ISSN : 0974-7419 Volume 14 Issue 6

Analytical CHEMISTRY

An Indian Journal

Full Paper

ACAIJ, 14(6) 2014 [220-227]

Compatible spectrophotometric methods for the determination of two antihypertensive mixtures containing hydrochlorothiazide in pharmaceutical preparations

Maha Farouk, Omar Abd El-Aziz, Marwa H.El-Taweel*

Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Ain Shams University, Abbassia, Cairo, 11566, (EGYPT)

E-mail : marwa.eltaweel@hotmail.com

ABSTRACT

Simple, rapid, accurate and precise spectrophotometric methods have been developed and subsequently validated for simultaneous determination of telmisartan (I) and bisoprolol fumarate (II), separately, in binary mixtures with hydrochlorothiazide (HCTZ). Isosbestic point technique was the first method adopted for determination of (I) and (HCTZ) mixture, by utilizing zero-order and first derivative spectra (D^1) at 266.0 and 323.0 nm for the total concentration and (I), respectively. The second method was ratio subtraction technique used for determination of bisoprolol fumarate in presence of hydrochlorothiazide at 224.4 nm, using hydrochlorothiazide (10 $\mu g.ml^{-1}$) as a divisor. Dual wavelength technique was the third way used for hydrochlorothiazide determination in presence of bisoprolol fumarate, by using the absorbance difference at 266.0 and 277.8 nm. The proposed methods have been validated according to International Conference Harmonization (ICH) guidelines and were found to be valid and suitable for the assay of the cited drugs in raw materials and in combined dosage forms. All the obtained results for the mentioned drugs were statistically compared with those of reference and United State Pharmacopeia for [(I) and (HCTZ)] and [(II) and (HCTZ)] and no significant differences were found.

KEYWORDS

Telmisartan;
Bisoprolol fumarate;
Hydrochlorothiazide;
Isosbestic point;
Ratio subtraction;
Dual wavelength spectrophotometric methods.

INTRODUCTION

Telmisartan (I) is 4'[(1, 4'-Dimethyl-2'-propyl [2, 6'-bi-1H-benzimidazol]-1'-yl) methyl] [1, 1'-biphenyl]-2-carboxylic acid, acts as an angiotensin II receptor blockers, used mainly for treatment of hypertension[1] (Figure 1a), Bisoprolol fumarate (II), 1-[4-(2-Isopropoxyethoxymethyl) phenoxy]-3-isopropylaminopropan-2-ol fumarate (2:1) salt, is a selective β1 adrenergic receptor blocker [2].It is given in the management of hypertension and angina pectoris[1] ((Figure 1b) and hydrochlorothiazide (HCTZ) is 6-Chloro-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulphonamide 1, 1-dioxide, a drug belonging to thiazide diuretics class of antihypertensive drugs. It is effective in the treatment of hypertension[2] ((Figure 1c).

Alone or in combination with HCTZ, (I) has been determined by derivative spectrophotometric[3],

spectrofluorimetric[3], electrochemical[4,5], immunoassay[6], titrimetric[7], capillary zone electrophoretic[8-10], high performance thin layer chromatographic[3] and high performance liquid chromatographic[11-16] methods.

In literatures, Several methods were reported for determination of (II) including electrochemical methods alone[17] or in combination with other β blockers[18,19], high-performance liquid chromatographic methods in pharmaceutical formulations[20] with other cardiovascular drugs[21] and HCTZ[22,23]. Also, (II) could be determined in waste water[24], in river water[25], in human plasma, in urine alone[26-29] or in combination with HCTZ [30,31] and other cardiovascular drugs[32,33].

The main goal of this work is to establish simple, rapid, accurate, precise, and low cost spectrophotometric methods for the simultaneous determination of (I) and (II), separately, in binary mixtures with HCTZ, which can be adopted for the routine quality control analysis of the investigated drugs without prior separation in raw material and in pharmaceutical preparations.

Ratio subtraction technique was the second adopted method having the following theory:

A mixture of two drugs X and Y with overlapping spectra can be resolved by ratio subtraction, if the spectrum of one drug, say (Y) is extended more than the other, say (X) can be determined by dividing the spectrum of the mixture by a certain concentration of Y as a divisor (Y'). The division will give a new curve that is represented by:

X / Y' + Constant

If the constant is subtracted, then the new curve obtained is multiplied by Y', the original curve of X is obtained.

This can be summarized in the following equations:

(X+Y) / Y' = (X / Y') + (Y / Y') = X / Y' + Constant

X / Y' + Constant - Constant = X / Y'

X / Y' x Y' = X

The constant can be determined directly from the curve (X+Y) / Y' by the straight line that is parallel to the wavelength axis in the region where Y is extended.

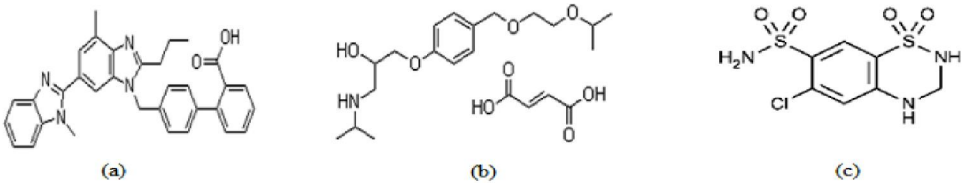

Figure 1 : Chemical structure of (I), (II) and (HCTZ).

EXPERIMENTAL

Instrumentation

Double-beam UV-Visible spectrophotometer (Shimadzu, Japan) model UV-1601 PC with quartz cell of 1 cm path length, connected to a computer fitted with UVPC personal spectroscopy software version 3.7 (Shimadzu) was used. The spectral bandwidth was 0.2 nm and the wavelength scanning speed was 1000 nm.min^{-1}. The measurements were done at 25.0 ˚C, using $\Delta\lambda$= 4 nm and scaling factor of 10 for computing first derivative (D^1).

Materials and reagents

(I) was kindly supplied by National Organization for Drug Control and Research-Egypt and certified to contain 99.90%. Micardis plus® tablets: batch No. (203232A) manufactured by Boehringer Ingelheim Pharmaceutical Company. Each tablet was labeled to contain 40 mg (I) and 12.5 mg HCTZ. (II) and HCTZ were kindly provided by Hikma Pharmaceuticals-Egypt and certified to contain 99.70% and 99.75%, respectively. Concor plus® tablets: batch No. (130781) manufactured by Serono Pharmaceutical Company. Each tablet was labeled to contain 5 mg (II) and 12.5 mg HCTZ.

Methanol (Sigma- Aldrich, Germany) and Sodium hydroxide (BDR) (Prolabo), aqueous 0.1M were used. All chemicals and reagents used through this work are of spectroscopic analytical grade.

Standard solutions and laboratory prepared mixtures:

Stock standard solutions:

For (I)/HCTZ mixtures, two stock standard solutions each having a concentration 1.00 mgml^{-1} were prepared in 0.1M NaOH and for (II)/HCTZ mixtures, two stock standard solutions each having a concentration

Full Paper

1.00 mgml^{-1} were prepared in methanol, respectively.

Working standard solutions

Aliquots of (I), (II) standard stock solutions were transferred separately into 100 ml volumetric flasks and the volumes were completed to the mark with 0.1 M NaOH and methanol, respectively to obtain 50.0 μg ml^{-1} used as working solution each. Also, working solutions of HCTZ were prepared by the same way, once with 0.1 M NaOH and the other with methanol, to obtain the last mentioned concentration.

Procedures

Isosbestic method

From (I) and (HCTZ) working solutions, accurately measured volumes were transferred separately into a series of 10-ml volumetric flasks, diluted to the volume with 0.1M NaOH. The zero order absorption spectra of each solution were recorded versus diluent and stored; then the absorbance at the isosbestic point (A_{iso}) was measured at 266.0 nm for both drugs. Two calibration curves were constructed relating the absorbance at the selected wavelength to the corresponding drug concentration. While for (I), the amplitude of first derivative spectrum (D^1) at 323.0 nm were recorded, using $\Delta\lambda$ = 4 nm and scaling factor of 10, plotted against the corresponding concentrations and then the regression equations were computed.

Ratio subtraction method

The overlapping spectra of a binary mixture, (II) with hydrochlorothiazide (HCTZ) were resolved by adopting the ratio subtraction technique. The spectra of (II) working standard solutions were scanned from 200–400 nm and stored in the computer. The spectra of the laboratory-prepared mixtures were divided (absorbance at each wavelength) by the spectrum of 10.0 μg ml^{-1} of (HCTZ). The absorbance in the plateau region was subtracted at wavelength above 305 nm (the constant). The obtained curves were multiplied (absorbance at each wavelength) by the spectrum of 10.0 μg ml^{-1} of (HCTZ). The absorbance of the ratio subtraction was computed at 224.4 nm, plotted versus concentrations, and the regression equation was then computed.

Dual wavelength method

Also, the overlapping between (II) and (HCTZ) spectra was resolved by adopting the dual wavelength technique, where the absorbance difference of the spectra (ΔA) corresponding to (HCTZ) was recorded at λ= 266.0 and 277.8 without any interference of (II). The calibration curve was constructed and the linear regression equation was then computed.

Application to pharmaceutical formulations

Twenty tablets from each Micardis plus® and Concor plus® tablets were individually weighed to get the average weight of the tablets, respectively. A sample of the powdered tablets, equivalent to one tablet of the mixed contents was separately transferred into a 50-ml volumetric flask using about 25 ml 0.1 M NaOH (in Micardis plus®) and methanol (in Concor plus®), shaking for 15 minutes and completed to volume with the same solvent. The contents of the flask were mixed well and filtered. Aliquots of filtrate were further diluted with the same mentioned solvents and then proceeds as described under (3-1.1. – 3.1.3.).

To check the validity of the proposed methods, the standard addition technique was applied and the procedures mentioned under (3.1.1. – 3.1.3.) were adopted.

RESULTS AND DISCUSSION

Spectrophotometric characteristics

The zero order spectra of the selected drug mixtures shown in (Figure 2 and 3) exhibit high degree of spectral overlapping which interfered with their simultaneous determination. This spectral overlapping is sufficient to demonstrate the resolving power of the proposed spectrophotometric methods which can resolve bands overlapping, without prior separation.

Isosbestic method

Isosbestic point is the wavelength at which the absorbance of two or more species is the same. At this point, the mixture of drugs acts as a single component and gives the same absorbance value as of the pure one. The zero order spectra of (I), (HCTZ) and their mixture showed three isosbestic points (Figure 2), where the absorbance value (A_{iso}) corresponding to the mixture could be recorded at 266.0 nm. While (I) could be detected at 323.0 nm, without interference from

(HCTZ) by adopting first derivative spectrophotometric technique (D^1) as shown in (Figure 4). Finally, (HCTZ) concentration could be calculated by subtraction.

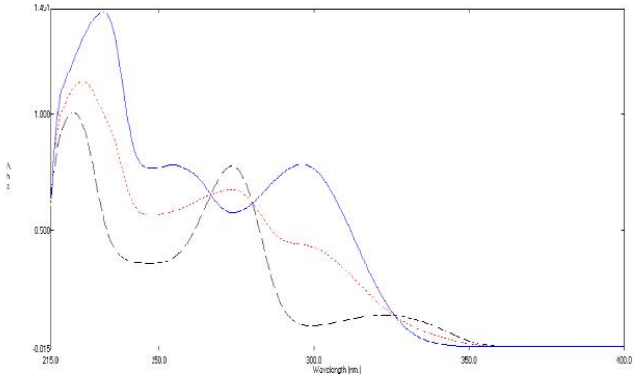

Figure 2 : Zero order spectra for (I) (—), (HCTZ) (- - -) (16.00 µg.ml^{-1}, each) and their mixture (….) (8.00 µg.ml^{-1}, each) using 0.1M NaOH as a blank.

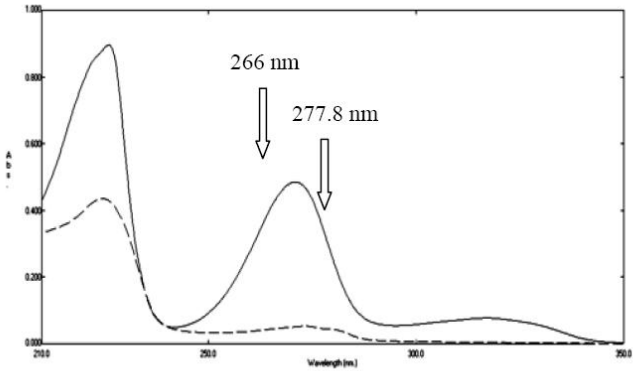

Figure 3 : Zero order spectra for (II) (- - -), (HCTZ) (—) (10.00 µg.ml^{-1}, each) using methanol as a blank

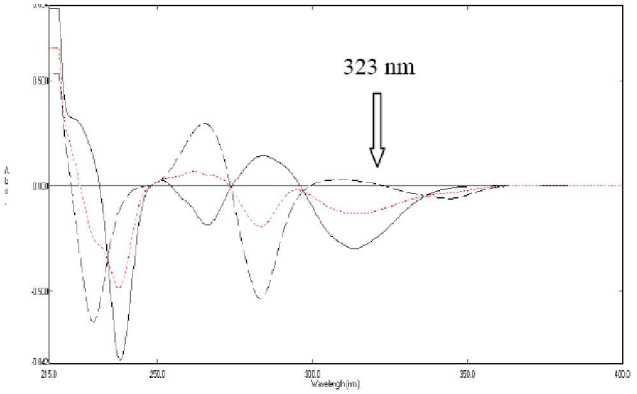

Figure 4 : First order derivative spectra for (I) (—), (HCTZ) (- - -) (16.00 µg.ml^{-1}, each) and their mixture (….) (8.00 µg.ml^{-1}, each).

Ratio subtraction method:

The spectrum of (HCTZ) is extended more than that of (II) which permits the use of ratio subtraction technique, where 224.4 nm was selected as optimum working wavelength for the determination of (II) in presence of (HCTZ) without any interference, as shown in (Figure 5-7).

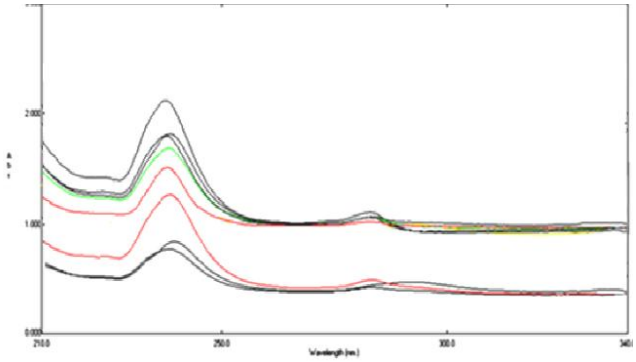

Figure 5 : Zero order absorption spectra for different mixtures of (II) and (HCTZ), using (10 µg.ml^{-1}) (HCTZ) as a divisor.

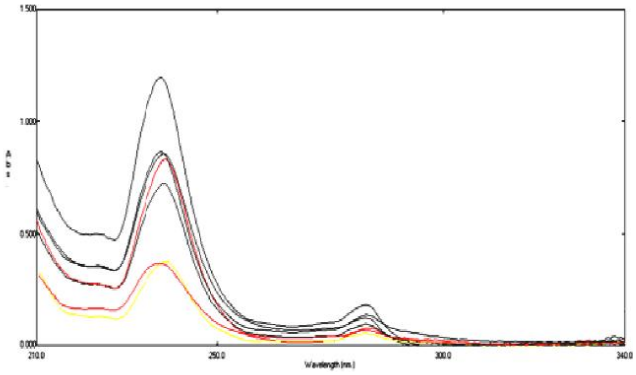

Figure 6 : Zero order absorption spectra for different mixtures of (II) and (HCTZ), using (10 µg.ml^{-1}) (HCTZ) as a divisor, after subtraction of constant.

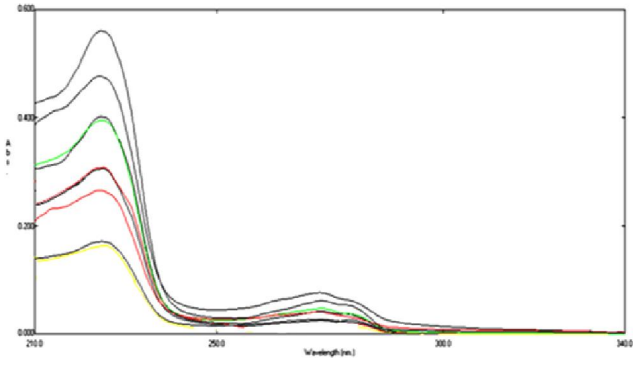

Figure 7 : Zero order absorption spectra for different mixtures of (II) and (HCTZ), using (10 µg.ml^{-1}) (HCTZ) as a divisor, after subtraction of constant and multiplying with the divisor.

Careful choice of the divisor is of great importance, as variable concentrations of (HCTZ) were tried and the best chosen one was 10.00 µg.ml^{-1}, which yielded best compromise in sensitivity, repeatability and signal

to noise ratio.

Dual wavelength method

The absorbance values of (II) were the same at 266.0 and 277.8 nm, so the absorbance difference between these two wavelengths will give the value representing (HCTZ) concentration without interference, as shown in (Figure 3).

Method validation

ICH-guidelines for method of validation were followed, where all the validation parameters are shown in (TABLE 1).

Linearity

A linear correlation was obtained between the absorbance and the concentration of the investigated drugs (I), (II) and (HCTZ). The statistical parameters and regression equations calculated are shown in (TABLE 1). The results obtained showed that linearity of the calibration graphs and the compliance with Beer's law were validated, as illustrated by the high values of correlation coefficients of regression equations and the small values of intercepts.

Limits of detection and quantification

In accordance with the formulae given by Miller[34], the limit of detection, LOD = 3.3 S/b and the limit of quantification, LOQ = 10 S/b, where S is the standard deviation of response and b is the slope of the calibration graph. The detection and quantification limits were calculated and presented in (TABLE 1).

Accuracy

The accuracy of the proposed methods was tested by analyzing freshly prepared solutions of the studied drugs in triplicate. The recovery percent and standard deviations showed in (TABLE 1), revealed an excellent accuracy.

Precision

The intra- and inter-assay precisions were estimated by analyzing three-level QC samples triplicate on the same and on three consecutive days, respectively. The results showed good precision expressed as percentage relative standard deviation, as showed in (TABLE 1).

Selectivity

This was assessed through analysis of different synthetic mixtures, containing '(I) and (HCTZ)' and '(II) and (HCTZ)' with different ratios by the proposed mentioned methods. The concentration of each drug was calculated from the corresponding regression equation and then the mean recovery percentages and the relative standard deviations were calculated. The results showed good selectivity as shown in (TABLE 1).

TABLE 1 : Results of assay validation obtained by the proposed spectrophotometric methods

Parameters	Isosbestic point			Ratio subtraction	Dual wavelength
	(I)		(HCTZ)	(II)	(HCTZ)
	λ_{266}	λ_{323}	λ_{266}	$\lambda_{242.4}$	ΔA
Linearity range ($\mu g.ml^{-1}$)	2 - 40	2 - 40	2 - 40	2 - 30	1 - 20
Intercept (a)	0.0081	0.0028	0.0073	-0.0004	-0.0039
Slope (b)	0.0411	0.0135	0.0417	0.0425	0.0125
Correlation coefficient (r)	0.9998	0.9999	0.9999	0.9999	0.9999
Accuracy [a]	99.76± 0.83	99.99± 0.44	99.74 ±0.70	100.03 ± 0.82	99.80 ± 0.52
Repeatability [c]	100.43 ± 0.78	99.72 ± 1.03	99.18 ± 0.82	99.23 ± 1.37	100.70 ± 0.69
Intermediate precision [c]	99.76± 1.12	100.54± 0.62	99.16 ± 0.81	99.90 ± 1.85	99.45 ± 0.97
Selectivity [b]	100.23 ± 1.21		99.31 ± 0.75	99.81 ± 0.79	99.62 ± 0.89
S_a	0.00517	0.001035	0.00405	0.00196	0.00049
S_b	0.000271	0.0000542	0.000199	0.000119	0.0000515
LOD ($\mu g.ml^{-1}$)	0.42	0.25	0.32	0.15	0.13
LOQ ($\mu g.ml^{-1}$)	1.26	0.77	0.97	0.46	0.39

Regression equation: A = a + bc, where A is the absorbance, a is the intercept, b is the slope and c is the concentration. S_a: standard deviation of intercept, S_b: standard deviation of slope.; [a] Mean ± SD, [b] Mean ± RSD%, [c] Mean ± RSD%.

Assay of pharmaceutical preparations and standard addition technique

The proposed methods were applied for the determination of the studied drugs in the pharmaceutical preparations. The results were satisfactory and with good agreement with the labeled amount. Moreover, to check the validity of the adopted proposed methods, a standard addition method was applied by adding known amounts of the studied drugs to the previously analyzed tablets. The recoveries were calculated

TABLE 2 : Quantitative determination of (I) and (HCTZ) in the pharmaceutical preparation and application of standard addition technique by the proposed method

Pharmaceutical preparation	Isosbestic point %Recovery*		Standard addition technique			
			(I)		(HCTZ)	
	(I)	(HCTZ)	Added ($\mu g.ml^{-1}$)	% Recovery*	Added ($\mu g.ml^{-1}$)	% Recovery*
Micardis 40 plus®	99.75 ± 0.90	99.6± 0.68	5	99.4	1	99.00
			6	100.67	2	98.50
			8	99.75	2.5	100.40
			9	100.60	3	98.27
			10	100.50	4	99.25
	Mean ± SD			100.18 ± 0.56		99.08 ± 0.83
	RSD%			0.56		0.84

* Average of three determinations

TABLE 3 : Quantitative determination of (II) and (HCTZ) in the pharmaceutical preparation and application of standard addition technique by the proposed methods.

Pharmaceutical preparation	Ratio subtraction			Dual wavelength		
	% Recovery*	Standard addition		% Recovery*	Standard addition	
		Added ($\mu g.ml^{-1}$)	% Recovery*		Added ($\mu g.ml^{-1}$)	% Recovery*
Concor 5 plus®	98.20 ± 0.28	4	101.20	100.18 ± 0.75	5	99.71
		5	99.40		6	98.52
		6	101.07		7	98.43
		10	100.30		8	99.90
	Mean ± SD		100.49 ±0.83			99.25 ± 0.72
	% RSD		0.83			0.73

* Average of three determinations.

TABLE 4 : Statistical comparison of the results obtained by the proposed methods and the reported and USP methods for determination of the examined drugs.

Value	(I)		(HCTZ)		(II)		(HCTZ)	
	Isosbestic point	Reference method*	Isosbestic point	Reference method*	Ratio subtraction	USP method	Dual wavelength	USP method
Mean	99.76	99.27	99.74	99.48	100.03	100.68	99.80	99.57
SD	0.83	0.51	0.70	0.38	0.82	0.83	0.52	1.00
n	8	6	7	6	6	6	7	6
Variance	0.68	0.26	0.49	0.14	0.67	0.69	0.27	1.00
t –value	1.38 (2.18)	-	0.85(2.26)	-	1.37 (2.23)	-	0.52 (2.36)	-
F-value	2.68 (4.88)	-	3.42(4.95)	-	1.02 (5.05)	-	3.62 (4.38)	-

Values in parenthesis are the theoretical values of t and F at P = 0.05.; * Inertsil ODS-C18 (250mmX4.6mm) using (40: 60, v/v) phosphate buffer of pH 3.0 and acetonitrile in an isocratic program with flow rate 1.0 ml/min and UV detection was performed at 271 nm. The retention times observed were 5.79 min and 2.85 min for telmisartan and hydrochlorothiazide respectively.

by comparing the concentration obtained from the spiked samples with that of each pure drug. The results of the analysis are shown in (TABLE 2 and 3) suggested that there is no interference from any excipients.

Comparison of the proposed methods with the reported and pharmacopeial methods

The results obtained by applying the proposed methods were compared statistically with those obtained from a reported method[35] for (I) and (HCTZ) mixture and united state pharmacopeial method[23,36] for (II) and (HCTZ) mixture, where each of the calculated *t* and F values were less than those tabulated ones, indicating that there is no significant difference between the proposed and those reported and pharmacopeial methods, as shown in (TABLE 4).

CONCLUSION

The proposed methods provide new, simple, rapid, accurate and reproducible quantitative analysis for determination of '(I) and (HCTZ)' in a binary mixture, (II) in presence of (HCTZ) and (HCTZ) in presence of (II), without any interference from excipients or prior separation.

REFERENCES

[1] S.C.Sweetman, (Ed); Martindale: The Complete Drug Reference, 36th Edition., Pharmaceutical Press; London, **(2009)**.

[2] R.Finkel, M.A.Clark, L.X.Cubeddu; Lippincott's Illustrated Reviews: Pharmacology, 4th Edition, Edition. R.A.Harvey and P.C.Champe, Lippincott Williams & Wilkins, **(2009)**.

[3] L.I.Bebawy, S.S.Abbas, L.A.Fattah, H.H.Refaat; Il Farmaco., **60(10)**, 859-867 **(2005)**.

[4] H.Yi, W.Huang; Colloids Surf., B., **58(2)**, 237-241 **(2007)**.

[5] M.Xu, J.Song, Y.Liang; J.Pharm.Biomed. Anal., **34(3)**, 681-687 **(2004)**.

[6] C.Hempen, L.Gläsle-Schwarz, U.Kunz, U.Karst; Anal. Chim. Acta., **560(1-2)**, 35-40 **(2006)**.

[7] S.H.Patil, M.V.Janjale; J.Pharm. Anal., **2(6)**, 470-477 **(2012)**.

[8] S.Hillaert, W.Van den Bossche; J.Pharm. Biomed. Anal., **31(2)**, 329-339 **(2003)**.

[9] S.Hillaert, T.R.M.De Beer, J.O.De Beer, W.Van den Bossche; J. Chromatogr., A., **984(1)**, 135-146 **(2003)**.

[10] M.Zhang, F.Wei, Y.F.Zhang, J.Nie, Y.Q.Feng; J. Chromatogr., A., **1102(1-2)**, 294-301 **(2006)**.

[11] N.Ferreirós, G.Iriarte, R.M.Alonso, R.M.Jiménez; Talanta., **73(4)**, 748-756 **(2007)**.

[12] L.Kristoffersen, E.L.Øiestad, M.S.Opdal, M.Krogh, E.Lundanes, A.S.Christophersen; J. Chromatogr., B., **850(1-2)**, 147-160 **(2007)**.

[13] T.Yan, H.Li, L.Deng, Y.Guo, W.Yu, J.P.Fawcett, D.Zhang, Y.Cui, J.Gu; J. Pharm. Biomed. Anal., **48(4)**, 1225-1229 **(2008)**.

[14] I.Salama; Bulletin of Faculty of Pharmacy, Cairo University., **49(1)**, 19-24 **(2011)**.

[15] V.K.Gupta, R.Jain, O.Lukram, S.Agarwal, A.Dwivedi; Talanta., **83(3)**, 709-716 **(2011)**.

[16] V.B.Ravi, J.K.Inamadugu, N.R.Pilli, V.Sreenivasulu, V. Ponneri; J. Pharm. Anal., **2(5)**, 319-326 **(2012)**.

[17] R.N.Goyal, A.Tyagi, N.Bachheti, S.Bishnoi; Electrochim. Acta., **53(6)**, 2802-2808 **(2008)**.

[18] B.Nigović, M.Marušić, S.Jurić; J. Electroanal. Chem., **663(2)**, 72-78 **(2011)**.

[19] S.S.M.Hassan, M.M.Abou-Sekkina, M.A.El-Ries, A.A.Wassel; J. Pharm. Biomed. Anal., **32(1)**, 175-180 **(2003)**.

[20] X.Zhang, J.Ouyang, W.R.G.Baeyens, S.Zhai, Y.Yang, G.Huang; J. Pharm. Biomed. Anal., **31(6)**, 1047-1057 **(2003)**.

[21] P.Modamio, C.F.Lastra, E.L.Mariño; J.Pharm. Biomed. Anal., **14(4)**, 401-408 **(1996)**.

[22] S.Shaikh, O.A.Thusleem, M.S.Muneera, J.Akmal, A.V.Kondaguli, K.Ruckmani; J. Pharm. Biomed. Anal., **48(3)**, 1055-1057 **(2008)**.

[23] S.J.Joshi, P.A.Karbhari, S.I.Bhoir, K.S.Bindu, C.Das; J. Pharm. Biomed. Anal., **52(3)**, 362-371 **(2010)**.

[24] P.P.Vázquez, M.M.Galera, A.S.Guirado, M.M.P.Vázquez; Anal. Chim. Acta., **666(1-2)**, 38-44 **(2010)**.

[25] M.Martínez Galera, M.D.Gil García, M.J.Culzoni, H.C.Goicoechea; J.Chromatogr., A., **1217(13)**, 2042-2049 **(2010)**.

[26] T.Suzuki, Y.Horikiri, M.Mizobe, K.Noda; J. Chromatogr., B: Biomed. Sci. Appl., **619(2)**, 267-273 **(1993)**.

[27] E.Caudron, S.Laurent, E.M.Billaud, P.Prognon; J. Chromatogr., B., **801(2)**, 339-345 **(2004)**.

[28] L.Ding, X.Zhou, X.Guo, Q.Song, J.He, G.Xu; J. Pharm. Biomed. Anal., **44(2)**, 520-525 **(2007)**.

[29] J.Bhatt, G.Subbaiah, S.Kambli, B.Shah, M.Patel,

A.Saxena, A.Baliga, S.Nigam, H.Parekh, G.Yadav; J. Chromatogr., B., **852(1-2)**, 374-381 **(2007)**.

[30] K.U.Bühring, A.Garbe; J. Chromatogr., B: Biomed. Sci. Appl, **382**, 215-224 **(1986)**.

[31] M.F.Tutunji, H.M.Ibrahim, M.H.Khabbas, L.F.Tutunji; J. Chromatogr., B., **877(16-17)**, 1689-1697 **(2009)**.

[32] S.Li, G.Liu, J.Jia, Y.Liu, C.Pan, C.Yu, Y.Cai, J.Ren; J. Chromatogr., B., **847(2)**, 174-181 **(2007)**.

[33] O.Gonzalez, G.Iriarte, E.Rico, N.FerreirÃ³s, M.I.Maguregui, R.M.Alonso, R.M.Jiménez; J. Chromatogr., B., **878(28)**, 2685-2692 **(2010)**.

[34] J.N.Miller, J.C.Miller; 'Statistics and Chemometrics for Analytical Chemistry', 4th Edition, Prentice Hall; Harlow, England, **(2000)**.

[35] T.G.Swamy, K.Nagaraju, A.L.Rao; Int. J. Drug Dev. Res., **3(4)**, 362-368, Oct-Dec **(2011)**.

[36] 'United States Pharmacopoeia 30 - National Formulary 25', United States Pharmacopoeial Convention Inc.; Maryland, **(2007)**.