

Review

Stability Indicating method for Estimation of Mefloquine Hydrochloride in Pharmaceutical Formulation

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Abstract: A simple, selective, rapid, precise and accurate reverse phase high pressure liquid chromatogram method has been developed and validate stability indicating RP-HPLC method for the estimation of Mefloquine hydrochloride in pharmaceutical formulations. Used developed and validate Hplc method for force degradation study. On the basis of reversed phase -HPLC mode (analyte) stationary phase with C18 bonded phase I.e waters symmetry c18 ,250 mm x 4.6 mm,5u was selected .Column with mobile phase consisting of 1.5 gm of sodium hydrogen sulphate mono hydrate(25) and 75 ml of methanol ,Detection was carried out at 280 nm and flow rate was 0.7 ml/Min .The developed method was validated for linearity accuracy, precision, selectivity, specificity, forced degradation studies, robustness ruggedness. By using the develop and validate RP- hplc method expedient study was carried out which is necessary in this case because the concentration of drug in the tablet formulation is very less than the other excipients.

Keywords: Mefloquine Hydrochloride, Rp -Hplc

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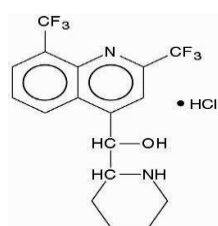
Introduction:

Mefloquine Hydrochloride 4-Quinoline methanol, α -2-piperidinyl-2-8-bis(trifluoromethyl)- monohydrochloride, (R*,R*)-(\pm)-DL- erythro- α -2-piperidyl-2-8-bis (trifluoromethyl)-4- quinoline methanol monohydrochloride. Methanol monohydrochloride is an antimalarial agent.

It is having molecular formula C₁₇H₁₆F₆N₂O. HCl. It is soluble in lower alcohol (methanol, ethanol), dimethylformamide, methyl acetate, sparingly soluble in isopropanol, slightly soluble in water. Mefloquine hydrochloride is white to almost white crystalline powder .

A recent literature survey revealed that few methods are developed and validated for the Mefloquine hydrochloride. The present work describes development and validation in compliance with ICH guidelines .

Mefloquine hydrochloride



Experimental

Chemicals and Reagents :

Mefloquine hydrochloride used as reference standard was provided by Mission viva care, Pithampur, India. And was used without further purification. Methanol (HPLC grade) and sodium hydrogen sulphate monohydrate were purchased from Merck (Mumbai, India). The water used was double glass distilled and membrane filtered HPLC grade water. Tablets of MFQH of brand name CANFAL 250mg (claimed to contain MFQH 250mg) manufactured by Lupin Pharmaceutical Ltd, India and placebo, prepared at Mission Vivacare Ltd, R & D Department Pithampur, MP, India.

The MFQH Related substance-A ((R,S)-[2,8 bis (trifluoromethyl) quinoline-4-yl][(2R,S)-Piperidine- 2-yl] methanol hydrochloride. Related substance -B ((R,S)-[2,8-bis(trifluoromethylquinoline-4-yl) [[pyridine-2-yl]methanol and Related substance-C ((R,S)-[2,8-bis(trifluoromethylquinoline-4-yl) [[pyridine-2-yl]methanone) were provided by Mission viva care Ltd, Pithampur, MP, India.

Material and Methods:

Mobile phase

Filtered and degassed mixture of buffer and methanol was prepared in the ratio of 25:75 where buffer was (1.5gm of sodium hydrogen sulphate monohydrate in 1000ml of water used and 0.45 μ nylon filter paper was used.

Chromatographic Conditions:

On the basis of reversed phase HPLC mode and number of carbon present in the molecule (analyte) stationary phase with C18 bonded phase i.e Waters Symmetry C18 250 mm x 4.6mm, 5 μ was selected. Photo diode array detector was selected, 280 nm wavelength was selected as detection wavelength. The flow rate was 0.7ml/Min, and injection volume 10 μ l and at 25 $^{\circ}$ C column temperature and run time 25 minutes.

Standard preparation :

Standard stock solution was prepared by dissolving 50 mg of MFQH reference standard to a 50 ml volumetric flask and dissolved and dilute up to the mark with diluent. pipette out 5ml of above solution in volumetric flask and make up volume with diluent

Assay procedure for commercial formulations :/

Twenty tablets were weighted and crushed to fine powder in to 50 ml volumetric flask, mobile phase were added and sonicated until tablets were dissolved and kept aside to achieve at room temperature and made up volume with diluent. further diluted 5 ml of above solution and transfer to 50 ml of volumetric flask and made-up the volume with diluent.

Procedure: 10 μ l of standard preparation and sample preparation were separately injected and the chromatogram were recorded.

Linearity and Range :

The result of the method was found to be linear in the range of 50 μ g/ml to 150 μ g/ml of MFQH and the peak areas recorded for all peaks and plotted peak versus concentration. Coefficient of correlation for MFQH was 0.9995.

.Results of linearity study are shown in Table 1 and Figure 1

Table 1: Results for Linearity Study

Linearity Level	Standard Concentration	Concentration of MFQH (ppm)	Mean area (n = 3)	Regression Coefficient (r ²)
Level – 1	50%	50	1852527	0.9995
Level – 2	80%	80	3051177	
Level – 3	100%	100	3705053	
Level – 4	120%	120	4389845	
Level – 5	150%	150	5557580	

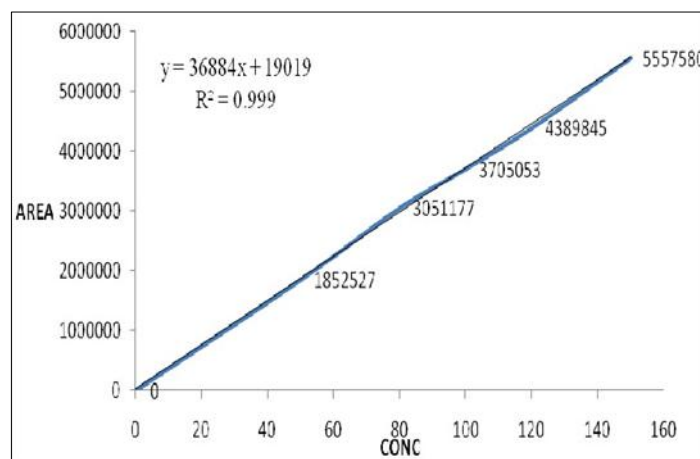


Figure 1 Linearity of MFQH

Accuracy:

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out at the three levels of 50, 100, and 150 % and percentage recovery were calculated and presented in the Table no 2.

Recovery was within range of $100 \pm 2\%$ which indicates accuracy of the methods standard and sample solutions.

Precision :

The accuracy of the method was demonstrated by interday and intraday version studies. The intra day studies 3 repeated injections of standard solutions were made in a day and the response of the factor of the drug peaks and percentage RSD were calculated and found to not more than 0.12% of MFQH. In the inter day variations studies 3 repeated injections standard and sample solution were made on 3 consecutive days and response of the factor of drug peaks and percentage RSD were calculated and found not more than 0.24 % of MFQH. The data obtained indicates that the developed method is precise.

Results of accuracy study shown in **Table 02**

Table2: Results for Recovery Studies

Level	Preparation	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean % Recovery	% RSD
50 %	Preparation-1	24.97	25.01	100.2	100.0	0.15
	Preparation-2	25.01	25.00	100.0		
Level	Preparation-3	25.05	25.03	99.9	100.0	0.15
100 %	Preparation-1	50.12	48.53	96.8	97.0	0.26
	Preparation-2	50.01	48.66	97.3		

Level	Preparation-3	49.86	48.38	97.0		
150 % Level	Preparation-1	75.07	73.52	97.9	98.1	0.75
	Preparation-2	74.98	74.13	98.9		
	Preparation-3	75.02	73.08	97.4		

Method Precision (Repeatability):

Results of repeatability study shown in Table 3.

Table 3: Results of Method Precision

Sample Preparation	% Assay of MFQH
Test solution -1	99.67
Test solution -2	99.59
Test solution -3	99.55
Test solution -4	99.38
Test solution -5	99.48
Test solution -6	99.69
Mean	99.56
SD	0.12
% RSD	0.12

Intermediate Precision (Ruggedness):

Results of ruggedness study are shown in Table 4.

Table 4: Results for Intermediate Precision

Analysis performed during method precision study	
Analyst: Analyst-I	HPLC ID No.: EAR040
Make : Waters Symmetry, C18, 250 mm □ 4.6 mm, 5 □	
Column serial number. : 0402471K	
Sr. No.	% Assay of MFQH
Test solution-1	99.67
Test solution-2	99.59
Test solution-3	99.55
Test solution-4	99.38
Test solution-5	99.48
Test solution-6	99.69
Analysis performed during intermediate precision study	

Analyst: Analyst-II	
HPLC ID No.: EAR039	
Make : Waters Symmetry, C18, 250 mm □ 4.6 mm, 5 □	
Column serial number : 0502481L	
Test solution-1	99.44
Test solution-2	99.56
Test solution-3	100.05
Test solution-4	99.42
Test solution-5	99.64
Test solution-6	99.83
Mean of twelve samples	99.66
SD	0.24
% RSD	0.24

Robustness:

Results of robustness study are shown in Table 05 to 17.

Table 5: Result for Flow rate 0.63 mL/min.

Parameter	Test Solution	%Assay for MFQH
Method Precision	1	99.67
	2	99.59
	3	99.55
	4	99.38
	5	99.48
	6	99.69

Change in flow rate 0.63 mL/ min.	1	99.65
	2	99.38
Mean		99.55
SD		0.12
% RSD		0.12

Table 6: Results for Flow rate 0.77 mL/min.

Parameter	Test solution	%Assay for MFQH
	1	99.67
	2	99.59
	3	99.55
	4	99.38
	5	99.48
	6	99.69
	1	99.21
	2	98.99
Mean		99.44
SD		0.24
% RSD		0.24

Table 7: Results for Wavelength 278

Parameter	Test solution	%Assay for MFQH
Method Precision	1	99.67
	2	99.59
	3	99.55
	4	99.38
	5	99.48
	6	99.69

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Change in wavelength 278 nm.	1	98.47
	2	97.65
Mean		99.18
SD		0.69
% RSD		0.69

Table 8: Results for Wavelength 282 nm.**4.2.3 Specificity:**

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc. Lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedure.

Selectivity:**Placebo solution preparation:**

Prepared blank preparation, prepared placebo preparation, standard preparation, sample preparation for MFQH 250 mg tablet, related compound solution A, B, C and related compound spiked sample solution as per the method.

Weighed accurately 254 mg of placebo and transfer it into 50 mL volumetric flask add about 10 mL of diluent, sonicate at for about 10 min with intermittent shaking, keep to achieve room temperature make up the volume with diluent. Pipette out 5 mL of the above solution and transfer to 50 mL volumetric flask and make up the volume with diluent.

Force degradation:

Forced degradation study was performed on placebo blend and MFQH tablet 250 mg. The degradation study was carried out as per the tabulated below table

Table 9: Forced Degradation Conditions

Sr. No.	Stress Study	Degrading Agents	Exposure Period
1	Acid Degradation	5N HCl (5mL)	60°C for 4 h
2	Base Degradation	5N NaOH (5mL)	60°C for 4 h
3	Oxidative Degradation	50% H ₂ O ₂ (5mL)	60°C for 4 h
4	Heat Degradation (Solid state)	-	60°C for 4 h

**Stability Indication Method For Estimation Of
Pharmaceutical formulation .**

Mefloquine Hydrochloride In

Sr. No.	Stress Study	Degrading Agents	Exposure Period
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5	Heat Degradation (Solution state)	-	60°C for 4 h
6	Humidity Degradation	-	75% RH for 4 h
7	Photolytic Degradation	UV/visible Light	6 day

Peak purity is demonstrated by comparing purity angle with purity threshold .peak is considered as to be pure and purity criteria threshold which implies method to be specific. The peak due to the degradation product of placebo blend and MfQH tablet 250mg are found to be well resolved from MFQH peak , hence it is concluded that method is specific.

The peak due to degradation products of placebo blend and MFQH tablet 250 mg are found to be well resolved from the MFQH peak . Hence it is concluded that the method is specific.

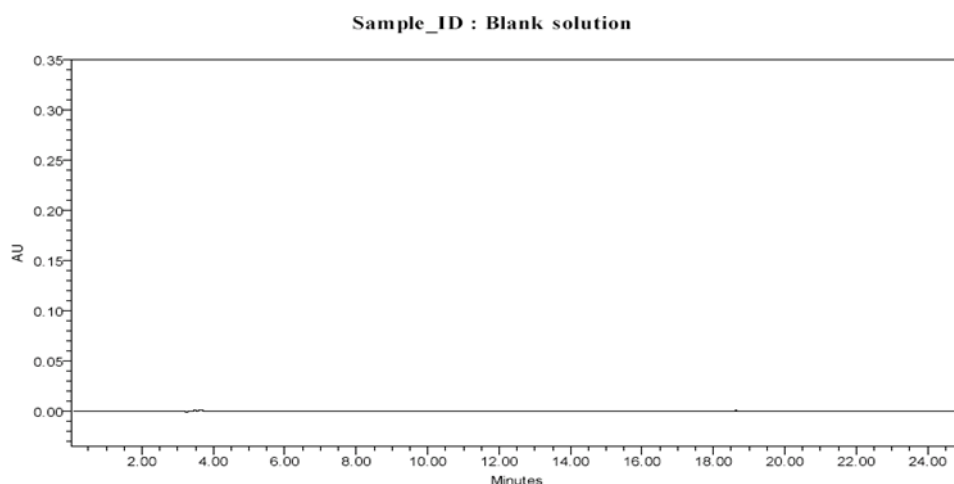


Figure 2 Chromatogram of Blank

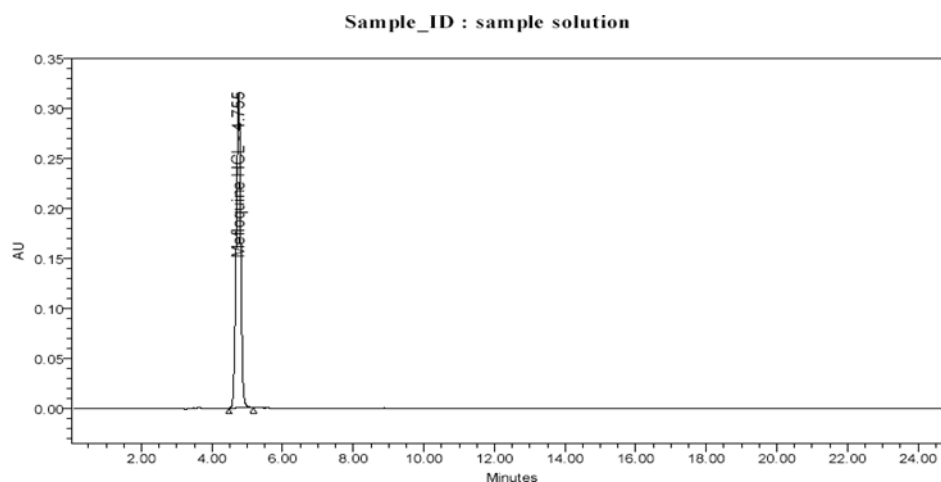


Figure 3 Chromatogram of MFQH Sample Solution

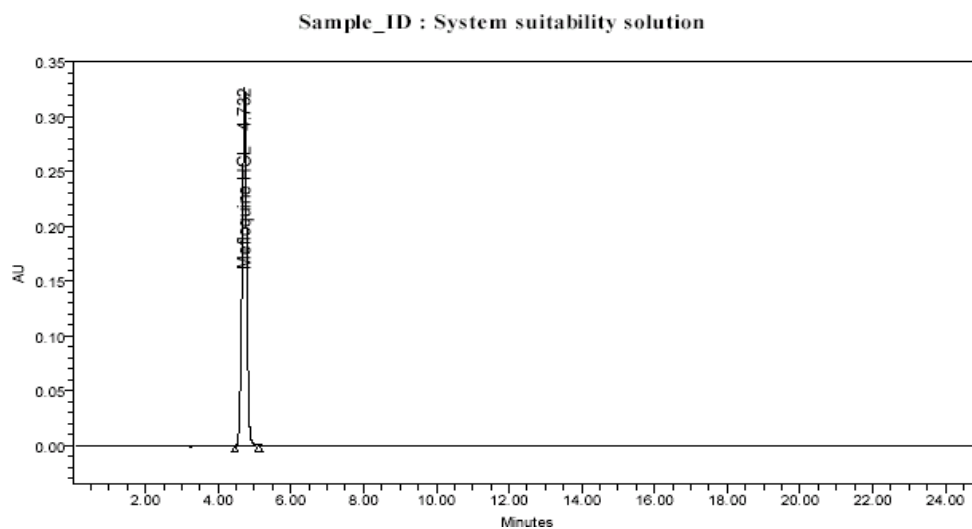


Figure 4 Chromatogram of MFQH Test Solution

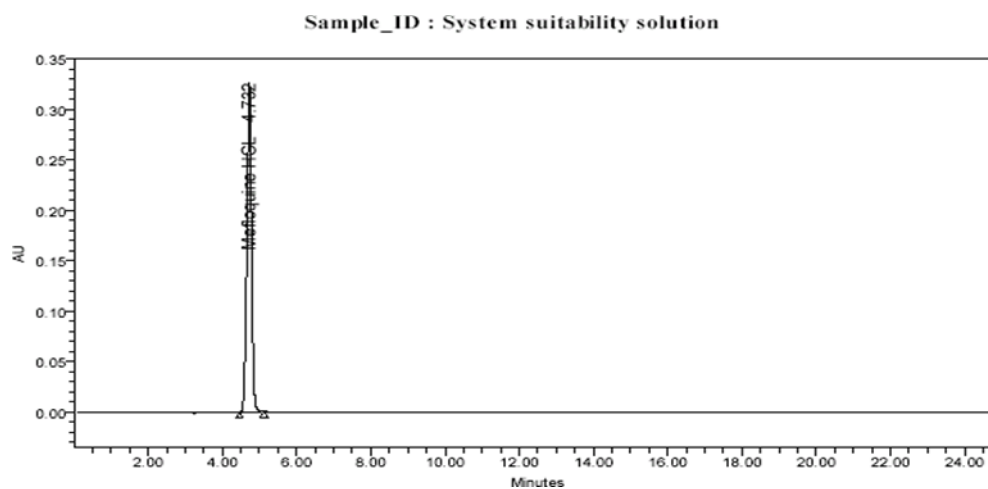


Figure 5 Chromatogram of MFQH Test Solution II

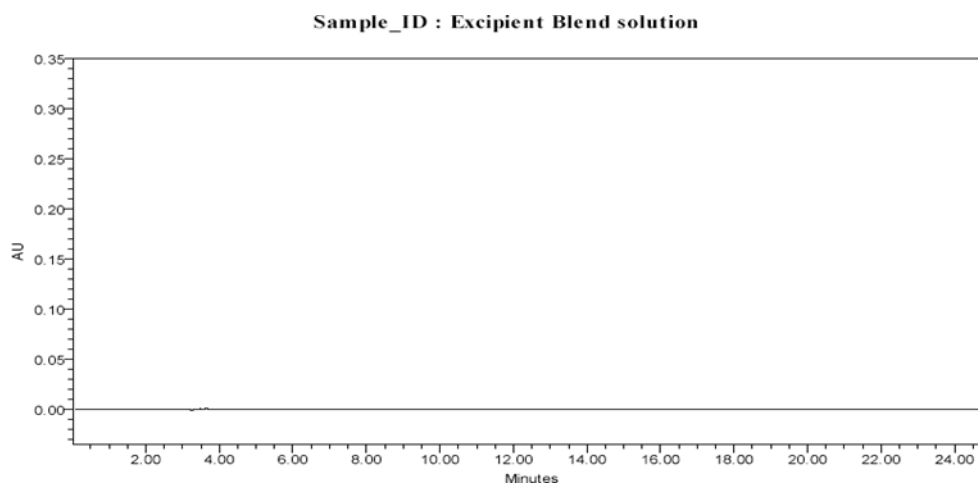


Figure 6 Chromatogram of Placebo Solution

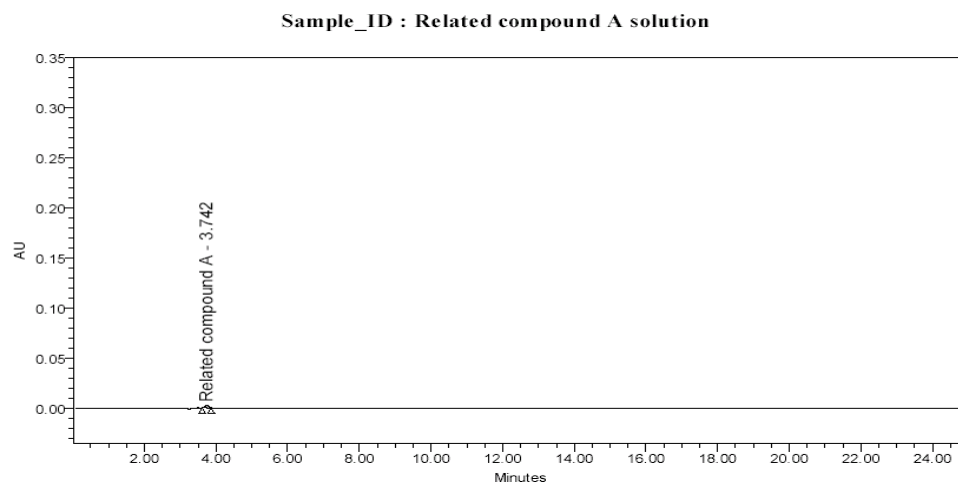


Figure 7 Chromatogram of Related Compound-A

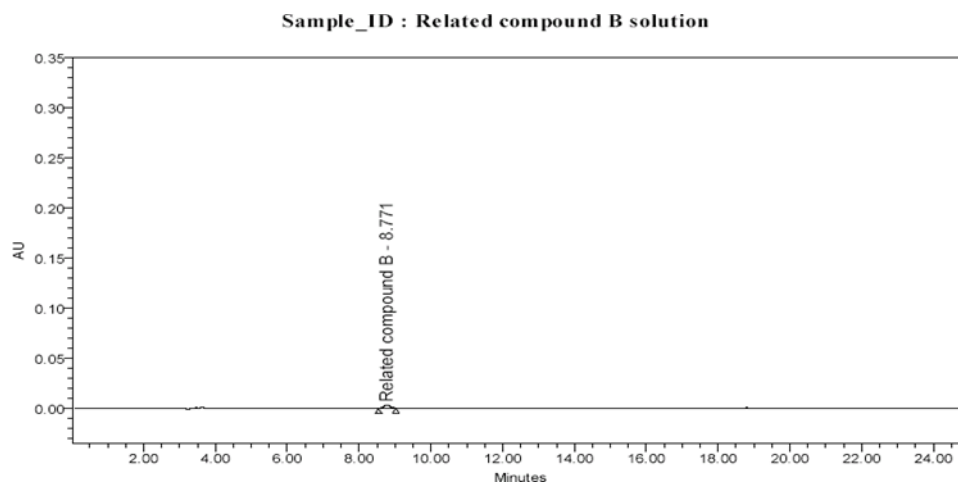


Figure 8 Chromatogram of Related Compound-B

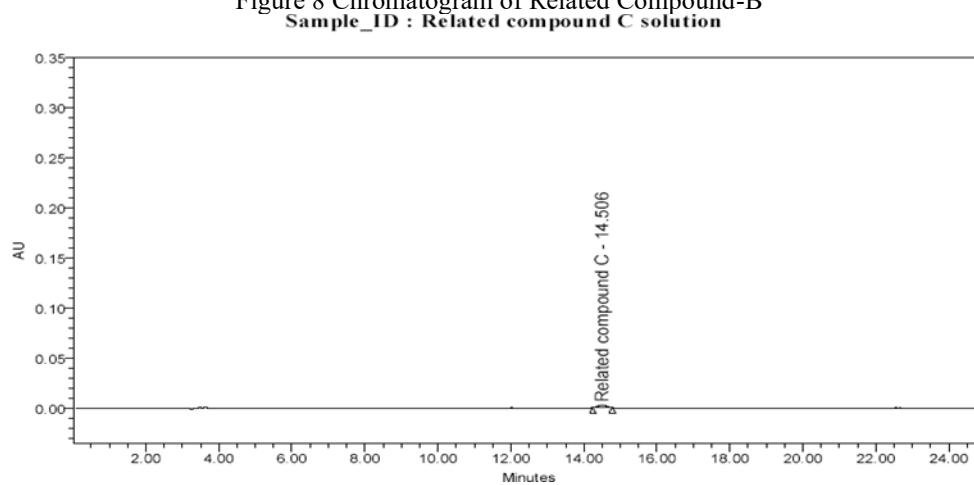


Figure 9 Chromatogram of Related Compound

Sample_ID : Spiked sample solution

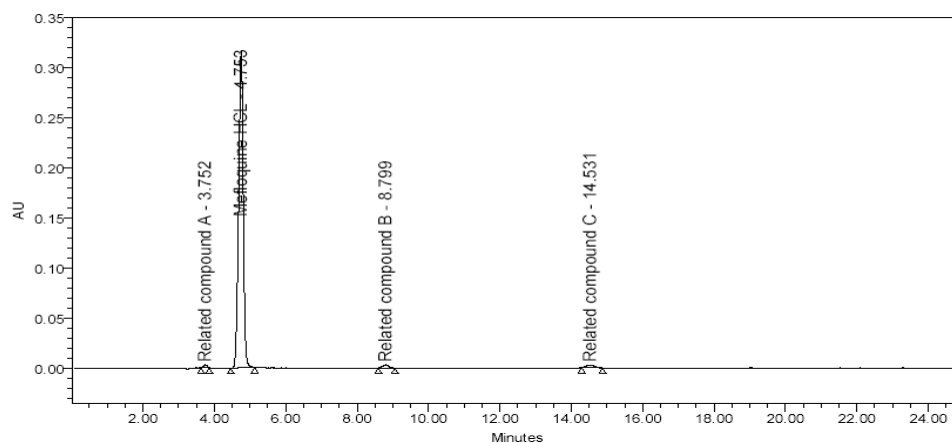


Figure 10 Chromatogram of Spiked MFQH Sample Solution

Sample_ID : sample solution

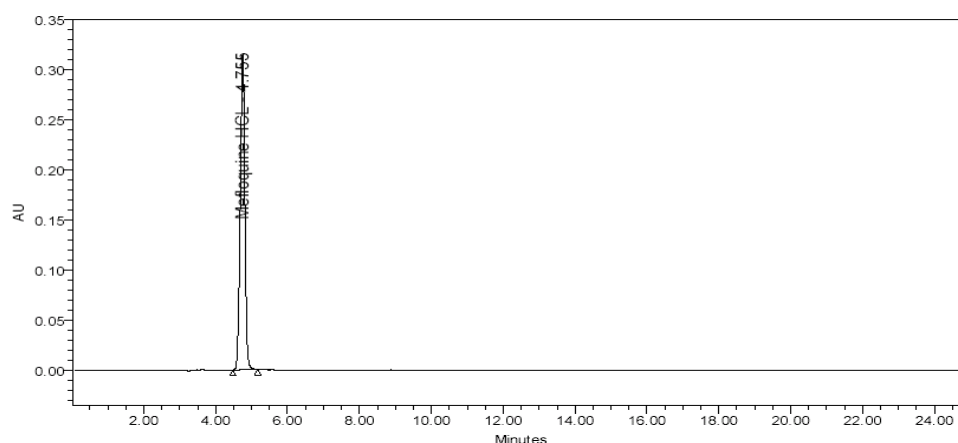


Figure 11 Forced Degradation MFQH System Suitability Solution.

Sample_ID : Excipient Blend - Photolytic degradation

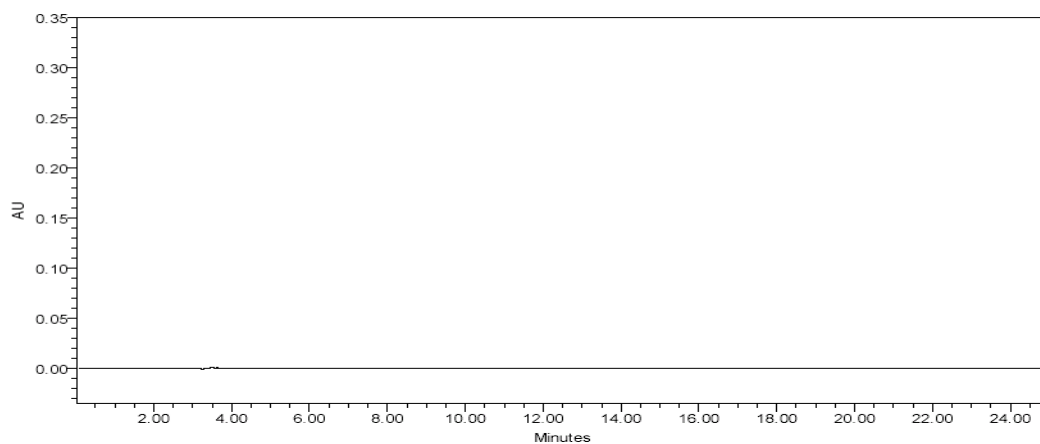


Figure 12 Forced Degradation Placebo blend Photolytic Degradation

Table 8: Results Forced Degradation Study of MFQH Tablet 250 mg

Sr. No.	Stress Study	% Degradation (Approximate)	MFQH Purity Angle	MFQH Purity Threshold	Purity Criteria
1	Untreated Sample	--	71.162	90.156	Pass
2	Acid Degradation	NIL	70.187	91.157	Pass
3	Base Degradation	18.1	71.198	90.182	Pass
4	Oxidative Degradation	NIL	71.258	93.154	Pass
5	Heat Degradation (Solid)	NIL	70.157	92.155	Pass
6	Heat Degradation (Solution state)	NIL	71.118	91.132	Pass
7	Humidity Degradation	NIL	72.120	93.142	Pass
8	Photolytic Degradation	NIL	71.789	92.852	Pass

Conclusion:

Multidrug administration is associated with clinically significant interaction, especially of narrow therapeutic index drugs, either at the pre-absorption and post absorption stage. This can limit the desired therapeutic effect of either the drug molecule. The pharmaceutical dosage form is recently introduced in the Market for the treatment of malaria.

Extensive literature survey revealed that various analytical methods were developed for the estimation of MFQH but not yet stability indicating RP-HPLC method was developed for the MFQH in the bulk and pharmaceutical formulations. So the main aim of the present work was to develop a simple, fast and accurate and precise stability indicating Reverse phase high performance liquid chromatography method.

Result and Discussion:

RP-HPLC method for determination of MFQH was developed. % assay was found between 96.92-98.06, linearity was observed in the concentration range of 50-150 µg/ml and correlation coefficient was found to be 0.9995. The recovery studies were carried out at 50, 100 and 150% level and % recovery for MFQH was found. All the parameters were passed as per compliance of the ICH guidelines.

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