METHODDEVELOPMENT AND VALIDATION OF AVAPRITINIB BY REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH STABILITY STUDIES

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Abstract:

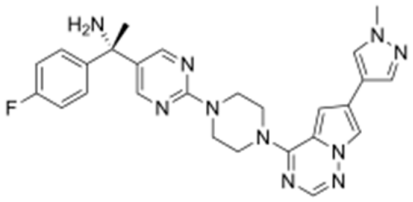
Hence, proposed analytical method was simple, novel, economical, rapid, sensitive, reproducible and accurate for the Avapritinib. A method was set up for synchronous estimation of a Avapritinib by RP-HPLC system. The chromatographic conditions were viably created for the unit of Avapritinib by using Inertsil - ODS C18(250 x 4.6 mm, 5µ), stream is 1.0 ml/min, convenient stage extent was Methanol: Acetonitrile (30:70), recognizable proof wave length was 225nm.The Linearity was found to be 20-70ug/ml for Avapritinib. Correlation coefficient was found to be 0.999 for Avapritinib respectively. Accuracy was found to be 100.3%,100.4%,100.4% for Avapritinib. LOD and LOQ was found to be 0.0048 & 0.0146. The %RSD for all the validation parameters were within the limit.

Key Words: Avapritinib, Catchphrases, RP-HPLC, Acetonitrile, Methanol, Water.

Introduction:

An analytical technique was created to estimate the drug substance of avapritinib using liquid chromatography. At room temperature, the chromatographic separation was accomplished using a C18 column (Xterra R18 150*4.6, 5um). Using a mobile phase of 0.1% v/v formic acid in water: acetonitrile (40:60), the separation was accomplished. The UV detector was set to 296 nm, and the flow rate was 1.0 ml/min. Avapritinib was shown to have a retention time of 2.71 minutes. The accuracy, linearity, precision, and selectivity of the suggested approach were all confirmed. Every validation parameter fell within the permissible bounds. For avapritinib, the test procedures were shown to be linear between 50 and 150µg/ml.

Material and Methods:



Chemical Formula : $C_{26}H_{27}FN_{10}$

Avapritinib is a selective kinase inhibitor that negatively modulates the action of cell transporters to resensitize them to other chemotherapies. It has a long duration of action as it is given once daily. Patients should be counselled regarding the risk of intracranial hemorrhage, CNS effects, and embryo-fetal toxicity. A 300mg oral dose of avapritinib reaches a C_{max} of 813ng/mL with a T_{max} of 2.0-4.1h and an AUC of 15400h*ng/mL

Mechanism of action: Avapritinib has a negative modulating effect on the transporters ABCB1 and ABCG2, which mediate the multidrug resistance phenotype of some cancers. This modulation may be due to interactions of avapritinib with the drug binding pocket of these transporters. Cancerous cells become less sensitive to the effects of chemotherapy drugs such as paclitaxel when these transporters are negatively modulated.

Chromatographic condition: A mobile phase consisted of Acetonitrile: Methanol (70:30) V/V ration was pumped at a flow rate of 1 ml/min. The elution was monitored at 225 nm and the injection volume was 20 µL. The validation of the method was done following the ICH guidelines

Instrumentation: Waters Model NO.2690/5 series Compact HPLC system with an Inertsil-C18 ODS column. And systronics UV spectrophotometer, SARTORIOUS electronic balance, and the Fast Clean Sonicator. For Solvents HPLC Grade Methanol. HPLC Grade Buffer (KH2PO4) are Used.

Preparation of Solutions:

Mobile Phase: Acetonitrile: Methanol (70:30) V/V. After 30 minutes of sonication, pass the mobile phase through 0.45-micron filter paper.

Preparation of Stock solution: Take 100mg Avapritinib working standard in 100ml volumetric Flask add methanol sonicate it 30 minutes, (That is 1000ppm solution). Further dilution takes 4ml of above solution in 100ml V.F add Methanol up to mark sonicate it 10minets (That 40ppm solution).

Selection of mobile phase for method Optimization and experimental condition

Several trial has been taken for the proper optimization of RP HPLC method by changing different mobile phase with different ratio. And finally the mobile phase for optimised condition was selected and given follows. And the optimised parameters was for Avapritinib.

Stage of stationary (column) : ODS C18 Inertsil (250 x 4.6 mm, 5 µ)

Phase of Mobility : Acetonitrile: Methanol (70:30)

The flow rate in milliliters per minute: 1.0 ml/min

Duration of operation (minutes) : 6 Min

Temperature in the column (°C) : Ambient

The injection loop's volume (l) : 20

Wavelength of detection (nm) : 225 nm

Medication RT (min) : 3.519 min

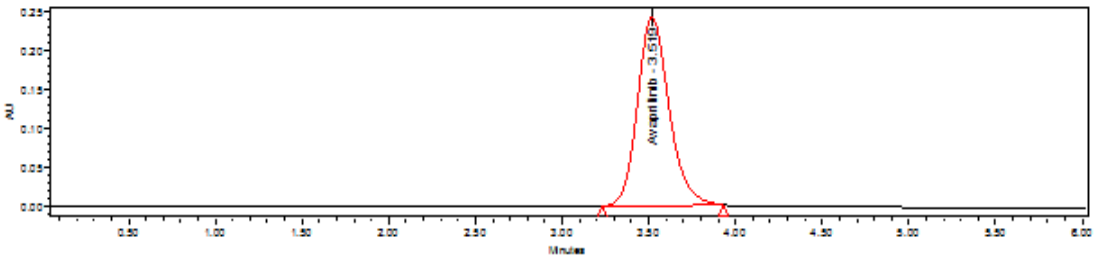


Figure 01: Chromatogram for the marketed formulation

This optimized method was validated in terms of linearity, accuracy, precision, specificity, limit of detection, limit of quantification and solution stability as per ICH guidelines.

System Suitability:

Injection	RT	Peak Area	USP Plate	USP Tailing
1	3.521	645478.48	10621	1.101
2	3.523	64.5449.32	10630	1.103
3	3.520	645455.29	10632	1.101
4	3.518	6454423.23	10645	1.103
5	3.520	645480.63	10650	1.102
Mean	3.5204	645457	10635	1.102
SD	0.001817	23.5654	…….	……
%RSD	0.051602	0.00365	…….	……

Table: 01 System suitability data

Results: It showing no more than 2.0% RSD for the major peak retention periods from each of the five replicate injections of the standard solution and it should be no more than 2.0% variation in the peak area responses of the major peak from each of the five replicate injections of the standard solution. The avapritinib peaks' tailing factor (T) is NMT 2.0.

Linearity:

Avapritinib working standard is used to generate a series of solutions with concentrations ranging from 20 ppm to 70 ppm of the target concentration

Concentration (ppm)	Avg Peak Area	Statistical Analysis	
0	0	Slope	16054
20	322716.85	y-Intercept	1854
30	484074.36	Correlation Coefficient	0.999
40	645432.45		

50	806790.56	
60	968148.84	
70	1120456.85	

Table: 02 Linearity Data

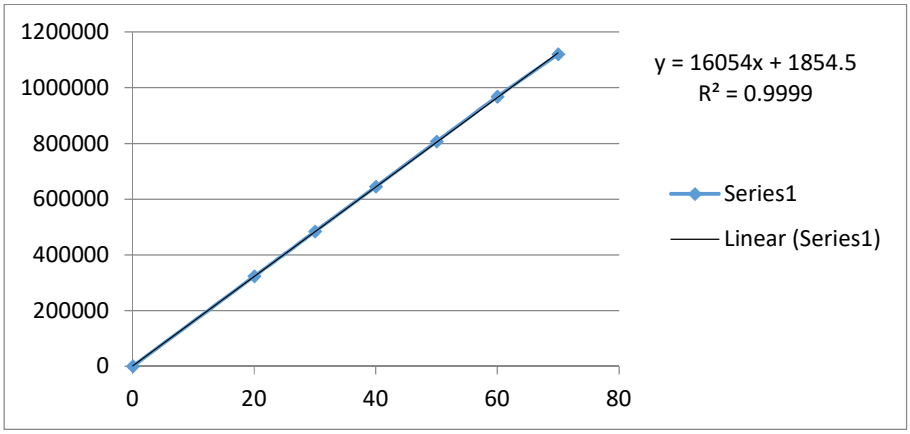


Fig: 2 Concentration vs. Response in a Linearity Plot

Results: The correlation coefficient is 0.9990. The percentage of the y-intercept must be ±2.0. The RSD percentage for Level 1 and Level 6 within the limits 2.0%.

Accuracy:

An investigation into accuracy was done. In accordance with the test protocol, the drug assay was carried out in triplicate, putting an equal amount of avapritinib into each volumetric flask for each spike level. This allowed for the determination of the concentration of avapritinib to be 50%, 100%, and 150% of the amount indicated. Avapritinib's average recovery percentage was computed.

Concentration %Spiked level	**Area**	**Amount**	**Amount found**	**% Recovery**	**Statistical Analysis Recovery %**	
50%	322742.02	20	19.98	99.94	Mean	100.32
50%	322769.61	20	20.10	100.51		
50%	322728.59	20	20.10	100.52	%RSD	0.336
100%	645512.85	40	40.09	100.23	Mean	100.42
100%	645489.56	40	40.20	100.51		
100%	645530.51	40	40.20	100.52	%RSD	0.1651
150%	968148.53	60	60.19	100.31	Mean	100.44
150%	968125.94	60	60.30	100.50		
150%	968165.54	60	60.30	100.51	%RSD	0.1104

TABLE-03: Data of Accuracy

Results: At every spike level, the average percentage of Avapritinib recovery is with the limits (98.0% and 102.0 %)

PRECISION

Reproducibility:

a. System precision: Five injections of a standard solution made in accordance with the test protocol.

b. Accurate procedure: Six sample preparations were made separately, utilizing a single test method, and each solution was injected.

	Injection	Peak Area of Avapritinib	% Assay
Concentration 40ppm	1	645440.56	100.22
	2	645480.24	100.22
	3	645471.28	100.22
	4	645423.23	100.21
	5	645462.64	100.22
	6	645470.63	100.22
Statistical Analysis	Mean	645455.59	100.22
	SD	23.3268	0.00363
	%RSD	0.00361	0.00362

TABLE-04 Data of Repeatability (System precision)

Results: Avapritinib's percentage relative standard deviation over the six units showing within limits 2.0%

Method of precision

	Injection	Peak Area of Avapritinib	% Assay
Concentration 40ppm	1	645522.29	100.23
	2	645499.64	100.23
	3	645531.85	100.23
	4	645481.56	100.22
	5	645539.93	100.23
	6	645510.45	100.23

Statistical Analysis	Mean	645514.28	100.23
	SD	21.5888	0.00336
	%RSD	0.00334	0.00335

Table-5: Data of Repeatability (Method precision & Analyst one)

Results: Avapritinib's percentage relative standard deviation over the six units showing within limits 2.0%.

Intermediate precision

	Injection	**Peak Area of Avapritinib**	**% Assay**
Concentration 40ppm	1	645413.13	100.21
	2	645440.85	100.22
	3	645482.67	100.22
	4	645429.99	100.22
	5	645461.16	100.22
	6	645466.66	100.22
Statistical Analysis	Mean	645449.07	100.22
	SD	25.7209	0.00400
	%RSD	0.00398	0.00399

Table06: Intermediary precision data (Analyst 2)

Results: The assay deviation for each individual assay of avapritinib should be NMT2.0% for both analysts, relative standard deviation showed within the range.

Robustness:

Impact of flow rate variation: To ascertain the impact of flow rate variation, research was done. A standard solution that was produced in accordance with the test procedure was introduced into the HPLC system at 1.0 and 1.2 milliliters per minute. After evaluation, the system suitability parameters for the flow rates of 1.0 and 1.2 milliliters per minute were found to be within tolerances.

Flow 0.8 ml	Std Area	Tailing factor	Flow 1.0 ml	Std Area	Tailing factor	Flow 1.2 ml	Std Area	Tailing factor
	640125.54	1.119		645444.52	1.123		650184.85	1.136
	640181.45	1.123		645489.23	1.125		650136.85	1.138
	640144.44	1.125		645512.12	1.123		650148.36	1.137
	640138.84	1.129		645463.52	1.124		650163.36	1.137
	640152.15	1.131		645475.57	1.125		650196.65	1.136
Avg	640148.48	1.125	Avg	645476.99	1.124	Avg	650166.01	1.137
SD	20.8325	0.0047	SD	25.6012	0.001	SD	24.8123	0.0008
%RSD	0.003254	0.4242	%RSD	0.00396	0.0889	%RSD	0.00381	0.0735

Table:06 Information on the Impact of Flow Rate Variation

Results: Variation in Flow should be the Tailing Factor of Avapritinib with in the standards.

Limit of detection (LOD) and limit of quantification (LOQ):

The linearity plot is used to determine the LOD and LOQ:

$$LOD = 3.3\,\sigma / S$$

$$= \frac{3.3 \times 23.5654}{16054} = 0.0048$$

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

$$= \frac{10 \times 23.5654}{16054} = 0.0146$$

Studies on Force Degradation:

Hydrolysis of acid:

An aliquot of stock solution (1 mg/ml) of avapritinib was added to 10 ml of methanol and 0.1 M HCl, and the combination was refluxed at 60°C for around six hours to achieve acid-induced, forced degradation. After allowing the solution to come to room temperature, 0.1 M NaOH was added to neutralize it to pH 7, and 100 ml of the mobile phase was added to dilute it to a final concentration of 10 µg/ml.

Alkaline hydrolysis:

An aliquot of stock solution (1 mg/ml) of avapritinib was added to 10 ml of methanol and 0.1 M NaOH, and the mixture was refluxed at 60°C for around six hours to achieve forced degradation in alkaline media. After allowing the solution to come to room temperature, 0.1 M HCl was added to neutralize it to pH 7, and 100 ml of the mobile phase was added to dilute it to a final concentration of 10 µg/ml.

Degradation by oxidation:

An aliquot of stock solution (1 mg/ml) of avapritinib was added to 10 ml of 30% H2O2 solution, and the mixture was refluxed at 60°C for roughly six hours in order to examine the impact of oxidizing conditions. After allowing the solution to come to room temperature, the mobile phase was added and diluted to 100 ml, resulting in a final concentration of 10 µg/ml.

Heat-related deterioration

Approximately 50 mg of avapritinib was kept at 100°C in a hot air oven for a full day in order to examine the impact of temperature. After dissolving it in 10 milliliters of methanol, the mobile phase was added to get the volume down to 50 milliliters. After adding the mobile phase to the previously prepared solution, the final concentration of the mixture was 10 µg/ml of avapritinib.

$$\% \text{ Degradation} = \frac{Au - At}{Au} \times 100$$

Where: Au=Untreated Solution Area, At= Solution Treating Area.

Forced Degradation for Avapritinib

Mode of Degradation	Condition	Peak Area	% Degradation as compared with Control
Control sample	No treatment	645478	-
Acid	0.1 M HCl	611735	5.2276
Base	0.1 M NaOH	631891	2.1049
Oxidative	30% H_2O_2	630142	2.3759
Thermal	100°C	620867	3.8128

Table:07 Forced Degradation for Avapritinib

CONCLUSION

Various parameters were examined in order to develop the analytical methodology. First off, it was found that avapritinib had a maximum absorbance of 225 nm. A great peak region was obtained by setting the injection volume at 20µl. In this job, the Inertsil C18 column was used, and ODS selected a good peak form. It was found that the ambient temperature was suitable for the kind of pharmaceutical solution. The flow rate was set at 1.0 ml/min due to the good peak area, sufficient retention length, and good resolution. A variety of mobile phase ratios were examined; however, because to its symmetrical peaks and good resolution, the mobile phase containing a Methanol: Acetonitrile (30:70) ratio was selected. Consequently, this mobile phase was utilized in the intended investigation. It was found that the system and technique both had precise and well-within-range accuracy. The linearity investigation yielded the correlation coefficient and curve fitting. The analytical method was demonstrated to be linear for both medications throughout a range of 20–70 ppm of the target concentration. The analysis passed the ruggedness and robustness testing. In both cases, the relative standard deviation was very good.

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