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RP-HPLC Method Development and Validation for The Analysis of Pharmaceutical Drug – AXITINIB

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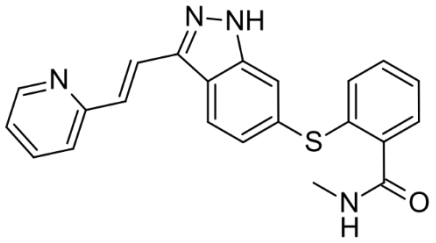
ABSTRACT
A simple, selective, linear, precise and accurate RP-HPLC method was developed and validated for rapid assay of AXITINIB. Isocratic elution at a flow rate of 1.2 ml /min was employed on a symmetry C18 column at ambient temperature. The mobile phase consisted of Acetonitrile : 0.1M Acetic Acid 50:50 (v/v). The UV detection wavelength was at 254 nm. Linearity was observed in concentration range of 10-50 ppm. The retention time for AXITINIB was 3.0 min. The method was validated as per the ICH guidelines. The proposed method can be successfully applied for the estimation of AXITINIB.

Keywords : AXITINIB, Method Development, Validation, 254nm, 3.0min.

I. INTRODUCTION

DRUGS: AXITINIB

STRUCTURE:



IUPAC NAME	*N*-Methyl-2-[[3-[(*E*)-2-pyridin-2-yletheny l]-1*H*-indazol-6-yl]su lfanyl]benzamide
FORMULA	$C_{22}H_{18}N_4OS$
MOLACULAR WEIGHT	386.469 g/mol
CLASSIFICATION OF DRUGS	Anticancer Drugs

Axitinib (AG013736; trade name Inlyta) is a small molecule tyrosine kinase inhibitor developed by Pfizer. It has been shown to significantly inhibit growth of breast cancer in animal (xenograft) models[2] and has shown partial responses in clinical trials with renal cell carcinoma (RCC)[3] and several other tumour types.[4]

It was approved for RCC by the U.S. Food and Drug Administration after showing a modest increase in progression-free survival,[5] though there have been reports of fatal adverse effects.[6]

II. EXPERIMENTAL

Chemicals and reagents

All HPLC SOLVENTS used like Acetonitrile, Acetic Acid and water which are of HPLC grade were purchased from Merck and Finar laboratory

Instrumentation and analytical conditions

The analysis of the drug was carried out on Shimadzu HPLC model (VP series) containing LC-20AT (VP series) pump, variable wave length programmable UV/visible detector SPD-20A and rheodyne injector (7725i) with 20µl fixed loop. Chromatographic

analysis was performed using phenolex C-18 column with 250 x 4.6mm internal diameter and 5µm particle size. Shimadzu electronic balance (AX-200) was used for weighing. Isocratic elution with, Acetonitrile, 0.1MAcetic Acid 50:50(v/v) was selected with a flow rate of 1.2 ml /min .The detection wavelength was set at 254 nm with a run time of 10 min. The mobile phase was prepared freshly and it was degassed by sonicating for 5 min before use. The column was equilibrated for at least 30min with the mobile phase flowing through the system. The column and the HPLC system were kept at ambient temperature.

Preparation of Stock, working standard solutions and Sample solutions

100 mg of Axitinib was weighted and transferred into a 100 ml volumetric flask. Water was added and sonicated to dissolve it completely and made up to the mark with the same solvent. Further 10 ml of the above stock solution was pipette into a 100ml volumetric flask and diluted up to the mark with water. The contents were mixed well and filtered through Ultipor N66 Nylon 6, 6 membrane sample filter paper. The calibration curve was plotted with the concentrations of the 10-50ppm solutions. Calibration solutions were prepared and analyzed immediately after preparation.

Table-1. Chromatographic conditions for AXITINIB

Sr. No	TEST H.P.L.C CONDITIONS	RESULT
1	Elution	ISOCRATIC
2	A.P.I Conc.	10 ppm
3	Mobile Phase	Acetonitrile:0.1M Acetic Acid(50:50)
4	pH	3.2
5	Column	C18
6	Wavelength	254 nm
7	Flow Rate	1.2ml/min
8	Runtime	10 Min
9	Retention Time	3.0
10	Area	4015.000
11	Th.Plates	4950
12	Tailing Factor	1.007
13	Pump Presure	105 kgf

Method Validation procedure

The objective of the method validation is to demonstrate that the method is suitable for its Intended purpose as it is stated in ICH guidelines. The method was validated for linearity, precision, accuracy, specificity, and limit of detection, limit of quantification, robustness and system suitability.

Linearity

S.NO	CONC	AREA
1	10 ppm	4015.000
2	20ppm	8220.001
3	30ppm	12360.203
4	40ppm	16496.096
5	50ppm	20519.000

Table 2: Linearity of Axitinib

The developed method has been validated as per ICH guidelines. Solutions of Axitinib in the mass concentration range of 10ppm to 50ppm as injected into the chromatographic system. The chromatograms were developed and the peak area was determined for each concentration of the drug solution. Calibration curve of Axitinib was obtained by plotting the peak area ratio versus the applied

concentrations of Axitinib. The linear correlation coefficient was found to be 0.9999.

Table 3. Linear Regression Data for Calibration curve

Drug	Axitinib
Concentration range	10 - 50ppm
Slope (m)	412.6
Intercept (b)	-111
Correlation coefficient	0.9999

Precision

Repeatability of the method was checked by injecting replicate injections of 30ppm of the solution for five times on the same day as intraday precision study of and the RSD was found to be 0.6356 for intraday and 0.6451for interday

Table 4 : Precision parameters of Axitinib

INJECTION	CONCENTRATION	INTRA DAY	INTER DAY
1	30 ppm	12360.990	12416.555
2	30ppm	12413.150	12512.200
3	30ppm	12295.050	12309.870
4	30ppm	12390.257	12425.272
5	30ppm	12420.000	12509.699
6	30ppm	12218.995	12361.444
	%RSD	0.6356	0.6451

Accuracy

The accuracy of the method was determined by calculating recovery of Axitinib by the method of standard addition. Known amount of Axitinib (0.5) was added to a pre-quantified sample solution and the amount of Axitinib was estimated by measuring the peak area ratios and by fitting these values to the straight line equation of calibration curve. The recovery studies were carried out three times over the specified concentration range and amount of Axitinib was estimated by measuring the peak area ratios by fitting these values to the straight line equation of calibration curve.

Specificity

The specificity of the method was determined by comparing test results obtained from analysis of sample solution containing excipients with that of test results those obtained from standard drug.

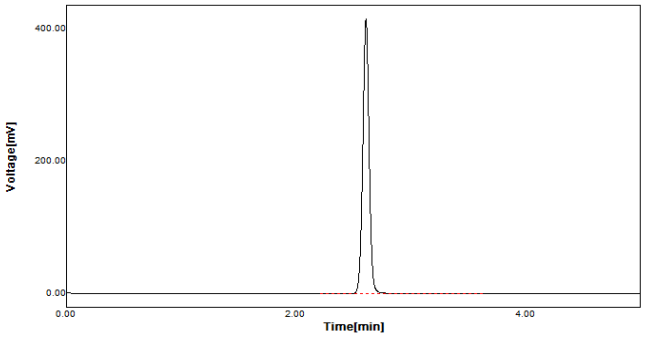


Figure : Typical chromatogram of Axitinib

LOD and LOQ

The limit of detection and limit of quantification were evaluated by serial dilutions of Axitinib stock solution in order to obtain signal to noise ratio of 3:1 for LOD and 10:1 for LOQ as per ICH guide-lines. Obtained results are shown in table 5.

Table 5: Results of LOD and LOQ.

Parameter	Measured
LOD	1.0ppm
LOQ	3.0ppm

Robustness

To determine the robustness of the method, two parameters from the optimized chromatographic Conditions were varied. First, Instrument and place were changed and second mobile phase concentration was changed Acetonitrile-0.1M Acetic Acid (48: 52, v/v) and Acetonitrile-0.1M Acetic Acid (52:48v/v). Results of Robustness are shown in table 6& 7.

Table 6: Robustness parameters

Parameter	Modification
M.PHASE	Acetonitrile-0.1M Acetic Acid (48: 52, v/v)
pH	3.5
WAVELENGTH	254 nm
R.T	3.0 Min

Table 7: Robustness results

Accuracy	Precision
4073.255	4033.612
4071.897	4034.520
4072.333	4033.917
4072.511	4033.827
4071.944	4035.111
RSD: 0.013	RSD: 0.015

System Suitability Parameter:

System suitability tests were carried out on freshly prepared standard stock solutions of Axitinib and it was calculated by determining the standard deviation of Axitinib standards by injecting standards in five replicates at 5 minutes interval and the values were recorded in Table 8.

Table 8: System suitability parameters of Axitinib

Parameters	Values
λ max (nm)	254 nm
Correlation coefficient	0.9999
Retention time	2.850min
Theoretical plates	4950
Tailing factor	1.009
Limit of detection	1.0 ppm
Limitof quantification	3.0 ppm

III. RESULT AND DISCUSSION

Optimization of the chromatographic conditions

The nature of the sample, its molecular weight and solubility decides the proper selection of the stationary phase. The drug Axitinib being non-polar is preferably analyzed by reverse phase columns and accordingly C18 column was selected. So the elution of the compound from the column was influenced by polar mobile phase. Different mobile phases were tried but satisfactory separation, well resolved and good symmetrical peaks were obtained with the mobile phase Acetonitrile: 0.1M Acetic Acid (50:50).The retention time of Axitinib was found to be 3.0 min, which indicates a good base line. The RSD values for accuracy and precision studies

obtained were less than 2% which revealed that developed method was accurate and precise.

IV. CONCLUSION

A validated RP-HPLC method has been developed for the determination of Axitinib in bulk form. The proposed method is simple, rapid, accurate, precise and specific. Its chromatographic run time of 6 min allows the analysis of a large number of samples in short period of time. Therefore, it is suitable for the routine analysis of Axitinib in pharmaceutical analysis.

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