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Short communication

Separation of *N*-alkyl phenothiazine sulfones by HPTLC using an optimum mobile phase

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Abstract

In high performance thin layer chromatography some form of optimization is necessary if complete separation of all components is required. The selection of mobile phase composition is one of the most important components of an optimization strategies. The aim of this paper is the separation of the *N*-alkyl phenothiazine sulfones by high performance thin layer chromatography using an optimum mobile phase system. The optimum composition of mobile phase (toluene–ethyl ether–chloroform, 30:50:20, v/v) are given by the maximum of objective function (F_{obj} = 10.6110).

Keywords: *N*-alkyl phenothiazine sulphones; High performance thin layer chromatography; Optimum mobile phase; Chromatographic response function

1. Introduction

The great amount of research on the mode of action and the metabolism of phenothiazine derivatives is a natural result of their application in human and veterinary medicine. The major metabolites in the metabolism of the phenothiazine-based drugs are the corresponding sulfoxides or 7-hydroxylated derivatives [1,2]. In some cases phenothiazine sulphones might also appear as minor metabolites.

Some analytical methods are used for the separation of phenothiazine derivatives such as spectrophotometry [3], capillary electrophoresis [4], high performance liquid chromatography [5], thin layer chromatography [6], paper chromatography [7], etc.

This paper presents the separation of *N*-alkyl phenothiazine sulfones by high performance thin layer chromatography (HPTLC) using an optimum mobile phase. The phenothiazine derivatives are shynthezed by new method at the biochem-

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istry department of our faculty [8]. These compounds are very similar structures and they differed only by one carbon atom.

2. Experimental

2.1. Materials

All solvents were of analytical grade and were obtained from 'Reactivul' Bucharest, Romania. Solutions (0.1%) of *N*-alkyl phenothiazine sulfones were prepared in chloroform. Chromatography was performed on 10 cm × 20 cm glass HPTLC plates precoated with silica gel 60 F_{254} (Merck, Darmstadt).

2.2. Chromatography

For every solvent systems tested solutions (0.2 μl) of *N*-alkyl phenothiazine sulfones were applied to the plates by means of a capillary pipette. Plates were developed to a distance of 180 mm, at room temperature, by the ascending technique, in a saturated N-chamber. The mobile phases were toluene–ethyl ether–chloroform mixtures of different compositions. Detection were performed in UV light, $\lambda = 254$ nm.

Samples were scanned in the zigzag reflectance mode with a 1.2×1.2 mm^2 slit at $\lambda = 254$ nm by use of a Shimadzu (Columbia, MD) CS-9000 dual wavelength flying-spot scanner.

3. Results and discussion

To reflect the quality of a chromatogram by a single number, a function must be selected; such a function is denoted an objective function [9]. The conditions for the separation are selected such that the numerical value of objective function becomes a maximum or possibly a minimum. A great number of objective functions have been designed and tested [10–13] because no single objective function will ever be entirely satisfactory in all cases. In our opinion, the preferred objective function is that which contains all the qualities of optimum chromatogram.

The objective function used in this work was [9,14]:

$$F_{\text{obj}} = an + b\overline{IR_s} + 10c/IE + d/(I_p + \varepsilon) \quad (1)$$

where a, b, c and d are arbitrary weighting factors (in present work $a = 1$, $b = 1$, $c = 0.1$, $d = 10$); n is the number of components observed as peaks; I is the quantity of information [15]; $\overline{R_s}$ is the mean resolution ($\overline{IR_s}$—informational power [12]; IE is the informational energy [13]; I_p is the performance index [11]; ε is the very small, arbitrary value (10^{-5}). These simple functions could be calculated with following equations:

$$I = -\sum_k p_k \log_2 p_k \quad (2)$$

$$IE = \sum_k p_k^2 \quad (3)$$

$$I_p = \sqrt{\frac{\sum(\Delta hR_{f,i+1} - \Delta hR_f)^2}{n(n+1)}} \quad (4)$$

where p_k is the probability of finding a peak in a group; $\Delta hR_{f,i+1}$ is the measured interval between any two adjacent peaks and ΔhR_f is the measured interval for an ideal separation.

The number of components entirely separated, n, is a useful quality but this number alone is not entirely satisfactory. The amount of information, I, and the informational energy, IE, illustrate the multicomponent separation using discontinuities of probabilities related to some arbitrary 'groups' of retention parameter values. They are not affected by peaks widths and therefore not very sensitive. The performance index, I_p, reflects the uniformity of the separation and this function is very useful criterion in mobile phase optimization.

Seven chromatographic runs were performed using the composition given in Table 1, and all chromatographic measurements were obtained in duplicate; reproducibility was better than ± 1.0%. The experimental results are presented in Table 2 and the values of individual functions and of objective function are listed in Table 3.

The measured data were used as input for a computer program written in Turbo Pascal [14]. The F_{obj} values were fitted to a second-order polynomial:

Table 1
The composition of mobile phases

Mobile phase	Toluen (v/v)	Ethyl ether (v/v)	Chloroform (v/v)
1	40	0	0
2	0	0	40
3	0	40	0
4	0	20	20
5	10	0	20
6	20	20	0
7	10	20	20

Table 3
The values of individual objective functions and chromatographic response function

No.	I_p	I	$\overline{R_s}$	IE	n	F_{obj}
1	5.7222	0.5983	0.1235	0.5918	1	4.5111
2	6.4883	1.2770	0.3787	0.3061	1	6.2915
3	8.1984	1.4751	0.8319	0.2653	2	8.2160
4	7.0280	1.4751	0.6766	0.2653	2	8.1901
5	5.7485	0.6830	0.1145	0.5102	1	4.7778
6	7.5636	1.4751	0.9141	0.2653	3	9.4397
7	7.2054	1.5498	0.7505	0.2245	3	10.0056

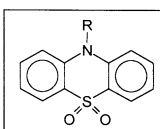
$$F_{obj} = a_1x_1 + a_2x_2 + a_3x_3 + a_{12}x_1x_2 + a_{13}x_1x_3 + a_{23}x_2x_3 + a_{123}x_1x_2x_3 \quad (5)$$

where x_i is the volume fractions of the solvents and a_i are coefficients. The coefficients were determined by means of a Turbo Pascal program [14] and the values of these coefficients are the following:

$$a_1 = 4.51110; \quad a_2 = 6.29150; \quad a_3 = 8.21600;$$
$$a_{12} = -4.14105; \quad a_{13} = 12.30460;$$
$$a_{23} = 3.74540; \quad a_{123} = 64.00100$$

The F_{obj} values for all mobile phase compositions within the solvent triangle could be calculated using the coefficients listed below with Eq. (5). The

Table 2
The experimental results for the *N*-alkyl phenothiazine sulfones (*w*—mm)

	**CH_3**		**C_2H_5**		**C_3H_7**		**C_4H_9**		**C_5H_{11}**		***iso*-C_5H_{11}**		**C_7H_{15}**	
	1		**2**		**3**		**4**		**5**		**6**		**7**	
No.	**hR_f**	**w**	**hR_f**	**w**	**hR_f**	**w**	**hR_f**	**w**	**hR_f**	**w**	**hR_f**	**w**	**hR_f**	**w**
1	3.4	4.6	4.7	5.4	6.1	5.0	5.9	3.2	8.1	5.1	8.3	5.6	7.7	4.7
2	26.7	4.5	27.0	5.5	33.2	5.6	37.6	5.9	42.5	8.0	42.6	7.1	44.8	5.7
3	48.3	6.8	59.4	5.4	81.2	4.2	87.6	3.3	91.4	3.5	90.8	3.1	91.7	1.6
4	59.6	6.0	67.2	5.8	76.8	5.1	81.8	3.9	83.7	3.6	83.6	4.2	86.9	3.4
5	22.4	5.1	22.0	5.8	27.3	7.6	26.2	3.9	26.2	5.5	23.8	5.7	23.3	4.1
6	43.0	9.1	51.7	5.0	64.2	4.9	70.7	3.9	74.6	4.0	73.9	4.5	79.8	4.1
7	54.2	5.8	58.5	5.3	70.0	4.6	73.8	3.9	78.0	4.3	77.1	4.4	84.7	3.4

optimum mobile phase composition was given by the maximum F_{obj} value. We found that the optimum mobile phase composition was toluene–ethyl ether–chloroform (30:50:20, v/v; $F_{obj} = 10.6110$).

Using the optimum mobile phase composition an additional experiment was performed to verify that separation of all the peaks was satisfactory. The chromatogram obtained is presented in Fig. 1. It can be seen from this figure that the using of the optimum mobile phase composition allowed the good separation of all components.

4. Conclusions

This objective function allowed the optimization of the mobile phase and the optimum composition of mobile phase can be determined. Using this mobile phase all components of the mixture can be separated even they are similar structures.

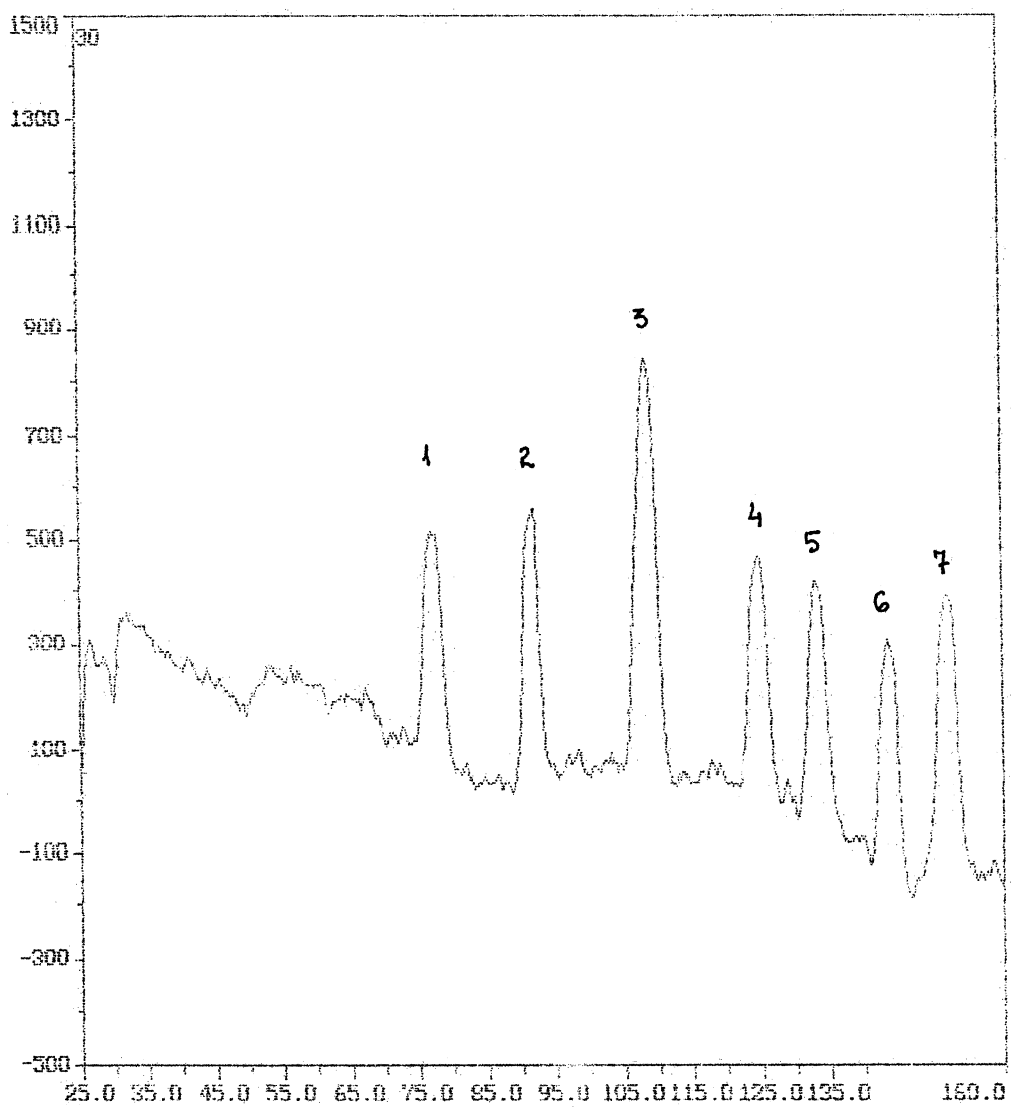


Fig. 1. The chromatogram obtained with optimum mobile phase.

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