Research Article

Development and Validation of UV Spectrophotometric Method for the Determination of Pazopanib Hydrochloride in Pharmaceutical Dosage Form

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ABSTRACT

A precise, accurate, simple, cost effective, the ultraviolet spectrophotometric method has been developed for the determination of Pazopanib hydrochloride in the Pharmaceutical dosage form. Pazopanib hydrochloride shows the highest λmax at 273 nm. The Pazopanib hydrochloride follows linearity in the concentration range of 2-10 µg /mL with a decorous correlation coefficient value of 0.999. The precision of the method was studied as an intra-day and inter-day study. The % RSD value is < 2, which indicates that the process is precise. The % recovery was found to be in the range lies between 99.27 - 101.38 %. The percentage assay of Pazopanib hydrochloride (Votrient) obtained was 99.445 %. The Proposed spectrophotometric method was validated as per the ICH Q2 (R1) guidelines. The proposed UV method is accurate, precise and reproducible. Hence this rapid method can be viable for the quality control analysis of Pazopanib hydrochloride in the pharmaceutical dosage form.

Keywords: Pazopanib Hydrochloride, Validation, Ultraviolet spectroscopy, Method development.

INTRODUCTION

The chemical name for Pazopanib hydrochloride is 5-[[4-[(2, 3-dimethylindazol-6-yl)-methyl amino] pyrimidin-2-yl] amino]-2-methylbenzenesulfonamide. It has a molecular formula, $C_{21}H_{23}N_7O_2S.HCl$ and a molecular weight of 473.991. Pazopanib hydrochloride is used to treat advanced Kidney cancer. It is also used to treat advanced soft tissue sarcoma that has been treated with other anticancer drugs. Pazopanib hydrochloride works by decreasing the blood supply to the cancer tumor to slow tumor growth. As per the Literature Survey, it is revealed that the drug has been estimated by LC-MS/MS[1-5], UPLC-Q-TOF/MS[6], HPLC-UV[7-8],Ultra Violet[9] But only a few UV – Spectroscopic method and Liquid Chromatography analysis have been reported for the estimation in bulk and pharmaceutical dosage forms. Generally, HPLC has proven to be useful in diagnostic purposes and the pharmaceutical industry[10-12].

The aim and objective of the present work were to develop and validate a simple, Precise, sensitive spectroscopy method for Pazopanib hydrochloride in its bulk and tablet dosage form. Chemical Structure of Pazopanib Hydrochloride is shown in figure 1.

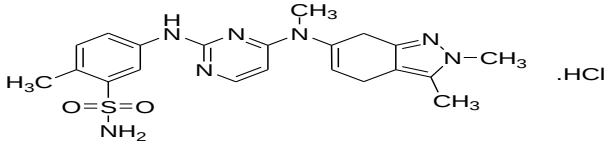

Figure 1: Chemical Structure of Pazopanib Hydrochloride

MATERIALS AND METHODS

Instrument

A double beam ELICO SL 210 UV spectrophotometer containing two matched quartz cells with a one cm light path was taken for measuring of absorbance of Pazopanib hydrochloride. Essaevibra AJ (0.1 mg sensitivity) balance was used for weighing. Ultra Sonicator bath Model no - 91250, PCI Ltd., Mumbai were used in this present study.

Chemicals and reagents

Pazopanib hydrochloride was procured from Hetero Drugs Ltd., Hyderabad, and Telangana, India. The Pazopanib Hydrochloride tablets containing 200 mg labelled claim of Pazopanib hydrochloride (Votrient) tablets were used for this study. ACN and CH_3OH were procured from E. Merck specialties, private Ltd., Mumbai, India.

Selection of the solvent

Plentiful trials were executed to find out the suitable solvent system for dissolving the Pazopanib hydrochloride. The solvents such as acetonitrile, DMSO, methanol, and triple distilled water were tried based on the solubility of the drug. Pazopanib hydrochloride is soluble in solvents such as ACN, methanol, and DMSO; thus, methanol was selected because methanol is cheap that why we are selected through the experiment.

Selection of detection wavelength

To determine the optimum λmax of Pazopanib hydrochloride, 10 µg /ml of the Pazopanib hydrochloride solution was prepared in CH_3OH and scanned in the Ultraviolet wavelength range of 200 - 400 nm. When methanol is used as a solvent, It was observed that the drug showed maximum absorbance at 273 nm, when Methanol: acetonitrile (50:50) solvent ratio is used the UV maximum λ_{max} was obtained at 271.4 nm. 273 nm was used as absorbance maximum and for the estimation of Pazopanib hydrochloride.

Standard preparation solution

A stock solution of Pazopanib hydrochloride at 100 µg/mL is prepared in CH_3OH by sonication. Dilution in methanol is made up of 2 - 10 µg/ ml concentrations.

Preparation of Calibration curve

From the above prepared Pazopanib hydrochloride stock solution, appropriate dilutions were prepared to get the final concentration of 2, 4, 6, 8, and 10 µg/ ml and absorbance was taken at λ_{max} 273 nm. Average of such five sets of values were taken for standard calibration plot, and the calibration curve was plotted. The aliquots of concentration ranging from 2-10 µg/ ml concentrations were used. The linearity was calculated by the least square regression method Calibration data of Pazopanib hydrochloride is depicted in Table 1 and Linear regression data is tabulated in table 1a. The summary output of Pazopanib hydrochloride by ANOVA is shown in table 1b. Figure 2 shows the calibration curve of Pazopanib Hydrochloride and Figure 2a shows the ultraviolet overlain spectra of Pazopanib Hydrochloride.

Table 1: Calibration data of Pazopanib hydrochloride

S. No	Concentration (µg/ml)	Absorbance
1	2	0.1333
2	4	0.2714
3	6	0.4139
4	8	0.5657
5	10	0.6969

Table 1a: Linear regression data

Parameter	Results
Detection wavelength (λ_{max})	273 nm
Beer's law limits (µg/ml)	2-10
Molar absorptivity (L. $mole^{-1}$ cm^{-1})	32120.24
Sandell's sensitivity (µg /cm^2/0.001 absorbance unit)	0.014738
Regression equation (Y = mx+ c)	0.0703x-0.0049
Slope (m)	0.0703
Intercept (c)	0.0049
The standard error of slope (S_m)	0.000683706
The standard error of intercept (S_c)	0.004140045
Standard error of estimate (S_e)	0.00572029
Correlation coefficient (r^2)	0.999

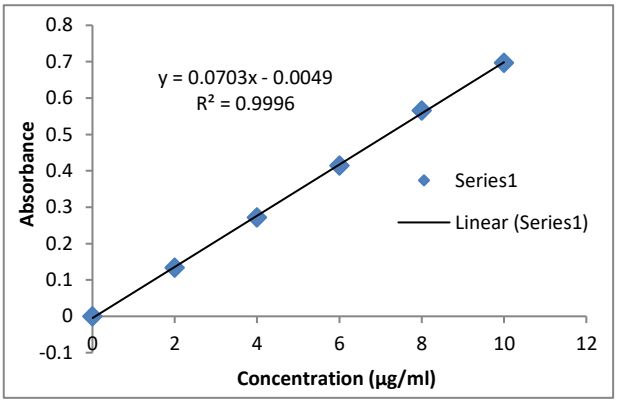

Figure 2: Calibration curve of Pazopanib Hydrochloride.

Table 1b: Summary output of Pazopanib hydrochloride (ANOVA):

	A	B	C	D	E	F	G	H	I
1	SUMMARY OUTPUT								
2									
3	*Regression Statistics*								
4	Multiple R	0.999811127							
5	R Square	0.999622289							
6	Adjusted R Square	0.999527862							
7	Standard Error	0.005720294							
8	Observations	6							
9									
10	ANOVA								
11		*df*	*SS*	*MS*	*F*	*Significance F*			
12	Regression	1	0.346396366	0.3463964	10586.116	5.3506E-08			
13	Residual	4	0.000130887	3.272E-05					
14	Total	5	0.346527253						
15									
16		*Coefficients*	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
17	Intercept	-0.004861905	0.004140045	-1.17436	0.3053933	-0.01635651	0.0066327	-0.01635651	0.006632702
18	X Variable 1	0.070345714	0.000683706	102.88885	5.351E-08	0.06844744	0.07224399	0.068447442	0.072243986
19									
20									
21									

Figure 2a: Ultra-violet Overlain Spectra of Pazopanib Hydrochloride

Method development and validation[13-16]

A different types of solvents were tested for solubility for Pazopanib hydrochloride solvents such as Methanol, DMSO, acetonitrile, and distilled water at 10 µg/ml concentrations. Nevertheless, Pazopanib hydrochloride was soluble and stable in methanol for a minimum of 24 hours at room temperature. Therefore, methanol solvent was used for the detection of wavelength and preparation of standard and working concentration. To ensure the planned method of the pharmaceutical formulation, an assay of Pazopanib hydrochloride 200 mg tablets was utilized at working concentration. Assay for working concentration of the sample at 273 nm was analyzed. A UV spectrophotometric method is validated according to ICH Q2 (R1) guidelines for validation of analytical procedures. The process was validated for parameters such as precision, accuracy, specificity, linearity, ruggedness, robustness, LOD and LOQ.

Precision

Precision studies were carried out to check the reproducibility of the method. Repeatability was determined by six replicates of the same concentration of the sample and measuring the absorbance. The intra-day precision study was carried out by analyzing the prepared drug solution at three different times (8-hour samples) in a day. The same procedure was followed for three different days to determined inter-day precision.

The results were reported as % RSD. System precision is shown in table 2.

Method precision

Method precision was estimated by conducting the assay of a sample under the test of repeatability (intraday precision) and intermediate precision performed during three successive days for three times. Eventually, the mean, SD, and % Relative standard deviation was determined. Intraday and interday precisions are shown in table 2a and table 2b respectively.

Table 2: Results of system precision

S. No	Absorbance
1	0.2714
2	0.2713
3	0.272
4	0.273
5	0.274
Mean	0.27234
Standard deviation	0.001148
% Relative Standard deviation	0.421547

Table 2a: Results of Method precision (Intraday precision)

Concentration (µg/ml)	Sample absorbance	Mean absorbance ± S. D	% RSD
4	0.271 0.274 0.273	0.272667 ± 0.001	0.560217
6	0.413 0.412 0411	0.412 ± 0.001	0.242718
8	0.565 0.564 0.563	0.564 ± 0.001	0.177305

Table 2b: Results of method precision (Interday precision)

Concentration (µg/ml)	Sample absorbance	Mean absorbance ± S. D	% RSD (n=3)
4	0.275 0.274 0.276	0.275 ± 0.001	0.3636
6	0.414 0.413 0.415	0.414 ± 0.001	0.241546
8	0.565 0.566 0.565	0.565 ± 0.001	0.176991

Accuracy (recovery studies):

Recovery studies of Pazopanib hydrochloride were carried out by utilizing a standard addition method in which estimation of % mean recovery of the sample by % method at 3 different levels (80 %, 100 %, and 120 %, i.e., 4 µg/ml, 6 µg/ml, 8 µg/ml). These 80 to 120 levels of the sample solutions were prepared as per the procedure given in the methods from the dilutions used for linearity (6 µg/ml). At each level, three analyses were performed. % mean recovery was calculated as shown in table 7. The accepted limits of recovery are 98 % - 101 %. In fact, the amount of Pazopanib hydrochloride was found, and % recovery was estimated. Accuracy results of Pazopanib Hydrochloride is shown in 3.

Table 3: Accuracy results of Pazopanib Hydrochloride

Level (%)	Absorbance	% Recovery	Mean % Recovery	% RSD
80	0.268	98.64	99.27	1.09
80	0.268	98.64		
80	0.276	100.53		
100	0.412	99.75	100.46	0.88
100	0.413	100.18		
100	0.419	101.452		
120	0.554	100.86	101.38	0.460
120	0.565	101.75		
120	0.567	101.55		

Ruggedness

Ruggedness is done by performing the proposed method on different instruments. In addition to that, this method is carried out by two various analysts and performing the technique on different days to check the reproducibility Results of ruggedness is shown in table 4.

Table 4: Results of ruggedness

Analyst	Concentration (µg/ml)	Sample absorbance	Mean absorbance± SD	% RSD
Analyst 1	10	0.693 0.695 0.696	0.6946±0.001	0.219
Analyst 2	10	0.697 0.699 0.698	0.698±0.001	0.1432

Limit of detection (LOD) and Limit of quantification (LOQ):

LOD is the lowest amount of analyte in the sample that can be detected. LOQ is the lowest amount of the analyte in the sample that can be quantitatively determined by suitable precision and accuracy. LOD and LOQ were determined by the following equation. LOD = 3.3σ/S, LOQ = 10 σ/S. where σ is the standard deviation of the Y-intercept of the calibration curve and S is the slope of the regression equation. The LOD and LOQ values were found to be 0.1924 µg/ml, 0.5832 µg/ml respectively.

Robustness

Analysis was carried out using concentration 10 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 Table 4a.

Table 4a: Robustness studies of Pazopanib hydrochloride

S.NO	Absorbance at 273 nm	Absorbance at 271 nm
1	0.693	0.697
2	0.695	0.699
3	0.696	0.698
Mean	0.694	0.698
Total SD	0.001	0.001
Total % RSD	0.21	0.143

Solution Stability

The Solutions of Pazopanib hydrochloride (Concentration 10 µg/ml) were tested for their stability at ambient temperatures. The absorbance values for 8 hrs, 16 hrs, 24 hrs, 32 hrs, 48 hrs, was reproducible, and absorbance variation was found to be less than 2 % in both conditions. Solution Stability studies of Pazopanib hydrochloride is shown in table 4b.

Table 4b: Solution Stability studies of Pazopanib hydrochloride

Time(hrs)	Absorbance 10 µg/ml standard in ambient conditions
0	0.6969
8	0.6986
16	0.6968
24	0.6968
32	0.6957
48	0.6936

Analysis of marketed formulation

The developed method was applied to analyze commercially available Pazopanib hydrochloride tablets (Votrient). The tablet was having the content of Pazopanib hydrochloride equivalent to 200mg. Ten tablets were weighed and weight equivalent to 100 mg was dissolved in methanol. By frequent shaking, volume was made up to mark with methanol. The solution was then filtered through Whatman filter paper #41. This filtrate was diluted suitability with solvent to get the solution of 5µg/ml concentration. The absorbance was measured against the solution blank. The amount of Pazopanib hydrochloride was calculated from the calibration curve. The readings were taken in triplicate. The assay results are shown in Table 5.

Table 5: Result of assay of pharmaceutical formulation of Pazopanib Hydrochloride

Table formulation	Label amount(mg/tab)	Amount obtained by proposed method	% Recovery (Amount found)
Tablet (n=3)	200	198.89±0.11	99.445

RESULTS AND DISCUSSION

The ultraviolet spectra of Pazopanib hydrochloride were scanned in the region between 200-400 nm. The overlay spectra of Pazopanib hydrochloride at different concentrations were absorbed maximum at 273 nm, which was selected as the detection wavelength. The response of the Pazopanib hydrochloride was found to be linear in the concentration range of 2-10 µg/ml with a good correlation coefficient of r^2 = 0.999. The system precision, intermediate precision results, i.e., inter-day and intra-day precision of Pazopanib hydrochloride, are tabulated in tables 2a and 2b respectively. The % RSD less than 2 in all precision results which indicates that the method was precise. In this recovery, study accuracy was carried out by using a standard addition method at three different concentration levels (80 %, 100 %, and 120 %). The mean percentage recovery at each level should be 99.27-101.38 %. All the results are well within the acceptance criteria, and results indicate that the method is accurate. Ruggedness was performed to check the reproducibility which showed the % RSD less than 2 which indicates that the method was rugged. The developed method was eventually applied for the quantification of Pazopanib hydrochloride in tablets. The mean % assay values were found to be 99.445 %. The amount of the drug in the tablet sample was in good agreement with the label claim of the formulation.

CONCLUSION

The UV method was developed for the estimation of Pazopanib hydrochloride. In this study, the precision and accuracy % RSD was < 2 % in all cases. This method provides reproducible results with high precision, accuracy, and was capable of analyzing Pazopanib hydrochloride in low concentrations. However, this UV method is simple, quick, sensitive. The results proved that this method is successfully feasible for routine quality control testing of Pazopanib hydrochloride in tablet formulation.

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