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METHOD DEVELPOMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF ARTESUNATE AND MEFLOQUINE IN PURE AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, rapid, accurate and precise development and validation of RP-HPLC method for the simultaneous estimation of Artesunate and Mefloquine in bulk and combined tablet dosage form. forms. ThermoBDS column (250mm×4.6mm, id) 5 µm particle size was used as stationary phase. The mobile phase used was Acetonitrile:Buffer POT.Phos. Buffer (60:40v/v) at pH7.2. A flow rate of 0.7 ml/min was delivered. UV detection was set at 230 nm. Retention time for ART and MEF was found to be 2.91 min and 9.37 min respectively. The linearity was observed over the range of 4-20mcg/ml and 5-25mcg/ml for ART and MEF respectively. The correlation co-efficient found to be 0.998 and 0.998 for ART and MEF respectively. Moreover, the %RSD for repeatability, Inter and intra-day precision was found to be less than 2% which reveals method is precise. The %recovery was found to be 101.00% for ART and 100.25% for MEF. The assay percentage was found to be 100.68% and 103.65% for ART and MEF respectively. All the validation parameters were checked according to ICH guidelines.

KEYWORDS: Artesunate and Mefloquine, RP-HPLC Method, Validation.

INTRODUCTION

MEFLOQUINE

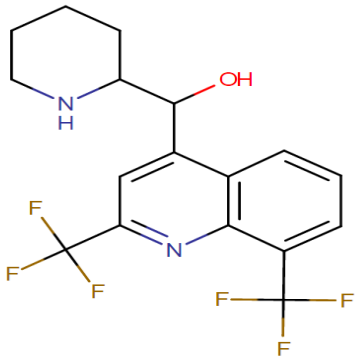

Fig. 1. Structure of Mefloquine

Mefloquine is an anti-malarial drug. This compound belongs to the quinolines and derivatives. These compounds containing a quinoline moiety, which consists of a benzene ring fused to apyrimidine ring to form benzo[b]azabenzene.Chemically it is a [2,8-bis(trifluoromethyl)quinoline-4-yl](piperidin-2-yi)methanol. It is active against plasmodium .falciparum and p.vivax. It acts by forming toxic complexes with heme, that damage membranesand interact with other plasmodial components. It is available in different brand names like Lariam,Mephaquine or Mefliam ect.[1]

ARTESUNATE

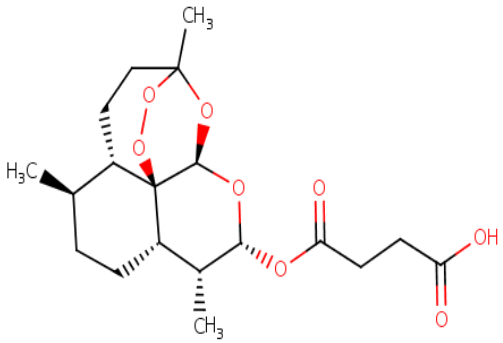

Fig No2 Structure of Mefloquine

Artesunate is an anti-malarial drug. It is a partof artemisinin group of drug.It is semi-syntheticderivative of artemisinin. Chemically it is a (3R5As,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 used for sever malaria. The mechanism action is, it involves damage to the parasite membrane by formation of carbon-centered free radicals, which are generated by the

breakdown of Malaria is a mosquito-borne infectious disease of humans and other animals caused by parasitic protozoans (a type of unicellular microorganism) of the genus Plasmodium. Commonly,the disease is transmitted via a bite from an infected female Anopheles mosquito, which introduces theorganisms from its saliva into a person's circulatory system.[2,3] Malaria parasites belong to thegenus Plasmodium .In humans, malaria is caused byP. falciparum, P. malariae, P. ovale, P. vivax andP. knowlesi. Among those infected, P. falciparum is themost common species identified (~75%) followedby P. vivax (~20%). In this case the combination of Quinoline Methanols and Artemisinin its derivatives i.e Artemether, Artesunate.[4,5]

MATERIALS AND METHODS

Younglin (S.K) Isocratic solvent delivery system,UV detector model no Acme 9000 ,Auto chro 3000 rheodyne injector with a 20-µl fixed loop, UV–visible detector, C18 ThermoBDS column (particle size 5µm; 250mm×4.6mm) A Micro analytical balance (Shimadzu), Ultrasonic cleaner, Nylon membrane filters (0.22 µm, 47 mm D).

Materials and Reagents

ART and MEF were procured as gift sample from Swapnroop Pharmaceutical Laboratory, Aurangabad. Methanol HPLC Grade, Water HPLC Grade, Mumbai ,India.

Selection of Analytical Wavelength

ART and MEF were scanned in UV as well different trails were taken in RP-HPLC at different wavelength in which both ART and MEF show reasonably good response at 230

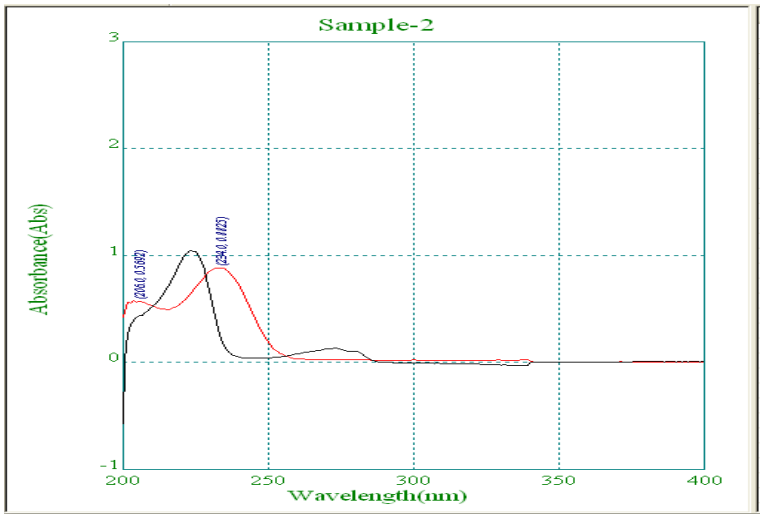

Fig. 3.Iso-absorptive point of Artesunate and Mefloquine. Optimization of mobile phase

Mixed standard solution containing 25 ppm ART and 10 ppm MEF was chromatographed using mobile phases.

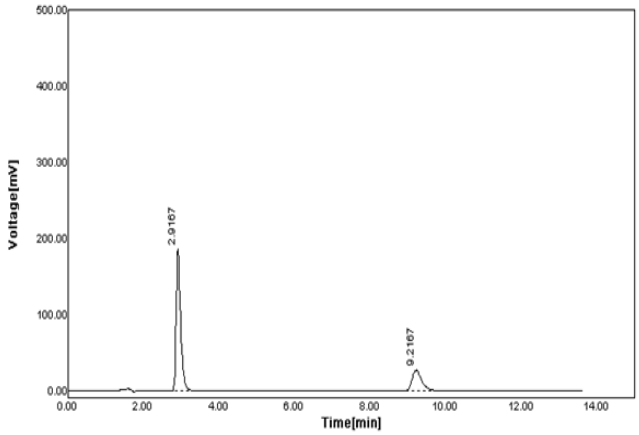

Figure no. 4: Optimised chromatogram of ART and MEF, (pH 7.0): Methanol :Water (60:40v/v).

Preparation of Standard Solutions

a) Preparation of Standard Stock Solution of ART (2500µg/ml)

25 mg of standard ART was weighed and transferred to a 10 ml volumetric flask and dissolved in 10 ml Methanol. The flask was sonicate and volume was made up to the mark and 0.1 ml pipette out the transffered the with mobile phase diluents in 10ml volumetric flask to give a solution containing 2500µg/ml ART.

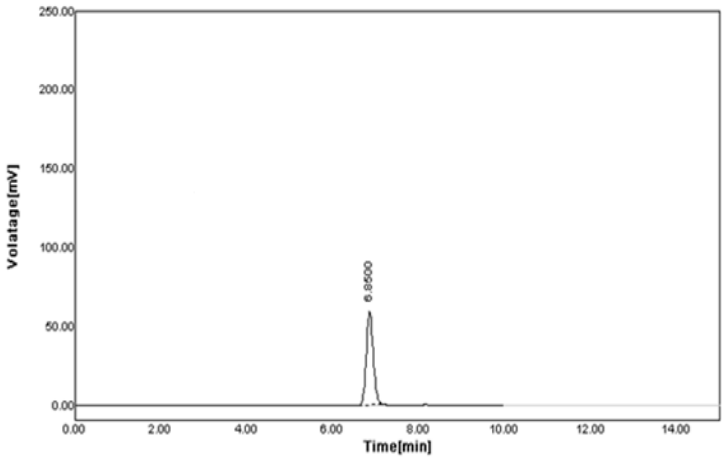

Fig. 5: Chromatogram of standard Artesunate

b) Preparation of Standard Stock Solution of MEF (1000µg/ml)

10 mg of standard MEF was accurately weighed and transferred to a 10ml volumetric flask and dissolved in 10 ml Methanol. The flask was sonicate 10 min and volume was made up to

the mark 0.1 ml pipette out the transffered the with mobile phase diluents in 10ml volumetric flask with mobile phase to give a solution containing 1000µg/ml MEF.

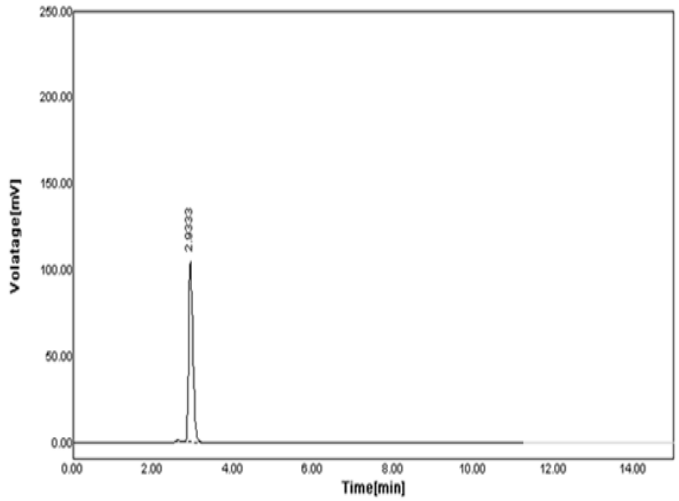

Fig. 6: Chromatogram of standard Mefloquine

Method Validation

The developed method is validated with respect to validation parameters like linearity & range, precision, accuracy, Robustness, etc.

RESULTS AND DISCUSSION

1) Linearity and Range

Calibration curve for ART (4-20µg/ml)

Chromatogram for following concentrations of 4,8,12,16,20µg/ml for ART at and at flow rate of 0.7ml/min was taken. Peak areas were obtained as tabulated in Table 1.0 and the graph of calibration curve was obtained as shown in Figure 4.0

Table no. 1: Linearity Range for ART and MEF

Sr. No.	**Concentrationµg/ml** Artesunate	**Area** Artesunate
1	4	145.26
2	8	306.25
3	12	477.12
4	16	620.84
5	20	766.66

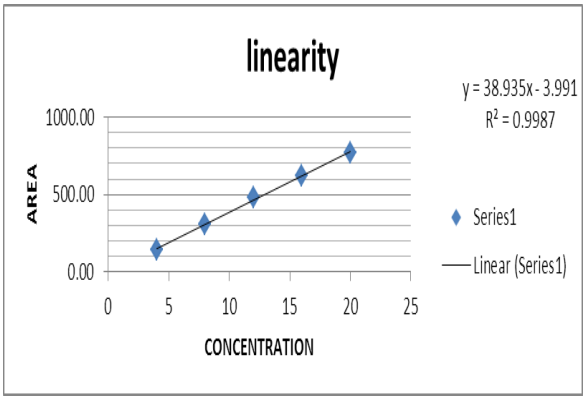

Fig.7: Calibration graph of Mefloquine

Table 2. Regression equation data for Artesunate

Regression Equation Data Y=mx+c	
Slope(m)	38.93
Intercept(c)	3.991
Correlation Coefficient	0.998

Calibration Curve for MEF (5-25µg/ml)

Chromatogram for following concentrations of 5,10,15,20,25µg/ml for MEF at and at flow rate of 0.7ml/min was taken. Peak areas were obtained as tabulated in Table 1.0 and the graph of calibrationcurve was obtained as shown in Figure 4.0

Table.3. Linearity of Mefloquine

Sr. No.	CONC.	avg.area
1	5	143.21
2	10	282.64
3	15	418.94
4	20	551.48
5	25	661.56

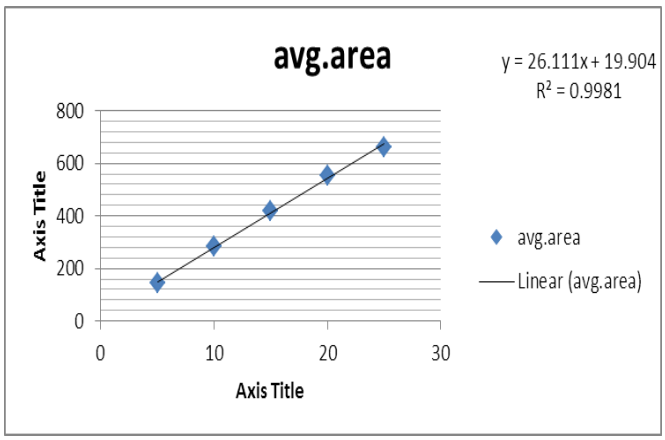

Fig.8.: Calibration graph of Mefloquine

Table No.4. Regression equation data for Mefloquine

Regression Equation Data Y=mx+c	
Slope(m)	26.11
Intercept(c)	19.90
Correlation Coefficient	0.998

2. Accuracy

Recovery studies were performed to validate the accuracy of developed method. To preanalyzed tablet solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed (Table No.12). Statistical validation of recovery studies shown in (Table No. 5)

Table No.5. Recovery studies of Artesunate and Mefloquine.

Level of Recovery (%)	80		100		120	
	ARTS.	**MEFLO**	**ARTS.**	**MEFLO.**	**ARTS.**	**MEFLO.**
Amount present (mg)	8 8	10 10	8 8	10 10	8 8	10 10
Amount of Std. Added (mg)	6.4 6.4	8 8	8 8	10 10	9.6 9.6	12 12
Amount Recovered (mg)	6.62 6.51	7.86 8.03	8.29 8.17	9.94 10.06	9.62 9.85	11.76 12.01
% Recovery	103.46 101.73	98.35 100.04	103.62 101.17	99.48 100.60	98.05 100.11	98.05 100.11

Table No. 6. Statistical Validation of Recovery Studies.

Level of Recovery (%)	Drug	Mean % Recovery	Standard Deviation*	% RSD
80	**ARTS.**	14.57	0.08	0.53
	MEFLO.	17.95	0.12	0.67
100	**ARTS.**	16.23	0.08	0.52
	MEFLO.	20.00	0.08	0.42
120	**ARTS.**	17.74	0.16	0.92
	MEFLO.	21.89	0.18	0.81

3. System suitability parameters

To ascertain the resolution and reproducibility of the proposed chromatographic system for estimation of Artesunate and Mefloquine system suitability parameters were studied.The result shown in below Table No.21

Table.7. Result of System Suitability Parameters.

System Suitability Parameters	Proposed Method	
	MEFLO.	ARTS.
Retention Time	2.9500	6.8167
Area	479.3480	420.9179
Theoretical Plate Number	2714.5	6441.7
Taling Factor	1.500	1.364

4. Precision:-

Table No.8. Inter-day precision study Artesunate

Sr No.	CONC	I	II	Mean	Amt Found	% AmtFnd	SD	RSD
1	8	309.84	302.78	306.31	7.97	99.63	4.99	1.63
2	12	479.83	480.86	480.35	12.44	103.67	0.73	0.15
3	16	621.05	625.39	623.22	16.16	101.04	3.07	0.49

Table No.9. Intra-day precision study Artesunate

Sr No.	CONC	I	II	Mean	Amt Found	% AmtFnd	SD	RSD
1	8	311.84	307.78	309.31	8.04	100.59	4.99	1.63
2	12	472.83	470.56	471.35	12.21	101.75	0.73	0.15
3	16	617.05	619.11	618.08	15.97	99.87	3.07	0.49

Table No.10. Inter-day precision study Mefloquine

Sr No.	CONC	I	II	Mean	Amt Found	% AmtFnd	SD	RSD
1	10	284.51	288.72	286.62	10.21	102.15	2.98	1.04
2	15	422.45	427.46	424.96	15.51	103.42	3.54	0.83
3	20	558.89	566.69	562.75	20.79	103.95	5.52	0.98

Table No.11. Intra-day precision study Mefloquine

Sr No.	CONC	I	II	Mean	Amt Found	% AmtFnd	SD	RSD
1	10	282.51	278.72	280.62	9.9854	99.8	2.98	1.04
2	15	412.45	417.46	414.96	15.13	100.86	3.54	0.83
3	20	548.89	546.69	547.75	20.21	101.08	5.52	0.98

5..Robustness

The mobile phase composition was changed in ± 1 ml proportion and the flow rate was varied by ± 0.1 ml min^{-1}, of optimized chromatographic condition. The results of robustness studies are shown in TableNo.(12,13)

Table No.12. Result of Robustness Study of Artesunate

Parameters	Conc.	Amount of detected(mean ±SD)	%RSD
Mobile phase composition-(61+39)	12	6.69±0.09	1.16
Mobile phase composition-(59+41)	12	6.96±0.67	1.19
Wavelength change232nm	12	2.37±0.89	0.42

Wavelength Change 230nm	12	2.02 ± 0.86	0.34
Flow rate change(0.9ml)	12	6.34± 0.71	1.36
Flow rate change(1.1ml)	12	3.10± 0.54	0.59

Table No .13. Result of Robustness Study of Mefloquine

Analysis of marketed formulation

Parameters	Conc.	Amount of detected(mean ±SD)	%RSD
Mobile phase composition-(61+39)	15	24.93±0.92	1.19
Mobile phase composition-(59+41)	15	7.42±0.67	0.56
Wavelength change232nm	15	4.53±0.89	0.27
Wavelength Change 230nm	15	32.81± 1.82	1.77
Flow rate change(0.9ml)	15	5.69± 1.48	0.47
Flow rate change(1.1ml)	15	4.68± 0.97	0.26

PROCEDURE

For analysis of the tablet dosage form, 20 tablets were weighed individually and their average weight was determined after that they were crushed to fine powders and power equivalent to 119.5 mg was taken and transferred to 50 ml volumetric flask and diluted with methanol.(STOCK I). from the above solution 0.2ml was taken and diluted to 10ml.The solutions were shaken vigorously for 10 min and filtered through 0.45 µg nylon membrane filters. Then volume was made up to mark with methanol:water..The amounts of Artesunate and Mefloquine.per tablet were calculated by extrapolating the value of area from the calibration curve. Analysis procedure was repeated five times with tablet formulation. Result is shown in (Table No. 6.)

Table. No. 14. Analysis of marketed formulation.

Sr.no	Amount present in mg		Amount found in mg		% Label claim	
	Artesunate	Mefloquine	Artesunate	Mefloquine	Artesunate	Mefloquine
1	16	20	16.05	20.64	100.31	103.20
2	16	20	16.17	20.82	101.06	104.10
Mean	_	_	_	_	100.69	100.69
SD	_	_	_	_	0.02	0.64
%RSD	_	_	_	_	0.02	0.61

From above, analysis of marketed formulation %Lable claim was found to be 100.69 for artesunate and mefloquine.

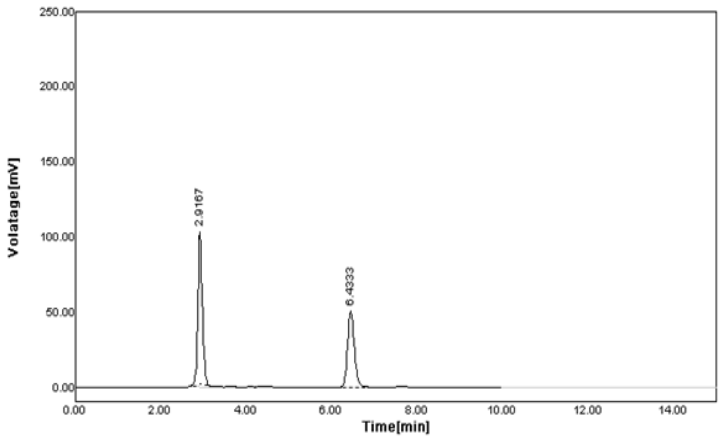

Fig.9: Chromatogram of Artesunate and Mefloquine in tablet formulation.

Table. No. 15. Details of chromatogram of Artesunate and Mefloquine in tablet formulation

Sr.no	Name of drug	RT (min)	Area	Theoretical Plates	Tailing factor
1	Mefloquine	2.9167	621.05	3465.8	1.2500
2	Artesunate	6.4333	558.89	6828.2	1.2000

CONCLUSION

The developed RP-HPLC method was found to be simple, sensitive, accurate and precise and can be used for routine analysis of Artesunate and Mefloquine. The developed methods were validated as per ICH guidelines.

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