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A New Validated RP-HPLC Method for the Estimation of Bosentan in Tablet Dosage Forms

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ABSTRACT

An accurate and precise high performance liquid chromatographic method was developed for quantitative estimation of bosentan in tablet dosage forms. Chromatographic separation of the drug was achieved on a Kromasil C_{18} column (150 x 4.6 mm; 5μ) by eluting with a mobile phase consisting of phosphate buffer (pH 4.0) and acetonitrile (30:70 v/v) at a flow rate of 1.0 mL/min. The drug in the eluate was monitored by U V detection at 270 nm. The retention time obtained for the drug was 3.54 min. The calibration curve plotted was linear over the range of 25-175 μg/mL of the drug. The validation of the method was done following the ICH guidelines. The proposed method could be applied for determination of bosentan in its tablet dosage forms without any interference from excipients. The method is suitable for routine quality control analysis of bosentan formulations.

Keywords: Bosentan, Estimation, Tablets, HPLC

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INTRODUCTION

Bosentan (4-tert-butyl-N-[6-(2-hydroxy ethoxy)-5-(2-methoxy phenoxy)-2-(pyrimidin-2-yl) pyrimidin-4-yl] benzene-1-sulphonamide) is a dual endothelin receptor antagonist drug used in the treatment of pulmonary artery hypertension (Figure 1). It is a competitive antagonist of endothelin-1 receptor acting on both endothelin A and endothelin B present in the smooth muscle of pulmonary blood vessels. Bosentan is also used to treat digital ulcers in patients with systemic sclerosis [1, 2, 3].

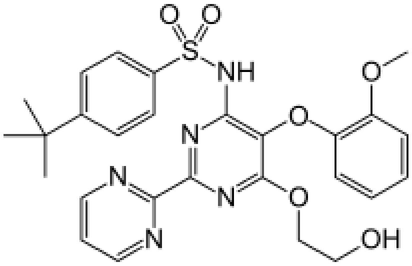

Figure 1: Structure of bosentan

A literature survey revealed that so far only a few analytical methods based on spectrophotometry[4-5], LC-MS[6] and HPLC[7] have been reported for the determination of bosentan in human plasma and formulations. Hence, an attempt was made to develop an efficient and selective HPLC method for the analysis of bosentan in bulk drug samples and in tablet dosage forms. The developed method was duly validated as per ICH guidelines[8].

MATERIALS AND METHODS

Drugs, chemicals, and solvents

The pure reference sample of bosentan was obtained from MSN Laboratories Limited, Hyderabad. The commercial tablets of bosentan `Bosentas 125`, manufactured by Cipla Limited were purchased from the local market. Potassium dihydrogen orthophosphate, ortho phosphoric acid, HPLC grade acetonitrile, triethylamine and water were procured from Rankem Fine Chemicals Ltd., Mumbai.

Equipment and chromatographic conditions

A Waters Alliance liquid chromatograph (Model: 2695) fitted with a U V diode array detector (Model: 2996) and running on Empower2 data handling software was employed in the study. A Kromasil C_{18} column (150 x 4.6 mm; 5µm) was used for analyzing the drug. All the chromatographic runs were carried out using a mobile phase consisting of phosphate buffer and acetonitrile (30:70 v/v) in isocratic mode at a flow rate of 1.0 mL/min. The injection volume of the samples was 10 µL. The detector wavelength was set at 270 nm. Under these optimized conditions, the retention time obtained for bosentan was 3.54 min.

Preparation of Phosphate buffer (pH 4.0)

The phosphate buffer was prepared by dissolving 6.8 g of potassium dihydrogen ortho phosphate in 1000 mL of water along with 1ml of triethylamine and adjusting the pH of the solution to 4.0 using orthophosphoric acid. It was then filtered through a 0.45µ membrane filter and sonicated.

Preparation of the mobile phase

The optimized mobile phase consisted of a mixture of the above-mentioned phosphate buffer (pH 4.0) and acetonitrile in a ratio of 30:70 v/v.

Preparation of the diluent

A mixture of the phosphate buffer (pH 4.0) and acetonitrile in a ratio of 50:50 v/v was used as the diluent.

Preparation of the working standard solution of bosentan

12.5 mg of the reference sample of bosentan was transferred into a 10 ml clean dry volumetric flask. 7.0 ml of acetonitrile was added, sonicated for 5 minutes and made up to volume with the same solvent to make the standard stock solution. 5.0 mL of this standard stock solution was transferred in to a 50 mL volumetric flask and diluted up to mark with the diluent. This solution (125 µg/mL) was used as the working standard.

Calibration curve

Solutions of bosentan at different concentration levels including the working standard concentration were prepared in the diluent. Ten microlitres of each concentration was injected three times into the HPLC system (n=3). The response was read at 270 nm and the corresponding chromatograms were recorded. From these chromatograms, the mean peak areas at the different concentration levels were calculated and the linearity plot of the mean peak areas over concentration was constructed.

Estimation of the drug from the tablet dosage form

Five tablets (Bosentas 125) were crushed into a fine powder and transferred into a 500 mL volumetric flask. 300 mL of the diluent was added to it and sonicated for 25 min. Then, the volume was made up with the diluent and the contents filtered through a 0.45µ nylon filter. From the filtered solution, 1.0 ml was pipetted out into a 10 ml volumetric flask and diluted to mark with the diluent. This solution was then chromatographed six times. From the chromatograms obtained, the average drug content in the formulation was calculated.

RESULTS AND DISCUSSION

During the method optimization studies, various combinations and proportions of the solvents and buffers were examined on Kromasil C_{18} column for efficient separation of bosentan. Using a mobile phase consisting of a mixture of phosphate buffer (pH 4.0) and acetonitrile in 30:70 v/v ratio, a good resolution and baseline separation for the drug peak was obtained. All the chromatographic conditions were optimized after evaluating the column efficiency parameters like theoretical plates and tailing (Table 1). Under these optimized conditions, the retention time obtained for bosentan was 3.54 min (Figure 2). The method was then validated as per the ICH guideline. The proposed method was also found to be applicable for the estimation of bosentan in tablet formulations.

Table 1: Optimized chromatographic conditions

Stationary Phase	Kromasil C18 (150 x 4.6 mm, 5µm)
Mobile Phase	Phosphate buffer : Acetonitrile =30:70 v/v
Flow Rate	1.0 mL/min
Column Temperature	25°C
Injection Volume	10 µL
Detection Wavelength	270 nm
Run Time	8 min
Retention Time	3.54 min

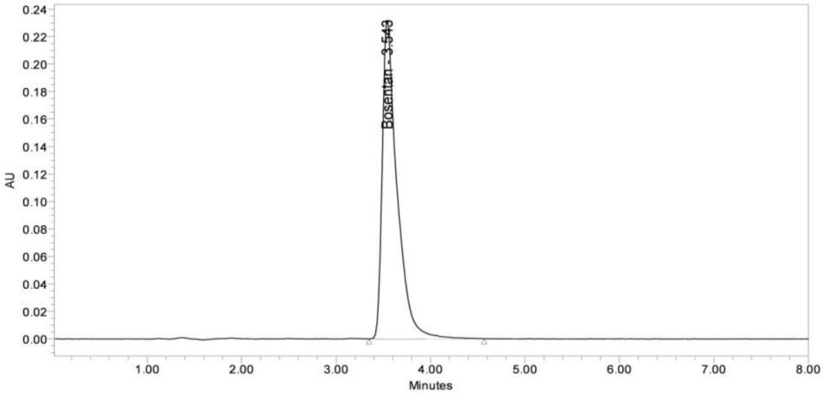

Figure 2: Representative chromatogram showing separation of bosentan from working standard solution

Specificity

A good analytical method should be able to measure the analytes accurately in the presence of probable interferences from blank and excipients. Figure 2 shows good chromatographic baseline separation of bosentan from working standard solution. Figure 3 demonstrates that no interference was found at the retention time of bosentan in its dosage form due to excipients.

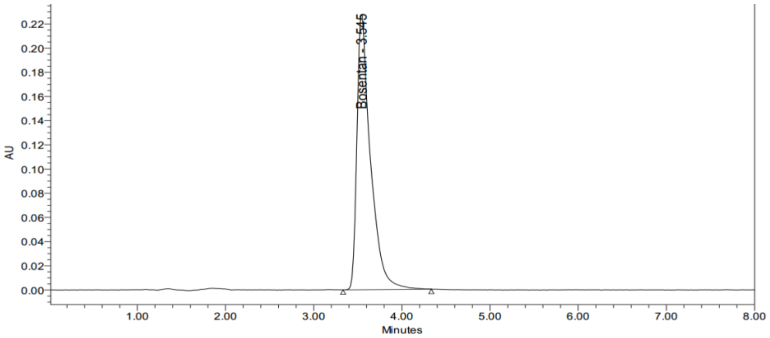

Figure 3: chromatogram obtained from analysis of bosentan from tablet dosage form

Linearity

The calibration curve (n=3) constructed for the drug was linear over the concentration range of 25 – 175 µg/mL (Figure 4). The regression of the plot was computed by the least squares method. The relevant correlation coefficient is greater than 0.99 and the %RSD for each concentration studied was less than 2.

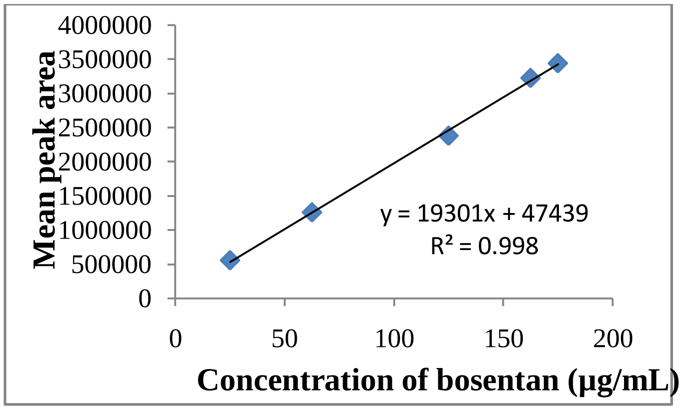

Figure 4. Linearity plot of bosentan.

Accuracy and precision

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out and the percentage recovery and standard deviation of the percentage recovery were calculated and presented in Table 2. The high percentage of recovery indicates that the proposed method is highly accurate. The precision of the method was demonstrated by inter-day and intra-day variation studies. Six replicate injections of sample solutions were given and the percent RSD was calculated and the results are shown in Table 3.

Table 2: Accuracy data of the proposed method

Analyte	Amount of the analyte taken (µg/mL)	Mean recovery (µg/mL) ± SD	% Mean recovery ± SD
Bosentan	50	51.03 ± 0.337914	102.0666 ± 0.337914
	100	100.02 ± 0.93508	100.0247 ± 0.93508
	150	148.43 ± 0.79281	98.95415 ± 0.52854

Table 3: Precision data

S.No.	**Intra-day precision**	**Inter-day precision**
1	1239846	1216598
2	1223570	1225784
3	1219789	1217859
4	1219985	1235478
5	1224567	1224875
6	1231256	1244965
Average	1226502	1227593
SD	7752.93	10865.18
%RSD	0.632	0.885

System suitability parameters

System suitability parameters were determined with six replicate injections of the standard sample solutions and are presented in Table 4.

Table 4: System suitability parameters

Parameter	**Value**
Retention time (min)	3.54
Tailing factor	1.1
Theoretical plates	4586
HETP	0.03271

Method suitability

The commercial tablet formulation, (Bosentas 125 mg) was analyzed by the proposed method. The recovery obtained (99.6%) by the proposed method was found to be in good agreement with the labeled amount of the drug, which confirms the suitability of the method for the analysis of bosentan in tablet dosage forms.

CONCLUSION

The proposed RP -HPLC method is sensitive, precise and accurate and can be used for the routine determination of bosentan in its tablet dosage forms.

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