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Analytical Method Development and Validation of RP-HPLC Method for Estimation of Pazopanib Drug Sample and It's Dosage Form

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Abstract: The study focuses on developing and verifying a cost-effective Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) technique for quantifying Pazopanib HCl in both bulk and tablet forms. The study aims to develop a cost-effective approach for routine quality control analysis by utilizing the simplicity and wide accessibility of HPLC. A Shimadzu C18 column (5 µm, 250 mm × 4.6 mm) was successfully used to separate Pazopanib HCl via chromatography. The mobile phase consisted of a mixture of Potassium dihydrogen phosphate in water (pH 2.9, corrected with Phosphoric Acid) and Acetonitrile in a ratio of 25:75 v/v. The isocratic elution mode was utilized with a flow rate of 1.0 mL/min, a column temperature of 25°C, and an injection volume of 20 µL. Pazopanib hydrochloride had a retention period of 2.8 minutes when measured at an isobestic wavelength of 215 nm. The described RP-HPLC technique showed exceptional specificity, accuracy, precision, linearity, and durability, rendering it a viable instrument for Pazopanib HCl tablets' regular quality control analysis. This method is both efficient in terms of analytical performance and economically profitable, making it very suitable for frequent pharmaceutical analysis.

Introduction

Pazopanib HCl is a powerful and specific inhibitor of multi-targeted receptor tyrosine kinases. It is used to treat advanced soft tissue sarcomas, bone sarcomas, and renal cell carcinoma by blocking angiogenesis and inhibiting the development of cancer cells. The main inhibitory effects are shown on c-kit, platelet endothelial growth factor receptors -α and -β, and vascular endothelial growth factor receptors-1, 2 and 3. Pazopanib HCl is categorized as a BCS class II medication, with a log P value of 3.2 and low oral bioavailability. It has a low solubility in water, measuring 0.33 mg/ml. The chemical structure is shown as 5-[[4-[(2,3-dimethyl-2H-indazol-6-yl) methylamino]]-2-pyrimidinyl] amino] on hydrochloride, 2-methyl benzenesulfonamide. The compound's empirical formula is $C_{21}H_{23}N_7O_2S$•HCl, and its molecular weight is 473.99 grams per mole. In addition to its proven uses, current studies indicate that it is useful in the treatment of non-small cell lung cancer (Koylu et al., 2022; Verheijen et al., 2022).

Studies have utilized sophisticated analytical methods, such as LC-MS/MS (Verheijen et al., 2016; Verheijen et al., 2018; Minocha et al., 2012; Sparidans et al., 2012; Pressiat et al., 2018) UPLC-QTOF/MS (Patel et al., 2015), and High-Performance Liquid Chromatography – UV (Escudero-Ortiz et al., 2015; Sharada et al., 2016), to quantify pharmaceuticals. In addition, Ultra Violet techniques (Sharada and Babu, 2016; Chaitanya and Pawar, 2015) have been used to estimate this medication.

HPLC has been beneficial in diagnostics and pharmaceuticals. Although several RP-HPLC methods have been documented for estimating Pazopanib HCl in tablet dosage forms and biological fluids, such as those by Shabada et al. (2017), Buralla et al. (2020), Ghode et

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al. (2020), and Sankar et al. (2021), there is still a lack of an economical and validated method for estimating Pazopanib HCl in bulk drugs and tablet forms. The current study seeks to address this deficiency by creating and verifying a cost-effective RP-HPLC technique for the precise measurement of Pazopanib HCl in both its raw form and tablet formulations.

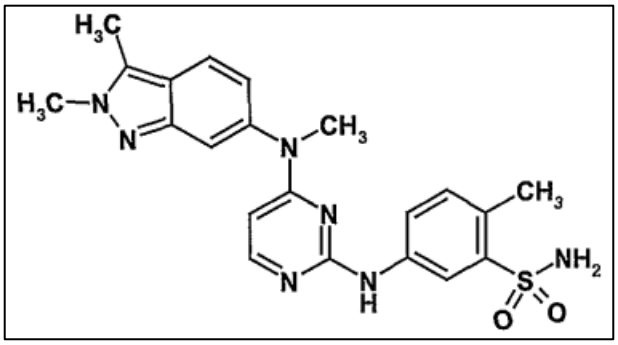

Figure 1. Chemical structure of Pazopanib HCl

Materials and Methods

Materials

Pazopanib HCl was a gift sample from Biocon Pharmaceuticals Ltd. A 200 mg tablet of Pazopanib HCl was purchased from a local market. HPLC-grade phosphoric acid and HPLC-grade acetonitrile were purchased from Merck. High-purity water was prepared using the Milli Q purification system, and AR-grade potassium dihydrogen phosphate was purchased from Merck Pvt. Ltd. in Mumbai, India.

Biocon Pharmaceuticals Ltd. kindly supplied a gift sample of pazopanib HCl. 200 mg tablet of Pazopanib HCl was purchased from a nearby store. HPLC-grade acetonitrile and phosphoric acid were purchased from Merck. The Milli Q filtration system produced high-purity water. The AR-grade potassium dihydrogen phosphate supplier was Merck Pvt. Ltd. in Mumbai, India.

Instrumentation

The HPLC system used was a Shimadzu UHPLC, LC 20 AD equipped with a quaternary pump and detector (SPD-20 A type). Openlab EZ Chrome was used to manipulate the instrument's settings. Pazopanib HCl was separated by chromatography using a C18 column (250 mm × 4.6 mm, 5 µm) and a mobile phase that included acetonitrile (25:75% v/v) and phosphate buffer pH 2.9 in isocratic elution mode at a flow rate of 1.0 mL/min. The injection volume was 20 µL, and the column temperature was maintained at 25°C.

Preparation of solution

Preparation of buffer solution

Potassium dihydrogen phosphate (0.68%) should be measured and transferred into a 1000 mL volumetric flask. After adding 700 mL of water and sonicating to aid in dissolving, dilute the solution with more water to the necessary amount. Mix the mixture well and use orthophosphoric acid to get the pH down to 2.9 ± 0.05.

Preparation of mobile phase

Prepare a mixture of Buffer pH 3.5 and Acetonitrile in the ratio of 250:750 v/v respectively, and mix well. Used mobile phase as diluent and blank.

Preparation of standard solution

Pazopanib HCl standard should be measured precisely and then added to a 100 mL dry volumetric flask. After adding around 70 mL of the diluent and using a sonicator to dissolve the contents for fifteen minutes, dilute the solution with diluent until it reaches the necessary volume and thoroughly mix. In addition, mix 100 mL of diluent well with 2.0 mL of the stock solution that was previously specified. Pass a 0.45 PTFE filter through the sample solution. After discarding the first 4.0 mL of filtrate, gather the sample.

Preparation of sample solution

After carefully weighing 100 mg of powdered pazopanib HCl, it was put in a 100 ml volumetric flask. After adding a mobile phase, the mixture was exposed to sonication for ten minutes while being periodically shaken. The solution was then diluted to volume using the mobile phase. The resulting mixture was filtered using a 0.22 µl syringe filter. Two millilitres were diluted with 100 milliliters of mobile phase, and impurities were eliminated from the final solution using a membrane filter.

Method Validation

The developed method was validated as per ICH guidelines for the following parameters.

System suitability

System suitability evaluations ensure that the method can produce results with sufficient accuracy and precision. The repeatability of the proposed approach was tested for system suitability and additional characteristics like retention time, the number of theoretical plates, and asymmetry variables were examined and determined to be satisfactory.

Specificity

The ability to evaluate the analyte in the presence of components that could be anticipated to be present is known as specificity. By contrasting the retention time and spectrum of the tablet solution with those of the standard solution, the specificity of the method for determining the presence of Pazopanib HCl in the tablet dosage form was established. We looked for impurity and excipient interference in the sample spectrum.

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Linearity

The capacity of the method to produce test findings that are directly correlated with the analyte concentration in the sample is known as linearity. Aliquots of the standard solution were collected to create sample solutions with drug concentrations ranging from 10 to 60 µg/ml. The solutions were examined one by one. A linear fit was proven using Linear Regression analysis by graphing the peak area versus the Pazopanib HCl concentration.

Limit of detection (LOD) and Limit of quantitation (LOQ)

Based on the slope of the calibration curve (S) at levels and the standard deviation of the response of the curve, the LOD and LOQ were computed. Based on the slope of the calibration curve at levels and the standard deviation of the response of the curve, the LOD Pazopanib HCl was calculated. The results showed that the technique could identify and measure the drug.

Accuracy (Recovery)

Accuracy was determined over the range of 50% to 150% of the sample concentration.

Precision

On the same day and 3 days (day 1, day 2, and day 3) for 3 different concentrations of Pazopanib HCl, intra-day and inter-day precision tests were carried out. The appropriate response is thought to be executed three times. Report the data as relative standard deviation (RSD) at (15, 35, and 55 µg/ml). Pazopanib HCl (5, 10, and 15 µg/ml) responses were evaluated as part of reproducibility experiments, and the results are expressed as relative standard deviation (RSD).

Robustness

The robustness of the developed method is evaluated by its capacity to remain unaffected by small but deliberate changes in the method parameter. The robustness was studied by varying the flow rate at ±0.1 ml/min and wavelength at ±0.1 ml/min. The result expressed in % Assay, the acceptance criteria for robustness is the absolute difference of % Assay is not greater than 2%.

Results and Discussion

The RP-HPLC technique for Pazopanib HCl was developed using a straightforward approach that focused on optimizing the mobile phase, flow rate, injection volume, and run-time. This optimization was based on the analysis of theoretical plates, asymmetry factor, and peak area.

Method development

The mobile phase consisted of a blend of Phosphorus buffer with a pH of 2.9 and acetonitrile in a ratio of 25:75 % v/v. The mobile phase flowed at a rate of 1 mL per minute. The injection volume was 20 µl, and the wavelength used was 215 nm. The sample and column temperatures were at room temperature. The 5-minute sample run produced a chromatogram that was deemed good, and the system suitability parameters were acquired correctly. Enhanced chromatographic conditions and system suitability parameters were developed for the determination of PZP using the proposed RP-HPLC technique.

Method validation

Establishing documented evidence, known as validation, is a procedure that offers a high level of assurance that a certain action will consistently generate a desired outcome or product that satisfies its pre-established requirements and quality attributes. ICH guidelines were followed in the method's validation.

System suitability

Tests to ensure the approach can produce findings with sufficient accuracy and precision are known as system suitability parameters. The specifications for system suitability are often created following the conclusion of method development and validation. The theoretical plates, retention time, and tailing factor, among other system suitability variables, were examined and found to be satisfactory. Table 1 displays the findings and the system suitability attributes.

Table 1. System suitability parameters.

Name	Area	Retention Time	Theoretical plates	Asymmetry
Results	2852356	2.846 min	2800	1.147
Limit	-------	----	NLT 1500	NMT 2.0

Specificity

To demonstrate specificity in this context, it must be demonstrated that the presence of excipients had no impact on the assay. An analytical method's specificity was determined by its capacity to quantify the target analyte precisely and without the interference of placebo and blank samples. The data demonstrates that retention time in standard and sample is the same for Pazopanib peak and no interference in blank and placebo at the retention time of peak.

Table 2. Result for Specificity.

Component	Retention time (min)	Area	Theoretical Plates	Asymmetry
Blank	-		-	-
Standard solution	2.843	2999930	2766	1.187
Sample solution	2.843	3098727	2782	1.137

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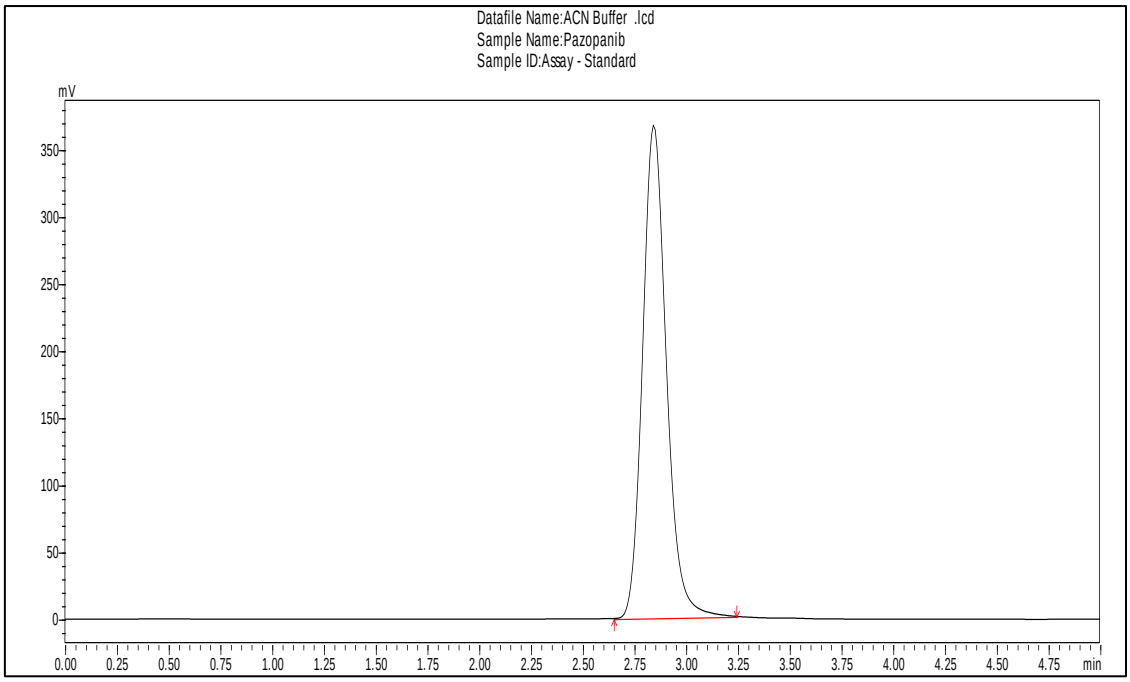

Figure 2. Chromatogram of Standard Solution.

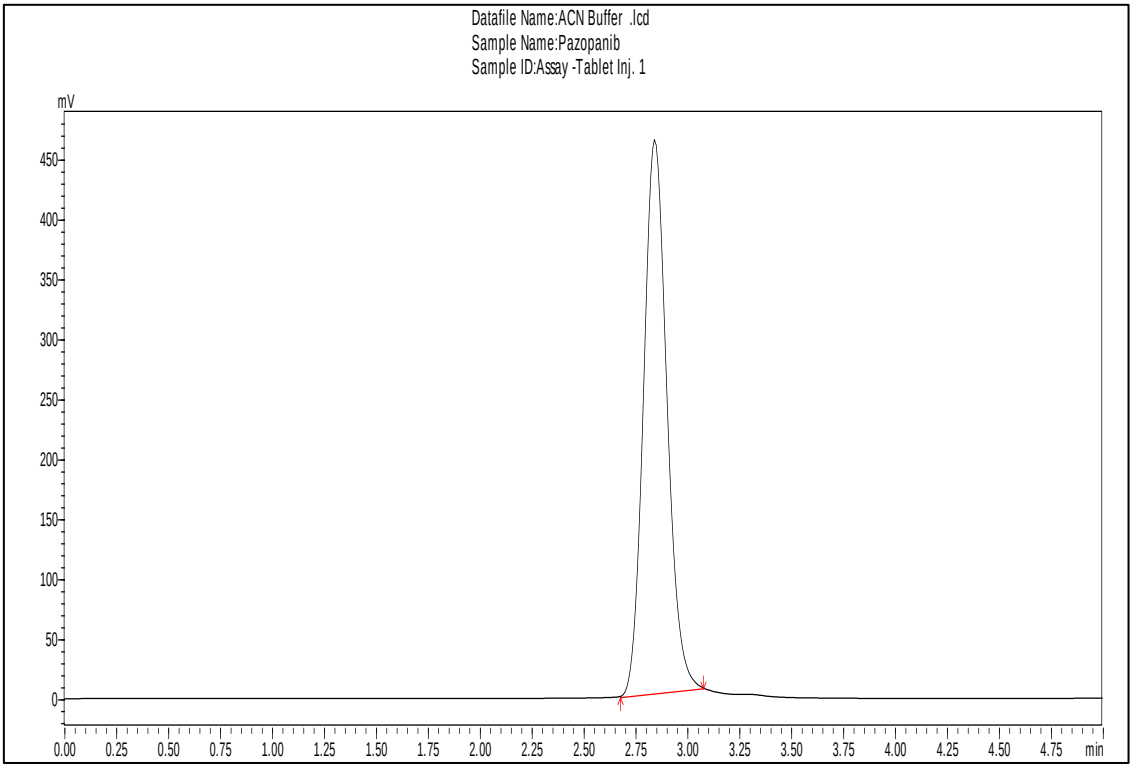

Figure 3. Chromatogram of Sample solution.

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Linearity

PZP's linearity was assessed at 10 and 60 µg/mL concentrations. Figure 4 displays the PZP calibration graph. Table 3 displays the linearity data.

Table 3. Results for Linearity.

Level (%)	Volume taken from Linearity stock solution (mL)	Total volume (mL)	Concentration (µg/mL)	Peak Area
50	1	100	10	1654454
100	2	100	20	2922103
150	3	100	30	4287906
200	4	100	40	5529529
250	5	100	50	6834191
300	6	100	60	7949543
			Regression (r^2)	0.9998
			The slope of the regression line	129669
			Y-intercept	355567

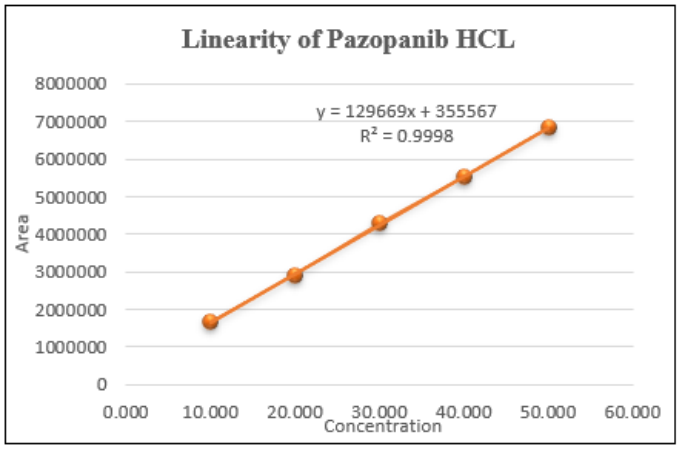

Figure 4. Linearity Plot.

Limit of detection (LOD) and Limit of quantitation (LOQ)

$$LOD = 3.3(Sy/S) = \frac{3.3\ X\ 69286}{127010} = 1.80\mu g/ml$$

$$LOQ = 10(Sy/S) = \frac{10\ X\ 69286}{127010} = 5.45\mu g/ml$$

The values obtained for the Limit of Detection (LOD) and Limit of Quantitation (LOQ) indicated that the method was susceptible to detecting and quantifying the drug.

Accuracy (Recovery)

Accuracy was determined over the range of 50% to 150% of the sample concentration. The accuracy of the analytical method expressed as % recovery determines the degree of closeness between the obtained and the true values. The overall % recovery was observed to be in the range of 102-102.7% for Pazopanib HCL.

Table 3. Results for Accuracy.

Theoretical Conc. (µg/ml)	Area Obtained	Recover Conc. (µg/ml)	% Recovery
30	3913217	30.81	102.7
40	5182008	40.80	102.0
50	6500033	51.17	102.3

Precision

Precision is expressed as % RSD, and % RSD NMT 2% is considered acceptable. Since the % assay and % RSD results were well within the acceptable range, the method is reproducible and accurate. The proposed method was found to have an overall % RSD for intra-day and inter-day precision of less than 2%.

Robustness

Small and purposeful changes to chromatographic conditions and assay settings were made to test this. These modifications affected system appropriateness and injection results for standard and sample solutions. Table 6 shows that wavelength and flow rate adjustments had no influence on retention time, theoretical plate, or asymmetry. This indicated that the analytical approach was reliable and could analyze both substances simultaneously. The created approach also satisfied the restrictions.

Conclusion

A simple specific precise and accurate RP-HPLC method was developed in this proposed development study for the estimation of Pazopanib HCl in pharmaceutical Dosage form. The developed HPLC method has a considerably shorter run time, less time required to prepare solutions and is easy to use and its simplicity. All the chromatographic parameters were optimized for a rugged and robust analytical method to optimise chromatography. The method was validated per regulatory requirements and ICH guidelines proposed method regarding linearity, accuracy, precision, and reproducibility. The validation data shows that the developed method has good reproducibility, and the RP-HPLC method is accurate, precise, specific, reproducible, and sensitive. The proposed method can be used for the routine analysis and quality control assays of Pazopanib HCl in drug samples and its dosage form. This method is recommended for future bioanalytical analyses because it can be easily modified to estimate Pazopanib HCl in various biological samples. The method that was developed for Pazopanib HCl is simple, rapid, cost-effective, and can be commercially used in industry.

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Table 4. Results for Intraday precision.

Sr. No.	Concentration	Area			Area Mean	% RSD
		Set I	Set II	Set III		
1	15µg/ml	2330687	2358932	2338470	2342696	0.62%
2	35µg/ml	4722101	4747668	4805400	4758389	0.89%
3	55µg/ml	7467742	7470091	7469814	7469215	0.02%

Table 5. Results for Inter day precision.

Sr. No.	Concentration	Area			Mean Area	% RSD
		Day 1	Day 2	Day 3		
1	15µg/ml	2330687	2314146	2343013	2329282	0.62%
2	35µg/ml	4722101	4738335	4807831	4756089	0.95%
3	55µg/ml	7467742	7475422	7468926	7470696	0.055%

Table 6. Results for Robustness.

Change in parameter	Condition	Area	Absolute difference of % Assay
Control	As per method	2834987	NA
Change in flow rate 1.0 ml/min (±0.1 ml/min)	0.9 ml/min	2313832	0.74
	1.1 ml/min	2805403	1.04
Change in wavelength (±2 nm)	213 nm	2838271	-0.11
	217 nm	2831863	0.11

Conflict of Interest

The authors declare no known conflict of interest for this article.

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