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Analytical Method Development and Validation for Estimation of Bosentan in Bulk and Tablet Dosage Form by High Performance Thin Layer Chromatography

S.H.Rizwan[1*], V.Girija Sastry[2] , Shaik Gazi[1], Nikath Jahan[1]
1. Department of Pharmaceutical Analysis,Deccan School of Pharmacy, Hyderabad
2. Department of Pharmaceutical Chemistry, College of Pharmaceutical Sciences, Andhra University

ABSTRACT

A simple, sensitive and accurate method employing HPTLC has been developed and validated as per ICH guidelines for determination of Bosentan in bulk and Tablet dosage form. The absorption maxima of the drug were found to be 288 nm in methanol. The method was validated as per ICH guidelines. The linearity range was found to be 50-300 ng/ml with a regression coefficient of 0.98.Subsequent validation parameters like precision, repeatability in terms of Interday and Intraday precision and recovery studies were evaluated with satisfactory results, the % RSD for these parameters was found to be 1.73%,1.30-0.48%,1.80 – 0.45 % , 1.54 – 1.48 % respectively with an Rf value of 0.23.

Keywords: Bosentan, High Performance Thin Layer Chromatography, Validation

*Corresponding Author Email: visitrizwan@rediffmail.com
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INTRODUCTION

Bosentan is a Dual Endothelin receptor antagonist used in the treatment of Pulmonary artery hypertension (PAH). Chemically it is 4-tertbutyl-N-[6-(2-hydroxyethoxy)-5-(2-methoxy-Phenoxy)-2-(Pyrimidin-2-yl) pyrimidin-4-yl] benzene - 1- sulphonamide.[1] It belongs to the category of Endothelian receptor antagonists (ERA) which are selective ERA-A blockers, ERA-B blockers and Dual endothelin blockers. Bosentan belongs to the last category; other examples of this class are Macitentan and Teazosentan. It works by stopping the action of Endothelin a natural substance that causes blood vessels to narrow and prevents normal blood flow in people having PAH.[2] It is manufactured in India by Lupin and Cipla Pharmaceuticals under the brand name of Lupibose and Bosentas respectively.

Literature survey reveals that analytical works have been reported using RP-HPLC [9,10,13,14], LC-MS [11,12] but cost effective analytical techniques like UV, Colorimetry [3,4,5,6] and HPTLC [7,8] are very few. Therefore the aim of the present work is to develop a cost effective, simple and reliable method employing HPTLC and validating the same as per ICH guidelines.

MATERIALS AND METHOD

Chemicals and reagents

Bosentan bulk drug was obtained as a gift sample from Chandra Labs, Hyderabad, Telangana. Analytical Grade Reagents such as HPLC grade Water, Methanol, Toluene and Triethylamine from Merck Distribution agency, Hyderabad, India.

Instrumentation

Chromatographic analysis of drug was performed on Merck TLC plates precoated with silica gel 60/UV_{254} (20 X 10 CM) with 0.2 mm layer thickness .The samples were applied onto the plates as a band with the width of 5 mm using AS-30 sample applicator (Desaga , Switzerland) with a 10 µl sample syringe (Hamilton , Switzerland) . Linear ascending development chromatography was carried out on the plate using a twin trough glass chamber (20 x10 cm). Later, Densitometry scanning was performed using densitometer CD-60 TLC scanner (Desaga) with proquant software and a UV cabinet fitted with a dual wavelength UV lamp. (254 nm & 366 nm).

Chromatographic Condition

Wavelength was selected by scanning standard solution across the UV range of 200 nm – 400 nm. Bosentan showed maximum absorbance at 288 nm; therefore photometric measurements were read at this wavelength using a deuterium lamp by means of Camag CD-60 TLC scanner , also using the DESAGA software.

To carry out HPTLC analysis Pre coated silica gel aluminium plate 60F-254 were pre washed with methanol, Activation was done in an oven at 50^0 C for 15 min.

Mobile phase consisted of a mixture of Toluene: Methanol: Triethylamine (8:2:0.1 v/v/v/).

The chamber required a saturation time of 30 mins, migration distance observed was 0.23 in approx less than 8 mins.

Development distance: 80 mm

Development time: 20 min

Detection at 288 nm

Preparation of Standard Stock Solution

Standard stock solution of Bosentan was prepared by dissolving 10 mg of drug to obtain a concentration of 10 ppm.

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, and transferred to a 100 ml volumetric flask. The contents of the flask were sonicated for 30 min to dissolve the active ingredients completely. The solution was filtered through a whatman filter paper no.41. This solution contains 100 mcg/ml of test solution and was used for further application.

METHOD VALIDATION

The above optimized HPTLC method was then validated for the parameters listed under ICH Q2B guidelines.[15]

Linearity and Range

A Calibration curve was plotted using peak area vs concentration in the range of 50 – 300 ng/band for Bosentan. Working standard solution is applied in the range of 50 ng/band to 300 ng/band to get above range of linearity. The Rf value obtained at 288 nm was found to be 0.23.

Precision

Precision of the method was determined in the terms of Intraday & Interday variation using % RSD. Intraday precision was assessed by analysing test drug solution (100 ng/band) within the calibration range , three times on the same day . Interday precision was assessed by analysing Test drug solution within calibration range on three different days.

Accuracy

Recovery studies were carried out by applying the standard addition method. A known amount of standard was added to the corresponding 50%, 100%, and 150% of preanalysed test solution of Bosentan. The recovery studies were carried out in triplicate.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ were separately determined basis of standard calibration curve. The residual standard deviation of the regression line or the standard deviation of y- intercepts of regression lines was used to calculate LOD and LOQ. Following formulae were used; LOD= 3.3×D/S and LOQ= 10×D/S, where, D is the standard deviation of the y-intercepts of regression line and S is the slope of the calibration curve.

Robustness study

Robustness of the method was performed for the various factors like Mobile Phase Composition and Wavelength. The data clearly show that the proposed method is robust at small but deliberate changes.

Solution stability study

The stability of the test solution was evaluated. The solution was stored at ambient temperature and tested at intervals of 24 and 48 hours. The responses for the aged solution were evaluated using a freshly prepared standard solution.

Specificity

The specificity of the method was determined by analyzing standard drug and test samples. The spot for Bosentan in the samples was confirmed by comparing the Rf to that of standard drug.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

Several mobile phases were tried to resolve the spot for Bosentan. Finally optimized mobile phase is Toluene: Methanol: Triethylamine (8:2:0.1 v/v/v) which gives Rf values of 0.23 for Bosentan and detection wavelength is 288 nm. The UV spectrum for the drug is represented in Figure.1, with overlain spectra of the chromatogram in Figure.3.

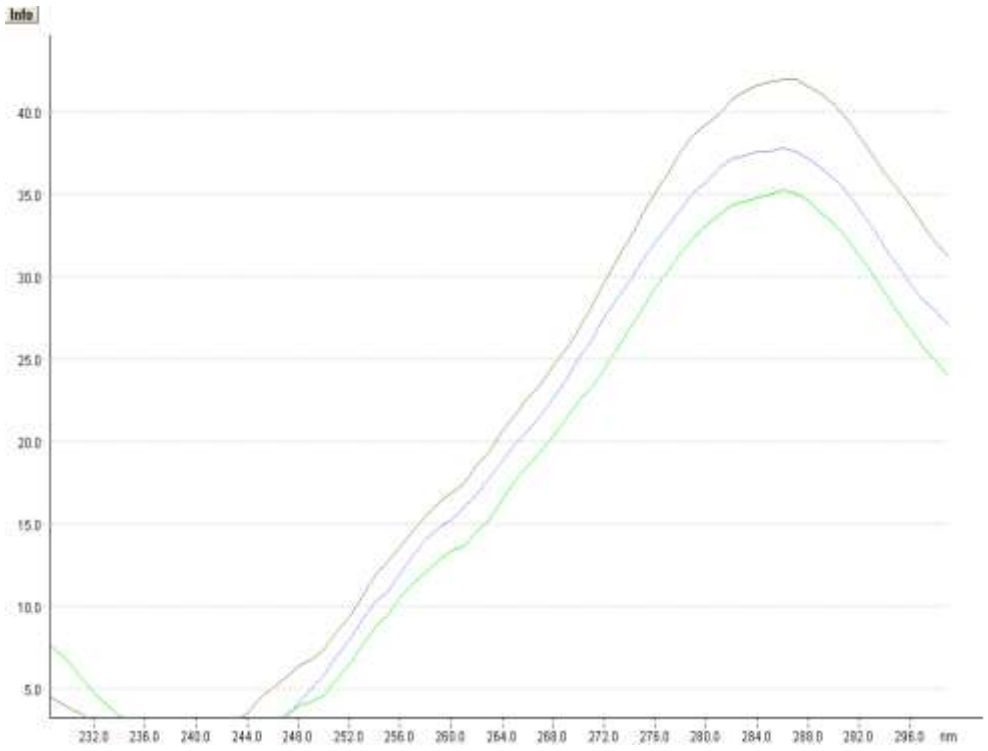

Figure 1: UV- Spectrum of Bosentan in Methanol at 288 nm.

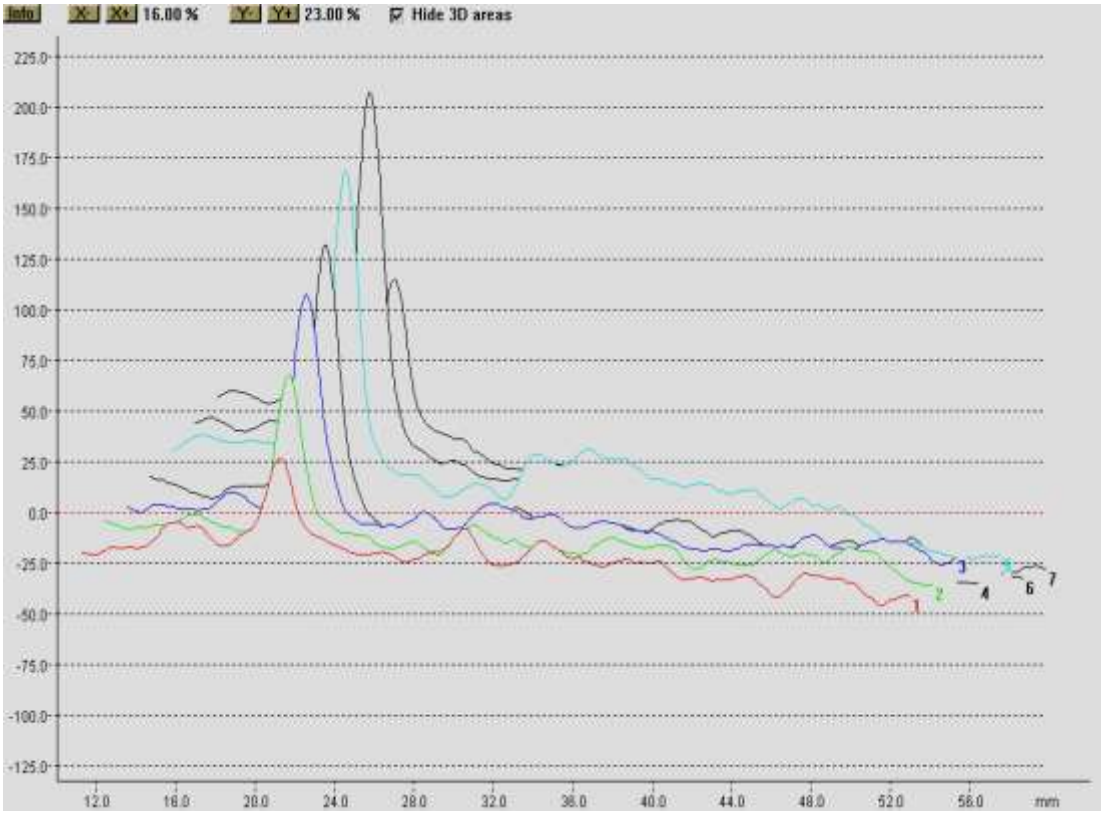

Figure 3 – Overlain representation of Bosentan Chromatogram

Linearity and Range

The response for the drugs was found to be linear in the concentration range of 50-300 ng/band for

Bosentan with correlation coefficient of 0.988. The linear regression equation obtained is y = 1.4311x + 27.4363. The graph for linearity is represented in Figure.2

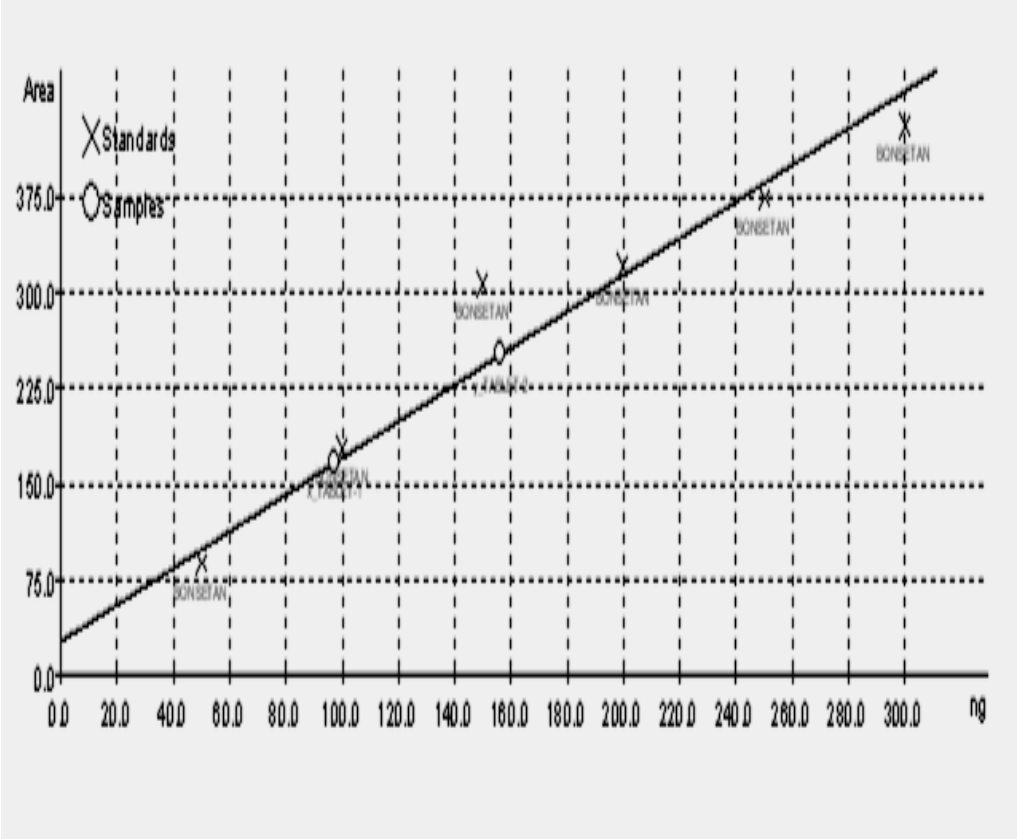

Figure 2 - Linearity Graph of Bosentan

Precision

Precision was calculated in terms of Repeatability and Intermediate precision. Repeatability was performed by applying six replicates of sample. For Intermediate Precision Interday and Intraday precision was performed by determining the corresponding responses in triplicate on the same day and on three different days thus confirming precision of method. The % RSD for repeatability was found to be 1.73%. The % RSD for Intermediate precision was found to be 1.80 %, thus confirming precision of the method. Table 1 and Table 2 represent the data of Precision and repeatability studies respectively.

Table 1: **Precision Data for Bosentan**

S.NO:	**Concentration ng/ml**	**Area Response**
1	100	166.5
2	100	170.28
3	100	168.60
4	100	165.27
5	100	175.3
	Average	168.032

S.D.	2.90
% R.S.D.	1.73

SD= Standard Deviation, **RSD**= Relative Standard Deviation

Table 2: **Intermediate Precision Data for Bosentan**

Drug	**Concentration (ng/ml)**	**Interday Precision**		**Intraday Precision**	
Bosentan		**Mean Peak Area**	**% RSD**	**Mean Peak Area**	**% RSD**
	100	168.16	1.30	162.05	1.80
	200	332.15	0.77	328.56	0.77
	300	498.25	0.48	487.98	0.45

Recovery Studies

Accuracy or Recovery studies were performed using the standard addition method on the drug sample at three different levels and finally calculating the average of 3 observations. % RSD for Accuracy was observed to be between 1.54% - 1.48 %. Table 3 represents the data for accuracy studies of Bosentan in formulation.

Table 3: Accuracy data for Bosentan

Drug	**Amount of Test Solution (ng/band)**	**Amount of Std added (ng/band)**	**Peak Area***	**% Recovery***	**%RSD***
Bosentan	50	100	197.25	92.08	1.54
	100	100	328.59	96.47	1.47
	150	100	475.32	94.62	1.48

***=Average of three determinations**

Limit of Detection and Limit of Quantification

The LOD was calculated by standard formula as per ICH guidelines was found to be 7.84 ng/ml for Bosentan. The LOQ value was found to be 23.75 ng/ml.

Robustness study

Slight change in the chromatographic condition of the developed method does not affect the acceptance result. So method is found to be robust.

Solution stability study

The solution stability study at different time intervals showed that standard and test solutions of Bosentan were stable up to 72 hr at ambient temperature as no significant difference was found in the assay results for the drug.

Specificity

The specificity of method was determined by the complete separation of Bosentan peak in the presence of tablet excipients. The peak purity of Bosentan was assessed by comparing their respective spectra at the peak start, peak apex and peak end positions of the spot. Figure.4

represents the chromatograms of both Bosentan bulk drug and formulation, highlighting no interference of excipients.

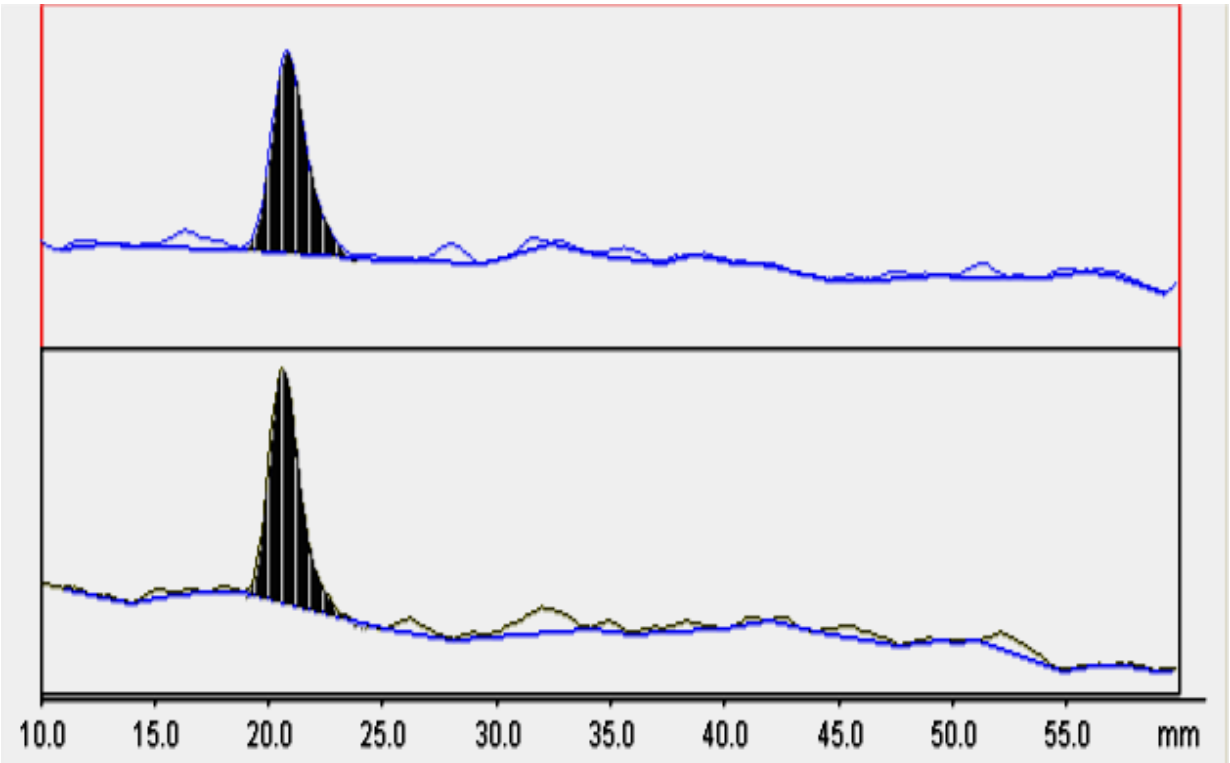

Figure.4 – Chromatographic representation of Bosentan

Assay of dosage form

% Assay of Bosentan was found to be 98.24±0.92 respectively in the dosage form. Table 4 represents the data of percentage assay observed.

Table 4: Assay for Bosentan

Drug	Amt of drug(mg)		%Label claimed	%RSD
	Labelled	Estimated		
Bosentan	62.5	59.62	96.24	0.92

***= Average of six determination**

Table 5: Summary of validation parameters

Bosentan Parameters	Result
Linearity Range (ng/spot)	50-300 ng/spot
Regression equation	Y= 1.4311x + 27.4363.
Co-relation Co-efficient	0.98
% Recovery	96.47 %
Precision (% RSD)	1.73
Inter-Day (% RSD)	1.30
Intra-Day (% RSD)	1.80
Limit of Detection (ng/spot)	7.84 ng/ml
Limit of Quantification (ng/spot)	23.75 ng/ml

CONCLUSION

Proposed study describes an HPTLC method for the estimation of Bosentan in bulk and pharmaceutical dosage form. The method has been found to be good because of use of an economical and readily available mobile phase and UV detection. The method gives good resolution for this drug with a short analysis time. The method was validated and found to be simple, sensitive, accurate and precise. Percentage recovery shows that the method is free from interference of the excipients used in the formulation. Moreover no pre-treatment of sample is required. Therefore, the proposed method can be used for routine analysis of Bosentan in bulk and formulation dosage form. The summary of the validation parameters is represented in Table 5.

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