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Research Article

Development and Validation of UV Spectroscopic and HPTLC Methods for the Determination of Bosentan from Tablet Dosage Form

A. Suganthi*, Sai Lakshmi. C. H, Sruthi Vinod, T. K. Ravi
Department of Pharmaceutical Analysis, College of Pharmacy,Sri Ramakrishna Institute of Paramedical Sciences, 395,Sarojini Naidu Road, Coimbatore -641 044, India

***Corresponding author**
Dr. A. Suganthi
Email: suganlemu@gmail.com

Abstract: Two simple, sensitive and precise UV spectroscopic and HPTLC methods were developed and validated for the determination of bosentan in tablet dosage form. UV spectroscopic method: For the determination of bosentan, the stock solution of 100 µg/ml of drug was prepared in dichloromethane. The measurement of absorbance was noted at 274 nm. The calibration curve was obtained in the range 10-40 µg/mL. The slope, intercept and correlation coefficient values were found to be 0.02975, -0.0063 and 0.9992, respectively. HPTLC method: For the determination of bosentan by HPTLC method, a precoated silicagel 60 F_{254} on aluminium sheets and a mobile phase system comprising of methanol: ammonia (10: 1 drop, v/v) were selected. After development the plate was scanned and quantified at 288 nm. Linearity was found in the concentration range of 10 to 80 ng/spot (r = 0.9991). Limit of detection was found to be 1 ng/spot and limit of quantification was found to be 4 ng/spot. Low relative standard deviation and good % recovery values of both the methods showed that the developed methods were highly precise, accurate and free from interference present in formulation.
Keywords: Bosentan, HPTLC, UV spectroscopy, Method Validation, ICH guidelines

INTRODUCTION

Bosentan is a dual endothelin receptor antagonist used in the treatment of pulmonary artery hypertension [1]. Chemically it is 4-tert-butyl-N-[6-(2-hydroxy ethoxy)-5-(2-methoxy phenoxy)-2-(pyrimidin-2-yl) pyrimidin-4-yl] benzene-1-sulfonamide with empirical formula $C_{27}H_{29}N_5O_6S$ and molecular weight 569.64 (Fig. 1).

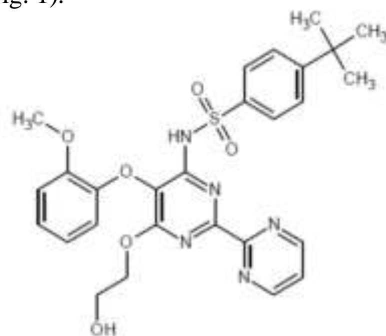

Fig. 1: Chemical structure of bosentan

Literature survey revealed that bosentan can be estimated by RP-HPLC [2-5] and LC-MS [6]. Among the various methods available UV and HPTLC methods continued to be very popular, because of their simplicity, specificity and low cost. Therefore, the need for fast, low cost, selective method is obvious, especially for the routine quality control analysis of pharmaceutical formulation containing bosentan. The purpose of the present work was to develop simple and reliable UV- spectroscopic and HPTLC methods for determination of bosentan from tablet dosage form. The methods have been optimized and validated as per the ICH guidelines [7].

MATERIALS AND METHODS

Chemicals and Reagents

Pharmaceutical grade of bosentan was used without further purification. The marketed formulations Lupibose- 62.5 mg (Lupin) tablets were purchased from local market. All chemicals and reagents used were of analytical grade. UV spectral measurement were recorded using Jasco V-630 Spectrophotometer and HPTLC was performed in Camag HPTLC System equipped with Linomat 5 sample applicator twin trough plate development chamber, TLC Scanner 3 with WinCATS software.

Instrumentation and analytical condition

The UV method was performed on Jasco V-630 Double Beam UV/Vis Spectrophotometer at 274 nm. One centimetre quartz cells were used for measuring absorbance. The HPTLC study was conducted on 20x10 cm aluminium sheet precoated with 250 µm layers of

silicagel $60F_{254}$ *(E.* Merck, Darmstadt, Germany). Before used the plates were pre washed with methanol and activated at 110ºC for 5 min. Samples were applied to the plate as bands that were 6mm wide and 6mm apart by means of CAMAG (Muttenz, Switzerland) Linomat 5 sample applicator equipped with a 100 µL syringe (Hamilton, Bonaduz, Switzerland). Linear ascending development was performed in a twin trough glass chamber of 20 x 100 cm (Model 022.5253 CAMAG), methanol: ammonia (10:1drop v/v) mobile phase after saturation of chamber with mobile phase vapour for 20 min. The development distance was 80 mm and the development time was approximately 15 min. After development, the plates were dried. The scanning was performed using TLC scanner 3 in the absorbance mode 288nm for all measurements having slit dimension was 5×0.45 mm. The source of radiation utilized was deuterium lamp emitting a continuous UV spectrum between 200 to 400 nm.

UV method

Preparation of standard solutions and calibration graph*:* Stock solution of bosentan (100µg/ml) was prepared by dissolving10 mg of pure drug in 100 ml of dichloromethane. Adequate dilutions were prepared from the standard stock solution to get a concentration ranging from 5-40 µg/ml of bosentan using dichloromethane. Absorbance of the solutions was measured at 274 nm. The measured absorbance of each solution was plotted against the corresponding concentration. Preparation of sample solution: Ten tablets, each containing 62.5 mg of bosentan were weighed and average weight was calculated. Weight equivalent to 10mg of bosentan was weighed, transferred to 100 mL standard flask, extracted with dichloromethane and made up with the same solvent and filtered through Whatman filter paper. Suitable aliquots of formulation solutions were prepared, scanned in UV region and absorbances were noted at274nm.

HPTLCmethod

Preparation of standard solution: Stock solution of bosentan (100 µg/mL), was prepared in methanol and applied as bands on a TLC plate in a range of 0.1 to 0.8 µl using the Linomat 5 sample applicator. The plate was developed and scanned using the established conditions. Each amount was analysed six times and peak areas were recorded. Calibration curves were plotted as peak area versus concentration. Preparation of sample solution: Ten tablets, each containing 62.5 mg of bosentan were weighed and average weight was calculated. Weight equivalent to 10 mg of bosentan was weighed, transferred to a 100 mL volumetric flask, extracted and made up to volume with methanol and filtered through a Whatmann filter paper. With the fixed chromatographic conditions, 0.1- 0.8 µL from standard stock solution of bosentan (100 µg/mL) and suitable volumes from sample solution were also applied on the plate and the peak areas were measured.

Method validation [7]

The methods were validated according to ICH Q2B guidelines for validation of analytical procedures in order to determine the linearity, sensitivity, accuracy and precision for each analyte.

Linearity and Range

Eight point calibration curves were generated with appropriate volumes of working standard solution for both UV and HPTLC methods. In case of UV, the concentration range selected was 5-40 µg/mL whereas for HPTLC, 10 to 80 ng/spot was taken to study the linearity.

Precision and Accuracy

Precision and accuracy were determined using the concentration of 10, 20 and 30µg/ml for UV and 40 and 50 ng/spot for HPTLC. The precision of the assay was determined by intra-day and inter-day precision and reported as % RSD.

To study the accuracy of the proposed methods, and to check the interference from excipients present in the dosage form, recovery experiments were carried out by the standard addition method. Accuracy is the % analyte recovered by assay from a known added amount.

Specificity

The method specificity was assessed by comparing the chromatograms (HPTLC) obtained from the drug and the commonly used excipients with those obtained from the blank. Here the excipients included lactose, starch and magnesium stearate. The drug to excipient ratio used was similar to that in commercial formulation.

Limit of detection (LOD) and Limit of quantification (LOQ)

These parameters are measured with acceptable accuracy and precision for both the developed methods. The S/N ratios 3:1 and 10:1 were considered as LOD and LOQ, respectively.

RESULTS AND DISCUSSION

UV method [8-11]

Bosentan is having π electrons in its structure and hence absorbs electromagnetic radiation between 225-400 nm. This character is used for its determination by UV spectroscopic method. Solubility of drug was checked in solvents like methanol, ethanol, and dichloromethane. Among these, bosentan was freely soluble in dichloromethane and gave a good spectrum with acceptable absorbance. Hence dichloromethane was selected as solvent. A solution containing bosentan (20 µg/mL) was prepared and scanned in UV region,

from which a wavelength of 274 nm was selected for further studies (Figure 2).

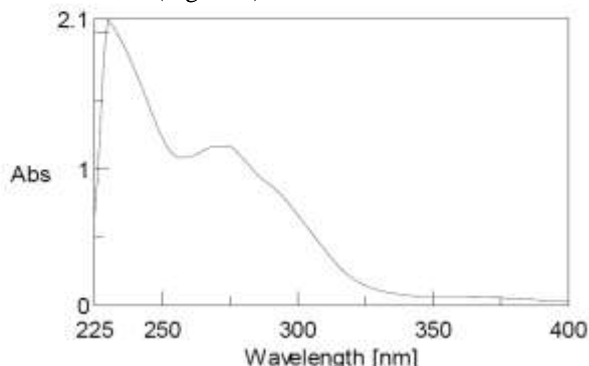

Fig. 2: UV spectrum for Standard Bosentan showing the absorbance maximum at 274 nm

An eight point calibration curve was constructed with working standards and was found linear (r = 0.9992) over their calibration ranges (Figure 3). Intra and inter-day variations were measured at six replicates and the results are given as % RSD (Table 1).Figure 4 show UV spectrum of formulation (20 µg/mL). In order to demonstrate the validity and applicability of the proposed method, recovery studies were performed by analysing pre analysed sample formulation. Recovery of the method was greater than 99% shows that method is highly accurate (Table 2).

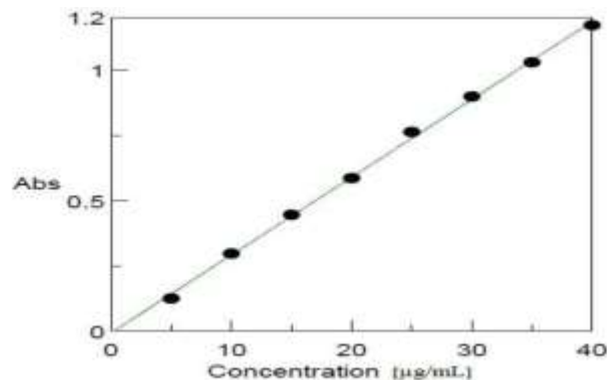

Fig. 3: Calibration graph of bosentan by UV method(5-40 µg/ml)

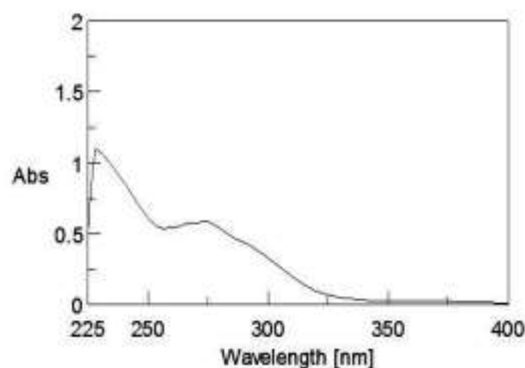

Fig. 4: UV spectrum of formulation (20 µg/mL)

Table 1: Precision studies for Bosentan by UV Spectroscopic method

Concentration (µg/ml)	Average absorbance	%RSD*
Intraday		
10	0.2946	0.4921
20	0.5732	1.338
30	0.8961	0.173
Interday		
10	0.2934	0.665
20	0.5864	0.234
30	0.8974	0.436

*Average of six determinations.

Table 2: Recovery studies of Bosentan by UV and HPTLC methods

Method	Level	% Recovery	% RSD*
UV	50%	99.24	0.518
	100%	99.85	1.137
HPTLC	50%	100.26	0.518
	100%	99.53	1.137

*Average of six determinations.

Table 3: Precision studies for Bosentan by HPTLC

Concentration (ng/spot)	Amount found (ng/spot)	%RSD*
Intraday		
40	39.89	0.870
50	49.74	0.604
Interday		
40	40.22	1.078
50	49.20	0.616

*Average of six observations

HPTLC method [12]

Various solvents like methanol, ethanol, chloroform, carbon tetra chloride, toluene, dichloromethane were tried along with ethylacetate, diethylamine and ammonia or glacial aceticacid. The mobile phase methanol with ammonia gave a good symmetrical peak, hence it was selected for the study (R_f = 0.76±0.02). After development of the plate bands were scanned over the range of 200-400nm (spectrum scan speed 100mm/s) the maximum absorbance was seen at 288nm which was used for the measurement of peak area.

When the saturation time was less than 20 min the development of the drug has taken more time with shift in R_f value and after 20 min. the development has taken in a straight line with a reproducible R_f value and resolution. Hence the chamber saturation time was fixed as 20 min. when the pre conditioning time was less than 20min, the appearance of secondary front was observed. However, after 20min. of plate saturation, the precision and reproducibility of peak area was found to be good. Hence, the plate equilibration time was fixed as 20min. Instead of ammonia (peak modifier) glacial acetic acid and triethyl amine were tried which results in poor peak symmetry. The optimum migration distance was 8 cm. Calibration curve was plotted with peak areas of standard drug versus concentrations (Figure 5). Linearity was found in the concentration range of 10 to 80 ng/spot (r=0.9991).

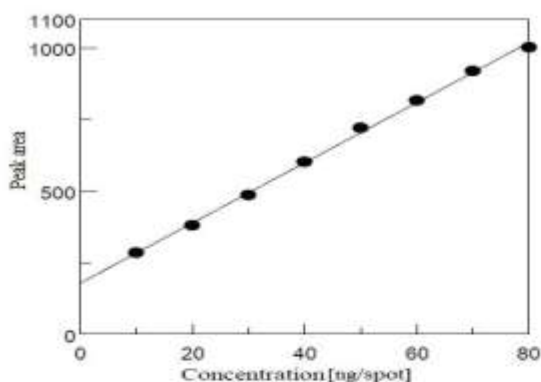

Fig. 5: Calibration graph of bosentan by HPTLC method (10-80 ng/spot)

Limit of detection was found to be 1 ng/spot and limit of quantification was found to be 4 ng/spot. Figure 6 shows standard chromatogram of bosentan (80ng/spot) while figure 7 shows chromatogram of bosentan formulation (40ng/spot). Accuracy and precision were determined using the concentration of 40 and 50 ng/spot for six times. The results were tabulated in table 2 & 3. The % recovery values (>99%) and low relative standard deviation show that methods are highly accurate and precise.

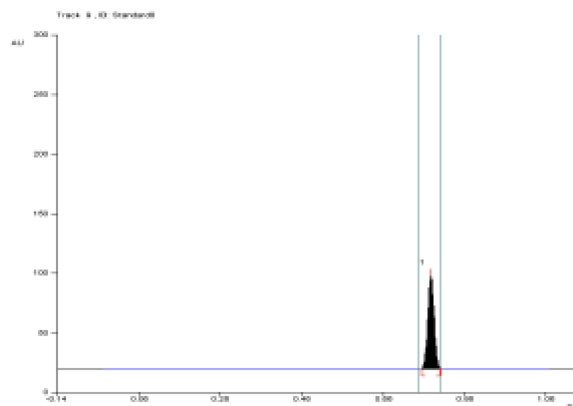

Fig. 6: Standard Chromatogram of Bosentan (80 ng/spot)

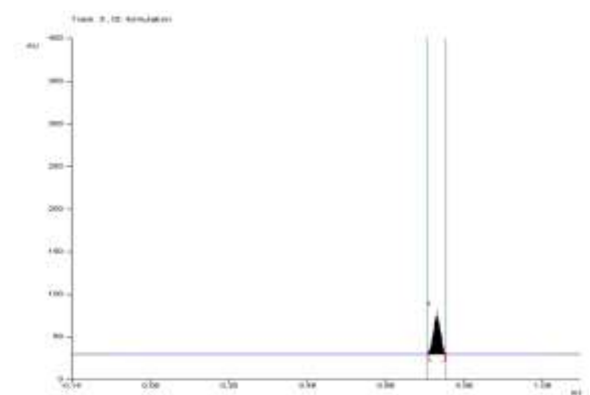

Fig. 7: Chromatogram of Bosentan formulation (40 ng/spot)

CONCLUSION

The results of analysis have been validated statistically and recovery studies confirmed the reproducibility and accuracy of the proposed methods which were carried out by following ICH guidelines. Analysis of authentic sample containing bosentan showed no interference from the common additives and excipients. Both the methods were found to be equally simple, sensitive and precise. Hence, recommended procedure is well suited for the assay and evaluation of drugs in commercial tablets. It can be easily and conveniently adopted for routine quality control analysis.

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