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A New RP-HPLC method for stability indicating assay of pazopanib hydrochloride in tablet dosage form: method development, validation, and degradation kinetics

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Abstract: This study reports the development and validation of a novel, rapid, and stability-indicating reversed-phase high-performance liquid chromatography (RP-HPLC) method for the quantification of pazopanib in tablet formulations. Chromatographic separation was achieved on an Inertsil ODS-3V column (150 mm × 4.6 mm, 5 µm) using an isocratic mobile phase of 0.1% trifluoroacetic acid and acetonitrile (70:30, v/v) at a flow rate of 1.0 mL/min with UV detection at 268 nm. The method demonstrated excellent resolution of pazopanib from its degradation products under forced degradation conditions, including acid/base hydrolysis, oxidation, photolysis, thermal, and humidity stress, confirming its stability-indicating capability. Validation in accordance with ICH Q2(R1) guidelines showed linearity over 25.71, 51.41, 82.26, 102.82, 123.38, and 154.23 µg/mL ($r^2 > 0.999$), precision with %RSD < 2.0, accuracy with recovery > 99%, and robustness under deliberate variations in chromatographic parameters. The limit of detection (LOD) and limit of quantification (LOQ) confirmed high sensitivity, and solution stability studies established analyte stability over 24 hours at 25 °C. With a runtime of 20 minutes and cost-efficient operation, this RP-HPLC method provides accurate, precise, and selective quantification of pazopanib in pharmaceutical dosage forms.

Keywords: Pazopanib hydrochloride; stability indicating RP-HPLC method; forced degradation study; assay; analytical method validation.

1. Introduction

Pazopanib, originally developed by GlaxoSmithKline, is a multitargeted tyrosine kinase inhibitor (TKI) with significant antitumor potential [1]. It has received regulatory approval from both the US FDA and the European Union for the management of advanced renal cell carcinoma (RCC) as well as advanced soft tissue sarcoma (STS). The drug demonstrates inhibitory effects against a wide variety of receptors, which include factors like vascular endothelial growth factor receptors (VEGFR-1, -2, 3), platelet-derived growth factor receptors (PDGFR-α, PDGFR-β), fibroblast growth factor receptors (FGFR-1, FGFR-3), as well as other kinases such as Kit, interleukin-2 receptor, inducible T-cell kinase (Itk), leukocyte-specific protein tyrosine kinase (Lck), and colony-stimulating factor receptor (c-Fms) [2]. Pazopanib hydrochloride in active pharmaceutical ingredient form exists as a white to yellow crystalline

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powder with low solubility, hence classifying it as a Biopharmaceutics Classification System (BCS) class II drug. Pazopanib's formulation is commercially marketed under the brand name VOTRIENT (Novartis), which is available in tablet forms of 200 mg and 400 mg for oral administration, with a maximum recommended daily dose of 800 mg. Additionally, apart from the active pharmaceutical ingredient (API), excipients such as magnesium stearate, microcrystalline cellulose, povidone, sodium starch glycolate, and film-coating agents may vary depending on the dosage strength present in these tablets. The adverse effects of Pazopanib that are reported include hepatotoxicity, hypertension, hypothyroidism, arterial thromboembolism, and genotoxicity, some of which may arise from impurities present in the formulation or drug substance [3]. A detailed study of pazopanib and its metabolites in various biological specimens was performed and published, utilizing multiple analytical techniques. One of the methods employed was a UPLC-MS/MS technique that was established and validated for monitoring pazopanib and its metabolites in clinical samples, using in-house metabolites produced through microsomal incubations [2]. The other was, oxidation of Pazopanib, catalyzed by metalloporphyrins, leading to metabolite formation, and the primary resulting metabolites were analyzed using the LC-MS method [4]; For the determination of Pazopanib along with other tyrosine kinase inhibitors in different plasma various LC-MS/MS assay methods were reported [5-10]; The analysis of Pazopanib along with other antitumor agents in the human plasma was performed by an established and validated micellar liquid chromatographic technique [11]. An HPLC-DAD technique was developed for the simultaneous measurement of four tyrosine kinase inhibitors in various human plasma samples [12,13]; There are some reports also available for synthesis pathway, process related impurities route synthesis and, manufacturing control of genotoxic impurities in API: Numerous potential impurities have been suggested, developed, and characterized using various analytical methods [1,14]; A study was established for control of process related genotoxic impurities by impurity fate mapping in manufacturing process of Pazopanib API [15]; A UPLC-Q-TQF/MS along with *in-silico* toxicity predictions was used to conduct a forced degradation investigation on pazopanib hydrochloride drug compounds in solution and its proposed degradants [16]. A few analytical methods were published for the Pharmaceutical Dosage form of Pazopanib: An HPLC-UV method was developed and validated for the determination of pazopanib in bulk drug, tablets formulation, and in human plasma [17]; Quality by Design based RP-HPLC Method was developed and validated for Simultaneous Estimation of Pazopanib in Bulk and Pharmaceutical Dosage Forms [18]; A Capillary zone electrophoresis method was developed and validated for separation of Pazopanib with other anticancer drugs in pharmaceutical formulation [19]. Although pazopanib hydrochloride has been previously subjected to stability studies, the analytical scope and intended application of the present work are fundamentally different from those in earlier reports. A previously published study by us [20] described a gradient RP-HPLC method with an extended run time of approximately 50 min, employing a complex mobile phase system for comprehensive impurity profiling and structural elucidation of degradation products. Comparison of chromatographic conditions and sensitivity of reported methods for Pazopanib is summarized in Table 1. The investigation was primarily research-oriented and focused on the identification and characterization of five novel oxidative degradants using advanced hyphenated and spectroscopic techniques, including LC–MS/MS, FT-IR, and NMR. In contrast, the objective of the present study was to develop a rapid, simple, and reliable isocratic RP-HPLC method with a significantly shorter run time (~20 min), specifically tailored for routine quality-control analysis of pazopanib tablets. As per the literature survey, there is no comprehensive study that has been conducted to identify, quantify, and perform forced degradation studies on pazopanib in pharmaceutical dosage form using the RP-HPLC method. This study aims to establish a reliable and specific stability-indicating chromatographic method for separating and quantifying pazopanib in tablet form. The structure of Pazopanib Hydrochloride is as given below in Figure1 [21-23].

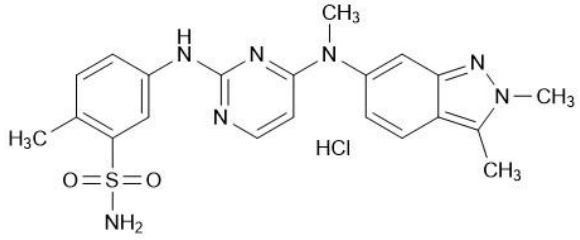

Figure 1. Structure of pazopanib hydrochloride

2. Experimental

2.1. Standards, Chemicals, and Reagents

The reference standards of Pazopanib, possessing a purity of approximately 99.2% were procured from Zydus Lifesciences Limited, situated in Ahmedabad, Gujarat, India. The chemicals and solvents utilized in this experiment were HPLC grade. Merck (Darmstadt, Germany) provided hydrochloric acid, trifluoracetic acid, sodium hydroxide, hydrogen peroxide, orthophosphoric acid, and acetonitrile. The Millipore Milli-Q water purification system, based in Bedford, MA, USA, produced HPLC-grade water.

2.2. Chromatographic Conditions, Equipment, and Software

Chromatographic separation characterized by exceptional resolution between the analyte peak and impurities was accomplished employing an Inertsil ODS-3V column (150mm x 4.6mm, 5µm particle size) manufactured by G.L. Sciences Ltd, Japan. The estimation and quantification of Pazopanib along with its degradants was performed using an Agilent HPLC system (1260 series), which comprised a quaternary solvent manager, an autosampler, and a diode array detector. The output signals in the form of chromatograms were monitored and processed utilizing the Chromeleon software (version 7.2, Dionex). The mobile phase containing 0.1% TFA buffer and Acetonitrile in a ratio (70:30, % v/v) with a flow rate of 1.0 mL/min for 20 minutes using Isocratic elution mode was employed. The temperature of the column oven was maintained at 45°C, and an injection volume of 5µL. The detection wavelength selected was 268 nm to achieve optimal response for the analyte. To prepare solutions, a diluent of water, acetonitrile, and orthophosphoric acid in a 50:50:0.1% v/v/v ratio was used because of its high solubility and stability.

Table 1. Comparison of chromatographic conditions and sensitivity of reported methods for pazopanib

No	Method	Column	Mobile Phase	Run time (Mins)	LOD/LOQ (µg/mL)	Ref.
1	UHPLC Q-TOF MS	Waters Acquity UPLC HSS T3 (100 × 2.1mm, 1.7 µm)	M.P: A- pH 5.0 ammonium acetate buffer M.P: B- Acetonitrile	7	1.38/4.06	[16]
2	HPLC-UV	Nucleosil CN (250 × 4.6mm, 5.0 µm)	pH 4.5 Sodium Acetate buffer: Acetonitrile (40:60%v/v)	8	0.27/0.82	[17]
3	RP-HPLC	Phenomenex Enable C18 column	pH 5 Phosphate buffer: Acetonitrile (40:60%v/v)	5	0.011/0.032	[18]
4	RP-HPLC	YMC Triart C18 (250mm x 4.6mm) 5µm	M.P: A- 0.1% PCA buffer: THF (95:05 %v/v) M.P: B- 0.1% PCA buffer, THF: methanol: acetonitrile (10:10:10:70 v/v/v/v, %v/v).	50	0.15/0.50	[20]
5	RP-HPLC	Inertsil ODS-3V column (150mm x 4.6mm, 5µm	M.P: 0.1 % TFA buffer and Acetonitrile (70:30, %) isocratic	20	0.12/0.36	Current Studdy

2.3. Standard and Sample Preparation

Standard and sample solutions for Pazopanib were meticulously prepared in a diluent at concentrations of 100 µg/mL. Initially, 20 intact Pazopanib hydrochloride tablets were weighed, and their average weight was obtained. Subsequently, weigh 5 tablets and transfer them into a 500 mL volumetric flask. Approximately 350 mL of diluent was added, and the mixture was sonicated for 30 minutes with intermittent shaking every 5 minutes for 30 seconds. The volume was adjusted up to the mark with diluent and thoroughly mixed; then 5 mL of this solution was diluted to 100 mL with solvent and mixed. The resulting solution was filtered using a 0.45µm Millipore PVDF syringe filter, after removal of the first 5 mL of filtrate.

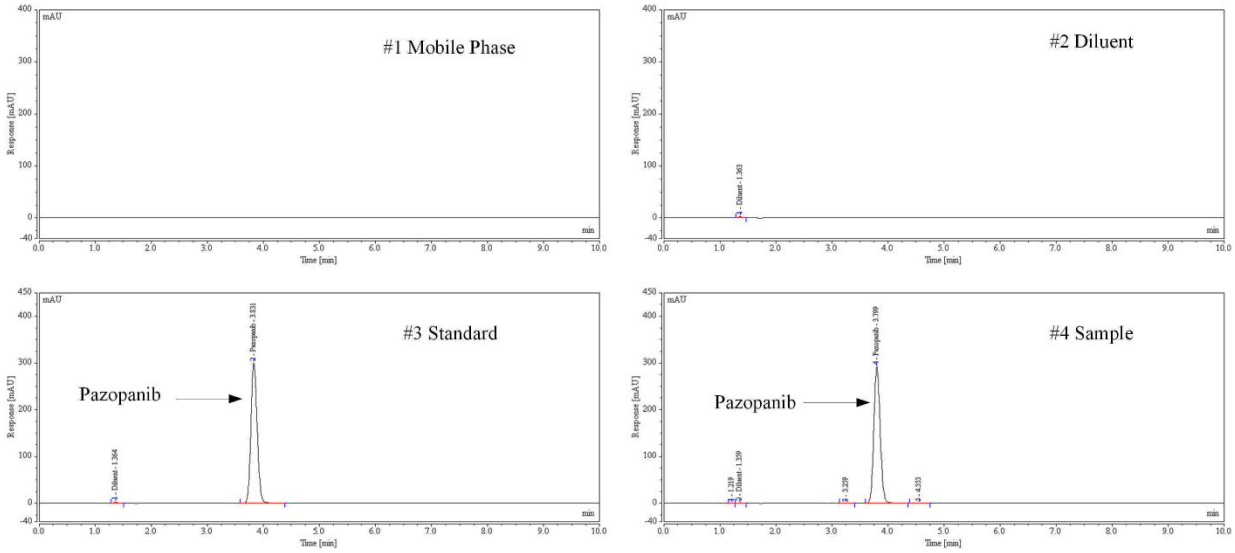

Figure 2. HPLC chromatograms of (a) Mobile phase preparation, (b) Diluent preparation, (c) Standard preparation, and (d) Sample preparation

For performing alkaline hydrolysis, the sample was treated with 5 mL of 1 N sodium hydroxide solution at 90°C for 3 hours. Subsequently, the above solution was neutralized by adding 5 mL of 1 N hydrochloric acid solution. Acid hydrolysis was induced by adding 5 mL of 5 N hydrochloric acid solution at a temperature of 90°C for 3 hours, then neutralizing with 5 mL of 5 N sodium hydroxide solution. For oxidative degradation, the sample was subjected to a temperature of 90°C for 3 hours by adding 10 mL of 30% hydrogen peroxide solution. For inducing thermal degradation, all samples were subjected to a hot air oven at 100°C for 5 days. To perform humidity degradation, the samples were placed in a humidity chamber at 50°C/80%RH (Relative Humidity) for 5 days. Photolytic degradation was performed by subjecting the samples to light and UV radiation in a photolytic chamber, with a total exposure of 2,968,040 Lux hours and 103,959 watt-hours per square meter [24, 26-28].

The chromatograms demonstrate the various stages of the analysis, which include the mobile phase, blank preparation, standard solution, sample solution, and degradation solution, are provided in Figures 2-4.

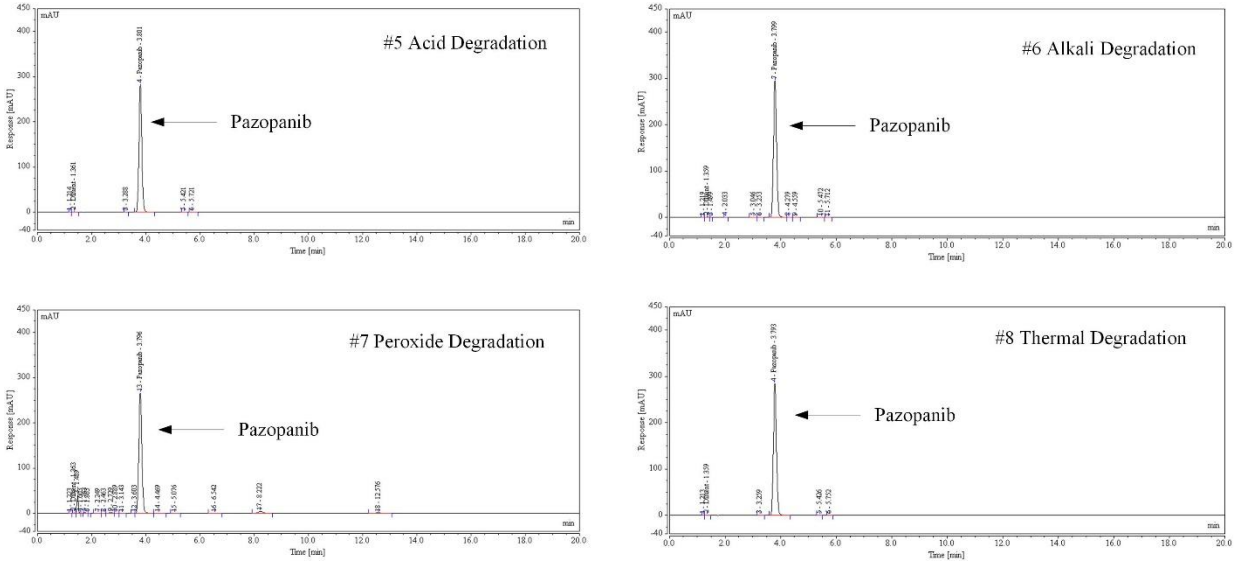

Figure 3. HPLC chromatograms of (a) acid degradation, (b) alkaline degradation, (c) peroxide degradation, and (d) thermal degradation

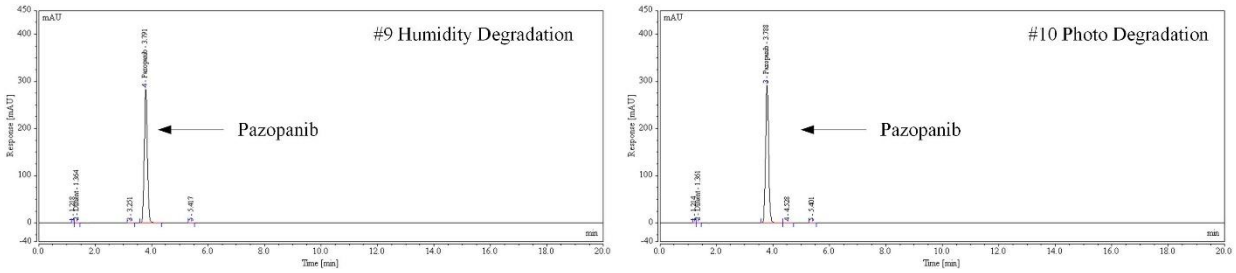

Figure 4. HPLC chromatograms of (a) humidity degradation and (b) photo degradation

2.4. Method Validation

Analytical method validation involves evaluating a method's suitability and continuous reliability for its intended application throughout the drug's lifecycle. Validating an analytical method requires ensuring that it consistently produces accurate results that are unaffected by external influences, which includes assessing a variety of performance parameters, including precision, accuracy, specificity, selectivity, linearity, robustness, and ruggedness, as well as establishing limits of detection (LOD) and quantification (LOQ). The validation of the analytical method mentioned here followed the guidelines provided by the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) in document Q2 (R1) [25].

2.4.1. System Suitability and Precision

System suitability in terms of chromatography refers to evaluating the system's resolution, column efficiency, and repeatability to ensure its appropriateness for a given analysis. In this experiment, the system suitability was demonstrated using five replicate injections of a standard solution harnessing the proposed approach. Later, to evaluate the system's performance, the chromatograms were analyzed.

2.4.2. Linearity

Linearity is the ability of a method to give test results directly proportional to the analyte concentration for a given range or through mathematical transformation. To determine the linearity of the method, a

minimum of five calibration points, within a defined range, were set up and plotted using a precise mathematical transformation technique. For the calculation of Pearson's correlation coefficient, which is the measure of the strength and direction of the linear relationship between the concentration of analytes and the corresponding test results, the regression equation derived using the least squares method was employed.

2.4.3. Establishment of Limit of Detection (LOD) and Limit of Quantification (LOQ)

In analytical chemistry, the Limit of Detection (LOD) and the Limit of Quantification (LOQ) are important parameters that determine the lowest concentration or amount of an analyte that can be detected and accurately quantified using an analytical method. LOD is the lowest concentration or amount of an analyte in a sample that may be steadily detected with a given level of confidence. Whereas LOQ is the lowest concentration or amount of an analyte in a sample that can be accurately quantified with a specified level of precision and accuracy. LOQ is a crucial parameter for assuring that the analytical data is reliable and valid and is used to determine the lowest concentration at which a sample can be quantified within accepted limits of error. To confirm the LOD and LOQ values for Pazopanib, a factor from the standard deviation of the response in relation to the slope of the calibration curve exhibiting linearity was obtained.

2.4.4. Precision

A crucial parameter in analytical chemistry to examine the repeatability and reproducibility of an analytical procedure is Precision. It assesses the degree of agreement or variation in the findings obtained from repeated measurements of the same sample with the same procedure under the same circumstances. To validate the precision of the developed method, intraday and interday precision attributes were evaluated. Intraday precision is used to examine the method's performance over a single day, for which six sample solutions from a homogeneous batch were analyzed independently using the suggested analytical approach. Inter-day precision helps to analyze the method's consistency over different days and instrumentation configurations. Various analyzers were used with different HPLC instruments and column batches, and on separate days, six independent sample solutions were injected and tested. Precision studies ensure the analytical method's reliability and robustness by analyzing the agreement and variation in the results.

2.4.5. Accuracy

Accuracy is an important parameter for analytical method validation as it examines the extent to which the results obtained from an analytical method match the true or known value of the analyte being measured. It is obtained by the nearness between the measured value and the reference value of the analyte. To test the analytical method's accuracy for this research, a recovery study was utilized. Pazopanib was added to the placebo sample at established levels to generate recovery sample solutions. Pazopanib was spiked at the 50%, 100% and 150% levels in the solution. Measured Pazopanib levels in spiked solutions were compared to solutions with known added amounts to determine the accuracy of the analytical method.

2.4.6. Robustness

Robustness includes intentionally making slight, deliberate changes to method parameters and comparing the findings to the original conditions, which is helpful to prove the robustness of the analytical method. In this research, robustness was evaluated by purposefully modifying the chromatographic conditions, which included changes in the flow rate (0.9 and 1.1 mL/min), column oven temperature (40°C and 50°C), and the composition of the buffer to solvent phase (± 2% absolute solvent ratio). The impact of assessing these deliberate modifications aided in the evaluation of the robustness of the analytical method.

2.4.7. Solution Stability

Solution stability is an important parameter to be considered for the development and validation of an analytical method. It refers to a solution's capacity to preserve its chemical and physical properties over time. The study assessed the stability of standard and sample solutions at regular intervals by determining the percent relative standard deviation (%RSD) for Pazopanib's peak response. The observation showed unchanged chromatographic conditions, and later chromatograms of all samples were analyzed for contamination caused by interactions between the analyte and placebo matrix during solution stability.

2.4.8. Specificity

Specificity refers to its capacity to distinguish and selectively quantify the target component in complicated product mixes containing other interfering substances/components. To validate the analytical method's capacity to indicate stability, forced degradation studies were used, which are essential in assessing the potential impurities that may arise throughout the entirety of a drug product's lifecycle. The structure of potential degradation products that may be encountered during accelerated and long-term stability analysis was influenced by the experimental design of forced degradation studies. These studies aid in selecting appropriate manufacturing procedures and excipients for creating stable dosage forms [10,11]. Forced degradation studies were performed on the tablet dosage form under various stress conditions, including acid and alkaline hydrolysis, oxidative, photolytic, thermal, and humidity stress. The degraded samples were injected into both assay and related-substances chromatographic systems [20] at the defined sample concentration to accurately determine mass balance. Additionally, the chromatographic run time for the assay method was extended to 20 min to ensure adequate retention and detection of unknown degradation peaks.

3. Results and Discussion

3.1. Optimization of Chromatographic and System Suitability Conditions

The chromatographic approach intends to separate active components, contaminants, and placebo influence. An essential aspect of the stability indicating method is achieving the separation and resolution from unknown impurities. Initially, the analytical method development for the separation of Pazopanib commenced by employing a mobile phase consisting of pH 6.5 buffer and methanol as solvents. Considering Pazopanib's pKa value of 5.07, the pH of the buffer was selected to be 6.5. It was determined that maintaining the mobile phase pH close to the drug substance's pKa value resulted in poor peak shape and inadequate ionization, leading to run-to-run variability [29,30].

The ACE 5 C18 column was chosen as the stationary phase for its easy availability, selectivity, and pH compatibility. However, development trials on this column were discontinued due to the unsatisfactory peak shape of Pazopanib. Optimization endeavors were undertaken by employing diverse C18 column brands, namely Hypersil-ODS, Kromasil, and Phenomenex, to attain satisfactory peak shape and separation from impurities. Unfortunately, these efforts did not yield any enhancements in the outcomes. To assess the impact of buffer pH on contaminants and analyte retention time, the pH was modified. When the sample was injected with a buffer pH of 3.0, co-elution of an unknown peak with the main peak was observed, along with an asymmetric peak of pazopanib. To address this, 0.1% TFA was incorporated as an ion-pairing reagent to enhance selectivity and achieve good resolution between components. The organic phase consisted of acetonitrile, which yielded promising results.

To meet system suitability criteria, additional experiments were conducted, modifying the flow rate, solvent composition, and column oven temperature. A satisfactory resolution was accomplished between Pazopanib and impurities with symmetric peak shapes using a mobile phase composition of 0.1% TFA buffer and Acetonitrile (70:30, v/v%) as mobile phase, on an Inertsil OSD-3V column (150mm × 4.6 mm, 5 µm). The flow rate was set to 1.0 mL/min, and the column oven temperature was kept at 45°C [31,32].

The pre-optimized chromatographic conditions were implemented on an Inertsil OSD-3V column (150mm × 4.6 mm, 5 µm) - a hybrid silica-based ODS column known for its high carbon loading and suitability for low pH conditions. The sample solution was injected to determine its impact on the stationary phase. The retention times for Pazopanib were determined at approximately 3.83 minutes. Notably, the peak shapes for Pazopanib exhibited significant enhancement, leading to a resolution exceeding 2.0 between adjacent compounds during forced degradation. Pazopanib indicated over 5000 theoretical plates with a tailing factor below 1.1. The proposed method underwent validation in compliance with current ICH stipulations [33], and the results about system suitability are outlined in Table 2.

Table 2. System suitability/system precision

Name	**System suitability parameters**		
	Peak Area	**Theoretical plates**	**Tailing factor**
Pazopanib	2411.243	5131	1.1
	2411.303	5082	1.1
	2418.406	5104	1.2
	2420.518	5091	1.2
	2424.874	5106	1.1
	2411.243	5131	1.1
Average	2416.265	5107.50	1.1
%RSD	0.243	0.395	4.56

3.2. Method Validation

3.2.1. Specificity

The analytical method's specificity was verified by forced degradation trials, which assessed potential interferences from the diluent and placebo at active component retention durations. The stability of Pazopanib was observed during acid, alkaline, thermal, and UV degradation experiments. However, degradation was successfully achieved through peroxide degradation, using 10mL of 30% hydrogen peroxide with heating in a water bath at 90°C for 3 hours. Peak purity analysis revealed that the degradation samples' purity values were above 990, indicating no co-eluting peaks or interference. The total percentage of impurities was determined using the related-substances chromatographic method previously reported [20]. The percentage mass balance, calculated as the sum of total impurities and assay, was found to be greater than 95% under all degradation conditions. Chromatograms depicting the forced degradation studies can be found in Figures 3 to 4. The results of the specificity and forced degradation studies are summarized below.

The unstressed sample showed a total impurity level of 0.09% with an assay of 100.2%. Acid degradation carried out using 5 mL of 5 N hydrochloric acid at 90 °C for 3 h resulted in 0.13% total impurities and an assay of 99.2%, yielding a mass balance of 99.3%. Alkaline degradation performed with 5 mL of 1 N sodium hydroxide at 90 °C for 3 h showed 0.27% total impurities and an assay of 97.4%, with a mass balance of 97.7%. Oxidative degradation using 10 mL of 30% hydrogen peroxide at 90 °C for 3 h produced the highest level of degradation, with total impurities of 11.3% and an assay value of 85.6%, resulting in a mass balance of 96.9%. Thermal degradation, achieved by storing the sample in a hot air oven at 100 °C for 5 days, showed 0.10% total impurities and an assay of 98.9%, corresponding to a mass balance of 99.0%. Humidity stress conducted at 50 °C and 80% RH for 5 days resulted in 1.1% total impurities and an assay of 98.4%, with a mass balance of 98.5%. Photolytic degradation performed in a photostability chamber for 2,968,040 lux hours and 103,959 Wh/m² showed minimal degradation, with total impurities of 0.07% and an assay of 99.1%, giving a mass balance of 99.2% as mentioned in Table S1.

3.2.2. Limit of Detection (LOD) and Limit of Quantification (LOQ)

To determine the limit of detection (LOD) and limit of quantitation (LOQ) for an analyte, a factor was calculated by dividing the standard deviation of the response by the slope of the linear calibration curve. [34-39]

3.2.3. Linearity

Linearity, defined as the ability of an analytical method to elicit test results that are directly proportional to the concentration of analyte within a given range, was evaluated for pazopanib across six concentration levels corresponding to 25–150% of the nominal sample concentration. Calibration solutions were prepared in the concentration range of 25.7050–154.2301 µg/mL. The peak area response showed a consistent and proportional increase with concentration, yielding mean peak areas of 598.612 ± 1.668 at 25.7050 µg/mL (25%), 1205.841 ± 2.730 at 51.4100 µg/mL (50%), 1926.579 ± 1.926 at 82.2560 µg/mL (80%), 2409.473 ± 2.548 at 102.8201 µg/mL (100%), 2907.539 ± 1.396 at 123.3841 µg/mL (120%), and 3611.099 ± 0.942 at 154.2301 µg/mL (150%) as shown Table S2. Regression analysis was performed using the least squares method, resulting in a correlation coefficient (r) of 1.0000 and a coefficient of determination (R^2) of 1.0000, indicating excellent linearity over the evaluated range. The calibration curve exhibited a slope of 23.4832 with a y-intercept of 2.8695, and the y-intercept bias at the 100% concentration level was 0.12 as shown in Table S3. These results confirm that the method is linear for the quantification of pazopanib within the established range of 25.71–154.23 µg/mL as represented in Figure S1 [34-39].

3.2.4. Precision

This study aimed to assess the repeatability and reproducibility of the analytical method for a homogeneous sample. The intra-day precision investigation involved the analysis of %RSD values for Pazopanib derived from six individual sample preparations. To determine the ruggedness of the analytical method, six sample preparations were injected on a distinct HPLC instrument (Shimadzu LC-2050C - quaternary solvent manager with rinse solution unit, an autosampler, UV detector with simultaneous monitoring of 2 wavelengths) by another analyst on different days, employing different batches of HPLC columns. During the intra-day precision study, the %RSD value for the analyte was determined to be below 5.0%, and the inter-day precision study provided further evidence of the method's ruggedness, as shown in Table 3. [34-39]

3.2.5. Accuracy

This parameter was established to evaluate the drug's extraction efficacy in a diluent at different concentrations. The method’s accuracy was tested by assessing placebo samples spiked with pazopanib at three distinct concentration levels, from 50% to 150% of the sample concentration. The %recovery for pazopanib at all concentration levels exceeded 90%, signifying the accuracy of the proposed method in quantifying Pazopanib in drug products. The accuracy study's method validation results can be found in Table 3. [34-39]

Table 3. Precision and accuracy data

Name	**Parameters**				
	Intraday Precision Assay (n=6) (%RSD)	**Inter-day Precision Assay (n=6) (%RSD)**	**%Recovery [a]**		
			50% [b]	**100% [b]**	**150% [b]**
Pazopanib	100.8 (0.6)	100.2 (0.9)	99.6 (0.3)	99.8 (0.2)	99.8 (0.2)
Acceptance criteria	< 2.0	< 2.0	98.0-102.0	98.0-102.0	98.0-102.0

[a] Mean (% RSD) for three determinations at each level were given in parenthesis.
[b] Amount of Pazopanib API Spiked to placebo.

3.2.6. Robustness

Robustness testing was performed in accordance with ICH Q2 (R1) guidelines to determine the reliability of the developed RP-HPLC method under small but deliberate variations in chromatographic conditions. The selected parameters- flow rate, column temperature, and organic phase composition were chosen because they represent the most common sources of minor variability in routine laboratory analysis and could potentially influence retention time, peak shape, and quantification of Pazopanib. [34-39] To justify the robustness of the method, the following observations were made based on the results:

3.2.6.1. Flow Rate Variation (±10%)

Although the retention time shifted proportionally with changes in flow rate (3.60 min at +10% and 4.41 min at –10%), the peak area, tailing factor, and theoretical plates remained within acceptable limits. The %RSD values (0.17–0.26%) indicate consistent quantification and no significant effect on accuracy or precision.

3.2.6.2. Temperature Variation (±5°C)

Small changes in temperature resulted in slight variations in retention time (3.81–4.12 min), but chromatographic performance remained stable, with tailing factor consistently at 1.1 and theoretical plates remaining >4900. These results demonstrate that the method is not sensitive to minor temperature fluctuations that commonly occur in laboratory environments.

3.2.6.3. Organic Phase Composition Variation (±2%)

As expected, slight changes in organic composition affected retention time (3.26 min at +2% and 5.13 min at −2%). However, the peak area remained consistent (2413.15–2420.70), tailing factor stayed at 1.1, and theoretical plates remained above 4300, confirming that the assay performance was unaffected. This highlights the method's resilience to solvent composition variability during mobile phase preparation.

Across all tested conditions, system suitability parameters remained well within acceptable limits, and no significant deviations were observed in assay results. The %RSD values for peak area remained below 0.30%, demonstrating excellent reproducibility. These results collectively justify that the developed RP-HPLC method is robust. Minor deliberate variations in flow rate, column temperature, and organic composition do not impact the accuracy, precision, or overall chromatographic performance of

pazopanib. This ensures method reliability during routine quality-control analysis. The proposed method exhibits robustness as it satisfies system suitability criteria under all altered circumstances, as documented in Table 4.

Table 4. Summary of the Robustness study during method validation

Condition	RT Pazopanib (min)	Area Mean ± SD	%RSD	Tailing factor of Pazopanib	Theoretical plates of Pazopanib
Optimized condition	4.02	2416.13 ± 4.14	0.17	1.1	5131
Flow rate, +10%	3.60	2449.20 ± 6.45	0.26	1.1	4828
Flow rate, -10%	4.41	2384.10 ± 3.95	0.17	1.2	5285
Column temperature, +5°C	3.81	2412.53 ± 3.94	0.16	1.1	4988
Column temperature, -5°C	4.12	2422.57 ± 5.40	0.22	1.1	5085
Organic composition (+2%)	3.26	2413.15 ± 6.38	0.26	1.1	4384
Organic composition (-2%)	5.13	2420.70 ± 3.17	0.13	1.1	5804

3.2.7. Solution Stability

To assess the stability of the drug during experimentation, the drug solution was analysed at predetermined intervals over 24 hours while maintained at an auto-sampler temperature of 25 °C. Our observations revealed that the percentage level of Pazopanib within the as such sample solution did not decrease during this timeframe. Additionally, the analyte's peak response in the standard and sample preparation remained within an acceptable range for 24 hours at a temperature of 25 °C in the auto-sampler. The results indicate that both the standard and sample preparations remain stable for a duration of 24 hours at an auto-sampler temperature of 25 °C.

4. Conclusions

The current study is report that deals with the development and quantification of a simple, novel, rapid, sensitive, selective, and cost-efficient stability-indicating reversed-phase high-performance liquid chromatography (RP-HPLC) method for assessing Pazopanib in tablet formulations. The proposed method was successfully validated in accordance with the prevailing International Council for Harmonisation (ICH) guidelines. Validation results indicate excellent separation between adjacent impurities from the main component throughout the forced degradation analysis, confirming the stability-indicating nature of the developed method. The proposed analytical method exhibits rapid performance, demonstrates linearity (with correlation coefficients exceeding 1.00 and y-bias -0.12), showcases precision (with intra-day and inter-day precision achieving %RSD values below 2.0), demonstrates accuracy (with analyte recovery exceeding 99.0%), proves specificity (as no interference was detected during forced degradation), and exhibits robustness (as the system suitability criteria were fulfilled under various altered conditions). The RP-HPLC method was successfully developed and validated for routine quality-control analysis of pazopanib tablets. The method demonstrates novelty by serving as a stability-indicating assay with short run time and reliable quantification of pazopanib hydrochloride and its degradation behaviours, which has not been previously reported. This study is clearly distinct from earlier work that focused on impurity profiling, as the present investigation is dedicated to assay quantification and stability assessment of the active drug. All chromatographic, validation, and degradation data reported here are newly generated, ensuring the originality and independent contribution of this method to the existing literature.

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Conflict of Interest

Conflict of interest: The authors affirm that no conflicts exist about the dissemination of this research manuscript.

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