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Development of a Dissolution Method Validation Technique Using UV-spectrophotometry for Bosutinib 500 mg Tablet

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ABSTRACT

Bosutinib is a tyrosine kinase inhibitor used in the treatment of chronic myelogenous leukemia. Any validated dissolution study of bosutinib by UV-spectrophotometric method is yet to be published in any literature. Thus, the current study is designed to propose a validated dissolution method of bosutinib 500 mg tablet using UV-spectrophotometric method. The dissolution parameters were set according to the updated dissolution guideline set by FDA. Validation parameters such as specificity, linearity and range, accuracy, precision, robustness, and system suitability were checked according to ICH guidelines. After confirming linearity and specificity of the method, accuracy was indicated afterwards by the %relative standard deviation (%RSD) value of 0.8%. The precision of the method was confirmed by intra-day repeatability (average 96.2%, %RSD 1.6) and inter-analyst intermediate precision (analyst 1: 96.2%, analyst 2: 91.91%, %RSD 2.8) studies. Further, the method was not affected by deliberately changing parameters such as wavelength. System suitability study showed that %RSD of absorbance was less than 2.0 (0.1%) while experimenting with different replicates. In summary, the study indicated that the proposed validation method for the dissolution study of bosutinib is simple, cost-effective, and replicable in any laboratory setting.

Keywords: Bosutinib, Dissolution, Method development, Method validation, UV-spectrophotometric method.

INTRODUCTION

Bosutinib (BTN) is a newly approved antineoplastic agent used against several forms of cancers such as leukemia of white blood cells in patients not responsive to other treatment strategies[1,2]. Initially, bosutinib had been explored as a dual retardant of Src and Abl proto-oncogene tyrosine kinases[3,4]. However, later investigations revealed it as an inhibitor of Bcr-Abl tyrosine kinase, the receptor involved in generating cancer cells[4,5]. Besides, it was also found that bosutinib has a strong serine threonine or tyrosine kinase inhibiting capability and mutation has been altered by interacting with both active and inactive form of Abl kinase domain[6-8]. The drug might also be effective against gastrointestinal stromal tumor and overexpression of some growth factors[9]. BTN

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is often used to treat elder patients with chronic myelogenous leukemia after achieving certifications from regulatory bodies, namely, Food and Drug Administration (2012) and European Medical Agency (2013) (10-13). The optimum therapeutic dose of bosutinib is 500 mg orally[14].

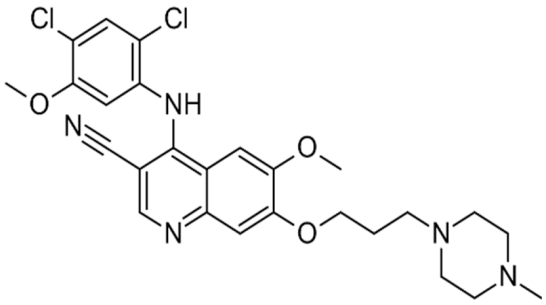

Fig. 1. Chemical structure of bosutinib

As a newly discovered drug, BTN is yet to be included in any pharmacopoeia. However, FDA established some dissolution criteria for this drug[15,16]. A validation method of BTN about the estimation of the drug in bulk in tablet form had been published already[17]. But, there are no existing published regarding the dissolution method validation of the drug yet. The reproducibility of the dissolution method can be ensured by several validation parameters, such as accuracy, linearity, specificity, precision, etc[18]. The current study had been designed to explore a simple and efficient technique of UV-spectrophotometric analysis of dissolution study from marketed tablet preparation of BTN according to International Council for Harmonisation (ICH) and FDA guidelines[19].

MATERIALS AND METHODS

Samples, Reagents and Instrumentation

Test sample (bosutinib monohydrate 516.98 mg as bosutinib 500 mg tablet), bosutinib monohydrate working standard, placebo (microcrystalline cellulose 140 mg, povidone (K25) 14.5 mg, poloxamer (188) 21.75 mg, croscarmellose sodium 29 mg & magnesium stearate 29 mg) and hydrochloric acid (HCl) (37%) were collected from a local source. Purified water was prepared in the laboratory. All the reagents used in this study were analytical grade. The weight of the standard and samples were measured using a sartorius analytical balance. An ultrasonic bath for sonication and dissolution tester (Electrolab, India) was used for this experiment. Finally, UV-Visible spectrophotometer (UV-1800, Shimadzu, Japan) was used for the analysis of the blank, standard, placebo, and sample solutions.

Spectrophotometric conditions

Standard solution and sample solutions were measured with UV absorbance at the wavelength of 266nm by an UV-spectrophotometer (Apparatus: Quartz cell (1 cm)) using dissolution medium as blank solution (0.1 N HCl). Dissolution media was used as diluent.

Optimization of dissolution conditions

The dissolution conditions were preset according to the FDA guidelines[16]. The USP apparatus-II (paddle) was used as dissolution apparatus, dissolution medium was 900 mL 0.1 N HCl and rotation speed was 50rpm. The temperature of 37°C ± 0.5°C after 45 min of dissolution. By calculating the mean percent dissolved drug from the sample (6 units) taken at 10 min, 15 min, 20 min, 30 min and 45 min time interval from the dissolution media the dissolution profile curve was prepared[16].

Preparation of Standard, Sample and Placebo Solution

To prepare the stock solution, 11 mg of bosutinib monohydrate working standard was taken and sonicated with diluents and cooled. Then, it was filtered through filter paper (pore size: about 11µm) and the filtrate was collected. Standard solution of 5.5 µg/mL of bosutinib monohydrate was prepared from the stock solution. On the other hand, samples were collected from dissolution medium at specified time intervals to be compared with standard.

For specificity study, placebo solution were prepared, sonicated and filtered by the same method as mentioned above.

Validation parameters

Currently three strengths of bosutinib tablet (100 mg, 400 mg & 500 mg) are available global market. Although the validation study can also applicable for all dosage form by using the same analytical setups and same concentration ranges, the validation was conducted with the highest strength of bosutinib tablet (500 mg per tablet). The following parameters were evaluated: specificity, linearity, range, accuracy, intra-day precision (repeatability), intermediate precision

and robustness. All the parameters were evaluated according to ICH guidelines[19].

System suitability

Standard solution was used as system suitability solution. The absorbance of system suitability solution (standard solution) was measured five times and then the %RSD was calculated.

Filter compatibility study

The filter compatibility study was conducted by analyzing each standard and sample passing both through particle retention filter paper (Whatman® qualitative filter paper, cellulose filter circles, pore size: about 11 µm). Duplicate measurements were made for each sample. The recovery of analyte for each individual sample and standard and average recovery for each filter were calculated.

RESULTS AND DISCUSSION

Validation of the dissolution curve

Dissolution profile shows that, in the end of 45 minutes, Mean %of drug dissolution is 96.20% (Figure 2).

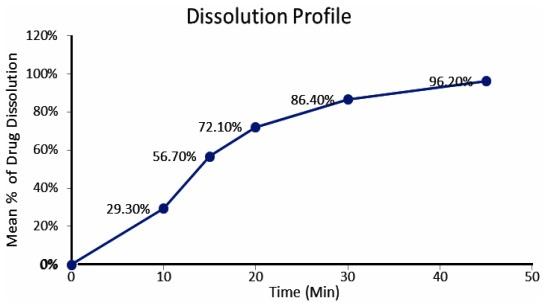

Fig. 2. Dissolution profile of bosutinib monohydrate

Validation parameters

Specificity

The spectrum of blank, placebo, standard, and sample solution in a UV-spectrophotometer from 200-400nm range was recorded to measure specificity, with dissolving media serving as a baseline correction. Spectrum measurements for the blank, placebo, standard, and test samples reflect the target analyte specificity. No peak was observed for the blank and placebo solutions indicating no spectrophotometric interference by additives. In the spectrum of standard and sample, the highest absorbance was found at 266nm, which is the specified maximum wavelength for bosutinib monohydrate found in the previous studies[17].

Linearity and Range

To study linearity, ascending concentrations of samples were taken. The absorbance of analytes were plotted (Y-axis) against its respective concentration (X-axis) in a regression curve. The regression coefficient (R^2) value was determined with the help of MS Excel®. The lower limit of quantitation was 2.8µg/mL while the upper limit of quantitation was within the linear range (8.3µg/mL). % of nominal value and different concentration of standard (µg/mL) are shown in Table 1 respectively. The linear regression coefficient (R^2) results 0.999 and slope of the regression line was 0.079.

Accuracy

According to the dissolution method specified in USP, a tolerance value (Q) is required for each product[20]. From the specification of the product monograph, the Q value of the bosutinib monohydrate was 70% dissolved within 45 minutes. Considering this, 70% of 5.5 µg/mL, i.e., 3.9 µg/mL was considered as 100% nominal value for accuracy study (Table 2). From the recovery study it was apparent that the nominal value was within the specified limit for accuracy study. The %RSD was found 0.8 which is also within the limit specified in the ICH guidelines[19].

Table 1: Linearity and range for the dissolution study of bosutinib

% of Nominal value	Conc. of Standard (µg/mL)	Absorbance	R^2 Limit	Result	y-intercept	Slope of the regression line
50%	2.8	0.219				
60%	3.3	0.26				
80%	4.4	0.347	Not Less	0.999	-0.002	0.079
100%	5.5	0.433	Than 0.980			
120%	6.6	0.525				
140%	7.7	0.61				
150%	8.3	0.656				

Conc.: Concentration, R^2 : Regression Coefficient.

Table 2: Accuracy study for bosutinib dissolution test

% of Nominal value	Amount of Bosutinib added (mg)	Replicate	Amount recovered	%Recovery	Limit
80%	6.16	1	6.2	100.7	
		2	6.09	98.8	
		3	6.23	101.1	
100%	7.7	1	7.73	100.4	
		2	7.73	100.4	95.0 to 105.0%
		3	7.7	100	
120%	9.24	1	9.22	99.8	
		2	9.15	99	
		3	9.18	99.3	
			Mean.	100	
			% RSD	0.8	

Abs.: Absorbance, RSD: Relative standard deviation

Repeatability

Repeatability study were carried out to see whether the method can stick to similar results albeit variations in sample preparations in a single day (intra-day precision)[21]. From Table 3, it is evident that all the sample studied showed more than 90% dissolution within specified time limit, which is well above the minimum limit for percent dissolution[19]. The criteria for %RSD was also met as only 1.6% standard deviation was observed.

Table 3: Determination of repeatability (intra-day precision) of the dissolution method

Sample no.	Weight of std.	Abs. of sample	Abs. of std.	%Dissolution	Limit (%Dissolution)	%RSD	Limit (%RSD)
1	11	0.448	0.442	94.1	Not less than 75%	1.6	Not more
2		0.459		96.4	in 45 minutes		than 10.0
3		0.467		98.1			
4		0.461		96.9			
5		0.462		97.1			
6		0.45		94.6			
Average of % dissolution				96.2			

Std.: Standard, Abs.: Absorbance, Disso. : Dissolution, RSD: Relative standard deviation.

Intermediate precision

According to ICH guidelines for validation, intermediate precision can be calculated by inter-day, inter-analyst or inter-equipment precision studies[19]. In the current study, two different repeatability experiment was carried out independently by two analyst on two different days (n=6 for each) and expressed as method precision (n=12). Average% dissolution by analyst 1 was 96.2% (and for analyst 2, the average% dissolution was 91.91%. The% RSD of the results from the separate studies was found 2.8% (Table 4).

Table 4: Determination of intermediate precision by different analyst

Analyst no.	Weight of std.	Abs. of sample	Abs. of std.	%Disso.	Limit (% Disso.	%RSD	%RSD of 12 Sample	Limit (%RSD)
Analyst 1	11	0.448	0.442	94.1	Not less than 75%	1.6	2.8	Not more
		0.459		96.4	in 45 minutes			than 10.0
		0.467		98.1				
		0.461		96.9				
		0.462		97.1				
		0.45		94.6				
Analyst 2	11	0.438	0.448	90.8		1.2		
		0.438		90.8				
		0.439		91				
		0.438		92.9				
		0.438		92.9				
		0.439		93.1				

Std.: Standard, Abs.: Absorbance, Disso. : Dissolution, RSD: Relative standard deviation.

Robustness

By varying one parameter (wavelength) by using same concentration of repeatability sample and standard solution, variation of the dissolution was observed. %dissolution was calculated from the corresponding absorbance for the concentration. limit (%dissolution) was not less than 75% in 45 min and %dissolution in 90.2 in 264nm, 94.1 in 266nm and 95.6 in 268nm (Table 5).

Table 5: Robustness study for bosutinib dissolution method

Parameter	Changes 264nm	Wavelength 268nm	Nominal maximum Wavelength 266nm	Limit (% Dissolution)
Absorbance of Standard	0.439	0.444	0.442	Not less than 75% in 45 minutes
	0.439	0.445		
Absorbance of Sample	0.452	0.457	0.45	
	0.453	0.457		
% Dissolution	90.2	95.6	94.1	

System suitability

The equilibrium condition of UV spectrophotometer was checked by measuring absorbance of standard solution repeatedly until the relative standard deviation is not more than 2.0 for five consecutive measurements. System suitability was established before starting validation parameters as follows: The spectrophotometric absorbance were automatically measured and visually inspected for an acceptable data analysis. The %RSD of responses of bosutinib monohydrate from five replicates measurement will not be more than 2.0 and results 0.1.

Filter compatibility study

Filter compatibility study was done to confirm that the filtration can remove the insoluble materials and there is no adsorption of bosutinib by the filter materials[22]. The %RSD for absorbance by the filtered and unfiltered standard solution was 0.3% and for sample solution it was 0.1%. Thus, the method was filter compatible for the intended filter paper (11µm) according to guidelines[22].

CONCLUSION

Bosutinib, a novel treatment against CML and other types of tumor, is one of the new drugs approved by US FDA. It is yet to be included in USP and a study on its dissolution method validation is not yet published. With an aim to establish a validated method of dissolution for bosutinib 500 mg tablet by ICH guidelines. UV-spectrophotometric method was used in the current study. Specificity showed no placebo effect on the sample. The study was found to be accurate and precise with repeatability and intermediate precision parameters are well within limits set by ICH. The method was also robust with minimal effect with changing wavelength. Different replicates showed similar results in the system suitability study. Overall, the current study proposes a validated simple, effective and specific dissolution method of bosutinib 500mg tablet using UV-Visible spectrophotometer which can be used in future researches and pharmaceutical industries as well.

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Conflict of Interest

The authors declare that they have no conflict of interests regarding this manuscript.

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