Research Article

Development and Validation of Stability Indicating HPTLC Method for Estimation of Bosentan Monohydrate in Tablet Dosage Form

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ABSTRACT

A sensitive, accurate, and precise stability-indicating HPTLC method has been developed for the estimation of Bosentan monohydrate in the presence of its degradation products. The drug was subjected to acidic, alkaline, oxidative and photo-degradation conditions. The method employed Chloroform: methanol: ammonia solution (8.5: 1.5: 0.1 v/v/v) as a mobile phase and Silica Gel G 60 F_{254} TLC plates as stationary phase. Quantitatively the spots were scanned at 270 nm. The R_f Value of drug was found to be 0.49 ± 0.02. The linearity was obtained in range 200 – 1000 ng/spot. The LOD and LOQ were found to be 17.42 ng/spot and 54.43 ng/spot respectively. The percentage recovery was found in the range of 98.90 – 100.30%. One degradation product was separated in each acidic, photolytic and oxidative condition; two degradation products were separated in alkaline degradation condition by HPTLC method. The method was validated according to the ICH guidelines Q2 (R1). The developed method was successfully applied for quantitative analysis of Bosentan monohydrate in its marketed formulation.

Keywords: Bosentan monohydrate (BST), HPTLC, Stability Indicating Method, Forced Degradation Study

1. INTRODUCTION

Bosentan is chemically 4-tert-butyl-N-[6-(2-hydroxyethoxy)-5-(2-methoxyphenoxy)-2-(pyrimidin-2-yl) pyrimidin-4-yl] benzene-1-sulfonamide (Figure 1) [1]. Bosentan is a competitive antagonist of endothelin-1 at the endothelin-A (ET-A) and endothelin-B (ET-B) receptors. Under normal conditions, endothelin-1 binding of ET-A or ET-B receptors causes constriction of the pulmonary blood vessels. By blocking this interaction, Bosentan decreases pulmonary vascular resistance. It is used in the treatment of pulmonary artery hypertension (PAH). Bosentan has slightly higher affinity for ET- A than ET-B.

Bosentan API and tablet is not official in any pharmacopoeias. The literature review reveals that few analytical and bio-analytical methods have been reported for its quantitative estimation in pharmaceutical formulations and biological fluids, which include spectrophotometry, HPLC, Stability indicating HPLC and bio-analytical methods [2-13].

Stability indicating HPTLC method is not reported. Therefore in the present study attempts were made to develop precise and accurate stability indicating HPTLC method for estimation of Bosentan monohydrate in its tablet dosage form.

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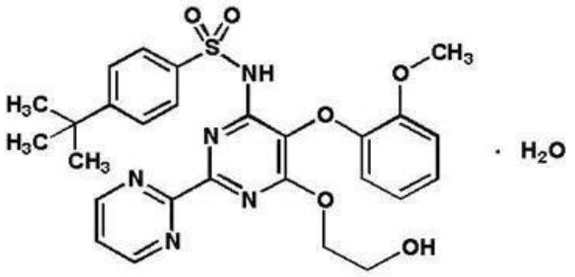

Figure 1: Structure of Bosentan

2. MATERIALS AND METHODS

2.1. Instruments

The HPTLC system (Camag Switzerland) consisting of Linomat V semiautomatic spotting device, TLC Scanner IV (Camag Muttenz, Switzerland), twin-trough developing chamber (10 x 10cm), UV cabinet with dual wavelength UV lamps, Win-CATS software, syringe (100 µl capacity, Hamilton) were used for chromatographic study. Electronic analytical balance (Shimadzu AUX-220) was used for all the weighing.

2.2. Reagents and Materials

Bosentan (98.99%) was received as a gift sample from Cipla Ltd., Mumbai. Methanol, chloroform, ammonia solution were purchased from s.d. Fine-Chem Limited, Mumbai, India. Bosentan tablets were purchased from local market.

2.3. Chromatographic Conditions

Chromatographic separation was performed on 10 × 10 cm aluminum backed plates pre-coated with 250 µm layer of silica gel 60 F_{254} (E. Merck, Darmstadt, Germany). The TLC plates were pre-washed with methanol and dried in oven at 50 ^{0}C for 30 min. Samples were spotted on TLC plate 15 mm from the bottom edge by Linomat V semi-automatic spotter using following parameters: band width, 6 mm; track distance, 11.6 mm; application rate, 0.1µl/s. The TLC plate was developed in twin trough chamber using chloroform: methanol: ammonia solution (8.5: 1.5: 0.1, v/v/v) as mobile phase at temperature, 25 ± 2 ˚C; chamber saturation time, 15 min; migration distance, 75 mm. The TLC plate was scanned and analyzed by TLC Scanner IV and WinCATS software using following parameters: slit dimension, 4 × 0.30 mm; scanning speed, 20 mm/sec; detection wavelength, 270 nm.

2.4. Preparation of standard solutions

2.4.1. Preparation of stock solution of standard BST

Accurately weighed quantity of BST (10 mg) was transferred into 10 ml volumetric flask, dissolved in 5 ml of methanol and diluted up to the mark with the same to obtain a stock solution having strength of 1,000µg/ml.

2.4.2. Preparation of working standard solution

The stock solution (0.5 ml) was withdrawn and transferred into 10 ml volumetric flask and diluted up to the mark with methanol (50µg/ml).

2.5. Preparation of Degradation solutions

BST was degraded in acid, alkaline, oxidative and photolytic stress conditions.

2.5.1. Acid Hydrolysis

The stock solution (5 ml) was transferred into 10 ml volumetric flask, diluted up to the mark with 2N HCl (500µg/ml). This solution was heated for 6 hours at 80^{0}C. After bringing to room temperature, aliquot of 1 ml of this solution was taken in 10 ml volumetric flask and then neutralized with 2N NaOH. Volume was made up to the mark with methanol (50µg/ml).

2.5.2. Alkaline Hydrolysis

The stock solution (5 ml) was transferred into 10 ml volumetric flask, diluted up to the mark with 2N NaOH (500µg/ml). This solution was heated for 6 hours at 80^{0}C. After bringing to room temperature, aliquot of 1 ml of this solution was taken in 10 ml volumetric flask and then neutralized with 2N HCl. Volume was made up to the mark with methanol (50µg/ml).

2.5.3. Oxidative Degradation

The stock solution (5 ml) was transferred into 10 ml volumetric flask, diluted up to the mark with

6% H_2O_2 (500µg/ml). This solution was heated for 4 hours at 80^0C. After bringing to room temperature, aliquot of 1 ml of this solution was taken in 10 ml volumetric flask and volume was made up to the mark with methanol (50µg/ml)

2.5.4. Photolytic Degradation

The stock solution (5 ml) was transferred into 10 ml volumetric flask, diluted up to the mark with methanol (500µg/ml). The solution was exposed to sun light for 24 hours. Aliquot of 1 ml of this solution was taken in 10 ml volumetric flask and volume was made up to the mark with methanol (50µg/ml).

A volume of 10 µl of each of the resulting solution was applied to TLC plate, developed and scanned as described under section 2.3.

2.6. Preparation of Calibration curve

From working standard solution (50µg/ml) aliquots of 4, 8, 12, 16 and 20 µl were spotted on the TLC plate, dried, developed and analyzed as described under section 2.3. The calibration curve of peak area versus respective concentration was plotted and correlation coefficient and regression line equation were computed.

2.7. Validation of Developed HPTLC Method

2.7.1. Linearity

The linearity response for BST was determined by analyzing five independent levels of calibration curves in the range of 200 – 1000 ng/spot.

2.7.2. Precision

2.7.2.1. Repeatability of measurement of peak area

Working standard solution (12 µl) was spotted on a TLC plate and analyzed as described under section 2.3. The separated spot of BST was scanned for seven times without changing plate position and RSD for measurement of peak area was computed.

2.7.2.2. Repeatability of sample application

Working standard solution (12 µl) was spotted on a TLC plate seven times. The areas of seven spots were measured and the RSD of peak area was calculated.

2.7.2.3. Intermediate Precision

Intraday Precision

It was determined by applying freshly prepared solution of BST (200-1000 ng/spot), three times on same day. RSD of peak area at each level was calculated.

Inter-day Precision

It was determined by applying freshly prepared solution of BST (200-1000 ng/spot) three times on different days. RSD of peak area at each level was calculated.

2.7.3. Specificity

The specificity of the method was ascertained by analyzing standard drug and sample. The band in sample was confirmed by comparing the R_f value and UV spectra of the band with that obtained from the standard. The peak purity was assessed by comparing the spectra acquired at three different positions on the band, i.e. peak start (s), peak apex (m) and peak end (e).

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

The limits of detection and quantification of the developed method were calculated from the standard deviation of the intercepts and mean slope of the calibration curves of BST using the formula as given below.

$LOD = 3.3\ \alpha/S$ $LOQ = 10\ \alpha/S$

Where, α is the standard deviation of the intercepts of the five calibration curves and S is the mean slope of the five calibration curve.

2.7.5. Accuracy

Accuracy was determined in terms of percent recovery. The proposed method was applied to determine BST in pharmaceutical dosage form.

Table 1: Preparation of Solutions for Accuracy Study

BST from tablet (mg)	Std. BST spiked (mg)	Concentration of BST in final solution (µg/ml)	Volume of solution spotted	Total BST (ng/spot)
10	-	50	6 µl	300
10	8	90	6 µl	540
10	10	100	6 µl	600
10	12	110	6 µl	660

Table 2: Recovery data of BST from formulation (Bosentas Tablet)

Amount of BST in tablet (mg)	Amount of standard BST added (mg)	Total amount of BST (mg)	Total peak area	Recovered amount of BST	% Recovery
10	-	10	4278.18	-	-
10	8	18	6734.13	7.91	98.90
10	10	20	7337.5	9.92	99.21
10	12	22	7989.66	12.03	100.30

The recovery experiment was carried out in triplicate by spiking previously analyzed samples with three different concentrations of standards at 80%, 100% and 120%. Preparation of solutions for accuracy study is given in Table 1.

2.8. Analysis of Marketed Formulation

Marketed tablet formulation (Bosentas) was analyzed using developed method. Twenty tablets were individually weighed and powdered. Powder equivalent to 10mg of BST was accurately weighed and transferred into 100 ml volumetric flask and 50 ml methanol was added. This solution was sonicated for 15 min and volume was made up to the mark with methanol. The solution was filtered. After filtration, the solution aliquot (5 ml) was transferred in 10 ml volumetric flask and diluted up to the mark with methanol. The solution (10 µl) was applied in triplicate on TLC plate and percentage of BST was calculated from the calibration curve.

3. RESULTS AND DISCUSSION

3.1. Selection of Wavelength

The working standard solution of BST (50µg/ml) was scanned between 200-700 nm and spectrum was recorded against methanol as blank in UV-Visible spectrophotometer. The absorbance maximum (λ_{max}) was found to be 270 nm which was selected as detection wavelength for BST.

3.2. Optimization of mobile phase

Numbers of trials were taken by changing the ratio of mobile phase with respect to achieve good resolution between BST and degradation products obtained in different degradation conditions with minimum tailing. The mobile phase Chloroform: Methanol: Ammonia solution (8.5: 1.5: 0.1, v/v/v) gave good resolution with Rf values of 0.49±0.02.

3.3. Forced Degradation Study

The degradation study indicated that BST was susceptible to acidic, alkaline, oxidative and photolytic degradation. BST gets degraded into one degradation product in acidic, photolytic, oxidation conditions and two degradation products under alkaline condition. The chromatogram of alkaline degraded sample of BST showed two additional peak at Rf value 0.38 and 0.70 (Fig.3). The chromatograms of the acidic, oxidative and photo degraded sample of BST showed one additional peak at Rf value 0.64, 0.34 and 0.59 respectively (Fig. 2). The results for stress degradation study are summarized in Table 3.

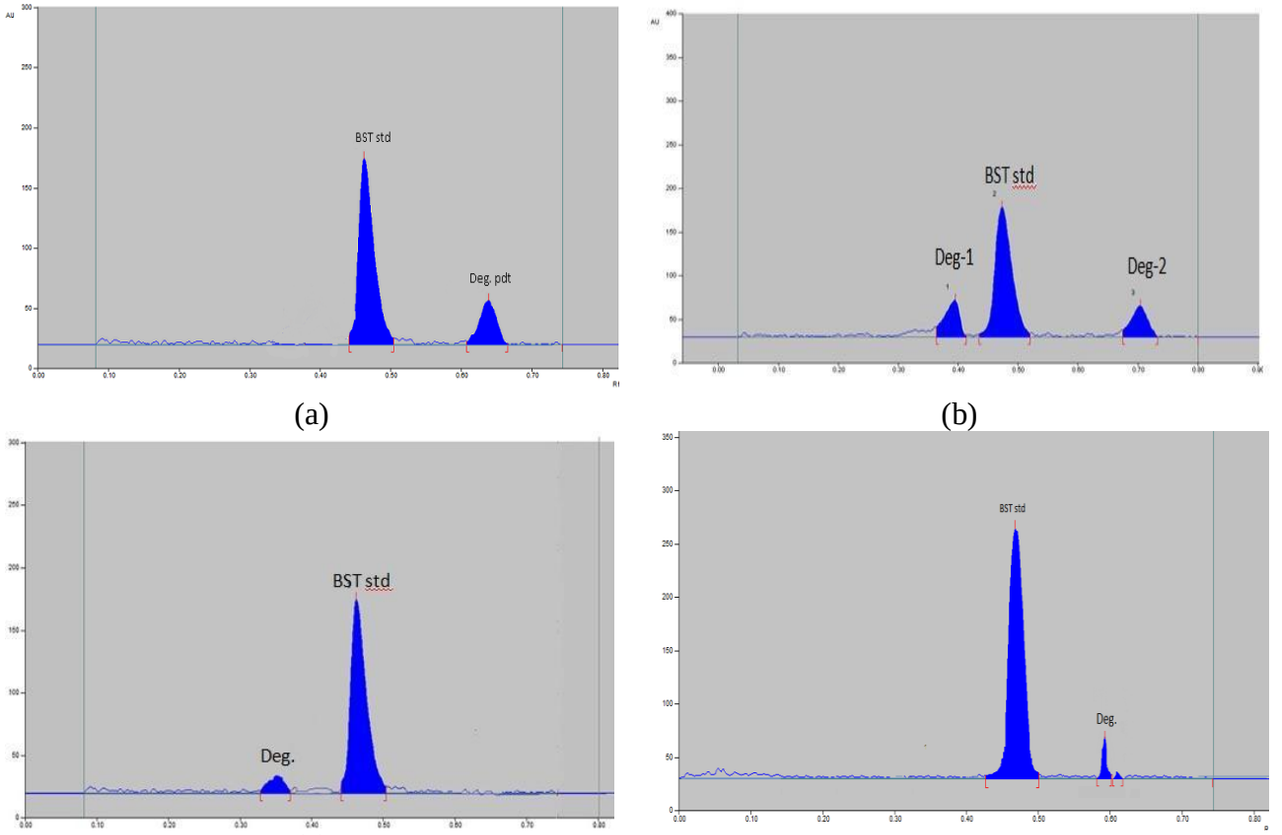

Figure 2: Chromatograms obtained from forced degradation studies. (a) Acidic hydrolysis (2N HCl at 80^{0}C for 6 hr); Deg (Rf: 0.64), BST (Rf: 0.49), (b) Alkaline hydrolysis (2N NaOH at 80^{0}C for 6 hr); Deg 1 (Rf: 0.38), Deg 2 (Rf: 0.70), BST (Rf: 0.49), (c) Oxidative degradation (6% H_2O_2 at 80^{0}C for 4 hrs); Deg (Rf: 0.34), BST (Rf: 0.48), (d) Photolytic degradation (exposed to sunlight for 24 hrs); Deg (Rf: 0.59), BST (Rf: 0.48)

3.4. Calibration curve of BST

Calibration curve of peak area versus respective concentration is shown in Figure 3. Linearity was determined from regression line equation.

3.5. Validation of Developed HPTLC Method

3.5.1. Linearity and Range

The linearity for BST was found in the range of 200-1000 ng/spot. Correlation coefficient for calibration curve of BST was found to be 0.9982. The regression line equation for BST is as following: y = 10.279 x + 1242.8.

3.5.2. Precision

The RSD for repeatability of sample application and for measurement of peak area of BST was found to be 0.76 and 0.31 respectively. The RSD for intra-day and inter-day precision of BST was found to be in range of 1.3 – 1.9 and 2.1 – 2.4 respectively.

3.5.3. Specificity

Comparison of chromatograms of standard BST and BST from tablet formulation showed identical R_f values of 0.49 ± 0.02 (n=3). Comparison of the UV absorbance spectra scanned at peak start (s), peak apex (m) and peak end (e) positions of individual spots of BST showed a high degree of correlation confirming the purity of the corresponding spots. Overlain spectra of BST and sample BST (Tablet) is given in Figure 4.

Table 3: Summary of forced degradation study of Bosentan Monohydrate

Stress type	Stress conditions	% Drug degradation
Acid hydrolysis	2N HCl at 80ºC, heated for 6 hr	18.96%
Alkali hydrolysis	2N NaOH at 80ºC, heated for 6 hr	30.09%
Oxidative degradation	6% H_2O_2 at 80ºC for 4 hr	9.43%
Photolytic degradation	Direct sunlight for 24 hr	11.27%

Table 4: Summary of Validation Parameters

Parameters	Results
Linearity Range	200-1000 ng/spot
Correlation Co-efficient (R^2)	0.9982
Precision (%RSD)	
Repeatability (n=7)	
Repeatability of measurement	0.31
Repeatability of sample application	0.76
Intermediate precision	
Intraday precision (n=3)	1.3 – 1.9
Inter day precision (n=3)	2.1 – 2.4
% Recovery	98.90 – 100.30 %
Limit of Detection (LOD)	17.42 ng/spot
Limit of Quantification (LOQ)	54.43 ng/spot

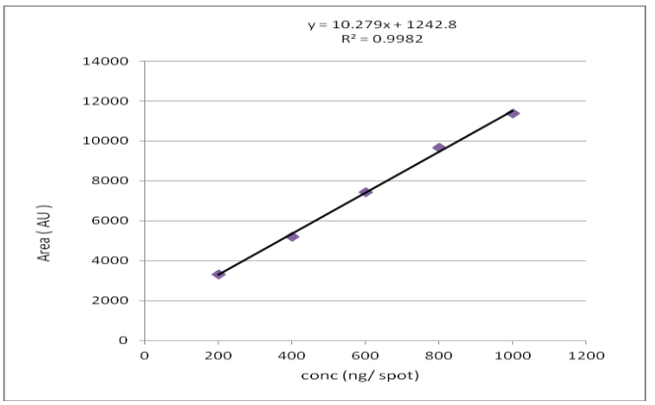

Figure 3: Calibration curve of BST (200 – 1000 ng/spot)

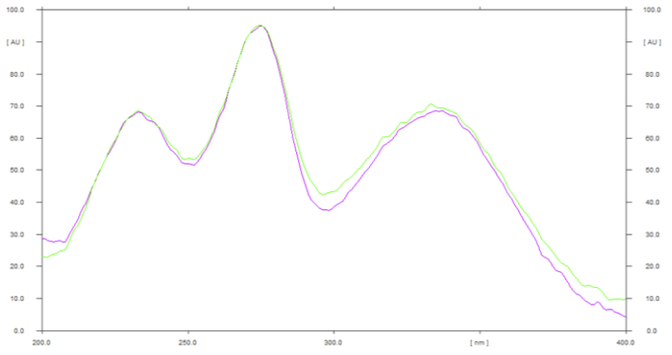

Figure 4: Overlay spectra of standard BST and sample BST (Tablet)

3.5.4. LOD and LOQ

The LOD and LOQ for BST were found to be 17.42 ng/spot and 54.43 ng/spot respectively.

3.5.5. Accuracy

Accuracy of the method was confirmed by recovery study from marketed formulation at three level of standard addition. The % recovery of BST was found to be in range of 98.90 - 100.30%. Results of recovery study are given in Table 2.

3.6. Analysis of marketed Formulation

The drug content was found to be 99.13 ± 0.89 percent of label claim. There was no interference from the excipients commonly present in the tablet.

The summary of results of validation parameters are given in Table 4.

4. CONCLUSION

The proposed stability indicating HPTLC method for the estimation of BST was precise, accurate, and selective. The methods were found to be linear in the concentration range of 200-1000

ng/spot. The developed method was free from interference of the excipients present in tablet dosage form.

As the method could effectively separate the drug from their degradation products; it can be employed as a stability indicating one.

5. ACKNOWLEDGEMENT

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