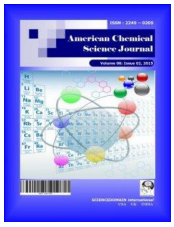

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Application of HPTLC-densitometry by Derivatization and Stability Indicating LC for Simultaneous Determination of Mefloquine Hydrochloride and Artesunate in Combined Dosage form

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SMS with PSSK and SM designed the study, performed the method development, wrote the protocol, and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

This paper presents simultaneous quantification of mefloquine hydrochloride (MEFQ) and artesunate (ARTS) by HPTLC and RP-HPLC methods in combined tablet formulation. In RP-HPLC method, the drugs were resolved using a mobile phase of methanol-phosphate buffer (70:30, v/v) with pH adjusted to 3.2 using phosphoric acid on Symmetry C_{18} (250 × 4.6 mm and 5 µm) column in isocratic mode. Quantification was achieved with UV detection at 220 nm for MEFQ and 313 nm for ARTS based on peak area with linear calibration curves at concentration ranges of 12.5–75.0

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and 2.5–15 µg/mL for MEFQ and ARTS respectively. In HPTLC method, the chromatograms were developed using a mobile phase of toluene-ethyl acetate-acetone (2.5:1.0:0.5, v/v/v) in pre-coated plate of silica gel 60 F_{254}. It is a single method with two steps in which after the development of chromatogram, MEFQ was detected at 285 nm. Then ARTM was derivatized and detected at 525 nm. Recovery values of 97.36–98.80%, %RSD <2 and r value >0.9994 shows that the developed methods were accurate and precise. In HPLC, the binary drug mixture was exposed to thermal, photolytic, acid, alkali and oxidative stress. The methods distinctly separated the drugs and degradation products even in actual samples. In conclusion, the proposed HPLC and HPTLC methods were simple, precise, rapid and accurate. Both methods have the potential to determine these drugs simultaneously from dosage forms without any interference of excipients.

Keywords: Mefloquine hydrochloride; artesunate; HPLC; TLC-densitometry; tablet dosage form.

1. INTRODUCTION

Nowadays, pharmaceutical products used to treat malaria often come in the form of combined formulation to obtain a synergistic effect and to reduce adverse effect. Mefloquine hydrochloride in combination with artesunate is the fixed-dose artemisinin-based combination therapy currently available to treat uncomplicated malaria [1,2]. Such a combination dosage form will be adhering to effective therapy and enhancing better patient compliance. Mefloquine hydrochloride (MEFQ) chemically, (±) Erythro-α-(2-piperidyl)-2, 8-bis (trifluoro-methyl)-4-quinoline methanol hydrochloride (Fig. 1a) is acted by inhibiting chloroquine resistant *P. falciparum* at the asexual intraerythrocytic stage. Arteunate (ARTS) chemically, (3R, 5aS, 6R, 8aS, 9R, 10S, 12R, 12aR)-Decahydro-3, 6, 9-trimethyl-3, 12 epoxy-12*H*-pyrano [4-3-j] 1, 2- benzodioxepin-10-ol hydrogen succinate (Fig. 1b) is a derivative of artemisinin, classified as counterfeit antimalarial medicine and used in large number of malaria patients in China, including those with both chloroquine-sensitive and chloroquine-resistant strains of *P. falciparum* [3,4]. MEFQ and ARTS have been reported to be quantified individually or in combination with other drugs in formulation and biological fluids by various techniques [5–32] and one LC method [33] was reported for simultaneous quantification of these drugs. To our knowledge there are no stability indicating LC or HPTLC methods were reported that permit the simultaneous quantification of MEFQ and ARTS in combined dosage form. The aim of the present work was to develop and validate [34,35] new simple, rapid, selective, cost effective HPTLC and stability indicating HPLC methods for simultaneous determination of MEFQ and ARTS in pharmaceutical formulation.

2. EXPERIMENTAL

2.1 Chemicals and Reagents

Mefloquine and Artesunate reference standards were obtained from Zydus Cadila (Ahmadabad, India). HPLC grade methanol, acetonitrile and distilled water were obtained from Merck Chemicals (Mumbai, India). Analytical grade methanol, potassium dihydrogen-o-phosphate, phosphoric acid, toluene, ethyl acetate, acetone, anisaldehyde and sulphuric acid were obtained from S.D Fine Chemicals (Mumbai, India). Falcigo plus (Zydus Cadila, Ahmadabad, India) labeled to contain 250 mg MEFQ and 50 mg ARTS was purchased from local pharmacy. High purity water was prepared by using Millipore Milli Q plus purification system.

2.2 Chromatographic Conditions

The HPLC (Shimadzu, Japan) instrument was equipped with a model series Shimadzu LC-10 ATVP pump, Rheodyne-7725 injector with 20 µl loop and a Shimadzu SPDM-20 A diode array detector. Separation was made on a Symmetry C_{18}, 250 × 4.6 mm column (5 µm particle size). The detection wavelength was set at 220 and 313 nm. Data acquisition was performed on a model Class-VP software.

In HPTLC, chromatographic separation of drugs was performed on Merck TLC plates (Germany) pre-coated with silica gel 60 F_{254} (10.0 × 10.0 cm with 250 mm layer thickness). Time for chamber saturation was optimized to 10 minutes. Sample and standard zones were applied to plates as bands by means of Camag 100 µl sample syringe (Hamilton, Switzerland) with a Linomat 5 applicator (Camag, Switzerland). The plates were left to equilibrate for 3 minutes in a 10.0 × 10.0 cm horizontal chamber (Camag, Switzerland) and then developed to a distance of

80 mm using toluene-ethyl acetate-acetone (2.5:1.0:0.5, v/v/v) as mobile phase. Separation was obtained within 10 minutes and before detection, the plates were dried at 60ºC for 4 minutes to eliminate mobile phase.

Initially for detection of MEFQ, densitometric scanning was carried out using TLC scanner (Camag, Switzerland) in the absorbance / reflectance mode at 285 nm. Artesunate shows very weak UV absorbance property. Therefore subsequent to this scanning, TLC plates were derivatized with anisaldehyde-sulphuric acid reagent for 4 seconds and heated for 3 minutes at 110ºC. This post chromatographic treatment of artesunate spot was considered to produce light-absorbing compound on the layer. Thus both MEFQ and ARTS can be developed and scanned in a single plate before and after derivatization. winCATS software (V 1.4.2, Camag, Switzerland) was used for scanner control and data processing. The sources of radiation used were deuterium-tungsten lamp. The whole procedure took not more than 30 minutes. The R_f values of MEFQ and ARTS were found to be 0.26±0.02 and 0.59±0.01 respectively.

2.3 Standard Solutions and Calibration Graphs

2.3.1 For HPLC method

Standard stock solutions prepared by dissolving 50 mg of MEFQ and 50 mg ARTS in 50 mL methanol. The mixed standard solutions were prepared by dilution of stock solution with methanol to reach a concentration range 12.5–75 µg/mL for MEFQ and 2.5–15 µg/mL for ARTS. Triplicate 20 µl injections were made for each concentration and chromatographed under conditions mentioned above. The peak area of each concentration was plotted against corresponding concentration to obtain calibration graph.

2.3.2 For HPTLC-densitometric method

Standard stock solutions were prepared separately by dissolving 25 mg of MEFQ and 25 mg of ARTS in 25 mL methanol. The mixed standard solutions were prepared by dilution of stock solution with methanol to reach a concentration range 12.5–100 µg/mL for MEFQ and 2.5–25 µg/mL for ARTS. A total of 10 µl of each solution was applied as bands to HPTLC plate, and analyzed in triplicate. The peak area of each concentration was plotted against corresponding concentration to obtain calibration graph.

2.4 Sample Preparation

Twenty tablets of Falcigo plus (Zydus Cadila, Ahmadabad, India) were weighed and the average weight was determined. The tablets were ground to fine powder. An amount equivalent to label claim of each active ingredient was accurately weighed and transferred to suitable volumetric flask. The volume was adjusted with methanol for both HPLC and HPTLC methods. The resultant solution was sonicated for 5 minutes and filtered through 0.45 µ nylon filter (Millipore, Milford, USA). For HPLC, suitable aliquots were transferred to 10 mL volumetric flask and completed to volume with methanol to have a final concentration of 50 µg/mL of MEFQ and 10 µg/mL ARTS respectively. From this final solution, 20 µl was injected. The procedure was repeated 5 times for each brand. For HPTLC, aliquots were made up to volume using methanol to have a final concentration of 5.0 µg/mL MEFQ and 1.0 µg/mL ARTS, respectively. From this final solution 10 µl was spotted as bands to furnish a concentration of 500 ng/band MEFQ and 100 ng/band ARTS, respectively.

2.5 Specificity

The specificity of both the methods was assessed by analyzing standard and sample solutions. In HPLC, the average retention time ± standard deviation for MEFQ and ARTS were found to be 3.4±0.03 and 7.3±0.02 min respectively for 10 replicates. The peaks obtained were sharp and have clear baseline separation. Other parameters like retention time (R_t), capacity factor (k), tailing or asymmetrical factor (T) were also determined. In HPTLC, the bands of MEFQ and ARTS from pharmaceutical formulations were confirmed by comparing R_f values as well as UV spectra of separated bands with those from standard. The peak purity spectra shows a value of 0.99 indicated the method specificity, as there was no interference from any impurities in the separation and determination of MEFQ and ARTS peaks. The peak purity spectra is the UV spectra obtained across the peak should be superimposable. The derived peak purity index (P) ranged from 0.9989 to 0.9999 for both compounds. The purity of each compound was confirmed by analyzing the UV spectrum at the start, apex and end of peaks.

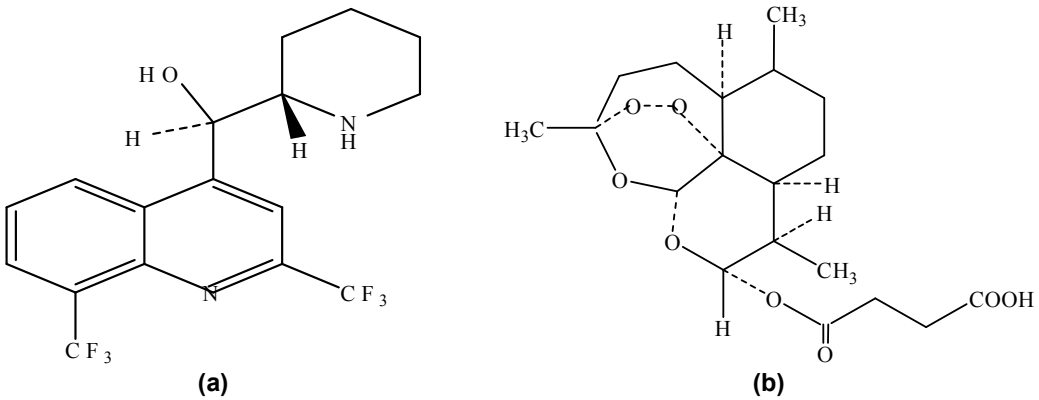


Fig. 1. The chemical structure of (a) Mefloquine and (b) Artesunate

2.6 Linearity and Sensitivity

In case of HPLC, linearity was studied by injecting six concentrations of standard MEFQ (12.5–75 µg/mL) and ARTS (2.5–15 µg/mL) in triplicate. In HPTLC, a series of combination solutions and standard curves were prepared over a concentration range from 125–1000 ng/band of MEFQ and 25–200 ng/band of ARTS from stock solution. In both methods, peak area versus concentration data was performed by least square linear regression analysis, whereby slope, intercept and correlation coefficient were determined. For both methods, sensitivity was determined with respect to LOD and LOQ. The LOD and LOQ parameters were determined from regression equations of MEFQ and ARTS; LOD = 3.3 × SD/s, LOQ = 10 × SD/s; where 'SD' is the standard deviation of response and 's' slope of calibration curve.

2.7 Precision and Accuracy

The precision of the methods was determined by repeatability (intraday precision) and intermediate precision (interday precision) studies and expressed as RSD of a series of measurements. Intraday precision was evaluated by six replicate readings at three concentration levels within the linearity range. Interday precision was performed by comparing the results on three different days. Recovery studies by standard addition method were performed in view to justify accuracy of the proposed methods. Previously analyzed samples containing MEFQ and ARTS were spiked with standard MEFQ and ARTS and the mixtures were analyzed in triplicate (n = 3) by proposed methods. Precision was calculated from percentage relative standard deviation (RSD, %) for repeated measurements, whereas accuracy expressed as % of recovery.

2.8 Robustness

Robustness of HPLC method was determined by deliberately varying certain parameters like flow rate, volume of acetonitrile in mobile phase and pH of mobile phase by ±0.1. For HPTLC, the conditions altered were mobile phase composition, development distance, time of spotting and detector wavelength. For all changes in conditions the samples were analyzed in triplicate. When the effect altering one set of conditions was tested, the other conditions were held constant at optimum values.

2.9 System Suitability

The system suitability test was performed to confirm that the LC system to be used was suitable for intended application. A standard solution containing 50 µg/mL of MEFQ and 10 µg/mL ARTS were injected six times. The parameters such as retention time, capacity factor, theoretical plates, tailing factor and % RSD were determined.

2.10 Forced Degradation Studies

To evaluate the stability indicating property of developed HPLC method, 1.0 mL of MEFQ and 0.3 mL of ARTS from previously mentioned stock solutions of standards, were transferred separately into 25 mL standard flask containing 5 mL each of 0.1M hydrochloric acid, 0.1M sodium hydroxide and 3% hydrogen peroxide (v/v), respectively. The mixture was refluxed at 60ºC for 1 hour and completed to volume with

methanol. For dry heat degradation, the solution was refluxed at 60ºC for 1 hour, and for photolytic degradation, the solution was exposed to UV-light (254 nm) in a photostability chamber for 24 hrs. The resulting solutions were run under optimized chromatographic conditions.

3. RESULTS AND DISCUSSION

3.1 Reversed Phase High Performance Liquid Chromatography

A satisfactory separation was obtained when using methanol-phosphate buffer (70:30, v/v) and pH was adjusted to 3.2 using phosphoric acid under isocratic conditions, and at a flow rate of 1.0 mL/min. From the overlain spectra (Fig. 2), it was observed that MEFQ exhibited strong absorbance at 313 nm, while that of ARTS at 220 nm. Therefore detection was carried out by UV dual detector at 220 and 313 nm. Peaks were well defined, resolved, and almost free from tailing. Retention times of MEFQ and ARTS were observed at 3.40±0.03 and 7.30±0.02 respectively (Fig. 3).

System suitability tests were also carried out to verify reproducibility, and results are summarized in Table 1. For quantitative applications, linear calibration graphs were obtained with correlation coefficients of 0.9989 and 0.9992 for MEFQ and ARTS, respectively. Calibration plots were linear from 12.5–75 µg/mL for MEFQ and 2.5–15 µg/mL for ARTS. Limits of detection (LOD) were 3.57 µg/mL for MEFQ and 1.39 µg/mL for ARTS, limit of quantification (LOQ) were found to be 8.69 µg/mL for MEFQ and 2.27 µg/mL for ARTS, which showed good sensitivity of the proposed method. The low RSD (< 2.0%) values of intraday and interday precision for MEFQ and ARTS revealed that the proposed method is precise (Table 2). % RSD of recovery study was found to be 0.61 to 1.50, which indicated that the method is accurate (Table 2). Upon slight variation in the selected parameters, insignificant difference in peak area and retention time was observed. The resolution between MEFQ and ARTS and its major degradation products were found to be ≥ 2.0 indicating robustness of the method (Table 3). The assay results for an average of five determinations of tablets (Falcigo Plus (250 mg MEFQ and 50 mg ARTS)) were shown in Table 4. The mean assay values were 100.43% for MEFQ and 99.58% for ARTS, respectively. The results of quantitative analysis of tablets indicate that the proposed method can be used for routine quantitative and quality control analysis of MEFQ and ARTS in pharmaceutical dosage forms.

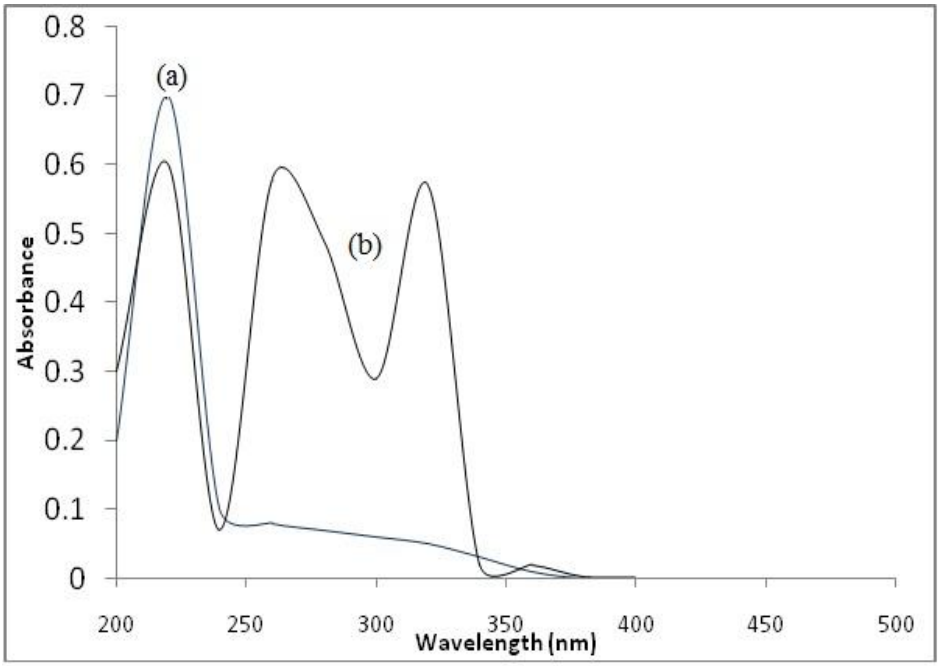


Fig. 2. Typical overlaid UV-spectra of (a) ARTS (10 µg/mL) and (b) MEFQ (10 µg/mL) in methanol

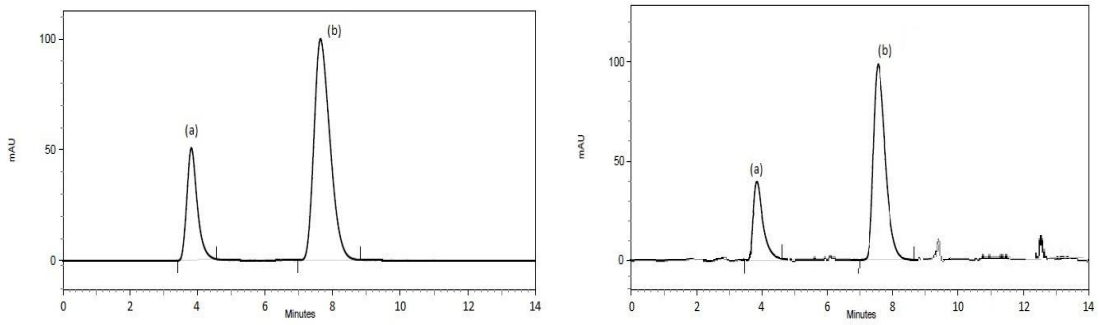


Fig. 3. Chromatogram obtained from (a) MEFQ (Rt 3.40) and (b) ARTS (Rt 7.30) for standard and sample

Table 1. Data for calibration graphs and system suitability (n = 3)

Parameter	RP-LC		HPTLC	
	MEFQ	ARTS	MEFQ	ARTS
Linearity range	12.5-75 µg/mL	2.5-15 µg/mL	125-1000 ng/band	25-200 ng/band
Regression equation	y=99.68x+2251	y=493x+1651	y=26.63x+1357	y=75.84x+1201
Slope (SD)	1.3870	1.4410	157.27	117.65
Intercept (SD)	0.8560	1.0230	9.73	8.51
Correlation coefficient (r)	0.9999	0.9992	0.9998	0.9994
LOD	3.57 µg/mL	1.39 µg/mL	14.93 ng/band	5.67 ng/band
LOQ	8.69 µg/mL	2.27 µg/mL	85.73 ng/band	14.79 ng/band
System suitability				
Asymmetry	1.01	0.92		
No. of theoretical plates	3217	6984		
Resolution	5.321	-		
Tailing factor	0.21	0.18		

MEFQ: mefloquine, ARTS: artesunate, SD: standard deviation, LOD: limit of detection, LOQ: limit of quantification

Table 2. Intraday and inter-day precision and accuracy by HPLC method

Added conc. (µg/mL)	Precision studies		Accuracy studies	
	Measured conc. (µg/mL) Mean ± SD; % RSD		Theoretical conc.(µg/mL)	Measured conc. (µg/mL) ± SD; % RSD (n = 6)
	Intraday (n = 6)	Interday (n = 6)		
MEFQ				
12	11.37±0.143, 1.25	11.91±0.034, 0.29	18.5	18.39±0.214, 1.16
36	35.47±0.386, 1.09	35.83±0.201, 0.56	50.0	49.48±0.354, 0.71
72	70.71±1.147, 1.62	70.36±1.001, 1.42	72.5	72.41±0.614, 0.85
ARTS				
3	2.82±0.020, 0.71	2.89±0.054, 1.86	4.0	3.93±0.024, 0.61
9	8.75±0.026, 0.28	8.75±0.037, 0.42	7.2	7.18±0.063, 0.88
15	14.46±0.104, 0.72	14.55±0.128, 0.88	12.5	12.26±0.184, 1.50

MEFQ: mefloquine, ARTS: artesunate

Typical chromatograms obtained following the assay of stressed samples of MEFQ and ARTS show significant degradation in acid (R_t 5.28, 9.35 and 11.05), alkali hydrolysis (R_t 1.01 and 10.06) and in oxidation (R_t 2.01, 9.58 and 10.45). Thermal (R_t 5.08 and 9.40) and photolytic stress (5.25 and 9.40) conditions show insignificant degradation for MEFQ and ARTS. From the peak purity profile studies, it was confirmed that peak of the degradation product was not interfering with the response of drugs (Table 5). It confirms that, degradation products can be separated from the drugs by this method (Fig. 4).

Table 3. Results of robustness study by HPLC

Parameter	% RSD	
	MEFQ	ARTS
Change in pH of mobile phase		
pH 4.0	0.81	0.45
pH 3.8	0.87	0.52
pH 4.2	0.91	0.43
Change in temperature		
20ºC	0.88	0.87
25ºC	1.04	0.72
30ºC	1.20	0.65
Change in flow rate		
0.8 mL/min	0.91	0.75
1.0 mL/min	0.96	0.59
1.2 mL/min	0.87	0.88
Change in wavelength		
314	0.67	-
312	0.52	-
219	-	0.44
221	-	0.59

MEFQ: mefloquine, ARTS: artesunate

3.2 High Performance Thin Layer Chromatography

Preliminary experiments were carried out to optimize parameters affecting simultaneous estimation of both the drug using HPTLC. The solvent type, solvent ratio and detection wavelength, were varied to determine the chromatographic conditions giving best separation. Mobile phase consisting of toluene-ethyl acetate-acetone (2.5:1.0:0.5, v/v/v) was found to give best sensitivity, efficiency and peak shape. The UV-spectra of both analytes were determined independently and in combination. It was observed that ARTS cannot be detected by UV-densitometric method because of weak UV absorption. Therefore after development of both components in the single plate, MEFQ was detected at 285 nm and ARTS was detected at 525 nm after derivatization with anisaldehyde-sulphuric acid reagent. Under the optimum conditions, the retention factors obtained for MEFQ and ARTS was 0.26±0.02 and 0.59±0.01 respectively (Fig. 5). It can also assumed from peak purity spectra (Fig. 6) that the method is specific for these components.

The calibration plots were linear in the concentration range between 125–1000 ng/band for MEFQ and 25–200 ng/band for ARTS, respectively. The LOD and LOQ obtained by this method were 14.93 and 85.73 ng/band for MEFQ and 5.67 and 14.79 ng/band for ARTS, respectively. Table 1 shows linearity parameters of calibration curve. The % RSD values were 0.76 to 1.38 and 0.67 to 1.41 for intra- and interday precision, respectively (Table 6). Recovery study performed at three different concentrations in triplicate shows good recoveries; 98.08 to 98.80 for MEFQ and 97.36 to 98.16 for ARTS, respectively (Table 6). Results of robustness study are depicted in Table 7. The retention factor (0.26±0.02 and 0.59±0.01) and assay (%) were not significantly affected (Fig. 7). RSD (%) value in all robustness parameter was found to be < 2%. The validated HPTLC method was applied for simultaneous determination of MEFQ and ARTS in commercial tablets. The results are depicted in Table 4 indicate that each drug in tablet corresponds to requirements of label claim. The low RSD value (< 2%) confirmed the suitability of method for routine analysis of MEFQ and ARTS in pharmaceutical dosage form.

Table 4. Results of analysis of tablets

Method	RP-LC		HPTLC	
	MEFQ	ARTS	MEFQ	ARTS
Label claim (mg/tablet)	250	50	250	50
% Mean (n = 5)	100.43	99.58	99.70	100.80
Standard deviation	1.231	0.410	1.641	0.851
Standard error	0.550	0.183	0.733	0.381
RSD (%)	1.23	0.41	1.65	0.84

MEFQ: mefloquine, ARTS: artesunate

Table 5. Stability study by HPLC method

Degradation condition	R_t	Area of degradation products (%)
HPLC		
Acid	5.28, 9.35, 11.05	13.20, 9.51, 9.20
Base	1.01, 10.06	10.37, 8.46

Degradation condition	R_t	Area of degradation products (%)
Oxidative	2.01, 9.58, 10.45	7.05, 9.48, 3.54
Heat	5.08, 9.40	8.25, 9.13
Photo	5.25, 9.40	11.80, 14.52

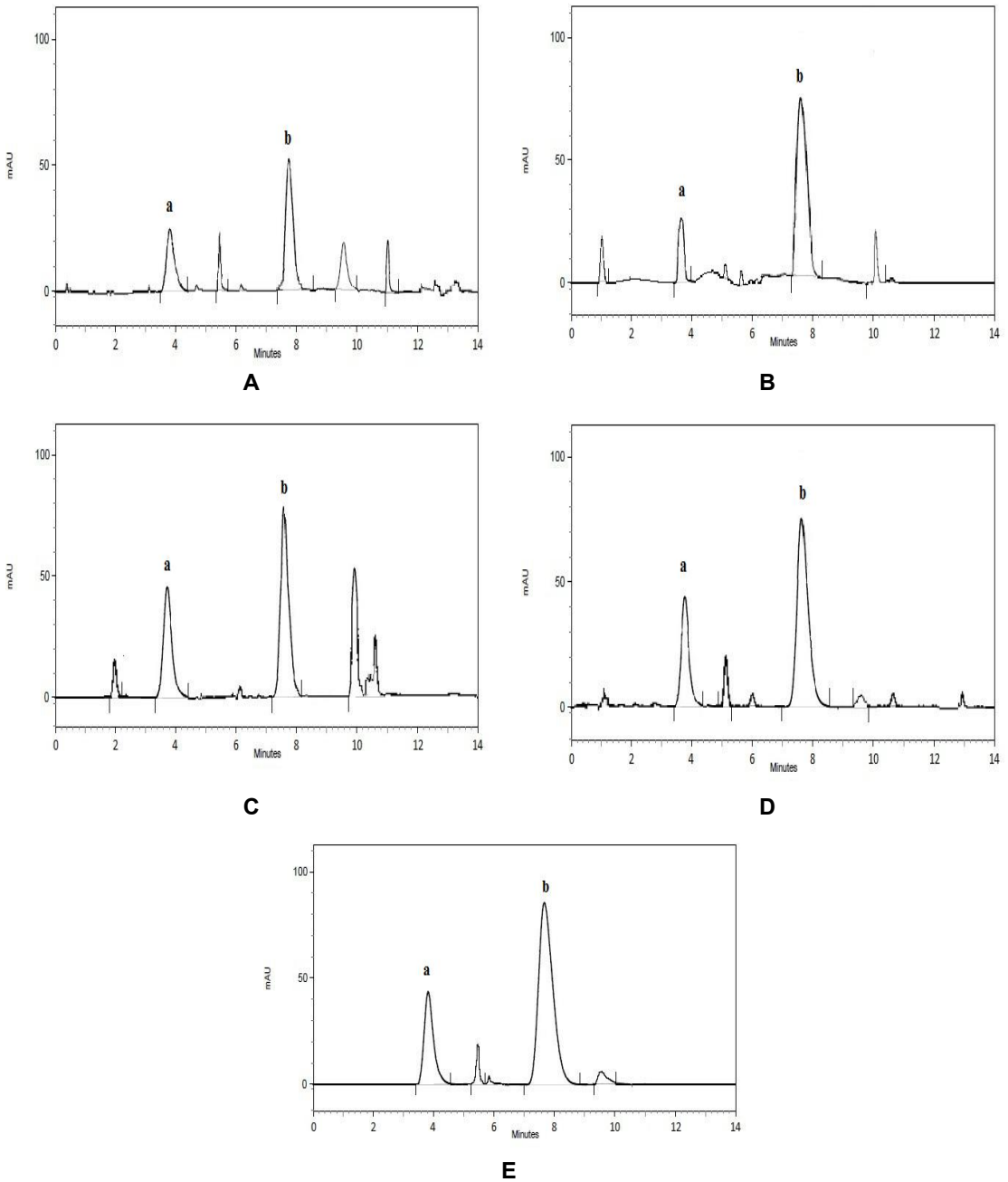


Fig. 4. HPLC chromatograms obtained for (a) ARTS and (b) MEFQ from degradation study: showing acid hydrolysis (0.02 M HCl, 60ºC, 1 hr) (A); alkaline hydrolysis (0.02 M NaOH, 60ºC, 1 hr) (B); oxidative degradation (3% H_2O_2, 60ºC, 1 hr) (C); dry heat degradation (60ºC, 1 hr) (D) and photolytic degradation (UV-chamber, 254 nm, 24 hrs) (E)

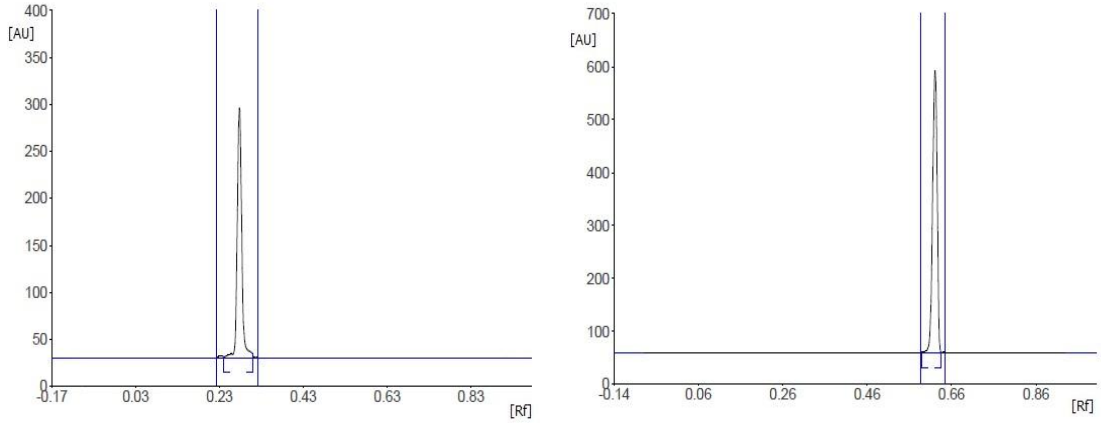
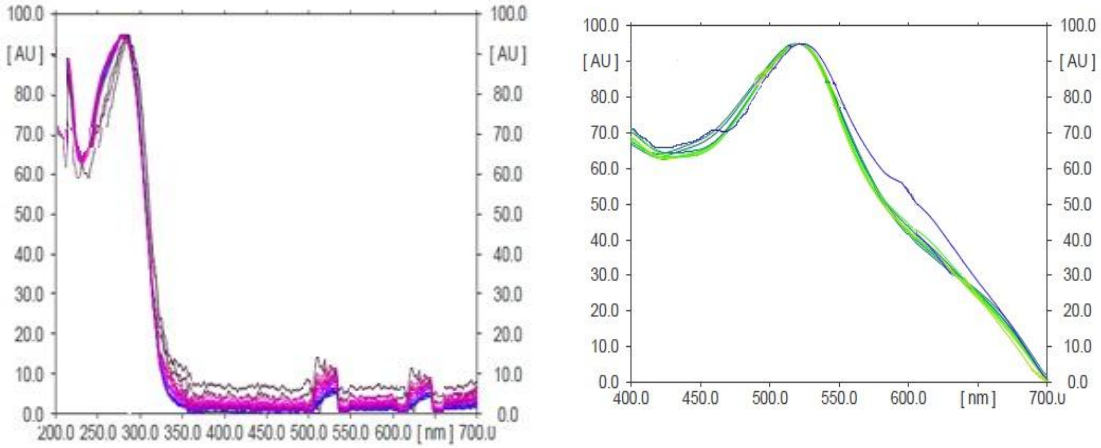


Fig. 5. Densitogram obtained from MEFQ (R_f 0.26) and ARTS (R_f 0.59)



(a) **(b)**

Fig. 6. Peak purity spectra of (a) mefloquine and (b) artesunate

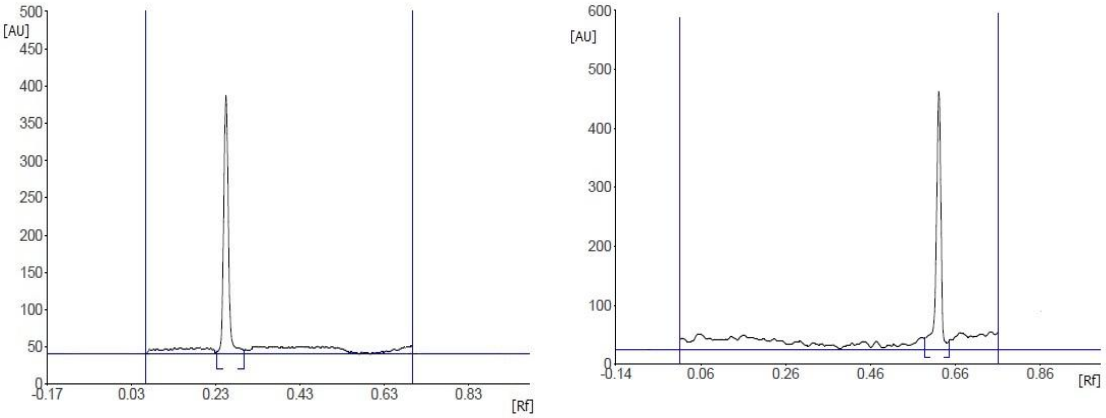


Fig. 7. Densitogram obtained from sample MEFQ (R_f 0.26) and ARTS (R_f 0.59)

Table 6. Intraday and interday precision and accuracy by HPTLC method

Added conc. (ng/band)	Precision studies		Accuracy studies	
	Measured conc. (ng/band) Mean ± SD; % RSD		Added conc. (ng/band)	% Recovery ± SD; % RSD (n = 6)
	Intraday (n = 6)	Inter-day (n = 6)		
MEFQ				
125	121.21±1.21; 1.00	121.51±1.43; 1.19	62.5	98.08±3.21; 1.75
625	622.10±5.71; 0.92	621.85±6.95; 1.12	125.0	98.52±4.07; 1.65
1000	978.25±7.39; 0.76	973.95±6.56; 0.67	187.5	98.80±5.86; 1.90
ARTS				
25	24.91±0.21; 0.84	24.89±0.35; 1.41	12.5	97.36±0.43; 1.18
125	123.40±1.10; 0.89	121.11±1.35; 1.11	25.0	97.50±0.59; 1.21
200	195.44±2.70; 1.38	197.86±2.21; 1.11	37.5	98.16±0.75; 1.22

MEFQ: mefloquine, ARTS: artesunate

Table 7. Results of robustness study by HPTLC

Condition		Retention factor (R_f)		Assay (%)		% RSD	
		MEFQ	ARTS	MEFQ	ARTS	MEFQ	ARTS
Mobile phase composition (v/v)							
Toluene-ethyl acetate-acetone	(2.6:1.1:0.5)	0.27	0.58	100.71	100.51	1.32	1.71
	(2.5:0.9:0.4)	0.26	0.58	97.32	99.32	0.86	1.42
	(2.4:1.0:0.6)	0.27	0.57	98.53	98.71	0.93	1.31
Development distance (cm)	6	0.28	0.59	97.32	99.86	1.97	1.32
	9	0.29	0.61	98.32	98.52	0.51	0.89
Time of spotting to chromatogram (min)	9	0.26	0.60	97.66	97.31	1.65	1.33
	11	0.25	0.57	98.71	99.33	1.62	1.10
Detection wavelength (nm)	280 & 520	0.26	0.58	99.53	100.61	1.97	0.52
	290 & 530	0.27	0.59	100.11	97.52	1.33	0.72

MEFQ: mefloquine, ARTS: artesunate

4. CONCLUSION

The proposed methods were found to be sensitive, reproducible, and accurate for analysis of MEFQ and ARTS in tablet dosage form. HPLC method envisages stability behavior of MEFQ and ARTS individually and in combination as per ICH guidelines. The HPTLC method has several advantages such as simultaneous analysis on the same plate, short system equilibrium time, multiple and or repeated scanning of chromatograms, large sample capacity, short run time, minimum solution composition and no need of prior solvent filtration or degassing. Therefore, the proposed methods could be used as stability indicating liquid chromatographic and HPTLC by derivatization for simultaneous determination of mefloquine hydrochloride and artesunate in bulk drug and in pharmaceutical formulations.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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