METHOD DEVELOPMENT AND VALIDATION OF MACITENTAN IN PHARMACETICAL TABLET DOSAGE FORMS BY RP-HPLC

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Abstract: A Specific, precise, accurate RP-HPLC method has been developed and validated for the quantitative analysis of Macitentan in tablet formulation. An isocratic separation was achieved using a Zorbax SB C8 (150mm×4.6mm); 5µm particle size column with a flow rate of 1.0ml/min and PDA detector at 215nm. The mobile phase consisted of 0.1%OPA and Acetonitrile (40:60% v/v). The Diluent consisted of pH6.8Phosphate buffer and Acetonitrile in the ratio of 20:80%v/v. The method was validated for specificity, linearity, precision, accuracy and robustness. The specificity of the method was determined by assessing interference from the placebo and by stress testing the drug product (forced degradation). The method was linear over the concentration range 30–150 ppm (r^2 = 0.9999). The accuracy of the method was between 98.9–101.6%. The method was found to be Robust and suitable for the quantitative analysis of Macitentan in a tablet formulation. Degradation products resulting from the stress studies did not interfere with the detection of Macitentan peak in chromatogram, demonstrating the stability-indicating power of method.

IndexTerms - **Stability indicating method, Macitentan, RP-HPLC.**

I. INTRODUCTION

Macitentan is an endothelin receptor antagonist that prevents the binding of ET-1 to ETA and ETB Receptors Macitentan displays high affinity and sustained occupancy of the ET receptors in human pulmonary arterial smooth muscle cells. Swelling of fingers and hands, unusual tiredness or weakness, pale skin, noisy and rattling breathing are side effects of Macitentan. It is mainly used to treat pulmonary arterial hypertension.
The IUPAC name of Macitentan is N-[5-(4-Bromophenyl)-6-[2-[(5-bromo-2-pyrimidinyl) oxy] ethoxy]-4-pyrimidinyl]-*N′*-propylsulfamide.

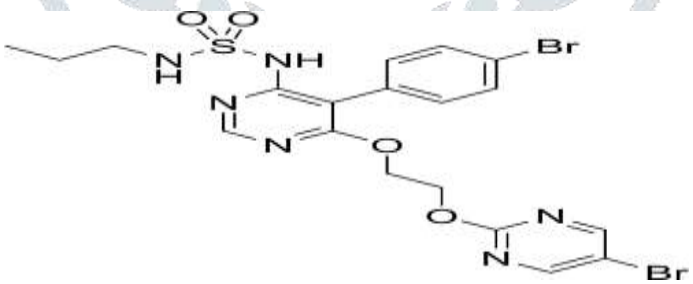

Figure1: Structure of Macitentan

During literature survey of Macitentan it was observed that the published methods for Macitentan by RP-HPLC were not precise showing fluctuations in retention time of Macitentan peak in system suitability parameters. It was observed that most of the assay methods for Macitentan were reverse phase gradient methods. Hence the objective of the present work is to develop a new isocratic stability indicating assay method by using RP-HPLC technique.

II. Materials and Methods:

a) Standards and Samples:

The Macitentan working standard and tablets were provided by Aizant drug research solutions pvt.Ltd.

The make and grade of the materials used were listed in below table1.

Chemical/reagent	Make	Grade
Water	Millipore	Milli-Q
Acetonitrile	Fischer-scientific	HPLC
Ortho-phosphoric acid	Merck	GR
NaOH	Merck	GR
Potassium dihydrogen phosphate	Rankem	AR

Table No. 1: Chemicals and reagents

Equipments:

Instruments used for the present study:
1. HPLC Waters model: Alliance 2695 with 2996 PDA detector, Empower2 software.
2. UV- Spectrophotometer (Shimadzu), analytical balance (Sartorius), Sonicator (power sonic 420) were used for this work.

Chromatographic parameters:

The chromatographic separation was achieved by using Zorbax SB C8 (150mm×4.6mm, 5µm) column. The mobile phase comprising of 0.1% OPA and Acetonitrile (40:60% v/v).The flow rate was maintained as 1.0 mL /min. The column temperature was maintained at 25˚C. The injection volume was 10 µL with sample compartment temperature 5°C.Run time was 15min. The λ max at 215 nm.

Diluent:

Buffer preparation (pH6.8 Phosphate buffer):

Weighed and transferred about1.36 g of Potassium dihydrogen phosphate into 1000 mL of purified water, adjusted the pH to 6.8 with 2M NaOH solution.

Diluent was prepared by mixing 200mL of pH 6.8 Phosphate buffer and 800mL of acetonitrile in the ratio of 20:80(%v/v).

Preparation of standard Solution:

Weighed and transferred 50 mg of Macitentan working standard into 100 mL volumetric flask, added 70 mL of diluent and sonicated to dissolve the material completely, made the volume up to the mark with diluent.

Piptted out 5mL of above solution and transferred into 25 mL volumetric flask and made the volume up to the mark with diluent.

Preparation o test solution:

Weighed and transferred 20 Macitentan tablets powder after crushing (equivalent to 10mg of Macitentan) into 200mL volumetric flask, added about 40 mL of pH 6.8 buffer and sonicated for 5 minutes with intermittent shaking, further added 100 mL of Acetonitrile and sonicated for another 25 minutes with intermittent shaking. Cooled the solution to room temperature and made the volume up to the mark with diluent and mixed well filtered through 0.45µ nylon filter.

Pipette out 5 mL of filtered solution into 25 mL volumetric flask, and made the volume up to the mark with diluent and mixed well.

III. Results and Discussions:

Method Development:

The method development was initiated in isocratic mode of separation with initial composition of mobile phase consisting of 0.1%OPA and acetonitrile in the ratio of (50:50%v/v) using different columns. Based upon trials a ratio of mobile phase consisting of 0.1%OPA and acetonitrile in the ratio (40:60%v/v) using Zorbax SB C8 (150mm×4.6mm, 5µm) column was finalized for the evaluation of Macitentan in Macitentan tablets. The blank and standard chromatograms were represented in Fig.2 and 3, and system suitability parameters were summarized in Table.2.

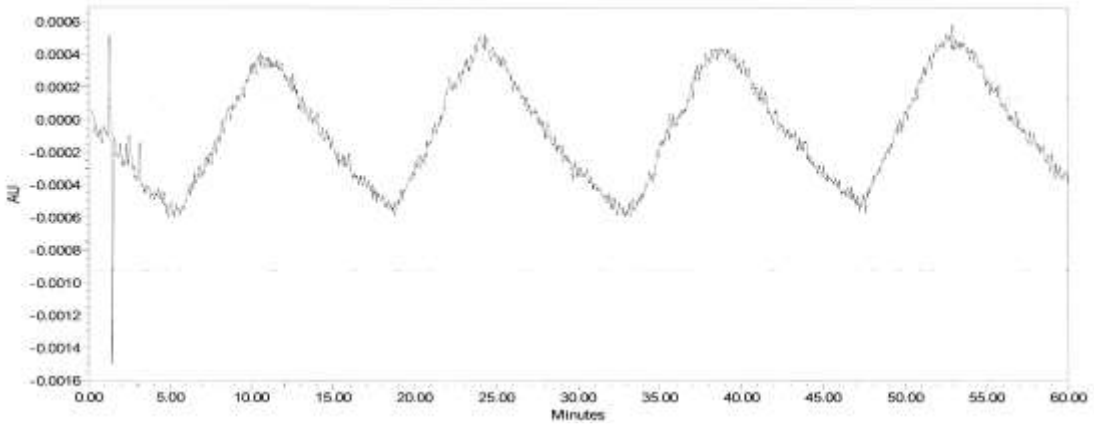

Fig.2: Blank chromatogram

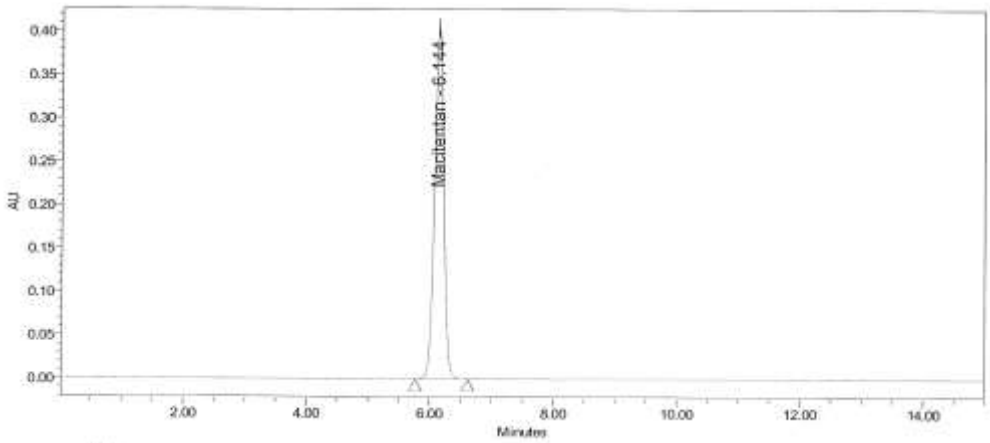

Fig.3: Standard chromatogram

System suitability parameter	Results
Theoretical plates(N)	4758
Tailing factor	1.0
% RSD for area of Macitentan peak obtained from five replicate injections	0.3
% Recovery of Macitentan	100.0

Table No. 2: System suitability parameters of Macitentan

METHOD VALIDATION:

The developed and optimized HPLC method was validated according to ICH guidelines for the following parameters.

1. Specificity
2. Precision
3. Accuracy
4. Linearity
5. Forced degradation
6. Robustness

Specificity:

Blank, Placebo, Impurity Spiked, Un spiked and degraded drug product solution were injected and compared with as such sample; No peak interference was observed in the blank, placebo, Impurity spiked, Un spiked and degraded sample at the retention time of Macitentan peak.

Descriptive chromatograms were represented in Fig. 4, 5 and 6.

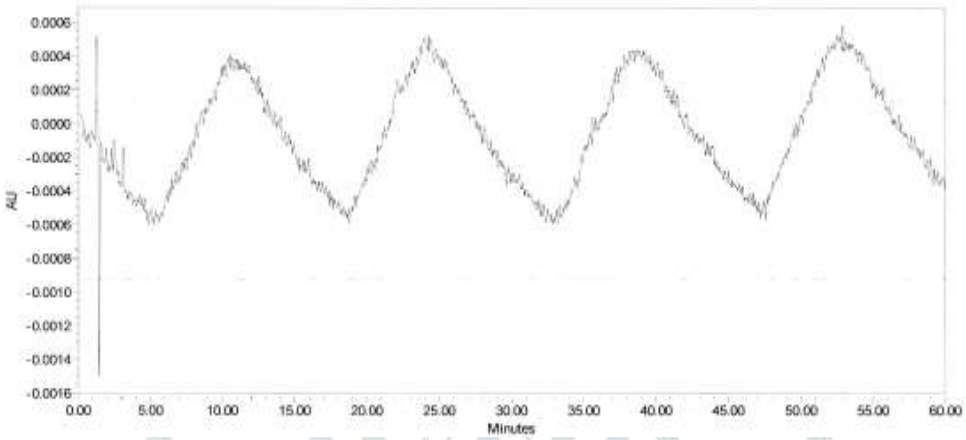

Fig.4: Blank chromatogram

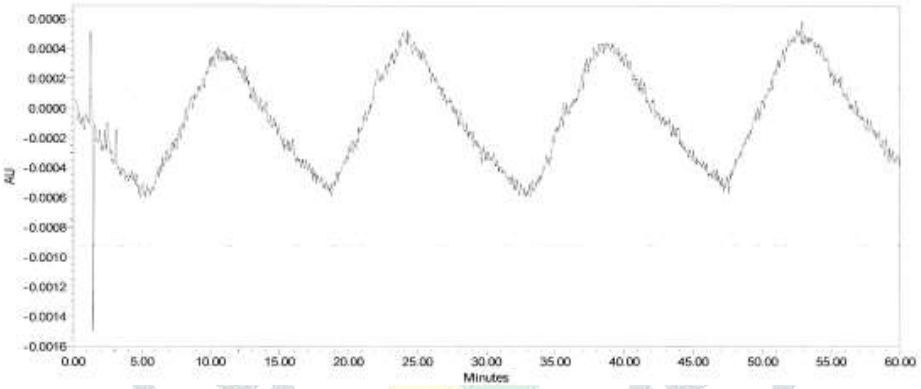

Fig.5: Placebo chromatogram

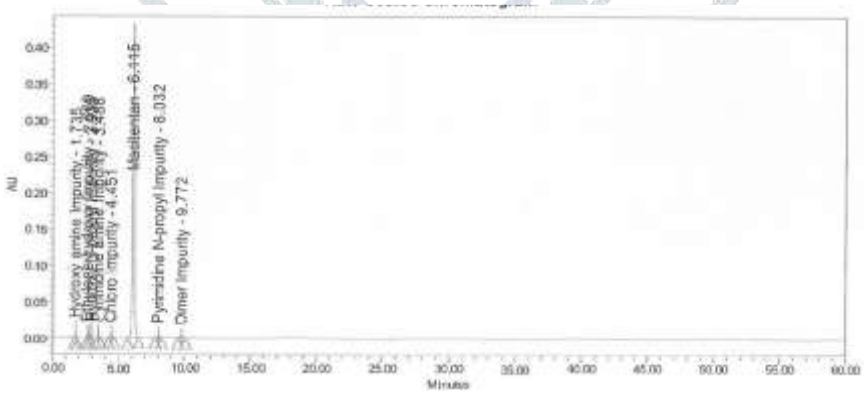

Fig.6: Spiked sample chromatogram

Precision:

Precision was evaluated by carrying out six different samples and the results were found to be within the pre established acceptance criteria. The results were tabulated in Table 3.

S.No	%Assay
1	102.2
2	102.6
3	102.6
4	102.6
5	101.7
6	101.5
Mean	**102.0**
%RSD	**0.4**

Table No. 3: Precision

Intermediate precision (Ruggedness):

The intermediate precision for Macitentan compound was carried out using six sample preparations on different day, by different analyst, using different HPLC and different lot of column. The results were observed to be meeting the pre established acceptance criteria and results were summarized in Table 4.

S.No	%Assay
1	101.2
2	101.5
3	101.1
4	100.0
5	101.2
6	101.5
Mean	101.0
%RSD	**0.8**

Table No. 4: Intermediate precision

Accuracy:

Accuracy study was performed at different concentrations i.e. 30%, 50%, 100%, 120% and 150% of target sample concentration % individual recovery, mean recovery, amount added and amount recovered were calculated. The accuracy results were summarized in Table 5.

Spike Level (%)	Amount added (mg)	Amount recovered (mg)	%Recovered	Mean % recovered
30	29.95	29.63	98.9	**98.9**
	29.97	29.57	98.7	
	30.01	29.61	98.7	
50	49.99	50.08	100.2	**100.2**
	50.11	50.04	99.9	
	50.02	50.27	100.5	
100	99.92	100.13	100.2	**100.6**
	99.97	100.44	100.5	
	99.94	100.93	101.0	
120	120.7	121.80	101.4	**101.6**
	120.00	121.99	101.7	
	120.07	122.27	101.8	
150	149.89	152.57	101.8	**101.6**
	149.98	152.42	101.6	
	149.89	152.57	101.8	

Table No. 5: Accuracy data of Macitentan

Linearity:

Five linear concentrations of Macitentan were prepared and analyzed. Square of correlation coefficient was observed to be 0.9999. The results were summarized in table 6 and figure 6.

% Linearity level	Concentration (ppm)	Mean area
30	29.92	1198449
50	49.87	2025300
100	99.74	4063033
120	119.68	4859540
150	149.61	6147178
Correlation coefficient(R^2)	0.9999	

Table No.6: Linearity of Macitentan

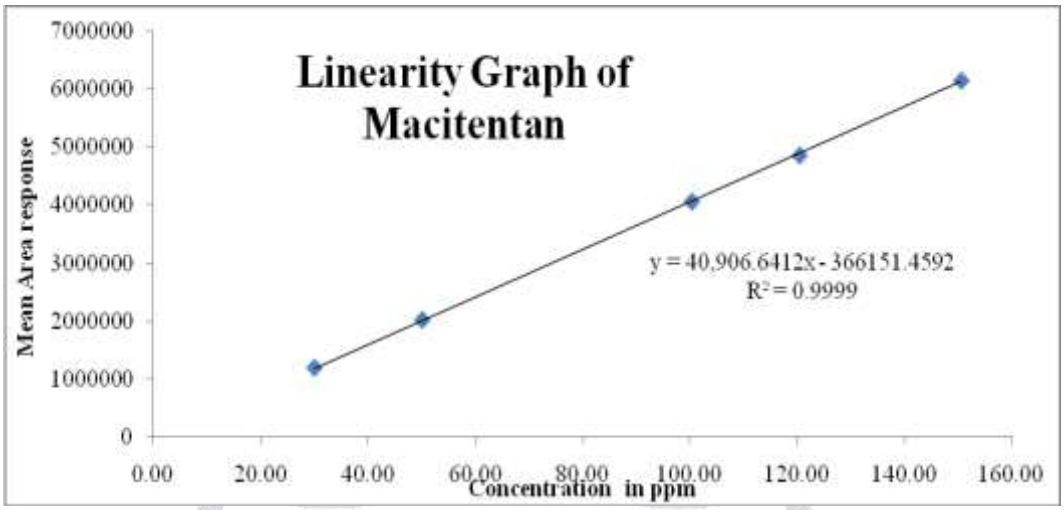

Fig No.6: Linearity graph of Macitentan

Robustness:

Small but deliberate changes in method parameters like flow rate, column oven temperature, wavelength and filters were made but no recognized change in the results were observed and were within range as per ICH guidelines. The results were tabulated in Table 7.

Condition	Difference from initial condition	% RSD
Flow rate plus	0.1 mL/min	0.6
Flow rate minus	0.1 mL/min	0.4
Temperature plus	5°C	0.5
Organic content plus	35:65	0.1
Organic content minus	45:55	0.1
Wavelength plus	2 nm	0.4
Wavelength minus	2 nm	0.4

Table No. 7: Robustness of Macitentan

Interference from Degradation products (Forced degradation studies):

Degradation condition	% Assay	Amount degraded	Peak purity of Macitentan peak
Control	102.1	N/A	Passed
Acid	96.7	5.4	Passed
Base	93.5	8.6	Passed
Peroxide	86.9	15.2	Passed
Water	98.0	4.1	Passed
Thermal	100.4	1.7	Passed
Photolytic	101.6	0.5	Passed
Humidity	102.3	0.2	Passed

Table No.8: Forced degradation data of Macitentan

Degradation studies were performed with the drug product and samples were injected. Assay of the injected samples were calculated. Samples in all stressed conditions passed the limits of forced degradation study. The results were tabulated in Table 8 and descriptive figures were represented from Fig. 7 to 13.

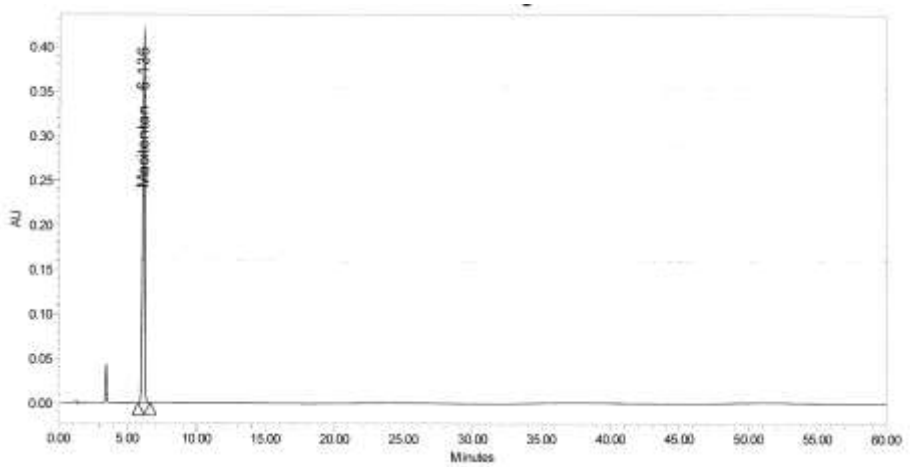

Fig No.7: Acid degraded sample of Macitentan

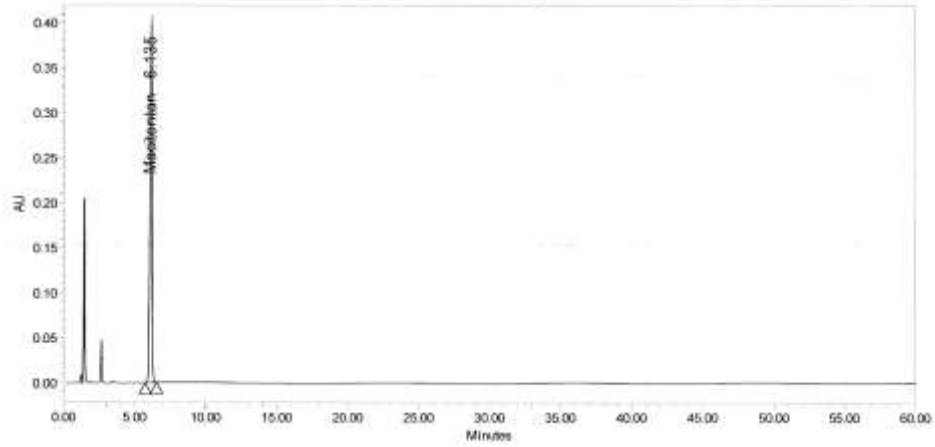

Fig No.8: Base degraded sample of Macitentan

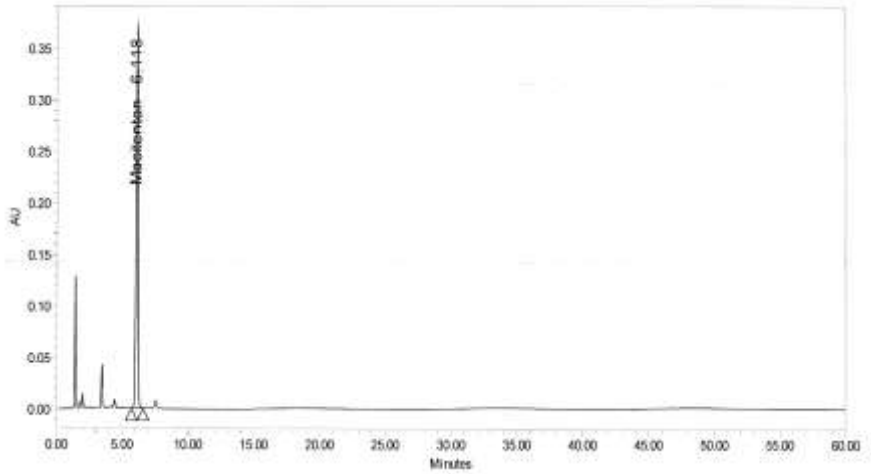

Fig No. 9: Peroxide degraded sample of Macitentan

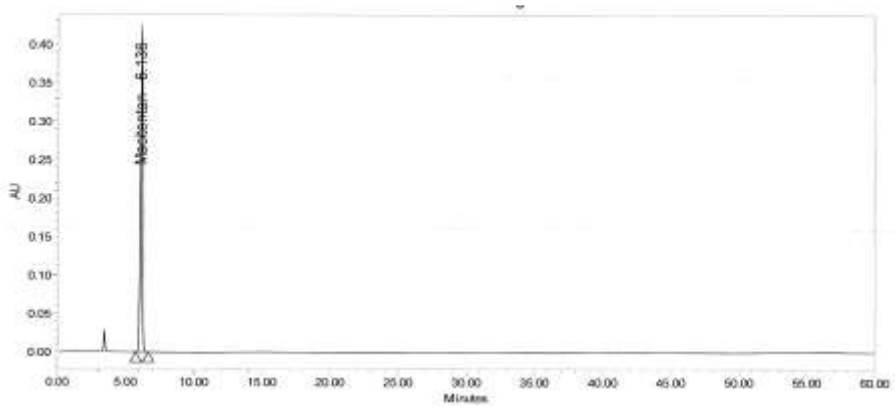

Fig No. 10: water degraded sample of Macitentan

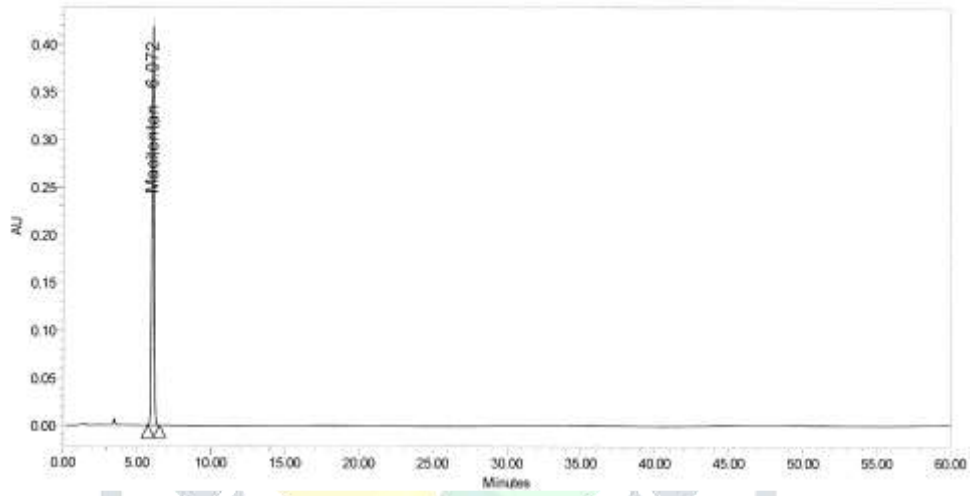

Fig No. 11: Thermal degraded sample of Macitentan

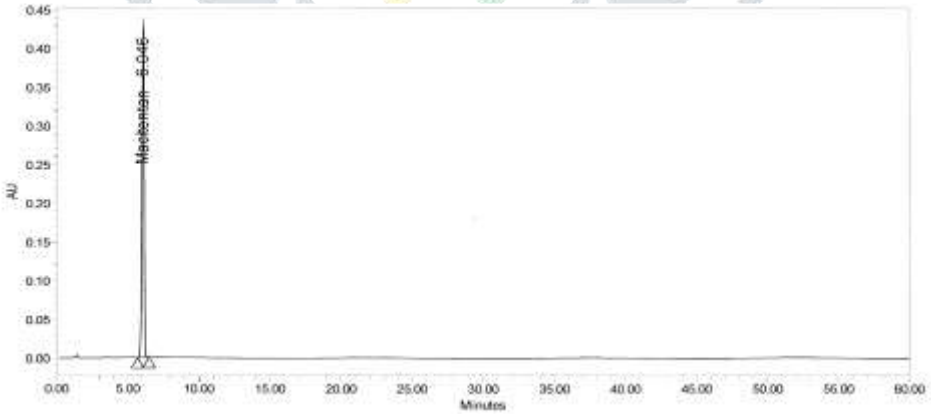

Fig No. 12: Humidity degraded sample of Macitentan

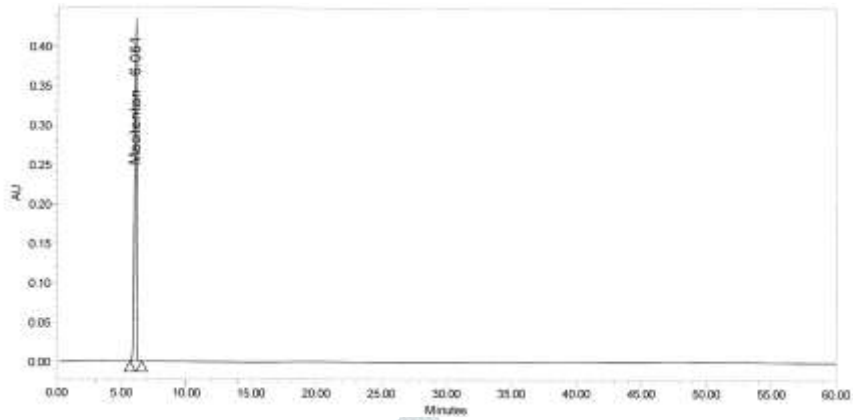

Fig No. 12: Photolytic degraded sample of Macitentan

Conclusion:

A simple, accurate and reproducible isocratic reverse phase HPLC method was developed for the estimation of Macitentan in Tablet formulations. The developed method was optimized prior to validation studies in terms of stationary phase, mobile phase composition, and flow rate and column oven temperature.

The developed method was validated as per ICH Q2A (R1) guidelines. The method was found specific, accurate, precise, linear, rugged and robust. This method can be used for routine quality control analysis of Macitentan in its tablet formulation.

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