RESEARCH ARTICLE

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE DETERMINATION OF ERLOTINIB HYDROCHLORIDE BULK AND IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT:

A simple, precise, and accurate RP – HPLC method was developed and validated for the determination of Erlotinib Hydrochloride in bulk and tablet dosage form. Gradient elution at a flow rate of 1.0 mL/min was employed on zorbax XDB C18 (150 X 4.6 I.D., 5µm particle size) at ambient temperature. The mobile phase consisted of Acetonitrile and 0.02M ammonium acetate, adjusted to pH 3.3 with acetic acid. Acetonitrile and water (50:50) was used as diluents. The UV detection wavelength was 247nm and 20µL of sample was injected. The retention times of Erlotinib Hydrochloride was found to be 4.576 min. The linearity was obtained in the range of 50 – 150 µg/mL. The % RSD for precision and accuracy of the method was found to be less than 1%. The method was validated as per the ICH guideline. The proposed method was suitable for the analysis of Erlotinib Hydrochloride in tablet formulation for quality control purpose.

Key Words: Erlotinib Hydrochloride, RP – HPLC, UV, %RSD, Method validation

INTRODUCTION:

Erlotinib hydrochloride is an Anti neoplastic agent, which is used for lung and various other types of cancer. Erlotinib hydrochloride targets the epidermal growth factor receptors. Chemically Erlotinib Hydrochloride is N-(3-ethynylphenyl)-6,7-bis(2-methoxyethoxy)quinazolin-4 amine[1]. Erlotinib Hydrochloride acts by inhibiting the intracellular phosphorylation of tyrokinase associated with the epidermal growth factor receptor (EGFR). EGFR is expressed on the cell surface of normal cells and cancer cells. Erlotinib Hydrochloride is available as an oral agent that blocks transduction of propagation signals mediated by the EGRF. It also has the potential to cause drug-drug interaction when given in conjugation with agents that are classified as CYPA4 inducers.

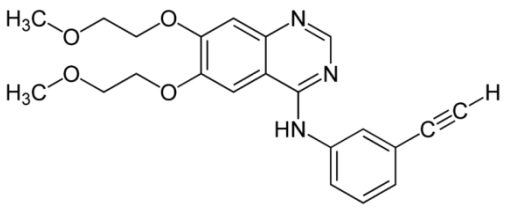

Figure 1: Structure of Erlotinib Hydrochloride

Literatura review suggest few HPLC [2-4],HPTLC[5], spectroscopic [6], Stability indicating HPLC [7] determinations were performed. After doing in depth study in the present research work, it was found that the present research work is having various advantages over the previous work. The advantages include less retention times of the component, with good resolution and with more number of theoretical plates. The % RSD of robustness was found to be less. The results obtained from the validation suggest that the method was found to be precise, accurate, linear and robust enough and the method was found to be economical. The aim of the present study is to develop a simple, precise, accurate, sensitive HPLC method for the determination of Erlotinib hydrochloride in bulk and tablet dosage form. The method was validated in compliance with ICH guidelines (International Conference on Harmonization, Geneva. 1996)[8].

EXPERIMENTAL WORK:

Chemicals and reagents

HPLC grade acetonotrile, methanol, from Rankem pvt Ltd. Ammonium acetate, acetic acid of analytical grade were used. Millipore grade water was used. The reference standard samples of Erlotinib hydrochloride was provided was provided by LAURUS LABS LTD, Hyderabad. Tarceva tablet dosage form containing 100 mg of Erlotinib Hydrochloride was purchased from the local market.

Instrumentation and analytical conditions

The analysis was carried out by using LC-2010 HCT Shimadzu, equipped with auto sampler and UV detector with Empower-2 software. Double beam UV-Visible spectrophotometer (Perkin Elmer), digital balance (metler tolado), vacuum pump (Gelman science), pH meter (poloman)

Chromatographic conditions

A Zorbax XDB C18 column (150 X 4.6 mm I.D, 5 µ) was used for separation. The mobile phase consists of Acetonitrile and 0.02M ammonium acetate, adjusted to pH 3.3 with acetic acid. Acetonitrile and water (50:50) was used as diluents. Flow rate was delivered at 1.0 mL/min with detection wavelength at 247 nm. A 20 µL was injected to the chromatographic system with ambient temperature.

Preparation of Standard stock solution:

An accurately weighed quantity of 100 mg of Erlotinib hydrochloride into 100mL volumetric flask.

Dissolve in about 30 mL of diluent and sonicate for about 5 mins until all the contents were dissolved, then the volume was made upto the mark with diluent. The concentration of Erlotinib hydrochloride was found to be 1000 µg/mL.

Preparation of sample solution:

Weigh accurately about 20 tablets and powdered. An equivalent amount of 100 mg of Erlotinib hydrochloride was taken into 100 mL volumetric flask. Add about 30 mL of diluents and sonicate for 5 mins until all the contents were dissolved. Make upto the mark with diluents. Filter the contents by using 0.45 µ membrane under vacuum. The concentration of Erlotinib hydrochloride was found to be 1000µg/mL.

Validation procedure

The objective of the method validation is to demonstrate whether the method was suited for the intended purpose. The method was validated as per the ICH guidelines. The method was validated for linearity, precision (repeatability, intermediate precision), accuracy, specificity, robustness, ruggedness, limit of detection, limit of quantification. A calibration graph was constructed by taking six different concentrations, ranging from 50 – 150 µg/mL. The peak area was calculated and calibration curve was constructed by taking peak area and concentration on both the axis. The linearity was evaluated by linear regression analysis. The precision studies were demonstrated by two parameters inter day and intraday precision. Intraday precision was performed by injecting six replicated injections to the chromatographic system on the same day and calculated the %RSD. The inter day precision was performed by injecting six replicated injections at two consecutive days. From the peak area of the chromatograms, the %RSD was calculated. The accuracy was determined by adding a known amount of the standard to the sample, and the percentage recovery was estimated. The robustness was determined by incorporating deliberate changes into the method conditions like the change in flow rate, pH, and gradient. Ruggedness was performed by carrying out the proposed method with two different analysts.

RESULTS AND DISCUSSION

Selection of wavelength

UV spectrum was obtained by preparing a solution by taking diluents and scanned between 200 to 400 nm. Erlotinib Hydrochloride shows λmax at 247 nm. So it is selected as a detection wavelength.

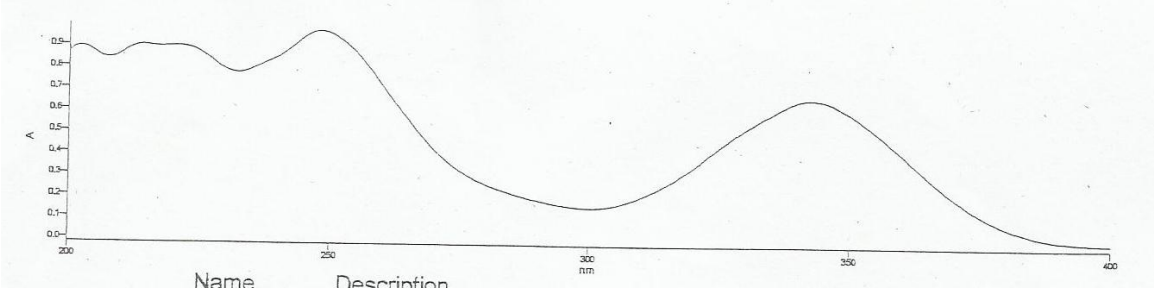

Figure 2: UV – Spectrum of Erlotinib Hydrochloride

Development and optimization of the HPLC method:

For getting an optimized chromatographic condition, a Zorbax XDB C18 column (150 X 4.6 mm I.D, 5µ) was used as stationary phase. The mobile phase consists of Acetonitrile and 0.02M ammonium acetate, adjusted to pH 3.3 with acetic acid. Acetonitrile and water (50:50) was used as diluents. Flow rate was delivered at 1.0 mL/min with detection wavelength at 247 nm. The run time was found to be 4.576 min for Erlotinib hydrochloride.

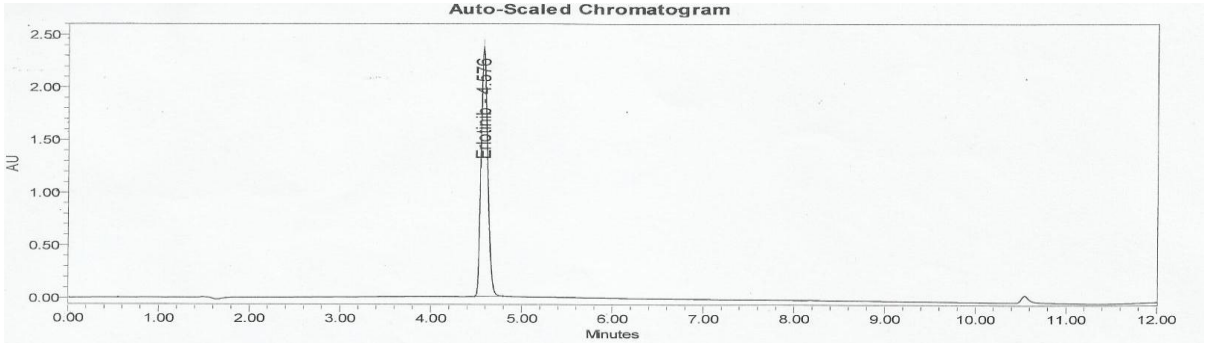

Figure 3: Optimized chromatogram of Erlotinib hydrochloride

METHOD VALIDATION[9]:

LINEARITY:

Linearity studies were performed by taking serial dilutions of 0.5, 0.75, 1.0, 1.2, 1.5 mL from the stock solution into various 10 mL flasks and made up to the volume with diluent. These concentrations were injected into the chromatographic system and record the response. Erlotinib hydrochloride shows linearity in the range of 50 – 150 µg/mL. The calibration graph was plotted by taking peak

area on the Y axis and concentration of standard solution on X axis. The degree of linearity was determined by calculating the correlation coefficient. The slope, intercept and correlation coefficient of Erlotinib hydrochloride was found to be 12503, 56891 and 0.999 respectively.

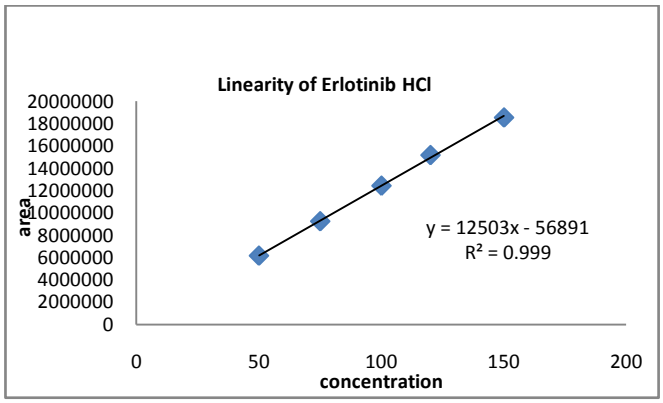

Figure 4: Calibration curve of Erlotinib Hydrochloride

Table 1: Linearity Data

S.No	Concentration(µg/mL)	Peak Area
1.	50	6173871
2.	75	9257219
3.	100	12441322
4.	120	15184469
5.	150	18550680

Precision

Six individual preparations containing 100mg/100 mL of stock solution were prepared from that, Pipette out 1.0 mL from each stock solution into a six 10 mL volumetric flask, and made upto the mark with diluent, mix well and the solutions contain 100µg/mL of Erlotinib hydrochloride.

Table 2: Precision Data

S.NO	Concentration (µg/mL)	Area of Erlotinib HCl	Stastical Analysis (n=6)
1.	100	12530617	**Mean** = 12390441 **SD** =76641.8 **%RSD** = 0.61
2.	100	12419412	
3.	100	12305124	
4.	100	12383824	
5.	100	12304657	
6.	100	12399014	

Table 3: Intraday Precision Data

S.NO	Concentration (µg/mL)	Area of Erlotinib HCl	Stastical Analysis (n=6)
1.	100	12340712	**Mean** = 12375323 **SD** =37668.006 **%RSD** = 0.30
2.	100	12418417	
3.	100	12419025	
4.	100	12329012	
5.	100	12346057	
6.	100	12398715	

Table 4: Interday Precision

S.NO	Concentration (µg/mL)	Area of Erlotinib HCl	Stastical Analysis
1.	100	12430617	**Mean** = 12353775 **SD** =41375.508 **%RSD** = 0.33
2.	100	12319412	
3.	100	12305124	
4.	100	12343824	
5.	100	12344657	
6.	100	12379014	

Accuracy:

Accuracy is usually reported as percent recovery by assay, using the proposed analytical procedure, of known amount of analyte added to the sample.

To determine the accuracy of a method, stability of a method recovery studies were performed by comparing the known quantity of standard with that of the sample. The percentage recovery can be calculated from the respective chromatograms. From the results we can determine that the method was accurate.

Table 5: Accuracy Data

S.No	%Spiking level	Amount added (mg)	Area	Amount Recovered (mg)	% Recovery	SD
1.	80	80	22511646	79.45	99.16	±0.277
2.	100	100	24995758	99.35	99.45	±0.163
3.	120	120	27512966	119.45	99.6	±0.340

Limit of Quantification:

The limit of quantification (LOQ) is defined as the lower concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under the stated operational conditions of the method.

Various determinations were performed by taking different concentrations in their decreasing order from stock solution such as 10µg/mL, 1µg/mL, 0.5 µg/mL, and 0.04µg/mL. The LOQ value of Erlotinib hydrochloride was found to be 0.04µg/mL according to the s/n value. The s/n value of LOQ was found to be 11

Limit of Detection (LOD):

The limit of detection (LOD) is defined as the lowest concentration of an analyte in a sample that can be detected, not quantified. The limit of detection can be performed by injecting the lowest amount of the analyte and observed for the accurate, precise and acceptable method development.

The limit of detection for Erlotinib hydrochloride was found to be 0.01 µg/mL, according to the s/n value. The s/n value was found to be 3.0

ROBUSTNESS

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in the analytical procedure parameters. For determining the robustness of the method which was developed, the conditions which were used in the method development were changed. The method must be robust enough so that it can remain unaffected by the deliberate change in the method conditions.

i) Effect of flow rate

Robustness of a method can be determined by changing the flow rate conditions. The initial flow rate was set at 1.0 mL per min. The flow rate was altered to 0.8 mL/min, and 1.2 mL/ min. Observed for any changes.

ii) Effect of pH:

Robustness of a method can be determined by changing the pH of the mobile phase. The optimized pH condition was 3.3. The pH was altered to 3.2 and 3.4 and observed for any changes.

RUGGEDNESS

Ruggedness as the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of normal test condition such as different labs, different analyst. Ruggedness is a measure of reproducibility of test results under normal expected operational conditions from laboratory and from analyst to analyst. The ruggedness was performed by two different analysts, and observed the analyst to analyst variation.

Table 6: Ruggedness Data

Analyst	Peak area of Erlotinib HCl
Analyst 1	12530617
Analyst 2	12490217
Mean	12510417
S.D	20200
%RSD	0.161

Assay[10]:

Inject 20 µl of standard and sample solution into the chromatographic system and measure the area of Erlotinib hydrochloride and calculate the percentage assay by using the following formula.

$$\frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{AVG.WT}{Label\ \ claim} \times 100$$

Where

AT = Peak area of test preparation

AS = peak area of standard preparation

WS = weight of working standard in mg

WT = weight of sample taken in mg

DS = dilution of standard solution

DT = dilution of test solution

P = percentage purity of working standard.

Table 8: Assay Data

Drug name	Label claim(mg)	Amount found(mg)	%Amount Found
Erlotinib HCl	100	100.76	100.76

CONCLUSION

A precise RP – HPLC method was developed for the determination of Erlotinib hydrochloride. The shorter run time elutes Erlotinib hydrochloride with good resolution, and symmetry. The method was validated as per the ICH guidelines and the method was found to be simple, precise, linear, accurate, rugged and robust enough.

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