



**RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF
PAZOPANIB HYDROCHLORIDE IN BULK AND TABLET**

Chethan S.* and Jose Gnana Babu C.

Department of Pharmaceutical Analysis, Bharathi College of Pharmacy, Bharathinagara, K.M.Doddi, MaddurTaluk,
Mandya District, Karnataka, India – 571 422.

***Corresponding Author: Chethan S.**

Department of Pharmaceutical Analysis, Bharathi College of Pharmacy, Bharathinagara, K.M.Doddi, MaddurTaluk, Mandya District,
Karnataka, India - 571 422.

Article Received on 07/03/2019

Article Revised on 28/03/2019

Article Accepted on 17/04/2019

ABSTRACT

A simple, novel, accurate, precise, linear, rapid and economical HPLC method was developed for the estimation of Pazopanib. The chromatographic separation was achieved using a Genesis 5 μ C18 (100 x 4.60mm 5 μ) column and isoceatic elution, a mobile phase comprising of Acetonitrile: 0.02MmAmmonium acetate (60: 40) at P^H3.0 was adjusted with water. The flow rate was 1.0 ml/min with detection at 220 nm using a UV detector and drug eluted with retention time of 1.67 min. The calibration curves were linear ($r^2=0.999$) in the concentration range of 0.1-1.4 μ g/ml. The limit of detection and limit of quantitation were found to be 0.3548 and 1.0644 μ g/ml respectively. Thus the simple, novel, economical, accurate, precise and rapid HPLC method was developed for estimation of Pazopanib and validated as per ICH guidelines. Hence the method holds good for routine analysis Pazopanib in pure and pharmaceutical dosage form.

KEYWORDS: Pazopanib Hcl, ICH guidelines, RP-HPLC, Validation.

INTRODUCTION

Pazopanib Hydrochloride is the hydrochloride salt of a small molecule inhibitor of multiple protein tyrosine kinases with potential antineoplastic activity. Pazopanib selectively inhibits vascular endothelial growth factor receptors (VEGFR)-1, -2 and platelet derived growth factor receptor (PDGF-R), which may result in inhibition of angiogenesis in tumours in which these receptors are upregulated.^[1]

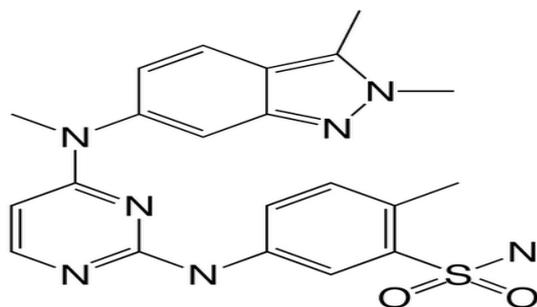


Figure.1: Chemical structure of Pazopanib Hcl.

Pazopanib is an 5-(4-((2, 3-dimethyl-2H-indazol-6-yl) (methyl) amino) pyrimidin-2-yl amino)-2-methyl benzene sulfonamide hydrochloride, Molecular Formula C₂₁H₂₃N₇O₂S.HCl, Molecular Weight 437.522g/mol, Characteristic, White Crystalline powder Uses, treatment for advanced/metastatic renal cell carcinoma and

advanced soft tissue sarcomas [anticancer].Solubility, methanol Brand name, Votrient-400mg.

Literature Survey revealed that the drug has been estimated by UV^[3-22] spectrophotometric, RP- HPLC^[23-34] and HPTLC^[35] method has been reported so far.

The aim of present work was to develop and validate a novel, rapid, simple, precise, and specific RP-HPLC method for estimation of Pazopanib in its bulk and tablet dosage form.

MATERIAL AND METHODS

Instrumentation

Chromatographic separation was performed on a Shimadzu LC-20AD HPLC system comprising a PDA detector, Shimadzu LC-20AD pump and Genesis 5 μ C18 (100 x 4.60mm, 5 μ) column. A manually operating Prominent auto sampler SIL-20 ACHT injector 20 μ l (20 μ l injection valve) was used for injecting sample and standard solution. Data was compiled using Shimadzu LC Solution software.

Chemicals and reagents

➤ Pazopanib Hcl pure form was obtained as gifted sample from pharma industry and its pharmaceutical dosage form Votrient labelled claim 400mg (manufactured by Cipla Private limited) were purchased from local pharmacy, Mandya.

- Acetonitrile, 0.02Mm Ammonium acetate and water are available in the Laboratory of Novetous pvt. limited, Bangalore.
- All the chemicals used in this investigation are HPLC grade.

Selection of mobile phase

Based on sample solubility, stability and suitability various mobile phase compositions were tried to get a good resolution and sharp peaks. The standard solution was run in different mobile phases. From the various mobile phases, acetonitrile:0.02Mm Ammonium acetate at P^H 3.5 (60:40 v/v) was chosen with detection wavelength 220nm, since it gave sharp peak with good symmetry within limits.

Preparation of mobile phase

Mobile phase was prepared by mixing acetonitrile: 0.02Mm Ammonium acetate at P^H 3.5 (60:40) This solution was sonicated for 10mins and filtered using a 0.2 μ membrane filter.

Chromatographic conditions

The optimized parameters which were used as a final method for the estimation of pazopanib Hcl represented in the Table 1.

Preparation of standard stock solution

Weigh accurately about 100 mg of pazopanib Hcl pure drug and then transferred into 100ml volumetric flask, a portion of diluent is added and sonicated for 10 min to dissolve it completely. The volume is made up to the mark with diluent (stock solution-1). From the above solution pipette out 1.0 ml into 10 ml volumetric flask and made up to the mark with diluent (stock solution-2), From the above solution pipette out 1.0 ml into 10 ml volumetric flask and made up to the mark with diluent (stock solution-3), from this solution pipette out 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 ml individual volumetric flask and add diluent up to the mark, this gives 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 μ g/ml concentrations.

Preparation of sample solution

Ten tablets of pazopanib Hcl 400 mg were weighed and powdered, the primary stock solution was prepared by dissolving a weight equivalent to 100 mg of pazopanib Hcl in 100ml volumetric flask by using HPLC grade methanol and the solution was sonicated for 10 minutes. The concentration of this solution gives (1000mg/ml). This solution was filtered using a 0.2 μ membrane filter. The above solution 10ml was pipetted out into a 100ml volumetric flask and the volume was made up to the mark with acetonitrile, and used for the analysis in RP-HPLC method, all the dilutions are made by acetonitrile.

Flow rate selection

Different flow rates in between 0.98 to 1.02 ml/min were studied. A flow rate of 1.0 ml/min gave an optimal signal to noise ratio with a reasonable separation time.

RESULTS AND DISCUSSION

System suitability

20 μ l of the standard solution was injected under optimized chromatographic conditions to evaluate the system suitability. Parameters such as number of theoretical plates (N), tailing factor (T), retention time (tR), asymmetry and area were determined. The obtained values indicate good performance of system shown in Fig-2. The values of system suitability parameters were shown in Table- 2.

Method validation

The method is validated according to the ICH guidelines.^[36-38]

Specificity

Specificity of the HPLC method was checked for interference of impurities, degradants or excipients in the analysis of sample solution and was determined by injecting a volume of 20 μ l sample solution and the chromatogram was recorded. There is no interference of impurities, excipient on the peak of Pazopanib hcl indicating the high specificity of method.

Linearity and Range

Calibration curve was plotted for different concentrations of working standards prepared from standard drug solution of pure drug, shown in Fig-3 and showed linearity over a concentration range of 0.1-0.6 μ g/ml shown in Table-3, along with regression parameters in Table-4. Each calibration was injected six times. The calibration curve was performed in six replicates.

Precision

The precision of the analytical method was determined by intraday and interday precision. The sample solution was prepared as per the test method. In intraday precision, the same concentration of sample solution was injected 6 times in the same day and in interday precision, injecting six solutions of same concentration for six different days in a week. The results of precision were tabulated in table-5. The average and standard deviation of mean area were taken and %RSD was calculated and reported. %RSD values were within the limits and the method was found to be precise.

Accuracy

The accuracy of the method was determined by recovery studies by the determination of % mean recovery of the drug at three different levels (80%, 100% and 120%). At each level, three determinations were performed. A known amount of standard pure drug was added to pre analyzed tablet powder and the sample was then analysed by developed method. Results of recovery studies were reported in table-6. The observed data were within the range, which indicates good recovery values.

Robustness

The robustness of analytical method was carried by varying the parameters deliberately from the optimized

chromatographic conditions like P^H of mobile phase (variation in ± 0.1 units), flow rate (variation in ± 0.02 ml/min.), wavelength (variation in ± 2 nm). The observed results were within the limit. The results were shown in table-7.

Ruggedness

Ruggedness was determined between different analysts. The value of %RSD was found to be <2 , showed

ruggedness of developed analytical method. The values were shown in Table-8.

Limit of detection and Limit of quantitation

The LOD and LOQ of the present method were calculated based on standard deviation of the response and slope of linearity curve. LOD and LOQ values of Pazopanib hcl were shown in Table-9.

RESULTED DIAGRAMS AND TABULAR COLUMNS

Table 1: Optimized chromatographic conditions

Mobile phase	Acetonitrile: 0.02Mm Ammonium acetate at P ^H 2.8 with (80:20v/v)
Stationary phase	Genesis 5 μ C18 (100 x 4.60mm, 5 μ)
Wavelength	220nm
Run time	5min
P ^H of mobile phase	1.67
Injector	(100 x 4.60mm, 5 μ)
Flow rate	1.0 ml per min
Injection volume	20 μ l
Temperature	Ambient
Mode of operation	Isocratic gradient elution

Table 2: Results of System suitability studies.

System suitability parameters	Acceptance criteria	Results
Retention time(tR)	-	1.67
Number of theoretical plates(N)	$N \geq 2000$	17719
Tailing factor(T)	$T \leq 2.0$	1.16

Table 3: Linearity data.

SL.NO	Concentration in μ g/ml	Peak area
01	1	24217.8
02	2	42635.2
03	3	60885.8
04	4	79959.7
05	5	98802.9
06	6	116782

*indicates average of six determinations,

Table 4: Regression parameters.

Regression Parameters	Pazopanib Hcl
Regression Equation	$Y = 18583x + 5507$
Slope (b)	18583
Intercept (a)	5507
Correlation Coefficient (r^2)	0.999

Table 5: Results of Precision studies.

Sl.no	Concentration (μ g/ml)	Intraday precision (area)	Inter-day precision (area)
1.	4	82647.572	79881.230
2.	4	80537.068	79871.746
3.	4	79942.762	79061.621
4.	4	79782.281	79695.010
5.	4	79678.742	80647.532
6.	4	79869.748	79452.982
Mean		80409.7	76768.35
Std. dev.*		1136.82	529.281
%RSD		1.41	0.6894

*indicates average of six determinations, RSD indicates relative standard deviation

Table 6: Results of Accuracy studies.

Level of recovery	Amount of formulation	Amount of Pure drug	Total amount of drug	%Recovery±SD	%RSD
80	2	1.2	3.2	99.17±477.649	0.634
100	2	2	4.0	99.99±143.125	0.178
120	2	2.8	4.8	100.76±551.519	0.570

Table 7: Results of Robustness studies.

Concentration (0.6µg/ml)			
Parameters	Factors	Mean area* ±Standard deviation	%RSD
p ^H	3.5	79828.57±440.769	0.5521
	4.0	79821.55±131.55	0.1648
	4.5	80061.064±321.049	0.4010
Temperature	29	79820.01±274.158	0.3434
	30	80168.15±496.278	0.6190
	31	79587.705±431.813	0.542
Flow rate (ml/min)	0.98	79397.64±740.784	0.9330
	1.00	79732.872±701.068	0.8792
	1.02	79981.60±603.33	0.7543

*indicates average of six determinations,

Table 8: Results of Ruggedness studies

Analysts	Mean area* ± Standard deviation	%RSD
Analyst 1	79963.45±205.255	0.2567
Analyst 2	79885.42±58.52	0.0732

*indicates average of six determinations,

Table 9: Results of LOD and LOQ.

Parameters	Results
LOD (µg/ml)	0.9475
LOQ (µg/ml)	2.842

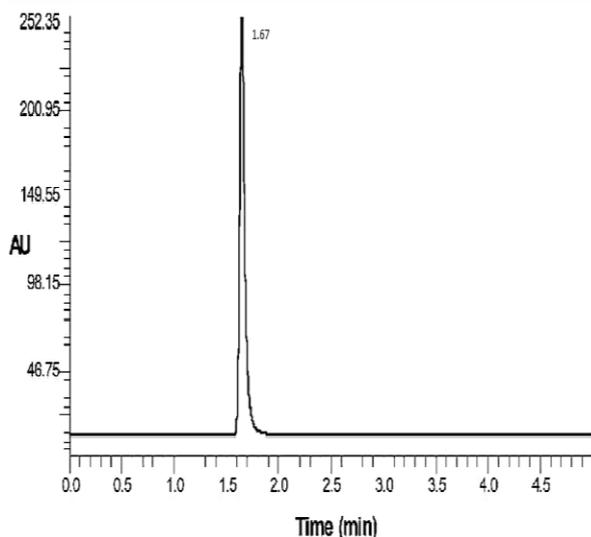


Fig 2: Chromatogram of Pazopanib Hcl.

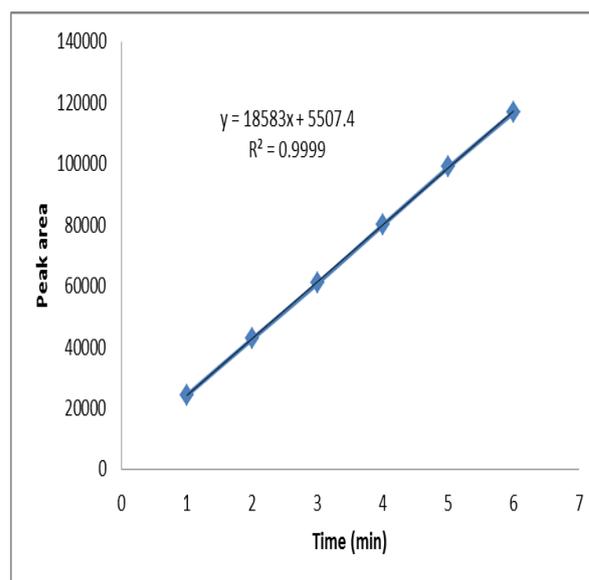


Fig.3: Linearity graph of Pazopanib Hcl.

CONCLUSION

From the above it can be concluded that all validation parameters (precision, accuracy, linearity, LOD, LOQ and Ruggedness) met the predetermined acceptance criteria as mentioned in ICH guidelines. The developed HPLC method is simple, rapid, accurate, precise and shown good linearity. Hence it can be applied for routine analysis of Pazopanib Hcl in bulk and its dosage forms.

ACKNOWLEDGMENT

We like to thanks management, principal, teaching staff, nonteaching staff and my dear Friends of Bharathi College of Pharmacy and for their continuous co-operation and support.

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