Acta Scientific Pharmaceutical Sciences (ISSN: 2581-5423)

Volume 3 Issue 7 July 2019

Review Article

Stability Indicating LC Method for the Estimation of Macitentan in Bulk and its Pharmaceutical Dosage Form

Patel Kesha Manojbhai[1], Chhaloitya Usmangani K[1]*, Yadav Preeti[1] and Shah Dimal A[2]

[1]*Department of Pharmaceutical Chemistry and Analysis, Indukaka Ipcowala college of Pharmacy, Phase IV, ADIT Campus, New Vallabh Vidyanagar, Gujarat, India*

[2]*Department of Pharmaceutical Chemistry and Analysis, Babaria Institute of Pharmacy, Vernama, Vadodara, Gujarat, India*

***Corresponding Author:** Chhaloitya Usmangani K, Department of Pharmaceutical Chemistry and Analysis,Indukaka Ipcowala College of Pharmacy,New Vallabh Vidyanagar – 388121, Gujarat, India.

Received: May 23, 2019; **Published:** June 13, 2019

DOI: 10.31080/ASPS.2019.03.0310

Abstract

An accurate, sensitive, selective and precise HPLC method was developed for the development and validation of Macitentan in bulk and its pharmaceutical dosage form. The HPLC was carried out with mobile phase containing Acetonitrile:Water (95:5v/v). UV - Visible Spectroscopic determination was carried out at an absorption maximum of 217 nm using acetonitrile as a solvent. The linearity were found to be in the range of 0.1-20 µg/ml. Validation of proposed method has been carried out with respect to linearity, accuracy, precision, specificity, selectivity and robustness. For acid and alkali hydrolysis, chemical oxidation, dry heat degradation and photolytic degradation conditions was performed by use of stock solution of Macitentan, and quantification has been achieved by proposed LC method. Our thought of interest that the proposed methods will be useful for the routine quality control analysis and quantification of drug in bulk and pharmaceutical dosage form.

Keywords: Macitentan; HPLC; Validation; Forced Degradation

Introduction

Macitentan (MACI) is an orally non-peptide, active and potent dual endothelin receptor antagonist. Macitentan is chemically, N-{[5-(4-bromophenyl)-6-{2-{5-bromopyrimidin-2- yl) oxy} ethoxy} pyrimidin-4yl}sulfamoyl}(propyl)amine. The empirical formula of MACI $C_{19}H_{20}Br_2N_6O_4S$ and molecular weight of 588.27g/mol. Macitentan treats symptoms of pulmonary arterial hypertension which is high blood pressure in the main artery that transfers blood from the right side of ventricle to the lungs. Macitentan works by relaxing these blood vessels and increasing the supply of blood to the lungs which reduce the work load of the heart. The symptoms of PAH include breathlessness, fatigue, weakness, chest pain, fainting and edema [1,2].

The International Conference on Harmonization (ICH) guideline entitled 'Stability testing of new drug substances and products' requires the stress testing to be carried out to elucidate the stability characteristics of the active substance [3]. Susceptibility to oxidation, hydrolysis and photolysis are the required tests. An ideal stability indicating method is one that quantifies the drug and resolves its degradation products.

Methods for the estimation of macitentan have been reported [3,4]. Present study involves High Performance Liquid Chromatography and Stability indicating HPLC method for the estimation of MACI in their dosage form as per ICH guidelines.

Materials and Methods

Instruments

The liquid chromatographic system consists of Waters series 2998 (Shelton, USA) equipped with a PDA detector, series 515 quaternary isocratic pump and manual injector rhysodine valve with 20 µL fixed loop. The analytes were monitored at 217 nm. Chromatographic analysis was performed on Thermo Scientific C18 column having 250 mm× 4.6 mm i.d. and 5µm particle sizes. All the drugs and chemicals were weighed on Mettler Toledo electronic balance (ME204/A04, METTLER TOLEDO Group).

Chemicals and Reagents

Analytically pure Macitentan obtained from reputed Pharmaceutical industry as gift samples. Acetonitrile (HPLC grade) of SRL Private Ltd. Marketed formulation Macitent (MSN Laboratories Pvt. Ltd., India) containing 10 mg of Macitentan, was procured from local pharmacy.

Experimental work

Selection of detection wavelength

The solution of Macitentan was processed in Acetonitrile at a concentration of 10µg/ml. It was examined in the wavelength range of 400 - 200 nm. Analytical wavelength of 217 nm was preferable for perseverance of Macitentan.

Optimization of Mobile phase

The mobile phase containing acetonitrile: water (95:5v/v) showed satisfactory results at a flow rate of 1 ml/min. MACI shows 2.9 minutes retention time where total time of analysis was 5 minutes.

Preparation of Mobile phase

Mobile phase was prepared by taking Acetonitrile: Water (95:5v/v) in 100 ml volumetric flask. The mixture was sonicated for 20 min for degassing the mixture prior to use. This solution was used as mobile phase.

Preparation of standard stock solution

Accurately weigh 10 mg of MACI and transfer to 10ml volumetric flask containing few ml of acetonitrile and makeup the volume upto the mark with acetonitrile which gives the solution having concentration of 1000 µg/ml of MACI. Pipette out an aliquot from the stock solution and dilute with mobile phase to obtain working standard of 100 µg/ml of EDA.

Calibration curve

Pipette out appropriate volume of aliquot from working standard stock solution and transferred to different volumetric flask of 10 ml and volume was adjusted with the mark with the mobile phase to give a solution containing 0.1, 0.5, 1, 5, 10, 15 and 20 µg/ml of MACI. Each solution was analyzed by the proposed method and the chromatogram was recorded. Calibration curve were constructed by plotting concentration v/s peak area and regression equation was computed.

Validation of RP-HPLC method

Validation of the developed HPLC method was carried out according to the International Conference on Harmonization (ICH) guidelines Q2 (R1) [7].

Linearity

Linearity was studied by preparing standard solution of 6 different concentrations of 0.1, 0.5, 1, 5,10, 15 and 20 µg/ml for MACI. Each concentration was repeated 5 times and linearity was assessed in terms of slope, intercept and regression coefficient of MACI. The calibration curves were developed by plotting concentration v/s peak area (n = 5).

Accuracy

The accuracy was determined by calculating recovery of MACI by standard addition method. Known amount of MACI (0%, 80%, 100% and 120%) were added to pre quantified sample solution and the amount of MACI were estimated by putting the value of peak area to the straight line equation of calibration curve.

Precision

Precision was calculated in terms of intraday and interday precisions. Intraday precision was determined by analyzing sample solution of MACI (0.1, 5 and 20 µg/ml) at three levels covering low, medium and high concentration of the calibration curve three times on the same day (n = 3). Now, interday precision was determined by analyzing sample solution of MACI (0.1, 5 and 20 µg/ml) at three levels covering low, medium and high concentration over a period of three days (n = 3).The peak areas obtained were used to calculate mean and %RSD values. The repeatability studies were carried out by estimating the response of 10 µg/ml of MACI six times (n = 6) and results are reported in terms of %RSD.

Limit of detection and limit of quantification

The limit of detection (LOD) is defined as the lowest concentration of analyte that can reliably be differentiated from background levels. The limit of quantification (LOQ) of an individual analytical procedure is the lowest amount of analyte that can be quantitatively determined with suitable precision and accuracy. LOD and LOQ were calculated using the following equation as per ICH guidelines:

$LOD = 3.3 \times \sigma / S$; $LOQ = 10 \times \sigma / S$

Where, σ is the standard deviation of y-intercepts of regression lines and S is the slope of the calibration curve.

Robustness

Small change in the detection wavelength, flow-rate introduced and the temperature effect on the results were examined. The mean and %RSD of peak were calculated.

Solution stability

Stability of sample solution were studied at room temperature for 24 hrs.

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components, which may be expected to be present.

Typically, it is include impurities, degradants, and preservatives etc. Preservative like sodium chloride was added to the drug and was prepared. The drug was analyzed from prepared injection using proposed method.

Analysis of marketed formulation

Twenty tablets were weighed and powdered; powder contain equivalent to 10 mg of Macitentan was transferred in to 10 ml volumetric flask containing few ml of acetonitrile and sonicated for 20 minutes. The solution was filtered through Whatman filter paper No. 41 and the volume was adjusted up to the mark with acetonitrile. This will produce sample solution containing Macitentan 1000 µg/ml. From the above solution, 1 ml aliquot was pipette out and transfer in to another 10ml volumetric flask and diluted to the mark with acetonitrile. This will produce sample solution containing Macitentan 100 µg/ml. From the above solution, 1 ml aliquot was pipette out and transfer in to another 10 ml volumetric flask and diluted to the mark with acetonitrile. This will produce sample solution containing Macitentan 10 µg/ml. The Rt of the solution was measured at 217 nm and the quantification was carried out by keeping this values to the straight line of calibration curve.

Forced degradation study

Stress degradation study using acid and alkali hydrolysis, chemical oxidation, dry heat degradation and photo degradation study was carried out and interference of the degradation products was investigated.

Alkali hydrolysis

To study forced degradation in basic medium 10.0 mg of Macitentan was transferred to 10 ml volumetric flask and 2 ml of 0.01 M NaOH was added to the flask. The content of the flask kept at room temperature for 1hour. Solution was neutralized with 0.01 M HCl using pH strip and volume was adjusted to the mark with acetonitrile. Aliquot (1 ml) was pipetted out into 10 ml volumetric flask, diluted with mobile phase to obtain final concentration of 10 µg/ml of Macitentan. The solution was analysed under proposed chromatographic conditions and chromatogram recorded. The amount of Macitentan was computed using regression equation.

Acid hydrolysis

To study forced degradation in acidic medium 10.0 mg of Macitentan was transferred to 10 ml volumetric flask and 2 ml of 0.01M HCl was added to the flask. The flask was kept at room temperature for 1hour. Solution was neutralized with 0.01 M NaOH using pH strip and volume was adjusted to the mark with acetonitrile. Aliquot (1 ml) was pipetted out into 10 ml volumetric flask, diluted with mobile phase to obtain final concentration of 10 µg/ml of Macitentan. The solution was analysed under proposed chromatographic conditions and chromatogram recorded. The amount of Macitentan was computed using regression equation.

Oxidative stress degradation

To perform oxidative stress degradation study, 10.0 mg of Macitentan was transferred to 10 ml volumetric flask and 2 ml of 3% hydrogen peroxide was added. The flask was kept at room temperature for 1hour and volume was adjusted to the mark with acetonitrile. Aliquot (1 ml) was pipetted out into 10 ml volumetric flask, diluted with mobile phase to obtain final concentration of 10 µg/ml of Macitentan. The solution was analysed under proposed chromatographic conditions and chromatogram recorded. The amount of Macitentan was computed using regression equation.

Study photolytic (UV light) degradation

Analytically pure 10 mg of drug were exposed to UV light for 48 hrs. The solids were allowed to cool and transferred to volumetric flask (10 ml) and dissolve in few ml of acetonitrile. Volume was made up to the mark with the acetonitrile. Solution was further diluted with the mobile phase to obtain final concentration of 10 µg/mlof Macitentan. The solution was analysed under proposed chromatographic conditions and chromatogram recorded. The amount of Macitentan was computed using regression equation.

Dry heat degradation

To study dry heat degradation, 10.0 mg of Macitentan was transferred to 10 ml volumetric flask and was exposed in oven at 80oC for 4 hrs. The solid was allowed to cool and dissolved in few ml of acetonitrile. Volume was made up to the mark with the acetonitrile. Solution was further diluted by mobile phase to obtain final concentration of 10 µg/ml of Macitentan. The solution was analysed under proposed chromatographic conditions and chromatogram recorded. The amount of Macitentan was computed using regression equation.

Result and Discussion

Selection of wavelength

An ideal wavelength is the one that gives good response of detection wavelength. Therefore, analytical wavelength of 217 nm was selected for estimation of Macitentan.

Optimization of mobile phase

The standard solution containing 10 µg/ml of MACI was chromatographed with use of different composition of mobile phases.

As a Mobile Phase Acetonitrile: Water (95:5 v/v) gave sharp symmetric peak with tailing factor (Figure 1) 2.9 therefore it was selected as a mobile phase for determination of MACI. The flow rate was maintained at 1.0 ml/min. Overlay chromatograms of MACI (0.1 - 20 µg/ml) are shown in figure 2.

Figure 1: Chromatogram study of MACI (10 µg/ml) using Acetonitrile:water (95:5 v/v) as mobile phase.

Figure 2: Overlay chromatogram of MACI (0.1 – 20 µg/ml).

Method Validation

Linearity and calibration curve

The calibration curve of Macitentan was found to be between 0.1 - 20 µg/ml having correlation coefficient of 0.997 (Figure 3). The data of calibration curve and regression analysis of calibration curve are reported in table 1.

Precision

The intra-day and inter-day precision were carried out and it was found to be 0.64 - 1.21 and 0.34 - 1.27 %RSD for Macitentan. Instrumental precision was determined by performing injection repeatability test and the %RSD was found to be 1.14%.

Concentration (µg/ml)	Area ± SD (n = 5)	%RSD
0.1	23912.8 ± 220.90	0.92
0.5	64183.4 ± 587.03	0.91
1	108868.4 ± 1317.8	1.21
5	445175.2 ±6143.035	1.37
10	837557.2 ±15500.35	1.85
15	1131419 ± 13084.6	1.15
20	1536698 ±14649.03	0.9

Table 1: Result of Calibration reading at 217nm for MACI.

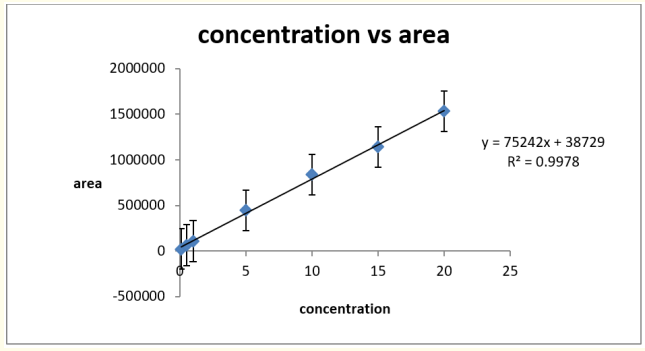

Figure 3: Calibration curve of MACI (0.1 – 20 µg/ml).

Accuracy

The accuracy of the method was determined by calculating recoveries of Macitentan, where a known amount of standard was spiked into pre-analyzed sample solutions. The recoveries were found to be 98.28 - 101.86% for Macitentan (Table 2).

Amount of drug from formulation (µg/ml)	Amount of standard drug spiked (µg/ml)	Amount of drug found (n = 3) (µg/ml)	% Recovery ± SD
8	0	8.02	100.25 ± 0.01
8	6.4	14.53	101.62 ±0 .11
8	8	16.14	101.86 ± 0.10
8	9.6	17.46	98.28 ± 0.09

Table 2: Result of accuracy study.

Limit of detection and limit of quantification

The LOD and LOQ were carried out by visual method, where LOD for Macitentan was found to be 0.005 µg/ml and also LOQ was found to be 0.01 µg/ml.

Robustness

The %RSD was found to be less than 2% after introducing small, deliberate changes in parameters like change in flow rate, mobile phase ratio and detection wavelength in the developed HPLC method, confirming its robustness.

System suitability

System suitability test was carried out and results are reported in table 3.

Parameter	Macitentan
Retention time (min)	2.9
Capacity factor(k')	1.00
Theoretical plates (N)	3364
Tailing factor (T_f)	1.00

Table 3: Result of system suitability.

Parameters	Macitentan
Range(µg/ml)	0.1-20
Retention time	2.9
Detection limit (µg/ml)	0.005
Quantitation limit(µg/ml)	0.01
Accuracy(%)	98.28-101.86
Precision (%RSD)	
Intra-day (n = 3)	0.64 - 1.21
Inter-day (n = 3)	0.34 - 1.27
Instrument precision (%RSD)	
Repeatability (n = 6)	1.14
Specificity	Specific

Table 4: Summary of validation parameters of proposed method.

Analysis of marketed formulation

The proposed method is applied to the determination of Macitentan in their dosage form. The % amount of drug found to be more than 98%. The result showed in table 5.

Formulation (Macitent)	Amount of drug taken (µg/ml)	Amount of drug found(µg/ml) (n=3) ± SD	%of drug found (n=3) ± SD
	10	9.99 ± 0.01	99.91 ± 0.01

Table 5: Analysis of marketed formulation.

Forced degradation study

Forced degradation study was carried out by subjecting the drug to acid and alkali hydrolysis, chemical oxidation, dry heat degradation and photolytic conditions. The MACI was found to be susceptible to degraded in acid and alkali hydrolysis (Figure 4 and 5) and chemical oxidative (Figure 6) conditions. The MACI was found to be stable dry heat degradation and photolytic (UV – Visible Light) condition (Figure 7 and 8). Summary of data derived from forced degradation study by proposed LC Method is shown table 6.

Figure 4: Chromatogram of Acid (0.01 M HCl) degraded Maci (10 µg/ml).

Figure 5: 3D-Chromatogram of Acid (0.01 M HCl) degraded Maci (10 µg/ml).

Figure 6: Chromatogram of Alkali (0.01M NaOH) degraded Maci (10 µg/ml).

Figure 7: 3D-Chromatogram of Alkali (0.01M NaOH) degraded Maci (10 µg/ml).

Figure 8: Chromatogram of chemical oxidation (3%H_2o_2) degraded Maci(10 µg/ml).

Figure 9: 3D-Chromatogram of chemical oxidation (3%H_2o_2) degraded Maci (10 µg/ml).

Figure 10: Chromatogram of dry heat degradation of Maci (10 µg/ml).

Figure 11: 3D-Chromatogram of dry heat degradation of Maci (10 µg/ml).

Figure 12: Chromatogram of photolytic degradation of Maci (10 µg/ml).

Figure 13: 3D-Chromatogram of photolytic degradation of Maci (10 µg/ml.)

Condition	Time (hr)	%Amount of Macitentan	Rt value of degradants of Macitentan
Base 0.01 M NaOH	1hrs (RT)	89.41%	1.5, 3.5
Acid 0.01 M HCl	1hrs (RT)	96.29%	1.6,2.7
3% Hydrogen Peroxide	1hrs (RT)	89.98%	2.7,3.6
UV – Visible Light	48hrs	96.21%	1.5
Dry Heat	4hrs	96.18%	1.5,4.06

Table 6: Summary of data derived from forced degradation study by proposed LC method.

Conclusion

The proposed study, describes LC method was developed for the determination ofMACI. The method was validated and found to be simple, sensitive, accurate and precise. Statistical analysis proved that method was repeatable and selective for the analysis of MACI without any interference from the excipients. As the stability indicating HPLC method separate drug from its degradants. Therefore, it can be conveniently used for routine quality control analysis. The method was successfully validated in accordance with ICH guidelines.

Acknowledgement

The authors are grateful to Indukaka Ipcowala College of Pharmacy and SICART, for their support and providing us with research facilities.

Bibliography

1. Wilkins MR. "Pulmonary hypertension: the science behind the disease spectrum". *European Respiratory Review* 21.123 (2012): 19–26.

2. Montani D., *et al*. "Pulmonary arterial hypertension". *Orphanet Journal of Rare Diseases* 8 (2013): 97.

3. ICH- Guidelines Q1A (R2). "Stability Testing of New Drug Substances and Products, International Conference on Harmonization, November Geneva, Switzerland (1996).

4. MazoorAhmed., *et al*. "RP-HPLC method development and validation for estimation of Macitentan in tablet dosage form". *World Journal of Pharmacy and Pharmaceutical Sciences* 4.1 (2014): 881-887.

5. Aziz Unnisa., *et al*. "Development and Validation of RP-HPLC-PDA Method for the Estimation of Macitentan in Bulk and Tablet Dosage Forms". *Indo American Journal of Pharm Research* 09 (2014): 3836-3843.

6. Vijaya Krishna c., *et al*. "Development and Validation of First order derivative spectrophotometric method for estimation of Macitentan in bulk and tablet dosage form". *International Journal of Universal Pharmacy and Bio-science* 4.1 (2015): 269-275.

7. ICH [Validation of Analytical Procedures: Methodology (Q2R1)], International Conference on Harmonization, Food and Drug Administration, USA, 1996 & 2005.

Volume 3 Issue 7 July 2019