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EFFECTS OF PEAK TAILING ON COMPUTER OPTIMISATION PROCEDURES FOR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

II. AN OPTIMISATION ROUTINE FOR TAILED PEAKS

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SUMMARY

An optimisation program is proposed as a means of compensating for the loss of resolution resulting from peak tailing. A mobile phase search area of ternary compositions of water with two of the three solvents, methanol, acetonitrile, and tetrahydrofuran, is defined by isoeluotropic binary mobile phases of water with each of the above three modifiers. Retention and peak shape (τ/σ) data are obtained for each solute in the mixture to be separated, using the three isoeluotropic binary mobile phases. These data are used to interpolate retention and peak shape data for intermediate ternary mobile phases. The area overlap for each peak pair is calculated, and the resolution of a pair of Gaussian peaks with the same degree of area overlap is determined. This value is then used for calculation of an optimisation criterion and the ultimate selection of the optimal mobile phase. The program was computer-validated by using a hypothetical case. It was then experimentally tested by using a mixture of six compounds, giving both symmetrical and tailed peaks. In both cases, the use of the proposed program resulted in the selection of a mobile phase that gave a chromatogram superior to that obtained without considering peak tailing effects.

INTRODUCTION

Computer optimisation of mobile phase composition in high-performance liquid chromatography (HPLC) involves selection of an optimal mobile phase on the basis of the quality of the chromatogram produced by that mobile phase. Quality is assessed using a of mathematical criterion which assigns a numerical value to the chromatogram, dependent on the degree of separation achieved. The optimisation criterion is therefore a crucial parameter in determining the ultimate success of the optimisation process.

Schoenmakers[1] has recently reviewed optimisation criteria, and has defined "elemental criteria" as those which may be used to quantify the separation between a pair of adjacent peaks in a chromatogram. Elemental criteria include peak-valley ratio (P)[2,3], valley-to-top ratio (P_v)[4], fractional peak overlap (FO), resolution (R_s),

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and the separation factor (S)[5,6]. Some elemental criteria have been compared[7,8] and it is apparent that their characteristics differ markedly. R_s and S do not reflect changes in the peak shape or the ratio of peak areas, but their values are relatively easily calculated and are transferable to other columns. On the other hand, P, P_v, and FO are often more difficult to measure, but they accurately reflect the actual separation and vary with changes in peak shape and area ratio. Ease of calculation is of paramount importance in the practical implementation of optimisation procedures, and therefore R_s is by far the most commonly employed elemental criterion.

Since a chromatogram generally consists of more than two peaks, the quality of the entire chromatogram must be assessed by some combination of elemental criteria for adjacent peak pairs. A simple example is use of the R_s value for the peak pair in the chromatogram with the poorest resolution (*i.e.* $R_{s_{min}}$) as an indicator of the quality of the entire chromatogram. Summation of elemental criteria, such as R_s and S, has been suggested[9,10], but this sum is strongly influenced by the largest value of the elemental criterion in a chromatogram, and therefore it may not accurately indicate the degree of separation of less-resolved peak pairs. Product criteria, in which the values of the elemental criteria for each peak pair are multiplied, have also been proposed[5,11]. These have proven to be particularly useful, especially when the product is normalised to account for the length of the chromatogram. Composite criteria which consider factors other than separation, such as analysis time, have also been suggested[12].

The chief drawback of R_s as an elemental criterion, and hence of the composite criteria that use R_s for their calculation, is that peaks are considered to be Gaussian in shape. The utility of R_s in optimisation procedures would therefore be enhanced considerably if a new criterion could be devised based on R_s values (and thus taking advantage of the simplicity of calculation for this parameter), which can also make allowances for non-Gaussian peaks. In this paper, we propose such a criterion which expresses the resolution of tailed peaks in terms of the equivalent area overlap of Gaussian peaks. The new procedure is first computer-validated and then applied to the separation of a mixture of six aromatic compounds.

EXPERIMENTAL

Instrumentation

The optimisation programs used in this work were operated on a Macintosh Plus microcomputer (Apple, Cupertino, CA, U.S.A.) with 1 Mb RAM, fitted with an external disk drive and an Apple Imagewriter II printer. The liquid chromatograph consisted of a Waters Millipore (Milford, MA, U.S.A.) Model M590 pump, Model U6K injector, Model M441 UV detector (operated at 254 nm), and a Model M730 data module. The column was a Waters reversed-phase C_{18} Nova-Pak column (150 × 3.9 mm I.D.).

Reagents

Binary and ternary mobile phases used for the optimisation procedure were prepared by measuring the required volumes of chromatography-grade solvents and water with a burette into a suitable container, mixing the resultant solution thoroughly, filtering through a 0.45-μm membrane filter, and degassing in an ultrasonic bath before use. The mobile phases also contained 5 mM sodium heptanesulphonate

(Ajax Chemicals, Sydney, Australia) and 1% acetic acid. Analytical-grade solutes were obtained from the following sources: toluene from May & Baker (Dagenham, U.K.), *p*-iodophenol from Fluka (Buchs, Switzerland), and doxepin and propranolol from Sigma (St. Louis, MO, U.S.A.). N-*n*-Butyl-2-phenethylamine hydrochloride and 2,2′-diphenethylamine hydrochloride were synthesised and checked for purity as previously reported[13].

Optimisation software (prior to any changes)

The optimisation method was based on the iterative procedure reported by Schoenmakers *et al.*[5] and Drouen *et al.*[6]. In this procedure, retention data obtained for three isoeluotropic binary mobile phases are used to predict retention times for the ternary solvent mixtures formed from linear combinations of the binary mobile phases. All possible chromatograms within a mobile phase search area bound by the three isoeluotropic binary mobile phases are then assessed on the basis of a mathematical criterion[14] and the optimal mobile phase is selected. Retention data for this mobile phase are then measured and added to the data file in the computer. The calculation of the criterion is repeated, and a new optimal mobile phase selected. This process continues until the same optimum is selected in successive calculations or a previously measured mobile phase composition is assigned to be the optimum. Full details of the operational procedure and theoretical basis of this method are given elsewhere[5,6,15].

RESULTS AND DISCUSSION

Effects of peak tailing on calculations of criteria

As outlined in the Introduction, the ability of an optimisation procedure to locate the optimal mobile phase is dependent on an accurate assessment of the quality of a chromatogram through the use of a mathematical criterion. The criteria used in this work were the resolution product, ΠR_s, or the relative resolution product, r, which can be defined as[1]

$$\prod R_s = \prod_{i=1}^{n-1} R_{s_{i,i+1}} \tag{1}$$

$$r = \prod_{i=1}^{n-1} \left[\frac{R_{s_{i,i+1}}}{\frac{1}{n-1} \sum_{i=1}^{n-1} R_{s_{i,i+1}}} \right] \tag{2}$$

R_s for adjacent peaks (denoted by i and $i+1$) is calculated from the equation

$$R_s = \frac{\sqrt{N}}{2} \frac{(t_{R_{i+1}} - t_{R_i})}{(t_{R_i} + t_{R_{i+1}})} \tag{3}$$

where t_R represents the retention time of a peak, and N is the efficiency of the chromatographic column used. Whilst eqn. 3 assumes that the peaks involved are Gaussian in shape, it is nevertheless very useful for optimisation procedures, since it

permits R_s to be calculated on the basis of column efficiency and retention times for solutes in the mixture to be optimised. If a relationship between retention time and mobile phase composition is known or assumed, it becomes possible to predict retention times for any desired mobile phase composition and, hence, to calculate a criterion value for the chromatogram that would be produced with that particular mobile phase. In this way, the optimal mobile phase can be selected.

This approach operates well when the component solutes of the mixture to be separated give symmetrical peaks for which the R_s values calculated from eqn. 3 accurately reflect the actual degree of separation of the two peaks concerned. When peak tailing is evident, the value of R_s calculated for each peak pair from eqn. 3, and hence the value of the optimisation criterion, will be unchanged, despite the fact that the area overlap of the peaks may have increased. This is illustrated in Fig. 1, which shows a chromatogram of five fully resolved Gaussian peaks (Fig. 1A), whilst Fig. 1B shows five tailed peaks ($\tau/\sigma = 3.0$ for each peak) eluted at the same retention times. Despite the fact that the separation is clearly poorer in Fig. 1B, both chromatograms have identical values for the ΠR_s and r criteria.

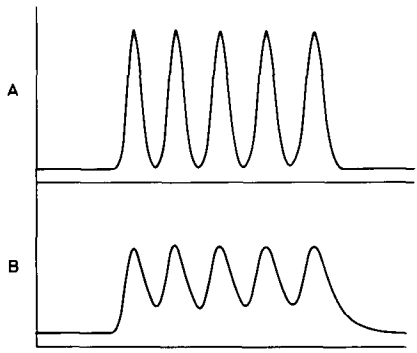

Fig. 1. Effect of peak tailing on area overlap. Retention times are the same for both chromatograms, but peaks are Gaussian in A and have $\tau/\sigma = 3.0$ in B.

Characteristics of tailed peaks in optimisation procedures

The aim of our work was to devise an optimisation program which would be capable of reliable prediction of optimal mobile phases for mixtures of solutes giving tailed peaks. The characteristics of tailed peaks that are of importance to optimisation procedures have been evaluated[16], and the results obtained can be summarised as:

(i) When a peak pair is considered, tailing of the leading peak causes a significant increase in the area overlap between the peaks, whereas tailing of the trailing peak generally results in a decrease in area overlap. When evaluating the separation of such a peak pair for an optimisation procedure, it is therefore necessary to consider the tailing exhibited by the leading peak only, and the trailing peak can be assumed to be Gaussian.

(ii) If the components of a peak pair are of different heights, the trends in area overlap are similar to those observed for peaks of equal height, except that a swamping region is introduced within which total area overlap is maintained until R_s reaches

a threshold value, dependent on the height ratio of the two peaks concerned. In the R_s region that is of most concern to optimisation procedures (*i.e.* 1–1.5), disparate height ratios do not exert a significant effect on area overlap. This factor, and the limitation on computer memory available for the optimisation program, lead to the necessity to assume that all peaks in a chromatogram are of equal height. Some loss of accuracy can be expected as a result of this assumption.

(iii) Peak asymmetry values, as given by τ/σ ratios, differ in binary mobile phases [water with methanol, acetonitrile or tetrahydrofuran (THF)]. A linear relationship between τ/σ and the mobile phase composition exists for ternary mobile phases, formed from linear combination of the above binary mobile phases. Thus, if the τ to σ ratio is known for each solute in the binary mobile phase compositions used to define the mobile phase search area in the optimisation, it becomes possible to calculate τ to σ ratios for ternary mobile phases within that search area.

(iv) The exponentially modified Gaussian function, which was used to generate tailed peaks in this study, is a good approximation for the actual peak shapes obtained experimentally.

Optimisation software for tailed peaks

Based on the above results, a new procedure was developed for the calculation of peak separation values, based on area overlap, which could be used for the determination of realistic criteria in cases of solutes giving tailed peaks. To achieve this goal, the optimisation program currently in use in our laboratory was modified in two ways.

In the first modification, the operator is asked to provide geometric information for each peak in the mixture, measured for each of the isoeluotropic binary mobile phases. The widths of the leading half (A) and the trailing half (B) of the peak, measured at 10% of peak height, are used to calculate the peak asymmetry ($B/A_{0.1}$) and, thence, the τ to σ ratio, from previously reported equations[16] or from a calibration curve, such as that shown in Fig. 2. The slope of an assumed linear relationship between τ/σ and Φ, the mobile phase composition, is then calculated for ternary mobile phases, comprising the search area defined by linear combinations of the isoeluotropic binary mobile phases. This permits calculation of τ to σ ratios for each solute in any mobile phase composition within the search area.

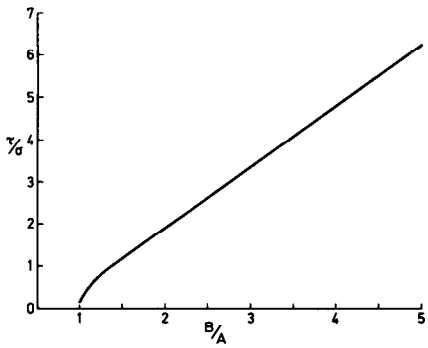

Fig. 2. Calibration plot for graphic conversion of asymmetry factors (B/A) into τ/σ values.

TABLE I

FOURTH-ORDER POLYNOMIAL COEFFICIENTS FOR EQUATIONS DESCRIBING THE RELATIONSHIP BETWEEN R_s AND PERCENTAGE AREA OVERLAP FOR PEAK PAIRS IN WHICH THE FIRST PEAK HAS THE INDICATED VALUE OF τ/σ

The coefficients are for the equaton $Ax^4 + Bx^3 + Cx^2 + Dx + E = 0$.

τ/σ	A	B	C	D	E	*Correlation coefficient*
0.0	−24.42	52.10	44.37	−168.48	100.38	0.9999
0.1	−42.56	91.03	22.09	−166.39	100.15	0.9999
0.2	−44.70	94.65	21.06	−167.36	100.20	0.9999
0.3	−21.90	40.15	58.45	−172.29	99.76	0.9999
0.4	−23.44	48.54	44.64	−164.31	99.90	0.9999
0.5	−28.40	71.64	10.41	−148.30	101.16	0.9999
0.6	−21.04	52.74	23.01	−148.13	100.58	0.9999
0.7	−10.36	18.54	57.23	−157.84	100.38	0.9999
0.8	−12.75	32.73	30.16	−141.33	100.65	0.9999
0.9	− 9.90	22.02	41.68	−143.50	100.16	0.9999
1.0	− 6.84	15.30	41.07	−138.88	101.58	0.9997
1.1	−10.03	31.30	13.91	−122.49	101.24	0.9997
1.2	− 3.71	5.00	47.66	−134.39	100.46	0.9999
1.3	− 4.01	8.65	36.74	−125.34	100.96	0.9998
1.4	− 6.43	20.17	18.71	−114.98	100.86	0.9999
1.5	− 1.67	−0.27	44.54	−123.61	100.74	0.9997
1.6	− 4.78	15.34	19.44	−109.25	100.30	0.9999
1.7	− 2.37	7.06	23.49	−105.20	100.79	0.9995
1.8	− 0.93	−1.35	38.78	−113.64	100.82	0.9997
1.9	− 1.28	1.79	30.39	−106.18	100.82	0.9996
2.0	− 0.73	−1.08	33.99	−106.29	101.25	0.9995
2.1	− 1.93	6.67	17.86	− 94.85	100.90	0.9995
2.2	− 0.19	−3.72	36.96	−105.30	100.99	0.9997
2.3	− 0.38	−2.21	32.77	−101.29	101.16	0.9996
2.4	0.12	−4.59	35.13	−100.21	101.35	0.9995
2.5	− 0.08	−2.90	30.21	− 95.13	101.10	0.9995
2.6	− 0.04	−2.97	29.43	− 93.36	101.51	0.9995
2.7	0.02	−3.47	30.49	− 93.54	101.21	0.9997
2.8	0.16	−4.11	30.89	− 92.98	101.85	0.9997
2.9	0.16	−3.97	29.55	− 90.10	101.53	0.9996
3.0	0.13	−3.52	27.56	− 86.83	100.74	0.9997
3.1	− 0.02	−2.20	23.72	− 83.43	101.52	0.9995
3.2	− 0.08	−1.61	21.60	− 80.75	101.56	0.9995
3.3	0.20	−3.89	27.49	− 85.23	101.51	0.9997
3.4	0.19	−3.72	26.32	− 82.91	101.28	0.9998
3.5	0.20	−3.74	26.02	− 82.12	101.79	0.9997
3.6	0.18	−3.40	24.38	− 79.47	101.46	0.9997
3.7	0.16	−3.12	23.14	− 77.58	101.52	0.9997
3.8	0.14	−2.85	22.09	− 76.24	101.95	0.9996
3.9	0.12	−2.61	20.81	− 73.93	101.59	0.9997
4.0	0.13	−2.67	20.90	− 73.49	100.95	0.9997
4.1	0.12	−2.57	20.28	− 72.34	101.23	0.9998
4.2	0.11	−2.36	19.28	− 70.73	101.27	0.9998
4.3	0.11	−2.32	18.96	− 70.15	101.79	0.9997
4.4	0.10	−2.21	18.18	− 68.35	101.40	0.9998
4.5	0.08	−1.91	16.85	− 66.34	101.22	0.9997
4.6	0.08	−1.94	16.77	− 65.77	101.48	0.9998
4.7	0.08	−1.81	16.08	− 64.50	101.51	0.9998

TABLE I *(continued)*

τ/σ	*A*	*B*	*C*	*D*	*E*	*Correlation coefficient*
4.8	0.06	−1.63	15.32	− 63.57	101.99	0.9996
4.9	0.08	−1.80	15.72	− 63.18	101.11	0.9999
5.0	0.07	−1.73	15.36	− 62.63	101.60	0.9998
5.1	− 0.21	0.74	8.29	− 55.49	100.79	0.9998
5.2	− 0.22	0.91	7.52	− 54.22	100.81	0.9999
5.3	− 0.24	1.09	6.78	− 52.98	100.83	0.9999
5.4	− 0.25	1.25	6.07	− 51.78	100.84	0.9999
5.5	− 0.24	1.16	6.38	− 51.95	100.96	0.9999
5.6	− 0.28	1.56	4.69	− 49.43	100.81	0.9999
5.7	− 0.28	1.68	4.12	− 48.38	100.82	0.9999
5.8	− 0.29	1.81	3.52	− 47.31	100.83	0.9999
5.9	− 0.31	1.95	2.90	− 46.26	100.83	0.9999
6.0	− 0.21	1.08	5.52	− 48.47	100.41	0.9999
6.1	− 0.09	0.14	7.52	− 49.40	100.53	0.9999
6.2	− 0.13	0.51	6.34	− 47.97	100.52	0.9999
6.3	− 0.14	0.61	5.87	− 47.07	100.53	0.9999
6.4	− 0.14	0.70	5.41	− 46.20	100.55	0.9999
6.5	− 0.15	0.79	4.94	− 45.28	100.51	0.9999
6.6	− 0.16	0.87	4.54	− 44.47	100.53	0.9999
6.7	− 0.16	0.96	4.10	− 43.64	100.54	0.9999
6.8	− 0.17	1.03	3.73	− 42.87	100.55	0.9999
6.9	− 0.17	1.11	3.34	− 42.10	100.56	0.9999
7.0	− 0.17	1.13	3.14	− 41.48	100.57	0.9999
7.1	− 0.18	1.19	2.80	− 40.77	100.59	0.9999
7.2	− 0.18	1.27	2.43	− 40.03	100.59	0.9999
7.3	− 0.19	1.33	2.10	− 39.35	100.60	0.9999
7.4	− 0.15	0.97	3.19	− 40.18	100.29	0.9999
7.5	− 0.16	1.04	2.89	− 39.49	100.29	0.9999
8.0	− 0.17	1.19	1.83	− 36.83	100.35	0.9999
8.5	− 0.18	1.39	0.67	− 34.11	100.38	0.9999
9.0	− 0.16	1.31	0.32	− 32.16	100.41	0.9999
9.5	− 0.16	1.27	0.33	− 31.22	100.03	0.9999
10.0	− 0.09	0.77	1.15	− 30.40	100.12	0.9999

Secondly, use is made of the relationships observed between % area overlap and R_s for a peak pair in which the leading peak exhibits tailing. These relationships have been reported previously[16] for τ/σ values in the range 0 to 10.0, and can be fitted[17] to fourth-order polynomial curves. The data for these polynomial functions are given in Table I, and are stored as part of the optimisation program. Thus, if the retention times of the components of a peak pair and τ/σ for the first-eluted peak are known, then R_s can be calculated from eqn. 3, and the percentage area overlap of the two peaks can be calculated by solving the equation in Table I for the appropriate τ/σ value.

This calculated area overlap can then be substituted into the polynomial expression for two Gaussian peaks (*i.e.* $\tau/\sigma = 0$ in Table I) to determine the R_s value which would be exhibited by two Gaussian peaks with the same degree of area overlap. The value calculated in this manner is described as the "Gaussian equivalent resolution", R_s'. Fig. 3 gives a graphic representation of the process involved for a peak

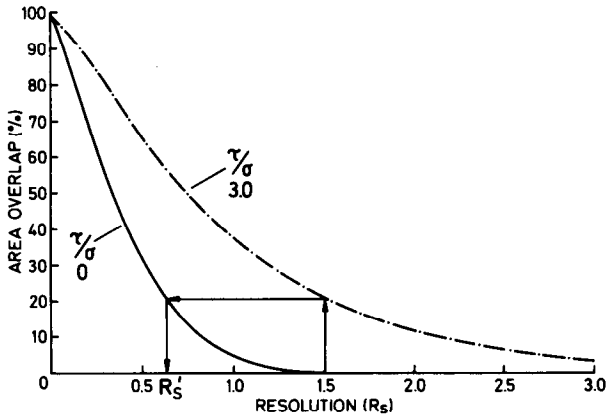

Fig. 3. Calculation of R_s', the Gaussian equivalent resolution, for a pair of peaks where $R_s = 1.5$ and the leading peak is tailed by $\tau/\sigma = 3.0$.

pair in which the first peak has $\tau/\sigma = 3.0$, the R_s calculated from eqn. 3 is 1.5 and the value of R_s' for the same peak pair is 0.63. Criteria values may then be calculated from R_s' values rather than R_s values, and criteria calculated in this way are identified in this paper as $\Pi R_s'$ and r'. The predicted optimal mobile phase composition is then determined and reported, together with a listing of the calculated R_s' values for each peak pair. A flow diagram of the entire process is presented in Fig. 4.

This modified optimisation program provides the operator with the option of including or excluding the consideration of peak tailing, so that when all solutes in a mixture give symmetrical peaks, it is not necessary to enforce the additional calculation time required in case of peak tailing.

Computer validation of the proposed procedure

It can be envisaged that peak tailing effects would exert the greatest influence in the situations described below, and it is in these cases that the modified optimisation procedure could be expected to show the most benefit:

(i) When the degree of tailing of solutes in the mixture varies widely; *e.g.*, one or two tailing solutes in a mixture, where solutes give symmetrical peaks.

(ii) When the order of elution of a tailed solute alters over the search area. Here, mobile phases in which a tailed peak is eluted last in a critical peak pair may be preferred to those in which the tailed peak is eluted first.

(iii) When solutes under consideration exhibit a significantly smaller degree of tailing with two of the modifiers, then ternary combination of those modifiers with water may be favoured.

In each of these situations, the use of R_s' values for criteria calculations would provide a more accurate appraisal of peak separation than that gained from the use of R_s values. A hypothetical retention file for three solutes in methanol–acetonitrile–water ternary mobile phases was used to validate the modified optimisation program. Table II lists the retention and peak shape data, and shows that only one solute (solute 1) exhibits peak tailing. The optimum was selected by using the relative resolution product criterion which gives preference to chromatograms in which peaks are evenly

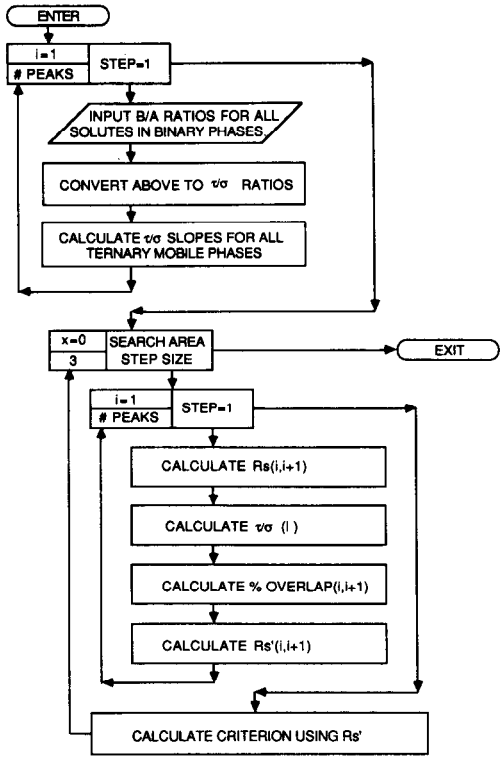

Fig. 4. Flow diagram of the steps incorporated into the optimisation program to compensate for peak tailing.

spaced. Using Eqn. 3 to calculate R_s values, the predicted optimal mobile-phase composition is calculated to be methanol–acetonitrile–water (17.5:22.5:60), with a criterion value of $r = 1$. Assuming Gaussian peak shapes, the optimal chromatogram is shown in Fig. 5a, whilst the actual chromatogram for this mobile phase composition is given in Fig. 5b. In its unmodified form, the optimisation software cannot

TABLE II

RETENTION AND PEAK SHAPE DATA FOR A HYPOTHETICAL TEST MIXTURE USED TO VALIDATE THE MODIFIED OPTIMISATION PROCEDURE

Solute	*Mobile phase: methanol–acetonitrile–water*			
	50:0:50		*0:35:65*	
	Retention time (min)	*Peak shape data (τ/σ)*	*Retention time (min)*	*Peak shape data (τ/σ)*
1	6.5	3.3	6.7	3.3
2	7.1	0	8.2	0
3	9.8	0	8.9	0

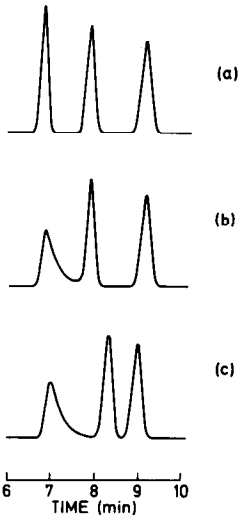

Fig. 5. Hypothetical test case for evaluation of the modified optimisation program. The data file in Table II was used. (a) Optimal chromatogram selected when peak tailing is not considered. (b) Actual chromatogram for this mobile phase. (c) Actual chromatogram selected by the modified program. Solute 1 has $\tau/\sigma = 3.3$.

distinguish between these two chromatograms, and does not recognise the area overlap existing between peaks 1 and 2 in Fig. 5b. When the same optimisation is repeated with the modified program, the predicted optimum mobile phase is acetonitrile–water (35:65), with a criterion value of $r' = 0.95$. The optimal chromatogram is shown in Fig. 5c, in which there is no area overlap between adjacent peaks. The chromatogram selected by the modified program shows superior resolution, despite the fact that the criterion values given above suggest that the reverse should be true.

Experimental validation of the proposed procedure

The performance of the modified software was evaluated by using a real sample mixture, comprising N-butylphenethylamine (N-BuPEA), 2,2′-diphenethylamine (di-PEA), propranolol, *p*-iodophenol (p-I-phenol), doxepin, and toluene. The optimisation search area of isoeluotropic mobile phases in which these solutes are eluted in the approximate capacity factor range $1 \leqslant k' \leqslant 10$ was found to be bounded by the binary mobile phases methanol–water (60:40), acetonitrile–water (44:56) and THF–water (42:58). Retention and peak shape data for each solute in these binary mobile phases were determined and are shown in Table III.

Optimisation without consideration of peak tailing yielded an optimal mobile phase composition of methanol–THF–water (36:17:47). The chromatogram obtained with this mobile phase is shown in Fig. 6. This separation attained a criterion value of $r = 0.84$, but it is clearly deficient in resolution between the two tailed peaks of propranolol and doxepin. The modified optimisation program was applied to the same search area and predicted the methanol–water (60:40) binary mobile phase to be optimal, giving a criterion value of $r' = 0.63$. The chromatogram obtained with this optimal mobile phase is shown in Fig. 7. It is clearly superior to the chromatogram shown in Fig. 6. This improvement is attributable partly to the exploitation of changes

TABLE III

RETENTION AND PEAK SHAPE DATA FOR TEST SOLUTES USED FOR EXPERIMENTAL VALIDATION OF THE MODIFIED OPTIMISATION PROCEDURE

Solute identities are given in the text.

Solute	*Mobile phase: methanol–acetonitrile–THF–water*					
	60:0:0:40		*0:0:42:58*		*0:44:0:56*	
	Retention time (min)	*Peak shape data (τ/σ)*	*Retention time (min)*	*Peak shape data (τ/σ)*	*Retention time (min)*	*Peak shape data (τ/σ)*
N-BuPEA	2.75	5.2	1.66	5.0	2.01	5.3
di-PEA	3.86	5.5	1.66	5.3	2.01	5.3
Propranolol	5.1	6.8	2.1	6.3	3.06	5.5
p-I-phenol	3.45	0	3.36	0	3.43	0
Doxepin	7.38	7.5	2.1	7.2	7.0	6.3
Toluene	6.46	0	5.13	0	8.15	0

in the order of elution of the solutes, particularly the tailed peak of doxepin, and is typical of case (ii) described in the previous section. Not only is the resultant chromatogram improved in terms of area overlap, but the optimal mobile phase was selected in one iteration of the optimisation procedure less than that required when peak tailing effects were not considered. It is clear that the modified program can be used successfully when tailed peaks are encountered, at the cost of some increase in computation time.

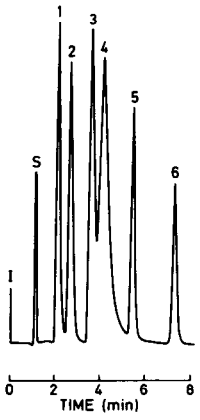

Fig. 6. Chromatogram obtained for the test mixture with the mobile phase selected by the unmodified optimisation program [*i.e.*, methanol–THF–water (36:17:47)]. The data file in Table III was used. Solute identities: 1 = N-BuPEA; 2 = diPEA; 3 = propranolol; 4 = doxepin; 5 = p-I-phenol; 6 = toluene; S = solvent peak.

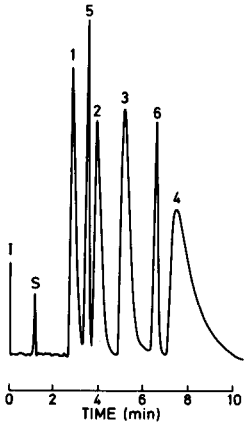

Fig. 7. Chromatogram obtained for the test mixture with the mobile phase selected by the modified optimisation program [*i.e.* methanol–water (60:40)]. The data file in Table III was used. Solute identities as for Fig. 6.

CONCLUSIONS

It has been demonstrated that when peak tailing effects are taken into consideration, better predictions of optimal mobile phase composition can be achieved. The proposed modifications are not extensive and with the aid of the coefficients listed in Table I, any existing optimisation program in which R_s values are used for the calculation of criterion values can be modified. Manual application of the techniques outlined in this paper is also possible when the calibration graph shown in Fig. 2 for conversion of $B/A_{0.1}$ values to τ to σ ratios is used, together with manual solution of the equations presented in Table I or use of the graphic relationships between R_s and percentage area overlap which we have reported previously[16].

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