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Research Article

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SPECTROPHOTOMETRIC DETERMINATION OF BOSENTAN AND ITS APPLICATION IN PHARMACEUTICAL ANALYSIS

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ABSTRACT

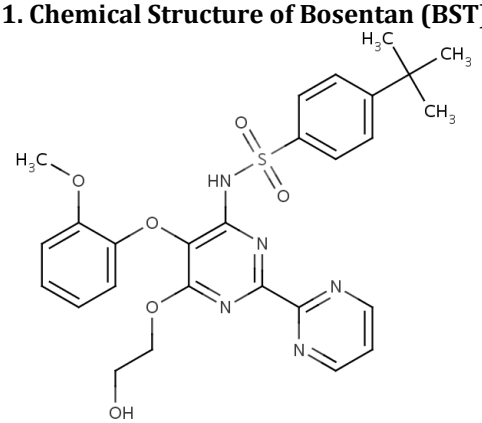
Three novel, simple, precise and accurate spectrophotometric methods have been described for the estimation of Bosentan in pharmaceutical dosage forms. Bosentan absorbing maximally at (λ_{max}) 269.0 nm (Method A) and obeys the Beer-Lambert's law in the concentration range of 1-120 µg/ml. Method B is a first derivative method (minima at 291 nm) and Method C is based on Area under Curve (AUC) method (259-279 nm) and obeys Beer-Lambert's law in the concentration range of 5-120 and 2-120 µg/ml respectively. The methods were validated according to ICH guidelines and can be successfully applied for the determination of Bosentan in pharmaceutical dosage forms.

Keywords: Bosentan, Derivative spectroscopy and AUC.

INTRODUCTION

Bosentan[1] (BST) chemically, 4-tert-butyl-*N*-[6-(2-hydroxyethoxy) -5- (2-methoxyphenoxy) -2- (pyrimidin-2-yl) pyrimidin-4-yl] benzene-1-sulfonamide (Figure **1**) having molecular formula, $C_{27}H_{29}N_5O_6S$. Bosentan is a non-peptidic endothelin receptor antagonist, which is under development for the treatment of hypertension and chronic heart failure. Bosentan (Figure **1**) acts on the human endothelian receptors, endothelin-A (ET-A) and endothelin-B (ET-B) receptors.

Figure 1. Chemical Structure of Bosentan (BST)



ET_A is present in smooth muscle cells and mediates vascoconstriction and proliferation. ET_B is present in astrocytes and neurons, endothelial/epithelial cells and certain smooth muscle cells, and can mediate, endothelium-dependent relaxation, vascoconstriction, and brochoconstriction. Metabolism of bosentan occurs mainly in the liver by the action of cytochrome P (CYP) 450 3A4 and 2C9, which produces three metabolites: the hydroxy lated (hydroxy) metabolite, the demethylated (phenol) metabolite and the hydroxylated and demethylated (hydroxy-phenol) metabolite.[2-5] Since the LOQ and the specificity of the HPLC-UV assay was not sufficient for monitoring kinetic profiles, a liquid chromatography tandem mass spectrometric (LC-MS-MS) method with a detection limit of 0.5 ng/ml and a run cycle time of 5 min using 0.5 ml plasma was developed. The LC-MS-MS method used, after protein precipitation, liquid-liquid extraction under basic conditions with subsequent separation on narrow bore HPLC and tandem mass spectrometric detection.[6]

Bosentan is not official in any pharmacopoeia. On detailed literature survey it is found that HPLC[7-10] methods and only one spectrophotometric[11] method have been developed so far and therefore the authors made an attempt to develop a simple and precise spectroscopic method for the determination of bosentan in pharmaceutical dosage forms.

MATERIALS AND METHODS

Chemicals and Reagents

Bosentan was supplied as gift sample by MSN Laboratories, India (% purity 99.98) and the commercial formulations Bosenta (62.5 mg) and Bozena (125 mg) were purchased from the local market.

Instrumentation

A double beam UV-VIS spectrophotometer (UV-1800, Shimadzu, Japan) connected to computer loaded with spectra manager software UV Probe was employed with spectral band-width of 1nm wavelength accuracy of ± 0.3 nm with a pair of 10 mm matched quartz cells. All weights were taken on electronic balance (Denver, Germany).

Preparation of stock and working standard solutions

The stock solution of BST was prepared by dissolving accurately 25 mg of drug in methanol in a 25 ml volumetric flask to obtain a concentration of 1000 µg/ml.

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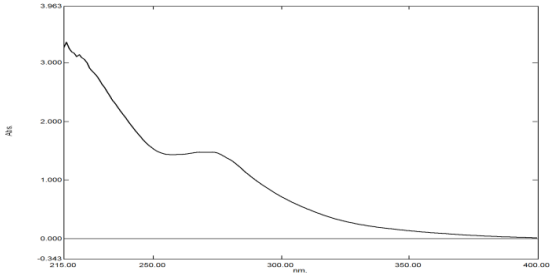
Solutions containing 1-120 µg/ml of the drug were prepared by further dilution from the stock and working standard solution (100 µg/ml).

Method A (Zero-order spectrometry)

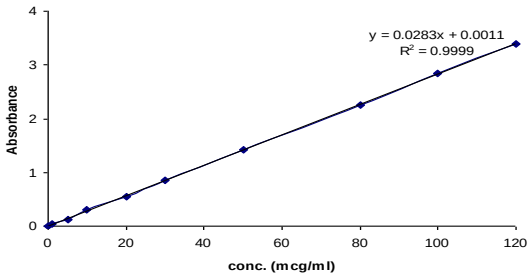
A series of standard solutions ranging from 1-120 µg/ml were prepared and scanned over the range of 400 nm to 200 nm against reagent blank. The λ_{max} was found to be at 220 nm and 269 nm (Figure **2**).

Figure 2. UV Absorption spectrum of Bosentan (50 µg/ml)



But the present study was carried out at 269 nm as the results were in good agreement with Beer-Lambert's law. Calibration curve was constructed by plotting concentration (µg/ml) on the x-axis and the corresponding absorbance on the y-axis (Figure **5A**) and the optical characteristics were shown in Table **1**.

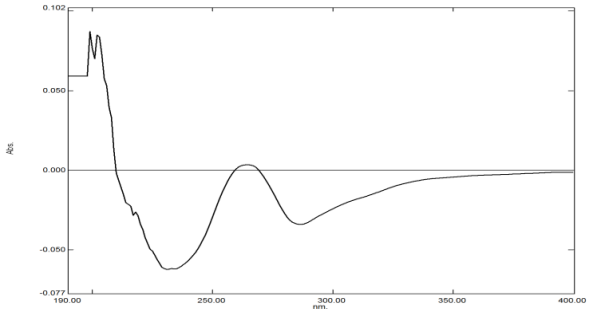
Figure 5A. Calibration curves of Bosentan (Method A)



Method B (First-Derivative spectrometry)

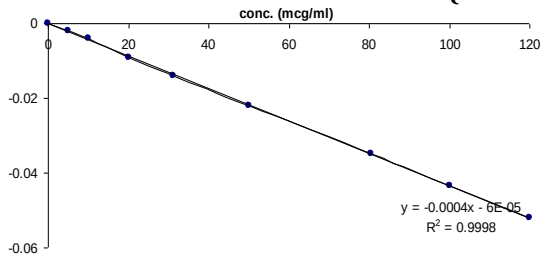
The entire above obtained zero-order spectrums were transformed to get first-order derivative spectra (Figure **3**) and the minima values of the derivative spectra were recorded.

Figure 3. First order Derivative spectrum (D_1) of Bosentan (50 µg/ml)



Calibration curve was constructed by plotting concentration (µg/ml) on the x-axis and the corresponding dA/dλ values on the y-axis (Figure **5B**) and the optical characteristics were shown in Table **1**.

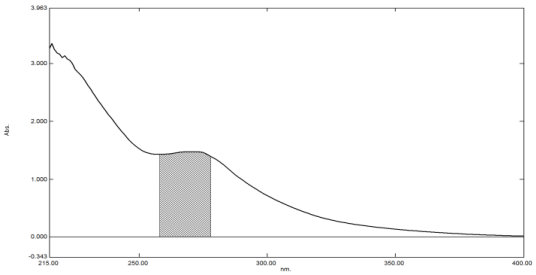
Figure 5B. Calibration curves of Bosentan (Method B)



Method C (AUC)

In this method the absorbance of the above solutions were recorded in the selected wavelength region (259-279 nm) (Figure **4**).

Figure 4. UV Absorption spectrum of Bosentan (50 µg/ml) [wave length selected for AUC 259 – 279 nm]



Calibration curve was constructed by potting concentration against AUC (Figure. **5C**) and the optical characteristics were shown in Table **1**.

Figure 5C. Calibration curves of Bosentan (Method C)

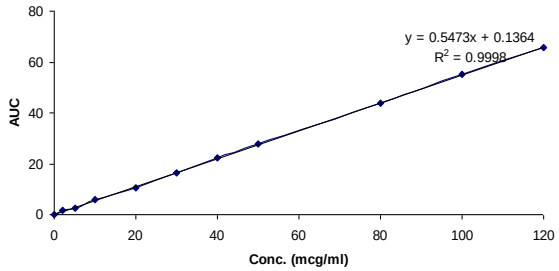


Table 1. Optical characteristics

Parameters	Method A	Method B	Method C
Beer-Lambert's range (µg/ml)	1-120	5-120	2-120
Wave length (nm)	269	291	259-279
Molar extinction coefficient **(Litre/mol^{-1}cm^{-1})**	1.90306 x 10^5	6.6119 x 10^2	3.6616 x 10^5
Sandell sensitivity (µg/cm^2/0.001 absorbance unit)	0.01358	0.8333	0.0014
Slope	0.0283	0.0004	0.5473
Intercept	0.0011	-	0.1364
Correlation coefficient	0.9999	0.9998	0.9998

Estimation of Bosentan in tablets

Twenty tablets of two different brands were purchased from the local market weighed, finely powdered and powder equivalent to 25 mg of the active ingredient was transferred to a 25 ml volumetric flask and dissolved in methanol, sonicated for 30 minutes and filtered through 0.42 mm Wattmann filter paper. Different sample solutions were prepared and analyzed against reagent blank as described above and the results were given in Table **2**.

Table 2. Analysis of commercial formulation (Tablets)

Formulation	Labeled amount (mg)	Amount found (mg)	% Recovery
Brand I	62.5	62.4775	99.964 ± 0.113
Brand II	125	124.8	99.84 ± 0.214

Method validation

Precision: The Intra-day and inter-day (n=3) precision were determined at three different levels (5, 10, 50 µg ml^{-1}) and the % recovery as well as % RSD values were calculated.

Accuracy: Recovery studies were carried out as per ICH guidelines[12] by adding different amounts (80%, 100%, 120%) of bulk samples of BST to the pre-analyzed formulation and the % recovery as well as % RSD values were calculated.

RESULTS AND DISCUSSION

Bosentan obeys Beer-Lambert's law in the concentration range of 1-120 µg/ml, 5-120 µg/ml and 2-120 µg/ml in method A, B and C respectively. The %RSD values in precision study were found to be in the range 0.315-0.527 (Intraday) and 0.472-0.893 (Interday) which are less than 2% indicating that the mehtod is more precise. The %RSD values in accuracy study were found to be less than 2% (0.78-1.69 %) indicating that the method is more accurate. Therefore the present methods can be employed for the estimation of bosentan in pharmaceutical formulations successfully.

CONCLUSION

Solid dispersion preliminary solubility analysis was carried out for the selection of carriers and solid dispersion was prepared with mannitol, PEG 4000 and PVP-K30. These solid dispersions were analysed for the solubility and Invitro dissolution profile, solid dispersion of drug with PEG 4000 had shown enhanced solubility with improved dissolution rate. In present study solid dispersion prepared with PEG 4000 shows the presence of amorphous form confirmed by the characterization study. The study also shows that dissolution rate of Atorvastatin can be enhanced to considerable extent by solid dispersion technique with PEG.

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