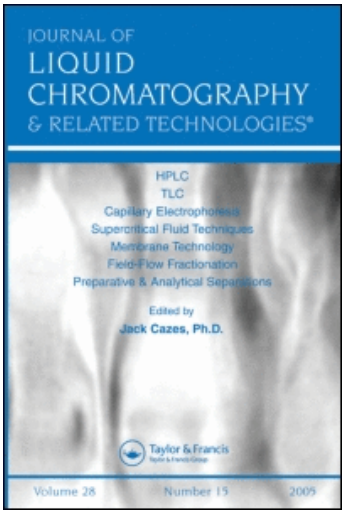

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Vučićević, Katarina , Popović, Gordana , Nikolic, Katarina , Vovk, Irena and Agbaba, Danica(2009) 'An Experimental Design Approach to Selecting the Optimum HPLC Conditions for the Determination of 2-Arylimidazoline Derivatives', Journal of Liquid Chromatography & Related Technologies, 32: 5, 656 — 667
To link to this Article: DOI: 10.1080/10826070802711113
URL: http://dx.doi.org/10.1080/10826070802711113

Journal of Liquid Chromatography & Related Technologies®, 32: 656–667, 2009

ISSN: 1082-6076 print/1520-572X online
DOI: 10.1080/10826070802711113

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Abstract: In order to improve the chromatographic resolution (Rs) with a good analysis time, experimental designs were applied for multivariate optimization of the experimental conditions of an isocratic reversed phase high performance liquid chromatographic (RP-HPLC) method used for the simultaneous determination of seven 2-arylimidazoline derivatives, acting as vasoconstrictors and antihypertensives. Optimal conditions for the separation of the seven 2-arylimidazolines were obtained using a mixture of acetonitrile–triethylamine phosphate buffer (pH 4.0; 25 mM) (30:70, v/v) as mobile phase at 25°C. Here the 2^3 full factorial experimental design was employed to optimize RP-HPLC separation of the 2-arylimidazolines. The suggested RP-HPLC method is applicable for routine analysis of the 2-arylimidazoline derivatives under the same chromatographic conditions, which can shorten the time and the costs of the analysis as well.

Detailed description of the method development and optimization given in this paper will enhance the reproducibility of the established experimental conditions and simplify the process of some future methods developments.

Keywords: Alpha agonist, Experimental design, Optimization of HPLC method

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INTRODUCTION

The 2-(arylmethyl)-imidazoline derivates, such as tetrahydrozoline, naphazoline, oxymetazoline, and xylometazoline, are selective α_1-agonists that are in commertial use as vasoconstrictors in the treatment of hypotension, shock, and nasal congestion. On the other hand, the 2-(arylamino)-imidazoline or 2-imino-imidazoline, are centrally acting antihypertensives. The hypotensive effect of the ligands, such as clonidine and moxonidine, is attributed both to 2-adrenergic receptors and imidazoline I_1-receptors.[1,2]

The aim of this study is to separate seven 2-arylimidazoline derivatives, acting as vasoconstrictors and antihypertensives, using reverse phase high performance liquid chromatography (RP-HPLC), with acetonitrile–triethylamine phosphate buffer as mobile phase.

Many chromatographic and electrophoretic methods have been developed for examination of the individual I_1-R ligands.[2–6]

Considering that these substances are available as commercial drugs, most literature data are focused on their determination using various analytical techniques. Spectrophotometric methods were developed for single component analysis,[3,4] as well as for simultaneous determination of mixtures containing naphazoline and antazoline,[5,6] naphazoline and diphenhydramine,[7] naphazoline and chlorpheneramine,[8] naphazoline and tetramethylthionine.[9] Also, ion association complexes of naphazoline, tolazoline, and xylometazoline with $[Co(NO_2)_6]^{3-}$ and $[Fe(CN)_6]^{3-}$ were precipitated and determined by atomic emission and atomic absorption spectrometry.[10]

Finally, different separation techniques, such as capillary electrophoresis, high performance thin layer chromatography (HPTLC), and HPLC, were applied for determination of components of mixtures containing one or two selected 2-arylimidazoline derivatives.[11–16]

Experimental design is used for experimental screening, design, and optimization of the HPLC method. In the novel literature there are many experimental design applications in development and optimization of the analytical method, especially in the area of separation science. The optimization of the investigated analytical factors can be carried out by applying different forms of experimental design, such as full factorial design, composition design, fraction factorial design, or artificial neural networking.[17–29]

The objective of the study was to examine application of different chromatographic conditions for the analysis of the selected ligands.

Since there is no data concerning the simultaneous determination of structurally similar drugs containing imidazoline moiety, the aim of this work was to develop and optimize an RP-HPLC method for their efficient separation. To the best of our knowledge, the presented chemometric study is the first attempt to design and optimize an RP-HPLC model

for separation and determination of the seven 2-arylimidazoline derivatives. The full factorial design was employed to examine the influence of temperature, pH, and composition of mobile phase on the RP–HPLC separation of the seven 2-arylimidazoline derivatives.

The use of multivariate techniques for optimization of analytical procedures is generally performed by preliminary evaluation using factorial designs, with the objective of selecting the significant variables that affect the analytical method and, afterward, determination of optimal values for these variables in this method.

In this paper, a method for simultaneous determination of the seven 2-arylimidazolines by RP-HPLC/UV was optimized using full three level factorial designs. In the optimization, the response was established considering resolution among the peaks of the tetrahydrozoline, naphazoline, and tramazoline and, also, analysis time.

EXPERIMENTAL

Reagents

All used reagents were HPLC and analytical grade purity. Acentonitrile (supergradient HPLC grade, Lab Scan (Dublin, Ireland), water (HPLC grade), triethylamine, Merck (Darmstadt, Germany), and 85% phosphoric acid Carlo Erba (Milano, Italy) were used for the preparation of the mobile phase. Clonidine and tramazoline were obtained from Zdravlje (Leskovac, Serbia and Montenegro). Moxonidine was obtained from Solvay pharmaceuticals (Hannover, Germany). Naphazoline was obtained from Panfarma (Belgrade, Serbia and Montenegro), Oxymetazoline was obtained from Lek (Ljubljana, Slovenia). Xylometazoline was purchased from Dolder AG (Basel, Switzerland). Tetrahydrozoline was obtained from Hemomont (Podgorica, Serbia and Montenegro).

Apparatus

The chromatographic system, Hewlett Packard 1100 series (Waldbronn, Germany) consisted of a HP 1100 pump, UV/VIS detector, and HP ChemStation integrator. UV detection was carried out at 230 nm. The flow rate was 1 mL min^{-1}. The samples were introduced through a Rheodine injector valve with a 20 μL sample loop produced by Hewlett Packard (Waldbronn, Germany). The separation analysis of tested substances was carried out on the Zorbax C_{18} 250 × 4.6 mm, 5 μm column (Agilent Technologies, Waldbronn, Germany). Mobile phase consisted of various ratios of acetonitrile and 25 mM triethylamine phosphate

buffer with different pH values. For the statistical analysis program, Origin 6.1 (Microcal Software, Inc. Northampton, USA) was used.

Solutions

Solutions of the investigated substances were prepared in the mixture acetonitrile–water (50:50, v/v) in the concentration of 0.1 mg mL^{-1}.

Optimization Strategy

The optimization was performed in two steps. First, a full three level factorial design was carried out for optimization of the variables of mobile phase (polarity and pH) and temperature. Maximum and minimum levels of each factor were chosen in agreement with data obtained in previous experiments. The experimental data were processed by using the XNUMBERS, Ver. 4.7 program.[30]

RESULTS AND DISCUSSION

Factorial designs are widely used in experiments involving several factors where it is necessary to study the joint effect of these factors on a response. The important descriptions of the full factorial experimental design are the number of factors involved and the number of levels for each factor. Based on previous experiments and obtained pK_a values, factors were defined and their influence on the observed system was investigated.

Method development for determination of the seven 2-arylimidazoline derivatives was performed by employing a full factorial experimental design. Based on the preliminary experimental data, the effect of temperature (a), the pH of the mobile phase (b), and the percentage of organic modifier (c) were chosen for the study (Table 1). The response of the system was observed through the chromatographic separation.

This investigation has been focused on studying the influence of the pH value of mobile phase, the concentration of acetonitrile in the mobile phase, and temperature, which affect a complete separation of the chromatographic peaks of these compounds, applying multivariate optimization methods, in order to improve the chromatographic resolution between the 2-arylimidazoline derivatives.

The data of the full factorial design performed for optimization of the pH value of mobile phase, the concentration of acetonitrile in the mobile phase, and temperature are described in Table 1. The observed factors were applied into 3–factor–2–level (2^3) factorial experimental design, with their "high" (+) and "low" (−) values.

Table 1. Factors and levels used in the full three-level factorial design

	Levels	
Factors	(−)	(+)
(a) temperature (°C)	25	40
(b) pH of the mobile phase	4.0	7.0
(c) acetonitrile (%)	30	40

The results of this factorial were evaluated, using a nonlinear mathematical model, to investigate the effects of pH, the concentration of acetonitrile in the mobile phase, and temperature (inputs) on chromatographic separation (output).

In order to evaluate the pure experimental error, an experiment was also performed in the center of the experimental domain (pH 5.5, percentage of acetonitrile 35%, and temperature 30°C). Retention times collected under different experimental conditions (pH, solvent, and temperature) were correlated to the three variables and their interactions and mathematical model was constructed.[31–36]

Matrix of the performed RP-HPLC experimental conditions (1–9) is presented in Table 2.

In the performed experimental design, the influence of the acidity of the mobile phase, the concentration of acetonitrile in the mobile phase, and temperature on separation and retention time of the seven 2-arylimidazoline derivatives was studied (Figure 1).

Obtained results for the retention factors for each experimental condition (1–9) are presented in Table 3.

Table 2. Matrix of the experiment

Number of the experiment	% acetonitrile	pH of the mobile phase	t (°C)
1	−	−	−
2	−	−	+
3	−	+	−
4	−	+	+
5	+	−	−
6	+	−	+
7	+	+	−
8	+	+	+
9	0	0	0

Table 3. Retention factors of investigated substances

Compound	Number of the experiment								
	1	2	3	4	5	6	7	8	9
Moxonidine	0.26	0.26	0.78	0.60	0.20	0.19	0.54	0.53	0.30
Clonidine	0.56	0.53	2.46	1.78	0.37	0.36	1.45	1.42	0.66
Tetrahydrozoline	0.85	0.82	2.46	1.94	0.49	0.48	1.79	1.72	0.89
Naphazoline	1.19	1.11	3.00	2.48	0.58	0.55	2.12	1.99	1.11
Tramazoline	1.58	1.53	4.66	3.36	0.75	0.73	2.77	2.62	1.48
Oxymetazoline	4.76	4.47	6.16	6.69	1.51	1.46	4.54	4.16	3.38
Xylometazoline	9.48	8.93	17.42	16.14	2.68	2.59	10.01	9.12	6.76

The results in Table 3 show that the most significant factor is the polarity of the mobile phase. In Figure 2, can also be seen that the chromatographic resolution increases with the polarity of the mobile phase. In addition, the decrease of percentage of acetonitrile induced longer retention times and peak tailing.

In the performed experimental design, a nonlinear mathematical model for the evaluation of the influence of the investigated factors on

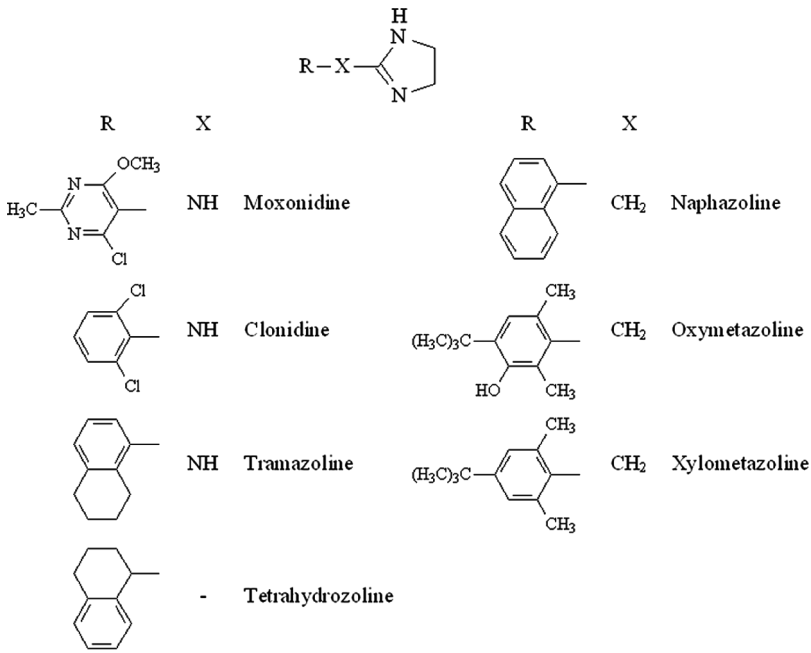


Figure 1. Chemical structure of investigated 2-arylimidazoline derivatives.

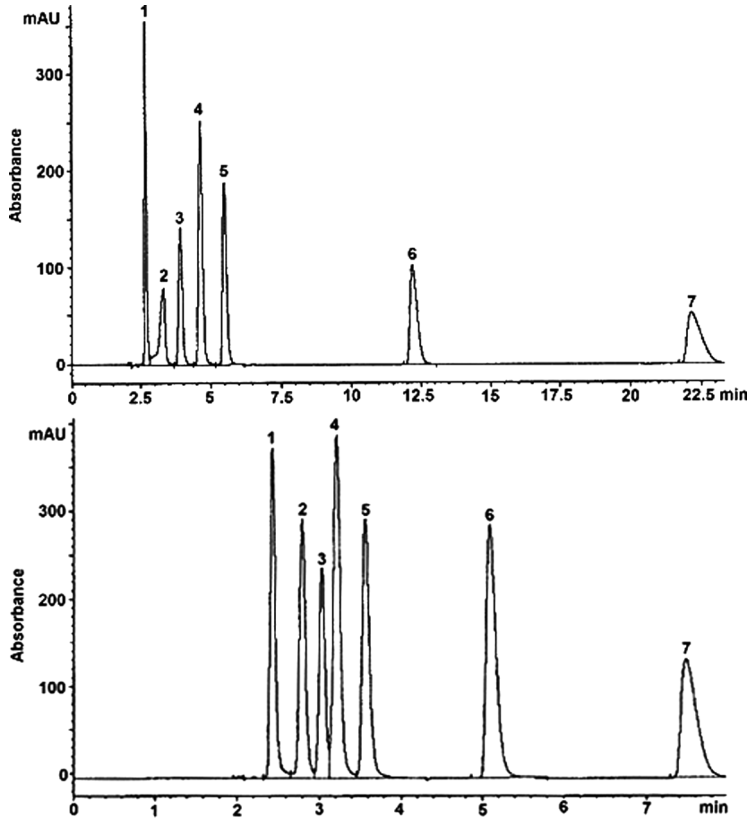


Figure 2. Chromatograms of moxonidine (1), clonidine (2), tetrahydrozoline (3), naphazoline (4), tramazoline (5), oxymetazoline (6) and xylometazoline (7); Upper: Mobile Phase is acetonitrile – triethylamine phosphate buffer (pH 4.0; 25 mM) (30:70, v/v) at 25°C. Lower: Mobile Phases acetonitrile – triethylamine phosphate buffer (pH 4.0; 25 mM) (40:60, v/v) at 25°C.

chromatographic retention parameters was employed. The form of a nonlinear model is:

$$y = b_o + b_1x_1 + b_2x_2 + b_3x_3 + \cdots + b_{N-1}x_{N-1} + b_Nx_N + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 + \cdots + + b_{(N-1)Nx(N-1)}x_N + b_{123}x_1x_2x_3 + \cdots + b_{(N-2)(N-1)N}x_{(N-2)}x_{(N-1)}x_N$$

where y-the estimated response, b_0-the average experimental response,

The coefficients b_1 to b_N present estimated effects of the factors considered. The influence of the coefficients on the performance of the method is called main effect.

Table 4. Matrix for calculating coefficients

Number of the experiment	Coefficients							
	b_1	b_2	b_3	b_{12}	b_{13}	b_{23}	b_{123}	b_0
1	−	−	−	+	+	+	−	+
2	+	−	−	−	−	+	+	+
3	−	+	−	−	+	−	+	+
4	+	+	−	+	−	−	−	+
5	−	−	+	+	−	−	+	+
6	+	−	+	−	+	−	−	+
7	−	+	+	−	−	+	−	+
8	+	+	+	+	+	+	+	+
Divisor	4	4	4	4	4	4	4	8

The coefficients b_{12} to $b_{(N-1)N}$ are called the interaction terms.

From the formula given above, it can be seen that the factorial design provides information about the effects of the considered factors on the chromatographic retention parameters and importance of interaction between the factors for the retention parameters.

The coefficients calculating matrix of the experiment is shown in Table 4.

The coefficient's values of mathematical model are presented in Table 5.

The obtained values for coefficients of the mathematical model for investigated compounds retention factors show the percentage of organic modifier, and pH of mobile phase (b_2 and b_3) has the greatest impact on chromatographic behavior. The effect of percentage of acetonitrile on retention behavior of the tested substances can be noticed from the obtained chromatograms presented in Figure 2.

Table 5. Coefficients of nonlinear mathematical model for retention factors

Compound	Factor effect							
	b_0	b_1	b_2	b_{12}	b_3	b_{13}	b_{23}	b_{123}
Moxonidine	0.4193	−0.0446	−0.1072	0.0402	0.3852	−0.0432	−0.0428	0.0433
Clonidine	1.175	−0.1920	−0.4329	0.1647	1.322	−0.1669	−0.2528	0.1565
Tetrahydrozoline	1.319	−0.1602	−0.3999	0.1124	1.319	−0.1372	−0.0484	0.1071
Naphazoline	1.628	−0.1869	−0.6334	0.1119	1.537	−0.1336	−0.0492	0.0862
Tramazoline	2.250	−0.3847	−1.064	0.293	2.201	−0.3442	−0.2474	0.2780
Oxymetazoline	4.220	−0.0479	−2.604	−0.1664	2.340	0.1248	0.5316	−0.2862
Xylometazoline	9.559	−0.7249	−6.871	0.1875	7.273	−0.4029	−0.3031	−0.0414

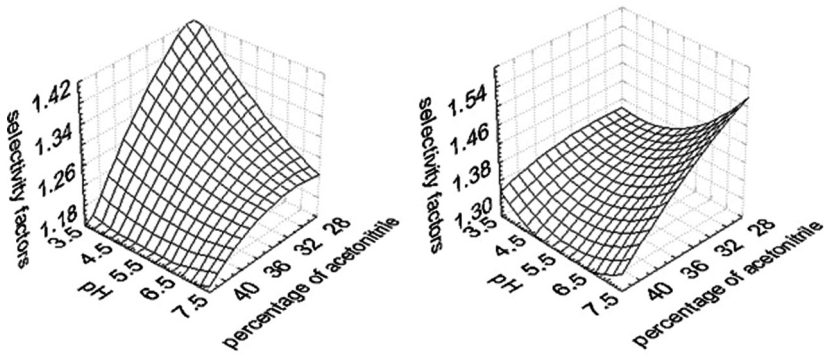
Table 6. Selectivity factors and resolution factors of tested compounds in chromatographic system with: Mobile phase I, consisting of acetonitrile – triethylamine phosphate buffer (pH 4.0; 25 mM) (30:70, v/v) at 25°C and Mobile phase II, consisting of acetonitrile – triethylamine phosphate buffer (pH 4.0; 25 mM) (40:60, v/v) at 25°C

	Mobile phase I		Mobile phase II	
	Selectivity factor	Resolution factor	Selectivity factor	Resolution factor
Moxonidine/Clonidine	2.182	4.2649	1.905	4.826
Clonidine/Tetrahydrozoline	1.5096	3.2114	1.316	3.019
Tetarhzdrozoline/Naphazoline	1.4011	4.6693	1.178	2.118
Naphazoline/Tramazoline	1.3316	5.3530	1.298	3.813
Tramazoline/Oxymetazoline	3.0042	30.5647	2.000	13.59
Oxymetazoline/Xylometazoline	1.9904	24.0415	1.780	14.26

The highest values for b_2, b_3, and b_{23} coefficients, computed by the nonlinear mathematical model (Table 5), indicated that the percentage of acetonitrile and pH of mobile phase have the strongest influence on the chromatographic separation. In order to optimize chromatographic separation, response surface methodology was used. Obtained values for selectivity factors ($\alpha = k_2/k_1$) and resolution factors ($RS = 2(t_2 - t_1)/(\omega_1 + \omega_2)$) under defined chromatographic conditions are presented in Table 6. Both factors, selectivity and resolution, were significantly higher with use of mobile phase-1 (Figure 2, upper chromatogram), than with use of mobile phase-2 (Figure 2, lower chromatogram) for all examined compounds.

The most critical compounds for separation were tetrahydrozoline/naphazoline and naphazoline/tramazoline combinations, with lowest values of selectivity and resolution factors (Table 6). The selectivity factor, as the most specific parameter, was selected for evaluation of the optimal chromatographic conditions for effective separation of the tetrahydrozoline/naphazoline and naphazoline/tramazoline peaks.

The selectivity factor was calculated for the tetrahydrozoline/naphazoline and naphazoline/tramazoline peaks, at different pH (3.5–7.5) and concentration of acetonitrile (28–42%) of the mobile phase. The computed selectivity factors are depicted as a function of the pH and acetonitrile concentration of the mobile phase (Figure 3a for pair tetrahydrozoline/naphazoline and Figure 3b for pair naphazoline/tramazoline). From the obtained 3D surface diagram, it was concluded that the optimal separation, with the highest selectivity factors, is



(a) (b)

Figure 3. 3D diagrams for tetrahydrozoline/naphazoline (a) and for naphazoline/tramazoline (b).

achieved with use of mobile phase acetonitrile–triethylamine phosphate buffer (pH 4.0; 25 mM) (30:70, v/v) at 25°C.

CONCLUSION

Optimal chromatographic separation requires separation of all components of the sample in a reasonable time. Thus, the retention time that should be as low as possible with high peak resolution and selectivity factors. In this study, optimization of the simultaneous chromatographic separation of seven structurally similar alpha agonists was achieved using full factorial design. Selectivity and optimization of separation was achieved by controlling the influence of pH and amount of organic modifier on the effective separation of the seven 2-arylimidazoline derivatives.

The experiment performed under the optimised conditions: mobile phase acetonitrile–triethylamine phosphate buffer (pH 4.0; 25 mM) (30:70, v/v) at 25°C gives the chromatogram of (Figure 2 – upper), in which, within 24 min, all seven 2-arylimidazoline derivatives are well separated.

The suggested RP-HPLC method is applicable for routine analysis of the 2-arylimidazoline derivatives under the same chromatographic conditions, which can bring to shortening the time and the costs of the analysis as well.

ACKNOWLEDGMENT

This work was supported by the Ministry for Science, Technology, and Environmental Protection of Serbia, contract No. 142071.

REFERENCES

1. Griffith, R.K. *Burger's Medicinal Chemistry and Drug Discovery,* 6th, Ed., Vol. VI; Wiley: New York, 2003.
2. Lemke, T.L.; Williams, D.A. *Foy's Principles of Medicinal Chemistry,* 5th, Ed.; Lippincott Williams & Wilkins: USA, 2002.
3. Zivanov-Stakic, D.; Panic, Lj.; Agbaba, D. Farmaco. **1990**, *45*, 381–383.
4. Manzoori, J.L.; Amjadi, M. Indian J. Chem., Sect. A. **2003**, *42*, 2988–2992.
5. Souri, E.; Amanlou, M.; Farsam, H.; Afshari, A. Chem. Pharm. Bull. **2006**, *54*, 119–122.
6. Al-Momani, I.F. Orient. J. Chem. **2001**, *17*, 17–22.
7. Santoni, G.; Mura, P.; Pinzauti, S.; Gratteri, P.; La Porta, E. Int. J. Pharm. **1989**, *50*, 75–78.
8. Kelani, K.M. J. AOAC Int. **1998**, *81*, 1128–1134.
9. Joseph-Charles, J.; Bertucat, M. Anal. Lett. **1999**, *32*, 373–382.
10. Khalil, S. Mikrochim. Acta. **1999**, *30*, 181–184.
11. Marchesini, A.F.; Williner, M.R.; Mantovani, V.E.; Robles, J.C.; Goicoechea, H.C.J. Pharm. Biomed. Anal. **2003**, *31*, 39–46.
12. Su, S.M.; Yu, Y.Q. J. Chin. Pharm. Sci. **2005**, *14*, 173–175.
13. Agbaba, D.; Milojevic, Z.; Eric, S.; Aleksic, M.; Markovic, G.; Solujic, M. J. Planar Chromatogr. **2001**, *14*, 322–325.
14. Andermann, G.; Richard A. J. Chromatogr. **1984**, *298*, 189–192.
15. Sa'Sa', S.I.; Al-Momani, I.F.; Jalal, I.M. Anal. Lett. **1990**, *23*, 953–971.
16. Milojevic, Z.; Agbaba, D.; Eric, S.; Boberic-Borojevic, D.; Ristic, P.; Solujic, M. J. Chromatogr. A. **2002**, *949*, 79–82.
17. Rodriguez-Nogales, J.M.; Garcia, M.C.; Marina, M.L. J. Chromatogr. A. **2006**, *1104*, 91–99.
18. Wang, Y.; Harrison, M.; Clark, B.J. J. Chromatogr. A. **2006**, *1105*, 77–86.
19. Ferreiros, N.; Iriarte, G.; Alonso, R.M.; Jimenez, R.M. Talanta. **2006**, *69*, 747–756.
20. De Luca, M.; Ioele, G.; Risoli, A.; Ragno, G. Microchem. J. **2006**, *83*, 24–34.
21. Wang, Y.; Harrison, M.; Clark, B.J. J. Chromatogr. A. **2006**, *1105*, 199–207.
22. Cutroneo, P.; Beljean, M.; Phan Tan Luu, R.; Siouffic, A.-M. J. Pharm. Biomed. Anal. **2006**, *41*, 333–340.
23. Safa, F.; Hadjmohammadi, M.R. Anal. Chim. Acta. **2005**, *540*, 121–126.
24. Shan, Y.; Seidel-Morgenstern, A. J. Chromatogr. A. **2005**, *1093*, 47–58.
25. Novotna, K.; Havlis, J.; Havel, J. J. Chromatogr. A. **2005**, *1096*, 50–57.
26. De Aragao, N.M.; Veloso, M.C.C.; Bispo, M.S.; Ferreira, S.L.C.; De Andrade, J.B. Talanta. **2005**, *67*, 1007–1013.
27. Sena, M.M.; Chaudhry, Z.F.; Collins, C.H.; Poppi, R.J. J. Pharm. Biomed. Anal. **2004**, *36*, 743–749.
28. Li, W.; Nadig, D.; Rasmussen, H.T.; Patel, K.; Shah, T. J. Pharm. Biomed. Anal. **2005**, *37*, 493–498.
29. Zhang, Y.; Wu, H.L.; Ding, Y.J.; Xia, A.L.; Cui, H.; Yu, R.Q. J. Chromatogr. B. **2006**, *840*, 116–123.
30. XNUMBERS, Ver.4.7, March 2006, Multi Precision Floating Point Computing and Numerical Methods for EXCEL/2000 XP.

31. El-Gindy, A.; Emara, S.; Mostafa, A. J. Pharm. Biomed. Anal. **2006**, *41*, 421–430.
32. Gennaro, M.C.; Marengo, E.; Gianotti, V.; Angioi, S. J. Chromatogr. A. **2002**, *945*, 287–292.
33. Ficarra, R.; Calabro, M.L.; Cutroneo, P.; Tommasini, S.; Melardi, S.; Semreen, M.; Furlanetto, S.; Ficarra, P.; Altavilla, G. J. Pharm. Biomed. Anal. **2002**, *29*, 1097–1103.
34. Wsol, V.; Fell, A. J. Biochem. Biophys. Meth. **2002**, *54*, 377–390.
35. Gonzalez-Barreiro, C.; Lores, M.; Casais, M.C.; Cela, R. J. Chromatogr. A. **2000**, *896*, 373–379.
36. Marengo, E.; Gianotti, V.; Angioi, S.; Gennaro, M.C. J. Chromatogr. A. **2004**, *1029*, 57–65.

Received April 14, 2008
Accepted October 22, 2008
Manuscript 6322