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Simultaneous optimisation of gradient time, gradient shape and initial composition of the mobile phase in the high-performance liquid chromatography of homologous and oligomeric series

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Abstract

A general approach for optimisation of non-linear gradient elution in reversed-phase and in normal-phase chromatography was suggested. This should be suitable especially for separations of more complex samples containing compounds with repeat units such as members of homologous or oligomeric series where more or less regular retention increase between the adjacent peaks is observed. The approach is based on predictive calculations of the retention and of the resolution of the individual pairs of compounds in the sample mixture.

Isocratic or gradient-elution retention data acquired in a few initial runs under different conditions are employed to determine the parameters of retention equations describing the dependence of the retention factor, *k,* on the composition of the mobile phase and on the number of repeat structural units in homologous or oligomeric series.

The approach allows us to optimise simultaneously the initial composition of the mobile phase, the time (volume) and the shape of the gradient to achieve required resolution for all components of the sample mixture in minimum time. Using non-linear gradients, band spacing and peak capacity in the chromatogram may be improved with respect to the elution with linear gradients. For better practical convenience, optimised curved shape of the gradient can be substituted by corresponding multisegmented linear profile.

The approach is illustrated by examples of reversed-phase gradient-elution separation of *n*-alkyl-3,5-dinitrobenzoates and of normal-phase gradient-elution separation of lower oligostyrenes on a silica gel column.

Keywords: Optimisation; Gradient elution; Alkyl dinitrobenzoates; Oligostyrenes

1. Introduction

In contemporary practice of High Performance Liquid Chromatography (HPLC), linear gradients are most frequently used. The differences in retention of sample compounds often lead to irregular spacing of peaks in chromatogram, even under optimised isocratic or gradient conditions. In such a case, spacing of peaks can be improved and time of analysis decreased by optimising subsequently several parts of the chromatogram showing largest differences in band spacing. This optimisation results in a step gradient consisting of several subsequent isocratic steps with different composition of mobile phase, or in segmented gradients with a few subsequent linear gradient steps of different duration and steepness.

In reversed-phase gradient-elution chromatography, the Dry-Lab computer simulation approach is probably the most widespread approach to optimisation of operation parameters [1,2]. Here, the re-

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tention data from two initial gradient runs are used to adjust subsequently the steepness and the range of the gradient and if necessary, other working parameters. This approach can be adopted also to optimise segmented gradients. Simplex optimisation can also be used for this purpose [3].

We have developed a scheme for simultaneous optimisation of the gradient steepness and initial composition at the start of gradient elution in reversed-phase [4] and in normal-phase [5] gradient-elution chromatography. This scheme is generally applicable in various chromatographic systems and can be adapted to optimisation of nonlinear curved gradients.

In present work, the approach for optimisation of non-linear gradients is presented and illustrated by examples of reversed-phase separation of homologous compounds and of normal-phase separation of oligomers. The separation of such series can be improved by using non-linear gradients with respect to linear gradient elution. Optimisation of the individual segments would be difficult because of large number of necessary segments. Hence, simultaneous optimisation of the entire gradient program using curved gradient profile seems more practical, as the optimised curved gradient can be readily substituted by a multisegmented linear gradient with large number of subsequent segments without affecting significantly the appearance of resulting chromatogram.

2. Theoretical

Various modes of gradient-elution HPLC can be optimised using the same general approach. Predictive optimisation is based on calculation of retention characteristics with various gradient functions, i.e., dependences of c on the time elapsed, t, or on the volume of the eluate, V, from the start of the gradient run. For this purpose, the dependence of the retention factor, k, on the concentration(s) of strong eluent(s), c, in the mobile phase under isocratic conditions should be known [6]. The retention volumes, bandwidths and resolution can be calculated in dependence on gradient parameters from adequate equations, which can be used to determine the parameters necessary to achieve required resolution in minimum analysis time.

In contemporary HPLC, linear gradients are most often used, where the concentration of the strong eluent in a two-component mobile phase, c, follows a linear gradient function:

$$c = A + BV \tag{1}$$

where A is the initial concentration of the strong solvent at the start of the gradient, $A = c_0$ and $B = (c_g - A)/V_g$ is the slope (steepness) of the gradient controlled by the gradient volume, V_g. necessary to achieve the final concentration, C_g.

In reversed-phase systems, the dependence of the retention factor k on the concentration of organic solvent in water, c, can usually be described by the equation [7,8]:

$$\log k = \log k_0 - cm \tag{2}$$

k_0 in Eq. (2) is the k extrapolated to pure water as the mobile phase and m is the 'solvent strength parameter' characterising the change in log k per unit concentration change of the organic solvent (modifier) in the mobile phase. In systems where Eq. (2) applies, the gradient elution retention volumes, V_R, can be calculated as [6–8]:

$$V_R = \frac{1}{mB} \log \left[2.31\, mBV_0 \cdot \frac{k_0}{10^{mA}} + 1 \right] + V_0 \tag{3}$$

In normal-phase (or in ion-exchange) systems the dependence of the retention factor k on c – the concentration of a more polar solvent in a less-polar one (normal-phase systems), or on the molarity of an electrolyte in water or in aqueous–organic solvent (ion-exchange systems) – usually has the following form, which differs from Eq. (2) applying for reversed-phase systems [9–11]:

$$k = k_0 / c^m \tag{4}$$

Here, k_0 is the k in the pure polar solvent (for normal-phase systems) or in the one-molar electrolyte (for ion-exchange systems) and m is the change in log k per one unit of log c. In these systems, the retention volumes, V_R, for linear gradients can be calculated from Eq.(5) [6,12]:

$$V_R = \frac{1}{B}[(m+1)Bk_0V_0 + A^{(m+1)}]^{(1/m+1)} - \frac{A}{B} + V_0 \quad (5)$$

For all HPLC systems, bandwidths w and resolution R_S in gradient elution can be calculated introducing the appropriate instantaneous retention factor k_f at the elution of peak maximum from the relationships $c_f = f(V_R)$, $k = f'(c_f)$ applying for the chromatographic mode and gradient function used [6,13]:

$$w = \frac{4V_0(1+k_f)}{\sqrt{N}}; \quad R_S = \frac{V_{R(2)} - V_{R(1)}}{W} \quad (6a,b)$$

f is so-called gradient function, e.g., described by Eq. (1) for linear gradients and by Eqs. (9) or (11) for non-linear gradients (see below) and f' is the retention-mobile phase composition relationship, which can be described by simple Eqs. (2) or (4) or by more complex equations. $V_{R(1)}$, $V_{R(2)}$ are the retention volumes of sample compounds with adjacent peaks, N is the number of theoretical plates and V_0 is the hold-up volume of the column.

The optimisation of analytical gradient elution consists in the determination of a combination of maximum A and minimum V_g (maximum B) for resolution of all pairs of sample compounds equal to or higher than the minimum required resolution in minimum analysis time [4,5].

Non-linear (curved) gradients are seldom used in practice. For most samples, gradients composed of several subsequent linear segments can be used, with two to four segments often being adequate. However, as it will be shown later, for various samples, especially for mixtures of polymers containing various numbers of a regular repeat structural unit, gradients with continuously changing profiles may improve the spacing of the peaks of sample compounds in the chromatogram and increase the peak capacity or decrease the time of separation. For such samples, optimisation of a curved gradient can be more promising than developing an optimised segmented gradient program, as smooth shape change is likely to fit more readily to regular retention changes between adjacent peaks in a series of homologues or oligomers. Curved gradient profiles can be described by various gradient functions, f. Most commercially available liquid chromatographs can control the mobile phase composition according to a program consisting of a large number of linear segments between a large number of points on the time scale, which can closely simulate any curved gradient profile. However, for the convenience sake, the present optimisation strategies for segmented gradients are limited to gradient profiles composed by only a few subsequent segments. Often a multi-segmented gradient is very useful for optimum separation. Subsequent optimisation of many segments could be very tedious in such a case. Instead, a simple and more practical approach is offered by the optimisation of a curved gradient. The optimised curved gradient profile can be then easily substituted by a multi-segmented linear gradient.

In a homologous or in an oligomeric series, the retention regularly changes with the number of repeat units, n. The dependence of retention factors of the members of such series on n and on the concentration of the strong solvent in the mobile phase, c, can be described by simple equations, both in reversed-phase systems [14]:

$$\log k = a_0 + a_1 n - (m_0 + m_1 n)c \quad (7)$$

and in normal-phase and ion-exchange systems [15]:

$$\log k = a_0 + a_1 n - (m_0 + m_1 n)\log c \quad (8)$$

where a_0, a_1, m_0, m_1 are constants depending on the type of the oligomeric series, on the column packing and on the nature of the components in the mobile phase.

Using isocratic elution, only limited number of compounds can be separated because of overresolution at high n, resulting in long retention times. Gradient elution is necessary for the separation of more complex samples. Here, spacing of peaks in the chromatogram depends on the initial concentration, A, on the gradient time, t_g (or on gradient steepness, B) and on a parameter that can adequately characterise the gradient shape (curvature), K. In HPLC with linear gradients, the retention volumes, V_R, and the resolution, R_S decrease with increasing A and B (decreasing t_g), which results in better peak spacing than under isocratic conditions, but in most cases a constant resolution and really equidistant peak spacing in the chromatogram hardly can be achieved.

In normal-phase chromatography, a convenient

curved gradient function allows characterisation of various concave down (convex) and concave up gradient profiles:

$$c = (A^{(1/K)} + BV)^K \quad (9)$$

where $A = c_0$ is the initial concentration of the strong eluent at the start of the gradient and $B = [c_g^{(1/K)} - A^{(1/K)}]/V_g$ is the steepness (slope) of the gradient. In this gradient function, the coefficient K characterises the curvature of the gradient profile. The gradients are concave up for $K > 1$, linear for $K = 1$ and concave down for $K < 1$, as it is illustrated in Fig. 1. The gradient shape is more curved as the difference of K from unity increases. For this type of gradient, the retention volumes of compounds for which Eq. (4) applies under isocratic conditions can be calculated from the equation:

$$V_R = \frac{1}{B}[(Km+1)Bk_0V_0 + A^{\frac{(Km+1)}{K}}]^{\frac{1}{(Km+1)}} - \frac{A^{\frac{1}{K}}}{B} + V_0 \quad (10)$$

This equation can be used also in reversed-phase chromatography with the gradient function:

$$c = K \log(A^{(1/K)} + BV) \quad (11)$$

where $A = 10^{c_0}$ (c_0 is the initial concentration at the start of the gradient) and $B = [10^{(cg\ K^{-1})} - A^{(1/K)}]/V_g$ is the steepness (slope) of the gradient. This logarithmic gradient function is limited to the concave down gradients. With increasing K, gradients become more concave down.

3. Experimental

An HP 1090M liquid chromatograph equipped with a UV diode-array detector, operated at 230 nm, an automatic sample injector, a 3DR solvent delivery system, a thermostatted column compartment and a Series 7994A workstation (Hewlett-Packard, Palo Alto, CA, USA) was used to acquire the elution data. Two glass cartridge columns in series, 150 mm × 3.3 mm I.D. each, packed with silica gel Separon SGX, 5 μm ($V_0 = 2.35$ ml) obtained from Tessek, Prague, Czech Republic were used for normal-phase experiments and a stainless steel column, 300 mm × 4.2 mm I.D., packed in the laboratory with Silasorb C_{18} 5 μm, Lachema, Brno, Czech republic ($V_0 = 3.16$ ml)

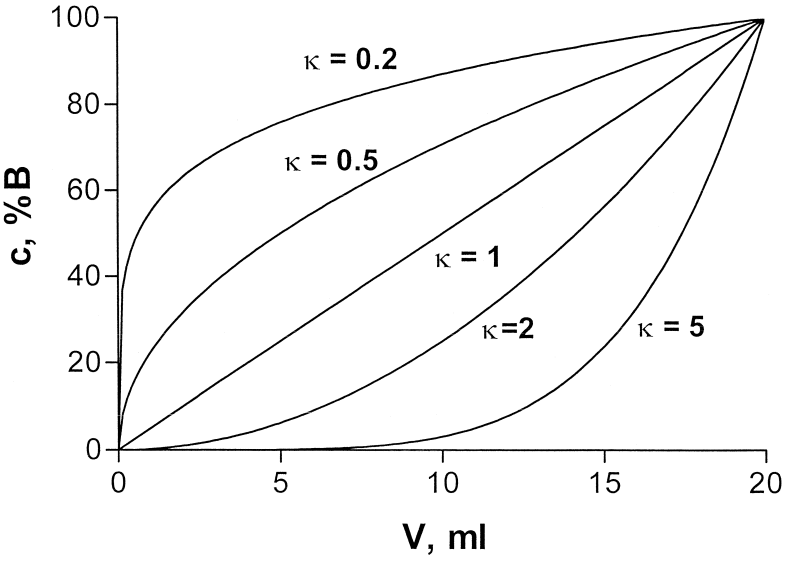

Fig. 1. Examples of linear, concave up and concave down gradients from 0 to 100% B in 20 min (at 1 ml min^{-1}) described by Eq. (9) with various values of the shape parameter K.

was used for reversed-phase experiments. The flow-rate of the mobile phases was kept at 1 ml min^{-1} and the temperature at 40°C in all experiments.

Methanol, *n*-heptane and dioxane, all of HPLC grade, were purchased from Baker, (Deventer, Netherlands). Deionized water was doubly distilled in glass with addition of potassium permanganate. All solvents were filtered using a Millipore 0.45 μm filter and degassed by ultrasonication before the use. Mobile phases were prepared directly in the HP 1090M instrument from the components continuously stripped by a stream of helium.

Homologous 3,5-dinitrobenzoates of *n*-alcohols were prepared by derivatisation with 3,5-dinitrobenzoylchloride as described elsewhere [14] and were dissolved in methanol to provide adequate response of the UV detector. A polystyrene standard of a nominal molecular mass 2350 (Waters, Milford, MA, USA) was dissolved in dioxane *n*-heptane (50:50). Five μl sample volumes were injected in each experiment.

The columns were first equilibrated with the mobile phase and then the retention volumes, V_R, of the sample compounds were measured under isocratic conditions in mobile phases with different concentrations of methanol in water or of dioxane in *n*-heptane. The parameters of the retention Eqs. (7) and (8) were determined from the isocratic retention factors, $k=(V_R/V_0-1)$ as described previously [16] and are listed in Table 1. In gradient elution experiments, a 5-min reversed gradient and a 5-min equilibration time were used after the end of each experiment to re-equilibrate the column. Column hold-up volumes, V_0. were determined using trichloroethylene as the marker in normal-phase systems and uracil in reversed-phase systems.

All optimisation calculations and modelling of chromatograms were performed in the form of spreadsheet using the Quattro Pro 5.0 table editor.

Table 1
Parameters of Eq. (7) for *n*-alkyl-3,5-dinitrobenzoates on a C_{18} column in methanol–water mobile phases (I) and of Eq. (8) for oligostyrenes on silica gel in dioxane *n*-heptane mobile phases

System	Parameter			
	a_0	a_1	m_0	m_1
I	1.713	0.517	2.773	0.446
II	−0.916	0.0171	0.302	0.1122

4. Results and discussion

Appropriate selection of the concentration of the strong eluting component in the mobile phase at the start of the gradient, *A*, is important because its influence on the resolution and on the time of analysis is equally significant as that of the gradient steepness, *B*. Our previous results have shown that optimum initial concentration, *A*, usually is not significantly affected by the gradient time, t_g [4].

We developed the strategy where the gradient parameters *A* and *B* are optimised simultaneously [17]. With a pre-set final concentration of the strong solvent, c_g, that should be achieved at $V=V_g$, the slope *B* of the gradient is a function of the initial concentration *A*:

$$B=\frac{(c_G-A)}{V_g} \tag{12}$$

Then, the elution volume V_R can be calculated as a function of the initial concentration, *A* from appropriate equation for the chromatographic mode and gradient function used, e.g., from Eqs. (3), (5) or (10). The resolution of all pairs of compounds with adjacent peaks is calculated using Eq. (6b) and is plotted versus *A* in the form of a 'window diagram' to select optimum initial conscentration of the strong eluent at the start of the gradient. There are two possibilities of optimisation:

(1) The 'window diagram' may be used to search for the largest value of *A* at which the desired resolution (e.g., $R_S=1.5$) is achieved for all compounds in the sample mixture. This approach in most cases automatically minimises the time of the analysis, as the retention volumes decrease with increasing *A*.

(2) Another possibility is to search the value of *A* that yields maximum possible resolution for the 'critical' pair of compounds showing the worst (minimum) resolution in the sample mixture on a given column. This maximised R_S is often lower than the desired resolution and in such a case the pre-set gradient volume or the column plate number

can be increased to further improve the resolution in the mixture. Similar approach can be used to diminish the 'maximised minimum resolution' if it is excessively large and to decrease the time of analysis.

In both optimisation approaches, with optimum value of A having been established, the gradient slope B can be calculated for the pre-set gradient volume V_G and final concentration c_g from Eq. (12).

If necessary, the approach can be repeated for various pre-set gradient volumes, V_g, for additional refinement of optimised separation.

The adaptation of this procedure to non-linear gradients is rather straightforward. In the modified approach, the additional gradient shape parameter, K, must be included. For this purpose, a simple optimisation spreadsheet program was developed. First, a pre-set gradient time is selected. Then, maps of both retention volumes of all sample compounds and of resolution in dependence on the initial concentration of the strong eluent in the mobile phase (A, c_0-in the range from 0 to maximum possible concentration) and on the gradient shape parameter, K (in the range from 0.01 to 10), were constructed using Eqs. (10), (6a) and (6b). From the retention and the resolution maps, the combination of the initial concentration and of the shape parameter, K was selected for shortest retention time of the last eluted compound keeping the resolution of all sample compounds equal to or greater than the desired value (e.g., $R_S = 1.5$). If maximum resolution for one or more pairs of adjacent peaks is lower than the desired resolution over the whole optimised space in the map, the optimisation calculations are repeated at a higher pre-set gradient time until the result is satisfactory. The procedure is rapid and yields equation describing optimised curved gradient profile, which, if necessary, can be substituted by a multi-segment gradient with the start and the end of each segment given by a point of the optimised curved gradient function and the duration of each linear segment of e.g. 0.5 or 1 min.

This approach is illustrated by two examples:

(I) Reversed-phase separation of 15 homologous *n*-alkyl-3,5-dinitrobenzoates on a C_{18} column using non-linear gradients of methanol in water (parameters of Eq. (7) in Table 1 (I)];

(II) Normal-phase separation of 30 lower oligostyrenes on two silica gel columns in series using non-linear gradients of dioxane in *n*-heptane (parameters of Eq. (8) in Table 1 (II)].

For the two separation problems, the columns have the efficiency of approximately 5000 theoretical plates at the flow-rate 1 ml min^{-1}. Minimum resolution of 1.5 between the adjacent peaks was required and the conditions were searched for to yield best spacing of peaks in the chromatogram and minimum separation time.

First, linear gradients were optimised for the two separations using our previous approach. With optimised gradient slope and initial concentration, it was possible to achieve the reversed-phase separation of the homologous mixture in 18 min and the mixture of oligostyrenes in 27 mins. The parameters of the optimised gradients are given in Table 2 and the resulting optimised separation is shown in Figs. 2A and 3A.

The effects of gradient nonlinearity on the separation was investigated using curved gradient functions (Eq. (11)) for reversed-phase separation and (Eq. (9)) for normal-phase separation. In both cases, the retention volumes were calculated from Eq. (10) and bandwidths and resolution from Eqs. (6a), (6b).

Fig. 4 illustrates the effect of the pre-set gradient volume on the retention of the first and of the last eluted homologous *n*-alkyl 3,5-dinitrobenzoates (*n* from 1 to 15) in reversed-phase nonlinear gradient elution chromatography. The elution volume of the methyl ester is practically independent of the pre-set gradient volume, V_g but the retention volume of the pentadecyl ester decreases with decreasing V_g, which means that both the spacing of the peaks in the chromatogram and the separation time are improved with steeper gradients. The dependences in Fig. 4 are plotted for simultaneously optimised initial concentration of methanol, A, and shape parameter, K, yielding minimum time of separation and the resolution of at least 1.5 for all adjacent peaks in the chromatogram. To achieve the required R_S, certain minimum value of the shape parameter K is necessary for a pre-set V_g. This minimum value, K_{min} is also plotted in Fig. 4 and corresponds to the optimum gradient shape, as the analysis time increases at $K > K_{min}$. As the pre-set gradient volume decreases, K_{min} increases and the separation time decreases. The gradient volume, V_g, cannot be de-

Table 2
Parameters c_0 (initial concentration of methanol or dioxane, respectively, in % (v/v)·10^{-2} e.g., $c_0 = 0.83$ for 83% methanol), B and K of optimised linear (L, Eq. (1)) and non-linear gradient functions (N, Eq. (11) for reversed-phase system, Eq. (9) for normal-phase systems) and corresponding gradient volumes, V_g, retention volumes of the first, $V_{R(1)}$ and of the last, $V_{R(15)}$ or $V_{R(30)}$ retention volumes (all in ml) and maximum resolution $R_{s(max)}$ (for pair 14/15 in system I and for pair 9/10 in system II). Optimisation for minimum resolution 1.5 or more, $N = 5000$, flow-rate 1 ml min^{-1}. Systems and sample solutes as in Table 1

System	Parameter							
	Gradient	c_0	B	K	V_g	$V_{R(1)}$	$V_{R(15)}$ / $V_{R(30)}$	$R_{s(max)}$
I	L	0.83	0.0113	–	15	4.2	17.3	2.2
	N	0.59	8.2*10^5	0.14	15	4.5	14.2	2.2
II	L	0.07	0.0232	1	40	3.2	25.7	2.6
	N	0.01	0.0217	0.58	46	3.3	21.3	2.0

creased deliberately as at too low a gradient volume, either the resolution drops below the desired value, or some more retained compounds would be eluted after the end of the gradient. This means that optimum separation conditions are found at minimum acceptable V_g and at corresponding K_{min} and A. The optimised non-linear gradient separation is shown in Fig. 2B and the parameters of the optimised gradient function are listed in Table 2.

Fig. 5 illustrates the effect of the gradient shape parameter, K on the resolution of the last pair of compounds and on the retention times of the first and of the last eluted sample solutes at a fixed pre-set gradient volume 60 ml. With increasing K, i.e., with more concave down gradients, the analysis time increases and strongly retained compounds are over resolved. The resolution in the series increases from less retained towards later eluted homologues under various separation conditions, but this increase is minimised for the gradient with optimised shape (from $R_S = 1.5$ for the first pair of compounds to 2.20 for the last pair).

Another example illustrates optimisation of the separation of a mixture of thirty oligostyrenes on a silica gel column by non-linear gradient of dioxane in *n*-heptane. The initial concentration of dioxane, A, the gradient volume, V_g, and the shape parameter, K, were optimised simultaneously in a similar way as the reversed-phase separation of homologous dinitrobenzoates using Eqs. (6a), (6b) and (10), except for Eq. (9) being the gradient function. Minimum time of this normal-phase separation was required with the resolution of all adjacent peaks of at least 1.5. Fig. 6 shows the effect of optimised shape parameter, K_{opt}, at corresponding optimum gradient volumes, V_g, on the retention volumes of the first and last (30th) oligostyrene. Unlike the reversed-phase separation, approximately equal resolution of the first and of the last pair of compounds is achieved, but the resolution increases towards the center of the chromatogram and is maximum for the oligomers with nine and ten oligostyrene units. This irregular peak distribution in normal-phase gradient-elution chromatography is due to complex dependence of the gradient retention volumes on the number of repeat units n in oligomeric series. Even though the dependences of the parameters log k_0 and m of Eq. (4) on n are approximately linear, the effect of n on the retention volumes and on the peak distribution is complex and cannot be predicted a-priori because it depends on the nature of the polar solvent in the chromatographic system, which controls the contributions of both the repeat unit and of the end groups in an oligomeric series. The dependence of this maximum resolution on K_{opt} is also shown in Fig. 6. From this figure, the optimised conditions correspond to the minima on the $R_{s\,max}$ and $V_{R(30)}$ versus K_{opt} plots.

The parameters of the optimised separation are given in Table 2 and the optimised separation of oligostyrenes with non-linear gradient of dioxane in *n*-heptane is shown in Fig.3B. Optimised non-linear gradients decrease the analysis time for homologous alkyldinitrobenzoates from 18 to 15 min and for oligostyrenes from 27 to 22 min, which correspond to approximately 20% improvement with respect to

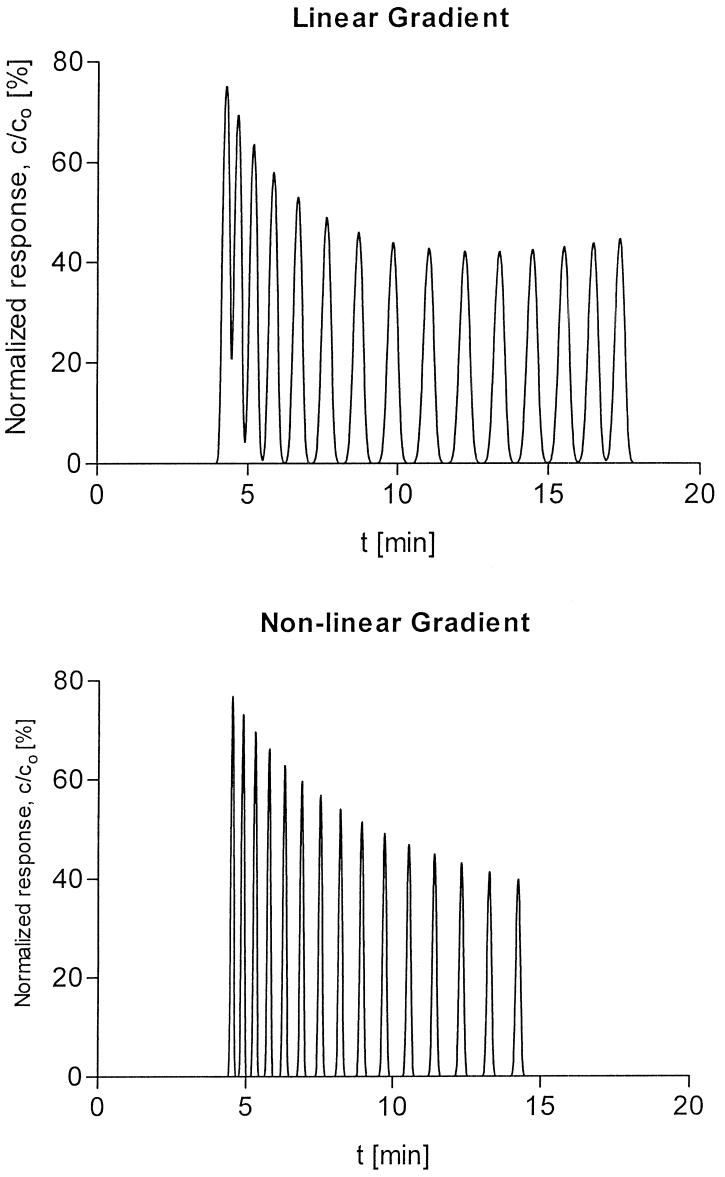

Fig. 2. Optimised gradient-elution separation of 15 homologous *n*-alkylbenzenes on a C_{18} column using linear and non-linear gradient of methanol in water at flow-rate of 1 ml min^{-1}. Other conditions in Table 2 and in the Experimental part. Normalised response relates to the initial concentration in sample injected.

optimised linear 1 gradients. It should be noted that V_g does not mean the real volume used in the experimental run, it is only an auxiliary quantity, as real gradient is terminated immediately after the elution of the last compound, i.e., at its V_R + approx. 0.5 ml. Fig. 7 compares the optimised profiles of linear and of non-linear gradients for these separations. With a fixed time of separation, corresponding increase in peak capacity can be obtained, which is probably more important in practice.

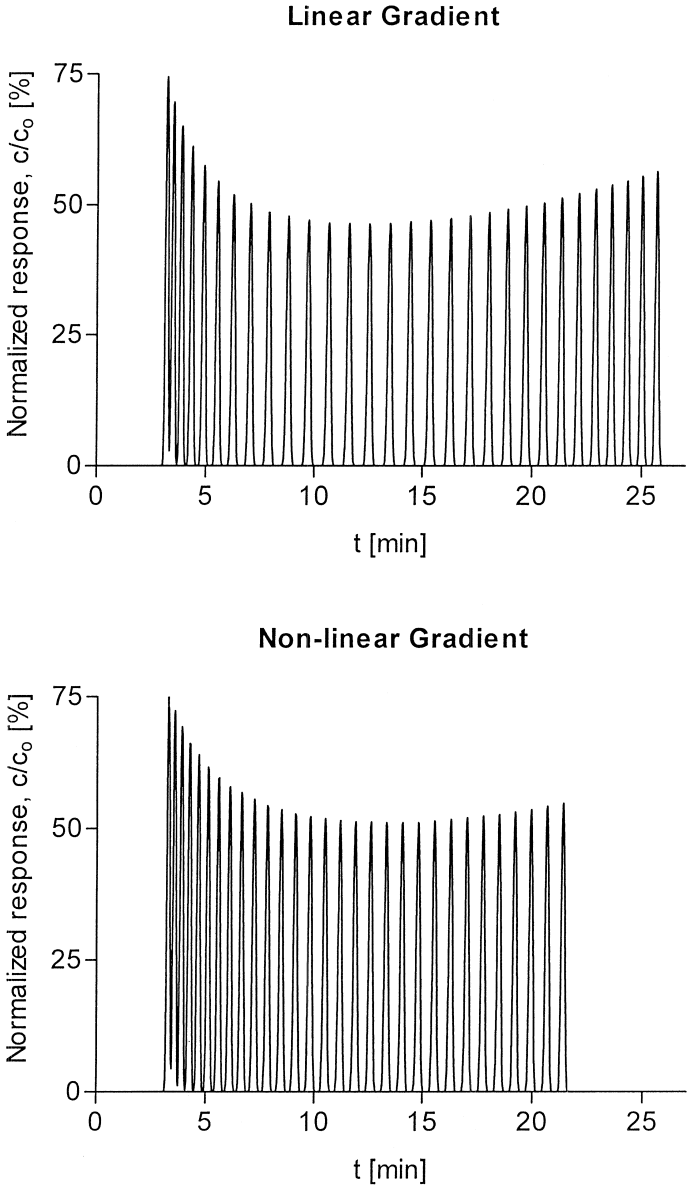

Fig. 3. Optimised gradient-elution separation of 30 lower oligostyrenes on two silica gel columns in series using linear and non-linear gradient of dioxane in *n*-heptane at flow-rate of 1 ml min^{-1}. Other conditions in Table 2 and in the Experimental part. Normalised response relates to the initial concentration in sample injected.

5. Conclusions

Simultaneous optimisation of the initial concentration of strong eluent and of the gradient time is essential. Additional optimisation of the curvature of a non-linear gradient may improve peak capacity and spacing in the chromatogram and decrease the analysis time under both reversed-phase and normal-phase conditions, especially for separations of homologous and of oligomeric compounds. Work is

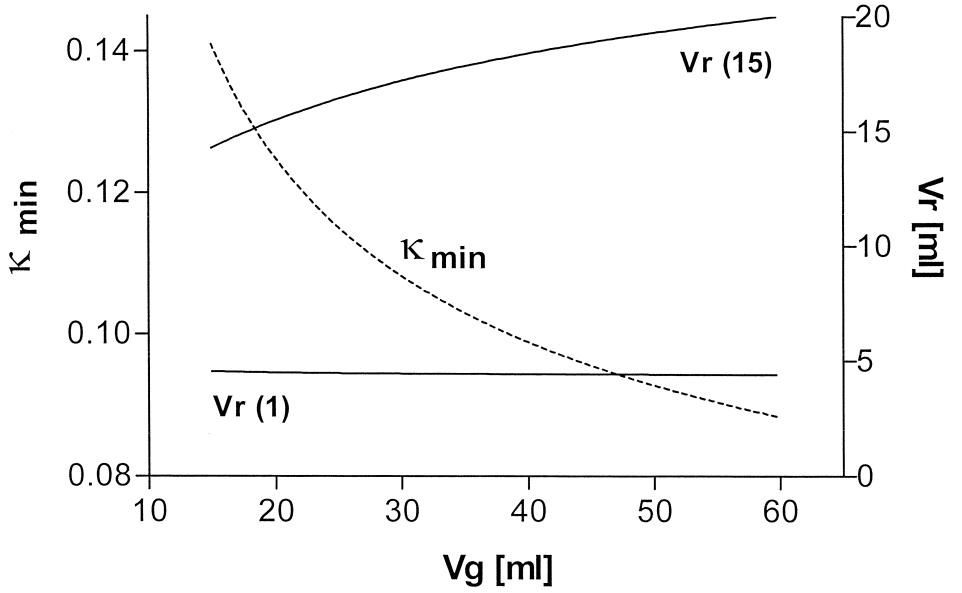

Fig. 4. Effect of the gradient volume, V_g, ml on the optimised shape parameter, K_{min} and on the retention volumes, V_R, of the first (1) and last (15) *n*-alkyl-3,5-dinitrobenzoate on a C_{18} column ($V_0 = 3.16$ ml, $N = 5000$) with non-linear gradients of methanol in water optimised for minimum resolution ≥ 1.5. Flowrate = 1 ml min^{-1}.

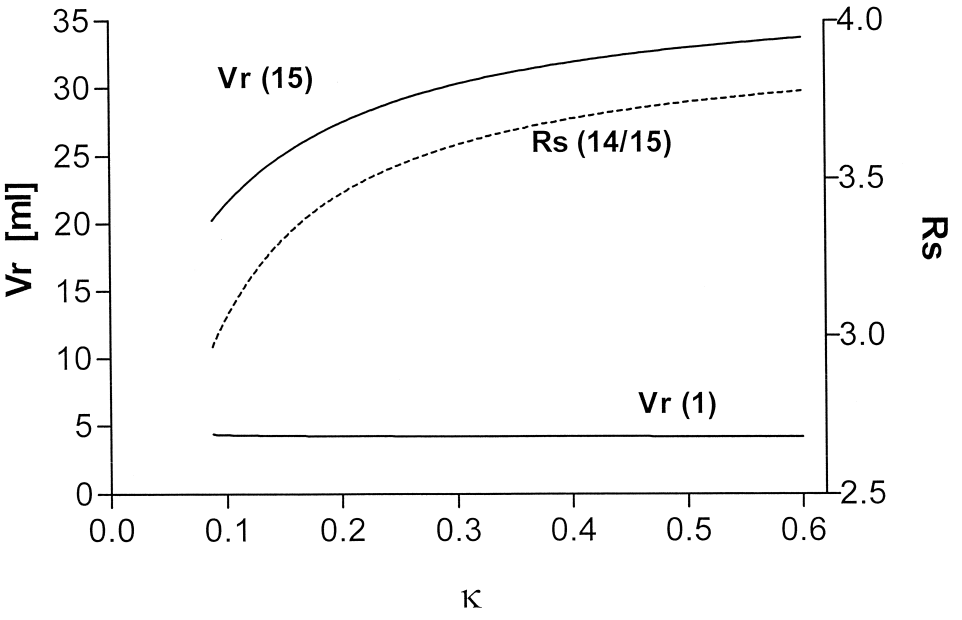

Fig. 5. Effect of the shape parameter, *K* on the retention volumes of the first (1) and last (15) *n*-alkyl-3,5-dinitrobenzoate and on the resolution of the last pair of compounds at a fixed gradient volume, $V_g = 60$ ml and optimum $A = 84\%$ methanol on a C_{18} column ($V_0 = 3.16$ ml, $N = 5000$) with non-linear gradients of methanol in water optimised for minimum resolution ≥ 1.5. Flowrate = 1 ml min^{-1}.

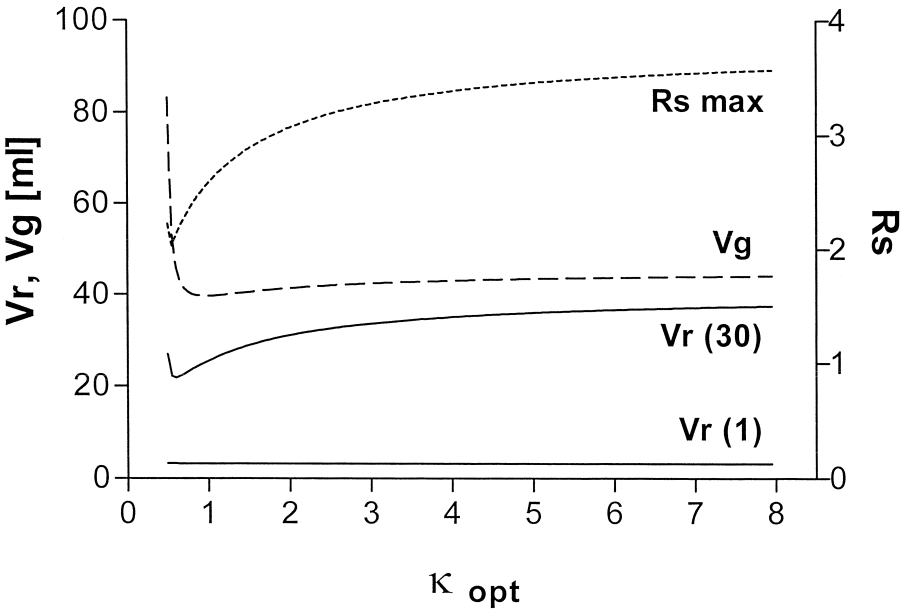

Fig. 6. Gradient volume, V_g, retention volumes of the first (1) and last (30) oligostyrene and maximum resolution, $R_{s\,max}$ of the oligomers with nine and ten oligostyrene units at the optimised shape parameter, K_{opt} in gradient elution separation of 30 lower oligostyrenes on two silica gel columns in series ($V_0 = 2.35$ ml, $N = 5000$) with non-linear gradients of dioxane in *n*-heptane optimised for minimum resolution ≥ 1.5. Flowrate = 1 ml min^{-1}.

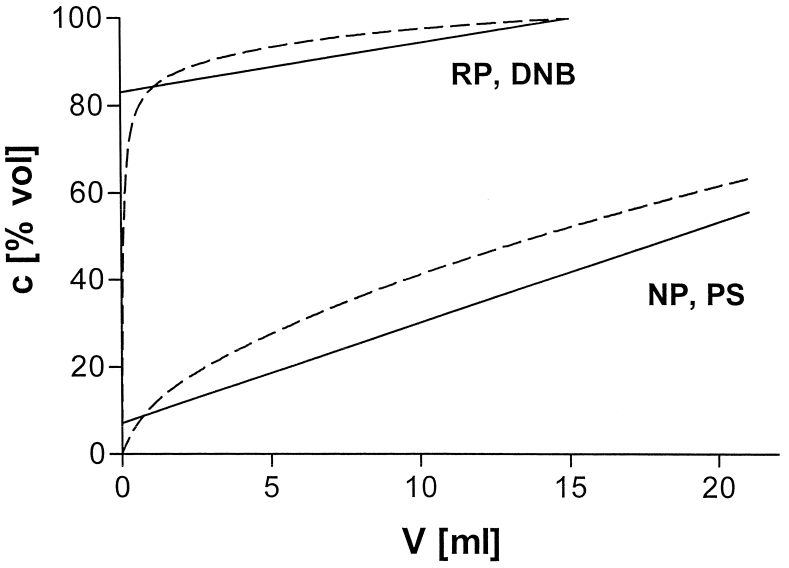

Fig. 7. Optimised profiles of linear and non-linear gradients of methanol in water for reversed-phase separation of 15 *n*-alkyl-3,5-dinitrobenzoates (RP, DNB) and of linear and nonlinear gradients of dioxane in *n*-heptane for normal-phase separation of 30 lower oligostyrenes on silica gel (NP, PS).

in progress to verify more general validity of the present approach for other types of compounds.

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References

[1] J.W. Dolan, D.C. Lommen, L.R. Snyder, J. Chromatogr. 485 (1989) 91.
[2] L.R. Snyder, J.J. Kirkland, J.L. Glajch (Eds.), Practical HPLC Method Development, 2nd ed, Wiley, New York, 1997, p. 448.
[3] J.C. Berridge, J. Chromatogr. 485 (1989) 3.
[4] P. Jandera, J. Chromatogr. 485 (1989) 113.
[5] P. Jandera, J. Chromatogr. A 797 (1998) 11.
[6] P. Jandera, J. Churáček, Adv. Chromatogr. 19 (1980) 125.
[7] L.R. Snyder, J.W. Dolan, J.R. Gant, J. Chromatogr. 165 (1979) 3.
[8] P. Jandera, J. Churáček, L. Svoboda, J. Chromatogr. 174 (1979) 35.
[9] L.R. Snyder, Anal. Chem. 46 (1974) 1384.
[10] E. Soczewiński, W. Golkiewicz, Chromatographia 4 (1971) 501.
[11] P. Jandera, J. Churáček, J. Chromatogr. 91 (1974) 207.
[12] P. Jandera, J. Churáček, J. Chromatogr. 91 (1974) 223.
[13] P. Jandera, J. Churáček, Gradient Elution in Liquid Column Chromatography, Elsevier, Amsterdam, 1985.
[14] P. Jandera, J. Chromatogr. 314 (1984) 13.
[15] P. Jandera, J. Rozkošná, J. Chromatogr. 362 (1986) 325.
[16] P. Jandera, M. Kučerová, J. Holíková, J. Chromatogr. A 762 (1997) 15.
[17] P. Jandera, M. Špaček, J. Chromatogr. 366 (1986) 107.