Research Article

NEW STABILITY INDICATING UPLC METHOD FOR THE ESTIMATION OF AFATINIB DIMALEATE IN BULK AND PHARMACEUTICAL DOSAGE FORM

Jahnvi Bandla ^{*1}, S. Ganapaty ²

¹Department of Pharmaceutical Analysis and QA, Faculty of Pharmacy, Vishnu Institute of Pharmaceutical Education and Research, Narsapur, Medak-502313, Telangana, India

²Department of Pharmacognosy and Phytochemistry, GITAM Institute of Pharmacy GITAM University, Rushikonda, Visakhapatnam-530045, Andhra Pradesh, India

*Corresponding Author Email: Jahnvi.bandla@gmail.com

Article Received on: 16/04/19 Approved for publication: 22/05/19

DOI: 10.7897/2230-8407.1007224

ABSTRACT

The aim of the present study is to develop and validate a stability indicating method for the estimation of afatinib dimaleate in bulk and pharmaceutical dosage form using ultra-performance liquid chromatography (UPLC). The drug was eluted by using acquity UPLC waters C18 (150mm x 2.1mm, 1.7 μ) column with mobile phase consisting of 0.01N potassium dihydrogen orthophosphate and acetonitrile in the ratio 50:50%v/v run on isocratic mode at a flow rate of 0.3ml/min. The column oven temperature was maintained at 30°C. The drug, afatinib was detected at 250nm. The retention time for afatinib was found to be 1.3min. The developed method was validated in accordance to international conference on harmonization (ICH) guidelines. The method obeyed Beer's law in the concentration range of 10 μ g/ml – 60 μ g/ml with correlation coefficient of 0.9997. The % Relative Standard Deviation (RSD) and % recovery were found to be 0.4 and 99.70% - 100.27% respectively. The method was found to be rugged, robust and stable in solution. When drug was exposed to various stress conditions, it was found to be stable as net degradation was within the limits. The developed method can be used for quality control of afatinib in pharmaceutical dosage form.

Keywords: Afatinib dimaleate, method development, stability indicating, UPLC, validation.

INTRODUCTION

Afatinib dimaleate¹⁻⁴ (Fig. 1), is chemically known as (Z)-but-2-enedioic acid;(E)-N-[4-(3-chloro-4-fluoroanilino)-7-[(3S)-oxolan-3-yl]oxyquinazolin-6-yl]-4-(dimethylamino)but-2-enamide. Its molecular formula is C₃₂H₃₃ClFN₅O₁₁ and molecular weight is 718.083g/mol. It is white to brownish yellow powder and soluble in water. It has pKa values of 8.81 and 12.49. It is an anticancer drug used for the treatment of patients with metastatic non-small lung cancer. It acts as an irreversible covalent inhibitor of receptor tyrosine kinases epidermal growth receptor (EGFR) and erbB-2 (HER2).

Ultra performance liquid chromatography (UPLC)⁵⁻⁷ is a relatively new technique giving new possibilities in liquid chromatography, especially concerning the decrease of time and solvent consumption. UPLC chromatographic system is designed in a special way to withstand extreme system back pressures.

Literature survey reveals that there is only one method i.e., reverse phase high performance liquid chromatography (RP-HPLC) method⁸ developed for the estimation of afatinib in bulk and pharmaceutical dosage form.

As there is no method with UPLC, the aim of the present study is to develop and validate a stability indicating UPLC method for the estimation of afatinib in bulk and pharmaceutical dosage form.

Fig. 1: Chemical structure of Afatinib dimaleate

MATERIAL AND METHODS

Chemicals and Reagents: Afatinib working standard was procured from spectrum labs, Hyderabad as gift sample. The gilotrif tablets were purchased from local pharmacy. All the chemicals used were of analytical reagent grade purchased from Merck, Mumbai. All the solvents used were of HPLC grade purchased from Sigma-Aldrich, Mumbai.

Chromatographic conditions and Instruments: The acquity UPLC system equipped with binary solvent manager, sample manager, ultra violet (UV) detector and water C18 (150mm x 2.1mm, 1.7 μ) column was used for the determination of afatinib. The optimized conditions include 0.01N potassium dihydrogen orthophosphate and acetonitrile (50:50%v/v) as mobile phase run on an isocratic mode at flow rate 0.3ml/min. The column was maintained at 30°C and detection was done at 250nm. Other

equipment includes pH meter, ultrasonic bath sonicator and weighing balance.

Preparation of mobile phase: Dissolve 1.36g of potassium dihydrogen orthophosphate into 1000ml of distilled water. Adjust the pH of the solution to 3 using dilute ortho-phosphoric acid. This gives 0.01N potassium dihydrogen orthophosphate buffer. Mixture of above buffer solution with acetonitrile in the ratio of 50:50%v/v was used as mobile phase.

Preparation of standard and sample solutions: Dissolve 40mg of Afatinib working standard in 100ml of diluent. Dilute 1ml of the above stock solution to 10ml with diluent. Average weight of 20 tablets (Gilotrif) was calculated and an amount equivalent to 40mg of drug was dissolved in 100ml of diluent. Filter and dilute 1ml of the above solution to 10ml with diluent.

Method Validation: The developed method was validated⁹ for the following parameters.

Specificity: The specificity of the method was determined by comparing the drug solution with the placebo solution and observed for the interference of retention time of placebo peak with that of drug peak.

Accuracy: Accuracy of the method was determined by % recovery. The drug solution along with sample was prepared in three concentration levels 50%, 100% and 150%. Then the % recovery was calculated.

Precision: Precision of the method was determined by injecting the standard solution six times into the UPLC system and % RSD was calculated.

Linearity: Linearity of the method was determined by preparing series of dilutions ranging from 10µg/ml – 60µg/ml and injecting them into UPLC system.

Ruggedness: Ruggedness was determined by injecting the standard solution into UPLC six times for different days. The %RSD was calculated.

Robustness: Robustness of the method was determined by varying the optimized analytical conditions such as mobile phase composition by $\pm 5\%$, flow rate by ± 0.1 ml/min and column oven temperature by $\pm 5^\circ\text{C}$.

Solution stability: Solution stability was determined by analyzing the standard drug solution after storage for 24 hours under laboratory conditions.

Forced degradation studies: Forced degradation studies¹⁰ were carried out for drug by exposing the drug solution to the various stress conditions such as acidic (2N hydrochloric acid for 30mins at 60°C), basic (2N sodium hydroxide for 30mins at 60°C), peroxide (20% hydrogen peroxide for 30 min at 60°C), neutral (refluxing the drug in water for 6 hours at 60°C), photolytic (105°C for 6h) and thermal (exposing the drug solution to UV Light by keeping the beaker in UV Chamber for 7 days or 200 Watt hours/m² in photo stability chamber) conditions.

RESULTS

Fig. 2: UV spectrum of Afatinib

Fig. 3A: Standard chromatogram

Fig. 3B: Sample chromatogram

Fig. 4: Linearity plot of Afatinib

Table 1: System suitability and validation parameter results

Parameter	Results	
Precision (%RSD, n)	0.4	
Accuracy (% Recovery, n)	99.70% – 100.27%	
Specificity	Specific, No interference	
Linearity range (µg/ml)	10 – 60	
Correlation coefficient, r	0.9997	
Limit of Detection (µg/ml)	0.07	
Limit of Quantitation (µg/ml)	0.23	
Ruggedness (%RSD, n)	Day 1	Day 2
	0.4	0.5
Robustness (%RSD, n)	Less flow rate	More flow rate
	0.6	0.8
	Less mobile phase (Organic)	More mobile phase (Organic)
	0.6	1.3
	Less column temperature	More column temperature
	0.8	0.8
Solution stability	Day 1	Day 2
% RSD, n	0.4	0.5
% Assay, n	99.60	99.70
USP Plate count	3177	
USP tailing factor	1.23	

n – number of samples, i.e., six samples for estimation

Table 2: Forced degradation studies results

Stress condition	% Assay	% area of degradation peak	% Degradation
2N HCl for 30min at 60°C	95.13	3.20	4.87
2N NaOH for 30min at 60°C	95.41	7.16	4.59
20% H ₂ O ₂ for 30min at 60°C	96.27	-	3.73
Water for 6h at 60°C	99.26	-	0.74
UV light 200wts/h or 7 d	97.49	-	2.51
105°C for 6h	96.74	-	3.26

Fig. 5A: Acid degradation chromatogram

Fig. 5B: Base degradation chromatogram

Fig. 5C: Peroxide degradation chromatogram

Fig. 5D: Water stress study chromatogram

Fig. 5E: Photo stability degradation chromatogram

Fig. 5F: Dry heat study chromatogram

DISCUSSION

For the development of method for the determination of afatinib, initially various mobile phases and columns were tried to elute the drug. Acquity UPLC water C18 (150mm x 2.1mm, 1.7µ) column maintained at 30°C and 0.01N potassium dihydrogen phosphate and acetonitrile in the ratio 50:50%v/v was used as mobile phase run on an isocratic mode at a flow rate of 0.3ml/min. The detection wavelength 250nm was selected based on the UV spectrum of afatinib as shown in figure 2.

Prepared standard solution and sample solution were injected into the UPLC system and system suitability parameters were noted in table 1 and chromatograms were shown in figure 3A and 3B respectively.

The method was found to be specific when compared with placebo solution, as there is no interference of retention time of placebo peak with that of drug peak. The method is said to obey Beer's law in the concentration range of 10 µg/ml – 60 µg/ml with correlation coefficient of 0.9997, thus indicating the method to be linear. The linearity graph was shown in figure 4.

The % recovery was found to be 99.70% - 100.27% and %RSD was found to be 0.4, indicates that the method is accurate and precise. The limit of detection (LOD) and limit of quantitation (LOQ) were found to be 0.07 µg/ml and 0.23 µg/ml respectively. The method was found to be rugged, robust and stable in solution even after 24 hours.

Forced degradation studies were performed and net degradation was noted. The net degradation was found to be within the limits indicating that the method is stable when drug was exposed to various stress conditions. The chromatograms were shown in figure 5 and results were summarized in table 2.

CONCLUSION

A stability indicating UPLC method was developed for the estimation of afatinib dimaleate in bulk drug and pharmaceutical dosage form. The developed method was validated in accordance to the ICH guidelines. The method was found to be accurate, precise, specific, linear, rugged, robust and stable in solution. From the forced degradation studies it was concluded that the drug is stable in extreme stress conditions. As the run time is less, this method can be used for routine analysis of afatinib in pharmaceutical dosage form.

ACKNOWLEDGEMENT

The authors are thankful to the spectrum labs, Hyderabad for providing the afatinib as the gift samples and also for providing required facilities to carry out this work.

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Cite this article as:

Jahnavi Bandla and S. Ganapaty. New stability indicating UPLC method for the estimation of afatinib dimaleate in bulk and pharmaceutical dosage form. *Int. Res. J. Pharm.* 2019;10(7):87-90 <http://dx.doi.org/10.7897/2230-8407.1007224>

Source of support: Nil, Conflict of interest: None Declared

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