Research Article

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A NEW UV SPECTROPHOTOMETRIC METHOD DEVELOPMENT AND VALIDATION FOR ERLOTINIB BY DERIVATIVE SPECTROSCOPY

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Abstract

Erlotinib is as an oral anti cancer agent. It is white to cream color amorphous powder, soluble in water, methanol and partially soluble in acetonitrile, acetone, ethyl acetate and hexane. In this study a simple, accurate and precise UV spectrophotometric method was developed in pure and pharmaceutical formulations. The drug showed maximum absorbance at 246 nm in methanol. The developed method was validated for linearity, precision, LOD and LOQ. The linearity of the drug was found at the concentration range of 2 -10 µg/ml. The results of analysis have been validated as per International Conference on Harmonization (ICH) guidelines. The percentage recovery for Erlotinib was in the range of 99.93 %. w/w. Hence the proposed method can be easily and conveniently used for the estimation of Erlotinib in bulk and pharmaceutical dosage form.

Keywords: Erlotinib, Anti cancer, UV spectroscopy.

Introduction

Erlotinib, N-(3-ethynylphenyl)-6, 7-bis (2-methoxyethoxy) 4-Quinazolinamine (Figure 1), is a new drug used for the treatment of lung cancer. The mechanism of action involved is an Epidermal Growth Factor Receptor inhibitor. It specifically targets the epidermal growth factor receptor (EGFR) tyrosine kinase, which is highly expressed and occasionally mutated in various forms of cancer. It binds in a reversible fashion to the adenosine triphosphate (ATP) binding site of the receptor. For the signal to be transmitted, two members of the EGFR family need to come together to form a homo dimer.[1-5] These then use the molecule of ATP to autophosphorylate each other, which causes a conformational change in their intracellular structure, exposing a further binding site for binding proteins that cause a signal cascade to the nucleus. By inhibiting the ATP, autophosphorylation is not possible and the signal is stopped.[6,7]

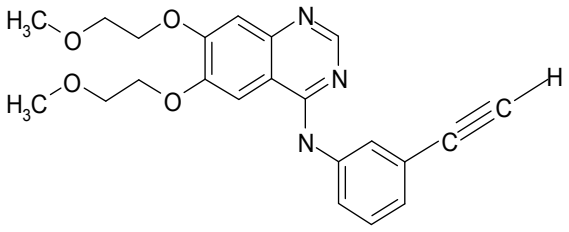

Fig. No. 01: Chemical structure of Erlotinib

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A few analytical methods have been reported for its quantitative estimation in pharmaceutical formulations by HPLC and colorimetric method[8,9]. The objective of the work is to develop a new UV spectrophotometric method for estimation and validation of Erlotinib in bulk and tablet dosage form with good accuracy, simplicity, precision and cost effective.

Materials and methods

Chemicals

Working standards of pharmaceutical grade Erlotinib (< 99%) was obtained as generous gifts from Ajanta Pharmaceutical Pvt Ltd, Mumbai, India. A tablet containing Erlotinib hydrochloride equivalent to Erlotinib 150 mg was procured from local market. Methanol was purchased from Merck chemicals, Mumbai, India.

Instruments

UV/VIS Spectrophotometer (Perkin elmer lambda-25) used with 1 cm path length quartz cell, analytical balance (Shimadzu) and ultra sonicator (Pci, 1.5 L50) were used.

Selection of wavelength

The known concentration of Erlotinib was weighed separately and dissolved in volumetric flasks using methanol. The resulting solution was scanned in the range of 200 nm to 400 nm. The maximum absorbance was found at 246 nm using derivative spectroscopy with characteristic peak as shown in the Figure 2.

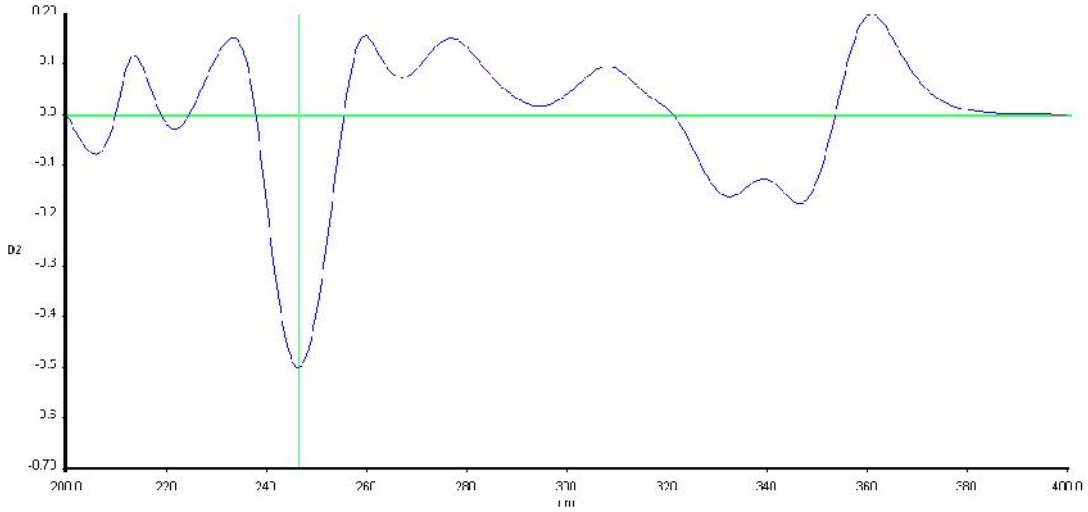

Fig. No. 02: λ_{max} of Erlotinib by derivative spectrum

Preparation of Standard Stock Solutions

Reference standard of Erlotinib 25mg was transferred to 25ml of volumetric flask and dissolved with solvent. The flask was sonicated for 10 min and made up the volume with solvent to obtain standard stock solution. The concentration was found to be 1000µg/ml of Erlotinib.

Working standard solution

From the stock solution, 1ml was taken into the 10 ml volumetric flask and made up to the mark using solvent. It contains 100µg/ml of Erlotinib**.** Further pipette out 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with solvent. The concentration was found to be 10µg/ml of Erlotinib.

Sample preparation

Accurately weigh 10 tablets and powder equivalent to 25mg of Erlotinib was weighed and transferred into a dry clean volumetric flask. Add about 5ml of solvent and sonicated to dissolve it completely and make volume up to the mark with the same solvent. From the stock solution, 1ml was taken into the 10ml volumetric flask made up to the mark using solvent to contain 100µg/ml of Erlotinib**.** Further pipette out 1ml of the above solution into a 10ml volumetric flask and dilute up to the mark with solvent. The concentration was found to be 10 µg/ml of Erlotinib.

Table No. 01: Assay results for Erlotinib

S.No	Particulars	Erlotinib
1	Standard absorbance	1.2992
2	Sample absorbance	1.2038
3	Average weight	351.5mg
4	Sample weight	108.69
5	Standard weight	50mg
6	Potency	99.8%
7	Amount found	149.52mg
8	Label claim	150mg
9	% Purity	99.68%

Method validation

The simultaneous equation method was validated by evaluating linearity, accuracy, method and system precision, limit of detection (LOD), limit of quantification (LOQ) and ruggedness were performed accordance with ICH guideline Q2 (R1).[10]

Linearity

From the standard stock solution of concentration 100 µg/ml, 0.2, 0.4, 0.6, 0.8, 1ml was transferred to five 10ml flasks and made up the volume with solvent. The concentration of Erlotinib was found to be 2-10 µg/ml. The calibration curve was shown in Fig 3. The results of linearity were shown in Table 1and Figure 3.

Table No. 02: Regression data for Erlotinib

Concentration (ppm)	Absorbance	Statistical Analysis
2	0.3012	Slope = 0.115 Intercept = 0.054 Correlation coefficient = 0.997
4	0.4991	
6	0.7252	
8	0.9938	
10	1.2038	

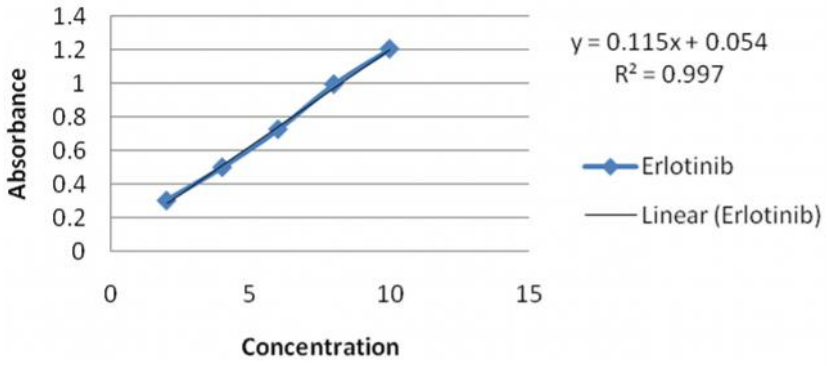

Fig. No. 03: Calibration curve of Erlotinib

Accuracy

Accuracy of the method was carried out by standard addition method at three levels of concentrations (80%, 100%, and 120%). The results of recovery (%) and %RSD were shown in Table 3.

Table No. 03: Accuracy results for Erlotinib

Level	Amount added (ppm)	Absorbance	Mean absorbance (n = 3)	Amount recovered (mg)	% mean recovery
80%	5	1.5605 1.5609 1.5604	1.5606	12.96	99.72
100%	5	1.8047 1.8042 1.8043	1.8044	14.99	99.93
120%	5	2.0292 2.0294 2.0296	2.0294	16.85	99.16

Method and system precision

Precision of the method was verified by repeatability (system precision) and intermediate (method precision) studies. Repeatability studies were performed by three replicate absorbance's of Erlotinib on the same day. The studies were replicated on different days to determine intermediate precision. The results of the system and method precision were shown in Table 4 and 5.

Table No. 04:
Method precision results for Erlotinib

S.No	Absorbance
1	1.2038
2	1.2035
3	1.2039
Mean	**1.203733**
SD	**0.000208**
RSD %	**0.017293**

Table No. 05:
System precision results for Erlotinib

S.No	Day 1	Day 2	Day 3
1	1.2038	1.2042	1.2101
2	1.2035	1.2044	1.2105
3	1.2039	1.2049	1.2109
Avg.	**1.203733**	**1.2045**	**1.2105**
S.D	**0.000208**	**0.000361**	**0.0004**
%R.S.D	**0.017293**	**0.029934**	**0.033044**

Limit of detection (LOD) and Limit of quantification (LOQ)

The limit of detection (LOD) is the lowest amount of analyte in a sample that can be detected, but not necessarily quantified, under the stated experimental conditions. LOD & LOQ was calculated by using standard deviation and slope values obtained from calibration curve.

L.O.D. ($3.3 \times \sigma/m$) 10.4475µg/ml
L.O.Q. ($10 \times \sigma/m$) 31.659 µg/ml

Results and Discussion

The proposed method for Erlotinib showed the maximum absorbance at wavelength of 246 nm. Linearity was observed in the concentration range of 2-10 µg/ml. The concentration of the drug present in the tablet was determined by the single component analysis at 246 nm. The drug content was found to be 99.68% for Erlotinib. The correlation coefficient (r^2) value of the drug was found to be 0.997. The % RSD for three replicates was found to be less than 2.0% for both method and system precision. The LOD & LOQ value for Erlotinib was found to be 10.44 µg/ml and 31.65 µg/ml respectively. The percentage recovery value for Erlotinib was in the range of 99.16-99.93%. It indicates that there was no interference from the excipients present in laboratory mixture. The results of assay validation of the proposed method show that they are accurate and precise.

Conclusions

An economic, simple and rapid UV Spectrophotometric method has been developed for the determination of Erlotinib in tablet dosage forms. The methods were validated for linearity, precision, accuracy, and LOD and LOQ. Therefore, the proposed method could be applied for the routine analysis of pharmaceutical dosage forms containing Erlotinib.

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