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DEVELOPMENT OF NEW VALIDATED ANALYTICAL METHODS FOR THE ESTIMATION OF BOSENTAN IN BULK AND PHARMACEUTICAL DOSAGE FORM BY UV SPECTROPHOTOMETRY

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ABSTRACT

The present study describes a simple, accurate, precise, specific and highly sensitive methods for the determination of Bosentan present in pharmaceutical dosage forms. Bosentan exhibits maximum absorbance (λ_{max}) at 269 nm (Method A) and Method B is an area under curve(AUC)method 264-274nm.The drug obeys the Beer-Lambert's law in the concentration range of 2.5-15 µg/ml in two methods. The methods were validated and can be successfully applied to estimate Bosentan in pharmaceutical dosage forms. The proposed methods were validated in terms of linearity, precision and accuracy the present work provides an accurate and sensitive method for the analysis of Bosentan in bulk and tablet formulation.

KEY WORDS: Bosentan , UV-Vis Spectrophotometry, AUC, tablets.

INTRODUCTION

Bosentan is competitive antagonist of endothelin-1 receptor acts on endothelin A and endothelin B present on smooth muscles of pulmonary blood vessels [1, 2, 3]. Bosentan used in the treatment pulmonary hypertension. Chemically it contain 4-tetrabutyl-N-[6-(2-hydroxyethoxy)-5-(2-methoxyphenoxy)-2-(pyrimidin-2-yl)-pyrimidin-4-yl]-benzene-1-sulfonamide[2] with molecular weight 551.61g.Molecular formula $C_{27}H_{29}N_5O_6S$. It is white powder and highly soluble in methanol .Bosentan available as tablet formulation - Tracleer, Lupibose, Bosentan (125mg, 62.5mg).

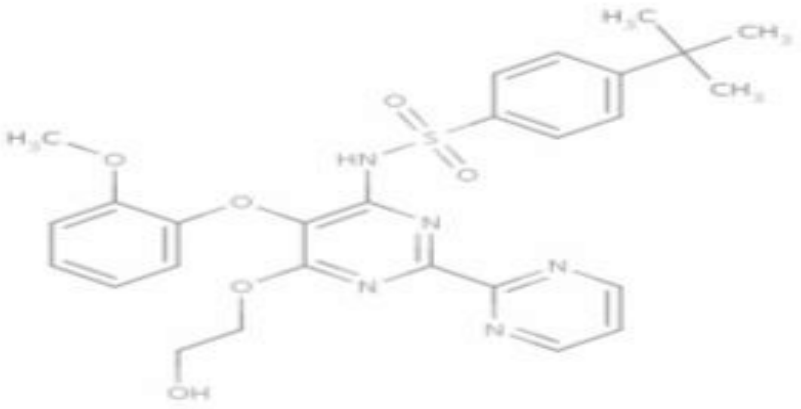

Fig 1: Chemical structure of Bosentan

MATERIALS AND METHODS

Drug substance and Reagents: Bosentan (working standard 99.77%) was obtained as gift sample from Aurabindo pharmaceutical company, Hyderabad, India. Pharmaceutical tablet formulation of TRACLEER 125mg purchased from local pharmacy. Methanol (A.R Grade; Qualigens), 0.1N NaoH and distilled water used for the study.

Instrumentation: UV Visible spectrophotometer (Shimadzu Model 1800) was employed with spectral bandwidth of 1nm attach with computer loaded with Shimadzu UV PC software (UV Probe) version 2.31 and using pair of 10mm matched quartz cells, Analytical balance ELICO.

Preparation of standard stock solution: working standard Bosentan 10mg was weighed accurately and transferred to a 10ml volumetric flask and dissolved in 5 ml of methanol. The flask was shaken for 5 min and volume was made up to the mark with methanol to give a solution of 1000 µg/ml. It was further diluted with 0.1N NaoH to get the concentration of 100 µg/ml. From this solution a series of aliquots were prepared for further method development.

Method A: Absorption Maxima Method: For the selection of analytical wavelength, 10µg/ml solution of Bosentan was prepared by appropriate dilution of standard stock solution and scanned in the spectrum mode from 200 nm to 400 nm. From the spectrum λ_{max} of Bosentan 269 nm was selected for the analysis. The calibration curve was prepared in the concentration range of 2.5-15µg/ml at 269 nm. The calibration curve for Bosentan was plotted in the concentration v/s absorbance and regression equation was calculated.

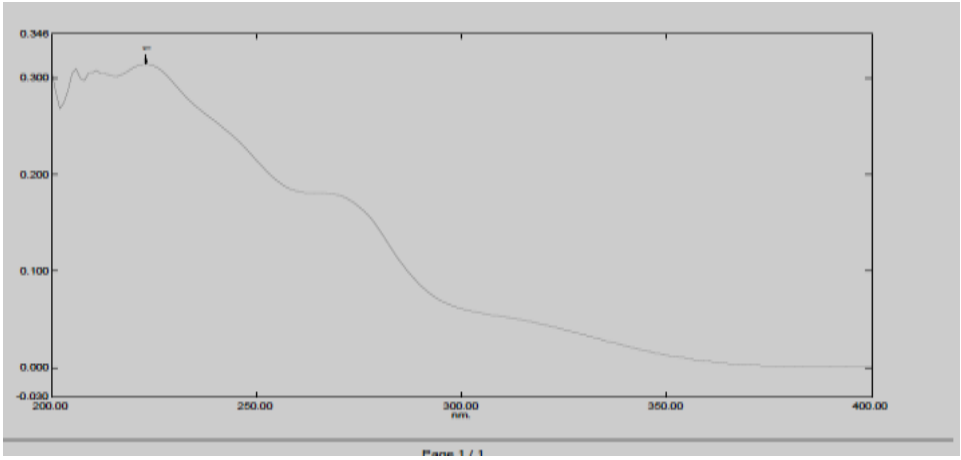

Fig.2: Absorption maxima spectrum of Bosentan

Table 1: Calibration Curve data of Bosentan by Absorption Maxima Method

S.NO	Concentration (µg/ml)	Absorbance
1	2.5	0.18
2	5	0.369
3	7.5	0.501
4	10	0.699
5	12.5	0.827
6	15	1.039

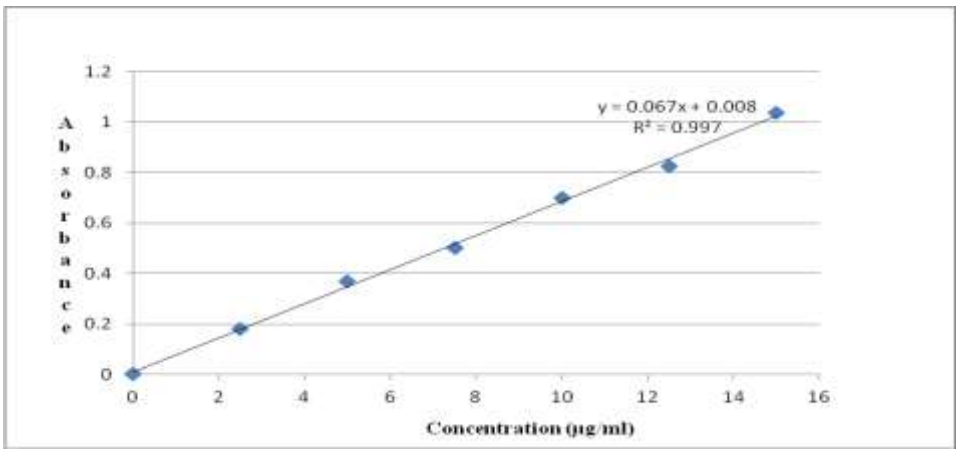

Fig .3: Calibration graph of Bosentan

Method B: Area under Curve method: For the selection of analytical wavelength, 10µg/ml solution of Bosentan was prepared by appropriate dilution of standard stock solution and scanned in the spectrum mode from 200nm to 400 nm. Area under curve (AUC) method involves the calculation of integrated value of absorbance with respect to the wavelength between two selected wavelengths 264 nm- 274 nm. Area calculation processing item calculates the area bound by the curve and the horizontal axis. The horizontal axis is selected

by entering the wavelength range over which the area has to be calculated. The wavelength range is selected on the basis of repeated observations so as to get the linearity between area under curve and concentration. From this regression equation was calculated

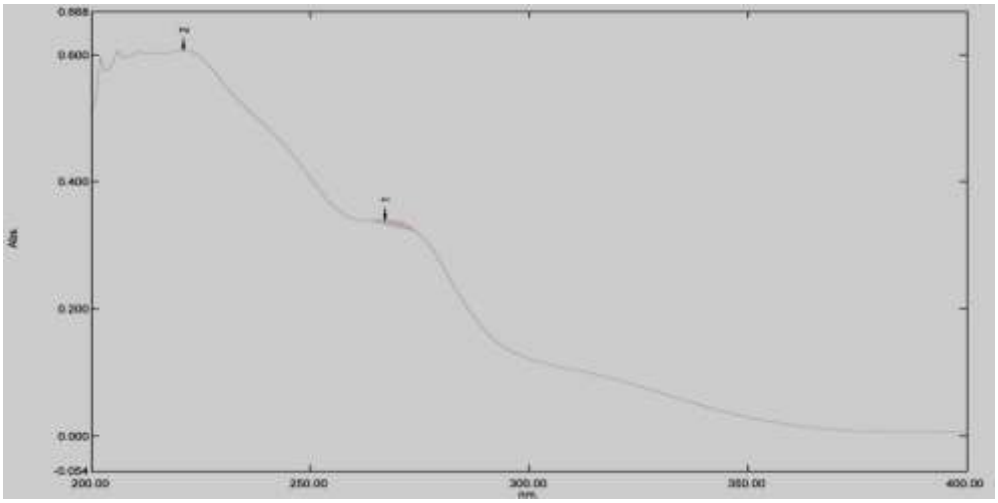

Fig. 4: UV- Spectrum of Bosentan indicating AUC

Table 2: Calibration curve data of Bosentan by using AUC

S.NO	Concentration (µg/ml)	α+β Values
1	2.5	1.852
2	5	3.525
3	7.5	5.243
4	10	6.992
5	12.5	8.516
6	15	10.350

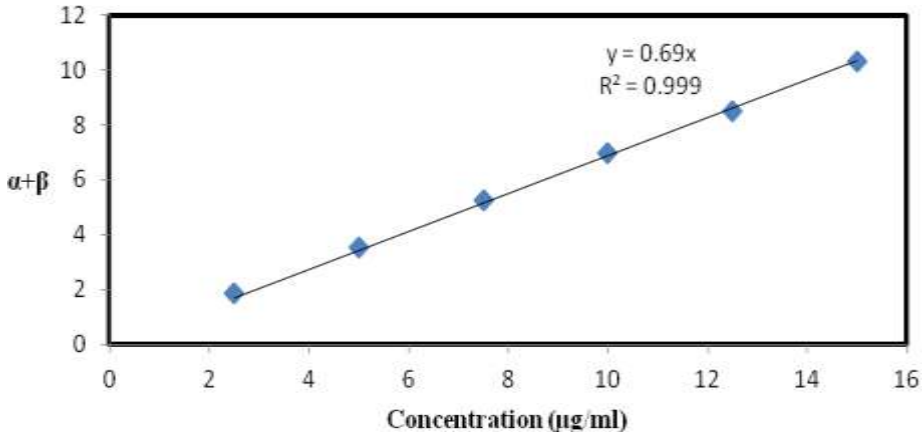

Fig .5: AUC Calibration graph of Bosentan

Estimation of Bosentan in Tablet Formulation

For the estimation of Bosentan in the commercial formulations, twenty tablets each containing 125 mg of Bosentan were weighed and average weight was calculated. The tablets were crushed and powdered in glass mortar. For the analysis of drug, quantity of powder equivalent to 10mg of bosentan was transferred to10 ml volumetric flask and dissolved in 5 ml of methanol. The flask was shaken for 5 min and volume was made up to the mark with methanol to give a solution of 1000 µg/ml. It was filtered with whatmann filter paper no.41. Further dilutions of the stock solution were made up with 0.1N NaoH to get required concentration. In method A, the concentration of bosentan was determined by measuring absorbances of sample solutions at 269 (λ_{max} of bosentan). In method B, the concentration of bosentan was determined by measuring absorbances of sample solutions in wavelength range of 264-274 nm. Results of tablet analysis was shown in table No-3.The assay procedure was repeated six times (n=6).

Table 3: Result of Marketed Formulation Analysis

Proposed methods	Label claim (mg)	Mean	Std. deviation	%RSD
A	125 mg	98.05	0.0632	0.064
B	125 mg	98.07	0.0924	0.094

Method Validation

The methods were validated according to ICH guidelines Q2(R1) to study linearity, accuracy and precision.

Linearity

A linear relationship was found for the two methods between the absorbance and the concentration of bosentan in the range of 2.5-15mcg/ml. The correlation coefficient for these methods A,B were 0.997,0.999 indicating linearity(r^2>0.999). table no.4

Table 4: Linearity studies of the proposed methods

S.NO	PARAMETER	Method A	Method B
1	Linearity (µg/ml)	2.5-15	2.5-15
2	Linearity Equation	Y= 0.067x + 0.008	Y= 0.673X + 0.175
3	Slope ± SD	0.067± 0.00226	0.673± 0.0242
4	Intercept ± SD	0.008± 0.001628	0.175± 0.0002054
5	Correlation coefficient	0.997	0.999

Precision

The precision of the method was expressed in terms of % Relative Standard Deviation (%RSD). The % RSD values for two methods A, B, found to be less than 2 for intra day and inter day precision, the precision results showed good reproducibility. The results were expressed in table 5.

Table 5: Intraday and Interday precision data of Bosentan

Concentration taken((µg/ml)	Intraday precision %RSD (n=3)	Interday precision %RSD (n=3)	
		Day I	Day II
5	0.36	0.85	0.86
7.5	0.68	0.89	0.90

Accuracy

Accuracy is expressed as the closeness of the results from standard samples to that of the actual known amounts. To determine the accuracy of the proposed method, recovery studies were carried out by established at 80,100,120% levels by addition of standard drug of Bosentan to pre-analysed samples. The solutions were suitably diluted in the range and then each of the dilution was observed six times. The percentage recovery of the drug was calculated. The results were shown in the table No.6.

Table - 6: Recovery data of Bosentan.

Concentration taken (µg/ml)	Spiked level (%)	Amount added (mg)	Amount found (mg) (n=6)		% Recovery	
			A	B	A	B
5	80	4	9.10	8.95	102.2	99.33
5	100	5	10.19	10.02	101.9	100.02
5	120	6	10.80	10.95	98.4	99.6

RESULTS AND DISCUSSION

For quatitative estimation of Bosentan in bulk and tablet dosage form two validated methods were proposed for method A, the absorbance maxima was found to be at 269 nm and for method B area under the curve in the range of 264-274 nm were selected for the analysis. The % assay by the two methods were found to be in the range 98.05-98.6% for Bosentan. No interference was observed from the pharmaceutical excipients. The % recovery obtained for absorption maxima and area under the curve was found to be in the range of 100.83%, 99.65%. Hence, the proposed methods were validated in terms of linearity, precision and

accuracy the present work provides an accurate and sensitive method for the analysis of Bosentan in bulk and tablet formulation.

Table 7: summary of optical characteristics and validation parameters

S.NO	Parameter	Method A	Method B
1	λ_{max} (nm)	269 nm	264-274 nm
2	Beer's range (µg/ml)	2.5-15	2.5-15
3	Correlation coefficient(r^2)	0.997±0.0015	0.999±0.00176
4.	Regression equation	Y=0.067x + 0.008	Y=0.673X + 0.175
5.	% Recovery	100.83%	99.65%.

CONCLUSION

The two spectrophotometric methods were developed and validated as per ICH guidelines. The standard deviation and % RSD calculated for the proposed methods are within limits, indicating high degree of precision of the methods. The results of the recovery studies performed indicate the methods to be accurate. The proposed methods were found to be simple, economical, rapid, precise and accurate for the determination of Bosentan in tablet dosage form. Thus, it can be easily and conveniently adopted for routine quality control of tablet analysis.

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