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RESEARCH ARTICLE

New Simple and Economical Spectrophotometric Method for Estimation of Artemether in Pharmaceutical Dosage Forms

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ABSTRACT

A new simple, sensitive, precise and economical spectrophotometric method of analysis for artemether both as a bulk drug and in capsule formulations was developed and validated. The method employed methanol as solvent and 1 N methanolic HCl was used to derivatize drug. This derivatized product was then estimated at 254 nm. The linear regression analysis data for the calibration plots showed good linear relationship with r^2 = 0.9997 in the concentration range 4-36 µg/ml. The mean value of correlation coefficient, slope and intercept were 0.9998±0.000116, 0.0307±0.000133 and 0.0337±0.001945 respectively. The method was validated for precision, accuracy and recovery studies. LOD and LOQ for artemether were found to be 0.2297 (µg/ml) and 0.696 (µg/ml) respectively. The method has been successfully applied in the analysis of marketed formulations.

KEY WORDS Spectrophotometric analysis, artemether

INTRODUCTION:

Artemether ((3*R*, 5*aS*, 6*R*, 8*aS*, 9*R*, 10*S*, 12*R*, 12*aR*)-Decahydro-10-methoxy-3, 6, 9-trimethyl-3, 12-epoxy-12*H*pyrano [4,3-j]-1, 2-benzodioxepin) (Fig. 1) is a semisynthetic polyoxygenated amorphene containing a peroxide bridge that confers potent antimalarial activity[1]. It is the O-methyl ether prodrug of dihydroartemisinin and a derivative of artemisinin (qinghaosu), the principal antimalarial constituent of the Chinese herb *Artemisia annua* (qing hao)[2]. Artemether is active against the erythrocytic stage of multidrug-resistant strains of *Plasmodium falciparum*.

The antimalarial activity has been attributed to chemical activation of the drug within the food vacuole of the intraerythrocytic stage of the parasite; it is proposed that reductive cleavage of the peroxide bridge by heme liberated during digestion of hemoglobin generates free radicals, which induce oxidative stress and alkylate heme and vital parasite proteins[3]. An interaction with membrane phospholipids has also been suggested[4]. The peroxide group in these compounds appears essential for activity, and the peroxide group is retained in the active metabolite, dihydroartemisinin[5].

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Because of the promising activity exhibited by artemether against multidrug-resistant strains of *P. falciparum*, several researchers have focused on the development of various analytical methods to determine artemether in different matrices, such as plant extracts, serum, pharmaceutical formulations. These methods include gas chromatography–mass spectrometry (GC–MS)[6], high-performance liquid chromatography (HPLC) based on UV absorption[7–10], chemiluminescence and electrochemical detection[11], high-performance thin-layer chromatography (HPTLC)[12–14] and the capillary electrophoresis techniques[15]. Gabriëls and Plaizier-Vercammen have reported determination of artemether by using normal phase thin-layer chromatography (NPTLC)[12] using pure chloroform as the mobile phase and also the use of reverse phase thin-layer chromatography (RPTLC)[13] using acetonitrile–water as mobile phase.

However, one spectrophotometric method already published in International Pharmacopoeia link: *http://whqlibdoc. who .int/publications/2003/9241545364_part4.pdf*[10], involves ethanol as solvent and time taken for the drug to derivatize was 5 hours at 55º C. In the presented study methanol was used as solvent and conditions of derivatization were optimized at 60 ºC for 3 hours.

The aim of the present work is to develop and validate[15] an accurate, specific, economical and reproducible UV Spectrophotometric method for determination of artemether as bulk drug and in solid dosage forms.

Drug was found to be freely soluble in methanol hence this solvent was chosen for proceeding study.

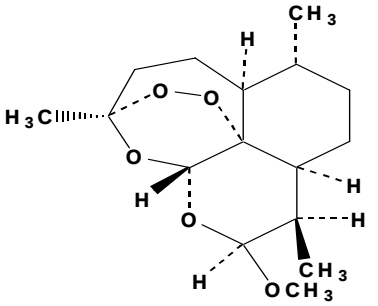

Fig. 1. Chemical structure of artemether

MATERIAL AND METHOD:

All the absorbance measurement measurements were made on Shimadzu UV-1601 UV/Visible double beam spectrophotometer with 10 mm matched cells. Whatman filter no. 42 was used to filter solutions. The ART standard was provided by Oasis lab, Ahmedabad. All chemicals were of analytical grade. Methanol was purchased from Merck ltd, Mumbai. Commercially available formulations were procured from local market.

Optimization of Parameters

It was found that artemether reacts with conc. HCl at room temperature to yield a product which has λ_{max} at 254 nm. Increase in temperature speed up the reaction. Hence, studies were carried out to establish most favorable conditions for the formation of product. The influence of the concentration as well as volume of the acid on the reaction has been studied. Effect of different concentrations and different volumes of acid, time and temperature were studied.

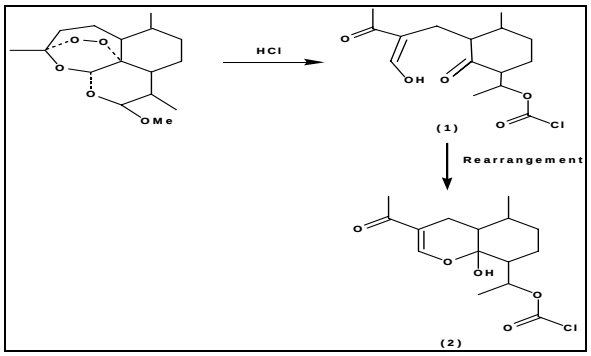

Figure 2: Proposed mechanism of reaction of ART with HCl

The selection criteria was based on the minimum strength and volume of acid that gives maximum absorbance, volumetric flasks should not be open up during heating due to vapour pressure of methanol. Temperature was maintained by immersing thermometer in the water bath.

Preperation of standard solution:

800 mg of Artemether accurately weighed was dissolved in about 50 ml of methanol and diluting with 100 ml with same solvent. 5 ml of this solution was again diluted to 100 ml with the same solvent. 1 M methanolic HCl was prepared by diluting 170 ml conc. HCl with methanol upto 2 liters. The prepared HCl was then titrated by standard IP method and normality was found to be 0.9976.

METHOD

Suitable aliquots of the standard solution of ART (0.1-0.9 ml) were taken in 10 ml volumetric flasks. To each flask added 5 ml of prepared methanolic HCl. Flasks were shaken for few seconds and heated on the water bath for 3 hours at temperature 60 ± 2 ºC. The solutions were allowed to cool at room temperature and volume was then made up to the mark with methanol to prepare a series of standard solutions containing 4-36 µg/ml. Absorbance of the complex was measured at 254 nm against blank.

Blank was prepared by heating 5 ml methanolic HCl in the same condition and diluting up to the 10 ml with methanol.

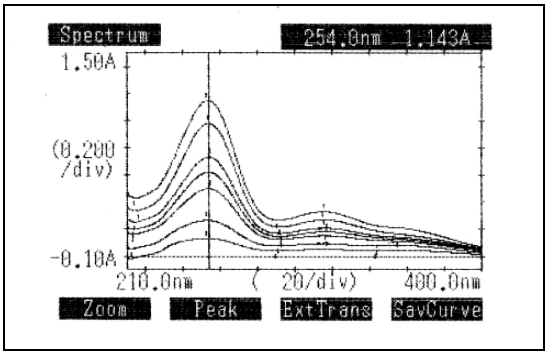

Figure 3: Overlain spectrum of 4, 8, 16, 20, 24, 32 and 36 µg/ml of ART solution after treatment with HCl in the proposed method.

Estimation of ART in capsules

Twenty capsules (of same respective batch number) of two pharmaceutical companies Larither (IPCA labs) and Falcidol (Skymax) were taken and their weight was calculated. Powder inside the capsule shell was carefully removed and empty capsule shells were weighed. The weight of empty capsule shells were deducted from the weight of the filled capsules and divided by twenty to get the average weight of powder per capsule.

The quantity of powder equivalent to 40 mg of Artemether was transferred to 100 ml volumetric flask and mixed with 50 ml of methanol and solution was sonicated for 10 minutes then after volume was made up to 100 ml with same solvent. The solution was filtered through Whatmann filter paper No. 42. From the filtrate, (0.5 ml) was transferred to four different 10 ml volumetric flasks and volume was made up to 10 ml with methanol 5 ml of previously prepared stock solution of 1 M HCl was added and solution was heated on the water bath for three hours at temperature 60 ± 2º. The solution was allowed to cool at room temperature after heating and then diluted up to the mark with methanol. Reference standard of ART was also treated in the same way at each concentration level and absorbance was noted at 254 nm against blank.

Table 1: Summary of optical and regression parameters

Linearity range (µg/ml)	4-36
λ_{max}	254 nm
Beers' law limit (µg/ml)	4 – 36
Regression equation Slope	Y = 0.0307x + 0.0337 0.0307
Intercept	0.0337
Correlation coefficient	0.9998

Estimation of artemether in injectables (40 mg/ml)

1 ml of the solution inside injection of artemether (Malither- Maan Pharmaceuticals) was transferred to five different 100 ml volumetric and shacked with 50 ml methanol for 25 minutes and volume was made with same solvent. 0.5 ml of each of this solution was transferred to 10 ml volumetric flasks and estimation of drug was done by proposed method.

METHOD VALIDATION

Accuracy of the method was determined by the recovery studies in the capsule formulation of ART. Recovery studies were carried out by addition of known quantities of standard drug solution to pre-analyzed sample at three different concentrations. Also the experiment was repeated three times in a day to determine intraday precision and on three different days to determine interday precision. The percent coefficient of variance (% CV) was calculated at each concentration level. The values of method validation are given in Table 2. The proposed method obeys beer's law in the concentration range of 4-36 µg/ml. in this method, the correlation coefficient (r^2) was found to be 0.9997, the slope was 0.0307(±0.000133) and intercept 0.0337(±0.001945). Limit of detection (LOD) and limit of quantitation (LOQ) were calculated by repeating the blank measurements six times at 254 nm. LOD and LOQ for artemether were found to be 0.2297 (µg/ml) and 0.696 (µg/ml) respectively. It can be concluded that the developed method is sensitive.

RESULTS AND DISSCUSSION:

The proposed method is simple and precise and do not suffer from any interference due to common excipients of capsule. Beer's law is obeyed in the concentration range of 4-36 µg/ml. Method were validated in terms of accuracy, and precision. The accuracy of the method was proved by performing recovery studies in the commercially available formulations. Values greater than 99% indicate that proposed method is accurate for the analysis of drug. The precision of method was checked in terms of interday and intraday, where methods were repeated on three different days and also repeated on three different time periods in the same day. The results given in Table 2 showing % CV of less than 1 % at each level clearly indicate that the method is precise enough for the analysis of the drug. Summary of optical and regression parameters are shown in Table 1.

Table 2: Summary of validation parameters

Specificity		% interference = 0.4203 %
Range (µg/ml)	Linear range Working range Target range Target concentration	12-28 0.696 – 36 16, 20 and 24 20
Precision (%RSD)	Repeatability Intra day Inter day	0.198 0.329 – 1.092 0.439 – 0.965
Accuracy (% recovery)		99.19 – 99.88
LOD (µg/ml)		0.2297
LOQ (µg/ml)		0.696

Table 3: Estimation of artemether in capsule dosage form

S. N0	Brand Name	Label claim (mg)	Concentration found (mg)		% drug found		% RSD
			Mean	SD	Mean	SD	
1.	A	40	39.905	0.0622	99.763	0.155	0.156
2.	B	40	39.805	0.079	99.512	0.197	0.199

A= Larither, B= Falcidol

Table 4: Estimation of artemether in injectables

S. N0	Brand Name	Label claim (mg)	Concentration found (mg)		% drug found		% RSD
			Mean	SD	Mean	SD	
1.	Malither	40	39.543	0.0865	98.760	0.216	0.219

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