

Original Article

## DEVELOPMENT AND VALIDATION OF UV-VISIBLE SPECTROPHOTOMETRIC METHOD FOR ANALYSIS OF BOSENTAN IN SPIKED HUMAN PLASMA

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### ABSTRACT

**Objective:** The aim of the present study is to develop and validate a simple, efficient, economical and accurate UV-visible spectrophotometric method for estimation of bosentan in spiked human plasma.

**Methods:** The analyte was extracted by Liquid-liquid Extraction (LLE) procedure using acetonitrile and chloroform. Absorbance of the analyte in the extract was measured at 270 nm using ethanol as a diluent. The developed method was validated for linearity, accuracy and robustness.

**Results:** The proposed method was found to be linear in the range of 6 to 18 µg/ml. The correlation coefficient ( $r^2$ ) was found to be 0.99. The results revealed that the linearity, accuracy and robustness of the developed method were within the acceptable range.

**Conclusion:** The analytical technique presented here demonstrates shorter and easier sample preparation method, decreased analysis time and reduces the need for complicated or expensive equipment. The sample preparation method used in this study can also be further extended to higher end analytical techniques and other biological samples for quantification of bosentan.

**Keywords:** Bosentan, Liquid-liquid extraction, Plasma, UV-Visible spectrophotometer, Acetonitrile

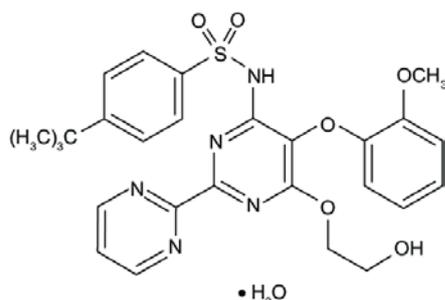
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### INTRODUCTION

Bosentan monohydrate chemically 4-tert-butyl-N-[6-(2-hydroxyethoxy)-5-(2-methoxyphenoxy)-2-pyrimidin-2-ylpyrimidin-4-yl] benzene sulfonamide hydrate is dual Endothelin Receptor Antagonist (ERA) with molecular formula of  $C_{27}H_{29}N_5O_6 \cdot S \cdot H_2O$  [1]. It is used to treat Pulmonary Arterial Hypertension (PAH) and chronic heart failure. It is a specific and competitive antagonist of endothelin-1 which is potent endogenous vasoconstrictor acting on both endothelin A and endothelin B receptors of the smooth muscles of pulmonary blood vessels leading to high blood pressure [2, 3]. Bosentan has a slightly higher affinity for ETA receptors than for ETB receptors. 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 [4]. Bosentan blocks the binding of endothelin to its receptors, thereby eliminating endothelin's deleterious effects [5].

A literature search revealed that there are several quantitative analytical methods for estimation of bosentan in biological samples; these methods include gradient RP-HPLC, SPE-LC-MS/MS etc., which are expensive and complex to perform in conventional bioanalytical laboratories [6-9]. Thus, it was decided to develop and validate an alternate simple, rapid, economical and robust method which was based on LLE for sample preparation and UV-visible Spectrophotometer for determination of bosentan in spiked human plasma.

### Structure of bosentan



### MATERIALS AND METHODS

#### Equipment

The instrument used was Elico SL 210 Double Beam UV-VIS Spectrophotometer [10-14] with silicon photo diode detector. The data acquisition was done on Spectratreats software. Other equipment used in the study was REMI R-8C centrifuge.

#### Materials

Pharmaceutical grade bosentan was obtained as a gift sample from Hetero Drug Laboratories, Hyderabad, India. Blood was collected from individuals not taking drug and plasma was separated and frozen in polypropylene micro-tubes at -20°C. Acetonitrile, chloroform and ethanol used in the study were of AR grade [15, 16]. All the chemicals were purchased from SD Fine Chemicals, Mumbai, India.

#### Methods

##### Preparation of standard stock solution

Accurately 10 mg of bosentan was weighed and transferred into 100 ml volumetric flask and dissolved in ethanol. Then the volume was made up to the mark with ethanol to produce 100 µg/ml concentration stock solution.

##### Preparation of working standard solutions

From the standard stock solution 0.6, 0.9, 1.0, 1.2, 1.5 and 1.8 ml aliquots were pipetted into separate 10 ml volumetric flasks and the volume was made up to the mark with ethanol to get working standard solutions with concentrations of 6, 9, 10, 12, 15 and 18 µg/ml respectively.

##### Determination of working wavelength ( $\lambda_{max}$ )

The  $\lambda_{max}$  (wavelength of maximum absorption) of bosentan was determined by scanning 10 µg/ml solution of the drug (ethanol used as diluent) using UV-Visible spectrophotometer within the wavelength region of 220 to 400 nm against ethanol as blank. The absorption curve (fig. 1) showed characteristic absorption maxima at 270 nm for Bosentan.

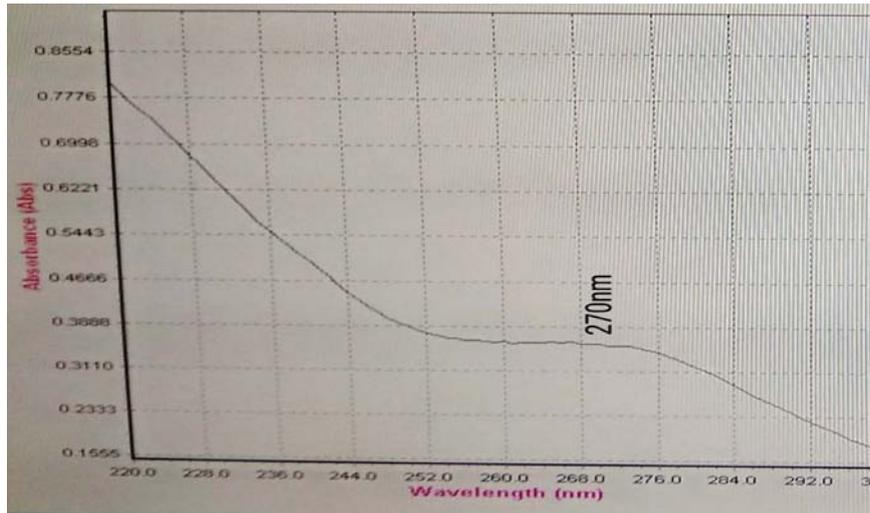


Fig. 1: UV spectrum of standard bosentan solution

**Sample preparation**

**Spiking procedure**

Appropriately 3 ml of plasma samples was placed in polypropylene tubes with conic bottom and aliquots of bosentan standard solution were spiked at the range 6 to 18 µg/ml.

**Extraction procedure**

Liquid-liquid Extraction (LLE) method was used. A mixture of 1.5 ml of acetonitrile and 0.25 ml of chloroform was added to the sample solution. A cloudy solution was obtained which was subjected to centrifugation for 5 min at 3000 rpm. The supernatant was collected and evaporated to dryness. The residue was dissolved in 10 ml ethanol and absorbance was measured at 270 nm using UV-Vis spectrophotometer.

**Method validation**

The method validation was done as per the FDA guidelines. The linearity, accuracy, recovery, and robustness parameters were tested.

**Linearity**

Linearity of the method was studied by analyzing six standard solutions covering the range of 6 to 18µg/ml. From the primary stock solution 0.6 ml, 0.9 ml, 1.0 ml, 1.2 ml, 1.5 ml and 18 ml of aliquots were pipetted into 10 ml volumetric flasks and made up to the mark with ethanol to give concentrations of 6µg/ml, 9µg/ml, 10µg/ml, 12µg/ml, 15µg/ml and 18µg/ml. Calibration curve showing concentration versus absorbance was plotted by measuring absorbance of the prepared working standard solutions at 270 nm using ethanol as blank. The linearity data is presented in table 1 and calibration curve is shown in fig. 2.

Table 1: Linearity data of bosentan

Concentration (µg/ml)	Absorbance
6	0.264
9	0.3642
10	0.3901
12	0.4707
15	0.5651
18	0.6756

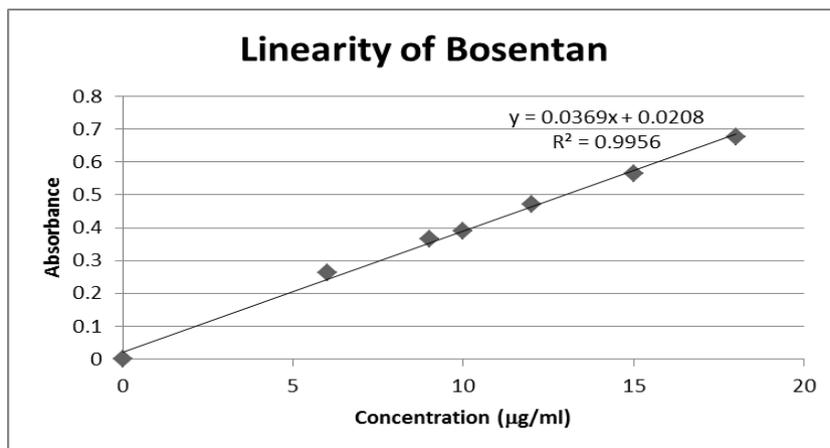


Fig. 2: Calibration curve of bosentan

**Accuracy (Recovery study)**

The accuracy of the method was determined by calculating the recoveries of bosentan by the standard addition method at three levels. Known amounts of standard solutions of bosentan were added at 50%, 100% and 150% concentration to pre quantified sample solution of bosentan and the amount of drug recovered was estimated. Each level has been analyzed in triplicate. Results are

shown in table 2. The recovery was found to be in between the predefined acceptance criteria of 80.0–120.0%.

**Robustness**

Robustness of the method was determined by deliberately altering the  $\lambda_{max}$ . Absorbance of the standard solution was measured at  $\lambda_{max} \pm 1$  nm. The results of the study (table 3) represented no significant changes in the absorbance illustrating the robustness of the method.

**Table 2: Accuracy study data**

Concentration added	Mean % recovery	% RSD
50%	99.9	0.007124
100%	100.02	0.005436
150%	98.92	0.009791

**Table 3: Robustness study data**

S. No.	269 nm	270 nm	271 nm
1	0.450	0.457	0.454
2	0.451	0.457	0.453
3	0.451	0.456	0.454
Mean	0.450666	0.456666	0.453666
SD	0.000546	0.000321	0.000275
%RSD	0.091341	0.061352	0.042576

**DISCUSSION**

The wavelength of 270 nm was chosen because it was the  $\lambda_{max}$  of bosentan. LLE was performed using chloroform as it has maximum extraction efficiency compared to other solvents like dichloromethane.

**CONCLUSION**

A simple, easier, rapid and economical LLE process with UV-Visible spectrophotometric measurement was developed and validated for analysis of bosentan in spiked human plasma. The analytical technique presented in this study decreases analysis time and also the need for complicated or expensive equipment. Therefore, this technique can be used for routine quantitative analysis of bosentan in plasma and other biological samples and can also be further extended to other higher end analytical techniques.

**AUTHORS CONTRIBUTIONS**

All the author have contributed equally

**CONFLICT OF INTERESTS**

Declare none

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