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 choused 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.

[1] C. Cimpoiu, L. Jäntschi, T. Hodisan. Journal of Planar Chromatography - Modern TLC, 11 (1998) 191-194.

[2] C. Cimpoiu, L. Jäntschi, T. Hodisan. Journal of Liquid Chromatography and Related Technologies 22 (1999) 1429-1441.

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 with the number of variable use 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 Semyonovich Tsvet, during his research on plant pigments [1]. An important task in separation of compounds from a mixture by chromatography is chousing 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 previous 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 quantitative measure of the choused 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 choused chromatographic parameter depends on the composition of mobile phase through a dependence equation, which can be one of two forms:

$$M6(x_1,x_2,x_3) = a_1x_1 + a_2x_2 + a_3x_3 + a_4x_1x_2 + a_5x_1x_3 + a_6x_2x_3 \quad \text{Eq.(1)}$$

$$M7(x_1,x_2,x_3) = a_1x_1 + a_2x_2 + a_3x_3 + a_4x_1x_2 + a_5x_1x_3 + a_6x_2x_3 + a_7x_1x_2x_3 \quad \text{Eq.(2)}$$

where x_1, x_2, x_3 are molar fraction of the three solvents ($x_1 + x_2 + x_3 = 1$), M6 and M7 are estimators and then predictors of choused chromatographic parameter, and a_1, a_2, a_3, a_4, a_5, a_6, a_7 are coefficients first determined based on the best estimation of choused chromatographic parameter and then used in prediction of used parameter for any composition of mobile phase.

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 M_{ob} matrix with one (Eq.(6)-Eq.(10)) ore more than one (Eq.(3)-Eq(5)) rows, one for each experiment. The elements of M_{ob} 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 determine solution for $p \geq 6$ for Eq.(1) and respectively for $p \geq 6$ for Eq.(2).

Optimization procedure

For each row of M_{ob} matrix is build a system with p linear equations with six or seven terms (Eq.(1), Eq.(2)) in a_j coefficients as following example:

$$M_{ob}(j) = a_1x_1 + a_2x_2 + a_3x_3 + a_4x_1x_2 + ... \quad \text{Eq.(11)}$$

where x_i are molar fraction of each solvent ($I = 1, 2, 3$) which enter into the composition of the e_j eluent ($j = 1, 2, \ldots, p$).

To the above describe system (Eq.(11)) the least squared method is applied for construction of the system with unique determine solution MMCP, which is obtained by applying the following formula:

$$MMCP(k,0) = M2(MOB,A(k)),\ MMCP(k,l) = M2(A(k),A(l)) \quad \text{Eq.(12)}$$

where $(k,0) = 1, 2, \ldots, 6$ for Eq.(1) and $(k,0) = 1, 2, \ldots, 7$ for Eq.(2), $A(k)$ is the series of terms known from Eq.(11), $M2$ calculate the mean for the product of MOB series and $A(k)$, and $MMCP$ is the extended matrix of system of linear equations which is used in determination of a_k coefficients.

For determination of the solution for Eq.(12) is applied the Gaussian method. The solution for the system from Eq.(1) and Eq.(2) are:

$$A0 = (a_{01}, a_{02}, \ldots, a_{06}) \quad \text{- for Eq.(1)}$$
$$A0 = (a_{01}, a_{02}, \ldots, a_{07}) \quad \text{- for Eq.(2)} \quad \text{Eq.(13)}$$

At one time as the coefficients $A0$ are determined, their values are used for prediction of the chromatographic parameter of interest by using one of the equations Eq.(1) or Eq.(2).

For example if Y is the choused chromatographic parameter, the MOB matrix (the predictor of Y) has more than one row as well as the estimator of Y. If z is the number of MOB matrix rows (and implicit the number of predictors) then we can state the estimator of choused chromatographic parameter $\hat{Y}$ as a followings:

$$\hat{Y} = (\hat{y}_1, \ldots, \hat{y}_z) \quad \text{Eq.(14)}$$

The optimum is obtained by application of a maximization or minimization function (as is for example the characterization of a separation of many compounds through the worst separation of two compounds):

$$\hat{y}_o = opt(\hat{Y}), \text{ where opt = "max" or opt = "min"} \quad \text{Eq.(15)}$$

Moving through all domains of possible values for the composition of the mobile phase, the optimum point is identified, this being the optimum composition of mobile phase (x_1, x_2, x_3):

$$(\cdot,\cdot,\cdot) \mid \hat{Y}(\cdot,\cdot,\cdot) = opt\{\ \hat{Y}(i/100,j/100,k/100) \mid i=0..100,\ j=0..100-i,\ k=100-i-j\} \quad \text{Eq.(16)}$$

The optimization procedure was included into a program and applied on three sets of previous studied compounds (two sets of steroids [6,7] and one set of N-alkyl phenothiazine sulfone [8]). All three sets of compounds were studied previously through high performance thin-layer chromatography with three solvents.

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.academicdirect.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 chousing 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:

$$\Delta rf = a_1x_1+a_2x_2+a_3x_3+a_4x_1x_2+a_5x_1x_3+a_6x_2x_3+a_7x_1x_2x_3 \quad \text{Eq.(17)}$$

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

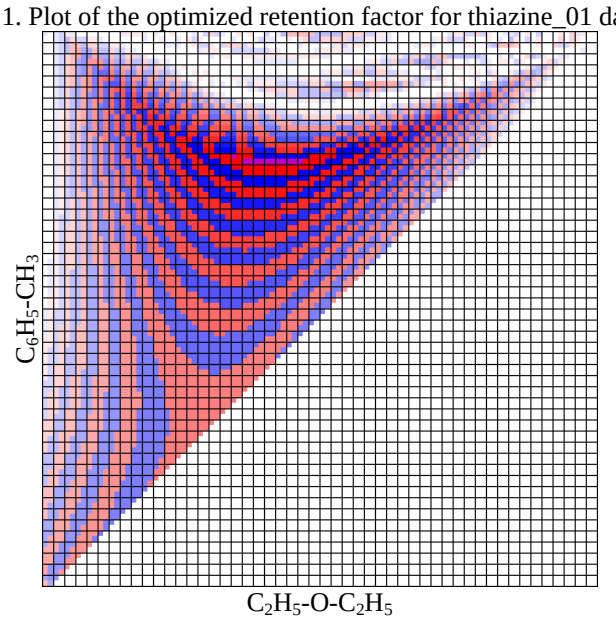
Table 1. Characteristics of the optimum mobile phase

Abb.	Optimum mobile phase	
	Previously	Optimization
steroids_01	73: 26: 1	91: 0: 9
steroids_02	52.5: 25.5: 22	90: 10: 0
thiazine_01	30:50:20	41: 23: 36

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 ($p > 0.05$).

The plot generate by the application, for the data set thiazine_01 obtained with the Eq.(17) for a Z_{min} equal with 0.001 created by the use of 25 colors is in figure 1.

Figure 1. Plot of the optimized retention factor for thiazine_01 data set



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. Mobile Phase Optimization Program proved to assure accurate results regarding the retention factors analyzed on three sets on compounds. The program can become a useful instrument in characterization of HPTLC parameters, opening the possibility of development of online library of optimized HPTLC parameters.

ACKNOWLEDGEMENT

The research was partly supported by UEFISCSU Romania through ET/46 project.

REFERENCES

[1] E. M. Senchenkova, Tsvet (or Tswett), Mikhail Semenovich (1872 - 1919). In: Dictionary of scientific biography, Charles Scribner Sons, New York. 13 (1976) 486-488.; [2] M. Mulja, G. Indrayanto, In: Encyclopedia of Chromatography, Marcel Dekker, New York, (2001) 794–797.; [3] E. Loeser, S. Babiak, P. Zhu, G. Yowell, M. Konigsberger, P. Drumm, *Chromatographia* 63 (2006) 345-351.; [4] Y.P. Zhang, Y.J. Zhang, W.J. Gong, A.I. Gopalan, K.-P. Lee., *J. Chromatogr., A* 1098 (2005) 183-187.; [5] C. Cimpoiu, L. Jäntschi, T. Hodisan, *J. Planar Chromatogr.--Mod. TLC* 11 (1998) 191-194.; [6] C. Cimpoiu, L. Jäntschi, T. Hodisan, *J. Liq. Chromatogr. Relat. Technol.* 22 (1999) 1429-1441.; [7] L. Jäntschi, S. Hodisan, Claudia Cimpoiu, Ionela Ceteraş. *Acta Universitatis Cibiniensis Seria F Chemia* 8 (2005) 67-76.; [8] C. Cimpoiu, S. Hodisan, M. Toşa, C. Paizs, C. Majdik, F.-D. Irimie, *J. Pharm. Biomed. Anal.* 28(2002) 385-389.