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# A NEW MATHEMATICAL MODEL FOR THE OPTIMIZATION OF THE MOBILE PHASE COMPOSITION IN HPTLC AND THE COMPARISON WITH OTHER MODELS

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## ABSTRACT

Mobile phase optimization is highly important in planar chromatography. Solvent selection based on experience and chromatographic intuition can be very time consuming when applied to complex mixtures. In these cases, more systematic strategies are needed. This paper presents a new mathematical model for the optimization of the mobile phase composition used for the separation of a mixture of 1,4-benzodiazepines and a comparison of this mathematical approach with other models.

## INTRODUCTION

Mobile phase composition is highly important in thin layer chromatography (TLC). From the early days of chromatography as an analytical tool for the separation and determination of multi-component mixtures, great effort has been made in the field of optimization because of specific problems encountered in liquid chromatography.

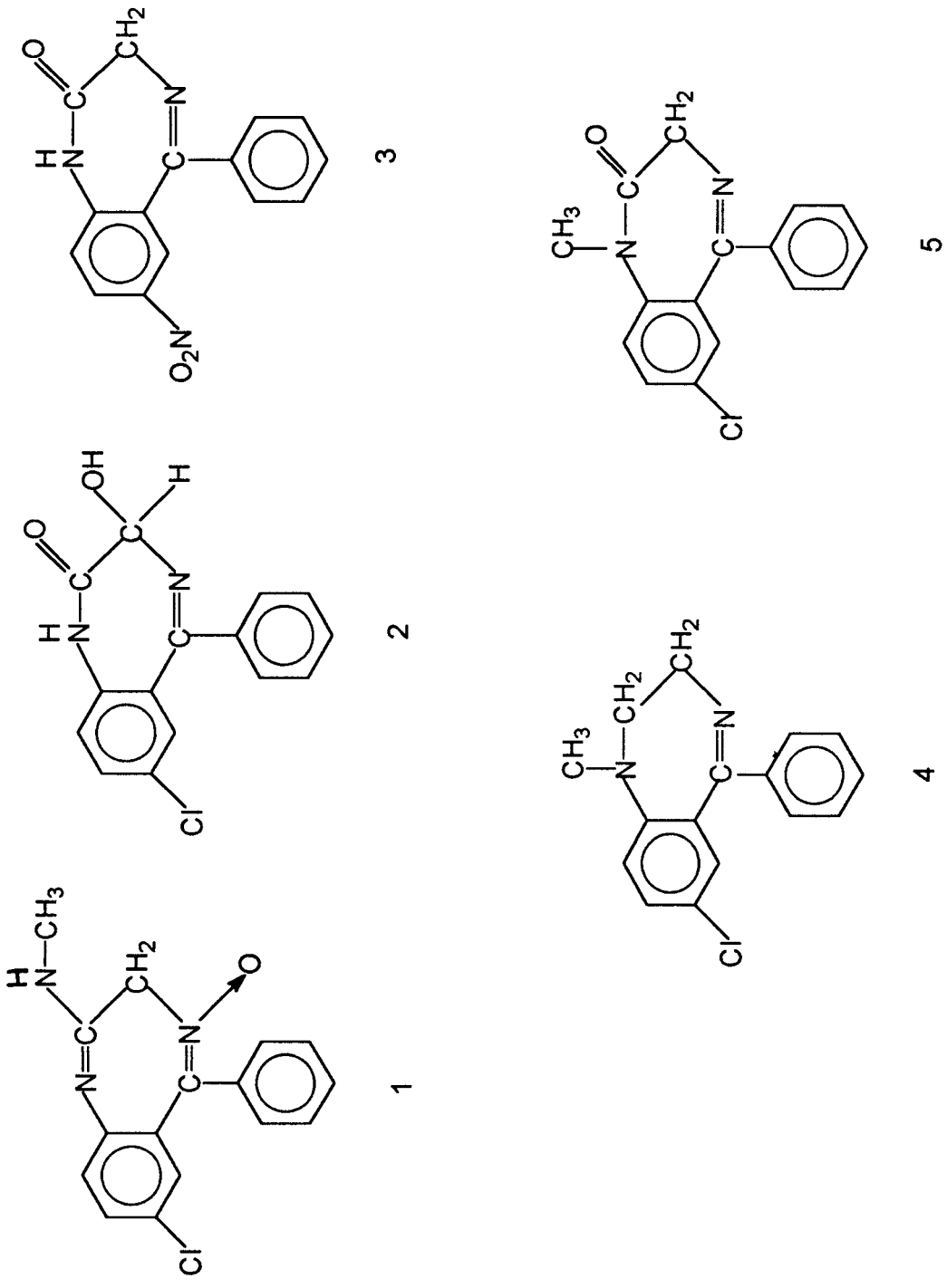


**Figure 1**. The chemical structures of 1,4-benzodiazepines: 1-chlordiazepoxide; 2-oxazepam; 3-nitrazepam; 4-medazepam; 5-diazepam.

**Table 1**

**Composition of the Mobile Phase**

| No. | Chloroform (mL) | Acetone (mL) | Iso-Propanol (mL) |
|---|---|---|---|
| 1 | 100 | 0 | 0 |
| 2 | 0 | 100 | 0 |
| 3 | 0 | 0 | 100 |
| 4 | 50 | 50 | 0 |
| 5 | 50 | 0 | 50 |
| 6 | 0 | 50 | 50 |
| 7 | 33 | 33 | 33 |

**Table 2**

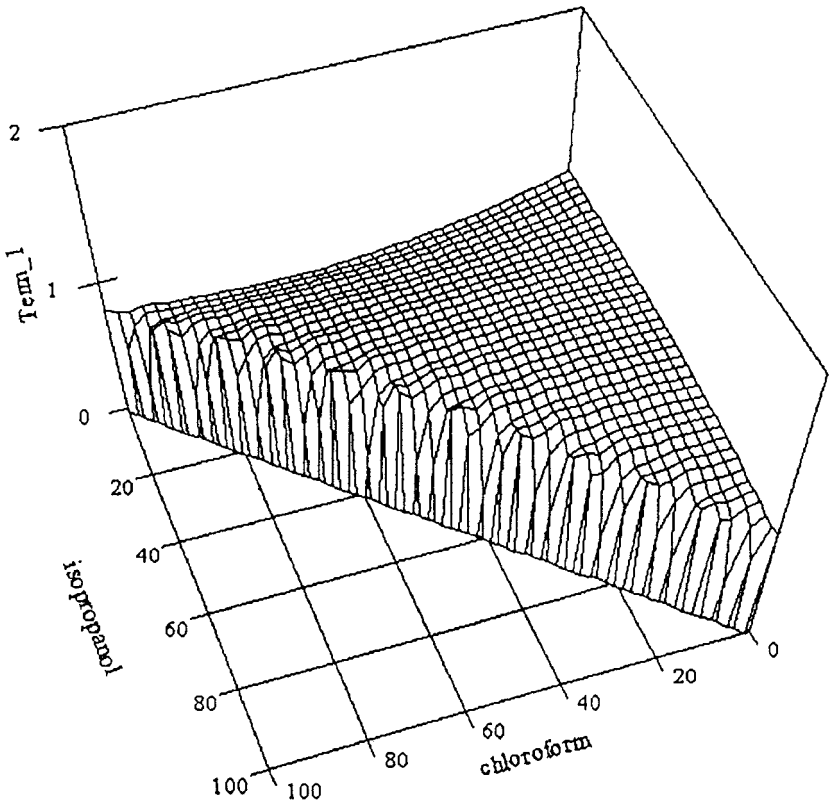
**The Experimental Results and the $F_{obj}$ Values for These Experiments**

| Mobile Phase | $w_1$ mm | $hR_{f1}$ | $hR_{f2}$ | $w_2$ mm | $hR_{f3}$ | $w_3$ mm | $hR_{f4}$ | $w_4$ mm | $hR_{f5}$ | $w_5$ mm | $F_{obj}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2.8 | 5.6 | 5.5 | 3.0 | 14.2 | 2.5 | 25.2 | 2.4 | 27.2 | 2.1 | 8.467 |
| 2 | 4.4 | 60.5 | 71.4 | 4.0 | 90.3 | 2.7 | 82.5 | 2.8 | 87.7 | 3.0 | 12.627 |
| 3 | 4.3 | 82.3 | 93.1 | 2.3 | 95.0 | 2.5 | 92.8 | 2.2 | 93.9 | 2.4 | 4.854 |
| 4 | 6.0 | 46.9 | 65.0 | 3.3 | 77.4 | 2.9 | 85.3 | 3.4 | 84.2 | 3.2 | 13.097 |
| 5 | 3.4 | 83.0 | 85.8 | 3.9 | 93.7 | 1.9 | 92.4 | 2.2 | 95.4 | 1.7 | 7.733 |
| 6 | 4.0 | 83.1 | 91.2 | 3.1 | 93.8 | 2.6 | 91.5 | 2.9 | 92.3 | 3.0 | 4.774 |
| 7 | 3.4 | 82.9 | 87.8 | 3.0 | 92.6 | 2.0 | 92.5 | 2.3 | 92.5 | 2.0 | 6.909 |

With the widespread availability of computers in analytical laboratories, the topic became more and more preferred,[1-5] and today several reviews can be consulted both for LC as well as for TLC[6,7] and a very few for more general cases.[8]

The most important factor that must be considered in the optimization of TLC systems is the composition of mobile phase, and this is often the only component seriously considered. In the literature, the principles for the choice of the mobile phase system for different classes of substances are described.

But there are also cases when the systems selected, if they are correctly used, do not give the expected results. In this case the research worker may resort to "the art of separation[9]" or to the methods of optimization.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chloroform
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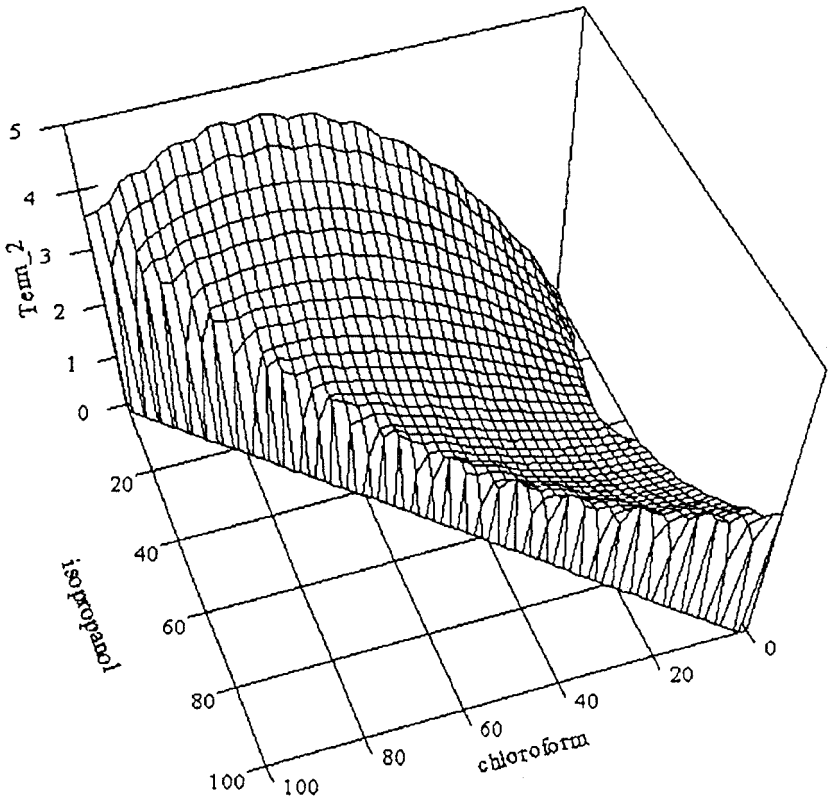
20
40
60
80
100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80
100
chloroform
0
20
40
60
80
100

**Table 3**

**The Coefficient Values Calculated with the Computer Program**

| Coefficient | Value |
|---|---|
| $a_1$ | 12.436802 |
| $a_2$ | 11.776420 |
| $a_3$ | 4.406722 |
| $a_{12}$ | 27.064013 |
| $a_{13}$ | -14.837945 |
| $a_{23}$ | -15.434747 |
| $a_{123}$ | -43.30400 |

**Table 4**

**The Compositions of Mobile Phase Obtained with Different Methods**

| Method | Chloroform (mL) | Acetone (mL) | *Iso*-Propanol (mL) |
|---|---|---|---|
| Simplex | 84 | 13 | 3 |
| Prisma | 85 | 10 | 5 |
| Quality factor | 86 | 13 | 1 |

In the literature, either simple methods or more sophisticated methods for mobile phase optimization are described, and some of these methods were realized with computers. An intuitive, trial and error approach to solvent selection is often acceptable when mixtures containing only a small number of components are to be separated.

Solvent selection based on experience and chromatographic intuition can be very time-consuming when applied to complex mixtures. For these mixtures, more systematic strategies were elaborated, such as the "Simplex" method,[10,11] the "Prisma" model,[12,13] the "window diagram" method,[14] the "overlapping resolution map" method,[15,16] etc.

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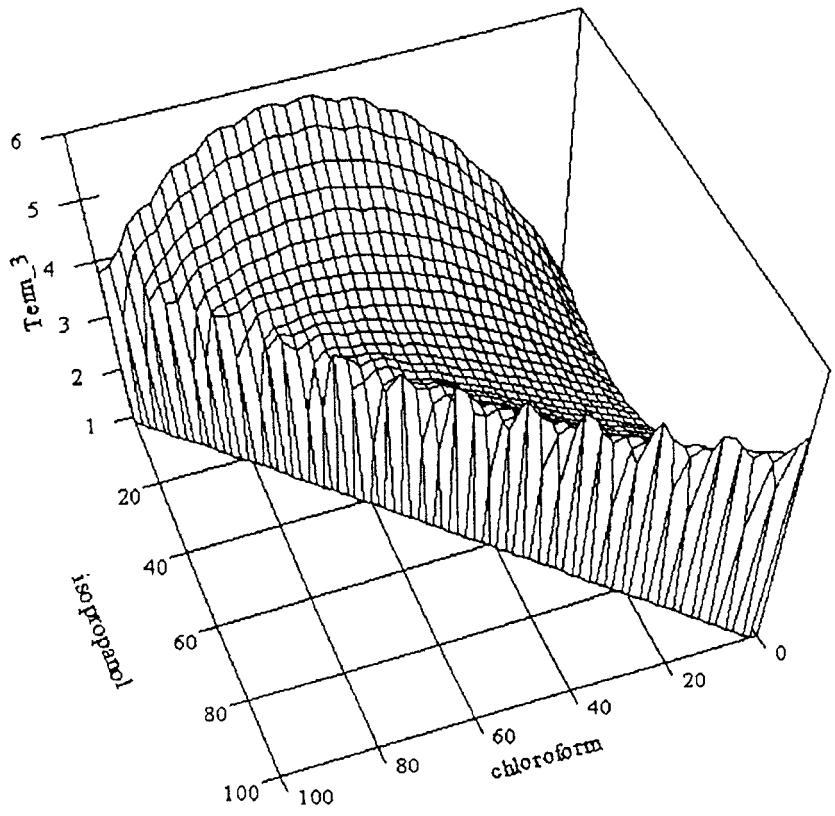
**Figure 2 (left)**. The three-dimensional representation of terms 1 and 2 of equation 1 *versus* volume fractions of solvents.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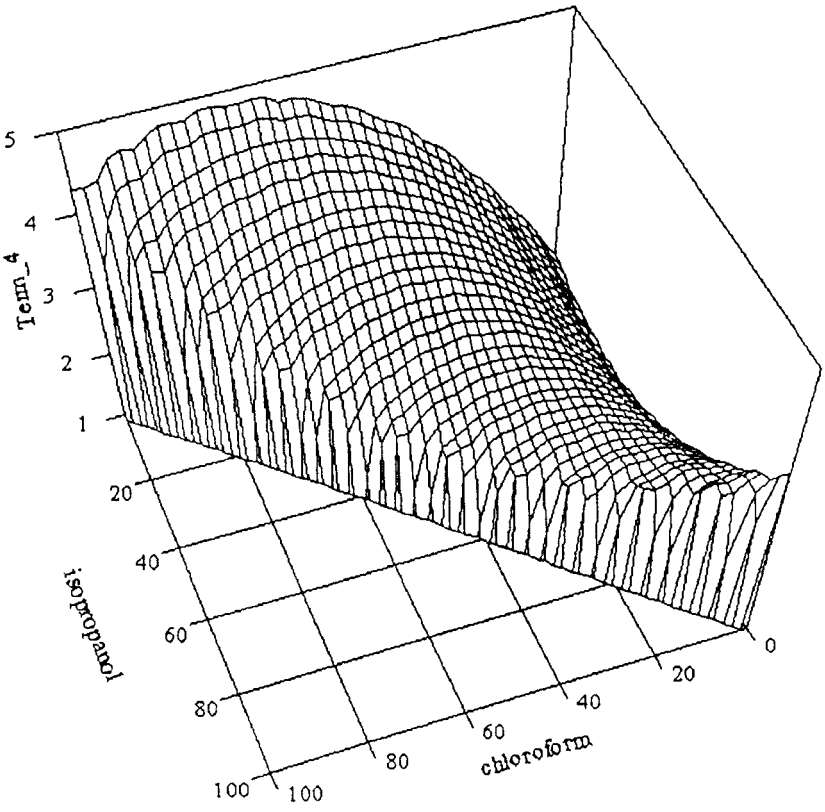
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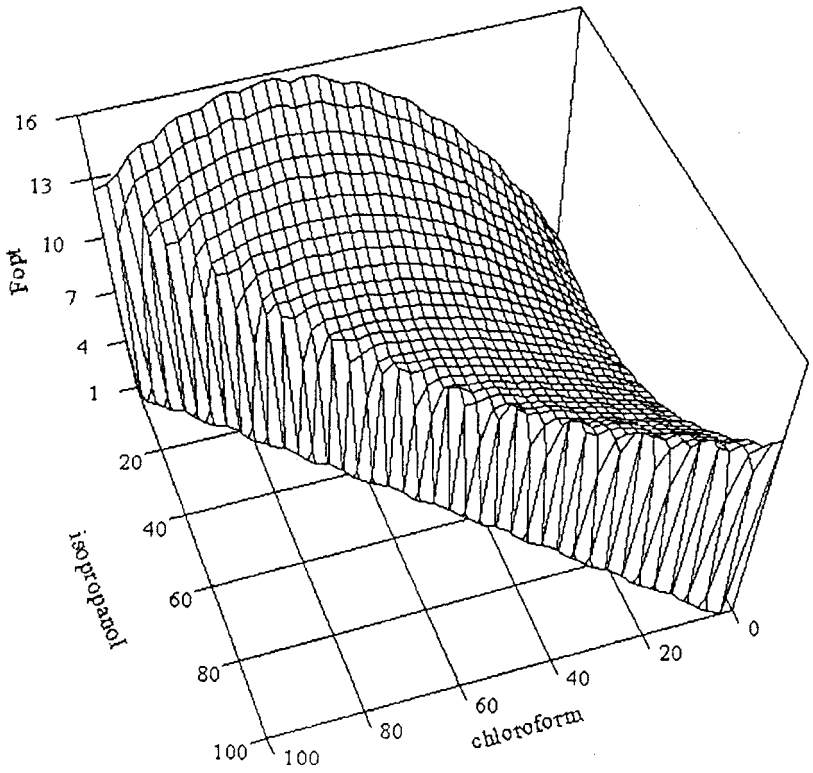


**Figure 4**. The three-dimensional representation of function $F_{obj}$ *versus* volume fractions of solvents.

This paper presents a new mathematical model for the optimization of the mobile phase composition used for the separation of a mixture of 1,4-benzodiazepines, a group of drugs with strong anticonvulsant and tranquilizing to hypnotic effect.[17] Because the 1,4-benzodiazepines are widely used in therapy, great attention has been devoted to their analysis, and HPTLC has been used with many mobile phases for their separation.

## EXPERIMENTAL

### Materials

The solutions of 1,4-benzodiazepines (1 mg $mL^{-1}$) were prepared in methanol. All solvents were of analytical grade and were obtained from

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**Figure 3 (left)**. The three-dimensional representation of terms 3 and 4 of Equation 1 *versus* volume fractions of solvents.

"Reactivul" (Bucharest, Romania). Chromatography was performed on 5 x 10 cm glass HPTLC plates pre-coated with silica gel $F_{254}$ (Merck).

### Chromatography

The solutions (0.2 μL) of compounds were applied to the plates using a micropipet. The plates were developed at room temperature, in a saturated N-chamber, by the ascending technique. The development distance was about 70 mm and the time required for this was ca.15 min. The mobile phases were chloroform-acetone-isopropanol mixtures of different compositions.

### Densitometry

Samples were scanned in the zigzag reflectance mode with a 1.2 x 1.2 mm slit at λ = 254 nm by use of a Shimadzu (Columbia, MD) CS-9000 dual wavelength flying-spot scanner.

## RESULTS AND DISCUSSION

A new mathematical model for the optimization of mobile phase used for the separation of 1,4-benzodiazepines mixture was applied. The mixture contained chlordiazepoxide (1), oxazepam (2), nitrazepam (3), medazepam (4), and diazepam (5), and the structural formulae of the components are presented in Fig. 1.

The complex method for mobile phase optimization had in view to find the maximum or minimum of a function called "objective function" or "chromatographic response function (CRF)" which reflects the quality of separation in a single number. While no one CRF will ever be entirely satisfactory in all cases and for all chromatographers, a great number of CRFs have been designed and tested.[18]

In our opinion, the preferred CRF is a combined function in the form of a weighted sum of simple functions that satisfied the conditions for the "optimum" chromatograms.

The CRF used in the present paper is:[18]

$$F_{obj} = a/(I_p + \varepsilon) + b \cdot \overline{IR_S} + 10c/IE + d \cdot n \qquad (1)$$

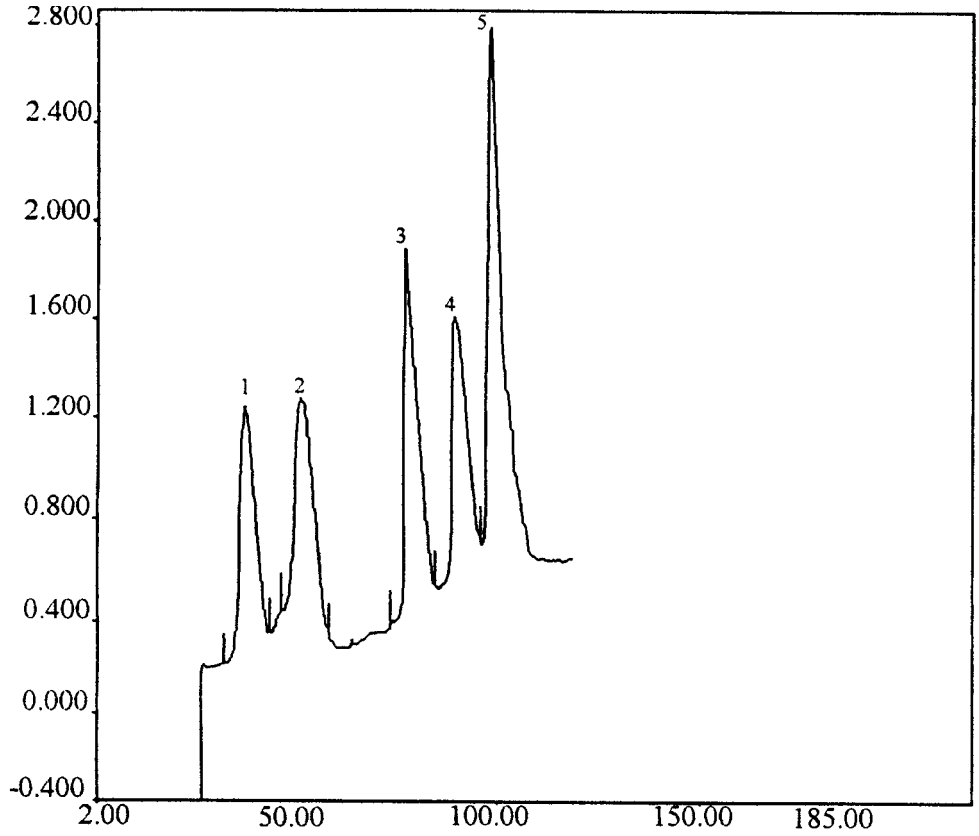


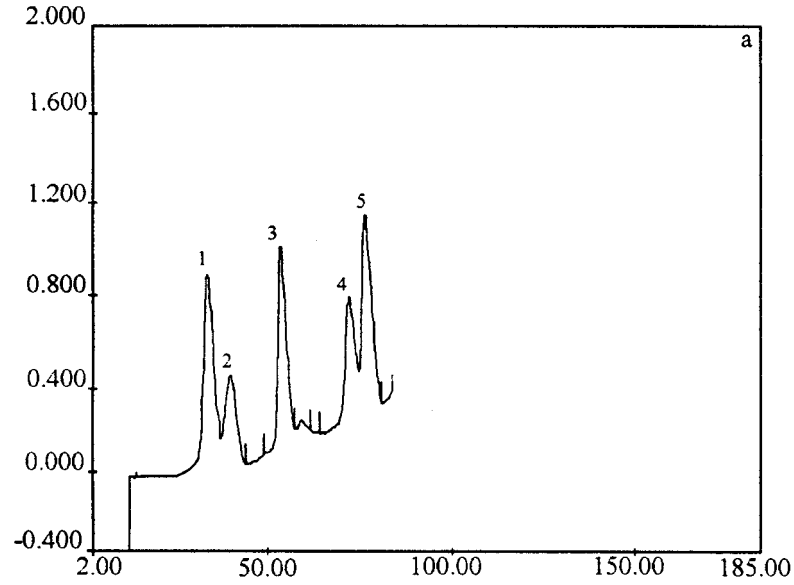

**Figure 5**. The densitograms obtained with optimum mobile phase.

where: a, b, c, and d are arbitrary weighting factors – in our case a = 10, b = 1, c = 0.1, d = 1; n is the number of components observed as peaks (zones); I is the amount of information; $R_s$ – the mean resolution of all adjacent peaks; IE – informational energy; $I_p$ – performance index; and ε a very small, arbitrary value ($10^{-5}$).

The functions I, Rs, IE, and $I_p$ were calculated with following equations:

$$I = -\sum_{k} p_k \log_2 p_k \tag{2}$$

$$R_s = \frac{2\Delta R_f}{w_1 + w_2};\ \overline{R_S} = \frac{\sum R_{S,i}}{n-1} \tag{3}$$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1.200
0.800
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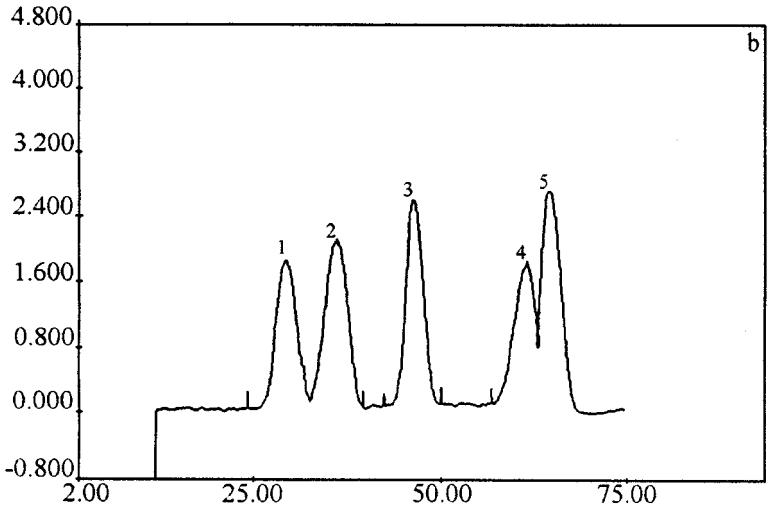
50.00
100.00
150.00
185.00
1
2
3
4
5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1
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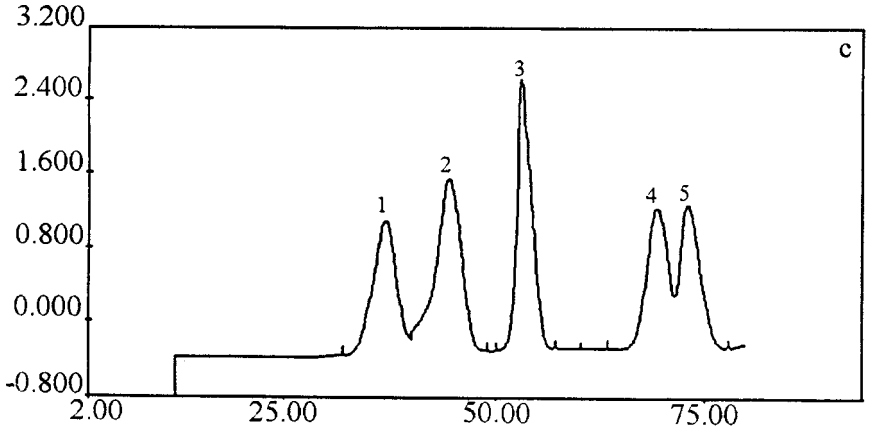
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2.400
1.600
0.800
0.000
-0.800
2.00
25.00
50.00
75.00
1
2
3
4
5

$$IE = \sum_{k} p_k^2 \tag{4}$$

$$I_p = \sqrt{\frac{\sum \left(\Delta hR_{f,i} - \Delta hR_{f,t}\right)}{n(n+1)}} \tag{5}$$

where $p_k$ is the probability of finding a peak in a group; $\Delta hR_{f,i}$ is the measured interval between any two adjacent peaks; and $\Delta hR_{f,t}$ is the measured interval for an ideal separation.

In a previous paper, a mixture of chloroform, acetone, and isopropanol was chosen as mobile phase using Snyder's classification of solvents and preliminary chromatographic runs.[19] The optimization procedure is begun with seven chromatographic runs that were carried out using the composition of mobile phase listed in Table 1. The measured data were used as input for our computer program written in Turbo Pascal. The experimental results and the values of $F_{obj}$ are listed in Table 2.

The $F_{obj}$ values were fitted into a second-order polynomial:

$$F_{obj} = a_1X_1 + a_2X_2 + a_3X_3 + a_{12}X_1X_2 + a_{13}X_1X_3 + a_{23}X_2X_3 + a_{123}X_1X_2X_3 \tag{6}$$

where $X_i$ is the volume fractions of solvents and $a_i$ are coefficients. The coefficients were determined with aid of our program, which solves systems of equations with a single and unique solution. The coefficient values are presented in Table 3.

The $F_{obj}$ diagram was obtained either by calculated values or by the overlapping of the individual term plots. The individual term plots are shown in Fig. 2 and Fig.3 and the final diagram is shown in Fig. 4. From these figures, it can be seen that the introduction of a greater number of factors, associated with a "good" separation, made the final result safer.

The optimum composition of mobile phase was given by the maximum of surface, and it was 73:1:26. Using this mobile phase composition, an additional experiment was performed to verify that a satisfactory separation of all the peaks could be achieved. The densitogram of this separation is presented in Fig. 5.

**Figure 6 (left)**. The densitograms obtained from (a) "Quality factor" method, (b) "Simplex" method and (c) "Prisma" method.

The result obtained with this mathematical model was compared with those obtained with "Simplex" method,[19] the "Prisma" method,[19] and the "Quality factor" model.[20] The optimum mobile phase compositions obtained with these methods are presented in Table 4, and the corresponding densitograms are shown in Fig. 6.

From Fig. 5 and Fig. 6, it can be seen that the optimum separation obtained with this new mathematical model is better than the others, especially for compounds 4 and 5. In the case of the "Simplex" method, we think that a "local" optimum was obtained. The composition of mobile phase can not be modified more precisely in the case of polar compounds with the "Prisma" method. In the case of the "Quality factor" model, the separation was worse because of the fact that we have considered only the resolution, $R_S$, respectively the quality factor Q ($Q = \min R_{si}$, $(i = 1 \div n-1)$), as an analytical parameter for the optimization.

We can conclude that, with this new mathematical model, there is obtained a "global" optimum. The separations that we have obtained with these four optimization methods are similar and major differences do not exist between them.

The mathematical model proposed is a rapid and versatile optimization method, a "global" optimum is obtained without much difficulty, and only seven different mobile phase systems need to be examined.

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