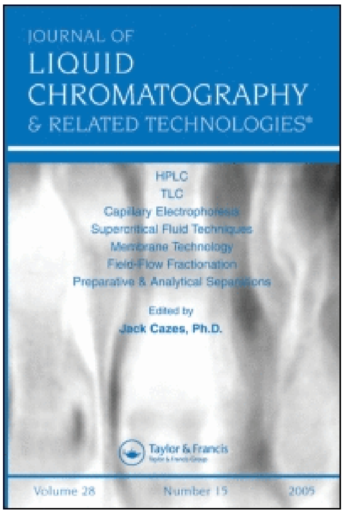

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EFFECT OF TEMPERATURE ON HPLC SEPARATIONS USING C_1, C_4, C_8, and C_{18} ALKYL CHAIN BONDED SILICA COLUMNS*

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ABSTRACT

The effect of temperature on retention in reversed phase high performance liquid chromatography was studied using a C_1, C_4, C_8 and C_{18} alkyl chain bonded columns and four groups of compounds: (1) anthraquinone, methyl anthraquinone and ethyl anthraquinone; (2) naphthalene and biphenyl; (3) dimethyl phthalate and diethylphthalate, and (4) p-nitroaniline and caffiene. The results indicated that the mechanism of separation using the four groups of solutes and the four columns at temperature ranges of 0°C to 77°C is the same and that a linear relationship was obtained for a plot of

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lnk' vs 1/T. Enthalpy values were observed in the following orders $C_{18}>C_8 = C_4>C_1$ depending on the solutes studied. The results also showed that α for a pair of adjacently eluting solutes decreased by approximately 10% with increase in temeperature from 30°C to 70°C. The greatest decrease in α (25%) was observed between 0°C and 27°C. It was also observed that in order to achieve the same retention time on all the columns for a certain compound, the volume of organic modifier should be different for each column, $C_{18}>C_8>C_4>C_1$. Finally, the best separation was obtained at lower rather than higher temperature.

INTRODUCTION

Several studies have been published on the effect of temperature on retention in high performance liquid chromatography (HPLC). Chemielowiec and Sawatzky (1) studied the effect of temperature on the separation of polynuclear aromatic hydrocarbons (PAH) on a C_{18} reversed phase column using a mobile phase of methanol/water. Snyder (2) discussed the role of temperature on selectivity in reversed phase (RP) LC. Gant et al. (3) presented a systematic approach to optimizing resolution (R_s) in RPLC with emphasis on the role of temperature. The separations were performed on C_8 RP columns, and mobile phase of methanol/water. Melander et al. (4) studied the dependence of retention on temperature and eluent composition, using C_{18} RP column and a mobile phase of acetonitrile/water. Diasio and Wilburn (5) studied the effect of temperature on the retention of five fluorouracil metabolites on C_{18} RP column using a mobile phase of 0.002 M phosphate buffer (pH 5.7). Colin et al. (6) studied the role of the temperature in RP HPLC using pyrocarbon containing adsorbents. They concluded that temperature does not appear to be a very important parameter in their system, and that selectivity decreased slightly with increase in separation temperature. They also found that solvent elutropic strength is not sensitive to temperature. The major effect of temperature is the increase in the diffusion coefficients resulting in an increase in the optimum solvent flow rate. Another study (7) dealt with the influence of temperature on the retention behavior of members of homologous series on RP C_{18} using methanol/water eluents. Atamna and Grushka (8) studied the effect of changes of mobile phase and temperature, and mobile phase velocity and temperature on optimization by isochronal analysis. They observed that α increased with increase in temperature (50% methanol/water) while α decreased with increase in temperature (60% methanol/water) for the same compounds, methylbenzoate and benzene.

To date, a search of the literature reveals that there has not been a study comparing the effect of temperature and mobile phase on the retention using different alkyl chain RP bonded supports. This study, therefore, compares the separation and retention of four groups of compounds using a C_1, C_4, C_8 and C_{18} bonded silica column at temperatures ranging from 0°C to 77°C, using a mobile phase of methanol/water. The columns used had the same dimensions and were manufactured from the same batch of silica under the same experimental conditions.

The different alkyl chain length columns were selected in order to gain some insight into the behavior of the alkyl chain under different temperature and mobile phase conditions, which will in turn aid us in understanding the mechanism of separation in reversed phase HPLC; does the longer alkyl chain column give better or worse separation than a shorter chain column at a higher temperature than at room temperature? Can we predict the behavior of the alkyl chain (stretched or curled) and its effect on the separation process? What is the effect of the percent organic modifier on the separations using C_1, C_4, C_8 and C_{18} columns at the same temperature? This study will try to answer the questions raised.

EXPERIMENTAL

Materials

The solutes used in this study were purchased from Aldrich Chemical Co., Inc. (Milwaukee, WI). Methanol was glass distilled uv grade (Burdick and Jackson, Muskegon, MI). Water was deionized glass distilled.

Apparatus

A Hewlett-Packard Model 1090 Liquid Chromatography equipped with a heater (9), a photodiode array detector, an automatic injector, strip chart recorder, a Hewlett-Packard Model 3392A integrator and a Hewlett-Packard Model 85 computer/controller was used. Prepacked RP C_1, C_4, C_8, C_{18} columns, 100 mm x 4.6 mm, 5 micron spherical particles, (Advanced Separation Technologies, Whippany, NJ) were used.

Procedures

Retention times were measured after system equilibration for 1 hour. Column dead time (t_{Ro}) was measured by injecting uracil solution. All solutes were reagent grade and solutions were prepared in methanol/water. Subambient temperatures were achieved by using a water bath.

RESULTS AND DISCUSSION

The C_1, C_4, C_8 and C_{18} columns were all manufactured and packed under the same experimental conditions from the same batch of silica in order to avoid any column irregularities due to column packings and preparation. The peak shapes obtained were all symmetrical and no tailing of the peaks was observed. The columns were washed before use with methanol then equilibrated for 1 hour at every temperature studied. At the beginning the experiment was run at the temperature range of room temperature to 77°C. Later, the experiments were run from 0°C to 77°C. k' was calculated according to the equation $k' = (t_r - t_{ro})/t_{ro}$ where t_r and t_{ro} are retention time of the solute and uracil (non retained peak) respectively. This article will not deal with the theoretical relationships between entropy ($\Delta S°$), enthalpy ($\Delta H°$), capacity factor (K'), free energy ($\Delta G°$) and temperature (T), where T is absolute temperature, as this has been discussed previously (2-4). The relationship between k', $\Delta H°$ and T is given by the following equation

$$d(\log k')/d(1/T) = \Delta H°/4.57 \qquad (I)$$

Snyder (2) stated that "solutes of similar structure have $\Delta H°$ increases linearly with log k'. If the linear dependence of $\Delta H°$ on log k' is exact, then solute retention order will be unchanged as separation temperature is varied".

Equation (II) gives the relation between k', $\Delta H°$, $\Delta S°$ and Φ (the phase ratio) in the column (4):

$$\ln k' = -\Delta H°/RT + \Delta S°/R + \Phi \qquad (II)$$

where R is the gas constant. Equation II means that a change in either column temperature or mobile phase composition, methanol/water in this study, or both should give optimum separation. Therefore, we will examine our results with the above two equations in mind.

The dependence of retention on temperature is apparent from figures 1-4, where plots of the natural logarithm of the capacity factor against the reciprocal of the absolute temperature for anthraquinone (A), methylanthraquinone (MA) and ethylanthraquinone (EA) gave linear relationships. The eluent was 55% methanol/water. The figures show a decrease of retention with increase in temperature. They also show that the linear relationships for each of the compounds are nearly parallel on each of the columns. This is due to the fact that the three solutes have similar chemical properties. However, on each column the intercepts for the compound are different, see for example figure 5. The intercept is related to the retention of the solute at 55% methanol/water. It was expected,

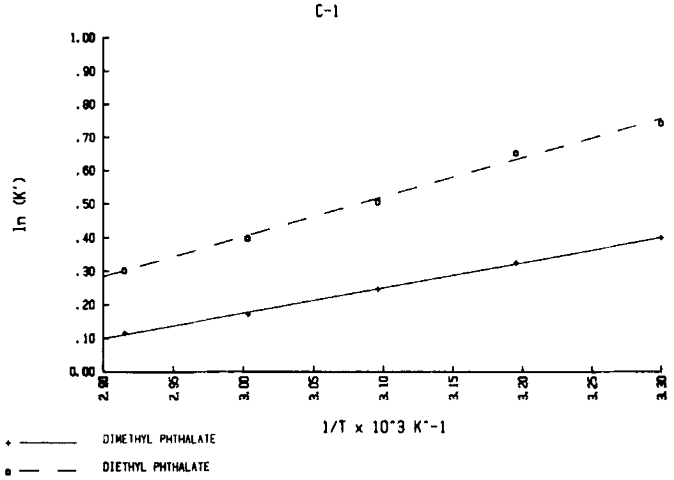

Figure 1. Effect of temperature on the capacity factor of anthraquinone, methylanthraquinone and ethylanthraquinone using a C_1 RP column, 4.6 x 100 mm, and a mobile phase of 55% MeOH/H_2O at a flow rate of 1 ml/min.

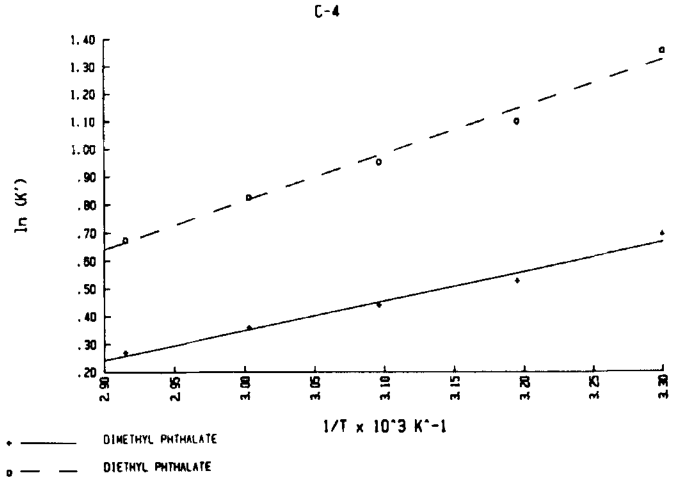

Figure 2. Same as Figure 1, but C_4 RP column was used.

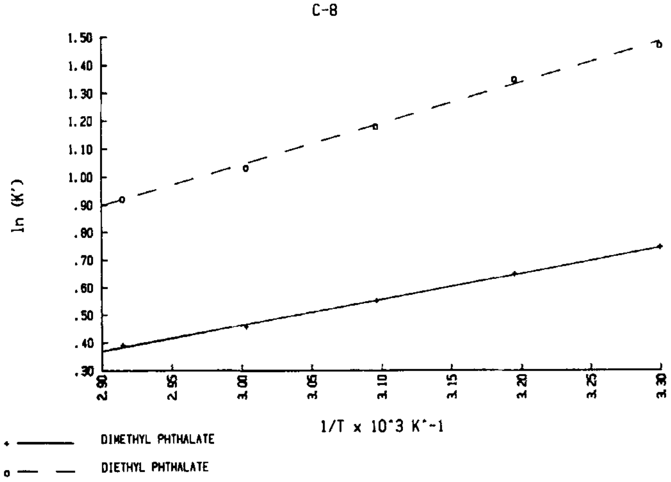

Figure 3. Same as Figure 1, but C_8 RP column was used.

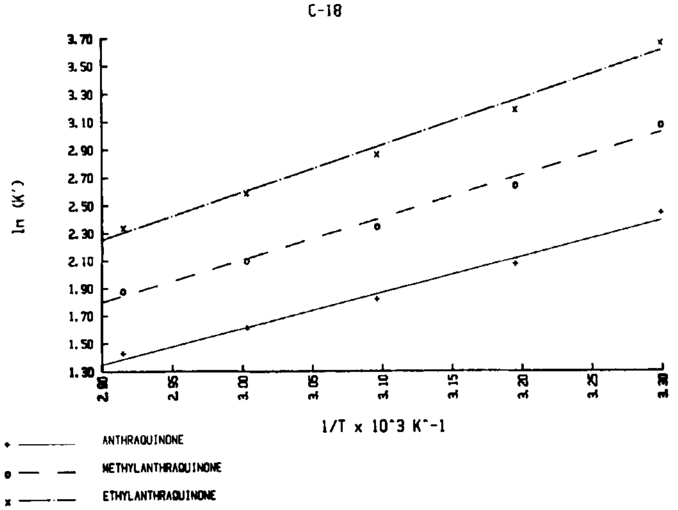

Figure 4. Same as Figure 1, but C_{18} RP column was used.

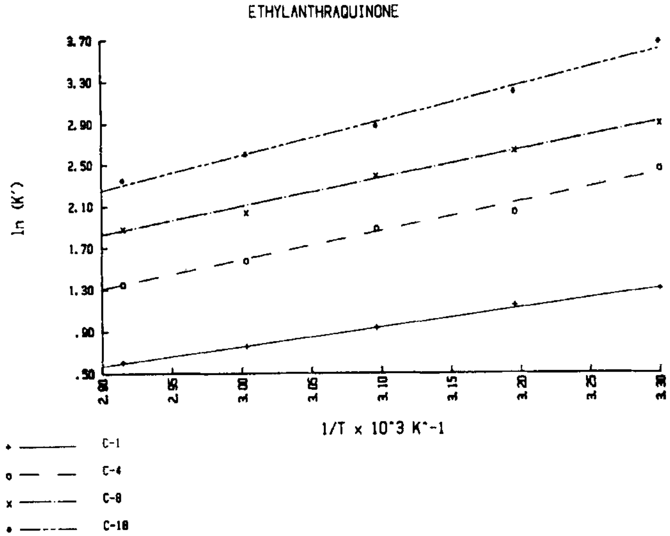

Figure 5. Effect of temperature on the capacity factor of ethylanthraquinone using a C_1, C_4, C_8, C_{18} RP columns, 4.6 x 100 mm, and a mobile phase of 55% $MeOH/H_2O$ at a flow rate of 1 ml/min.

based on previous experience (10), that at this eluent composition the longest retention of the anthraquinones will be on the C_{18} column and the shortest retention on the C_1 column. The slope of the line gives the value for ΔH°. Table I lists the ΔH° values of the anthraquinones on each column. The results can be examined in two ways; (a) how did ΔH° values compare between the anthraquinones on the same column, and (b) how did ΔH°, for each anthraquinone, vary between the C_1, C_4, C_8 and C_{18} columns. That is (a) changes in ΔH° due to chemical structural changes of the solutes and (b) changes due to the column's alkyl chain differences using the same eluent but varying the temperature. Table I shows (a) that ΔH° values increased with the substitution on the ring, the longer the side chain the larger is ΔH°, such that EA>MA>A on each of the four columns; (b) that the ΔH° values where lowest on the C_1 column, but were the same on C_4 and C_8 but increased

Table I

ΔH° values for A, MA and EA on C_1, C_4, C_8 and C_{18} RP columns using 55% $MeOH/H_2O$ as the eluent

Column	ΔH° (Kcal/Mole) A	MA	EA
C_1	1.26	1.54	1.85
C_4	2.04	2.44	2.81
C_8	2.07	2.45	2.75
C_{18}	2.61	3.07	3.41

by more than 20% on the C_{18} column. The lowest ΔH° value on C_1 column may be attributed to interaction with residual silinol group on the silica, while with C_{18} column the solutes are farthest away and prevented by the C_{18} alkyl chain from interacting with the residual silinol groups on the silica.

Figure 6 shows the plot of MA retention at room temperature against mobile phase composition. Similar plots were obtained for A and EA. The results revealed two interesting observations: (a) the retention times at 60% methanol/water are inversely proportional to the column's alkyl chain length; and (b) in order to get the same retention time (say 10 min) on C_1, C_4, C_8 and C_{18} the volume of methanol in the eluent is directly proportional to the column's aliphatic chain length, the longer the column the higher the percentage of methanol at the same temperature. The results also show that resolution is inversely proportional to the separation temperature. Best resolution is obtained at the lowest temperature. A plot of the logarithm of temperature against percentage of methanol gives a linear relationship (Figure 7).

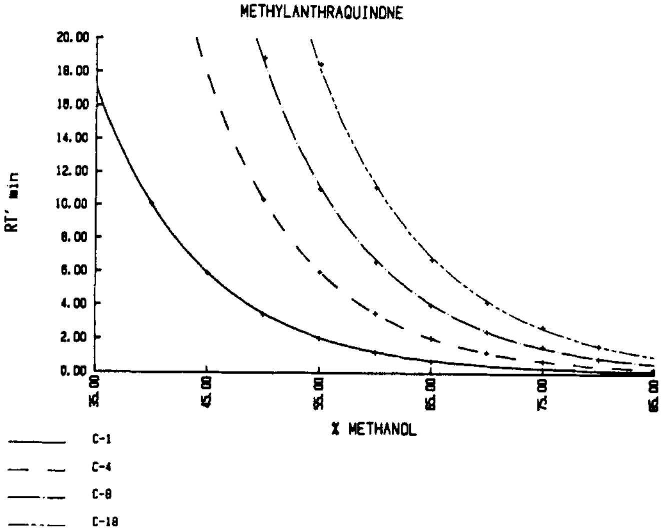

Figure 6. Effect of volume of methanol in the eluent on the retention of methyl anthraquinone using C_1, C_4, C_8 and C_{18} RP columns.

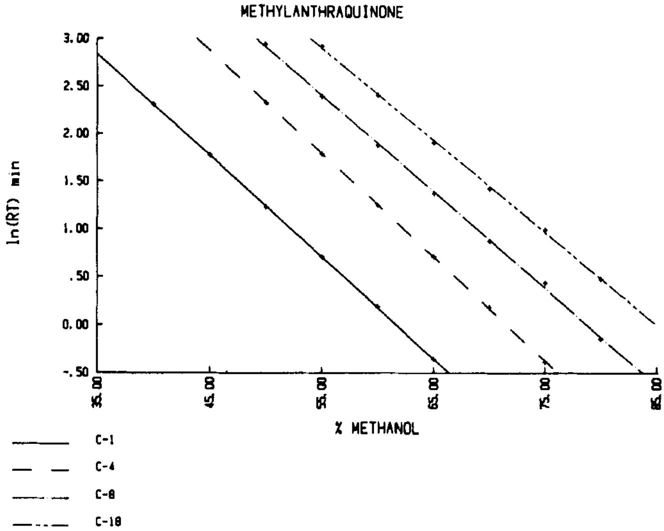

Figure 7. A plot of the volume of methanol in the eluent against the natural logarithm of retention time for methyl anthraquinone using a C_1, C_4, C_8 and C_{18} RP columns.

The next group to investigate was a mixture of naphthalene (N) and biphenyl (B). The plots of the logarithm of capacity factor against inverse temperature for the C_1, C_4, C_8 and C_{18} columns is given in figures 8-11, respectively, and show linear relationships. Again it is clear that the results obtained with this group of compounds are similar to those obtained with the anthraquinones. Figure 12 is a plot of corrected retention times for N against percent methanol in the eluent at room temperature. The plot shows that there is a larger difference in the retention of these compounds on C_1 than either of the other three columns and that the difference in retention using C_4, C_8 and C_{18} is comparable. This was also observed when the retention of the anthraquinones on the four columns were compared. This may be due to stronger interaction between the solute and alkyl chain, the longer the alkyl chain the stronger the interaction and as a result longer retention times, and/or the effect of residual silinol groups on the surface of the silica particles, which are shielded by the longer alkyl chain.

The third group of compounds that was used to investigate the effect of temperature and eluent on retention was a mixture of dimethylphthalate (MP) and diethylphthalates (EP). A linear relationship was attained when the logarithm of the capacity factor was plotted against the inverse temperature, Figures 13-16. Again the results of this group are the same as those obtained for the other two groups of solutes. A plot of retention time against a percent methanol is given in Figure 17, which shows that the solutes will not be retained by any of the four columns at or above 80% methanol in the mobile phase. Other conclusions are the same as mentioned earlier for the other two groups of solutes.

The results given above, although indicated differences in the enthalpy, which were not very large in most cases, did not really draw the line and enable us to conclude what is really happening to the alkyl chain at that temperature, i.e., is it stretched or curled? Also, all the columns

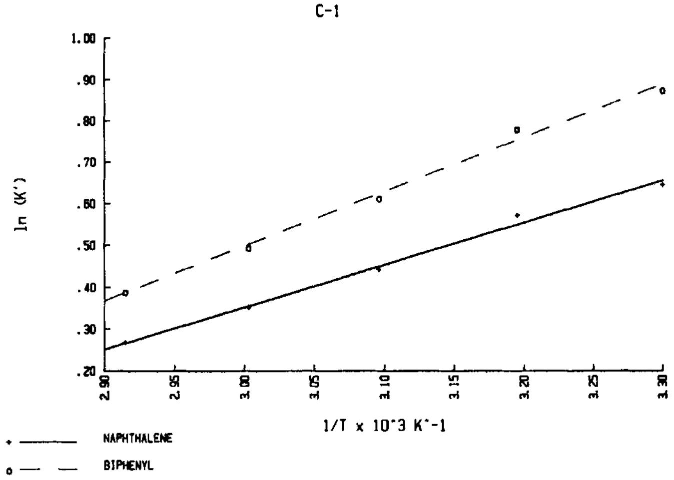

Figure 8. Effect of temperature on the capacity factor of naphthalene and biphenyl using a C_1 RP column, 4.6 x 100 mm, and a mobile phase of 55% MeOH/H_2O at a flow rate of 1 ml/min.

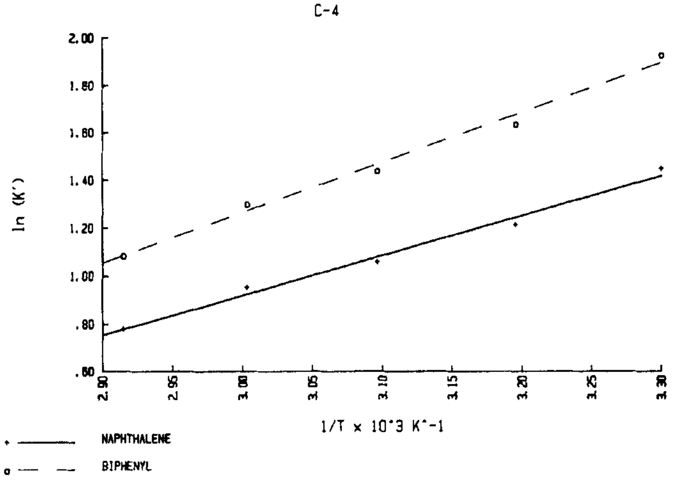

Figure 9. Same as Figure 8, but C_4 RP column was used.

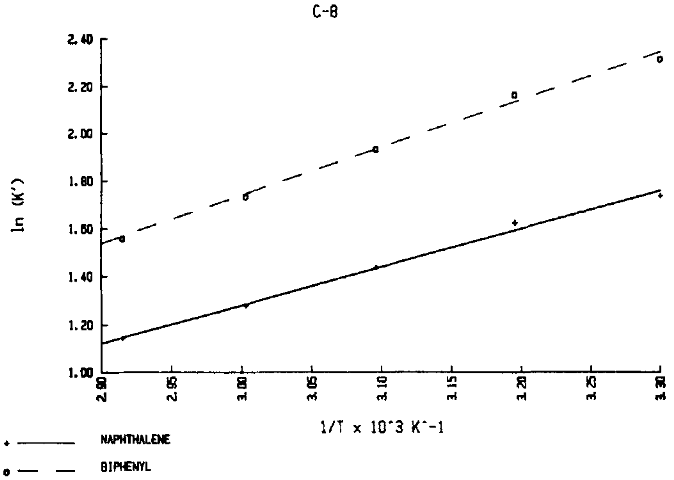

Figure 10. Same as Figure 8, but C_8 RP column was used.

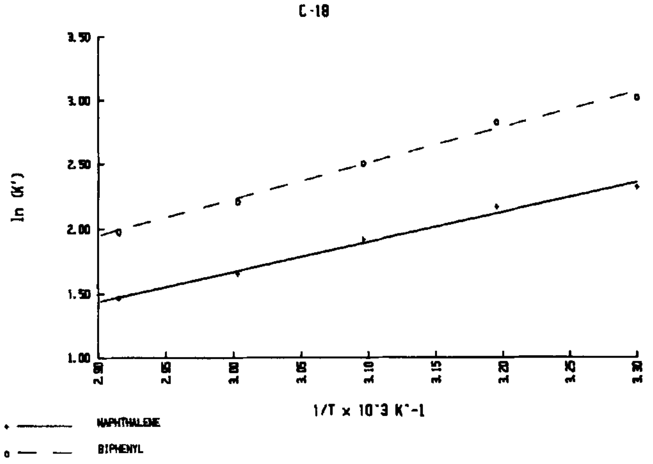

Figure 11. Same as Figure 8, but C_{18} RP column was used.

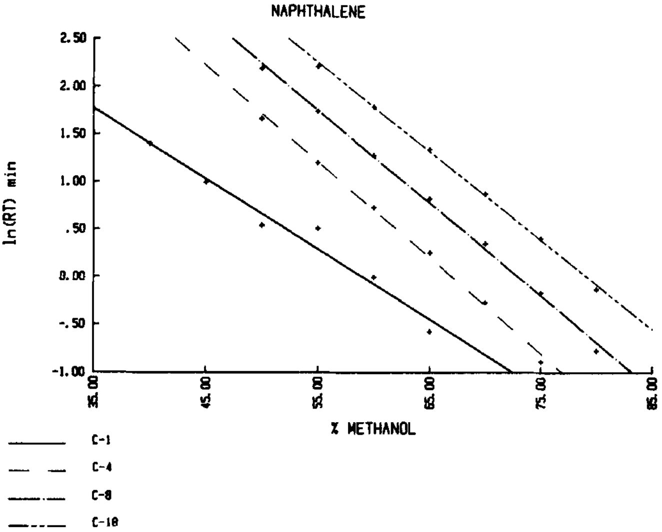

Figure 12. A plot of volume of methanol in the eluent against the natural logarithm of retention time for naphthalene using a C_1, C_4, C_8 and C_{18} RP columns.

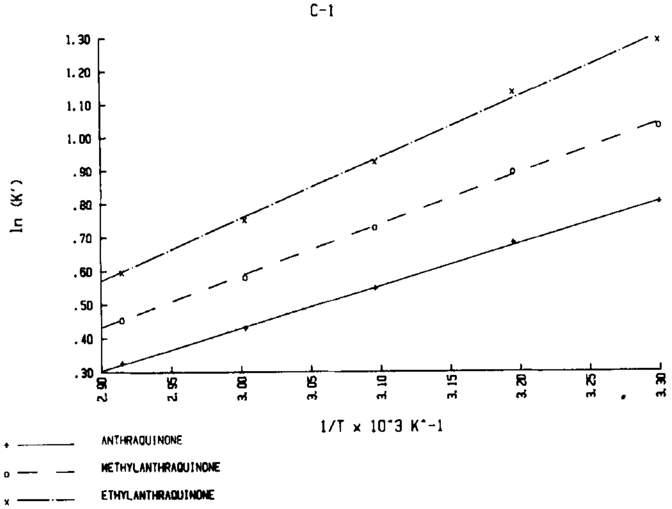

Figure 13. Effect of temperature on the capacity factor of dimethylphthalate and diethylphthalate using a C_1 RP column, 4.6 x 100 mm, and a mobile phase of MeOH/H_2O at a flow rate of 1 ml/min.

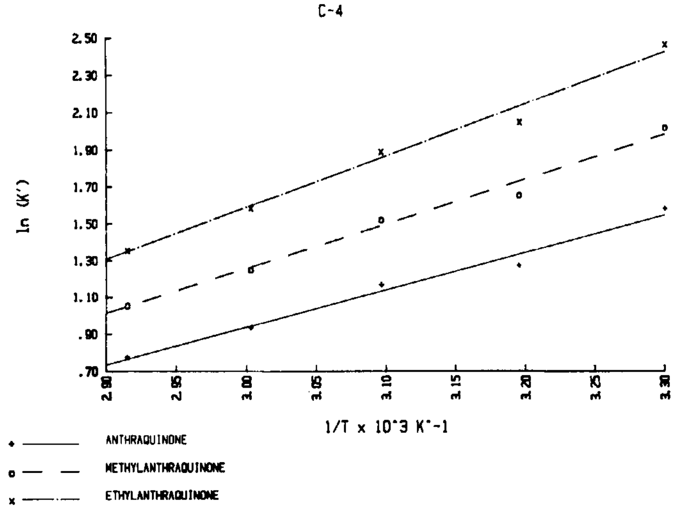

Figure 14. Same as Figure 13, but a C_4 RP column was used.

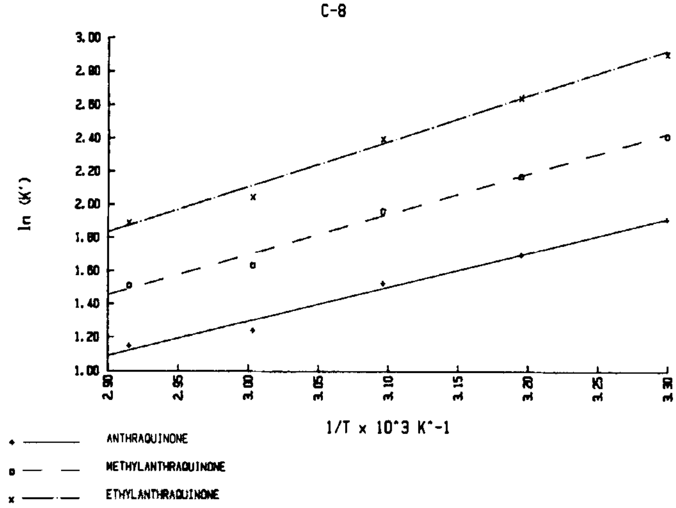

Figure 15. Same as Figure 13, but a C_8 RP column was used.

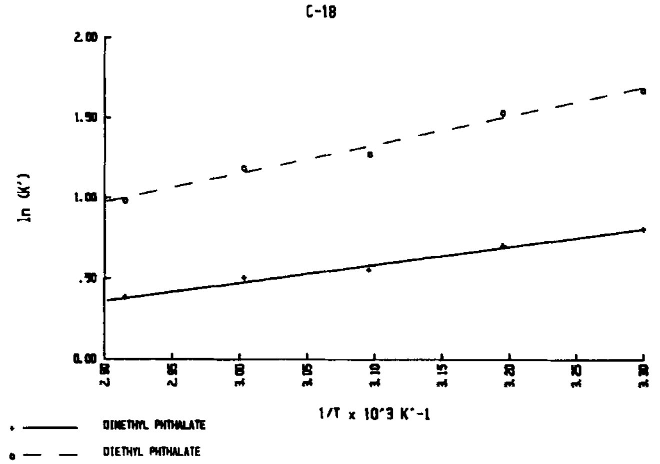

Figure 16. Same as Figure 13, but a C_{18} RP column was used.

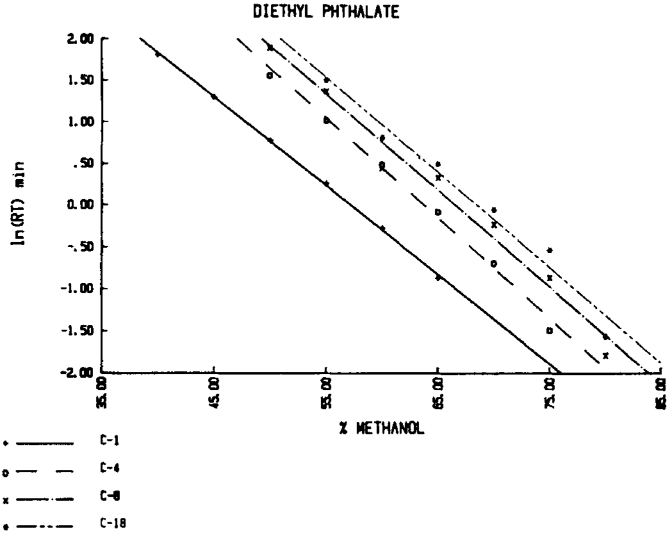

Figure 17. A plot of the volume of methanol in the eluent against the natural logarithm of retention for diethylphthalate using a C_1, C_4, C_8 and C_{18} RP columns.

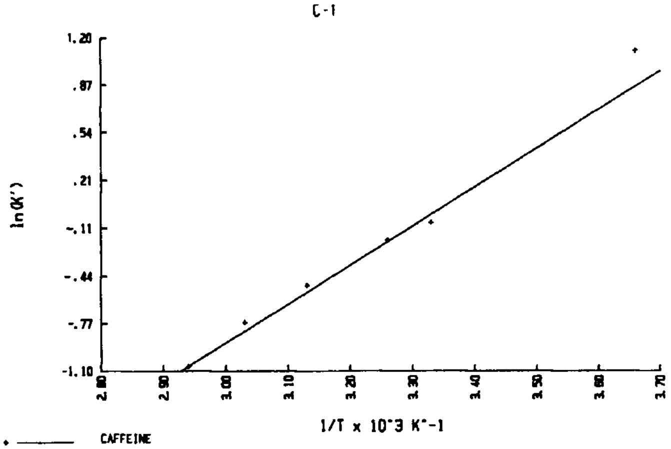

Figure 18. Effect of temperature on the capacity factor of caffeine using a C_1 RP column and a mobile phase of 10% MeOH/H_2O at a flow rate of 1 ml/min.

gave linear relationships when lnk' was plotted vs. 1/T. It was expected that some break in this linear relationship would be observed, but did not happen. It was suggested to us (11) that if (a) the experiment was conducted from subambient rather than room temperature, and (b) the volume of methanol was decreased in the eluent a non linear relationship may be observed. These two suggestions were accepted and the experiments were conducted from 0°C to 77°C and the volume of methanol in the mobile phase was decreased to 10%. Two new compounds, caffeine (C) and p-nitroaniline (NA), were selected based on their retention times on the four columns. P-nitroaniline was not well retained on the C_1 column to give meaningful data and caffeine gave very small retention times, 4.9 min. at 0°C and a fraction of a min. at above 47°C. Although, it was possible to generate a plot of lnK' vs. 1/T for caffiene, figure 18, a scatter, larger than expected, was obtained due to the small values of t_R. Figures 19-21 give a plot of lnk' vs. 1/T for C and NA on C_4, C_8 and C_{18}, respectively. Again a linear

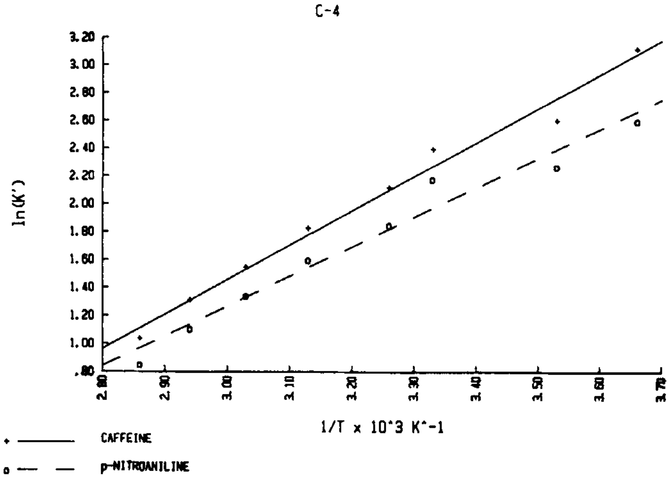

Figure 19. Effect of temperature on the capacity factor of caffiene and p-nitroaniline using a C_4 RP column and a mobile phase of 10% $MeOH/H_2O$ at a flow rate of 1 ml/min.

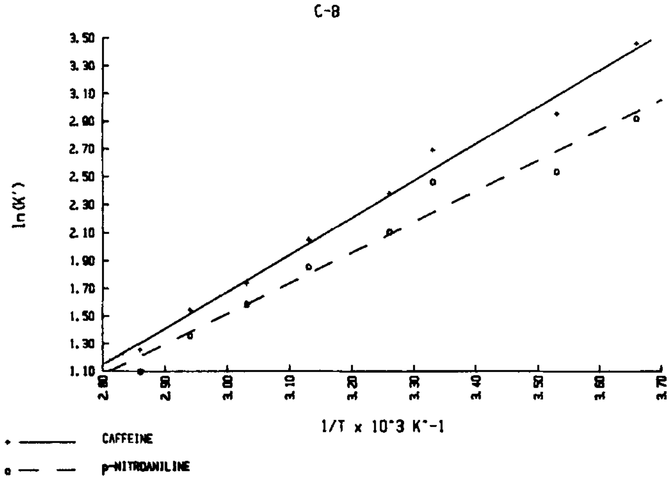

Figure 20. Same as Figure 19, but C_8 RP column was used.

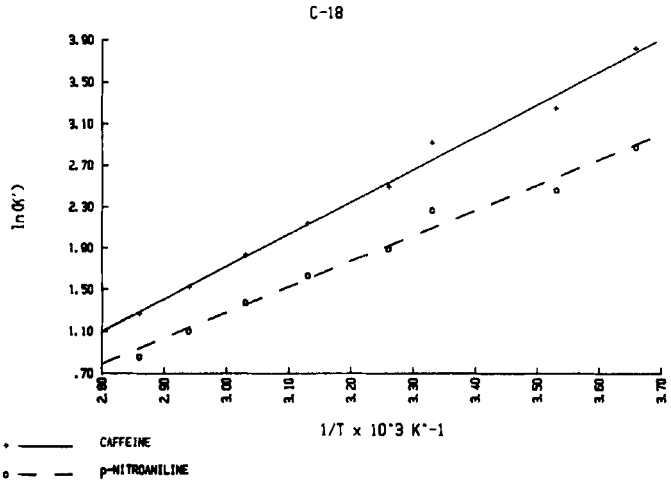

Figure 21. Same as Figure 19, but C_{18} RP column was used.

Table II

Summary of (ΔH°) and Y-intercepts for all compounds used in this study using C_1, C_4, C_8 and C_{18} RP columns and a mobile phase of 55% MeOH/H_2O and 10% MeOH/H_2O (C and NA)

ΔH° (KCal/mole)	A	MA	EA	MP	EP	N	B	C	NA
C_1	1.26	1.54	1.85	0.76	1.19	1.01	1.31	2.69	-
C_4	2.04	2.14	2.81	1.06	1.72	1.68	2.12	2.48	2.14
C_8	2.07	2.45	2.75	0.94	1.48	1.56	3.03	2.66	2.21
C_{18}	2.61	3.07	3.40	1.12	1.81	2.33	2.83	3.14	2.47
Y-intercept									
C_1	-3.35	-4.03	-4.80	-2.1	-3.16	-2.67	-3.43	-8.97	-
C_4	-5.17	-6.05	-6.84	-2.84	-4.34	-4.10	-5.10	-5.97	-5.14
C_8	-4.92	-5.65	-6.14	-2.35	-3.38	-3.52	-4.34	-6.31	-5.10
C_{18}	-6.23	-7.91	-7.61	-2.89	-4.26	-5.32	-6.26	-7.69	-6.13

Table III

α values for A, MA, EA, N, B, DMP and DEP at different temperatures and columns using 55% MeOH/H_2O as the eluent

Column	T(°C)	α(NA/A)	α(EA/A)	α(B/N)	α(EP/MP)
C_1	30	1.27	1.20	1.26	1.42
	40	1.24	1.27	1.23	1.39
	50	1.20	1.22	1.19	1.30
	60	1.17	1.19	1.16	1.26
	70	1.09	1.15	1.13	1.21
C_4	30	1.56	1.57	1.62	1.96
	40	1.47	1.49	1.53	1.79
	50	1.43	1.45	1.47	1.68
	60	1.38	1.40	1.42	1.61
	70	1.33	1.35	1.36	1.56
C_8	30	1.67	1.64	1.80	2.08
	40	1.62	1.60	1.73	2.02
	50	1.56	1.55	1.65	1.89
	60	1.49	1.51	1.59	1.79
	70	1.45	1.46	1.53	1.71
C_{18}	30	1.91	1.81	2.04	2.40
	40	1.79	1.73	1.96	2.34
	50	1.72	1.68	1.81	2.08
	60	1.65	1.63	1.76	1.99
	70	1.59	1.59	1.69	1.86

relationship was obtained and no break in the line was observed. Therefore, one may conclude, based on the above data, that the mechanism of separation, using C_1, C_4, C_8 and C_{18} bonded RP columns, is the same in each case, however, the enthalpy and intercept values are different. The trend for enthalpy values in most cases is $C_{18}>C_8=C_4>C_1$, depending on the solutes studied (Table II). Another observation is that the ratio of α for a pair of adjacently eluting compounds at 30°C to 70°C (Table III) decreased slightly, 10% with increase in temperature. These results are in agreement with those observed by Colin et al. (12). The greatest decrease in α was between 0° and 27°C, Table IV, which indicates that the separation factor

Table IV

α values for caffeine and p-nitroaniline at different temperatures and columns using 55% MeOH/H_2O as the eluent

Column	C_4	C_8	C_{18}
T(°C)	(C/NA)	(C/NA)	(C/NA)
0	1.67	1.71	2.54
10	1.39	1.50	2.17
27	1.23	1.24	1.89
37	1.30	1.30	1.80
47	1.25	1.20	1.64
57	1.22	1.16	1.56
67	1.22	1.19	1.51
77	1.19	1.16	1.48

(column efficiency) for the tested solutes was less at higher than at lower temperatures and that the C_8 and C_{18} columns were the least efficient at the higher temperatures. The results also showed that the C_{18} column required the highest volume of methanol in the mobile phase, while C_1 required the least, which is predictable based on their hydrophobic properties. Also, in order to achieve the same retention time on all the columns for a certain compound, the volume of organic modifier should be different for each column, $C_{18}>C_8>C_4>C_1$. Finally, the best separation is obtained, as can be judged from the solutes in this study, at the lower than higher temperature.

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