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Research Article

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Development and validation of a stability indicating RP-HPLC method for the determination of Pazopanib in bulk drug and its pharmaceutical dosage form

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ABSTRACT

A specific and accurate HPLC method is developed for the determination of pazopanib in bulk drugs and in solid tablet dosage form. Best symmetric peak shape obtained with Phenomenox Kinetex -C18 (100 X 4.6 mm, 2.6μ) column in an isocratic mode, with retention time 20min.The mobile phase used was potassium dihydrogen phosphate buffer : Methanol 60:40(v/v) with flow rate 0.8 ml/min and wavelength monitored at 263 nm. As per ICH guidelines method has validated. Method has found linear in the range of 5-45 μg/ml. The LOD and LOQ were found to be 0.05 and 0.1 μg/ml respectively. Method was found specific with respective of diluents, excipients and degradants.

Key words: Pazopanib (PPB), Limit of Detection (LOD), Limit of quantitation (LOQ)

INTRODUCTION

Pazopanib HCl (PPB) chemically, 5-[[4-[(2, 3- dimethyl-2H-indazol-6- yl) methylamino]-2- pyrimidinyl] amino]- 2-methylbenzenesulfonamide monohydrochloride. (Figure: 1). The empirical formula is $C_{21}H_{23}N_7O_2S.HCl$ & the molecular weight is 473.99 gms/mol. It is a potent and selective multitargeted receptor tyrosine kinase inhibitor of VEGFR- 1, VEGFR-2, VEGFR-3,PDGFR-a/β1, and c-kit that blocks tumor growth and inhibits angiogenesis [7]. It has been approved for renal cell carcinoma and soft tissue sarcoma and also active in ovarian cancer. Pazopanib also appears effective in the treatment of non-small cell lung carcinoma. Literature survey reveals a few chromatographic methods to determine PPB in tablet dosage form and also in biological fluids. HPLC methods are useful in the drug samples and also in pharmaceutical dosage forms. The availability of an HPLC method with high sensitivity and selectivity will be very useful for the determination of PPB in pharmaceutical formulations. The aim of the study was to develop a simple, precise and accurate reverse phase HPLC method for the estimation of PPB in bulk and finished dosage forms which are available in the market.

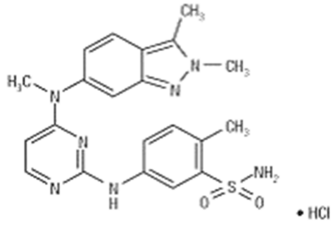

Figure No.1 Pazopanib

EXPERIMENTAL SECTION

Chemicals and Reagents: Pazopanib HCl was obtained as a gift sample from M/s GlaxoSmithKline Pharmaceuticals Ltd. (GSK RxIndia), Mumbai. Methanol (Ranchem) and water (Milli Q) used were of HPLC grade (Merck). PotassiumDihydrogen Orthophosphate and Ortho- Phosphoric Acid were obtained from Merck, Mumbai. Commercially available tablets (Votrient®- GSK RxIndia) were procured from local market.

2.2 ***Chromatography Instrument:***
Quantitative HPLC was performed on liquid Chromatography, Alliance 2695, PDA detector module equipped with automatic injector with injection volume 0.8µl, and 2489 UV-Visible Detector. An Phenomenox Kinetex, C18 column (100 X 4.6 mm, 5µ) was used. The HPLC system was equipped with Empower 3 Software. The column was maintained at 40ºC and eluted under isocratic conditions over 20.0 min at a flow rate of 0.8 ml/min. All chemicals and reagents used for the study were of analytical grade and procured from Merck Specialties Private Limited, Mumbai. Water used for analysis is Milli-Q grade.

Preparation of Buffer
Weigh and transferred about 1.36 g of Potassium dihydrogen orthophosphate into a beaker containing1000 ml of water and dissolved. Adjusted the pH of the solution to 3.0±0.05 with orthophosphoric acid. Filtered and degassed the solution through 0.45 µm membrane filter.

Preparation of diluent
Prepare a degassed mixture of Buffer and methanol in the ratio of 50:50%v/v.

Preparation of Standard solution
Accurately weighed and transferred about 25mg of Pazopanib Hydrochloride working standard into a 50ml volumetric flask, then 30ml of diluent was added and sonicated to dissolve. Diluted to volume with diluent and mix. Transfer 5.0ml of above solution into a 50ml volumetric flask, diluted to volume with diluent and mix.

Preparation of Sample solution
Twenty tablets (Votrient®- GSK Rx India) were weighed, and then powdered. A sample of the powdered tablets, equivalent to mixture containing concentration of each 0.2 mg/mL of PPB active ingredients, was mixed with 15 ml of Buffer: methanol in ratio of 50:50 v/v as diluent in 50 ml volumetric flask. The mixture was allowed to stand for 30 minr with intermittent sonication (maintain the sonicator bath temperature between 20 to 25ºC) to ensure complete solubility of the drug, and then filtered through a 0.45 µm membrane filter, dilute the volume with diluent and mix.

5ml of the above filtered sample solution was taken in 50 ml volumetric flask and made up to 50 ml with diluent to get a concentration of 200 µg/mL of PPB.

Method development and validation
Preliminary studies like solubility, polarity, UV absorbtivity etc are study for initial development of method conditions. Various solvents with different ratios has been tried with different reverse phase columns. Later optimization of methods conditions has been carried out to evaluated system suitable chromatogram.

RESULTS AND DISCUSSION

HPLC method development and optimization
To optimize the chromatographic conditions, different combinations of Buffer and methanol compositions were tested. The effect of the flow rate was studied in the range 0.8 to 1.2 mL.min^{-1}. With acetonitrile content as a mobile phase prolonged analysis time was observed. Mobile phase with Potassium dihydrogen phosphate: Methanol: 60:40 (v/v) composition was therefore used at a flow rate of 0.8 mL min−1, for further studies. Under these conditions, the analyte peak obtained was well defined and free from tailing (Figure.2). The retention time (RT) was about 5 min.

The short retention time achieved implied that many samples can be run using a small quantity of mobile phase, thus minimizing analysis time and cost per analysis. The optimized chromatographic conditions for the determination of Pazopanib are represented in Table 1. After completion of method development and optimization method was

validated as per ICH guideline [1] such as linearity, precision, specificity and accuracy, limit of detection (LOD), limit of quantitation (LOQ) and robustness.

Table 1: Optimized chromatographic conditions of Pazopanib

Column	Phenomenox Kinetex -C18, C18 column (100 X 4.6 mm, 5µ)
Pump mode	Isocratic
Wavelength	267 nm
Mobile phase	Buffer :Methanol 60:40 (v/v)
Diluent	Buffer : Methanol (50:50 v/v)
Injection Volume	10µl
Column Temp	40ºC
Flow rate	0.8ml/min
Retention Time	4.8 minutes
Run time	20 minutes

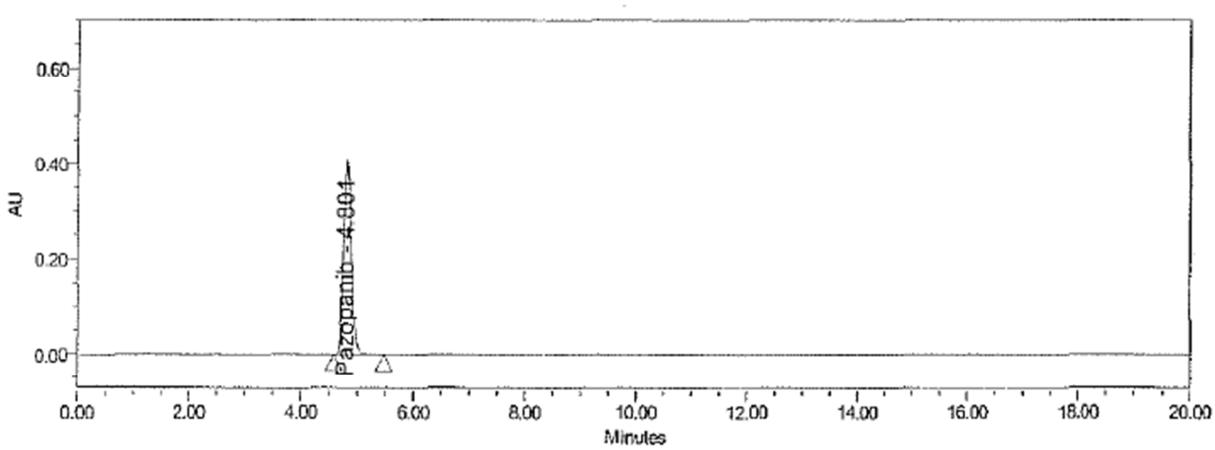

Figure no: 2 Chromatogram showing the Pazopanib at Retention time 4.8min

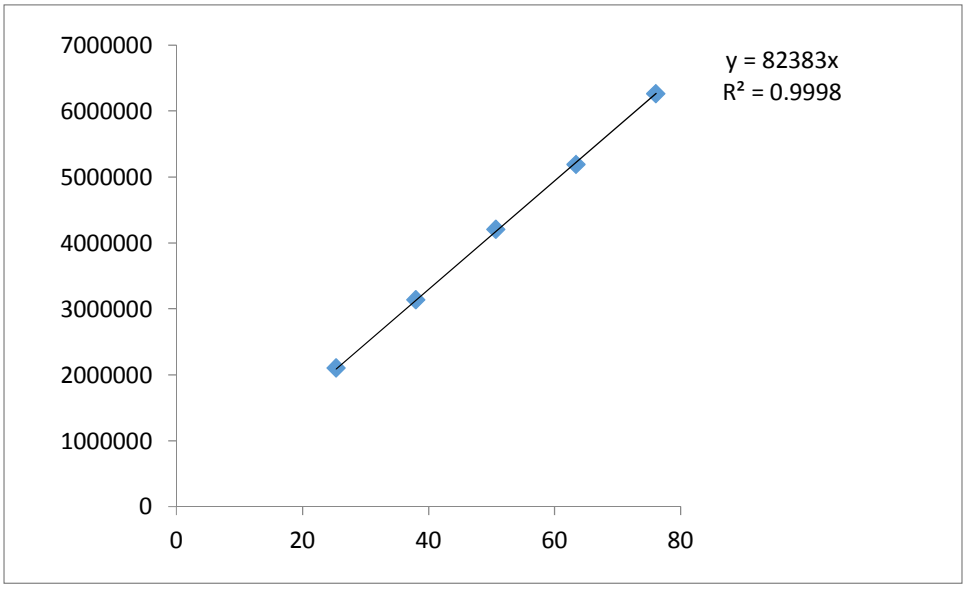

Figure 3: Calibration curve for Pazopanib

VALIDATION

Linearity and Range

Aliquots of standard PPB stock solution was taken in different 10 ml volumetric flasks and diluted up to the mark with the mobile phase such that the final concentrations of PPB was in the range of 25-75 µg/mL. Each of these drug

solutions (10 µL) was injected into the column, and the peak areas and retention times were recorded. Evaluation was performed with UV detector at 267 nm and the Calibration graph was obtained by plotting peak area versus concentration in µg/mL of PPB **(Figure: 3)**.

The plot of peak areas of each sample against respective concentration of PPB was found to be linear in the range of 25-75 µg/mL with correlation coefficient of 0.999.

Accuracy

Accuracy of proposed method was ascertained on the basis of recovery studies performed by standard addition at different level of labeled claim (50%, 100% and 150%) of standard. Percentage of recovery for each case was calculated and was found to be 98.5 to 99.3. This was found to be well within the acceptance criteria of 98-102%.

Table 2: Accuracy studies of Pazopanib

Accuracy Level		Amount added (in mg)	Amount found (in mg)	% Recovery	Mean % Recovery
50%	Sample 1	49.79	49.23	98.9	99.1
	Sample 2	49.87	49.54	99.3	
	Sample 3	49.92	49.53	99.2	
100%	Sample 1	99.38	98.48	99.1	99.2
	Sample 2	99.09	98.21	99.1	
	Sample 3	99.18	98.49	99.3	
150%	Sample 1	149.43	147.60	98.8	98.8
	Sample 2	149.53	147.98	99.0	
	Sample 3	149.71	147.52	98.5	

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Limit of Detection (LOD) of the method was determined as the lowest concentrations of active pharmaceutical ingredients producing a signal-to-noise (S/N) ratio of about 3. The Limit of Quantitation (LOQ) was determined as the lowest concentrations of active pharmaceutical ingredients capable of being quantified with acceptable accuracy and precision producing signal-to-noise (S/N) ratio of about 10.

Precision

The system precision of the proposed method was determined by injecting standard solution for five times and measured the area for them in HPLC. The method precision of the proposed method was determined by injecting six sample solutions into HPLC prepared individually. The % RSD for the areas of system precision and method precision were calculated and results are found to be 0.06 and 0.28 which were within the limit of 2.0%

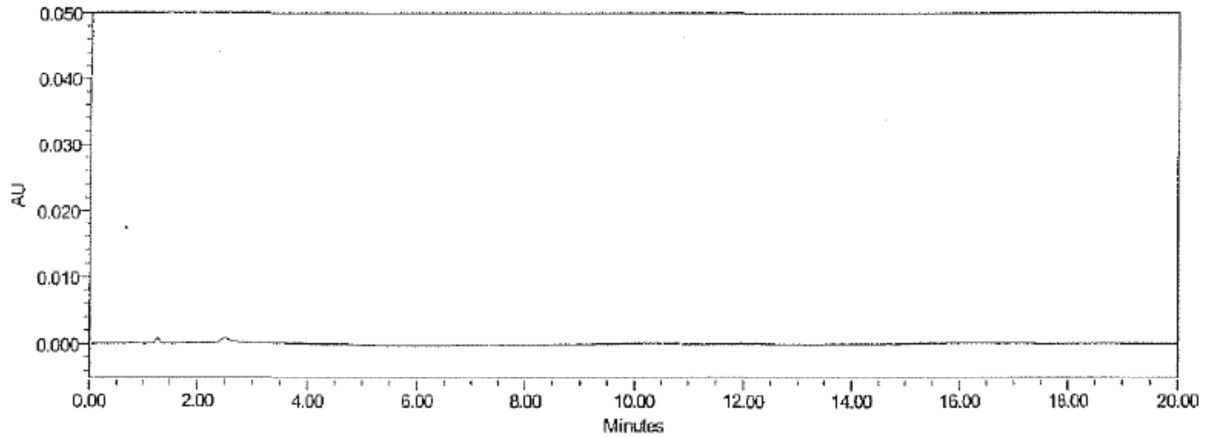

Figure: 4 Blank chromatogram

Robustness

Robustness was studied by change in chromatographic conditions , Mobile phase variation, pH of the buffer. The percent relative standard deviation (% RSD) was calculated and it was found to within the range of 0.05 – 0.52 which is well within the range of %RSD 2.

Specificity

Prepared blank solutions was inject into the chromatographic system. Blank chromatograms show no peak at the retention time of Pazopanib due to impurities.

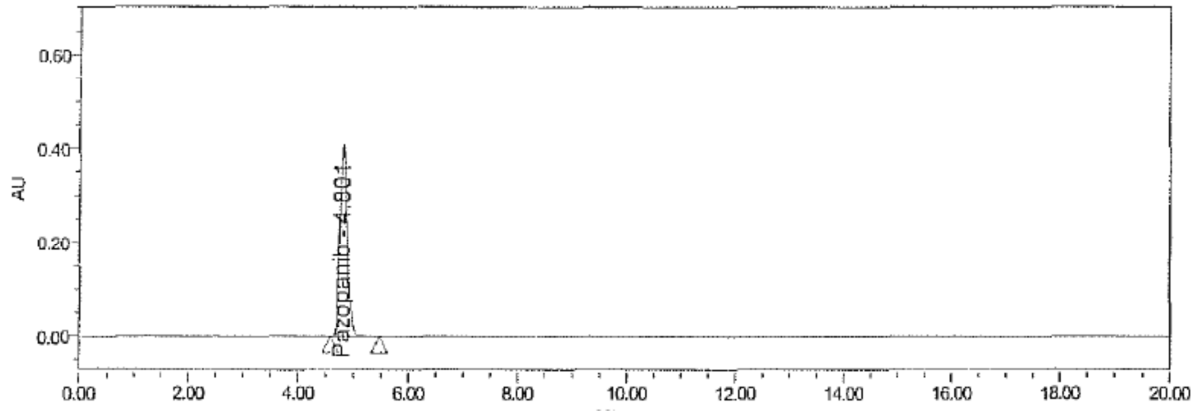

Figure: 5 Chromatogram of standard solution

Forced Degradation studies

Forced degradation studies i.e. Acid, Base and peroxide degradations have been carried to establish specificity and stability indicating nature of the method.

From the studies found that there is no possible degradation occurs during acid, base and peroxide degradations. Also there is no co-eluting peaks with the Pazopanib. Peak purity of PPB has passed. The Purity angle was found to be less than the purity threshold. Results of the forced degradation studies have been provided in table 3.

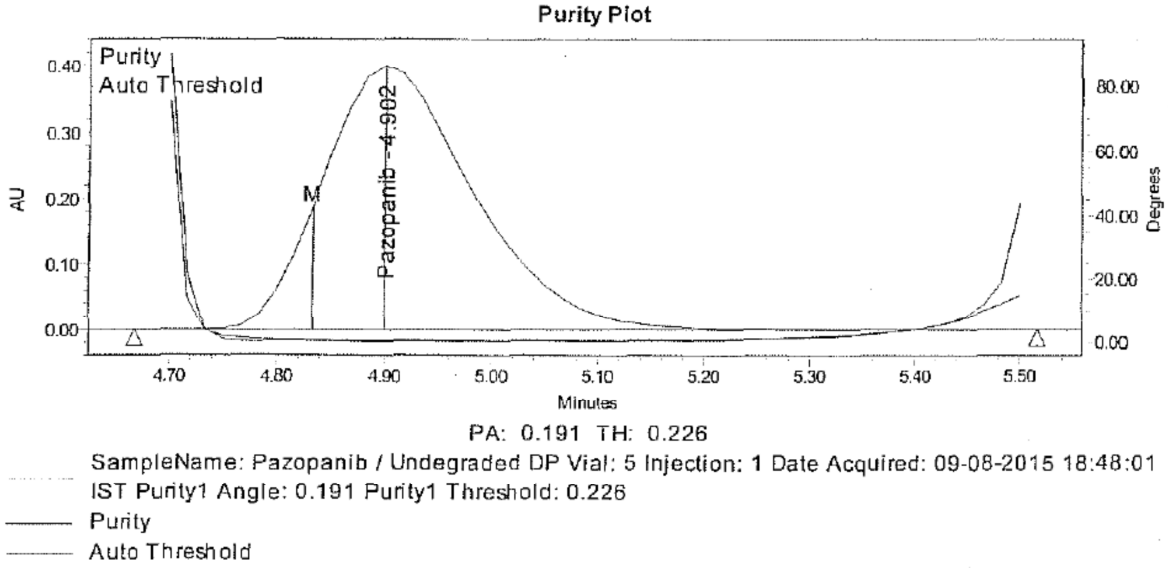

Figure: 6 Purity plot of Acid degradation sample solution

Table 3: Forced degradation data of Pazopanib Tablets

Degradation Condition	% Assay	% Degradation	Purity angle	Purity threshold	Remarks
Acid degradation					
Acid with 5N HCL stressed for 2 hrs at 80 ºC	98.7	No degradation	0.191	0.226	Passed
Base degradation					
5N NaoH, stressed for 2 hours at 80 ºC	98.5	No degradation	0.205	0.225	Passed
Peroxide degradation					
5% H_2O_2 stressed for 2 hours at 80 ºC	98.3	No degradation	0.215	0.227	Passed

CONCLUSION

A simple Specific stability indicating liquid chromatographic method is developed for the quantification of Pazopanib Pharmaceutical dosage forms. This method is validated and it is found to be Specific, precise, accurate, Robust and linear for the detection and quantification of Pazopanib. The method is stability-indicating and can be used for routine analysis of production sample and to check the stability samples of Pazopanib in Pharmaceutical dosage forms

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