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Stability Indicating RP-UHPLC Method Development and Validation for the Simultaneous Estimation of Artesunate and Mefloquine HCl in Synthetic Mixture and Tablet Dosage Form

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ABSTRACT

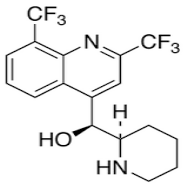
Combination of Mefloquine Hydrochloride and Artesunate was used for the treatment of Malaria. A simple, rapid, economical, precise and accurate Stability indicating RP-UHPLC method for simultaneous estimation of Mefloquine Hydrochloride and Artesunate in their combined synthetic mixture form has been developed. The method has shown adequate separation for Mefloquine Hydrochloride and Artesunate from their degradation products. The separation was achieved by Solas 1.9 um C18 120 A,2.1mm id X 100mm column and Buffer (Potassium Phosphate, pH 4.0): Acetonitrile (35:65) as mobile phase, at a flow rate of 0.3 ml/min. Detection was carried out at 215 nm. These drugs were subjected to hydrolysis, oxidation, photolysis and thermal to apply stress conditions. Stability indicating UHPLC method was developed and validated. Retention time of Mefloquine Hydrochloride and Artesunate were found to be 0.870 min and 1.523 min respectively. The method has been validated for linearity, accuracy and precision. Linearity observed for Mefloquine Hydrochloride 5.00-15.00 µg/ml and for Artesunate 11.00-33.00 µg/ml. The drug was subjected to stress condition of hydrolysis, oxidation, photolysis and thermal degradation. The proposed method was successfully applied for the simultaneous estimation of both the drugs in commercial Combined synthetic mixture form. The UHPLC methods were found to be simple, accurate, robust and reproducible. In UHPLC method Degradation products produced as a result of stress studies did not interfere with the detection of Mefloquine Hydrochloride and Artesunate and the assay can thus be considered stability-indicating and can be successfully applied for routine QC analysis.

INTRODUCTION

Chemically Mefloquine Hydrochloride was(RS)-[2,8bis(trifluoromethyl)quinoline-4-yl] [(2SR)-piperidin-2-yl methanol hydrochloride] (fig-1)[1] and Artesunate was 4-oxo-4-{[(1R,4S,5R,8S,9R,10S,12R,13R)-1,5,9-trimethyl-11,14,15,16-tetraoxatetracyclo[10.3.1.0^{4},^{13}.0^{8},^{13}]hexadecan-10-yl]oxy}butanoicacid(fig-2)[2].

Both of the Drugs are active against *P. falciparum malaria* parasite. Artesunate is active against the severe malaria and also active against cerebral malaria.

Literature survey reveals that through Ultra High Performance Liquid Chromatography for determination of mefloquine HCl and artesunate by simultaneous estimation in synthetic mixture and tablet dosage form are not established or either established and reported in single dosage form and nor in combination form by using UHPLC, HPLC, HPTLC and with other method also. This paper describes a Simple, accurate, precise, fast, sensitive and validated, RP-UHPLC method for simultaneous estimation of Artesunate and mefloquine in synthetic mixture and in tablet dosage form. This proposed method is optimized and validated as per ICH guidelines[4-10].



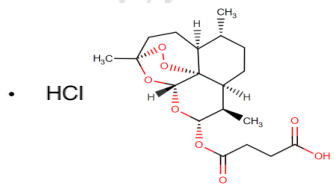


Figure No. 1: Structure of Mefloquine HCL Figure No. 2: Structure of Artesunate

MATERIALS AND METHODS

Drugs and chemicals

A standard drug sample of ART and MEF HCL are procured from Yash pharma and marketed formulation Falcigo plus tablet formulation containing MEF HCL 100mg and ART 220mg was used, solved are HPLC grade was used.

Standard stock solution of ART and MEF HCL

Accurately weighed separately quantity of 10.00 mg Mefloquine HCl and 22.00 mg Artesunate API were transferred into 100 ml volumetric flask and dissolved in HPLC grade

methanol using ultrasonication and diluted up to mark to give a stock solution having concentration of 100 µg/ml Mefloquine HCl and 220 µg/ml Artesunate[11-12].

Preparation of Sample Stock Solution

Take synthetic mixture equivalent to 10.00 mg of Mefloquine HCl or 22.00 mg of Artesunate was transferred to 100ml volumetric flask. Then 60 ml methanol was added and sonicated for 5 mins to ensure complete solubilization of drug. After sonication, volume was made up to the mark with methanol. Filter the stock solution with Whatman filter paper no 42 and the final filtrate is collected as sample stock solution[11-12].

Detection of Wavelength

UV spectra of Mefloquine HCl and Artesunate were taken in Methanol and λmax was observed using SPD-20A, UV-VIS Spectrophotometer. At 215 nm both drug give good response and also degradation product detected at this wavelength. So, 215 nm was selected for stability indicating simultaneous estimation of Mefloquine HCl and Artesunate in tablet dosage form.

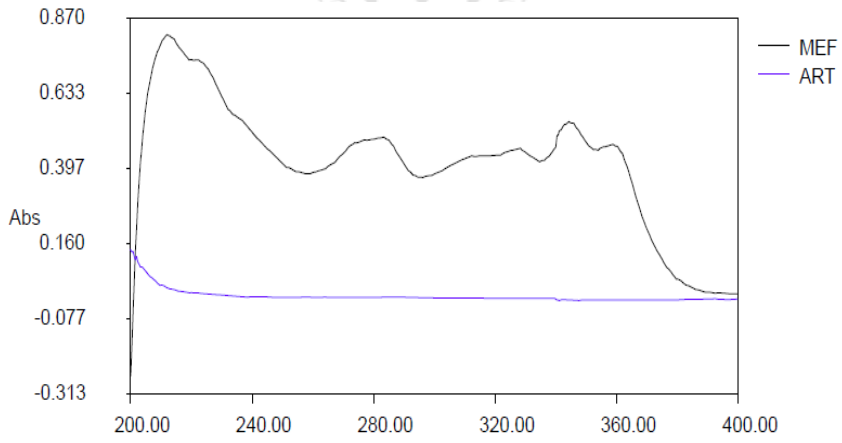


Figure No. 3: UV Spectra of ART and MEF HCL

Analysis of Market Formulation

Take synthetic mixture equivalent to 10.00 mg of Mefloquine HCl or 22.00 mg of Artesunate was transferred to 100ml volumetric flask. Then 60 ml methanol was added and sonicated for 5 mins to ensure complete solubilization of drug. After sonication, volume was made up to the mark with methanol. Filter the stock solution with Whatman filter paper no 42 and the final filtrate is collected as sample stock solution. 1 ml of this solution was diluted to 10 ml

with mobile phase. The solution was injected 10 µl. The areas of resulting peak were measured at 215 nm.

Table No. 1: Analysis of marketed formulation

Label claim		Assay (% of label claim*) Mean ± S. D.	
Mefloquine HCl	**Artesunate**	**% Mefloquine HCl**	**% Artesunate**
100 mg	220 mg	101.536 ± 0.701	96.236 ± 0.709

Method Validation

The method was developed and validated according to the analytical procedure as per ICH guidelines for validation of ICH guidelines for validation of Analytical procedures in order to Determine Linearity, Precision, LOD, LOQ, and accuracy for the Analyte.

RESULTS AND DISCUSSION

Linearity

The linearity for Mefloquine HCl and Artesunate were assessed by analysis of combined standard solution in range of 5.0-15.0 µg/ml and 11.0-33.0µg/ml respectively. The graph of peak area obtained versus respective concentration was plotted.

Acceptance criteria: Value of r^2 should be nearer to 1 or equal to 1.

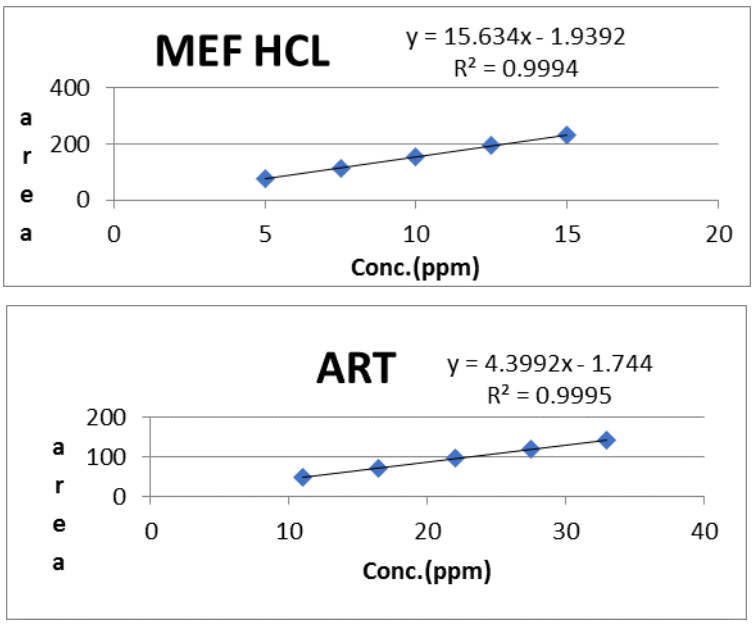


Figure No. 4: calibration curve of MEF HCL and ART

Table No. 2: Linearity study of MEF HCL

Sr. No.	Stock solution of Artesunate (ml)	ml make up	Concentration (µg/ml)	area
1	0.5	10	11	47.175
2	0.75	10	16.5	69.655
3	1	10	22	95.25
4	1.25	10	27.5	120.263
5	1.5	10	33	142.849

Table No. 3: Linearity study of ART

Sr. No.	Stock solution of Mefloquine (ml)	ml make up	Concentration (µg/ml)	area
1	0.5	10	5	77.414
2	0.75	10	7.5	113.061
3	1	10	10	154.58
4	1.25	10	12.5	195.147
5	1.5	10	15	231.794

Precision:

Repeatability:

Standard solution containing Mefloquine HCl (10.00 µg/ml) and Artesunate (22.00µg/ml) was injected six times and areas of peaks were measured and % R.S.D. was calculated.

Acceptance criteria: % RSD of Area should not be more than 2.0%.

Table No. 4: Repeatability Data for Mefloquine HCl

Mefloquine HCl				
Sr. No.	**Conc (µg/ml)**	**Area**	**Mean ± S.D (n=6)**	**% R.S.D**
1.	10.00	154.929	154.624±0.836	0.541
		155.916		
		154.278		
		153.821		
		155.061		
		153.739		

Table No. 5: Repeatability data for Artesunate

Artesunate				
Sr. No.	**Conc (µg/ml)**	**Area**	**Mean ± S.D (n=6)**	**% R.S.D**
1.	22.00	93.189	94.826 ±0.806	0.850
		95.057		
		95.25		
		95.057		
		95.249		
		95.154		

Intraday precision: Standard solution containing (5.0, 10.0, 15.0µg/ml) of Mefloquine HCl and (11.0, 22.0, 33.0 µg/ml) of Artesunate were analyzed three times on the same day and % R.S.D was calculated.

Acceptance criteria: % RSD of Area should not be more than 2.0%.

Table No. 6: Intraday precision data for Estimation of Mefloquine HCl and Artesunate

Sr. No.	**Mefloquine HCl**			**Artesunate**		
	Conc. (µg/ml)	**Area Mean ± S.D. (n=3)**	**% R.S.D**	**Conc. (µg/ml)**	**Area Mean ± S.D. (n=3)**	**% R.S.D**
1	5.00	76.405 ± 1.147	1.501	11.00	46.82 ± 0.249	0.533
2	10.00	154.625 ± 1.170	0.756	22.00	94.346 ± 0.579	0.613
3	15.00	232.042 ± 2.239	0.965	33.00	141.821 ± 0.808	0.569

Interday precision: Standard solution containing (5.0, 10.0, 15.0µg/ml) of Mefloquine HCl (11.0, 22.0, 33.0 µg/ml) of Artesunate were analyzed three times on the different day and % R.S.D was calculated.

Acceptance criteria: % RSD of Area should not be more than 2.0%.

Table No. 7: Interday Precision data for Estimation of Mefloquine HCl and Artesunate

Sr. No.	Mefloquine HCl			Artesunate		
	Conc. (µg/ml)	Area Mean ± S.D. (n=3)	% R.S.D	Conc. (µg/ml)	Area Mean ± S.D. (n=3)	% R.S.D
1	5.00	76.720 ± 0.601	0.783	11.00	46.634 ± 0.530	1.137
2	10.00	153.073 ± 1.806	1.179	22.00	94.563 ± 0.698	0.738
3	15.00	230.871 ± 2.988	1.294	33.00	141.403 ± 1.281	0.905

Accuracy

Accuracy of the method was confirmed by recovery study from marketed formulation at three level of standard addition. The results are shown in table 7 and 8. Percentage recovery for Mefloquine HCl was **98.32-100.52%**, while for Artesunate, it was found to be in range of 98.86-**101.09** %.

Table No. 8: Recovery data for Mefloquine HCL

Sr. No.	Conc. Level (%)	Sample amount (µg/ml)	Amount Added (µg/ml)	Amount recovered (µg/ml)	% Recovery	% Mean Recovery ± S.D
1	80 %	5	4	3.93	98.32	99.54 ± 1.12
2		5	4	4.02	100.52	
3		5	4	3.99	99.79	
4	100 %	5	5	4.96	99.15	99.53 ± 0.82
5		5	5	5.02	100.47	
6		5	5	4.95	98.96	
7	120 %	5	6	6.00	100.06	99.65 ± 0.42
8		5	6	5.95	99.21	
9		5	6	5.98	99.67	

Table No. 9: Recovery data for Artesunate

Sr. No.	Conc. Level (%)	Sample Amount	Amount Added	Amount recovered (µg/ml)	% Recovery	% Mean Recovery ± S.D
1	80 %	11	8.8	8.76	99.51	100.31 ± 0.79
2		11	8.8	8.90	101.09	
3		11	8.8	8.83	100.33	
4	100 %	11	11	10.92	99.28	99.63 ± 0.43
5		11	11	11.02	100.15	
6		11	11	10.94	99.46	
7	120 %	11	13.2	13.20	100.01	99.53 ± 0.61
8		11	13.2	13.05	98.86	
9		11	13.2	13.17	99.78	

Robustness:

The effect of changes was found to be within the acceptance criteria as shown in table no. 10 and table no. 11. The % RSD should be less than 2%.

Table No. 10: Robustness data for Mefloquine HCl

Sr. No.	Area at Flow rate (- 0.2 ml/min)	Area at Flow rate (+ 0.2 ml/min)	Area at pH (-0.2)	Area at pH (+0.2)	Area at Mobile phase(-2)	Area at Mobile phase (+2)
1	159.445	151.698	158.151	146.779	158.957	149.725
2	162.18	152.623	160.294	146.371	160.014	150.861
3	162.645	150.529	161.245	148.399	161.245	151.494
% R.S.D	1.071	0.692	0.991	0.728	0.715	0.594

Table No. 11: Robustness data for Artesunate

Sr. No.	Area at Flow rate (- 0.2 ml/min)	Area at Flow rate (+ 0.2 ml/min)	Area at pH (- 0.2)	Area at pH (+ 0.2)	Area at Mobile phase (-2)	Area at Mobile phase (+2)
1	98.298	91.957	97.15	90.344	96.027	91.819
2	97.933	93.057	97.722	91.151	97.722	92.964
3	99.152	93.625	98.298	91.441	98.298	93.344
% R.S.D	0.635	0.913	0.587	0.624	1.212	0.856

CONCLUSION

This method was found to be simple, accurate, robust and reproducible. In UHPLC method Degradation products produced as a result of stress studies did not interfere with the detection of Mefloquine HCl and Artesunate the assay can thus be considered stability-indicating and can be successfully applied for routine QC analysis.

From the above study, we can conclude that the Mefloquine HCl and Artesunate. The linearity was investigated in the range of 5.00-15.00µg/mL (r^2 = 0.9994) for Mefloquine HCl and 11.00-33.00µg/ml (r^2 = 0.9995) for Artesunate.

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