New Spectrophotometric Method for the Determination of Bosentan - An Anti-Hypertensive Agent in Pharmaceutical Dosage Forms

A. NARENDRA^{*}, D. DEEPIKA[#] AND M. MATHRUSRI ANNAPURNA[§]

^{*}Department of Pharmaceutical analysis and Quality Assurance, Roland Institute of Pharmaceutical Sciences, Berhampur, Orissa, India.

[#]Royal College of Pharmacy and Health Sciences, Berhampur, Orissa, India.

[§]GITAM Institute of Pharmacy, GITAM University, Visakhapatnam, India.

naren_angirekula@yahoo.com

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Abstract: A new, simple and sensitive spectrophotometric method in ultraviolet region has been developed for the determination of Bosentan in bulk and in pharmaceutical formulations. Bosentan exhibits absorption maxima at 273 nm with apparent molar absorptivity of 1.3293×10^4 L/mol.cm in methanol and octane 1-sulfonic acid solvent mixture. Beer's law was found to be obeyed in the concentration range of 0.1-100 µg/mL. The method was validated as per the ICH guidelines.

Keywords: Bosentan, Methanol, Octane-1-sulfonic acid, ICH.

Introduction

Bosentan¹ (BST) is chemically, 4-tert-butyl-N-[6-(2-hydroxyethoxy) -5- (2-methoxyphenoxy) -2- (pyrimidin-2-yl) pyrimidin-4-yl] benzene-1 sulfonamide (Figure 1). It has a molecular formula, C₂₇H₂₉N₅O₆S with molecular weight 551.614.

Bosentan (BST) is a dual endothelin receptor antagonist important in the treatment of pulmonary artery hypertension. Bosentan is used to treat pulmonary hypertension by blocking the action of endothelin molecules that would otherwise promote narrowing of the blood vessels and lead to high blood pressure. Endothelin-1 (ET-1) is a neurohormone, the effects of which are mediated by binding to ET_A and ET_B receptors in the endothelium and vascular smooth muscle. ET-1 concentrations are elevated in plasma and lung tissue of patients with pulmonary arterial hypertension, suggesting a pathogenic role for ET-1 in this disease. Bosentan is a specific and competitive antagonist at endothelin receptor types ET_A and ET_B. Bosentan has a slightly higher affinity for ET_A receptors than for ET_B receptors. ET is present in astrocytes and neurons, endothelial / epithelial cells and certain smooth muscle cells, and can mediate both, endothelium-dependent relaxation, vasoconstriction, and

brochoconstriction. Metabolism of bosentan (I) occurs mainly in the liver by the action of cytochrome P (CYP) 450 3A4 and 2C9, which produces three metabolites: the hydroxylated (hydroxy) metabolite (II), the demethylated (phenol) metabolite (III), and the hydroxylated and demethylated (hydroxy-phenol) metabolite (IV)²⁻⁶.

Bosentan is not official in any pharmacopoeia. On detailed literature survey it is found that very few HPLC⁷⁻¹⁰, LC-MS¹¹ and spectrophotometric¹²⁻¹⁵ methods were developed for the determination of Bosentan. The aim of the present work is to develop new spectrophotometric method for the determination of BST in pharmaceutical dosage forms and to validate as per ICH guidelines¹⁶.

Figure 1. Chemical structure of Bosentan (BST).

Experimental

Apparatus

All spectral study was performed using a double beam UV-VIS spectrophotometer (UV-1800, Shimadzu, Japan) was employed with a pair of 10 mm matched quartz cells.

Chemicals and reagents

Methanol (AR) (Merck), octane-1 sulfonic acid sodium salt (Spectrochem Pvt. Ltd.) and ortho phosphoric acid (AR) (Merck) were used in the present study. Bosentan (BST) was obtained from Cipla Ltd. (India) and was used as such without further purification.

Recommended procedure and calibration curve

Preparation of stock solution

BST (25 mg) was accurately weighed and dissolved in methanol in a 25 mL volumetric flask to prepare stock solution (1000 µg/mL).

Preparation of buffer solution

4.35 grams of octane 1- sulfonic acid was dissolved in 800 ml of water and the pH was adjusted to 3.5 with o-phosphoric acid in a 1000 ml volumetric flask.

The stock solution was further diluted suitably with methanol and octane 1-sulfonic acid (pH 3.5) (50:50 v/v) solvent mixture to get a working standard solution (100 µg/mL). A series of solutions (0.1-100 µg/ml) were prepared and scanned against reagent blank. The absorption maximum was recorded as 273 nm (Figure 2) and the absorbance values of all the above mentioned solutions were noted at that λ_{max} . A calibration curve of BST was plotted with

concentration of the drug solution on the x-axis and the corresponding absorbance values on the y-axis.

Figure 2. Absorption spectrum of Bosentan ($10 \mu\text{g ml}^{-1}$).

Assay Procedure for tablets

Bosentan gift sample was supplied by Cipla Laboratories (India) and the commercial formulations available are BOSENTAS (62.5 mg and 125 mg), BOZETAN (62.5 mg and 125 mg) and LUPIBOSE (62.5 mg and 125 mg) and were purchased from the local market.

Twenty tablets were finely powdered and powder equivalent to 25 mg of Bosentan was extracted with methanol, sonicated for half an hour and filtered through Whatman 0.42 mm filter paper and suitable dilutions were made from the filtrate as described earlier in a 25 ml volumetric flask and analyzed against reagent blank.

Precision

The Inter-day precision was determined on three different days at three different levels (10, 20 and $50 \mu\text{g mL}^{-1}$) and the Intraday precision was determined at three different levels (10, 20 and $50 \mu\text{g mL}^{-1}$) by the same analyst.

Accuracy

Recovery studies were carried out by adding different amounts (80%, 100%, 120%) of bulk samples of BST to the pre-analyzed formulation and the mixture was analyzed for the drug content using the proposed method. The percentage recovery was calculated.

Results and Discussion

Bosentan obeys Beer-Lambert's law in the concentration range of 0.1-100 $\mu\text{g/ml}$. The optical characteristics were given in Table 1.

Table 1. Optical characteristics of BST.

Parameters	Values
Beer-Lambert's range ($\mu\text{g/ml}$)	0.1-100
λ_{max} (nm)	273.0
Molar extinction coefficient ($\text{Litre mol}^{-1}\text{cm}^{-1}$)	1.3293×10^4
Sandell sensitivity ($\mu\text{g/cm}^2/0.001$ absorbance unit)	0.04149
Regression equation	$y=ax+b$
Slope (a)	0.025
Intercept (b)	0.007
Correlation coefficient (r)	0.999

The % RSD values in precision study were found to be in the range 0.347-0.698 (Intraday) and 0.632-0.901 (Interday) which are less than 2% indicating that the method is more precise. The % RSD values in accuracy study were found to be less than 2% (0.831-1.052 %) indicating that the method is more accurate. Therefore the present methods can be employed for the estimation of Bosentan in pharmaceutical formulations successfully. The statistical analysis of commercial formulations has been shown in Table 2.

Table 2. Analysis of commercial formulations (Tablets).

S. No	Formulation	Labeled claim (mg)	Amount found (mg)	% Label claim $\pm$ SD*
1	Brand I	62.5	62.478	99.96 ± 0.121
2	Brand II	62.5	62.439	99.90 ± 0.312

*Average of six determinations.

Conclusions

The present method is simple, precise, accurate and can be employed for the determination of Bosentan in pharmaceutical formulations successfully.

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