

RESEARCH ARTICLE

# A controlled, efficient and robust process for the synthesis of an epidermal growth factor receptor inhibitor: Afatinib Dimaleate

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**Abstract:** A simple, controlled, robust and scalable three-stage manufacturing process of Afatinib Dimaleate was assessed and optimized leading to improved yield and quality. The synthetic process involves sequence of reactions as nitro-reduction, amidation and salification. The developed and optimized route was demonstrated on 300 g scale with over all isolated yield of 84% for Afatinib free base. The developed process has the capability to control not only the process related impurities but also the degradation impurities. One new impurity was identified during the process development studies and characterized as acetamide impurity, chemically known as (S)-N-(4-((3-chloro-4-fluorophenyl) amino)-7-((tetrahydrofuran-3-yl) oxy) quinazolin-6-yl) acetamide. Other impurities were identified as degradation impurities, Process impurities and were labeled as 1-(4-((3-chloro-4-fluorophenyl) amino)-7-(((S)-tetrahydrofuran-3-yl) oxy) quinazoline-6-yl)-5-Hydroxypyrrolidin-2-one (hydroxy impurity), Afatinib N-Oxide impurity and N4-(3-chloro-4-fluorophenyl)-7-[[3(S)-tetrahydro-3-furanyl] oxy]-4,6-quinazolinediamine (Intermediate-1).

**Keywords:** Afatinib Dimaleate, HPLC, degradation impurities, NMR, LC-MS, new process impurity, improved process

## 1 Introduction

Over the years many quinazoline derivatives were reported as epidermal growth factor receptor (EGFR) signal transduction pathway inhibitors and Afatinib Dimaleate<sup>[1]</sup> is one of them which is powerful, irreversible tyrosine kinase inhibitor of EGFR, with IC<sub>50</sub> value (half-maximal inhibitory concentration) of 0.5nM, exhibits potent anti-tumor activity against non-small cell lung cancer (NSCLC) (<https://www.medchemexpress.com>). Afatinib [BIBW 2992; N-[4-[(3-chloro-4-fluorophenyl) amino]-7-[[3(S)-tetrahydro-3-furanyl]oxy]-6-quinazolinyl]-4-(dimethylamino)-2-butenamide] is an ATP-competitive anilinoquinazoline derivative harboring a reactive acrylamide group.<sup>[2-4]</sup> Afatinib Dimaleate is approved by the FDA as a first line

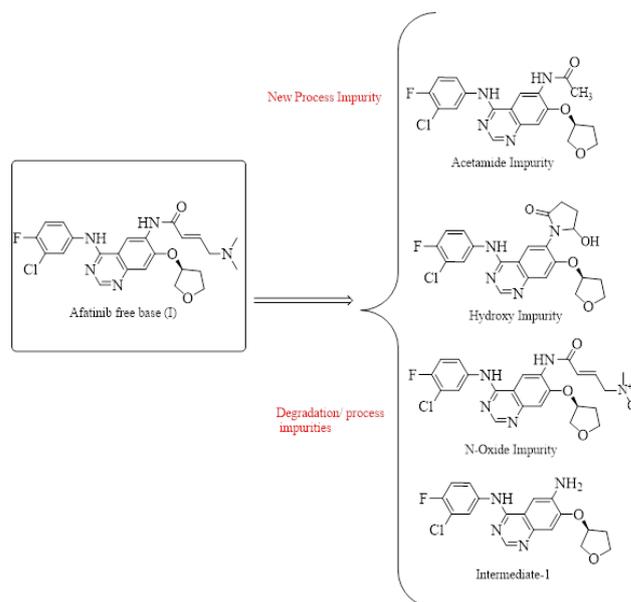


Figure 1. Graphical abstract

### Key features of the article:

1. Improved process for the synthesis of Afatinib Dimaleate.
2. Identification, synthesis and characterization of one new process impurity by NMR, LC-MS and HPLC.
3. Forced degradation studies as per ICH guidelines to have control on process impurities.
4. Developed HPLC method which is highly sensitive, specific, selective and robust for analysis.

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treatment of patients detected with metastatic non-small cell lung cancer (NSCLC) with common epidermal growth factor receptor (EGFR) mutations as detected by an FDA-approved tests (Gilotrif FDA Label). It was designed to covalently bind and irreversibly block enzymatically active ErbB receptor family members.<sup>[5]</sup>

As per prior art methods very few synthetic procedures are reported for Afatinib Dimaleate and none of process has discussed clearly about the process impurities and their control measures.<sup>[6]</sup> Impurities in active pharmaceutical ingredient (API) are highly undesirable and in some cases can prove to be harmful to the patient. The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) Q7 is a guidance for API manufacturers, mentions that impurities to be maintained below set limits.<sup>[7]</sup> Thus, it is pertinent to identify and characterize the impurities in API to develop suitable process where in their levels can be kept within permissible limits (FDA guidelines for good manufacturing practices for API). The impurity profile study should be carried out for any bulk drug to identify and characterize all the unknown impurities that are present at a level of above 0.05%. A comprehensive study has been undertaken to isolate and characterize these impurities by spectroscopic techniques. This research article describes the improved process for the synthesis of Afatinib Dimaleate, identification, isolation, synthesis and characterization of impurities that are present in the range of 0.08%-0.30% by area percent in the Afatinib Dimaleate.

During the analysis of laboratory batches of Afatinib Dimaleate by high performance liquid chromatography (HPLC) four different and major impurities were observed, one of them was identified as new process impurity, two of them as major degradant impurities and one of them as both process impurity or degradation impurity. The impurities were in the range of 0.08-0.30% along with drug substance. In regulatory terms, the level of impurities in drug substance is quite important for the drug approval and can show a significant impact on the quality and safety of drug. Thus, impurity profiling is the most concerning task in the modern pharmaceutical analysis especially when it comes to oncology drugs.<sup>[8]</sup>

None of processes reported in the prior art has explained the controlled measures for the listed impurities. Based on these views, our focus was to develop a highly effective, optimized and efficient process which should have all the control measures for these impurities, synthesize and characterize the new impurity and to control the degradation impurities in the drug substance. The structure of new impurity was presumed based on the liquid chromatograph-mass spectrometer (LC-MS)/MS

data and confirmed its synthesis followed by spectroscopic analysis such as <sup>1</sup>H NMR, <sup>13</sup>C NMR, mass and IR. In addition to this, an effective and sensitive HPLC method was developed to separate and quantify all the related substance of Afatinib Dimaleate. To our knowledge this is the first study that comprehensive analysis of the potential impurities and degradation products in Afatinib Dimaleate has been done, including their synthesis and characterization.

## 2 Experimental

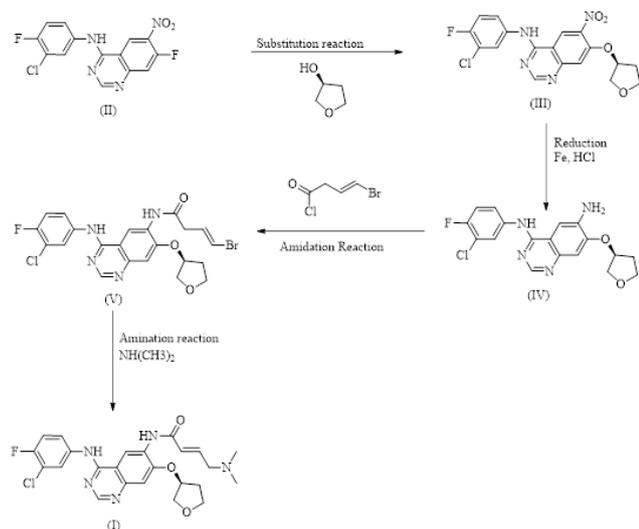
### 2.1 Chemicals and Reagents

The Afatinib Dimaleate was used from in-house sources as synthesized in Chemical Research and Development laboratory of Oncogen Pharma (Malaysia) Sdn Bhd. HPLC grade methanol, acetonitrile, OPA (85%), TEA and other chemical reagents were purchased from Merck & JT Baker. The solvent N, N-dimethylacetamide from sigma Aldrich (with appropriate specification). Milli-Q-Purified water by Milli-Q plus purification system from Millipore (Bradford, USA), was used during experimental studies. The process used deoxygenated water which was generated by purging nitrogen gas in the Milli-Q-water for 3 h. All the general chemicals were brought either from Merck Sdn Bhd., Malaysia or local chemical supplier. Solvents from Polyscientific Enterprise Sdn. Bhd. Malaysia. IR spectra were recorded with KBr pellets using Shimadzu FTIR Tracer-100 spectrophotometer, <sup>1</sup>H NMR and <sup>13</sup>C NMR were recorded in solvents CDCl<sub>3</sub>, DMSO-d<sub>6</sub> and CD<sub>3</sub>OD at 300MHz and 75MHz respectively using Bruker instrument. All the chemical shift values are reported in  $\delta$  units downfield from TMS as internal standard. Differential scanning calorimetry (DSC) were performed using T. A. instrument model no. DSCQ20. X-Ray Diffraction pattern (XRD) analysis were performed using PANalytical instrument model no. Empyrean. Melting point were recorded using BUCHI melting point apparatus with model no. M-565.

### 2.2 Prior art method for the synthesis of Afatinib Dimaleate

The prior art method<sup>[9,10]</sup> for the synthesis of Afatinib Dimaleate involves the series of reaction wherein the compound 4-[(3-chloro-4-fluorophenyl) amino]-6-nitro-7-fluoro quinazoline **5**, was used as a starting material, substitution reaction of **5** with S-(3)-hydroxy tetrahydrofuran **6**, in the presence of catalytical amount of potassium tert. butoxide resulted in 4-[(3-chloro-4-fluorophenyl) amino]-6-nitro-7-(S)-(tetrahydrofuran-3-

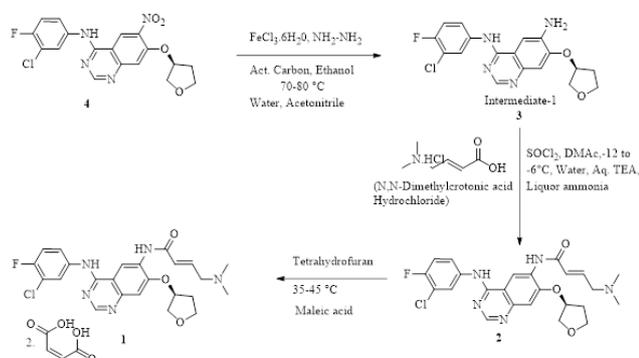
yl) oxy] quinazoline **7**, which on reduction at 6<sup>th</sup> position of nitro group to resulted in corresponding amine **8**, which reacts with bromo crotonyl chloride to get intermediate **9**. Amination reaction of the **9** with dimethylamine affords Afatinib free base **2**. The entire reaction sequence is depicted below [Figure 2](#).



**Figure 2.** Prior art scheme

### 2.3 Synthetic Process for Afatinib Dimaleate

Synthesis of Afatinib Dimaleate was followed as per the scheme shown below:



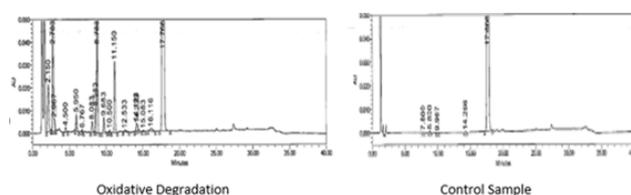
**Figure 3.** Synthetic scheme for Afatinib Dimaleate

### 2.4 Forced Degradation (FD) studies

Based the FD data it was easy to identify the possible degradants and their conditions and thus it became easy to establish their control measure in the process. The HPLC purity of control sample used for the study was 99.94% with impurities at about RRT 0.44 (0.01%), 0.50 (0.03%) and 0.81 (0.01%). All the FD study performed is summarized below as:

### 2.5 Oxidative Degradation

The Afatinib Dimaleate was treated with 5% hydrogen peroxide solution for 2 h at 70 °C. Few unknown peaks were observed at different RRT's with area percent ranging from 1.6% to 3.17%. During the laboratory development batches, one impurity at about RRT 0.49 was in concordant with impurity observed during oxidative degradation. Thus, based on the LC-MS data of that impurity it was concluded as Afatinib-N-oxide (3.17%) with m/z 524.2 (the actual molecular weight is 501.4), so it was confirmed as sodiated adduct of Afatinib-N-oxide. Thus, as per this analysis one of the impurities identified which need to be controlled in the process was Afatinib-N-Oxide. The HPLC chromatogram after before and after the oxidative degradation studies is shown in [Figure 4](#):



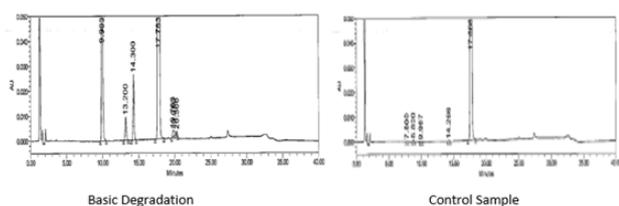
**Figure 4.** HPLC Chromatograms: Oxidative Degradation Vs Control Sample

### 2.6 Degradation under basic condition

The Afatinib Dimaleate was treated with 0.5N aqueous sodium hydroxide solution for 2 h at 70 °C. Different peaks were observed at about RRT 0.56 (11.03%), 0.74 (0.58%), 0.80 (1.51%) and 1.11 (0.20%). Among the list of impurities, two major impurities at about RRT 0.58 and 0.84 were in concordant with impurities observed regularly during laboratory development batches. Based on the LC-MS data of these RRT's, impurity at about RRT 0.56 was observed as hydroxy impurity with m/z 459.2 (the actual molecular weight is 458.1), so it was confirmed as protonated mass of hydroxy impurity and impurity at RRT 0.84 was observed as intermediate-1 with m/z of 375.1 (the actual molecular weight is 374.09), so it was confirmed as protonated mass of intermediate-1. Thus, as per this analysis two impurities identified which need to be controlled in the process was hydroxy impurity and intermediate-1. The HPLC chromatogram after before and after the basic degradation studies is shown in [Figure 5](#):

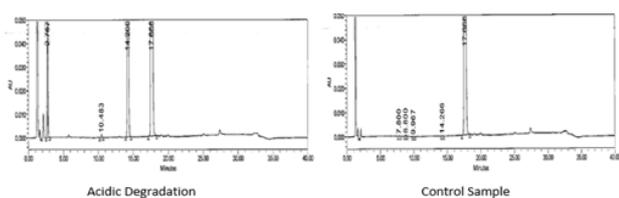
### 2.7 Degradation under acidic condition

The Afatinib Dimaleate was treated with 1.0N aqueous Hydrochloric acid solution for 4 h at 70 °C. Only



**Figure 5.** HPLC Chromatograms: Basic Degradation Vs Control Sample

one peak was observed as major degradant at about RRT 0.81 (9.20%). Since the impurity at RRT 0.81 was in concordant with the impurity observed constantly during laboratory development batches, so LC-MS was not performed. It is evident by the FD studies that intermediate-1 is the major degradant during both acidic and basic hydrolysis and is also the process impurity during the reaction. Thus, it needs to be controlled in such a way that the resulting sample should comply with ICH guidelines with actual limit not more than 0.10%. The HPLC chromatogram after before and after the acidic degradation studies is shown in Figure 6:



the precipitated solid and washed the solid with water (250 ml.). Suck dried the solid sufficiently under vacuum not less than 700 mm Hg and under nitrogen blanketing. Treated the sufficiently suck dried solid (moisture content NMT 5%) with mixed solvent system [dissolved the solid in tetrahydrofuran (1250 ml) at 25-30 °C and precipitated the solid by dropwise addition of water (3125ml) at 25-30 °C, gradually cooled the reaction mass to ambient temperature and further to 0-10 °C. Filtered the solid and washed the solid with tetrahydrofuran (50 ml.)]. Dried the solid under vacuum not less than 700 mm Hg without heating. Yield: 294.9 g (91% on theoretical basis), HPLC 99.87% with all impurities less than 0.10% by area percent. <sup>1</sup>H-NMR (DMSO-d<sub>6</sub>): δ 2.15 (s, 1H), 2.35 (m, 1H), 3.10 (d, 2H), 3.79 (td, 1H), 3.94 (q, 1H), 4.01 (d, 2H), 5.29 (d, 1H), 6.60 (d, 1H), 6.80 (dt, 1H), 6.85 (t, 1H), 7.42 (t, 1H), 7.80 (m, 1H), 8.13 (dd, 1H), 8.52 (s, 1H), 8.96 (s, 1H), 9.44 (s, 1H), 9.81 (s, 1H); <sup>13</sup>C-NMR (DMSO-d<sub>6</sub>): 32.32, 45.06, 59.69, 66.51, 71.92, 78.70, 107.92, 108.87, 116.18, 116.46, 122.34, 123.43, 125.72, 127.46, 136.80, 142.08, 148.59, 151.46, 153.10, 153.75, 154.68, 156.68; Mass (M+H): 486.0; IR (cm<sup>-1</sup>): 1620, 1674, 1577, 1427, 1215, 1249, 1149, 1531, 3317, 817 and 2862. The developed process not only resulted in high quality Afatinib free base with all the impurities less than 0.10% but also resulted in novel polymorph<sup>7,8</sup> as designated by the following 2-theta values as tabulated in Table 1:

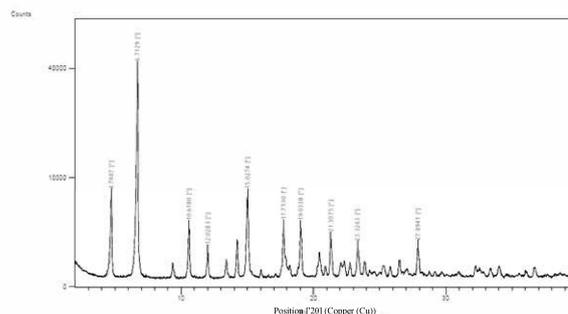
**Table 1.** 2-Theta values

| 2-theta values | d-spacing | Intensity |
|----------------|-----------|-----------|
| 4.75           | 18.61     | 16.82     |
| 6.71           | 13.17     | 100.00    |
| 10.62          | 8.33      | 7.00      |
| 12.02          | 7.35      | 2.31      |
| 15.02          | 5.89      | 16.93     |
| 17.75          | 4.99      | 7.24      |
| 19.03          | 4.66      | 7.04      |
| 21.30          | 4.17      | 4.55      |
| 23.32          | 3.81      | 2.91      |
| 27.89          | 3.19      | 3.05      |

The XRD diffractogram is shown in the Figure 7.

## 2.10 Synthesis of (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 (Afatinib Dimaleate, 1)

In a clean and dry glass assembly charged tetrahydrofuran (2000 ml) and (E)-N-[4-(3-chloro-4-fluoroanilino)-7-[(3S)-oxolan-3-yl]oxyquinazolin-6-yl]-4-(dimethylamino)but-2-enamide, 2 (250.0 g, 0.5145



**Figure 7.** XRD Diffractogram of novel Afatinib free base

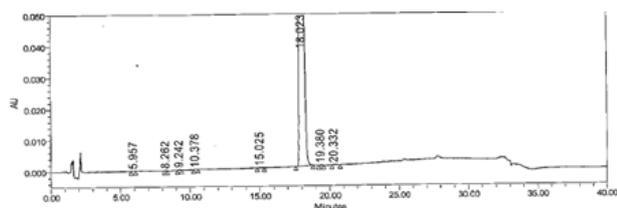
moles) to get suspension. Heated the reaction mass to 35-45 °C to get clear solution and added solution of maleic acid in tetrahydrofuran (122.45g, 1.054 moles in 750 ml tetrahydrofuran) maintaining the temperature. After precipitation of solid, gradually cooled the reaction mass to ambient temperature and further to 10-15 °C. Maintained the reaction mass at 10-15 °C for 60-90 mins, filtered the solid and washed with tetrahydrofuran (250ml). Dried the solid under vacuum not less than 700 mm Hg at 45-50 °C for 16 h. (m/c 0.45%). Yield: 328.8g (89% on theoretical basis), HPLC Purity: 99.90%. <sup>1</sup>H-NMR (DMSO-d<sub>6</sub>): δ 2.36 and 2.14 (m, m, 2H), 2.83 (s, 6H), 3.78 (m, 1H), 4.01 and 3.92 (m, m, 5H), 5.32 (m, 1H), 6.14 (s, 4H), 6.80 (m, 2H), 7.28 (s, 1H), 7.44 (t, 1H), 7.78 (m, 1H), 8.09 (m, 1H), 8.59 (s, 1H), 8.96 (s, 1H), 9.76 (s, 1H), 10.03 (bs, 1H); <sup>13</sup>C-NMR (DMSO-d<sub>6</sub>): 32.31, 42.11, 56.84, 66.51, 71.91, 78.93, 107.22, 108.57, 116.30, 116.59, 116.74, 118.58, 118.83, 122.71, 122.80, 123.93, 127.22, 131.59, 132.22, 133.35, 136.31, 136.35, 147.41, 151.80, 153.47, 153.60, 155.02, 156.99, 162.34, 166.92; Mass (M+H): 486.0; IR (cm<sup>-1</sup>): 1616, 1681, 1573, 1427, 1492, 1192, 3344, 1249, 1149, 1527, 3317, 817, 1458, 2862 and 1350.

All the impurities discussed in the article is observed during the stage-02 (amidation step). Typical HPLC chromatogram of Afatinib dimaleate with all the process impurities formed in the process is shown in Figure 8. Detailed description of impurities observed during the process development studies of Afatinib dimaleate is discussed below:

## 2.11 Process impurities and their Structure elucidation

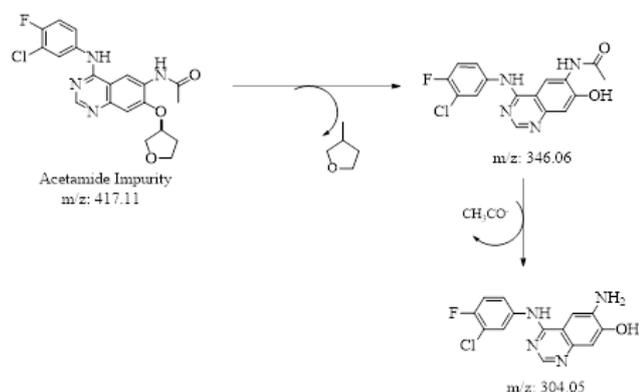
### 2.11.1 (S)-N-(4-((3-chloro-4-fluorophenyl) amino)-7-((tetrahydrofuran-3-yl) oxy) quinazolin-6-yl) acetamide (acetamide impurity)

During initial developmental batches a particular impurity at RRT 0.93 were observed constantly. The



**Figure 8.** HPLC chromatogram of Afatinib Dimaleate

knowledge about the fragmentation pattern of impurities could acquire structural information and therefore taken further studies using MS/MS. Based on the initial characterization by LC-MS, the impurity manifested protonated molecular mass of  $m/z$  417.11 (M+H) and two daughter ions as  $m/z$  346.06 and  $m/z$  304.05. The entire fragment pattern is shown below with chemical structures in [Figure 9](#):



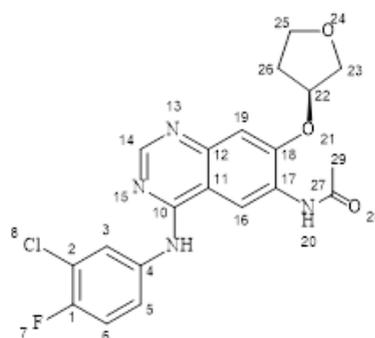
**Figure 9.** MS/MS of acetamide Impurity

The acetamide impurity was formed by reaction between acetic acid and intermediate-1. The speculation of impurity formation with acetic acid was confirmed by adding catalytic amount of acetic acid in amidation reaction which led to same impurity at about RRT 0.93. The source of acetic acid was identified as solvent N, N-dimethylacetamide which was the reaction media for amidation step. By titrimetric analysis and gas chromatography it was confirmed that catalytic amount of acetic acid was present in the solvent N, N-dimethylacetamide. Thus, the content of acetic acid was controlled in N, N-dimethylacetamide up to a level of 0.002%. However, that catalytical amount was not reflecting the actual percentage of impurity forming in the reaction mass. Even by controlling the amount of acetic acid in N, N-dimethylacetamide acetamide impurity was still observed. Later it was postulated that the N, N-dimethylacetamide is degrading into acetic acid in the reaction mass under acidic pH (source of acidity was excess of thionyl chloride) and presence of moisture (either

from raw material or intermediate-1). To prove this postulation, a reaction was performed with excess quantity of thionyl chloride and elevated content of acetamide impurity was observed in the isolated solid. Thus, by controlling the acidity of reaction mass (by using the optimal quantity of thionyl chloride) and moisture in raw material and intermediate-1, this acetamide impurity was controlled up to a level of less than 0.04%.

## 2.12 Synthesis of Acetamide Impurity

In a clean and dry glass assembly added, dichloromethane (50 ml) followed by intermediate-1 (5.0 g) and acetic anhydride (1.49g, 0.0145 moles). Heated the reaction mass to 35-40 °C for 45-60 mins and monitored the reaction mass on HPLC. Distilled the reaction mass to dryness on rotavapor and slurried the distilled residue with n-heptane (50 ml). Filtered the solid, washed with n-heptane (25 ml). Dried the material in oven at vacuum not less than 660 mm Hg till constant weight. Yield: 3.0 g (60% on w/w basis). HPLC : 98.74%. The HPLC chromatogram of the prepared acetamide impurity is shown in [Figure 10](#) and was characterized by NMR ( $^1\text{H}$  and  $^{13}\text{C}$ ), ([Figure 11](#), [Figure 12](#)), Mass ([Figure 13](#)) and IR ([Figure 14](#)) as per the structure given below:



$^1\text{H-NMR}$  (DMSO- $d_6$ ): NH (20)  $\delta$  9.77 (s, 1H), C14-H 9.33 (s, 1H), C16-H 8.81 (s, 1H), C19-H 8.52 (s, 1H), C3-H 8.13 (dd, 1H), C5-H 7.80 (m, 1H), C6-H 7.41 (t, 1H), NH (9) 7.16 (s, 1H), C22-H 5.27 (d, 1H), C23 and C25-Hb (3.94 (q, 1H), C31-H 3.10 (d, 1H), C26-Ha 2.33 (m, 1H), C29-H and C26-Hb 2.15 (s, 3H and 1H);

$^{13}\text{C-NMR}$  (DMSO- $d_6$ ): C10 (168.68), C27 (156.67), C18 (153.73), C14 (153.1), C1 (151.46), C12 (148.61), C4 (136.74), C17 (127.62), C28 (123.44), C2 (122.34), C5 (118.71), C3 (118.46), C6 (116.46), C11 (116.18), C19 (108.8), C16 (107.92), C22 (78.61), C23 (71.93), C25 (66.5), C29 (32.34); **Mass (M+H)**: 417.11; **IR** ( $\text{cm}^{-1}$ ): 1624, 1681, 1531, 1577, 1431, 1207, 1027, 1141, 3529, 3298, 817, 2881.

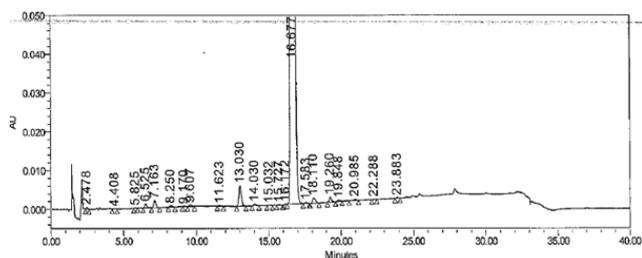


Figure 10. HPLC Chromatogram of acetamide impurity

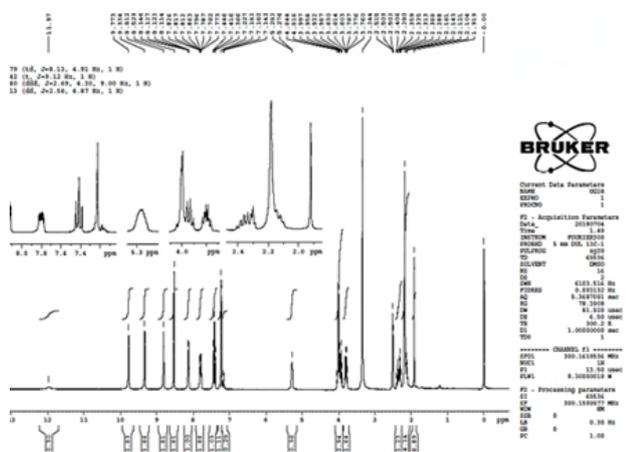


Figure 11. <sup>1</sup>H-NMR of acetamide impurity

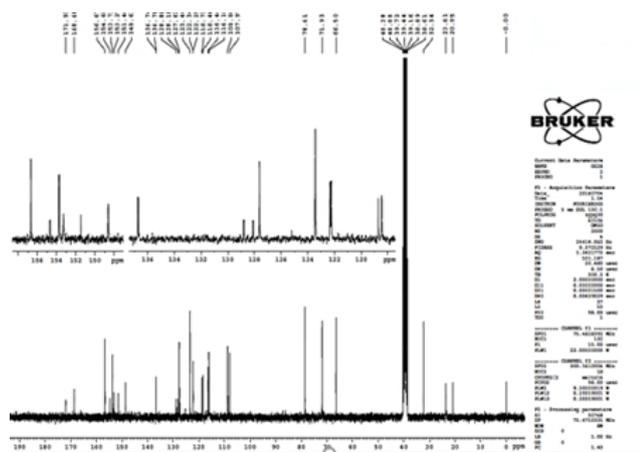


Figure 12. <sup>13</sup>C-NMR of acetamide impurity

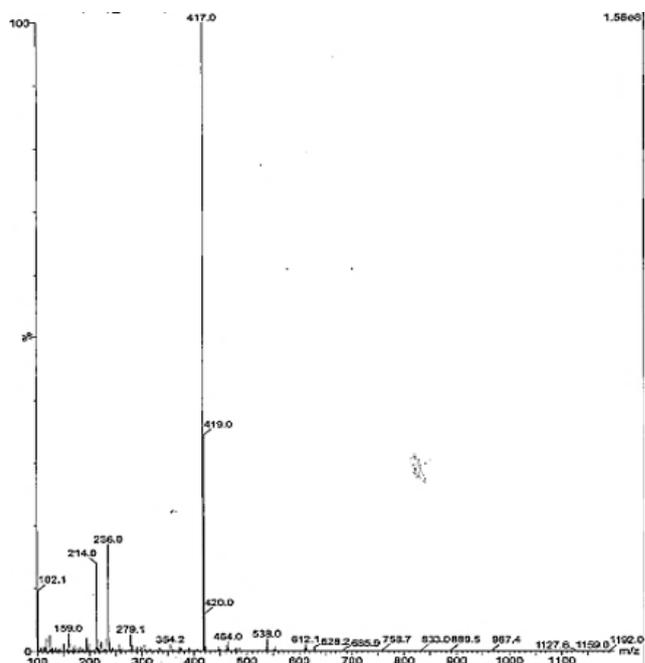


Figure 13. Mass chromatogram of acetamide

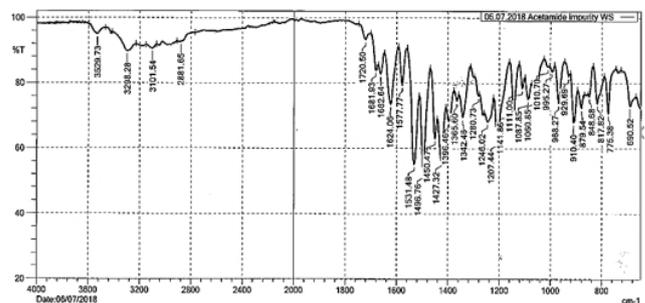


Figure 14. IR spectrum of acetamide impurity

### 2.12.1 1-(4-((3-chloro-4-fluorophenyl) amino)-7-((S)-tetrahydrofuran-3-yl) oxy) quinazoline-6-yl)-5-Hydroxypyrrolidin-2-one (hydroxy impurity)

As per the developed analytical method hydroxy impurity elutes at about RRT 0.58. The initial characterization was based on the LC-MS data. The mass spectrophotometer manifested protonated molecular mass of hydroxy impurity at  $m/z$  459.2 (M+H). The typical mass chromatogram is shown below in Figure 15:

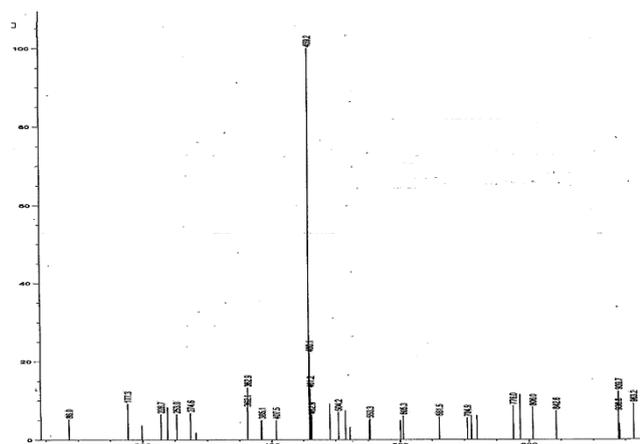


Figure 15. Mass chromatogram of Hydroxy impurity

### 2.12.2 Afatinib N-oxide

As per the developed analytical method Afatinib-N-oxide elutes at RRT 0.50. The initial characterization was based on the LC-MS data. The knowledge about the fragmentation pattern of impurities could acquire structural information and therefore taken further studies using MS/MS. The mass spectrophotometer manifested molecular mass of Afatinib-N-oxide at  $m/z$  524.2 (M+Na) The mass chromatogram is represented in the Figure 16 as below:

### 2.13 High Performance Liquid Chromatography (analytical)

A waters HPLC system equipped with alliances 2695 series low pressure quaternary gradient pump along with photo diode array detector and auto sampler has been used for the analysis of samples. The data was collected and processed using waters "Empower 2" software. An Inertsil C18 (150\* 4.6 mm, 5-Micron, GL Sciences, Japan) column was employed for the separation of impurities from Afatinib Dimaleate. The column eluent was monitored at 254 nm. A simple gradient reverse-phase HPLC method was optimized for the separation of impurities from Afatinib Dimaleate active pharmaceutical ingredient where the mobile phase was a mixture of 2

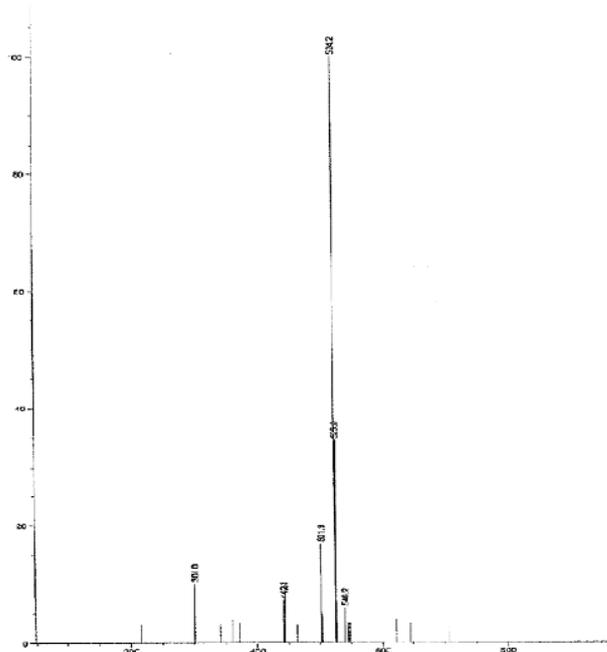


Figure 16. Mass chromatogram of Afatinib-N-oxide

mmol L<sup>-1</sup> ammonium acetate and acetonitrile (composition of mobile phase 0.02M potassium dihydrogen phosphate and 1.0 g/L 1-octanesulphonic acid sodium salt and acetonitrile). Chromatography was performed at room temperature using a flow rate of 1.0 mL min<sup>-1</sup>. The chromatographic run time was 40 min. The result was analysed weight/weight (w/w) with respect to reference standard and all the total impurities were complying the ICH guidelines. The developed method was validated as per ICH guidelines with respect to precision, accuracy, linearity, robustness, specificity and system suitability.

### 2.14 Mass Spectrometry (LC-MS/MS)

LC-MS/MS analysis has been performed on API 2000, Mass Spectrometer. The analysis was performed in positive ionization mode with turbo ion spray interface. The parameters for ion source voltage IS = 5500 V, declustering potential, DP = 70 V, focusing potential, FP = 400 V, entrance potential, EP = 10 V were set with nebulizer gas as air at a pressure of 40 psi and curtain gas as nitrogen at a pressure of 25 psi. An Inertsil C18 (150 \* 4.6 mm, 5-Micron, GL Sciences, Japan) column was used for the separation. The mobile phase is a mixture of 2 mmol L<sup>-1</sup> ammonium acetate and acetonitrile with a flow rate of 1.0 mL min<sup>-1</sup>.

### 2.15 NMR spectroscopy

The <sup>1</sup>H and <sup>13</sup>C NMR experiments were carried out at frequencies of 300 MHz and 75 MHz respectively,

in DMSO-d<sub>6</sub> at 25 °C temperature on a Varian-400 FT NMR spectrometer. <sup>1</sup>H and <sup>13</sup>C chemical shifts are reported on the δ scale in ppm, relative to tetra methyl silane (TMS) δ 0.00 and CDCl<sub>3</sub> at 77.0 ppm in <sup>13</sup>C NMR respectively.

### 3 Results and discussion

Initial studies for the synthesis of Afatinib involves lot of efforts starting from selection of reducing agents to selection of solvent for amidation, selection of chlorinating agent for N, N-dimethylcrotonic acid hydrochloride, appropriate reaction temperature for chlorination and amidation, isolation procedure for Afatinib free base as it is highly unstable and degrades easily in the presence of moisture and oxygen. The main challenge was to isolate the Afatinib free base in highly pure and stable form, its purification (if required) and stability over the period. For the synthesis of Afatinib free base **2**, the entire study was divided in three parts, part-1 was the selection of solvent and chlorinating agent for the conversion of N, N-dimethylcrotonic acid hydrochloride to acid chloride, part-2 was the selection of solvent for amidation and addition mode and part-3 was the isolation of Afatinib free base. For part-1, based on the nature of reaction, all protic solvents were ruled out, during exploratory studies solvents such as toluene, ethyl acetate, acetonitrile and thereof were ruled out, the options left were polar aprotic solvents such as N, N-dimethylformamide, N-methylpyrrolidone (NMP) and N, N-dimethylacetamide. Out of these solvents only N, N-dimethylacetamide was feasible and effective, rest solvents were either charring the reaction or resulting in incomplete conversion. To select chlorinating agents, wide variety of reagents were available such as thionyl chloride, oxalyl chloride and thereof but with oxalyl chloride complete conversion of reaction was never achieved. Thus, based on effectiveness and cost thionyl chloride was opted as chlorinating reagent. By optimal quantification all other parameters such as reaction temperature, mode and rate of addition of chlorinating agent, the complete conversion of N, N-dimethylcrotonic acid hydrochloride to acid chloride was achieved in 1.0-1.5 h. For part-2, during exploratory studies it was observed that mixture of solvent is not capable for complete conversion (from **3** to **2**) so single solvent reaction was finalized and only N, N-dimethylacetamide was used throughout the reaction. Later the addition pattern was studied whether solution of **3** in acid chloride reaction mass or vice-versa. Based on exploratory studies, addition of acid chloride solution to **3** was generating lot of impurities (since acid chloride is highly unstable) and reaction time was longer (more

than 3 h), so the idea was dropped, and the only feasible option was to add solution of **3** in acid chloride solution. By doing this, as soon as the addition of **3** was completed reaction complies with conversion rate of more than 99.5% and all the process as well degradation impurities were well within the controllable limit. For part-3, during the exploratory studies it was observed that quenching of thionyl chloride with water would be tedious during the scale up, thus it was suggested to reduce the pH of reaction mass followed by quenching with water. To follow this, aqueous triethylamine was used instead of water which would not only reduce the pH but would also quench the reaction mass. Since, **2** is in its hydrochloride form and to isolate it in pure form as base further pH adjustment is required. Lot of organic and inorganic bases were explored but based on the degradation data under basic condition, it was suggested to have that base which should not facilitate the degradation of **2**. Thus, based on the scientific logic and available data, 10-15% liquor ammonia was used and after pH adjustment, nitrogen was purged to remove the excess ammonia. This approach not only avoided the degradation of compound but also made the isolation of product in pure form with HPLC purity around 99.59% by area percent. Later, the **2** was treated with tetrahydrofuran and water to get compound with HPLC purity more than 99.80% and all listed process impurities less than 0.10%.

As per above establishment, synthesis of Afatinib dimaleate was completed in three steps starting from (S)-N-(3-Chloro-4-fluorophenyl)-6-nitro-7-((tetrahydrofuran-3-yl) oxy) quinazolin-4-amine **4**. The yield in every step is quantitative. The entire protocol is depicted in figure-1

#### 3.1 Conclusion

Afatinib Dimaleate is a potent aromatase inhibitor drug used in the treatment of cancer diseases. The present research work describes an improved process wherein all impurities (known and unknown) are controlled to a level of 0.10%. New HPLC method was developed for the detection and separation of four process related impurities from Afatinib Dimaleate. The reported process explains the formation of new process impurity which needs to be controlled to achieve the material as per regulatory guidelines. All the four impurities detected using the new HPLC method and were characterized using LC-MS and NMR data.

#### References

- [1] Walter C, Lahiri S, Bhaskar B M, et al. Polymorphic forms of Afatinib free base and Afatinib Dimaleate. 2015, WO2016/199076 A3.

- [2] Solca F, Dahl G, Zoepfel A, *et al.* Target binding properties and cellular activity of Afatinib (BIBW 2992), an irreversible ErbB family blocker, *The Journal of Pharmacology and Experimental Therapeutics*, 2012, **343**(2): 342-350. <https://doi.org/10.1124/jpet.112.197756>.
- [3] Matoram RG, Channaveerayya H, Yadav M D, *et al.* Novel salts and polymorphic forms of Afatinib. 2011, WO 2012/121764 A1.
- [4] Wang X, Goldstein D, Crowe PJ, *et al.* Next-generation EGFR/HER tyrosine kinase inhibitors for the treatment of patients with nonsmall-cell lung cancer harboring EGFR mutations: a review of the evidence. *OncoTargets and Therapy*, 2016, **9**:5461-5473.
- [5] Himmelsbach F. Quinazoline derivatives and pharmaceutical compositions containing them. US Patent RE43431 E, 2012.
- [6] Gilotrif (Afatinib)-FDA label (2003) [https://www.accessdata.fda.gov/drugsatfda\\_docs/label/2018/201292s014lbl.pdf](https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/201292s014lbl.pdf)
- [7] ICH Q7 Good Manufacturing Guidelines for API Manufacturing <https://www.fda.gov/downloads/Drugs/%E2%80%A6/Guidances/ucm073497.pdf>
- [8] Handoo S, Arora V, Khera D, *et al.* A comprehensive study on regulatory requirements for development and filling of generic drugs globally. *International Journal of Pharmaceutical Investigation*, 2012, **2**(3): 99-105. <https://dx.doi.org/10.4103/2230-973X.104392>
- [9] Soyka R, Rall W, Schnaubelt J, *et al.* Process for Preparing amino crotonyl compounds. US8426586 B2, 2006.
- [10] Wissner A, Tsou R H, Johnson D B, *et al.* Substituted quinazoline derivative. US Patent 6251912, 1998.