COLORIMETRIC DETERMINATION OF METOCLOPRAMID HYDROCHLORIDE AND TRANEXAMIC ACID USING 9-CHLOROACRIDINE REAGENT

Theia'a N. Al-Sabha[a], Maha T. Al-Obaidi[b] and Thabit S. Al-Ghabsha[c]

Keywords: metoclopramide hydrochloride, tranexamic acid, 9-chloroacridine, spectrophotometry.

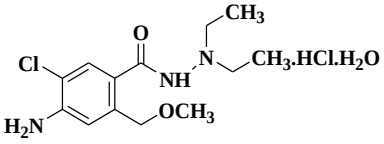
A simple, sensitive and accurate spectrophotometric method is developed for the quantitative determination of metoclopramide hydrochloride and tranexamic acid drugs. The method is based on the interaction between these drugs and 9-chloroacridine reagent. The spectra of the products show maximum absorption at 470 and 479 nm and Beer's law is obeyed in the concentration range of 2-50 and 1-40 μg mL^{-1} with molar absorptivity values 8.50×10^3 and 7074×10^3 L mol^{-1} cm^{-1} for above drugs respectively. The average percent recoveries are 99.90 % and 98.60 % respectively, and relative standard deviation (RSD) is ≤ 0.456 % for both drugs. In addition, the stability constant has been determined and the reaction mechanism is proposed. The method has been applied successfully for the assay of metoclopramide hydrochloride and tranexamic acid in pharmaceutical formulations and compared favourably with the amounts mentioned in formulations.

*Corresponding Authors
E-Mail: dr_theiaa@yahoo.co.uk
[a] Dept. of Chemistry , College of Education , University of Mosul-Iraq
[b] Dept. of Chemistry , College of Science, University of Mosul –Iraq
[c] College of Pharmacy, University of Mosul –Iraq

Introduction

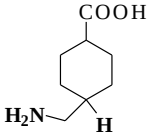
Metoclopramide hydrochloride (MCP.HCl), [4-amino-5-chloro-N-(2-diethylaminoethyl)-2-methoxybenzamide hydro-chloride] [I] is used as an anti-emetic in the treatment of some forms of nausea and vomiting and to increase gastrointestinal motility. It is of little benefit in the prevention or treatment of motion sickness or in the treatment of nausea and vertigo due to Meniere disease or other labyrinth disturbance,[1] also it is used to relieve certain stomach and esophagus problems such as diabetic gastroparesis and gastroesophageal reflux disorder.[2]

Tranexamic acid (TRA), [Trans 4-(aminomethyl)-cyclohexanecarboxylic acid] [II] is used as antifibrinolytic and haemostatic in the treatment of bleeding associated with excessive fibrinolysis, such as haemorrhage following prostatectomy, tonsillectomy and menorrhagia.[3]



$C_{14}H_{22}ClN_3O_2.HCl.H_2O$
M.wt =354.3 gm/mol

I



$C_8H_{15}NO_2$
M.wt =157.2 gm/mol

II

Various analytical techniques have been reported for determination MCP.HCl and TRA in pure or dosage forms. Chromatographic methods,[4-8] titrimetry,[9] voltammetry,[10] atomic absorption,[11] flow injection[12,13] and ion selective electrode[14] are used for determination of MCP.HCl, whereas chromatographic[15-17] and conductometric[18] methods are used for determination of TRA. Of course, the above mentioned techniques are sensitive but expensive. Spectrophotometry is the technique of choice even today due to its inherent simplicity. Many spectrophotometric procedures have been applied for the determination of MCP.HCl and TRA using different reagents such as phenothiazine as coupling reagent and ferric nitrate as oxidizing reagent,[19] o-phenanthroline or bipyridyl in the presence of Fe(III) or Ce(IV) as oxidizing reagents,[20] dibenzoyl methane,[21] aniline as coupling reagent[22] and p-dimethylaminocinnamaldehyde[23] in addition to other spectrophotometric methods[24-28] used for determination of MCP.HCl. Vanilline,[29] acetylacetone and formaldehyde,[30] 2,6-dichloroquinone-4-chlorimide,[31] ascorbic acid,[32] 1,2-naphthoquinone-4-sodium-sulphonate,[33] DDQ and TCNQ[34] p-nitroaniline[35] and potassium ferricyanide in the presence of ferric chloride[36] reagents are used for determination of TRA. Some of these methods are time-consuming, extraction procedures or heating require strictly controlled reaction conditions. Others are less sensitive. The present work describes a simple and sensitive spectrophotometric method for assay of MCP.HCl and TRA drugs in pure and dosage forms using 9-chloroacridine as chromogenic reagent without any derivatization or catalysis.

Experimental

Apparatus

Shimadzu UV-1650 PC UV-Visible spectrophotometer, equipped with a 1.0-cm path length silica cell**,** is used for spectral measurements**.** Philips PW (9421) pH-meter with a

combined glass electrode was used for pH measurements**,** All calculations in the computing process were done in Microsoft Excel for Windows.

Reagents

All chemicals used are of the highest purity available which are provided by BDH, Fluka and Molekula. 9-Chloroacridine (Eastman chemical Co.) was used as the chromogenic reagent. Absolute ethanol is used. Sodium hydroxide (1×10^{-2} M) and hydrochloric acid (1×10^{-2} M) solution are prepared by appropriate dilution of the concentrated NaOH (1 M) or HCl (1 M) solutions with distilled water. 9-chloroacridine reagent (1×10^{-3} M) (9-CA) solution is prepared by dissolving 0.0053 g of 9-chloroacridine in absolute ethanol and then the volume is completed to 25 ml in a volumetric flask. The solution is freshly prepared and used immediately.

Metoclopramide hydrochloride (MCP.HCl) and tranexamic acid (TRA) (100 µg mL^{-1})

The solutions are prepared by dissolving 0.01g of pure MCP.HCl and TRA (provided from SDI) in distilled water (the solutions were equivalent to 100 µg mL^{-1} for each drug).

Recommended procedure

To a series of 5 ml calibrated flasks, increasing volumes of the working MCP.HCl or TRA solutions (100 µg mL^{-1}) were transferred to cover the concentration range 2-50 or 1-40 µg mL^{-1} for MCP.HCl or TRA respectively, followed by the addition of 1.5 ml of 1×10^{-3} M 9-CA . The solutions were diluted to the mark with methanol. The solutions were kept in a water bath at room temperature for 5 and 15 min, for MCP.HCl and TRA respectively, and the absorbance was measured at 470 and 479 nm respectively against the respective blank reagent.

Procedure for MCP.HCl and TRA assay in dosage forms

Tablet

Weighed and finely powdered 10 tablets (each containing 10 mg MCP.HCl and 500 mg TRA), an accurately weighed amount of powder, equivalent to one tablet, was dissolved in 10 ml ethanol, shaked to increase the solubility and filtered into 100 ml calibrated flask, then the solution was made to the volume with the distilled water (the solution was equivalent to 100 µg mL^{-1} for MCP.HCl and 5000 µg mL^{-1} for TRA). A suitable volume was diluted with distilled water and followed the recommended procedure.

Syrup

The content of MCP.HCl syrup (5 mg/5 mL) was homogenized well and 10 ml of syrup was quantitatively transferred into 100 ml volumetric flask and completed to the mark with distilled water. An aliquot of diluted drug was taken and treated as mentioned in the recommended procedure.

Injection

For the analysis of injection, 2ml vial containing 10 mg/2 ml of MCP.HCl and 1ml containing 250 mg/5 mL TRA were transferred into 100 ml volumetric flask separately, and diluted up to the mark with distilled water. Working standard was prepared by suitable dilution and the recommended procedure was followed.

Results and discussion

In the preliminary investigation work, it was found that 9-CA reagent reacted selectively with MCP.HCl and TRA in alcoholic medium of ethanol and produced an orange colored solutions immediately with maximum absorption at 470 and 479 nm for above drugs respectively. Whereas the reagent blank which shows low absorbance at these wavelengths but have a maximum absorption at 386 nm (Fig. 1). However, the wavelength of maximum absorption 470 and 479 nm for MCP.HCl and TRA respectively were used in all subsequent experiments.

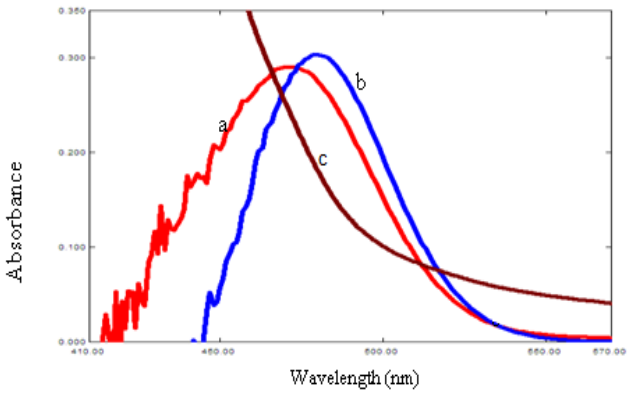


Figure 1. Absorption spectra of a) MCP.HCl (6.0 µg mL^{-1}), b) TRA (6.0 µg mL^{-1}) products with 9-CA (1×10^{-3} M) against reagent blank and c) Reagent blank against ethanol.

Selecting optimum reaction conditions

The effect of various parameters on the absorption intensity of the colored products were investigated and the reaction conditions were optimized.

Effect of solvent

A number of mixtures were prepared by mixing 0.3 and 0.5 ml of MCP.HCl and TRA solutions, containing 100 µg of the drug mL^{-1}, with 1 ml of 1×10^{-3} M 9-CA and the volume was made to 5 ml with solvents such as water, methanol, ethanol, acetone, acetonitrile and tetrahydrofuran in calibrated flasks as given in Table 1. It was observed that the mixtures prepared from the solution of 9-CA in methanol and drug solutions in water made to 5 ml with the methanol gave maximum absorbance for MCP.HCl and TRA at 470 and 479 nm respectively, Therefore, this combination of solvents and wavelengths was chosen in the subsequent experiments.

Table 1. Effect of solvent on color intensity of drug-9-CA products.

Drug dissolved in	9-CA solvent	Dilution by	MCP.HCl		TRA	
			λ_{max} (nm)	Abs.	λ_{max} (nm)	Abs.
water	methanol	water	309	0.051	473	0.151
water	methanol	methanol	479	0.191	470	0.327
methanol	methanol	methanol	548	0.001	471	0.045
water	ethanol	water	458	0.035	473	0.112
water	ethanol	ethanol	439	0.112	472	0.268
ethanol	ethanol	ethanol	454	0.009	470	0.031
water	acetone	water	464	0.031	463	0.067
water	acetone	acetone	435	0.092	428	0.170
acetone	acetone	acetone	416	0.012	421	0.021
water	acetonitrile	water	472	0.025	472	0.092
water	acetonitrile	acetonitrile	435	0.145	475	0.181
acetonitrile	acetonitrile	acetonitrile	417	0.030	473	0.033
water	THF	water	499	0.02	473	0.023
water	THF	THF	419	0.103	476	0.133
THF	THF	THF	307	0.093	473	0.015

Effect of pH

The effect of pH on the colour intensity in the pH range between 1.32 and 12.0, obtained by suitable addition of 0.01 M of HCl and NaOH, was examined. It was found that the sensitivity of products decreased on addition of HCl or NaOH. However; the pH of the final dilution, measured in the absence of HCl and NaOH, was found 9.42. Different buffer solutions (bicarbonate, borate, tetraborate and ammonium of pH 9.5) were also examined. These showed a negative effect on the absorbance of the products (Fig. 2).

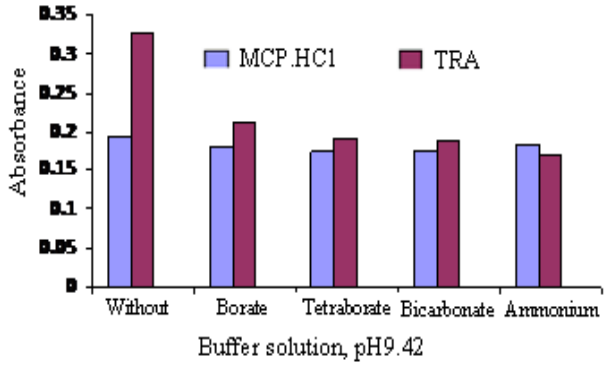


Figure 2. Effect of buffer solutions on the absorbance of 6 µg mL^{-1} MCP.HCl and 10 µg mL^{-1} TRA products with 9-CA reagent

Effect of reagent concentration

Different volumes of 9-CA (1×10^{-3} M), in the range 0-2.0 ml, were added to solutions containing same amounts of MCP.HCl and TRA (6 µg mL^{-1}) separately, and making the final volume to 5 ml. The absorbance was measured at 470 and 479 nm at room temperature against their respective reagent blank, respectively. It was evident that the absorbance increases with increasing 9-CA concentration and reached maximum value when 1.5-1.7 ml of 9-CA was used for both drugs (Fig.3). Finally, 1.5 ml of 9-CA was used in subsequent experiments.

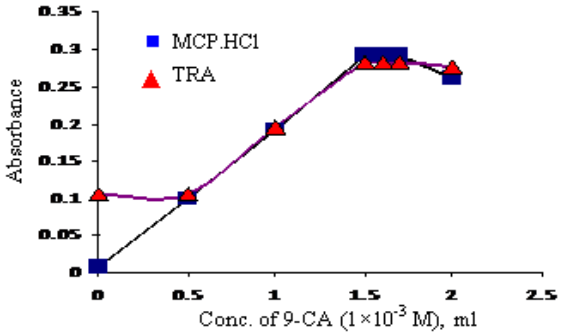


Figure 3. Effect of 9-CA reagent concentration

Effect of temperature and development time

The effect of temperature on the rate of reaction for drug–9-CA products was studied at room temperature (25 °C), 40 °C and 0 °C at the previous optimum reaction conditions. The results indicated that the products were formed after the addition of reagent immediately and reached its maximum absorbance at room temperature after 5 and 15 min and remained constant for 85 and 55 min for MCP.HCl and TRA after which the absorbance decreased indicating dissociation (Fig. 5).

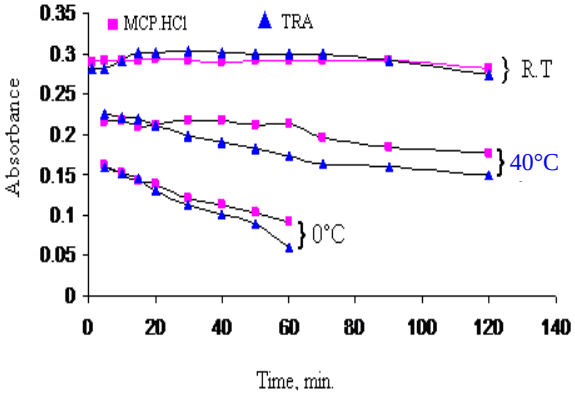


Figure 5. Effect of temperature and developing time for the absorption of 6 µg mL^{-1} MCP.HCl and 6 µg mL^{-1} TRA.

Effect of surfactants

Effect of various surfactants, SDS, CTAB, Tween-80 and Triton X-100, of 0.2 % concentration was examined on the absorption intensity of the drug – 9-CA products. It was found that the chosen surfactants had a negative effect on the absorbance of the products.

Quantitation

The results for the determination of MCP.HCl and TRA by 9-CA reagent are summarized in Table 2. The Beer's law limits and molar absorptivity values were evaluated and the results indicated that the method is sensitive. The linearity was represented by the regression equation and the corresponding correlation coefficient for drugs determined by the proposed method represents excellent linearity. The relative standard deviation (RSD) and accuracy (average recovery %) for the analysis of four replicates of each three different concentrations for paracetamol indicated that the method is precise and accurate. Limit of detection (LOD) and limit of quantitation (LOQ) were calculated according to the following equations:

$$LOD = \frac{3.3\sigma}{b} \quad \text{and} \quad LOQ = \frac{10\sigma}{b}$$

where σ is the standard deviation of five reagent blank determinations and *b* is the slope of the calibration curve. The results obtained are in the accepted range below the lower limit of Beer's law range.

Study of interferences

The extent of interference by some excipients which often accompany pharmaceutical preparations were studied by measuring the absorbance of solutions containing fixed amount of drug (10 μg mL^{-1}) and various amounts of diverse species in a final volume of 5 ml. It was found that the studied excipients did not interfere seriously even in the presence of 30 fold excess (Table 3). However; an error of 5.0 % in the absorbance readings was considered tolerable. This indicated that the method was free from interferences.

Table 2. Summary of optical characteristics and statistical data for the proposed method

Parameter	MCP.HCl	TRA
λ_{max} (nm)	470	479
Linear range (μg mL^{-1})	2-50	1-40
Molar absorptivity (L mol^{-1} cm^{-1})	8.503×10^3	7.074×10^3
LOD (μg mL^{-1})	0.110	0.055
LOQ (μg mL^{-1})	0.368	0.182
Sandell sensitivity (μg cm^{-2})	0.0417	0.0222
Average recovery (%)*	99.90	98.60
Correlation coefficient	0.996	0.998
Regression equation (*Y*) **		
Slope, *a*	0.024	0.045
Intercept, *b*	0.105	0.007
RSD*	≤0.456	≤0.279

* Average of five determinations, ** $Y = a\,X + b$, where *X* is the concentration of MCP.HCl and TRA in μg mL^{-1}.

Analytical applications

The proposed method was successfully applied to determine MCP.HCl and TRA in pharmaceutical tablet, syrup and injection preparations using three concentrations for each drug. The average recovery % was in the range 99.48 - 102.5 % for MCP.HCl and 96.67 -101.29 % for TRA indicating that the method is accurate (Table 4). The obtained results were compared statistically by a Student's *t*-test for accuracy and a variance ratio *F*-test for precision with the official method procedures[37,38] at the 95 % confidence level with four degrees of freedom, As cited in Table 5, the results showed that the experimental *t*-test and *F*-test were less than the theoretical value (t = 3.182, F = 9.12), indicating that there was no significant difference between the proposed method and official method.

Stoichiometry and stability constant

The molar ratio of the products formed between each of MCP.HCl and TRA with *9-CA* reagent were investigated by applying the continuous variation (Job's) and mole ratio methods.[39] The results indicated that products were formed in the ratio of 1:1 (Figure 6). This finding supports that these products are formed through amino group present in MCP.HCl and TRA. The stability constant (K_{st}) of the products were determined according to the previous ratio and found 2.962×10^4 and 8.026×10^4 L mol^{-1} respectively, indicating good stability.

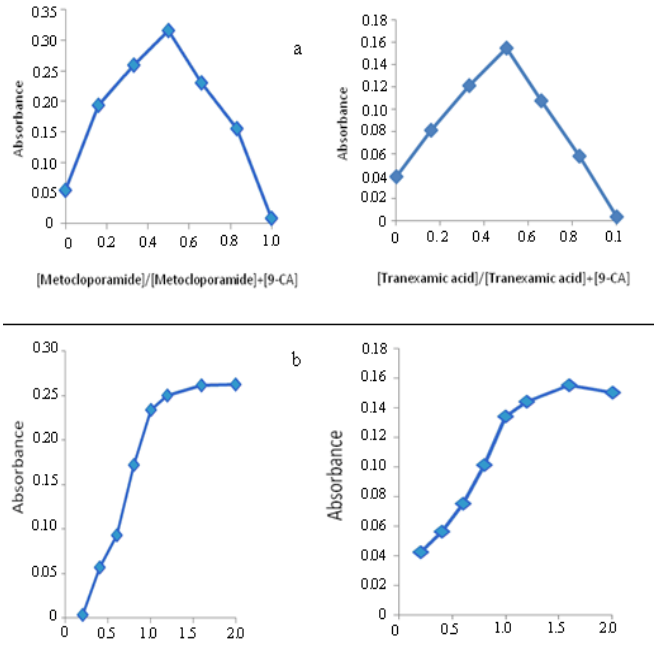


Figure 6. Continuous variation (a) and mole ratio (b) plots for drug– 9-CA products

Reaction mechanism

The colour produced from the interaction of MCP.HCl and TRA with 9-CA suggested that a free amino function in the molecule is necessary for the reaction. This finding is in agreement with the interaction of primary aromatic amines with the acridine[40,41] to form highly coloured solutions. The reaction mechanism has been postulated in Scheme 1.

Table 3. Effect of excipients on the determination of MCP.HCl and TRA

Exciepient	Recovery % of 10 µg mL^{-1} of target compounds in the presence of exciepient in µg mL^{-1}							
	MCP.HCl				TRA			
	50	100	200	300	50	100	200	300
Glucose	99.86	98.29	100.85	102.27	99.60	98.19	101.41	100.81
Lactose	100.28	101.70	102.27	101.98	98.99	99.60	97.38	100.40
Arabic gum	99.86	98.29	100.85	102.27	100.40	100.81	102.62	103.02
Sodium chloride	99.15	99.43	97.73	103.13	98.99	100.81	101.01	101.41
Sucrose	99.72	98.30	97.73	98.01	98.58	100.40	99.66	100.81
Starch	98.58	96.31	98.01	97.73	101.01	103.02	98.99	97.38

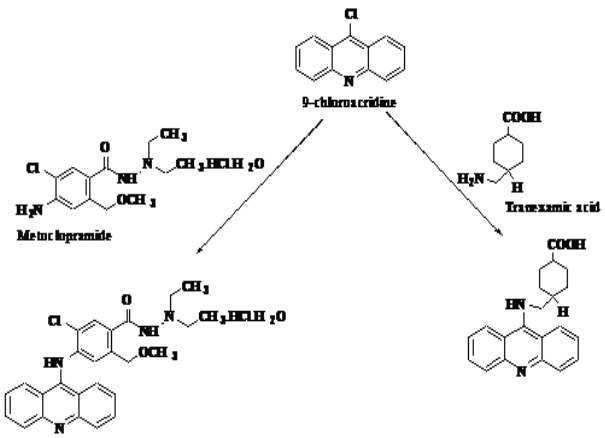
Table 4. Results of Assay of MCP.HCl and TRA in pharmaceutical preparations using the proposed method

Average recovery (mg)	Drug content Found * (mg)	Recovery* (%)	Amount present (µg.ml^{-1})	Certified value	Pharmaceutical preparation
10.2	10.25	102.5	10	10mg	Meclodin tablet[a]
	10.15	101.5	14		
	10.2	102.0	18		
5.03	5.09	101.7	10	5 mg/5 ml	Clopram syrup[b]
	4.97	99.48	14		
	5.02	100.46	18		
10.09	10.13	101.30	10	10 mg/2 ml	Metoclopramide.HCl injection[c]
	10.03	100.30	14		
	10.12	101.16	18		
505.64	508.0	101.16	10	500 mg	Transamine tablet[d]
	506.43	101.29	14		
	502.5	100.5	18		
245.86	249.50	99.8	10	250 mg/5 ml	Transamine injection[d]
	246.42	98.57	14		
	241.67	96.67	18		

*Average of four determinations, [a]Samarra drug industries , Iraq (S.D.I-Iraq), [b]The Arab pharmaceutical manufacturing Co. Ltd., Sult-Jordan, [c]Nanjnghuaxinbiopharm. CO. Ltd, manufactured in china, [d]Actavis, Istanbul, Turkey.

Table 5. Comparison of the proposed method with official method

F_{test}	t_{exp}	Recovery (%)		Pharmaceutical preparation	Drug
		Standard method[36,37]	Present method		
4.5	1.36	101.29	102.5	Meclodin tablet	MCP.HCl
5.9	2.1	101.29	99.48	Clopram syrup	
4.9	0.15	101.29	101.16	Metoclopramid. HCl injection	
5.1	0.23	100.40	100.46	Transamine tablet	TRA
1.53	1.57	100.42	99.83	Transamine injection	



9–[(2-chloro-4- (N-methyl-N-ethylamido) -5- methoxy)]amino-acridine

9-(cyclohexane carbcoxylic acid -4-y1) methyl aminoacridine

Scheme 1. Proposed reaction mechanism of 9-CA with MCP.HCl and TRA

Conclusion

A simple, selective and sensitive spectrophotometric method has been developed for the determination of microgram amounts of MCP.HCl and TRA based on their reaction with 9-CA reagent to form colored product having maximum absorption at 470 and 479 nm in methanolic medium respectively. The proposed method was applied successfully for the assay of the pharmaceutical formulations as tablet, syrup and injection.

References

[1]Martindal "*The Extra Pharmacopoeia*", 46th ed., The Pharmaceutical Press, London, **2000**, 1200.

[2]Ponte, C. D. and Nappi, J. M., *Am. J. Hosp. Pharm.*, **1981**, *38*, 829.

[3] Bennet, P. N. and Brown, M. J., *Clin. Pharm.*, **2008,** 522.

[4]Fatmi, A. A., Williams, G. V. *Drug. Dev. Ind. Pharm.*, **1989**, *15*, 1365.

[5]Ross, L. M., Eadie, M. J., Bochner, F., . Hooper, W. D. and Tyrer, J. H., *J. Chromatogr. Biomed. Appl.*, **1980**, *9*, 175.

[6]Riggs, K. W., Szetz, A., Runak, D. W., Mull, A. E., Abbote, B. F. S and Axeison, J. E., *J. Chromatogr. B. Biomed. Appl.*, **1994**, *660*, 315.

[7]Bryson, S. M. and Gibert, L. M., *J. Clin. Pharm. Therap.*, **2008**, *9*, 263.

[8]Vejendla, R., Kudidhi1,V., Sravanthi, S., Taruni, G., Musthafa, M. D., *Int. J. Pharm. Phytopharmacol. Res.*, **2013**, *3*, 83.

[9]Jawan, B. and Owas, L. *Int. J. Pharm.*, **1986**, *28*, 44.

[10]Wang, Z. H., Zhary, H. Z., Zhou, S. P. and Dong, J., *Talanta*, **2001**, *53*, 1133.

[11]Park, M. K., Lim, B. R., Yu, K. S. and Yong, K. H., *Yakhar Hoe Chi*, **1978**, *22*, 27.

[12]Buna, M., Haron, J., .Prognen, P. and Mah, F., *Analyst*, **1996**, *121*, 1551.

[13]Al-Arfa, N. A., *Talanta*, **2004**, *62*, 255.

[14]Al-Haideri, A. A., Abdulla, N. I. and Malih, I. J. K., *Iraqi J. Pharm Sci.*, **2012**. *21*, 70.

[15]Patil, K. R., Rane, V. P., Sangshetti, J. N. and Shinde, D. B., *Eurasian J. Anal. Chem.*, **2010**, *5*, 204.

[16]Khuhawar, M. Y. and Rind, F. M. A., *Chromatographia*, **2001**, *53*, 709.

[17]Tampubolon, H. B. and Sumarlik, E., *J. Liq. Chromatogr. Related. Techn.*, **2005**, *28*, 3243.

[18]Elazazy, M. S., El-Mammli, M. Y., Shalaby, A. and Ayad M. M., *Chem. Anal. (Warsaw)*, 2008, *53*,725.

[19]Al-Abachi, M. Q. and Al-Ward, H. S., *Natl. J. Chem.*, **2002**, *7*, 363.

[20]Amin, A. S. and Ragab, G. H., *Anal. Sci.,* **2003**, *19*, 747.

[21]Revanasiddappa, H. D. and Manju, B., *J. Pharm. Biomed. Anal.*, **2001**, *25*, 631.

[22]Shah J., Rasul J. M., Azam K., M. and Amin S., *J. Anal. Chem.*, **2005**, *60*, 633.

[23]Moussa, B. A., *J. Pharm. Biomed. Anal.*, **2000**, *23*, 1045.

[24]El-Gendy, A. E., *Spectroscopy Lett.*, **1992**, *25*, 1297.

[25]Fan, J., Wang, A. J., Feng, S. L. and Wang, J. J., *Talanta*, **2005,** *66*, 236.

[26]Abdel-Gawad, F. M., El-Guindi, N. M., *Anal. Lett.*, **1995**, *28*, 1437.

[27]Herrero, M. R., Romero, A. M. and Calatayud, J. M., *Talanta*, **1998**, *47***,** 223.

[28]Ramappa, P. G. and Revanasiddappa, H. D., *Indian Drugs*, **1999**, *36*, 381.

[29]Rind, E. M., Laghari, M. G., Memon, A. H., Mughal, U. R., Almai, F., Meman, N., Khuhawar, M. Y. and Maneshwari, M. L., *Yaoxue XueBao*, **2009**, *44*, 125.

[30]Abulghasem, El A. K. and Mohamed, A. A., *Chem. Pharm. Bull.*, **2007**, *55*, 364.

[31]Kariem, El R. A., Jabbir, M. A. A., *World App. Sci. J.*, **2012**, *19*, 1263.

[32]Mohamed, M. A., Kariem, E. A. and Jabbir, M. A. A., *J. Anal. Techn.*, **2012**, *1*, 2.

[33]Agarwa, S. L. and Murthy, R., *Int. J. Res. Pharm. Biomed. Sci.*, **2012**, *3*, 2.

[34]Raza A., *Anal. Lett.*, **2006**, *39*, 2217.

[35]Dhamra, M. Y., *J. Educ. Sci.*, **2011**, *24*, 21.

[36]Mohite, P. B., Pandhare, R. B., Khanage, S. G., and Bhaskar, V. H., *Asian J. Res. Chem.*, **2009**, *2*, 273.

[37]*British Pharmacopoeia*, CD-Rom. The Stationery Office, London, **2010**.

[38]*British Pharmacopoeia* on CD-Rom, 3rd Edn., System Simulation Ltd. The Stationery Office, London, **2000**.

[39]Hargis, L.G.. "*Analytical Chemistry, Principles and Techniques*", Prentice- Hall Inc., New Jersey. **1988**, 424- 427.

[40]Stewart J. T., Ray, A. B. and Fackler, W. B., *J. Pharm. Sci.*, **1969,** *58*, 1261.

[41]Gammans, R. E., Stewart, J. T. and Sternson, L. A., *Anal. Chem.*, **1974**, *46*, 62.

Received: 13.04.2015.
Accepted: 05.06.2015.