International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304
Vol.8, No.4, pp 569-579, 2015

# Stability Indicating Method Development and Validation of Bosentan in Bulk Drug and Formulation by Rp-Hplc Method

**S.H.Rizwan*[1], V.Girija Sastry[2], Q.Imad[3]**

[1]Deccan School of Pharmacy, Osmania University, Hyderabad, India
[2]College of Pharmaceutical Sciences, Andhra University, Vizag, India
[3]MESCO College of Pharmacy, Osmania University, Hyderabad, India

**Abstract:** A simple and selective stability indicating RP-HPLC Method is developed and validated for the quantification of Bosentan in bulk and tablet dosage form. Quantification was achieved by using the mobile phase (Mixed phosphate buffer of pH 6.8: Acetonitrile) in the ratio of 55:45 on an Inertsil C-18 column (250*4.6 3.5µm). The flow rate was 1.0 ml/min. Measurements were made at a wavelength of 220nm. The average retention time for Bosentan was found to be 4.40 min. The proposed method was validated for selectivity, precision, linearity and accuracy. The method was found to be linear within 50-150µg/ml. All validation parameters were within the acceptable range. The developed method was successfully applied to estimate the amount of Bosentan in tablet dosage form and to study the stability of the product in various stress conditions as per ICH guidelines.
**Keywords**: BOSENTAN, RP-HPLC method, INERTSIL C18 column,KH2PO4, Acetonitrile and Validation and stability.

## 1. Introduction

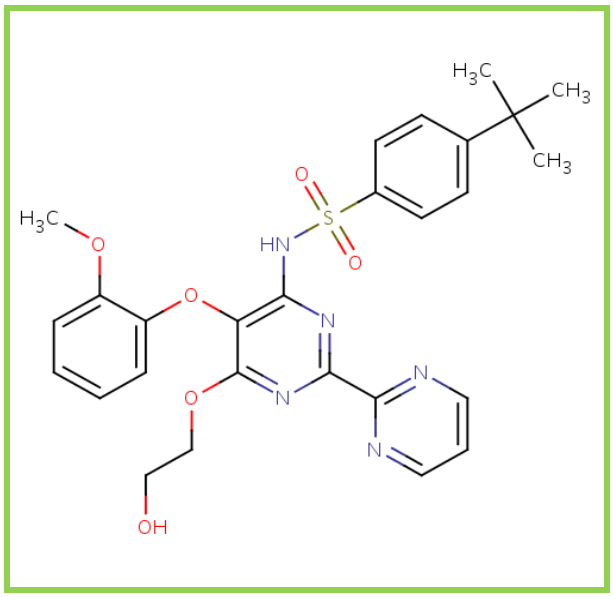
Bosentan is a dual endothelin receptor antagonist important in the treatment of pulmonary artery hypertension (PAH). Chemically it is 4-tertbutyl-N-[6-(2-hydroxyethoxy)-5-(2-methoxy-Phenoxy)-2-(Pyrimidin-2-yl) pyrimidin-4-yl] benzene - 1- sulphonamide. Bosentan is used to treat pulmonary hypertension by blocking the action of endothelin molecules that would otherwise promote narrowing of the blood vessels and lead to high blood pressure.

Endothelin-1 (ET-1) is a neurohormone, the effects of which are mediated by binding to ET-A and ET-B receptors in the endothelium and vascular smooth muscle. ET-1 concentrations are elevated in plasma and lung tissue of patients with pulmonary arterial hypertension, suggesting a pathogenic role for ET-1 in this disease. Bosentan is a specific and competitive antagonist at endothelin receptor types ETA and ETB. Bosentan has a slightly higher affinity for ETA receptors than for ETB receptors.

Bosentan belongs to a class of drugs known as endothelin receptor antagonists (ERAs). Bosentan blocks the binding of endothelin to its receptors, thereby negating endothelin's deleterious effects. It is manufactured in India by Lupin and Cipla Pharmaceuticals under the brand name of Lupibose and Bosentas respectively. Several analytical methods have been described in the literature for the determination of Bosentan in pharmaceutical dosage forms and biological fluids. UV-spectrophotometric methods [5-8] employing different solvents have been reported for the assay of the drug in pharmaceutical dosage forms. Khan et al. [9] developed a stability indicating HPLC method for the determination of related substances of BSN. Jadhav et al. [10] proposed a stability indicating gradient reverse phase liquid chromatographic method for the determination of process and degradation impurities in Bosentan. These methods are not applied to pharmaceutical dosage

forms. Two narrow-bore liquid chromatography with ion spray tandem mass spectrometric detection methods (LC-MS) were reported by Lausecker et al. [11, 12] The first method is applied for the determination of Bosentan in human plasma whereas the second method is useful for simultaneous determination of Bosentan and its three main metabolites in plasma, serum, bile, and liver samples from man, dog and rat. The reported LC-MS methods are expensive.There are few reports on the application of HPLC with UV detection [13-15] for the assay of Bosentan in bulk drug and pharmaceutical dosage forms. The present method is an attempt to develop an alternate easy, selective and robust method including study of the drug under stress degradation parameters as per ICH guidelines.

**Structure of Bosentan:**




## Materials and Method:

**Instruments:**

The chromatographic technique was performed on a Shimadzu LC20-AT Liquid chromatography with SPD-20A prominence UV-visible detector and Spinchrom software, reversed phase C18 column (Inertsil C18 column (250*4.6 3.5µm)) as stationary phase, Electron corporation double beam UV-visible spectrophotometer (vision pro-software), Ultrasonic cleaner, Shimadzu analytical balance AY-220,Vacuum micro filtration unit with 0.45µ membrane filter was used in the study.

**Materials**:

Bosentan was obtained as gift sample from Chandra lab, Prashanthinagar, Kukatpally, Hyderabad, India. The purity of the drug was evaluated by obtaining its melting point and ultraviolet (UV) and infrared (IR) spectra. No impurities were found. The drug was used without further purification.

HPLC-grade Acetonitrile , Water and other reagents were of standard Quality . Potassium Phosphate monobasic and Dibasic (AR grade) was obtained from Merck.

A tablet formulation of Bosentan (62.5mg) was procured from local market.

**Determination of Working Wavelength (λmax):**

10 mg of Bosentan was weighed and transferred in to 100ml volumetric flask and dissolved in methanol and then made up to the mark with methanol and diluted to produce 10 µg /ml of solution by diluting 0.1ml to 10ml with methanol. The wavelength of maximum absorption ($\lambda_{max}$) for 10 µg/ml solution of the drug in methanol was scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against methanol as blank. The absorption curve shows characteristic absorption maxima at 220 nm for Bosentan. (Figure No:1)

**Preparation of mobile phase:**

A mixture of 550 volumes of Phosphate buffer pH 6.8 and 450 volumes of Acetonitrile was prepared. The mobile phase was sonicated for 10 min to remove gases.

**Stock and Working Standard solutions**

Weigh accurately 100 mg of Bosentan in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. This was taken as primary stock solution. From above stock solution 100 µg/mL of Bosentan is prepared by diluting 1 ml to 10ml with mobile phase. Five working standard solutions were prepared for calibration by adding defined volumes of the standard stock solution and diluting with mobile phase to get concentrations in the range of 50, 75,100,125 and 150 mcg respectively and were used for recording chromatogram.

**Tablet sample Preparation**

20 tablets (each tablet contains 62.5 mg) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solution of Bosentan was prepared by dissolving weight equivalent to 100 mg of Bosentan in sufficient mobile phase. The solution was sonicated for 5 min and filtered using 0.45-micron syringe, diluted to 100 ml with mobile phase. Further dilutions were prepared in 5 replicates of 100 µg/ml of Bosentan was made by adding 1 ml of stock solution to 10 ml of mobile phase.

5 replicates of each of sample and standard solutions were injected and their average peak areas were taken. Results shown in above table no.6

**Selection of Detection Wavelength:**

The UV spectrum of diluted solutions of various concentrations of Bosentan in mobile phase was recorded using UV spectrophotometer. The wavelength of maximum absorbance was observed at 220 nm. This wavelength was used for detection of Bosentan.

## Method Validation:

**Linearity:**

Linearity was studied by analyzing five standard solutions covering the range of 50-150µg/ml. From the primary stock solution 0.5ml, 0.75ml, 1.0ml, 1.25ml and 1.5 ml of aliquots are pipetted into 10 ml volumetric flasks and made up to the mark with the mobile phase to give concentrations of 50µg/ml, 75µg/ml, 100µg/ml, 125µg/ml and 150µg/ml.

Calibration curve showing concentration versus peak area was plotted by injecting the above prepared solutions and the obtained data was subjected to regression analysis using the least squares method. The calibration data is presented Table No.1 - and calibration curve is shown in Fig 1.1

**Table No: 1 – Calibration Data for Bosentan**

| | Calibration Data for Bosentan | | |
|---|---|---|---|
| | **mcg** | **Retention Time** | **Area** |
| | 50 | 4.3 | 3354.939 |
| | 75 | 4.3 | 4724.278 |
| | 100 | 4.3 | 6343.813 |
| | 125 | 4.3 | 7967.887 |
| | 150 | 4.3 | 9577.078 |

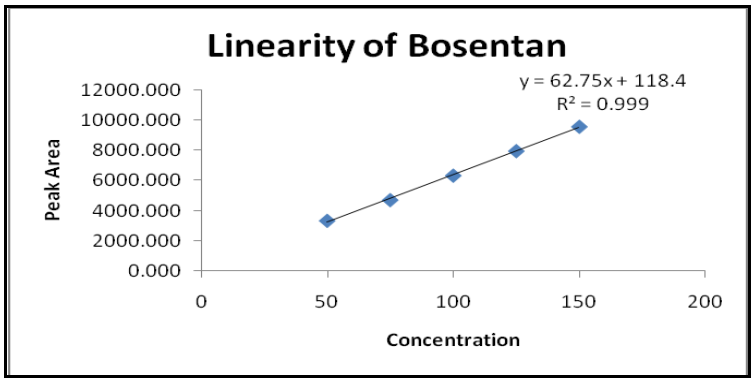



**Figure No.1 Calibration curve of Bosentan**

**Optimized chromatographic conditions and system suitability parameters o f proposed rp-hplc method for Bosentan**

**System Suitability:**

The chromatographic systems used for analysis must pass the system suitability limits before sample analysis can commence. Set up the chromatographic system , allow the HPLC system to stabilize for 40 min. Inject blank preparation (single injection) and standard preparation (six replicates) and record the chromatograms to evaluate the system suitability parameters like resolution , tailing factor, theoretical Plates, and & % RSD for peak area of six replicates . The system suitability data is reported in Table -2.

**Table No: 2 – System Suitability Parameters**

| **Parameter** | **Chromatographic Condition** |
|---|---|
| Instrument | Shimadzu(LC 20 AT VP) |
| Column | Inertsil ODS 3V(250x4.6mm) 5µm |
| Detector | Shimadzu SPD – 20A prominence UV-VIS detector |
| Mobile Phase | 55:45 – Phosphate buffer PH 6.8 and ACN |
| Flow Rate | 1.0 ML/MIN |
| Detection Wavelength | 220 NM |
| Run Time | 7 min |
| Retention time | 4.3 min |
| Theoretical Plates | 3354 |
| Tailing Factor | 1.5 |

**Method precision (Repeatability)**:

The precision of the instrument was checked by repeated injections of a concentration and measurement of peak areas and retention times of solutions (n = 6) for Bosentan without changing the parameter of the proposed chromatographic method. Table -3.

**Table No: 3 Method Precision (Repeatability)**

| **Injection** | **Bosentan** | | |
|---|---|---|---|
| | **Concentration** | **Retention Time** | **Area** |
| 1 | 100 mcg/ml | 4.357 | 6393.834 |
| 2 | 100 mcg/ml | 4.340 | 6347.254 |
| 3 | 100 mcg/ml | 4.350 | 6517.941 |
| 4 | 100 mcg/ml | 4.403 | 6397.585 |
| 5 | 100 mcg/ml | 4.347 | 6384.045 |
| 6 | 100 mcg/ml | 4.347 | 6382.426 |

| | | | |
|---|---|---|---|
| AVG | | 4.3573 | 6403.848 |
| SD | | 0.0230 | 58.670 |
| %RSD | | 0.53 | 0.92 |

**Intermediate precision (Reproducibility)**:

The method was validated for both Intraday and Interday precision The Intermediate Precision of the method was checked by injecting replicate injections six times with same concentrations on the same day as intraday precision and, on three different days with three different concentrations as Interday Precision study . The proposed method was validated by analyzing the corresponding responses of Bosentan. The result was reported in terms of relative standard deviation. Intraday precision Table – 4 and Interday precision Table – 5

**Intermediate Precision:**

**Table No: 4 Intraday Precision**

| Drug | Concentration µg/ml | % RSD |
|---|---|---|
| Bosentan | 100 | 0.93 |

**Table No: 5 Interday Precision**

| Drug | Concentration mcg/ml | Mean % Recovery ± %RSD |
|---|---|---|
| Bosentan | 50 | 99.61 |
| | 75 | 97.02 |
| | 100 | 98.34 |

**n=6 (No. of Replicates)**

**Limit of detection and limit of quantification:**

The limit of detection (LOD) and limit of quantification (LOQ) were separately determined based on standard deviation of the y-intercept and the slope of the calibration curve by using the equations (2) and (3), respectively.

**LOD = 3.3 δ/S ……. (3)**
**LOQ =10 δ/S …….. (4)**

Where, σ = the standard deviation of the response, S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte. Results are shown in Table – 6.

**LOD & LOQ:**

**Table No: 6 LOD and LOQ values**

| Parameter | mcg | Area |
|---|---|---|
| **Limit of Detection** | 2.08 | 132.09 |
| **Limit of Quantitation** | 6.30 | 400.28 |

**Accuracy (recovery study):**

The accuracy of the method was determined by calculating the recoveries of Bosentan by the standard addition method. Known amounts of standard solutions of Bosentan were added at 50% concentration to pre quantified sample solutions of Bosentan (50,100,150 µg/ml) and the amount of drug recovered was estimated. Results are shown in Table – 7.

**Table No: 7 Recovery Data**

| % Conc. | Area | Amount Added(mcg/mL) | Amount Recovered(mcg/mL) | % Recovery | Mean % Recovery |
|---|---|---|---|---|---|
| 50% Accuracy | 4588.583 | 75 | 74.93 | 99.91 | 100.17 |
| | 4708.246 | | | | |
| | 4863.171 | | | | |
| 100% Accuracy | 6452.054 | 100 | 100.08 | 101.08 | |
| | 6389.113 | | | | |
| | 6395.962 | | | | |
| 150% Accuracy | 7993.801 | 150 | 150.41 | 99.54 | |
| | 7818.674 | | | | |
| | 7980.726 | | | | |

**Specificity:**

In an assay, demonstration of specificity requires that it can be shown that the procedure is unaffected by the presence of impurities or excipients. In practice, this can be done by spiking the drug substance or product with appropriate levels of impurities or excipients and demonstrating that the assay results are unaffected by the presence of these extraneous materials. There should be no interference of the diluents, placebo at retention time of drug substances.

**Assay Results:**

**Table No: 8 Assay results**

| Sample | Batch No: | Label Claim | % Amount Found | Average |
|---|---|---|---|---|
| Bosentan | 1 | 62.5 mg | 99.7 | 99.23 |
| | 2 | | 99.8 | |
| | 3 | | 98.2 | |

**Acceptance criteria:** The percentage assay should be in the limits of 90-110%.

**Robustness**:

Robustness is the measure of a method remain unaffected by small, deliberate changes in method parameters like flow rate and detection wavelength on assay of the analyte of interest. Here the detection wavelength varied ±2nm and flow rate was varied ±0.2 ml/min. The results were shown in Table – 9

**Robustness study:**

**Table No: 9 Robustness study**

| Chromatographic change | | Bosentan | |
|---|---|---|---|
| | | Theoretical plates | Asymmetry |
| **Flow rate** | 1ml/min | 2887 | 1.6 |
| | 0.8ml/min | 3227 | 1.6 |
| | 1.2ml/min | 2885 | 1.5 |
| **Wavelength** | 220nm | 2882 | 1.6 |
| | 222nm | 2889 | 1.6 |
| | 218nm | 2891 | 1.6 |

**Ruggedness:**

The ruggedness of the method was studied by analyzing the sample and standard preparations by two analysts. The %RSD assay values between two analysts was calculated i.e.,(limit <2%). This indicates the method was rugged. The results were shown in Table – 10

**Ruggedness Study:**

**Table No: 10 Ruggedness study**

| Analyst | Sample | %Assay | %RSD |
|---|---|---|---|
| Analyst-1 | Bosentan | 99.96% | 0.12% |
| Analyst-2 | | 99.85% | |

**Forced Degradation Study:**

Forced degradation studies were performed to evaluate the stability indicating properties and specificity of the method. All solutions for use in stress studies were prepared at an initial concentration of 1 mg/ml of Bosentan and refluxed for 30 min at 80 ºC. All samples were then diluted in mobile phase to give a final concentration of 100 µg/ml and filtered before injection.

**Acidic degradation:**

Acidic degradation was performed by treating the drug solution (1 mg/mL) with 0.1 N hydrochloric acid for 30 min in a thermostat maintained at 80 ºC, cooled and then the stressed sample was neutralized and diluted with mobile phase as per the requirement before injecting in to the HPLC system.

**Alkaline degradation:**

Alkaline degradation was performed by treating the drug solution (1 mg/mL) with 0.1 N sodium hydroxide for 30 min in a thermostat maintained at 80 ºC, cooled and then the stressed sample was neutralized and diluted with mobile phase as per the requirement before injecting in to the HPLC system.

**Oxidation degradation:**

Oxidation degradation was performed by treating the drug solution (1 mg/mL) with 3 % H2O2 for 30 min in a thermostat maintained at 80 ºC, cooled and then the stressed sample was diluted with mobile phase as per the requirement before injected in to the HPLC system.

Increased the %H2O2 concentration 3 to 5%, 10% and up to 30% for 30 min in a thermostat maintained at 80 ºC, cooled and then the stressed sample was diluted with mobile phase as per the requirement before injected in to the HPLC system.

**Photolytic degradation:**

The drug solution (1 mg/mL) for photo stability testing was exposed to UV light (365 nm) chamber for 4 hours and then analyzed.

**Thermal degradation:**

The drug solution (1 mg/mL) was heated in a thermostat maintained at 80 ºC for 30 mins, cooled and then the stressed sample was diluted with mobile phase as per the requirement before injected in to the HPLC system.

## Results and Discussion:

In RP HPLC method, the primary requirement for developing a method for analysis is that the using different solvents and buffers and columns to get better retention time and theoretical plates, and better cost effective and time saving method than the previously developed methods. . The Maximum uv absorbance was found to be 220nm (Figure No: 2.3) by scanning in UV region.The chromatographic method was optimized with mobile phase consisting of Mixed phosphate buffer: Acetonitrile (55:45) and C18 INERTSILC18column. All the validation parameters were studied at a the wavelength 220nm.Accuracy was determined by calculating the recovery (Table No.3) and the results were in acceptable range (limit 98-102%). The method was successfully used to determine the amount of BOSENTAN present in the Tablet. The results obtained were in good agreement with the corresponding labelled amount (Table No.3). The method was linear in the

concentration range of 50 to 100 µg/ml forBOSENTAN(Table No.1).Precision was calculated as repeatability and intra and inter day variations (% RSD) for thedrug (Table No.7). Robustness and ruggedness results were in acceptable range (Table No.4 and Table No.5).As the drug peak was well separated even in the presence of degradation products the method is more specific. The percentage of degradation in all types of degradations is less than 2.0 % and therefore it is concluded that Bosentan is more resistant towards acidic, alkaline, oxidative, thermal and photolytic degradations. Summary of all validation parameters for method is given in Table No.8. By observing the validation parameters, the method was found to be simple, sensitive, accurate and precise. Hence the method can be employed for the routine analysis of Bosentan in bulk and tablet dosage form

**Stability Data:**

**Table No: 11 Stability Data**

| **S.NO:** | **Compound** | **% Degradation** | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Untreated | Acid | Base | Oxidation | Neutral | UV | Heat |
| 1 | Bosentan | 99.23% | 6.8 | 3.59 | 28 | 3.59 | 0.38 | 0.55 |

**Validation parameters of evaluated method:**

**Table No.12: Validation Summary**

| **S. No** | **Parameter** | **Value Obtained of Bosentan** |
|---|---|---|
| 1. | Accuracy(%Recovery) | 100.17% |
| 2. | Linearity concentrations Range(µ g/mL)<br>Regression coefficient (R2 value) | 50-150 µ g/mL<br>0.999 |
| 3. | LOD(mcg/mL) | 2.14 |
| 4. | LOQ(mcg/mL) | 6.48 |
| 3. | Precision (% RSD)<br>Method precision(Repeatability) (%RSD, n = 6) | 0.53-0.92 |
| 4. | Robustness | Met with system suitability criteria |
| 5. | Ruggedness(%RSD analyst to analyst variation) | 0.12% |

**(a) S.D. =Standard deviation (b) LOD = Limit of detection (c)LOQ = Limit of quantification**
**(d) RSD = Relative standard deviation**

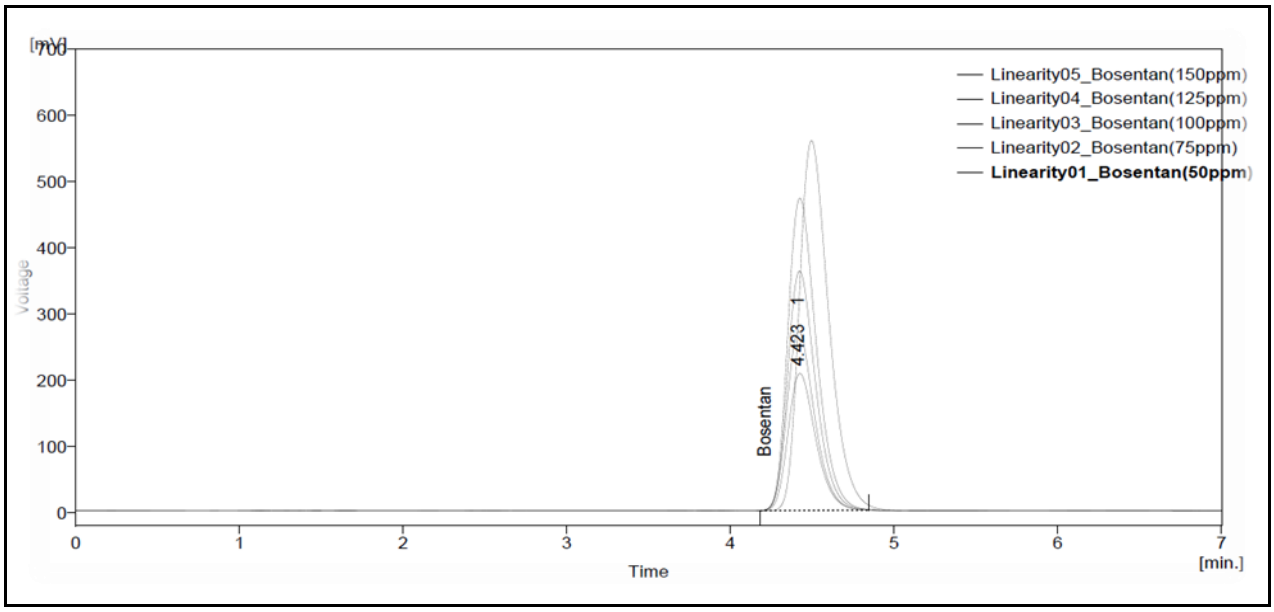



**Figure No.2: linearity chromatogram of Bosentan**:

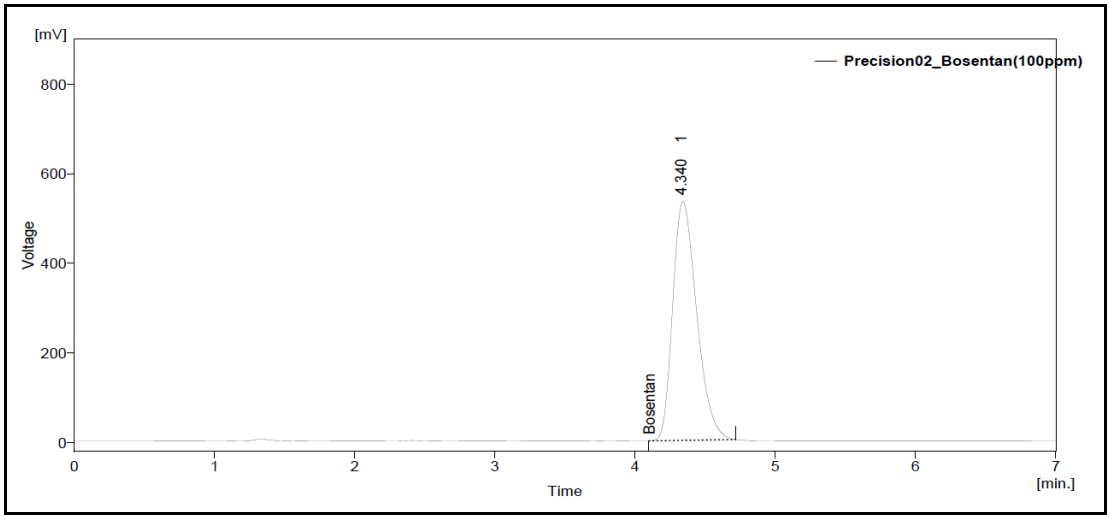



**Figure 3 : Chromatogram of Bosentan**

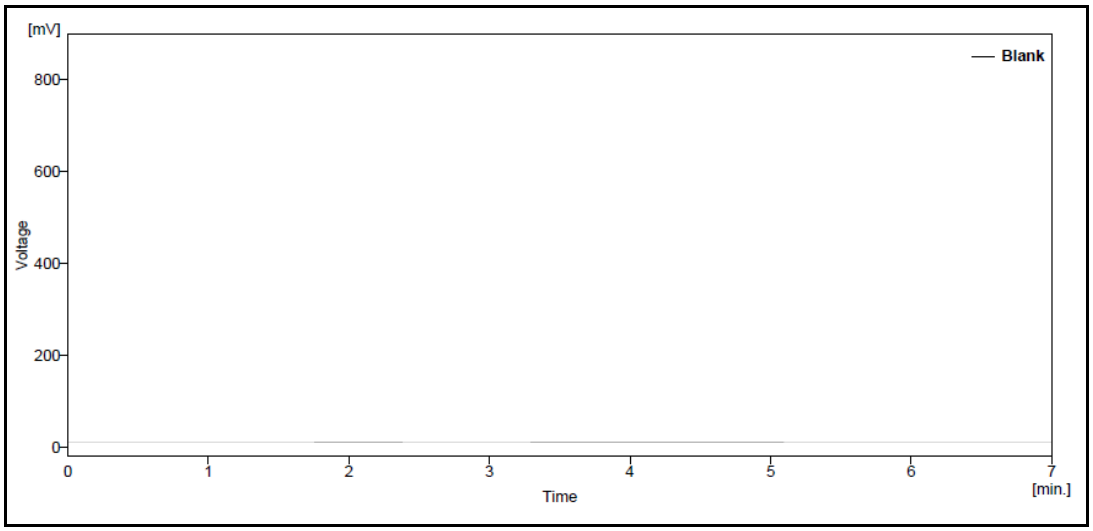



**Figure NO.4: Placebo chromatogram**

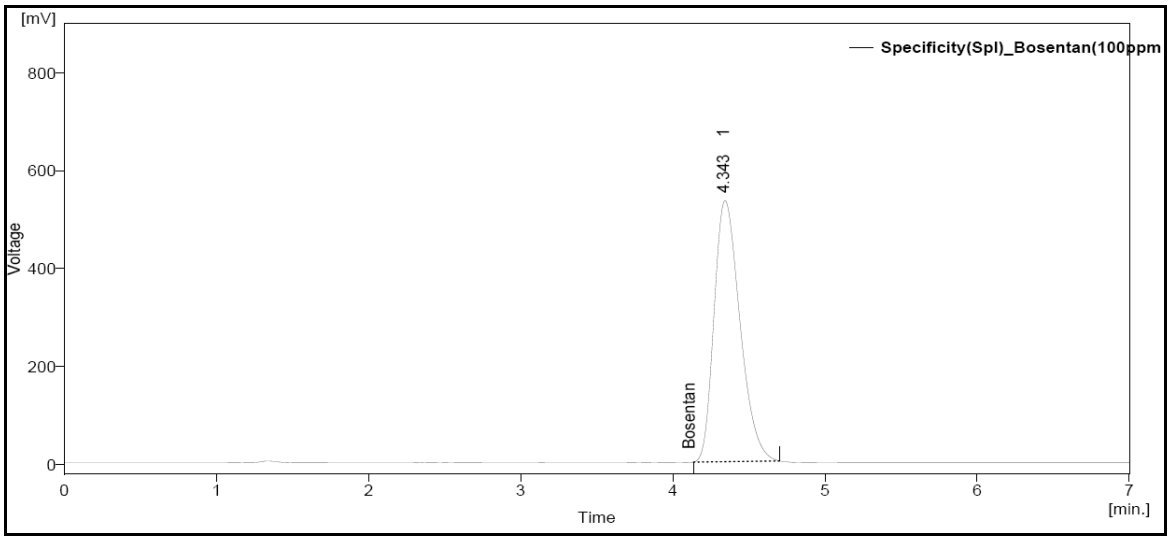



**Figure 5: Bosentan sample**

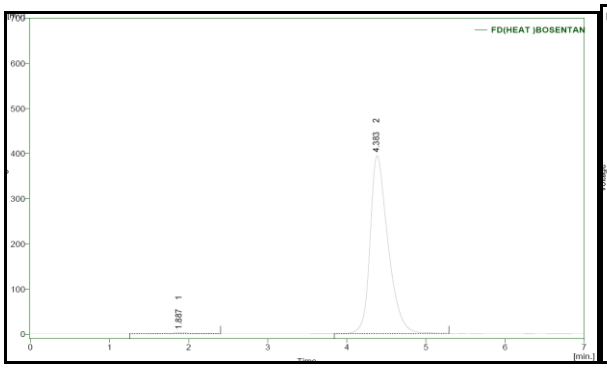



**Figure 6: Heat Degradation**

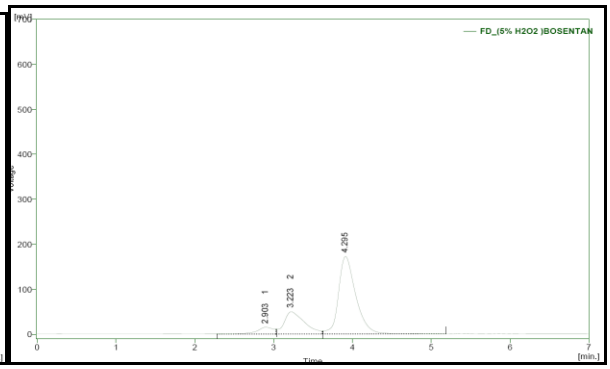



**Figure 7: Oxidative Degradation**

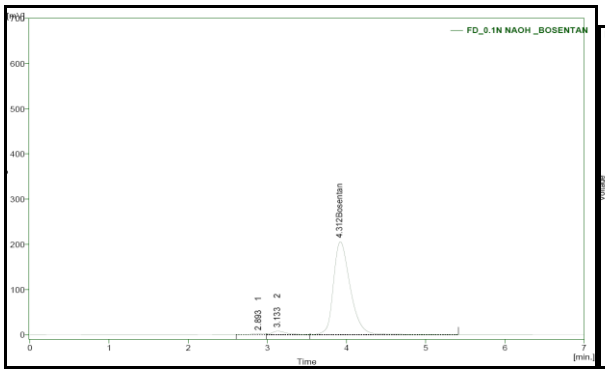



**Figure 8: Alkali Degradation**

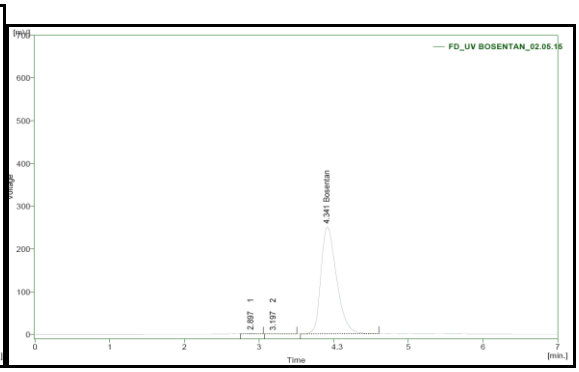



**Figure 9: UV Degradation**

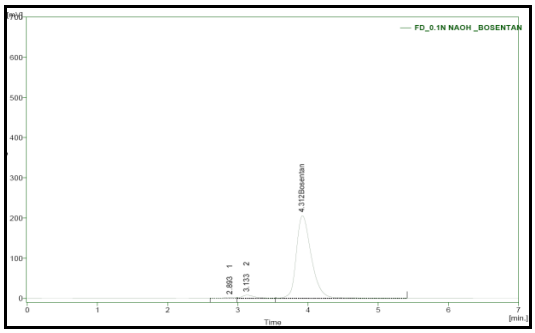



**Figure 10: Acid degradation**

## Conclusion:

The proposed estimation by RP-HPLC method was found to be simple, sensitive, accurate and precise for determination Bosentan in Bulk and tablet. The method utilizes easily available and cheap solvent for analysis of Bosentan hence the method was also economical for estimation of Bosentan from Tablet .The common excipients and other additives are usually present in the Tablet mixture do not interfere in the analysis of Bosentan, hence it can be conveniently adopted for routine quality control analysis of the drug in pharmaceutical formulation.

## Acknowledgement:

The authors are highly thankful to Chandra labs, Kukatpally, Hyderabad, India for providing all the facilities to carry out this work.

## References:

1. Annapurna MM, Bisht SPS, Kumar BVVR, Kumar VR, Narendra A. Spectrophotometric determination of bosentan and its application in pharmaceutical analysis. Int. J. Comp. Pharma. 2011; 2 (1): 1-2.
2. Ashok Kumar A, Anil Kumar A, Sankar DG. Development, estimation and validation of bosentan in bulk and in its pharmaceutical formulation by UV-VIS spectroscopic method. Int. J. Pharma. Bio. Sci. 2011; 2(2): 225-230.
3. Kumar D, Sreenivas SA, Samal HB, Dey S, Priyanka Y. Method development and estimation of bosentan monohydrate in bulk and pharmaceutical dosage forms using UV-Visible spectrophotometer. J. Pharma. Res. 2011; 4(6): 1713-1715.
4. Narendra A, Deepika D, Annapurna MM. New spectrophotometric method for the determination of bosentan - An anti-hypertensive agent in pharmaceutical dosage forms. E-J. Chem. 2012; 9(2): 700-704.
5. Khan MA, Sinha S, Todkar M, Parashar V, Swamy K. Development and validation of a stability indicating analytical method for the related substances of bosentan drug substance by HPLC. American J. Sci. Ind. Res. 2012; 3(2): 69-80.
6. Jadhav SA, Landge SB, Jadhav SL, Niphade NC, Bembalkar SR, Mathad VT. Stability-indicating gradient RP-LC method for the determination of process and degradation impurities in bosentan monohydrate: An endothelin receptor antagonist. Chromatogr. Res. Int. 2011; 2011(ID 929876): 1-5.
7. Lausecker B, Hopfgartner G. Determination of an endothelin receptor antagonist in human plasma by narrow-bore liquid chromatography and ion spray tandem massspectrometry. J. Chromatogr. A. 1995; 712(1): 75-83.
8. Lausecker B, Hess B, Fischer G, Mueller M, Hopfgartner G. Simultaneous determination of bosentan and its three major metabolites in various biological matrices and species using narrow bore liquid chromatography with ion spray tandem mass spectrometric detection. J. Chromatogr. B. Biomed. Sci. Appl. 2000; 749(1): 67-83.
9. Muralidharan S, Kumar JR. Simple estimation of bosentan in tablet formulation by RP-HPLC. American J. Anal. Chem. 2012; 3(11): 715-718.
10. Reddy TK, Younus Md, Reddy YR, Kumar GA, Sravan S. RP-HPLC method development and validation of bosentan
11. ICH, Q2A validation of analytical procedure: Methodology International Conference on Harmonization, Geneva, October 1994.
12. ICH, Q2B Validation of analytical procedure: Methodology International Conference on Harmonization, Geneva, March 1996.
13. wikipedia.org/wiki/Bosentan
14. http://www.medlineindia.comwww.drugbank.ca/drugs/DB00808
15. www.drugbank.ca/drugs/DB04861
16. http://www.irjponline.com/admin/php/uploads/1029_pdf.pdf

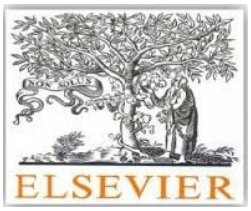
*****

# International Journal of PharmTech Research

## log on to - www.sphinxsai.com

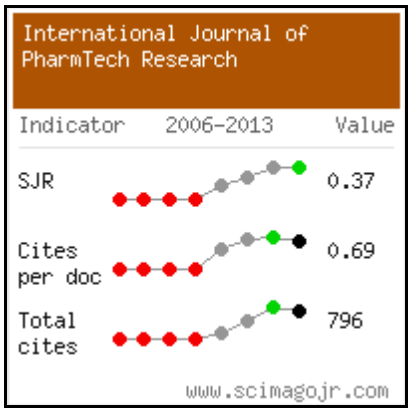
**Indexed/Abstracted/ Ranked by**

**Elsevier SCOPUS- scimagojr.**





Powered by Scopus




**International Journal of PharmTech Research is an open access Bimonthly Journal, 7.5 Years old. It contains more than 2200 published papers since 2009.**

**Subject areas:** This journal publishes the Research and Review papers of the following subject/areas. Pharmaceutics, Pharmaceutical Chemistry, Biopharma, Pharmacology, Pharmacy Practice, Pharmacognosy, Analytical Chemistry, Biotechnology, Microbiology, Biochemistry, , Medicinal Science, Clinical Pharmacy, Medichem, and applied related subject areas.

**[1] RANKING:**

**It has been ranked from India (subject: Pharma Sciences) from India at International platform, by SCOPUS- scimagojr.**

**It has topped in total number of CITES AND CITABLE DOCUMENTS.**

**Find more by clicking on SCOPUS-scimagojr SITE....AS BELOW.....**

**http://www.scimagojr.com/journalrank.php?area=3000&category=0&country=IN&year=2013&order=tc&min=0&min_type=tc**

**Please log on to - www.sphinxsai.com**

*****