METHOD DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR ESTIMATION OF BOSUTINIB IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

An accurate, simple, precise, rapid and robust RP-HPLC method has been developed for estimation of bosutinib in bulk and pharmaceutical dosage form. The separation was achieved on C18 column (250 mm×4.6mm, 5µm), using Acetonitrile: Methanol: water (80:5:15) as a mobile phase, at flow rate 1.0 ml/min. Detection was carried out at 246 nm and drug eluted with a retention time were 5.63 min. Beer's law was obeyed with the concentration range of 20-100µg/ml with correlation coefficient 0.999. The method has been validated according to ICH guidelines for linearity, accuracy, precision, robustness, range, LOD and LOQ. The method was found to be accurate, simple, precise, robust and rapid. The proposed method was convenient for quantitative routine analysis and quality control of bosutinib in bulk and pharmaceutical dosage form.

Keywords- bosutinib, RP-HPLC, Validation.

INTRODUCTION

Bosutinib is a cancer medication prescribed to treat leukemia[1]. It operates by inhibit protein associated with cancer cell growth in order to relieve symptoms, prevents the spread of cancer cells and aid other treatments. The U.S. FDA approved bosutinib in 2012 to treat chronic myelogenous leukemia (CML), blood and bone marrow disease that usually affects older adults. The drug is designed to inhibit tyrosine kinases such as bcr-abl and src used to treat chronic myelogenous leukemia (CML)[2,3].

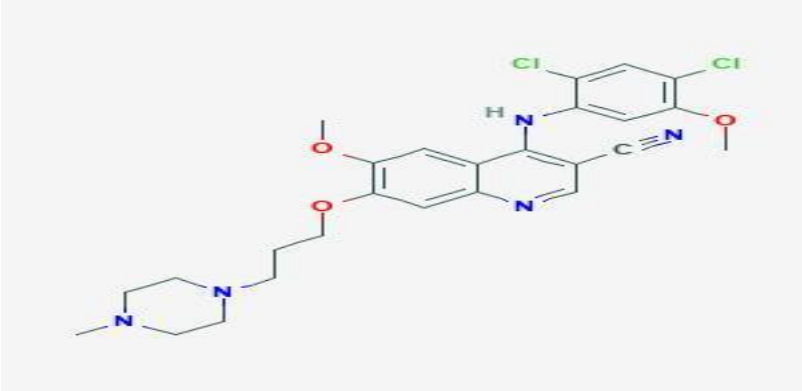

Fig. No. 1 Chemical structure of Bosutinib

The chemical name of bosutinib is 4-(2, 4-dichloro-5-methoxyanilino)-6-methoxy-7-[3-(4-methylpiperazin-1yl) propoxy] quinoline-3-carbonitrile. It has a molecular formula of $C_{26}H_{29}CL_2N_5O_3$ and molecular weight of 530.45 g/mol. It has the structure formula (fig.1). Bosutinib is a yellow crystalline powder which in soluble in dimethyl sulfoxide, ethanol, and acetonitrile[4,5].

The literature survey revealed that the drug has been estimated by liquid chromatography method in biological fluids like human plasma and ultraviolent detection, RP-HPLC method for pharmaceutical formulation, RP-HPLC method in bulk form has been reported so far.

The aim of present work is to develop and validate simple, accurate, robust, rapid and precise RP-HPLC method for estimation of bosutinib in bulk and pharmaceutical dosage form.

MATERIALS AND METHODS

Instruments

Chromatographic separation was performed on water 2695 HPLC system equipped with C18 column (250mm × 4.6mm, 5.0 µm), Single pump, degasser, Dual λ absorbance detector and injector with 10 µl loop volume.

Chemicals and reagents

Bosutinib pure form was obtained from cubic lab Ankleshwar. The Acetonitrile (HPLC grade), Water (HPLC grade), Methanol (HPLC grade), Orthophosphoric acid (AR grade) were used.

Preparation of mobile phase

The mobile phase consisted of Acetonitrile: Methanol: Water (80:05:15 v/v/v) and pH adjusted to 7.0 with ortho phosphoric acid was used. The elution mode used was Isocratic. Mobile phase was filtered through a 0.22 µm nylon membrane filter and degassed prior to use.

Preparation of standard stock solution

A standard stock solution of Bosutinib (1000 µg/ml) was prepared by dissolving 100 mg of drug in 100 ml of volumetric flask and dissolve with 100 ml of Acetonitrile. The obtained concentration of bosutinib was 1000 µg/ml. The resulting solution was sonicated for 15 min.

Optimization of analytical wavelength

Optimization of analytical wavelength was done by scanning bosutinib standard solution in the range of 200-400 nm. By observing the spectra of standard solution λ_{max} at 246 nm were taken for trials to develop UV method.

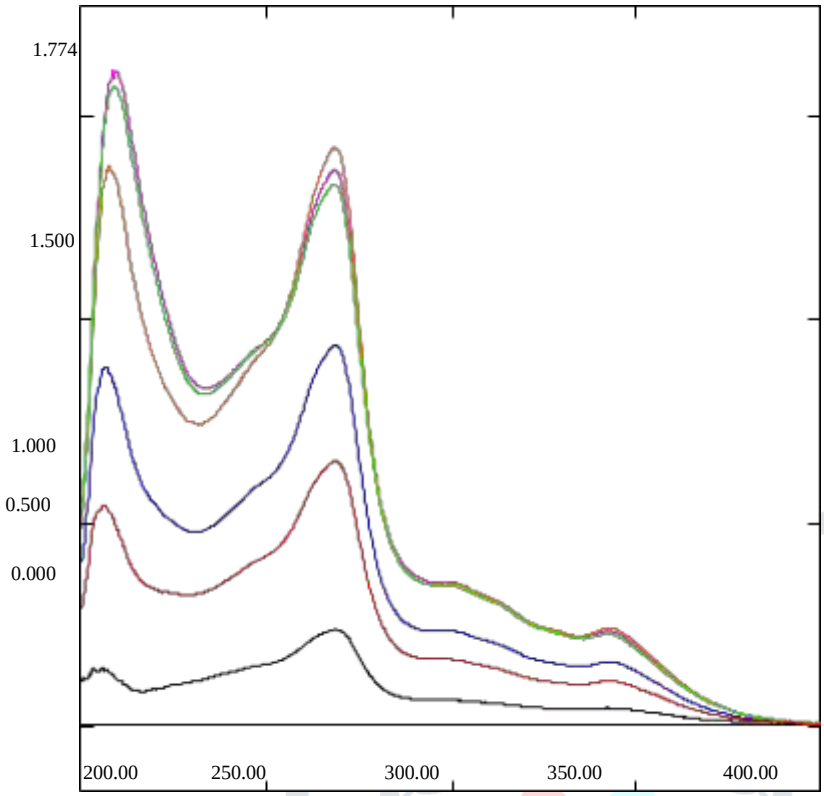

Fig 2. UV spectra of bosutinib

Optimized chromatographic conditions

Optimization of chromatographic conditions were done by performing different trials by taking different mobile phase and varying their compositions, flow rates. Finally an optimized chromatogram was obtained. The system suitability parameters were.

Retention Time (min) : 5.63

Peak Area : 689554

Theoretical plates : 8653

Asymmetry (10%) : 0.26

Resolution : 9.56

Procedure for calibration curve

The calibration curve was prepared in a concentration range of 20-100 µg/ml by taking 0.2-1.0 ml of standard stock in 10 ml volumetric flask and volume was made up to the mark with mobile phase. The resulting solution were filtered through 0.45 µ membrane filter paper and the filtrate was used for analysis.

Estimation of bosutinib in tablet dosage form

The synthetic mixture of bosutinib was prepared. Below all ingredients were shift and blend to make uniformity of mixing. Take Powder equivalent about 10 mg BST in 100 ml volumetric flask. Dissolve Content in 25 ml of Acetonitrile and Sonicated for 15min. Dilute up to 100 ml with Solvent shake vigorously, Filter solution through Whatman filter paper No. 42 and further dilute. The sample formulations were injected and chromatograms were recorded at 246 nm. The amount of drug estimated from the calibration curve.

Sr. No	**Drug-Excipient Name**	**Quantity** (mg)	**Quantity** (mg)
1	Bosutinib	10	200
3	Starch	30	600
4	Magnesium Stearate	10	200
5	Lactose	20qs	400qs
Total		100	2000

RESULT AND DISCUSSION

A reverse phase HPLC method was developed by keeping in mind system suitability parameters i.e. tailing factor (T), number of theoretical plate (N), run time and cost effectiveness. The optimized method developed resulted in the elution of bosutinib at 5.63 min. fig 3 represents standard chromatogram of bosutinib. The total run time is 10 min. The system suitability tests are the integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time (R_t), number of theoretical plates (N), and peal asymmetric factor were evaluated for six replicate injections of the standard at working concentration. The results given in table 1.

In order to test the applicability of the developed method to a synthetic mixture was chromatographed at working concentration 60 µg/ml. The sample peak was identified by comparing the retention time with the standard drug. System suitability parameters were within the acceptance limits, ideal for the chromatographed sample. Integration of standard peak area was done and drug concentration was determined using the peak area concentration relationship obtained in the standardization step. The protocol affords reproducible assay of the drug in the sample ranging between 98-102%, which is the standard level in the any pharmaceutical quality control.

The obtained chromatogram showed no interference from excipients fig 3. The system suitability parameters were within the limits. The developed method was simple, economical and the retention time that showed that the method was rapid. The developed method was validated as per ICH guidelines.

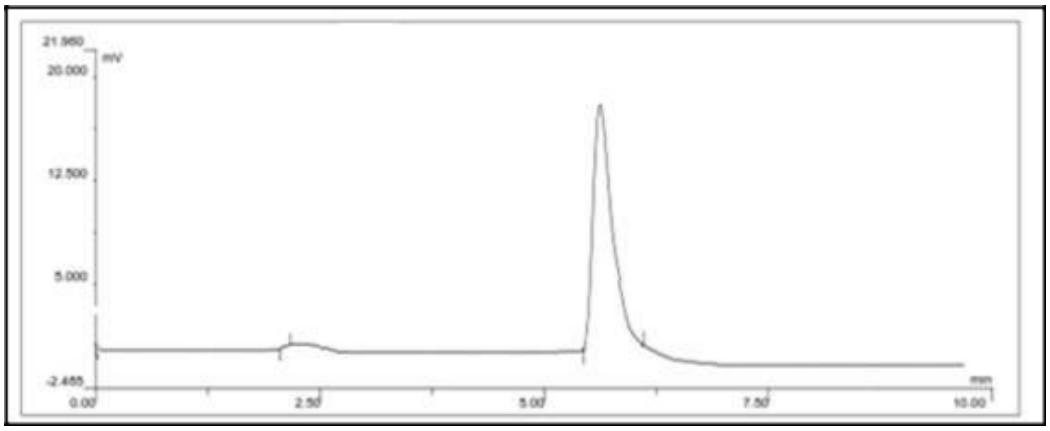

Fig 3. Chromatogram of bosutinib

PARAMETERS	Obser BST*	IP'2010 Specification
Retention Time (min)	5.63	RSD<1%
Peak Area	689554	-
Theoretical plates	8653	Not less than 2000
Asymmetry (10%)	0.26	Not greater than 2
Resolution	9.56	>2

Table 1 Result from system suitability studies

METHOD VALIDATION

Validation is the analytical method is the process that establishes by the laboratory studies in which performance characteristics of the method meet the requirements for the intended analytical application. RP-HPLC developed the method was validated according to International Conference on Harmonization (ICH) guidelines for validation of analytical procedures. The method was validated for the parameters like system suitability, specificity, linearity, precision, accuracy, robustness, and ruggedness, limit of detection (LOD) and limit of quantification (LOQ)[6,7].

Specificity

Specificity was checked for the interference of excipients in the analysis of sample solution and was determined by injecting sample solution with added excipients under optimized chromatographic conditions to demonstrate separation of bosutinib in from excipients. There is no interference of excipient peak on the peak of bosutinib indicating the high specificity of method[8,9].

Linearity

Calibration curve was plotted for different concentration of sample solution prepared from stock solution. Figure 4 shows linearity over the concentration range of 20-100 µg/ml shown in table 02. Each sample was injected six times and calibration curve was constructed by plotting the peak area verses drug concentration.

Concentration (µg/ml)	Peak area
20 µg/ml	30782.8
40 µg/ml	62367.5
60 µg/ml	95638
80 µg/ml	126485.7
100 µg/ml	158640.2

Table 2 linearity data for bosutinib

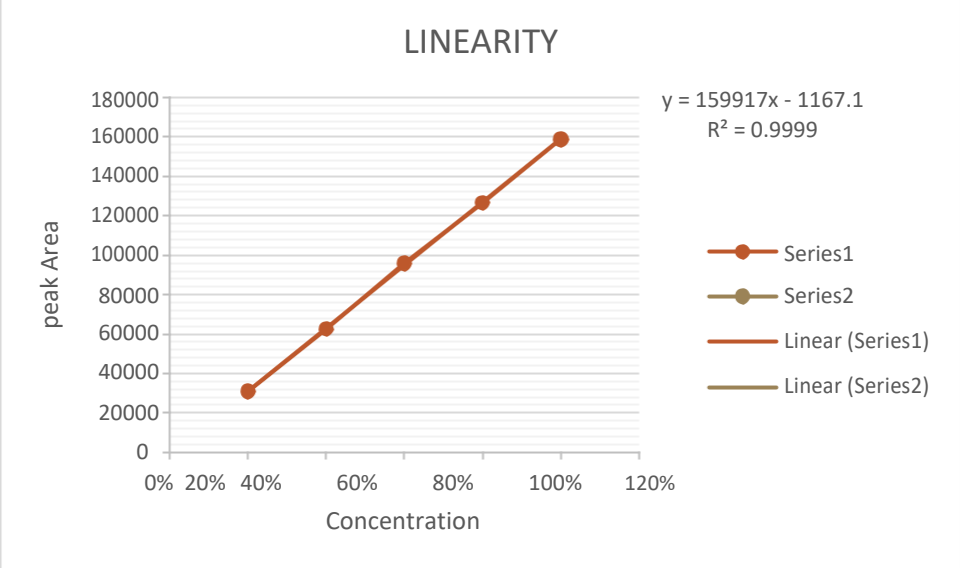

Figure 4. Calibration curve for bosutinib

Regression parameter	Bosutinib
Regression equation	y = 159917x - 1167.1
Slope	159917
Intercept	1167.1
Correlationcoefficient (r^2)	0.9999

Table 3. Regression parameters table for bosutinib

Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The precision was Intraday and inter day precision[10].

Intraday precision- it is determined by analyzing three different concentration solution of bosutinib within the same day with specific interval. Intraday precision is estimated by analyzing 40µg/ml, 60µg/ml, 80µg/ml, 100µg/ml within the same day.

Interday precision- it is determined by analyzing three different concentration of solution of bosutinib in three different days over the period of week. Inter day precision is estimated by above mention concentration for three different days over the period of week.

Sr no	40 µg/ml	60 µg/ml	80 µg/ml
1	62245	95645	126494
2	62344	95630	126483
3	62350	95633	126583
AVG	62313	95636	126520
SD	58.96609	7.937254	54.83612
% RSD	0.094629	0.008299	0.043342

Table 4. Intraday precision

Sr. No	Day 1			Day 2			Day 3		
	40 µg/ml	60 µg/ml	80 µg/ml	40 µg/ml	60 µg/ml	80 µg/ml	40 µg/ml	60 µg/ml	80 µg/ml
1	62224	95630	126500	62324	95621	126510	62201	95625	126460
2	62360	95645	126440	62332	95660	126502	62231	95631	126450
3	62350	95700	126450	62360	95601	126555	62331	95645	126440
AVG	62311.33	95658.33	126463.3	62338.67	95627.33	126522.3	62254.33	95633.67	126450
SD	75.79798	36.85557	32.1455	18.90326	30.00556	28.57155	68.06859	10.2632	10
%RSD	0.121644	0.038528	0.025419	0.030323	0.031378	0.022582	0.10934	0.010732	0.007908

Table 5. Interday precision

Accuracy

The accuracy of the method was obtained by spiking 80, 100 and 120% of bosutinib standard concentration, in which the amount of marketed formulation kept constant and the amount of pure drug was varied. Solution were prepared in triplicates and accuracy was indicated by % recovery which was between 99.83 to 100.18%. The result were shown in table 6.

Level	Amount taken	Amount added	Total amount	Found area	Concentration	% recovery
80 µg/ml	20	16	36	56390	35.99	99.97
	20	16	36	56450	36.03	100.08
	20	16	36	56299	35. 94	99.83
100 µg/ml	20	20	40	62698	39.94	99.85
	20	20	40	62760	39.97	99.85
	20	20	40	62771	39.98	99.92
120 µg/ml	20	24	44	69322	44.08	100.18
	20	24	44	69110	43.94	99.86
	20	24	44	69185	43.99	99.98

Table 6. Accuracy of bosutinib

Robustness

Robustness was carried by varying three parameters deliberately from the optimized chromatographic conditions like mobile phase, flow rate and pH. The RSD was found to be < 2, shown in table 7.

Parameters	values	Peak area	AVG	SD	% RSD
PH	7.0	689554	689553.3	295.4426	0.042847
	7.01	689545			
	6.95	689561			
Flow rate	1.0	689554	689535.3	133.665	0.019384
	1.1	689231			
	0.9	689821			
Mobile phase composition (ACN:methanol:water)	80:05:15	689554	689546.3	8.020806	0.001163
	82:03:15	689676			
	70:10:20	689409			

Table 7. Robustness results of bosutinib

Limit of detection and limit of quantification

The LOD and LOQ of the present method were calculated based on standard deviation of the response and slope of linearity curve[11,12]. The LOD & LOQ values of bosutinib were found to be 0.00062 ug/ml and 0.001879 ug/ml.

CONCLUSION

Thus, the developed method was found to easy, simple, rapid, robust, selective and precise for the routine estimation of bosutinib in bulk and pharmaceutical dosage form.

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