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Original Article

A New HPTLC Method for Analysis of Artemisinin Derivatives (Artemether and Lumefantrine) in Bulk Drug and Liposomal Formulation: Stress Degradation Study

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ABSTRACT

Purpose: A stability indicating high performance thin layer chromatography (HPTLC) method has been established and validated for analysis of two anti-malarial drugs, ART and LUM in bulk drug as well as nanoliposomal formulation. **Materials and Methods:** Study was performed on pre-coated silica gel HPTLC plates using toluene:ethyl acetate:amonia (2:6.5:0.5 v/v/v) as the mobile phase. Densitometric analysis was carried out in the reflectance mode at 269nm for LUM and 519nm for ART. The method is specific for analyte constituents examined and characterized by high sensitivity. **Results:** The correlation coefficients of calibration curves were found to be 0.997 and 0.998 in the concentration range of 20-120 and 100-300 ng spot-1 for ART and LUM, respectively. The method had an accuracy of 100.5 % for ART and 100.4% for LUM. **Conclusion:** The method had the potential to determine these drugs simultaneously from bulk drug as well as nanoliposomal formulation without any interference of the excipients. As the method could effectively separate the drug from its degradation products, it can be employed as a stability indicating one. Moreover, the proposed HPTLC method was utilized to investigate the kinetics of acid and base degradation process. Arrhenius plot was constructed and activation energy was calculated.

Key words: HPTLC, Artemether, Lumefantrine, Nanoliposome, Validation, Degradation kinetics.

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INTRODUCTION

Combination therapy of artemether (ART) and lumefantrine (LUM) is well-established for the treatment of uncomplicated malaria to obtain a synergistic effect and to reduce adverse effects and are the first fixed-dose artemisinin-based combination therapy currently available.[1] Combination therapy of ART with LUM decreases the probability of development of resistance to ART; therefore, it is vital to supervise the therapeutic efficacy of the combination and thus offer advance caution in case of slight changes in efficacy.[2]

ART is a semisynthetic polyoxygenated amorphene which is chemically [(3R, 5aS, 6R, 8aS, 9R, 10S, 12R, 12aR)-decahydro-10-methoxy-3,6,9-trimethyl-3,12-epoxy-12H-pyrano(4,3-j)-1,2-benzodioxepin] (Figure 1a), hold a peroxide bridge that confers potent antimalarial activity. It is powerful and fast acting blood schizonticide, highly efficacious in treating chloroquine-resistant falciparum malaria along with complicated falciparum malaria and cerebral malaria. Chemical activation of drug within the food vacuole of the intraerythrocytic stage of the parasite is endorsed for its antimalarial activity. It is anticipated that reductive cleavage of peroxide bridge by heme liberated for the period of digestion of hemoglobin generates free radicals, which provoke oxidative stress, alkylate heme and vital parasite proteins.[3,4]

On the other hand, LUM is a synthetic aryl-amino alcohol which is chemically (z)-2,7–dichloro–9–((4-chlorophenyl)methylene)–alpha–((dibutylamino)methyl)-9h–fluorene–4–methanol (Figure 1b) and is active against erythrocytic stages of Plasmodium falciparum. It is a blood schizonticide, inhibits the formation of b-hematin by forming a complex with haeme and, as a result, inhibits the synthesis of nucleic acid and protein.

Several methods including liquid chromatography for the determination of LUM alone in biological fluids (serum/plasma) have been reported.[5-10] ART alone has also been analyzed by HPLC-UV detection,[11-13] HPLC with electrochemical detection[14-16] and HPTLC methods.[17-19] ART has also been analyzed by gas chromatography mass spectrometry and capillary electrophoresis technique in pharmaceutical formulation and plasma[20,21] Only a few HPLC methods have been reported for analysis of LUM and ART in combination using UV detection[22] and mass spectrometry.[23,24] Many of these methods need special equipment and are expensive and slow. HPTLC is becoming a routine analytical technique due to its advantages of low operating cost, high sample throughput and need for minimum sample clean up. The major advantage of HPTLC is that several samples can be run simultaneously using a small quantity of mobile phase unlike HPTLC, thus lowering the analysis time and cost per analysis. In a combination containing LUM and ART, LUM shows UV absorbance and ART shows weak UV absorbance due to the lack of chromophore. ART shows considerable UV absorbance only below 220nm. Therefore, it is poorly detected by a standard spectrophotometric method. Hence, it became an enormous challenge for the simultaneous determination of LUM and ART by HPTLC using densitometric determination. To overcome this problem, after chromatographic development of both the drug in the same plate, LUM was detected at 269nm and ART at 519nm after derivatization with an anisaldehyde sulfuric acid reagent, thus enabling simultaneous determination of both compounds in combined form.

The aim of the present work is to develop an accurate, specific, repeatable and stability indicating HPTLC method for simultaneous determination of ART and LUM in the presence of its degradation products. Acid-induced degradation kinetics was investigated by quantitation of drug by validated stability-indicating HPTLC method.

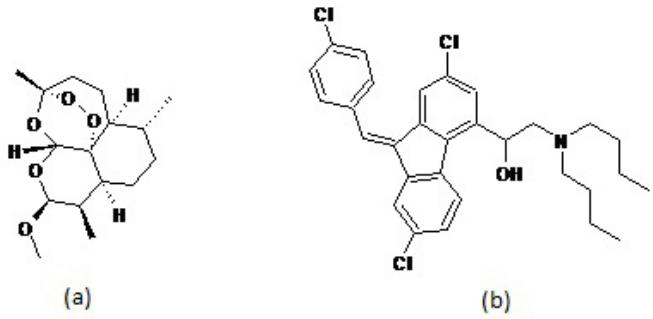

Figure 1: Structure of (a) artemether and (b) lumefantrine.

MATERIALS AND METHODS

ART and LUM were procured as a gift sample from IPCA Laboratories Ltd. (Mumbai, India). All the solvents used were of chromatographic grade, other chemicals were of analytical reagent (AR) grade and purchased from Merck Chemicals, India. Aluminium backed silica gel $60F_{254}$ plates (20×10 cm: 200 µm thickness) were purchased from E. Merck (Darmstadt, Germany).

Instrumentation and chromatographic conditions

The samples were spotted to the plates, 10 mm from the bottom and 10 mm from the side edges in the form of bands or streaks with band length of 6 mm with a Camag microlitre syringe on precoated silica gel aluminium plate $60F_{254}$, (20×10 cm: with 250 mm thickness; E. Merck, Germany) using a Linomat V (CAMAG, Muttenz, Switzerland). The mobile phase consisting of toluene:ethyl acetate:amonia (2:6.5:0.5 v/v/v) was used in each chromatographic run. Linear ascending development technique was carried out in a 20×10 cm twin trough chambers. The optimized chamber saturation time for the mobile phase was 20 min at room temperature (25±2°C) and relative humidity of 60±5%. The development includes chromatogram run of 8 cm, 20 ml of mobile phase and time duration of 10 min. The slit dimension 3×0.45 mm and scanning speed 20 mm/sec was chosen as optimized equipment parameters. A constant application or spraying rate of 160 nL/sec and scanning speed 20 mm/sec were employed. Subsequent to the development, TLC plates were dried in a current of air with the help of air dryer. Initially, for detection of LUM, densitometric scanning was carried out using TLC scanner in the absorbance/reflectance mode at 269 nm. Subsequent to this scanning, TLC plates were derivatized with anisaldehyde–sulfuric acid reagent for 4 sec and heated for 3 min at 110°C. Densitometric scanning was performed in absorbance/reflectance mode at 519 nm. Thus, both LUM and ART can be developed and scanned in a single plate prior to and after derivatization with the anisaldehyde sulfuric acid reagent. Densitometric scanning was performed on CAMAG TLC scanner III, using tungsten lamp as a radiation source and operated by winCATS software (Version 1.2.0).

Preparation of standard solutions and calibration standard

Standard stock solutions were prepared separately by dissolving accurately weighed 10 mg of both ART and LUM in 100 mL of methanol to obtain a concentration of 1000 µg/mL. The standard stock solutions were suitably diluted with methanol to obtain combination solution containing ART (20-120 ng/ml) and LUM (100-600 ng/ml). 10 µL of each solution were spotted as bands in the plate to furnish a concentration of 20-120 ng/spot^{-1} for ART and (100-600 ng spot^{-1}) for LUM, respectively. QC samples as low, medium and high at concentration level of 20, 60 and 120 ng spot^{-1} were taken for ART and 100, 300 and 600 ng spot^{-1} were considered for LUM to carry out validation of the method.

Method validation

The method was validated in compliance with ICH guidelines for linearity range, precision, accuracy, recovery, limits of detection (LOD) and quantification (LOQ).

Linearity

Linear relationship between peak area and concentration of the drugs was evaluated over the concentration range expressed in ng spot^{-1} by making five replicate measurements in the concentrations range of 60–120 ng spot^{-1} for ART and 100–600 ng spot^{-1} for LUM, respectively.

Precision and accuracy

The precision and accuracy of the method was validated for intraday precision at intervals of 3 hr and inter-day precision on 3 consecutive days. Precision was measured at three different samples at concentrations of low, medium and high QC levels of LUM and ART by spotting the samples in triplicate. Precision was expressed as the coefficient of variation (CV, %) of measured concentrations for each calibration level whereas accuracy was expressed as percentage recovery [(Drug found/drug applied) × 100].

Recovery studies

Recovery studies were carried out by spiking three different known amounts of the standard substances to the drug product (standard addition method). Hence, 30, 60 and 90 ng spot^{-1} of ART and 150, 300 and 450 ng spot^{-1} of LUM were spiked to the sample that contained 60 and 300 ng spot^{-1} of ART and LUM, respectively.

Robustness of the method

The effect of deliberate variations in parameters like mobile phase composition, volume of the mobile phase, saturation time of TLC plate and detection wavelength were evaluated in this study. The effect of these changes on R_f values, assay percentage and RSD percentage were evaluated. Mobile phases prepared from Toluene/Ethyl acetate/Ammonia in different proportions (1.75:7:0.5 and 2.5:6:0.5, v/v/v) were used for chromatography. Mobile phase volume and duration of saturation investigated were 12 ± 2 ml (10, 12 and 14 ml) and 20 ± 10 min (10, 20 and 30 min), respectively.

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

The limits of detection and quantification of the developed method were calculated from the standard deviation of the y-intercepts and slope of the calibration curves of ART and LUM using the formulae as given below.

Limits of Detection = 3σ/S

Limits of Quantification = 10 σ/S

where σ is the standard deviation of the y-intercepts and S is the slope of the calibration curve.

Analysis of ART and LUM in developed nanoliposome

To determine the content of ART and LUM in nanoliposomes prepared in our laboratory. An accurately weighed quantity of formulation equivalent to 20 mg ART and 120 mg LUM was extracted with 100

ml methanol by sonication for 20 min and volume was made up to 100 ml. The resulting solution was centrifuged at 5000 rpm for 10 min and supernatant was analyzed for drug content. One microlitre of the filtered solution was applied to a TLC plate followed by development and scanning as described earlier. The analysis was repeated in triplicate. The possibility of excipient interference in the analysis was studied.

Stability studies

To evaluate the stability indicating properties of the developed HPTLC method, forced degradation studies were carried out in accordance to the ICH guidelines. The standard drugs were subjected to acid, base, oxidation, wet heat, dry heat and photo-degradation studies.

Acid- and base-induced degradation study

To 5 mL methanolic stock solutions of ART and LUM, 5 ml each of HCl and NaOH (0.001 M) were added separately in 25 mL volumetric flasks. The mixtures were refluxed at 60°C for 1 h. The forced degradation was performed in the dark to exclude the possible degradation effect of light. The resulting solutions (20 ng $sopt^{-1}$ and 100 ng $sopt^{-1}$ for ART and for LUM, respectively) were applied to TLC plates and the chromatograms were run as described above.

Hydrogen peroxide-induced (oxidation) degradation study

5 mL H_2O_2 (50% w/v) was added separately to 5 mL methanolic stock solutions of ART and LUM in 25 mL volumetric flasks. The solution was heated in boiling water bath for 10 min to remove completely the excess of hydrogen peroxide and then refluxed at 60°C for 1 h. The resulting solutions (20 ng $sopt^{-1}$ and 100 ng $sopt^{-1}$ for ART and for LUM, respectively) were applied to TLC plates and the chromatograms were run as described above.

Wet heat and dry heat degradation study

For wet heat degradation study, 5 mL stock solutions of each drug were transferred to 25 mL volumetric flasks separately. To each 5 mL methanol was added and the samples were refluxed for 8 h on boiling water bath. For dry heat degradation study, the standard powder drugs were placed in an oven at 100°C for 24 h. Appropriate dilutions were prepared in methanol and then analyzed under the optimized chromatographic conditions.

Photo-degradation study

For the photo-degradation study, the standard powder drugs were exposed to UV light in a photo-stability chamber for 24 h. Appropriate dilutions were prepared in methanol and then analyzed under the optimized chromatographic conditions.

Study of acid-induced degradation kinetics

Accurately weighed 50 mg of each drug was dissolved in 100 ml methanol. 15 ml of this standard solution were transferred into 100 ml of double-neck round-bottom flask. To it 15.0 ml of 1 N HCl was added to get final concentration of 250 µg/ml and refluxed at different temperatures (40, 50, 60, 70 and 80°C). At specified time intervals the contents of the flask (100 µl) were quantitatively transferred to 10 ml volumetric flasks with the help of microsyringe. Then 1 µl were spotted to TLC plate and estimated by developed method by one-point standardization using external standard. The experiment was carried out in triplicate. The concentration of the remaining drug was calculated for each temperature and time interval. Data were further processed and degradation kinetics constants were calculated.

RESULTS AND DISCUSSION

HPTLC method optimization

The TLC procedure was optimized with a view to develop a stability indicating assay method to quantify ART and LUM simultaneously. Different solvent systems were tried for the separation of pure drug as well as degraded products on the TLC plates. For the selection of appropriate mobile phase for the effective separation of both the drug, several runs were made by using mobile phases containing solvents of varying polarity, at different concentration levels. Different mobile phase systems like toluene:ethyl acetate, chloroform:ethyl acetate, chloroform:ethyl acetate:acetic acid, toluene:ethyl acetate: ammonia at different concentration levels were tried. Among the different mobile phase combinations employed, the mobile phase consisting of toluene:ethyl acetate:ammonia in the ratio of 2:6.5:0.5 v/v/v gave the best resolution with sharp well defined peaks with R_f values of 0.70±0.02 and 0.52±0.02 for ART and LUM, respectively (Figure 2). Resolution between spots of standard and depredates appeared better when TLC plates were saturated with conc. ammonia vapors for 20 min in TLC chamber prior to application. It was required to eliminate the edge effect and to avoid unequal solvent evaporation losses from the developing plate that can lead to various types of random behavior usually resulting in generally lack of reproducibility in R_f values.

Method validation

Linearity

Peak areas were found to have better linear relationship with the concentration than the peak heights. For ART, the regression coefficient (r^2) was found to be 0.997 and for LUM the r^2 was 0.998. Calibration graphs were constructed in the concentration range of 20–120 ng $spot^{-1}$ for ART and 100–600 ng $spot^{-1}$ for LUM, respectively. The correlation coefficients, y-intercepts and slopes of the regression lines of the two drugs were calculated and are presented in Table 1. No significant difference was observed in the slopes of standard plots (ANOVA, $P > 0.05$).

Precision and accuracy

Intra-day and inter-day precision as coefficient of variation (%CV) and accuracy of the assay as percentage recovery for ART and LUM at three QC levels are presented in Table 2. Intra-day precisions ($n = 6$) for ART and LUM were ≤0.99% and ≤0.84%, however the inter-day precisions were ≤1.12% and ≤0.62%, respectively, which demonstrated the good

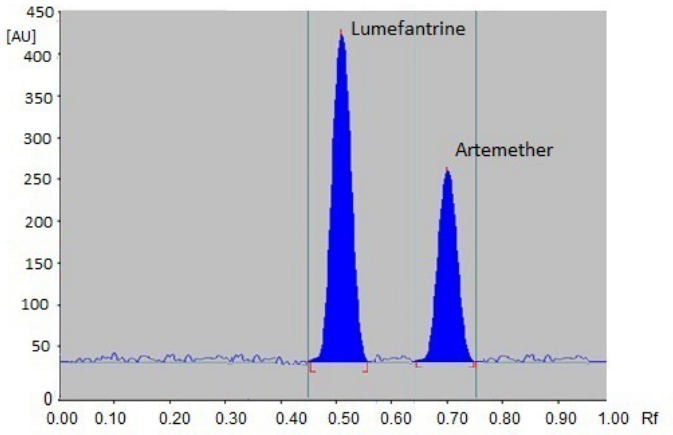

Figure 2: Chromatogram of standard ART (20 ng spot-1), Peak 1: R_f: 0.70± 0.02 and LUM (100 ng $spot^{-1}$), Peak 2: R_f: 0.52± 0.02, mobile phase- 2:6.5:0.5 v/v/v.

Table 1: Summary of linear regression data.

Parameters	ART	LUM
Linearity range	20-120	100-600
Linear regression equation	y=118.4x + 52.03	y=176.7x + 2183
Slope ± SD	118.4±0.252	176.7 ± 0.426
Intercept ± SD	52.03 ± 0.725	2183 ± 1.236
Correlation coefficient (r^2)	0.997	0.998
Limit of detection (LOD)	1.73	5.19
Limit of quantification (LOQ)	5.24	15.72

Table 2: Precision and accuracy of the proposed method (*n*=3).

Drug	Nominal Conc. (ng spot^{-1})	Conc. found (ng spot^{-1}) ±SD	Precision (% CV)	Accuracy (%)	Conc. found (ng spot^{-1}) ±SD	Precision (% CV)	Accuracy (%)
		Intra-day batch			Inter-day batch		
ART	20	19.84 ± 0.16	0.81	99.2	20.04 ± 0.13	0.65	100.2
	60	59.6 ± 0.43	0.72	99.3	59.48 ± 0.22	0.34	99.1
	120	119.75 ± 1.18	0.99	99.8	119.18 ± 1.34	1.12	99.3
LUM	100	99.63 ± 0.84	0.84	99.6	99.83 ± 0.54	0.54	99.8
	300	299.75 ± 2.12	0.71	99.9	299.17 ± 1.86	0.62	99.7
	600	598.94 ± 2.78	0.46	99.8	599.62 ± 2.75	0.46	99.9

precision of proposed method. Intra-day accuracy for ART and LUM were 99.2-99.8% and 99.6-99.8%, however inter-day accuracy for ART and LUM were 99.1-100.2% and 99.7-99.9%, respectively. These values are within the acceptable range, so the method was accurate, reliable and reproducible.

Recovery studies

The recovery studies were carried out at 50%, 100% and 150% of the test concentration as per ICH guidelines. The percentage recovery of ART and LUM at all the three levels was found to be satisfactory (Table 3). For ART, the % recovery was found between 98.4% and 101.7% and for LUM between 99.8% and 100.3%, respectively.

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

The limits of detection and quantitation were found to be 1.73 and 5.19 ng spot^{-1} for ART and 5.24 and 15.72 ng spot^{-1} for LUM, respectively, indicating the sensitivity of the developed method.

Robustness of the method

To determine the robustness of the method, the experimental conditions were deliberately altered and the retention factor (R_f), assay percentage and RSD (%) were evaluated. Conditions altered were the mobile phase composition, mobile phase volume, duration of saturation and detection wavelength. Assay of ART and LUM for all deliberate changes of conditions was within 97.4–100.4%. The low values of %RSD (less than 2%) indicated robustness of the method. The summary of results is shown in Table 4.

Table 3: Recovery of the proposed method (*n*=3).

Drug	Recovery level (%)	Initial content (ng spot^{-1})	Amount added (ng spot^{-1})	Amount detected (ng)	Recovery (%)	RSD %
ART	50	60	30	29.53 ± 0.44	98.4	1.49
	100		60	61.02 ± 0.78	101.7	1.27
	150		90	89.53 ± 0.44	99.4	0.49
LUM	50	300	150	149.62 ± 1.23	99.8	0.82
	100		300	299.17 ± 2.56	99.7	0.85
	150		450	451.39 ± 2.92	100.3	0.65

Table 4: Robustness of the method (*n*=3).

Parameters	Retention factor (R_f)		Assay (%)		RSD (%)	
	ART	LUM	ART	LUM	ART	LUM
Mobile phase composition (v/v/v)						
1.75:7:0.5	0.72	0.51	99.5	98.6	0.67	0.72
2:6.5:0.5	0.70	0.52	100.3	99.8	0.47	0.73
2.5:6:0.5	0.71	0.51	98.6	100.1	0.72	0.37
Mobile phase volume (ml)						
10	0.71	0.50	100.3	97.4	0.29	0.83
12	0.70	0.52	99.8	98.5	0.49	0.56
14	0.72	0.51	100.3	99.4	0.62	0.48
Saturation time (min)						
10	0.72	0.51	100.4	98.5	1.24	0.95
20	0.70	0.52	99.4	99.7	0.73	0.48
30	0.71	0.52	99.2	98.6	0.73	0.62
Detection wavelength (nm)						
267/517	0.70	0.51	99.8	97.6	0.82	0.97
269/519	0.70	0.52	99.9	100	0.72	0.39
271/521	0.71	0.51	98.4	99.6	0.29	0.69

Analysis of ART and LUM in developed nanoliposome

Two spot at R_f 0.70 and 0.52 corresponds to ART and LUM were observed in the chromatogram extracted from nanoliposomal formulation. No interference from the excipients present in the formulation was observed. The drug content was found to be 99.8±0.52% with a %RSD and SEM of 0.56 and 0.42, respectively. It may be, therefore, concluded that degradation of ART and LUM have not occurred in the prepared formulation and there was no interaction with the excipients. The low %RSD and SEM further indicates the suitability of the method for routine analysis of the drug in novel pharmaceutical formulations.

Stability studies

The results of the forced degradation study of ART and LUM using toluene:ethyl acetate:ammonia in the ratio of 2:6.5:0.5 v/v/v as the mobile phase system are summarized in Table 5.

Table 5: Stability studies for the developed method.

Degradation condition	No. of degradation products	R_f value
Acid	4	0.17, 0.34, 0.42, 0.83
Base	3	0.20, 0.23, 0.84
Oxidative	4	0.15.0.28, 0.35, 0.63
Wet Heat	3	0.13, 0.21, 0.90
Dry Heat	2	0.18, 0.87
Photo	3	0.25, 0.43, 0.85

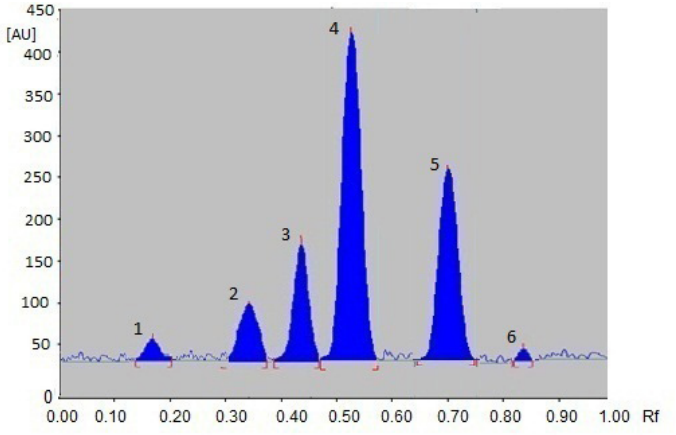

Figure 3: Chromatogram of acid treated ART and LUM (peak 1: degraded, R_f: 0.17; peak 2: degraded, R_f: 0.34; peak 3: degraded, R_f: 0.42; peak 4: LUM, R_f: 0.70; peak 5: ART, R_f: 0.52; peak 6: degraded, R_f: 0.83).

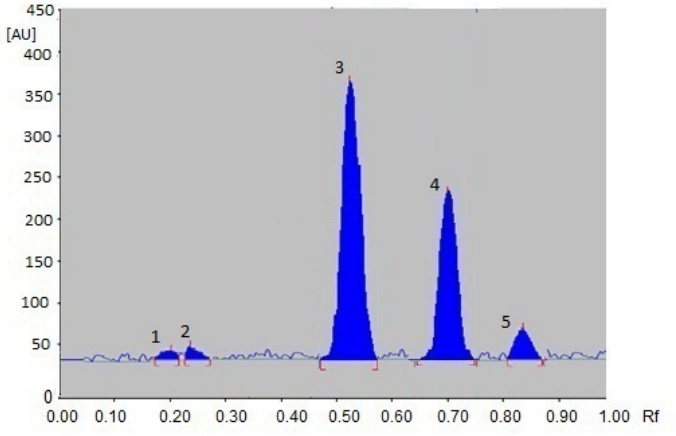

Figure 4: Chromatogram of base treated ART and LUM (peak 1: degraded, R_f: 0.20; peak 2: degraded, R_f: 0.23; peak 3: LUM, R_f: 0.70; peak 4: ART, R_f: 0.52; peak 5: 0.84).

Acid- and base-induced degradation study

Acid induced degradation study ART and LUM, both were found to undergo acid degradation very rapidly. The reaction in HCl showed extensive degradation for LUM with additional peaks at R_f values of 0.17, 0.34, 0.42. For ART additional peaks were observed with R_f values 0.83 (Figure 3). In base induced degradation study, LUM and ART showed additional peaks at R_f values 0.20, 0.23 and 0.84, respectively (Figure 4).

Hydrogen peroxide-induced (oxidation) degradation study

In the oxidative degradation study, it was found that both ART and LUM were extremely liable to degradation. LUM exhibited degradation peaks at R_f values 0.15. 0.28, 0.35 and for ART at R_f values 0.63. The densitogram for the oxidative degradation study is shown in (Figure 5).

Wet heat and dry heat degradation study

The wet degradation studies suggested that both LUM and ART were labile to wet degradation and showed additional peaks at R_f values of 0.13, 0.21 and 0.90, respectively (Figure 6). In the dry heat degradation study, LUM and ART showed additional peaks at R_f value at 0.18 and 0.87, respectively (Figure 7).

Photo-degradation study

LUM and ART both showed additional peaks at R_f value 0.25 0.43 and 0.05, 0.85, respectively, in the photo-degradation study (Figure 8).

Degradation kinetics

The kinetic of degradation of ART and LUM was investigated in conc. HCl. Each experiment was repeated three times at each temperature and

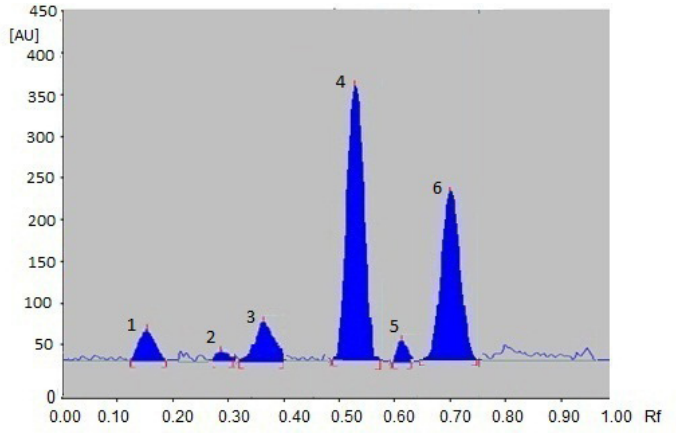

Figure 5: Chromatogram of peroxide treated ART and LUM (peak 1: degraded, R_f: 0.15; peak 2: degraded, R_f: 0.28; peak 3: degraded, R_f: 0.35; peak 4: LUM, R_f: 0.70; peak 5: degraded, R_f: 0.83; peak 6: ART, R_f: 0.52).

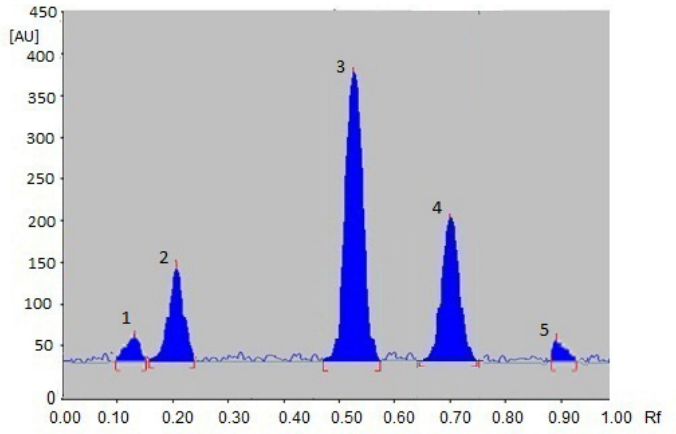

Figure 6: Chromatogram of wet heat degraded ART and LUM (peak 1: degraded, R_f: 0.13; peak 2: degraded, R_f: 0.21; peak 3: LUM, R_f: 0.70; peak 4: ART, R_f: 0.52; peak 5: degraded, R_f: 0.90).

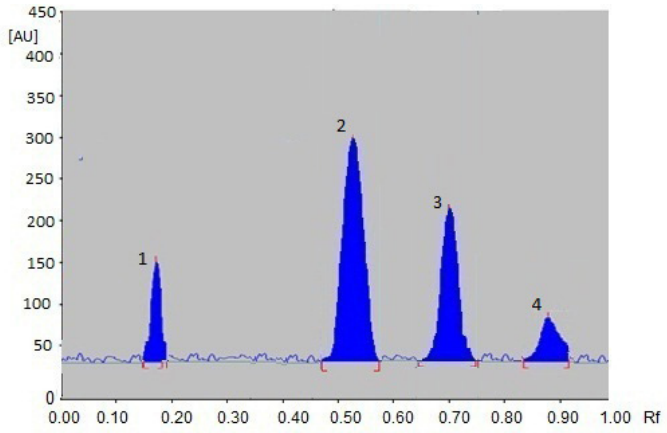

Figure 7: Chromatogram of dry heat degraded ART and LUM (peak 1: degraded, R_f: 0.18; peak 2: LUM, R_f: 0.70; peak 3: ART, R_f: 0.52; peak 4: degraded, R_f: 0.87).

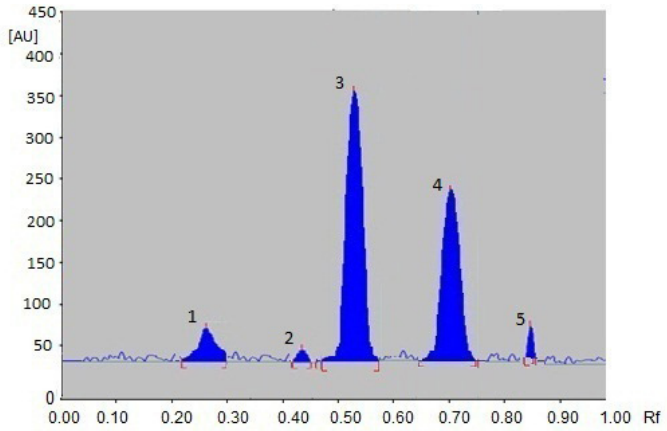

Figure 8: Chromatogram of photo degraded ART and LUM (peak 1: degraded, R_f: 0.25; peak 1: degraded, R_f: 0.43; peak 3: LUM, R_f: 0.70; peak 4: ART, R_f: 0.52; peak 5: degraded, R_f: 0.85).

time interval. The mean concentration of ART and LUM was calculated for each experiment. A decrease in concentration with increasing time was observed.

At the selected temperatures (40, 50, 60, 70 and 80°C for acidic degradation), the degradation process for ART and LUM followed pseudo first-order kinetic (Figure 9 and 10). Apparent first-order degradation rate constant, half-life ($t_{1/2}$) and t_{90} (i.e. time where 90% of original concentration of the drug is left) were obtained from the slopes of the straight lines at each temperature (Table 6). Data obtained from first-order kinetics treatment were further subjected to fitting in Arrhenius equation:

$$\log K = \log A - Ea/2.303\ RT$$

Where K is rate constant, A is frequency factor, E_a is energy of activation (Cal mol^{-1}), R is gas constant (1.987 cal deg^{-1} mol^{-1}) and T is absolute temperature (^oK). A plot of (2 + log K_{obs}) values versus ($1/T \times 10^3$) the Arrhenius plot was obtained (Figure 11), which was found to be linear in the temperature range of 40-80°C.

CONCLUSION

The developed HPTLC technique was precise, specific, accurate and stability-indicating. Statistical analysis proves that the method is suitable

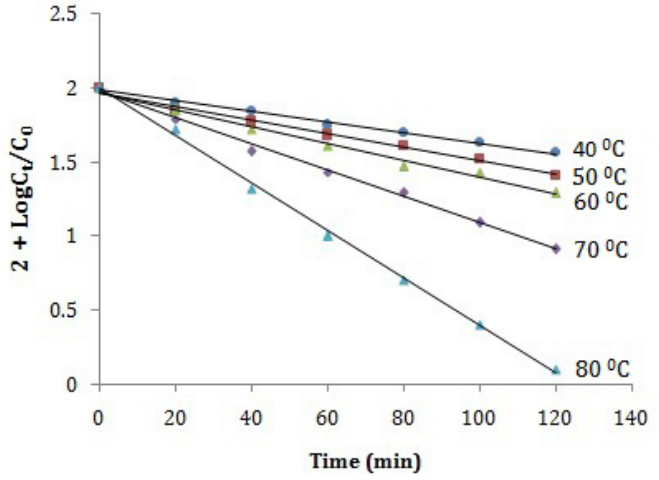

Figure 9: Pseudo first-order plots for the degradation of ART with conc. HCl at various temperatures; C_t = Concentration at time t; C_0 = Concentration at time zero.

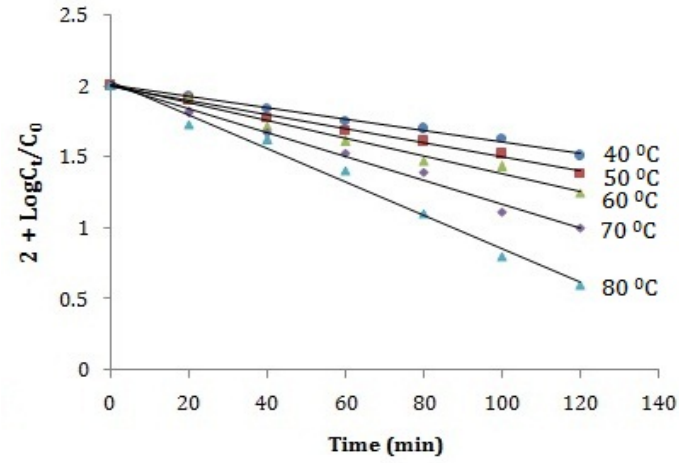

Figure 10: Pseudo first-order plots for the degradation of LUM with conc. HCl at various temperatures; C_t = Concentration at time t; C_0 = Concentration at time zero.

Table 6: Details of degradation kinetic data at 25°C.

Parameters	ART	LUM
k_{25} (h^{-1})[a]	0.342	0.489
$t_{1/2}$ (h)[b]	2.02	1.42
t_{90} (h)[c]	0.31	0.21

[a] = Degradation rate constant; [b] = Half-life; [c] = Shelf Life (Time left for 90% potency)

for the analysis of simultaneous estimation of ART and LUM. This study is a typical example of development of a stability-indicating assay, established following the recommendations of ICH guidelines. It is one of the rare studies where forced decomposition was done under all different suggested conditions and the degradation products were resolved. It is proposed for the analysis of the drug and degradation products in stability samples in industry. Further, it can be concluded that the impurity present in the drug could be due to hydrolysis or oxidation during processing

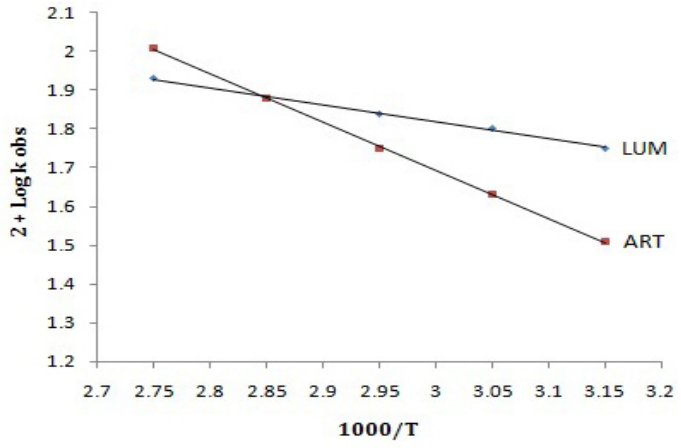

Figure 11: Arrhenius plot for the degradation of ART and LUM in presence of Conc. HCl.

and storage of the said drug. The above results showed the suitability of proposed method for acid-induced degradation kinetic study of ART and LUM. The degradation rate constant (K25°), half-life ($t_{1/2}$) and shelf life (t_{90}) of ART and LUM can be predicted for acid degradation process. It may be extended for quantitative estimation of said drug in plasma and other biological fluids. The method, however, is not suggested to establish material balance between the extent of drug decomposed and formation of degradation products. As the method separates the drug from its degradation products, it can be employed as a stability-indicating one.

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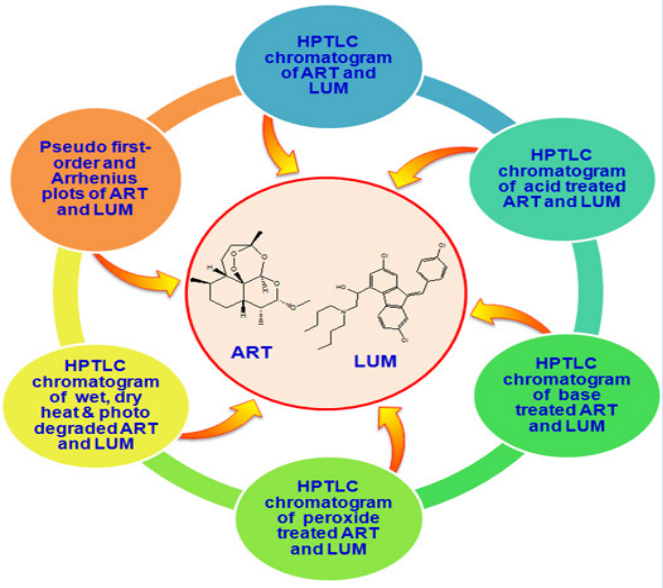
CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Shakeel K, Raisuddin S, Ali S, Imam SS, Rahman MA, Jain GK, *et al.* Development and *in-vitro*/*in-vivo* Evaluation of Artemether and Lumefantrine Co-loaded Nanoliposomes for Parenteral Delivery. J Liposome Res. 2017;29(1):35-43.
2. Price R, Uhlemann AC, Vught MV, Brockman AL, Hutagalung R, *et al.* Molecular and Pharmacological Determinants of Therapeutic Response to Artemether Lumefantrine in Multidrug Resistant Falciparum Malaria. Clin Infect Dis. 2006;42(11):1570-7.
3. Meshnick SR, Taylor TE, Kamchongwongpaisan S. Artemisinin and Antimalarial Endoperoxides: From Herbal Remedy to Targeted Chemotherapy. Microbiol Rev. 1996;60(2):301-5.
4. Bryant BJ, Knights KM, Salerno E. Pharmacology for Health Professionals; Mosby Elsevier: Australia. 2011.
5. Annerberg A, Singtoroj T, Tipmanee P, White NJ, Day NPJ, Lindegardh N. High Throughput Assay for Determination of Lumefantrine in Plasma. J Chromatogr B. 2005;822(1-2):330-3.
6. Lindegardh N, Annerberg A, Blessborn D, Bergqvist Y, Day NPJ, White NJ. Development and Validation of Bioanalytical Method using Automated Solid Phase Extraction and LC-UV for Simultaneous Determination of Lumefantrine and Its Desbutyl Metabolite in Plasma. J Pharm Biomed Anal. 2005;37(5):1081-7.
7. Blessborn D, Romsingh S, Annerberg A, Sundquist D, Bjorkman A, Lindegardh N, *et al.* Development and Validation of Automated Solid Phase Extraction and Liquid Chromatography Method for Determination of Lumefantrine in Capillary Blood on Sampling Paper. J Pharm Biomed Anal. 2007;45(2):282-7.
8. Ntale M, Ogwal-Okeng JW, Mahindi M, Gustafsson LL, Beck OA. Field Adapted Sampling and HPLC Quantification Method for Lumefantrine and Its Desbutyl Metabolite in Whole Blood Spotted on Filter Paper. J Chromatogr B. 2008;876(2):261-5.
9. Kiran RP, Vipul PR, Jayaprakash NS, Devanand BS. A Stability Indicating LC Method for Lumefantrine. Chromatographia. 2009;69:375-9.
10. Insaf FK, Ulla A, Leneh HA, Deogratius M, Michael A, Lotte H. Measurement of Lumefantrine and Its Metabolite in Plasma by HPLC with UV Detection. J Pharm Biomed Anal. 2011;54(1):168-72.
11. Thomas CG, Ward SA, Edwards G. Selective Determination in Plasma of Artemether and Dihydroartemisinin by High Performance Liquid Chromatography with UV Detection. J Chromatogr B. 1992;583(1):131-6.
12. Muhia DK, Mberu EK, Watkins WM. Differential Extraction for Artemether and Dihydroartemisininfrom Plasma and Determination by High Performance Liquid Chromatography. J Chromatogr B. 1994;660(1):196-9.
13. Chimanuka B, Grabriels M, Detaevernier MR, Plaizer-Vercammen JA. Preparation of b Artemether Liposome, Their HPLC-UV Evaluation and Relevance for Clearing Recrudescent Parasitemia in Malaria Infected Mice. J Pharm Biomed Anal. 2002;28(1):13-22.
14. Navratnam V, Mansor LK, Chin AM, Nair NK. Determination of Artemether and Dihydroartemisinin in Blood Plasma by HPLC for Application in Pharmacological Studies. J Chromatogr B. 1995;669(2):289-94.
15. Karbwang J, Na-Bangchang K, Molunto P, Banmairuroi V, Congpuong K. Determination of Artemether and Its Major Metabolite Dihydroartemisinin in Plasma using HPLC with Electrochemical Detection. J Chromatogr B. 1997;690(1):259-65.
16. Sandrenan N, Sioufi A, Godbillon J, Netter C, Donkar M, Vanvalkenburg C. Determination of Artemether and its Metabolite Dihydroartemisinin in Plasma using HPLC and Electrochemical Detection in Reductive Mode. J Chromatogr B. 1997;691(1):145-53.
17. Gabriels M, Plaizer-Vercammen JA. Densitometric TLC Determination of Artemisinin and Derivative Artemether and Artether. J Chromatogr Sci. 2003;41(7):359-66.
18. Gabriels M, Plaizer-Vercammen JA. Development of Reversed Phase TLC Method for Artemisinin and Its Derivatives. J Chromatogr Sci. 2004;42(7):341-7.
19. Nitin GT, Mangal SN. Validated HPTLC Method of Analysis for Artemether and its Formulation. J Pharm Biomed Anal. 2007;43(3):839-44.
20. Mohammed SS, Khalid SA, Ward SA, Wan TSM, Tang HPO, Zheng M, *et al.* Simultaneous Determination of Artemether and Its Major Metabolite Dihydroartemisinin by Gas Chromatographic-Mass Spectrometry-Selected Ion Monitoring. J Chromatogr B. 1999;731(2):251-60.
21. Avery BA, Venkatesh KK, Avery MA. Rapid determination of artemisinin and related analogues using high-performance liquid chromatography and an evaporative light scattering detector. J Chromatogr B Biomed Sci Appl. 1999;730(1):71-80.
22. DaCosta CI, Nogueira FH, Pianetti GA. Simultaneous Determination of Artemether and Lumefantrine in Fixed Dose Combination Tablet by HPLC with UV Detection. J Pharm Biomed Anal. 2008;48(3):951-4.
23. Hodel EM, Zanolari B, Mercier T, Biollaz J, Keiser J, Olliaro P, *et al.* A Single LC-Tandem Mass Spectrometry Method for the Simultaneous Determination of 14 Antimalarial Drugs and Their Metabolite in Human Plasma. J Chromatogr B. 2009;877(10):867-86.
24. Isabela C, Jose A, DeLeonardo S, Karini T, Femanda BBC, Josianny MM, *et al.* Liquid Chromatography-Tandem Mass Spectrometry for the Simultaneous Quantitation of Artemether and Lumefantrine in Human Plasma: Application for a Pharmacokinetic Study. J Pharm Biomed Anal. 2011;54(1):114-20.

PICTORIAL ABSTRACT



SUMMARY

The present research work described about the development of stability indicating high performance thin layer chromatography (HPTLC) method for analysis of two anti-malarial drugs (artemether and lumefantrine) in bulk as well as liposomal formulation. Study was performed on pre-coated silica gel HPTLC plates using toluene:ethyl acetate:amonia as the mobile phase. Densitometric analysis was carried out in the reflectance mode at 269nm for LUM and 519nm for ART. The proposed method was utilized to investigate the kinetics of acid and base degradation process. Arrhenius plot was constructed and activation energy was calculated. As the method separates the drug from its degradation products, it can be employed as a stability-indicating one. The present method can be suitably applied for the routine analysis of both artemether and lumefantrine in both bulk drug and in liposomal formulation.

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