Original Article

Bioanalytical Method Development And Validation Of Pazopanib Hydrochloride By Uv- Visible Spectrometry And Rp- Hplc In Human Plasma

Abhishek Amod Gupta[1], Ramanlal N. Kachave[2*], Shruti K. Patil[3], Raj K. Keservani[4*]

[1]Assistant Professor, Department of Pharmacology, Government Pharmacy Institute, Agamkuan, Patna, Aryabhatta Knowledge University, Gyan Parisar, Mithapur, Patna, Bihar, 800 007, India
[2]Department of Pharmaceutical Chemistry, Amrutvahini College of Pharmacy, Sangamner, Affiliated Savitribai Phule Pune University, Maharashtra, India. orcid: 0000-0002-9225-5881
[3]Department of Pharmaceutical Quality Assurance, Amrutvahini College of Pharmacy, Sangamner, Affiliated Savitribai Phule Pune University, Maharashtra, India.
[4]Faculty of B. Pharmacy, CSM Group of Institutions, Prayagraj, Uttar Pradesh, 212111, India. orcid: 0000-0003-2604-7440
*Corresponding author: Ramanlal N. Kachave (ramanlalkachave26@gmail.com); Raj K. Keservani (rajksops@gmail.com)
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Abstract

Background: Pazopanib Hydrochloride is Anti-Cancer agent used in the treatment of renal cell cancer and Von Hippel Lindau Disease. Newly method development and validation of Pazopanib Hydrochloride in Human Plasma was carried out using the chromatographic method.

Methods: The UV Spectrophotometric developed with chromatographic method involved LC C_{18} column, using acetonitrile and water containing 0.1 % formic acid in ratio of 80:20:0.1 v/v/v as mobile phase, a flow rate of 1 ml/min, and UV detection at 270 nm.

Results: The retention time of plasma & Pazopanib Hydrochloride was found to be 2.2 & 4.4 min, respectively. Linearity was acceptable in the concentration range for UV-visible Spectrometric method 5-25 µg/ml and for RP-HPLC 1-15 µg/ml. The inter day & intraday precision was found less than 2 for UV-visible spectrometry and for RP-HPLC % CV was found less than 15%. The accuracy was found to be 1.8812 and for RP-HPLC mean recovery was found to be 4.8845.

Conclusion: we conclude that the method can be useful for routine analysis, bioanalytical studies and therapeutic drug monitoring.

Keywords: Pazopanib hydrochloride, UHPLC, Validation of Pazopanib Hydrochloride.

1. Introduction:

Pazopanib is anti-neoplastic agent used in the treatment of the advance renal cell carcinoma, soft tissue sarcoma & Von Hippel Lindau disease.Pazopanib was approved by the US food and drug administration for medicinal use in 19 Oct 2009.Pazopanib is selected multitargeted receptor tyrosine kinase inhibitor which block the growth of tumor and inhibit the angiogenesis.It belongs to the class of organic compound alkyldiarylamines.Pazopanib pharmacologically targeted on the VEGFR 1,2,3 (vascular endothelial growth factor), PDGFR α/β (platelet derived growth factor). Recently it used in the treatment of the Von Hippel Lindau disease – rare autosomal dominant disease caused due to germline mutation of VHL gene.

The recommended dose of orally administered pazopanib is 400 mg or 800 mg daily. Due to ease administration of drug it shows better quality of life.[3] the protein binding of pazopanib is 90-99 % and it is metabolized by CYP3A4.[4]

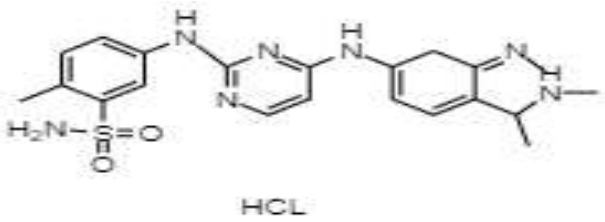

Figure 1: Structure of Pazopanib Hydrochloride

2. Methods and Materials:

2.1 Instruments:

Shimadzu 1800 UV Spectrophotometer containing two quartz cell with 1 cm path length was used for the measurement of pazopanib hydrochloride. Agilent technology 1260 Infinity II HPLC was used for the method development of pazopanib hydrochloride. Shimadzu LC 2030 C UHPLC was used for bioanalytical validation of pazopanib hydrochloride. Shimadzu balance (0.01mg sensitivity) was used for weighing. Ultra sonicator bath used for the work. REMI CM- 12 Plus was used for the centrifugation. Separation and quantification done by using Agilent 5 HC – C18 [250 X4.6 mm, 5 µm].

2.2 Reference samples, chemicals and Reagent:

Pazopanib Hydrochloride procured from INVOCHEM Laboratory, Mumbai, Maharashtra, India.400mg of pazopanib Hydrochloride tablets used for study. Methanol (HPLC Grade), CAN (HPLC Grade) was obtained from Merck, Mumbai, India, DMSO, Water (Milli Q).

2.3 Chromatographic Condition:

Agilent 5 HC- C18 [250 X 4.6 mm,5 µm] was used as stationary phase. The isocratic mobile phase consisting of mixture of Acetonitrile and water with 0.1 % formic acid in the ratio 80:20 % (v/v), was used for the analysis.

2.4 Sample preparation:

2.4.1 Preparation of stock solution (100 µg/ml) :

Weigh accurate 10 mg of pazopanib and transferred to 100 ml of volumetric flask and dissolved in methanol to obtained stock solution.

2.4.2 Plasma sample preparation :

Before processing, stored plasma sample were allowed to thaw at room temperature. The plasma samples were centrifuged at 4000 rpm for 10 min, an aliquot (0.5 ml) was pipetted into 10 ml of polypropylene tube and 1.5 ml of acetonitrile was added. The mixture was vertex and after standing for 2 min at room temperature, the mixture was centrifuged at 5000 rpm for 15 min at 4 °c. The supernant was carefully transferred into vial and injected into LC system.

2.4.3 Selection of Solvents:

Abundant trials are carried out for determination of solubility of pazopanib by dissolving in suitable solvent system. The solvent such as water, acetonitrile, DMSO, methanol etc. the pazopanib soluble in organic solvents like acetonitrile, methanol, DMSO, etc. and insoluble in water. Therefore, methanol was selected because it is cheap.

2.4.4 Selection of wavelength:

To determine the optimal λ max of pazopanib hydrochloride, 10 µg/ml of PZH solution was prepared from above stock solution by diluting with distilled water. Scan UV wavelength range in between 200- 400 nm. It shows the maximum absorbance at 249 nm.

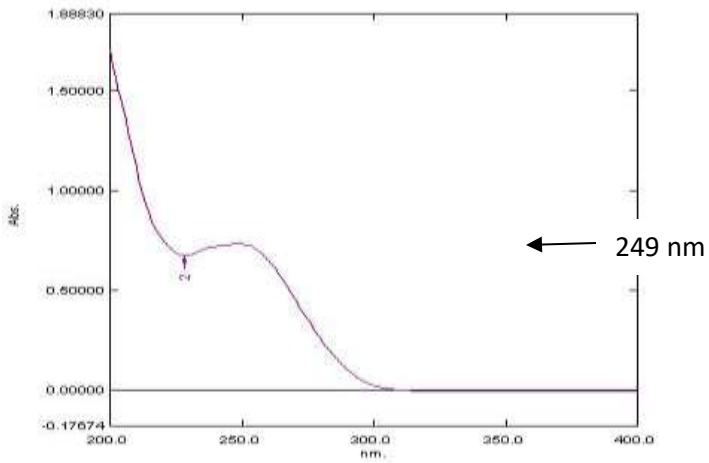

Figure 2: UV Spectra of Pazopanib Hydrochloride

2.4.5 Optimization method:

The RP-HPLC was optimized to developed for estimation of pazopanib hydrochloride in spiked human plasma the chromatogram shown in fig.

For the method optimization trials are carried out on different mobile phases ratios, the acceptable retention time, theoretical plates, and good resolution was observed with Acetonitrile : Water : 0.1% formic acid (80:20:0.1%) using column Agilent 5 HC C18 [250 X 4.6 mm, 5µm]

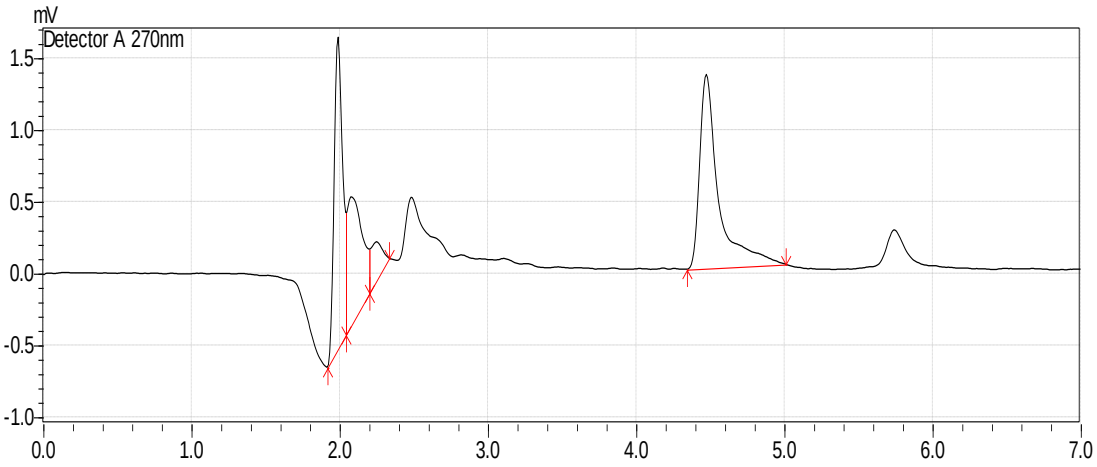

Figure 3: chromatogram of spiked concentration15 µg/ml

2.5 Result and discussion:

PART I

2.5.1 Validation of UV Spectrometric Method:

1. Linearity and Range:

For determination of linearity on UV Spectrophotometer prepared the dilution from above stock solution in 10 ml volumetric flask. Ranges from 5-10 µg/ml and volume makeup with distilled water. Calibration curve plotted with observed absorbance against concentration.

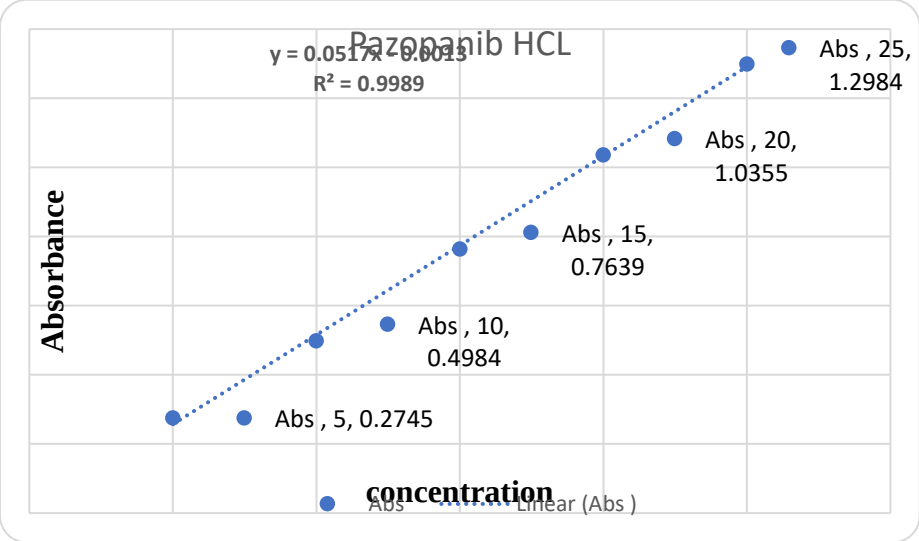

Figure 4: Linearity of UV Spectrophotometer

Table 1: linearity of UV Spectrophotometer

Parameter	**Pazopanib**
Linearity range	5-25µg/ml
Regression equation	Y= 0.0517-0.0013
Correlation coefficient (r2)	0.9989
Slope	0.0517
Y –intercept	0.0013

2.Precision:

In method validation precision is used to check the reproducibility. There are three types of precision as follows;

3.Intraday:

This method carried out by taking three replicates of each three concentration of 5, 10, 15 ug/ml. The results of this method reported in following table:

4.Inter day:

This method was performed as intermediate precision, it is used to check the reproducibility of drug. Analysis was carried out in laboratory at three different concentration at three replicates. The result for interday reported in following table:

5.Repeatability:

Repeatability performed for one concentration at six replicates. The result reported in following table:

Table 2: Precision

Validation parameter	**% mean**	**SD**	**% RSD**
Intra day (Moring)	93.33	1.78	1.90
Evening	93.85	1.65	1.75
Inter day	92.1	1.66	1.80

6. LOD& LOQ:

LOQ is the quantitatively determination the lowest amount analyte in the sample.

LOD is the lowest amount of analyte detected in the sample. The found values of LOD & LOQ are reported in following table:

Table 3: LOD & LOQ

	FORMULA	RESULT µg/ml
LOD	3.3* SD/ SLOPE	0.0967
LOQ	10* SD/SLOPE	0.2932

7.Accuracy:

The accuracy was performed on three concentration 80 %, 100%, 120% at three replicates. The results are reported in following table:

Table 4: Accuracy

Validation parameter	**% mean**	**SD**	**% RSD**
80%	86.68	1.76	2.0
100%	105.4	1.90	1.80
120%	110.5	1.99	1.80

8. Robustness:

The robustness was performed at two different wavelength using two different concentration 5 & 25 ug/ml. the results are reported in following table:

Table 5: Robustness At 248 nm&251 nm

Validation parameter	% mean	SD	% RSD
248 nm	94.85556	1.866599	1.967833
251 nm	92.40889	1.565589	1.694198

PART II

2.5.2 Bioanalytical validation of RP-HPLC:

1. Linearity & Range:

For determine the linearity on UHPLC prepared the serial dilution from above stock solution in 10 ml of volumetric flask. Range from 1-15 µg/ml volume make up with mobile phase acetonitrile: water in ratio 70:30 v/v. The spiked sample prepared from above plasma sample preparation procedure. Calibration Curve Plotted from observed peak area against Concentration.

Table 6: Peak Area

Concentration µg/ml	Peak Area
1	12680
2	21574
4	41395
6	54164
8	75718
10	99097
12	124212
15	150038

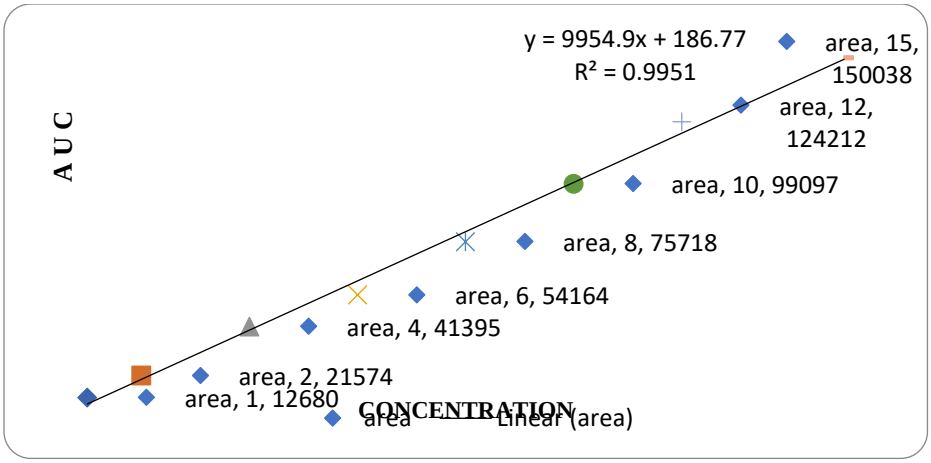

Figure 5: Linearity of PZH

Table 7: Linearity

Parameter	**Pazopanib**
Linearity range	1-15 µg/ml
Regression equation	Y= 9954.9+186.7
Correlation coefficient (r2)	0.9951
Slope	9954.9
Y –intercept	186.7

2.Precision:

The precision was performed as interday&intra day precision by injecting the three injection of each three concentration. The resultas of % RSD are reported in following table:

Table 9: Intra day precision

Validation parameter	% mean	SD	% CV
Intra day	94.83	12.3203	12.9920
Inter day	95.19	1.3464	1.4144
Repeatability	94.1435185	1.62780701	1.72906965

3. LOD & LOQ:

LOQ is the quantitatively determination the lowest amount analyte in the sample.

LOD is the lowest amount of analyte detected in the sample. The found values of LOD & LOQ are reported in following table:

Table 10: LOD & LOQ

	FORMULA	RESULT µg/ml
LOD	3.3* SD/ SLOPE	0.5714
LOQ	10* SD/SLOPE	1.7316

4. Recovery:

The recovery was determined by capering the extracted samples with plasma and unextracted samples % CV of recovered samples are reported in following table:

Table 11: Recovery

Conc. of drug in sample	**Std. drug solution added**	**Recovered amount**			**mean**
		R1	**R2**	**R3**	
8	4	3.37	3.65	4.02	92
8	8	7.91	8.10	7.97	99.96
8	12	13.79	13.899	13.66	91.91
				Mean	94.62333
				SD	4.621908
				% CV	4.884533

5. Robustness:

Robustness are carried out by changing the chromatographic conditions like flow rate by using three different concentration at three injection.The result are reported in following table:

Table 12: Robustness at 1 ml/min& 0.8 ml/min

Validation parameter	**% mean**	**SD**	**% CV**
1 ml/min	97.43889	9.246205	9.489235
0.8 ml/min	96.24444	11.64695	12.10143

6. Stability:

The stability test of the analyte was designed to cover expected condition concerning the handling of clinical samples. The stabilities of the analyte in human plasma were investigated under various storage and processing conditions. The results in summarized in table.

- Regarding long term stability of the analyte in matrix stored in freezer:

  The QC samples should be stored in freezer under same condition and at least for the same duration as the study samples.

Table 13: Result of Stability

Validation parameter	% mean	SD	% CV
Stability at freeze thaw	95.32333	0.527457	0.553335
Stability at room temperature	95.19111	1.800606	1.891569

CONCLUSION:

The simple, accurate, precise and rapid both UV Spectrophotometric method and bioanalytical RP- HPLC methods were developed and validated as per ICHQ2(R1) and ICH M10 guidelines. both method was time – effective and cost effectiveThe simple liquid- liquid extraction method was used for bioanalytical method development. Theoverall results of validation parameters of UV Spectrometric as well as bioanalytical RP- HPLC method meets with acceptable limits, we conclude that the method can be useful for routine analysis and for bioanalytical studies, therapeutic drug monitoring.

DECLARATIONS

Consent for publication

All the authors approved the manuscript for publication.

Availability of data and material

All required data is available.

Competing interests

All authors declare no competing interests.

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