VALIDATED DERIVATIZATION SPECTROFLUOROMETRIC METHOD FOR ARTESUNATE AND ARTEMETHER IN TABLET DOSAGE FORM

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ABSTRACT
A validated derivatization spectrofluorometric method was developed for the determination of artesunate and artemether in tablet dosage form. Artesunate and artemether are semi synthetic drugs obtained as a derivative of artemisinin. Both of the drugs did not have native fluorescence with a fused sesquiterpene lactone ring system varying in the substitution at C10. The drug artemether was treated with acetic anhydride and sulphuric acid and artesunate was treated with acetic acid and sulphuric acid in fixed ratio and heated to convert the compounds into a fluorescent moiety. The peroxide linkage present in the fused lactone ring system is converted into a unsaturated group for artemether and artesunate respectively. The method was validated according to the ICH guidelines for validation of analytical procedures. The linearity was found in the concentration range of 100 to 500 ng/ml for artesunate and between 100 to 800 ng/ml for artemether. The assay values were found between 98.2 to 100.67 % for both the compounds with a %RSD of 0.022842 and 0.4740 respectively. The % RSD of intraday and interday assay was found to be less than 1. Hence the reported method was found to be precise and accurate for the quantitative determination of artesunate and artemether in tablet dosage form. The interference studies was done with the excipients present in the formulations and found to have no interferences. The solution of artesunate was found to be stable for 2 hours and for artemether was found to be stable for 1 hour.

Key words: Spectrofluorimetry, Artemether, Artesunate, Derivatization, Validation.

INTRODUCTION
Artesunate and artemether are semi synthetic drugs derived from the natural anti malarial drug artemisinin which is traditional Chinese medicine. Chemically artemether is (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 artesunate is(3R,5aS,6R,8aS,9R,10S,12R,12aR)– Decahydro-3,6,9-trimethyl-3,12-epoxy12H-pyrano[4,3-j]-1,2benzodioxepin-10-ol hydrogen. The artesunate has a hydrogen succinate at C10 position and artemether has methoxy at C10 position. They both have similar peroxide linkage in the fused heptane ring. The compounds do not have any native fluorescence and absorption in the UV visible region due to the lack of unsaturation group. The fluorescence was developed by treating them with acid and heat. Artesunate was treated with acetic acid and sulphuric acid (2:1) and heated at 100° C developing greenish brown fluorescence. Artemether was treated with acetic anhydride and sulphuric acid (5:1) developing a greenish yellow fluorescence. The method was validated according to ICH guidelines. The literature survey reveals that few methods such as Colorimetry [1], Differential pulse polaroigraphy [2], HPLC with post column alkali and pre column acid derivatization [3] and UV detection [4] were reported for Artemether. Methods like HPLC [5,6], UV Spectrophotometry with simulated intestinal fluid [7] and LC-MS method [8] were reported for artemesunate. The presence of peroxide bridge in the structure of the compound offers the advantage of the use of acids to

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derivatize both the drugs in a unsaturated compound.

**Artesunate**

![Artesunate structure]

**Artemether**

![Artemether structure]

**Instruments and reagents used**

Shimadzu UV-Visible spectrophotometer 1201, Jasco 750 FP spectrofluorimeter and shimadzu AY220 – Analytical single pan balance were used to do the work. The solvents and acids used are of analytical grade procured from s.d.fine chemicals Ltd., Mumbai. The pure drug was procured commercially. The tablets were procured from the local market.

**Preparation of standard stock solutions**

10 mg of each of artesunate and artemether pure drug was accurately weighed and transferred to 10 ml volumetric flask individually. To the drug artesunate acetic acid and sulphuric acid (2:1) was added by placing the flask in a ice bath. The artemether solution was prepared by adding acetic anhydride and sulphuric acid (5:1) by placing in ice bath. Both the solutions were brought to the room temperature and kept in the boiling water bath (100°C) for 5 minutes with shaking at regular intervals. Then the solutions were brought to the room temperature and made up to the volume with methanol cautiously.

**Preparation of test stock solutions**

20 tablets of each of artesunate and artemether was weighed separately and made into a fine powder. Tablet powder of 10 mg equivalent of the each drug was weighed accurately and transferred to a 10 ml volumetric flask. The fluorescence was developed in a same manner as for the standard drug for both the drugs. The volume was made with methanol and the solution was filtered through a whatmann filter paper.

**Validation**

**Specificity**

The method developed was checked for its specificity by analyzing the excipients such as starch, lactose, talc and magnesium separate separately in the similar procedure used for the pure drug and no specific absorption peak and fluorescence found at the wavelength used for both the drugs.

**Linearity and range**

From the standard stock solution further dilutions were done to give a concentration range of 100 -500 ng/ml for artesunate and 100-800 ng/ml for artemether. Linearity of both the drugs were found to obey the Beer-Lambert’s law within the above mentioned range above which there was decrease in the fluorescence intensity. The values are reported in table no 1.

**Accuracy**

The test stock solution was further diluted to give a concentration of 300 ng/ml and emission spectra was obtained by fixing the excitation wavelength at 438 nm for artemether and 303 nm for artesunate. The assay was repeated 5 times and the average is reported in the table no 1.

**Precision**

Intraday and interday assay studies were performed. The intraday assay studies was done within the same day using 300ng/ml test solution. The interday studies were done using the same concentration for three successive days. The % RSD calculated was found to be below 1 and the recovery values were within the limits of 99.05 and 101.45 %.

**Limit of detection and limit of quantitation**

The limit of detection and limit of quantitation was done experimentally and found to be 50 ng/ml and 100 ng/ml for artesunate and 75 ng/ml and 100 ng/ml for artemether respectively.

**Stability**

The stability was checked for both the drugs for every 15 minutes under similar experimental conditions and the fluorescence was found to be stable for 2 hours for artesunate and one hour for artemether.

**RESULTS AND DISCUSSIONS**

Artesunate and artemether have no reported spectrofluorimetric method for its analysis in pharmaceutical formulations. The literature survey reveals the development of stability studies, UV spectroscopic method and post column derivatisation HPLC method. The present study was validated according to ICH guidelines for validation of analytical procedures. So the
reported method can be used for the routine analysis of artesunate and artemether in tablet dosage form.

Both the drugs as such do not have an absorption in the UV-Visible region due to the absence of unsaturation in the structure. The present method involves the derivatization of the artesunate and artemether in a fluorescent compound by treating with acetic acid and sulphuric acid in the ratio of 2:1 for artesunate and acetic anhydride and sulphuric acid in the ratio of 5:1 and heating at 100°C for 5 minutes under controlled temperature conditions.

Table 1. Validation parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Artesunate</th>
<th>Artemether</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity Range</td>
<td>100-500 ng/ml</td>
<td>100-800 ng/ml</td>
</tr>
<tr>
<td>Correlation coefficient(r²)</td>
<td>0.99885</td>
<td>0.99961</td>
</tr>
<tr>
<td>Regression equation</td>
<td>y=-0.5+0.607*X</td>
<td>y=0.964+0.407*X</td>
</tr>
<tr>
<td>Slope(b)</td>
<td>0.607</td>
<td>0.40702</td>
</tr>
<tr>
<td>y-intercept(a)</td>
<td>-0.5</td>
<td>0.96429</td>
</tr>
<tr>
<td>Assay values(% RSD)</td>
<td>0.022842</td>
<td>0.4740</td>
</tr>
<tr>
<td>Accuracy studies</td>
<td>98.2 to 100.67 %</td>
<td>99.62-100.33%</td>
</tr>
<tr>
<td>Precision</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intraday (% RSD) (n=6)</td>
<td>0.3176</td>
<td>0.3086</td>
</tr>
<tr>
<td>Interday(% RSD) (n=6)</td>
<td>0.2520</td>
<td>0.7621</td>
</tr>
<tr>
<td>LOD &amp;LOQ</td>
<td>50 ng/ml and 100 ng/ml</td>
<td>75 ng/ml and 100 ng/ml</td>
</tr>
<tr>
<td>Stability of fluorescence</td>
<td>2 hours</td>
<td>1 hour</td>
</tr>
</tbody>
</table>

Figure 1. UV Spectrum of Artesunate
Various solvents like chloroform, acetonitrile, 0.1 M acetic acid and methanol were scanned between 400-200 nm for the absorbance spectra. Methanol was found to be the most suitable solvent as it gave an absorption spectrum at 438 nm for artemether and 303 nm for artemesunate. The various experimental conditions such as ratio of acetic acid and sulphuric acid for artemesunate, ratio of acetic anhydride and sulphuric acid for artemether, heating time and temperature were fixed.

The excitation wavelength was fixed at 438 nm and 303 nm for artemesunate and artemether based on the absorption spectrum obtained. The emission wavelength was fixed at 508 nm and 609 nm for artemether and artemesunate. The solution of artemesunate was found to be stable for 2 hours and for artemether was found to be stable for 1 hour.

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