

## A VALIDATED STABILITY INDICATING RP-HPLC METHOD FOR ESTIMATION OF AVAPRITINIB IN BULK AND TABLET DOSAGE FORM

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### ABSTRACT

**Objective:** The present investigation aims to develop a simple, cost effective, and reliable stability-indicating RP-HPLC assay method for analysis of avapritinib in bulk and the tablet dosage form.

**Methods:** The method was developed using Acetonitrile and Methanol in a ratio of 70:30 (v/v) as the mobile phase, flowing at a rate of 1 ml/min, in an isocratic mode. A reverse-phase column (Symmetry C18 column, 250 mm x 4.6 mm, 5 µm) was used as the stationary phase. The detection of the compound was made using a UV detector set at 245 nm. The validation and force degradation studies of the method were done following the International Conference on Harmonization (ICH) guidelines.

**Results:** The method was validated using the prescribed parameters like system suitability, LOD, LOQ, accuracy, precision, robustness, specificity etc. The relative standard deviation (% RSD) of the peak area observed in each case was found within the accepted range (<2%). The tailing factor and the plate count were found to be less than 2 and more than 2000 respectively in each observation. The coefficient of correlation ( $r^2$ ) value in the linearity study was found to be 0.9997. The drug was found to be quantified accurately in presence of its degraded products.

**Conclusion:** The developed simple and economic method is a suitable option for the qualitative and quantitative study of avapritinib in bulk and tablets even in the presence of its degraded products, which may arise because of oxidation, hydrolysis, thermal and photolytic decomposition.

**Keywords:** Avapritinib, RP-HPLC, Method development, Forced degradation

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### INTRODUCTION

Avapritinib, (fig. 1) a tyrosine kinase inhibitor, has been approved recently by Food and Drugs Administration (FDA, USA) as a breakthrough therapy for the treatment of adults with unresectable or metastatic gastrointestinal stromal tumors (GIST) [1]. Avapritinib is also indicated for the treatment of adults with advanced systemic mastocytosis, aggressive systemic mastocytosis, systemic mastocytosis with an associated hematological neoplasm, and mast cell leukemia (MCL) [2, 3]. Systemic mastocytosis (SM) is a rare hematological neoplasm characterized by clonal expansion and multifocal accumulation of neoplastic mast cells affecting various tissues, predominantly bone marrow, skin, and visceral organs [4, 5]. Around 15% of patients with malignant mastocytosis develop mast cell leukemia, a very rare variant of acute myeloid leukemia [6, 7].

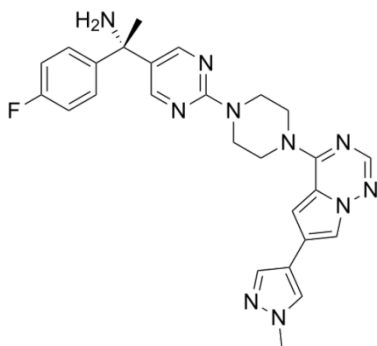


Fig. 1: Chemical structure of avapritinib

As a newer drug, very few pieces of information were found in the literatures, about the methods used for its estimation in bulk and pharmaceutical dosage forms. Two methods were available for the

analysis of avapritinib in biological fluids using LC-MS [8, 9] and one for the analysis of avapritinib in bulk and dosage forms using HPLC [10]. The ICH guideline entitled "stability testing of new drug substances and products" requires elucidation of the inherent stability of drugs through stress testing [11]. Thus, as per the ICH guideline, a specific and stability-indicating procedure should be available for the analysis of drugs [12, 13]. The stability study provides information about proper storage conditions for bulk and formulation so that appropriate precautions can be taken during the process of formulation in order to avoid possible instabilities [14]. We propose a new, simple, and stability-indicating method that would allow the estimation of avapritinib in bulk and dosage forms quite accurately in the presence of its degradation products.

### MATERIALS AND METHODS

Pure Avapritinib was taken from Medvitaz Pharma Solutions, New Delhi, as a gift sample. The test sample (Ayvakit Tablets, Blueprint Medicines Corporation) was purchased from the local market. HPLC grade water was purchased from Sd fine-Chem ltd; Mumbai, HPLC grade methanol and acetonitrile was purchased from Loba Chem; Mumbai. All other analytical grade chemicals used were purchased from Loba Chem; Mumbai. The work was done using RP-HPLC (WATERS, with Empower 2 Software) with UV-Visible Detector.

#### Preparation of mobile phase

The mobile phase was prepared by mixing 700 ml of Acetonitrile with 300 ml of Methanol to get a volume of 1000 ml (Acetonitrile: Methanol=70:30 v/v). The mixture was degassed using an ultrasonic bath prior to use in HPLC.

#### Preparation of standard solution

Accurately weighed and transferred 10 mg of Avapritinib working standard into 10 ml of clean, dry volumetric flasks. About 7 ml of acetonitrile was added to it and sonicated to facilitate dissolution. The volume was made up to 10 ml with acetonitrile to prepare the Stock solution. The stock solution was diluted to prepare the working standard solution of Avapritinib (10 µg/ml).

### Preparation of test solution

Twenty tablets (Ayvakit 100 mg, Blueprint Medicines) were weighed accurately, and the average weight was determined. A quantity of the crushed tablets equivalent to 10 mg of avapritinib was taken in 8 ml of acetonitrile, sonicated for 15 min and volume was made up to 10 ml. A tenfold dilution of the solution was made and the particulate excipients were separated by membrane filtration. The

filtrate was degassed on ultrasonic bath and diluted with the mobile phase to get a working test solution of avapritinib (10 µg/ml).

### Method development

The RP-HPLC method employed a U. V detector for the detection and quantification of HPLC column eluants. The UV spectrum of avapritinib was recorded, from which the  $\lambda_{\max}$  value was observed to be 245 nm (fig. 2).

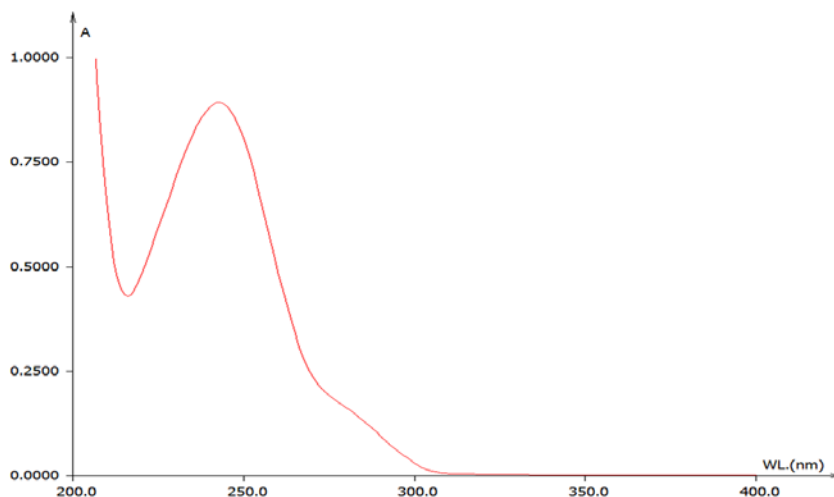


Fig. 2: UV spectrum of avapritinib

Thus, the method development and its subsequent validation process were accomplished by setting the detector at 245 nm. Various chromatographic parameters like columns, mobile phase composition and flow rate and column temperatures were tested during the development process [15-18]. After the selection of the suitable mobile and stationary phases, other secondary parameters were optimized. The optimized chromatographic condition is given in table 1.

Table 1: The optimised chromatographic condition

Parameters	Values
Column	Symmetry C18 (250 mm X 4.6 mm, 5 µm)
Mobile Phase	Methanol: Acetonitrile (70:30% v/v)
Flow Rate	1.0 ml/minute
Wave length of detection	245 nm
Injection volume	10 µl
Run time	7 min
Column temperature	Ambient

### Method validation

Only a validated method should be employed for the estimation of drugs. The developed method was validated following the ICH Q2 (R1) guidelines and other literatures using the prescribed validation parameters [19-30].

### System suitability

Prior to the start of laboratory studies to demonstrate method validity, a system suitability test must be done to check the analytical system is performing properly. For this, avapritinib standard solution was prepared and analyzed by HPLC, in six replicates. The mean, standard deviation (SD), and relative standard deviation (% RSD) of the peak areas of avapritinib were calculated.

### Linearity and range

To check the linearity, a series of concentrations were prepared from the stock solution. Each solution was analyzed by HPLC, in

three replicates, to observe the peak area. The mean peak area was plotted versus the concentration to get the calibration curve.

### Accuracy

Accuracy of this new method was checked by recovery studies, where three different concentrations (80%, 100%, and 120% of standard avapritinib solution) were analyzed by HPLC, in three replications. The linear regression equation obtained from the calibration curve was used to calculate the amount recovered (percentage recovery value).

### Precision

#### Repeatability

Six replicates of the standard solution of Avapritinib were analyzed by HPLC. The peak area and retention time of each trial were observed. The SD and % RSD of the observations were calculated.

#### Intermediate precision

The Intermediate Precision is conducted for checking the reproducibility within an intra-day set of results and within inter-day results. In the intra-day process, the working standard solution was injected at different intervals of time in a single day. In the inter-day process, the working standard solution was injected on different days. The variation in results was observed and expressed in SD and % RSD.

### Method robustness

The robustness of the developed method was checked by changing the important chromatographic parameters deliberately to a little extent. The flow rate was altered by  $\pm 0.2$  ml/min, the detection wavelength by  $\pm 2$  nm and the proportion of acetonitrile in the mobile phase by  $\pm 5\%$ . The variation in results was observed and expressed in % RSD.

### Limit of detection (LOD) and limit of quantitation (LOQ)

The LOD and LOQ were calculated from the slope (s) of the calibration plot and the SD ( $\sigma$ ) of the peak areas using the formulae  $LOD = 3.3 \sigma/s$  and  $LOQ = 10 \sigma/s$ .

### Assay of avapritinib tablets

The test solution was analyzed by HPLC, in six replicates, and the observations were recorded. The observed mean peak area was compared with the standard solution by the help of the calibration curve equation to determine the percentage of avapritinib in the given tablets.

### Forced degradation studies

Forced degradation study (Stress testing) is performed to check the stability of drugs in the finished products, establishing the degradation pathways and to resolve stability related problems. Performed early in the drug development cycle, it helps in deciding suitable dosage forms and storage conditions. The stability study was performed by applying the physical stress (acid, alkali, hydrogen peroxide, heat, and light) to the product [31-35].

#### Acid hydrolysis

10 mg of avapritinib was taken with 30 ml of HCl (0.1 N) in a round bottom flask and refluxed at 60 °C in a water bath for 4 h. After attaining room temperature, it was neutralized using NaOH (0.1 N) solution. The mobile phase was used to dilute it to a concentration of 10 µg/ml prior to analysis by HPLC. This experiment was repeated for three times to observe the degradation profile in an acidic medium.

#### Basic hydrolysis

10 mg of avapritinib was taken with 30 ml of NaOH (0.1 N) in a round bottom flask and refluxed at 60 °C in a water bath for 4 h. After attaining room temperature, it was neutralized using HCl (0.1 N) solution. The mobile phase was used to dilute it to a

concentration of 10 µg/ml prior to analysis by HPLC. This experiment was repeated for three times to observe the degradation profile in an acidic medium.

#### Thermal degradation

10 mg of avapritinib was taken with 30 ml of HPLC grade water and refluxed at 60 °C in a water bath for 4 h. After attaining room temperature, a concentration of 10 µg/ml was prepared with the mobile phase and analyzed by HPLC in three replicates.

#### Photolytic degradation

Approximately 10 mg of avapritinib was exposed to UV radiation of 254 nm for 24 h. A 10 µg/ml concentration of the UV exposed drug was prepared with the mobile phase and analyzed by HPLC in three replicates.

#### Oxidation with (3%) H<sub>2</sub>O<sub>2</sub>

Accurately weighed 10 mg of avapritinib was taken in 30 ml of H<sub>2</sub>O<sub>2</sub> (3%) and a little mobile phase was added to it to make it soluble and then kept as such in the dark for 24 h. Suitable dilution was made with the mobile phase to prepare a 10 µg/ml concentration and analyzed by HPLC in three replicates.

### RESULTS AND DISCUSSION

Development of a simple method for the estimation of avapritinib in bulk and tablet dosage forms was attempted. The stability of avapritinib was also checked through forced degradation studies following ICH guidelines. The developed stability-indicating method was validated and found suitable for qualitative and quantitative analysis of avapritinib. A sample chromatogram of avapritinib recorded in optimized chromatographic conditions is given in fig. 3.

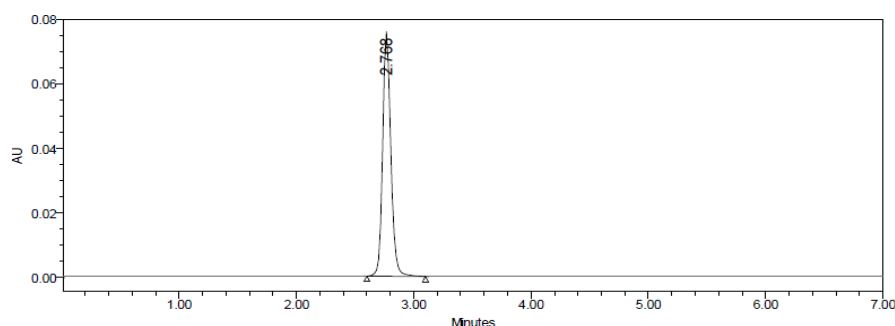


Fig. 3: A sample chromatogram of avapritinib in the optimised chromatographic conditions

### System suitability

Six replicates of freshly prepared standard solution were analyzed by HPLC. The retention time, tailing factor, theoretical plates, and

peak area were observed from each injection. The qualifying parameters such as mean tailing factor, mean theoretical plates, and % RSD of the peak area were found within the acceptance limits (table 2).

Table 2: Result of system suitability test of avapritinib

System suitability parameters	*Observed value [mean±SD (% RSD)]	Acceptance limit
USP tailing factor	1.36±0.019 (1.39)	NMT 2
USP plate count	5821.5±116.34 (1.99)	NLT 2000
Peak area	756207.8±1347.976 (0.18)	% RSD NMT 2

\*Data expressed as mean±SD; n=6

Table 3: Linearity concentrations of avapritinib

S. No.	Concentration (in µg/ml)	*Mean peak area±SD (% RSD)
1	0	0
2	6	457896±914.26 (0.19)
3	8	607574±1883.47 (0.31)
4	10	752268±1053.17 (0.14)
5	12	896587±1882.83 (0.21)
6	14	1036579±2487.78 (0.24)

\*n=3

**Linearity range, LOD and LOQ**

An excellent linear response was obtained for avapritinib within 6-14 µg/ml (table 3). The linear regression equation observed from

the graph as;  $y = 74143x + 7294.9$  with  $r^2$  value of 0.9997 (fig. 4). The lower concentration level at which the drug can be detected reliably (LOD) and quantified (LOQ) were found to be 0.507 and 1.539 µg/ml, respectively.

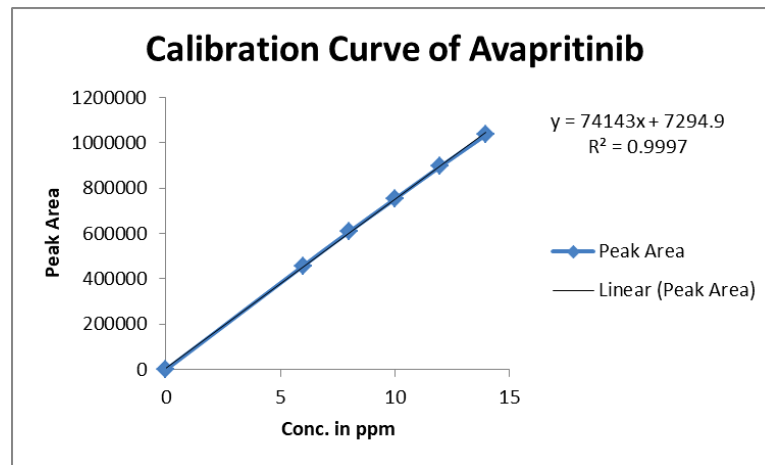


Fig. 4: Calibration curve of avapritinib

**Accuracy**

The amounts of avapritinib recovered after injection of each concentration (80%, 100%, and 120% of standard solution) were observed and expressed in percentage recovery value with the help

of the linearity equation;  $y = 74143x + 7294.9$ . The overall recovery of avapritinib was found to be 100.41% ( $\pm 0.18$ ) which is within the acceptance range (98 to 102 %). Thus, the method can be used for the quantification of avapritinib quite accurately. The results are shown in table 4.

Table 4: Result of accuracy study

Level	Amount injected	Mean peak area	Mean amount recovered	% Recovery	*% Recovery (mean $\pm$ SD)
80 %	8 µg/ml	601648	8.016 µg/ml	100.20	100.41 $\pm$ 0.18
100 %	10 µg/ml	752398.33	10.049 µg/ml	100.49	
120 %	12 µg/ml	901849.33	12.065 µg/ml	100.54	

\*n=3

**Precision**

The repeatability of the method was checked by observing the retention time, theoretical plate count, tailing factor, and peak area, obtained from

six replicate injections of Avapritinib. The observed % RSD of the peak areas was within the limit (% RSD<2). The mean theoretical plate number was found more than 2000, and the mean tailing factor was less than 2 which justifies the repeatability of the method (table 5).

Table 5: Results of repeatability study

Retention time	Theoretical plates	Tailing factor	Peak area	% RSD of peak area
2.792 $\pm$ 0.008	5930.333 $\pm$ 54.46	1.37 $\pm$ 0.014	746495 $\pm$ 1268.126	0.17

Data expressed as mean $\pm$ SD; n=6

The intermediate precision was also calculated, injecting the standard avapritinib solution on a same day (Intra-day precision) and at the same intervals on different days (Inter-day precision).

The mean % assay was found to be 99.73 ( $\pm 0.089$ ) and 99.66 ( $\pm 0.1$ ) in intra-day and inter-day observations respectively with % RSD within the accepted range (% RSD<2) (table 6 and 7).

Table 6: Results of intra-day precision study

S. No.	Concentration	Area	% Assay	*% assay	% RSD
1	10 µg/ml	751587	100.38	99.73 $\pm$ 0.089	0.08
2	10 µg/ml	738845	98.66		
3	10 µg/ml	749857	100.15		
4	10 µg/ml	749698	100.13		
5	10 µg/ml	741859	99.07		
6	10 µg/ml	748948	100.03		

\*Data expressed as mean $\pm$ SD; n=6

Table 7: Results of inter-day precision study

S. No.	Concentration	Area	% Assay	*Mean % assay	% RSD
1	10 µg/ml	743945	99.35	99.66±0.1	0.1
2	10 µg/ml	739991	98.82		
3	10 µg/ml	749272	100.07		
4	10 µg/ml	750157	100.19		
5	10 µg/ml	741994	99.09		
6	10 µg/ml	752408	100.49		

\*Data expressed as mean±SD; n=6

#### Method robustness

The flow rate was altered by ± 0.2 ml/min, the detection wavelength was altered by ± 2 nm and the acetonitrile proportion in the mobile phase was altered by ± 5%. The observed % RSD was found less than 2%, justifying the method is robust and suitable for the estimation of Avapritinib (table 8).

#### Assay of avapritinib tablets

The test solution was analyzed by HPLC, in six replicates and the individual observations were recorded. The observed mean peak area was compared with the area observed for standard solution with the help of the regression equation obtained from calibration curve. The % Purity of Ayvakit Tablets (Blueprint Medicines Corporation) containing Avapritinib was found to be 99.875%

(±0.746). A sample chromatogram of Avapritinib extracted from tablets is shown in fig. 5 and the results are shown in table 9.

Table 8: Results of method robustness test

Change in parameter	%RSD
Flow (1.2 ml/min)	0.17
Flow (0.8 ml/min)	0.19
Methanol: Acetonitrile (75:25% v/v)	0.26
Methanol: Acetonitrile (65:35% v/v)	0.29
Wavelength of Detection (247 nm)	0.37
Wavelength of detection (243 nm)	0.31

Data expressed as % RSD; n=3

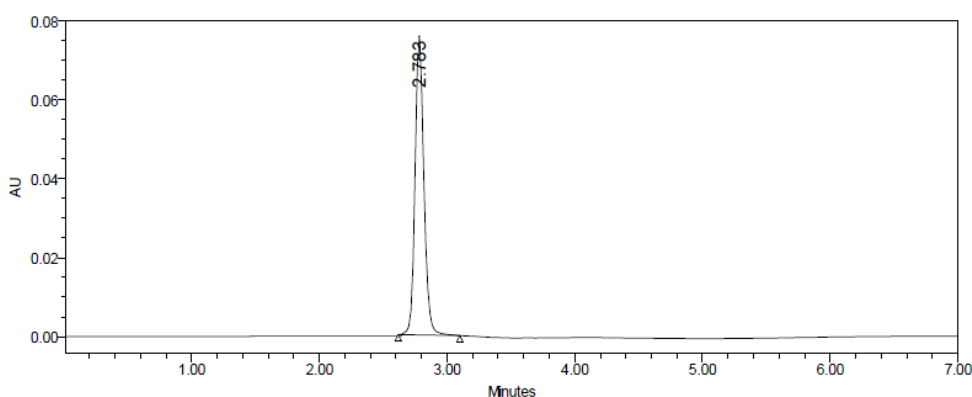


Fig. 5: A sample chromatogram of avapritinib extracted from tablets

Table 9: Assay of avapritinib tablets

Brand name of tablets	Labelled amount (mg)	*Amount found (mg)	*% assay	% RSD
Ayvakit Tablets	100	99.875±0.234	99.769%±0.746	0.74

\*Data expressed as mean±SD; n=6

Table 10: Results of forced degradation studies of avapritinib

Stress condition	Time	*% Assay	*% Degradation
Acid hydrolysis (0.1N HCl)	4 h	87.93±1.16	11.87±0.44
Basic hydrolysis (0.1N NaOH)	4 h	94.15±0.87	5.64±0.19
Thermal degradation (60 °C)	6 h	96.81±0.92	3.28±0.32
UV (254 nm)	24 h	98.33±0.16	1.92±0.09
3% Hydrogen peroxide	24 h	89.34±0.74	10.38±0.21

\*Data expressed as mean±SD; n=3

#### Forced degradation studies

The results observed in the stress studies indicated the specificity of the developed method. Avapritinib was found to be relatively stable in each condition with less degradation. The results are given in table 10.

The proposed method is reliable in terms of accuracy, precision, robustness, reproducibility, and sensitivity. The validation and stability studies performed for avapritinib prove that the proposed method is stability-indicating and can suitably be used for qualitative and quantitative analysis of the drug. Moreover, this stability

indicating method may be applied in all kinds of stability studies of Avapritinib. Being a newer drug, very few methods are available in the literature for estimation of avapritinib. The two methods reported for analysis of avapritinib in the biological sample (plasma) [8, 9] use mass spectrometers (LC-MS) for detection of avapritinib, which increases the cost of the equipment by many folds. The usefulness of these methods for routine assay of avapritinib is far away from the point of cost effectiveness and simplicity. The method reported for routine analysis and stability studies of avapritinib [10] detects the analyte at 296 nm which may pose problems related to specificity because the  $\lambda_{\text{max}}$  of the compound is 265 nm. Moreover the reported method used formic acid as a part of solvent system which might be detrimental to the column used in HPLC, on long run. This developed method does not use any buffer solution in the mobile phase which is directly related to the cost of the stationary phase and this makes it better than the reported methods.

## CONCLUSION

The proposed assay method using HPLC technique is simple, specific, precise, accurate, robust, and stability-indicating. Reproducibility of the results, as evidenced by the statistical analysis, proves that the method is quite appropriate for the estimation of avapritinib in bulk and formulation with no interference from the excipients. Also, the above results indicate the applicability of this method for hydrolytic, oxidative, thermal, and photolytic degradation studies. The mobile phase used in the method is devoid of any buffer in, which is directly related to the cost of the stationary phase. This method can be employed for qualitative and quantitative analysis of avapritinib present in different matrices and for many purposes like characterization, dissolution testing, content uniformity testing, to name a few.

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None

## AUTHORS CONTRIBUTIONS

All authors have contributed equally.

## CONFLICT OF INTERESTS

Declared none

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