Article published on 2024-8-6

Stability Indicating UPLC Method Development and Validation for the Quantitative Estimation of Pazopanib in Pure form and Marketed Pharmaceutical Dosage form

Nerella Naveen Kumar[1*], Dr. Shailesh Sharma[2]

[1]Research scholar, Department of Pharmacy, Shyam university, Dausa-303511, Rajasthan, India
[2]Principal and Professor, Department of Pharmacy, Shyam university, Dausa-303511, Rajasthan, India

***Correspondence Author:**
Nerella Naveen Kumar
Research scholar, Department of Pharmacy, Shyam university, Dausa-303511, Rajasthan, India

Chinese Journal of Applied Physiology, 2024: e20240018

Abstract An analytical, accurate, precise, specific, efficient and simple Ultra-Performance Liquid Chromatography method has been developed and validated for the determination of Pazopanib in bulk and was applied on marketed Pharmaceutical Dosage form. The mobile phase used for the chromatographic runs consisted of 0.1% OPA Buffer and Acetonitrile in the ratio of 30:70% v/v. The separation was achieved on a BHEL UPLC column using isocratic mode. Pazopanib Drug peak were well separated and were detected by a PDA detector at 256 nm. The developed method was linear at the concentration range 6–14 µg/ml for Pazopanib. The method has been validated according to ICH guidelines with respect to system suitability, specificity, precision, accuracy and robustness. The LOD and LOQ for the Pazopanib were found to be 0.5853 µg/ml and 1.7738µg/ml respectively. The developed method is simple, precise, specific, accurate and rapid, making it suitable for estimation of Pazopanib in bulk and marketed pharmaceutical dosage form dosage form.

Keywords Pazopanib, UPLC, Accuracy, Precision, Robustness, ICH Guidelines

INTRODUCTION

Pazopanib is a second generation Tyrosine Kinase Inhibitor (TKI).1 It used in the treatment of ovarian, renal, colon, neck and head, lung and prostate cancer.2,3 Pazopanib is a potent and selective multi-targeted, tyrosine kinase inhibitor of vascular endothelial growth factor receptor-1 (VEGFR-1), VEGFR-2, VEGFR-3 and PDGFR-α/β1.4 It also behaves like a stem cell growth factor receptor (c-kit) that blocks tumor growth and ceases angiogenesis.5 Literature survey reveals several analytical methods have been developed for estimation of Pazopanib

DOI: 10.62958/j.cjap.2024.018
www.cjap.ac.cn

Published by CJAP editorial office and Asian BioMed Innovation Press

in pharmaceutical dosage forms and biological samples including HPLC,6,7 simultaneous estimation of Pazopanib by HPLC.8,9 However, these reported chromatographic methods for estimation of Pazopanib possess multiple drawbacks like sample preparation, low sensitivity, complex mobile phase mixture, strict monitoring of critical method parameters like mobile phase, flow rate, column temperature, flow gradient, maintenance of pH, etc. This calls for the development of a simple, rapid, sensitive, efficient and reliable UPLC method for quantification of Pazopanib in bulk and pharmaceutical dosage forms. The validation of the proposed method was carried out according to ICH guideline ICH Q2 (R1).10 Molecular formula and molecular weight of Pazopanib are C21H23N7 O2 S and 437.52gm/mol.11 It is soluble in water and acetonitrile. Chemically Pazopanib (Figure 1) is known as 5[{4(2,3-dimethyl-2H-indazol-6-yl) methylamino}2-pyrimidinyl]2-methylbenzenesulfonamide.

MATERIALS AND METHODS

Chemical and reagents Reference standard of Pazopanib was used to develop the new UPLC method. Acetonitrile was obtained from Sd Fine chem. Ltd (India). Water for UPLC was prepared using Milli Q Water (Merk). Pazopanib HCl is commercially available as Votrient® marketed by GSK Rx India with a labeled claim of 200mg per tablet.

METHOD DEVELOPMENT

Chromatographic Parameters

Equipment: Ultra performance liquid chromatography equipped with Auto Sampler and PDA detector
Column: BHEL UPLC COLUMN
Elution Mode: Isocratic
Flow rate: 0.25 mL per min
Wavelength: 256 nm
Injection volume: 5 µl
Column temperature: Ambient
Run time : 2 min

Preparation of 0.1% OPA Buffer pH-3

To prepare 0.1% OPA buffer solution, by adding 1ml of ortho phosphoric acid in 1000ml water. Adjust this solution to pH 3 by using sodium hydroxide.

Preparation of Mobile Phase

Mix a mixture of 0.1% OPA buffer 300 ml (30%) and 700 ml Acetonitrile UPLC (70%) and degas in ultrasonic water bath for 5 minutes. Filter through 4.5 µ filter under vacuum filtration.

Diluents Preparation

0.1% OPA buffer: Acetonitrile (30:70) ratio.

Wave length Selection

UV spectrum of 10µg/ml Pazopanib in diluents (mobile phase composition) was recorded by scanning in the range of 200nm to 400nm. From the UV spectrum wavelength selected as 256 nm. At this wavelength the drug shows good absorbance.

UV Graph

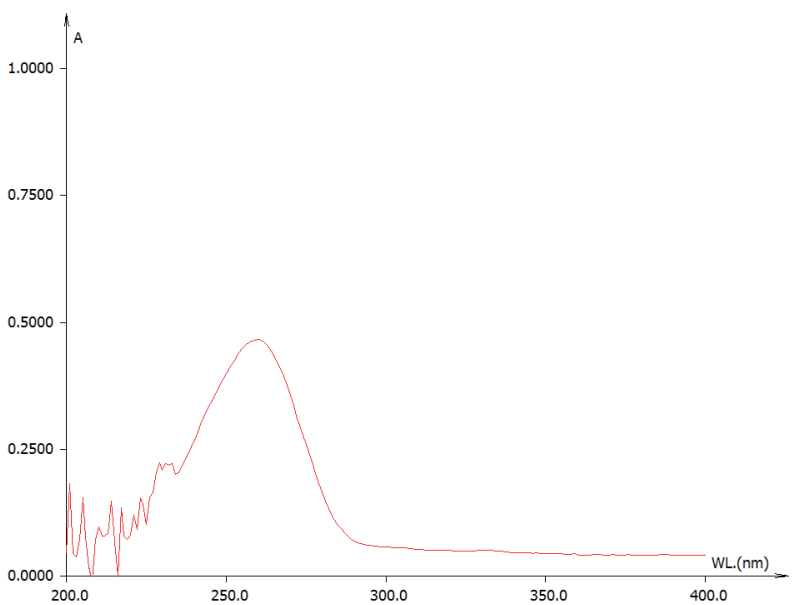

Figure 1: UV Spectrum of Pazopanib (256nm)

Preparation of the Pazopanib Standard & Sample Solution

Standard Solution Preparation

Accurately weigh and transfer 10 mg of Pazopanib is taken into a 10ml clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the diluent. (Stock solution)

Further pipette 0.1ml of Pazopanib of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Sample Solution Preparation

Accurately weigh and transfer equivalent to 10 mg of Pazopanib sample is taken into a 10ml clean dry volumetric flask add diluents and sonicate to dissolve it completely and make volume up to the mark with the diluent. (Stock solution)

Further pipette 0.1ml of Pazopanib of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.

Procedure

Inject 5 µL of the standard, sample into the chromatographic system and measure the areas for the Pazopanib peaks and calculate the % Assay by using the formula.

Optimized Chromatographic Conditions

Equipment: Ultra performance liquid chromatography equipped with Auto Sampler and PDA detector
Column: BHEL UPLC COLUMN
Mobile Phase: 0.1% OPA Buffer: Acetonitrile (30:70% v/v)
Flow rate: 0.25 mL per min
Wavelength: 256 nm
Injection volume: 5 µl
Column temperature: Ambient
Run time: 2 min

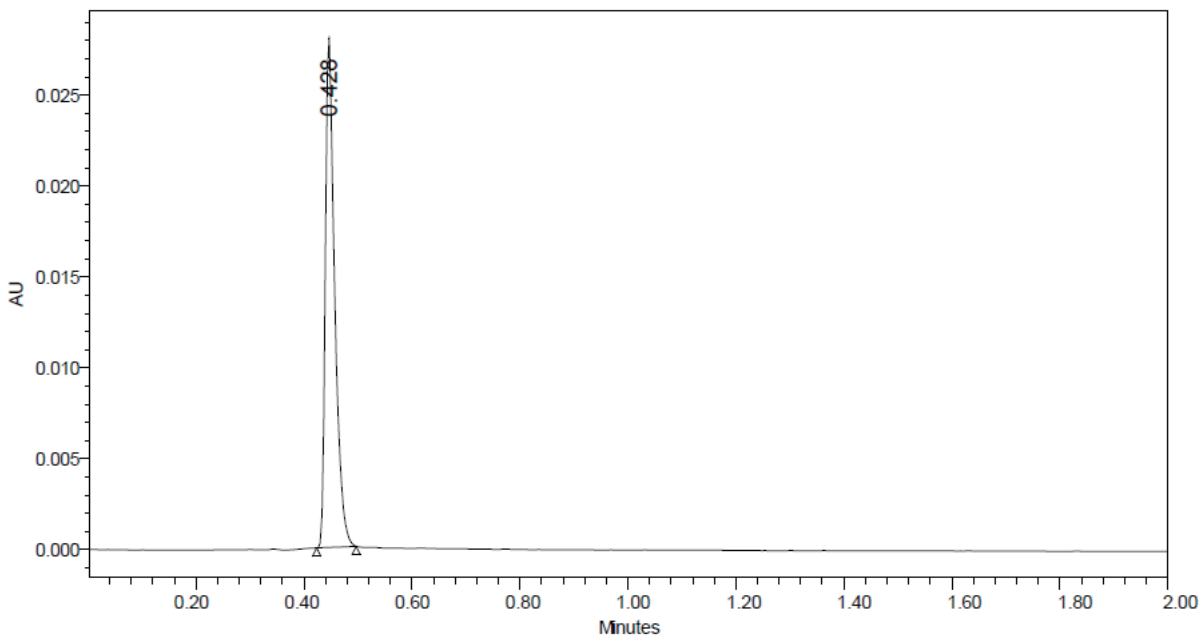

Figure 2: Optimized Chromatogram of Pazopanib

Table 1: Results of Optimized Chromatogram of Pazopanib

S.No.	Name	Retention Time (min)	Area (µV*sec)	Height (µV)	% Area	USP Plate Count	USP Tailing
1	Pazopanib	0.428	511519	273160	100.00	3559.77	1.34

METHOD VALIDATION

System Suitability

√ Tailing factor for the peaks due to Pazopanib in Standard solution should not be more than 2.0.

√ Theoretical plates for the Pazopanib peaks in Standard solution should not be less than 2000.

Table 2: Results of System Suitability for Pazopanib

S. No.	Peak Name	RT (min)	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Pazopanib	0.435	515867	275841	3652.48	1.36
2	Pazopanib	0.438	516854	275486	3568.75	1.38
3	Pazopanib	0.434	515752	275864	3695.49	1.37
4	Pazopanib	0.436	514986	275684	3745.28	1.39
5	Pazopanib	0.435	515874	275468	3865.42	1.34
6	Pazopanib	0.436	516423	275649	3598.47	1.36
Mean			515959.3			
Std. Dev.			635.8596			
% RSD			0.123238			

Acceptance Criteria

√ %RSD of five different sample solutions should not more than 2.

√ The %RSD obtained is within the limit, hence the method is suitable.

Specificity

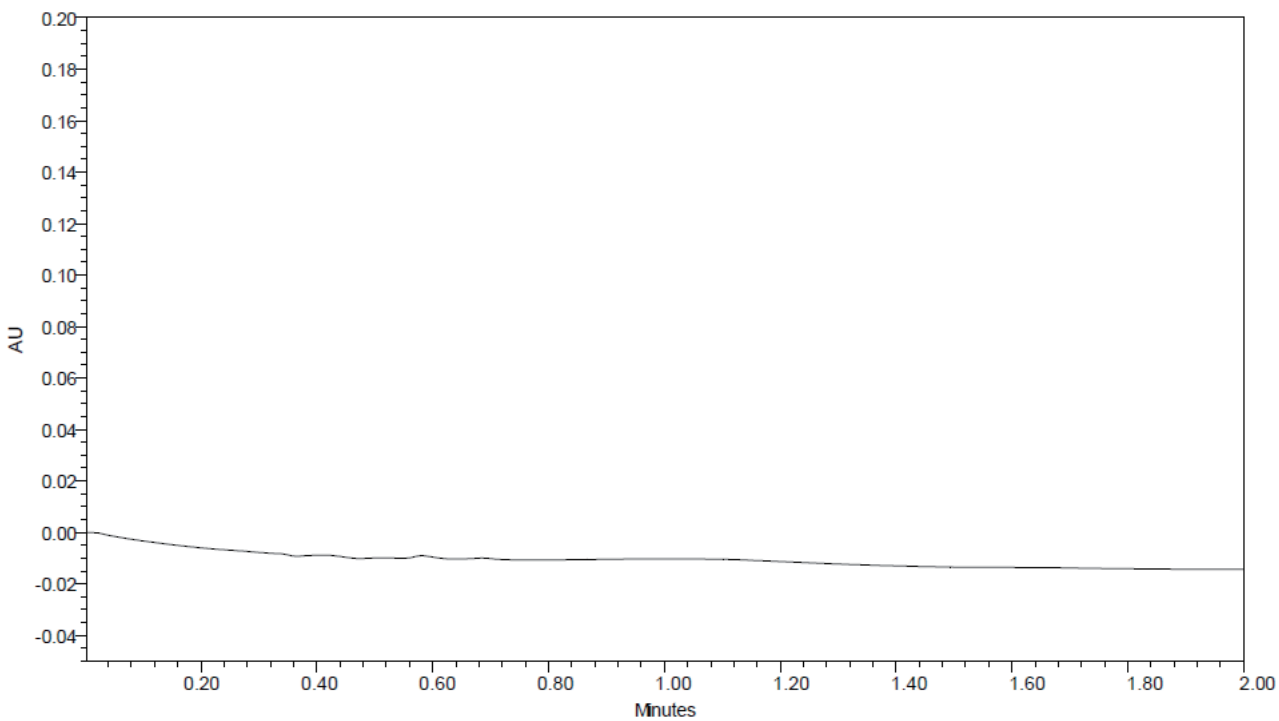

Figure 3: Blank Chromatogram (Mobile Phase Preparation)

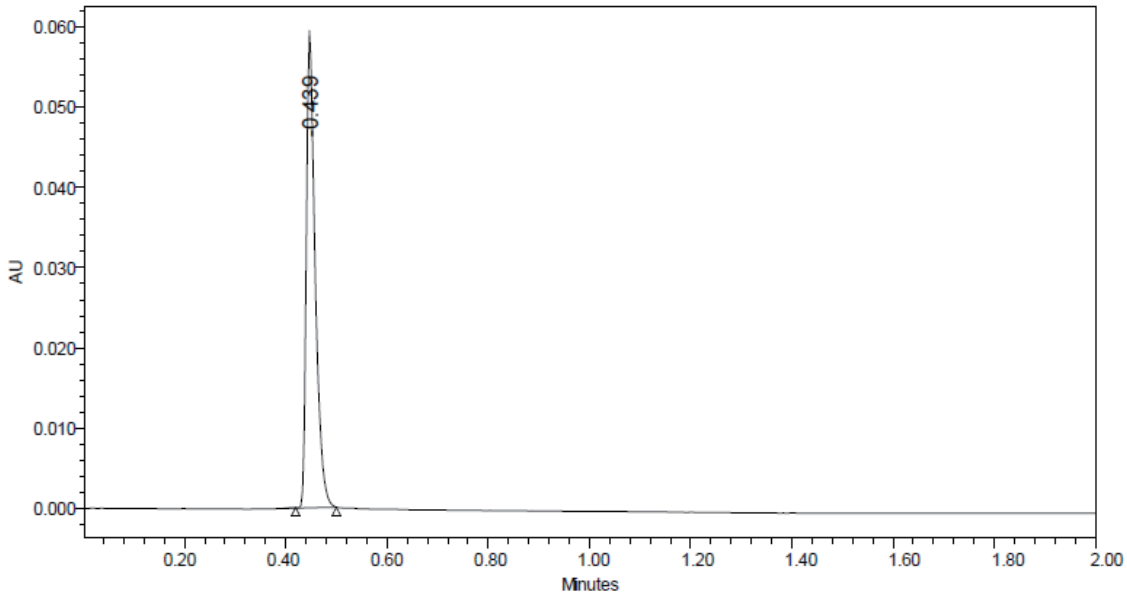

Figure 4: Standard Chromatogram of Pazopanib

Precision

Preparation of stock solution

Accurately weigh and transfer 10 mg of Pazopanib is taken into a 10ml clean dry volumetric flask add diluents and sonicate to dissolve it completely and make volume up to the mark with the diluent. (Stock solution)

Further pipette 0.1 ml of Pazopanib of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure

The standard solution was injected for six times and measured the area for all six injections in UPLC. The %RSD for the area of Six replicate injections was found to be within the specified limits. The results are summarized Pazopanib

Acceptance Criteria

√ %RSD for sample should be NMT 2.

√ The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Table 3: Results of Repeatability for Pazopanib

S. No.	Peak name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Pazopanib	0.436	516854	273564	3568.69	1.39
2	Pazopanib	0.435	514857	274865	3685.47	1.35
3	Pazopanib	0.436	515863	274981	3598.78	1.37
4	Pazopanib	0.434	516985	278685	3659.84	1.34
5	Pazopanib	0.438	514256	279863	3785.24	3.46
6	Pazopanib	0.435	517854	275258	3692.41	3.47
Mean			516111.5			
Std. Dev			1373.246			
%RSD			0.266075			

Repeatability

Intermediate Precision/Ruggedness

Preparation of Stock Solution

Accurately weigh and transfer 10 mg of Pazopanib is taken into a 10ml clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the diluent. (Stock solution)

Further pipette 0.1ml of Pazopanib of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day by using different make column of same dimensions.

Procedure

The standard solution was injected for six times and measured the area for all six injections in UPLC. The %RSD for the area of six replicate injections was found to be within the specified limits. The results are summarized Pazopanib

Table 4: Results of Intermediate Precision for Pazopanib

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USPTailing
1	Pazopanib	0.438	518659	278543	3658.75	1.39
2	Pazopanib	0.439	518696	275465	3768.45	1.38
3	Pazopanib	0.435	519632	278564	3898.38	1.34
4	Pazopanib	0.434	518748	274584	3785.96	1.38
5	Pazopanib	0.434	519865	274869	3648.74	1.37
6	Pazopanib	0.435	518523	273542	3895.24	1.36
Mean			519020.5			
Std. Dev.			573.5604			
% RSD			0.110508			

Acceptance Criteria

The % RSD for the area of six standard injections results should not be more than 2%.

Accuracy

Preparation of Standard stock solution

Accurately weigh and transfer 10mg of Pazopanib is taken into a 10ml clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the diluent. (Stock solution)

Further pipette 0.05ml of Pazopanib of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent

Table 5: The Accuracy Results for Pazopanib

Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	266301.7	5	5.018	100.360	
100%	528178.7	10	10.002	100.020	100.195%
150%	792391	15	15.031	100.206	

Acceptance Criteria

The % Recovery for each level should be between 98.0 to 102.0%

Accuracy for 50%

Table 6: Results of Accuracy for concentration-50%

S.No.	Name	RT	Area	Height	USP Plate Count	USP Tailing	Injection
1	Pazopanib	0.434	265864	136582	3869.85	1.38	1
2	Pazopanib	0.436	266898	136487	3798.46	1.39	2
3	Pazopanib	0.428	266143	136825	3988.75	1.37	3

Table 7: Results of Accuracy for concentration-100%

S.No.	Name	RT	Area	Height	USP Plate Count	USP Tailing	Injection
1	Pazopanib	0.435	527868	284573	3968.45	1.39	1
2	Pazopanib	0.439	528679	286574	3947.46	1.38	2
3	Pazopanib	0.436	527989	285425	3899.96	1.36	3

Accuracy For 150%

Table 8: Results of Accuracy for concentration-150%

S.No.	Name	RT	Area	Height	USP Plate Count	USP Tailing	Injection
1	Pazopanib	0.435	793574	402546	3986.85	1.41	1
2	Pazopanib	0.439	791245	401698	3989.96	1.43	2
3	Pazopanib	0.436	792354	402857	3979.59	1.46	3

LINEARITY

Accurately weigh and transfer 10 mg of Pazopanib is taken into a 10ml clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the diluent. (Stock solution)

Further pipette 0.1ml of Pazopanib of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent

Preparation of stock solution

Accurately weigh and transfer 10 mg of Pazopanib is taken into a 10ml clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the diluent. (Stock solution)

Preparation of Level – I (6ppm of Pazopanib):

0.06 ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with Diluents.

Preparation of Level – II (8ppm of Pazopanib):

0.08 ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with Diluents.

Preparation of Level – III (10ppm of Pazopanib)

0.1 ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with Diluents.

Preparation of Level – IV (12ppm of Pazopanib)

0.12 ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with Diluents.

Preparation of Level – V (14ppm of Pazopanib)

0.14 ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with Diluents.

Procedure

Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

Table 9: Linearity Results: (for Pazopanib)

S. No.	Linearity Level	Concentration	Area
1	I	6	323566
2	II	8	421274
3	III	10	528589
4	IV	12	632787
5	V	14	736598
Correlation Coefficient			0.999

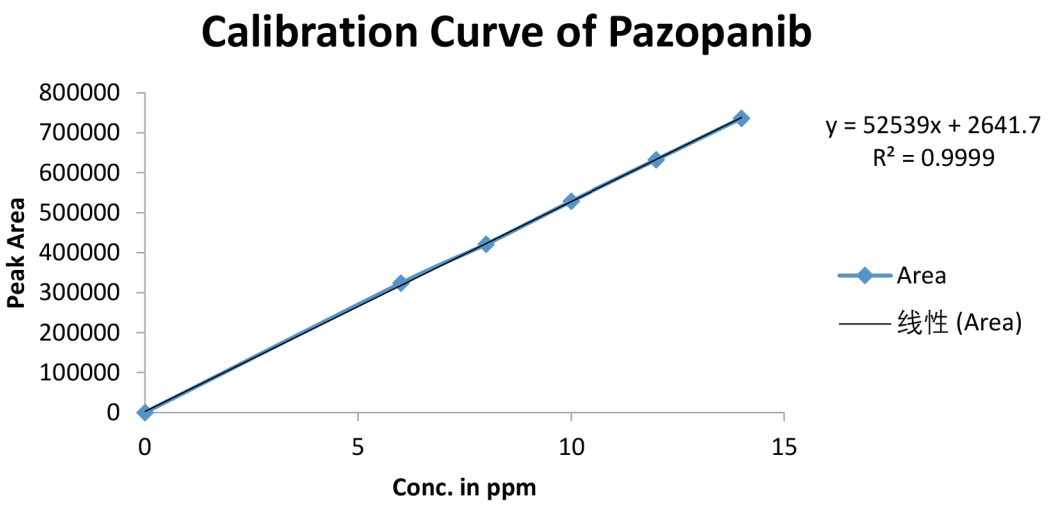

Figure 5: Calibration Curve of Pazopanib

Acceptance Criteria

Correlation coefficient should be not less than 0.999.

ROBUSTNESS

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition Temperature Variation was made to evaluate the impact on the method.

a). The flow rate was varied at 0.225 ml/min to 0.275 ml/min.

Standard solution 10 ppm of Pazopanib prepared and analysed using the varied flow rates along with method flow rate.

The results are summarized

On evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ± 10%.

The method is robust only in less flow condition.

Table 10: System suitability results for Pazopanib

S. No.	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.225	3912.96	1.33
2	0.25	3559.77	1.34
3	0.275	3777.23	1.37

* Results for actual flow (0.25ml/min) have been considered from Assay standard.

b). The Organic composition in the Mobile phase was varied from 60% to 80%

Standard solution 10µg/ml of Pazopanib was prepared and analysed using the varied Mobile phase composition along with the actual mobile phase composition in the method.

The results are summarized

On evaluation of the above results, it can be concluded that the variation in 10% Organic composition in the mobile phase affected the method significantly. Hence it indicates that the method is robust even by change in the Mobile phase ±10.

Table 11: System Suitability Results for Pazopanib

S.No.	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	3456.84	1.50
2	*Actual	3559.77	1.34
3	10% more	3658.61	1.20

LOD and LOQ

LOD: The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

LOD= 3.3 × σ / s

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

LOQ: The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

LOQ = 10×σ/S

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Observation: On the evaluation of above results the LOD and LOQ for the Pazopanib was found to be 0.5853 µg/ml and 1.7738µg/ml respectively.

Assay of Marketed Formulation

Twenty Tablets were taken and the I.P. method was followed to determine the average weight. Above weighed Tablets were finally powdered and triturated well. A quantity of powder equivalent to 25 mg of drugs were transferred to 25 ml volumetric flask, make and solution was sonicated for 15 minutes, there after volume was made up to 25 ml with same solvent. Then 10 ml of the above solution was diluted to 100 ml with mobile phase. The solution was filtered through a membrane filter (0.45 µm) and sonicated to degas. The solution prepared was injected in five replicates into the HPLC system and the observations were recorded.

Assay % = (AT/AS) × (WS/DS) × (DT/WT) × (P/100) × Avg. Wt = mg

Where:

AT = Peak Area of drug obtained with test preparation

AS = Peak Area of drug obtained with standard preparation

WS = Weight of working standard taken in mg

WT = Weight of sample taken in mg

DS = Dilution of Standard solution

DT = Dilution of sample solution

P = Percentage purity of working standard

Result & Discussion: The %Purity of Marketed Formulation of Pazopanib was found to be 99.536%.

Standard Solution

Table 12: Results of Standard Solution-1

S.No.	Name	Retention Time (min)	Area (µV*sec)	Height (µV)	% Area	USP Plate Count	USP Tailing
1	Pazopanib	0.435	511519	273160	100.00	3559.77	1.34

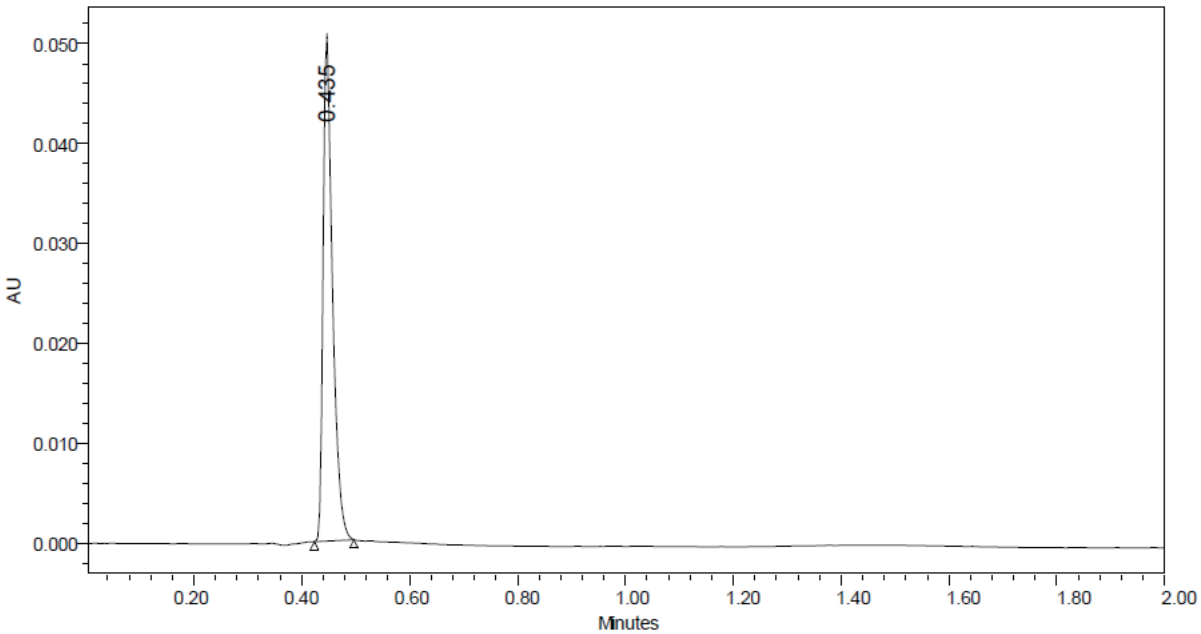

Figure 6: Chromatogram of Standard Solution-1

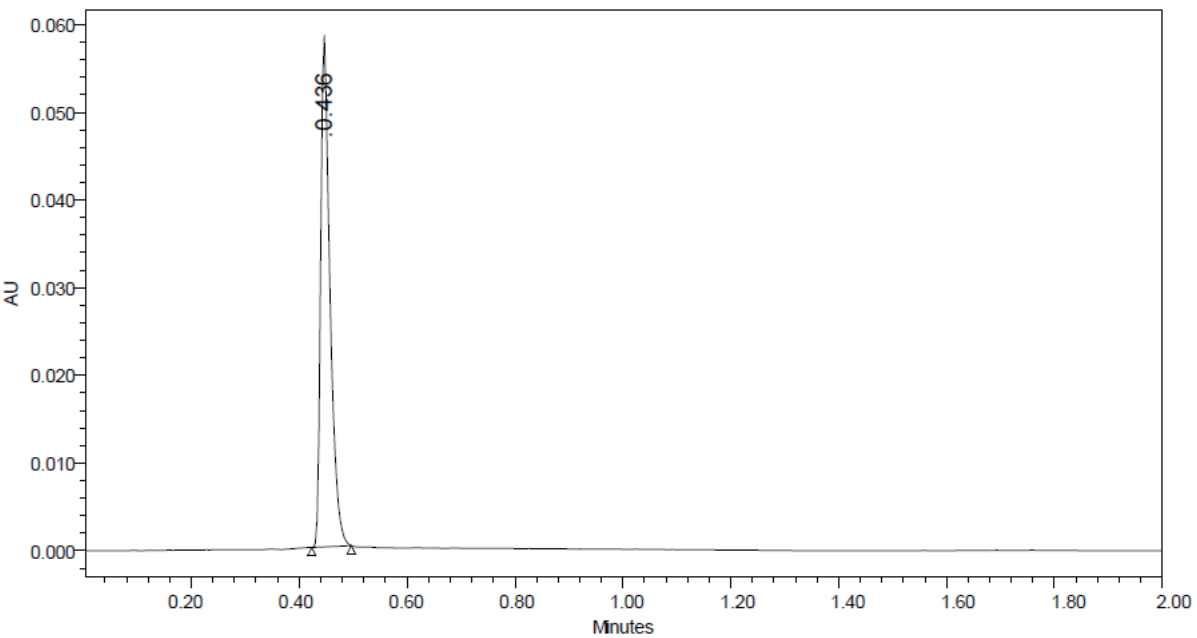

Figure 7: Chromatogram of Standard Solution-2

Table 13: Results of Standard Solution-2

S.No.	Name	Retention Time (min)	Area (µV*sec)	Height (µV)	% Area	USP Plate Count	USP Tailing
1	Pazopanib	0.436	515752	275864	100.00	3490.36	1.31

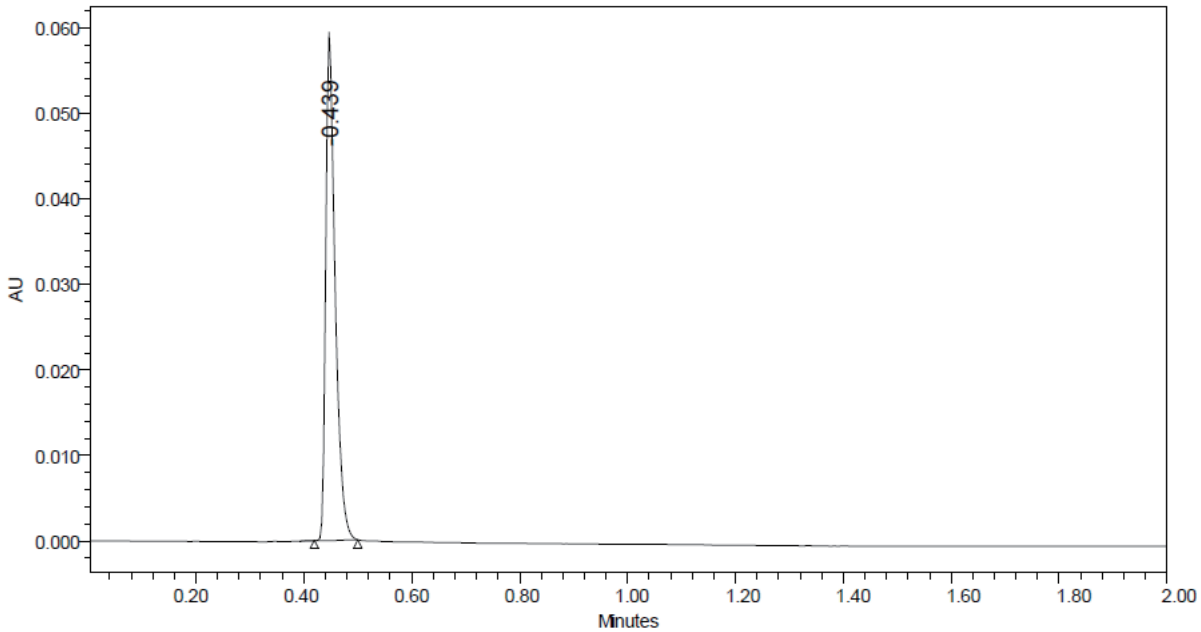

Figure 8: Chromatogram of Standard Solution-3

Table 14: Results of Standard Solution-3

S.No.	Name	Retention Time (min)	Area (µV*sec)	Height (µV)	% Area	USP Plate Count	USP Tailing
1	Pazopanib	0.439	518732	277410	100.00	3464.48	1.32

Sample Solution

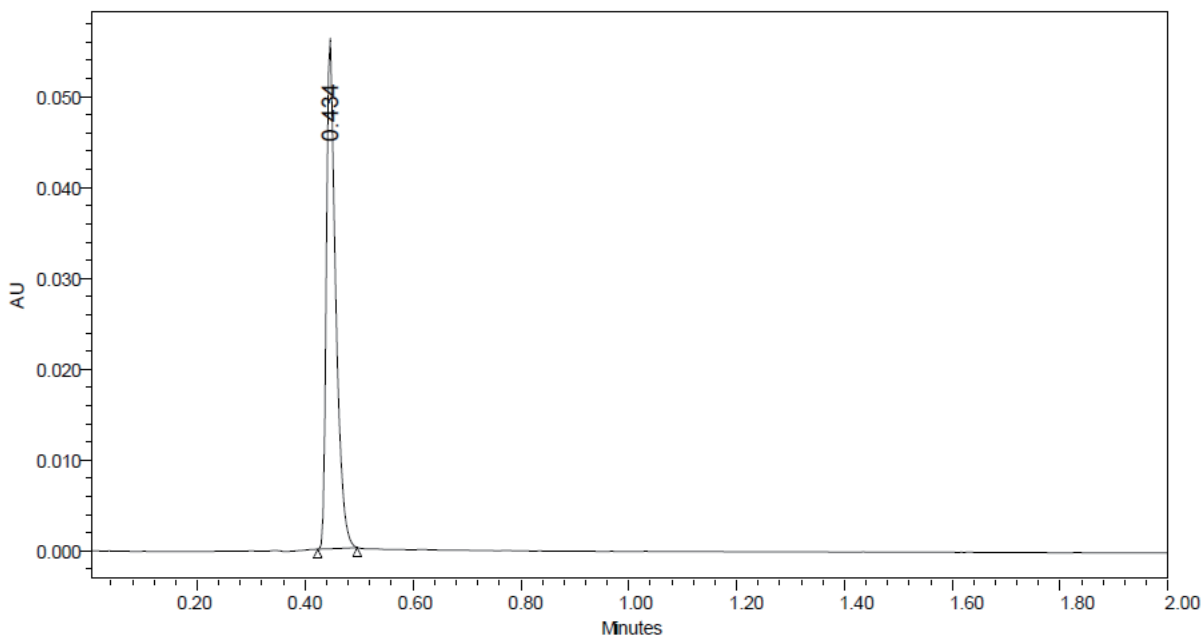

Figure 9: Chromatogram of Sample Solution-1

Table 15: Results of Sample Solution-1

S.No.	Name	Retention Time (min)	Area (µV*sec)	Height (µV)	% Area	USP Plate Count	USP Tailing
1	Pazopanib	0.434	511928	302502	100.00	3491.06	1.36

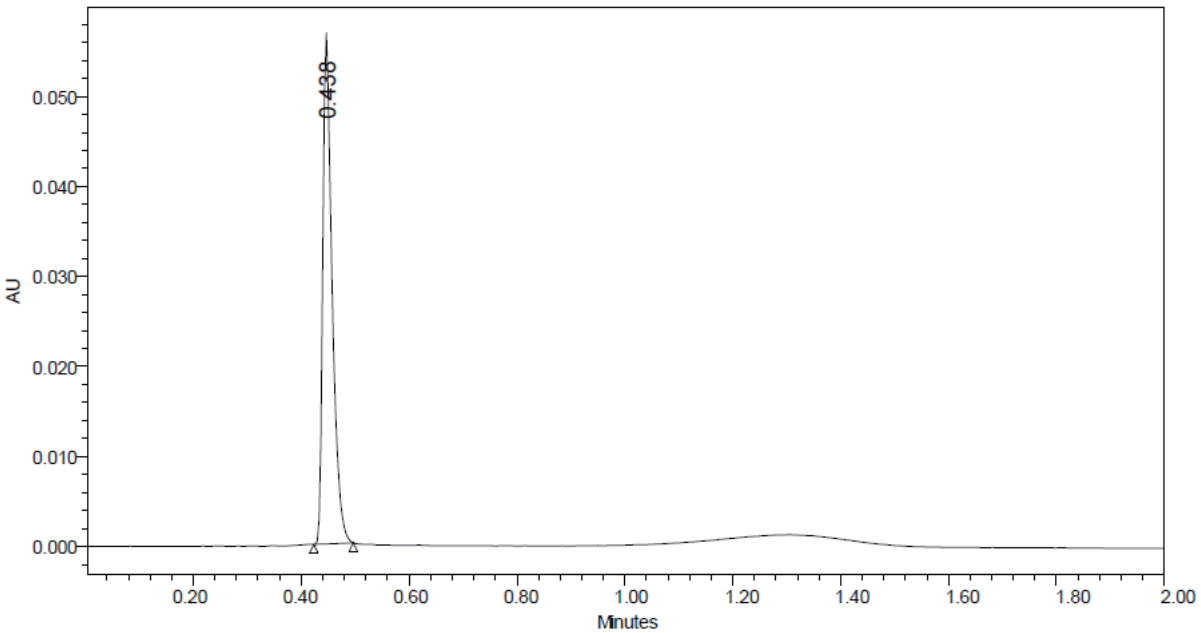

Figure 10: Chromatogram of Sample Solution-2

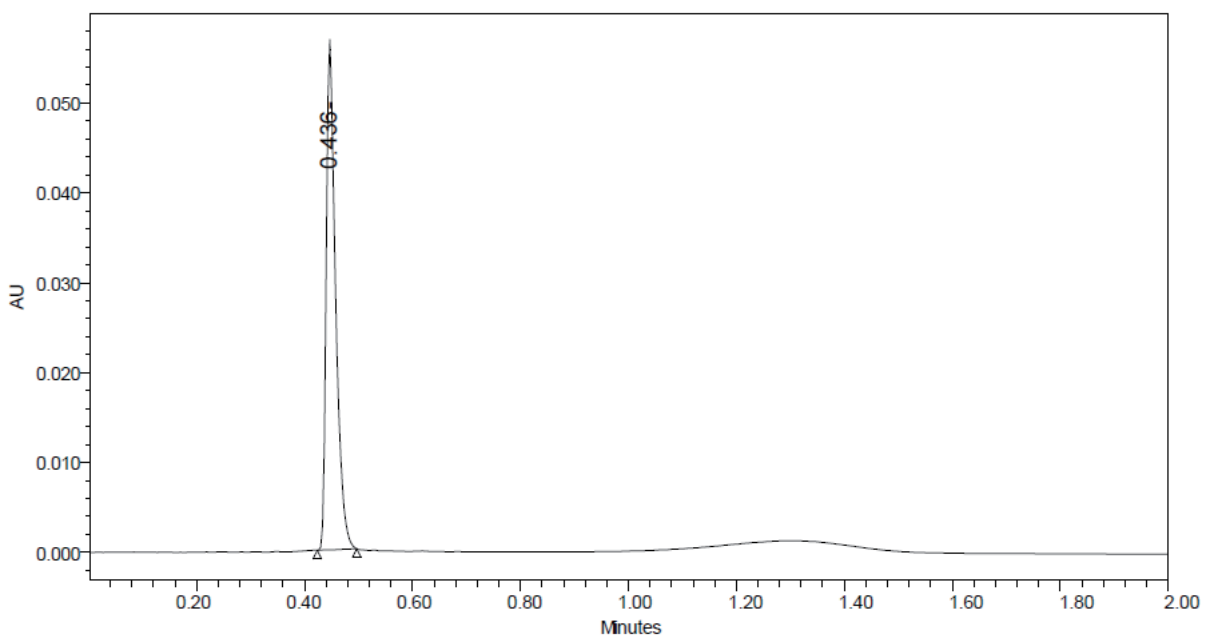

Figure 11: Chromatogram of Sample Solution-3

Table 16: Results of Sample Solution-2

S.No.	Name	Retention Time (min)	Area (µV*sec)	Height (µV)	% Area	USP Plate Count	USP Tailing
1	Pazopanib	0.438	512846	303651	100.00	3433.64	1.39

Table 17: Results of Sample Solution-3

S.No.	Name	Retention Time (min)	Area (µV*sec)	Height (µV)	% Area	USP Plate Count	USP Tailing
1	Pazopanib	0.436	514838	305831	100.00	3428.12	1.38

Table 18: Results of Degradation Studies

Sample Name	Pazopanib	
	Area	% Degraded
Standard	511519	0.000
Acid	496859	2.866
Base	495047	3.221
Peroxide	499327	2.384
Thermal	490258	4.157
Photo	509289	0.436

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate UPLC method was developed for the quantitative estimation of Pazopanib in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatization or purification steps. Pazopanib was found to be freely soluble in water, Soluble in DMSO (17 mg/ml at 25° C), water (<1 mg/ml at 25° C), ethanol (<1 mg/ml at 25° C), and 30% PEG400/0.5% Tween80/5% propylene glycol.0.1% OPA Buffer: Acetonitrile (30:70% v/v) was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise.The results expressed in Tables for UPLC method was promising. The UPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Pazopanib in bulk drug and in Pharmaceutical dosage forms.

Conflict of interest

None to declare for all authors.

References

1. Escudero-Ortiz V, Perez-Ruixo JJ. Development and validation of an HPLCUV method for Pazopanib quantification in human plasma and application to patients with cancer in routine clinical practice. The Drug Monit. 2015;37(2):172-9.
2. Fierce B. Pazopanib Shows Encouraging Activity In Several Tumour Types, Including Soft Tissue Sarcoma And Ovarian Cancer. 2015. Web. 3 June 2015.
3. Sleijfer S, Ray-Coquard I, Papai Z, LeCesne A, Scurr M, Schoffski P, et al. Pazopanib, a multikinase angiogenesis inhibitor, in patients with relapsed or refractory advanced soft tissue sarcoma: A phase II study from the European Organisation for Research and Treatment of Cancer-soft tissue and bone sarcoma group (EORTC study 62043). Journal of Clinical Oncology. 2009;27(19):3126-32.
4. Tugues S, Koch S, Gualanli L, Xiujuan L. Vascular endothelial growth factors and receptors: Anti-angiogenic therapy in the treatment of cancer.

Molecular Aspects of Medicine. 2011;32(2):88-111.
5. Saharinen P, Eklund L, Pulkki K, Bono P, Alitalo K. VEGF and angiopoietin signaling in tumor angiogenesis and metastasis. Trends Mol Med. 2011;17(7):347-67.
6. Khalil NY, Darwish IA, Alshammari MF, Wani TA. ICH Guidelines-compliant HPLC-UV Method for Pharmaceutical Quality Control and Therapeutic Drug Monitoring of the Multi-targeted Tyrosine Kinase Inhibitor Pazopanib. S Afr J Chem. 2017;70(70):60-6.
7. Santhosh illendula, Yusra Azeez, CH.V.Suresh, K.N.V.Rao. RP-HPLC method development and validation for the quantitative estimation of pazopanib in pureform and marketed pharmaceutical dosage forms. Journal for innovative development in pharmaceutical and technical science. 2024;7(5):17-25
8. Chaitanya D, Prasanna KK, Harini U, Lingam M, Pawar KM. 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. Journal of Chemical and Pharmaceutical Research. 2015;7(12):950-60.
9. Khan A, Venkateswara RJ, Ravi PP, Suresh KS, Sujana K. Estimation of Pazopanib Hydrochloride in Tablet Dosage Forms by Rp-Hplc. International Journal of Advances in Pharmaceutical Analysis. 2013;3(1):24-9.
10. Ramu B, Chittela KB. High Performance Thin Layer Chromatography and Its Role Pharmaceutical Industry [Review]. Open Sci. J. Biosci. Bioeng. 2018;5(3):29– 34.