Stability Indicating Rp-Hplc Method Development and Validation for Estimation of Avapritinib in Pure Form and Marketed Pharmaceutical Tablet Dosage Form

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ABSTRACT
A simple, rapid, specific and accurate reverse phase high performance liquid chromatographic method has been developed for the validated of Avapritinib in bulk as well as in marketed pharmaceutical dosage form. This separation was performed on a Symmetry ODS C18 (4.6×250mm, 5µm) column with Methanol: Phosphate Buffer (35:65) v/v as mobile phase at a flow rate of 1.0 mL min−1 with UV detection at 235 nm; the constant column temperature was Ambient. The runtime under these chromatographic conditions was less than 8 min. The retention time of Avapritinib was found to be 2.257min. The calibration plot was linear over the concentration range of 6–14 µg mL−1 with limits of detection and quantification values of 1.2 and 3.6 ng mL−1 respectively. The mean % assay of marketed formulation was found to be 99.86%, and % recovery was observed in the range of 98-102%. Relative standard deviation for the precision study was found <2%.The developed method is simple, precise, specific, accurate and rapid, making it suitable for estimation of Avapritinib in bulk and marketed pharmaceutical dosage form dosage form.
Keywords: Avapritinib, RP-HPLC, Validation, Accuracy, Robustness, ICH Guidelines.

I. 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 was granted FDA approval on 9 January 2020 [1].Avapritinib is indicated for the treatment of unresectable, metastatic gastrointestinal stromal tumours with a platelet-derived growth factor receptor alpha exon 18 mutations. 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. 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 [2]. Negative modulation of these transporters, resensitizes cancerous cells to treatment with chemotherapeutic agents like paclitaxel.Avapritinib is used to treat a certain type of gastrointestinal stromal tumor (GIST; a type of tumor that grows in the wall of the stomach, intestine [bowel], or esophagus [tube that connects the throat with the stomach]) in adults that has spread to other parts of the body or that cannot be removed by surgery [3]. The IUPAC Name of Avapritinib is (1S)-1-(4-fluorophenyl)-1-[2-[4-[6-(1-methylpyrazol-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 shown in following figure-1.

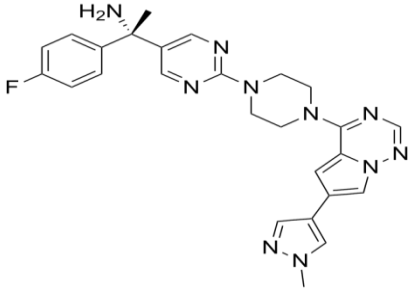

Fig-1: Chemical Structure of Avapritinib

Based on the previously mentioned analytical techniques, our primary goal is to develop an efficient, rapid, sensitive, selective, linear, and accurate RP-HPLC approach for Avapritinib determination [28-29]. The process was evaluated using ICH Guidelines [19, 27]. Linearity, accuracy, precision, specificity, the limit of detection (LOD), and the limit of quantification (LOQ) are performed and utilized to evaluate the drug concentration of Avapritinib in bulk and various pharmaceutical products by ICH Q2R1 guidelines [19, 27].

II. EXPERIMENTAL WORK

Table-1: Instruments Used

S. No.	Instruments and Glass wares	Model
1	HPLC	WATERS Alliance 2695 separation module, Software: Empower 2, 996 PDA detectors.
2	pH meter	LabIndia
3	Weighing machine	Sartorius
4	Volumetric flasks	Borosil
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil
7	Digital Ultra Sonicator	Labman

Table-2: Chemicals Used

S. No.	Chemical	Brand Names
1	Avapritinib	Synpharma Research Lab, Hyderabad
2	Water and Methanol for HPLC	LICHROSOLV (MERCK)
3	Acetonitrile for HPLC	Merck

HPLC Method Development:

Preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Avapritinib working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

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

Procedure:

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 [19].

Mobile Phase Optimization:

Initially the mobile phase tried was Methanol and Methanol: Water with varying proportions. Finally, the mobile phase was optimized to Methanol: Phosphate Buffer in proportion 35:65% v/v.

Optimization of Column:

The method was performed with various C18 columns like, X- bridge column, Xterra, and C18 column. Symmetry ODS C18 (4.6 x 250mm, 5µm) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

Preparation of Buffer and Mobile Phase:

Preparation of Potassium Dihydrogen Phosphate (KH2PO4) Buffer (pH-3.6):

Dissolve 6.8043 of potassium dihydrogen phosphate in 1000 ml HPLC water and adjust the pH 3.6 with diluted orthophosphoric acid. Filter and sonicate the solution by vacuum filtration and ultra-sonication [4].

Preparation of Mobile Phase:

Accurately measured 350 ml (35%) of Methanol, 650 ml of Phosphate buffer (65%) were mixed and degassed in digital ultra sonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration [5].

Diluent Preparation:

The Mobile phase was used as the diluent.

Method Validation Parameters

System Suitability

Accurately weigh and transfer 10 mg of Avapritinib working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 0.1ml of the above Avapritinib stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure:

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Specificity:

Preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Avapritinib working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 0.1ml of the aboveAvapritinib stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Preparation of Sample Solution:

Weight 10 mg equivalent weight ofAvapritinib 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.
Further pipette 0.1ml of Avapritinib above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure:

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

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

Linearity and Range:

Accurately weigh and transfer 10 mg of Avapritinib working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Preparation of Level – I (6ppm of Avapritinib):

Take 0.6ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluents and sonicate the solution for bubble entrapment using ultrasonicator.

Preparation of Level – II (8ppm of Avapritinib):

Take 0.8ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluents and sonicate the solution for bubble entrapment using ultrasonicator.

Preparation of Level – III (10ppm of Avapritinib):

Take 0.1ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluents and sonicate the solution for bubble entrapment using ultrasonicator.

Preparation of Level – IV (12ppm of Avapritinib):

Take 0.12ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluents and sonicate the solution for bubble entrapment using ultrasonicator.

Preparation of Level – V (14ppm of Avapritinib):

Take 0.14ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluents and sonicate the solution for bubble entrapment using ultrasonicator.

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.

Precision

Repeatability

Preparation of Avapritinib Product Solution for Precision:

Accurately weigh and transfer 10 mg of Avapritinib working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

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

Procedure:

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 [6].

Intermediate Precision:

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different daysby maintaining same conditions.

Procedure:

Analyst 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.

Analyst 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.

Accuracy:

For Preparation of 50% Standard Stock Solution:

Accurately weigh and transfer 10 mg of Avapritinibworking standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.05ml of the above Avapritinib stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

For Preparation of 100% Standard Stock Solution:

Accurately weigh and transfer 10 mg of Avapritinib working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

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

For Preparation of 150% Standard Stock Solution:

Accurately weigh and transfer 10 mg of Avapritinib working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.15ml of the above Avapritinib stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

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 [7].

Robustness:

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

For Preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Avapritinibworking standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

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

Effect of Variation of Flow Conditions:

The sample was analyzed at 0.9 ml/min and 1.1 ml/min instead of 1ml/min, remaining conditions are same. 10µl 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. Methanol: Phosphate Bufferwas taken in the ratio and 40:60, 30:70 instead (35:65), remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

III. RESULTS AND DISCUSSION

Optimization of Analytical Method Development:

RP-HPLC method has been developed for estimation of Avapritinibin bulk and marketed pharmaceutical dosage form. The separation has achieved by Symmetry ODS C18 (4.6mm×250mm, 5µm)Column and mobile phase is consists of Methanol and Phosphate buffer pH 3.6 (35:65% v/v) at a flow rate of 1.0 ml/min. The detection has carried out at 235 nm. The retention time has been found to be 2.257min for Avapritinib in fig-2.

Optimized Chromatographic Condition:

Mobile phase ratio : Methanol: Phosphate Buffer (35:65%) v/v
Column : Symmetry ODS C18 (4.6×250mm, 5µm)
Column temperature : Ambient
Wavelength : 235nm
Flow rate : 1ml/min
Injection volume : 10µl
Run time : 8min

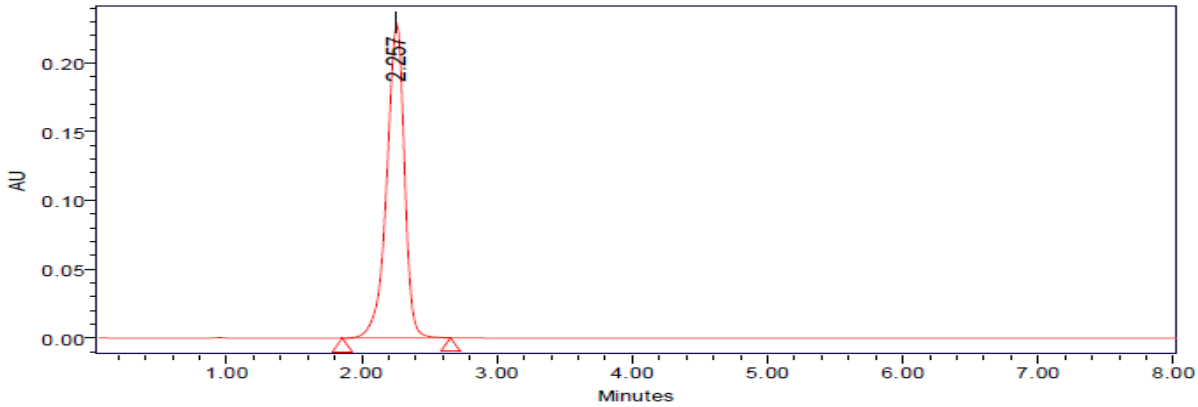

Fig-2: Chromatogram for Optimized Condition

Table-3: Results of Optimized Chromatographic Condition

S.No.	Name	RT	Peak Area	Height	USPTailing	USPPlate Count
1	Avapritinib	2.257	1658242	185421	1.24	6569

Method Validation: The validation has been done according to ICH guidelines. The proposed method has validated with respect to specificity, linearity, the limit of detection (LOD), limit of quantitation (LOQ), precision, accuracy, and robustness [19, 27].

System Suitability: In the system, suitability injected standard solution and reported USP tailing and plate count values are tabulated in Table 4 [8].

Table-4: Results of System Suitability for Avapritinib

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Avapritinib	2.277	1652847	185647	6589	1.24
2	Avapritinib	2.277	1653658	186254	6587	1.26
3	Avapritinib	2.267	1654521	185475	6584	1.28

4	Avapritinib	2.265	1653564	186594	6582	1.29
5	Avapritinib	2.277	1658745		6895	1.24
Mean			1654667			
Std.Dev			2355.764			
%RSD			0.142371			

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 [9, 10]. Analytical method was tested for specificity to measure accurately quantitatesAvapritinib in drug product.

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

= 99.40%

The % purity of Avapritinibin pharmaceutical dosage form was found to be 99.40%.

Linearity

For the Avapritinib solution, the area of the linearity peak vs. various concentrations (µg/ml) has been calculated as 6, 8, 10, 12, and 14, respectively [11]. A linearity test was performed between 6 and 14µg/ml, and the correlation coefficient were higher than 0.9996 (Table 5 and Fig-3).

Table-5: Data for Linearity of Avapritinib

Concentration µg/ml	Average Peak Area
6	1078475
8	1461129
10	1808358
12	2211573
14	2593778

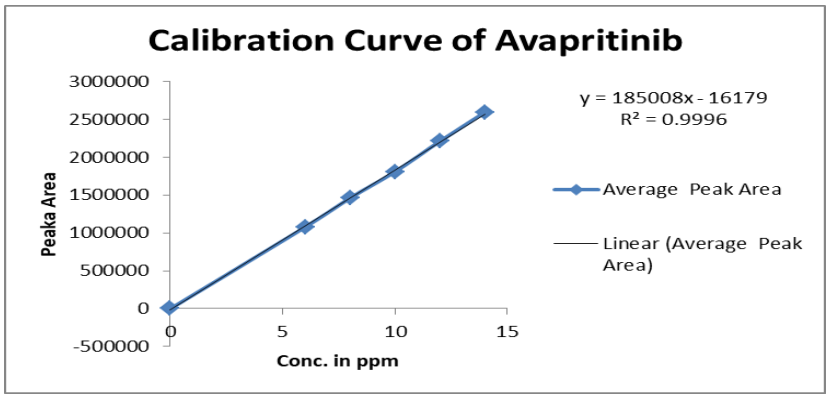

Fig-3: Linearity Curve of Avapritinib

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) = 18500

Intercept (c) = 16179

Correlation Coefficient (r) = 0.999

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

Conclusion: Correlation Coefficient (r) is 0.99, and the intercept is 0.16179. These values meet the validation criteria [12].

Precision:

The precision 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 [13-14].

Repeatability: Obtained Six (6) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD.

Table-6: Results of Repeatability for Avapritinib

S. No.	Peak Name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Avapritinib	2.293	1658954	186958	1.26	6785
2	Avapritinib	2.276	1658745	187548	1.27	6854
3	Avapritinib	2.286	1659865	189854	1.26	6852
4	Avapritinib	2.277	1653254	186985	1.25	6784
5	Avapritinib	2.280	1654781	189542	1.24	6895
6	Avapritinib	2.293	1661324	187586	1.28	6965
Mean			1657821			
Std. Dev			3120.433			
%RSD			0.188225			

Intermediate Precision:
Analyst1:

Table-7: Results of Intermediate Precision for Avapritinib

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP PlateCount	USP Tailing
1	Avapritinib	2.274	1678541	186589	6587	1.26
2	Avapritinib	2.258	1685985	186598	6321	1.26
3	Avapritinib	2.267	1685745	186985	6385	1.25
4	Avapritinib	2.270	1685987	187854	6580	1.26
5	Avapritinib	2.264	1698526	187549	6721	1.27
6	Avapritinib	2.265	1685943	186598	6637	1.26
Mean			1686788			
Std.Dev			6463.466			
%RSD			0.383182			

Analyst2:
Table-8: Results of Intermediate Precision Analyst 2 for Avapritinib

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Avapritinib	2.277	1665847	167481	6854	1.25
2	Avapritinib	2.255	1658989	167854	6785	1.26
3	Avapritinib	2.265	1659845	167895	6854	1.24
4	Avapritinib	2.255	1665964	167854	6895	1.26
5	Avapritinib	2.253	1659863	168585	6459	1.25
6	Avapritinib	2.252	1665986	167859	6456	1.26
Mean			1662749			
Std.Dev			3501.766			
%RSD			0.210601			

Accuracy: The accuracy of the method has evaluated in triplicates by recovery studies at three different concentration levels 80%, 100%, and 150% known amount of standard drug concentration were added to the sample. Then, the amount of drug recovered is calculated in terms of % recovery [15-17]. The accuracy data and results were shown in Table 9.

Table-9: The Accuracy Results for Avapritinib

%Concentration (at Specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	109068.3	5	5.021	100.420%	100.72%
100%	202187	10	10.054	100.540%	
150%	297032.3	15	15.181	101.206%	

Limit of Detection

The detection limit of an individual analytical procedure is the lowest amount of analyte in a samplewhich can be detected but not necessarily quantitated as an exact value [18].

$$LOD = 3.3 \times \sigma / s$$

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Result:

= 0.95µg/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 [20].

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

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Result:

= 2.9µg/ml

Robustness:

The robustness 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 Avapritinibwas injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution; tailing factor, asymmetric factor, and plate count [21-23].

Table-10: Results for Robustness

Parameter used for Sample Analysis	Peak Area	Retention Time	Theoretical Plates	Tailing Factor
Actual Flow rate of 1.0 mL/min	1658242	2.312	6569	1.24
Less Flow rate of 0.9 mL/min	1854215	2.458	6865	1.35
More Flow rate of 1.1 mL/min	1758468	2.032	6254	1.32

Stability Studies:

The specificity 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 [24-26].

Table-11: Results of Forced Degradation Studies for Avapritinib

S.No.	Stress Condition	Peak Area	% of Degraded Amount	% of Active Amount	Total % of Amount
1	Standard	1658242	0	100%	100%
2	Acidic	1331734.15	19.69	80.31	100%
3	Basic	1594233.85	3.86	96.14	100%
4	Oxidative	1394747.34	15.89	84.11	100%
5	Thermal	1575827.37	4.97	95.03	100%
6	Photolytic	1345331.73	18.87	81.13	100%
7	Water	1360090.08	17.98	82.02	100%

SUMMARY

The analytical method was developed by studying different parameters.First of all, maximum absorbance was found to be at 235nm and the peak purity was excellent. Injection volume was selected to be 10µl which gave a good peak area. The column used for study was Symmetry ODS C18 (4.6×250mm, 5µm)because it was giving good peak. Ambient temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0ml/min because of good peak area and satisfactory retention time. Mobile phase is Methanol: Phosphate Buffer pH-3.6 in the ratio of 35:65 v/v was fixed due to good symmetrical peak. So this mobile phase was used for the proposed study. Methanol was selected because of maximum extraction sonication time was fixed to be 10min at which all the drug particles were completely soluble and showed good recovery. Run time was selected to be 8min because analyze gave peak around 2.276 and also to reduce the total run time.The percent recovery was found to be 98.0-102 was linear and precise over the same range. Both system and method precision was found to be accurate and well within range. The analytical method was found linearity over the range of 6-14ppm of theAvapritinib target concentration. The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory.

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Avapritinib in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatization or purification steps. Methanol: Phosphate Buffer (35:65) v/v was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise.The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Avapritinib in bulk drug and in Pharmaceutical dosage forms.

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