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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF ARTEMETHER AND LUMEFANTRINE IN BULK AND PHARMACEUTICAL DOSAGE FORM

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Keywords:

Artemether, Lumefantrine, RP-HPLC, Simultaneous determination, Analytical Method development

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ABSTRACT: A simple, sensitive, specific, precise, and accurate stability-indicating reverse phase liquid chromatographic method was developed to simultaneously determine Artemether and Lumefantrine in bulk drugs and pharmaceutical formulations. The chromatographic analysis was performed by Microsorb MV100-5 C-18 column (250 x 4.6mm, 5 µm), with a mobile phase consisting of buffer and acetonitrile in the ratio of 65:35v/v, orthophosphoric acid used as a buffer (pH 3.5), at a flow rate of 1 ml/min and eluents monitored at 254nm. The method was validated for linearity, accuracy, precision, robustness, and application for assay as per ICH guidelines. The retention times of Artemether and Lumefantrine were 2.67 and 4.62 min, respectively. The calibration curves of peak area versus concentration, which were linear from 530µg/ml for Artemether and 60-210µg/ml for Lumefantrine had a regression coefficient (r^2) greater than 0.999 & precise (R.S.D. < 2.0%). The method had the requisite accuracy, precision, and robustness for simultaneous determination of Artemether and Lumefantrine in tablets.

INTRODUCTION: Malaria is the world's most important parasitic infection, ranking among the major health and developmental challenges for the world's poor countries [1]. One of the greatest challenges facing malaria control worldwide is spreading and intensifying parasite resistance to antimalarial drugs. Artemisinin-based combination therapy (ACT) is increasingly being advocated as a promising treatment.

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ACT is based on using two drugs with different modes of action: an artemisinin-derivative that causes rapid and effective reduction of parasite biomass and gametocyte carriage and a partner drug that has a longer duration of action [2]. Artemether (ART) is chemically, (3R, 5aS, -6R, 8aS, 9R, 10S, 12R, 12aR) Decahydro-10-methoxy-3, 6, 9-tri-methyl-3, 12epoxy-12H-pyrano[4,3-j]-1,2 benzodiazepine and is used as antimalarial agent.

Lumefantrine (LUM) is chemical, 2, 7-Dichloro-9-[(4chlorophenyl) methylene]-α-[(dibutyl amino) methyl]-9H-fluorene-4-methanol and is used in the treatment of uncomplicated falciparum malaria. ART-LUM is an ACT widely used nowadays and consists of a registered fixed-dose combination of

ART (20 mg) and LUM (120 mg) in tablets [3]. ART has a rapid onset of action and is rapidly eliminated from the plasma (half-life of two to three hours [4]. LUM is cleared more slowly and has a longer elimination half-life (approximately 4.5 days) [5]. The rationale behind this combination is that ART initially provides rapid symptomatic relief by reducing the number of parasites present before LUM eliminates any residual parasites. ART-LUM also reduces gametocyte carriage and thus should have an impact on malaria transmission [6].

The increasing use of ART-LUM association as an effective treatment for resistant malaria demands the need of analytical methods to simultaneously quantify these drugs in tablets in order to evaluate its quality. Some papers have described the analysis of ART in plasma based on HPLC with electrochemical [7–9] or mass spectrometry detection [10]. Few methods are available to assay ART in pharmaceutical products [11, 12].

The quantitative determination of LUM in plasma has been described using HPLC with UV detection [13, 15]. Due to the low molar absorptivity of ART in the UV region (210 nm) and the lower concentration of this drug in the tablets compared to LUM, standard addition of ART was carried out to improve its detection, which gives the need for the simultaneous quantitation of ART and LUM. The validated method was applied to the analysis of fixed-dose combination tablets containing 40mg ART and 240 mg of LUM. The proposed method is the objective of the present investigation was to develop & validate simple, accurate, reproducible, stability-indicating, and suitable for routine determination of ART and LUM in tablet dosage form with the RP-HPLC method.

EXPERIMENTAL REQUIREMENTS:

MATERIALS:

Instrument used:

Instrument	Specifications
HPLC System	Youngling (S.K) gradient system
Pump:	SP930D Gradient solvent delivery
Detector:	system
Data processor:	UV Detector
Column:	Autochro-3000 database software
	Phenomenex Gemini C_{18} (5 µm, 250 mm X 4.6 mm i.d.)
Weighing Balance:	Shimadzu Aux - 120
Digital pH Meter:	Systronicsµ pH System 362
Ultrasonicator:	ENERTECH Electronics Pvt. Ltd.

Reagent and Chemicals: Reference standards of Artemether (ART) was obtained as a gift sample from Dr. Reddy's Laboratories, Hyderabad, India, while Lumefantrine (LMF) was obtained as generous gift from Micro Labs Ltd., Bangalore, India. The pharmaceutical formulation was purchased from the local market (Brand: LUMERAX-20DT labelled claim Artemether 80mg and Lumefantrine 480mg make Ipca Laboratories Pvt. Ltd.). The HPLC grade solvents used were procured from E-Merck (India) Ltd., Mumbai. HPLC grade Acetonitrile, Phosphate buffers, and other chemicals (Merck, Mumbai, India) were used in the analysis. HPLC grade water was prepared using Millipore purification system.

Selection of Chromatographic Parameters:

Selection of Chromatographic Mode: The reverse-phase HPLC was selected for separation because it is more convenient and rugged than other forms of liquid chromatography and is more likely to result in a satisfactory final separation.

Selection of Stationary Phase: Based on reversed-phase HPLC mode and a number of carbon present in molecule (analyte) stationary phase with C_{18} bonded phase *i.e.*, RP Phenomenex - Gemini C_{18} (250 mm x 4.6 mm I.D.) with particle size 5 µm was selected.

Selection of Mobile Phase: The selection was made on the basis of a literature survey. After assessing the solubility of drug in different solvents as well on the basis of the literature survey, various combinations of mobile phases were screened with respect to resolution, theoretical plate capacity factors, and other system suitability parameters. Finally, the separation was performed with freshly prepared mobile phase consist of Acetonitrile: 50 mM potassium dihydrogen phosphate (pH-2.5) in the ration of 60:40 and pH up to 2.5 with isocratic programming at a flow rate of 1.0 ml/min. with injection volume of 20 µL and ambient temperature was maintained during the entire process to obtain symmetric peaks of ART and LMF.

Selection of Detector and Detection Wavelength: Ultraviolet (UV) detector was selected as it is reliable and easy to set at the correct wavelength. From the spectra of drug 215 nm, wavelength was selected as the detection wavelength.

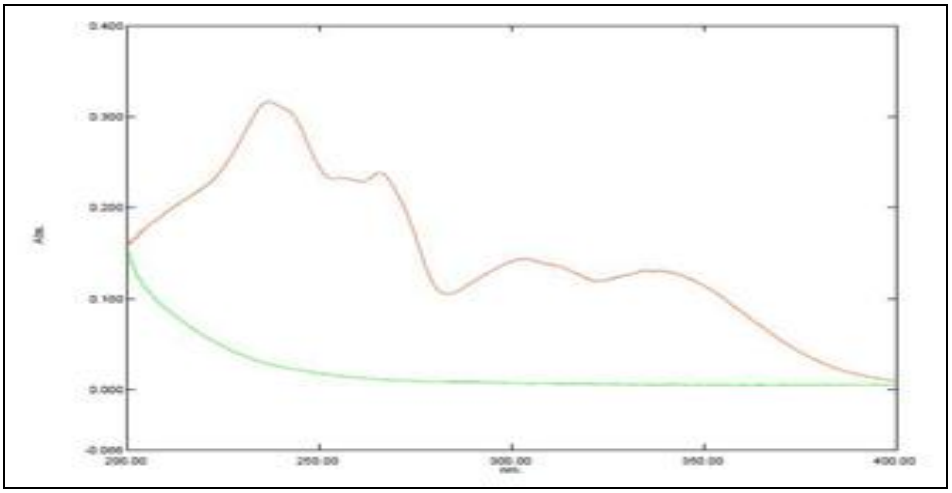

TABLE 1: FINAL CHROMATOGRAPHIC CONDITIONS

Chromatographic mode	Chromatographic condition
Standard solution	400 µg/ml for ART and 2400 µg/ml for LMF.
HPLC System	Youngling (S.K) gradient system
Pump	SP930D Gradient solvent delivery system
Detector	UV detector
Data processor	Autochro-3000 database software
Stationary phase	Phenomenex Gemini C_{18} (5 µm, 250 mm X 4.6 mm i.d.)
Mobile phase	Acetonitrile: 50 mM potassium dihydrogen phosphate (pH-2.5) in the ration of 60:40 with isocratic programming
Detection wavelength	215 nm
Flow rate	1 ml/min.
Sample size	20 µl
Column temperature	25 °C

Preparation of Standard Stock Solution: All solutions were prepared on a weight basis. Solution concentrations were also measured on weight basis to avoid using an internal standard pharmaceutical formulation available in the market with a proportion of 1:6.

The standard stock solution was prepared by dissolving separately 10 mg of ART and 60 mg of LMF in 25 ml clean, dry volumetric flask. Dissolved and diluted with methanol up to the mark and filtered through 0.45 µm membrane filter. This gives the concentration of stock solution 400 µg/ml for ART and 2400 µg/ml for LMF.

Optimization of Chromatographic Parameters: Optimization in HPLC is the process of finding a set of conditions that adequately separate and enable the quantification of the analytes from the endogenous material with acceptable accuracy, precision, sensitivity, specificity, cost, ease, and speed.

Optimization of Mobile Phase Strength: The mobile phase was chosen after several trials with methanol and phosphate buffer in various proportions. A mobile phase consisting of methanol: phosphate buffer (70:30, v/v) was selected to achieve symmetrical peak and sensitivity.

The effects of flow rates in the ranges of 0.5 to 1.5 ml/min were examined at variable pH ranging from 2.5 to 8. A 1 ml/min flow rate gave reasonable retention time; using reverse phase C18 column, the retention times were 8.15 and 10.23 min for ART and LMF, respectively. The total time of analysis was less than 12 min.

TABLE 2: OPTIMIZATION OF MOBILE PHASE STRENGTH

S. no.	Mobile phase strength [Acetonitrile: KH_2PO_4 v/v]	R_t of ART [min]	R_t of LMF [min]
1	100:0	7.20	8.53
2	80:20	8.10	9.90
3	60:40	8.10	10.23
4	40:60	7.15	7.83

Optimization of Detection Wavelength: UV detector was selected as it is reliable and easy to set at the correct wavelength. Fixed concentrations of analyte were analyzed at different wavelengths. As per the response of the analyte, 215 nm was selected.

Linearity Studies: From the prepared standard stock solutions of both, 0.05 ml, 0.1, 0.15, 0.2, 0.25 and 0.30 ml were transferred to 10 ml volumetric flask and volume made up to the mark with the optimized mobile phase to obtain a concentration of 2-10 µg/ml for ART, while 12-60 µg/ml for LMF respectively. A volume of 20 µL of each sample was injected with the help of a Hamilton Syringe. All measurements were repeated five times for each concentration, and a calibration curve was constructed by plotting the peak area vs. the drug concentration. The observations are shown in **Tables 4** and **5,** while the calibration curve is shown in **Fig. 1** and **2**.

TABLE 4: LINEARITY STUDY OF ART

S. no.	Concentration of ART [µg/ml]	Mean peak area [n=5]	± SD	%RSD
1	2	1262.40	24.99	1.98
2	4	2275.80	28.97	1.27
3	6	3241.60	32.39	1.00
4	8	4237.80	29.22	0.69
5	10	5117.40	32.52	0.64

TABLE 5: LINEARITY STUDY OF LMF

S. no.	Concentration of LMF [µg/ml]	Mean peak area [n=5]	± SD	%RSD
1	12	1838.40	41.82	2.27
2	24	2845.60	43.71	1.54
3	36	3841.60	34.70	0.90
4	48	4810.80	72.49	1.51
5	60	5862.80	84.32	1.44

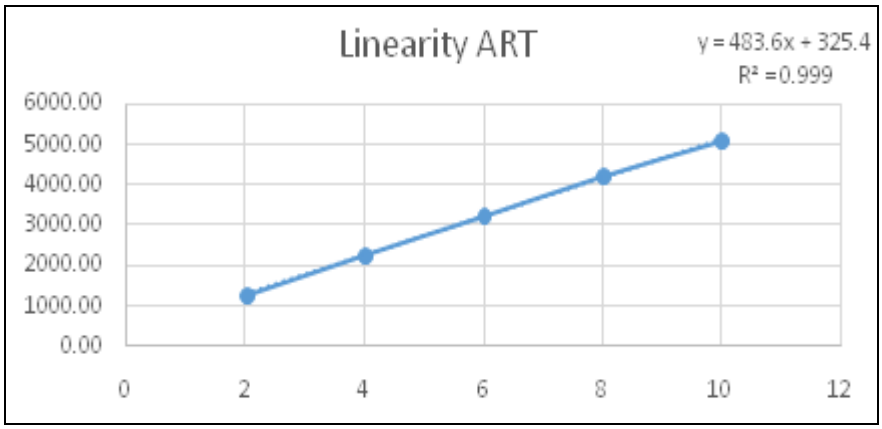

FIG. 1: LINEARITY OF ART. y = 483.6x + 325.4 Correlation coefficient = 0.9994, Slope = 483.6, Intercept =325.4

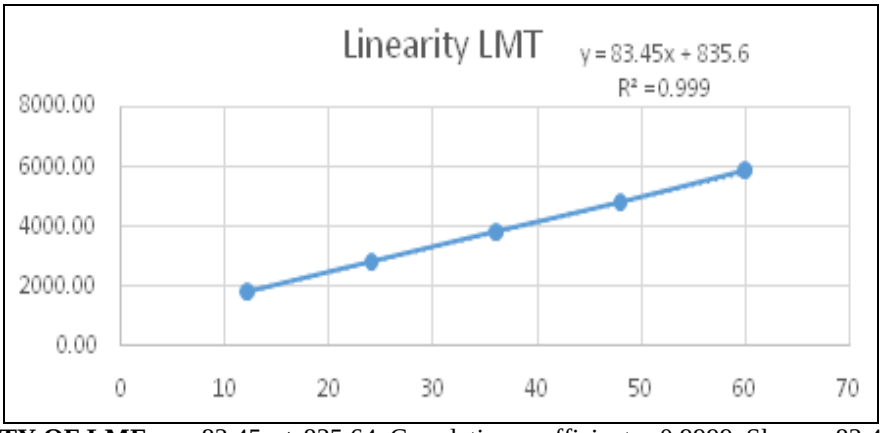

FIG. 2: LINEARITY OF LMF. y = 83.45x + 835.64. Correlation coefficient = 0.9999, Slope = 83.45, Intercept = 835.6

Application of the Proposed Method to Bulk Sample: Accurately weighed quantities of 10 mg ART and 60 mg LMF were transferred to 250 ml volumetric flask separately. These were dissolved in methanol and volume was adjusted up to mark. The solution was further diluted to get a concentration 6 µg/ml of ART and 36 Accurately weighed quantity of 10 mg ART and 60 mg LMF were transferred to 250 ml volumetric flask separately. These were dissolved in methanol, and volume was adjusted up to mark. The solution was further diluted to get a concentration 6 µg/ml of ART and 36 µg/ml of LMF, and these were subjected to the proposed method, and the amount of ART and LMF were determined. The procedure was repeated six times; results are shown in **Table 6** for ART and **Table 7** for LMF. Chromatogram of laboratory mixture showed in **Fig. 3**.

TABLE 6: ANALYSIS OF BULK SAMPLE ART

Component	Amount taken ART [µg/ml]	Amount found ART [µg/ml]	Amount found ART [%]
MET	6	76.14	101.52
	6	74.93	99.91
	6	75.78	101.03
	6	76.38	101.84
	6	74.57	99.43
	6	75.65	100.87
	Mean ± SD	75.57 ± 0.65	100.77 ± 0.93
	% RSD	0.84	0.92

TABLE 7: ANALYSIS OF BULK SAMPLE LMF

Component	Amount taken LMF [µg/ml]	Amount found LMF [µg/ml]	Amount found LMF [%]
LMF	15	14.87	99.11
	15	15.34	102.29
	15	15.42	102.78
	15	15.27	101.80
	15	14.83	98.87
	15	15.42	102.78
	Mean ± SD	15.19 ± 1.80	101.27 ± 1.80
	% RSD	1.71	1.78

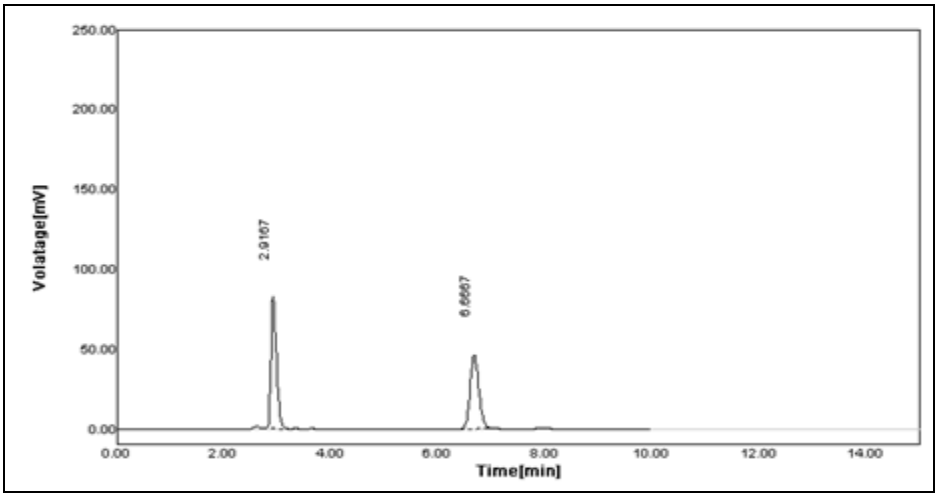

FIG. 3: CHROMATOGRAM OF ART AND LMF

Application of Proposed Method to Tablet Formulations: To determine the contents of drugs in conventional tablets (Brand: tenglyn-M tablet labelled claim Artemether 500 mg and Lumefantrine 20 mg per tablet make Zydus). Twenty tablets were weighed, their mean weight determined, and they were finely powdered. Powder equivalent to 500 mg ART was transferred into a 100 ml volumetric flask containing 50 ml methanol. In that solution standard, Lumefantrine was added to have the concentration proportion 1:5. The resulting solution was sonicated for 30 min and diluted to 100 ml with methanol. The solution was filtered using 0.45 µm filter (Milli filter, Milford,

MA). Excipients were separated by filtration. The solution was further diluted with an optimized mobile phase to get concentration75 µg/ml of ART and 15 µg/ml of LMF which were subjected to the proposed method and the amount of ART and LMF were determined. The assay procedure was repeated six times; results are shown in Table 8 and the chromatogram of tablet solution in **Fig. 4**.

TABLE 8: ANALYSIS OF TABLET FORMULATION

Drugs	Label claim [mg]	Amount found [mg]	Amount found [%]
ART	500	501.23	100.646
	500	497.17	99.434
	500	499.87	99.974
	500	501.34	100.268
	500	497.67	99.534
	500	498.78	99.756
	Mean ± SD	499.343 ± 1.76	99.86 ± 0.34
	%RSD	0.344	0.345
LMF	20	19.72	98.6
	20	20.24	101.2
	20	20.17	100.85
	20	19.83	99.15
	20	20.15	100.75
	20	20.25	101.25
	Mean ± SD	20.06 ± 0.22	100.30 ± 1.134
	%RSD	1.13	1.25

Brand name: LUMERAX-20DT
Batch no: DJ1505

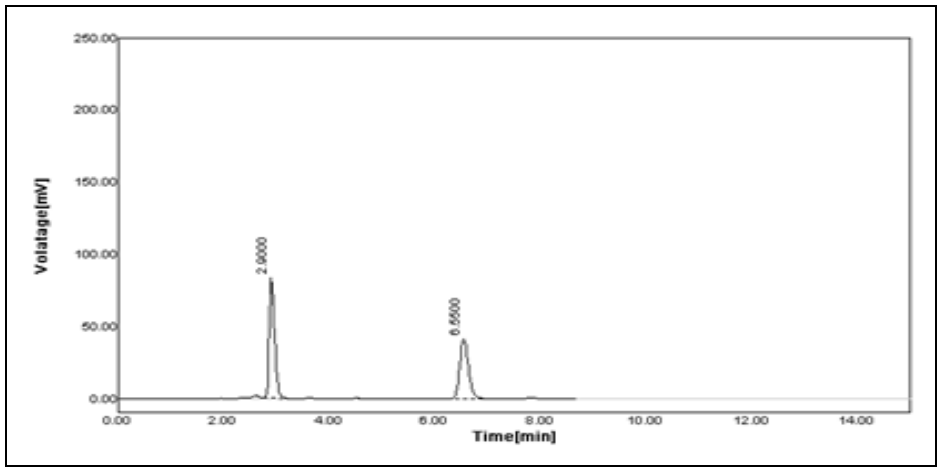

FIG. 4: CHROMATOGRAM OF TABLET SOLUTION

RESULT AND DISCUSSION: The chromatographic parameters were initially evaluated using a Microsorb-MV 100-5 C-18 column (250 x 4.6mm, 5 µm). Under these conditions, the retention factors obtained for ART and LUM were 2.67 and 4.62, respectively.

The mobile phase composed of acetonitrile and 0.01M potassium dihydrogen orthophosphate buffer (65:35) promoted an adequate separation, and a short run time (10min), and so, this condition was adopted in subsequent analysis ART shows UV absorption only in the initial wavelengths of the spectrum (200–220 nm), due to the absence of chromophores in its structure. Nevertheless, the ART absorptivity is considerably low in this region, resulting in HPLC-UV methods with poor sensitivity. Hence, an RP HPLC method was developed by means of ART standard addition to the sample solutions. This approach allowed an adequate ART detection and consequently.

Validation of Proposed Method: The proposed method was validated as per ICH guidelines. The solutions of the drugs were prepared as per the earlier adopted procedure given in the experiment.

Accuracy: It was done by recovery study using the standard addition method at 80%, 100%, and 120 % level; known amount of standard ART and LMF were added to pre analyzed sample (75 µg/ml of ART; 15µg/ml of LMF) and subjected them to the proposed HPLC method. Results are shown in **Table 9** and **Table 10.**

TABLE 9: RESULTS OF RECOVERY STUDIES OF ART

Drug	Initial amount [µg/ml]	Amount added [µg/ml]	Amount recovered ± S.D. [µg/ml, n = 3]	% Recovery	% RSD
MET	**75**	0	75.29 ± 0.67	100.39	0.89
	75	60	59.89 ± 0.89	99.81	1.49
	75	75	74.69 ± 1.09	99.58	1.45
	75	90	90.49 ± 1.28	100.55	1.42

TABLE 10: RESULTS OF RECOVERY STUDIES OF LMF

Drug	Initial amount [µg/ml]	Amount added [µg/ml]	Amount recovered ± S.D. [µg/ml, n = 3]	% Recovery	% RSD
LMF	15	0	15.15 ± 0.27	100.99	1.83
	15	12	12.07 ± 0.20	100.57	1.67
	15	15	15.08 ± 0.24	100.51	1.58
	15	18	17.84 ± 0.18	99.11	1.02

Precision: Precision is the measure of how close the data values are to each other for a number of measurements under the same analytical conditions.

TABLE 11: RESULTS OF REPEATABILITY (ART)

S. no.	Concentration [µg/ml]	Peak area
1	75	628
2	75	611
3	75	623
4	75	618
5	75	621
6	75	636
	Mean ± SD	622.833 ± 8.56
	% RSD	1.37

Repeatability: It is measured by multiple injections of a homogenous sample of 75 µg/ml of ART and 15 µg/ml of LMF indicates the performance of the HPLC instrument under chromatographic conditions. Results are shown in **Table 11** and **Table 12.**

TABLE 12: RESULTS OF REPEATABILITY (LMF)

S. no.	Concentration [µg/ml]	Peak area
1	15	374
2	15	380
3	15	386
4	15	378
5	15	366
6	15	382
	Mean ± SD	377.66 ± 6.97
	% RSD	1.84

Intraday and Interday Precision: Intraday precision was determined by analyzing the three different concentrations 50 µg/ml, 75 µg/ml and 100 µg/ml of ART, 10 µg/ml, 15 µg/ml and 20 µg/ml of LMF for three times in the same day. Day-to-day variability was assessed using the above-mentioned three concentrations analyzed on three different days over a period of one week. This result shows the reproducibility of the assay. The % RSD values are shown in **Table 13** and **Table 14.**

Robustness: To evaluate robustness, a few parameters were deliberately varied. The parameters include a variety of flow rates, percentage of methanol using 75 µg/ml solution of ART and 15 µg/ml of LMF.

TABLE 13: RESULTS OF PRECISION STUDIES OF ART (INTRADAY AND INTERDAY)

Drug	Conc. [µg/ml]	Intraday Amount Found [µg/ml]		Interday Amount Found [µg/ml]	
		Mean ± SD	% RSD [n= 3]	Mean ± SD	% RSD [n= 3]
MET	50	49.87 ± 4.16	0.34	49.51 ± 8.50	0.69
	75	74.37 ± 10.21	0.55	74.77 ± 7.64	0.41
	100	99.50 ± 6.66	0.27	99.50 ± 9.45	0.38

TABLE 14: RESULTS OF PRECISION STUDIES OF LMF (INTRADAY AND INTERDAY)

Drug	Conc. [µg/ml]	Intra-day Amount found [µg/ml]		Inter-day Amount found [µg/ml]	
		Mean ± SD	% RSD [n= 3]	Mean ± SD	% RSD [n= 3]
LMF	10	9.77 ± 2.00	0.29	10.13 ± 2.00	0.28
	15	15.25 ± 3.06	0.27	15.09 ± 5.57	0.50
	20	20.39 ±5.51	0.35	19.65 ± 5.03	0.34

TABLE 15: ROBUSTNESS EVALUATION OF THE HPLC METHOD FOR ART

Chromatographic conditions	T	K'	N
A: Mobile phase pH	Tailing	Capacity Factor	Theoretical Plate
2.8	1.26	1.23	2683.9
3.0	1.22	1.27	2683.5
3.2	1.21	1.33	2625.5
Mean ± SD	1.23 ± 0.02	1.27 ± 0.05	2678.63 ± 36.80
B: Flow rate (ml/min.)			
0.90	1.23	0.98	2723.8
1.0	1.16	1.08	2818.9
1.1	1.15	1.09	2768.7
Mean ± SD	1.18 ± 0.04	1.05 ± 0.06	2770.47 ± 47.57
C: Percentage methanol in mobile phase (v/v)			
60	1.09	1.22	2646.2
70	1.06	1.13	2687.4
80	1.19	1.18	2638.3
Mean ± SD	1.11 ± 0.06	1.17 ± 0.04	2657.3 ± 26.36

TABLE 16: ROBUSTNESS EVALUATION OF THE HPLC METHOD FOR LMF

Chromatographic conditions	T	K'	N
Chromatographic conditions	T	K'	N
A: Mobile phase pH	Tailing	Capacity Factor	Theoretical Plate
5	1.28	0.99	7591.4
6	1.23	1.09	7632.5
7	1.25	1.15	7414.7
Mean ± SD	1.25 ± 0.02	1.07 ± 0.02	7546.2 ± 111.72
B: Flow rate (ml/min.)			
0.90	1.26	0.76	7587.3
1.0	1.29	1.10	7668.8
1.1	1.22	0.88	7423.5
Mean ± SD	1.25 ± 0.03	0.91 ± 0.17	7593.2 ± 72.82
C: Percentage methanol in mobile phase (v/v)			
90	1.18	0.87	7623.8
70	0.94	0.95	7667.3
50	1.23	0.87	7433.2
Mean ± SD	7.296 ± 2.95	1.213 ± 0.73	7574.77 ± 124.51

Sensitivity: The sensitivity of the proposed method wer estimated in terms of Limit of Detection (LOD) and Limit of Quantitation (LOQ).

LOD = 3.3 SD/S and LOQ = 10 SD/S, where SD is the residual standard deviation and S is the slope of the line. LOD and LOQ were found to be 1.656 and 5.019 for ART; 0.617 and 1.869 for LMF, respectively.

Specificity and Selectivity: The analytes should have no interference from other extraneous components and be well resolved.

Specificity is a procedure to detect quantitatively the analyte in presence of component that may be expected to be present in the sample matrix, while selectivity is the procedure to detect the analyte qualitatively in presence of components that may be expected to be present in the sample matrix.

The method is quite selective. There was no other interfering peak around the retention time of ART and LMF; also, the baseline did not show any significant noise.

Ruggedness: From stock solutions, sample solutions of ART (75 µg/ml) and LMF (15 µg/ml) were prepared and analyzed by two different analysts using similar operational and environmental conditions.

The Peak area was measured for the same concentration solutions six times. The results are shown in **Table 17** and **Table 18.**

TABLE 17: RESULTS OF RUGGEDNESS

Analyst	Amount found of ART [%]	%RSD [n=3]
I	99.40	0.98
II	99.58	1.13

TABLE 18: RESULTS OF RUGGEDNESS

Analyst	Amount found of LMF [%]	%RSD [n=3]
I	99.60	1.27
II	99.80	1.31

System Suitability test: System suitability testing is essential for the assurance of the quality performance of the chromatographic system.

Earlier prepared solutions for chromatographic conditions were tested for system suitability testing. Results are shown in **Table 19** and **Table 20.**

TABLE 19: SYSTEM SUITABILITY TEST FOR ART

System suitability parameters	Proposed method
Retention time (R_t)	2.9333
Capacity factor (K')	1.18
Theoretical plate (N)	2838.7
Tailing factor (T)	1.16

TABLE 20: SYSTEM SUITABILITY TEST FOR LMF

System suitability parameters	Proposed method
Retention time (R_t)	6.9167
Capacity factor (K')	0.99
Theoretical plate (N)	74.65.8
Tailing factor (T)	0.95

Procedure for Forced Degradation Study: Forced degradation of each drug substance and the drug product was carried out under acidic, basic, oxidative stress, thermolytic and photolytic, conditions.

Thermal degradation of the drug was carried out in solid-state. While remaining, all studies were carried out in solution form.

Solutions were prepared by dissolving the drug with either distilled water, aqueous hydrochloric acid, aqueous sodium hydroxide, or aqueous hydrogen peroxide solution, which is further diluted with mobile phase to achieve a concentration of 150 µg/ml each of ART and 30 µg/ml for LMF.

These solutions were kept for 1 h. For thermal stress, drug samples were placed in a controlled-temperature oven at 50°C for 1 hr. Solutions of drug substances and drug products were also kept at 80 °C for 48 h. For photolytic stress, drug samples in the solution state were irradiated with UV radiation having peak intensity at 254 and 366 nm. The degradation studies **Fig. 5** to **8** were tabulated in **Table 21**.

TABLE 21: FORCED DEGRADATION OF ART AND LMF

Sample Exposure condition	Total Number of products with their Rt	ART		LMF	
		Degradation remained (150 µg/ml)	Recovery (%)	Degradation remained (30 µg/ml)	Recovery (%)
Acidic, 1N, 1 h	5 (2.95, 4.80, 6.05, 7.08, 7.65)	136.224	90.81	28.25	94.18
Basic, 1N, 1 h	6 (2.61, 2.80, 2.95, 3.38, 4.51, 7.20)	122.22	81.48	13.28	44.29
Per oxide, 30 %, 1 h	4 (2.63, 2.83, 4.76, 7.03)	128.50	85.67	20.92	69.73
Heat, 50 °C, 1 h	3 (2.61,2.81,6.766)	136.58	91.05	22.20	74.01

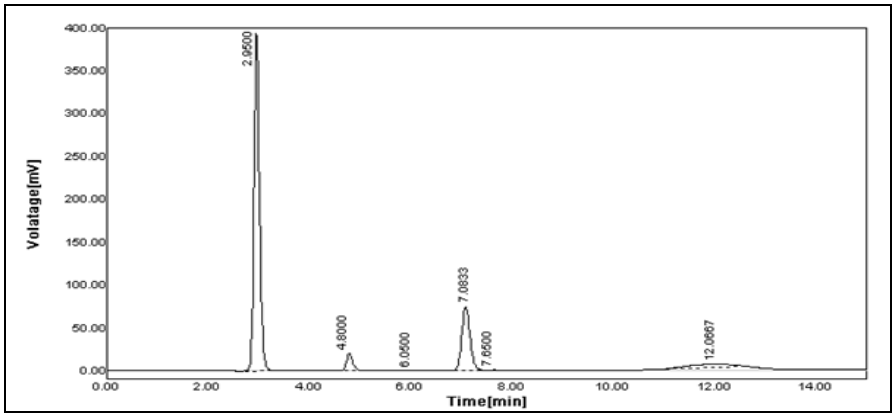

FIG. 5: ACIDIC DEGRADATION (1N, HCL) AFTER 1 H

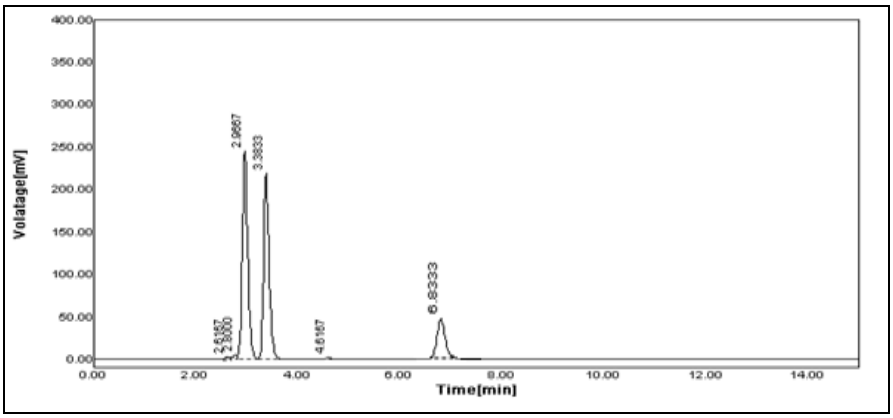

FIG. 6: ALKALINE DEGRADATION (1N NAOH) AFTER 1 H

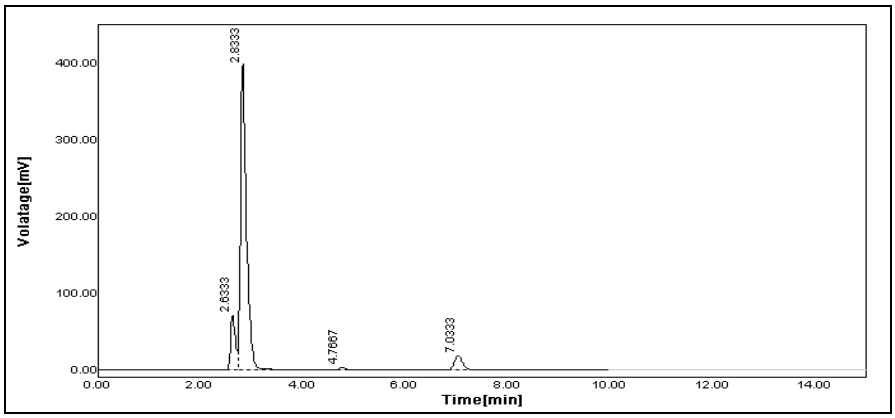

FIG. 7: PER OXIDE DEGRADATION (30% H2O2) AFTER 1 H

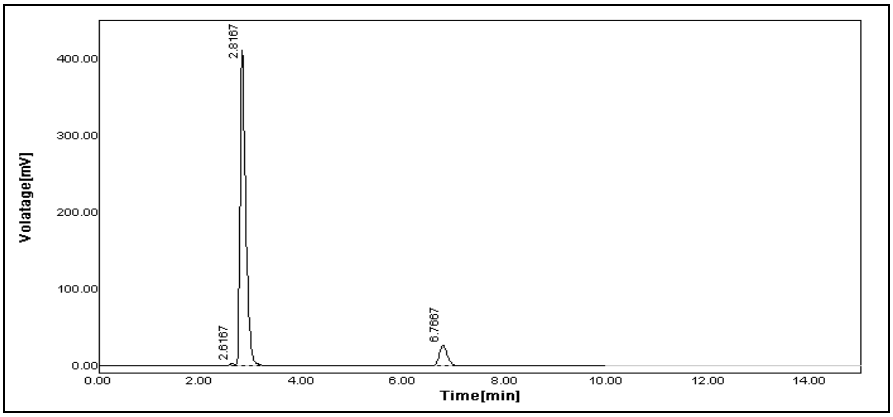

FIG. 8: HEAT DEGRADATION AT 50 °C FOR 1 H

CONCLUSION: The proposed RP-HPLC method is rapid, specific, accurate, and precise for quantifying ART and LUM from its tablet dosage form. The method has been better because of its wide range of linearity, use of readily available mobile phase, and lack of extraction procedures.

All these factors make this method suitable for quantifying ART and LUM in tablet dosage forms. The method can be successfully used for routine analysis of ART and LUM in bulk drugs and pharmaceutical dosage forms.

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