J. Flow Injection Anal., Vol. 22, No. 2 (2005) 129–133

Flow injection kinetic spectrophotometric method for the determination of famotidine in pharmaceutical preparations

Nawel Helali, Nafaâ Adhoum and Lotfi Monser*

Institut National des Sciences Appliquées et de Technologie, Département de Chimie, Centre Urbain Nord B.P.N° 676, 1080 Tunis Cedex, Tunisia,

Abstract

A new simple flow injection kinetic spectrophotometric method has been developed for the determination of famotidine in pharmaceutical preparations. The method is based on a kinetic investigation of the oxydation reaction of the drug in alkaline potassium permanganate. The absorbance of the produced green coloured manganate species was monitored at 610 nm.

Flow injection variable parameters such as reagent concentration, injected volume, reactor length and flow rate were carefully investigated and optimised. The determination of famotidine in the range of 1-50 mg L^{-1} was possible with a correlation coefficient of 0.9981 and a detection limit of 0.22 mg L^{-1}. The precision of the method was within 2% for 30 mg L^{-1} famotidine (n=5). The flow injection method can be satisfactorily applied to the determination of famotidine in pharmaceutical preparations with a sampling frequency of 60 samples h^{-1}.

***Keywords**:* Flow injection, famotidine, Potassium permanganate, Spectrophotometer, pharmaceutical formulations.

1. Introduction

Famotidine (FMT) N^2-aminosulphonyl-3-[[[2-[(diaminomthylene) amino]thiazol-4-yl] methyl] thio] propanamidine is a potent histamine H_2 receptor antagonist that widely used in the treatment of gastric and duodenal ulcers [1]. According to the European and US Pharmacopoeia [2,3], the drug is determined in pharmaceutical preparations by potentiometric titration and HPLC methods. Thin layer chromatographic method has been reported for the determination of the related impurities of FMT [2,3].

Various liquid chromatographic methods have been reported for the individual and simultaneous determination of FMT and other analogue antihistaminic products in pharmaceutical preparations, in human serum and in urine [4-12]. FMT has been also determined in pharmaceutical preparations using potentiometric [13,14], voltammetric [15], spectrophometric [16-22], quantitative NMR [23] and Capillary electrophoresis [24] methods. These analytical methods have been used with varying degrees of success and convenience, to meet the sensitivity and selectivity requirements of these analyses. Flow injection analysis (FIA) has proved [25,26] to be a suitable technique for on line analysis because of its low reagent and sample consumption and to its simplicity, high sampling frequency and the repeatability of its results.

In this work, an efficient and simple FIA method was developed for the determination of FMT in pharmaceutical formulations. The method is based on the reaction of the drug with alkaline potassium permanganate. The experimental parameters that influence the development and stability of the colour were carefully investigated and the suitability of the proposed method for the determination of FMT was tested by analysing several FMT containing drugs.

2. Materials and Methods

2.1. Reagents and standards

FMT was obtained from the pharmaceutical society Ibn Al Baytar (Tunis, Carthage, Tunisia). A 200 mg L^{-1} stock standard solution of FMT was prepared by dissolving appropriate amount of the drug in distilled water. Working solutions of lower concentrations were freshly prepared by appropriate dilution of the stock standard solution. Potassium permanganate (0.1 M) and sodium hydroxide (2 M) solutions were prepared daily in distilled water, by weighting an appropriate amounts of analytical grade reagents (Prolabo, France).

2.2. Apparatus

The flow injection system employed is shown in Fig.1. The reagent solutions were delivered by a peristaltic pump (Gilson Minipuls, Anachem, Luton Bedfordshire, UK). The sample was introduced into the sodium hydroxide stream using an Omnifit injection valve to which a variable sample loop is attached. A silicone rubber tubes (0.8 mm i.d.), PTFE joints and tubing were used for connections. The absorbance of the resulting solution was measured at 610 nm using a Beckman spectrophotometer equipped with a 1 cm path length flow cell.

2.3. Preparation of FMT in formulations

Two brands of commercial drugs (Famodine 20 and Famodine 40) were purchased from local pharmacy. Ten FMT tablets were finely powdered and weighed. An accurately weighed quantity of the mixed powder containing an equivalent to 20 mg of FMT was dissolved in 100 mL of water. The solution was then introduced into an ultrasonic bath for 10 min, filtered and diluted to furnish FMT solutions of final concentration ranging from 5-20 mg L^{-1}. The sample is injected into the sodium hydroxide (0.5 M) carrier with the aid of a rotary valve with a loop of 100 µL. Permanganate solution (2.5×10^{-3} M) as the other stream, joined the sample plug before the reaction coil. The resulting reaction product is formed in the reaction coil and then directed to the detector. The signal corresponding to FMT is measured at 610 nm.

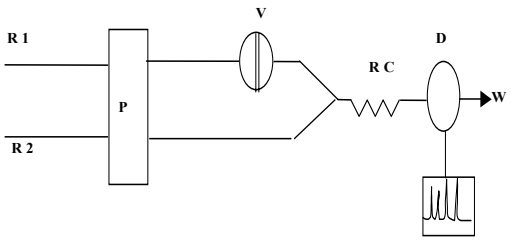


Figure 1. Flow injection system used for determination of FMT. R1: sodium hydroxide, R2: potassium permanganate, P: pump, V: injection valve, RC: reactor coil, D: detector, W: waste.

3. Results and Discussion

3.1. Optimisation of the flow system

The reaction between FMT and potassium permanganate in alkaline medium yields a green colour due to the production of manganate ions, which absorbs at 610 nm. The absorbance of the colour formed was found to be proportional to the concentration of FMT. The various experimental parameters affecting the development of the reaction product were optimised by changing each variable in turn, while keeping all others constant. The initial FIA parameters used during optimisation were the following: sodium hydroxide concentrations was (0.5 M), overall stream flow rate was 2 mL min^{-1}, reactor length (1.4 m) and injection volume of 100 µL.

3.1.1. Influence of chemical parameters

The following chemical parameters were studied in order to improve the sensitivity of the proposed flow injection method: concentration of potassium permanganate and sodium hydroxide.

Concentration of potassium permanganate

The effect of potassium permanganate concentration on the reaction was studied over the range 0.3 x 10^{-3} – 20 x 10^{-3} M. The absorbance values were monitored by injecting 30 mg L^{-1} standard solution of FMT. Figure 2 shows that the maximum absorbance was obtained at concentration of 2.5 x 10^{-3} M. Higher concentrations of potassium permanganate yielded lower absorbance values, probably due to decomposition of the product which is attributed to the possible oxidation of manganate ions by permanganate to form solid brown manganese dioxide.

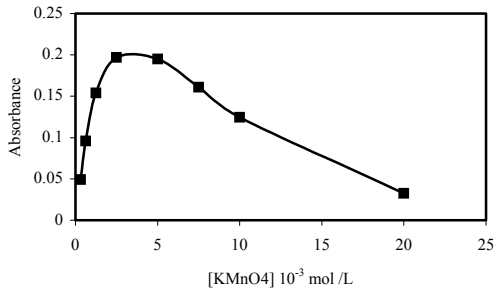


Figure 2. Effect of potassium permanganate concentration on the absorbance of FMT. NaOH = 0.5 M, overall flow rate = 2 mL/min, reactor length = 1.4 m and injection volume =100 µ

Concentration of sodium hydroxide

The effect of sodium hydroxide concentration was investigated between 0.1 – 1.25 M. Figure 3. shows that the absorbance values increased with increasing sodium hydroxide concentration to reach a maximum at 1M. No further changes in the absorbance were measured when NaOH concentration was increased above this concentration. This indicates a complete oxidation of FMT. Therefore, a concentration of 1M was chosen for further investigations.

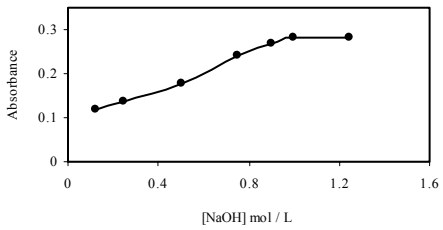


Figure 3. Effect of sodium hydroxide concentration on the absorbance of FMT. $KMnO_4$ = 2.5 x 10^{-3} M, flow rate = 2 mL/min, reactor length = 1.4 m and injection volume =100 µL

3.1.2. Influence of manifold parameters

Several experiments were conducted in order to establish the best experimental conditions for operating the flow injection manifold, such as the flow rate of acceptor and donor stream, sample injection volume, the reaction coil length and the temperature.

Flow rate

The flow rate of the carrier stream is a very important variable to a FIA determination because it influences the dispersion of the sample zone and thus the sensitivity of the determination. The effect of the overall flow rate (NaOH and potassium permanganate) on signal response was studied in the range 1.25 – 3.25 mL/min under the optimal chemical variables. It was observed (Fig.4) that the peak height increased by increasing the overall flow rate reaching a maximum at 1.7 mL/min. At higher flow rates, lower absorbance values were obtained due to the short sample residence time. Therefore, a flow rate of 1.7 mL/ min was selected as a compromise between signal sensitivity and sample residence time.

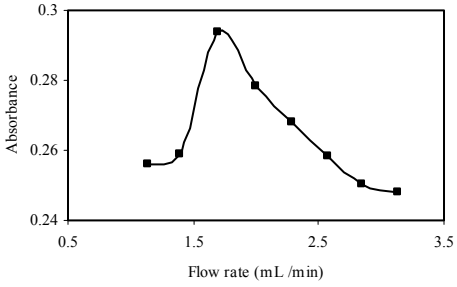


Figure 4. Effect of stream flow rates on the absorbance of FMT. NaOH = 1.0 M, $KMnO_4$ = 2.5 x 10^{-3} M, reactor length = 1.4 m and injection volume =100 µL

Sample injection volume

The sample loop volume was varied from 50-200µL by changing the length of the sample loop in the injection valve. As can be seen in Fig.5. the detection response increased no linearly with increasing the sample loop volume until it reaches a maximum at 135 µL, after which the peak heights remains almost constant. A

sample loop volume of 135 µL was chosen for further study as a compromise between absorbance and dispersion.

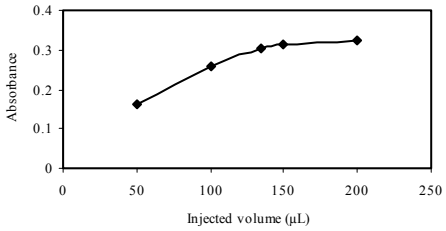


Figure 5. Effect of injected volume on the absorbance of FMT. NaOH = 1.0 M, $KMnO_4$ = 2.5 x 10^{-3} M, flow rate = 1.7 mL/ min, reactor length = 1.4 m

Reactor length

The influence of reaction coil length on the detector response was studied in the range 0.25 m to 8.5 m. As can be seen from Fig.6, maximum absorbance values were obtained for reactor coil length varying between 0.6-3.5 m. Above these values we observe a decrease in the absorbance due to the dispersion phenomena. Therefore, a 0.6 m reactor length was selected as optimal for further experiments.

Temperature

The effect of temperature was studied in the range of 25-70°C. As expected (Fig.7), the rate of reaction increased with increasing temperature. However, 25°C was selected as optimum temperature due to the low reproducibility of absorbance values obtained at higher temperatures due to the formation of bubbles in the system.

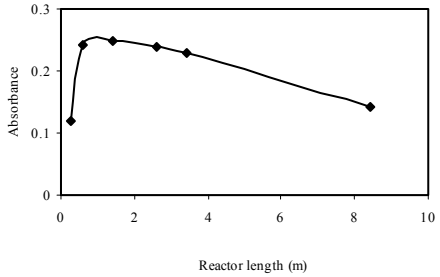


Figure 6. Effect of reactor length on the absorbance of FMT. NaOH = 1.0 M, $KMnO_4$ = 2.5 x 10^{-3} M, flow rate = 1.7 mL/ min, and injection volume =135 µL

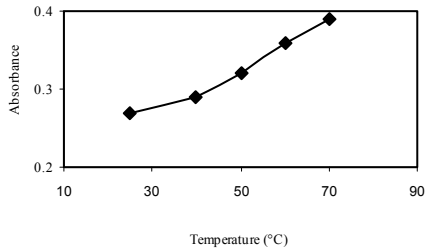


Figure 7. Effect of temperature on the absorbance of FMT. NaOH = 1.0 M, $KMnO_4$ = 2.5 x 10^{-3} M, flow rate = 1.7 mL/ min, and injection volume =135 µL, reactor length = 0.6 m.

3.2. Evaluation of the method

The flow injection system (Fig.1) was used for preparing the calibration graph, using the optimum parameters (Table 1). A linear calibration graph (Fig.8) was obtained in the concentration range 1-50 mg L^{-1} FMT with a regression coefficient of 0.9981. The equation of the line was A = 0.0079 [FMT] + 0.0564. The detection limit calculated as three times the baseline noise was 0.22 mg L^{-1}. The precision of the method expressed as relative standard deviation (RSD) was determined by replicate analysis (n=5) of 30 mg L^{-1} in both interday and intradays reproducibilities. The RSD of interday and intradays were 0.91 % and 1.22 % respectively. The recovery was determined by spiking the FMT drug formulations with a known amount of FMT (5, 10 and 20 mg L^{-1}). Average recovery was ranged between 97 and 102%.

Table 1. Studied chemical and physical parameters and their optimal values

		Range studied	Optimal value
Chemical variables	$KMnO_4$ (M)	(0.3-20) 10^{-3}	2.5. 10^{-3}
	NaOH (M)	0.1- 1.25	1
Physical variables	Flow rates (mL/ min)	1.25-3.25	1.70
	Sample volume (µL)	50-200	135
	Reactor length (m)	0.25-8.45	0.60
	Temperature (°C)	25-80	25

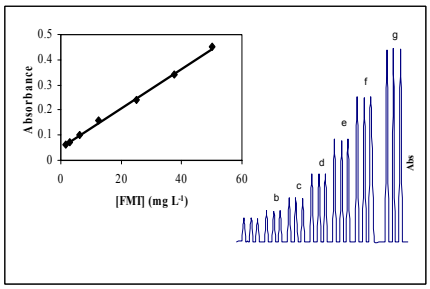


Figure 8. Typical spectrophotometric response of FMT with permanganate solution using the optimum FI operating conditions : (a) 1.5 mgL^{-1}, (b) 3.1 mgL^{-1}, (c) 6.2 mgL^{-1}, (d) 12.5 mgL^{-1}, (e) 25 mgL^{-1}, (f) 37.5 mgL^{-1}, (g) 50 mgL^{-1}

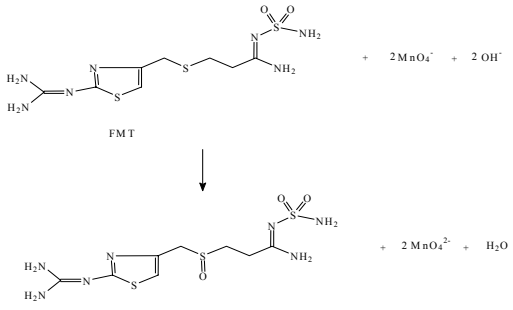
3.3. Analysis of Famotidine drug

In order to apply the proposed method to the analysis of FMT drug, the selectivity of this method has been evaluated by studying the effect of frequently encountered excipients such as Magnesium stereate, Sodium laurylsulfate, Avicel pH 102 and Aerosyl 200 that can be found in famotidine containing formulations. Different amounts of the possible interferences were added to a solution containing 20 mg L^{-1} FMT. The relative error was calculated by comparing the peak height obtained with a pure FMT with that of solutions containing interfering compounds (Table 2). No significant interference was observed from the presence of these additives even when mass ratios were greater than that contained in pharmaceutical products.

The proposed method was applied to the determination of FMT in commercial available formulations. The obtained results (Table 3) demonstrate that the content of drug in each of these formulations correspond to each drug label. The percent recoveries with respect to the amount claimed were found to be 99.5 and 101%. The accuracy of the method was further investigated by comparing the results obtained with the new developed method to that obtained with the official EU Pharmacopeia. Table 3 shows an excellent agreement between the two methods.

Table 2. Effect of excipients on the determination of Famotidine.

Ratio FMT/ placebo (w/w)	Error (%)
1:1	0
1:2	0
1:3	0
1:4	0.9
1:6	2.5
1:8	9.0



Scheme 1. Proposed reaction between FMT and $KMnO_4$.

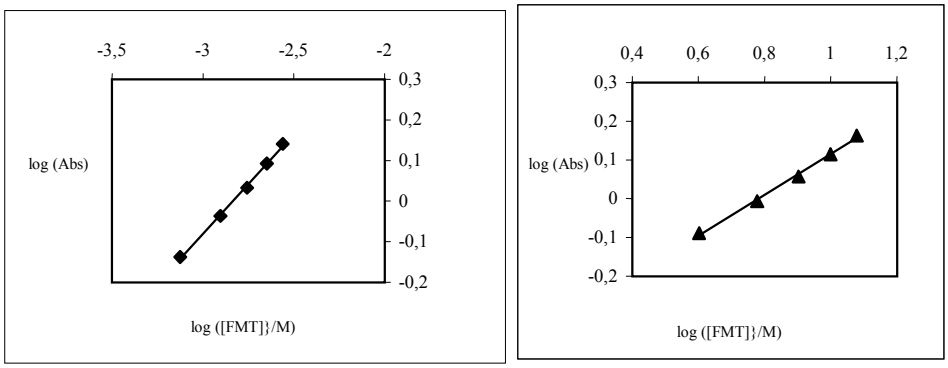
Table 3 Famotidine contents in drug tablets expressed as % with respect to label amount claimed.

Drug	Famotidine (mg) claimed	found with proposed method	found with official method (pharmacopea)	%
Famodine 20	20	20.10	20.03	99.65
Famodine 40	40	39.80	40.10	100.75

3.4. Stoichiometry and reaction mechanism

In order to determine the stoichiometry ratio between $KMnO_4$ and FMT in the reaction, the limiting logarithmic method was used by performing two sets of experiments [27]. In the first case, $KMnO_4$ concentration was varied while keeping a constant concentration of FMT. In the second, $KMnO_4$ concentration was kept constant while varying the concentration of FMT. The ratio may be found by plotting the logarithms of the absorbances measured at a fixed time in both cases versus respective varied concentration (Fig. 9).

The slope of the curve in first case yields the number of moles of $KMnO_4$ and the second gives the number of moles of FMT. The molar ratio of the reaction $KMnO_4$ / FMT was found to be 1:1. Based on this result and to the presence of the thioether linkage in famotidine formulation and its ability to oxidation to sulphoxide [28], a hypothetic reaction pathway may be proposed (scheme 1).



(1) (2)

Figure 9. Limiting logarithmic plot for molar ratio of reaction $KMnO_4$ / FMT: (1) log Absorbance vs. log [$KMnO_4$]; (2) log Absorbance vs. log [FMT].

3.5. Kinetic of the reaction

The rate of the reaction was determined by studying the absorbance evolution with time for various solutions containing different amounts of FMT in presence of a large excess of permanganate (Fig. 10). The result shows that the reaction rate increases upon increasing FMT concentration. In presence of a great excess of permanganate, the reaction obeys the following equation:

$\log (\text{rate}) = k' [\text{FMT}]^n = dA/dt$

Where k' is the pseudo-order constant, n is the order of the reaction, A is the absorbance, and t is the time in seconds. Taking logarithms of initial rates and concentrations, the above equation is transformed into:

$\log (\text{initial rate}) = \log k' + n \log [\text{FMT}]_0$

$= \log (\Delta A/\Delta t)$

This enables the determination of the pseudo-order constant from the intercept of log initial rate vs. log $[FMT]_0$ plot. The relation of log initial rate versus log $[FMT]_0$ was linear with a correlation coefficient $R^2 = 0.9991$ and a regression equation (Fig.11) of log (initial rate) = 1.7 + 0.97 log $[FMT]_0$. Hence k'= 50.1 s^{-1} and the reaction is pseudo-first order (n = 0.97) with respect to the FMT concentration, which is coherent with previous results.

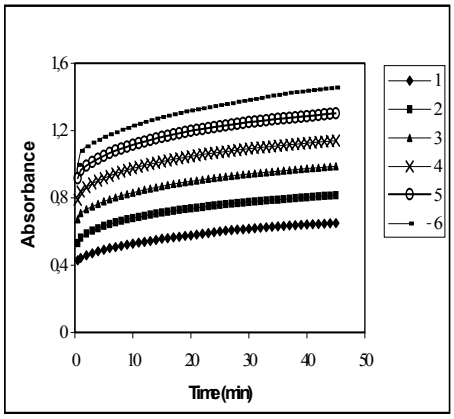


Figure 10. Absorbance vs. times curves for the reaction between FMT and $KMnO_4$: $KMnO_4$ 2.5 $x10^{-3}$ mol L^{-1} and FMT (1) 5.93 x 10^{-6} mol L^{-1}; (2) 1.18 $x10^{-5}$ mol L^{-1}; (3) 1.78$x10^{-5}$ mol L^{-1}; (4) 2.37 $x10^{-5}$ mol L^{-1};(5) 2.96 $x10^{-5}$ mol L^{-1}; (6) 3,56 $x10^{-5}$ mol L^{-1}.

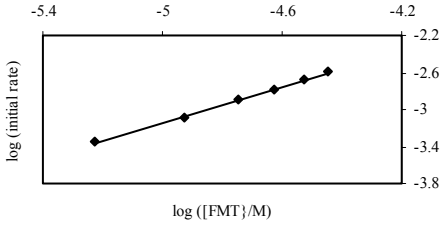


Figure 11. log rate (dA/dt) of the reaction between FMT and $KMnO_4$ vs. log FMT concentration

4. Conclusion

The proposed FIA method has been successfully applied for the determination of FMT in pharmaceutical forms with a precision and accuracy similar to the official method. This the advantages of simplicity, speed, repeatability and the use of inexpensive equipment. The method is useful for the quality control and routine analysis and for the kinetic determination of FMT in pharmaceuticals, since there is no interference from the excipients and additives.

References

1. Goodman, Gilman's, The Pharmacological Basis of Therapeutics, 9th edition, McGraw-Hill, New York 1996, pp. 904-907
2. *European Pharmacopeia*, 3ed edition, Council of Europe, Strasbourg 1997, pp. 864-866.
3. *The United State Pharmacopeia,* 23ed edition, United StatesT Pharmacopeial Convention, Rockville, MD 1995, pp. 651-652
4. J. Novakovic, *J. Chromatogr. A*, **864** , 193 (1999)
5. H. Chih, H. Hsi-Min, H. Shu-Ying, S. Ching-Yih, C. Ber-Lin, *Drug Development and Industrial Pharmacy,* **25** 379,(1999)
6. H. Maurer, *J. Chromatogr.B*, **531**, 369 (1990)
7. B. Carkir, U. Tosun, F. Sahin, *Pharm. Sci.,* **493,** 493 (1997) L. Zhong, K.J. Yeh, *J. Pharm. Biomed. Anal.*, **16**, 1051(1998)
8. L. Zhong and K. Yeh, *J. Pharm. Biomed. Anal*., **16**, 1051 (1998)
9. M. Campanero, I. Bueno, M. Arangoa, M. Escolar, E. Quetglas, A. Lopez-Ocariz, J. Azanza, *J. Chromatogr. B*, **763**, 21(2001)
10. T. Dowling, R. Frye, *J. Chromatogr. B*, **732**, 239 (1999)
11. S. Wanwimoluk, A. Zoest, C. Hung, *J. Chromatogr. B*, **572**, 227 (1991)
12. N. Helali, F. Darghouth, L. Monser**,** *Chromatographia*, **60**, 455 (2004)
13. M. Ayad, A. Shalaby, H. Abdellatef, H. Elsaid, *J. Pharm. Biomed. Anal*., **29**, 247 (2002)
14. J. Petkovic, D. Minic, Z. Koricanac, *J. Jovanovic, Pharmazie*, **58**, 163 (1998)
15. S. Skrzypek, W. Ciesielski, A. Sokolowski, S. Yilmaz, D. Kazmierczak, *Talanta* **66,** 1146, (2005)
16. A. Abu Zuhri, R. Shubietah, G. Badah, *J. Pharm. Biomed. Anal.,* **21**, 459 (1999)
17. K. Kelani, A. Azziz, M. Hegazy, L. Abdel Fattah, *Spectros. Lett.,* **35**, 543 (2002)
18. A. Amin, S. Shama, I. Ahmed, E. Gouda, *Anal. Lett*., **35**, 1851 (2002)
19. S. Nassr, M. Brunet, P. Lavoie, J. Brazier, *J. Liq. Chromatogr.*, **24**, 265 (2002)
20. N. Rahman, M. Kasihif, *Il Farmaco* **58**, 1045 (2003)
21. N. Rahman, M. Kashif, *Anal. Sciences*, **19**, 907 (2003)
22. M. Walash, M. Sharaf-El-Din, M. Metwally, M. Shabana, *J. Chinese Chem. Soc.,* **52**, 71 (2005)
23. M. Baranska, K. Czarniecki, L. Proniewicz, *J. Mol. Struct*.,**27,** 347 (2001)
24. T. Perez-Ruiz, C. Martinez-Lorenzo, V. Tomas, E. Bravo, R. Galera, *J. Pharm. Biomed. Anal*., **30**,1055 (2002)
25. B. Kamath , K. Shivram and A. Shah; *J. Pharm. Biom. Anal*., **12**, 343 (1994)
26. B. Kamath, K. Shivram and S. Vangani, *Anal. Letters* **26**, 2183 (1993)
27. J. Rose**,** Advanced Physico-Chemical Experiments, Pitman, London 1964, p 67
28. E. Hassen and E. Belal, *J. Pharm. Biomed. Anal*., **27,** 31 (2002)

(Received July 22, 2005)
(Accepted August 31, 2005)