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A NEW ANALYTICAL NOVEL RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF ARTESUNATE AND MEFLOQUINE IN BULK AND MARKETED PHARMACEUTICAL DOSAGE FORMS

G. Haritha[1], Arunabha Mallik[2], Thallapalli Uddipa[3], Kadagoni Pravalika[4]

[1]Associate Professor, Department Of Pharmaceutical Analysis, Marri Laxman Reddy Institute Of Pharmacy, Dundigal, Hyderabad, Telangana, India.

[2]Associate Professor, Department Of Pharmaceutical Analysis, Marri Laxman Reddy Institute Of Pharmacy, Dundigal, Hyderabad, Telangana, India.

[3,4]Department Of Pharmaceutical Analysis, Marri Laxman Reddy Institute Of Pharmacy, Dundigal, Hyderabad, Telangana, India.

Corresponding Author: G. Haritha

E-Mail: haritha.shyam1@gmail.com

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ABSTRACT

A simple, reproducible and efficient reverse phase high performance liquid chromatographic method was developed for simultaneous determination of Artesunate and Mefloquine in pure form and marketed combined pharmaceutical dosage forms. A column having Symmetry (C18) (150mm x 4.6mm, 5µm) in isocratic mode with mobile phase containing Methanol: Phosphate Buffer (pH-3.8) (28:72% v/v) was used. The flow rate was 1.0 ml/min and effluent was monitored at 252 nm. The retention time (min) and linearity range (ppm) for Artesunate and Mefloquine were (1.791, 3.442min) and (10-30, 10-50), respectively. The method has been validated for linearity, accuracy and precision, robustness and limit of detection and limit of quantitation. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.86µg/ml and 2.58µg/ml for Artesunate and 1.28µg/ml 3.84µg/ml for Mefloquine respectively. The developed method was found to be accurate, precise and selective for simultaneous determination of Artesunate and Mefloquine in bulk and pharmaceutical dosage forms.

Keywords: Artesunate And Mefloquine, RP-HPLC, Validation, Accuracy, Precision.

1. INTRODUCTION

Artesunate is an artemisinin derivative that is the hemisuccinate ester of the lactol resulting from the reduction of the lactone carbonyl group of artemisinin. It is used, generally as the sodium salt, for the treatment of malaria[1]. It has a role as an antimalarial, a ferroptosis inducer and an antineoplastic agent. It is an artemisinin derivative, a sesquiterpenoid, a dicarboxylic acid monoester, a cyclic acetal, a semisynthetic derivative and a hemisuccinate. Artesunate is indicated for the initial treatment of severe malaria. The World Health Organization recommends Artesunate as first line treatment for severe malaria[2]. Artesunate was developed out of a need for a more hydrophilic derivative of [artemisinin]. Artesunate was granted FDA approval on 26 May 2020. The IUPAC name of Artesunate is 4-oxo-4-[[(1R,4S,5R,8S,9R,10S,12R,13R)-1,5,9-trimethyl-11,14,15,16-tetra oxa tetra cyclo [10.3.1.04,13.08,13] hexadecan-10-yl] oxy] butanoic acid[3]. The Chemical Structure of Artesunate is shown in following figure-1.

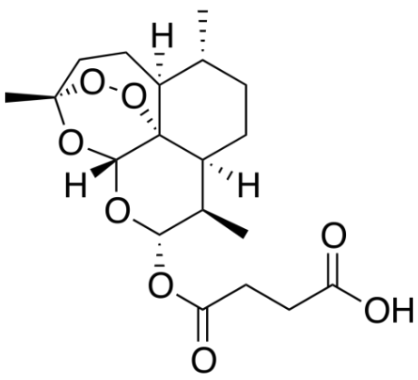

Fig 1: Chemical Structure of Artesunate

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Mefloquine is an antimalarial agent used in the prophylaxis and treatment of malaria caused by Plasmodium falciparum and Plasmodium vivax. Mefloquine, commonly known as Lariam, is an antimalarial drug used for the prevention and treatment of malaria caused by infection with Plasmodium vivax and Plasmodium falciparum[4]. The drug was initially discovered by the Walter Reed Army Institute of Research (WRAIR) during a malaria drug discovery program between 1963 until 1976. It was approved by the FDA in 1989, and was first marketed by Hoffman Laroche.[5] This drug has been the subject of widespread controversy due to concerns regarding neurotoxic effects; product information warns of potential serious neuropsychiatric effects[6]. The IUPAC name of Mefloquine is [2, 8-bis (trifluoro methyl) quinolin-4-yl]-piperidin-2-ylmethanol. The Chemical Structure of Mefloquine is shown in follows

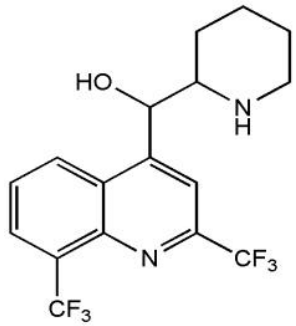

Fig 2: Chemical Structure of Mefloquine

2. MATERIALS AND METHODS

Instruments Used:

Table 1: Instruments Used

S.No.	Instruments and Glass wares	Model
1	HPLC	WATERS Alliance 2695 separation module, software: Empower 2, 996 PDA detector.
2	pH meter	Lab India
3	Weighing machine	Sartorius
4	Volumetric flasks	Borosil
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil
7	Digital Ultra Sonicator	Labman

Chemicals Used:

Table 2: Chemicals Used

S.No.	Chemical	Brand Names
1		Zydus Cadila
2	Mefloquine	Zydus Cadila
3	Water and Methanol for HPLC	LICHROSOLV (MERCK)
4	Acetonitrile for HPLC	Merck

HPLC Method Development:

Preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Artesunate and Mefloquine working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.2ml of the above Artesunate and 0.3ml of the Mefloquine stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure:

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines[14,15].

Mobile Phase Optimization: Initially the mobile phase tried was Methanol: Water and Water: Acetonitrile and Methanol: Phosphate Buffer: ACN with varying proportions. Finally, the mobile phase was optimized to Methanol: Phosphate Buffer in proportion 28:72 (pH-3.8) v/v respectively.

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Optimization of Column:

The method was performed with various columns like C18 column, Symmetry and Zodiac column. Symmetry (C18) (150mm x 4.6mm, 5µm) Column was found to be ideal as it gave good peak shape and resolution at 1ml/min flow[7].

Preparation of Potassium dihydrogen Phosphate (KH2PO4) buffer (pH-3.8):

Dissolve 6.8043 of potassium dihydrogen phosphate in 1000 ml HPLC water and adjust the pH 3.8 with diluted orthophosphoric acid. Filter and sonicate the solution by vacuum filtration and ultra sonication.

Preparation of Mobile Phase:

Accurately measured 280 ml (28%) of Methanol, 720 ml of Phosphate buffer (72%) were mixed and degassed in digital ultra sonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration[8].

Diluent Preparation:

The Mobile phase was used as the diluent.

Method Validation Parameters

System Suitability

Accurately weigh and transfer 10 mg of Artesunate and 10mg of Mefloquine working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent[9]. (Stock solution)

Further pipette 0.2ml of the above Artesunate and 0.3ml of the Mefloquine stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Procedure:

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Specificity:

Preparation of Standard Solution:

Accurately weigh and transfer 10mg of Artesunate and 10mg of Mefloquine working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.2ml of the above Artesunate and 0.3ml of the Mefloquine stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent[10].

Preparation of Sample Solution:

Take average weight of one Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight of Artesunate and Mefloquine sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette 0.2ml of the sample solution from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

The mean and percentage relative standard deviation were calculated from the peak areas[11].

Procedure:

Inject the three replicate injections of standard and sample solutions and calculate the assay by using formula:

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

Linearity:

Accurately weigh and transfer 10 mg of Artesunate and 10mg of Mefloquine working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Preparation of Level – I (10ppm of Artesunate & 10ppm of Mefloquine):

Pipette out 0.1ml of Artesunate and 0.1ml of Mefloquine stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – II (15ppm of Artesunate & 20ppm of Mefloquine):

Pipette out 0.15ml of Artesunate and 0.2ml of Mefloquine stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – III (20ppm of Artesunate & 30ppm of Mefloquine):

Pipette out 0.2ml of Artesunate and 0.3ml of Mefloquine stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent[12].

Preparation of Level – IV (25ppm of Artesunate & 40ppm of Mefloquine):

Pipette out 0.25ml of Artesunate and 0.4ml of Mefloquine stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – V (30ppm of Artesunate & 50ppm of Mefloquine):

Pipette out 0.3ml of Artesunate and 0.5ml of Mefloquine stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Procedure:

Inject each level into the chromatographic system and measure the peak area.

Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient[13].

Precision:

Repeatability:

Preparation of Artesunate and Mefloquine Product Solution for Precision:

Accurately weigh and transfer 10 mg of Artesunate and 10mg of Mefloquine working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.2ml of the above Artesunate and 0.3ml of the Mefloquine stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits[16].

Intermediate Precision:

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.

Procedure:

Day 1:

The standard solution was injected for Six times and measured the area for all Six injections in HPLC. The %RSD for the area of Six replicate injections was found to be within the specified limits.

Day 2:

The standard solution was injected for Six times and measured the area for all Six injections in HPLC. The %RSD for the area of Six replicate injections was found to be within the specified limits[17].

Accuracy:

For preparation of 50% Standard stock solution:

Accurately weigh and transfer 10 mg of Artesunate and 10mg of Mefloquine working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.1ml of the above Artesunate and 0.15ml of the Mefloquine stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

For preparation of 100% Standard stock solution:

Accurately weigh and transfer 10 mg of Artesunate and 10mg of Mefloquine working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.2ml of the above Artesunate and 0.3ml of the Mefloquine stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

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For preparation of 150% Standard stock solution:

Accurately weigh and transfer 10 mg of Artesunate and 10mg of Mefloquine working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.3ml of Artesunate and 0.45ml of Mefloquine from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure:

Inject the Three replicate injections of individual concentrations (50%, 100% and 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Artesunate and Mefloquine and calculate the individual recovery and mean recovery values[18].

Robustness:

The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results. .

For preparation of Standard solution:

Accurately weigh and transfer 10 mg of Artesunate and 10mg of Mefloquine working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.2ml of the above Artesunate and 0.3ml of the Mefloquine stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Effect of Variation of Flow Conditions:

The sample was analyzed at 0.9 ml/min and 1.1 ml/min instead of 1ml/min, remaining conditions are same. 20µl of the above sample was injected and chromatograms were recorded.

Effect of Variation of Mobile Phase Organic Composition:

The sample was analyzed by variation of mobile phase i.e. Methanol: Phosphate Buffer was taken in the ratio and 33:64, 23:77 instead (28:72), remaining conditions are same. 20µl of the above sample was injected and chromatograms were recorded[19].

3. RESULTS AND DISCUSSION

Development of Analytical Method:

Optimized Chromatographic Conditions:

Mobile phase ratio : Methanol: Phosphate Buffer (pH-3.8) (28:72%v/v)
Column : Symmetry (C18) (150mm x 4.6mm, 5µm) Column
Column temperature : Ambient
Wavelength : 252nm
Flow rate : 1.0ml/min
Injection volume : 20µl
Run time : 8minutes

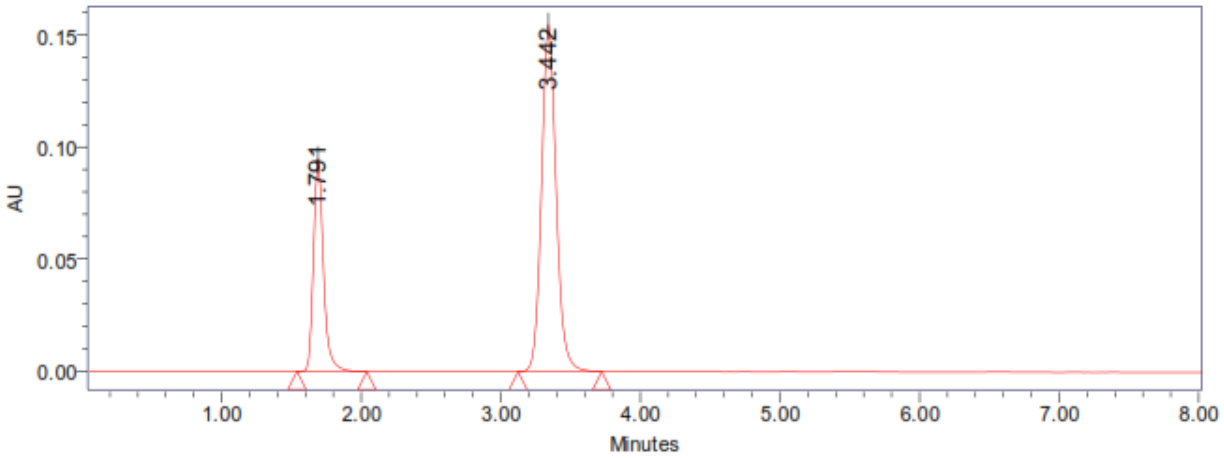

Fig 3: Optimized Chromatogram

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Analytical Method Validation:

The method was validated for linearity, accuracy, precision and limit of detection, and limit and quantitation[20-22].

Specificity (Assay)

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components.

Analytical method was tested for specificity to measure accurately quantities Artesunate and Mefloquine in drug product[23].

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

Observation: The % purity of Artesunate and Mefloquine in pharmaceutical dosage form was found to be 100.154%.

Linearity: The standard solutions of Artesunate and Mefloquine were prepared in the concentration range of 10 - 30 µg/ml (n=5) and 10 - 50 µg/ml (n=5) respectively. Each sample was subjected to chromatographic analysis starting from lower to higher concentration. A standard curve was constructed by plotting chromatographic peak area against the drug concentration and its linearity was statistically confirmed[24].

Table 3: Chromatographic Data For Linearity Study For Artesunate:

Concentration µg/ml	Average Peak Area
10	292985
15	430752
20	565265
25	693487
30	821584

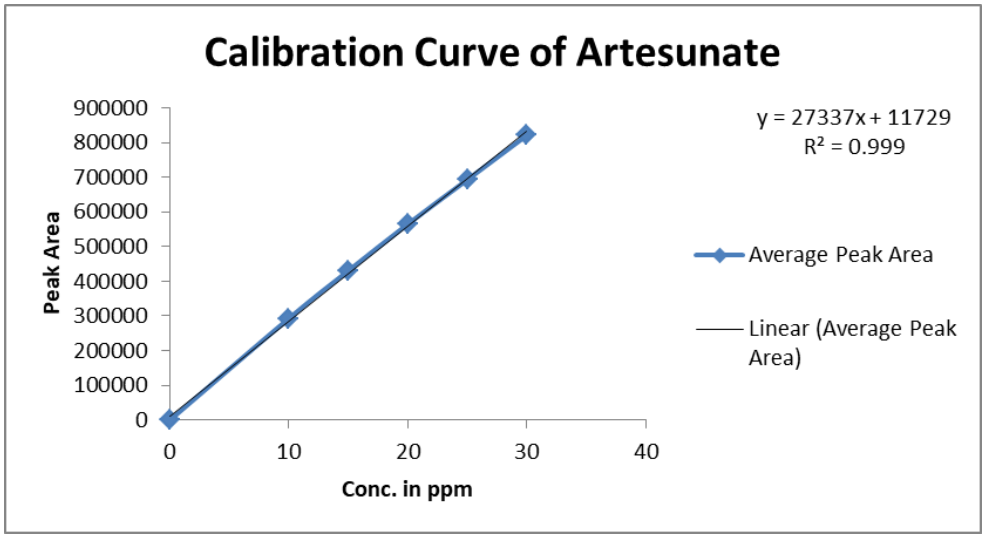

Fig 4: Chromatogram Showing Linearity Level

Linearity Plot: The plot of Concentration (x) versus the Average Peak Area (y) data of Artesunate is a straight line.

Y = mx + c

Slope (m) = 27337

Intercept (c) = 11729

Correlation Coefficient (r) = 0.999

Validation Criteria: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

Conclusion: Correlation Coefficient (r) is 0.99, and the intercept is 11729. These values meet the validation criteria.

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Table 4: Chromatographic Data For Linearity Study For Mefloquine:

Concentration µg/ml	Average Peak Area
10	2828756
20	5485784
30	7999859
40	10656542
50	13085985

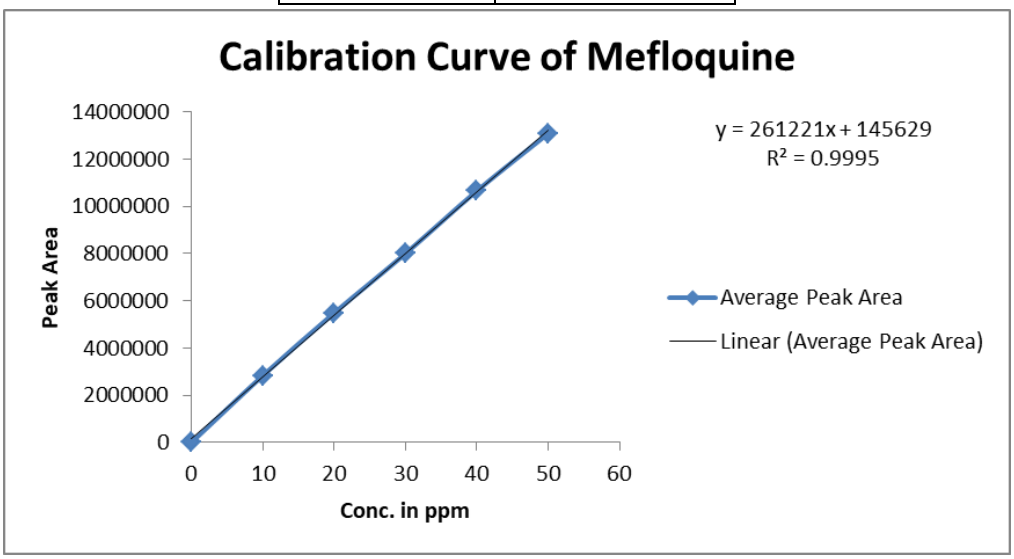

Fig 5: Chromatogram Showing Linearity Level

Linearity Plot: The plot of Concentration (x) versus the Average Peak Area (y) data of Mefloquine is a straight line.

Y = mx + c

Slope (m) = 26122

Intercept (c) = 14562

Correlation Coefficient (r) = 0.9994

Validation Criteria: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

Conclusion: Correlation Coefficient (r) is 0.99, and the intercept is 14562. These values meet the validation criteria.

Precision: Precision of the developed method was assessed by analyzing the standard solutions of Artesunate and Mefloquine (n=6) in the strengths of 20 µg/ml, and 30 µg/ml (nominal drug concentrations) and measuring peak areas of their chromatograms. The within-run precision, i.e., repeatability of the method within a batch was determined by estimating the nominal drug concentrations in the same run on the same day, while the between-run precision, i.e., repeatability of the method over time was determined by estimating them on three different days[25]. The precision was measured as the percent coefficient of variation.

Repeatability:

Table 5: Results of Repeatability for Artesunate:

S. No.	Peak Name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Artesunate	1.792	548698	7458	7569	1.10
2	Artesunate	1.791	548955	7485	7546	1.10
3	Artesunate	1.790	548745	7469	7592	1.09
4	Artesunate	1.790	549856	7463	7519	1.10

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5	Artesunate	1.789	546587	7495	7535	1.09
Mean			**548568.2**			
Std Dev			**1202.217**			
% RSD			**0.2191554**			

Table 6: Results of Repeatability for Mefloquine:

S. No.	Peak Name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Mefloquine	3.435	7768958	43659	8659	1.12
2	Mefloquine	3.428	7765984	43856	8647	1.13
3	Mefloquine	3.419	7785469	43658	8675	1.12
4	Mefloquine	3.414	7785498	43549	8652	1.12
5	Mefloquine	3.408	7769852	44526	8692	1.13
Mean			**7775152**			
Std Dev			**9539.236**			
%RSD			**0.122689**			

Intermediate Precision:

Day 1:

Table 7: Results of Intermediate Precision Day1 for Artesunate

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Artesunate	1.787	556985	75986	7695	1.11
2	Artesunate	1.789	558649	75986	7642	1.12
3	Artesunate	1.789	557847	75689	7683	1.12
Mean			**557827**			
Std. Dev			**832.1803**			
% RSD			**0.149183**			

Table 8: Results of Intermediate Precision Day1 for Mefloquine

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Mefloquine	3.482	7856982	44586	8758	1.13
2	Mefloquine	3.477	7845285	44758	8769	1.14
3	Mefloquine	3.477	7854633	44986	8728	1.13
Mean			**7852300**			
Std. Dev			**6187.659**			
% RSD			**0.078801**			

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Day 2:

Table 9: Results of Intermediate Precision Day 2 for Artesunate

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Artesunate	1.790	536598	7365	7459	1.08
2	Artesunate	1.789	534875	7358	7436	1.07
3	Artesunate	1.793	534698	7349	7482	1.08
Mean			**535390.3**			
Std. Dev			**1049.608**			
% RSD			**0.196045**			

Table 10: Results of Intermediate Precision Day 2 for Mefloquine

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Mefloquine	3.474	7698521	42568	8582	1.11
2	Mefloquine	3.473	7685985	42698	8546	1.10
3	Mefloquine	3.478	7645897	42365	8574	1.10
Mean			**7676801**			
Std. Dev			**27487.83**			
% RSD			**0.358064**			

Accuracy: The accuracy of the method was assessed by determination of the recovery of the method at 3 different concentrations (50%, 100% and 150% concentration) by addition of known amount of standard to the placebo. For each concentration three sets were prepared[26].

Table 11: The Accuracy Results for Artesunate

%Concentration (at Specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	286080.7	10	10.035	100.350%	100.291%
100%	561215	20	20.100	100.500%	
150%	833959.7	30	30.077	100.023%	

Table 12: The Accuracy Results for Mefloquine

%Concentration (at Specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	408328	15	15.074	100.493%	100.163%
100%	798306.3	30	30.003	100.010%	
150%	1189915	45	44.994	99.986%	

Limit of Detection for Artesunate and Mefloquine

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value[27].

$$\mathbf{LOD = 3.3 \times \sigma / s}$$

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Result:

Artesunate = 0.86µg/ml

Mefloquine = 1.28µg/ml

Quantitation Limit for Artesunate and Mefloquine

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined[28].

LOQ = 10×σ/S

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Result:

Artesunate = 2.58µg/ml

Mefloquine = 3.84µg/ml

Robustness: The robustness was performed for the flow rate variations from 0.9 ml/min to 1.1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Artesunate and Mefloquine. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%. The standard and samples of Artesunate and Mefloquine were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count[29].

Table 13: Results for Robustness -Artesunate

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 0.9mL/min	545265	1.794	7564	1.09
Less Flow rate of 0.8mL/min	625486	1.867	7856	1.13
More Flow rate of 1.0mL/min More Flow rate of 0.9mL/min	526548	1.744	7425	1.12
Less organic phase (about 5 % decrease in organic phase)	536548	1.831	7265	1.06
More organic phase (about 5 % Increase in organic phase)	514875	1.874	7169	1.08

Table 14: Results for Robustness-Mefloquine

Parameter used for Sample Analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 0.9mL/min	7768545	3.440	8695	1.12
Less Flow rate of 0.8mL/min	7985695	3.721	8948	1.13
More Flow rate of 1.0mL/min	7458642	3.097	8452	1.12
Less organic phase (about 5 % decrease in organic phase)	7685421	6.242	8365	1.10
More organic phase (about 5 % Increase in organic phase)	7569864	2.402	8254	1.09

Stability Studies:

The specificity of the method can be demonstrated by applying stress conditions using acid, alkaline, peroxide, thermal, UV, water degradations. The sample was exposed to these conditions the main peak of the drug was studied

for peak purity that indicating the method effectively separated the degradation products from the pure active ingredient[30-31].

Table 15: Results of Forced Degradation Studies of Artesunate

S.No.	Stress Condition	Peak Area	% of Degraded Amount	% of Active Amount	Total % of Amount
1	Standard	545265	0	100%	100%
2	Acidic	389537.316	28.56	71.44	100%
3	Basic	501899.409	7.97	92.03	100%
4	Oxidative	176393.227	32.35	67.65	100%
5	Thermal	117831.766	21.61	78.39	100%
6	Photolytic	212489.770	38.97	61.03	100%

Table 16: Results of Forced Degradation Studies of Mefloquine

S.No.	Stress Condition	Peak Area	% of Degraded Amount	% of Active Amount	Total % of Amount
1	Standard	7768545	0	100%	100%
2	Acidic	5706773.157	26.54	73.46	100%
3	Basic	7093458.439	8.69	91.31	100%
4	Oxidative	5424774.973	30.17	69.83	100%
5	Thermal	1545163.600	19.89	80.11	100%
6	Photolytic	2522446.561	32.47	67.53	100%

4. CONCLUSION

The analytical method was developed by studying different parameters. First of all, maximum absorbance was found to be at 252 nm and the peak purity was excellent. Injection volume was selected to be 20µl which gave a good peak area. The column used for study was Symmetry (C18) (150mm x 4.6mm, 5µm) Column because it was giving good peak. An ambient temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0ml/min because of good peak area and satisfactory retention time. Mobile phase is Methanol: Phosphate Buffer (pH-3.8) (28:72% v/v) was fixed due to good symmetrical peak. So, this mobile phase was used for the proposed study. Run time was selected to be 8 min because analyze gave peak around 1.792, 3.446 ± 0.02min respectively and also to reduce the total run time. The percent recovery was found to be 98.0-102% was linear and precise over the same range. Both system and method precision were found to be accurate and well within range. The analytical method was found linearity over the range 10-30mg/ml of Artesunate and 10-50mg/ml of Mefloquine of the target concentration. The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory. Stability study correspondingly confirmed the specificity of the method. As a part of peak purity study, peak threshold was found to be higher than angle and no flag for both the analytes was observed. Degradation study revealed that Artesunate and Mefloquine were degraded in oxidative and photolytic condition only.

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