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SPECTROPHOTOMETRIC METHODS FOR THE DETERMINATION OF ERLOTINIB IN PURE AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

Three visible spectrophotometric methods have been developed for the determination of Erlotinib either in pure form or in their pharmaceutical formulations. The developed methods are based on reaction of Erlotinib with Phenol red, Bromocresol green and Erichrome black T. They are quantified spectrophotometrically at their absorption maximum at 418 nm (Method A), 424nm (Method B) and 333nm (Method C). Beer's law was obeyed in the concentration ranges of 40-80 µg/ml, 20-40 µg/ml and 10-50 µg/ml for the three methods respectively. The colors were found to be stable for more than 4 hours. The proposed methods were successfully applied for determination of the Erlotinib in their pharmaceutical formulations and the results compared favorably to that of reference methods, hence are recommended for quality control and routine analysis.

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Key Words

Spectrophotometer,
Pharmaceutical dosage forms,
Erlotinib, Phenol red,
Bromocresol green and
Erichrome black T.

INTRODUCTION

Erlotinib hydrochloride N (3Ethynylphenyl) [6,7bis(2methoxyethoxy) quinazolin- 4-yl] is a drug used to treat non-small cell lung cancer, pancreatic cancer and several other types of cancer. It is a tyrosine kinase inhibitor, which acts on the epidermal growth factor receptor (EGFR). Erlotinib specifically targets the (EGFR) tyrosine kinase, which is highly expressed and occasionally mutated in various forms of cancer Lung cancer is the leading cause of cancer-related mortality, worldwide, for both men and Women¹. Tyrosine kinase receptors are over-expressed or deregulated in various types of solid tumours, including non-small cell lung cancer (NSCLC)² Erlotinib received approval from the US Food and Drug Administration in November 2004 for the treatment of NSCLC after the failure of more than one or two courses of previous chemotherapy^{3,4} Erlotinib is a small molecule with the chemical name N-(3-ethynylphenyl)-6,7- bis (2- methoxyethoxy)-4-quinazolinamine that reversibly and selectively inhibits the intracellular autophosphorylation of tyrosine kinase in association with epidermal growth factor receptor (EGFR) (Figure 1). Erlotinib is available as an oral agent that blocks transduction of propagation signals mediated by the EGFRs in a concentration-dependent manner⁵. Erlotinib has demonstrated inter-patient variability in food and drug pharmacokinetic clinical studies^{6,7}, it also has the potential to cause drug-drug interactions when given in conjunction with agents that are classified as CYP3A4 inducers⁸. Literature survey reveals few HPLC⁹⁻¹¹, LC-MS¹² methods reported for the estimation of Erlotinib hydrochloride in blood serum and in ocular fluids. Stability-indicating assay method using hydrophilic interaction liquid chromatography (HILIC) has also been reported¹³. However there is no simple and accurate method reported for the determination of erlotinib in pharmaceutical formulation by UV spectrophotometry

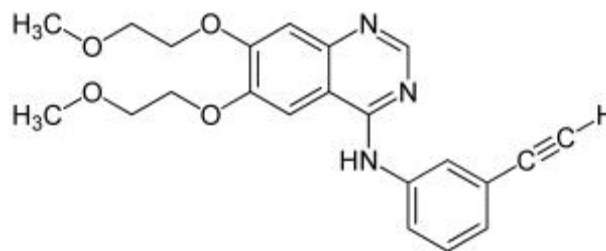


Figure 1: Structure of erlotinib

MATERIALS AND METHODS:

Instruments used: An Elico model SL 164 UV-Visible double beam spectrophotometer with 1 cm matched quartz cell was used for recording spectra and absorbance measurements.

Reagents: All reagents used were of analytical grade and were obtained from s.d. fine chemicals, Mumbai. Erlotinib was kindly supplied by Hetero drugs Pvt.Ltd. (India). Erlotinib tablets were purchased from a local market.

EXPERIMENTAL:

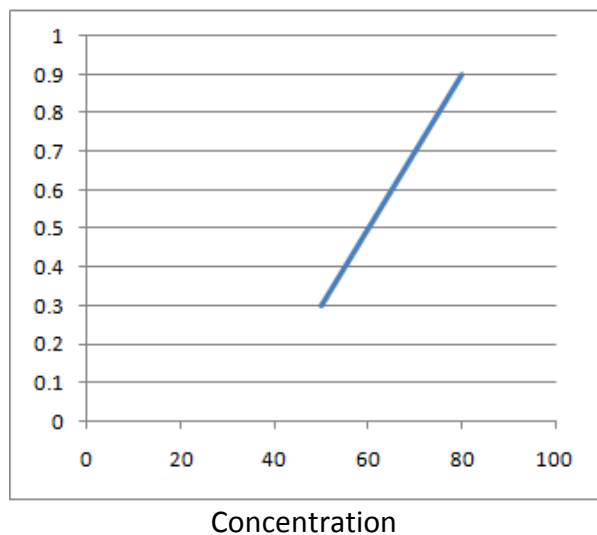
Preparation of standard Erlotinib solution

Standard solution of Erlotinib (1000µg/ml) was prepared by dissolving 100mg in methanol and diluting to the mark in a 100ml volumetric flask.

Working standard solution of 100µg/ml was prepared by further dilution of the above standard stock solution

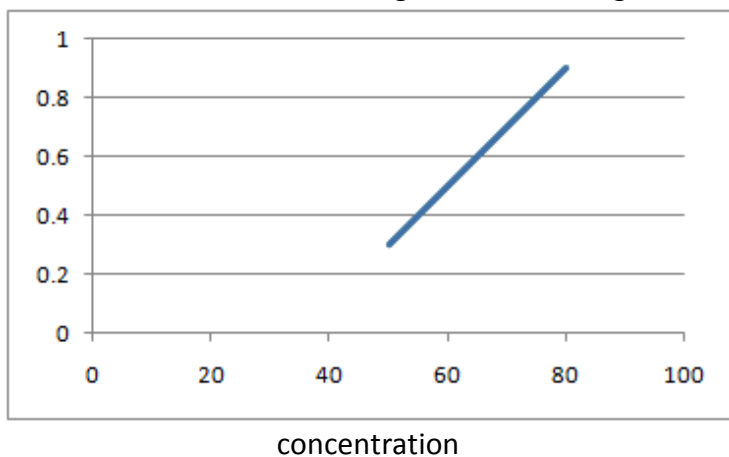
Method A: Using phenol red

Aliquots of the working standard solution of Erlotinib (40-80µg/ml) were prepared and from that 1ml of sample was accurately measured and transferred in to a series of volumetric flasks by means of a micro burette. To each of the above aliquots, 1ml of 90µg/ml of phenol red solution in methanol was added and mixed thoroughly. The volume was brought up to 5ml mark with methanol, mixed thoroughly and after 10 min absorbance of each species was measured at 418nm against reagent blank. A calibration graph was constructed by plotting the absorbance against the concentration of the drug as shown in Fig. 1.



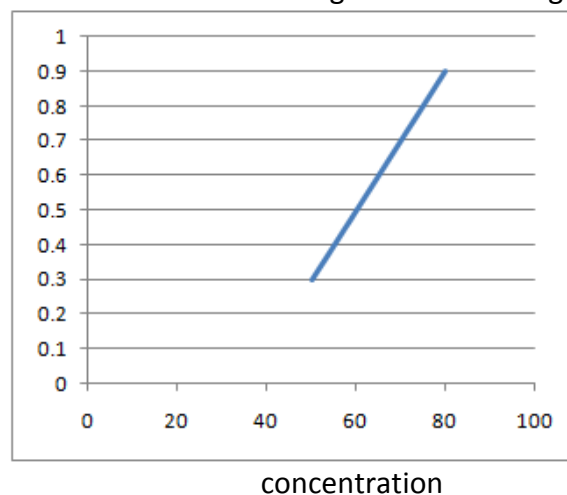
Method B: Using bromocresol green

Aliquots of the working standard solution of Erlotinib (20-40 $\mu\text{g}/\text{ml}$) were prepared and from that 1ml of sample was accurately measured and transferred in to a series of volumetric flasks by means of a micro burette. To each of the above aliquots, 1ml 190 $\mu\text{g}/\text{ml}$ of bromocresol green solution in methanol was added and mixed thoroughly. The volume was brought up to 5ml mark with methanol, mixed thoroughly and after 10 min absorbance of each species was measured at 424 nm against reagent blank. A calibration graph was constructed by plotting the absorbance against the concentration of the drug as shown in Fig. 2.



Method C: Using erichrome black T

Aliquots of the working standard solution of Erlotinib (10-50 $\mu\text{g}/\text{ml}$) were prepared and from that 1ml of sample was accurately measured and transferred in to a series of volumetric flasks by means of a micro burette. To each of the above aliquots, 1ml of 30 $\mu\text{g}/\text{ml}$ of erichrome black T solution in methanol was added and mixed thoroughly. The volume was brought up to 5ml mark with methanol, mixed thoroughly and after 10 min absorbance of each species was measured at 333 nm against reagent blank. A calibration graph was constructed by plotting the absorbance against the concentration of the drug as shown in Fig. 3.



RESULTS AND DISCUSSION:

Determination of absorption maximum

Erlotinib was treated with, phenol red, bromocresol green and erichrome black T. To determine the absorption maxima, 100 $\mu\text{g}/\text{ml}$ of drug concentration was prepared from that dilution was made to 80 $\mu\text{g}/\text{ml}$ of drug solution. 1ml of 80 $\mu\text{g}/\text{ml}$ of drug solution with 1ml of 90 $\mu\text{g}/\text{ml}$ of phenol red solution were mixed the volume was made up to 5ml with methanol. The λ_{max} was seen at 418nm. Similarly 1ml of 40 $\mu\text{g}/\text{ml}$ of drug solution was mixed with 1ml of 190 $\mu\text{g}/\text{ml}$ of bromocresol green. The volume was made up to 5ml with methanol. λ_{max} was found to be 424nm. Similarly 1ml of 50 $\mu\text{g}/\text{ml}$ of drug solution was mixed with 1ml of 30 $\mu\text{g}/\text{ml}$ of crystal violet. The volume was made up to 5ml with methanol. λ_{max} was found to be 333nm.

Fig-4: Absorption spectrum of allylestrenol with 1, 2-naphthoquinoe sulphonic acid sodium

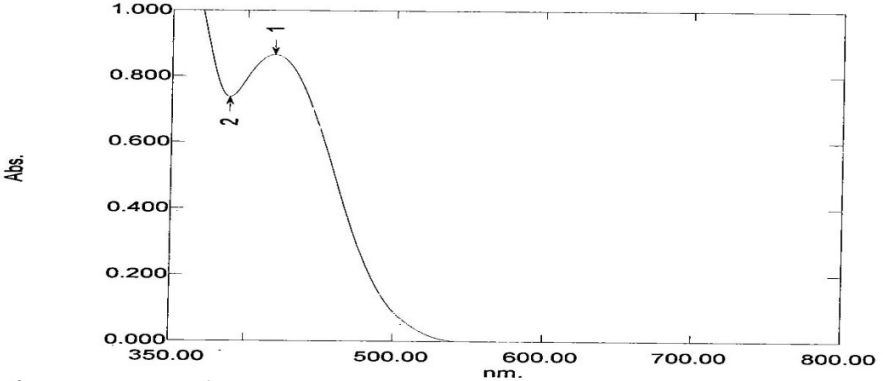


Fig-5: Absorption spectrum of Allylestrenol with Bromocresol green

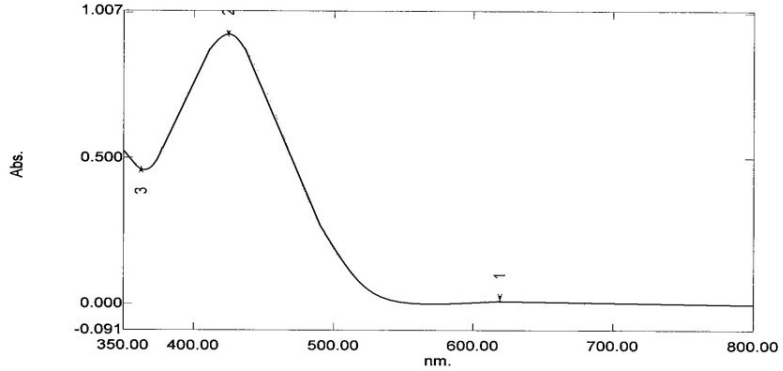
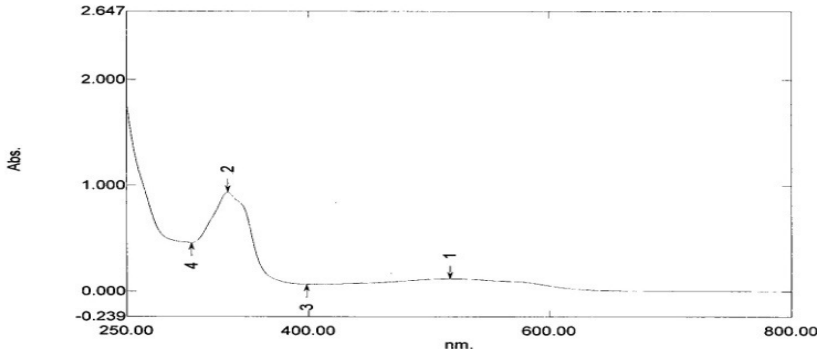


Fig-6: Absorption spectrum of Allylestrenol with crystal viole



Optimization of reaction conditions

Optimum reaction conditions for quantitative determination of drug were achieved through a number of preliminary experiments. This was done by measuring the absorbance's of a series of solutions at 418, 424 and 333 nm (Method A, B and C) by varying one and fixing the other parameters. These conditions were incorporated in the procedure.

Table1. Analytical parameters of spectrophotometric methods.

PARAMETERS	METHOD A	METHOD B	METHOD C
λ_{\max}	418nm	424nm	333nm
Beers law limit($\mu\text{g/ml}$)	40-80 $\mu\text{g/ml}$	20-40 $\mu\text{g/ml}$	10-50 $\mu\text{g/ml}$
Sandells sensitivity($\mu\text{g/ml}$)	0.0479	0.0465	0.0297
Regression equation*			
Slope	0.0203	0.0189	0.0377
Intercept	0.0006	0.0107	0.0122
Correlation coefficient	0.9986	0.9981	0.9979
Stability of coloured products	4	4	4
% rang of error	0.143	0.258	0.138
Limit of dection ($\mu\text{/ml}$)	0.408	0.783	0.419
Limit of quantification($\mu\text{/ml}$)	0.119	0.5218	0.0553

** $Y=bX+c$ where Y is the absorbance and X is the concentration of the drug in $\mu\text{g/ml}$ * average of six determinations

Table 2. Determination of erlotinib in formulations by the proposed and Reference Method

Tablet Brand name	Labeled Amount	%Recovery	Method A	Method B	Method C
erlotinib	100mg	99.91	99.23	99.33	99.69
	600mg	99.90	99.20	99.35	99.65

Optical characteristics and validation of the method

Optical characteristics such as Beer's law limits and Sandell's sensitivity, for erlotinib, are given in Table 1.

Stability

The resultant colored products of the proposed methods were found to be stable for more than four hours, which was sufficient time to make proper determination of erlotinib drug.

Data of the regression analysis using the least squares method made for the calibration curves are also

given in the same table. The accuracy and precision of the proposed methods were checked by analyzing

six replicate samples within the Beer's law range containing the same amount of each drug. The lower values of RSD indicate the good precision and reproducibility of the methods. The validity of the proposed procedure for the determination of drug in their pure state was checked by analyzing this drug using the proposed methods. The results obtained for pure drug were reproducible with low relative standard deviations (RSD). The LOD and LOQ for allylestrenol by the proposed method were determined by using calibration standards.

Applicability of the method

The applicability of the proposed spectrophotometric methods for the assay of erlotinib was tested by analyzing various available commercial formulations. The results given in Table 2 of the analysis showed that the data are consistent with the label claim of the formulations. The calibration curves showed a linear response over the concentration ranges used in the general procedures. The RSD values for the reproducibility and recovery studies show that the methods are precise and accurate. In addition it is observed that there is no interference (Table 3) from the excipients used in the formulations. Hence, these methods can be adopted for the routine quality control of erlotinib in bulk and in formulations.

CONCLUSIONS:

To summarize, our studies showed a possibility to use phenol red, bromocresol green and erichrome black T reagents for the spectrophotometric determination of erlotinib. The determination procedures are characterized by low detection limits, simplicity, cheap and good reproducibility. The statistical analyses showed that the data from the proposed methods are in good agreement with those of the reported methods. The color reaction does not require stringent conditions nor any specific reagent or buffer. The colored species was stable for more than four hours, which is sufficient time for the analyst to perform the analysis. Moreover they do not require any pretreatment

of the drug and tedious extraction procedure. Hence, the data presented in the thesis by spectrophotometric methods for the determination of erlotinib in its pure and dosage form demonstrate that the proposed methods are accurate, precise and linear. Thus it can be extended for routine analysis of erlotinib in bulk and pharmaceutical dosage.

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