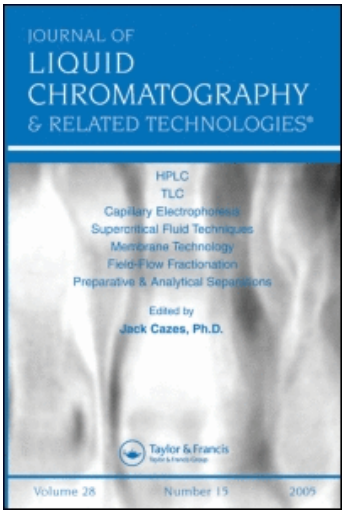

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

The Evaluation and Sequential Use of Resolution Based Criteria in the Optimization of the Separation of a Limited Subset of Components by HPLC

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To cite this Article Barth, Akos , Billiet, Hugo A. H. and De Galan, Leo(1989) 'The Evaluation and Sequential Use of Resolution Based Criteria in the Optimization of the Separation of a Limited Subset of Components by HPLC', Journal of Liquid Chromatography & Related Technologies, 12: 1, 173 — 197
To link to this Article: DOI: 10.1080/01483918908049195
URL: http://dx.doi.org/10.1080/01483918908049195

JOURNAL OF LIQUID CHROMATOGRAPHY, 12(1&2), 173-197 (1989)

THE EVALUATION AND SEQUENTIAL USE OF RESOLUTION BASED CRITERIA IN THE OPTIMIZATION OF THE SEPARATION OF A LIMITED SUBSET OF COMPONENTS BY HPLC

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ABSTRACT

Some resolution based optimization criteria are adapted by using weighting factors to express the quality of separation of a limited subset of components in samples containing more solutes. Criteria are evaluated in detail with respect to the successive objectives of the analyst: satisfactory resolution for the peaks of interest, the peaks make the best use of the available separation space, and the analysis time is at minimum (while the resolution is maintained at the required level). A sequential use of the appropriate optimization criteria is suggested to achieve the separation of the peaks of interest with a satisfactory resolution in the shortest possible analysis time.

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INTRODUCTION

The systematic optimization of separations in HPLC is often directed to achieve complete resolution of all components in the sample mixture. However, in a number of situations the analyst might be interested to quantitate only a limited subset of key components in a sample containing more solutes. Thus, in many practical cases, especially when attempting to analyze a limited number of solutes within complex matrixes, separation of the components of interest from all their nearest neighbours may be achievable while the complete separation of all components may not. The term 'limited optimization' used throughout this paper refers to such separation problems.

The application of experimental solvent optimization procedures to limited optimization problems requires: (i) the recognition of all relevant peaks in subsequent chromatograms; (ii) optimization criteria which refer only to the separation of the peaks of interest; (iii) check on peak purity.

Peak recognition is mandatory not only for limited optimization, but in all 'predictive' optimization procedures, in which the criterion response surface is calculated from the retention surfaces of the individual solutes. Currently used peak recognition techniques were recently reviewed by Strasters et al. [1] in connection with optimization strategies.

Many different optimization criteria have been suggested in the literature and applied in optimization procedures [2,3], but no systematic evaluation has been presented for limited optimization. Schoenmakers [3,4] has shown that the selection of criteria should be suited to the goals of the chromatographer. Different criteria were defined [4] that aim for the lowest analysis time, the best distribution of peaks, the minimum required number of plates etc. These criteria can be used individually or (to combine more goals) sequentially.

Another possible approach is the use of the Multi-Criteria Decision Making (MCDM) procedure suggested by Smilde et al. [5], which results in a graph of resolution vs. analysis time, from which the user can make a balanced choice between either criterion. In this paper the sequential approach is favoured because the MCDM plots do not provide information on the ruggedness of the resolution optimum, since neighbouring pareto-optimal points (offering similar resolution and analysis time) can correspond to completely different eluent compositions. It seemed therefore appropriate to examine what goals and criteria apply for limited optimizations. Two assumptions have been made in this study.

First, the total number of components is known or estimated [6] and has a reasonable upper limit ($n<20$). As was shown by Herman et al. [7], above 15 components the probability of succesful separation of all components rapidly decreases because of the modest peak capacity in currently used RP HPLC systems. Therefore no attempt was made in this study to evaluate such multicomponent criteria, which include the number of observed peaks [2] trying to separate as many as possible.

Second, we assume that all the components in the sample can be tracked in sequential chromatograms, as needed for all predictive optimization methods. Only resolution based optimization criteria are evaluated, which can be calculated from the retention data of the individual solutes.

In this paper, five resolution based optimization criteria have been adapted and tested for limited optimization. The possible goals of the analyst and the behaviour of the corresponding criteria are discussed using computer simulated optimization examples. The possibilities of criteria selection to obtain satisfactory resolution for the peaks of interest are examined in detail along with two other goals: (i) the peaks make the best use of the available separation space; (ii) the analysis time is at minimum.

EXPERIMENTAL

Conditions For Computer Simulations

The various criteria are evaluated by examining one-dimensional computer simulated optimization problems.

The simulation programs were written in PRO/BASIC language and run on a Waters 840 Data Management System (Digital Equipment Corporation, Maynard, MA, USA). The capacity factors of solutes were generated from the 6 coefficient multilinear equations of 32 solutes in RP HPLC, given in ref. [8]. However, for the sake of simplicity in the examples presented the logarithm of the solute capacity factors is assumed to be a linear function of the optimizaton parameter (e.g. binary eluent composition) X.

RESULTS AND DISCUSSION

1. Comparison Of Criteria

In Fig. 1 a fictional one-parameter optimizaton problem is shown. The logarithm of the capacity factor (ln k') of the five solutes is a linear function of the eluent composition X. The analysis time changes drastically with the optimization parameter X, in order to demonstrate the behaviour of all criteria in a general example. Only one solute (number 3) is of interest and it has to be separated from the other four.

The response surfaces of the different optimization criteria are calculated from these retention data, as in the interpretive methods. Each component has to be identified in the sequential simulations (chromatograms) to assign the corresponding weighting factors (see discussion below).

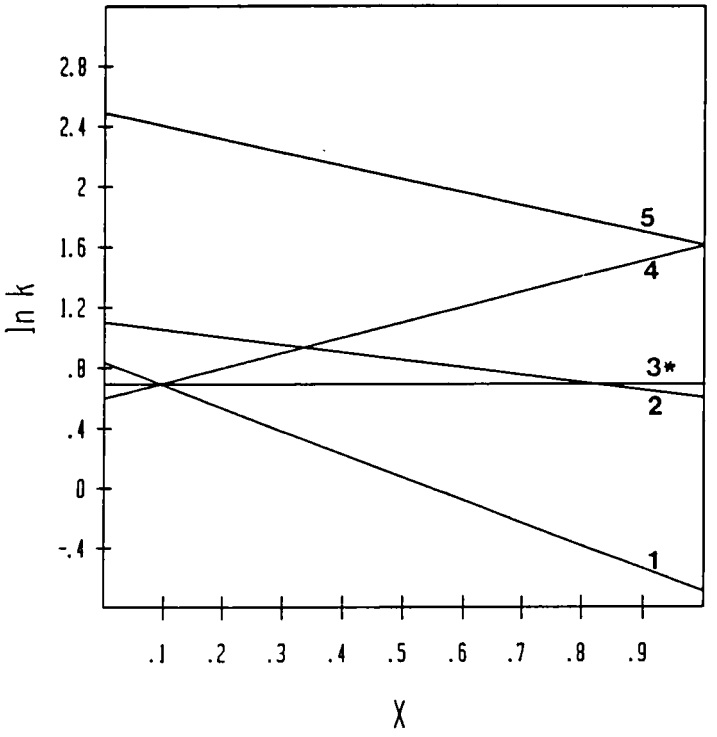

FIGURE 1: *Example I. Limited optimization problem involving five solutes, where only one (solute 3 (*)) is of interest. Solute retention (ln k) is assumed to be a linear function of the parameter (X) to be optimized.*

Simulated chromatograms corresponding to different compositions X are collected in fig. 2. A hold up time of 1.0 minute and column plate count of 3600 was assumed in all calculations.

2. Optimization for the Required Resolution

Generally the primarily goal of chromatographic selectivity optimization is to separate the solutes of interest from all the other (unimportant) components of the sample mixture.

A direct approach is to calculate the resolution (Rs) between the peaks of interest and their nearest neighbours, and set the minimum resolution criterion (Rs_{min}) equal to the lowest value. Limited optimization can be seen as a special case of assigning weighting factors to the different solutes in the optimization criteria. The relevant resolution for the (i+1)th peak pair can be determined from the column plate count (N), capacity factors (k) and weighting factors (w) of solutes i and i+1, respectively:

$$Rs_{i,i+1} = \frac{\sqrt{N}}{2} * \frac{k_{i+1} - k_i}{k_{i+1} + k_i + 2} \qquad (1)$$

AND

$$(w_i \text{ OR } w_{i+1}) = 1, \; w_0 = 0, \; k_0 = 0 \qquad (2)$$

where w_i is the weighting factor of the ith solute with a value of 0 for the unimportant and 1 for the important peaks; (w_{i+1} OR w_i) is the logical OR function of the weighting factors.

The separation from a possible solvent peak is promoted by the introduction of an imaginary peak at t-zero (i.e. $k_o=0$). This peak is assigned to be not of interest ($w_o=0$) and considered only if the first eluting solute peak is of interest.

The preferred value for the weighting factors are 1 and 0 for the important and the unimportant peaks, respectively. Other values for these weighting factors are not recommended, since the separation of a given solute rarely (or not at all) could be defined e.g. twice more important ($w_i=2$) than the separation of an other one.

The response surface of the minimum resolution criterion (Rs_{min}), calculated from the retention data of fig. 1, is shown

in fig. 3. The chromatogram at X=0.33, corresponding to the maximum criterion value is shown in fig. 2/b.
During selectivity optimization the Rs_{min} criterion can be used in two diferent ways (see also ref. [3] for detailed discussion):

(i) The optimization can be carried out to find conditions where the Rs_{min} for the peaks of interest is as high as possible. For limited optimization this approach is in fact similar to the critical band method, a graphical procedure described by Colin et al. [9] earlier.

The optimum results in excellent separation for the important solute (3) as shown in fig. 2/b. However, this method has shortcomings when the retention of the last eluting solute varies significantly with the optimization parameter (X).

Chromatograms at X=0 and X=1 (see Figs. 2/a and 2/e) represent the same Rs_{min} value, but the criterion reveals nothing about the total analysis time or the use of the separation space.

Obviously, in those cases where the overall retentions vary largely, the maximum of the Rs_{min} criterion may result in unnecessary long chromatograms.

(ii) The above problem can be solved by setting a threshold value for the resolution, above which the result is acceptable (e.g. $Rs_{req}=1$). In the parameter range (ΔX) where $Rs_{min} \geq Rs_{req}$, a sequential optimization can be carried out to adjust the analysis time. This approach seems preferable when the capacity factors are expected to change considerably.

A note on the required resolution (Rs_{req}) is appropriate here. The definition of the limit of satisfactory resolution may include the needs of the analyst in terms of what is considered to be a safe resolution for the peaks of interest. When the

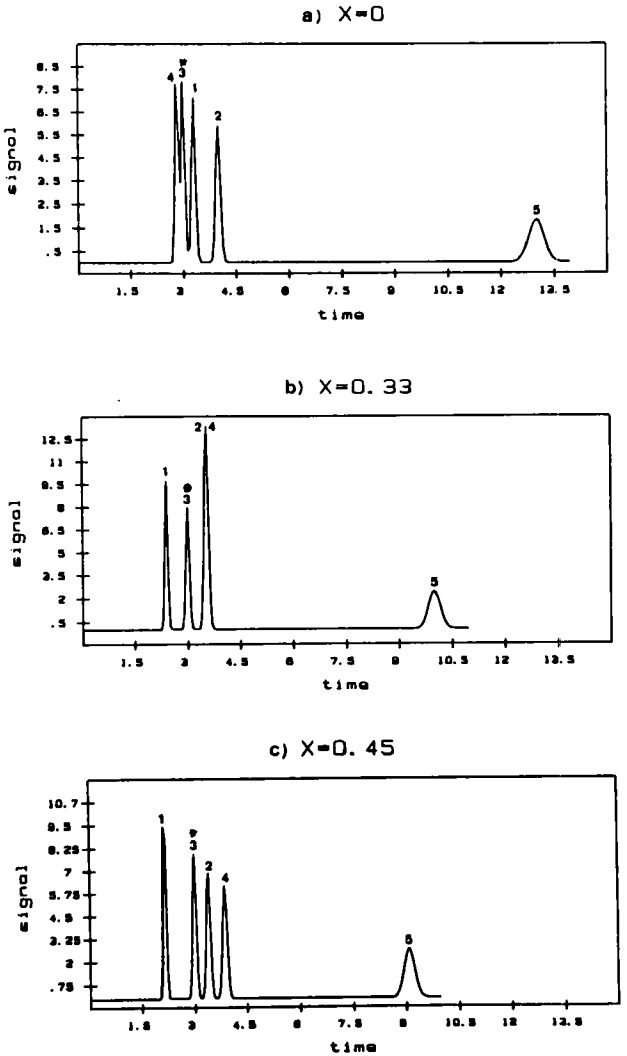

FIGURE 2: *Simulated chromatograms of the five component solute mixture shown in fig. 1 at different values of parameter X. Void time of 1.0 min and plate count of 3600 were assumed.*

(continued)

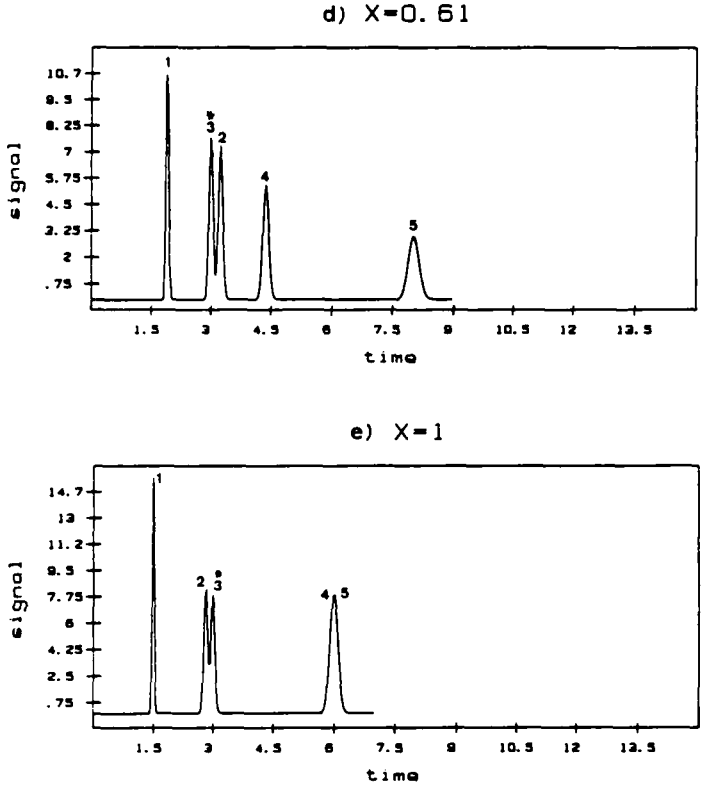

FIGURE 2 (continued)

peaks have different height and/or asymmetry in the chromatogram, it seems reasonable to require a higher minimum resolution limit. Recently, Schoenmakers et al. [10] suggested a method to correct the resolution values for these special cases. The use of such corrected resolution values can provide more realistic measures of the quality of such separations as well, and the level of Rs_{req} does not have to be overestimated.

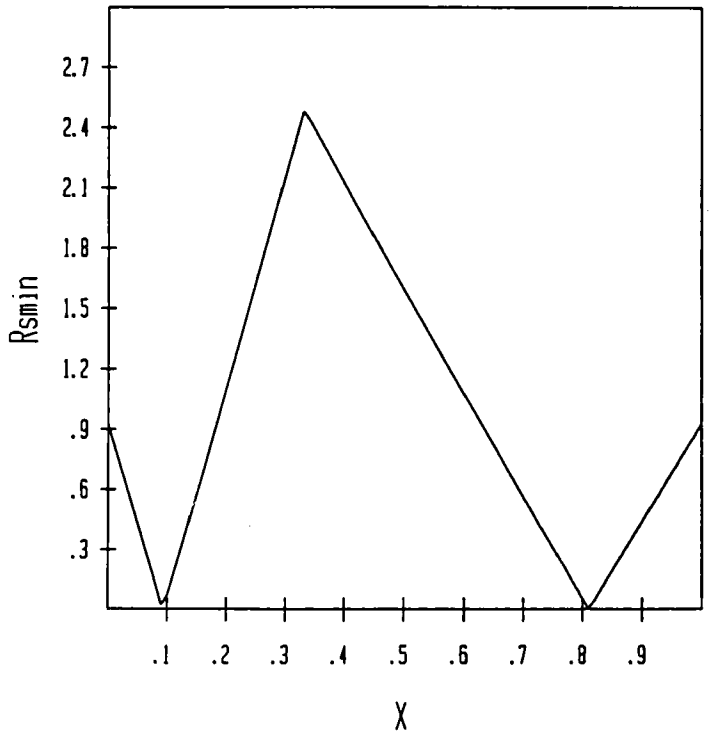

FIGURE 3: *Response surface of the minimum resolution criterion* (Rs_{min}) *as a function of the optimization parameter X, calculated from retention data of fig. 1.*

3. Optimization for the Use of the Separation Space

The maximal use of the separation space can be defined as (equally) high resolution for the peaks of interest and coelution of all the unimportant peaks in the chromatogram. This can be advantageous in practice, since it provides a rugged optimum and allows to quantitate several key components in a complex sample. It may also allow to decrease the analysis time by adapting the length of the column.

In the 'general' case, when all peaks are of interest, a number of product type criteria have been suggested [2,3] to find the ideal distribution of the components throughout the chromatogram.

Two typical product type criteria will be considered below:

The calibrated , normalized resolution product (r^*) suggested by Drouen et al. [11] has been modified by Schoenmakers [3] to suit limited optimization.

$$r^* = \frac{\prod_{i=1}^{n} (Rs_{i,i-1} * Rs_{i,i+1})^{w_i/2}}{\left[\frac{\sum_{i=0}^{n=1} Rs_{i,i+1}}{P}\right]^P} \qquad (3)$$

where n - the number of all components in the sample,

p - the number of the peaks of interest

w_i - the weighting factor of the ith solute in the sample

The $Rs_{i,i-1}$ refers to resolution between the ith peak and the preceding one and $Rs_{i,i+1}$ to that between the ith peak and the following one (both defined after eq. 1).

Since w_i is 0 or 1, therefore

$$p = \sum_{i=0}^{n=1} w_i \qquad (4)$$

As a result of weighting factors the product includes only the relevant resolutions (power of 0 or 0.5), while the sum is taken over all pairs of peaks. The sum is divided by the number of peaks of interest ($p \leq n$) and it provides the highest possible resolution for the relevant peaks, as a normalizaton factor.

This resolution product equals 1 in the ideal situation, when all the relevant peaks are equally well resolved, while the irrelevant ones contribute nothing to the overall length of the chromatogram, but coelute with the imaginary peak at t-zero.

However in this form of the expression the resolution does not take a natural value between the last peak and the

(imaginary) following one (see indexing of eq. 3). The average value represented by the denominator of eq. (3) was suggested for use [3].

A modified version is given bellow, which eliminates this problem:

$$r_w^* = \frac{\prod_{i=0}^{n-1} Rs_{i,i+1}^{(w_i \text{ OR } w_{i+1})}}{\left[\frac{\sum_{i=0}^{n-1} Rs_{i,i+1}}{P}\right]^{swor}} \qquad (5)$$

where $swor = \sum_{i=0}^{n-1} (w_i \text{ OR } w_{i+1})$, $w_o=0$ and $k_o=0$.

The second criterion, the Chromatographic Optimization Function (COF) suggested by Glajch et al. [12] originally includes both resolution and analysis time. However in order to compare with the other criterion, only the resolution part is considered here:

$$COF = \sum_{i=1}^{n} (w_i * \ln (\frac{Rs_i}{Rs_{req}})) \qquad (6)$$

where the index i refers to a peak pair rather than a given solute.

Equation (6) can be rewritten as:

$$COF = \sum_{i=0}^{n-1} ((w_i \text{ OR } w_{i+1}) * \ln (\frac{Rs_{i,i+1}}{Rs_{req,i}})) \qquad (7)$$

Assuming that the same resolution is required for all the components of interest:

$$COF = \ln \left(\frac{\prod_{i=0}^{n-1} Rs_{i,i+1}^{(w_i \text{ OR } w_{i+1})}}{Rs_{req}^{swor}} \right) \qquad (8)$$

When the required resolution is unity, COF is identical with the natural logarithm of the simple non-normalized resolution product.

For comparability with the r_w^* criterion, Rs_{req} was set to 1, and the exponential of COF (i.e. the product of relevant

resolutions) was calculated. The response surfaces for the two criteria are shown in fig. 4.

The highest value of r_w^* occurs at X=1, an optimum corresponding to the chromatogram in fig. 2/e. Clearly, in spite of the shorter analysis time the separation at X=1.0 (Rs_{min}=0.9) can not be favoured against the optimum found for the minimum resolution criterion where Rs_{min}=2.48 (fig. 2/b). The 'optimum' at X=1.0 is clearly preferred by r_w^*, even against its secondary maximum at X=0.45 (see chromatogram in fig. 2/c).
This behaviour is caused by two facts:

(i) r_w^* points to the maximal, but not necessarily equal, use of the separation space by the peak(s) of interest. Thus, a large resolution on one side of peak 3 (between 3 and coeluting 4,5) can easily overbalance a small resolution on its other side (between 3 and 2), assigning the optimum to a much less acceptable separation shown in Fig. 2/e.

(ii) The chromatogram at X=1 is much shorter (i.e. the separation space is much smaller) than any of the others, which decreases the value of the denominator in eq. (5) and results in a high criterion value.

Although less outspoken, a similar behaviour is observed for the other product type criterion (COF). The composition X=1 is considered to be highly acceptable again and another maximum found at X=0.4 defines a chromatogram between the ones shown in Figs. 2/b and 2/c.

Unfortunately, these ambiguities remain at other ratios of p/n, although the predictions improve when more solutes are of interest. The basic problem is to define a comprehensive normalization factor for the resolution product.

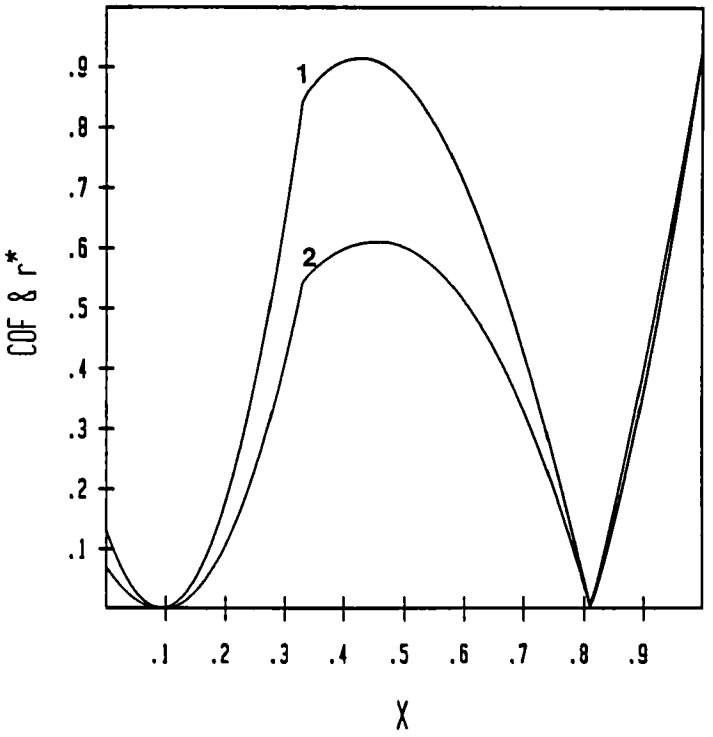

FIGURE 4: *Response surfaces of the product type criteria. Curve 1: Exponential of the Chromatographic Optimization Function (COF), with the required resolution set to 1 (see discussion for details); curve 2: calibrated normalized resolution product* (r^*_w) *modified for limited optimization. Criteria are calculated from retention data of fig. 1 and normalized to give maximum values between 0.5 and 1.*

As a conclusion, the product type criteria can not be preferred over the simple Rs_{min} criterion in limited optimization.

4. Optimization for the Minimum Analysis Time

When the resolution for the peaks of interest is higher than (or equal to) the required minimum resolution, the analysis time may be the next important factor to be optimized.

- The chromatographer may seek for the shortest analysis time on the given column, which is used in the optimization process.
- The length of the column can be shortened, while leaving the flow rate and the particle diameter of the packing unaltered (e.g. using and coupling cartridge type columns).
- In principle, analysis time may be reduced by working at higher flow rates. However, one must consider the higher column pressure and eluent consumption. When the analysis is to be performed only a few times and the resolution is high enough to compensate for the lower column efficiency, the magnitude of flow rate increase can be estimated (setting also an upper limit for the column pressure) from the reduced plate height vs. reduced flow relationship of the column.
- Variation of flow rate, column length and particle diameter simultaneously, can also result in a significant gain of analysis time (see ref. [4] for detailed discussion). However, alteration of the particle diameter of the stationary phase can also influence its chromatographic properties in RP HPLC, resulting in a need to reoptimize for separation selectivity.

Therefore, because of practical reasons, criteria formulated only for the first two cases are considered in the following discussion, using guidelines given by Schoenmakers [3,4].

(i) Optimization on the final column

Provided, that the user is optimizing on the final column, the shortest possible analysis time (at constant flow rate and diameter) at sufficient resolution, can be found by using the threshold separation criterion [3,4]:

$$S_k = 1 / k_{last} \qquad \text{if } Rs_{min} \geq Rs_{req} \qquad (9)$$

$$S_k = 0 \qquad \text{if } Rs_{min} < Rs_{req} \qquad (10)$$

where k_{last} is the capacity factor of the last peak in the chromatogram and Rs_{min} is calculated as given by eq.(1) and (2).

(ii) Optimizing when the column length is a variable

If the length of the column may be altered after the completion of the selectivity optimization process, but preferably leaving the flow and particle diameter unaltered, the required analysis time criterion (T_{nefd}) [3,4] may be used for the optimization.

$$1 / T_{nefd} = Rs_{min}^2 / (1 + k_{last}) \qquad (11)$$

Using this criterion the analysis time can be adjusted by shortening the length of the column. The value Rs'_{min} obtained at the maximum of the $1/T_{nefd}$ criterion is used to calculate the minimum column length (in fact column plate count) needed to establish the required resolution:

$$L_{ne} = \left(\frac{N_{ne}}{N_c}\right) * L_c = \left(\frac{Rs_{req}}{Rs'_{min}}\right)^2 * L_c \qquad (12)$$

where L_c , N_c , Rs'_{min} are the actual, L_{ne} , N_{ne} , Rs_{req} the needed column length, plate count, and resolution, respectively.

The response surfaces for these two criteria (calculated from the retention data of fig. 1) are plotted in fig. 5 using a relative scale. The S_k criterion predicts an optimum at X=0.61, with a satisfactory resolution (Rs_{min}=1) and analysis time of 8 minutes. The simulated chromatogram at this composition is shown in fig. 2/d. The optimum for the required analysis time criterion ($1/T_{nefd}$) is coinciding with that for the minimum resolution criterion (X=0.33). Since for that eluent composition Rs_{min}=2.47, according to eq. (11) the column length can be reduced more than fourfold resulting in an analysis time of only 2 minutes with a minimum resolution of 1 for component 3 !

This example demonstrates that the more flexibility exists in realizing the optimum on another column, the better the result can be. Consequently, the term 'shortest possible analysis time' covers two different optima, depending on the (chromatographic hardware) possibilities of the analyst.

5. The Sequential Use of Resolution Based criteria

In the evaluation of different resolution criteria, we have clarified that the minimum resolution criterion and two different analysis time criteria are applicable in limited optimizations.

A general strategy is proposed here to select and use these three criteria both in 'full' and 'limited' optimizations. We will discuss how to optimize for the goal "satisfactory resolution in the 'shortest possible' analysis time", applying the above three criteria sequentially.

First, the optimization is recommended to be carried out using the minimum resolution criterion (Rs_{min}), between the peaks

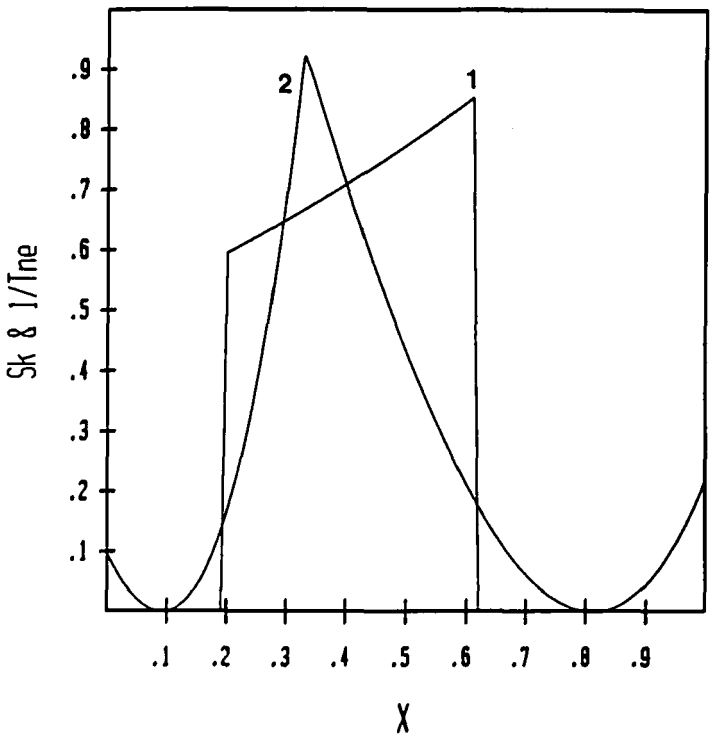

FIGURE 5: *Response surfaces of the analysis time criteria. Curve 1: threshold separation criterion* (S_k); *curve 2: required analysis time criterion* ($1/T_{nefd}$). *Required resolution was assumed to be 1 for* S_k. *Criteria are calculated from retention data of fig. 1 and normalized to give maximum values between 0.5 and 1.*

of interest and their nearest neighbours including the solvent peak at t-zero. Weighting factors of 1 should be assigned to the important, 0 to all the unimportant peaks.

The chromatographer may define what resolution is required (Rs_{req}). This may refer to the use of the separation space, in terms of what is considered to be a safe resolution for the peaks of interest.

However, for a given chromatographic system the highest possible resolution, at optimum conditions (Rs_{min}) can still be lower than the required value.

Threshold type criteria (like S_k for example) can overlook the optimum in such a case (especially at the beginning of the optimization process), since variations in resolution under a certain threshold are ignored. Nevertheless, such variations are very significant during optimization, because:

(i) improvements in resolution in the lower range could help to direct the optimization procedure into the right direction;

(ii) on a different (more efficient) column the separation with the optimum, though low value for Rs_{min} could (easily) be realised.

Therefore it is strongly suggested, that the optimization should be carried out for Rs_{min} with a continuous check whether the observed (predicted) resolution exceeds Rs_{req}, but not to include this required resolution as a threshold value at this stage of the optimization procedure. Once the optimum for Rs_{min} has been found with confidence, it can be decided if the optimization could be continued to achieve the secondary goal, i.e. the minimum analysis time, or further improvements are needed to reach the Rs_{req}.

If $Rs_{min} > Rs_{req}$ and the optimization is carried out on the final column, the minimal analysis time on that column can be found by using the threshold separation criterion (S_k). If the column length is a variable (columns or cartridges of different lengths, or bulk packing material of the same type and particle diameter are at disposal) AND the analysis time is a major factor, the optimization can be continued for the required analysis time criterion ($1/T_{nefd}$), while leaving the flow rate

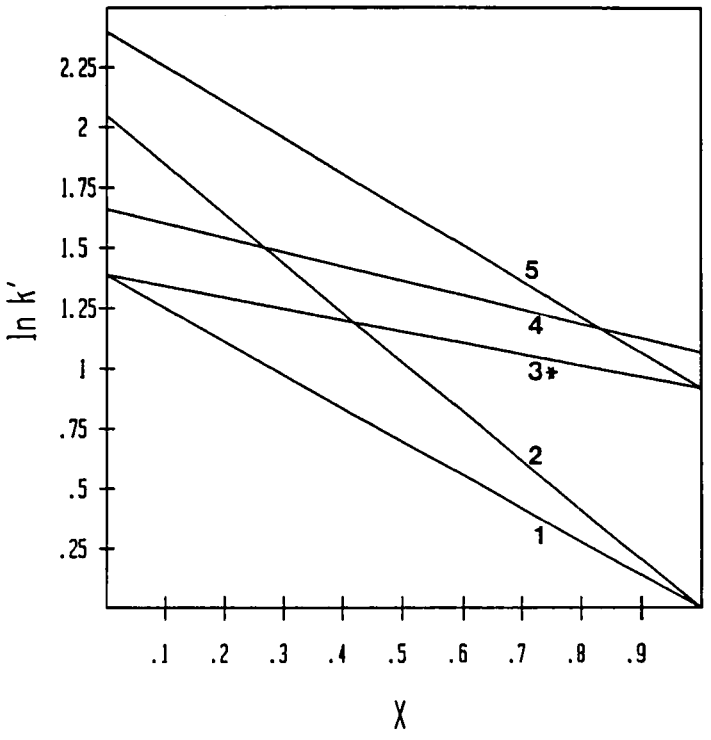

FIGURE 6: *Example II. Limited optimization problem involving five solutes, only solute 3 (*) is of interest. Solute retention (ln k) is assumed to be a linear function of the parameter (X) to be optimized.*

and the packing particle diameter unchanged. Conversely, this latter criterion can also be used to calculate the column length or plate count increase, needed when $Rs_{min} < Rs_{req}$.

It must be pointed out, that the optimization process might be headed into different directions, depending on the selection of the secondary criterion, to optimize for the analysis time. An example is given below, when the use of these criteria results in three different optimal compositions.

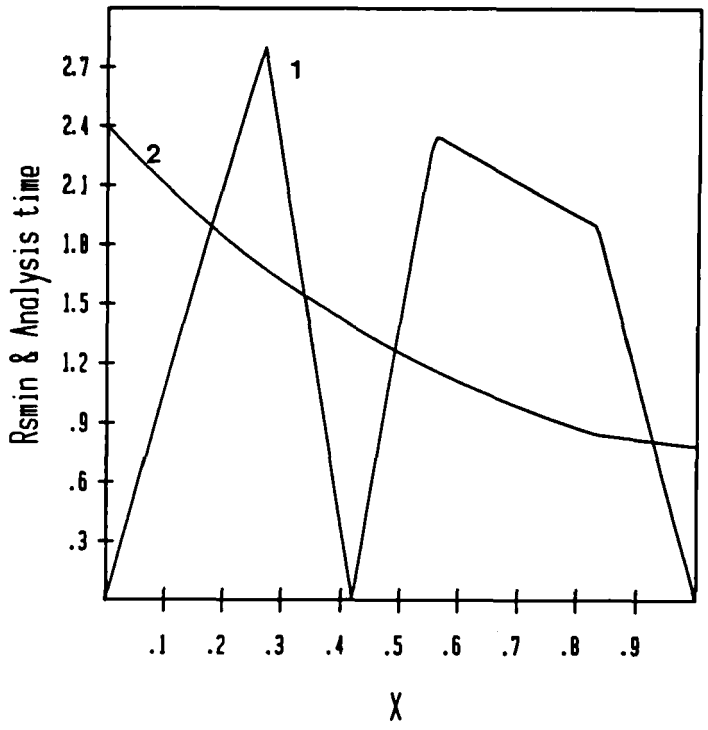

FIGURE 7: *Response surface of the minimum resolution criterion* (Rs_{min}) *calculated from retention data of fig. 6 (curve 1) and the (normalized) retention time of the last eluting component (curve 2).*

The behaviour of the Rs_{min}, S_k and $1/T_{nefd}$ criteria is demonstrated again on a simple separation problem. The ln k' vs. X composition plots of the five solutes and the calculated response surfaces for the three criteria are shown in Figs. 6-8. Again, out of five solutes only one solute (number 3) is of interest.

(i) The optimum for the Rs_{min} (see fig. 7, curve 1) is 2.8 at X=0.27. The retention time plot of the last eluting peak (see curve 2 in fig. 7) indicates an analysis time of 8.4 minutes.

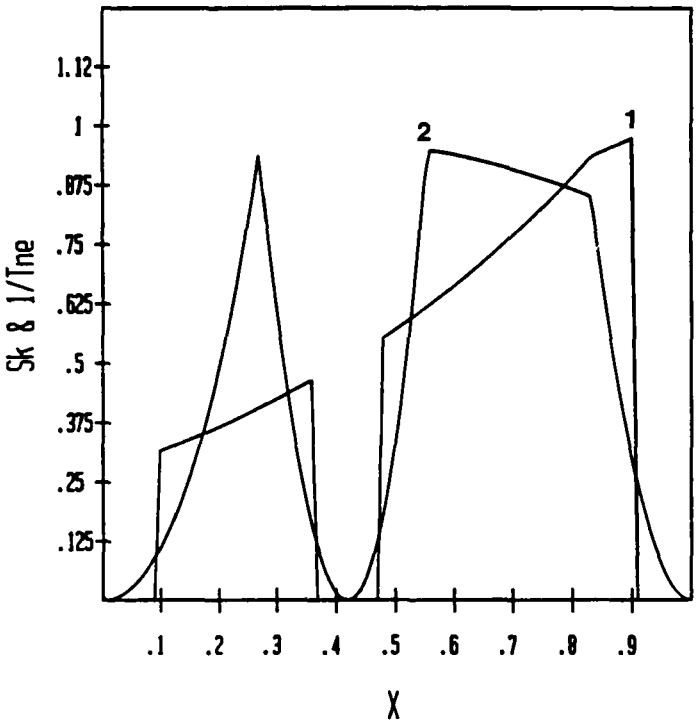

FIGURE 8: *Response surfaces of two optimization criteria calculated from retention data of fig. 6. Curve 1: threshold separation criterion* (S_k); *curve 2: required analysis time criterion* ($1/T_{nefd}$). *Required resolution was assumed to be 1 for* S_k. *Criteria are normalized to give maximum values between 0.5 and 1.*

(ii) The optimum for S_k (see fig. 8, curve 1) is at X=0.9, providing the shortest possible analysis time (4.0 min) on the given column, with a satisfactory (required) resolution of Rs_{req} =1.0.

(iii) X=0.56 is predicted by criterion $1/T_{nefd}$ (see fig.8, curve 2) as the optimal composition. Although, at the given column

it provides somewhat longer analysis time (5.8 min) than the S_k criterion, the higher resolution (Rs_{min}=2.34) at this optimum allows the shortening of the length by a factor of 5.5, i.e. this separation can be realized with satisfactory resolution of 1.0 in about 1.1 minutes, if the column can be shortened to the appropriate length (at constant flow & particle diameter).

The gain in analysis time is really dramatic in this case, and the optimum approaches the originally described goal (having satisfactory resolution in minimum analysis time) to the highest extent. Naturally, there are practical limits of the column length variation in HPLC, and the realisation may strongly depend on the hardware possibilities of the chromatographer. However, the overall column length can easily be adapted by using cartridge type columns, and if the analysis will be performed on a routine base, it may be worthwhile to pack a shorter column for the given separation.

It may appear that the sequential way of using criteria needs more measurements. However, one might be satisfied already with the optimum found for the first (Rs_{min}) criterion. A better result (shorter analysis time) may need more effort. On the other hand, all the criteria can be calculated from the beginning of the optimization procedure. If optimum is found not only for Rs_{min}, but for (one of) the other two criteria (i.e. the predicted optimal composition is within the confidence interval of an earlier measured one during the search), the option to realise any of these optima can become available without any further measurements.

CONCLUSIONS

Five resolution based criteria were carefully selected and tested for limited optimization using computer simulated optimization

examples. The criteria were adapted to qualify the separation of several key components in a sample containing more solutes, by assigning weighting factors of 1 (important) or 0 (unimportant) for each component of the sample.

It was concluded that limited optimization of several key components in a mixture should preferentially be carried out in a sequential way, i.e. first satisfactory resolution, second acceptable analysis time should be found.

The definition of limit of the satisfactory resolution may include the requirements of the analyst on the use of the separation space, since product type criteria fail to provide satisfactory results, when not all components are of interest.

It has been demonstrated that the optimization process might be headed into different directions, depending on the selection of the secondary criterion, to optimize for the analysis time. A general strategy was suggested to use three criteria in a sequential way, both for 'full' or 'limited' optimizations, adapting the analysis time criteria to the goals and possibilities of the analyst.

ACKNOWLEDGEMENTS

The financial support of Millipore-Waters Chromatography Division, Milford, Mass., USA, throughout the course of this work is gratefully aknowledged. The authors also would like to thank the useful discussions on optimization criteria with P.J. Schoenmakers.

REFERENCES

[1] Strasters J.K., Billiet H.A.H., de Galan L., Vandeginste B.G.M., Kateman G.: Automated peak recognition from photodiode array spectra in liquid chromatography, J. Liq. Chrom., in press.

[2] Berridge J.C.: Techniques for the Automated Optimization of HPLC Separations, Wiley-Interscience, Chichester, 1986, Chapter 2.

[3] Schoenmakers, P.J.: The Optimization of Chromatographic Selectivity, A Guide for Method Development, Elsevier, Amsterdam, 1986, Chapter 4.

[4] Schoenmakers P.J.: Criteria for comparing the quality of chromatograms with great variations in capacity factors, J. Liq. Chrom. 10, 1865, 1987.

[5] Smilde A.K., Knevelman A., Coenegracht P.M.J.: Introduction of multi-criteria decision making in optimization procedures for high performance liquid chromatographic separations, J. Chromatogr. 369, 1, 1986.

[6] De Galan L., Herman D.P., Billiet H.A.H.: The determination of starting conditions for modifier optimization in reversed-phase HPLC, Chromatographia , 24 108, 1987.

[7] Herman D.P., Billiet H.A.H., de Galan L.: Statistical basis to select appropriate starting eluent compositions for solvent optimization in isocratic reversed-phase liquid chromatography, Anal. Chem., 58, 2999, 1986.

[8] Schoenmakers P.J., Billiet H.A.H., de Galan L.: Systematic study of ternary solvent behaviour in reversed-phase liquid chromatography, J. Chromatogr. 218, 261, 1981.

[9] Colin H., Kristulovic A., Guiochon G., Bounine J.P.: The importance of dead volume in the optimization of separations in reversed-phase liquid chromatography using ternary eluents, Chromatographia, 17, 209, 1983.

[10] Schoenmakers P.J., Strasters J.K., Bartha A.: Correction of the resolution function for non-ideal peaks, submitted to J. Chromatogr.

[11] Drouen A.C.J.H., Billiet H.A.H., Schoenmakers P.J., de Galan L.: An improved optimization procedure for the selection of mixed mobile phases in reversed phase liquid chromatography, Chromatographia, 16, 48, 1982.

[12] Glajch J.L., Kirkland J.J., Squire K.M., Minor K.M.: Optimization of solvent strength and selectivity for reversed-phase liquid chromatography using an interactive mixture-design statistical technique, J. Chromatogr. 199, 57, 1980.