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Research article

Analytical method development and validation of Erlotinib hydrochloride in bulk and pharmaceutical dosage form by RP-HPLC

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Abstract

A simple, precise and accurate method for the estimation of Erlotinib in bulk and pharmaceutical dosage forms by reverse phase high performance liquid chromatography method was developed. A reverse phase Grace C18 column (250 cm · 4.6 mm · 5 µm) with mobile phase consisting of potassium dihydrogen orthophosphate, acetonitrile and methanol (50:30:20 V/V) having pH 4.0 which was adjusted by using orthophosphoric acid. The flow rate was 1 mL min^{-1} and the effluents were monitored at 247 nm. The retention time was found to be 5.83 min. The drug shows good linearity within the range of 10–60 µg mL^{-1}. The inter-day and intra-day variation was found to be less the 2%. The mean recovery of drug from solution was 101.10%. The results of analysis have been validated according to ICH guideline requirements. The method can be applied for the estimation of Erlotinib in pharmaceutical dosage form.

Introduction

Erlotinib (ETB), chemically known as N-(3-ethynylphenyl)-6,7-bis(2-methoxyethoxy)-4-quinazolin amine (Figure 1). Similar to gifitinib it is a selective inhibitor of tyrosine kinase receptor intracellular further inhibits autophosphorylation in association with epidermal growth factor receptor (EGFR). It was approved by the U. S. Food and Drug Administration (USFDA) in 2004 for the treatment of locally advanced or metastatic non small-cell lung cancer (NSCLC) [1]. Erlotinib is available in markets as conventional tablets with a trade name of Tarceva. It has shown survival benefits in the treatment of lung cancer in phase III trials. Some high-performance liquid-chromatographic (HPLC) methods with ultraviolet (UV) has been developed. Some methods with tandem mass spectrometry (MS=MS), each with its own advantages and limitations, have been reported for the assay of ETB in human plasma [2-6]. A HPLC assay method for ETB in bulk and pharmaceutical formulations has also been reported, so far. The present article describes the quantitative determination and validation of ETB in bulk drug and formulations. The proposed method is simple and specific because it can determine ETB in the presence of its degradation products, excipients, and additives.

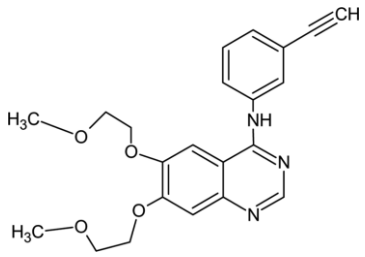

Figure 1. Structure of erlotinib.

Experimental procedure

Instruments

High performance liquid chromatography (SHIMADZU SCL-10 VP) with one SCL-10 AT VP pump was used for analysis. UV/VIS detector SPD-10 AVP, Grace C18 column (250 mm x 4.6 mm with a particle size 5 µm) was used isocratic mode. HPLC system was equipped with the software class-VP 1c solutions (Shimadzu) and an auto-sampler.

Reagents and chemicals

Erlotinib hydrochloride was obtained as a gift sample from Cipla Pvt. Ltd. All reagents and solvents used were of analytical and HPLC grade including methanol, acetonitrile, potassium dihydrogen orthophosphate, orthophosphoric acid and monobasic sodium phosphate purchased from SD fine-chem Ltd., Mumbai, India. Deionized water was obtained by double distillation and purification through milli-Q water purification system.

The 0.45 µm membrane filter was obtained from Wockhardt Pvt. Ltd., Aurangabad.

Buffer preparation

Potassium dihydrogen orthophosphate (2.72 g) was dissolved in 100 mL of HPLC grade water and adjusted to pH 4.0 with dilute orthophosphoric acid. The solution was filtered through 0.45 µm membrane filter and degassed. It was used as a diluent for the preparation of sample and standard solution.

Method

Selection of wavelength

Accurately weighed quantity of erlotinib (50 mg) was taken in a 50 mL volumetric flask containing 25 mL of methanol, sonicated further for 10 min and filtered through 0.45 µm membrane filter. The volume was makeup to 50 mL with methanol and scanned between 200 and 400 nm by UV spectroscopy using methanol as a blank.

Chromatographic conditions

The experiments were performed with isocratic elution. The mobile phase consisted of a mixture of Potassium dihydrogen phosphate buffer (0.02 M), acetonitrile and methanol in the ratio of 50:30:20 v/v. The pH 4.0 was adjusted by using 0.1% orthophosphoric acid which was filtered through a membrane filter. The mobile phase was degassed before running at a flow rate of 1.0 mL min^{-1}. The column temperature was ambient at 25°C. The 20 µL volume of sample was taken up by auto-sampler and were detected using UV detector at a wavelength of 247 nm.

Method development

The chromatographic conditions were optimized by selecting various variable that might affect the system, such as mobile phase constituents, pH, flow rate and solvent ratio. Many solvent systems were tried for the development of a suitable HPLC method for estimation of erlotinib in bulk and pharmaceutical formulations [7]. After an extensive literature survey it was observed that most the mobile phase constituents contain phosphate buffer, acetonitrile and methanol. Here in this method we have developed a new mobile phase constituting phosphate buffer pH 4 as a major part of mobile phase [8]. The mobile phase tried for this purpose were phosphate buffer (pH 4), acetonitrile and methanol (50:30:20 v/v), methanol: water (70:30 v/v), buffer: acetonitrile (40:60 v/v), methanol: water: acetonitrile (25:40:35 v/v/v) and acetonitrile: methanol: phosphate buffer (20:40:40 v/v/v). The effect of flow rate and pH were also studied on the symmetry and resolution. The extraction of drug from the formulation was performed for quantification of a drug by extraction using different solvents [9].

Calibration curve

Various concentrations (10-60 µg/ml) were made for the preparation of calibration curve from the stock solution. The mobile phase after filtration through 0.45 µm membrane filter was delivered at 1.0 ml/min for standardization of column, the baseline was continuously monitored during the process. The UV-vis spectroscopy scan of ETB was performed between 200-400 nm and detection wavelength was selected at 247 nm. The prepared dilutions were injected serially and areas under the peak were calculated for every dilution. Stability of drug in solution was analyzed repeated analysis of samples during the experimentation process on the same day and also after 48 h of storage at laboratory bench condition and in the refrigerator.

Sample solution

Twenty tablets of Erlonat and Tarceva each containing 150 mg of ETB were accurately weighed, averaged and finely powdered and weight equivalent to one tablet was weighted and transferred to a 100 mL volumetric flask. Diluent (100 mL) was added to the volumetric flask and was sonicated for 10 min for complete dissolution of the drug in the solution, filtered through a 0.45 µm membrane filter and made up to the volume with diluent. Filtrate (10 mL) was taken in a 150 mL volumetric flask and the volume as make up with same diluent to the final concentration of 100 µg mL^{-1} (Figure 3 and Table 2).

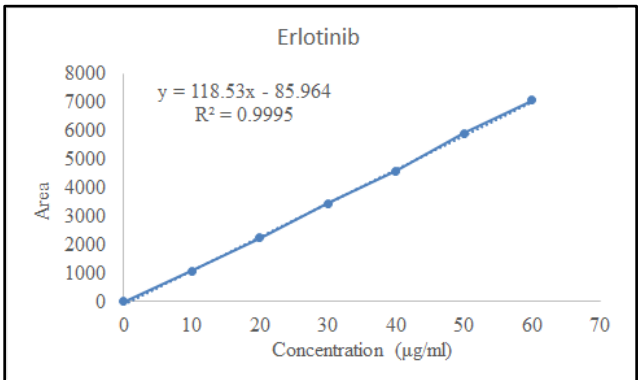

Figure 2. Calibration curve of ETB.

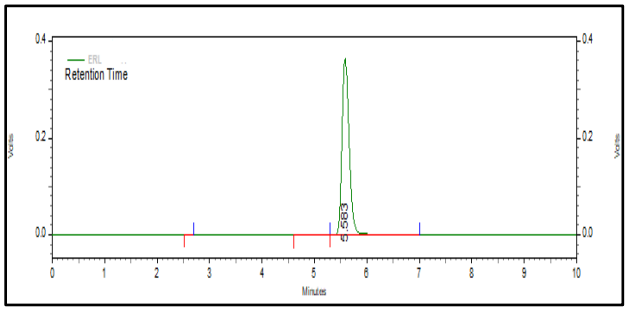

Figure 3. Chromatographic peak of ETB.

Method validation

Linearity
For quantitative analysis of ETB, the calibration curves were plotted for each concentration ranges. The linearity ranges for ETB found to be 10-60 µg/ml respectively.

Precision
The reproducibility of developed method was determined by performing tablet assay at different time intervals (3 hour interval) on same day (Intra-day precision) and on three different days (Inter-day precision) of ETB.

Accuracy as recovery
The accuracy was determined by standard addition method. Three different levels (80%, 100% and 120%) of standards were spiked to commercial tablet in triplicate. The mean of percentage recoveries and the % relative standard deviation (% RSD) was calculated.

Specificity
The specificity was analyzed by using mixture of generally used tablet excipients such as lactose, starch, magnesium stearate (blank placebo). It is calculated as a percentage recovery of each component in the presence of other interfering substances.

Robustness
Robustness of the method was determined small and deliberate variation in wavelength of the analysis. Ruggedness was estimated by analyzing a sample with two different analysts. The pH of buffer solution was deliberately changed slightly from pH 3.5 to 5.

Assay of commercial dosage form
The quantity equivalent to 10 mg tablet powder was weighed accurately and transferred into 10 ml volumetric flask and dissolved in 5ml acetonitrile and volume is make up to the mark. The solution is ultrasonicated for 30 minutes and then filtered through Whatman filter paper (No. 41). It gives 1000 µg/ml of ETB (stock solution). 0.25 ml stock solution was taken and transfer into 10ml volumetric flask. Volume was made with mobile phase up to the mark. It gives 25 µg/ml of ETB the sample was injected corresponding to 25 µg/ml of ETB and peak area was recorded.

Results and discussion

Development and optimization of the HPLC method
The proposed HPLC method was optimized with a view to developed a suitable analytical method for estimation of ETB. Mobile phase with different combinations were made to run the chromatograms such as methanol: water (70:30 v/v), acetonitrile: water (60:40 v/v), buffer: acetonitrile (40:60 v/v), methanol: water: acetonitrile (25:40:35 v/v/v), acetonitrile: ammonium acetate (90:10 v/v). The chromatogram obtained with phosphate buffer: acetonitrile and methanol (50:30:20 v/v/v) solvent system was found to have very good symmetry with Rt (5.83±0.01) and sharp well defined peak. The mixture of phosphate buffer: acetonitrile and methanol (50:30:20 v/v/v) was chosen as mobile phase. The drug was stable in the mixture of mobile phase for a period of 48 h in a laboratory at room temperature and under refrigerator condition.

Method validation

Linearity
A 50:30:20 v/v/v mixture of acetonitrile, phosphate buffer (pH 4) and methanol was used and dilution was made in the range of 10- 60 µg/ml for ETB. The calibration graph constructed by plotting concentration of the drug against peak area. A linear correlation in the concentration range of 10-60 µg/ml for ETB was obtained. Calibration curve was shown in Figure 2. The regression equations of this curves was computed and regression coefficient was found to be 0.995.

LOD and LOQ
The limit of detection and limit of quantification for ETB was found to be 0.148 µg/ml and 0.364 µg/ml respectively, which indicate the sensitivity of the method.

Precision
Precision of the method was performed by intra-day and inter-day studies. The % RSD values obtained from peak area for ETB was observed and shown in the Table 2a and 2b.

Accuracy
The accuracy of the method was determined by recovery studies and the percentage recovery was calculated. The recoveries of ETB were found to be in the range of 99-102%. The proposed liquid chromatographic method was applied to the determination of ETB in their dosage forms. The results for ETB comparable with the corresponding labeled amounts. From the amount of drug percentage recovery was calculated. The relevant results are furnished in Table 3.

Specificity
The specificity of the method was tested by some impurities and mixture of generally used tablet excipients such as lactose, starch, magnesium stearate (blank placebo). It is calculation of the percentage recovery of each component in the presence of other interfering substances. The results are presented in the Table 4,

which confined that separation of analytes from other interfering excipients was completed.

Robustness

Robustness was studied by determining effects of small variation of mobile phase composition (±1%), Wavelength and Flow rate, no significant change in ***R*F** value and relative standard deviation of peak area indicating the robustness of method. Results are shown in Table 5.

Table 1. Summary of Validation Parameters by HPLC.

Sr. No.	Parameters	Readings
	System suitability	
1	Retention time (mins)	5.83
2	Theoretical plate count	6481
3	Tailing factor	1.074
4	Linearity range (µg mL^{-1})	10-60
5	Detection limit (µg mL^{-1})	0.148
6	Quantitation limit (µg mL^{-1})	0.364
	Regression data	
7	Slope	118.53
8	Intercept	85.599
9	Correlation coefficient	0.9995

Table 2 (a). Precision study of ETB (Interday).

Sr No.	Conc.	Mean Peak Area	Amount found	% Amount found	SD	% RSD
1	10	1072.04	10.04	100.04	1.42	0.74
2	15	1625.27	15.15	101.00	2.09	0.38
3	20	2235.32	19.96	99.80	1.24	0.79

Table 2 (b). Precision study of ETB (Intraday).

Sr No.	Conc.	Mean Peak Area	Amount found	% Amount found	SD	% RSD
1	10	1074.05	10.01	100.39	1.26	0.18
2	15	1587.21	14.80	98.69	5.19	0.37
3	20	2235.08	19.99	99.95	2.54	0.58

Table 3. Accuracy by the recovery study of ETB.

Statistics	Level of Recovery		
	80 %	100 %	120 %
Amount Present (µg/ml)	10	10	10
Amount of Standard Added (µg/ml)	8	10	12
Mean Peak Area	1929.32	2236.49	2460.12
Total Amount Recover (µg/ml)	17.99	20.02	22.0046
% Recovery	99.98	100.11	100.21
SD	0.03	0.04	0.02
% RSD	0.12	0.21	0.35
Mean % Recovery	100.10		
Mean SD	0.021		
Mean % RSD	0.18		

Table 4. Specificity study of ETB.

Added (µg/ml)	Recovered (µg/ml)	Recovery %
7.5	7.58	101.06
7.5	7.62	101.60
7.5	7.59	101.20
7.5	7.49	99.86
Mean= 7.57;	% RSD=0.72	

Table 5. Robustness study for ETB.

Flow Rate	**Flow-0.8ml**		**Flow Rate**	**Flow-1.2 ml**	
Sr. No.	Conc. (µg/ml)	Area	Sr. No.	Conc. (µg/ml)	Area
1	20	2234.85	1	20	2235.08
2	20	2235.07	2	20	2234.86
Mean		2234.96	Mean		2234.97
SD		1.45	SD		2.15
% RSD		0.14	% RSD		0.24
Mobile phase Volume :	45:30:25		Mobile phase Volume :	45:35:20	
Sr. No.	Conc.(µg/ml)	Area	Sr. No.	Conc.(µg/ml)	Area
1	20	2235.14	1	20	2235.42
2	20	2234.77	2	20	2234.88
Mean		2234.95	Mean		2235.15
SD		2.47	SD		3.51
% RSD		0.37	% RSD		0.54
Wavelength Change	249		Wavelength Change	245	
Sr. No.	Conc. (µg/ml)	Area	Sr. No.	Conc. (µg/ml)	Area
1	20	2235.44	1	20	2236.12
2	20	2235.62	2	20	2236.01
Mean		2235.53	Mean		2236.06
SD		2.97	SD		5.31
% RSD		0.38	% RSD		0.49

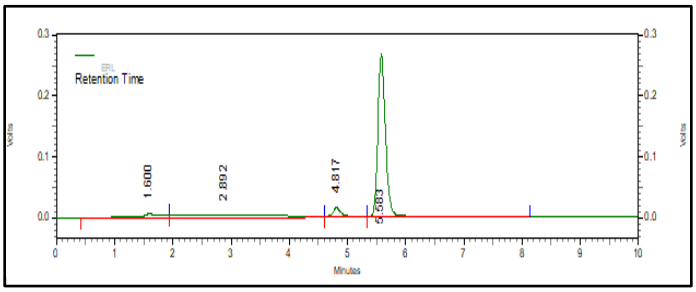

Figure 4. HPLC peak of ETB.

The method was used for determination of ETB in (Tarceva pharma, India). The results obtained (Table 3) shows that percentage recoveries were high and % RSD value were low, which confirm that method is suitable for routine estimation of ETB in its pharmaceutical formulations. Figure 3.

A typical chromatogram obtained from analysis of branded formulation. Stability of ETB in solution was checked by determining the percentage deviation of the amounts present in solution after 48 h at room temperature (Figure 4) in comparison with the amount of zero time. After the competition of 48 h, results obtained showed no significant variation, the percentage deviation was less than 2% of the initial amount. This is confirming that good stability of each component in the mixture over a period of 48 h.

Conclusion

The RP-HPLC method was developed, validated and applied to pharmaceutical analysis for estimation of ETB in bulk and tablet formulation. This HPLC method using common reagents and simple sample preparation procedure is particularly appropriate for analysis of ETB in pharmaceutical dosage form. This method has advantages of accuracy, precision, simplicity, sensitivity and quantification of ETB compared with other reported methods. The retention time is 5.83 min only so many samples also be analyzed in short period of time.

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