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Research Article

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Development and validation of stability indicating RP-HPLC method for the determination of pazopanib hydrochloride in bulk drug and its pharmaceutical dosage form

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ABSTRACT

A simple, sensitive, reproducible and stability indicating RP-HPLC method for the determination of Pazopanib hydrochloride in bulk and pharmaceutical dosage form has been developed and validated. Chromatography was carried out by isocratic technique on a reversed phase C18 ZORBAX Eclipse Plus column (100 x 4.6 mm x 3.5µ particle size) containing mobile phase of Phosphate buffer P^H 2.5 (adjusted with diluted ortho phosphoric acid) and methanol in the ratio of 55:45% v/v at a flow rate of 1.0mL/min. The analyte was monitored using PDA detector at 270nm wave length. The retention time was found to be 3.18 min for Pazopanib hydrochloride. The proposed method was found to be linear in the concentration range of 30-80µg/mL with a regression factor $\geq r^2$ 0.999. The developed method was validated according to ICH Q2B guidelines. Stress condition tests which covered acid, alkali, peroxide, photolytic and thermal degradation was carried and to prove the specificity of the method and the degradation was achieved. Hence this developed method can be applied for the stability indicating RP-HPLC method for the determination of Pazopanib hydrochloride in bulk and pharmaceutical dosage forms and also in routine quality control analysis.

Key words: Pazopanib, RP-HPLC, Forced degradation, Method Validation

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INTRODUCTION

Pazopanib Hydrochloride (Fig.1), chemically is 5-[[4-[(2, 3-dimethyl-2H-indazol-6yl) methyl amino]-2-pyrimidinyl] amino]-2-methylbenzenesulfonamide monohydrochloride. Pazopanib is a novel multi-target orally administered drug. It is a potent inhibitor of vascular endothelial growth factor (VEGF) receptors (VEGFR)-1, -2, and -3, platelet-derived growth factor (PDGFR)-α and –β, and stem cell factor receptor [1]. Pazopanib inhibits both tumor growth and angiogenesis through suppressing these targets. It was multi-target tyrosine kinase inhibitor (TKI). In preclinical studies, it has shown anti-tumor activity against several human tumor xenografts, including renal, prostate, colon, lung, melanoma, head and neck, and breast cancer and also showed desirable pharmacokinetics and oral bioavailability in animal models [2]. Pazopanib has been approved for advanced/metastatic renal cell carcinoma and advanced soft tissue sarcomas [3] by multiple regulatory administrations worldwide, including FDA and EMA.

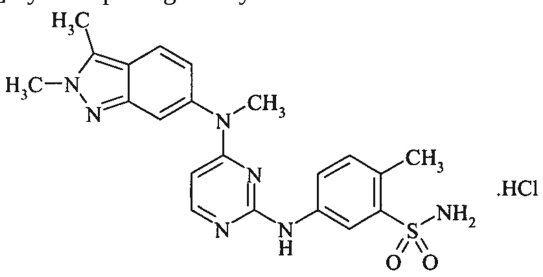

Fig. 1: Chemical structure of Pazopanib Hydrochloride

Literature survey revealed that very few analytical methods [4-6] were reported in biological fluids but no stability indicating method in pharmaceutical dosage form was reported so far. Hence author has planned to develop a simple and sensitive stability indicating RP-HPLC method for the determination of Pazopanib in bulk and pharmaceutical dosage forms.

EXPERIMENTAL SECTION

Chemicals and regents:
Pazopanib tablets (Glaxo Smithkline-Votrient) was purchased from local pharmacy. Methanol & Water (HPLC grade Solvents), Potassium dihydrogen phosphate, ortho phosphoric acid (OPA) were purchased from Fisher Scientific.

Instrumentation:

Table-1

Equipment	Apparatus
HPLC system	Agilent 1260 infinity series, with DAD detector, manual injector of 20 µl
Software	Open lab version A.02.01(1.3.3)

Preparation of 2.5 pH Phosphate buffer:
Accurately weighed about and transferred 1.36 gm of KH_2PO_4 in a 1000mL volumetric flask containing 900mL of HPLC grade water and then sonicated for 5 min and finally made up to the volume with HPLC grade water. To this 1mL of tri ethyl amine was added and P^H was adjusted to 2.5 by using diluted OPA and finally filtered through 0.45µ membrane filter.

Preparation of Mobile Phase:
55 parts of phosphate buffer (P^H 2.5) and 45 parts of methanol was mixed properly and degassed.

Preparation of Diluent:
Equal parts of each phosphate buffer (P^H 2.5) and methanol was mixed properly and degassed.

Preparation of Standard Solution:
Accurately weighed about and transferred 25mg of Pazopanib into a 25mL volumetric flask, added about 15mL of diluent, sonicated for 10 min and finally made up to volume with diluent to get 1mg/mL solution. From the above solution 5mL was pipette out into 100 ml volumetric flask and further diluted with diluent up to the mark to get the final concentration of 50µg/ml which was used as a working standard.

Preparation of Sample:
10 tablets of Pazopanib were taken (each containing 200mg of Pazopanib), weighed and grounded to a fine powder. Then quantity equivalent to 100mg of Pazopanib was transferred into 100mL volumetric flask, 60mL of diluent was added, sonicated for 10 min and finally the volume was made up to mark with diluent. Filtered and from the filtrate, 5mL was pipette out into 100mL volumetric flask, volume made up to mark with diluent to get the final concentration 50µg/mL of Pazopanib.

Method Development & Optimization:
To develop a precise, accurate and stability indicating RP-HPLC method for estimation of Pazopanib Hydrochloride, different mobile phases, solvent-buffer ratios and P^H of buffer were tried to proposed the final chromatographic conditions. The peak shape and symmetry of Pazopanib was good at flow rate of 1.0mL/min with mobile phase in ratio of 55:45%v/v (P^H 2.5 buffer: methanol) and the retention time was found to be 3.18 min. The developed method was successfully applied for quantitative determination of Pazopanib Hydrochloride.

Optimized Chromatographic Conditions:

Table -2

Parameter	Optimized condition
Flow rate	1.0 ml/ min
Mobile Phase composition	55:45 % v/v (Phosphate buffer pH 2.5: Methanol)
Wave length	270 nm
Injection Volume	20 µl
Run time	5 min

Method Validation:
The optimized RP-HPLC method was validated for system suitability, specificity, precision, linearity, accuracy and robustness according to ICH guidelines.

System suitability:
System suitability was performed by injecting six replicates of 50µg/mL standard solution of Pazopanib Hydrochloride into the chromatographic system. System suitability parameters were then evaluated for tailing factor, theoretical plates, retention time and peak area from the standard chromatograms. The system was deemed to be suitable for use if tailing factor was less than 2.0, theoretical plates were greater than 2000, and %RSD of peak area and retention times were less than 2.0. The results are shown in table-3.

Specificity:
Specificity is the ability to assess unequivocally the analytes in the presence of compounds that may be expected to present, such as impurities, degradation products, and matrix components. The specificity of the method was demonstrated by injecting standard and sample solutions of Pazopanib at a concentration of 50µg/mL into the chromatographic system, the chromatograms are shown in Fig.2 and Fig.3.

Precision:
The precision of the assay was studied with respect to both intra-day (repeatability) and inter-day (intermediate precision). Repeatability was calculated by injecting six replicates of standard solutions into the chromatographic system on the same day and on the second day for intermediate precision. The method was found to be precise with low % RSD values of retention time and peak areas. The results obtained are shown in the Table 4.

Linearity:
The linearity of Pazopanib was studied by preparing six different concentrations of standard solution in the range of 30-80µg/mL of Pazopanib and each concentration was injected in five replicates and mean value of peak area was taken for calibration. The calibration curve was found to be linear with correlation coefficient of 0.999 with regression equation y= 18020x + 91975. The results are shown in Fig.4.

Accuracy:
The accuracy of the test method for Pazopanib was calculated by recovery studies at three different concentrations i.e. 50%, 100% and 150% levels by using standard addition method and each concentration was injected three times into the chromatographic system. The accuracy of an analytical method should be established across its range. The results obtained are shown in the Table 5.

Robustness:
Robustness of the proposed method was determined by analyzing the standard and sample solutions by varying the physical parameters like flow rate, mobile phase composition and pH of the buffer. The results obtained are shown in the Table 6.

Force Degradation:
Stress testing of drug substance was carried out to identify any likely degradation products, by establish the degradant pathways and the intrinsic stability of the drug molecule and to develop and validate the stability indicating power of the procedure used.

Acid Degradation:
To 5 mL of the stock solution of Pazopanib, 5 mL of 0.1N Hydrochloric acid solution was added and kept in water bath at 60ºC for 30 min, to this 5ml of 0.1N NaOH solution was added to neutralize and made up the volume with diluent to get the final concentration of 50µg/mL in a 100mL volumetric flask. From this, 20µL of the solution was injected and chromatograms were recorded to assess the stability of sample as shown in Fig 5.

Base Degradation:
To 5 mL of the stock solution of Pazopanib, 5mL of 0.1N NaOH solution was added and kept in water bath at 60ºC for 30 min, to this 5mL 0.1N Hydrochloric acid solution was added to neutralize and made up the volume with diluent to get the final concentration of 50µg/mL in a 100mL volumetric flask. From this, 20µL of the solution was injected and chromatograms were recorded to assess the stability of sample as shown in Fig 6.

Peroxide Degradation:
To 5mL of the stock solution, 5mL of 3% H_2O_2 solution was added and kept in water bath at 60ºC for 30 min and then made up the volume with diluent to get the final concentration of 50µg/mL in a 100mL volumetric flask. From this, 20µL of the solution was injected and chromatograms were recorded as shown in Fig 7.

Thermal Studies:
Small quantity of drug sample was placed in an oven at 105ºC for 48 hours, from this 25mg of thermal stressed drug powder transferred into the 25mL volumetric flask to get 1000µg/mL solution after diluted with a diluent. From the above solution, 5 mL was pipette out and transferred into 100 mL volumetric flask and then volume was made up with diluent to get the final concentration of 50µg/mL. 20µL of the above solution was injected and chromatograms were recorded to assess the stability of the sample as shown in Fig 8.

Photolytic Studies:
Small quantity of Pazopanib was exposed to U.V light by keeping the petridish in UV chamber for 7 days or 200 watt hours/m in a photo stability chamber. From this, 25 mg of stressed drug powder transferred into the 25mL volumetric flask to get 1000µg/mL solution after diluted with a diluent. From the above solution, 5 mL was pipette out and transferred into 100mL volumetric flask and then volume was made up with diluent to get final concentration of 50µg/mL. 20µL of the above solution was injected and chromatograms were recorded to assess the stability of the sample as shown in Fig 9.

RESULTS AND DISCUSSION

The detection response was measured at 270 nm with the retention time of 3.18 min for Pazopanib. System suitability results are shown in Table 3. The obtained standard and sample chromatograms are shown in the Fig 2 and Fig 3. Linearity was established in the concentration range of 30-80µg/mL, the calibration curve of Pazopanib was described by the equation y= 18020x + 91975, with correlation coefficient of 0.999 as in the Fig 4. The assay results and validation summary for the marketed samples are given in the Table 4. The accuracy data are reported in Table 5. The results of robustness studies are given in the Table 6.

System suitability studies:

Table 3: System suitability studies of Pazopanib

Parameter	Results
Retention time (min)	3.18
Tailing factor	1.29
Theoretical plates	4495
% RSD of peak area*	0.83

Specificity studies:

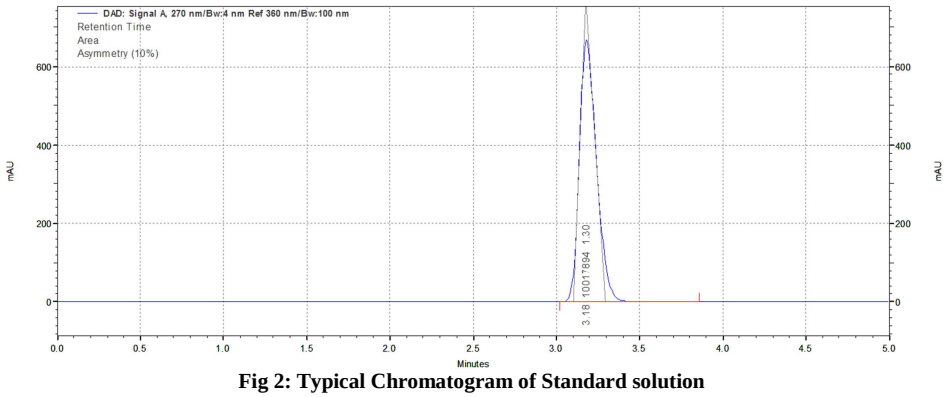

Fig 2: Typical Chromatogram of Standard solution

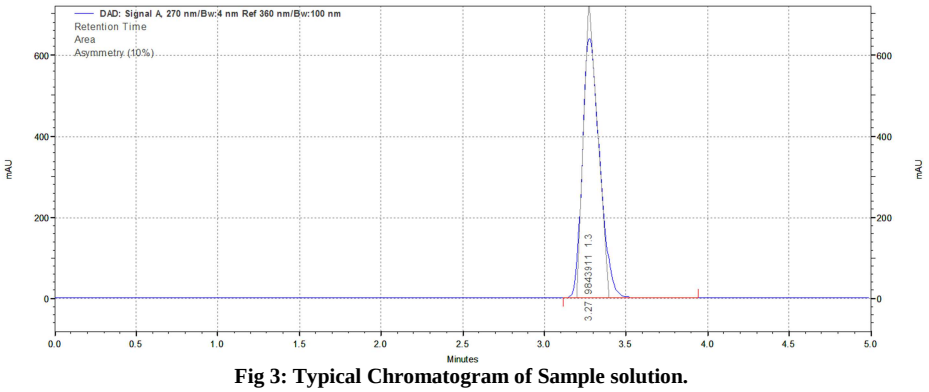

Fig 3: Typical Chromatogram of Sample solution.

Table 4: Inter & Intraday Precision of the proposed RP-HPLC method

Precision	%RSD of 6 Replicates
Retention time Intra-day	0.27
Peak area Intra-day	0.83
Retention time Inter-day	0.59
Peak area Inter-day	0.88
% Assay found	99.80%

Linearity:

The calibration curve was found to be linear in the concentration range of 30-80µg/mL for Pazopanib, with correlation co-efficient of 0.999.

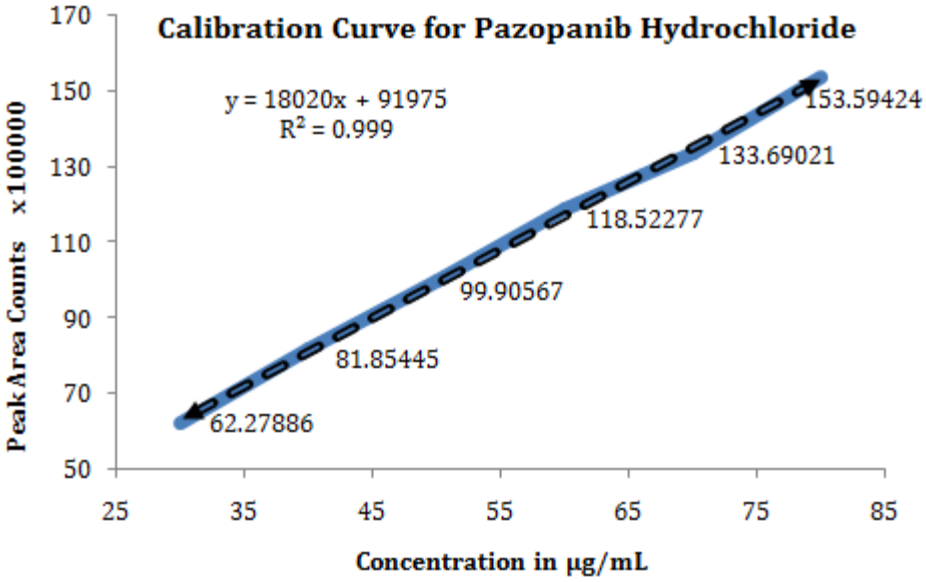

Fig 4: Linearity graph for Pazopanib

Accuracy (Recovery studies):

Table 5: Accuracy data for Pazopanib

Sample	Test concentration level	Triplicate Mean Peak area	Mean% recovery
Pazopanib	50%	5972066	99.41
	100%	10023880	100.11
	150%	14125902	100.77

** Mean of three standard injections*

Robustness:

The developed method for Pazopanib was found to be robust for deliberate changes with variations of flow rate, P^H of the buffer and mobile phase organic composition.

Table 6: Results of robustness study of Pazopanib

Parameter	Change Level	Pazopanib			
		RT in min.	Peak Area	USP Tailing	USP plate count
Flow Rate (±0.1mL/min)	0.8 ml/min	3.5	9649924	1.23	4098
	1.2 ml/min	2.86	10468274	1.26	4614
P^H of the Buffer	P^H 2.3	3.22	10251169	1.26	4454
	P^H 2.7	3.19	10015213	1.30	4364
Mobile phase composition	65:35 %v/v	3.46	10286837	1.32	4392
	45:55 %v/v	2.85	10360463	1.25	4467

Degradation studies of Pazopanib:

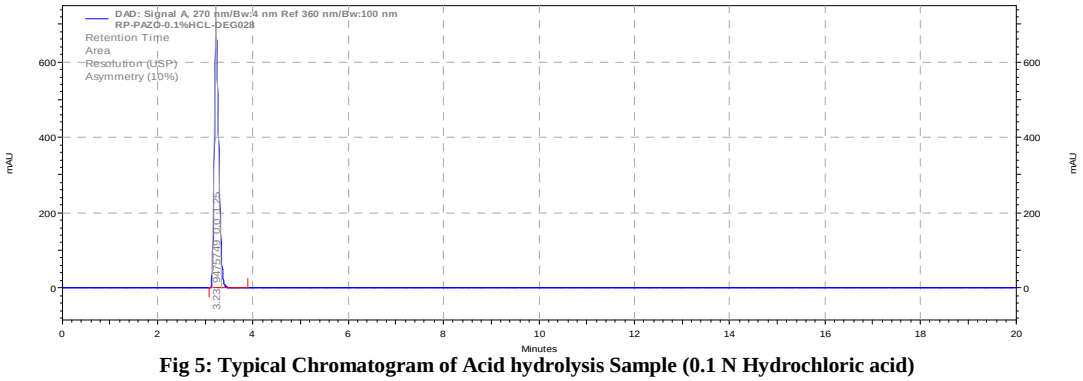

Fig 5: Typical Chromatogram of Acid hydrolysis Sample (0.1 N Hydrochloric acid)

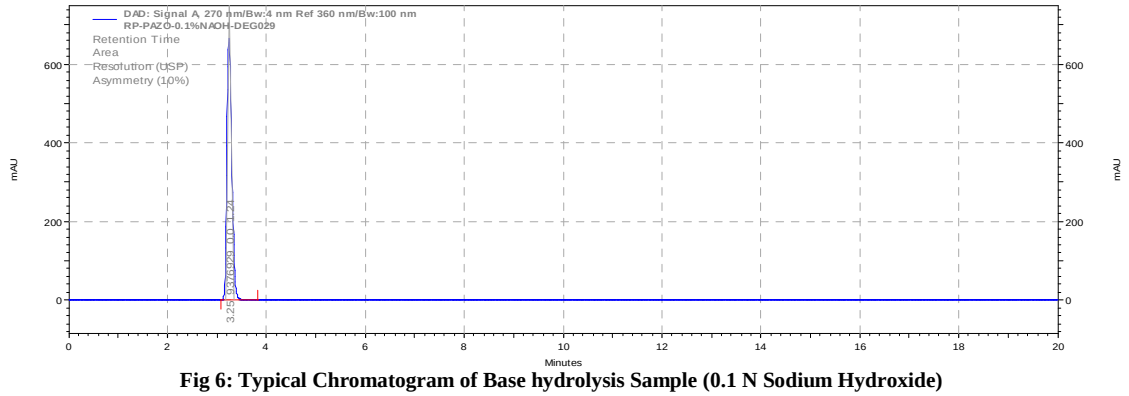

Fig 6: Typical Chromatogram of Base hydrolysis Sample (0.1 N Sodium Hydroxide)

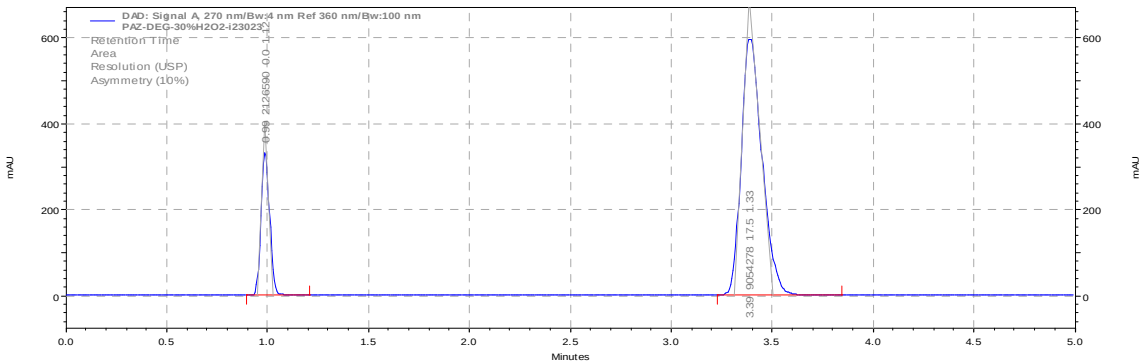

Fig 7: Typical Chromatogram of Oxidation stressed Sample (3%H_2O_2)

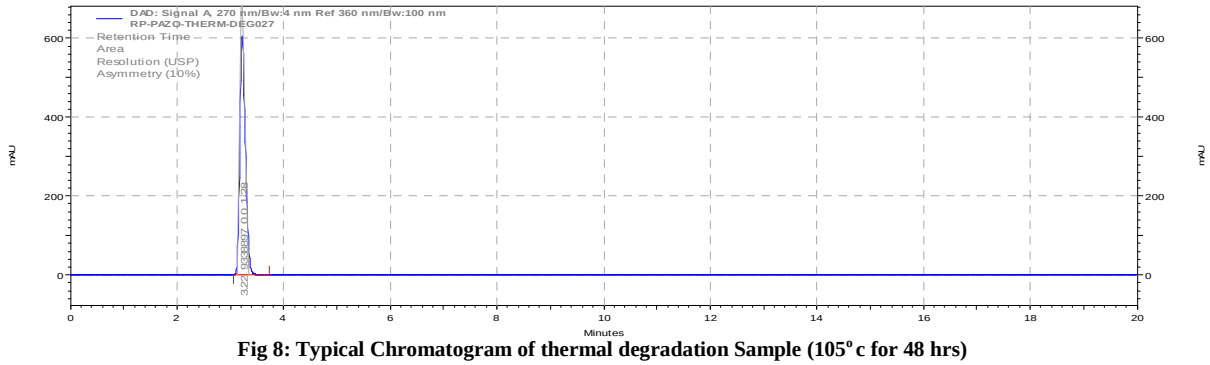

Fig 8: Typical Chromatogram of thermal degradation Sample (105°c for 48 hrs)

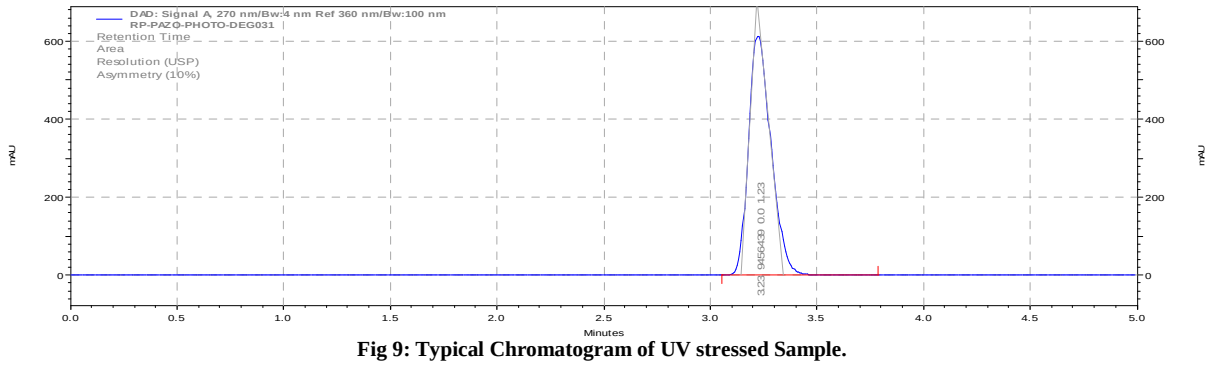

Fig 9: Typical Chromatogram of UV stressed Sample.

Table 7: Degradation studies of Pazopanib

Stress condition	Peak area	Degradation % assay	% net degradation
Acid Hydrolysis	9475749	94.64	5.27
Base Hydrolysis	9376929	93.65	6.17
Peroxide degradation	9054278	90.43	9.39
Thermal degradation	9338897	93.27	6.55
UV exposure	9456439	94.45	5.37

CONCLUSION

The results of statistical parameters and recovery studies reported were found to be satisfactory as per ICH guidelines. From this study, it is concluded that the proposed stability indicating RP-HPLC method was found to be accurate, precise, sensitive and useful for the routine analysis of Pazopanib Hydrochloride in bulk and pharmaceutical dosage forms.

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REFERENCES

[1] S. Tugus; S. Koch; L. Gualanli; L. Xiujuan. *molecular aspects of medicine,* **2011,** 88-111.
[2] G. Sonpavde; T. E. Hutson. *current Oncology report,* **2007,** 115-119.
[3] M. Minocha; Vkhurana; A. K. Mitra. *J chromatogr B analyt technol biomed life sci.,* **2012,** 85-92.
[4] P. Ravi pratap; A. S. Khan; J. Venkateswarao; K.Sujana. *International journal of advances in pharmaceutical analysis*, **2013,** 24-29.
[5] V O Escudero; J J perez Ruixo; B Valenzuela. *Ther Drug Monit.*, **2015,** 172-179.
[6] S. B Dharmesh; S.K Alireza. *Journal of Pharmaceutical and Biomedical Analysis*, 52(4), **2010,** 493-507.