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Original Article

Development and validation of new UV-spectrophotometric methods for analysis of bosentan in different simulated body fluid media

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ABSTRACT

In the present study a simple, accurate, precise, economical, reproducible and specific UV- Spectrophotometric method for estimation of bosentan (BST) in simulated body fluid and in pharmaceutical formulations has been developed. The drug shows the same, maximum λ_{max} at 269 nm in different media. The correlation coefficient (r^2) of linearity range of developed methods in range of 3-30 µg/ml of drug were found to be 0.9963, 0.9967, 0.9914 and 0.9947 in distilled water, simulated tears, phosphate buffer and in methanol respectively. The reproducibility of presented method was found to be within the range i.e. less than 2% RSD. The limit of detection (DL) were found to be 1.30 µg/ml, 0.12 µg/ml, 2.02 µg/ml and 0.15 µg/ml where as limit of quantification (QL) were found to be 3.97 µg/ml, 0.37 µg/ml, 0.57 µg/ml and 0.48 µg/ml respectively in different- different solvent system. The proposed method was validated statically according to ICH guidelines in reference to specificity, linearity, range, accuracy, precision and robustness. Thus proposed method were validated and found to be accurate and specific for the estimation of BST in bulk, in pharmaceutical formulations and in simulated fluids.

Keywords: UV-Spectrophotometric method, bosentan, simulated tears, ICH-guideline, validation.

INTRODUCTION

Bosentan (BST) is chemically 4-tertbutyl-N-[6-(2-hydroxyethoxy)-5-(2-methoxyphenoxy)-[2,2`]-bipyrimidinyl-4-yl]-benzenesulfonamide (Fig.1) that is used for the treatment of pulmonary hypertension in chronic heart failure. It acts by antagonizing the bending of endothelins to endothelin-A and endothelin-B receptors. BST is one of the latest cardiovascular drugs facing study of phase-IV of clinical trial. Pulmonary arterial hypertension (PAH) is a severe and progressive disease that has no cure and can lead to heart failure and death. At present available therapies target the three major pathways, endothelin-1, nitric oxide, and prostacyclin pathways, of PAH that involved in development and progression of PAH. Combination therapy using this drug is a key management method for PAH; however, the risks and benefits of this strategy are unclear. Prostacyclin and its analogs, which can be administered intravenously, subcutaneously or by inhalation, are generally reserved for patients with serve disease or for those who are progressing on oral drugs. In addition, beraprost sodium, and oral prostacyclin analog, is available for the treatment of mild PAH in some countries. The uses of these drugs in combination are adjusted on the basis of patient condition or the treatment environment. There for, there is no standard procedure for combination therapy concerning PAH [1-5]. At present the reported drug BST is not official in any pharmacopeia [6-8], but it was approved by US-FDA in 2001. After that BST became the first oral drug used for the successful treatment of pulmonary arterial hypertension [9]. In view of different reported analytical methods to analyze the BST in different pharmaceutical dosage form were; UV- Spectrophotometric method [10-13], HPLC with UV detector [14-16], HPLC with mass detector [17-19] and Electrochemical detection [20]. The reported literature does not reveal any UV-Spectrophotometric methods together to determine the drug in bulk and in different simulated body fluids. Some of the reported UV-method was developed with single solvent system, 0.1N HCl but not collectively with other simulated body fluids. Thus the objective of present work is to establish the conditions for quantitative determination of BST in different simulated body

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fluid media like distilled water, tears, plasma and methanol.

MATERIALS AND METHODS

Chemicals and reagents: The drug BST was obtained as a kind gift from KPS, Clinical Services, Pvt. Ltd. Greater Noida (Mfg. by Indoco Remedies Pvt. Ltd. Mumbai, INDIA). The marketed formulation 'Bosentas' was purchased from the local market. Other chemicals Dimethyl sulfaoxide (DMSO), Disodium hydrogen phosphate, potassium dihydrogen phosphate, sodium chloride, sodium bicarbonate, calcium chloride, hydrochloric acid and sodium hydroxide were purchased from Qualigens (Fischer), Mumbai, India. In house prepared double distilled water was used as a general solvent during experiment.

Instrumentation: A UV-Visible double beam spectrophotometer (Pharmaspec-1700, Shimadzu, Japan) with 1cm matched quartz cells was used for the absorbance measurement that was connected to computer that was loaded with UV-Probe software having spectral bandwidth of 1 nm, with wavelength accuracy of ± 0.3 nm. The electronic balance (Vibra, DJ-150S-S, Shinko, Denshi, Japan), sonicator and borosil glass apparatus were used during this experiment.

Preparation of standard and sample stock solutions: The standard stock solution of BST was prepared by dissolving 30 mg of drug in DMSO in a 100 mL of volumetric flask, to produce standard stock solution of 300 µg/ml. The final stock solution was prepared by diluting 1 ml of 300 µg/ml to 10ml to get a dilution of 30µg/ml. The aliquots at range from 0.5-5.0 µg/ml of standard stock solution were taken in 10 ml of volumetric flask separately to get the concentration range of 3-30µg/ml. The absorbance of each diluted standard diluted sample was measured at 269 nm against the respective medium as a blank. The calibration curves were prepared by plotting graph between absorbances and concentrations.

Preparation of diluting media

Phosphate buffer saline: Phosphate buffer saline of pH 7.4 was made by dissolving 2.38 g of disodium hydrogen phosphate, 0.19 g of potassium dihydrogen phosphate and 8.0 g of sodium chloride in sufficient distilled water to produce 1000 ml. The pH was maintained by concentrated hydrochloric acid and sodium hydroxide solution.

***Simulated tears*:** A simulated tear of pH 7.4 was prepared by dissolving 0.2 gm of sodium bicarbonate, 0.008 gm calcium chloride and 0.67 gm of sodium chloride in volumetric flask to produce 100 ml. The pH was maintained by concentrated hydrochloric acid and sodium hydroxide solution.

Method validation

The methods were validated according to International Conference on Harmonization [21, 22] (ICH) guidelines for validation of analytical procedures with respect to specificity, linearity, range, accuracy, precision, and robustness.

Specificity: The specificity of the method was analyzed by scanning of the drug solution in different media like distilled water, simulated tears, phosphate buffer saline and methanol to determine the λ_{max} of the sample.

Linearity and range: The linearity was established across the range and the absorbance of standard stock solution in the range of 3-30 µg/ml was measured at 269 nm. The calibration curves were prepared by plotting graph between average (n=3) absorbance's and concentrations. Linearity was determined by the methods of least square of regression method. The specified range was selected from linearity studies and the methods for specified range was established by analyzing all 3 µg/ml, 6 µg/ml and 9 µg/ml of cocentration at 80%, 100% and 120% concentration in replicate and absorbance were measured at 269 nm. The range was expressed as percentage recovery with SD and % RSD.

Accuracy: Accuracy can be analyzed by percentage recovery of added marketed drug solution (6, 9 and 12 µg/ml) to fixed concentration of standard drug solution (10µg/ml) that results 16 µg/ml, 19 µg/ml and 22 µg/ml after diluting with different-different simulated fluids respectably distilled water. The accuracy was reported as percentage recovery by the assay of known amount of sample in the standard solution.

Precision: Repeatability precision was calculated by analyzing six determinates at 100% of the marketed BST solution of 15 µg/ml. The repeatability precision was expressed as %RSD. While the intermediate precision was evaluate on the standard solution of strength 9 µg/ml on same day, also on two consecutive weeks.

Limit of detection (DL) and limit of quantification (QL): The detection limit (DL) and the quantitation limit (QL) were based on the slope of the calibration curve and standard deviation of Y-intercept of regression line.

Robustness: The robustness of the method was evaluated by analyzing the standard solution of BST on same day, also on two consecutive days. The robustness was expressed as percentage amount recovery, SD and % RSD. Robustness established by analyzing the sample strength of 3 µg/ml, 6 µg/ml and 9 µg/ml of standard stock solution.

RESULTS AND DISCUSSION

The proposed method was validated according to the guidelines of International Conference on Harmonization (ICH). The method discussed in this analysis provides a simple, accurate, economical and convenient for the analysis of BST by UV-Spectroscopy. The absorbance spectra of BST in different media like distilled water, simulated tears, phosphate buffer saline and methanol solution were shown in figure 2 a-d respectively. The average λ_{max} was found to be 269 nm. Thus proposed methods were found to be specific and selective. In the developed method, linearity was observed in the concentration range of 3-30 µg/ml. Linear absorbance versus concentration gives regression equation;Y=0.0294X−0.0459, Y=0.0288X−0.0113, Y=0.0323X−0.0259 and Y=0.0288X−0.0113 with a more than 0.99 correlation coefficient, in different media (Table 1). The linear regression equation with a high correlation coefficient indicates a good linearity between absorbance and selected concentration. The precision of the method was validated on the basis of selected three concentrations with 3-replicates of each in different simulated media (Table 2). In each set of media there was more than 95% recovery (with less than 0.05% confidence interval) within the limit of percentage relative deviation (<2%), proves highly precision of the method. In a another set of experiment precision by repeatability (n=6) gives high recovery and less than 2% RSD again proves high precision of the method (Table 3). The intermediate precision and robustness for selected sample 9 µg/ml was found to be satisfactory when analyzed on same day, also on the two consecutive weeks. The percentage recovery (>95%) and % RSD (<2%) proves the high stability and robustness of the method (Table 4). The specified range test of the proposed method for each 6, 9 and 12 µg/ml samples were studied at 80%, 100% and 120% and calculated for their percentage recovery with SD and % RSD. High recovery and %RSD within the range (< 2%) proves the specific range test within limit (Table 6).

CONCLUSION

The methods were found to be very simple, rapid, precise, accurate, economical and sensitive. The validated UV method can be used for the drug analysis in bulk and in solid dosage forms in distilled water, simulated tears, phosphate buffer and in methanol. Thus proposed method will be suitable for the analysis of BST in different pharmaceutical formulations.

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Table 1: Regression, validation and system suitability parameters of drug

Parameters	Media 1	Media 2	Media 3	Media 4
Linearity range (µg/ml)	3-30	3-30	3-30	3-30
Regression equation	Y=0.0294X−0.0459	Y=0.0288X−0.0113	Y=0.0323X−0.0259	Y=0.0288X−0.0113
Correlation coefficient (r^2)	0.9963	0.9967	0.9914	0.9947
Molar absorptivity (ε) [L/mol/cm]	0.34×10^{-2}	0.33×10^{-2}	0.35×10^{-2}	0.27×10^{-2}
Sandell sensitivity µg/cm^2/0.001abs unit	0.1621×10^{-2}	0.1670×10^{-2}	0.1574×10^{-2}	0.2041×10^{-2}
95% Confidence interval for slope	<0.001	<0.0001	<0.0001	<0.0001
95% Confidence interval for intercept	0.0044	0.0658	0.506	0.4412
Standard error of slope	0.006	0.007	0.0010	0.007
Standard error of intercept	0.0117	0.0121	0.0187	0.0139
Standard error of estimate	0.0172	0.0178	0.0273	0.0204
Repeatability (%RSD)	0.601	0.801	1.33	1.154
DL (µg/ml)	1.3	0.12	2.02	0.15
QL (µg/ml)	3.97	0.37	0.57	0.48

Media 1: Distilled water; Media 2: Simulated tear (pH = 7.4); Media 3: Phosphate buffer saline (pH = 7.4); Media 4: Methanol; % RSD: Percentage residual standard deviation of six determinates; DL: Detection limit QL: Quantitation limit

Table 2: Precision analysis of method

Media	Concentration (µg/ml)	Percentage mean recovery ± SD	%RSD
Media 1	3	94.20 ± 3.40	1.203
	6	97.90 ± 8.50	1.447
	9	94.20 ± 3.90	0.46
Media 2	3	95.30 ± 3.50	1.22
	6	96.70 ± 3.10	1.22
	9	91.90 ± 4.70	0.568
Media 3	3	89.20 ± 5.00	1.867
	6	96.90 ± 3.80	0.654
	9	93.50 ± 5.30	0.634
Media 4	3	96.60 ± 4.00	1.6
	6	98.18 ± 9.20	1.562
	9	98.50 ± 4.00	0.456

% RSD: Percentage residual standard deviation of three determinates
SD: Standard deviation

Table 3: Precision by repeatability method

Media	Concentration (µg/ml)	Percentage mean recovery ± SD	%RSD
Media 1	15µg/ml	103.00 ± 2.29	0.601
Media 2	15µg/ml	98.00 ± 3.10	1.154
Media 3	15µg/ml	102.00 ± 3.00	0.801
Media 4	15µg/ml	97.00 ± 1.90	1.330

Table 4: Intermediate precision and stability

Media	Time (days)	Concentration (µg/ml)	Mean percentage recovery ± SD	%RSD
Media 1	1	9	97.30 ± 3.00	1.00
	7	9	96.00 ± 4.00	1.90
	14	9	101.60 ± 4.30	0.47
Media 2	1	9	98.30 ± 1.25	1.50
	7	9	97.90 ± 3.50	0.405
	14	9	104.60 ±1.35	1.46
Media 3	1	9	92.90 ± 1.00	1.28
	7	9	95.60 ± 7.50	0.87
	14	9	102.40 ± 1.30	1.40
Media 4	1	9	97.80 ± 9.20	1.08
	7	9	99.90 ± 5.30	0.59
	14	9	104.10 ± 7.20	0.76

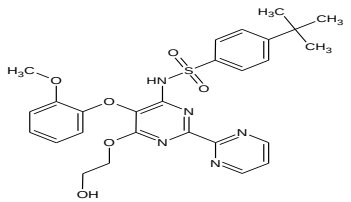

Figure 1: Chemical structure of bosentan (BST)

Table 5: Accuracy determination

Media	Standard (µg/ml)	Added sample (µg/ml)	Final conc. (µg/ml)	Perecentage mean recovery ± SD	% RSD
Media 1	10	6	16	101.00 ± 5.00	0.361
	10	9	19	103.00 ± 2.10	1.401
	10	12	22	100.00 ± 1.10	0.538
Media 2	10	6	16	102.00 ± 5.00	0.322
	10	9	19	102.00 ± 1.40	0.729
	10	12	22	102.00 ± 6.00	0.397
Media 3	10	6	16	102.00 ± 2.10	1.297
	10	9	19	101.00 ± 6.00	0.351
	10	12	22	101.00 ± 5.00	0.294
Media 4	10	6	16	104.00 ± 3.00	0.610
	10	9	19	101.00 ± 2.00	0.406
	10	12	22	101.00 ± 1.50	0.708

Table 6: Range investigation of method

Media	Conc. (µg/ml)	80% Concentration (µg/ml)		100% Concentration (µg/ml)		120% Concentration (µg/ml)	
		%Recovery ± SD	%RSD	%Recovery ±SD	%RSD	%Recovery ±SD	%RSD
Media 1	6	80.70±5.10	1.070	98.30±8.30	1.480	122.10±7.00	0.955
Media 2	6	84.40±6.40	1.270	97.90±3.50	0.608	120.40±3.90	1.900
Media 3	6	78.80±6.80	1.440	96.90±6.80	1.217	119.50±6.60	0.920
Media 4	6	84.50±3.90	0.760	99.10±6.00	1.073	116.70±5.70	1.840

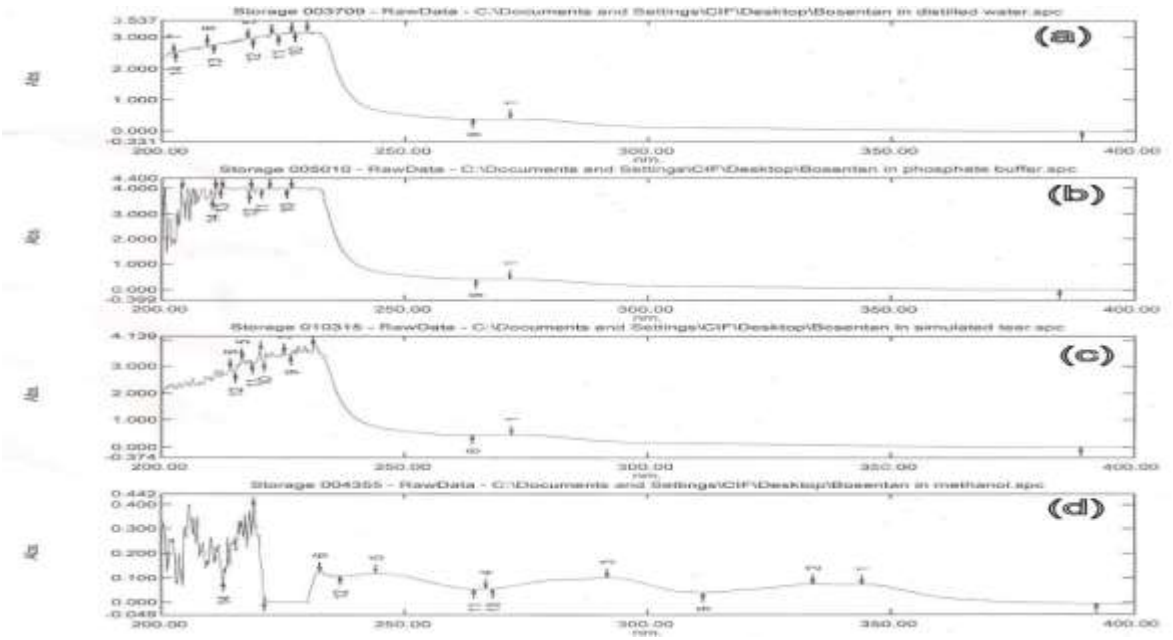

Figure 2: Absorbance spectrum of BST in different media i.e. (a) distilled water (b) phosphate buffer saline (c) simulated tears (d) methanol

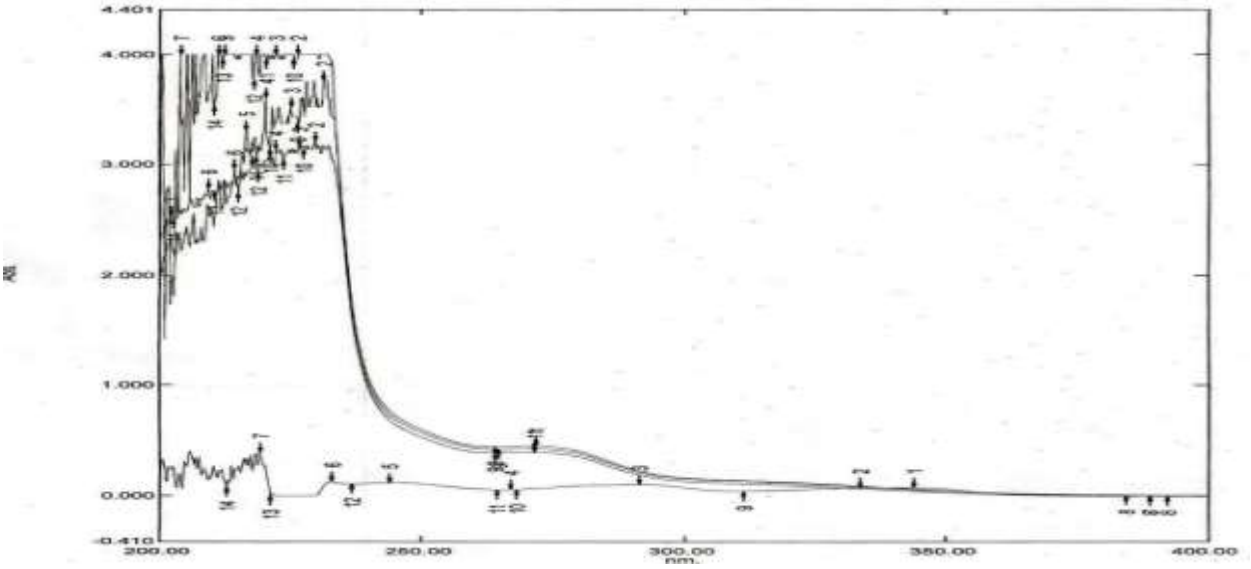

Figure 3: Comparative absorption spectrum of BST in different media

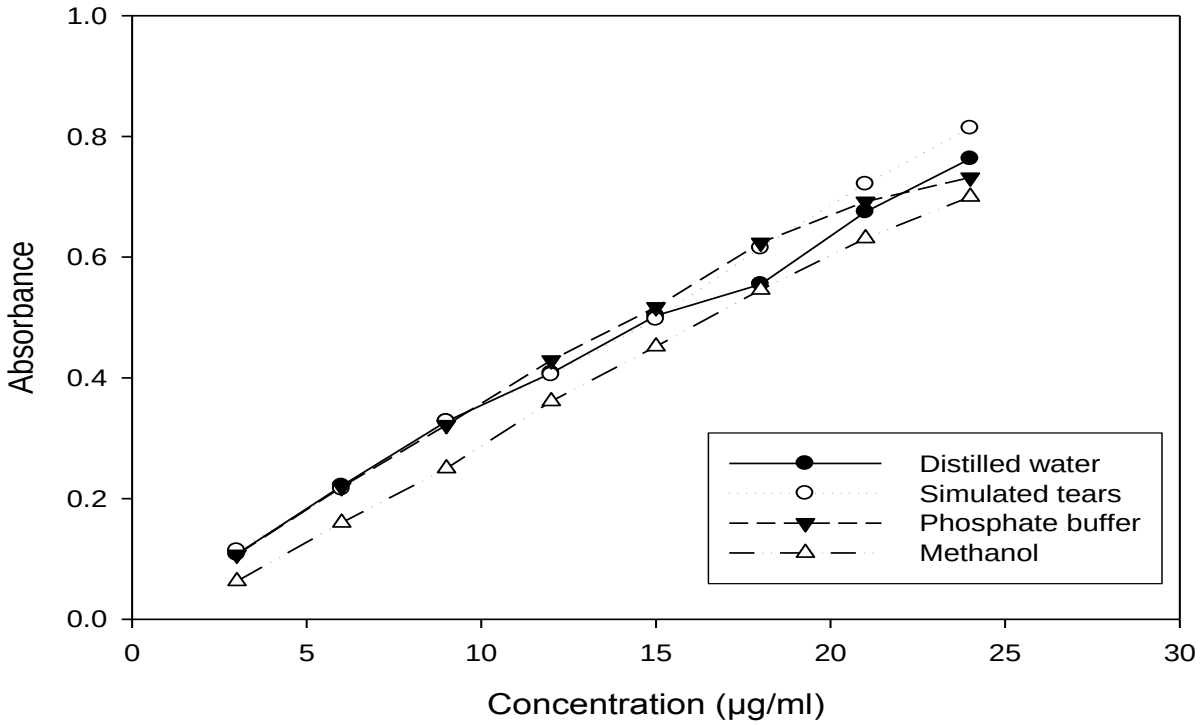

Figure 4: Calibration curve of BST in different simulated body fluids

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