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Short communication

# Simultaneous determination of artemether and lumefantrine in fixed dose combination tablets by HPLC with UV detection

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ABSTRACT

This paper describes the development and validation of a HPLC-UV method (210 nm) for the simultaneous quantitation of artemether and lumefantrine in fixed dose combination tablets. The method showed to be linear ($r^2 > 0.99$), precise (R.S.D. < 2.0%), accurate (recovery of 101.07% for artemether and 101.58% for lumefantrine), specific and robust. Four batches of artemether–lumefantrine tablets were assayed by the validated method. The artemether contents in the tablets varied from 98.61% to 103.35%, while lumefantrine contents were 97.92–100.48%.



## 1. Introduction

Malaria is the world's most important parasitic infection, ranking among the major health and developmental challenges for the poor countries of the world [1]. One of the greatest challenges facing malaria control worldwide is the spread and intensification of parasite resistance to antimalarial drugs. The limited number of such drugs has led to increasing difficulties in the development of antimalarial drug policies and adequate disease management [2].

Artemisinin-based combination therapy (ACT) is increasingly being advocated as promising treatment. ACT is based on the use of two drugs with different modes of action: an artemisinin-derivative that causes rapid and effective reduction of parasite biomass and gametocyte carriage and a partner drug that has a longer duration of action [3].

Artemether–lumefantrine is an ACT widely used nowadays and consists of a registered fixed dose combination of artemether (20 mg) and lumefantrine (120 mg) in tablets (Fig. 1). The rationale is that artemether will rapidly reduce parasitemia, resulting in symptomatic relief, and lumefantrine will eliminate the remaining parasites [4]. World Health Organization (WHO) recommends this association as first line therapy for falciparum malaria in endemic areas [5].

The increasing use of artemether–lumefantrine association as an effective treatment for resistant malaria demands the need of analytical methods to simultaneously quantify these drugs in tablets in order to evaluate its quality. Some papers have described the analysis of artemether in plasma, based on HPLC with electrochemical [6–8] or mass spectrometry detection [9]. Few methods are available to assay artemether in pharmaceutical products [10,11]. The quantitative determination of lumefantrine in plasma has been described using HPLC with UV detection [12–15]. However, there is no method reported regarding the simultaneous quantitation of artemether and lumefantrine.

Hence, the aim of this study was to develop and validate a HPLC method, using UV detection, to simultaneously quantify artemether and lumefantrine in fixed dose combination tablets. Due to the low molar absorptivity of artemether in the UV region (210 nm), and the lower concentration of this drug in the tablets compared to lumefantrine, standard addition of artemether was carried out to improve its detection. The validated method was applied to the analysis of tablets containing the artemether–lumefantrine association (20 + 120 mg).

## 2. Experimental

### 2.1. Reagents and materials

Artemether and lumefantrine reference standards were purchased from Dafra Pharma (Turnhout, Belgium). Coartem® (Novartis, Basel, Switzerland) tablets were kindly donated by

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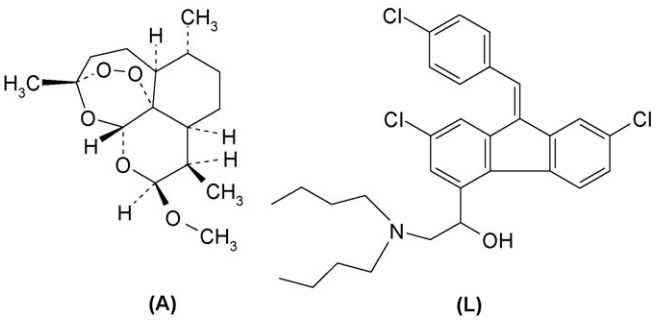


**Fig. 1.** Chemical structures of artemether (A) and lumefantrine (L).

Brazilian Health Ministry and Co-artesiane® tablets were purchased from Dafra Pharma (Turnhout, Belgium). Ultra-pure water was obtained from a Millipore system (Bedford, MA, USA). Acetonitrile, trifluoroacetic acid and chloroform (HPLC grade) were obtained from Tedia (Fairfield, OH, USA).

### 2.2. Instrumental and analytical conditions

The HPLC analyses were carried out on an Agilent 1200 system (Palo Alto, CA, USA), composed of a quaternary pump, autosampler, diode array detector (DAD) and HP ChemStation software. The columns evaluated were a Zorbax SB-Ciano (150 × 4.6 mm i.d.; 5 μm particle size) from Agilent (Santa Clara, CA, USA) and a Symmetry $C_{18}$ (250 × 4.6 mm i.d.; 5 μm particle size) from Waters (Milford, MA, USA), both maintained at 30 °C. UV detection was performed at 210 nm. UV spectra from 190 to 400 nm were online recorded for peak identification. The injection volume was 20 μl. An isocratic mobile phase containing acetonitrile and 0.05% trifluoroacetic acid (60:40, v/v) was used at a flow rate of 1.0 ml/min. The separation of artemether and lumefantrine was evaluated in different proportions of these solvents and, for each condition, retention factor ($k$) and resolution ($R$) were calculated. In order to determine $k$, $t_0$ was estimated by injecting a 0.01% (w/v) $NaNO_3$ solution in mobile phase onto the chromatograph. The optimized condition was achieved using the Zorbax SB-Ciano column and a mobile phase composed of acetonitrile and 0.05% trifluoroacetic acid (60:40), pH 2.35.

### 2.3. Preparation of standard solutions

#### 2.3.1. Artemether–lumefantrine standard solution

Approximately 40 mg of artemether and 30 mg of lumefantrine reference standards were accurately weighed and transferred to a 100 ml volumetric flask. Chloroform (2 ml) was added to ensure complete solubilization, followed by the addition of 80 ml of acetonitrile. The volume was filled to the mark with 0.05% trifluoroacetic acid, to obtain a solution at 400 μg/ml of artemether and 300 μg/ml of lumefantrine.

#### 2.3.2. Artemether stock solution

Approximately 175 mg of artemether reference standard was accurately weighed and transferred to a 100 ml volumetric flask. Chloroform (2 ml) was added to ensure complete solubilization and the flask volume was completed with acetonitrile. The final concentration was 1750 μg/ml of artemether.

#### 2.3.3. Artemether work solution

An aliquot of 10 ml of *artemether stock solution* was transferred to a 50 ml volumetric flask and the volume was adjusted with mobile phase, to obtain a solution at 350 μg/ml of artemether.

### 2.4. Analysis of fixed dose combination tablets

Three different batches of Coartem® and one batch of Co-artesiane® were analyzed using the validated method. Artemether standard was added to the samples, with the aim of increasing the peak area of artemether in the chromatograms and thereby improving the detection of this compound. Due to the poor solubility of lumefantrine, chloroform was added to ensure the complete solubilization of the samples. For the analysis, six replicates of each batch were assayed. The tablets were weighed and finely powdered. An accurately weighed portion of the powder, equivalent to about 25 mg of artemether and 150 mg of lumefantrine, was transferred to a 100 ml volumetric flask followed by the addition of 5 ml of chloroform. The solution was sonicated for 3 min and diluted with acetonitrile to volume. An aliquot of 10 ml of this solution was transferred to a 50 ml volumetric flask and 10 ml of the *artemether stock solution* (described in Section 2.3) was added. The volume was adjusted with mobile phase. An *artemether work solution* was also prepared by diluting 10 ml of the *artemether stock solution* to 50 ml with mobile phase. To calculate artemether content in the tablets, the peak area of artemether obtained in the *artemether work solution* was subtracted from the peak area of artemether obtained in the sample solutions. The difference corresponds to the amount of artemether originally present in the tablet samples.

### 2.5. Validation

#### 2.5.1. Linearity

Standard solutions containing 750 μg/ml of lumefantrine were prepared, in triplicate. Aliquots of these solutions were diluted in mobile phase to five different concentrations, corresponding to 150, 225, 300, 375 and 450 μg/ml of lumefantrine. For the artemether, solutions were prepared using the standard addition procedure. Stock sample solutions containing 250 μg/ml of artemether were prepared in triplicate. An aliquot of these sample solutions was diluted in mobile phase and 10 ml of *artemether stock solution*, corresponding to 350 μg/ml of artemether, was added. Therefore, the final concentrations were 375, 387.5, 400, 412.5 and 425 μg/ml of artemether. Calibration curves for concentration versus peak area were plotted for each compound and the obtained data were subjected to regression analysis using the least squares method with a weighting factor of $1/x$.

#### 2.5.2. Precision

The intra-day precision was evaluated by analyzing six sample solutions ($n = 6$), at the final concentration of analyses (400 μg/ml of artemether and 300 μg/ml of lumefantrine). Similarly, the inter-day precision was evaluated in three consecutive days ($n = 18$). The artemether and lumefantrine concentrations were determined and the relative standard deviations (R.S.D.) were calculated.

#### 2.5.3. Accuracy

Artemether and lumefantrine reference standards were accurately weighed and added to a mixture of the tablet excipients, at three different concentration levels (300, 400 and 500 μg/ml of artemether and 225, 300 and 375 μg/ml of lumefantrine). At each level, samples were prepared in triplicate and the recovery percentage was determined.

#### 2.5.4. Specificity

Spectral purities of artemether and lumefantrine chromatographic peaks were evaluated using the UV spectra recorded by a diode array detector. In addition, a solution containing a mixture

**Table 1**
Chromatographic parameters for artemether and lumefantrine at different mobile phase compositions using a Zorbax SB-Ciano column

| Mobile phase composition, Acetonitrile:0.05% trifluoroacetic acid | Artemether retention factor ($k$) | Lumefantrine retention factor ($k$) | Resolution ($R$) |
|---|---|---|---|
| 80:20 | 0.32 | 0.28 | 0.00 |
| 75:25 | 0.38 | 0.36 | 0.00 |
| 70:30 | 0.48 | 0.56 | 0.00 |
| 65:35 | 0.63 | 0.91 | 0.54 |
| 60:40 | 0.86 | 1.53 | 2.38 |
| 55:45 | 1.14 | 2.19 | 3.23 |

of the tablet excipients was prepared using the sample preparation procedure and injected onto the chromatograph, to evaluate possible interfering peaks.

*2.5.5. Robustness*

Six sample solutions were prepared and analyzed under the established conditions and by variation of the following analytical parameters: flow rate of the mobile phase (0.8, 1.0 and 1.2 ml/min), acetonitrile proportion in mobile phase (58%, 60% and 62%), mobile phase pH (2.15, 2.35 and 2.55) and column temperature (25, 30 and 35 °C). The artemether and lumefantrine contents were determined for each condition and the obtained data were submitted for statistical analysis (ANOVA test).

*2.5.6. Detection and quantitation limits*

Combined standard solutions were prepared by sequential dilutions and injected onto the chromatograph, at decreasing concentrations, in the range of 0.13–15 μg/ml of artemether and 0.10–11.25 μg/ml of lumefantrine. The limit of detection was defined as the concentration for which a signal-to-noise ratio of 3 was obtained and, for quantitation limit, a signal-to-noise ratio of 10 was considered.

## 3. Results and discussion

The chromatographic parameters were initially evaluated using a Symmetry $C_{18}$ column and a mobile phase composed of acetonitrile and 0.05% trifluoroacetic acid (80:20). Under these conditions, the retention factors obtained for artemether and lumefantrine were 5.05 and 0.61, respectively. In spite of achieving a good resolution, this condition exhibited a long run time, since artemether peak was eluted after 9 min. Hence, the Symmetry $C_{18}$ was substituted by a Zorbax SB-Ciano column. Using this column, different proportions of mobile phase solvents were evaluated, to obtain an adequate resolution between artemether and lumefantrine peaks (Table 1). The mobile phase composed of acetonitrile and 0.05% trifluoroacetic acid (60:40) promoted an adequate separation ($R$ = 2.38), and a short run time (5 min), and so, this condition was adopted in subsequent analyses (Fig. 2A).

Artemether shows UV absorption only in the initial wavelengths of the spectrum (200–220 nm), due to the absence of chromophores in its structure. Nevertheless, the artemether absorptivity is considerably low in this region, resulting in HPLC-UV methods with poor sensitivity. Hence, a HPLC method with UV detection was developed by means of artemether standard addition to the sample solutions. This approach allowed an adequate artemether detection and consequently quantitation at 210 nm.

### 3.1. Validation

*3.1.1. Linearity*

A linear correlation was found between the peak areas and the concentrations of artemether and lumefantrine, in the assayed range. The regression analysis data are presented in Table 2. The regression coefficients ($r^2$) obtained were higher than 0.99 for both compounds, which attest the linearity of the method.

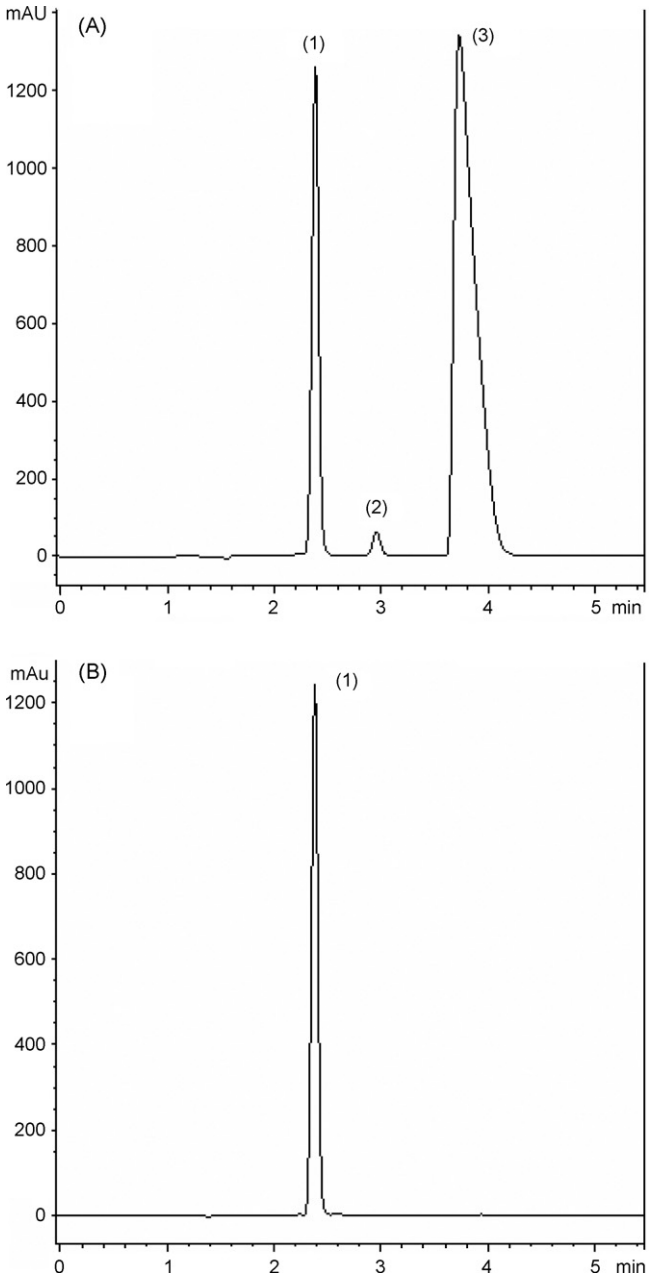


**Fig. 2.** Chromatograms obtained for (A) a sample solution at 400 μg/ml of artemether and 300 μg/ml of lumefantrine and (B) a mixture of the tablet excipients, using a Zorbax SB-Ciano column (150 × 4.6 mm i.d.; 5 μm) and a mobile phase composed of acetonitrile and 0.05% trifluoroacetic acid (60:40), at a flow rate of 1.0 ml/min. Detection was performed at 210 nm. Peak assignation: (1) chloroform; (2) artemether and (3) lumefantrine.

*3.1.2. Precision*

Mean contents of artemether and lumefantrine in the intra-day precision analysis ($n$ = 6) were 405.95 μg/ml (R.S.D. = 1.23%) and 297.03 μg/ml (R.S.D. = 0.89%), respectively. For the inter-day precision ($n$ = 18), the mean contents obtained were 406.92 μg/ml (R.S.D. = 1.07%) and 297.35 μg/ml (R.S.D. = 0.89%) for artemether and lumefantrine, respectively. R.S.D. values, lower than 2.0%, assure the precision of the method.

**Table 2**
Calibration curve data for artemether and lumefantrine

| Regression parameters | Artemether | Lumefantrine |
|---|---|---|
| Regression coefficient, $r^2$ | 0.9984 | 0.9998 |
| Slope ± standard error | 1.08 ± 0.01 | 56.80 ± 0.24 |
| Intercept ± standard error | −100.61 ± 4.74 | 192.65 ± 76.25 |
| Relative standard error (%) | 0.25 | 0.57 |
| Concentration range (μg/ml) | 375–425 | 150–450 |
| Number of points | 5 | 5 |

*3.1.3. Accuracy*

It was investigated by means of addition of artemether and lumefantrine reference standards to a mixture of the tablet excipients. Artemether mean recovery ($n = 9$) was 101.07% (R.S.D. = 0.52%) and lumefantrine mean recovery was 101.58% (R.S.D. = 0.25%), demonstrating the accuracy of the method.

*3.1.4. Specificity*

Peak purities higher than 99.0% were obtained for artemether and lumefantrine in the chromatograms of sample solutions, demonstrating that other compounds did not co-elute with the main peaks. The chromatogram obtained with the mixture of the tablet excipients showed no interfering peaks in the same retention time of artemether and lumefantrine (Fig. 2B).

*3.1.5. Robustness*

Statistical analysis showed no significant difference between results obtained employing the analytical conditions established for the method and those obtained in the experiments in which variations of some parameters were introduced. Thus, the method showed to be robust for changes in mobile phase flow rate from 0.8 to 1.2 ml/min, acetonitrile proportion from 58% to 62%, mobile phase pH in the range of 2.15–2.55 and column temperature from 25 to 35 °C.

*3.1.6. Detection and quantitation limits*

According to the determined signal-to-noise ratio, artemether and lumefantrine presented limits of detection of 5 and 0.1 μg/ml and limits of quantitation of 15 and 0.5 μg/ml, respectively. However, the objective of the method is the simultaneous quantitation of artemether and lumefantrine, so that the values obtained for artemether should be considered as the limit of method sensitivity. Hence, the detection limit established was 5 μg/ml of artemether and 3.75 μg/ml of lumefantrine and the quantitation limit was 15 μg/ml of artemether and 11.25 μg/ml of lumefantrine, the same compounds proportion found in the sample solutions injected onto the chromatograph.

*3.2. Analysis of fixed dose combination tablets*

Samples of fixed dose combination tablets (Coartem® and Co-artesiane®) containing 20 mg of artemether and 120 mg of lumefantrine were analyzed using the validated method. The results obtained are presented in Table 3. All analyzed batches presented artemether and lumefantrine contents very close to the labeled amount. The artemether content in the tablet samples varied from 98.61% to 103.35%, while lumefantrine content varied from 97.92% to 100.48%.

The development of simple and reliable methods is essential to assure the identification and quantitative determination of antimalarial drugs, since the problem of counterfeit or substandard antimalarials is well established all over the world. The use of these poor quality drugs might contribute to the development of plasmodium resistance in endemic areas due to the exposition to anti-infective subtherapeutic doses [10,11]. The quality control of the antimalarial pharmaceutical preparations marketed nowadays may help to assure the treatment efficacy and avoid the development of resistance to antimalarial drugs.

**Table 3**
Contents of artemether and lumefantrine in the fixed dose combination tablets ($n = 6$)

| Sample tablet | Batch | Content (%) ± S.D. | |
|---|---|---|---|
| | | Artemether | Lumefantrine |
| Coartem® | A | 101.69 ± 1.09 | 99.02 ± 0.63 |
| | B | 103.35 ± 1.25 | 97.92 ± 0.24 |
| | C | 98.61 ± 0.59 | 98.89 ± 0.53 |
| Co-artesiane® | A | 101.71 ± 1.00 | 100.48 ± 0.23 |

S.D. = standard deviation.

## 4. Conclusion

This study was the first report of simultaneous determination of artemether and lumefantrine in fixed dose combination tablets. The developed method showed to be a simple and suitable technique to quantify these antimalarials and might be employed for quality control analysis, as well as in further studies in other matrices, such as plasma. The artemether–lumefantrine tablets analyzed by the validated method showed adequate quality and drug contents in concordance with the labeled amount.

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