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Research Article

Development of a Validated UV Spectrophotometric Method for the Estimation of Ambrisentan in Pure and Marketed Formulations

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Abstract: A simple, accurate, rapid and sensitive UV spectrophotometric method has been developed for the estimation of Ambrisentan (ABS) in bulk as well as in marketed formulations. Ambrisentan is used in the treatment of pulmonary hypertension. It is available in the market in tablet dosage forms with strength 5mg and 10mg. The present research work involves the estimation of Ambrisentan using 0.1 N NaOH as a solvent. An absorption maximum (λ max) was found to be 263.5 nm. It obeys the Beer's law in the concentration range of 10-50µg/ml with a correlation coefficient value of 0.9993. The %RSD was found to be below 2.0 for repeatability, Intra and Inter-day precision indicated that the method was highly precise. The LOD and LOQ were found to be 0.522 & 1.944 µg/ml revealed that the method was sensitive. The percentage recoveries were found to be 98.62 - 100.05 %, indicating the accuracy of the method and absence of interference of the excipients present in the formulation. Validation of the method has been carried out statistically as per ICH guidelines and the results were found to be satisfactory. The proposed method was simple, fast, specific, accurate, precise, rugged and reproducible and hence can be applied for routine quality control analysis of Ambrisentan in pharmaceutical formulations.
Keywords: Ambrisentan, Spectrophotometric method, tablet dosage forms, Validation, ICH guidelines

INTRODUCTION

Ambrisentan, an endothelin receptor antagonist that is selective for the endothelin type-A (ETA) receptor. Chemically it is (+)-(2S)-2-[(4, 6-dimethylpyrimidin-2-yl) oxy]-3-methoxy-3, 3-diphenylpropanoic acid [1,2]. It is used in the treatment of primary pulmonary hypertension ie., high blood pressure in lungs. It has a molecular formula of $C_{22}H_{22}N_2O_4$ and a molecular weight of 378.42. It contains a single chiral center determined to be the (S) configuration. Ambrisentan works by blocking the effects of a substance called endothelin, which is made by the body in increased amounts in patients with PAH. Endothelin causes blood vessels to narrow (constrict). It also causes overgrowth of the muscle in the walls of the blood vessels in the lungs. By blocking the action of endothelin, ambrisentan can reduce blood pressure in the lungs and improve activity level and well-being in PAH patients. Short-term randomized studies demonstrate that ambrisentan improves exercise capacity and delays clinical worsening in PAH patients. Long-term open label trials of the drug showed sustained benefit in exercise capacity [3-4].

The review of literature revealed that few analytical methods have been reported like chiral HPLC [5,6], LC/MS/MS [7] in biological fluids and spectrophotometric method [8] in pharmaceutical dosage form but no simple UV Spectrophotometric method in pharmaceutical dosage forms has been reported so far. Hence author has planned to develop and validate a novel, simple, precise and rapid UV Spectrophotometric method for the estimation of Ambrisentan in pharmaceutical dosage forms.

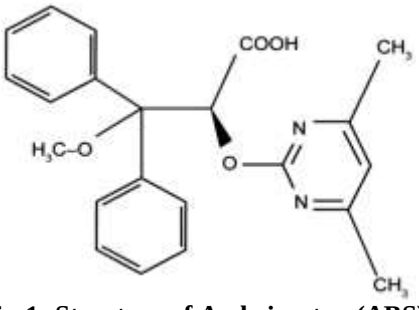

Fig-1: Structure of Ambrisentan (ABS)

MATERIALS AND METHODS

Instrumentation:

ELICO SL 210 UV Double beam Spectrophotometer having Spectra Treats (3.11.1) Software with a pair of 1cm matched quartz cells was employed to measure absorbance of all solutions.

Digital balance (Essae) of 1 mg sensitivity was used for weighing.

Chemicals and Reagents:

Ambrisentan reference standard was received as a gift sample from MSN Laboratories Ltd., Hyderabad. The commercial tablet dosage form of endobloc containing 10mg of Ambrisentan manufactured by Cipla Ltd., Mumbai was procured from Paras distributors, Chennai. Methanol (Merck, Mumbai) and double distilled water (in house laboratory) were used in the following experimental work.

Preparation of standard stock solutions of Ambrisentan (ABS):

Accurately weighed 25 mg of ABS was transferred into a 25 ml volumetric flask, added 15 ml of 0.1 N NaOH to dissolve. The volume was adjusted upto the mark with 0.1 N NaOH to obtain concentration of 1mg/ml. Further 5ml of above stock solution was diluted into a 50 ml volumetric flask with double distilled water to obtain 100 µg/ml working standard concentration. Aliquots were further diluted in double distilled water to obtain linearity solutions of concentration ranging from 10-50µg/ml.

Analysis of Ambrisentan (ABS) in marketed formulations:

20 tablets were weighed and crushed to a fine powder. A quantity of the tablet powder equivalent to 10 mg of ABS was accurately weighed and transferred into a 10 ml volumetric flask, dissolved in 0.1 N NaOH and then made up to the volume with 0.1 N NaOH . The resulting solution was filtered through Whatmann filter paper no. 41. Further dilutions were carried out with double distilled water to obtain a solution of final concentration of 10µg/ml respectively.

Determination of absorption maxima of ABS:

The absorption maxima of ambrisentan (ABS) was measured by scanning solution of concentration of 10µg/ml at wave length range from 200-400nm. The λ_{max} of ABS was found to be 263.5 nm as shown in figure 2. The absorbance of linearity solutions were recorded at the selected wavelength (263.5nm) as shown in figure 3.

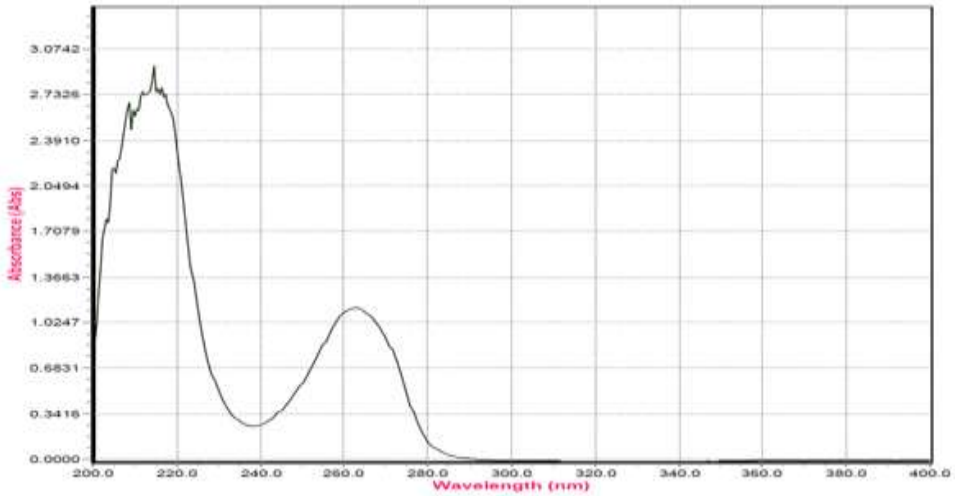

Fig-2: Absorption spectrum of ABS (10µg/ml in 0.1 N NaOH)

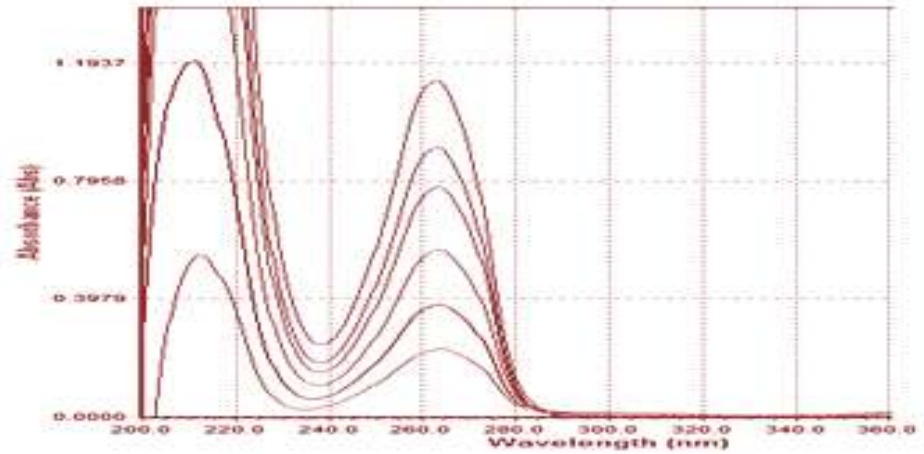

Fig-3: Overlay spectrum of ABS (10-60 µg/ml)

METHOD VALIDATION

The developed method has been validated as per ICH Q2 (R1) guidelines [9] by means of the following parameters:

Linearity:

The linearity was evaluated by analyzing different concentrations of ABS solutions. Appropriate aliquots (1.0-5.0 ml) of ABS working standard solution (100µg/ml) were transferred into the series of 10 ml volumetric flasks and the volume was made up to the mark with double distilled water. All absorbance were measured at 263.5nm against 0.1 N NaOH as blank solvent. A calibration curve was constructed by plotting absorbance versus concentration. The graph was found to be linear (Figure 4) and regression equation was calculated. The results were shown in Table 1.

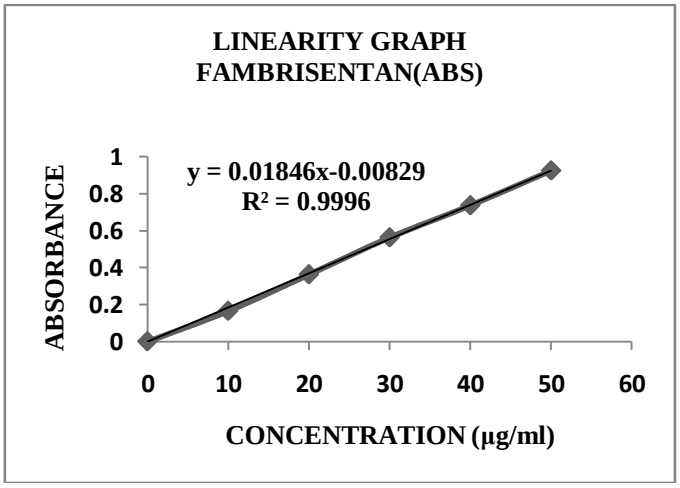

Fig-4: Calibration curve of ABS at 263.5nm

Table -1: Linearity Studies of ABS

S.No.	Parameters	Results (ABS at 263.5 nm)
1	Beer's law limit (µg/ml)	10-50
2	Regression equation	y= 0.01846x-0.00829
3	Correlation coefficient (r^2)	0.9996
4	Intercept (a)	- 0.00829
5	Slope (b)	0.01846

Precision:

The repeatability of the proposed method was ascertained by performing six independent assays of test sample. In intra-day study was carried out on the same day at an interval of two hours whereas inter-day study was carried out on three different days. The results of statistical analysis were given in Table 2.

Table -2: Precision Studies of ABS

Drug	Repeatability % RSD*	Inter-day precision % RSD*	Intra-day Precision % RSD*
Ambrisentan	0.32	0.52	0.53

***Mean of six determinations**

Accuracy:

The accuracy of the proposed method was determined by recovery studies using standard addition method. The % recovery studies of ABS were carried out in triplicate (50, 100 and 150%) by spiking previously analyzed samples of the tablet with standard drug solutions. The results of accuracy studies were shown in Table 3 and assay in Table 4.

Table -3: Accuracy Studies of ABS

Drug	Level of % Recovery	% Recovery ± SD*	% RSD*
ABS	50	98.62±0.24	0.24
	100	100.05±0.20	0.21
	150	99.77±0.280	0.28

***Mean of three determinations**

Table -4: Assay of Ambrisentan (ABS) in Formulation

Drug	Labeled Amount (mg)	Amount found (mg)	% Assay
ABS	10	9.927	99.27%

The optical characteristics such as Beer's law limit, molar absorptivity, Sandell's sensitivity, Correlation coefficient, slope and intercept, % Relative Standard Deviation (Precision) , % Range of error (at 0.05 and 0.01 confidence limits) were calculated and are summarized in Table 5.

Table -5: Optical characteristics and Precision of proposed UV Spectrophotometric method for Ambrisentan (ABS)

S.No.	Parameter	Results
1	λ max	263.5 nm
2	Beer's law limit (µg/ml)	10-50
3	Apparent Molar absorptivity (L mole^{-}	0.708 x 10 ^{4}
4	Correlation coefficient (r)	0.9996
	Slope (b)	0.01846
	Intercept (a)	- 0.00829
5	Regression equation (Y = a + bC)	Y=0.01846x-
6	Repeatability (% RSD)*	0.32
7	Sandell's sensitivity (µg cm^{-2} / 0.001	0.060
8	Limit of Detection (LOD)	0.522 µg/ml
9	Limit of Quantitation (LOQ)	1.944 µg/ml

***Mean of six determinations**

RESULTS AND DISCUSSION

In this proposed method the absorbance of ABS were measured against 0.1 N NaOH as a solvent blank at 263.5nm. ABS obeyed Beer-Lambert's law in the concentration range of 10-50µg/ml with correlation coefficient (r^2) of 0.9996. % RSD for inter-day and intra-day precision were found to be 0.52 and 0.53 respectively. % RSD for repeatability was found to be 0.32 for ABS. As the % RSD values of precision studies were found to be below 2.0 % indicated highly precise method. % Recovery values of ABS were found to be 98.62-100.05% respectively and it indicates that the proposed method was accurate. Tablet dosage form was analyzed and the result of assay of the drug was in good agreement with the label claim of formulation as indicated by % assay with 99.27% for ABS. All the results were found to be within the limits and therefore the proposed method was found to be free from interferences due to excipients in the tablet dosage form.

CONCLUSION

A simple, sensitive, rapid and economic UV spectrophotometric method was developed and validated for the assay of Ambrisentan (ABS) in tablet formulation. This method produced high recoveries with good linearity and precision. It can be concluded that the proposed method is a good approach for obtaining reliable results and found to be suitable for the routine analysis of Ambrisentan (ABS) in tablet formulation.

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