Highly selective separation of Mefloquine HCl and its diastereomer by using conventional RP-LC: A green analytical chemistry approach

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Running title: Green RP-LC method for separation of Mefloquine HCl and its diastereomer.

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Abstract

The concept of green analytical chemistry has been generating great interest in the field of pharmaceutical analysis for the separation of analytes on order to avoid use of organic solvents without compromising in chromatographic performance as well as to protect the environment. In the present research, principles of green chemistry were adopted to develop rapid, green, and robust stability indicating the RP-LC method for the separation of mefloquine hydrochloride and its diastereomer impurity by using mass-compatible mobile phases. The separation was achieved on Acquity UPLC BH C8 (100 x 2.1 mm),1.7µm column with an isocratic elution of 0.1% trifluoro acetic acid in water and ethanol in a ratio of 57:43% v/v as mobile phase at 0.2 $mLmin^{-1}$ flow rate with UV detection at 220 nm with a column oven temperature of 50°C. The method was aimed to be eco-friendly and also an alternative to conventional chromatographic methods. The proposed method was validated as per the ICH Q2 (R1) guidelines and was found to be rapid, linear, precise, and accurate. The proposed validated method is deemed to be fit for the determination of diastereomer impurity in mefloquine hydrochloride bulk drugs (API) or finished dosage forms.

Key words: Mefloquine HCl, Diastereomer, Green analytical chromatography.

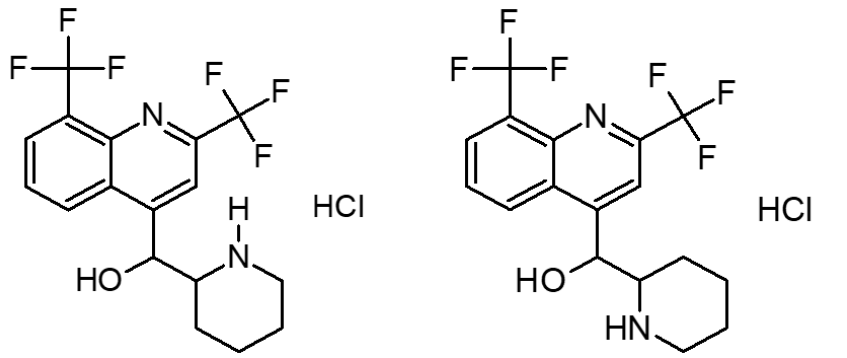
Abbreviations: Green Analytical Chemistry (GAC), Mefloquine HCl (MFQ), Reverse phase liquid chromatography (RP-LC), Ultra-high performance liquid chromatography (UHPLC), Liquid chromatography mass spectrometry (LCMS), Quadrupole Time of Flight (Q-TOF).

1. Introduction

Green chemistry has been attracting a lot of attention and been explored exhaustively across the globe, among which a new domain of Green Analytical Chemistry (GAC) has been derived and emerging with the analytical chemistry. Recently, it has emerged as one of the most interesting and acceptable field among researchers of analytical chemistry in order to focus on the deteriorating effects of the solvents/reagents on environment as well as the health and safety of analysts. The objective of GAC mainly focusses on the elimination or reduction of hazardous chemicals from analytical processes keeping in view the environment and health of personnel without compromising on the performance of the method [1,2]. Thus, developing green RP-LC methods provides an alternative and more eco-friendly way of analyzing the samples. In order to achieve the goal, the common procedures involve substituting the conventional organic solvents such as acetonitrile and methanol with greener solvents [3]. Among the various solvents under the "green category', ethanol has been the favourite and most explored alternative solvent because of its availability and safety index [4]. Based on the principles of green chemistry, some strategies were implemented to achieve greener liquid chromatography methods. This study aims to reduce solvent consumption by lowering the column length, internal diameter, and column particle size.

Mefloquine HCl, a hydrochloride salt of quinoline derivative is commonly used in the treatment of malaria caused by *Plasmodium falciparum* and *Plasmodium vivax*. Mefloquine hydrochloride (MFQ HCl) is chemically a hydrochloride salt of (RS)- [2,8 bis(trifluoromethyl) quinolin-4-yl] [(2SR)-piperidin-2-yl] methanol [5]. The chemical structures of mefloquine hydrochloride and diastereomer (Impurity-1) are shown in Fig. 1. In United Stated Pharmacopeia (USP) monographs, the available related substance procedure uses higher concentrations of ion pair reagents like tetra heptyl ammonium bromide, sodium hydrogen sulfate and hazardous organic solvents like

acetonitrile and methanol for separation of MFQ HCl using HPLC [6]. The quantification of impurities plays a critical role in assessing the quality and safety of a drug substance and/or drug product [7-10]. Therefore, developing stability indicating methods with high accuracy and precision is required in order to quantify the impurities present in the final drug product/substance.



a) Mefloquine HCl **b) Mefloquine HCl Diastereomer**

Fig.1. Chemical structures of Mefloquine HCl (a) and its diastereomer (b)

The present work has been focused to develop a stability indicating green RP-LC method for impurity quantification in MFQ HCl using mass-compatible mobile phases. A thorough literature review revealed reverse phase liquid chromatography methods were reported for MFQ HCl identification and determination in biological fluids and plasma [11-14] simultaneous determination of MFQ HCl [15] or in combination with artesunate in its formulations [16-20] and there are very few reported methods for related substances determination in MFQ HCl [6].

To the best of our knowledge, stability indicating green RP-LC method using eco-friendly eluent like ethanol has not been reported in the literature for separation and quantification of mefloquine hydrochloride diastereomer (impurity-1) in the MFQ HCl drug substance. Therefore, the current research work was aimed to develop a simple, selective, robust, and stable indicating green RP-LC method coupled with UV detection for the rapid analysis of the related substance in MFQ HCl.

2. Experimental

2.1. Materials and Reagents

Mefloquine hydrochloride (MFQ HCl) pure sample and its diastereomer (Impurity-1) were obtained from the United States Pharmacopeial Convention-India (P) Ltd., Hyderabad, India. The analytical reagent (AR) grade reagents such as trifluoro acetic acid, 30% v/v hydrogen peroxide (H_2O_2), hydrochloric acid (HCl), and sodium hydroxide (NaOH) were purchased from Merck (Merck, Mumbai, India), HPLC grade solvent ethanol (EtOH) was purchase from Honeywell research chemicals (Shanghai, China). The HPLC grade purified water was collected from the sartorius Milli Q water system (Arium pro-VF) to prepare all solutions.

2.2. Instrumentation

MFQ HCl and its diastereomer impurity were separated using a Waters UHPLC system (Waters Corp., Milford, MA, USA), coupled with a PDA detector equipped with a quaternary pumping system with degasser, column temperature, and an autosampler temperature regulation facility controlled by Empower 3 software. Mass (m/z) confirmation studies were performed using SYNAPT G2 Q-TOF mass spectrometer (Waters Corporation). The mass detection uses a quadrupole time-of-flight mass analyzer (Q-TOF) with an electrospray ionization (ESI) source. The data was acquired and processed by using Masslynx 4.1 software using the same chromatographic conditions.

2.3. Chromatographic conditions

A good separation between MFQ HCl and its diastereomer (Impurity-1) was achieved with Acquity UPLC BEH C8, 100 mm x 2.1 mm; 1.7 µm particle size column and a mobile phase consisting of 0.1% trifluoro acetic acid in water and ethanol in an isocratic ratio of 53:47 % v/v.

The flow rate of the mobile phase was set at 0.2 mL/min and 50°C column oven temperature along with 3 µL of injection volume. The detection of MFQ HCl and its diastereomer (Impurity-1) was performed at 220 nm using a photodiode array (PDA) detector. The final chromatographic separation is shown in Fig.2. A Q-TOF MS instrument was used to demonstrate the orthogonal detection for the same analysis and the mass spectra are shown in Fig.3. The typical operating source conditions for MS scan in ESI positive mode were optimized as follows: Capillary (kV):4.00; cone (v): 30.00; extractor (v):2.00; Source Temperature (°C):100; Desolvation temperature (°C): 500; Gas flow: 650 Lit/Hr, Cone Gas Flow: 50 Lit/Hr. The chromatographic data were monitored and processed using Empower 3. Mass spectrometry data were acquired using Masslynx 4.1 software. The mobile phase was used as the diluent.

2.4. Sample solutions preparations

Preparation of sample solution

A sample stock solution was prepared with 5 mg of accurately weighed MFQ HCl and transferred into a 25 mL volumetric flask, 15 mL of mobile phase was added, sonicated to dissolve, and made up the volume with the mobile phase to obtain a concentration of 200 µg/mL.

Preparation of MFQ HCl and its impurity stock solution

A stock solution of MFQ HCl and its diastereomer (Impurity-1) of 200 µg/mL individually was prepared by dissolving an appropriate amount in the mobile phase. Further diluted this solution 10 times to achieve a concentration of 20 µg /mL and this stock solution was used for validation studies.

Preparation of organic impurities (OI) standard preparation

Organic Impurity (OI) standard solution consists of MFQ HCl, and its diastereomer (Impurity-1) at 0.2 µg/mL (0.1% level with respect to sample concentration of 200 µg/mL) concentration was prepared by diluting 100 µL of the above stock solution to 10 mL with diluent for organic impurities determination.

Preparation of Organic impurities spiked sample solution

5 mg of MFQ HCl was transferred into a 25 mL volumetric flask and dissolved using 15 mL mobile phase. 250 µL of the above organic impurities (OI) standard stock solution was spiked to this solution, mixed well and made up the volume with the mobile phase to obtain a concentration of 200 µg/mL of MFQ HCl and 0.2 µg/mL of its diastereomer (Impurity-1).

Forced degradation studies of MFQ HCl

The MFQ HCl was subjected to degradation conditions (acid, base hydrolytic degradation, oxidative, photolytic, thermal, and humidity) as per ICH Q1A (R2) guidelines [21]. Initially, the MFQ HCl stock solutions (1.0 mg/mL) were prepared by dissolving in a small amount of mobile phase and diluted with acid (0.1 N HCl), base (0.1 N NaOH), and 3% hydrogen peroxide (H_2O_2). Photolytic degradation studies were carried out by exposing the sample to ultraviolet and visible lights at 200 $Whrm^{-2}$ and 1.2 million lux hours respectively. Thermal degradation was performed by exposing the sample at 105 °C whereas humidity stress studies were carried out at 85 % RH & 85° C for 3 days.

2.5. Chromatographic method validation

The developed UHPLC–PDA method for MFQ HCl drug substance was validated in compliance with ICH Q2 (R1) guidelines [22]. Specificity, linearity, and range were determined as part of the validation study. Accuracy and precision were also confirmed.

System suitability (SST)

The system suitability requirements were evaluated by injecting the organic impurity standard solution consisting of 0.2 µg/mL of MFQ HCl and its diastereomer (Impurity-1) in six replicate injections. % RSD not more than 5.0%, and the resolution not less than 3.0 between diastereomer and MFQ HCl were set as the requisite SST parameters, and the results are captured in Table 2.

Specificity

Specificity studies were conducted to prove the method's capability to resolve the principal compound(s) in the presence of all other interferences. The peak purity analysis of degradation product and main compound(s) manifests the method's specificity. All the peaks were evaluated using mass spectrometry as an orthogonal detection for identification and further confirming the specificity of the proposed analytical method.

Linearity

Five levels of linearity solutions were prepared ranging from 50 to 150% of the median analyte concentration (200 µg/mL) for MFQ HCl and its diastereomer (Impurity-1) at five levels. The calibration curve (least-squares linear regression) was plotted using the % area against the concentration expressed in µg/mL and recorded the correlation coefficient value to show the linearity of the developed method.

Accuracy

The accuracy of the related substance method was calculated at three different levels 50%,100%, and 150 % of the analyte concentration (200 µg/mL). For accuracy determination, six samples were prepared at 100% level and three for 50 and 150%.

Limit of detection (LOD) and limit of quantification (LOQ)

The sensitivity of the method was evaluated by injecting the lower concentrations of MFQ HCl and its impurity. The S/N method was used and Limit of detection (LOD) was estimated at 3:1 and the Limit of quantification (LOQ) at 10:1 respectively, to determine the sensitivity of the developed method.

Robustness

The Robustness study was conducted by making changes in the flow rate ± 10% of the actual value (0.20 mL/min), column oven temperature ±5°C (50°C) from its original temperature. The resolution between diastereomer (Impurity-1) and MFQ HCl was measured.

3. Results and Discussion

3.1. Mobile phase and sample preparation with green solvents

A RP-HPLC method generally comprises of an aqueous component along with reagents/additives and an organic component which is considered as the non-aqueous part. Over the years, acetonitrile and methanol have been commonly used as the favorite organic solvents in RP-LC due to the well-known advantages offered by them such as low viscosity, water-compatibility and lower UV cut-off wavelength. However, with respect to environment and personnel health/ safety they have a detrimental impact and hence have been classified as hazardous solvents. Green RP-LC methods are developed by replacing acetonitrile and methanol with greener ones. Ethanol is considered to be one of the best alternatives, due to its low toxicity, bio-sourcing, and biodegradability. Although, it poses few drawbacks w.r.t baseline drift during gradient (especially at lower wavelength), and increased viscosity when mixed with water, leading to elevated column backpressures. Therefore, the present study focused on the conventionally used isocratic elution

mode and studied various compositions of the mobile phase at lower flow rates by considering the higher backpressure and column oven temperature to achieve a method compatible with the HPLC system. Green solvents with minimal environmental impact under acidic modifiers and organic solvents were investigated. Also in the sample preparation, mobile phase solvents were selected in order to maintain the concept of GAC approach. However, the solvents for the samples were chosen according to the solubility and eluotropic strength requirement.

3.2. Optimization of chromatographic conditions

The RP-UHPLC procedure optimized was an eco-friendly stability-indicating method. Utilizing the fundamentals of green chemistry, various solvent(s) and mobile phase(s) were considered for the method development resulting in a green and environmentally friendly UHPLC method. MFQ HCl and its diastereomer (Impurity-1) structures are identical mirror images of each other, and therefore their separation was the most critical factor in the method development. The method development included various systematic approaches for selection of eluent and stationary phases.

The physicochemical properties of the analytes (MFQ HCl and Impurity-1) were considered for the mobile phase selection. Acquity BEH C8 column produced better separation among the peaks. The drug and its diastereomer (Impurity-1) gave a good response at 220 nm and was thus set as the detection wavelength. Therefore, the final method was fixed with 0.1% v/v trifluoro acetic acid in water as acidic modifier and ethanol as the organic modifier with an isocratic ratio of 57:43 % v/v with 0.2 mL/min flow rate, and the column oven temperature at 50°C. The resolution between MFQ HCl and its diastereomer (Impurity-1) was good, and the respective chromatogram is shown in Fig. 2. Mass spectrometry analysis confirms the mass values using the same chromatographic conditions and the related spectrum shown in Fig. 3.

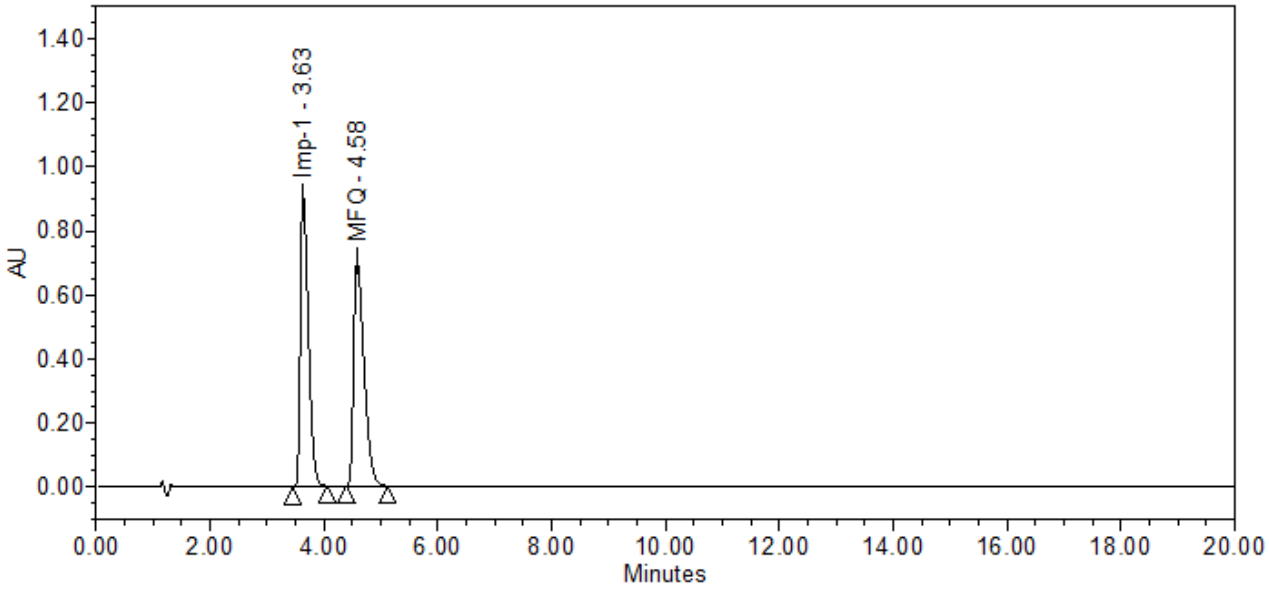


Fig. 2. RP-UHPLC chromatogram of Mefloquine HCl and its diastereomer (Impurity-1)

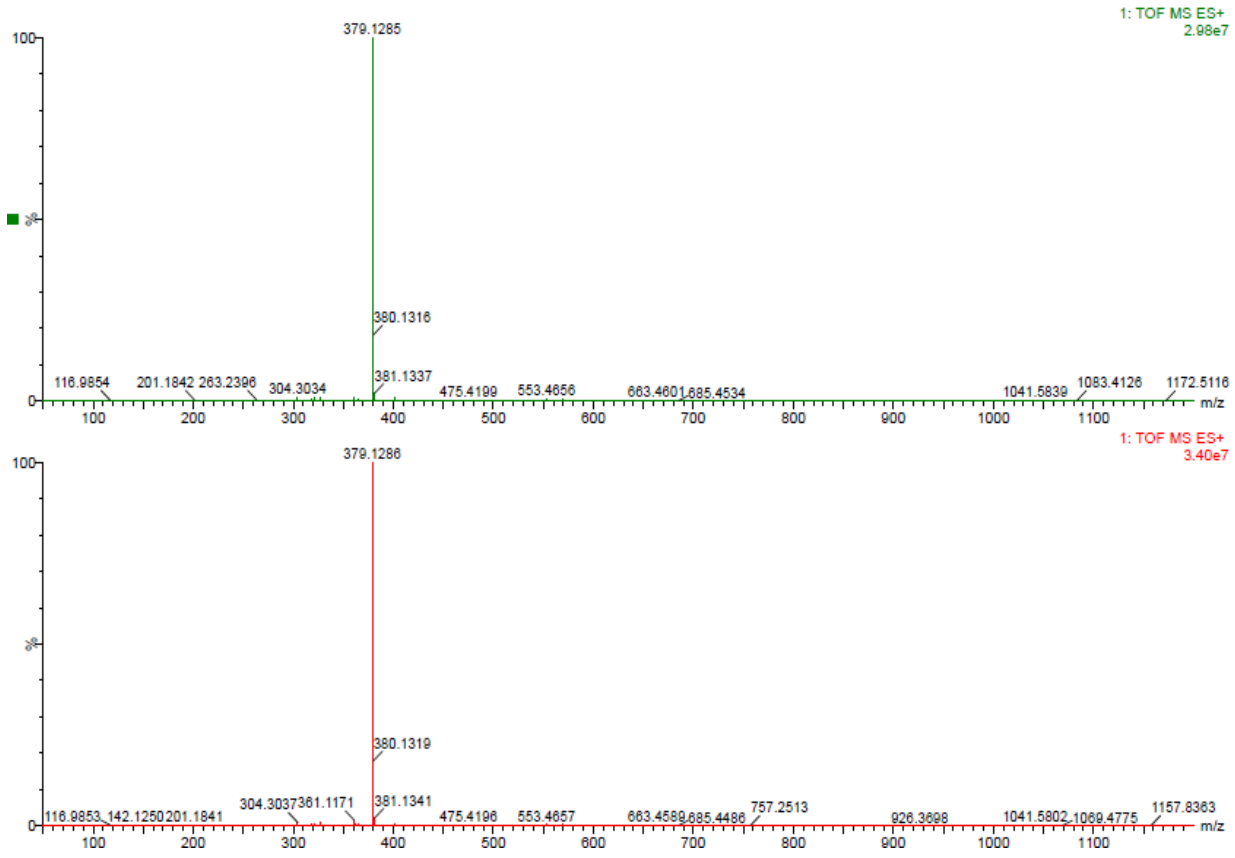


Fig. 3. LC-QTOF-MS spectra of $[M+H]^+$ ions Mefloquine and its diastereomer (Impurity-1)

4. Method Validation

The proposed green UHPLC method was validated as per the ICH Q2 (R1) guidelines for system suitability, accuracy, linearity, limit of detection (LOD), the limit of quantification (LOQ), precision, selectivity, robustness, and specificity.

4.1. System suitability

Drug impurities at 0.1% level of drug concentration were solubilized for preparation of the system suitability solution. Six consecutive injections were evaluated for retention time, peak area, resolution, and USP tailing factor and their %RSD were calculated. Results of system suitability are tabulated in Table 2.

4.2. Specificity: Forced degradation studies

Ability of the method to measure analyte in the presence of other interferences is termed as specificity. The selectivity of this method considers the separation of MFQ HCl with its impurity. The peak purity of all the samples was determined using a photodiode array (PDA) and a mass detector. The purity angle is less than the purity threshold for all peaks indicating the specificity of the developed method. Of all stress conditions, the drug was susceptible to alkaline (4% loos of MFQ HCl) condition and stable under rest of the conditions. Table 1 summarizes all degradation conditions and their results. The overlaid chromatogram of the base degradation sample is depicted in Fig. 4.

Table 1

Summary of forced degradation conditions and degradation information

Degradation study	Exposure conditions	Degradation Information
Acid degradation	1 N HCl at room temperature for 3 days	ND
Base degradation	1 N NaOH at room temperature for 3 days	MFQ HCl Diastereomer
Peroxide degradation	3% H_2O_2, at room temperature for 3 days	ND
Thermal degradation	Thermal at 105° C for t 48 hours	ND
UV light	200 $Whrm^{-2}$	ND
Fluorescent light	1.2 million lux hours	ND
Humidity degradation	Humidity at 85% RH&85 °C for 3 days	ND

ND: Not Detected

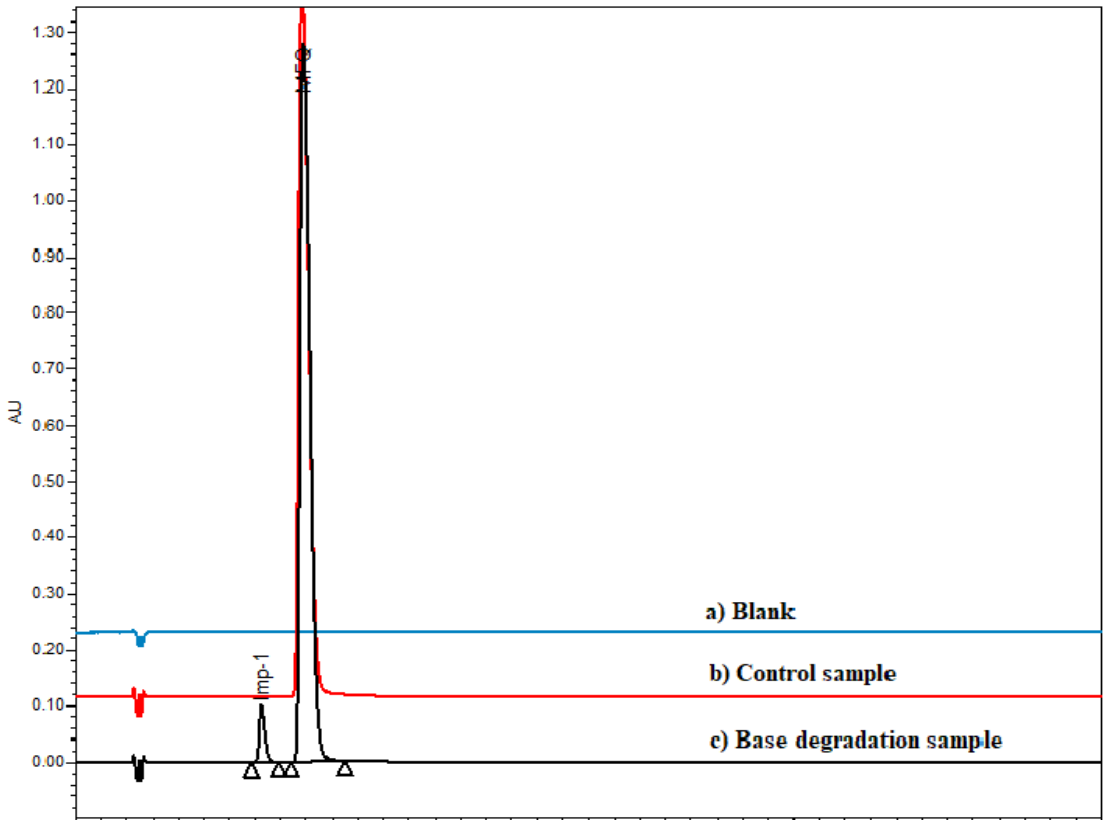


Fig. 4. Overlaid UHPLC chromatograms of a) Blank, b) control sample and c) base degradation sample

4.3. Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ for the standard solutions were found to be 0.02 and 0.05 µg/mL respectively, which are equivalent to 0.01% and 0.025% of the analytical concentration (200 µg/mL) of MFQ HCl. The S/N for LOQ standard solutions was greater than 10, and all the S/N for LOD solutions were greater than 3. These results suggested that the developed UHPLC method has good sensitivity for quantifying related compounds in MFQ HCl (results in Table 2).

4.4. Linearity

The correlation coefficient (r^2) was found to be greater than 0.999 in linear calibration plot for the related substance method when tested over a calibration range of 50 to 150%. A calibration plot for the impurities was determined with a range of 0.05 to 0.15 % for MFQ HCl and its impurity. The correlation coefficient obtained was greater than 0.999 and the summary of results are shown in Table 2 and respective linearity chromatograms in Fig.5.

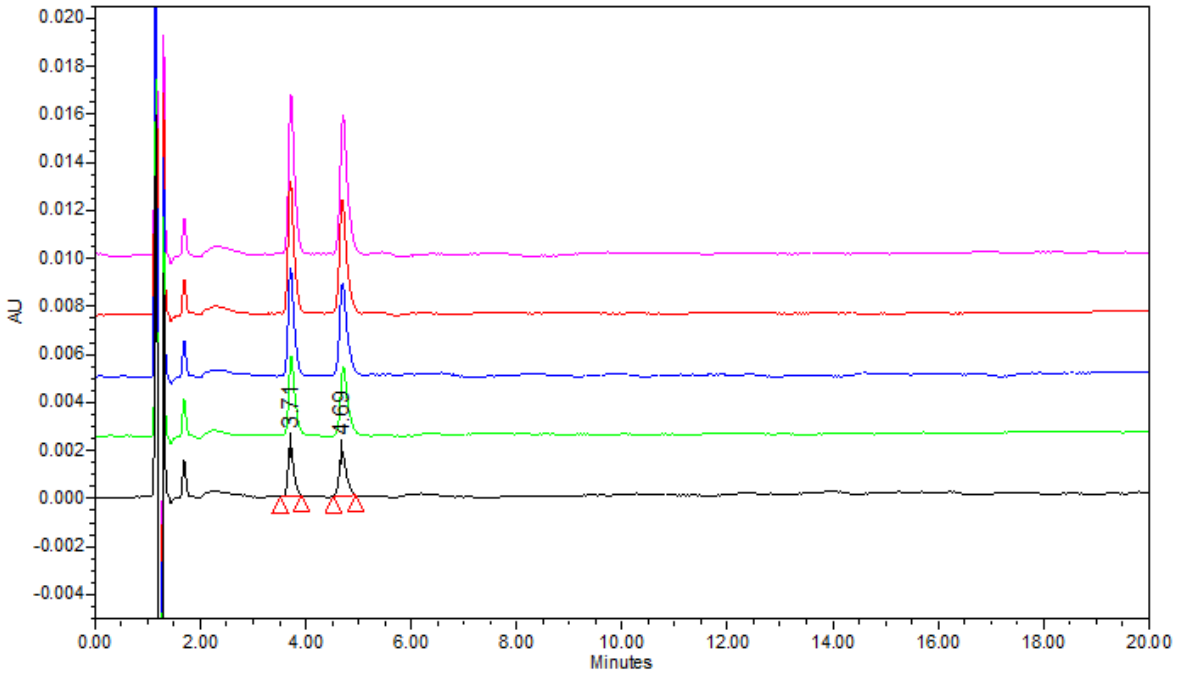


Fig. 5. Overlaid Linearity chromatograms

Table 2

System suitability, linearity, LOD and LOQ data of MFQ HCl and its diastereomer (Impurity-1)

Compound	% RSD for Peak area	LOQ Conc. (µg/mL)	LOD Conc. (µg/mL)	Linearity & Range (µg/mL)	Correlation (r^2 value)
(Impurity-1)	0.48	0.05	0.02	0.10-0.30	0.9992
MFQ HCl	0.94	0.05	0.02	0.10-0.30	0.9995

LOQ: Limit of Quantitation LOD: Limit of Detection

4.5. Precision

Repeatability and intermediate precision are the major expressions for method precision. Repeatability of the method was demonstrated by analyzing six preparations of the same concentration in the same equipment on the same day. The % RSD of the peak area was below 1.0%. For intermediate precision, a different analyst, and instruments on different days were considered. The % RSD of each peak area was within the acceptable limit. The results are tabulated in Table 3 indicating the method to be precise.

4.6. Accuracy

Accuracy is defined as a measure of closeness of experimental value to the true value. The samples at three different levels (50%, 100%, and 150%) of impurities were spiked to mefloquine hydrochloride and used to validate the method for accuracy. Recoveries in terms of percentage were calculated and found to be within the specified limit of 80.0–120.0% recovery, which confirms the accuracy (Table 3).

Table 3

Accuracy and precision data for mefloquine hydrochloride diastereomer (Impurity-1)

Amount spiked at different levels*	Concentration (µg/mL)	% Recovery# (Precision)	% Recovery# (intermediate Precision)
at 50% level	0.10	101.27	100.32

at 100% level	0.20	101.21	99.74
at 150% level	0.30	101.03	100.36
* Amount of impurity spiked with respect to nominal concentration of 0.2 mg/mL of MFQ HCl			
# Mean % recovery for six determinations at 100 % and three determinations for other levels			

4.7. Robustness

Robustness is the capability of the analytical method to remain unaffected when a deliberate change in parameters are made. The performance of the validation by changing the optimized temperature ±5°C (nominal temp 50°C) and flow rate±10% of flow (0.2 mL/min). The effect of mobile phase composition variation of about ± 5% on resolution keeps other parameters unaffected. Even after altering the method, criterion results were unchanged, proving the robustness of the developed method.

5. Conclusion

This study was carried out with an aim to develop environmentally benign alternatives to hazardous chemicals and processes in the field of drug/pharmaceutical analysis. The present research is directed in a way to open up new avenues in greening of the chromatographic methods in the field of pharmaceutical analyses. A selective method with good separation was achieved using the UHPLC procedure for mefloquine hydrochloride and its diastereomer. The proposed method was found to be sensitive, precise, and accurate with shorter run time. With all the above mentioned advantages the proposed method finds a superior position in the application for the routine analysis of mefloquine hydrochloride and its related substances in commercially available dosage forms.

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Disclosure statement/Conflicts of Interest

The authors declare no conflicts of interest.

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