Egypt. J. Anal.Chem, Vol. 22, PP. 84-96 (2013)

Validated Spectrophotometric Methods for the Determination of Rosuvastatin Calcium

Sawsan A. Abdel-Razeq[(1)] ,Maha A.Marzouk[(2)] and Marwa M. Soliman[(3)]

Analytical Chemistry [(1,3)]and Pharmaceutics [(2)] Departments, Faculty of Pharmacy (Girls), Al-Azhar University, Cairo, Egypt.

E-mail address: razeqhegab@yahoo.com

Summary: Three simple and accurate spectrophotometric methods are developed for the determmination of Rosuvastatin-Ca. The first one is atomic absorption spectrometry for determination of drug through its calcium content at 422.7 nm .The second method depends on the reaction of drug as an n-donor with three π-acceptors; dichloro-dicyanobenzoquinone, tetracyanoquinodimethane and dichlorophenol indophenol. Highly colored radical anions are obtained in the polar solvents used having λ_{max} 460, 841 and 640 nm, respectively. The third method is based on the formation of red ion- association complex between rosuvastatin-Ca and basic fuchsin in presence of phosphate buffer (pH=8.3±0.1) which is extracted in $CHCl_3$ and measured at 484 and 555 nm. Beer's law is obeyed for the cited drug within the concentration ranges of 100-500, 40-160, 5-50, 10-80 and 4-20 μg mL^{-1} rosuvastatin-Ca for AAS and the four reagents used, respectively. The proposed methods have been successfully applied to the analysis of the studied drug in pharmaceutical formulations with mean recoveries range from 97.29±0.82 to 100.59±1.98. The methods are also validated according to ICH guidline.

Introduction

Rosuvastatin-Ca is an antihyperlipidemic agent chemically known as 6-heptenoic acid,7-[4-((4-flurophenyl)-6-(1-methylethyl)-2-[methyl(methylsulphonyl)amino]-5-pyrimidinyl]-3,5-dihydroxy-, calcium salt (2:1),(3R,5S,6E). It acts by inhibiting the enzyme 3-hydroxy-3-methyl glutryl-coenzyme A (HMG-CO A) reductase[(1)]. Reviewing the literature, only few analytical techniques have been published for its estimation in pharmaceutical formulations and in biological fluids including HPTLC[(2)], LC-MS[(3)], HPLC[(4-9)] and spectrophotometry[(10-12)] . The main target of this work is to develop simple and efficient spectrophotometric methods for the determination of the drug in bulk powder and pharmaceutical formulations.

Experimental

Apparatus

Perkin Elmer A/Analyst 400 atomic absorption spectrometer (USA), Shimadzu UV/Vis spectrophotometer PC – 1601, (Tokyo, Japan) and Jenco pH meter (608) with jenway double junction glass electrode, (England) were used.

Samples

Pure rosuvastatin, B.N. RCM2MHAO2A, was kindly supplied by Hikma Pharmaceutical Company, Egypt. Suvikan® tablets, B.N. 003, labeled to contain 20 mg rosuvastatin-Ca per tablet, a product of Hikma Pharmaceutical Company, Egypt, were purchased from local market.

Chemicals and reagents

All chemicals and reagents used were of analytical grade and solvents were of spectroscopic grade.

Methanol, acetonitrile and chloroform (Sigma – Aldrich, USA). KH_2PO_4, NaOH and anhydrous Na_2SO_4 (EL-Nasr Co., Egypt).

Dichloro-dicyanobenzoquinone, DDQ (Merck, Germany), 0.15% and $1x10^{-2}$ M solutions in acetonitrile, the later was prepared by dissolving 227.01 mg in 100 mL acetonitrile.

Tetracyanoquinodimethane, TCNQ (Merck, Germany), 0.2% and $1x10^{-2}$ M solutions in acetonitrile, the later was prepared by dissolving 204.2 mg in 100 mL acetonitrile.

Dichlorophenol indophenol, DCPI (Winlab, France), 0.1% and $1x10^{-3}$ M solutions in chloroform. They were prepared by dissolving 0.1 g or 0.0268 g in 30 mL water. The solution was quantitatively transferred to a separator and then rendered acidic with 5 mL 2 N HCl. DCPI was extracted with four successive 20 mL portions of chloroform. The chloroform extracts were pooled through anhydrous Na_2SO_4 into a 100 mL volumetric flask and diluted to volume with chloroform [13].

Basic fuchsin (Diamond fuchsin), BF (Merck, Germany), 0.2% and $0.5x10^{-3}$ M solutions in water, the later was prepared by dissolving 5 mg in 100 mL water.

Phosphate buffer solution (pH 6-10), prepared by mixing 50 mL of 0.2 M KH_2PO_4 with different volumes of 0.2 M NaOH and diluting to 200 mL with water[14].

Standard solutions

1 mg mL^{-1} solution of pure rosuvastatin-Ca was prepared in H_2O for AAS.

1 mg mL^{-1} drug solution was prepared in methanol and further diluted with the same solvent to obtain 0.5 and 0.1 mg mL^{-1} solutions.

$1x10^{-2}$ M drug solution was prepared by dissolving 1.004 g drug in 100 mL methanol and diluted to $0.5x10^{-3}$ M or 1 $x10^{-3}$ M using methanol.

General Procedure

A- Atomic absorption spectrometry (AAS) method

Aliquots from standard drug solution (1 mg mL^{-1}) in H_2O equivalent to 100-500 µg mL^{-1} rosuvastatin-Ca (4.1-20.5 Ca^{+2}) was transferred into a series of 10-mL volumetric flask. Then volumes were adjusted to the mark with H_2O and atomic absorbance of calcium content was measured at 422.7 nm.

B- Charge transfer complexation

Aliquots from standard drug solution (0.5 mg mL^{-1}) in methanol equivalent to 0.4-1.6 or 0.05-0.5 mg rosuvastatin-Ca into one of two series of 10-mL volumetric flask. The volume was completed to 2 mL with methanol and 1 mL of 0.15% DDQ or 0.2% TCNQ in acetonitrile was added and set aside for 20 min or 10 min, respectively. Then volumes were completed to the mark with acetonitrile and absorbance was measured at 460 nm for DDQ or 841 nm for TCNQ against a similarly prepared reagent blank.

For DCPI- volumes of standard drug solution (1 mg mL^{-1}) in methanol equivalent to 0.1-0.8 mg of the drug were mixed with 2.5 mL of 0.1% DCPI solution in $CHCl_3$ in a series of 10-mL volumetric flasks and completed to the mark with methanol. The absorbance of bluish violet colored product was measured at 640 nm against a reagent blank.

C- Ion- pairing

Accurate volumes of standard methanolic drug solution (0.1 mg mL^{-1}) equivalent to 0.04-0.2 mg were delivered into a series of 125-mL separating funnels. One mL of phosphate buffer solution (pH=8.3±0.1) was added followed by 2 mL of 0.2% BF to each funnel and the aqueous phase was adjusted to 10 mL with water. Extraction was carried out using 2×10 mL $CHCl_3$, filtered through anhydrous Na_2SO_4 into a 25-mL volumetric flask and adjusted to volume with $CHCl_3$. The absorbance of the red colored product was measured at 484 nm and 555 nm against a reagent blank.

Application to pharmaceutical formulation

Twenty Suvikan® tablets were weighed and finely ground. Amounts of fine powder equivalent to 100 mg of rosuvastatin-Ca were weighed and introduced in a 100-mL volumetric flask and extracted by shaking with 70 mL H_2O or methanol for about 10 min. The clear aqueous filtrate labeled to contain 1 mg mL^{-1} drug was analyzed by AAS. While methanolic filtrate claimed to contain 1 mg mL^{-1} rosuvastatin-Ca was assayed by DCPI method. Then diluted with methanol to obtain solutions containing 0.5 mg mL^{-1} or 0.1 mg mL^{-1} to be analyzed using DDQ and TCNQ or BF, respectively as detailed under "General procedure".

Results and discussion

Atomic absorption spectrometry (AAS) method

Determination of drug directly in water through its calcium content at 422.7 nm. A hollow cathode lamp for Ca with slit width 1.2 nm, current lamp 4 mA and air-acetylene flame was used.

Charge transfer complxation method

Rosuvastatin-Ca as a nitrogenous compound was expected to act as an n-donor for some π-acceptors. Upon reacting it with DDQ and TCNQ in 2:8 methanol-acetonitrile or with DCPI in 2.5:7.5 $CHCl_3$-methanol, charge-transfer complexes were obtained. Then complete charge-transfer from donor to acceptor was facilitated by polar solvents used to give orange, green or bluish violet radical anions absorbing maximally at 460, 841 and 640 nm, respectively; Figures (1,2).

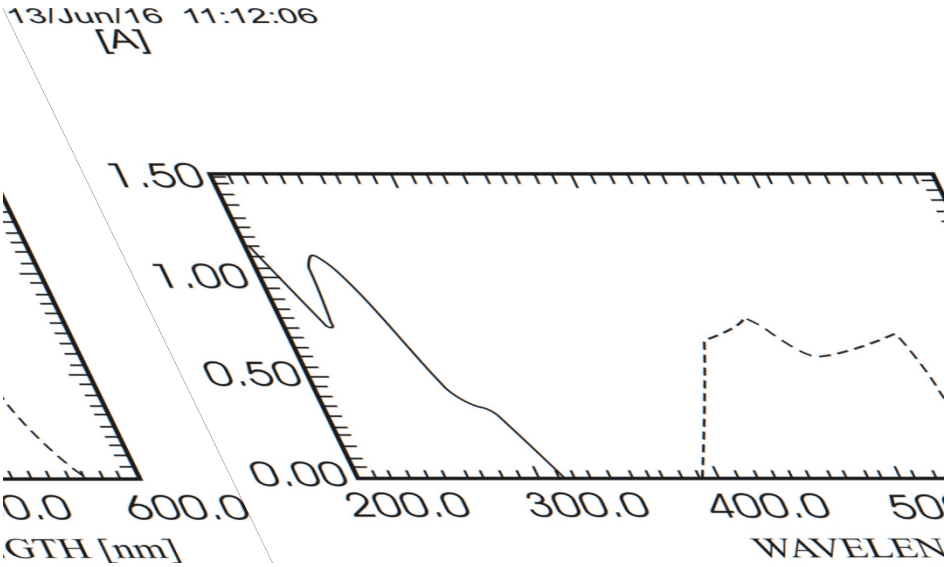


Figure (1): Absorption spectra of 25 µg mL^{-1} rosuvastatin-Ca in methanol (−) and 120 µg mL^{-1} rosuvastatin-Ca -DDQ reaction product in methanol- acetonitrile (2:8) (- -).

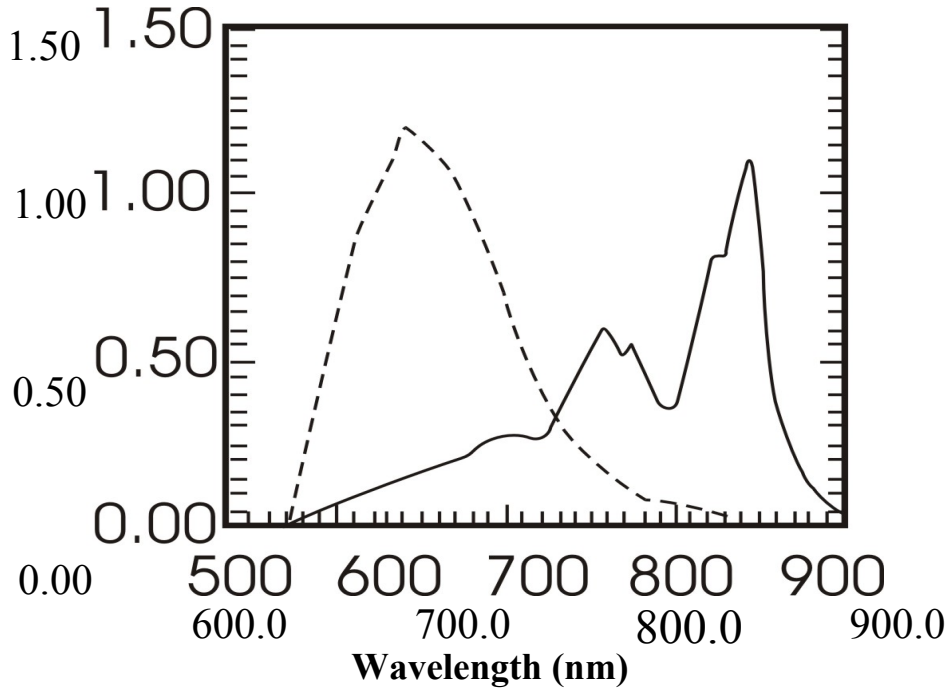


Figure (2): Absorption spectra of 42.5 µg mL^{-1} rosuvastatin-Ca -TCNQ reaction product in methanol-acetonitrile (2:8) (−) and 70 µg mL^{-1} rosuvastatin-Ca -DCPI reaction product in $CHCl_3$- methanol(2.5:7.5) (- -).

The different experimental parameters were studied and optimized as follow:

Effect of solvent

Rosuvastatin-Ca is only soluble in methanol while acetonitrile is the solvent of choice for DDQ and TCNQ and $CHCl_3$ is excellent for DCPI. Upon trying methanol, ethanol, $CHCl_3$ and acetonitrile as diluent for the reaction mixtures , acetonitrile gave highest sensitivity for DDQ and TCNQ, thus a ratio of 2:8 methanol-acetonitrile was used for the two chromogens. While methanol gave maximum colour intensity for DCPI, hence a ratio of 2.5:7.5 $CHCl_3$-methanol was applied for this chromogen.

Effect of reagent volume

One mL of 0.15% DDQ and 0.2% TCNQ solutions in acetonitrile or 2.5 mL of 0.1% DCPI solution in $CHCl_3$ were found to be sufficient for maximum sensitivity at the relevant maxima.

Effect of reaction time and stability of the color

The color started to develop immediately with DDQ and TCNQ, reaching its maximum after 20 min or 10 min and remained stable for further 20 min or 60 min, respectively. Whereas, for DCPI, the reaction was spontaneous and the bluish violet colour remained stable for one hour.

Ion- pairing method

Rosuvastatin-Ca contained a negatively charged carboxylic group reacted with basic fuchsin (BF) to yield a red ion-pair in $CHCl_3$ exhibiting 2 λ_{max} at 484 and 555 nm; Figure (3).

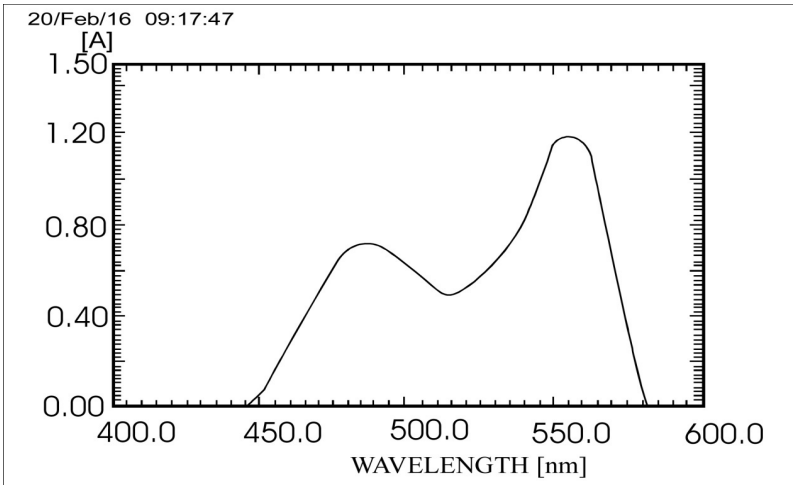


Figure (3): Absorption spectra of 15 µg mL^{-1} rosuvastatin-Ca -BF ion-pair in $CHCl_3$.

The reaction conditions were optimized as follow:

Effect of pH and buffer volume

Rosuvastatin-Ca was allowed to react with BF in presence of phosphate buffer of different PH values (6-10). Maximum colour intensity was obtained using phosphate buffer of pH (8.3±0.1). Different volumes (0.5-2 mL) of the later buffer were studied and 1 mL was found to be optimum.

Effect of dye volume

Different volumes (0.5-3.0 mL) of 0.2% BF in water were allowed to react with definite concentration of drug. 2 ml of 0.2% dye solution gave maximum intensity at 484 and 555 nm.

Effect of reaction time

The ion-pair formation was found to be instantaneous and red colour obtained was stable for one hour.

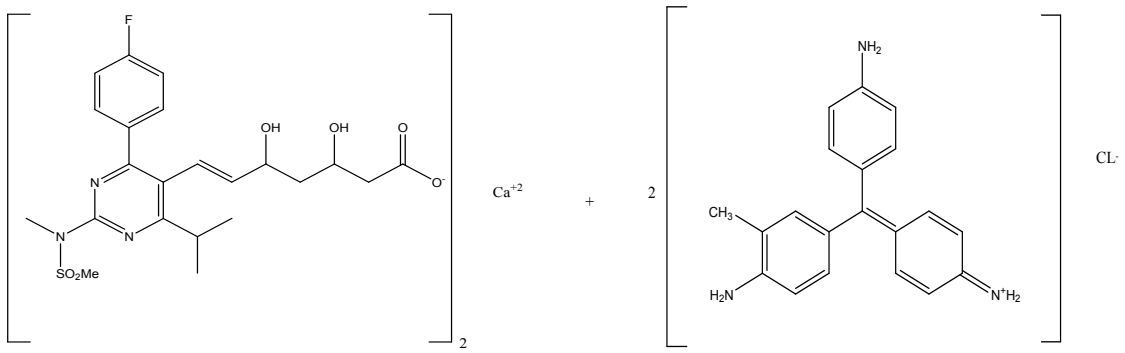
Stoichiometry of the reaction

Job›s method[15] was applied using $1x10^{-2}$, $1x10^{-3}$ and $0.5x10^{-3}$ M solutions of rosuvastatin-Ca for the charge transfer complexes and ion-pairing, respectively. 1:2 ratio between the

drug and DDQ, TCNQ and BF reagents due to presence of two molecules of drug form calcium salt which reacted to give 1:2 ratio or 1:1 ratio between the drug and DCPI reagent were predicted suggesting the following mechanism:

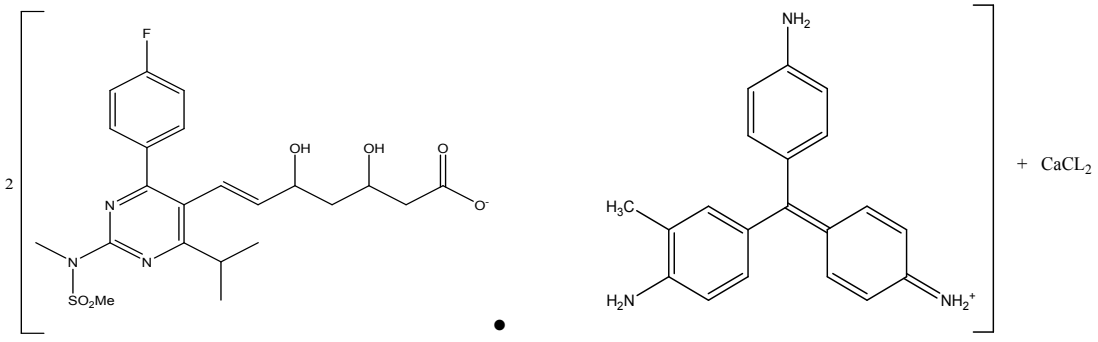
$$D + A \longrightarrow D{\rightarrow}A \longleftrightarrow D^{\bullet+} + A^{\bullet-}$$

donor acceptor complex coloured radical anion



rosuvastatin-Ca Basic fuchsin

Phosphate buffer (pH 8.3±0.1)



Ion-pair complex of rosuvastatin-Ca with Basic fuchsin

Scheme (1): The suggested reaction mechanism of rosuvastatin with BF.

Validation of the methods

Linearity

Under the above optimum experimental conditions, Beer's law was found to be obeyed in the ranges of 100-500 rosuvastatin-Ca equivalent to 4.1-20.5 µg mL^{-1} Ca^{+2} for AAS and 40-160, 5-50, 10-80 and 4-20 µg mL^{-1} rosuvastatin-Ca for DDQ, TCNQ, DCPI and BF, respectively. The regression parameters are represented in, Table (1).

The relative sensitivities of the products using the four mentioned chromogens were compared by calculating A(1%,1cm). The ion-pair with BF was found to be the most sensitive; 768 at 555 nm, Table (1).

Accuracy and Precision

The accuracy of the methods ranged between 99.90 and 100.97%. The intraday precision calculated as RSD amounted to 0.21-1.83% while the intermediate precision over a period of two weeks was 0.15-2.08%, Table (1).

Table (1): Assay and validation parameters of the proposed spectrophotometric methods for the determination of rosuvastatin-Ca.

Parameter	AAS	DDQ	TCNQ	DCP	BF	
$\lambda_{max\ (nm)}$	422.7	460	841	640	484	555
Linearity range (μg ml^{-1})	100-500*	40-160	5-50	10-80	4-20	4-20
A(1%,1cm)	19.5	67	252	173	473	768
Regression parameters**						
Slope (b)	0.0019	0.0067	0.0250	0.0168	0.0470	0.0752
Intercept (a)	0.0094	0.0006	0.0028	0.0195	0.0045	0.0038
Correlation coefficient (r^2)	0.9993	0.9996	0.9999	0.9993	0.9990	0.9997
Accuracy(R%±S.D) ***	100.97±1.04	100.57±0.33	99.96±0.80	100.38±1.66	100.30±0.68	99.90±1.40
Precision (RSD%)						
Intraday	0.21-0.99	0.66-1.32	0.56-1.76	0.30-1.83	0.65-1.25	0.30-1.75
intermediate	0.53-1.23	0.83-2.08	0.56-1.26	1.02-1.46	0.37-1.74	0.15-1.75

* (4.1-20.5 Ca^{+2})

**Regression equation (y = a + bc)

*** n = 9

Application to Pharmaceutical formulation

Good recoveries of rosuvastatin-Ca from its Suvikan tablets were obtained ranging between; 97.29-100.59±0.82-1.99%, indicating non interfering excipients and additives. These results were statistically analyzed and found to be in accordance with those of a compendial method[10] which involved UV- measurement of the drug solution in methanol at 243 nm, Table (2).

The validity of the proposed methods was further assessed by applying the standard addition technique showing mean% recoveries of 99.1±1.06 - 102.9±1.79, Table (2).

Table (2): Statistical analysis of the results obtained by the proposed and reported method(10) .

Parameter	AAS	DDQ	TCNQ	DCPI	BF		Reported method(10)
					484 nm	555 nm	
N	5	5	5	5	5		5
Mean %	99.09	97.29	98.78	98.77	99.66	100.40	98.53
S.D	0.82	1.16	1.18	0.93	1.99	1.73	1.35
Variance	0.67	1.35	1.39	0.86	3.96	2.99	1.82
t-test	0.79	1.56	0.31	0.327	1.74	1.16	
F-test	2.71	1.35	1.31	2.11	2.17	1.64	
Standard addition Mean± RSD%	-	100.74±0.76	100.29±1.17	99.69±1.89	99.12±1.07	99.58±1.45	

The theoritical t- and F- values at P=0.05 are 2.31 and 6.39 , respectively.

Ref (10) : UV measurement of the drug solution in methanol at 243 nm.

Conclusion

The demand for simple, accurate and precise methods for the determination of rosuvastatin-Ca was achieved throughout this work by the development of AAS and spectrophotometric methods, either through charge-transfer complexation of the drug with DDQ, TCNQ and DCP or ion association with basic fuchsin. The simplicity of the assays could allow for their use in routine and quality control analysis.

References

1- The Merck Index 14th Ed, Merck and Co INC, Whitehouse Station, N.J.USA. p 1428 (2006).

2- R. Sane, S. Kamat, N. Menon, Inamdar, R. Shafi and R. Mander; J. Planar chromatogr-Modern TLC.', 18(103) 194 (2005).

3- K. Trivedi, R. Kallem, R. Mullangi and R. Snnivas; J.Pharm. Biomed. Anal., 39(3-4) 661 (2005).

4- K. Hull, D. Penman, K. Smith and D. Marten; J. Chromatogr. B: Anal. Technol. Biomed. Life Sci., 772(2) 219 (2002).

5- K. Hull, D. Marten, J. Warwick and E. Thomas; J. Pharm. Biomed. Anal., 35(3) 609 (2004).

6- S. Vittal, R. Shitut, R. Kumar , A. Vinu and R. Mullangi; Biomed. Chromatogr., 20(11) 1252 (2006).

7- A. Oudhoff, T. Sangster, E. Thomas and D. Wilson; J. Chromatogr. B. Anal. Technol. Biomed. Life Sci., 832(2) 191 (2006).

8- R. Nirogi, K. Mudigonda and V. Kandikere; J.Pharm. Biomed. Anal., 44(2) 379 (2007).

9- K. Lan, X. Jiang, Y. Li, L. Wang, J. Zhou, Q. Jiang and L. Ye; J.Pharm. Biomed. Anal., 44(2) 540 (2007).

10- Alkagupta, P. Mishra and K. Shah;E-J. chem., 6(1) 89 (2009).

11- K. Maroyhu and S. Dannana; E-J. chem., 4(1) 46 (2007).

12- S. D. Patel, R.G. Shah, R. R. Parmar, N.A. Mahajan and A.D. Shah; Int. J. Instit. Pharm. Life Sci., 2(2) 112 (2012).

13- M. Abdel Salam, A.S. Issa and M.S. Mahrous; Anal.Letter, 19(23) 2207 (1986).

14- United States Pharmacopoeia XXV; National Formulary XX; US Pharmacopoeial Convention: Rockville, MD, p 2340 (2002).

15- D.S. Harris; Quantitative chemical analysis, 6th ED., W. H. Freeman and Company, USA, Chapter 19, (2003).