

CHROM. 18 574

PREDICTION OF RETENTION OF HYDROXYL AROMATICS IN REVERSED-PHASE LIQUID CHROMATOGRAPHY WITH SLOPE-INTERCEPT RELATIONSHIPS

HOWARD A. COOPER and ROBERT J. HURTUBISE*

Department of Chemistry, University of Wyoming, Laramie, WY 82071 (U.S.A.)

(First received November 20th, 1985; revised manuscript received February 19th, 1986)

SUMMARY

Methanol–water compositions were used to empirically predict the retention of unknown hydroxyl aromatic compounds at different mobile phase compositions on a μ Bondapak C₁₈ column. A single experimental k' value was needed to predict k' values at other mobile phase compositions for an unknown compound. The method of predicting retention was based on two linear relationships derived from the experimental data. A linear relationship was obtained from plots of $\log k'$ as a function of the volume fraction of methanol in the mobile phase for standard hydroxyl compounds. Then a linear relationship was obtained by plotting the slope as a function of the intercept from the slope and intercept values derived from the $\log k'$ plots. Together, the two linear relationships permitted the prediction of retention at different mobile phase compositions for compounds structurally similar to the original data set.

INTRODUCTION

The exact mechanism governing solute retention in reversed-phase high-performance liquid chromatography (RP-HPLC) is of considerable research interest. A number of aspects have been considered for solute retention in RP-HPLC^{1–17}. At present, the most widely accepted mechanism and most extensive treatment for solute retention in RP-HPLC is the solvophobic (hydrophobic) model developed by Horváth and co-workers^{1,4,5,18–20}. The solvophobic model is a rigorous thermodynamic model which accounts for both mobile and stationary phase interactions. However, it is assumed that mobile phase interactions predominate in the RP-HPLC retention mechanism.

The consequence of such a rigorous model, although important, is the introduction of several physical parameters which are unknown and difficult to ascertain. For such models, this limits their practical application for predicting solute retention. As a result, a number of approaches have been developed which are to varying degrees less theoretical. These approaches have implemented a variety of factors including interaction indices^{21–24}, quantitative structure–retention relationships^{25–27},

molecular connectivity², solubility parameter⁷⁻⁹, hydrophobicity²⁸, molecular structural features²⁹⁻³² and carbon chain length^{33,34}, among others, for the prediction of retention in RP-HPLC.

In this work, we present an empirical method for predicting solute retention based on slope-intercept relationships. The prediction of retention for a number of mono- and dihydroxyl aromatic compounds using RP-HPLC is described. Linear relationships were obtained for $\log k'$ as a function of the volume fraction of methanol in the mobile phase. In addition, a linear relationship was obtained by plotting the slope as a function of the intercept for the slope and intercept values obtained from the linear $\log k'$ relationships. Combined, the two linear relationships allow the prediction of retention at most mobile phase compositions for unknown compounds which are structurally similar to the original data set.

EXPERIMENTAL

High-performance liquid chromatography

The liquid chromatograph used was a Waters Assoc. (Milford, MA, U.S.A.) Model ALC/GPC 244 equipped with a Model 6000A pump controlled by a Model 680 Automated Gradient Controller operating in the isocratic mode. A U6K injector, a dual channel free standing UV detector set at 254 nm and 280 nm, a dual channel 10-mV strip chart recorder, and a Hewlett-Packard Model 3390A integrator were also used with the liquid chromatograph.

Columns

The column investigated was a 30 cm \times 3.9 mm I.D. prepacked C₁₈ column obtained from Waters Assoc. The C₁₈ column was packed with μ Bondapak C₁₈ and consisted of octadecyl groups chemically bonded to Waters 10- μ m porous silica.

Reagents

HPLC-grade methanol was obtained from Fisher Scientific (Pittsburgh, PA, U.S.A.) and was prefiltered through a Millipore (Bedford, MA, U.S.A.) type FH 0.5- μ m filter. Distilled water was prefiltered through a Millipore Milli-Q water purification system. The binary solvent mixtures were degassed by stirring overnight. The hydroxyl aromatic standards were obtained from commercially available sources and were purified when necessary.

Chromatographic systems

Reversed-phase, μ Bondapak C₁₈ with methanol-water mobile phases at 1.0 ml/min.

Hydroxyl aromatic standards

Solutions of 1-12 mg/ml of the hydroxyl aromatic standards were prepared in 2-propanol, chloroform or tetrahydrofuran depending on the solubility of the individual standards. The retention volumes of the hydroxyl standards were determined by injecting 1.0-6.0 μ l of the standard solution onto the chromatographic systems described above. Peak splitting was observed for 99 + % 1,4-dihydroxybenzene when it was injected onto the above chromatographic systems. The peak splitting was not

studied further and the largest peak obtained was used to determine the capacity factor for this compound. The capacity factor k' was calculated by $k' = (V_R - V_M)/V_M$, where V_R is the retention volume (ml) and V_M is the column void volume (ml). The column void volume was obtained by eluting methanol and was determined to be 2.80 ml for the μ Bondapak C₁₈ column using the UV detector.

Computer

Calculations and plots of data were obtained with a Hewlett-Packard Model 87 computer programmed in BASIC and equipped with a Model 82908A 64K Expansion Memory Module, a Model 82901M Flexible Disc Drive, and a Model 82905B Printer.

RESULTS AND DISCUSSION

For reversed-phase chromatographic systems it was shown by Schoenmakers *et al.*³⁵ that $\log k'$ behaves as a quadratic function of the volume fraction of the organic modifier (ϕ_m).

$$\log k' = A\phi_m^2 + B\phi_m + C \quad (1)$$

A similar equation in terms of mole fraction of the organic modifier was derived earlier³⁶. The coefficients A , B , and C have been defined previously³⁵. Snyder *et al.*³⁷ have pointed out that for most practical applications eqn. 1 can be simplified to

$$\log k' = \log k_w - S\phi_m \quad (2)$$

where $\log k_w$ (C) is the log of the capacity factor in pure water and the constant S (B) is related to the strength of the organic modifier³⁷. However, researchers have experimentally found that a linear relationship exists between the slope (S) and intercept ($\log k_w$) terms of eqn. 2 for a given data set and certain chromatographic systems³⁸⁻⁴⁰. This is illustrated by eqn. 3.

$$S = -p \log k_w - q \quad (3)$$

The coefficients p and q are the linear regression coefficients for the slope and intercept, respectively, for eqn. 3. The significance of eqn. 3 is that coupled with eqn. 2 a means of empirically predicting retention at most mobile phase compositions for unknown compounds is at hand. The general equation for predicting k' values for an unknown compound at different mobile phase compositions in terms of the volume fraction of the mobile phase (ϕ_m) is obtained by substituting eqn. 3 and eqn. 2 and is shown by eqn. 4

$$\log k' = (1 + p\phi_m)C + q\phi_m \quad (4)$$

where C is $\log k_w$. Eqn. 4 has been defined and discussed earlier⁴¹. To predict the retention for an unknown compound at different mobile phase compositions, a single experimental determination of k' at a given composition is needed. This leaves a single unknown in eqn. 4, the intercept value C for a given compound, since p and q are known from a standard data set.

TABLE I
LOG *k'* VALUES FOR MONOHYDROXYL AROMATICS ON μ BONDAPAK C₁₈ WITH METHANOL-WATER MOBILE PHASES

No. Compound	Methanol-water (v/v)										
	70:30	67.5:32.5	65:35	62.5:37.5	60:40	70:30	65:35	50:50	40:60	30:70	
<i>Monohydroxyl compounds</i>											
1 1-Acenaphthenol	-0.10	-0.06	0.01	0.10	0.16						
2 5H-Dibenz[<i>a,f</i>]cycloheptene-5-ol	0.06	0.12	0.24	0.31	0.38						
3 7,12-Dimethyl-9-hydroxybenz[<i>a</i>]anthracene	0.75	0.96	1.12	1.24	1.33						
4 2-Hydroxybenzo[<i>c</i>]phenanthrene	0.49	0.63	0.78	0.87	0.98						
5 3-Hydroxybenzo[<i>c</i>]phenanthrene	0.55	0.64	0.79	0.89	0.99						
6 1-(1-Hydroxymethyl)pyrene	0.47	0.55	0.70	0.78	0.88						
7 1-(Hydroxymethyl)benzo[<i>a</i>]pyrene	0.76	0.85	1.00	1.12	1.21						
8 4-Hydroxymethylpyrene	0.27	0.42	0.54	0.64	0.71						
9 9-Hydroxyphenanthrene	0.24	0.30	0.41	0.50	0.58						
10 13-Hydroxypicene	1.08	1.17	1.36	1.48	1.59						
11 1-Hydroxypyrene	0.44	0.52	0.60	0.74	0.81						
12 4-Hydroxypyrene	0.43	0.50	0.59	0.72	0.79						
13 1-Indanol	-0.31	-0.28	-0.22	-0.16	-0.13						
14 5-Indanol	-0.13	-0.08	-0.01	0.07	0.11						
15 1-Naphthol	-0.13	-0.09	-0.02	0.08	0.12						

Prediction of retention for hydroxyl aromatic compounds

Twenty-one monohydroxyl and fourteen dihydroxyl aromatic compounds were investigated with a μ Bondapak C_{18} column with several methanol-water mobile phase compositions. Table I gives the compounds investigated and the chromatographic data. Table II gives the least-squares slope and intercept values from $\log k'$ vs. ϕ_m plots (eqn. 2) and the corresponding linear correlation coefficients for all the compounds. Table III gives the least-squares slope and intercept values from the slope-intercept graphs of the data in Table II using eqn. 3 for various sets of compounds. Fig. 1 shows the graph of the slope and intercept values obtained from eqn. 2 for the monohydroxyl aromatics. The straight line in Fig. 1 is defined by eqn. 3. In addition, it is apparent from Table III that mono- and dihydroxyl aromatics

TABLE II

SLOPE, INTERCEPT AND CORRELATION COEFFICIENT VALUES ON μ BONDAPAK C_{18} FROM $\log k'$ vs. ϕ_m GRAPHS

Compound No.	Slope	Intercept	Correlation coefficient
1	-2.75	1.81	0.992
2	-3.28	2.35	0.993
3	-5.75	4.81	0.988
4	-4.94	3.96	0.995
5	-4.51	3.70	0.997
6	-4.17	3.39	0.996
7	-4.71	4.04	0.996
8	-4.43	3.39	0.992
9	-3.54	2.71	0.996
10	-5.32	4.80	0.994
11	-3.80	3.09	0.994
12	-3.76	3.05	0.995
13	-1.93	1.04	0.994
14	-2.52	1.63	0.994
15	-2.64	1.71	0.993
16	-2.92	1.83	0.992
17	-3.12	2.21	0.996
18	-3.48	2.72	0.996
19	-2.31	1.45	0.996
20	-2.89	2.06	0.996
21	-3.57	2.94	0.996
22	-2.83	1.73	0.991
23	-3.61	2.00	0.997
24	-1.85	0.53	0.997
25	-2.03	0.42	0.998
26	-1.56	0.37	0.996
27	-3.34	1.83	0.995
28	-3.10	1.50	0.996
29	-3.35	1.81	0.998
30	-3.16	1.78	0.998
31	-2.75	1.24	0.990
32	-3.18	1.58	0.996
33	-4.39	2.84	0.997
34	-1.94	0.48	0.993
35	-2.44	0.86	0.998

TABLE III

SLOPE-INTERCEPT REGRESSION DATA AND CORRELATION COEFFICIENT VALUES FOR DATA IN TABLE II

	<i>p</i>	<i>q</i>	<i>r</i>
All compounds	-0.822	-1.49	0.963
Monohydroxyl	-0.947	-0.990	0.992
Dihydroxyl	-1.05	-1.40	0.979
Monohydroxyl*	-0.943	-0.998	0.991
Dihydroxyl**	-1.00	-1.48	0.978

* Monohydroxyl "unknowns" deleted (1, 2, 6, 7, 8, 13, 18 and 19).

** Dihydroxyl "unknowns" deleted (23, 26, 27, 30 and 31).

should be considered separately, in terms of eqn. 3, since better linear correlation coefficients are obtained when they are not grouped together. This result may be attributed to the slope values in Table II being dependent on both the chromatographic system and the solutes under study³⁸. In addition, it should be emphasized that Schoenmakers *et al.*³⁸ have shown that no correlation existed for eqn. 3 when acetonitrile-water mobile phases were used.

On the basis of the results presented in Table III, eqn. 4 should give accurate prediction of retention at essentially any mobile phase composition for unknown compounds that are similar to the original data set. Predicting the retention of an

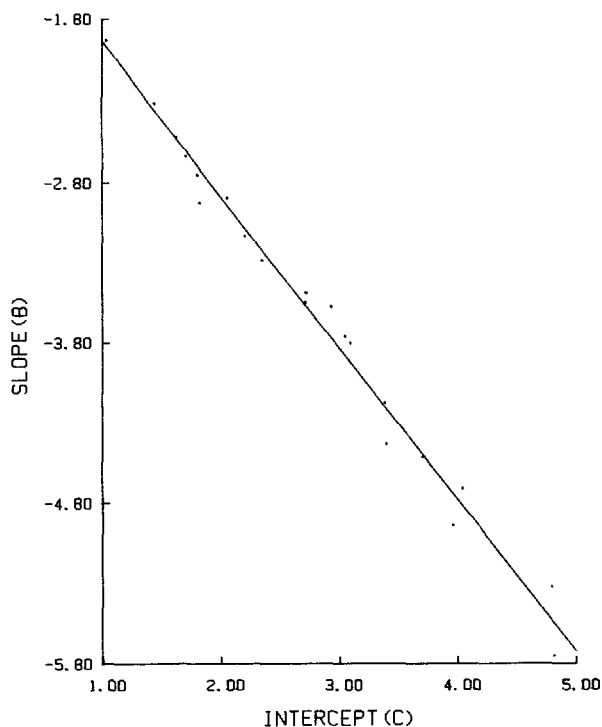


Fig. 1. Graph of slope and intercept values obtained from eqn. 2 for monohydroxyl aromatics.

unknown compound at different mobile phase compositions requires a single determination of $\log k'$ at a given mobile phase composition for the unknown. Consequently, substitution of the experimental values for $\log k'$ and φ_m into eqn. 4 leaves a single unknown; the intercept value, C , which is readily calculated and apparently unique for a given compound. Eqn. 4 can be used to predict $\log k'$ values at different φ_m values for an unknown using the p and q values obtained as defined in Table III. Furthermore, the intercept value, C , can be used to obtain the slope for the unknown via eqn. 3 and therefore the calculated linear $\log k'$ relationship for the unknown (eqn. 2).

Eqn. 4 was used to predict the retention of eight "unknown" monohydroxyl compounds. The "unknown" compounds were the hydroaromatic and aliphatic alcohol types listed in Table I (compounds 1, 2, 6, 7, 8, 13, 18 and 19). These compounds were chosen because they are generally far less acidic than hydroxyl aromatic compounds and thus should challenge the predictive method to a greater degree. These compounds were omitted from the least-squares slope-intercept plot in Fig. 1. The values for the slope (p), intercept (q) and correlation coefficient with these compounds omitted are shown in Table III. The above values for the slope (p) and intercept (q) were used in eqn. 4 to predict k' at a φ_m of 0.60 from k' at a φ_m of 0.70 and *vice versa* for the "unknown" compounds. This situation represents predicting k' values from both compositional extremes investigated. The experimental k' values at a φ_m of 0.70 and 0.60 were obtained from Table I. Table IV shows the results of predicting k' values for the "unknowns" at both compositional extremes investigated. The results in Table IV show a very good correlation between the predicted k' values (k'_{pred}) and the experimental k' values (k'_{exp}) calculated from the linear regression

TABLE IV

COMPARISON OF k'_{exp} AND k'_{pred} VALUES FOR UNKNOWN MONOHYDROXYL COMPOUNDS ON μ BONDAPAK C_{18}

S_e = Standard error of estimate⁴⁴.

Compound No.	$k' (\varphi_m = 0.60)$ from $k' (\varphi_m = 0.70)$			$k' (\varphi_m = 0.70)$ from $k' (\varphi_m = 0.60)$		
	k'_{exp} *	k'_{pred} **	Relative error	k'_{exp} *	k'_{pred} **	Relative error
1	1.44	1.45	0.69	0.77	0.79	2.60
2	2.43	2.35	3.29	1.14	1.16	1.75
6	7.65	7.85	2.61	2.93	2.85	2.73
7	16.6	18.2	9.64	5.63	5.21	7.46
8	5.43	4.81	11.3	1.96	2.12	8.16
13	0.76	0.80	5.26	0.49	0.47	4.08
18	4.29	4.57	6.53	1.93	1.80	6.74
19	1.14	1.19	4.39	0.67	0.65	2.99
			Average 5.46			Average 4.56
	$k'_{\text{pred}} = (1.09 \pm 0.04)k'_{\text{exp}} - 0.34 \pm 0.29$			$k'_{\text{pred}} = (0.92 \pm 0.02)k'_{\text{exp}} + 0.10 \pm 0.06$		
	$S_e = 0.58$ and $r = 0.996$			$S_e = 0.11$ and $r = 0.998$		

* Values obtained from linear regression data in Table II

** Values obtained from eqn. 4.

data in Table II for the "unknown" compounds. For the "unknown" compounds, the average relative error was less than 5.5% for the predicted k' values at all values of φ_m investigated. In terms of predicted retention volumes, the average relative error was less than 6.1% for the predicted k' values at all values of φ_m investigated. The individual relative errors for all "unknowns" were less than 12.5% in terms of retention volumes. However, the relative error for an individual compound depends on the composition (φ_m) at which $\log k'$ was experimentally determined. For example, predicting k' at a φ_m of 0.60 from k' at a φ_m of 0.70 resulted in a 9.64% relative

TABLE V

COMPARISON OF k'_{exp} AND k'_{pred} VALUES FOR TWO UNKNOWN MONOHYDROXYL COMPOUNDS AT ALL VALUES OF φ_m

φ_m	Compound 1			Compound 8		
	k'_{exp} *	k'_{pred} **	Relative error	k'_{exp} *	k'_{pred} **	Relative error
<i>k' (φ_m) from k' ($\varphi_m = 0.70$)</i>						
0.70	0.77	0.79	2.06	1.96	1.85	5.61
0.675	0.90	0.92	1.11	2.52	2.29	9.13
0.65	1.05	1.07	1.90	3.26	2.83	13.2
0.625	1.23	1.25	1.62	4.20	3.70	11.9
0.60	1.44	1.45	0.69	5.43	4.81	9.64
<i>k' (φ_m) from k' ($\varphi_m = 0.675$)</i>						
0.70	0.77	0.75	2.60	1.96	2.09	6.63
0.675	0.90	0.88	2.22	2.52	2.60	3.17
0.65	1.05	1.02	2.86	3.26	3.25	0.31
0.625	1.23	1.18	4.07	4.20	4.04	3.81
0.60	1.44	1.37	4.86	5.43	5.04	7.18
<i>k' (φ_m) from k' ($\varphi_m = 0.65$)</i>						
0.70	0.77	0.75	2.60	1.96	2.19	11.7
0.675	0.90	0.87	3.33	2.52	2.74	8.73
0.65	1.05	1.01	3.81	3.26	3.43	5.21
0.625	1.23	1.17	4.88	4.20	4.28	1.90
0.60	1.44	1.36	5.56	5.43	5.35	1.47
<i>k' (φ_m) from k' ($\varphi_m = 0.625$)</i>						
0.70	0.77	0.80	3.90	1.96	2.21	12.8
0.675	0.90	0.93	3.33	2.52	2.76	9.52
0.65	1.05	1.09	3.51	3.26	3.45	5.82
0.625	1.23	1.27	3.25	4.20	4.32	2.86
0.60	1.44	1.48	2.78	5.43	5.41	0.37
<i>k' (φ_m) from k' ($\varphi_m = 0.60$)</i>						
0.70	0.77	0.79	2.06	1.96	2.12	8.16
0.675	0.90	0.92	1.11	2.52	2.65	5.16
0.65	1.05	1.07	1.90	3.26	3.30	1.23
0.625	1.23	1.24	0.81	4.20	4.12	1.90
0.60	1.44	1.45	0.69	5.43	5.14	5.34
		Average	2.70			Average 6.11

* Values obtained from linear regression data in Table II.

** Values obtained from equation 4.

error for compound 8, whereas predicting k' at a φ_m of 0.60 from k' at a φ_m of 0.625 resulted in 0.37% relative error (see Table V). In general, for all the compounds investigated a lower relative error resulted when predicting k' values at high φ_m values from k' values at low φ_m values. Table V shows the relative error to be expected for predicting k' values at all of the φ_m values investigated from k' values for each respective φ_m value for two typical compounds. Table V clearly shows that the relative error for each determination depends on the φ_m value at which k' was experimentally determined.

In addition, it was generally observed that the best prediction of k' values for the monohydroxyl "unknowns" was obtained for adjacent compositions. Table VI shows the results of predicting k' at a φ_m of 0.60 from k' at a φ_m of 0.625, the next lower (adjacent) value of φ_m in Table I. The results in Table VI are improved in relation to the comparable results shown in Table IV. The data for the dihydroxyl compounds in Table VI are discussed below.

Analogous to the previous discussion, eqn. 4 was used to predict the retention of five "unknown" dihydroxyl compounds. The "unknowns" were compounds 23, 26, 27, 30 and 31 with the hydroxyl groups *ortho*, *meta* or *para* to one another or substituted on separate aromatic rings. Table III gives the slope (p) and intercept (q) values from the slope-intercept plot with the above compounds deleted. These values were used to predict k' at a φ_m of 0.30 from k' at a φ_m of 0.70 and *vice versa* for the "unknown" dihydroxyl compounds. The experimental k' values at a φ_m of 0.70 and 0.30 were obtained from Table I. Table VII shows the results of predicting k' values for the dihydroxyl "unknowns" at both compositional extremes investigated. It is evident from Table VII that predicting k' values at low φ_m values from k' values at high φ_m values for the dihydroxyl "unknowns" leads to substantial relative error. The result in this case is apparently caused by the extremely small k' values at φ_m

TABLE VI

COMPARISON OF k'_{exp} AND k'_{pred} VALUES PREDICTED FROM ADJACENT COMPOSITIONS FOR MONO- AND DIHYDROXYL UNKNOWN ON μ BONDAPAK C₁₈

Monohydroxyl compound	k' ($\varphi_m = 0.60$) from k' ($\varphi_m = 0.625$)			Dihydroxyl compound	k' ($\varphi_m = 0.30$) from k' ($\varphi_m = 0.40$)		
	k'_{exp} *	k'_{pred} **	Relative error		k'_{exp} *	k'_{pred} **	Relative error
1	1.44	1.48	2.78	23	8.29	7.02	15.3
2	2.43	2.44	0.41	26	0.80	1.00	25.0
6	7.65	7.75	1.31	27	6.65	5.57	16.2
7	16.6	17.5	5.42	30	6.82	6.75	1.03
8	5.43	5.41	0.37	31	2.58	2.38	7.75
13	0.76	0.78	2.63				Average 13.1
18	4.29	4.52	5.36				
19	1.14	1.18	3.51				
			Average 2.72				

$$k'_{\text{pred}} = (1.05 \pm 0.01) k'_{\text{exp}} - 0.09 \pm 0.07$$

$$S_e = 0.14 \text{ and } r = 0.999$$

$$k'_{\text{pred}} = (0.84 \pm 0.08) k'_{\text{exp}} + 0.31 \pm 0.43$$

$$S_e = 0.48 \text{ and } r = 0.988$$

* Values obtained from linear regression data in Table II.

** Values obtained from eqn. 4.

TABLE VII

COMPARISON OF k'_{exp} AND k'_{pred} VALUES FOR UNKNOWN DIHYDROXYL COMPOUNDS

Compound No.	$k' (\varphi_m = 0.30)$ from $k' (\varphi_m = 0.70)$			$k' (\varphi_m = 0.70)$ from $k' (\varphi_m = 0.30)$		
	k'_{exp}^*	k'_{pred}^{**}	Relative error	k'_{exp}^*	k'_{pred}^{**}	Relative error
23	8.29	5.99	27.7	0.29	0.35	20.6
26	0.80	2.02	153	0.19	0.14	26.3
27	6.65	7.55	13.5	0.31	0.34	9.68
30	6.82	10.9	59.8	0.37	0.34	8.10
31	2.58	3.11	20.5	0.20	0.23	15.0
			Average 54.9			Average 15.9
$k'_{\text{pred}} = (0.87 \pm 0.40) k'_{\text{exp}} + 1.53 \pm 2.32$			$k'_{\text{pred}} = (1.12 \pm 0.45) k'_{\text{exp}} - 0.02 \pm 0.13$			
$S_c = 2.56$ and $r = 0.780$			$S_c = 0.07$ and $r = 0.819$			

* Values obtained from linear regression data in Table II.

** Values obtained from eqn. 4.

equal 0.70 (see Table I) which are close to the column void volume and thus lead to a significant error in predicted k' values. However, the converse shows reasonable relative errors in view of the small k' values as shown in Table VII. In fact, the average relative error for predicting k' values for the dihydroxyl "unknowns" is less than 16% (3.2% in terms of retention volume) if the predicted k' values are calculated from k' at a φ_m of 0.30. Therefore, the experimental k' values should be well away from the column void volume for accurate prediction of retention. However, the predicted k' values at a φ_m of 0.70 calculated from k' values at a φ_m of 0.30 are generally not that analytically useful since they are less than one. Table VI shows the results of predicting k' at a φ_m of 0.30 from k' at a φ_m of 0.40, the next lower (adjacent) value of φ_m in Table I. The results in Table VI show good agreement between the k'_{exp} and k'_{pred} values with an average relative error in k' of 13.1%. In terms of retention volumes (V_R) the average relative error was 9.10% for this case. The individual relative errors for all dihydroxyl "unknowns" were less than 14% in terms of retention volumes. In general, the best prediction of k' values for the dihydroxyl "unknowns" was achieved for adjacent compositions. This was also concluded earlier for the monohydroxyl "unknowns".

CONCLUSIONS

Overall, it is apparent that the empirical method of predicting retention gives very good estimates of retention for monohydroxyl aromatic compounds but somewhat poorer estimates for dihydroxyl compounds chromatographed on μ Bondapak C₁₈ with methanol-water mobile phases. It appears that the method is best suited for individual compound classes with $1 < k' < 10$. More work is required to determine the generality of the method. However, additional work in this laboratory has shown that the method described is also applicable to cyanopropyl bonded phases and to nitrogen containing compounds chromatographed with methanol-water mo-

bile phases. On the other hand, it appears that the method may fail for acetonitrile-water systems as discussed earlier³⁸.

In practice, the empirical method presented here could be used for the prediction of retention of unknowns in complex mixtures of structurally similar compounds. This could possibly aid in the development of separation methods and characterization techniques for complex mixtures such as coal liquids. Furthermore, the relationships presented here could be used to determine the optimal shape of the solvent gradient used in gradient elution³⁸ and to estimate isocratic retention from gradient elution data⁴². Consequently, the relationships discussed here would be useful in the determination of optimal mobile phase conditions of complex hydroxyl aromatic mixtures⁴³ and probably other mixtures.

Jandera^{24,45} has discussed slope-intercept relationships and other relationships for predicting retention of homologous series and the use of homologous series as calibration standards for predicting the retention of other types of compounds. The method presented in this work does not depend on a set of homologous series, but does depend on structurally similar compounds. Additional work would be needed to determine if a homologous set of compounds would improve the predictive accuracy of the method discussed in this paper.

ACKNOWLEDGEMENT

Financial support for this project was provided by the U.S. Department of Energy under contract No. DE-AC22-83PC60015.

REFERENCES

- 1 Cs. Horváth, W. Melander and I. Molnar, *J. Chromatogr.*, 125 (1976) 129.
- 2 B. L. Karger, J. R. Gant, A. Hartkopf and P. H. Weiner, *J. Chromatogr.*, 128 (1976) 65.
- 3 R. M. McCormick and B. L. Karger, *J. Chromatogr.*, 199 (1980) 259.
- 4 A. Nahum and Cs. Horváth, *J. Chromatogr.*, 203 (1981) 53.
- 5 K. E. Bij, Cs. Horváth, W. R. Melander and A. Nahum, *J. Chromatogr.*, 203 (1981) 65.
- 6 R. N. Nikolov, *J. Chromatogr.*, 286 (1984) 147.
- 7 R. Tijssen, H. A. H. Billiet and P. J. Schoenmakers, *J. Chromatogr.*, 122 (1976) 185.
- 8 P. J. Schoenmakers, H. A. H. Billiet and L. de Galan, *Chromatographia*, 15 (1982) 205.
- 9 B. L. Karger, L. R. Snyder and C. Eon, *J. Chromatogr.*, 125 (1976) 71.
- 10 J. J. Kirkland, *J. Chromatogr. Sci.*, 10 (1972) 129.
- 11 T. Hanai and K. Fujimura, *J. Chromatogr. Sci.*, 14 (1976) 140.
- 12 H. Colin and G. Guiochon, *J. Chromatogr.*, 158 (1978) 183.
- 13 H. Colin, N. Ward and G. Guiochon, *J. Chromatogr.*, 149 (1978) 169.
- 14 R. P. W. Scott and P. Kucera, *J. Chromatogr.*, 142 (1977) 213.
- 15 J. H. Knox and A. Pryde, *J. Chromatogr.*, 112 (1975) 171.
- 16 C. H. Lochmuller and D. R. Wilder, *J. Chromatogr. Sci.*, 17 (1979) 574.
- 17 D. C. Locke, *J. Chromatogr. Sci.*, 12 (1974) 433.
- 18 Cs. Horváth and W. Melander, *J. Chromatogr. Sci.*, 15 (1977) 393.
- 19 Cs. Horváth, W. Melander and I. Molnar, *Anal. Chem.*, 49 (1977) 142.
- 20 W. R. Melander and Cs. Horváth, in Cs. Horváth (Editor), *High-Performance Liquid Chromatography: Advances and Perspectives*, Academic Press, New York, Vol. 2, 1980, pp. 113-319.
- 21 P. Jandera, H. Colin and G. Guiochon, *Anal. Chem.*, 54 (1982) 435.
- 22 H. Colin, G. Guiochon and P. Jandera, *Anal. Chem.*, 55 (1983) 442.
- 23 H. Colin, G. Guiochon and P. Jandera, *Chromatographia*, 17 (1983) 83.
- 24 P. Jandera, *J. Chromatogr.*, 314 (1984) 13.
- 25 S. P. Assenza and P. R. Brown, *J. Chromatogr.*, 282 (1983) 477.

- 26 I. Molnár and Cs. Horváth, *J. Chromatogr.*, 145 (1978) 371.
- 27 B.-K. Chen and Cs. Horváth, *J. Chromatogr.*, 171 (1979) 15.
- 28 T. Hanai and J. Hubert, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 6 (1983) 20.
- 29 J. F. Schabron, R. J. Hurtubise and H. F. Silver, *Anal. Chem.*, 50 (1978) 1911.
- 30 K. Jinno and K. Kawasaki, *Chromatographia*, 17 (1983) 445.
- 31 M. N. Hasan and P. C. Jurs, *Anal. Chem.*, 55 (1983) 263.
- 32 R. J. Hurtubise, T. W. Allen and H. F. Silver, *J. Chromatogr.*, 235 (1982) 517.
- 33 P. Dufek, *J. Chromatogr.*, 299 (1984) 109.
- 34 E. Grushka, H. Colin and G. Guiochon, *J. Chromatogr.*, 248 (1982) 325.
- 35 P. J. Schoenmakers, H. A. H. Billiet, R. Tijssen and L. de Galan, *J. Chromatogr.*, 149 (1978) 519.
- 36 P. Jandera and J. Churáček, *J. Chromatogr.*, 91 (1974) 207.
- 37 L. R. Snyder, J. W. Dolan and J. R. Gant, *J. Chromatogr.*, 165 (1979) 3.
- 38 P. J. Schoenmakers, H. A. H. Billiet and L. de Galan, *J. Chromatogr.*, 185 (1979) 179.
- 39 T. L. Hafkenscheid and E. Tomlinson, *J. Chromatogr.*, 218 (1981) 409.
- 40 W. E. Hammers, G. J. Meurs and C. L. de Ligny, *J. Chromatogr.*, 247 (1982) 1.
- 41 T. L. Hafkenscheid and E. Tomlinson, *J. Chromatogr.*, 264 (1983) 47.
- 42 P. J. Schoenmakers, H. A. H. Billiet and L. de Galan, *J. Chromatogr.*, 205 (1981) 13.
- 43 H. A. Cooper and R. J. Hurtubise, *J. Chromatogr.*, 324 (1985) 1.
- 44 R. B. Davis, J. E. Thompson and H. L. Pardue, *Clin. Chem.*, 24 (1978) 611.
- 45 P. Jandera, *Chromatographia*, 19 (1984) 101.