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# Development UV-visible spectrophotometric method for simultaneous estimation of artemether and lumefantrine

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## ABSTRACT

The novel method for simultaneous estimation of Artemether (ATM) and Lumefantrine (LUM) was developed using alcoholic solubilization technique. Methanol was used as a solvent because both drugs are completely soluble in it. Methanol did not interfere in the spectroscopic determination of ATM and LUM which have maximum absorbance at 258 nm and 235 nm respectively. ATM and LUM follow Beer-Lambert's law in range of 1-6 µg/mL and 6-36 µg/mL respectively. LOD and LOQ values of ATM and LUM were found to be 0.050 and 0.166 µg/mL and 0.145 and 0.484 µg/mL respectively. The proposed method is recommended for routine analysis since it is rapid, simple, accurate, precise, sensitive and specific.



## KEYWORDS

Artemether;
Lumifantrine;
Simultaneous estimation.

## INTRODUCTION

The combination of artemether and lumefantrine is an artemisinin-based combination therapy (ACT) used in the treatment of acute uncomplicated malaria produced by *plasmodium falciparum*.

Chemically ATM 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. Artemether is an antimalarial drug used in the treatment of multi-drug resistant strains of falciparum malaria and *P. Vivax*. ATM is effective against the blood flukes. Artemether has been shown to have significant anticancer and antitumor activities.

Chemically LUM is 2, 7-Dichloro-9-[(*Z*)-*p*-chlorobenzylidine]-alpha [(dibutylamino) methyl]-fluorene-4-methanol. Both ATM and LUM act by killing the parasites. Structures of ATM and LUM represented in Figure 1 and 2 respectively.

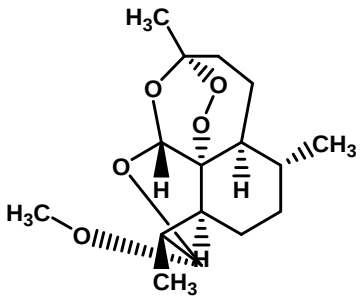


**Figure 1: Structure of artemether**

Standard drug sample of Artemether and

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

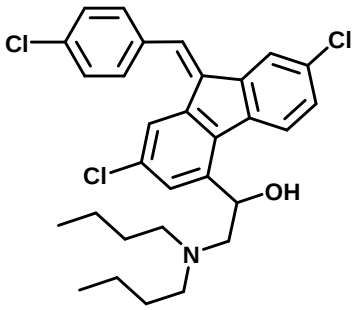


**Figure 2 : Structure of lumefantrine**

UV- spectrophotometer UV-1800 (Shimadzu, Japan) with spectral bandwidth of 2 nm and 10 mm matched quartz cells were used for development analytical method over the range of 200-400 nm.

Marketed formulation *Lumerax* tablet containing ATM 80 mg and LUM 480 mg was used as sample; purchased from local market.

## EXPERIMENTAL

### Preparation of standard stock solutions

100 mg of each pure drug was accurately weighed and transferred to individual 100 mL volumetric flask containing 70 mL methanol and diluted to 100 mL with methanol. Further dilutions carried out to get final concentration of 10 µg/mL of ATM and 100 µg/mL of LUM.

### 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. ATM and LUM showed absorbance maxima at 258 nm (Figure 3) and at 235 nm (Figure 4) respectively. Figure 5 represents the overlain spectra of both the drugs.

### Selection of analytical concentration ranges

From the standard stock solution of ATM, appropriate aliquots were pipetted out into 10 mL volumetric flasks and dilutions were made with methanol to obtain working standard solutions of concentrations 1- 6 µg/mL. Absorbance for these solutions were measured at 258 nm (TABLE I) and a calibration curve of absorbance against concentration was plotted (Figure 6)

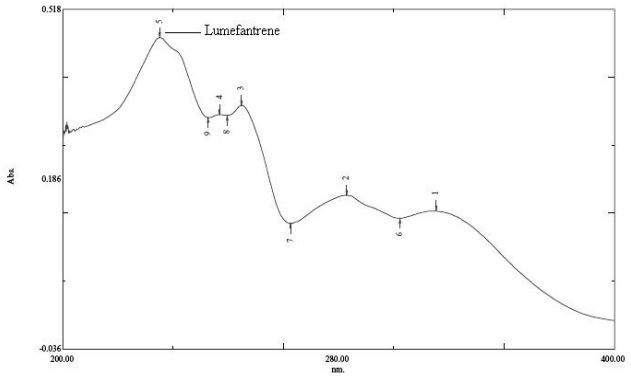


**Figure 3: UV spectrum of LUM**

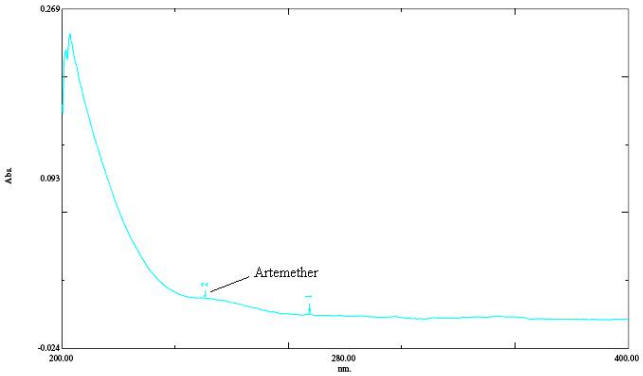


**Figure 4: UV spectrum of ATM**

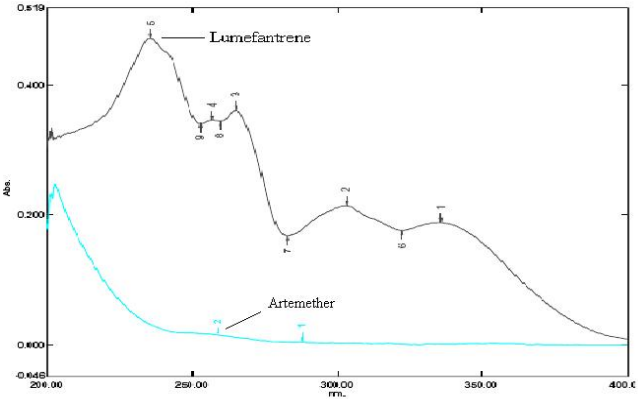


**Figure 5: Overlain spectrum of the ATM and LUM**

Similarly, a series of standard solutions of concentration 6 - 36 µg/mL were prepared for LUM and their absorbance were measured at 235 nm (TABLE 1). A standard calibration curve of absorbance against concentration was plotted (Figure 7). Both drugs followed the Beer-Lamberts law in the range of 1-6 µg/mL and 6-36 µg/mL for ATM and LUM respectively. TABLE 2 summaries the optical characteristics of both the drugs.

**TABLE 1: Standard calibration table for ATM and LUM**

| Sr. No. | For Artemether | | For Lumefantrine | |
|---|---|---|---|---|
| | Conc. (µg/mL) | Abs.* at 258 nm | Conc. (µg/mL) | Abs.* at 235 nm |
| 1. | 1 | 0.010 | 6 | 0.332 |
| 2. | 2 | 0.021 | 12 | 0.582 |
| 3. | 3 | 0.030 | 18 | 0.912 |
| 4. | 4 | 0.041 | 24 | 1.250 |
| 5. | 5 | 0.051 | 30 | 1.532 |
| 6. | 6 | 0.060 | 36 | 1.834 |

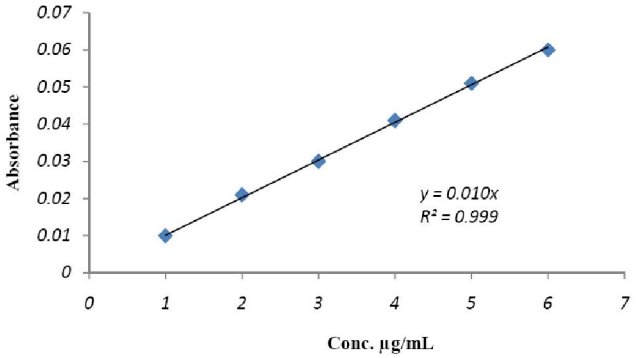


**Figure 6 : Calibration curve of ATM**

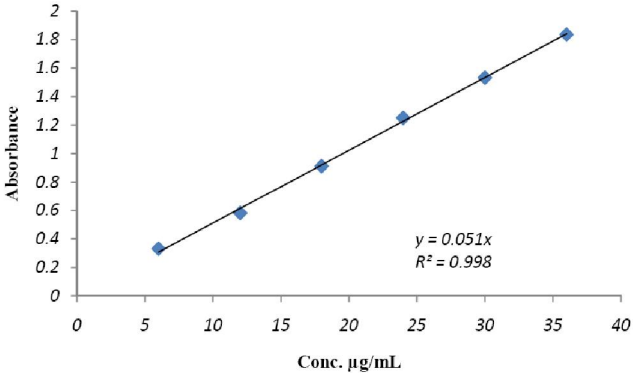


**Figure 7 : Calibration curve of LUM**

## Determination of absorptivity coefficients at analytical wavelengths

The absorptivity coefficients for two drugs were determined at both the selected wavelengths. The values obtained as mean of six independent determinations were used for forming the simultaneous equations.

The simultaneous equations formed were:

$$A_1 = 48.46 \times C_1 + 11 \times C_2 \text{ at } 235nm \quad (1)$$

(For Lumefantrin)

$$A_2 = 10.33 \times C_1 + 51.08 \times C_2 \text{ at } 258 nm \quad (2)$$

(For Artemether)

Where $A_1$ and $A_2$ are the absorbance of sample solution at 235 nm and 258 nm respectively and $C_1$ and $C_2$ are the concentrations of lumefantrin and artemether respectively (g/l) in the sample solution. By solving two simultaneous equations, the concentration of lumefantrin ($C_1$) and artemether ($C_2$) in sample solutions can be obtained.

**TABLE 2 : Optical characteristics and other parameters**

| Parameters | ATM | LUM |
|---|---|---|
| Working wavelength (nm) | 258 | 235 |
| Linearity range (µg/mL) | 1-6 | 6-36 |
| Molar absorptivity | 10 | 55.33 |
| Limit of detection (µg/mL) | 0.05 | 0.145 |
| Limit of quantitation (µg/mL) | 0.166 | 0.484 |
| Y= mx + c | | |
| Slope | 0.010 | 0.051 |
| Intercept | 0.007 | 0.012 |
| Regression Coefficient | 0.999 | 0.999 |

## Analysis standard containing ATM and LUM

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 1:6 containing 2, 4 and 6 µg/mL of ATM and 12, 24 and 36 µg/mL of LUM 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 258 nm and 235 nm. The concentrations of ATM and LUM present in mixed standards were calculated using equation 1 and 2. (TABLE 4) Good results were obtained and hence the method was applied to the marketed tablet formulation.

## 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 80 mg of ATM and 480 mg of LUM was weighed and transferred to a 100 mL volumetric flask containing 70 mL methanol 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 to give the stock solution containing 800 µg/mL of ATM and 4800 µg/mL of LUM. Various dilutions of the tablet stock

solutions were scanned and the absorbance of these solutions were measured at 235 nm and 258 nm respectively and the concentrations of the two drugs in the sample solutions were calculated using equations (1) and (2). The analysis procedure was repeated six times. The results of marketed tablet formulation are given in TABLE 5.

**TABLE 3 : Absorbance of mixed standards containing ATM and LUM**

| Sr. No. | Mixed Standards | | Abs. at 258 nm | Abs. at 235 nm |
|---|---|---|---|---|
| | Conc. of ATM (µg/mL) | Conc. of LUM (µg/mL) | | |
| 1. | 2 | 12 | 0.022 | 0.584 |
| 2. | 4 | 24 | 0.043 | 1.252 |
| 3. | 6 | 36 | 0.062 | 1.839 |

**TABLE 4 : Results of mixture containing ATM and LUM**

| Sr. No. | Amount Present * (µg/mL) | | Amount Found* (µg/mL) | | % Amount Found* | |
|---|---|---|---|---|---|---|
| | ATM | LUM | ATM | LUM | ATM | LUM |
| 1 | 2 | 12 | 1.99 | 12.01 | 99.50 | 100.08 |
| 2 | 4 | 24 | 3.97 | 23.86 | 99.25 | 99.41 |
| 3 | 6 | 36 | 6.01 | 35.86 | 100.16 | 99.61 |

***each value is a mean of six observations**

**TABLE 5 : Results of marketed tablet formulation**

| Sr. No. | Label Claim (mg/tab) | | Amount Found (mg/tab) | | % of Label Claim | |
|---|---|---|---|---|---|---|
| | ATM | LUM | ATM | LUM | ATM | LUM |
| 1 | 80 | 480 | 79.96 | 479.91 | 99.95 | 99.98 |
| 2 | 80 | 480 | 80.12 | 479.87 | 100.15 | 99.97 |
| 3 | 80 | 480 | 79.89 | 480.12 | 99.86 | 100.02 |
| 4 | 80 | 480 | 79.69 | 479.60 | 99.61 | 99.91 |
| 5 | 80 | 480 | 79.78 | 480.03 | 99.72 | 100.00 |
| 6 | 80 | 480 | 99.84 | 479.97 | 99.80 | 99.99 |
| | | | | Mean | 99.84 | 99.97 |
| | | | | SD | 0.1881 | 0.0376 |
| | | | | % RSD | 0.1884 | 0.0376 |

**formulation: Lumerax (Ipca pharmaceutical ltd, mumbai)**

## Linearity and range

The linearity for ATM and LUM were determined at six concentration levels, ranging from 1-6 µg/mL and 6-36 µg/mL respectively using working standards.

## Accuracy (Recovery studies)

Recovery studies were carried out at three levels i.e. 80, 100 and 120 % of the label claim of the Tablet formulation as per ICH guidelines.

To perform recovery studies at 80 % of the test concentration (sample containing 80 mg of ATM and 480 mg of LUM) was weighed and transferred to a 100 mL volumetric flask. To it, 64 mg of standard ATM and 384 mg of standard LUM was added, the mixture was mixed thoroughly then 70 mL methanol 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 80 mg of ATM and 480 mg of LUM was weighed. To it, 80 mg of standard ATM and 480mg of standard LUM was added and at 120 % level, 96 mg of standard ATM and 576 mg of standard LUM was added to the tablet powder equivalent to 80 mg of ATM and 480 mg of LUM. Added 70 mL methanol 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.

## Precision of method

Precision of the method was verified by using stock solutions in the ratio of 1:6 containing 4 µg/mL of ATM and 24 µg/ml of LUM. 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 8.

## Limit of detection and limit of quantitation

The Limit of Detection (LOD) is the smallest concentration of the analyte that gives the measurable response. LOD was calculated using the following formula and shown in TABLE 2

**LOD = 3.3 (σ / S)**

**TABLE 6 : Results of recovery studies**

| Level of (%) Recovery | Amount present (mg/tab) | | Amount of standard added (mg) | | Total amount recovered (mg) | | % Recovery* | |
|---|---|---|---|---|---|---|---|---|
| | ATM | LUM | ATM | LUM | ATM | LUM | ATM | LUM |
| 80 | 80 | 480 | 64 | 384 | 149.3 | 897.20 | 99.91 | 99.98 |
| 100 | 80 | 480 | 80 | 480 | 159.76 | 959.25 | 99.85 | 99.92 |
| 120 | 80 | 480 | 96 | 576 | 176.01 | 1056.05 | 100.00 | 99.97 |
| | | | | | | Mean | 99.92 | 99.95 |
| | | | | | | SD | 0.0616 | 0.0262 |
| | | | | | | % RSD | 0.0616 | 0.0262 |

***each value is the mean of three observations**

**TABLE 7 : Results of intermediate precision**

| Formulation | Parameter | Intra-day precision* | Inter-day precision* |
|---|---|---|---|
| ATM | % Mean | 99.86 | 100.04 |
| | SD | 0.3092 | 0.1357 |
| | RSD | 0.3096 | 0.1356 |
| LUM | % Mean | 99.79 | 99.91 |
| | SD | 0.3724 | 0.1006 |
| | RSD | 0.3731 | 0.1006 |

***each value is a mean of six observations**

**TABLE 8 : Summary of validation parameters**

| Parameters | | ATM | LUM |
|---|---|---|---|
| Linearity Range (µg/mL) | | 1-6 | 6-36 |
| Correlation coefficient ($r^2$) | | 0.999 | 0.999 |
| Precision (RSD) | Intraday* | 0.3096 | 0.3731 |
| | Interday* | 0.1356 | 0.1006 |
| Accuracy (%) | | 99.92±0.0612 | 99.95±0.0262 |
| Repeatability * | | 99.84±0.1881 | 99.97±0.0376 |
| LOD (µg/mL) | | 0.05 | 0.145 |
| LOQ (µg/mL) | | 0.166 | 0.484 |

***Mean of six determinations**

Where, S = slope of the calibration curve & σ = standard deviation of the response. The Limit of Quantification (LOQ) is the smallest concentration of the analyte, which gives a response that can be accurately quantified. LOQ was calculated using the following formula and shown in TABLE 2.

**LOQ = 10 (σ / S)**

Where, S = slope of calibration curve & σ = standard deviation of the response.

## RESULTS AND DISCUSSION

In the present work, new simultaneous estimation method was developed for the simultaneous spectroscopic estimation of ATM and LUM in commercially available tablet dosage form.

The concentrations range of 1-6 µg/mL of ATM and 6-36 µg/mL of LUM respectively. Mixed working standard of drugs and two set of wavelengths gave optimum accuracy, precision, time, economy, and sensitivity for this method. The proposed procedure was successfully applied to the determination of ATM and LUM in the commercially available tablets dosage form, and the results are shown in TABLE 5.

The recovery studies were carried out at different concentrations by spiking a known concentration of standard drug to the pre-analyzed sample and contents were reanalyzed by proposed methods. The method was validated statistically for range, linearity, precision, accuracy, repeatability, LOD, and LOQ (TABLE 2). Accuracy was ascertained on the basis of recovery studies. Precision was calculated as interday and intraday variation for both the drugs. The contents estimated using the proposed method was found in agreement with the labeled amount. The relative standard deviations were found to be within the limit, indicating good accuracy, precision, and repeatability of the proposed method.

## CONCLUSION

The first novel method for simultaneous estimation of ATM and LUM was developed using alcoholic solubilization technique. ATM and LUM were 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 ATM and LUM having maximum absorbance at 258 nm and

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235 nm respectively. ATM and LUM follows Beer-Lambert's law in range of 1-6 µg/mL and 6-36 µg/mL shows ATM and LUM can be estimated in Methanol. Commercial formulation containing ATM and LUM were analyzed proposed method. Mean assay values in Lumerax were found to be 99.84±0.1884 and 99.97±0.0376 respectively. The accuracy of method was determined by recovery studies. Pure ATM and LUM were added to the preanalyzed tablet powder at three different levels viz. 80, 100, 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 99.92±0.0616 % and 99.95±0.0262 % in Lumerax 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 258 and 235 were found to be 0.05 and 0.145 µg/mL and 0.166 and 0.484 µg/mL respectively.

We propose the present method for routine analysis of ATM and LUM in pharmaceutical preparations as this method is simple accurate and precise.

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