A New Method for Mobile Phase Optimization in High-Performance Thin-Layer Chromatography (HPTLC)

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Ternary mobile phase
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Summary

A new method has been used to optimize a ternary mobile phase used for separation of mixtures of organic compounds by high-performance thin-layer chromatography (HPTLC). Computer programs have been written to assist prediction of the optimum mobile phases. The method was found to be a rapid and efficient tool in mobile phase optimization.

1 Introduction

In (HP)TLC some form of optimization is usually necessary if complete separation of all components in a sample is required. One of the most important problems is the optimization of the mobile phase.

The optimization of multicomponent mobile phases can be performed in different ways. Solvent selection based on experience and chromatographic intuition is suitable for the separation of simple mixtures. For complex mixtures this procedure can be very time-consuming and a more statistical and systematic strategy should be used. During the last decade several optimization procedures have been described for (HP)TLC; these include use of window diagrams [1,2], the simplex method [3–6], the prisma model [7–9], and the overlapping resolution map (ORM) [10,11].

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This paper presents a new method for optimization of ternary mobile phases. Solvents are chosen from the *Snyder* classification [12] and optimization is achieved by use of a method based on a new evaluation criterion, *Q*. The advantage of the method is its wide applicability. In this work it was used for the separation of a mixture of 1,4-benzodiazepines, compounds which have strong anticonvulsant and tranquilizing to hypnotic effects. Because these compounds are widely used in therapy, much attention is devoted to their analysis [13]. The compounds studied were chlordiazepoxide, oxazepam, nitrazepam, medazepam, and diazepam; the structures of the compounds are given in **Figure 1.**

2 Experimental

2.1 Materials

All solvents were obtained from Reactivul Bucharest, Romania. Solutions (1 mg mL^{-1}) of 1,4-benzodiazepines were prepared in methanol. Chromatography was performed on 5 cm × 10 cm glass HPTLC plates precoated with silica gel 60 F_{254} (Merck).

2.2 Chromatography

Solutions (0.2 μL) of the 1,4-benzodiazepines were applied to the plates by means of a capillary pipet. The plates were developed at room temperature, by the ascending technique, in a saturated *N*-chamber. The development distance was approximately 70 mm and the time required for development was approximately 15 min. Chloroform–acetone–*i*-propanol mixture of different composition were used as mobile phases.

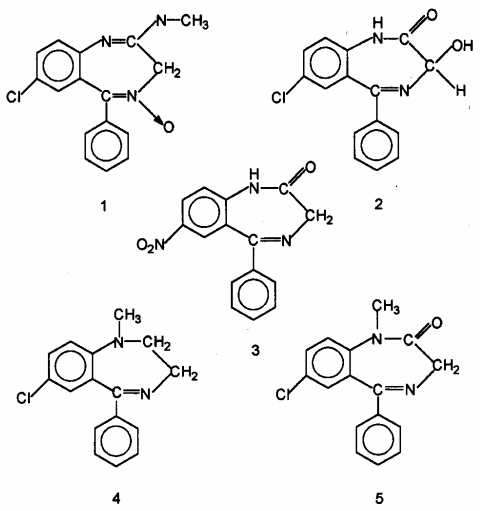

Figure 1

The structural formulas of the benzodiazepines: 1, chlordiazepoxide; 2, oxazepam; 3, nitrazepam; 4, medazepam; 5, diazepam.

2.3 Densitometry

Densitometric measurements were performed at λ = 254 nm using a Shimadzu CS-9000 dual-wavelength flying-spot scanner. Plates were scanned in zig–zag reflectance mode with a 1.2 mm × 1.2 mm slit.

3 Results and Discussion

The method, which was used for optimization of the mobile phase used for separation of a mixture of 1,4-benzodiazepines, is based on a questioning logic algorithm presented in **Figure 2**. The first step in any optimization procedure is optimization of the separation potential of the mobile phase [14]. After preliminary chromatographic runs the three solvents, chloroform, acetone, and *i*-propanol were chosen from the *Snyder* classification and used to prepare mobile phases (step 2). Seven chromatographic runs were performed using the compositions given in **Table 1**. We did not consider optimization of the strength of the solvent system (step 3).

All chromatographic measurements were obtained in duplicate; reproducibility was better than ±2%. The experimental results are listed in **Table 2**.

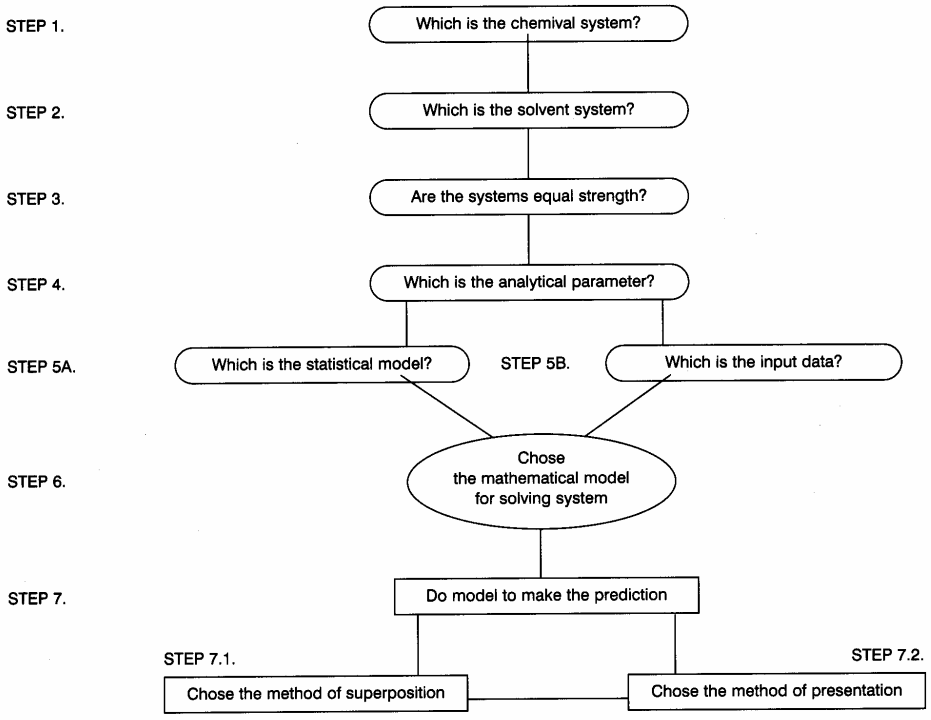

Figure 2

The questioning logic algorithm.

Table 1

Mobile phase compositions in terms of volume percentages.

Mixture	Chloroform	Isopropanol	Acetone
1	33.3	33.3	33.3
2	10	10	80
3	10	80	10
4	80	10	10
5	50	0	50
6	50	50	0
7	0	50	50

Table 2

R_F values of the compounds for each of the mobile phases listed in Table 1.

Compound	Mobile phase						
	1	2	3	4	5	6	7
Oxazepam	0.817	0.715	0.844	0.485	0.377	0.821	0.774
Medazepam	0.845	0.788	0.765	0.551	0.585	0.853	0.847
Chlordiazepoxide	0.889	0.870	0.858	0.670	0.725	0.919	0.869
Nitrazepam	0.919	0.808	0.845	0.787	0.773	0.929	0.915
Diazepam	0.939	0.870	0.880	0.828	0.822	0.951	0.924

As analytical parameter for the optimization model we chose the resolution, R_S (step 4), which could be calculated from the experimental results by use of the equation:

$$R_S = 2\Delta R_F / (w_1 + w_2) \quad (1)$$

where $\Delta R_F = R_{Fi+1} - R_{Fi}$ and w_i is the width of ith peak.

The values calculated for R_S (step 5B) are presented in **Table 3**.

The R_S values were fitted to a second-order polynomial (step 5A):

$$R_S = a_1x_1 + a_2x_2 + a_3x_3 + a_{12}x_1x_2 + a_{23}x_2x_3 + a_{13}x_1x_3 + a_{123}x_1x_2x_3 \quad (2)$$

where x_i are the volume fractions of the solvents and a_i are coefficients. The coefficients for each pair of peaks were determined by means of a *Pascal* program which solves a system of equations giving a single definite solution (step 6). The values obtained for the coefficients are listed in **Table 4**.

The R_S values for all mobile phase compositions within the solvent triangle could be calculated using the coefficients listed in Table 4 with eq. (2) (step 7). These values were used to calculate the quality factor, Q, for all the pairs of peak, by use of the equation (step 7.1):

$$Q = \min(R_{Si}, i=1,...,n-1) \quad (3)$$

The individual quality-factor plots were then superimposed to give the final diagram which is shown in **Figure 3** (step 7.2). We also used a second criterion—for all the peaks in the final chromatogram the resolution should be greater than 1 ($R_S \geq 1$).

Table 3

Resolution between adjacent peaks in the chromatograms obtained using the mobile phases listed in Table 1.

Peak pair	Mobile phase						
	1	2	3	4	5	6	7
1	0.857	1.351	1.250	1.179	3.578	0.535	1.204
2	0.540	0.326	0.028	2.814	2.483	1.225	0.459
3	0.727	0.977	0.300	3.280	0.902	0.225	1.029
4	0.452	0.000	0.476	1.000	0.974	0.516	0.169

Table 4

Coefficients of eq. (2) for the four pairs of peaks.

Peak pair	Coefficient						
	a_1	a_2	a_3	a_{12}	a_{23}	a_{13}	a_{123}
1	0.541	1.855	0.514	−2.651	0.079	12.203	−31.937
2	3.682	−0.281	−0.378	−1.900	3.155	3.325	−26.358
3	6.055	0.671	1.541	−12.552	−0.307	−11.585	18.559
4	1.168	0.635	−0.444	−1.542	0.294	2.448	−3.627

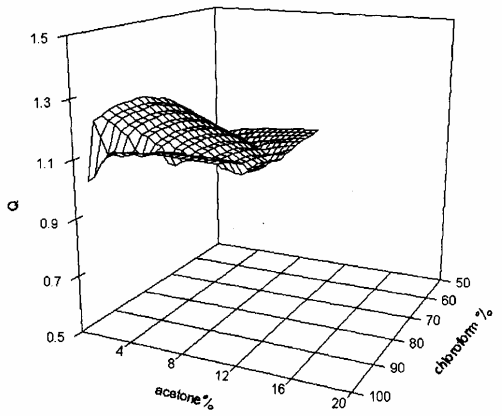

Figure 3

Three-dimensional representation of the dependence of the quality factor, Q, on the volume fractions of the solvents ($R_s \geq 1$).

The optimum mobile phase composition was given by the maximum of the surface. From Figure 3 we found that the optimum mobile phase composition was 86:1:13. Using this composition an additional experiment was performed to verify that separation of all the peaks was satisfactory. The chromatogram obtained is presented in **Figure 4**.

These results demonstrated that this method of optimization is rapid and versatile and that overall analysis time can be reduced. The optimum mobile phase composition can be

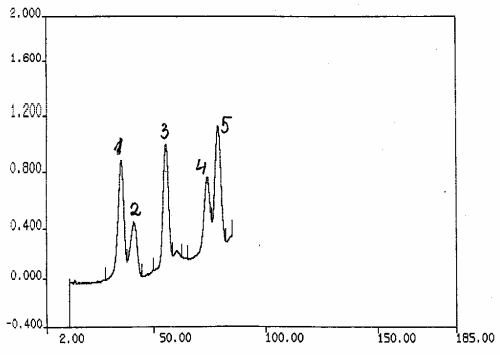

Figure 4

The final chromatogram obtained by use of the optimum mobile phase.

determined without much difficulty and only seven different mobile phase systems need to be examined.

4 Conclusion

This optimization method is original because of the evaluation criterion chosen–the worst separation between two peaks. From this it follows that the optimum separation is that giving the most homogenous distribution of the peaks.

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Author Keywords

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