PAPER DETAILS

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ORIGINAL ARTICLE

A Validated HPLC Method for Separation and Determination of Mefloquine Enantiomers in Pharmaceutical Formulations

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ABSTRACT

A simple, rapid and validated method for separation and determination of mefloquine enantiomers was developed. Mefolquine was separated and quantitated on cyclodextrin chiral column Quest-CM carboxymethyl-BCD (250x4mm i.d., 5µm particle size) using a mixture of acetonitrile: 1% triethylammonium acetate buffer (pH = 4.5) (20:80 výv) as a mobile phase at 20 °C and a flow rate of 1 mL /min. The UV-detector was set at 240 nm. The applied HPLC method allowed the separation and quantification of mefloquine enantiomers with good linearity ($r > 0.999$) in the studied range. The relative standard deviations (RSD) were 0.865 and 0.907 for the mefloquine enantiomers with accuracy of 100.00 and 100.68. The limit of detection and limit of quantification of mefloquine enantionmers were found to be 5 and 15 µg/mL, respectively. The method was validated through the parameters of linearity, accuracy, precision and robustness. The HPLC method was applied for the quantitative determination of mefloquine in pharmaceutical formulations.

Keywords Chiral separation, antimalarials, mefloquine, pharmaceutical analysis.

1. INTRODUCTION

Although many chiral drugs are clinically used as racemates, it has been demonstrated that in most cases the two enantiomers of a chiral drugs have different pharmacological activities [1]. Mefloquine (MFQ), rac-erythro- -(2- piperidyl)-2,8- bistrifluoromethyl)-4-quinolone methanol, Figure 1, with two asymmetric carbon atoms has been used as a racemic mixture in prophylaxis and treatment of the resistant strains of *Plasmodium faciparum*. Various studies demonstrated that MFQ pharmacokinetics are highly stereoselective [2].

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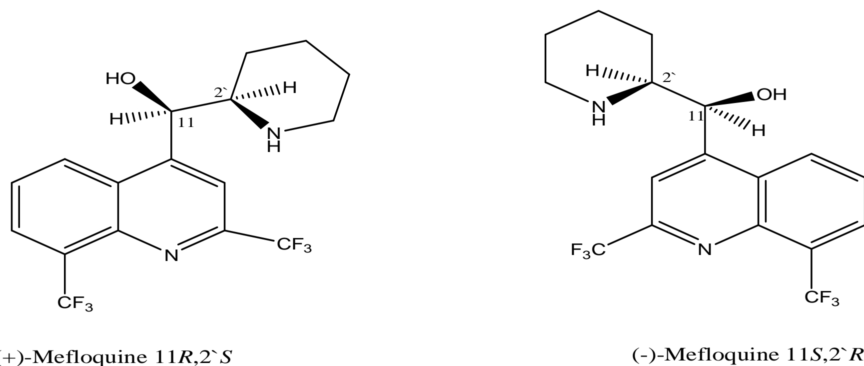


(+)-Mefloquine 11*R*,2`*S*

(-)-Mefloquine 11*S*,2`*R*

Figure 1. Stereochemical structure of mefloquine enantiomers

The peak concentration and the area under the curve (AUC) of the (-)-MFQ have been significantly higher than those of the (+)-MFQ in blood and plasma after oral administration [3-5]. Higher concentration of the (+)-MFQ in rat plasma after oral administration has also been reported [6].

There are conflicting reports about the antimalarial activity of MFQ enantiomers. No significant difference is observed between antimalarial activities of enantiomers against *Plasmodium bergheior* and *Plasmodium yoelli* in rodents [4,7]. In vitro activity of MFQ enantiomers on two chloroquine resistant and susceptible strains of *Plasmodium falciparum* showed similar activities for both enantiomers [8]. In another report, the (+) -enantiomer of MFQ has shown 1.69-1.81 times more active than the (-)-MFQ against chloroquine sensitive (*Sierra leone D-6*) and chloroquine resistant (*Indochina w-2*) strains of *Plasmodium falciparum in vitro* [9]. Because mefloquine enantiomers displayed different pharmacological activities, the objective of this work is to develop a validated method of separation and enantioselective analysis of mefloquine enantiomers in the pharmaceutical tablet formulation. All the validation parameters are performed including accuracy, precision and robustness besides linearity, limit of quantification (LOQ) and limit of detention (LOD) using HPLC on cyclodextrin chiral column Quest-CM carboxymethyl-BCD (250x4mm i.d., 5μm particle size). The mobile phase used was acetonitrile: 1% triethylammonium acetate buffer (pH = 4.5) in the ratio 20: 80 (výv).

2. EXPERIMENTAL

Chemicals

Mefolquine hydrochloride was obtained from Aldrich (Schnelldorf, Germany, Cat. No. M2319). Methanol (HPLC-grade) was obtained from Merck (Darmstadt Germany). Acetonitrile and triethylamine of analytical grade were delivered from Sigma chemicals (St. Louis, MO, USA).

Pharmaceutical preparation, Lariam® Tablets, was labeled to contain 250 mg mefloquine per tablet, manufactured by Roche Company. B.N. 1163BO1.

Instrumentation and Analytical Conditions

The HPLC unit was a Agilent 1100 series apparatus equipped with a quaternary pump, a vacuum degasser, a column oven, a diode array UV-detector, and a HP Chemstation. The column used was cyclodextrin chiral column Quest-CM carboxymethyl-BCD (250x4mm i.d., 5µm particle size) as a gift obtained from Cyclolab Ltd., Budapest, Hungary.

The mobile phase consisted of acetonitrile, 1% triethylammonium acetate buffer pH= 4.5 (20:80% výv). The flow rate was 1mL/min. All the samples were measured at wavelength 240 nm at 20 ^{0}C.

Preparation of the standard solutions

Mefloquine reference standard (50 mg) was accurately weighed, transferred to 50 mL volumetric flask, and dissolved in 20 mL methanol, and then completed to volume with methanol (final concentration 1mg /mL). The resulting solution was sonicated for 10min and diluted to volume. All solutions were freshly prepared.

Determination of Mefloquine Enantiomers

For construction of the calibration graph, take aliquot portions (0.5-5 mL) of 1mg/mL mefloquine standard solution into a series of 25 mL measuring flasks, and complete to volume with methanol. Inject 20 µL of the solution from each flask and record the chromatograms, maintaining the flow rate at 1mL/min and wavelength 240nm. Measure the ratio of peak area corresponding to concentration of each. Construct a calibration graph representing the relation between concentration and peak area. Concentration of unknown samples could be derived from the calibration graph or calculated from the following regression equation.

Enantiomer **1**: $Y = 0.0018X + 0.0981$ $r = 0.9990$

Enantiomer **2**: $Y = 0.0018X + 0.0841$ $r = 0.9997$

Where: Y= Peak area of sample.

X= concentration of mefloquine in µg/mL.

r = Correlation coefficient.

During the chromatographic analysis, the following parameters were measured.

k_1 and k_2: Capacity factors of the first and second eluted enantiomers and were 0.49 and 1.76, respectively.

: selectivity factor, $= k_2 / k_1 = 3.61$

R_s: resolution factor was found to be 2.61, calculated according to the following equation, $R_s = 2(t_2 ó t_1) / w_1 + w_2$.

Where w is the baseline band width obtained by drawing tangents to the inflexion points of the chromatographic peak.

Detetermination of Mefloquine Enantiomers in Lariam ® Tablets

Weight 10 tablets individually , grind in a mortar and weigh powdered tablets equivalent to 50 mg of mefloquine. Extract with 30mL methanol using a magnetic stirrer and complete to volume with methanol then filter. Determine mefloquine concentration by taking 0.5-5 mL into 25mL measuring flasks. Complete to volume with methanol and proceed as previously described before.

3. METHOD VALIDATION

The methods were validated according to the International Conference on Harmonization guidelines for validation of analytical procedures (ICH, 1996). ANOVA was used to verify the validity of the methods.

Linearity

The calibration curve was obtained with six concentrations of the standard solution 50-500 µg/mL. The solutions were prepared in triplicate. The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method.

Precision

The precision of the assay was determined by repeatability (intra-day) and intermediate precision (inter-day). Intra-day precision was evaluated by assaying the sample, at the same concentration and during the same day-six sample solutions (100µg/mL) were prepared and assayed. The intermediate precision (inter-day) was studied by comparing the assays on different days (3-days).

Accuracy

The accuracy of an analytical method is determined by how close the test results obtained by that method come to the true value. It can be determined by application of the analytical procedure to an analyte of known purity (for the drug substance) or by recovery studies, where a known amount of standard is spiked in the placebo (for drug product). In this study, a number of different solutions were prepared with a known added amount of drug substance and injected in triplicate. Percent recoveries of response factor (area and concentration) were calculated as shown in Table 1, which indicates the accuracy of the proposed method.

Table 1. Intra- Day and inter- Day Accuracy and Precision Data of HPLC Method for Mefloquine

Theoretical Concentration µg/mL	Intra-Day		Inter- Day	
	Accuracy %	Precision [a] (RSD %)	Accuracy %	Precision [a] (RSD %)
Enantiomer **1**				
100	100.64	0.32	100.23	0.56
200	100.92	0.43	100.64	0.67
300	99.99	0.59	100.43	0.89
Enantiomer **2**				
100	100.33	0.55	99.82	0.72
200	100.68	0.63	100.34	0.81
300	100.74	0.69	100.20	0.98

[a] Mean of five determinations for each concentration.

Robustness

The robustness of the HPLC method was determined by analysis of samples under a variety of conditions by making small changes in the mobile phase composition, in the flow rate (0.8-1.2 mL/ min), in the temperature of the column (18-25 C), and in the wavelength (240-260 nm).

Limit of Detection and Limit of Quantification

LOD is defined as the lowest concentration of an analyte in a sample that can be detected, but not necessarily quantified and LOQ was defined as the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy.

4. RESULTS AND DISCUSSION

Currently, there is a great interest within the pharmaceutical laboratories to develop single isomer formulations and also analytical methods to determine the enantiomeric purity of drugs.

This research deals with the enantiomeric separation and quantitation of mefloquine enantiomers in bulk and in pharmaceutical tablets using a new cyclodextrin based weak cation-exchange chiral column Quest-CM carboxymethyl-BCD (250x4mm i.d., 5µm particle size) stationary phase as a chiral selector.

The chromatographic conditions were optimized in order to provide a reliable assay performance. Mobile phase selection was based on peak parameters, runtime, ease of preparation and cost. A typical chromatogram is shown in Figure 2 for the analysis and separation of a sample solution of mefloquine enantiomers

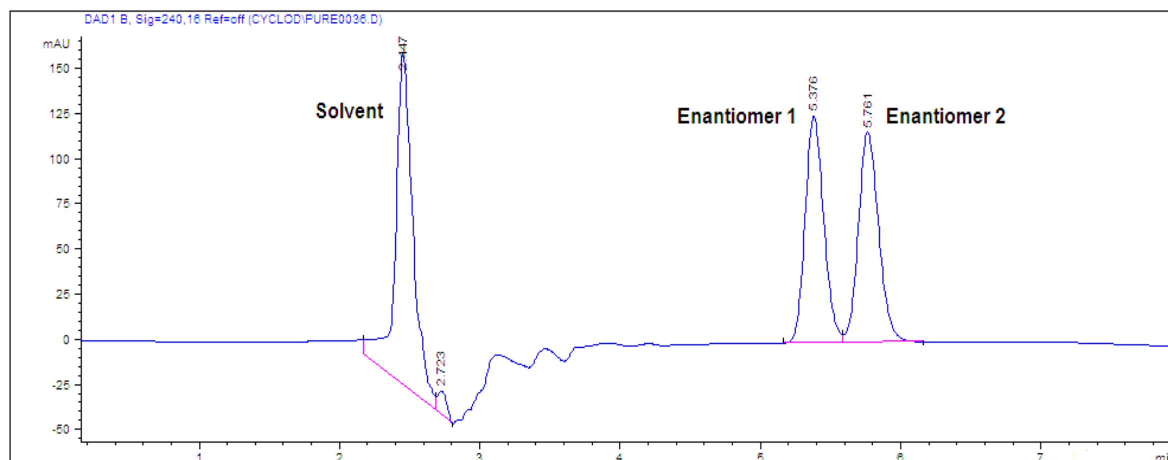
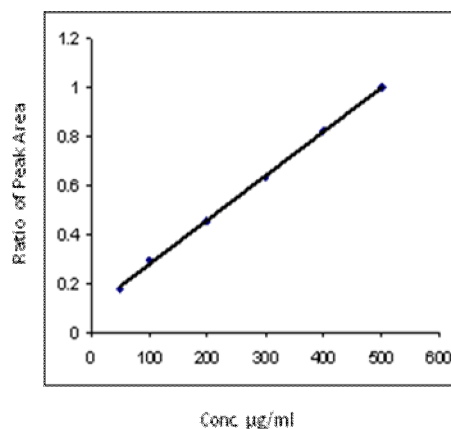


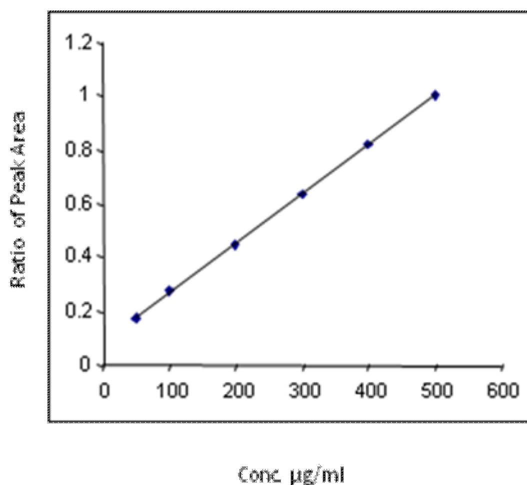
Figure 2 Chromatogram of Mefloquine 300µg/ml on a cyclodextrin chiral column Quest-CM carboxymethyl-BCD (250x4mm i.d., 5µm particle size) using a mixture of acetonitrile, 1% triethylammonium acetate buffer pH= 4.5 (20: 80% v∕v) as a mobile phase and flow rate 1ml/min at 240 nm.)

The retention time was observed at 5.38min for enantiomer **1** and 6.76min for enantiomer **2**. The LOD and LOQ were obtained using the slope and standard deviations of the intercept from three curves and determined by the linear regression line and were 5 and 15µg/mL, respectively. These values were also used in an experimental assay confirming the calculation.

The calibration curves for mefloquine enantiomers were constructed by plotting concentration versus the ratio of peak area and showed good linearity in the 50 -500 µg / mL range as shown in Figures 3 and 4.



F,gure 3. Linearity of concentration of mefloquine enantiomer **1** to peak area of mefloquine enantiomer **1** (500µg/ml) as an external Standard.



F,gure 4 Linearity of concentration of mefloquine enantiomer **2** to peak area of mefloquine enantiomer **2** (500µg/ml) as an external standard

The representative linear equations were:

Y = 0.0018 X + 0.0981 for enantiomer **1** and Y= 0.0018X + 0.084 for enantiomer **2** with high correlation coefficients r = 0.9990 and r= 0.9997, respectively.

Accuracy and precision of the proposed method were assessed by performing triplicate analyses of the standard solutions.

Three different concentrations, diluted with the mobile phase, were prepared in the linear range of the calibration curve and analyzed to determine intra-day variability and accuracy.

The inter- and intra-day precisions were calculated as the RSD%. The results and the mean values were shown in Table 1 demonstrating good precision and accuracy.

When chromatographic conditions were intentionally altered, no significant effect was observed in the chromatogram, confirming the robustness of the method.

The intraday-precision obtained by the proposed method showed a RSD of 0.45 and 0.62 % for both enantiomer **1** and enantiomer **2**, respectively.

Interóday variability was calculated and showed a RSD of 0.71 and 0.84% for both enantiomers, respectively, as shown in Table 1.

Results of the determination of mefloquine in Lariam®, the pharmaceutical tablets formulation, are shown in Tables 2 and 3.

Table 2 Results of Determination of Mefloquine in Lariam® Tablets by HPLC

Sample (µg)	Experimental Amount [a] (µg)	%	(RSD%)
Enantiomer **1**			
	100.24	100.24	
100	99.95	99.95	0.16
	99.98	99.98	
Enantiomer **2**			
	99.89	99.89	
100	99.78	99.78	0.16
	100.10	100.10	

[a] Mean of five determination for each concentration.

Table 3 Results of Standard Addition of Authentic Mefloquine to Lariam® Tablets.

Added Authentic µg/mL	Found Autheutic µg/mL for Enantiomer 1	Recovery % (X) for Enantiomer 1	Found Authent µg/mL for Enantiomer 2	Rcovery % (X) for Enantiomer 2
100	100.12	100.12	100.32	100.32
150	149.8	99.87	150.21	100.14
200	200.42	100.21	199.90	99.95
300	300.31	100.10	299.86	100.10
400	399.98	100.00	400.34	100.09
Mean ± RSD		100.06 ± 0.13		100.12 ± 0.13

The tablets excipients did not interfere with the analysis of mefloquine entantiomers and it was found that the accuracy of the HPLC method for enantiomers **1** and **2** was 100.00±0.865 and 100.68±0.907%, respectively. The results are expressed in Table 4.

Table 4 Determination of authentic mefloquine via the suggested HPLC method.

Added Authentic µg/ml	Found Authentic µg/ml of Enantiomer 1	Recovery % of Enantiomer 1	Found Authentic µg/ml of Enantiomer 2	Recovery % of Enantiomer 2
200	199.94	99.97	199.76	99.88
300	296.61	98.87	303.28	101.09
400	403.83	100.96	399.88	99.97
500	501.06	100.21	508.83	101.77
Mean ± RSD[a]		100.00 ± 0.87		100.68 ± 0.91

[a] Average of at least three separate determination.

The proposed analytical method was compared with reference method using statistical analysis [10]. The calculated t-value for both enantiomers (t cal =0.26) and (t cal=0.62) were found to be less than the tabulated t-value (t tab = 2.45) and (t tab= 2.45) at a 1% significance level, respectively Table 5. The calculated F-value for both enantiomers (F cal =2.99) and (F cal=2.68) were found to be less than the tabulated F-value (F tab = 9.28) and (F tab=9.28) at a 1% significance level, respectively Table 5.

Table 5 Statistical Comparison of the Results obtained by Adopting the Proposed Method as Compared with the Reference Method [a] for Analysis is of Mefloquine

Technique	Mean ± RSD	n	Variance	Student (t) test	F
Reference method	100.20 ± 1.492	4	2.24	-	-
HPLC for Enantiomer **1**	100.00 ± 0.865	4	0.75	0.26 (2.45)[a]	2.99 (9.28)[b]
HPLC for Enantiomer **2**	100.68 ±0.907	4	0.83	0.62 (2.45)[a]	2.68 (9.28)[b]

[a] Quautitative UV spectrophotometry in ethanol using A (1%,1cm) at 282.5 nm for the determination of mefloquine.
[b] The figures in parenthesis are the theoretical t and F values at (P = 0.05).

The methanolic solution of mefloquine was stable all through the period required for analysis and did not show sign of degradation products.

The proposed method described discusses a fully validated analytical procedure for mefloquine enantiomers in pharmaceutical tablets formulations.

5. CONCLUSION

The proposed HPLC method described a quantitative determination and separation of mefloquine enantiomers in bulk drug and in pharmaceutical tablets formulations. The proposed HPLC method is fast, precise, accurate, and efficient and can be applied for routine analysis in quality control laboratories.

6. ACKNOWLEDGMENTS

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