

Short Communication

Optimization of the separation of technical aldicarb by semi-preparative reversed-phase high-performance liquid chromatography

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ABSTRACT

A computer-assisted method is presented for optimization of separation of technical aldicarb pesticide by semi-preparative reversed-phase HPLC. The optimization of the expected separation is based on a polynomial estimation from five preliminary experiments. A statistical scanning technique was used for optimization and the designated resolution was established for chromatographic performance measurement in this method. Excellent agreement was obtained between the predicted and experimental results.

INTRODUCTION

In recent years, various procedures have been described for the selection of the optimum mobile phase composition in HPLC and there are several reviews on this subject [1–5]. The sequential simplex method [6,7], window diagrams [8,9], overlapping resolution maps [10,11], the Prisma model method [12,13] and iterative mixture design [14,15] have been suggested as optimization methods for mobile phase composition in HPLC. Recently we described a computer-assisted optimization of binary mobile phase composition, pH and ion concentration selectivity using a scanning technique [16] and a simplex optimization of the experimental parameters in pre-

parative liquid chromatography has been also described [17].

In this paper, a computer-assisted method is presented for the optimization of the selection of the composition of the mobile phase in semi-preparative reversed-phase HPLC. The successful separation of technical aldicarb (a synthetic pesticide) is presented as an example. Excellent agreement was obtained between the predicted and experimental results. For the optimization the computer program OS-PLC (Optimization System for Preparative Liquid Chromatography) was developed.

EXPERIMENTAL

Materials

Technical aldicarb and its impurities were prepared in our Organic Synthesis Laboratory. Solutions with a concentration of 2.3 mg/ml in the mobile phase solvent were used for injection. Before

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TABLE I

k' VALUES OF ALDICARB AND IMPURITIES MEASURED BY SEMI-PREPARATIVE HPLC WITH DIFFERENT MOBILE PHASE COMPOSITIONS

Mobile phase: methanol-water with volume fractions (X_s) of methanol from 0.3 to 0.7.

No.	Compound	X_s				
		0.30	0.40	0.50	0.60	0.70
1	Aldicarb sulphoxide	1.279	1.161	1.148	1.085	1.050
2	Aldicarb sulphone	1.534	1.237	1.211	1.089	1.050
3	Unknown A	2.508	1.832	1.754	1.475	1.240
4	Unknown B	3.168	1.989	1.869	1.475	1.240
5	Propionaldoxime	4.473	2.756	2.540	1.869	1.416
6	Aldicarb	5.752	3.004	2.734	1.893	1.429

use, all solvents were redistilled, filtered through a 0.45- μ m filter and vacuum degassed.

Apparatus

All computer studies were carried out on a Model HP-220 computer (Hewlett-Packard) with an HP-9133A disk drive, HP-2225A printer and HP-7470A graphics plotter. The OS-PLC program was written in HP basic 4 language. Alternatively, an IBM-XT personal computer with True BASIC language was used.

The reversed-phase HPLC system was composed of Series 4 liquid chromatograph (Perkin-Elmer) with a Perkin-Elmer LC-75 UV detector and C-R1B data system (Shimadzu). A Spherisorb C₁₈ column (300 mm \times 8 mm I.D.) (Dalian Institute of Chemical Physics, Dalian, China) was used.

Chromatography

Methanol-water in different proportions (Table I) was used as the mobile phase. The injection volume was 200 μ l. Experiments were run at 20°C with a flow-rate of 2.5 ml/min. The UV detector was used at 240 nm.

RESULTS AND DISCUSSION

The principle of the method is based on the relationship between the capacity factor, k' , of each solute and the mobile phase composition. In a binary solvent system, the capacity factor of a solute is related to the volume fraction, X_s , in the following manner:

$$k' = A_0 + A_1 X_s + A_{11} X_s \quad (1)$$

where A_0 , A_1 and A_{11} are constants of the given solute. It is necessary to determine experimentally the constants for each solute in the binary solvent system. Five preliminary experiments were used for solving eqn. 1 to yield A_0 , A_1 and A_{11} .

Minimum resolution ($R_{s \min}$) was used as the criterion for separation. The predicted k' values of the solutes were used to calculate the R_s values for adjacent pairs. If n is the peak number, only $n-1$ pairs were calculated at each solvent composition and the minimum resolution, *i.e.*, the least separated pair of peaks, was selected. Then a diagram of $R_{s \min}$ versus solvent composition (X_s) was obtained. The maximum $R_{s \min}$ was selected and this shows that the solvent composition will give a better separation for the least separated pair, so all other peak pairs will have higher R_s values. The result of chromatographic selectivity optimization is the separation of the solutes of interest from all the other (unimportant) components of the sample mixture.

Obviously, sometimes the separation of the least

TABLE II

COEFFICIENTS A_0 , A_1 AND A_{11} FOR ALDICARB AND IMPURITIES

No. ^a	A_0	A_1	A_{11}	r
1	1.6022	-1.3626	0.8286	0.9775
2	2.4722	-4.1160	3.0000	0.9727
3	4.3271	-7.7573	4.8643	0.9744
4	6.7848	-15.8986	11.5286	0.9713
5	9.5170	-21.8082	14.8071	0.9756
6	14.4074	-38.3070	28.5500	0.9700

^a See Table I.

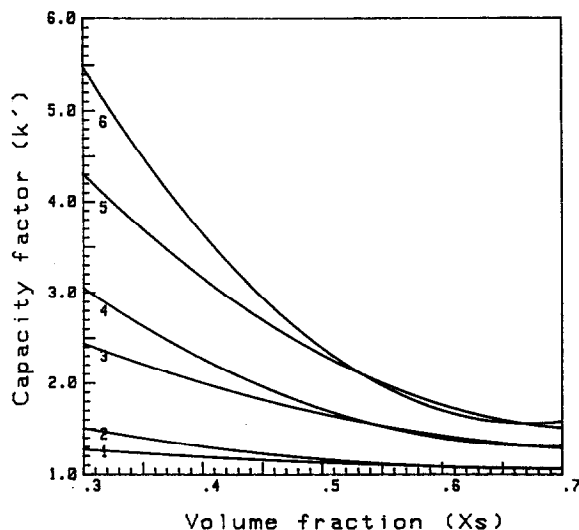


Fig. 1. Capacity factor, k' , versus mobile phase composition for aldicarb and impurities. For compounds (1-6), see Table I.

the general resolution criterion that only the least separated pair of peaks is considered is a disadvantage. A direct approach is to calculate the resolution only between the peaks of interest and their nearest neighbours, and set the minimum resolution criterion (R_{s_min}) equal to the lowest value. This is the designated resolution that was used in this study. The results concern only the required separation. The maximum R_{s_min} is then selected, and this shows that the solvent compositions will give a better separation for the peaks of interest. The entire procedure described above is performed by the software (OS-PLC). The mobile phase optimization procedure was evaluated with the separation of technical aldicarb.

The k' values of five preliminary experiments are given in Table I and the values of A_0 , A_1 , A_{11} in Table II. Fig. 1 shows the plots of k' for each composition as a function of the volume fraction of methanol. The computer scanning technique was used for optimizing the binary solvent composition (X_s from 0.3 to 0.7). Fig. 2 gives the corresponding diagrams. The maximum R_{s_min} is 0.79, and the optimum X_s is 0.30. Obviously an X_s of less than 0.30 will give a resolution of more than 0.79, but the analysis time is then too long. Note that in Fig. 1 the least separated pair is compounds 1 and 2, if we consider the peak of interest (compound 6, aldi-

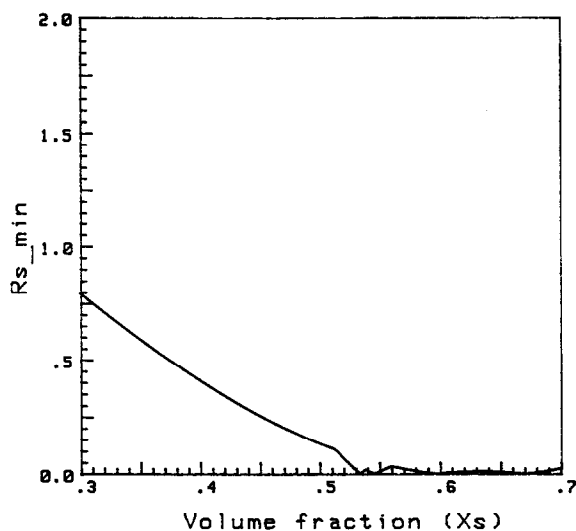


Fig. 2. Relative resolution map for aldicarb and impurities.

carb) and their nearest neighbours for separation. The results are shown in Fig. 3. The resolution for aldicarb separation is 1.57. The experimental result under optimum conditions is shown in Fig. 4. There is good agreement between the predicted and experimental results.

Obviously, in practical application the method

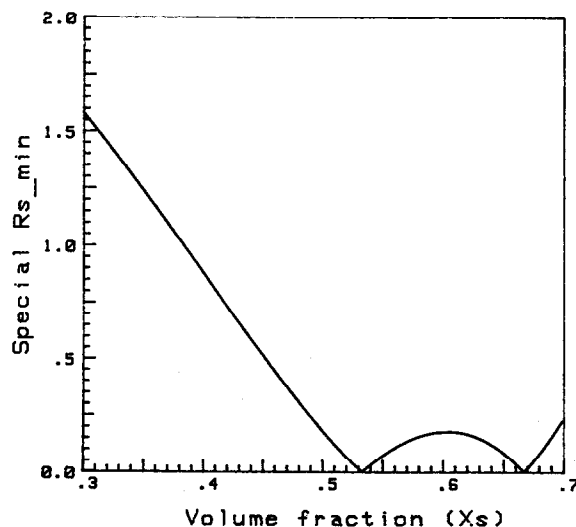


Fig. 3. Relative designated resolution map for aldicarb and impurities.

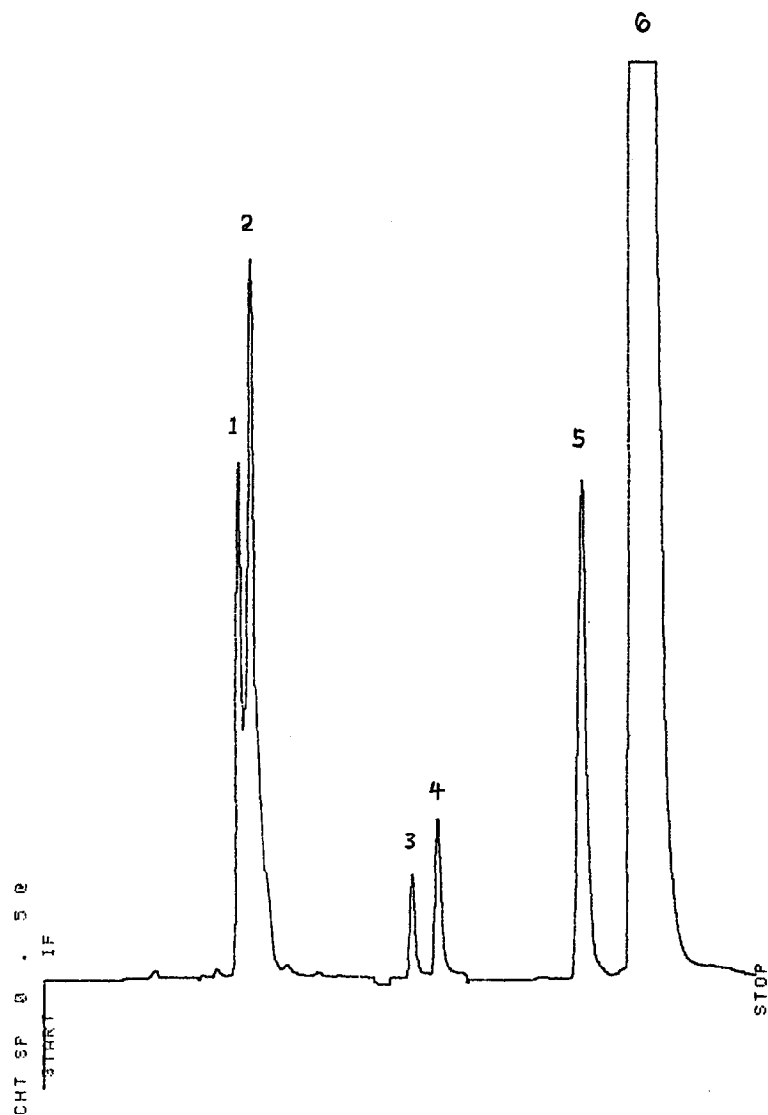


Fig. 4. Experimental chromatogram at optimum condition $X_s = 0.30$ ($200 \mu\text{l}$, 2.3 mg/ml).

can be used to increase the injection volume for aldicarb for preparations under overload conditions, for which Snyder *et al.* [18] combined certain features of the Knox-Pyper model with their own model of preparative HPLC so as to allow convenient and reliable computer simulations to be carried out under conditions of mass overload. The use of the program PREPSIMX in conjunction with the Knox-Pyper model made it possible to draw a

number of general conclusions relating to optimum conditions for preparative HPLC. Here $200\text{-}\mu\text{l}$ solutions with a concentration of 43.8 mg/ml in the mobile phase solvent were used for injection. The results are shown in Fig. 5. The maximum amount of sample that can be injected on to the column is much smaller than when there is higher resolution. A yield of aldicarb of 87.1% was achieved in 30 min . The amount of sample required is not obtained

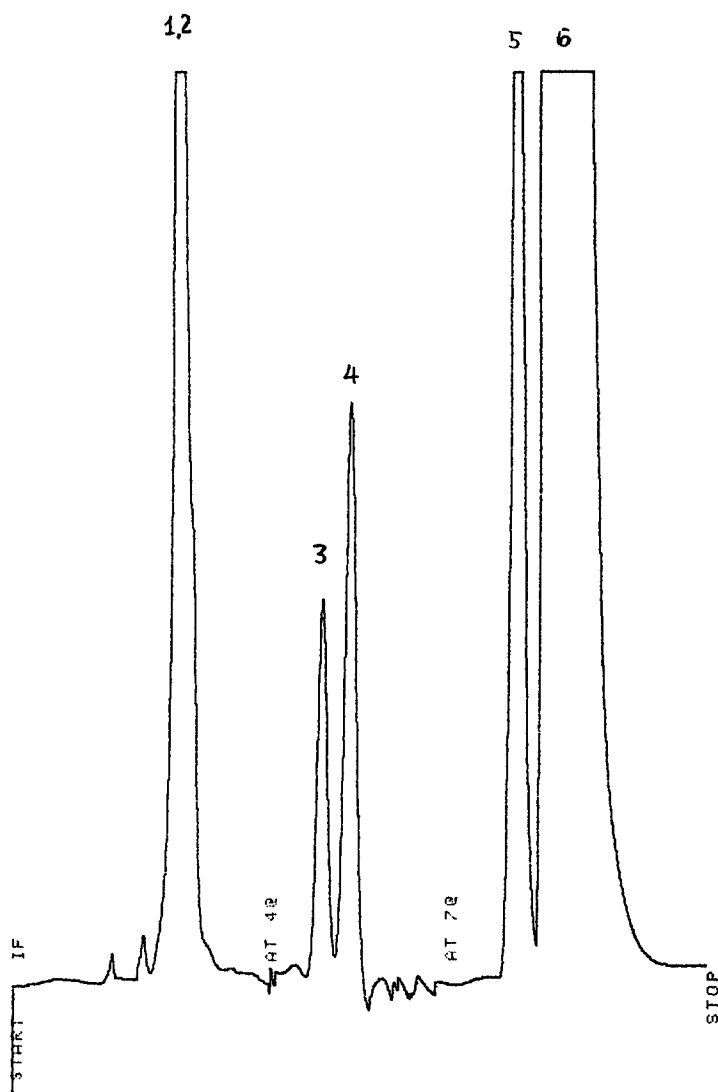


Fig. 5. Experimental preparative chromatogram at optimum condition $X_s = 0.30$ (200 μ l, 43.8 mg/ml).

in one injection, hence repetitive injections must be made and the eluate fractions collected and combined.

CONCLUSIONS

We have shown that it is possible to apply optimum conditions for the separation of technical aldicarb in semi-preparative reversed-phase HPLC using the computer scanning technique. The designat-

ed resolution was used as the criterion. Excellent agreement was obtained between the predicted data and the experimental results.

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