

METHOD DEVELOPMENT AND VALIDATION OF ARTESUNATE AND MEFLOQUINE HYDROCHLORIDE IN BULK AND DOSAGE FORM BY HPTLC

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ABSTRACT

A simple, specific, accurate and precise HPTLC method has been developed for the determination of Artesunate and Mefloquine Hydrochloride in bulk dosage form by using HPTLC plates (Merck) precoated with silica gel 60F₂₅₄ on aluminum sheets, with a band width of 5 mm using Camag 20 µl syringe and Camag – Linomat IV Sample Applicator. The sample was analysed using Methanol: Chloroform in the ratio of 4 : 1 v/v and detected by absorbance mode at 230nm. The assay was performed and validated extensively for specificity, linearity, accuracy, precision, recovery, limit of quantization and detection. The proposed method was precise, rapid, accurate, specific and reproducible.

KEY WORDS: Validation parameters, Method Development, Artesunate, Mefloquine, High Performance Thin Layer Chromatography (HPTLC).

1. INTRODUCTION

Artesunate is chemically (3R,5As,6R,8As,9R,10S,12R,12aR)-Decarbohydro-3,6,9-trimethyl-3,12-epoxy-12H-pyrano(4,3-j)-1,2-benzodioxepin-10-ol. It is a part of Artemisinin group of drugs that treat malaria. It is a semi-synthetic derivative of Artemisinin that is given by injection. It is the drug of choice for severe malaria both in children and adults where there is low transmission. Artemisinins exert their antimalarial action by perturbing redox homeostasis in malaria parasites.

Mefloquine hydrochloride is [(2, 8- bis(trifluoromethyl)quinolin-4-yl)-(2-piperidyl)methanol.HCl. It is an orally administered medication used in the prevention and treatment of malaria. The exact mechanism of action is uncertain. However, it is proposed to share a similar mechanism of action with Chloroquine, which is inhibition of Heme polymerase.

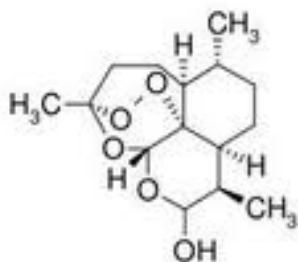


Figure 1: Chemical Structure of Artesunate

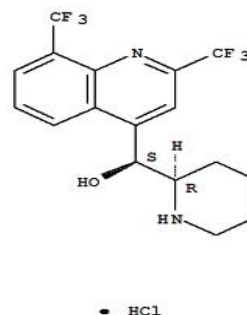


Figure 2: Chemical Structure of Mefloquine hydrochloride

2. MATERIALS AND METHODS

Instruments employed: HPTLC system consists with Camag Linomat IV Semi Automated Sample Applicator, With Camag 100 µl Syringe; Camag Twin Trough Chamber (20 x 10); Camag TLC Scanner III with Wincat software. Shimadzu Digital Electronic Balance and Ultra Sonicator was used.

Preparation of standard stock solutions: For Artesunate, standard stock solution was prepared by dissolving 10mg of drug in 10ml of methanol, to give a concentration of 1mg/ml.

For Mefloquine hydrochloride, standard stock solution was prepared by dissolving 10mg of drug in 10ml of methanol, to give a concentration of 1mg/ml.

Linearity: Dilutions were made from the stock solutions to get mixed standard 1000 $\mu\text{g/ml}$ of each Artesunate and Mefloquine hydrochloride. This solution, containing 4-14 $\mu\text{g/spot}$ for Artesunate and 8.8-30.8 $\mu\text{g/spot}$ for Mefloquine hydrochloride was then applied as spots on same HPTLC plate. After developing the plate with selected mobile phase, it was then dried with hair drier. Then areas under curve of Artesunate and Mefloquine hydrochloride, in those six mixed standard concentrations were measured at 230 nm. The measured peak areas were plotted against concentrations and the corresponding data were taken.

System precision: System precision was done by repeating the area under curve values obtained using six spots of 4 $\mu\text{l/spot}$ each of the sample solution containing 10 and 22 $\mu\text{g/spot}$ of both Artesunate and Mefloquine hydrochloride respectively and the results are taken.

Assay

Preparation of sample solution: Accurately weigh 20 capsules of Artesunate and Mefloquine hydrochloride combination each containing 100mg of Artesunate and 220mg of Mefloquine hydrochloride as per label claim. A weight (avg.weight) equivalent to 100mg of Artesunate and 220mg of Mefloquine hydrochloride was taken in a 100ml standard flask dissolved in water. Shake well for 30mins, Ultrasonicate and filtered using Whatmann filter paper and finally made up to the mark with methanol. The stock solution prepared above contains 1000 $\mu\text{g/ml}$ of Artesunate and 1000 $\mu\text{g/ml}$ of Mefloquine hydrochloride.

Estimation: The solution prepared above was applied as spots of 8 μl , 10 μl and 12 $\mu\text{l/spot}$ on the same HPTLC plate. After development of plate on the selected mobile phase, it was dried in hot air oven. Then the peak areas of Artesunate and Mefloquine hydrochloride in those three concentrations were measured at 230 nm.

Recovery studies: To ensure the reliability of the method, recovery studies were carried out by mixing known quantity of standard drug with the preanalyzed sample formulation and the contents were reanalyzed by the proposed method. A dilution of 1000 : 1000 $\mu\text{g/ml}$ of Artesunate and Mefloquine hydrochloride respectively were prepared, and 0.8 ml, 1 ml and 1.2 ml from this solution was added individually to sample formulation having 100 $\mu\text{g/ml}$ Artesunate and 220 $\mu\text{g/ml}$ of Mefloquine hydrochloride respectively. The peak areas of Artesunate and Mefloquine hydrochloride in those three dilutions were measured at 230 nm and the results obtained are tabulated.

RESULTS AND DISCUSSION

Linearity and Range: From the HPTLC densitograms it was found that Artesunate and Mefloquine hydrochloride obeys Beer's law, and the concentration lies between 4-14 $\mu\text{g/spot}$ for Artesunate and 8.8-30.8 $\mu\text{g/spot}$ for Mefloquine hydrochloride as shown in figure.3

Figure.3: HPTLC densitogram showing linearity of Artesunate and Mefloquine hydrochloride in mixed standards

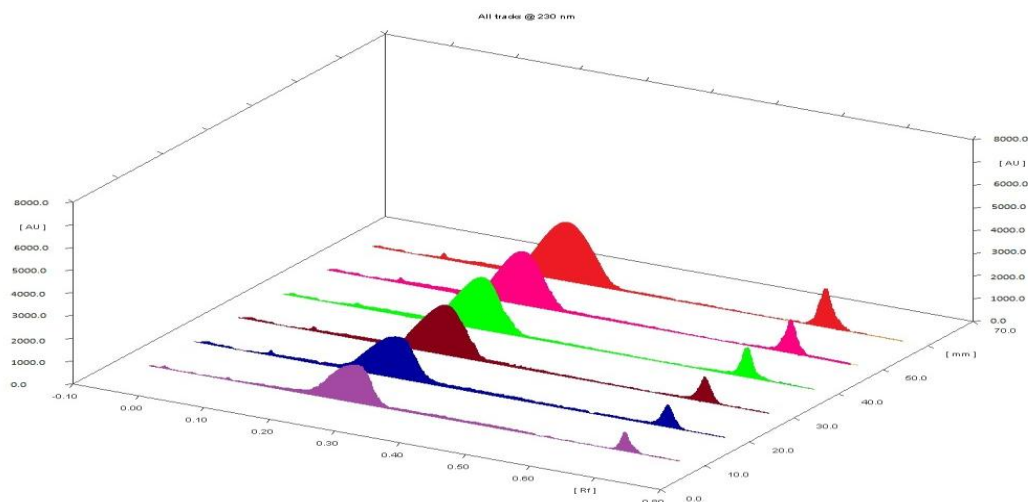


Table.1: Peak area values obtained for Artesunate and Mefloquine hydrochloride in mixed standards

Artesunate			Mefloquine hydrochloride		
Amount (µg/spot)	Rf values	Peak area	Amount (µg/spot)	Rf values	Peak area
4	0.23	2016.71	8.8	0.67	719.16
6	0.23	2904.91	13.2	0.67	848.54
8	0.23	3732.82	17.6	0.67	964.76
10	0.23	4554.04	22	0.67	1089.34
12	0.23	5339.32	26.4	0.67	1209.72
14	0.23	6039.07	30.8	0.67	1298.64

Figure.4: Calibration curve of Artesunate in HPTLC

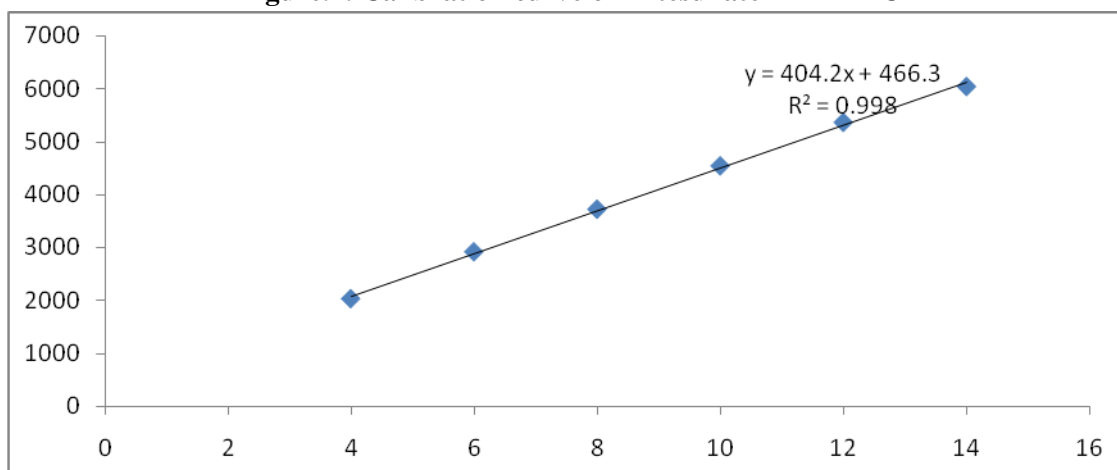
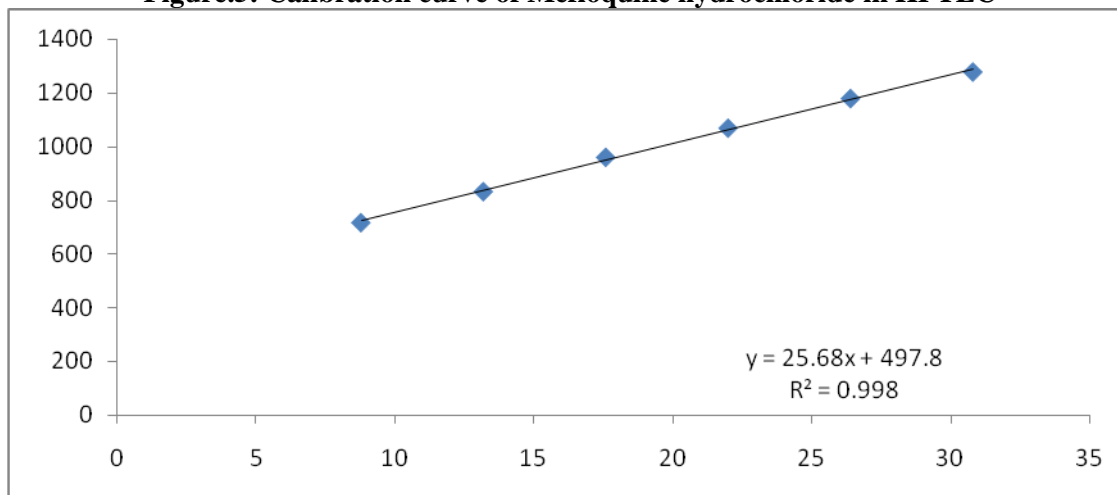


Figure.5: Calibration curve of Mefloquine hydrochloride in HPTLC



Assay of the marketed formulation:

Table.2: Percentage purity of the marketed formulation in assay.

Drug	Label claim (mg)	Amount estimated (mg)	Percentage purity
Artesunate	100	99.97	99.97
Mefloquine hydrochloride	220	220.03	100.01

Figure.6: HPTLC densitogram of Artesunate-Mefloquine hydrochloride marketed formulation

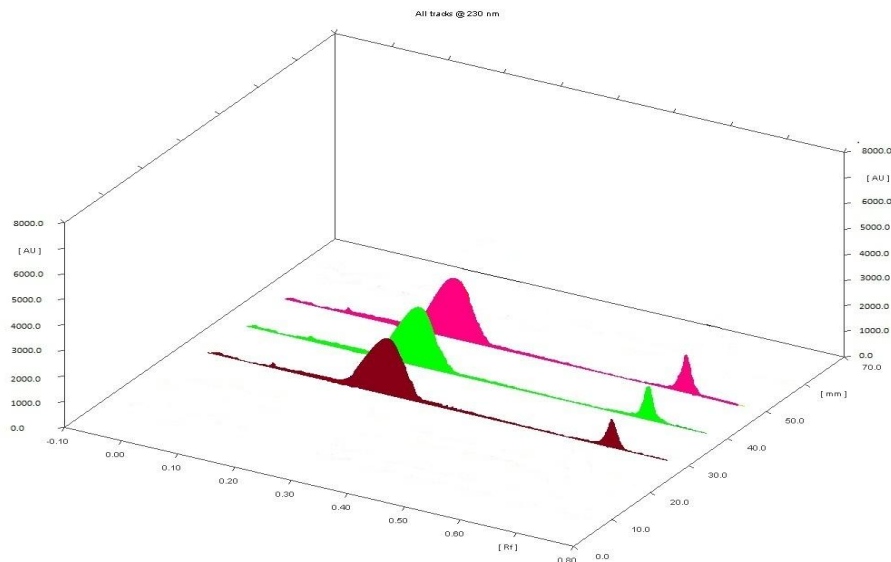
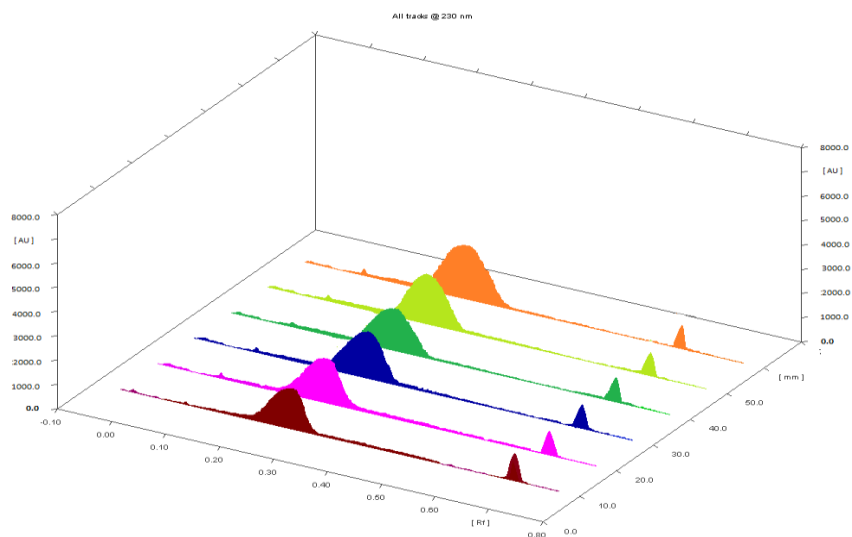


Table.3: Peak area values of Artesunate and Mefloquine hydrochloride mixed standard

Sample No.	Conc. of Artesunate	Mixed Std ($\mu\text{g}/\text{spot}$) Mefloquine hydrochloride	Peak area Artesunate	Mefloquine hydrochloride
1	10	22	4320.81	1149.14
2	10	22	4298.51	1147.64
3	10	22	4257.06	1129.39
4	10	22	4367.82	1168.39
5	10	22	4307.26	1137.82
6	10	22	4344.37	1157.62

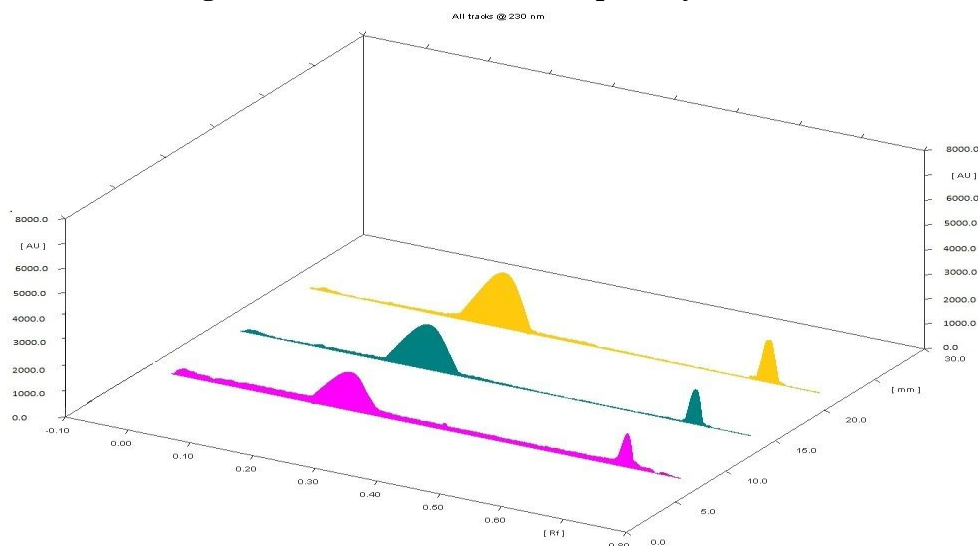
Figure.7: HPTLC densitogram of Artesunate-Mefloquine hydrochloride mixed standard.



The % RSD values for three replicate samples of Artesunate and Mefloquine hydrochloride were found to be 0.88 and 1.19 respectively, which are well within the acceptance criteria limit of < 2%.

Table.4: Percentage Recovery of the Marketed Formulation

Drug	S No.	Amount of Drug Present	Amount of Std Drug added	Amount Recovered	Percentage Recovery
Artesunate	1	100	80	181.67	100.93
	2	100	100	196.40	98.20
	3	100	120	218.61	99.37
Mefloquine hydrochloride	1	220	176	396.99	100.25
	2	220	220	439.95	99.99
	3	220	264	486.85	100.59

Figure.8: HPTLC densitogram of Artesunate and Mefloquine hydrochloride in recovery samples.

The percentage recovery of Artesunate in the marketed formulation tablets was found to be in the range of 99.20 % - 100.93 % and for Mefloquine hydrochloride was found to be in the range of 99.99 % - 100.59 %, which were well within the acceptance limit of 97% - 103% w/w.

CONCLUSION

Today HPTLC is rapidly becoming a routine analytical technique due to its advantages of low operating costs, high sample throughput and the need for minimum sample preparation. The major advantage of HPTLC is that several samples can be run simultaneously using a small quantity of mobile phase-unlike HPLC - thus reducing the analysis time and cost per analysis. The developed HPTLC technique is precise, specific and accurate. Statistical analysis proves that the method is suitable for the analysis of Artesunate and Mefloquine Hydrochloride in pharmaceutical dosage form. Hence this was adopted for the routine analysis and separation of Mefloquine Hydrochloride and Artesunate in combined dosage form.

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