Biological Forum – An International Journal **15(4): 475-478(2023)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Rp HPLC Method Development for Pazopanib in mixture and tablet Form

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(Received: 18 February 2023; Revised: 12 March 2023; Accepted: 19 March 2023; Published: 20 April 2023)
(Published by Research Trend)

ABSTRACT: Analytical method development involves screening various column and eluent conditions, method optimization includes iterative testing of various separation conditions of the HPLC method and is performed to achieve the best possible resolution, speed, and reproducibility, robustness testing and method validation. Pazopanib is a class of drugs called kinase inhibitors with potent antineoplastic effects and is used in the treatment of kidney and soft tissue sarcomas. This work includes the development of a simple, accurate, precise and reproducible liquid chromatography (RP HPLC) method for the determination of pazopanib in tablet dosage. Isocratic elution was performed at a flow rate of 1.0 mL/min on a Kromasil C18 column (250 mm × 4.6 mm, 5 µm) at 25 °C. The mobile phase was methanol: 0.025% TFA in water (60:40) v/v. The UV detection wavelength is 273 nm and the injection volume is 20 µL. Pazopanib has a retention time of approximately 2.83 minutes. According to ICH guidelines, the process has been validated for various parameters such as suitability, efficiency, recovery and robustness. The validated RP HPLC method is specific, precise, and accurate, and has been successfully used to identify pazopanib and its commercial samples.

Keywords: HPLC, pazopanib, ICH guideline, method development, validation.

INTRODUCTION

Pazopanib is a second generation tyrosine kinase inhibitor (TKI) (Escudero-Ortiz *et al.,* 2015). It is 5-(pyrimidin-2yl) amino 2 methyl benzene sulfonamide substituted by (methyl) amino at position 4 (2, 3 dimethylindazole 6 yl) cancer, lung cancer and cancer blood prostate, pazopanib is a potent and selective multi target tyrosine kinase inhibitor that inhibits vascular endothelial growth factor receptor (VEGFR 1), VEGFR 2, VEGFR PDGFR α/β1 (Fierce, 2015; Sleijfer *et al.,* 2009; Tugues *et al.,* 2011). It also acts as a receptor for stem cell growth factors (stopping tumor growth and angiogenesis) (Saharinen *et al.,* 2011). A review of the literature showed that several methods such as Rp HPLC have been reported to produce a compound or a single dosage form of pazopanib (Buralla & Parthasarathy 2020; Sankar *et al.,* 2021) and UV spectroscopy (Chaitanya *et al.,* 2015). Therefore, this study aims to develop a simple, fast, sensitive, efficient and reliable HPLC method for the quantification of pazopanib in bulk and pharmaceutical form. The plan has been validated in accordance with ICH guidelines ICH Q2 (R1). Molecular formula and molecular weight of C21H23N7O2S and 437.52 g/mol.

MATERIALS AND METHODS

The LC system has the following components: Chromatography was performed on Kromasil C18 at 5 µm dimensions (250 mm × 4.6 mm diameter) using Spinchrom software. A Shimadzu electronic balance (AX 200) was used. Analytical pure pazopanib was received as a gift sample from Glenmark Ltd. (Mumbai, India). Methanol, water (E. Merck, Mumbai, India) was the HPLC grade. Tablets 200 mg of Pazoci were purchased from the local market.

Preparation of stock solutions: Pazopanib Standard stock solution was prepared by dissolving 10.83 mg Pazopanib hydrochloride (Equivalent to 10 mg of Pazopanib) into a 20 mL clean and dried volumetric flask, added about 15 mL of water to dissolve it completely and made volume up to the mark with water (500 PPM). Further diluted 2 ml of stock solution to 10 mL with mobile phase (100 PPM). It was prepared in mobile phase of each trial and injected in development trials.

Selection of analytical wavelength for HPLC method development: The analytical wavelength is chosen from the maximum absorption wavelength of the spectrophotometric analysis, which is 273 nm.

Sample preparation: Weigh 20 tablets of in a mortar and grind into a fine powder. Mix the contents thoroughly with wax paper. Measure powder equivalent to 100 mg of pazopanib and transfer to a clean and dry 100 mL volumetric flask. Add 70 mL of water and sonicate for 10 minutes with intermittent shaking. After 10 minutes, allow the solution to cool to room temperature and bring to the mark with water. Discard 3

5 mL of the first filter by passing the solution through an appropriate 0.45 µ syringe filter. Dilute 0.2 ml of the filtered product to 10 ml with the mobile phase. (20 mcg pazopanib), inject the resulting drug, record the chromatogram and save the results.

Accuracy. Take clean and dried 9 volumetric flasks of 100 mL. Weighed aprrox 201.76 mg of placebo and transferred in each 100 mL volumetric flask. Weighed Pazopanib hydrochloride API as per accuracy level and transferred in same 100 ml volumetric flask. Add 70-75 ml of water sonicate it for 10 minutes with intermittent shaking. Allowed to cool the solution at room temperature and made the volume up to the mark with water. Filter the solution through suitable 0.45 µ Nylon syringe filter discarding 5mL of filtrate. Further dilute 0.2 ml of filtrate to 10 ml with mobile phase.

Acceptance criteria. 1. % Recovery and average recovery for each sample should be in the range of 98-102%.

2. The relative standard deviation should not be more than 2.0%

Precision. Intraday and interday precision studies of pazopanib at 3 different pazopanib concentrations (5, 10, 15 µg/ml) on the same day and 3 days (days 1, 2, and 5), and results are reported as standard deviation (RSD), Table 2). Reproducibility studies were performed by measuring triplicate responses to 3 different pazopanib concentrations (5, 10, 15 µg/ml), and results are reported as standard deviation (RSD).

Specificity. Incorporate common excipients (starch, microcrystalline cellulose and magnesium stearate) into pre weighed

Solution. Chromatograms were obtained with appropriate dilution and the amount of drug was determined.

Limits of detection and quantification. Calibration curves were prepared using pazopanib concentrations of 3-30 µg/ml. The standard deviation of the y-intercept of the regression line is determined and kept in the equation below, which is used to determine the detection and quantification limit. Detection limit = 3.3σ/s; quantitation limit = 10σ/s; where σ is the standard deviation of the y intercept of the regression line and s is the slope of the curve.

Robustness. The method was carried by changing the concentration of organic phase ±% and changing the pH ±0.2. Monitor the stability of the drug placed in the mobile phase at a temperature of 35 °C for 24 hours.

RESULTS AND DISCUSSION

The mobile phase was optimized according to the resolution, asymmetry factor and peak area obtained with pazopanib. Mobile phase methanol: 0.025% TFA in v/v in water (60:40) adjusted to pH 4 was found to be satisfactory and gave symmetrical and well resolved peaks. Pazopanib has a retention time of 2.83 minutes, respectively (Fig. 1). The asymmetry factor is 1.5. The ultraviolet spectrum shows that the drug has absorption at 273 nm, so 273 nm is chosen as the detection wavelength of liquid chromatography. The resolution system was analyzed at 200 nm and 400 nm. Determine the wavelength of the maximum absorption of the solution. Pazopanib exhibited absorbance at 209, 243, 273 and 307 nm. As shown in Fig. 2. 209 nm was not chosen because it is close to the cut off wavelength. Based on research data, 273 nm was chosen as the wavelength of pazopanib most of the time. Therefore, 273 nm was accepted as the benchmark for further evaluation. The calibration curve was obtained by plotting the area peak versus pazopanib concentration in the range of 2-30 µg/ml, and it was found to be linear with r= 0.9982. (Fig. 3). The detection limit and quantitation limit were 0.348 µg/ml and 1.056 µg/ ml, respectively which suggest that a nanogram quantity of both the compounds can be estimated accurately. The validation parameters are summarized in Table 1. The system suitability parameters are shown in Table 2. The liquid chromatographic method was applied to the determination of pazopanib in single dosage form. The results were comparable with the corresponding labeled amounts (Table 3).

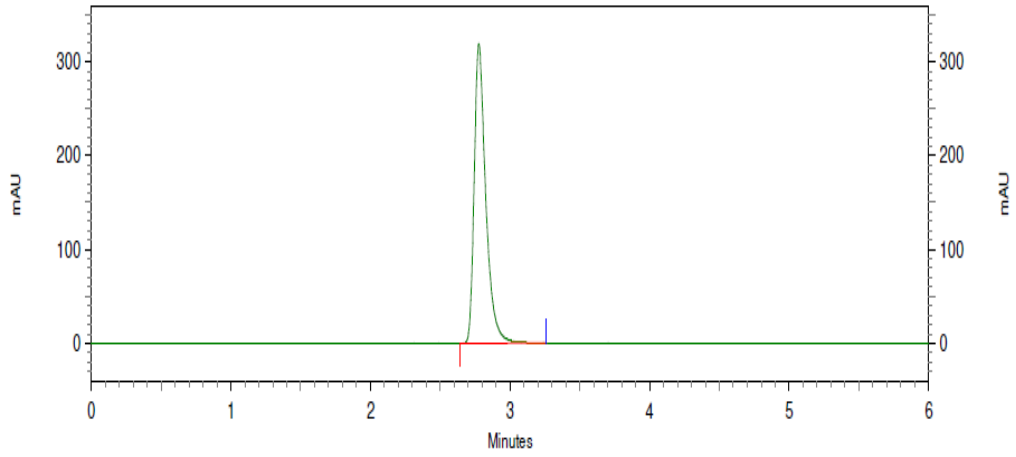

Fig. 1. Typical chromatogram of Pazopanib.

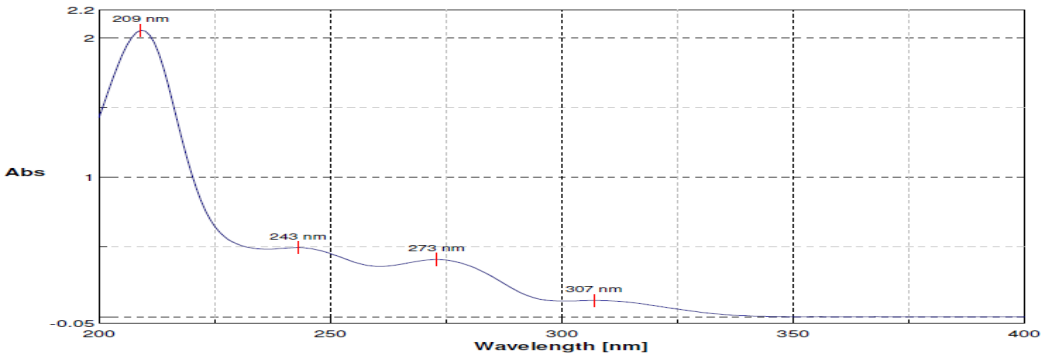

Fig. 2. UV spectrum of Pazopanib.

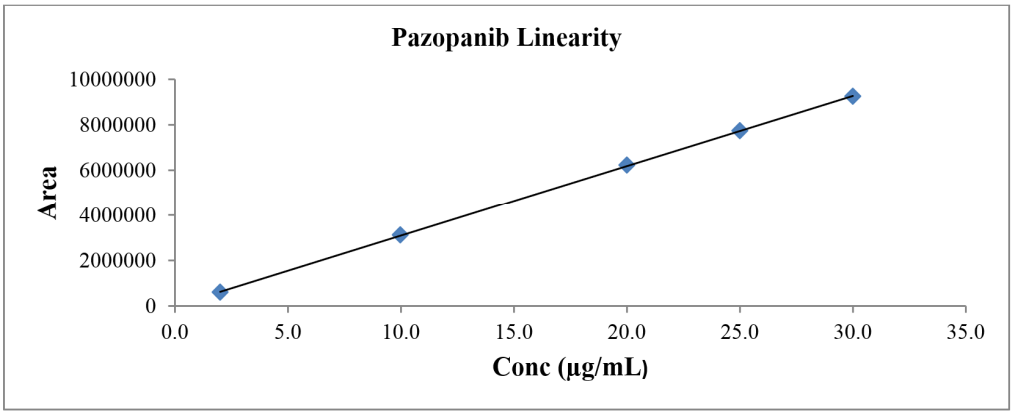

Fig. 3. Calibration curve of Pazopanib.

Table 1: Summary of validation parameters.

Parameters	Pazopanib
Detection limit (µg/ ml)	0.348
Quantitation limit (µg/ ml)	1.056
Accuracy (%)	98.02-100.68
Precision (RSDa, %)	
Intraday (n=3)	0.91-1.23
Interday (n=3)	1.01-1.57
Repeatability (RSDa, n=3)	0.56-0.9

Table 2: System suitability test parameters for Pazopanib.

System suitability Parameters	**Pazopanib**
Retention time (min)	2.79
Resolution	5.89
Theoretical plates	5639
Tailing factor (asymmetric factor)	1.5

Table 3: Assay results of combined dosage form using proposed method.

Trial No.	**Labelled amount (mg)**	**Amount obtained (mg)b**	**% Recovery**
1	200	199.2	99.6
2	200	201	100.5
3	200	200.7	100.35

DISCUSSION

In most of analytical method development for Pazopanib were carried out by using acetonitrile: phosphate buffer as a mobile phase. Acetonitrile is expensive solvent which will cost a method development, therefore method was developed using mobile phase methanol: 0.025% TFA in water (60:40) v/v, wavelength 273 nm, flow rate 1.0 ml/min. The column used was a Kromasil C18 column (250 mm × 4.6 mm, 5 mm). The method is

simple, powerful and accurate, well insulated and usable for commercial analysis.

CONCLUSION

The study described a new RP HPLC method that uses a simple phase to quantitatively measure pazopanib content compared to the reported. The method provides good resolution of both compounds with a short measurement time (<10 minutes). The method is simple, precise, accurate and straightforward. Recovery data showed that the method was not affected by the excipients used in the formulation. Therefore, this method can be used for routine analysis of pazopanib in dosage form.

FUTURE SCOPE

The method which is developed for pazopanib is a simple, rapid with low cost and can be commercially used in industry.

Acknowledgement. The authors are thankful to Dr. S. B. Bhawar Principal, Pravara Rural College of Pharmacy Pravaranagar for providing research facilities.
Conflict of interest. None.

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How to cite this article: Godge R. K. and Ghorpade R. S. (2023). Rp HPLC Method Development for Pazopanib in mixture and tablet Form. *Biological Forum – An International Journal, 15*(4): 475-478.