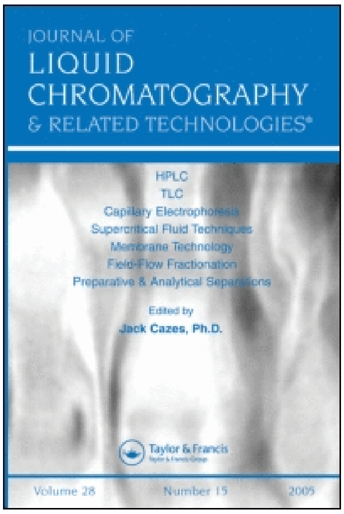

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

OPTIMIZATION OF THE MOBILE PHASE COMPOSITION IN NP-TLC WITH THE CHEMICALLY BONDED 3-CYANOPROPYL STATIONARY PHASE

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To cite this Article Prus, W. and Kowalska, T.(1999) 'OPTIMIZATION OF THE MOBILE PHASE COMPOSITION IN NP-TLC WITH THE CHEMICALLY BONDED 3-CYANOPROPYL STATIONARY PHASE', Journal of Liquid Chromatography & Related Technologies, 22: 1, 15 — 27
To link to this Article: DOI: 10.1081/JLC-100101640
URL: http://dx.doi.org/10.1081/JLC-100101640

J. LIQ. CHROM. & REL. TECHNOL., 22(1), 15-27 (1999)

OPTIMIZATION OF THE MOBILE PHASE COMPOSITION IN NP-TLC WITH THE CHEMICALLY BONDED 3-CYANOPROPYL STATIONARY PHASE

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ABSTRACT

We investigated the dependence between retention of five different test solutes and the mobile phase composition in thin-layer chromatographic systems with the chemically bonded 3-cyanopropyl stationary phase and binary 2-propanol - n-hexane eluents. Then, we calculated regression parameters for the selected three models of solute retention (applicable to the adsorption liquid chromatography mode), first upon all 36 experimental data points and, then, upon a restricted number of four experimental data points. Finally, the relevant conclusions were drawn regarding efficiency of the retention models considered in predicting solute retention (and consequently, in optimization of separation selectivity) based upon the results of an absolutely minimal introductory experiment.

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INTRODUCTION

Chromatographic techniques are known for offering some quite extraordinary possibilities for analytical and preparative separations of mixtures of compounds. However, making a full use of this outstanding capacity of chromatography can occasionally be obtained only after an excessively time-consuming procedure of optimizing the working conditions of the chromatographic process. The main goals of such optimization procedure are: (i) enhancement of the quality of separation and (ii) shortening of the time of analysis. One factor which doubtlessly contributes to the quality of separation is selectivity. Many parameters of the chromatographic system affect this factor, some of them playing a pronounced role, while others affect selectivity to a small extent. Among the factors which strongly affect selectivity of separations in liquid chromatography is composition of mobile phase.

The simplest approach to optimization of separation selectivity, i.e., the trial-and-error method, has a number of drawbacks, the most important among them being an excessively long optimization time and an alarmingly huge consumption of reagents and other materials necessary for the seemingly endless repetitions of slightly modified experiments. There are numerous other approaches to the same issue, and one of them is the so-called interpretative strategy.[1,2] This particular approach allows one to predict the optimum conditions for running the chromatographic process based upon the data derived from a relatively simple introductory experiment.

The key role in the interpretative strategy of optimization of separation selectivity is played by the possibility of predicting solute retention, based upon a suitable retention model. It is obvious that the important demand of the laboratory praxis is to attain such a possibility with the simplest and shortest experiment possible. Hence, a sort of uncertainty arises as to the predicitve power of the available retention models, if implemented with a rigidly limited number of the experimental data.

If we consider retention models which differ among themselves in the algebraic sense, it becomes particularly interesting and even challenging to compare their efficiency in predicting solute retention and to determine how sensitive they are to minimization of the introductory experiment.

It is the aim of this paper to compare three selected models of solute retention, applicable to the adsorption liquid chromatography mode (i.e., the models described by Soczewiński[3] and Kowalska,[4] and the so-called 'cubic' model[5]) with respect to their efficiency in a case of a maximally-reduced pilot experiment.

THEORY

When we talk about optimization of the quantitative composition of a given mixed mobile phase under the notion of the '*retention model*', we normally understand the equation which couples together the retention parameter of a solute with the magnitude which describes the eluent's composition. If we assume that in the chromatographic system described in this paper (composed of silica with chemically bonded 3-cyanopropyl ligands / 2-propanol + n-hexane) adsorption predominates in the overall retention mechanism, then the retention parameter of a given solute can be described with aid of several models proposed by different authors.

One of the earliest relationships, specially derived for the adsorption liquid chromatography mode, coupling together the retention parameter of a solute ($\log k = R_M$) with the mole fraction of the mobile phase component able to intermolecularly interact with stationary phase (X_S), is the main equation of the Soczewiński model of solute retention:[3]

$$\log k = C + n \log X_S \tag{1}$$

where C and n are the equation constants.

Another model, which originates from the assumptions about association of the mobile phase components through intermolecular interactions,[4] was proposed by Kowalska as a bilinear equation which describes the relationship between the retention parameter of a given solute (R_F) and the quantitative composition of the mobile phase (expressed by the volume fractions of its components, x):

$$R_F = A\sqrt{x} + B(1 - x) + C \tag{2}$$

where A, B, and C are the equation constants.

The third model, which is recommended owing to its mathematical properties as a 3rd degree continuous function, describes the relationship between the retention parameter of a given solute (R_F) and the mobile phase composition expressed by the volume fractions of its components (x):[5]

$$R_F = a x^3 + b x^2 + c x + d \tag{3}$$

where a, b, c, and d are the equation constants.

Due to a specific property of the parameter R_F, which basically is the dependence between the numerical value and the variance of this magnitude (i.e., the higher is the measured R_F value, the higher also becomes its variance), and, in order to at least partially limit the influence of this property upon our calibrations, we first had to transform model relationships considered in this paper and given as eqs (1) - (3) to the formulas given below (as eqs (4) - (6)):

$$\ln R_F = -\ln\left(1+10^{C+n\log X_S}\right) \tag{4}$$

$$\ln R_F = \ln\left(A\sqrt{x}+B(1-x)+C\right) \tag{5}$$

$$\ln R_F = \ln(ax^3 + bx^2 + cx + d) \tag{6}$$

To evaluate an agreement between the experimental retention data ($R_{fi}^{ex.}$) and those calculated from eqs (4) - (6) ($R_{Fi}^{calc.}$), we used a statistical magnitude of the root mean square error (*RMSt*), defined as:

$$\mathrm{RMSt} = \sqrt{\frac{\sum_{i=1}^{n}\left(\ln R_{Fi}^{ex.} - \ln R_{Fi}^{calc.}\right)^2}{n}} \tag{7}$$

where n denotes the number of the above pairs of the data.

EXPERIMENTAL

Statistical evaluations were performed upon the laboratory experimental results, obtained under the following conditions.

Stationary phase: glass-backed HPTLC plates containing silica gel chemically bonded 3-cyanopropyl ligands (E. Merck, Darmstadt, Germany; cat. # 12571).

Mobile phases: 2-propanol + n-hexane binary mixtures of different volume proportions (volume fractions of 2-propanol ranged from 0.05 to 1.00).

Test solutes: 1-naphthol, 1-naphthylamine, quinoline, 8-methylquinoline, and 4-methylquinoline.

Standard solutions of the test solutes (2 % w/v) were prepared in 2-propanol. Aliquots, 1 µL, of each standard solution were spotted and the plates were left until evaporation of the solvent from each spot was completed. Then the chromatograms were developed to the distance of 9.5 cm with use of a

Table 1

Regression Parameters for the Three Retention Models*

		Retention Model	
Test Solute	**Equation (4)**	**Equation (5)**	**Equation (6)**
1-Naphthol	C = -0.8759	A = 2.499	a = 2.098
		B = 1.367	b = -4.553
	n = -1.296	C = -1.622	c = 3.257
			d = 0.09480
1-Naphthylamine	C = -0.04912	A = 1.113	a = 1.237
		B = 0.5334	b = -2.441
	n = -0.7166	C = -0.6022	c = 1.661
			d = 0.07859
Quinoline	C = -0.2820	A = 1.055	a = 0.5641
		B = 0.3342	b = -1.494
	n = -0.8639	C = -0.3559	c = 1.483
			d = 0.1343
8-Methylquinoline	C = -0.6026	A = 1.541	a =1.387
		B = 0.8783	b = -2.898
	n = -0.7116	C = -0.7693	c = 1.976
			d = 0.3280
4-Methylquinoline	C = -0.1279	A = 0.7761	a = 0.6774
		B = 0.1527	b = -1.444
	n = -0.8215	C = -0.1551	c = 1.289
			d = -0.1064

---

* Given by eqs (4) - (6) and, for the five test solutes considered, obtained from calibrations performed on all 36 experimental data points.

horizontal chromatographic chamber of the DS type. After drying the plates at room temperature, the chromatographic spots were visualized with use of UV light and in iodine vapours. For each test solute, R_F values were obtained for 16 different quantitative compositions of the 2-propanol + n-hexane eluent, yielding 36 different results. For each test solute and each quantitative mobile phase composition, at least two independent determinations of R_F were carried out.

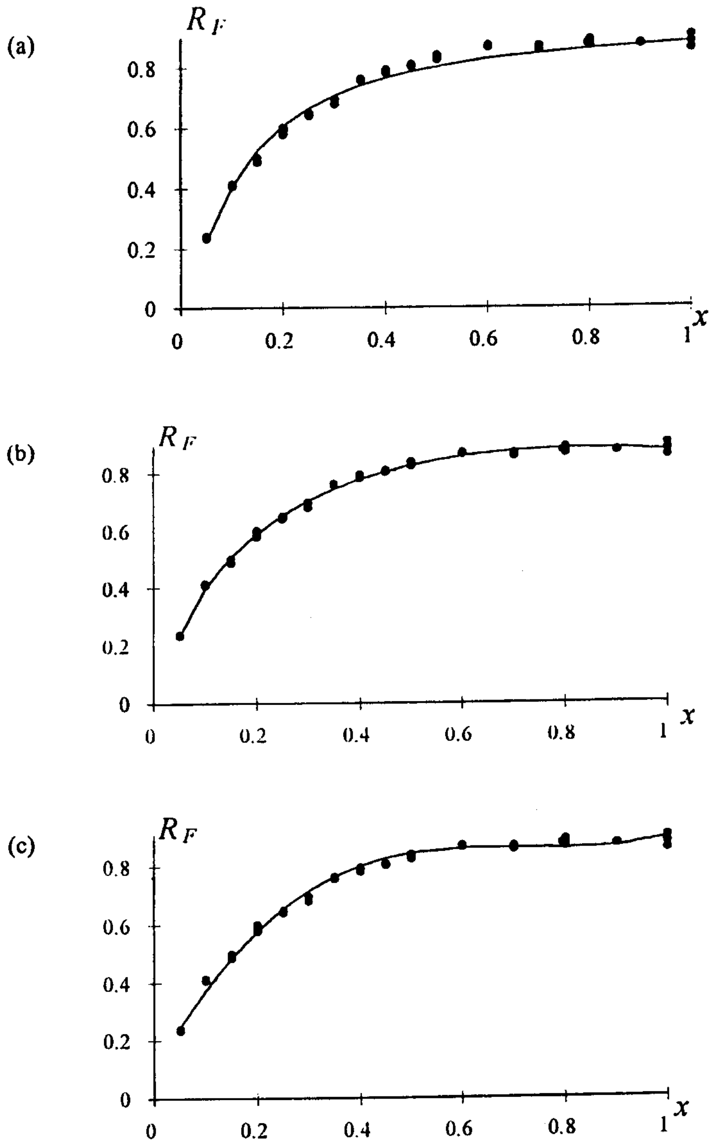

Figure 1. The experimental (dots) and calculated [from (a) eq. (4), (b) eq. (5), and (c) eq. (6) on the basis of all 36 available data points] (line) relationship between the parameter R_F and the volume fraction of 2-propanol in the binary 2-propanol + n-hexane mixture for 1-naphthol.

Table 2

A Comparison of the Numerical Values of *RMSt**

Test Solute	Equation (4)	Retention Model Equation (5)	Equation (6)
1-Naphthol	0.0327	0.0183	0.0305
1-Naphthylamine	0.0604	0.0597	0.0528
Quinoline	0.0705	0.0521	0.0463
8-Methylquinoline	0.0250	0.0255	0.0314
4-Methylquinoline	0.0763	0.0527	0.0530
Average Value	0.0530	0.0417	0.0428

---

* Obtained for each individual test solute and the retention model considered from calibrations performed on all 36 experimental data points, and the respective mean *RMSt* values, valid for each indivual retention model separately.

RESULTS

Fitting of all 36 experimental data points to the three retention models given by eqs (4) - (6) resulted in the respective regression parameters of these relationships as shown in Table 1. Shown in Table 2 are the *RMSt* values calculated for each individual test solute and the retention model studied, as well as, the mean *RMSt* values calculated for each individual retention model and the whole population of the examined solutes. An additional possibility of evaluating the quality of agreement between the experimental R_F values and those calculated (for all 36 experimental data points) with aid of the consecutive retention models is offered by a selection of the respective plots, given in Fig. 1. The examples shown in Fig. 1 refer solely to the case of 1-naphthol, but they are representative for all of the solutes studied.

An alternative approach was also tried, namely fitting of four experimental data points only for each individual test solute (corresponding to the volume fractions of 2-propanol equal to 0.05, 0.25, 0.45, and 0.90) to the compared retention models, which resulted in another set of the regression parameters of these relationships (see Table 3) and in a collection of the *RMSt* values, analogous to that from Table 2 (see Table 4).

Table 3

Regression Parameters for the Three Retention Models*

	Retention Model		
Test Solute	**Equation (4)**	**Equation (5)**	**Equation (6)**
1-Naphthol	$C = -0.8747$	$A = 2.546$	$a = 2.657$
		$B = 1.4058$	$b = -5.243$
	$n = -1.289$	$C = -1.675$	$c = 3.467$
			$d = 0.06943$
1-Naphthylamine	$C = -0.06868$	$A = 0.8586$	$a = 0.7139$
		$B = 0.2714$	$b = -1.535$
	$n = -0.7328$	$C = -0.2899$	$c = 1.305$
			$d = 0.09848$
Quinoline	$C = -0.2871$	$A = 0.9686$	$a = 0.3256$
		$B = 0.2427$	$b = -1.119$
	$n = -0.8054$	$C = -0.2376$	$c = 1.360$
			$d = 0.1447$
8-Methylquinoline	$C = -0.6508$	$A = 1.645$	$a = 1.993$
		$B = 0.9337$	$b = -3.745$
	$n = -0.7606$	$C = -0.8547$	$c = 2.319$
			$d = 0.2932$
4-Methylquinoline	$C = -0.1306$	$A = 0.7298$	$a = -0.3029$
		$B = 0.09352$	$b = -0.1478$
	$n = -0.7688$	$C = -0.08273$	$c = 0.9178$
			$d = 0.1245$

---

* Given by Eqs. (4) – (6), and for the five test solutes considered, obtained from calibrations performed on the minimal set of four experimental data points only (corresponding to the volume fractions of 2-propanol equal to 0.05, 0.25, 0.45, and 0.90).

Similar to the preceding case, we attempted to visualize the quality of agreement obtained between the experimental and the calculated R_F values based on the four data points (see plots in Fig. 2). Again, the comparison was made for 1-naphthol as the test solute, but the conclusions which can be drawn from this comparison are valid for the remaining test solutes as well.

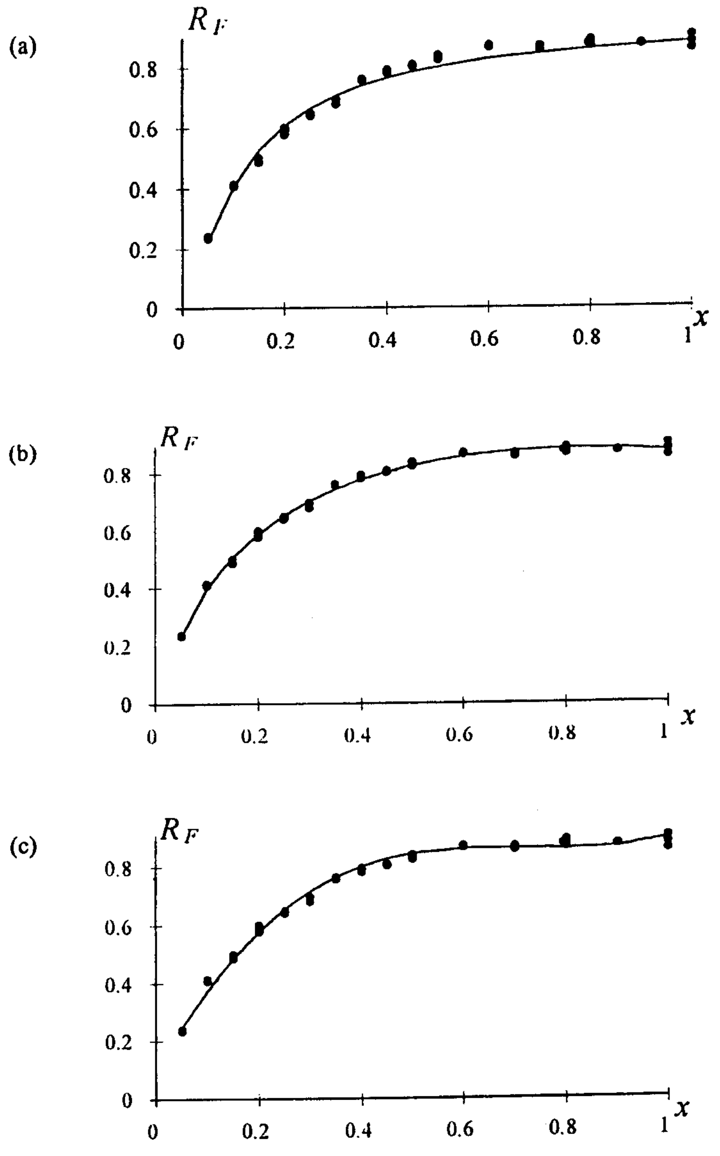

Figure 2. The experimental (dots) and calculated [from (a) eq. (4), (b) eq. (5), and (c) eq. (6) on the basis of a minimum number of the 4 data points] (line) relationship between the parameter R_F and the volume fraction of 2-propanol in the binary 2-propanol + n-hexane mixture for 1-naphthol.

DISCUSSION

In the first series of calculations performed (see Tables 1 and 2 and Fig.1), we used for our calibrations the maximum number of 36 data points, representing retention of the investigated test solutes in the chromatographic systems considered.

If the prediction of solute retention can, in this case, be regarded as interpolation, then the numerical values of *RMSt* given in Table 2 span the following magnitude ranges:

- for eq. (4) from 0.0250 (8-methylquinoline) to 0.0763 (4-methylquinoline);
- for eq. (5) from 0.0183 (1-naphthol) to 0.0597 (1-naphthylamine);
- for eq. (6) from 0.0305 (1-naphthol) to 0.0530 (4-methylquinoline).

On the basis of the above results, one could easily conclude that the Kowalska model of solute retention and the 3rd degree continuous function perform slightly better than the Soczewiński model, especially if we consider the respective average *RMSt* values (equal to 0.0417, 0.0428, and 0.0530). The differences among these three average *RMSt* values remain within the same magnitude order and, therefore, cannot be considered as very significant. However, a comparison of performance based on discrepancies between the whole populations of the obtained experimental and calculated results does not seem fully justified, especially if we consider further utilization of these approaches for optimization of separation selectivity in the framework of the so-called interpretative strategy.

From the practical standpoint, it is a well recognized fact that the introductory experiment ought to be limited to an absolutely necessary minimum. The scope of such an experiment largely depends on the optimization strategy chosen and, in the case of the interpretative strategy, on the assumed retention model. In the case of the models discussed in this paper, it suffices to limit our experiment to obtaining four retention parameters for each investigated test solute and for four different quantitative proportions of the binary mobile phase. In TLC, this goal can easily be attained by developing four chromatograms for each test solute considered.

In the second series of calculations performed (see Tables 3 and 4, and Fig. 2), we used for our calibrations the minimum number of four data points, representing retention of the investigated test solutes in the chromatographic systems considered. Again, if the prediction of solute retention also in this case can be regarded as interpolation, then the numerical values of *RMSt* given in Table 4 span the following magnitude ranges:

Table 4

A Comparison of the Numerical Values of *RMSt**

Test Solute	**Equation (4)**	**Retention Model** **Equation (5)**	**Equation (6)**
1-Naphthol	0.0339	0.0192	0.0387
1-Naphthylamine	0.0645	0.0767	0.0760
Quinoline	0.0880	0.0611	0.0549
8-Methylquinoline	0.0286	0.0327	0.0305
4-Methylquinoline	0.0870	0.0592	0.0795
Average Value	0.0604	0.0498	0.0559

---

* Obtained with each individual test solute and the retention model considered for 32 experimental data points (corresponding to the volume fractions of 2-propanol from 0.05 to 0.90) upon calibrations performed on the four experimental data points only (corresponding to the volume fractions of 2-propanol equal to 0.05, 0.25, 0.45, and 0.90), and the respective mean *RMSt* values, valid for each individual retention model separately.

- for eq. (4) from 0.0286 (8-methylquinoline) to 0.0880 (quinoline);
- for eq. (5) from 0.0192 (1-naphthol) to 0.0767 (1-naphthylamine);
- for eq. (6) from 0.0305 (8-methylquinoline) to 0.0795 (4-methylquinoline).

On the basis of the above results, once again we could easily conclude that the Kowalska model of solute retention performs the best (the average *RMSt* value equals to 0.0498), the Soczewiński model performs the worst (the average *RMSt* value equals to 0.0604), and performance of the 3rd degree continuous function can be considered as an intermediate (the average *RMSt* value equals to 0.0559). Again though, the differences among these three average *RMSt* values remain within the same magnitude order and, therefore, cannot be viewed as very significant.

However, in a comparison of the retention models with respect to their predictive power, on the basis of the experimental results contaminated with random errors, one should additionally consider relationship between gradual limitation of an introductory experiment and an increase of the respective *RMSt* values (where *RMSt* is assumed as a measure of a given model's performance). In the particular case of our experiment, reduction of the experiment from the initial 36 data points to an absolute minimum of four different quantitative mobile phase compositions gave the following results: the average *RMSt* value increased by 0.0074 in the case of the Soczewiński model, by 0.0081 in the case of the Kowalska model, and by 0.0131 in the case of the 3rd degree continuous function. Thus, it can be stated that the Kowalska and the Soczewiński models show a comparable and somewhat better stability towards minimization of the introductory experiment than the 3rd degree continuous function.

An additional and general remark can also be added to our discussion: If calibration is aimed at prediction of the dependent variable (in our case of the retention parameter, normally obtained from an experimental measurement), then it seems clear to expect that the ideal model of solute retention be represented by the simplest possible mathematical function (i.e., by a straight-line, or a close-to-linear relationship), additionally having a justified physicochemical background. However, the transformed models given by eqs. (4) - (6) are more complicated than the above assumed ideal and, moreover, the 3rd degree continuous function lacks a confirmed physicochemical background.

Further, it can be stated that, in the case of these models which are represented by the more complicated mathematical functions, an agreement between the experimental data and the computed ones is the best if the abundant sets of the experimental results are used for calibrations. This regularity was well confirmed by the results originating from our TLC experiment, and specifically when applying the third model (in its transformed shape given by eq. (6), and the only one curvilinear even before transformation). In this case, we observed a very fine reconstitution of the experimental results; thus, it can be agreed that the discrepancy between the numerical values of this function and the respective real data is similar to the discrepancy between the experimental values burdened with random errors and the 'pure', i.e., error-free data.

The results shown in Table 4 (i.e., the respective *RMSt* and the average *RMSt* values) give evidence of a remarkably good (and also comparable) predictive power of the retention parameter on the basis of the four data points only with all three retention models considered, which is the proof of their usefulness for implementation in the interpretative strategy of the separation selectivity optimization. The results presented in this paper, and an extensive discussion thereof, furnish a good example of certain crucial issues which can be encountered by chromatographers who, in their work, intend to apply the interpretative strategy for optimization of separation selectivity and who are then faced with a problem of selecting a retention model most suitable for this particular purpose.

REFERENCES

1. P. J. Schoenmakers. *Optimization of Chromatographic Selectivity. A Guide to Method Development*. *Journal of Chromatography Library, Vol. 35*, Amsterdam: Elsevier (1986).

2. R. B. Laub, J. H. Purnell, P. S. Williams. *Anal. Chim. Acta*, **95**, 135-143 (1977).

3. E. Soczewiński. *Anal. Chem*., **41**, 179-182 (1969).

4. T. Kowalska. *Chromatographia*, **30**, 298-300 (1990).

5. V. Coman, C. Măruţoiu, S. Puiu. *J. Chromatogr. A*, **779**, 321-328 (1997).

Received March 10, 1998
Accepted April 21, 1998
Manuscript 4823-TLC

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