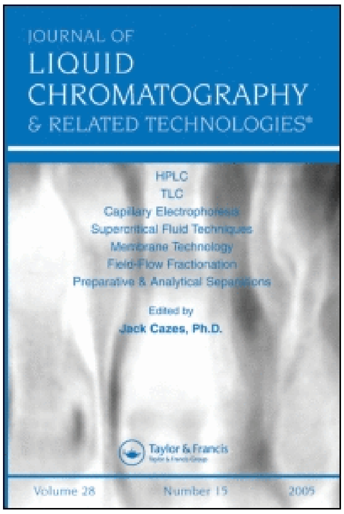

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COMPUTER-ASSISTED OPTIMIZATION OF PH AND ION CONCENTRATION SELECTIVITY IN HPLC USING A MIXTURE DESIGN SIMPLEX METHOD

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ABSTRACT

A computer–assisted mixture design simplex method is presented for optimization of two–factor (pH and ion concentration) simultaneous selectivity for the optimal separation in reversed–phase HPLC. The method is based on two–factor selectivity rectangle concept with a special polynomial estimated from nine preliminary runs. Then connect to general simplex method for optimization. Double criteria simulation system (DCSS) is established for the measurement of chromatographic performance by this method. The validity of the optimization strategy is proved by applying it to an actual mixture and as compared with the general simplex method about thirty eight experiments can be omitted.

INTRODUCTION

Today HPLC is routine method for solving many practical analytical problems. In addition to the broadening of its applications, advaces have been made in our fundamental understanding of the separation mechanisms in HPLC. However, in spite of this most routine HPLC analyses are still being developed in a non–systematic mannar, and very often the results are not as good as might be expected from this powerful technique.

In recent years, several procedures have been described for the selection of the optimal mobile phase composition in HPLC. Sequential simplex method [1] [2], window diagrams [3] [4], overlapping resolution maps (ORM) [5] [6], "PRISMA" model method [7] [8] and iterative mixture designs [9] [10] has been suggested as optimization methods of moblie phase composition selectivity in HPLC. Recently we have described a computer–assisted optimization of binary moblie phase composition, pH and Ion concentration selectivity [11]. In most of the previously published papers only one factor was optimized in HPLC except simplex method. The results of the optimization of single factor very often are not as good as might be expected from the optimal separation. Sometime, the multifactor optimization can be obtained more better separation. But sequential simplex method requires more experments and local optimum may be found which are distinct disadvantages.

In this paper a computer–assisted mixture design simplex method is presented for optimization of two–factor (pH and ion concentration) simultaneous selectivity for the optimal separation of a mixture of five compounds ((1) 3,4–dihydroxybenzoic acid, (2) benzoic acid, (3) 4–hydroxybenzaldehyde, (4) 4–nitrophenol and (5) 2–nitrophenol) in reversed–phase HPLC. The principle of method is based on a special polynomial between capacity factor k' and two factors which are estimated from nine preliminary experiments according to the full factorial design, then proceed on general simplex method. Double criteria simulation system (DCSS) was established for the measurement of chromatogaphic performance. Excellent agreement is obtained between predicted and experimental results and compared with original simplex method about thirty eight experiments can be omitted.

For the optimization we have developed the SDO_L (Simplex Difactor Optimization for HPLC) computer program.

EXPERIMENTAL

Materials

Compounds 3, 4–hydroxybenzoic acid, benzoic acid, 4–hydroxybenzaldehyde, 4–nitrophenol and 2–nitrophenol were obtained from Chemical Reagent Factory of Beijing (Beijing, P. R. China). Compounds dissolve in acetone and then dilute with eluent solvent.

Approximate 0.1mg / ml concentration of the solutions in eluent solvent were used for injection. Before use all solvents were distilled and filtered through a 0.45 μ filter and vacumm–degassed.

The mobile phase components and their different ratios are given in Table 1. The compositions of the four constituents were: A. methanol, B. water, C. 0.015 M tetrabutyl ammonium bromide and D. pH buffer solution (combinations of 3.6 ml 0.2 M sodium acetate solution and 16.4 ml acetic acid solution dilute to 400 ml).

Apparatus

All computer studies were carried out on a Model HP–220 computer (Hewlett packard, Palo Alto, CA USA) with a HP–9133A disk drive, HP–2225A printer and HP–7470A graphics plotter.

SDO_L program was written in HP BASIC 4 language, alternately an IBM–XT personal computer with True BASIC language was used.

The reversed–phase HPLC system was composed of a Model series 4 liquid chromatograph (Perkin–Elmer, Norwalk, CT USA) with a Perkin–Elmer LC_ 75 UV detector and C–R1B data system (Shimadzu, Tokyo, Japan).

The μ Bondapak C_{18} column (Waters Assoc., Milford, MA USA) was 300 mm × 3.9 mm.

TABLE 1. The k′ values of five compounds measured by HPLC using different mobile phase composition

Mobile phase composition * *					Compound No. *				
No.	A	B	C	D	1	2	3	4	5
	%				k′				
1	50.0	50.0	0	0	0.94	0.98	1.48	2.20	2.54
2	50.0	47.5	2.5	0	0.97	1.50	1.50	2.18	2.68
3	50.0	45.0	5.0	0	1.02	1.08	1.54	2.27	2.81
4	50.0	47.5	0	2.5	1.08	1.70	1.50	2.21	2.70
5	50.0	45.0	2.5	2.5	1.12	1.80	1.49	2.16	2.72
6	50.0	42.5	5.0	2.5	1.11	1.90	1.48	2.13	2.63
7	50.0	45.0	0	5.0	1.16	1.84	1.60	2.34	2.90
8	50.0	42.5	2.5	5.0	1.15	2.04	1.54	2.18	2.70
9	50.0	40.0	5.0	5.0	1.16	1.89	1.52	2.11	2.57

* Compound No. (1) 3, 4–dihydroybenzoic acid, (2) benzoic acid, (3) 4–hydroxybenzaldehyde, (4) 4–nitrophenyl and (5) 2–nitrophenyl.

* * A, B, C and D refer the text.

Chromatography

Quanternary mobile phase components A, B, C and D were composed in different ratio as shown in Table 1. The flow was 1.0 ml / min and 2 μl samples were injected. The column was thermostted at 25 ℃ and detector was used at 250 nm.

RESULTS AND DISCUSSION

The principle of computer–assisted mixture design simplex method is based on the effect of two variables (pH and ion concentration) in HPLC as well as their possible interaction. A full fractorial design (Figure 1) was adopted as

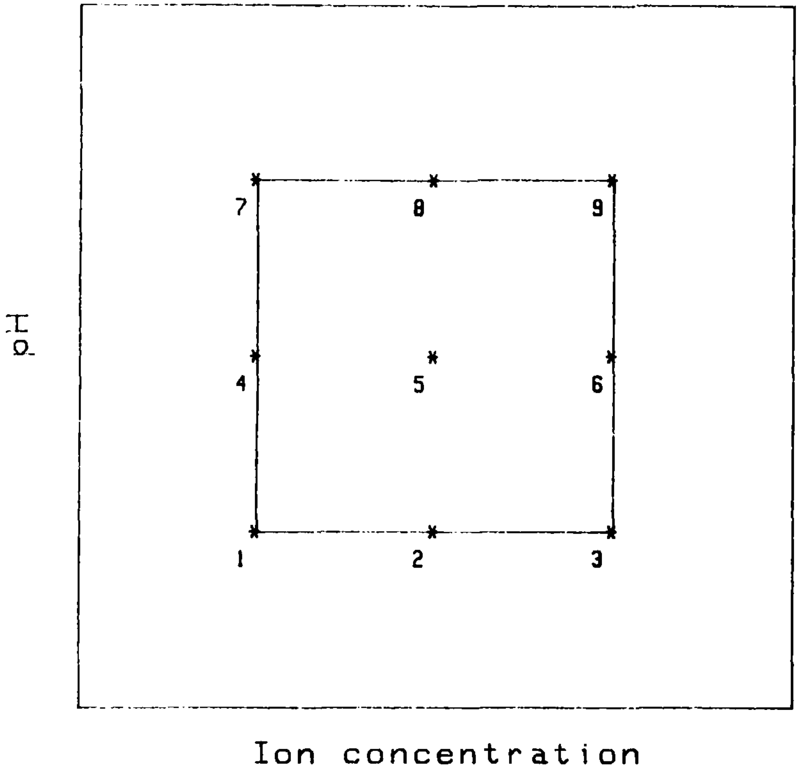

FIGURE 1. A full fractorial design for two-factor effects.

the optimization strategy. The nine preliminary experiments were carried out and the capacity factor values measured. These values were then substituted into the following equation, in order to establish the values of the constants:

$$k' = b_0+b_1X_1+b_2X_2+b_{11}X_1^2+b_{22}X_2^2+b_{12}X_1X_2 \qquad (1)$$

where k' is the capacity factor, X_1, X_2 are ion concentration and pH, b_0, b_1, b_2, b_{11}, b_{22} and b_{12} are constants characteristic of a given compound. Equation (1) means that X_1 and X_2 are given the capacity factor can be determined for the chromatographic conditions.

Since SDO_L method considers only two factors, only three initial experiments were required which can be selected from the nine preliminary experiments to perform the simplex process and obtain the maximum R_S value. The analysis time can be controlled by limiting the k′ value.

However, optimization criterion have a great influence on the success of an optimization. To solve this problem, double criteria simulation system (DCSS) was established for measurement chromatographic performance in the present paper which was used optimization criteria of two kind with simulation chromatogram technique. The principle is described in below.

Minimal resolution was used as the first criterion for separation; which can be effect by three independent factors:

$$R_S = \frac{1}{4}\,\frac{(\alpha-1)}{\alpha}\,(\sqrt{N})\,\frac{k'}{1+k'} \tag{2}$$

Where α is the selectivity factor two peaks and N is the column plate number. Note that the predicted k′ value of the solutes are used to arrange in order before calculation of every R_S, then analysis of resolution only adjacent pairs not for all pair peaks; the result is requirded acceptable. Minimal resolution, was selected in each simplex iterative proceeding. The result was obtained the largest resolution for the worst separated pair of peaks. All the other pairs of peaks give larger resolution values.

TABLE 2. The values of coefficient b of five compounds and correlation coefficient, r.

No. *	b_0	b_1	b_2	b_{11}	b_{22}	b_{12}	r
1	0.938	0.017	0.073	0.000	−0.006	−0.003	0.9948
2	1.044	0.200	0.348	−0.034	−0.039	−0.002	0.9637
3	1.482	0.003	−0.009	0.002	0.006	−0.006	0.9930
4	2.194	−0.015	−0.009	0.006	0.007	−0.012	0.9906
5	2.546	0.058	0.056	−0.001	0.003	−0.024	0.9836

* Compounds No. refer Table 1.

TABLE 3. The result of double criteria for selection initial condition in SDO_L method.

No.	Initial condition		R_s criterion			$\prod R_s$ criterion		
	C%	D%	R_s	C%	D%	$\prod R_s$	C%	D%
1	2.5 5.0 2.5	2.5 2.5 5.0	1.01	5.00	2.52	3.13	5.00	0.00
2	2.5 2.5 5.0	0.0 2.5 2.5	1.01	5.00	2.52	3.38	4.53	0.00
3	2.5 2.5 0.0	0.0 2.5 2.5	1.06	0.95	0.00	2.94	1.25	0.00
4	2.5 2.5 0.0	5.0 2.5 2.5	1.07	0.69	2.72	2.48	0.58	2.73
5	2.5 5.0 0.0	5.0 2.5 2.5	1.01	5.00	2.52	3.38	4.53	0.00
6	0.0 2.5 0.0	0.0 2.5 5.0	0.83	0.00	4.10	3.13	5.00	0.00
7	2.5 0.0 2.5	0.0 2.5 5.0	1.06	0.95	0.00	2.94	1.25	0.00
8	5.0 0.0 2.5	0.0 2.5 5.0	0.70	5.00	0.00	3.13	5.00	0.00
9	0.0 5.0 2.5	0.0 2.5 5.0	0.99	5.00	2.50	2.94	1.10	0.00

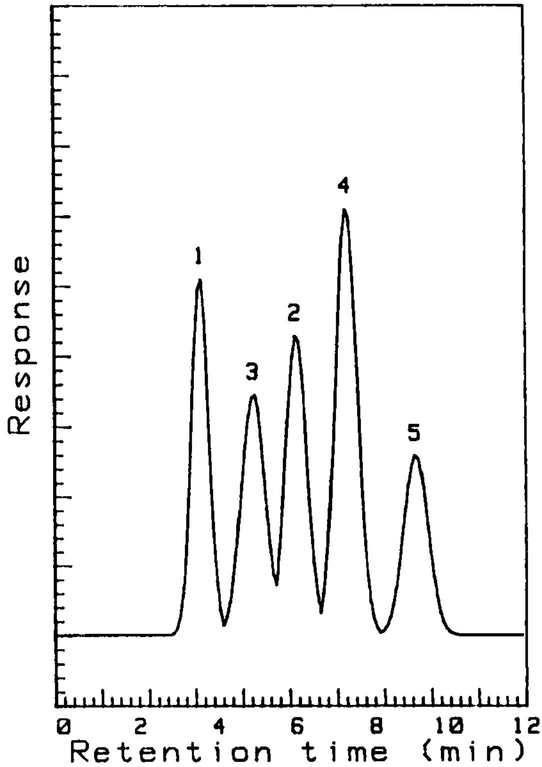

FIGURE 2. Initating chromatogram at C = 0.69 %, D = 2.72 %.

The fact, that only the worst separated pair of peaks is considered, however, is a disadvantage, since the overall chromatogram may look just as bad, while other conditions may be possible where one pair of peaks is only partly separated but all others are separated well.

Obviously, the disadvantage is partly overcome with the kind of response function as an optimization criterion. The resolution product, $\prod R_S$, was used as the second optimization criterion which was defined by Drouen et al. [9].

$$\prod R_S = \prod_{i=1}^{n-1} R_{S(i,i+1)} \tag{3}$$

where $R_{S(i,i+1)}$ = resolution between peak and its next neighbour and n = num-

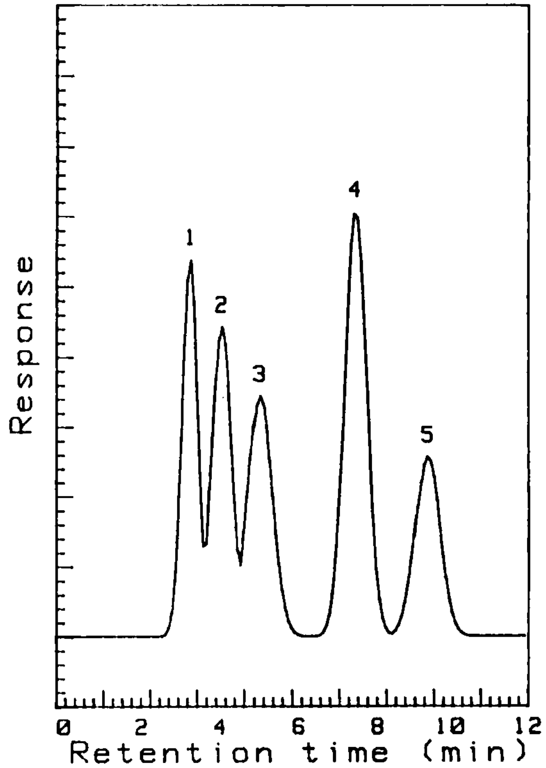

FIGURE 3. Initating chromatogram at C = 4.53 %, D = 0.00 %.

ber of peaks. This criterion aims at an even distribution of all peak over the chromatogram. But the serious drawback of all response funtions is the loss of individual peak information.

So the criteria of two different kind have its advantage and disadvantage. Therefore the optimization criteria of two different kinds were incorporated into the method which can be constructed to supplement each other. Then an evaluation of the results of two criteria can be employed computer–assisted simulation technique [12], which can be simulated the chromatogram at any given two–factor composition of mobile phase and the separation of peaks as related to composition can be predicted.

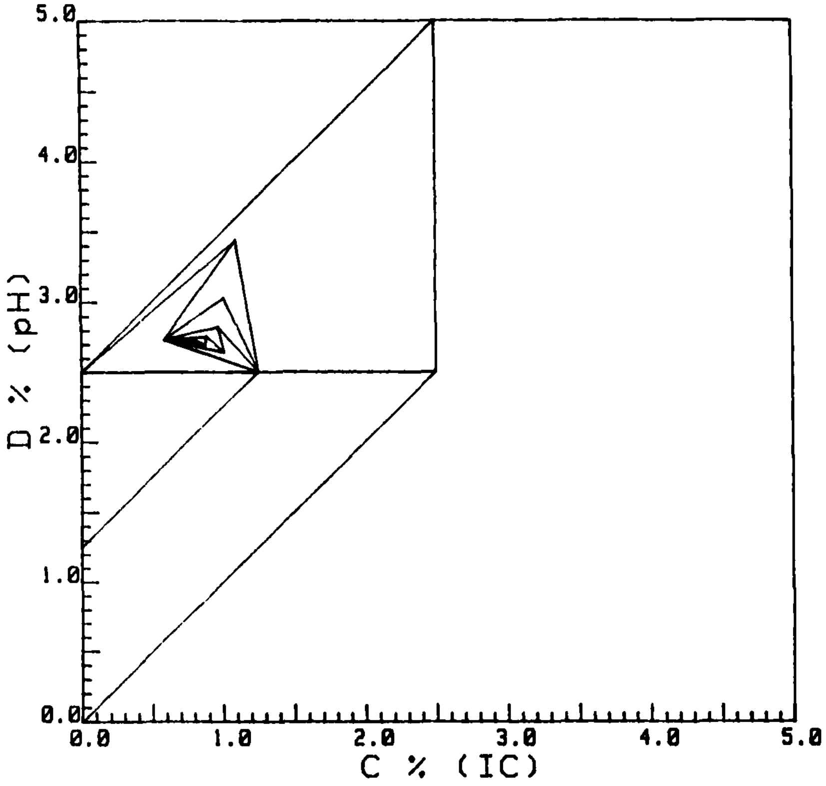

FIGURE 4. Simplex optimization using minimal resolution for criterion.

Imitating chromatograms were obtained from the result of optimal criterion of two kind to find these mobile phase compositions, then accrding to the practical need selecte one.

A mixture of five compounds (Table 1) was applied for the optimization of the two–factor (pH and ion concentration) in reversed– phase HPLC. Table 1 shows the experimental k′ values and Table 2 lists the coefficients of b. The experimental boundary condition are:

C% (ion concentration): 0–5
D% (pH) : 0–5

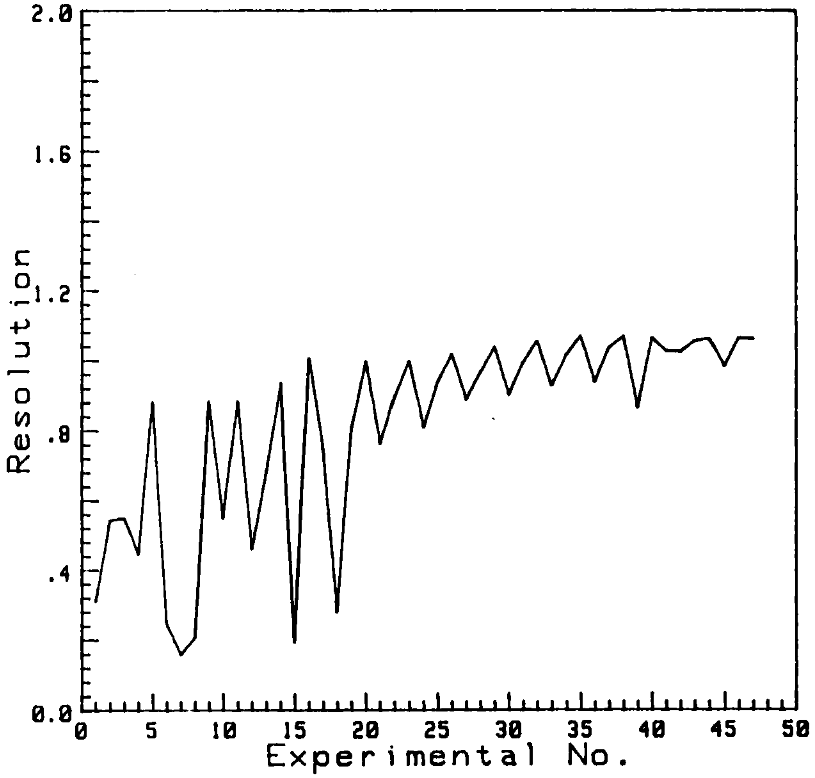

FIGURE 5. Simplex iterative process vs. resolution.

Three initial experiments were required which was selected nine times repeatedly from the nine preliminary experiments to perform the simplex process. The result can be reduced finding local optimization to obtain the optimal condition which was shown in Table 3.

According to criterion of minimal resolution the maximum R_S is 1.07 that corresponding to initial condition is No. 4 (Table 3) and the optimal condition of two–factor is 0.69% C and 2.72% D. The result of criterion of resolution product give the maximum $\prod R_S$ is 3.38 at the initial condition No. 2 and 5 (Table 3) which give the same two–factor optimal condition: C% = 4.53, D% = 0. Then we could simulate the chromatograms at the

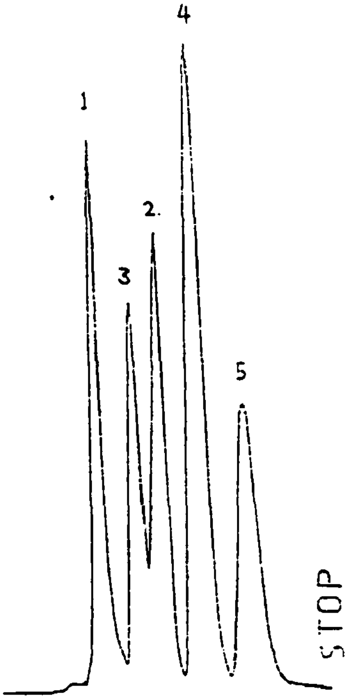

FIGURE 6. Experimental chromatogram at optimum condition. C = 0.69 %, D = 2.72 %.

two–factor composition of No. 4 and 5. Figures 2 and 3 show the imitating chromatograms, respectively. Clearly , the result of No. 4 is better than No. 5. So the No. 4 was selected for the experimental separation and corresponding to the initial experimental condition are:

1. C% (IC) = 2.5, D% (pH) = 5.0
2. C% (IC) = 2.5, D% (pH) = 2.5
3. C% (IC) = 0.0, D% (pH) = 2.5

The result of simplex process were shown in Figures 4 and 5 from using minimal resolution criterion. Note that forty eight iterative process were performed by the computer. The maximum R_s is 1.07. It is the highest resolution for the worst separated pair of peak. The optimum condition of two factor is 0.69% C and 2.72% D. Figure 6 shows the experimental result at the optimal condition. There is a good agreement between the predicted and experimental results.

The result of SDO_L method as compared with the general sequential simplex method about thirty eight experiments can be omitted. In addition SDO_L can be selected repeatedly the simplex initial experiments from the nine preliminary experiments without any additional experiment. The result can be reduced finding local optimization to obtain the maximum R_S.

Therefore the SDO_L method have distinct advantage comparing with the general sequental simplex method for two–factor optimization in HPLC.

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