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AUTOMATED OPTIMISATION OF REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY SEPARATIONS

AN IMPROVED METHOD USING THE SEQUENTIAL SIMPLEX PROCEDURE

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SUMMARY

In previous publications it has been shown that the sequential simplex procedure can be used to direct the optimisation of a wide variety of separations in liquid chromatography. It is now shown that the performance of simplex optimisation in reversed-phase chromatography can be improved by restricting the area over which it searches for the optimum mobile phase composition. The restricted area is determined from an initial gradient elution separation of the mixture under study.

INTRODUCTION

There is an increasing number of schemes being developed to aid in the optimisation of high-performance liquid chromatography (HPLC) separations. Overlapping resolution mapping[1,2], window diagrams[3,4], iterative mixture designs[5–7] and the sequential simplex procedure[8,9] are most commonly used.

The sequential simplex procedure, during which the chromatographic response surface is explored by successive experiments and "climbed" until the optimum separation is reached, has proved to be successful in a wide variety of situations[8–11]. As a general optimisation method, the simplex procedure can be used for mobile phase optimisation (both normal-[11] and reversed-phase[8]), for isocratic and gradient separations[8,10] and for the optimisation of secondary effects, such as temperature and flow-rate[9]. The procedure is well suited to automated optimisation, particularly for well behaved separations, as no assumptions about the separation or about solute behaviour are made and solute identification is not necessary.

This ability to optimise a separation with little prior knowledge gives many advantages to the use of the simplex procedure. However without the input of any preliminary data a number of the experiments conducted will provide little information useful in improving the quality of the separation: this may not influence the optimum located but will slow the procedure down. Additionally, while an optimum may be located, there is no guarantee that the global optimum will be found. With no prior information the optimum located may depend upon the starting positions

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of the initial simplex. If, however, the simplexes can be constrained in a relatively small area within which the global optimum lies, then the simplex procedure should reliably and quickly locate that optimum.

What is required is a means of defining this search area. For reversed-phase separations this can be achieved by carrying out a gradient elution separation and then calculating the area of factor space which the simplexes should explore to yield an optimised isocratic separation. The use of a gradient elution separation to predict the conditions necessary to achieve an isocratic separation has been described[5,12,13], although the further optimisation of the isocratic separation necessitated knowledge of the identities of the detected peaks. We have investigated the use of the approach described by Snyder *et al.*[13], which is a computationally simple method, for defining the area in which the optimum isocratic separations lie, following this with a simplex search in that area.

By combining an initial gradient separation with a calculation of a restricted search area it was hoped that a sequential search of this area would more reliably and more rapidly locate the true separation optimum. In this paper we demonstrate that this is indeed the case for a reversed-phase, ternary, isocratic separation. In a situation where a sequential search of all available compositions fails to locate an optimum, a constrained search reliably locates the true optimum and its success does not depend on the places from which the search is started.

THEORY

The equations described by Snyder *et al.*[12,13] were used to calculate the appropriate experimental conditions for the initial gradient separation going from 0 to 100% methanol. The gradient steepness φ' (fraction % per min) was calculated from

$$\varphi' = 0.2/3t_0 \tag{1}$$

where t_0 is the column dead-time in minutes. Having carried out the gradient separation, the predicted value of the capacity factors (k_0) of both the first and last detected peaks in a totally aqueous mobile phase were calculated from*:

$$k_0 = \frac{1}{0.46}\left[10^{\left(\frac{(0.2\ (t_g\ -\ t_0)}{t_0}\right)} - 1\right] \tag{2}$$

where t_g is the elution time (min) of the last detected component.

Next the capacity factors (k_f) of the first and last components in the mobile phase composition present at the exit of the column as the compounds eluted were estimated from

$$k_f = \frac{1}{0.46 + 1/k_0} \tag{3}$$

The actual volume fraction of modifier (φ_f) present in the mobile phase at the time of elution (t_g) is obtained from

$$\varphi_f = \varphi'(t_g - t_0 - t_d) \tag{4}$$

* But see ref. 14 for a further discussion of this value.

where t_d is the delay time of the system, the time taken for a requested change in mobile phase to be registered at the top of the column. Knowing these values, the values of the solvent strength parameter S for the first and last eluted components were calculated from

$$S = (\log k_0 - \log k_f)/\varphi_f \tag{5}$$

The calculation of composition required for a given isocratic retention time was achieved by rearranging eqn. 5. The calculation of φ_f for the last eluted component then gives the proportion of methanol necessary to elute this compound in the desired retention time. It is a simple matter to calculate the corresponding amount of tetrahydrofuran that would be required from ref. 15,

$$\varphi_{\text{tetrahydrofuran}} = 0.577\varphi_{\text{methanol}} \tag{6}$$

This gives the minimum amount of organic modifier required to elute all peaks within a specified time and hence the maximum amount of water that will be needed.

A similar procedure can be employed for the first eluted peak but methanol alone is considered, the balance of water being calculated. Should the amount of water required to achieve a minimum retention of the first peak turn out to be higher than the value calculated for the last eluted peak, this signifies that an isocratic separation is not possible and optimisation can be halted. If all is well these calculations provide a constrained region within which the global optimum will be. It is acknowledged that the constrained region will not completely prevent experiments being conducted which will not elute all the sample components but the number of such experiments will be greatly reduced compared with a completely unconstrained optimisation.

Quality criterion

A requirement for automated optimisation is that the controlling computer must be able to judge the quality of a given separation according to a suitable criterion. This criterion should take into account the number of peaks detected in a chromatogram, their resolutions and the separation time[8,16,17]. The choice of a quality criterion is difficult: many have been described[16]. In previous studies[8], however, the chromatography response function (CRF; eqn. 7) was found to direct reliably simplex optimisations towards the optimum separation.

$$\text{CRF} = \sum_{i=1}^{L} R_i + L^a - b|T_A - T_L| + c(T_1 - T_0) \tag{7}$$

where R is the resolution between adjacent peak pairs, L is the number of peaks detected, T_A is a specified analysis time, T_L is the retention time of the last-eluted peak, T_1 is the retention time of the first-eluted peak, T_0 is a specified minimum retention time and a, b and c are operator-selectable weightings.

If the response surface is not well behaved, or the quality criterion is not unequivocally related to the response surface, a sequential experimental technique may enounter difficulties in finding the global optimum. A separation described as giving

rise to problems associated with a poorly behaved response surface and optimum is that of the five sulphonamides listed in Table I[17]. These compounds were selected to evaluate the modified optimisation scheme, since it was suggested[17] that the simplex optimisation of these compounds is likely to be unreliable.

TABLE I

SOLUTES USED AND PEAK IDENTITIES

Solute name	*Peak identity*
Sulphisomidine	1
Sulphaguanidine	2
Sulphanilamide	3
Sulphacetamide	4
Sulphadiazine	5

EXPERIMENTAL

Apparatus and reagents

All experiments were performed using an Analyst 7800 gradient chromatograph, comprising two ConstaMetric III pumps, a SpectroMonitor D variable-wavelength ultraviolet absorbance detector, set at 254 nm, and an automatic injector (Laboratory Data Control, Stone, U.K.). For ternary mobile phases a third ConstaMetric III pump was added and the whole system was controlled by a Chromatography Control Module, also from LDC.

The columns used for all studies were 15 × 0.46 cm I.D., containing Ultrasphere I.P. (octadecylsilane) from Beckman, High Wycombe, U.K. A pre-column, 4.5 × 0.46 cm I.D., containing Ultrasphere octyl (Beckman) was placed ahead of the main column.

HPLC-grade methanol and acetonitrile were obtained from Rathburn Chemicals, Peebles, U.K., and water was freshly glass distilled. Acetic acid (1%, v/v) was added to all solvents. Solutes were used as received and were dissolved in mixtures of methanol and water at approximately 0.1 mg/ml.

Software

Unconstrained optimisations were carried out using the program TERNOPT which has been described previously[8]. The revised procedure has been incorporated into a BASIC program, entitled FASTOPT, written for the Chromatography Control Module and occupying some 25 kilobytes of memory. Fig. 1 is a flow chart of the program.

The program FASTOPT begins by running an initial gradient according to conditions calculated to produce maximum selectivity[12]. From such a separation, the maximum and minimum levels of water that would be required to bracket the optimum isocratic separation are calculated, for any organic modifier with an eluting power in the range encompassed by methanol and tetrahydrofuran. The minimum amount of water is found from calculating the methanol–water composition that should give a capacity factor of 0.25 for the first detected peak. The maximum

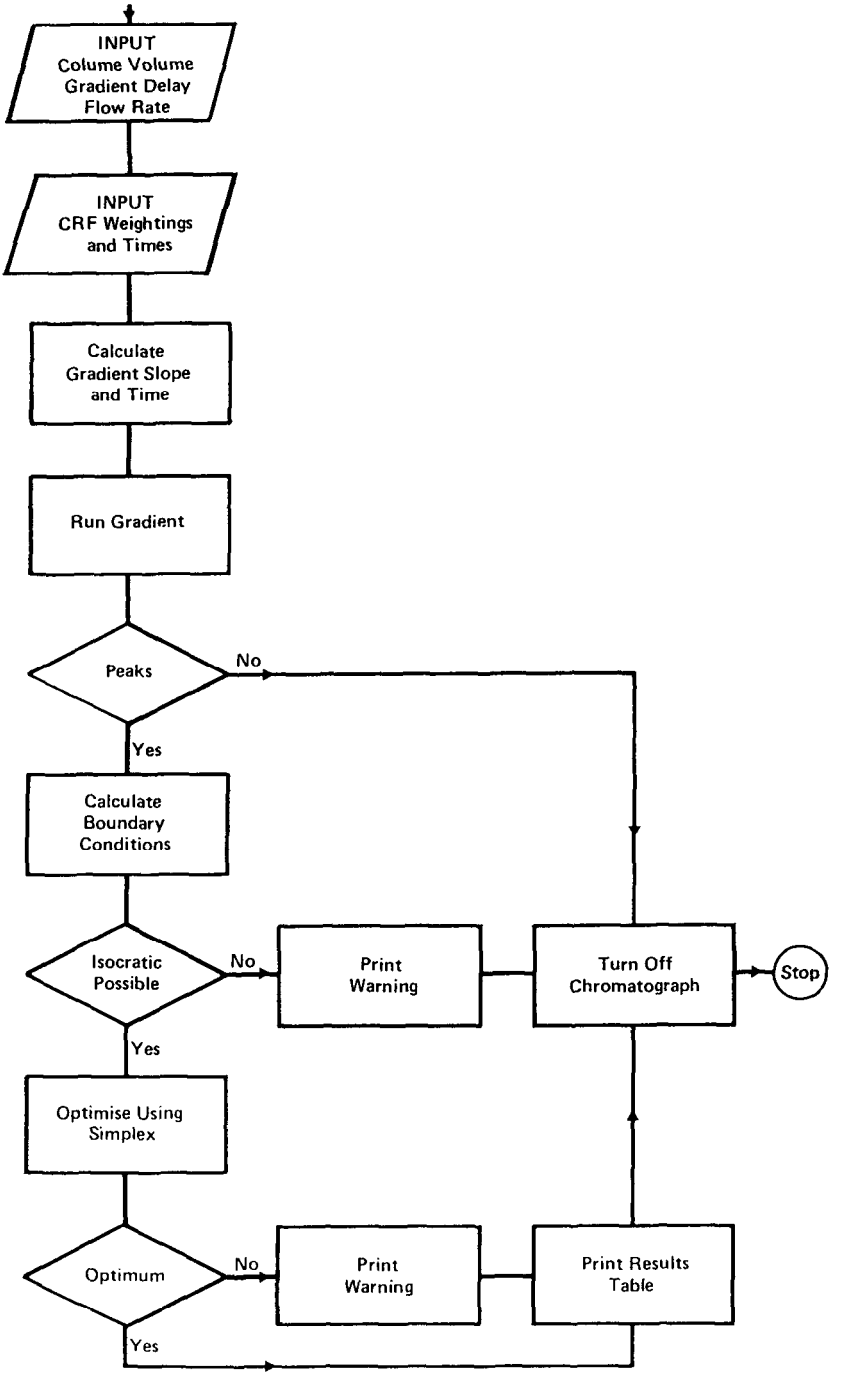

Fig. 1. Structure of program FASTOPT.

amount of water is found from calculating the tetrahydrofuran–water composition required to give a capacity factor corresponding to the specified maximum desired retention time. The results of these calculations then restrict the levels of the two organic modifiers that can be used, and within this range the initial simplex is located according to the method of Yarbro and Deming[18].

Response surface mapping was carried out by determining the value of the CRF, with weighting factors *a*, *b* and *c* set to 2, 0.5 and 0.5 respectively, at intervals over the range 0–45% of each organic modifier for ternary separations with methanol and acetonitrile. The response surface was plotted using a FORTRAN program, entitled 3-D SIMPLEPLT, running on a D.E.C. VAX 11/780 computer.

RESULTS AND DISCUSSION

The unconstrained optimisation of the sulphonamide separation, carried out using TERNOPT, was found to be unreliable, as predicted[17]. Using a flow-rate of 1.5 ml/min, weightings for *a*, *b* and *c* of 2, 0.5 and 0.5 respectively, with minimum and maximum times of 2 and 12 min, the program TERNOPT failed (3 analyses) to locate any optimum within its constraint of a maximum of 30 experiments. The criterion of an optimum having been located was that three moves of the simplex should not differ by more then 3% of the total variable span. This unreliability of the program TERNOPT precluded any detailed evaluation of its performance for the sulphonamide mixture under study and so all subsequent studies were carried out with FASTOPT.

The column dead volume, determined by injecting water under isocratic elution conditions with mobile phases containing 10–30% methanol, was found to be 2.1 ml.

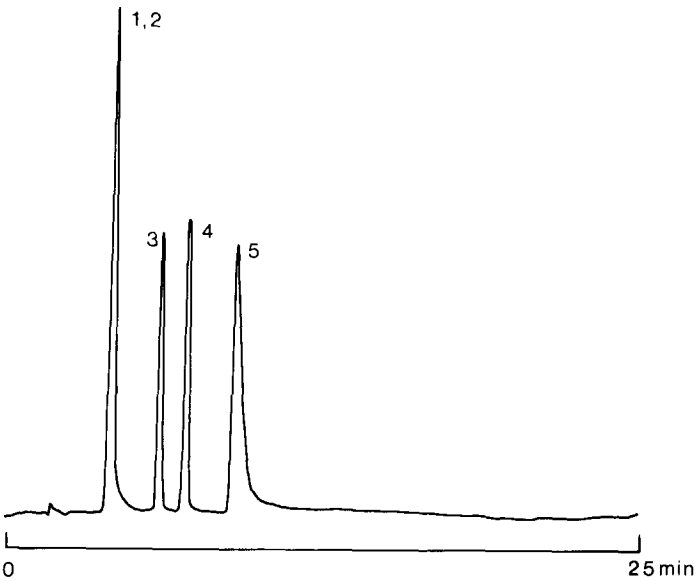

Fig. 2. Initial gradient separation of 5 sulphonamides (for peak identities see Table I). 0–100% methanol in 21 min, 1.5 ml/min. For other conditions see text.

The gradient delay volume was determined as 0.9 ml. All separations were carried out at a flow-rate of 1.5 ml/min from which the slope of the gradient was calculated as 4.8%/min: this gives a total gradient time of 21 min, but the program FASTOPT allows for the gradient separation to continue at 100% methanol for an additional 0.2 times the gradient duration, *i.e.* 25 min in total. A typical initial gradient separation carried out under these conditions is shown as Fig. 2. Table II summarises the results of a number of optimisations carried out under the control of FASTOPT. In

TABLE II

OPTIMA LOCATED USING FASTOPT

A = Water, B = methanol, C = acetonitrile.

Analysis No.	*Water range (%)*	*Weighting (b, c)*	*Optimum (%)*			*No. of experiments*
			A	*B*	*C*	
1	72–91	0.5	86.0	6.6	7.4	24
2	81–92	0.5	83.1	1.2	15.7	14
3	72–91	0.5	85.4	6.7	7.9	26
4	76–100	0.5	85.7	2.2	12.1	22
5	76–91	0.5	85.7	3.5	10.8	17
6	75–91	0.1	88.1	4.7	7.2	19
7	76–91	0.1	83.7	4.6	11.8	24
8	75–91	0.1	88.6	1.2	10.2	24
9	73–91	0.1	88.6	1.9	9.5	21
		Mean	86.1	3.6	10.3	21
		σ_{n-1}	2.0	2.2	2.7	4

all cases a minimum retention time was requested of 2 min, a maximum of 12 min, with an analysis time of 15 min. Parameter *a* of the CRF was set at 2, but *b* and *c* were set as shown in Table II for the individual experiments. In every case, an optimum was located close to the global optimum: slight variations are to be expected due to changes in experimental conditions over the time of investigation (3 months). The position of the global optimum can be deduced from inspection of the response surface, shown as Fig. 3: it lies approximately at water–methanol–acetonitrile (85:4:11). A chromatogram obtained under the optimum conditions is shown as Fig. 4.

The results summarised in Table II also indicate that the location of the global optimum is not dependent upon the values of the weighting parameters *b* and *c*, except in so far as the global optimum will move slightly as the emphasis on time parameters is changed. The movements of the simplexes during a constrained optimisation are shown as Fig. 5 for analysis No. 6.

The sensitivity of the constrained optimisation to its starting points was investigated by locating the initial simplex at different positions within one constrained region. From Table III it can be seen that the procedure is insensitive to that initial starting position. Examining the response surface (Fig. 3) of the separation indicates some of the reasons for the contrast in results between unconstrained and constrained

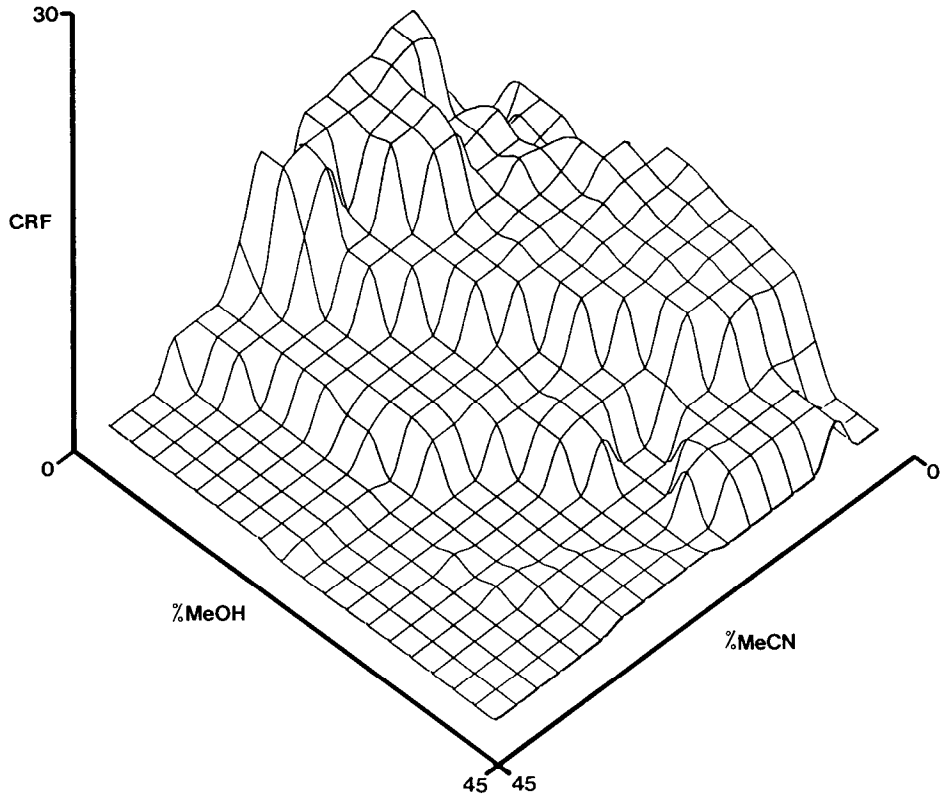

Fig. 3. Chromatographic response surface using CRF as quality criterion.

optimisations. The actual area of optimum separation is extremely small and, therefore, it is likely to be missed by an unconstrained optimisation. Additionally, there are a number of plateaux around the optimum, that is local optima, upon which the simplexes may halt. The constrained optimisations, however, all search only a small area in which the global optimum must be and so always locate it. Note that the response surface, as defined by the use of the CRF, is in fact smooth and well behaved and does not suffer from the multiple optima that have been experienced with other quality criteria for a similar separation[17].

CONCLUSIONS

The sequential simplex procedure is a powerful algorithm for directing the optimisation of HPLC separations without any prior assumptions. However, its performance and reliability can be improved greatly by constraining the simplex search area to a small region containing the global optimum of the separation. It has been shown that, for reversed-phase chromatography, an initial gradient separation can be used to define this restricted search area. The gradient is run under standardised conditions for maximum peak resolution, but it is necessary only to establish the

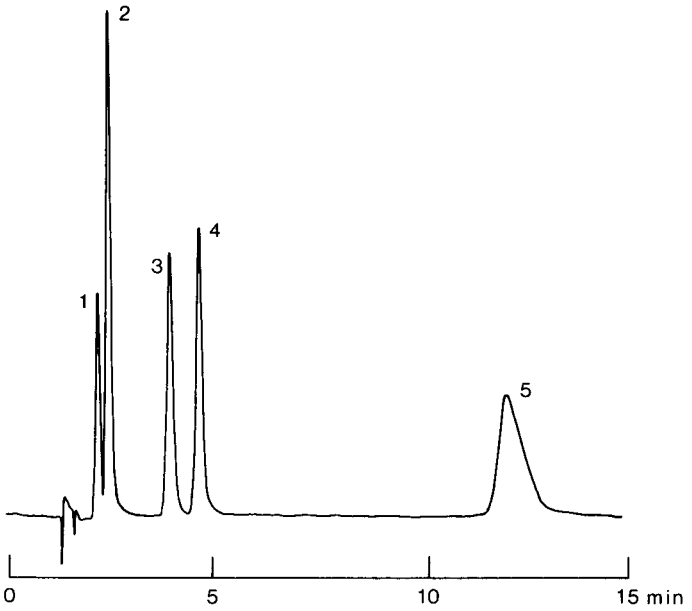

Fig. 4. Optimised separation of sulphonamides. Mobile phase, water–methanol–acetonitrile (85:4:11). For other conditions and peak identities see text and Table I.

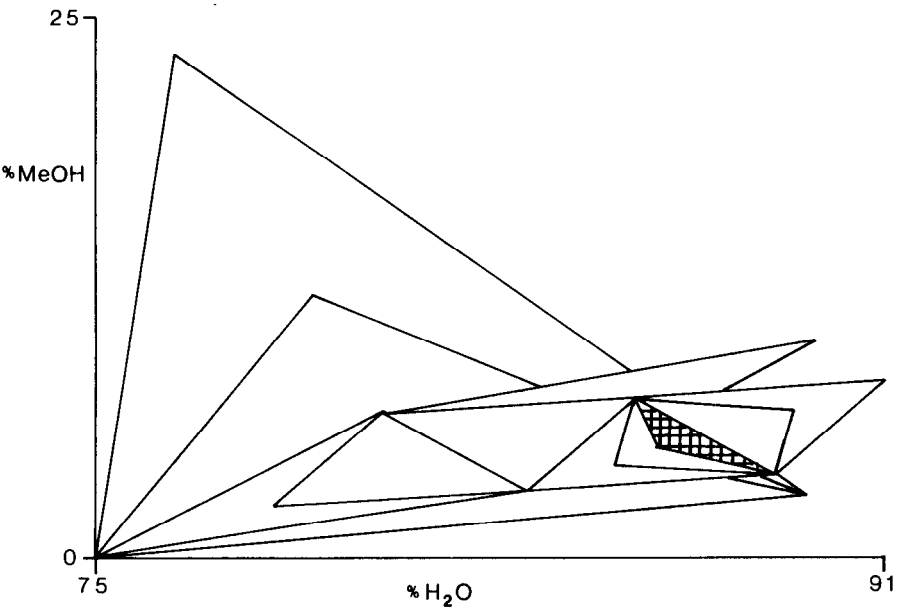

Fig. 5. Movements of simplexes during analysis No. 6 of Table II. The simplex at the optimum is shown hatched.

TABLE III

SENSITIVITY OF OPTIMUM TO LOCATION OF INITIAL SIMPLEX

A = Water, B = methanol, C = acetonitrile.

Analysis No.	*Starting points (%)*			*Weighting (b, c)*	*Optimum (%)*			*No. of experiments*
	A	*B*	*C*		*A*	*B*	*C*	
1	76	24	0	0.5	85.7	3.5	10.8	17
	89.5	8.1	2.4					
	77.5	3.1	19.4					
2	76	24	0	0.1	81.4	5.6	13.0	17
	89.5	8.1	2.4					
	77.5	3.1	19.4					
3	76	0	24	0.5	84.0	3.0	13.0	20
	89.5	2.4	8.1					
	77.5	19.4	3.1					
4	76	0	24	0.5	82.8	4.9	12.3	16
	89.5	2.4	8.1					
	77.5	19.4	3.1					
5	76	0	24	0.5	83.8	7.4	8.8	26
	89.5	2.4	8.1					
	77.5	19.4	3.1					
6	76	0	24	0.1	83.7	4.6	11.8	24
	89.5	2.4	8.1					
	77.5	19.4	3.1					
				Mean	83.6	4.8	11.6	20
				σ_{n-1}	1.4	1.6	1.6	4

retention times of the first- and last-eluted components. These times are used for the calculation of the area in which the global optimum must lie. The precise location of this area is not critical, since subsequent simplex optimisation then quickly and reliably locates the optimum separation by maximising the value of a chromatography response function, a quality criterion which does not require that individual peaks be identified or tracked during the optimisation. The whole procedure can be carried out entirely automatically using a suitable computer-controlled chromatograph.

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