Research Article CODEN: IJPRNK ISSN: 2277-8713
Sourabh Kheechi, IJPRBS, 2014; Volume 3(1): 200-211 IJPRBS

INTERNATIONAL JOURNAL OF
PHARMACEUTICAL RESEARCH AND BIO-SCIENCE

DEVELOPMENT AND VALIDATION OF FIRST ORDER DERIVATIVE UV-SPECTROSCOPIC METHOD FOR SIMULTANEOUS ESTIMATION OF ARTESUNATE AND MEFLOQUINE AS API AND IN MARKETED TABLET DOSAGE FORM.

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Accepted Date: 13/01/2014; Published Date: 27/02/2014

Abstract: A simple, economic, sensitive, precise and accurate first derivative spectrophotometric method has been developed for determination of Artesunate and Mefloquine as API and in tablet dosage form. The quantitative determination of the Artesunate and Mefloquine was carried out using the first derivative values measured at 222 nm and 241 nm respectively. The method was validated as per ICH guidelines. Statistical analysis of the result has been carried out revealing high accuracy and good precision. The proposed method was successfully applied to the determination of Artesunate and Mefloquine pharmaceutical formulations without any interference from common excipients. Calibration curve of Artesunate and Mefloquine was linear in concentration range of 10-70 µg/ml and 20-140 µg/ml with correlation coefficient value of 0.9994 and 0.9991 . The slope of Artesunate and Mefloquine was found to be 0.0001. The results of analysis validated statistically and by recovery studies. The developed method was found to be sensitive, specific, accurate, precise and cost effective quality control tool for the routine analysis of Artesunate and Mefloquine as API and in tablet dosage form.

Keywords: Artesunate, Mefloquine, First Derivative Spectrophotometry, Validation.

PAPER-QR CODE

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How to Cite This Article:

Sourabh Kheechi, IJPRBS, 2014; Volume 3(1): 200-211

INTRODUCTION

The development of simple and cost-effective analytical protocols for artemisinin and its derivatives in both pure form and in dosage form has become very important in the face of increasing supply and demand for this antimalarial agent. The results obtained from the studies show that UV absorption of artesunate could be employed for the assay of the drug especially in poorly equipped laboratories like those found in most developing countries. Artemisinin-based combination therapy (ACT) is widely promoted as a strategy to counteract the increasing resistance of P. falciparum to anti-malarial drugs, prevent disease transmission and reduce the risk of drug resistance. ACT can rapidly reduce the parasite biomass, and may also prevent transmission of P. falciparum by acting against gametocytes. Several formulations of ACT are currently available for the treatment of uncomplicated malaria. Studies showed that artesunate-mefloquine is effective and safe for the treatment of uncomplicated malaria.[1-4]

Artesunate is a member of drug class known as statins. It is a derivative of artemisinin and has antimicrobial property. The compound is an active ingredient in the Chinese herb Artemisia annua and has been used in malarial studies. Artesunate has been tested against 55 cell lines and have demonstrated anti-cancer activity also. Research shows that conditional expression of CDC25A heightens the sensitivity of tumor cells to Artesunate. Mefloquine is useful for the prevention of malaria in all areas except for those where parasites may have resistance to multiple drugs. It is typically taken for one to two weeks before entering an area with malaria. Doxycycline and atovaquone/proguanil provide protection within one to two days and may be better tolerated. If a person becomes ill with malaria despite prophylaxis with mefloquine, the use of halofantrine and quinine for treatment may be ineffective. [5-9]

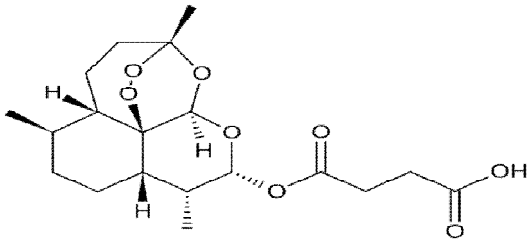

Fig. 1: Chemical structure of Artesunate

Mefloquine is a clinically useful anti-malarial compound active against strains of the plasmodium parasite. The behavioral effects of each of the enantiomers of mefloquine were assessed. The (+)-(11R,12S)-enantiomer showed higher activity against Plasmodium falciparum. Progress has been made toward the enantioselective synthesis of the (+)-(11R, 12S)-8-chloromefloquine analog. An enantiometically pure product was obtained by using hydrozirconation followed by a zinc-palladium catalyzed Negishi cross-coupling reaction and a

sharpless dihydroxylation as key steps. A series of mefloquine analogs have been developed to facilitate future structure-activity relationships and the development of new antimalarial agents. A recent study suggested that there is an increase in the potency when electron-withdrawing groups are attached at both the 2- and 8-positions of the quinoline ring. According to this concept, six new 8-position derivatives were synthesized via a palladium mediated coupling reaction.[10-12]

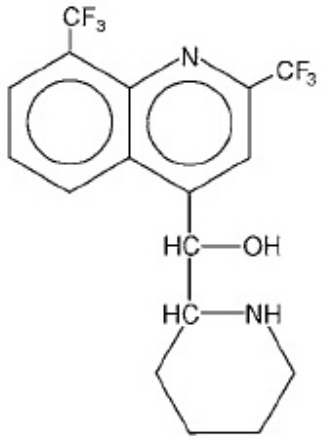

Fig. 2: Chemical structure of Mefloquine

Artemisinin-based combination therapy (ACT) is widely promoted as a strategy to counteract the increasing resistance of P. falciparum to anti-malarial drugs, prevent disease transmission and reduce the risk of drug resistance. ACT can rapidly reduce the parasite biomass, and may also prevent transmission of P. falciparum by acting against gametocytes. Several formulations of ACT are currently available for the treatment of uncomplicated malaria. Studies showed that Artesunate- Mefloquine is effective and safe for the treatment of uncomplicated malaria.[1-15]

Review of literature revealed that many analytical methods have been reported for determination of Artesunate in combination with other drugs by RP-HPLC. However one method is reported for simultaneous estimation of this drug combination with the help of UV spectrophotometry but derivative UV spectroscopy and differential UV spectroscopy ware not reported so far for this combination.[16-18]

2. EXPERIMENTAL

2.1 Chemicals and Reagents

Artesunate was obtained as gift sample from Aster Pharmaceuticals, Mumbai (India) and Mefloquine was obtained as gift sample from Intas Pharmaceuticals, Dehradun (India). A

commercial tablet formulation was purchased from the local market. Methanol of analytical grade was used.

3. MATERIALS AND METHODS

3.1 Instrument

A double beam UV-VIS Spectrophotometer (UV 1800, Shimadzu, Japan) Spectral bandwidth of 1 nm and wavelength accuracy of ±0.5 nm with a pair of 10 mm matched quartz cells. All weights were taken on Digital electronic balance Sartorius, CP225D.

3.2 Preparation of Standard Stock Solution of Artesunate

Accurately weighed 10.0 mg of Artesunate and was transferred to 100 ml volumetric flask, dissolved in about 50 ml of methanol and volume was up-to 100 ml with methanol to obtain stock solution of drug concentration of 100 µg/ml.

3.3 Preparation of Standard Stock Solution of Mefloquine

Accurately weighed 20.0 mg Mefloquine and was transferred to 100 ml volumetric flask, dissolved in about 50 ml of methanol and volume was up-to 100 ml with methanol to obtain stock solution of drug concentration of 200 µg/ml.

3.4 Determination of λ_{max} of Artesunate

The 1 ml of standard stock solution was diluted to 10 ml with the help of methanol to get the concentration of 10 µg/ml, scanned in the range of 200 to 400 nm against methanol as a blank. The spectrum was recorded and λ_{max} was found to be 241 nm.

3.5 Determination of λ_{max} of Mefloquine

1 ml of standard stock solution was diluted to 10 ml with the help of methanol to get the concentration of 20 µg/ml, scanned in the range of 200 to 400 nm against methanol as a blank. The spectrum was recorded and λ_{max} was found to be 222 nm.

3.6 Preparation of calibration curve for Artesunate

From standard stock solution of Artesunate 1, 2, 3, 4, 5, 6 and 7 ml solutions were pipette out in a series of seven 10 ml volumetric flasks. The volumes in each flask were made up to 10 ml with solvent (methanol), to obtain final solutions contained 10, 20, 30, 40, 50, 60 and 70 µg/ml of drug. The spectrum was recorded. The amplitude (D value) was measured at 222 (Table 1) and Calibration curve was plotted (Fig. 5).

3.7 Preparation of calibration curve for Mefloquine

From standard stock solution of Mefloquine 1, 2, 3, 4, 5, 6 and 7 ml solutions were pipette out in a series of seven 10 ml volumetric flasks. The volumes in each flask were made up to 10 ml with solvent (methanol), to obtain final solutions contained 20, 40, 60, 80, 100, 120 and 140 µg/ml of drug. The spectrum was recorded. The amplitude (D value) was measured at 241 (Table 2) and Calibration curve was plotted (Fig. 8).

3.8 Estimation of Artesunate and Mefloquine in tablet dosage form

The powder of 20 Artesunate and Mefloquine tablets (label claim 100 mg + 200 mg) of the same batch no. were triturated and mixed properly. Accurately weighed 59.3 mg powder (equivalent to 10 mg of Artesunate and 20 mg Mefloquine) was transferred in 100 ml volumetric flask containing small quantity of reference solvent (methanol). Ultrasonic water bath was used for 20 minutes to complete dissolution. The solution were diluted to volume and filtered through Whatman filter paper no. 40. Further suitable dilutions were made to obtain six replicates of 10 µg/ml and 20 µg/ml of Artesunate and Mefloquine respectively. These solutions were analyzed and percent recovery of Artesunate and Mefloquine tablet was determined.

3.9 Method Validation

3.9.1 Specificity: Excipients present in selected tablet formulation were used to spike into a pre-weighed quantity of drug. The amplitude (D value) was measured and calculations were determined the quantity of the each drug.

3.9.2 Linearity: A calibration curve was constructed at optimum experimental conditions using D values versus concentration in the range of 10-70 µg/ml for Artesunate and 20-140 µg/ml for Mefloquine. The regression equation (y=0.0001x+0.0004) for Artesunate and (y=0.0001x+0.0008) for Mefloquine was obtained, where 'y' is amplitude of the peak and 'x' is the concentration of each of the sample in µg/ml.

From calibration curve data, high value of the correlation coefficient (0.9994 and 0.9991 for Artesunate and Mefloquine respectively) was found and the values of the intercept on ordinate, which is close to zero for each drug, shows very good linearity of the calibration graph and adherence of the method to Beer's law.

3.9.3 Precision: For Intraday and Interday precisions of the method, solutions of Artesunate and mefloquine were prepared at three concentration levels 32, 40, 48 (µg/ml) and 64, 80, 96 (µg/ml) for each in triplicate respectively. These solutions were analyzed respectively three times within one day and three consecutive days and the result was reported in terms of relative standard deviation(RSD).

3.9.4 Accuracy: The accuracy of the method is based on recovery study. The technique of standard addition was used to assess accuracy of the method. For this purpose a concentration of 10, 20, 30 µg/ml and 20, 40, 60 µg/ml was selected for Artesunate and Mefloquine respectively to prepare the standard sample of the blank drug. The amplitude of the sample matrix of 40 µg/ml and 80 µg/ml for Artesunate and Mefloquine respectively and after standard addition was measured in triplicate. The result was reported in terms of % recovery.[19]

4. RESULTS AND DISCUSSION

According to the International Conference on Harmonization, the main objective of the validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose, and the parameters that need to be selected are the responsibility of the analyst. The solubility of Artesunate and Mefloquine in methanol, so it was used in this method. Artesunate and Mefloquine in methanol shows absorption maxima at 241 and 222 nm in first order derivative spectrum (Fig.3&5)respectively. The amplitude (D value) for Artesunate and Mefloquine was measured at 222 and 241 nm for calibration curve (Table 1) respectively. The response for Artesunate and Mefloquine was found to be linear in the concentration range of 10–70 µg/ml and 20-140 µg/ml (Fig. 4&6).The % mean recovery data for estimation of Artesunate and Mefloquine in tablet dosage form are summarized in Table 3.The optical characteristics of the method and regression analysis of the calibration curve are shown in Table 4&5. The results of validation parameters are shown in Table 6. The recovery of Artesunate and Mefloquine was found to be good. Excipients used in the specificity study did not interfere with response of the drug at its analytical wavelength. Also, no significant change in response of Artesunate and Mefloquine solutions was observed. Hence, the method is sensitive, specific and robust for estimation of Artesunate and Mefloquine. The proposed spectrophotometric methods were applied to the determination of Artesunate and Mefloquine in its pharmaceutical formulations.

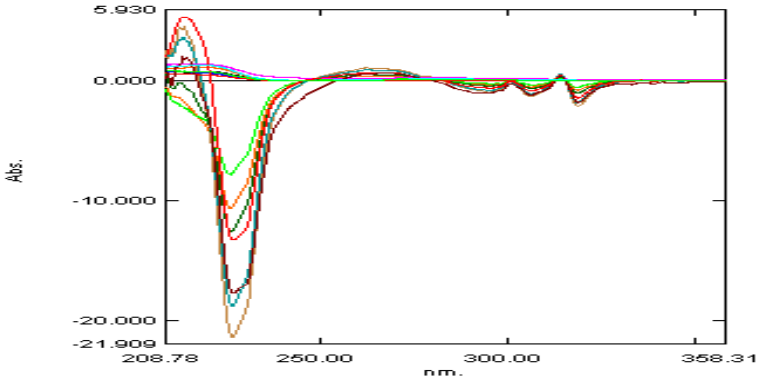

Fig. 3: First derivative UV spectrum of Artesunate

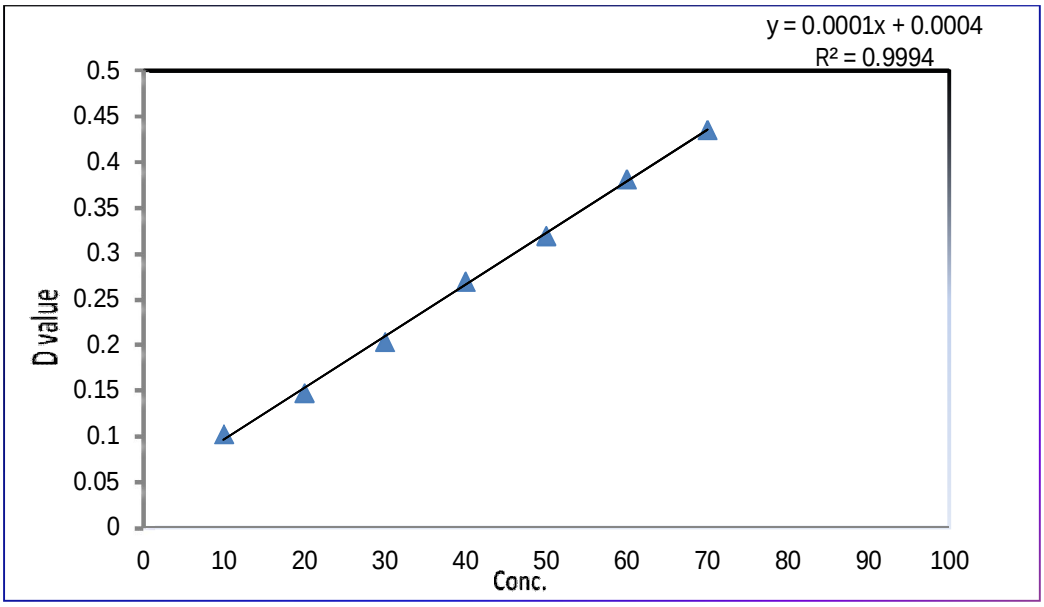

Fig. 4: Calibration curve of Artesunate at 222 nm

Table 1: Calibration curve data for Artesunate

S. No.	Conc. (µg/ml)	D value (amplitude)
1.	10	0.00102
2.	20	0.00149
3.	30	0.00208
4.	40	0.00269
5.	50	0.00319
6.	60	0.00381
7.	70	0.00435

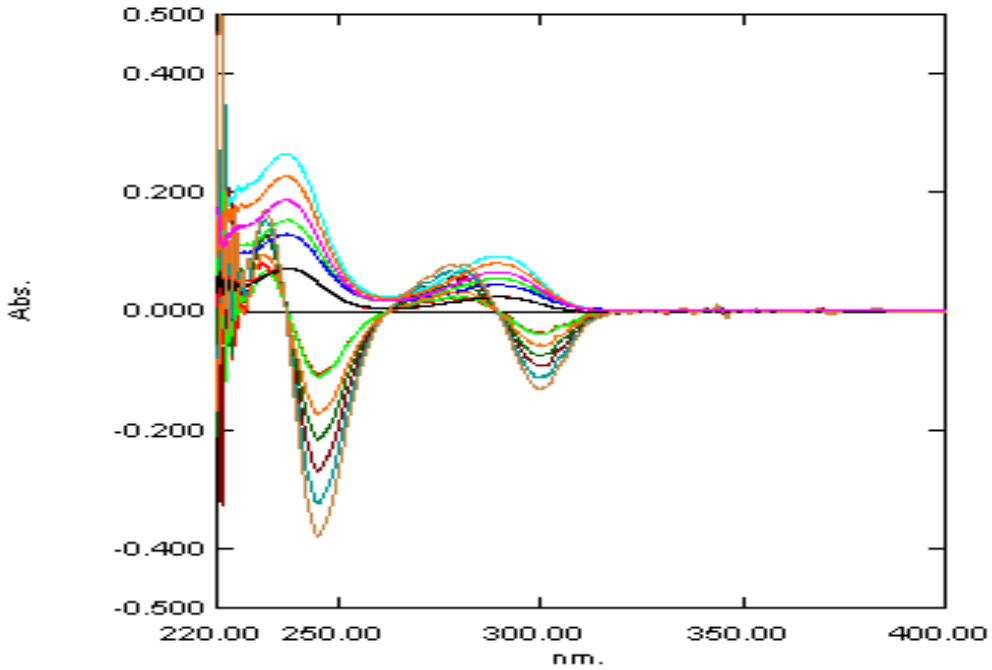

Fig. 5: First derivative UV spectrum of Mefloquine

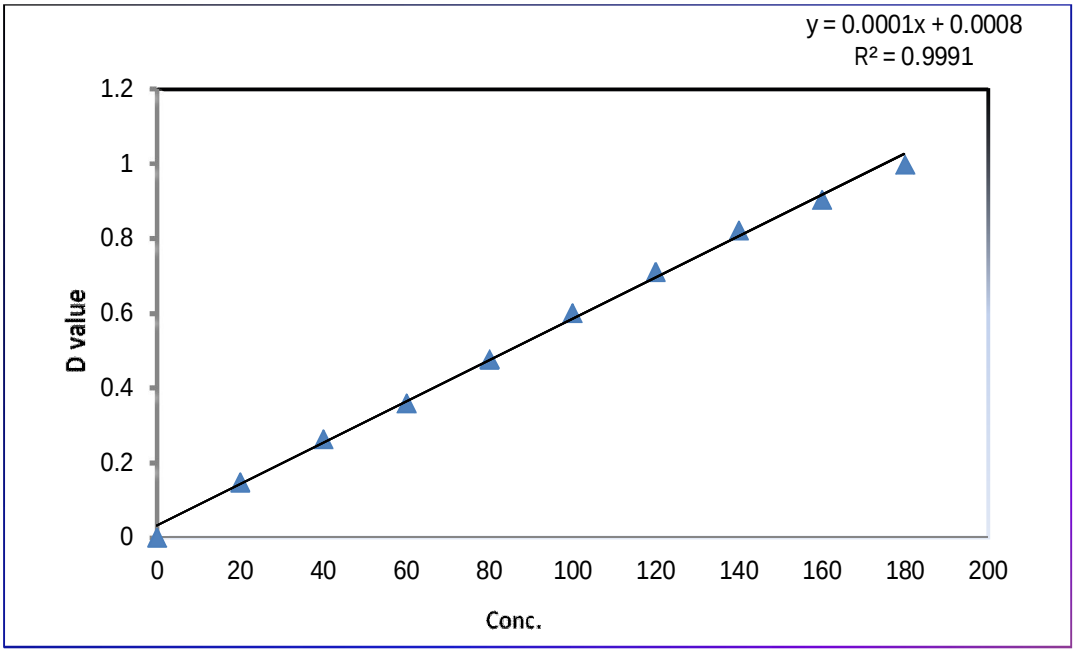

Fig. 6: Calibration curve of Mefloquine at 241 nm

Table 2: Calibration curve data for Mefloquine

S. No.	Conc. (µg/ml)	D value (amplitude)
1.	20	0.00198
2.	40	0.00287
3.	60	0.00404
4.	80	0.00514
5.	100	0.00621
6.	120	0.00741
7.	140	0.00851

Table 3: Assay result of Artesunate and Mefloquine in tablets

Label claim	Amount found (mg/tab.)	Standard deviation	% Mean Recovery
Artesunate- 100 mg	99.23	0.776	99.23
Mefloquine- 200mg	198.17	0.547	99.08

Table 4: Beer's law data and Regression characteristic of Artesunate

S. No.	Parameters	Values
1.	Beer's law limit	10-70 (µg/ml)
2.	Molar absorptivity	3.6 lit. gm^{-1} cm^{-1}
3.	Regression equation (Y = a + bc)	0.0001x+0.0004
4.	Correlation coefficient (r^2)	0.9994
5.	Slope (b)	0.0001
6.	Intercept (a)	0.0004

Table 5: Beer's law data and Regression characteristic of Mefloquine

S. No.	Parameters	Values
1.	Beer's law limit	20-140 (μg/ml)
2.	Molar absorptivity	6.6 lit. gm^{-1} cm^{-1}
3.	Regression equation (Y = a + bc)	0.0001x+0.0008
4.	Correlation coefficient (r^2)	0.9991
5.	Slope (b)	0.0001
6.	Intercept (a)	0.0008

Table 6: Validation parameters of Artesunate and Mefloquine (ART and MEF)

Validation parameters		Drug	Values
Specificity		ART	0.242
		MEF	0.175
Precision (% RSD)	Repeatability	ART	0.374
		MEF	0.634
	Intraday	ART	0.133
		MEF	0.108
	Interday	ART	0.368
		MEF	0.319
Range	Working Range	ART	1.0 to 70 (μg/ml)
		MEF	2.0 to 140 (μg/ml)
	Linearity Range	ART	10 μg/ml to 70 μg/ml
		MEF	20 μg/ml to 140 μg/ml

	Target Concentration	ART	40μg/ml
		MEF	80μg/ml
	Target Range	ART	32μg/ml, 40 μg/ml, 48 μg/ml
		MEF	64μg/ml, 80 μg/ml, 96 μg/ml
Accuracy (% recovery)		ART	99.95 ± 0.130
		MEF	100.09 ± 0.313
LOD (μg/ml)		ART	0.33
		MEF	0.66
LOQ (μg/ml)		ART	1.00
		MEF	2.00
Percent mean recovery for Tablets		ART , MEF	99.23 ± 0.776 , 99.08 ± 0.547

5. CONCLUSION

The method was validated and found to be sensitive, specific, economic, accurate and precise. Hence, the method can be used successfully for routine analysis of pharmaceutical dosage form of Artesunate and Mefloquine.

6. ACKNOWLEDGMENT

Authors are grateful to the Director, Lachoo College of Science and Technology, Pharmacy Wing for providing necessary facilities to carry out the research work and Aster Pharmaceuticals and Intas Pharmaceuticals for providing the gift sample of the drugs.

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