CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

http://doi.org/10.5281/zenodo.1206381

Available online at: http://www.iajps.com *Research Article*

METHOD DEVELOPMENT AND VALIDATION OF AFATINIB IN BULK AND PHARMACEUTICAL DOSAGE FORM BY UV-SPECTROSCOPIC METHOD

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Abstract:

A new simple, accurate, rapid, precise, reproducible and cost effective spectrophotometric method for the quantitative estimation of Afatinib in bulk and pharmaceutical dosage form. The developed visible spectrophotometric method for the quantitative estimation of Afatinib is based on measurement of absorption at maximum wavelength 246 nm using Sodiumcitrate with Water as a solvent. The stock solution of Afatinib was prepared, and subsequent suitable dilution was prepared in distilled water to obtained standard curve. The standard solution of Afatinib shows absorption maxima at 246 nm. The drug obeyed beer lambert's law in the concentration range of 5 - 25 µg/ml with regression 0.999 at 246 nm. The overall % recovery was found to be 99.03% which reflects that the method was free from the interference of the impurities and other excipients used in the bulk and marketed dosage form. The low value of % RSD was indicative of accuracy and reproducibility of the method. The % RSD for inter-day and intra-day precision was found to be 0.298 and 0.2941respectively which is<2% hence proved that method is precise. The results of analysis have been validated as per International Conference on Harmonization (ICH) guidelines. The developed method can be adopted in routine analysis of Afatinib in bulk and tablet dosage form.

Keywords: *Afatinib, UV Visible Spectrophotometry, Method development, Validation, ICH guidelines, Sodium citrate , Accuracy, Precision.*

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Please cite this article in press Santhosh Illendula et al., ***Method Development and Validation of Afatinib in Bulk and Pharmaceutical Dosage Form by UV-Spectroscopic Method,*** *Indo Am. J. P. Sci, 2018; 05(03).*

INTRODUCTION:

Afatinib (*N*-[4-[(3-chloro-4-fluorophenyl)amino]-7-[[(3*S*)-tetrahydro-3-furanyl]oxy]-6-quinazolinyl]-4-(dimethylamino)-(2*E*)-2-butenamide; Tovok; Gilotrif; Boehringer Ingelheim Pharmaceuticals, Inc; Figure 6) through single usage, or in combination therapy along with other kinase inhibitors, may delay or suppress the emergence of drug resistance caused by certain drug-induced ErbB (proto-oncogene B of the avian erythroblastosis virus) mutations. Structures of most of the transmembrane, EGFR tyrosine kinase inhibitors, including afatinib (approved by FDA in 2013 for non–small cell lung carcinoma), have the 4-(fluorophenylamino)quinazoline moiety, which exhibits strong hydrogen bonding interactions with the active site amino acid residues [1-8]. Afatinib is also being evaluated for other types of cancers such as head and neck and breast cancers [9-12].

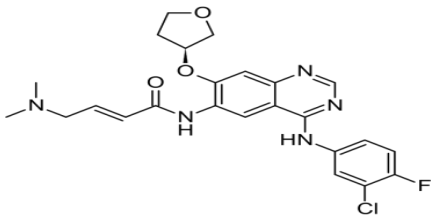

Fig.1: Structure of Afatinib

Afatinib is a potent inhibitor of ErbB family EGFR tyrosine kinases. In humans, there are four structurally similar EGFRs, which are HER-1 (ErbB-1), HER-2 (ErbB-2), HER-3 (ErbB-3), and HER-4 (ErbB-4). Aberrant signaling through these receptor kinases is responsible for various epithelial cancers, and thus targeting the ErbB family of growth factor receptors plays a key role in the development of therapeutics for epithelial cancers [13-20]. Afatinib is an irreversible inhibitor of EGFR tyrosine kinases, and forms a covalent adduct with the active site sulfhydryl group through Michael addition reaction (vide infra).

MATERIALS AND METHODS:

Chemicals and Reagents: Methanol, Ethanol, Acetone, Dimethylsulphoxide, Sodium citrate with water.

Instruments:

SHIMADZU UV-1601 UV – Vis spectrophotometer, Electronic Balance **(CITIZEN BALANCE BL-220H),** Ultra Sonicator **(ANALYTICAL),** and P^H Analyzer **(ELICO),** Distillation unit **(BOROSIL),** Vaccum filteration unit**(BOROSIL).**

Reagents and Solutions

Diluent preparation: Take 2.58gm of Sodium Citrate and add 60ml of water. Dissolve and make up to 100ml in a volumetric flask.

Preparation of Standard Solutions

Accurately weighed 100mg of Afatinib was weighed accurately and transferred into 100ml volumetric flask. About 10 ml of diluent was added and sonicated to dissolve. The volume was made up to the mark with same solvent. The final solution contained about 100µg/ml of Afatinib. Working standard solution of Afatinib containing 10µg/ml for method . Finally add those above solutions and prepare the final solution is about 10µg/ml.

Preparation of Sample Solutions .

Take 20 Tablets average weight and crush in a mortar by using pestle and weight powder 100 mg equivalent weight of Afatinib sample into a 100ml clean dry volumetric flask, dissolve and make up to volume with diluent. Further dilution was done by transferring 0.1 ml of the above solution into a 10ml volumetric flask and make up to volume with diluent.

Determination of wavelength of maximum absorbance for Afatinib

The absorbance of the final solution scanned in the UV spectrum in the range of 200 to 400nm against solvent mixture as blank.

Optimization Optimization of selection of Solvent

It is well known that the solvents do exerts a profound effect on the quality and the shape of the peak. The choices of solvents for UV method development are: Methanol, Ethanol, Acetonitrile, Dimethyl Sulphoxide, Sodium citrate etc. First optimize the different solvents. From that solvents methanol satisfied the all the optimized conditions.

Wavelength Selection

The standard solutions are prepares by transferring the standard drug in a selected solvent and finally diluting with the same solvent or Diluent. That prepared solution is scanned in the UV visible wavelength range of 200-400nm.This has been performed to know the maxima of Afatinib. While scanning the Afatinib solution we observed the maxima at 246 nm. The visible spectrum has been recorded on (SHIMADZU UV-1601 make UV – Vis spectrophotometer model UV-1601.The scanned visible spectrum is attached in the following page.The λ_{max} of the Afatinib was found to be 246 nm in diluents as solvent system.

METHOD VALIDATION

1. Accuracy: ***Recovery study:*** To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of pure drug of Afatinib were taken and added to the pre-analyzed formulation of concentration 10µg/ml. From that percentage recovery values were calculated. The results were shown in Table-1.

2. Precision:

Repeatability

The precision of each method was ascertained separately from the peak areas & retention times obtained by actual determination of six replicates of a fixed amount of drug. Afatinib (API) the percent relative standard deviations were calculated for Afatinib is presented in the Table-2.

Intermediate Precision:

Intra-assay & inter-assay:

The intra & inter day variation of the method was carried out & the high values of mean assay & low values of standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Afatinib revealed that the proposed method is precise. The results were shown in Table-3.

3. Linearity & Range:

The calibration curve showed good linearity in the range of 5-25µg/ml, for Afatinib (API) with correlation coefficient (r^2) of 0.999 (Fig-2). A typical calibration curve has the regression equation of y = 0.055x + 0.004 for Afatinib.

Standard solutions of Afatinib in the concentration range of 5 µg/ml to 25 µg/ml were obtained by transferring (5,10,15 and 20,25 ml) of Afatinib stock solution (100ppm) to the series of clean & dry 10 ml volumetric flasks. The volumes in each volumetric flask were made up with the solvent system and mixed.

The absorbances of the solutions were measured at 246 nm against the solvent system as blank and calibration curve is plotted. The Lambert-Beer's Law is linear in concentration range of 5 to 25 µg/ml at 246 nm for Afatinib. The results were shown in Table-4.

4. Method Robustness:

Robustness of the method was determined by carrying out the analysis under different Wavelength i.e. at 244nm,246nm and 248nm.. The respective absorbances of 10µg/mlwere noted SD < 2%) the developed UV-Spectroscopic method for the analysis of Afatinib (API). The results were shown in Table-5.

5. LOD & LOQ:

The LOD and LOQ were calculated by the use of the equations LOD = 3.3 × σ / S and LOQ = 10 × σ / S where σ is the standard deviation of intercept of Calibration plot and S is the average of the slope of the corresponding Calibration plot.

The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 0.080 & 0.24 µg/ml respectively.

6. ASSAY OF AFATINIB IN DOSAGE FORM:

AFATINIB 20mg

Assay of marketed tablet formulation Brands :

Afatinib was procured from the local market as tablets of strength having 20mg, marketed with brand names of GILOTRIF. These marketed formulations were manufactured by the .Boehringer Ingelheim Pharmaceuticals, respectively.

Weighed accurately about twenty tablets and calculate the weights of individual tablets and finally calculate the average weight. They were triturated to fine powder by using a mortar and pestle. The powdered tablet equivalent to 20mg of Afatinib was dissolved in 15ml of diluent with the help of sonication process and the final volume was made upto the mark with the diluent in 25 ml volumetric flask. The resulted solution was filtered using whatman filter paper (0.45µm). This final solution was further diluted to obtain 10µg/ml concentration of the solution by using diluents used as a solvent and observed by UV analysis. This procedure was repeated in triplicate. The data are shown in Table-6.

$$\text{Amount Present} = \frac{\text{Sample Absorbance}}{\text{Standard Absorbance}} \times \frac{\text{Standard Dilution}}{\text{Sample Dilution}} \times \frac{\text{Potency}}{100} \times \text{Average weight}$$

$$\%\text{Content} = \frac{\text{Amount Present}}{\text{Label Claim}} \times 100.$$

RESULTS AND DISCUSSION:

The standard solutions of Afatinib in Sodiumcitrate with Water (10µg/ml) subjected to a scan individually at the series of wavelengths of 400 nm to 800 nm. Absorption maximum of Afatinib was found to be at 246 nm. Therefore, 246 nm was selected as λ_{max} of Afatinib for the present study. The calibration curve of Afatinib was found to be linear in the range of 5-25 µg/ml at 246 nm. Therefore, it was clear that Afatinib can be determined without interference of

any irrelevant substance in single component pharmaceutical products. The used technique was initially attempted on bulk drugs in their synthetic sample and concentrations were estimated.

The % recovery was carried out at 3 levels, 80%, 100% and 120% of Afatinib standard concentration. Three samples were prepared for each recovery level. The solutions were then analyzed, and the percentage recoveries were found to be satisfactory within the acceptable limits as per the content of the label claim for marketed tablet dosage form. The newly developed method was validated according to the ICH guidelines and the method validation parameters.

The developed method was subjected to do the various method validation parameters such as specificity, accuracy, precision, linearity and range, limit of detection and limit of quantification, robustness and ruggedness etc.

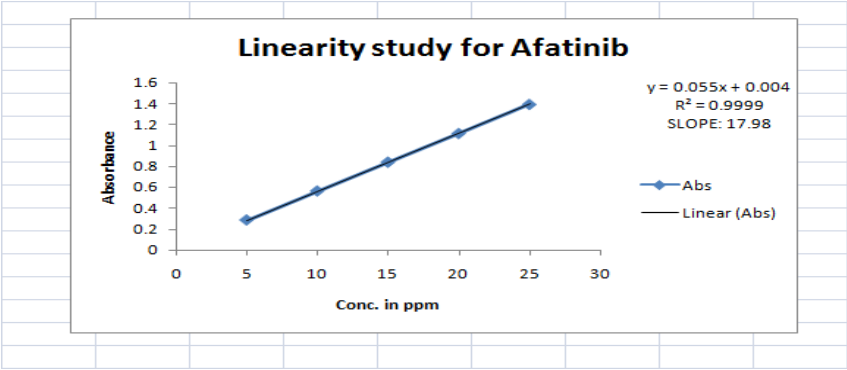

Fig-2: Calibration curve of Afatinib (API).

Table-1: Results of accuracy:

Level of Recovery	Sample Conc. (µg/ml)	Amount Recovered (µg/ml)	% Recovery	Mean % Recovery
80%	8	7.960	99.5	99.2
80%	8	7.984	99.8	
80%	8	7.881	98.5	
100%	10	9.718	97.18	98.4
100%	10	9.891	98.91	
100%	10	9.913	99.13	
120%	12	11.941	99.5	99.5
120%	12	11.916	99.3	
120%	12	11.973	99.7	

Acceptance criteria: correlation coefficient should not be less than 0.990.

2. Precision:

Repeatability:

Table-2: Results of Repeatability

S.No.	Conc. (µg/ml)	Wavelength (nm)	Absorbance
1	10	246	0.576
2	10	246	0.576
3	10	246	0.578
4	10	246	0.583
5	10	246	0.581
6	10	246	0.578
Mean ± S.D.			0.576
Standard Deviation			0.00404
% RSD			0.7013

Table-3: Results of intra-Day & inter-Day

Con. taken (µg/mL)	Observed Conc. Of Afatinib (µg/ml) by the proposed method			
	Intra-Day		Inter-Day	
	Absorbance	**Statistical Analysis**	Con. found (µg/mL)	**Statistical Analysis**
10	0.581	Mean = 0.58 SD = 0.00173 %RSD = 0.298	0.577	Mean = 0.578 SD = 0.0017 %RSD = 0.2941
10	0.578		0.580	
10	0.581		0.579	

Table-4: Results of Linearity

Concentration (µg/ml)	Absorbance (n=6)
5	0.282
10	0.561
15	0.839
20	1.115
25	1.395

Acceptance criteria: correlation coefficient should not be less than 0.990

Table-5: Result of Method Robustness Test

Wavelength(-2)(244nm)

Concentration(µg/ml)	Absorbance	Statistical Analysis
10	0.551	Mean = 0.5545 SD = 0.0100059 % RSD = 1.80
10	0.561	
10	0.553	
10	0.571	
10	0.548	
10	0.543	

Wavelength(246nm)

Concentration(µg/ml)	Absorbance	Statistical Analysis
10	0.581	Mean = 0.574 SD = 0.009 % RSD = 1.56
10	0.563	
10	0.568	
10	0.571	
10	0.574	
10	0.588	

Wavelength(248nm)

Concentration(µg/ml)	Absorbance	Statistical Analysis
10	0.533	Mean = 0.53783 SD = 0.0053 % RSD = 0.98
10	0.538	
10	0.545	
10	0.531	
10	0.542	
10	0.538	

Table-6: Assay Results of Marketed Formulations

Formulations	Actual concentration of Afatinib [Label Claim] (µg/ml)	Amount obtained of Afatinib (µg/ml)	% Afatinib
GILOTRIF	20	19.816	99.08

CONCLUSION:

From the experimental studies it can be concluded that first UV-Spectroscopic method is developed for Afatinib in marketed pharmaceutical dosage form. The developed method for the drug (Afatinib) was found to be accurate and precise.

The great features of spectrophotometric methods are their simplicity, economical and rapidity. The results of method validation showing that the developed analytical procedure is suitable for its intended purpose and meets the Guidelines given by the ICH. The developed method was successfully applied for the routine analysis of Afatinib in bulk and pharmaceutical dosage form in the future.

ACKNOWLEDGEMENT:

The authors are greatful to the management of Nalanda College of Pharmacy, Nalgonda for providing the facilities to carry out the present research work

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