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RP-HPLC-PDA method for the analysis of ambrisentan in bulk drug and pharmaceutical dosage forms

Balakrishna M, Aziz Unnisa*, Prabhakar Reddy and Suma CH.

Department of Pharmaceutical Analysis, KVSR Siddhartha College of Pharmaceutical Sciences, Vijayawada, Andhra Pradesh, India.

* **Corresponding Author:** E-Mail: khushiazeez@yahoo.co.in

ABSTRACT

The aim of the present work was to develop and validate a simple, efficient and economical method for the analysis of Ambrisentan in bulk drug and pharmaceutical dosage forms by reverse phase high pressure liquid chromatography. A Phenomenex C_{18} reverse phase column (250 x 4.6mm, 5µm) with mobile phase containing ortho phosphoric acid (0.05%v/v): methanol (20:80%v/v) was used at isocratic mode and eluents were monitored at 262nm. The retention time of Ambrisentan was 5.8min. The method showed a good linearity in the concentration range of 5-25µg/mL with a correlation coefficient of 0.999. The validation characteristics included specificity, linearity, limit of detection, limit of quantification, precision, robustness and stability. Validation acceptance criteria were met in all cases. The percent recoveries ranged between 99.3-101.3, RSD < 2%. The method could be successfully used for the analysis of Ambrisentan in bulk drug and pharmaceutical dosage forms.

Keywords: Ambrisentan. RP-HPLC. PDA Detection. Method development and Validation.

1. INTRODUCTION

Ambrisentan (AMB) is anti-hypertensive drug. Chemically it is (2*S*)-2-[(4, 6-dimethylpyrimidin-2-yl) oxy]-3-methoxy- 3, 3-diphenylpropanoic acid and used in the treatment of pulmonary hypertension. It functions as an endothelin receptor antagonist, and is selective for the type A endothelin receptor (ET_A) [1-3].

Various analytical methods have been reported in the literature for quantitative determination of AMB using enantio selective liquid chromatography[4], Stability indicating RP-HPLC assay method and its related substances[5], Spectrophotometry[6-7] and LC-MS[8]. However, the so far reported HPLC methods for the estimation of AMB used high percent of organic solvents. Literature survey reveals that there were no validated RP-HPLC/PDA methods reported for the estimation of AMB in bulk drug and pharmaceutical dosage forms.

Hence, the present investigation was aimed at developing a validated RP-HPLC-PDA method for the analysis of AMB in bulk drug and pharmaceutical dosage forms which is simple, precise and economical.

2. MATERIAL AND METHODS

2.1. Chemicals

AMB was gift sample from Cipla India Limited. Ortho phosphoric acid, water and methanol were purchased from E. Merck, Mumbai, India. All the solvents and reagents were of HPLC grade. Endobloc tablet containing Ambrisentan 10mg (manufactured by Cipla India Limited) was commercially purchased.

2.2. Equipment

A Shimadzu Prominence HPLC system provided with DGU-20A3 degasser, LC-20AD binary pumps, SIL-20AHT auto sampler, and SPD-M20A PDA detector was used. Data acquisition was carried out using LC solutions software. The chromatographic analysis was performed on Phenomenex C_{18}- RP column (250 × 4.6mm,5µm).

2.3. Chromatographic Conditions

Mobile phase consisting of Ortho phosphoric acid (0.05%v/v): methanol (20:80%v/v) was used in isocratic mode and the mobile phase was filtered through nylon disc filter of 0.45µm (Millipore) and sonicated for 3 min before use. The flow rate was 1 mL/min and the injection volume was 10µL. PDA detection was

performed at 262nm and the separation was achieved at ambient temperature.

2.4. Preparation of stock and standard solutions

The stock solution of AMB strength 1mg/mL was prepared by dissolving 10mg of drug in methanol and volume was adjusted to the mark with the same. An appropriate volume of the stock solution was then further diluted with ortho phosphoric acid (0.05%v/v) to get the required concentrations of standard solutions at a concentration range of 5-25µg/mL.

2.5. Validation of the HPLC method

The proposed method was validated as per ICH guidelines[9].

2.5.1. Linearity

A linear relationship was evaluated across the range of the analytical procedure with a minimum of five concentrations. A series of standard dilutions of AMB were prepared over a concentration range of 5-25µg/mL (5, 10, 15, 20, 25µg/mL) from stock solution and injected in triplicate. Linearity is evaluated by a plot of peak areas as a function of analyte concentration, and the test results were evaluated by appropriate statistical methods where by slope, intercept, and regression (R^2) correlation coefficients (R) were calculated.

2.5.2. Precision

Precision is the measure of closeness of the data values to each other for a number of measurements under the same analytical conditions. Repeatability was assessed by using a minimum of six determinations at 100% of the test concentration. The standard deviation and the relative standard deviation were reported for precision. Less than 2% RSD for peak areas indicates the precision of the developed method.

2.5.3. Specificity

The specificity of the method was determined by comparing the chromatograms obtained from the drug substance with that obtained from the tablet solution. The overlay of diluent, placebo, standard and sample were presented. The retention times of drug substance and the drug product were observed. Absence of interference of excipients in the tablet indicates the specificity of the proposed method.

2.5.4. Accuracy

Accuracy was established across the specified range of the analytical procedure. To ascertain the accuracy of the proposed method recovery studies were performed by the standard addition method by spiking 80%, 100%, 120% of the known quantities of standard within the range of linearity to the synthetic solution of drug product (10µg/mL) and these solutions were analyzed by developed method in triplicate. The % recovery and the %RSD were calculated at each level of addition.

2.5.5. Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ were calculated based on calibration curves. They were expressed as LOD = (3.3 ×σ)/m; LOQ= (10.0×σ)/m (Where, σ is the standard deviation of the y-intercepts of the three regression lines and m is mean of the slopes of the three calibration curves).

2.5.6. Robustness

To determine the robustness of the method developed, the experimental conditions were deliberately altered and the chromatographic parameters viz., tailing factor, no. of theoretical plates and % assay were recorded. The flow rate of the mobile phase was 1mL/min. To study the effect of change in mobile phase (±2% v/v), effect of flow rate, the flow rate was changed by 10%, and the effect of wavelength was studied by changing wavelength by ±2nm.

2.5.7. System suitability

System suitability was carried out by injecting a standard concentration at different injection volumes in the range of 10-50µL. The system suitability test parameters were noted and % RSD was calculated.

2.5.8. Assay

Twenty tablets were weighed and finely powdered, the powder equivalent to 10mg of AMB was accurately weighed and transferred into a 10mL volumetric flask, dissolved in methanol, vortexed for 5min and volume was adjusted up to the mark with methanol. The above solution was centrifuged and then filtered using Nylon disposable syringe filter (13 mm, 0.45µm). An aliquot of filtrate was diluted with water and analyzed in triplicate. The amount present in the each tablet was quantified by comparing the area of standard AMB with that of the sample.

3. RESULTS AND DISCUSSION

Literature survey reveals only a single RP-HPLC method reported for the estimation of AMB in bulk drug and pharmaceutical dosage forms.

Hence, the present investigation was aimed at developing a validated RP-HPLC-PDA method for the analysis of AMB in bulk drug and pharmaceutical dosage forms which is simple, precise and more economical.

3.1. Method Development

Mobile phase optimization was initially carried with Inertsil ODS C_{18} column (250 x 4.6 mm×5µm) using water and methanol as mobile phase in different ratios. AMB eluted at 8.4min using 1 mL/min flow rate, the peak obtained was not symmetrical. In other trial ortho phosphoric acid and methanol (40:60 %v/v) was used and the peak observed had good symmetry, but the retention time was high. A few more trials were performed by varying the mobile phase composition to lower the retention time. Using the mobile phase composition of orthophosphoric acid and methanol (20:80%v/v) and the flow rate of 1mL/min the peak was obtained at the retention time of 5.8 min with good symmetry, using the mobile phase composition of orthophosphoric acid and methanol (20:80%v/v) and the flow rate of 1mL/min. A standard chromatogram of AMB under these conditions is shown in figure 1.

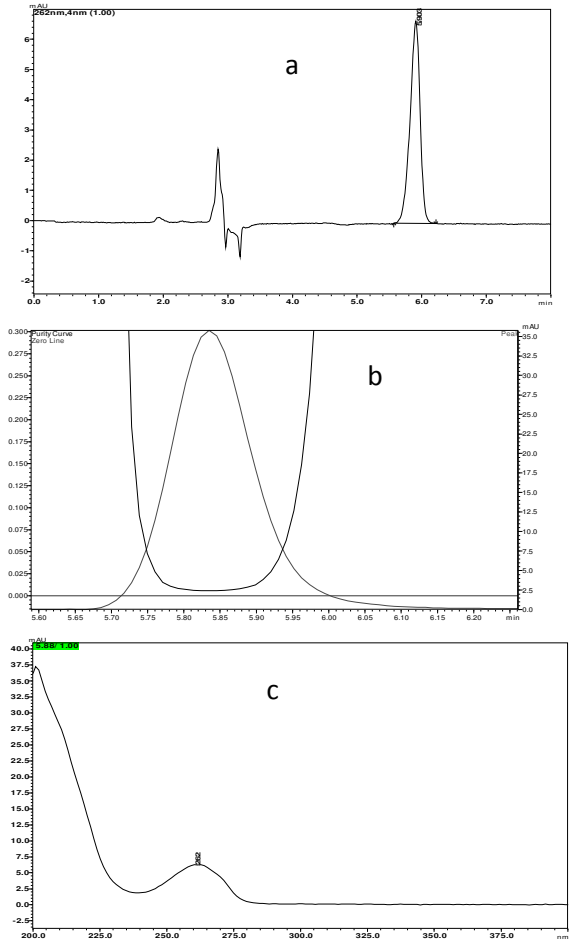


Figure - 1: Standard Chromatogram (a) of AMB (10 µg/mL) with Peak purity index (b) and UV spectrum (c).

For quantitative analytical purpose wavelength was set at 262 nm, which provided better reproducibility with minimum or no interference. The method was validated as per ICH guidelines. The peak purity index was found to be greater than 0.9999 and this indicating peak purity of the drug sample used in the analysis and shown in figure 1 along with UV spectra.

3.2 Method validation

The method has been validated as per ICH-Guidelines for following parameters.

3.2.1. Linearity

The range of reliable quantification was set at the concentrations of 5-25µg/mL of AMB. This range was selected based on 80-120% of the standard concentration used for accuracy and were analyzed in triplicate. Peak areas and concentrations were subjected to least square regression analysis to calculate regression equation. The correlation coefficient (R) was found to be 0.999 indicating a linear response over the range used. The data from the calibration curve was given in table 1.

3.2.2. Precision

Precision studies were carried out in terms of repeatability. Repeatability of standard application was assessed by using six replicates of concentration at 10µg/mL level and the data was given in table 1. The % RSD was found to be below 2 for peak areas, this shows the closeness of the data values to each other, indicating the precision of the method and shown in figure 2.

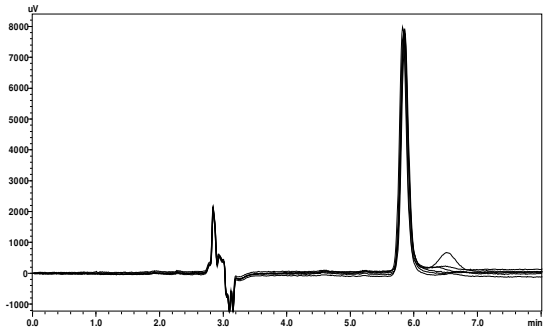


Figure - 2: Overlay of method precision chromatograms of sample solution of AMB (10µg/mL).

3.2.3. Accuracy

Accuracy of the proposed method was ascertained by performing recovery studies by standard addition method by spiking the known quantities of standard at 80%, 100%, 120% to the drug product solution of 10µg/mL and these solutions were analyzed in triplicate in each level of addition. The %RSD and the %Recovery were within the acceptable limit in all cases. It is evident from the results of accuracy study given in table 1, that the proposed method enables very accurate quantitative estimation of AMB.

Table - 1: Linearity, Precision and Accuracy data of AMB	
Linearity (n=3)	Range 5-25 µg/mL y =41221x-99913 R=0.999 R^2=0.999
Precision (n=6)	**Average peak area of the standard sample (%RSD)**
	4586242(1.2%)
Accuracy (n=3) **Level of addition**	**Mean Percent Recovery (%RSD)**
80%	101.13(1.404%)
100%	99.36(0.058%)
120%	99.76(1.331%)

3.2.4. Specificity

The specificity of the method was established by injecting the solutions of diluent, placebo, standard, sample (Formulation) individually to examine any interference, from the overlay of chromatograms as shown in figure 3 and the 3D plots of placebo and formulation in figure 4. It can be inferred that there were no co-eluting peaks at the retention time of AMB, this shows that peak of analyte was pure and the excipients in the formulation did not interfere with the analysis and the peak purity indices for sample and standard was found to be greater than 0.9999 and this confirms specificity of the method.

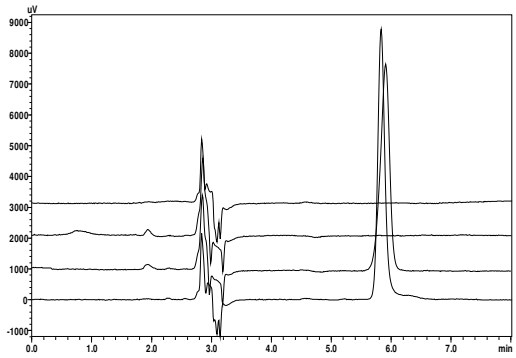
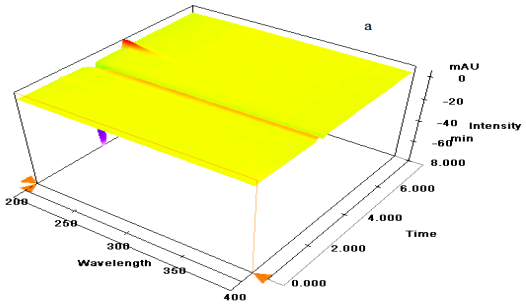


Figure - 3: Overlay of the Sample (a), Standard (b), Placebo (c) and Diluent (d) chromatograms



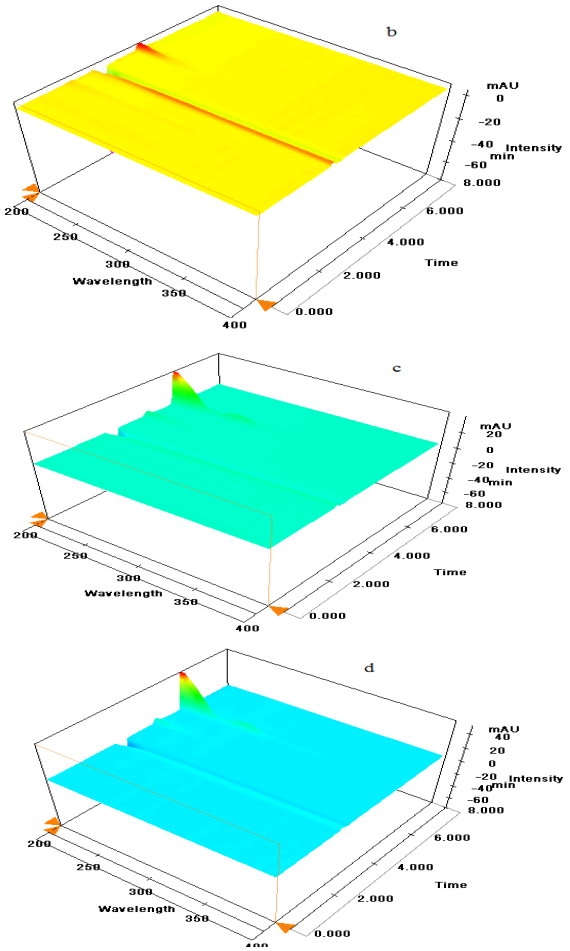


Figure - 4: 3D plots of Placebo (a), Diluent (b), Sample (c) and Standard (d).

3.2.5. Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ were determined based on statistical calculation from the calibration curves, where LOD = (3.3 ×σ)/m; LOQ= (10.0×σ)/m (σ is the standard deviation of the y-intercepts of the three regression lines and m is mean of the slopes of the three calibration curves).The limit of detection for AMB was found to be 0.404µg/mL, the drug peak could be detected without any base line disturbances at this concentration. The limit of quantification for AMB was found to be 1.225 µg/mL.

3.2.6. Robustness

As part of the robustness, a deliberate change in the flow rate, mobile phase and wavelength was made to evaluate the impact on the method. Retention times were significantly changed with flow rate, mobile phase and no change in the retention time was observed in wavelength change. Percent assay values were also estimated under these changed conditions and the results were given in table 2. The parameters like tailing factor, theoretical plate number and assay were not changed and were within the limits.

Table - 2: Robustness data

Chromatographic parameter		Retention time (min)	Theoretical plates (#)	Tailing factor (T_f)	%Assay
Flow rate	0.9	5.93	11111	1.37	101.5
	1.0	5.90	10139	1.36	100.5
	1.1	5.80	9253	1.34	100.0
Mobile phase	78-22	5.85	10586	1.31	99.0
	80-20	5.93	10139	1.37	100.0
	82-18	5.83	10120	1.34	99.5
Wave length (nm)	260	5.93	10136	1.37	99.8
	262	5.93	10139	1.37	100.1
	264	5.93	10142	1.37	99.7

These results indicated that the method is robust in terms of changed flow rate, mobile phase and wavelength.

3.2.7. System suitability

System suitability testing is an integral part of the analytical procedure. System suitability studies were carried out by injecting five times a 10µg/mL standard concentration of AMB at different injection volumes ranging from 10µL to 50µL. The %RSD values for system suitability test parameters like retention time [R_t = 5.92 (0.53)], tailing factor [T_f = 0.87 (1.24)] and theoretical plate number 6014 (1.3) were less than 2 indicating the present conditions were suitable for the analysis of AMB in tablets.

3.2.8. Assay

Assay of AMB tablets was performed by the proposed method and the % assay of the formulation was calculated as an average of 3 determinations, which was about 100.69 ± 1. These results indicate that the present HPLC method can be successfully used for the assay of AMB in bulk drug and pharmaceutical dosage forms.

3.2.9. Stability of the stock solution

The stability of the stock solution was determined by analyzing the samples under refrigeration (8±1°C) at different time intervals up to 48hrs. The % variation in assay values at different time intervals were found to be less than 2 of the initial zero time interval solution, thus indicating that the solutions were stable for a period of 48hrs when stored at 8°C.

4. CONCLUSION

In this work, a simple and efficient RP-HPLC-PDA method was developed for the analysis of AMB in bulk drug and pharmaceutical dosage forms. The method was validated fully as per International Conference on Harmonisation (ICH) Guidelines, and found to be applicable for routine quality control analysis for the estimation of AMB in tablets using isocratic binary mode of elution. The results of linearity, precision, accuracy and specificity, proved to be with in the limits. The method provides selective quantification of AMB without interference from diluents and placebo. Therefore, this method can be employed in quality control to estimate the amount of AMB in bulk drug and pharmaceutical dosage forms.

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