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A new analytical method development and validation of estimation of avapritinib by RP-HPLC

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Abstract
Aim of present study is to develop simple, precise, and accurate method for quantitative estimation of Avapritinib in bulk form and marketed pharmaceutical tablet dosage form. The method was achieved on Symmetry C18 ODS (4.6mm×250mm) 5µm particle size column with mobile phase containing composition of Acetonitrile and Phosphate buffer (0.01M, pH-3.2) in the ratio of 30:70v/v at a flow rate 1.0ml/min with detection wavelength at 246nm. The linearity was obtained in the concentration range of 6-14 µg/ml for Avapritinib. The suitability of this method was proved by validation in accordance with ICH Q2 (R1) guidelines. The method was found to be accurate with percent recovery was found to be 100.130%. The %RSD for method repeatability and for intermediate precision were found be within the limits i.e. 0.441, 0.258 and 0.373 respectively. The proposed method was found to be simple and sensitive for routine quality control application of Avapritinib used in bulk form and pharmaceutical tablet dosage form.

Keywords: Avapritinib, RP-HPLC, Method Development, Validation, Accuracy, Precision

Introduction

Avapritinib, or BLU-285, is a selective tyrosine kinase inhibitor of KIT and platelet derived growth factor receptor alpha indicated for the treatment of unresectable, metastatic gastrointestinal stromal tumours. It is one of the first medications available for the treatment of multidrug resistant cancers. Avapritinib shares a similar mechanism with [Ripretinib]. Avapritinib [1] was granted FDA approval on 9 January 2020. Avapritinib is indicated for the treatment of unresectable, metastatic gastrointestinal stromal tumours with a platelet-derived growth factor receptor alpha exon 18 mutations. Avapritinib [2] 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.3 Patients should be counseled regarding the risk of intracranial hemorrhage, CNS effects, and embryo-fetal toxicity. Avapritinib [3] 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. Negative modulation of these transporters, resensitizes cancerous cells to treatment with chemotherapeutic agents like paclitaxel. The IUPAC Name of Avapritinib is (1S)-1-(4-fluoro phenyl)-1-[2-[4-[6-(1-methyl pyrazol-4-yl) pyrrolo [2, 1-f] [1, 2, 4] triazin-4-yl] piperazin-1-yl] pyrimidin-5-yl] ethanamine. The Chemical Structure of Avapritinib is as following.

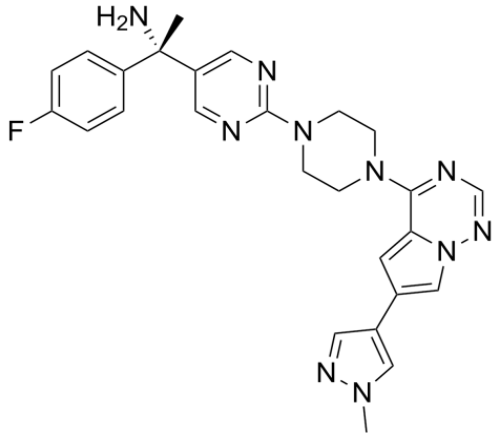

Fig 1: Chemical Structure of Avapritinib

Materials and Methods

Table 1: List of Equipments

S. No.	Instruments/Equipments/Apparatus
1.	HPLC WATERS with Empower2 Software with Isocratic with UV-Visible Detector.
2.	T60-LABINDIA UV – Vis spectrophotometer
3.	High Precision Electronic Balance
4.	Ultra Sonicator (Wensar wuc-2L)
5.	Thermal Oven
6.	Symmetry C_{18} Column, 250 mm x 4.6 mm and 5µm particle size
7.	P^H Analyser (ELICO)
8.	Vaccum Filtration Kit (Labindia)

Table 2: List of Chemicals used

S.No.	Name	Grade	Manufacturer/Supplier
1.	HPLC grade water	HPLC	Sd fine-Chem ltd; Mumbai
2.	Methanol	HPLC	Loba Chem; Mumbai.
3.	Ethanol	A.R.	Sd fine-Chem ltd; Mumbai
4.	Acetonitrile	HPLC	Loba Chem; Mumbai.
5.	DMSO	A.R.	Sd fine-Chem ltd; Mumbai
6.	DMF	A.R.	Sd fine-Chem ltd; Mumbai

Estimation of Avapritinib in bulk and pharmaceutical dosage form

Procedure

Preparation of Mobile Phase

Accurately measured 300 ml (300%) of HPLC Grade Acetonitrile and 700 ml of Phosphate buffer (70%) were mixed and degassed in a digital ultra sonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filter.

Preparation of 0.01M Potassium dihydrogen orthophosphate Buffer Solution

About 1.36086grams of Potassium dihydrogen orthophosphate was weighed and transferred into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC Grade water. The pH was adjusted to 3.20 with diluted orthophosphoric acid.

Diluent Preparation

Accurately measured 300 ml (300%) of HPLC Grade Acetonitrile and 700 ml of Phosphate buffer (70%) were mixed and degassed in a digital ultra sonicater[4] for 15 minutes and then filtered through 0.45 µ filter under vacuum filter.

Assay

Preparation of the Avapritinib standard solution

Preparation of standard solution: (Avapritinib)

Accurately weigh and transfer 10 mg of Avapritinib, working standard into a 10ml of clean dry volumetric flasks add about 7ml of diluent and sonicate to dissolve and removal of air completely and make volume up to the mark with the diluent. Further pipette 0.1ml of Avapritinib from stock solution in to a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure

Inject the samples by changing the chromatographic conditions [5] and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines [6].

Preparation of Sample Solution

Take average weight of Tablet and crush in a mortar by using pestle and taken weight 10 mg equivalent weight of Avapritinib 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.

Procedure

Further pipette 0.1ml of Avapritinib from above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Inject the three replicate injections of standard and sample solutions and calculate the assay [7] by using formula:

%ASSAY =

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

Analytical Method Validation

Validation

Validation [8-10] is a process of establishing documented evidence which provide a high degree of assurance that specific activity will consistently produce a desired result or product meeting its predetermined specification and quality characteristics.

System Suitability

System suitability is the evaluation of the components of an analytical system to show that the performance of a system meets the standards required by a method. A system suitability [11] evaluation usually contains its own set of parameters. For chromatographic assays, these may include tailing factor, resolution, precision, capacity factor time and theoretical plates.

Accuracy

For preparation of 50% Standard stock solution

Further pipette 0.05ml of Avapritinib from stock solutions in

to a 10ml volumetric flask and dilute up to the mark with diluent.

For preparation of 100% Standard stock solution
Further pipette 0.1ml of Avapritinib from stock solutions in to a 10ml volumetric flask and dilute up to the mark with diluent.

For preparation of 150% Standard stock solution
Further pipette 0.15 ml of Avapritinib from stock solutions in to a 10ml volumetric flask and dilute up to the 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 Avapritinib and calculate the individual recovery and mean recovery values [12].

Acceptance Criteria
The %RSD [13] for each level should not be more than 2.

Precision
Repeatability
Preparation of Avapritinib for Precision
Further pipette 0.1 ml of Avapritinib from stock solutions in to a 10ml volumetric flask and dilute up to the mark with diluent.
The standard solution was injected for five times and measured the area for all five injections in HPLC[14]. The %RSD for the area of five replicate injections was found to be within the specified limits.

Ruggedness
To evaluate the intermediate precision of the method, Precision [15] was performed on different days by maintaining same conditions.

Procedure
Day 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 [16].

Day 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.
The % RSD for the area of five standard injections results should be not more than 2%.

Linearity
Preparation of Level – I (6µg/ml of Avapritinib)
Further pipette 0.06 ml of Avapritinib from stock solutions in to a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of Level – II (8µg/ml of Avapritinib)
Further pipette 0.08 ml of Avapritinib from stock solutions in to a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of Level – III (10µg/ml of Avapritinib)
Further pipette 0.1ml of Avapritinib from stock solutions in to a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of Level – IV (12µg/ml of Avapritinib)
Further pipette 0.12ml of Avapritinib from stock solutions in to a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of Level – V (14µg/ml of Avapritinib)
Further pipette 0.14ml of Avapritinib from stock solutions in to a 10ml volumetric flask and dilute up to the 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 [17].
Acceptance Criteria: Correlation coefficient should be not less than 0.999.

Limit of Detection
The detection limit [18] is determined by the analysis of samples with known concentration of analyte and by establishing that minimum level at which the analyte can reliably detected.

Limit of Quantitation
The quantification limit [19] is generally determined by the analysis of sample with known concentrations of analyte and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision.

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

Effect of Variation of flow Rate
The sample was analyzed at 0.9 ml/min and 1.1 ml/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. Acetonitrile: Phosphate Buffer was taken in the ratio and 70:30, 75:25 instead of 65:35, remaining conditions are same. 20µl of the above sample was injected and chromatograms were recorded.

Forced Degradation Studies
The specificity [20] 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.
Acid Degradation Studies: To 1 ml of Avapritinib stock, 1 ml of 2N HCl was added and refluxed for 30 min at 60 ºC.

The resultant solution was neutralized with 1 ml 2N NaOH and makeup to final volume to obtain (10µg/ml) solution. Cool the solution to room temperature and filtered with 0.45µm membrane filter. A sample of 20µl was injected into the HPLC system, and the chromatograms were recorded to assess the stability of the sample.

Alkali Degradation Studies: To 1 ml of stock solution of Avapritinib 1 ml of 2N sodium hydroxide was added and refluxed for 30 min at 60 ºC. The resultant solution was neutralized with 1 ml 2N HCl and makeup to final volume to obtain (10µg/ml) solution. Cool the solution to room temperature and filtered with 0.45µm membrane filter. The sample of 20µl was injected into the system, and the chromatograms were recorded to an assessment of sample stability.

Oxidation Degradation Studies: To 1 ml of stock solution of Avapritinib 1 ml of 20% hydrogen peroxide (H_2O_2) was added separately. The solution was kept for 30 min at 60ºC. For HPLC study, the resultant solution was diluted to obtain (10µg/ml) solution. Cool the solution to room temperature and filtered with 0.45µm membrane filter. A sample of 20µl solution was injected into the system, and the chromatograms were recorded to assess the stability of the sample.

Dry Heat Degradation Studies: The 1 ml of standard drug solution was placed in the oven at 60ºC for 6h to study dry heat degradation. For HPLC study, the resultant solution was makeup to final volume to obtain (10µg/ml) solution. Cool the solution to room temperature and filtered through a 0.45µm membrane filter. A sample of 20µl solution was injected into the system, and the chromatograms were recorded for the assessment of sample stability.

Photo Degradation Studies: The photo stability of the drug was studied by exposing the stock solution to UV light for 1day or 200Watt-hours/m2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain (10µg/ml) solution and filtered with 0.45µm membrane filter. A sample of 20µl solution was injected into the system, and the chromatograms were recorded for the assessment of sample stability.

Water Degradation Studies: To 1 ml of stock solution of Avapritinib, 1 ml of distilled water was added. The solution was kept aside for 30 min at 60 ºC. For HPLC study, the resultant solution was diluted to obtain (10µg/ml) cool the solution to room temperature and filtered with 0.45µm membrane filter. A sample of 20µl was injected into the HPLC system, and the chromatograms were recorded for the assessment of sample stability.

Results and Discussion

Method Development

HPLC Instrumentation & Conditions: The HPLC system employed was **HPLC Waters** with Empower2 Software with Isocratic with UV-Visible Detector.

Selection of Wavelength

The detection wavelength was selected by dissolving the drug in mobile phase to get a concentration of 10µg/ml for individual and mixed standards. The resulting solution was scanned in U.V range from 200-400nm. The UV spectrum of Avapritinib was obtained and the Avapritinib showed absorbance's maxima at 246nm. The UV spectra of drug are follows:

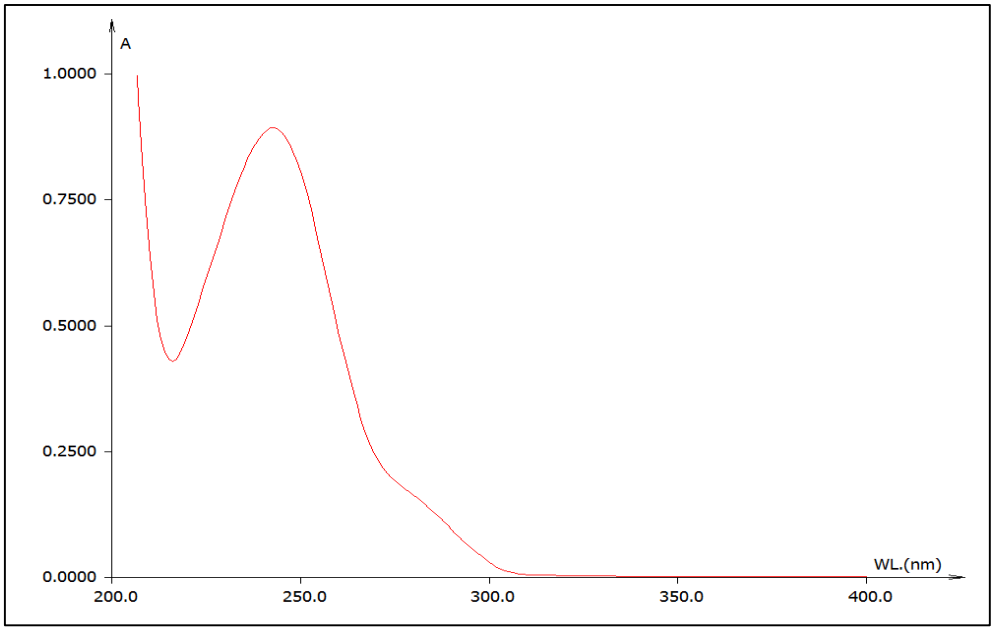

Fig 2: UV Spectrum of Avapritinib (246nm)

Observation: While scanning the Avapritinib solution we observed the maxima at 246nm. The UV spectrum has been recorded on T60-LAB INDIA make UV-Vis spectrophotometer model UV-2450.

Selection of Chromatographic Methods

The proper selection depends upon the nature of the sample, (ionic or ion stable or neutral molecule) its molecular weight and stability. The drugs selected are polar, ionic and hence reversed phase chromatography was selected.

Optimization of Column

The method was performed with various columns like Hypersil C_{18} column, X- bridge column and X-terra (4.6 ×150mm, 5µm particle size), Symmetry C18 ODS (4.6mm×250mm) 5µm particle size Column was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

Mobile Phase Optimization

Initially the mobile phase tried was Water: Methanol and Water: Acetonitrile and Methanol with TEA Buffer with varying proportions. Finally, the mobile phase was optimized to Acetonitrile: Phosphate buffer (0.01M, pH-3.2) in the ratio of 30:70 respectively.

Preparation of 0.01M Potassium dihydrogen orthophosphate Buffer Solution

About 1.36086grams of Potassium dihydrogen orthophosphate was weighed and transferred into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC Grade water. The pH was adjusted to 3.20 with diluted orthophosphoric acid.

Preparation of Standard Solution

10 mg of Avapritinib working standard was accurately weighed and transferred into a 10 ml clean dry volumetric flask. Add about 7 ml of diluents and sonicate to dissolve it completely and volume was made up to the mark with the same solvent which gave stock solution of 1000 ppm.

Further pipette 1 ml of the above stock solution into a 10 ml volumetric flask was diluted up to the mark with diluents (100 ppm solution).

Further 1 ml of prepared 100 ppm solution was pipetted into a 10 ml volumetric flask and was diluted up to the mark with diluents which gave 10 ppm Avapritinib working standard solution. The solution was mixed well and filtered through 0.45µm filter.

Preparation of Sample Solution

Twenty tablets were taken and the average weight was calculated as per the method prescribed in I.P. The weighed tablets were finally powdered and triturated well. A quantity of powder of Avapritinib equivalent to 10mg were transferred to clean and dry 10 ml volumetric flask and 7 ml of HPLC grade methanol was added and the resulting solution was sonicated for 15 minutes. Make up the volume up to 10 ml with same solvent. Then 1 ml of the above solution was diluted to 10 ml with HPLC grade methanol. One ml (0.1 ml) of the prepared stock solution diluted to 10 ml and was filtered through membrane filter (0.45µm) and finally sonicated to degas.

Optimized Chromatographic Conditions

Mobile phase	:	Acetonitrile: Phosphate buffer (0.01M, pH-3.2) (30:70v/v)
Column	:	Symmetry C18 ODS (4.6mm× 250mm) 5µm particle size
Flow rate	:	1 ml/min
Wavelength	:	246 nm
Column temp	:	Ambient
Injection Volume	:	20 µl
Run time	:	10 minutes

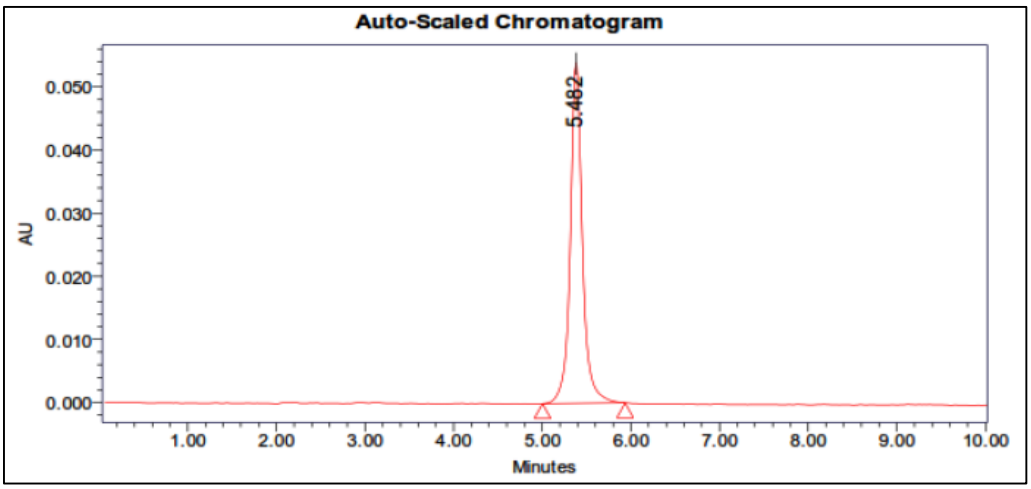

Fig 3: Optimized Chromatogram Condition

Validation of Method

System Suitability

The system suitability [21] parameters were found to be within the specified limits for the proposed method.

Table 3: Observation of System Suitability Parameters

S.No.	Parameter	Avapritinib
1.	Retention Time (min)	5.453
2.	Theoretical Plates	6967
3.	Tailing factor	1.12
4.	Peak Area (AUC)	647856

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 quantitates Avapritinib in drug product.

%ASSAY =

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

The % purity [22] of Avapritinib in present in the marketed pharmaceutical dosage form was found to be 99.85%.

Linearity

Table 4: Chromatographic Data for Linearity Study of Avapritinib

Concentration µg/ml	Average Peak Area
6	468784
8	615798
10	768759
12	925748
14	1078765

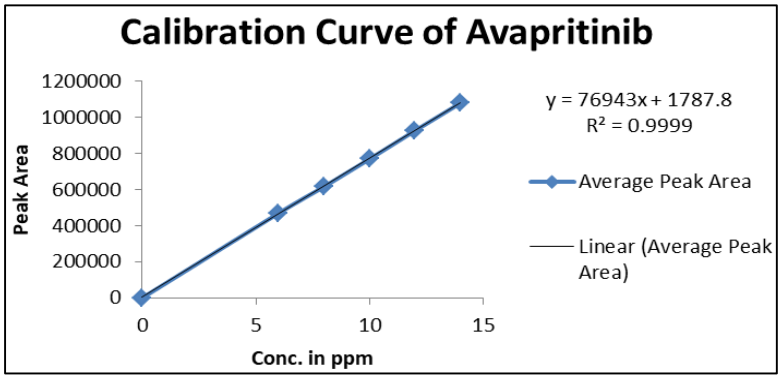

Fig 4: Calibration Curve of Avapritinib

Linearity Plot
The plot of Concentration (x) versus the Average Peak Area (y) data of Avapritinib is a straight line.

Y = mx + c
Slope (m) = 76943
Intercept (c) = 1787
Correlation Coefficient (r) = 0.99

Validation Criteria: The response linearity [23] is verified if the Correlation Coefficient is 0.99 or greater.

Conclusion: Correlation Coefficient (r) is 0.99, and the intercept is 76943. These values meet the validation criteria.

Precision
The precision [24] 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 5: Results of Repeatability for Avapritinib

S. No.	Peak Name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Avapritinib	5.419	645784	83685	6825	1.05
2	Avapritinib	5.405	642589	84932	6849	1.09
3	Avapritinib	5.478	643658	85847	6845	1.08
4	Avapritinib	5.466	648759	86295	6839	1.09
5	Avapritinib	5.493	649657	86587	6895	1.07
6	Avapritinib	5.466	647854	87853	6874	1.10
Mean			**646383.5**			
Std. Dev			**2853.319**			
%RSD			**0.441428**			

Intermediate Precision/Ruggedness
Analyst 1

Table 6: Results of Intermediate precision for Avapritinib

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USPTailing
1	Avapritinib	5.484	636854	84863	6758	1.09
2	Avapritinib	5.493	637489	84759	6726	1.08
3	Avapritinib	5.406	635762	84685	6749	1.09
4	Avapritinib	5.419	636984	84697	6698	1.07
5	Avapritinib	5.446	634856	84258	6728	1.08
6	Avapritinib	5.452	639689	84753	6699	1.08
Mean			**636939**			
Std. Dev.			**1649.149**			
% RSD			**0.258918**			

Analyst 2

Table 7: Results of Intermediate precision Analyst 2 for Avapritinib

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USPPlate Count	USPTailing
1	Avapritinib	5.491	628985	85698	6985	1.09
2	Avapritinib	5.482	624879	85479	6899	1.07
3	Avapritinib	5.416	625846	85748	6928	1.06
4	Avapritinib	5.482	623568	85647	6874	1.09
5	Avapritinib	5.495	628985	85246	6984	1.07
6	Avapritinib	5.427	628473	85924	6872	1.08
Mean			**626789.3**			
Std. Dev.			**2340.636**			
% RSD			**0.373433**			

Accuracy

Accuracy [25] at different concentrations (50%, 100%, and 150%) was prepared and the % recovery was calculated.

Table 8: The accuracy results for Avapritinib

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	386559	5	5.00	100.000%	100.130%
100%	768536	10	9.965	99.650%	
150%	1164522	15	15.111	100.740%	

Limit of Detection

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

LOD= 3.3 × σ / s

Where,
σ = Standard deviation of the response
S = Slope of the calibration curve

Result

= 0.487µg/ml

Quantitation Limit

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

LOQ=10×σ/S

Where,
σ = Standard deviation of the response
S = Slope of the calibration curve

Result

= 1.477µg/ml

Robustness

The robustness [26] 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 Avapritinib. The method is robust only in less flow condition. The standard of Avapritinib was 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 9: Results for Robustness of Avapritinib

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	648759	5.484	6845	1.08
Less Flow rate of 0.9 mL/min	635248	5.599	6786	1.09
More Flow rate of 1.1 mL/min	659865	4.576	6528	1.05
Less organic phase	625986	7.415	6689	1.03
More organic phase	615869	3.827	6354	1.01

Stability Studies

Table 10: Results of Forced Degradation Studies for Avapritinib

S.No.	Stress Condition	Peak Area	% of Degraded Amount	% of Active Amount	Total % of Amount
1	Standard	648759	0	100%	100%
2	Acidic	539378.232	16.86	83.14	100%
3	Basic	603540.497	6.97	93.03	100%
4	Oxidative	545217.063	15.96	84.04	100%
5	Thermal	616450.801	4.98	95.02	100%
6	Photolytic	533344.773	17.79	82.21	100%
7	Water	625079.296	3.65	96.35	100%

Summary
The method was developed to estimate Avapritinib using RP-HPLC; developed method was validated with parameters like linearity, accuracy, precision and robustness. Avapritinib were subjected to Forced Degradation like Acid degradation, Base Degradation, Oxidation Degradation, Thermal Degradation and Photolytic Degradation. Developed method was validated as per ICH guidelines.

Table 11: Summary of Validation Parameters of Avapritinib

Parameter	Avapritinib
Linearity range (n=5)	6-14µg/ml
Accuracy (%)	100.130%
LOD (µg/ml)	0.487µg/ml
LOQ (µg/ml)	1.477µg/ml
Repeatability (n=6) %RSD	0.441
Intraday (n=6) %RSD	0.258
Interday (n=6) %RSD	0.373
Robustness (% RSD)	0.284
%Assay	99.85%

Conclusion
Stability Indicating RP-HPLC method has been developed and validated for the estimation of Avapritinib in pure substances and marketed pharmaceutical dosage form. The methods are found to be specific as there was no interference of any co-eluting impurities after degradation study. The degraded products are well resolved, indicating the method can also be useful for determination of degraded products.
All the parameters and results are found within the acceptance limit as given in the validation protocol. So, we can conclude that developed RP-HPLC method is found to be specific, linear, accurate and robust. Therefore, method is to be specific with good resolution. Thus, the proposed method can be used in pharmaceutical analysis for forced degradation study and routine quality control samples for Avapritinib in pure substances and marketed pharmaceutical dosage form.

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