ISSN : 0974-7419 Volume 12 Issue 2

Trade Science Inc.

Analytical CHEMISTRY

An Indian Journal

Full Paper

ACAIJ, 12(2) 2013 [41-47]

Selective spectrophotometric methods for quantification of sitagliptin and metformin

Safa'a M.Riad*, Mamdouh R.Rezk, Ghada Y.Mahmoud, Abdel-Aziz El Bayoumi Abdel Aleem

Analytical Chemistry Department, Faculty of Pharmacy-Cairo University, Kasr El-Aini Street, ET-11562 Cairo, (EGYPT)

E-mail : s.mohany02@gmail.com

ABSTRACT

Four simple, accurate and precise spectrophotometric methods were developed and validated for the simultaneous determination of sitagliptin phosphate (STA) and metformin HCl (MTF) in their mixture. Among the developed methods were first derivative, second derivative, dual wavelength and ratio subtraction methods. The first derivative spectrophotometric method was used for the determination of STA in the range of 25-500 μg mL^{-1}, while the second derivative spectrophotometric method was used for the determination of STA in the range of 25-500 μg mL^{-1} in addition to MTF in the range of 2.5-35 μg m^{-1}. The dual wavelength and ratio subtraction methods were used for the determination of MTF in the range of 5-30 μg mL^{-1} and 2.5-30 μg mL^{-1}, respectively. The results were statistically compared with the reference ones. The developed methods were proved to be selective and accurate for the quality control and routine determination of the cited drugs in their mixture and in their pharmaceutical formulations.

KEYWORDS

Metformin;
Sitagliptin;
First derivative;
Second derivative;
Dual wavelength;
Ratio subtraction.

INTRODUCTION

Sitagliptin is [(2R)-1-(2,4,5-trifluorophenyl)-4-oxo-4-[3-(trifluoromethyl)-5,6dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]butan-2-amine], Figure 1.a. It is an orally active and selective inhibitor of dipeptidyl peptidase-IV that is used for the treatment of Type 2 diabetes[1]. Metformin hydrochloride is *N*,*N*-dimethylimidodicarbonimidic diamide hydrochloride, Figure 1.b. It is prescribed as an oral hypoglycemic agent, used in the management of non-insulin dependent diabetes mellitus[2].

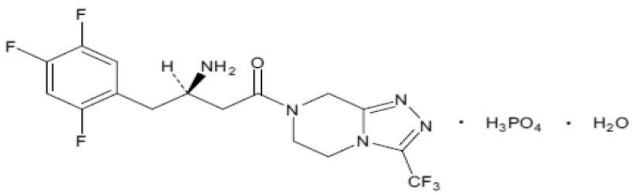

Figure 1a : Structural formula of sitagliptin phosphate (STA)

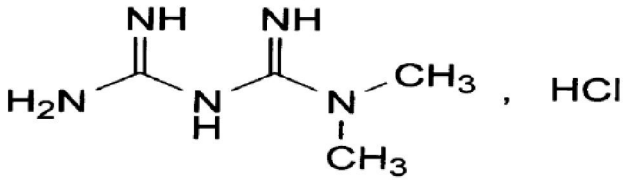

Figure 1b : Structural formula of metformin (MTF)

The literature survey reveals several analytical methods for quantitative estimation of sitagliptin alone[3], or in combinations[4,5], metformin alone[6-9] and in other combinations[10-15].

Full Paper

In modern analytical laboratories there is always a need for simultaneous determination of STA and MTF in the drug analysis. The present work aimed to develop rapid, simple and sensitive methods for selective quantification of STA and MTF in their pure forms or even in their pharmaceutical formulation. The methods described here include first derivative, second derivative, dual wavelength and ratio subtraction technique.

EXPERIMENTAL

Instruments

Spectrophotometer: shimadzu UV-1601 PC, dual-beam UV-Vis spectrophotometer (Japan), with matched 1-cm quartz cells, connected to an IBM-compatible PC and an HP-600 inkjet printer. Bundled, UV-PC personal spectroscopy software version 3.7 was used to process the absorption and the derivative spectra. The spectral bandwidth was 2 nm with wavelength-scanning speed of 2800 nm min^{-1}.

Materials and reagents

Materials

Reference sitagliptin phosphate (STA) standard was kindly donated by Merck & Co., Inc., Whitehouse station (New Jersey, USA). Its purity was found to be 98.87% ± 1.20 (n=6), according to a reference spectrophotometric method[16].

Reference metformin hydrochloride (MTF) standard was kindly supplied by Cid Co. (Cairo, Egypt). Its potency was found to be 99.39 ± 0.954 (n=6), according to the USP potentiometric method[17].

Pharmaceutical formulation

Janumet® tablets BN: 0426580, labeled to contain 50 mg STA as phosphate salt and 1000 mg MTF as hydrochloride salt.

Standard solutions

STA standard solution (1mg mL^{-1}) and MTF (0.1mg mL^{-1}) standard solution in distilled water.

All calculations and samples preparation of STA and MTF for reference material and pharmaceutical formulation were done on basis of the salt form. Solutions were freshly prepared on the day of analysis and stored in refrigerator to be used within 24 h.

Reagents

De-ionized water: Bi-distilled from "Aquatron" Automatic Water still A4000 was provided by Bibby sterillin Ltd. (UK).

PROCEDURES

First-derivative (^1D) method

Spectral characteristics of STA and MTF

Two aliquots 3 mL of STA (1mg mL^{-1}) and 3 mL of MTF (0.1mg mL^{-1}) aqueous solutions were separately transferred into two 10-mL volumetric flasks. The volume was completed with distilled water to obtain final concentration of 300 μg mL^{-1} of STA and 30 μg mL^{-1} of MTF. The zero-order and the first derivative (^1D) of the prepared solutions were measured and recorded.

Linearity

Portions equivalent to (0.25-5 mL) of STA standard stock solution (1 mg mL^{-1}) were separately transferred into a series of 10-mL volumetric flasks. Each flask was completed to the volume with distilled water to reach a concentration range of 25-500 μg mL^{-1}. The amplitudes of the first-derivative peaks of STA were measured at 274.8 nm with $\Delta\lambda$=4 nm and scaling factor =10.

Calibration graph was constructed by plotting $\Delta A/\Delta\lambda$ versus concentration. The regression equation was then computed for STA at the specified wavelength and used for determination of unknown samples containing the specified drug.

Second-derivative (^2D) method

Linearity

Portions equivalent to (0.25-5 mL) of STA standard stock solution (1mg mL^{-1}) and (0.25-3.5 mL) of MTF standard stock solution (0.1 mg mL^{-1}) were separately transferred into a series of 10-mL volumetric flasks. Each flask was completed to the volume with distilled water to reach the concentration range of 25-500 μg mL^{-1} and 2.5-35 μg mL^{-1} of STA and MTF, respectively. The amplitudes of the second-derivative peaks of STA were measured at 278.6 nm, while that of MTF were measured at 256.5 nm with $\Delta\lambda$ =8 nm and scaling factor =100. Calibration graphs were constructed by plotting the peak amplitude versus concen-

tration. The regression equations were then computed for STA and MTF at the specified wavelengths and used for determination of unknown samples containing them.

Dual wavelength method

The method depends on measuring the absorbance difference between two points on the mixture spectra. This absorbance difference is proportional to the concentration of the component of interest.

Linearity

Portions equivalent to (0.5-3.0 mL) of MTF standard stock solution (0.1 mg mL^{-1}) were separately transferred to a series of 10-mL volumetric flasks. Each flask was then completed with distilled water to reach a concentration range of 5-30 μg mL^{-1}. The amplitudes of the peaks were measured at 228.6 nm & 245.3 nm.

The difference in absorbance was plotted versus MTF concentration. The regression equation was then computed for MTF and used for its determination in unknown samples containing MTF alone or in combination with STA.

Ratio subtraction method

Two aliquots (3 mL) of STA (1mg mL^{-1}) and (1.5 mL) of MTF (0.1mg mL^{-1}) aqueous solutions were separately transferred into two 10-mL volumetric flasks. The volume was completed with distilled water to obtain final concentration of 300 μg mL^{-1} of STA and 15 μg mL^{-1} of MTF. The spectra were measured and recorded.

Linearity

Into a series of 10-mL volumetric flasks, (0.25-3 mL) aliquots of MTF stock solution (0.1 mg mL^{-1}) were transferred accurately and then completed to volume with distilled water. The spectra of the prepared standard solutions were measured and stored in the computer. The peak amplitudes were measured at 233 nm and calibration graph was obtained for MTF.

Different aliquots from MTF and STA stock solutions were transferred into 10-mL volumetric flasks to prepare mixtures containing different ratios of the studied drugs. The spectra of the laboratory-prepared mixtures were recorded and then divided (absorbance at each wavelength) by the spectrum of 300μg mL^{-1} STA. The absorbance of the plateau region in the resulting spectrum was subtracted at λ above 260 nm (the constant). The obtained spectra were then multiplied (absorbance at each wavelength) by the spectrum of 300μg mL^{-1} STA (the devisor). The obtained spectra were used for the determination of MTF from the corresponding regression equation.

Analysis of laboratory prepared mixtures

Laboratory prepared mixtures containing different ratios of STA and MTF were analyzed using the suggested methods. The concentration of each component was calculated from the corresponding regression equation.

Assay of pharmaceutical formulation (Janumet® ablets)

Twenty tablets were weighed and the average weight was calculated. Tablets were crushed to furnish a homogenous powder and 2.375gm of powdered tablets were dissolved by the aid of an ultrasonic bath for 2 hours and filtered through Whatman filter paper to prepare stock solutions and then procedures were completed as described under each method.

RESULTS AND DISCUSSION

First-derivative (^{1}D) method

Derivative spectrophotometry is a powerful tool in quantification of mixtures of drugs. A simple, rapid and selective spectrophotometric technique was proposed and applied for the determination of STA, either in raw material or in pharmaceutical formulation containing MTF. This was done by applying the first-derivative (^{1}D) ultraviolet spectrophotometry. The method could solve the problem of spectral bands overlapping between STA and MTF without sample pretreatment or separation steps of the two analyzed drugs. The absorption spectra of STA and MTF showed overlapping, little interference (Figure 2). When the first-derivative spectra (Figure 3) were examined, it was found that STA can be determined at 274.8nm, where MTF has no contribution. This allowed accurate determination of STA in presence of MTF. A linear relationship was obtained in the range of 25-500μg mL^{-1} for STA. The regression equation was computed and found to be:

Full Paper

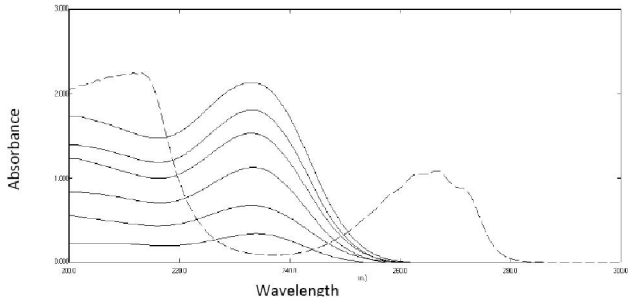

Figure 2 : Scanning profile of MTF5-30 µg mL^{-1} (—) and STA 300 µg mL^{-1}(---)

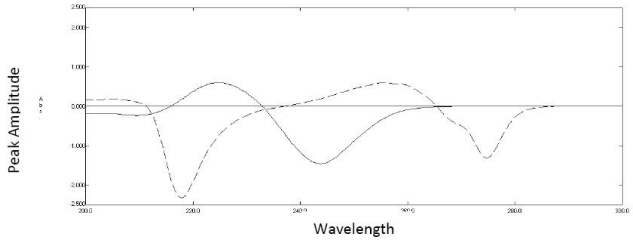

Figure 3 : First derivative spectra of (300µg mL^{-1}) of STA (….) and 30 µg mL^{-1} of MTF (—)

^{1}D = 0.0042 C + 0.0406 (r = 0.9997), at 274.8 nm

Where ^{1}D is the peak amplitude of the first-derivative curve ($\Delta A/\Delta\lambda$) at 274.8 nm, C is the concentration of STA (µg mL^{-1}) and r is the correlation coefficient. The precision of the proposed method was confirmed by the analysis of different samples in triplicates. The mean percentage recovery was found to be 99.31% at 274.8 nm.

Second-derivative (^{2}D) method

The second-derivative (^{2}D) ultraviolet spectrophotometry was applied for the simultaneous determination of MTF and STA, either in their mixture or in their pharmaceutical formulation. The method could solve the problem of spectral bands overlapping between STA and MTF without sample pretreatment or separation steps of the two analyzed drugs.

The absorption spectra of STA and MTF show overlapping and error probability that affect the use of direct spectrophotometry and first-derivative method (^{1}D) for their determination, especially at higher levels of MTF, When the second-derivative spectra were examined, (Figure 4) it was found that STA could be determined at 278.6 nm, where MTF has no absorbance and MTF could be determined at 256.5 nm where STA has no contribution (zero crossing). A linear relationship was obtained in the range of 25-500µg mL^{-1} for STA and 2.5-35 µg mL^{-1}for MTF. The corresponding regression equations were computed and found to be:

^{2}D = 0.0052 C + 0.0384 (r=0.9997), at 278.6 nm (1)

for STA

^{2}D = 0.0236 C - 0.0094 (r=0.9995), at 256.5 nm (2)

for MTF

Where ^{2}D is the peak amplitude of the second-derivative curve at the corresponding wavelengths, C is the concentration of STA and MTF (µg mL^{-1}) and r is the correlation coefficient.

The mean percentage recoveries were found to be 99.51% at 278.6 nm for STA and 100.48% at 256.5 nm for MTF

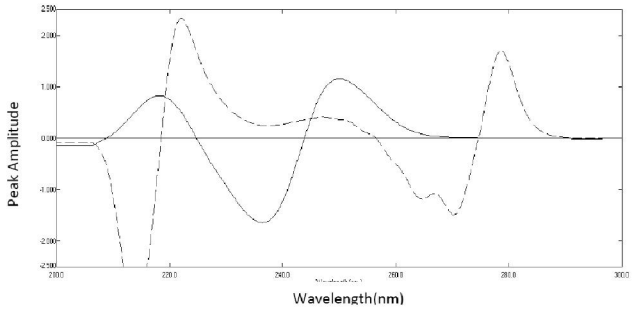

Figure 4 : Second derivative spectra of STA 300µg (---) and MTF 30 µg (—)

Dual wavelength method

The method depends on selecting two wavelengths at which STA shows the same absorbance while MTF has different absorbance values. The wavelengths selected for determination of MTF were 228.6 nm & 245.3 nm. At these wavelengths STA has the same absorbance values (Figure 2).

Linearity

Linearity of the proposed method was verified by analyzing six different concentrations in the range of 5-30µg mL^{-1} for MTF (Figure 2). Each concentration was made in triplicate. The regression equation of calibration curve was:

ΔA= 0.0345 C + 0.0365 r = 0.9997

Regression analysis of Beers plots showed good correlation in concentration range of 5-30 µg mL^{-1} for MTF.

Ratio subtraction

Spectral characteristics of STA and MTF

For a mixture of two drugs, X and Y with overlap-

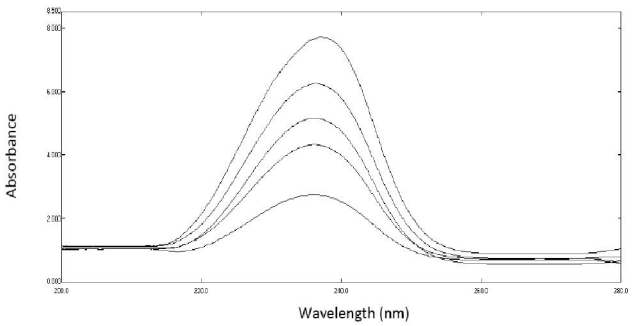

Figure 5 : Division spectra of laboratory-prepared mixtures of MTF(X) and STA(Y) using 300µg mL^{-1} of STA (Y') as the divisor and water as solvent

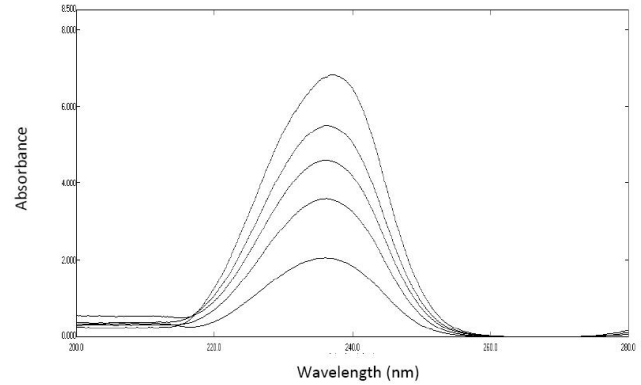

Figure 6 : Division spectra of laboratory-prepared mixtures of MTF (X) and STA (Y) using STA (Y') 300µg mL-1 as divisor and distilled water as solvent after subtraction of the constant

ping spectra, and the spectrum of Y is extended more than X, the determination of X can be done 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 represents

$$\frac{X}{Y'} + \text{Constant}$$

If we subtract this constant, then multiply the new curve obtained after subtraction by Y', we obtain the original curve of X.

This can be summarized in the following equations:

$$\frac{X+Y}{Y'} = \frac{X}{Y'} + \frac{Y}{Y'} = \frac{X}{Y'} + \text{constant}$$

$$\frac{X}{Y'} + \text{Constant} - \text{constant} = \frac{X}{Y'}$$

$$\frac{X}{Y'} * Y' = X$$

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

This method was applied for the determination of MTF (component X) in the presence of STA (component Y). The zero order spectra measured and stored in the PC were used for estimating the concentration of MTF. The resulting division spectra are shown in figure 5. Figure 6 shows the resulting spectra after subtraction of the constant value, shown above 260 nm and indicated by straight line parallel to the wavelength axis. The spectra obtained were then multiplied by the devisor spectrum (300 µg mL^{-1}) of STA to obtain the spectra of MTF. The method has the advantage of simple steps and avoiding derivative calculations and also one can ensure the validity of the results when obtaining the direct spectra of component X at the end of calculations.

Analysis of the laboratory-prepared mixtures

The suggested methods were applied for the determination of STA and MTF in their mixtures. The re-

TABLE 1

Methods	^{1}D method at 274.8 nm	^{2}D method	Dual wavelength method	Ratio subtraction method
Sitagliptin				
Mean ± S.D.	100.08 ± 1.44	101.69 ± 0.68	-	-
Metformin				
Mean ± S.D.	-	99.55 ± 1.26	100.47 ± 1.49	100.17 ± 1.51

TABLE 2

Preparation Janumet® Tablets BN: 0426580	^{1}D method at 274.8 nm	^{2}D method	Dual wavelength method	Ratio subtraction method
Sitagliptin				
Mean ± S.D.	99.53 ± 0.61	99.49 ± 0.75	-	-
Metformin				
Mean ± S.D.	-	100.36 ± 1.87	99.25 ± 0.77	100.11 ± 0.99

TABLE 3

Parameter	^{1}D-method	^{2}D-method		Dual wavelength method	Ratio subtraction method
	STA	STA	MTF	MTF	MTF
Range	25-500 µg	25-500 µg	2.5-35 µg	5-30 µg	2.5-30 µg
Slope	0.0042	0.0052	0.0236	0.0348	0.0797
Intercept	0.0406	0.0384	-0.0094	0.0279	-0.0586
Mean	99.31	99.51	100.48	101.60	100.12
SD	1.043	1.139	1.648	0.982	1.39
Variance	1.089	1.299	2.717	0.964	1.94
Coefficient of variation	1.050	1.145	1.640	0.966	1.39
Correlation coefficient (r)	0.9997	0.9997	0.9995	0.9977	0.9993
RSD (%)[a]	0.182-0.523	0.197-0.381	0.207-0.875	0.427-0.854	0.910-1.437
RSD (%)[b]	0.157-0.495	0.189-0.468	0.445-0.549	0.496-0.970	1.171-1.214

[a] the inter-day (n=6) relative standard deviation of 100-300 µg for STA and 10-20 µg for MTF; [b]the intra-day (n=6) relative standard deviation of 200-300 µg for STA and 10-20 µg for MTF

TABLE 4

Parameter	^{1}D-method	^{2}D-method	Reference Method[16]	^{2}D-method	Dual wavelength method	Ratio subtraction method	Official USP method[17]
	STA	STA	STA	MTF	MTF	MTF	MTF
Mean	99.31	99.51	99.87	100.48	100.03	100.12	99.30
S.D.	1.043	1.139	0.954	1.64	1029	1.39	1.20
Variance	1.089	1.299	0.91	2.717	1.687	1.94	1.44
N	11	6	6	8	6	7	6
F-test	1.20 (4.74)[a]	1.43 (5.05)[a]		2.98 (4.88)[a]	1.17 (5.05)[a]	1.34 (4.95)[a]	
Student's t-test	1.120 (2.131)[a]	0.594 (2.228)[a]		1.554 (2.179)[a]	1.825 (2.228)[a]	1.142 (2.201)[a]	

[a] the values in the parentheses are the corresponding theoretical t-and F-values at p = 0.05[18]

sults shown in TABLE 1 Detemination of sitagliptin and metformin in laboratory prepared mixtures illustrate good percentage recoveries and high precision

Application of the proposed methods to the pharmaceutical formulation

The proposed methods were successfully applied for the determination of STA and MTF in Janumet® tablets, showing good percentage recoveries, TABLE 2 Detemination of sitagliptin and metformin in Janumet® tablets.

Statistical analysis

Assay parameters and validation sheet for the determination of sitagliptin and metform are shown in TABLE 3 Assay parameters and validation sheet for determination of sitagliptin and metformin. Results of the suggested methods for determination of STA and MTF were statistically compared with those obtained by applying the reported methods[16,17], respectively. The calculated t- and F-values[18] were found to be less than the corresponding theoretical ones, confirming good accuracy and excellent precision TABLE 4 Statistical comparison for the results obtained by the proposed methods and reference methods for sitagliptin and metformin.

CONCLUSION

Unlike the most proposed HPLC-procedures, the proposed spectrophotometric methods are simple and not expensive. The reagents used in the proposed methods are cheap and readily available, just distilled water also offers clean chemistry. The procedures applied in each method do not involve any critical reactions or tedious sample preparations. This aspect of spectrophotometric analysis is of major interest in analysis since

it offers distinct possibility of assaying STA and MTF in their mixture and in their pharmaceutical formulation without interference from each other or due to the tablet excipients.

The suggested methods are found to be simple, accurate, selective and equally sensitive with no significant difference of the precision compared with the reported methods[16,17]. They could be applied for routine analysis of pure drugs or in its pharmaceutical formulation.

REFERENCES

[1] P.Aschner, M.S.Kipnes, J.K.Lunceford, M.Sanchez, C.Mickel, D.E.W.Herman; Diabetes Care, **29**, 2632 **(2006)**.

[2] N.A.Thornberry, B.Gallwitz; Best Pract.Res. Clin.Endocrinol.Metab., **23**, 479 **(2009)**.

[3] C.B.Sekaran, A.P.Rani; Int.J.Pharm.Pharm.Sci., **2**, 138 **(2010)**.

[4] R.I.El-Bagary, E.F.Elkady, B.M.Ayoub; Int.J. Biomed.Sci., **7**, 62 **(2011)**.

[5] R.I.El-Bagary, E.F.Elkady, B.M.Ayoub; Int.J. Biomed.Sci., **7**, 55 **(2011)**.

[6] M.S.Arayne, N.Sultana, M.H.Zuberi, F.A.Siddiqui; Indian J.Pharm.Sci., **71**, 331 **(2009)**.

[7] S.Ashour, R.Kabbani; Anal.Lett., **36**, 361 **(2003)**.

[8] I.H.I.Habib, M.S.Kamel; Talanta, **60**, 185 **(2003)**.

[9] S.S.M.Hassan, W.H.Mahmoud, M.A.F.EL-Mosallamy, A.H.M.Othman; Anal.Chim.Acta, **378**, 299 **(1999)**.

[10] M.A.Hegazy, M.R.El-Ghobashy, A.M.Yehia, A.A.Mostafa; Drug Test Anal, **1**, 339 **(2009)**.

[11] K.S.Lakshmi, T.Rajesh, S.Sharma, S.Lakshmi; Der.Pharma.Chemica., **1**, 238 **(2009)**.

[12] P.K.Chaturvedi, R.Sharma; Anal.Lett., **41**, 2133 **(2008)**.

[13] J.R.Patel, B.N.Suhagia, B.H.Patel; Indian J.Pharm. Sci., **69**, 844 **(2007)**.

[14] M.B.Shankar, V.D.Modi, D.A.Shah, K.K.Bhatt, R.S.Mehta, M.Geetha, et al; J AOAC International, **88**, 1167 **(2005)**.

[15] A.Aruna, K.Nancey; Indian Drugs, **37**, 533 **(2000)**.

[16] P.Parag, I.Md, B.Vinod, A.Yogesh; J.Pharm.Res., **4**, 871 **(2011)**.

[17] The United States Pharmacopeia (USP34), National Formulary (NF29), The United States Convention, Inc., Rockville, 1375-1376 **(2011)**.

[18] M.R.Spiegel, L.J.Stephens (Eds); Schaum Outline of Theory and Problems of Statistics, Schaum Outline Series, New York, **(1999)**.