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Analytical Method Development and Validation for The Determination of Erlotinib in API Form and Marketed Pharmaceutical Dosage Forms By RP-HPLC

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Abstract

A new, simple, rapid, precise, accurate and reproducible RP-HPLC method for estimation of Erlotinib in bulk form and marketed formulation. Separation of Erlotinib was successfully achieved on a Develosil ODS HG-5 RP C18, 5µm, 15cmx4.6mm i.d. column in an isocratic mode of separation utilizing Methanol: Phosphate buffer (0.02M, pH-3.6) in the ratio of 45:55% v/v at a flow rate of 1.0 mL/min and the detection was carried out at 255nm. The method was validated according to ICH guidelines for linearity, sensitivity, accuracy, precision, specificity, and robustness. The response was found to be linear in the drug concentration range of 12-28mcg/mL for Erlotinib. The correlation coefficient was found to be 0.9995 for Erlotinib. The LOD and LOQ for Erlotinib were found to be 5.004µg/mL and 15.164µg/mL respectively. The proposed method was found to be good percentage recovery for Erlotinib, which indicates that the proposed method is highly accurate. The specificity of the method shows good correlation between retention times of standard solution with the sample solution. Therefore, the proposed method specifically determines the analyte in the sample without interference from excipients of pharmaceutical dosage forms.

Keywords

Erlotinib, RP-HPLC, Accuracy, Precision, Robustness, ICH Guidelines.

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INTRODUCTION

Erlotinib is a tyrosine kinase receptor inhibitor that is used in the therapy of advanced or metastatic pancreatic or non-small cell lung cancer. Erlotinib therapy is associated with transient elevations in serum aminotransferase levels during therapy and rare instances of clinically apparent acute liver injury[1]. Erlotinib is a small molecule tyrosine kinase inhibitor that targets EGFR at an intracellular site and inhibits EGFR-mediated intracellular signaling by blocking the autophosphorylation of EGFR21. Thus, reducing the pEGFR levels plays an important role in Erlotinib action. The mechanism of clinical antitumor action of Erlotinib is not fully characterized. Erlotinib inhibits the intracellular phosphorylation of tyrosine kinase associated with the epidermal growth factor receptor (EGFR). Specificity of inhibition regarding other tyrosine kinase receptors has not been fully characterized. EGFR is expressed on the cell surface of normal cells and cancer cells[2]. Erlotinib is used to

treat certain types of non-small cell lung cancer that has spread to nearby tissues or to other parts of the body in patients who have already been treated with at least one other chemotherapy medication and have not gotten better[3]. The IUPAC name of N-(3-ethynyl phenyl)-6, 7-bis (2-methoxy ethoxy) quinazolin-4-amine. The Chemical Structure of Erlotinib is shown as follows.

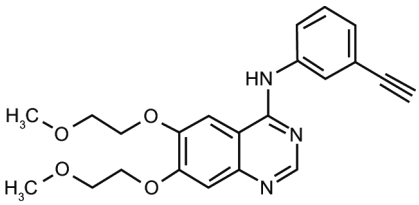

Fig-1: Chemical Structure of Erlotinib

MATERIALS AND METHODS

Materials, Reagents and Pharmaceutical Products

Erlotinib and analytical reagent grade Potassium Dihydrogen Phosphate were purchased from Sigma–Aldrich (Mumbai). Erlocip Tablet ® (Erlotinib 100 mg tablets) (Cipla Ltd) were obtained from a local pharmacy. Analytical reagent grade Ethanol, DMSO and DMF, Orthophosphoric Acid were obtained from Sd. fine-Chem ltd, Mumbai. HPLC grade acetonitrile, methanol and water were procured from Lichrosolv (Merck).

HPLC Method Development:

Preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Erlotinib working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.1ml of the above Erlotinib stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Preparation of Sample Solution:

Twenty capsules were taken, and the average weight was calculated as per the method prescribed in I.P. The weighed tablets were finally powdered and triturated well. A quantity of powder of Erlotinib equivalent to 10mg was transferred to a clean and dry 10 ml volumetric flask and 7 ml of HPLC grade methanol was added and the resulting solution was sonicated for 15 minutes. Make up the volume up to 10 ml with same solvent. Then 1 ml of the above solution was diluted to 10 ml with HPLC grade methanol. One ml (0.1 ml) of the prepared stock solution diluted to 10 ml and was filtered through membrane filter (0.45µm) and finally sonicated to degas.

Procedure:

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines[25,30].

Mobile Phase Optimization:

Initially the mobile phase tried was Methanol and Methanol: Water with varying proportions. Finally, the mobile phase was optimized to Methanol and Phosphate buffer (0.02M, pH-3.6) in proportion 45:55% v/v.

Optimization of Column:

The method was performed[4] with various C18 columns like, X- bridge column, Xterra, and C18 column. Develosil ODS HG-5 RP C18, 5µm, 15cmx4.6mm i.d. was found to be ideal as it gave good peak shape and resolution at 1.0ml/min flow.

Preparation of Potassium Dihydrogen Phosphate (KH2PO4) Buffer (0.02M) (pH-3.6):

Dissolve 2.72172g of potassium dihydrogen phosphate in 1000 ml HPLC water and adjust the pH 3.6 with diluted orthophosphoric acid. Filter and sonicate the solution by vacuum filtration and ultra-sonication.

Preparation of Mobile Phase:

Accurately measured 450 ml (45%) of Methanol and 550 ml of Phosphate buffer (55%) were mixed and degassed in digital ultra sonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

The Mobile phase was used as the diluent.

Validation Parameters

System Suitability

Accurately weigh and transfer 10 mg of Erlotinib working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.1ml of the above Erlotinib stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure:

The standard solution was injected five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits[6].

Specificity:

Preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Erlotinib working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.1ml of the above Erlotinib stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Preparation of Sample Solution:
Weight 10 mg equivalent weight of Erlotinib sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.
Further pipette 0.1ml of Erlotinib above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.
Procedure:
Inject the three replicate injections of standard and sample solutions[7] and calculate the assay by using formula:

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet.}}{\text{Label claim}} \times 100$$

Linearity and Range:
Accurately weigh and transfer 10 mg of Erlotinib working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Preparation of Level – I (12ppm of Erlotinib):
Take 0.12ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluents and sonicate the solution for bubble entrapment using ultrasonicator.
Preparation of Level – II (16ppm of Erlotinib):
Take 0.16ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluents and sonicate the solution for bubble entrapment using ultrasonicator.
Preparation of Level – III (20ppm of Erlotinib):
Take 0.2ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluents and sonicate the solution for bubble entrapment using ultrasonicator.
Preparation of Level – IV (24ppm of Erlotinib):
Take 0.24ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluents and sonicate the solution for bubble entrapment using ultrasonicator.
Preparation of Level – V (28ppm of Erlotinib):
Take 0.28ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluents and sonicate the solution for bubble entrapment using ultrasonicator.
Procedure:
Inject each level into the chromatographic system and measure the peak area.

Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient[8].

Precision

Repeatability

Preparation of Erlotinib Product Solution for Precision:

Accurately weigh and transfer 10 mg of Erlotinib working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.1ml of the above Erlotinib stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure:

The standard solution was injected six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Intermediate Precision:

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.

Procedure:

Analyst 1:

The standard solution was injected six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Analyst 2:

The standard solution was injected six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Accuracy:

For preparation of 80% Standard stock solution:

Accurately weigh and transfer 10 mg of Erlotinib working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.08ml of the above Erlotinib stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

For preparation of 100% Standard stock solution:

Accurately weigh and transfer 10 mg of Erlotinib working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.1ml of the above Erlotinib stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

For preparation of 120% Standard stock solution:

Accurately weigh and transfer 10 mg of Erlotinib working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.12ml of the above Erlotinib stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure:

Inject the Three replicate injections of individual concentrations (80%, 100%, 120%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found, and Amount added for Erlotinib and calculate the individual recovery and mean recovery values[9].

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

Preparation of 5.004µg/ml Solution (For LOD):

Accurately weigh and transfer 10 mg of Erlotinib working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.05004ml of the above Erlotinib stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Preparation of 15.164µg/ml Solution (For LOQ):

Accurately weigh and transfer 10 mg of Erlotinib working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.15164ml of the above Erlotinib stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Robustness:

The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results.

For preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Erlotinib working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.1ml of the above Erlotinib stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Effect of Variation of Flow Conditions:

The sample was analyzed at 0.9 ml/min and 1.1 ml/min instead of 1ml/min, remaining conditions are same. 20µl of the above sample was injected and chromatograms were recorded.

Effect of Variation of Mobile Phase Organic Composition:

The sample was analyzed by variation of mobile phase i.e. Methanol: Phosphate Buffer was taken in the ratio and 50:50, 40:60 instead (45:55), remaining conditions are same. 20µl of the above sample was injected and chromatograms were recorded.

RESULTS AND DISCUSSION

Method Development:

Selection of Wavelength:

The detection wavelength was selected by dissolving the drug in mobile phase to get a concentration of 10µg/ml for individual and mixed standards. The resulting solution was scanned in U.V range from 200-400nm. The UV spectrum[10] of Erlotinib was obtained and the Erlotinib showed absorbance's maxima at 255nm. The UV spectra of drug are follows:

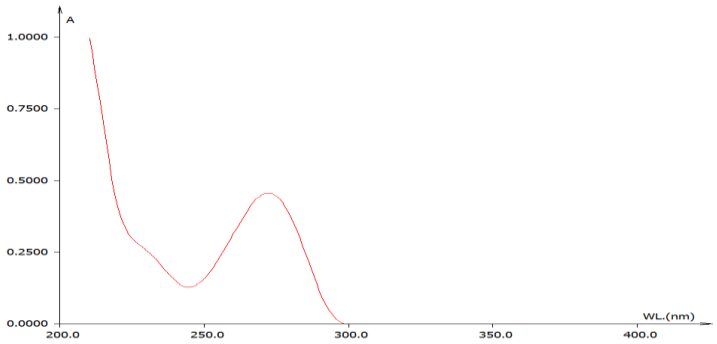

Fig-2: UV Spectrum of Erlotinib (255nm)

Observation: While scanning the Erlotinib solution we observed the maxima at 255nm. The UV spectrum has been recorded on T60-LAB INDIA make UV-Vis spectrophotometer model UV-2450.

Optimization of Method:

Optimized Chromatographic Conditions:

Table-3: Optimized Chromatographic Conditions:

Mobile phase	Methanol: Phosphate buffer (0.02M, pH-3.6) = 45:55 v/v
Column	Develosil ODS HG-5 RP C_{18}, 5µm, 15cmx4.6mm i.d.
Column Temperature	Ambient
Detection Wavelength	255 nm
Flow rate	1.0 ml/ min.
Run time	07 min.
Temperature of Auto sampler	Ambient
Diluent	Mobile Phase
Injection Volume	20µl
Type of Elution	Isocratic

Standard Solution:

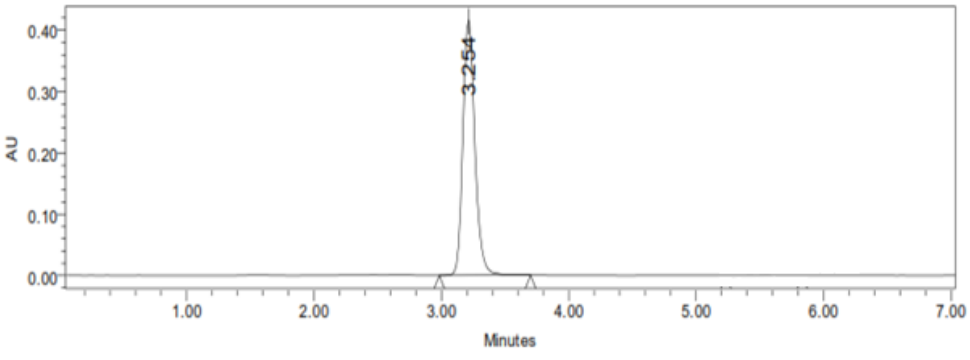

Fig-3: Chromatogram of Erlotinib in Optimized Chromatographic Condition

Method Validation

System Suitability: System suitability testing[11-14] is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations, and samples to be analyzed constitute an integral system that can be evaluated as such. The following system suitability test parameters were established. The data are shown in Table-4 & 5.

Table-4: Data of System Suitability Test

S.No.	Injection No.	RT	Area	USP Plate Count	USP Tailing
1	Injection 1	3.253	284568	7368	1.26
2	Injection 2	3.254	285684	7295	1.25
3	Injection 3	3.215	283659	7346	1.27
4	Injection 4	3.297	284754	7394	1.29
5	Injection 5	3.253	283695	7425	1.25
6	Injection 6	3.213	284578	7385	1.27
Mean			**284489.7**	**7368.833**	**1.265**
S. D			**752.5617**		
%RSD			**0.26453**		

Table-5: System Suitability Results for Erlotinib (Flow rate)

S.No.	Parameter	Limit	Result
1	Asymmetry	T ≤ 2	Erlotinib = 0.12
2	Theoretical plate	N > 2000	Erlotinib = 7258
3	Tailing Factor	(Tf) < 2	Erlotinib = 1.25

Specificity:

Specificity can be determined by comparing the chromatograms obtained from the drugs with the chromatogram obtained from the blank solution. Blank solution was prepared by mixing the excipients in the mobile phase without drug. Drug solutions were prepared individually and the sample containing three drugs was also prepared. Now these mixtures were filtered by passing through 0.45 µ membrane filter before the analysis. In this observation no excipient peaks were obtained near the drug in the study run time. This indicates that the proposed method[15] was specific.

The chromatograms representing the peaks of blank, Erlotinib and the sample containing the three drugs were shown in following figures respectively.

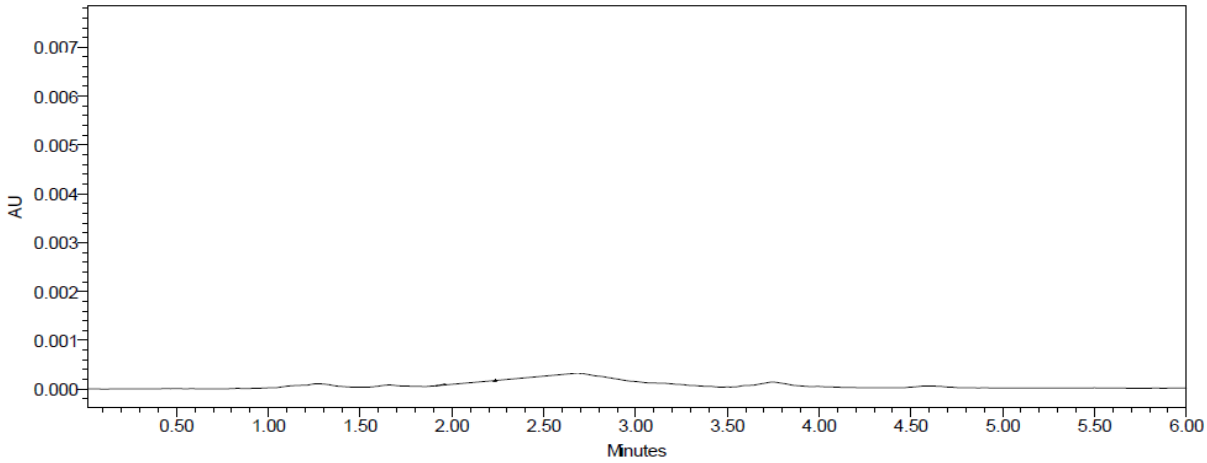

Fig-4: Chromatogram of Blank Solution

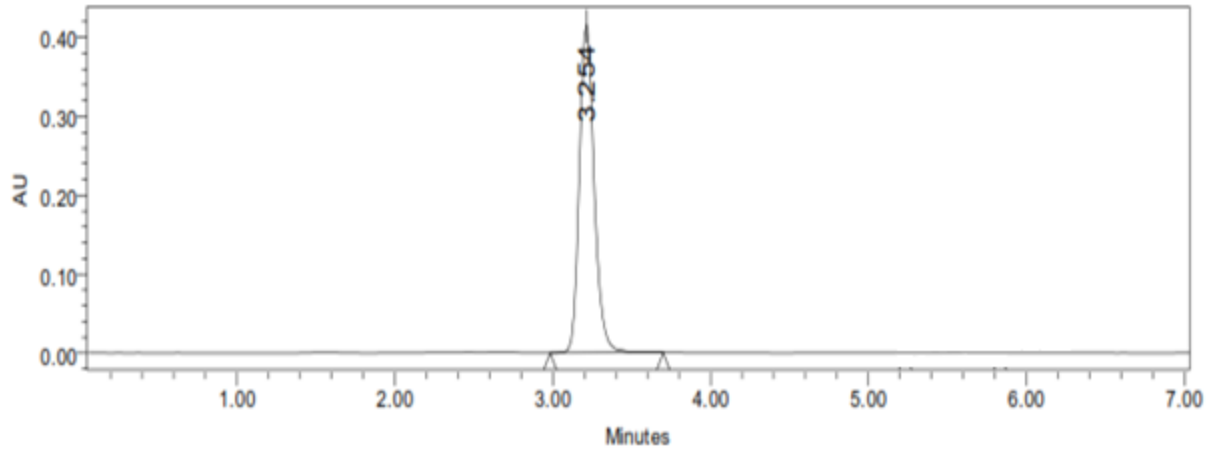

Fig-5: Chromatogram of Erlotinib Standard Solution

Observation:

In this test method blank, standard solutions were analyzed individually to examine the interference[16]. The above chromatograms show that the active ingredient was well separated from blank and their excipients and there was no interference of blank with the principal peak. Hence the method is specific.

Linearity:

To evaluate the linearity, serial dilution of analyte was prepared from the stock solution was diluted with mobile phase to get a series of concentration ranging from 0-28µg/ml for Erlotinib. The prepared solutions were filtered through Whatman filter paper (No.41). From these solutions, 20µl injections of each concentration were injected into the HPLC system

and chromatographed under the optimized conditions. Calibration curve[17] was constructed by plotting the mean peak area (Y-axis) against the concentration (X-axis).

Plotting of Calibration Graphs: The resultant areas of linearity peaks are plotted against Concentration.

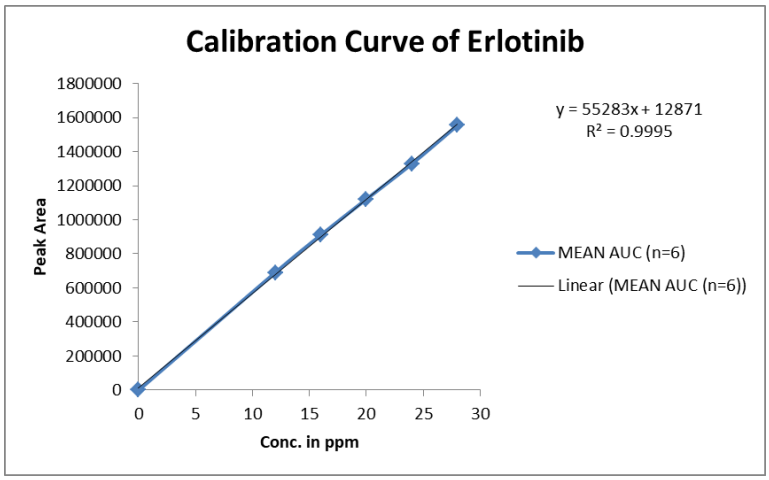

Fig-6: Standard Curve for Erlotinib

Observation: Linearity range[17] was found to be 0-28µg/ml for Erlotinib. The correlation coefficient was found to be 0.9995, the slope was found to be 55283 and intercept was found to be 12871 for Erlotinib.

Table-6: Linearity Readings for Erlotinib

CONC.(µg/ml)	MEAN AUC (n=6)
0	0
12	690316
16	910621
20	1121057
24	1328903
28	1554666

Linearity Plot:

The plot of Concentration (x) versus the Average Peak Area (y) data of Erlotinib is a straight line.

Y = mx + c

Slope (m) = 55283

Intercept (c) = 12871

Correlation Coefficient (r) = 0.9995

Acceptance/Validation Criteria: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

Conclusion: Correlation Coefficient (r) is 0.99, and the intercept is 12871. These values meet the validation criteria[18].

Accuracy:

Inject the three replicate injections of individual concentrations (80%, 100%, 120%) were made under the optimized conditions[19]. Recorded the chromatograms and measured the peak responses. Calculate the Amount found, and Amount added for Erlotinib and calculate the individual recovery and mean recovery values.

Accuracy at different concentrations (80%, 100%, and 120%) was prepared and the % recovery was calculated.

Table-7: Accuracy results of Erlotinib

Sample ID	Concentration (µg/ml)			%Recovery of Pure drug	Statistical Analysis
	Conc. Found	Conc. Recovered	Peak Area		
S_1 : 80 %	8	8.064107	458679	99.867	Mean= 100.4113%
S_2 : 80 %	8	7.843532	446485	100.637	S.D. = 0.473694346
S_3 : 80 %	8	8.19449	465887	100.73	% R.S.D.= 0.471753
S_4 : 100 %	10	9.892661	559767	99.41	Mean= 100.6646667%
S_5 : 100 %	10	9.978655	564521	100.868	S.D. = 1.166369295 R.S.D.=
S_6 : 100 %	10	10.19623	576549	101.716	1.158667
S_7 : 120 %	12	11.85907	668476	99.878	Mean= 100.4637%
S_8 : 120 %	12	12.16785	685546	100.69	S.D. = 0.51154309
S_9 : 120 %	12	12.18644	686574	100.823	% R.S.D. = 0.509181

Observation:
The mean recoveries were found to be 100.411, 100.664 and 100.463% for Erlotinib. The limit for mean % recovery is 98-102% and as both the values are within the limit, it can be said that the proposed method was accurate[20].

Precision:
The precision[21] of each method was ascertained separately from the peak areas obtained by actual determination of six replicates of a fixed amount of drug Erlotinib. The percent relative standard deviations[22] were calculated for Erlotinib are presented in Table-8.

i) Repeatability
Obtained Six (6) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD.

Table-8: Repeatability Results of Erlotinib

HPLC Injection Replicates	AUC for Erlotinib
Replicate – 1	285479
Replicate – 2	284571
Replicate – 3	286954
Replicate – 4	283261
Replicate – 5	285964
Replicate – 6	284259
Average	**285081.3**
Standard Deviation	**1318.666**
% RSD	**0.462558**

Observation: The repeatability study[23] which was conducted on the solution having the concentration of about 20µg/ml for Erlotinib (n=6) showed a RSD of 0.462558% for Erlotinib. It was concluded that the analytical technique showed good repeatability.

ii) Intermediate Precision / Ruggedness
To evaluate the intermediate precision[24] (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.

Procedure:
Analyst 1: The standard solution was injected six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Analyst 2:
The standard solution was injected six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits[26].

Intra Day (Day-1)/Analyst-1:

Table-9: Results of Ruggedness for Erlotinib (Analyst-1)

S.No.	Peak Name	RT	Peak Area	Theoretical Plates	Tailing Factor
1	Erlotinib	3.253	284568	7368	1.26
2	Erlotinib	3.254	285684	7295	1.25
3	Erlotinib	3.215	283659	7346	1.27
4	Erlotinib	3.204	286598	7457	1.22
5	Erlotinib	3.202	287965	7635	1.29
6	Erlotinib	3.297	285698	7459	1.28
Mean			**285695.3**		
Std. Dev.			**1508.898**		
% RSD			**0.528149**		

Inter Day (Day -2/Analyst-2)

Table-10: Results of Ruggedness for Erlotinib (Analyst-2)

S.No.	Peak Name	RT	Peak Area	Theoretical Plates	Tailing Factor
1	Erlotinib	3.297	294754	7394	1.29
2	Erlotinib	3.253	293695	7425	1.25
3	Erlotinib	3.213	294578	7385	1.27
4	Erlotinib	3.297	296534	7584	1.23
5	Erlotinib	3.210	296571	7745	1.24
6	Erlotinib	3.254	298698	7658	1.25
Mean			**295805**		
Std. Dev.			**1819.334**		
% RSD			**0.615045**		

Observation:
Intraday and interday studies[27] show that the mean RSD (%) was found to be within acceptance limit (≤2%), so it was concluded that there was no significant difference for the assay, which was tested within day and between days. Hence, the method at selected wavelength was found to be precise.

Robustness:
Robustness[28] is defined as the capacity of that method to be unaffected by even small deliberate changes that occur in the method parameters. The evaluation of robustness of a method is done by varying the chromatographic parameters such as pH, temperature, flow rate, mobile phase proportions change, ionic strength etc., and determining any possible effect on the results obtained by that method.

Table-11: Result of Method Robustness Test for Erlotinib

Parameter used for Sample Analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	283261	3.254	7258	1.25
Less Flow rate of 0.9 mL/min	315864	3.297	7569	1.29
More Flow rate of 1.1 mL/min	298542	3.212	7841	1.41
Less organic phase	279856	3.253	7965	1.27
More organic phase	306985	3.215	7458	1.28

Observation: Influence of small changes in chromatographic conditions such as change in flow rate (± 0.1ml/min), Temperature (±2^{0}C), Wavelength of detection (±2nm) & organic phase (±5%) studied to determine the robustness of the method are also in favour of (Table-11, % RSD < 2%) the developed RP-HPLC method for the analysis of Erlotinib (API).

LOD: The limit of detection (LOD) is the lowest concentration of analyte in a sample which can be detected, but not quantitated. LOD is a limit test that specifies whether an analyte is above or below a certain value. A signal-to-noise ratio of three-to-one is used to determine LOD. The results were shown in table-12.

L.O.D. = 3.3 (SD/S).

Where, SD = Standard deviation of the response

S = Slope of the calibration curve

Table-12: Results of LOD

	LOD
SD of Intercept	19518.16286
Slope	55283

Observation: The LOD was found to be 1.165µg/ml for Erlotinib.

LOQ: The Limit of Quantitation (LOQ) is defined as the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under the stated operational conditions of the method. Signal-to-noise ratio of ten-to-one is used to determine LOQ. The results were shown in table-13.

L.O.Q. = 10 (SD/S)

Where, SD = Standard deviation of the response

S = Slope of the calibration curve

Table-13: Results of LOQ

	LOQ
SD of Intercept	19518.16286
Slope	55283

Observation: The LOQ[29] was found to be 3.53µg/ml for Erlotinib.

Assay of Pharmaceutical Dosage form

Twenty tablets/Capsules were taken and the I.P. method was followed to determine the average weight. Finally, the weighed tablets are powdered and triturated well by using mortar and pestle. A quantity of powder which is equivalent to the 100mg of drugs were transferred to a clean and dry 100ml of volumetric flask and add 70 ml of mobile phase and the resulted solution was sonicated for 15 minutes by using ultra sonicator, Then the final volume was make up to the mark with the mobile phase. The final solution was filtered through a selected membrane filter (0.45 µm) and to sonicate to degas the mobile phase (Solvent system). From this above stock arrangement (1 ml) was exchanged to five distinctive 10 ml volumetric flagons and volume was made up to 10 ml with same dissolvable framework (Mobile stage).

The readied arrangements were infused in five repeats into the HPLC framework, and the perceptions were recorded.

A duplicate injection (Blank Solution) of the standard arrangement likewise infused into the HPLC framework and the chromatograms and peak zones were recorded and figured.

ASSAY:

Assay % =

$$\frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \text{Avg. Wt} = \text{mg/tab}$$

Where:

AT = Peak Area of drug obtained with test preparation

AS = Peak Area of drug obtained with standard preparation

WS = Weight of working standard taken in mg

WT = Weight of sample taken in mg

DS = Dilution of Standard solution

DT = Dilution of sample solution

P = Percentage purity of working standard

The assay was performed as explained in the previous chapter. The results which are obtained are following:

Table-14: Recovery Data for Estimation Erlotinib in Erlocip 100 Tablet

Brand Name of Erlotinib	Labelled amount of Drug (mg)	Amount (mg) found by the proposed method (n=3)	Assay %
Erlocip 100 Tablet (100mg) (Cipla)	100mg	99.695mg	99.598%

RESULT & DISCUSSION:

The amount of drug in Erlocip 100 Tablet was found to be 99.695 (± 0.789) mg/tab for Erlotinib & % Purity was 99.598 (± 0.695) %.

Forced Degradation Studies

Following protocol was strictly adhered to for forced degradation of Erlotinib Active Pharmaceutical Ingredient (API). The API (Erlotinib) was subjected to keep in some stress conditions[30] in various ways to observe the rate and extent of degradation that is likely to occur during storage and/or after administration to body. It is one type of accelerated stability studies of the drugs that is used to help us to determining the total fate of the drug that is likely to happen after long time storage, within a very short time as compared to the real time or long-term stability testing. The different types of forced degradation pathways/studies are studied here are acid hydrolysis, basic hydrolysis, thermal degradation, and oxidative degradation.

Table-15: Results of Force Degradation Studies of Erlotinib API

Stress Condition	Time (hours)	Assay of active substance	Assay of degraded products	Mass Balance (%)
Acid Hydrolysis (0.1N HCl)	24Hrs.	91.326	8.674	100.00
Basic Hydrolysis (0.IN NaOH)	24Hrs.	83.215	16.785	100.00
Thermal Degradation (60 ^{0}C)	24Hrs.	90.311	9.689	100.00
UV (254nm)	24Hrs.	81.322	18.678	100.00
3% Hydrogen Peroxide	24Hrs.	73.514	26.486	100.00

SUMMARY AND CONCLUSUION

To develop a precise, linear, specific & suitable stability indicating RP-HPLC method for analysis of Erlotinib, different chromatographic conditions were applied & the results observed are presented in previous chapters. Isocratic elution is simple, requires only one pump & flat baseline separation for easy and reproducible results. So, it was preferred for the current study over gradient elution. In case of RP-HPLC various columns are available, but here Develosil ODS HG-5 RP C_{18}, 5µm, 15cmx4.6mm i.d. column was preferred because using this column peak shape, resolution and absorbance were good. Detection wavelength was selected after scanning the standard solution of drug over 200 to 400nm. From the U.V spectrum of Erlotinib it is evident that most of the HPLC work can be accomplished in the wavelength range of 255 nm conveniently. Further, a flow rate of 1.0 ml/min & an injection volume of 20µl were found to be the best analysis. The result shows the developed method is yet another suitable method for assay which can help in the analysis of Erlotinib in different formulations.

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