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# RP-HPLC BASED DEVELOPMENT AND VALIDATION FOR PAZOPANIB IN BULK AND PHARMACEUTICAL DOSAGE FORM USING QUALITY BY DESIGN

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## ABSTRACT

**Objective:** Development of an accurate, sensitive, precise, robust, economical and quick isocratic reverse-phase high-performance liquid chromatography (RP-HPLC) method complying quality by design (QbD) and as per ICH guidelines for the analysis as well as quantification of Pazopanib in bulk and formulation. **Method:** The estimation of the Pazopanib with Dasatinib as an internal standard in bulk and pharmaceutical dosage forms by quality by designs, multicriteria decision-making approach. Chromatographic separations were carried out using Phenomenex® Gemini $C_{18}$ analytical column (150mm×4.6mm i.d., 5µm) and the mobile phase composed of potassium di-hydrogen ortho-phosphate (pH5) and acetonitrile at the ratio of 40:60% v/v with a flow rate of 1.2ml/min. the elutes were analyzed using PDA-UV detector at a wavelength of 290nm. The proposed method was developed and validated anticancer drug "Pazopanib" in pure form as well as pharmaceutical formulation. The method was optimized by utilizing Derringer's desirability functions. **Result:** In the present study, the chromatograms of Pazopanib showed a good resolution with a retention time of 2.19min. Pazopanib showed an excellent linearity with 0.998 of correlation coefficient. The detection (LOD) and quantification limits were 10.47ng/ml

and 31.74ng/ml, respectively. **Conclusion:** Thus the newly developed and validated method can be conveniently used for the quantification of Pazopanib in bulk and formulation.

**Keywords: Dasatinib, Pazopanib, Quality by Design, RP-HPLC, Response Surface Methodology, Tyrosine kinase inhibitors**

## INTRODUCTION

Pazopanib is a second generation tyrosine kinase inhibitor (TKI) **[1]**. It used for the treatment of ovarian, renal, colon, neck, 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 that several analytical methods have been developed for the estimation of Pazopanib in pharmaceutical dosage forms and biological samples including HPLC **[6, 7]**, simultaneous estimation of Pazopanib by HPLC **[8, 9]**. However, these possess multiple limitations 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. Hence, the present study aimed 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 respectively. 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-methyl benzene sulfonamide.

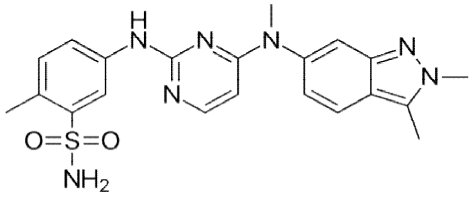


**Figure 1: Chemical structure of Pazopanib**

## 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 pore water purification system (Merk). Pazopanib HCl is commercially available as Votrient®

marketed by GSK $R_x$ India with a label claim of 200mg per tablet.

**Instrumentation**

The HPLC analysis was carried out using 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 0.1mg sensitivity, digital pH meter (DELUX model 101), a Sonicator (Systrinices, model 2200MH). Absorbance spectra were recorded using UV-VIS spectrophotometer (Systronices, India) employing a quartz cell of 1 cm of path length.

**Preparation of phosphate buffer pH 5**

Accurately weighed 0.68gm of potassium dihydrogen ortho phosphate and transferred into a 500ml volumetric flask. Added 400ml of Milli Q water, dissolved by sonication and the final volume was made up to 500ml using Milli Q water. The pH of the buffer solution was adjusted to 5±0.5 using diluted orthophosphoric acid. 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 Pazopanib into 10ml volumetric flask. Added 8ml of acetonitrile and sonicated for 5-10min. Finally the volume was made up with acetonitrile to achieve a final concentration of 1mg/1ml. 10µm/ml of working standard solution was prepared by taking suitable aliquot from standard stock solution and the volume was adjusted with acetonitrile.

**Selection of detection wavelength**

For RP-HPLC method, the analytical wavelength was determined from UV-spectra of Pazopanib and Dasatinib by using UV-VIS spectrophotometer. The solutions of both the drugs were scanned between 200 to 400nm against blank. Pazopanib and Dasatinib showed a significant absorbance at 290nm using PDA detector. The results are shown in **Figure 2**.

**Chromatographic Conditions**

The mobile phase was composed of phosphate buffer pH 5 and acetonitrile at a ratio of 40:60%v/v and the optimized chromatographic condition are shown in **Table 1**.

**Optimization of RP-HPLC-PDA method**

Initially, the trial and error method was applied to gain knowledge about the method performance as well as recognition of various vital independent variables and its effect on dependent variables. The Central-Composite design with response surface was employed

for the optimization of experimental conditions of the method. In the present investigation, experiments were planned and performed according to the Central Composite Design (CCD) **[11]**. In the proposed research, 20 experimental runs were performed and analyzed. The results of various parameters such as retention time, capacity factor, resolution factor and separation factors are in accord with the Central-Composite design. Further investigation was performed using response surface methodology (RSM) to evaluate the relationship between the dependent and independent variables using obtained data **(Table 2)**.

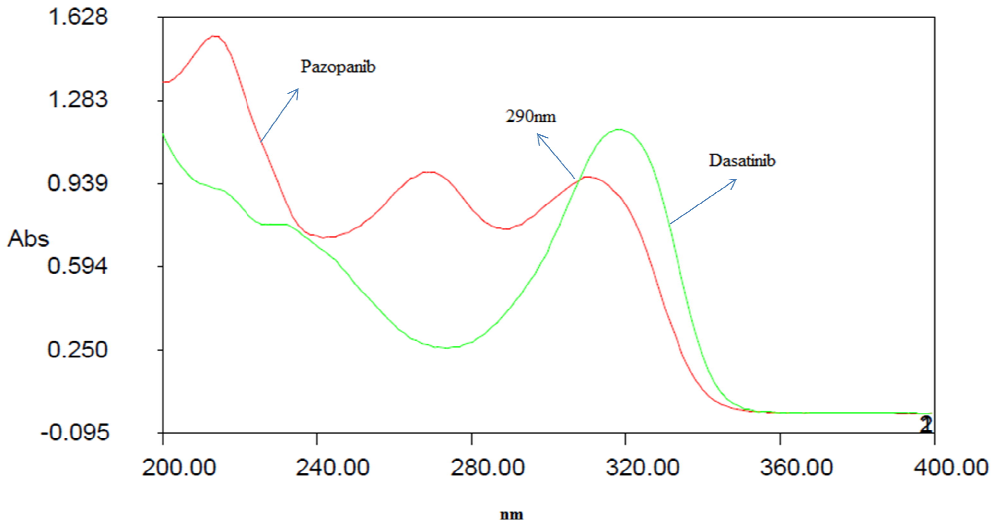



**Figure 2: UV spectra overlay of Pazopanib and Dasatinib**

**Table 1: Chromatographic conditions**

| Parameters | Methods |
|---|---|
| Stationary phase | Phenomenex enable $C_{18}$ column |
| Mobile phase | $KH_2PO_4$ buffer (pH 5): Acetonitrile (40:60) |
| Flow rate | 1.2ml/min |
| Run time (minutes) | 6 min. |
| Column temperature | Ambient |
| Volume of injection | 20µl |
| Detector | PDA |
| Detection of wavelength | 290nm |
| Drug $tR_2$ | 2.19minutes |

**Table 2: Experimental design and results of a central composite design**

| Run | Factor A (ACN %v/v) | Factor B (pH) | Factor C (Flow rate ml/min.) | Response 1 ($tR_2$) | Response 2 ($k_1$) | Response 3 ($R_{s\ 1,2}$) | Response 4 ($\alpha_{1,2}$) |
|---|---|---|---|---|---|---|---|
| 1 | 50 | 4 | 0.9 | 2.996 | 0.178 | 1.183 | 1.414 |
| 2 | 50 | 2.3 | 0.9 | 2.382 | 0.504 | 2.538 | 1.263 |
| 3 | 50 | 4 | 0.4 | 6.763 | 0.141 | 1.326 | 0.973 |
| 4 | 16 | 4 | 0.9 | 6.541 | 5.380 | 1.596 | 1.235 |
| 5 | 70 | 5 | 0.6 | 4.361 | 0.316 | 4.506 | 2.251 |
| 6 | 30 | 5 | 1.2 | 1.573 | 0.046 | 1.362 | 1.863 |
| 7 | 84 | 4 | 0.9 | 2.421 | 7.242 | 1.640 | 1.619 |
| 8 | 70 | 3 | 0.6 | 2.825 | 1.121 | 0.506 | 1.117 |
| 9 | 70 | 3 | 1.2 | 1.422 | 0.138 | 0.468 | 1.558 |
| 10 | 50 | 5.6 | 0.9 | 5.577 | 0.48 | 5.381 | 1.623 |
| 11 | 50 | 4 | 0.9 | 3.004 | 0.181 | 1.141 | 1.416 |
| 12 | 30 | 3 | 1.2 | 3.095 | 0.677 | 0.305 | 1.561 |
| 13 | 30 | 3 | 0.6 | 5.081 | 0.062 | 2.572 | 1.247 |
| 14 | 50 | 4 | 0.9 | 2.988 | 0.171 | 1.191 | 1.524 |
| 15 | 50 | 4 | 0.9 | 3.017 | 0.189 | 1.121 | 1.496 |
| 16 | 50 | 4 | 0.9 | 2.996 | 0.193 | 1.145 | 1.419 |
| 17 | 50 | 4 | 1.4 | 1.920 | 0.874 | 1.132 | 1.721 |
| 18 | 70 | 5 | 1.2 | 2.117 | 0.253 | 3.859 | 2.535 |
| 19 | 50 | 4 | 0.9 | 2.992 | 0.175 | 1.129 | 1.422 |
| 20 | 30 | 5 | 0.6 | 3.259 | 0.037 | 0.707 | 2.631 |

## METHOD DEVELOPMENT

The optimized chromatographic method was validated entirely as per ICH guidelines and Q2B. The calibration curves were tested using one-way ANOVA at 5% significance level. The model was also validated by analysis of variance (ANOVA) using design expert software, and the results are presented in **Table 3**. Based on press value, a quadratic model was selected for responses such as retention time, capacity, resolution, and separation factors of Pazopanib. The significant effects observed with a *p*-value less than 0.05, while the low standard deviation (% CV) and adjusted *R*-square value indicated a good relationship between the experimental data and those of the fitted model. The predicted *R*-square value was in acceptable concordance with the adjusted *R*-square value for all responses **(Table 3) [12]**. The normal probability plot shown in **Figure 3A** indicates that whether the residuals follow a normal distribution, in which case the points will follow a straight line. The points on this plot lies fairly close to the straight line, so the model seems appropriate. The plots of residuals versus predicted values are shown in **Figure 3B** which is a measure of how many standard deviations the actual value deviates from the value predicted. From this plot, it is possible to conclude that they were randomly distributed around zero and there is no evidence of outliers i.e. no point lies away from the mean value more

than three times the standard deviation. Since, the assumptions of normality and constant variance of the residuals were found to be satisfied, the fitted model for the $Rs_{(1,2)}$ can be accepted.

Response surfaces plots for $k_1$, $Rs_{(1,2)}$, $\alpha_{(1,2)}$ and $tR_2$ are illustrated in **Figure 4** (% ACN concentration was plotted against the pH. Flow rate held at constant at the center value). Analysis of perturbation as well as response plots of optimization models revealed that the factor A and B had the significant effect on separation of the analytes, whereas the factor C, i.e., the flow rate, is of less significance.

**Multi criteria decision making**

Derringer's desirability function was employed for global optimization of four responses and to select different optimal conditions for the analysis of drug.

**Optimal condition for formulation assay**

The criteria for the optimization of each individual response are shown in Table 4. Criteria have been optimized for selecting an optimum experimental condition for analyse routine quality control samples. As observed under criteria 1, the response $tR_2$ was minimized, in order to shorten the analysis time. On the other hand, $Rs_{1,2}$ was targeted at 5 to allow baseline separation of Pazopanib and Dasatinib. In order to separate the first eluting peak of Dasatinib from the solvent front, $k_1$ was targeted at 0.04. The importance ranged from 1 to 5, which gives emphasis to a target value. Following the conditions as well as restrictions as above, the optimization procedure was carried out and the global desirability function was calculated. From the results it was concluded that there was a set of coordinates producing high desirability value (D =0.814) when acetonitrile concentration of 60.430% v/v, pH of 5.0 and the flow rate of 1.2ml/min. The predicted response values corresponding to the later value of D where $k_1$ = 0.037, $Rs_{1,2}$ = 3.665, $\alpha_{1,2}$ = 2.059 and $tR_2$ = 2.194min. **(Table 5)**. Optimum conditions chosen for the assay were 0.01mM $K_2HPO_4$ (pH5) solution, acetonitrile (39.570:60.430 %v/v) and the flow rate of 1.2ml/min. The eluate was monitored using UV detector at 290nm. The prediction efficiency of the model was confirmed by performing the experiment under the optimal condition and the corresponding chromatograms are shown in **Figure 5**.

**Table 3: Response models and statistical parameters obtained from ANOVA for CCD**

| Responses | Regression model | Adjusted $R^2$ | Model *p*-value | % C.V | Adequate precision |
|---|---|---|---|---|---|
| $tR_2$ | +3.37-0.6745*A+0.312*B-1.13*C | 0.4866 | <0.0032 | 32.44 | 8.6771 |
| $k_1$ | +0.2536+0.3032*A-0.1017*B+0.0591*C-0.0046*AB-0.2091*AC+0.0396*BC+1.69*$A^2$-0.3632*$B^2$-0.3577*$C^2$ | 0.5165 | <0.0400 | 143.20 | 6.9925 |
| $R_{s(1,2)}$ | +1.17+0.3271*A+0.8321*B-0.1921*C+1.02*AB+0.1159*AC+0.2891*BC+0.0486*$A^2$+0.8764*$B^2$-0.0889$C^2$ | 0.8057 | <0.0007 | 34.76 | 11.541 |
| $\alpha_{(1,2)}$ | +1.59+0.0589*A+0.3224*B+0.1120*C | 0.3534 | <0.0185 | 21.94 | 6.9311 |

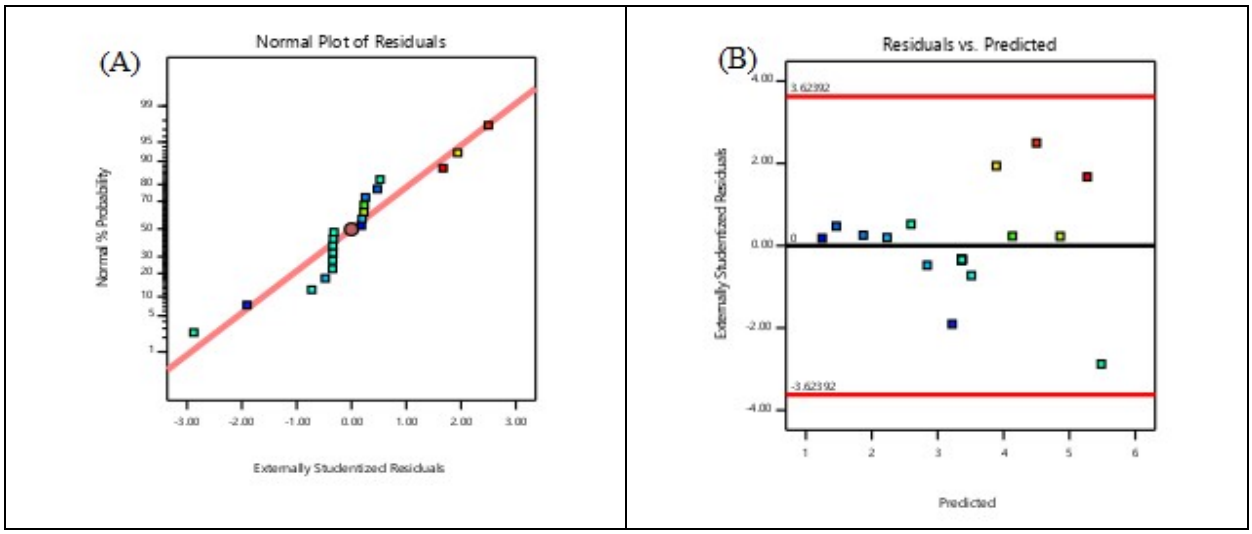



**Figure 3: Diagnostic plots for $Rs_{(1,2)}$ response.**
**Where, (A) Normal probability plot of residuals and (B) Plot of residuals versus predicted values**

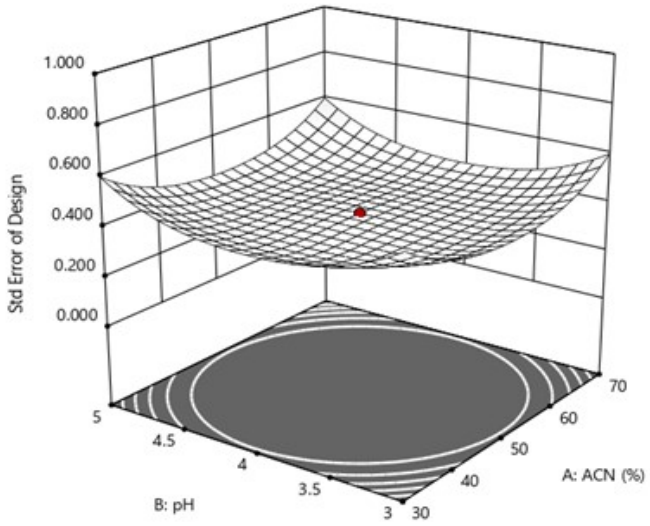



**Figure 4: Response surfaces related to acetonitrile, pH and flow rate:retention time of the last peak ($tR_2$), capacity factor first peak ($k_1$), resolution factor ($Rs_{1,2}$) and separation factor ($\alpha_{1,2}$).**

**Table 4: Criteria for the optimization of the individual responses for the analysis**

| Responses | Lower limit | Upper limit | Criteria 1 | | |
|---|---|---|---|---|---|
| | | | Goal | Importance | Weights |
| $tR_2$ | 1.422 | 6.763 | Minimize | 1 | 1 |
| $k_1$ | 0.037 | 7.242 | Minimize | 5 | 3 |
| $R_{s1,2}$ | 0.305 | 5.381 | In range | 5 | 1 |
| $\alpha_{1,2}$ | 0.973 | 2.631 | Maximize | 5 | 1 |

**Table 5: Comparison of observed and predictive values of different objective functions under optimal conditions**

| Optimum conditions | Acetonitrile (%) | pH | Flow rate (ml/min) | $k_1$ | $R_{s1,2}$ | $\alpha_{1,2}$ | $tR_2$ |
|---|---|---|---|---|---|---|---|
| Formulation | 60 | 5 | 1.2 | | | | |
| | Desirability value (D) = 0.814 | | | | | | |
| | Experimental value | | | 0.035 | 3.548 | 2.009 | 2.190 |
| | Predicted value | | | 0.037 | 3.665 | 2.059 | 2.194 |
| | Average % deviation | | | 4.324 | 3.190 | 2.420 | 0.180 |

## VALIDATION

The optimized chromatographic conditions were aggrandized applied to the validation of assertion for the system suitability, linearity, accuracy, precision, sensitivity, selectivity, and robustness. The optimized RP-HPLC method was validated as per the guidelines of the (ICH) Q2 (R1) for various parameters.

### System suitability

System suitability tests were referred for assessing chromatographic system before the sample analysis starts. The system suitability test was evaluated and the percent relative standard deviation (%RSD) was commencing less than 2% confine the appropriateness of strategy advancement.

### Linearity

For the linearity, the concentration ranges from 2 to 10µg/ml of Pazopanib was prepared. The calibrated graph was plotted by taking the peak area versus concentration. The correlation coefficient, intercept, slope, and linear regression analysis were done **[13]**. The results are shown in **Table 6 and Figure 5**.

### Sensitivity

With the formula 3.3 $\sigma/S$ and $10\sigma/S$, the limit of detection (LOD) and limit of quantitation (LOQ) was calculated respectively, where "$\sigma$" is the standard deviation of the response (y-intercept) and "S" is the slope of the linearity plot **[14]** and the results are shown in **Table 7**.

### Specificity

It was calculated by comparing test results from the analysis of a sample solution containing excipient with the results of standard drug **[15]**.

### Precision

It was calculated by different concentrations such as 2, 4 and 6µg/ml of Pazopanib samples analyzed in triplicates **[16]** and the results are shown in **Table 8**.

**Accuracy**

It is the closeness in the agreement between the accepted true value and the actual results obtained. Accuracy studies are usually evaluated by determining the sample of the analyte into the mixture of the samples to be analyzed. For accuracy studies, three different concentrations of solutions such as 8, 10 and 12µg/ml were used. After injecting each concentration, mean % recovery was calculated **[12]** and the results are shown in **Table 9**.

**RESULTS AND DISCUSSION**

**Linearity**

The results of method validation for linearity revealed that the above assay was linear over the concentration range between 2 to 10µg/ml for Pazopanib **(Table 6 and Figure 6)**. The regression coefficient was 0.998 for Pazopanib.

**Precision**

Precision was evaluated by the estimation of intraday precision by assay of three different concentrations of Pazopanib such as 2, 4 and 6µg/ml at various time intervals. The RSD (%) for intraday precision for Pazopanib were in the range between 1.03 to 2.09%, which was fall within the acceptable limit. The developed method exhibited good precision for the drug shown in **Table 8**.

**Accuracy**

The accuracy of the samples has been computed from measured concentrations of samples extrapolated from calibration curve mainly produced for the determination of the accuracy of the method. The results of accuracy studies for Pazopanib and Dasatinib are summarized in **Table 9**. It is clearly evident from the conclusion that %RSD of the compound was less than 2. Hence, the method can be considered as accurate.

**Specificity and selectivity**

Specificity and selectivity were studied for the examination of the presence of interfering components in the working solution of Pazopanib. The results indicate that the retention time of Pazopanib is at 2.19 minutes respectively. There is no variation in the retention time of the compound as compared to the standard drug. They are free from interference from formulation excipient and solvent. This indicates that the method was selected and specific for the determination of Pazopanib.

**Limit of detection and quantification**

The LOD and LOQ of Pazopanib were estimated as 10.47 and 31.74 respectively. The values indicated that the method was susceptible to quantify both the drugs **(Table 7)**.

**Application of the developed method**

The developed RP-HPLC method is sensitive and specific for the quantitative assurance of Pazopanib. The technique was approved for various parameters and, consequently, has been applied for the estimation of the drug in pharmaceutical dosage forms, such as tablets. Each tablet was analyzed in triplicate after extracting the drug as mentioned in the sample preparation of the experimental section. The recovered amount of Pazopanib was 99.78% **(Table 10)**.

None of the ingredients of tablet interfered with the analyte peak. The method was validated for linearity, precision, accuracy, sensitivity, system suitability as well as robustness. The developed method is convenient and effective for quality control as well as simultaneous routine analysis of Pazopanib in pharmaceutical dosage forms. The measured signal was shown to be precise, accurate, and linear over the concentration range tested with a retention time of 2.19 minutes and made the method economical due to lower solvent consumption. The % RSD for all parameters was observed under 2, which shows the validity of technique and assay results obtained by this method are in reasonable agreement. Chromatogram of Pazopanib is given in **Figure 5**.

**Table 6: Linearity test for Pazopanib**

| Conc. (µg/ml) | Ratio of Area (mAU) |
|---|---|
| 2 | 0.322 |
| 4 | 0.635 |
| 6 | 0.964 |
| 8 | 1.244 |
| 10 | 1.622 |

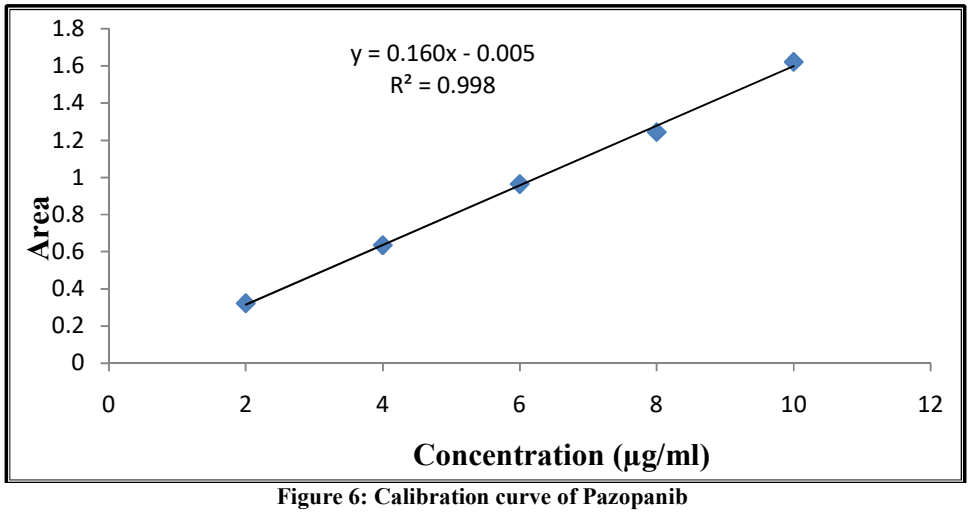



**Figure 6: Calibration curve of Pazopanib**

**Table 8: Precision of Pazopanib**

| Conc. (µg/ml) | Pazopanib (Drug) | Dasatinib (Internal Standard) | Ratio of Area (Drug/IS) | Mean ± SD | %RSD |
|---|---|---|---|---|---|
| 2 | 26685 | 81111 | 0.328994 | 0.322 ± 0.006 | 2.098 |
| | 26268 | 81528 | 0.322196 | | |
| | 25851 | 81945 | 0.315468 | | |
| 4 | 51329 | 79688 | 0.644125 | 0.635 ± 0.008 | 1.337 |
| | 50912 | 80105 | 0.635566 | | |
| | 50497 | 80522 | 0.627121 | | |
| 6 | 79181 | 81259 | 0.974427 | 0.964 ± 0.010 | 1.038 |
| | 78764 | 81673 | 0.964382 | | |
| | 78347 | 82090 | 0.954404 | | |

**Table 9: Accuracy for Pazopanib**

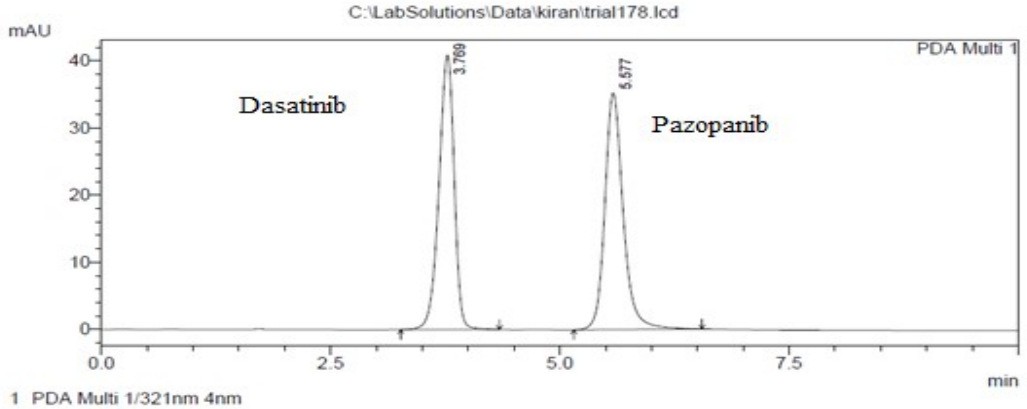
| Percentage (%) | Pazopanib | Dasatinib (Internal Standard) | Ratio of Area (Drug/IS) | Mean ± SD | %RSD | %Recovery |
|---|---|---|---|---|---|---|
| 80 | 231527 | 81847 | 2.828778 | 2.865± 0.032 | 1.138 | 99.65 |
| | 233286 | 81049 | 2.878333 | | | |
| | 235045 | 81321 | 2.890336 | | | |
| 100 | 261165 | 82592 | 3.16211 | 3.188 ± 0.024 | 0.774 | 99.78 |
| | 262924 | 81877 | 3.211207 | | | |
| | 264683 | 82937 | 3.191374 | | | |
| 120 | 287433 | 81721 | 3.517248 | 3.538 ± 0.019 | 0.543 | 100.65 |
| | 289192 | 81639 | 3.542327 | | | |
| | 290951 | 81842 | 3.555033 | | | |

**Table 7: LOD and LOQ values for Pazopanib**

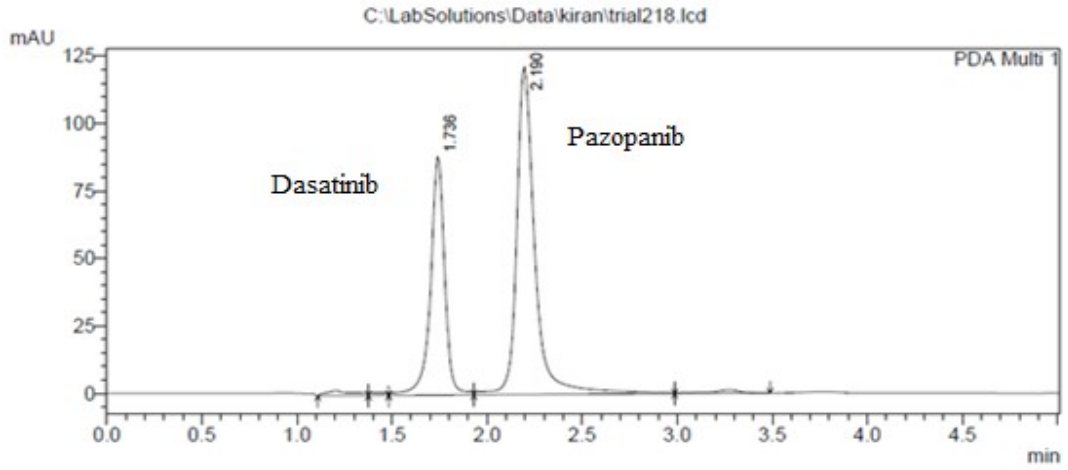
| | |
|---|---|
| LOD | 10.47ng/ml |
| LOQ | 31.74 ng/ml |

**Table 10: Assay of Votrient (Pazopanib) tablet**

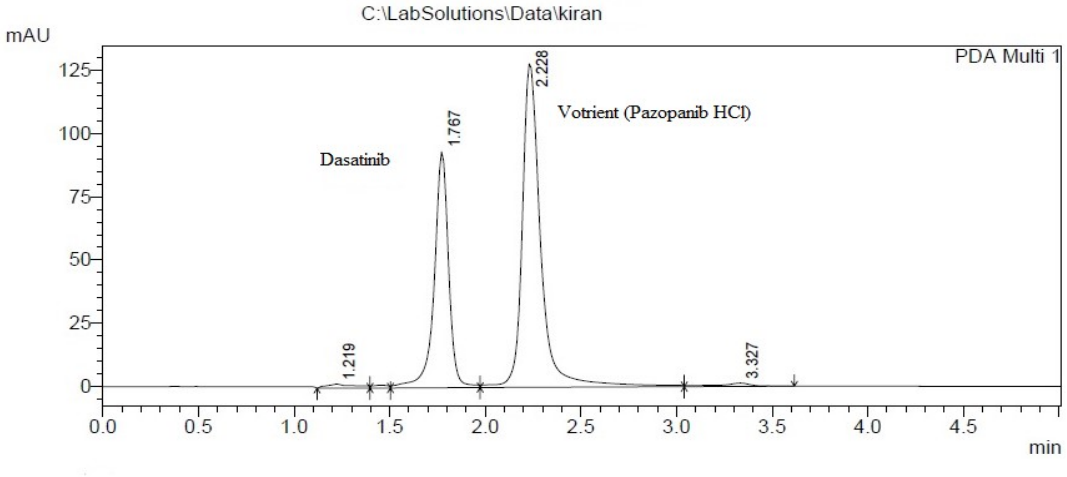
| Conc. (µg/ml) | Pazopanib | Dasatinib (Internal Standard) | Ratio of Area (Drug/IS) | Obtained Amount | Mean ± SD | %RSD | Label Amount | % Recovery |
|---|---|---|---|---|---|---|---|---|
| 10 | 131622 | 82368 | 1.5979 | 10.01 | 9.97± 0.04 | 0.400 | 200mg | 99.78 |
| | 131215 | 82775 | 1.5852 | 9.938 | | | | |
| | 130808 | 82182 | 1.5916 | 9.978 | | | | |




**(A) Before Optimization Condition**




**(B) After Optimization Condition**




**(C) Chromatogram of formulation (Votrient)**

**Figure 5: Chromatograms corresponding to bulk and formulation of Pazopanib and Dasatinib at 10µg/ml. (A) Before optimization, (B) After optimization and (C) Formulation**

## CONCLUSION

An efficient isocratic reversed-phase high-performance liquid chromatography method was developed, which was optimized and validated for the simultaneous estimation of second-generation TKI's namely Pazopanib in bulk and pharmaceutical formulations using Chemometrics Multi-Criteria Decision Making-Approach. This method reduced overall assay development time and provides essential information such as the sensitivity of various chromatographic factors and their interaction effects on the attributes of separation. Time of analysis, resolution and quality of the peaks was simultaneously optimized by applying useful tools of quality by design: Central Composite Design and Derringer's desirability function. The validation study supported the selection of the assay conditions by confirming that the assay was specific, accurate, linear, precise and robust. Therefore, the developed RP-

HPLC method can be used for routine quality control analysis of second-generation tyrosine kinase inhibitors.

**ABBREVIATIONS**

RP-HPLC: Reverse Phase High Performance Liquid Chromatography

TAP: Target Analytical Profile

CMA: Critical Method Attributes

CQA: Critical Quality Attributes

ACN: Acetonitrile

TKI: Tyrosine Kinase Inhibitor

RSM: Response Surface Methodology

CCD: Central Composite Design

QbD: Quality by Design

ATP: Analytical Target Profile

DOE: Design of Expert

LOD/DL: Limit of Detection

LOQ/QL: Limit of Quantification

PAZ: Pazopanib

DST: Dasatinib

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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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