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MOBILE PHASE OPTIMIZATION FOR THE REVERSED-PHASE LIQUID CHROMATOGRAPHY OF COMPLEX HYDROXYL AROMATIC MIXTURES

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SUMMARY

Mobile phases were optimized for the reversed-phase chromatography of complex hydroxyl aromatic mixtures using a method based on solubility parameter theory. Mixtures of twenty-one monohydroxyl aromatics and fourteen dihydroxyl aromatics were considered separately for the optimization. Optimal separation of the mono- and dihydroxyl aromatics was achieved with ternary mobile phases. However, complete resolution of the complex mixtures was not possible under the conditions investigated. Therefore, the limitations of the reversed-phase chromatographic systems were also defined.

INTRODUCTION

Hydroxyl aromatics are important in many chemical processes. They are used in the manufacture of resins, lacquers and adhesives as well as in the production of pharmaceuticals, dyes and pesticides. Hydroxyl aromatics are also environmental pollutants and are found in the waste effluents of coking, and pulp and paper plants. In addition, these compounds are important in coal liquefaction processes[1–7]. The separation and characterization of hydroxyl aromatic compounds are particularly essential for the development of modern coal liquefaction processes and for an understanding of coal liquefaction chemistry. However, hydroxyl aromatic fractions obtained from coal liquid samples are extremely complex. Generally, little work has been done in separating complex hydroxyl aromatic mixtures. Apparently, no work has been done in the systematic optimization of mobile phases for the separation of hydroxyl aromatics by high-performance liquid chromatography (HPLC).

Until recently, the determination of optimal mobile phase conditions was achieved by time consuming trial and error procedures. Various methods were developed to avoid those tedious processes. Morgan and Deming[8] reviewed a number of methods which are used to optimize chromatographic conditions. The chromatographic response function (CRF) has found utility in measuring chromatographic performance[9,10] by generalizing the concept of peak separation. Laub and co-workers[11–14] discussed the window diagram approach for improving separations by maxi-

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mizing the selectivity of the most difficult to resolve peak pairs. Recently, the window diagram approach was applied to HPLC[15,16]. Glajch *et al.*[17] described a systematic reversed-phase chromatographic mobile-phase optimization method. In general, the optimization method incorporated the Snyder solvent selectivity-triangle concept with a mixture-design statistical technique to optimize mobile phase strength and selectivity. Separation performance was evaluated by overlapping resolution mapping (ORM) plots which predicted the best mobile phase composition enabling a specified minimum resolution of all components in a mixture. This technique was extended to normal-phase[18] and gradient elution[19] systems.

Very recently, a new systematic reversed-phase optimization method emerged based on solubility parameter theory[20,21]. The optimization method relies on the assumption that an approximate linear relationship exists between log k' and a given volume fraction of a solvent in a ternary mobile phase. In addition, it was assumed that the ternary mobile phase was formed by mixing two iso-eluotropic binary mobile phases. With this approach, binary and ternary mobile phases offer the possibility of specific chromatographic effects which might enhance the resolution of a multicomponent mixture. The criteria for optimization are based on chromatographic parameters and are not dependent on statistical techniques. This allows the researcher to rely more on chromatographic results for determining optimal mobile phase conditions.

In this work, numerous mono- and dihydroxyl aromatics were used as model compounds to evaluate the solubility parameter optimization method for highly complex hydroxyl aromatic mixtures. Collectively, these compounds have not been investigated previously by HPLC. Moreover, little or no HPLC chromatographic data are available for many of these compounds. The optimization method provided a systematic method for determining optimal mobile phase conditions for complex hydroxyl aromatic mixtures. Furthermore, the approach defined the limitations of the chromatographic systems.

EXPERIMENTAL

High-performance liquid chromatograph

The liquid chromatograph used was a Waters Model ALC/GPC 244 equipped with a Model 6000A pump and a Model M-45 pump, both controlled by a Model 680 automated gradient controller for operation in the isocratic or gradient modes. A U6K injector, a dual channel free standing ultraviolet detector set at 254 nm and 280 nm, a Bascom-Turner Model 8120 electronic recorder, a dual channel 10-mV strip chart recorder, and a Hewlett-Packard Model 3390 A integrator were also used.

Columns

The columns employed were a 30 cm × 3.9 mm I.D. prepacked C_{18} column obtained from Waters Assoc. (Milford, MA, U.S.A.) and a 25 cm × 4.6 mm I.D. prepacked C_8 column obtained from Fisher Scientific (Pittsburgh, PA, U.S.A.). The Waters column was packed with μBondapak C_{18} and consisted of octadecyl groups chemically bonded to Waters 10-μm porous silica. The Fisher column was packed with Resolvex C_8 and consisted of octylsilane groups chemically bonded to Fisher Resolvex Sil (10-μm porous silica).

Reagents

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

Chromatographic systems

(1) Reversed-phase, μBondapak C_{18} with a methanol–water gradient at 1.0 ml/min.

(2) Reversed-phase, μBondapak C_{18} with a methanol–water mobile phase at 1.0 ml/min.

(3) Reversed-phase, μBondapak C_{18} with an acetonitrile–water mobile phase at 1.0 ml/min.

(4) Reversed-phase, μBondapak C_{18} with a tetrahydrofuran–water mobile phase at 1.0 ml/min.

(5) Reversed-phase, μBondapak C_{18} with a methanol–tetrahydrofuran–water mobile phase at 1.0 ml/min.

(6) Reversed-phase, μBondapak C_{18} with an acetonitrile–tetrahydrofuran–water mobile phase at 1.0 ml/min.

(7) Reversed-phase, Resolvex C_8 with an acetonitrile–tetrahydrofuran–water mobile phase 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 μl of the standard solution into the chromatographic systems described above. Peak splitting was observed for >99% 1,4-dihydroxybenzene when it was injected onto the above chromatographic systems. This 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). V_m was obtained by eluting methanol and was determined to be 2.80 ml for the μBondapak C_{18} column.

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

Theoretical considerations

Schoenmakers *et al.*[20,21] have discussed in detail the chromatographic use of

ternary mobile phases which are defined by two limiting iso-eluotropic binary mobile phases. It can be shown using solubility parameter theory that all ternary mixtures of water and two organic modifiers which possess a given polarity follow a straight line between two iso-eluotropic binary compositions in a three component phase diagram[20,22]. The optimization scheme developed by Schoenmakers *et al.*[20,21] assumes that log k' varies linearly with the composition of ternary mixtures formed by mixing two iso-eluotropic mobile phases. Deviations from the assumed linear relationships are expected and are experimentally observed due to specific solute–solvent effects[22]. Very recently, Colin *et al.*[23] have suggested the non-linear behavior observed with ternary solvent systems can be attributed partly to the relative compositions of the binary solvents used to prepare the ternary mixtures and the method used to determine column void volume.

The mobile-phase optimization criteria considered in this study have been discussed previously[20,21] and only a brief overview will be given. The optimization criterion, ΠR_s, corresponds to the product of resolution factors, R_s, for each pair of adjacent peaks in a chromatogram. The advantages of this optimization criterion were discussed earlier[20]. The most important advantage is the inclusion of peak band width in the criterion. The product ΠR_s is defined by eqn. 1[20].

$$\Pi R_s = \prod_{i=1}^{n-1} \frac{k_{i+1} - k_i}{k_{i+1} + k_i + 2} \tag{1}$$

Recently, this criterion was modified to account for differing chromatogram lengths[21]. The relative resolution product, r, takes into account changing chromatogram length with changes in solvent composition. This optimization criterion is defined by eqn. 2[21].

$$r = \prod_{i=1}^{n-1} R_{s_{i+1,i}} \Bigg/ \left[\left(\sum_{i=1}^{n-1} R_{s_{i+1,i}} \right) \Big/ (n-1) \right]^{n-1} \tag{2}$$

A further refinement of the optimization criterion, r, yields the optimization criterion, r^*, which aims at optimum analysis time and is defined by eqn. 3[21].

$$r^* = \prod_{i=0}^{n-1} R_{s_{i+1,i}} \Bigg/ \left[\left(\sum_{i=0}^{n-1} R_{s_{i+1,i}} \right) \Big/ (n-1) \right]^{n-1} \tag{3}$$

The latter two equations were misprinted earlier[21] and the corrected forms presented here have been verified[24].

The above optimization criteria were evaluated for the separation of individual groups of twenty-one mono- and fourteen dihydroxyl aromatics on a μBondapak C_{18} column. In the past, the optimization approach has been used for the separation of only five or six compounds[20,21].

TABLE I

LOG k' VALUES FOR HYDROXYL AROMATICS ON μBONDAPAK C_{18} WITH ISO-ELUOTROPIC BINARY MOBILE PHASES

Compound number	*Compound*	*Log k'*		
		Methanol–water (65:35)	*THF–water (40:60)*	*Acetonitrile–water (50:50)*
Monohydroxy compounds				
1	1-Acenaphthenol	0.01	0.26	0.04
2	5H-Dibenzo[*a*,*d*]cycloheptene-5-ol	0.24	0.68	0.41
3	7,12-Dimethyl-9-hydroxybenz[*a*]-anthracene	1.12	0.96	0.98
4	2-Hydroxybenzo[*c*]phenanthrene	0.78	0.81	0.77
5	3-Hydroxybenzo[*c*]phenanthrene	0.79	0.79	0.74
6	1-(1-Hydroxyethyl)pyrene	0.70	0.63	0.62
7	1-(Hydroxymethyl)benz[*a*]pyrene	1.00	0.69	0.86
8	4-Hydroxymethylpyrene	0.54	0.51	0.48
9	9-Hydroxyphenanthrene	0.41	0.74	0.48
10	13-Hydroxypicene	1.36	1.13	1.26
11	1-Hydroxypyrene	0.60	0.76	0.65
12	4-Hydroxypyrene	0.59	0.76	0.65
13	1-Indanol	−0.22	0.04	−0.20
14	5-Indanol	−0.01	0.48	0.11
15	1-Naphthol	−0.02	0.54	0.18
16	2-Naphthol	−0.07	0.46	0.11
17	3-Phenylphenol	0.18	0.62	0.31
18	1,2,3,4-Tetrahydro-4-hydroxy-4-methylphenanthrene	0.47	0.75	0.52
19	1,2,3,4-Tetrahydro-1-naphthol	−0.07	0.21	−0.03
20	5,6,7,8-Tetrahydro-1-naphthol	0.20	0.67	0.30
21	5,6,7,8-Tetrahydro-2-phenanthrol	0.62	0.83	0.62
		Methanol–water (40:60)	*THF–water (25:75)*	*Acetonitrile–water (30:70)*
Dihydroxy compounds				
22	*o*,*o*'-Biphenol	0.51	1.20	0.67
23	*p*,*p*'-Biphenol	0.51	0.96	0.34
24	1,2-Dihydroxybenzene	−0.22	0.34	−0.22
25	1,3-Dihydroxybenzene	−0.41	0.21	−0.39
26	1,4-Dihydroxybenzene	−0.21	−0.04	−0.52
27	1,3-Dihydroxynaphthalene	0.43	0.96	0.33
28	1,6-Dihydroxynaphthalene	0.24	0.81	0.16
29	1,7-Dihydroxynaphthalene	0.45	0.87	0.26
30	2,3-Dihydroxynaphthalene	0.50	0.98	0.40
31	2,6-Dihydroxynaphthalene	0.11	0.62	0.02
32	2,7-Dihydroxynaphthalene	0.28	0.73	0.13
33	2,5-Dihydroxyphenanthrene	1.03	1.30	0.79
34	2,6-Dihydroxytoluene	−0.31	0.40	−0.21
35	3,5-Dihydroxytoluene	−0.13	0.39	−0.18

Gradient elution

The mobile phase optimization procedure was initiated by using gradient elution to predict an isocratic methanol–water mobile phase, which would approximately give $1 < k' < 1$ for the complex mono- and dihydroxyl mixtures. Recent work in this laboratory showed that mono- and dihydroxyl aromatics can be group separated by a normal-phase chromatographic system. This work will be published later. The two groups of compounds were considered separately for the reversed-phase optimization method considered here. Table I gives the standard hydroxyl compounds comprising the two complex mixtures investigated.

Snyder *et al.*[25,26] have previously described a theoretical relationship for predicting isocratic mobile phases from gradient elution data. This method, due to its simplicity, was used in contrast to the graphical procedure developed by Schoenmakers *et al.*[27]. Also, this method was not constrained to a certain data set as with the approach by Schoenmakers *et al.*[27] and comparison of the two approaches yielded nearly identical results. Gradient elution affords a more rapid and practical means of predicting isocratic mobile phases compared to the isocratic trial and error approach. Thus, using the guidelines of Snyder and co-workers[28,29], a linear gradient of methanol–water was designed to effect complete elution of the complex hydroxyl aromatic mixtures from the μBondapak C_{18} column within the time of the gradient. The data obtained from the gradient experiments were then used to predict isocratic mobile phases of methanol–water (65:35 and 40:60) for the mono- and dihydroxyl mixtures, respectively. These predicted compositions eluted most of the components in the respective mixtures within the approximately desired k' range, namely, $1 < k' < 10$.

Iso-eluotropic binary mobile phases

Solubility parameter theory was used to calculate the compositions of various binary mobile phases that were iso-eluotropic, or in other words have the same polarity as the methanol–water binary mobile phases determined previously from gradient elution results. These calculations relied on the ability of the total solubility parameter to quantitate solvent polarity. Schoenmakers *et al.*[22,30] have discussed in detail the use of solubility parameter theory to calculate iso-eluotropic mobile phases. In this work, the total solubility parameter was used in calculating iso-eluotropic binary mobile phases of acetonitrile–water and tetrahydrofuran (THF)–water that were iso-eluotropic with the methanol–water binary mobile phases. The iso-eluotropic binary mobile phases investigated in this study for the monohydroxyl mixture were methanol–water (65:35), acetonitrile–water (50:50), and THF–water (40:60). For the dihydroxyl mixture the binary compositions studied were methanol–water (40:60), acetonitrile–water (30:70), and THF–water (25:75).

Mobile phase optimization for monohydroxyl aromatic compounds

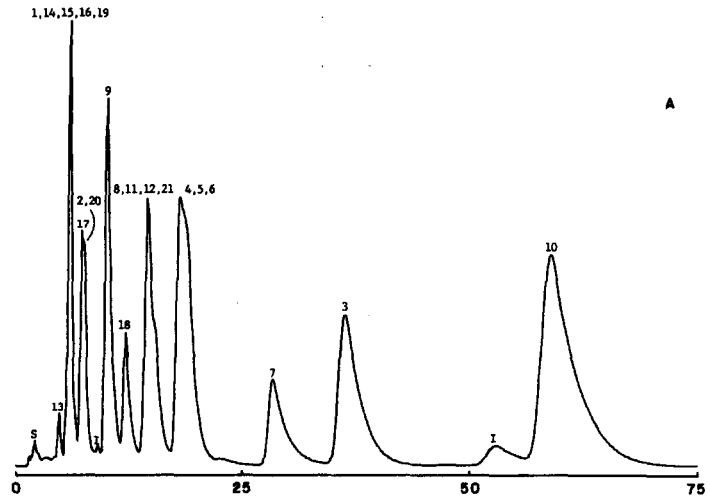
Twenty-one monohydroxyl aromatic compounds were investigated with a μBondapak C_{18} column with several binary and ternary mobile phases. Table I gives the compounds investigated and the chromatographic data obtained with the iso-eluotropic binary mobile phases discussed previously. Fig. 1 shows the chromatographic separation of the monohydroxyl aromatic mixture with the iso-eluotropic binary mobile phases. It is evident from Fig. 1 that none of the iso-eluotropic binary

mobile phases is capable of completely separating the monohydroxyl mixture. However, the same compounds did not overlap with a given binary mobile phase suggesting that a ternary mobile phase may provide better resolution of the mixture. In addition, iso-eluotropic three-component mobile phase diagrams were constructed by us for a number of hydroxyl aromatic compounds, and these diagrams supported the conclusions drawn by Schoenmakers and co-workers[20,22]. Therefore, it was assumed, the optimization scheme developed by Schoenmakers *et al.*[20,21] would be applicable for optimizing hydroxyl aromatics on μBondapak C_{18}. Based on the results discussed above, ternary compositions were investigated for optimizing the separation of the complex monohydroxyl mixture on μBondapak C_{18}.

In general, the optimization criterion, defined by eqn. 2, is the most important; however, all three criteria were used to judge the optimal mobile phase composition in this work[21]. Optimum conditions corresponded to maximizing these criteria with particular attention given to r[21]. Drouen *et al.*[21] have described an iterative routine to predict the optimal mobile phase composition taking into account the non-linear behavior of log k' as a function of mobile phase composition. Basically, in this procedure the solvent composition predicted by the optimization criterion, r, is actually shifted to a new composition which minimizes the difference between the assumed linear and the more appropriate quadratic relationship for log k' integrated over a range on either side of the predicted optimum[21]. Therefore, the next chromatogram is obtained with the shifted composition rather than the one predicted by the optimization criterion. The result is a more efficient convergence upon the true optimum mobile phase. The expression for calculating the shifted composition has been discussed previously[21] and was implemented in this work. In addition, a computer program was developed by us which calculated and constructed plots of log k' as a function of mobile phase composition from chromatographic retention data for any number of compounds. The program utilized the information from the above plots to calculate values for the three optimization criteria which were used in turn to construct a mobile phase selection diagram. Furthermore, the program calculated the mobile phase compositions corresponding to the maximum values of the optimization criteria.

In general, the optimization procedure used in this work can be outlined as follows: (a) construct a mobile phase selection diagram from the retention data obtained with three iso-eluotropic binary mobile phases, (b) determine the composition predicted by the maximum value of the criterion, r, and calculate the appropriate shifted composition, (c) obtain retention data with the shifted composition and incorporate this data into the previous mobile phase selection diagram, (d) determine the mobile phase composition predicted by the new maximum value of the criterion, r, from the partially corrected mobile phase selection diagram, and (e) calculate the appropriate shifted composition and repeat steps c through e until approximately no change in the optimal mobile phase composition occurs.

Fig. 2 shows the assumed linear relationships for some of the twenty-one monohydroxyl compounds and the computer calculated uncorrected mobile phase selection diagrams for all twenty-one monohydroxyl aromatics. The situation presented here is more complex than those previously considered because of the number of compounds investigated, the number of isomers present, and the fact that only hydroxyl aromatics were investigated[20,21]. The maximum value of the relative resolu-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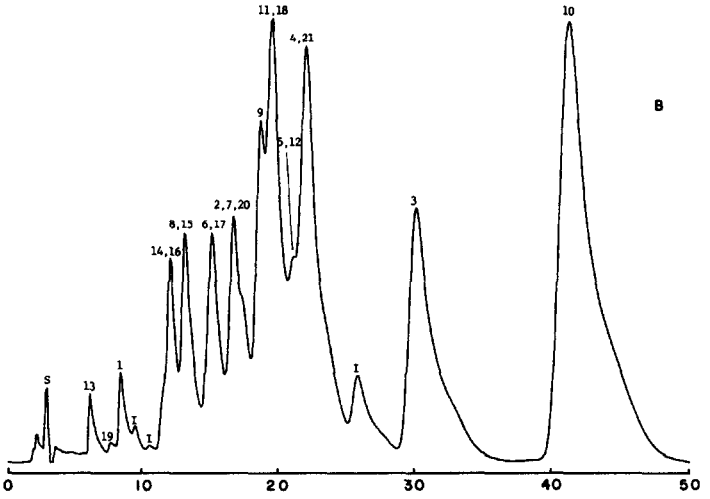
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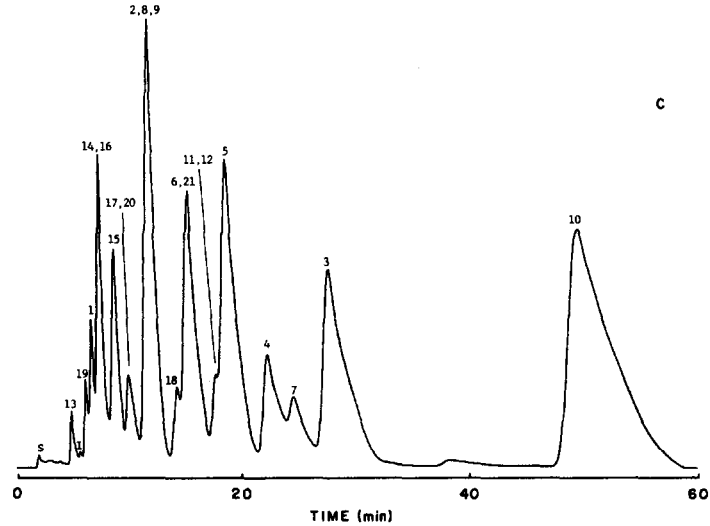
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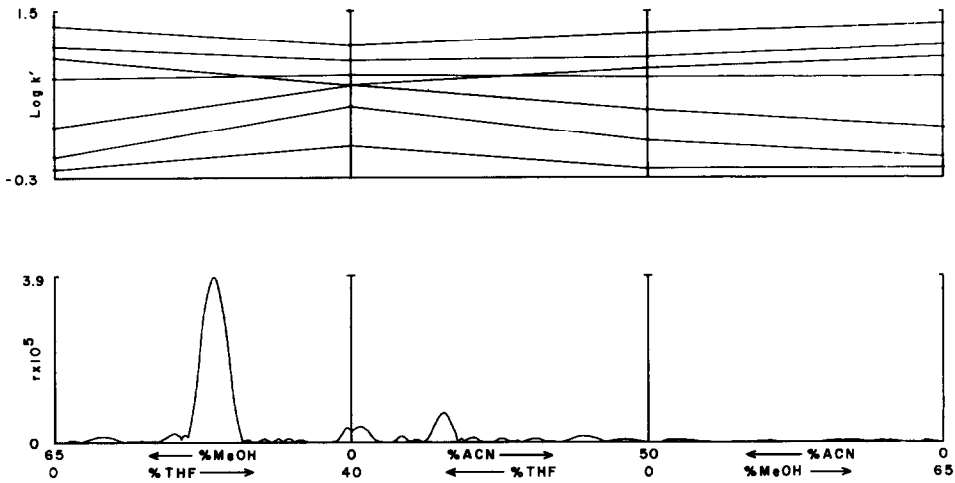
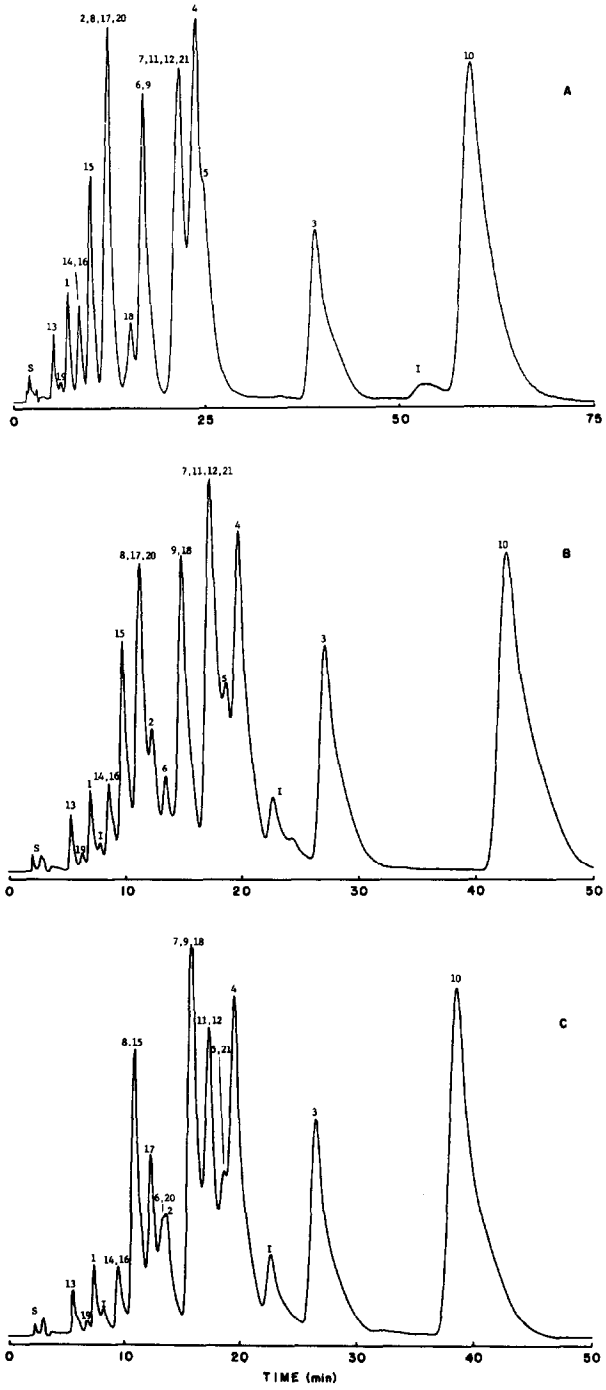


Fig. 2. Uncorrected mobile phase selection diagram for the relative resolution product, r, calculated from the chromatographic data of Fig. 1 for the monohydroxyl aromatic mixture. For clarity only seven of the twenty-one log k' relationships are shown in the top figures. MeOH = methanol; ACN = acetonitrile.

tion product, $r = 3.9 \cdot 10^{-4}$, which was obtained from Fig. 2, predicts a ternary composition of methanol–THF–water (30.0:21.5:48.5). However, using the iterative method of Drouen *et al.*[21], the chromatogram in Fig. 3A was obtained with a shifted composition which in this case was calculated to be identical to the above predicted composition (see Table II). This chromatogram showed little or no improvement over the chromatograms in Fig. 1. The new retention data in Fig. 3A was used to construct a partially corrected mobile phase selection diagram from which a new value of the criterion, r, was obtained. This process was repeated for each additional shifted mobile phase composition discussed below resulting in Fig. 4. Fig. 4 shows the non-linear behavior for log k' discussed earlier and the final corrected mobile-phase selection diagram.

The value of the criterion obtained from Fig. 4 for the shifted composition methanol–THF–water (30.0:21.5:48.5) was now significantly reduced ($r = 5.8 \cdot 10^{-8}$). However, from Fig. 2, a previous higher value for the criterion, $r = 6.6 \cdot 10^{-5}$, was recalled which defined a ternary composition of acetonitrile–THF–water (15.2:27.8:57.0) (see Table II). Again, the actual chromatogram (Fig. 3B) was obtained at a shifted composition of acetonitrile–THF–water (19.2:24.7:56.1). The resulting chromatogram (Fig. 3B) shows a definite improvement over previous separation attempts with a total of fourteen peaks now discernable and $r = 5.6 \cdot 10^{-6}$ (Table II). Using the retention data in Fig. 3B, an even higher criterion value was calculated ($r = 1.6 \cdot 10^{-4}$) which corresponded to a chromatogram (Fig. 3C) run at the shifted composition acetonitrile–THF–water (9.4:32.5:58.1). This chromatogram is clearly poorer than its predecessor, and this result is reflected by the concomitant

Fig. 1. Chromatograms of monohydroxyl aromatic mixture on μBondapak C_{18} with iso-eluotropic binary mobile phases methanol–water (65:35) (A), THF–water (40:60) (B), and acetonitrile–water (50:50) (C) at 1.0 ml/min. Peak numbers correspond to compounds in Table I. S and I are solvent and impurity peaks, respectively.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TIME (min)

Fig. 3.

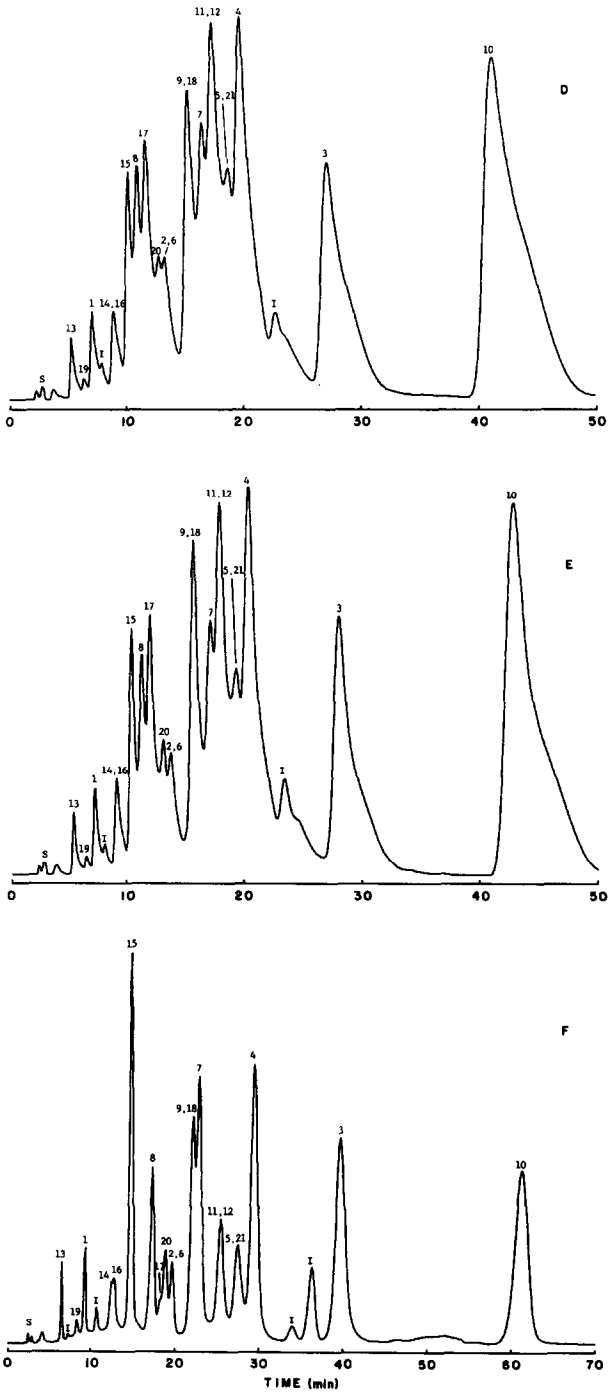

Fig. 3. Chromatograms of monohydroxyl aromatic mixture on μBondapak C_{18} with ternary mobile phases methanol–THF–water (30.0:21.5:48.5) (A), acetonitrile–THF–water (19.2:24.7:56.1) (B), acetonitrile–THF–water (9.4:32.5:58.1) (C), acetonitrile–THF–water (14.6:28.3:57.1) (D), acetonitrile–THF–water (15.2:27.8:57.0) (E), and acetonitrile–THF–water (15.2:27.8:57.0, on Resolvex C_8) (F) all at 1.0 ml/min. Peak numbers correspond to compounds in Table I. S and I are solvent and impurity peaks, respectively.

TABLE II

COMPARISON OF PREDICTED AND SHIFTED MOBILE PHASE COMPOSITIONS AND OPTIMIZATION CRITERIA VALUES FOR THE SEPARATION OF MONO- AND DIHYDROXY MIXTURES

	Predicted from optimization criterion (r)		*Calculated from chromatograms obtained at shifted compositions*				
	Composition	*r*	*Composition*	*Fig.*	*r*	ΠR_s	*r**
Monohydroxy mixture	Methanol–THF–water						
	30.0:21.5:48.5	$3.9 \cdot 10^{-4}$	30.0:21.5:48.5	3A	$5.8 \cdot 10^{-8}$	$4.6 \cdot 10^{-32}$	$3.1 \cdot 10^{-9}$
	Acetonitrile–THF–water						
	15.2:27.8:57.0	$6.6 \cdot 10^{-5}$	19.2:24.7:56.1	3B	$5.6 \cdot 10^{-6}$	$1.7 \cdot 10^{-31}$	$1.6 \cdot 10^{-7}$
	14.2:28.7:57.1	$1.6 \cdot 10^{-4}$	9.4:32.5:58.1	3C	$1.3 \cdot 10^{-7}$	$2.5 \cdot 10^{-34}$	$1.5 \cdot 10^{-9}$
	14.2:28.9:56.9	$1.0 \cdot 10^{-4}$	14.6:28.3:57.1	3D	$7.2 \cdot 10^{-5}$	$1.1 \cdot 10^{-30}$	$1.6 \cdot 10^{-6}$
	15.2:27.8:57.0	$9.8 \cdot 10^{-5}$	(15.2:27.8:57.0)*	3E	$(9.8 \cdot 10^{-5})$	$(1.7 \cdot 10^{-30})$	$(2.2 \cdot 10^{-6})$
Dihydroxy mixture	Methanol–THF–water						
	8.5:19.7:71.8	$8.1 \cdot 10^{-3}$	13.1:16.8:70.1	5D	$2.0 \cdot 10^{-3}$	$3.7 \cdot 10^{-16}$	$5.7 \cdot 10^{-4}$
	1.8:23.7:74.5	$2.6 \cdot 10^{-3}$	8.5:19.7:71.8	5E	$5.1 \cdot 10^{-3}$	$8.2 \cdot 10^{-16}$	$1.3 \cdot 10^{-3}$
	8.5:19.7:71.8	$5.1 \cdot 10^{-3}$					

* Represents final mobile phase composition and corresponding optimization factors.

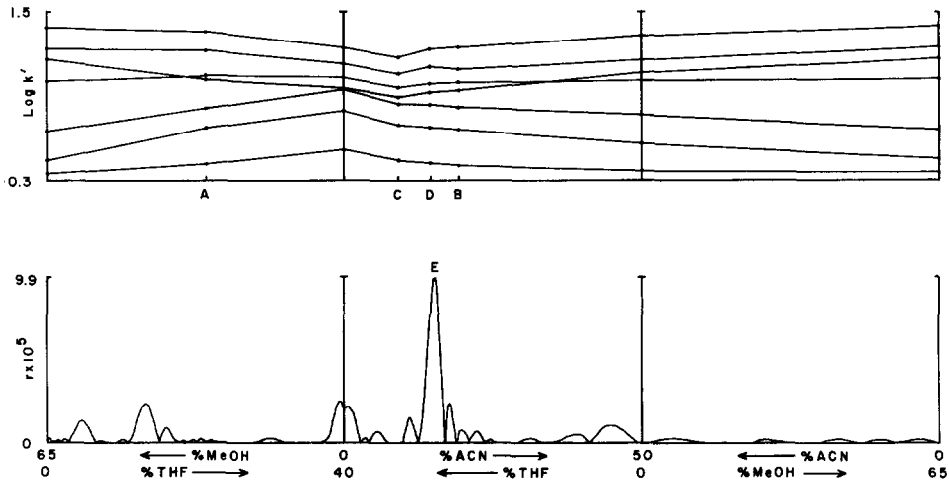

Fig. 4. Final corrected mobile phase selection diagram for the relative resolution product, r, calculated from the chromatographic data of Figs. 1 and 3 for the monohydroxyl aromatic mixture. A, B, C, D and E refer to the chromatograms in Fig. 3. For clarity only seven of the twenty-one log k' relationships are shown in the top figures.

decrease in r for the chromatogram ($r = 1.3 \cdot 10^{-7}$). The most likely reason for this result and consequently the larger predicted r values compared to shifted r values in Table II is the non-linearity of the log k' *versus* mobile phase composition plots (Fig. 4). However, a still higher value for the criterion was calculated ($r = 1.0 \cdot 10^{-4}$) for the mobile phase shown in Table II which resulted in a shifted composition of acetonitrile–THF–water (14.6:28.3:57.1). The resulting chromatogram (Fig. 3D) is significantly improved over the previous one shown in Fig. 3C with $r = 7.2 \cdot 10^{-5}$. In addition, the separation in Fig. 3D is clearly superior to that shown in Fig. 3B. This conclusion is substantiated by the higher criteria values (Table II) attributable to the chromatogram in Fig. 3D. To this end, a higher value for the criterion was obtained from Fig. 4, $r = 9.8 \cdot 10^{-5}$, corresponding to the shifted composition acetonitrile–THF–water (15.2:27.8:57.0). The resulting chromatogram (Fig. 3E) is very similar to the chromatogram in Fig. 3D.

Comparison of the respective optimization criteria values given in Table II for Fig. 3D and E suggests that the composition acetonitrile–THF–water (15.2:27.8:57.0) results in a slightly better separation for the monohydroxyl mixture. Furthermore, the compositions used in obtaining these chromatograms (Fig. 3D and E) are nearly identical; thus it can be assumed that the non-linearity between these compositions is minimal. Therefore, the optimization procedure ends with the chromatogram in Fig. 3E obtained with acetonitrile–THF–water (15.2:27.8:57.0) as the optimal mobile phase for the separation of the monohydroxyl mixture on μBondapak C_{18}. In fact, any of the three shifted optimization criteria in Table II suggest the same conclusion. This is partly a result of using the maximum values of the criterion, r, in establishing the predicted mobile phases. However, it is important to retain the criterion, ΠR_s, throughout the optimization since a maximum value of the criterion, r, does not necessarily mean an optimum separation[21]. On the other hand, r^* added little additional information not already predicted by r. Also, it should be noted that the

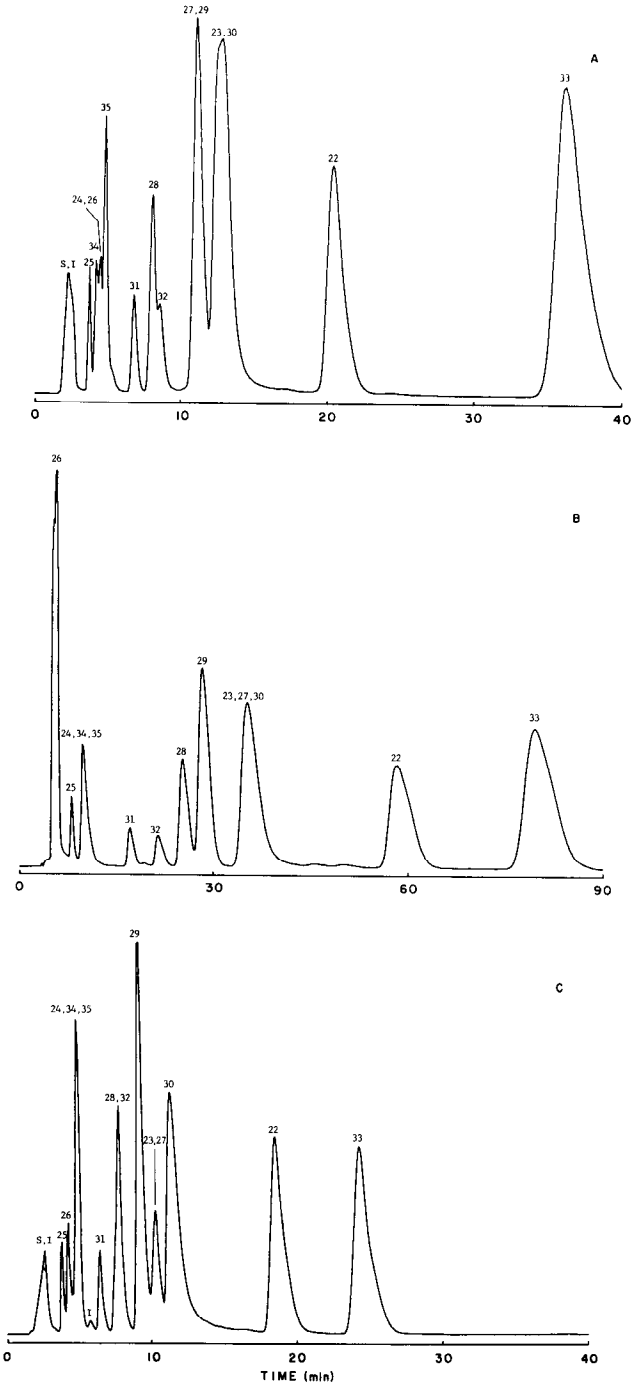
A
27,29
23,30
33
35
22
28
24,26
34
S,I
25
31
32
0
10
20
30
40
B
26
29
23,27,30
33
24,34,35
28
22
25
31
32
0
30
60
90
C
29
24,34,35
30
28,32
22
33
23,27
26
S,I
25
31
I
0
10
20
30
40
TIME (min)

Fig. 5.

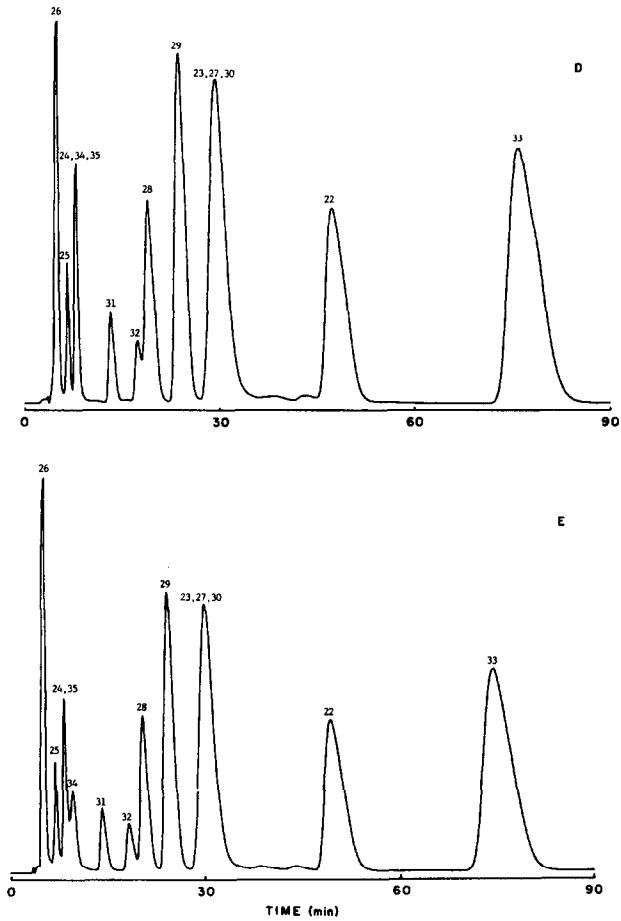

Fig. 5. Chromatograms of dihydroxyl aromatic mixture on μBondapak C_{18} with iso-eluotropic binary and ternary mobile phases methanol–water (40:60) (A), THF–water (25:75) (B), acetonitrile–water (30:70) (C), methanol–THF–water (13.1:16.8:70.1) (D), and methanol–THF–water (8.5:19.7:71.8) (E) all at 1.0 ml/min. Peak numbers correspond to compounds in Table I. S and I are solvent and impurity peaks, respectively.

optimal mobile phase composition predicted by Fig. 4, acetonitrile–THF–water (15.2:27.8:57.0), was predicted earlier by Fig. 2 (also see Table II). This most likely occurred because of the approximate linear relationship between log k' and mobile phase composition shown in the middle section of Fig. 4. Even though the final mobile phase composition was predicted earlier, it was necessary to use the iterative procedure because the final mobile phase could not be predicted *a priori*.

In an attempt to further improve the separation shown in Fig. 3E, the monohydroxyl mixture was chromatographed on a Fisher Resolvex C_8 column having approximately twice the number of theoretical plates as the μBondapak C_{18} column with the above optimum mobile phase acetonitrile–THF–water (15.2:27.8:57.0). The result (Fig. 3F) is a significant improvement in resolution and selectivity for the monohydroxyl mixture. A total of seventeen peaks are now evident

most with good resolution. Also, no change in elution order occurred in switching from the C_{18} to the C_8 column. In addition, the monohydroxyl aromatic mixture was chromatographed on a new μBondapak C_{18} column with the optimum ternary mobile phase resulting in a substantial improvement in resolution. This indicated that the original μBondapak C_{18} column had deteriorated somewhat with use. However, the C_8 column gave the best resolution of the three columns used.

Optimization of dihydroxyl aromatic compounds

Analogous to the previous discussion, fourteen dihydroxyl aromatic compounds were investigated with a μBondapak C_{18} column with several binary and ternary mobile phases. Table I gives the compounds investigated and the chromatographic data obtained with the iso-eluotropic binary mobile phases discussed previously. Fig. 5A–C shows the chromatograms of the mixture of dihydroxyl compounds with the binary mobile phases listed in Table I. Similar to the previous discussion, none of the iso-eluotropic binary mobile phases effected complete separation of the dihydroxyl mixture. The possibility of a ternary mobile phase improving the above separation was apparent since different compounds overlapped in each iso-eluotropic binary mobile phase.

The optimization method discussed above was applied to the dihydroxyl mixture to search for the optimum mobile phase for separating the mixture on μBondapak C_{18}. Fig. 6 shows the assumed linear relationships for some of the fourteen dihydroxyl compounds and the computer calculated mobile phase selection diagram for all fourteen dihydroxyl aromatics. The maximum value of the relative resolution product $r = 8.1 \cdot 10^{-3}$ obtained from Fig. 6 predicted a ternary composition of methanol–THF–water (8.5:19.7:71.8). In accordance with the discussion beforehand, the next chromatogram (Fig. 5D) was run at a shifted composition of methanol–THF–water (13.1:16.8:70.1) (see Table II). Fig. 5D with $r = 2.0 \cdot 10^{-3}$ shows no improvement over the best binary separation (Fig. 5B) for the dihydroxyl mixture.

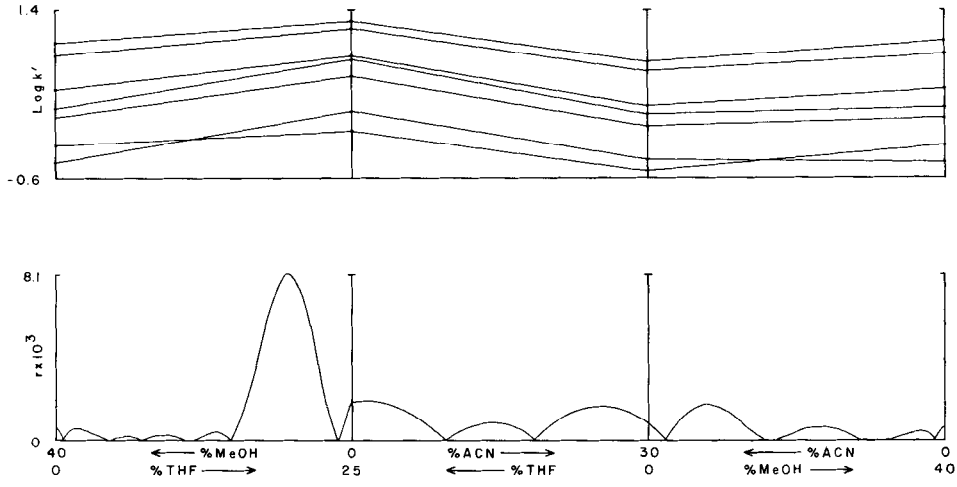

Fig. 6. Uncorrected mobile phase selection diagram for the relative resolution product, r, calculated from the chromatographic data of Fig 5A–C for the dihydroxyl aromatic mixture. For clarity only seven of the fourteen log k' relationships are shown in the top figure.

However, using the retention data in Fig. 5D a higher criterion value was obtained, $r = 2.6 \cdot 10^{-3}$, corresponding to the shifted composition methanol–THF–water (8.5:19.7:71.8). The resulting chromatogram (Fig. 5E) is improved compared to the previous one (Fig. 5D) with the criterion value now increased to $r = 5.1 \cdot 10^{-3}$. In addition, eleven peaks are evident in Fig. 5E compared to the ten discernable in Fig. 5D. At this point the optimization was terminated since the next predicted composition and the previous shifted composition were identical (see Table II). Furthermore, the compositions used in obtaining the chromatograms in Fig. 5D and E are not widely different; therefore, it can be assumed that the non-linearity between these compositions is small. Comparison of the optimization criteria values in Table II suggests that the composition used to obtain Fig. 5E gives optimum separation of the dihydroxyl mixture. Therefore, with a criterion value of $r = 5.1 \cdot 10^{-3}$ the chromatogram in Fig. 5E obtained with methanol–THF–water (8.5:19.7:71.8) was determined to be the optimal mobile phase for the separation of the dihydroxyl mixture on μBondapak C_{18}. Notice that this composition was predicted earlier in Fig. 6 (see also Table II). Apparently, this occurred because of the approximate linear relationship between log k' and mobile phase composition shown in Fig. 7. In addition, Table II shows that ΠR_s and r^* follow the same trend as r. As with the monohydroxyl aromatics, it was not possible to know beforehand that an earlier predicted mobile phase composition was the optimum.

Although complete resolution of the mono- and dihydroxyl mixtures was not possible, it was shown that the optimization scheme developed by Schoenmakers *et al.*[20,21] provides a systematic method for determining optimal mobile phase conditions for highly complex mixtures. It should be emphasized that the hydroxyl aromatic mixtures contained several isomers which added to the difficulty in separating all the components. Note that not all ternary systems were searched. This is due to the inherent ability of the optimization method to discriminate against inferior ter-

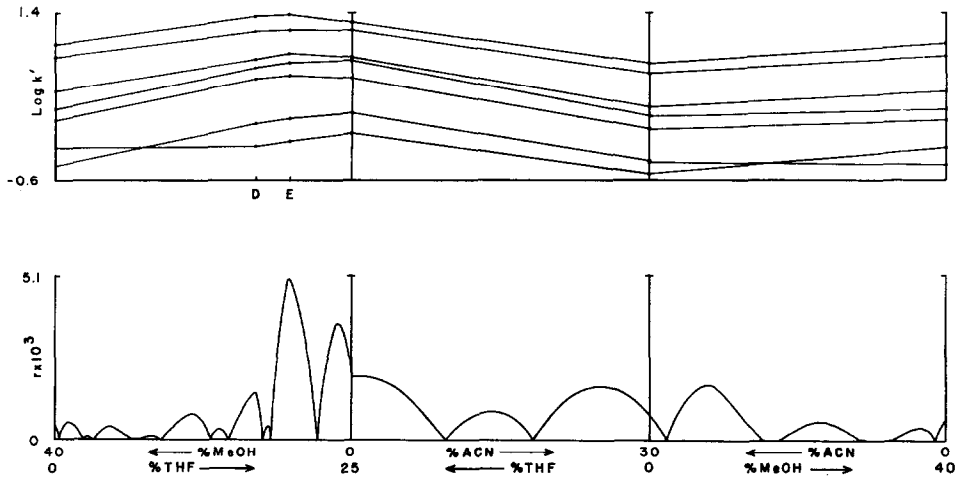

Fig. 7. Final corrected mobile phase selection diagram for the relative resolution product, r, calculated from the chromatograpahic data of Fig. 5 for the dihydroxyl aromatic mixture. D and E refer to the chromatograms in Fig. 5. For clarity only seven of the fourteen log k' relationships are shown in the top figure.

nary systems. This results in increased efficiency in searching for optimal conditions. Furthermore, the optimization method[20,21] defines the limitations of the reversed-phase chromatographic systems. In this case no single isocratic binary or ternary mobile phase was capable of effecting complete resolution of the mono- or dihydroxyl mixtures. However, the resolution of the poorest separated peak pair can be obtained for the optimum composition with the method described, thus allowing the prediction of the number of theoretical plates required for complete separation. This is an advantage shared with other optimization methods[11–14,17,18].

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