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**Research**

# Analysis of Artemisinin-Based Combination Therapy (ACT) in Bulk and Formulations by Fourier Transform Infrared Spectrophotometry

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## Abstract

A simple, rapid, and precise FTIR spectroscopic method was developed and validated for the simultaneous estimation of Artemether (ART) and Lumefantrine (LUM) in both bulk and pharmaceutical formulations. The method is based on measuring specific absorption bands: the ether functional group at 1113.10–1091.77 $cm^{-1}$ for ART and the phenyl substitution band at 893.65–857.08 $cm^{-1}$ for LUM. These characteristic peaks were not interfered with by any of the formulation excipients. Method validation was performed in accordance with ICH guidelines. Calibration curves demonstrated linearity in the concentration range of 17–470 mg for ART and 10–250 mg for LUM, with correlation coefficients ($r^2$) of 0.999 for both drugs. Precision, assessed by repeatability, showed % RSD values of 0.831% for both intraday and interday analyses. Accuracy was confirmed through recovery studies using the standard addition method at three concentration levels (80%, 100%, and 120%), yielding mean recoveries of 99.8 ± 0.556% for ART and 99.9 ± 0.094% for LUM. The % assay values obtained from the calibration curve were 99.0 ± 0.1% for ART and 99.9 ± 0.1% for LUM, indicating close agreement with labeled claims. The validated method is suitable for routine analysis of ART and LUM in bulk drug and tablet dosage forms.

**Keywords:** FTIR spectroscopy, Artemether, Lumefantrine, Simultaneous estimation, Method validation, Pharmaceutical formulation, Calibration curve, Recovery studies, Precision, Accuracy.

## INTRODUCTION

Artemether is chemically known as (3R, 5aS, 6R, 8aS, 9R, 10S, 12R, 12aR)-Dehydro-10-methoxy-3,6,9-trimethyl-3,12-epoxy-12H-pyrano[4,3-j]-1,2-benzodioxepine, while Lumefantrine is chemically described as 2-dibutylamino-1-[2,7-dichloro-9-(4-chlorobenzylidene)-9H-fluoren-4-yl]ethanol (racemate) (Fig. 1 and Fig. 2). Despite the widespread use of this combination therapy, only a limited number of analytical methods have been reported for the simultaneous determination of Artemether and Lumefantrine. The combination presents complex and overlapping absorption characteristics in UV spectroscopy, making simultaneous estimation challenging. However, their complementary pharmacokinetic profiles make them highly effective as a fixed-dose combination therapy for malaria.

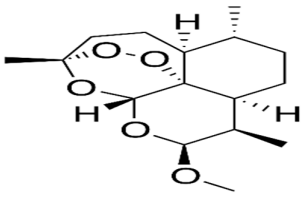
Artemether is rapidly absorbed, reaching peak plasma concentrations—along with its active metabolite, dihydroartemisinic (DHA) within approximately two hours of administration. This rapid action contributes to the quick reduction of the asexual parasite load and prompt symptom relief. In contrast, Lumefantrine is absorbed more slowly and has a prolonged terminal elimination half-life of approximately 3–4 days in malaria patients. This extended activity allows Lumefantrine to accumulate with repeated dosing, ensuring the eradication of any remaining parasites and preventing recrudescence after Artemether and DHA have been cleared from the body.

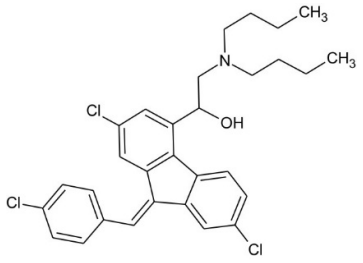
## MATERIALS AND METHODS

### Chemicals and Reagents

Artemether and Lumefantrine reference standards were kindly provided by Mylan Laboratories Ltd., Hyderabad. Various solid pharmaceutical formulations containing Artemether and Lumefantrine as active pharmaceutical ingredients (APIs) were obtained as gift samples from Alvizia Healthcare, Chandigarh.

**ARTEMETHER**






**LUMEFANTRINE**

### Instrumentation

Fourier Transform Infrared (FTIR) spectroscopic analysis was performed using a Bruker Optics FTIR spectrometer equipped with an Attenuated Total Reflectance (ATR) accessory (ZnSe crystal), operated through OPUS software and Quant Builder module for data acquisition and processing.

### Preparation of Standards and Samples

Calibration standards were prepared using accurately weighed quantities of Artemether and Lumefantrine in the concentration range of 17–470 mg for Artemether (Fig. 3) and 10–250 mg for Lumefantrine (Fig. 4). The samples were finely triturated to ensure homogeneity before analysis.

### Calibration Curve Construction

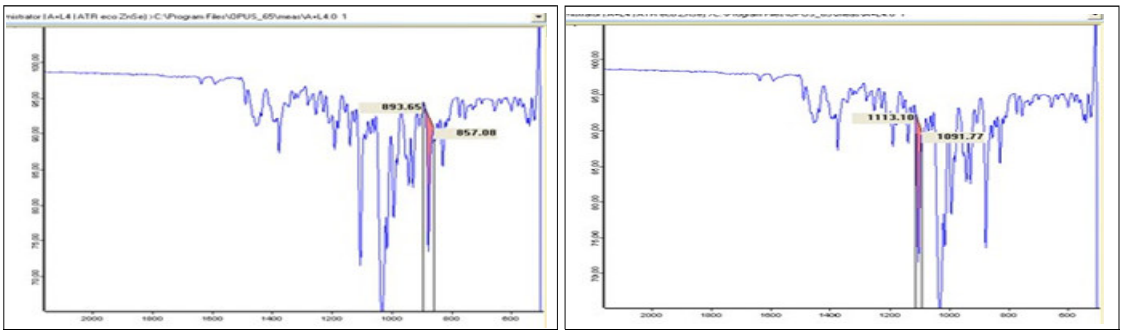
For each drug, calibration curves were constructed using five different concentrations, with each concentration measured in six replicates. The Area Under the Curve (AUC) was determined for the specific FTIR absorption bands: the ether functional group at 1113.10–1091.77 $cm^{-1}$ for Artemether (Fig. 5) and the phenyl ring substitution band at 893.65–857.08 $cm^{-1}$ for Lumefantrine (Fig. 6). The mean AUC values from six measurements were used to generate the calibration curves (Ref. 7–13).

### Data Analysis

All statistical analyses and calibration curve plotting were performed using OPUS version 6.0 software for Windows.

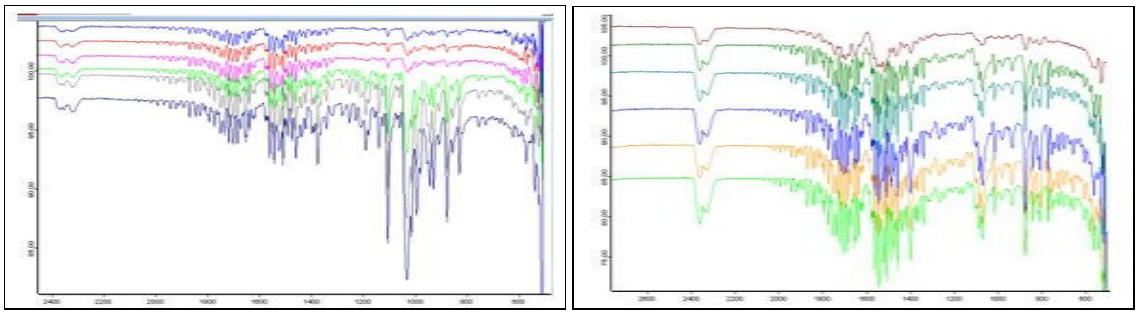
**Sample Preparation Procedure**

The developed FTIR method requires minimal sample preparation. The pharmaceutical formulations were accurately weighed and finely ground using a mortar and pestle until a uniform powder was obtained. No additional sample treatment was necessary. The samples were scanned in the range of 4000–400 $cm^{-1}$ using FTIR to obtain the characteristic spectra.




**Method Validation**

The developed method was validated in accordance with ICH guidelines for key analytical parameters including **accuracy**, **linearity**, and **precision**.



**Accuracy**

Accuracy was evaluated using the standard addition method. Known amounts of Artemether and Lumefantrine corresponding to 80%, 100%, and 120% of the label claim were added to pre-analyzed tablet samples. The spiked samples were thoroughly mixed and analyzed in six replicates. The percentage recovery was calculated to assess the accuracy of the method.

**Linearity**

Linearity was assessed by preparing solid-state samples of Artemether and Lumefantrine over a concentration range of 17–470 mg for Artemether and 10–250 mg for Lumefantrine. Ten different concentrations were analyzed in triplicate. The calibration curves were constructed by plotting the Area Under the Curve (AUC) versus concentration, and linear regression was performed to obtain correlation coefficients and regression equations.

**Precision**

Precision was evaluated in terms of repeatability and intermediate precision.

- **Repeatability**: Six replicates of five different concentrations of Artemether and Lumefantrine were analyzed on the same day (Day 1).
- **Intermediate Precision**: The same procedure was repeated on two consecutive days (Days 2 and 3) to assess inter-day variation. The relative standard deviation (%RSD) was calculated for both intraday and interday analyses.

**Analysis of Marketed Tablet Formulations**

The developed method was applied to determine the drug content in commercially available tablets of *ATMITHER-AL®*. Ten tablets were weighed to determine the average weight and finely powdered. A

representative quantity of the powder was triturated to ensure homogeneity. The analysis was carried out using three independently prepared samples, each analyzed in triplicate (Fig. 9).

**Results and Discussion**

The FTIR method is based on the identification and quantification of specific absorption bands that are unique to the active pharmaceutical ingredients and absent in the excipients. Artemether was quantified using its ether absorption band at 1113.10–1091.77 $cm^{-1}$, and Lumefantrine was identified by its phenyl substitution band at 893.65–857.08 $cm^{-1}$.

The method demonstrated excellent linearity over the studied concentration ranges. The linear regression equations were found to be:

- **Artemether**: $y = +84.78 - 193.1x$
- **Lumefantrine**: $y = +353.75 + 2129.1x$

The correlation coefficient ($r^2$) for both drugs was 0.999, indicating a strong linear relationship(Table 1).

**Precision** results showed that the %RSD was 0.831 for both intraday and interday analyses, which is well within the acceptable limit of ±2.0% (Tables 2–4), demonstrating the method's repeatability and robustness.

**Accuracy**, as determined by recovery studies at 80%, 100%, and 120% levels, yielded mean recoveries of 99.8 ± 0.556% for Artemether and 99.9 ± 0.094% for Lumefantrine (Table 5). These values fall within the acceptable range of 98–102%, confirming the method's reliability and accuracy.

When applied to the analysis of *ATMITHER-AL®* tablets, the assay results were 99.0 ± 0.1% for Artemether and 99.9 ± 0.1% for Lumefantrine, indicating good agreement with labelled content (Table 6).

**Table 1: Regression Analysis Data and Summary Of Validation Parameters For The Ftir Spectrophotometric Method**

| Parameters | | ART | LUM |
|---|---|---|---|
| Wavenumber range ($cm^{-1}$) | | 1113.10-1091.77 | 893.65-857.08 |
| Beer's law limit (mg) | | 17-470 | 10-250 |
| Regression equation (y = mx + c) | | Y = +84.78-193.1*x | Y = +353.75+2129.1*x |
| Slope (m) | | M = +84.78 | M = +353.75 |
| Intercept (c) | | C = -193.1*x | C = 2129.1*x |
| Correlation Coefficient ($r^2$) | | 0.999 | 0.999 |
| Accuracy (Recovery) | Level I | 178.9 | 287 |
| (n = 3) | Level II | 199.9 | 319.9 |
| | Level III | 221 | 353 |
| Method precision (Repeatability) (% RSD, n= 5), | | 0.621252 | 0.621252 |
| Interday (n = 3) (% RSD) | | 0.831 | 0.436 |
| Intraday (n = 3) (% RSD) | | 0.831 | 0.436 |
| Assay ± S. D. (n = 3) | | 99% ± 0.1 | 99.9% ± 0.1 |

*RSD = Relative standard deviation. LOD = Limit of detection. LOQ = Limit of quantification. S. D. is the standard deviation*

**Table 2: Repeatability Data for The Method (N=5)**

| Concentration (ART: LUM) (100 mg ) | ART | LUM |
|---|---|---|
| | **1113.10-1091.77 $cm^{-1}$** | **893.65-857.08 $cm^{-1}$** |
| 1 | 101.34 | 100.35 |
| 2 | 99.79 | 101.12 |
| 3 | 101.12 | 99.79 |
| 4 | 100.87 | 100.87 |
| 5 | 100.35 | 101.34 |
| Mean | 100.694 | 100.695 |
| SD | 0.625564 | 0.625564 |
| % RSD | 0.621252 | 0.621252 |

**Table 3: Results of Method Precision For Intra - Day Precision**

| Concentration (µg/ml) | | Observed value | | Mean ± SD | | % RSD | |
|---|---|---|---|---|---|---|---|
| **ART** | **LUM** | **ART** | **LUM** | **ART** | **LUM** | **ART** | **LUM** |
| | | 9.9 | 49.9 | | | | |
| 10 | 50 | 9.8 | 50 | 9.9 ± 0.1 | 49.6 ± 0.8090 | 1.63% | 1.01% |
| | | 10 | 48.9 | | | | |
| | | 49.9 | 150 | | | | |
| 50 | 150 | 50 | 149.9 | 49.9 ± 0.1 | 149.6 ± 0.8090 | 0.540% | 0.20% |
| | | 49.8 | 148.9 | | | | |
| | | 99.9 | 249.8 | | | | |
| 100 | 250 | 100 | 248.9 | 99.9 ± 0.1 | 249.6 ± 0.8090 | 0.325% | 0.10% |
| | | 99.8 | 250 | | | | |

**Table 4: Results Of Method Precision For Interday Precision**

| Concentration (µg/ml) | | Observed value | | Mean ± SD | | % RSD | |
|---|---|---|---|---|---|---|---|
| **ART** | **LUM** | **ART** | **LUM** | **ART** | **LUM** | **ART** | **LUM** |
| | | 9.9 | 49.9 | | | | |
| 10 | 50 | 9.8 | 50 | 9.9 ± 0.1 | 49.6 ± 0.8090 | 1.63% | 1.01% |
| | | 10 | 48.9 | | | | |
| | | 49.9 | 150 | | | | |
| 50 | 150 | 50 | 149.9 | 49.9 ± 0.1 | 149.6 ± 0.8090 | 0.540% | 0.20% |
| | | 49.8 | 148.9 | | | | |
| | | 99.9 | 249.8 | | | | |
| 100 | 250 | 100 | 248.9 | 99.9 ± 0.1 | 249.6 ± 0.8090 | 0.325% | 0.10% |
| | | 99.8 | 250 | | | | |

**Table 5: Recovery Data of Proposed Method**

| Drug | Accuracy level % | Actual Amount | Amount added | Amount recovered | % recovery | Mean ± SD | % RSD |
|---|---|---|---|---|---|---|---|
| | 80% | 100 | 80 | 178.9 | 99.3% | | |
| ART | 100% | 100 | 100 | 199.9 | 99.9% | 99.8 ± 0.556 | 0.55% |
| | 120% | 100 | 120 | 221 | 100.4% | | |
| | 80% | 160 | 128 | 287 | 99.6% | | |
| LUM | 100% | 160 | 160 | 319.9 | 99.9% | 99.9 ± 0.094 | 0.094% |
| | 120% | 160 | 192 | 353 | 100.2% | | |

*S. D. is Standard deviation, and n is number of replicates*

**Table 6: Assay of Artemether And Lumefantrine in Tablet Formulation**

| Formulations | Drug | Label claim (mg/tab) | Sample solution concentration (mg) | Amount found ± SD | % recover | % RSD |
|---|---|---|---|---|---|---|
| | ART | 80 | 20 | 19.8 ± 0.1 | 99% | 0.50% |
| I | LUM | 480 | 120 | 119.9 ± 0.1 | 99.9% | 0.08% |

## CONCLUSION

A simple, rapid, time-efficient, cost-effective, and precise FTIR spectrophotometric method was successfully developed and validated for the simultaneous estimation of Artemether and Lumefantrine in bulk and pharmaceutical formulations. Validation studies were performed in accordance with ICH guidelines, assessing parameters such as linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ), and assay. The results demonstrated that the method is highly reproducible and reliable, showing excellent agreement with the labelled content of the drugs. Furthermore, the method is free from interference by excipients, making it suitable for routine quality control and analytical applications in pharmaceutical industries for the combined dosage forms of Artemether and Lumefantrine.

**Acknowledgement**

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**Conflict of Interest**

The authors declare no conflict of interest.

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