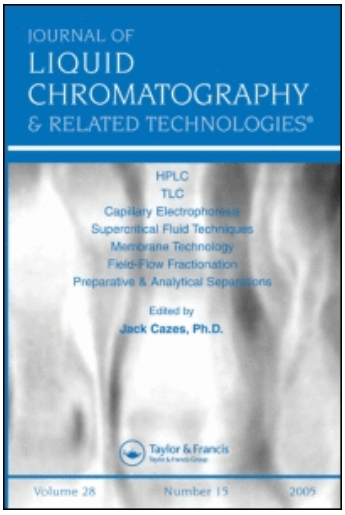

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Criteria for Comparing the Quality of Chromatograms with Great Variations in Capacity Factors

Peter J. Schoenmakers[a]
[a] Philips Research Laboratories, Eindhoven, The Netherlands

To cite this Article Schoenmakers, Peter J.(1987) 'Criteria for Comparing the Quality of Chromatograms with Great Variations in Capacity Factors', Journal of Liquid Chromatography & Related Technologies, 10: 8, 1865 — 1886
To link to this Article: DOI: 10.1080/01483918708066803
URL: http://dx.doi.org/10.1080/01483918708066803

JOURNAL OF LIQUID CHROMATOGRAPHY, 10(8&9), 1865-1886 (1987)

CRITERIA FOR COMPARING THE QUALITY OF CHROMATOGRAMS WITH GREAT VARIATIONS IN CAPACITY FACTORS

Peter J. Schoenmakers
Philips Research Laboratories
P. O. Box 80000
5600 JA Eindhoven
The Netherlands

ABSTRACT

Optimization procedures in chromatography require clear and unambiguous goals. Optimization criteria express such goals in mathematical terms. If the capacity factors vary as a function of the parameters to be optimized, criteria should be selected that allow the simultaneous optimization of the retention and the selectivity.

It is demonstrated that the result of an optimization process depends on the optimization criterion selected. For a simple (fictive) example five different criteria are seen to result in five different locations for the optimum. This example will be used to compare the different criteria and to formulate general recommendations for two different situations.

An optimization problem in (capillary) supercritical- fluid chromatography is discussed as a practical example.

INTRODUCTION

In the past four decades chromatographic techniques have been developed to solve an enormous number of separation problems. Gas chromatography and liquid chromatography are now approaching maturity. Many of the factors that

affect the chromatographic separation have been identified and investigated and are now understood. Instrumentation and columns allow an increasing degree of reproducibility. This should lighten the task of those involved in developing new separation methods or improving existing ones. Indeed, an increasing number of systematic procedures and software tools are becoming available to assist the chromatographer in reaching particular, well-defined separation goals.

Developments in this direction provide obvious benefits, but also create novel demands. Chromatographers are forced to define in clear and unambiguous terms the exact goal of their efforts. This implies statements on which components of a mixture need to be separated and to what extent. The process of optimization may then be defined as the realization of these goals in the shortest possible time, on the shortest possible column, with the highest possible sensitivity, etc. It is usually possible to obtain optimum conditions towards only one of these parameters. Moreover, each of them may require further refinement. For example, there may be a minimum analysis time on a given column, a different minimum time on the given column at a given flow rate, a minimum analysis time for a column with a given diameter (*i.e.* column diameter for open columns; particle diameter for packed columns) and flow rate but with a variable length, and yet another minimum analysis time if the column or particle diameter, the column length and the flow rate are all allowed to vary within the constraints of the maximum allowable pressure drop over the column.

An extensive discussion of optimization criteria to minimize the required analysis time in any of the above situations can be found in Ref.1 (chapter 4). However, there are several problems that have not yet been fully investigated. Some of the questions that remain to be answered are related to optimization problems in which

- the capacity factors are a strong function of the parameters to be optimized;
- programmed analysis is applied (*e.g.* solvent programming, temperature programming);
- only a few peaks need to be separated in a complex sample (this can be seen as a special case of the problem of assigning weighting factors to different sample components in optimization criteria);
- the shape of the peaks is highly non-ideal (solvent peaks form a specific example).

The first of these areas is the subject of this paper. In many recent optimization studies the parameter space (*i.e.* the collection of all possible combinations of experimental variables) was selected in such a way that the overall capacity factors would roughly remain constant. In liquid chromatography the Sentinel method of Glajch et al.(2) and the iterative method of Schoenmakers and Drouen (3,4) are examples of this approach. The former procedure has been modified to allow for mobile phases to be considered that give rise to widely varying capacity factors (so-called non-isoeluotropic mobile phase mixtures) (5). However, no special attention has been paid to the optimization criteria required. Atamna et al. (6,7) have recently discussed optimization procedures in which the analysis time was explicitly kept constant ("isochronal" optimization). This is achieved by varying several parameters at the same time. If one of these is the flow rate, then the capacity factors will vary and criteria need to be selected correspondingly.

A number of criteria have been suggested in Ref.1. The potentially useful ones are summarized in Table 1. These criteria will be compared and discussed on the basis of a simple, fictive example. The recommendations obtained from this comparison will then be used to demonstrate the possibility to optimize retention,

selectivity and efficiency for a real example taken from the field of supercritical-fluid chromatography (SFC).

COMPARISON OF CRITERIA

In order to discuss the various criteria defined in Table 1 we will use a fictive one-dimensional optimization problem. This is illustrated in Fig.1a. In this figure the logarithm of the capacity factor is plotted for three solutes as a function of the one parameter to be optimized (*e.g.* mobile phase composition or reciprocal temperature). The capacity factor of the last eluting component (k_{to}) is seen to vary greatly with varying x.

The data in Fig.1a and the definitions in Table 1 may be used to calculate response surfaces, which describe the variation of the optimization criterion (the response) as a function of the parameter to be optimized. Optimization procedures in which the response surface is calculated indirectly from the retention surfaces of the individual solutes can be called "interpretive methods". These methods are based on the assumption that the retention surfaces are very smooth in comparison with the response surfaces (compare Fig.1a with Figs.1b, c and d).

Minimum separation

The response surface for the minimum separation criterion (S_{min}) is shown in Fig.1b. S_{min} is the lowest value of the separation factor (S) observed for any pair of successive peaks in the chromatogram. The pair $i,i-1$ for which S is lowest ($S_{i,i-1} = S_{min}$) may be called the critical pair. S is proportional to the resolution R_s if the number of plates N is assumed to be constant, *i.e.* independent of the parameters to be optimized. Variations in N can be taken into account, as shown

TABLE 1

Summary of optimization criteria considered in this study. Optimum conditions correspond to the maximum value for each criterion. See text for explanation.

1. Minimum separation (S_{min})

$$S_{i,i-1} = \frac{k_i - k_{i-1}}{2 + k_i + k_{i-1}} \tag{1}$$

2. Threshold separation (and minimum capacity factor for the last peak; k_ω)

$$S_k = 1/k_\omega \qquad \text{for } S_{min} \geq \varepsilon \tag{2}$$
$$S_k = 0 \qquad \text{for } S_{min} < \varepsilon \tag{2a}$$

3. Calibrated normalized resolution product

$$r^* = \prod_{i=1}^{n} (S_{i,i-1}/\tilde{S}) \tag{3}$$

where $\tilde{S}$ is the average separation for all pairs of peaks:

$$\tilde{S} = \frac{1}{n} \sum_{i=1}^{n} S_{i,i-1} \tag{4}$$

4a. Required analysis time for conditions of constant flow and constant diameter (of open column or of particles in packed column).

$$\frac{1}{[t_{ne}]_{f,d}} = \frac{S_{min}^2}{1 + k_\omega} \tag{5}$$

4b. Time-corrected calibrated normalized resolution product for conditions of constant flow and constant diameter.

$$[r_{nt}^*]_{f,d} = \frac{\sqrt[n]{r^*}}{[t_{ne}]_{f,d}} \tag{6}$$

5a. Required analysis time for conditions of constant pressure drop over the column.

$$\frac{1}{[t_{ne}]_p} = \frac{S_{min}^4}{1 + k_\omega} \tag{7}$$

5b. Time-corrected calibrated normalized resolution product for conditions of constant pressure drop over the column.

$$[r_{nt}^*]_p = \frac{\sqrt[n]{r^*}}{[t_{ne}]_p} \tag{8}$$

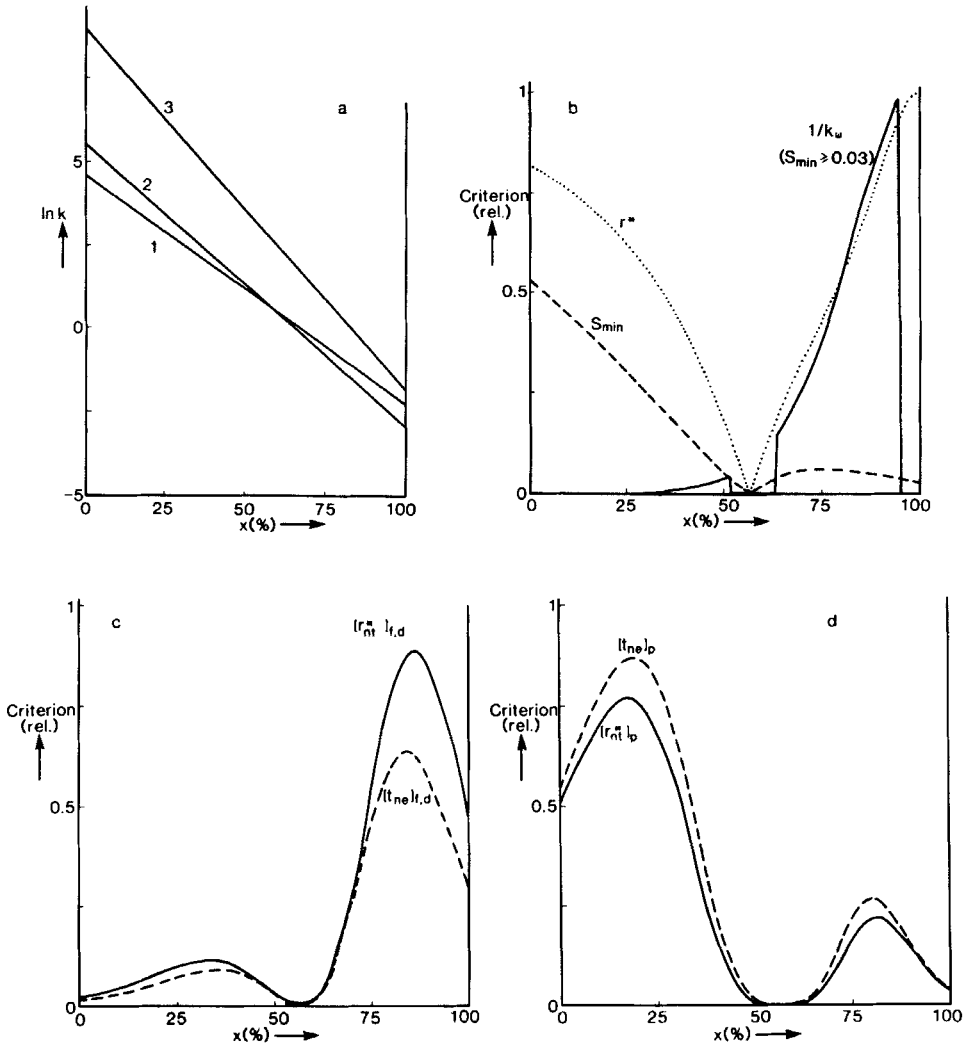

Figure 1

Schematic example of an optimization problem involving three solutes. Fig.1a: Retention surfaces, describing retention ($\ln k$) as a function of the parameter to be optimized (x). Figs.1b to 1d: Response surfaces, describing criteria as a function of x. Criteria are normalized to give maximum values between 0.5 and 1. Fig.1b: S_{min} (----), S_k for $\varepsilon = 0.03$ (—) and r^* (....). Fig.1c: $[r^*_{nt}]_{f,d}$ (—) and $[t_{ne}]_{f,d}$ (----). Fig.1d: $[r^*_{nt}]_p$ (—) and $[t_{ne}]_p$ (----).

by Svoboda (8). The number of plates needed (N_{ne}) can be related to the required resolution by

$$N_{ne} = (\frac{2R_s}{S_{min}})^2 \quad (9)$$

In aiming for the highest possible value of S_{min} , the chromatographer tries to realize the separation with the lowest possible number of theoretical plates. If $S_{min} = 0.03$, 10000 plates are required to achieve $R_s = 1.5$ for the least separated (critical) pair of peaks (see Eqn.9). If $S_{min} = 0.1$ only 900 plates would be required.

From Fig.1b it can be seen that in the present example the maximum value for S_{min} is observed for $x = 0$ (left axis). At this point the separation between the first two peaks is critical ($S_{min} = 0.424$). Under these conditions no more than 50 plates would be required to achieve baseline separation for symmetrical peaks. The values for the capacity factors (k) and the separation factors (S) are listed in Table 2a. The values for S_{min} and the required number of plates (N_{ne}) are given in Table 2b.

Based on the required number of plates we may proceed to calculate chromatographic conditions that would enable us (in principle) to realize the separation. The column length (L) may be calculated from

$$L = N_{ne}H = N_{ne}d_p h \quad (10)$$

where H is the plate height, d_p the particle size and h ($= H/d_p$) the reduced plate height. $h = 4$ is a reasonable assumption for practical HPLC conditions at which the reduced velocity (v) is equal to 20. v is defined as

$$v = \frac{ud_p}{\mathbf{D}_m} \quad (11)$$

TABLE 2

Optimum separation conditions for the example shown in Fig.1 according to a number of different criteria. Separation criteria are normalized by assuming a peak to be present in the chromatogram at $t = t_0$ ("anchor peak"). Chromatographic conditions are calculated with $h = 4$, $v = 20$, $\mathbf{D}_m = 10^{-9}$ m²/s and $\eta = 10^{-3}$ Pa.s , except for the $[r^*_{nt}]_p$ criterion, for which $h = 2$ and $v = 5$. Particle sizes were taken as small as possible, without exceeding a pressure limit of 200 bar. For all but the $[r^*_{nt}]_p$ criterion the available particle sizes were assumed to be 3, 5 and 10 μm.

a. Retention and separation data

Optimization criterion	x_{opt}	Capacity factor			Separation (x1000)		
		k_1	k_2	k_3	$S_{1,0}$	$S_{2,1}$	$S_{3,2}$
S_{min}	0	100	250	7480	980	424	935
S_k	0.945	0.08	0.15	0.27	38	30	51
r^*	0.995	0.05	0.10	0.16	25	24	24
$[r^*_{nt}]_{f,d}$	0.86	0.16	0.26	0.68	76	41	141
$[r^*_{nt}]_p$	0.175	30.0	56.2	1126	937	297	903

b. Calculated chromatographic conditions

Optimization criterion	S_{min}	N_{ne}	d_p	L	t_0	t_ω	Δp	F
			μm	mm	s	s	bar	%
S_{min}	0.424	50	3	0.6	0.09	673	4.4	1.4
S_k	0.03	10000	10	400	200	254	80	85
r^*	0.024	15756	10	630	315	365	126	91
$[r^*_{nt}]_{f,d}$	0.041	5302	5	106	26.5	44.5	170	69
$[r^*_{nt}]_p$	0.297	102	0.23	0.05	0.002	2.5	193	2.8

where u is the linear velocity and $\mathbf{D}_m$ the diffusion coefficient of the solute in the mobile phase. A combination of Eqns.10 and 11 yields an equation for the unretained time t_0:

$$t_0 = \frac{L}{u} = \frac{N_{ne} d_p^2 h}{v \mathbf{D}_m} \tag{12}$$

The analysis time (retention time for the last peak) can be found from

$$t_\omega = t_0(1 + k_\omega) \tag{13}$$

and finally the pressure drop over the column can be found from the Darcy equation, which for a packed column can be written as

$$\Delta p = \frac{1000 N_{ne} \eta h v \mathbf{D}_m}{d_p^2} \tag{14}$$

where Δp is the pressure drop in Pa, η the viscosity of the mobile phase (Pa.s), $\mathbf{D}_m$ is in m^2/s and d_p in m.

The calculated chromatographic conditions are listed in Table 2b. It is seen that for the conditions at which S_{min} reaches its maximum value ($x = 0$) an unpractically short column results. However, the required analysis time is still very large because of the high value of k_ω . Another serious problem can be identified if we define a sensitivity factor F as follows:

$$F = \frac{1 + k_\alpha}{1 + k_\omega} \tag{15}$$

If we assume equal areas for the first and the last peak, then F describes the ratio of the peak heights. At $x = 0$ we find an F value of 0.014, so that for equal peak areas the last peak is 70 times lower than the first one.

Threshold separation

The threshold separation criterion as defined in Table 1 can be interpreted as a function which aims at sufficient resolution in a minimum time. For a given column with N_c theoretical plates, the threshold ε can be determined by rearranging Eqn.9. If the required resolution is $R_{s,\,ne}$, then

$$\varepsilon = \frac{2R_{s,\,ne}}{\sqrt{N_c}} \tag{9a}$$

The highest value for S_k corresponds to the shortest possible analysis time if the column and operating conditions (flow rate) are left unaltered. Although analysis times may in principle be reduced by working at high flow rates, this is nor recommendable from a theoretical point of view, nor attractive in practice. Therefore, the threshold separation criterion can be seen as indicating the optimum conditions on a given column.

In Fig.1b S_k is plotted for $\varepsilon = 0.03$. According to Eqn.9a this implies that 10000 plates are required to realize $R_s = 1.5$. For the present example the optimum is found at $x = 0.945$ and small k values are obtained. Table 2b shows that on a given column with 10000 plates ($L = 40$ cm, $d_p = 10$ μm) a chromatogram with sufficient resolution can be obtained in just over 4 min. Because of the small k values, the value for F is high. A problem with such small k values will arise if a

signal (*e.g.* a solvent peak) is present at $t = t_0$. This is one of the remaining problems we have identified in the introduction.

Calibrated normalized resolution product

The calibrated normalized resolution product aims at achieving an ideal distribution of the peaks throughout the chromatogram. r^* equals 1 if the resolution between all pairs of peaks is the same, and equal to the resolution between the first peak and a hypothetical peak at $t = t_0$.

The distribution of peaks is virtually ideal at $x = 0.995$ for the present example ($r^* = 0.999$). However, the values for S under these conditions are low and hence a large number of theoretical plates is required (see Table 2b). Consequently, both the required column length and the analysis time are longer than was the case with the threshold separation criterion (S_k). This is in agreement with earlier observations that r^* should not be used if the overall capacity factors are expected to vary with changes in the experimental conditions (see Ref.1, p.155).

Time-corrected resolution product; conditions of constant flow and diameter

If the length of the column may be altered after the completion of the selectivity optimization process, but if the flow and diameter (of open columns or of particles in a packed column) will not be changed, then Eqns.5 and 6 may be used to find optimum separation conditions. As can be seen in Fig.1c, there is little difference between the response surfaces obtained for $[t_{ne}]_{f,d}$ and $[r^*_{nt}]_{f,d}$. The former criterion strictly minimizes the required analysis time. The second criterion pays some attention to an equal spreading of peaks over the chromatogram. The

contribution of r^* in Eqn.6 is, however, of secondary importance. Only when the required analysis time varies little between two sets of experimental conditions may the inclusion of an r^* factor turn the balance towards the chromatogram with a better spacing of peaks (see *e.g.* Ref.1, pp.156-157).

Using the optimum conditions for $[r^*_{nt}]_{f,d}$ ($x = 0.86$), a column of just over 10 cm length packed with 5 μm particles can be selected. This column leads to very acceptable results, with an analysis time of 44.5 s and a sensitivity for the last peak that is 69% of that for the first peak. This 5 μm column is within the constraints of a maximum allowable pressure drop of 200 bar. Alternatively, a column of 21 cm with 10 μm particles may be used, for which $t_\omega = 178$ s.

Time-corrected resolution product; conditions of constant pressure drop

The time-corrected resolution product under conditions of constant pressure drop may be used to establish optimum separation conditions using a packed column with particles of optimum diameter or an open column with an optimum inner diameter. For a packed column we can find from Eqn.14 an equation that expresses the optimum particle size as a function of the maximum allowable pressure drop:

$$d_p = \sqrt{\frac{1000 N_{ne} \eta h \nu \mathbf{D}_m}{\Delta p}} \tag{16}$$

At the conditions where $[r^*_{nt}]_p$ shows its maximum value ($x = 0.175$) the required number of plates is only 102 and therefore a very small particle size ($d_p = 0.23$ μm) is optimal. Despite a capacity factor of more than 1000, the last peak will be eluted in a few seconds, but the sensitivity factor F is unfavourable.

Discussion

The optimization problem illustrated in Fig.1a appears to be extremely simple. Only three peaks are assumed to be present in the sample and there is only one point at which the retention lines for two different solutes intersect. Nevertheless, the five different optimization criteria that were applied to the problem (considering either $[t_{ne}]_{f,d}$ and $[t_{ne}]_p$ or $[r'_{nt}]_{f,d}$ and $[r'_{nt}]_p$) give rise to five different locations for the predicted optimum! Apparently, even a simple optimization problem may turn out to be difficult if the capacity factor of the last peak is a strong function of the parameter to be optimized. Admittedly, the problem of Fig.1a is not as simple as it seems. Because the two lines for solutes 1 and 2 intersect at a point where the k values are roughly optimal (*i.e.* $1 < k < 10$), the optimum range for k cannot be used, and separation conditions have to be established at which the values for k are either higher or lower. In any case, the present example amply demonstrates the need for a careful consideration of optimization criteria for optimization problems in which k_ω varies considerably.

We have seen that for the present example S_{min} is not a useful criterion. Only for difficult separation problems, in which the required number of plates may be the critical factor, may S_{min} be applied sensibly. Such cases will identify themselves if the conditions suggested by other optimization criteria require numbers of theoretical plates that are beyond the practical limits.

The threshold separation criterion appears to provide useful results for optimization procedures which are conducted on the "final analytical column", *i.e.* if the entire optimization process is carried out on the column that will be used for the actual separation in practice. By selecting the threshold separation criterion

it is assumed that before the optimization process is started a flow rate has been selected for which the column performance and the pressure drop are acceptable.

The threshold separation criterion by definition strives towards the lowest possible k values at which the resolution is still acceptable. Therefore, this criterion may often require modification in order to avoid interference between relevant sample peaks and the solvent front.

The calibrated normalized resolution product is seen in the example of Fig.1 to lead to conditions at which the peaks are homogeneously distributed over the chromatogram, *i.e.* the S values are almost equal for every pair of successive peaks. However, the absolute values for S are low and therefore a large number of plates and a long analysis time are required. This will usually be too high a price to pay for a near-perfect chromatogram. Hence, r^* is not an attractive criterion if k_ω varies with x.

The response surfaces obtained for $[t_{ne}]_{f,d}$ and $[t_{ne}]_p$ closely resemble those obtained for $[r^*_{nt}]_{f,d}$ and $[r^*_{nt}]_p$, respectively. Either t_{ne} or r^*_{nt} may be preferred. t_{ne} results in the shortest possible analysis time. r^*_{nt} may yield a slightly better distribution of peaks in a time that will never be much above the minimum value. In most cases the selection of r^*_{nt} or t_{ne} will be a matter of preference.

The $[r^*_{nt}]_{f,d}$ criterion leads to a very acceptable result from a practical point of view. The chromatographic parameters listed in Table 2b can easily be realized. It is seen that by allowing the column length to vary and by allowing a choice of particle sizes (3, 5 or 10 μm), the analysis time may be reduced by a factor of six in comparison with the shortest possible time on the 40 cm column with 10 μm particles. Even if the particle size is kept constant (*e.g.* at10 μm), the analysis time will always be shorter if the column length is adapted according to the optimum conditions suggested by the $[t_{ne}]_{f,d}$ criterion, than it is for a given column at the

optimum conditions in terms of the threshold separation criterion. In other words, it is always favourable to allow for the column length to be adapted to the result of a selectivity optimization procedure.

It appears that for the separation problem illustrated in Fig.1 the optimum conditions suggested by the $[r^*_{nt}]_p$ are not very practical. The problem is so simple (the required number of plates is so low), that the current status of HPLC technology does not allow the separation to be performed under truly optimum conditions. Ironically, for more difficult separation problems (for which N_{pc} is larger) $[r^*_{nt}]_p$ may prescribe a more realistic set of conditions. With the estimates used for calculating the data in Table 2b ($h = 4$, $v = 20$, $\eta = 1$ cP and $\mathbf{D}_m = 10^{-9}$ m^2/s) and assuming a minimum practical particle size of 3 μm and a maximum practical pressure drop of 200 bar, we may calculate from Eqn.16 that separations which require more than 2000 plates can be performed with particles of (approximately) optimum diameters.

General recommendations

If possible, a chromatographic optimization process should lead to optimized separation and analysis time. This can best be achieved by first optimizing the chromatographic selectivity and by subsequently optimizing the column length and possibly the diameter of an open column or of the particles in a packed column.

If such variations in length and diameter are allowed, then it is to be recommended that two different criteria are calculated as a function of the parameters to be optimized (*i.e.* two response surfaces). Since these three parameters are closely related, this will only have a marginal effect on the required

computation time. First, the optimum should then be established in the response surface for $[r_{nt}^*]_p$ (or $[t_{ne}]_p$). At these conditions, the optimum (particle) diameter may be calculated. If the resulting value is beyond a certain practical limit (which should presently be set at 3 or 5 μm), then $[r_{nt}^*]_p$ is a useful criterion and the column length may be calculated.

If the optimum (particle) diameter is below the practical threshold or if variations in the particle size are not allowed by the chromatographer, then the optimum in $[r_{nt}^*]_{f,d}$ (or $[t_{ne}]_{f,d}$) may be found and the corresponding column length may be calculated.

Only if the required number of plates is extremely high (*i.e.* if the available number of plates becomes the limiting factor) may there be a case for selecting S_{min}. However, the optimum in terms of S_{min} will always lead to (much) longer analysis times than the optimum in terms of $[r_{nt}^*]_p$.

If the optimization process takes place on the final analytical column, then the threshold separation criterion ($1/k_\omega$ for $S \geq \varepsilon$) should be recommended. The value for ε can be calculated from Eqn.9a using the available number of plates of the column (N_c) and the required value for the resolution (*e.g.* 1.5).

APPLICATION

An example of an optimization process in which retention (capacity factors), separation and analysis time are all considered is demonstrated in Fig.2. The optimization problem concerns the composition of the mobile phase in supercritical-fluid chromatography (SFC). The data in this figure are taken from Ref.9.

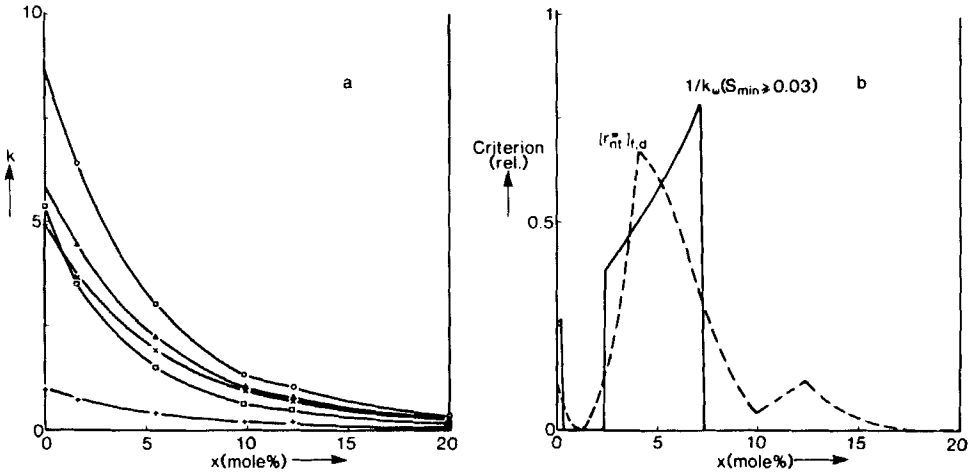

Figure 2
Example of an optimization problem in capillary SFC. Experimental data taken from Ref.9. Fig.2a: Retention surfaces (linear interpolation for ln k as a function of x in between data points). Solutes: decylbenzene (+), myristophenone (□), phenanthrene (×), phenanthridine (△), perinaphthenone (○). x = concentration of isopropanol in carbon dioxide. Temperature 120 °C, pressure 168 bar; 100 μm I.D. column coated with cross-linked polysiloxane (OV-17). Fig.2b (Normalized) Response surfaces: S_k for $\varepsilon = 0.03$ (—) and $[r^*_{nt}]_{f,d}$ (----).

Fig.2a shows the experimental retention data. Values in between the experimental data were obtained by linear interpolation (in terms of ln k). The requirements for models which describe experimental data with sufficient accuracy for optimization processes have been discussed elsewhere (10).

Fig.2b contains the response surfaces for the threshold separation criterion S_k and for $[r^*_{nt}]_{f,d}$. Table 3a gives the values for the capacity factors of the five peaks, Table 3b lists the separation factors and Table 3c shows the calculated chromatographic parameters at each of the two optima. The current status of column technology for capillary SFC (11) implies that 10000 plates may be obtained on a column of 6m length with an internal diameter of 100 μm

TABLE 3

Optimum separation conditions for the example shown in Fig.2 according to two different criteria. An imaginary ("anchor") peak is assumed to be present in the chromatogram at $t = t_0$. Chromatographic conditions are calculated for open columns in SFC with $h = 6$, $v = 50$, $\mathbf{D}_m = 2.10^{-8}$ m²/s, $\eta = 5.10^{-5}$ Pa.s and $d_c = 100$ μm. Solutes: DB = decylbenzene, M = myristophenone, P = phenanthrene, Pa = phenanthridine, Pn = perinaphthenone.

a. Retention data

Optimization	x_{opt}	Capacity factor				
criterion	mole %	DB	M	P	Pa	Pn
S_k	7.2	0.305	1.064	1.473	1.630	2.181
$[r'_{nt}]_{f,d}$	4.1	0.999	2.038	2.411	2.832	3.948

b. Separation data

Optimization	x_{opt}	Separation factor				
criterion	mole %	DB	M	P	Pa	Pn
S_k	7.2	0.132	0.225	0.090	0.031	0.095
$[r'_{nt}]_{f,d}$	4.1	0.200	0.339	0.058	0.058	0.127

c. Calculated chromatographic conditions

Optimization	x_{opt}	S_{min}	N_{ne}	L	t_0	t_ω	F
criterion	mole %			m	min	min	%
S_k	7.2	0.03	10000	6	10	31.8	41
$[r'_{nt}]_{f,d}$	4.1	0.058	2675	1.6	2.7	13.2	30

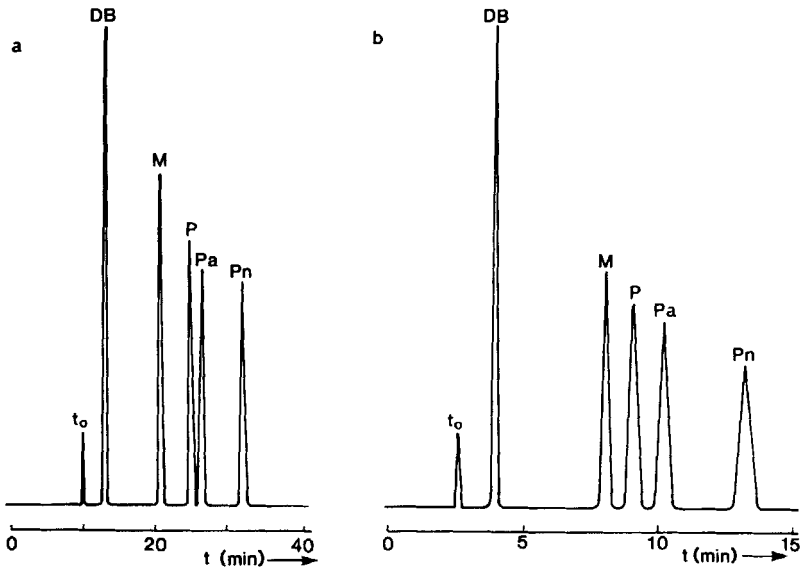

Figure 3
Resulting chromatograms for the two optima shown in Fig.2b. Fig.3a (left): Optimum in terms of S_k for $\varepsilon = 0.03$ ($L = 6$ m, $t_0 = 10$ min). Fig.3b (right): Optimum in terms of $[r^*_{nt}]_{f,d}$ ($L = 1.6$ m, $t_0 = 2.7$ min).

($h = 6$, $v = 50$). On such a given column the separation at the optimum for the threshold separation criterion may be performed in about 1/2 h. At the optimum for $[r^*_{nt}]_{f,d}$ the column may be shortened to about 1.6 m and the separation will take less than 15 min.

The two resulting chromatograms that correspond to the conditions of Table 3c. are simulated in Fig.3. Fig.3a shows the chromatogram that may be obtained on the original column. The separation in Fig.3b may be obtained on the shortened column. It should be noticed that the two chromatograms are normalized in terms of k values. The chromatogram on the right shows higher values for k, but the retention times are actually shorter, as is illustrated on the axes.

The calculation of the optimum conditions in terms of $[r^*_{nt}]_p$ is not as straightforward for capillary SFC as it is for HPLC with packed columns. SFC has not matured to the same extent as has LC, and therefore there are no uniformly accepted values for the chromatographic conditions. However, it is easily possible to demonstrate the validity of the present optimization scheme using some reasonable estimates and rewriting Eqn.14 in a form that is suitable for open columns with an inner diameter d_c:

$$\Delta p = \frac{32 N_{ne} \eta h v \mathbf{D}_m}{d_c^2} \qquad (14a)$$

In the present example $[r^*_{nt}]_p$ yielded the same optimum composition as $[r^*_{nt}]_{f.d}$. Hence, $S_{min} = 0.058$ and 2675 plates are required. If we maintain our practical conditions at the same values as used in Table 3 (*i.e.* $h = 6$ and $v = 50$), then assuming $\eta = 0.05$ cP, $\mathbf{D}_m = 10^{-4}$ cm^2/s and a maximum allowable pressure drop of no more than 1 bar, Eqn.16 suggests a column diameter of 16 μm, a column length of 26 cm and an analysis time (t_{ro}) of just over 20 seconds. As of yet, columns of 16 μm I.D. are not practical in capillary SFC, but columns as small as 25 μm I.D. have been demonstrated (12). For such a column $L = 40$ cm and $t_{ro} = 40$ s are obtained.

It is worth noting the exemplary character of these calculations. The present example is not to be seen as a general recommendation for the column diameter in capillary SFC. Indeed, very different results may be obtained in different situations. For instance, it is always better (in terms of the required analysis time) to work closer to the minimum in the *H vs. u* curve. At $h = 0.7$, $v = 1$ we find from Eqn.16 for the present example ($N_{ne} = 2675$) a column inner diameter of less than 1 μm. However, a larger number of required plates leads to larger column

diameters (*e.g.* 15 μm I.D. is optimal at $h = 0.7$, $v = 1$ for 10^6 theoretical plates; $L = 10$ m, $t_0 = 2.2$ h, $\Delta p = 1$ bar).

CONCLUSIONS

1. Optimization problems in which the capacity factor varies require a careful selection of the optimization criterion. It was shown that for a very simple (fictive) example five different criteria gave rise to five different optima.

2. If the optimization is carried out on the "final analytical column", then the threshold separation criterion (S_k) may be selected.

3. If the dimensions of the column are allowed to be varied after the selectivity optimization has been completed, then the $[r^*_{nt}]_p$ (or $[t_{ne}]_p$) criterion gives the best results (shortest analysis times). However, if this criterion suggest conditions that are unattractive (or impossible) in practice, then $[r^*_{nt}]_{f,d}$ (or $[t_{ne}]_{f,d}$) may be used.

4. The required analysis time will depend on the selected optimization criterion and will increase in the following order:
   $[t_{ne}]_p$ ($\simeq[r^*_{nt}]_p$) $<$ $[t_{ne}]_{f,d}$ ($\simeq[r^*_{nt}]_{f,d}$) $<$ S_k.
   Therefore, it is advantageous to allow the column length and/or diameter to vary.

5. It has been demonstrated, using an fictive example from HPLC and a real example from (capillary) SFC, that a judicious choice of an optimization criterion enables the chromatographer to optimize the separation and the analysis time simultaneously.

REFERENCES

1. P.J.Schoenmakers, *The Optimization of Chromatographic Selectivity, A Guide to Method Development*, Elsevier, Amsterdam, 1986.

2. J.L.Glajch, J.J.Kirkland, K.M.Squire and J.M.Minor, *J.Chromatogr.* **199** 57 (1980).

3. P.J.Schoenmakers, A.C.J.H.Drouen, H.A.H.Billiet and L.de Galan, *Chromatographia* **15** 688 (1982).

4. A.C.J.H.Drouen, P.J.Schoenmakers, H.A.H.Billiet and L.de Galan, *Chromatographia* **16** 48 (1982).

5. G.d'Agostino, F.Mitchell, L.Castagnetta and M.J.O'Hare, *J.Chromatogr.* **305** 13 (1984).

6. I.Atamna, E.Grushka, H.Colin and G.Guiochon, *Chromatographia* **19** 48 (1984).

7. I.Atamna and E.Grushka, *J.Chromatogr.* **355** 41 (1986).

8. V.Svoboda *J.Chromatogr.* **361** 25 (1986).

9. C.R.Yonker and R.D.Smith, *J.Chromatogr.* **201** 241 (1980).

10. P.J.Schoenmakers and T.Blaffert, *J.Chromatogr.* **382** 117-133 (1987).

11. S.M.Fields, R.C.Kong, M.L.Lee and P.A.Peaden, *HRC&CC* **7** 423 (1984).

12. S.M.Fields, R.C.Kong, J.C.Fjeldsted, M.L.Lee and P.A.Peaden, *HRC&CC* **7** 312 (1984).