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ORIGINAL ARTICLE

Analytical Method Development and Validation of Pazopanib Hydrochloride by UV- Visible Spectrometry

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ABSTRACT
The goal of this study was to develop and validate a simple, rapid, and economical UV spectroscopic method for the estimation of Pazopanib API and its tablet formulation. The study was meticulously designed to validate the developed methods in strict adherence to ICH guidelines, ensuring the reliability and standardization of the method. The quantification process was performed on a UV spectrophotometer. Different analytical parameters such as linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ), repeatability, and stability studies were determined. The solutions of standard and sample were prepared and scanned in the UV visible range of 400 to 200nm. Pazopanib shows the highest λmax at 273nm. The Pazopanib follows linearity in the 10- 20µg/mL concentration ranges with a superior correlation coefficient value of 0.999. The precision of the method was studied as an intraday and interday study. The %RSD value is <2, indicating the precise method. Degradation studies were performed, and the results are according to ICH guidelines. The proposed UV method is accurate, precise and reproducible. Hence, this rapid method can be viable for the quality control analysis of Pazopanib in its formulation.
Keywords: Pazopanib, Spectrophotometric method, Validation, Degradation studies, λmax.

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INTRODUCTION

Pazopanib (Fig.1.) is an anti-neoplastic agent used to treat advanced renal cell carcinoma, soft tissue sarcoma & Von Hippel Lindau disease. Pazopanib was approved by the US Food and Drug Administration for medicinal use on 19 Oct 2009. Pazopanib is a selected multitargeted receptor tyrosine kinase inhibitor that blocks tumour growth and inhibits angiogenesis. It belongs to the class of organic compound alkyl diary amines. Pazopanib is pharmacologically targeted on the VEGFR [1,2,3] (vascular endothelial growth factor) and PDGFR α/β (platelet-derived growth factor). Recently, it has been used to treat the Von Hippel Lindau disease – a rare autosomal dominant disease caused by germline mutation of the VHL gene. The recommended dose of orally administered pazopanib is 400 or 800 mg daily. The ease of administration of drugs shows a better quality of life. [3] the protein binding of pazopanib is 90-99 %, and CYP3A4 metabolises it. [4]

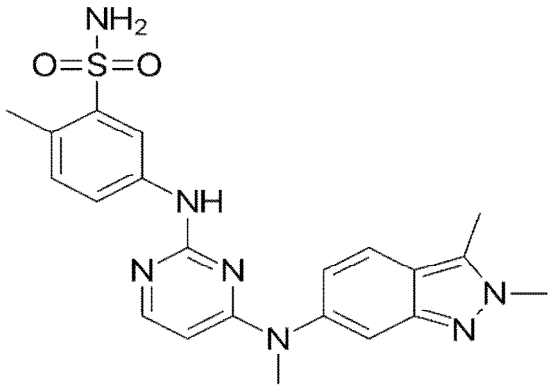

Fig.1. Chemical structure of Pazopanib

MATERIAL AND METHODS

Instruments and reagents

A double-beam UV-visible spectrometer with spectra manager software was used for the analysis. Quartz cells with a 3 cm length and a 1 cm path length were used for spectral measurement. Weighing balance (Mettler Toledo) with internal calibration mode was used for the accurate weighing. Pazopanib was obtained as a gift sample from the Life Research lab in Hyderabad. Methanol was purchased from Merck. All the chemicals of analytical grade were used for the proposed study.

Standard preparation

Weigh 10 mg of Pazopanib in a 100 ml volumetric flask and make its mark with ethanol. From the stock solution, prepare various concentrations of sample solution, such as 10 µg/ml, 12 µg/ml, 14 µg/ml, 16 µg/ml, 18 µg/ml, and 20 µg/ml.

Sample preparation

20 tablets (30 mg of Pazopanib) were weighed and taken into a mortar and crushed to fine powder and the powder to separate coating material then uniformly mixed Tablet stock solutions of Pazopanib (µg/ml) were prepared by dissolving weight equivalent to 29.7 mg of Pazopanib and dissolved in sufficient ethanol After that filtered the solution using 0.45-micron syringe filter and sonicated for 5 min and dilute to 100 ml with mobile phase. Further dilutions are prepared in 5 replicates of 10 µg/ml, 12µg/ml, 14 µg/ml, 16 µg/ml, 18 µg/ml, and 20 µg/ml are prepared by diluting 1 ml to 10 ml with mobile phase was made by adding 1 ml of stock solution to 10 ml of ethanol.

λmax Pazopanib

The wavelength of maximum absorption (λmax) of the drug, 10 µg/ml solution of the drugs in ethanol, was scanned using a UV-visible spectrophotometer within the wavelength region of 200-400 nm against methanol as a blank. The resulting spectra are shown in the figure, and the absorption curve shows characteristic absorption maxima 273nm for Pazopanib.

Preparation of Calibration curve

Weigh 10 mg of Pazopanib in a 100 ml volumetric flask and mark with ethanol from the stock solution to prepare various concentrations of sample solution. Such as 10µg/ml, 12 µg/ml. 14 µg/ml. 16 µg/ ml, 18 µg /ml, 20 µg/ml strength. An absorbance of every calibration standard was estimated at λmax 273 nm using fixed wavelength measurement mode. The calibration curves representing concentration vs. absorbance were plotted in Microsoft Excel 2016. The previously mentioned technique was rehashed multiple times with the goal that reproducible outcomes can be obtained

Method Validation

The developed UV method for the estimation of Pazopanib was validated in terms of parameters like linearity, range, precision, robustness, ruggedness, accuracy, limit of quantification (LOQ), and limit of detection (LOD) using predefined calibration standards as portrayed below [5-18]

Linearity and range

The linearity of the proposed UV method was established using six different calibration standards. Based on the analysis of calibration standards, calibration curves in terms of absorbance vs. concentration plots were developed and subjected to linear least square regression analysis. R square value was considered an important factor for establishing the linearity of the proposed method. The interval between the upper and lower concentration limits with acceptable linearity was reported to be the range of the proposed UV method.

Accuracy

The accuracy of the proposed UV method was evaluated using recovery studies after the standard addition of the analyte of interest. Three different solutions of Pazopanib were prepared in triplicate at levels of 50%, 100% and 150% of its predefined concentration. In predefined concentrations, different Pazopanib amounts were included (standard addition method), and accuracy was determined based on per cent recovery. For calculating the percent recovery, the following equation was utilised

%RC= (SPS-S/SP) × 100

Where,

% RC = Percent recovery

SPS = Amount found in the spiked sample

SP = Amount added to the sample

S = Amount found in the sample

Intra-day precision and Inter-day precision

The precision of the assay method was assessed in terms of repeatability by carrying out seven independent assays of the Pazopanib test arrangement and measuring the % RSD (intra-day). The method's intermediate precision was checked by performing the same methodology on three consecutive days.

Robustness

The robustness of a developed method is its capacity to remain unaffected by small changes in condition. To determine the method's robustness, the experimental conditions were deliberately altered, and the assay was evaluated. The effect of the detection wavelength was studied at ±2nm.

Limit of Quantification (LOQ)

In UV method development, LOQ was determined by utilising the following equation.

LOQ = 10xSD/S

Where S= slope

SD= Standard deviation of Y-intercepts

Limit of Detection (LOD)

In UV method development, LOD was determined by utilising the following equation.

LOD =3.3×SD/S Where, SD= Standard deviation of Y-intercepts S= Slope

Estimation of Pazopanib content in marketed formulation

Twenty Pazopanib tablets (Votrient), each containing 30mg of Pazopanib, were weighed, the average weight calculated, and powdered. A quantity equivalent to 30mg of the drug was weighed and transferred into a 50-ml volumetric flask, where it was dissolved in methanol to obtain a final concentration of 1000µg/ml. The volumetric flask was sonicated for about 15 minutes to affect the complete dissolution of the drug, and the solution was filtered.

RESULTS AND DISCUSSION

Method development and optimisation

Identifying the wavelength of maximum absorbance is a prerequisite for quantitative UV analysis. A solution representing an absorbance value less than 1 is generally considered suitable for determining the wavelength of maximum absorbance. Considering the prerequisite and the suitability, the maximum wavelength for the Pazopanib solution (10 µg/mL) was determined using the full scan mode of a UV-visible spectrophotometer (Figure 2). The full scan was processed using UV software, and the λmax was identified with the help of software. It was found to be 273 nm for Pazopanib

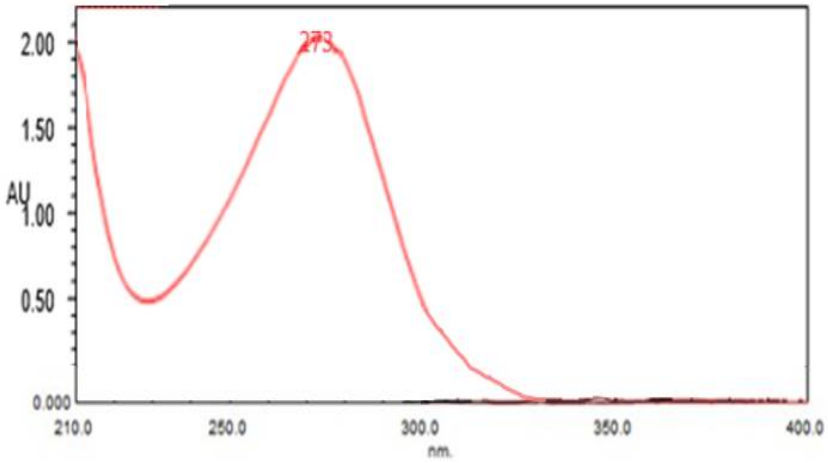

Fig.2. UV spectra of Pazopanib

Linearity and range

Standard solutions of Pazopanib were prepared in the concentration range of 10-20µg/ml by transferring 1, 1.2, 1.4, 1.6, 1.8 and 2.0ml of Pazopanib stock solution (100µg/ml) to the series of 10ml volumetric flasks. The volume in each volumetric flask was made with solvent and mixed. Calibration curves were plotted by taking concentration on the X-axis and absorbance on Y- the Y-axis. The correlation coefficient was found to be 0.999 at 273nm. The slope was found to be 0.025, and the intercept was found to be 0.033 at 273nm.

Table 1: Linearity of Pazopanib

S.no	Concentration	Absorbance
1	**10**	0.177
2	**12**	0.261
3	**14**	0.380
4	**16**	0.535
5	**18**	0.679
6	**20**	0.780

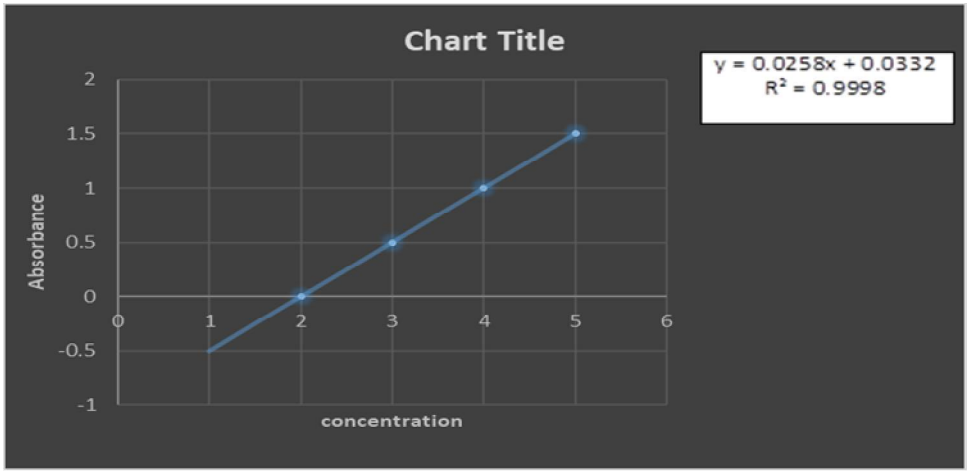

Fig.3. Calibration curve of Pazopanib

Accuracy

The accuracy studies were carried out at three levels, i.e., 50%, 100%, and 150%. To ensure the reliability of the above method, recovery studies were carried out by mixing a known quantity of the standard drug with the pre-analyzed sample formulation, and the contents were reanalyzed by the proposed method

Table 2: Accuracy results of Pazopanib

S.no	Spike level	%Recovery	Mean % Recovery
1	50%	97	99.7%
		98	
		90	
2	100%	105	102.3%
		99	
		103	
3	150%	102	106.6%
		108	
		97	

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

LOQ represents the lowest concentration that can be analyzed accurately and precisely. Generally, LOQ is the first calibration standard. LOD and LOQ of the proposed UV method were found to be 0.012 and 0.012 µg/ml, respectively

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The method's precision was determined regarding repeatability and intraday and interday precisions.

Intraday precision

Intraday precision was found by analyzing the intermediate concentration 10 times on the same day. Mean and standard deviation were then calculated.

Table 3: Intraday precision of Pazopanib

S. No.	Conc. (µg/ml)	Absorbance	
		Morning	Afternoon
1	10	0.008	0.234
2	10	0.082	0.210
3	10	0.086	0.205
4	10	0.126	0.276
5	10	0.008	0.275
6	10	0.076	0.247
	Average	0.064	0.241
	SD	0.042	0.029
	%RSD	0.65	0.120

Interday precision

Intraday precision was found by analyzing the intermediate concentration 10 times on 2 days. Mean and standard deviation were then calculated.

Table 4: Interday precision of Pazopanib

S NO	Concentration (µg/ml)	Absorbance(at 273 nm)	
		Day 1	Day 2
1	10	0.231	0.008
2	10	0.111	0.082
3	10	0.287	0.086
4	10	0.244	0.126
5	10	0.280	0.008
6	10	0.204	0.076
	Average	0.0226	0.064
	S.D	0.059	0.042
	%RSD	0.26	0.65

Robustness

The robustness of a developed method is its capacity to remain unaffected by small changes in condition. To determine the method's robustness, the experimental conditions were deliberately altered, and the assay was evaluated. The effect of the detection wavelength was studied at ±2nm.

Table 5: Robustness of Pazopanib at 273 nm

S.no	concentration	absorbance		
		271	273	275
1	10	0.350	0.351	0.347
2	10	0.244	0.245	0.246
3	10	0.413	0.414	0.285
4	10	0.373	0.383	0.363
5	10	0.286	0.367	0.381
6	10	0.393	0.293	0.356
	Average	0.3436	0.3421	0.3296
	S.D	0.065419	0.062258	0.013735
	%RSD	0.19039	0.18198	0.04167

Assay of formulation

Twenty Pazopanib tablets (votrient), each containing 30mg of Pazopanib, were weighed, average weight was calculated and powdered. A quantity equivalent to 30mg of the drug is weighed and transferred into a 50-volumetric flask and is dissolved in methanol to obtain a final concentration of 1000µg/ml. The volumetric flask was sonicated for about 15 minutes to affect the complete dissolution of the drug, and the solution was filtered. From the above mixture, 10ml of solution was taken and then diluted to 100ml with methanol in a volumetric flask to obtain a 100µg/ml concentration. this solution was diluted with methanol to get a 10µg/ml concentration of Pazopanib. This procedure was repeated at different concentrations of 2ppm and 6ppm. The absorbance was measured at wavelength 273nm. Then, the amount of drug in the formulation was calculated, and the results were reported.

Table 6: Assay of formulation of Pazopanib

sample	Label claim	%label claim±SD	%Assay
1	200mg	0.200±0.062	99%

CONCLUSION

This study aimed to develop and validate a simple, rapid, and economical UV spectroscopic method for estimating Pazopanib in its active pharmaceutical ingredient (API) form and tablet formulation, adhering to the International Council for Harmonisation (ICH) guidelines. The method development and validation included various analytical parameters such as linearity, precision, accuracy, limit of detection (LOD), quantification (LOQ), repeatability, and stability studies. The analysis was performed using a double-beam UV-visible spectrometer, and the solutions were prepared and scanned in the UV-visible range of 200-400 nm. Pazopanib exhibited the highest absorption at a wavelength (λmax) of 273 nm. The method demonstrated linearity in the10-20 µg/mL concentration range, with a high correlation coefficient 0.999. The method's precision was confirmed with %RSD values less than 2, indicating high precision. The method's accuracy was validated through recovery studies, which showed a mean recovery rate close to 100%, affirming the method's accuracy. In addition to linearity and precision, robustness was evaluated by deliberately altering experimental conditions, and the method proved robust. The LOD and LOQ were determined to be 0.012 µg/mL and 0.012 µg/mL, respectively, confirming the method's sensitivity. Pazopanib's proposed UV spectroscopic method is accurate, precise, reproducible, and robust. Therefore, it can be effectively utilized for its formulation's quality control analysis of Pazopanib, offering a viable option for routine analysis in pharmaceutical settings.

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