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Estimation of Bosentan Monohydrate in Tablet Dosage Forms by a New RP-HPLC Method

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ABSTRACT

A Simple, Sensitive, and rapid reverse phase high performance liquid chromatographic method has been developed for the determination of Bosentan monohydrate in bulk and tablet Dosage Forms. Chromatographic separation was achieved on a Zorbax SB-Phenyl (150×4.6 mm), 3.5 µm make: Aglient column with a 30:70 mixture of 0.02M sodium dihydrogen phosphate and HPLC grade Methanol as mobile phase, detection was at 225 nm, oven temperature is 30°C, injection volume is 20µL, flow rate is 1.5 mL/min and the expected retention of Bosentan monohydrate peak is about 5.2 minutes. Response was a linear function of concentration in the range 2-0.01 µg/mL for Bosentan monohydrate; correlation coefficiencent was 0.9999, respectively.LOD and LOQ for Bosentan monohydrate were found 0.01 µg/mL and 0.03 µg/mL based on signal to noise ratio(S/N). Accuracy (recoveries 90-98%) and reproducibility were found to satisfactory.

Key words: Bosentan monohydrate, S/N ratio, RP-HPLC method and validation.

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INTRODUCTION

Bosentan monohydrate (Figure 1) is used to treat pulmonary arterial hypertension (PAH), a life-threatening condition affecting the blood vessels from the heart to the lungs. Chemically, Bosentan monohydrate is designated as 4-tert-butyl-N-[6-(2-hydroxyethoxy)-5-(2-methoxyphenoxy)-2-(pyrimidin-2-yl) pyrimidin-4-yl] benzene-1-sulfonamide.

In this paper we describe a simple, sensitive, and validated RP-HPLC method[1,4,5] for determination of Bosentan monohydrate in tablet Dosage Forms. The method has been successfully used for quality control analysis of the drugs and other analytical purposes.

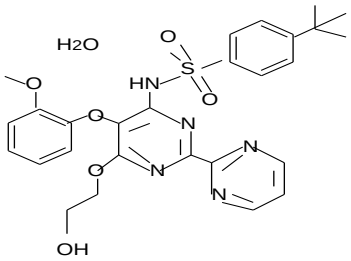

Figure 1: The structure of Bosentan monohydrate

MATERIALS AND METHODS

Apparatus and Chromatographic Conditions

Chromatographic separation was performed on a Shimadzu chromatographic system equipped with a LC-20AT pump and SPD-20A UV-VIS detector with 20µL fixed loop and analyzed by using LC-Solution software.

Zorbax SB-Phenyl (150×4.6 mm), 3.5 µm make: Aglient column with a 30:70 mixture of 0.02M sodium dihydrogen phosphate and HPLC grade Methanol as mobile phase was delivered at flow rate 1.5 mL/min. The mobile phase was filtered through 0.45µ membrane filter and sonicated for 5 min. An external standard method was used. UV detection was performed at 225 nm and column oven temperature is 30°C.Peak was confirmed by comparison of retention time with standard.

Reagents And Solutions

Preparation of standard solution

Accurately weighed 125.07 mg of reference standard of Bosentan monohydratein 250 mL volumetric flask and the volume was brought upto the mark using acetonitrile.

Preparation of sample solution

The commercial samples of tablet containing the drug namely Bosentas, 125 mg (Cipla) chosen for this purpose. One tablet, containing 125 mg of Bosentan monohydrate was weighed

accurately and transferred to a 250 ml volumetric flask with 50ml acetonitrile, shaken for 5min, then diluted to volume with acetonitrile to furnish a solution containing 500 µg/mL Bosentan monohydrate. After filtration the solution the solution was diluted with diluent as an acetonitrile to give a final concentration of 1 µg/mL Bosentan monohydrate.

Method Validation

Once the HPLC method development was over, the method was validated in terms of parameters like specificity, press ion, accuracy, linearity and range, LOD,LOQ, raggedness, robustness, stability etc. For all the parameters percentage relative standard deviation values ware calculated. The proposed HPLC method was validated as per ICH guidelines[6].

Linearity and range

Different known concentrations of Bosentan monohydrate (2.0 µg/mL – 0.01 µg/mL) were prepared in diluent by diluting the stock solution. Injected the standard solutions and measured the peak area. A calibration curve has been plotted for concentration of the standards injected versus area observed and the linearity of the method was determined from the correlation coefficient. The results were shown in Table: 2. the slope, intercept and correlation coefficient values were found to be 31282, 85.77 and 0.9999.

Precision

Precision was evaluated by carrying out three independent sample preparation of a single lot of formulation. The sample solution was prepared in the same manner as described in the sample preparation. Percentage relative standard deviation (% RSD) was found to be less than 1% for within a day and day to day variations, which proves that that method is precise. Results were shown in Table 3-4.

Accuracy

To study the reliability, suitability and accuracy of the method recovery experiments were carried out. A known quantity of the pure drug was added to the preanalysed sample formulation at the level of 50%, 100% and 150%, dissolved in diluents and made up to 100ml with same solvent. Further dilutions were made so that the each aliquot contained 0.03mg/L of Bosentan monohydrate. The contents were determined from the respective chromatograms. The concentration of the drug product in the solution was determined using assay method. The recovery procedure was repeated 10 times and % RSD was calculated by using the following formula. The contents of Bosentan monohydrate tablet found by proposed method are shown in Table 3; the lower values of RSD of assay indicate the method is accurate. The mean recoveries were in range of 90-98 % which shows that there is no interference from excipients. Table: 5.

% recovery = b-a/c

Where,

a-The amount of drug found before the addition of standard drug

b-The amount of drug found after the addition of standard drug

c- The amount of standard drug added

Repeatability of solution

A standard solution of drug substance was injected ten times and corresponding peak areas were recorded. The % RSD was found to be less than 1%.Table:6.

Specificity

Condition of HPLC method like percentage of organic solvent in mobile phase, ionic strength, pH of buffer flow rate etc, was changed. In spite of above changes no additional peaks were found, although there were shift retention times or little changes in peaks shapes.

Assay

20µl of standard and sample solutions were injected into an injector of RP-HPLC, from the peak area of standard amount of drug in sample were computed. The values are given in Table: 7.

Limit of detection and limit of quantification

The limit of Detection (LOD) and limit of Quantification (LOQ) of the development method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3). The LOD for Bosentan monohydrate found to be 0.01µg/mL The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified (signal to noise ratio of 10) The LOQ was 0.03 µg/mL for Bosentan monohydrate. It was concluded that the developed method is sensitive.

Ruggedness and robustness

The ruggedness of the method was determined by carrying out the experiment on different instruments like shimadzu HPLC and Agilent HPLC by different operators using different columns of similar types. The %RSD values with two different instruments shimadzu HPLC and Agilent HPLC, analyst and columns were 0.5-0.5, 0.6-0.5 and 0.4-0.3% respectively.

Robustness of the method was determined by making slight changes in the chromatographic conditions, such as changes in mobile phase, flow rate and column temperature. It was observed that ther were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method is rugged and robust. The robustness limit for mobile phase variation, flow rate variation, and temperature variation are well within the limit, which shows that the method is having good

system suitability and precision under given set of conditions and were within the acceptance criteria of not more than 2%.

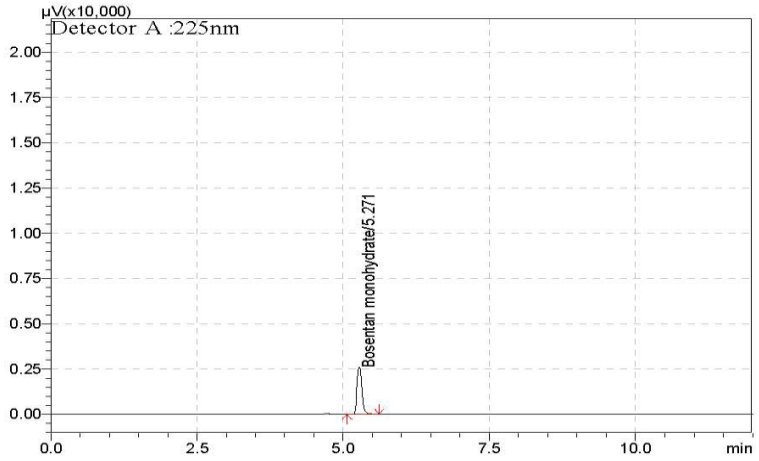

Figure 2: Chromatogram of standard (1.0 µg/mL)

RESULTS AND DISCUSION

UV spectrum[2,3] of Bosentan monohydrate was recorded from which 225 nm was selected as wavelength. Flow rate of 1.5 mL/min was selected. 30:70 mixture of 0.02M sodium dihydrogen phosphate and HPLC grade Methanol as mobile phase. The retention time was found to be 5.2 min. Bosentan monohydrate shown linearity in the range of 0.01-2 µg/mL, and the co-efficient was found to be 0.9999. Recovery studies were performed at 50%, 100% and 150%, levels. The sensitivity of method LOD and LOQ is shown in Table 2. The stability at room temperature and refrigeration was found to be 3 and 8.5 hrs respectively. Hence the proposed method is simple, accurate, and rapid and can be employed for routine analysis. The low standard deviation and good percentage recovery indicates the reproducibility and accuracy of the method.

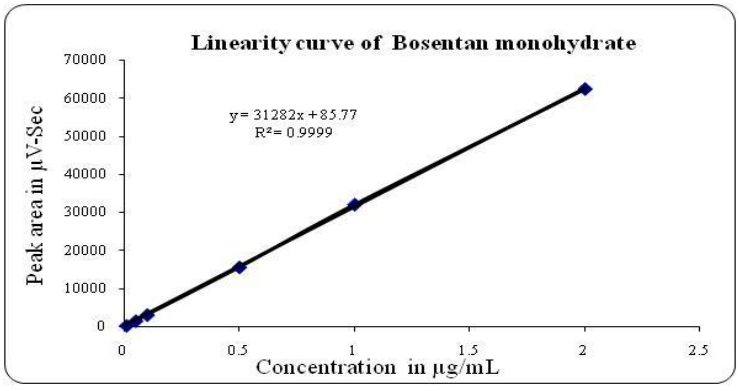

Figure 3 Linearity curve of Bosentan monohydrate

Regression analysis of the calibration curve for Bosentan monohydrate showed a linear relationship between the concentration and peak area with correlation coefficient higher than 0.999 in all curves assayed.

Table1: Optimized chromatographic conditions

Parameter	Optimized condition
Chromatograph	HPLC (Shimadzu system equipped with LC-20 AT pump and SPD-20A interfaced with LC Solution software
Column	Zorbax SB-Phenyl (150×4.6 mm), 3.5 µm make: Aglient
Mobile Phase	30:70 mixture of 0.02M sodium dihydrogen phosphate and HPLC grade Methanol
Flow Rate	1.5 mL/min
Detection	UV at 225 nm
Injection Volume	20µL
Column oven temperature	30ºC

Table 2: Validation Parameters

Parameters	Bosentan monohydrate
Linearity range	0.01-2 µg/mL
Correlation coefficient	0.9999
Slope	31282
Y Intercept	85.77

Table 3: Intraday Precession

Concentration (µg/mL)	Area	%RSD
0.03	923	
	912	1.41
	938	
0.3	9122	
	9236	1.04
	9314	
1	32985	
	32754	0.96
	32364	

The intraday precision was found to be within 1% RSD for conc.0.03, 0.3, 1.0µg/mL

Table 4:Interday Precision

Concentration (µg/mL)	Day	Area	% RSD
0.03	1	902	
	2	911	1.62
	3	931	
0.3	1	9082	
	2	9185	1.13
	3	9289	
1	1	31985	
	2	31744	1.14
	3	32464	

Intraday precision was performed for concentration of 0.03, 0.3 and 1.0 µg/mL For about three days and their peak, areas are shown in the table. The %RSD for concentration 0.03, 0.3, and 1.0 µg/mL was found to be within 2%

Table 5: Recovery studies

Level (µg/mL)	% Recovery	% RSD
0.03	90	0.74
0.3	97	0.52

Recovery studies were performed at 0.03 µg/mL and 0.3 µg/mL levels and the results were found to be within the limits mentioned as per ICH guidelines.

Table 6: Repeatability of injection

Con (mg/L)	Peak area	%RSD
	9281	
	9114	
	9296	
	9103	
0.3	9216	1.07
	9251	
	9112	
	9383	
	9229	
	9346	

Repeatability of injection was performed using 0.3 µg/mL sample for 10 times and corresponding peak areas were recorded. The % RSD peak was reported.

Table 7: Analysis of formulation

Amount of drug (mg)		% Label claim	%RSD (n=6)
Labeled	Estimated		
125	121.15	97	0.47

Analysis of formulation was performed using Bosentan monohydrate4 mg of tablet and the claim was found to be 97.

CONCLUSION

A convenient and rapid RP-HPLC method has been developed for estimation of Bosentan monohydrate in tablet dosage form. The assay provides a linear response across a wide range of concentrations. Low intra-day and inter-day % RSD coupled with excellent recoveries. The proposed method is simple, fast, accurate and precise for the simultaneous quantification of Bosentan monohydrate in dosage form, bulk drugs as well as for routine analysis in quality control.

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