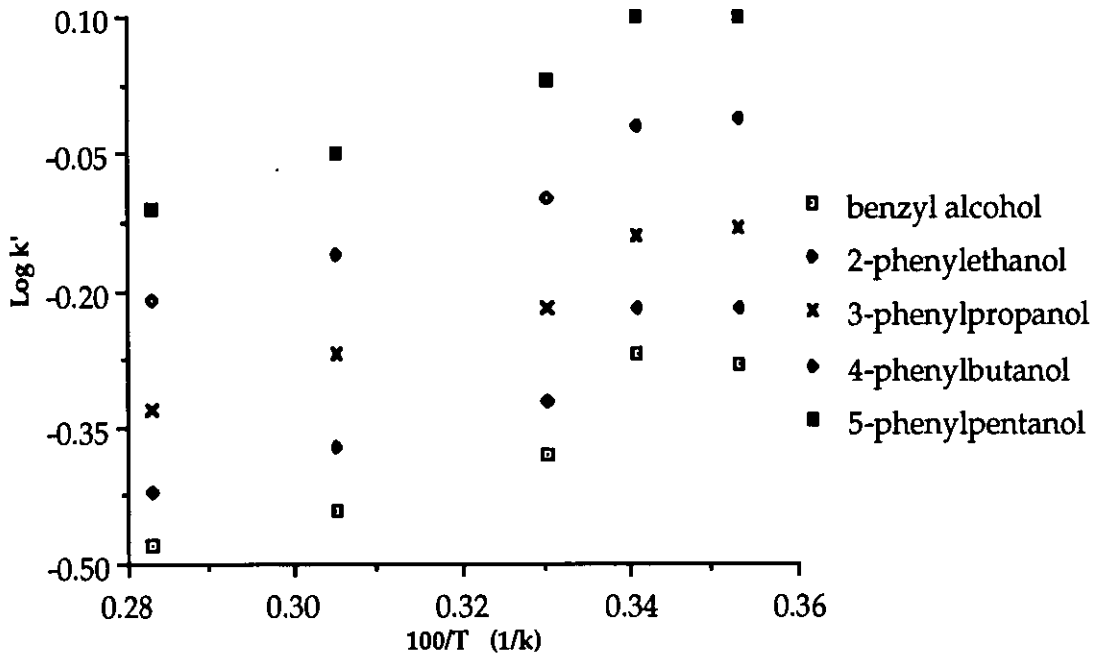

Figure 4.1.7 Log k' against 100/T for phenylalkanols (acetonitrile-water 70:30)

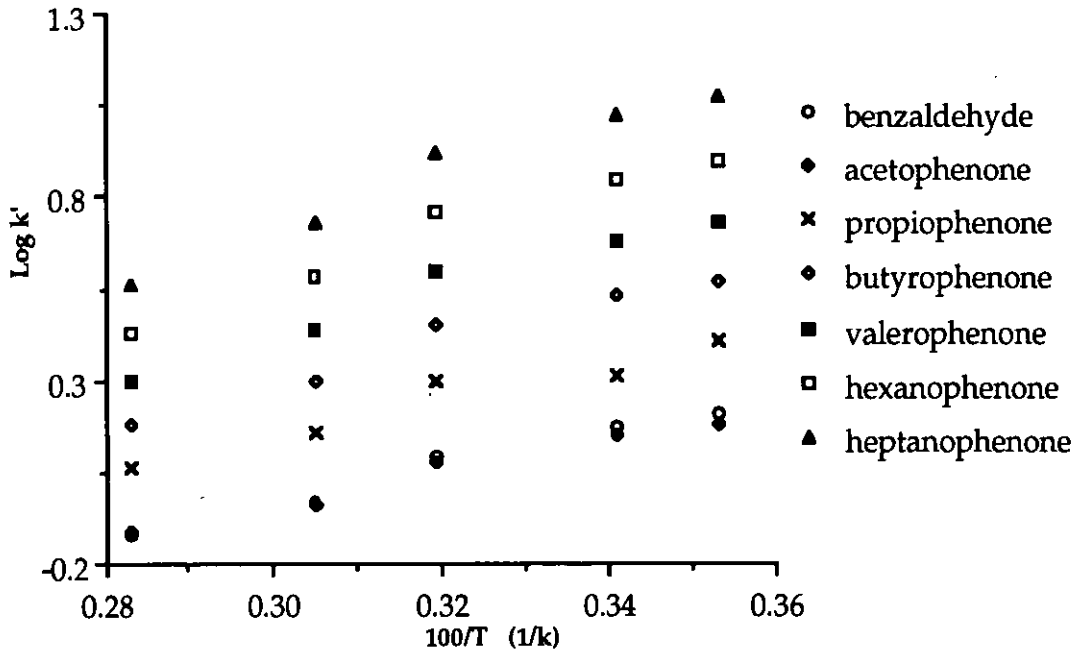

Figure 4.1.8 Log k' against 100/T for AAK (acetonitrile-water 70:30)

4.1.7) generally increased with the increase of the number of methylenes for the same group. The slopes of AAK were higher than those of phenylalkanols in both mobile phases reflecting their longer capacity factors. These indicated that the enthalpies of transfer for AAK from stationary phase to mobile phase were higher than phenylalkanols and AAK stayed in the column longer than phenylalkanols. This was because in reversed phase HPLC, the stationary phase is non-polar and the mobile phase is polar. Based on "like to like", the polar solutes should be eluted by mobile phase early and non-polar solutes would spent more time in the stationary phase and would be eluted later. This was also consistent with their capacity factors listed in Tables 4.1.1 to 4.1.4. However, it was found that the slopes of phenylalkanols in acetonitrile-water (70:30) seemed to be very similar. This might be caused by the short retention times, the deviation of void volume, or a damaged column. It was noted that the log k' of phenylalkanols at 10°C and 20°C were above the line that interpolates the data of 30°C to 80°C (Figure 4.1.7). The experiment started from 30°C to 80°C then back to 10°C, 20°C and 30°C. Comparing the data at 30°C before and later. it was found that the retention time became longer, efficiencies and peak shapes became worse. Such deteriorating results were possibly caused by column damage.

The correlations of phenylalkanols in acetonitrile were much lower than in methanol (Table 4.1.7). However it was noted that the correlations of both phenylalkanols and AAK roughly increased with the increase of the number of methylenes. It was also noted that the correlations of AAK were better than that of phenylalkanols with the same eluent, and for the same solute the correlation was better in methanol than in acetonitrile. It was interesting to note that the correlation seemed to increase with increasing retention time. Therefore the worse results were for those solutes with shorter retention time which could be caused by either deviations in measurement of void volume or other system errors.

4.1.3 Relationship between Temperature and Efficiency in RP-HPLC

Many workers have studied the relationship between efficiency and temperature in RP-HPLC. Mixed results have been reported which showed beneficial effect (50,92), no effect (93) and negative effect (94,95) on the efficiency. In this study, two series of homologous, in methanol and acetonitrile aqueous solvents were examined with constant flow rate and various temperature from 10°C to 80°C. The efficiency of each of the peaks was calculated based on Equation (1.5). The results were shown in Tables 4.1.1 to 4.1.4 and Figures 4.1.9 to 4.1.12. It was found that the efficiencies of both series of homologous increased with increasing temperature, until reaching a maximum value (see Figures 4.1.9 to 4.1.12). When the temperature was increased further the efficiency began to decrease. Different solutes reached their maximum value at different temperatures which depended on the property of solutes and the mobile phase used. It was also noted that the efficiencies of both groups at 10°C and 20°C were quite low. The measurements were made after the high temperature runs. The reason might be that the column was damaged after hundreds of runs. Comparing the values of efficiencies measured at 30°C before and after temperature study, it was found that the efficiencies obtained later were much lower. The maximum difference could reach 900. (e.g. for 5-phenylpentanol, $N_{1MeOH} = 1985$, $N_{2MeOH} = 1086$).

The effect of increasing temperature on the efficiency of the column can be analysed as follows. There are two main factors that contribute to band broadening and efficiency. One is microporosity (51), another is diffusion (87). These two contributions conflict with each other. At low temperatures, the rate of diffusion is very low, the band broadening and low efficiency are mainly caused by restricted movement in microporoes which also causes severe peak tailing. With increasing temperature, the rate of diffusion is increased, the molecules will enter and leave the micropores more easily, therefore the effect of the microporosity decreases, and on balance the band broadening and efficiency are improved. However, when

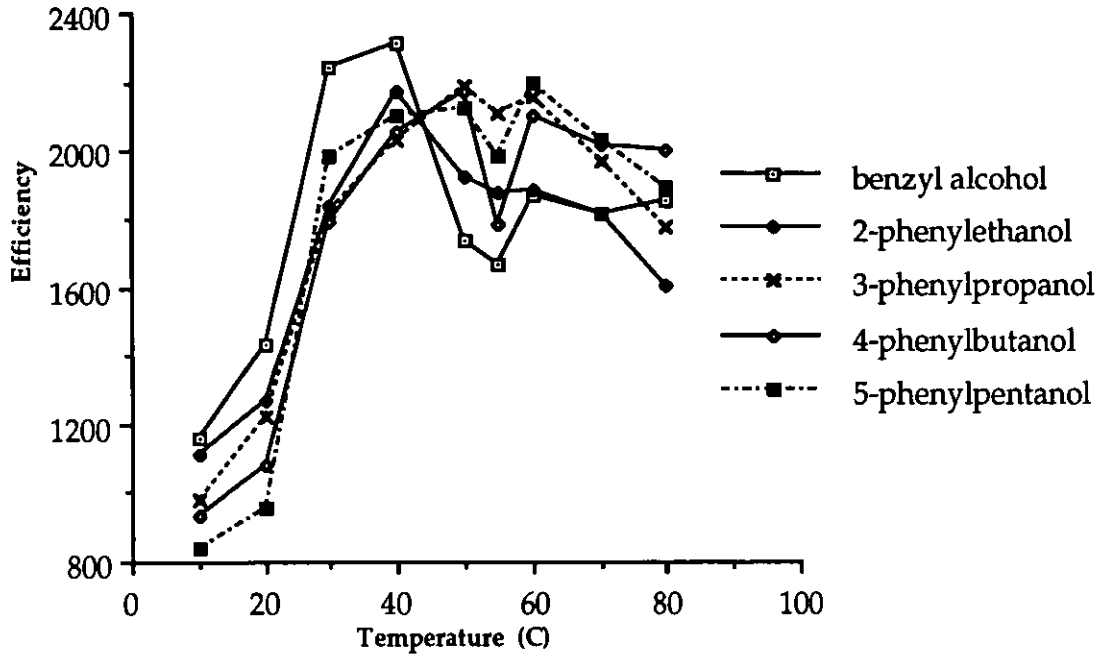

Figure 4.1.9 Efficiency against temperature for phenylalkanols (methanol-water 90:10)

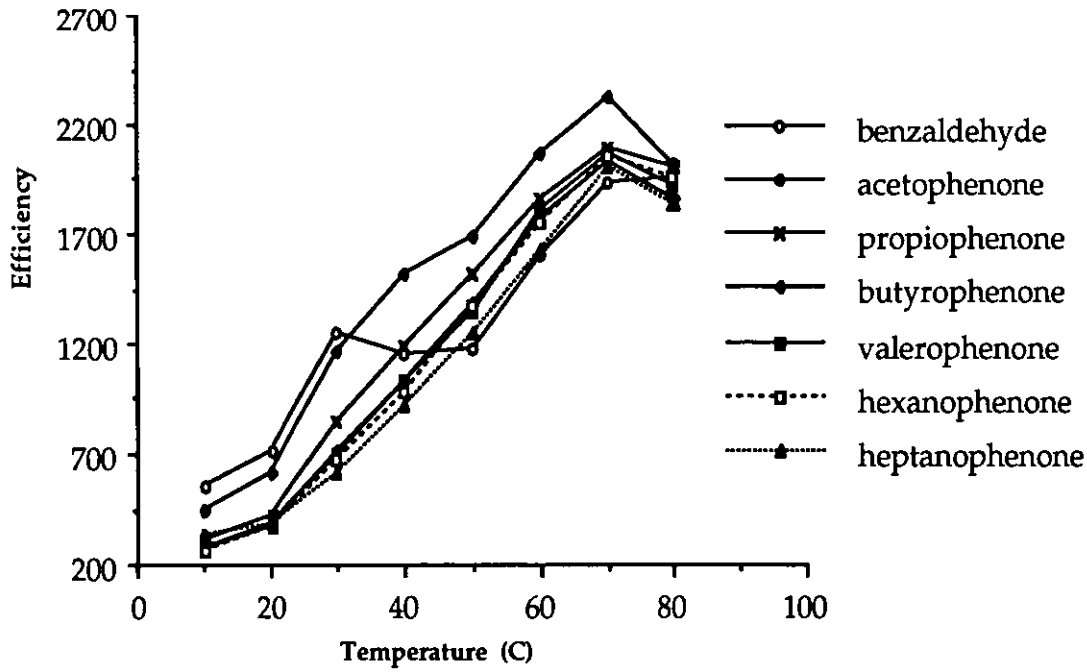

Figure 4.1.10 Efficiency against temperature for AAK (methanol-water 90:10)

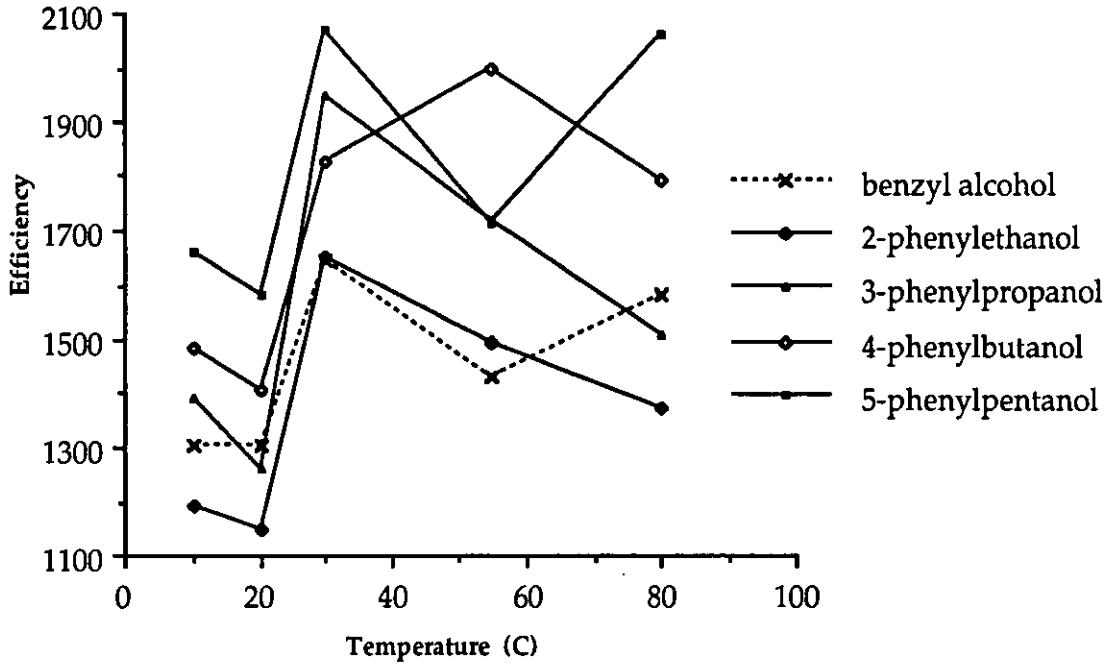

Figure 4.1.11 Efficiency against temperature for phenylalkanols (acetonitrile-water 70:30)

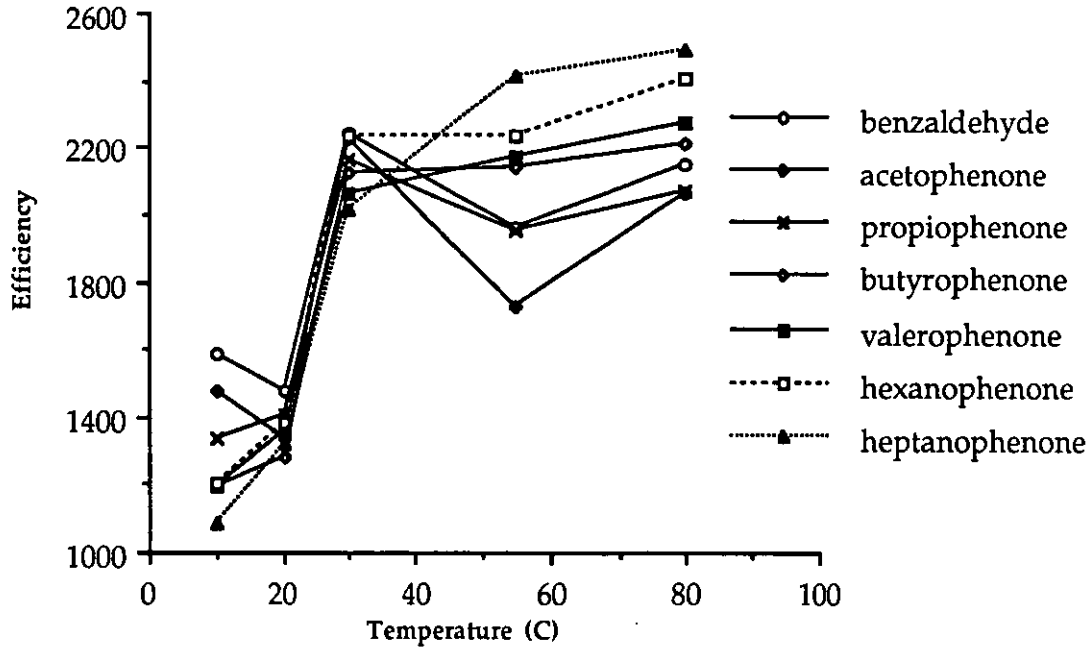

Figure 4.1.12 Efficiency against temperature for AAK (acetonitrile-water 70:30)

temperature increases further the rate of diffusion will be so high that the band broadening will increase and efficiency will decrease again. As noted previously by Smith and co-workers (86), the efficiencies in the present study were markedly dependent on the organic component in the eluent. In the methanol-water (90:10) relative poor efficiencies were given especially at lower temperature. In contrast the better efficiencies were obtained in acetonitrile-water (70:30). It was found (86) that with THF eluent the compounds also showed higher efficiency. The results obtained on ODS column were completely different from those on PS-DVB column. This unusual behaviour suggest that there are special interactions between column material and eluent used.

It has been reported (39,43,45) that methanol would shrink the PS-DVB column material, acetonitrile and THF could swell it. When using methanol as solvent, because of shrinking the particle size of packing material would be reduced, the effect of microporosity would increase and relative higher temperature would be needed to get the best efficiency. When using acetonitrile as solvent, because of swelling, the particle size would increase, the microporosity would not be as important as before, and relative lower temperature could provide the best efficiency. This has been confirmed in Figures 4.1.9 to 4.1.12. Besides, increasing temperature was more beneficial to compounds with long retention times. For phenylalkanols, the maximum efficiencies were reached at 40°C in methanol and 30°C in acetonitrile. The best efficiencies of AAK were only obtained at quite higher temperature, 70°C in methanol and 80°C in acetonitrile. However, the first three compounds were out of order. This is because in general, the compounds with longer retention times have relatively lower diffusion rate and are very difficult to get in and out of micropores. Increasing temperature can increase diffusion rate and reduce the effect of microporosity, it is more benefit for chromatographic procedures which use methanol as the mobile phase. The comparisons of four compounds selected from phenylalkanols and AAK in methanol-water (90:10) and acetonitrile-water (70:30) are shown in Figure 4.1.13 and Figure 4.1.14. It was found

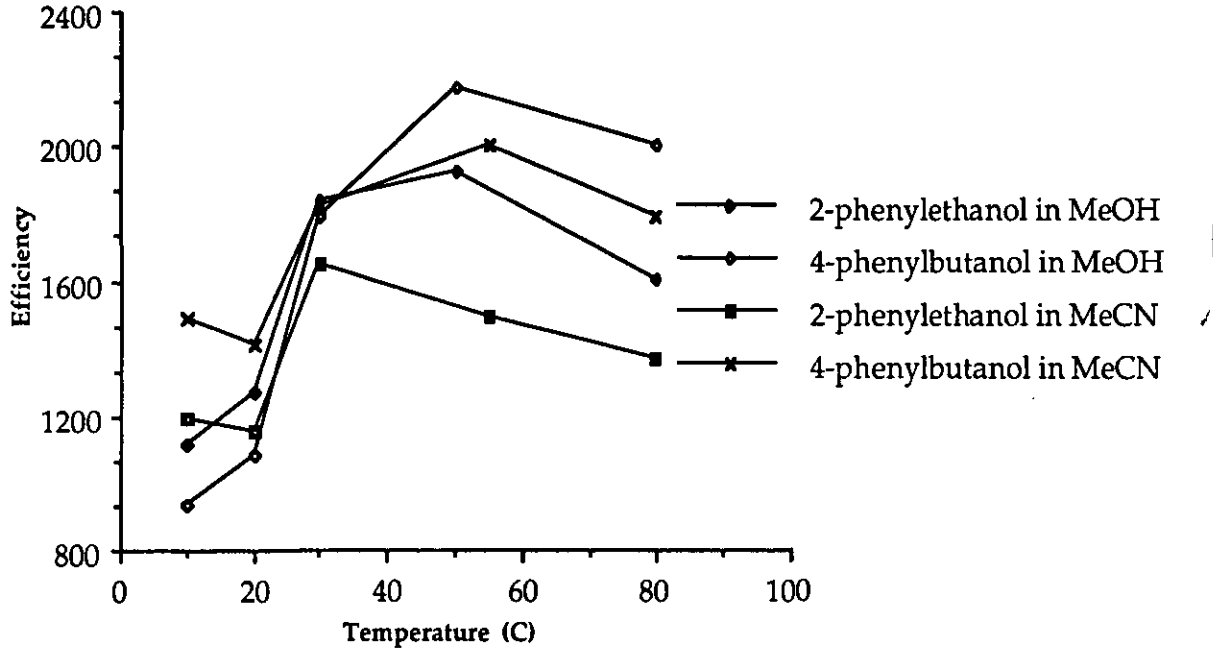

Figure 4.1.13 Composition of efficiencies of phenylalkanols in methanol-water (90:10) and acetonitrile-water (70:30)

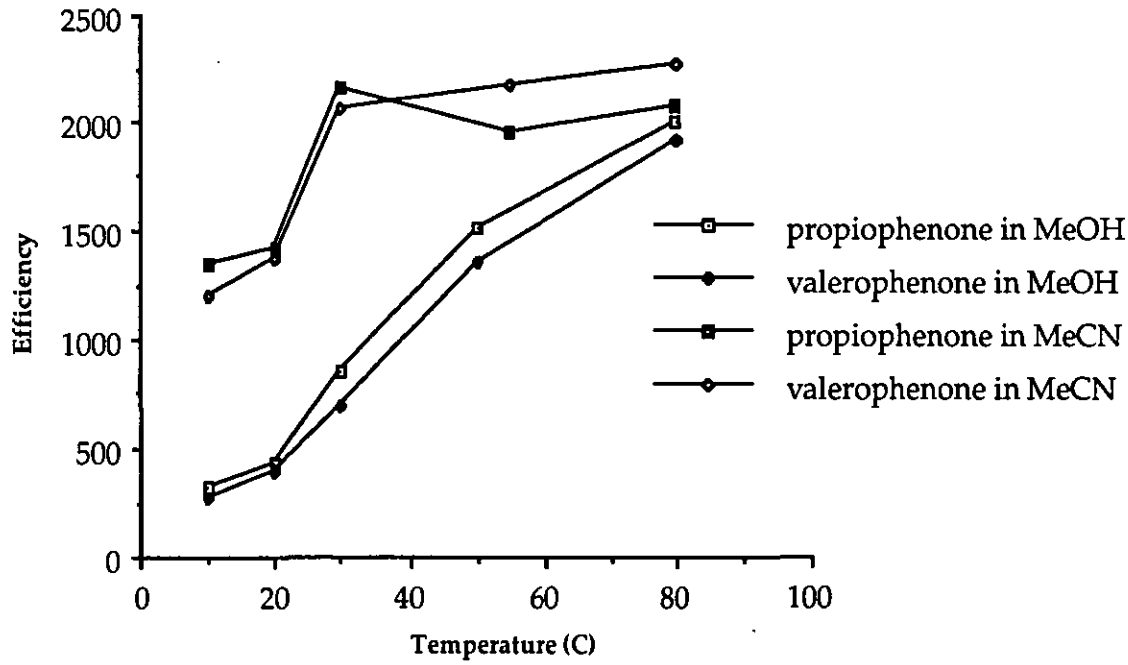

Figure 4.1.14 Composition of efficiencies of AAK in methanol-water (90:10) and acetonitrile-water (70:30)

that the efficiencies of AAK in acetonitrile were much higher than in methanol, but this phenomenon did not happen for phenylalkanols. It was also found that the increasing temperature give more benefit to the efficiencies of AAK when methanol used as eluent. However, it was not so apparent for phenylalkanols. The reason may be related to the interaction between stationary phase and solutes as mentioned by Wheals (87) and the abnormal behaviour for phenylalkanols was not clear.

It was interesting to note that, in generally, the efficiencies of both homologous series reduced with their carbon number. This interesting feature of PS-DVB columns can be predicted in the theory. Hawkes (96) proposed that in Van Deemter equation (1.7) the term "eddy diffusion" is used by chemical engineers to describe a phenomenon related to turbulence, but turbulence is a type of flow that probably never occurs in chromatographic practice. The longitude diffusion term B/u only dominates at very low flow rate. The mass transfer term C can be divided into two parts respectively, that is, the term C_S due to the mass transfer of stationary phase, and the term C_M due to the mass transfer of mobile phase. Thus equation (1.7) becomes

$$h = A + B/u + (C_M + C_S)\,u\,, \qquad (4.4)$$

As simple adsorption-desorption kinetics are very fast, C_S should make a negligible contribution to C. The mobile phase mass transfer C_M is proportional to the square of the particle diameter d_p and the square of the column diameter d_c. For spherical particles an equation can be given as follows

$$C_M = [\,(1 + k' - F)^2\, d_p^2\,]/[\,30\,(1 - F)\, r_p\, (1 + k')^2\, D_M\,] \qquad (4.5)$$

where D_M is the coefficient of molecular diffusion, k' is the capacity factor, r_p is obstruction factor of particles and F is fraction of the mobile phase that is outside the

porous particles. The above equation can also be written as

$$C_M = [1 - F/(1 + k')]^2 d_p^2 / [30(1 - F) r_p D_M], \qquad (4.6)$$

For a given column, F, d_p and r_p are constants, k' and D_M are variables. When k' increases, the term $[1 - F/(1 + k')]^2$ becomes larger. Furthermore, for longer retained compounds, usually the diffusion is slow, so the diffusion coefficient D_M are relatively small. As a result C_M and thus h becomes larger for longer retained compounds, therefore the efficiencies are low. The performance of the PS-DVB column fit above explanation well. However, this explanation is not suitable for ODS column since for ODS column the efficiency usually increase with the capacity factor.

4.1.4 Relationship between Temperature and Peak Asymmetry in RP-HPLC

The relationship between temperature and peak asymmetry is shown in Tables 4.1.1 to 4.1.4 and Figures 4.1.15 to 4.1.18.

Colin and co-workers (93) reported that peak symmetry seemed to be independent of the column temperature. However, different results were obtained in this work. Basically, peak asymmetry decreased with increasing temperature, especially for weakly retained compounds, then it increased with further increasing temperature. These can be explained as follows. In a PS-DVB column, there are micropores in the stationary phase, some solutes can be trapped in the pores which cause peak asymmetry. For the larger size compounds, once the molecules are trapped in the pores, it will be difficult for them to leave. Therefore for these compounds, asymmetry is very severe. Increasing temperature can increase the diffusion rate, thus it can help trapped molecules to get out. At high temperature, the rate of diffusion is high, the trapped compounds can leave micropores relatively

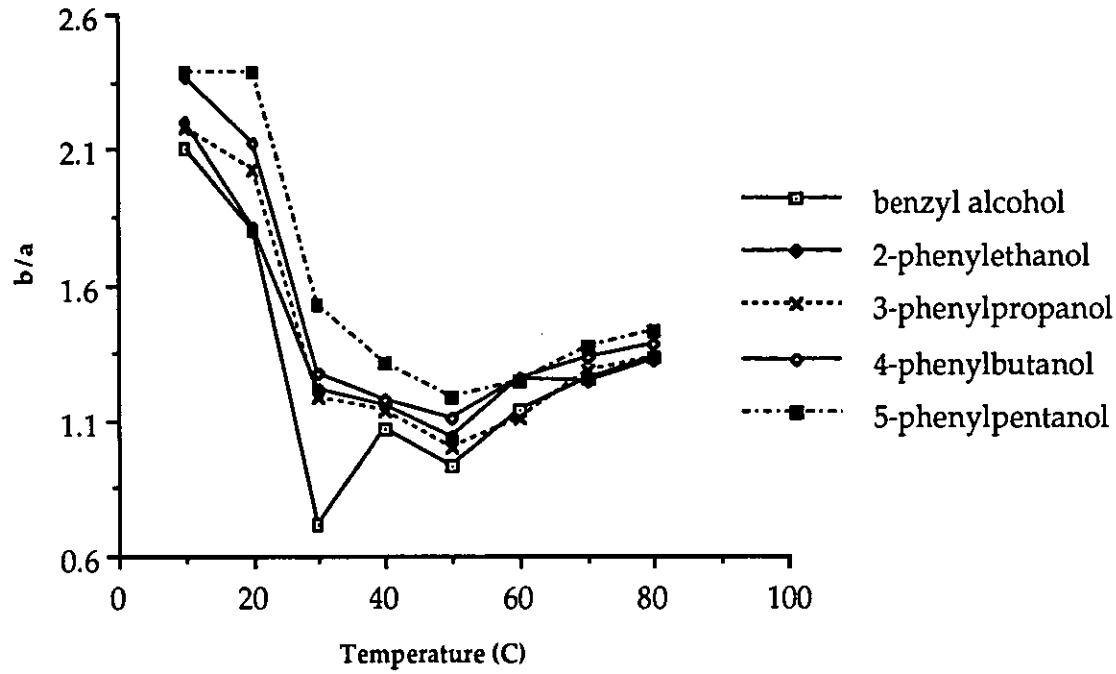

Figure 4.1.15 Asymmetry (b/a) against temperature for phenylalkanols (methanol-water 90:10)

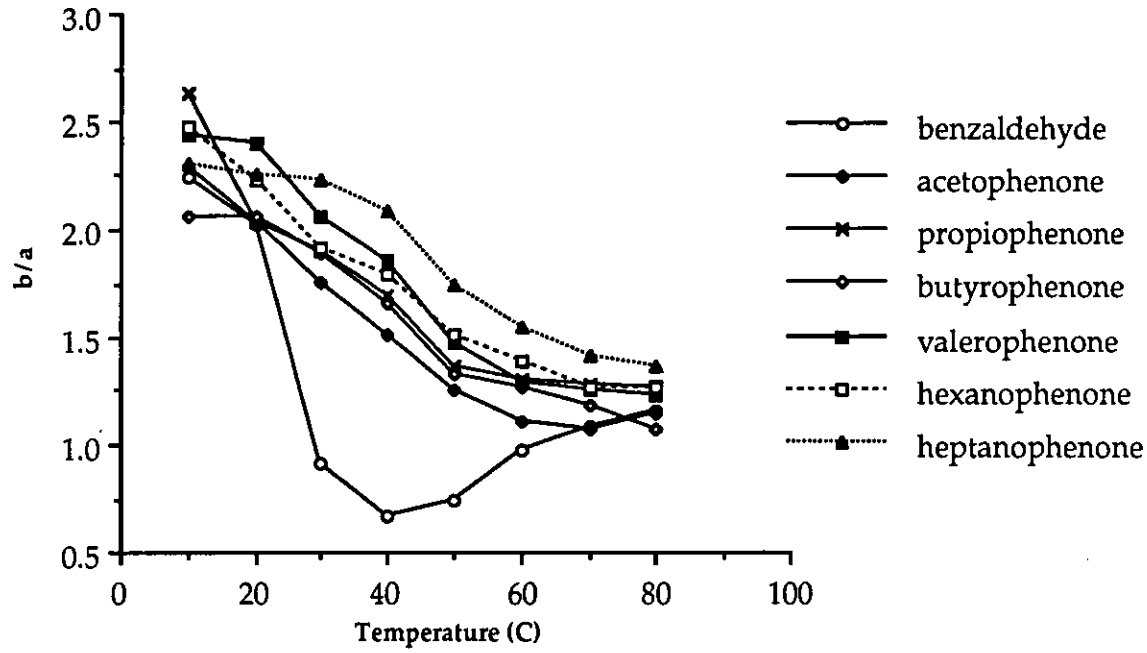

Figure 4.1.16 Asymmetry (b/a) against temperature for AAK (methanol-water 90:10)

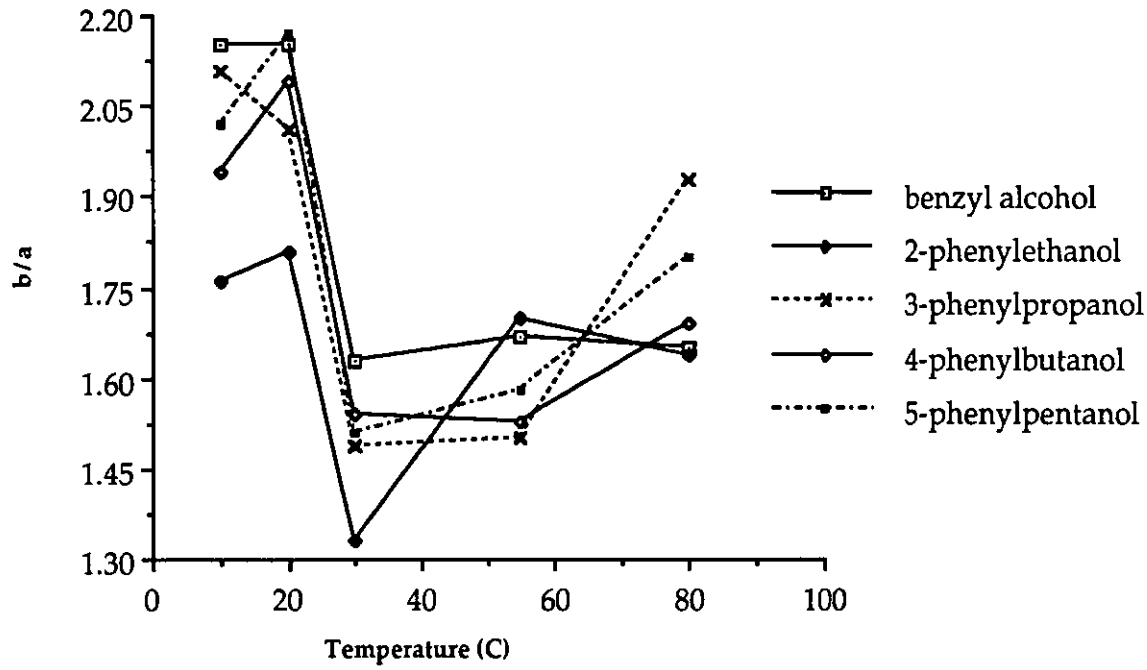

Figure 4.1.17 Asymmetry (b/a) against temperature for phenylalkanols (acetonitrile-water 70:30)

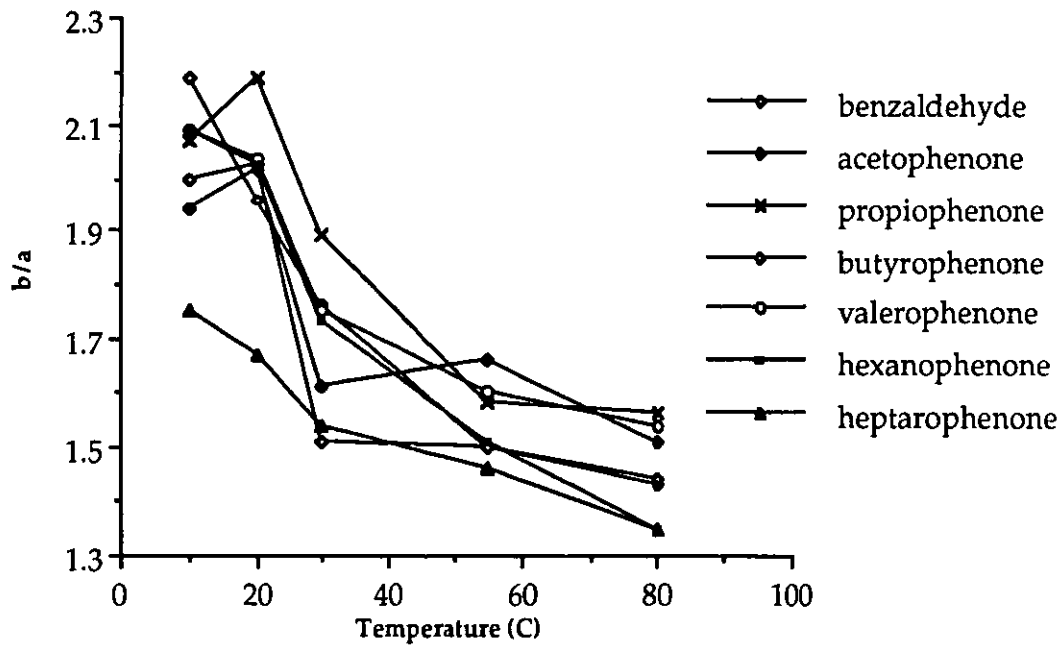

Figure 4.1.18 Symmetry against temperature for AAK (acetonitrile-water 70:30)

freely, but the axial diffusion rate is high as well which causes band broadening.

4.2 The effect of flow rate in RP-HPLC

A good general description of the relationship between the linear flow velocity, u and the column efficiency expressed as the plate height, H, is given by the Van Deemter equation (4.4). Giddings (97) suggested a more useful equation where u and H are replaced by their reduced values. The reduced eluent velocity, υ, is calculated from

$$\upsilon = u D_M / d_p \qquad (4.7)$$

and the reduced plate height (h) from

$$h = H / d_p \qquad (4.8)$$

where d_p is the particle size and D_M is the diffusion coefficient in the mobile phase. Giddings' equation eliminates the dimension of the linear relationship between h and υ and is easier to compare different column sizes, packings and eluents.

Knox (98) built another equation for reduced plate height and reduced velocity

$$h = A'\upsilon^{1/3} + B'/\upsilon + C'\upsilon. \qquad (4.9)$$

Chen *et al.* (99) compared Van Deemter equation with Knox equation and found that the former gives more physically meaningful results than the latter. Therefore equation (4.6) were used in this study. Because the diffusion coefficient D_M is difficult to calculate, and for microporous phases the actual diffusion coefficient is

much smaller than D_M due to the restricted diffusion in the micropores (51), so the relationship between the reduced plate height and the linear velocity of the eluent was investigated using acetonitrile as solvent since it was thought that the effect of micropores was weaker when using acetonitrile as eluent (Tables 4.2.1 and 4.2.2 and Figures 4.2.1 and 4.2.2). It was found that for AAK the optimum flow rate was about 1 ml/min, and flow rate of 0.5 ml/min was more suitable for phenylalkanols except for benzyl alcohol.

It was also found (Figure 4.2.1 and Figure 4.2.2) that the longitudinal diffusion term dominated the reduced plate height before optimum flow rate, and after that the mass transfer term took over. Generally, shorter retained compounds have higher diffusion rates and better efficiency. So their position of the curve should be lowest in the plot of h *vs* u. In the present case, the steep slope was obtained in C term for benzyl alcohol and abnormal behaviour were also obtained for other compounds (Figure 4.2.1 and Figure 4.2.2). A similar result on PS-DVB column in SEC were reported by Nevejans and co-workers (51), who explained this effect as restricted permeation.

Some work was also done with methanol as solvent for the two series of homologous (Table 4.2.3 and Table 4.2.4), the results showed that the efficiency decreased when the flow rate changed from 1 ml/min to 0.5 ml/min for these two groups (except the last two compounds of AAK). It has been reported that, at the optimal flow rate, the plate height with methanol were two or three times larger than with acetonitrile in microbore HPLC (100). In present case, however, the efficiency of phenylalkanols in acetonitrile at optimal flow rate was similar to that in methanol at 1 ml/min.

4.3 The effect of temperature in normal phase - HPLC

It was reported that the PS-DVB column has weak polarity (101). On the other

Table 4.2.1 The effect of flow rate for the retention of phenylalkanols on PS-DVB column with acetonitrile-water (70:30) as eluent, T = 30°C

Flowrate		Compounds[a]					
ml/min		1	2	3	4	5	6
	t_R	22.28	23.72	26.38	28.04	32.75	15.75 (min)
0.1	N	2487	1697	1053	900	952	
	b/a	2.27	1.85	2.08	1.86	2.61	
	h	12.10	17.70	28.50	33.30	31.50	
	t_R	4.40	4.54	4.97	5.50	6.36	3.09 (min)
0.5	N	2244	1781	2049	1862	2383	
	b/a	1.52	1.85	1.71	1.76	1.68	
	h	13.40	16.80	14.60	16.10	12.60	
	t_R	2.21	2.30	2.50	2.79	3.22	1.56 (min)
1.0	N	1645	1649	1948	1825	2071	
	b/a	1.63	1.33	1.49	1.54	1.51	
	h	18.20	18.20	15.40	16.40	14.50	
	t_R	1.93	2.02	2.22	2.48	3.00	1.28 (min)
1.2	N	1512	1346	1501	1491	1679	
	b/a	1.90	1.52	1.70	1.77	1.91	
	h	19.80	22.30	20.00	20.10	17.90	
	t_R	1.52	1.58	1.73	1.95	2.27	1.07 (min)
1.5	N	923	952	966	1168	1268	
	b/a	1.80	1.61	1.79	1.75	1.85	
	h	32.50	31.50	31.10	25.70	23.70	

[a] 1. benzyl alcohol 2. 2-phenylethanol 3. 3-phenylpropanol 4. 4- phenylbutanol 5. 5-phenylpentanol 6. sodium nitrate

Table 4.2.2 The effect of flow rate for the retention of alkyl aryl ketones on PS-DVB column with acetonitrile-water (70:30) as eluent, T = 30°C

flowrate		Compounds[a]							
ml/min		1	2	3	4	5	6	7	8
	t_R	37.11	36.25	49.70	63.76	83.91	114.76	159.64	5.45 (min)
0.1	N	1324	1879	1940	1819	1904	1892	1950	
	b/a	1.84	1.87	2:12	2.04	2.08	2.15	1.99	
	h	22.70	16.00	15.50	16.50	15.80	15.90	15.40	
	t_R	6.81	6.82	9.22	11.69	15.31	20.67	28.78	3.09 (min)
0.5	N	1774	1601	1869	1780	1853	1867	1819	
	b/a	2.30	1.97	2.14	2.13	1.90	1.88	1.81	
	h	16.90	18.70	16.10	16.90	16.20	16.10	16.50	
	t_R	3.46	3.40	4.62	5.89	7.69	10.45	14.51	1.55 (min)
1.0	N	2242	2228	2163	2123	2062	2235	2014	
	b/a	1.51	1.61	1.89	1.76	1.75	1.73	1.54	
	h	13.40	13.50	13.90	14.10	14.50	13.40	14.90	
	t_R	3.11	3.05	4.26	5.57	7.46	10.41	14.78	1.28 (min)
1.2	N	1626	1639	1718	1649	1642	1729	1615	
	b/a	1.84	1.67	1.88	1.83	1.88	1.86	1.89	
	h	18.50	18.30	17.50	18.20	18.30	17.40	18.20	
	t_R	2.43	2.35	3.20	4.09	5.36	7.31	10.17	1.07 (min)
1.5	N	1170	1241	1508	1292	1427	1333	1401	
	b/a	1.74	1.73	1.79	1.80	1.87	1.87	1.64	
	h	25.60	24.20	19.90	23.20	21.00	22.50	21.40	

[a] 1. benzaldehyde 2. acetophenone 3. propiophenone 4. butyrophenone 5. valerophenone 6. hexanophenone 7. heptanophenone 8. sodium nitrate

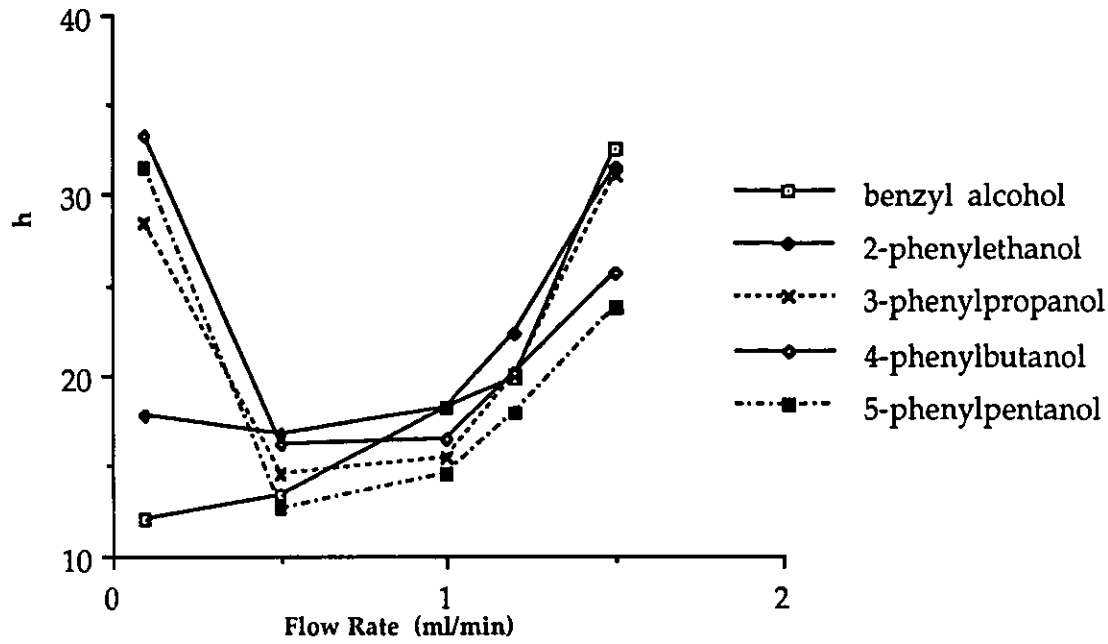

Figure 4.2.1 Reduced plate height (h) against flow rate for phenylalkanols (acetonitrile-water 70:30)

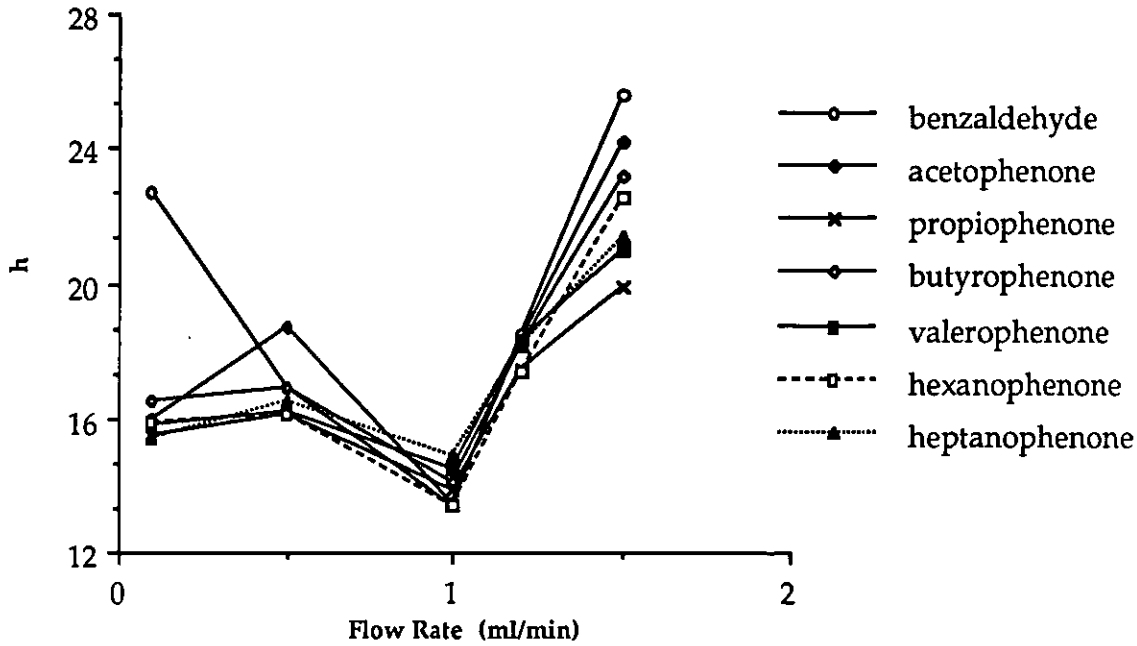

Figure 4.2.2 Reduced plate height (h) against flow rate for AAK (acetonitrile-water 70:30)

Table 4.2.3 The effect of flow rate for the retention of phenylalkanols on PS-DVB column with methanol-water (90:10) as eluent, T = 30 °C

Flowrate		Compounds[a]					
ml/min		1	2	3	4	5	6
	t_R	4.98	5.51	6.11	7.14	8.69	3.37 (min)
0.5	N	1865	1833	1623	1598	1563	
	b/a	1.90	1.99	2.12	2.24	2.42	
	h	16.10	16.40	18.50	18.80	19.20	
	t_R	2.46	2.74	3.05	3.61	4.45	1.59 (min)
1.0	N	2242	1840	1822	1795	1985	
	b/a	0.72	1.21	1.19	1.27	1.53	
	h	13.40	16.30	16.50	16.70	15.10	

[a] 1.benzyl alcohol 2. 2-phenylethanol 3. 3-phenylpropanol 4. 4- phenylbutanol 5. 5-phenylpentanol 6. sodium nitrate

Table 4.2.4 The effect of flow rate for the retention of alkyl aryl ketones on PS-DVB column with methanol-water (90:10) as eluent, T = 30°C

Flowrate		Compounds[a]							
ml/min		1	2	3	4	5	6	7	8
	t_R	11.19	11.81	16.94	21.49	28.61	40.77	59.29	3.37 (min)
0.5	N	955	936	738	640	634	680	663	
	b/a	0.94	2.60	2.34	2.38	2.49	2.63	2.49	
	h	31.40	32.10	40.70	46.90	47.30	43.90	45.20	
	t_R	5.50	5.79	8.45	10.83	14.60	21.07	30.94	1.59 (min)
1.0	N	1247	1158	848	711	694	674	615	
	b/a	0.92	1.76	1.90	1.89	2.06	1.92	2.43	
	h	24.10	25.90	35.40	42.20	43.20	44.50	48.80	

[a] 1. benzaldehyde 2. acetophenone 3. propiophenone 4. butyrophenone 5. valerophenone 6. hexanophenone 7. heptanophenone 8. sodium nitrate

Table 4.3.1 The effect of temperature on PS-DVB column for phenylalkanols (hexane as eluent)

Temperature	Compounds[a]				
T(^{o}c)	1	2	3	4	5
	Capacity factor				
30	2.95	1.96	2.09	1.91	1.71
40	2.12	1.47	0.49	1.40	1.24
50	1.65	1.16	1.16	1.09	0.97
60	1.27	0.88	0.87	0.79	0.69
	Efficiency				
30	261	343	283	315	308
40	359	454	440	456	417
50	510	648	626	583	639
60	721	1043	988	915	900
	Asymmetry				
30	4.65	5.09	5.08	5.03	5.24
40	4.62	4.93	4.52	4.48	4.18
50	4.42	4.30	4.46	4.60	4.40
60	3.85	4.01	4.06	4.05	3.77

[a] 1. benzyl alcohol 2. -phenylethanol 3. 3-phenylpropanol 4. 4-phenylbutanol 5. 5-phenylpentanol

Table 4.3.2 The effect of temperature on PS-DVB column for AAK with hexane as eluent

Temperature	Compounds[a]						
T(°c)	1	2	3	4	5	6	7
	Capacity factor						
30	1.26	1.15	0.79	0.58	0.48	0.41	0.35
40	1.05	0.97	0.68	0.50	0.41	0.35	0.30
50	0.98	0.90	0.66	0.49	0.41	0.36	0.32
60	0.66	0.63	0.46	0.34	0.26	0.21	0.19
	Efficiency						
30	775	866	1402	1506	1845	1927	2357
40	1077	1241	1741	1853	2335	2443	2253
50	1594	1582	2341	2706	2428	2427	2645
60	2026	2231	2773	3191	3568	3340	3463
	Asymmetry						
30	2.70	3.04	2.44	2.43	2.06	1.89	2.12
40	2.78	2.71	2.32	2.34	2.31	2.20	1.97
50	2.36	2.64	2.09	2.14	1.82	1.84	1.68
60	2.10	2.27	1.75	1.78	1.64	1.56	1.58

[a] 1. benzaldehyde 2. acetophenone 3. propiophenone 4. butyrophenone 5. valerophenone 6. hexanophenone 7. heptanophenone

mobile phase of NP-HPLC is non-polar, according to the principle of "like to like", it is easier for the non-polar solute to stay in the mobile phase than in the stationary phase during the distribution of solutes between the two phases. As less polar compounds AAK could be eluted quickly and had shorter retention time. than phenylalkanols which have a higher polarity. Within a homologous series, the polarity of compounds decreased with their carbon numbers, therefore compounds with larger carbon number were eluted earlier. The plots of log k' against carbon number are shown in Figure 4.3.1 and Figure 4.3.2. At the same temperature, for both groups of homologues, the compounds with carbon number of 8 behaved abnormally. 2-phenylethanol had a lower log k' than it should have if a systematic relationship held. The logarithm of the capacity factor of acetophenone was higher than expected. The similar problem was also seen in SFC. The reasons for these abnormalities were not clear. However, It is unlikely that they are caused by experimental error, since the experiments under the same temperature were finished within a day and the experiments at different temperatures were carried out on different days.

The correlations, slopes and intercepts for the two groups at various temperatures are shown in Table 4.3.3. It could be noted that neither set of homologous compounds fitted the linear relationship well even if the first two compounds were taken out from AAK. Therefore, it has been demonstrated that there did not seem to be a linear correlation between log k' and carbon number in NP-HPLC.

4.3.2 Relationship between Efficiency and Temperature in NP-HPLC

As noted earlier, increasing the temperature can increase the rate of diffusion, and make it easier for solute molecules to migrate in and out of pores, and can increase efficiency. However, it was found in RP-HPLC that improving efficiency by increasing temperature had its limitation and it was more beneficial for the compounds with longer retention time.

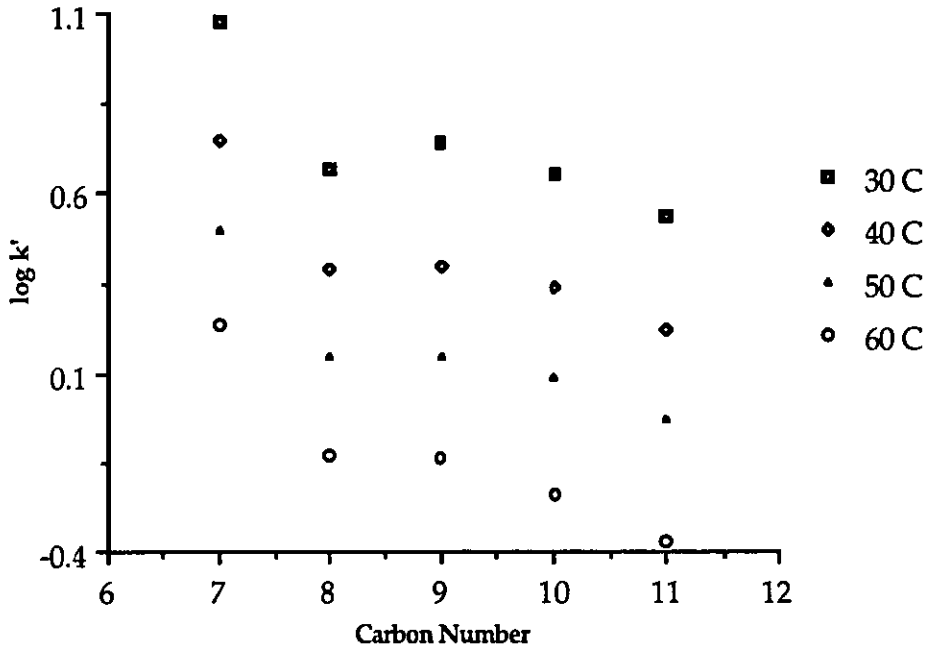

Figure 4.3.1 Log k' against carbon number on PS-DVB column for phenylalkanols (100% hexane)

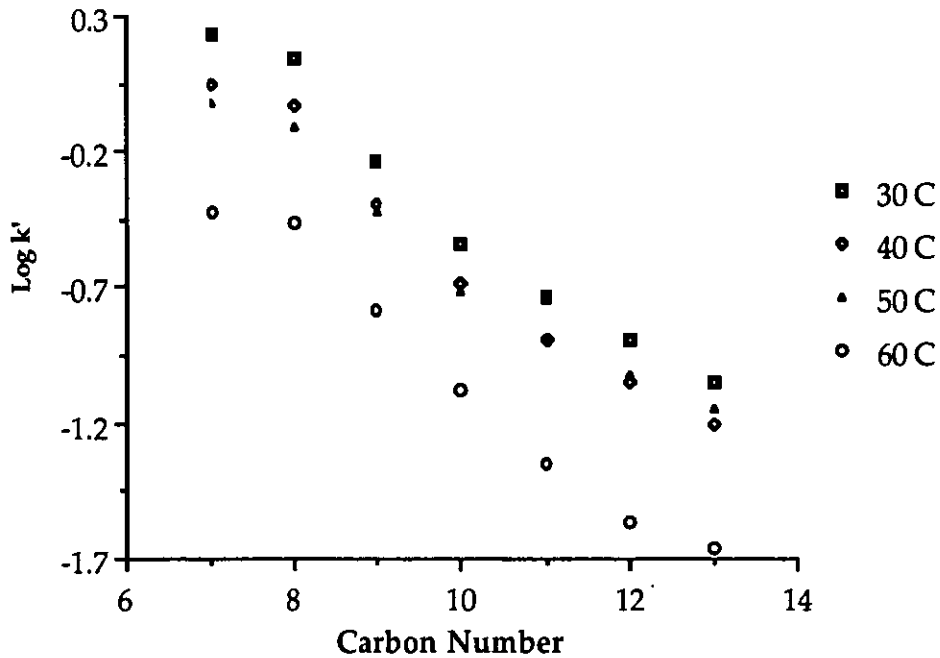

Figure 4.3.2 Log k' against carbon number on PS-DVB column for AAK (100% hexane)

Table 4.3.3 The correlations, slopes and intercepts of the plots of log k' vs carbon number for AAK and phenylalkanols on PS-DVB column in NP-HPLC

Temperature	AAK		
	Slope	Intercept	Correlation
30°C	-0.22821	1.8421	0.976
40°C	-0.22464	1.6464	0.977
50°C	-0.20179	1.4021	0.973
60°C	-0.23179	1.2736	0.976
	Taking benzaldehyde and acetophenone out of AAK		
30°C	-0.19700	1.4770	0.980
40°C	-0.19800	1.2340	0.977
50°C	-0.17500	1.0890	0.963
60°C	-0.22400	1.1780	0.970
	Phenylalkanols		
30°C	-0.11000	1.7260	0.718
40°C	-0.11100	1.4190	0.787
50°C	-0.11200	1.1800	0.804
60°C	-0.13300	1.0690	0.856

in NP-HPLC It was observed (Table 4.3.1 and Table 4.3.2) that the efficiency became higher when the temperature was increased. Although the efficiency of heptanophenone from 30°C to 40°C decreased and the efficiency for hexanophenone seemed to be similar at 40°C and 50°C, all the other data tended to increase when temperature was increased (Figure 4.3.3 and 4.3.4).

In addition, it was found that in RP-HPLC with methanol-water the efficiency seemed to decrease with increases in the number of the methylenes in the homologues (Table 4.1.1 to Table 4.1.4). However, it was observed in NP-HPLC (Tables 4.3.1 and 4.3.2) that efficiency of AAK were better than phenylalkanols, and within a homologous series, the efficiency increased with carbon number. Thus an interesting feature of the PS-DVB column, was that the efficiency of solutes became worse with increased capacity factor and this was found in RP-HPLC (Figure 4.3.5), and also in NP-HPLC (Figure 4.3.6). This effect might be caused by microporosity of PS-DVB column material. For compounds with longer retention times, their diffusion rate would be slow, some of them might even stay in the micropores and not get out. This would caused very serious band broadening and lower efficiency. It was noted that with methanol-water the plot changed less at a higher temperature and the efficiencies decreased less with increasing capacity factor (Figure 4.3.5) but with hexane, the changes of efficiencies were greater. These relationships were not very apparent with acetonitrile as eluent and the very different results are shown in Figure 4.3.7. Comparing Figures (Figure 4.3.5 to Figure 4.3.6). it was found that the efficiencies of compounds with similar retention times (capacity factor > 0.5) at the same temperature were higher in RP-HPLC than in NP-HPLC.

4.3.3 The Relationship between Log k' and Reciprocal of Temperature in NP-HPLC

The relationship between log k' and the reciprocal of temperature can be described by the equation (4.3) as a linear relationship. This relationship was fitted

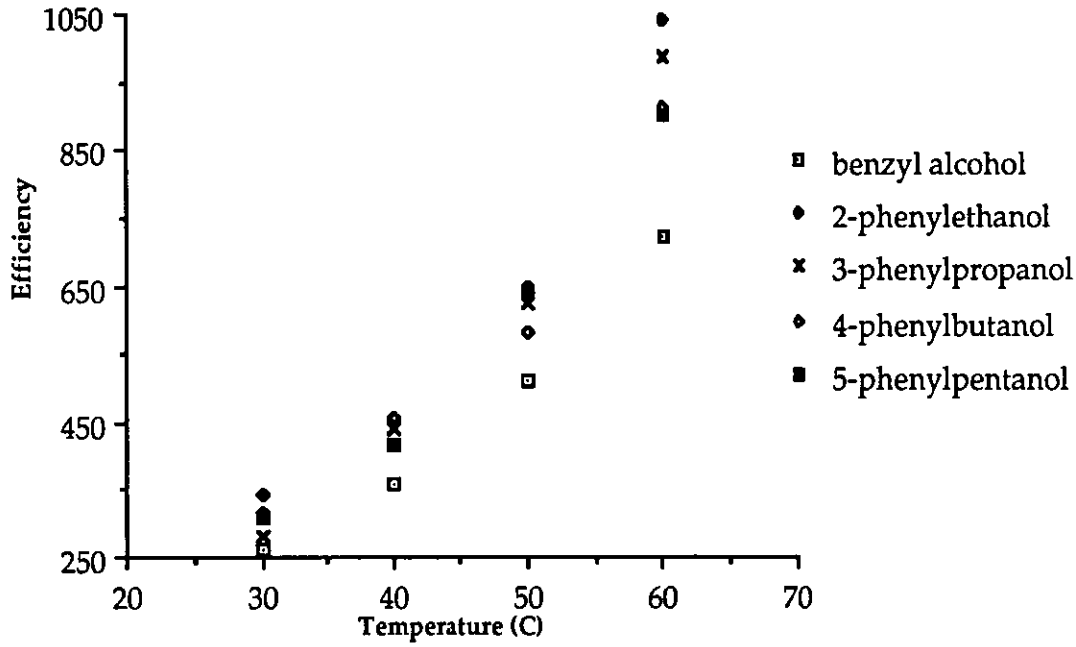

Figure 4.3.3 Efficiency against temperature on PS-DVB column for phenylalkanols (100% hexane)

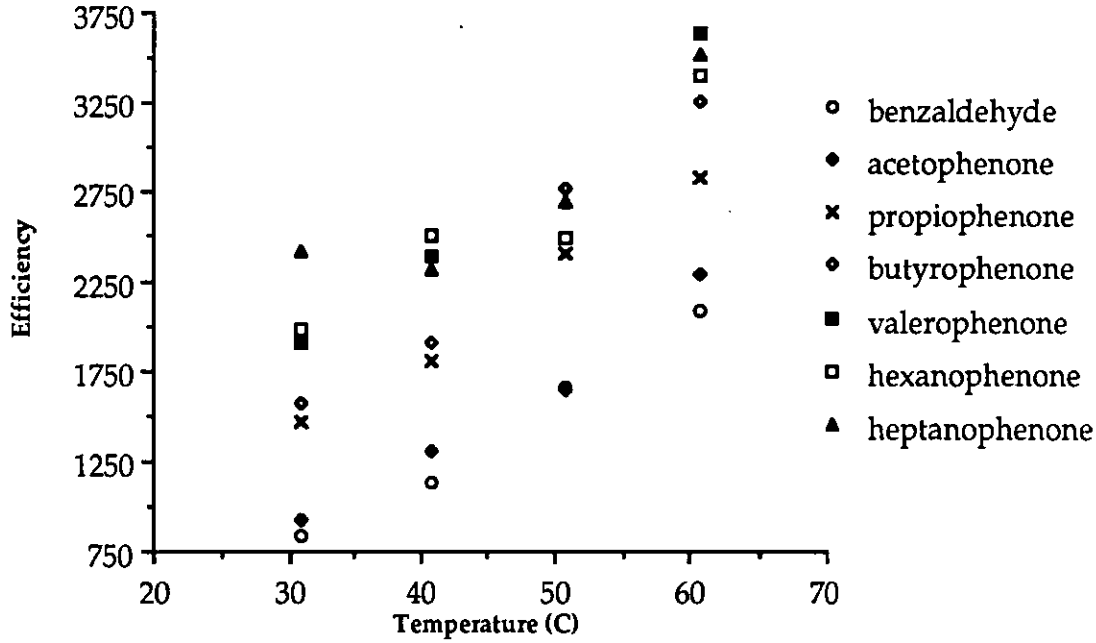

Figure 4.3.4 Efficiency against temperature on PS-DVB column for AAK (100% hexane)

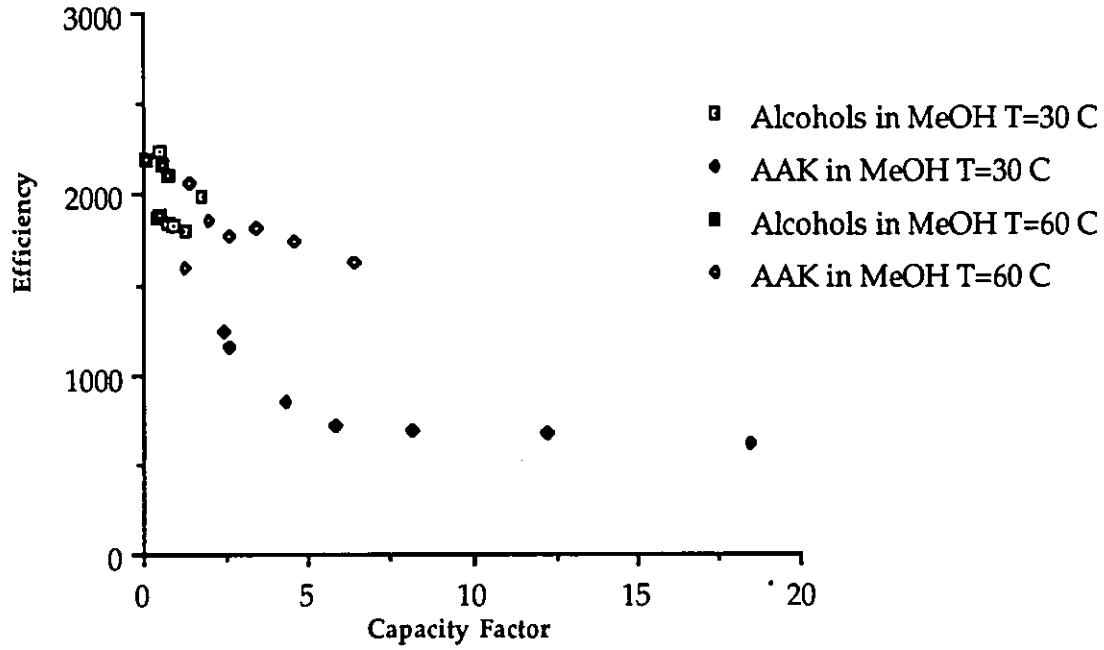

Figure 4.3.5 Capacity factor against efficiency in methanol-water (90:10) for AAK and phenylalkanols at two different temperature

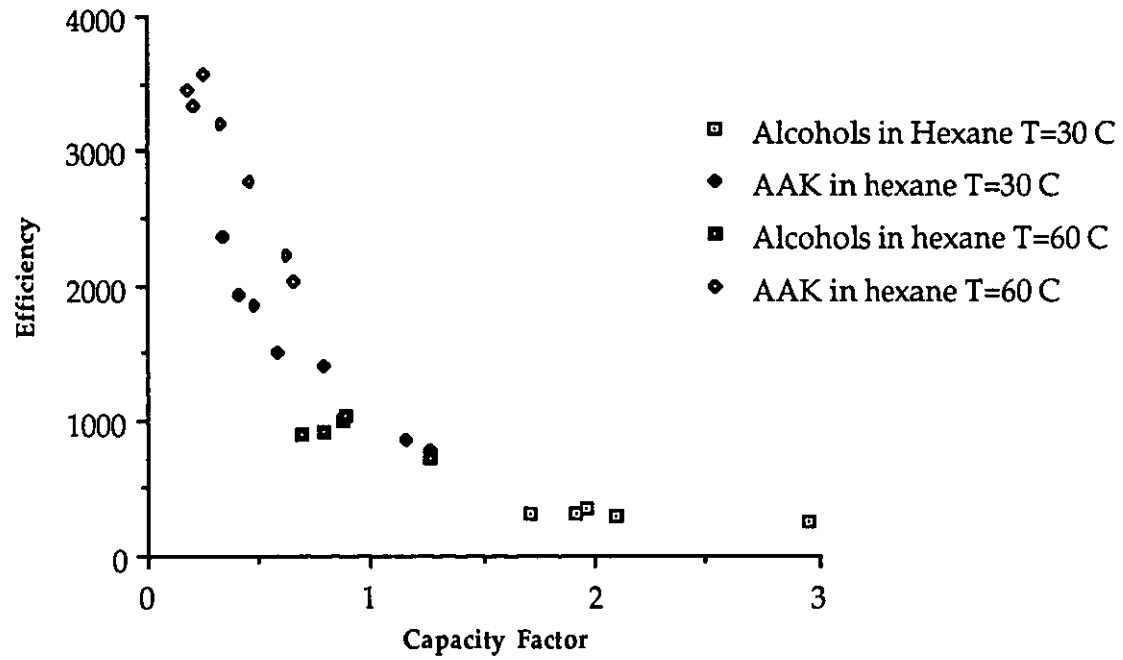

Figure 4.3.6 Capacity factor against efficiency in hexane for AAK and phenylalkanols at two different temperature

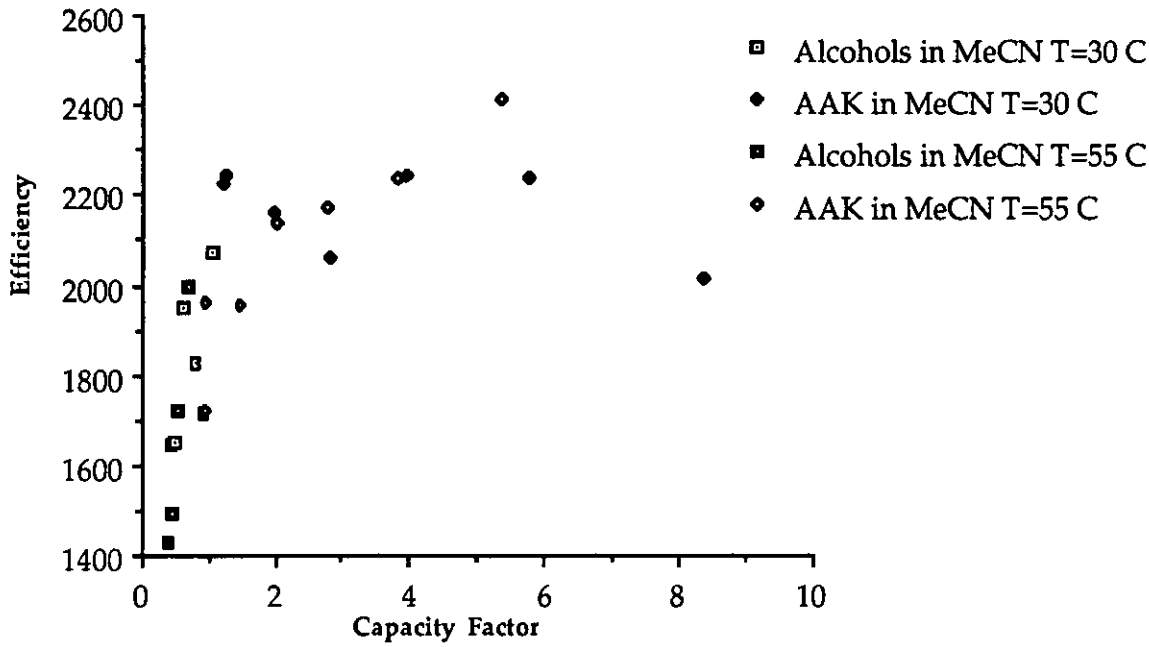

Figure 4.3.7 Capacity factor aganist efficiency in acetonitrile-water (70:30) for AAK and phenylalkanols at two different temperature

well in the present work for RP-HPLC, where two homologous series of AAK and phenylalkanols were used as standard samples, and methanol-water and acetonitrile-water were used as eluents.

In order to examine this relationship in NP-HPLC, the Van't Hoff plots were drawn (Figure 4.3.8 and 4.3.9). The slopes, intercepts and correlations of the plots were listed in Table 4.3.4. It was noted that phenylalkanols group fitted the linear relationship well, the correlations for this group were close to one, but very poor correlations were obtained for AAK. The reason for the poor correlation was not clear, but it might be that the AAK suffered from deviations in measurement of the void volume because of their shorter retention times. It could be seen that AAK had abnormal capacity factors at 50°C, and the values of log k' at 50°C were higher than expected. The retention times of AAK decreased with increasing temperature (Table 4.3.2). However it was noted that the retention time of iso-octane at 50°C was lower than it was at 60°C, this might be caused by experiment error.

4.3.4 Relationship between Symmetry and Temperature in NP-HPLC

It was reported in RP-HPLC (Section 4.1.4) that for longer retained compounds, the molecules had slow diffusion. Therefore their peak shapes were poorer than shorter retained compounds. Increasing temperature can increase the diffusion rate and make it easier for solute molecules to move in and out of pores, thus reduce retention times and improving peak shapes. The values of the peak symmetry within a range of temperature were measured and the results were shown in Table 4.3.1 and Table 4.3.2 and Figure 4.3.10 and Figure 4.3.11. As expected the peak shape tended to improve with increasing temperature except in a few cases. The polar phenylalkanols showed worse peak shapes than AAK.

4.3.5 The Effect of Adding Polar Organic Solvent Modifier in NP-HPLC

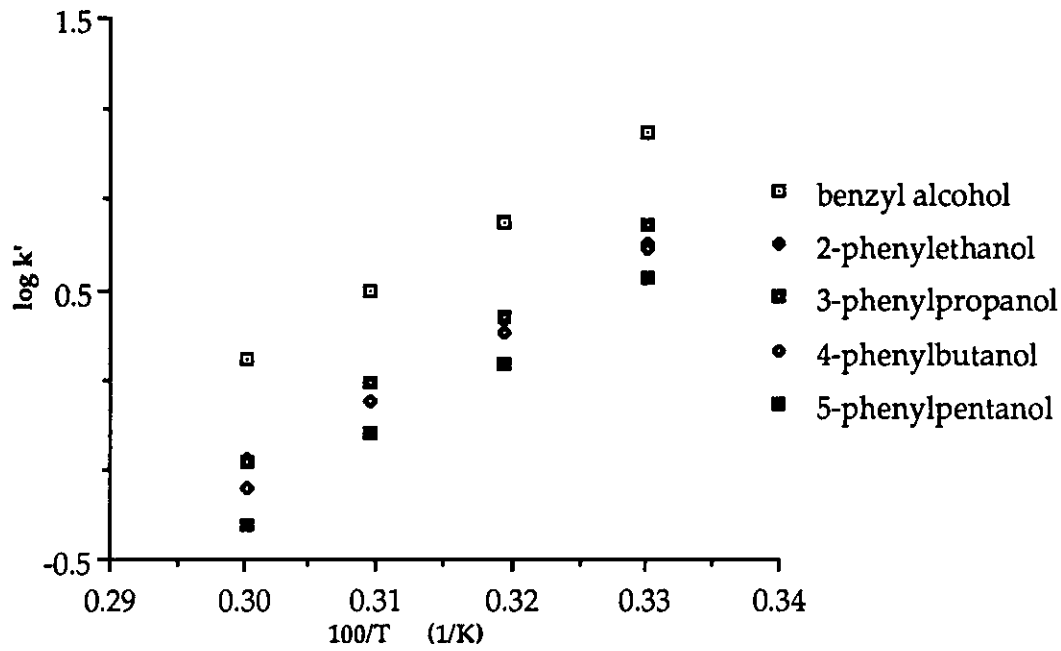

Figure 4.3.8 Log k' against 100/T on PS-DVB column for phenylalkanols (100% hexane)

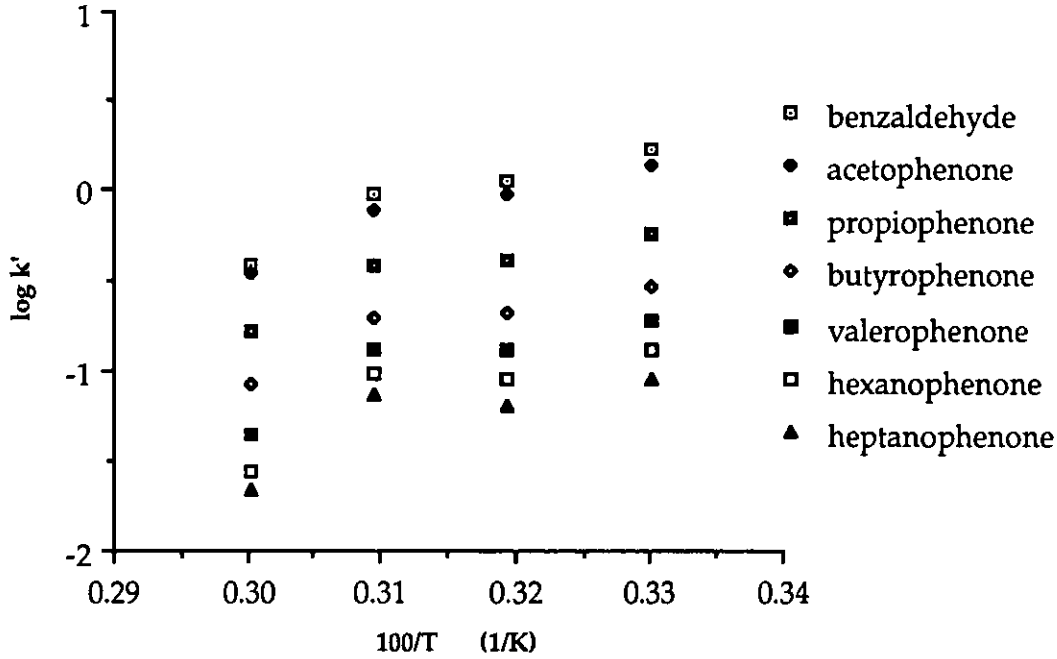

Figure 4.3.9 Log k' against 100/T on PS-DVB column for AAK (100% hexane)

Table 4.3.4 The slopes, intercepts and correlations of the plots of log k' vs 100/T on PS-DVB column in NP-HPLC (hexane as eluent)

Compounds	Slope	Intercept	Correlation
benzaldehyde	20.233	-6.4103	0.891
acetophenone	18.846	-6.0487	0.912
propiophenone	16.508	-5.6551	0.851
butyrophenone	16.401	-5.9189	0.836
valerophenone	18.569	-6.8117	0.789
hexanophenone	19.730	-7.3420	0.733
heptanophenone	17.618	-6.8098	0.687
benzyl alcohol	27.977	-8.1663	0.998
2-phenylethanol	26.622	-8.1121	0.998
3-phenylpropanol	29.175	-8.8984	0.998
4-phenylbutanol	29.433	-9.0572	0.996
5-phenylpentanol	30.038	-9.3677	0.995

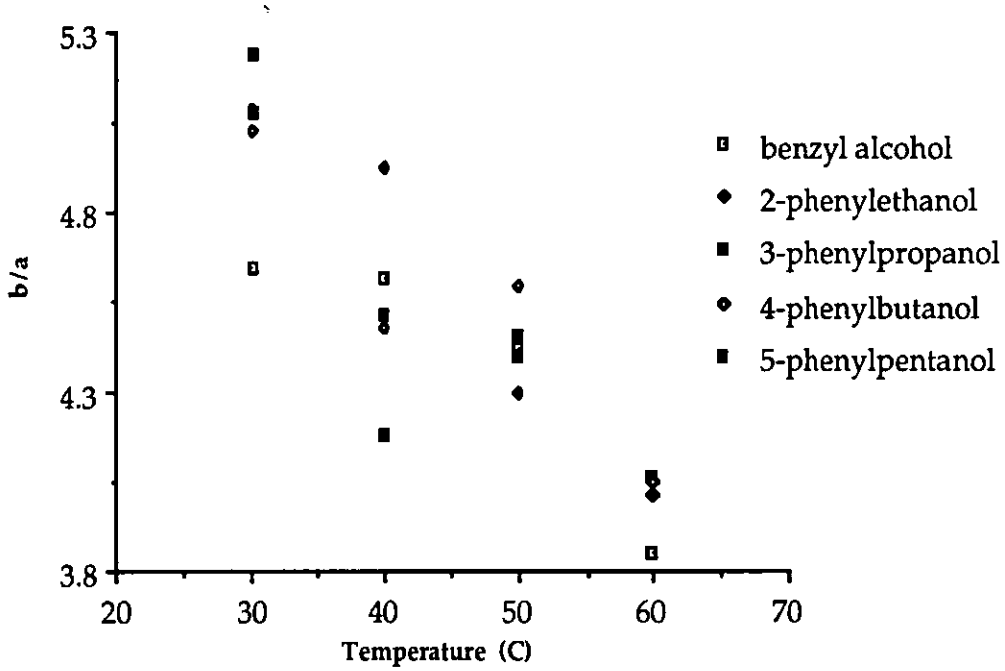

Figure 4.3.10 Asymmetry (b/a) against temperature on PS-DVB column for phenylalkanols (100% hexane)

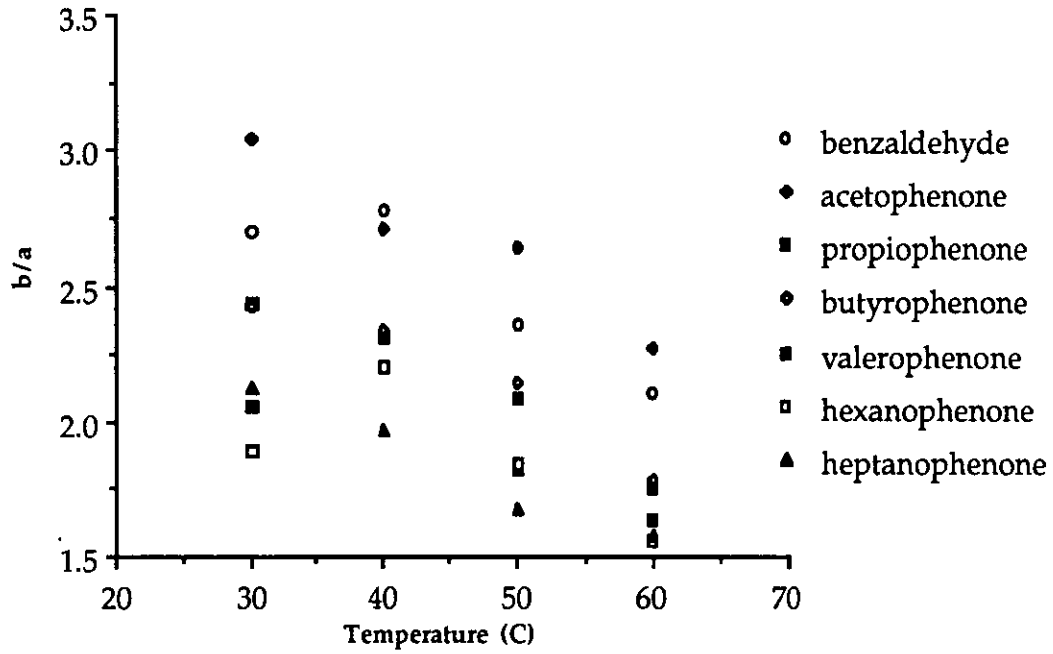

Figure 4.3.11 Asymmetry (b/a) against temperature on PS-DVB colum for AAK (100% hexane)

The separations in NP-HPLC can be characterised by the following aspects (103).

1. increased retention with increased solute polarity.
2. decreased retention with increased mobile phase polarity.
3. solute retention mechanism dominated by interaction of polar stationary phase sites with solute polar groups.
4. mobile phase usually less polar than the stationary phase.

The use of a polar organic solvent modifier in non-polar solvent can reduce the retention time and improve peak shape. In this experiment, a low level of iso-propanol (0.2%) was added in the non-polar solvent (hexane) to keep normal-phase conditions and improve retention mechanism. The results of adding organic modifier are shown in Table 4.3.5. Comparing table 4.3.5 with Table 4.3.1 and Table 4.3.2 at 30^{o}C, it was found that the addition of the polar organic modifier reduced the retention times, increased the efficiencies and improved peak asymmetry. The improvements were more apparent for polar compounds such as phenylalkanols than for AAK, which were less polar.

Table 4.3.5 The effect of polar organic modifier on PS-DVB column in NP-HPLC (T=30°C) for AAK and phenylalkanols (hexane + 0.2% iso-propanol as eluent)

Compound	Retention (min)	Capacity factor	Efficiency	Asymmetry
benzaldehyde	3.27	1.11	1090	2.72
acetophenone	3.14	1.03	1107	2.48
propiophenone	2.67	0.72	1507	2.46
butyrophenone	2.35	0.52	1775	2.03
valerophenone	2.22	0.43	2203	2.01
hexanophenone	2.11	0.36	2310	1.63
heptanophenone	2.04	0.32	2253	1.94
benzyl alcohol	4.77	2.08	489	3.79
2-phenylethanol	3.66	1.36	821	3.48
3-phenylpropanol	3.71	1.39	863	3.89
4-phenylbutanol	3.52	1.27	854	3.81
5-phenylpentanol	3.26	1.10	1017	3.82
iso-octane	1.55			

Chapter 5. Conclusion for the Study on PS-DVB Column

The effect of increasing temperature on retention time, efficiency and peak symmetry on PS-DVB column has been studied. The retention times and capacity factors decreased with increasing temperature. Efficiency and peak symmetry were improved by the increase of temperature. However, it has been noted that increasing temperature as a method of improving efficiency and symmetry had its limitation. It was more beneficial for compounds with longer retention times. On the other hand, poorer selectivity might be obtained at high temperatures.

The difference of the polarity of the mobile phase of RP-HPLC and NP-HPLC caused different eluent order. For example, AAK were retained longer in RP-HPLC compared with phenylalkanols, but were retained less in NP-HPLC. The efficiencies of compounds with similar retention times at the same temperature were higher usually in RP-HPLC than in NP-HPLC. Furthermore, because of the limitation of the non-polarity of mobile phase, the selectivity of solvents in the NP-HPLC was not as wide as in RP-HPLC.

In NP-HPLC, it was found that the use of a low level of polar organic modifier in non-polar solvent could reduce the retention times and improve peak shape and column efficiency.

It was interesting to note that the efficiency on the PS-DVB column decreased with increasing retention time in both NP-HPLC and RP-HPLC. However, the efficiency on ODS column usually increases with increasing retention time as extra column dead volume less significant.

Chapter 6. Introduction to Retention Prediction in RP-HPLC

6.1 Introduction

HPLC is used for the separation of mixtures of compounds. If it is possible to determine the retention behaviour of each component of the mixture then the experiment conditions can be optimised, and better resolution, peak shape and column efficiency can be obtained. Based on this ideal, a number of retention prediction methods have been developed in HPLC. The details will be discussed in the following section.

6.2 Retention prediction in HPLC

There have been many methods to predict retention in RP-HPLC. The simplest ones are those in which the retention is calculated by extrapolation or interpolation between experimental data. These basic empirical methods do not require a knowledge of the molecular structure of the compounds being separated, but there are deviations especially for those values derived by extrapolation. More useful prediction methods have been based on additive structural properties of the solute. These prediction methods generally exclude any specific interactions between the solute and hydrocarbonaceous stationary phase, and assume that the mobile phase has a dominant role in the retention process. This solvophobic theory of retention was proposed by Horvath (104,105) and has been widely used. Other retention prediction mechanisms have also been proposed but few have been used in practice because of the more complex parameters that would be required.

6.2.1 Retention Prediction by Solvent Strength and pH

Many optimisation methods use mobile phase relationships to predict the

retentions of compounds (106). In RP-HPLC separations, it is generally observed that there is a linear relationship between log k' and mobile phase composition for a particular organic modifier (equation 6.1)

$$\log k' = \log k_W - S\,\phi \qquad (6.1)$$

Where k' is the capacity factor of the solute, k_W is the extrapolated value of k' for pure water as mobile phase, ϕ is the volume fraction of the organic modifier in the mobile phase and the S is a constant for a given solute and organic modifier. This linear relationship has led to the prediction of the behaviour of compounds on changing eluent compositions. In fact these methods are interpolation or extrapolation methods rather than "prediction".

Two methods which are related to the molecular structure of the compound have been proposed by Baty *et al.* (107) and Cooper (108). In these two methods, a set of calibration standards structurally closely related to the analyte were used to obtain equations which describe the change in retention with eluent composition. Using the calibration line it was possible to calculate the retention of "unknown" compounds from a single measured capacity factor. However, these methods are basically also extrapolation or interpolation methods as they hardly make an use of the molecular structure.

Lewis and co-workers (109,110) used a computer simulation software (Drylab I/mp) to predict HPLC separation as a function of changes in mobile phase pH. However, HPLC method development based on selecting an optimum mobile phase pH is only effective for samples that contain acidic or basic solutes, whose ionisation and retention change as a function of pH.

6.2.2 Retention Prediction Using Molecular Size and Shape Parameters

A linear relationship between carbon number and logarithm of the capacity

factor for the members of a homologous series has been verified for many different homologous series (2,52,54,73,86,88). This relationship has also formed the basis of most retention index scales in both GC and LC. To predict the retention of the solute it is necessary to measure the retention of two or more members of the same homologous series. The retention of the rest of the homologous can be calculated either by extrapolation or interpolation. The retention of any member of a homologous series at any temperature and any eluent composition can also be calculated from the retentions of two members of the same homologous series. However, it was found that this was insufficient to calculate the retention of 54 alkylbenzenes and polymethylbenzenes by using carbon number only (111). Therefore the combinations of the carbon number with the number of double bonds and rings were proposed by some workers (112,113).

Molecular connectivity index is another more useful size and shape descriptor for retention prediction. It is based on graph theory and provide a description of the shape of the molecule. A linear relationship between molecular connectivity index and log k' has been reported (114-118). A few workers have extended the method to predict the retention of compounds from their calculated values (113-118). However, it has been found that molecular connectivity index was insufficient to account for the elution order of branched chain alkanes (119) alkylbenzenes (17,111), and diols (120).

6.2.3 Retention Prediction Using Octanol-Water Partition Coefficients

The molecular interactions are probably related to solubility. A relative solubility in water expressed as an octanol-water partition coefficient (log P_{oct})has been proposed mathematically as Hansch's π- constant (121) (equation 6.2).

$$\log P_{Ph\text{-}X} = \log P_{Ph\text{-}H} + \pi \qquad (6.2)$$

Where log P_{Ph-X} is the partition coefficient of compound with substituent (X) and log P_{Ph-H} is the partition coefficient of its parent compound. This constant was later used as Rekker's hydrophobic fragmental constant (122). Rekker's hydrophobic fragmental constant has been used to optimise reversed-phase liquid chromatography (123), and Hammett's equation has been used to predict the retention time of ionisable compounds (123).

The partition coefficient (log P) calculated by Rekker's method has a linear relationship with log k' measured in reversed-phase liquid chromatography (124) (equation 6.3)

$$\log k' = Y*\log P + m \qquad (6.3)$$

where Y and m are constants in a given system. Good correlation coefficients for the linear relationship between log k' and log P have been reported for many groups of compounds (125,126). However, it has been noted that hydrogen-bonding and non-hydrogen bonding species need to treated separately (127,128).

Using the observed linear relationship, it should be possible to calculate the capacity factor of a compound from its known log P. However, complications have arisen as the slope of the relationship between log P and log k' was dependent on the column material, the eluent composition and the type of the compounds being studied. Braumann (126) has reported that there should be unit slope between log k' and log P and the values obtained should be independent of the stationary phase at 0% organic modifier (129). However, it is unusual to use 100% water in practice because of the long retention times that would be obtained.

Miyake *et al.* (130) investigated the relationship between log k' and log P of compounds with hydrogen bonding on ODS and gly-CPG (glyceryl-coated controlled-pore glass) columns and found that log k' increased with increase in log P_{oct} on both columns. The introduction of a hydrogen-bonding term was necessary to obtain a single correlation between log k' and log P_{oct} for a wide variety of

compounds.

6.2.4 Retention Prediction by Other Methods

There are also many other methods apart from those discussed above, such as methods based on gradient elution, molecular surface area, aqueous solubility, solvatochromic parameter, group contributions, thermodynamic parameters and computer based prediction methods etc. In a review, Hanai (123) discussed the existing methods and proposed using linear relationship between enthalpy and log k' to predict retention behaviour of a "unknown" compound. However, the correlation of this relationship is not very high.

6.3 The use of retention indices in RP-HPLC

The capacity factor is the commonest way to report the retentions of compounds in RP-HPLC. Although it should be independent of the column dimensions and eluent flow rate, it is susceptible to the measurement of void volume and temperature, and has poor reproducibility. On the other hand retention indices have been shown to be robust to small changes in the experimental conditions (18).

Most retention index scales are based on the linear relationship between the carbon number and logarithm capacity factor for a homologous series. A successful standard homologous series should have a reasonably strong UV chromophore and cover the polarity range of typical analytes. The standards should not be ionised under the experimental conditions (18). n-Alkanes are among the compounds suggested as standards because of their use in GLC. However, this series are found to be not generally applicable in HPLC because of the problem of the lack of a UV chromophore and their limited polarity range. Some other homologous series have been suggested, such as n-alkylbenzenes, alkan-2-ones, esters, polycyclic hydrocarbons, nitroalkanes and alkyl aryl ketones. Of these the alkyl aryl ketone

scale proposed by Smith (14) has been widely used.

In recent years retention indices have been introduced into retention prediction. Although they do change over a wide eluent range and are sensitive to selectivity changes between columns, these changes are considerably less than the corresponding changes in the capacity factors (43). Retention prediction schemes based on retention indices should therefore be more robust than schemes based on capacity factors.

Mockel *et al.* (131-134) used the n-alkane scale to describe the relationship between retention and molecular surface area. Shalaby (135) calculated retention indices from the octanol-water partition coefficient of the analyte using the alkan-2-one retention index scale.

6.4 Introduction to current work

A method of calculating the retention of a compound based on its molecular structure has been developed by Smith and co-workers (136-141). Alkyl aryl ketones was used as the retention index scale, and the retention index of a analyte were calculated as the summation of the parent index, the substituent indices, and terms to account for the interaction between substituents - (the interaction indices). The retention index of a compound at a particular eluent composition could therefore be calculated using equation 6.4.

$$I = I_P + \Sigma I_{S,\,Ar\text{-}X} + \Sigma I_{S,\,Al\text{-}X} + I_{S,\,R} + I_{I,\,Y\text{-}Z} \qquad (6.4)$$

Where: I_p = retention index of a parent compound,

$I_{S,\,Ar\text{-}X}$ = substituent index of the aromatic substituent,

$I_{S,\,Al\text{-}X}$ = substituent index of the aliphatic substituent,

$I_{S,\,R}$ = substituent index of saturated alkyl chain,

$I_{I,\ Y\text{-}Z}$ = interaction indices to account for interactions between substituents.

Each term in the above equation was calculated from retention index increments and was related to the organic modifier concentration of the eluent using a quadratic equation. Thus the retention index could be calculated at any eluent composition.

The retention index increment of aliphatic substituents could be calculated from the difference between the retention index and the predicted value of the corresponding unsubstituted alkylbenzene, which is calculated as the sum of the parent index (I_p), the methylene increments ($I_{S,CH2}$) and as appropriate the interaction correction ($I_{I,PhCH2R}$) for methylene substitution on the benzylic carbon (equation 6.5).

$$\delta I_X = I_{Ph(CH2)n\text{-}X} - [\ I_P + nI_{S,\ CH2} + I_{I,\ PhCH2R}\ (\text{if } n>1)\ ] \qquad (6.5)$$

The retention index increment of the aromatic substituent ($I_{S,\ Ar\text{-}X}$) can be calculated using equation 6.6.

$$\delta I = I_{Ph\text{-}X} - I_{Ph\text{-}H} \qquad (6.6)$$

The retention index increment could then be used to determine the regression equation describing the change in $I_{S,\ Ar\text{-}X}$ with eluent composition. The substituent index at any eluent composition could be calculated using this regression equation.

For the compounds with more than one substituent on aromatic ring (e.g. XC_6H_4Y), their interaction increments (δI) were calculated from the difference between the measured retention index of this compound and the sum of the parent retention index and substituent indices for X and Y

$$\delta I = I_{XPhY} - (I_P + I_{S,X} + I_{S,Y}) \qquad (6.7)$$

A single parent compound, benzene, was selected throughout the study as its substituent derivatives can be readily detected. The substituent indices have been examined for 14 aromatic functional groups and 12 aliphatic functional groups in methanol and acetonitrile buffer eluent at different composition by Smith and Burr (136-141) and the coefficients of the regression equations were held in a database. An expert system CRIPES was also developed as an interface to these data (140). The substituent indices of some aromatic and aliphatic functional groups in THF have been determined by Smith and Wang (142).

There was a particular need to develop a more general set of values to predict interaction between substituents, as the values obtained initially were empirical and limited in specific compounds, to pairs of substituents which has been tested empirically. A simplified prediction model for interactions has been proposed by Leo (143, 144)

$$\log P = \Sigma\pi + (\rho_Y\sigma_X + \rho_X\sigma_Y) - 0.29F_O + 0.63F_{HB} - 0.15F_{a\phi} \qquad (6.8)$$

Where σ is Hammett constant, ρ, F_{HB} and F_o represent the susceptibility constant, intramolecular hydrogen bonding, and negative *ortho* effects, respectively. $F_{a\phi}$ is the presence of alkyl-aryl system. Although a single intramolecular hydrogen bonding term was used, this was found to be insufficient to account for the observed hydrogen bonding effects between *ortho* -hydroxyl and amide groups (141).

Smith and Burr (141) proposed a prediction model in which each substituent is associated with a set of terms that can reflect their mutual interactions. An equation (6.9) was derived for $I_{I,X-Y}$ based on equation 6.8.

$$I_{I,X\text{-}Y} = (\sigma_X\rho_Y^* + \sigma_Y\rho_X^*) + F_{HB}^* + F_o^* . \qquad (6.9)$$

In each instance each term are expressed in retention index units and could be directly related to the Hammett constant through a common regression equation for the eluent composition, e.g. $\rho^* = \rho\ (ax^2 + bx + c)$.

However, some commonly found functional groups had not been included because of the lack of standards and time. The retention index increments of compounds determined at different THF buffer compositions by Smith and Wang (142) had not been included in the worksheet of the VP-expert system and the knowledge base of the program was not developed with THF buffer as eluent. Therefore, the first stage of the present work was to examine additional compounds, and to add the values to the database. The development of the knowledge base of the VP-expert system was considered as the second task of this study, some new rules would be set and other information would be added if necessary.

The retention increments of disubstituted compounds in THF had also not been examined. In this study a set of compounds would also be selected to try to obtain the relationships with Hammett constant, so that the disubstitution interactions of other compounds could be calculated.

In most cases the substituents would not have an isolated effect on the retention. The observed retention always is the result of combined effects of the substituents. Therefore interaction terms may not be simply additive. There are various interactions which can occur between substituents on an aromatic ring such as hydrogen bonding and electronic effects involving electron donating and accepting groups. The size of these interactions will depend on the position of substituents and their type. When substituents are in *ortho* position steric interactions can occur and if one of the substituent is a hydroxyl group (or an amino group) there is the possibility of intramolecular hydrogen bonding (143). The effect of hydrogen-bonding of the compounds in THF would also be investigated in this study.

Chapter 7. Experimental for Retention Prediction Study

7.1 Chemicals

7.1.1 Mobile Phase Components

Methanol, acetonitrile and THF were HPLC grade (FSA Laboratory Supplies, Loughborough) and distilled water was used to adjust composition of mobile phase. Sodium dihydrogen orthophosphate and disodium hydrogen orthophosphate were AR grade (FSA Laboratory Supplies, Loughborough).

7.1.2 Retention Index Standards

Acetophenone, propiophenone, butyrophenone, valerophenone, hexanophenone, heptanophenone were from various sources (see Chapter 3).

7.1.3 Model and Test Compounds

The model and test compounds were laboratory grade purchased from various sources. 4-Phenylbutyramide and methyl 4-phenylbutyrate were prepared from phenylbutyryl chloride by reaction with ammonium hydroxide and methanol, respectively.

Void volume marker was the same as described in chapter 3 for RP-HPLC.

7.2 HPLC Equipment

The HPLC equipment was the same as described in Chapter 3. The column was Spherisorb ODS 2 (Batch 23/151, Phase Separations, Queensferry, U.K.), which was the same batch as used in earlier study (138-142).

7.3 Experimental Procedure

7.3.1 Mobile Phase Preparation

The aqueous phase was a pH 7 buffer prepared from 1.5g of sodium dihydrogen orthophosphate and 1.3g of disodium hydrogen orthophosphate dissolved in 1L distilled water (as ref. 126 = pH 7.0). The buffer was diluted 10 fold at 80% acetonitrile organic modifier to prevent precipitation of the buffer salts. The preparation of mobile phases with different composition were the same as in chapter 3.

7.3.2 Solute and Void Volume Preparation

The processes of the preparation of the solute and void volume were the same as in Chapter 3.

7.3.3 Column Testing

A column was repacked when the peak shape deteriorated. During the study three columns were packed in the laboratory using the same batch of packing material. The columns were packed by using an upward slurry packing method at a pressure of 6000-6500 psi. The packing material (1.8g) was suspended in 5 ml of propan-2-ol and packed in propan-2-ol. Methanol-water (50:50) was used to condition the column.

The columns were tested for efficiency using an eluent containing methanol-water (70:30). The test solution containing benzamide, acetophenone, benzophenone and biphenyl. The efficiency was measured on the biphenyl peak and was around 2500 for each 100mm long column.

To ensure that the retentive capacity of the different columns was comparable,

after packing each column was tested at a single eluent composition (methanol-water 60:40) using a set of model compounds. For each eluent composition the column performance was monitored on a daily basis by testing the retention index of two model compounds, phenol and toluene, and comparing the results with those in Smith and Burr (136-141).

7.4 The Calculation of Data

7.4.1 Capacity Factor

The calculation of capacity factors were the same as described before (Chapter3).

7.4.2. Retention Indices

Retention indices were obtained for the test solutes by substitution of their capacity factors into a linear least squares regression equation which was obtained from the plot of log k' vs 100 x carbon number for the alkyl aryl ketone standards which had been run as part of the same sequence of injections. A computer program was written in BASIC to carry out the calculation.

Parent retention indices were calculated from the published quadratic regression equation relating the change in the retention index of the parent (benzene) to the methanol 40-80%, acetonitrile 30-80% and THF 20-60% (136-142).

7.4.3 Substituent Indices

Retention index increment for a substituent were calculated using the equation 7.1.

$$\delta I = I_X - I_P. \qquad (7.1)$$

Where I_X is the retention index of a substituted compound and I_P is the calculated parent index. Substituent indices (I_S) were calculated from quadratic regression equations relating the change in the retention index increments to eluent composition.

7.4.4. Interaction Increments

Interactions between substituents were measured using disubstituted compounds. The interaction increments were calculated from the difference between the measured retention index of those compounds (I_{PhXY}) and the sum of the parent index (I_P) and the substituent indices of the substituents present ($I_{S,X}$, $I_{S,Y}$) (equation 7.2).

$$\delta I = I_{Phxy} - (I_P + I_{S,X} + I_{S,Y}) \qquad (7.2)$$

Chapter 8 Retention Prediction in RP-HPLC

During the study of retention prediction, three columns were used and each was packed in the laboratory using the same batch of packing material. In order to confirm the reproducibility of retention indices on the different columns and to make sure that the columns were packed correctly, the retention of a set of test compounds were measured at a single eluent composition on each column (methanol-water 60:40). The results are shown in Table 8.1. It was noted that the capacity factors on different columns changed significantly. The biggest change happened between column A and column C, which could reach over 20%. The retention indices showed very strong stability, the differences were only within 6 units and these were very close to those found by Smith and Burr (137). This further confirmed the choice of the retention indices as a robust retention measurement for compounds in this study.

8.1 Determination of substituent indices of compounds

To extend the database for the expert system (CRIPES), eight compounds with aromatic functional groups and seven compounds with aliphatic functional groups were tested in either methanol, or acetonitrile, or THF buffer eluents under a range of different compositions. Benzene was used as a parent compound. The calculations of retention indices of compounds were based on alkyl aryl ketones (acetophenone to heptanophenone) retention index scale.

8.1.1 The Substituent Indices of Aromatic Compounds

There were eight aromatic compounds containing additional functional groups to be examined in this study. In addition to these compounds, phenol and toluene have been examined in the previous study and would be used in the present study

Table 8.1 Reproducibility of capacity factors and retention indices on different columns (A-C) packed with the same batch of Spherisorb ODS-2 using the same experimental condition (methanol-water 60:40) and comparison with previous study

Compounds	Capacity factor			Retention index			
	A	B	C	A	B	C	ref. 135
acetophenone	1.91	1.96	1.77				
propiophenone	3.56	3.68	3.22				
butyrophenone	6.32	6.60	5.59				
valerophenone	11.90	12.60	10.40				
hexanophenone	22.97	24.61	19.59				
heptanophenone	44.85	48.67	38.72				
benzyl alcohol	0.96	0.96	0.88	694	693	691	691
benzyl cyanide	1.45	1.48	1.32	759	760	763	756
methyl phenylacetate	2.78	2.86	2.46	863	863	864	862
toluene	8.12	8.37	7.29	1033	1030	1043	1039
benzene	4.26	4.35	3.84	931	929	939	938
phenol	0.94	0.95	0.84	691	690	689	691
benzyl bromide	7.17	7.64	6.40	1014	1016	1021	1020

as reference compounds for monitoring the reproducibility of retention indices and experimental conditions. Some of the combinations of analytes and modifiers had been measured previously and were not examined here. Retention times and capacity factors were very small at 90 % methanol, 90 % acetonitrile and 70 % THF and it has been suggested that 90 % methanol might cause a selectivity change in the system (136). As a result it was decided to restrict the composition ranges up to 80 % for methanol and acetonitrile and 60% for THF eluent in this study.

The capacity factors of these compounds are shown in Tables 8.1.1 to 8.1.3. It can be noted that the capacity factors decreased as the proportion of the organic modifier increased. The regression equations and correlations of alkyl aryl ketones are listed in Table 8.1.4. These values were used to calculate retention indices (Table 8.1.5 to Table 8.1.7), which showed some changes across wide composition ranges. However, the relative changes were considerably less than the corresponding changes in capacity factors, and the effects of the changes of retention indices were not certain.

A comparison of retention indices for aniline (Ph-NH_2, ref.136), N-ethylaniline (Ph-NHC_2H_5) and N,N-dimethylaniline (Ph-$N(CH_3)_2$) showed that the retention indices increased with number of substituents. As expected , the difference between the amino group (-NH_2) and N-ethyl group (-NHC_2H_5) in methanol and acetonitrile buffer eluent was about 200 units, the difference increased to 250 units at higher proportions of methanol as mentioned by Smith and Wang (142) in THF. However, the difference was not apparent in acetonitrile. The retention indices for isomer N-ethylaniline (-NHC_2H_5) and N,N-dimethylaniline (-$N(CH_3)_2$) (Table 8.1.5 and Table 8.1.6) were different. Although their molecular weight are the same, N,N-dimethylaniline had a longer retention. The difference of retention indices between these two groups was not significant in THF (within 12 units, data of N-ethylaniline from ref. 142. e.g; 20%, 943; 30%, 976; 40%, 994; 50%, 1000; 60%, 1004), but it was marked in methanol and acetonitrile buffer eluents. The difference increased to 108 units in acetonitrile and 141 units in methanol with proportion of modifier. This suggested that the influence of eluent on these substituents was

Table 8.1.1 The capacity factors of compounds containing aromatic substituents over a range of methanol-buffer compositions

	Capacity factor				
	Eluent composition (% methanol)				
Compound	40	50	60	70	80
benzene sulphonamide	0.82	0.53	0.33	0.23	0.30
N-ethylaniline	11.55	6.06	2.88	1.42	0.94
N,N-dimethylaniline	22.27	11.23	5.18	2.51	1.51
fluorobenzene	16.30	8.43	3.91	1.86	1.09
acetanilide	2.39	1.41	0.79	0.46	0.46
naphthalene	91.17	34.78	12.41	4.87	2.36
phenol	2.48	1.57	0.88	0.52	0.48
toluene	34.07	17.04	7.25	3.28	1.78
acetophenone	7.14	3.58	1.75	0.94	0.72
propiophenone	16.72	7.43	3.20	1.52	0.96
butyrophenone	38.04	14.85	5.59	2.37	1.28
valerophenone	94.54	32.06	10.39	3.86	1.81
hexanophenone	244.39	71.44	19.82	6.42	2.58
heptanophenone	639.61	161.06	37.17	10.79	3.78

Table 8.1.2 The capacity factors of compounds containing aromatic substituents over a range of acetonitrile-buffer compositions

	Capacity factor					
	Eluent composition (% acetonitrile)					
Compound	30	40	50	60	70	80
benzene sulphonamide	1.25	0.82	0.62	0.46	0.31	0.24
N-ethylaniline	15.66	8.07	3.99	2.30	1.36	0.86
N,N-dimethylaniline	23.65	11.71	5.55	3.15	1.84	1.18
fluorobenzene	17.62	8.86	4.24	2.40	1.40	0.86
acetanilide	1.94	1.16	0.80	0.59	0.42	0.32
naphthalene	73.59	27.69	10.48	4.96	2.66	1.56
phenol	2.75	1.66	1.09	0.73	0.49	0.35
toluene	34.21	15.71	6.85	3.68	2.08	1.28
acetophenone	6.22	3.53	2.17	1.32	0.87	0.63
propiophenone	15.12	7.37	3.70	2.10	1.28	0.82
butyrophenone	33.18	14.07	5.99	3.12	1.78	1.10
valerophenone	75.87	27.51	9.93	4.74	2.51	1.48
hexanophenone	176.65	55.04	16.70	7.32	3.63	2.02
heptanophenone		109.92	28.67	11.42	5.30	2.77

Table 8.1.3 The capacity factors of compounds containing aromatic substituents over a range of THF-buffer compositions

	Capacity factor				
	Eluent composition (% THF)				
Compound	20	30	40	50	60
N,N-dimethylaniline	56.87	22.27	9.25	4.85	2.04
phenol	11.66	6.07	2.98	1.84	0.94
toluene	106.60	37.24	13.56	6.56	2.57
acetophenone	10.90	4.92	2.51	1.71	0.99
propiophenone	32.45	11.85	5.15	2.99	1.39
butyrophenone	87.23	25.71	9.13	4.56	1.86
valerophenone	251.05	54.48	16.11	6.87	2.39
hexanophenone		118.81	27.93	10.19	3.01
heptanophenone		250.92	46.83	14.76	3.78

Table 8.1.4 The regression equations and correlation of AAK in different eluents ($I = a + b \times \log k'$, $I = 100Cn$)

Composition (%)	Regression Equation	Correlation
	Methanol-buffer	
40	y = -5.2633 + 8.9714E-3x	0.999
50	y = -4.8300 + 7.5857E-3x	0.999
60	y = -4.3580 + 6.1171E-3x	0.999
70	y = -3.7680 + 4.6886E-3x	0.996
80	y = -3.0247 + 3.3171E-3x	0.997
	acetonitrile-buffer	
30	y = -4.7800 + 8.2900E-3x	1.000
40	y = -4.1847 + 6.8314E-3x	1.000
50	y = -3.3720 + 5.1686E-3x	1.000
60	y = -3.1327 + 4.2771E-3x	1.000
70	y = -2.9913 + 3.5743E-3x	0.999
80	y = -2.8587 + 2.9686E-3x	0.999
	THF-buffer	
20	y = -5.9220 + 1.0410E-3x	1.000
30	y = -4.6107 + 7.8229E-3x	0.999
40	y = -3.6320 + 5.7971E-3x	0.997
50	y = -2.7627 + 4.2343E-3x	0.995
60	y = -2.0713 + 2.6457E-3x	0.994

Table 8.1.5 The retention indices of aromatic compounds over a range of methanol-buffer compositions

	Retention index				
	Eluent composition (% methanol)				
Compound	40	50	60	70	80
benzene*	885	913	938	961	982
benzene sulphonamide	564	554	531	490	550
N-ethylaniline	860	874	886	878	894
N,N-dimethylaniline	932	956	981	1000	1035
fluorobenzene	898	918	935	936	939
acetanilide	684	682	673	637	677
naphthalene	1089	1105	1124	1141	1171
phenol	688	696	691	665	692
toluene	980	1011	1036	1057	1087

* calculated retention index of benzene (from ref.136)

Table 8.1.6 The retention indices of aromatic compounds over a range of acetonitrile-buffer compositions

	Retention index					
	Eluent composition (% acetonitrile)					
Compound	30	40	50	60	70	80
benzene*	910	927	940	951	958	963
benzene sulphonamide	603	583	560	550	510	481
N-methylaniline	908	919	919	926	924	912
N,N-dimethylaniline	958	973	983	1001	1008	1020
fluorobenzene	923	932	931	938	932	912
acetanilide	656	635	610	609	593	579
naphthalene	1095	1099	1107	1107	1111	1111
phenol	698	687	670	660	638	609
toluene	1002	1015	1024	1036	1041	1047

* calculated retention index of benzene (from ref. 136)

Table 8.1.7 The retention indices of aromatic compounds over a range of THF-buffer compositions

	Retention index				
	Eluent composition (% THF)				
Compound	20	30	40	50	60
benzene*	944	966	994	1026	1063
N,N-dimethylaniline	957	986	1009	1026	1051
phenol	805	819	815	797	760
toluene	1017	1052	1077	1096	1138

* calculated retention index of benzene (from ref. 142)

different.

The calculated retention index of benzene calculated from the quadratic least squares relationship between the experimental retention index of benzene and the proportion of modifier in the eluent was used as the reference values (parent index value) for the database (136). Using the calculated parent index (I_P) values for benzene, the effect of each substituent can be calculated as the retention index increments by the following equation (from ref. 136)

$$\text{Retention index increment } (\delta I) = I_{Ph\text{-}X} - I_{Ph\text{-}H} \qquad (8.1)$$

The results of the retention index increments of the substituents at 40 to 80 % methanol, 30 to 80 % acetonitrile and 20 to 60 % THF buffer eluent are listed in the Table 8.1.8 to Table 8.1.10. The values for the aromatic substituent (Ph-X) of the mixed alkyl aryl substituents (Ph-X-R) were calculated by excluding the contributions from the aliphatic groups (e.g. -Me, I_S = 100, -Et, I_S = 200). The coefficients of the quadratic equation between the retention index increment and the percentage of composition of eluent were obtained at each eluent (Table 8.1.11 to Table 8.1.13). These coefficients would be used in the prediction to calculate the substituent index (I_S) values as $I_S = ax^2+bx+c$.

It was noted (Table 8.1.8 to Table 8.1.10) that the retention index increment for the methyl group changed with the eluent. They decreased with the increase of the strength of eluent. The smallest values were obtained in THF buffer eluent that agreed with the results by Smith and Wang (142).

It was also noted that the values of retention index increments of substituents were related to the increments of Hansch constant (π). The correlations between the π values for aromatic substituents and the corresponding I_S values at different eluent compositions have been determined (Table 8.1.14). As reported by Smith and Burr (136), the approximately linear relationship were obtained in methanol

Table 8.1.8 The retention index increments of aromatic substituents over a range of methanol-buffer compositions (from equation 8.1)

	Retention index increment					
	Methanol (%)					
Substituent	40	50	60	70	80	π^*
SO_2NH_2	-321	-359	-407	-471	-432	-1.82
NHC_2H_5	-25	-39	-52	-83	-88	0.08
NHR	-225	-239	-252	-283	-288	
$N(CH_3)_2$	47	43	43	39	53	0.18
NR_2	-153	-157	-157	-161	-147	
F	13	5	-3	-25	-43	0.14
$NHCOCH_3$	-201	-231	-265	-324	-205	-0.97
NHCOR	-301	-331	-365	-424	-305	
OH	-197	-217	-247	-296	-290	-0.67
CH_3	95	98	98	96	105	0.56
CH=CH-CH=CH	204	192	186	180	189	

* data from ref.136

Table 8.1.9 The retention index increments of aromatic substituents over a range of acetonitrile-buffer compositions (from equation 8.1)

	Retention index increment						
	Acetonitrile (%)						
Substituent	30	40	50	60	70	80	π*
SO_2NH_2	-307	-344	-380	-401	-448	-482	-1.82
NHC_2H_5	198	192	179	175	166	149	0.08
NHR	-2	-8	-21	-25	-34	-51	
$NH(CH_3)_2$	248	246	243	250	250	257	0.18
NR_2	48	46	43	50	50	57	
F	13	5	-9	-13	-26	-51	0.14
$NHCOCH_3$	-254	-292	-330	-342	-365	-384	-0.97
NHCOR	-354	-392	-430	-442	-465	-484	
OH	-212	-240	-270	-291	-320	-354	-0.67
CH_3	92	88	84	85	83	84	0.56
CH=CH-CH=CH	185	172	167	156	153	148	

* data from ref.136

Table 8.1.10 The retention index increments of aromatic substituents over a range of THF-buffer compositions (from equation 8.1)

	Retention index increment					
	THF (%)					
Substituent	20	30	40	50	60	π*
NR_2	-187	-180	-185	-200	-212	0.18
OH	-139	-147	-179	-229	-303	-0.67
CH_3	73	86	83	70	75	0.56

* data from ref. 136

Table 8.1.11 The coefficients of substituent index equation for aromatic substituents
Methanol-buffer (40:60 to 80:20) ($I_S = ax^2 + bx + c$, x = % methanol)

Substituent	Coefficient		
Ph-X	a	b	c
SO_2NH_2	0.0986	-15.170	138
NHR	0.0000	-1.700	-155
NR_2	0.0243	-2.854	-76
F	-0.0243	1.494	-8
NHCOR	0.0521	-9.267	-7
OH	0.0236	-5.479	-10
CH_3	0.0071	-0.677	112
CH=CH-CH=CH	0.0300	-4.020	317

Table 8.1.12 The coefficients of retention index equation for aromatic substituents
acetonitrile-buffer (30:70 to 80:20) ($I_S = ax^2 + bx + c$, x = % acetonitrile)

Substituent	Coefficient		
Ph-X	a	b	c
SO_2NH_2	-0.0052	-2.882	-218
NHR	-0.0070	-0.168	-191
NR_2	0.0102	-0.937	-133
F	-0.0145	0.400	12
NHCOR	0.0277	-5.562	-214
OH	0.0046	-2.264	-141
CH_3	0.0059	-0.803	11
CH=CH-CH=CH	0.0086	-1.666	227

Table 8.1.13 The coefficients of retention index equation for aromatic substituents THF-buffer (20:80 to 60:40) ($I_S = ax^2 + bx + c$, x = % THF)

Substituent	Coefficient		
Ph-X	a	b	c
NHR	-0.0343	2.043	-213
OH	-0.1071	4.471	-185
CH_3	-0.0186	1.366	56

Table 8.1.14 Regression coefficients for p compared to calculated substituent indices (from Tables 8.1.8 and 8.1.9) $I_S = a\pi + b$

Composition	Regression equation	Correlation
Methanol (%)		
40	y = -19.03 + 182.31x	0.960
50	y = -28.85 + 199.23x	0.963
60	y = -40.09 + 220.94x	0.963
70	y = -63.13 + 248.83x	0.956
80	y = -50.57 + 218.41x	0.905
Acetonitrile (%)		
30	y = 52.14 + 234.78x	0.753
40	y = 40.96 + 252.58x	0.764
50	y = 27.00 + 268.80x	0.772
60	y = 23.18 + 279.70x	0.776
70	y = 12.41 + 298.76x	0.791
80	y = -0.01 + 312.35x	0.791

containing eluent, but the correlation were poor in acetonitrile.

The retention index increments of naphthalene over the range of methanol and acetonitrile (Table 8.1.8 and Table 8.1.9) were compared with those of 1-phenyl-1-propene (data from ref.139. In MeOH: 40%, 145; 50%, 141; 60%, 139; 70%, 136; 80%, 111. In MeCN: 30%, 126; 40%, 117; 50%, 109; 60%, 106; 70%, 103; 80%, 115). The results showed that the values of naphthalene were higher than 1-phenyl-1-propene, but much less two times of values of 1-phenyl-1-propene. This suggested that the influence of -CH=CH-CH=CH- differed from two ethylene (-CH=CH-) and this two substituents should be treated separately, although their molecular weight were same. The performance of -CH=CH-CH=CH- also differed from four methyl groups because the value of each methylene was defined as 100 (136).

8.1.2 The Substituent Indices of Aliphatic Compounds

In this section the retention indices of aliphatic substituents will be discussed. The capacity factors (Table 8.1.15 to Table 8.1.17) and retention indices (Table 8.1.18 to Table 8.1.20) of seven compounds containing aliphatic substituents were measured in either 40-80 % methanol, 30-80 % acetonitrile or 20-60 % THF. It was noted that the capacity factors showed systematic reductions with increased modifier in the eluent. The changes in retention indices were not as marked as for the capacity factors but all the compounds showed some dependence on the eluent composition. The retention indices of 4-phenylbutyramide showed a particularly strong dependence, with a change with the range of composition of 173 units in methanol, 138 units in acetonitrile and 268 units in THF.

It was found (138) that the defined value of 100 units for the addition of a methylene group to an alkyl chain substituted on benzene was generally valid but it was not suitable for the addition of a methylene group to a benzylic carbon. It was therefore necessary to apply an interaction index correction of -12 units to exclude

Table 8.1.15 The capacity factors of compounds containing aliphatic substituents over a range of methanol-buffer compositions

	Capacity factor				
	Eluent composition (% methanol)				
Compound	40	50	60	70	80
4-phenylbutyramide	6.06	2.84	1.30	0.66	0.52
hydrocinnamaldehyde	5.31	2.86	1.53	0.96	0.69
acetophenone	7.14	3.58	1.75	0.94	0.72
propiophenone	16.72	7.43	3.20	1.52	0.96
butyrophenone	38.04	14.85	5.59	2.37	1.28
valerophenone	94.54	32.06	10.39	3.86	1.81
hexanophenone	244.39	71.44	19.82	6.42	2.58
heptanophenone	639.61	161.06	37.17	10.79	3.78

Table 8.1.16 The capacity factors of compounds containing aliphatic substituents over a range of acetonitrile-buffer compositions

	Capacity factor					
	Eluent composition (% acetonitrile)					
Compound	30	40	50	60	70	80
4-phenylbutyramide	3.19	1.54	0.91	0.62	0.42	0.32
hydrocinnamaldehyde	5.55	3.22	1.96	1.47	0.81	0.61
acetophenone	6.22	3.53	2.17	1.32	0.87	0.63
propiophenone	15.12	7.37	3.70	2.10	1.28	0.82
butyrophenone	33.18	14.07	5.99	3.12	1.78	1.10
valerophenone	75.87	27.51	9.93	4.74	2.51	1.48
hexanophenone	176.65	55.04	16.70	7.32	3.63	2.02
heptanophenone		109.92	28.67	11.42	5.30	2.77

Table 8.1.17 The capacity factors of compounds containing aliphatic Substituents over a range of THF-buffer compositions

	Capacity factor				
	Eluent composition (% THF)				
Compound	20	30	40	50	60
1-phenyl-1-propene	286.42	94.89	24.57	9.57	3.10
3-phenyl-1-propene	47.05	14.17	5.39	2.76	1.17
4-phenylbutyramide	9.27	2.79	1.15	0.76	0.49
methyl-4--phenylbutyrate	155.00	34.35	10.86	4.94	1.90
methyl phenylethyl ether	35.95	14.15	6.14	3.48	1.64
4-phenyl-2-butanone	15.48	7.62	2.94	1.66	0.89
hydrocinnamaldehyde	9.65	4.93	2.61	1.78	1.06
acetophenone	10.90	4.92	2.51	1.71	0.99
propiophenone	32.45	11.85	5.15	2.99	1.39
butyrophenone	87.23	25.71	9.13	4.56	1.86
valerophenone	251.05	54.48	16.11	6.87	2.39
hexanophenone		118.81	27.93	10.19	3.01
heptanophenone		250.92	46.83	14.76	3.78

Table 8.1.18 The retention indices of compounds containing aliphatic substituents over a range of methanol-buffer compositions

	Retention index				
	Eluent composition (% methanol)				
Compound	40	50	60	70	80
4-phenylbutyramide	787	774	755	714	716
hydrocinnamaldehyde	773	775	783	795	800

Table 8.1.19 The retention indices of compounds containing aliphatic substituents over a range of acetonitrile-buffer compositions

	Retention index					
	Eluent composition (% acetonitrile)					
Compound	30	40	50	60	70	80
4-phenylbutyramide	717	676	635	620	593	579
hydrocinnamaldehyde	783	784	782	824	778	798

TAble 8.1.20 The retention indices of compounds containing aliphatic substituents over a range of THF-buffer compositions

	Retention index				
	Eluent composition (% THF)				
Compound	20	30	40	50	60
1-phenyl-1-propene	1113	1171	1179	1186	1210
3-phenyl-1-propene	939	928	916	893	843
4-phenylbutyramide	783	721	651	589	515
methyl-4-phenylbutyrate	1053	1042	1039	1030	1025
methyl phenylethyl ether	913	928	939	948	968
4-phenyl-2-butanone	888	885	873	873	848
hydrocinnamaldehyde	787	794	792	789	806

the effect of interaction between benzene and secondary methylene group in methanol and acetonitrile eluents. In the case of THF eluent the interaction index correction would be -14 units (142).

The retention index increment (δI) was the difference between the retention index and the predicted value of the corresponding unsubstituted alkylbenzene, which was calculated as the sum of the parent index for benzene, the methylene increments and as appropriate the interaction correction for methylene substitution on the benzylic carbon

$$\delta I_X = I_{Ph(CH2)n-X} - (I_P + nI_{S,CH2} + I_{I,PhCH2R}) \qquad (8.2)$$

Some substituents, such as NHR, NR_2, COR and OR, also contained saturated alkyl groups which did not directly attached to the aromatic ring. The increments for these corresponding functional groups could be calculated by subtracting 100 units for each aliphatic methylene group. It was noted (Table 8.1.21 to Table 8.1.23) that the differences of retention increments of each substituent across the eluent composition were more significant in THF and less in methanol and acetonitrile. However, the amide group in 4-phenylbutyramide was an exception because of its large change in methanol and acetonitrile.

The effect of introducing a double bond into a alkyl chain has been examined in THF by using 1-phenyl-1-propene ($PhCH=CHCH_3$) and 3-phenyl-1-propene ($PhCH_2CH=CH_2$). These two compounds differed only in the position of the double bond, but their capacity factors and retention indices showed significant difference. This indicated that the retention was dependent on the position of the double bond relative to the ring. Therefore these two compounds were treated separately as an aliphatic unsaturated functional group ($PhRCH=CH_2$) and an aromatic unsaturated functional group (PhCH=CHR). It was found that the double bonds caused a significant reduction in the retention compared to the addition of two methyl

Table 8.1.21 The retention index increments of aliphatic substituents over a range of methanol-buffer compositions (from equation 8.2)

	Retention index increment				
	Methanol (%)				
Substituent	40	50	60	70	80
Substituents on propylbenzene					
$CONH_2$	-386	-427	-471	-535	-554
CHO	-300	-326	-343	-354	-370
Substituents on ethylbenzene					
$CONH_2$*	-368	-403	-459	-486	-530
CHO*	-195	-261	-293	-327	-300

* data from ref. 138

Table 8.1.22 The retention index increments of aliphatic substituents over a range of acetonitrile-buffer compositions (from equation 8.2)

	Retention index increment					
	Acetonitrile (%)					
Substituent	30	40	50	60	70	80
Substituents on propylbenzene						
$CONH_2$	-481	-539	-593	-619	-653	-672
CHO	-315	-331	-346	-315	-368	-353
Substituents on ethylbenzene						
$CONH_2$*	-455	-513	-555	-582	-578	-577
CHO*	-229	-243	-259	-261	-268	-281

* data from ref. 138

Table 8.1.23 The retention index increments of aliphatic substituents over a range of THF-buffer compositions (from equation 8.2)

	Retention index increment				
	THF (%)				
Substituent	20	30	40	50	60
CH=CH	69	105	85	60	47
$CH=CH_2$	-105	-138	-178	-233	-320
$CONH_2$	-447	-531	-629	-723	-834
CO_2R	-177	-250	-341	-382	-424
OR	-317	-324	-341	-364	-381
COR	-256	-281	-321	-353	-415
CHO	-343	-358	-388	-423	-443

groups. The similar results were obtained in methanol and acetonitrile buffer eluent, but they were not as marked as those in THF buffer eluent. The reason for the very large values for 3-phenyl-1-propene are unclear.

If the double bond made no contribution to the retention of these compounds, three carbon chain would be expected to contribute 286 to the retention index in the THF.

$$\delta I_{chain} = 3 \times \delta I_{CH2} + \Pi_{PhCH2\text{-}R} = 3 \times 100 - 14 = 286 \qquad (8.3)$$

In practice the contribution of retention indices of side chain were considerably different from this value.

The coefficients for the quadratic equations (Table 8.1.24 and Table 8.1.26) relating the increments to the eluent composition were calculated. The data of 4-phenylbutyramide and hydrocinnamaldehyde were used to replace the previous measured coefficients (138) as the longest available alkyl chain. A comparison of retention index increments of these data and those obtained earlier with shorter alkyl chain showed that the increment increased with increasing chain length (see Table 8.1.21 and Table 8.1.22), this indicated that the interactions between aromatic ring and substituents were changeable with the distance and the use of data of longest alkyl chain can give the better description of the substituents.

8.1.3 The Substituent Indices of Disubstituted Compounds

In the previous sections the parent retention index, the substituent indices and interaction indices were used to predict the retention indices of mono-substituted alkylbenzenes and benzenes at various eluent compositions using a quadratic relationship. This method of prediction involves a lot of experiments and data processing. These were then used to determine I_I for specific pair analytes by comparing measured and calculated values. However, it would be necessary to

Table 8.1.24 Coefficients of substituent index equation for aliphatic substituents methanol-buffer (40:60 to 80:20) ($I_{R,X} = ax^2 + bx + c$, x = % methanol)

Substituent	Coefficient		
Ph-R-X	a	b	c
$CONH_2$	0.0171	-6.497	-150
CHO	0.0186	-3.909	-175

Table 8.1.25 Coefficients of substituent index equation for aliphatic substituents acetonitrile-buffer (30:70 to 80:20) ($I_{R,X} = ax^2 + bx + c$, x = % acetonitrile)

Substituent	Coefficient		
Ph-R-X	a	b	c
$CONH_2$	0.0491	-9.182	-251
CHO	0.0005	-0.830	-294

Table 8.1.26 Coefficients of substituent index equation for aliphatic substituents
THF-buffer (20:80 to 60:40) ($I_{R,X} = ax^2 + bx + c$, x = % THF)

Substituent	Coefficient		
Ph-R-X	a	b	c
CH=CH	-0.0737	4.996	6
$CH=CH_2$	-0.0879	1.779	-108
$CONH_2$	-0.0357	-6.803	-296
CO_2R	-0.0629	0.769	-252
OR	-0.0186	-0.194	-304
COR	-0.0471	-0.129	-235
CHO	-0.0107	-1.793	-300

measure every possible combinations of analytes to determine all increments. A simpler way of prediction of disubstituents interactions was suggested by Leo (equation 6.8, ref. 144) and used in methanol and acetonitrile eluents with some success by Smith and Burr (141). In this study this method was tried for disubstituents aromatic compounds in THF.

It was reported (141) that for the *meta* - and *para* - substituted phenols, as its σ_X values for OH are relatively small and are assumed to be zero, the general equation of calculation of retention index increment can be written as

$$\delta I = \sigma_Y \rho_X^* = \sigma_Y \rho_X (ax^2 + bx + c) \qquad (8.4)$$

Where σ_Y is Hammett constant of substituent, σ_X is the susceptibility of OH and $ax^2 + bx + c$ is a common regression equation for the eluent composition. It was found that there was a close relationship between the empirical interaction increments δI and σ_Y. Therefore the values of the coefficients a, b, c in equation could be calculated by the ratios $\delta I / \rho_X \sigma_Y$. The mean values of the ratio from the different substituents were virtually independent of the percentage of methanol and acetonitrile and the ratios at the methanol and acetonitrile buffer eluents could be represented by a single value, except for halogens which were represented by a simpler linear expression.

Based on these results a selected group *meta* - and *para* - isomers including 2 halogen isomers were selected to examine the ratios between retention increment (δI) and Hammett constant (σ_Y) times susceptibility (ρ_X). THF-buffer 20:80 to 60:40 were used as eluent. The retention increments of other isomers over THF-buffer eluent composition would be calculated by using these examined ratios.

The capacity factors of compounds (Table 8.1.27) were determined over a range of eluent composition. In each instance, the retentions of homologous series of alkyl

Table 8.1.27 The capacity factors of disubstituent isomers in THF-buffer eluent

	Capacity factor				
	Eluent composition (% THF)				
Compound	20	30	40	50	60
methyl 2-hydroxybenzoate	35.62	14.98	4.72	2.34	1.15
2-hydroxyphenol	3.85	2.66	1.41	0.99	0.58
2-hydroxyacetophenone	16.37	8.16	3.15	1.72	0.94
3-hydroxyacetophenone	4.58	2.67	1.28	0.84	0.51
4-hydroxyacetophenone	3.63	2.16	1.02	0.70	0.46
3-methoxyphenol	7.71	4.24	1.80	1.06	0.60
4-methoxyphenol	4.72	2.99	1.44	0.93	0.56
3-nitrophenol	23.90	10.69	3.25	1.55	0.81
4-nitrophenol	11.35	5.24	1.84	0.96	0.65
3-bromophenol	56.75	18.67	4.60	2.00	0.90
4-bromophenol	52.44	17.25	4.37	1.95	0.88

aryl ketones , acetophenone to heptanophenone, were also measured and these values were used to calculate the retention indices of the compounds (Table 8.1.28). Although the capacity factors changed significantly with the eluent composition, the retention indices were usually relatively constant across the composition range. For most of the substituents the retention indices of the *meta* - and *para* - isomers were similar (within 50 units). However, as in methanol and acetonitrile buffer eluents the difference of retention indices between 3- and 4- nitrophenol isomers were considerable (80-140 units). The capacity factors and retention indices for 4-nitrophenol were abnormally low. As the pK_a of 4-nitrophenol is 7.15 (137), this compound may be partially ionised at the working pH of 7.00 which would result in a large differences between the isomers.

To calculated the size of interactions between substituents, the summation of the parent index and substituent indices were calculated (Table 8.1.29). The difference between the experimental retention indices and the summation has been used to calculate the interaction increments (δI) using the equation (8.5)

$$\delta I = I_{exp} - (I_P + \Sigma I_S + I_{S,R}) \qquad (8.5)$$

at each eluent composition (Table 8.1.30). The values of ρ and σ (Table 8.1.31) were obtained from the literature (121,144,146). The ratios $\delta I/1.06\sigma_Y$ ($\rho_{OH} = 1.06$) were calculated for each inducers substituent (NO_2, Cl, Br, CN) over THF-buffer eluent composition range and the relationships between these ratios and THF-buffer eluent composition were showed in Figure 8.1.1 to Figure 8.1.2. The mean values of the ratios from different substituent were virtually independent of the percentage of THF and suggested that the relationship for THF-buffer eluents could be represented by a single value rather than a quadratic expression, hence $\rho^*_{meta\text{-}X} = (155\text{-}1.4x)\rho_X$ and $\rho^*_{para\text{-}X} = (215\text{-}2.64x)\rho_X$. Using these ratios, the predicted interactions are in

Table 8.1.28 The retention indices of disubstituent isomers in THF-buffer eluent

	Retention index				
	Eluent composition (% THF)				
Compound	20	30	40	50	60
methyl 2-hydroxybenzoate	995	980	977	968	968
2-hydroxyphenol	737	737	719	724	653
2-hydroxyacetophenone	894	895	888	882	876
3-hydroxyacetophenone	755	737	700	677	602
4-hydroxyacetophenone	730	707	652	622	552
3-methoxyphenol	812	802	771	744	667
4-methoxyphenol	758	752	723	706	635
3-nitrophenol	934	932	894	853	810
4-nitrophenol	854	833	775	715	709
3-bromophenol	1029	1011	967	925	852
4-bromophenol	1020	1000	955	920	843

Table 8.1.29 Retention index calculated as the sum of parent index and substituent indices

	The sum of retention indicesx				
	Eluent composition (% THF)				
Compound	20	30	40	50	60
I_P+2I_{OH}	690	656	590	488	353
$I_P+I_{OH}+I_{COR}$	571	538	488	418	331
$I_P+I_{OH}+I_{OR}$	700	686	654	600	526
$I_P+I_{OH}+I_{NO2}$	784	757	706	627	523
$I_P+I_{OH}+I_{Br}$	957	925	882	825	758
$I_P+I_{OH}+I_{CO2R}$	671	630	574	500	411

Table 8.1.30 The interaction increments of disubstituent isomers in THF-buffer eluent

	Retention index increment				
	Eluent composition (%)				
Compound	20	30	40	50	60
OH+2CO_2R	324	350	403	468	557
OH+2OH	47	81	129	236	300
OH+2COR	323	357	400	464	545
OH+3COR	184	199	212	259	271
OH+4COR	159	169	164	204	221
OH+3OR	112	116	117	144	141
OH+4OR	58	66	69	106	109
OH+3NO_2	150	175	188	226	287
OH+4NO_2	70	76	69	88	186
OH+3Br	72	86	85	100	94
ON+4Br	63	75	73	95	85

Table 8.1.31 The values of σ and ρ used in calculation of increments

Substituent	$\sigma meta$ [a]	$\sigma para$ [a]	ρ [b]
CH_3	-0.07	-0.17	0.00[c]
phenyl	0.06	-0.01	0.00[c]
inducers			
CN	0.56	0.66	0.00
NO_2	0.71	0.78	0.00
Br	0.39	0.23	0.00
Cl	0.37	0.23	0.00
Bi-directional			
CHO	0.35	0.42	0.44
CO_2CH_3	0.37	0.45	0.27
$COCH_3$	0.38	0.50	0.27
$CONH_2$	0.28	0.36	0.72
OCH_3	0.12	-0.27	0.50
responders			
OH	0.12	-0.37	1.06
NH_2	-0.16	-0.66	1.08

[a] from ref. 121
[b] from ref. 144
[c] from ref. 146

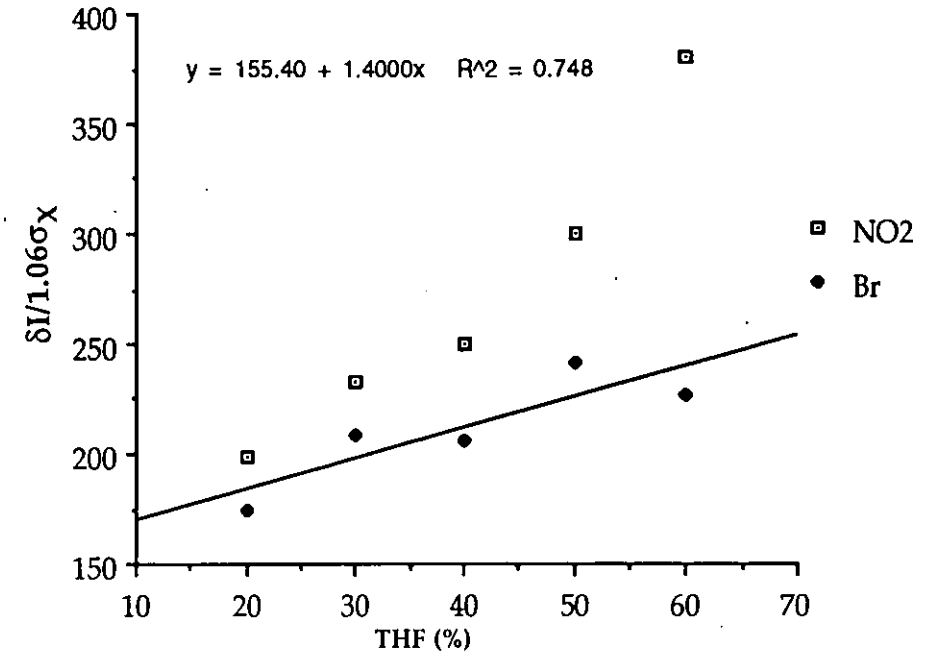

Figure 8.1.1 The relationship between $\delta I/1.06\sigma_X$ and THF-buffer compositions for *meta* - isomers

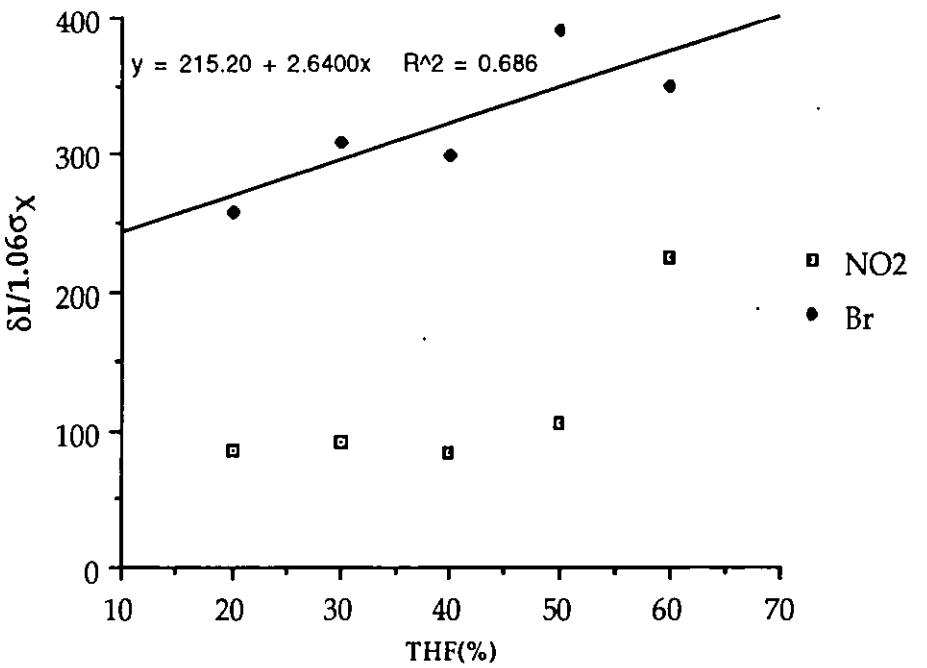

Figure 8.1.2 The relationship between $\delta I/1.06\sigma_X$ and THF-buffer compositions for *para* - isomers

Table 8.1.31. Comparing the predicted values (Table 8.1.32) with the values from Table 8.1 29, it was found that in most instance the predicted I_I and experimental values were not close and the difference tend to be bigger with the proportion of THF since the experimental interaction increments (Table 8.1.30) were not constant across the eluent ranges unlike those in methanol and acetonitrile buffer eluents analysed by Smith and Burr (141). Considering the data of σ and ρ (Table 8.1.30) were obtained in 50 % ethanol (121), the effect of ethanol and THF on the retentions of compounds are considerably different. Therefore this was presumed that the Hammett constants was not suitable when THF was in the eluent.

8.2 The effect of hydrogen bonding in THF

To determine the effect of hydrogen bonding in THF, the *ortho* - phenol isomers were also examined in which there were *ortho* - hydroxyl and carbonyl groups (OH + OH, OH + COR, OH + CO_2R). It was noted that the retention of *ortho* - hydroxyacetophenone was very different from *meta* - and *para* - isomers. The largest interactions (Table 8.1.29) were observed with the carbonyl substituents capable of hydrogen bonding, such as 2-hydroxyacetophenone and methyl 2-hydroxybenzoate, which also differed markedly from the 4- and 3- isomers. This is because these compounds can undergo strong intramolecular hydrogen bonding and the polarity of solute is decreased by intramolecular hydrogen bonding (140). As in methanol and acetonitrile buffer eluents (141) the interactions of 2-hydroxyphenol in THF-buffer eluent were smaller than those *ortho* - compounds with the carbonyl group. The sizes of interactions of these compounds with carbonyl group in THF were larger than those in methanol and acetonitrile, but for the compounds with hydroxy group the size of interactions in THF were slightly higher than that in methanol and slightly lower than that in acetonitrile.

8.3 The use of CRIPES

Table 8.1.32 Predicted interaction index values for *meta-* and *para* - substituted phenols

	Interaction terms[a]				
	THF (%)				
Substituent	20	30	40	50	60
OH+3COR	80	86	92	98	104
OH+4COR	115	127	138	149	161
OH+3OR	34	37	39	42	45
OH+4OR	-126	-139	-151	-164	-176
OH+3NO_2	138	148	159	169	180
OH+4NO_2	221	243	265	287	309
OH+3Br	76	81	87	93	99
OH+4Br	65	72	78	85	91

VP-expert is an expert system development tool written in Microsoft C to run on an IBM compatible PC with a minimum of 300K of memory. It is a rule base system that operates in the backward chaining or goal-driven mode. There are several features which make it particularly suitable for use with the present application. The most important is its capability to handle mathematical functions. VP-Expert can also communicate with compatible external spreadsheets and databases. Therefore the regression coefficients for the parent, substituent and interaction indices can be held outside the main program and updated easily. It is also easy to expand the program for other different eluents.

Smith and Burr (140) have used this program to calculate and predict the retention indices of substituted benzenes in methanol and acetonitrile buffer (pH = 7) eluents on Spherisorb-ODS and Hypersil-ODS. In this study some new rules will be added into the knowledge base, and the coefficients obtained in Section 8.1.1 to 8.1.3 will be stored into the worksheet, as well as those from previous work in this laboratory in THF eluent (142). so that the prediction can be carried out for THF. The program can now be used for consultations in three eluents, namely, methanol, acetonitrile and THF with a range of composition.

To use CRIPES, the user will be asked some questions by the expert system. The user is required to choose the answers from the several choices given by the expert system. An example of the questions is given in Figure 8.3.1. After answering all the questions, the expert system will retrieve the data from the data base and calculate the retention indices and capacity factors using appropriate rules. The final results will be given in the form of Figure 8.3.2.

8.3.1 The Test of CRIPES

To make sure that the program was capable of extracting the appropriate data from the spreadsheet and gave a satisfactory consultation. CRIPES was tested to calculate the retention indices of a number of compounds. The results (Table 8.3.1 to

Table 8.3.3) showed that the calculated data were very close to the experimental data, the differences were within 10 units. These indicated that the CRIPES can work well and correct results can be given. It is ventually hoped to include terms within CRIPES to calculate interactions from Hammett coefficients, although as noted earlier that the values in THF will be unreliable.

Which column do you want the result for?

Spherisorb-ODS Hypersil-ODS

Input name of solute

Toluene

How many aromatic substituents are present in toluene?

1

Which, if any, of these aromatic substituents are present?

COR	CHO	OR
CO_2R	CH_3/	$CONH_2$
OH	CH-CHR	NHR
NR_2	NHCOR	ANOTHER

How many aliphatic substituents present in Toluene?

Is the alkyl chain directly attached to the aromatic ring?

YES/ NO

How many aromatic CH_3 present?

1

How many saturated carbon atoms in the alkyl chain?

1

Printout required?

YES NO

Figure 8.3.1 An example of questions for calculating retention index of toluene by CRIPES

Column:	Sperisorb-ODS					
Solute:	Toluene					
Eluent	Retention index			Approx. capacity factor		
Comp(%)	MECN	MEOH	THF	MECN	MEOH	THF
20	990	921	946	72.69	122.94	28.04
30	1010	954	973	30.62	72.89	11.66
40	1027	984	1004	13.37	44.54	5.17
50	1041	1012	1039	6.24	28.56	2.52
60	1051	1038	1077	3.19	19.38	1.40
70	1059	1061	1120	1.83	14.16	0.93
80	1063	1082	1166	1.18	11.22	0.80
90	1065	1100	1216	0.87	9.75	0.94

Figure 8.3.2 An example of the results print out by CRIPES

Table 8.3.1 Comparison of retention indices between experimental values and calculated values by CRIPES at a range of methanol-buffer eluent

Compound	Eluent composition (% methanol)									
	40		50		60		70		80	
	exp.	cal.	exp.	cal.	exp.	cal.	exp.	cal.	exp.	cal.
phenol	688	678	696	683	691	680	665	669	692	650
toluene	980	984	1011	1012	1036	1037	1057	1061	1087	1081
benzenesulphonamide	564	573	554	538	531	520	490	520	550	537
4-phenylbutyramide	787	790	774	768	755	747	714	728	716	709
N-ethylaniline	860	861	874	872	886	881	878	887	894	891
N,N-dimethylaniline	932	932	956	954	981	978	1000	1005	1035	1033
fluorobenzene	898	897	918	918	935	932	936	938	939	938
naphthalene	1089	1088	1105	1103	1124	1121	1141	1143	1171	1168
acetanilide	684	690	682	672	673	662	637	660	677	667
hydrocinnamaldehyde	773	878	775	839	783	823	795	833	800	865

Table 8.3.2 Comparison of retention indices between experimental values and calculated values by CRIPES at a range of acetonitrile-buffer eluent

Compound	Eluent Composition (% acetonitrile)											
	30		40		50		60		70		80	
	exp.	cal.	exp.	cal.	exp.	cal.	exp.	cal.	exp.	cal.	exp.	cal.
phenol	698	698	687	684	670	671	660	660	638	650	609	640
toluene	1002	1010	1015	1027	1024	1041	1036	1051	1041	1059	1047	1063
benzenesulphonamide	603	600	583	585	560	566	550	542	510	514	481	482
4-phenylbutyramide	717	716	676	675	635	641	620	614	593	594	579	580
N-ethylaniline	908	908	919	918	919	924	926	925	924	922	912	914
N,N-dimethylaniline	958	958	973	973	983	986	1001	999	1008	1010	1020	1020
fluorobenzene	923	921	932	932	931	936	938	935	932	928	912	915
naphthalene	1095	1095	1099	1101	1107	1106	1107	1109	1111	1111	1111	1112
acetanilide	656	654	635	635	610	618	609	603	593	591	579	873
hydrocinnamaldehyde	1002	1010	1015	1027	1024	1041	1036	1051	1041	1059	1047	1063

Table 8.3.3 Comparison of retention indices between experimental values and calculated values by CRIPES at a range of THF-buffer eluent

Compound	Eluent composition (% THF)									
	20		30		40		50		60	
	exp.	cal.	exp.	cal.	exp.	cal.	exp.	cal.	exp.	cal.
phenol	805	819	819	818	815	802	797	770	760	722
toluene	1017	946	1052	973	1077	1004	1096	1039	1138	1077
1-phenyl-1-propene	1113	1123	1171	1163	1179	1192	118	1210	1210	1217
3-phenyl-1-propene	939	939	928	939	916	927	893	900	843	859
4-phenylbutyramide	783	786	721	727	651	665	589	599	515	530
methyl-4-phenylbutyrat	1053	1071	1042	1074	1039	1068	1030	1054	1025	1031
methyl phenyl ethyl ether	913	917	928	933	939	948	948	964	968	981
hydrocinnamaldehyde	787	792	794	796	792	801	789	808	806	817
N,N-dimethylaniline	957	960	986	991	1009	1018	1026	1042	1051	1063

Chapter 9 Conclusion for the Retention Prediction in RP-HPLC

The retention indices for 18 mono- or disubstituents aromatic compounds and 8 aliphatic compounds have been examined based on alkyl aryl ketones retention index scale. Their substituents indices and interaction indices have been expressed as quadratic equations, covering a wide range of methanol, acetonitrile and THF eluents composition which can be used to predict retention in multisubstituted compounds. The coefficients of the substituents indices and interaction indices were determined and stored in the worksheet of VP-Expert system and can be used to predict the retention indices of other substituents. Some new rules have been added into an existing knowledge base of CRIPES to make it working under THF buffer eluent. New data for a few substituents in methanol and acetonitrile which were not considered at previous work (136-142) also made CRIPES more complete.

A general model equation of expert system proposed by Smith and Burr (141) was tried to calculated the retention increments of disubstituents in THF-buffer eluent using a set of selected disubstituted compounds based on previous experience (141). It was found that the use of Hammett substituent constant had its limitation and was not suitable if THF was eluent.

The effect of intramolecular hydrogen bonding in THF was investigated and it was found that some *ortho* phenol isomers with hydroxy and carboxyl groups can form hydrogen-bonds, which would reduce the polarity of solute and give longer retention and larger retention increments

The revised CRIPES has been tested. The results showed that the calculated data were very close to experimental data.

9.1 Future work

CRIPES can give correct consultations and calculate the capacity factors and retention indices of substituted benzenes in three commonly used RP-HPLC

solvents with pH = 7 buffer solution. The use of other pH buffer solutions would be possible if we could calculate the substituent index for those compounds which could be ionised.

The main task for the future is to study in particular the interactions between substituents and try to discover any underlying rules. It may be possible to find out a common model which is able to calculate the interactions of substituents in any eluent.

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