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Original Article

Validated RP-HPLC Method Development of Pazopanib in Bulk and its Pharmaceutical Dosage Form

Kiran Kumar Buralla[1], Varadarajan Parthasarathy[2,*]

[1]Department of Pharmacy, Annamalai University, Annamalainagar, Cuddalore, Tamil Nadu, INDIA.
[2]Department of Pharmacy, Director, Centre for Cell Biology and Drug Discovery, Annamalai University, Annamalainagar, Cuddalore, Tamil Nadu, INDIA.

ABSTRACT

Objectives: An accurate, sensitive, precise and rapid method for analysis and quantification of Pazopanib by Reverse Phase High Performance Chromatography (RP-HPLC) was developed and validated. Pazopanib in bulk and formulations were analyzed and quantification. **Methods:** Pazopanib in bulk and formulations were analyzed on Phenomenex enable C_{18} column (15x4.6mm, 5µm particle size) as stationary phase. Mobile phase was composed of acetonitrile and phosphate buffer (pH 5) in the ratio of 60:40%v/v at a flow rate of 1.2ml/min. elutes were analyzed using PDA detector at a detection wavelength of 290nm. The proposed method was validated by ICH guidelines, Validation of Analytical Procedures: Text and Methodology Q_2 (R1). **Results:** In this study, the chromatographic peaks of Pazopanib showed good resolution with retention time of 2.190min. Pazopanib showed an excellent linearity with 0.998 of correlation coefficient. Other validation parameters including precision, specificity, accuracy and robustness demonstrated good reliability in the quantification of Pazopanib. **Conclusion:** Thus the newly developed and validated method can be conveniently used for the quantification of Pazopanib in bulk and formulation. The method can also be applied to multi-component drug analysis.

Key words: Pazopanib, RP-HPLC, PDA, Precision, Accuracy.

Correspondence
Dr. V. Parthasarathy, Ph.D., Post. Doc. Res.,
Professor, Department of Pharmacy, Director, Centre for Cell Biology and Drug Discovery Annamalai University, Annamalainagar-608002, Cuddalore, Tamilnadu, INDIA.
Phone no: +91 9443512724
E-mail: vapartha@yahoo.com
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INTRODUCTION

Pazopanib is a second generation Tyrosine Kinase Inhibitor (TKI).[1] It used in the treatment of ovarian, renal, colon, neck and head, lung and prostate cancer.[2,3] Pazopanib is a potent and selective multi-targeted, tyrosine kinase inhibitor of vascular endothelial growth factor receptor-1 (VEGFR-1), VEGFR-2, VEGFR-3 and PDGFR-α/β1.[4] It also behaves like a stem cell growth factor receptor (c-kit) that blocks tumor growth and ceases angiogenesis.[5]

Literature survey reveals several analytical methods have been developed for estimation of Pazopanib in pharmaceutical dosage forms and biological samples including HPLC,[6,7] simultaneous estimation of Pazopanib by HPLC.[8,9] However, these reported chromatographic methods for estimation of Pazopanib possess multiple drawbacks like sample preparation, low sensitivity, complex mobile phase mixture, strict monitoring of critical method parameters like mobile phase, flow rate, column temperature, flow gradient, maintenance of pH, etc. This calls for the development of a simple, rapid, sensitive, efficient and reliable HPLC method for quantification of Pazopanib in bulk and pharmaceutical dosage forms. The validation of the proposed method was carried out according to ICH guideline ICH Q2 (R1).[10]

Molecular formula and molecular weight of Pazopanib are $C_{21}H_{23}N_7O_2S$ and 437.52gm/mol.[11] It is soluble in water and acetonitrile. Chemically Pazopanib (Figure 1) is known as 5[{4(2,3-dimethyl-2H-indazol-6-yl) methylamino}2-pyrimidinyl]2-methylbenzenesulfonamide.

MATERIALS AND METHODS

Chemical and reagents

Reference standard of Pazopanib was used to develop the new RP-HPLC method. HPLC grade Acetonitrile was obtained from Sd Fine chem. Ltd (India). Water for RP-HPLC was prepared using Milli Q Water (Merk). Pazopanib HCl is commercially available as Votrient® marketed by GSK Rx India with a labeled claim of 200mg per tablet.

Instrumentation

The HPLC analysis was carried out with a Shimadzu HPLC system (Tokyo, Japan) with two LC-20AD separation modules and SPD-m20A PDA detector, a Rheodyne injector (model 7125, USA). The chromatographic and integrated data were recorded using LC solution data acquisition software. An electronic weighing balance with a 0.1 mg sensitivity, digital pH meter (DELUX model 101), a Sonicator (Systrinices, model 2200MH). Absorbance spectra were recorded using a UV-VIS spectrophotometer (Systronices, India) employing a quartz cell of 1 cm of path length. The mobile phase was composed of Acetonitrile and phosphate buffer pH 5 in the ratio of 60:40%v/v. the optimized chromatographic condition are shown in Table 1.

Preparation of phosphate buffer pH 5

Accurately weighed 0.68gm of phosphate buffer (potassium dihydrogen ortho phosphate) and transferred into a 500ml volumetric flask. Added 400ml of Mille Q water, dissolved by Sonication and the final volume was made up to 500ml using Mille Q water. The pH of the buffer solution was adjusted to 5±0.5 using orthophosphoric acid (dilute). Filtered through membrane filter (0.45µm) prior to use.

Preparation of standard solution of Pazopanib

Stock standard solution of Pazopanib was prepared by transferring 10mg of drug in to 10ml of volumetric flask. Added 8ml of acetonitrile and was sonicated for 5-10min. finally the volume was made up with acetonitrile which gives 1mg/1ml. 10µm/ml of working standard solution was prepared by taking suitable aliquot from standard stock solution and volume was made up with acetonitrile.

Assay procedure

Ten tablets (Votrient) were weighed and then powdered, which is equivalent to 100mg of Pazopanib into a 10ml of volumetric flask and

added 8ml of acetonitrile and sonicated for 5-10min. The volume made up to 10mL with acetonitrile and mixed. Solution was filtered by 0.45µm filter to remove particulate matter, if any. The filtered solution was further diluted for analysis, to get a test concentration of 10µg/ml. Assay results are tabulated in Table 2.

METHODS VALIDATION AND RESULTS AND DISCUSSION

The developed RP-HPLC method was validated as per ICH guidelines.

Linearity

Stock solution of Pazopanib (1mg/ml) was suitably diluted with Acetonitrile to get concentration in the linearity range of 2 to 10µg/ml. A sample volume of 20µl was injected onto the column in triplicate, for each solution. Chromatograms, peak area and retention times of each solution were recorded. Calibration curve of Pazopanib was prepared by selecting concentration (µg/ml) on x-axis and average peak areas on y-axis (Figure 2 and Table 3). The calibration curve data was further subjected to stastical analysis to find out the slope intercept and correlation of coefficient. R^2 for Pazopanib was found to be 0.998 (Table 4). Figure 3 are the chromatogram of Pazopanib (10µg/ml).

Accuracy

Accuracy, which is the measure of closeness of the experimental value to the true value, was determined by standard addition method. To a pre-analyzed sample formulation a known quantity of standard was added at three levels (80, 100 and 120% of the assay concentration). The experimental was performed in triplicates. The % recoveries were calculated for all the concentrations. Results are summarized in Table 5.

Precision

Method precision was determined in terms of repeatability (intra-day) and intermediate precision (inter-day) studies by measuring the peak area and retention time of three different concentrations (2, 4 and 6µg/ml) of Pazopanib. Repeatability was performed by repeated injection of three different concentrations from single batch under the same experimental conditions on the same day. From the results, RSD values for retention time were less than 2%, while RSD values for peak area were less than 2% for the intra-day assay precision. Precision results are expressed in Table 6.

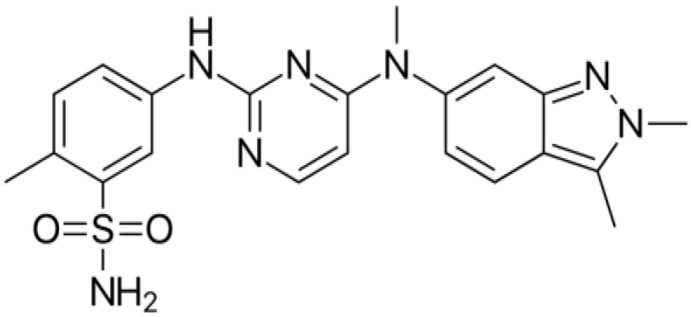

Figure 1: Chemical structure of Pazopanib.

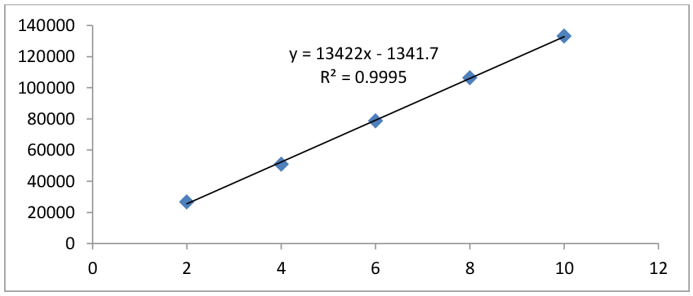

Figure 2: Calibration curve for Pazopanib.

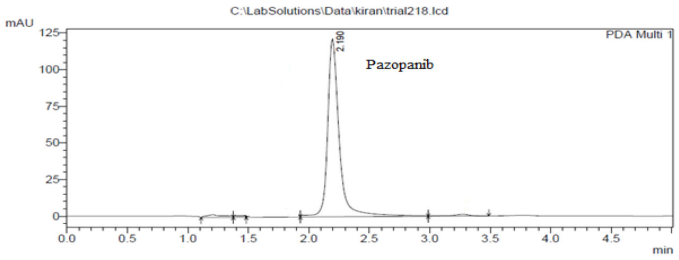

Figure 3: Optimized chromatogram of Pazopanib.

Table 1: Chromatographic conditions.

Parameters	Methods
Stationary phase	Phenomenex enable C_{18} column
Mobile phase	Acetonitrile: Phosphate buffer pH 5 (60:40)
Flow rate	1.2
Run time (minutes)	6
Column temperature	Ambient
Volume of injection	20µl
Detector	PDA
Detection of wavelength	290
Drug re tR	2.190

Table 2: Assay of formulation.

Brand name	Available form	Label claim	Amount found	Assay
Votrient	Tablet	200mg	199.9mg	99.78%

Table 3: Calibration curve of Pazopanib.

Concentration (µg/ml)	Peak area
2	26485
4	50912
6	78764
8	106449
10	133134

Table 4: Linearity data of Pazopanib.

Parameters	Pazopanib
Linearity	2-10µg/ml
Regression equation	Y=13422x-1341
Slope	13422
Intercept	1341
Correlation coefficient	0.999
Retention time	2.190min

Table 5: Accuracy study for Pazopanib.

Percentage	Pazopanib	Sdv	%RSD	%Recovery
	231527			
80%	233286	0.032632	1.138665	99.65
	235045			
	261165			
100%	262924	0.024699	0.774692	99.78
	264683			
	287433			
120%	289192	0.019227	0.543418	100.65
	290951			

Table 6: Precision values for Pazopanib.

Conc	Drug Area	Sdv	%RSD
	26485		
2	26068	220.16	0.838
	26399		
	51329		
4	50912	416.00	0.817
	50497		
	79591		
6	78764	633.16	0.803
	78347		

Table 7: Limit of detection and quantification.

Parameter	Pazopanib
LOD	10.43nanogram/ml
LOQ	31.63nanogram/ml

Sensitivity

Sensitivity of the method was determined from limit of detection (LOD) and limit of quantification (LOQ). The LOD and LOQ were determined using the calibration curve and results are summarized in Table 7.

$$LOD= 3.3xD/S \text{ and } LOQ= 10xD/S,$$

Where,

D = standard deviation of Y intercept of regression line

S = slope of the calibration curve

System suitability tests

The test was carried out by making six replicate injections of a standard solution containing 10µg/ml of Pazopanib and analyzing each solute for their peak area, theoretical plates (N), tailing factor (T) and asymmetric factors (As).

Robustness

Robustness of the method was studied to evaluate the effect of small but deliberate variation of the chromatographic conditions on the method parameters. Robustness was determined by changing individually the flow rate (1.2±0.1ml/min.), organic solvent (60±0.5%) and ionic strength of buffer (5±0.2).

CONCLUSION

The RP-HPLC method developed was accurate, precise, reproducible and specific. The method is economical and utilizes a mobile phase which can be easily prepared. The method is less time consuming. All these merits make this method suitable for quantification of Pazopanib in bulk and its pharmaceutical dosage forms without interference.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest. The article does not contain any studies with animals or human participants performed by any of the authors.

Role of the Funding Source

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ABBREVIATIONS

RP-HPLC: Reverse Phase High Performance Liquid Chromatography; **ACN:** Acetonitrile; **TKI:** Tyrosine Kinase Inhibitor; **LOD/DL:** Limit of Detection; **LOQ/QL:** Limit of Quantification; **PAZ:** Pazopanib; **DST:** Dasatinib.

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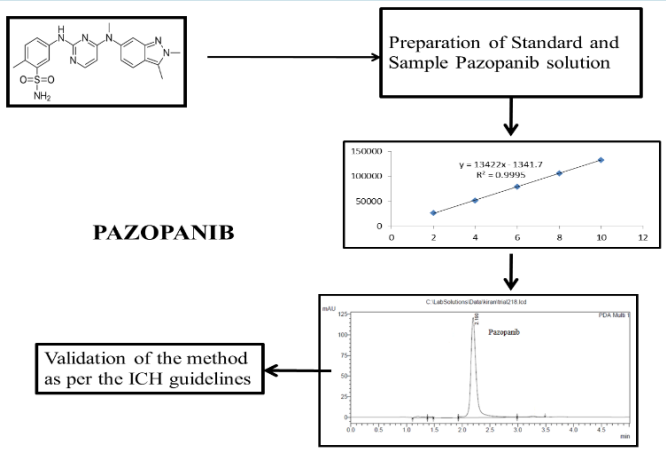
PICTORIAL ABSTRACT



SUMMARY

•Simple, sensitive, precise and rapid RP-HPLC method for the analysis of Pazopanib in bulk and pharmaceutical dosage form was developed.
•The developed method was validated according to the ICH guidelines.

ABOUT AUTHORS

Varadarajan Parthasarathy: Currently working as a Professor in the Department of Pharmacy at the Annamalai University, Chidambaram. He has obtained his Ph.D. (University of Sheffield, U.K), Post Doct. Research (Harvard University, U.S.A). His main research interests are in the areas of Molecular Biology, Immunology, Pharmacology and Bio-Analytical method development for the Synthetic drugs and pharmaceutical formulations.

Kiran Kumar Buralla: Is a Research Scholar in the Department of Pharmacy at the Annamalai University, Chidambaram. His research area of intresets is analytical method development and validation for synthetic and pharmaceutical dosage formulations.