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COMPUTER-ASSISTED OPTIMIZATION IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD DEVELOPMENT

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SUMMARY

Computer-assisted optimization in high-performance liquid chromatography has been encouraged by a need to define method development strategies for automated chromatographic instruments. Several algorithms have been developed for the optimization of various aspects of chromatographic performance. Additional experimental designs have been used to understand the influence of factors on the performance of the chromatographic system.

INTRODUCTION

A recurrent objective in the development of high-performance liquid chromatographic (HPLC) methods is optimization, the attainment of the best performance from a system by adjusting a set of experimental factors. Over the years, a number of optimization strategies have been applied to and developed specifically for chromatographic systems. Concurrent with the development of these strategies, computers were being introduced to the general field of chromatography. The chief benefits of computers in chromatographic method development have been (a) the evolution of highly automated instruments that can obtain chromatographic information unattended and (b) the implementation of optimization strategies as memory-resident algorithms. Thus, today, computer-assisted optimization in HPLC method development can be done automatically with a high degree of success.

This paper reviews some aspects of the history of computer-assisted optimization in HPLC method development.

AUTOMATED METHOD DEVELOPMENT

In the 1960s, great emphasis was placed on the automation of instrumentation for *routine* analyses[1–4]. Less emphasis was placed on instrumentation for the automated *development* of analytical methods[5]. This was largely because the required experiments in automated method development are investigative or non-routine in nature and include widely varying factor levels that are usually refined on the basis of results from preceding experiments[6]. Nonetheless, by the late 1960s and early 1970s, it was felt that if the conditions for a particular non-routine investigative experiment

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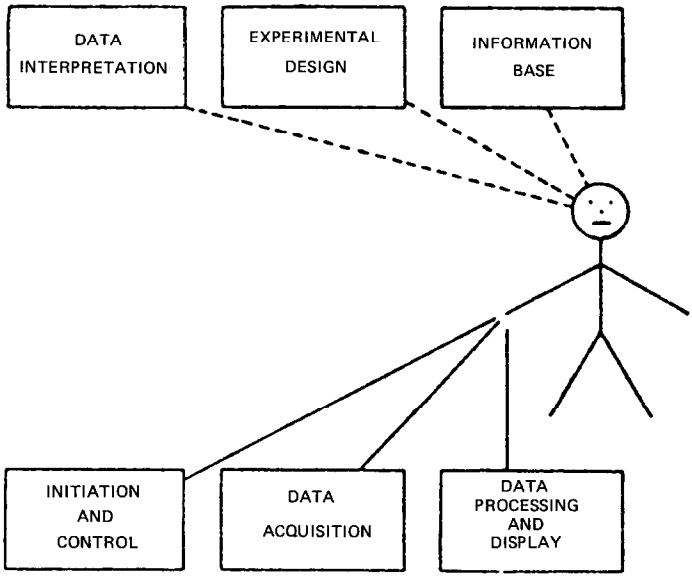

Fig. 1. Procedures employed for manual development of an analytical method. (Reprinted with permission from ref. 6.)

could be established, then the actual execution of that experiment could be carried out by routine procedures[6].

Fig. 1 is a pictorial representation of the procedures conventionally employed for the manual development of an analytical method. The information base, experimental design and data interpretation all require or make use of thought or decision-making processes. Initiation and control of the experiment, data acquisition and data processing and display are routine procedures that can be performed manually but had been automated to a greater or lesser extent by the late 1960s.

Fig. 2 is a pictorial representation of the general procedures proposed by several groups in the 1960s for the automated development of an analytical method. In this situation, it is still the experimenter's responsibility to provide the information base, but once provided, the experimenter is free to undertake other tasks. The instrumental system will automatically design experiments by interpreting data obtained from the information base and execute the experiments with automated initiation and control of the experiments, data acquisition and data processing and display until the development is considered complete[6].

In the late 1960s and early 1970s, emphasis in the automated development of analytical methods was on producing hardware that would allow the scheme of Fig. 2 to be realized. As it turns out, this was a straightforward engineering endeavor and was accomplished relatively quickly with the technology that was available at that time. Completely automated systems were soon available.

Once analytical chemists possessed the computerized hardware to carry out automated method development, they were required to tell the computer how to accomplish that method development. It is probably fair to say that this caused a crisis,

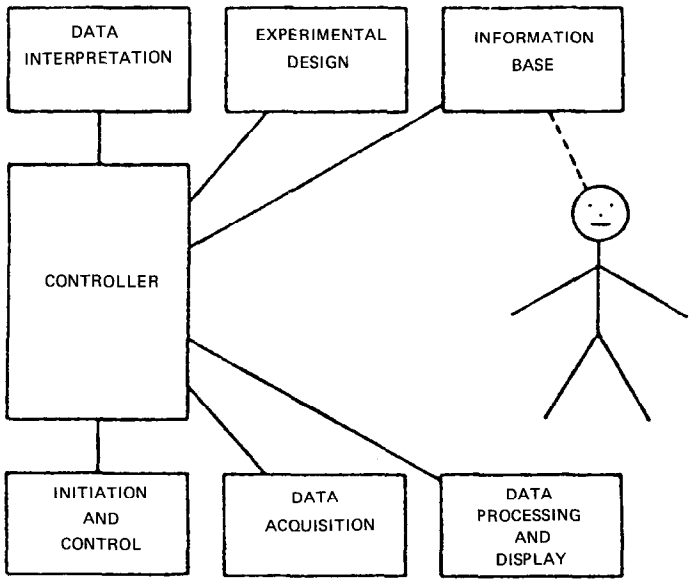

Fig. 2. Procedures conceived for automated development of an analytical method. (Reprinted with permission from ref. 6.)

for with the exception of Wilson[7,8] and a few others, no one seems to have considered a systematic strategy for developing an analytical method. Nonetheless, it soon became obvious that three things were required for successful analytical method development: (1) obtaining a response, (2) improving that response and (3) understanding that response. In the field of chromatography, this led naturally to three areas of intense research that continue to this day: (1) defining chromatographic performance, (2) developing chromatographic optimization strategies and (3) applying classical experimental designs to understand the operational influence of chromatographic variables and make chromatographic methods rugged with respect to them.

CHROMATOGRAPHIC PERFORMANCE

One goal of chromatographic method development is to obtain adequate separation of all components of interest in a reasonable analysis time[9]. For the general case of a multi-component separation, there is no universally accepted measure of performance. Common practice had been to maximize the separation of the pair of peaks that is currently most difficult to resolve[10–12]. This procedure is not always successful for multi-component separations[13–15]. Improving the separation of a given pair of components will not necessarily improve the overall separation. The separation of other pairs of components might decrease. Further, the amount of time required for the analysis might become unreasonably long.

Many approaches to the problem of multicomponent separations use measures of performance that are related to the information content of a chromatogram[16]. One popular measure of chromatographic information is Kaiser's easily evaluated "peak separation"[17–19], which is applicable to any two adjacent peaks and can therefore be

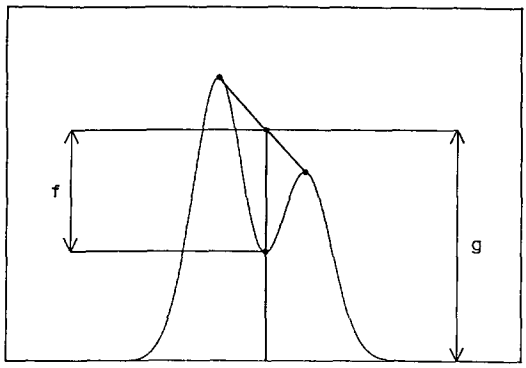

Fig. 3. Peak separation function, $P = f/g$. (Reprinted with permission from ref. 9.)

generalized to the multi-component case. As shown in Fig. 3 for two components, the peak separation (P) is given by the depth of the valley (f) below a straight line connecting the two adjacent peak maxima, divided by the height of the straight line above the baseline at the valley (g), *i.e.*,

$$P = f/g \tag{1}$$

Morgan and Deming[9] suggested that if some function of the peak separation of adjacent peaks is to be used in a multi-component measure of performance, it must provide greater sensitivity to highly overlapped peaks and lesser sensitivity to components that are adequately resolved. The logarithm of the peak separation satisfies these requirements: when adjacent peaks are greatly overlapped, the peak separation is very small, the logarithm is a large negative number and the sensitivity to change in peak separation is large; when there is little overlap, the peak separation is close to unity, the logarithm is near zero and the sensitivity to change in peak separation is small. Generalization to multi-component separation is accomplished by summing the logarithms of the peak separation for all j pairs of adjacent peaks:

$$CRF = \sum_{i=1}^{j} \ln(P_i) = \ln(P_1 P_2 \cdots P_i) \tag{2}$$

where P_i is the peak separation (eqn. 1) of the ith pair of adjacent peaks. This measure of performance was referred to by Morgan and Deming as the "chromatographic response fuction" (CRF), and was used to optimize successfully several multi-component separations.

The simple definition of chromatographic performance given by eqn. 2 has been rightly criticized, elaborated and expanded over the years by many research groups[20–36], usually to satisfy a need for a more specific measure of chromatographic performance. It appears unlikely that a single, generally appropriate chromatographic response function will be found. Instead, a choice may be made from the several chromatographic response functions that now exist. Alternatively, a still different chromatographic response function might be defined. In any case, care must be taken

that the chromatographic response fuction is appropriate for the task at hand and will perform as expected; Smith *et al.*[37] have intentionally shown what happens when the *CRF* is inappropriate. The paper by Wittkowski and Luethe[29] is also of particular interest because of its use of desirability functions as introduced by Harrington[38]; desirability functions are equivalent to fuzzy set concepts as introduced by Zadeh[39] and used recently by Otto[40] and others[41].

A second popular measure of chromatographic information is the relative retention, α:

$$\alpha = (t_2 - t_0)/(t_1 - t_0) \tag{3}$$

where t_2 is the retention time of the slower eluting component, t_1 is the retention time of the faster eluting component and t_0 is the time equivalent of the void volume. The relative retention is especially useful because it automatically corrects (normalizes) for increases in the widths of normal peaks at longer elution times. Although the relative retention is defined for only a single pair of peaks, graphical techniques in which all possible relative retentions are plotted simultaneously are useful in assessing overall chromatographic performance[42].

SEQUENTIAL SIMPLEX OPTIMIZATION

In 1975, two radically different papers on multi-component chromatographic optimization appeared in the Munich Symposium issue of this journal: Laub and Purnell[42] introduced an ingenious technique called "window diagrams" for the single-factor global optimization of the separation of mixtures in gas chromatography; Morgan and Deming[9] applied the well known sequential simplex method for the two-factor local optimization of the separation of mixtures in gas chromatography.

Sequential simplex optimization was originally proposed by Spendley *et al.*[43] in 1962 as a means of improving the performance of industrial processes. It was offered as a more efficient alternative to classical evolutionary operation (EVOP) approaches[44]. It is interesting that Spendley *et al.* were specifically interested in developing a computer algorithm that could be used to optimize processes automatically. The original fixed-size algorithm was later modified by Nelder and Mead[45] to give it the capability of accelerating in favorable directions of search and decelerating in unfavorable directions. This variable-size algorithm has been modified by King[46] to eliminate the inefficient "massive contraction" rule.

Long[47] was probably the first to apply the sequential simplex method to the optimization of an analytical method. Before being applied to the optimization of chromatographic separation, the sequential simplex had been used extensively in analytical methods development[48–54] and other areas[55]. It continues to be an important method of optimization in general analytical chemical method development[21,56–64].

Fig. 4 shows the progress of a variable-size simplex as it is used to adjust temperature and flow-rate in the time-constrained optimization of a two-component gas chromatographic separation[9]. The simplex adapts itself to the contours of the response surface and keeps the separation within the allowable 30-min time constraint. Fig. 5 shows the chromatograms from the optimization.

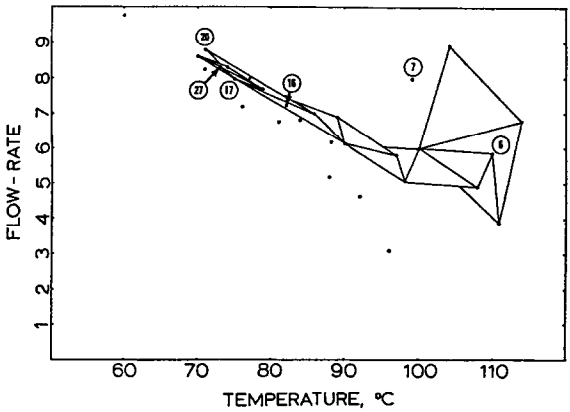

Fig. 4. Simplex progress for two-component system, 30-min time constraint. (Reprinted with permission from ref. 9.)

Although the sequential simplex has been used extensively to improve the separation of components in chromatographic systems[20,24,65–85], it must be remembered that it is a local optimization technique, that is, it will "climb" to the top of whatever "hill" it finds itself on in the response surface. For optimization problems that have only one optimal set of conditions, the simplex will find that optimum very efficiently. For optimization problems that have several locally optimal sets of conditions (one of which is the global, or overall, optimum), the simplex will find a local optimum but there is no guarantee that this will be the global optimum. It has been suggested that a large, variable-size simplex might be more likely to collapse onto the global optimum, but again there is no guarantee that this will be the case. Others[86] have suggested that there is some evidence that the global optimum is usually the broadest optimum, and therefore there is a greater likelihood that the simplex will converge to this optimum; there is merit to this argument, but there is still no guarantee that the simplex will converge to the global optimum.

It is probably best to consider the sequential simplex method as a means of fine-tuning a separation, a method that can be used in the later stages of chromatographic method development.

WINDOW DIAGRAMS

The window diagram technique of Laub and Purnell[42] has been especially useful because it offers a means of predicting the region of the global or overall optimum. Multiple optima exist in chromatographic systems because of peak order reversal. In the case of two components, A and B, the elution orders A–B and B–A both represent satisfactory chromatography, but the intermediate condition of coelution represents unsatisfactory chromatography; thus, as the chromatographic conditions (*e.g.*, pH in HPLC) are changed to cause peak-order reversal, good chromatography changes to bad chromatography, which eventually becomes good (but different) chromatography again. By plotting the relative retention (α) as a function of the chromatographic variables (*e.g.*, pH), the local optima can be visualized and the global optimum identified.

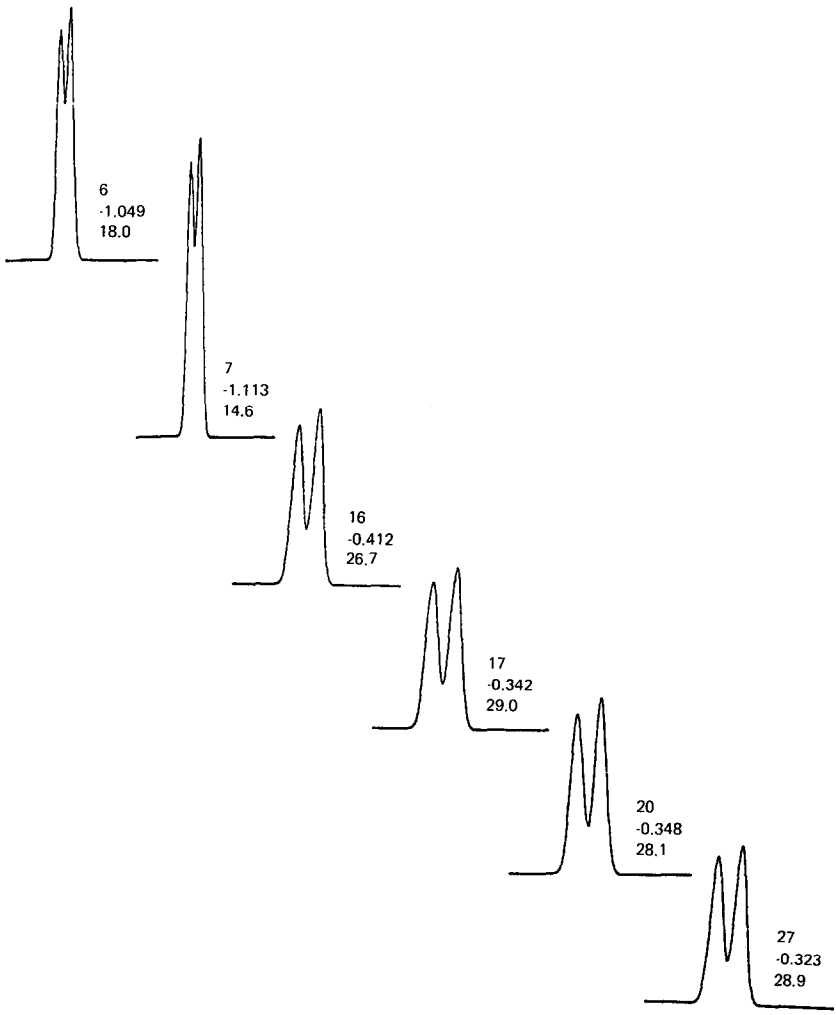

Fig. 5. Representative chromatograms from the optimization of two-component system, 30-min time constraint. Vertex number, *CRF* and analysis time are given at the right of each chromatogram. (Reprinted with permission from ref. 9.)

Fig. 6 shows the retention times of five weak organic acids as a function of pH in a study by Deming and Turoff[87]. When the relative retention of all possible pairs of compounds are plotted, the "spaghetti plot" of Fig. 7 results. Following Laub and Purnell[42] and letting optimum chromatography be defined appropriately as the best separation of the worst separated pair of peaks, the gobal optimum occurs at those conditions (pH) which correspond to the top of the tallest (shaded) window. The resulting chromatograms are shown in Fig. 8. A similar study involving nine cinnamic acids and related compounds was carried out by Price *et al.*[88].

These ideas can be applied to multi-factor HPLC systems in which more than one factor is varied. Graphical representations of these multi-factor applications can

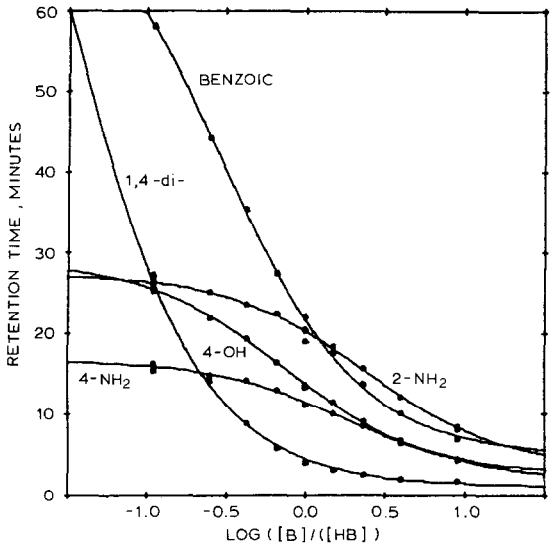

Fig. 6. Retention time *vs.* (pH − 4.71) for five weak acids. Solid lines are predicted behavior, dots are observed behavior. (Reprinted with permission from ref. 87.)

be found in several papers[63,89–91]. Similar approaches based on statistical mixture designs[92] (also called "simplex mixture" or "simplex lattice" designs, which are different from the "sequential simplex" designs discussed earlier) have been used by Glajch and co-workers[93–97] and others[98–101] and are reported elsewhere in this volume.

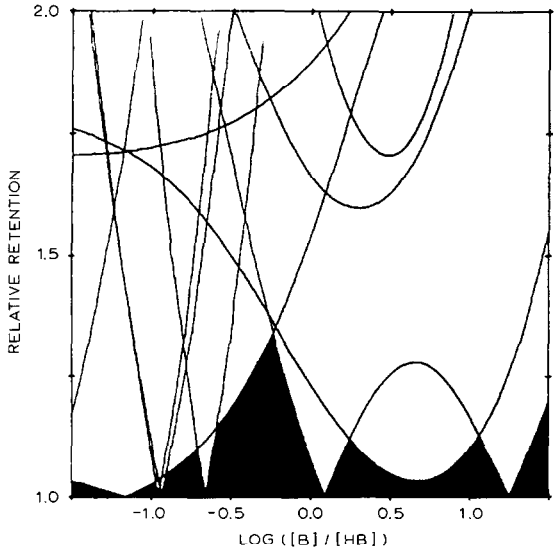

Fig. 7. Window diagram for all ten pairs of five weak acids. (Reprinted with permission from ref. 87.)

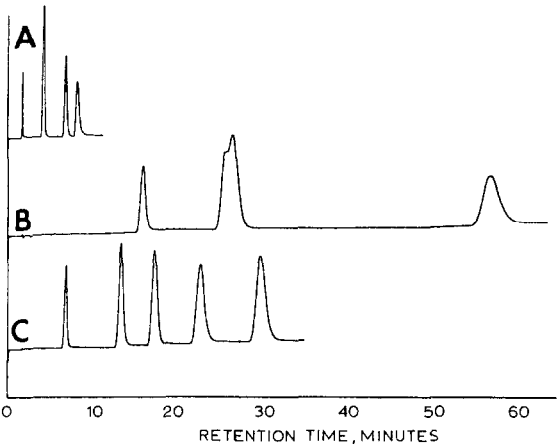

Fig. 8. Chromatograms of mixture of five weak acids. (A) pH = 5.66; (B) pH = 3.76; (C) pH = 4.44. (Reprinted with permission from ref. 87.)

COMMENTS

Computers assist the optimization of HPLC methods by two equally important means: (a) by controlling highly automated instruments that can obtain chromatographic information unattended and (b) by implementing various optimization strategies as memory-resident algorithms. Although both areas will undoubtedly continue to advance, it is likely that both areas are becoming "mature technologies".

The basic difficulty with chromatographic optimization is that the ubiquitous possibility of reversal of elution order gives rise to the ubiquitous possibility of multiple optima.

Gradient search techniques (such as the sequential simplex method) are now realized to be less useful in the *earlier* states of chromatographic optimization when it is important to locate the global optimum. Gradient search techniques are probably most useful in the *later* stages of chromatographic optimization when "fine-tuning" of multiple factors is important to achieve truly optimal performance from the system.

Techniques based on classical response surface mapping designs coupled with appropriate mathematical models (techniques such as the window diagram method) are probably most useful in the earlier stages of chromatographic optimization when it is important to locate the region of the global optimum. If the model fits well, then the prediction of the global optimum will be accurate; if the model does not fit well, then the prediction of the global optimum will be less accurate and fine-tuning might be necessary to achieve a truly optimal response. This fine-tuning might be accomplished by a second iteration of the mapping design, or by a gradient technique such as the sequential simplex.

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