International Research Journal of Education and Technology
Peer Reviewed Journal
ISSN 2581-7795

DEVELOPMENT AND VALIDATION OF REVERSE PHASE-HPLC METHOD FOR THE QUANTITATIVE DETERMINATION OF AXITINIB IN API FORM AND MARKETED PHARMACEUTICAL DOSAGE FORMS

CHENNUPATI V SURESH[1]*, HEENA[2] , SANTHOSH ILLENDULA[3], K N VENKATESWARA RAO[4]

1. Professor & Head of Department, Department of Pharmaceutical Analysis, Nalanda College of Pharmacy, Cherlapally (v), Nalgonda (Dt), Telangana (St), India, 508001
2. Post Graduate Scholar, Department of Pharmaceutical Analysis, Nalanda College of Pharmacy, Cherlapally (v), Nalgonda (Dt), Telangana (St), India, 508001
3. Associate Professor, Department of Pharmaceutical Analysis, Nalanda College of Pharmacy, Cherlapally (v), Nalgonda (Dt), Telangana (St), India, 508001
4. Professor & Principal, Nalanda College of Pharmacy, Cherlapally (v), Nalgonda (Dt), Telangana (St), India, 508001

Corresponding Author mail ID:Sureshchennupati@rediffmail.com

ABSTRACT

A new, simple, rapid, precise, accurate and reproducible RP-HPLC method for estimation of Axitinib in bulk form and marketed formulation. Separation of Axitinib was successfully achieved on a Symmetry ODS C_{18} (4.6 x 250mm, 5µm) column in an isocratic mode of separation utilizing Acetonitrile : Methanol in the ratio of 80:20% v/v at a flow rate of 1.0 mL/min and the detection was carried out at 272nm. The method was validated according to ICH guidelines for linearity, sensitivity, accuracy, precision, specificity and robustness. The response was found to be linear in the drug concentration range of 10-50mcg/mL for Axitinib. The correlation coefficient was found to be 0.999 for Axitinib. The LOD and LOQ for Axitinib were found to be 1.1µg/mL and 3.2µg/mL respectively. The proposed method was found to be good percentage recovery for Axitinib, which indicates that the proposed method is highly accurate. The specificity of the method shows good correlation between retention times of standard solution with the sample solution. Therefore, the proposed method specifically determines the analyte in the sample without interference from excipients of pharmaceutical dosage forms.

Keywords: Axitinib, RP-HPLC, Accuracy, Robustness, Linearity, ICH Guidelines.

International Research Journal of Education and Technology
Peer Reviewed Journal
ISSN 2581-7795

INTRODUCTION

Axitinib is an indazole substituted at position 3 by a 2-(pyridin-2-yl)vinyl group and at position 6 by a 2-(N-methyl amino carboxy) phenyl sulfanyl group. Used for the treatment of advanced renal cell carcinoma after failure of a first line systemic treatment. It has a role as an anti neoplastic agent, a tyrosine kinase inhibitor and a vascular endothelial growth factor receptor antagonist. It is a member of indazoles, a member of pyridines, an aryl sulfide and a member of benzamides. Axitinib is a second generation tyrosine kinase inhibitor that works by selectively inhibiting vascular endothelial growth factor receptors (VEGFR-1, VEGFR-2, VEGFR-3). Through this mechanism of action, Axitinib blocks angiogenesis, tumor growth and metastases. It is reported to exhibit potency that is 50-450 times higher than that of the first generation VEGFR inhibitors. Axitinib is an indazole derivative. It is most commonly marketed under the name Inlyta® and is available in oral formulations. Axitinib is a Kinase Inhibitor. The mechanism of action of Axitinib is as a Receptor Tyrosine Kinase Inhibitor. Axitinib is used alone to treat advanced renal cell carcinoma (RCC, a type of cancer that begins in the cells of the kidneys) in people who have not been treated successfully with another medication[3].The IUPAC name of Axitinib is N-methyl-2-[[3-[(E)-2-pyridin-2-ylethenyl]-1H-indazol-6-yl]sulfanyl]benzamide. The Chemical Structure of Axitinib is shown in fig-1.

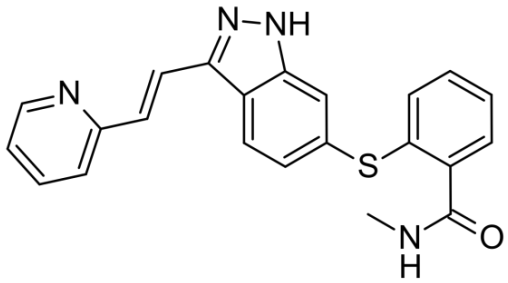

Fig-1: Chemical Structure of Axitinib

EXPERIMENTAL

Table-1: Instruments Used

S.No.	Instruments and Glass wares	Model
1	HPLC	WATERS Alliance 2695 separation module,

		Software: Empower 2, 996 PDA Detector.
2	pH meter	Lab india
3	Weighing machine	Sartorius
4	Volumetric flasks	Borosil
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil
7	Digital ultra sonicator	Labman

Table-2: Chemicals Used

S.No.	Chemical	Brand Names
1	Axitinib (Pure)	Local Market
2	Water and Methanol for HPLC	LICHROSOLV (MERCK)
3	Acetonitrile for HPLC	Merck

Preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Axitinib working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.3ml of the above Axitinib stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.(20)

Preparation of Sample Solution:

Take average weight of the Powder and weight 10 mg equivalent weight of Axitinib sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette 0.3ml of the above Axitinib stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure:

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization:

Initially the mobile phase tried was methanol: Water and ACN: Water with varying proportions. Finally, the mobile phase[6]was optimized to ACN: Methanol 80:20% v/v) respectively.

Optimization of Column:

The method was performed with various C18columns like Symmetry, Zodiac and Xterra. Symmetry ODS C_{18} (4.6 x 250mm, 5µm)Column was found to be ideal as it gave good peak shape and resolution[7] at 1ml/min flow.

Preparation of mobile phase:

Accurately measured 800 ml (80%) of HPLC Acetonitrile and 200 ml of Methanol (20%) were mixed and degassed in a digital ultrasonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

The Mobile phase was used as the diluent.

Method Validation Parameters

System Suitability

Accurately weigh and transfer 10 mg of Axitinib working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.3ml of the above Axitinib stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure:

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Specificity:

Preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Axitinib working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.3ml of the above Axitinib stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Preparation of Sample Solution:

Take average weight of the Powder and weight 10 mg equivalent weight ofAxitinib sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette 0.3ml of the above Axitinib stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure:

Inject the five replicate injections of standard and inject the three replicate injections sample solutions and calculate the assay by using formula:

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

Linearity:

Accurately weigh and transfer 10 mg of Axitinib working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Preparation of Level – I (10ppm of Axitinib):

Take 0.1ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

Preparation of Level – II (20ppm of Axitinib):

Take 0.2ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

Preparation of Level – III (30ppm of Axitinib):

Take 0.3ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

Preparation of Level – IV (40ppm of Axitinib):

Take 0.4ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

Preparation of Level – V (50ppm of Axitinib):

Take 0.5ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

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.

Precision

Repeatability

Preparation of Axitinib Product Solution for Precision:

Accurately weigh and transfer 10 mg of Axitinib working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Take 0.3ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

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

Intermediate Precision:

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different daysby maintaining same conditions.

Procedure:

Analyst 1:

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

Analyst 2:

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

Accuracy:

For preparation of 50% Standard stock solution:

Accurately weigh and transfer 10 mg of Axitinib working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Take 0.15ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

For preparation of 100% Standard stock solution:

Accurately weigh and transfer 10 mg of Axitinib working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Take 0.3ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

For preparation of 150% Standard stock solution:

Accurately weigh and transfer 10 mg of Axitinib working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Take 0.45ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

Procedure:

Inject the Three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Axitinib and calculate the individual recovery and mean recovery values.

Limit of Detection (LOD) andLimit of Quantification (LOQ):

Preparation of 0.597µg/ml solution (LOD):

Accurately weigh and transfer 10 mg of Axitinib working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.00597ml of the above Axitinib stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Preparation of 1.811µg/ml solution (LOQ):

Accurately weigh and transfer 10 mg of Axitinib working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.01811ml of the above Axitinib stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Robustness:

The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results. .

For preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Axitinib working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Take 0.3ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

Effect of Variation ofFlow Conditions:

The sample was analyzed at 0.9ml/min and 1.1ml/min instead of 1ml/min, remaining conditions are same. 20µl of the above sample was injected and chromatograms were recorded.

Effect of Variation of Mobile Phase Organic Composition:

The sample was analyzed by variation of mobile phase i.e. ACN: Methanol was taken in the ratio and 75:25, 85:15 instead of 80:20, remaining conditions are same. 20µl of the above sample was injected and chromatograms were recorded.

RESULTS AND DISCUSSION

Optimization of Method

Column : Symmetry ODS C_{18} (4.6 x 250mm, 5µm)

Column temperature : Ambient

Wavelength	:	272 nm
Mobile phase ratio	:	ACN: Methanol (80:20% v/v)
Flow rate	:	1.0mL/min
Injection volume	:	20 µl
Run time	:	8 minutes

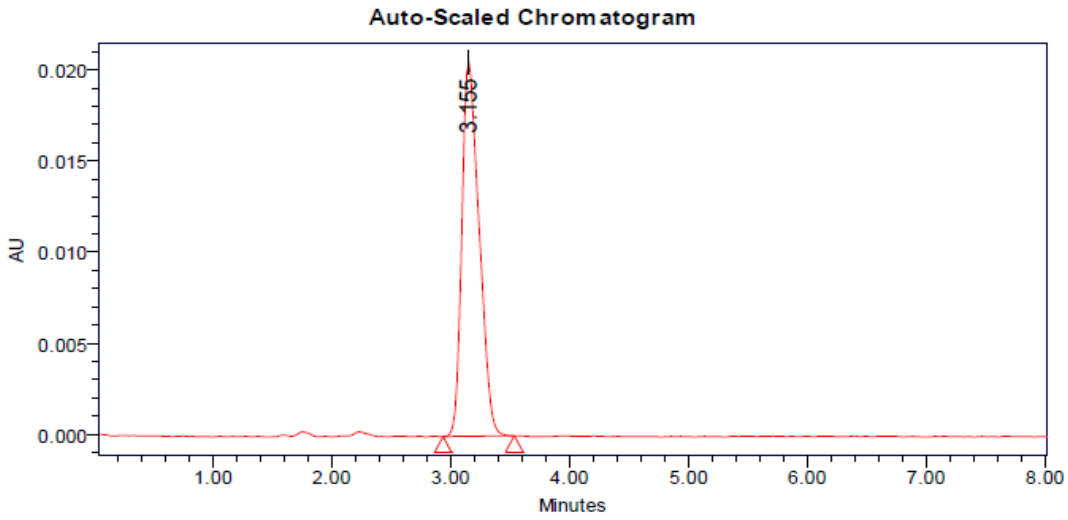

Fig-2: Optimized Chromatographic Condition

Validation of Analytical Method:

The validation of an analytical method confirms the characteristics of the method to fulfill the requirements of the application domain. The method was validated according to the ICH guidelines for specificity, linearity, precision, recovery, and stability.

System Suitability:

A standard solution of Axitinib working standard was prepared as per procedure and injected 5 times into the HPLC system. Then, the system suitability parameters were evaluated from standard chromatograms obtained. The % relative standard deviations (RSD) of retention time, tailing factor, theoretical plates, and peak areas from five replicate injections was within range and results were shown in Table 3.

International Research Journal of Education and Technology
Peer Reviewed Journal
ISSN 2581-7795

Table-3: Results of System Suitability for Axitinib

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Axitinib	3.192	225645	20584	6286	1.38
2	Axitinib	3.146	225847	20965	6358	1.39
3	Axitinib	3.123	228656	20758	6285	1.41
4	Axitinib	3.167	228547	20859	6278	1.40
5	Axitinib	3.158	229658	20968	6395	1.42
Mean			227670.6			
Std.Dev.			1810.899			
%RSD			0.795403			

Specificity

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components.

Analytical method was tested for specificity to measure accurately quantitatesAxitinib in drug product.

Table-4: Results of Assay (Standard)for Axitinib Standard

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Axitinib	3.146	220136	20568	6125	1.36
2	Axitinib	3.123	220187	20653	6132	1.38
3	Axitinib	3.192	220175	20548	6129	1.34
4	Axitinib	3.164	220196	20698	6187	1.35
5	Axitinib	3.181	220134	20548	6159	1.35

Mean			220165.6			
Std.Dev.			28.91885			
%RSD			0.013135			

Table-5: Peak Results for Assay Sample

S.No.	Name	RT	Area	Height	USPTailing	USPPlateCount	Injection
1	Axitinib	3.170	224596	20469	1.35	6098	1
2	Axitinib	3.174	224658	20489	1.34	6108	2
3	Axitinib	3.170	224585	20458	1.35	6107	3

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

= 99.24%

The % purity of Axitinib in pharmaceutical dosage form was found to be 99.24%.

Linearity

To demonstrate linearity of the assay method, five standard solutions with concentrations of about 10-50 ppm of Axitinib was injected. Then, a graph was plotted between concentrations and peak area. Linearity plot was shown in Fig. 3.

Table-6: Statistical Data for Linearity

Concentration µg/ml	Average Peak Area
10	78683

20	146545
30	213584
40	279895
50	346568

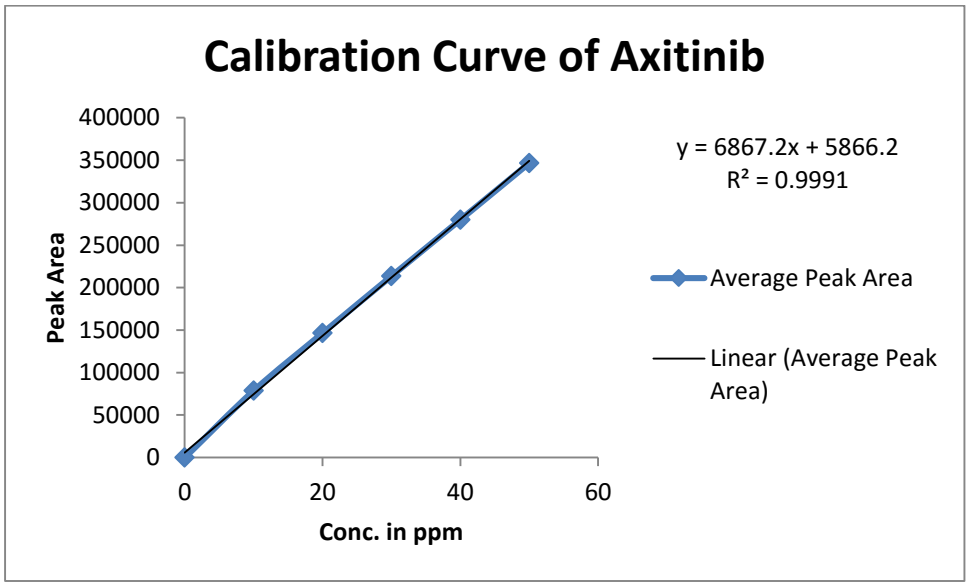

Fig-3: Calibration Curve of Axitinib

Linearity Plot:

The plot of Concentration (x) versus the Average Peak Area (y) data of Axitinib is a straight line[25].

$Y = mx + c$

Slope (m) =6867

Intercept (c) = 5866

Correlation Coefficient (r) = 0.99

Validation Criteria: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

Conclusion: Correlation Coefficient (r) is 0.99, and the intercept is 5866. These values meet the validation criteria.

Precision:

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

Repeatability

Obtained Six (6) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD.

Table-7: Results of Method Precision for Axitinib:

S. No.	Peak name	Retention time	Area(µV* sec)	Height (µV)	USP Plate Count	USP Tailing
1	Axitinib	3.165	225645	20562	6125	1.36
2	Axitinib	3.163	225847	20645	6129	1.36
3	Axitinib	3.158	226542	20534	6135	1.35
4	Axitinib	3.167	226598	20564	6189	1.36
5	Axitinib	3.171	226584	20549	6138	1.35
6	Axitinib	3.181	226859	20685	6179	1.37
Mean			226345.8			
Std.Dev			482.1068			
%RSD			0.212996			

International Research Journal of Education and Technology
Peer Reviewed Journal
ISSN 2581-7795

Intermediate Precision:

Analyst 1:

Table-8: Results of Ruggedness for Axitinib

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP PlateCount	USP Tailing
1	Axitinib	3.165	226534	20653	6235	1.35
2	Axitinib	3.163	226542	20598	6198	1.36
3	Axitinib	30158	225989	20653	6254	1.36
4	Axitinib	3.167	226512	20548	6281	1.35
5	Axitinib	3.171	226531	20653	6199	1.36
6	Axitinib	3.171	225898	20658	6253	1.35
Mean			226334.3			
Std.Dev.			304.2622			
%RSD			0.13443			

Analyst2:

Table-9: Results of Intermediate Precision Analyst 2 for Axitinib

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate	USP Tailing
1	Axitinib	3.173	225487	20542	6253	1.35
2	Axitinib	3.134	225484	20532	6098	1.36
3	Axitinib	3.161	225364	20541	6254	1.35
4	Axitinib	3.174	226513	20534	6235	1.36
5	Axitinib	3.199	225487	20549	6199	1.36

6	Axitinib	3.199	226532	20451	6235	1.35
Mean			225811.2			
Std.Dev.			553.0524			
%RSD			0.244918			

Accuracy

Three concentrations of 50%, 100%, and 150% were injected in a triplicate manner then % recovery and % RSD were calculated and shown in Table 10.

Table-10: The Accuracy Results for Axitinib

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	109283.3	15	15.060	100.40%	100.42%
100%	212732	30	30.124	100.413%	
150%	316263.3	45	45.201	100.446%	

Limit of Detection for Axitinib

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

$$LOD = 3.3 \times \sigma / s$$

Where σ = Standard deviation of the response

S = Slope of the calibration curve

Result: = 0.597µg/ml

Limit of Quantitation for Axitinib

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

$$LOQ=10\times\sigma/S$$

Where σ = Standard deviation of the response

S = Slope of the calibration curve

Result:= 1.811µg/ml

Robustness

The robustness was performed for the flow rate variations from 0.9 ml/min to 1.1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Axitinib. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%. The standard and samples of Axitinib were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.

Table-11: Results for Robustness

Parameter used for Sample Analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	225645	3.155	6125	1.36
Less Flow rate of 0.9 mL/min	236586	3.488	6452	1.38
More Flow rate of 1.1 mL/min	219865	2.877	6098	1.42
Less organic phase	235848	4.705	6126	1.43
More organic phase	241245	2.090	6324	1.39

Forced Degradation Studies

The specificity of the method can be demonstrated by applying stress conditions using acid, alkaline, peroxide, thermal, UV, water degradations. The sample was exposed to these conditions the main peak of the drug was studied for peak purity that indicating the method effectively separated the degradation products from the pure active ingredient.

Table-12: Results of Forced Degradation Studies for Axitinib

S.No.	Stress Condition	Peak Area	% of Degraded Amount	% of Active Amount	Total % of Amount
1	Standard	225645	0	100%	100%
2	Acidic	190015.65	15.79	84.21	100%
3	Basic	187353.04	16.97	83.03	100%
4	Oxidative	190985.92	15.36	84.64	100%
5	Thermal	183020.65	18.89	81.11	100%
6	Photolytic	181034.98	19.77	80.23	100%
7	Water	210549.34	6.69	93.31	100%

SUMMARY AND CONCLUSION

The analytical method was developed by studying different parameters. First of all, maximum absorbance was found to be at 272nm and the peak purity was excellent. Injection volume was selected to be 20µl which gave a good peak area. The column used for study was Symmetry ODS C_{18} (4.6 x 250mm, 5µm)because it was giving good peak. Ambient temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0ml/min because of good peak area and satisfactory retention time. Mobile phase is Acetonitrile and Methanol (80:20% v/v)was fixed due to good symmetrical peak. So this mobile phase was used for the proposed study. Run time was selected to be 8.0min because analyze gave peak around 3.155 and also to reduce the total run time. The percent recovery was found to be 98.0-102 was linear and precise over the same range. Both system and method precision were found to be accurate

and well within range. The analytical method was found linearity over the range of 10-50µg/ml of the Axitinib target concentration. The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory.

REFERENCES

1. https://go.drugbank.com/drugs/DB06626
2. https://pubchem.ncbi.nlm.nih.gov/compound/Axitinib
3. https://en.wikipedia.org/wiki/Axitinib
4. Sharma BK. Instrumental methods of chemical analysis, Introduction to analytical chemistry, 23th ed .Goel publishing house Meerut, 2004, P12-23.
5. H.H. Willard, L.L. Merritt, J.A. Dean, F.A. Settle. Instrumental methods of analysis, 7th edition, CBS publishers and distributors, New Delhi. 1986, P.518-521, 580-610.
6. Berry RI, Nash AR. Pharmaceutical process validation, Analytical method validation, Marcel Dekker Inc. New work, 1993; 57:411-28
7. Anthony C Moffat, M David Osselton, Brian Widdop. Clarke's analysis of drugs and poisons, Pharmaceutical press, London, 2004, P.1109-1110, 1601-1602.
8. L. R. Snyder, J.J. Kirkland, and J. L. Glajch, Practical HPLC Method Development, John Wiley & Sons, New York, 1997.
9. K., Robards (1994). Principles and practice of modern chromatographic methods. Haddad, P. R., Jackson, P. E. Amsterdam: Elsevier/Academic Press.
10. ICH Guidance. Validation of analytical methods - definition and terminology. Q2A. Geneva: International Conference on Harmonization. Nov 2005.
11. ICH Guidance, Validation of analytical procedures - methodology. Q2B. Geneva: International Conference on Harmonization. Nov 2005.
12. CH.V. Suresh, S. Greeshma, Santhosh Illendula ; A new analytical Method development and validation of estimation of avapritinib by RP-HPLC , International Journal of Multidisciplinary Research and Growth Evaluation, 2023; 04(01) : 175-182
13. Santhosh Illendula, A. Navyasree, CH. Ganesh, K.Sneha & KNV Rao ; Method development and validation of Axitinib in bulk and pharmaceutical dosage form by UV spectroscopic method, Indo American Journal of Pharmaceutical Sciences, 2019 ; 06(03): 6221-6227.

14. 1B. Jala Chandra Reddy and 2N. C. Sarada*, Development and validation of Stability Indicating RP-HPLC Method for the Determination of Axitinib in Bulk and its Pharmaceutical Formulations, Scholars Research Library Der Pharmacia Lettre, 2016, 8 (11): 97-106.
15. Ami R Patel1, Dr. V G Patel2, RP-HPLC Method Development and Validation for the Analysis of Pharmaceutical Drug – AXITINIB, © 2019 IJSRST | Volume 6 | Issue 4 | Print ISSN: 2395-6011 | Online ISSN: 2395-602X, July-August-2019 [6 (4): 367-371].
16. M. Sunil1*, A. Ramanjaneyulu2, A. Harshavardhan2, P. Suvarna Raj2, N. Manikya Bai2, T. Aswani2, Determination, Development and Validation of Method for Simultaneous Axitinib Pharmaceutical Dosage form by a Reverse Phase HPLC, Research & Review: Drugs and Drugs Development, Volume 1 Issue 2, Page 28-42.
17. Santhosh Illendula, Naveen Kumar Singhal ; A Review: Novel analytical method development & validation for the determination of selected anti cancer & anti viral drugs, World Journal of Pharmacy & Pharmaceutical Sciences 2022; 11(07): 533-566
18. Santhosh Illendula, M. Sanjana & Rajeswar Dutt ; A validated stability indicating RP_HPLC method development for the estimation of pomalidomide in bulk & pharmaceutical dosage form , International Journal of Pharmacy and Biological sciences, 2019: 09(01): 63-72.
19. Narottam Pal[1] *, Tayyaba Mahtab[2], Sumaiyya Saleem[3], Sayeeda Tabasum[2] and A. Srinivasa Rao[4], HPLC Method Development and Validation for the Determination of Axitinib in Tablet Dosage Form, European Journal of Biomedical and Pharmaceutical sciences, ejbps, 2019, Volume 6, Issue 8, 337-344.
20. Bolleddu R, Venkatesh S, Bhongiri B, Varanasi S. Establishment of Quality Parameters for Flowers of Karanja [Pongamia pinnata (L.) Pierre] through Powder Microscopy and Phytochemical Studies. J Drug Res Ayurvedic Sci 2018; 3 (4):228-233.