

DEVELOPMENT AND VALIDATION OF AN RP-HPLC METHOD FOR BOSUTINIB IN BULK FORM

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ABSTRACT

An accurate, Precise, Simple and Economical High Performance Liquid Chromatographic method for the estimation of Bosutinib in bulk form has been developed. The method so developed is Reverse Phase High Performance Liquid Chromatographic method using Primesil C18 column (Length: 250nm, Diameter: 4.6nm, Particle size: 5 μ) with a simple methanol and Sodium Phosphate Buffer 10 mm ph 6.5 mixed in a proportion of 85:15 v/v as mobile phase. The retention time for Bosutinib was found to be 7.433 min. The linearity for the method was observed in a concentration range of 10-50 μ g/mL with the correlation coefficient of 0.998. The method so developed was validated in compliance with the regulatory guidelines by using well developed Analytical method validation tool which comprises with the analytical method validation parameters like Linearity, Accuracy, Method precision, Specificity, System suitability, Robustness and Ruggedness. The results obtained were well within the acceptance criteria.

Keywords: Bosutinib, RP-HPLC, 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 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 RP-HPLC method has been developed and validated.

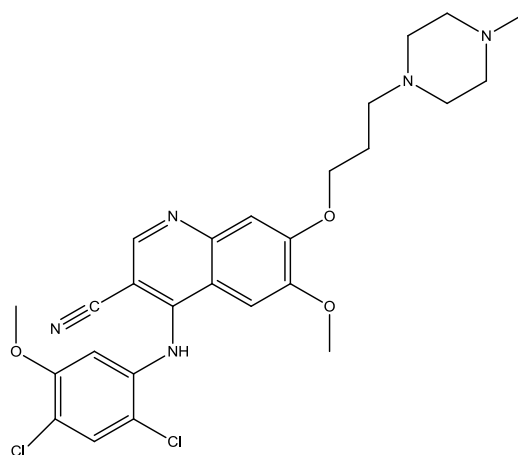


Fig. 1: Chemical Structure of Bosutinib

MATERIALS AND METHOD

Instrumentation

Chromatographic separation was achieved using a C-18 column (250mm x 4.6mm i.d., 5µm particle size) of Younglin (S.K) Gradient system that is equipped with UV Detector

Materials Required

Bosutinib pure standard was purchased from Swapnaroop drug agency (India). Methanol and Water of HPLC grade were purchased from Merck (India) and Qualigens (India) respectively. Bosutinib tablets available under the brand name Bosulif (100mg, Pfizer Ltd) were purchased and used. Optimized Conditions The mobile phase with methanol and Sodium Phosphate Buffer in the ratio of 85:15 %v/v was employed in isocratic mode at a flow rate of 0.7 ml/min .The run time was 10 mins and 20µL of the sample was injected for every run into the column. The wavelength of the UV detector was set at 266nm.

Chromatographic conditions

A mixture of phosphate buffer and methanol in the ratio of 85:15 V/V was found to be the most suitable mobile phase for ideal chromatographic separation of Bosutinib. The solvent mixture was filtered through 0.45 µ membrane filter and sonicated before use. It was pumped through the column at a flow rate of 0.7mL/min. Injection volume was 20µL and the column was maintained at ambient temperature. The column was equilibrated by pumping the mobile phase through the column for at least 30 minutes prior to the injection of the drug solution. The detection of the drug was monitored at 266 nm. The run time was set at 10min.

Preparation of Standard Stock Solution

Accurately about 10mg of Bosutinib was weighed and transferred to a 10mL volumetric flask. 5mL of methanol was added to the flask and sonicated to dissolve it. The volume was then made up to the mark with methanol to get a standard solution of Bosutinib at a concentration of 1000µg/mL

Preparation of sample Solutions

Working solutions for HPLC injections were prepared on daily basis. Aliquots of the standard stock solution were taken and diluted with the mobile phase to get solutions in a concentration range of 10-50 µg/mL.

Linearity

Several aliquots of standard solution of Bosutinib was taken in different 10 mL volumetric flasks and diluted up to the mark with diluents such that the final concentrations of Bosutinib were in the range of 10 to 50 µg/mL. Evaluation of the drug was performed with UV detector at 266 nm, peak area was recorded for all the peaks. The correlation coefficient value of Bosutinib was 0.998. The results show that an excellent correlation exists between peak area and concentration of drug within the concentration range indicated. The data is tabulated in table 1.

System suitability

System suitability parameters like retention time, theoretical plates and tailing factor were calculated and compared with standard values.

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

The precision of the method was determined by intra and inter day precision studies. This was evaluated by injecting three independent sample preparations of Bosutinib from a single formulation at three different concentration levels on the same day (Intra day) and on three different days (Inter day).The %RSD was then calculated. The data is represented in table 3.

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

Robustness was established by introducing small changes in the HPLC optimized conditions which include mobile phase ratio (± 1), flow rate ratio (± 0.1) and wavelength(± 1). This was studied using two replicates at a concentration level of 40µg/mL of Bosutinib.

RESULTS AND DISCUSSION

The proposed method was found to be simple. Linearity was observed in the concentration

range of 10-50 μ g/mL with the regression equation $y=49.85x+ 44.21$ and the correlation coefficient of 0.999. System suitability parameters indicates high column efficiency with large number of theoretical plates (>2000). The tailing factor was found to be 1.21 which is does not exceed the critical value (2). The average retention time was found to be 7.433. 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 0.24 -1.29. The % RSD was found to be 0.199-1.21 for intraday and 0.197-0.66 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 0.297 μ g/mL and 0.901 μ g/mL respectively. The RSD for the % assay of sample was calculated for each parameter in robustness and was found to be less than 2% confirming the robustness of the method.

Table 1: Data for Linearity

Conc.(μ g/mL)	Avg. peak Area
10	518.20
20	1083.73
30	1512.37
40	2064.20
50	2520.65

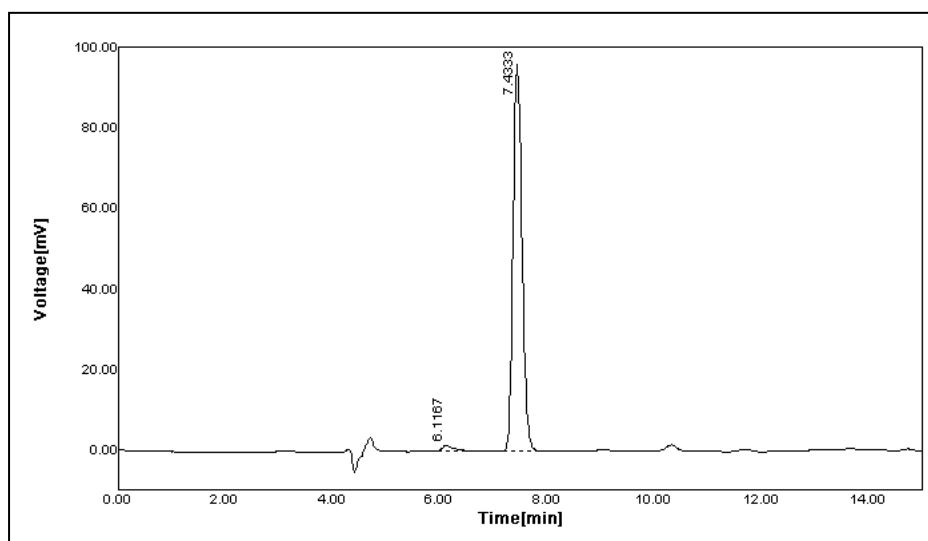
Table 2: Recovery Studies for Bosutinib

% Spike Level	Amount Added(μ g/mL)	Peak Area	Amount Found(μ g/mL)	%Recovery	Mean % Recovery
80	8	939.44	17.95	99.48	98.58
80	8	932.27	17.81	97.68	
100	10	1036.8	19.91	99.11	98.94
100	10	1035.11	19.87	98.77	
120	12	1124.73	21.67	97.29	98.06
120	12	1134.37	21.86	98.83	

Table 3: Data for Precision

Sr. No.	Conc.(μ g/ml)	Inter day		Intra day	
		Mean Area \pm SD	%RSD	Mean Area \pm SD	%RSD
1	10	545.18 \pm 3.60	0.66	544.03 \pm 6.59	1.21
2	30	1567.62 \pm 3.09	0.20	1545.30 \pm 14.27	0.92
3	50	2531.00 \pm 10.34	0.41	2556.72 \pm 5.10	0.20

*Mean area of two injections.

**Fig. 2: Chromatogram of Bosutinib**

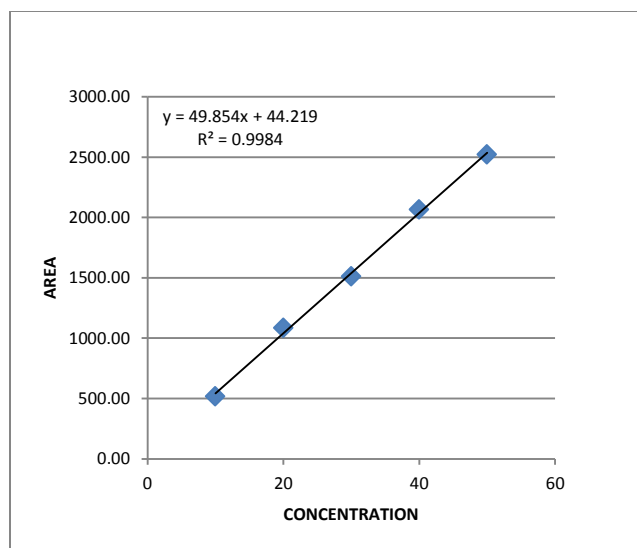


Fig. 3: Calibration Curve of Bosutinib

CONCLUSION

A validated RP-HPLC method was developed for the determination of Bosutinib in tablet dosage form and bulk forms. 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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