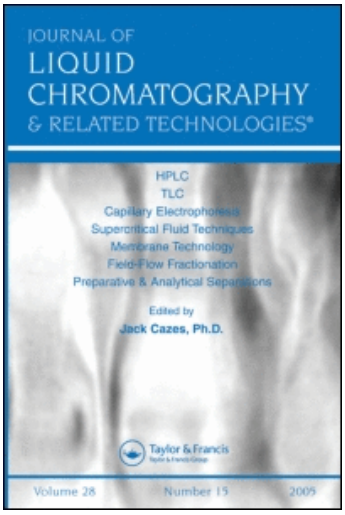

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A SYSTEMATIC STATISTICAL METHOD OF SOLVENT SELECTION FOR OPTIMAL SEPARATION IN LIQUID CHROMATOGRAPHY

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ABSTRACT

A systematic method is described for selecting the optimum ternary mobile phase for both thin layer and high performance liquid chromatography. The statistical data analysis employs overlapping resolution mapping in which a contour plot is made by plotting resolution against solvent composition. The computer analysis predicts optimum mobile phase regions, from which the analyst can select the least viscous, and cheapest, mobile phase. Peak crossover is taken into consideration. Good agreement was observed between predicted and experimental data. The method is simple and easy to apply to liquid chromatography.

INTRODUCTION

The selection of a solvent system which will give optimum resolution in liquid chromatography (adsorption, partition or ion exchange) is not a simple matter. The most important considerations are the properties of the material being separated and the solid phase. The mobile phase can be selected only when these two factors have been defined. When the solvent is a binary, ternary...etc. mixture, solvent-solute and solvent-solvent interactions must be taken into consideration. A trial-and-error procedure is generally used to

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find a mobile phase which will satisfactorily resolve all the components of the mixture. When the mobile phase is composed of more than one solvent the task of selection becomes complex. In TLC, unlike HPLC, the process of solvent selection is less time consuming because the analyst can spot as many plates as he has developing tanks and develop them in different solvent mixtures or use a unit like the Selecta Sol or the Vario KS-Chamber in which up to 16 different solvents can be simultaneously tried on a 20 X 20 cm plate (1). Although it has been shown that TLC solvents can be used as mobile phases for HPLC (2,3), this simple approach is by no means a systematic one leading to the selection of an optimum mobile phase. We define an optimum mobile phase as that solvent mixture which would give base-line-separation of all the components of a sample mixture in the minimum amount of time.

Glajch et al (4), Belinky (5), and others (6-8) described a systematic solvent optimization procedure which employs statistical methods of data analysis. Our study describes a systematic approach to selecting a ternary solvent mixture based on a plot of pair resolution versus solvent composition, and overlapping resolution mapping (ORM) data analysis similar to that employed by Glajch et al (4). Peak crossover is taken into consideration. The method is simple and can be applied to both partition and adsorption liquid chromatography. In addition to solvent optimization, the answers obtained give the analyst the opportunity to select (a) the least viscous mobile phase (least back pressure), (b) the cheapest solvent mixture and (c) the shortest retention times.

EXPERIMENTAL

Materials: All solvents were glass distilled (Burdick and Jackson). The chemicals were analytical grade (Aldrich Chemical Co.) and used without further purification.

Apparatus: A modular HPLC system consisting of Laboratory Data Control (LDC) constametric I and II Pumps attached to an LDC Gradient Master, a Chromatronix

dual-channel uv absorbance detector, a Rheodyne injector, and a strip-chart recorder operated at 0.2 in/min was used.

The RP-8 and RP-18 reverse phases columns were all 250 mm X 4.6 mm prepacked with 10 μm particle size materials (Merck). 10 μl samples were injected. Experiments were run at room temperature using a mobile phase flow rate of 1.2 ml/min. Retention times, peak widths (W) and resolution (R_S) were determined by a 3352A Laboratory Data System (Hewlett-Packard) linked through a Hewlett-Packard 1865 A/D converter to the UV detector output of the liquid chromatograph. The output from the data system was recorded on a 9866A thermal line printer (Hewlett-Packard). Silica gel and reverse phase (RP-8 and RP-18) TLC plates were purchased from Whatman, Inc. Standard TLC tanks and equipment were used. Plates were spotted with 5 μl disposable micropipettes.

Procedure: A combination of the three initial solvents is devised according to Table 1. Other combinations may also be used. The initial solvents maybe pure

Table 1

Combination of solvents A, B and C used in this study to predict optimum mobile phase compositions.

% SOLVENT A	% SOLVENT B	% SOLVENT C
100	0	0
0	100	0
0	0	100
50	50	0
50	0	50
0	50	50
33	33	33
67	16	16
16	67	16
16	16	67

or a mixture of two organic solvents (normal phase) or a mixture of water/organic modifier (reverse phase). After selecting the solvents and proportions to be used (Table 1), 10 data points, one for each solvent combination are collected. These are used to calculate the resolutions of each pair of compounds in the mixture. If no peak crossover takes place the resolution between each pair (1-2, 2-3, 3-4.....etc) is used. If peak crossover does occur the resolution between all the peaks is calculated (1-2, 1-3, 1-4, 2-3, 2-4, 3-4..etc.), and used in determining the optimum mobile phase.

Two computer programs are used to predict optimum solvent composition. The first (Appendix 1) is a FORTRAN program (PEAKIN) which rearranges resolutions to correct for crossover, and if necessary, prints a table similar to Table 2 or Table 3, and produces a data file suitable for use in the next program. The second program (Appendix 2) is a SAS (Statistical Analysis System - version 79.5) route (9). A DATA paragraph converts the three-dimensional solvent compositions to a two-dimensional triangle representation as used by Snee (10). The data is fitted into a cubic model for a three dimensional system. The parameters of the cubic equation for each set of peak resolutions are computed using the general

Table 2

Mobile phase ratios and resolutions obtained for aflatoxins B_1, B_2, B_1 and G_2 using silica gel plates and acetone:chloroform (10:90), methanol:chloroform (5:95) and ethyl acetate:chloroform (30:70).

SOLVENT	MOBILE PHASES RATIOS									
10% ACETONE	1.00	0.0	0.0	0.0	0.5	0.50	0.33	0.16	0.16	0.67
5% MEOH	0.0	1.00	0.0	0.50	0.50	0.0	0.33	0.16	0.67	0.16
30% ETHYL-ACETATE	0.0	0.0	1.00	0.50	0.0	0.50	0.33	0.67	0.16	0.16
$R_s(B_1 - B_2)$	6.20	9.60	7.10	6.70	7.50	7.90	7.10	5.30	6.70	7.50
$R_s(B_2 - G_1)$	4.20	5.80	3.30	6.20	5.80	3.70	5.00	4.50	6.30	5.00
$R_s(G_1 - G_2)$	5.80	0.40	5.00	3.80	2.50	4.10	5.00	4.80	3.30	4.50

Table 3

Solvent compositions and resolutions obtained for the peak pairs N - M, M - E and E - D on reverse phase TLC plates using 95% CH_3OH:H_2O, 80% CH_3CN:H_2O and 75% 2-ethoxyethanal:H_2O.

SOLVENT	MOBILE PHASE RATIOS									
95% CH_3OH	1.00	0.0	0.0	0.50	0.50	0.0	0.33	0.67	0.16	0.16
80% CH_3CN	0.0	1.00	0.0	0.50	0.0	0.50	0.33	0.16	0.67	0.16
75% 2ETHO	0.0	0.0	1.00	0.0	0.50	0.50	0.33	0.16	0.16	0.67
R_S(N - M)	6.3	7.4	7.1	7.5	7.9	7.9	7.5	7.5	7.0	8.3
R_S(M - E)	4.1	5.9	7.1	5.9	5.4	6.7	5.4	4.5	7.1	7.5
R_S(E - D)	2.9	1.5	3.3	2.5	3.4	2.9	2.1	3.8	2.5	2.9

linear model (GLM) procedure. The PRINT procedure lists predicted resolutions of each peak pair for all solvent combinations varying each solvent from zero to 100 percent by 2% increments (see for example Table 4). Contour plots of the region where the predicted resolution above a desired level determined by the analyst are produced (see for example Figs. 1-3) using the PLOT procedure. The union of these plots showing the region where all resolutions are above this level, Fig. 4, and plots showing the area of maximum total resolution, Fig. 5, are also produced using PLOT. A flow chart of the procedure is shown in Fig. 6. The programs are run on an IBM model 370/168, and uses 210 K of core.

Ideally, where a combination of three modifiers and a base solvent is used the region of the optimum mobile phase mixture found from the ORM calculations will be in the center of the triangle. If one of the modifiers (A) is not ideal, the optimum mixture will be composed of the other two modifiers (B and C), with only a small amount of A. Therefore, the optimum region can indicate which of the three modifiers is a poor choice. Examples will be described later. The base solvents are water for reverse phase and hexane for normal phase (4). Other solvents for normal phase are also used.

Table 4

A sample of computer tabulation of mobile phase compositions and resolutions obtained using ORM calculations.

SOLVENT STUDY - A=CH3OH,B=CH3CN,C=THF

OBS	A	B	C	PEAK1	PEAK2	RESPRED
12741	0.8	0.10	0.10	1	2	1.6178
12742	0.8	0.10	0.10	1	3	6.4093
12743	0.8	0.10	0.10	1	4	8.9504
12744	0.8	0.10	0.10	1	5	10.5934
12745	0.8	0.10	0.10	2	3	4.7915
12746	0.8	0.10	0.10	2	4	7.3326
12747	0.8	0.10	0.10	2	5	8.9709
12748	0.8	0.10	0.10	3	4	2.5411
12749	0.8	0.10	0.10	3	5	4.1808
12750	0.8	0.10	0.10	4	5	1.6383
12751	0.8	0.12	0.08	1	2	1.3231
12752	0.8	0.12	0.08	1	3	6.5454
12753	0.8	0.12	0.08	1	4	8.8780
12754	0.8	0.12	0.08	1	5	10.7395
12755	0.8	0.12	0.08	2	3	5.2223
12756	0.8	0.12	0.08	2	4	7.5548
12757	0.8	0.12	0.08	2	5	9.4134
12758	0.8	0.12	0.08	3	4	2.3326
12759	0.8	0.12	0.08	3	5	4.1920
12760	0.8	0.12	0.08	4	5	1.8586
12761	0.8	0.14	0.06	1	2	1.0106
12762	0.8	0.14	0.06	1	3	6.6990
12763	0.8	0.14	0.06	1	4	8.8448
12764	0.8	0.14	0.06	1	5	10.9383
12765	0.8	0.14	0.06	2	3	5.6884
12766	0.8	0.14	0.06	2	4	7.8341
12767	0.8	0.14	0.06	2	5	9.9269
12768	0.8	0.14	0.06	3	4	2.1457
12769	0.8	0.14	0.06	3	5	4.2389
12770	0.8	0.14	0.06	4	5	2.0928
12771	0.8	0.16	0.04	1	2	0.6804
12772	0.8	0.16	0.04	1	3	6.8703
12773	0.8	0.16	0.04	1	4	8.8509
12774	0.8	0.16	0.04	1	5	11.1913
12775	0.8	0.16	0.04	2	3	6.1899
12776	0.8	0.16	0.04	2	4	8.1705
12777	0.8	0.16	0.04	2	5	10.5114
12778	0.8	0.16	0.04	3	4	1.9806
12779	0.8	0.16	0.04	3	5	4.3213
12780	0.8	0.16	0.04	4	5	2.3409
12781	0.8	0.18	0.02	1	2	0.3324
12782	0.8	0.18	0.02	1	3	7.0592
12783	0.8	0.18	0.02	1	4	8.8963
12784	0.8	0.18	0.02	1	5	11.4970
12785	0.8	0.18	0.02	2	3	6.7268
12786	0.8	0.18	0.02	2	4	8.5639
12787	0.8	0.18	0.02	2	5	11.1670
12788	0.8	0.18	0.02	3	4	1.8370
12789	0.8	0.18	0.02	3	5	4.4394

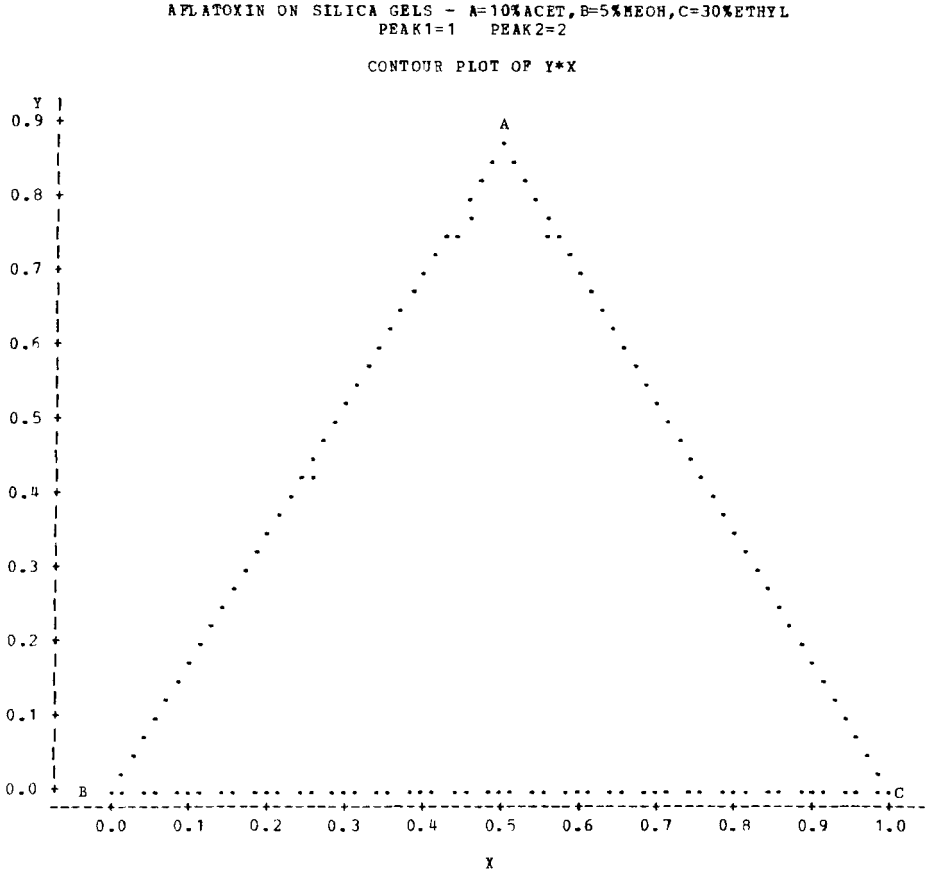

Fig. 1 Contour plot of aflatoxins B_1 and B_2, A = 10% acetone/chloroform, B = 5% methanol/chloroform, C = 30% ethylacetate/chloroform

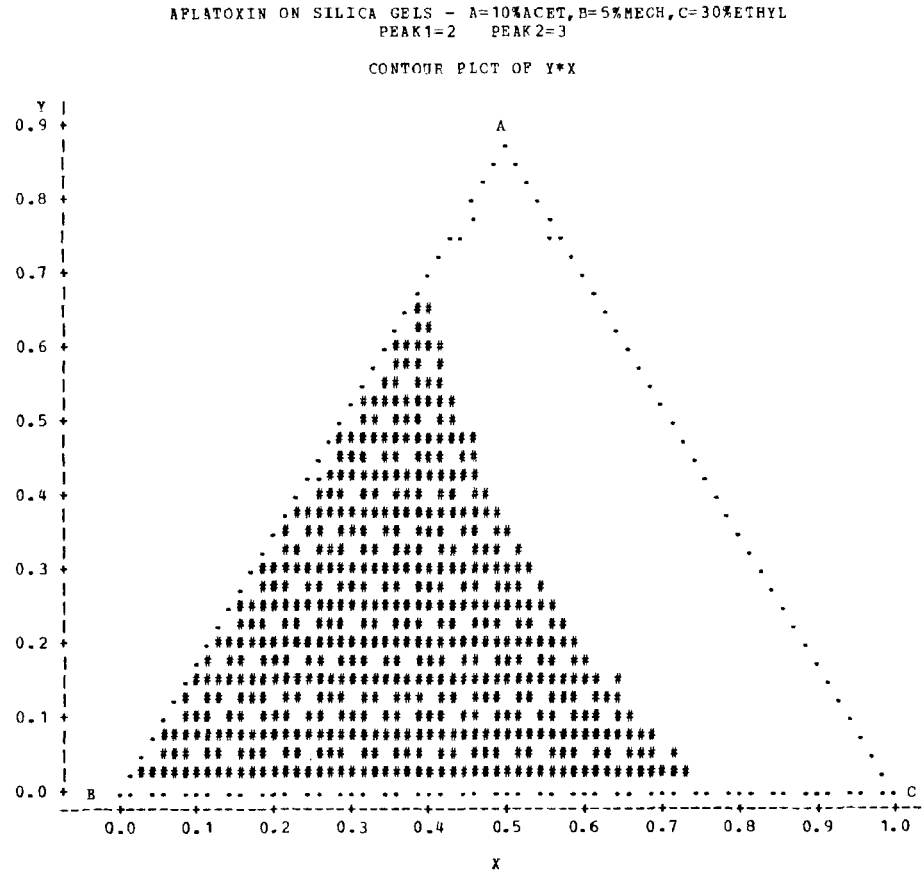

Fig. 2 Contour plot of aflatoxins B_2 and G_1, solvents as in Fig. 1. Shaded area designate mobile phase compositions that would give resolution greater than 5.15.

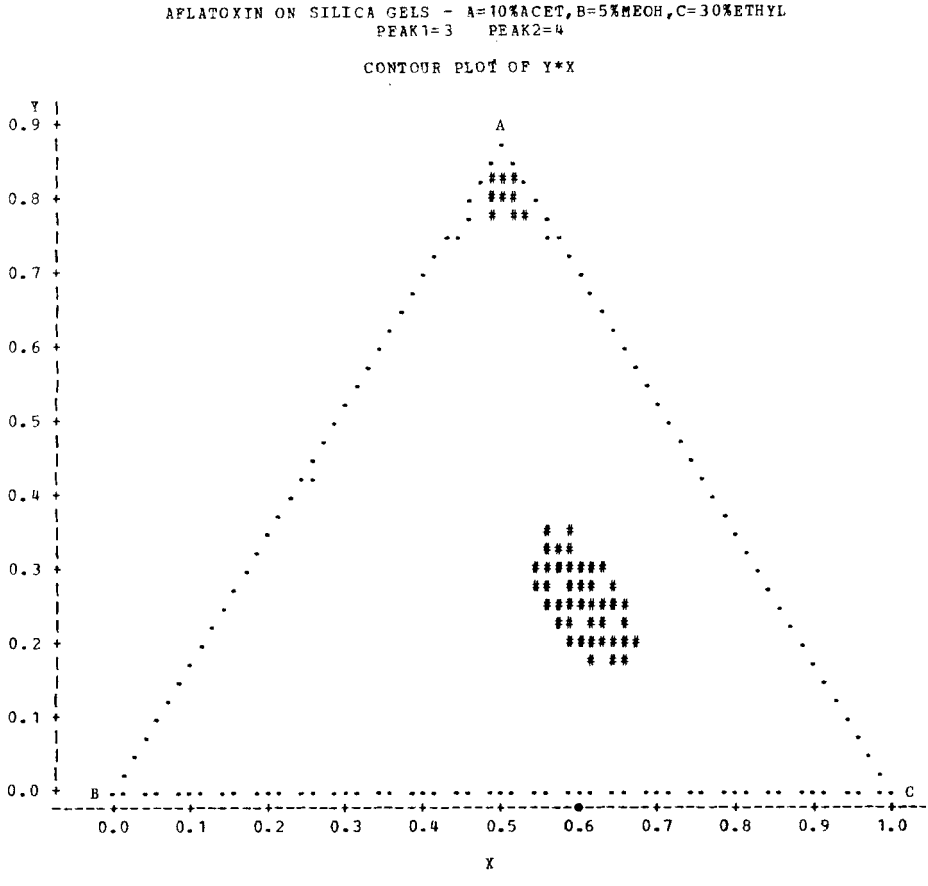

Fig. 3 Contour plot of aflatoxins G_1 and G_2, solvents as in Fig. 1. Shaded area designate mobile phase compositions that would give resolution greater than 5.15.

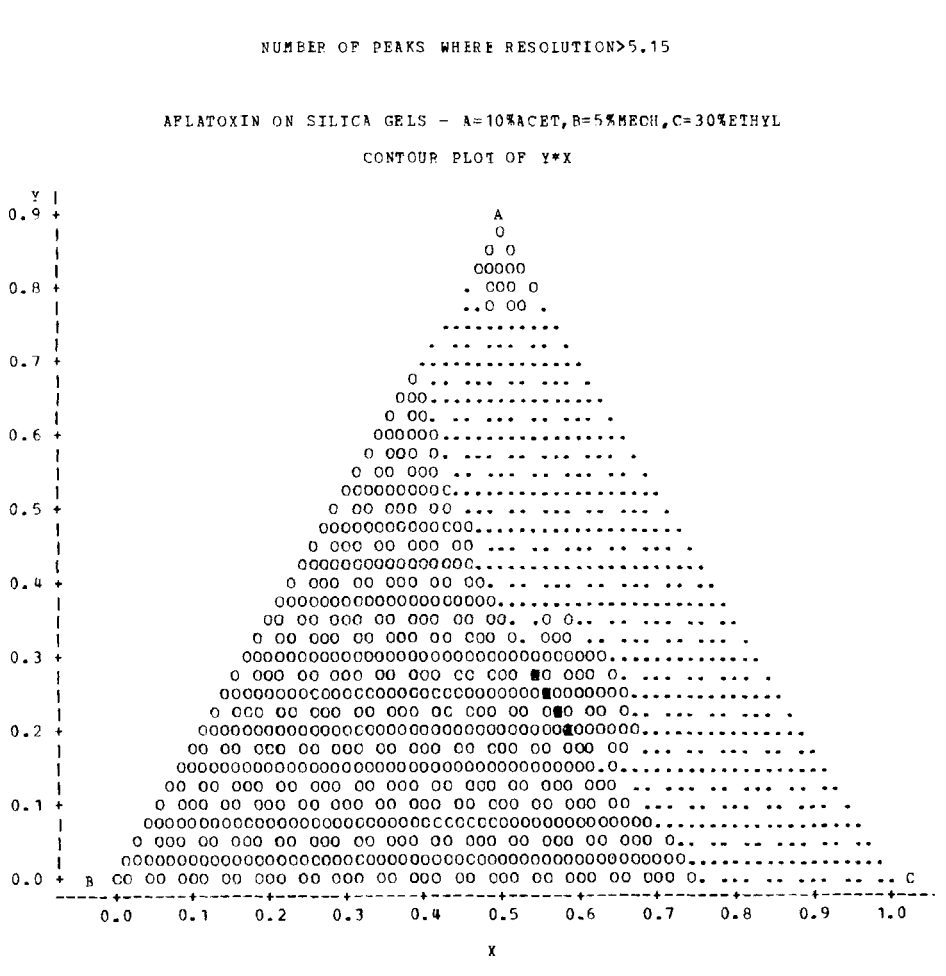

Fig. 4 Contour plot showing the area of optimum solvent composition where the resolution between each pair of the four aflatoxins is greater than 5.15 (■). Solvents as in Fig. 1.

S T A T I S T I C A L A N A L Y S I S S Y S T E M

SUM OF PEAK RESOLUTIONS FROM EACH MIX

AFLATOXIN ON SILICA GELS - A=10%ACET,B=5%MEOH,C=30%ETHYL

CONTOUR PLOT OF Y*X

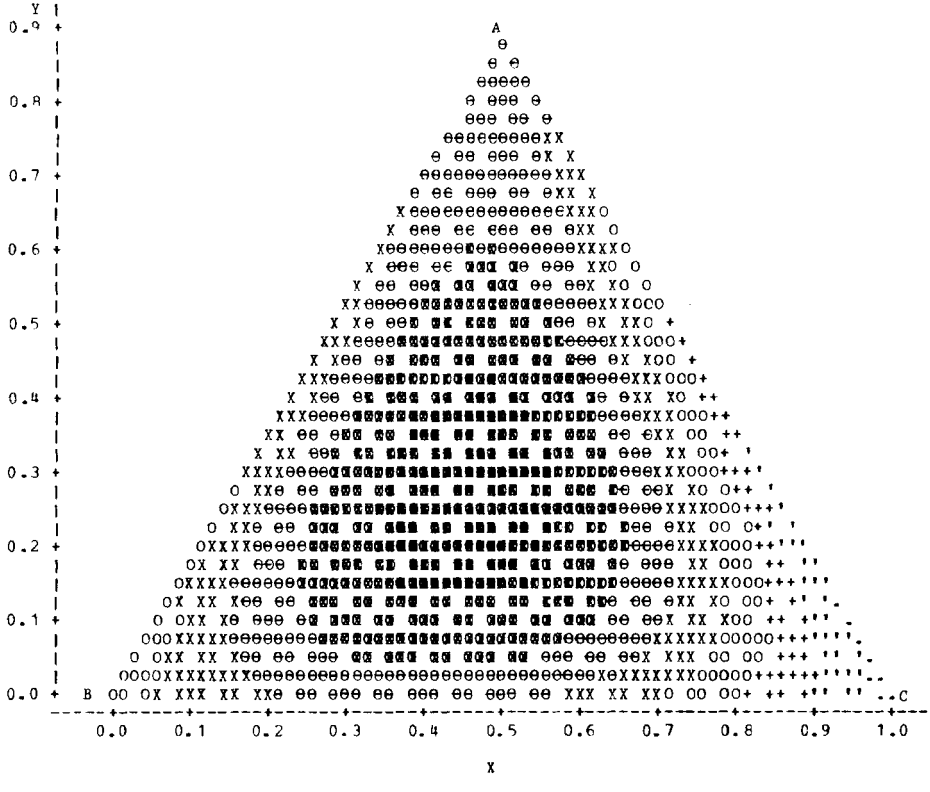

Fig. 5 Contour plot of area of maximum total resolution (A) between the four aflatoxins. Solvents as in Fig. 1.

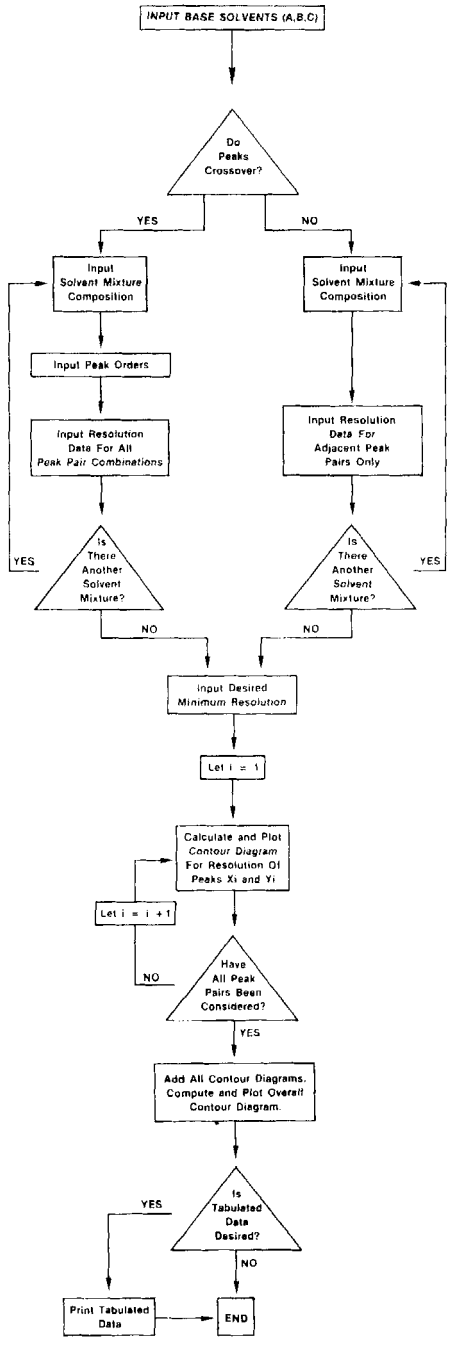

Fig. 6 Flow chart of the procedure used in the present study.

Another criterion is that any pair of the component mixture should be resolved in at least one of the three mobile phases selected, otherwise base line resolution of that pair in the final (optimum) mobile phase may not be possible. The solvents selected therefore, should have different chemical properties, hydrogen bonding, proton-donor or acceptor, dipole-dipole interaction...etc. To achieve that, Synder's solvent groups (11) were used.

THEORETICAL CONSIDERATIONS

Selection of the solvent in thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) is based on the elutropic series. However this series is based on values obtained for pure solvents. Selection of a binary, ternary etc... solvent mixture and predicting the separation results and elution times of the components of a mixture may not be that simple. In reverse and normal phase liquid chromatography elution times of the solute are a function of the properties of the stationary and mobile phases. Snyder (12) gave the following equation for calculating the polarity of a binary solvent mixture:

$$P' = \Phi_1 P_1 + \Phi_2 P_2 \qquad (I)$$

ϕ_1 and ϕ_2 are the volume fractions of solvents 1 and 2, P_1 and P_2 are the polarity of pure solvents 1 and 2 and P' is the polarity of the mixture. This relation does not apply to normal phases where the calculations are more complicated (12).

The relation between retention time and solvent strength is described by the following equation (12):

$$\log K'_1/K'_2 = \alpha A_S (\varepsilon^\circ_2 - \varepsilon^\circ_1) \qquad (II)$$

Where K' is the capacity factor, and ε° is the solvent strength parameter of solvents 1 and 2. A_S is molecular area of adsorbed sample and α is adsorbent surface activity function, and

$$K' = (R_t - R_{to})/R_{to} \qquad (III)$$

It was found that the relation in equation (II) does not always hold (13-15). For example, when two mobile phases (acetonitrile/water and methanol/water)

which have the same solvent strength (calculated according to eq. I) were used to elute the same solutes (naphthalene and biphenyl) on the same solid phase the retention times were not the same (15). A term was later added to eq (II) which accounted for solvent-solute interactions (13,14).

Since different solvents give different selectivities (11,16-18), changing the solvent composition may result in different elution orders depending on the properties of the sample mixture and the solvent chosen. For a mobile phase mixture, solvent strength (polarity) in general determines elution distance of the solutes (i.e. R_t), while mobile phase composition determines its selectivity. The composition of the mobile phase would determine the degree of separation (α), between two adjacent peaks i and ii, where

$$\alpha = K'_{ii}/K'_{i} \qquad \text{(IV)}$$

Based on Snyder's theory (12), Saunders (17) presented a graphical representation based on ε° for selecting a solvent for adsorption liquid chromatography. The application of these graphs is rapid and provides a reasonable first approximation to a solvent mixture appropriate for a given sample. It must be stressed that the results are approximate, and in some cases, the solvent mixture will not be ideal (17).

For a given sample and adsorbent, log K' varies linearly with ε°. This is generally true for K' values between 1 and 10, which is an acceptable working range allowing separation of a component from the mixture, but does not lead to dilution of the sample or long retention times. Solvent strength (polarity) gives a general indication of solute retention but it may not predict the correct retention times (15,16,19).

In their work Glajch et al (4) used eq (I) to select solvents that gave the same K' values. We have used solvent strength to predict approximate retention times, which in turn were used to predict the resolution (R_s) between two adjacent peaks;

$$R_s = (R_{t2} - R_{t1}) \,/\, 1/2(W_1 + W_2) \qquad \text{(V)}$$

Where R_t is the time of elution of peak maximum, and W is the baseline width of the peak in units of time.

Also, resolution in liquid chromatography has been defined (16) by the following equation:

$$R_S = 1/4\ (\alpha - 1)\ (N)^{1/2}\ (K'/1 + K') \qquad (VI)$$

where the number of theoretical plates, N is defined as:

$$N = 16\ (R_t/W)^2 \qquad (VII)$$

Note that all the above factors are a function of R_t (equations III-VII)

The three terms in eq. (VI) should be optimized to achieve maximum resolution. However, if the experimental conditions (flow rate, column dimensions and particle size and properties of sample) are kept constant the only parameter effecting separation is the mobile phase. The composition of which will determine not only the retention times of the solutes but also their order of elution. It is important to have a solvent which will give reasonable retention times for all components of the mixture, R_t between 5 - 40 min, in HPLC, and an R_f value of 0.2 - 0.7 in TLC.

The resolution values for HPLC were calculated according to eq. (V). For TLC, resolution was defined as $R_{fn} - R_{f(n-1)}$. We found this to be simple and human error is eliminated from the measurement of spot width. Otherwise a densitometer should be used to scan the spots and calculate R_S as defined in eq. (V).

RESULTS AND DISCUSSION

The basis for statistical data analysis in both cases was the work of Snee (10). However, in the present work a cubic equation was used where nine data points were required for an answer and the tenth point allows for goodness of fit. In their work (4) a quadratic equation was used where seven data points were required for solvent optimization, and three for checking the system. Belinky (5) on the other hand used 17 data points: he used acetonitrile, methanol and water to form four solvent systems (pure acetonitrile, pure methanol, 60% methanol and 70% acetonitrile) from which an optimum mobile phase was selected. This is time consuming when the mixture contains more than four components.

The separation of alfatoxins B_1, B_2, G_1 and G_2 on silica gel TLC plates was used to test the method and to see if the optimum mobile phase selected from the ORM calculations would optimize the resolution between the aflatoxin pairs B_1-B_2, B_2-G_1 and G_1-G_2. As base solvent, chloroform was selected based on literature data (20). The solvent combinations used are listed in Table 2 along with the resolution between the peaks of the adjacent pairs. Note that the resolution between aflatoxin B_1 and B_2 is always equal to or greater than 5.30, no matter what solvent composition is used as the mobile phase. However this is not the case for aflatoxin pairs B_2-G_1 and G_1-G_2. Table 2 also indicates that no solvent combination gives a resolution greater than 5 between the three pairs of aflatoxins. Our aim therefore, is to find a mobile phase which would maximize the resolution between the four aflatoxins, and which would give a resolution value greater than 5.15 between each of the aflatoxin pairs. The contour plots generated from the data in Table 2 is shown in Figs. 1-3. Fig. 1 shows that any solvent combination would give a minimum resolution of 5.15 between aflatoxins B_1 and B_2. Fig. 2 shows (the shaded area) where the minimum resolution between aflatoxins B_2 and G_1 is equal to or greater than 5.15. Fig. 3, the shaded area shows that solvent combination which would produce a minimum resolution of 5.15 between aflatoxins G_1 and G_2. Fig. 4 is th contour diagram for the resolution of the four aflatoxins obtained from the union of the diagrams of the individual aflatoxin pair resolutions Figs. 1-3. The four ⊠ in the center of the triangle (Fig. 4) gives the solvent combination where the resolution between each pair of the four aflatoxins is greater than 5.15. The O indicate areas of mobile phase combinations which will give a resolution greater than 5.15 between 3-4 aflatoxins. The dotted areas indicate mobile phase compostions which will give a resolution greater than 5.15 for 2-3 aflatoxins. Fig. 5 gives the area of maximum total resolution, i.e. the sum of the resolutions between the four aflatoxins. This is not necessarily the best resolution between each pair. The ⊠ in the center of the triangle corresponds to mobile phase compositions which would give maximum resolution. Good correlation was obtained between

predicted and experimental values for the separation of the aflatoxins. Other examples will follow.

Reverse phase C_{18} plates were used for the separation of naphthalene (N), 1-methylnaphthalene (M), 1-ethylnaphthalene (E), and 1,3-dimethylnaphthalene (D). Solvents used and resolutions obtained are listed in Table 3. The contour plot, Fig. 7, predicts an optimal solvent mixture (M) containing only methanol and 2-ethoxyethanol. These two solvents, therefore, are mainly responsible for the separation, while acetonitrile does not help. This means that CH_3CN/H_2O (80/20) is a bad choice. If the resolutions obtained are unsatisfactory, the analyst may choose to vary the ratios of CH_3 CN/H_2O or an entirely different organic modifier. Table 5 shows the predicted and experimental resolutions obtained using reverse phase C_{18} plates and different mobile phases of various compositions.

It is also possible to select one solvent (B) which gives better resolution of the components of a mixture than the other two solvents (A & C). The contour plot will show a bias toward solvent (B), Fig. 8. In this case, other solvents should be substituted for A & C. These examples show that the initial selection of the individual mobile phases is an important step which can lead to good resolution using the three organic modifiers.

HPLC results indicated that this solvent selection system can be successfully applied to mobile phase optimization. Peak crossover due to different solvents can easily be handled by this method for both HPLC and TLC. Figs. 9-11, show the separation of a napthalene, biphenyl, anthraquinone, methyl- and ethylanthraquinone mixture. Note the peak crossover in each of the solvents used. Fig. 12 shows the separation using the predicted mobile phase mixture on reverse phase C_8 column.

Although peak crossover occurred in each of the solvents used in HPLC, Figs. 9-11 this was not the case in TLC. Only when 42% THF/water was used did peak crossover occur, (Table 6) as shown by the negative R_S values. This may be due to differences of carbon loading and manufacturing processes of the plates and the columns.

Table 5

Predicted and experimental peak pair resolutions for N - M, M - E and E - D, using reverse phase TLC plates and selected mobile phases from the contour plot.

Selected Mobile Phase	Compound	Predicted Resolution	Experimental Resolution
95% Methanol: 75% 2-Ethoxyethanol	D		
(2:3)	E	8.1	8.1
	M	5.8	5.4
	N	3.5	3.6
95% Methanol: 75% 2-Ethoxyethanol	D		
(3:2)	E	7.9	7.9
	M	5.1	5.0
	N	3.5	4.2
95% Methanol: 75% 2-Ethoxyethanol	D		
(1:1)	E	8.1	7.5
	M	5.4	5.9
	N	3.5	3.5
95% Methanol: 42%	D		
80% Acetonitrile: 4%	E	8.1	7.9
75% Ethoxyethanol: 54%	M	5.7	5.0
	N	3.4	3.5

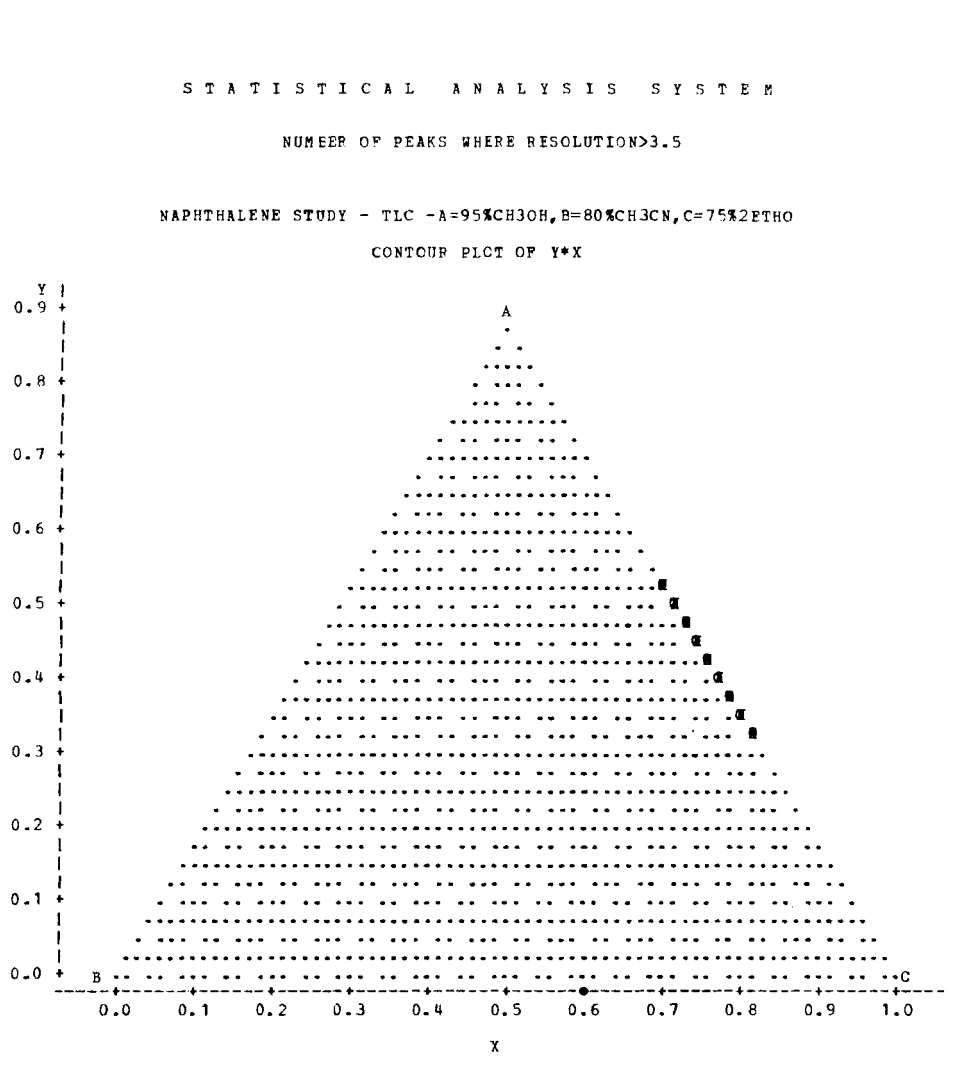

Fig. 7 Contour plot of the naphthalenes N, M, E and D using reverse phase TLC plates and 95% CH_3OH/H_2O (A), 80% CH_3CN/H_2O (B) and 75% 2-ethoxy ethanol/H_2O (C). Shaded circles (■) designate mobile phase composition that would give resolution greater than 3.5.

S T A T I S T I C A L A N A L Y S I S S Y S T E M

NUMBER OF PEAKS WHERE RESOLUTION>2.25

ANTHRA & NAPTH-BI - HPLCS - A=64%CH3CN,B=42%THP,C=72%MEOH

CONTOUR PLOT OF Y*X

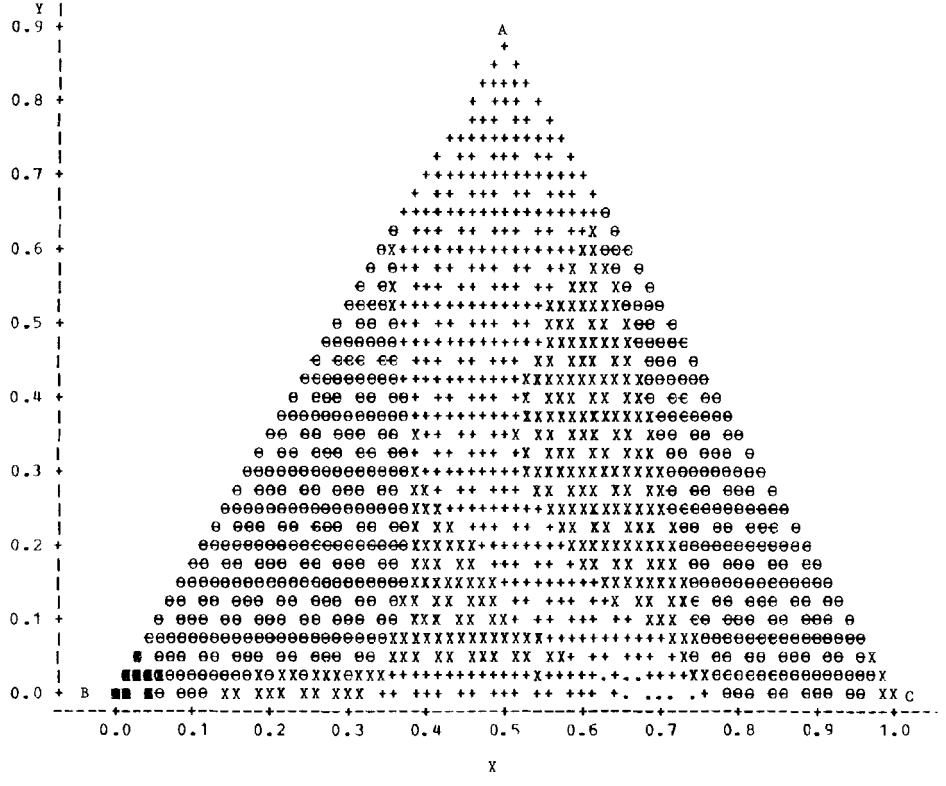

Fig. 8 Contour plot of naphthalene, biphenyl, anthraquinone, methyl- and ethyl anthraquinone, using reverse phase TLC plates and 64% CH_3CN/H_2O (A), 42% THF/H_2O (B) and 72% CH_3OH/H_2O (C). Shaded circles (●) are optimum mobile phase compositions.

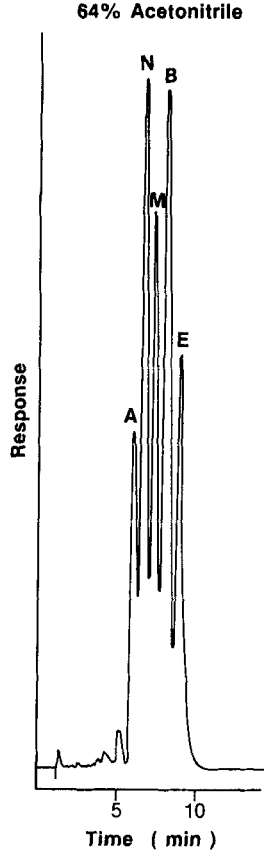

Fig. 9 HPLC separation of anthraquinone (A), naphthalene (N), 1-methylnaphthalene (M), 1-ethylnaphthalene (E), and 1-3-dimethylnaphthalene (D) on reverse phase C_8 column using 64% CH_3CN/H_2O, at a mobile phase flow rate of 1.2 ml/min.

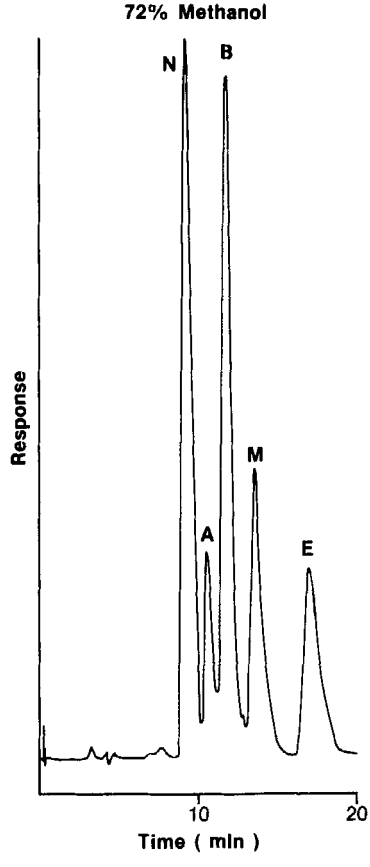

Fig. 10 Same as Fig. 9 but the mobile phase used is 72% CH_3OH/H_2O.

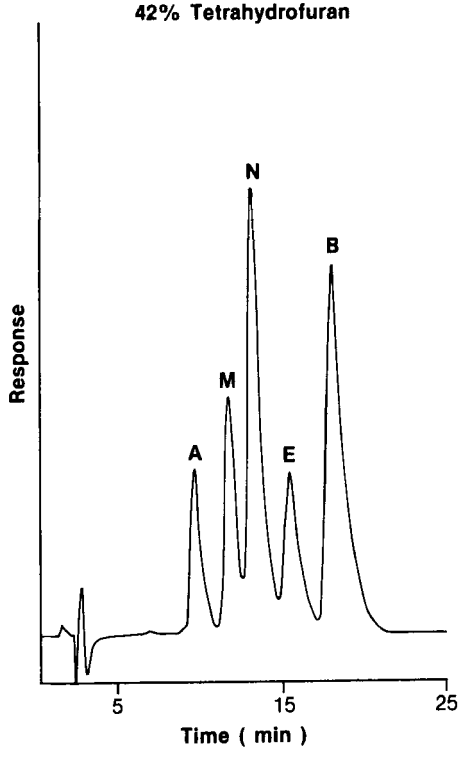

Fig. 11 Same as Fig. 9 but the mobile phase used is 42% THF/H_2O.

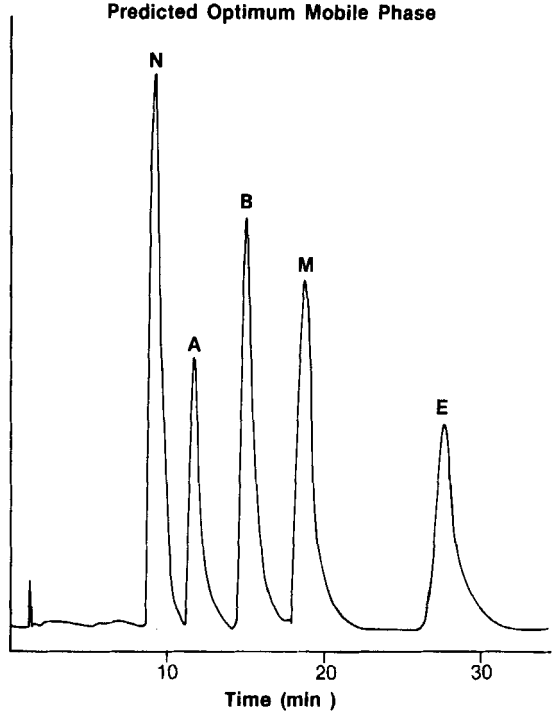

Fig. 12 Separation of A, N, M, B and E on reverse phase C_8 column using a predicted mobile phase of 64% CH_3CN:72% CH_3OH:42% THF (10:67:23), at a flow rate of 1.2 ml/min.

Table 6

Solvent combinations and peak pair resolutions for N, A, B, M, and E, and experimental resolutions obtained using reverse phase C_8 HPLC column and 72% CH_3OH:H_2O, 64% CH_3CN:H_2O and 42% THF:H_2O.

Solvent	Mobile Phase Ratios									
72% CH_3OH	1.00	0.0	0.0	0.50	0.50	0.0	0.33	0.67	0.16	0.16
64% CH_3CN	0.0	1.00	0.0	0.50	0.0	0.50	0.33	0.16	0.67	0.16
42% THF	0.0	0.0	1.00	0.0	0.50	0.50	0.33	0.16	0.16	0.67
R_S(N - A)	0.8	4.6	2.1	0.4	4.2	5.0	3.7	2.1	4.2	5.5
R_S(N - B)	7.5	6.7	1.7	7.1	4.2	5.8	4.5	5.4	6.2	3.4
R_S(N - M)	9.2	11.7	4.2	9.2	8.8	10.8	5.4	9.2	11.2	10.5
R_S(N - E)	11.7	13.8	3.3	12.1	7.9	10.8	5.4	10.4	12.1	8.8
R_S(A - B)	6.7	2.1	-0.4	6.7	0.0	0.8	0.8	3.3	2.0	-2.1
R_S(A - M)	8.4	7.1	2.1	8.8	4.6	5.8	1.7	7.1	7.0	5.0
R_S(A - E)	10.9	9.2	1.3	11.7	3.7	5.8	1.7	8.3	7.9	3.3
R_S(B - M)	1.7	5.0	2.5	2.1	4.6	5.0	0.9	3.8	5.0	7.1
R_S(B - E)	4.2	7.1	1.75	5.0	0.9	5.0	0.9	5.0	5.9	5.4
R_S(M - E)	2.5	2.1	-0.8	2.9	-0.9	0.0	0.0	1.2	0.9	-1.7

Table 7 shows good agreement between the predicted and experimental resolution of the five components of the mixture using RP-8 TLC plates.

CONCLUSION

The method described here employs statistical data analysis to predict optimum ternary mobile phase compositions in a systematic and straightforward manner in contrast to operator intuition. The initial selection of the three solvents, of which the final mixture is composed, is important and will affect the degree of separation and resolution of adjacent peaks. The method is easily applied to both TLC and HPLC. Good agreement was observed between predicted

Table 7

Predicted and experimental peak pair resolutions for N, A, B, M, and E, using reverse phase C_8 TLC plates and selected mobile phases from the contour plot.

SELECTED MOBILE PHASE	COMPOUND	COMPOUND	PREDICTED RESOLUTION	EXPERIMENTAL RESOLUTION
72% CH_3OH:64% CH_3CN 1:9	N	A	3.4	4.7
	N	B	6.8	7.7
	N	M	11.1	12.0
	N	E	13.5	14.1
	A	B	3.4	3.5
	A	M	7.8	7.3
	A	E	10.1	9.4
	B	M	4.3	4.3
	B	E	6.7	6.4
	M	E	2.3	2.1
72% CH_3OH:64% CH_3CN 16:84	N	A	2.7	2.9
	N	B	6.9	7.0
	N	M	10.8	11.6
	N	E	13.3	13.7
	A	B	4.1	4.1
	A	M	8.1	8.7
	A	E	10.5	10.8
	B	M	3.9	4.6
	B	E	6.4	6.7
	M	E	2.5	2.6

and experimental data. To the best of our knowledge, this is the first systematic statistical method of solvent selection for normal and reverse phase TLC.

ACKNOWLEDGEMENT

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APPENDIX 1

```
C                                                                       0001
C PROGRAM PEAKIN:                                                       0002
C       THIS PROGRAM ACCEPTS PEAK RESOLUTIONS FROM UP TO 20 SOLVENT     0003
C       MIXTURES, CORRECTS FOR CROSSOVER IF NECESSARY, AND OUTPUTS A    0004
C       DATA FILE THAT IS PASSED TO A SAS ROUTINE TO ANALYZE THE BEST   0005
C       SOLVENT MIXTURE TO USE FOR MAXIMUM PEAK RESOLUTION OF ALL       0006
C       PEAKS.                                                          0007
C                                                                       0008
C       IF PEAKS DO NOT CROSSOVER FOR DIFFERENT MIXTURES, ONLY          0009
C       RESOLUTIONS FOR ADJACENT PEAKS ARE USED.  IF CROSSOVER DOES     0010
C       OCCUR, ALL PEAK RESOLUTIONS ARE USED                            0011
C                                                                       0012
C       CARD INPUT ORDER:                                               0013
C            TITLE                                                      0014
C            NAMES OF SOLVENTS - 3A8                                    0015
C            CROSSOVER OPTIONS - 'CROSS' OR 'NOCROSS'                   0016
C            # OF PEAKS                                                 0017
C            REPEAT AS NEEDED:                                          0018
C                SOLVENT COMPOSITION - SHOULD ADD TO 1.0 - 3F5          0019
C                IF 'NOCROSS' -                                         0020
C                    RESOLUTIONS OF ADJACENT PEAKS  -  ONE/CARD         0021
C                         RESOL 1 - 2                                   0022
C                         RESOL 2 - 3                                   0023
C                             :                                         0024
C                         RESOL N-1 - N                                 0025
C                                                                       0026
C                IF 'CROSS' -                                           0027
C                    ORDER OF PEAKS - 2013                              0028
C                    RESOLUTIONS OF ALL PEAKS  -  ONE/CARD              0029
C                         RESOL 1 - 2                                   0030
C                         RESOL 1 - 3                                   0031
C                             :                                         0032
C                             :                                         0033
C                         RESOL 1 - N                                   0034
C                         RESOL 2 - 3                                   0035
C                             :                                         0036
C                         RESOL 2 - N                                   0037
C                             :                                         0038
C                         RESOL N-1 - N                                 0039
C                                                                       0040
```

```
      DOUBLE PRECISION A,B,C                                                    0041
      DIMENSION DATA(190,20),ACOMP(20),BCOMP(20),CCOMP(20),NORD(20)             0042
      DIMENSION TITLE(20)                                                       0043
      DATA CROSS/'CROS'/                                                        0044
      INTEGER P1,P2,P11                                                         0045
C                                                                               0046
      READ(5,15) TITLE                                                          0047
15    FORMAT(20A4)                                                              0048
      READ(5,10)A,B,C                                                           0049
10    FORMAT(3A8)                                                               0050
      READ(5,11) OPTION                                                         0051
11    FORMAT(A4)                                                                0052
      COPT=0                                                                    0053
      IF(OPTION.EQ.CROSS) COPT=1                                                0054
      READ(5,*)NPEAK                                                            0055
      NPEAK1=NPEAK-1                                                            0056
      NPOINT=0                                                                  0057
100   READ(5,12)A1,B1,C1                                                        0058
12    FORMAT(3F5.0)                                                             0059
      IF(A1+B1+C1.LE.0) GO TO 1000                                              0060
      NPOINT=NPOINT+1                                                           0061
      ACOMP(NPOINT)=A1                                                          0062
      BCOMP(NPOINT)=B1                                                          0063
      CCOMP(NPOINT)=C1                                                          0064
      IF(COPT.EQ.1) GO TO 500                                                   0065
C                                                                               0066
C INPUT NO CROSSOVER PEAK RESOLUTIONS                                           0067
      DO 150 P1=1,NPEAK1                                                        0068
      P2=P1+1                                                                   0069
      NPTR=(P1-1)*NPEAK+(P2-1)                                                  0070
150   READ(5,*) DATA(NPTR,NPOINT)                                               0071
      GO TO 100                                                                 0072
C                                                                               0073
C INPUT CROSSOVER PEAK RESOLUTIONS                                              0074
500   READ(5,13)NORD                                                            0075
13    FORMAT(20I3)                                                              0076
      DO 550 I=1,NPEAK1                                                         0077
      I1=I+1                                                                    0078
      DO 550 J=I1,NPEAK                                                         0079
      P2=NORD(J)                                                                0080
      P1=NORD(I)                                                                0081
      IF(NORD(I).LT.NORD(J)) GO TO 510                                          0082
      P1=NORD(J)                                                                0083
      P2=NORD(I)                                                                0084
510   NPTR=(P1-1)*NPEAK+(P2-1)                                                  0085
550   READ(5,*) DATA(NPTR,NPOINT)                                               0086
      GO TO 100                                                                 0087
C                                                                               0088
C PRINT TABLE OF RESULTS                                                        0089
1000  WRITE(6,20) TITLE                                                         0090
20    FORMAT('1'//1X,20A4//' DATA ENTERED BY COMPOSITION'//)                    0091
      WRITE(6,21)A,(ACOMP(I),I=1,NPOINT)                                        0092
21    FORMAT(1X,A8,20F6.2)                                                      0093
      WRITE(6,21)B,(BCOMP(I),I=1,NPOINT)                                        0094
      WRITE(6,21)C,(CCOMP(I),I=1,NPOINT)                                        0095
      WRITE(6,22)                                                               0096
22    FORMAT(/2X,'P1  P2  -  RESOLUTIONS:'/)                                    0097
      IF(COPT.EQ.1) GO TO 2000                                                  0098
      DO 1050 P1=1,NPEAK1                                                       0099
      P2=P1+1                                                                   0100
      NPTR=(P1-1)*NPEAK+(P2-1)                                                  0101
      WRITE(6,23)P1,P2,(DATA(NPTR,I),I=1,NPOINT)                                0102
23    FORMAT(2I4,1X,20F6.2)                                                     0103
C                                                                               0104
C OUTPUT DATA TO FILE                                                           0105
      DO 1050 I=1,NPOINT                                                        0106
      WRITE(11,24)P1,P2,ACOMP(I),BCOMP(I),CCOMP(I),DATA(NPTR,I)                 0107
24    FORMAT(2I4,3F6.2,F10.3)                                                   0108
1050  CONTINUE                                                                  0109
      STOP                                                                      0110
2000  DO 2050 P1=1,NPEAK1                                                       0111
      P11=P1+1                                                                  0112
      DO 2050 P2=P11,NPEAK                                                      0113
      NPTR=(P1-1)*NPEAK+(P2-1)                                                  0114
      WRITE(6,23)P1,P2,(DATA(NPTR,I),I=1,NPOINT)                                0115
      DO 2050 I=1,NPOINT                                                        0116
      WRITE(11,24)P1,P2,ACOMP(I),BCOMP(I),CCOMP(I),DATA(NPTR,I)                 0117
2050  CONTINUE                                                                  0118
      STOP                                                                      0119
      END                                                                       0120
```

APPENDIX 2

```
*************************************************************************************

  SAS ANALYSIS OF SOLVENT MIXTURE RESOLUTION DATA

  CHANGE 'XXX' TO CUTOFF RESOLUTION
  CHANGE 'ASSAY TITLE' TO TITLE OF ASSAY

*************************************************************************************

  DATA SET1;
     INPUT PEAK1 PEAK2 A B C RESOL;
     INFILE PEAK;

  PROC SORT;
      BY PEAK1 PEAK2;

  DATA SET2;
     SET SET1; BY PEAK1 PEAK2;
          X=1.232*A+1.732*C-(1-B)*.732;
          Y=A*.866;
      OUTPUT;
      IF LAST.PEAK2 THEN DO;
         RESOL=.;
         DO A=0 TO 1 BY .02;
            DO B=0 TO 1-A BY .02;
               C=1-A-B;
                  X=1.232*A+1.732*C-(1-B)*.732;
                  Y=A*.866;
                  OUTPUT;
            END;
      END;
  END;

  PROC GLM; BY PEAK1 PEAK2;
       MODEL RESOL=A B C A*B A*C B*C A*B*C/NOINT;
       OUTPUT OUT=SET3 P=RESPRED;

  DATA SET4;
       SET SET3;
       IF RESPRED>XXX THEN GTRES=1;
              ELSE GTRES=0;
       EDGE=GTRES;
       IF A=0 OR B=0 OR C<.01 THEN DO; EDGE=2; END;
       OUTPUT;

  PROC PLOT; BY PEAK1 PEAK2;
       PLOT Y*X=EDGE / CONTOUR=3 S1=' ' S2='#' S3='.'
                                HPOS=80 HAXIS=0 TO 1 BY .1
                               VAXIS=0 TO .9 BY .1;
       TITLE4 GTRES=1 - RESOLUTION>XXX   GTRES=0 - RESOLUTION<=XXX;
       TITLE8  ASSAY TITLE;

  PROC SORT;
       BY A B C PEAK1 PEAK2;

  PROC PRINT;
       VAR A B C PEAK1 PEAK2 RESPRED;

  PROC SORT;
       BY A B C;

  DATA TOTAL;
       SET SET4 END=EOF; BY A B C;
       IF FIRST.C THEN DO; TOTGTRES=0; TOTRESOL=0; END;
       IF RESOL=. THEN DO;TOTGTRES+GTRES; TOTRESOL+RESPRED; END;
       IF LAST.C THEN DO; IF TOTRESOL>0 THEN OUTPUT; END;
       IF EOF THEN DO; IF TOTRESOL>0 THEN OUTPUT; END;

  PROC PLOT;
       PLOT Y*X=TOTGTRES/CONTOUR=8 HPOS=80 HAXIS=0 TO 1 BY .1
                         VAXIS=0 TO .9 BY .1;
       TITLE4 NUMBER OF PEAKS WHERE RESOLUTION>XXX;
       TITLE8  ASSAY TITLE;

  PROC PLOT;
       PLOT Y*X=TOTRESOL/CONTOUR=8 HPOS=80 HAXIS=0 TO 1 BY .1
                         VAXIS=0 TO .9 BY .1;
       TITLE4 SUM OF PEAK RESOLUTIONS FROM EACH MIX;
       TITLE8  ASSAY TITLE;
```