ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Bio-analytical Method Development and Validation for Avapritinib in Rat Plasma by LC-MS/MS

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Abstract:
To establish a simple, sensitive and validated method for the analysis of avapritinib in rat plasma by using LC-MS/MS. Method is developed by using ibrutinib as internal standard. The chromatographic separation is done on X-Bridge phenyl (150x4.6mm, 3.5µm) column with an isocratic mobile phase of 0.1% Ortho Phosphoric acid (OPA) and Acetonitrile (50:50) and pumped at a flow stream of 1.0ml/min at ambient temperature. The proposed method showed fine calibration curve in the range of 5-100 ng/ml with r^2 – 0.99924. The %CV values of intraday precision and inter day precision were found to be within the limits. The drug was found to be stable throughout the freeze thaw, auto sampler, bench top and long term stability studies. The liquid chromatography tandem mass spectrometry method was successfully validated in accordance with the acceptable limits. The newly designed and validated approach was simple, fast and applied effectively in rat plasma.

Key words: LC-MS/MS, Avapritinib, Ibrutinib, Development, Validation, US-FDA guidelines.

1. INTRODUCTION

Avapritinib

Avapritinib, sold under the brand name Ayvakit, is a medication used for the treatment of tumors [1, 2, 3] due to one specific rare mutation [4, 5]. It is specifically intended for adults with unresectable or metastatic gastrointestinal stromal tumor (GIST) [6,7, 8, 9] that harbour a platelet-derived growth factor receptor alpha (pDGFRA) [10, 11, 12] exon 18 mutation. Common side effects are edema[13] (swelling), nausea [14], fatigue/asthenia (abnormal physical weakness or lack of energy), cognitive impairment [15], vomiting, decreased appetite [16], diarrhea [17], hair colour changes, increased lacrimation [18] (secretion of tears), abdominal pain, constipation [19], rash and dizziness [20]. Avapritinib is a kinase inhibitor [21]. Avapritinib was approved based on the results from the phase I NAVIGATOR.

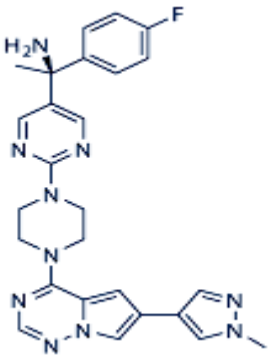

Fig. No. 1: Structure of Avapritinib

2. MATERIALS AND METHODS

2.1 Chemicals and reagents

The reference avapritinib (99.9% purity) and Ibrutinib (99.9% purity) standards were obtained from Cadila Health care Ltd, Ahmedabad, India. HPLC grade acetonitrile, ortho phosphoric acid were got from Merck chemical division (Mumbai, India). Purified HPLC water was obtained from Milli-Q (Milli Q system, USA) water purification system.t

2.2 Instrumentation

An HPLC system (Waters Alliance e2695 model) connected with mass spectrometer QTRAP 5500 triple quadrupole instrument (Sciex) was used. Data processing was performed with Empower 2.0 software.

2.3 Chromatographic Condition

Mobile phase was a mixture of 0.1% Ortho phosphoric acid and acetonitrile (50: 50 v/v). Mobile phase was used as diluent. A flow stream of 1.0ml/min is used with isocratic elution. The injection volume was 10 µl.

2.4 Preparation of working standard solutions

The stock solution of avapritinib (200 ng/ml), ibrutinib (200 ng/ml) were prepared using diluent. Working standard solutions of avapritinib were made through serial dilution of the stock standard solution with diluents, and the avapritinib ranging from 5-100 ng/ml working standard solutions of the ibrutinib (50 ng/ml) was made by diluting aptly stock standard solutions of ibrutinib with diluents. All prepared standard solutions are stored at 4°C and brings to normal room temperature before using it.

Preparation of Sample solution: For sample preparation, 500 µl of acetonitrile and 500 µl of internal standard, 500 µl of standard stock and 1000 µl of diluents to precipitate all the proteins and mix in the vortex cyclo mixture. Centrifuge at 500 rpm for 30min. Collect the supernatant solution in HPLC vial and inject into the chromatogram.

2.5 Method Validation

2.5.1 Selectivity

Selectivity was performed by analyzing the rat plasma samples from six different rats to test for interference at the retention time of analytes.

2.5.2 Matrix effect

The matrix consequence of rat plasma on the analysis of avapritinib was evaluated through comparison of peak areas of avapritinib in extracted blank plasma with that of

obtained from avapritinib standard solutions. The matrix effect was studied at LQC and HQC levels in 3 replicates.

2.5.3 Precision and Accuracy

The intra-day accuracy and precision were tested in six replicates in a single set using samples from HQC, MQC, LQC and LLOQ concentration samples on 3 successive separate batches. The precision was expressed by % CV and the accuracy by % recovery.

2.5.4 Recovery

Avapritinib recovery was evaluated by comparing peak areas of extracted LQC, MQC and HQC samples with spiked LQC, MQC and HQC samples after extraction. Internal standard recovery was analyzed by equating peak areas of the extracted samples to spiked samples after extraction.

2.5.5 Stability

The stability of avapritinib in rat plasma was evaluated by analyzing HQC and LQC samples under different storage conditions.

- Analyzing the samples immediately after preparation (Stability initial)
- Analyzing the samples following 24h of storage at room temperature (Bench top)
- Analyzing the samples after one month of storage of at -28°C (Freeze thaw stability)
- Analyzing the samples following one month of storage at -80°C (Wet extract stability)

3. Results And Discussion

Electro spray ionization (ESI) having maximum response over atmospheric pressure chemical ionization (APCI) mode selected in this method. The optimization of instrument to give sensitivity and signal stability during in-fusen of the analyte in the continuous flow of mobile phase to electro spray ion source operated at both polarities at flow rate of 10 µl/min. Avapritinib give more response in positive ion mode when compared to negative ion mode.

To obtain the best chromatographic condition, different columns like $C_{18,}$ C_8 and CN and mobile phases composed of tri ethyl amine of pH-2.5 adjusted with OPA and acetonitrile were tested. The best chromatographic separation occurred on X-Bridge phenyl column with a mobile phase consisting of 0.1% OPA and acetonitrile in 50:50 ratio at a flow rate of 1ml/min.

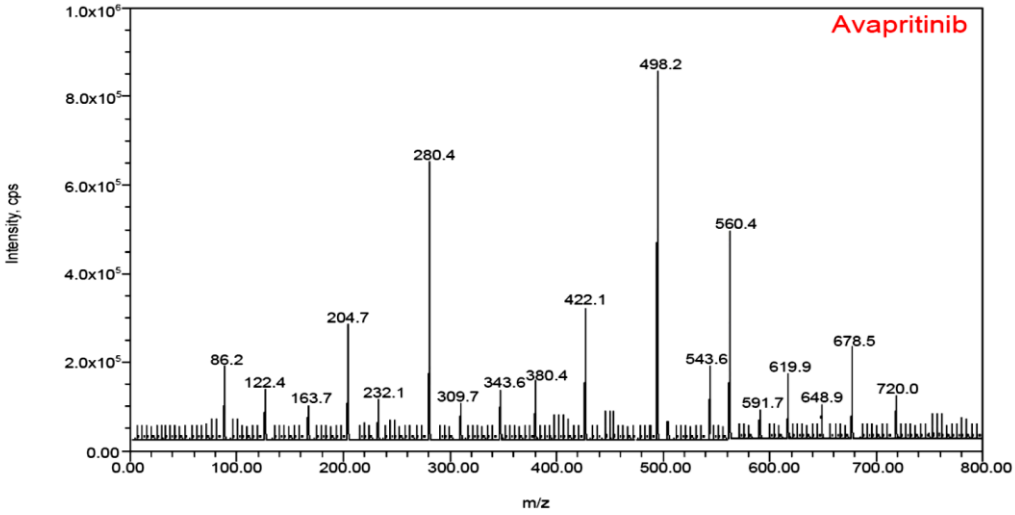

Fig. No. 2: Mass spectra of Avapritinib

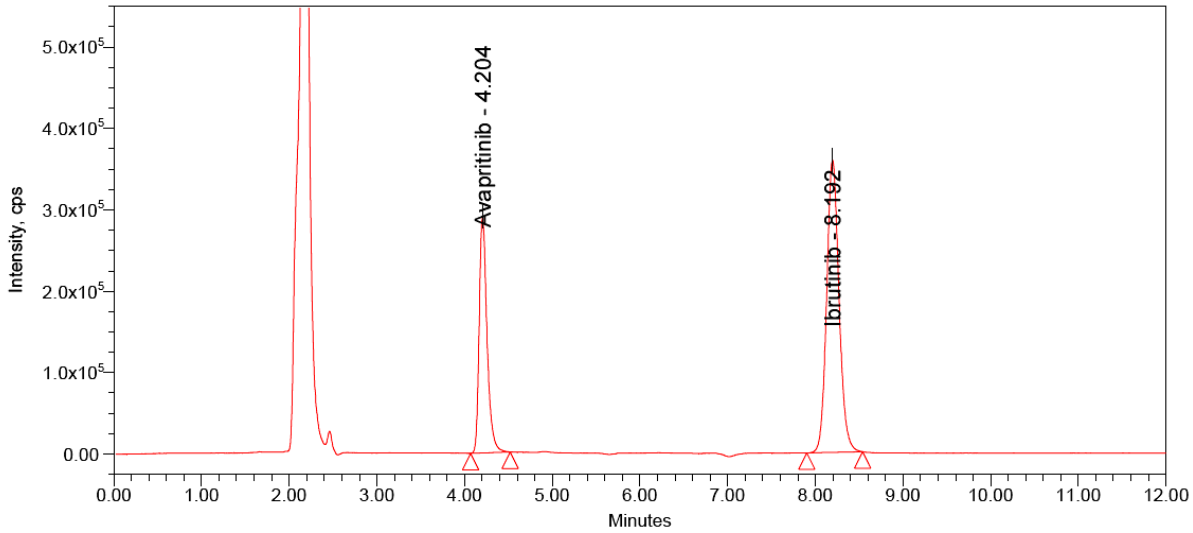

Fig. No. 3: Chromatogram of MQC

3.1 Bio-Analytical Process Validation

Representative chromatogram obtained from blank spiked with a lower limit of quantification (LOQ) sample. The mean % interference observed at the retention time of analytes between six different lots of rat plasma, including hemolyzed and lipedemic plasma containing K_2EDTA as an anti-coagulant was 0.00% avapritinib, which was within acceptance criteria. Six replicates of extracted samples at the LLOQ level in one of the plasma sample having least interference at the retention time of avapritinib was prepared and analyzed. The % CV of the area ratios of these six replicates of samples was 0.7% for avapritinib.

3.1.1 Matrix effect

The percent average recoveries for avapritinib at LQC and HQC levels were 99.87% with %CV is 0.7%. The % recovery of avapritinib at LQC and HQC levels were 99.68% and 99.94% respectively. These values indicated that there was no significant effect of the matrix on the bioanalytical methodology for simultaneous evaluation of avapritinib.

3.1.2 Linearity

The calibration curve of avapritinib was linear through the concentration range of 5-100 ng/ml. Equation of avapritinib calibration curve was, Peak area ratio = 0.0168x+0.00085; r^2 = 0.99924.

The correlation coefficient values demonstrated better linearity of avapritinib in the studied concentration range.

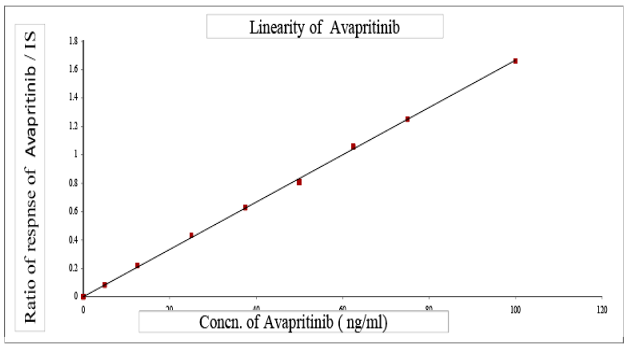

Fig. No. 4: Calibration plot for avapritinib

3.1.3 Precision and accuracy

The inter-run and accuracy were determined by pooling all individual assay results of replicate quality control over five separate batch runs analyzed on four different days. The inter-run precision (%CV) was <5% and inter-run accuracy was in between 85 and 115 for avapritinib. All these data presented in table 2 indicate that the method is precise and accurate.

3.1.4 Recovery

Six aqueous (sample spiked reconstitution-solution) at low, medium and high quality control concentration levels for avapritinib was prepared for recovery determination and the areas obtained for extracted samples of the same concentration levels from a precision and accuracy batch run on the same day. The mean recovery for avapritinib was 99.64% with a precision of 1.27%. This indicates that the extraction efficiency for avapritinib.

3.1.5 Re-injection Reproducibility

Re-injection reproducibility exercise was performed to check whether the instrument performance remain unchanged after hardware deactivation due to any instrument failure during real subject sample analysis. The change was less than 2.0 at LQC and HQC concentration levels. Hence batch can be re-injected in the case of instrument failure during real subject sample analysis. Furthermore, sample were prepared to be re-injected after 24hrs, which shows % change less than 2.0% at LQC and HQC concentration levels, hence batch can be re-injected after 24hrs in the case of instrument failure during real subject sample analysis.

3.1.6 Stability

The results of avapritinib stability tests was summarized in table 3. The findings showed that avapritinib is duarable in rat plasma under various storage conditions studied, including stored at room normal temperature for 24h and at -20°C for 30days and at -80°C.

Table 1: Linearity data of Avapritinib

S. No.	Avapritinib	
	Conc. (µg/ml)	Area
Linearity-1	5	0.312
Linearity-2	12.5	0.834
Linearity-3	25	1.626
Linearity-4	37.5	2.362
Linearity-5	50	3.026
Linearity-6	62.5	3.965
Linearity-7	75	4.689
Linearity-8	100	6.225
Slope	0.0168	
Intercept	0.00085	
CC	0.99924	

Table 2: Within run and between run precision and accuracy for avapritinib

Nominal Conc. (ng/ml)	Within run			Between run		
	Mean (ng/ml)	Precision (%CV)	Accuracy	Mean (ng/ml)	Precision (%CV)	Accuracy
5	5.264	0.36	99.68	5.314	0.55	98.67
25	25.487	0.28	99.54	25.647	0.69	99.95
50	50.632	0.57	100.25	50.497	0.48	100.45
75	75.421	0.49	99.64	100.354	0.62	99.28

Table 3: Stability of avapritinib

Stability experiments		Spiked plasma concentration (n=6, ng/ml)	Concentration measured (n=6, ng/ml)	%CV
Bench top stability	LQC	25	25.364	0.63
	HQC	75	75.214	0.75
Auto sampler stability	LQC	25	25.648	0.28
	HQC	75	75.326	0.49
Long term stability	LQC	25	25.441	0.38
	HQC	75	75.628	0.25
Freeze-thaw stability	LQC	25	25.369	0.49
	HQC	75	75.824	0.46
Wet extract stability	LQC	25	25.346	0.27
	HQC	75	75.284	0.16
Dry extract stability	LQC	25	25.136	0.34
	HQC	75	75.263	0.28
Short term stability	LQC	25	25.348	1.11
	HQC	75	75.429	0.16

4. CONCLUSION

In this investigation, a simple and sensitive LC-MS/MS method was established and validated to quantify avapritinib in the sample plasma of rats. The method involved simple single step method using acetonitrile for sample preparation. The results showed satisfactory recovery as well as a lack of major matrix effects. The validation results are well within the criteria of acceptance.

Conflicts Of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Acknowledgement

The authors are thankful to research guide Mr. Kantipudi Rambabu for supporting and Shree Icon Pharmaceutical Laboratories for providing laboratory facilities.

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