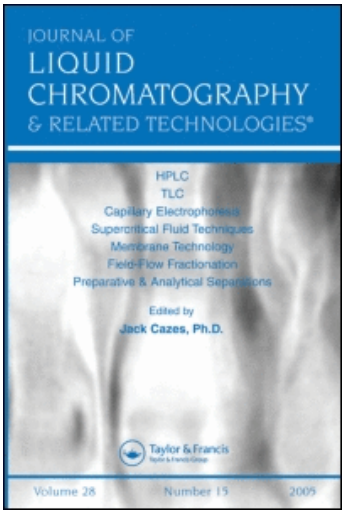

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Prediction of Retention in Reversed Phase Ion-Pair Chromatography Using Sodium Dodecyl Sulphate as Pairing Ion

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PREDICTION OF RETENTION IN REVERSED PHASE ION-PAIR CHROMATOGRAPHY USING SODIUM DODECYL SULPHATE AS PAIRING ION

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ABSTRACT

A 3 x 3 factorial design has been applied in the study of the chromatographic behaviour of thirteen protonated amines in reversed phase HPLC using sodium dodecyl sulphate as the pairing ion. The maximum capacity factors (log k'_{max}) of the test compounds and the mobile phase pairing ion concentrations that produce the retention maxima (log P_{max}), have been correlated to the pK_a, hydrophobicity and connectivity index of the solute, as well as the mobile phase acetonitrile and tetrabutylammonium (TBA) contents. The two dependent variables log k'_{max} and log P_{max}, have been shown to be governed mainly by the hydrophobicity of the solutes and, the acetonitrile and mobile phase TBA concentration. The mathematical models generated have been employed to predict experimental conditions for retention optimization of diltiazem and deacetylated diltiazem. The predicted log k'_{max} and log P_{max}, have been found to be in agreement with those obtained experimentally.

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INTRODUCTION

Retention optimization using statistical techniques is currently the method of choice in the development of HPLC analysis (1-5). In the case of ion-pair chromatography, the optimization process becomes less straight forward. This is probably due to its theoretical complexity and the lack of a commonly accepted mathematical model (6-8). The parabolic relationship that is regularly observed between the k' of an ionic solute and the mobile phase pairing ion concentration further complicates the eluent selection process (9, 10). Thus, retention and separation of ionic solutes using ion-pair chromatography are usually achieved by empirical optimization methods (6, 8, 11). The chromatographic condition selected often contains a mobile phase pairing ion concentration that produces a maximum solute retention (k'_{max}) (12, 13). This approach offers the advantage that retention of the ionic solutes is relatively insensitive to small changes in the mobile phase pairing ion concentration. It has been demonstrated that for a given pairing ion, the maximum capacity factor (k'_{max}) and the pairing ion concentration that allows k'_{max} (P_{max}) are dependent on the hydrophobicity of the solute (7), the organic modifier content (9) and the mobile phase counterion concentration (10, 12, 14). The pH of the eluent and the dissociation constant (pK_a) of solute have also been shown to have significant effect on the retention of an ionic solute in ion-pair chromatography (15-17). In addition it has been observed that there is a strong correlation between the connectivity index (χ), an empirical structural parameter of a solute and its k' in reversed phase HPLC (18, 19).

This study was undertaken to investigate the relationship of the two dependant variables in ion-pair chromatography, k'_{max} and P_{max}, with the hydrophobicity parameter ($\log k'_w$), pK_a and χ of the solute, as well as the organic modifier content and mobile phase counterion concentration. Since reversed phase ion-pair chromatography is employed extensively in the analysis of basic solutes, all compounds investigated in this study were organic amines. The pH of the eluents was adjusted to 2 to ensure complete solute ionization. Sodium dodecyl sulphate (SDS) was chosen as the pairing-ion because of its popularity in ion-pair chromatography and its ready availability (9). For similar reasons acetonitrile was chosen as the organic modifier. In view of the widespread popularity of using organic amines to modify the retention behaviour of basic solutes in reversed phase HPLC (12, 20, 21), tetrabutylammonium bromide (TBA) was used as the organic counterion in this investigation.

MATERIALS AND METHODS

Solute Selection

A solute mixture containing adrenaline hydrogentartrate (A), methyldopa (B), *m*-aminophenol (C), phenylalanine, *o*-anisidine (E), trimethoprim (F), 5-amino-acridine (G), chlortetracycline (H), proflavine hemisulphate (I), lignocaine (J), labetalol (K), amitriptyline (L) and nortriptyline (M) was used to examine the effects of solute properties on the k'_{max} and P_{max}, of the ion-pair chromatographic mobile phase. Large differences in hydrophobicity, chemical structure and acidity exist among this set of solutes. Compounds B, K, L and M were obtained from Glaxo N. Z. Ltd (Palmerston North, New Zealand). The rest of the solutes were purchased from BDH (Poole, UK).

Apparatus and Materials

The chromatographic system consisted of a Waters M6000A pump and a fixed wavelength (254 nm) M441 UV detector (Milford, MA, USA). Samples were introduced into the column via a Rheodyne 7125 injector (Cotati, CA, USA) fitted with a 20 µl loop. The chromatographic columns were either a 34 mm x 2.1 mm I.D. or a 100 mm x 2.1 mm I.D., slurry packed with 5 µm ODS-Hypersil (Shandon, Cheshire, UK). Tetrabutylammonium bromide (TBA) was purchased from Sigma (St Louis, MO, USA). Acetonitrile (*ACN*) was obtained from J.T. Baker (Philpsburg, NJ, USA). The disodium hydrogenphosphate, orthophosphoric acid and sodium dodecyl sulphate (SDS) were supplied by BDH (Poole, UK). Water was double glass distilled and Milli-Q filtered. All chemicals were of AnalaR or B.P. grade.

Determination of the Solute Parameters

The extrapolated capacity factors of the solutes in 100% pH 2 aqueous buffer ($\log k'_w$) (22) were used to describe the solute hydrophobic property. The k' of the solutes were measured in 3 mobile phases containing 20, 30 and 40% acetonitrile buffer (20 mM Na_2HPO_4), adjusted to pH 2 with orthophosphoric acid respectively. The retention data for each solute were then fitted to the following equation:

$$\log k' = \log k'_w - A(ACN\ \%\ v/v) \qquad (1)$$

to estimate the log k'_w where A is a constant for a given solute. The connectivity index (χ) for each solute was calculated as described by Kier et al (23). Solute dissociation constants were obtained from the literature (24-26).

Study Design

A 3 x 3 factorial design (27) was employed to investigate the effects of acetonitrile volume fraction (*ACN* % v/v) and the mobile phase counterion concentration (mM TBA) on the k'_{max} of the solutes and P_{max} of the pairing ion. The combinations of the nine mobile phases used are displayed in Table 1. Each of the mobile phases contained 20 mM Na_2HPO_4 and was adjusted to pH 2 by orthophosphoric acid. A fixed volume of solvent was recycled, pairing ion was adjusted by adding weighed amounts of sodium dodecyl sulphate (SDS) directly to the eluent and reequilibrating.

The k'_{max} and P_{max} for each solute with various eluents were obtained from the plots of k' versus mobile phase SDS concentration (7). The experiments were conducted in a temperature controlled room at 28 ± 2 °C. The nine eluents shown in Table 1 were randomized for testing. The retention time of the test compounds under every chromatographic condition was measured in triplicate and if an individual measurement differed from the mean by more than 5%, further replicate measurements were performed. The data obtained were analysed using the SAS computer package (28).

TABLE 1
Compositions of Mobile Phase

Mobile Phase*	Composition		
	ACN (% v/v)	H_2O (% v/v)	TBA (mM)
1	10	90	0
2	10	90	5
3	10	90	10
4	35	65	0
5	35	65	5
6	35	65	10
7	60	40	0
8	60	40	5
9	60	40	10

*Each of the mobile phase contained 20 mM Na_2HPO_4 and was adjusted to pH 2 by orthophosphoric acid.

RESULTS AND DISCUSSION

Figure 1 shows the variation of the k' of each test compound with the volume fraction of acetonitrile content in the absence of mobile phase SDS and TBA. As seen from this figure, linear relationships, as described in equation 1, was obtained for all elutes. The solute properties for compounds A-M are presented in Table 2.

Examples of the plots of solute k' versus the eluent SDS concentration under the nine experimental chromatographic conditions are shown in Figure 2. As seen from this figure, parabolic relationship exist between the k' of the solute and the mobile phase SDS concentration. The k'_{max} and P_{max} for each solute obtained from the nine experimental conditions are shown in Table 3 and Table 4 respectively. Results presented in Table 4 show that the P_{max} of each eluent is dependent on the solute properties. This further casts doubt on the explanation that the decrease in k' in ion-pair chromatography is due to micelle formation (29). In this case the P_{max} should be identical for a given mobile phase.

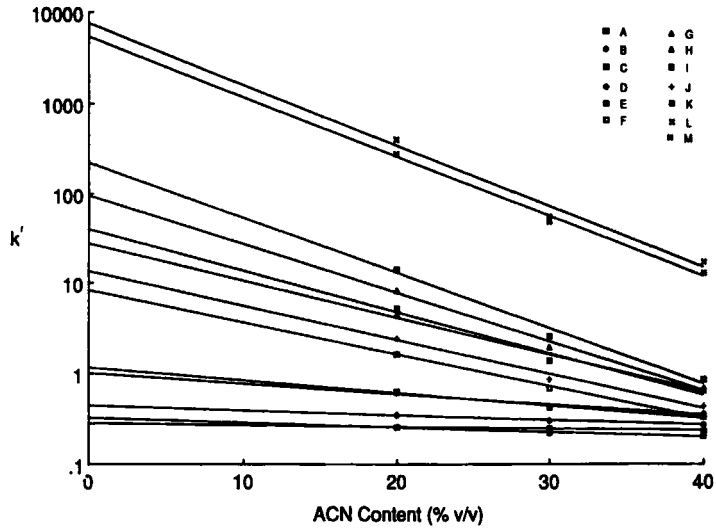

FIGURE 1

Retention behaviour of compounds A-M as a function of the eluent acetonitrile (*ACN*) content at pH 2.

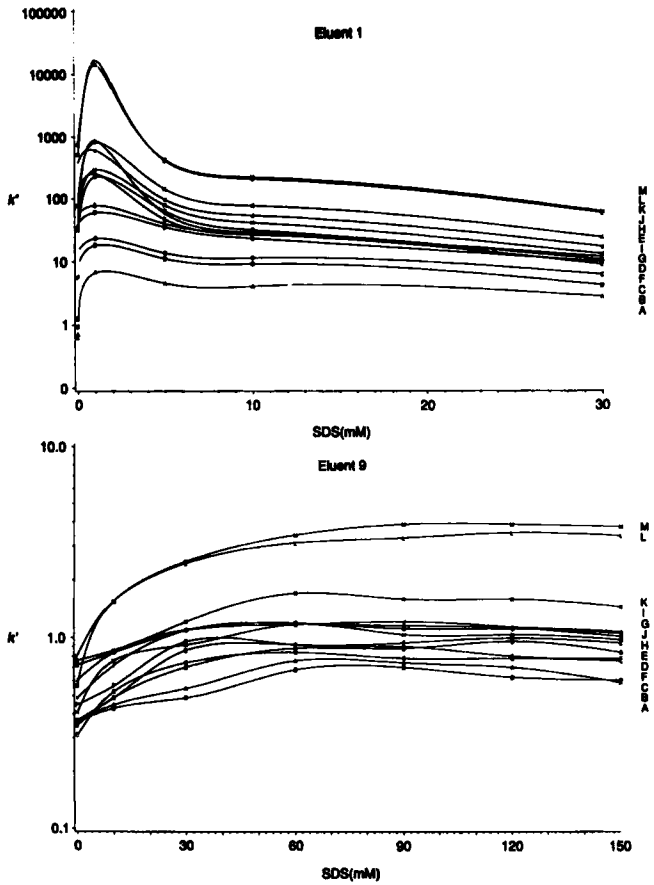

FIGURE 2

Variation of solute k' values with mobile phase SDS concentration in eluents 1 and 9.

The observed k'_{max} and P_{max} of each of the 13 compounds (A-M), obtained from the nine eluents, was fitted simultaneously using multivariate regression (30), to a second order polynomial with respect to the five independent variables, i.e k'_w, pK_a and χ of the solutes, ACN volume fraction and TBA concentration (31). Initial attempts to use a linear model without transformation were unsuccessful. It was identified that good correlation could only be achieved after the logarithmic

TABLE 2

Properties of Compounds A-M*

Compound	log k'_w	*pKa*	*x*
A (adrenaline)	-0.5569	8.7	6.15
B (methyldopa)	-0.3465	10.6	6.83
C (*m*-aminophenol)	-0.4880	8.2	3.92
D (phenylalanine)	0.0057	9.2	5.70
E (*o*-anisidine)	0.0636	9.5	4.34
F (trimethoprim)	0.9156	7.2	10.08
G (5-aminoacridine)	1.4322	4.5	7.36
H (chlortetracycline)	1.9705	9.3	15.39
I (proflavine)	1.5893	9.7	7.72
J (lignocaine)	1.1262	7.0	8.08
K (labetalol)	2.3315	7.4	11.47
L (amitriptyline)	3.8791	9.4	10.25
M (nortriptyline)	3.7288	9.7	9.90

* Data obtained from references 24-26.

TABLE 3

k'_{max} of Compounds A-M Derived from Using the Nine Mobile Phase conditions

Compound	Mobile Phase*								
	1	2	3	4	5	6	7	8	9
A	7.9	3.4	2.8	1.6	0.8	1.1	0.4	0.2	0.4
B	23	4.6	3.5	2.0	0.9	1.3	0.2	0.3	0.5
C	28	8.6	4.7	2.6	1.2	1.2	0.3	0.2	0.4
D	82	5.9	3.7	3.7	1.5	1.6	0.4	0.4	0.5
E	104	5.0	3.0	4.5	0.6	1.1	0.6	0.4	0.5
F	303	33	14	5.2	1.7	1.6	0.5	0.4	0.6
G	357	207	92	12	4.0	3.6	1.1	0.1	0.8
H	434	99	43	11	3.6	2.6	0.9	0.5	0.6
I	1030	82	118	13	4.3	2.9	1.0	0.5	0.6
J	718	30	14	11	3.7	2.7	1.0	0.6	0.7
K	1150	502	223	29	11	7.8	0.8	0.7	0.8
L	17493	745	261	160	28	18	2.6	0.3	1.2
M	14406	997	356	140	36	22	1.9	0.2	1.0

*Compositions of the mobile phase are listed in Table 1.

TABLE 4

P_{max} (mM) of Compounds A-M Derived from Using the Nine Different Mobile Phase conditions

Compound	Mobile Phase *								
	1	2	3	4	5	6	7	8	9
A	1.3	1.8	2.4	17	51	6.3	76	77	95
B	1.2	2.1	1.6	17	26	10	13	71	101
C	1.4	2.2	2.0	27	59	26	109	117	101
D	1.3	2.6	2.6	31	38	22	90	128	116
E	1.3	3.3	3.2	25	95	53	83	110	105
F	1.0	1.5	1.4	22	71	32	85	101	90
G	0.8	1.3	1.1	20	20	30	75	112	92
H	0.8	1.3	1.0	13	23	22	86	102	93
I	0.8	1.4	1.0	16	26	28	96	151	95
J	1.0	1.5	1.4	18	26	30	94	105	90
K	0.8	1.0	0.9	17	20	19	93	105	98
L	0.7	0.7	0.6	18	17	18	98	97	105
M	0.7	0.8	0.7	12	19	18	103	98	104

* Compositions of the mobile phase are listed in Table 1.

transformation of k'_{max}, P_{max}, k'_w, *ACN %* v/v and (1+TBA (mM)). After deleting the statistically non-significant terms ($p > 0.05$), the following model of the following form were obtained.

$$\log k'_{max} \text{ or } \log P_{max} = A + a \log k'_w + b(\log k'_w)^2 + c\, pKa + d\chi + e\chi^2 + f \log (ACN\ \%v/v) + g(\log(ACN\ \%\ v/v))^2 + h\log(1 + \text{TBA}(mM)) + i(\log(1+\text{TBA}(mM)))^2 + j\log(ACN\%v/v)\log(1 + \text{TBA}(mM)) + k\log k'_w\chi + l\log k'_w\log(ACN\%v/v) + m\log k'_w\log(1 + \text{TBA}(mM)) + pKa\chi \quad (R^2 = 0.97) \quad (2)$$

Where A is the intercept and a-n are coefficients. However, it was also established that correlation exists between the chromatographically determined hydrophobic parameter ($\log k'_w$) and the pK_a and χ of the solute (see equation 3).

$$\log k'_w = 4.6 - 1.18\,pK_a - 0.06\,\chi^2 + 0.16\,pK_a\,\chi \quad (3)$$
$$(R^2 = 0.76)$$

For this reason the pK_a and χ were removed from the model in the second regression analysis. After the deletion of the statistically non-significant terms the following models were obtained :

$$\log k'_{max} = 0.17 + 1.24\log k'_w + 3.98\log (ACN\ \%v/v)$$
$$-2.42(\log(ACN\%v/v))^2 - 2.60\log(1 + \text{TBA}(mM))$$
$$+ 0.44(\log (1 + \text{TBA}(mM)))^2$$
$$+ 1.19\log(ACN\%v/v)\log(1 + \text{TBA}(mM))$$
$$- 0.57\log k'_w\log(ACN\%v/v) - 0.11 \log k'_w\log(1 + \text{TBA}(mM))$$
$$(R^2 = 0.96) \quad (4)$$

$$\log P_{max} = -1.41 - 0.23\log k'_w + 1.03\log(ACN\%v/v)$$
$$+ 0.49(\log(ACN\%v/v))^2 + 0.8\log (1 + \text{TBA}(mM))$$
$$- 0.44(\log(1+\text{TBA}(mM))^2$$
$$-0.18\log(ACN\%v/v)\log(1 + \text{TBA}(mM))$$
$$+ 0.13\log k'_w\log(ACN\%v/v)$$
$$- 0.01\log k'_w\log(1 + \text{TBA}(mM)) \quad (5)$$
$$(R^2 = 0.97)$$

Equation 4 indicates that in general the log k'_{max} of an ionic solute in ion-pair chromatography, increases with its hydrophobicity (log k'_w) but decreases upon the addition of organic modifier and organic counterion content in the mobile phase. This is in agreement with results previously obtained (8, 9, 12, 31).

The P_{max} on the other hand decreases with the log k'_w of the solute but increases with the organic modifier content of the mobile phase. Such phenomena has also been observed previously (29, 31). The mobile phase organic counterion concentration however has a complex effect on the P_{max} of the solute. The presence of interaction terms in both equations 4 and 5 indicates that empirically optimized ion-pair chromatography may have some difficulties in locating the optimal condition. Replacement of log k'_w by the lipophilic index (log k^{o}_{w}) (31) resulted in a rather complicated models but did not increase the accuracy of the predictions.

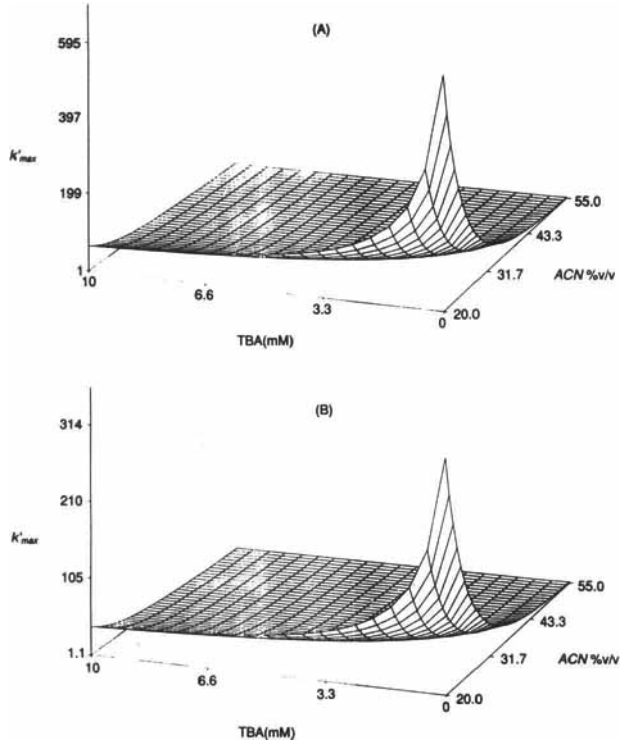

FIGURE 3

(A) k'_{max} map for diltiazem as a function of mobile phase TBA concentration, and of acetonitrile (*ACN*) content.

(B) k'_{max} map for deacetylated diltiazem as a function of mobile phase TBA concentration, and of *ACN* content.

In order to evaluate the accuracy of the derived equations (equations 4 and 5), the model was employed for the retention optimization of two compounds, diltiazem and its major metabolite, deacetylated diltiazem (32). The log k'_w for diltiazem and deacetylated diltiazem were determined as outlined in the experimental section and found to be 3.07 and 2.63, respectively.

Using SDS as pairing ion the k'_{max} maps of diltiazem and deacetylated diltiazem as a function of mobile phase organic counterion (TBA) concentration and *ACN*

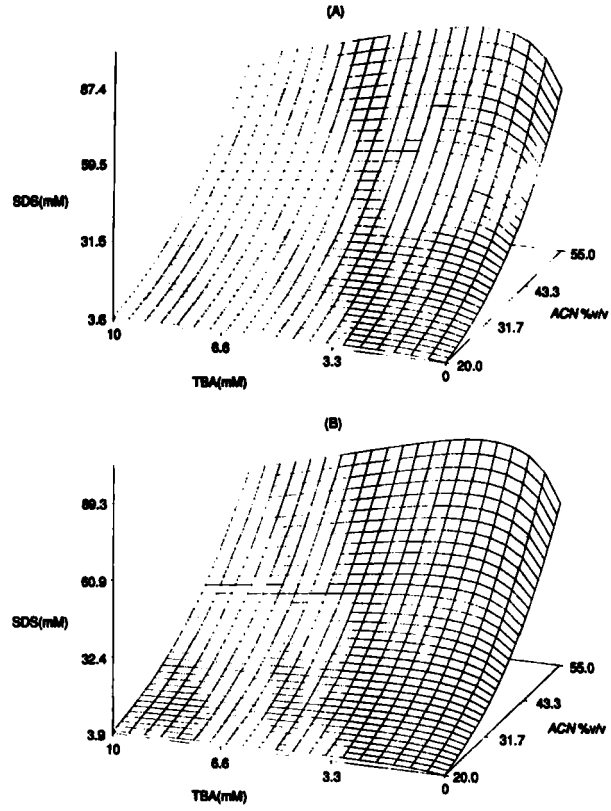

FIGURE 4

P_{max} map for diltiazem (A) and for deacetylated diltiazem (B), as a function of mobile phase TBA concentration, and of acetonitrile (*ACN*) content.

content are presented in Figure 3. Their corresponding P_{max} maps are shown in Figure 4. Contour plots of the k'_{max} of diltiazem and deacetylated diltiazem between 4 and 20 were generated and are displayed in Figure 5. Using a value of 3000, for N the resolution plot (Figure 6) was generated using the following equation:

$$R_s = \frac{\sqrt{N}}{4} \frac{\alpha - 1}{\alpha} \frac{k'}{k' + 1} \qquad (6)$$

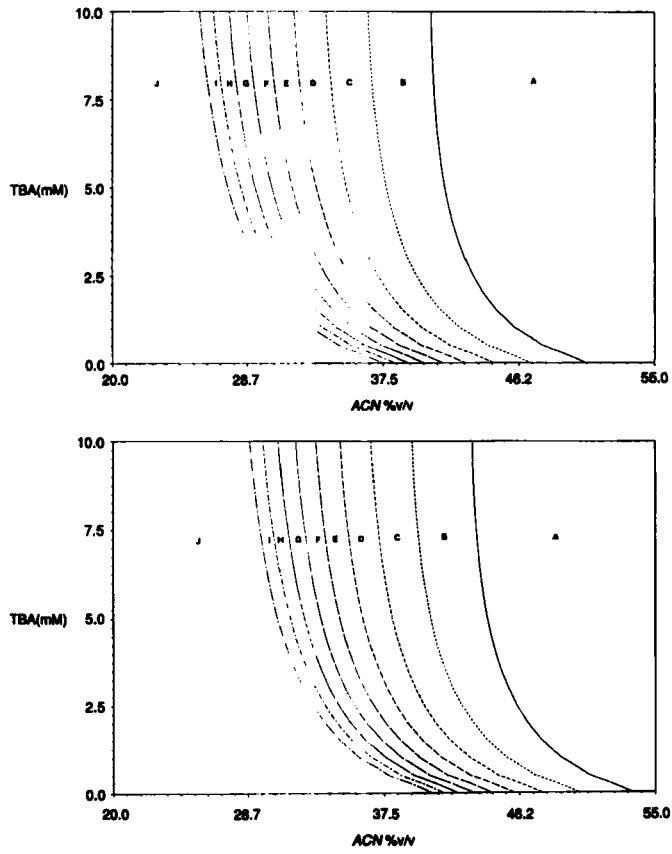

FIGURE 5

Experimental conditions that will provide the k'_{max} of diltiazem (*upper panel*) and of deacetylated diltiazem (*lower panel*), between 4 and 20.

Key for k'_{max} value: A = 0-4; B = 4-6; C = 6-8; D = 8-10; E = 10-12; F = 12-14; G = 14-16; H = 16-18; I = 18-20; J = over 20.

This reveals that resolution greater than 3 between diltiazem and deacetylated diltiazem can be achieved in most of the experimental region (see Figure 6).

Thus the chromatographic conditions were selected based on the retention times of the solutes. Examination of Figure 3 indicates that retention of deacetylated diltiazem with k' around 5 can be achieved with *ACN* ranges of 38 - 42 % v/v and

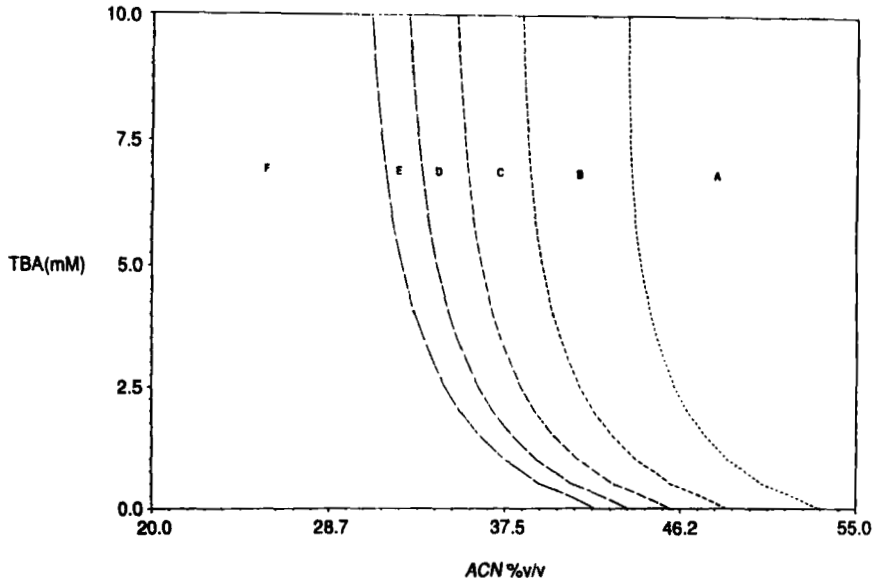

FIGURE 6

Predicted resolution map of diltiazem and deacetylated diltiazem.
Key for resolution (R_s) value: A = 1-3; B = 3-5; C = 5-7; D = 7-9; E = 9-11; F = over 11.

with TBA from 2.5 to 5 mM. Under the same conditions the k'_{max} for diltiazem will be in the region of 7. For convenience an eluent containing 3 mM TBA, 40 % *ACN* - buffer (20 mM Na_2HPO_4 at pH 2) was selected. The estimated k'_{max} and P_{max} for diltiazem with the above eluent, using equations 4 and 5 are 7.6 and 34 mM SDS, respectively. Similarly the respective estimated k'_{max} and P_{max} for deacetylated diltiazem are 5.9 and 36 mM SDS. The experimentally determined k' for these two solutes as function of mobile phase SDS concentration are shown in Figure 7. The k'_{max} and P_{max} obtained from this figure for diltiazem and deacetylated diltiazem are 8.1 and 37 mM SDS, and 6.8 and 43 mM SDS respectively. The predicted k'_{max} and P_{max} for the two compounds are within 17% of the experimentally obtained values, which confirms the predictive power of the models.

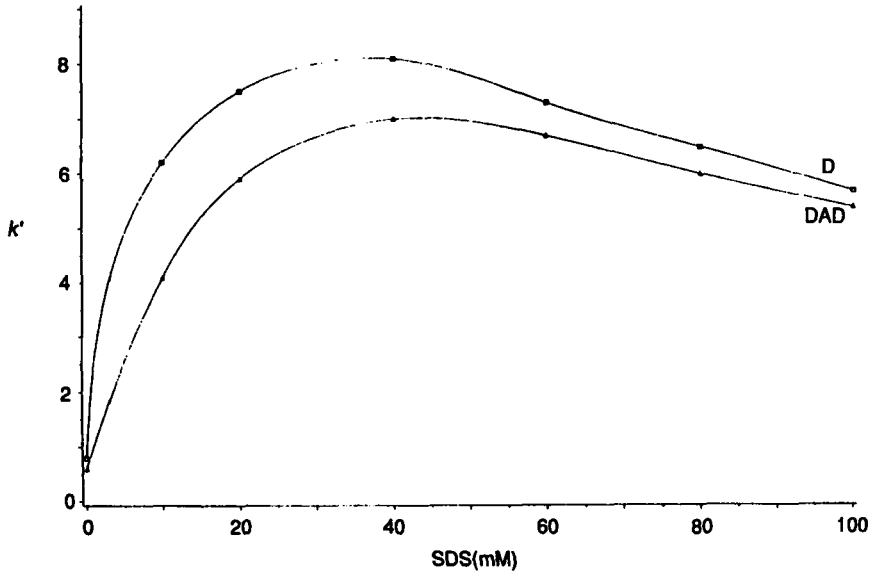

FIGURE 7

Plots showing the variation of k' with mobile phase pairing ion, SDS concentration for diltiazem (D) and deacetylated diltiazem (DAD).

In summary, the present study using a set of ionic compounds with large differences in solute properties, indicate that the log k'_{max} and log P_{max} of ionic solutes in ion-pair chromatography are governed by the solute hydrophobicity (logk'_w), mobile phase organic modifier and counterion concentration. Once these parameters are determined retention optimization of ionic solutes using ion-pair chromatography can be achieved using relatively simple mathematical models and statistical techniques.

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