RESEARCH ARTICLE

A VALIDATED REVERSE PHASE HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF ARTEMETHER AND LUMEFANTRINE IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A simple, sensitive and precise reverse phase high performance liquid chromatographic method has been developed for the simultaneous estimation of Artemether and Lumefantrine in pharmaceutical dosage forms. The mobile phase consisted of Acetonitrile: buffer (0.1% v/v ortho phosphoric acid, pH – 3) in the ratio of 60:40 v/v delivered at a flow rate of 1.5 ml / min and wavelength of detection at 303 nm. The retention times of Artemether and Lumefantrine were 13.888 min and 7.207 min respectively. The developed method was validated according to ICH guidelines. The proposed method can be used for determination of these drugs in combined dosage forms.

Key words: Artemether, Lumefantrine, RP-HPLC.

INTRODUCTION

Artemether is chemically (3R,5aS,6R,8aS,9R,10S,12R,12aR-Decahydro-10-methoxy-3,6,9-trimethyl-3,12-epoxy-12H-pyrano[4,3-j]1,2-benzodioxepin) and is used as antimalarial agent. Lumefantrine is chemically 2,7-Dichloro-9-[4-chlorophenyl]methylen] α-(dibutylamino)methyl]-9H-fluorene-4-methanol and is used in the treatment of uncomplicated falciparum malaria. Both of these drugs are available in combined tablet dosage form with the label claim of Artemether 20mg and Lumefantrine 120mg per tablet. Literature survey revealed that a few analytical methods have been reported for the simultaneous determination of Artemether and Lumefantrine in pure drug, pharmaceutical dosage forms and in biological samples using liquid chromatography [3-14], high performance thin layer chromatography [15] and Polarography [16] either in single or in combined forms. The present work describes the development

and validation of reverse phase high performance liquid chromatographic (RP-HPLC) method, which can quantify these components simultaneously. Confirmation of the applicability of the developed method was validated according to the International Conference on Harmonization (ICH) [17] for the determination of Artemether and Lumefantrine in bulk and in tablet dosage form.

EXPERIMENTAL

Reagents and Chemicals:

Artemether API and Lumefantrine API were obtained as gift sample from Aristo Pharmaceuticals Pvt. Ltd., Mumbai. The branded formulations (tablets) (Combither tablets containing 20 mg of Artemether and 120 mg of Lumefantrine) were procured from the local market. Acetonitrile, Potassium dihydrogen orthophosphate, Water and orthophosphoric acid used were of HPLC grade and purchased from Merck Specialities Private Limited, Mumbai, India.
Instrumentation:

Chromatographic separation was performed on a Shimadzu chromatographic system equipped with a LC-20AT pump; variable wavelength programmable UV/VIS detector, SPD-20A and Rheodyne injector (7725i) with 20µl fixed loop.

Chromatographic conditions:

Symmetry C<sub>18</sub> (250 x 4.6mm, 5µ) was the column used for separation. Mobile phase consisting of a mixture of Acetonitrile and Buffer (0.1% v/v orthophosphoric acid, pH-3) in the ratio 60:40 v/v was delivered at a flow rate of 1.5 ml/min with detection at 303 nm. The mobile phase was filtered through a 0.45µ nylon filter and sonicated for 15 min. Analysis was performed at ambient temperature.

Pharmaceutical formulation:

Commercial tablets were procured randomly from the local market.

Method development:

Acetonitrile and water in different proportions were tried and finally Acetonitrile: Buffer (0.1% v/v orthophosphoric acid, pH-3) (60: 40 v/v) was selected as an appropriate mobile phase which gave good resolution and acceptable system suitability parameters. The chromatogram of working standard solution is shown in fig 1.

PROCEDURE:

Preparation of standard solution:

A standard stock solution was prepared by accurately weighing about 4 mg of Artemether and 24 mg of Lumefantrine standard and transferred into 100 ml volumetric flask; added about 60ml of mobile phase and sonicated for about 5 minutes to dissolve and made up to volume with mobile phase.

Calibration curve:

Accurately measured volumes of working standard solution of Artemether and Lumefantrine were transferred into a series of 100ml volumetric flasks and diluted appropriately with mobile phase. 20µl of each solution was injected under operating chromatographic conditions described above. Calibration curves were obtained by plotting the response (area of drug peak) versus concentration of drug. Regression equations were calculated. The method was found linear over a concentration range of 20µg/ml to 60 µg/ml for Artemether and 120µg/ml to 360µg/ml for Lumefantrine.

Figure 1

Typical Chromatogram of Artemether and Lumefantrine
Procedure for analysis of tablets:

Weigh and powder not less than ten tablets. Accurately weigh and transfer tablet powder equivalent to 4 mg of Artemether and 24 mg of Lumefantrine into 100 ml volumetric flask, add about 60 ml of acetonitrile and sonicate for 30 minutes with intermediate shaking. Make up the volume with buffer (40 ml). Filter a portion of the solution through 0.45 µm membrane filter and discard first few ml of the filtrate. With the optimized chromatographic conditions, a steady baseline was recorded, the mixed working standard solution was injected and the chromatogram was recorded. The retention times of Artemether and Lumefantrine were found to be 13.888 and 7.207 min respectively. The proposed method was found to be specific and no interference from common tablet excipients like lactose, starch etc. was observed. The response factors of the standard solutions and sample solutions were calculated. The assay was calculated from the equation of regression line for each drug. The assay procedure was repeated for 6 times and the percentage of individual drug in the formulation was calculated. The results of analysis shows that the amount of drug was in good agreement with the label claim of formulation (Table 1).

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Analyte</th>
<th>Label claim (mg)</th>
<th>% label estimated*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tablet</td>
<td>Artemether</td>
<td>20</td>
<td>99.23</td>
</tr>
<tr>
<td></td>
<td>Lumefantrine</td>
<td>120</td>
<td>99.10</td>
</tr>
</tbody>
</table>

*mean of six determinations

METHOD VALIDATION

Linearity:

The method was linear in the range of 20 µg/ml to 60 µg/ml for Artemether and 120 µg/ml to 360 µg/ml for Lumefantrine. Linear regression data was given in Table 2.

Precision:

The precision of the method was demonstrated by inter day and intraday variation studies. In the intraday studies, solutions of standard and sample were repeated thrice in a day and percent relative standard deviation (%RSD) for response factor was calculated. The intraday %RSD of Artemether and Lumefantrine were found to be 1.06 and 0.10 respectively.

In the interday variation studies, injections of standard and sample solutions were made on three consecutive days and %RSD was calculated. The interday %RSD for Artemether and Lumefantrine were found to be 0.79 and 0.74 respectively. From the data obtained the developed RP-HPLC method was found to be precise.

Accuracy:

The accuracy of the method was determined by recovery experiments. Known concentration of working standard was added to the fixed concentration of the pre-analyzed tablet solution. Percent recovery was calculated by comparing the area before and after the addition of working standard. Percent recovery was performed in the same way. The recovery studies were performed in triplicate. This standard addition method was performed at 50%, 100%, 150% level and the percentage recovery was calculated. Percent recovery was within the range of 98.46 to 99.86 for Artemether and 98.63 to 99.09 for Lumefantrine which indicates that the method was accurate.
Table 2 Linear regression data for calibration curves

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Artemether</th>
<th>Lumefantrine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity range (µg/ml)</td>
<td>20 – 60</td>
<td>120 – 360</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.9999</td>
<td>0.9999</td>
</tr>
<tr>
<td>Slope</td>
<td>572</td>
<td>26365</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.006</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Robustness:

Robustness of the method was checked by making slight deliberate changes in chromatographic conditions like mobile phase ratio, pH of buffer, flow rate. It was observed that there were no marked changes in chromatograms, which demonstrated that the developed RP-HPLC method is robust.

Solution stability:

In order to demonstrate the stability of both standard and sample solutions, the solutions were analyzed over a period of 12 hours at room temperature. The results show that, the retention time and peak area of Artemether and Lumefantrine remained unchanged (%RSD less than 0.2) and no significant degradation within the indicated period was observed. This indicates that both solutions were stable for at least 12 hours, which was sufficient to complete the analytical procedure.

RESULTS AND DISCUSSION

The proposed method was found to be linear in the concentration range of 20 to 60 µg/ml for Artemether and 120 µg/ml to 360 µg/ml for Lumefantrine. The method was specific since excipients in the formulation did not interfere in the estimation of Artemether and Lumefantrine. Accuracy of the method was indicated by recovery values from 98.46 to 99.86 % for Artemether and 98.63 to 99.09 % for Lumefantrine. Precision is reflected by %RSD values less than 2. These low values suggest sensitivity of the developed method. Validation parameters were summarized in Table 3.

Table 3 Summary of validation parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Artemether</th>
<th>Lumefantrine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean % recovery</td>
<td>98.46-99.86</td>
<td>98.63-99.09</td>
</tr>
<tr>
<td>Precision (% RSD)</td>
<td>1.06</td>
<td>0.10</td>
</tr>
<tr>
<td>Interday (n=3)</td>
<td>0.79</td>
<td>0.74</td>
</tr>
<tr>
<td>Intraday (n=3)</td>
<td>1.01</td>
<td>1.15</td>
</tr>
<tr>
<td>Retention time (min)</td>
<td>13.888</td>
<td>7.207</td>
</tr>
<tr>
<td>Theoretical plates</td>
<td>17578</td>
<td>20012</td>
</tr>
<tr>
<td>Tailing factor</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CONCLUSION

The developed RP-HPLC method was simple, sensitive, precise and accurate and hence can be used in routine for the simultaneous determination of Artemether and Lumefantrine in bulk as well as in pharmaceutical preparations.

REFERENCES


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