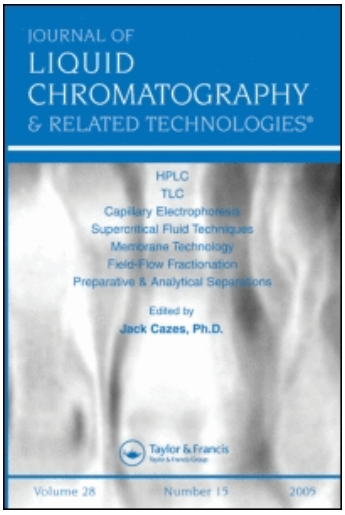

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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P. Jandera[a]; B. Prokeš[a]
[a] University of Chemical Technology, Pardubice, Czechoslovakia

To cite this Article Jandera, P. and Prokeš, B.(1991) 'Predictive Optimization of the Separation of Phenylurea Pesticides using Ternary Mobile Phase Gradients in Reversed-Phase HPLC', Journal of Liquid Chromatography & Related Technologies, 14: 16, 3125 — 3151
To link to this Article: DOI: 10.1080/01483919108049379
URL: http://dx.doi.org/10.1080/01483919108049379

JOURNAL OF LIQUID CHROMATOGRAPHY, 14(16&17), 3125-3151 (1991)

PREDICTIVE OPTIMIZATION OF THE SEPARATION OF PHENYLUREA PESTICIDES USING TERNARY MOBILE PHASE GRADIENTS IN REVERSED-PHASE HPLC

P. JANDERA AND B. PROKEŠ
University of Chemical Technology
Nám. Legií 565
532 10 Pardubice, Czechoslovakia

ABSTRACT

The method for optimization of ternary mobile phase gradients in HPLC developed earlier is illustrated on a practical example of reversed-phase separation of phenylurea pesticides. Predictive calculation methods were used to optimize subsequently "solvent strength", "selectivity" and "combined solvent strength - selectivity" gradients. The merits of the three types of ternary gradients for this specific separation problem are discussed to elucidate the basic principles of the optimization strategy. The predicted retention data are compared with the experimental results and the limitations of the approach are shown. The present method makes it possible to reduce significantly the number of experiments and calculations necessary to perform the optimization in comparison to a systematic search of the optimized parametr space.

INTRODUCTION

Gradient elution is preferred technique for improving the separation of sample mixtures with a wide reten-

tion range in column liquid chromatography[1]. In addition to the empirical "trial-and-error" approach, a variety of systematic methods are available, some of which can be readily used or adapted to optimize the gradient profile[1-2]. These methods, including both simplex optimization procedure[3] or computer-assisted predictive calculation methods[4-20], have been applied mainly to continuous or stepwise (segmented) binary solvent gradients. Snyder and co-workers[21-30] developed a computer-simulated prediction approach for optimization of gradient elution based on the theory of linear solvent strength gradients, which has become available as the Dry Lab G (and Dry Lab S) programs.

For some complex separations, ternary or quaternary solvent gradients may yield better selectivity and resolution than binary gradients, but their optimization is much more complex, as it requires simultaneous optimization of at least four to six experimental parameters, i.e., initial concentrations, gradient slopes and, possibly gradient curvatures for two or three stronger eluting components of the mobile phase in a weak solvent. A systematic predictive computer simulation over the full parametr space would require several hundreds or thousands of calculations[13]. So many simulated chromatograms would be difficult to compare for the selection of optimum results. Therefore, a simplified strategy for the optimization of multisolvent gradients is desirable.

For this purpose, the experimental design, originally introduced for the optimization of selectivity in reversed-phase isocratic chromatography with quaternary mobile phases using "overlapping resolution mapping" (ORM) approach, was later extended to the optimization of a ratio of methanol, acetonitrile and tetrahydrofuran in water in course of quaternary gradients[31-33].

A similar strategy was used to optimize the separation of PTH-amino acid derivatives with a ternary gradient of methanol and tetrahydrofuran in a phosphate buffer[34].

We have proposed predictive optimization methods for various specific types of ternary gradients in reversed-phase chromatography, based on the computer assisted predictive calculations of elution volumes, band widths and resolution of sample solutes, using the retention data acquired in binary mobile phases[12,13,35]. The calculations make use of the following relationship between the solute capacity factors k´and concentrations φ_x, φ_y of the two organic solvents X and Y in a ternary aqueous--organic mobile phase[35]:

$$\log k' = \frac{a_x.\varphi_x + a_y.\varphi_y}{\varphi_x + \varphi_y} - m_x.\varphi_x - m_y.\varphi_y \qquad /1/$$

The net elution volume, V_g', in gradient-elution chromatography with linear ternary gradients of both X and Y according to the partial gradient functions (dependencies of the individual actual concentrations φ_x, φ_y of the solvents X, Y in the mobile phase at the column inlet on the volume of the eluent passed through the column from the start of the gradient, V):

$$\varphi_x = A_x + B_x.V \qquad /2/$$

$$\varphi_y = A_y + B_y.V \qquad /3/$$

can be calculated using Eqn. /4/:

$$V_g' = \frac{1}{m_x.B_x + m_y.B_y} \cdot \log \left[2.31 V_m \cdot (m_x.B_x + m_y.B_y) \cdot .10^{(a_G - m_x.A_x - m_y.A_y)} + 1 \right] \qquad /4/$$

Here, V_m is the column dead volume and a_x, m_x, a_y, m_y are the respective coefficients of semilogarithmic relationships plotted to the experimental data measured in binary mixtures water-organic solvent in concentration φ[4-6,8,9]:

$$\log k' = a - m \cdot \varphi \qquad /5/$$

Finally, a_G is an auxiliary variable:

$$a_G = \frac{(A_x + B_x \cdot \frac{V'_g}{2}) \cdot a_x + (A_y + B_y \cdot \frac{V'_g}{2}) \cdot a_y}{A_x + A_y + (B_x + B_y) \cdot \frac{V'_g}{2}} \qquad /6/$$

For more details concerning the derivation, see ref. 35. To calculate V'_g from Eqns. /4/ and /6/, an iterative method should be used. Band widths, w_g, are calculated from:

$$w_g = \frac{4V_m}{\sqrt{N}} \cdot (1 + k'_f) \qquad /7/$$

after the introduction of $k' = k'_f$ from Eqn. /1/ for φ_x and φ_y corresponding to the volume of the eluate at the time of elution of the band maximum, i.e. for $V = V_g$. k'_f is the k' value of a solute at the time of elution and N is column plate number. To calculate the resolution R_s of the solutes 1 and 2, with adjacent bands, the definition equation is used[13]:

$$R_s = 2 \frac{V'_{g,2} - V'_{g,1}}{W_2 + W_1} \qquad /8/$$

Ternary gradients may be classified into three basic types: "solvent strength", "selectivity" and "combined selectivity-solvent strength" gradients[12].

Ternary "solvent strength" gradients make use of a pre-set concentration ratio of two organic solvents X and Y in aqueous mobile phases, $g = \varphi_x : \varphi_y$, which is kept constant during the gradient elution, while the elution strength is increased by increasing the sum of the concentrations, $\varphi_T = \varphi_x + \varphi_y$, in a linear manner. The separation selectivity is relatively constant in course of a "solvent strength" gradient and the purpose of such a gradient is to affect primarily the absolute retention of sample solutes, the adequate separation selectivity of which may be attained at an optimized solvent ratio in a ternary mobile phase. "Solvent strength" gradients are controlled by a single gradient function

$$\varphi_T = A + B.V \qquad /9/$$

and a simple equation for binary gradients can be used to calculate the net elution volumes, $V_g^{'12,13}$:

$$V_g' = \frac{1}{mB} .\log \left[2.31mBV_m \, . \, 10^{(a-mA)} + 1 \right] \qquad /10/$$

with

$$a = a_T = \frac{a_x.g + a_y}{1 + g} \qquad /11/$$

and

$$m = m_T = \frac{m_x.g + m_y}{1 + g} \qquad /12/$$

In ternary "selectivity" gradients, the sum of concentrations of the organic solvents X and Y, $\varphi_T = \varphi_x + \varphi_y$, is kept constant during the gradient elution, but ratio of concentrations, $g = \varphi_x : \varphi_y$ is changed with time (or with the volume of the eluate, V, so that:

$$\varphi_x = A_x + B_x.V \qquad \text{and} \qquad /13/$$

$$\varphi_y = A_y + B_y.V = \varphi_T - A_x - B_x.V \qquad /14/$$

This means that an increase in φ_x should be compensated for by an equivalent decrease in φ_y per unit volume of the eluate, to hold φ_T constant during the gradient. As A_x, B_x determine A_y and B_y, the retention can be described in a similar way as with binary solvent gradients and Eqn. /10/ can be used for calculation of V_g', but with different meaning of the parameters a and m:

$$a = a_x - m_x.\varphi_T \qquad /15/$$

$$m = \frac{a_y - a_x}{\varphi_T} + m_x - m_y \qquad /16/$$

and $B = B_x = -B_y$; $A = A_x - \varphi_T = -A_y$ /17,18/

In contrast to a "solvent strength" gradient, the primary factor affecting the separation during a "selectivity" gradient is a change in selectivity with time, although the elution strength also changes to some extent (increases if X is a stronger eluent than Y and decreases in the opposite case)[12,13]. A "selectivity" gradient may improve the separation of a sample mixture containing a pair or a group of relatively weakly retained compounds with a good separation selectivity in binary mobile phases composed of the solvent Y and water, but a poor selectivity in binary mobile phases containing the solvent X and water, while the opposite applies to another pair or group of sample solutes with a relatively stronger retention[35].

A "solvent strength" and a "selectivity" ternary gradient should be attempted first to resolve a sample and only if these fail, a "combined selectivity-solvent strength" gradient is to be used as a last resort, because it is much more complex to describe and complex Eqns. /4/ and /6/ should be used to predict the retention.

It was the purpose of the present work to illustrate the application of the theory of ternary gradients on a practical example of separation of a mixture of phenylurea pesticides and related compounds.

MATERIALS AND METHODS

Apparatus

An HP 1090M liquid chromatograph was used, equipped with a UV diode-array detector, operated at 230 nm (reference wavelength 550 nm), an automatic sample injector, a 3DR solvent delivery system, a thermostatted column compartment with temperature set at 40°C, a Series 79994A workstation and an HP 2225 Think-Jet printer (Hewlett-Packard, Avondale, PA, U.S.A.). The column, stainless steel, 300x4.2mm i.d.was packed in the laboratory with octadecylsilica Silasorb SPH C18, 7.5μm (Lachema, Brno, Czechoslovakia).

Chemicals

Methanol, spectroscopic grade (Lachema), acetonitrile, spectroscopic grade (Janssen, Beerse, Belgium) and water (deionized and doubly distilled in glass with an addition of potassium permanganate) were mixed in the instrument in appropriate volume ratios to prepare isocratic and gradient mobile phases.

Samples of phenylurea pesticides were obtained from the Cental Control and Testing Agricultural Institute (Brno, Czechoslovakia): hydrxymetoxuron (HY), desfenuron (DF), fenuron (FE), metoxuron (MX), monuron (MU), monolinuron (ML), chlortoluron (CT), metobromuron (MB), diuron (DU), linuron (LU), chlorbromuron (CB) and neburon (NB). The chemical structures of these compounds are given in ref. 11. Sample solutions were prepared either in mobile phases or in pure methanol.

Procedures

Sample volumes of 5 μl were injected. The elution volumes, V_R, were calculated from the elution times at peak maxima and the flow rate of the mobile phase measured using a stop watch and a burette at the outlet from the detector. The capacity factors of sample solutes were calculated as $k' = V_R/V_m - 1$ from the arithmetic means of two or three experimental V_R values for each isocratic mobile phase tested (40,50,60,70 and 80% (v/v) methanol and 40,50,60,70 and 80% (v/v) acetonitrile in water). The column dead volumes, V_m, were measured in each mobile phase as the elution volume of D_2O measured using a differential refractometer R 401 (Waters Ass., Milford, MA., U.S.A.) and an AD convertor Model 760 (Nelson)). Linear regression was used to determine the constants a and m of Eqn. /5/ for the two sets of experimental data in methanol - water and in acetonitrile - water mobile phases (Table 1).

RESULTS AND DISCUSSION

Table 1 surveys the constants a_x, a_y and m_x, m_y of Eqn. /5/ for methanol - water and acetonitrile - water mobile phases. The log k'vers. φ plots are linear in the mobile phase composition range tested, as it is documented by the values of correlation coefficients, R in this table. This means that the constants in Table 1 can be used as the basis of predictive optimization calculations using the present theory.

The first attempts at separation of a mixture of twelve phenylurea herbicides using binary mobile phases showed that the reversed-phase separation of CT, ML, MB and DU presented a difficult problem. The resolution of ML from CT is better in acetonitrile - water than in

TABLE 1

The constants a and m of Eqn. /5/ of substituted phenylurea compounds in acetonitrile - water (x) and methanol - water (y) mobile phases.
Column: Silasorb SPH C18, 7.5 μm, 300x4.2 mm, V_m = 2.38 cm^3; R - correlation coefficients of the fit of Eqn. /5/ to the experimental retention data in 40 - 80% acetonitrile and 40 - 80% methanol.

Solute	acetonitrile - water			methanol - water		
	a_x	m_x	R	a_y	m_y	R
HY	0.080	1.313	0.9986	0.906	3.337	-
DF	0.108	1.154	0.9970	1.018	2.480	0.9998
FE	0.418	1.468	0.9927	1.164	2.625	0.9998
MX	0.910	2.073	0.9933	1.662	3.152	0.9992
MU	1.051	2.115	0.9925	1.861	3.158	0.9997
ML	1.546	2.553	0.9925	2.129	3.321	0.9998
CT	1.214	2.147	0.9989	2.363	3.589	0.9994
MB	1.631	2.608	0.9938	2.257	3.402	0.9996
DU	1.576	2.599	0.9899	2.427	3.568	0.9992
LU	1.822	2.664	0.9981	2.746	3.814	0.9994
CB	1.765	2.535	0.9999	2.876	3.917	0.9997
NB	2.223	2.959	0.9996	3.567	4.551	0.9992

methanol - water mobile phases, whereas the opposite applies to the pair HY - DE. Therefore, a ternary mixture methanol - acetonitrile - water could possibly improve the separation. The elution order, separation selectivity and resolution in the group CT - ML - MB - - DU depends not only on the concentration ratio of methanol and acetonitrile, but also on the sum of con-

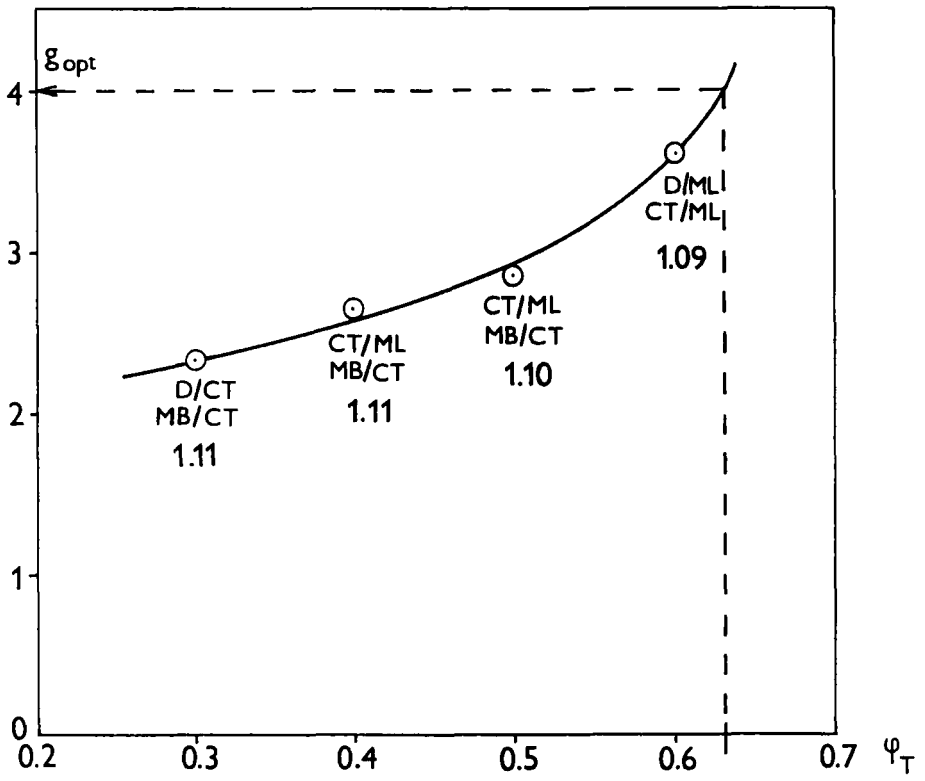

Figure 1. The plot of optimized ratio of the concentrations of acetonitrile to methanol, g_{opt}, in dependence on the sum of these concentrations, φ_T, in a reversed-phase linear ternary "solvent strength" gradient. The numbers at each point indicate the maximized minimum resolution for the resp[ective two pairs of phenylurea herbicides. Column as in Table 1.

centrations of these solvents in a ternary mobile phase, so that a precise control of the mobile phase composition is very important. Moreover, there are great differences in retention of the least (HY) and the most (NB) strongly retained sample components and gradient elution is necessary to accomplish the separation in a reasonable time. A ternary gradient could be

useful to achieve an adequate separation of the sample mixture.

To optimize the separation, a ternary "solvent strength" gradient was investigated first, with a constant pre-set concentration ratio of methanol and acetonitrile, $g = \varphi(ACN) / \varphi(MeOH)$. To select g, relative retentions were calculated for the pairs of solutes with adjacent peaks as a function of g for various sums of concentrations, φ_T = 30,40,50 and 60% v/v, using Eqn. /1/. For each φ_T, the optimum value of g, g_{opt}, was selected which yields maximized minimum relative retention, and plotted as a function of φ_T in Fig. 1, where the pairs of compounds most difficult to resolve are indicated. The minimum relative retention is fairly independent of φ_T, but $(g_{opt})^{-1}$ is shifted to higher values with increasing sum of concentrations of the organic solvents, φ_T. From the previous theory of gradient elution[1,4,5] it follows that the most important factor for resulting separation is the actual composition of mobile phase at the time of elution of a solute. For the present group of sample solutes (parameters m in between 2 - 4, the elution is accomplished in mobile phases with instantaneous φ_T about 60 - 65% (k'_f approximately 2 - 2.5), which corresponds to the value of $(g_{opt})^{-1}$ between 3.5 and 4. Thus, g_{opt} = 0.25 was selected for further optimization.

To optimize the "solvent strength" gradient, similar approach was used as for the simultaneous optimization of the gradient slope, B, abd the initial concentration of the organic solvent, A, in a linear binary gradient applied to reversed-phase systems[11-13]. A constant gradient time, t_G, and gradient volume, $V_G = t_G \cdot F_m$, from the start to the end of the gradient was pre-selected, V_G = 45 cm^3. (F_m is the flow-rate of the mobile phase). V_G relates the gradient slope to the concentration chan-

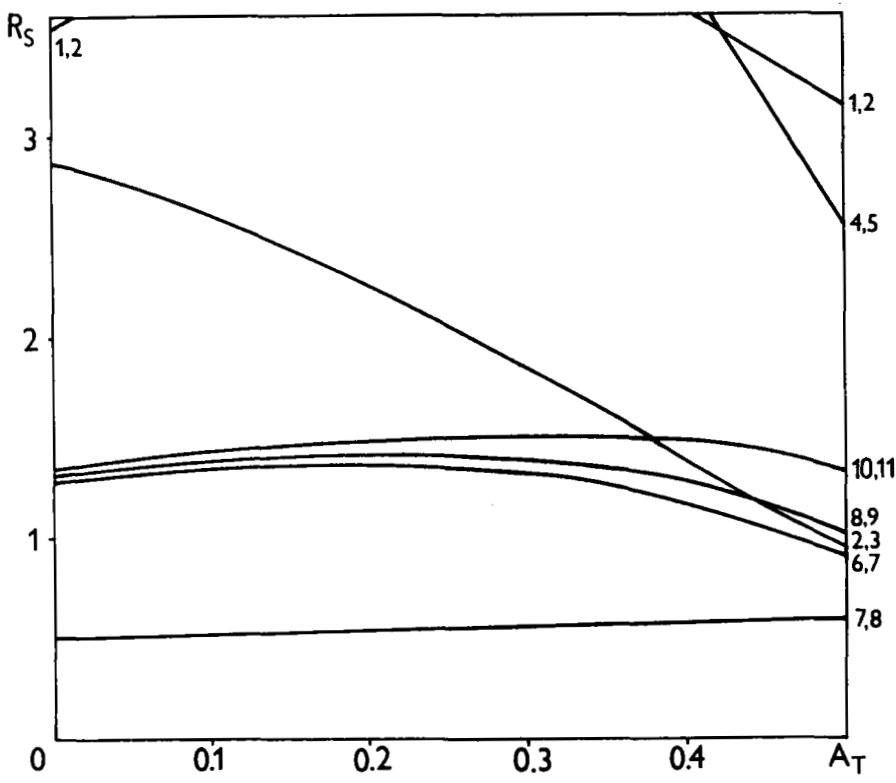

Figure 2. The "resolution map" for the optimization of ternary "solvent strength" gradients. R_s for the individual pairs of compounds plotted in dependence on the sum of the concentrations of acetonitrile and methanol, A_T(%v/v.10^{-2}), at the start of the gradient for g_{opt} = 0.25. Column as in Table 1. Compounds: 1 - HY; 2 - DF; 3 - FE; 4 - MX; 5 - MU; 6 - ML; 7 - CT; 8 - MB; 9 - DU; 10 - LU; 11 - CB; 12 - NB. V_0 = 45 cm^3.

ge from the initial, A_T, to the final, φ_G, concentration sum φ_T^{11-13}:

$$B = \frac{\varphi_G - A_T}{V_G} \qquad /19/$$

(φ_T was set to 1.0, i.e., 100% acetonitrile + methanol.) It has been shown earlier[12] that the selection of V_G is not critical for the result of optimization. A "resolu-

tion map" was calculated using Eqns. /10-12,7,8/ and plotted in Fig. 2 in dependence on A_T for g_{opt} = 0.25 and N = 3000 (Fig.2). As expected, no change in the order of elution occurs when A_T is changed and the dependence of resolution of the solute pairs 6,7 (ML/CT), 8,9 (MB/DU) and 10,11 (LU/CB) on A_T is relatively insignificant in the range of A_T from 0 to 0.3 (30% acetonitrile + methanol). For higher A_T values the resolution decreases and best separation is predicted for A_T between 0.2 to 0.3. The resolution for the pair 7,8 (CT/MB) is low and approximately constant over the whole range of A_T values tested.

To verify this prediction, three experiments were performed using a more efficient column and ternary "solvent strength" gradients in the predicted optimum range of A_T, for 20, 25 and 30% acetonitrile + methanol (Fig.3). There are no significant differences in resolution between the individual chromatograms, except for a slightly impaired separation of the pairs of compounds 2,3 (DF/FE) with increasing A_T, which is in agreement with the resolution map in Fig.2. To check the selection of g_{opt}, two additional experiments were run at higher values of g, 0.43 and 0.67, resp., with the optimized value of A_T = 0.2 (Fig.4). The separations are inferior in comparison to those with g_{opt} = 0.25 in Fig.3, namely the resolution of compounds 6 and 7 (ML/CT) is poor. As the separation of the group of compounds 6 - 9 (ML, CT,MB and DU) is stil not satisfactory even with the optimized "solvent strength" gradient, application of a ternary "selectivity" gradient was tested in the next step.

For the "selectivity" gradient, the same gradient time (volume) V_G = 45 cm^3 was pre-set as for the "solvent strength" gradient. As the separation selectivity

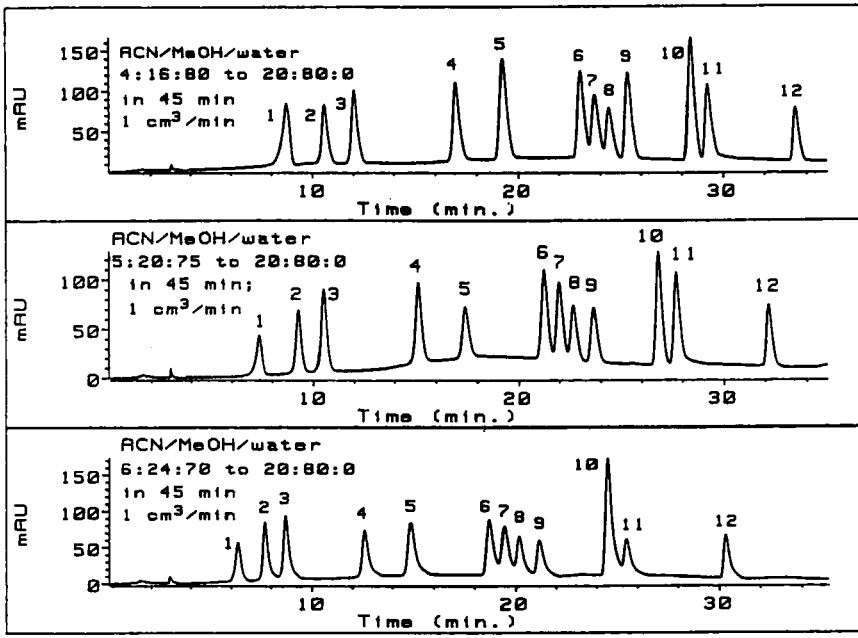

Figure 3. "Solvent Strength" gradients, g = 0.25.

for the later eluted sample components is better in acetonitrile - water mobile phases, but the early eluted compounds are better resolved in methanol - water mobile phases, a "selectivity" gradient of increasing concentration of acetonitrile and decreasing concentration of methanol should be used. From the data in Table 1, it can be calculated that the elution of the compounds 10,11 (LU/CB) with V_g = 40 to 45 cm^3 can be expected in mobile phases with φ_T = 0.4 (40% v/v) for a column with $V_M \cong 3$ cm^3. Neburon is eluted later, but it can be separated easily from the other sample compounds and a gradient step with increasing concentration of aceto-

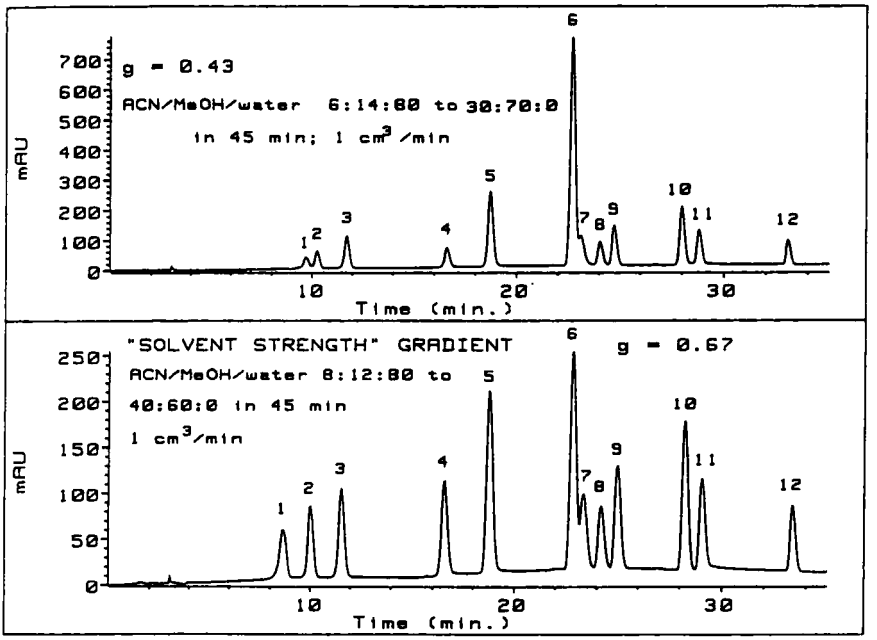

Figure 4. "Solvent Styrength" gradients, g = 0.43 and g = 0.67.

nitrile can be used to speed up its elution after the end of the "selectivity" gradient step.

To optimize the "selectivity" gradient of increasing acetonitrile concentration, resolutions of the individual pairs of sample solutes were calculated as a function of the initial concentration of acetonitrile, A_x, using Eqns. /10,13-19/ from the data in Table 1 with pre-set V_G = 45 cm^3 and $A_T = \varphi_T$ = 0.4. Fig. 5 shows the calculated resolution map, indicating a significant dependence of separation selectivity on A_x. Reversals of the elution order are predicted for compounds 6 and 7 (CT/ML) at the initial composition 12% acetonitrile + 28% methanol and for compounds 8 and 9 (MB/DU) at 24% acetonitrile + 16% methanol. Three maxima of minimum re-

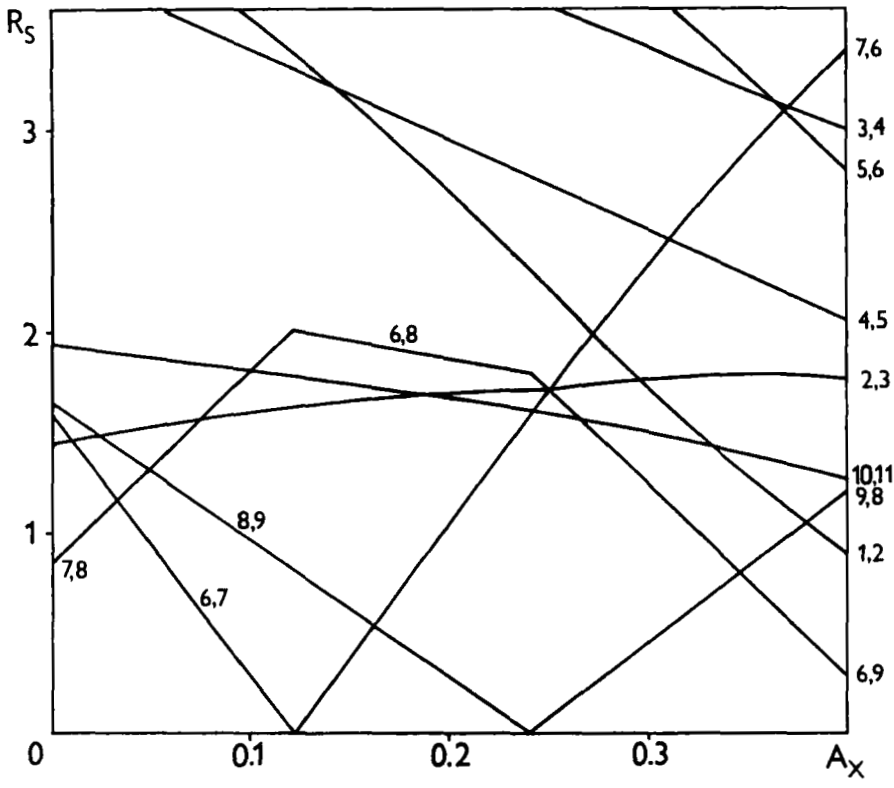

Figure 5. The "resolution map" for the optimization of ternary "selectivity" gradients. R_s for the individual pairs of compounds are plotted in dependence on the concentration of acetonitrile, A_x(% v/v.10^{-2}) at the start of the gradient for φ_T = 0.4 (40% v/v). Column as in Table 1; numbers of compounds as in Figure 2. V_0 = 45 cm^3.

solution are predicted from the resolution map at A_x = 0.03, 0.16 and 0.35, i.e., at the initial concentration 3, 16, and 35% acetonitrile, the highest maximum corresponding to the lowest of these concentrations. Two chromatograms near to the predicted optimum are shown in Fig. 6. In agreement with the predicted resolution map, the resolution of the compounds 6 and 7 (ML/CT) for A_x = 0 is improved at a cost of slightly decreased reso-

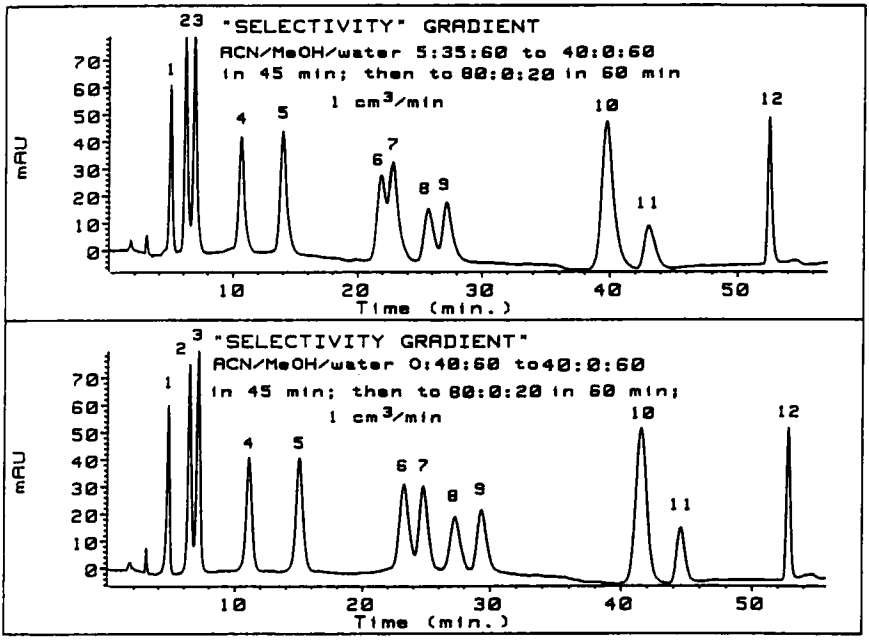

Figure 6. "Selectivity Gradients."

lution of the compounds 7 and 8 (CT/MB) in comparison with the chromatogram obtained at A_x = 0.05 (5% acetonitrile). Fig. 7 compares two non-optimized chromatograms. These confirm the predicted behaviour (Fig. 5), i.e., the gradient with A_x = 0.2 (20% acetonitrile) shows practically no separation of the compounds 8 and 9 (MB/DU) and poor separation of compounds 6 and 7 (ML/CT). The gradient starting at 30% acetonitrile (A_x = 0.3) shows a better separation of the "critical" group of compounds 6-9, with lowest separation of the pairs 6 and 9 (ML/DU) and 9 and 8 (DU/MB), but a slightly impaired separation of compounds 1 and 2 (HY/DF). The resolution of compounds 2 and 3 (DF/FE) is slightly improved for

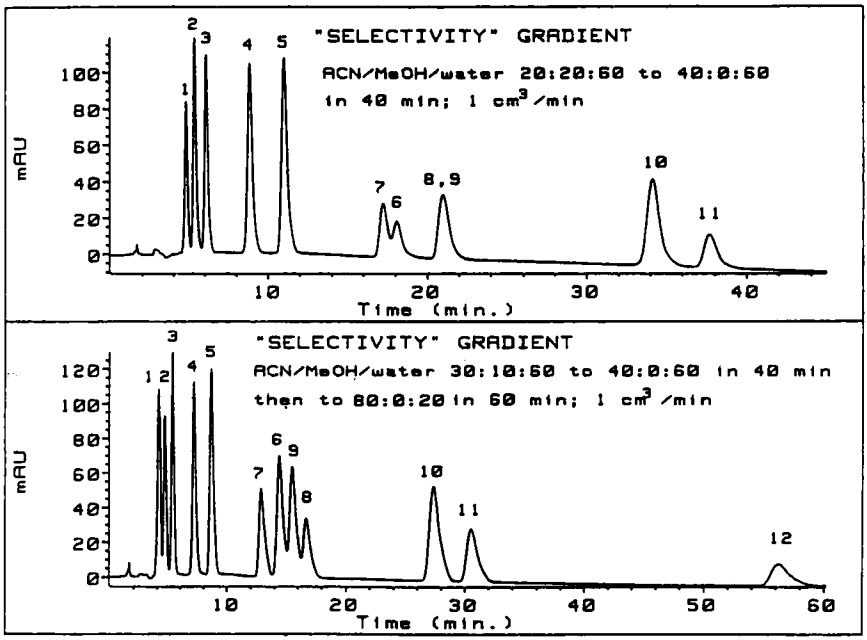

Figure 7. "Selectivity Gradients."

gradients with A_x = 0.2 - 0.3 in comparison to the gradients with A_x = 0 - 0.05.

Finally, a "combined selectivity-solvent strength" gradient was optimized to find if it can offer a better separation than the "selectivity" gradient. First, a binary gradient of acetonitrile was selected to yield the best separation of the group of compounds 6 - 11, provided zero initial concentration of acetonitrile A_x = 0 and the pre-set time of gradient 45 min (V_G = 45 cm^3 at F_m = 1 cm^3/min). Calculated simulated chromatograms showed the best resolution of this group of compounds for the gradient with final concentration 40% acetonitrile in 45 min (φ_G = 0.4). On this gradient, a gradient of decreasing concentration of methanol was superimpo-

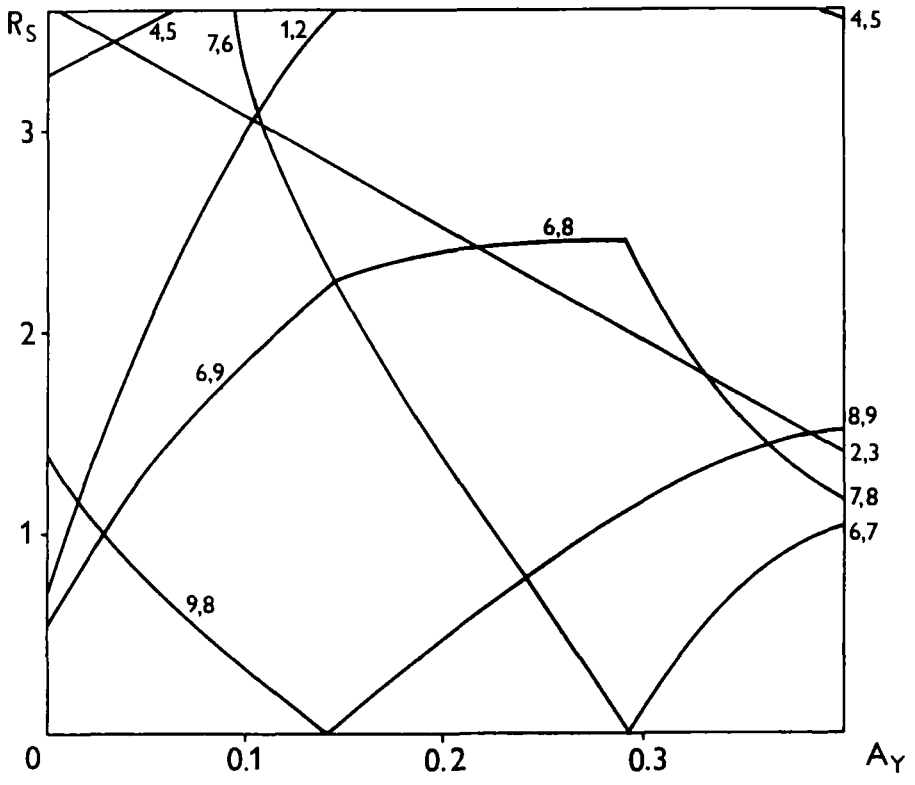

Figure 8. The "resolution map for the optimization of ternary "combined selectivity-solvent strength" gradients. R_s for the individual pairs of compounds are plotted in dependence on the concentration of methanol, A_y(% v/v.10^{-2}) at the start of the gradient for zero initial concentration of acetonitrile, $A_x = 0$. Final conditions: 40% acetonitrile, 0% methanol; V_G = 45 cm^3. Column as in Table 1; numbers as in Figure 2.

sed, which should achieve zero concentration of methanol in 45 min. The map of resolution was calculated for the individual pairs of sample solutes in dependence on the initial concentration of methanol, A_y, using Eqns. /4, 6-8/, (Fig. 8). Like for the "selectivity" gradient, reversalsin the order of elution were observed for the compounds 8 and 9 (MB/DU) at A_y = 0.14 and for the compounds 6 and 7 (ML/CT) at A_y = 0.29. Three maxima of

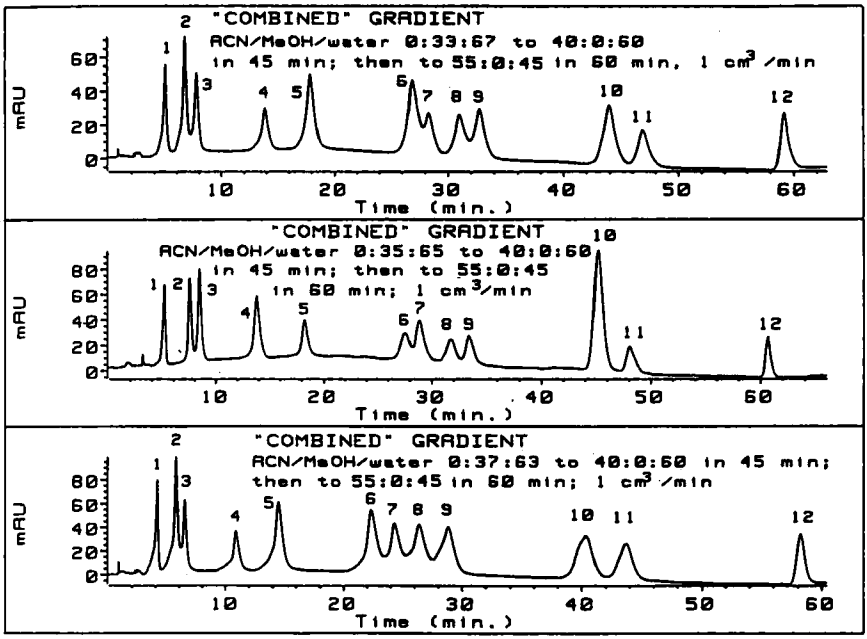

Figure 9. "Combined Gradients."

minimum resolution were predicted for A_y = 0.03, 0.24 and 0.35 - 0.4, the last yielding the highest R_S(min). Fig. 9 shows three chromatograms obtained in the experiments with "combined" ternary gradients starting in the optimum region at 33, 35 and 37% methanol. In this order, the resolution of the compounds 6 and 7 (ML/CT) and 8 and 9 (MB/DU) increases at the cost of impairing resolution of the compounds 7 and 8 (CT/MB) and the resolution of the compounds 2 and 3 (DF/FE) decreases in agreement with the predicted resolution map (Fig. 8). The best separation is achieved using the gradient from 0% acetonitrile and 35% methanol to 40% acetonitrile and 0% methanol in 45 min. This optimized "combined" gradient is very close to the optimized "selectivity"

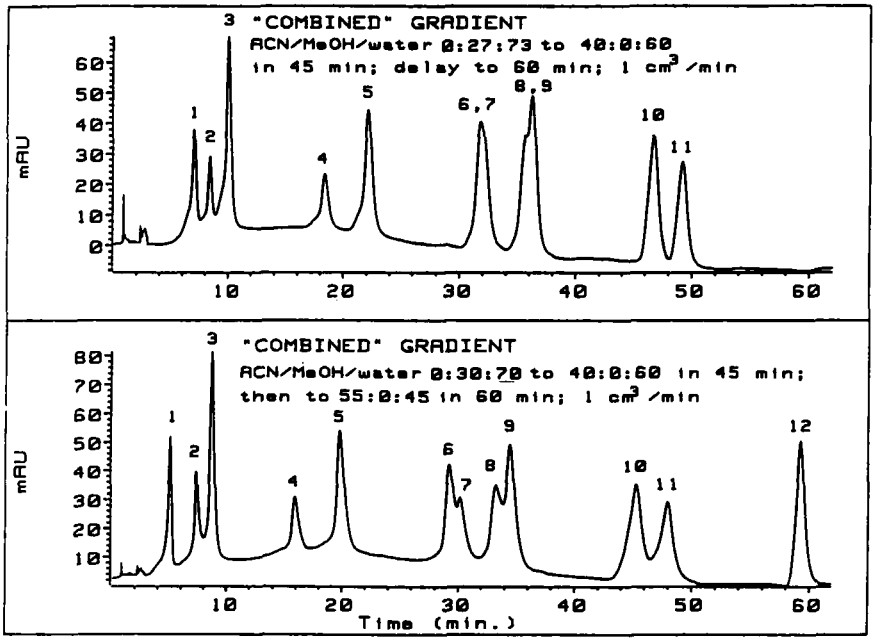

Figure 10. "Combined Gradients."

gradient in Fig.6 as both the gradient profile and the resolution achieved are concerned. Thus, the "combined" gradient did not yield a significant improvement in separation of sample compounds in comparison to the optimized "selectivity" gradient. To verify the predicted resolution map on non-optimized gradients, Fig. 10 shows the chromatograms obtained with "combined" gradients starting at 27 and 30% methanol and 0% acetonitrile, run in 45 min to 40% acetonitrile and 0% methanol. The first chromatogram shows expected poor resolution of the compounds 6 and 7 (ML/CT) and 8 and 9 (MB/DU) and the first of this pair of compounds is predicted to be unresolved

TABLE 2

Predicted (p) and experimental (e) elution volumes, V_g, in cm^3, of substituted phenylureas.

A: Ternary "selectivity" gradient from 0% acetonitrile + 40% methanol to 40% acetonitrile + 0% methanol in 45 min (Eqns. /10, 15-16/ are used for predictive calculations).

B: Ternary "solvent strength" gradient from 6% acetonitrile + 24% methanol to 20% acetonitrile + 80% methanol in 45 min (Eqns. /10-12/ are used for predictive calculations).

Column as in Table 1; flow rate 1 cm^3/min.

	A		B	
Solute	$V_g(p)$	$V_g(e)$	$V_g(p)$	$V_g(e)$
HY	4.81	4.66	5.77	6.36
DF	6.95	6.31	7.79	7.71
FE	7.71	7.00	8.78	8.73
MX	11.27	10.91	12.15	12.63
MU	15.04	14.82	14.71	14.90
ML	21.62	22.95	18.22	18.74
CT	24.39	24.49	19.21	19.46
MB	25.09	26.97	19.63	20.18
DU	27.66	29.04	20.69	21.19
LU	38.34	41.16	24.03	24.55
CB	42.23	44.23	25.16	25.47
NB	-	-	29.92	30.33

in the second chromatogram, in agreement with the experiment.

To illustrate the precision of predictive calculations, two examples of the experimental and calculated elution volumes at different profiles of ternary gradients are shown in Table 2. The average deviation of the calculated elution volumes from the experimental values is approximately 5 - 7% rel., which does not allow to predict the exact values of resolution of adjacent peaks accurately enough for a perfect fit of the predicted and experimental chromatograms. Nevertheless, the examples shown in this work demonstrate that accurate elution order is predicted and the range of the values of gradient parameters likely to yield the optimum separation is estimated closely enough to make it possible to optimize satisfactorily ternary gradients after two or three additional "fine-tuning" runs in the predicted optimum parameter range.

GLOSSARY OF THE TERMS USED

a - experimental constant in the eqn. /5/

a_G - mean value of the constants a_x, a_y in the ternary mobile phase - see eqn. /6/

a_T - value of a at the beginning of the ternary gradient - see eqn. /11/

a_x, a_y - experimental constants a for the binary mobile phases water - org. solvent x and water - org. solvent y, resp.

$g = \varphi_x : \varphi_y$ - ratio of the concentrations of the more efficient eluting components in the ternary mobile phase

g_{opt} - optimized g

k' - capacity factor of the solute

k'_f - instantaneous k' at the time of elution of band maximum

m - experimental constant in the eqn. /5/

m_T - value of m at the beginning of the ternary gradient - see eqn. /12/

m_x, m_y - experimental constants m for the binary mobile phases water - org. solvent x and water - org. solvent y, resp.

t_G - time of the gradient, i.e. the time from the start till the end of the gradient elution, in min.

w_1, w_2 - bandwidths of the solute compounds 1 and 2, resp., in cm^3

w_g - bandwidth of the solute under gradient-elution conditions, in cm^3

A - φ at the beginning of the gradient elution with a linear binary gradient

A_x, A_y - φ_x and φ_y at the beginning of the gradient elution using a linear ternary gradient

B - slope of the linear binary gradient in volume per cents.10^{-2} per 1 cm^3 of the eluate

B_x, B_y - slopes of the changes of φ_x and φ_y, resp., during a linear ternary gradient, in volume per cents.10^{-2} per 1 cm^3 of the eluate

F_m - flow rate of the mobile phase, in $cm^3.min^{-1}$

N - theoretical plate number of the column used

R_s - resolution of the solute compounds 1 and 2

V - volume of the eluate, in cm^3

V_G - gradient volume, i.e. the volume of the eluate from the start till the end of the gradient elution

V'_g - net elution volume of the solute under gradient-elution conditions

V'_{gi} - V'_g of the sample compound the retention of which should be minimized

V_m - column dead volume, in cm^3

V_R - elution volume under isocratic conditions, in cm^3

φ - concentration of the more efficient eluting component in the binary mobile phase, in volume per cents.10^{-2}

φ_G - φ at the end of the gradient elution

$\varphi_T = \varphi_x + \varphi_y$ - concentration sum of the more efficient eluting components x and y in the ternary mobile phase

φ_x, φ_y - concentrations of the more efficient eluting components x and y, resp., in the ternary mobile phase, in volume per cents.10^{-2}

REFERENCES

1. Jandera,P. and Churáček,J., Gradient Elution in Column Liquid Chromatography, Elsevier, Amsterdam, 1985.
2. Schoenmakers,P.J., Optimization of Chromatographic Selectivity, Elsevier, Amsterdam, 1986.
3. Berridge,J.C., J.Chromatogr., 244, 1, 1982.
4. Snyder,L.R., Dolan,J.W. and Gant,J.R., J. Chromatogr., 165, 3, 1979.
5. Dolan,J.W., Gant,J.R. and Snyder,L.R., J. Chromatogr., 165, 31, 1979.
6. Jandera,P. and Churáček,J., J.Chromatogr., 91, 223, 1974.
7. Jandera,P. and Churáček,J., J. Chromatogr., 170, 1, 1979.
8. Jandera,P., Churáček,J. and Svoboda,L., J. Chromatogr., 174, 35, 1979.
9. Jandera,P. and Churáček,J., Adv. Chromatogr., 19, 125, 1980.
10. Jandera,P. and Churáček,J., J. Chromatogr., 192, 19, 1980.

11. Jandera,P. and Špaček,M., J. Chromatogr., 366, 107, 1986.
12. Jandera,P., J. Liq. Chromatogr., 12, 117, 1989.
13. Jandera,P., J.Chromatogr., 485, 113, 1989.
14. Schmidt,J. and Mc.Clain C.,J. Chomatogr., 419, 1, 1987.
15. Schmidt,J., J. Chromatogr., 485, 421, 1989.
16. Baba,Y., Yoza,N. and Ohashi,S., J. Chromatogr., 348, 27, 1985.
17. Baba,Y., Yoza,N. and Ohashi,S., J. Chromatogr., 350, 119, 1985.
18. Baba,Y., J. Chromatogr., 485, 143, 1989.
19. Baba,Y. and Ito,M.K., J.Chromatogr., 485, 647, 1989.
20. Parente,E.S. and Wetlaufer,D.B., J. Chromatogr., 355, 29, 1986.
21. Stout,R.W., Sivakoff,S.I, Ricker,R.D and Snyder,L. R., J. Chromatogr., 353, 439, 1986.
22. Snyder,L.R., Dolan,J.W. and Quarry,M.A., Trends Anal. Chem., 6, 106, 1987.
23. Snyder,L.R. and Quarry,M.A., J. Liq. Chromatogr., 10, 1789, 1987.
24. Dolan,J.W., Snyder,L.R. and Quarry,M.A., Chromatographia, 24, 261, 1987.
25. Ghrist,B.F.D., Cooperman,B.S. and Snyder,L.R., J. Chromatogr., 459, 1, 1988.
26. Ghrist,B.F.D. and Snyder,L.R., J. Chromatogr., 459, 25, 1988.
27. Ghrist, B.F.D. and Snyder,L.R., J.Chromatogr., 459, 43, 1988.
28. Snyder,L.R., Quarry,M.A. and Glajch,J.L., Chromatographia, 24, 33, 1987.
29. Dolan,J.W., Lommen ,D.C. and Snyder,L.R., J. Chromatogr., 485, 91, 1989.
30. Stuart,J.D., Lisi,D.D. and Snyder,L.R., J. Chromatogr., 485, 657, 1989.

31. Glajch,J.L. and Kirkland,J.J., Anal. Chem., 54, 2593, 1982.
32. Kirkland,J.J. and Glajch,J.L., J. Chromatogr., 255, 27, 1983.
33. Glajch,J.L. and Kirkland,J.J., J. Chromatogr., 485, 51, 1989.
34. Glajch,J.L. and Kirkland,J.J., J. Chromatogr. Sci. 25, 4, 1987.
35. Jandera,P., Churáček,J. and Colin,H., J. Chromatogr., 214, 35, 1981.