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REVIEW

SIMPLEX OPTIMIZATION OF HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC SEPARATIONS

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CONTENTS

1. INTRODUCTION

The sequential simplex method was first proposed in 1962 by Spendley *et al.*[1] as a development of EVolutionary OPeration (EVOP) following consideration of how EVOP might be automated. The simplex procedure is a hill-climbing method whose direction of advance is dependent solely on the ranking of responses. The calculations and decisions that guide the procedure are rigorously specified yet almost trivially simple. The great advantages of the simplex procedure in the optimization of liquid chromatographic separations are that it is able to optimize many interdependent variables with no prior knowledge about the mode of separation or the complexity of the sample. Nor does it require any pre-conceived model of the retention behaviour of solutes and so does not require that the solutes be identified or recognized in individual separations. The method has the further advantages of permitting the introduction of new variables during the optimization process for the price of just one additional experiment per variable and one can also assess the progress of the optimization *during* rather than at the end of the experimental sequence. The procedure is therefore relatively efficient, multi-factor and has an empirical feedback which should permit rapid attainment of the experimental optimum.

There are, however, significant disadvantages associated with simplex optimization. The ranking of responses requires that the quality of the chromatogram from each experiment be assessed: this is potentially a major stumbling block since it requires an optimization, or response, function which can direct the algorithm towards the "optimum", yet it is extremely difficult either to know just how to describe what constitutes such an optimum or to express that knowledge in a way which is readily

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describable by a simple mathematical equation. As it is a "blind" optimization method, the simplex procedure is generally unable to assess the quality of a located optimum. For example, peak elution orders may change in successive separations and the procedure would not be expected to decide unambiguously which elution order should be pursued to provide the global optimum. Thus several local optima may be located and the simplexes will move towards the most favourable local optimum rather than continue to search for the global optimum. It has also to be recognized that the simplex procedure can require relatively large numbers of experiments to locate optimum separation conditions.

2. SIMPLEX PROCEDURE

2.1. Selection of algorithm

Although there are now many variants of the simplex procedure, they are all based on the basic procedure of Spendley *et al.*[1]. A simplex is defined as a geometric figure having one more point (vertex) than the number of variables being optimized. Thus, for two variables a simplex is a triangle and for three variables the simplex is a tetrahedron. Although it is difficult to visualize a simplex for more than three

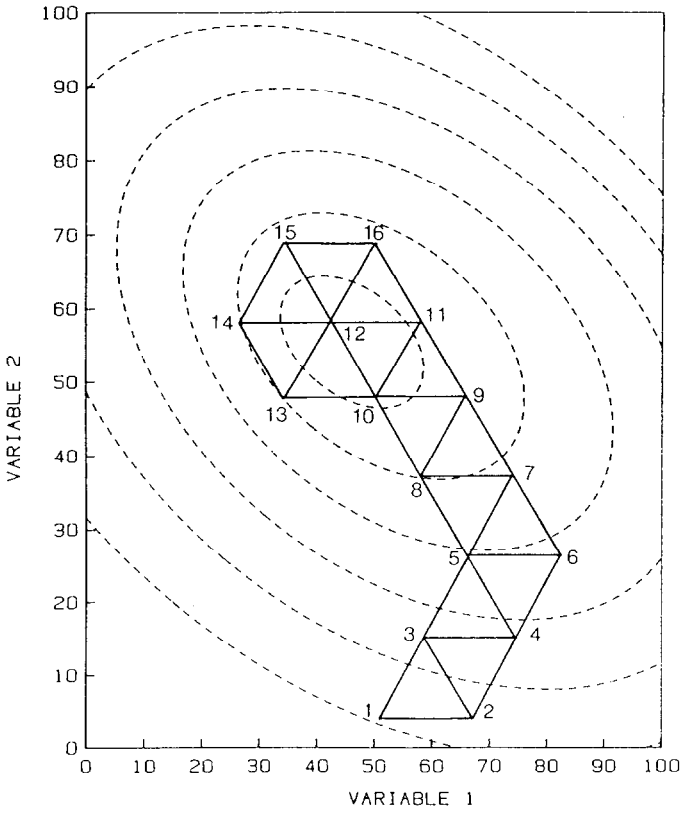

Fig. 1. Fixed-step size simplex optimization of two variables. Initial simplex is 123 and optimum region is at vertex 12. Reproduced from ref. 3 with permisson.

variables, the mathematics do not become significantly more complex and the procedure is easily handled by manual or digital computation. Fig. 1 shows a two-variable (dimension) simplex as it moves, with fixed step sizes, across a response surface. The optimization proceeds by rejection of the vertex which has the worst experimental response and reflecting its coordinates through the mid-point of the hyperplane. More detailed descriptions of the process have been published recently[2,3].

Unfortunately, this original, fixed-step size procedure suffers from several severe limitations. In particular, progress across the response surface is made at a constant rate because the step size is fixed; this fixed size also means that the optimum may not be precisely located since the simplexes will be forced (in two dimensions at least) to circle around it. Additionally, the fixed-step size simplex is prone to failure on a response surface ridge, in that it will become stranded and not make progress towards the optimum. These limitations have been largely overcome in the modified procedure of Nelder and Mead[4]. They introduced two new operations to the fixed-step size procedure, namely expansion and contraction. These operations allow the simplexes to expand and thus accelerate towards the optimum region when, having located it approximately, the simplexes contract and reduce the search region until the optimum is precisely located. Fig. 2 shows a variable-step size simplex on the same response surface as in Fig. 1.

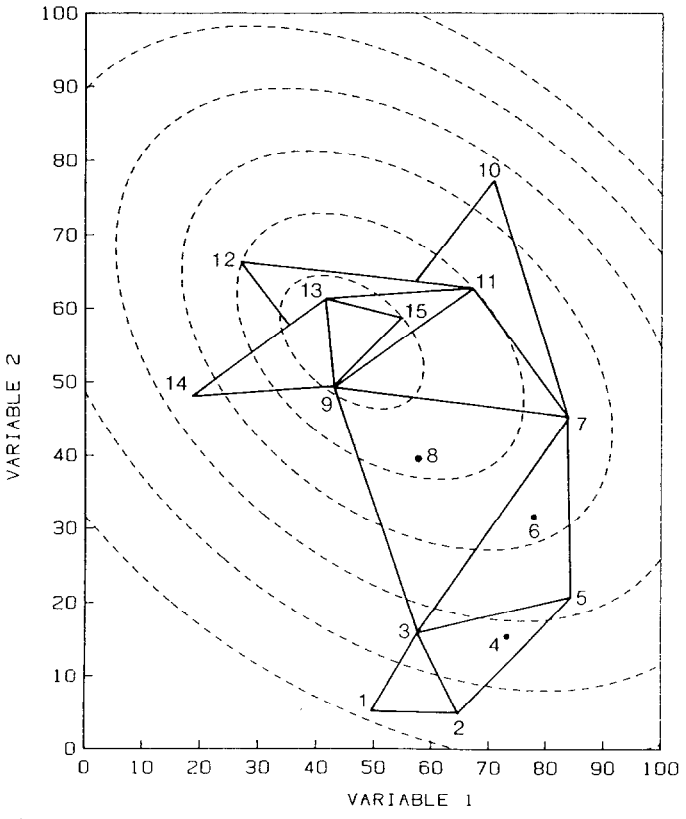

Fig. 2. Variable-step size simplex. (From ref. 3.)

There are now further developments of the modified procedure, intended to increase speed and/or reliability. The basic modified procedure uses a fixed rate of expansion and contraction (usually 2 and 0.5, respectively), but the super-modified simplex (SMS)[5] determines optimum values for the coefficients according to a parabolic fit through the worst vertex, the reflected vertex and the mid-point. The weighted centroid method (WCM)[6] does not assume that the most rapid progress will be made by reflection through the centre of the hyperface and instead carries out a reflection biased towards the best remaining vertex.

For the optimization of high-performance liquid chromatographic (HPLC) separations, the modified procedure is likely to be the algorithm of choice as it is able to locate the optimum more precisely and will usually be more efficient than the fixed-step size simplex. Additionally, as the simplex sizes change, it is not necessary to make initial judgements about how large a step should be taken. The response surfaces generated by most response functions are often irregular and the newer algorithms such as the weighted centroid method or the super-modified simplex may not offer significant advantages. They can be less efficient owing to the noise often encountered in chromatography and they do require more complex calculations.

2.2. Response functions for simplex optimization

Sequential simplex procedures rely on the results from previous experiments to define future experimentation. If those results are ambiguous it will be impossible to locate even a local optimum. The selection of an appropriate function and method of assessment of chromatographic quality is paramount for the successful utilization of a sequential simplex procedure. Further, the procedure is poor at dealing with other than smooth response surfaces. It is very difficult to climb a mountain "blind" if it is criss-crossed with crevasses. An optimization function needs to be defined that results in a smooth and unambiguous response surface across which the simplexes can move towards the global optimum, not being distracted by local optima. The optimization function must also represent, as a simple number, the chromatographer's definition of an optimum separation, in itself a complex requirement since this assessment will almost certainly be a multi-criterion decision. Very few workers using simplex optimization, or indeed other separation optimization methods, explicitly define what the desired optimum is. For example, most practising chromatographers want a method that "works". For one sample sensitivity may be critical, whereas for another throughput (speed) may be more important. Most criteria is use today are aimed at generating a chromatogram of more or less evenly spaced peaks within a predetermined time constraint. Infrequently considered are requirements such as robustness or cost.

The functions used successfully to date for simplex optimization are, in general, based on sums of terms, usually reflecting resolution and analysis time and a selection are presented in Table 1. Ideally, there should be some indication of peak elution order changes in order that the global optimum can be searched for, but this has yet to be introduced directly into a response function. Not surprisingly, there is no ideal response function that meets all demands for sequential simplex optimizaton. Further discussions on optimization criteria can be to be found in refs. 7 and 8. It remains to be seen whether the newer response functions that have been designed for separations complicated by non-ideal peak shapes (*e.g.*, a solvent peak or matrix peak)[9] or when

TABLE 1
CHROMATOGRAPHIC OPTIMIZATION FUNCTIONS USED WITH SEQUENTIAL SIMPLEX OPTIMIZATION

Function[a]	*Variables*	*Ref.*
$P_{inf} = \sum_i {}^2\log S_i$	Binary mobile phase	11
$F_{obj} = \Sigma[10(1.5-R_i)]^2$	Ternary mobile phase	12
$F_{obj} = \Sigma 100e^{1.5-R_i}+(t_m-t_n)^3$	Ternary mobile phase	13
$CRF = \Sigma\ln(P^i/P_d) + a(t_m-t_n)$	Gradient parameters and flow-rate	14
	Binary mobile phase	15
$CRF = \Sigma\ln(f_i/g_i) - 100(M-n)$	Concentration of organic modifier, pH	16
$CRF = \Sigma R_i + n^a - b \mid t_m-t_n \mid - c(t_0-t_1)$	Composition of ternary mobile phase, temperature, flow-rate, pH	17,18
$CRF = \Sigma r_i + n - (t_m-t_n)$ (for $t_m-t_n > 1$)	Composition of ternary mobile phase	19
$Y = p/M$	Gradient parameters (S/b)	20

[a] P_{inf} = informing power, S_i = peak overlap, R_i (P_i) = actual resolution (peak separation) and R_d (P_d) = desired resolution (peak separation), t_n = retention time of last peak and t_m = desired retention time, t_0 = void time and t_1 = retention time of the first eluted peak and f and g = peak separation factors[1]; N = noise, M = number of peaks expected, n = number of peaks detected and p = number of peaks separated with a given resolution; CRF = chromatographic optimization function and Y = the extent of separation; the parameters a, b and c are selectable weightings.

the objective is to separate a small number of components from a complex sample[10] can be used successfully in simplex optimization procedures.

3. APPLICATIONS

3.1. Isocratic separations

The first published use of the sequential simplex procedure in liquid chromatography appeared in 1975[11]. This seminal example considered the ion-exchange separation of inorganic cations and, while demonstrating the value of the procedure, served to highlight the fundamental importance of deriving an appropriate optimization criterion. Not until 1977 did the first truly HPLC optimization example appear, when the normal-phase optimization of phospholipids was described[21]. However, once again the difficulties associated with the selection of optimization criterion were recognized. In these two examples, the authors used the fixed-step size simplex, which worked well for two-variable optimization but, on the introduction of a third variable[21], the authors met the additional problem of not being able to recognize when an optimum point on the response surface had been located causing the simplexes to "circle".

The simplex algorithm is conceptually simple and relatively easy to implement on a computer. The incentive for computer implementation of the procedure is small unless many variables are being handled, as the calculations required are relatively trivial. However, with the arrival in the late 1970s of commercially available microcomputer-controlled chromatographs came the opportunity to automate separation optimization. The first example of the fully automated use of the modified simplex algorithm for HPLC separation optimization was published in 1982 by

Berridge[17]. A response function was developed with the aim of providing greater flexibility and meeting the needs of fully automated optimization:

$$CRF = \Sigma R_i + n^a - b \mid t_m - t_n \mid - c(t_0 - t_1) \quad (1)$$

It was demonstrated that binary and ternary mobile phase optimization could be achieved without operator intervention, for both reversed-phase and normal-phase[22] separations. In the normal-phase example, the difficulties of recognizing the location of the global optimum were appreciated and differentiation[23], in the time domain, was used to indicate incomplete peak resolution and trigger a new optimization using new criteria or a new mobile phase component.

These early examples were then expanded to consider the combined optimization of a ternary mobile phase, flow-rate and temperature[24], and it was shown that pH, ion-pair concentration, flow-rate and temperature could be optimized automatically, in less than 40 experiments, to yield an acceptable separation of five- and six-component mixtures. These examples are of interest as the variables being considered are strongly interdependent. Trial-and-error and univariate optimization methods are particularly prone to failure when the variables are interdependent, but the simplex procedure is particularly suited to such situations.

Forty experiments is still a large number to conduct, even if the system is under automatic control. The number of experiments can be reduced, and the chances of successfully locating the global optimum increased, if the search area is constrained. Constraining of the search area can be achieved in reversed-phase separations by carrying out an initial gradient separation in order to estimate the likely range of solvent strength required to achieve a desired analysis time. Berridge and Morrissey[18] showed that constraining the simplex search in this manner was indeed effective in both reducing the number of experiments required and increasing the likelihood of locating the global optimum. A microcomputer-controlled chromatograph was employed and the whole process, including the extrapolation from the gradient separation to initial isocratic conditions, was automated successfully. Computer software (in BASIC) for the gradient to isocratic calculation and for the simplex optimization itself has been published[7].

De Smet *et al.*[15] used a similar idea to constrain the variable space during a simplex optimization. In developing a normal-phase separation for sulphonamides, an initial gradient separation was conducted to establish the desired solvent strength after which a small number of isocratic experiments were carried out in order to establish the most appropriate starting conditions for subsequent optimization.

Two examples of the off-line use of the computer methods incorporating the simplex algorithm for isocratic separation optimization are the optimization of a quaternary mobile phase and flow-rate for alkaloids[13], and a ternary mobile phase for the normal-phase separation of carotenoids[12]. In both instances the super-modified simplex procedure[5] was used. Nickel and Deming[16] have reported the successful automated optimization of the separation of 19 PTH-amino acids. Wright *et al.*[25] used automated implementation of the modified procedure as part of a much more complex investigation into the development and optimization of the separation of the antifungal drug tioconazole and its process-related impurities. A single-column anion separation optimization considered eluent concentration, pH and flow-rate[26].

The software was written in C and is based on the algorithm of Nelder and Mead[4] but with six modifications, including variable initial step size, irregular simplex shape, omission of massive contraction and function maximization only.

Other examples of simplex procedures applied to HPLC separation include the optimization of a binary mobile phase and temperature for flavones[27], a binary mobile phase for nitroaromatics and flavones[28] and pH and percentage of methanol in the eluent for an ion-pair separation of naphthylamines[29].

None of the examples cited above considered in detail two of the most frequently encountered problems that limit the usefulness of simplex procedures in HPLC optimization, namely the occurrence of elution order changes and the existence of local optima (which may arise from such elution order changes or may be due to coeluted peaks or non-eluted components). Indeed, there can be no truly optimized separation of an "unknown", as the number of peaks will not be known.

By using the extra information provided by multi-channel detection systems, and in the case of HPLC this will usually be a linear diode-array detector, Wright *et al.*[30] showed that it is possible to estimate the number of peaks that are present in a mixture before a complete separation has been achieved, the extent of elution of the components can be checked and the elution orders examined to check for peak cross-overs. The number of peaks present in the original sample can be estimated through checking peak homogeneity for each chromatogram and updating the maximum number of possible peaks as more become discovered.

While complex chemometric techniques have been used to establish peak homogeneity and/or deconvolute peaks into their component elution profiles[31], for simply estimating peak numbers use can be made of on-board capability usually provided by the detector's integration software to detect coelution of components. For example, where some separation between two components occurs, but without any observable resolution, the relative contributions of the two components will change throughout the elution profile. Where there is a sufficient spectral difference between the two components, both peak shape and retention time will be dependent on the detection wavelength. A sufficient difference in the shift of peak retention time with wavelength can provide an early indication of peak inhomogeneity and allow an updating of an estimate of the total number of peaks such that the search process can be continued as appropriate.

Now that more information is known about the sample under investigation, it is possible to halt the optimization process on the basis of the closeness of the actual response to a maximum possible response, rather than the more usual method of halting optimization when the simplex size reduces below a predefined limit or the response fails to change significantly.

To overcome some of the anomalies generated by using a multi-criterion *CRF* in simplex optimization, Wright *et al.*[30] simplified the *CRF* (eqn. 1) to

$$CRF = \Sigma R_i + n - (t_m - t_n) \qquad (t_m - t_n > 1) \tag{2}$$

The time constraint for the first eluted peak is deleted and that for the last eluted peak is considered only if the difference between its retention time and the target retention time is greater than 1 min. The term for the number of peaks (n) is not raised to a power to ensure that, when the time term is included, it has a significant influence on the *CRF*

value. The net effect of this *CRF* is to force the maximum number of peaks to be searched for with the last being positioned close to the target analysis time. Also, this *CRF* is designed to give a maximum value when the separation meets or exceeds a predetermined "development" requirement. If the maximum value (CRF_{max}) is reached during the course of optimization, the procedure is halted on the basis of response.

The algorithm is shown in Fig. 3. Note that the optimization procedure includes a check on the peak elution order. If different elution orders for the vertices are detected, the chromatographer can be warned of the existence of local optima or new optimization(s) can be started around each optimum. If all vertices indicate the same elution order, a greater degree of confidence can be attached to the result of the optimization.

This procedure has been completely automated and was used for the optimization of three binary, isoeluotropic pseudo-components with a seven-component sample[30]. The sample and mobile phase components had been chosen deliberately to challenge the optimization process. While a separation of all components was achieved, it represented a local optimum as the multiple peak cross-overs that occurred prevented the simplex procedure from reliably locating the global optimum.

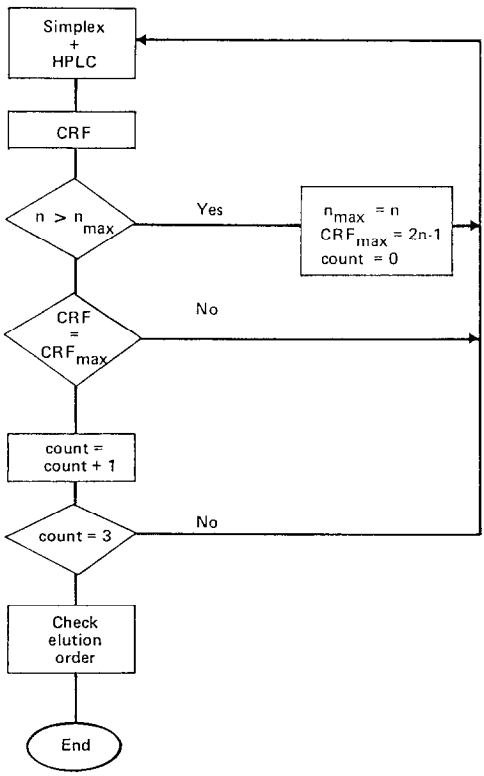

Fig. 3. Optimization algorithm for simplex procedure which uses extra information provided by multi-channel detection to estimate number of components present. (From ref. 19.)

3.2. Gradient separations

Being a general optimization method, the simplex procedure can be used to optimize gradient separation parameters. Watson and Carr[14] recognized the need to develop more advanced optimization criteria and developed a function incorporating both peak separation and time terms:

$$CRF = \Sigma\ln(P_i/P_d) + a(t_m - t_n) \tag{3}$$

which was used for the succesful optimization of up to five variables, namely two solvents, gradient shape, gradient duration and mobile phase flow-rate. Computer assistance was used, the algorithm being the modified procedure and an optimum separation was achieved in twenty experiments. The same *CRF* was used by Fast *et al.*[32] for univariate and multivariate optimization of the separation of steroid mixtures. In this instance the calculations were done manually, some adjustments to the results being required since the controlling microcomputer could not treat the gradient settings as a continuous variable; the simplex procedure assumes completely continuous variables but can handle discrete variables if they vary by uniform, constant steps.

Berridge[17] was able to automate the optimization of multilinear gradient segments such that an adequate separation of four components was achieved in fifteen experiments. The algorithm and control procedures were essentially the same as those used for the optimization of isocratic separations; this shows the ability of the simplex procedure to act as a general, but ignorant, optimization method.

Off-line simplex optimization of gradient separations was described by Sabaté *et al.*[33]. The variables flow-rate, initial modifier content, rate of modifier change and initial isocratic time were examined to improve the separation between 2,4-dinitrophenyl hydrazones of isolated carbonyl compounds in beer. The optimization program (OPEX) was described, but details of the simplex algorithm itself were not published.

Another method for gradient optimization is to optimize the gradient steepness in terms of S/b (Solvent strength, b = constant; b = 0.2 for 10-μm and 0.1 for 5-μm packings). The experimental gradient steepness is calculated from Snyder's linear solvent strength (LSS) equation:

$$\varphi' = b/St_0 \tag{4}$$

where φ' is the volume fraction change per unit time. This approach can be used to optimize multi-segment gradient elution profiles[20].

3.3. Other uses of simplex procedures in HPLC method development

In a mathematical, rather than truly experimental, optimization investigation, Svoboda[34] compared the use of simplex optimization with a grid search technique for the reversed-phase separation of twelve nucleotides. Organic modifier content, buffer concentration, pH, column length and separation time were considered. Retention as a function of these variables was described by a set of parabolic equations and then the factor space was searched by the two techniques. The simplex procedure was found to more effective with all five variables; when the number of variables was reduced, the

grid search became more efficient. The full algorithm of the modified simplex procedure employed was given; it includes a four-fold simplex size reduction in the case that an optimum is not located.

Cela and Pérez-Bustamante[35] showed that simplex optimization was an effective procedure for the deconvolution of overlapping chromatographic peaks, assuming each peak to have a Gaussian profile. The speed and reliability of the modified procedure were compared with those of the weighted centroid method, the latter being found to be faster and less dependent on the initial simplex location. The algorithm was published and the procedure was implemented on a very simple personal computer, having only 16K RAM.

Finally, method development and optimization are not solely concerned with the improvement of chromatographic parameters; sample treatment prior to chromatography should also be considered. Halfpenny and Brown[36] used the modified simplex procedure to establish the optimum values for pH and substrate concentration for a complex HPLC enzyme assay.

4. CONCLUSIONS

The simplex procedure is a versatile, general optimization method with wide applicability to HPLC separation optimization. No assumptions need be made about the sample under investigation, either its composition or behaviour. Interdependent variables can be optimized and it is easy to see how the optimization is proceeding and halt it on the basis of separation quality (response) or when a pre-determined simplex size has been reached. Further, it is not necessary to identify or track peaks in successive chromatograms.

The simplex method is easy to use through manual calculations, but computer assistance in the calculations simplifies the process. Complex programs are not required; indeed, it is possible to use a simple spreadsheet[37]. The simplex procedure is also one of the easiest optimization procedures to link to the on-line optimization of HPLC separations through microcomputer-controlled chromatographs and at least two commercial manufacturers[38,39] implemented simplex procedures on their instruments.

There are however, drawbacks with the simplex procedure that limit its utility for HPLC separation optimization. Most notable is the problem of locating a local, rather than the global, optimum. This will inevitably be the case when elution orders change during the optimization, although methods to determine this are now available. Other disadvantages are the large number of experiments required (typically 15–30) and that, having conducted these experiments, little information is available about the robustness of the separation. As a technique for computer-aided method development in the future, it is likely to be superseded by the faster, more predictive methods which use regression methods or even structure–retention data to predict separation. However, it may still be useful to retain it as a potential method, useful in circumstances when the predictive methods may fail. Such an approach has been suggested by Fell *et al.*[40], who incorporated the modified simplex procedure as a part of an expert system for eluent optimization.

5. SUMMARY

Since its inception in 1962, the sequential simplex procedure has found wide applicability for the optimization of a wide variety of liquid chromatography separations. This paper describes the basic agorithms and the chromatographic response functions necessary for the successful application of the procedure. Application of the simplex procedure to selectivity optimization, using manual and on-line computer systems, are given with particular consideration to the major limitation of the procedure, the difficulty of finding the global optimum. Both isocratic and gradient separations in normal, reversed-phase and ion chromatography are discussed. The use of the procedure for other chromatographic applications is considered.

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