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DERIVATIVE ULTRA-VIOLET SPECTROPHOTOMETRIC METHOD FOR THE SIMULTANEOUS ESTIMATION OF ARTEMETHER AND LUMEFANTRINE IN BULK AND FORMULATIONS

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ABSTRACT

A derivative Ultra Violet Spectrophotometric method was developed to estimate Artemether and Lumefantrine simultaneously in bulk as well as in formulations The method is based on comparing area of a first derivative curve of Artemether and Lumefantrine. The area between 215.80-245.0 nm for Artemether and area between 263.60-283.40 nm for Lumefantrine was fixed as analytical wavelength for the determination in the first derivative mode (n=5) The calibration curve of both the drugs was plotted. The linear relationship between concentration and absorbance of both drugs were evaluated over the concentration range in1-9 µg/ml and 6-54 µg/ml for ART and LUM, respectively. The linearity ranges for ART and LUM were replicated for five times. The sensitivity of method was measured in terms of Limit of Detection (LOD) and Limit of Quantification (LOD). Intra-day precision was carried out by performing three replicates (at morning, afternoon and evening) of three different concentration (5, 7 and 9 µg/ml for ART; 30, 42, 54 µg/ml for LUM) on the same day and percent relative standard deviation (%RSD) was calculated. To ascertain the accuracy of proposed method, recovery studies were carried out by standard addition method by adding known amount of standard; 4, 5 and 6µg/ml for ART and 24,30 and 3 6 µg/ml to the marketed tablet (5 µg/ml ART and 30µg/ml LUM) at 80, 100 and 120 % level. The validated method was applied for the assay of commercial tablet of ATMITHER AL the results 97.1%±0.1 for ART and 95.5%±0.1 for LUM in combination.

KEYWORDS: Derivative Spectrophotometry, Lumefantrine, Artemether, Simultaneous estimation.

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-chlorobenzylidine)-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.[4-7] Artemether and Lumefantrine exhibit complementary pharmacokinetic profiles. Artemether is absorbed quickly. Peak concentrations of Artemether and its main active metabolite, dihydro artemisinin (DHA) occur at approximately two hours post-dos, leading to 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.

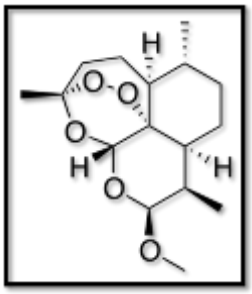

Fig 1: Chemical Structure of Artemether.

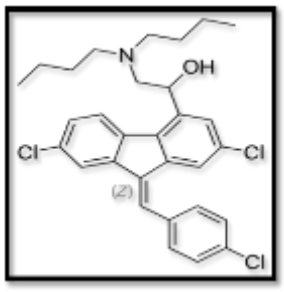

Fig 2: Chemical Structure of Lumefantrine.

MATERIALS AND METHODS

Artemether and Lumifantriene was obtained as a gifted sample from Mylan Laboratories, Hyderabad.

Instrumentaion

UV-Visible Spectrophotometer, SHIMADZU 1700 *PharmaSpec/UVProbe*®.

Standards and Samples

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

Preparation of standard stock solutions

An accurately weighed quantities (100mg each) of ART and LUM are dissolved in 50 ml of acetonitrile and transferred in 100ml volumetric flask. Volume was made up to mark with acetonitrile to obtain stock solution of 1000 µg/ml concentration. Further 1ml of stock solutions was diluted to 10ml with Acetonitrile (ACN) to obtain 100µg/ml working standard solution and was used for optimization of volume of concentrated hydrochloric acid (con. HCl) and reaction time (10 minutes) for derivatization of ART.

Selection of analytical concentration range

From the standard stock solution of ART, appropriate aliquots were pipetted out into 10 mL volumetric flasks and dilutions were made with ACN to obtain working standard solutions of concentrations 1- 9 µg/mL. Absorbance for these solutions were measured at 242nm (**Figure 1**) similarly, a series of standard solutions of concentration 6 - 54 µg/mL were prepared for LUM and their absorbance were measured at 234nm (**Figure3**). A standard calibration curve of absorbance against concentration was plotted. Both drugs followed the Beer-Lamberts law in the range of 1-9 µg/mL (**Figure 2**) and 6-54 µg/mL (**Figure 4**) for ART and LUM respectively.

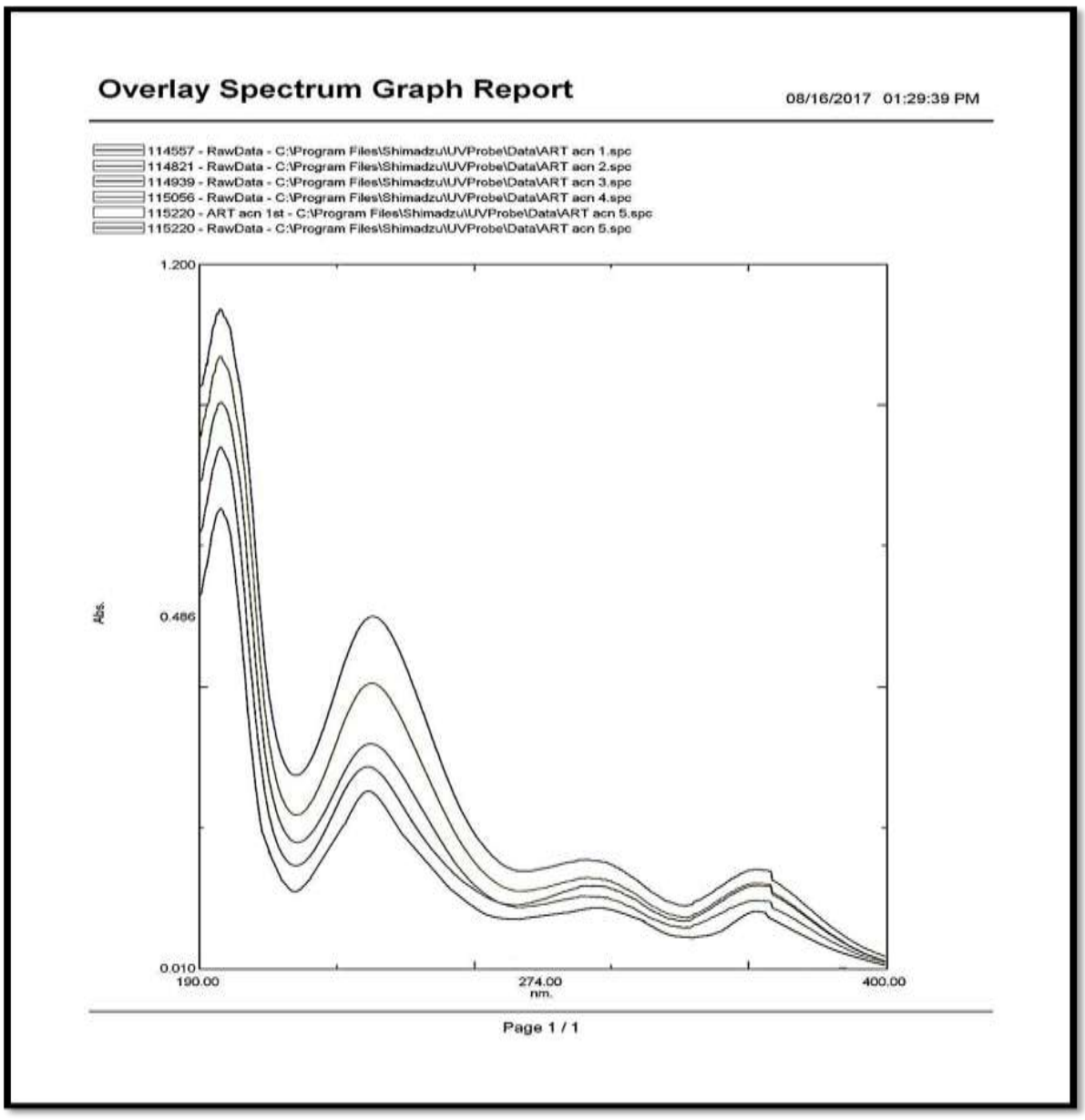

Figure 1: Zero order standard spectrum of derivatised ART.

Standard Table Report

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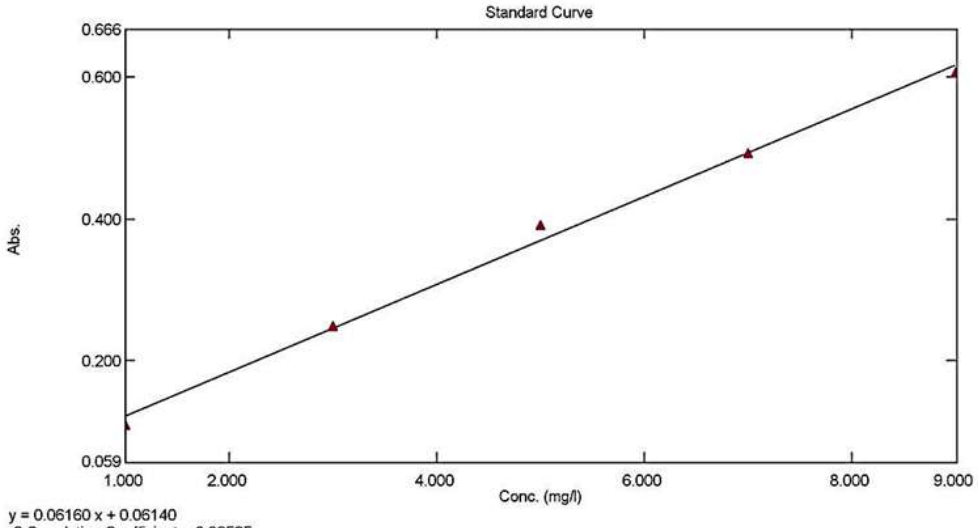

y = 0.06160 x + 0.06140
r2 Correlation Coefficient = 0.99525
Residual Standard Deviation = 0.01346

Standard Table

	Sample ID	Type	Ex	Conc	WL242.0	Wgt.Factor	Comments
1	1	Standard		1.000	0.110	1.000	
2	2	Standard		3.000	0.250	1.000	
3	3	Standard		5.000	0.390	1.000	
4	4	Standard		7.000	0.492	1.000	
5	5	Standard		9.000	0.605	1.000	
6							

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Figure 2: Calibration graph of derivatised ART..

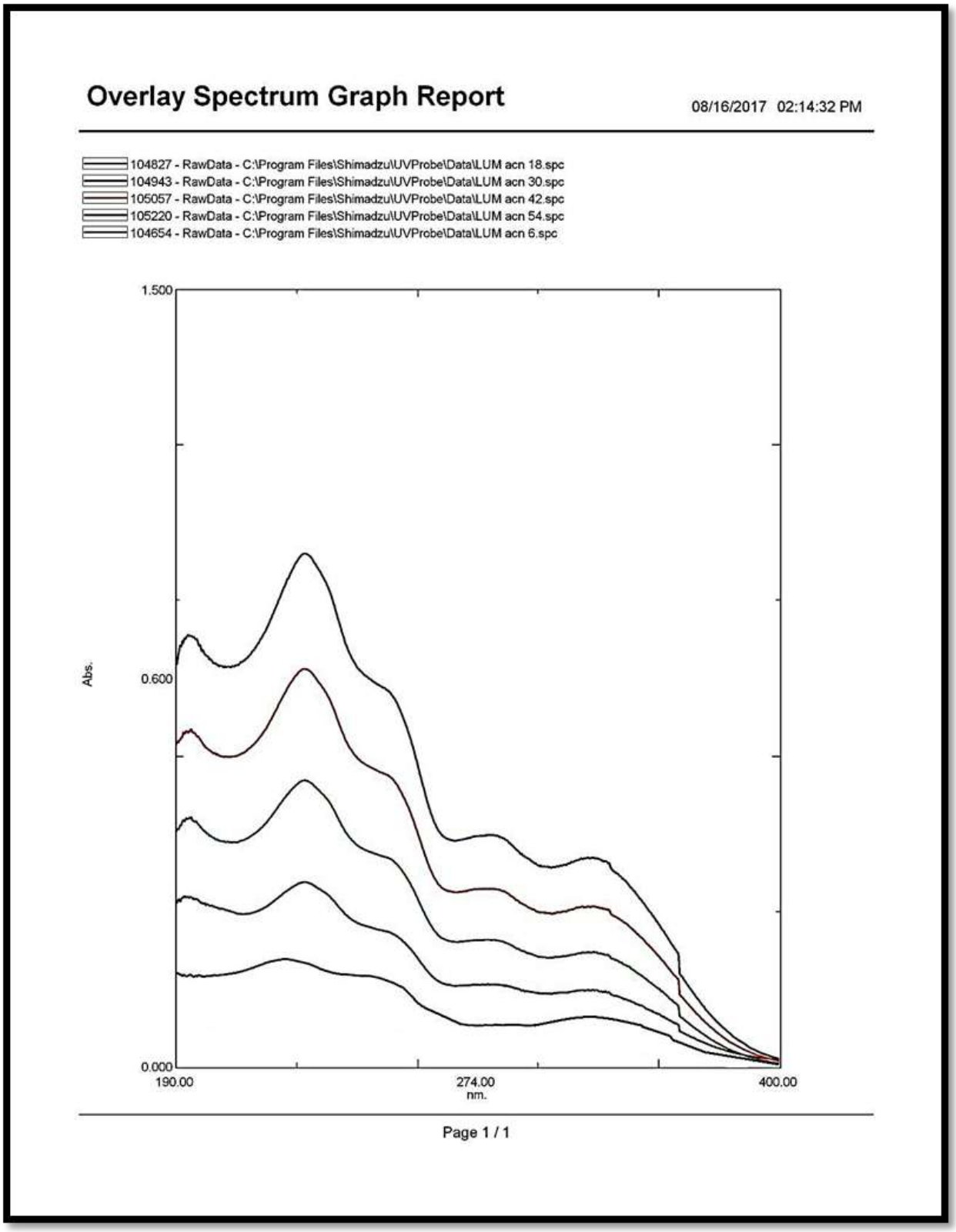
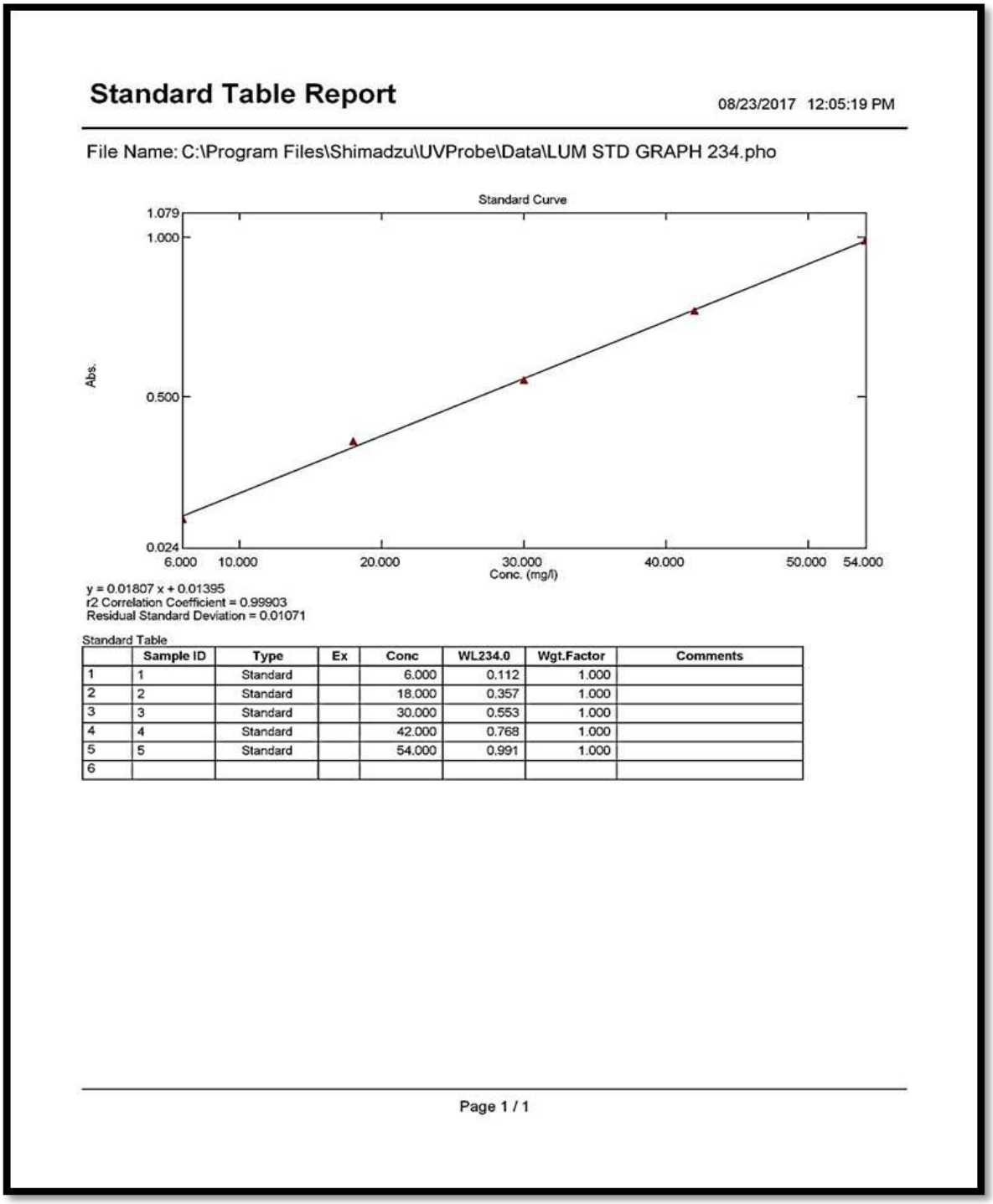


Figure 3: Zero order standard spectrum of LUM.



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y = 0.01807 x + 0.01395
r2 Correlation Coefficient = 0.99903
Residual Standard Deviation = 0.01071

Standard Table

	Sample ID	Type	Ex	Conc	WL234.0	Wgt.Factor	Comments
1	1	Standard		6.000	0.112	1.000	
2	2	Standard		18.000	0.357	1.000	
3	3	Standard		30.000	0.553	1.000	
4	4	Standard		42.000	0.768	1.000	
5	5	Standard		54.000	0.991	1.000	
6							

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Figure 4: Calibration graph of LUM.

First order derivative spectrophotometric method

Stock solutions of 1000 µg/ml of ART and LUM were prepared by dissolving accurately weighed quantity of 100 mg of stated drugs in to 100 ml volumetric flasks separately; it was dissolved and diluted up to the mark with ACN. Further dilutions were performed after addition of optimized volume of conc. HCl and both the drug were allowed to react with conc. HCl for the optimized reaction time and then working standard solution concentration 1, 3, 5, 7 and 9 µg/ml for ART. Similarly from the standard stock solution of LUM subsequent dilution were made with ACN to obtain working standard solutions of concentrations 6, 18, 30, 42, and 54µg/ml.

The area between 215.80-245.0 nm for Artemether and area between 263.60-283.40 nm for Lumefantrine was fixed as analytical wavelength for the determination in the first derivative mode with N=5 (**Figure 6, 8**) The calibration curve of both the drugs was plotted (**Figure 7, 9**).

Analysis of marketed formulations

Twenty ATMITHER AL tablets (Artemether-80 mg and Lumefantrine 480 mg) manufactured by Alvizia Healthcare, Chandigarh were accurately weighed and finely powdered. A quantity equivalent to 80 mg of ART and 480 mg of LUM was transferred to 100 ml volumetric flask and mixed with 50 ml of ACN and the solution was sonicated for 30 min and then the volume was made up with the same solvent. The solution was filtered through Whatmann filter paper. Then 1 ml filtrate was taken and transferred to another 10 ml of volumetric flask; further derivatized using optimized derivatizing conditions i.e. volume of conc. HCl (1ml) and reaction time 10 min. Then volume was made up to the mark with ACN. For analysis of both the drugs above solution was appropriately diluted to obtain concentration 1-9 µg/ml for ART and 6-54 µg/ml for LUM Sample solutions were prepared in triplicate and analyzed according to above mentioned procedure. (**Figure 10 & 11**).

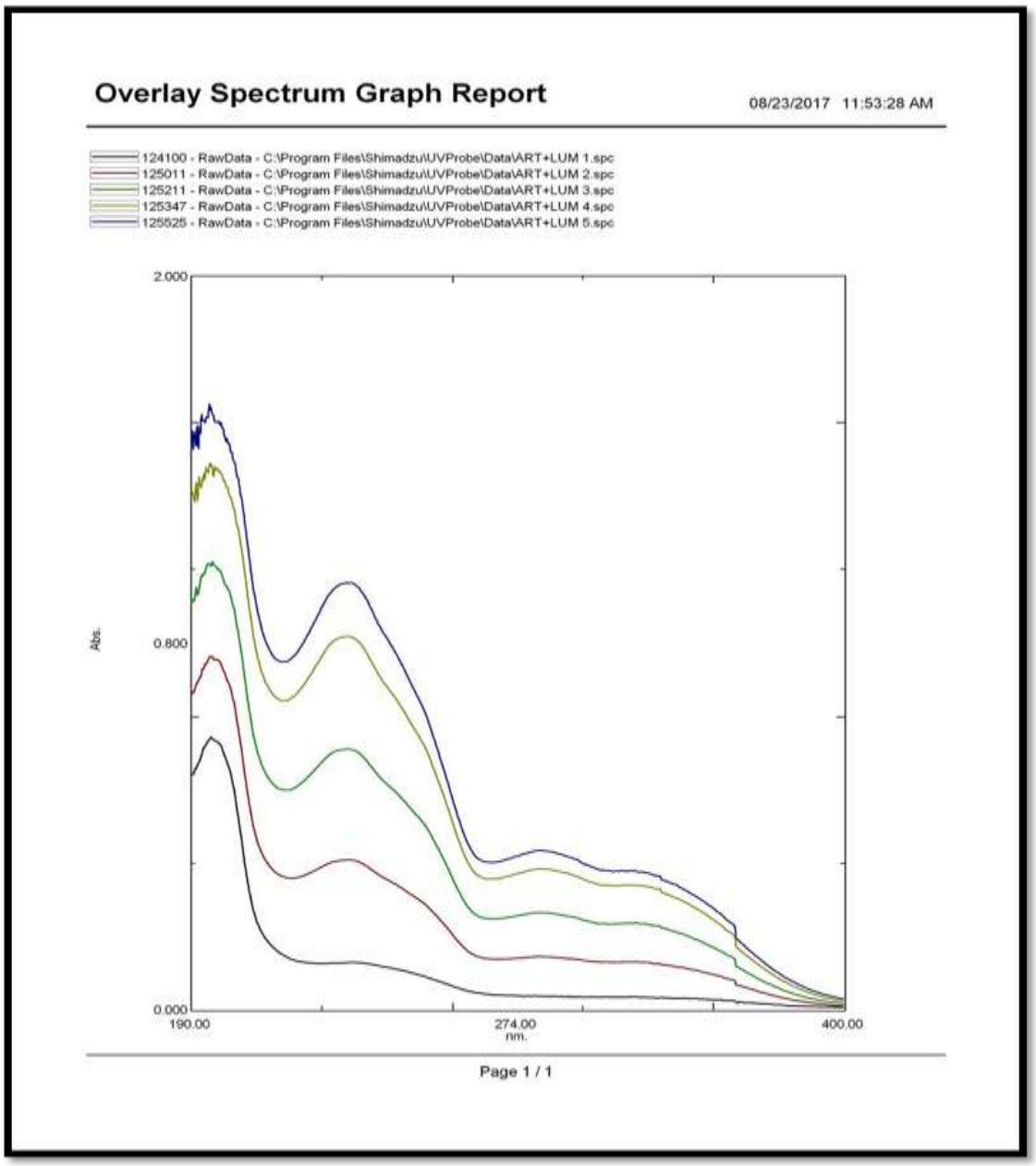

Figure 5: Zero order spectrum of ATMITHER AL tablet.

Method validation

The method was validated with respect to linearity, precision, accuracy, LOD and LOQ in accordance with ICH guidelines.[8-11]

Linearity

The linear relationship between concentration and absorbance of both drugs were evaluated over the concentration range in1-9 µg/ml and 6-54 µg/ml for ART and LUM, respectively. The linearity ranges for ART and LUM were replicated for five times.

Sensitivity

The sensitivity of method was measured in terms of Limit of Detection (LOD) and Limit of Quantification (LOD). LOD and LOQ of the developed method were calculated from the standard deviation of the response (σ) and slope of the calibration curve (S) of each drug using the formula, Limit of detection = 3.3*σ/S; Limit of quantitation = 10*σ/S.

Precision

The precision of developed method was evaluated by performing Intra-day and Inter-day precision studies. Intra-day precision was carried out by performing three replicates (at morning, afternoon and evening) of three different concentration (5, 7 and 9 µg/ml for ART; 30,42,54 µg/ml for LUM) on the same day and percent relative standard deviation (%RSD) was calculated. Inter-day precision study was assessed by mentioned concentrations of ART and LUM on three different days in triplicate and % RSD was calculated.

Accuracy

To ascertain the accuracy of proposed method, recovery studies were carried out by standard addition method by adding known amount of standard; 4, 5 and 6µg/ml for ART and 24,30 and 3 6 µg/ml to the marketed tablet (5 µg/ml ART and 30µg/ml LUM) at 80, 100 and 120 % level. The mean % recovery was calculated. Also, accuracy was expressed as the bias, i.e. the difference between the results obtained and the reference value. Recovery studies were performed in triplicate.

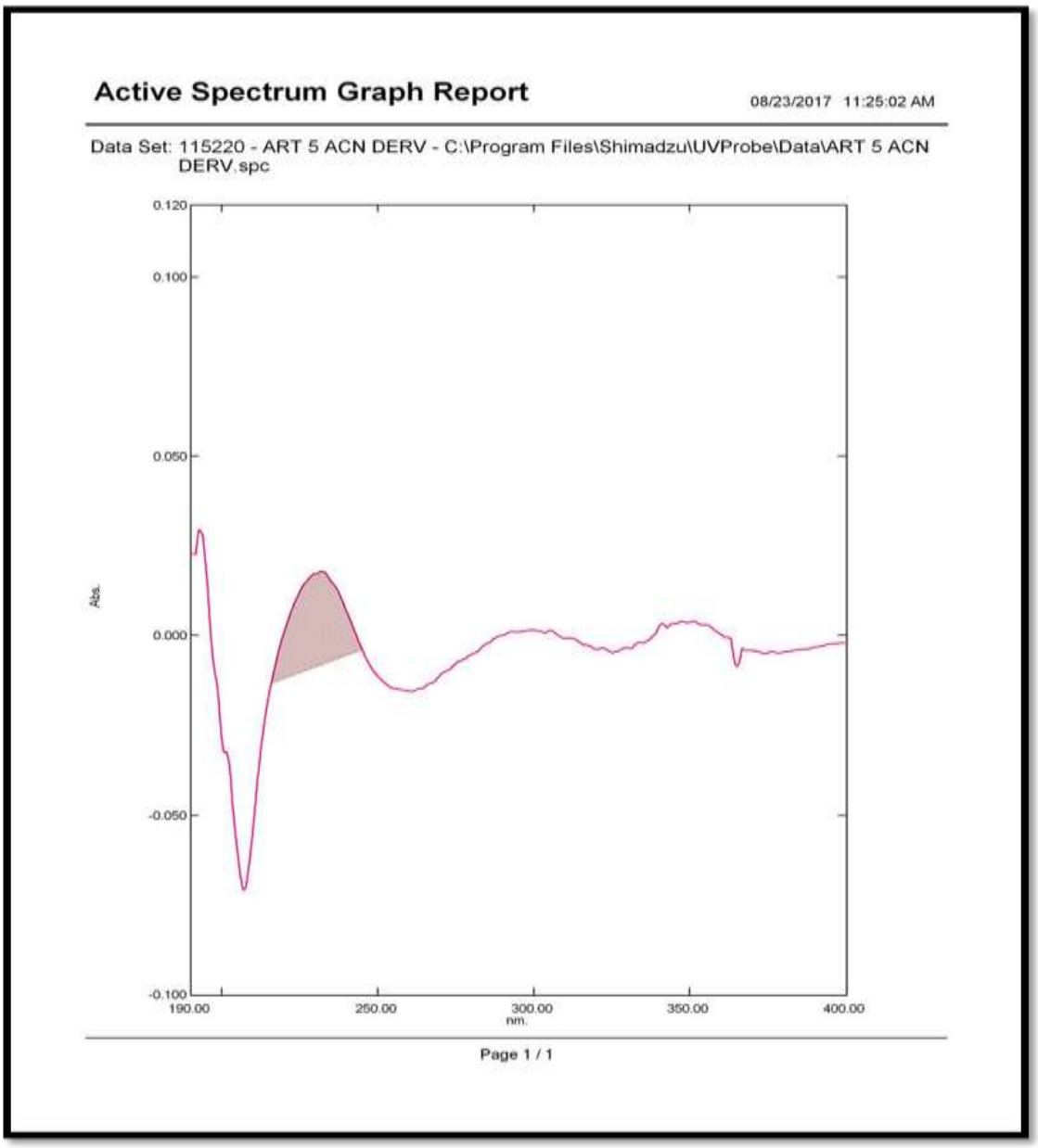

Figure 6: UV derivative spectrum of standard ART.

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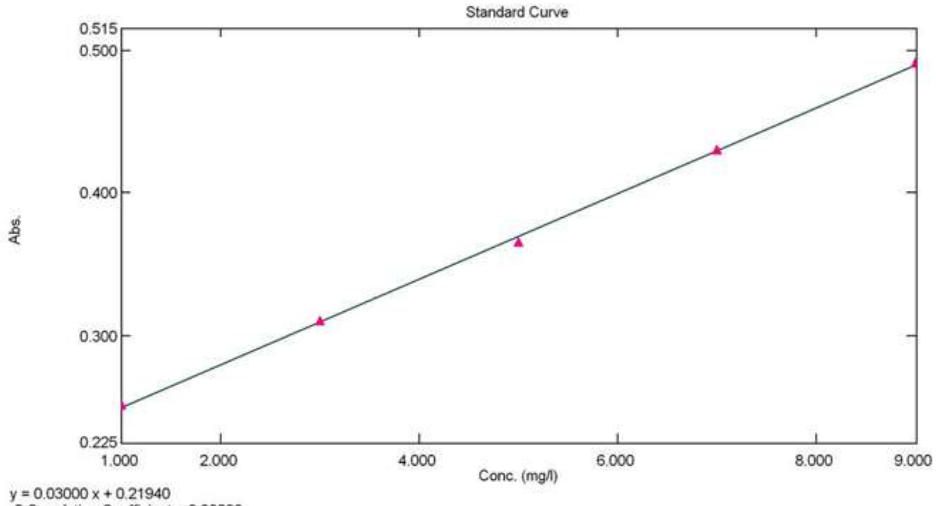

y = 0.03000 x + 0.21940
r2 Correlation Coefficient = 0.99930
Residual Standard Deviation = 0.00251

Standard Table

	Sample ID	Type	Ex	Conc	WL215.8_245.0	Wgt.Factor	Comments
1	1	Standard		1.000	0.251	1.000	
2	2	Standard		3.000	0.310	1.000	
3	3	Standard		5.000	0.365	1.000	
4	4	Standard		7.000	0.430	1.000	
5	5	Standard		9.000	0.491	1.000	
6							

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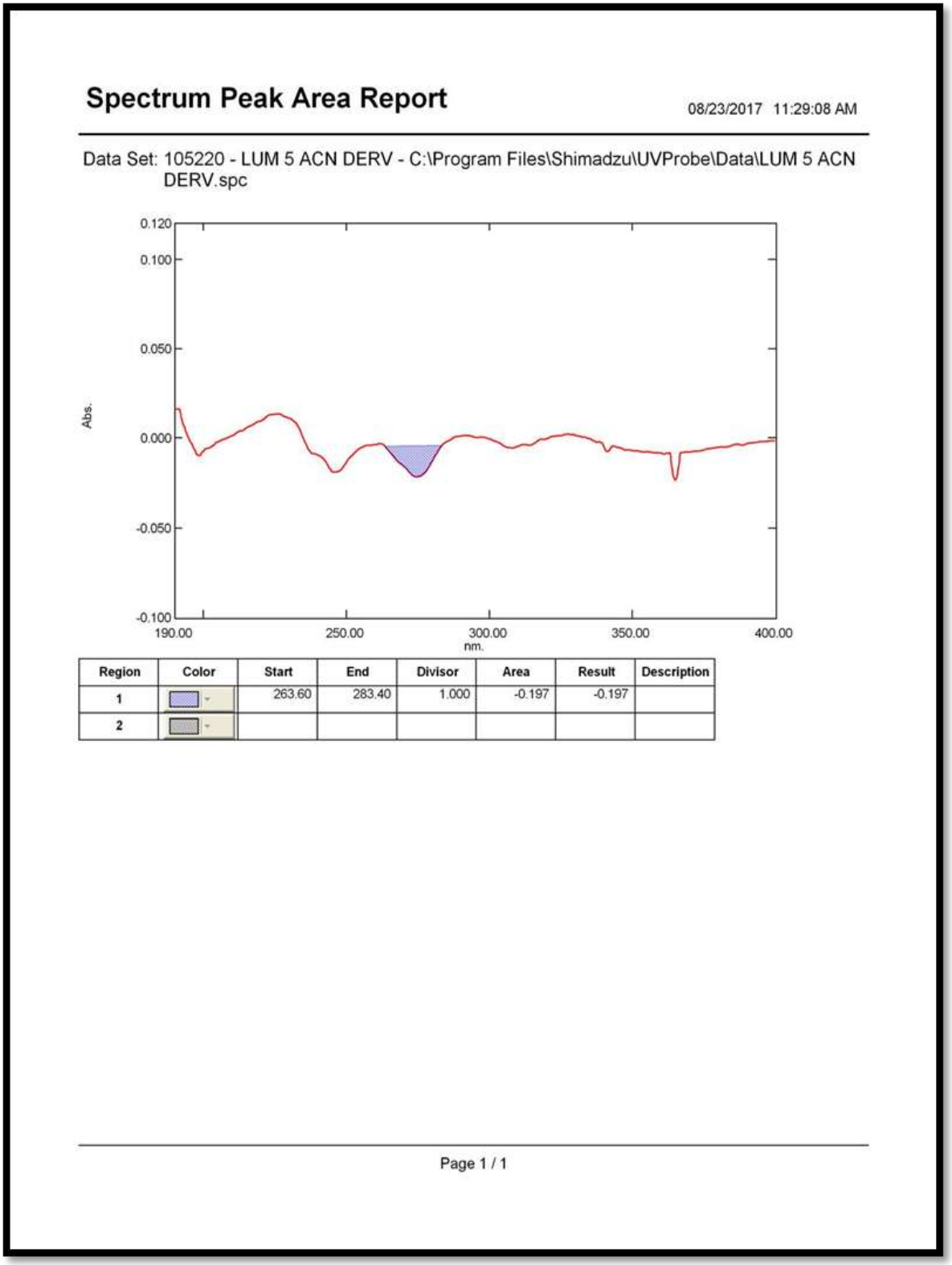
Figure 7: Linearity data of standard ART.



Spectrum Peak Area Report

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Data Set: 105220 - LUM 5 ACN DERV - C:\Program Files\Shimadzu\UVProbe\Data\LUM 5 ACN DERV.spc

Region	Color	Start	End	Divisor	Area	Result	Description
1		263.60	283.40	1.000	-0.197	-0.197	
2							

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Figure 8: UV derivative spectrum of standard LUM.

Standard Table Report

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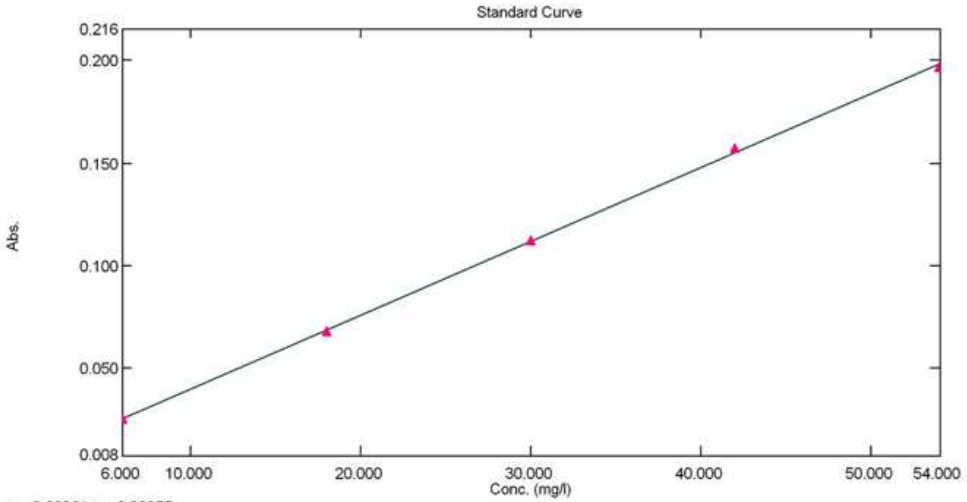

y = 0.00361 x + 0.00355
r2 Correlation Coefficient = 0.99969
Residual Standard Deviation = 0.00121

Standard Table

	Sample ID	Type	Ex	Conc	WL263.6_283.4	Wgt.Factor	Comments
1	1	Standard		6.000	0.025	1.000	
2	2	Standard		18.000	0.068	1.000	
3	3	Standard		30.000	0.112	1.000	
4	4	Standard		42.000	0.157	1.000	
5	5	Standard		54.000	0.197	1.000	
6							

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Figure 9: Linearity data of standard LUM.

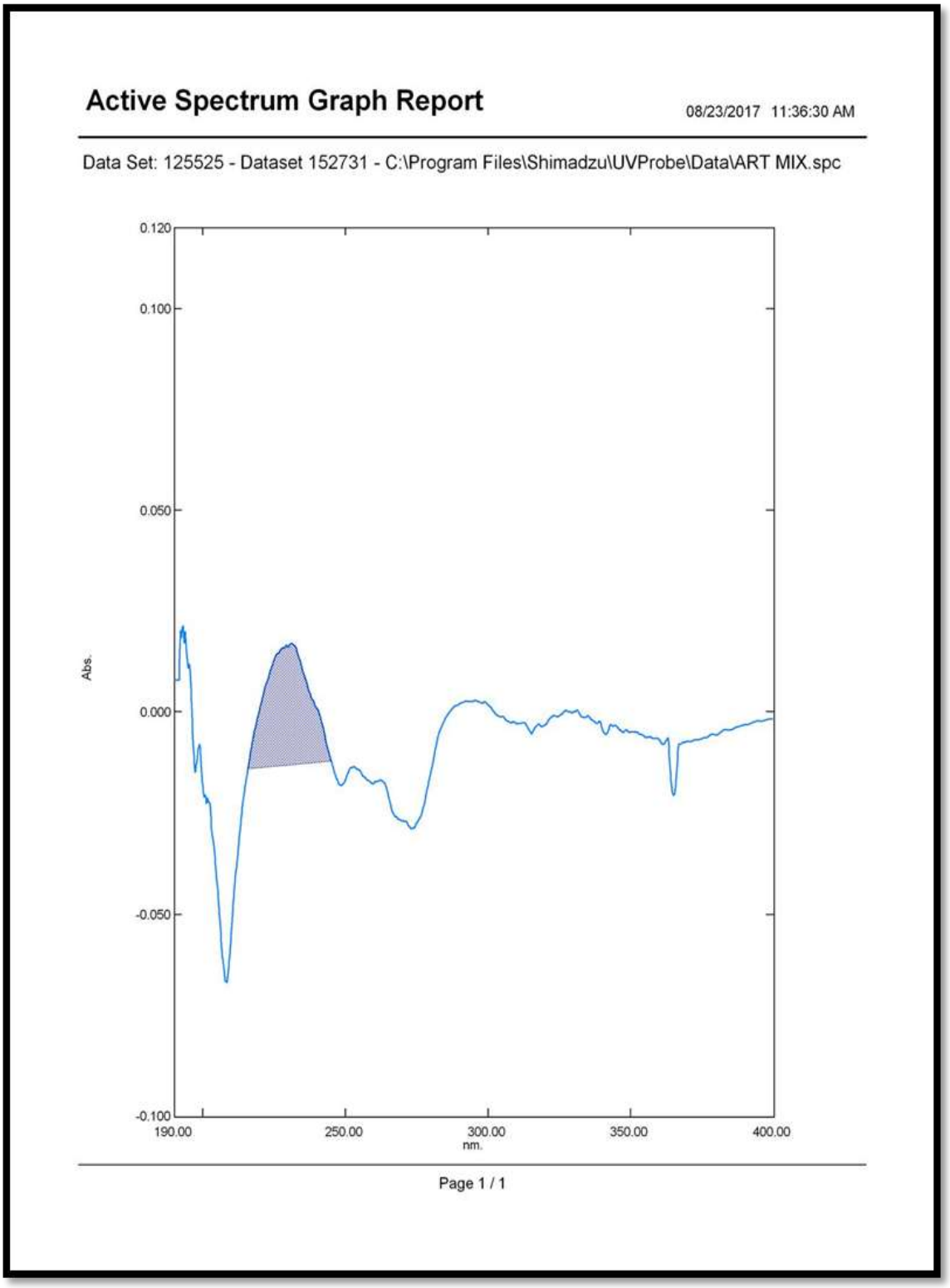

Figure 10: UV spectrum Area of ART of tablet formulation.

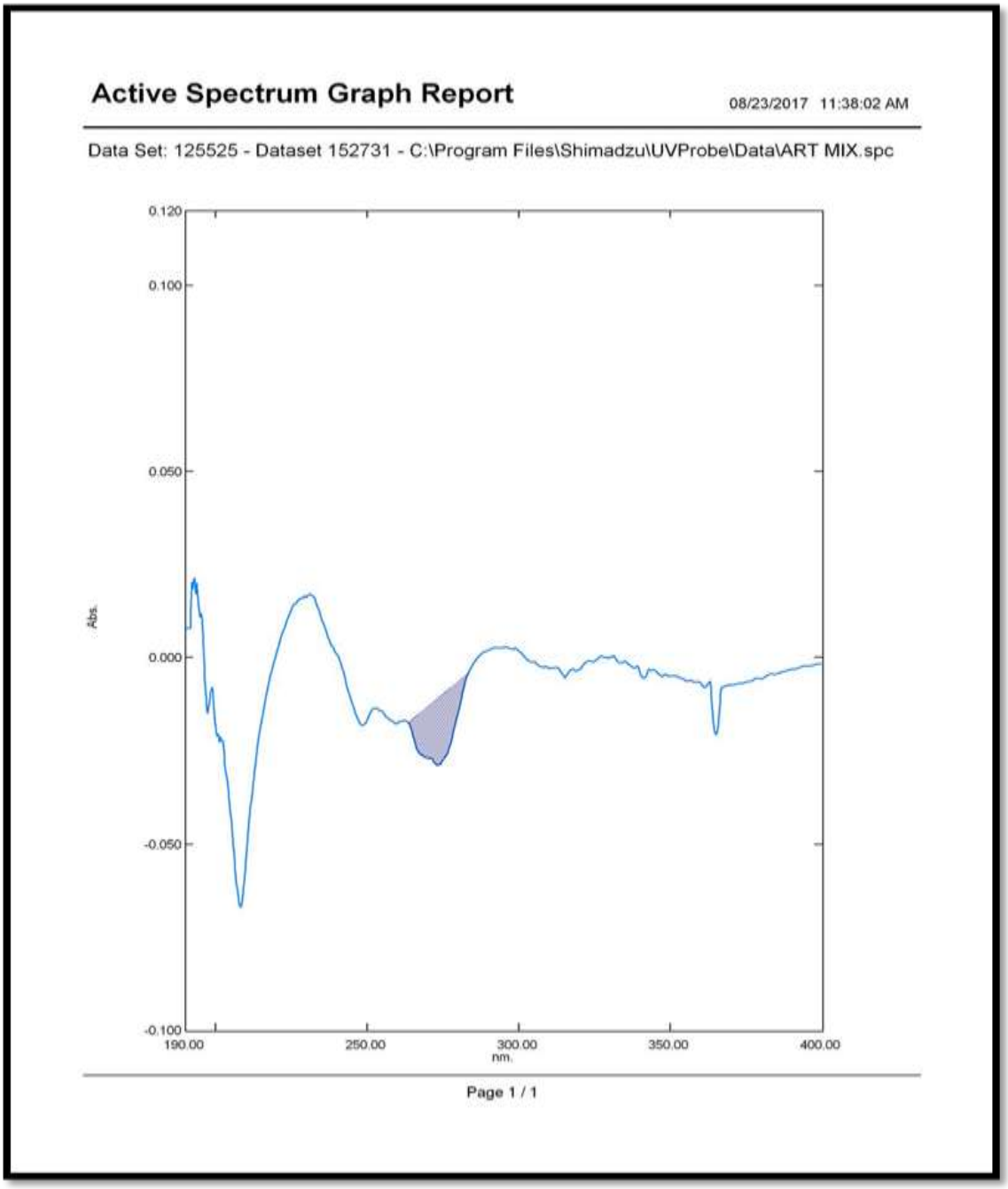

Figure 11: UV spectrum Area of LUM of tablet formulation.

RESULTS AND DISCUSSION

Linearity was observed with concentrations of Artemether over the range of 1-9 µg/ml within this range, graphs of area against concentration of ART gave R2 values of 0.99930 in all instances. This shows that there is linear relationship between the ART concentrations and the area values at 215.80-245.00 nm(Table 1).

Also, with respect to accuracy, there was a percentage recovery close to 100% when ART reference sample was mixed with tablet excepients and analyzed. The average percentage recovered was 100.8%(Table 2).
The method proved to be precise. In both the intra-day and inter-day precision, the method presented RSD values lower than 2.0%, assuring a good precision. The intra-day and inter- day precision assessment yielded % RSD values of 0.246% and 0.226% intraday precision respectively(Table 3).

The method proved to be specific for ART as tablet excepients and LUM did not interfere with the analysis.

The LOD and LOQ values obtained from the calibration curve were 0.0377µg/ml and 0.1144 µg/ml respectively.

Lumefantrine
Linearity was observed with concentrations of LUM over the range of 6 -54 Mg/ml Within this range, graphs of

area against concentration values of Artemether gave straight lines with R2 values of above 0.99969 in all instances. This shows that there is linear relationship between the Artemether concentrations and the area values between 263.60-283.40 nm.

Also, with respect to accuracy, there was an average percentage recovery of 100.4% of LUM when LUM reference sample was mixed with tablet excepients and analyzed.

A good precision was observed with the method. The intra-day and inter-day precision assessment yielded % RSD values of 0.54% and 0.61% respectively. (**Table 4 & 5**).

Calculating the LOD and LOQ from the calibration curve gave their values as 1.124 µg/ml and 3.408 µg/ml respectively.

The validated method was applied for the assay of commercial tablet of ATMITHER AL the results 97.1%±0.1 for ART and 95.5%±0.1 for LUM in combination. (**Table 6**).

The advantages of this method of analysis is that fixed dose artemether-lumefantrine combination tablets can be analyzed using this method. This is because of artemether and lumefantrine cannot interferes each other at the selected wavelength of 215.80-245 for ART and 263.60-283.40 for LUM respectively. The major limitation of this method is that, ART does not show any significant absorption in the effective wavelength region of the UV-Visible spectroscopy and also lacks specific chemical groups, so the significant UV absorption is achieved by treating the drug with derivatizing agent.

Table 1: Regression analysis data and summary of validation parameters for the first Derivative Spectrophotometric method.

Parameters		**ART**	**LUM**
Wavelength range (nm)		215.80-245.0 nm	263.60-283.40
Beer's law limit (µg/ml)		1-9 µg/ml	6-54 µg/ml
Regression equation (y = mx + c)		Y=0.03000x+0.21940	Y=0.00361x+0.00355
Slope (m) Intercept (c)		M=0.03000 C=0.21940	M=0.00361 C=0.00355
Correlation Coefficient (r2)		0.99930	0.99969
Accuracy (Recovery) (n = 3)	Level I	8.98	99.9
	Level II	9.98	100
	Level III	12	101.5
Method precision (Repeatability) (% RSD, n = 5),		0.618%	1.11%
Interday (n = 3) (% RSD)		0.246 %	0.61 %
Intraday(n = 3) (% RSD)		0.226%	0.54 %
LOD (µg/ml)		0.0377µg/ml	1.124µg/ml
LOQ (µg/ml)		0.1144µg/ml	3.408µg/ml
Assay ± S. D. (n = 6)		97.1±0.1	99.5±0.1

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

Table 2: Repeatability Data for the first order derivative spectrophotometric Method. (n=5).

Concentration (ART:LUM) (1:6 µg /ml)	**ART**	**LUM**
	(215.80-263.60-283.40)	**(263.60-283.40 nm)**
1	0.345	0.143
2	0.343	0.144
3	0.345	0.142
4	0.342	0.141
5	0.340	0.140
Mean	0.343	0.142
SD	0.00212	0.00158
%RSD	0.618%	1.11%

Table 3: Results of method precision for intra - day precision.

Concentration (µg/ml).		Sample area		Mean area± SD		% RSD	
ART	LUM	ART	LUM	ART	LUM	ART	LUM
5	30	0.345	0.143	0.344±0.001	0.142±0.001	0.29	0.70
		0.344	0.141				
		0.343	0.142				
7	42	0.474	0.200	0.473±0.001	0.201±0.001	0.21	0.49
		0.473	0.201				
		0.472	0.202				
9	54	0.546	0.220	0.547±0.001	0.221±0.001	0.18	0.45
		0.547	0.221				
		0.548	0.222				

Table 4: Results of method precision for inter- day precision.

Concentration (µg/ml).		Sample area		Mean area± SD		%RSD	
ART	LUM	ART	LUM	ART	LUM	ART	LUM
5	30	0.345	0.143	0.345±0.001	0.144±0.001	0.28	0.69
		0.344	0.144				
		0.346	0.145				
7	42	0.474	0.200	0.474±0.001	0.202±0.0014	0.21	0.69
		0.473	0.202				
		0.475	0.204				
9	54	0.546	0222	0.545±0.0014	0.222±0.001	0.25	0.45
		0.544	0.223				
		0.547	0.221				

Table 5: Recovery data of proposed method.

Drug	Accuracy Level (%)	Actual amount (µg/ml)	Amount added (µg/ml)	Amount found (µg/ml)	% Recovery (Average)	Mean ±SD	%RSD
ART	80%	5	4	8.98	99.7	100.8±1.87	1.86
	100%	5	5	9.98	99.8		
	120%	5	6	11.33	103		
LUM	80%	30	24	53.99	99.9	100.4±0.9	0.896
	100%	30	30	60	100		
	120%	30	36	67	101.5		

S. D. is Standard deviation and n is number of replicate

LOD and LOQ

LOD and LOQ values for ART found to be 0.0377 and 0.1144 µg/ml at wavelength between 215.80-245.0 nm, and LUM were found to be 1.124 µg/ml and 3.408 µg/ml at wavelength between 263.60-283.40 nm. Low value of LOD & LOQ indicates that the method is sensitive.

Table 6: Assay of artemether and lumefantrine in tablet formulation.

Formulation	Drug	Label claim(mg/tab)	Sample solution concentration(µg/ml)	Amount found ±SD	% recovery	% RSD
I	ART	80	7	6.8±0.1	97.1%	1.4%
	LUM	480	42	41.8±0.1	99.5%	0.23%

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 Derivative UVSpectrometry 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.

ACKNOWLEDGEMENT

The Authors are thankful to Mylan laboratories Ltd, hyderabad. Who supplied Artemether and Lumefantrine standard for the present study The different solid pharmaceutical formulations having ART and LUM as API were obtained as gift sample from Alvizia Healthcare, Chandigarh.

CONFLICT OF INTEREST

Authors do not have any conflict of interest.

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