Int. J. Chem. Sci.: 7(3), 2009, 1642-1652

VIS-SPECTROPHOTOMETRIC METHODS FOR THE DETERMINATION OF NATEGLINIDE

A. PRAMEELA RANI*, C. BALA SEKARAN[a], N. ARCHANA, P. SIVA TEJA and B. ARUNA

Department of Pharmaceutics, K. V. S. R. Siddhartha College of Pharmaceutical Sciences, VIJAYAWADA – 520 010 (A. P.) INDIA
[a]Department of Biotechnology, J. K. C. College, GUNTUR – 522 006 (A. P.) INDIA

ABSTRACT

Two spectrophotometric methods for the determination of nateglinide (NTG) in either pure form or in its pharmaceutical formulations are described. The first method is based on the reaction of 3-methylbenzothiazolin-2-one hydrazone (MBTH) with nateglinide in the presence of ferric chloride in acidic medium. The resulting blue complex absorbs at λmax 630 nm. The second method describes the charge transfer reaction between the drug and ρ-chloranilic acid (ρ-CA) to yield a purple colored product with λmax at 480 nm. The reaction conditions were optimized to obtain maximum color intensity. The absorbance was found to increase linearly with increasing the concentration of NTG; the systems obeyed the Beer's law in the range 2–10 and 5–25 μg mL^{-1} for MBTH and ρ-CA methods, respectively. LOD and LOQ values for MBTH and ρ-CA methods were 0.91, 2.7 and 2.6, 7.9 μg mL^{-1}, respectively. The correlation coefficient values were found to be 0.9999 for both the methods. No interference was observed from common excipients present in pharmaceutical formulations. The proposed methods are simple, sensitive, accurate and suitable for quality control applications.

Key words : Nateglinide, Charge transfer, Spectrophotometry, Pharmaceutical formulation.

INTRODUCTION

Nateglinide [N-(trans-4-isopropylcyclohexylcarbonyl)-D-phenylalanine] is a novel, highly physiologic, mealtime glucose regulator recently approved for the treatment of type II diabetes Mellitus. Nateglinide has a rapid onset and short duration of insulinotropic[1] action that results in reduction of mealtime glucose rise and lowers the post absorptive potential for hypoglycemia in humans and experimental animals[2, 3]. Nateglinide lowers blood glucose by stimulating the release of insulin from the pancreas. It achieves this by closing ATP-dependent potassium channels in the membrane of the β cells[4, 5]. This

* Author for correspondence; E-mail: balumphil@gmail.com, Call: 91-866-2538492

depolarizes the β cells and causes voltage-gated calcium channels to open. The resulting calcium influx induces fusion of insulin-containing vesicles with the cell membrane and insulin secretion occurs. Literature survey revealed that few analytical methods have been reported for the estimation of PDE; they include, UV spectrophotometric[6], Vis-spectrophotometric[7, 8], HPLC[9], LC–MS[10] and micellar electro kinetic chromatography method[11].

In the present work, two simple, sensitive, economical and accurate spectrophotometric methods have been developed for the quantitative estimation of nateglinide in bulk and pharmaceutical formulations. Both the reagents, MBTH and ρ-CA, are used for the first time in NTG analysis.

EXPERIMENTAL

Apparatus

An ELICO Model SL-159 double beam, UV-VIS spectrophotometer (Elico India Ltd., India) with 1.0 cm matched quartz cells was used for absorbance measurements.

Reagents and materials

Nateglinide (gift sample from local pharmaceutical industry), 3-methylbenzothia-zolin-2-one hydrazone (MBTH) (Himedia Laboratories Pvt. Ltd., India, certified to be 99.0%) and ρ-chloranilic acid (ρ-CA) (S. d. Fine Chem., India, certified to be 98.5%) were used. All other chemicals and solvents used were of analytical reagent grade.

The following dosage forms containing nateglinide were purchased from local commercial sources : Glinate and natelide equivalent to 60 mg nateglinide.

Standard solutions

Accurately weighed 100 mg of nateglinide was dissolved in 100 mL of methanol (MBTH method) and 100 mL of acetonitrile (ρ -CA method) to give a concentration of 1 mg/mL. The final concentration was brought to 100 μg mL^{-1} for MBTH and ρ -CA methods with the same solvents used for stock preparation.

Aqueous solutions (0.2%) of MBTH and ρ -chloranilic acid (0.3%) in acetonitrile were freshly prepared.

Analytical procedures

Method A (MBTH) : Aliquots of 0.5-2.5 mL nateglinide solution were transferred into 10 mL calibrated flasks. To each 0.7% $FeCl_3$ in 0.5% HCl (1.0 mL), an aqueous solution of MBTH (1.5 mL) was added. The solutions were swirled and allowed to stand for 5 min. The flasks were made up to the mark with water. The absorbance was measured at 630 nm against the corresponding reagent blank and calibration graph was constructed.

Method B (ρ-CA) : Aliquots of 0.2-1.0 mL of nateglinide solution were transferred into a series of 10 mL volumetric flasks followed by 2 mL of 0.3% ρ-CA. The mixture was shaken, diluted to volume with acetonitrile and the absorbance was measured at 480 nm against an appropriate blank prepared simultaneously and calibration graph was constructed.

Validation

The linearity, slope and the intercepts were calculated using the regression equation. Precision and accuracy of the proposed methods were tested by carrying out the determination of five replicates of pure and commercial samples of the drug, whose concentration was within Beer's law range. Values of the standard deviation (SD), relative standard deviation (RSD) and range of error at 95% confidence level were calculated[12]. These two methods have been applied to various pharmaceutical formulations and recovery studies have been made by the standard-addition method.

Intra-day precision and intra-day error of the methods were assessed from the results of five replicate analyses on the pure drug solution. The mean values and relative standard deviation values for replicate analysis at three different concentration levels were calculated.

Accuracy of the methods was determined by recovery studies via the standard-addition method.

The limit of detection (LOD) and limit of quantification (LOQ) were calculated according to the current ICH guidelines[13] as the ratio of 3.3 and 10 standard deviation, respectively and the slope of the calibration line.

Assay of nateglinide pharmaceutical formulations

Twenty tablets were powdered and mixed thoroughly. An amount equivalent to 100 mg of the nateglinide was dissolved in methanol (MBTH method) or acetonitrile (ρ-

CA method) and filtered. The filtrate was made up to 100 mL with the same solvents. Aliquots of these solutions were analyzed as previously mentioned using MBTH and ρ-CA. The nominal content of the tablets was calculated either from a previously plotted calibration graph or using the regression equation.

RESULTS AND DISCUSSION

These methods are based on (i) the oxidative coupling reaction of the drug with 3-methylbenzothiazolin-2-one hydrazone (MBTH) in the presence of ferric chloride in acidic medium and (ii) the charge transfer reaction between the drug and ρ-chloranilic acid (ρ-CA) in acetonitrile medium.

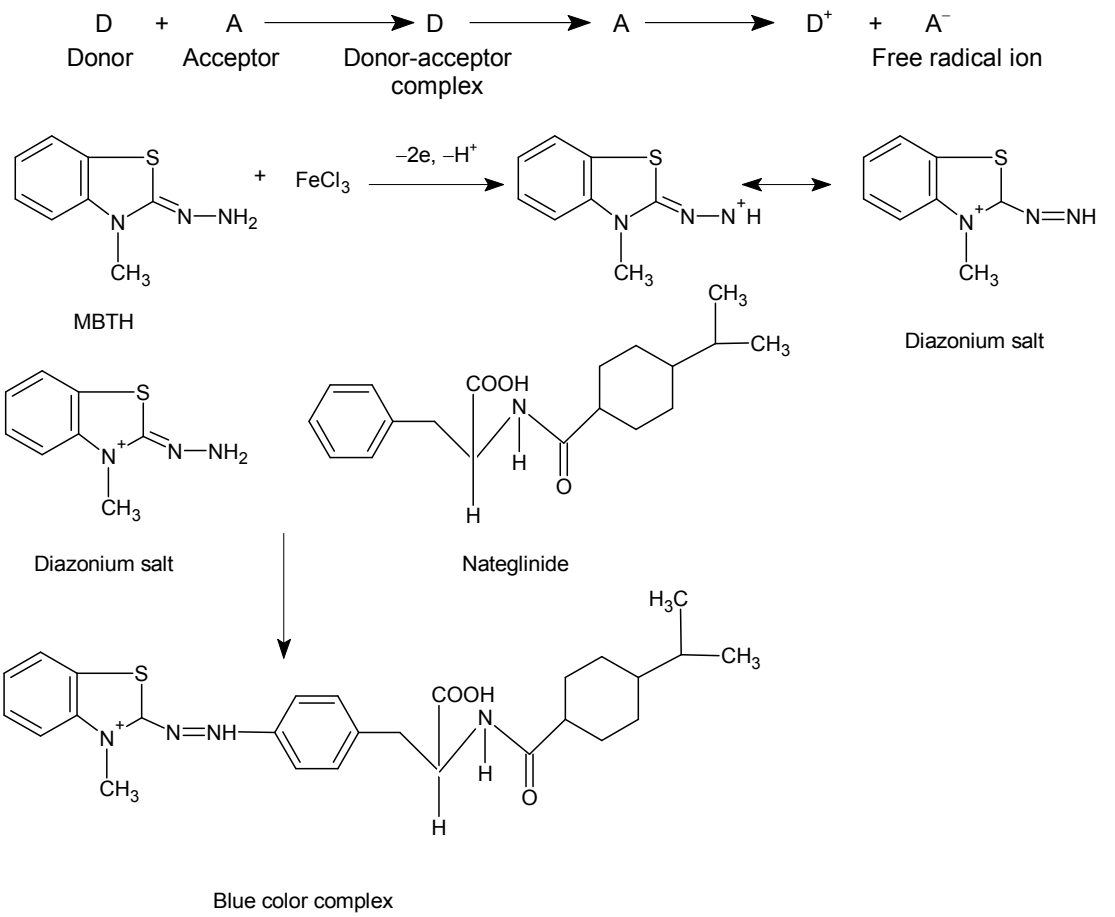
Reaction products

The bluish colored product (NTG-MBTH) and purple colored product (NTG - ρ-CA) shows absorbance maxima at 630 nm and 480 nm, respectively. The above mentioned blanks have practically negligible absorption in both systems.

In the MBTH method, the drug reacts with MBTH in the presence of $FeCl_3$ in acidic medium to give a blue colored product. Actually, this is an iron catalyzed oxidative coupling reaction of MBTH with the drug. Under the reaction conditions, on oxidation, MBTH loses two electrons and one proton forming an electrophilic intermediate, which is the active coupling species. This intermediate undergoes electrophilic substitution with the drug to form the colored product.

The charge-transfer (CT) reaction has been widely studied. Many drugs are easy to be determined by spectrophotometry based on color CT complexes formed with electron acceptors[14, 15]. ρ- CA is a π electron acceptor as a result of the strong electron withdrawing halo- and cyano- groups conjugated with the π -system[16,17]. The nateglinide has secondary amino group, which acts as n-electron donor. Therefore, the nateglinide react with electron acceptor to form CT complex or radical anions. Interaction of nateglinide with ρ-CA in acetonitrile instantaneously yields an intense purple color, absorbing at a maximum of 480 nm. The new absorption band was formed as the result of the formation of CT complexes [16, 17].

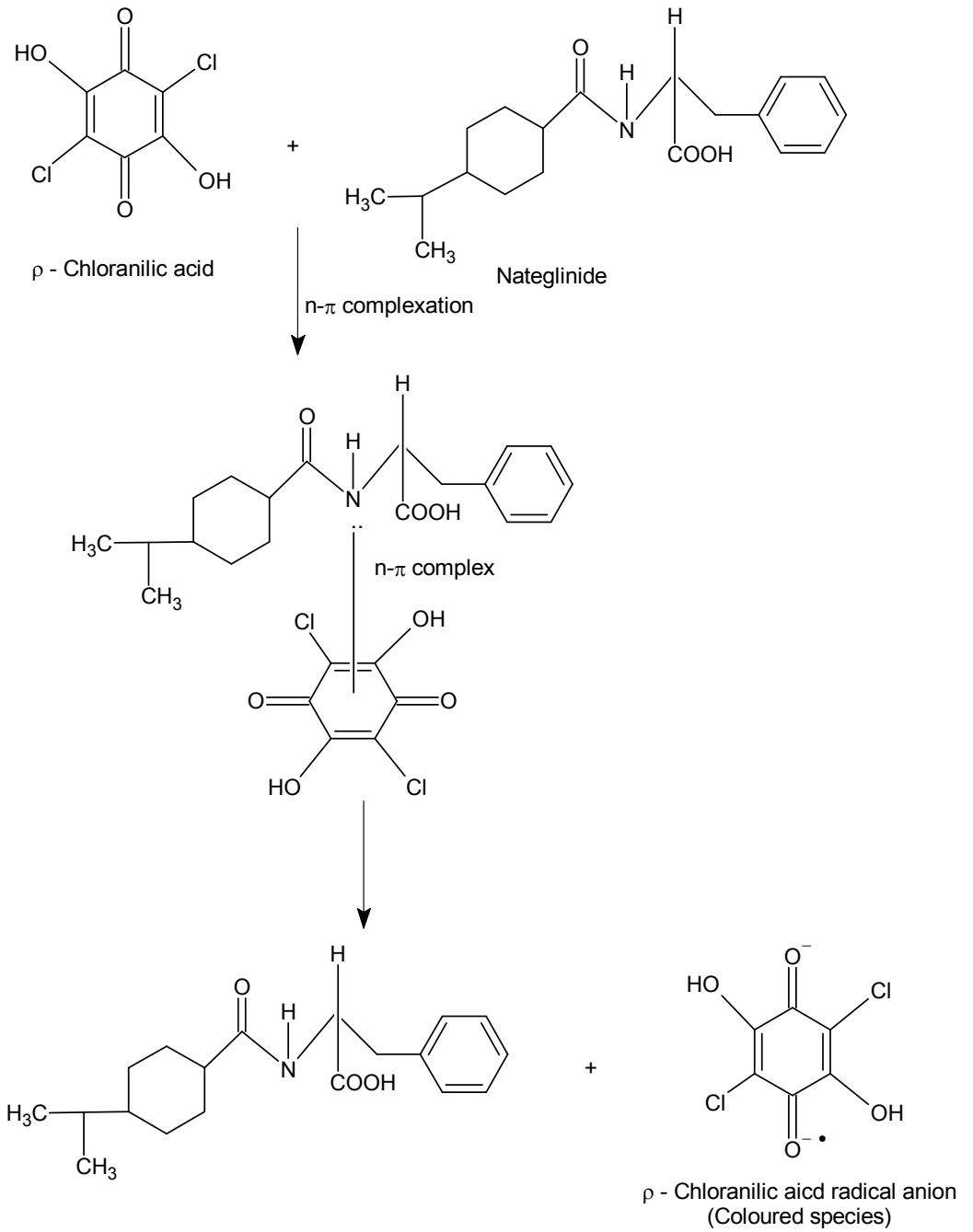
The CT complexes are formed through the lone pair of electrons donated by the nateglinide as n- donor and the charge transfer reagent as an electron acceptor in which a partial ionic bond ($D^+ A^-$) is assumed to be formed.



Scheme 1

This interaction was particularly strong on using ρ- CA so that it involves a complete transfer of electronic charge with the formation of a free radical anion ($A^{-\bullet}$). These radical anions formed were the predominant chromogens in the reaction. The dissociation of the donor-acceptor complex in those reactions was promoted by the high ionizing power of the solvent, acetonitrile[18].

The reaction mechanisms (NTG-MBTH) and (NTG - ρ-CA) for both methods are shown in Schemes 1 and 2, respectively. The colored products were found to be stable for 5 and 10 hours, respectively, at room temperature.


HO
Cl
Cl
OH
O
ρ - Chloranilic acid
+
H
O
H
N
COOH
H3C
CH3
Nateglinide
n-π complexation
n-π complex
HO
Cl
Cl
OH
O
O
HO
Cl
Cl
OH
O⁻
O⁻ •
ρ - Chloranilic aicd radical anion
(Coloured species)

Scheme 2

Optimization of reaction conditions

The different experimental parameters affecting the formation of the complex were extensively studied to determine the optimal conditions for the assay procedure.

The optimum reagent concentration for MBTH method was determined by adding various volumes of 0.2% MBTH and 0.7% ferric chloride in 0.5% hydrochloric acid to the fixed concentration of nateglinide. To achieve maximum color intensity, 1.5 mL of 0.2% MBTH, 1mL 0.7% ferric chloride in 0.5% hydrochloric acid were sufficient to develop maximum color intensity.

The optimum reagent concentration for ρ-CA method was determined by adding various volumes of ρ- CA to the fixed concentration of nateglinide. It was found that 2 mL of the reagent solution was enough to develop the absorbance to its maximum intensity. Different solvents like acetonitrile, dichloromethane, chloroform and dioxane have been tried in order to achieve maximum sensitivity and product stability. Among them, the acetonitrile was selected as the best for the ρ- CA charge-transfer complex formation.

Room temperature is recommended for both the methods (MBTH and ρ-CA) for the absorbance measurements of colored products.

Figures of merit

Linear correlations were found between absorbance at λmax and NTG concentration and are described by the regression equations :

$A = -0.0098 + 0.0017\ x$; $R = 0.9999$, $n = 5$ (MBTH method)

$A = 0.0037 + 0.0051\ x$; $R = 0.9999$, $n = 5$ (ρ-CA method)

Where A is the absorbance and x is the concentration in mg mL^{-1}, R is the correlation coefficient and n is the number of measurement levels. Beer's law is obeyed for 2–10 and 5–25 μg mL^{-1} for the MBTH and ρ-CA methods, respectively. Calculated apparent molar absorptivity values were found to be 3.552×10^4 and 1.66×10^4 Litre/$mole^{-1}$ cm^{-1} for the MBTH method and ρ-CA methods, respectively.

The LOD values were found to be 0.91 and 2.7 μg mL^{-1} for NTG with MBTH and with ρ-CA, respectively. The LOQ values were 2.6 and 7.9 μg mL^{-1} for NTG with MBTH and with ρ-CA, respectively. These values indicate that the MBTH method is more

sensitive than the ρ-CA method.

Table 1 summarizes the intra-day precision and intra-day error data for the assay of NTG in pure drug solution by the proposed methods. The intra-day RSD values ranged from 0.36–0.76% for MBTH method and 0.48 – 1.02% for ρ-CA method. The inter-day RSD values ranged from 0.24–0.96% for MBTH method and 0.86 – 1.20% for ρ-CA method. The percentages of error for both the methods are less than 2%. These values reflect the usefulness of the method in routine estimation.

The accuracy was assessed by analyzing the pharmaceutical formulation containing the nateglinide and the percent recovery of the active ingredient was calculated, which was found to be in the range of 99.66 ± 0.64 to 100.25 ± 0.49 for MBTH method and 100.08 ± 0.73 to 100.16 ± 0.42 for ρ-CA (Table 2), indicating that the co-formulated substances such as talc, starch, gum acacia, lactose, dextrose, hydroxyl methyl cellulose, sodium alginate and magnesium stearate did not interfere in the assay. Even precisionwise, the proposed methods are satisfactorily, with low RSD values ranging from 0.42 to 0.73%.

Table 1 : Intra-day precision and intra-day error of the proposed methods

Concentration of nateglinide (mg/mL)	Observed concentration of nateglinide(µg/mL)							
	Intra-day				Inter-day			
	Mean*	% Error	% RSD	% Recovery	Mean*	% Error	% RSD	% Recover
MBTH method								
10	9.99	0.10	0.36	99.9	10.15	1.50	0.96	101.50
20	20.06	0.30	0.42	100.3	19.90	0.50	0.53	99.50
30	29.92	0.27	0.76	99.73	30.05	0.16	0.24	100.16
ρ-CA method								
10	10.05	0.50	0.48	100.5	9.85	1.50	1.20	98.5
20	19.75	1.25	1.02	98.75	19.83	0.85	0.86	99.15
30	30.32	1.06	0.96	101.06	30.14	1.33	1.17	101.33

* For five determinants

Table 2 : Assay of drug in pharmaceutical formulations

Formulations	Labeled amount(mg)	% Found** ± S. D			
		Reference method*	Proposed method	t-test	F-test
MBTH method					
Tablet I	60	99.83 ± 0.24	99.66 ± 0.64	1.98	4.51
Tablet II	60	100.16 ± 0.56	100.25 ± 0.49	1.53	5.13
ρ-CA method					
Tablet I	60	99.83 ± 0.24	100.08 ± 0.73	1.72	3.79
Tablet II	60	100.16 ± 0.56	100.16 ± 0.42	1.48	5.83

** Recovery amount was the average of five determinants

* UV method developed in the laboratory

Tabulated t-value at 95% confidence level is 2.306

Tabulated F-value at 95% confidence level is 6.39

Table 3: Recovery of nateglinide in pharmaceutical formulations by standard addition technique

Amount of drug added (µg)	Mean amount (µg) Recovered(n = 5)	Mean % of Recovery(n = 5)	% RSD
MBTH method			
200	202.10	101.05	0.62
400	399.45	99.86	0.49
P-CA method			
200	200.15	100.07	0.43
400	398.75	99.68	0.75

Application to dosage forms

The proposed methods were applied to the analysis of NTG in pharmaceutical dosage forms and the results were statistically compared with UV method by calculating the Student's t- and F-values. The evaluated t- and F-values were less than the tabulated

values at the 95% confidence level for eight degrees of freedom[18], as revealed by the results compiled in Table 2. This actually suggests that the proposed methods are accurate and precise as the UV method.

In order to further ascertain the accuracy and reliability of the methods, recovery experiments were performed by the standard-addition method. Pre-analyzed formulation was spiked with pure NTG at two different levels and the total was found by the proposed methods. The percent recovery of pure drug added was in the range of 99.86–101.05% for MBTH and 99.68–100.07% for ρ-CA. RSD ranged from 0.49 to 0.62% and 0.43 to 0.75% for MBTH and ρ-CA, methods respectively. The results of this study summarized in Table 3 indicate that neither the accuracy, nor the precision of the methods is affected by the coformulated substances.

CONCLUSION

The proposed methods were found to be simple, economical, selective and sensitive. The statistical parameters and recovery study data clearly indicate the reproducibility and accuracy of the methods. Analysis of the authentic samples containing nateglinide showed no interference from the common excipients. Hence, these methods could be considered for the determination of nateglinide in the quality control laboratories.

ACKNOWLEDGEMENTS

The authors are grateful to the Management of Siddhartha Academy, Vijayawada and J. K. C. College, Guntur for their continuous support and encouragement and for providing the necessary facilities.

REFERENCES

1. C. Saloranta, K. Hershon, M. Ball, et al., J. Clin. Endocrinol. Metab., **87**, 4171–4176 (2002).
2. S. Fujitani, T. Ikenoue, M. Akiyoshi, T. Maki and T. Yada, Metabolism, **45**, 184–189 (1996).
3. M. Hanefeld, K. P. Bouter, S. Dickinson and C. Guitard, Diabetes Care, **23,** 202–207 (2000).
4. K. Bokvist, M. Hou, K. Bushard, et al., Eur. J. Pharmacol., **386**, 105–11 (1999).
5. K. Bokvist, M. Hou, C. R. Poulsen, et al., Diabetologia., **41**, A139 (1998).

6. A. Rajasekaran, S. Murugesan, M. K. Abdul Hathi, S. N. Ajith, G. Aruna Devi, G. Sowmiya and P. Dharmar, Indian J. Pharma. Sci., **66(6)**, 806-807 (2004).

7. G. V. V. S. G. Sagar, N. Rao and B. S. Sastry, Indian J. Pharma. Sci., **66(3)**, 356-358 (2004).

8. G. Vidya Sagar, N. V. S. Gangadhara Rao and B. S. Sastry, Indian J. Pharma. Sci., **66(2)**, 219-221 (2004).

9. M. Sankalia Jolly, G. Sankalia Mayur, Vijay B. Sutariya and C. Mashru Rajashree, J. Pharma. Biomed. Anal., **44(1)**, 196-204 (2007).

10. He. Zhonggui, Chen. Xiaoyan, Zhong Dafang, Zhao Chunshun, Liu. Xiaohong and Zhang Ruhua, Biomed. Chromato., **18(8)**, 532–537 (2004).

11. Yan. Hongyuan, Yang Gengliang, Qiao Fengxia and Yi Chen, J. Pharma. Biomed. Anal., **36 (1),** 169-174 (2005).

12. D. Harvey, Modern Analytical Chemistry, 1st Ed., McGraw-Hill Book Co, Singapore, (2000) pp. 38–44.

13. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH Harmonised Tripartite Guidelines, Validation of Analytical Procedures : Text and Methodology Q2 (R1), Current Step 4 Version, Nov. (1996), Geneva, Nov. (2005).

14. Z. A. El-Sherif, A. O. Mohamed, M. I. Walash and F. M. Tarras, J. Pharm. Biomed. Anal., **22**, 13 (2000).

15. A. A. M. Moustafa, J. Pharm. Biomed. Anal., **22**, 45 (2000).

16. S. Görög, Ultraviolet-Visible Spectrophotometry in Pharmaceutical Analysis; CRC Press Inc. Florida, (1995) p. 188.

17. R. Foster, Organic Charge-Transfer Complexes, Academic Press, London, (1969).

18. H. F. Askal, G. A. Saleh and N. M. Omar, Analyst, **116**, 387 (1991).

Accepted : 13.04.2009