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Development and Validation of Analytical Method for Artemether by HPLC

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This paper describes the development and validation of a HPLC method (254 nm) for the quantitation of artemether in pure form and pharmaceutical formulations. The method showed to be linear ($r^2 > 0.99$), precise (RSD < 9.73 %), accurate (recovery of 99.83 % for artemether), specific and robust. Three batches of artemether capsules were assayed by the validated method. The artemether contents in the capsules varied from 99.12-100.65 %.

Key Words: Artemether, Antimalrials, Capsules, HPLC-UV.

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 is increasingly being advocated as promising treatment. Artemisinin is based on the use of drug which has an artemisinin derivative that causes rapid and effective reduction of parasite biomass and gametocyte carriage[3].

Artemether is widely used nowadays and consists of a registered fixed dose of artemether (80 mg) in capsules (Fig. 1). The artemether will rapidly reduce parasitemia, resulting in symptomatic relief[4]. It also produce seminiferous epithelium interstitial leydig cell in the testicle[5] World Health Organization (WHO) recommends this drug as first line therapy for falciparum malaria in endemic areas[6].

The increasing use of artemether as an effective treatment for resistant malaria demands the need of analytical methods to quantify the drug in capsules in order to evaluate its quality. Some papers have described the analysis of artemether in plasma, based on HPLC with electrochemical[7-11], stability indicating[12] mass spectrometry detection[13,14]. Few methods are available to assay artemether in pharmaceutical products[15,16]. However, there is no method reported regarding the quantitation of artemether.

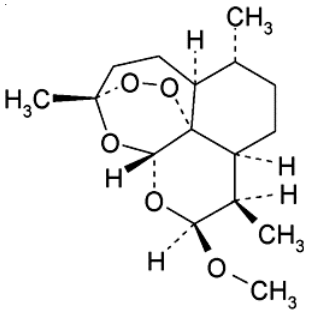

Fig. 1. Chemical structure of artemether

Hence, the aim of this study is to develop and validate a HPLC method, using UV detection to quantify artemether in pure form and pharmaceutical formulations. Due to the low molar absorptivity of artemether, the UV region was found to be at 254 nm. The validated method was applied for the analysis of capsules containing artemether (80 mg).

EXPERIMENTAL

Artemether reference standards were purchased from Ipca Laboratories, Mumbai. Capsules were purchased from local pharmacies which was manufactures by Ajanta Pharma (Mumbai, India) Amether®. Ultra-pure water was obtained from a Millipore system (Bedford, MA, USA). Acetonitrile (HPLC grade) was obtained from E-Merck (India) Ltd.-Mumbai-India. All other chemicals used in the analysis were AR grade.

The HPLC analyses were carried out on shimadzu LC10 (Shimadzu Corporation, Kyoto, Japan) system with UV

detector, Hypersil C_{18} (250 mm × 4.6 mm, 5 µm) column from Shimadzu, Kyoto, Japan. UV detection was performed at 254 nm. UV spectra from 190-400 nm were online recorded for peak identification. The injection volume of sample was 20 µL. An isocratic mobile phase containing acetonitrile and 0.01 M potassium dihydrogen orthophosphate buffer (45:55), at the pH 6.9 was carried out with the flow rate of 1 mL/min.

Preparation of standard solution: Approximately 50 mg of artemether reference standards was accurately weighed and transferred to a 50 mL volumetric flask, 10 mL of acetonitrile was added to ensure the complete solubilization and the volume was adjusted with the mobile phase. Further dilutions were made to get the final concentration of 1 mg/mL of artemether.

Analysis of fixed dose capsules: Three different batches of Amether® 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. For the analysis, six replicates of each batch were assayed. The capsules were weighed and finely powdered. An accurately weighed portion of the powder, equivalent to about 80 mg of artemether was transferred to a 100 mL volumetric flask followed by the addition of 25 mL of acetonitrile. The solution was sonicated for 3 min and diluted with mobile phase to volume. Further dilutions were made to get the final concentration of 30 µg/mL of artemether.

Validation

Linearity: Standard solutions containing 1 mg/mL of artemether was prepared, in triplicate. Aliquots of these solutions were diluted in mobile phase to six different concentrations, corresponding to 10, 20, 30, 40, 50, 60 µg/mL of artemether. Calibration curves for concentration *versus* peak area were plotted for artemether and obtained data were subjected to regression analysis using the least squares method with a weighting factor of 1/x.

Precision: The intra-day precision was evaluated by analyzing six sample solutions (n = 6), at the final concentration of analyses (30 µg/mL) of artemether. The inter-day precision was evaluated in three consecutive days (n = 18). The artemether concentrations were determined and the relative standard deviations (RSD) were calculated.

Accuracy: Artemether reference standards were accurately weighed and added to a mixture of the capsule excipients, at three different concentration levels (30, 40 and 50 µg/mL of artemether). At each level, samples were prepared in triplicate and the recovery percentage was determined.

Specificity: Spectral purities of artemether chromatographic peaks were evaluated using the UV spectra recorded by a UV detector. In addition, a solution containing a mixture of the capsule excipients was prepared using the sample preparation procedure and injected on to the chromatograph, to evaluate possible interfering peaks.

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 and buffer as mobile phase (60:40, 50:50, 40:60, 45:55), mobile phase pH (5.0, 6.8, 6.9) and column temperature (23, 25 and 27 ºC). The artemether contents was determined for each condition and the obtained data was submitted for statistical analysis (ANOVA test).

Detection and quantitation limits: Standard solution was prepared by sequential dilutions and injected onto the chromatograph, at decreasing concentrations, in the range of 0.13-15 µg/mL of artemether. 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.

RESULTS AND DISCUSSION

The chromatographic parameters were initially evaluated using a Hypersil C_{18} (250 mm × 4.6 mm, 5 µm) column and a mobile phase composed of acetonitrile and 0.01 M potassium dihydrogen orthophosphate buffer (45:55). Using this column, different proportions of mobile phase solvents were evaluated, to obtain a good peak (Table-1). Under these conditions the retention factor obtained for artemether was 7.03 and a short run rime (5 min) and so, this condition was adopted in subsequent analysis (Fig. 2).

TABLE-1
PARAMETERS FOR ARTEMETHER AT DIFFERENT MOBILE PHASE COMPOSITION USING HYPERSIL C_{18} (250 mm × 4.6 mm, 5 µm) COLUMN

Mobile phase composition acetonitrile:buffer	Artemether retention factor (k)
60:40	6.32
50:50	6.47
40:60	6.84
45:55	7.01

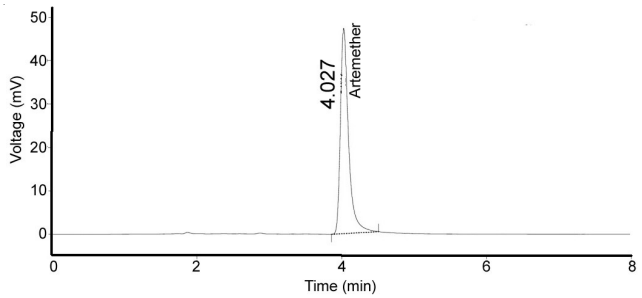

Fig. 2. Chromatogram of artemether

After the evaluation of the artemether UV spectrum in the range of 200-400 nm, the wavelength of 254 nm was selected for detection, due to the adequate molar absorptivity of artemether in this region and the higher selectivity of this wavelength regarding possible interfering compounds or solvents in the sample.

Validation

Linearity: A linear correlation was found between the peak areas and the concentrations of artemether in the assayed range. The regression analysis data were presented in Table-2. The regression coefficients (Y = 31.916 x - 17.336) obtained were higher than 0.99 for the artemether (Fig. 3), which attest the linearity of the method.

TABLE-2
CALIBRATION CURVE DATA FOR ARTEMETHER

Regression parameters	Artemether
Regression coefficient (r^2)	0.99
Slope ± standard error	31.91 ± 0.012
Intercept ± standard error (%)	-17.33 ± 0.34
Relative standard error (%)	0.21
Concentration range (µg/mL)	10-60
Number of points	6

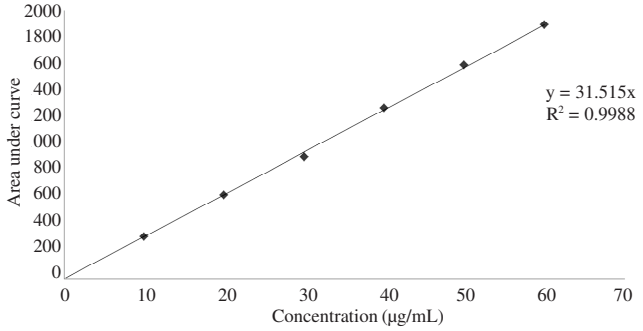

Fig. 3. Linearity profile of artemether

Precision: Mean contents of artemether in the intra-day precision analysis (n = 6) was 30 µg/mL (RSD = 1.12 %). For the intra-day precision (n = 18) the mean contents obtained for artemether was 30.2 µg/mL (RSD = 0.23 %). RSD values lower than 2.0 % assure the precision of the method.

Accuracy: It was investigated by means of addition of artemether reference standards to a mixture of the capsule excipients. Artemether mean recovery (n = 9) was 99.83 % (RSD = 0.039 %) demonstrating the accuracy of the method.

Specificity: Peak purities higher than 99.0 % were obtained for artemether 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 capsule excipients showed no interfering peaks in the same retention time of artemether.

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 0.8-1.2 mL/min, acetonitrile: buffer proportion from (60:40, 50:50, 40:60, 45:55), mobile phase pH (5, 6.8, 6.9) and column temperature (23, 25 and 27 ºC).

Detection and quantitation limits: According to the determined signal-to-noise ratio, artemether presented limits of detection of 0.14 µg/mL and limits of quantitation of 0.42 µg/mL, where the compounds proportion found in the sample solutions injected onto the chromatograph. However, the objective of the method is the quantitation of artemether, so that the values obtained for artemether should be considered as the limit of method sensitivity.

Analysis of capsules: Samples of fixed dose capsules Amether® containing 80 mg of artemether was analyzed using the validated method. The results obtained were presented in Table-3. All the analyzed batches presented artemether contents were very close to the labeled amount. The artemether content in the capsule samples varied from 99.12-100.65 %.

TABLE-3
CONTENTS OF ARTEMETHER IN THE FIXED DOSE CAPSULES (n = 6)

Sample capsules	Batch	Content (%) + SD artemether
Amether®	A	99.12 ± 0.23
	B	100.65 ± 0.14
	C	99.63 ± 0.56

SD: Standard deviation.

The development of simple and reliable method is essential to assure the identification and quantitative determination of antimalarial drugs, since the problem of counterfeit or sub-standard 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 antiinfective sub therapeutic doses[12,13]. 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.

Conclusion

This study is the first report of development and validation of artemether in pure form and pharmaceutical formulations. The developed method showed to be a simple and suitable technique to quantify the antimalarial and might be employed for quality control analysis, as well as in further studies in other matrices, such as plasma. The artemether capsules analyzed by the validated method showed adequate quality and drug contents in concordance with the labeled amount.

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