Research Article

Development and Validation of a Stability-indicating Method for the Determination of Pazopanib Hydrochloride by RP-HPLC

P. Ravisankar*, M. Nithya Satya, K. Saisneha lata, A. Bhavani Sailu, Sk. Rijwana, K. Harsha Sri, K. Pujitha
Department of Pharmaceutical Analysis, Vignan Pharmacy College, Vadlamudi, Guntur-522 213, Andhra Pradesh, India.
***Corresponding author's E-mail:** banuman35@gmail.com

Received: 06-03-2021; **Revised:** 25-04-2021; **Accepted:** 30-04-2021; **Published on:** 15-05-2021.

ABSTRACT

A precise, accurate, highly sensitive, rapid, and reproducible stability HPLC method was developed and validated for the estimation of Pazopanib Hydrochloride (PAZO) in bulk and tablet dosage form. Decent quality chromatographic separation of Pazopanib Hydrochloride was done by using Eclipse plus C_{18} column (4.5 mm i.e. X 150 mm, 3.5µm particle size) (based on 99.99 % ultra-high purity silica) using mobile phase that containing 0.1 % Orthophosphoric acid: Acetonitrile (55:45 % v\v) at a flow rate of 1.0 mL/minute. The wavelength λ_{max} of PAZO used for the detection was found to be 271.4 nm. The retention time for Pazopanib was found to1.43 minutes. The PAZO was linear in the concentration range of 2-10 µg/mL (r^2 = 0.9999) for HPLC method. The regression equation for PAZO was found to be Y = 700955 x + 28022 for HPLC. The LOD and LOQ were found to be 0.1675 µg/mL, 0.0552 µg/mL for the HPLC method, respectively. The developed methods are validated in pursuance of ICH Q2 (R1) guidelines. The method was linear, precise, accurate with recoveries in the range of 98 – 102 %, and minimum values of % RSD indicate the accuracy of the method. The % assay of the PAZO was found to be 99.85 ± 1.2, which was in good agreement with the labeled claim. Pazopanib was subjected to stressed conditions like acidic, basic, oxidative, photolytic, and thermal conditions. The degradation results were found satisfactory. The developed gradient RP-HPLC method can be successfully practiced for the routine quality control analysis of PAZO in pharmaceutical tablets and routine quality control analysis.

Keywords: Pazopanib Hydrochloride, forced degradation, RP-HPLC, Method validation.

QUICK RESPONSE CODE →

DOI: 10.47583/ijpsrr.2021.v68i01.035

DOI link: http://dx.doi.org/10.47583/ijpsrr.2021.v68i01.035

INTRODUCTION

The chemical name for PAZO Fig. 1**,** 5-[[4-[(2, 3-dimethylindazol-6-yl)-methyl amino] pyrimidin-2-yl] amino]-2-methylbenzenesulfonamide. It has the molecular formula $C_{21}H_{23}N_7O_2S.HCl$ and a molecular weight of 473.991 PAZO are used to treat advanced Kidney cell Carcinoma and it is also used to treat advanced soft tissue and it is also potent a selective multi-targeted receptor tyrosine kinase inhibitor that blocks tumor growth and inhibits angiogenesis.

As per the Literature Survey, it is revealed that the drug has been estimated by LC-MS/MS[1-5], UPLCQ-TOF/MS[6], High-Performance Liquid Chromatography -UV[7-11], Ultra Violet[12-13] But only a few stability-indicating methods for the determination of PAZO HPLC analysis has been reported for the estimation in pharmaceutical dosage forms. Mostly, HPLC has proven to be valuable in diagnostic purposes and the pharmaceutical industry[14-16].

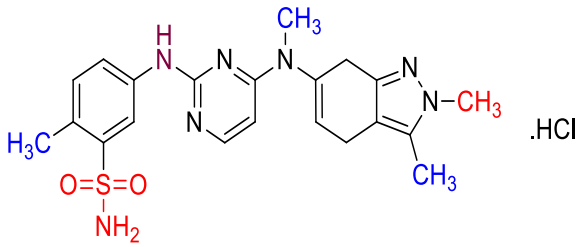


Figure 1: Chemical Structure of Pazopanib hydrochloride

MATERIALS AND METHODS

Chemicals and Reagents

A sample of PAZO was gifted from Hetero Labs Ltd., Hyderabad, India PAZO (Pazopanib Hydrochloride 200 mg.) was purchased from a local pharmacy. HPLC grade Methanol, HPLC grade Acetonitrile, High purity water was prepared using Milli Q purification system from Millipore, Bangalore, India, and AR grade Sodium Hydroxide, AR grade Hydrogen Peroxide were purchased from Merck specialties Pvt. Ltd., Mumbai, India.

Instrumentation

For UV detection of the sample, an ELICO SL-210 UV spectrophotometer with 1 cm matched quartz cells were used for all spectral and absorbance measurements, and solutions were prepared in methanol. For HPLC, the chromatographic system consists of Agilent HPLC quaternary-1260 Infinite- II

series, Eclipse plus C_{18} column, 1260 series with diode array detector was used for higher data quality for more confidence. For homogenizing the solution prepared, Ultra-Sonicator of Spectral labs, model UCB 40 was used. For weighing of the sample and excipients, Radwag analytical balance AS 82/220.R_2) (0.01g), was used. For measuring the pH of the prepared solutions pH meter, the Systronics model – 802 was used. The data was acquired and processed by utilizing EZ chrome elite software.

Method development and optimization of chromatographic conditions

For HPLC development, a variety of mobile phases containing HPLC grade water, acetonitrile, methanol in different ratios with or without buffers, and also various flow rates were performed. A good symmetrical peak was found when the mobile phase containing a mixture of 0.1 % Orthophosphoric acid: Acetonitrile (55:45 % v\v)

Selection of detection wavelength

To estimate the maximum λ_{max}, PAZO 100 µg/ml of working standard solution was prepared and scanned in a UV wavelength range of 200 - 400 nm utilizing as a blank. It was observed that the drug showed maximum absorbance at 273 nm in methanol. 0.1% Orthophosphoric acid: acetonitrile in the ratio of 55:45 v/v, in which the detection wavelength was found to be 271.4, so for HPLC this wavelength was chosen as the detection wavelength for the determination of PAZO. As a matter of fact, in this wavelength exquisite symmetric peak is obtained. The overlay spectrum of PAZO is shown in Fig. 1a.

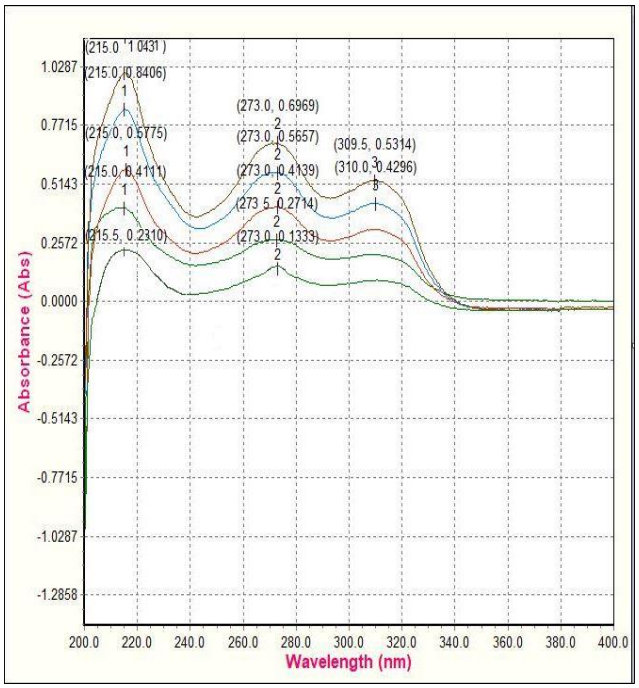


Figure 1a: Overlay Spectrum of Pazopanib Hydrochloride for UV method

Preparation of the mobile phase

The mobile phase was prepared by mixing 0.1 % Orthophosphoric acid and acetonitrile in the proportion of 55: 45 v/v. The prepared mobile phase was filtered through a 0.45 µm nylon membrane filter and degassed by sonication.

Preparation of Stock and Working standard solution

Precisely weighted 100 mg of PAZO was transferred into 100 mL volumetric flask, dissolved, and diluted up to the mark with mobile phase to get a stock solution containing 1.0 mg/mL of PAZO. Aliquots of stock solution were diluted with mobile phase to attain the calibration standard solutions over the range of 2, 4, 6, 8, and 10 µg/mL.

Preparation of sample solution

For the assay of pharmaceutical formulation, 20 tablets of PAZO marketed formulation (Votrient 200 mg) were weighed, the average weight was calculated, and a quantity of tablet powder equivalent to 100 mg of PAZO was accurately weighed and transferred into a 100 mL volumetric flask containing 30 mL of the mobile phase. The solution was ultra-sonicated for about 15 minutes, filtered through a What man filter paper (0.45 µm) nylon filter and the filtrate was made up to volume with the mobile phase. The concentration was 1mg/mL. Transfer 1 ml of the filtered sample solution to 10 mL volumetric flask and made up to volume with mobile phase to get a solution of 100 µg/mL. The solution is further diluted with mobile phase to obtained the required concentrations. This is used as a working solution for the preparation of the assay. Then 0.2 ml of this solution is transferred into a 10 ml volumetric flask and made up to volume to obtain 2 µg/mL which is used for the assay. The assay results are presented in Table 4. Representative in Assay results of PAZO by HPLC. Solutions were injected as per the above chromatographic conditions and peak areas were recorded. The quantifications were carried out by keeping these values to the straight-line equation of the calibration curve.

Method development optimization

The optimized HPLC conditions of several mobile phases with different compositions were tested to develop an optimization of chromatographic conditions like tailing factor, peak shape, and the number of theoretical plates. For the selection of the mobile phase, primarily methanol: acetonitrile, ethanol: water, acetonitrile: water has been tested for different compositions. Eventually, the gradient mode and mobile phase containing a mixture of 0.1 % Orthophosphoric acid: Acetonitrile (55:45 % vlv) at a flow rate of 1 mL/ minute was found to be satisfactory and proper system suitability parameters obtained. Optimized chromatographic conditions, system suitability parameters for estimation of PAZO by proposed gradient RP-HPLC method are depicted in Table 1**.**

Table 1: Optimized chromatographic conditions for the proposed HPLC method

Parameter	Chromatographic conditions
Instrument	Agilent HPLC quaternary-1260 Infinite- II series
Column	Eclipse plus C_{18} column (4.5 mm i.d. X 150 mm, 3.5 µm particle size) (based on 99.999 % ultra-high purity silica)
Detector	1260 Diode Array Detector.
Mobile phase	0.1 % Orthophosphoricacid:Acetonitrile (55:45 %v\v).
Flow rate	1 mL/minute
Detection wavelength	In methanol, the wavelength was 273 nm, in HPLC mobile phase 271.4 nm.
Run time	5 minutes
Temperature	Room temperature (25 °C).
The volume of injection loop	10 µL
Retention time (R_t)	1.403 minutes
Theoretical plates [th.pl] (Efficiency)	3863
Theoretical plates per meter [t.p/m]	77360
Tailing factor (asymmetry)	1.12

Method Validation [17-20]

Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its pre-determined specifications and quality characteristics. The method was validated as per ICH guidelines.

System suitability

System suitability parameters can be defined as tests to ensure that the method can generate results of acceptable accuracy and precision. The requirements for system suitability are usually developed after method development and validation have been completed. The system suitability parameters like theoretical plates, retention time, tailing factor, were studied and found satisfactory. The results show the system suitability parameters in Table 1a.

Table 1a: System suitability parameters

System Suitability parameters	Limits	Pazopanib Hydrochloride
Tailing factor (T)	≤ 2.0	1.12
Number of theoretical plates	NLT 2000	3863
Theoretical plates per meter (N)*	-	77,360
Retention time*	-	1.430 minutes
SD for peak area for RT		0.00158
% RSD	NMT 2.0	0.112

* Average of five determinations, SD = Standard deviation, RSD = relative standard deviation.

The result is well within the acceptance criteria, and the study concludes the suitability of the analytical system for the analysis.

Precision

System precision

For RP-HPLC, six replicate injections of standard 10 µg/mL and blank were injected into the HPLC system. The system precision responses are represented in table 2.

Table 2: System Precision

Injection No.	Area Response
1	6978057
2	6979165
3	6978054
4	6977163
5	6978274
6	6979385
Mean	6978350
% RSD	0.011683

Method precision & intermediate precision

For method precision, six test preparations were analyzed as per the methodology representing a single batch, and the assay was determined for the same. The % RSD for assay of six test preparations should not be more than 2.0. The results are well within acceptance criteria, and the % RSD observed for assay values indicates the precision of the method.

For intermediate precision, six sample preparations were made and analyzed as per the method by different analysts using various instruments and different columns on different days. The % RSD values for assay of 6 test preparations should not be more than 2.0. Cumulative % RSD of 12 determinations, ie., method and intermediate precision should not be more than 2.0. The results are well within acceptance criteria, and the % RSD observed for

assay indicates the precision of the method. The resultant values for method precision & Intermediate precision are depicted in Table2a.

Table 2a: Complied data of method precision & intermediate precision

Injection No.	Method Precision	Intermediate Precision
1	98.2	99.2
2	99.5	99.5
3	98.3	98.1
4	99.3	98.4
5	99.0	99.3
6	98.6	99.5
Mean	98.81667	99.00
% RSD	0.54087	0.60
Cumulative	**RSD**	1.1205

Precision at different levels

Precision at different levels of the analytical method was determined in the concentration range of 50 %, 100 %, 150 %, their values are portrayed in Table 2b.

Table 2b: Precision at 50 %, 100 %, 150 % (Precision at different levels)

S. No.	50 %	100 %	150 %
1	1405868	2895311	427689
2	1407879	2874522	428864
3	1416558	2881294	428513
4	1405689	2887523	428567
5	1404567	2891485	427614
6	1406776	2886454	428954
Mean	1407890	2886098	428366.8
% RSD	0.31179	0.25609	0.13530

Linearity

The linearity of PAZO was determined in the concentration range of 2 to 10 µg/mL. The calibration graph of PAZO is shown in Fig. 2. The linearity data is presented in Table 2c, and the summary output of ANOVA study of PAZO is presented in Fig. 2a.

Table 2c: Linearity data of Pazopanib Hydrochloride by HPLC

S. No.	Concentration	Area
1.	0	0
2.	2	1405868
3.	4	2895311
4.	6	4231585
5.	8	5685969
6.	10	6978057
7.	Intercept	28022
8.	Slope	70095

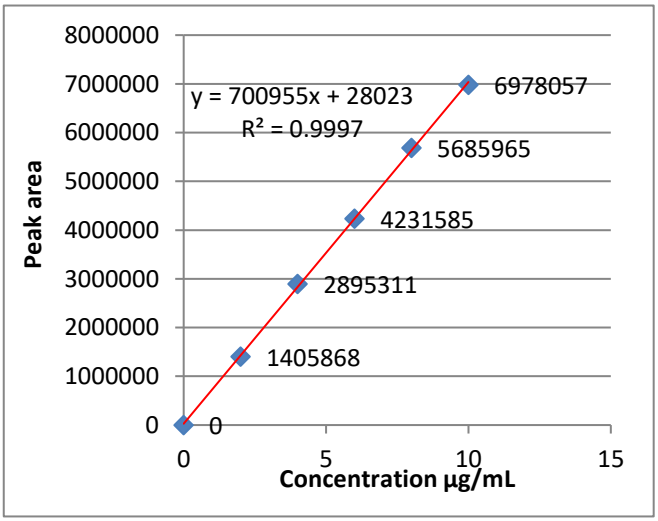


Figure 2: Calibration graph of Pazopanib Hydrochloride by RP- HPLC

	A	B	C	D	E	F	G	H	I
1	SUMMARY OUTPUT								
2									
3	*Regression Statistics*								
4	Multiple R	0.9998333							
5	R Square	0.999666627							
6	Adjusted R Square	0.999583284							
7	Standard Error	53548.44877							
8	Observations	6							
9									
10	ANOVA								
11		*df*	*SS*	*MS*	*F*	*Significance F*			
12	Regression	1	3.43937E+13	3.43937E+13	11994.56569	4.1681E-08			
13	Residual	4	11469745461	2867436365					
14	Total	5	3.44051E+13						
15									
16		*Coefficients*	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
17	Intercept	28022.66667	38755.52189	0.723062555	0.509659968	-79579.9124	135625.25	-79579.9124	135625.25
18	X Variable 1	700955	6400.263795	109.5197046	4.16813E-08	683185.019	718724.98	683185.019	718724.98

Figure 2a: The summary output of ANOVA study of Pazopanib Hydrochloride

Accuracy (Recovery studies)

A known amount of drug was spiked with placebo at three different levels in triplicate preparations. The samples were then analyzed as per the proposed standard method. The accuracy studies of PAZO are mentioned in Table 2d.

Robustness

The robustness of the method was determined for the system suitability and assay value under variable conditions. The robustness of the analytical method was established by demonstrating its reliability against deliberate changes in the chromatographic conditions. The robustness of the method of PAZO is mentioned in Table 3.

Table 2d: Accuracy study of Pazopanib Hydrochloride

% Recovery	Level	Amount Added (mg)	Amount recovered (mg)	% Recovery
50 %	1	12.13	12.08	99.4
	2	12.19	12.10	99.1
	3	12.07	11.95	99.5
100 %	1	24.50	24.10	98.1
	2	24.63	24.48	99.2
	3	24.70	24.51	99.6
150 %	1	36.90	36.76	99.7
	2	37.01	36.80	99.7
	3	36.79	36.71	99.4

LOD and LOQ

Limit of Detection is the lowest concentration in a sample that can be detected but not necessarily quantified under the stated experimental conditions. The limit of Quantitation is the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy. The LOD and LOQ are shown in Table 3a.

Table 3a: LOD and LOQ results of Pazopanib Hydrochloride by HPLC

Limit of Detection (LOD)	0.1675 µg/mL
Limit of Quantitation (LOQ)	0.0552 µg/mL

Forced degradation studies:

Forced degradation studies such as acid, base, peroxidase, dry heat, UV light have been carried to establish specificity and stability-indicating the nature of the method. The studies found that there is not much degradation occurs during the acid, base, peroxidase degradations. In fact, there are no co-eluting peaks with the Pazopanib. Peak purity of PAZO was found to be less than the purity threshold. Results of the forced degradation studies have been tabulated in Table 3b.

Analysis of Pazopanib Hydrochloride in tablet formulation

The developed and validated method was successfully applied for the determination of PAZO in their tablet dosage form. The assay result Table 4 shows that the amount of the drug was in excellent agreement with the labeled value of the formulation. The representative sample chromatogram of PAZO is shown in Fig. 4.

Table 3: Robustness results of Pazopanib Hydrochloride.

Parameters	Optimized	Used	Retention time (min)	Plate count $	Peak asymmetry #	Remarks
Flow rate (± 0.2 mL/min)	1.0 mL/min	0.8 mL/min	1.51 min	4756	1.249	*Robust
		1.0 mL/min	1.40 min	3848	1.127	*Robust
		1.2 mL/min	1.25 min	2736	1.016	*Robust
Detection wavelength (±5 nm)	271nm	260 nm	1.40 min	3847	1.249	*Robust
		271nm	1.43 min	3848	1.127	*Robust
		270 nm	1.40 min	3846	1.016	*Robust
Mobile phase composition 0.1% orthophosphoricacid:CAN (55:45 % v/v)	55:45 v/v	55:45 v/v	1.40 min	3848	1.127	*Robust
		50:50 v/v	1.40 min	3847	1.016	*Robust

Acceptance criteria (Limits): #Peak Asymmetry < 1.5, $ Plate count > 2000, * Significant change in Retention time.

Table 3b: Forced degradation studies

S.No	Stress conditions	% Assay	Peak Purity Angle	Peak purity threshold
1	2N HCl for 30 minutes at 60°C	95.20	0.157	0.261
2	2N NaOH for 30 minutes at 60°C	97.12	0.222	0.264
3	20% H_2O_2 for 30 minutes at 60^0C	93.90	0.212	0.266
4	Dry heat 105^0C for 6hrs	98.64	0.058	0.262
5	UV light 200 wts/hr or 7days	99.04	0.067	0.326

Table 4: Assay results of Pazopanib Hydrochloride by HPLC

S. No.	Formulation	Labeled claim	Amount found*	Mean % recovery ± SD	% RSD
1	Pazopanib Hydrochloride tablets (Votrient)	200 mg/tablet	198.8 mg/tablet	99.4 ± 12	**1.13**

* Average of six determinations, SD denotes standard deviation; RSD denotes % relative standard deviation.

RESULTS AND DISCUSSION

Since the above-mentioned PAZO is polar, and RP-HPLC method was used. The column for the separation was a C_{18} column that has an internal diameter of 4.5 mm, length of 150 mm, and 3.5µm particle size. Multiple numbers of trials were performed using various buffer solutions with various compositions of methanol, ethanol, acetonitrile, and HPLC grade water and variable flow rates. Eventually, optimum separation was obtained with a mixture of 0.1 % Orthophosphoric acid: Acetonitrile (55:45 % v/v). The mobile phase flow rate was adjusted at 1mL/min, and the detection wavelength was set at 271.4 nm. Thus, a proper chromatographic peak was obtained with excellent symmetry and the least peak tailing. The chromatograms of various concentrations were shown in Fig. 3 – Fig. 3d.

System suitability was accompanied as per the methodology system suitability solution, and six replicate of standard preparation was injected into HPLC. The tailing factor was found to be 1.122. The number of theoretical plates was 3863; the number of theoretical plates per meter was 77,360. The retention time was found out to be 1.430 minutes, and the % RSD was calculated to 0.112. The results were well within the acceptance criteria, and the study concludes the suitability of the analytical system for analysis.

The precision of the method was examined by using System precision, Method, and Intermediate precisions. Various levels of concentration were taken in six replicate samples. For Method and Intermediate precisions, the %RSD was found to be 0.54087 and 0.60606. The % RSD of the System precision was found to be 0.01168. The precision at different levels was mentioned in Table 3b. The results are well within the acceptance criteria, and the % RSD observed for the replicate injections indicates the precision of the HPLC used, assay values indicate the precision of the method.

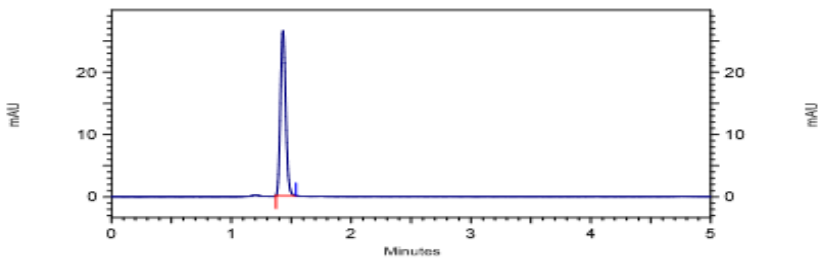
The linearity of PAZO was determined in the concentration range of 2 µg/mL to 10 µg/mL of the test concentration. The squared correlation coefficient value was found to be 0.999, which is well within the limit. The results of the linearity studies were mentioned in Table 2c.

To determine the accuracy of the PAZO, the drug was spiked with a placebo at three different levels in triplicate preparations. The results of accuracy are mentioned in the Table, and the results of precision at accuracy are given in the Table. The mean % recovery at each level was found out to be within limits i.e., 98.0 % to 102.0 %

The robustness of the HPLC was determined for the suitability and assay value under multiple variable conditions like Flow rate change, Wavelength change, and change in mobile phase composition. The results are mentioned in Table 3.

The Limit of Detection and Limit of Quantitation of PAZO were found out to be 0.1675 µg/mL and 0.0552 µg/mL, respectively, which is represented by Table 3a.

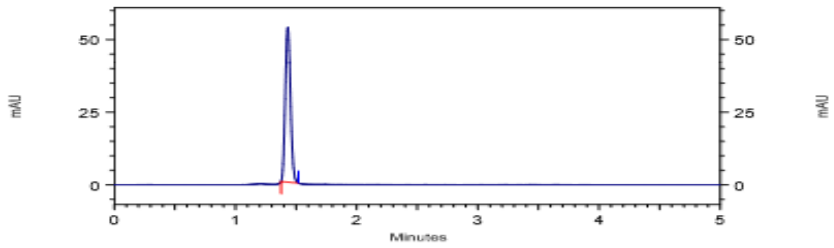
The % assay of the PAZO was found to be 99.4 ± 1.2, (Table 4) which was in good agreement with the labeled claim. The method was specific and has no interference observed when the PAZO was determined in presence of excipients.



VWD: Signal A, 271 nm Results

Retention Time	Area	Theoretical plates (USP)	Asymmetry
1.430	1364103	4948	1.17478
Totals	1364103		

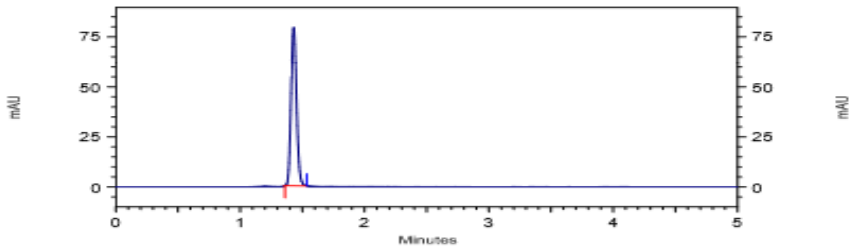
Figure 3: Standard chromatogram of Pazopanib Hydrochloride (2 µg/mL)



VWD: Signal A, 271 nm Results

Name	Retention Time	Area	Asymmetry (10%)	Theoretical plates (USP)
PAZOPANIB	1.430	2722615	1.15074	4982
Totals		2722615		

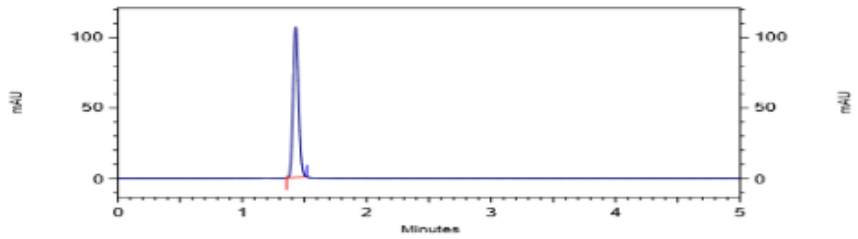
Figure 3a: Standard chromatogram of Pazopanib Hydrochloride (4 µg/mL)



VWD: Signal A, 271 nm Results

Name	Retention Time	Area	Asymmetry (10%)	Theoretical plates (USP)
PAZOPANIB	1.430	4096869	1.11325	4931
Totals		4096869		

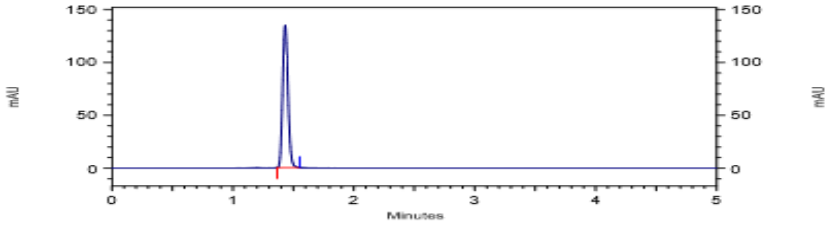
Figure 3b: Standard chromatogram of Pazopanib Hydrochloride (6 µg/mL)



VWD: Signal A, 271 nm Results

Name	Retention Time	Area	Asymmetry (10%)	Theoretical plates (USP)
PAZOPANIB	1.430	5505696	1.11159	4947
Totals		5505696		

Figure 3c: Standard chromatogram of Pazopanib Hydrochloride (8 µg/mL)



VWD: Signal A, 271 nm Results

Name	Retention Time	Area	Asymmetry (10%)	Theoretical plates (USP)
PAZOPANIN HCL	1.433	6978057	0.00000	
Totals		6978057		

Figure 3d: Standard chromatogram of Pazopanib Hydrochloride (10 µg/mL)

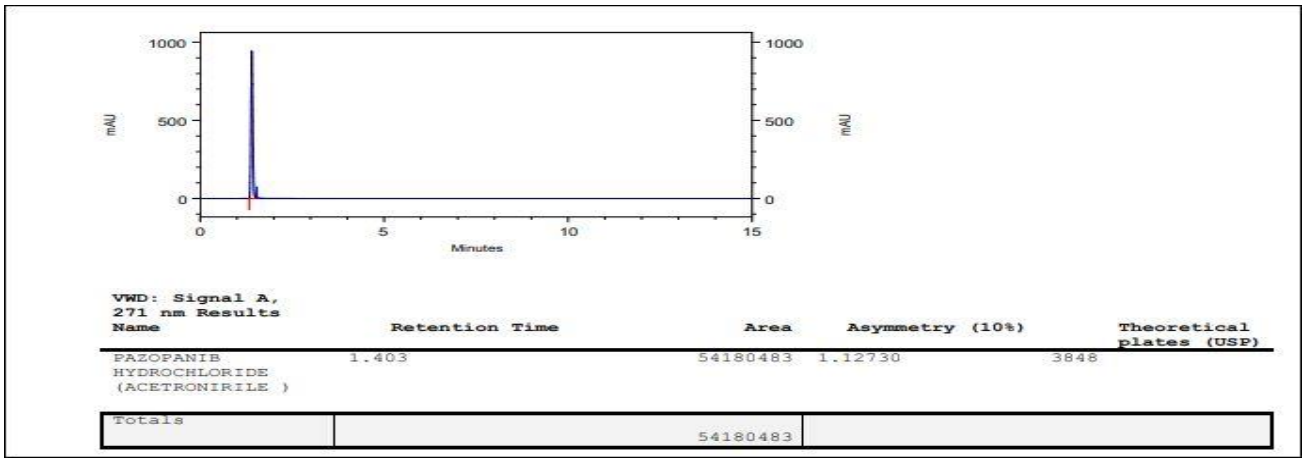


Figure 4: Sample chromatogram of Pazopanib Hydrochloride (Votrient)

CONCLUSION

The existent study demonstrated a validated Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method for the estimation of PAZO available as the tablet dosage form. 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 determination of drugs in the tablet dosage form. The method was wholly validated and showed satisfactory results. The method was free from the interference of the other active ingredients and additives used in the formulation. The RP-HPLC method for the estimation of PAZO has various advantages like less solvent consumption, low retention time, good peak symmetry, precision, accuracy, and robustness. 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 quality control analysis of PAZO in active pharmaceutical preparations.

Acknowledgement: The authors are thankful to Hetero Labs for providing the sample of PAZO. We are very grateful to Dr. L. Rathaiah, esteemed chairman of Vignan group of institutions, Vadlamudi, Guntur, for providing us the essential laboratory facilities to carry out the current investigation.

REFERENCES

1. Remy B Verheijen, BasThijssen, Hilde Rosing, Jan H M Schellens, Lianda Nan, Nikkie Venekamp, Jos H Beijnen, Neeltje Steeghs, Alwin D R Huitema, Fast and Straight forward method for the quantification of Pazopanib in human plasma using LC-MS/MS, Therapeutic Drug Monitoring, 2018;40(2):230-236. doi: 10.1097/FTD.0000000000000479.
2. Remy B Verheijen, Sander Bins, Development and clinical validation of an LC-MS/MS method for the quantification of Pazopanib in DBS. bioanalysis, 2016;8(2):123-34.
3. Mukul Minocha, Varun Khurana, Determination of Pazopanib (GW-786034) in Mouse Plasma and Brain Tissue by Liquid Chromatography-Tandem Mass Spectrometry (LC/MS-MS), J Chromatogr B Analyst Technol Biomed Life Sci. 2012;90:85-92.
4. Rolf W Sparidans, Tahani T A Ahmed, Liquid Chromatography-Tandem Mass Spectrometric Assay for Therapeutic Drug Monitoring of the Tyrosine Kinase inhibitor Pazopanib in Human Plasma. J Chromatogr B Analyst Technol Biomed LIFE Sci, 2012;905:137-40.
5. Claire Pressiat, Huu-Hien Huynh, Alain Ple, Development and Validation of a Simultaneous Quantification Method of Ruxolitinib, Vismodegib, Olaparib, and Pazopanib in Human Plasma Using Liquid Chromatography Coupled With Tandem Mass Spectrometry, The Drug Monit. Journal, 2018;40(3):337-343.
6. Prinesh N Patel, Pradipbhai D Kalariya, Characterization of Forced Degradation Products of Pazopanib Hydrochloride by UHPLCQ-TOF/MS and in Silico Toxicity Prediction. Journal of Mass Spectrometry, 2015;50(7):918-28.
7. Vanesa Escudero-Ortiz, Development and Validation of an HPLC-UV Method for Pazopanib Quantification in Human Plasma and application to Patients with Cancer in Routine Clinical Practice, Therapeutic Drug Monitoring.2015;37(2):172-9.
8. Musty Sharada, Ravichandra Babu R, Determination and characterization of process impurities in Pazopanib hydrochloride drug Substance, International Journal of Pharmacy and Pharmaceutical Sciences, 2016;8:0975-1491.
9. Ravi Sankar P, Saisneha Latha K, Bhavani Sailu A, Taheera SK, Madhuri B, Development and Validation of RP-HPLC Method for the Determination of Pazopanib Hydrochloride (A Tyrosine Kinase Inhibitor) in Pharmaceutical Dosage Form, Research J. Pharm. and Tech 2021;14(3):1549-1554.

10. Chaitanya G, Prasanna Kumar K, Harini U, Lingam M, and K. M. Pawar A, Development and validation of rapid RP HPLC-PDA method for the analysis of Pazopanib hydrochloride in bulk, dosage forms and in in vitro dissolution samples, India Journal of Chemical and Pharmaceutical Research, 2015;7(12):950-960.

11. Ravisankar P, D Rao G, N Kumar M, Chaitanya MK, An improved RP-HPLC method for the quantitative determination of capecitabine in bulk and pharmaceutical tablet dosage form, Journal of Chemical and Pharmaceutical Research, 2013;5(3):249-260.

12. Chaitanya G and Pawar AKM, Development and Validation of UV spectrophotometric method for the determination of Pazopanib hydrochloride in bulk and tablet formulation, Journal of Chemical and Pharmaceutical Research, 2015;7(12):219-225.

13. Saisneha Latha K, Ravisankar P, Srinivasa Babu P, Development and validation of UV spectrophotometric method for the determination of Pazopanib hydrochloride in pharmaceutical dosage form, Int. J.Pharm. Sci.Rev, Res., 2020;61(2):13-18.

14. Ravi Sankar P, Sai Snehalatha K, Tabassum Firdose Shaik, Srinivasa Babu P, Applications of HPLC in Pharmaceutical Analysis, Int.J.Pharm.Sci.Rev.Res. 2019;59(1):117-124.

15. Bokai Ma, XinLei Gou, Application of high-performance liquid chromatography in food and drug safety analysis, Journal of food safety and quality, 2016;7:295-4298.

16. Ravi Sankar P, Rajyalakshmi G, Devadasu Ch, Devala Rao G, Instant tips for a right and effective approach to solve HPLC troubleshooting, Journal of Chemical and Pharmaceutical Sciences, 2014;7(3):191-206.

17. Ravisankar P, Naga Navya Ch, Pravallika D, Navya Sri D, A review on step-by-step analytical method validation, IOSR Journal of Pharmacy, 2015;5:7-19.

18. Ravisankar P, Gowthami S, Devala Rao G, A review on analytical method development, Indian journal of research in pharmacy and Biotechnology, 2014;2:1183-1195.

19. Ravisankar Panchumarthy, Anusha S, Supriya K, Ajith Kumar U, Fundamental chromatographic parameters, Int. J. Pharm. Sci. Rev, Res. 2019;55(2):46-50. Ravisankar P, Sai Geethika A, Racgana G, Srinivasa Babu P, Bhargavi J, Bioanalytical method validation: A comprehensive review, Int.J. Pharm. Sci. Rev. Res, 2019;56(1),50-58.

20. ICH Q2 (R1), Validation of analytical procedures, Text, and methodology, International Conference on Harmonization, Geneva, 2005,1-17.

Source of Support: None declared.

Conflict of Interest: None declared.