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Research Article

Development and Validation of Stability indicating method for the estimation of Axitinib in tablet dosage forms by UPLC

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ABSTRACT

A Stability Indicating Ultra-Performance Liquid Chromatography method was developed and validated for quantification of Axitinib in tablets. The chromatographic separation was done in an isocratic mode using the STD RP-18 Endcapped (50mm × 4.6mm, 2µ particle size) column. The mobile phase 0.1% OPA and acetonitrile 55:45 (%v/v) at the flow rate of 0.2mL/min and at ambient temperature was used. The wavelength used for detection was 249nm. The retention time for Axitinib was found to 1.03min. Axitinib was linear in the concentration range of 12.5µg/mL to 75µg/mL respectively. The developed method was validated and found to be accurate, specific and robust. The drug was subjected to the stressed conditions like acidic, basic, oxidative, photolytic, thermal and neutral conditions. The degradation results are found satisfactory. This method can be applied for the estimation of Axitinib in pharmaceutical dosage forms.

Introduction

Axitinib (Fig.1), chemically designated as *N*-Methyl-2-[[3-[(*E*)-2-pyridin-2-ylethenyl]-1*H*-indazol-6-yl]sulfanyl]benzamide, is a white to light yellow powder, slightly soluble in methanol, ethanol and water and has a pKa of 4.8. It is used in the treatment of advanced renal cell Carcinoma by acting as tyrosine kinase receptors VEGFR-1 (vascular endothelial growth factor receptor), VEGFR-2, and VEGFR-3 blocker [1-3]. According to the literature survey, very few methods were developed using HPLC [4-6]only. As no UPLC method was developed, the proposed method aimed to develop and validate a stability indicating method for the estimation of Axitinib in pharmaceutical dosage form using UPLC.

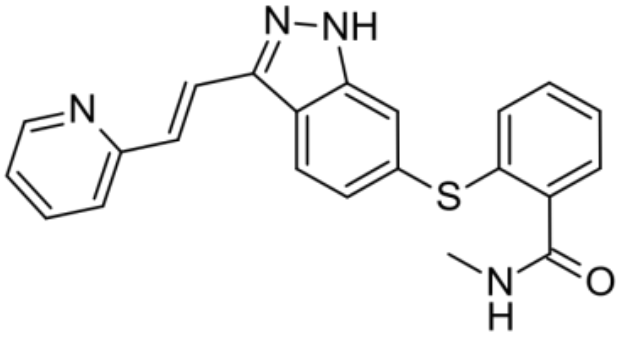

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Fig.1: Chemical structure of Axitinib

Material and Methods

Reagents and Chemicals

Axitinib standard drug was supplied as gift sample by spectrum labs, Hyderabad (India). The Axitinib Tablets (Inlyta) were purchased from local pharmacy. All the solvents used were of HPLC grade and purchased from Merck, Mumbai, India. All the chemicals used for developing method were of AR grade and purchased from sigma Aldrich.

Instruments and Chromatographic conditions

Water ACQUITY UPLC system equipped with Binary solvent manager, a sample manager with STD RP-18 End capped (50mm × 4.6mm, 2µ particle size) column maintained at room temperature, a solvents tray and UV detector was used for the estimation of Axitinib in pharmaceutical dosage form. All the parameters of UPLC were controlled by Empower software. Other instruments used were electronic balance, digital pH meter and Ultrasonic bathsonicator. The mobile phase composition 0.1% Orthophosphoric acid and acetonitrile (55:45%v/v) was used on isocratic mode at a flow rate of 0.2mL/min. The detection wavelength used was 249nm.

Preparation of standard solution and sample solution

5mg of Axitinib working standard was accurately weighed and transferred into a 10mL volumetric flask. 7mL of diluent was added, sonicated to dissolve and make up to final volume with diluent. From the above stock solution, 1mL was pipetted into a 10mL volumetric flask and the volume was made up to mark with diluent.

20 Tablets (Inlyta) were weighed accurately and the average weight was calculated. The tablets were crushed and fine powder was collected. An amount equivalent to 5mg of Axitinib was weighed and transferred into 10mL volumetric flask. 7mL of diluent was added and sonicated for 30min with intermediate shaking. Volume was made up with diluent. The above solution was filtered using UPLC filters. 1mL of the above solution was pipette into 10mL volumetric flask and made up with diluent.

Method validation [7]

System suitability

Inject standard solution into the chromatographic system and calculate the parameters such as % relative standard deviation (RSD), tailing factor and plate count.

Linearity

Serial dilutions of standard Axitinib in the concentration range of 12.5µg/mL and 75µg/mL were prepared and injected into the UPLC. A linearity graph was plotted between concentration and peak areas.

Accuracy

The solutions were prepared in three different concentration levels of 50%, 100% and 150%, injected into UPLC and % recoveries were calculated.

Precision

The precision of the method was determined by Intra and Inter-day precision studies. The standard solution was injected six times on the same day (intra-day) as well as on different day (inter-day) and the % RSD was calculated.

Specificity

The specificity of the method was determined by injecting the placebo solution and comparing with standard solution for the interference with Axitinib peak.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

LOD and LOQ are determined by standard deviation (SD) and slope of the calibration curve. The limiting values are calculated as per the following equations: LOD = (3.3 × SD)/ Slope and LOQ = (10 × SD)/ Slope.

Robustness

Robustness of the method was determined by varying the optimum chromatographic conditions such as mobile phase ratio (±10%), flow rate (±0.1mL/min) and column oven temperature (±5ºC). The system suitability parameters were calculated and recorded.

Forced degradation studies

The drug solution was subjected to the various stress conditions such as acidic (2N Hydrochloric acid, 60 ºC for 30 min), basic (2N sodium hydroxide, 60 ºC for 30 mins), oxidative (20% hydrogen peroxide, 60 ºC for 30 min), neutral (refluxingthedruginwaterfor6hrsatatemperature of 60ºC), photolytic (exposing the drug solution to UV light by keeping the beaker in UV Chamber for 7 days or 200Watt hours/m^2 in photo stability chamber) and thermal (drug solution was placed in an oven at 105ºC for 6 hours) conditions.

Results and Discussion

The main aim of the study was to develop a stability indicating UPLC method for the estimation of Crizotinib in capsule dosage form and to validate the method. Initially various mobile phase compositions were tried to elute the drug. Mobile phase ratio and flow rate were selected based on peak parameters and retention time. Standard solution of 50µg/mL was prepared and scanned in the range of 200 – 400nm for detecting the maximum absorption wavelength and it was found to be 249nm (Fig.2).

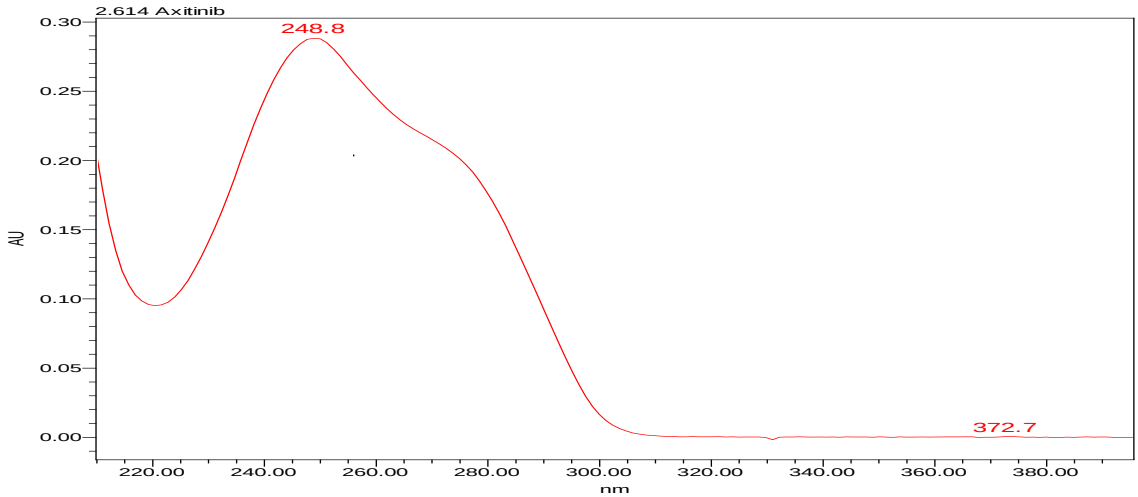


Fig.2: UV spectrum of Axitinib

After considering the entire system suitability parameters mobile phase 0.1% OPA and acetonitrile (55:45%v/v) run in isocratic mode and flow rate 0.2ml/min was selected. The retention time of Axitinib was found to be 1.028 min. The system suitability parameters are calculated from standard chromatogram (Table No. 1 and Fig.3)

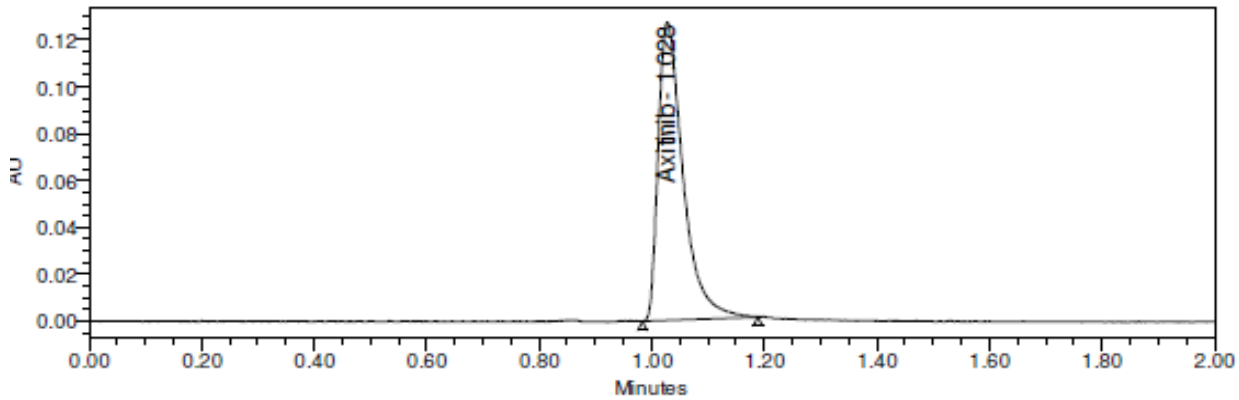


Fig.3: Standard chromatogram of Axitinib

For the estimation of linearity of method, concentrations ranging from 12.5µg/mL to 75µg/mL were prepared and a linearity graph (Fig.4) was plotted using concentration against peak area. The regression equation was found to be y = 8007.8x + 5131.2, with a correlation coefficient of 0.9997, indicating that a good linearity was observed .

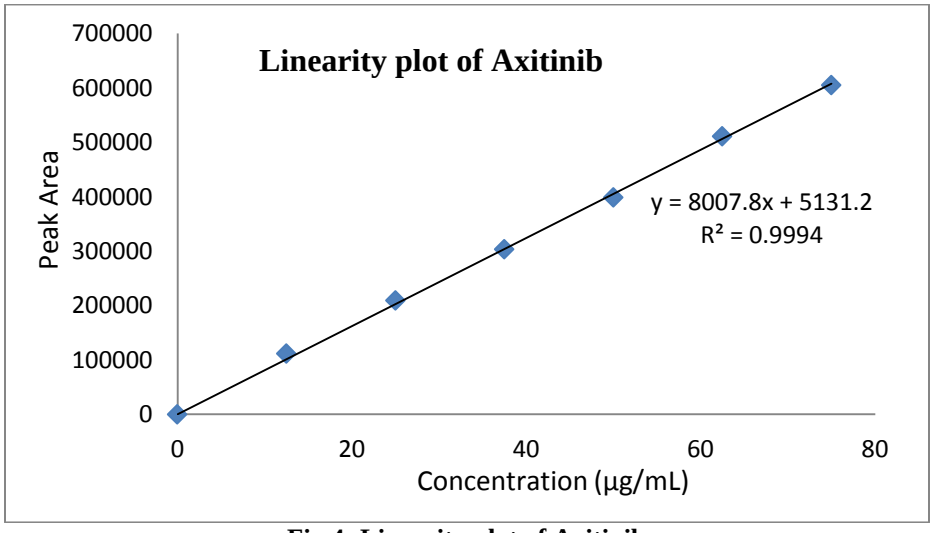


Fig.4: Linearity plot of Axitinib

The % recovery of Axitinib was found to be 99.49% - 99.80% and % RSD was found to be 0.3. As the results were found to be within the limits, indicates that the method was accurate and precise. The LOD and the LOQ for Axitinib were found to be 0.26µg/mL and 0.80µg/mL respectively. The method was found to be rugged, robust and stable up to 24hrs. The method was found to be specific, as there is no interference of placebo peaks with the retention time of Axitinib as shown in Fig.5.

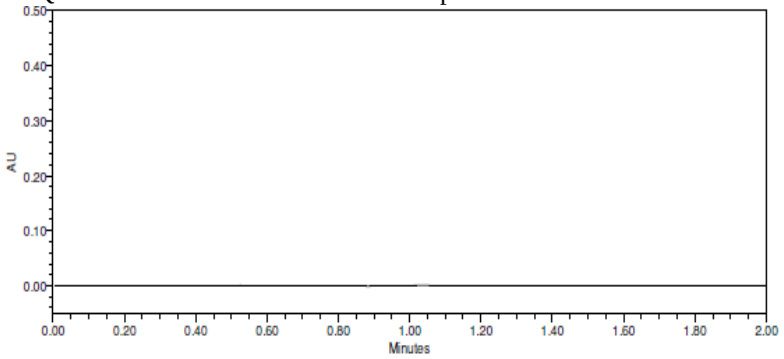


Fig.5: Placebo chromatogram

Table No. 1 System suitability and validation parameter results

Parameters	Axitinib
Specificity	Specific, No interference
Precision (%RSD)	0.3
Accuracy (% recovery)	99.49% - 99.80%
Linearity range (µg/mL)	12.5-75
Correlation coefficient, r	0.9997
Limit of Detection (µg/mL)	0.26
Limit of Quantitation (µg/mL)	0.80
Ruggedness (%RSD)	0.4
Robustness	Robust
Stability	Stable
USP Plate count	2821
USP tailing factor	1.81

The stability of an analytical method was determined by forced degradation studies, in which the stability of the method was carried out by performing Acid stress study, Base stress study, Peroxide stress study, Water stress study, UV light exposurestudy and Dry heat stress study. The net degradation was found to be within the limits. The results and chromatograms were summarized in Table No. 2 and Fig.6.

Table No. 2 Result of Forced degradation studies

S.No.	Stress condition	% Assay	% area of degradation peak
1	2N HCL for 30mins at 60°C	95.94	2.27
2	2N NaOH for 30mins at 60°C	97.94	0.17
3	20% H_2O_2 for 30mins at 60^0C	98.10	-
4	Water for 6hrs at60^0C	99.36	-
5	UV light 200wts/hr or 7 days	99.61	-
6	105^0C for 6hrs	99.48	-

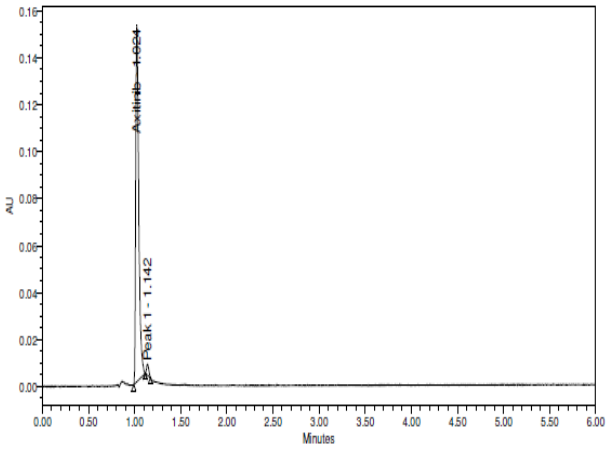


Fig.6a:Acid degradation chromatogram

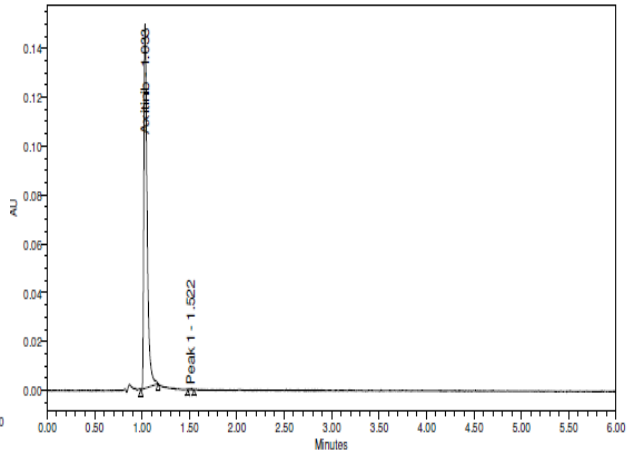


Fig.6b:Base degradation chromatogram

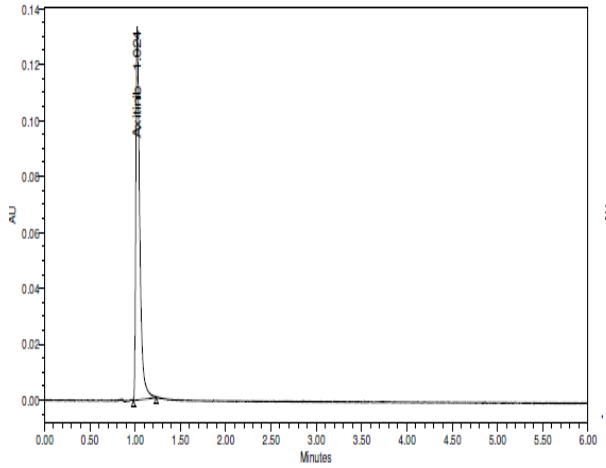


Fig.6c: Peroxide degradation chromatogram

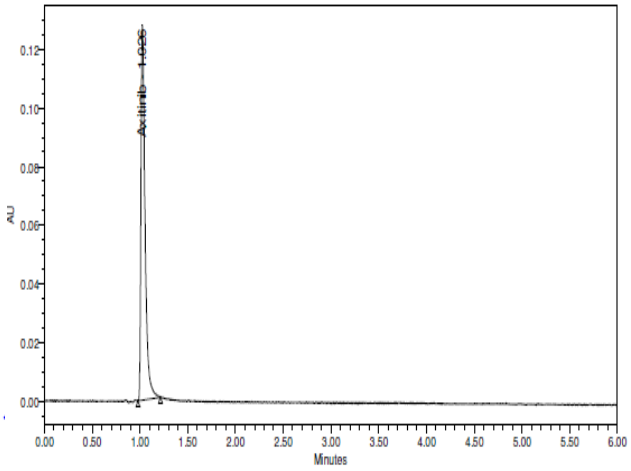
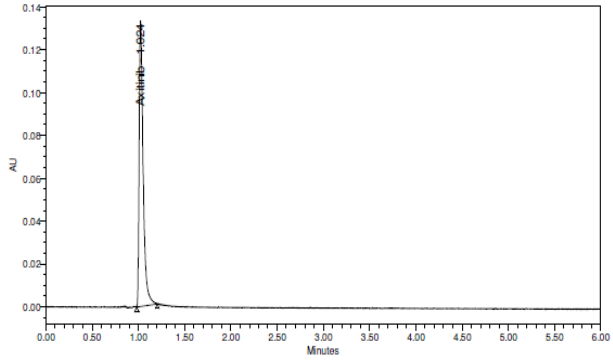


Fig.6d: Water stress study chromatogram



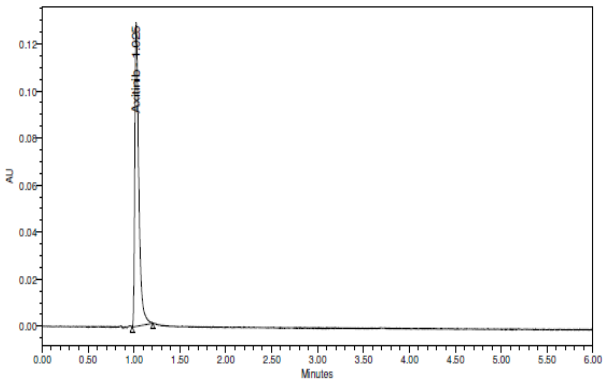


Fig.6e: Photo stability degradation chromatogram Fig.6f: Dry heat study chromatogram

Conclusion

A specific, accurate stability indicating method was developed for the estimation of Axitinib in pharmaceutical dosage form using UPLC. The method was validated by using various validation parameters and the method was found to be linear, precise, accurate, specific and robust. From the degradation studies conducted it is concluded that Axitinib was more stable at more concentrations of acid, base, peroxide, thermal, UV and water stress study conditions. The run time was 2min which enables rapid quantitation of many samples in routine and quality control analysis of capsule formulations.

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