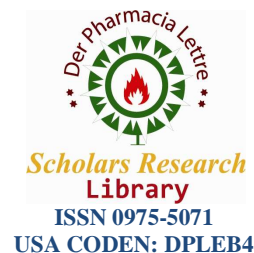




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Development and validation of stability indicating method for the estimation of pazopanib hydrochloride in pharmaceutical dosage forms by RP-HPLC

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ABSTRACT

A stability indicating reverse phase high performance liquid chromatography (RP-HPLC) method was developed and validated in pharmaceutical dosage form using Inertsil C18 (250mm × 4.6mm, 5 μ) column in isocratic mode with a mobile phase consisting of 0.1% ortho phosphoric acid and acetonitrile in the ratio 55:45 (%v/v). The detection was monitored using PDA detector at 269nm with a flow rate of 1mL/min and the column oven temperature was maintained at 30°C. The retention time was found to be 5.1mins. A good linear response was observed in the concentration range of 25 μ g/mL – 150 μ g/mL with a correlation coefficient of 0.999. The method was validated as per ICH guidelines. Pazopanib hydrochloride was subjected to stress conditions including acidic, alkaline, oxidation, photolysis, thermal and neutral degradation, and the results showed that the net degradation was found to be within the limits.

Keywords: Pazopanib hydrochloride, RP-HPLC, stability indicating, method validation.

INTRODUCTION

Pazopanib hydrochloride[1,2] (figure 1) is chemically designated as 5-(4-((2, 3-dimethyl-2H-indazol-6-yl) (methyl) amino) pyrimidin-2-yl amino)-2-methyl benzene sulfonamide hydrochloride. It is a potent and selective multi-targeted receptor tyrosine kinase inhibitor that blocks tumour growth and inhibits angiogenesis. It is used for renal cell carcinoma and soft tissue sarcoma. This compound belongs to the class of organic compounds known as alkyldiarylamines. These are tertiary alkylarylamines having two aryl and one alkyl groups attached to the amino group. According to the literature survey, it was found that only few analytical methods like Spectrophotometry method[3] and RP-HPLC methods[4-6] were developed for the estimation of Pazopanib hydrochloride in pharmaceutical dosage form. The main objective of the aimed method was to develop and validate the stability indicating method for the estimation of Pazopanib hydrochloride in pharmaceutical dosage form by RP-HPLC.

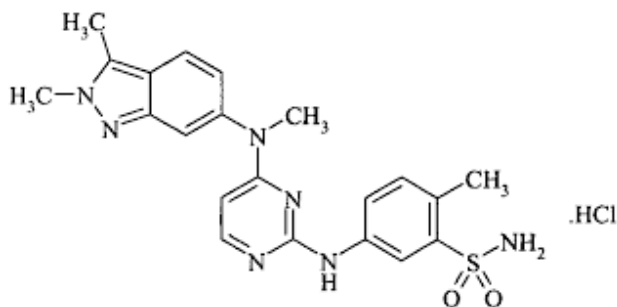


Figure 1: Chemical structure of Pazopanib hydrochloride

MATERIALS AND METHODS

Chemicals and Reagents: Pazopanib hydrochloride working standard was received as gift samples. Pazopanib hydrochloride tablets 100mg were purchased from local pharmacies. Purified water was obtained from Millipore system. Acetonitrile (HPLC grade) was obtained from E-Merck. All other chemicals used in the analysis were of AR grade.

Instrumental and Analytical conditions: The HPLC analysis was performed using a Waters 2998 model equipped with an autosampler, Photo Diode Array detector and running on empower software. Column used was Inertsil C-18 (250mm × 4.6mm, 5 μ particle size). The detection wavelength was 269nm. The injection volume of sample was 10 μ L. An isocratic mobile phase containing 0.1% ortho phosphoric acid and acetonitrile in the ratio 55:45 (%v/v) was carried out with the flow rate of 1mL/min. Column oven temperature was maintained at 30°C.

Preparation of Buffer: (0.1% Ortho Phosphoric Acid)

Dilute 1mL of ortho phosphoric acid with 1000mL of milli-Q water in 1000mL volumetric flask.

Preparation of Mobile phase:

Mixture of Buffer and Acetonitrile in the ratio 55:45 (%v/v) respectively

Preparation of Diluent:

Mixture of Water and Acetonitrile in the ratio 50:50 (%v/v) respectively

Preparation of standard solution: 50mg of Pazopanib hydrochloride working standard was accurately weighed and transferred into a 25mL volumetric flask. 15mL of diluent was added, sonicated to dissolve and make up to final volume with diluent. From the above standard stock solution, 1mL was pipetted into a 10mL volumetric flask and the volume was made upto mark with diluent.

Preparation of sample solution: 20 Tablets (Votrient) were weighed accurately and the average weight was calculated and tablets were crushed to fine powder. An amount equivalent to 50mg of Pazopanib hydrochloride was weighed and transferred into 25mL volumetric flask. 15mL of diluent was added and sonicated for 30min with intermediate shaking. Volume was made up with diluent. The above solution was filtered using HPLC filters. 1mL of the above solution was pipette into 10mL volumetric flask and made up with diluent.

METHOD DEVELOPMENT

Inertsil C-18 column (250mm × 4.6mm, 5 μ) as stationary phase with a mobile phase of 0.1% O-Phosphoric acid and acetonitrile (55: 45%v/v) at a flow rate 1mL/min and a detection wavelength of 269nm afforded the best separation of drug. The standard solution and sample solution prepared as above were injected into the 10 μ L loop and the chromatograms were recorded as shown in the figure 2, 3 and 4 respectively. The retention time of drug was found to be 5.1min. The % Assay of Pazopanib hydrochloride was calculated.

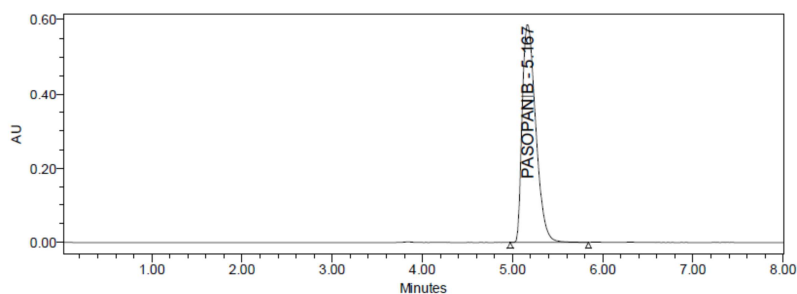


Figure 2: Standard Chromatogram

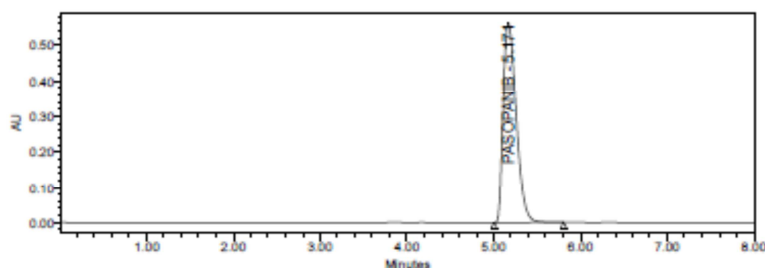


Figure 3: Sample Chromatogram

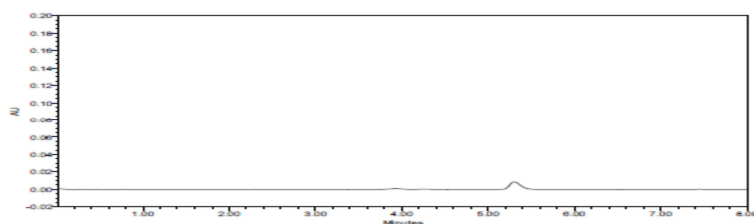


Figure 4: Blank Chromatogram

METHOD VALIDATION [7]**SPECIFICITY: (Placebo interference)**

The study of placebo interference from excipients was conducted. Placebo interference was checked for two strengths in duplicate, equivalent to about the weight of placebo as per the test method. The results were summarized in table 2 and chromatogram was recorded as shown in figure 6.

ACCURACY: (Recovery)

To determine the accuracy of the test method samples were prepared by spiking Pazopanib hydrochloride raw material with the equivalent amount of placebo at 50%, 100% and 150% of the target concentration. Samples were prepared at each concentration levels in triplicate. The average % recovery of Pazopanib hydrochloride was determined. The results were summarized in table 2.

PRECISION:

Precision of the test method was determined by injecting six samples of standard Pazopanib hydrochloride solution. The %RSD was determined. The results were summarized in table 2.

LINEARITY:

A series of Standard solutions were prepared and injected into the HPLC system. A graph was plotted to “concentration” versus “peak area” in linearity section. The results and graph were summarized in table 2 and figure 7.

LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTIFICATION (LOQ):

For determining LOD and LOQ, initially standard deviation and slope of calibration curve was calculated. Then by using these values as per formula maintained in ICH guideline these parameters were evaluated. The results were summarized in table 2.

RUGGEDNESS: (Analyst to analyst/ System to system variability)

Ruggedness of assay method was conducted on Pazopanib hydrochloride tablets using two different systems by different analysts and analyzed under similar conditions as per the test method. The results were summarized in table 2.

ROBUSTNESS:

Standard solution was prepared as per the test method, injected into HPLC system and analyzed using varied mobile phase composition ($\pm 10\%$ of actual organic phase composition), flow rate (± 0.2 mL/min of actual flow rate) and column oven temperature ($\pm 5^\circ\text{C}$ of actual column oven temperature). The system suitability parameters were evaluated as per the test method. The results were summarized in table 2.

FORCED DEGRADATION STUDIES:**Acid stress study:**

To 1 mL of Pazopanib hydrochloride stock solution, 1 mL of 2N hydrochloric acid was added and refluxed for 30 mins at 60°C . The resultant solution was diluted to obtain $200\mu\text{g/mL}$ solution and $10\mu\text{L}$ solution was injected into the chromatographic system and the chromatogram was recorded to assess the stability of sample as shown in figure 8a. The result was summarized in table 3.

Base stress study:

To 1 mL of Pazopanib hydrochloride stock solution, 1 mL of 2N sodium hydroxide was added and refluxed for 30 mins at 60°C . The resultant solution was diluted to obtain $200\mu\text{g/mL}$ solution and $10\mu\text{L}$ was injected into the chromatographic system and the chromatogram was recorded to assess the stability of sample as shown in figure 8b. The result was summarized in table 3.

Peroxide stress study:

To 1 mL of Pazopanib hydrochloride stock solution, 1 mL of 20% hydrogen peroxide (H_2O_2) was added. The solutions were kept for 30 min at 60°C . For HPLC study, the resultant solution was diluted to obtain $200\mu\text{g/mL}$ solution and $10\mu\text{L}$ was injected into the system and the chromatogram was recorded to assess the stability of sample as shown in figure 8c. The result was summarized in table 3.

Dry heat exposure study:

The standard drug solution was placed in oven at 105°C for 6h to study dry heat degradation. For HPLC study, the resultant solution was diluted to $200\mu\text{g/mL}$ solution and $10\mu\text{L}$ was injected into the system and the chromatogram was recorded to assess the stability of the sample as shown in figure 8d. The result was summarized in table 3.

UV light exposure study:

The photochemical stability of the drug was also studied by exposing the drug solution to UV Light by keeping the beaker in UV Chamber for 7 days or $200\text{ Watt hours/m}^2$ in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain $200\mu\text{g/mL}$ solution and $10\mu\text{L}$ was injected into the system and the chromatogram was recorded to assess the stability of sample as shown in figure 8e. The result was summarized in table 3.

Water stress study:

Stress testing under neutral conditions was studied by refluxing the drug in water for 6 hrs at a temperature of 60°C . For HPLC study, the resultant solution was diluted to $200\mu\text{g/mL}$ solution and $10\mu\text{L}$ were injected into the system and the chromatogram was recorded to assess the stability of the sample as shown in figure 8f. The result was summarized in table 3.

RESULTS

Selection of detection wavelength

From the UV spectrum, suitable wavelength considered for monitoring the drug was 269nm.

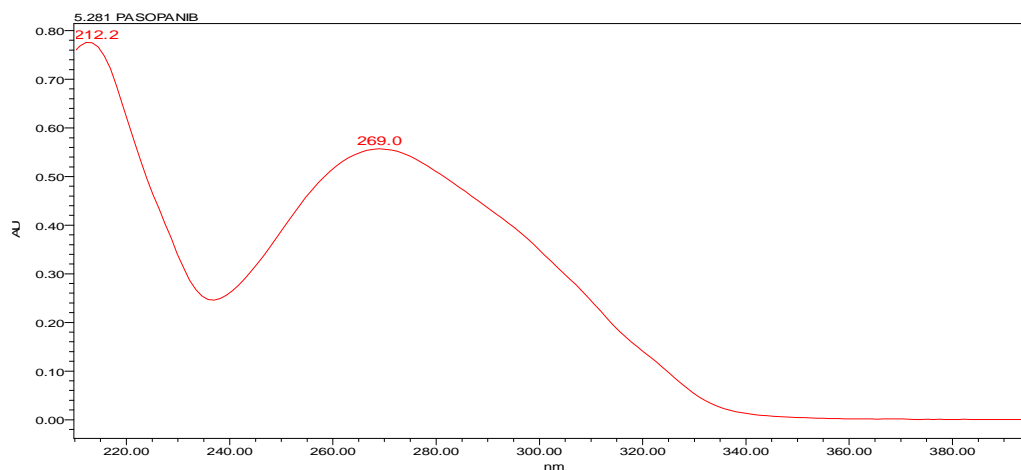


Figure 5: UV Spectrum of Pazopanib hydrochloride

SYSTEM SUITABILITY:

Table 1: System suitability parameters

S. No.	System Suitability Parameter	Observed value	Acceptance criteria
1	% RSD	0.32	NMT 2.0
2	USP Tailing factor	1.47	NMT 2.0
3	USP Plate Count	5744	NLT 2000

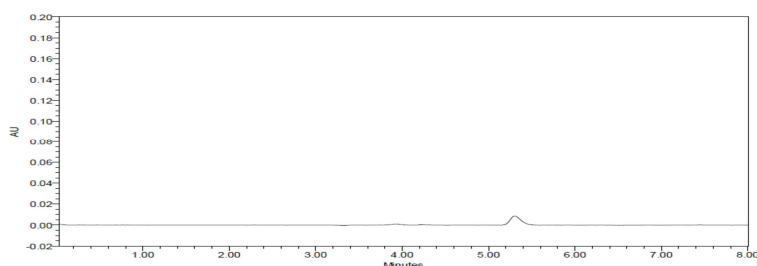


Figure 6: Placebo Chromatogram

Table 2: Results of Method Validation parameters

Parameter		Result
Specificity		Specific
Linearity	Concentration range, µg/mL	25- 150
	Regression equation, y=mx+c	y=55078x
	Slope, m	55078
	Correlation coefficient, r	0.999
Accuracy (% recovery) n=3	Level I, 50%	99.94
	Level II, 100%	99.53
	Level III, 150%	99.99
Precision (%RSD), n=6		0.32
Ruggedness (%RSD), n=6		< 2
Robustness (%RSD)	Variation in flow rate (±0.2mL/min)	< 2
	Variation in organic phase composition (±10%)	< 2
	Variation in column temperature (±5%)	< 2
Limit of Detection (LOD), µg/mL		2.48
Limit of Quantification (LOQ), µg/mL		7.53
Forced degradation studies (net degradation)		< 50%

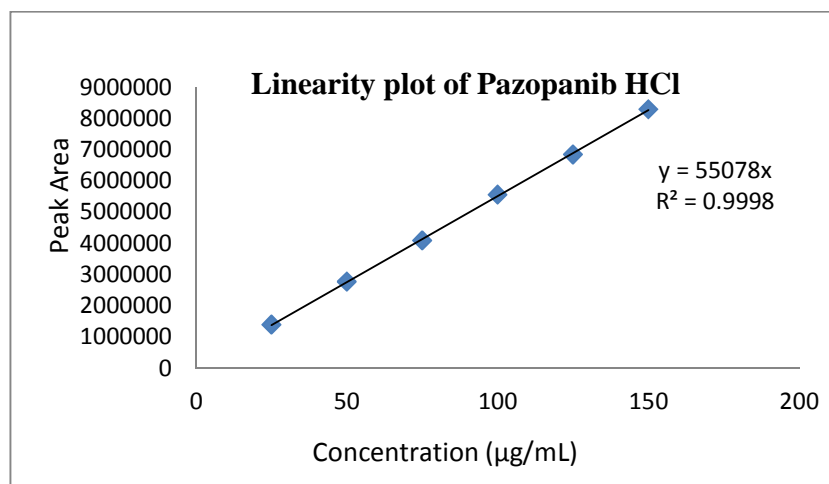


Figure 7: Linearity plot of Pazopanib hydrochloride

Table 3: Forced degradation studies

S.No.	Stress condition	% Assay	% area of degradation peak	Peak purity Angle	Peak purity threshold
1	2N HCL for 30mins at 60°C	95.34	1.56	0.058	0.264
2	2N NaOH for 30mins at 60°C	97.13	1.29	0.046	0.262
3	20% H ₂ O ₂ for 30mins at 60°C	93.94	2.41	0.054	0.262
4	105°C for 6hrs	98.64	-	0.053	0.269
5	UV light 200wts/hr or 7days	99.04	-	0.061	0.324
6	Water for 6hrs at 60°C	99.15	-	0.058	0.265

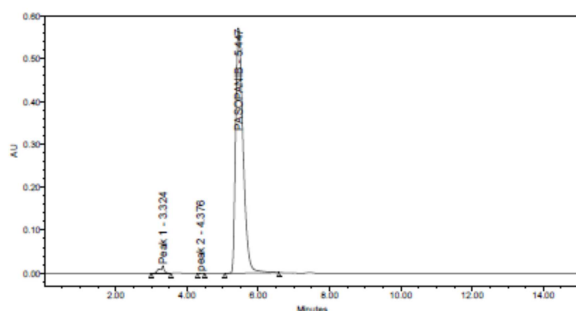


Figure 8a: Acid stress study chromatogram

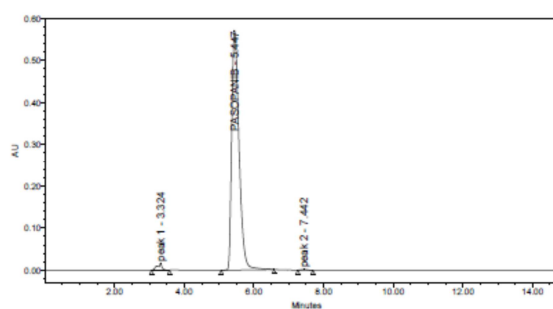


Figure 8b: Base stress chromatogram

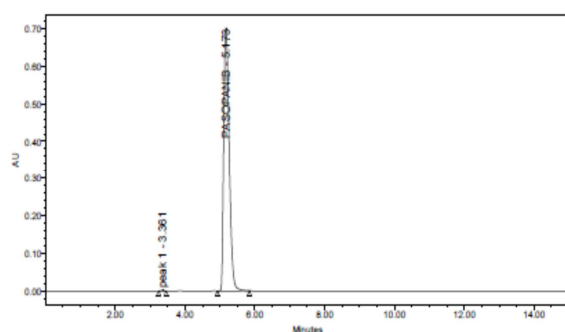


Figure 8c: Peroxide stress chromatogram

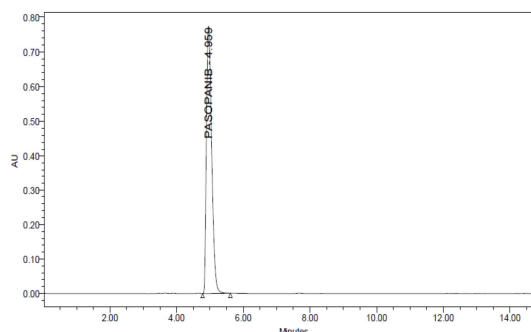


Figure 8d: Dry heat study chromatogram

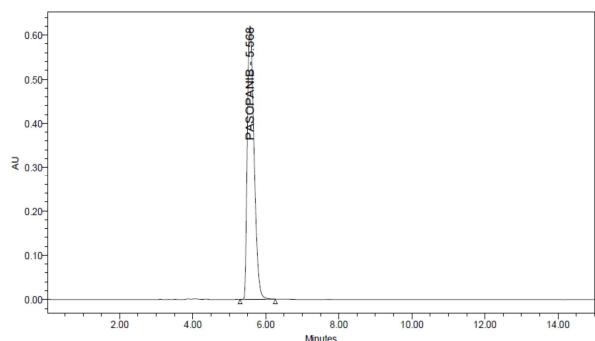


Figure 8e: UV degradation chromatogram

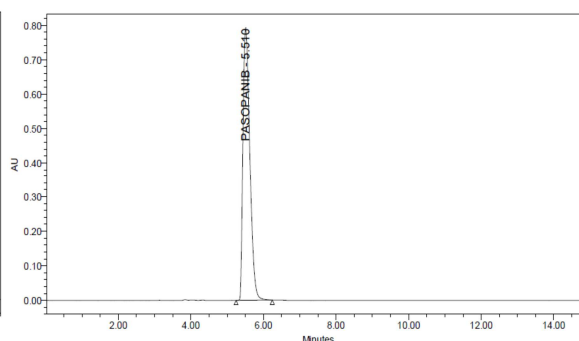


Figure 8f: Water Stress chromatogram

DISCUSSION

Initially, various mobile phase compositions were tried to elute the drug. Mobile phase ratio and flow rate were selected based on peak parameters (height, capacity, theoretical plates, tailing or symmetry factor), run time and resolution.

The solution of 10ppm of Pazopanib hydrochloride in diluent (Acetonitrile: Water, 50:50) was prepared and the solution was scanned in the range of 200-400nm. At 269nm, the drug showed maximum absorbance and better detector response. After considering the entire system suitability parameters mobile phase 0.1% O-Phosphoric acid buffer and acetonitrile (55:45%v/v) run in isocratic mode and flow rate 1.0mL/min was selected. The retention time of Pazopanib hydrochloride was found to be 5.1min. The system suitability parameters are calculated.

The calibration was linear in concentration range of 25-150 µg/ml, with correlation coefficient 0.999, indicates that the concentration of Pazopanib hydrochloride obeys Beer's-Lambert's law.

The method was found to be specific as there is no interference of placebo peak at the retention time of Pazopanib hydrochloride peak. Accuracy was confirmed by recovery studies by proposed method. The percentage recovery of Pazopanib hydrochloride was found to be 99.53% - 99.99%. Hence the developed method was found to be accurate. To evaluate Precision study, %RSD was calculated. The %RSD value was found to be 0.32. These results showed reproducibility of the assay.

The limit of detection and the limit of quantification were found to be 2.48µg/mL and 7.53µg/mL. To evaluate ruggedness study, %RSD was calculated and found to be less than 2. This indicates that the method is rugged. The values in the robustness evaluation study, indicated that the method was quite robust.

The stability of an analytical method was determined by forced degradation studies. The net degradation was found to be within the limits. Purity angle is found to be less than purity Threshold.

CONCLUSION

A simple, stability indicating method for the estimation of Pazopanib hydrochloride has been developed by RP-HPLC. The developed method was validated as per ICH guidelines. The proposed method shows good agreement with all validation parameters. The optimized method is precise, accurate, specific, rugged, robust and stable. A linear relation is observed between the concentration and the result. The developed method can be used for the analysis of routine quality control sample.

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REFERENCES

- [1] S. Tugus; S. Koch; L. Gualanli; L. Xiujuan. *Molecular Aspects of Medicine*, **2011**, 88-111.
- [2] G. Sonpavde; T. E. Hutson. *Current Oncology report*, **2007**, 115-119.
- [3] S. Susena; K. Vanitha Prakash; P. Ravi Pratap; B. Umashankar; E. Manasa. *International Journal of Pharmaceutical, Chemical and Biological Sciences*, **2013**, 3, 3, 533-537.
- [4] V Escudero-Ortiz; JJ Pérez-Ruixo; B Valenzuela. *Therapeutic Drug Monitoring*, **2015**, 37, 2, 172-179.
- [5] Ravi Pratap Pulla; Asadulla Khan; J. Venkateswara Rao; Suresh Kumar Sudam; K. Sujana. *International Journal of Advances in Pharmaceutical Analysis*, **2013**, 3, 1, 24-29.
- [6] K. Vinodh Kumar; K. Puspha; D. Gowri Sankar. *Journal of Chemical and Pharmaceutical Research*, **2015**, 7, 8, 114-120.
- [7] ICH, Validation of Analytical Procedures: Text and methodology Q2 (R1): International Conference on Harmonization, IFPMA, Geneva, **2005**.