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FTIR SPECTROPHOTOMETRIC METHOD FOR THE SIMULTANEOUS ESTIMATION OF ARTEMETHER AND LUMEFANTRINE IN BULK AND FORMULATIONS

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Keywords:

FTIR, Artemether, Lumefantrine, Simultaneous method

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ABSTRACT: A FTIR method was developed to estimate Artemether and Lumefantrine simultaneously in bulk as well as in formulations. It is based on the measurement of absorption of radiation at the absorption band of ether at 1113.10-1091.77 cm^{-1} for ART and phenyl substitution band at 893.65-857.08 cm^{-1} for LUM, because those absorption bands did not occur in excipients present in a pharmaceutical preparation. The proposed method was validated as per ICH guidelines. The calibration curve was obtained for a series of concentration in the range of 17-470 mg for ART and 10-250 mg for LUM, and it was found to be linear. The linear regression equation was y = +84.78-193.1*x for ART and y = +353.75+2129.1*x for LUM with correlation coefficient value 0.999 for ART and LUM which were within the acceptance criteria. The precision was measured regarding repeatability and % RSD was calculated and was found to be 0.831 for intraday and 0.831 for inter-day precision. Recovery was carried out standard addition method at three different levels which are 80%, 100%, and 120%. The % recovery was calculated and was found to be 99.8 and ± 0.556 for ART and 99.9 ± 0.094 for LUM. The % assay was calculated from the standard calibration curve. The results 99 ± 0.1 for ART and 99.9 ± 0.1 for LUM presented good agreement within the labelled content. Thus the method developed in the present investigation is simple, sensitive, rapid and precise. Hence, the developed method can be successfully applied for the estimation of ART and LUM in bulk and tablet dosage form.

INTRODUCTION: Artemether[1-2] is chemically (3R, 5As, 6R, 8As, 9R, 10S, 12R, 12aR)-Dehydro-10-methoxy-3,6,9-trimethy-3,12-epoxy-12*H*-pyrano [4,3-*j*]-1,2-benzodioxepine and Lumefantrine[3] is 2-dibutylamino-1-[2,7-dichloro-9-(4-chlorobenzylidi-ne)-9H-fluoro-4-yl]-ethanol (racemate) **Fig. 1** & **Fig. 2**. Only very few methods has been reported for determination of this combination. Combination shows wavy absorption patterns in UV spectroscopy [4].

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Artemether and Lumefantrine exhibit complementary pharmacokinetic profiles. Artemether is absorbed quickly. Peak concentrations of Artemether and its main active metabolite, dihydroartemisinin (DHA) occur at approximately two hours post-dose, leading to a rapid reduction in asexual parasite mass and prompt resolution of symptoms. Lumefantrine is absorbed and cleared more slowly (terminal elimination half-life 3-4 days in malaria patient's) and accumulate with successive doses, acting to prevent recrudescence by destroying any residual parasites that remain after Artemether and DHA have been cleared from the body.

MATERIALS AND METHODS: Artemether and Lumefantrine were obtained as a gifted sample from Mylan Laboratories, Hyderabad.

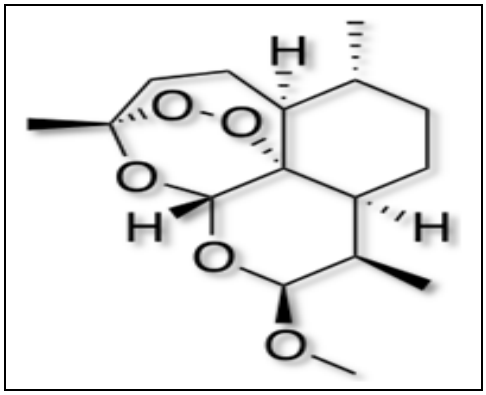

FIG. 1: CHEMICAL STRUCTURE OF ARTEMETHER

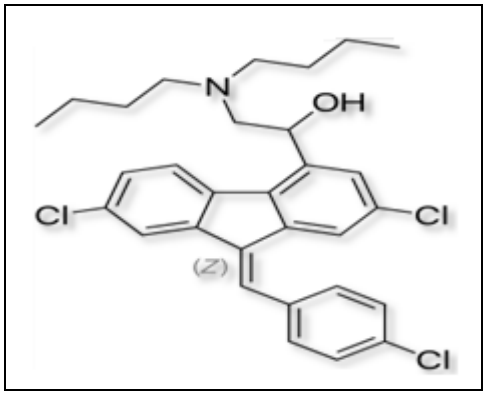

FIG. 2: CHEMICAL STRUCTURE OF LUMEFANTRINE

Instrumentation: FTIR from Bruker Optics ATR, ZnSe equipped with OPUS software and Quant Builder

Standards and Samples: Artemether and Lumefantrine standard for the present study to establish calibration was obtained as a gift sample from Mylan Laboratories Ltd., Hyderabad. The different solid pharmaceutical formulations having ART and LUM as API were obtained as a gift sample from Alvizia Healthcare, Chandigarh.

Calibration Curve: Calibration curve were prepared for five different concentrations of the drugs in the range of 17-470 mg ART **Fig. 3** and 10-250 mg **Fig. 4** for LUM. An appropriate quantity of drugs was triturated to ensure sample homogeneity. Each calibration standard was analyzed in the replicates of six. Area Under Curve (AUC) corresponding to the ether peak around 1113.10-1091.77 cm^{-1} for ART **Fig. 5** and Phenyl Ring Substitution Bands around 893.65 - 857.08 cm^{-1} for LUM **Fig. 6** was used for the quantification, and the average of six measurements were used to obtain the calibration curve [7-13]. All the statistical calculations and calibration curve plotting were carried out using Opus version 6.0 software for windows.

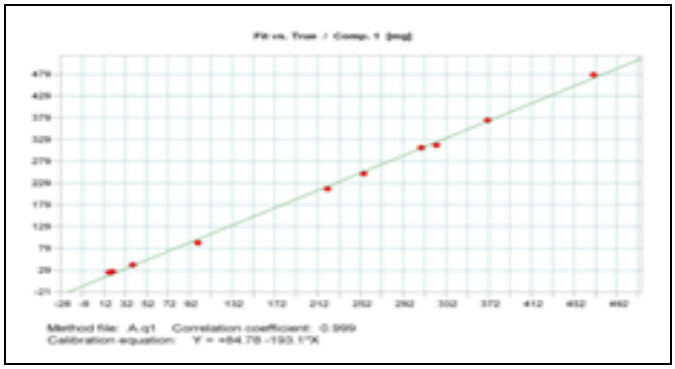

FIG. 3: LINEARITY DATA OF STANDARD ART

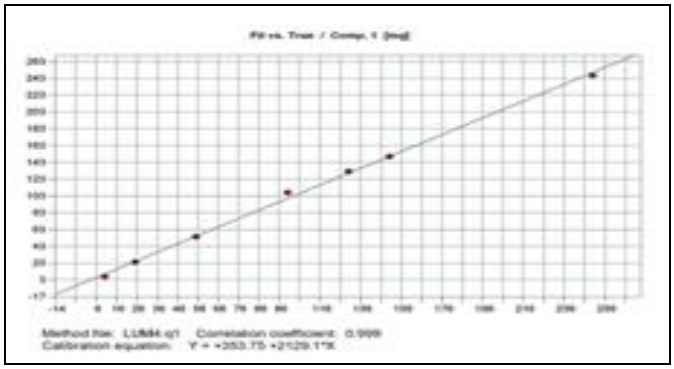

FIG. 4: LINEARITY DATA OF STANDARD LUM

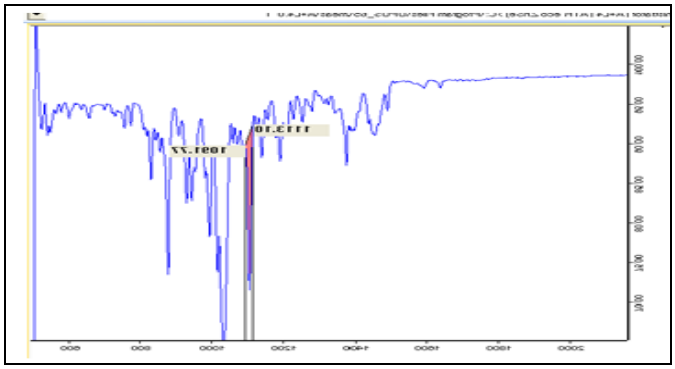

FIG. 5: AUC REGION OF ARTEMETHER

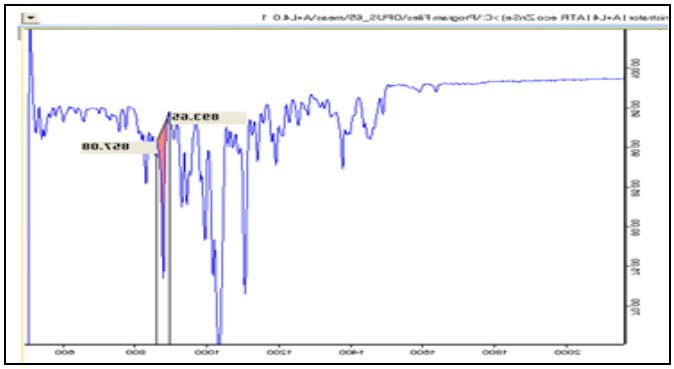

FIG. 6: AUC OF LUMEFANTRINE

Sample Preparation Procedure: In this method except grinding no prior sample treatment is required for FT-IR run. The pharmaceutical samples were accurately weighed and grinded in a mortar until a fine powder was obtained. These are scanned from 4000 to 400 cm^{-1} on FTIR to record spectra.

Method Validation: The developed method was validated for precision, accuracy, and linearity.

Precision: Repeatability and intermediate precision studies assessed the precision of method. Repeatability studies were performed by analyzing six samples of five different concentrations (17-470 mg) of ART **Fig. 7** and LUM (10-250 mg) **Fig. 8** 3 times on the same day (day 1).

The intermediate precision of the assay method was evaluated by repeating studies interday (on day 2 and 3).

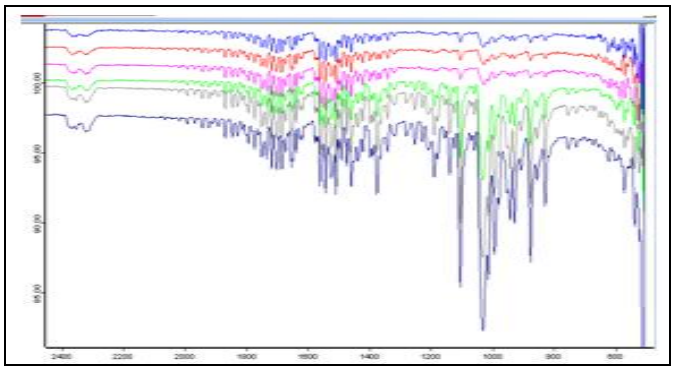

FIG. 7: OVERLAY SPECTRUM OF STANDARD ART

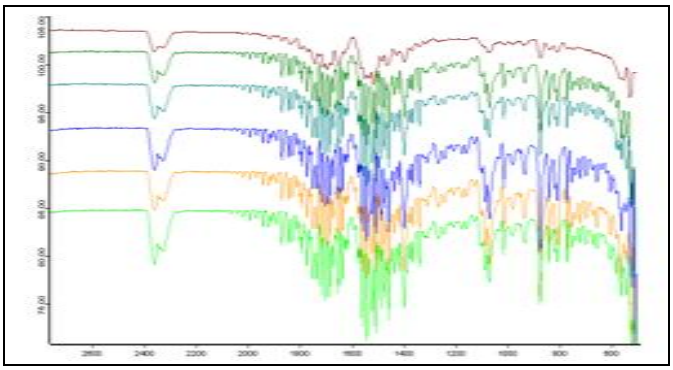

FIG. 8: OVERLAY SPECTRUM OF STANDARD LUM

Accuracy: The accuracy of the assay method was evaluated by the standard addition method with the recovery of pure drug from excipients at three different quantities (80, 100 and 120% w/w). To the pre-analyzed tablet powder, known the amount of ART and LUM standard powder corresponding to 80, 100 and 120% of label claim was added. The sample was mixed thoroughly and analyzed by making in six replicate.

Linearity: The linearity of the calibration curve was assessed by linear regression. The solid-state sample in the concentration range of 17-470 mg for ART and 10-250 mg for LUM were prepared as described in the calibration curve. The linearity of the method was studied by analyzing the samples of ten different concentrations of ART and LUM in three replicates.

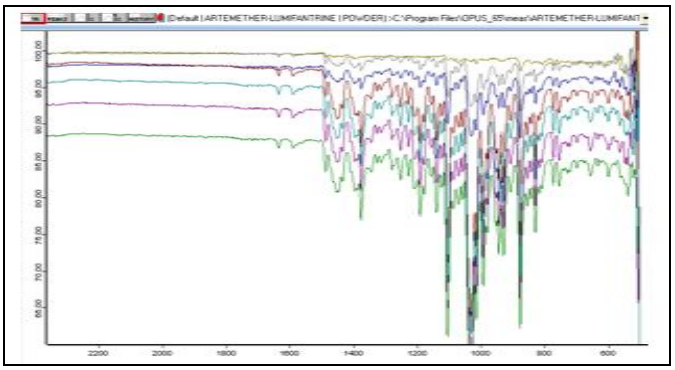

FIG. 9: SPECTRA OF TABLET FORMULATION

Analysis of Marketed Tablet Formulations: ATMITHER AL® of tablets are used to determine the drug content. Ten tablets were weighed accurately, their average weight determined, and finely powdered. An appropriate quantity of each tablet powder samples was mixed thoroughly by triturating. The analysis was carried out using three samples which were analyzed in 3 replicates **Fig. 9**.

RESULTS AND DISCUSSION: The method is based in the measurement of absorption of radiation at the absorption band of ether at 1113.10-1091.77 cm^{-1} for ART and phenyl substitution band at 893.65-857.08 cm^{-1} for LUM, because those absorption bonds did not occur in excipients present in a pharmaceutical preparation. The proposed method was validated as per ICH guidelines. The calibration curve was obtained for a series of concentration in the range of 17-470 mg for ART and 10-250 mg for LUM, and it was found to be linear. The linear regression equation was y = +84.78-193.1*x for ART and y = +353.75+2129.1*x for LUM with correlation coefficient value 0.999 for ART and LUM which were within the acceptance criteria **Table 1**.

The precision was measured regarding repeatability, which was determined by a sufficient number of sample within the day (intraday) **Table**

2 and next consequent three days for inter-day precision **Table 3**. For each cases % RSD was calculated and was found to be 0.831 for intraday and 0.831 for inter-day precision **Table 4**. These values were well within the acceptance limit ± 2.0%. This showed that the precision of the method was satisfactory, good.

Accuracy found out by recovery study from prepared samples (three replicates) with a standard solution. Recovery was carried out standard addition method at three different levels which are 80%, 100%, and 120%. The % recovery was calculated and was found to be 99.8 and ± 0.556 for ART and 99.9 ± 0.094 for LUM. This was found to be well within the acceptance criteria of 98 - 102%. This showed that the recovery of ART and LUM by proposed method was satisfactory **Table 5**.

The validated method was applied for the assay of commercial tablets of ATMITHER-AL. The % assay was calculated from the standard calibration curve. The results 99 ± 0.1 for ART and 99.9 ± 0.1 for LUM presented good agreement within the labeled content **Table 6**. Thus, the method developed in the present investigation is simple, sensitive, rapid and precise. Hence, the developed method can be successfully applied for the estimation of ART and LUM in bulk and tablet dosage form.

TABLE 1: REGRESSION ANALYSIS DATA AND SUMMARY OF VALIDATION PARAMETERS FOR THE FTIR SPECTROPHOTOMETRIC METHOD

Parameters		ART	LUM
Wavenumber range (cm^{-1})		1113.10-1091.77	893.65-857.08
Beer's law limit (mg)		17-470	10-250
Regression equation (y = mx + c)		Y = +84.78-193.1*x	Y = +353.75+2129.1*x
Slope (m)		M = +84.78	M = +353.75
Intercept (c)		C = -193.1*x	C = 2129.1*x
Correlation Coefficient (r^2)		0.999	0.999
Accuracy (Recovery) (n = 3)	Level I	178.9	287
	Level II	199.9	319.9
	Level III	221	353
Method precision (Repeatability) (% RSD, n = 5),		0.621252	0.621252
Interday (n = 3) (% RSD)		0.831	0.436
Intraday (n = 3) (% RSD)		0.831	0.436
Assay ± S. D. (n = 3)		99% ± 0.1	99.9% ± 0.1

RSD = Relative standard deviation. LOD = Limit of detection. LOQ = Limit of quantification. S. D. is the standard deviation

TABLE 2: REPEATABILITY DATA FOR THE METHOD (N=5)

Concentration (ART: LUM) (100 mg)	ART	LUM
	1113.10-1091.77 cm^{-1}	893.65-857.08 cm^{-1}
1	101.34	100.35
2	99.79	101.12
3	101.12	99.79
4	100.87	100.87
5	100.35	101.34
Mean	100.694	100.695
SD	0.625564	0.625564
% RSD	0.621252	0.621252

TABLE 3: RESULTS OF METHOD PRECISION FOR INTRA - DAY PRECISION

Concentration (µg/ml)		Observed value		Mean ± SD		% RSD	
ART	LUM	ART	LUM	ART	LUM	ART	LUM
		9.9	49.9				
10	50	9.8	50	9.9 ± 0.1	49.6 ± 0.8090	1.63%	1.01%
		10	48.9				
		49.9	150				
50	150	50	149.9	49.9 ± 0.1	149.6 ± 0.8090	0.540%	0.20%
		49.8	148.9				
		99.9	249.8				
100	250	100	248.9	99.9 ± 0.1	249.6 ± 0.8090	0.325%	0.10%
		99.8	250				

TABLE 4: RESULTS OF METHOD PRECISION FOR INTERDAY PRECISION

Concentration (µg/ml)		Observed value		Mean ± SD		% RSD	
ART	**LUM**	**ART**	**LUM**	**ART**	**LUM**	**ART**	**LUM**
		9.9	49.9				
10	50	9.8	50	9.9 ± 0.1	49.6 ± 0.8090	1.63%	1.01%
		10	48.9				
		49.9	150				
50	150	50	149.9	49.9 ± 0.1	149.6 ± 0.8090	0.540%	0.20%
		49.8	148.9				
		99.9	249.8				
100	250	100	248.9	99.9 ± 0.1	249.6 ± 0.8090	0.325%	0.10%
		99.8	250				

TABLE 5: RECOVERY DATA OF PROPOSED METHOD

Drug	Accuracy level %	Actual Amount	Amount added	Amount recovered	% recovery	Mean ± SD	% RSD
	80%	100	80	178.9	99.3%		
ART	100%	100	100	199.9	99.9%	99.8 ± 0.556	0.55%
	120%	100	120	221	100.4%		
	80%	160	128	287	99.6%		
LUM	100%	160	160	319.9	99.9%	99.9 ± 0.094	0.094%
	120%	160	192	353	100.2%		

S. D. is Standard deviation, and n is number of replicates

TABLE 6: ASSAY OF ARTEMETHER AND LUMEFANTRINE IN TABLET FORMULATION

Formulations	Drug	Label claim (mg/tab)	Sample solution concentration (mg)	Amount found ± SD	% recover	% RSD
	ART	80	20	19.8 ± 0.1	99%	0.50%
I	LUM	480	120	119.9 ± 0.1	99.9%	0.08%

CONCLUSION: As method development procedure, validation studies were also performed for the same, parameters were observed as linearity, precision, accuracy, limit of detection, limit of quantification and assay. Hence, we conclude that the simple, rapid, less-time consuming, cost-effective and precise method was developed and validated by FTIR with Artemether and Lumefantrine.

The result of the analysis by the proposed method is highly reproducible and reliable and it is in good agreement with the label claim of the drug. The method can be used for the routine analysis of the ART and LUM in combination without any interference of excipients.

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CONFLICT OF INTEREST: Authors do not have any conflict of interest.

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