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*Research Article*

# Simultaneous Spectrophotometric Estimation of Artesunate and Mefloquine

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A simple, rapid, precise, and accurate UV-visible spectrophotometric method has been developed for the simultaneous determination of Artesunate in combination with Mefloquine. For developing the method, methanol was used as a solvent. Artesunate and Mefloquine showed $\lambda_{max}$ at 240 nm and 222 nm, respectively. The proposed method was validated as per ICH guideline. The linearity range of Artesunate and Mefloquine were 10–60 and 20–120 $\mu$g/mL, respectively. 99.91 ± 0.2740 and 99.56 ± 0.2067 these value represent the percent recovery of Artesunate and Mefloquine respectively The correlation coefficients of Artesunate and Mefloquine were 0.999, and 0.999, respectively. The relative standard deviation for six replicates was always less than 2%. The statistical analysis proves that the method is suitable for the analysis of Artesunate and Mefloquine as the bulk drugs and in pharmaceutical formulation without any interference from the excipients.

## 1. Introduction

A simple, rapid, precise, and accurate UV-visible spectrophotometric method has been developed for the simultaneous determination of Artesunate in combination with Mefloquine. ART and MEF are poorly water soluble drugs; therefore, methanol was used as a solvent as it is completely soluble in it. Methanol did not interfere in the spectroscopic determination of ART and MEF having maximum absorbance at 240 nm and 222 nm, respectively.

Chemically Artesunate is (3R,5aS,6R,8aS,9R,10S,12R, 12aR)-Decahydro-3,6,9 trimethyl-3,12-epoxy-12H-pyrano [4,3-j]-1,2benzodioxepin-10-ol, hydrogen succinate, while Mefloquine is DL-erythro-α-2-piperidyl-2,8-bis(trifluoromethyl)-4quinolinemethanol monohydrochloride; (R*, S*)-(±)-& alpha;-2-piperidinyl-2,8-bis(trifluoromethyl)-4-quinolinemethanol monohydrochloride. Structures of ART and MEF are shown in Figure 1. Artesunate and its active metabolite dihydroartemisinin are potent blood schizonticides, active against the ring stage of the parasite. Artesunate is ideal for the treatment of severe malaria, including cerebral malaria [1, 2].

Standard drug sample of Artesunate and Mefloquine was pursued as a gift sample from Cipla Ltd. and Macleoids Ltd. All chemicals and solvents of AR grade were purchased from Qualigens fine Chemicals, Mumbai, India.

UV-spectrophotometer UV-1800 (Shimadzu, Japan) with spectral bandwidth of 2 nm and 10 mm matched quartz cells were used for the development analytical method over the range of 200–400 nm. Marketed formulation *Falcigo Plus* tablet containing ART 100 mg and MEF 200 mg was used as sample, purchased from local market. Calibrated glassware was used throughout the work.

## 2. Experimental

*2.1. Preparation of Standard Stock Solutions.* An accurately weighed quantity of about 10 mg of pure drug of ART was dissolved in methanol and diluted to 100 mL. (Concentration 100 $\mu$g/mL).

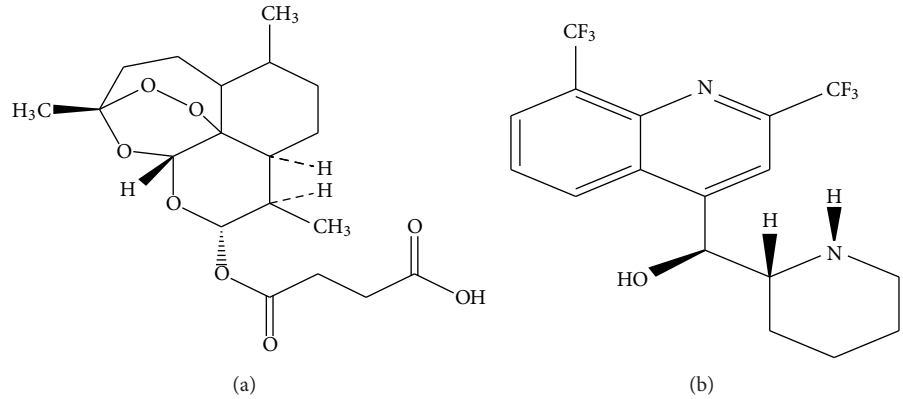


Figure 1: (a) Structure of Artesunate and (b) structure of Mefloquine.

Table 1: Standard calibration table for ART and MEF.

| Sr. no. | For Artesunate | | For Mefloquine | |
|---|---|---|---|---|
| | Conc. ($\mu$g/mL) | Abs.* at 240 nm | Conc. ($\mu$g/mL) | Abs.* at 222 nm |
| (1) | 10 | 0.037 | 20 | 0.132 |
| (2) | 20 | 0.07 | 40 | 0.28 |
| (3) | 30 | 0.107 | 60 | 0.397 |
| (4) | 40 | 0.139 | 80 | 0.548 |
| (5) | 50 | 0.175 | 100 | 0.676 |
| (6) | 60 | 0.208 | 120 | 0.796 |

*Each value is a mean of six observations.

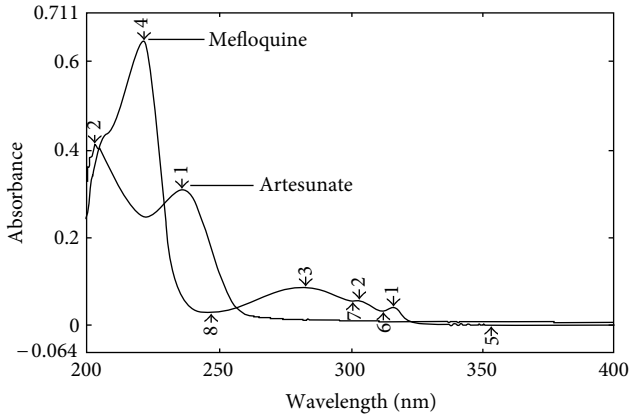


Figure 2: Overlay spectrum of the ART and MEF.

Similarly, accurately weighed quantity of about 20 mg of pure drug of MEF was dissolved in methanol and diluted to 100 mL. (Concentration 200 $\mu$g/mL).

*2.2. Selection of Analytical Wavelengths.* Appropriate dilutions were prepared for each drug from the standard stock solution and scanned in the spectrum mode from 400 nm to 200 nm. ART and MEF showed absorbance maxima at 240 nm (Figure 2) and at 222 nm (Figure 3), respectively. Figure 4 represents the overlay spectra for ART and MEF.

Table 2: Optical characteristics and other parameters.

| Parameters | ART | MEF |
|---|---|---|
| Working wavelength (nm) | 240 | 222 |
| Linearity range ($\mu$g/mL) | 10–60 | 20–120 |
| Molar absorptivity | 3.7 | 6.6 |
| Limit of detection ($\mu$g/mL) | 0.54 | 0.45 |
| Limit of quantitation ($\mu$g/mL) | 1.79 | 1.48 |
| $Y = mx + c$ | | |
| Slope | 0.003 | 0.006 |
| Intercept | 0.0001 | 0.0003 |
| Regression coefficient | 0.999 | 0.998 |

*2.3. Selection of Analytical Concentration Ranges.* From the standard stock solution of ART, appropriate aliquots were pipetted out into 10 mL volumetric flasks and dilutions were made with methanol to obtain working standard solutions of concentrations 10–60 $\mu$g/mL. Absorbance for these solutions were measured at 240 nm (Table 1) and a calibration curve of absorbance against concentration was plotted as shown in (Figure 3).

Similarly, a series of standard solutions of concentration 20–120 $\mu$g/mL were prepared for MEF and their absorbance was measured at 222 nm (Table 1). A standard calibration curve of absorbance against concentration was plotted (Figure 4). Both drugs followed the Beers-Lamberts law in the range of 10–60 $\mu$g/mL and 20–120 $\mu$g/mL for ART and MEF,

Table 3: Absorbance of mixed standards containing ART and MEF.

| Sr. no. | Mixed Standards | | Abs. at 240 nm | Abs. at 222 nm |
|---|---|---|---|---|
| | Conc. of ART ($\mu$g/mL) | Conc. of MEF ($\mu$g/mL) | | |
| (1) | 10 | 20 | 0.039 | 0.134 |
| (2) | 30 | 60 | 0.112 | 0.399 |
| (3) | 60 | 120 | 0.204 | 0.790 |

Table 4: Results of mixture containing ART and MEF.

| Sr. no. | Amount present* ($\mu$g/mL) | | Amount found* ($\mu$g/mL) | | % Amount found* | |
|---|---|---|---|---|---|---|
| | ART | MEF | ART | MEF | ART | MEF |
| (1) | 10 | 20 | 9.98 | 19.89 | 99.80 | 99.45 |
| (2) | 30 | 60 | 29.85 | 59.76 | 99.50 | 99.60 |
| (3) | 60 | 120 | 59.93 | 119.65 | 99.88 | 99.70 |

*Each value is a mean of six observations.

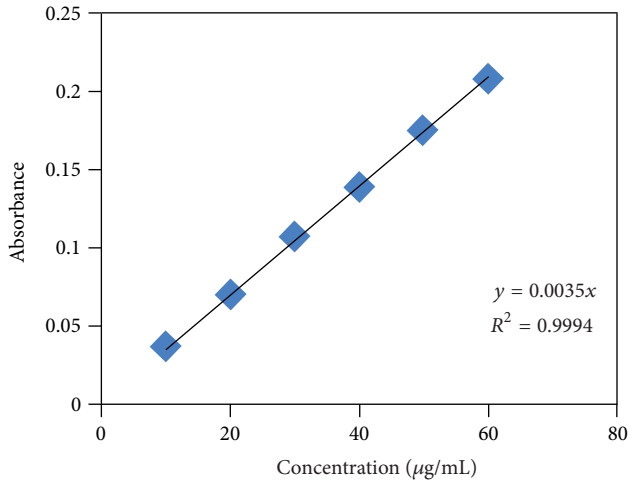


Figure 3: Calibration curve of ART.

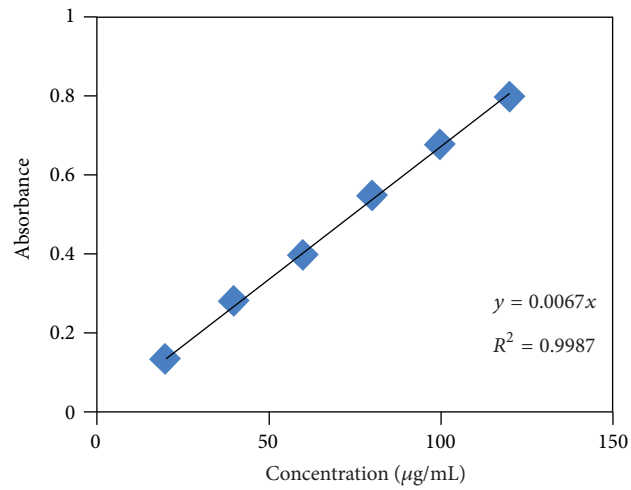


Figure 4: Calibration curve of MEF.

respectively. Table 2 summaries the optical characteristics of both drugs.

*2.4. Determination of Absorptivity Coefficients at Analytical Wavelengths.* The absorptivity coefficients for the two drugs were determined at both the selected wavelengths. The values obtained as the mean of six independent determinations were used for forming the simultaneous equations.

The simultaneous equations formed were as follows:

$$A_1 = 6.95 \times C_1 + 4.2 \times C_2 \quad (1)$$

at 222 nm (For Mefloquine)

$$A_2 = 3.5 \times C_1 + 6.58 \times C_2 \quad (2)$$

at 240 nm (For Artesunate), where $A_1$ and $A_2$ are the absorbance of sample solution at 222 nm and 240 nm, respectively, and $C_1$ and $C_2$ are the concentrations of Mefloquine and Artesunate, respectively, (in $gL^{-1}$) in the sample solution. By solving the two simultaneous equations, the concentration of mefloquine ($C_1$) and artesunate ($C_2$) in sample solutions can be obtained.

*2.5. Analysis Standard Containing ART and MEF.* The method was checked by analyzing a solution containing known concentration of both drugs. The mixed standards in the Beer-Lambert's range for each drug in the ratio of 1 : 2 containing 10, 30, and 60 $\mu$g/mL of ART and 20, 60, and 120 $\mu$g/mL of MEF, respectively, were prepared by diluting appropriate volumes of standard stock solutions. The scanning of mixed standard solutions was carried out in the range of 400 nm to 200 nm in spectrum mode (Table 3). The absorbance of mixed standard solutions was measured at 240 nm and 222 nm. The concentrations of ART and MEF present in mixed standards were calculated using (1) and (2) (Table 4). The results obtained were good and hence the method was applied to the marketed tablet formulation.

*2.6. Procedure for Analysis of Tablet Formulation.* Twenty tablets were weighed accurately; the average weight was determined and then triturated to a fine powder. A quantity equivalent to 100 mg of ART and 200 mg of MEF was weighed and transferred to a 100 mL volumetric flask containing

Table 5: Results of marketed tablet formulation.

| Sr. no. | Label claim (mg/tab) | | Amount found (mg/tab) | | % of Label claim | |
|---|---|---|---|---|---|---|
| | ART | MEF | ART | MEF | ART | MEF |
| (1) | 100 | 200 | 99.63 | 198.72 | 99.63 | 99.36 |
| (2) | 100 | 200 | 99.70 | 199.44 | 99.70 | 99.72 |
| (3) | 100 | 200 | 100.02 | 198.88 | 100.02 | 99.44 |
| (4) | 100 | 200 | 99.91 | 199.96 | 99.91 | 99.98 |
| (5) | 100 | 200 | 99.86 | 198.96 | 99.86 | 99.48 |
| (6) | 100 | 200 | 99.57 | 200.36 | 99.57 | 100.18 |
| | | | | Mean | 99.78 | 99.69 |
| | | | | SD | 0.1754 | 0.3287 |
| | | | | % RSD | 0.1757 | 0.3297 |

Formulation: *Falcigo Plus* (Zydus Cadila Pharmaceutical Ltd., Ahmadabad).

Table 6: Results of recovery studies.

| Level of (%) recovery | Amount present(mg/tab) | | Amount of standard added (mg) | | Total amountrecovered(mg) | | % Recovery* | |
|---|---|---|---|---|---|---|---|---|
| | ART | MEF | ART | MEF | ART | MEF | ART | MEF |
| 80 | 100 | 200 | 80 | 160 | 179.28 | 359.24 | 99.59 | 99.79 |
| 100 | 100 | 200 | 100 | 200 | 199.8 | 398.46 | 99.89 | 99.61 |
| 120 | 100 | 200 | 120 | 240 | 220.6 | 436.90 | 100.26 | 99.29 |
| | | | | | | % Mean | 99.91 | 99.56 |
| | | | | | | SD | 0.2740 | 0.2067 |
| | | | | | | RSD | 0.2742 | 0.2076 |

*Each value is the mean of three observations.

70 mL methanol, and the contents were sonicated for 20 min with methanol to dissolve the active ingredients. Volume was made up to 100 mL with methanol and filtered through Whatman filter paper no. 41 to give the stock solution containing 1000 $\mu$g/mL of ART and 2000 $\mu$g/mL of MEF. Various dilutions of the tablet stock solutions were scanned and the absorbance of these solutions were measured at 240 nm and 222 nm, respectively, and the concentrations of the two drugs in the sample solutions were calculated using (1) and (2). The analysis procedure was repeated six times. The results of marketed tablet formulation are given in Table 5.

*2.7. Recovery Studies.* Recovery studies were carried out at three levels that is, 80, 100, and 120% of the label claim of the Tablet formulation as per ICH guidelines [3, 4].

To perform recovery studies at 80% of the test concentration, sample containing 100 mg of ART and 200 mg of MEF was weighed and transferred to a 100 mL volumetric flask. To it, 80 mg of standard ART and 160 mg of standard MEF was added, the mixture was mixed thoroughly. Then 70 mL of methanol was added and the contents were sonicated for 20 min with methanol to dissolve the active ingredients, and the volume was made up to 100 mL with methanol and filtered through Whatman filter paper no. 41.

Similarly, to perform recovery studies at 100% of the test concentration, tablet powder containing 100 mg of ART and 200 mg of MEF was weighed. To it, 100 mg of standard ART and 200 mg of standard MEF was added and at 120% level, 120 mg of standard ART and 240 mg of standard MEF was added to the tablet powder equivalent to 100 mg of ART and 200 mg of MEF. Then 70 mL of methanol was added, the contents were sonicated for 20 min with methanol to dissolve the active ingredients, and the volume was made up to 100 mL with methanol and filtered through Whatman filter paper no. 41.

From the stock solutions prepared at each level, suitable aliquots were pipetted out and diluted to 10 mL with methanol and were analysed as per the procedure for tablet formulations. The results of the recovery studies were also validated statistically. The results of recovery studies are given in Table 6.

*2.8. Precision of Method.* Precision of the method was verified by using stock solutions in the ratio of 1 : 2 containing 60 $\mu$g/mL ART and 120 $\mu$g/mL of MEF. System repeatability was done by repeating the assay three times of six replicate dilutions of the same concentration after every two hours on the same day for intraday precision. Interday precision was carried out by performing the assay of six sample sets after 24 hours and 48 hours. The results of intermediate precision are given in Table 7.

## 3. Conclusion

The novel method for simultaneous estimation of ART and MEF was developed using alcoholic solubilization technique. ART and MEF follow Beer-Lambert's law in range

Table 7: Results of intermediate precision.

| Formulation | Parameter | Intraday precision* | Interday precision* |
|---|---|---|---|
| ART | Mean | 99.59 | 99.69 |
| | SD | 0.3177 | 0.3723 |
| | % RSD | 0.3190 | 0.3734 |
| MEF | Mean | 100.21 | 99.49 |
| | SD | 0.5657 | 0.2502 |
| | % RSD | 0.5645 | 0.2515 |

of 10–60 $\mu$g/mL, and 20–120 $\mu$g/mL shows ART and MEF can be estimated in Methanol. Commercial formulation containing ART and MEF were analyzed by proposed method Mean assay values in Falcigo Plus were found to be 99.78 ± 0.1754 and 99.69 ± 0.3287, respectively. The accuracy of method was determined by recovery studies. Pure ART and MEF were added to the preanalyzed tablet powder at three different levels, namely, 80, 100, and 120% of labeled claims as per the ICH guidelines. Three replicate analyses were carried out at each level. The mean recovery was found to be 99.91 ± 0.2740% and 99.56 ± 0.2067% in Falcigo Plus samples, respectively, indicating that the method has required accuracy and there was no interference from the common excipients present in tablets. The RSD value below 2% indicated that the method has required precision. LOD and LOQ values at 240 and 222 were found to be 0.54 and 0.45 $\mu$g/mL and 1.79 and 1.48 $\mu$g/mL, respectively.

Thus, the developed method was simple, accurate, and precise and can be used for routine analysis of ART and MEF in pharmaceutical preparation.

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