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VISIBLE SPECTROPHOTOMETRIC DETERMINATION OF GEFITINIB IN BULK DRUG AND PHARMACEUTICAL FORMULATIONS

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ABSTRACT

Two new simple, sensitive and cost effective visible spectrophotometric methods were developed for the estimation of gefitinib in both bulk drug samples and tablets. These two methods are based on the formation of ion pair complexes of the drug with two acidic dyes namely bromocresol green (Method A) and bromophenol blue (Method B) in acidic buffer solution followed by their extraction in organic solvent (chloroform). The absorbance of the organic layer for both the methods was measured at their respective absorption maxima at 538 nm for bromocresol green and 429.5 nm for bromophenol blue against the corresponding reagent blank. The methods obeyed Beer's law between 10-100 µg/mL for bromocresol green and 1-8 µg/mL for bromophenol blue. The interference studies also revealed the common excipients and other additives usually present in pharmaceutical dosage forms did not interfere in the proposed methods. The methods were statistically evaluated and **t**he proposed methods are precise, accurate, sensitive, and cost effective and can be used in routine analysis in quality control laboratories.

Key words: Gifitinib, Spectrophotometric.

INTRODUCTION

Gefitinib is an antineoplastic agent that is currently indicated for the continued treatment of patients with locally advanced or metastatic non-small cell lung cancer. It is chemically N - (3 - chloro - 4 - fluoro-phenyl) - 7 -methoxy- 6 - (3 - morpholin - 4 - ylpropoxy) quinazolin-4-amine. The drug is official in Martindale. The Complete Drug Reference [1].Chemical structure of gefitinib is shown in Fig. 1.

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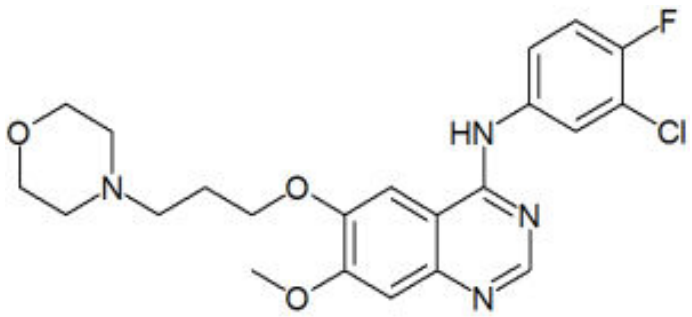

Fig. 1: Chemical structure of gefitinib

Gefitinib inhibits the epidermal growth factor receptor (EGFR) tyrosine kinase by binding to the adenosine triphosphate (ATP)-binding site of the enzyme. Thus, the function of the EGFR tyrosine kinase in activating the Ras signal transduction cascade is inhibited and malignant cells are inhibited. Gefitinib is the first selective inhibitor of the EGFR tyrosine kinase, which is also referred to as Her1 or ErbB-1. EGFR is over expressed in the cells of certain types of human carcinomas - for example in lung and breast cancers. Over expression leads to inappropriate activation of the apoptotic Ras signal transduction cascade, eventually leading to uncontrolled cell proliferation[1].

Few HPLC methods for quantitative determination of gefitinib were reported in the literature. Majority of these HPLC methods were applied in the determination of gefitinib and it's metabolites in biological fluids [2-7] and are mainly useful for therapeutic monitoring of the drug. No visible spectrophotometric method for quantitative determination of gefitinib in bulk drug samples and formulations was reported. The objective of this research was to develop and validate rapid, economical and sensitive visible spectrophotometric method for quantitative determination of gefitinib in bulk drug samples and tablets. Gefitinib contains secondary amino group in the molecular structure making it possible to form the ion-pair complexes with acidic dyes namely bromocresol green (BCG) and bromophenol blue (BPB).

EXPERIMENTAL

Instruments

Pharmaspec -1700 Ultraviolet-Visible spectrophotometer (double beam) was used for all spectral measurements. Digisun Model DI-707 pH meter was used for all the pH measurements.

Chemicals and reagents

All the chemicals used were of analytical grade. BCG (0.1% w/v), BPB (0.1% w/v), phthalate buffer of pH 2.2, chloroform, dimethyl sulfoxide (DMSO).

BCG: 100 mg of bromocresol green was dissolved in 0.72 mL of 0.1 N NaOH and 20 mL of methanol. After solution was affected, sufficient distilled water was added to produce 100 mL**.**

BPB: 100 mg of bromophenol blue was approximately weighed and taken in a 100 ml volumetric flask. To this 1.5 mL of 0.1N NaOH and 20 mL of methanol were added. The solution was then diluted with distilled water to make up the volume to 100 mL.

(This solution was treated with methanol to remove methanol soluble impurities.)

Phthalate buffer of pH 2.2 was prepared as per Indian Pharmacopoeia.

Chloroform: AR grade chloroform was used directly.

Procedure

Preparation of standard drug solution:

10 mg of gifitinib was dissolved in 10 mL of DMSO (concentration 1 mg/mL). Aliquots of standard drug solution were diluted to 10 mL from the stock solution.

Preparation of sample solution

A quantity of the powder from tablets equivalent to 10 mg of the drug was dissolved in DMSO, filtered and volume was made up to 10 mL with DMSO.

BCG Method

Aliquots of standard drug solution (0.1-1.0 mL) were added to 5 mL of phthalate buffer of pH 2.2 contained in a separating funnel followed by 0.5 mL of 0.1% w/v BCG solution**.**

The solution was extracted with chloroform and collected chloroform layer was dried over anhydrous sodium sulfate. Volume was made up to 10 mL. A linear graph was obtained at 538 nm against reagent blank prepared simultaneously.

BPB Method

100 µg/mL solution is prepared by taking 1 mL standard solution and made up to 10 mL with DMSO.

Aliquots of standard drug solution (0.1-0.8 mL) were added to 3 mL of phthalate buffer of pH 2.2 contained in a separating funnel followed by 1.0 mL of 0.1% w/v BPB solution.

The solution was extracted with chloroform and collected chloroform layer was dried over anhydrous sodium sulfate. Volume was made up to 10 mL. A linear graph was obtained at 429.5 nm against reagent blank prepared simultaneously.

RESULTS AND DISCUSSION

Analytical data and method validation

The optimum conditions were established by varying one parameter at a time and keeping the others fixed and observing the effect on absorbance of chromogen.

The optimum pH required for complexation, effect of dye concentration and efficiency of the solvent to extract the ion pair were studied with respect to maximum sensitivity, colour stability, adherence to Beer's law and other optimum conditions are incorporated in the procedure (Table 1).

The optical characteristics such as absorption maxima, Beer's law limits, molar absorptivity and Sandell's sensitivity are also presented in Table 1.

The regression analysis using the method of least squares was made to evaluate the slope (m), intercept (b) and correlation coefficient (r) obtained from different concentrations and the results are presented in Table 1**.** The graph showed negligible intercept as described by the regression equation $y = mx + b$, where y is the absorbance and x is the concentration in µg/mL.

Commercially available tablets of gefitinib were analyzed by the proposed method and as additional check on the accuracy of the method, recovery experiments were also conducted by spiking known amounts of pure drug in preanalysed formulation and the recovery was calculated in each of the case using the regression line equation developed under the linearity experiment. The results of recovery experiments are given in Table 2 for

Methods A and B**.** The interference studies revealed the common excipients and other additives usually present in pharmaceutical dosage forms did not interfere in the proposed methods.

The proposed visible spectrophotometric methods enable quantitative determination of gefitinib in bulk drug samples and tablets. Efficient visible spectrophotometric detection at the respective absorption maxima was found to be suitable without any interference from tablet excipients. The calibration curves were linear over a concentration range from 10-100 µg/mL for BCG and 1-8 µg/mL for BPB. The relative standard deviations (R.S.D.) were less than 1% and average recovery was around 100.23%. Analytical results of samples were in accordance with those of standard solution in the same concentrations. The proposed method is fast, precise, accurate, sensitive and efficient and can be used in routine analysis in quality control laboratories.

Table 1: Optical characteristics, precision and accuracy of the method

S. No	Parameter	BCG	BPB
1	λ_{max} (nm)	538	429.5
2	Beer's law range (µg/mL)	10-100	1-8
3	Molar extinction coefficient (L. mole^{-1} cm^{-1})	0.23 x 10^{6}	0.3 x 10^{6}
4	Sandell's sensitivity (µg/cm^2/0.001)	0.097	0.0076
5	Regression equation (y = mx + b)*	0.0095	0.1259
	Slope (m)	0.026	0.0152
	Intercept (b)		
6	Correlation coefficient (r)	0.9984	0.9986
7	Precision (% Relative standard deviation)	0.1617	0.1559

*y = mx + b, where y is the absorbance unit and x is the concentration in µg/mL.

Table 2: Determination of gefitinib in tablets

S. No	Sample (Tablets)	Reagent	Label claim (mg)	Amount *(mg) Found by proposed method	% Recovery**
1	1	BCG	250	251.90	100.76%
		BPB	250	250.00	100.00%
2	2	BCG	250	250.00	100.00%
		BPB	250	250.78	100.31%

*Average of three determinations **After spiking the sample

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