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Research Article

DEVELOPMENT AND VALIDATION OF AN UV-SPECTROMETRIC METHOD FOR ESTIMATION BOSUTINIB IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

A novel, safe and sensitive method of Spectrophotometric estimation in UV-region has been developed for the Bosutinib. The method have been developed and validated for the Bosutinb using Methanol as diluents, which does not show any interference in Spectrophotometric estimations. All the parameters of the analysis were chosen according to ICH [Q2 (R1)] guideline and validated statistically using RSD and %RSD along with neat chromatograms.

Keywords: Bosutinib, UV-Spectrometric Method, Method development, Validation.

INTRODUCTION

Bosutinib is used in treating Chronic Myelogenous Leukemia (CML), Bosutinib, functions as a dual inhibitor of SRC and ABL kinases, and preclinical studies demonstrated a high antiproliferative activity in human CML cell lines and a number of other malignancies. It is the first member of a new class of agents that act by inhibiting particular tyrosine kinase enzymes, instead of non-specifically inhibiting rapidly dividing cells. It is a protein tyrosine kinase created by the Philadelphia chromosome abnormality in chronic myeloid leukemia. The usual tablet dose is 100mg and 500mg. As a very novel and recently synthesized drug, there are only a few references for Bosutinib. As there is no other or few analytical methods available for the estimation of this drug in the bulk and pharmaceutical dosage forms. The chemical structure of Bosutinib is given in Fig 1. Chemically it is 4-(2,4-dichloro-5-methoxyanilino)-6-methoxy-7-[3-(4-methylpiperazin-1-yl)propoxy]quinoline-3-carbonitrile with empirical formula $C_{26}H_{29}Cl_2N_5O_3$. In the present work simple, accurate and precise UV-Spectrometric method has been developed and validated.

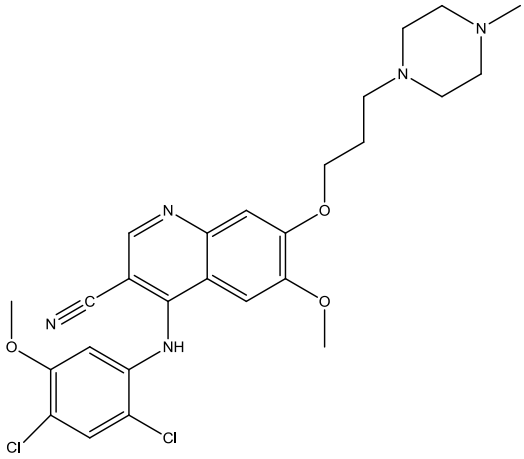

Fig. 1: Chemical Structure of Bosutinib

MATERIALS AND METHOD

Instrumentation

UV-Visible double beam spectrophotometer with matched quartz cells (1cm), Model: Shimadzu Corp. A114550**09290**, Make: Shimadzu

Materials Required

Bosutinib pure standard was purchased from Swapnaroop drug agency (India). Methanol analytical grade were purchased from Dipa enterprises. Bosutinib tablets available under the brand name Bosulif (100mg, Pfizer ltd.) were purchased and used.

Preparation of Standard Stock Solution

10mg of Bosutinib was weighed accurately and transferred to a 10ml volumetric flask containing some amount of methanol. Volume was made up to the mark using methanol to obtain the resulting solution of 1000 µg/mL. The absorbance of the latter was recorded using UV visible spectrophotometer in range 200-400nm.

Preparation of sample Solutions for calibration curve

From stock solution 0.05, 0.1, 0.15, 2.0,2.5 and 3.0 mL solutions were pipetted out and diluted up to 10ml using solvent mixture to obtain resultant solutions of 5, 10, 15, 20, 25 and 30µg/ml. Absorbance for each of these solution was recorded in triplicate and calibration curve was constructed considering mean absorbance of each test solution. From the calibration curve equation of line, correlation coefficient and intercept were determined.

Accuracy

The recovery studies for the method were carried out by standard addition method. It was evaluated at three concentration levels (80,100 and 120%) and the percentage recoveries were calculated. The data is tabulated in table 2.

Precision

From the calibration range three QC standards were define viz. 8, 18 and 20µg/mL as LQC, MQC and NQC respectively. The solutions for QC standards were prepared by diluting stock solution of 0.8, 1.8 and 2.0 ml solutions up to 10 mL. Absorbance of each QC standard was recorded for intraday and inter day precision in triplicates as per ICH guidelines Q_2R_1

Limit of Detection and Limit of Quantification

The Limit of Detection (LOD) and Limit of Quantification (LOQ) were determined based on the standard deviation of the response and the slope of the calibration curve. The sensitivity of the method was established by the LOD and the LOQ values.

Robustness

10µg/mL solution was selected for robustness study for the parameters like wavelength. Wavelength was subjected to minor variation of ± 1 (viz. 266 ± 1). The absorbances for each of these wavelengths were recorded in triplicate. The variation should not be more than 5% RSD.

RESULTS AND DISCUSSION

The proposed method was found to be simple. Linearity was observed in the concentration range of 5-30µg/mL with the regression equation y=0.108x-0.051 and the correlation coefficient of 0.999. No interference was seen from any of the components of the pharmaceutical dosage form indicating the specificity of the method. The recovery studies were performed and the % RSD was found to be in the range 0.017 -1.3. The % RSD was found to be 0.017-1.3 for intraday and 0.04-0.1 for inter day precision studies. Thus the method was found to be accurate and precise as the %RSD was not more than 2%. The limit of detection and limit of quantification for Bosutinib were found to be 0.25µg/mL and 0.76µg/mL respectively. The RSD for the % assay of sample was calculated for parameter in robustness and was found to be less than 2% confirming the robustness of the method.

Table 1: Determination of Wavelength

Sr. No.	Wavelength (in nm)	Absorbance
1	**266.0**	**1.366**
2	296.0	0.321
3	341.0	0.224

Table 2: Data for Linearity

Concentration (µg/ml)	Absorbance (at 266 nm)
5	0.056
10	0.160
15	0.280
20	0.385
25	0.496
30	0.594

Table 3: Percent Accuracy

Sr. No.	Concentration (PPM)	Mean absorbance	Amount recover (mean measured concentration)	% Assay	Limit (95-105%)
1	8	0.306	7.8	100.5	Pass
2	18	0.742	18.2	102.9	Pass
3	28	1.176	27.9	100.7	Pass

Table 4: Data for Precision

Sr. No.	Conc.(µg/ml)	Intra day		Inter day	
		Mean Absorbance*±SD	%RSD	Mean Absorbance*±SD	%RSD
1	8	0.306**±0.04**	1.3	0.304**±0.004**	0.1
2	18	0.745**±0.02**	0.2	0.744**±0.008**	0.1
3	20	1.162**±0.017**	0.017	1.174**±0.005**	0.04

***Mean area of two injections.**

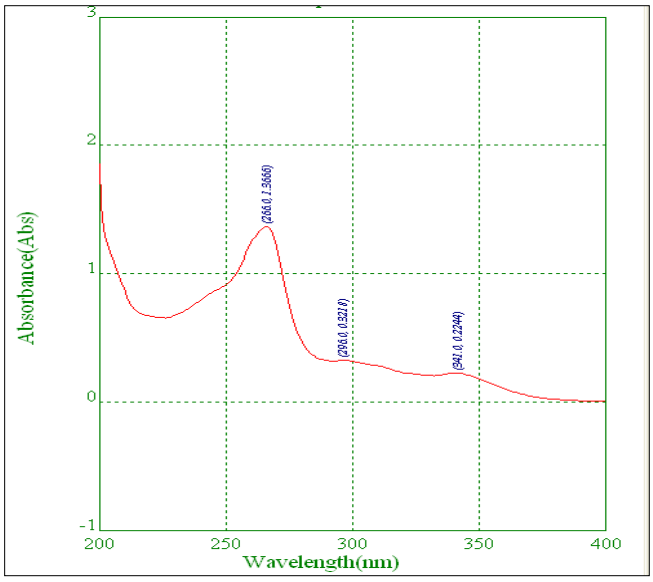

Fig. 2: UV spectrum of Bosutinib (sample)

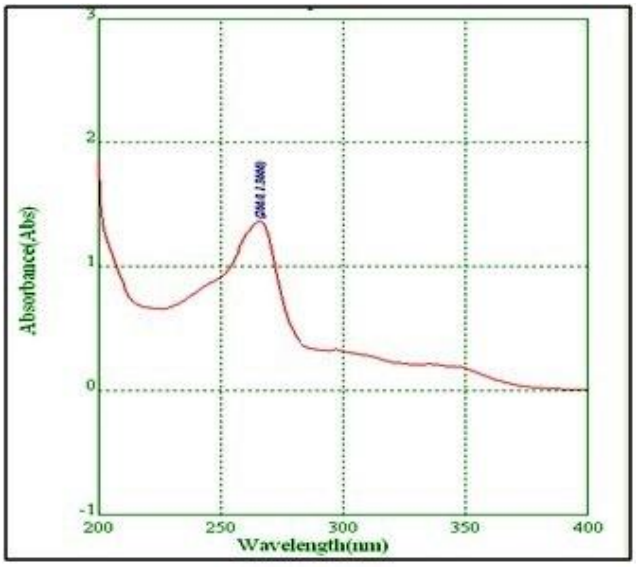

Fig. 3: UV spectrum of Bosutinib (standard)

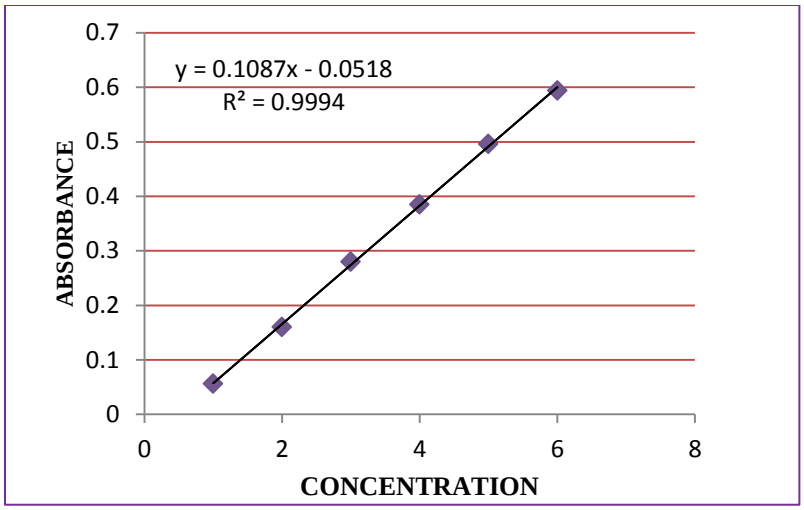

Fig. 4: Calibration Curve of Bosutinib

CONCLUSION

A validated UV-Spectrometric Method was developed for the determination of Bosutinib in bulk forms and tablet dosage form. As the proposed method is simple, rapid, accurate, precise and specific it can be employed for the routine analysis of Bosutinib in pharmaceutical dosage forms.

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