WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 6.805
Volume 5, Issue 12, 417-424. Review Article ISSN 2277– 7105

RP-HPLC METHOD FOR ESTIMATION OF BOSUTINIB IN BULK FORM AS PER ICH GUIDELINES

Vidya L. Chaudhari* and Amol A. Kulkarni

Siddhant College of Pharmacy, Pharmaceutical Quality Assurance Department, Sudumbare, Pune-412109.

Article Received on
14 Oct. 2016,

Revised on 04 Nov. 2016,
Accepted on 24 Nov. 2016

DOI: 10.20959/wjpr201612-7485

***Corresponding Author**
Vidya L. Chaudhari
Siddhant College of Pharmacy, Pharmaceutical Quality Assurance Department, Sudumbare, Pune-412109.

ABSTRACT

An accurate, Precise, Simple and Economical High Performance Liquid Chromatographic method for the estimation of Bosutinib in bulk form has been developed. The drug were resoled using a methanol and potassium dehydrogenase Phosphate Buffer 10 mm ph. 5.9 mixed in a proportion of 90:10 v/v as mobile phase on an Primesil C18 column (Length: 250nm, Diameter: 4.6nm, Particle size: 5µ). The linearity for the method was observed in a concentration range of 10-50µg/mL with the correlation coefficient of 0.998. The retention time for Bosutinib was found to be 6.9 min. Detection wavelength chosen was 266 nm. The accuracy studies of RP-HPLC method was performed at three different levels, i.e. 80%, 100%, 120% and recovery was found to be in the range of 99.58 to 101.25% for Bosutinib respectively. 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 4-[(2,4-4dichoro-5-methoyphenyl)amino]-6-methoxy-7-[3(-methylpiperazin-1-yl)propoxy]quinoline- 3-Carbonitrile (fig.1). Bosutinib is a Pale yellow powder with molecular formula $C_{26}H_{29}Cl_2N_5O_3$, Bosutinib has a molecular weight of 530.446 g/mol. Bosutinib is used or treatment of chronic myeloid leukemia (CML), also known as “chronic

myelogenous leukemia," is a type of cancer that starts in the blood-forming cells of the bone marrow and invades the blood. 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.

Literature survey revealed that Bosutinib was very few analytical methods have been reported for the estimation of Bosutinib. In the present work the authors have developed a simple, rapid, precise, accurate and robust stability indicating liquid chromatographic method for the determination of Bosutinib in tablets as per ICH guidelines. The drug substance is soluble in ethanol and methanol.

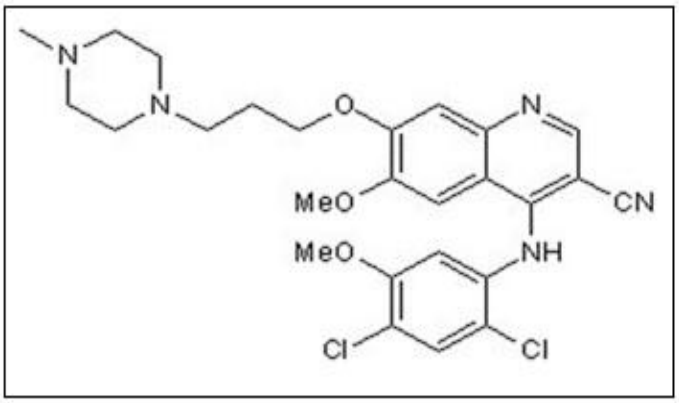

Fig 1. Chemical structure of Bosutinib

MATERIAL AND METHODS

- **Experimental selection**

Drug purchased from local market, Methanol (Merck), Acetonitrile (Merck), Purified water (NA), Ortho- phosphoric acid (Merck).

- **Instruments**

Jasco HPLC system, pump (Model PU 2080 Plus), Rheodyne sample injection port with 20µl loop, Column (Hypersil BDS C18), Detector (MD 2010 PDA), Software (Borwin- PDA (version 1.5), Shimdazu Model AY-120 Balance, Jasco Model V-550 UV-Visible Double beam spectrophotometer with single Monochromator, Elga Lab (PURELAB UHQ-II) water purification system.

- **Selection of analytical wavelength**

From the standard stock solution further dilutions were done using methanol and scanned

over the range of 200 – 400 nm. The spectrum was obtained. It was observed that the drug showed considerable absorbance at 266 nm (Fig.2).

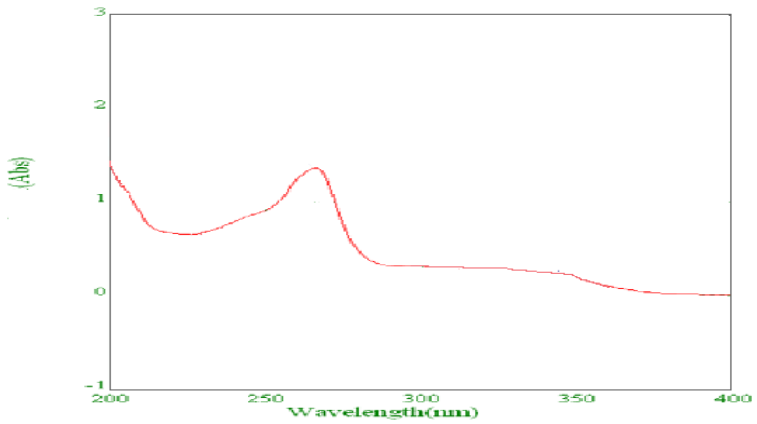

Fig.2: UV Spectrum of Bosutinib (10µg/ml Preparation of Mobile Phase

- **Buffer Preparation**

0.1 M potassium dihydrogen phosphate buffer was prepared by dissolving 1.360 gm of phosphate in sufficient water to produce 1000 ml with pH 5.9 adjusted by Orthophosphoric - phosphoric acid.

- **Solvent mixture**

Prepare mixture by mixing methanol and 0.01 M potassium dihydrogen phosphate buffer (pH 5.9) in the ratio of 90:10 v/v.

- **Preparation of mobile phase**

Mobile phase was prepared by mixing methanol and potassium dihydrogen phosphate buffer (adjust pH. 5.9 With OPA) in the ratio 90:10 v/v. It was then filtered through 0.45 µm membrane filter paper using vacuum filtration assembly and then sonicated on ultrasonic water bath for 15 min.

- **Preparation of standard stock solution**

Standard stock solution of Bosutinib was prepared by dissolving 10 mg of drug in 10 ml of Methanol to get concentration of 1000 µg/ml (A). From the standard stock solution, working standard solution was prepared in mobile phase containing 100 µg/ml of Bosutinib (B). Further dilution was made in mobile phase to get final solution of Bosutinib (8µg/ml).

- **Selection of mobile phase**

The standard solution of Bosutinib (10µg/ml) was injected into the HPLC system and run in different solvent systems. Different mobile phases like methanol and water, methanol and acetate buffer, methanol and acetonitrile, methanol and potassium dihydrogen phosphate buffer in varying proportion of mobile phase components, varying conditions of pH were tried in order to obtain the desired system suitability parameters for the Bosutinib. After several trials, methanol and potassium dihydrogen phosphate buffer 10 mm pH 5.9 with opa in the ratio of 90: 10 v/v was chosen as the mobile phase, which gave good resolution and acceptable peak parameters.

Optimized chromatographic conditions

Sr. No.	**Parameter**	**Conditions used for Analysis**
1	Column	Hypersil BDS C18 (4.6 x 250 mm)5 µm particle size)
2	Mobile phase	Methanol: potassium dihydrogen phosphate buffer with ph. 5.9 with opa (90:10v/v)
3	Flow rate	1 ml/min
4	Detection Wavelength	266 nm
5	Sample Volume	20µl
6	Column temperature	Ambient

Method validation

- **Linearity**

Linearity was tested by injecting the linearity solutions prepared in the range of concentrations 10-50µg/ml. Each sample in two replicates was analyzed and peak areas were recorded. The response factors were plotted against the corresponding concentrations of Bosutinib to obtain the calibration curve. Figure 3 and 4 represents the linearity and calibration curve for Bosutinib respectively. Result of linearity shown in table no.1.

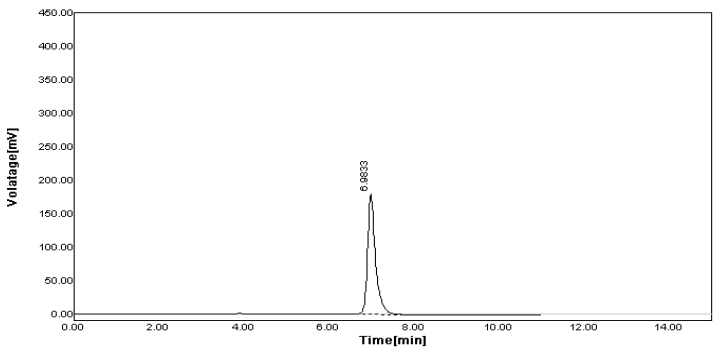

Fig: 3.2 Chromatogram of Bosutinib

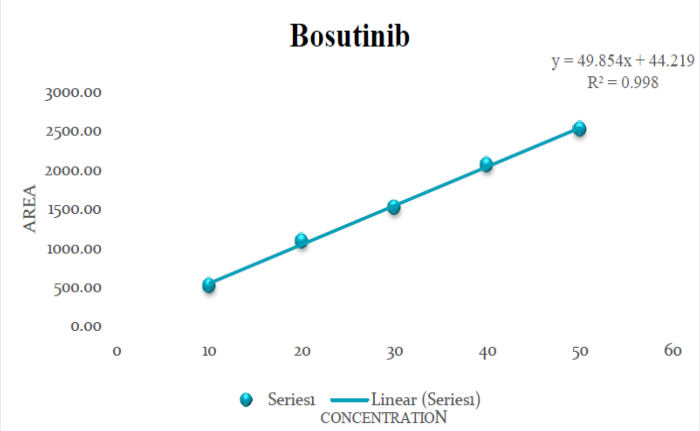

Fig.4: Calibration curve for Bosutinib

- **System suitability**

System suitability parameters were calculated and compared with standard values.

- **Accuracy**

To check accuracy of the method, recovery studies were carried out by adding standard drug to sample (Excipients) at three different levels 80%, 100% and 120%. Basic concentration of sample chosen was 10 µg/ml of standard. These solutions were injected into HPLC system in triplet to obtain the chromatogram.

The drug concentrations were calculated by using linearity equation of Bosutinib. The results obtained are shown in (Table 2).

- **Precision**

The precision of the method was demonstrated by intra-day and inter-day studies. In the intra-day studies, 2 replicates of 2 standard solutions (10, 30and 50µg/ml) were analyzed in a same day and percentage RSD was calculated (Table 3). For the inter-day variation studies, 6 replicates of 3 standard solutions (10, 30 and 50µg/ml) were analyzed on 2 consecutive days and percentage RSD was calculated (Table 4).

- **Robustness**

Robustness of the method was determined by carrying out the analysis under conditions during which flow rate, Concentration (Strength) of buffer, mobile phase ratio were altered and the effects on the peak area were noted. The robustness of the method evaluated by assessing the effect of variations in method parameters on peak areas showed low RSD values (less than 2%) indicating robustness of the method (Table 5).

Table 1: Linearity of Bosutinib

Conc. (µg/ml)	Replicates	Area	Mean	SD	%RSD
10	1	374.218			
	2	378.31	376.26	2.89	0.77
20	1	635.2			
	2	633.46	634.33	0.48	0.19
30	1	913.97			
	2	922.22	918.10	5.83	0.64
40	1	1183.82			
	2	1194.84	1189.33	7.79	0.66
50	1	1421.2			
	2	1399.53	1410.37	15.32	1.08

Table 2: Recovery studies of Bosutinib

Level	Theoretical Conc. (µg/ml)	Area	Recovered Conc. (µg/ml)	% Recovery
80%	8	440.79 441.28	7.95 7.96	99.37 99.56
100%	10	545.29 543.80	10.05 10.02	100.79 100.21
120%	12	650.80 654.10	12.16 12.23	101.40 101.95

Table 3: Intra-day precision study of Bosutinib

Concentration (µg/ml)	Area	Mean Area	SD	% RSD
10	535.09	530.32	6.74	1.27
525.56				
30	744.31	749.26	7	O.93
754.21				
50	999.83	996.01	5.40	0.54
992.2				

Table 4: Inter-day precision of Bosutinib

Concentration (µg/ml)	Area	Mean Area	SD	% RSD
10	478.85	476.80	2.91	0.61
474.74				
30	669.48	670.57	1.55	O.61
671.67				
50	867.61	872.40	6.77	0.78
877.18				

Table No 5. Robustness study

Drug			% RSD Found For Robustness Study(peak area)			
	Flow Rate (9 ml/min)		Wavelength (266)		Mobile phase ratio (90:10)	
	0.9	1.1	265	267	91: 14	89: 16
Bosutinib	0.88	0.12	0.92	0.32	0.08	0.33

Table No. 6: Summary of validation parameter

Sr. No.	Validation parameters	Bosutinib
1.	Linearity Equation (r2) Range	Y=49.85X+44.21 r2 = 0.998 10-50µg/ml
2.	Precision (% RSD) Interday Intraday	 0.58 1.27
3.	Accuracy	% Recovery
	80	99.55
	100	100.00
	120	101.25
4.	Limit of Detection	0.29µg/ml
5.	Limit of Quantitation	0.90µg/ml
6.	Specificity	Specific
7.	Robustness	Robust

CONCLUSION

The developed method was validated according to the ICH guidelines. The linearity, precision, range, robustness were within the limits as specified by the ICH guidelines. Hence the method was found to be simple, accurate, precise, economic and reproducible. So the proposed methods can be used for the routine quality control analysis of Bosutinib in bulk drug as well as in formulations.

REFERENCES

1. Richat A, Joseph B and Daryl S, Effect of rifampin on the pharmacokinetics of bosutinib, a dual Src/Abl tyrosine kinase inhibitor, when administered concomitantly to healthy subjects.
2. Kuna A. K, Kumar K. J, Rp-Hplc Method Development And Validation Of Imatinib Mesylate In Tablet dosage Form, International Journal Of Pharmacy And Pharmaceutical Science, Nov 2011; 162-165, 0975- 1491.
3. Sowjanya G, Annapurna M. M and Sriram A. V, Development And Validation Of Stability Indicating Rp- Hplc Method For The Determination Of Nilotinib (A Tyrosine Kinase Inhibitor), Indo American Journal Of Pharmaceutical Research, 2013; 4541-4551.

4. Shreedevi A and Lakshmana Rao A., Development and Validation of Novel HPLC Method for the Estimation of Dasatinib In Bulk and Pharmaceutical Dosage forms, International journal Of Research in Pharmacy and Chemistry, 2013; 724-729.
5. Ramachandran B and Naidu NVS, Validation of RP-HPLC Method for Estimation of Dasatinib in Bulk and Its Pharmaceutical Dosage Forms. International journal of pharmacy and biological sciences. 2014; 61-68, 2230-7605.
6. Abbas R, Leister C, Gaaloul M. El, Chalon S, Sonnichsen D. Ascending single-dose study of the safety profile, tolerability, and pharmacokinetics of bosutinib coadministered with ketoconazole to healthy adult subjects. Clin Ther, 2012; 34: 2011–9.
7. Phani RS, Prasad KRS and Reddy U. Scientific approach for RP-HPLC method development: complete review. J Caribbean Journal of Science and Technology. 2016; 2: 896-903, 2249-5347.
8. Khopkar S. M, Basic concepts of analytical chemistry, 2nd ed.; New age International Ltd. Publishers, New Delhi, 1998; 178-179.
9. United State Pharmacopeia, USP 29/NF 24, US Pharmacopeial Convention, Inc, Rockville MD, 2006; 555-556 1777, 1861, 3050-3052.
10. Chakravarthy VK and Sankar DG. Development and validation of RP-HPLC method for estimation of Erlotinib in bulk and its pharmaceutical formulation. J Rasayan. 2011; 4: 393-399, 0974-1496.
11. Sethi P. D, High Performance Liquid Chromatography, Quantitative Analysis of Pharmaceutical Formulations 1st ed.; CBS Publishers and Distributors, New Delhi, 2007; 1-11, 116-120.
12. Ivaturi R, Manikya Sastry T, Satyaveni S. Development and validation of a stability indicating hplc method for the determination of Nilotinib Hydrochloride in bulk and pharmaceutical dosage form. International Journal of Pharmacy and Pharmaceutical Sciences. 2016; 8(9): ISSN- 0975-1491,41-48.
13. BOSULIF® (bosutinib). Full Prescribing Information, Pfizer Labs, New York, NY, 2012.