

Research Article

Development and validation of UV-Method for simultaneous estimation of Artesunate and Mefloquine hydrochloride in bulk and marketed formulation

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Abstract: A simple, accurate, precise and economical Uv-method for estimation of Artesunate and Mefloquine HCL was developed. For analysis of Artesunate and Mefloquine HCl, 242nm and 256nm wavelengths were used respectively. The linearity range for Artesunate and Mefloquine HCl were 20-140µg/ml & 50-350µg/ml. Results of analysis were validated statistically, recovery studies for ART were 98.33-100.33 & for MEF 101.33-101.92. The LOD and LOQ were found to be 0.13µg/ml and 0.39µg/ml for ART. For MEF the LOD and LOQ values were found to be 1.53µg/ml & 2.21µg/ml the method was validated as per ICH guidelines.

Keywords: Artesunate, Mefloquine HCL, UV-Spectroscopy.

INTRODUCTION

Chemically ART was (3R,5aS,6R,8aS,9R,10S,12R,12aR)- Decahydro-3,6,9-trimethyl-3,12-epoxy-12 H-pyrano[4,3-j]-1,2-benzodioxepin-10-ol, hydrogen succinate [1] (Fig.1) and chemically MEF was 2-piperidylidyl-2,8-bis (trifluoromethyl)-4-quinoline methanol hydrochloride [2] (Fig.2) both drugs were active against *P. Falciparum* and *P. vivax* [3-4]. Both of drugs were active against the ring stage of the parasite. Artesunate is ideal for the treatment of severe malaria, including cerebral malaria [3-4].

Literature survey reveals High Performance Liquid Chromatographic (HPLC) [5], ART and MEF estimation by RP-HPLC method and for determination of ART in human plasma either as single or in combination with other drugs [6-8]. Colorimetric method for estimation of ART is also reported with Amodiaquine [6].

No, method has been reported for simultaneous estimation of both drug by UV-Spectrophotometric method. This paper describes a simple, accurate, sensitive and validated UV-Spectrophotometric method for simultaneous quantification of these compounds as the bulk drug and in combined tablet dosage forms. The proposed method is optimized and validated as per the International Conference on Harmonization (ICH) guidelines [9-10].

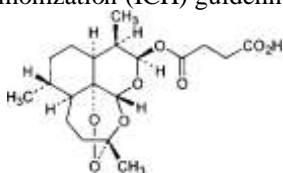


Fig.no.1 Chemical structure of ART

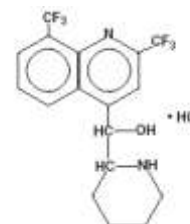


Fig.no.2 Chemical structure of MEF

MATERIAL AND METHODS

Drugs and Chemicals

A standard drug samples of ART and MEF were purchased from Zeal Pharma, Mumbai. All chemicals and solvents used of AR grade. Marketed formulation Falcigo plus tablet containing ART 100mg and MEF 200mg was used.

Standard stock solution of ART and MEF

An accurately weighed quantity of about 10 mg of ART & MEF was taken in 10 ml volumetric flask dissolved in sufficient quantity of methanol and diluted to 10 ml with the same solvent so as to get the concentration of 1000 µg/ml.

Determination of λ max of ART and MEF

The standard solution of ART was scanned in the range of 200-400 nm and the λ max was found to be 242 nm against methanol. Similarly, the standard solution of MEF was scanned in the range of 200-400 nm and the λ max was found to be 256 nm against methanol.

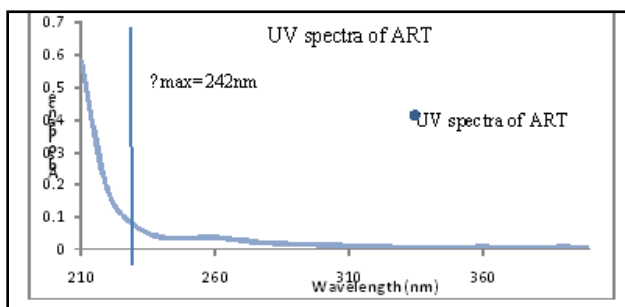


Fig.no.3 : λ max of ART

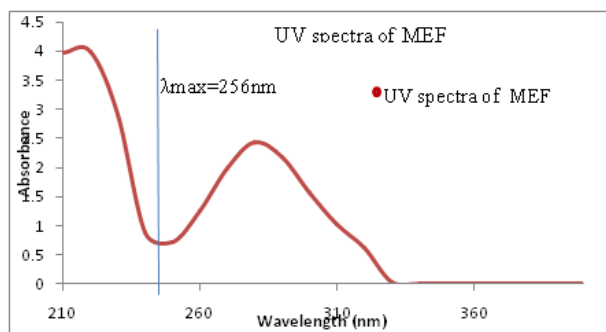


Fig.no.4: λ max of MEF

Analysis of Marketed formulation

Twenty tablets were weighed accurately; the average weight was determined and then triturated to a fine powder. A quantity equivalent to 100 mg of ART and 200 mg of MEF was weighed and transferred to a 100 ml volumetric flask containing 70 ml methanol and the contents were sonicated for 20 min with methanol to dissolve the active ingredients. Volume was made up to 100 ml with methanol and filtered through Whatman filter paper no. 41 to give the stock solution containing 1000 $\mu\text{g/ml}$ of ART and 2000 $\mu\text{g/ml}$ of MEF. Various dilutions of the tablet stock solutions were scanned and the absorbances of these solutions were measured at 242 nm and 256 nm respectively.

Table No.1: Analysis of Marketed formulation

Drug Label claim	% found \pm RSD	% recovery \pm RSD
Artesunate 100mg	99.69 \pm 0.3297	99.36 \pm 0.4183
Mefloquine hydrochloride 200mg	99.75 \pm 0.2345	99.41 \pm 0.3214

Method validation

The method was developed and validated according to analytical procedure as per the ICH guidelines [9-10] for validation of analytical procedures in order to determine linearity, precision, LOD, LOQ and accuracy for the analyte .

RESULT AND DISCUSSION

A) LINEARITY

From the standard stock solutions containing 1000 $\mu\text{g/ml}$ of ART and 1000 $\mu\text{g/ml}$ of MEF dilutions

were made to prepare range of standard solutions having different concentrations of ART (20-140 $\mu\text{g/ml}$) and MEF (50-350 $\mu\text{g/ml}$). The absorbances were measured at 242 nm (λ max of ART) and at 256 nm (λ max of MEF) respectively. The results obtained are shown in Table no.2.

The linearity of the relationship between absorbances and concentration was determined by plotting the calibration curve for ART and MEF respectively .The calibration curves are shown in figure 2 and 3

Table No.2: Linearity study for ART and MEF

Sr. no.	Conc . ($\mu\text{g/ml}$)	Absorbance of ART	Conc . ($\mu\text{g/ml}$)	Absorbance of MEF
		242nm		256 nm
1	20	0.031	50	0.124
2	40	0.035	100	0.233
3	60	0.039	150	0.353
4	80	0.044	200	0.478
5	100	0.049	250	0.607
6	120	0.055	300	0.710
7	140	0.060	350	0.830
		R=0.9954		R=0.9994

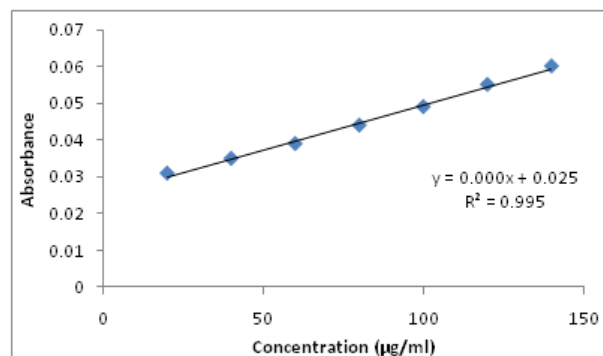


Fig No.5: Calibration curve of Artesunate at 242nm

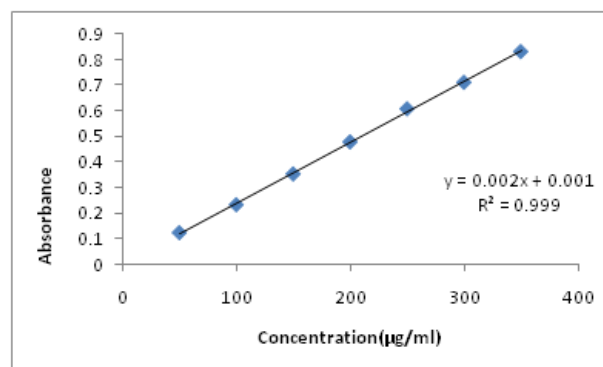


Fig.No.6: Calibration curve of Mefloquine at 256nm

B) PRECISION

Repeatability of method was established by analyzing various replicates samples of ART and MEF. Precision was carried out by performing Interday

variation and intraday variation. In Interday variation the sample was analyzed on three consecutive days. In intraday variation the absorbances were measured three times in a day.

The results for intraday precision are shown in Table no.3 & 4 and for Interday precision are shown in Table no. 5 & 6.

Table No.3: Intraday Precision for ART

Conc. (µg/ml)	% of label claim			Mean % of label claim	± SD	%RSD
	Trial 1	Trial 2	Trial 3			
80	98.44	98.96	99.44	98.94	0.277128	0.28172
100	101.19	100.2	101.19	100.86	0.525389	0.51767
120	98.7	98.57	98.7	98.65	0.075056	0.07686

Table No.4: Intraday Precision for MEF

Conc. (µg/ml)	% of label claim			Mean % of label claim	± SD	% RSD
	Trial 1	Trial 2	Trial 3			
180	99.36	99.85	99.96	99.72	0.319427	0.32032
200	100.06	100.28	100.06	100.13	0.127017	0.12685
220	100.15	99.95	100.28	100.12	0.166233	0.16603

Table No.5: Interday Precision for ART

Conc.(µg/ml)	% of label claim			Mean % of label claim	± SD	%RSD
	Day 1	Day 2	Day 3			
80	99.44	98.96	99.44	99.28	0.277128	0.2939
100	99.23	98.56	98.86	98.88	0.315119	0.3297
120	98.7	98.29	98.7	98.56	0.236714	0.2455

Table No. 6: Interday Precision for MEF

Conc. (µg/ml)	% of label claim			Mean % of label claim	± SD	%RSD
	Day 1	Day 2	Day 3			
180	99.36	99.85	99.97	99.72	0.32316	0.32406
200	100.06	100.06	100.17	100.09	0.06350	0.06345
220	99.55	99.65	99.85	99.68	0.15275	0.15324

C) LIMIT OF DETECTION

The Limit of Detection (LOD) is the smallest concentration that can be detected but not necessarily quantified as an exact value. LOD was calculated using the following formula:

$$\text{LOD} = \frac{3.3 \times \text{Std.deviation of } y \text{ intercept}}{\text{Slope of calibration curve}}$$

The limit of detection of ART and MEF are as follows:

ART: 0.13 µg/ml

MEF: 1.53 µg/ml

D) LIMIT OF QUANTITATION

The Limit of Quantification (LOQ) is the lowest amount of analyte in the sample that can be quantitatively determined with suitable precision and accuracy. LOD was calculated using the following formula:

$$\text{LOQ} = \frac{10 \times \text{Std.deviation of } y \text{ intercept}}{\text{Slope of calibration curve}}$$

The limit of detection of ART and MEF are as follows:

ART: 0.39 µg/ml

MEF: 2.21 µg/ml

Table No.7: Linear regression analysis of calibration curves with their respective Absorptivity values.

Parameter	ART	MEF
Detection of wavelength	242 nm	256 nm
Beer's law limit (µg/ml)	20-140	50-350
Correlation coefficient(r)	0.9954	0.9994
Slope	0.0002	0.0024
Intercept	0.0251	0.0013
LOD(µg/ml)	0.13	1.53
LOQ(µg/ml)	0.39	2.21

E) RECOVERY STUDIES

Recovery studies were carried out by standard addition method at three levels, 80%, 100%, and 120%. In this method a known amount of standard drug solution were added to tablet solution and absorbances were measured at 242 nm and 256 nm (λ_{max} of ART and MEF respectively) and the concentration of both drugs can be determined. At each level three determinations were performed and results were obtained. The results for recovery studies were given in Table 8, 9 & 10.

Table No.8: Recovery study of ART and MEF

Level of % Recovery`	Amount present		Amount added		Absorbance values		% Recovery	
	1. ART	MEF	ART	MEF	242 nm	256nm	ART	MEF
80	100	200	80	180	0.892	0.998	100.33	101.94
	100	200	80	180	0.891	0.997	100.41	102.02
	100	200	80	180	0.889	0.995	100.27	101.80
100	100	200	100	200	0.896	1.003	98.50	101.69
	100	200	100	200	0.894	1.001	98.65	101.68
	100	200	100	200	0.895	1.002	97.85	101.46
120	100	200	120	220	0.900	1.007	98.54	101.64
	100	200	120	220	0.903	1.011	98.09	101.51
	100	200	120	220	0.905	1.013	98.68	101.69

Table No.9: Statistical Validation for recovery studies of ART

Level of % Recovery	% Mean Recovery	SD	% RSD
80	100.33	0.07023	0.07000
100	98.33	0.42524	0.43245
120	98.46	0.30827	0.31317

Table No.10: Statistical Validation for recovery studies of MEF

Level of % Recovery	% Mean Recovery	SD	% RSD
80	101.92	0.11135	0.10925
100	101.61	0.13452	0.12794
120	101.33	0.09291	0.09144

The linearity of ART and MEF was observed in the range of 20-140µg/ml and 50-350µg/ml respectively with correlation coefficient 0.9954 & 0.994 for ART and MEF respectively. Detection wavelength used was 242nm for ART and 256nm for MEF. The LOD and LOQ values for ART were 0.13µg/ml & 0.39µg/ml. For MEF the LOD and LOQ values were 1.53µg/ml & 2.21µg/ml.

CONCLUSION

A simple, precise, accurate and economic UV was developed and validated for estimation of Artesunate and Mefloquine from bulk and marketed formulation (Falcigo plus). The method was validated as per ICH guidelines by using various validation parameters such as Linearity, accuracy, precision, LOD, LOQ, range and robustness. The LOD and LOQ were found to be 0.13µg/ml and 0.39µg/ml for ART. For MEF the LOD and LOQ values were found to be 1.53µg/ml & 2.21µg/ml the method was validated as per ICH guideline.

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