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Research Article

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Development and validation of UV spectrophotometric method for the determination of pazopanib hydrochloride in bulk and tablet formulation

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ABSTRACT

The present work discuss the development and validation of a simple, accurate and cost efficient UV spectrophotometric method for Pazopanib hydrochloride (PAZ).The optimum conditions and solvents for the analysis of the drug were established through scouting trails using different solvent compositions. PAZ showed maximum sensitivity, good percent recovery, good spectral properties in methanol compared to other solvent/solvent mixtures. Absorption maxima (λ max) of PAZ was found to be 214nm in methanol. The percentage recovery of PAZ was found to be 99.69±0.873. Beers law was obeyed in the concentration range of 5.5-7.5µg/mL and calibration curve has shown a linear relationship between the absorbance and concentration with in this range. The line equation Y = 0.2016x - 0.5804 with R^2 of 0.9933 was obtained from the linearity studies. Validation was performed according to ICH guidelines for linearity, accuracy, precision, LOD and LOQ. The sample solution was stable up to 36 hours. The proposed method can be used for the analysis of Pazopanib hydrochloride in bulk and tablet formulation for quality control purposes.

Keywords: Pazopanib hydrochloride, UV spectrophotometric method, ICH Analytical Method Validation, Tablets.

INTRODUCTION

Pazopanib is a second generation tyrosine kinase inhibitor (TKI) and is generally present in its white to yellow solid hydrochloride salt form, with the chemical formula 5[[4[(2,3-dimethyl-2H-indazol-6-yl)methylamino]-2-pyrimidinyl]amino]-2-methylbenzenesulfonamide-mono hydrochloride. It has molecular formula $C_{21}H_{23}N_7O_2S.HCL$ and a molecular weight of 473.99[1,2] .

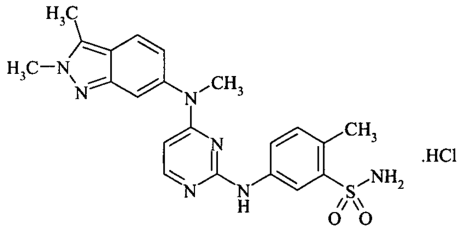

Figure.1 Structure of Pazopanib hydrochloride

Pazopanib is a multityrosine kinase inhibitor that blocks tumor growth and inhibits angiogenesis. It inhibits vascular endothelial growth factor receptor (VEGFR)1, VEGFR2, VEGFR3, platelet derived growth factor receptor

(PDGFR) α and β, fibroblast growth factor receptor (FGFR) 1 and 3,cytokine receptor (Kit), interleukin 2 receptor, inducible T cell kinase (Itk), leukocyte specific protein tyrosine kinase (Lck) and transmembrane glycoprotein receptor tyrosine kinase (cFms). It is approved by numerous regulatory administrations worldwide like FDA**,** EMA, MHRA and TGA for use as a treatment for advanced/metastatic renal cell carcinoma (RCC) and advanced soft tissue sarcomas in patients who have been treated with chemotherapy. Also it is found to be therapeutically active against ovarian and non-small cell lung cancer [3, 4]. Due to the rarity of advanced or metastatic RCC, pazopanib has been designated as an orphan drug on 24th March, 2009 by the Therapeutic Goods Administration (TGA), Australia[5]. Literature review reveals one extractive spectrophotometric method and very few liquid chromatographic methods have been reported for quantitative estimation of PAZ in tablet dosage forms and biological fluids [6,7,8,9,10,11,12]. However, no validated UV spectrophotometric method was reported so far for the estimation of PAZ in bulk, pharmaceutical dosage forms. Hence, the present work aimed at the development of a new simple,sensitive and validated UV spectrophotometric method for the analysis of PAZ in bulk, pharmaceutical dosage forms.

EXPERIMENTAL SECTION

Instrumentation and software: Thermo Fischer Scientific UV/Visible double beam spectrophotometer UV10 with a spectral bandwidth of 2nm and wavelength accuracy of ± 0.2 nm was used for the study and 1.0 cm matched quartz cells were used for analytical method development and validation. VISIONlite version 5.0 installed on windows 7 operating system was used for data acquisition. Thermo Scientific Micropipette of variable volume 10-1000 µL ,PCI Analytics 3.5L Ultrasonicator and Mettler Toledo analytical balance ME 204 were used for the sample preparation.

Reagents: Pazopanib hydrochloride was obtained as a gift sample from MSN Laboratories Pvt. Ltd, Hyderabad. Distilled water was used for the study was of double distilled grade and all chemicals like Methanol, Potassium dihydrogen phosphate, Hydrochloric acid, Sodium hydroxide were purchased from Merck (P) Ltd. Mumbai are of analytical grade.

Standard solution of Pazopanib hydrochloride: 10 mg of Pazopanib hydrochloride standard was accurately weighed and transferred to a 10 ml volumetric flask. Few ml of selected solvent methanol was added to it and sonicated and the volume was made up to the mark with Methanol to obtain a concentration of 1 mg/mL.

Preparation of sample solution: Contents from 20 tablets were taken, accurately weighed and powdered. Tablet powder equivalent to 200 mg of pazopanib in to a 100ml volumetric flask was taken. Initially 25 ml of methanol was added and the mixture was allowed to stand for 1 hr with intermittent sonication to ensure complete solubility of the drug, and then filtered through a 0.45m membrane filter, followed by adding methanol to obtain a stock solution of 2000µg/ml. Transfer for 5ml of this solution to a 100 ml of volumetric flask and made upto sufficient volume with solvent to give 100µg/ml solution. The solution was suitably diluted so as to obtain a concentration in the linearity range and absorbance was measured against blank at 214 nm. Result of analysis is shown in Table 9.

Selection of detection wavelength for maximum absorbance (λmax):
From the stock solution, 10ml was transferred to a 100ml volumetric flask and made up the volume with methanol to give a 100µg/mL solution. From the above stock solution, pipette out 0.6 ml in to 10ml volumetric flask and finally made up the volume with methanol, to produce a concentration of 6µg/mL. The sample was then scanned in UV spectrophotometer from a range of 200-400 nm against methanol solvent as blank, heavy spectral noise was found from 320 nm to 400 nm. Hence UV scan range of 200 nm to 320 nm was selected for the method and the wavelength corresponding to maximum absorbance was found at 214 nm (figure.2).

Preparation of standard calibration curve
For the preparation of standard calibration curve, concentration of 5.5-7.5µg/mL were prepared by pipetting out 0.55,0.60,0.65,0.70,0.75ml from the 100µg/mL solution in to a 10ml volumetric flask and made up the volume with above said solvent. The absorbance of each solution was measured at 214 nm against solvent as blank. Calibration curve of the drug was then plotted by taking the absorbance obtained on y-axis and the concentration of the solution on x-axis (Figure3).

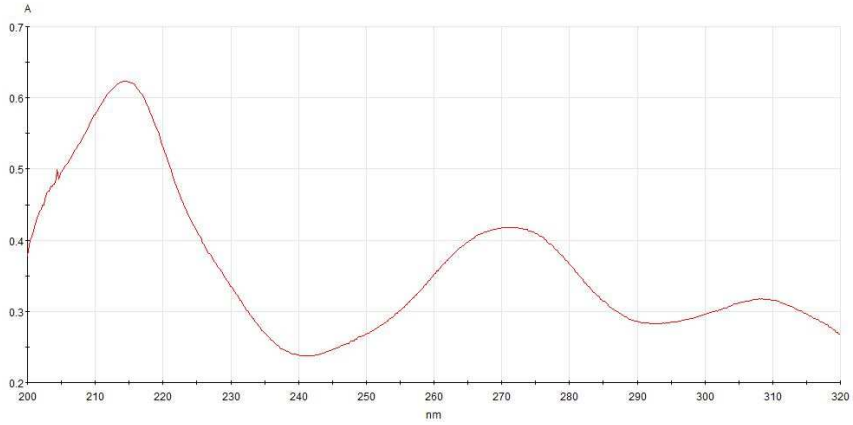

Figure.2 UV Spectrum of Pazopanib HCl in methanol

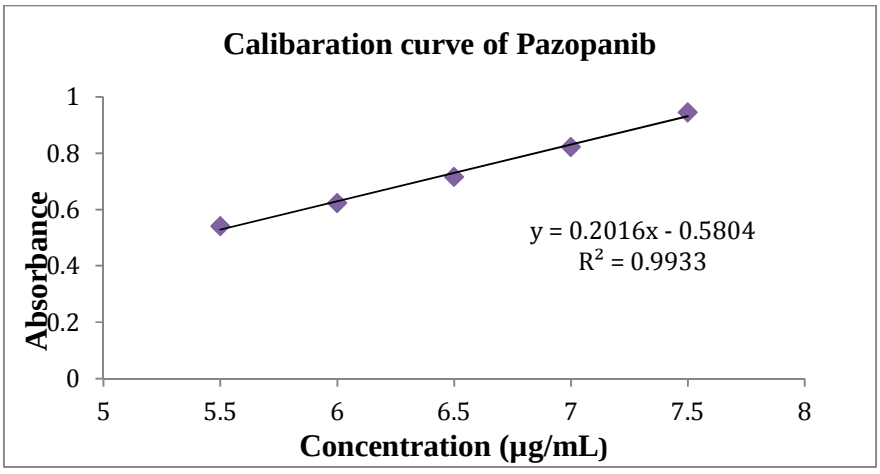

Figure.3 Calibration curve of Pazopanib

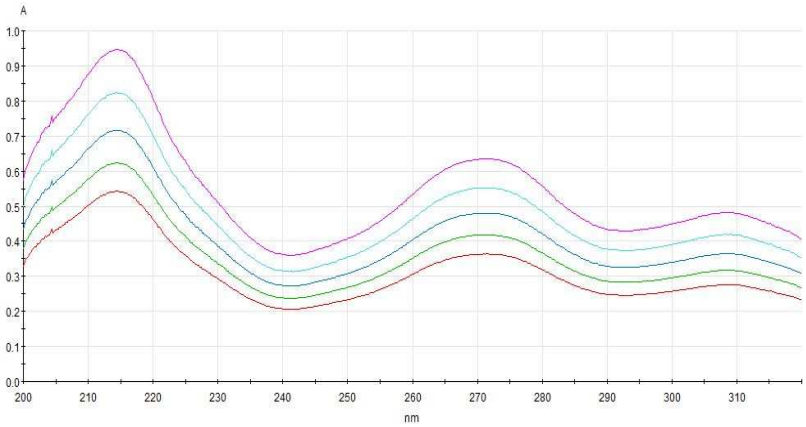

Figure.4 Overlay spectra of linearity for Pazopanib

Validation : The method was validated for several parameters like linearity, accuracy, precision, Ruggedness, Robustness, Limit of detection(LOD), Limit of quantification(LOQ) according to ICH guidelines.

Linearity: The linearity of the analytical method was its ability to elicit test results which are directly proportional to analyte concentration in samples within a given range. The aliquots of concentration ranging 5.5-7.5 µg/mL

concentrations were used. The linearity was calculated by the least square regression method with correlation coefficient 0.9933 shown in Table 1.

Table.1 Linearity table of Pazopanib

Concentration (µg/mL)	Absorbance
5.5	0.542
6	0.623
6.5	0.716
7	0.823
7.5	0.946

Precision: Precision studies were carried out to check the reproducibility of the method. Repeatability was determined by preparing six replicates of same concentration of the sample and measuring absorbance. Intraday precision study was carried out by analyzing the prepared drug solutions at three different times (8 hour samples) in a day. The same procedure was followed for three different days to determine interday precision. The results were reported as %RSD in (Table 3)

Table.2 Repeatability studies of Pazopanib

Concentration (µg/mL)	Absorbance	Statistical Analysis
6.5	0.716	Mean: 0.716 SD: 0.001 %RSD: 0.25
6.5	0.718	
6.5	0.714	
6.5	0.714	
6.5	0.716	
6.5	0.718	

Table.3 Intraday precision studies

Concentration (µg/mL)	Absorbance 1	Absorbance 2	Absorbance 3	Average %RSD
6.5	0.716	0.717	0.716	0.23
6.5	0.718	0.718	0.718	
6.5	0.714	0.717	0.715	
6.5	0.714	0.716	0.716	
6.5	0.716	0.717	0.711	
6.5	0.718	0.718	0.718	
Mean	0.716	0.717	0.715	
SD	0.001	0.000	0.002	
%RSD	0.25	0.10	0.36	

Table.4 Interday precision studies

Concentration (µg/mL)	RSD			Average %RSD
	Day1	Day2	Day3	
6.5	0.23	0.42	0.36	0.33

Accuracy (Recovery): For the accuracy of the proposed method, recovery studies were performed by the standard addition method at three different levels (50%, 100% and 150% of final concentration). A known amount of standard pure drug was added to pre-analyzed tablet powder and the sample was then analyzed by the proposed method. Results of recovery studies were found to be satisfactory and reported in Table 5.

LOD and LOQ: Limit of detection (LOD) is the lowest amount of analyte in the sample that can be detected. Limit of quantification (LOQ) is the lowest amount of analyte in the sample that can be quantitatively determined by suitable precision and accuracy. LOD and LOQ were determined by the following equation LOD=3.3 σ/s, LOQ =10 σ/s Where σ is standard deviation of y intercept of calibration curve and s is slope of regression equation. The LOD and LOQ values were found to be 0.24 µg/mL and 0.75 µg/mL respectively.

Ruggedness: Ruggedness was determined by carrying out analysis by two different analysts and the respective absorbance was noted and the results were indicated as % RSD Table 6.

Table.5 Accuracy studies of Pazopanib

Level of addition (%)	% Recovery	Statistical Analysis		
		Mean	SD	%RSD
50	98.54	98.73	0.778	0.79
50	98.07			
50	99.59			
100	100.16	99.93	1.009	1.09
100	98.83			
100	100.81			
150	101.19	100.43	0.653	0.65
150	100.10			
150	100.02			
Overall Mean Recovery,SD and %RSD		99.69	0.873	0.88

Table.6 Ruggudness studies of Pazopanib hydrochloride

Analyst 1		
Concentration (µg/mL)	Absorbance	Statistical Analysis
7.5	0.945	Mean: 0.945 SD: 0.002 %RSD: 0.25
7.5	0.946	
7.5	0.941	
7.5	0.946	
7.5	0.944	
7.5	0.948	
Analyst 2		
7.5	0.941	Mean: 0.945 SD: 0.002 %RSD: 0.28
7.5	0.943	
7.5	0.945	
7.5	0.946	
7.5	0.947	
7.5	0.948	

Robustness: Analysis was carried out using concentration 7.5 µg/mL standard at two different wavelengths, room temperature to determine the robustness of the method and the respective absorbance was measured. The results were indicated as %RSD in **Table7**

Table.7 Robustness studies of Pazopanib hydrochloride

Absorbance			
S.No.	213 nm	214 nm	215 nm
1	0.946	0.946	0.945
2	0.944	0.944	0.944
3	0.941	0.945	0.945
4	0.944	0.946	0.940
5	0.944	0.949	0.949
6	0.940	0.947	0.944
Mean	0.943	0.946	0.944
Total SD	0.001		
Total %RSD	0.16		

Table.8 Solution stability studies of Pazopanib hydrochloride

Time (Hrs)	Absorbance of 6.5µg/mL standard in ambient conditions	Absorbance of 6.5µg/mL standard in refrigerated conditions
0	0.716	0.716
8	0.715	0.716
16	0.716	0.715
24	0.716	0.714
32	0.714	0.713

Solution Stability: The solutions of Pazopanib hydrochloride (Concentration 6.5µg/mL) were tested for their stability at ambient temperatures and refrigerated temperatures (2-8°C) . The absorbance values for 8 hrs,16 hrs,24

hrs,32 hrs was reproducible and absorbance variation was found to be less than 2% in both conditions.

Determination of active ingredients in tablets: The proposed method was applied to analyze commercially available pazopanib hydrochloride tablets (Votrient® marketed by GSK Rx India). The tablet was having content of Pazopanib hydrochloride equivalent to 200mg. Ten tablets were weighed and weight equivalent to 100mg was dissolved in methanol. By frequent shaking volume was made up to mark with methanol. The solution was then filtered through Whattman filter paper #41. This filtrate was diluted suitably with solvent to get the solution of 5µg/mL concentration. The absorbance was measured against solution blank. Amount of pazopanib hydrochloride was calculated from the calibration curve. The readings were taken in triplicate.

Table.9 Assay of Pazopanib hydrochloride in Tablet formulations

Tablet formulation	Labeled Amount (mg/tab)	Amount obtained by proposed method	% Label Claim
Tablet (n=3)	200	198.89±0.21	99.44

RESULT AND DISCUSSION

During scouting trails for method development of the drug, different solvents were tested such as water, methanol, 0.1N HCl, 0.1N NaOH and Phosphate buffer (pH7.4). Due to greater solubility and reproducible readings of maximum absorbance, methanol was taken into consideration for further work. By serial dilution, different dilutions of standard drug having concentration 5,5.5,6,6.5,7,7.5/mL were prepared and calibration curve was plotted. The data were statistically validated by means of the least square regression method and results were presented in Table 1. The detection and quantization limits, LOD (k=3. 3) and LOQ (k=10) were calculated to be 0.24 µg/mL and 0.75µg/mL respectively. The precision (measurements of intraday and interday) results showed good reproducibility with percent relative standard deviation (% RSD) is below 2.0. This indicated that method is highly precise. The evaluation of accuracy of the method was performed by the standard addition method at three different levels 50%,100% and 150% of final concentration and total mean recovery of all 9 determinations was found to be 99.69±0.873.This indicated the accuracy of the proposed method.

The proposed method was also applied for the assay of Pazopanib hydrochloride in tablet formulation (in triplicate) and the results as tabulated in Table 9. The results obtained were in good agreement with the label claims.

Table.10 Summary of Validation Parameters

S.No	Parameter	Result
1.	Absorption Maxima (nm)	214
2.	Linearity Range (µg/mL)	5.5-7.5
3.	Standard Regression Equation	y = 0.2016x - 0.5804
4.	Correlation Coefficient (R^2)	0.9933
5.	Slope	0.2016
6.	Intercept	0.5804
5.	Accuracy(% Recovery ±SD)	99.69±0.873
6.	LOD (µg/mL)	0.24
7.	LOQ (µg/mL)	0.75

CONCLUSION

The developed method can be concluded to be simple, accurate, reliable and economical. The proposed method is specific without and interference of excipients and hence can be used for the routine analysis of Pazopanib hydrochloride in bulk and in pharmaceutical formulation.

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