Journal of Chromatography, 298 (1984) 1–20
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 16,789

A SIMPLE PRAGMATIC OPTIMIZATION PROCEDURE FOR SOME PARAMETERS INVOLVED IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC SEPARATIONS: COLUMN DESIGN, TEMPERATURE, SOLVENT FLOW-RATE AND COMPOSITION

J. P. BOUNINE
Rhone Poulenc Santé, Centre de Recherches de Vitry, 13 Quai Jules Guesde, 94400 Vitry sur Seine (France)
and
G. GUIOCHON* and H. COLIN
Ecole Polytechnique, Laboratoire de Chimie Analytique Physique, Route de Saclay, 91128 Palaiseau Cedex (France)
(Received April 2nd, 1984)

SUMMARY

A simple approach to optimize separations in liquid chromatography is described. The method is based on the existence of linear relationships between the retention parameters (k' or $\log k'$) and the variables to be optimized (solvent composition and flow-rate, temperature, column length and particle size), both in normal- and reversed-phase chromatography. The validity of these linear relationships is discussed and justified by some experimental results. The methodology developed allows any type of optimization with various constraints, such as minimum time of analysis with a certain minimum resolution between each peak and a column inlet pressure lower than a given limit.

INTRODUCTION

The search for efficient optimization procedures is a fundamental aspect of liquid chromatography (LC), which has become very important with the development of the routine application of LC.

For some time, most attention was devoted to the optimization of the column design parameters (column length, particle size) and of the solvent flow-rate[1–4]. Little attention was paid to the rôle of the solvent composition, probably because its influence on peak resolution was not clearly understood. This situation has changed recently. A number of advances have been made in the understanding of the influence of solvent composition on retention, and various schemes for the optimization of mobile phase composition have been proposed[5–8]. Automatic equipment, controlled by microcomputers, is available which permits a programmed, stepwise change in the composition of a multisolvent eluent between successive analyses. Optimum conditions for the achievement of a given separation can then be selected, either by the

0021-9673/84/$03.00

analyst using his best judgment in interpolating results or by the computer using optimization algorithms. Usually, the column design and operating parameters are not considered in these schemes, which are already quite complex and time-consuming because of the amount of data required.

There is, accordingly, a need for a more exhaustive optimization procedure which would take account of all parameters but would be simple to perform. Optimization procedures can be classified into four main categories:

empirical methods,

graphic methods such as window diagrams (which are refined, systematic versions of empirical methods),

statistical methods such as the SIMPLEX techniques,

theoretical methods.

In the first three approaches, retention data are measured in a part of the multidimensional space spanned by the various parameters. Then, more or less systematic procedures, either manual (empirical, graphic, etc.) or computerized (Simplex, etc.) are used to approach the optimum conditions, by successive trial and error. In the last case, theoretical relationships between retention times and experimental parameters are assumed, measurements are carried out to determine the values of the different constants appearing in these relationships and the optimum conditions are derived from these values.

The present work is a contribution to the development of optimization techniques belonging to this last group. Obviously the degree of complexity of these methods is directly related to the complexity of the relationships used in the model, and a compromise is made between the accuracy of the prediction and the number of measurements necessary to acquire the relevant data. We have chosen to sacrifice accuracy to some extent by using only linear relationships. Therefore, in theory, only two measurements are necessary for each parameter. The acumen of the analyst in the selection of the data points and the precision of the measurements will have a direct bearing on the quality of the results.

THEORETICAL

Principle

We have chosen to base our procedure on the exclusive use of linear relationships. This requires a number of approximations: some are conventional, others are not and may seem bold to many. They are acceptable, however, within some range of the corresponding parameters. Furthermore, in all applications, the set of relationships has always lead to surprisingly good results.

Various indicators have been used to characterize a separation: the resolution between two closely eluted compounds, the analysis time, the pressure drop, the detection limit (or the extent of sample dilution during the analysis) or a combination of these criteria such as the chromatographic separation function[9], etc. It is clear, however, that it is extremely difficult and regrettable to reduce the quality of a separation to a single number because this necessarily corresponds to a loss of information.

An interactive procedure based on preliminary experiments is preferable. With such an approach, the analyst can:

specify some constraints (*i.e.*, the minimum resolution, the analysis time, etc.)

ask the computer some specific questions (*i.e.*, the resolution between two particular solutes under some particular experimental conditions) or obtain a simulated chromatogram

adjust his requirements to achieve a compromise he will consider as good, using information difficult to quantitate such as solvent cost and toxicity, detector noise under some particular conditions, etc.

A large number of parameters can be optimized. Intuitively, and this is supported by experience, the larger this number the better is the result, but also the larger is the number of preliminary measurements to be carried out. The critical parameters are listed in Fig. 1, on the outer circle, while the basic criteria of the quality of a chromatographic separation are listed on the inner circle. On the intermediate circle are important constants determined by the parameters on the outer circle and which mediate their influence on the quality of the separation. The most important relationships existing between the parameter are indicated by arrows.

For instance, a change in column temperature results in a change of the column capacity factor and the mobile phase viscosity. The former results in a change of resolution, analysis time and detection limits, while the latter results in a change in pressure drop (or velocity) and efficiency, and therefore an additional change in resolution and detection limits. These interactions between so many parameters make the problem complex, even though each single relationship involved is rather simple.

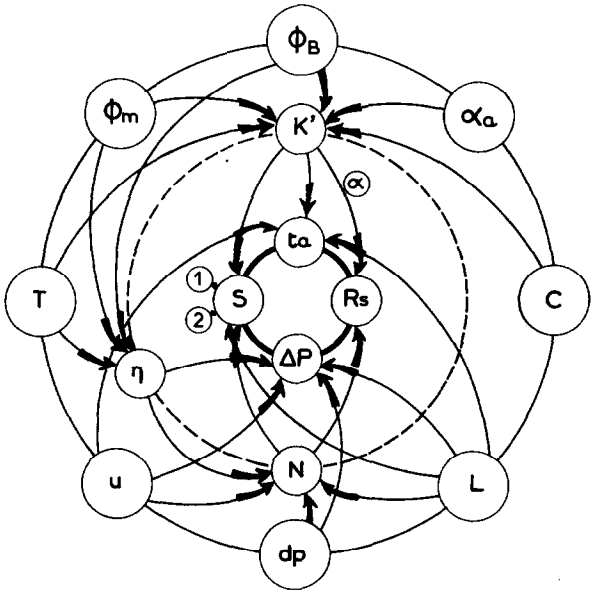

Fig. 1. Interactions between variables, intermediate parameters and criteria of the quality of a separation. Variables: φ_B = volume fraction of the strongest solvent; φ_M = volume fraction of a modifying solvent; T = temperature; u = flow velocity; d_p = particle size; L = column length; α_a = adsorbent activity; C = carbon content of an alkyl-bonded silica, or counter-ion concentration. Intermediate parameters: k' = capacity factor; N = column efficiency; η = solvent viscosity; α = selectivity factor. Criteria of quality: t_a = analysis time; R_s = resolution; ΔP = pressure drop; S = response factor (peak height) for weakly concentrated solute but sample available in large amount (1) and for limited quantity of sample (2).

Accordingly, we distinguish two steps in our optimization: the derivation of the relationships between the criteria of analysis quality and the intermediate parameters, and the derivation of the basic relationships between the column design and operating parameters and these intermediate parameters.

Derivation of the quality criteria equations

These criteria are the resolution between two successive compounds, the analysis time, the detection limits and the column pressure drop.

Resolution. The resolution between two successive compounds A_i and A_{i+1} is given by the classical definition

$$R_s = 2 \cdot \frac{t_{R_{i+1}} - t_{R_i}}{W_{i+1} + W_i} = \frac{\sqrt{N}}{2} \cdot \frac{k'_{i+1} - k'_i}{k'_{i+1} + k'_i + 2} \tag{1}$$

(*cf.*, List of Symbols and Fig. 1). The derivation of the second part of eqn. 1 assumes that the two peaks are symmetrical; however, perfectly symmetrical peaks are rarely obtained. For this reason, the program can take peak asymmetry into account if the analyst so desires. Then the apparent efficiency is defined from the width at half-height:

$$N_i = 5.54 \left(\frac{t_R}{W_{1/2}} \right)^2 \tag{2}$$

The asymmetry, A_S, is also defined at half-peak height by the ratio l_2/l_1 (*cf.*, Fig. 2). In the optimization, it is assumed that the two peak halves correspond to gaussian profiles with different standard deviations. This is not exactly true for the second half which usually presents an exponential decay[10]. In most cases, however, the approximation is acceptable. The plate number in eqn. 1 must thus be replaced by the average plate number, N_a, corresponding to the second half of the first peak and the first half of the second peak, according to:

$$N_a = \frac{1}{8} \left[N_i \left(\frac{1 + A_{s,i}}{A_{s,i}} \right) + N_{i+1} \left(\frac{1 + A_{s,i+1}}{A_{s,i+1}} \right) \right] \tag{3}$$

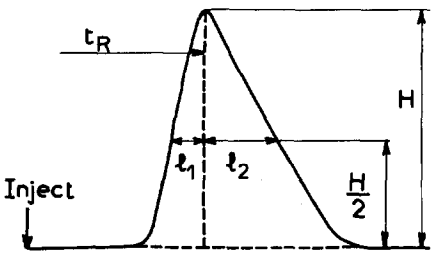

Fig. 2. Characteristic parameters of an eluted peak.

The paradox of a better resolution between strongly unsymmetrical peaks than between symmetrical ones for a given plate number has already been underlined by Kirkland *et al.*[11]. It has limited effect in practice as the column efficiency is usually low for strongly unsymmetrical peaks.

Analysis time. Assuming that the end of a peak is reached 4σ after the maximum, the time of analysis can be calculated from the capacity ratio of the last solute, k'_n, and the asymmetry of the last peak:

$$t_A = (1+k'_n)\, t_o \left[1 + \frac{2\, A_{s,n}}{(1+A_{s,n})\sqrt{N_n}} \right] \tag{4}$$

Detection limits. These depend both on the detector characteristics (sensitivity and noise) and on the dilution of the sample during the chromatographic analysis. Only the last factor is influenced by the column parameters. There are basically two kinds of problems in trace analysis, depending upon whether the sample size is limited or not.

When a large amount of sample is available, it is possible to inject the appropriate amount of sample to reach the maximum acceptable degree of column overload, defined either by a drop in the plate number or by a change in capacity factor. The concentration of solute that produces the stationary phase overload depends only on the chromatographic system characteristics and on the level of acceptable loss of performance, but not on the column diameter. If the diameter were to be changed, a different volume of sample would need to be injected but the required concentration would remain the same. This procedure permits one to account for situations which are typical of compounds less strongly retained than either the trace components considered (plate overload) or the main components of the mixture (stationary phase overload).

If the maximum sample size which can be introduced in the column is larger than the amount of sample available, the column diameter is too large and some excessive sample dilution will occur, hence a loss in sensitivity, which can be avoided, at least in theory, by turning to a narrower column. This simple reasoning assumes that the detector response and noise are unchanged by a marked decrease in flow-rate and that the detector cell volume does not contribute markedly to band broadening[12].

At any rate, it is possible to calculate the peak height using the equation:

$$y_M = S\, C_M = \frac{4\, Sm\sqrt{N}}{\pi\, \varepsilon_c\, (1+k')L\, d_c^2\sqrt{2\pi}} \tag{5}$$

The detection limits can be optimized by either arranging the peak height to be maximal or placing some constraint on the amount of dilution. If the maximum allowed sample size can be injected, however, there is little place for further optimization.

Inlet pressure. This is related to the column parameters by the integrated Darcy law:

$$\Delta P = u \eta L / k^o d_p^2 \tag{6}$$

The pressure is of secondary importance as long as it is markedly smaller than the maximum pressure at which the pump can work. This determines the upper limits of the column length and solvent velocity and the lower limit of the particle size which can be used. The calculation of ΔP requires the knowledge of the solvent viscosity. A table of the viscosity of the most frequently used solvents is introduced into the computer memory and the viscosity at intermediate compositions is interpolated linearly as a function of volume fraction between pure solvents. This is acceptable for normal phase chromatography. For the solvents used in reversed-phase chromatography, a table of viscosity as a function of composition is introduced and again values of the viscosity at intermediate concentrations are interpolated linearly. If viscosities are not available at various temperatures, the program provides for a 1% decrease in the viscosity per °C between 10 and 70°C[13].

Derivation of the column efficiency

Since no theoretical equation can predict *a priori* the efficiency of a chromatographic column, it is preferable to use as simple a relationship as possible between the plate height and the eluent velocity. A simple empirical relationship is easier to use than a theoretical expression which would need the determination of several parameters difficult to measure.

Since, in practice, columns are always used at relatively large reduced velocities, where the contribution to the plate height due to axial dispersion is small, we use an old, empirical, two-term equation:

$$H = A d_p + C d_p^2 u \tag{7}$$

The coefficients A and C may change from one compound to the other and are functions of the mobile phase composition. Since we are always dealing with the same compounds during the optimization of the experimental conditions of an analysis, only the relative variations of H with experimental conditions are important. For example, because the diffusion coefficient is inversely proportional to the solvent viscosity it can be assumed as a first approximation that the plate height is proportional to the solvent viscosity:

$$H = H_o \eta/\eta_o \tag{8}$$

This does not account completely for the change in plate height associated with a change in solvent composition, because of the variation of the capacity factor which itself induces a change in the HETP. In practice, this variation is small and again can be well accounted for by a linear relationship

$$H = H_o (1 + a \varphi_B) \tag{9}$$

where φ_B is the volume fraction of the stronger solvent. If measurements are carried out at two different solvent compositions, the following equations can be written

$$H_2 = H_1 + a\,(\varphi_{B2} - \varphi_{B1}) \tag{10a}$$

or

$$H_2 = H_1\,[1 + a_1\,(\varphi_{B2} - \varphi_{B1})] \tag{10b}$$

where $a_1 = a/H_1$.

In addition to its influence on viscosity and retention, already accounted for through eqns. 8 and 10, the temperature has a residual effect on the plate height which can be taken into account using the following linear relationship:

$$\log H_2 = [1 + a_2\,(T_2 - T_1)] \log H_1 \tag{11}$$

In summary, two measurements of the plate height at different mobile phase velocities, using the same solvent composition and temperature, permits the calculation of constants A and C in eqn. 7. Combination of eqns. 7–11 gives:

$$\log H_2 = [a_2(T_2 - T_1) + 1] \log \left\{ (Ad_p + Cd_p^2 u)\,[1 + a_1(\varphi_{B2} - \varphi_{B1})]\,\frac{\eta}{\eta_o} \right\} \tag{12}$$

A third measurement carried out at the same temperature with a different solvent composition and a last one at a different temperature permits the successive determination of a_1 and a_2, which are usually small.

Derivation of the column capacity factor

The column capacity factor depends mainly on the composition of the solvent mixture used, and on some properties of the stationary phase (specific surface area in normal phase chromatography, ligand density and chain length in reversed-phase chromatography).

A simple way to optimize the mobile phase composition is first to determine empirically the eluotropic strength which gives convenient column capacity factors. Then the selectivity, *i.e.*, relative retentions, is adjusted at constant eluotropic strength. Using this approach, Glajch *et al.*[8] determined a map of relative retentions as a function of the composition of mixtures of water with methanol, acetonitrile and tetrahydrofuran from which they derived the composition giving maximum selectivity. This is a three-dimensional extension of the window-diagram method of Laub and Purnell[14–16]. It has the disadvantage of requiring a large number of experimental measurements: ten at constant eluotropic strength, plus those necessary to adjust the eluotropic strength to a convenient value. This approach is difficult to use with complex mixtures because of the problems associated with solute identification. Separate measurements must be carried out on the pure components (which must be identified and available) or on simple mixtures of them. Finally this method does not take into account the column parameters, the pressure, the temperature and the detection sensitivity.

Another approach involves a change of the mobile phase polarity which causes a simultaneous change in the capacity factors and selectivities[17–19]. When the composition of a two-solvent mixture changes regularly, the variation of the capacity factors of most compounds can be correctly accounted for by simple logarithmic relationships. As each one of the two solvents itself can be a mixture, there is no practical limit to the complexity of the mixtures which can be studied.

The polarity parameter, P, as defined by Snyder[20], permits the derivation of the capacity factor for all compositions from one measurement

$$\log k'_{(2)} = \log k'_{(1)} + (P_{(1)} - P_{(2)}) \tag{13}$$

where k'_1 and k'_2 are the column capacity factors observed with the solvents of polarity P_1 and P_2, respectively. For solvent mixtures, the polarity parameter is related to the volume fraction, φ_i, of each component:

$$P_{(m)} = \sum_i \varphi_i P_i \tag{14}$$

Eqns. 13 and 14 are very useful to determine the mixture composition permitting the adjustment of capacity factors in a given range or close to a preset value. They cannot be used, however, for an accurate assessment of relative retention, hence of the resolution between two peaks. To obtain more precise relationships one must distinguish between normal and reversed-phase chromatography.

Normal phase chromatography. The classical model developed by Snyder[21–23] for normal phase liquid–solid chromatography assumes that the solute–solvent interactions are weaker than the solute–adsorbent interactions. The energy involved in this process (free energy of adsorption) results from the replacement of solvent molecules by a given solute molecule which occupies the same area on the adsorbed layer. The contribution to the free energy of bulk solution interactions is thus neglected. The capacity factor is related to the void volume, the solvent eluotropic strength, the adsorbent activity and the molar surface area of the solute by:

$$\log k' = \log V_0 + \alpha(S^\circ - A_s \varepsilon^\circ) \tag{15}$$

This equation is not easy to use in practice. Although eluotropic strengths have been tabulated, the solute molecular surface area and the adsorbent activity are difficult to determine. A simple empirical relationship is thus preferable. Such relationships have been suggested, involving a linear dependence of the capacity factor, or its inverse, on the concentration of the strongest solvent in a binary mixture, or a proportionality of the capacity factor to some arbitrary power of this concentration.

The serious problem in normal phase chromatography is to maintain a constant adsorbent activity, while changing solvent composition and possibly temperature. The adsorbent activity depends on the amount of water adsorbed on the support. This in turn depends on the solubility of water in the solvent, which increases with increasing solvent polarity. It has been shown with various solvent mixtures that the column capacity factor is related only to the water content of the solvent, through

$$k' = a + \frac{b}{X_{H_2O}} \tag{16}$$

where a and b are *ad hoc* constants and X_{H_2O} is the mole fraction of water. The nature of the polar solvent is irrelevant or very nearly so. For instance, this is true for mixtures of isooctane, diisopropyl ether and methanol.

When working with isohydric solvents, a simpler relationship can be used. Such solvents have the property of being in equilibrium with a gas phase having a given partial pressure of water. Accordingly, after equilibration with an adsorbent, they all give a sorbed layer with the same water concentration. Table I gives the water content of a set of isohydric solvents. As is seen, the water content varies widely from one solvent to another. The water content of a binary solvent mixture is given by:

$$X_{1,2} = X_1 \varphi_1 + X_2 \varphi_2 \tag{17}$$

It depends essentially on the amount of polar solvent in the mixture. Unless mixtures of solvents with relatively similar water solubilities are used, or mixtures with a very low concentration of the polar solvent, the water content of the weak solvent can be neglected in the calculation of the water mole fraction introduced in eqn. 16. Accordingly, when optimizing the composition of a mixture of a non-polar (or weakly polar) solvent and a polar solvent of constant water content, the volume fraction of the polar solvent can be used to replace the water mole fraction (to which it is proportional) and eqn. 16 can be rewritten:

$$1/k' = a' + b' \varphi_B \tag{18}$$

Here a' is the inverse of the capacity factor in the pure low polarity solvent and b' is the difference $1/k'_p - a'$ where k'_p is the capacity factor in the polar solvent. This assumes that eqn. 18 is satisfied over the whole range of solvent composition. This is the case only when the water concentration in the polar solvent is markedly below

TABLE I

ELUENT STRENGTH AND ISOHYDRIC WATER CONTENT OF SOME SOLVENTS

Solvent	*Eluent strength*	*Isohydric water content*	
		% Volume	*Mole fraction*
Methanol	0.73	5.2	$1.1 \cdot 10^{-1}$
Isopropanol	0.63	0.7	$2.9 \cdot 10^{-2}$
Acetonitrile	0.50	0.22	$6.4 \cdot 10^{-3}$
Ethyl acetate	0.45	0.06	$3.3 \cdot 10^{-3}$
Dioxane	0.43	0.14	$6.6 \cdot 10^{-3}$
1,2-Dichloroethane	0.38	0.007	$3.1 \cdot 10^{-4}$
Tetrahydrofuran	0.35	0.13	$5.9 \cdot 10^{-3}$
Diisopropyl ether	0.22	0.008	$6.3 \cdot 10^{-4}$
Cyclohexane	0.03	0.0004	$2.4 \cdot 10^{-5}$

saturation and when it is completely miscible, in all proportions, with the apolar solvent. When these conditions are not met, eqn. 18 remains valid in some range of φ_B but the parameters a' and b' loose their physical meanings.

Depending on the analyst, the program can use either eqn. 16 or 18. In either case, only two independent analyses are required to determine the parameters appearing in these equations. When, for the sake of selectivity improvement, two polar isohydric solvents are mixed with a low polarity solvent, eqn. 18 can be extended to:

$$1/k' = a' + b'_1 \varphi_1 + b'_2 \varphi_2 \tag{18a}$$

Temperature is not usually a parameter considered in the optimization of analysis by normal phase chromatography, and is not taken into account in this treatment. The same algorithm as used in reversed-phase chromatography could be employed when necessary, but it must be kept in mind that the activity of the adsorbent depends on the temperature.

Reversed-phase chromatography. It is usually accepted that the column capacity factor is related to the composition of a water–organic solvent mixture through the relationship

$$\log k' = a + b \varphi_B \tag{19}$$

where φ_B is the volume fraction of the organic solvent mixture. Eqn. 19 gives good results over a large range of compositions ($0.1 < \varphi_B < 0.8$) with methanol and acetonitrile, whereas the linearity is often much poorer with tetrahydrofuran (THF). At low or high organic solvent compositions ($\varphi_B < 0.1$ or $\varphi_2 > 0.8$) better results are obtained with a second-degree polynomial. In practice, however, most analyses are carried out in the intermediate concentration range where eqn. 19 is an excellent approximation.

In optimizing the mobile phase composition, several different concentrations may have to be determined in addition to the water/organic solvent (usually methanol or acetonitrile) ratio: the concentration of a second organic solvent, often called modifier (typically THF, diisopropyl ether), the concentration of a ligand, a counter ion, as well as the pH or the ionic strength. These parameters are used to modify the selectivity as explained above. Since solution thermodynamics are yet unable to predict accurately the retention or relative retention changes associated with a modification in the mobile phase composition, empirical relationships such as eqn. 19 have to be used.

If the eluotropic strength of the modifier is not too different from that of the organic solvent, an extended form of eqn. 19 can be used:

$$\log k' = a + b_1 \varphi_B + b_2 \varphi_c \tag{20}$$

Three experimental measurements are necessary to determine the three parameters in eqn. 20 for each solute. If more measurements are made, the possible curvature of the ($\log k'$, φ_B, φ_c) surface can be accounted for by introducing second-order terms in eqn. 20.

Influence of temperature. Temperature has a complex effect on the results of

optimization, since viscosity and diffusion coefficients as well as capacity ratios are temperature dependent. It is an important parameter in the optimization of a separation, which should be considered more often, at least everytime the separation is not easy or straightforward.

It follows from thermodynamics that there is a simple relationship between capacity factor and temperature:

$$\log k' = a + b/T \tag{21}$$

Combination with eqn. 20 gives

$$\log k' = a_1 + b_1 \varphi_B + b_2 \varphi_c + (c + d_1 \varphi_B + d_2 \varphi_c)(1/T_i - 1/T) \tag{22}$$

where a_1, b_1 and b_2 are determined at temperature T_i.

Accordingly, the use of temperature as an experimental parameter to be optimized doubles the number of measurements to be made: three, at two different temperatures.

Multiparameter optimization

A complete optimization involves a large number of parameters: column length, particle size, mobile phase velocity, composition of a ternary mobile phase (two parameters) and temperature. The criterion selected will usually be the minimization of analysis time, with a number of possible constraints on the resolution, the inlet pressure and the sensitivity (peak height).

Statistical methods such as SIMPLEX require a large number of determinations before optimization is achieved. When the relationship between the function to be optimized and the variables is complex (as is the case between the analysis time, the temperature and the mobile phase composition) convergence may, sometimes, be a problem. The use of simple relationships permits a faster determination of the optimum conditions, but the result will be only as good as the accuracy of the derivation of the empirical parameters introduced in the equations of the model.

The use of a chromatographic response function or chromatographic optimization function seems attractive at first. There is a large measure of arbitrariness in the choice of a compromise between resolution and time. Actually, a resolution value larger than 2.5 is not worth much and we would rather sacrifice it for a gain in analysis time. However, when the resolution is below 1 it becomes a very highly considered quality and no decrease in resolution is acceptable. It is very difficult to derive a convenient relationship for the highly non-linear function thus defined. This is illustrated, for instance, when the computer selects optimum conditions under which two peaks are not resolved but the analysis time is so short that a compensation occurs in the optimization criterion for the analysis of a complex mixture. The solution may be acceptable or not. This problem can be overcome if a minimum resolution between any two adjacent peaks is introduced in the optimization scheme.

Accordingly, a computer program has been written which permits the calculation of four functions for any set of values of the six parameters of a separation (L, d_p, u, φ_A, φ_B, T). These functions are the analysis time, a certain resolution (see below), the inlet pressure and the peak height of a selected component. The analyst must tell the computer the acceptable range for each of the six parameters and the

steps to be used for each of them. Calculations are carried out for each of the nodes of the six-dimensional space thus defined.

The resolution can be chosen in several different ways. The computer first calculates the resolution between each pair of peaks. It can then just select the smallest value obtained, although in most practical situations it is not always necessary to give the same importance to all possible pairs of compounds. In pharmacokinetic investigations, for instance, it is necessary to separate the drug under study from all other interferences (which requires generally a resolution of 1.5 or more between this peak and those eluted just before and just after), whereas a less complete separation of the metabolites (R *ca.* 0.9–1.0) is most often acceptable and no separation of the other compounds present in the sample is really necessary. The program can take these requirements into account, as the analyst can specify a minimum value of the resolution between any peak and its two closest neighbours and select different values for different peaks. Finally, one resolution can be maximized or the analysis time minimized within the various resolution constraints chosen.

Accordingly, the analyst has a choice of the optimization criterion which can be the shortest analysis time, the largest value of resolution (between any two compounds, around one compound, between the less well separated pair of compounds), the highest peak for a given compound or the lowest inlet pressure. The optimization is made while applying some constraints on the other functions: maximum value of the analysis time or the pressure or the height of a given peak, or minimum value of the resolution.

It is also possible to look for all combinations of parameters which will lead to a chromatogram satisfying one specification for each of the four functions, time, resolution, peak height and pressure. The computer prints the number of solutions and if they are too numerous, some specifications can be tightened. The computer can also list them in tabular form.

During the phase of data introduction, the analyst is guided by the "user friendly" computer program, which requires for each experimental parameter the range of value to be explored and the step. Failure to answer these questions results in an optimization which does not take this parameter into account. Experimental plans indicating the number of measurements to be made, or data to be introduced, are displayed. Data can be either entered through the keyboard or from files, since the computer can also be used for data acquisition or reduction. The number of experiments to be run prior to an optimization to permit calculation of the parameters of the equations of the model is a maximum of seven for each compound since the parameters for the variation of peak efficiency and retention with solvent composition and temperature can be derived from the same analyses. A number of compounds can usually be injected together for faster determinations.

In addition to printing the optimum experimental conditions, the computer also plots the corresponding chromatogram on the printer plotter, including the individual peaks. The analyst can then decide whether this result is satisfactory and accept it, or whether the specifications have to be modified. This is especially useful when compounds of very large relative concentration are eluted close together, as the resolution originally selected may turn out to be insufficient. A comparison with the chromatogram actually obtained under the same conditions sometimes reveals the presence of unsuspected compounds.

EXPERIMENTAL

A synthetic blend of C_8–C_{12} alkylbenzenes was eluted on a 15-cm Nucleosil 5 C_{18} (Macherey-Nagel, F.G.R.) column, the temperature of which was controlled by water circulation using a D3 thermostated bath (Haake, Karlsruhe, F.G.R.). The pumping system was a Model 6000 A from Waters (Milford, MA, U.S.A.). Solutes were injected with a 7125 valve (Rheodyne, Berkeley, CA, U.S.A.) and detected with a UV detector Model 440 from Waters.

A blend of phenothiazines (levomepromazine, chlorpromazine, 3-chlorophenothiazine, dimethothiazine, propericiazine, oxomemazine; Rhône-Poulenc Santé, Paris, France) was eluted on 5- and 10-cm Spherosil XOA 600, 6 μm, columns (Prolabo, Paris, France), using a SP 8000 chromatograph (Spectra-Physics, San Jose, CA, U.S.A.).

A 9835 B computer (Hewlett-Packard, Palo Alto, CA, U.S.A.) was used to develop the program. Simulations and curves were plotted on a 7225 A Hewlett-Packard printer-plotter.

RESULTS AND DISCUSSION

The first experiments were carried out to ascertain the range of validity of the relationships between the capacity factors, the solvent composition and the temperature. Then an application to an actual drug analysis problem was studied.

Capacity factors and solvent composition

The column capacity factors of the alkylbenzenes were measured with eluents of different acetonitrile–water compositions. Table II gives the coefficients of the linear relationship between log k' and the volume fraction of acetonitrile, and the corresponding regression coefficient. The coefficients a_1, b_1 and b_2 of eqn. 20 are given in Table III. Finally, in Table IV the values of the capacity factors measured experimentally and calculated using eqn. 20 and the coefficients in Table III are compared for two different mobile phases. The differences are relatively small and can be explained by experimental errors (flow-rate and temperature fluctuations).

TABLE II

PARAMETERS OF THE RELATIONSHIPS $\log k' = a_1 + b_1 \varphi_B$ FOR DIFFERENT COMPOUNDS (SEE TEXT)

Peak number	a_1	b_1	*Correlation coefficient,* r^2
1	1.358	−0.0131	0.8862
2	5.993	−0.0520	0.9951
3	7.856	−0.0668	0.9975
4	9.188	−0.0780	0.9981
5	9.774	−0.0816	0.9986
6	11.16	−0.0932	0.9991
7	12.19	−0.1016	0.9992
8	13.00	−0.1066	0.9989

TABLE III

VALUES OF THE PARAMETERS a, b_1 AND b^2 IN EQN. 20

Peak number	a	b_1	b_2	*Correlation coefficient,* r_2
1	0.159	−0.0131	−0.00859	0.9207
2	1.273	−0.0520	−0.02048	0.9910
3	1.797	−0.0668	−0.02633	0.9926
4	2.167	−0.0780	−0.02676	0.9932
5	2.390	−0.0816	−0.03239	0.9941
6	2.781	−0.1016	−0.03285	0.9996
7	3.062	−0.1016	−0.03717	0.9996
8	3.424	−0.1066	−0.04007	0.9996

Eqn. 20 is quite satisfactory over the range investigated, which is in agreement with a wealth of published experimental data.

Capacity factors, solvent composition and temperature

Table V gives the capacity factors of alkylbenzenes eluted with a given water–acetonitrile mixture at different temperatures. The coefficients of eqn. 21 determined by a least squares fit are also given. The value of the regression coefficient, very close to 1, justifies the use of only two measurements for the determination of $\log k_i$ and C.

The coefficients of eqn. 19 were also determined at different temperatures (one organic solvent only), which permits the calculation of the coefficients of eqn. 22 with $b_2 = d_2 = 0$ since only one organic solvent is used here.

Knowing the parameters of eqn. 22, it is possible to predict the capacity factors for all temperatures and compositions, at least within the range of parameter values investigated. The results of such predictions are given in Table VI, together with the experimental data. The agreement is excellent for the retained compounds, well within the expected range of experimental errors.

TABLE IV

COMPARISON BETWEEN CALCULATED AND MEASURED CAPACITY FACTORS IN TWO DIFFERENT MOBILE PHASES

Peak number	*Water–acetonitrile–THF (20:40:40)*			*Water–acetonitrile–THF (5:90:5)*		
	$k'_{calc.}$	$k'_{meas.}$	*% error*	$k'_{calc.}$	$k'_{meas.}$	*% error*
1	1.13	0.91	1.0	1.07	1.01	2.8
2	2.24	2.29	1.3	2.57	2.4	4.8
3	3.30	3.31	0.3	3.96	3.66	6
4	5.0	4.91	1.4	5.14	5.13	0.2
5	5.09	5.43	5.6	6.36	5.9	6.2
6	7.86	8.0	1.6	8.44	8.43	0.1
7	9.04	9.37	3.3	10.4	10.5	1
8	11.8	12.3	3.6	14.5	14.3	0.8

TABLE V

CAPACITY FACTORS OF ALKYLBENZENES AT DIFFERENT TEMPERATURES

Mobile phase: water–acetonitrile (10:90, v/v).

Capacity factors k' at T_i (°C)									*log k = log k'$_i$ + c(1/T_i−1/T)*		
30	*35.5*	*40*	*45*	*50*	*60*	*70*	*80*	*90*	*log k'$_i$*	*c*	*r²*
3.17	2.97	2.78	2.61	2.44	1.92	1.90	1.70	1.50	1.160	−1.392	0.982
5.28	4.79	4.39	4.03	3.69	3.13	2.68	2.32	2.0	1.668	−1.777	0.999
7.52	6.87	6.31	5.81	5.32	4.50	3.82	–	–	2.028	−1.767	0.999
9.28	8.14	7.27	6.50	5.83	4.67	3.82	–	–	2.230	−2.310	0.999
13.4	11.9	10.7	9.62	8.59	6.99	5.67	4.66	3.84	2.613	−2.296	0.999
17.7	15.6	13.9	12.3	10.9	8.69	6.92	5.58	4.56	2.884	−2.477	0.999
24.7	21.3	18.6	16.3	14.2	11.1	8.58	6.79	5.42	3.219	−2.786	0.999

Optimization of a separation

The separation of a phenothiazine mixture has been reported previously[24]. The experimental conditions were selected by a skilled analyst on a purely empirical basis and it was felt that little improvement in the quality of the separation could be expected from further work, *i.e.*, that a reduction in the analysis time could be achieved only through a decrease in the resolution.

Optimization of this analysis was carried out, in order to achieve the minimum analysis time under the conditions that the resolution between two successive peaks always be larger than 2 and the inlet pressure be lower than 200 atm. (There is no problem of detection limit in this case.) Fig. 3 and Table VII show the results obtained by progressively increasing the number of experimental parameters with which the optimization was carried out.

When only the column length is optimized, the result obtained is similar to that given by the empirical approach. When the column length and the solvent ve-

TABLE VI

COMPARISON BETWEEN CALCULATED AND MEASURED CAPACITY FACTORS FOR TWO SETS OF SIMULTANEOUS VARIATIONS OF TEMPERATURE AND SOLVENT COMPOSITION

Initial conditions: 90% (v/v) acetonitrile; 30.0°C.

Peak	*75% φ_B, 90°C*			*97% φ_B, 20°C*		
	k'$_{calc.}$	*k'$_{meas.}$*	*% error*	*k'$_{calc.}$*	*k'$_{meas.}$*	*% error*
2	2.9	3.23	10	2.5	2.41	3.7
3	5.15	5.13	0.4	3.63	3.76	3.5
4	8.06	7.97	1.1	4.98	4.83	3.1
5	8.05	8.11	0.7	6.43	6.35	1.2
6	13.23	13.11	0.9	7.96	8.03	0.9
7				10.9	10.2	0.7
8				14.7	14.1	0.4
Mean			2.7			1.9

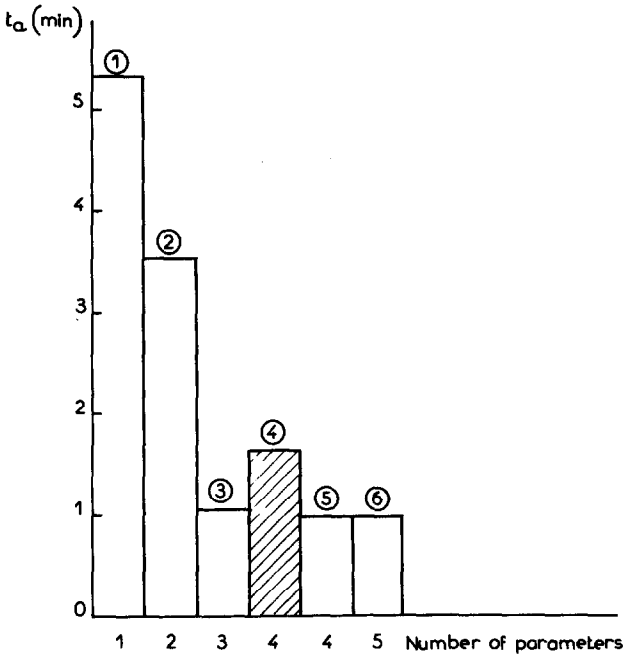

Fig. 3. Influence of the number of parameters used for the optimization of the analysis time. In this example, the analysis time is optimized, for a given minimum resolution and a maximum pressure. Parameters employed: L (1); L, u (2); L, u, d_p (3); L, u, solvent B, T (4); L, u, solvent B, d_p (5); L, u, solvent B, d_p, T (6).

locity are optimized, the computer selects a longer column with a larger velocity than did the analyst (this is permitted by the upper pressure limit) and the analysis time is reduced by one third, from 5.4 to 3.6 min.

If particle size is included in the optimization, the computer selects the smallest size available (3 μm), a still larger flow velocity and a shorter column. The analysis time is then only 1.01 min, a more than five-fold decrease compared to the empirical

TABLE VII

CHANGE IN ANALYSIS TIME WITH THE NUMBER OF PARAMETERS INTRODUCED IN THE OPTIMIZATION

Solvent = mixture of A + B; A = isooctane–diisopropyl ether (1:1, v/v); B = diisopropyl ether–methanol (1:1, v/v) containing 5.2% water. Values in parentheses indicate initial conditions.

t_a (min)	L (cm)	Flow-rate (ml/min)	d_p (μm)	Solvent B (%, v/v)	T (°C)
5.6	(10)	(1)	(6)	(35)	(20)
3.5	15	2	(6)	(35)	(20)
1.1	5	2	3	(35)	(20)
1.6	5	2	(6)	25	(20)
0.99	5	2	3	35	20

approach! Further small or negligible improvements are achieved by optimizing also the solvent composition and temperature.

In other cases the computer does not always select the smaller particle size (because of the pressure limit). It should also be emphasized that the order in which the parameters were introduced in Fig. 3 is arbitrary. For example, if the particle size may not be optimized, the computer elects to change the mobile phase composition (25 rather than 35% solvent B), use a shorter column (the k' values are larger) and a large flow velocity. The analysis time is 1.66 min, appreciably shorter than that obtained with the empirical approach, but longer than that which the use of small particles permits.

Figs. 4 and 5 show some of the chromatograms obtained under the experimental conditions suggested by the computer, compared to those predicted. The only significant difference results from a marked peak asymmetry, as often observed on silica, and which was not taken account of in this simulation, although this could have been done (see above). This situation is worse on the fast chromatogram because of the detector and recorder response times. Accordingly, the signal does not return to the baseline between the first three peaks as predicted, but the overall result is very satisfactory.

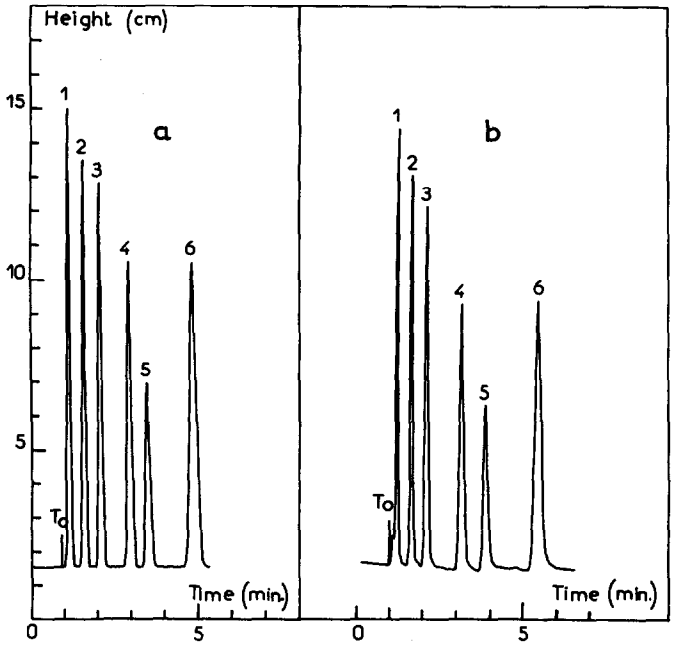

Fig. 4. Comparison between the actual and the simulated chromatograms of a phenothiazine mixture (see text). Chromatogram a: computer simulation. Required conditions: shortest analysis time; resolution between any two peaks to be greater than 2; pressure drop to be less than 200 bar. Parameter optimized: column length, optimum value L = 10 cm. Chromatogram b: actual chromatogram corresponding to the conditions of a. Operating conditions: L = 10 cm (Spherosil XOA 600), d_p = 6 μm; u = 0.1 cm/sec; weaker solvent (A), isooctane–diisopropyl ether (1:1, v/v); strongest solvent (B), diisopropyl ether–methanol (1:1, v/v) containing 5.2% water; mobile phase, A–B (65:35, v/v); T = 30°C; ΔP = 30 bar; t_a = 5.4 min. An unknown impurity is eluted just before peak 1. Peak identification: 1 = 3-chlorophenothiazine; 2 = levomepromazine; 3 = chlorpromazine; 4 = dimethothiazine; 5 = propericiazine; 6 = oxomemazine.

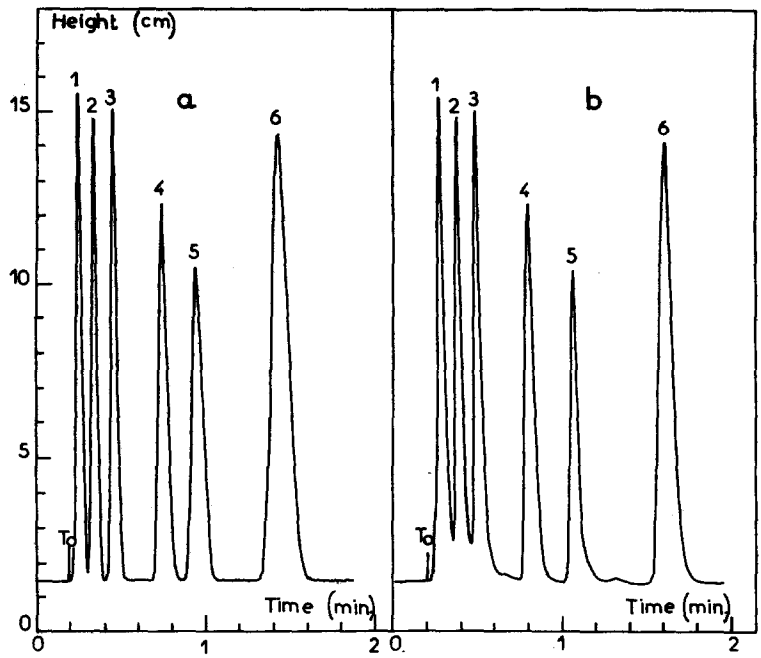

Fig. 5. Multiparameter optimization. Comparison between computer simulation and the actual chromatogram. Chromatogram a: computer simulation of the chromatogram after optimization. Required conditions as in Fig. 4. Parameters optimized: column length, flow-rate, volume fraction of the strongest solvent, temperature. Optimum conditions: L = 5 cm; u = 0.45 cm/sec; φ_B = 0.25; T = 30°C; t_a = 1.65 min. Chromatogram b: chromatogram obtained experimentally. Peak tailing arises from a combination of the dead volumes (use of a thermostated oven), detector response time (1 sec) and recorder response time (0.4 sec). Peak identification as in Fig. 4.

CONCLUSIONS

In spite of the use of simplified, quasi-empirical relationships, it appears possible to optimize simultaneously all the important parameters of a chromatographic separation in a relatively short time. Impressive improvements over the results of an empirical optimization can be expected because the computer can explore rapidly but systematically the entire domain indicated by the analyst. This is made possible by the specific choice of linear relationships which allow rapid calculations. The large improvements arise from the near impossibility for the human mind to consider the complex interaction of six parameters even if they are simply related.

The computer can also be used to adjust the experimental conditions to a change in specifications when a different instrument is used or, for example, when going from a quantitative to a qualitative analysis via liquid chromatography–mass spectrometry (LC–MS) to identify unknowns neglected in the quantitative analysis.

The main barrier to a generalized application is the necessity to carry out a minimum of seven analyses under different experimental conditions (including changes in solvent composition and temperature). These analyses can be performed simultaneously using mixtures of all compounds, provided all the peaks can be iden-

tified, which is not always straightforward unless one uses LC–MS or a diode-array UV spectrophotometer. Further test runs on mixtures of only some of the compounds is more time-consuming and may even be impossible when unknowns are considered.

SYMBOLS

a, a_1, a_2, A, a', b, b'_1, b'_2, b_1, b_2, c, d_1, d_2, c', d'_1, d'_2	Constants in linear equations
A_s	Molecular area of adsorbed solute
$A_{s,i}$	Asymmetry of peak i
C_M	Concentration at the peak maximum
d_c	Column diameter
d_p	Particle diameter
H	Height equivalent to a theoretical plate
H_1, H_2	Values of H in solvents 1 and 2, respectively
H_o	Initial value of H
k^0	Permeability constant
k'_i	Capacity factor of peak i
k'_n	Capacity factor of the last peak
$k'_{(1)}$, $k'_{(2)}$	Capacity factors in solvents 1 and 2
k'_a, k'_p	Capacity factors in apolar and polar solvents
L	Column length
l_1, l_2	First and second segments of the peak width at half-height
m	Amount of injected sample
N	Average efficiency of column, in theoretical plates number
N_i	Apparent efficiency of peak i
N_a	Average plate number between two successive non-symmetrical peaks
N_m	Apparent efficiency of the last peak
ΔP	Pressure drop
$P_{(1)}$, $P_{(2)}$	Polarity parameters of solvents 1 and 2
$P_{(m)}$	Polarity parameter of a mixture of solvents
R_s	Resolution between two successive peaks
S	Response factor of the detector
S°	Solute adsorption free energy
T	Temperature
t_A	Analysis time
t_R	Retention time
$t_{R,i}$	Retention time of peak i

t_o	Elution time of a non-retained compound
u	Solvent velocity
V_o	Volume of adsorbed solvent
W_i	Base width of peak i
$W_{1/-2}$	Width at half-height
X_{H_2O}	Mole fraction of water
X_1, X_2, $X_{1,2}$	Water concentrations of solvents 1, 2 and their mixture, respectively
Y_M	Peak height
α	Adsorbent activity coefficient
ε_c	Adsorbent porosity
ε°	Eluent strength parameter
η	Solvent viscosity
η_0	Initial solvent viscosity
φ_A	Volume fraction of solvent A

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