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DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC AND CHROMATOGRAPHIC METHOD FOR THE ESTIMATION OF BOSENTAN IN BULK AND FORMULATIONS

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ABSTRACT

Objective: Objective of the present analytical research work was to develop and validate Spectrophotometric method and Ultra Performance Liquid Chromatographic method (UPLC Method) for the Bosentan bulk and tablets dosage form. **Methods:** A spectrophotometric method and a UPLC method have been developed and validated for estimation of BST in pharmaceutical oral dosage form. **Method A (UV spectrometry Method):** The stock and working standard solutions of the drugs were prepared in methanol. Standard solutions were scanned over the range of 400-200nm in spectrum mode of spectrophotometer at medium scanning speed using UV spectrophotometer. The maximum absorbance for Bosentan was found at 269nm. **Method B (UPLC Method):** The UPLC Method for Bosentan was developed using UPLC Acquity BEH C18 column (100 mm × 2.1 mm × 1.7 µm), as stationary particle, isocratic mode. ACN: Water (80:20v/v) as mobile phase. Mobile phase was maintained data flow rate of 0.3ml/min and detection was carried out at 273nm. Both the methods were validated in accordance with ICH guidelines. **Results:** Bosentan was found to be linear in the concentration range of 10-30µg/ml for spectrophotometric method and10-50µg/ml for UPLC method. Retention time was found to be 3.7min for Bosentan. The amount of Bosentan in marketed formulation by spectrophotometric method was found to be 100.0%, the amount of Bosentan in marketed formulation by UPLC method was found to be 99.99%. **Interpretation and Conclusion:** Results of assay and validation study were found to be satisfactory. So, the methods can be successfully applied for the routine analysis of Bosentan.

KEYWORDS

Bosentan, Anti-hypertensive, UPLC, UV –Vis and Method development.

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INTRODUCTON

Bosentan is an endothelin receptor antagonist which is marketed by the trade name Tracleer by Actelion Pharmaceuticals Pvt. Ltd. Bosentan is used in the treatment of pulmonary hypertension followed by blocking the action of endothelin molecules.

Bosentan is chemically 4-*tert*-butyl-*N*-[6-(2-hydroxyethoxy)-5-(2-methoxyphenoxy)-2-(pyrimidin-2-yl) pyrimidin-4-yl] benzene-1-

sulfonamide, it is a selective mixed endothelin A and endothelin B receptor antagonist used to treat pulmonary hypertension. Increased endothelin concentrations are highly corresponds with the disease severity. Bosentan causes decrease in the level of both pulmonary and systemic vascular resistance which results in an elevated cardiac output without increasing the heart rate. It is recommended for the treatment of pulmonary arterial hypertension (PAH) to enhance exercise capability and symptoms in patients with WHO functional class III and also some improvements shown in patients with WHO functional class II. The drug is also indicated to reduce a number of new digital ulcers in patients with systemic sclerosis and ongoing digital ulcer disease. Researchers study suggested that major achievement gain in the reduction of these impurity levels can by means of three consecutive crystallization (2 from methanol: isopropyl acetate and 1 from ethanol: water); however, no additional data were published. Therefore, it is very necessary to find an analytical method suitable for determination of Bosentan which is very precise, accurate and economical.

MATERIAL AND METHODS

Spectrophotometric Method

Development of Spectrophotometric Method

Selection of Solvent

Solutions of BST (100µg/ml) was prepared in different solvents like methanol and water. These solutions were scanned in UV-Visible Region (200nm to 800nm) and intensity of absorption and wavelength of absorption were studied.

Preparation of Standard Stock Solution

Standard stock solution was prepared.

Selection of Wavelength Range

From the stock solutions, 1.0ml of BST was transferred to 10ml volumetric flask and the volume was adjusted to the mark with MeOH to obtain Strength 10µg/ml. The solution was scanned in the UV range 200-400nm.

Preparation for Calibration Curve

Calibration curve were prepared and graph was plotted.

Analysis of Tablets

For analysis of commercial formulation, twenty tablets were weighed, average weight determined and crushed into fine powder. An accurately weighed quantity of powder equivalent to 10mg of BST was transferred into 100ml volumetric flask containing 50ml methanol, shaken manually for 10 min, volume was adjusted to mark with same solvent and filtered through Whitman filter paper. The absorbance of sample solution was recorded at recorded at 269nm.

Validation of Spectrophotometric Method

Linearity and Range

The linearity of analytical method for BST was determined by studying standard calibration curves. The range of analytical method was decided from the interval between upper and lower level of calibration curves by plotting the log curve.

Accuracy

Accuracy of the method was assessed by standard addition method at three different concentration levels i.e. 80%, 100, 120. Standard concentration of 7, 10 and 13µg/ml was added into 10µg/ml of tablet concentration. The % recovery was then calculated by using formula

% Recovery = A - B/ C,

Where,

A = Total amount of drug estimated

B = Amount of drug found on pre analysed basis

C = Amount of Pure drug added

Precision

The precision of an analytical method was studied by performing intermediate precision.

Intra-day Precision

Intra-day precision was determined by analyzing the 10, 15, 20µg/ml of BST for three times in the same day.

Inter-day Precision

Inter-day precision was determined by measuring the 10, 15, 20µg/ml of BST for three consecutive days.

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

Detection limit and quantitation limit were determined based on the standard deviation of y-

intercepts of calibration curves and average slope of calibration curves.

$$LOD = 3.3 \times \frac{\text{Standard deviation of intercept}}{\text{Slope}}$$

$$LOD = 10 \times \frac{\text{Standard deviation of intercept}}{\text{Slope}}$$

Ruggedness

Ruggedness of the method was checked by two different analysts keeping same experimental and environmental conditions. An appropriate concentration 10, 20µg/ml of BST was subjected to analysis and concentration was determined. This procedure was repeated three times.

Chromatographic Method

Development of Chromatographic method

Description

The sample of Bosentan was observed for its color and texture.

Solubility

The sample of Bosentan was taken in test tubes and observed for solubility in various solvents like alcohol and water.

Chemicals and Reagents

Analytically pure samples of Bosentan were kindly supplied by Cipla Pvt. Ltd, Water (HPLC Grade), ACN (HPLC Grade) Research Chem lab and (MeOH AR grade) FINAR Ltd were used for the method development.

Instrument Used

Electronic Weighing Balance (Shimadzu AY-220), Ultrasonicator (Bio-Technics India ICO 900/2000), Cellulose Acetate Filter, 0.45µm (Nylon66), UPLC System (Agilent Technologies).

Selection of Mobile Phase

The selection was made on the basis of literature survey. After assessing the solubility of drug in different solvents as well on the basis of literature survey, Methanol and water were selected as a first choice.

Selection of Analytical Wavelength

To investigate the appropriate wavelength for determination of BST, the solution of the same in the MeOH were scanned separately by UV-Visible spectrophotometer in the range of 190-400nm and the spectrum were recorded.

Preparation of Mobile Phase

Mobile Phase A

HPLC grade MeOH was degassed in sonicator for 15 min.

Mobile Phase B

HPLV grade water

Preparation of Standard Stock Solution

Standard stock solution was prepared by dissolving10 mg of Bosentan in 100ml methanol that gives concentration of 100g/ml of Bosentan and labeled as Standard stock Bosentan.

Preparation of Calibration Curve

Analysis of tablets

To determine the content of BST in conventional tablets; the twenty tablets were weighed, their mean weight determined and they were finely powered and powder equivalent10.0 mg BST was transferred into a 100mL volumetric flask containing 50mL methanol, sonicated for 30 min and diluted to 100mL with methanol (100µg/mL). The resulting solution was filtered, using 0.22µm filter and 30µg/mL was injected into system. The amount of BST was determined. The assay procedure was repeated for six times and Calculated using following equation.

$$Ct = \frac{Rt \times Cs}{Rs}$$

Where, Ct and Cs = Concentration of Sample and Standard Solution, respectively.

Rt and Rs = Peak Area for Sample and Standard Solution, respectively.

Validation of UPLC Method

Linearity

The linearity of analytical method for BST was determined by studying standard calibration curves. The range of analytical method was decided from the interval between upper and lower level of calibration curves by plotting the log curve.

Accuracy

Accuracy of the method was assessed by standard addition method at three different concentration levels i.e. 80%, 100, 120%. Standard concentration of 7, 10 and 13µg/ml was added into 10µg/ml of tablet concentration. The % Recoveries was calculated by applying regression equation.

Precision

The precision of an analytical method was studied by performing intermediate precision.

Intra-day Precision

Intra-day precision was determined by analyzing the standard solutions of BST (20, 30, 40µg/ml) and at three different time intervals on same day.

Inter-day Precision

Inter-day precision was determined by analyzing the combined standard solution of Bosentan (20, 30, 40µg/ml) on three consecutive days. The results were reported in terms of %RSD.

Limit of Detection and Limit of Quantitation

Detection limit and quantitation limit were determined based on the standard deviation of y-intercepts of calibration curves and average slope of calibration curves.

$$LOD = 3.3 \times \frac{\text{Standard deviation of intercept}}{\text{Slope}}$$

$$LOD = 10 \times \frac{\text{Standard deviation of intercept}}{\text{Slope}}$$

Robustness

Standard stock solution of BST (100µg/ml) were used and analyzed at different flow rate (0.1, 0.3, 0.5ml/min).

Ruggedness

Ruggedness of the method was checked by two different analysts keeping same experimental and environmental conditions. An appropriate concentration 10, 20µg/ml of BST was subjected to analysis and concentration was determined. This procedure was repeated three times.

System Suitability

Standard solution of BST (50µg/ml) was prepared and analyzed. Chromatograms were studied for different parameters such as tailing factor, resolution and theoretical plates to see that whether they complies with the recommended limit or not.

RESULTS AND DISCUSSION

UV-Visible Spectrophotometric Methods

Linearity study

Standard solution having concentration range of 10, 15, 20, 25, 30µg/ml of BST was prepared. Absorbances of these solutions were recorded at 269nm. Calibration curve was plotted, absorbance *vs* concentration. Figure No. 3, Table No.1.

Assay of Marketed Formulation

Using this method, the marketed formulation was analyzed. Sample solution containing 30µg/ml. The amount of drug present in the marketed formulation was calculated. The mean % assay of BST was found to be 100.00%. Table No.3.

Validation Parameters

Validation of the method was performed in accordance to ICH guidelines. Accuracy of the method was determined at 80%, 100% and 120% level by standard addition method and percentage recovery BST were found to be in the range of 99.35–100.20%. Precision of the method was determined by %RSD of intra-day precision, inter-day precision. It was found to be less LOD and LOQ of BST was found to be 0.0522 and 0.1582µg/ml, respectively. Table No.4, 5A, 5B, 6 and No.7.

Chromatographic Method

Selection of Analytical Wavelength

The standard solutions of BST (100µg/ml) in mobile phase were scanned in the UV region of 190 - 400 nm and the overlain spectra were recorded. It was observed that BST drugs showed the absorbance at 273nm. So, the wavelength of detection used was 273nm.

Linearity Study

BST was found to be linear in the concentration range of 10-50µg/ml. Figure No.5, Table No.8 and Table No.9.

Assay of Marketed Formulation

Amount of drugs present in the marketed formulation equations. Amount of BST found in the range from 99.99% and SD ±0.015. Table No.10.

Validation Parameters

This method was validated in accordance to ICH guidelines. Percentage of recoveries of BST was found in the range from 98.65 **-** 101.69%. Precision of the method was determined by % RSD found among intra-day precision, inter-day precision. LOD and LOQ of BST were found to be 0.356 and 1.081µg/ml, respectively. For robustness study, the effect of change in wavelength and flow rate (± 0.2 ml/min) on the Mean peak area, % RSD and %

Assay were studied. Percentage RSD of each peak in all variables was found to be less than 2 %. Table No.11, 12A, 12B, 13, 14, 15.

Table No.1: Data of calibration curve by UV

S.No	Conc. (µg/ml)	Absorbance
1	10	0.160
2	15	0.321
3	20	0.498
4	25	0.632
5	30	0.795

Table No.2: linear regression analysis by UV

S.No	Parameters	Absorption Maxima Spectrophotometric Method
1	ƛ max (nm)	269nm
2	Beer's law limit (µg/ml)	10-30
3	Regression equation [y]	+ 0.1512
4	Slope [m]	0.0316
5	Intercept [c]	0.1512
6	Correlation coefficient [r2]	0.9985
7	Limit of detection (LOD) (µg/ml)	0.0522
8	Limit of Quantitation (µg/ml)	0.1582

Table No.3: Result of tablet analysis

S.No	Drug Name	Mean*	SD	%RSD
1	Bosentan	100.00	0.0959	0.0958

Table No.4: Result of Accuracy study

S.No	Level of addition	% Mean recovery*	SD	%RSD
1	80%	99.44	0.1183	0.1189
2	100%	100.03	0.2041	0.2040
3	120%	99.89	0.1592	0.1593

Table No.5A: Result of intraday precision

S.No	Conc. (µg/ml)	Mean	SD	%RSD
1	10	0.1616	0.00108	0.6693
2	15	0.3246	0.0004612	0.1269
3	20	0.4963	0.000817	0.1646

Table No.5B: Result of interday precision

S.No	Conc. (µg/ml)	Mean	SD	%RSD
1	10	0.1616	0.00108	0.6693
2	15	0.3256	0.000185	0.2513
3	20	0.4963	0.000817	0.1646

Table No.6: Result of robustness study

S.No	Parameters	Change In Wavelength (± 2nm)			
		Wavelength (267nm)		Wavelength (271nm)	
		20ppm	30ppm	20ppm	30ppm
1	Mean (n=5)	0.495	0.792	0.496	0.794
2	SD	0.00082	0.00102	0.00084	0.00182
3	%RSD	0.1743	0.1287	0.1693	0.2292

Table No.7: Result of ruggedness study

S.No	Parameters	Change In Analyst			
		Analyst I		Analyst II	
		10ppm	20ppm	10ppm	20ppm
1	Mean (n=5)	0.162	0.497	0.160	0.498
2	SD	0.000117	0.000802	0.000106	0.000827
3	%RSD	0.0722	0.1613	0.0662	0.1660

Table No.8: Result of calibration curve

S.No	Concentration µg/ml	Area
1	10	925.78
2	20	1781.58
3	30	2785.22
4	40	3750.22
5	50	4791.23

Table No.9: Linear regression analysis

S.No	Parameters	Ultra-Performance liquid Chromatography method
1	ƛ max (nm)	273nm
2	Beer's law limit (µg/ml)	10-50
3	Regression equation [y]	96.995x ± 103.06
4	Slope [m]	96.995
5	Intercept [c]	103.06
6	Correlation coefficient [r2]	0.999
7	Limit of detection (LOD) (µg/ml)	0.3568
8	Limit of Quantitation (µg/ml)	1.081

Table No.10: Assay result by UPLC

S.No	Drug Name	Mean*	SD	%RSD
1	Bosentan	99.99	0.01549	0.01549

Table No.11: Result of Accuracy by UPLC

S.No	Level of addition	% mean recovery*	SD	%RSD
1	80%	99.7	0.6265	0.6284
2	100%	99.58	0.667	0.6695
3	120%	100.21	0.8728	0.8703

Table No.12A: Result of intraday precision

S.No	Conc. (µg/ml)	Mean*	SD	%RSD
1	20	1782.87	5.728	0.3212
2	30	2792.15	2.560	0.0917
3	40	3777.13	6.125	0.1621

Table No.12B: Result of Interday precision

S.No	Conc. (µg/ml)	Mean*	SD	%RSD
1	20	1771.53	1.917	0.1082
2	30	2768.15	2.894	0.1045
3	40	3763.80	2.678	0.0711

Table No.13: Result of robustness study

S.No	Parameters	Flow Rate mL/min	Conc. (µg/ml)	Mean* Area	SD	%RSD
1	Change in flow rate	0.1	30	2733.63	1.89	0.06913
2		0.5	40	3778.23	2.78	0.07357

Table No.14: Result of Ruggedness

S.No	Analyst	Conc. (µg/ml)	Mean* Area	SD	%RSD
1	Analyst-I	10	928.78	0.987	0.1062
2	Analyst-II	20	1763.58	1.235	0.07002

Table No.15: Result of system Suitability

S.No	Conc. (µg/ml)	Retention Time/min	Theoretical Plates	Asymmetry factor
1	50	3.761	9895	1.25
2	50	3.766	9970	1.25
3	50	3.769	9897	1.23
4	50	3.768	9887	1.25
5	50	3.770	9975	1.23
Mean		3.766	9925.4	1.242
SD		0.001837	21.87	0.00547
%RSD		0.04878	0.2203	0.4410

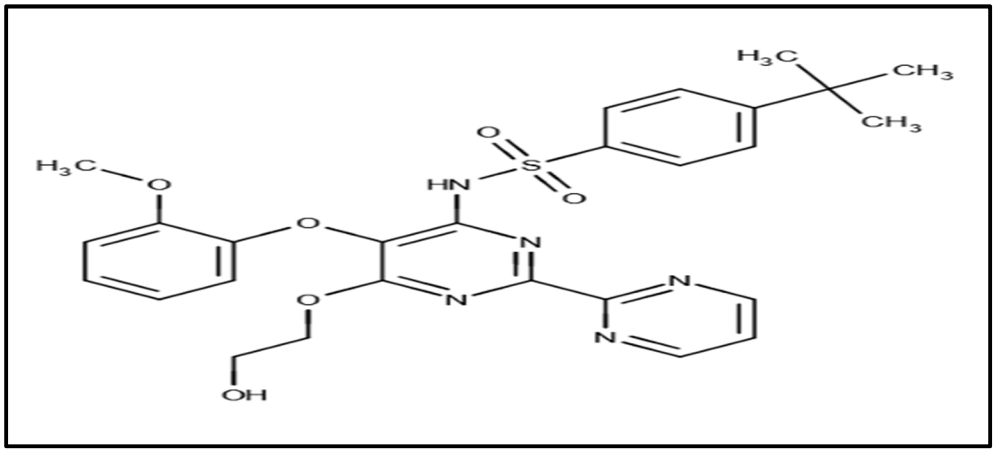

Figure No.1: Structure of Bosentan

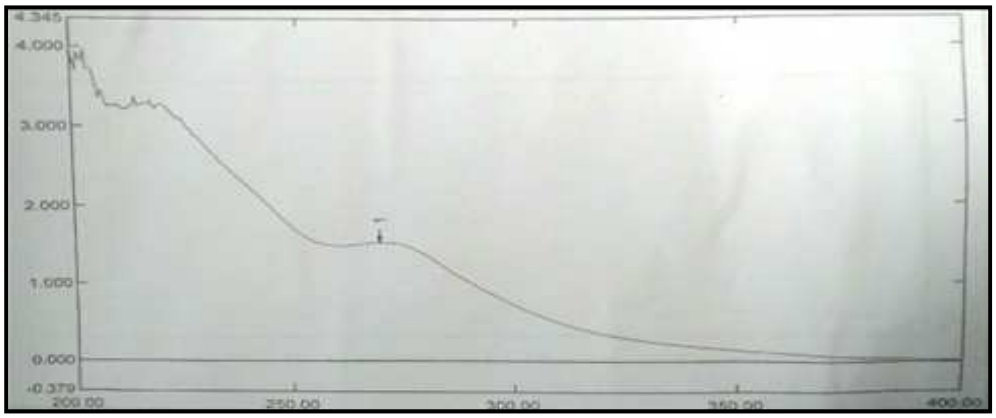

Figure No.2: UV spectra of Bosentan

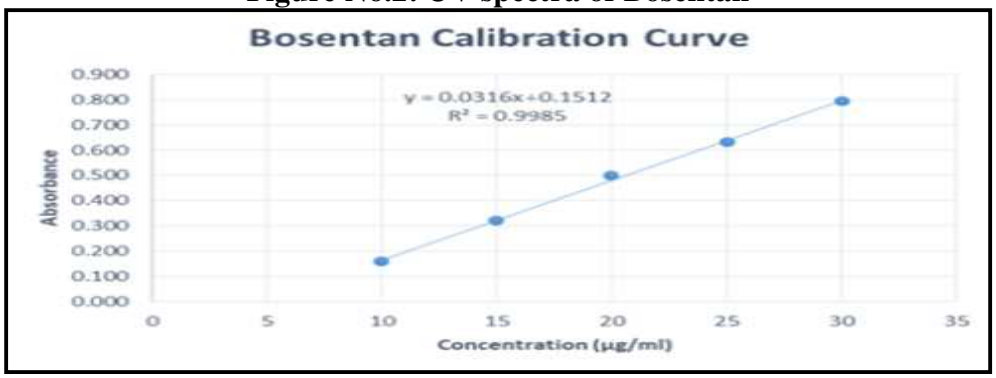

Figure No.3: Calibration curve by UV

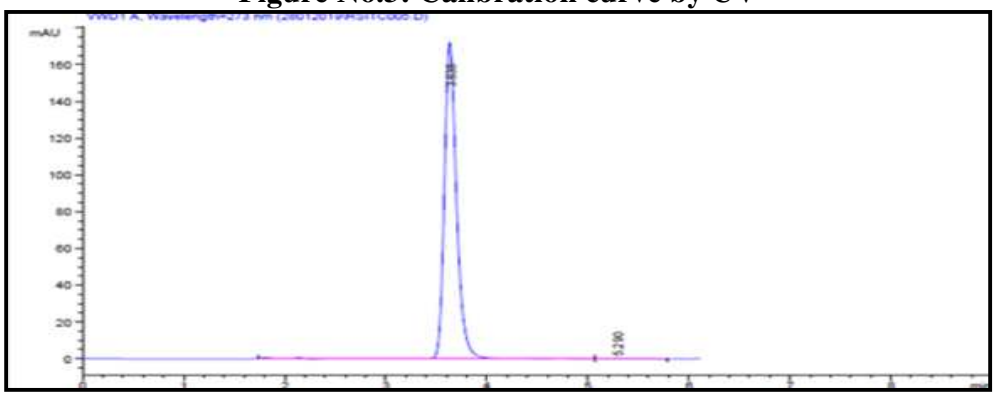

Figure No.4: Typical chromatograph of Bosentan by UPLC at Optimized condition

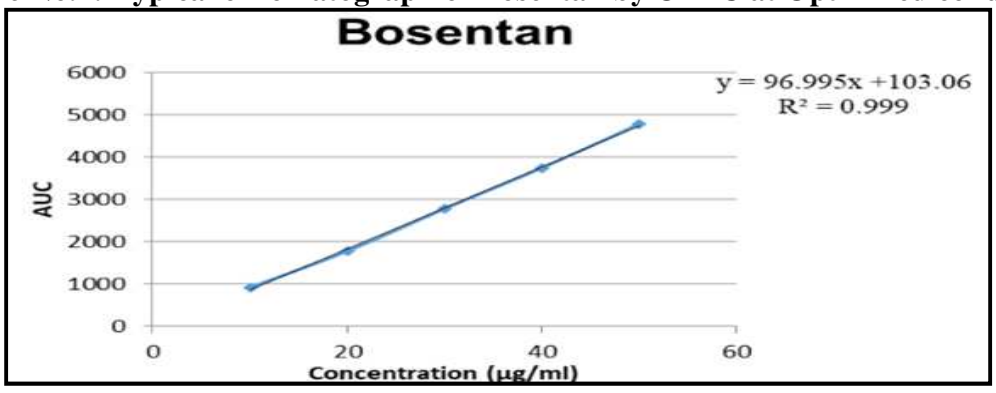

Figure No.5: Calibration curve by UPLC

CONCLUSION

In the present investigation, the developed and validated, UV Spectrophotometric method were found to be simple, economical and rapid method. UPLC was found to more precise, accurate, rugged and robust for determination of Bosentan. The excipients usually present in the pharmaceutical formulation did not interfere with determination of Bosentan. Developed method can be successfully used in laboratory to measure the concentration of API in specific dosage form. This method is also beneficial for the formulation and development department. These methods are always useful for analysis, purity testing and assay. The consumption of time and chemicals is less as compare to other tedious method. This is new concept for the validation of method development and method transferin pharmaceutical companies. The results and the statistical parameters demonstrate that the proposed UV spectrophotometric and UPLC method is simple, rapid, specific, accurate and precise.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

BIBILIOGRAPHY

1. Lavudu P, Avula P R, Are P C, Sekaran C B. Determination of Bosentan in Pharmaceutical dosage forms by high performance liquid chromatography, *International Journal of Drug Delivery,* 5(2), 2013, 146-151.
2. Rizwan S H, Sastry V G, Imad Q. Stability Indicating Method Development and Validation of Bosentan in Bulk Drug and Formulation by RP-HPLC Method, *International Journal of Pharm Tech Research,* 8(4), 2015, 569-579.
3. ICH, Q2 (R1), Validation of Analytical Procedure: text and methodology, *International Conference on Harmonization, Geneva,* 2005, 1-13.
4. Jagtap N S, Vanjari S S and Bhosale S D. Stability indicating Spectrophotometric method of Bosentan in Bulk and Pharmaceutical dosage form, *World Journal of Pharmacy and Pharmaceutical Sciences,* 5(7), 2016, 1420-1427.
5. Annapurna M M, Pavani S, Anusha S and Mahanti H. Validated Stability Indicating RP-HPLC Method for the determination of Bosentan in presence of degradation products, *Indo American Journal of Pharmaceutical Research,* 3(6), 2013, 4528-4540.
6. Beckett A H and Stenlake J B. Practical Pharmaceutical Chemistry, Part-II, *CBS Publishers and Distributors, New Delhi,* 4th Edition, 2004, 85-174.
7. Willard H H, Merrist L L, Dean A J, Frank A and Settle A F. Instrumental Methods of Analysis, *CBS Publishers and Distributors, New Delhi,* 7th Edition, 1986, 2-3, 585-587
8. Skoog D A, Holler F J and Nieman T A. Principle of Instrumental Analysis, *Thomson Publication Limited, London,* 5th Edition, 2004, 103.
9. Sethi P D. High Performance Liquid Chromatography, Quantitative Analysis of Pharmaceutical Formulations, *CBS Publishers and Distributors, New Delhi,* 1st Edition, 2001, 3-11, 116-120.
10. Siddappa K and Hanamshetty P C. Development and Validation of High Performance Liquid Chromatography (HPLC) method for the Determination of Bosentan in Pure and Formulated Forms, *Scholars Research Library Der Pharmacia Lettre,* 8(8), 2016, 404-411.
11. Shahul H M, Jat R K, Indulekha V N. Method Development and its Validation for quantitative determination of Bosentan in tablet dosage form by RP-HPLC, *Journal of*

Drug Delivery and Therapeutics, 7(2), 2017, 85-95.

12. Toomula N, Kumar A, Kumar S, Bheemidi V S. Development and Validation of Analytical Methods for Pharmaceuticals, *Journal of Analytical and Bioanalytical Techniques,* 2(5), 2011, 1-4.
13. Lakshmi S R P. A Review on Chromatography with High Performance Liquid Chromatography (HPLC) and its Functions, *Research and Reviews: Journal of Pharmaceutical Analysis,* 4(1), 2015, 1-15.
14. Swartz M E. UPLC: An introduction and review, *J Liq Chromatogr Rel Technol,* 28(7-8), 2005, 1253-1263.

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