Journal of Pharmaceutical Research Vol. 9, No. 4, October 2010 : 167-171.

SIMULTANEOUS SPECTROPHOTOMETRIC METHODS FOR ESTIMATION OF CEFPODOXIME PROXETIL AND POTASSIUM CLAVULANATE IN TABLET DOSAGE FORM

Thomas AB*, Dighe SB, Kothapalli LP, Nanda RK, Jagdale SN, Deshpande AD
Department of Pharmaceutical Chemistry, Pad. Dr. D.Y.Patil Institute Of Pharmaceutical Sciences and Research, Pimpri, Pune-411 018, Maharashtra, India.

Received on : 15.07.2010 Revised : 18.10.10 Accepted : 20.10.10

ABSTRACT
Three UV spectrophotometric methods have been developed for the simultaneous estimation of Cefpodoxime proxetil (Cef) and Potassium clavulanate (Pot.clav.) in combined tablet dosage forms. The first method involves Q-absorbance ratio method, the sampling wavelengths selected are, 315.0nm (isoabsorptive point) and 233.0 nm (λmax of Cef). The second method is the First order derivative method, the sampling wavelengths selected are 269.0 nm and 240.0 nm for estimation of Cef and Pot.clav. respectively. In both the methods, the linearity range for both Cef and Pot.clav. was in the concentration range of 1.5-50 µg mL^{-1}. The third method based on ratio derivative spectrophotometry, involves measurement of absorbances at the amplitudes in the first order derivative of the ratio spectra at 246.0 nm and 327.0 nm for Cef and Pot.clav. respectively over the concentration range of 2.5-50 µg $mL^{-1}$for both the drugs. The results of the analysis were validated statistically and recovery studies carried out as per ICH guidelines. The developed methods are rapid, precise, accurate, rugged and can be employed for the routine estimation of Cefpodoxime proxetil and Potassium clavulanate in both bulk and tablet dosage form.

Keywords: *Cefpodoxime proxetil; Potassium clavulanate; Q-Absorbance ratio method; First order derivative spectroscopy; Ratio spectra derivative spectrophotometry*.

INTRODUCTION

Cefpodoxime proxetil (Cef) is an antibacterial agent which is official in United State Pharmacopoeia and British Pharmacopoeia[1-2]. Potassium clavulanate (Pot.clav.),a â-lactamase inhibitor is official in British Pharmacopoeia[2]. The bactericidal activity of Cef results from its inhibition of bacterial cell wall synthesis. It is used in the treatment of acute otitis media, pharyngitis and tonsillitis. Due to the similarity in chemical structure, Pot. clav. acts as a competitive inhibitor of beta-lactamases secreted by certain bacteria thereby helping to restore the antimicrobial activity of Cef [3-5].

Literature survey reveals several methods such as, U.V. spectroscopy[6-9], HPLC [10-14] and HPTLC [15-16] which have been reported for the estimation of individual drugs as well as in combination with other drugs. Not a single UV, HPLC or HPTLC method is reported so far for the simultaneous analysis of Cef and Pot.clav. in their combined dosage form. A combination of Cef and Pot.clav. is now available in combined tablet dosage form for the treatment of bacterial infections. So a need was felt to develop new methods to analyze the drugs simultaneously. This paper describes three UV spectrophotometric methods for the simultaneous determination of Cef and Pot.clav. in tablet formulation using Q-absorbance ratio method, first order derivative spectroscopy and ratio spectra derivative spectrophotometry.

EXPERIMENTAL

Material and Methods

A Shimadzu UV/Visible spectrophotometer, Model 1700 (Japan) was employed with spectral bandwidth of 2nm and wavelength accuracy of ±0.5nm, with automatic wavelength correction employing a pair of quartz cells. A Shimadzu electronic analytical balance (AX-200) was used for weighing the sample. An ultrasonic cleaner (Art No.400014CL) was used for sonicating the tablet sample solution. Analytical pure samples of Cef (%purity-99.98) and Pot.clav.(%purity-98.70) (Emcure Pharmaceuticals,Pune, India) were used in the study. The pharmaceutical dosage form employed in this study was Cepodem XP 325 tablet (Ranbaxy Pharmaceuticals Ltd.,New Delhi, India) labeled to contain 200 mg of Cef and 125 mg of Pot. clav. per tablet.

Preperation of standard stock solutions

Standard stock solutions (100 µg mL^{-1}) of Cef and Pot.clav. were prepared by dissolving separately 10 mg of each drug in 100 mL methanol: water mixture (60:40%v/v).

***Correspondence :** dypharmachem@yahoo.co.in

Preperation of sample stock solutions

Twenty tablets were weighed and crushed to fine powder. An accurately weighed powder sample equivalent to 10 mg of Cef was transferred to a 100 ml volumetric flask and dissolved in 50 ml of methanol: water mixture(60:40%v/v). After the immediate dissolution, the volume was made up to the mark with the same solvent. The solution was sonicated for about 30 min and was then filtered through Whatmann filter paper No.41. The solution was suitably diluted with distilled water to obtain sample solutions containing Cef and Pot.clav. in the concentrations ratio of 10:6.25 µg mL^{-1} respectively.

Method A: Q-Absorbance ratio method[17]

Standard solutions (10 ug mL^{-1}) of Cef and Pot.clav. were scanned in the spectrum mode of the instrument from 400-200 nm. From the overlain spectra of the two drugs (Fig.1), 315.0 nm (iso-absorptive point) and 233.0 nm (λmax of Cef) were selected for analysis. The optical characteristics and regression values for the calibration curve is presented in Table 1.Mixed standards of Cef and Pot.clav.in the concentration ratio of 10:6.25 ug mL^{-1} were prepared and their absorbances were measured at the selected wavelengths. The concentration of the two drugs in mixed standards were calculated employing equation 1 and 2 using the ratio of the absorbances and the mean absorptivity coefficients of Cef and Pot.clav.at the selected wavelengths.

$C_{Cef} = Qm - Qy/ Qx - Qy \times A_1/ ax_1$ ..... (1)

$C_{Pot.clav.} = Qm - Qx/ Qy - Qx \times A_1/ ay_1$ ..... (2)

Where $Qm = A_2/A_1$, $Qx = ax_2/ax_1$ and $Qy = ay_2/ay_1$

A_1 and A_2 are the absorbances of mixed standard and tablet sample solutions at 315.0 nm and 233.0 nm. ax_1 (11.71), ax_2 (37.37) and ay_1 (11.71), ay_2 (15.32) are mean absorptivities of Cef and Pot.clav. at 315.0 and 233.0 nm respectively.

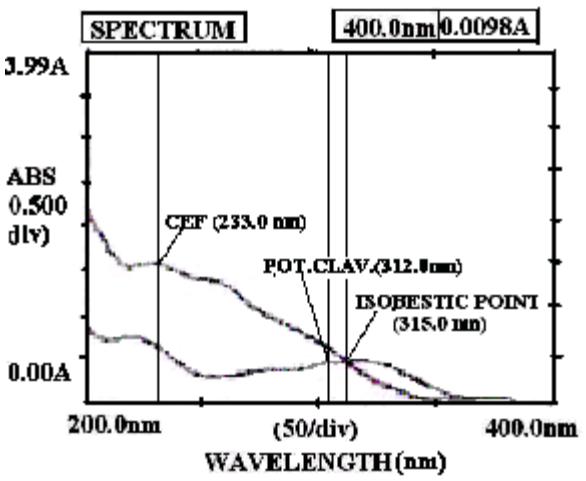

Fig. 1: *Overlain Spectra of Cef and Pot.clav. in Q-Absorbance ratio method*

Table 1: *Optical Characteristics and Validation Data of Cefpodoxime proxetil and Potassium clavulanate*

Parameters	Cefpodoxime proxetil			Potassium clavulanate		
	Method-A	Method-B	Method-C	Method-A	Method-B	Method-C
Working wavelengths	233.0 nm	269.0 nm	248.0 nm	—	240.0 nm	327.0 nm
Beer-Lambert's Law range ($\mu g/mL^{-1}$)	1.5-50	1.5-50	2.5-50	—	1.5-50	2.5-50
Precision*						
Inter day (%RSD)						
Intra day (%RSD)	0.8212	0.4450	1.2780	0.2792	0.8223	1.2290
LOD ($\mu g/ml$)*	0.8455	0.8558	1.4500	0.8428	0.6238	0.8235
LOQ ($\mu g/ml$)*	0.0709	0.1956	0.2117	0.2449	0.1477	0.1576
Regression Values:	0.2151	0.5927	0.6417	0.7422	0.4478	0.4775
I. Slope*						
II. Intercept*	0.0350	0.0044	0.0018	—	0.0041	0.0032
III. Regression coefficient(r^2)*	0.0108	0.0012	0.0012	—	0.0008	0.0041
Rugedness* (%RSD)	0.9976	0.9981	0.9988	—	0.9976	0.9944
Different Instruments						
Different Analyst	0.232	0.691	0.812	0.702	0.744	0.767
	0.377	0.420	0.548	0.782	0.803	0.813

** Average of six estimations, Method-A: Q- Absorbance ratio method, Method-B: First order derivative method, Method-C: Ratio spectra derivative spectrophotometric method*

Estimation from marketed preparations

Suitable dilutions of tablet sample solutions were scanned in the range of 400 – 200 nm and their absorbances were recorded at the selected wavelengths. The concentrations of each drug in sample solutions were calculated using equations (1) and (2). The results of the analysis and statistical validation data of the tablet formulation are given in Table 2.

Table 2: *Statistical Validation Data of Tablet Formulation*

Methods	*Tablet content*	% Amount found*	% RSD *
A	Cef	99.63	1.202
	Pot.clav.	99.62	0.656
B	Cef	100.05	0.609
	Pot.clav.	99.71	1.172
C	Cef	99.45	0.962
	Pot.clav.	99.68	0.786

** Average of six estimations*

Tablet Formulation: Cepodem XP 325, Ranbaxy pharmaceutical, India

Method-A: Q- Absorbance ratio method, Method-B: First order derivative method, Method-C: Ratio spectra derivative spectrophotometric method

Cef- Cefpodoxime proxetil, Pot.clav.- Potassium clavulanate

Method B: First order derivative method

The standard stock solutions were prepared as discussed in Method A. Suitable dilution of both drug solutions (10 ug mL^{-1} for both Cef and Pot.clav.) were scanned between 400 to 200 nm using the spectrum mode of the instrument. The absorption spectra thus obtained were derivatised from first to fourth order. The first order derivative spectra were selected for the analysis of both the drugs. From the overlain derivative spectra, 269.0 nm and 240.0 nm were selected for the estimation of Cef and Pot.clav. respectively (Fig 2). Mixed standards of Cef and Pot.clav. in the concentration ratio of 10:6.25 ug mL^{-1} were prepared and their absorbances were measured at the selected wavelengths in the first order derivative mode. The absorbances were plotted against concentration to

obtain standard calibration curves. Cef and Pot.clav. both exhibited linearity with absorbances in the concentration range of 1.5-50 ug mL^{-1} at their respective wavelengths. Co-efficients of correlation were found to be 0.9981 and 0.9976 for Cef and Pot.clav. respectively. A set of two simultaneous equations were established using the mean absorptivities of Cef and Pot.clav. at the selected wavelengths.

$A1 = (-4.00)\ C_{CEF} + (0.0)\ C_{POT.CLAV.}$ ..... (3) at 269.0 nm (ë1)

$A2 = (-2.30)\ C_{CEF} + (-4.30)\ C_{POT.CLAV.}$ ..... (4) at 240.0 nm (ë2)

Where,

- 4.00 and -2.30 are mean absorbtivity values of Cef at $ë_1$ and $ë_2$ respectively.

0.0 and -4.30 are mean bsorbtivity values of Pot.clav. at ë1 and ë2 respectively.

A1 and A2 are the absorbance of mixed standard at ë1 and ë2 respectively.

C_{CEF} and $C_{POT.CLAV.}$ are the concentrations of Cef and Pot.clav. respectively.

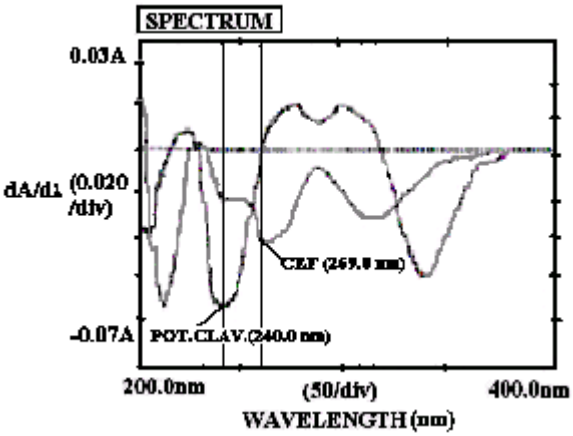

Fig. 2: *Overlain Spectra of Cef and Pot.clav. in First order derivative method*

Estimation from marketed preparations

The tablet sample solutions were scanned in the spectrum mode in the range of 400 to 200 nm. The absorbances of the sample solutions were recorded at 269.0 nm and 240.0 nm in the first order derivative mode. By using equations (3) and (4), the unknown concentration of the drugs in sample solutions were obtained. The analysis procedure was repeated six times with the same batch of tablets. The results of the tablet analysis and its statistical validation data are given in Table 2.

Method C: Ratio spectra derivative spectrophotometric method [18-21]

This method involves dividing the zero order spectras of pure drugs by the individual zero order spectra of each of the drugs. The zero order divisor spectras thus obtained are derivitised to obtain ratio derivative spectras that are independent of the concentration of the drug used as the divisor. By appropriate dilutions of standard stock solutions, working standards containing Cef at increasing concentrations (2.5-50 ug mL^{-1}) were prepared and scanned in the wavelength range of 400-200 nm. The ratio spectras [Fig 3(A)] obtained by dividing each of the zero order spectras of Cef with the stored spectrum of standard solution of Pot.clav. (50 ìg ml^{-1}) were further derivitised to obtain the ratio first order derivative spectras[Fig (3B)]. From the ratio derivative spectra, 246.0 nm was selected for the quantification of Cef in a mixture containing Cef and Pot.clav. Similarly, for Pot.clav., the ratio spectras were obtained by dividing the zero order spectras of standard solutions of Pot.clav. at different concentrations (2.5-50 ug mL^{-1}) with the stored spectrum of standard Cef (40 ug mL^{-1}) as the divisor[Fig 4(A)]. From the derivative of the ratio spectra [Fig 4(B)], 327.0 nm was selected for the quantification of Pot.clav. in a mixture of Cef and Pot.clav. The measured analytical signals at these wavelengths were plotted against concentrations to obtain standard calibration curves. Cef and Pot.clav. both exhibited linearity with absorbances in the concentration range of 2.5-50 ug mL^{-1} at their respective wavelengths. Co-efficients of correlation were found to be 0.9988 and 0.9944 for Cef and Pot.clav. respectively. The standard calibration curve equations were employed to obtain the concentrations of Cef and Pot.clav. in mixed standard and tablet sample solutions.

C_{CEF} = d/dë[A Cef / A Pot.clav.] – (0.0012) / (0.0018)..... (5) at 246.0 nm (ë3)

$C_{POT.CLAV.}$ = d/dë [A Pot.clav./ A Cef] – (0.0041) / (0.0032)..... (6) at 327.0 nm (ë4)

C_{CEF} and $C_{POT.CLAV.}$ are concentration of Cef and Pot.clav. respectively.

A_{CEF} and $A_{POT.CLAV.}$ are absorbance of Cef and Pot.clav. respectively.

0.0012 and 0.0018 are the intercept and slope of Cef respectively at 246.0 nm.

0.0041 and 0.0032 are the intercept and slope of Pot.clav. respectively at 327.0 nm.

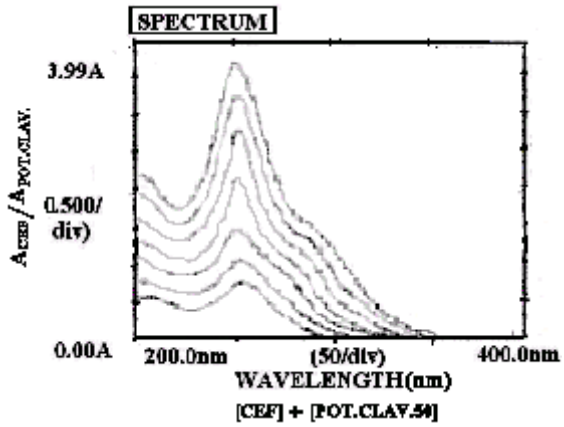

Fig. 3(A): *Zero order overlain ratio spectra of Cef at the concentration level of 2.5-50 ì g mL^{-} in Ratio derivative method*

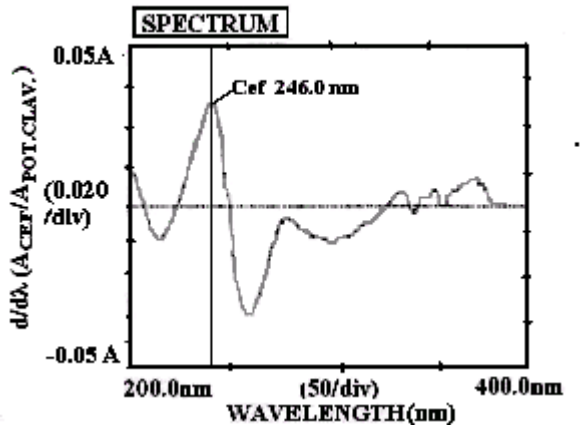

Fig. 3(B): *First Order Derivative of Ratio spectra of Cef (2.5-50 ì g mL^{-1}) ÷ Pot.clav.as divisor(50 ug mL^{-1})*

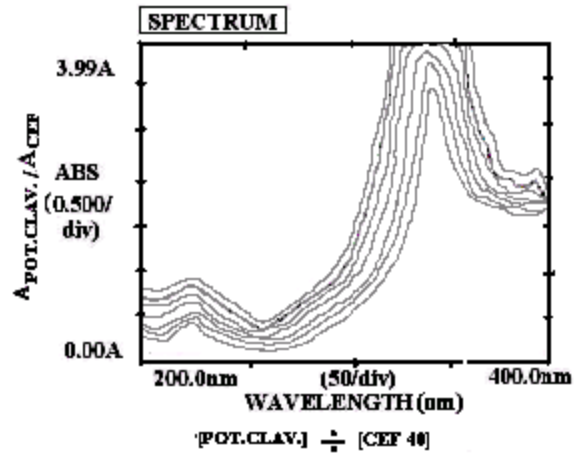

Fig. 4(A): *Zero order overlain ratio spectra of Pot.clav. at the concentration level of 2.5-50 ì g mL^{-1} in Ratio derivative method*

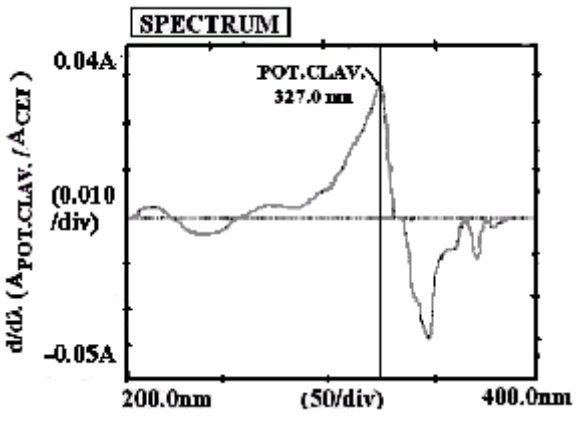

Fig.4(B) : *First Order Derivative of Ratio spectra of Pot.clav. (2.5-50 ì g mL^{-1}) ÷ Cef as divisor (40 ug mL^{-1})*

Estimation from marketed preparations

The sample solutions containing Cef and Pot.clav. in the concentrations ratio of 10:6.25 µg mL^{-1} were prepared and analyzed as per the procedure for mixed standards. By using equations (5) and (6) the concentrations of each drug in sample solutions were calculated. The results of the analysis and statistical validation data of the tablet formulation are given in Table 2. The developed methods were validated as per ICH guidelines[22].

RESULTS AND DISCUSSION

The optical characteristics and regression values of the calibration curves for the developed methods are presented in Table 1. The mean % content of Cef and Pot.clav. in tablet formulation by the developed methods were 99.71% and 99.67% respectively (Table 2). The mean % recoveries of Cef and Pot.clav. were found to be 99.72% and 99.89% respectively indicating high degree of accuracy of the developed methods (Table 3). The results of the ruggedness study indicated that the developed methods were unaffected by variation in instruments and analysts. The results of the proposed methods were statistically evaluated using Turkey-Kramer one way ANOVA which showed P>0.05. The calculated F value was 0.626 and 0.012 for Cef and Pot.clav. respectively which is less than the standard F value(2.91), indicating that there exists no significant difference between the developed methods for the analysis of Cef and Pot.clav. in both bulk and tablet formulation.

Table 3 : *Statistical Validation of Recovery Studies*

Level of % recovery	Methods	% Recovery*		%RSD*	
		Cef	Pot.clav.	Cef	Pot.clav.
80	A	100.10	100.32	0.098	0.414
	B	99.93	99.99	0.227	0.233
	C	99.46	98.90	0.942	0.587
100	A	99.87	100.29	0.076	0.588
	B	99.92	100.19	0.208	0.461
	C	99.07	99.41	0.685	0.890
120	A	100.06	100.05	0.182	0.111
	B	99.83	100.12	0.052	0.301
	C	99.23	99.78	0.504	0.436

**Average of three estimations at each level of recovery.*
Method-A: Q- Absorbance ratio method, Method-B: First order derivative method, Method-C: Ratio spectra derivative spectrophotometric method
Cef- Cefpodoxime proxetil, Pot.clav. - Potassium clavulanate

CONCLUSION

Three simple UV spectrophotometric methods (Q-Absorbance ratio method, First order derivative method and ratio spectra derivative spectrophotometry) were developed for their simultaneous estimations. The standard deviation and RSD calculated for the methods are low (less than 2% as required by ICH guidelines), indicating high degree of precision of the methods. The % recoveries was between 98-102% indicating high degree of accuracy of the proposed methods. Also the results of Turkey-Kramer one way ANOVA indicated that there exists no significant difference between these

methods for the analysis of Cef and Pot.clav. in bulk and formulation. However the ratio derivative method offers the advantage of analyzing individual drugs without any interference of the other drug present in the mixture. The developed methods are rapid, precise, accurate, rugged and can be employed for the routine estimation of Cef and Pot.clav. in both bulk and tablet dosage form.

ACKNOWLEDGEMENTS

The authors express their gratitude to Pad. Dr. D. Y. Patil Institute of Pharmaceutical Sciences and Research, Pune, MH, India, for providing necessary facilities, and to Emcure Pharmaceutical, Pune, India, for the generous gift samples of pure Cef and Pot.clav.

REFERENCES

1. The United States Pharmacopoeia 29, (2004), National Formulary 22, United State Pharmacopoeia Convention Inc., Rockville, MD, p 378, 466, 3087.
2. British Pharmacopoeia ,London: The Stationary Office 2008; II:1777-1779.
3. Sweetman SC. In *Martindale: The complete drug reference.* 32nd edition. London: Pharmaceutical Press, 2002, p 172, 190.
4. Tripathi KD. In *Essentials of Medical pharmacology*. 2008 ; p 702-703.
5. Saathoff N, Lode H, Neider K. Antimicrobial agents and chemotherapy. 1992; 36: 796-800.
6. Rao YS, Chowdary KP, Rao S. Chem. Anal. 2004; 49: 111.
7. Hesham S, Gamal AS. Journal of Pharmaceutical and Biomedical Analysis. 2002; 28**:** 1205-1213.
8. Hesham S. Analytica Chimica Acta. 2004; 515: 333-341.
9. Ewa BG. Journal Microchimica Acta. 136: 31-34.
10. Malathi S, Dubey RN , Venkatnarayanan R. IJPS. 2009, 71(1):102-105.
11. Ming JW, Zou WB, Xue J, Hu CQ. Chromatographia. 65: 69-75.
12. Kakumanua VK, Arora VK, Bansal AK. Journal of Chromatography B. 2006; 835: 16-20.
13. Khan, Sharif IU, Ashfaq S, Asghar M, Nadeem M. Journal of AOAC International. 2008; 91: 4.
14. Sengar MR, Gandhi SV, Patil UP, Rajmane VS. International Journal of ChemTech Research**.** 1: 1105-1108.
15. Darji BH, Shah NJ, Patel AT, Patel NM. IJPS. 2007; 69(2): 331-333
16. Date AA, Nagarsenker MS. Chromatographia journal. 2007; 66: 11-12.
17. Beckett AH, Stenlake JB. In *Practical Pharmaceutical Chemistry*. 4th Edn. Part 2, p 284-299.
18. Ahmad AK, Kawy MA, Nebsen M. Journal of Pharmaceutical and Biomedical Analysis. 2002, 30 (3) : 479-489.
19. Sabnis SS, Dhavale ND, Jadhav VY. Eurasian Journal of Analytical Chemistry. 2008, 3(2): 236-244.
20. Erk N. Journal of Pharmaceutical and Biomedical Analysis. 1999, 21(2): 429-437.
21. Dinc E, Gamze K ,Feyyaz O. Journal of Pharmaceutical and Biomedical Analysis. 2000; 22 (6): 915-923.
22. ICH, Q2 (R1), Harmonised tripartite guideline, Validation of analytical procedures: Text and Methodology, International Conference on Harmonization ICH, Geneva, Nov 2005.