QBD APPROACH TO ANALYTICAL RP- HPLC METHOD DEVELOPMENT AND VALIDATION OF ERLOTINIB IN PHARMACEUTICAL DOSAGE FORM

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Abstract: *By using desired and specified requirements, quality by design (QbD) aims to attain a certain predictable quality. Understanding components and the impacts of their interactions through a chosen set of tests is a highly helpful QbD component. The current work outlines the creation of a thorough science- and risk-based HPLC technique for the analysis of Erlotinib active pharmaceutical ingredient (API) utilizing a quality by design methodology. The three essential elements of the RP-HPLC method—the column, pH, and mobile phase—are systematically explored in this article to create an effective experimental design. The approach was linear as specified. (r (2)=0.999). The results for accuracy, ruggedness, and robustness were all within the prescribed ranges (1% for system precision and 2% for other characteristics). Erlotinib may be routinely analyzed using the suggested approach in quality control labs.*

Keywords*:* *Erlotinib, RP-HPLC method, Quality by design (QbD), Analytical Method Development and Validation.*

INTRODUCTION

Quality by Design (QbD) is a concept first outlined by well- known quality expert Joseph M. Juran in various publications. While Quality by Design principles have been used to advance product and process quality in every industry and particularly the automotive industry, they have most recently been adopted by the U.S. Food and Drug Administration (FDA) as a vehicle for the transformation of how drugs are discovered, developed and commercially manufactured. Since first initiated by the U.S. Food and Drug Administration (FDA) in its "Pharmaceutical cGMPs for the twenty-first century". Quality by Design (QbD) has become an important concept for the pharmaceutical industry that is further defined in the International Conference on Harmonisation (ICH) guidance on pharmaceutical development as "a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management". The scientific understanding gained during the method development process can be used to devise method control elements and to manage the risks identified. High-performance liquid chromatography (HPLC), particularly Reversed Phase HPLC (RP-HPLC) is the most popular analytical technique in the pharmaceutical industry. The quality of HPLC methods has become increasingly important in a QbD environment. For the purpose of QbD for HPLC methods, robustness and ruggedness should be verified early in the method development stage to ensure method performance over the lifetime of the product. The aim of the analytical method is to separate and quantify the main compound while meeting the method performance criteria based on regulatory requirements, such as specificity, linearity, accuracy, precision, sensitivity, robustness, and ruggedness. [1-7]

Erlotinib (ERL), chemically known as N-(3-ethynylphenyl)-6, 7-bis (2-methoxyetho -xy) quinazolin-4-amine. Erlotinib is an epidermal growth factor receptor inhibitor (EGFR inhibitor) and used to treat nonsmall cell lung cancer (NSCLC)), the oral epidermal growth factor receptor (EGFR) tyrosine-kinase

inhibitor (TKI). Erlotinib is an established second-line treatment for advanced NSCLC.1- 2 The molecule structure is shown in Figure-1. [8,9]

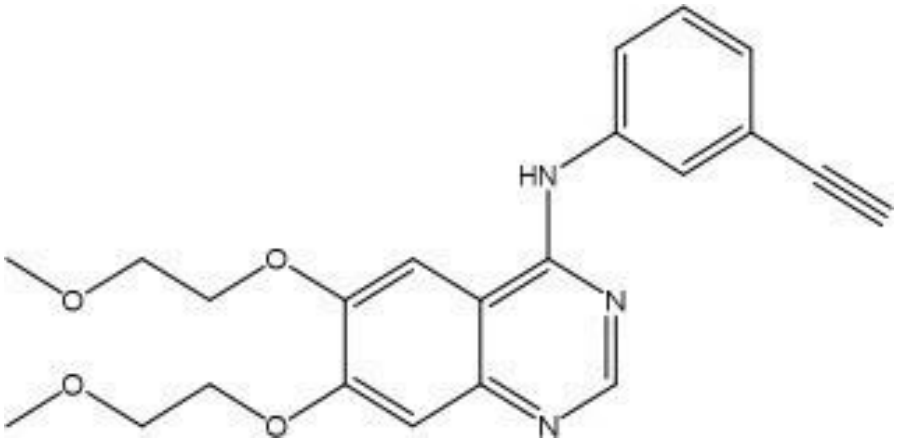

Figure 1: Erlotinib structure

MATERIALS AND METHODS [10-12]

Material

Erlotinib standard is procured as a gift sample fromAlkem Laboratories Ltd. Chemicals utilized for method development are of HPLC grade includes Acetonitrile, water were purchased from Merck (India) Ltd.

Methods

1. Organoleptic Characteristics

It is a white to pale yellow crystalline, non-hygroscopic powder.

2. Solubility-

The solubility of erlotinib was determined. 10mg drug was dissolved in water, methanol, ethanol, DMF, DCM, acetone and DMSO. Depending on the visual observation for drug particles in the solvents, the results were obtained.

3. Melting point determination

It was determined by melting point apparatus (Superfit, India) after filling the drug powder in capillary tubes (heat-sealed at one end).

4. Differential Scanning Calorimetry (DSC) of Erlotinib

Approximately 5mg of erlotinib was weighed and placed in the crucible. The crucible was sealed using small lid and crimped and analyzed at temperature range between 30- 400˚C at heating rate 10˚C per minute. Then the sample was placed in the DSC apparatus and endothermic or exothermic nature of the drug substance was analyzed.

5. Infrared spectroscopy of Erlotinib

IR spectra of the drug was obtained by scanning in the range between 400 to 400 cm-1. The interpretation was seen form literature available. The spectrum was recorded and compared with standard spectrum.

6. UV Spectroscopy: (Determination of λ maximum)

Linearity shall be established by demonstrating that the absorbance obtained is directly proportional to the concentration of the standard solution. The standard solutions are to be prepared at 6 different concentration levels ranging of working concentration and finding the response at each concentration level for assay.

Procedure

Accurately weighed 50mg of erlotinib was transferred into a clean and dry 50ml volumetric flask, a minimum required volume of 0.1M HCl was added, the volumetric flask was shaken gently to dissolve whole amount of the drug and the volume was made up to 100 ml with 0.1M HCl to obtain 100µg/ml stock solution. The aliquots 0.5 ml, 1.0 ml, 1.5 ml, 2.0, 2.5, 3.0, 3.5,4.0, 4.5, 5.0 ml were taken in 10 ml volumetric flasks and volumes were made up to the mark with 0.1M HCl. The resulting concentrations ranged from 5-50 µg/ml. The absorbance of each concentration was determined at 248nm in a UV-Visible spectrophotometer (Jasco- V630) against 0.1M HCl as blank. The standard curve was prepared between absorbance and concentration.

Instrumentation

HPLC system used was JASCO system equipped with model PU 4180 RHPLC pump, Rheodyne sample injection port (20 µl), JASCO UV-4075 UV-VIS detector and ChromNAV CFR chromatography software (version 2.0). Separation was carried out on HiQSil C18 (250 mm × 4.6 mm, 5 µm) column.

Chromatographic conditions

An Jasco gradient HPLC system with manual injector and UV was used for the purpose of separation. The separation was carried out on HiQSil C18 (250 mm × 4.6 mm, 5 µm) column.
by using mobile phase vary in the ratio for the development of the HPLC method.

Determination of Lambda maximum

Preparation of stock solution of Erlotinib

Erlotinib (50 mg) in a 25mL volumetric flask and 25 mL of 0.1M HCl to it and it was vortexed (Eltek) for 2 minutes. This was the main stock accounting for concentrations of 1000 µg/mL. A diluted solution was used to scan in UV-Spectrophotometer in the range of 200-400nm, taking 0.1M HCl as blank.
The lambda maximum for Erlotinib was found to be 248nm.

Preparation of mobile phase

The preparation of mobile phase was done by mixing Acetonitrile with HPLC grade water in the ratio of 65:35. Removal of gases was carried out in ultrasonic Acetonitrile bath for 15 minutes. Filtered the solution through 0.45µ filter.

Diluent preparation

Mobile phase used as diluents.

Preparation of standard stock solution

50mg of Erlotinib standard was transferred into 50ml volumetric flask, dissolved & make up to volume with mobile phase. Further dilution was done by transferring 1 ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase and performed the subsequent dilutions.

Preparation of test solution

50mg equivalent of Erlotinib standard was transferred into 50ml volumetric flask, dissolved & make up to volume with mobile phase. Further dilution was done by transferring 1 ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase and performed the subsequent dilutions.

Selection of analytical wavelength

It is the characteristic of a compound which helps to provide the electronic structure of the compound or analyte. The structural analysis of Erlotinib was carried out under UV ranging from 200-400nm using the standard solution.

METHODOLOGY

The optimization of chromatographic conditions was carried out on HiQSil C18 (250 mm × 4.6 mm, 5 µm) column. The separation was done by utilizing Acetonitrile: water 65:35% v/v ratio, the volume of sample was 20µl. The flow rate was maintained at 1.5ml/min. The detection of drug Erlotinib was done at 248nm.

Method Validation [13-15]

Linearity:

The linearity of the developed method was studied over the concentration ranges between 5- 30µg/ml. The aliquots of 5, 10, 15, 20, 25, 30µg/ml were prepared by diluting standard stock solution of 0.5 3 ml with mobile phase. The obtained concentrations were injected into the chromatographic system. Calibration curve of Erlotinib was constructed by plotting peak area versus used concentration of Erlotinib. To assure the concentration range studied is linear the regression equation and correlation coefficient were evaluated.

Accuracy

Accuracy was carried out by % recovery studies at three different concentration levels. To the pre-analyzed sample solution of Erlotinib, a known amount of standard drug powder of Erlotinib was added to 80, 100, 120% level.

Precision method

By studying the changes in the inter-day and intra-day determined the precision of the method. In the intra-day studies, six repeated injections of standard solution was made and % RSD were calculated. In the inter-day variation studies, six repeated injections of standard solution were made for six consecutive days and %RSD were calculated.

Limit of Detection and Limit of Quantitation

Based on the standard deviation of response of the calibration curve the LOD and LOQ of the drug was determined separately.

Robustness

Robustness of the method was tested by small but deliberate variations of flow rate, mobile phase composition and wavelength.

RESULT & DISCUSSION

1. Organoleptic Characteristics

Table 1: Active Pharmaceutical Drug

Sr. No.	Name	Description
1.	Erlotinib	white to pale yellow solid

2. Solubility

Erlotinib had a very slightly soluble characteristic by dissolving approximately 10mg/mL at a pH of 2.

Table 2: The solubility profile of erlotinib is mentioned below in the table.

Solvent	Solubility parameter
DMSO, ethanol	Soluble
Water	very slightly soluble

Methanol	slightly soluble
acetonitrile, acetone, ethyl acetate and hexane	practically insoluble

3. Melting point determination

It was determined by melting point apparatus. The melting point was found to be 228˚C, which is complying as mentioned in the monograph given in official pharmacopoeia.

4. Differential Scanning Calorimetry (DSC) of Erlotinib

Figure shows the DSC thermogram of pure drug Erlotinib. The onset was seen at 226.78˚C, peak at 230.42˚C and end set at 235.19˚C. The peak of the endotherm was similar to the melting point of the drug.

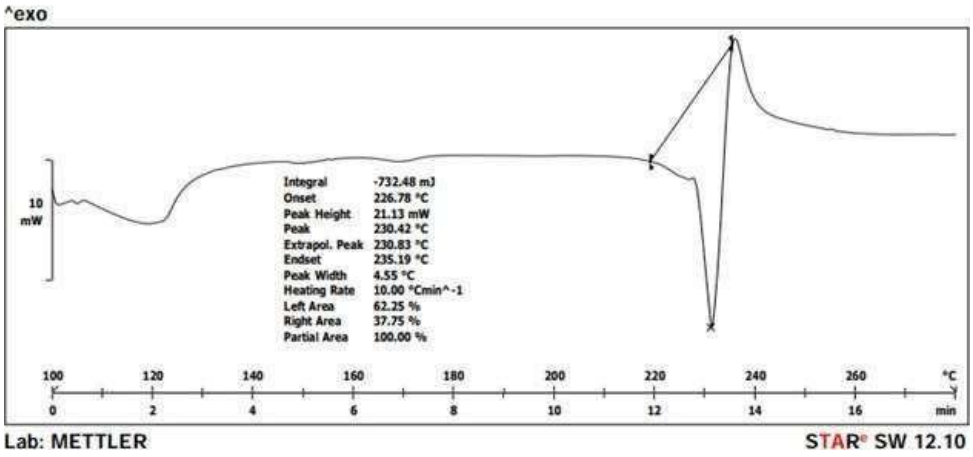

Figure 2: DSC thermogram of pure drug Erlotinib

5. Infrared spectroscopy of Erlotinib HCl

IR spectra of the drug was obtained by scanning in the range between 400 to 400 cm-1. The interpretation was seen form literature available. The spectrum was recorded and compared with standard spectrum.

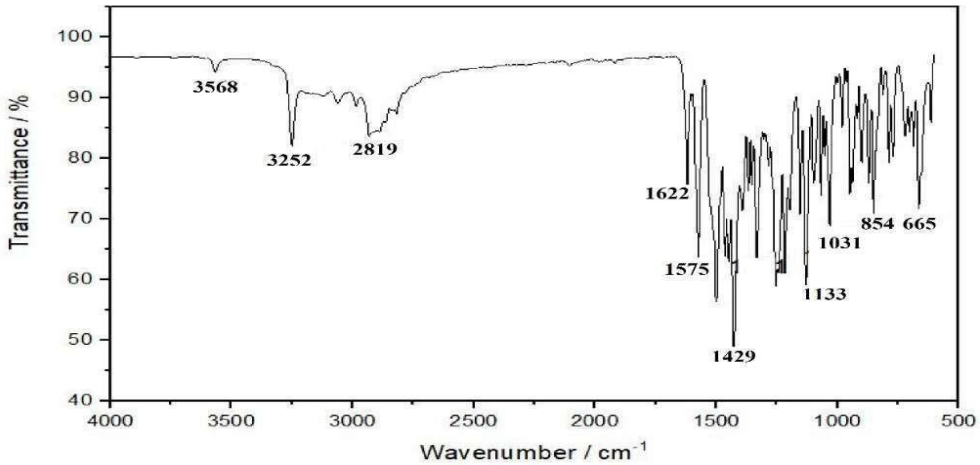

Figure 3: FTIR spectra of pure drug Erlotinib

FTIR analysis of Erlotinib showed typical bands at 3568 cm− 1 indicated the O-H bond stretch, 1622 cm− 1 indicated N-H bending of the amines, The C-O bond stretch was seen at 1133cm− 1 and the 1030 cm−1 bending was C=H bending.

Method Development

The proposed chromatographic method was found to be suitable for effective separation of Erlotinib with good resolution, peak shape given in the figure. The mobile phase composed of Acetonitrile: Water in ratio of 65:35 % v/v, at a flow rate of 1.5 ml/min was selected as it gavewell resolved peaks of standard Erlotinib. The optimum wavelength 248nm selected for detection and quantitation.

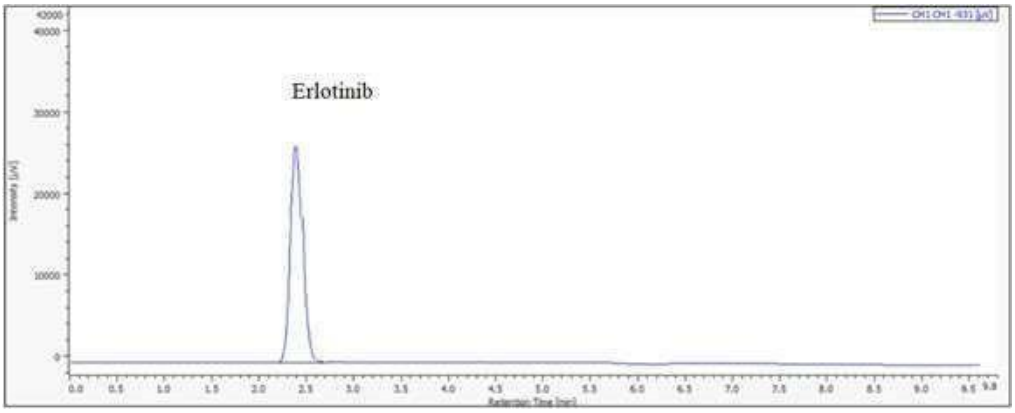

Figure 4: HPLC Chromatogram with resolved peak of Erlotinib

Method Validation

Linearity

The calibration curves were found be linear for the concentration range of 05-30ppm. The standard working curve equation for drug was found to be y = 1377.5x - 1470.8 with correlation coefficient value r^2 = 0.9978. The results of linearity are given in Table and Figure.

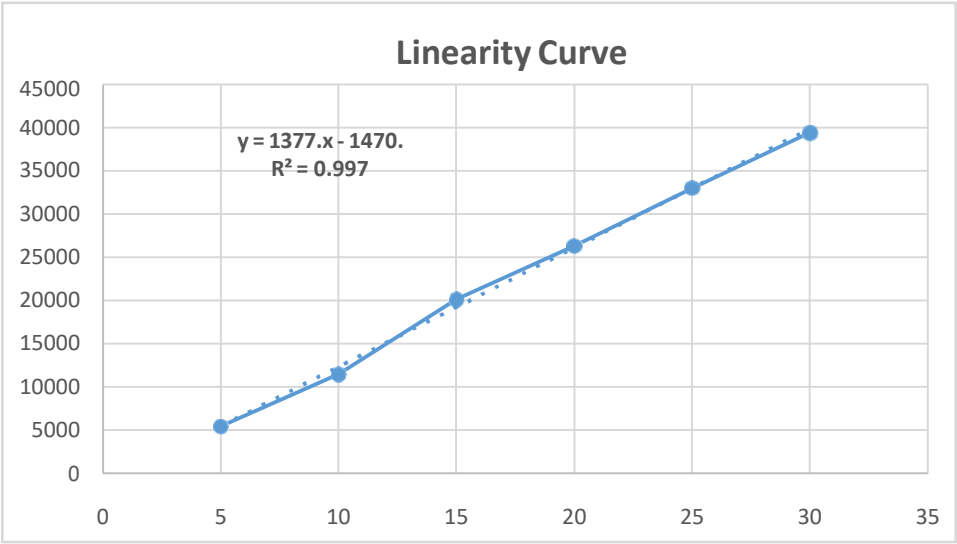

Figure 5: Linearity curve of standard Erlotinib

Recovery studies

The mean % recovery at 80, 100, 120 % of the test concentration along with its statistical validation for drug Erlotinib given in Table. The % recovery at 80, 100, and 120 % is given below. It was confirmed that the developed method was accurate as the percent recovery was in the range of 100%.

Table 3: Recovery data of Erlotinib

Level (%)	Drug Conc (mg)	Amt recovered (mg)	% Recovery
80	8	9.9	99.21
100	10	10.23	100.23
120	12	11.98	101.98

Precision

The repeatability of sample application and measurement of peak area were expressed in terms of % RSD and was found to be less than 2.0%. The results of precision studies are shown in Table.

Table 4: Precision study (intra- day) of Erlotinib

Conc µg/mL	Area	AVG	SD	%RSD
10	11723	11691	201.910871	1.72706245
	11875			
	11475			
15	21456	21464.333	19.857828	0.09251547
	21450			
	21487			
20	27354	27400.667	76.53975	0.27933536
	27489			
	27359			

Conc, Concentration; AVG, average; SD, Standard deviation; RSD, Relative standard deviation

Table 5: Precision study (inter-day) of Erlotinib

Conc µg/mL	Area	AVG	SD	%RSD
10	11459	11517.667	232.616279	2.01964761
	11320			
	11774			
15	22564	22318.667	301.558176	1.35114781
	22410			
	21982			
20	27110	27228.667	291.702474	1.07130649
	27561			
	27015			

Conc, Concentration; AVG, average; SD, Standard deviation; RSD, Relative standard deviation

Limit of Detection (LOD) and Limit of Quantification (LOQ)

This data showed that the sensitivity of method to determine the drug Erlotinib. The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 1.91 & 2.34 µg/m/ respectively.

Robustness

Robustness of method was measured by multiple injections of a homogenous sample containing Erlotinib by changing flow rate 1.3 mL/min and 1.7 mL/min, mobile phase composition Acetonitrile: Acetonitrile ratio 64:36 and 66:34, wavelength i.e. 245nm and 247nm. The method was found to be robust in the range of deliberate changes made.

Table 6: Robustness study with change in flow rate of Erlotinib

Flow rate mL/min	Conc µg/mL	Area	AVG	%RSD
1.3	20	25301	25563	1.44969
1.3		25987		
1.3		25401		
1.7	20	25692	25709.33	0.96251
1.7		25471		
1.7		25965		

Conc, Concentration; AVG, average; SD, Standard deviation; RSD, Relative standard deviation

Table 7: Robustness study with change in concentration of mobile phase of Erlotinib

Mobile phase (Acetonitrile: 01% OPA)	Conc µg/mL	Area	AVG	%RSD
64:36	20	26087	25732.67	1.22994
64:36		25633		
64:36		25478		
66:34	20	25478	25661	0.77821
66:34		25631		
66:34		25874		

Conc, Concentration; AVG, average; SD, Standard deviation; RSD, Relative standard deviation

Table 8: Robustness study with change in Wavelength of Erlotinib

Wavelength nm	Conc µg/mL	Area	AVG	%RSD
259	20	26547	26293.67	0.86812
259		26104		
259		26230		
261	20	26895	26645	0.91142
261		26630		
261		26410		

CONCLUSION

As can be seen from the validation findings, the RP-HPLC test technique for erlotinib created using the QbD methodology is linear, accurate, exact, repeatable, and specific. The created approach simply

identifies stability and may be utilised for quality control to establish the test in routine Erlotinib product development, production, and stability samples.

CONFLICT OF INTEREST

None declared by authors.

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