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Method development and validation of anti-cancer drug Erlotinib in marketed pharmaceutical dosage form by RP-HPLC

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Abstract

A novel reversed-phase high performance liquid chromatographic (HPLC) technique for the determination of Erlotinib and its Pharmaceutical Dosage form has been optimized and validated as per ICH Guidelines. Erlotinib was separated on a Symmetry ODS (C18) RP Column, 250 mm x 4.6 mm, 5µm with a composition of Phosphate Buffer and Methanol in the ratio of 46:54%v/v (pH-3.2) adjusted by addition of 0.1% orthophosphoric acid solution) as mobile phase at a flow rate of 1.0 mL min-1. The effluent was monitored by UV detection at 206 nm. Calibration plots were linear in the range of 60 to 140 µg mL-1 and the LOD and LOQ were 0.08 and 0.24 µg mL-1, respectively. The whole technique is developed and validated as per International Council for Harmonization (ICH) guidelines. The proposed method is robust, sensitive, rapid and successful and helpful in the regions where regulatory agencies recommend HPLC analytical method.

Keywords: Erlotinib, RP-HPLC, Accuracy, Precision, Robustness, LOD and LOQ

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. Erlotinib [1] is a quinazoline derivative with antineoplastic properties. Competing with adenosine triphosphate, Erlotinib reversibly binds to the intracellular catalytic domain of epidermal growth factor receptor (EGFR) tyrosine kinase, thereby reversibly inhibiting EGFR phosphorylation and blocking the signal transduction events and tumorigenic effects associated with EGFR activation. Erlotinib is a quinazoline compound having a (3-ethynylphenyl) amino group at the 4-position and two 2-methoxyethoxy groups at the 6- and 7-positions. It has a role as an antineoplastic agent, a protein kinase inhibitor and an epidermal growth factor receptor antagonist. It is a member of quinazolines, a terminal acetylenic compound, an aromatic ether and a secondary amino compound. Erlotinib [2] is an inhibitor of the epidermal growth factor receptor (EGFR) tyrosine kinase that is used in the treatment of non-small cell lung cancer, pancreatic cancer and several other types of cancer. It is typically marketed under the trade name Tarceva. Erlotinib binds to the epidermal growth factor receptor (EGFR) tyrosine kinase reversibly at the adenosine triphosphate (ATP) binding site of the receptor. Recent studies demonstrate that Erlotinib [3] is also a potent inhibitor of JAK2V617F, which is a mutant form of tyrosine kinase JAK2 found in most patients with polycythemia Vera (PV) and a substantial proportion of patients with idiopathic myelofibrosis or essential thrombocythemia. This finding introduces the potential use of Erlotinib in the treatment of JAK2V617F-positive PV and other myeloproliferative disorders. The IUPAC Name of Erlotinib is N-(3-ethynyl phenyl)-6, 7-bis (2-methoxy ethoxy) quinazolin-4-amine. The Chemical Structure of Erlotinib is follows

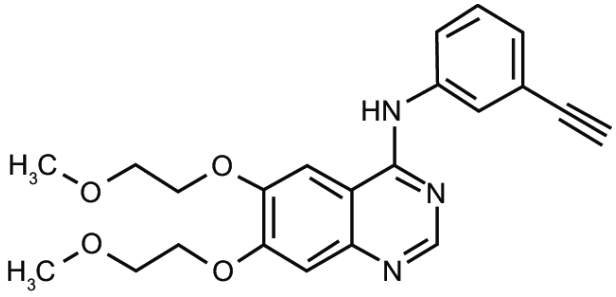

Fig 1: Chemical Structure of Erlotinib

Literature survey [31-35] reveals a few UV Spectrophotometric, UPLC and LC-MS methods reported for the determination of Erlotinib and its bulk and pharmaceutical dosage forms and metabolites in biological fluids. A few methods are reported for the determination of Erlotinib in pharmaceutical formulations by HPLC, HPTLC, UV spectrophotometric, and colorimetric methods; however HPLC method has not been reported for its estimation till date. Hence an attempt has been made to develop and validate a rapid and sensitive HPLC method for the estimation of Erlotinib as recommended by the International Conference on Harmonization (ICH) guidelines. The method was validated by parameters such as linearity, accuracy, precision, LOD, LOQ, robustness.

Experimental

Table 1: List of Instrument used

S. No.	Instruments/Equipments/Apparatus
1.	**HPLC** with Empower2 Software with Isocratic with UV-Visible Detector (Waters).
2.	**T60-LAB INDIA** UV – Vis spectrophotometer
3.	Electronic Balance **(SHIMADZU ATY224)**
4.	Ultra Sonicator **(Wensar wuc-2L)**
5.	Thermal Oven
6.	Symmetry ODS RP C_{18},5µm, 15mm x 4.6mm i.d.
7.	P^H Analyzer **(ELICO)**
8.	Vacuum filtration kit **(BOROSIL)**

Table 2: List of Chemicals used

S.N.	Name	Specifications		Manufacturer/Supplier
		Purity	Grade	
1.	Doubled distilled water	99.9%	HPLC	Sd fine-Chem ltd; Mumbai
2.	HPLC Grade Water	99.9%	HPLC	Sd fine-Chem ltd; Mumbai
3.	Methanol	99.9%	HPLC	Loba Chem; Mumbai.
4.	Hydrochloric Acid	99.9	A.R.	Sd fine-Chem ltd; Mumbai
5.	Acetonitrile	99.9%	HPLC	Loba Chem; Mumbai.
6.	Sodium Hydroxide	99.9	A.R.	Sd fine-Chem ltd; Mumbai
7.	Ethanol	99.9	A.R.	Sd fine-Chem ltd; Mumbai
8.	Octanol	99.9	A.R.	Sd fine-Chem ltd; Mumbai

Method Development

Selection of Wavelength

The standard & sample stock solutions [4] were prepared separately by dissolving standard & sample in a solvent in mobile phase diluting with the same solvent.(After optimization of all conditions) for UV analysis. Itscanned in the UV spectrum in the range [5] of 200 to 400nm. This has been performed to know the maxima of Erlotinib, so that the same wave number can be utilized in HPLC UV detector[7] for estimating the Erlotinib. The scanned UV spectrum is attached in the following page.

Sample & Standard Preparation for the UV-Spectrophotometer Analysis

25 mg of Erlotinib standard was transferred into 25 ml volumetric flask, dissolved & make up to volume with mobile phase.Further dilution was done by transferring 0.5 ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase [8].

Preparation of 0.01M Potassium Dihydrogen Orthophosphate Solution

About 1.36086grams of Potassium dihydrogen orthophosphate was weighed and transferred into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC Grade water. The pH was adjusted to 3.20 with diluted orthophosphoric acid.

Preparation of Mobile Phase

460ml of Phosphate buffer (0.05M) pH 3.20 and 540ml of HPLC Grade Methanol were mixed well and degassed in ultrasonic water bath [9] for 15 minutes. The solution was filtered through 0.45 µm filter under vacuum filtration.

Method Validation

Method validation [10-16] is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. The proposed method was validated according to the ICH guidelines [30].

System Suitability Tests

The chromatographic systems[17] used for analysismust pass the system suitability limits beforesample analysis can commence. It is used toverify that the reproducibility of the system isadequate for the analysis to be performed. Parameters like %RSD, tailing and theoreticalplates[18] are to be taken in to consideration.

Linearity & Range

The response was found linear [19] over a concentration range of 60-140µg/ml for Erlotinib. Accurately measured stock solution of 0.6,0.8,1.0.1.20 & 1.40ml were transferred to a series of 10 mL of volumetric flasks and diluted upto the mark with mobile phase such that the final concentrations are 60, 80, 100, 120 & 140 µg/mL for Erlotinib. These solutions were injected into chromatographic system, peak area was determined for each concentration of drug solution. Plot a calibration graph [20] of peak area versus concentration (on X-axis concentration and on Y-axis Peak area).The correlation coefficients (r), slopes and Y-intercepts of the calibration

curve were determined.

Accuracy

The accuracy [21] of the method was determined interms of % recovery. A known quantity of the pure drug was added to the pre analysed sample formulation as 80%, 100% and 120% levels. The recovery studies were carried out 3 times of each level and the percentage recovery [22] was calculated.

Precision

The precision [23] of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Five consecutive injections of 100µg/ml concentration were given on the same day and the values of relative standard deviation (RSD) were calculated to determine intraday precision. The study was also repeated on different day to determine inter day precision.

LOD and LOQ

The limit of detection [24] (LOD) is the lowest detectable concentration of the analyte, while limit of quantitation[25] (LOQ) is the lowest quantifiable concentration. The LOD and LOQ were calculated using the ICH guidelines equation as LOD = 3.3 x σ/S and LOQ = 10 xσ/S, where σ is the standard deviation of y intercepts of regression lines and S is the slope of the calibration curve.

Robustness of the Method

The optimum chromatographic conditions set for this method have been slightly modified to evaluate the method robustness [26]. The small changes made include the flow rate (±0.05 ml/min) and the organic content in the mobile phase (±10%).

Results and Discussion

Method Development

Wavelength Selection

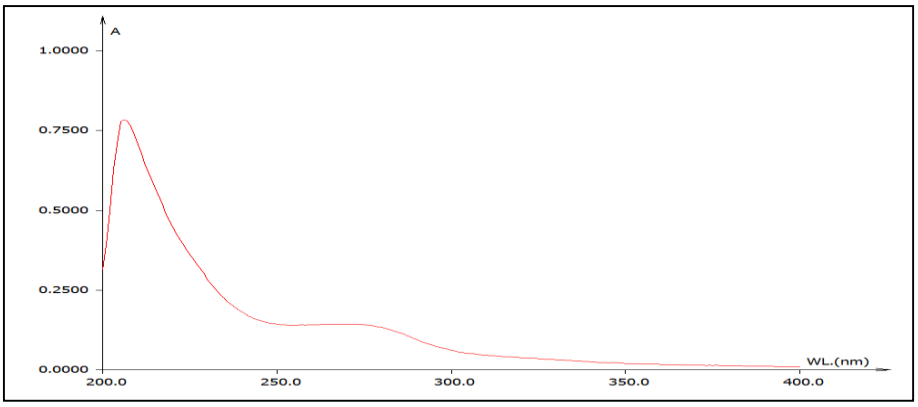

Figure 2: UV spectrum for Erlotinib

Observation: While scanning the Erlotinib solution we observed the maxima at 206nm.

Summary of Optimized Chromatographic Conditions

The Optimum Chromatographic conditions [27] obtained from experiments can be summarized as below:

Table 3: Summary of Optimised Chromatographic Conditions

Mobile phase	**Phosphate Buffer : Methanol = 46:54 (pH-3.2)**
Column	Symmetry ODS (C_{18}) RP Column, 250 mm x 4.6 mm, 5µm
Column Temperature	Ambient
Detection Wavelength	206 nm
Flow rate	1.0 ml/ min.
Run time	08 min.
Temperature of Auto sampler	Ambient
Diluent	Mobile Phase
Injection Volume	10µl
Type of Elution	Isocratic
Retention time	3.526 minutes

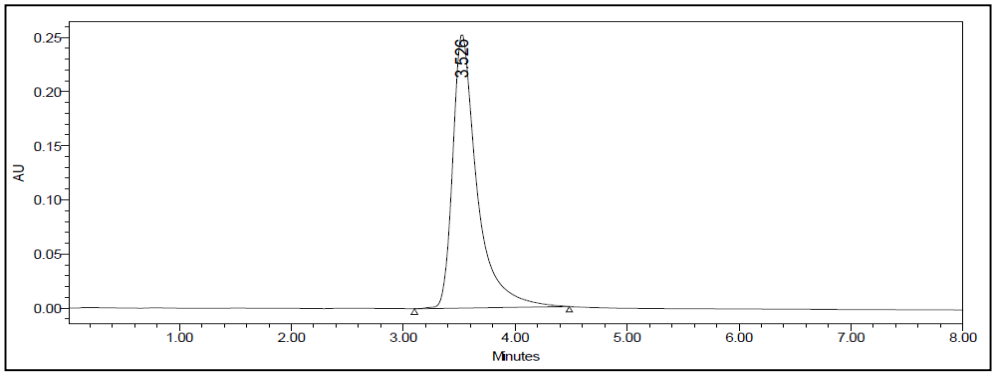

Fig 3: Chromatogram of Erlotinib in Optimized Condition

Validation of Method

1. Accuracy

Recovery study

To decide the exactness of the proposed strategy, recuperation contemplates were completed by including diverse sums (80%, 100%, and 120%) of unadulterated medication of ERLOTINIB were taken and added to the pre-examined plan of fixation 100µg/ml. From that rate recuperation esteems were figured. The outcomes were appeared in table-4.

Table 4: Accuracy Readings

Conc. In ppm	Conc. Found	Peak Area		% Recovery
80	80.461	3959294		100.576
80	80.095	3941634		100.118
80	80.194	3946409		100.242
			Avg.	100.312
			S.D	0.236888
			%RSD	0.236151
Conc. In ppm	**Conc. Found**	**Peak Area**		% Recovery
100	100.932	4948323		100.932
100	99.879	4897463		99.879
100	100.030	4904741		100.030
			Avg.	100.2803
			S.D	0.569388
			%RSD	0.567796
Conc. In ppm	**Conc. Found**	**Peak Area**		% Recovery
120	120.019	5870480		100.015
120	119.907	5865040		99.922
120	119.794	5859590		99.828
			Avg.	99.92167
			S.D	0.0935
			%RSD	0.093574

2. Precision

2.1. Repeatability

The accuracy of every technique was found out independently from the pinnacle regions and maintenance times gotten by real assurance of six recreates of a fixed amount of drug Erlotinib (API). The percent relative standard deviation was calculated for Erlotinib are presented in the table-5.

Table 5: Repeatability Readings

HPLC Injection Replicates of Erlotinib	Retention Time (Minutes)	Peak Area
Replicate – 1	3.639	3948323
Replicate – 2	3.622	3935751
Replicate – 3	3.575	3979135
Replicate – 4	3.525	3971013
Replicate – 5	3.526	3919463
Replicate – 6	3.523	3974741
Average		**3954738**
Standard Deviation		**24108.89**
% RSD		**0.609621**

2.2. Intermediate Precision

2.2.1. Intra-assay & inter-assay

The intra & inter day variation [28] of the method was carried out & the high values of mean assay & low values of standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Erlotinib revealed that the proposed method is precise.

Table 6: Results of Intra-Assay & Inter-Assay

Conc. Of Erlotinib(API) (µg/ml)	Observed Conc. of Erlotinib (µg/ml) by the proposed method			
	Intra-Day		Inter-Day	
	Mean (n=6)	% RSD	Mean (n=6)	% RSD
80	79.35	0.88	80.36	0.56
100	100.57	0.65	99.86	0.36
120	119.87	0.93	120.18	0.87

3. Linearity & Range

The calibration curve showed good linearity in the range of 0 – 140 µg/ml, for Erlotinib (API) with correlation coefficient (r^2) of 0.999 (Fig-4). A typical calibration curve has the regression equation of y = 48313x + 71968 for Erlotinib.

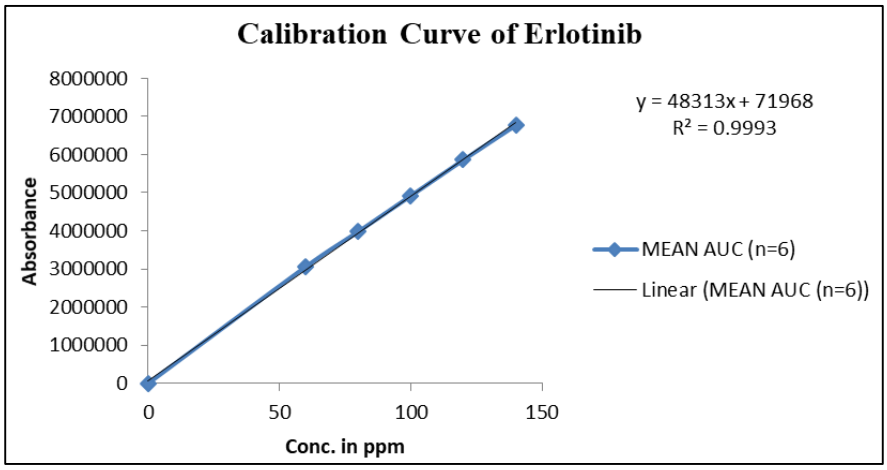

Fig 4: Calibration Curve of Erlotinib (API)

Table 7: Linearity Results

CONC.(µg/ml)	MEAN AUC (n=6)
0ppm	0
60ppm	3059294
80ppm	3979280
100ppm	4919463
120ppm	5859590
140ppm	6770480

4. Method Robustness

Impact of little changes in chromatographic conditions, for example, change in Flow rate (± 0.1ml/min), Wavelength of location (±2nm) and organic phase content in mobile phase (±5%) concentrated to decide the Robustness of the technique are additionally for (Table-8, % RSD < 2%) the created RP-HPLC strategy for the examination of Erlotinib (API).

Table 8: Result of Method Robustness Test

Change in parameter	% RSD
Flow (1.1 ml/min)	0.61
Flow (0.9 ml/min)	0.75
More Organic	0.69
Less Organic	0.81
Wavelength of Detection (208 nm)	0.89
Wavelength of detection (204 nm)	0.99

5. LOD & LOQ

The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 0.08 & 0.24µg/ml respectively.

6. System Suitability Parameter

Framework appropriateness testing is a necessary piece of numerous explanatory methodologies. The tests depend on the idea that the gear, hardware, logical tasks and tests to be examined comprise a necessary framework that can be assessed thusly. Following framework reasonableness test parameters were built up. The information is appeared in Table-9.

Table 9: Data of System Suitability Parameter

S. No.	Parameter	Limit	Result
1	Resolution	Rs > 2	9.34
2	Asymmetry	T ≤ 2	Erlotinib=0.16
3	Theoretical plate	N > 2000	Erlotinib=3065
4	Tailing Factor	T<2	Erlotinib=1.55

7. Estimation of Erlotinib in Pharmaceutical Dosage Form

Twenty pharmaceutical dosage forms were taken and the I.P. technique was taken after to decide the normal weight. Above measured tablets were at long last powdered and triturated well. An amount of powder comparable to 25 mg of medications were exchanged to 25 ml volumetric jar, make and arrangement was sonicated for 15 minutes, there after volume was made up to 25 ml with same dissolvable. At that point 10 ml of the above arrangement was weakened to 100 ml with mobile phase. The arrangement was separated through a film channel (0.45 µm) and sonicated to degas. The arrangement arranged was infused in five repeats into the HPLC framework and the perceptions were recorded.

A copy infusion of the standard arrangement was likewise infused into the HPLC framework and the pinnacle zones were recorded. The information is appeared in Table-10.

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 medication acquired with test readiness
AS = Peak Area of medication acquired with standard readiness
WS = Weight of working standard taken in mg
WT = Weight of test taken in mg
DS = Dilution of Standard arrangement
DT = Dilution of test arrangement
P = Percentage virtue of working standard

Table 10: Recovery Data for estimation Erlotinib in Erlocip 100

Brand name of Erlotinib	Labelled amount of Drug (mg)	Mean (± SD) amount (mg) found by the proposed method (n=6)	Assay % (± SD)
Erlocip 100 Tablet (100 mg) (Cipla)	100mg	99.869 (± 0.789)	99.698 (±0.758)

Result & Discussion: The amount of drug in Erlocip 100 Tablet was found to be 99.869 (± 0.789) mg/tab for Erlotinib & % assay [29] was 99.698%.

Summary and Conclusion

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 Symmetry ODS (C_{18}) RP Column, 250 mm x 4.6 mm, 5µm column was preferred because using this column peak shape, resolution and absorbance were good. Recognition wavelength was chosen in the wake of checking the standard arrangement of medication more than 200 to 400nm. From the U.V range of Erlotinib it is apparent that a large portion of the HPLC works can be refined in the wavelength scope of 206 nm helpfully. Further, a stream rate of 1 ml/min and an infusion volume of 10µl were observed to be the best examination. The outcome demonstrates the created strategy is amazingly, one more appropriate technique for test and steadiness related contamination thinks about which can help in the examination of Erlotinib in various definitions.

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