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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING UV SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF BOSENTAN IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

Objective: Development of a simple, sensitive, accurate, precise and robust stability indicating UV spectrophotometric method for the quantitative determination of Bosentan in bulk and pharmaceutical formulation. **Method:** The method is developed using methanol as a solvent. The stock solution of Bosentan was prepared in methanol and subsequent dilutions were done in methanol. The standard solution of Bosentan showed absorption maxima at 272 nm. The calibration curve was constructed by plotting absorbance v/s concentration (µg/ml). Correlation coefficient has been measured.**Result:** Beer's law has been followed for the drug in the concentration range of 5-25µg/ml. To determine the precision of the method Bosentan solutions at concentration 05, 15, 25 µg/ml were analyzed each in triplicate. The method was found to be precise. The % RSD values for interday precision at concentration 05, 15, 25 µg/ml was found to be 0.994, 0.012, 0.990 respectively and for intraday precision it was 0.450, 0.321, 0.126 respectively. Accuracy was found as (100.12-100.35%), precision as (%RSD 0.994-0.990), however the method was effectively functional to the pharmaceutical dosage form comprising the Bosentan deprived of any interference through the excipients. According to ICH guidelines these outcomes from analysis has been validated. **Conclusion:** Forced degradation studies comprises the influence of temperature, oxidation, neutral, photolysis and susceptibility to hydrolysis through a extensive range of pH values, were accepted out approving to the ICH necessities which can be used for the routine and quality control analysis of Bosentan bulk as well in pharmaceutical formulations.

***Keywords*:** Bosentan, stability indicating, forced degradation.

INTRODUCTION:

In the course of the pharmaceutical expansion of a new drug, it is essential to choose as early as promising formulation which having finest stability features. Guidelines regards with stability testing are provided by International Commission for Harmonization (ICH), which highlights the stress testing environments with prime purpose of evaluating the consequence of severe circumstances on the drug. Such kinds of outcomes are playing a crucial part in the estimation of a drug product shelf life throughout initial periods of its pharmaceutical growth. These results not only important for estimation of shelf life of drug but may also help as monitors for improved drug design, drug formulation and drug analysis.[1-6]

Bosentan is chemically known as 4-tert-butyl-N-[6- (2-hydroxyethoxy)-5-(2-methoxyphenoxy)-2-(pyrimidin-2-yl) pyrimidin-4-yl] benzene-1-sulfonamide, monohydrate, It is a selective endothelin-receptor antagonist (Fig. 1).Mechanism of action of Bosentan is a competitive antagonist of endothelin-1 at the endothelin-A (ET-A) and endothelinB (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. Bosentan has a slightly higher affinity for ET-A than ET-B. Bosentan is important in the treatment of pulmonary artery hypertension (PAH). Bosentan is used to treat pulmonary hypertension by blocking the action of endothelin molecules that would otherwise promote narrowing of the blood vessels and lead to high blood pressure[7-9].

The literature review revealed a simple UV spectroscopic method development and validation of Bosentane in tablet dosage form[10] Zero-derivative spectrometry, First-Derivative spectrometry and Area under Curve [11]. Literature survey also tells about the RP HPLC estimation of Bosentan Monohydrate,stability indicating RP-HPLC method for development and validation of Bosentan in pure and tablet dosage from [12-14].

Until there are, no UV stability indicating method has been described for the analysis of this drug in its pharmaceutical formulations. Amongst the several approaches existing for the determination of drugs, but still spectrophotometry is actual popular, due to its simplicity, specificity and low cost.

In current study a simple, sensitive, selective, sensitive, economical, accurate and reproducible analytical method with superior finding range for estimation of Bosentan in pure form as well from pharmaceutical dosage form was developed and validated. Based on forced degradation studies, the method was also tested for its stability indicating capability permitting to the ICH requirements which can be used for the routine and quality control analysis of Bosentan in bulk and pharmaceutical formulation.

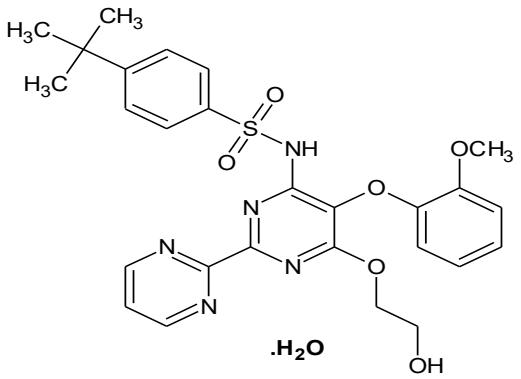

Fig.1: Chemical structure of Bosentan

MATERIAL AND METHODS:

Chemicals and reagents:

Bosentan was achieved as a gift sample from Spectrum Lab Hyderabad, India. All solvents and other chemicals used for above said work, were of analytical reagent grade procured from Research lab, Mumbai. Double distilled water used throughout experiment.

Instrumentation:

A projected work was conceded out on Lab India UV/VIS double beam spectrophotometer (model 3000+) with 1 cm matched quartz cells was used for all spectral measurements. All weighing was done on electronic balance (Sansui-Vibra MK-1890S).

Selection of Solvents:

On the basis of solubility methanol is used during the experiment as a solvent to dissolve the drug.

Preparation of Standard Stock Solution of Bosentan:

10 mg of Bosentan was precisely weighed and transmitted to 100 ml volumetric flask and dissolved in around 20 ml of methanol. The volume was completed up to the mark along with methanol to give 100 µg/ml stock solution.

Preparation of calibration curve for Bosentan:

By scanning a suitable standard solution in the UV-VIS spectrophotometer in the wavelength range of 200-400 nm, the λ max of the drug was determined. Aliquots (0.5, 1,1.5,2 and 2.5ml) from standard solution of Bosentan were pipetted out in to a sequence of five volumetric flasks and the volume was made up to 10 ml with methanol. The absorbance was measured at 272 nm contrary to blank. The calibration curve was constructed by plotting absorbance v/s concentration (µg/ml). Correlation coefficient has been measured. The summary of analytical parameters and calibration curve data are presented in Table 1 and Table 2 respectively.

Estimation of Bosentan:

05 tablets of Bosentas® (Cipla Ltd.) were weighed correctly and powdered. Powder equivalent to one tablet weight was taken in to 100 ml volumetric flask and added solvent, sonicated for 30 min with intermediate shaking and made up to

mark with methanol. Firstly it was filtered by 0.45µm Whatman filter paper. At last concentration of 100 µg/ml of Bosentan has been prepared. This solution again filtered by the filter paper to eliminate certain un-dissolved excipients. After filtration, from this 2 ml was taken and diluted to 10 ml with methanol water which provides 20 µg/ml solution whereas absorbance of this solution was measured at 272 nm.

Method Validation:

The method was validated according to ICH Q2B guidelines to determine the Linearity, sensitivity, precision, and accuracy of the analyte [15]. Linearity of the recommended method was determined by determining the absorbance of the standard solutions in the concentration range of 5-25µg/ml and execution smallest square regression analysis. In addition, the accuracy of the proposed method has been patterned by standard addition method and recovery studies were passed out at 80%, 100% and 120% of required concentration. The percentage analytical recovery was calculated by comparing the concentration resulted with the addition of spiked samples with actual expected theoretical increase in concentration. Intra-day precision was determined by carrying out the analysis for six concentrations at two different time interval in a day. Similarly interday precision was determined by performing analysis on two consecutive days. LOD and LOQ of the proposed methods were calculated [16-19]. Recovery of the analyte of interest from a given matrix can be used as a measure of the accuracy or the bias of the method [20-23].

Stability Studies of Bosentan:

Stability studies were accomplished by forced degradation study of Bosentan and it comprises the study of consequence of photolysis, oxidation, temperature and prone to hydrolysis through a wide range of pH values. For acidic hydrolysis 0.1, 1.0 N HCl, for basic hydrolysis 0.1, 1 N NaOH, for neutral hydrolysis distilled water, for oxidation study 0.1%, 1% and 3% H_2O_2 was used. For carrying out photolysis studies the drug was treated by sunlight for 3 days and thermal stress was applied by heating the drug at 60^0C for 2 hrs

RESULTS AND DISCUSSION:

The development of a simple, economic, sensitive, and accurate analytical method for the quantitative determination of samples has been decrease needless sample preparations and the charge of materials as well labor. The λ max of Bosentan was taken in methanol is shown in (Fig 2).

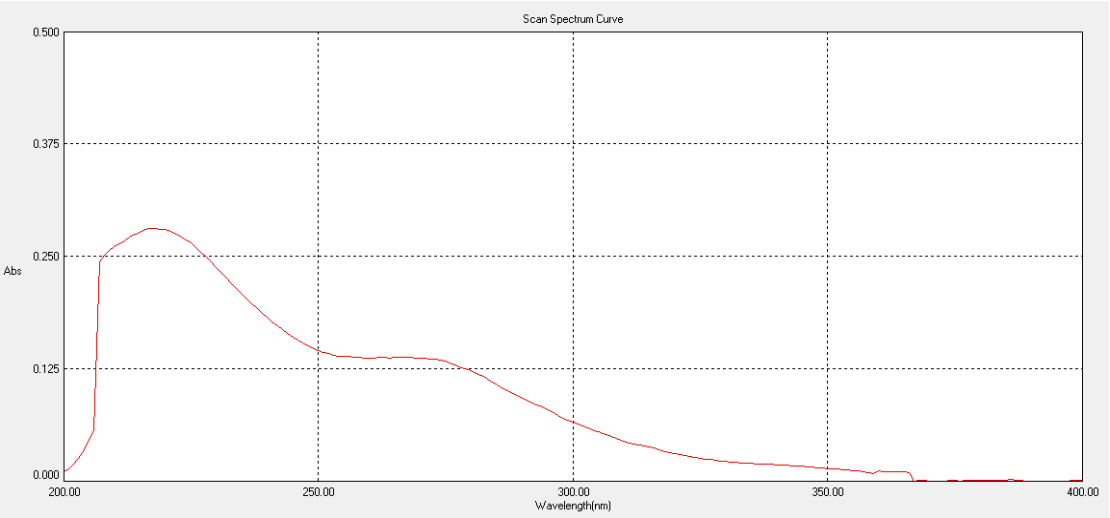

Fig. 2 Uv spectrum of Bosentan at 272 nm

For the analysis of Bosentan λ max was measured at (272 nm) by making scans of the Bosentan solutions in the whole UV region. Data for calibration curve was made in the series of concentrations of 5-25 µg/ml. Beer's law was followed above this concentration range (Fig 3). Overlain spectra for 5-25 µg/ml concentrations shown in (Fig 4).

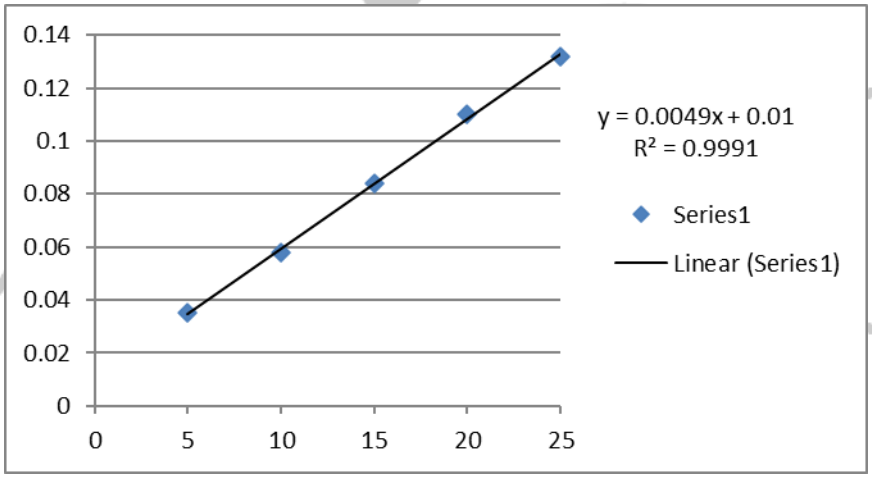

Fig.3: Calibration curve of Bosentan at 272 nm

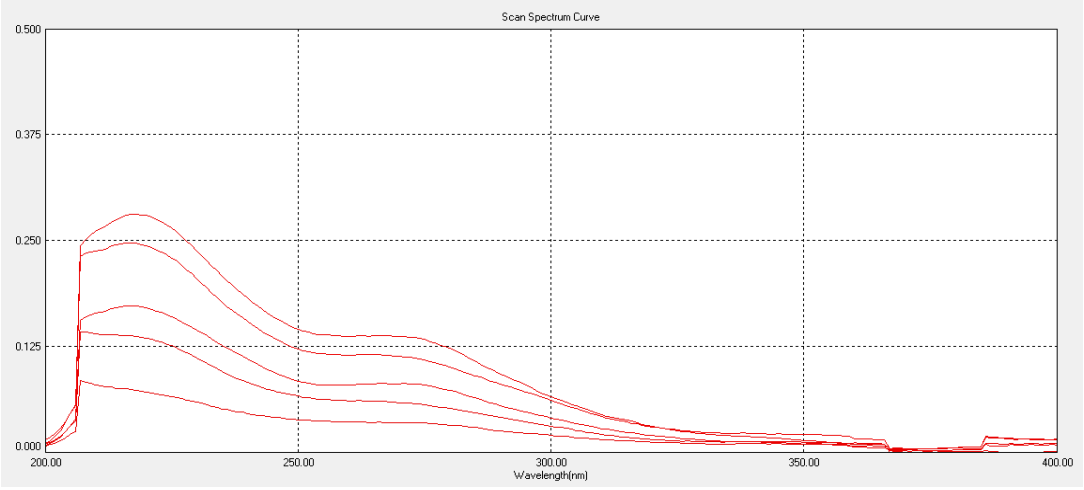

Fig.4: Overlain spectra of Bosentane for 5-25 µg/ml concentrations at 272 nm

The regression equation was found to be Y=0.0049x+0.010. The correlation coefficient (r) of the standard curve was established 0.9991. The summary of analytical parameters and calibration curve data are presented in (Table 1 and Table 2) respectively.

Table 1: Optical characteristics of the proposed method

Parameters	Result
Measured wavelength (λ max)	272 nm
Beers law limit (µg/ml)	5-25 ppm
Regression equation (y = m x + c)	Y=0.0049x+0.01
Slope	0.0049
Intercept	0.010
Correlation coefficient (r)	0.9989
LOD µg/ml	0.17
LOQ µg/ml	0.85

Table 2: Calibration curve data for Bosentan

Sr. No.	Conc. (µg/ml)	Absorbance
1	5	0.035
2	10	0.058
3	15	0.084
4	20	0.110
5	25	0.132

Performing repeat examines of the standard solutions was used to evaluate the accuracy and precision of the recommended methods (Table 3 and Table 4). The LOD and LOQ were found to be 0.17µg/ml and 0.85 µg/ml respectively.

Table 3: Result of recovery studies

Level of % recovery	% Mean* recovery	S.D.	R.S.D	SE
	Bosentan			
80	100.12	0.610	0.617	0.338
100	100.11	0.510	0.517	0.301
120	100.35	0.475	0.465	0.291

*** Mean of three determinations at each, SE- Standard error**

To examine the accuracy of the projected technique and to check the interference due to excipients used in dosage forms, standard addition method has been used for recovery experiments. 100.12-100.35 is the average of recovery result has been concluded. The recommended methods can be effectively useful for assay in tablet dosage forms without any interference (Table 3).

Methanol was used for the performing calibration range and analyzed with the relevant calibration curves to determine the intra- and inter-day variability.

To determine the precision of the method Bosentan solutions at concentration 05, 15, 25 µg/ml were analyzed each in triplicate. Solutions for the standard curves were prepared fresh daily. The method was found to be precise. The % RSD values for interday precision at concentration 05, 15,

25 µg/ml was found to be 0.994, 0.012, 0.990 respectively and for intraday precision it was 0.450, 0.321, 0.126 respectively. Results are shown in (Table 4).

Table 4: Statistical validation for interday and intraday precision

Parameters	Concentrations (µg/ml)		
	05	15	25
Intraday*			
% mean±S.D	2.04±0.209	50.22±0.602	79.77±0.769
%RSD	0.994	0.012	0.990
SE	0.0353	0.0405	0.0437
Interday*			
% mean±S.D	20.08 ± 0.32	50.77±0.16	80.47±0.119
%RSD	0.450	0.321	0.126
SE	0.0299	0.0456	0.0424

*Denotes average of three determinations, SE- Standard error

The use of this technique is elucidated in the experimental segment. The achieved consequences establish the validity and accuracy of the recommended method for the determination of Bosentan in tablet dosage form.The stability studies indicates that considerable alterations were detected by treating the drug with acid, basic hydrolysis, oxidation, however no significant degradation was obtained under neutral, sun light, thermal stress conditions. The results are summarize in (Table 5).

Table5: Result of forced degradation study ofBosentan

Sr. No.	Conditions applied	Conc. taken	Average Conc. Found	Observation
1	Acidic hydrolysis (0.1, 1 N HCl)	20 µg/ml	12.08 µg/ml	Degraded
2	Basic hydrolysis (0.1, 1 N NaOH)	20 µg/ml	15.35 µg/ml	Degraded
3	H_2O_2 (0.1, 1, 3%)	20 µg/ml	11.12 µg/ml	Degraded
4	Neutral (H_2O)	20 µg/ml	19.94 µg/ml	Stable
5	Thermal stress (60^0 C, 2 hrs)	20 µg/ml	Change in λ max	Stable
	Sunlight treatment 1 day	20 µg/ml	19.97 µg/ml	Stable
6	Sunlight treatment 2	20 µg/ml	19.91 µg/ml	Stable
	Sunlight treatment 3 day	20 µg/ml	19.88 µg/ml	Stable

These results disclose that the developed method was simple, sensitive, sensitive, inexpensive, accurate and reproducible and subsequently, can be applied to the determination of Bosentan tablet in pharmaceuticals deprived of any interference from the excipients. Based on forced degradation studies according to the ICH requirements, this method can be used for the routine and quality control analysis of Bosentan in raw material and pharmaceutical formulations.

CONCLUSION:

A simple, sensitive, accurate, precise, reproducible and cost effective stability indicating UV spectrophotometric method has been established for quantitative determination of Bosentanin bulk and pharmaceutical formulation. This method decreases general method development period. Forced degradation studies comprises the influence of temperature, oxidation, neutral, photolysis and susceptibility to hydrolysis through extensive

range of pH values, were accepted out approving to the ICH necessities which can be used for the routine and quality control analysis of Bosentan bulk as well in pharmaceutical formulations.

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CONFLICT OF INTEREST:

The authors declare no conflict of interest.

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