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RESEARCH ARTICLE

PHARMACEUTICAL ANALYSIS

DEVELOPMENT, ESTIMATION AND VALIDATION OF BOSENTAN IN BULK AND IN ITS PHARMACEUTICAL FORMULATION BY UV-VIS SPECTROSCOPIC METHOD.

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ABSTRACT

The present study describes a simple, accurate, precise, specific and highly sensitive method for the determination of bosentan present in pharmaceutical dosage forms. The method is validated for the determination of bosentan in bulk and tablet dosage form. **Bosentan** is a dual endothelin receptor antagonist used in the treatment of pulmonary artery hypertension (PAH). The solvent used was methanol: water (60:40) and the λmax or the absorption maxima of the drug was found to be 270nm. A linear response was observed in the range of 10-90µg/ml with a regression coefficient of 0.9993. The method was then validated for different parameters as per the ICH (International Conference for Harmonization) guidelines. This method can be used for the determination of Bosentan in quality control of formulation without interference of the excipients.

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KEYWORDS

Bosentan, λmax, ICH, UV-VIS spectroscopy

INTRODUCTION

Bosentan is competitive antagonist of endothelin-1 receptor acts on endothelin A and endothelin present smooth mussels of pulmonary blood vessels[1, 2, 3]. Bosentan used in the treatment pulmonary hypertension and digital ulcers in patients with systemic sclerosis .chemically it contain 4-tertbutyl-N-[6-(2-hydroxyethoxy)-5-(2-methoxyphenoxy)-2-(pyrimidin-2-yl) pyrimidin-4-yl] benzene-1-Sulfonamide [2]Molecular weight 551.61. Molecular formula C27H29N5O6S.it is white to yellowish powder soluble in very low in water (1mg/100ml) and highly soluble in (43 mg/100ml) P H 7.5(3, 4). Bosentan available as tablets formulation as tracleer, Lupibose, Bosentas (125 mg, 62.5 mg) literature surveys reveals that bioanlytical methods are developed for analysis of metabolite of bosentan in blood plasma but there is no one RP-HPLC method available for analysis of drug present in formulation[5,6,7,8]

Present work explains that simple accurate and precise spectrophotometric method for the method for estimation of drug present in the pure and its tablets.

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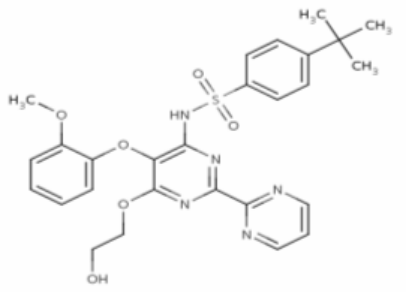


CHEMICAL STUCTURE OF BOSENTAN

MATERIALS AND METHODS

The instrument used for the study was SHIMAZDU UV Visible (UV 1800) spectrophotometer with 1cm matched pair quartz cells. The solvent used was methanol and water (60:40).

METHOD DEVELOPMENT

Solubility Test: Solubility test for the drug Oseltamivir was performed by using various Solvents. The solvents include Water, Methanol, Ethanol, Acetonitrile, and 0.1N Hydrochloric Acid (HCl), 0.1 N Sodium Hydroxide (NaOH) and Chloroform. However, Methanol: water (60:40) was chosen as a solvent for developing the method.

Determination of λmax:

Preparation of Stock Solution: Standard stock solution of Oseltamivir was prepared by dissolving 10mg of Bosentan in 10ml of methanol to produce a concentration of 1000µg/ml. 1ml of this stock solution was taken and then diluted up to 10ml by using Methanol: water (60:40) to produce a concentration of 100µg/ml which is the standard stock solution.

Preparation of Working Standard Solution: From the above stock solution, Working standard solutions are prepared from 10 to 100 ppm and the solutions are scanned in spectrophotometer from 200 nm to 400 nm.All the solutions are having the λmax at 270 nm (fig.1).

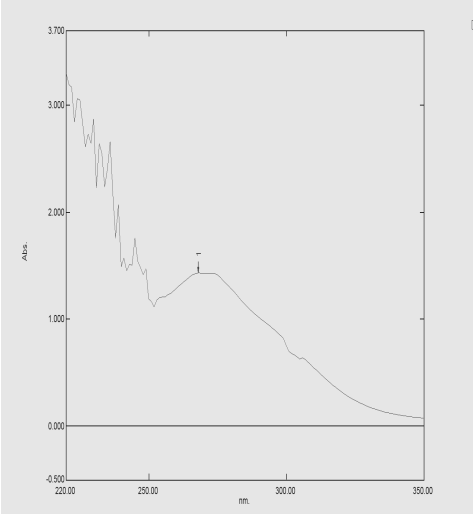
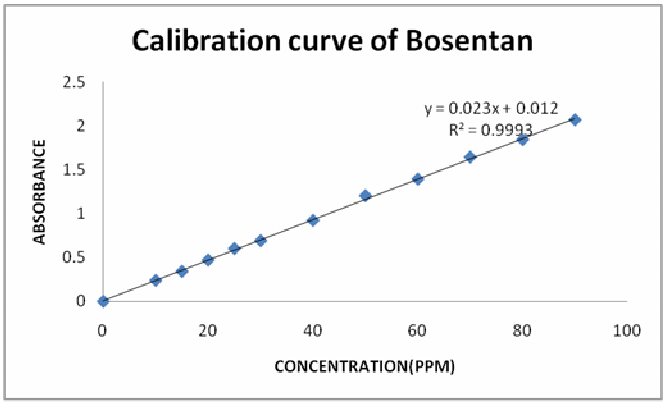


Fig 1
UV Spectrum of Bosentan in methanol: water (60:40)

Preparation of Calibration Curve:
1ml of the 100µg/ml solution was diluted to 10ml by using methanol and water(60:40) to produce 10µg/ml solution. 2ml, 3ml, 4ml and 5ml of 100µg/ml solution were diluted to 10ml using methanol: water (60:40) to produce20µg/ml, 30µg/ml, 40µg/ml, 50µg/ml solutions respectively. Then the construction of calibration curve was done by taking the above prepared solutions of different concentration ranging from 10-90µg/ml. Then, the calibration curve was plotted by taking concentration on x-axis and absorbance on y-axis (in fig.2). The curve showed linearity in the concentration range of 10-90µg/ml. The correlation coefficient (r^2) was found to be 0.9993.

Fig 2
Calibration curve of bosentan.
(Abs. - absorbance, Conc. - concentration)



ASSAY OF BOSENTA TABLETS (62.5 mg)

A quantity of powder equivalent to 50mg of Oseltamivir was taken in a 50ml volumetric flask and it was dissolved and diluted up to the mark with methanol: water(60:40). The resultant solution was ultrasonicated for 5 minutes. The solution was then filtered using Whatmann filter paper No.40. From the filtrate, appropriate dilutions were made in Methanol water (60:40) to obtain the desired concentration (50µg/ml). This solution was then analysed in UV and the result was indicated by % recovery given in table 2.

Table
2 Amount of Bosentan in tablets

Formulation	Labelled amount (mg)	Amount found	%Recovery
Brand I	62.5	62.4775	99.964 ± 0.113

Each value is average of three determinations ± standard deviation.

METHOD VALIDATION

Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics.The method was validated for different parameters like Linearity, Accuracy and Precision.

Linearity: Various aliquots were prepared form the stock solution (100µg/ml) ranging from 10-100µg/ml. The samples were scanned in UV-VIS Spectrophotometer using methanol: water (60:40) as blank. It was found that the selected drug shows linearity between the 10-90µg/ml (table 1).

Table 1
Linearity table of Bosentan in Working Standard

Concentration (µg/ml)	Absorbance
0	0
10	0.238
20	0.341
30	0.47
40	0.601
50	0.69
60	0.921
70	1.202
80	1.391
90	1.642

Accuracy: The accuracy of the method was determined by preparing solutions of Different concentrations that is 80%, 100% and 120% in which the amount of marketed formulation (Bosenta-62.5 mg) was kept constant (10mg) and the amount of pure drug was varied that is 8mg, 10mg and 12mg for 80%, 100% and 120% respectively. The solutions were prepared in triplicates and the accuracy was indicated by % recovery (table 4).

Table 4
Accuracy Readings

Sample ID	Concentration (µg/ml)		%Recovery of Pure drug
	Pure drug	Formulation	
S_1 : 80 %	8	10	99.8
S_2 : 80 %	8	10	98.2
S_3 : 80 %	8	10	100.5
S_4 : 100 %	10	10	98.95
S_5 : 100 %	10	10	99.28
S_6 : 100 %	10	10	99.26
S_7 : 120 %	12	10	99.3
S_8 : 120 %	12	10	99.7
S_9 : 120 %	12	10	99.9

Precision: The precision of the proposed method was ascertained by actual determination of eight replicates of fixed concentration of the drug within the Beer's range and finding out the absorbance by the proposed method. From these absorbances, Mean, Standard deviation, % RSD was calculated. The readings were shown in Table 3**.**

Table 3
Precision readings

Concentrations (µg/ml)	Absorbance	Statistical Analysis
20	0.475	
20	0.471	
20	0.475	Mean = 0.474
20	0.477	
20	0.469	SD = 0.0034
20	0.479	
20	0.475	%RSD = 0.670
20	0.474	

TABLE 5
OPTICAL CHARACTERISTICS

Parameters	VALUE (Methanol: water (60:40)
Absorbance maximum (λ_{max}) in nm	270
Beers law limit (µg/ml)	10-90
Molar Absorptivity (L/Mol/cm)	4.314
Slope	0.0203
Intercept	0.012
Correlation coefficient	0.9993
LOD(µg/ml)	1.892
LOQ(µg/ml)	6.872

RESULTS AND DISCUSSIONS

From the optical characteristics of the proposed method, it was found that Bosentan obeys linearity within the concentration range of 10-100 µg/ml. From the results shown in Table 3, it was found that the % RSD is less than 2, which indicates that the method has good reproducibility. From the results shown in accuracy Table 4, it was found that the percentage recovery values of pure drug from the pre analyzed solution of formulation were in between 99.9-100.5 which indicates that the proposed method is accurate and also reveals that the commonly used excipients and additives in the pharmaceutical formulations were not interfering in the proposed method.

CONCLUSION

The proposed method was simple, sensitive and reliable with good precision and accuracy. The proposed method is specific while estimating the commercial formulations without interference of excipients and other additives. Hence, this method can be used for the routine determination of Bosentan in pure samples and pharmaceutical formulation.

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