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Research Article

# HPLC Method Development and Validation for Determination of Artemether in Pharmaceutical Dosage Forms

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## Abstract

A fast, precise and simple HPLC method was developed for estimation of artemether in pure and pharmaceutical dosage forms. The chromatographic separation was conducted on Shimadzu (Prominence LC 20 HPLC) connected with Detector is ELSD with nitrogen pressure = 360 Kpa; and temp = 37°C; Gain = 7; using column C18; Waters $\mu$Bondapak, (300×3.9 mm, 10 $\mu$m). The isocratic mobile phase consisted of acetonitrile: water in ratio of (90: 10, v/v). The mobile phase was delivered to the system at a flow rate of 1.5 ml/min. An injection volume of 20 $\mu$l was used for Artemether. Column Temperature was 37°C. The detection was carried out using ELSD detector. The calibration curve of Artemether in mobile phase was linear with correlation coefficient (r2) of 0.9987; over a concentration range of 1100–2000 mg/L; with a retention time of 2.52 min. The percentage recovery of artemether was 96.34%. The relative standard deviation (RSD) was found to be less than 2. The proposed method was validated and successfully applied for determination of artemether in tablet dosage form.

**Keywords:** HPLC, artemether, ELSD detector, method validation, dosage form, anti-malarial

## Introduction

Artemether is chemically, (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 is used in the treatment of falciparum malaria Figure 1 [1].

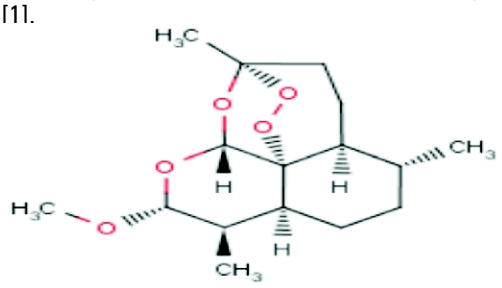


**Figure 1.** Chemical structure of artemether

The increasing use of artemether as an effective treatment for resistant malaria demands the need of analytical methods for the quality control of these drugs in tablets. Some methods have been reported for determining the presence of either artemether in various pharmaceutical and biological matrices [2–10]. However, few methods are reported for determination of artemether in biological [11, 12] and formulation matrices [13-15]. Hodel et al. developed an HPLC–ESI–MS/MS method for the quantitation of 14 antimalarial drugs in human plasma including artemether [11]. Recently, César et al. (2011) improved the method of Hodel et al., which involves the absence of the drying step in sample preparation and a shorter chromatographic run time [12]. Nevertheless, ultraviolet detection is not adequate for artemether quantitation in a biological matrix due to its low sensitivity and selectivity. Hence, the aim of this study was to develop and validate a HPLC-ELSD method to quantify artemether in tablet dosage form.

## Materials and Methods

### Materials

All chemicals and reagents used were HPLC grade. Pure standards of Artemether were obtained from Hetero Drugs, Hyderabad. Acetonitrile was HPLC grade from Romil chemicals. Water for chromatography was from Merck.

### Determination of artemether in tablet dosage form

#### *Chromatographic condition*

Shimadzu LC prominence 20 (HPLC) connected with ELSD detector was used. Shimadzu Lab solutions software was used for data acquisition. Column C18 (Waters) (300 x 3.9 mm, 10 $\mu$m) was used as a stationary phase. The isocratic mobile phase consisted of acetonitrile: water in ratio of (90:10, v/v). The mobile phase was delivered at a flow rate of 1.5 ml/min. An injection volume of 20 $\mu$l was used. The detection was carried out by ELSD detector with run time 3.5 minutes. The column was maintained at 37°C temperature.

#### *Preparation of stock and working standard solution*

A 625 mg of artemether working standard was weighed and transferred into a 250 ml clean and dry volumetric flask. The volume was completed with acetonitrile; then sonicated for 5 min until artemether was dissolved. The stock solution was then diluted with diluents to give concentration range of 1100-2000 mg/L.

### Analytical method validation

#### *Selectivity*

It is an indication for the selectivity of the method. The method is to be selective, if the main peak retention time is well resoluted from any other peak by resolution of minimum 2. This was done by injecting placebo and comparing it with that of standard and the test samples.

#### *Linearity*

It is defined by the correlation coefficient, which must not be less than 0.99, using peak area responses.Linearity for single point standardization should extend to at least 20% beyond the specification range and include the target concentration. This was done by preparing five different concentrations; then making three replicates of each concentration. The linear working range was determined from the standard calibration curve.

**Intraday Precision**

Multiple analyses were conducted on a suitable number of portions of a homogeneous sample. This was performed by assaying multiple aliquots with the same concentration starting from the first step to the final step of analysis. The analytical precision of the method was determined by the relative standard deviation.

**Inter-day Reproducibility (Method Ruggedness)**

The degree of reproducibility was determined by analysis of samples from homogeneous lot of materials, under different but the same test conditions. The method is to be rugged, at any item if the pooled %RSD of the total number of replicates that have been made in this item is within the acceptance criteria. Three replicates of a single sample of powder material were used for each determination. On the first day, three replicates were done; while on the second day, three replicates; then finally on third day. Another replicates of freshly prepared test from the same sample were analyzed, using the same conditions.

**Accuracy and Recovery**

Accuracy was evaluated by spiking standard solution. The measurements were made at the concentration of standard mix, which was found to be the target concentration, and at suitable intervals around this point. The test samples was spiked with known quantities of standard artemether using three determinations over five concentrations level covering the specified range (i.e. five concentrations and three replicates). Relative recoveries of artemether used in the standards were evaluated by comparing their peak area with those obtained from the calibration curve equation

**Specificity**

The ELSD chromatograms of the artemether in standard and sample were recorded. In the chromatograms of the formulations, some additional peaks were observed which may be due to excipients present in the formulations. These peaks however did not interfere with the standard peaks, which demonstrate that the assay method is specific. Furthermore, the purity of the peaks was studied by peak purity studies. The results revealed that the peak is free from interferences, which shows that the HPLC method is specific.

**Linearity**

Each of the concentrations (1100, 1250, 1500, 1750 and 2000 mg/L) was injected in triplicate to get reproducible response. The calibration curve was plotted as concentration of the respective drug versus the response at each level. The proposed method was evaluated by its correlation coefficient and intercept value calculated in the statistical study. They were represented by the linear regression equation:

$Y_{artemether} = 2978.27x - 2275388.5$ ($r^2 = 0.9987$), where Y= Artemether concentration and $r^2$ = Correlation coefficient. Slopes (m) and intercepts (c) were obtained by using regression equation ($Y = mx + c$) and least square treatment of the results used to confirm linearity of the method developed.

**Quantification limit**

The limit of detection (LOD) and limit of quantification (LOQ) of the developed method was determined by injecting progressively low concentrations of the standard solutions using the developed methods. The LOD is the lowest concentration of the analyte that can be detected with signal to noise ratio (3:1) and LOQ is the lowest concentration that can be quantified with acceptable precision and accuracy with signal to noise ratio (10:1). The LOD of artemether found to be 50 mg/L. The LOQ of artemether found to be 150 mg/L.

**Solution stability**

In this study, the mobile phase, the standard solutions, and the sample solution were subjected to long term (3 days) stability studies. The stability of these solutions was studied by performing the experiment and looking for changes in separation, retention, and asymmetry of the peaks which were then compared with the pattern of the chromatogram of freshly prepared solutions

**System suitability**

The resolution, capacity factor, theoretical plates/meter, Rt values and peak symmetry were calculated for the standard solutions. The values obtained demonstrated the suitability of the system for the analysis of the above drug combinations System suitability parameters might fall within ± 4% standard deviation range during routine performance of the method.

**Results and Discussion**

The proposed HPLC-ELSD method was found to be simple and very rapid. This method could be used in quality control test in pharmaceutical industries. The response for the detector was determined to be linear over the range of 1100-2000 mg/L (1100, 1250, 1500, 1750 and 2000) for artemether as shown in Fig. 2. The chromatogram of artemether is shown in Figure 3 (retention time: 2.52 min).

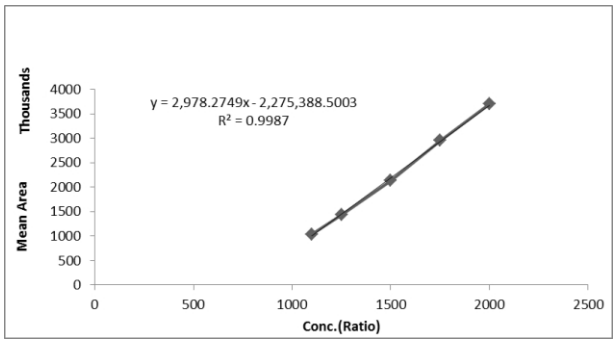


**Figure 2.** Calibration curve of standard artemether

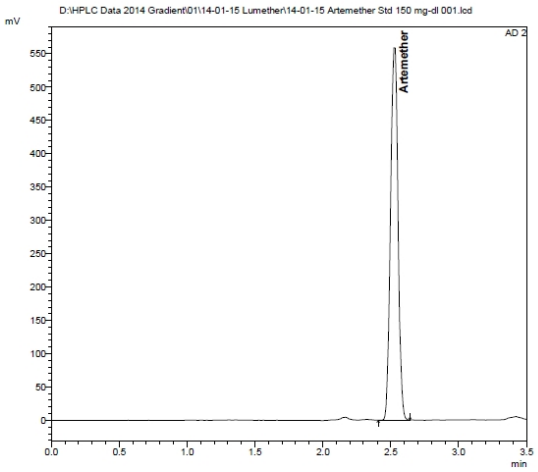


**Figure 3.** HPLC Chromatogram of artemether

**Conclusion**

This method is simple, specific, selective, easy and precise. The time required to perform the analysis is short. Low limit of quantification and limit of detection makes this method suitable for use in quality control. This method enables determination of artemether because of good separation and resolution of the chromatographic peaks. The method was found to be accurate, precise, linear, and rugged.

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**Conflict of interest**

The authors declare no conflict of interest.

**References**

[1].Sweetman SC. Martindale: The Complete Drug Reference, Antimalarials, Artemisinin derivatives, Artemether Pharmaceutical Press: London, UK, 37th ed., Volume A, 2011, pp. 650-52.

[2].Green MD, Mount DL, Wirtz RA. Authentication of artemether, artesunate and dihydroartemisinin antimalarial tablets using a simple colorimetric method. Trop Med Int Health 2001; 6:980-82.

[3].Atemnkeng MA, De Cock K, Plaizier Vercammen J. Quality control of active ingredients in artemisinin-derivative antimalarials within Kenya and DR Congo. J Trop Med Int Health 2007; 12: 68–74.

[4].Arun R, Smith A. Development of analytical method for lumefantrine by UV spectrophotometry. Asian J Chem 2011; 23: 1764-66.

[5].Karbwang J, Na-Bangchang K, Congpuong K, Molunto P, Thanavibul A. Pharmacokinetics and bioavailability of oral and intramuscular artemether. Eur J Clin Pharmacol 1997; 52: 307–10.

[6].Navaratnam V, Mansor SM, Chin LK, Mordi MN, Asokan M, Nair NK. Determination of artemether and dihydroartemisinin in blood plasma by high-performance liquid chromatography for application in clinical pharmacological studies. J Chromatogr B. 1995; 669: 289–94.

[7].Karbwang J, Na-Bangchang K, Molunto P, Banmairuroi V, Congpuong K. Determination of artemether and its major metabolite, dihydroartemisinin, in plasma using high-performance liquid chromatography with electrochemical detection. J Chromatogr B. 1997; 690: 259–65.

[8].Sandrenan N, Sioufi A, Godbillon J, Netter C, Donker, M, Valkenburg V. Determination of artemether and its metabolite, dihydroartemisinin, in plasma by high-performance liquid chromatography and electrochemical detection in the reductive mode. J Chromatogr B 1997; 691: 145–53.

[9].Souppart, C., Gauducheau, N., Sandrenan, N. and Richard, F. Development and validation of a high-performance liquid chromatography-mass spectrometry assay for the determination of artemether and its metabolite dihydroartemisinin in human plasma. J Chromatogr B 2002; 774: 195–03.

[10]. Shi B, Yu Y, Li Z, Zhang L, Zhong Y, Su S, Liang S. Quantitative analysis of artemether and its metabolite dihydroartemisinin in human plasma by LC with tandem mass spectrometry. Chromatographia 2006; 64: 523–30.

[11]. Hodel EM, Zanolari B, Mercier T, Biollaz J, Keiser J, Olliaro P, Genton B. A single LC-tandem mass spectrometry method for the simultaneous determination of 14 antimalarial drugs and their metabolites in human plasma. J Chromatogr B 2009; 877: 867–86.

[12]. César IC, de Aquino Ribeiro JA, de Souza Teixeira L, Bellorio KB, de Abreu FC, Moreira JM et al. Liquid chromatography-tandem mass spectrometry for the simultaneous quantitation of artemether and lumefantrine in human plasma: application for a pharmacokinetic study. J Pharm Biomed Anal 2011; 54: 114-20.

[13]. César IC, Nogueira FHA, Pianetti GA. Simultaneous determination of artemether and lumefantrine in fixed dose combination tablets by HPLC with UV detection. J Pharm Biomed Anal 2008; 48: 951–54.

[14]. Sridhar B, Hanumantha Rao K, Sai Srinivas TV, Seshu madhuri V, Madhuri K, Seshagiri Rao JV. method for the simultaneous estimation of Artemether and Lumefantrine in pharmaceutical dosage forms. J Adv Pharm Sci 2010; 1: 95-99.

[15]. Sunil J, Sanjith Nath M, Samba Moorthy U. HPLC method development and validation for simultaneous estimation of Artemether and Lumefantrine in pharmaceutical dosage forms. Int J Pharm Pharmaceut Sci 2010; 2: 93-96.