Computer-Assisted Optimization of Two-Step Development High-Performance TLC

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Abstract

A computer-assisted method is presented for the optimization of two mobile phase compositions and the selection of development distance of a mixture of nine components in two-step development high-performance thin-layer chromatography. Optimization of the separation over the experimental region is based on two special polynomials estimated from preliminary runs of two groups. Excellent agreement is obtained between predicted and experimental results using R_f difference as the selection criterion and a three-factor statistical scanning technique.

Introduction

In recent years, studies on the systematic optimization of mobile phase composition have gained widespread acceptance. There are a number of schemes being developed for the optimization of thin-layer chromatographic separations. The following methods have been suggested: overlapping resolution maps (1); sequential simplex (2,3); window diagram (4); PRISMA (5); computer-assisted comprehensive optimization (6); two methods that use statistical techniques to design optimized multicomponent solvent systems (7,8); and integration optimization (9).

Complex mixtures containing components with a wide range of retardation factors cannot be separated by general optimization methods. A technique for optimizing mobile phase composition in stepwise gradient thin-layer chromatography (TLC) (10) and two-dimensional TLC (11,12) has been published.

The multiple development technique is a modern method that enables high spot capacities to be attained. It is used for the analysis of complex mixtures (13). However, this technique is being studied in a nonsystematic manner, and often, the results are not as good as might be expected.

In this paper, a computer-assisted statistical scanning method is proposed for optimizing the separation of a mixture of nine components in two-step development TLC. The principle of the method is based on two special polynomials between the R_f values and mobile phase composition, which can be estimated using from four to six preliminary experiments of two groups. This is followed by a three-dimensional computer scanning technique that involves the following parameters: volume fraction of first-step development, Xs(1); volume fraction of second-step development, Xs(2); and first-step development distance, Z1.

For optimization, we developed the TSDO_T (Two-Step Development Optimization for TLC) computer program.

Experimental

Materials

The following nine components (six pesticides and three unknown compounds) were used in two-step development high-performance TLC: unknown A; metalaxyl; pyridaphenthion; 2,3,6-trimethylpyrazine; prometryn; unknown B; thiobencarb; parathion; and unknown C. Approximately 1 mg/mL of a sample in ethyl acetate was used for spotting. *n*-Hexane, ethyl acetate, methylene chloride, and chloroform were obtained from Second Chemical Reagent Factory of Tainjin (Tainjin, P.R. China), and all solvents were redistilled before use.

Apparatus

TLC was performed on precoated silica high-performance TLC plates from Merck (Darmstadt, Germany). A Nanomat applicator (Camag; Muttenz, Switzerland) was used with a Pt-Ir pointed glass capillary. Plates were developed in a closed chamber (Camag). A Model HP-220 microcomputer (Hewlett-Packard; Palo Alto, CA) was used for data processing. The TSDO_T program was written in HP BASIC 4 language; an IBM personal computer equipped with QBASIC was also used.

Chromatography

Plates were developed with several different volume fractions of the first (*n*-hexane–ethyl acetate) and second (methylene chloride–chloroform) mobile phases (see Tables I and II).

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Samples (200 nL) were spotted by means of a Pt-Ir pointed glass capillary. A typical development required the solvent front to reach 50 mm from the origin. After development, the plate was dried thoroughly with warm air, and the positions of the components were revealed by iodine.

Optimization of conditions in two-step development experiments was achieved in the following way. The first step was developed with a 0.25-volume fraction of *n*-hexane–ethyl acetate. A typical development required the solvent front to move 35 mm (Z1 = 0.7) from the origin. At the end of the first development, the plate was removed from the tank, and the solvent was evaporated using a stream of warm air. The second development was performed with a 0.08-volume fraction of methylene chloride–chloroform. The solvent front was required to move 50 mm (Z2 = 1) from its original position. After development, the plate was dried thoroughly with warm air, and the positions of the components were revealed by iodine.

Table I. R_{f1} Values of Nine Components at Different Xs(1) of Ethyl Acetate*

Components	$R_{f1} \times 100$				
	0.2	0.4	0.6	0.8	1.0
Unknown A	1.1	2.0	3.0	2.9	3.6
Metalaxyl	7.7	21.5	41.2	50.4	54.5
Pyridaphenthion	7.9	24.0	44.4	56.8	61.9
2,3,6-Trimethylpyrazine	20.1	32.6	37.2	40.2	43.9
Prometryn	31.1	51.8	66.9	70.5	71.3
Unknown B	31.1	57.0	67.4	69.9	72.3
Thiobencarb	45.2	60.9	73.4	76.2	76.8
Parathion	46.9	64.6	76.6	76.6	79.1
Unknown C	61.9	73.0	76.6	76.6	79.1

*Mobile phase composition: *n*-hexane–ethyl acetate (v/v).

Table II. R_{f2} Values of Nine Components at Different Xs(2) of Chloroform*

Components	$R_{f2} \times 100$				
	0.0	0.2	0.5	0.8	1.0
Unknown A	1.0	1.2	1.5	1.6	2.0
Metalaxyl	4.1	4.0	4.7	9.9	11.1
Pyridaphenthion	16.3	14.7	17.9	22.4	25.8
2,3,6-Trimethylpyrazine	17.7	17.0	18.8	19.8	20.8
Prometryn	20.6	16.0	19.4	24.6	29.8
Unknown B	44.4	42.7	42.7	44.8	47.9
Thiobencarb	71.8	70.9	70.4	70.4	70.6
Parathion	56.7	56.9	56.8	61.8	65.1
Unknown C	83.1	82.4	79.7	79.8	78.2

*Mobile phase composition: methylene chloride–chloroform (v/v).

Results and Discussion

The principle of the optimization method is based on the relationships between R_{f1} (R_f from first development) and Xs(1) (volume fraction of ethyl acetate) and between R_{f2} (R_f from second development) with Xs(2) (volume fraction of chloroform) in the following manner:

$$R_{f1} = a_0 + a_1 \text{Xs}(1) + a_{11}\text{Xs}(1)^2 \qquad \text{Eq 1}$$

$$R_{f2} = b_0 + b_1\text{Xs}(2) + b_{11}\text{Xs}(2)^2 \qquad \text{Eq 2}$$

where a_0, a_1, a_{11}, b_0, b_1, and b_{11} are constants characteristic of given components. It is necessary to determine these values experimentally for each component in a given volume fraction. Equation 1 or 2 may be used to predict R_f values of samples components for any composition of the mobile phase.

Markowski and Soczewinski (14) derived Equation 3 for the R_f value of a solute chromatographed under two-step development conditions:

$$R_{fg} = Z1\ R_{f1} = (Z2 - Z2\ R_{f1})R_{f2} \qquad \text{Eq 3}$$

where Z1 and Z2 are the distances in the first and second step development, respectively; R_{fg} is the final R_f value; R_{f1} is R_f of the first-step development with the volume fraction Xs(1); and R_{f2} is the R_f of the second-step development with the volume fraction Xs(2). From Equations 1, 2, and 3, the R_{fg} in any Z1, Z2, Xs(1), and Xs(2) can be predicted. In this study, the second step development distance, Z2, is equal to 1. Then Z1, Xs(1), and Xs(2) are used for simultaneous optimization with the computer scanning tech-

Table III. Values of Coefficients a_x and the Correlation Coefficient (r)

Components	a_0	a_1	a_{11}	r
Unknown A	0.000	0.062	–0.027	0.9763
Metalaxyl	–0.166	1.252	–0.533	0.9940
Pyridaphenthion	–0.183	1.349	–0.538	0.9962
2,3,6-Trimethylpyrazine	0.086	0.687	–0.343	0.9887
Prometryn	0.029	1.595	–0.916	0.9984
Unknown B	0.035	1.653	–0.980	0.9896
Thiobencarb	0.230	1.247	–0.713	0.9980
Parathion	0.246	1.291	–0.757	0.9911
Unknown C	0.516	0.636	–0.371	0.9725

Table IV. Values of Coefficients b_x and the Correlation Coefficient (r)

Components	b_0	b_1	b_{11}	r
Unknown A	0.010	0.007	0.002	0.9755
Metalaxyl	0.040	–0.016	0.093	0.9731
Pyridaphenthion	0.158	–0.033	0.137	0.9903
2,3,6-Trimethylpyrazine	0.174	0.007	0.029	0.9650
Prometryn	0.197	–0.149	0.254	0.9778
Unknown B	0.444	–0.107	0.142	0.9990
Thiobencarb	0.717	–0.044	0.033	0.9929
Parathion	0.569	–0.060	0.144	0.9898
Unknown C	0.832	–0.066	0.018	0.9637

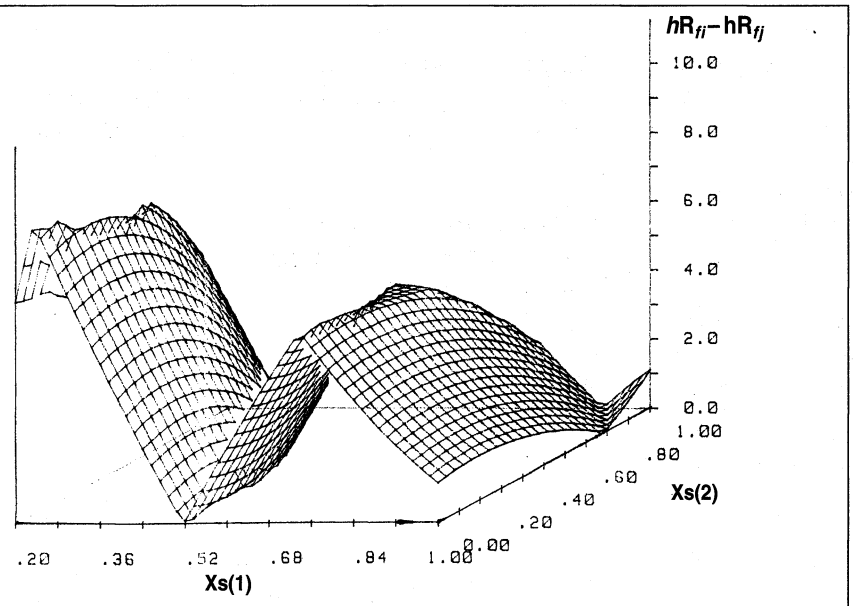

Figure 1. TSDO_T stereo ΔR_f contour map for the nine components in two-step development high-performance TLC.

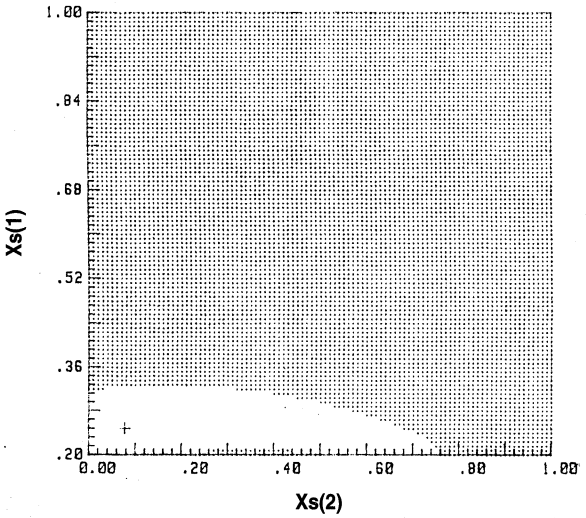

Figure 2. TSDO_T planar ΔR_f contour map for the nine components in two-step development high-performance TLC.

Table V. Comparison of Predicted (P) and Experimental (E) Values for Nine Components Under Optimum Conditions

Components	hR_{fg} (P)	hR_{fg} (E)	E%
Unknown A	2.0	1.9	5.3
Metalaxyl	1.4	12.1	5.8
Pyridaphenthion	22.6	21.0	7.6
2,3,6-Trimethylpyrazine	31.1	28.8	8.3
Prometryn	39.7	38.8	8.0
Unknown B	58.8	56.0	5.0
Thiobencarb	81.3	77.0	5.3
Parathion	72.4	65.2	11.0
Unknown C	90.6	84.2	7.6

* For Z1 = 0.7, Xs(1) = 0.248, and Xs(2) = 0.08.

nique in three dimensions.

In our previous work (4,6,7,9), the difference between R_f values was used as the separation criterion for optimization methods. This value is also used in the present method as an indication of chromatographic performance.

$$\Delta R_{fg} = |R_{fgi} - R_{fgj}| \qquad \text{Eq 4}$$

The R_{fg} values predicted for the solutes are used to calculate the ΔR_{fg} values for every pair of spots under each condition of the two-step development. The method requires that the individual spot positions be mapped as the conditions change. The TSDO_T program used for this procedure calculates ΔR_{fg} for adjacent pairs of spots only, not for all possible pairs; the results show that the largest ΔR_{fg} value was obtained for the worst pair of spots, so for all other pairs of adjacent spots, the ΔR_{fg} value will be higher. This two-step development optimization procedure was evaluated for the high-performance TLC separation of a mixture of nine components. The R_{f1} and R_{f2} values for each of the nine components are presented in Tables I and II, and values for coefficients *a* and *b* are listed in Tables III and IV

The following results were obtained using this optimization procedure: Z1 = 0.7, Xs(1) = 0.248, and Xs(2) = 0.08, which gave a ΔhR_{fg} of 8.5.

The stereo and planar ΔR_{fg} contour maps are produced by fixing Z1 at 0.7 and plotting the values of ΔR_{fg} for the worst separated pair of spots as a function of Xs(1) and Xs(2) (Figures 1 and 2). Note that in Figure 2, the white region is designated as the optimum two-step development condition, where the value of ΔR_{fg} between all possible pairs of spots is equal or better than the desired value (6.0). The point of the maximum ΔhR_{fg} value (8.5) is marked. In practice, the optimum separation was obtained. Table V lists the predicted and measured results using optimum conditions; they are in good agreement.

Optimazation of the two-step development technique has been compared with that of one-step development optimization using a one-dimensional scanning technique (9). The results are shown in Figures 3 and 4. Obviously the general optimization method (one-step development) obtains maximum ΔR_{fg} (1.8) with *n*-hexane ethyl acetate. It achieves a ΔhR_{fg} of 4.0 with methylene chloride–chloroform. All are less than the two-step development. Therefore, the TSDO_T method has distinct advantages over the general optimization method for the separation of a complex mixture.

Conclusion

It is possible to optimize the separation of a complex mixture by using computer-assisted two-step development high-per-

formance TLC. The R_f difference was used as the criterion. Excellent agreement was obtained between the predicted data and the experimental results.

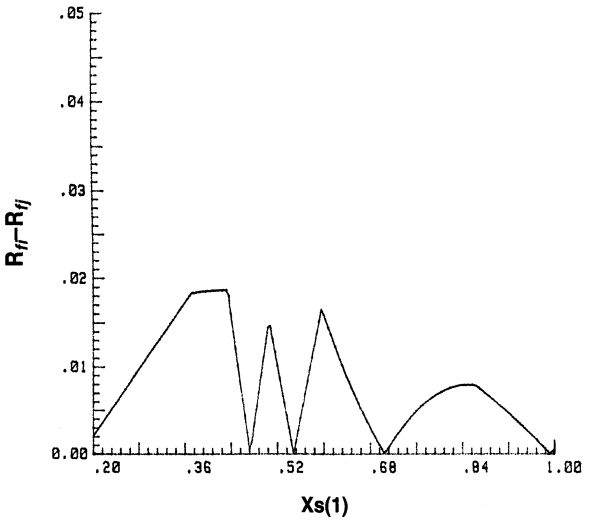

Figure 3. Response surface of ΔR_f as a function of Xs(1) for nine components in high-performance TLC.

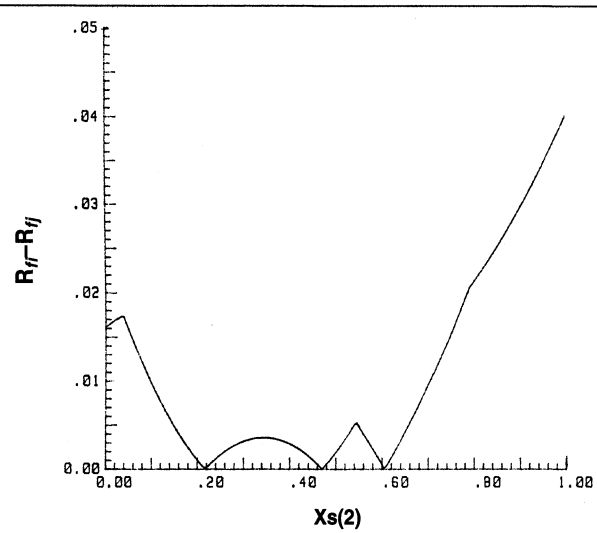

Figure 4. Response surface of ΔR_f as a function of Xs(2) for nine components in high-performance TLC.

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