DEVELOPMENT AND VALIDATION OF RP-HPLC-PDA METHOD FOR THE ESTIMATION OF MACITENTAN IN BULK AND TABLET DOSAGE FORMS.

Aziz Unnisa1,2*, Syed. Sadath Ali3, Santosh Kumar.S1
2Research scholar, Sunrise University, Alwar, Rajasthan, India.
3Azad institute of Pharmacy and research, Lucknow, India.

ARTICLE INFO
Article history
Received 28/09/2014
Available online
30/09/2014

Keywords
Macitentan,
RP-HPLC-PDA,
Method Development,
Validation.

ABSTRACT
The aim of the present study was to develop RP-HPLC-PDA method for the estimation of Macitentan in bulk and pharmaceutical dosage forms. The method uses an Inertsil (250 mm x 4.6, 5 μm) with mobile phase consisting of Acetonitrile: 10 mM Ammonium acetate (60:40 v/v) in an isocratic mode with an injection volume of 10μL and the eluents were monitored at 255 nm. The retention time of Macitentan 5.578 min, it showed linearity in the concentration range of 2-10 μg/mL with a good correlation coefficient of 0.999. The validation parameters like specificity, system suitability, linearity, LOD (0.4042 μg/mL), LOQ (1.225 μg/mL), precision (%RSD >1), robustness were all within the limits stated in ICH guidelines. The developed RP-HPLC-PDA method is specific, accurate, robust and economic, hence it can be successfully applied in routine quality control for the determination of Macitentan in bulk and tablet dosage forms.


Copy right © 2014 This is an Open Access article distributed under the terms of the Indo American journal of Pharmaceutical Research, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

www.iajpr.com
INTRODUCTION

Macitentan (MCT) is a novel orally active dual endothelin receptor antagonist, with sustained receptor binding and enhanced tissue penetration resulting from an extensive and targeted drug discovery program[1-3]. The chemical name of macitentan is N-[5-(4-Bromophenyl)-6 - [2-[(5-bromo-2-pyrimidinyl) oxy]ethoxy]-4-pyrimidinyl]-N’propylsulfamide (Figure.1). Pulmonary arterial hypertension (PAH) is a progressive, not curable disease affecting 50 adults per million with a higher incidence in women than in men [4]. Recently, doses of 3 and 10 mg macitentan were demonstrated to reduce the risk of morbidity/mortality events in PAH patients in the event-driven outcome phase III [5, 6].The apparent elimination half-life (t½) was approximately 15 h[7-9]. After multiple dosing, macitentan attains steady-state by Day 3, with an accumulation of approximately 1.5-fold [10].

As MCT is a potent drug, its precise, accurate and sensitive quality control in pure and tablets dosage form is of vital importance. It was found from the literature survey that no official or unofficial method has been reported for estimation of the drug alone or in combination with other drugs in pharmaceutical dosage forms or biological fluids. Hence, the present investigation was aimed at developing an optimised method for the estimation of the drug in bulk and in pharmaceutical formulations; the developed method was validated in accordance with ICH guidelines[11].

![Figure 1. Structure of Macitentan.](image-url)

MATERIAL AND METHODS

Materials

MCT pure sample was obtained from Dr.Reddy’s Laboratories, Hyderabad, India. Ammonium Acetate, water and Acetonitrile were purchased from Merck India, Mumbai. All the solvents used were of HPLC grade. The other chemicals and reagents were of AR grade.

Instrumentation

A Shimadzu Prominence HPLC system provided with DGU-20A3 degasser, LC-20AD binary pumps, SIL-20AHT autosampler and SPD-M20A PDA detector was used. Data was acquired using LC solutions software. The chromatographic analysis was performed on Inertsil C18 reverse phase column (150 × 4.6 mm, 5 µm).

Chromatographic Conditions

Mobile phase consisting of Acetonitrile:10mM Ammonium acetate (40:60 v/v) was used in isocratic mode, the mobile phase was filtered through nylon disc filter of 0.45 µm (Millipore) and sonicated for 3 min before use. The flow rate was set at 1.2 mL/min and the injection volume was 10µL. PDA detection was performed at 255 nm and the separation was achieved at ambient temperature.

Preparation of stock solutions

Accurately weighed quantity of MCT was dissolved in sufficient quantity of methanol in a 10 mL volumetric flask. The volume was made up to the mark with methanol to obtain a stock solution of 10 mg/mL of MCT.

Method validation

Linearity

A linear was evaluated across the range of the analytical procedure with five concentrations. A series of standard dilutions were prepared over a concentration range of 2-10 μg/mL for MCT and injected three times on to the column. Linearity was evaluated by a plotting peak area as a function of analyte concentration and the test results were evaluated by appropriate statistical methods where by slope, intercept, regression and correlation coefficient 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 determined by using a minimum of six determinations at 100 % of the test concentration (4µg/mL of MCT). The standard deviation and the relative standard deviation (RSD) were computed for precision.

Accuracy

Accuracy was established across the specified range of the analytical procedure. To ascertain the accuracy of the planned method recovery studies were performed by the standard addition method by spiking 80 %, 100 %, 120 % of the known quantities of
standards within the range of linearity to the synthetic solution of drug product (4 μg/mL of MCT), these solutions were analyzed by developed method in triplicate. The % recovery and the % RSD were calculated at each level of addition.

**Limit of Detection and Limit of Quantification**

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).

**Robustness**

To determine the robustness of the method developed, the experimental conditions were intentionally changed and the chromatographic parameters like capacity factor, tailing factor, no. of theoretical plates and % assay were recorded. The flow rate of the mobile phase was 1.2 mL/min. To study the effect of change in flow rate, it was altered by ± 0.2 mL/min and the effect of wavelength was studied by changing wavelength by ± 1 nm.

**System suitability**

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

**Assay**

Twenty tablets were weighed and finely powdered. The powder blend equivalent to 10mg of MCT was accurately weighed, transferred into a 10 mL volumetric flask, dissolved in methanol, vortexed for 5 min and volume was adjusted up to the mark. The above stock solution was centrifuged and filtered using Nylon disposable syringe filter (13 mm, 0.45 μm). From the filtrate 4 μL was transferred into a 2 mL centrifuge tube, diluted to 1 mL with Acetonitrile. The solution was injected in triplicate and analyzed. The amount of drug present in each tablet was quantified by comparing the area of standard with that of the sample.

**RESULTS AND DISCUSSION**

No methods were reported for the estimation of MCT in bulk and pharmaceutical dosage forms. Hence, the present study was focused to develop a simple, economic, specific and efficient RP-HPLC-PDA method for the simultaneous determination of MCT in bulk and in tablets.

**Method Development and Optimization**

In the present examination, different analytical columns with various stationary phases and mobile phase were tested to develop a highly sensitive LC method, for the analysis of MCT in bulk and formulations. Initial trials were carried out using Phenomenex C18 column (250 x 4.6 mm, 5μm) with methanol: water (50:50 v/v) at a flow rate of 1mL/min, the peak eluted at 4.1 min with good shape and symmetry etc; however, the peak response was too low. Same mobile phase was tried using Develosil RP Aqueous column (250 x 4.6 mm) but resulted in bad peak shape. In another trial mobile phase of Methanol: 0.02% v/v formic acid in water (50:50) at a flow rate of 1 mL/min was studied using same column but peak Fronting was observed. In the next trial mobile phase of acetonitrile: water (50:50 v/v) with drug working standard in acetonitrile, peak fronting was observed. Finally a mobile phase consisting of Acetonitrile: 10mM Ammonium acetate (60:40 v/v) resulted in good peak shape and response when compared to the previous trials. With these conditions the retention time of MCT was 5.578 min at a flow-rate of 1.2 mL/min with a backpressure of 112 kfg and the injection volume was 20 μL within run time 7 min. For quantification wavelength was fixed at 255 nm, which provided better reproducibility with least or no interference. The method was validated in accordance with ICH guidelines. The peak purity index was greater than 0.9999 (Figure.5.) indicating the purity of the drug sample peaks. The standard chromatogram obtained from the optimized method is shown in Figure.2.

**Figure.2. Typical chromatogram of standard solution containing MCT(4 μg/mL).**
Method validation
The method has been validated in accordance with ICH-Guidelines for following parameters

Specificity
The standard, sample, placebo and diluent solution were injected into the HPLC system following the test conditions, the chromatograms were recorded for each of the solutions and the response of the peaks were noted for examining interference. From the base shift overlay of the chromatograms in (Figure 3) and 3D plots of diluents, standard, placebo and formulation shown in (Figure 4), it can be found that there were no co-eluting peaks interfering with the drug peaks (Figure 5).
Linearity

The range of reliable quantification was set at the concentrations of 2-10 μg/mL for MCT. 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 for MCT demonstrating a good correlation coefficient. The data from the calibration curve was given in Table 1. The overlaid chromatogram and calibration curve were depicted in Figure. 6 and 7 respectively.

![Figure 5: Peak purity curve of Macitentan.](image1)

**Figure 5.** Peak purity curve of Macitentan.

Impurity: not detected, Peak purity index: 0.999998, Single point threshold: 0.988588, Minimum peak purity index: 11410

**Figure 6.** Overlaid chromatograms of standard solution of MCT at different concentration levels.

![Figure 6: Overlaid chromatograms of standard solution of MCT at different concentration levels.](image2)

**Table 1.** Linearity data of MCT.

<table>
<thead>
<tr>
<th>MCT concentration(μg/mL)</th>
<th>Peak Area(±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>28099.1±166.328</td>
</tr>
<tr>
<td>4</td>
<td>55869.8±798.408</td>
</tr>
<tr>
<td>6</td>
<td>83240.8±80.098</td>
</tr>
<tr>
<td>8</td>
<td>111677±64.133</td>
</tr>
<tr>
<td>10</td>
<td>137626±59.4334</td>
</tr>
<tr>
<td>Regression equation</td>
<td>y=13713x+1212.4</td>
</tr>
<tr>
<td>Correlation Coefficient( R)</td>
<td>0.9997</td>
</tr>
<tr>
<td>Regression coefficient( R²)</td>
<td>0.9997</td>
</tr>
</tbody>
</table>
Precision

Precision studies were performed in terms of repeatability. Repeatability of standard application was carried out by injecting six replicates of the mixed standard at concentration of 4μg/mL for MCT, the data was shown in Table 2. The % RSD was found to be below 2 for peak areas of MCT this shows the closeness of the data values to each other, indicating the precision of the method. Overlaid chromatogram of precision was shown in the following.

Table 2. Precision data of MCT.

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>SYSTEM PRECISION</td>
<td>58788±199.223</td>
<td>0.33</td>
</tr>
<tr>
<td>METHOD PRECISION</td>
<td>56753±222.7205</td>
<td>0.39</td>
</tr>
</tbody>
</table>

Accuracy

Accuracy of the method was confirmed by performing recovery studies following standard addition method by spiking the known quantities of standard (80%, 100% and 120%) to the mixture of drug product solution comprising of 4μg/mL of MCT, and these solutions were analyzed in triplicate at each level of addition. The %RSD and %Recovery were within the acceptable limits in all cases. It is clear from the results of accuracy study given in Table 3, that the proposed method enables very accurate quantitative estimation of MCT in commercial tablets.

Table 3. Accuracy data of MCT.

<table>
<thead>
<tr>
<th>Level of Recovery</th>
<th>Amount Present</th>
<th>Amount added(μg/mL)</th>
<th>% Recovery (Mean ± SD)</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>80%</td>
<td>4</td>
<td>3.2</td>
<td>100.237 ±0.41932</td>
<td>0.112</td>
</tr>
<tr>
<td>100%</td>
<td>4</td>
<td>4</td>
<td>100.0267±0.8528</td>
<td>0.8</td>
</tr>
<tr>
<td>120%</td>
<td>4</td>
<td>4.8</td>
<td>100.2933±0.5316</td>
<td>0.01</td>
</tr>
</tbody>
</table>

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

The limit of detection for MCT was found to be 0.4042μg/mL. The limit of quantification for MCT was 1.225μg/mL.

Assay

Assay of MCT formulation was performed by the proposed method and the % assay of the formulation was calculated as an average of three findings, which was about 100.031±0.117. These results indicate that the present HPLC method can be successfully used for the analysis MCT in bulk and dosage forms. The results were shown in Table 4.

Table 4. Assay data of MCT.

<table>
<thead>
<tr>
<th>Sample Number</th>
<th>MCT Peak Area</th>
<th>MCT % Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>55378.4</td>
<td>100.283</td>
</tr>
<tr>
<td>2</td>
<td>55442.8</td>
<td>99.017</td>
</tr>
<tr>
<td>3</td>
<td>555402.6</td>
<td>99.844</td>
</tr>
<tr>
<td>Average</td>
<td>33016.333</td>
<td>100.031</td>
</tr>
</tbody>
</table>
Robustness

Robustness was determined by analyzing the same sample at normal operating conditions and by changing the analytical conditions like wavelength of detection and flow rate of the mobile phase. Percent assay values were estimated under these modified conditions and the results were given in Table 5. Changes in the flow rate slightly affected the retention times of the MCT. However, the parameters like theoretical plate number, capacity factor and assay were not changed and were within reasonable limits. Similar results were obtained with the changed wavelength.

<table>
<thead>
<tr>
<th>Robustness Parameters</th>
<th>Retention time</th>
<th>Tailing factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>253nm</td>
<td>5.578</td>
<td>1.431</td>
</tr>
<tr>
<td>255nm</td>
<td>5.578</td>
<td>1.431</td>
</tr>
<tr>
<td>255nm</td>
<td>6.308</td>
<td>1.432</td>
</tr>
<tr>
<td>1mL/min</td>
<td>6.032</td>
<td>0.978</td>
</tr>
<tr>
<td>1.2mL/min</td>
<td>5.578</td>
<td>0.938</td>
</tr>
<tr>
<td>1.4mL/min</td>
<td>4.865</td>
<td>0.955</td>
</tr>
</tbody>
</table>

System suitability

System suitability studies were carried out by injecting mixed standard concentration of 4μg/mL (MCT) at different injection volumes ranging from 10μL to 50μL. The %RSD values for system suitability parameters like retention time \([R_t= 4.026 (0.764)\) for MCT], tailing factor \([T_f= 1.129 (0.618)\) for MCT] and theoretical plate number \([37.3056 (0.524)\) were found to be less than 1% indicating the present conditions were suitable for the analysis of MCT in tablets. The overlay of chromatograms for system suitability was given in Figure.8.

Figure.8. Base shift overlay Chromatograms of MCT System suitability data.

Stability of the analytical solution

The stability of the stock and standard solutions were determined by analyzing the samples under refrigeration (8±1°C) at different time intervals up to 48 hrs. The percentage variation in assay values at different time intervals were found to be less than 1% of the initial zero time interval solution, thus indicating that the solutions were stable. The results were shown in the Table 7.

<table>
<thead>
<tr>
<th>Time interval</th>
<th>%Variation in peak area</th>
</tr>
</thead>
<tbody>
<tr>
<td>0hrs</td>
<td>0</td>
</tr>
<tr>
<td>48hrs</td>
<td>0.935</td>
</tr>
</tbody>
</table>

CONCLUSION

In this work, a simple, efficient and economic RP-HPLC-PDA method has been developed for the determination of MCT in bulk and tablets. The method was validated, and validation acceptance criteria were met in all cases. Application of this method for determination of MCT from tablets showed that neither the degradation products nor the excipients interfered with the estimation of the drug, therefore this method was simple, specific and economical, hence it can employed successfully for the estimation of MCT in commercial tablet dosage forms.
ACKNOWLEDGEMENTS

The authors are grateful to Dr Reddy’s laboratories, India for providing gift samples of the drugs and also to the Siddhartha Academy of General and Technical Education, Vijayawada, for providing the necessary facilities to conduct this research work.

Competing Interests
The authors declare no conflict of interest.

REFERENCES