Research Article

Stability Indicating Method Development and Validation for Simultaneous Estimation of Mefloquine and Artesunate in Tablet Dosage Form

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Abstract: The main objective of this study was to develop a simple, efficient, specific, precise and accurate Stability indicating Reverse Phase High Performance Liquid Chromatographic method for estimation of Mefloquine and Artesunate in tablet dosage form.The chromatographic separation can be done by using reverse phase C18 column; Inert sil ODS (250mm× 4.6mm ×5μm).The mobile phase used was mixture of Phosphate buffer (13.6gm of Potassium dihydrogen Phosphate in 1000ml of water;(pH 4.2) Methanol in the ratio of 40:60(v/v) at isocratic mode and detection of eluents can be done using PDA detector 210-400nm as the detector.The optimized method contains the retention times of Mefloquine and Artesunate at 2.734 and 3.630 respectively with theoretical plate count of 4731 and 7411.The method shows a good linearity in the concentration range of 400-1200μg/ml for Mefloquine and 200-600μg/ml for artesunate with regression co efficient of 0.999 and 1.0 for Mefloquine and Artesunate. The % assay of Mefloquine and Artesunate were 99.10% and 99.70%.The LOD and LOQ were 2.895 and 9.650 μg/ml for Mefloquine and 2.7119 and 9.0395 μg/ml for Artesunate respectively. The % of recoveries of both drugs100%.The proposed stability indicating method was accurate, precise, robust, stable and specific. The developed method was validated in accordance with ICH guidelines and hence can be successfully applied to the stability indicating estimation of Mefloquine and Artesunate in tablet formulation.
Keywords: Mefloquine, Artesunate, RP-HPLC, Stability indicating, Validation.

INTRODUCTION:

Mefloquine hydrochloride 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 a pyrimidine 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 membranes and interact with other plasmodial components. It is available in different brand names like Lariam, Mephaquine or Mefliam ect[1].

Artesunate is an anti-malarial drug. It is a part of artemisinin group of drug.It is semi-synthetic derivative of artemisinin. Chemically it is a (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 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

ferrous protoporphyrin IX or covalent alkylation of proteins [2,3].
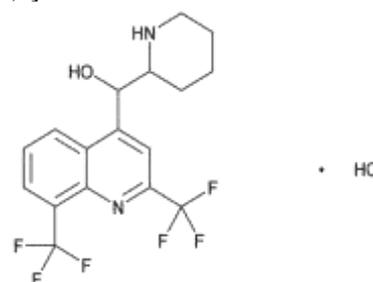

Fig.1: Chemical structure of Mefloquine HCl
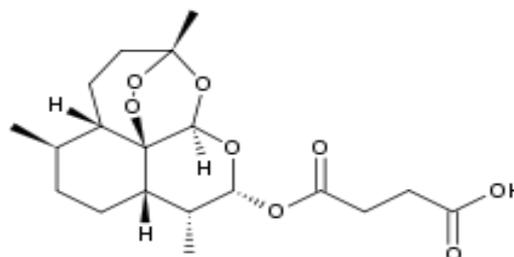

Fig.2: Chemical structure of Artesunate

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 the organisms from its saliva into a person's circulatory system. Malaria parasites belong to the genus Plasmodium .In humans, malaria is caused by P. falciparum, P. malariae, P. ovale, P. vivax and P. knowlesi. Among those infected, P. falciparum is the most common species identified (~75%) followed by P. vivax (~20%). In this case the combination of Quinoline Methanols and Artemisinin its derivatives i.e Artemether, Artesunate[4,5].

For simultaneous estimation of the drugs, which are present in multicomponent dosage forms, HPLC method is considered to be most suitable. It is a powerful and rugged method.Many methods have been reported in the literature for the estimation of Mefloquine and Artesunate individually and in combination[6-17]. However, there is no simple and shorter run times have been reported for the stability indicating method for estimation of Mefloquine and Artesunate. The present work was aimed to developing a fully validated RP-HPLC method for the stability-indicating method for estimation of Mefloquine and Artesunate in pure and tablet dosage form. It is more economical, simpler, precise and accurate than the previous methods.

MATERIALS AND METHODS:
Instrumentation:
Water's e 2695 separation module were used. Water's HPLC system consisting of 2690 pump model, auto sampler and inert sil ODS (250mm×4.6mm×5µ) column were used.PDA detectors -2998 model -210-400nm.The drug analysis data were acquired using Empower2 software.

Reagents and chemicals:
Mefloquine and Artesunate pure drug samples were provided by R.R drugs private Ltd, Hyderabad. Methanol and Water were of HPLC grade and purchased from Rankem India. Fixed dose combination tablets (Brand name: Falcigo plus) containing 200mg of Mefloquine and 100mg of Artesunate were procured from local pharmacy, Hyderabad, India.

Chromatographic conditions:
The mobile phase, a mixture of buffer and methanol (40:60v/v) pumped at a flow rate of 1ml/min in to the column Inert sil ODS (250mm×4.6mm×5µ).

Buffer preparation:
13.6gm of potassium dihydrogen phosphate is dissolved in 1000 ml of water.pH4.2.The mobile phase was filtered through 0.45µm filters to remove all fine particles and degassed it for 10min by sonication to remove gases. Samples of 10µl were injected into the HPLC system and the effluents were analysed by using a PDA detector, with a runtime of 30 mins.

Preparation of standard solution:
Weigh accurately 200mg of Mefloquine and 100mg of Artesunate in 50 ml volumetric flasks and make up the volume with methanol. From the above solution 5ml is pipette and diluted to 25ml with methanol.

Preparation of sample solution:
20 tablets were accurately weighed and the average weight was calculated. The tablets were grinded to fine power. Then the amount of powder equivalent to an average weight of a tablet was transferred to a 50ml volumetric flask, dissolved in methanol and shaken for about 10min then filtered through filter paper. From the filtered solution pipette out 5ml transferred into 25ml of volumetric flask .Make up with final concentration with methanol. Then 10µl of standard and sample solutions were injected into the column and chromatogram was recorded.

Development and validation of stability indicating HPLC method [20]:
The present study was conducted to obtain a new, affordable and cost-effective and convenient method for determination of Mefloquine and Artesunate in tablet dosage forms. The method was validated for the parameters like system suitability, accuracy, precision, linearity, specificity, robustness, and forced degradation studies.

System suitability:
A standard solution was prepared by using Mefloquine and Artesunate working standard as per the test method and was injected six times into the HPLC system.

The parameters namely USP plate count, peak asymmetry factor and resolution for the standard solutions were calculated.

Linearity:
The linearity of the method was determined by constructing calibration curves. Sample solutions of Mefloquine and Artesunate at different concentration levels (50%, 75%, 100%, 125%, and 150%) were used. Before injection of the solution, the column was equilibrated for at least 30 min with the mobile phase. The peak areas of the chromatograms were plotted against the concentrations of Mefloquine and Artesunate to obtain the calibration curves. The five concentrations of the solutions were subjected to regression analysis to calculate calibration equation and correlation coefficients. The mean area with its standard deviation and % relative standard deviation of peak areas were calculated.

Accuracy

Accuracy is the closeness in agreement between the accepted true value or a reference value and the actual results obtained. Accuracy studies are usually evaluated by determining the recovery of a spiked sample of the analyte into the mixture of the sample to be analysed. For this prepare three different concentrations of solution like 50%, 100%, 150%. For each concentration was injected and the mean % recovery was calculated.

Precision:

Method precision was determined by injecting six replicates of the drug sample solution. The retention times and peak areas of six replicates are recorded. The precision is expresses as the %RSD of peak areas and it should not be more than 2%.

Specificity:

The specificity was conducted to establish specificity of the proposed method by injecting blank and placebo using the chromatographic conditions. It was found that there is no interference due to blank and excipients in tablet formulation.

Robustness:

The robustness was assessed by altering the some chromatographic conditions such as by changing the flow rate from 0.8 to 1.2ml/min, temperature of the column (25ºC to 35ºC).

Limit of detection and Limit of quantitation:

Limit of detection and Limit of quantitation represents the concentration of analyte that would yield signal to noise ratio of 3 for LOD and 10 for LOQ respectively.LOD and LOQ was calculated from linear curve using formulae.

$LOD = 3.3^* \sigma$ / Slope
$LOQ = 10^* \sigma$ / Slope

Where σ= The standard deviation of the response and
    S=Slope of calibration curve

Forced degradation studies:

In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed for a period of 24hrs at room temperature. The results show that both solutions the retention times and peak areas of Mefloquine and Artesunate were almost similar and there is no significant within the time period. It indicated that both solutions were stable for 24hrs.It is sufficient to complete whole analytical process. The further forced degradation studies were conducted to demonstrate the stability of the proposed method.

Acid degradation studies:

Twenty tablets were weighed and finely powdered. An accurately weighed portion of powder sample equivalent to 200mg of Mefloquine and 100 mg of Artesunate was transferred into the 50ml of vol. flask add the diluent upto 15ml. The flask keeps for sonication about 30min for complete solubility of the drug at controlled temperature. For above solution adds 5ml of 0.1N HCl and refluxed for 60 minutes at 60ºC, cooled to room temperature. Then neutralized with 0.1N NaOH and make up the final volume with same diluent. Then this mixture was filtered through 0.45µ membrane filter .pipette 5ml of the above filtered sample solution into a 25ml vol. flask and diluted with a final volume with diluents.

Base degradation studies:

Twenty tablets were weighed and finely powdered. An accurately weighed portion of powder sample equivalent to 200mg of Mefloquine and 100mg of Artesunate was transferred into the 50ml of vol. flask adds the diluent upto 15ml. The flask keeps for sonication about 30minfor complete solubility of the drug at controlled temperature. For above solution add 5ml of 0.1N NaOH and refluxed for 60 minutes at 60ºC cooled to room temperature. Then neutralized with 0.1N HCl and make up the final volume with same diluents. Then this mixture was filtered through 0.45µ membrane filter. Pipette 5ml of the above filtered sample solution into a 25ml vol. flask and diluted with final a volume with diluents.

Peroxide degradation studies:

Twenty tablets were weighed and finely powdered. An accurately weighed portion of powder sample equivalent to 200mg of Mefloquine and 100mg of Artesunate was transferred into the 50ml of vol. flask add the diluent upto 15ml.The flask keeps for sonication about 30 min for complete solubility of the drug at controlled temperature. For above solution add 2ml of 3% peroxide was added, refluxed for 60 minutes at 60ºC cooled to room temperature and dilute to final volume with same diluent. Then filtered through 0.45µ membrane filter.Pipett out 5ml of the above filtered solution into 25ml vol. flask and diluted to final volume with diluent.

Thermal degradation studies:

Twenty tablets were weighed and finely powdered. The powder is exposed to heat at 105ºC for about 24 hrs. An accurately weighed portion of powder sample equivalent to 200mg of Mefloquine and 100mg of Artesunate was transferred into the 50ml of vol. flask add the diluent upto 15ml.The flask keeps for sonication about 30 min for complete solubility of the drug at controlled temperature. Cool the solution to room temperature and dilute to final volume with same diluent. Then filtered through 0.45µ membrane filter. Pipett out 5ml of the above filtered solution into 25ml vol. flask and diluted to final volume with diluent.

Photo degradation studies:

Twenty tablets were weighed and finely powdered. The powder is exposed to sunlight for 2 days. An accurately weighed portion of powder sample

equivalent to 200mg of Mefloquine and 100mg of Artesunate was transferred in to 50ml of vol. flask add the diluent up to 15ml.The flask keep for sonication about 30min for complete solubility of the drug at controlled temperature. Cool the solution to room

temperature and dilute to final volume with same diluent. Then filtered through 0.45µ membrane filter.Pipett out 5ml of the above filtered solution into 25ml of vol. flask and dilute to final volume with diluent.
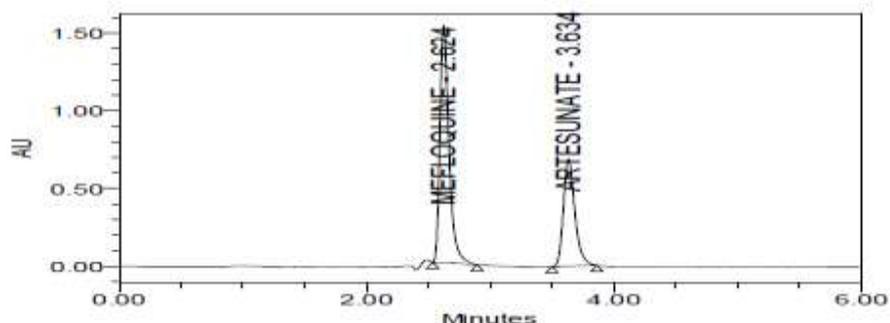

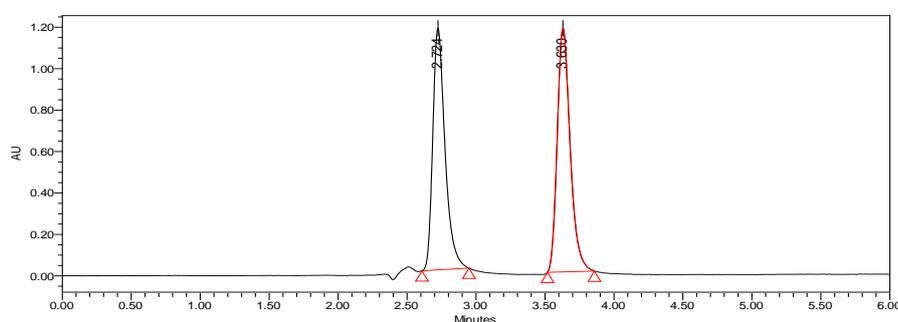**Fig.3: It shows chromatogram of Mefloquine and Artesunate in Standard preparation**

Fig.4: It shows chromatogram of Mefloquine and Artesunate in Sample preparation

RESULTS AND DISCUSSION:
 Method development and optimization:
The new HPLC method is optimized with a view to develop a stability indicating method of Mefloquine and Artesunate.13.6gm of potassium di hydrogen phosphate is dissolved in 1000ml of water. pH 4.2 mixed well and used as mobile phase. Mixture of buffer solution and methanol in the ration of 60:40v/v as mobile phase for trails on a inertsil ODS (250×4.6) mm, 5µ stationary phase with a 25cm length, 4.6mm ID and 5µm particle size. Flow rate was 1ml/min. Injection volume 10µl. Detetion at 291nm.The samples shows greater retention times (4.320&9.035) and pass the all system suitability parameters. In this trail retention times are high. In another trail changes were made on flow rate (1.2ml/min) was injected. With this trail low retention times were achieved (3.560&7.463) and peaks were having high resolution. Based on 2nd trail another trail was carried out by changing the mobile phase composition (40:60v/v).The retention times were improved to 2.281 and 3.068 and first peak shows more tailing (1.94) and passes all system suitability parameters. Based on this, next trail was carried out by changing the injection volume 5µl.With this trail tailing is reduced (1.57), retention times were 2.275 and 3.049 and passes all system suitability parameters. In this trail retention times are

very less, to improve the retention times the flow rate is reduced to 1ml/min in the next trail. In this trail it improves retention times 2.724 and 3.630 and good resolution was observed. Tailing was 1.40 and 1.29.Theoritical plates were 4731& 7411. Satisfactory results were observed in the last trail. So last trail was the optimized trail for this method.

Forced degradation studies:
Mefloquine and artesunate drugs were exposed to 0.1N HCl, 0.1N NaOH and 3% H_2O_2 at 60°c for 60min with continuous stirring. The drugs gradually undergone degradation within the time .No major degradation products were observed when mefloquine and artesunate stressed in photolytic and thermal conditions after 48hrs.From the degration studies % of degradation were calculated by using PDA detector.

System suitability:
This test was carried out to find out the resolution and reproducibility of the system for the analysis. The total results of system suitability studies summarized in Table 1.
In this studies %RSD value of retention times, peak areas, asymmetry and theoretical plate count were found to be less than 2% for both Mefloquine and Artesunate.

Table 1: It shows system suitability of Mefloquine and Artesunate

Parameters	Mefloquine	%RSD	Artesunate	%RSD
Peak area	7755626	0.3	4383185	0.1
Retention time	2.626	0.2	3.6238	0.1
Theoretical plates	6407	1.9	7708	0.8
Tailing factor	1.51	1.28	1.29	0.7

Linearity:

The linearity studies were determined at different concentration ranging from 400-1200µg/ml for Mefloquine and 200-600µg/ml for Artesunate. The regression coefficient was 0.999 for Mefloquine and 1.000 for Artesunate showing good linearity. This shows that the linearity of the calibration curve over the range studied.
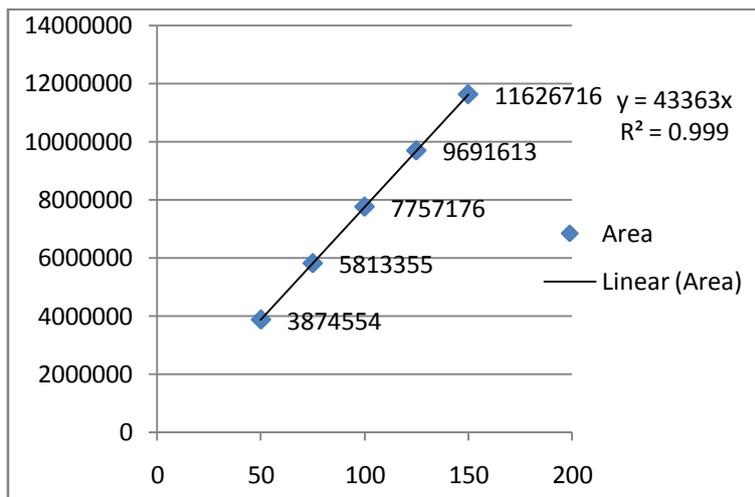

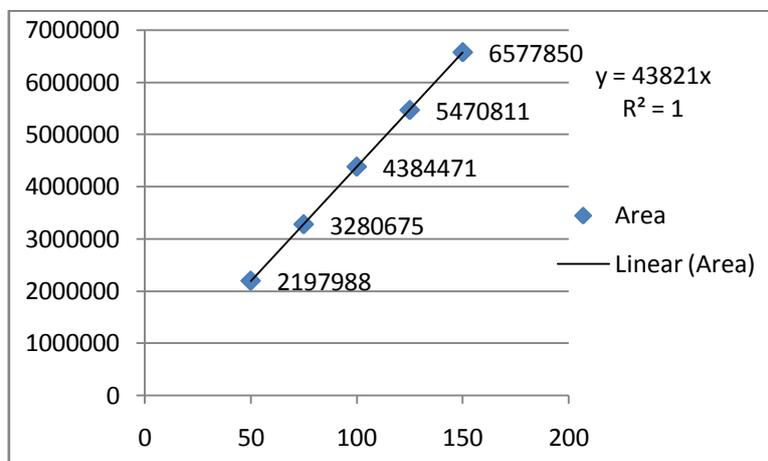**Fig.5: It shows linearity plot of Mefloquine**

Fig.6: It shows linearity plot of Artesunate

Accuracy:

The accuracy studies were determined at 3 different concentrations like 50%, 100%, 150%.The %recovery was calculated for both Mefloquine and Artesunate.

Table 2: It shows accuracy of Mefloquine and Artesunate

Drug	Concentration	% of recovery	Mean
Mefloquine	50%	99.95	
	100%	99.98	100.02
	150%	100.2	
Artesunate	50%	100.2	
	100%	100.08	100.09
	150%	100 .01	

Precision:
Method precision

Method precision was determined by injecting sample solutions of concentrations like Mefloquine (400µg/ml) and Artesunate(200µg/ml) for six times. The chromatograms were recorded for both Mefloquine and Artesunate and results are mentioned in Table 3.% RSD of retention times and peak areas were 0.11and 0.07 for Mefloquine and 0.14 and 0.12 for Artesunate. Method precision was observed that %RSD values for the retention times and peak areas of both Mefloquine and Artesunate were found to be less than 2%.

Table 3: It shows precision of Mefloquine and Artesunate

Drug	Parameter	Observation	% of RSD
Mefloquine	Retention time	2.6456	0.11
	Peak area	7757908	0.07
Artesunate	Retention time	3.621	0.14
	Peak area	4386215	0.12

Robustness:

In the robustness, the changes in the flow rate and Column temperature were made to evaluate impact on the method and retention times were significantly changed.

Table 4: It shows robustness of Mefloquine and Artesunate

S.no	Parameter	condition	RT	Theoretical plates	Tailing factor
1	Flow rate	0.8ml/min	3.355	5316	1.55
		1.2ml/min	2.238	4267	1.49
2	Temperature	25ºC	2.690	5526	1.50
		35ºC	2.682	5141	1.51

Specificity:

The specificity of the method was established by determining the interferences of the peaks of diluent or excipient. Here it shows that there is no interference of diluent peak in the main peaks.Fig.7 The specificity of the method was established by determining the interferences of the peaks of diluent or excipients.
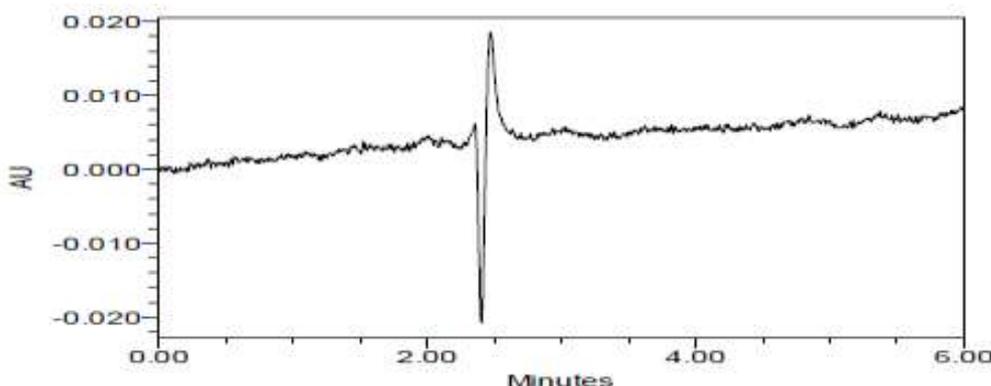

Fig.7: It shows the specificity of Mefloquine and Artesunate

Limit of detection and Limit of quantitation:

The LOD for this method was found to be 1.448µg/ml for Mefloquine and 1.3559µg/ml for Artesunate respectively. The LOQ for this method was found to be 4.825µg/ml for Mefloquine and 4.519µg/ml for Artesunate respectively.

Forced degradation studies:

The forced degradation studies were done at different conditions like acid, base, peroxide, heat and light. The results are shown in the Table 6.

Table 5: It shows LOD&LOQ of Mefloquine and Artesunate

Drug	Peak areas	LOD	Peak areas	LOQ
Mefloquine	1788369	1.448	3536950	4.825
Artesunate	1043028	1.3559	2010617	4.519

Table 6: It shows Forced degradation studies of Mefloquine and Artesunate

Stress condition	Degradation time	Mefloquine		Artesunate	
		%Assay	%Degradation	%Assay	%Degradation
Acid	1hr	77	-22	76	-24
Base	1hr	80	-19	74	-25
Peroxide	1hr	84	-15	82	-18
Light	48hr	86	-13	81	-19
Heat	24hr	90	-9	81	-19

CONCLUSION:

The proposed stability indicating RP-HPLC method has been evaluated over the accuracy, precision, linearity and forced degradation, proved to be more convenient and effective for the quality control and identity to Mefloquine and Artesunate in pharmaceutical dosage forms. Moreover,the lower solvent consumption along with the short analytical run time of 6 minutes. It leads to an environmentally friendly chromatographic procedure that allows the analysis of a large number of samples in a short period of time.

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