

AFATINIB-A COMPREHENSIVE REVIEW OF ANALYTICAL METHODS DEVELOPED IN PHARMACEUTICALS AND BIOLOGICAL MATRICES

HEMANTH KUMAR¹, LALIT KUMAR², RUCHI VERMA^{1*}

¹Department of Pharmaceutical Chemistry, Manipal College of Pharmaceutical Sciences, Manipal Academy of Higher Education, Manipal-576104 (Karnataka) India. ²Department of Pharmaceutics, National Institute of Pharmaceutical Education and Research, Hajipur-844102, Vaishali, Bihar, India

*Corresponding author: Ruchi Verma; *Email: ruchiverma.pharma@gmail.com

Received: 09 Jan 2024, Revised and Accepted: 15 May 2024

ABSTRACT

Afatinib is a selective irreversible ErbB family blocker that can be taken orally and has demonstrated broad-range effectiveness in preclinical studies against Epidermal Growth Factor Receptor (EGFR) mutations. The inhibition of ErbB receptors by afatinib may stop the development and spread of tumours because they are crucial for cellular proliferation and apoptosis. A modulator of the tyrosine kinase receptor known as afatinib treats specific types of metastatic non-small cell lung cancer. Afatinib has been found to produce acute liver injury, also apparent rare cases of death, and is associated with momentary elevations in serum aminotransferase levels at the time of therapy.

The analytical techniques for evaluating afatinib in pharmaceuticals and biological matrices are the main subject of this study. For each method, the important validation parameters such as linearity, detection system, retention time, mobile phase, limit of Detection (LOD), and limit of Quantification (LOQ) are examined. Additionally, the discussion includes important quality characteristics like sensitivity, specificity and technique utilised for sample preparation pertaining to bioanalytical methods.

Keywords: Afatinib, Analytical methods, Biological matrix, Mobile phase

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INTRODUCTION

Tyrosine Kinase Inhibitors (TKIs) have been authorised as monotherapy for the management of cancer in recent years. These specific drugs target tyrosine kinases, which are crucial for the transmission of growth impulses inside of cells. In 2013, Afatinib's authorization for use in the US was granted. Current indications include second-line treatment for individuals who have been treated and have refractory squamous cell lung cancer. Also, it is used as first therapy for metastatic Non-Small Cell lung Cancer (NSCLC) with Epidermal Growth Factor Receptor 2 (EGFR2) mutations. Afatinib pills frequently cause side effects, such as diarrhoea (loose stool), rashes, oral mucositis, anorexia nervosa, vomiting, dryness in skin, paronychia, and pruritus [1, 2]. Serious diarrhoea that causes dehydration and kidney failure is one of the uncommon but possibly dangerous side effects, along with bullous and exfoliative skin rashes, erythema multiforme major (Stevens-Johnson syndrome), which is a rare, serious disorder of the skin and mucous membranes., interstitial lung disease, and toxicity to the foetus. Other malignancies, including head and neck and breast cancers, are also being studied in relation to afatinib. There are four EGFRs in people that share a structural similarity: HER-1 (ErbB1), HER-2 (ErbB2), HER-3 (ErbB3), and HER-4. (ErbB-4) [4].

The FDA approved afatinib for treating people with locally advanced Non-Small Cell lung Cancer (NSCLC) whose tumours have mutations at exon 21 (L858R) substitution or by exon 19 deletions in EGFR. Therefore, it is important to consider all potential interactions as drug research for the treatment of cancer advances. Serum albumins' molecular and physiological characteristics have been thoroughly investigated. The perceived distribution volume and elimination rate of the various drugs may be significantly influenced by their interactions with one another. Therefore, research on the interaction between drugs and proteins will help to understand how drugs are metabolised and transported. The Bovine Serum Albumin (BSA), has a variety of physiological characteristics that involve binding, transporting, and delivering a variety of molecules [5, 6].

In comparison to afatinib oral solution, afatinib tablets exhibited a geometric mean bioavailability of 92%. The maximum plasma concentration (C_{max}) of afatinib tablets was attained 2-5 h after oral

treatment. With the injection of afatinib 20-50 mg, C_{max} and area under the plasma concentration-time curve values soared marginally high than dosage correspondingly. After 8 days of repeated treatment, steady-state plasma afatinib concentrations were achieved. When afatinib was taken with a rich meal, its exposure was decreased; afatinib should be avoided with food. *In vitro*, afatinib bound 95% of plasma protein [7, 8].

Afatinib is an oral medication that is only accessible in tablet form. Because absorption diminishes with high-fat meals, it is advisable to take this medicine on an empty stomach, either 1 h prior or 2 h after having meal. The pill should be taken whole with 8 ounces of water. The pill should not be crushed or dissolved. This tablet needs to be stored at ambient temperature [9].

The primary goal of this article is to gather various suggested methodologies adopted for the estimation of afatinib in formulations and to present a classified compiled review. The data presented is 10 y available data from the year 2011-2022. The literature was obtained from various databases i. e. science direct, scopus, taylor and francis, web of sciences, elsevier, springer, pubmed.

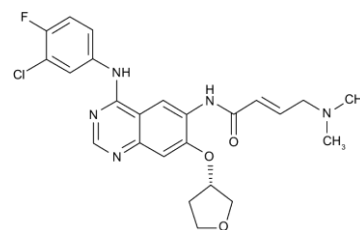


Fig. 1: Structure of afatinib

UV/VIS Spectrophotometric methods

When expensive, modern equipment like Gas Chromatography (GC) or High-Performance liquid Chromatography (HPLC) is not accessible, spectrophotometric methods, which are readily available

in laboratories, could be utilized to determine the presence of drugs [10]. The advantages of spectrophotometric methods over other

methods include their simplicity, economy, rapidity, ease of use, reduced expense, and reduced time requirements [11].

Table 1: Spectrophotometric methods reported for the quantification of afatinib

Drug	Matrix	Solvent/Reagent	Detection (nm)	Linearity (µg/ml)	LOD (µg/ml)	LOQ (µg/ml)	References
Afatinib-BSA	Serum	Methanol, Ultrapure water of 18.2 MΩ.	334-344	0.3-10	NA	NA	[12]
Afatinib	Tablets	Methanol, Ethanol, Acetone, Dimethylsulphoxide, Sodium citrate	246	5-25	0.080	0.24	[13]

Bioassay methods

For Afatinib bioanalysis, two distinct forms of heterogeneous fluorescent immunoassays were created and approved. These tests included a Kinetic Exclusion Assay (KinExA) utilizing a KinExA 3200 immunosensor and a microwell-based Fluorescence Immunoassay (FIA) utilizing a fluorescence plate reader. The same materials were used to create both FIA and KinExA: goat anti-mouse IgG labelled with fluorescein isothiocyanate (FITC-IgG) for generating signal.

Solid-phase immobilised Afatinib was conjugated with Bovine Serum Albumin protein (Afatinib-BSA), and mouse anti-Afatinib antibody.

The development and validation technique of a general ELISA for measuring afatinib in plasma samples outperforms every other chromatographic assay for afatinib currently in use in terms of ease of use, comfort, and high-throughput analysis. It is expected that the proposed ELISA will significantly aid in the therapeutic monitoring of afatinib in clinical settings.

Table 2: Bioassay methods for the quantification of Afatinib

Drug	Technique	Pre-treatment of plasma samples	Range (ng/ml)	LOD (ng/ml)	LOQ (ng/ml)	Accuracy (Recovery %)	Precision (RSD, %)	References
Afatinib-BSA and KLH	ELISA	Dilution with phosphate buffer saline	0.04-2000	2	4	100.9±1.42	3.5-7.1	[14]
Afatinib in Human plasma	ELISA	Dilution with phosphate buffer saline	7.5-122.88	24.6	30	96.9-105.0	3.5-5.7	[15]
Afatinib-BSA	Fluor immune assay	Dilution with phosphate buffer saline	0.008-200	0.4	1.2	101.8±1.8	4.2-8.4	[16]
Afatinib-BSA	Kinetic exclusion assay	Dilution with phosphate buffer saline	0.01-100	0.1	0.3	99.4±3.4	4.2-4.8	[16]

Chromatographic methods

Instrumentation for chromatography is managed and automated by HPLC. It offers instrument validation, reporting, data administration, and security features. manages all parameters associated with analysis ranging from sample to instrument, separation of components to reporting findings, thus resulting in increased productivity. The main aim of HPLC lies with identifying, measuring, and purification of analyte or compound. Analyses can be carried out quantitatively and qualitatively [17, 18].

HPLC is broadly used chromatographic method for drug quantification among the different analytical techniques. Additionally, HPLC is among the most trustworthy techniques for quantification due to its accuracy, robustness, and sensitivity. The

quantification of afatinib as a single dosage or in fixed-dose combinations is possible using HPLC methods.

The quantitative determination of biopharmaceuticals can be carried out with liquid Chromatography (LC) linked to tandem mass spectrometry has lately gained popularity as an alternative to conventional ligand-binding assays. The advantages of LC-MS/MS involve better precision and accuracy, greater selectivity, and generic applicability without the need to raise analyte-directed antibodies [19-22].

The quantification of drugs and their byproducts or metabolites present in biological fluids like plasma, urine, and serum using bioanalytical techniques is crucial for determining and interpreting the results of pharmacokinetic, toxicokinetic, and bioequivalence studies [23-25].

Table 3: Chromatographic methods available for the quantification of afatinib alone and in combination with another drug

Matrices	Method used	Column	Mobile phase	Detection system	Flow rate (ml/min)	CT (°C)	Linearity (µg/ml)	Rt min	References
Human plasma	UPLC-DAD	UPLC BEH C18 specification: (1.7 µm; 2.1 mm × 50 mm)	Ammonium formate buffer (4 mmol, pH 3.2 adjusting with formic acid) and acetonitrile (ACN)	268 nm	0.4	40	0.005-0.25	2.38	[26]
Standard and tablets	RP-HPLC	X-Terra RP-8 (250 mm × 4.6 mm) 5.0 µm	Aqueous KH ₂ PO ₄ buffer which was adjusted to pH 3.0 with (orthophosphoric acid and acetonitrile) and methanol (70:30, v/v).	258 nm	1	Ambient	0.12-0.36	10.55	[27]
Human plasma	Nexera X2 UHPLC	XBridge Shield RP ₁₈ 3.5 µm 2.1 × 50 mm (Waters, Milford, MA, USA) and XBridge Guard columns Shield RP ₁₈ 2.1 × 10 mm	mobile phase ration was 70:30 (v/v) mixture of 10 mmol ammonium hydroxide in acetonitrile and 1 mmol aqueous ammonium hydroxide (pH 10.5)	ESI/TMS	0.2	40	0.00005-0.1	2.7	[28]
Mouse plasma	LC-MS-MS	UPLC BEH C18 column (30 × 2.1 mm, d _p = 1.7 µm, Waters, Milford, USA) with an Aquity UPLC BEH C18 VanGuard	solvent A contained 0.1%(v/v) ammonium hydroxide in water and solvent B was acetonitrile.	ESI	0.6	40	0.005-0.5	1.04	[29]

Matrices	Method used	Column	Mobile phase	Detection system	Flow rate (ml/min)	CT (°C)	Linearity (µg/ml)	Rt min	References
Human Plasma	LC-MS/MS	pre-column (Waters, 5 × 2.1 mm, dp = 1.7 µm) Luna-PPF 100 Å column (50 × 2.0 mm; 3.0 µm)	Acetonitrile: water ratio (40:60, v/v) with 10 mmol ammonium formate buffer (pH 4.5) which was adjusted with formic acid	ESI	0.4	Ambient	0.005-0.5	1.28	[30]
Human plasma and urine	LC-MS/MS	Luna-PPF 100 Å C18 column (50 mm × 2.0 mm) 3.0 µm.	Acetonitrile and 0.01 M ammonium formate buffer of (pH 4.1) (50 × 50, v/v)	ESI/MRM	0.3	Ambient	0.001-0.1	1.50	[31]
Tablets	HPLC	X-terra RP-18 (4.6 × 250 mm) 5.0 µm.	Acetonitrile: potassium dihydrogen phosphate buffer pH 3.0 adjusted with orthophosphoric acid (60:40, v/v)	254 nm	1	Ambient	15-180	2.83	[32]
Tablet	UPLC	RP-18 column Endcapped (50 mm × 4.6 mm, 2µ)	0.1% OPA: acetonitrile 55:45 (%v/v)	249	0.2	Ambient	12.5-75	1.03	[33]
Rat Plasma	LC-MS/MS	Agilent Eclipse XDB-CN (100 × 2.1 mm, 3.5 µm)	Water: methanol (15:85, v/v) containing 0.1% formic acid.	ESI/TMS	0.5	35	0.005-0.2	0.74	[34]
Standard and tablets	LC-Q-TOF/MS/MS	Agilent Eclipse plus C18 (150 × 4.6 mm, 5 µ)	Ammonium acetate buffer (10 mmol, pH 6.7) with ACN.	258 nm	1	Ambient	10-350	7.3	[35]
Human plasma	UPLC-MS/MS	UPLC BEH C18 column 1.7 µm, 50 mm × 2.1 mm	Mobile phase A (10 mmol ammonium formate, of pH 4.5) and mobile phase B (pure acetonitrile acidified by formic acid 0.1%)	XEVO TQ-S	0.4	40	0.001-0.1	1.3	[36]
Human serum and plasma	LC-MS/MS	Waters XBridge Phenyl 3.5 µm (2.1 × 50 mm) column	Water: methanol (9:1, v/v) along with 10 mmol ammonium bicarbonate as phase A and methanol-water (9:1, v/v) containing 10 mmol ammonium bicarbonate as phase B	MRM	0.4	18	0.002-0.5	3	[37]
Human plasma	LC-MS/MS	C18 column (2.1 × 50 mm, 5 µm, Kinetex EVO, USA)	Mobile Phase A was of water (containing 0.1% ammonia) and mobile Phase B of acetonitrile: water (V: V = 95:5, containing 0.2% ammonia)	ESI	0.3	35	0.0001-0.025	1.34	[38]
PLGA Nanoparticles	UPLC	UPLC BEH C18 column (50 × 2.1 mm, 1.7µm, 130 Å)	Acetonitrile and phosphate buffer (adjusted to pH 7.55 using 10 mmol of potassium hydroxide) at 60:40 ratio.	254	0.25	Ambient	0.32-100	0.731	[39]
Human plasma	LC-MS/MS	Zorbax SB-C18 analytical column (2.1 mm × 100 mm, 3.5 µm, Agilent, USA)	0.1% formic acid in water (phase A) and 0.1% formic acid in acetonitrile (phase B)	MRM	0.3	35	0.005-0.4	1.3	[40]
Human serum	LC-MS/MS	Mastro2 C18 column size (2.2 µm, 50 mm × 2.0 mm, Shimadzu, Nakagyo-ku, Kyoto, Japan)	Water with 0.1% formic acid and acetonitrile with 0.1% formic acid	ESI-MS/MS	0.2	40	0.0025-0.125	2.9	[41]
Rat plasma	UPLC MS/MS	Aquity UPLC™ HSS T3 1.8-µm column	Solvent A is 10 mmol ammonium formate (pH 7 ± 0.2) containing 0.2% FA and 1% ACN; and solvent B, 50% ACN: MeOH containing 0.2% FA.	ESI	0.4	50	0.995	3.5	[42]
Human plasma	micellar liquid chromatography	Kromasil C18 column (AkzoNobel, Amsterdam, The Netherlands), with the following characteristics: 150 × 4.6 mm; 5 µm particle size; 10 nm pore size.	Aqueous solution of 0.07 M SDS – 6.0% v/v 1-pentanol, buffered at pH 7 with 0.01 M phosphate salt,	260	1 ml/min. 20-µl loop and a UV-Absorbance diode array detector (DAD) was used.	ambient	0.990	15.2	[43]

DISCUSSION

In 2013, FDA approved Tyrosine Kinase Inhibitor (TKI) afatinib. It is used primarily for treating metastatic non-small lung cancer. Additionally, it was approved for the treatment of squamous cell carcinoma of lung in 2016. It is included in the class of drugs known to be tyrosine kinase inhibitors [44-46]. It is typically sold in tablet dosage form [47]. The most popular and easy method for determining afatinib as a single drug and also along with combination of other drugs in both types i. e. bulk formulation and pharmaceutical formulations, is UV-visible spectrophotometric technique. Methanol, Ethanol, Acetone, Dimethylsulphoxide, and

Sodium Citrate are frequently used as solvents for spectrophotometric techniques to estimate afatinib in combination. Afatinib interaction with bovine serum albumin is an important study to estimate drug protein binding. It helps in understanding metabolism and mobility of the drug which is related to pharmacokinetic and pharmacokinetic profile of the drug.

For its estimation by HPLC in pharmaceuticals in combination with other drugs for biological matrix, few methods are described. HPLC is known to be the most versatile, useful and sensitive method for the separation and identification of analytes. However, there are several limitations like high operational cost. The time duration is

more and precision is also comparatively lower than other LC methods [48].

Bioassay methods for the quantification of afatinib involves technique as ELISA, Fluor immune assay, kinetic exclusion assay. For chromatographic methods such as RP-HPLC, IC-MS-MS, UPLC-DAD, UHPLC, spectrophotometric approaches have been reported for quantifying afatinib. In this review article, UV detector wavelengths range from 240 nm to 400 nm. Matrices used were human plasma and urine, rat plasma. Column used in the study are mentioned, Mobile phase composition for the respective methods and detection systems are provided. Along with this Flow rate, temperature, Rt for each study is also included in the table. The assays were microwell-based fluorescence immunoassay (FIA) and Kinetic Exclusion Assay (KinExA). KinExA was found to be sensitive and selective when compared. The higher sensitivity was due to high surface area of Polymethyl methacrylate bead when compared to FIA. Both the assays were found to be better when compared to the available chromatographic methods. For estimating the drug in various biological fluids as plasma, serum, and urine samples, numerous IC-MS/MS methods are available. Methods for determining afatinib by UPLC MS/MS Assay are rapid, sensitive, less time-consuming and less expensive. UPLC is widely used technique which has several advantages when compared to conventional HPLC methods. It utilizes short columns containing smaller particles, which results into short analysis time, better peak width, improved resolution, usage of less toxic chemicals and low concentration of analytes. Without losing sensitivity, injection volume also can be decreased. The limitation of UPLC involves usage of special instrument due to increased column back pressure [49].

Another method which was used like micellar liquid chromatography for the determination of afatinib in combination with other drugs. The advantages of this methods involves simplified method for the preparation of sample, easy handling by the operator, usage of less amount of hazardous chemicals, not expensive and many samples in a short time can be processed. These conditions could be achieved via micellar mobile phases solubility properties and the interaction nature of the micelles with the macromolecules and the analytical sample. Drawback with this method involves lower efficiency when compared to Reversed-Phase liquid Chromatography (RPLC) in case of hydro-organic mobile phases [50].

However, greener technologies for estimation of afatinib were not found. Usage of these technologies would avoid harmful solvents and chemicals.

CONCLUSION

In this review, a broad variety of analytical techniques for the estimation of afatinib are discussed, including UV-spectrophotometry, ELISA, fluor immunoassay, kinetic exclusion assay, and chromatography methods. To sum up, in upcoming projects, experienced formulators and analysts will work to adopt greener techniques for estimating afatinib that will use safe solvents rather than toxic ones. There are more IC-MS/MS-based techniques for determining afatinib in biological fluids, which might be more important for identifying therapeutic potential of afatinib, than there are methods based on UV-spectrophotometry and HPLC.

ACKNOWLEDGMENT

The authors are thankful to Manipal college of Pharmaceutical Sciences, Karnataka, India for providing facilities for the literature search.

AUTHORS CONTRIBUTIONS

Dr. Ruchi Verma-Drafting of Manuscript, Idea and Conceptualization, Supervision, Critical reviews

Hemanth Kumar-Collection of data, Drafting of manuscript

Dr. lalit Kumar-Idea and conceptualization, Critical reviews, supervision

CONFLICT OF INTERESTS

The authors have no conflict of interest to declare.

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