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Method development and Validation of Axitinib in Bulk and Pharmaceutical dosage form by RP- HPLC

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ABSTRACT

A simple, rapid, precise and accurate RP-Hplc method was developed and validated for the determination of Axitinib, in bulk and pharmaceutical dosage form. The separation is achieved on RP-HPLC using a PDA detector by incorporation of empower 2 software with a flow rate of 1.0ml/min using a mixture of Methanol and water (15:85% v/v) as mobile phase. The column used was Hypersil C18 (4.6×150mm, 5µ) at a wave length of 284nm. The retention time of the Axitinib was 3.515min. The linearity of the drug was 25-125µg/m and the method precision for the determination of assay was below 2.0%RSD.

The proposed method was validated and applied for the estimation of Axitinib in quality control of bulk and pharmaceutical dosage forms.

Keywords: Axitinib, RP-HPLC, validation.

INTRODUCTION

Axitinib is the drug used for the treatment of specific type of cancer. It is a protease inhibitor for the treatment of multiple myeloma .The IUPAC name of Axitinib is N-Methyl-2-[[3-[(E)-2-pyridin-2-ylethenyl]-1H-indazol-6-yl] sulfanyl] benzamide. Structure of Axitinib shown in Figure 1. Axitinib is a peptide analogue that reversibly inhibits the protein proteasome subunit beta type-5 (PSMB5), which is part of the 20S proteasome complex. Its empirical formula is C22H18N4OS. It is a white powder consists of molecular weight 361.03 and soluble in polar solvents like water, methanol etc.

On November 20, 2015, the U.S. Food and Drug Administration approved Axitinib for the treatment of multiple myeloma Axitinib is not official in any Pharmacopoeia. A literature survey on Axitinib revealed that, until now no analytical method was reported for its determination in bulk drug and its capsule dosage form. Since literature did not cite any method for determination of this drug from bulk drug as well as its formulation it was proposed to develop and validate a new method by using RP-HPLC method(2).

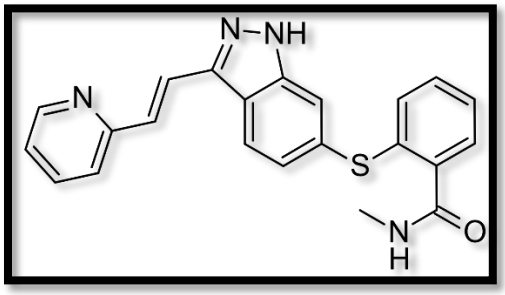

Fig 1. Axitinib (1)

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MATERIALS AND METHODS

The method development and validation was done by using waters Alliance 2895 seperation model with a PDA detector 996model.the data was acquired using Enpower 2 software and the column used was Hypersil C18, 150 x4.6mm, 5

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Instrumentation and Experimental conditions:

The method development and validation was done by using Waters Alliance 2895 seperation model with a PDA detector 996model.The data was acquired using Enpower 2 software and the column used was Hypersil C18, 150 x4.6mm,5µ. Labman Digital Ultra sonicator was used for enhancing dissolution of the drug. A Lab india make pH meter was used for pH adjustment.

Chemicals and Reagents:

Pure Axitinib gift sample was obtained from Sura Pharma Reaseach Lab, Hyderbaded. HPLC grade water, Methanol-HPLC, Acetonitrile-HPLC from Merck, Mumbai. Labman Digital Ultra sonicator was used for enhancing dissolution of the drug. A Lab india make pH meter was used for pH adjustment.

Preparation of Solutions:

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Preparation of Mobile phase:

Accurately measure 150 ml (15%) of HPLC Methanol and 850 ml of HPLC Water (85%) were mixed and degassed with a digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration. The Mobile phase was used as the diluent.

Preparation of Standard solution:

Weigh accurately 10 mg of Axitinib and transfer into a clean, dry 10ml volumetric flask and add 7ml of Methanol, sonicate to dissolve and removal of air completely and make the volume upto the mark with the diluent. Further pipette out 0.75ml of the above Axitinib stock solution into a 10ml volumetric flask and dilute up to the mark with diluent and filtered through 0.45µm membrane filter.

Preparation of Sample solution:

Each capsule was weighed and powered by using mortor and pestle and 10mg equivalent weight of Axitinib sample was weighed and transferred into 10ml of clean, dry volumetric flask. Then add 7ml of diluent and sonicate to dissolve for 20min. The volume was made upto 10ml with diluent.

Pipette out 0.75ml of the above solution into a 10ml volumetric flask and dilute up to the mark with diluent and filtered through 0.45µm membrane filter.

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 Water, Methanol: Water and Acetonitrile: Water with varying proportions. Finally, the mobile phase was optimized to Methanol: Water in proportion 15:85 v/v respectively.

Optimization of Column:

The method was carried out with various C18 columns like Phenomenex column, Xterra, and Symmetry C18 column. Hypersil C18 (4.6 x 150mm, 5µm) was found to be ideal as it gave good peak shape and resolution at 1.0ml/min flow the sample was injected using 10 µl fixed loop and PDA detector was set at a wavelength of 284nm and the run time is 6min.

RESULTS AND DISCUSSION

The method developed was validated as per ICH guidelines. The system suitability parmeters are performed and other parameters like Accuracy, Presicion, Linearty, Robustness, LOD, and LOQ are performed by using optimized chromatographic conditions. The Retention time of Axitinib was 3.513min. The standard chromatograph are shown in Figure: 2a.

System Suitability:

A standard solution was prepared by using Axitinib stock solution as per test method and was injected for 5 times into HPLC column. From the system suitability studies it is observed that % RSD values were within the specified limit i.e not more than 2. System suitability data was presented in table: 1

Specificity:

It is the ability to asses unequivocally the analyte in the presence of components that may be expected to be present. Excipients that are commonly used were spiked into a pre weighed quantity of drugs. Appropriate dilutions were injected into chromatographic system and the quantities of the drugs were determined. Results of the method are tabulated in table: 2

Linearity:

A series of solutions were prepared using Axitinib working standard solution at a concentration levels from 25-125µg/ml of target concentration and the peak area response of all solutions are measured. A graph was plotted against the Concentration (mg/ml) on X-axis versus area/response on Y-axis. The detector response was found to be linear with a correlation coefficient of 0.999. Linearity graph is shown in Figure: 3 linearity results of the method are tabulated in table:3

Precision:

Precision studies were performed (Method,Day to day). The results are reported in term of Relative standard deviation. The repeatability studies were carried out by estimsting response of 6 different concentrations of Axitinib and reported in terms of % RSD, % RSD was 0.13, for method precision it was 0.11 and for day to day precision it was 0.12. Precision results of the method are tabulated in table: 4

Accuracy:

Accuracy of the method was determined by calculating the recovery of Axitinib by the spiked method. Known quantity of Axitinib was added to a pre-determined sample solution and the amount of Axitinib was estimated by measuring peak areas. Mean % recovery values are within the limit (limit is 98-102%). Accuracy data was presented in table: 5 &5a

LOD and LOQ Limits:

The level of detection (LOD) and level of Quantification (LOQ) were conducted on the basis of standard deviation of the response and the slope .The LOD and LOQ for Axitinib were found to be 5.2 and 15.8 respectively.

Robustness:

Robustness is a measure of its capacity to remain unaffected by small, but deliberated variation in the method parameters and gives an indication of its reliability during normal usage. Robustness of the method was studied by injecting 0.9 ml min-1, 1.0 ml min-1, 1.1ml min-1 and mobile ratio variation from more organic phase to less organic phase ratio. The method passed robustness test with well % RSD. .Robustness data was presented in table: 6

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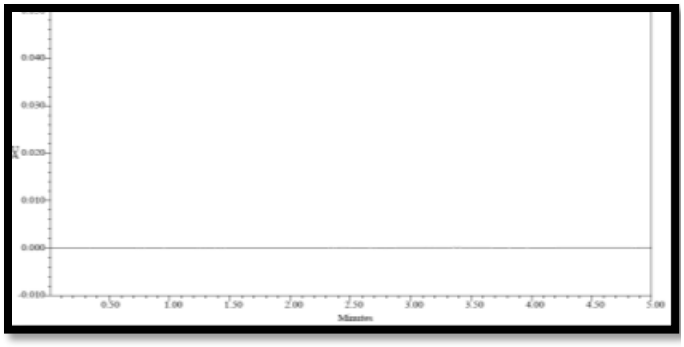

Fig 2: Chromatogram showing blank (mobile phase preparation)

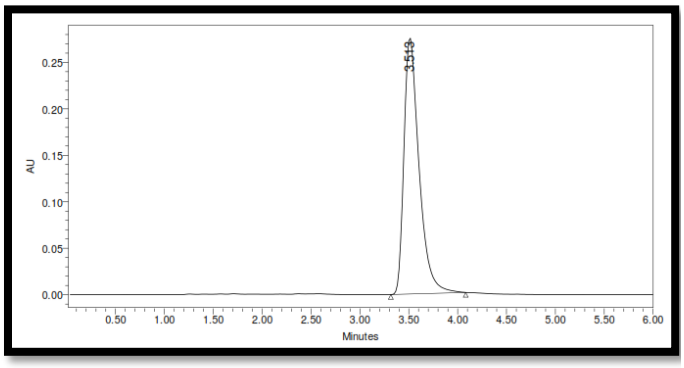

Fig 2 a: Optimized Chromatogram (Axitinib Standard)

System suitability:

Table 1: Results of system suitability for Axitinib

S.No	Peak Name	RT	Area	Height (µV)	USP Plate	USP Tailing
1	Axitinib	3.513	2947505	275462	7462	1.1
2	Axitinib	3.516	2958475	275361	7462	1.1
3	Axitinib	3.515	2965847	275144	6472	1.1
4	Axitinib	3.517	2952642	275837	7183	1.1
5	Axitinib	3.512	2951645	275948	7428	1.1
Mean			2955223			
Std.Dev			7114.704			
% RSD			0.24075			

Specificity:

Table 2: Peak results for Assay Standard

S.No	Name	RT	Area	Height	USP	USP Plate
1	Axitinib	3.518	2967593	275837	1.1	6583
2	Axitinib	3.517	2967399	275922	1.1	5938
3	Axitinib	3.515	2960183	271844	1.1	5883

Table 2a: Peak results for Assay sample

S.No	Name	RT	Area	Height	USP	USP Plate
1	Axitinib	3.511	2983744	275833	1.1	7584
2	Axitinib	3.511	2958374	275984	1.1	6294
3	Axitinib	3.514	2957262	275481	1.1	8194

% ASSAY =

Sample area / Standard area (X) Weight of standard/ Dilution of standard(X)Dilution of sample/Weight of sample (X) Purity/100 (X) Weight of Capsule/ Label claim (X) 100 = =2966460 / 2965058 × 10/75 × 75/0.0145 × 99.7/100 × 0.0174/12 × 100 =99.7

The % purity of Axitinib in pharmaceutical dosage form was found to be 99.7%

Linearty:

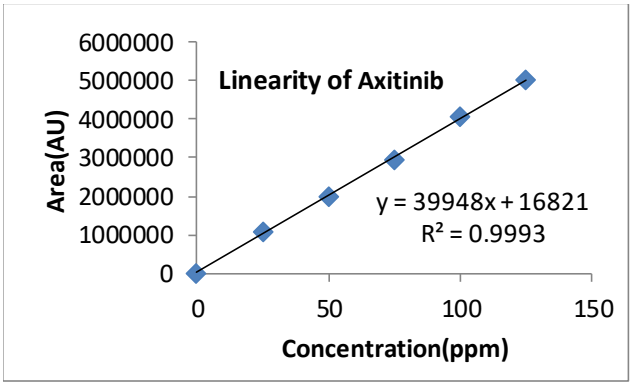

Fig: 3: Linearity graph of Axitinib

Table 3: For linearity data

Conc. Level (%)	Concentration(g/ml)	Average Peak Area
33	25	1083048
66	50	1973321
100	75	2955166
133	100	4063921
166	125	5006038

Precision

Table 4: Results of repeatability for Axitinib:

S. No	Peak name	RT	Area(µV *sec)	Height(µV)	USP Plate Count	USP Tailing
1	Axitinib	3.528	2958333	275983	7583	
2	Axitinib	3.516	2951049	275911	7593	1.1
3	Axitinib	3.514	2959294	275955	8674	1.1
4	Axitinib	3.519	2953391	275921	7958	1.1
5	Axitinib	3.512	2950744	275221	9745	1.1
Mean			2954562			
Std. dev			4028.083			
%RSD			0.136334			

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Table 4a: Results of Intermediate precision day1 for Axitinib

S.no	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate	USP Tailing
1	Axitinib	3.517	2957344	275838	7194	1.1
2	Axitinib	3.514	2951847	275629	8573	1.1
3	Axitinib	3.517	2950834	276931	7655	1.1
4	Axitinib	3.517	2957155	275623	7944	1.1
5	Axitinib	3.512	2950185	275184	7562	1.1
6	Axitinib	3.518	2951750	275193	7585	1.1
Mean			2953186			
Std.			3207.331			
%			0.108606			

Table 4b: Results of Intermediate precision Day 2 for Axitinib

S.no	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate	USP Tailing
1	Axitinib	3.513	2951848	275929	7937	1.1
2	Axitinib	3.511	2958275	275222	7284	1.1
3	Axitinib	3.516	2950185	275857	7684	1.1
4	Axitinib	3.518	2957462	275163	7917	1.1
5	Axitinib	3.511	2957541	275164	7585	1.1
6	Axitinib	3.519	2951164	275154	7192	1.1
Mean			2954413			
Std.			3715.025			
% RSD			0.125745			

Table 5: Results of Accuracy for concentration-50%, 100% and 150%

S.no	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate	USP Tailing
1	50%	3.519	1493763	275837	1.1	6453
2	50%	3.520	1493923	275819	1.1	7584
3	50%	3.519	1492575	275083	1.1	6785
4	100%	3.516	2983722	275829	1.1	7483
5	100%	3.518	2984722	275143	1.1	6935
6	100%	3.519	2967382	275063	1.1	6285
7	150%	3.517	4462011	275622	1.1	7483
8	150%	3.519	4469483	275922	1.1	7265
9	150%	3.520	4489583	276331	1.1	7194

Table 5a: The accuracy results for Axitinib

%Concentration (at specification Level)	Area	Amount Added(ppm)	Amount Found(ppm)	%Recovery	Mean Recovery
50%	1483420	37.5	36.96	98.5%	98.8%
100%	2978609	75	74.1	98.8%	
150%	4473692	112.5	111.56	99.1%	

Robustness:

Table 6: Results for Robustness

Parameter used for sample analysis	Area	RT	Theoretical	Tailing
Actual Flow rate of 1.0 mL/min	2955764	3.513	7483	1.1
Less Flow rate of 0.9 mL/min	2958393	3.897	6028	1.1
More Flow rate of 1.1 mL/min	2956411	3.218	6928	1.2
Less organic phase	2950683	3.707	6733	1.2
More organic phase	2957265	3.350	6285	1.1

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capsule dosage form. From the results obtained, we conclude that the suggested method showed high sensitivity, accuracy, and specificity. Moreover, this method was simple and economical and can be employed for the routine quality control of Axitinib in pharmaceutical dosage forms

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