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COMPUTER ROUTINE FOR SELECTION OF THE MOBILE PHASE SEARCH AREA IN OPTIMISATION METHODS

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SUMMARY

Computer-based methods for selection of the optimal mobile phase composition in reversed-phase liquid chromatography usually involve selection of a search area of mobile phases formed from linear combinations of isoeluotropic binary mobile phases of water with methanol, acetonitrile or tetrahydrofuran. A simple interactive computer routine is described which facilitates the determination of acetonitrile–water and tetrahydrofuran–water compositions which are isoeluotropic with any given methanol–water binary mobile phase. This routine is based on the assumption that the relationship between $\ln k'$ and the percentage of organic modifier in a binary solvent mixture can be approximated either by a single straight line or a series of line segments.

The proposed method is successfully applied to three groups of test solutes (diphenylamines, sympathomimetic amines and aromatic compounds) and it is shown that only minimal experimentation is required to locate isoeluotropic mobile phases for the solutes studied.

INTRODUCTION

Computer-based methods for the selection of the optimal experimental parameters for use in liquid chromatography have increased both in variety and complexity over recent years. These methods have been reviewed by several authors[1–3], and these reviews show that it is the mobile phase composition which is generally optimised, rather than other chromatographic parameters such as the column type, temperature or flow-rate.

In the case of reversed-phase liquid chromatography (RPLC), optimisation methods are generally focused on the selectivity effects which may arise when the nature of the organic modifier in the mobile phase is altered. Typically, only those mobile phases formed from binary, ternary or quaternary combinations of water with methanol, acetonitrile (ACN) or tetrahydrofuran (THF) are considered. The entire range of possible mobile phases formed in this way has been described as the mobile phase "domain"[4], and contains an infinite number of discrete mobile phases.

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In searching for the optimal mobile phase, the computer optimisation method can either consider all mobile phases in this domain, or select a small portion of the domain (designated as the "search area") on the basis that many mobile phases in the domain will prove unsatisfactory in terms of either inadequate or excessive retention of solutes. The first approach requires a great deal of computer time, whilst the success of the second approach is dependent on the selected search area containing the optimal mobile phase.

Consideration of the factors which influence the resolution of solutes in a chromatographic system suggests that the ideal retention range for elution of components of a mixture is when all solute capacity factors, k' fall within the limits $1 \leqslant k' \leqslant 10$ (ref. 5). When this condition applies, retention does not play a major role in the resolution of solutes, and this condition is therefore commonly employed as a basis for the selection of the optimisation search area. This selection may be conveniently performed by first locating a methanol–water binary mobile phase which satisfies the above retention condition and then converting this composition into isoeluotropic binary mobile phases of ACN and THF with water. This produces a search area bounded by three isoeluotropic binary mobile phases, and containing those ternary and quaternary mobile phases which can be produced by linearly combining the three binary mobile phases. A more comprehensive discussion of the background to the selection of the optimisation search area, and its relationship to the mobile phase domain, may be found elsewhere[4,6–8].

A crucial step in this process is the conversion of the empirically determined methanol–water binary mobile phase (which satisfies the retention condition) into isoeluotropic binary ACN–water and THF–water mobile phases. The essential requirement is that all three binary mobile phases are isoeluotropic, since if this is not the case, retention will exert an influence on the optimisation as well as the desired selectivity effects. Various equations have been proposed for calculating isoeluotropic binary mobile phases[9–12] and these are based on the retention behaviour of a wide range of solutes with varying functional groups in binary mixtures of water with methanol, ACN and THF. In a previous paper[6] we have shown that the mobile phases predicted by such equations may exhibit wide variation in retention characteristics, which in turn implies that the mobile phase search area obtained by application of these equations may contain mobile phases which are not isoeluotropic.

Since the success of the optimisation process rests on correct specification of the mobile phase search area, we have developed a simple computer routine which ensures that the binary mobile phases used to define the boundaries of the search area are isoeluotropic, within a user-defined margin of error. This routine is independent of the nature of the solutes used and in this paper, we have successfully applied it to three groups of solutes containing a variety of functional groups.

THEORY

In a study of the relationship between capacity factor and the volume fraction of organic modifier (φ) in binary mobile phases, Schoenmakers[12] proposed the following equation:

$$\ln k' = A\varphi^2 + B\varphi + C \tag{1}$$

Where A, B and C are constants with magnitudes dependent on the solute under consideration. However, since this relationship is of practical interest only over the restricted retention range given by $1 \leq k' \leq 10$, a linear approximation can be used to describe retention according to:

$$\ln k' = \ln k'_0 - S\varphi \tag{2}$$

It should be pointed out that the intercept $\ln k'_0$ has no physical significance since k'_0 is much smaller than the capacity factor of a solute in pure water. Moreover, any two mobile phase compositions (φ_1 and φ_2) giving capacity factors k'_1 and k'_2, respectively, are related by the equation:

$$\ln (k'_1/k'_2) = S(\varphi_2 - \varphi_1) \tag{3}$$

Eqn. 3 provides a convenient means for the calculation of a binary mobile phase composition which gives a required capacity factor, provided the value of S is known for the particular solute under consideration. This approach may be applied to the task of converting a given methanol–water binary mobile phase into isoeluotropic ACN–water and THF–water binary mobile phases, as required in the definition of an optimisation search area. To do this, only the capacity factor of the longest retained peak in a sample mixture need be considered, since it is this peak which establishes the length of the chromatographic run. The following experimental sequence is suggested.

(a) A binary methanol–water mobile phase (of composition φ_M) is determined empirically to satisfy the retention condition $1 \leq k' \leq 10$ for the components of the mixture under study. The value of $\ln k'$ for the last eluting solute is designated the target value to be attained by the isoeluotropic mobile phases of water and ACN or THF. φ_M is then converted into a predicted isoeluotropic binary ACN–water (composition φ_{ACN}) or THF–water (composition φ_{THF}) mobile phase using previously published transfer rules[12]. That is:

$$\varphi_{ACN} = 0.32\varphi_M^2 + 0.57\varphi_M \tag{4}$$

$$\varphi_{THF} = 0.66\varphi_M \tag{5}$$

For the purposes of illustration, only the ACN–water binary mobile phase will be considered further. However, the procedure described applies equally well to THF–water binary mobile phases. The predicted isoeluotropic ACN–water binary mobile phase is designated P_1.

(b) The sample mixture is chromatographed using composition P_1 as mobile phase and $\ln k'$ for the last eluting solute is determined (M_1). This is compared with the target $\ln k'$ value obtained for the same solute using the above methanol–water mobile phase (*e.g.* 2.303 for k' of 10) and if the difference (A_1) exceeds a user-defined error (for example, $\pm 5\%$), the subsequent steps described below are followed.

(c) Eqn. 3 is used to predict a new mobile phase composition (P_2) which gives the target value of $\ln k'$ for the last eluting solute. To enable this calculation to be performed, an arbitrary value of -20 is assigned to S. This value is intentionally

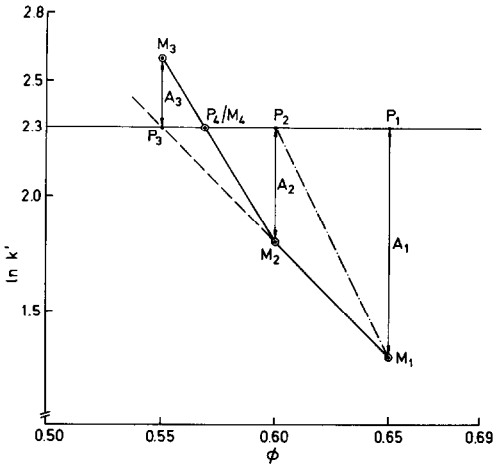

Fig. 1. Schematic illustration of the method for selection of isoeluotropic mobile phases. See text for discussion and Table I for values of the parameters P, M, A and S in the example illustrated.

chosen to be numerically greater than previously reported values[12] so that the newly predicted composition P_2 is not excessively solvent-poor or solvent-rich.

(d) The sample mixture is chromatographed using composition P_2 as mobile phase and ln k' for the last eluting peak determined (M_2). This value is again compared to the target value and if the difference (A_2) exceeds the defined error, M_1 and M_2 are used to define a new value of S and eqn. 3 is then employed to calculate a new mobile phase composition (P_3) giving the target value of ln k' for the last eluting solute.

(e) Step (d) above is repeated using composition P_3 as mobile phase and if necessary, a new value of S is determined and a new mobile phase composition (P_4) calculated. The process continues until the predicted mobile phase composition provides a value of ln k' for the last eluting solute which is sufficiently close to the target value to comply with the user-defined error.

Fig. 1 illustrates this process for a hypothetical case, and Table I lists numerical values for the parameters P, M, A and S for each iteration of the procedure.

EXPERIMENTAL

Instrumentation

The liquid chromatograph consisted of a Waters Assoc. (Milford, MA, U.S.A.)

TABLE I

NUMERICAL VALUES FOR THE PARAMETERS P, M, A AND S IN FIG. 1

Iteration	P	M	A	S
1	0.65	1.3	1.0	−20.0
2	0.6	1.8	0.5	−10.0
3	0.55	2.6	0.3	−16.0
4	0.57	2.3	0	−16.0

Model M6000A pump, Model U6K injector, Model M441 detector operated at 254 nm and Model M730 data module. A Waters Assoc. C_{18} Nova-Pak column (150 × 3.9 mm I.D.) was employed at a flow-rate of 1 ml/min, and under these conditions, the void volume of this column was determined to be 0.8 ml. The computing unit was an Apple II-plus microcomputer (Apple Computer, Cupertino, CA, U.S.A.), fitted with twin floppy disk drives and containing a Digicard 80-column expansion card (Macclagan Wright and Assoc., Eltham, Australia).

Reagents

Chromatographic grade solvents (Waters Assoc.) were used for the preparation of mobile phases. The desired amounts of organic modifiers and water were measured by burette and the resultant solution mixed thoroughly, degassed in an ultrasonic bath and filtered through a 0.45-μm membrane filter before use. All mobile phases were prepared freshly as required.

The reagents in the solute mixtures used to validate the computer routine were all of analytical grade and were used without further purification. N-methyl-2-phenethylamine hydrochloride, N,N-dimethyl-2-phenethylamine hydrochloride, N-*n*-butyl-2-phenethylamine hydrochloride and 2,2'-diphenylethylamine hydrochloride were synthesised as previously reported[6].

RESULTS AND DISCUSSION

Potential problems

There were two potential problems which could arise in the use of the proposed computer routine for the location of isoeluotropic mobile phases. The first was that the routine would require a large number of iterations before the user-defined error margin was reached, and the second was whether the routine would operate successfully when a change in solute elution order occurred at the end of the chromatogram.

In assessing the significance of the first of these potential problems, it is necessary to examine the applicability of eqns. 1 and 2 to the chromatographic system under study. When eqn. 1 describes accurately the retention behaviour of the last eluting solute in the test mixture, then it is clear that a number of iterations of the procedure will be needed in order to approximate the quadratic relationship between $\ln k'$ and φ with a series of line segments of varying slope (S) values. On the other hand, when the last eluting solute follows the retention pattern described by eqn. 2, only one iteration should be required in order to determine accurately S in the $\ln k'$ *versus* φ equation. When the same solute elutes last throughout the experiment, the number of iterations is therefore governed solely by which retention equation is followed, and in the case that eqn. 1 applies, by the degree of curvature exhibited in the $\ln k'$ *versus* φ relationship.

The second potential problem arises when a change in elution order at the end of the chromatogram results in a different solute eluting last at some stage of the experiment. This must invariably lengthen the process since a slope value pertinent to the new solute must be determined, and when this solute follows eqn. 1, further iterations must be expected.

TABLE II

RETENTION DATA FOR DIPHENYLAMINES

Key to solute identities: 1 = N-nitrosodiphenylamine; 2 = 4-nitrodiphenylamine; 3 = diphenylamine; 4 = 2-nitrodiphenylamine.

Mobile phase composition				*Retention time (min)*			
Methanol	*ACN*	*THF*	*Water*	*1*	*2*	*3*	*4*
65	0	0	35	4.7	5.4	6.1	10.4
0	50.6	0	49.4	5.9	6.1	7.4	10.0
0	0	42.9	57.1	10.7	17.9	20.1	21.8
0	0	46.8	53.2	7.0	10.4	12.1	13.1
0	0	48.6	51.4	6.1	8.5	9.8	10.7

Applications

The proposed procedure was examined using three groups of solutes as test mixtures. The first of these was a group of four diphenylamines, which were found to elute in the retention range $1 \leq k' \leq 10$ using methanol–water (65:35) as the mobile phase. The target value of ln k' was 2.303. An isoeluotropic ACN–water (50.6:49.4) binary mobile phase was predicted from the transfer rule (eqn. 4) and when retention data were obtained, this prediction was found to be accurate. However, this was not the case for the isoeluotropic THF–water mobile phase, where two iterations of the computer routine were required to locate the correct mobile phase composition. Retention data for this application are given in Table II and Fig. 2 shows a schematic illustration of the results obtained in the location of the THF–water mobile phase. Table II shows that the transfer rule for THF (eqn. 5) predicted a mobile phase which gave excessively long retention times, however two iterations of the computer routine located the isoeluotropic THF–water (48.6:51.4) binary mobile phase. Fig. 2. suggests

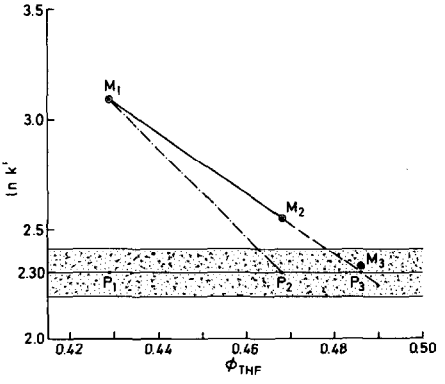

Fig. 2. Diagrammatic representation of the steps followed in the conversion of a methanol–water (65:35) mobile phase into an isoeluotropic binary THF–water mixture using diphenylamine solutes (see Table II). The hatched area represents the user-defined error margin (in this case ± 5%) within which mobile phases are considered to be isoeluotropic.

TABLE III

RETENTION DATA FOR SYMPATHOMIMETIC AMINES

Key to solute identities: 1 = phenylpropanolamine; 2 = phenethylamine; 3 = N-methyl-2-phenethylamine; 4 = amphetamine; 5 = N,N-dimethyl-2-phenethylamine; 6 = N-*n*-butyl-2-phenethylamine; 7 = 2,2'-diphenylethylamine.

Mobile phase composition				*Retention time (min)*						
Methanol	*ACN*	*THF*	*Water*	*1*	*2*	*3*	*4*	*5*	*6*	*7*
50	0	0	50	2.5	2.5	2.5	3.0	2.5	4.8	8.2
0	36.5	0	63.5	1.2	1.3	1.5	1.5	1.8	2.7	2.7
0	29.7	0	70.3	1.7	1.9	2.2	2.2	2.6	5.6	6.3
0	27.8	0	72.2	2.0	2.3	2.8	2.6	3.2	8.1	10.0
0	28.6	0	71.4	1.8	2.1	2.5	2.5	3.1	7.3	8.5
0	0	33.0	67.0	1.5	1.5	1.5	1.7	1.5	2.2	3.2
0	0	27.4	72.6	1.8	1.8	1.8	2.1	1.8	3.0	5.6
0	0	23.9	76.1	2.3	2.3	2.3	2.7	2.1	3.9	8.7

that the retention behaviour of the last eluting solute, 2-nitrodiphenylamine, is described adequately by eqn. 2.

The second application involved a group of seven sympathomimetic amines using ion-interaction RPLC and all mobile phases in this application contained 5 m*M* heptanesulphonate and 1% acetic acid. Table III lists the retention data obtained and it can be seen that the isoeluotropic ACN–water binary mobile phase was found

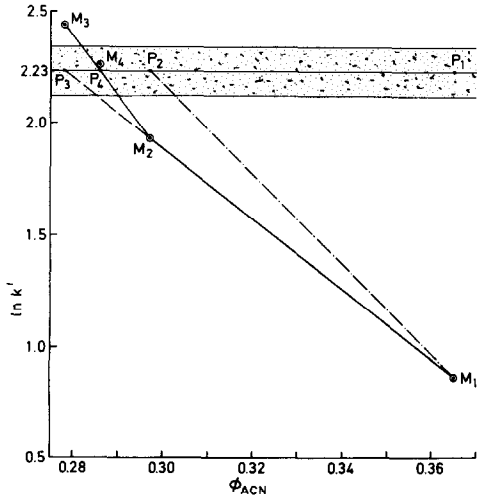

Fig. 3. Diagrammatic representation of the steps followed in the conversion of a methanol–water (50:50) mobile phase into an isoeluotropic binary ACN–water mixture using sympathomimetic amines as solutes (see Table III). The hatched area represents the user-defined error margin (see Fig. 2). Note that the mobile phases used in this application contained 5 m*M* heptanesulphonate and 1% acetic acid in addition to the stated concentrations of water and organic modifier.

TABLE IV

RETENTION DATA FOR AROMATIC COMPOUNDS

Key to solute identities: 1 = phenol; 2 = benzaldehyde; 3 = *o*-cresol; 4 = *m*-dinitrobenzene; 5 = benzene; 6 = *p*-iodophenol.

Mobile phase composition				*Retention time (min)*					
Methanol	*ACN*	*THF*	*Water*	*1*	*2*	*3*	*4*	*5*	*6*
46	0	0	54	2.7	3.7	4.7	4.7	9.2	12.2
0	33.0	0	67.0	2.6	4.2	4.2	7.3	10.1	8.8
0	32.0	0	68.0	2.7	4.4	4.4	7.9	11.0	9.8
0	0	30.4	69.6	5.3	5.3	6.7	18.4	15.0	19.0
0	0	32.7	67.3	4.1	4.1	5.3	14.0	10.4	14.0
0	0	35.0	65.0	3.8	3.8	4.7	12.1	8.8	12.2

after three iterations of the computer routine, whereas the THF–water mobile phase required two iterations. Fig. 3 illustrates the sequence followed in selection of the ACN–water mobile phase, where a target value of ln k' of 2.23 was used. In this case, it is clear that the retention behavior of 2,2'-diphenylethylamine is described by eqn. 1. Again, it is worth noting that the binary mobile phases predicted from the transfer rules (eqns. 4 and 5) were not isoeluotropic.

In the final application, the test mixture comprised a series of aromatic compounds. Retention data for these solutes are given in Table IV, from which it can be seen that one and two iterations, respectively, were required to locate the isoeluotropic ACN–water and THF–water binary mobile phases.

CONCLUSIONS

This study has shown that isoeluotropic binary mobile phases may be obtained using a simple computer routine based on the assumption that retention behaviour in binary solvent systems can be approximated by a linear relationship between ln k' and φ, or by a series of successive linear relationships.

This computer routine performs essentially the same steps as would be followed by an experienced chromatographer, but has the advantages of speed, accuracy and potential for automation. Moreover, the approach is independent of the natures of the solutes under examination and can be used with complex systems such as ion-interaction chromatography. When applied to the selection of the mobile phase search area in computer-assisted optimisation methods, the procedure provides a more reliable approach than the use of transfer rules or solvent weighting factors.

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