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OPTIMIZATION STRATEGIES FOR SOLUTES EXHIBITING PEAK TAILING

COMPARISON OF TWO APPROACHES

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SUMMARY

Two methods have recently been proposed for the improved calculation of resolution values when tailed peaks are present. Both are based on the exponentially modified Gaussian model, but use different approaches. One employs empirical relationships between calculated resolution and area overlap and the other is a mathematical approach in which a new resolution equation is derived. Optimization procedures were developed for both approaches and were compared using computer-generated peaks and actual solute mixtures. Both approaches gave superior mobile phase selection in comparison with the approach in which optimization was performed without consideration of peak tailing. Moreover, the performances of the two methods were almost identical in terms of the mobile phases selected, but slight differences in calculation time and computer memory requirements were noted. It is concluded that both methods may be used successfully in the optimization of mobile phase composition for solutes giving tailed peaks.

INTRODUCTION

Chromatographic optimization procedures require the measurement of the extent of separation exhibited by entire chromatograms. This may be achieved by first determining the degree of separation of each pair of peaks in the chromatogram. Mathematical expressions used for this purpose may be referred to as elemental criteria¹. The chromatographic resolution (R_s), calculated according to eqn. 1, is currently the most extensively used elemental criterion in chromatography. Ease of calculation in practice and transferability to other columns appear to be the major advantages of R_s over alternative elemental criteria such as peak valley ratios^{1,2}, valley-to-top ratios³ and fractional peak overlap. Eqn. 1 shows that the resolution between peaks i and j is defined by the respective retention times (t_i and t_j) and the width of the peaks (w_i and w_j), expressed in units of time:

$$R_s = \frac{2(t_j - t_i)}{(w_i + w_j)} \quad (1)$$

A composite criterion, which is used to determine the quality of a chromatogram as a whole, may now be defined as some combination of elemental criteria. Many composite criteria have been developed for use in optimization procedures. Some of these are fairly simple extensions of elemental criteria, such as the summation of R_s values for each adjacent pair of peaks^{4,5}. This sum is strongly influenced by the largest value of the elemental criterion in a chromatogram and therefore may not accurately indicate the degree of separation of less resolved peaks. Another example is the product criterion, where the elemental criteria for each peak pair in a chromatogram are multiplied^{6,7}. Normalization of this criterion aids in the handling of chromatograms of different lengths. Other composite criteria that consider other factors as well as separation have also been suggested⁸.

The greatest disadvantage of R_s calculation is that it does not reflect changes in peak shape or the ratio of peak areas. The composite criteria that are based on these R_s calculations are therefore subject to the same shortcomings. As it is generally agreed that the occurrence of Gaussian peaks in chromatography is very rare, it follows that composite criteria that use R_s will often give an erroneous estimate of the quality of a chromatogram. Use of such composite criteria in optimization procedures will therefore lead ultimately to the selection of an incorrect optimum when non-Gaussian peaks are present. If it were possible to maintain the simplicity of R_s calculation, while at the same time compensating for variations in peak shape and peak-area ratio, a more realistic composite criterion based on R_s would result.

Two procedures have recently been reported for this purpose. Both use the exponentially modified Gaussian (EMG) function as the tailed peak model. One procedure was developed by Sekulic and Haddad^{9,10} (method A) specifically for optimization procedures. The other reported by Schoenmakers *et al.*¹¹ (method B) is a mathematical approach that describes new resolution equations for non-Gaussian peaks. In this paper we first develop these new resolution equations into a full optimization procedure, which is then compared with our own approach.

THEORY

Outline of the general optimization strategy

The general optimization strategy (GOS) employed here follows the iterative semi-empirical approach reported by De Galan and co-workers^{6,12}. The aim of the procedure is to locate the mobile phase composition giving an optimum separation of a solute mixture as determined by a chosen criterion. This procedure may be summarized by the following sequence:

Step 1: A methanol–water binary mobile phase composition is located so as to elute all solutes within the capacity factor range $1 < k' < 10$.

Step 2: Acetonitrile–water and tetrahydrofuran (THF)–water binary mobile phase compositions which are isoelutotropic (same eluting strength) with the methanol–water binary mobile phase are found. This is achieved with the use of a procedure reported previously¹³.

Step 3: An optimization search area is then defined which consists of the above three binary isoelutotropic mobile phase compositions, together with all the ternary mobile phases formed from linear combinations of these binary mobile phases. Retention data are then obtained for the three isoelutotropic binary mobile phases and

are used to predict retention times for the ternary mobile phases by assuming a linear relationship between $\ln k'$ and the volume fraction of organic modifier in the mobile phase (φ).

Step 4: All chromatograms possible within the defined search area are then assessed for quality of separation on the basis of a composite criterion. The three most commonly used criteria in this laboratory are the following:

$$r = \prod_{i=1}^{n-1} \left[\frac{R_{s,i+1}}{\frac{1}{n-1} \sum_{i=1}^{n-1} R_{s,i+1}} \right] \quad (2)$$

$$\prod R_s = \prod_{i=1}^{n-1} R_{s,i+1} \quad (3)$$

$$\text{Max}[R_{s(\text{min})}] = \text{Max}[R_s \text{ for least resolved peak pair}] \quad (4)$$

The optimum mobile phase composition is selected on the basis of the highest calculated criterion value.

Step 5: Retention data for the optimum mobile phase composition are measured and added to the existing data file and step 4 is repeated until the same optimum composition is selected in successive calculations or a previously measured composition is selected. The optimization process is then considered to be complete.

Full details of the operational procedure and theoretical basis of this method are given elsewhere^{6,12,14}. It will be noticed that the above composite criteria employed in this optimization procedure are all based on the initial calculation of R_s between adjacent peak pairs in the chromatogram, using the assumption that all peak profiles are Gaussian.

Peak tailing compensation (method A)

This method is based on calculations of area overlap at different R_s values for peaks generated by the EMG function. It has been shown⁹ that tailing of the *first peak* in a peak pair has a profound effect on the area overlap of the two peaks, whereas distortion of the second (later eluted) peak did not significantly affect area overlap. It was also concluded that in the R_s region of most interest in optimization procedures (*i.e.*, $1.0 < R_s < 1.5$), no significant influence on R_s was observed when the areas of the two peaks were altered. Therefore, method A does not make compensations for differences in peak-area ratios.

A procedure was developed for calculation of the "Gaussian equivalent resolution" value, R'_s . This term represents the R_s value for two Gaussian peaks that exhibit the same amount of area overlap as for a peak pair in which the first-eluted peak is tailed. R'_s is calculated in the following manner.

(i) Empirically obtained fourth-order polynomial equations are used to describe the area overlap and R_s values for peak pairs in which the degree of tailing of the first peak is incrementally increased. The coefficients of these polynomial equations are listed elsewhere¹⁰.

(ii) Peak asymmetry ratios for individual solutes are calculated according to

$$A_s = \frac{b}{a} \quad (5)$$

where a is the width of the leading half of the peak and b is the width of the trailing half of the peak, both measured at 10% of the peak height. The asymmetry ratio is then used to calculate τ/σ for the peak, where σ represents the standard deviation and τ is the time constant of the generated peak. The conversion of asymmetry ratios to τ/σ ratios may be achieved using equations reported by Anderson and Walters¹⁵ (as in this instance) or Foley and Dorsey¹⁶.

(iii) The R_s value of each adjacent peak pair in a chromatogram is calculated using eqn. 1. This R_s value, together with the value of τ/σ for the first-eluted peak, is then used to calculate the area overlap of the peak pair by substitution into the appropriate polynomial equation from (i) above.

(iv) The R'_s value can then be obtained by substituting the above area overlap into the polynomial equation for a pair of Gaussian peaks. This sequence is represented graphically in Fig. 1. The broken line represents the fourth-order polynomial describing the relationship between area overlap and R_s for a pair of peaks whose leading peak has $\tau/\sigma = 3.0$. The solid line represents the same relationship for a pair of Gaussian peaks ($\tau/\sigma = 0$). The R_s value calculated for the tailed peaks by eqn. 1 is 1.5. Using $R_s = 1.5$ and the $\tau/\sigma = 3.0$ polynomial, the amount of overlap is determined to be approximately 20%. By solving the Gaussian polynomial ($\tau/\sigma = 0$) for this amount of overlap, the R'_s value is calculated to be 0.63. Note that the curves in Fig. 1 represent a peak pair in which both component peaks have the same area. When the two component peaks have different areas, the curves differ only marginally from those in Fig. 1. These curves are therefore applied in all cases.

(v) The R'_s values calculated in this manner are then used in the calculation of composite criteria and ultimately for the selection of the optimum mobile phase.

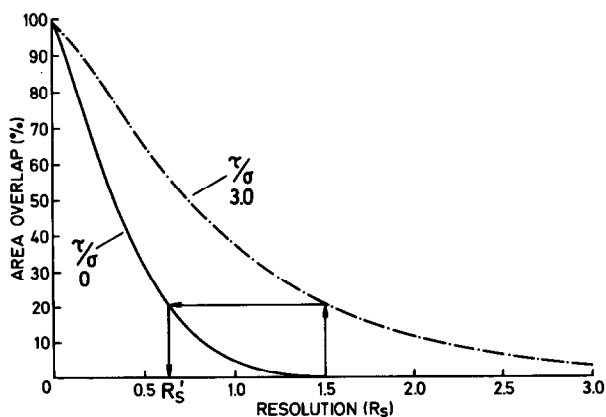


Fig. 1. Calculation of R'_s , the Gaussian equivalent resolution, for a pair of peaks (of equal areas) where $R_s = 1.5$ and the leading peak is tailed by $\tau/\sigma = 3.0$. The resultant R'_s value is calculated to be 0.63. Reproduced with permission from ref. 10.

In order to adapt this method of R_s calculation to optimization procedures, it was necessary to investigate the effect of mobile phase composition on the extent of tailing exhibited by solutes. This investigation⁹ concluded that measurement of asymmetry was necessary only in the three isoelutropic binary mobile phases, as asymmetry for any solute was found to vary linearly for intermediate ternary mobile phases. It was therefore possible to evaluate the extent of asymmetry exhibited by solutes over the entire optimization search area.

Peak tailing compensation (method B)

This approach is based on the premise that peaks of significantly varying peak heights (or areas) eluting next to each other will each be resolved to different extents. The larger peak will be less affected by overlap with the smaller peak and by analogy the smaller peak will be affected to a greater extent by overlap with the larger peak. A new resolution equation (eqn. 6) has been derived¹¹ that takes into consideration not only the retention times of the two peaks (t_i and t_j), but also the extent of asymmetry. This equation assumes that the two component peaks in a peak pair are of equal height.

$$R_s^i[i,j] = \frac{(t_j - t_i)(1 + A_{s_i}^i)(1 + A_{s_j}^i)\sqrt{N_i N_j}}{4A_{s_i}^i t_i (1 + A_{s_j}^i)\sqrt{N_j} + 4t_j (1 + A_{s_i}^i)\sqrt{N_i}} \quad (6)$$

The superscripts in this equation indicate which peak (i or j) was used as a reference for determining the point (13.5% of reference peak height) at which asymmetry measurements were made. The subscripts denote the peak to which each parameter refers. Fig. 2 illustrates the asymmetry parameters and the symbolism used. Eqn. 6 describes the R_s value calculated between peaks i and j when asymmetry measurements are made at 13.5% of the height of peak i . An analogous equation therefore results for the reverse situation, *i.e.*, the calculation of R_s between peaks i and

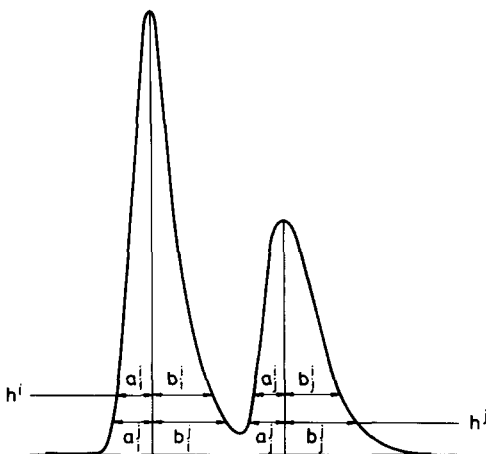


Fig. 2. Illustration of the asymmetry parameters required for the calculation of $R_s[i,j]$ as defined by eqns. 10 and 11. Reproduced with permission from ref. 11.

j with asymmetry measurements are made at 13.5% of the height of peak j . The reference height of 13.5% is used as it coincides with a peak width of 4σ and simplifies some of the mathematical derivations obtained.

In the case of a Gaussian peak, the number of theoretical plates (N) and the retention time can be used to obtain σ as follows:

$$N = \left(\frac{t}{\sigma}\right)^2 \quad (7)$$

At 13.5% of peak height, the width of the peak (w) is equal to 4σ , so eqn. 7 may be rewritten as

$$N = 16\left(\frac{t}{w}\right)^2 \quad (8)$$

and, as $w = a + b$,

$$N = 16\left(\frac{t}{a + b}\right)^2 \quad (9)$$

Incorporation of the factors a and b in eqn. 9 permit the evaluation of the plate count (N) for non-Gaussian peaks. By making measurements of a and b at the appropriate reference peak height, N_i^i and N_j^j in eqn. 6 may be evaluated.

The chief drawback of the new R_s equation (eqn. 6) is that eight measurements (a and b values for both peaks, determined at 13.5% of the height of each peak) are required for its calculation. A suitable approximation to simplify this calculation is to assume that peak asymmetry is independent of the reference peak height at which it is measured. Eqns. 10 and 11 utilize this assumption and also incorporate a correction factor for differences in heights between adjacent peaks. It is noteworthy that these two equations require measurements to be made on individual peaks at a reference height determined by that *same* peak.

$$R_s^i[i,j] = \frac{(t_j - t_i)(1 + A_{s_i})(1 + A_{s_j})\sqrt{N_i N_j}}{4A_{s_i}t_i(1 + A_{s_j})\sqrt{N_j} + 4t_j(1 + A_{s_i})\sqrt{N_i}\sqrt{1 + 0.5 \ln(h_j/h_i)}} \quad (10)$$

$$R_s^j[i,j] = \frac{(t_j - t_i)(1 + A_{s_i})(1 + A_{s_j})\sqrt{N_i N_j}}{4A_{s_j}t_i(1 + A_{s_j})\sqrt{N_j}\sqrt{1 + 0.5 \ln(h_i/h_j)} + 4t_j(1 + A_{s_j})\sqrt{N_i}} \quad (11)$$

where h_i and h_j are the peak heights.

Further simplifications are possible, but eqns. 10 and 11 are suitable for use in optimization procedures as they closely approximate eqn. 6 but require only four measurements for each peak. It is evident that two R_s values will be obtained for a pair of peaks as eqns. 10 and 11 assign an individual value to each peak. Use of the lower R_s value calculated for a pair of peaks has been recommended when all peaks in a solute mixture are of equivalent significance¹¹.

Computer optimization using method B

Some additional steps must be taken before eqns. 10 and 11 can be used in an optimization procedure. The parameters required for these equations must be known over the entire search area of mobile phase compositions. Retention data can be modelled in the same way as that used in method A, namely by assuming a linear relationship between $\ln k'$ and the volume fraction of organic modifier in the mobile phase. In addition, eqns. 10 and 11 require a knowledge of the peak-width parameters (a and b) and the peak heights (h_i and h_j) over the optimization search area.

Asymmetry measurements. In the preliminary work carried out for method A, it was shown that linear combinations of binary mobile phases produced linear combinations of peak asymmetry. Therefore, by measuring asymmetry in the three isoeluotropic binary mobile phases, linearity for this parameter may be assumed between the measured points.

Peak width. The value of N will vary over the optimization search area. In order to calculate the plate count (from eqn. 9), a true representation of the peak width ($a + b$) must be available for tailed peaks over the optimization search area. When peaks are Gaussian, this may be achieved by calculating σ from eqn. 7, as the peak width at 13.5% of the peak height is equal to 4σ . However, this relationship is no longer valid for tailed peaks. An empirical relationship between a , σ and A_s was therefore developed. This was achieved by computer generation of tailed peaks of known σ , followed by computer measurement of a . Curve fitting of these data yielded the following relationship:

$$\frac{a}{2\sigma} = 1 + 0.21(A_s - 1) - 0.031(A_s - 1)^2 \quad (12)$$

Note that when $A_s = 1$ (Gaussian peak), a becomes equal to 2σ again. Thence, from eqn. 5

$$b = A_s a = A_s [1 + 0.21(A_s - 1) - 0.031(A_s - 1)^2] 2\sigma \quad (13)$$

A comparison of the values of a and b obtained using eqns. 12 and 13 with those obtained by computer measurement is presented in Table I. It can be concluded that these equations provide an adequate estimate of the parameters a and b and thereby w , the width of a tailed peak. As asymmetry can be calculated in the search area, the peak width can therefore also be determined. When coupled with the retention time of each peak, this allows the calculation of N_i and N_j (from eqn. 9) over the optimization search area.

Peak height. As the injection profile of a solute is not varied for the duration of the optimization procedure, it should only be necessary to calculate the peak areas once if we assume that the detection sensitivity remains constant over the search area. This measurement of peak height can be made for each solute in the methanol-water binary mobile phase composition used at the start of the optimization procedure to define the search area. As peak height, τ/σ and retention time would now be available, areas of both Gaussian and tailed peaks may be calculated using equations describing the relevant peak model. Peak heights may then be calculated for any τ/σ and retention time as the value of the area is assumed to remain constant.

TABLE I

COMPARISON OF ASYMMETRY PARAMETERS OBTAINED BY MEASUREMENT AND CALCULATION (EQNS. 12 AND 13)

All peaks were generated using $N = 1000$ and $t = 10.0$ min for the parent Gaussian peak, and with the indicated τ/σ ratio.

τ/σ	Measured values		Calculated values	
	<i>a</i>	<i>b</i>	<i>a</i>	<i>b</i>
0	0.28	0.28	0.28	0.28
1	0.31	0.42	0.31	0.42
2	0.35	0.66	0.33	0.64
3	0.36	0.94	0.36	0.93
4	0.38	1.21	0.38	1.21
5	0.39	1.49	0.39	1.50

Using the above approaches, it is now possible to obtain all the parameters required for the calculation of R_s using eqns. 10 and 11 for the entire search area. Method B can therefore be incorporated into an optimization procedure.

EXPERIMENTAL

Instrumentation

The optimization programs used were operated on a Macintosh Plus micro-computer (Apple Computer, 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 Millipore Waters (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 employed was a Waters reversed-phase C_{18} Nova-pak column (150×3.9 mm I.D.). A Shimadzu SPD-6AV UV-VIS spectrophotometric detector operated at 224 nm was employed for the work involving pesticides.

Reagents

Binary and ternary mobile phases used for the optimization procedure were prepared by measuring the required volumes of chromatographic-grade solvents and water with a burette into a suitable container, with the resulting solution being mixed thoroughly, filtered through a $0.45\text{-}\mu\text{m}$ membrane filter and degassed in an ultrasonic bath before use.

Analytical-reagent grade solutes were obtained from the following sources: toluene from May and 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'-diphenylethylamine hydrochloride were synthesized and checked for purity as reported previously¹⁷. The mobile phases used for this group of solutes also contained 5 mM sodium heptanesulphonate (Ajax Chemicals, Sydney, Australia) and 1% acetic acid.

Deltaresmethrin, bioresmethrin, permethrin and phenothrin were obtained in the pure form from the Curator of Standards, Australian Government Analytical Laboratories (Melbourne, Australia).

RESULTS AND DISCUSSION

Optimization software

Table II summarizes the measurements required for optimization procedures which account for peak tailing using method A or B, together with those required when peak tailing is not considered. It should also be pointed out that method A requires the polynomial coefficients describing the relationships between R_s , area overlap and τ/σ .

The data input requirements for method A include retention times and asymmetry measurements (at 10% of the peak height) for each solute in the three isoelutotropic binary mobile phases. These data are used to interpolate retention times and asymmetry values for each solute in each ternary mobile phase in the search area. R'_s can then be calculated and used to determine the desired composite criterion for each mobile phase, from which the optimum is selected. Data entry requirements for method B include retention times, peak height and peak asymmetry (at 13.5% of the peak height). Peak-height measurements are made in the methanol-water binary mobile phase, *i.e.*, step 1 of the GOS. Measurements of asymmetry ratios need to be made for all solutes in all three isoelutotropic binary mobile phase compositions. This information is made available in step 3 of the GOS. Step 4 is then modified to calculate $R_s[i,j]$ values using eqns. 10 and 11. As in this work all peaks in a mixture are of equal significance, the smaller of the two calculated values ($\text{Min } R_s[i,j]$) is then incorporated in the subsequent calculation of the desired composite criterion.

Comparison of methods A and B using computer-generated EMG peaks

Peak pairs were generated in which the relative areas and the degree of tailing of the first-eluted peak were varied. These peak pairs were then used to calculate resolution in the various ways which have been discussed earlier. Table III shows the values obtained for R_s calculated using eqn. 1, R'_s (the "Gaussian equivalent resolution" of method A), $\text{Min } R_s[i,j]$ calculated using eqns. 10 and 11 of method B,

TABLE II
MEASUREMENTS REQUIRED FOR DIFFERENT OPTIMIZATION PROCEDURES

<i>Standard optimization procedure (peak tailing not considered)</i>	<i>Peak tailing compensation used</i>	
	<i>Method A</i>	<i>Method B</i>
Retention time for each solute in each isoelutotropic mobile phase composition (<i>i.e.</i> , 3 measurements per solute)	Retention time for each solute in each isoelutotropic mobile phase composition (<i>i.e.</i> , 3 measurements per solute)	Retention time for each solute in each isoelutotropic mobile phase composition (<i>i.e.</i> , 3 measurements per solute)
	Asymmetry of each solute in each isoelutotropic mobile phase composition (<i>i.e.</i> , 6 measurements per solute)	Asymmetry of each solute in each isoelutotropic mobile phase composition (<i>i.e.</i> , 6 measurements per solute)
		Peak height of each solute in the methanol-water binary mobile phase composition (<i>i.e.</i> , 1 measurement per solute)

TABLE III

COMPARISON OF R_s , R'_s , $\text{MIN } R_s[i,j]$ (FROM EQNS. 10 AND 11 AND $\text{MIN } R_s[i,j] (h_i = h_j)$ (FROM EQNS. 10 AND 11 BUT ASSUMING EQUAL PEAK HEIGHTS) FOR VARIOUS τ/σ AND PEAK-HEIGHT RATIOS

The data shown were calculated for two peaks with retention times of 15 and 18 min on a column having 1000 theoretical plates.

Peak-area ratio	τ/σ	R_s	R'_s	$\text{Min } R_s[i,j]$	$\text{Min } R_s[i,j] (h_i = h_j)$
1:1	0	1.44	1.44	1.41	1.44
	1	1.27	0.95	1.05	1.05
	2	1.19	0.65	0.73	0.75
	3	1.14	0.50	0.55	0.57
	4	1.11	0.41	0.44	0.45
4:1	5	1.09	0.34	0.36	0.38
	0	1.44	1.44	1.25	1.44
	1	1.27	0.95	0.91	1.05
	2	1.19	0.65	0.65	0.75
	3	1.14	0.50	0.50	0.57
4	1.11	0.41	0.40	0.45	
5	1.09	0.34	0.34	0.38	

and $\text{Min } R_s[i,j] (h_i = h_j)$, which is again calculated using eqns. 10 and 11 but without the peak-height correction component (*i.e.*, assuming the peak heights are equal). These calculations were made for Gaussian peaks and peaks having τ/σ in the range 1–5 for two peak-area ratios (1:1 and 4:1).

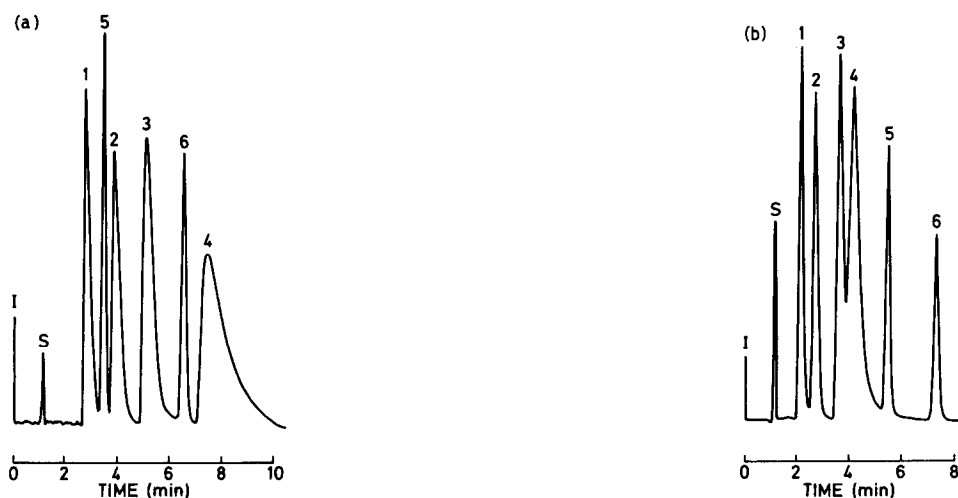


Fig. 3. Chromatograms obtained with the optimum mobile phase composition for aromatic solutes (a) using peak tailing correction and (b) without consideration of peak tailing (*i.e.*, using the GOS). Conditions: mobile phases, (a) methanol–water (60:40), (b) methanol–THF–water (36:17:47); flow-rate, 1.0 ml/min; detection, UV absorption at 254 nm (0.04 a.u.f.s.). Solutes: 1 = N-(*n*-butyl)-2-phenethylamine; 2 = 2,2'-diphenylethylamine; 3 = propranolol; 4 = doxepin; 5 = *p*-iodophenol; 6 = toluene; S = solvent peak. Reproduced with permission from ref. 10.

When the peak-area ratios are kept constant, the only variation in peak height results from the variation in retention times. The later the peak is eluted, the smaller is the peak height. Close examination of results in Table III shows that the $\text{Min } R_s[i,j]$ values obtained by method B are generally larger than R'_s calculated for method A for equal peak heights, but values from the two methods converge at a peak-area ratio of 4:1. Comparison of $\text{Min } R_s[i,j]$ results with those for $\text{Min } R_s[i,j] (h_i = h_j)$ at a peak-area ratio of 4:1 shows that the values of the latter criterion are significantly greater.

Experimental comparison of methods A and B

An experimental comparison of methods A and B was undertaken in order to determine whether the differences in calculated resolution noted above would prove significant in an actual optimization. This comparison was made using two separate solute mixtures, for which the separation was optimized with each of the composite criteria given in eqns. 2–4. The first mixture consisted of aromatic compounds that exhibited a large range of peak asymmetries, whilst the second mixture consisted of pesticides for which variation in peak-area ratios was evident.

Optimization applied to aromatic solutes. Table IV shows the retention, tailing and peak-height data obtained in the optimization of the separation of the aromatic solutes. The criterion used for both methods A and B was the normalized R_s product, r (eqn. 2), which has a value between 0 and 1, with the latter value being assigned to chromatograms with evenly spaced peaks and the former value to chromatograms containing complete overlap of at least one peak pair. Methods A and B located the same optimum mobile phase composition [*i.e.*, methanol–water (60:40)] at the second iterative step of the procedure. The criterion values for this mobile phase were 0.74 and 0.96 for methods A and B, respectively. Fig. 3a shows the chromatogram obtained with this optimum mobile phase composition. It is difficult to ascertain by visual inspection which of the above two criterion values provides a more realistic evaluation of this chromatogram. For comparison, the chromatogram obtained using the GOS

TABLE IV

RETENTION TIME, PEAK SHAPE AND PEAK HEIGHT DATA FOR THE OPTIMIZATION OF THE SEPARATION OF SOME AROMATIC SOLUTES

<i>Mobile phase</i>								
Methanol (%)	60	60	0	0	60	0	0	30
Acetonitrile (%)	0	0	44	0	0	44	0	0
Tetrahydrofuran (%)	0	0	0	42	0	0	42	21
Water (%)	40	40	56	58	40	56	58	49
<i>Compound</i>	<i>Peak height (cm)</i>	<i>Peak shape (τ/σ)</i>			<i>Retention time (min)</i>			
N- <i>n</i> -Butyl-2-phenethylamine	13.0	4.8	4.8	4.8	2.75	2.01	1.66	1.86
2,2'-Diphenylethylamine	10.5	5.1	4.8	4.8	3.86	2.01	1.66	2.30
Propranolol	11.0	5.4	5.1	5.0	5.10	3.06	2.10	3.06
<i>p</i> -Iodophenol	15.0	0	0	0	3.45	3.43	3.36	5.15
Doxepin	6.5	6.2	5.0	6.0	7.38	7.00	2.10	3.35
Toluene	10.5	0	0	0	6.46	8.15	5.13	6.91

TABLE V
RETENTION TIME, PEAK SHAPE AND PEAK HEIGHT DATA FOR THE OPTIMIZATION OF THE SEPARATION OF SOME PESTICIDE SOLUTES

<i>Mobile phase</i>												
	85	85	0	0	85	0	0	38.3	0	42.5	0	76.5
Methanol (%)	0	0	80	0	0	80	0	0	32	40	60	64
Acetonitrile (%)	0	0	0	53	0	0	29.2	31.8	0	0	13.3	10.6
Tetrahydrofuran (%)	15	15	20	47	15	20	32.5	36.2	17.5	26.7	25.4	15.5
Water (%)												
<i>Compound</i>	<i>Peak height (cm)</i>	<i>Peak shape (τ/σ)</i>	<i>Retention time (min)</i>									
Deltaresmethrin	8.5	0	0	0	4.01	4.63	6.04	11.34	5.81	5.52	4.98	4.96
<i>trans</i> -Permethrin	6.0	0.7	0	0	5.65	5.59	6.37	13.03	6.73	6.95	5.94	5.93
<i>cis</i> -Permethrin	3.5	0.7	0	0	6.99	6.56	6.77	13.03	7.70	8.34	6.94	6.93
Bioresmethrin	5.0	0	0	0	5.47	4.99	6.16	11.30	5.95	6.28	5.21	5.20
<i>trans</i> -Phenothrin	6.0	0.7	0	0	6.92	6.57	6.30	13.27	7.06	8.30	6.63	6.69
<i>cis</i> -Phenothrin	1.5	1.0	0	0	6.39	6.16	6.30	12.54	6.78	7.68	6.26	6.29

and the same criterion is shown in Fig. 3b. The optimum mobile phase in this instance was methanol-THF-water (36:17:47) and the criterion value for the optimum was 0.84.

The same optimization was then repeated using method B without peak-height correction. Once again, the same number of iterations was required to locate an identical optimum mobile phase, with a criterion value of 0.95. Table III showed a slight increase in $R_s[i,j]$ values calculated without the peak-height correction. In the case under consideration, almost the same criterion value is obtained (0.96 vs. 0.95) with and without peak-height correction. As this is a normalized criterion equation, small differences in calculated $R_s[i,j]$ values will have an even smaller effect on the resulting value of the criterion. Therefore, these results suggest that the peak-height correction component of eqns. 10 and 11 can be neglected when peak-height ratios for adjacent peaks are moderate (such as the maximum value of 2.3 listed in Table IV).

The same solute mixture was again optimized using the $\text{Max}[R_{s(\text{min})}]$ (eqn. 4) composite criterion and once again no differences were observed between methods A and B, even when the latter was employed without peak-height correction. The criteria values obtained were 1.08, 1.35 and 1.36 for method A, method B and method B without peak-height correction, respectively.

Optimization applied to pesticides. Table V contains the optimization data obtained for the pesticide mixture when the resolution product (eqn. 3) was employed as the composite criterion. Two iterations were required by both methods for the location of an optimum mobile phase composition of acetonitrile-THF-water (60:13.3:26.8), giving normalized R_s product criterion values of 1.96 and 1.52 for methods A and B, respectively. The smaller criterion value for method B results from the decrease in $R_s[i,j]$ values calculated for the smaller peaks in the chromatogram. It should be noted that the peak-height ratio reaches a maximum value of 4:1 for this solute mixture. The chromatogram obtained with the optimum mobile phase composition is presented in Fig. 4a. The chromatogram obtained when separation of

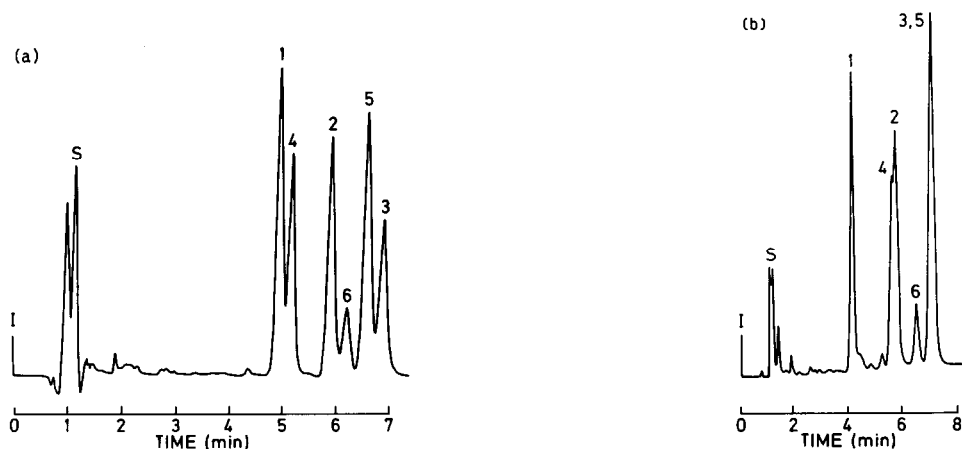


Fig. 4. Chromatogram obtained with the optimum mobile phase composition for pesticide solutes (a) using peak tailing correction and (b) without consideration of peak tailing (*i.e.*, using the GOS). Conditions: mobile phases, (a) acetonitrile-THF-water (60:13.25:26.75), (b) methanol-water (85:15); flow-rate, 1 ml/min; detection, UV absorption at 224 nm (0.04 a.u.f.s.). Solutes: 1 = deltamethrin; 2 = bioresmethrin; 3 = permethrin-*trans*; 4 = phenothrin-*cis*; 5 = phenothrin-*trans*; 6 = permethrin-*cis*; S = solvent peak.

the same mixture was optimized by the GOS is shown in Fig. 4b. The optimum mobile phase selected in this instance was methanol–water (85:15) and the criterion value was 2.76. Even with this criterion value, the actual chromatogram obtained showed virtually no resolution between permethrin-*trans* and phenothrin-*trans*.

Repeating the optimization using method B without peak-height correction gave the same optimum mobile phase composition, but required one further iteration of the procedure. The criterion value calculated with this approach was 2.22. As the peak-height correction component of eqns. 10 and 11 depends directly on the peak-height ratio, it follows that the calculated $\text{Min } R_s[i,j]$ value will decrease with increasing peak-height ratio. Thus, when the elution order of solutes of widely differing peak heights varies over the optimization search area, appropriate changes in the composite criterion are more readily identified when a peak-height correction is made. When this correction is neglected, an erroneous choice of the optimum mobile phase may occur, leading to an increase in the number of iterations required for the optimization procedure. The extra iteration in the above example is attributed to this cause.

Fig. 5 shows plots of the composite criterion over the entire search area for method A, method B and method B without peak-height correction. Although the shapes of the criteria plots are similar, the magnitude of the composite criterion was always lower when peak-height correction was used. Nevertheless, the reduction in the criterion value caused by peak-height correction was non-uniform over the search area (see Fig. 5). This may lead to the selection of a different optimum when peak-height correction is made, despite the fact that this was not observed in the above example. In view of this, peak-height correction should be included in $R_s[i,j]$ calculations where solutes are known to exhibit large variations in peak-height ratios. Neglecting to do

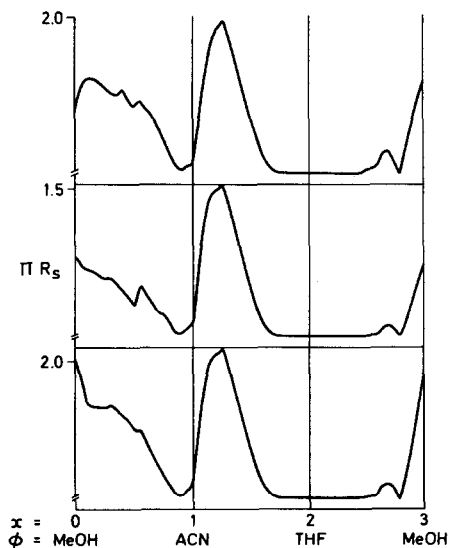


Fig. 5. Comparison of the criteria plots obtained for various optimizations of pesticide solutes when IIR_s was used as the criterion. Plots obtained using (top) method A, (middle) method B and (bottom) method B without peak-height correction. ACN = Acetonitrile; MeOH = methanol.

this for method B could result in an increase in the amount of experimentation required to reach the optimum mobile phase composition.

CONCLUSIONS

All of the optimizations conducted on tailed peaks in this work were also conducted simultaneously using the standard optimization procedure (GOS) without correction for peak shape. In every instance, the optimum mobile phase located by this method gave an inferior separation to that obtained when peak tailing was considered. Therefore, the first conclusions that can be reached are that peak tailing must be considered if the optimization is to be successful, and that both of the methods evaluated in this paper are suitable for this purpose. This supports our earlier findings on this subject^{9,10}.

The two approaches for compensation for peak tailing effects examined in this paper showed almost identical results in terms of the optimum mobile phases selected and the number of iterations required to complete the optimization process. However, slight differences between these methods were noted in the number of measurements needed for each solute (see Table II) and the computer calculation time and memory requirements. Method B has a slightly faster calculation time than method A under the programming conditions we have used and also uses less memory. We therefore conclude that the two methods give comparable performance, but method B has some advantages. It is possible that the utility of method B could be enhanced if a new criterion was developed to incorporate the R_s value obtained for *each* peak, instead of considering only the lower value for each peak pair (as we have done).

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