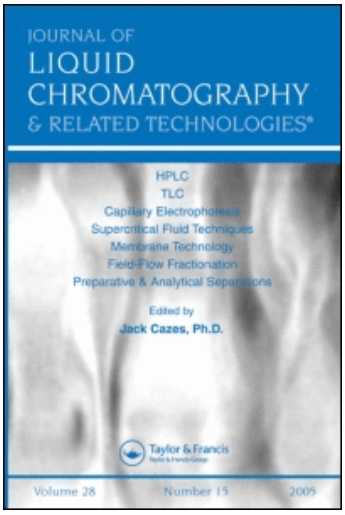

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A New Sequential Procedure for the Efficient and Automated Location of Optimum Conditions in High Performance Liquid Chromatography (HPLC)

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A NEW SEQUENTIAL PROCEDURE FOR THE EFFICIENT AND AUTOMATED LOCATION OF OPTIMUM CONDITIONS IN HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

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ABSTRACT

A new sequential Optimization Procedure by Search Point (OPSP) based on Hooke-Jeeves algorithm is developed. The procedure is an automated multifactor optimization of conditions for an HPLC separation. Its usefulness in computer-assisted method development is shown by the experimental mobile phase optimization of an isocratic reverse phase liquid chromatography separation of a mixture of six selected pesticides. The relative composition of a ternary mobile phase (Acetonitrile, Methanol, Water) was varied during the optimization process. An objective function (OF) which was used as the criterion of quality of the chromatographic

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separation is described. The perfomance of this new chromatographic method is evaluated either by plotting the map of the separation quality using a Grid Search method or by comparing the results with the ones obtained by the application of a Modified Simplex method, in both cases over the same triangular (Acetonitrile, Methanol, Water) parameter space. The optimum solvent composition for the satisfactory separation of pesticides by application of the OPSP was determined from only eleven chromatographic experiments while by applying the Modified Simplex or the Grid Search procedure the number of experiments were 15 and 36 respectively.

INTRODUCTION

The use of the computer as an aid in selecting adequate or optimum conditions in order to perform a given analytical separation within an acceptable analysis time has played an important role in the development of HPLC methods of analysis. This general topic continues to attract great interest as it has been attested by several recent books [1-5] and by the availability of the new commercial software offered by several HPLC manufacturers [2,6,7].

Optimization of the operating conditions neccesary to accomplish adequate separations by HPLC generally requires a formal experimental design coupled with the use of an optimization strategy. Most optimization techniques fall into two categories, viz., sequential experimental procedures and simultaneous experimental procedures [8,9]. Both approaches have been used to optimize the operating conditions of HPLC methods of analysis. The advantages and disadvantages of both approaches have been extensively described [1,3,4].

Quality criteria

Sequential procedures depend basically on two factors, the power of the directing algorithm (search algorithm) and the effectiveness of the quality criterion that is being used. The quality of a chromatographic separation must be expressed as a single numerical value or experimental response. The selection of a suitable criteria to achieve this may vary considerably from one example to another according to the different goals to be met in the optimization process. Often a compromise between conflicting goals has to be found. Optimization of chromatographic separation may therefore be considered as a multicriterion problem [10]. To solve such a problem it is necessary to translate such different analytical goals into objective functions [11] (OF) or desirability functions [12] (DF). These are complex functions incorporating a measure of the amount of separation by sum criteria (ΣRs, ΣP, etc.), product criteria (ΠRs, ΠP, etc.), etc. and other factors such as number of peaks, time requirements, etc. (by separate terms, penalty functions, etc.).

Search algorithm

The Basic Simplex method [13] (BSM) or Modified Simplex method [14] (MSM) unequivocally is the sequential method of choice to optimize HPLC separations. Nevertheless, the Simplex method has serious limitations mainly derived from its limited searching capability [15,16]. This fact leads to the development of different modifications such as Super Modified Simplex [17] (SMS), Weighted Centroid method [18] (WCM). Each of these search methods includes symmetry criteria [19] by which the search algorithm may continue to decide the next search direction after each step in the search process. These limitations are especially important to select mobile phase composition for HPLC due to the limitations in the experimental modification of solvent percentages. Therefore, the existence of

such problems or limitations of this search algorithm means that its implementation for the optimization of a particular analytical method may be very difficult in the hands of one who is not expert in these chemometrics methods.

In this paper a new sequential Optimization Procedure by Search Point (OPSP) based on Hooke-Jeeves algorithm [20] is described, and it is applied to the optimization of the composition of a ternary mobile phase for the HPLC separation of six selected pesticides. The result of the separation is compared with those obtained by application of a Modified Simplex and by application of a Grid Search design over the same parameter space. An objective function (OF) based on resolution (Rs) and time requirements is described and used as quality criterion. The major advantages of this multi-factor procedure are its high simplicity, efficiency, great search capacity, and the suitability for automation of the optimization process.

THEORY

The OPSP method is guided by calculations and decisions that are rigorously specified but trivially simple. The procedure is a hill-climbing method in which the direction and increment of advance is dependent only on the experimental responses and not on any particular values on an absolute scale. Only one experiment is needed for each step of the optimization process.

The algorithm

The objective of this sequential procedure is to force a point inside the selected factor space, named "search point", to move away from regions of poor response towards the region of optimum experimental response. In the present discussion a point at a given location within the factor space

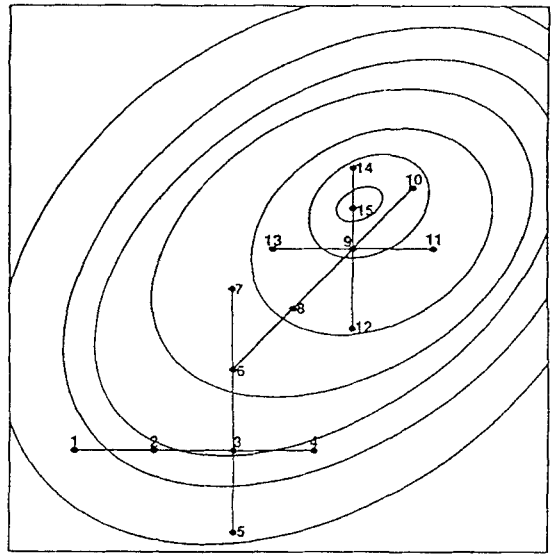

FIGURE 1. Illustration of a two-dimensional OPSP optimization. Concentric lines are contour lines.

corresponds to a specific ternary mobile phase composition. Movement implies a change in the composition of the ternary mobile phase and, thus, a point at a different location within the factor space. In Figure 1, a simple experiment beginning at point 1 is carried out to determine the experimental response. Subsequent movements or steps in the process are made according to a set of "rules". Figure 2 summarizes the rules of the OPSP procedure.

Rule 1.- After each experiment a movement is made according to any of the orthogonal or bisecants directions toward the limits of the factor space chosen (for non orthogonal experimental design, e.g. triangle, it will be considered as orthogonal the two firstly selected variables to locate the movements) by applying Rule 2 (orthogonal movement) or Rule 4 (bisecant movement). Figure 1 shows the progress of the "search point" in a two

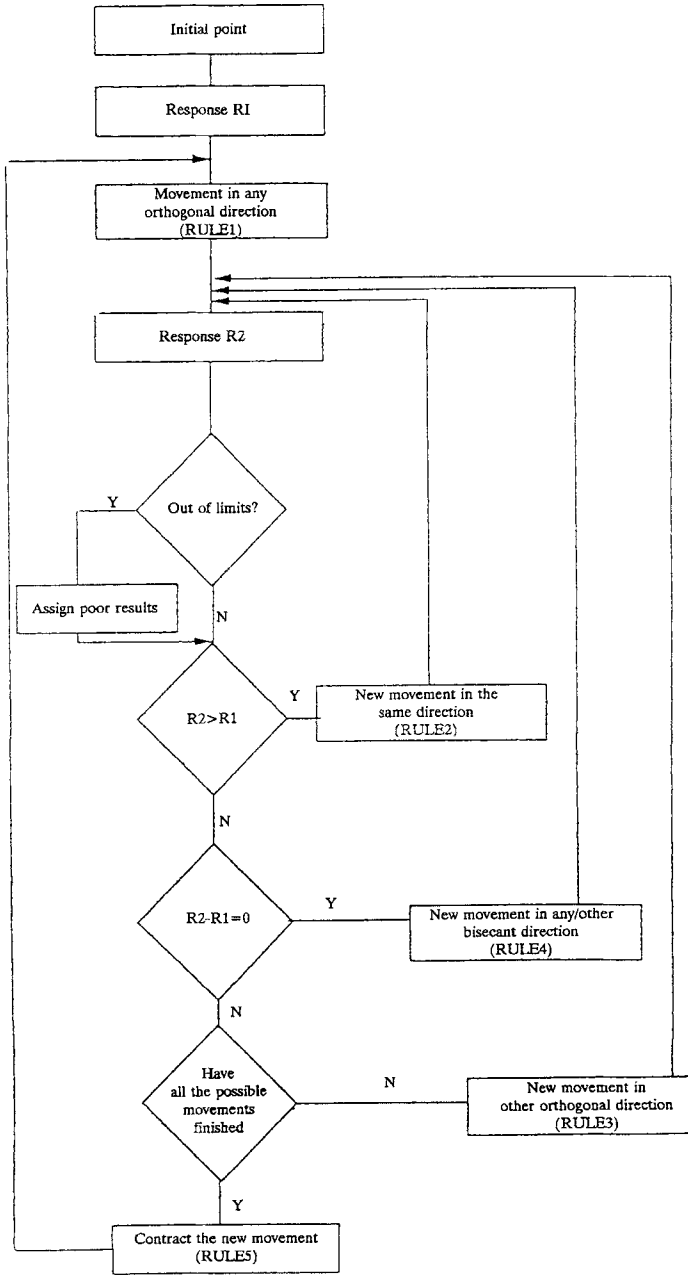

FIGURE 2. Flow chart of OPSP procedure.

variable factor space. Having carried out an experiment at point 1 at the beginning of the procedure, the experimental response is calculated by an adequate objective function and a new point, 2, is then calculated by application of a vector.

Rule 2.- Direction, sense and magnitude of the movement remain constant if the experimental response obtained is classified as better. For example, as shown in Figure 1 the movement of point 2 gives a new point, 3, and the movement of point 3 leads to point 4. This procedure is repeated until the optimum is located or until a violation of Rule 3, 4 or 5 occurs.

Rule 3.- If the new point has a worse response, do not apply Rule 2 but instead change the direction 90 degrees from the previous movement. As it can be seen in Figure 1, point 3 is followed by two sequential attempts before a better response, 6, is obtained.

Rule 4.- If the response at a new point differs very little or not at all from the previous point (R_2-$R_1 \approx 0$ or minor than a target value previously fixed from 0.01 to 1.00) do not apply Rule 2 but instead change the direction 45 degrees of the previous movement. As it can be seen in Figure 1, point 7 has an equal response than 6 and then it is followed by point 8.

Rule 5.- If the response at a new point is worse than the experimental responses in all the possible displacements from the previous point, then re-apply Rule 1 with a contracted increment. Referring to Figure 1, the movement of point 9 gives a new point, 15, as a result of a contracted vector displacement.

Increment of movement: The choice of the step sizes for Rule 2 and Rule 5 are arbitrary but usually a large increment (e.g. 1/6-1/8 of the range of a selected variable for Rule 2) is an advantage for two reasons. First,

to ensure some change in response and second a large step can more rapidly approach the optimum. In the OPSP program discussed here the increment can be fixed arbitrarily or generated automatically following the criterion mentioned above.

Location of the initial search point: Locating the initial search point is closely related to the size of the movement selected, but we considered a good practice to choose a central point over the domain of factor space to force the search point to back itself. In the OPSP program described here, location of the initial point can be fixed arbitrarily or generated automatically by application of a single mathematical relation among the limits of the search area selected.

End of Search criterion: One can choose among various criteria, such as the level of response, the number of experiments carried out or whether the different subsequent responses approaches the random noise and error.

EXPERIMENTAL

Instrumentation

High perfomance liquid chromatography system used includes a(n):

- 600E pump (Waters, Mildford Massachusetts),
- Rheodyne six-port injection valve with 20 µl loop,
- 990 photodiode array detector (Waters),
- 990 printer/plotter (Waters) and
- Olivetti microcomputer using a 991 software (Waters) implemented with the OPSP program developed by the authors.

TABLE 1

Pesticides studied in this work.

No	Pesticide studied	Aqueous solubility ($mg.\ l^{-1}$)	Use in Europe[21]
1	Metomyl	58×10^3	local
2	Dimethoate	25×10^3	major
3	Aldicarb	6×10^3	major
4	Dichlorvos	10×10^3	local
5	Carbofuran	700	local
6	Atrazine	30	major

HPLC separations were conducted using a Hypersil (Green Env.) 3x150 mm (5 μm particle size) C18 column (Shandon).

Chemicals and solvents

HPLC-grade solvents (Merck) were used in this work. Mobile phases were degassed with helium prior to use. Distilled water was obtained from a Millipore water purification Milli-Q system.

The pesticide standards (pestanal quality) listed in Table 1 were obtained from Riedel-de Haën (Seelze, German). Stock solutions were prepared by dissolving 20 mg of each purity certified pesticides in 100 ml of acetonitrile. A standard sample solution was prepared by mixing 200 μl of each of the stock solutions and making up to 10 ml with acetonitrile. All solvents and samples were filtered through a Millipore membrane filters (0.45 μm) before injection onto the column.

Chromatographic conditions

The following initial chromatographic conditions were used: the ternary mobile phase consisted of solvent A acetonitrile (AcN), solvent B methanol

(MeOH), and solvent C (water); flow-rate was 1 ml. min^{-1}; the UV detector was set at 210 nm; volume injected was 20 µl and column temperature was 30°C. The isocratic composition of the mobile phase in the reversed phase elution was optimized to obtain the best separation of pesticides. The void time was obtained by using methanol as the unretained component for all mobile phases. All chromatographic runs were duplicated. Reproducibility of retention times was ±0.5% or better.

Quality criteria

Objective Function. The first step in the optimization procedure was to select a suitable criteria to achieve an optimum judgement according to the different goals that have to be met in optimization procedure. The selection of an objective function (OF) based on penalty functions -p- type "infinite wall" was realized on the criteria given by Deming [11]. This function is defined as

$$OF = \Sigma (R_{i,j} \cdot P) \quad (I)$$

$$P = 1 \text{ for } Y > Y_{it};\ Y < Y_{ft} \quad (II)$$

$$P = 0 \text{ for } Y < Y_{it};\ Y > Y_{ft} \quad (III)$$

where $R_{i,j}$ is the resolution of each pair of peaks i and j. The maximum value assigned to $R_{i,j}$ is fixed in 1.5 in order to avoid that $\Sigma R_{i,j}$ is determined largely by the largest values of $R_{i,j}$.

P is the value of the penalty, y is the value of the retention time and Yt is the target retention time (in all the cases i is referred to the initial peak and f the last peak considered).

We assign a target value for Y_{it} =2 minutes and the second target retention time is Y_{ft} = 15 minutes as maximum acceptable retention time of the last peak considered. This objective function was used in the different optimization procedures applied in this work.

RESULTS AND DISCUSSION

Variable space selected

A water, acetonitrile, methanol triangular variable space is selected. Relying on literature data and previous experience, we observe that domains near the vertexes are of no interest because they have lower values of Y_{it} than 2 min or higher values of Y_{ft} than 15 min. So, in order to restrict the search area we select as esperimental boundary conditions: %AcN 20-70, %MeOH 0-65 and %Water 20-60. Therefore the variable space is restricted as shown in Figure 3 where the search region is darkened.

Over this search domain we apply the procedure proposed (OPSP computer program elaborated by the authors) to locate the coordinates of the optimum mobile phase (highest criterion value OF) to separate the standard mixture selected.

OPSP optimization

Inital point. The first point was automatically defined by the program using the equation,:

$$Vi = Vmin + Range\ (100 - \Sigma Vmin_{A,B,C}) / \Sigma Vmax_{A,B,C} - \Sigma Vmin_{A,B,C} \qquad (IV)$$

where Vi is the initial volume percentage selected of each variable (A, acetonitrile; B, methanol; and C, water), Range is the range of each

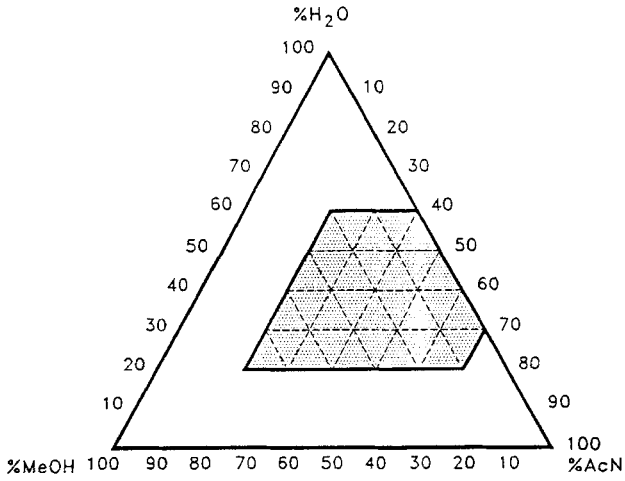

FIGURE 3. Graphical representation of search area (darkened) in the OPSP optimization.

variable (A,B,C), $\sum Vmin_{A,B,C}$ is the sum of minimum values of volume percentages of each variable and $\sum Vmax_{A,B,C}$ is the sum of maximum values of volume percentages of each variable. The initial value selected consists of the smallest possible value plus a factor proportional to the range of each variable. The initial experimental conditions are 39% AcN, 25% MeOH, 36% Water. The chromatogram of this initial experiment are shown in Figure 4.

Size of movements. The size of movements were automatically generated with sizes of 1/8 of the range of each variable considered for Rule 2 and Rule 4 and half ratios for Rule 5. The only two variables as criteria for the size and direction of orthogonal movements were the two first variables selected; %AcN and %MeOH.

OPSP movements. A set of experiments (Figure 5A) was therefore carried out guided by the search algorithm. A satisfactory separation (OF =

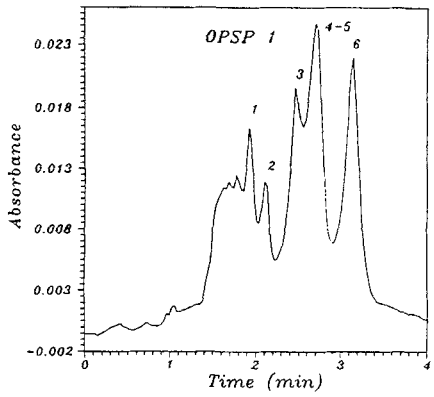

FIGURE 4. LC chromatogram of standard mixture of pesticides using a mobile phase corresponding to the initial point selected in the OPSP procedure. Chromatographic conditions are described in text. For peak identification see Table 1.

7.5) was achieved when eleven experimental chromatographic runs had been performed. This value OF =7.5 was the maximum possible, so we stop the movements at this point (see Table 2). The optimum conditions are 27% AcN, 17% MeOH and 56% Water. Figure 6A shows the chromatogram of the selected pesticides using these conditions.

Modified Simplex optimization

Initial Simplex. The location of the initial Simplex was (close to initial point of the OPSP procedure): vertex 1, 30% AcN: 30% MeOH: 40% Water; vertex 2, 40% AcN: 32% MeOH: 28% Water; and vertex 3, 32% AcN: 40% MeOH: 28% Water.

Size of movements. The size of the movements was calculated using a step size [22] of 10% (close to the sizes of the OPSP procedure) and a α value of 1. For constractions were used alpha values of 0.8, 0.6 and 0.5

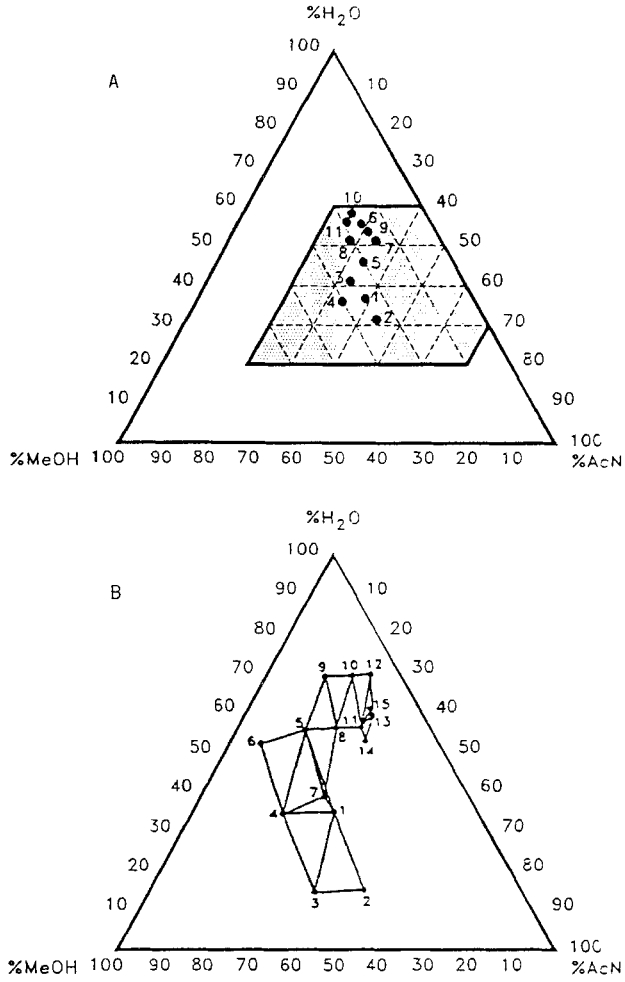

FIGURE 5. (A) Movements of the OPSP procedure across the variable space selected; (B) movements of the Modified Simplex procedure across the variable space selected; (C) Overview of the mobile phases compositions requiered in the Grid Search used.

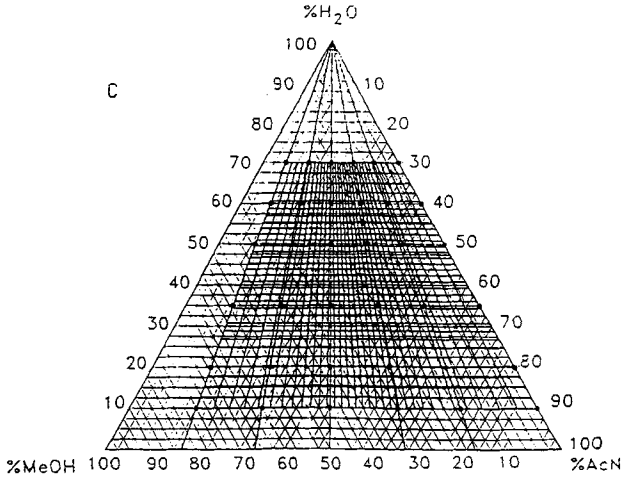

FIGURE 5 (continued)

TABLE 2
Experimental runs and results for the OPSP optimization.

Experience	%AcN:%MeOH:%H_2O	FO
1	39:25:36	3.18
2	44:25:31	3.02
3	34:25:41	3.42
4	34:30:36	2.88
5	34:20:46	3.84
6	29:15:56	5.04
7	34:15:51	3.67
8	29:20:51	5.74
9	31:15:54	4.99
10	27:15:58	6.36
11	27:17:56	7.50

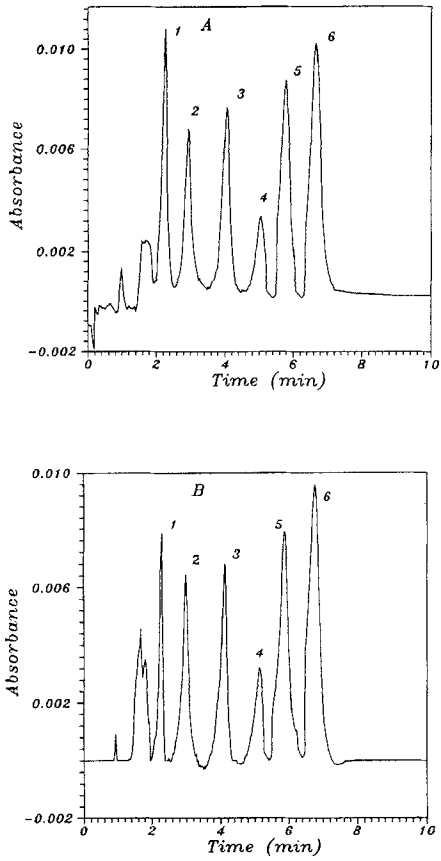

FIGURE 6. LC chromatograms of standard mixture of pesticides under optimum mobile phase obtained by: (A) the OPSP procedure; (B) the Modified Simplex procedure and (C) the Grid Search procedure. Chromatographic conditions are described in text. For peak identification see Table 1.

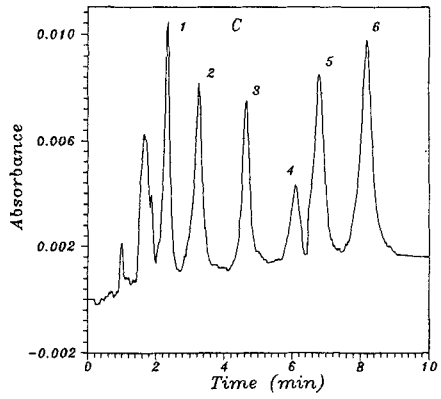

FIGURE 6 (continued)

TABLE 3
Experimental runs and results for the Modified Simplex optimization.

Initial vertexes	%AcN:%MeOH:%H_2O	α	FO
1	30:30:40	—	2.93
2	40:32:28	—	1.62
3	32:40:28	—	1.80
Movement N° (Vertex N°)	**%AcN:%MeOH:%H_2O**	**α**	**FO**
1 (4)	23:37:40	1.0	5.20
2 (5)	20:28:52	1.0	5.25
3 (6)	15:34:51	0.8	5.16
4 (7)	27:31:42	0.8	5.22
5 (8)	24:23:53	0.8	5.86
6 (9)	19:20:61	0.8	5.36
7 (10)	22:17:61	0.8	6.09
8 (11)	27:19:54	0.8	7.20
9 (12)	25:14:61	0.8	6.80
10 (13)	28:17:55	0.6	7.06
11 (14)	29:20:51	0.6	7.05
12 (15)	27:17:56	0.5	7.50

(see Table 3). These size values are close to the sizes used in the OPSP procedure.

Simplex movements. The movements of the simplexes are listed in Table 3 and them can be seen in Figure 5B. The best value of OF is obtained in the movement 12. So, 15 experiences have been necessary. Figure 6B shows the chromatogram of the selected pesticides using the optimum conditions.

Grid Search optimization

To check the results obtained by the OPSP procedure an additional set of 36 experiments was performed to draw the map of the separation quality using a grid of small increments over the same water-acetonitrile-methanol solvent triangle as shown in Figure 5C and with the mobile phases indicated in Table 4. The different OF values obtained let the graphic representation of the response surface as shown in Figure 7. The set experimental conditions corresponding to the top of the tallest "mountain" gives the best possible separation. Figure 7 predicts the global optimum in a narrow region near %AcN = 20-40, %MeOH = 10-20 and %Water = 45-65 which are in very close agreement with that obtained by the proposed procedure. Figure 6C shows the chromatogram of the selected pesticides using the best conditions.

Comparison of optimization methods applied

The results of the application of the OPSP, Grid Search and Simplex optimization are listed in Table 5 and optimum chromatograms obtained in each procedure are shown in Figure 6. It can be noted lower experiences number for OPSP procedure and an optimum separation in this case.

TABLE 4

Objective function (OF) values corresponding to the Grid Search chosen.

Experience	% AcN:% MeOH:%H2O	FO	Experience	% AcN:% MeOH:%H2O	FO
A1	5:25:70	3.00	D1	11:54:35	2.56
A2	10:20:70	3.00	D2	22:43:35	3.03
A3	15:15:70	3.00	D3	33:32:35	3.12
A4	20:10:70	4.50	D4	43:22:35	3.07
A5	25:5:70	7.00	D5	53:12:35	1.62
A6	30:0:70	6.74	D6	65:0:35	2.16
B1	7:33:60	5.09	E1	13:67:20	1.92
B2	13:27:60	4.00	E2	27:53:20	1.17
B3	20:20:60	6.68	E3	40:40:20	0.00
B4	27:13:60	7.28	E4	53:27:20	0.77
B5	33:7:60	6.36	E5	66:14:20	0.00
B6	40:0:60	6.45	E6	80:0:20	0.00
C1	8:42:50	5.14	F1	15:75:10	0.00
C2	17:33:50	6.09	F2	30:60:10	0.00
C3	25:25:50	5.70	F3	45:45:10	0.00
C4	33:17:50	5.15	F4	60:30:10	0.00
C5	41:9:50	4.56	F5	73:17:10	0.00
C6	50:0:50	4.89	F6	90:0:10	0.00

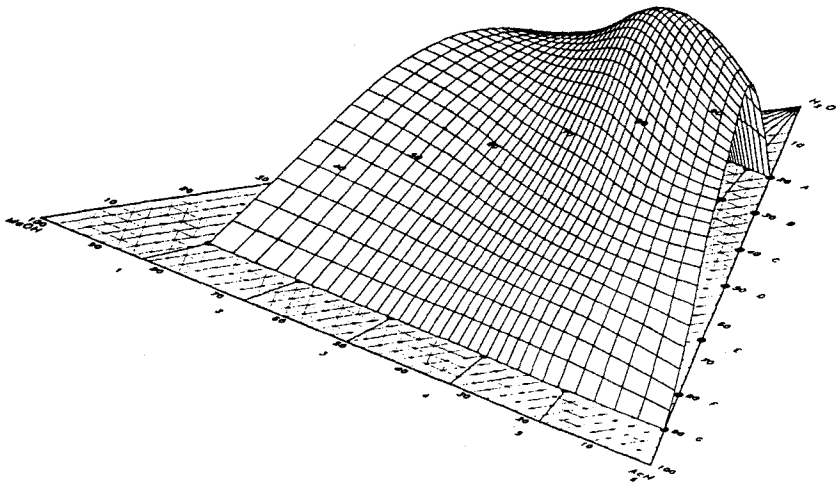

FIGURE 7. Pseudo-three dimensional response surface obtained by the Grid Search method.

TABLE 5
Movile phase composition optimum, experiences number and objective function (FO) for the three methods of optimization studied.

OPTIMIZATION METHOD	MOVILE PHASE COMPOSITION (AcN:MeOH:H_2O)	EXPERIENCES No	FO
GRID SEARCH	28:15:57	44	7.29
OPSP	27:17:56	11	7.50
MODIFIED SIMPLEX	27:17:56	15	7.50

CONCLUSIONS

A new sequential procedure (OPSP) has been developed and applied to the optimization of the chromatographic selectivity in the separation of selected pesticides by HPLC using isocratic reverse phase elution. The above results demonstrate that the OPSP procedure is an useful technique to find eluent compositions that give optimal separation. Althought the present study has involved only three factors and isocratic mode, the concepts are entirely general and can be extended to higher dimensional factor spaces, to other LC modes and to other operating parameters. The OPSP program can be requested from the authors.

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