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

Claudia Cimpolu, Lorentz Jantschi, and Teodor Hodisan*

Key Words:
Trinary mobile phase
Optimization
Evaluation criterion, Q
1,4-Benzodiazepines

Summary
A new method has been used to optimize a trinary 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].

This paper presents a new method for optimization of trinary 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 F₂₅₄ (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.

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2.3 Densitometry

Densitometric measurements were performed at \( \lambda = 254 \) nm using a Shimadzu CS-9000 dual-wavelength flying-spot scanner. Plates were scanned in zig-zag reflectance mode with a 1.2 mm x 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 \( \pm 2\% \). The experimental results are listed in Table 2.

STEP 1.
Which is the chemical system?

STEP 2.
Which is the solvent system?

STEP 3.
Are the systems equal strength?

STEP 4.
Which is the analytical parameter?

STEP 5A.
Which is the statistical model?

STEP 5B.
Which is the input data?

STEP 6.
Choose the mathematical model for solving system

STEP 7.
Do model to make the prediction

STEP 7.1.
Choose the method of superposition

STEP 7.2.
Choose the method of presentation

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

Figure 2
The questioning logic algorithm.
Table 1  
<table>
<thead>
<tr>
<th>Mixture</th>
<th>Chloroform</th>
<th>Isopropanol</th>
<th>Acetone</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>33.3</td>
<td>33.3</td>
<td>33.3</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>10</td>
<td>80</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>80</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>80</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>5</td>
<td>50</td>
<td>0</td>
<td>50</td>
</tr>
<tr>
<td>6</td>
<td>50</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>50</td>
<td>50</td>
</tr>
</tbody>
</table>

Table 2  
<table>
<thead>
<tr>
<th>Compound</th>
<th>Mobile phase 1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxytropin</td>
<td>0.817</td>
<td>0.715</td>
<td>0.844</td>
<td>0.485</td>
<td>0.377</td>
<td>0.821</td>
<td>0.774</td>
</tr>
<tr>
<td>Mezaxolam</td>
<td>0.845</td>
<td>0.788</td>
<td>0.765</td>
<td>0.551</td>
<td>0.585</td>
<td>0.853</td>
<td>0.847</td>
</tr>
<tr>
<td>Chlordiazepoxide</td>
<td>0.880</td>
<td>0.870</td>
<td>0.858</td>
<td>0.670</td>
<td>0.725</td>
<td>0.919</td>
<td>0.869</td>
</tr>
<tr>
<td>Nitrazepam</td>
<td>0.919</td>
<td>0.808</td>
<td>0.845</td>
<td>0.787</td>
<td>0.773</td>
<td>0.929</td>
<td>0.915</td>
</tr>
<tr>
<td>Diazepam</td>
<td>0.929</td>
<td>0.870</td>
<td>0.880</td>
<td>0.828</td>
<td>0.822</td>
<td>0.951</td>
<td>0.924</td>
</tr>
</tbody>
</table>

As an 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:

$$R_s = \frac{\Delta R_s}{w_i + w_j}$$

(1)

where $\Delta R_s = R_{s2} - R_{s1}$ 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):

$$R_s = a_1 x_1 + a_2 x_2 + a_3 x_3 + a_4 x_1 x_2 + a_5 x_1 x_3 + a_6 x_2 x_3$$

(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 peaks, by use of the equation (step 7.1):

$$Q = \min(R_{s0}), i=1, n-1$$

(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 ($R_s \geq 1$).

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 86.1:13:1. 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
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 homogeneous distribution of the peaks.

References


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Abstract

A new method has been used to optimize a ternary mobile phase used for separation of mixtures of organic
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Author Keywords

1,4-Benzodiazepines; Evaluation criterion, Q; Optimization; Ternary mobile phase

References (14)


8. Poole, C.F., Poole, S.K.
Elsevier, USA

9. □ Cimpoiu, C., Hodișan, T., Nașcu, H.
Comparative study of mobile phase optimization for the separation of some 1,4-benzodiazepines

10. □ Glajch, J.L., Kirkland, J.J.
Optimization of selectivity in liquid chromatography

11. □ Li, S.F.Y., Lee, H.K., Ong, C.P.
Optimization of mobile phase composition for high-performance liquid chromatographic separation by means of the overlapping resolution mapping scheme
doi: 10.1016/S0021-9673(01)91581-X
12. □ Snyder, L.R.  
**CLASSIFICATION OF THE SOLVENT PROPERTIES OF COMMON LIQUIDS.**  
(1978) *Journal of Chromatographic Science*, 16 (6), pp. 223-234. [Cited 143 times.](#)  

13. □ Klimes, J., Kastner, P.  

14. □ Nyiredy, Sz., Dallenbach-Tölke, K., Sticher, O.  