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Development and Validation of First Order Derivative UV Spectrophotometric Method for Simultaneous Estimation of Cefoperazone Sodium and Tazobactam Sodium In Pharmaceutical Formulation

Khushboo P. Patel[1]*, Dilip G. Maheshwari[1]

1.L. J. Institute of Pharmacy, Sarkhej Circle, S. G. Road, Ahmedabad- 382210, Gujarat.

ABSTRACT

A simple, accurate, reliable and reproducible first order derivative method was developed for the simultaneous determination of Cefoperazone sodium (CEFO) and Tazobactam sodium (TAZO) in pharmaceutical formulation. The linearity was carried out by using the concentration range 5-50 µg/ml for CEFO (275.2 nm ZCP of TAZO) and 2-50 µg/ml for TAZO (225 nm ZCP of CEFO) respectively. The correlation coefficient of CEFO and TAZO was found to be 0.999 and 0.998 respectively. At zero crossing point (ZCP) of CEFO (225nm) TAZO showed a measurable derivative absorbance, whereas at zero crossing point (ZCP) of TAZO (275.20nm) CEFO showed a appreciable derivative absorbance value. Precision study showed that %RSD was within range of acceptable limits (< 2%). The % recovery for CEFO and TAZO was found to be within range of 98-101% and 98-102%respectively. The percentage assay was found to be 101.05% and 99.41% for CEFO and TAZO. The result of analysis has been validated as per ICH guideline. This method has applied successfully for determination of CEFO and TAZO in its pharmaceutical formulation.

Keywords: Cefoperazone sodium, Tazobactam sodium, UV Spectrophotometry, First order derivative Spectrophotometry.

*Corresponding Author Email: khushboopatel122@gmail.com

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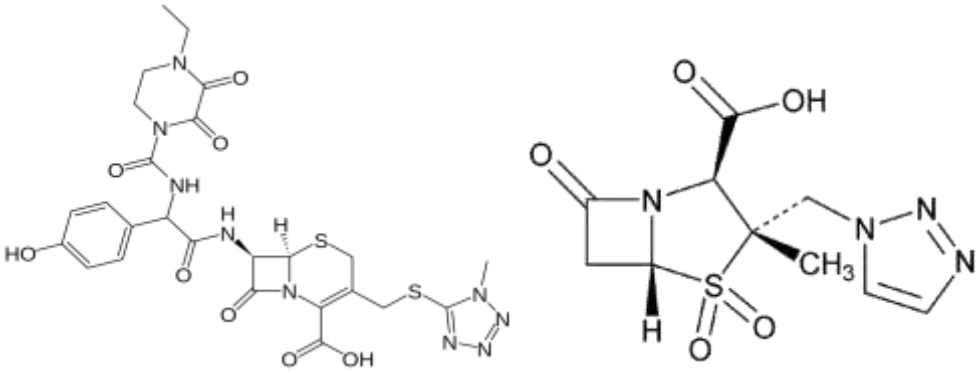
INTRODUCTION

Cefoperazone chemically (6*R*,7*R*)-7-[(2*R*)-2-{[(4-ethyl-2,3-dioxopiperazin-1-yl)carbonyl] amino}-2-(4-hydroxyphenyl)acetamido]-3-{[(1-methyl-1H-1,2,3,4-tetrazol-5-yl)sulfanyl]methyl}-8-oxo-5-thia-1-azabicyclo-oct-2-ene-2-carboxylic acid is a Third-generation cephalosporins[1]. Cefoperazone sodium is official in IP, BP and USP [2-5]. Tazobactam sodium 4-Thia-1-azabicyclo-heptane-2-carboxylic acid, 3-methyl-7- oxo-3-(1H-1, 2, 3-triazol-1-ylmethyl)-, 4, 4-dioxide, 2*S*-(2α,3β,5α)] is a Antipseudomonal penicillins (beta-lactamase inhibitor with antibacterial properties [6]. Tazobactam sodium is official in USP[7].

Both drugs are formulated to-gather in the form of sterile powder for injection for treatment of urinary tract infection, respiratory tract infection, gynecological infection and post operative infection[8-9]. The chemical structures of both drugs [1, 6] were shown in figure 1.



(a) (b)

Figure 1: Chemical structure of Cefoperazone sodium (a) and Tazobactam sodium (b).

Literature survey reveals that some methods have been developed for their determination by spectrophotometry and HPLC either alone or in combination, but no method has been developed for these combined dosage form by first order derivative UV spectrophotometry. The purpose of this work was to develop a simple, accurate and sensitive UV derivative method for determination of Cefoperazone sodium and Tazobactam sodium in pharmaceutical formulation.

MATERIALS AND METHODS

Instrument:

The instrument was double beam UV-visible spectrophotometer (Shimadzu, model 1800, Software: UVProbe 2.31) having two matched quartz cells with 1 cm path length. Sonication of sample solutions was done using ultrasonic cleaner (Sonica 2200MH).

Materials:

Cefoperazone sodium (CEFO) drug sample and Tazobactam sodium (TAZO) drug sample were

procured from E. G. Pharmaceutical. (Himachal Pradesh, India). Sterile powder for injection (Lupitum, Mfg by Lyka Labs Ltd. Gujarat, India and marketed by Lupin Ltd. Mumbai, India) was purchased from local market, containing Cefoperazone sodium 1gm and Tazobactam sodium 125 mg. Methanol (95%) was purchase from Astron chemicals Pvt, Ltd. India.

METHOD

Preparation of standard stock solution:

The stock solution having 1000µg/ml concentration of CEFO and TAZO were prepared separately by dissolving accurately weighed 100 mg of both drugs in 100 ml methanol. The working standard stock solution of 100µg/ml concentration of CEFO and TAZO were prepared seperately by diluting 10 ml of standard stock solution of respective drug with methanol in 100 ml volumetric flask up to the mark.

METHOD DEVELOPMENT (First order derivative):

Selection of scanning range and sampling wavelength:

The solution having 10 µg/ml concentrations of CEFO and TAZO were prepared separately by diluting 1 ml of standard working stock solution of respective drug with Methanol in 10 ml volumetric flask up to the mark. These solutions were scanned in UV range 200-400 nm. The λmax of CEFO and TAZO were found to be 228 nm and 276 nm respectively in normal UV spectra shown in figure 2.

Development of first order derivative spectra:

The spectral data was then processed to obtain first order derivative spectrum at wavelength interval of 2 nm for the range of 200-400nm. It was observed that CEFO shows ZCP at 225nm and TAZO shows ZCP at 275.20 nm. At ZCP of CEFO (225nm), TAZO showed a measurable dA/dλ, whereas at ZCP of TAZO (275.20nm), CEFO showed a measurable dA/dλ. Hence the wavelengths 275.20nm and 225nm were selected as analytical wavelengths for determination of CEFO and TAZO first order derivative method respectively shown in figure 3.

METHOD VALIDATION

The above proposed method was validated according to ICH Q2 R1 guidelines for validation of analytical procedures [10] in order to determine the linearity, Accuracy, Precision and Assay of marketed formulation.

Linearity and Range:

Calibration curve constructed was linear over a selected range of 5-10 µg/ml and 2-10 µg/ml for CEFO and TAZO respectively. The aliquots of both the drugs used in linearity studies were converted to first derivative spectra and the derivative absorbance at 275.20 nm and 225 nm for

CEFO and TAZO were measured respectively. The calibration curve of responses against concentration was plotted was shown in figure 4 and 5. Each concentration was repeated three times. Correlation coefficient and regression line equations for CEFO and TAZO were calculated and were shown in table 1.

Accuracy:

The accuracy of the developed method was determined by finding out the amount of recovery of Cefoperazone sodium and Tazobactam sodium. For the accuracy standard addition method was used where, as known amount of CEFO and TAZO were added to the known concentration (20µg/ml) of sterile powder for injection solution. The amount recovered was found by measuring the absorbance of the solution and was expressed as mean recovery of samples with upper and lower limits of percent relatives of standard deviation. Recovery was done at three different levels i.e. 80%, 100% and 120%, within the linearity range of both the drugs.

Precision

Repeatability (n=6):

For the repeatability study, from the working stock solution of both drugs, aliquot of 2 ml was transferred to a separate 10 ml volumetric flask and diluted upto mark with methanol such that it gives the concentration of 20 µg/ml of CEFO and TAZO both. The absorbance of the solutions was measured at 275.20 nm and 225 nm respectively. The procedure was repeated six times and % RSD was calculated and shown in table 3.

Intraday Precision (n=3):

From the working stock solution, aliquots of 0.5 ml, 3 ml, 5 ml of CEFO and 0.2 ml, 3 ml, 5ml of TAZO were transferred to separate 10 ml volumetric flask and diluted upto the mark with methanol to give concentration of 5, 30 and 50µg/ml for CEFO and 2, 30 and 50µg/ml for TAZO. The solutions were analyzed three times on the same day and % RSD was calculated and shown in table. 3.

Interday Precision (n=3):

From the working stock solution, aliquots of 0.5 ml, 3 ml, 5 ml of CEFO and 0.2 ml, 3 ml, 5ml of TAZO were transferred to separate 10 ml volumetric flask and diluted upto the mark with methanol to give concentration of 5, 30 and 50µg/ml for CEFO and 2, 30 and 50 µg/ml for TAZO. The solutions were analyzed three times on three different days and % RSD was calculated and were shown in table 3.

Limit of Detection (LOD) and Limit of Quantification (LOQ):

Limit of detection (LOD) is the minimum concentration of the analyte in the sample which can

be analyzed by the instrument. Limit of quantification (LOQ) is the minimum concentration of the analyte that can be reliably quantified. The Limit of detection (LOD) and Limit of quantification (LOQ) were measured using following formula. The values of LOD and LOQ for CEFO and TAZO were shown in table no. 5.

$$LOD = 3.3 \times (SD/Slope)$$

And

$$LOQ = 10 \times (SD/Slope)$$

Where,

SD = Standard deviation of the Y- intercepts of the 3 calibration curves.

Slope = Mean slope of the 3 calibration curves.

Assay of Pharmaceutical formulation:

Commercially available marketed formulation containing both Cefoperazone sodium and Tazobactam sodium (Lupitum) were used for the study. Sterile powder for injection equivalent to 100 mg of and 12.5 mg of Cefoperazone sodium and Tazobactam sodium was weighed. 87.5 mg pure Tazobactam sodium was added by standard addition method transferred in to a 100 ml volumetric flask to bring both drugs in 1:1 ratio. This stock solution was prepared in Methanol, sonicated for 15 min, the volume was adjusted up to the mark with same solvent. Then solution was filtered through whatman filter paper No. 41. This stock solution contains Cefoperazone sodium 1000 µg/ml and Tazobactam sodium 1000 µg/ml. 10 ml of this solution was transferred to 100 ml volumetric flask and diluted to mark with Methanol. Then the appropriate dilution of 20µg/ml was made using methanol as solvent. All the determinations were carried out in triplicate. The absorbance of the prepared solutions was measured at ZCP of CEFO and ZCP of TAZO and then the concentration of both the drug was calculated using calibration curve equation. The amount of the drug found in dosage form was shown in table 4.

RESULTS AND DISCUSSION:

The first order derivative method is useful for routine analysis of Cefoperazone sodium and Tazobactam sodium in pharmaceutical formulation. The derivative spectroscopy method applied has the advantage that it locates hidden peak in the normal spectrum. It eliminates the interference caused by the excipients and the degradation products present, if any, in the formulation. The zero order and first order spectra for Cefoperazone sodium and Tazobactam sodium were recorded and shown in the figure. The zero crossing point was found at the wavelength of 225 nm and 275.20 nm for CEFO and TAZO respectively (Figure 3).

Linearity and Range

The Beer- Lambert's concentration range was found to be 5-50 µg/ml for CEFO and 2-50 µg/ml for TAZO at 275.20 nm and 225 nm respectively. The correlation coefficient was found to be 0.999 for CEFO and 0.998 for TAZO (Table 1) for proposed method.

Precision

Precision was determined by studying repeatability, intraday and interday precision. The standard deviation and Relative standard deviation (%RSD) were calculated for both the drugs. The % RSD for proposed method were found to be not more than 2.0% which indicates good intermediate precision (Table 3).

LOD and LOQ

The values of LOD and LOQ were 1.7 µg/ml and 4.71µg/ml for CEFO and 0.53 µg/ml and 1.57 µg/ml for TAZO respectively (Table 5).

Accuracy

To study the accuracy of the proposed methods, and to check the interference from excipients used in the dosage forms, recovery experiments were carried out by the standard addition method. This study was performed by addition of known amounts of CEFO and TAZO to reanalyzed respective solutions of commercial injectable (Table 2).

Analysis of the Marketed Formulation:

The drug content was found to be 101.05%and 99.41% for CEFO and TAZO respectively. It may therefore be inferred that degradation of CEFO and TAZO had not occurred in the marketed formulations that were analyzed by this method. This method can be used for routine analysis of CEFO and TAZO in pharmaceutical dosage form (Table 4).

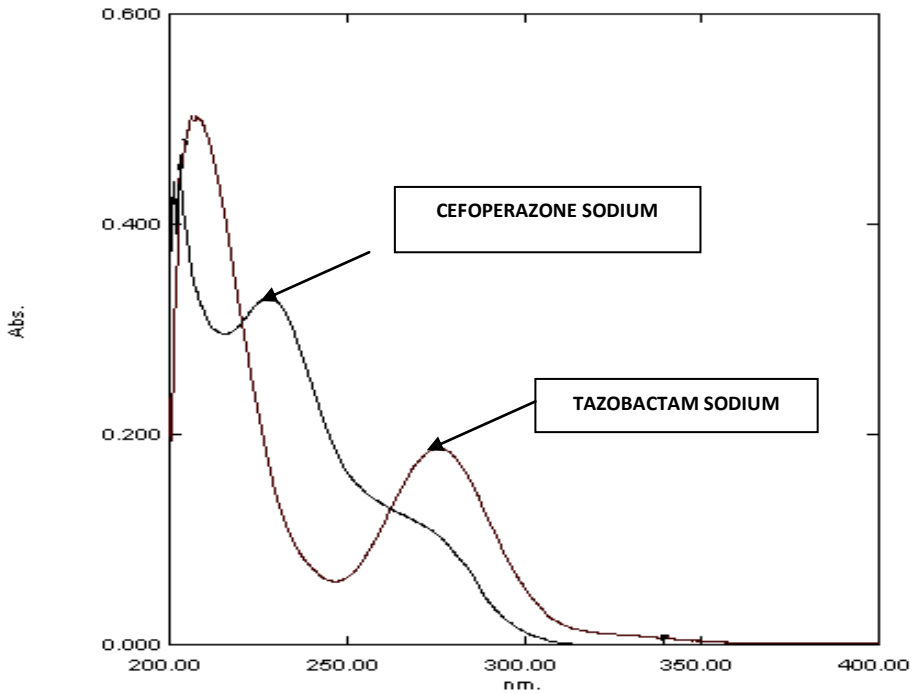


Figure 2: Overlain zero order spectra of CEFO (10µg/ml) and TAZO (10 µg/ml) in methanol

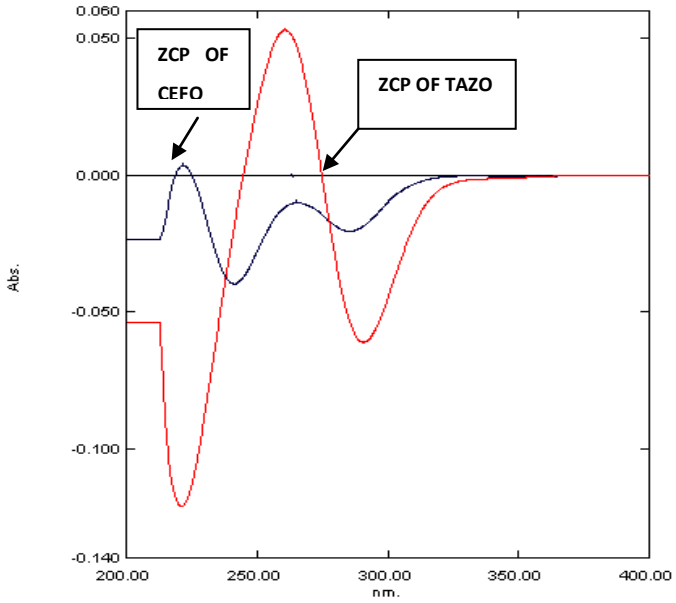


Figure 3: Overlain first order spectra of CEFO (10µg/ml) and TAZO (10µg/ml) in Methanol

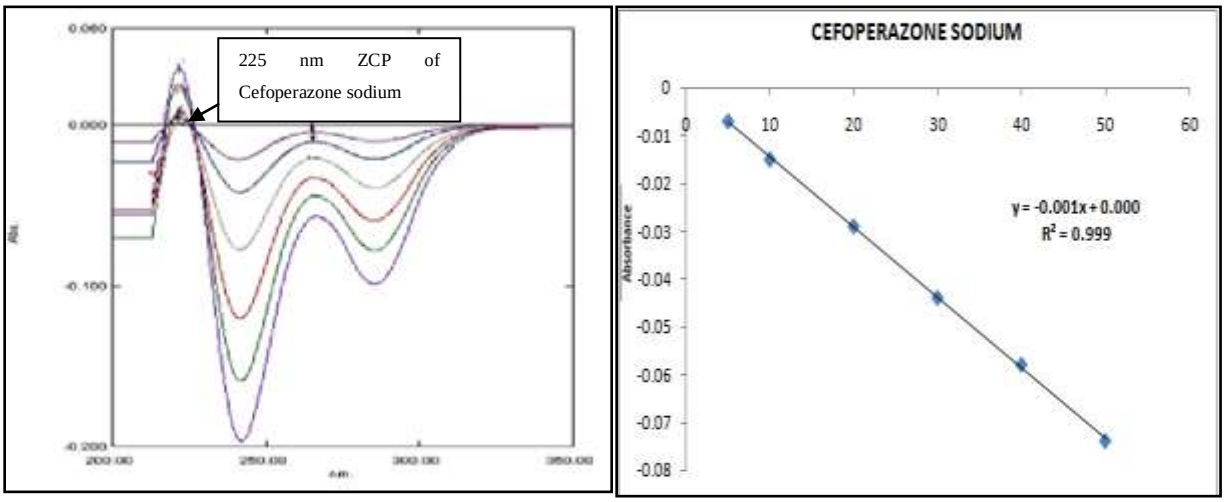


Figure 4: Linearity graph for first order derivative of CEFO

Table 1: Optical Characteristics

Parameters	Cefoperazone sodium 275.20 nm	Tazobactam sodium 225 nm
Beer's law limit (µg/ml)	5 - 10	2 - 70
Regression equation	Y = – 0.001x + 0.000	Y = – 0.006x – 0.059
Slope (m)	0.001	0.000
Intercept (c)	0.000	0.059
Correlation coefficient (R^2)	0.999	0.998

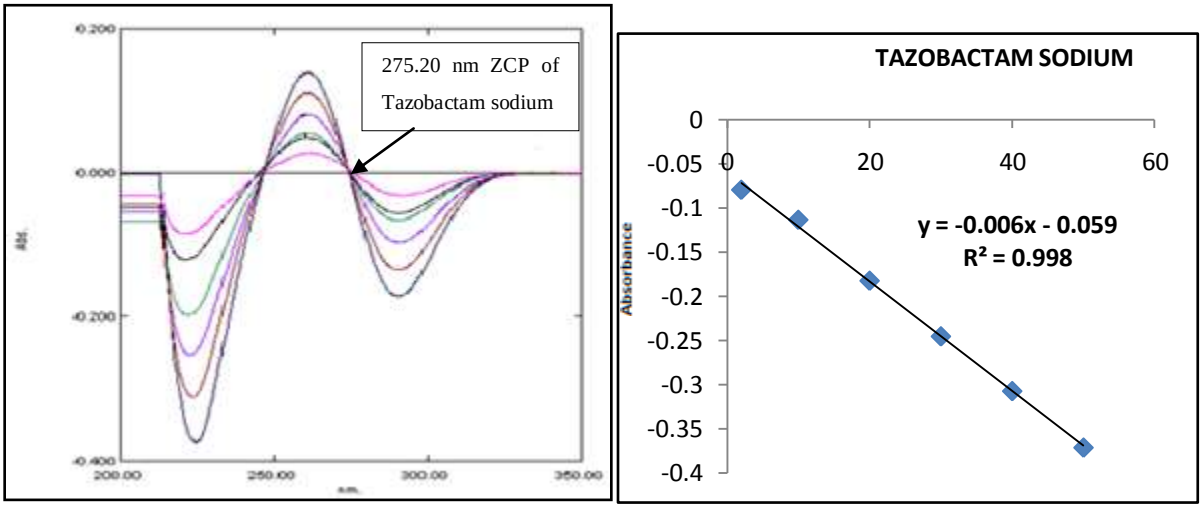


Figure 5: Linearity graph for first order derivative of TAZO

Table 2: Results of Recovery studies

Drug	Conc. of std drug	Recovery level (%)	Amount of drug added (µg/ml)	Amount of drug recovered (µg/ml)	% Recovery ± SD(n=3)
Cefoperazone sodium	20 µg	80 100	16 20	35.66 40.33	99.07 ± 1.309 100.83± 1.178

		120	24	43.66	99.24 ± 1.073
Tazobactam sodium	20 µg	80	16	54.22	99.44 ± 1.046
		100	20	59.90	101.13 ± 0.830
		120	24	66.42	101.28 ± 0.686

Table 3: Repeatability, Inter-day and Intra-day precision of Cefoperazone sodium and Tazobactam sodium

Drug	Concentration (µg/ml)	Average ABS ± SD	% RSD
Repeatability (n=6)			
Cefoperazone sodium	20	-0.029 ± 0.00047	1.60
Tazobactam sodium	20	-0.183 ± 0.00115	0.631
Intra-Day Precision (n=3)			
Cefoperazone sodium	5	-0.007 ± 0.00013	1.85
	30	-0.045 ± 0.000816	1.81
	50	-0.074 ± 0.000471	0.63
Tazobactam sodium	2	-0.080 ± 0.000816	1.02
	30	-0.244 ± 0.000816	0.33
	50	-0.382 ± 0.003742	0.98
Inter-Day Precision (n=3)			
Cefoperazone sodium	5	-0.008 ± 0.00015	1.88
	30	-0.044 ± 0.00047	1.05
	50	-0.075 ± 0.00081	1.08
Tazobactam sodium	2	-0.080 ± 0.00124	1.54
	30	-0.244 ± 0.00047	0.19
	50	-0.380 ± 0.00524	1.38

Table 4: Analysis of pharmaceutical formulation

Drug	Label claim (g)	Amount found (g)	% Drug found ±SD (n=3)
Cefoperazone sodium	1	1.010	101.05 ± 0.950
Tazobactam sodium	0.125	0.1242	99.41 ± 1.037

Table 5: Limit of detection (LOD) and Limit of Quantification (LOQ)

Parameters	Cefoperazone sodium	Tazobactam sodium
LOD (µg/ml)	1.75	0.53
LOQ (µg/ml)	4.71	1.57

CONCLUSION

First order derivative method was developed for simultaneous estimation of CEFO and TAZO in their combined formulation without prior separation. Spectra of CEFO were completely overlapped by TAZO and derivatization was used as a powerful tool for simultaneous determination. Method was found to be accurate, sensitive and precise as can be reflected from validation data. Developed methods were successfully applied for estimation of CEFO and TAZO in pharmaceutical formulation.

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REFERENCES

1. An encyclopedia of chemical, drugs and biological, The Merk index, 14th edition, Published by merk research laboratory, pp 317.
2. Government of India. Ministry of Health and Family welfare. Indian Pharmacopeia Vol. II. The controller of Publication, New Delhi; 2007: 255.
3. Government of India. Ministry of Health and Family welfare. Indian Pharmacopeia Vol. II. The controller of Publication, New Delhi; 2010: 1014-1015.
4. United State Pharmacopoeia, USP 30, NF 27, USP Convention INC. Rockville, Asian edition; 2009: 1661.
5. British Pharmacopoeia, British Pharmacopoeia Commission office, 6th Edn, version 15, Vol I, II. TSO information & Publishing solution, London; 2009: 1199.
6. An encyclopedia of chemical, drugs and biological, The Merk index, 14th edition, Published by Merk research laboratory, pp 1561.
7. United State Pharmacopoeia, USP 31, NF 26, USP Convention INC. Rockville, vol. III., 2011: 3323.
8. Mohan H., Textbook of Pathology; 6th edition; Jaypee Brothers Medical Publishers Pvt. Ltd.; 2010: 467-475,723.
9. Trevor AJ., Katzung BG., Katzung and Trevor's Pharmacology; 8th edition; Mc grow Hill Medical publisher, Newyork; 2009: 364-428.
10. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human use on Validation of Analytical Procedures: Text and Methodology ICH Q2 (R1), 2005.