Mobile Phase Optimization in Three Solvents High Performance Thin-Layer Chromatography: Methodology and Evaluation

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The optimization of the chromatographic mobile phase proved to be possible when the number of experimental determinations of separation parameters for each compound is obtained for more than one distinct compositions of mobile phase, at least equal with the number of variable use in the mathematical model [1,2]. Starting from this point of view a mobile phase optimization program based on an original mathematical approach was developed and its performances were tested by applying on three sets of compounds.

The original optimization procedure start from the idea that into a mixture of three solvents the quantitative measure of the choosed chromatographic parameter is dependent on composition of mobile phase through an equation of dependency with six or seven parameters, taking into consideration the molar fraction of the solvents. The optimization procedure was included into a program and applied on three sets of previous studied compounds (two sets of steroids and one set of N-alkyl phenothiazine sulfone) through high performance thin-layer chromatography with three solvents.

The mobile phase optimization process proved to be able to provide accurate, precise and reproducible method of characterization and analysis of chromatographic parameters.

MOBILE PHASE OPTIMIZATION IN THREE SOLVENTS HIGH PERFORMANCE THIN-LAYER CHROMATOGRAPHY: METHODOLOGY AND EVALUATION

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ABSTRACT

The optimization of the chromatographic mobile phase proved to be possible when the number of experimental determinations of separation parameters for each compound is obtained for more than one distinct compositions of mobile phase, at least equal to the number of variables in the mathematical model. Starting from this point of view a mobile phase optimization program based on an original mathematical approach was developed and its performances were tested by applying on three sets of compounds.

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INTRODUCTION

Chromatographic analysis, define as techniques used for the separation of a mixture of compounds by their distribution between two phases, was invented in 1901 by Russian botanist Mikhail Semenovich Tsvet, during his research on plant pigments [1]. An important task in obtaining the separation of compounds from a mixture by chromatography is choosing of the proper mobile phase [2], this task being time consuming. Researchers all over the world studied optimization methods for column liquid chromatography [3] and for high performance liquid chromatography [4].

Starting with results previously obtained in optimization of the mobile phase of chromatography separation [5,6], the aim of the poster is to present the performances of an original mathematical model for mobile phase optimization and its application in High Performance Thin-Layer Chromatography.

METHOD

The original optimization procedure start from the idea that into a mixture of three solvents the quantative measure of the chosen chromatographic parameter is dependent on composition of mobile phase through an equation of dependency with six or seven parameters, taking into consideration the molar fraction of the solvents.

Mathematical model

Into a mixture of three solvents, the quantitative measure of chosen chromatographic parameter depends on the composition of mobile phase through a dependence equation, which can be in two forms:

\[ M(k_1,k_2,k_3) = \sum_{i=1}^{4} a_i x_{1,i} + a_2 x_{2,i} + a_3 x_{3,i} + a_4 x_{1,i} x_{2,i} + ... \]

Eq.(11)

where \( x_{1,i}, x_{2,i}, x_{3,i} \) are molar fraction of the solvents. \( a_i \) and \( M(k_1,k_2,k_3) \) are estimators and then predictors of choosed chromatographic parameter, and \( a_1, a_2, a_3, a_4 \) are coefficients first determined based on the estimated of chromatographic parameter.

Starting from the above presented equations (Eq.(1) and Eq.(2)) chromatographic parameters were modeled through eight equations (Eq(3) – Eq(10)). By application of one of the equations Eq(3)–Eq(10) on a series of p experiments, result a matrix with one Eq(6)–Eq(10) row or more one Eq(3)–Eq(5) rows, one for each experiment. The elements of \( M(k_1,k_2,k_3) \) matrix represent the values of chromatographic parameter which is modeled by using one of Eq(1) or Eq(2). The optimization algorithm has a unique determinate solution for \( p \geq 6 \) for Eq(1) and \( p \geq 6 \) for Eq(2).

Optimization procedure

For each row of \( M(k_1,k_2,k_3) \) matrix build a system with \( p \) linear equations with six or seven terms \((Eq(1), Eq(2))\) in \( a_i \) coefficients as following example:

\[ M(k_1,k_2,k_3) = \sum_{i=1}^{4} a_i x_{1,i} + a_2 x_{2,i} + a_3 x_{3,i} + a_4 x_{1,i} x_{2,i} + a_5 x_{1,i} x_{3,i} + a_6 x_{2,i} x_{3,i} + a_7 x_{1,i} x_{2,i} x_{3,i} \]

Eq.(11)

Optimization of the mobile phase was considered a method to determine the best performing composition of mobile phase through the worst separation of two compounds.

RESULTS

The application of optimization of mobile phase at chromatographic separation which used mixture’s of three solvents was created, and can run on any computer connected to the Internet, being available at the following URL: http://vl.academicrodirect.org/molecular_dynamics/mobile_phase_opt/

The retention factor was considered as the most important parameter into chromatography and the results of optimization refer this factor. The optimum mobile phase previous reported was took into consideration in choosing the best performing optimization model for the three sets of compounds included into analysis.

In all three sets of compounds, the optimum mobile phase was obtained with the following generic equation:

\[ \Delta x_{1} = a_1 x_{1} + a_2 x_{2} + a_3 x_{3} + a_4 x_{1} x_{2} + a_5 x_{1} x_{3} + a_6 x_{2} x_{3} + a_7 x_{1} x_{2} x_{3} \]

Eq.(17)

The mobile phase characteristics obtained previously and through optimization procedure are in Table 1.

Table 1. Characteristics of the optimum mobile phase

<table>
<thead>
<tr>
<th>Abb.</th>
<th>Optimum mobile phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>steroids 01</td>
<td>73:26:1</td>
</tr>
<tr>
<td>steroids 02</td>
<td>52.5:25.5:22</td>
</tr>
<tr>
<td>thiazine 01</td>
<td>10:50:20</td>
</tr>
</tbody>
</table>

There were not identified statistically significant differences between experimental retention factor and the values obtained by the used of proposed optimization method, for none of studied sets of compounds. The plot generate by the application, for the data set thiazine_01 obtained with the Eq.(17) for a\(_{x1}\) equal with 0.003 created by the use of 25 colors is in figure 1.

CONCLUSIONS

The proposed optimization procedure opens a new pathway in analyzing and characterization of chromatographic parameters of HPTLC analysis which used a mixture of three solvents. The program can become a useful instrument in characterization of HPTLC parameters, opening the possibility of development of online library of optimized HPTLC parameters.

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REFERENCES


Figure 1. Plot of the optimized retention factor for thiazine 01 data set

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