

## Research Article

# Estimation of Bosentan in Pure and Tablet Dosage Form by RP-HPLC

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## ABSTRACT

A simple, rapid, sensitive and precise reverse phase high performance liquid chromatographic (RP-HPLC) method for the determination of bosentan was developed on a Waters 2695 HPLC system on a reverse phase Agilent XDB C18 column (150 mm × 4.6 mm, i.d., 5 μ) using a mobile phase mixture containing phosphate buffer (pH-5) and acetonitrile in 45:55 % v/v ratio. The flow rate was 1.0 mL/min and column effluents were monitored at 270 nm and bosentan eluted at 5.7 minutes. The method is linear in the concentration range of 25-150 μg/mL with a correlation coefficient of 0.999. The method was validated for the parameters like linearity, accuracy, precision, limit of detection and limit of quantification and system suitability studies. The developed RP-HPLC method was used for the assay of tablet dosage form.

**Keywords:** Bosentan, RP-HPLC method, validation, tablets.

## INTRODUCTION

Bosentan <sup>1</sup> is chemically, 4-tert-butyl-N-[6-(2-hydroxyethoxy) -5-(2-methoxyphenoxy) -2-(pyrimidin-2-yl) pyrimidin-4-yl] benzene-1-sulfonamide. Bosentan is a non-peptidic endothelin receptor antagonist, which is under development for the treatment of hypertension and chronic heart failure <sup>2</sup>. The literature survey reveals that there is a narrow bore HPLC and tandem mass spectrometric detection <sup>3</sup>, HPLC <sup>4-7</sup> methods and spectrophotometric <sup>8-10</sup> methods for the estimation of the drug, so the authors developed a simple, sensitive and accurate RP-HPLC method for the determination of bosentan in tablets.

## EXPERIMENTAL

### Apparatus

Waters 2695 HPLC system equipped with Agilent Eclipse XDB C18, 150 x 4.6 mm, 5μ column, Rheodyne injector with 10 μL loop, 2996 PDA detector and Empower-2 software was used.

### Reagents

Pottasium dihydrogen ortho phosphate of analytical grade, HPLC grade milli-Q water, methanol and acetonitrile were used. Bosentan was a gift sample from Aurobindo Pharma, Hyderabad, India. The tablets of bosentan were obtained from local pharmacy. Mobile phase was a mixture of phosphate buffer and acetonitrile in the ratio of 45:55 % v/v.

### Preparation of Buffer

6.8 g of Pottasium dihydrogen ortho phosphate was weighed accurately and transferred in to a 1000 mL volumetric flask, 900 mL of HPLC grade milli-Q water was added and dissolved it. After the complete dissolution, the volume was made up to the mark with the same (pH 5).

### Preparation of standard stock solution

100 mg of bosentan was weighed accurately and transferred in to a 100 mL volumetric flask. 50 mL of HPLC grade ethanol was added and dissolved. After complete dissolution the remaining volume was made up

to the mark with ethanol which gives 1000 µg/mL of bosentan.

#### Procedure for plotting calibration graph

Aliquots of the standard stock solution were further diluted with diluent in 10 mL volumetric flasks to get various concentrations range of 25-150 µg/mL of the drug. From this 10 µL of the each solutions were injected in to the HPLC system and chromatograms were recorded. The peak area of the chromatograms was taken in Y axis and concentration (µg/mL) of analyte was taken in X axis, calibration curve was plotted.

#### Procedure of determination of Bosentan in tablets

Twenty tablets were weighed accurately, average weight was calculated, powdered and then powder equivalent to 100 mg/mL of bosentan was transferred to a 100 mL volumetric flask. It was dissolved in ethanol and filtered through a membrane filter (0.2 µ). The filtered sample solution was suitably diluted and used for the analysis. After setting the chromatographic conditions and stabilizing the instrument to obtain a steady baseline, the sample solution was loaded in the 10 µL fixed-sample loop of the injection port. The sample solution was injected and a chromatogram was recorded. The injections were repeated six times and the chromatograms were recorded.

#### RESULTS AND DISCUSSION

The method was optimised based on trial and error method. The mobile phase phosphate buffer and acetonitrile in the ratio of 45:55 % v/v found to be better for the elution of the drug. Before an analytical method is applied to quality control, it is necessary to validate the method. The validation ensures that the procedure is suitable for its intended purpose. The method was validated for the parameters like linearity, accuracy, precision, limit of detection and limit of quantification and system suitability studies. The method is linear in the concentration range of 25-150 µg/mL. Linear regression data for calibration curve is shown in Table 1. A representative chromatogram for standard drug has been given in fig. 1.

Chromatogram for blank is shown in fig. 2. The peak area of the drug was calculated and the amount of drug present in tablet was estimated from the calibration curve. The result of analysis reported in Table 2. The LOD and LOQ were found to be 1.3344 µg/mL and 4.043 µg/mL, respectively. The precision of the method was determined by repeatability (intraday) and intermediate precision (inter day) and was expressed as % RSD of a series of measurement. The experimental values for the estimation of bosentan in tablets are presented in Table 2. The specificity test indicated that there was no interference in the drug peak. The chromatogram obtained through the injection of the placebo solution did not contain any other peak at the retention time of bosentan. To ensure reliability and accuracy of the method, a known quantity of drug was mixed with preanalyzed sample. Recovery studies were carried out by the proposed method. The results are shown in Table 2.

#### CONCLUSION

The HPLC method described here is simple, sensitive, specific and validated as per guideline. The accuracy studies prove that there is no any interference of additives in this method. This method may be used for the routine analysis of the drug in tablet formulation.

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**Table 1: Linear regression data for calibration curve**

Parameters	Values
Concentration range, µg/mL	25-150
Slope	18590.5
Intercept	-56630
Correlation coefficient	0.999

**Table 2: Assay, recovery results and precision studies**

Formulation	Labeled amount (mg/ tablet)	(% label claim* ± S.D	% Recovery	Precision (% RSD)	
				Intermediate (n=24)	Intra-day (n=6)
Bosentan Tablets	125	99.97 ± 0.1051	99.32 to 100.16	0.1198	0.1052

\* Average of six determinations.

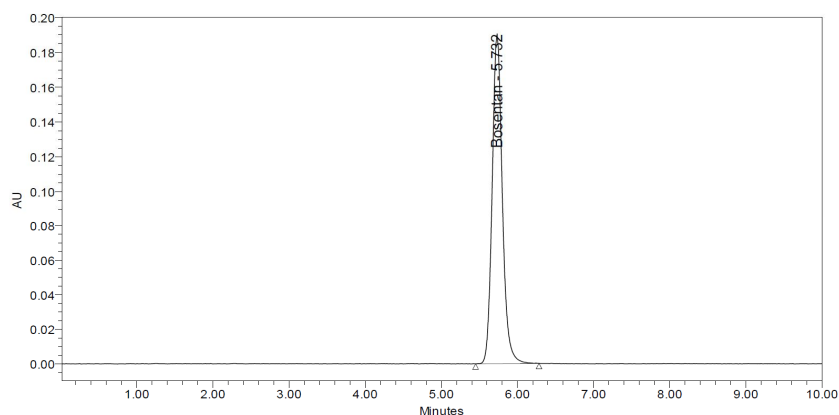


Fig. 1: A typical chromatogram of Bosentan standard solution

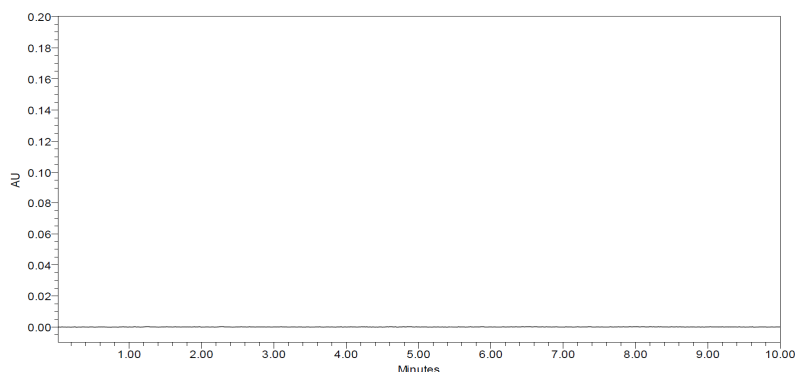


Fig. 2: Chromatogram of Blank

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