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

Kiran Kumar Buralla¹, Varadarajan Parthasarathy²,*

¹Department of Pharmacy, Annamalai University, Annamalainagar, Cuddalore, Tamil Nadu, INDIA.
²Department 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 C₁₈ 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 Q² (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, FDA, 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.²³ 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/β.¹ It also behaves like a stem cell growth factor receptor (c-kit) that blocks tumor growth and ceases angiogenesis.³

Literature survey reveals several analytical methods have been developed for estimation of Pazopanib in pharmaceutical dosage forms and biological samples including HPLC,⁴,⁶ simultaneous estimation of Pazopanib by HPLC.⁵ 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).⁹

Molecular formula and molecular weight of Pazopanib are C₁₁₀H₁₁₂N₁₀O₁₁ and 437.52gm/mol.¹⁰ 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 (Systronic, model 2200MH). Absorbance spectra were recorded using a UV-VIS spectrophotometer (Systronics, 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 ±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. 10µl/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 statistical 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.
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.

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ABBREVIATIONS

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

REFERENCES


PICTORIAL ABSTRACT

• 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.

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 interest is analytical method development and validation for synthetic and pharmaceutical dosage formulations.