Asian Journal of Chemistry Vol. 20, No. 3 (2008), 2105-2114

Simultaneous Kinetic-Spectrophotometric Determination of Levodopa and Carbidopa

M.A. KARIMI*†, M. MAZLOUM ARDAKANI‡ and H. AMIRYAN
Department of Chemistry, Payame Noor University of Ardakan, Ardakan, Iran
Fax: (98)(351)8253221
E-mail: m_karimi@pnu.ac.ir; ma_karimi43@yahoo.com; ma.karimi43@gmail.com

Simultaneous kinetic-spectrophotometric determination of binary mixture of levodopa and carbidopa by principal component regression (PCR) and partial least squares (PLS) calibration is described. The methods were based on the difference in the reduction rate of Fe(III) with levodopa and carbidopa in the presence of 2,2'-bipyridine (Bpy). The coloured complex of $[Fe(Bpy)_3]^{2+}$ resulted can be monitored at 520 nm. The results show that using PCR and PLS, levodopa and carbidopa can be determined simultaneously in the concentration ranges of 0.04-6.0 and 0.05-5.0 μg mL^{-1}, respectively. The root mean squares errors of prediction (RMSEP) of levodopa and carbidopa were 0.0161 and 0.0191 (for PLS), 0.0632 and 0.1313 (for PCR), respectively. Both the methods (PCR and PLS) were validated using a set of synthetic sample mixtures for simultaneous determination of levodopa and carbidopa in pharmaceutical preparation. The recoveries were satisfactory and statistically comparable to those obtained by the reference method of high performance liquid chromatography.

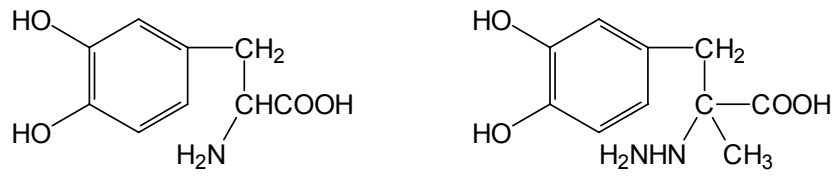
Key Words: Simultaneous determination, Levodopa, Carbidopa, Principal component regression, Partial least squares.

INTRODUCTION

Levodopa [3-(3,4-dihydroxyphenyl)-L-alanine], as an important neurotransmitter has been used for the treatment of neural disorders such as Parkinson's. In order to enhance its pharmacological effect, levodopa is generally associated with a peripherical aromatic decarboxylaze inhibitor such as carbidopa, [(S)-3-(3,4-dihydroxyphenyl)-2-hydrazino-2-methyl-propionic acid]. This combination is also used to treat tumors, spasms and poor muscle control caused by CO and manganese intoxication, as well as

†Department of Chemistry, Shahid Bahonar University of Kerman, Kerman, Iran.
‡Department of Chemistry, Yazd University, Yazd, Iran.

in opthalmology (amblicopia and strabismus)[1-4]. By administering levodopa combined with carbidopa, the concentration of dopamine is controlled at appropriate level effectively with generally reduced side effects[5]. In order to achieve better curative effect and lower toxicity, it is important to control the content of levodopa and carbidopa in pharmaceutical tablets. In recent years, considerable work has been done on their detection and quantification. Various methods such as, spectrofluorimetry[6], gas chromatography[7], high performance liquid chromatography[8], radio immunoassay[9], chemiluminescence[10] and voltammetric determination[11] have been reported in the literature on the determination of these compounds in various biological samples and pharmaceutical preparations. Many spectrophotometric methods have also been proposed. Some of them require long heating[12] or involve nonaqueous media[13]. Some other spectrophotometric methods have very narrow limits of detection[14]. Their simultaneous determination in pharmaceutical preparations and biological fluids has been traditionally achieved by HPLC[15-19]. In recent years, capillary electrophoresis (CE) has been alternatively used and several separation methodologies have been developed in order to resolve both compounds and their enantiomers[20,21]. As part of a program devoted to the development of chemometrics-assisted analytical methods for simple and rapid pharmaceutical quality control[22,23], we are currently exploring the use of kinetic-spectrophotometric data, as a valid alternative for the determination of mixtures of chemical compounds with similar structures and/or spectroscopic properties[24-26]. These methodologies allow generation of multivariate signals for which appropriate algorithms can be successfully applied, particularly principal component regression (PCR) and partial least squares (PLS) calibration, which have been shown to be reliably applied in other instances[27,28]. In this paper, we use PCR and PLS methods to determine simultaneously levodopa and carbidopa (Fig. 1) in pharmaceutical preparations based on their reduction rate difference with iron(III) in the presence of 2,2'-bipyridine (Bpy). This is done by monitoring the coloured complex between Fe(II) and Bpy at 520 nm.



Levodopa Carbidopa

Fig. 1. Structural formula of levodopa and carbidopa

EXPERIMENTAL

A GBC UV-Visible Cintra 6 Spectrophotometer model, with 1 cm glass cells was used for recording the kinetic spectrophotometric data. A Metrohm 780 pH meter furnished with a combined glass-saturated calomel electrode was calibrated with at least two buffer solutions at pH 3 and 9. The data were treated in an AMD 2000 XP (256 Mb RAM) microcomputer using MATLAB software. PLS and PCR analysis were performed using PLS and PCR toolboxes in MATLAB program. Version 7.0.

All chemicals were of analytical reagent grade and double distilled water was used throughout. Stock solutions (1000 μg mL^{-1}) of levodopa and carbidopa were prepared in 100 mL volumetric flasks by dissolving 0.1 g levodopa (ICN Biochemicals USA) and 0.108 g carbidopa (ICN Biochemicals USA) in water and diluting with water to the mark. Stock solution of 0.05 M Fe(III) was prepared in a 100 mL flask by dissolving 2.430 g ammonium ferric sulfate in water and diluting to the mark. Stock solution of 0.05 M 2,2'-bipyridine (Bpy) was prepared by dissolving 0.784 g of Bpy (Merck) in ethanol and diluting to 100 mL volumetric flask with water. Acetate buffer solution (1.0 M, pH 5.0) was prepared using acetic acid and NaOH solutions and adjusting its pH with a pH-meter.

Procedure and sample preparation: $[Fe(Bpy)_3]^{3+}$ complex solution as oxidizing agent, for both methods (PLS and PCR) has been prepared daily in a 100 mL volumetric flask by the addition of 2.5 mL of buffer solution (pH 5.0), 7.0 mL of Fe(III) solution (0.05 M) and 8.0 mL of Bpy solution (0.05 M) diluting with water to the mark. After thermostating this solution at 25 °C for 10 min, 2.4 mL of the solution was transferred into a glass cell of the spectrophotometer and the absorbance of this solution was zeroed before injecting the analyte(s). Then 100 μL of solution containing levodopa, carbidopa or mixture of both in the range of interest was injected by a microsyringe into the cell. The absorbance changes *vs.* time were recorded at 520 nm at the time intervals of 2 s. Simultaneous determination of levodopa and carbidopa using PCR and PLS methods was performed in their concentration ranges by recording the absorbance spectra for each solution from 0 to 200 s.

RESULTS AND DISCUSSION

The Fe(III)-2,2'-bipyridine (Bpy) system allows the spectrophotometric determination of a reducing agent, A_{red}, as follows[29]:

$$n[Fe(Bpy)_3]^{3+} + A_{red} \longrightarrow n[Fe(Bpy)_3]^{2+} + A_{ox}$$

The reaction is complete with the formation of an equivalent amount of $[Fe(Bpy)_3]^{2+}$ with respect to the *n*-electron reductant, A_{red}. The reduction of $[Fe(Bpy)_3]^{3+}$ to complex of $[Fe(Bpy)_3]^{2+}$ (with λ_{max} = 520 nm) is completed in the presence of suitable reducing agents such as levodopa and

carbidopa in few minutes. The reduction rate of $[Fe(Bpy)_3]^{3+}$ with levodopa and carbidopa is different. The difference provides the possibility of resolving their mixtures using PLS and PCR methods. Characteristics of calibration graphs of levodopa and carbidopa are given in Table-1.

TABLE-1
CHARACTERISTIC OF CALIBRATION GRAPHS FOR THE DETERMINATION OF LEVODOPA AND CARBIDOPA

Analyte	Slope (mL μg^{-1})	Intercept (n = 10)	Correlation coefficient ($\mu g\ mL^{-1}$)	Range ($\mu g\ mL^{-1}$)	Detection limit
Levodopa	0.25	0.0187	0.9993	0.04-6.00	0.04
Carbidopa	0.30	0.0434	0.9994	0.05-5.00	0.00

A series of experiments were conducted to establish the optimum analytical to achieve maximum sensitivity in the simultaneous determination of levodopa and carbidopa. The experimental parameters, such as reagents concentration, temperature and pH of solutions were optimized. Optimization process gave similar results for both individual analytes and mixture of three analytes.

Effect of Fe(III) and Bpy concentrations: The effect of Fe(III) and Bpy concentrations, in the ranges of 1.0×10^{-4}– 1.0×10^{-3} M and 5.0×10^{-4}–5.0×10^{-3} M were studied, respectively. At a constant concentration of Bpy equal to 1.5×10^{-3} M, Fe(III) concentration was varied in its range. With an increase in Fe(III) concentration, the reaction rate and absorbance increase up to 3.5×10^{-4} M for both levodopa and carbidopa, but at the higher concentrations of Fe(III), a decrease in reaction rate and amount of absorbance was observed. So, a concentration of 3.5×10^{-4} M Fe(III) was selected as the optimum concentration. The effect of Bpy concentration on the reaction rate and absorbance of levodopa and carbidopa at constant concentration of Fe(III) (3.5×10^{-4} M) was also studied. The increase of Bpy concentration up to 1.2×10^{-3} M, causes an increase in the reaction rate and absorbance of levodopa and carbidopa, but at higher concentrations of Bpy, a decrease in reaction rate and amount of absorbance was observed. Thus, for simultaneous determination of levodopa and carbidopa by both PCR and PLS methods, it was preferred to choose 1.2×10^{-3} M Bpy as the optimum concentration for further studies.

Effect of surfactants: The effect of three kinds of surfactants (anionic, cationic and nonionic) and their concentrations on the reaction rates of levodopa and carbidopa with $[Fe(Bpy)_3]^{3+}$ were investigated. The results showed that each three kinds of surfactants of sodium dodecyl sulfate (SDS), cetyltrimethyl ammonium bromide (CTAB) and Triton X-100 (TX-100)

causes a few decrease on the reaction rate of both species (levodopa and carbidopa). Therefore, any surfactant was not used for further studies in this work.

Effect of pH: The effect of pH over the ranges of 1.0 to 7.0 on the reaction rate of two compounds with Fe(III) in the presence of Bpy was studied. For both of levodopa and carbidopa, pH 5.0 has maximum absorbance, but at above pH 5.0, the absorbance and reaction rate decrease. Thus, pH 5.0 was chosen as an optimized pH value. It was found that concentration decrease of buffer (< 1.0 M) in the same pH, causes to decrease absorbance for levodopa and carbidopa.

Effect of temperature: The effect of temperature on the absorbance of levodopa and carbidopa with Fe(III) in the presence of Bpy was studied in the range of 20 to 70 °C. An increase in the temperature caused an increase in the reaction rates of both two analytes. However, for the sake of simplicity and better control of the temperature effects on the precision of determinations, 25 °C was chosen as the optimum temperature.

Absorbance-time behaviour: Under the optimized conditions, the reactions of levodopa and carbidopa with Fe(III)-Bpy system showed the different kinetic behaviours (Fig. 2). This difference in reaction rates allows designing multivariate methods to determine simultaneously levodopa and carbidopa. The linearity of the analytes was studied individually under the optimized conditions (Table-1).

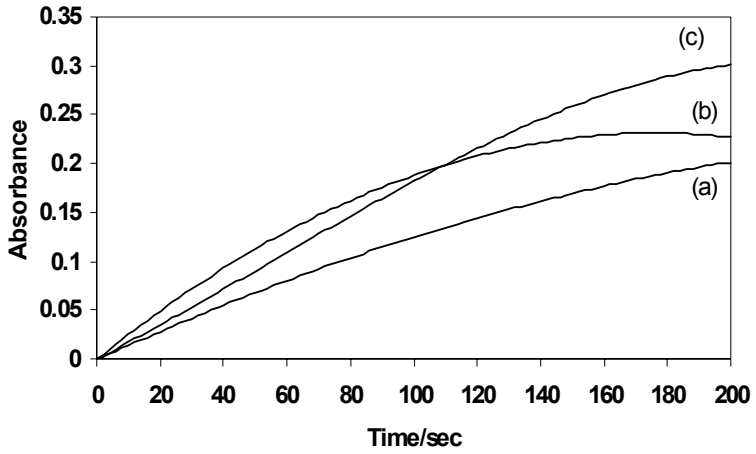


Fig. 2. Absorbance changes of Fe(III)/Bpy complex *vs.* time in the reaction with: 2.0 μg mL^{-1} of levodopa (a), 2.0 μg mL^{-1} of carbidopa (b) and mixture of them (c). Conditions: 3.5×10^{-4} M Fe(III), 1.2×10^{-3} M Bpy, pH 5.0, 25 °C

Multivariate calibration: Multivariate calibration methods such as PLS and PCR require a suitable experimental design of the standard belonging to the calibration set in order to provide good prediction. In this research, a synthetic set of 30 solutions containing different concentrations of levodopa and carbidopa were prepared. The concentration ranges were 0.04-6.0, 0.05-5.00 μg mL^{-1} for levodopa and carbidopa, respectively. A collection of 20 solutions (Table-2) were selected as the calibration set and the other 10 were used as prediction set (Table-3). Their composition was randomly designed to obtain more information from the calibration procedure. Changes in the absorbance of the solution were recorded during a time period of 200 s.

TABLE-2
CALIBRATION SET FOR CONSTRUCTING PLS AND PCR METHOD IN DETERMINATION OF LEVODOPA AND CARBIDOPA (μg mL^{-1})

Solutions	Carbidopa	Levodopa	Solutions	Carbidopa	Levodopa
1	0.1	0.3	11	1.0	0.3
2	0.1	0.6	12	1.0	0.6
3	0.1	0.9	13	1.0	0.9
4	0.1	1.2	14	1.0	1.2
5	0.1	1.5	15	1.0	1.5
6	0.5	0.3	16	2.0	0.3
7	0.5	0.6	17	2.0	0.6
8	0.5	0.9	18	2.0	0.9
9	0.5	1.2	19	2.0	1.2
10	0.5	1.5	20	2.0	1.5

TABLE-3
PREDICTION SET FOR CONSTRUCTING PLS AND PCR METHODS IN DETERMINATION OF LEVODOPA AND CARBIDOPA (μg mL^{-1})

Solution	Amount added (μg mL^{-1})		Predicted (μg mL^{-1})			
			PCR		PLS	
	Carbidopa	Levodopa	Carbidopa	Levodopa	Carbidopa	Levodopa
1	0.90	2.35	0.735	2.701	0.911	2.325
2	1.00	0.50	1.015	0.447	1.000	0.475
3	1.25	0.50	1.267	0.486	1.243	0.526
4	0.30	1.30	0.248	1.406	0.297	1.308
5	1.15	0.95	1.127	0.971	1.139	0.909
6	0.95	0.70	0.951	0.680	0.952	0.679
7	1.10	0.45	1.187	0.370	1.088	0.479
8	0.90	0.80	0.914	0.834	0.934	0.789
9	2.00	1.55	1.971	1.547	1.970	1.564
10	1.20	0.85	1.195	0.863	1.200	0.842

To select the number of factors in the PLS and PCR algorithm a cross-validation, leaving out one sample methods was employed[30]. The prediction error was calculated for each species for the prediction set. This error was expressed as the prediction residual error sum of squares (PRESS):

$$PRESS = \Sigma_{i=1}^{m}(\hat{C}_i - C_i)^2 \quad (1)$$

where m is the total number of calibration sample, $\hat{C}_i$ represents the estimated concentration and $\hat{C}_i$ is the reference concentration for the ith sample left out of the calibration during cross validation. Fig. 3 shows a plot of PRESS against the number of factors for mixture of components. To find minimum factors, the F-statistic was also used to carry out the significant determination[30]. The optimal number of factors for two components were obtained as 3 for both PLS and PCR methods.

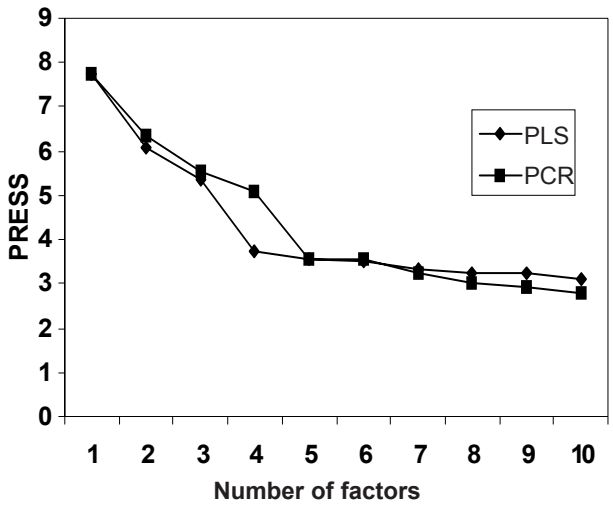


Fig. 3. Plot of PRESS against the number of factors for mixture of levodopa and carbidopa

Statistical parameters: For the evaluation of the predictive ability of a multivariate calibration model, the root mean square error of prediction (RMSEP) and relative standard error of prediction (RSEP) can be used[31-33]:

$$RMSEP = (\Sigma_{i=1}^{N}(\hat{C}_i - C_i)^2 / n)^{1/2} \quad (2)$$

$$RSE\ (\%) = (\Sigma_{i=1}^{N}(\hat{C}_i - C_i)^2 / \Sigma_{i=1}^{N}(C_i)^2 \times 100 \quad (3)$$

where $\hat{C}_i$ represents the estimated concentration, C_i and n are the actual analyte concentration and the number of samples, respectively.

The squares of correlation coefficient (R^2), which is an indication of the quality fit of all the data to a straight line is presented[33] by eqn. 4:

$$R^2 = \Sigma_{i=1}^{N}(\hat{C}_i - C')^2 / \Sigma_{j=1}^{N}(C_i - C')^2 \quad (4)$$

For each component, RSE, RMSEP and R^2 values of PLS and PCR methods have been summarized in Table-4. As shown in this table, results of PLS are in better statistical behaviour than PCR.

TABLE-4
STATISTICAL PARAMETERS CALCULATED FOR THE PREDICTION SET USING PLS AND PCR METHODS

Component	RSE (%)		RMSEP		R^2	
	PCR	PLS	PCR	PLS	PCR	PLS
Levodopa	2.54	1.42	0.0632	0.0161	0.9786	0.9988
Carbidopa	1.21	1.69	0.1313	0.0191	0.9916	0.9989

Interference studies: In order to assess the possible analytical applications of the proposed methods, the effects of common excipients used in pharmaceutical preparations were studied. Potential changes of a solution containing levodopa (0.1 μg mL^{-1}) and carbidopa (0.1 μg mL^{-1}) were analyzed for four times and then the effect of interfering species at different concentrations on the potential of this solution were studied. A species was considered as interference when its presence produced a variation in the concentration of the levodopa and carbidopa mixture more than 5 % relative error. The following excipients did not interfere in the maximum tested concentrations (μg mL^{-1}) shown in parentheses: starch (20), galactose (20), glucose (20), sucrose (20), riboflavin (10). Some other compounds with significant redox properties (*e.g.*, ascorbic acid) may interfere in the proposed procedure. But our goal was the determination of levodopa and carbidopa in tablets. In these tablets the other redox compounds are absent.

Application: The proposed methods were applied to determine simultaneously levodopa and carbidopa in several commercially available pharmaceutical formulations. 20 Tablets of each sample were accurately weighed and their solutions were prepared by dissolving them in water and filtering the solutions. The prepared solutions containing aliquot amounts of levodopa and carbidopa were analyzed (n = 4). The accuracy of proposed methods was determined by analyzing the recoveries of known amounts of analytes into samples and comparing test results from the proposed methods (PCR and PLS) with those obtained applying the reference method proposed in the US Pharmacopeia based on HPLC[34]. The quantitative results of this analysis were summarized in Table-5. Good agreement between results obtained and the nominal values labeled and reference method indicates that PCR and PLS can be applied successfully for simultaneous determination of levodopa and carbidopa in pharmaceutical samples. Although

commercial samples always contain excipients, the good recoveries confirmed that they do not seriously interfere under the present conditions. The comparison of the PLS and PCR results with those provided by HPLC using standard t-test statistics revealed no significant differences between the chemometrics-assisted methodologies and the reference technique (Table-5).

TABLE-5
PREDICTION RESULTS ON COMMERICAL SAMPLES WITH PLS, PCR AND COMPARISON WITH HPLC[34]

Commercial sample (nominal content)	Levodopa[c]				
	PCR mg (s) R %[d]	PLS mg (s) R %[d]	HPLC mg (s) R %[d]	t[e]	t[f]
Sample[a]	106.7(3.2)106.7	105.3(1.1)105.3	104.3(2.6)104.3	3.6	0.7
Sample[b]	260.4(2.0)104.2	259.6(2.3)103.8	262.8(1.8)105.1	1.2	2.2
	Carbidopa[c]				
Sample[a]	11.6(0.6)116.0	9.80(0.6)98	10.3(2.4)103.0	1.1	0.4
Sample[b]	23.4(0.7)93.6	25.6(1.0)102.4	25.5(2.1)102.0	1.9	0.1

[a](Levodopa, 100 mg; Carbidopa, 10 mg per tablet); Ramofarmin Co. Iran
[b](Levodopa, 250 mg; Carbidopa, 25 mg per tablet); Alborz Daruo Co. Iran
[c]The results are averages of four replicates and are given in mg per sample. Standard deviation (s) is in parenthesis.
[d]R% is the recovery percent calculated from the contents declared by the manufacturing laboratories.
[e]Calculated values of the statistical t coefficient for the PCR method comparison with HPLC. The critical value at 95 % confidence level and 6 d.f. is t (ν = 6, 95 %) = 2.45.
[f]Calculated values of the statistical t coefficient for the PLS method comparison with HPLC. The critical value at 95 % confidence level and 6 d.f. is t (ν = 6, 95 %) = 2.45.

Conclusion

In this work, it is shown that levodopa and carbidopa can be determined simultaneously in pharmaceutical formulations using kinetic spectrophotometric PCR and PLS methods without sample pretreatment. Both methods of PCR and PLS adopted well for simultaneous determination of two compounds, but PLS is rather than PCR method. Apparatus, reagents and software are accessible to most routine laboratories. Reproducibility and accuracy tests are successful and the recovery results are statistically comparable to those obtained by the reference Pharmacopeia method based on HPLC. Both methods are cheaper than chromatographic separation methods, furthermore, in these methods, we don't need to use complex pretreatment or toxic organic solvents. In other words, they belong to green chemistry.

ACKNOWLEDGEMENT

The authors wish to thank Payame Noor University of Ardakan for support of this work.

REFERENCES

1. W. Maruyama, M. Naoi and H. Narabashi, *J. Neurol. Sci.*, **139**, 141 (1996).
2. P. Benetello, M. Furlanut, M. Fortunato, F. Pea and M. Baraldo, *Pharmacol. Res.*, **35**, 313 (1997).
3. L.N. Johnson, M.E. Guy, G.B. Krohel and R.W. Madsen, *Ophthalmology*, **107**, 521 (2000).
4. P. Bhartiya, P. Sharma, N.R. Biswas, R. Tandom and S. Khokhar, *J. Pediat. Ophth. Strab.*, **6**, 368 (2002).
5. E.G.C. Clarke and A.C. Moffat, Clarke's Isolation and Identification of Drugs in Pharmaceuticals, Body Fluids and Post-mortem Material, Pharmaceutical Press, London, edn. 2 (1986).
6. K. Imai, *J. Chromatogr.*, **105**, 135 (1975).
7. C. Sharma, S. Mohanty, S. Kumar and N.G. Rao, *Analyst*, **121**, 1963 (1996).
8. L.H. Parsons, T.M. Kerr and F. Weiss, *J. Chromatogr. B*, **709**, 35 (1998).
9. L.J. Riceberg, H.V. Vaunakis and L. Levine, *Anal. Biochem.*, **60**, 551 (1974).
10. O. Nozaki, T. Iwaeda and Y. Kato, *J. Biolumin. Chemilumin.*, **11**, 309 (1996).
11. K.D. Kozminski, D.A. Gutman, V. Davila, D. Sulzer and A.G. Ewing, *Anal. Chem.*, **70**, 3123 (1998).
12. T. Aman, I.U. Khan, N. Aslam and I. Ahmed, *Anal. Lett.*, **31**, 1007 (1998).
13. I.A. Biryuk, V.V. Petrenko and B.P. Zorya, *Farm. Zh. (Kiw.)*, **2**, 57 (1992).
14. W.I. Mohamed and F.B. Salem, *Anal. Lett.*, **17**, 191 (1984).
15. K.A. Sagar and M.R. Smyth, *J. Pharm. Biomed. Anal.*, **22**, 613 (2000).
16. S. Ting, *J. Assoc. Off. Anal. Chem.*, **69**, 169 (1986).
17. E. Nissinen and J. Taskinen, *J. Chromatogr. B*, **231**, 459 (1982).
18. A. Tolokan, I. Klebovich, K. Balogh Nemes and G. Horvai, *J. Chromatogr. B*, **698**, 201 (1997).
19. R.S. Husain, R. Sekar and R. Nageswararao, *J. Chromatogr. A*, **687**, 351 (1994).
20. S. Fanali, V. Pucci, C. Sabbioni and M.A. Raggi, *Electrophoresis*, **21**, 2432 (2000).
21. L. Zhang, G. Chen, H. Oin and F. Yuzhi, *Anal. Chim. Acta*, **431**, 287 (2001).
22. A. Nepote, P. Damiani and A. Olivieri, *J. Pharm. Biomed. Anal.*, **31**, 621 (2003).
23. A.J. Nepote, L. Vera-Candiotti, M.R. Williner, P.C. Damiani and A.C. Olivieri, *Anal. Chim. Acta*, **489**, 77 (2003).
24. M.A. Karimi, M.M. Ardakani, R.B. Ardakani, O. Moradlou and F. Banifatemeh, *J. Chin. Chem. Soc.*, **54**, 15 (2007).
25. M.A. Karimi, R.B. Ardakani and F. Banifatemeh, *Asian J. Chem.*, **19**, 1971 (2007).
26. M.A. Karimi, M.R. Nateghi, M. Malekzadeh, F. Banifatemeh and O. Moradlou, *Asian J. Chem.*, **19**, 4533 (2007).
27. B.K. Lavine, *Anal. Chem.*, **70**, 209R (1998).
28. D. Özdemir and B. Öztürk, *Turk. J. Chem.*, **28**, 497 (2004).
29. A.A. Syed, A. Syeda and H.R.K. Mahesh, *IL Farmaco*, **60**, 47 (2005).
30. D.M. Haaland and E.V. Thomas, *Anal. Chem.*, **60**, 1193 (1988).
31. J. Ghasemi and M. Vosough, *Spectro. Lett.*, **35**, 153 (2002).
32. M. Oue and W. Wegscheider, *Anal. Chem.*, **57**, 63 (1985).
33. J. Ghasemi, A. Nikrahi and A. Niazi, *Turk. J. Chem.*, **29**, 669 (2005).
34. M.D. Rockville, United State Pharmacopeia XXIV, United State Pharmacopeia Convention, Suppl. NF., p. 19 (2000).

(*Received*: 30 April 2007; *Accepted*: 19 November 2007) AJC-6083