ANALYTICAL METHOD VALIDATION FOR DETERMINATION OF % ASSAY IN PAZOPANIB TABLETS 200 mg AND 400 mg BY HPLC

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ABSTRACT

Objectives: Development and Validation of an Accurate, Sensitive, Precise, and Quick Analytical Method for Determination of Percent Assay in Pazopanib Tablets 200 mg and 400 mg by Reverse Phase High Performance Chromatography.

Methods: On Inertsil ODS C18,150 x 4.6 mm, 5 µm as the stationary phase, Pazopanib in bulk and formulations were evaluated. At a flow rate of 1.0 ml/min, the mobile phase was made up of Buffer solution and Acetonitrile in a 75:25 (percent v/v) ratio, respectively. Using a PDA detector with a 275 nm detection wavelength, elutes were examined. According to ICH guidelines, Validation of Analytical Procedures: Text and Methodology Q2, the suggested method was approved (R1).

Results: The chromatographic peaks of Pazopanib in this investigation had good resolution with a retention duration of 3.8 min. With a 1.000 correlation coefficient, Pazopanib demonstrated remarkable linearity. The quantification of Pazopanib showed good reproducibility when other validation factors like precision, specificity, accuracy, and robustness were used.

Conclusion: Reverse Phase High Performance Chromatography (RP-HPLC) was used to quickly design and validate the Analytical Method Validation for Determination of Percent Assay in Pazopanib Tablets 200 mg and 400 mg.

Key words: PDA, RP-HPLC, Pazopanib, Accuracy and Precision.

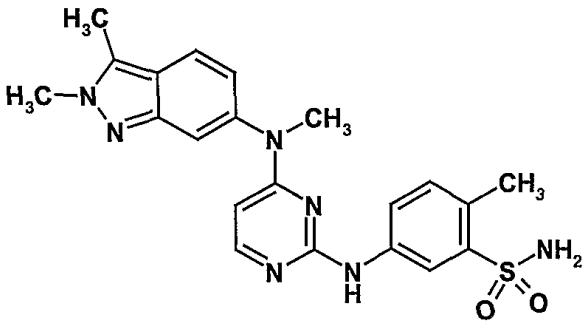
INTRODUCTION

Pazopanib is an inhibitor of the second generation of tyrosine kinases (TKI). Cancers of the head, neck, prostate, kidney, ovary, lung, and colon are among those it is used to treat. [2,3] PDGFR-1, VEGFR-1, VEGFR-2, VEGFR-3, and vascular endothelial growth factor receptor-1 are the five targets of the potent and selective multitargeted tyrosine kinase inhibitor Pazopanib (VEGFR-1). [4] It demonstrates traits similar to the stem cell growth factor receptor (c-kit), which prevents angiogenesis and slows tumor growth.

In accordance with ICH guideline ICH Q2, the proposed method validation was conducted (R1). [6] Pazopanib has the molecular weight and formula C21H23N7 O2 S, and 437.52gm/mol. [7] It

is dispersible in acetonitrile and water. The chemical name for Pazopanib is 5[4(2,3-dimethyl-2H-indazol-6-yl) methylamino-2-pyrimidinyl] 2-methylbenzenesulfonamide (Figure 1).

Figure 1: Structure of Pazopanib



MATERIALS AND METHODS

Chemical and reagents

The 200 mg and 400 mg Pazopanib Tablets are produced by Spectrum laboratories. Milli Q Water was utilized to prepare water for RP-HPLC (Merck). SD Fine Chem. Ltd. provided the ammonium acetate, glacial acetic acid, and acetonitrile AR grade (or) equivalent (India).

Instrumentation

The Waters HPLC system was used to conduct the HPLC analysis. Empower 3 data programme was used to record the chromatographic and integrated data. An electronic weighing scale with a 0.1 mg sensitivity, a quartz cell with a 1 cm path length, and a UV-VIS spectrophotometer from Systronics in India were used to capture the absorbance spectra. The buffer solution and acetonitrile were combined in the mobile phase in the ratio of 75:25 (percent v/v) respectively.

Preparation of Buffer solution:

Accurately 0.77g of ammonium acetate should be weighed and added to 1000 mL of water. The mixture should then be sonicated to completely dissolve the substance. Utilizing glacial acetic acid, bring the pH of the solution to 3.3 ±0.05. pass a 0.45-micron membrane filter through the fluid.

Preparation of Standard solution of Pazopanib:

Pazopanib Hydrochloride (Equivalent to 25mg of Pazopanib) Standard: Weigh and transfer approximately 27.0 mg accurately into a clean, dry 100-mL volumetric flask. Add approximately 60 mL of diluent, sonicate to completely dissolve the substance, then diluent to volume and well mix. Pipette 3.0 mL of the aforementioned solution into a dry, clean 25-mL volumetric flask. Dilute with diluent to volume, and thoroughly mix.

Assay procedure

Preparation of Sample solution 1:(For 200 mg and 400 mg strength)

Use a mortar and pestle to grind the weighing tablets into a fine powder. Pazopanib tablet powder, equivalent to 200 mg, should be accurately weighed and transferred into a clean, dry 200-mL volumetric flask. Diluent should then be added, and the mixture should be sonicated for 30 minutes with occasional shaking before being diluted to volume. The sample should be centrifuged at 3000 rpm for 15 minutes. Pipette 3.0 mL of the aforementioned supernatant sample solution into a dry, clean 100-mL volumetric flask, diluent to volume, and thoroughly mix. Discard the first 5 mL of the sample solution after filtering it with a 0.45 Nylon syringe filter.

Preparation of Sample solution 2: (For 200 mg and 400 mg strength)

Follow procedure as per the Preparation of sample solution-1.

Allow at least 30 minutes for the mobile phase solution to equilibrate the column. Inject the sample solution, the placebo standard solution, and the blank solution into the chromatographic equipment mentioned above. Figures 2 to 5 show the chromatograph's record and measurements of the peak responses.

Figure 2: Chromatogram of blank

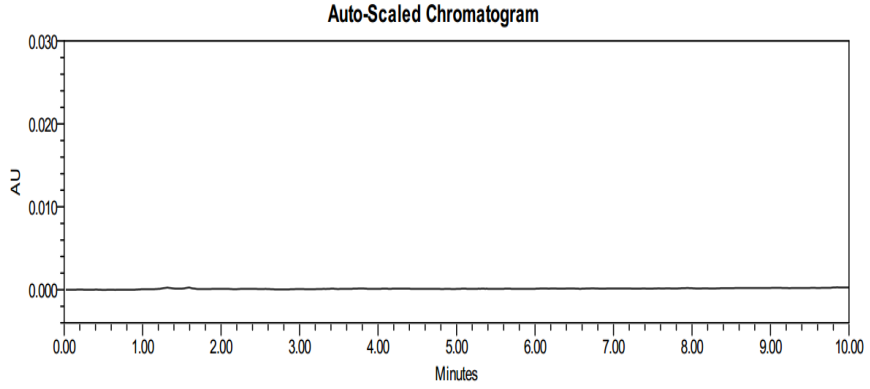


Figure 3: Chromatogram of Placebo

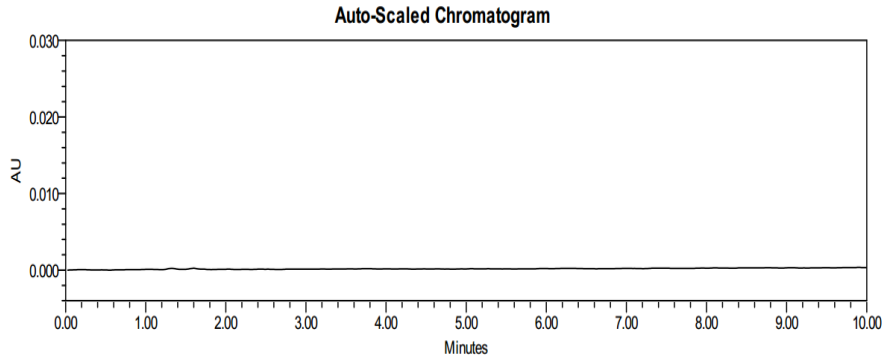


Figure 4: Chromatogram of Standard

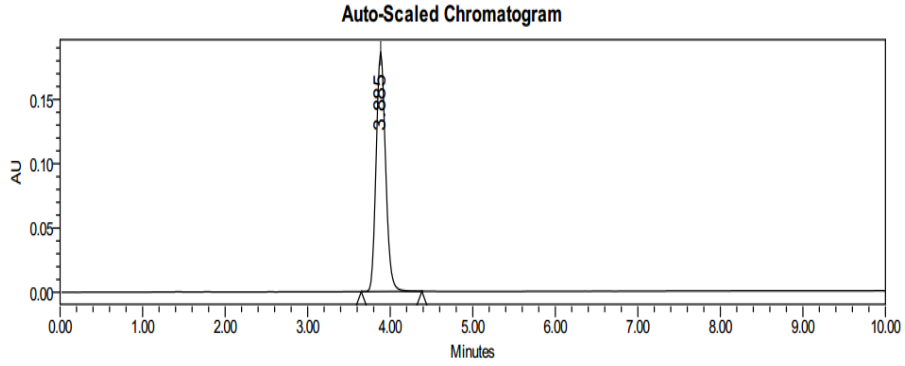
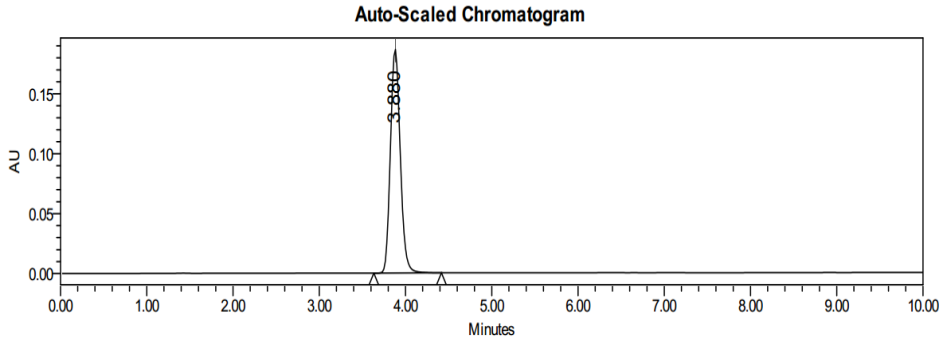


Figure 5: Chromatogram of sample



Chromatographic conditions:

Column	:	Inertsil ODS C18,150 x 4.6 mm, 5 µm
Flow rate	:	1.0 mL/minute
Injection volume	:	10µL
Column oven temperature	:	30°C
Sampler cooler temperature	:	5°C
Wavelength	:	275 nm
Run time	:	10 minutes
Elution mode	:	Isocratic

Validation of the method

Validation of the optimized HPLC method was carried out with the following parameters.

System Suitability

As per the Method Validation Protocol, prepared and injected system suitability standard solutions and evaluated the system Suitability parameters

System Precision

The system precision is measured by multiple injections of a homogenous standard solution indicates the performance of the HPLC instrument under the chromatographic conditions. A minimum of six injections of the standard solution is recommended. Injected six injections of standard solution for system precision and evaluated the system performance as per the method validation protocol requirement

Method Precision

The precision of the analytical method expresses the closeness of agreement between a series of measurements obtained from multiple samplings of the same homogeneous sample. Prepared six sample preparations as per the test method and injected into the HPLC system. Calculated the six preparations % Assay of % RSD

Intermediate Precision

To demonstrate ruggedness of test method, conduct system-to-system, column-to-column, day-to-day, and analyst to-analyst variability study on two HPLC systems (of the same or different manufacturer) by using different column of same brand by different analyst on different days. Prepared six sample preparations as per the protocol and injected into the HPLC system. Calculated the six preparations % Assay of % RSD.

Linearity

The linearity of analytical method was performed over the range 1X of 50% to 3X of 150 % of the target standard concentration. Injected duplicate injections at each level. Calculated the correlation coefficient, regression coefficient, slope, intercept and % Y-Intercept at 100 % bias.

Accuracy

Prepared accuracy solutions by using Pazopanib Hydrochloride API stock Solution with placebo in the range from 1X of 50% to 3X of 150% with respect to target test concentration. Injected in triplicate preparations at each level. Recorded the chromatograms. Calculated amount added in mg, amount found in mg, individual % recovery, mean % recovery and %RSD at each level.

Range

Based on the Precision, Linearity and Accuracy data it can be concluded that the method is precise, linear and accurate in the range of 1X of 50% to 3X of 150% with respect to target test concentration

Robustness

The robustness of analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in procedural parameters listed in the procedure documentation and indication of its suitability during normal usage. Performed system suitability as per the test method. Prepared the standard solutions and inject into HPLC as per protocol and checked the robustness of the method for following variations.

Solution stability of standard and sample solutions

Performed system suitability as per the protocol. Prepared standard and sample solutions as per the protocol kept these solutions at room temperature and in refrigerator (2 -8 °C) for Solution Stability Study and performed the analysis as per protocol at initial and next time point. Calculated the % Assay for samples against freshly prepared standard at each interval and calculated the % difference of Assay between initial to next time point. For standard calculate the similarity factor of aged standard against freshly prepared standard at each interval

Filter Compatibility

Performed system suitability as per the protocol. Prepared standard and sample solutions as per the protocol, for standard calculate the similarity factor of filtered standard against unfiltered standard. Calculated the % Assay for samples against freshly prepared standard and calculated the % difference of Assay between Centrifuged versus filtered sample

METHOD VALIDATION AND RESULTS

System suitability

The USP tailing factor from the first Standard Injection of Pazopanib peak in standard preparation-1 should be NMT 2.0. The USP theoretical plates from the first Standard injection of Pazopanib peak in standard preparation-1 should be NLT 2000.

The % RSD from the six replicate standard injections of Pazopanib peak responses in standard preparation-1 should be NMT 2.0%. The similarity factor of standard-2 against standard-1 should be between 0.98 to 1.02

Parameter	Result	Acceptance Criteria
USP tailingfactor	1.2	**NMT 2.0**
USP theoreticalplates	4574	**NLT 2000**
% RSD	0.25	**NMT 2.0%**
Similarityfactor	0.99	**Between 0.98 to 1.02**

System precision

The % RSD of Pazopanib peak obtained from six (n=6) replicate injections of standard solution-1 should be not more than 2.0%.

Method Precision

Assay results should meet thespecification limits. %RSD for six (n=6) replicate test preparations of assay results should be not more than 2.0.

Method Precision Results

Name of Sample	200 mg Strength %Assay	400 mg Strength %Assay
Preparation-1	98.5	100.0
Preparation-2	98.9	99.5
Preparation-3	99.2	100.2
Preparation-4	97.9	98.9
Preparation-5	99.9	99.3
Preparation-6	99.3	99.5
Average	**99.0**	**99.6**
SD	**0.69**	**0.47**
% RSD	**0.70**	**0.47**

Intermediate Precision

Name of Sample	400 mg Strength %Assay
Preparation-1	99.7
Preparation-2	98.6
Preparation-3	99.1
Preparation-4	99.0
Preparation-5	98.2
Preparation-6	99.5
Average	**99.0**
SD	**0.56**
% RSD	**0.56**

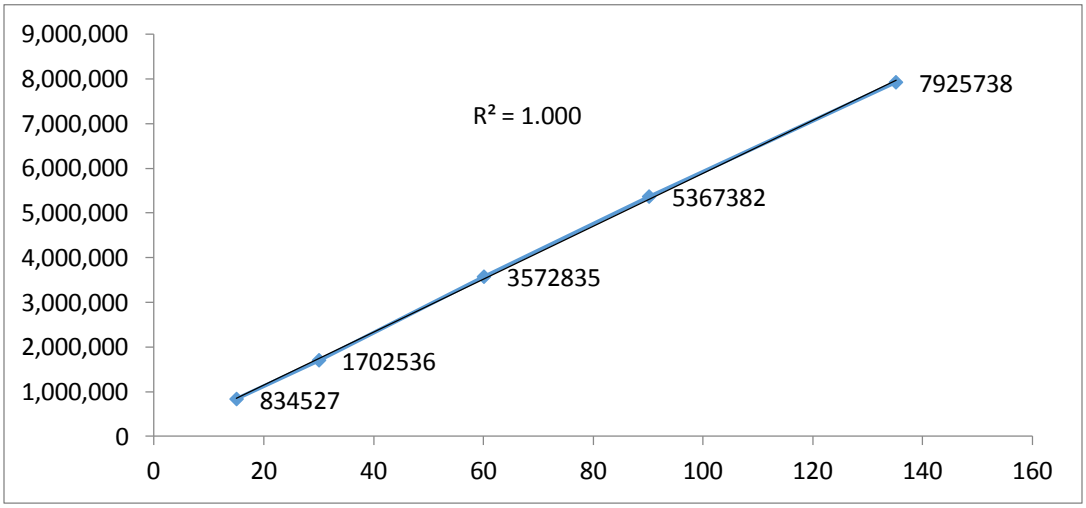
400 mg Strength Cumulative Results

Name of Sample	Method Precision	IntermediatePrecision
Preparation-1	100.0	99.7
Preparation-2	99.5	98.6
Preparation-3	100.2	99.1
Preparation-4	98.9	99.0
Preparation-5	99.3	98.2
Preparation-6	99.5	99.5
Overall Mean	**99.3**	
Overall SD	**0.57**	
Overall % RSD	**0.57**	

Linearity

The correlation coefficient (r) should be not less than 0. 999.Report the Slope, Regression coefficient, Intercept and Residual sum of Squares. % Y-intercept at 100 % bias should be NMT ± 2.0%.

Level	Concentration in (ppm)	Area
50% of 1X	15.041	834527
100% of 1X	30.065	1702536
100% of 2X	60.104	3572835
100% of 3X	90.184	5367382
150% of 3X	135.145	7925738
Correlation coefficient		**1.000**



Accuracy

The individual recovery of Pazopanib at each level should be in between 98.0% to 102.0%. The %RSD for the individual recovery at each level should be not more than 2.0**.**

Levels (%)	Mean % Recovery	% RSD
50% of 1X	99.9	0.5
100% of 2X	100.4	1.0
150% of 3X	98.7	0.4

Range

Establish the range of the method under the validation parameters of linearity, method precision and accuracy from 50% of 1X to 150% of 3X level. The range of the method is from 50% of 1X to 150% of 3X level. The method ranges from 15 ppm to 135 ppm

Solution stability

The standard solution is considered stable if the similarity factor should be between 0.98 and 1.02 from initial to next time point. The test solution is considered stable if the difference in %assay results from initial to next time point should be not more than 2.0%

Standard solution stability for RT and RF Condition

Time	Similarity factor	Acceptancecriteria
Initial	NA	**0.98-1.02**
24 Hours RT	1.00	
24 Hours 2-8 °C	0.99	
48 Hours RT	0.99	
48 Hours 2-8 °C	1.01	

Sample solution stability for RT and RF Condition

Time	% Assay	% Difference
Initial	**100.0**	NA
24 Hours RT	**99.5**	0.5
24 Hours 2-8 °C	**99.3**	0.7
48 Hours RT	**100.8**	0.8
48Hours 2-8 °C	**99.7**	0.3

Robustness

Effect of variation in flow rate:

System suitability parameters were analyzed as per the protocol by variation in flow rate at a Low flow rate (0.8 mL/min) and a High flow rate (1.2mL/min)

Parameter	Low	As such	High
Flow	0.8mL/min	1.0mL/min	1.2mL/min
RT	4.51	3.88	3.05
USP tailing factor	1.2	1.2	1.1
USP theoretical plates	4373	4574	3247
% RSD	0.4	0.3	0.3
Similarity factor	0.99	0.99	1.00

The method is considered robust for all above mentioned variations, if all the system suitability values meet the acceptance criteria set forth in the protocol. The above results reveal that the method is robust towards Low flow 0.8 mL/min and High flow 1.2 mL/min.

Effect of variation in column oven temperature:

System suitability parameters were analyzed as per the protocol by variation in Column oven temperature at a Low-temperature (30°C) and a High temperature (40°C).

Parameter	Low	As such	High
temperature	25°C	30°C	35°C
RT	4.01	3.88	3.59
USP tailing factor	1.4	1.2	1.3
USP theoretical plates	4057	4574	4470
% RSD	0.5	0.3	0.5
Similarity factor	0.99	0.99	0.98

The method is considered robust for all above mentioned variations, if all the system suitability values meet the acceptance criteria set forth in the protocol. The above results reveal that the method is robust towards Low Temperature 30°C and High temperature 40°C.

Effect of variation in Buffer pH

System suitability parameters were analyzed as per the protocol by variation in Buffer pH variation at Low pH Variation (3.0 ± 0.05) and High pH Variation (3.4 ± 0.05)

Parameter	Low	As such	High
Buffer pH Variation	**3.10 ± 0.05**	**3.30 ± 0.05**	**3.50 ± 0.05**
RT	3.39	3.88	3.95
USP tailing factor	1.3	1.2	1.2
USP theoreticalplates	3274	4574	4624
% RSD	0.4	0.3	0.3
Similarity factor	1.00	0.99	0.99

The method is considered robust for all above mentioned variations, if all the system suitability values meet the acceptance criteria set forth in the protocol. The above results reveal that the method is robust over the pH variation in the Low pH Variation (3.0 ± 0.05) in as such pH (3.2 ± 0.05) and High pH Variation (3.4 ± 0.05).

Filter study

The Similarity factor for filtered standard should be between 0.98 to 1.02 from the unfiltered standard. The %difference of % Assay compared from centrifuged versus filtered sample should be ±2.0%.

Standard Solution filter compatibility Results:

Name	Similarity factor	Acceptance Criteria
Unfiltered Standard	NA	**0.98-1.02**
0.45µm PVDF filter	1.02	
0.45µm Nylon filter	1.00	

Sample Solution filter compatibility Results:

Name	% Assay	%Difference
Centrifuge Sample	99.9	NA
0.45µm PVDF filter	96.0	3.9
0.45µm Nylon filter	100.0	0.1

CONCLUSION:

The present study demonstrated a validated Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method for the estimation of Pazopanib Hydrochloride in tablet dosage form 200 mg & 400 mg. The scope of the present work is to build up the linearity and optimization of the chromatographic conditions, to develop the RP-HPLC method for the estimation of the drug in the tablet dosage form 200 mg & 400 mg. The method was completely validated and showed satisfactory results. The method was free from the interference of the other active ingredients and additives used in the formulation. The results of the study indicate that the developed method was found to be accurate, precise, linear, sensitive, simple, economical, and reproducible, which has a short run time, which makes the method rapid. Hence it can be concluded that this method may be employed for the routine analysis of Pazopanib Hydrochloride in pharmaceutical preparations.

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