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Development of HPLC method for estimation of Ambrisentan from Immediate release tablets.

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ABSTRACT

The aim of the present work was to develop and validate a simple and efficient method for the analysis of Ambrisentan in pharmaceutical dosage forms by reverse phase high pressure liquid chromatography. A stainless steel column 150 mm long, 4.6 mm internal diameter filled with octasilyl silica chemically bonded with silica gel particles of 5 µm diameter was used for elution. The retention time of Ambrisentan was 4.451 min. The method showed a good linearity in the concentration range of 12.5-250 µg/mL with a correlation coefficient of 1.000. 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 method could be successfully used for the analysis of Ambrisentan in pharmaceutical dosage forms.

Keywords: Ambrisentan, Accuracy, Precision, Linearity, Mobile Phase and Validation

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INTRODUCTION

Ambrisentan (AMB) is anti-hypertensive drug used in the treatment of pulmonary hypertension. It functions as an endothelin receptor antagonist, and is selective for the type A endothelin receptor (ETA) [1-3]. Literature survey reveals that there are very few HPLC, UV methods were available[4-8]. Hence RP-HPLC method for estimation of Ambrisentan was developed. Hence, the present investigation was aimed at developing a validated RP-HPLC method for the analysis of AMB in pharmaceutical dosage forms which is simple, precise and economical.

MATERIALS AND METHOD

Chemicals

Ambrisentan was obtained as gift sample from Glenmark Pharmaceuticals.Potassium Dihydrogen Phosphate, Acetonitrile, Orthophosphoric acid and methanol were purchased from Merck.

Instrumentation

A High Performance Liquid Chromatographic system with gradient/isocratic elution capability, a Spectrophotometer UV detector and an auto sampler with a stainless steel column 150 mm long, 4.6 mm internal diameter filled with octasilyl silica chemically bonded with silica gel particles of 5 µm diameter.

Preparation of mobile phase

Buffer preparation: Prepare 20mM Potassium dihydrogen phosphate in water. Adjust with dilute Phosphoric acid to pH of 2.5

Mobile phase: Mix Buffer and Methanol in the ratio of 35:65 v/v and degas.

Diluent: Mix Water and Acetonitrile in the ratio of 50:50 v/v.

Chromatographic conditions:

Column	C18-150x4.6 mm, 5µmor equivalent.
Flow rate	: 1.0 mL/min
Detection	: UV, 260 nm
Injection Volume	: 20 µL
Data acquisition time	: 8 minutes
Column temperature	: 35°C
Pump mode	: Isocratic

Preparation of solutions:

Blank Preparation: Use diluent as blank solution.

Standard solution: Prepare a solution containing 0.1mg/mL of Ambrisentan in diluent.

Weigh accurately 25.0 mg of Ambrisentan working standard into a 50 mL clean, dry volumetric flask. Add 30 mL of diluent and sonicate to dissolve. Make up to volume with diluent and mix. Dilute 5 mL to 25 mL with diluent and mix. Label this standard solution as STD-I. Prepare the standard solution in duplicate and label as STD-II.

Sample solution: Prepare a solution containing 0.1mg/mL of Ambrisentan in diluent.

Weigh and transfer 10 tablets into 100 mL clean, dry volumetric flask. Add about 70 mL of diluent and sonicate for 15 minutes with intermittent shaking. Make up to volume with diluent and mix. Centrifuge the sample for 5 minutes at 2500 rpm. Filter through 0.45µm syringe filter by discarding first 5 mL of filtrate. Dilute 5 mL to 25 mL with diluent and mix.

Evaluation of System Suitability:

Inject the blank solution into the liquid chromatographic system and record the chromatogram. Inject the STD-I solution, five times into the liquid chromatographic system and record the chromatograms and evaluate the system suitability. Symmetry factor should not be more than 2.0 for the Ambrisentan peak from the standard chromatogram.

% RSD for Ambrisentan peak areas of five injections from STD-I should not be more than 2.0.

Inject STD-II solution in duplicate into the liquid chromatographic system and record the chromatogram. Calculate the similarity factor between two standard preparations. The similarity factor between two standard preparations should be between 0.98 to 1.02

Calculation of similarity factor:

$$\text{Similarity factor} = \frac{\text{Average area of STD-I}}{\text{Average area of STD-II}} \times \frac{\text{Weight of STD-II}}{\text{Weight of STD-I}}$$

Procedure:

Inject the sample solutions into the liquid chromatographic system and record the chromatogram. Retention time of Ambrisentan is about 4.5 minutes.

Calculation:

$$\%\text{Label claim} = \frac{At}{As} \times \frac{Ws}{50} \times \frac{5}{25} \times \frac{100}{W_t} \times \frac{25}{5} \times P \frac{AW}{LC}$$

Where,

At = Area of peak corresponding to Ambrisentan in sample solution chromatogram.

As = Average area of peak corresponding to Ambrisentan in STD-I chromatograms

W_s = Weight of Ambrisentan working standard used for STD-I in mg

P = % Assay of Ambrisentan working standard on as is basis

W_t= Weight of sample in mg

AW = Average weight of the tablets in mg

LC = Label claim of Ambrisentan film-coated tablets in mg

VALIDATION OF THE HPLC METHOD

The proposed method was validated as per ICH guidelines

Linearity:

A series of standard dilutions of Ambrisentan were prepared from stock solution. 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 (R2) correlation coefficients (R) were calculated.

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.

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 chromatograms of diluents, standard and sample were shown.

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 50%, 100%, and 150% of the known quantities of standard.

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.

System suitability

System suitability was carried out by injecting a standard concentration at different injection volumes. The system suitability test parameters were noted and % RSD was calculated.

RESULTS AND DISCUSSION

SPECIFICITY

Inference

The blank solution, placebo solution, standard solution, impurity solutions, sample solutions and Impurity spiked sample solutions are analyzed by HPLC system and checked for interference. There is no interference peak was observed due to blank, placebo and known Impurity at the retention time of Ambrisentan.

Acceptance criteria

No significant Interference of blank, placebo and known impurities should be observed at the retention time of analyte peak.

Peak purity should pass for analyte.

Table 1: Interference table for blank, placebo, standard and assay sample

S.No	Sample	Retention time (min)	Purity angle	Purity threshold	Peak Purity
1	Blank	-	-	-	-
2	Placebo solution	-	-	-	-
3	Standard solution	4.451	0.424	1.009	Pass
4	Assay sample	4.451	0.387	1.002	Pass

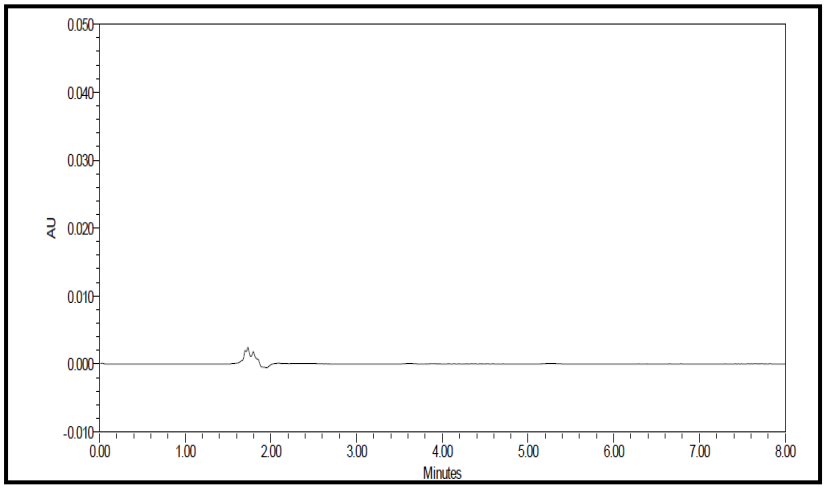

Figure 1: Blank solution – Chromatogram

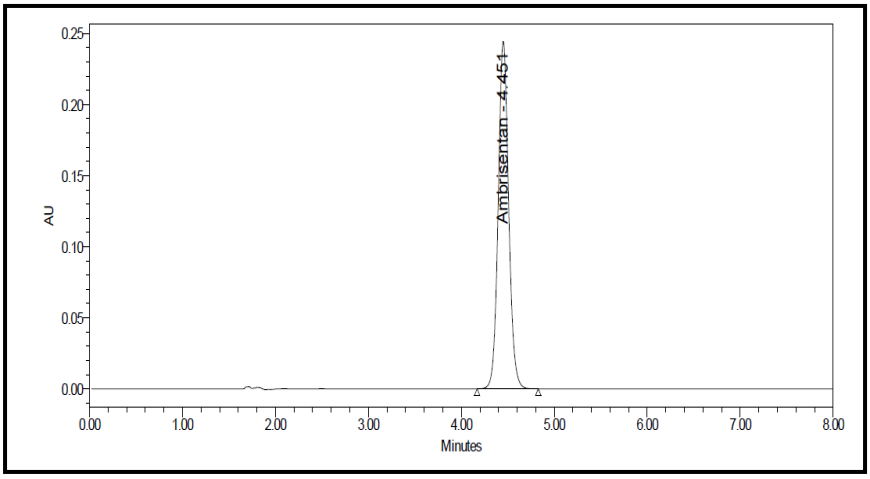

Figure 2: Standard Solution – Chromatogram

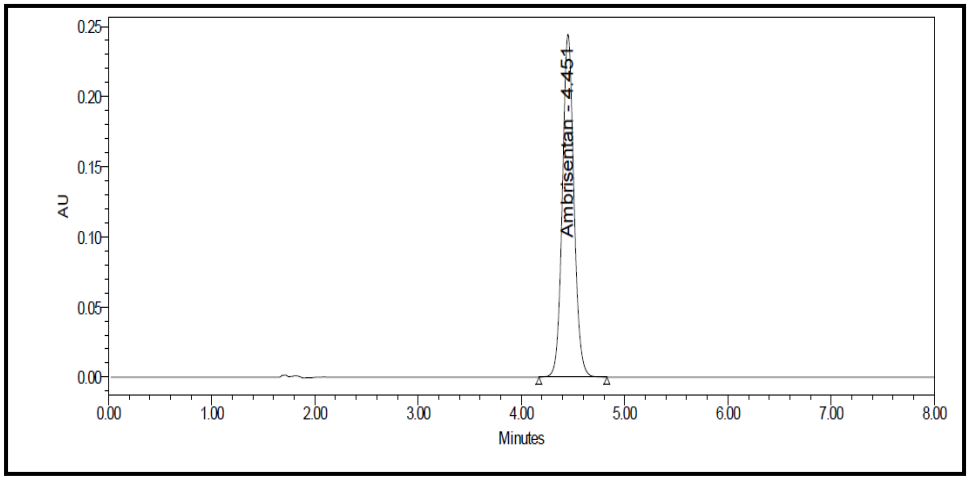

Figure 3: Standard solution – Peak purity

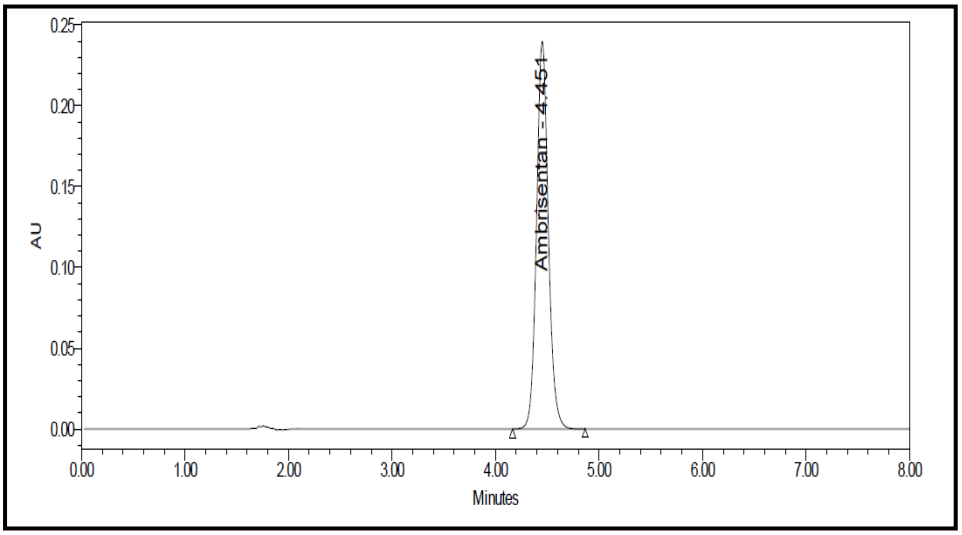

Figure 4: Chromatogram of Assay sample solution

Linearity

The linearity of response for Ambrisentan is determined at different concentration levels as shown in the following table and enclosed graphically. The results are calculated from linearity graph using the linearity equation: Y = BX + A (Where as B is the slope and A is the intercept)

Table 2: Linearity Table

S. No.	Concentration (mg/mL)	Area
1	0.0125	228965
2	0.0800	1465415
3	0.1000	1824748
4	0.1200	2199028
5	0.250	4545745
Correlation Coefficient [R]		1.0000
Regression Coefficient [R^2]		1.0000

Acceptance criteria

Correlation coefficient (R) should not be less than 0.99 within the specified range.

Regression coefficient should (R^2) be not less than 0.98.

The correlation coefficient is 1.0000 and the regression coefficient is 1.0000. The regression line of analysis shows linear relationship between concentration and response of Ambrisentan.

Precision

Repeatability

Injected six preparations of Ambrisentan Tablets into HPLC system. The results of analysis are shown below.

Table 3: Precision Table

Preparation No	% Label claim
1	100.4
2	100.8
3	100.7
4	100.8
5	99.9
6	100.5
Mean	100.6
Std Dev	0.34
% RSD	0.34

Acceptance criteria: % RSD for replicate analysis should not be more than 2.0.

The result indicates of precision of analytical method for the assay of Ambrisentan in Ambrisentan Tablets.

Accuracy for Assay

A known amount of analyte, both above and below the normal levels expected in the sample spiked with placebo and analyzed by the proposed HPLC method and the results are shown below

Table 4: Accuracy for assay

Level	Theoretical Concentration (mg/mL)	Experimental Concentration (mg/ml)	% Recovery
50%-T1	0.050	0.051	102.5
50%-T2	0.050	0.051	102.8
50%-T3	0.050	0.051	102.6
100%-T1	0.100	0.102	102.5
100%-T2	0.100	0.102	102.2
100%-T3	0.100	0.102	102.3
150%-T1	0.150	0.153	102.5
150%-T2	0.150	0.154	102.8
150%-T3	0.150	0.153	102.3
Mean			102.5
Standard deviation			0.21
% RSD			0.21
Minimum			102.2
Maximum			102.8

Table 5: Precision and Accuracy at 50% and 150%.

	Precision and Accuracy at 50% Level			Precision and Accuracy at 150% level		
S.No	**Theoretical Concentration (mg/mL)**	**Experimental Concentration (mg/ml)**	**% Recovery**	**Theoretical Concentration (mg/mL)**	**Experimental Concentration (mg/mL)**	**% Recovery**
1	0.050	0.0513	102.5	0.150	0.153	102.5
2	0.050	0.0514	102.8	0.150	0.154	102.8
3	0.050	0.0513	102.6	0.150	0.153	102.3
4	0.050	0.0515	102.9	0.150	0.154	102.6
5	0.050	0.0515	103.0	0.150	0.153	102.5
6	0.050	0.0516	102.9	0.150	0.154	102.6
Mean			102.8	Mean		102.6
Standard deviation			0.19	Standard deviation		0.16
% RSD			0.19	% RSD		0.16
Minimum			102.5	Minimum		102.3
Maximum			103.0	Maximum		102.8

Acceptance criteria:

% Recovery of analyte should be 97 to 103 within specified Range.

The % Recovery was found in between 97 to 103 for all accuracy level. The results indicate the precision and accuracy of analytical method is good with in the specified range.

Robustness

To establish the robustness of the HPLC method employed for analysis of assay of Ambrisentan in Ambrisentan Tablets, the method was challenged for various parameters like stability of analytical solutions, change in wavelength, effect of mobile phase flow, column oven temperature, mobile phase composition, filter interference and filter saturation volume.

Solution Stability

To establish the stability of analytical solutions of the proposed analytical method employed for analysis of Ambrisentan in Ambrisentan Tablets, stability of analytical solutions was evaluated during method validation. The observations in different conditions are tabulated below.

Table 6: Solution stability

Parameter	**Limit**	**Initial**	**On Bench Top**		**In Refrigerator**	
			48 hours	**72 hours**	**48 hours**	**72hours**
Similarity Factor with Fresh standard	0.98 to 1.02	0.99	1.00	0.99	1.00	0.99
% Label claim	95.0 to 105.0	100.8	102.1	101.5	103.1	101.2

Analytical solutions were stable up to 72 Hours on bench top and in Refrigerator.

Table 7: Effect of change of wavelength and Flow rate.

Details	Limit	Change of wavelength			Change of Flow rate		
		Initial (260nm)	Change-1 (258nm)	Change-2 (262nm)	Initial (1.0 ml/min)	Change-1 (0.8 ml/min)	Change-2 (1.2 ml/min)
% Label claim	95.0 to 105.0	102.0	101.0	102.0	101.2	101.5	100.2
Symmetry Factor	NMT 2.0	0.96	1.08	1.08	0.96	1.12	1.07
% RSD	NMT 2.0	0.28	0.11	0.11	0.28	0.15	0.08
Similarity Factor	0.98 to 1.02	1.00	1.00	1.00	1.00	1.00	1.00

Table 8: Effect of change of temperature and mobile phase.

Details	Limit	Change of column temperature			Change of Mobile Phase		
		Initial (35°C)	Change-1 (30°C)	Change-2 (40°C)	Initial (buffer: Methanol 30:60)	Change-1 (buffer: Methanol 30:70)	Change-2 (buffer: Methanol 40:60)
% Label claim	95.0 to 105.0	100.6	100.8	102.5	101.2	101.9	100.9
Symmetry Factor	NMT 2.0	0.96	1.09	1.10	0.96	1.14	1.05
% RSD	NMT 2.0	0.28	0.09	0.06	0.28	0.19	0.13
Similarity Factor	0.98 to 1.02	1.00	1.00	1.00	1.00	1.00	1.01

There is no significant variation observed in the results obtained by doing small variation in the process parameters.

System Suitability

The system suitability chromatograms were obtained during validation. System suitability parameters show no significant difference in the values during validation.

Table 9: System suitability

S.No	Parameter	Results		Limit	Pass/Fails
		System-1	System-2		
1	% RSD of Area / 5 injections	0.28	0.17	RSD $\leq$ 2.0	Pass
2	Symmetry Factor	0.96	1.32	Not more than 2.0	Pass
3	Similarity factor	1.00	1.00	Between 0.98 to 1.02	Pass

CONCLUSION

The experimentally obtained results meet the limits of specificity, linearity, range, precision, and accuracy, stability of analytical solutions, robustness and system suitability. Thus determination of Assay of Ambrisentan method of analysis for Ambrisentan Tablets can be adopted day to day analysis.

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