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Extractive colorimetric method development and validation for erlotinib in bulk and tablet dosage form

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ABSTRACT

Two simple, rapid, sensitive, precise and economic spectrophotometric methods have been developed for the estimation of Erlotinib in bulk and tablet formulation. During the course of study, it was observed that solution of the drug formed colored ion-pair complexes with Bromocresol Green (BCG) and Methyl Orange (MO) in phosphate buffer pH 2.5, and extracted in chloroform. This property of the drug was followed for the development of colorimetric methods for analysis of drug. The complex of etoricoxib with BCG and MO showed λ max at 418.5 nm and 424.4 nm respectively. The complex was stable up to 22 hrs and obeyed Beer's law over the concentration ranges of 10-1000 µg/ml. Correlation coefficient was found to be 0.9985. In addition we have determined the molar absorptivity, Sandell sensitivity and the optimum conditions for quantitative analysis of erlotinib. These methods were validated statistically. Recovery studies gave satisfactory results indicating that none of common additives and excipients interfere the assay method. The proposed methods are found to be simple, accurate and reproducible that was successfully applied for the analysis of tablet formulation.

Key words: Spectrophotometry, erlotinib, Bromocresol green, Methyl orange, Phosphate buffer, Ion-pair complex

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INTRODUCTION

Lung cancer is the leading cause of cancer-related mortality, worldwide, for both men and women. Tyrosine kinase receptors are over-expressed or deregulated in various types of solid tumours, including non-small cell lung cancer (NSCLC) (Baselga et al., 2002). 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 (Zielinski, 2004; Shepherd, et. al., 2005). 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) (Fig. 1). Erlotinib is available as an oral agent that blocks transduction of propagation signals mediated by the EGFRs in a concentration-dependent manner (Hidalgo et. al., 2001). Erlotinib has demonstrated inter-patient variability in food and drug pharmacokinetic clinical studies (Hidalgo et. al., 2003; Birner, 2003) it also has the potential to cause drug-drug interactions when given in conjunction with agents that are classified as CYP3A4 inducers. Based on the above data, the need to develop an analytical method to determine erlotinib concentrations in patients is expedient before attempting to draw any correlation between drug dose and biological effects. Our aim was the development and validation of a simple colourimetric
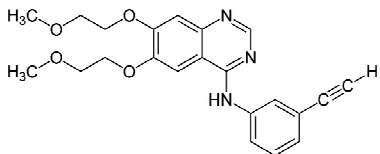
Fig 1: Molecular Structure of erlotinib.

method for the determination of erlotinib, which can be implemented easily for routine use in quality control laboratories.

MATERIALS AND METHODS

Apparatus and Instruments: Double beam UV-visible spectrophotometer (Shimadzu, model 160-A) having two matched quartz cells with 1cm light path. Shimadzu-Libror AEG-220 balance, Digital pH meter (Systronic 335),

Reagents

All chemicals used were of analytical reagent grade and double distilled water was used throughout. BCG and MO reagent supplied by BDH chemicals, India. Aqueous solution of BCG (0.5% w/v), and MO (0.05%) and buffer solutions pH 2.5 were prepared. Etoricoxib as generously supplied by M/s Systopic Laboratories Pvt. Ltd., New Delhi, India was used as such without further purification. The commercially available tablets of etoricoxib were procured from local market labeled to contain 60 mg etoricoxib/tablet.

Preparation of standard stock solution

Standard stock solution of Erlotinib was prepared by dissolving 100 mg in 100 ml of water to get concentration of 1 mg/ml. From this a working standard of 800 μg/ml.

Preparation of phosphate buffer pH 2.5

Dissolve 100 gm of potassium dihydrogen phosphate in 800ml of water adjust the pH to 2.5 with Hydrochloric acid and add sufficient water to produce 1000ml.

Preparation of 0.05% methyl orange dye solution for 25 ml:

Weigh 0.125 gm of dye sample and dissolve in 10ml of water and make up to 25ml.

Preparation of 0.5% Bromo Cresol Green dye solution for 25ml

Weigh 0.25 gm of dye sample and dissolve in 10ml of water and make up to 25ml and filter it using filter paper.

PROCEDURE

Method A

Working standard of drug solution of Erlotinib 50μg/ml (1 ml), Dye solution of Methyl Orange 0.05% (1ml), Phosphate buffer pH 2.5 (2ml) transferred into separating funnel, chloroform (10 mL) was added to the separating funnel and contents were shaken for 2 minutes. The two phases were allowed to separate and the absorbance of the chloroform layer was scaned in the range of

400-800 nm and the λmax was found to be 424.4 nm. Then calibration curve was plotted. The sample solutions prepared were also treated in similar manner and the exact amount of erlotinib present was deduced from the calibration graph.

Method B

Working standard of drug solution of Erlotinib 800μg/ml (0.5 ml), Dye solution of Bromo Cresol Green 0.5% (1ml), Phosphate buffer pH 2.5 (2ml) transferred into separating funnel, chloroform (10 mL) was added to the separating funnel and contents were shaken for 2 minutes. The two phases were allowed to separate and the absorbance of the chloroform layer was scanned in the range of 400-800 nm and the λmax was found to be 418.5 nm. Then calibration curve was plotted. The sample solutions prepared were also treated in similar manner and the exact amount of erlotinib present was deduced from the calibration graph.

Optimization of conditions

Condition under which reaction of etoricoxib with dyes fulfils the essential requirements was investigated. All conditions studied were optimized at room temperature ($32°\pm2°C$).

RESULTS AND DISCUSSION

The optical characteristics such as Beer's law limits, sandell's sensitivity, molar absorptivity, per cent relative standard deviation were calculated and the results are summarized in Table-1. Regression characteristics like slope, intercept, correlation coefficient and LOD, LOQ values were calculated and shown in Table-1. The optimum conditions for colour development for method A and B, have been established by varying the parameters one at a time and keeping the other parameters fixed and observing the effects of product on the absorbance of the coloured species and incorporated in the procedures.

Spectral characteristics

Absorption spectra of the pale yellow color ion-pair complex of MO and deep yellow colour ion-pair complex of BCG is shown in Fig. 2, with a maximum absorbance (λ) at 424.4 and 418.5 respectively. The colored complex was stable for at least 24 h at room temperature (25 ºC) determined by proposed method.
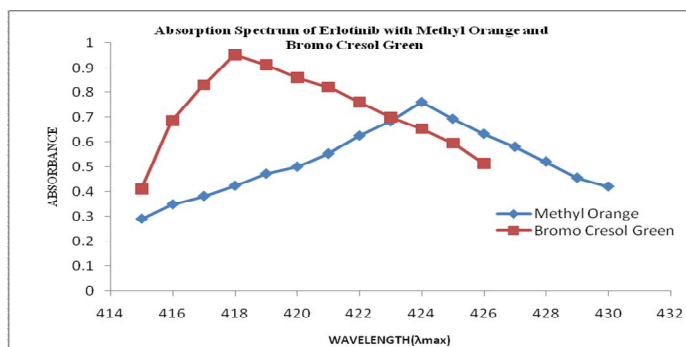

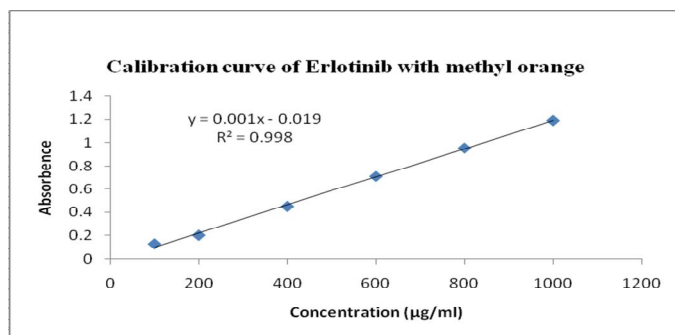**Fig 2:** Absorption spectrum of Erlonitib with methyl orange and Bromo cresol green.

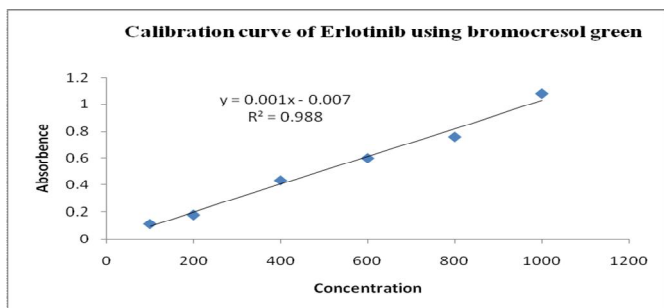**Fig 3:** Calibration curve of Erlotinib with methyl orange.

Fig 4: Calibration curve of Erlotinib using Bromocresol Green.

The molar absorptivity and sandell's sensitivity showed that the methods were sensitive. The optimum conditions for colour development had been established by varying the different parameters involved. The result of analysis of commercial formulation significantly showed low values for standard deviation, standard error of mean, coefficient of variance and percentage range of error (within 95% confidence limits), thus showed precision of the methods results shown in table 2. For testing the accuracy and reproducibility of the proposed methods, recovery studies were performed. The data obtained by recovery studies indicate non-interference from the excipients used in the formulation. The percentage recoveries were close to 100%. This study revealed that the common excipients and other additives such as lactose, starch, gelatine, talc and magnesium stearate that are usually present in the tablet dosage forms do not interfere in the analysis. Thus, it can be concluded that the proposed methods are found to be simple, rapid, sensitive and accurate that can be used for the determination of erlotinib in their pharmaceutical dosage forms in a routine manner.

Linearity

The linearity was determined for erlotinib. Solutions of the drug at six different concentrations with both the dyes were analyzed and calibration curves were constructed by concentration against the respective absorbance. The method was evaluated by determination of the correlation coefficient and intercept values. The results of linearity for Erlotinib with MO and Erlotinib with BCG are given in Table 3 and 4 respectively. From the calibration plots it was clear that the linear range for erlotinib with MO and BCG was found to be in between 10-1000 µg/ml, with the correlation coefficient 0.99

Table 1: Optical Characteristics and Precision Data.

Parameters	Methyl Orange	Bromo Cresol Green
λmax nm	424.4	418.5
Beers Law limit	100-1000 mcg/ml	100-1000 mcg/ml
Molar Absorbtivity (micrograms/cm2/0.001 Absorbance unit)	9.5×10^3	9.4×10^3
Sandell's sensitivity (micrograms/cm2/0.001 Absorbance unit)	0.266	0.266
Regression equation y Slope (m)	0.00127	0.00123
Intercept©		
Precision(% relative standard deviation)	0.01	0.042

Table 2: Recovery studies of erlotinib commercially available brands.

Table Brand	Labeled Amount (Mg)	Amount obtained(mg)* By proposed method		%Recovery By the Proposed Method *	
		Method A	Method B	Method A	Method B
Tarceva	25	23.6	24.2	94.4	96.8
	25	23.09	24.65	95.6	98.6

* Average of six determinations

Table 3: Absorbance of Erlotib with Methyl Orange.

S. No.	Concentration(µg)	Absorbance
1	100	0.1276
2	200	0.2013
3	400	0.4476
4	600	.0.7102
5	800	0.9553
6	1000	1.1901

Table 4: Absorbance of Erlotib in with Bromo Cresol Green

S. No	Concentration(µg)	absorbance
1	100	0.115
2	200	0.181
3	400	0.435
4	600	0.602
5	800	0.762
6	1000	1.078

CONCLUSION

Two simple Spectrophotometric Methods for the determination of ERLOTINIB in pure as well as in its dosage form were developed with Methyl Orange and Bromo Cresol Green the absorbance of chromogen was to be maximum at 424.4 nm and 418.5 nm against the corresponding reagent blank. The method is found to be simple, precise, economic and less time consuming. The method has been statistically evaluated and results obtained are accurate, precise and insensitive and free from the interferences of other additives present in the formulation.

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