Karajgi S. R. et al /J. Pharm. Sci. & Res. Vol. 12(2), 2020, 258-263

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Simultaneous Determination of Artemether and Lumefantrine by Area Under Curve UV Spectrophotometric Method

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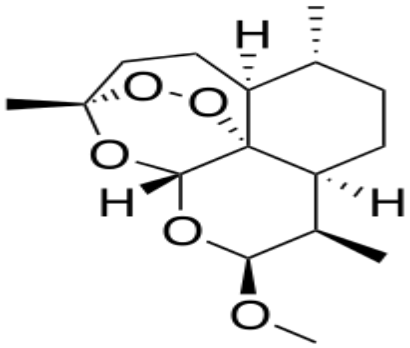
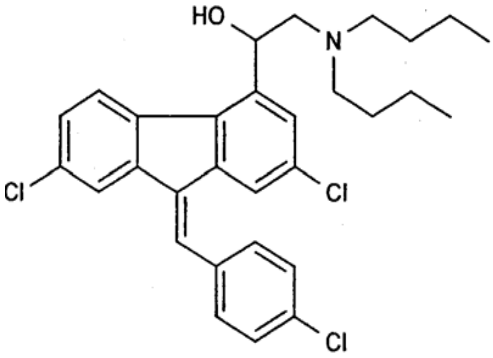
Abstract:
A new area under curve uv spectrophotometric method has been developed for the simultaneous determination of Artemether and Lumefantrine has been developed. The spectroscopic method for estimation of Artemether and Lumefantrine employed Area under curve method for analysis using Ethanol as solvent. Artemether has absorbance maxima 212 nm and Lumefantrine has absorbance maxima 232nm and both these drugs obey Beer's law in concentration range of 10 -30µg/ml for Artemether and 24 - 44µg/ml for Lumefantrine. The recovery studies ascertained the accuracy of the purposed method and the results were validated as per ICH guidelines. The results were found satisfactory and reproducible. The method was applied successfully for the estimation of Artemether and Lumefantrine in tablet dosage form without the interference of common excipients.

Key Words: Artemether, Lumefantrine; Area under curve; Simultaneous; Estimation

INTRODUCTION



Artemether



Lumefantrine

Artemether is a methyl ether derivative of artemisinin, which is a peroxide lactone withdrawn from the antimalarial plant Artemisia annua. It is an antimalarial for the treatment of multiple drug strains of Plasmodium falciparum malaria, It is the most essential medications required in a primary health system. Artemether is effective against the blood schizonts of both the malarial parasites P. falciparum and the P. vivax. It, however, may not be as good as artesunate for severe malaria. [1] Lumefantrine (or benflumetol) is an antimalarial drug. It is only used in combination along with artemether. The term "co-artemether" is formerly used to describe this combination. [2]. Artemether and lumefantrine both work by interrupting with the capability of the malaria parasites to convert haem into haemozoin. This produce levels of the toxic haem to increase, which put away the blood stages of the malarial parasites and blocks the infection from continuing. [3]. Review of literature revealed that there are very few methods reported for the estimation of Artemether and Lumefantrine in combined dosage forms. Eight HPLC methods [4-12], one UV spectrophotometric absorption factor correction method [13] and one UV simultaneous equation method were reported [13] and no area under curve method has been so far reported for simultaneous estimation of these drugs in combined dosage form. So, the aim and objective of present study were to develop an area under curve UV-Visible spectrophotometric analytical method for the estimation of Artemether and Lumefantrine in bulk and formulated dosage form in combination without prior separation and to establish a simple, sensitive, standard, reproducible method for the quality control of these drugs in combined dosage forms.

MATERIALS AND METHODS

Shimadzu 1800 spectronic double beam UV-visible spectrophotometer with 1 cm matched quartz cells, was used for all the measurements. Ethanol (95%) A.R. Grade (Qualigens, Fine chemicals) was used as the solvent. Commercial branded tablets were obtained from local market for assay and recovery studies.

Selection of Analytical Wavelength Ranges:

The standard solution of Artemether (10 µg/ml) and Lumefantrine (20 µg/ml) were scanned separately in the wave length range of 200 to 260nm for Artemether and 200-400nm for Lumefantrine and the absorption maximum was found to be 212nm and 262 nm for Artemether and Lumefantrine respectively in first derivative mode at N=5. (Figure 1 and Figure 2)

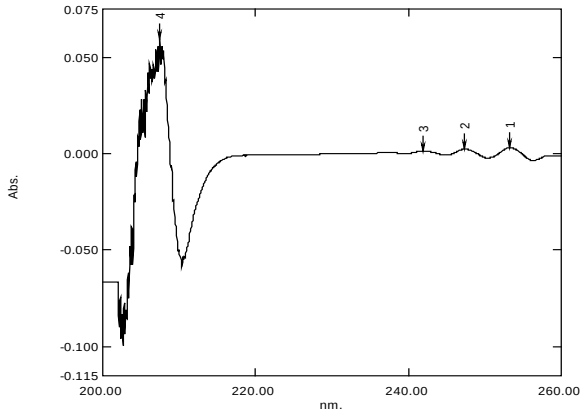


Figure 1: Spectra for Artemether of conc. 10 µg/ml showing λmax.

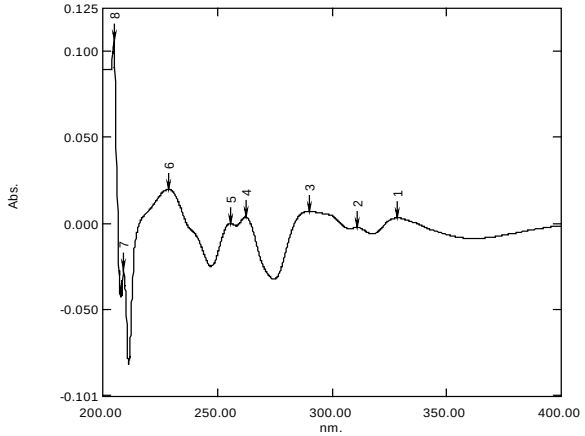


Figure 2: spectra for Lumefantrine of conc. 20 µg/ml showing λmax

Preparation of calibration curve:

From the standard stock solution of Artemether, appropriate aliquots were pipetted out in to 10 ml volumetric flasks and dilutions were made with ethanol to obtain working standard solutions of concentrations 10, 15, 20, 25 and 30 µg/ml. Similarly from the standard stock solution of Lumefantrine subsequent dilution were made with ethanol to obtain working standard solutions of concentrations 24, 28, 32, 36, 40 and 42µg/ml. The area between 210 to 214nm for Artemether and area between 260 to 264nm for Lumefantrine was fixed as analytical wavelength for the determination in the first derivative mode with N=5**.** (Table 1) The calibration curve of both the drugs was plotted. (Figure 3 to Figure)

Table 1: Calibration Data for Artemether and Lumefantrine

Sr. No	Artemether		Lumefantrine	
	Conc. (µg/ml)	Area between 210-214nm	Conc .(µg/ml)	Area between 260-264nm
1	0	0	0	0
2	10	0.0924	24	0.0128
3	15	0.1392	28	0.0149
4	20	0.1872	32	0.0171
5	25	0.2382	36	0.0192
6	-	-	40	0.0213

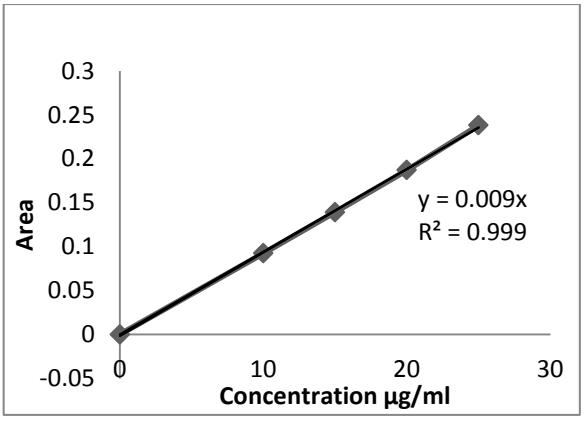


Figure 3: Calibration Plot of Artemether

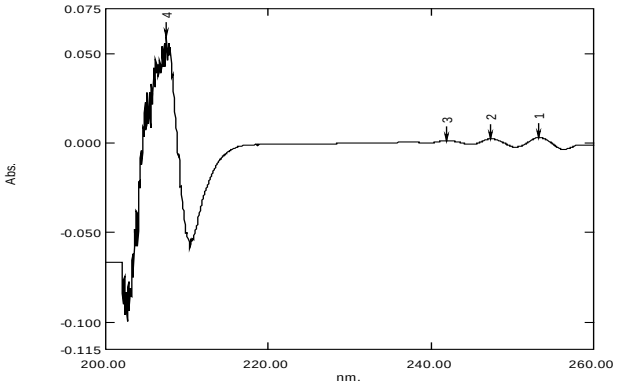


Figure 4: Area Under curve of Artemether 10 µg/ml

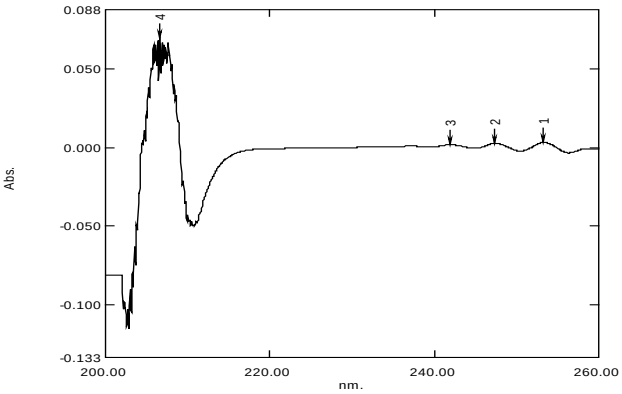


Figure 5: Area Under curve of Artemether 15 µg/ml

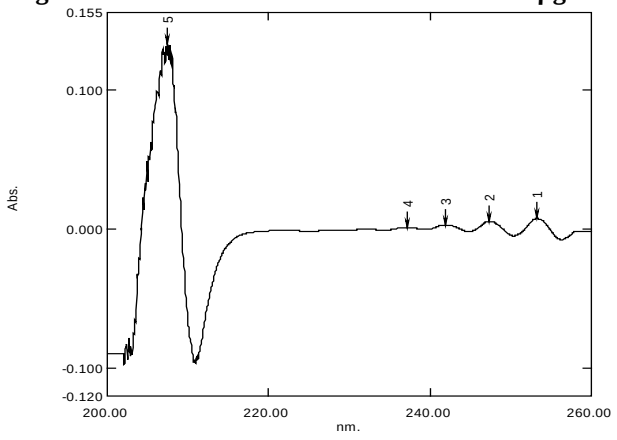


Figure 6: Area Under curve of Artemether 20 µg/ml

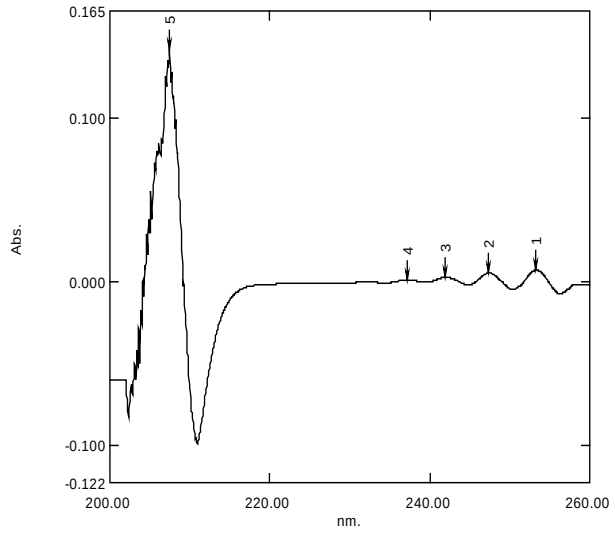


Figure 7: Area Under curve of Artemether 25 µg/ml

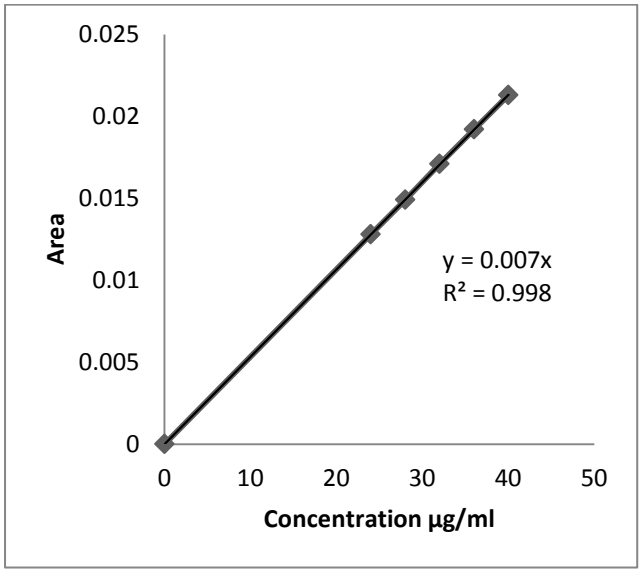


Figure 8: Calibration curve of Lumefantrine at 262 nm

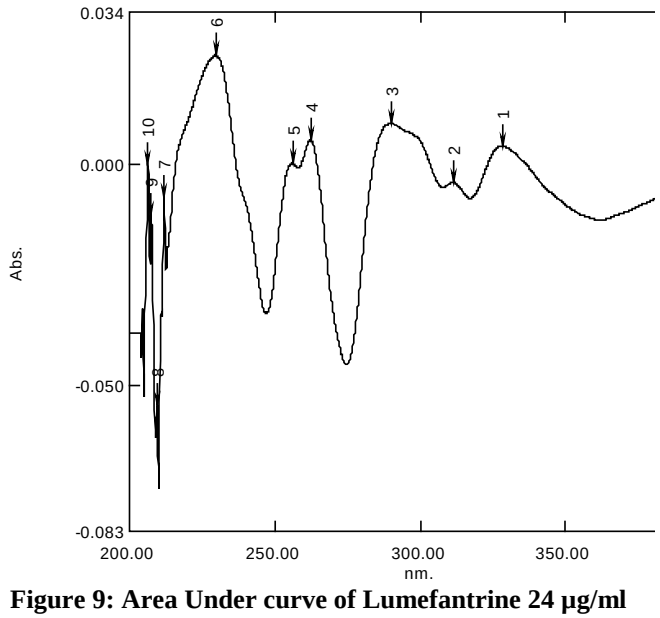


Figure 9: Area Under curve of Lumefantrine 24 µg/ml

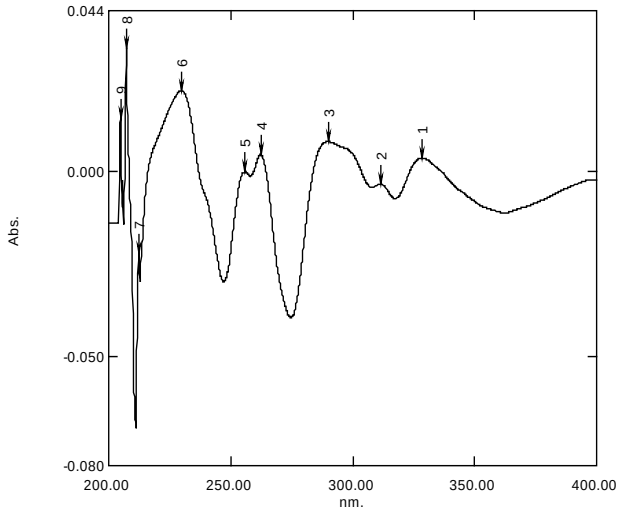


Figure 10: Area Under curve of Lumefantrine 28 µg/ml

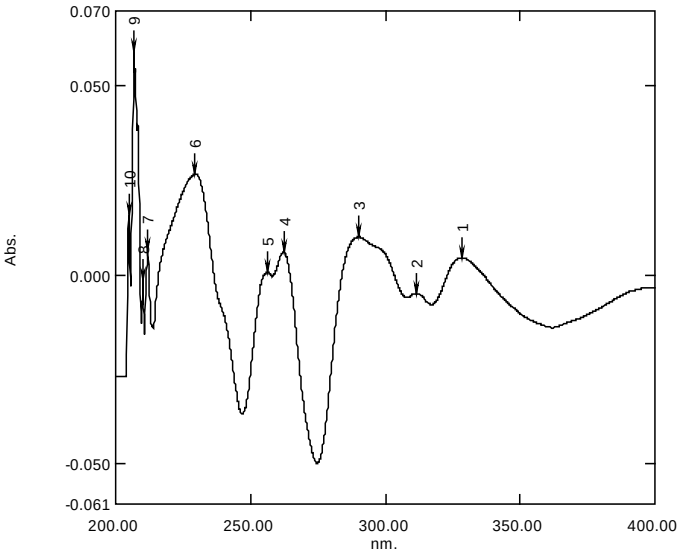


Figure 11: Area Under curve of Lumefantrine 32 µg/ml

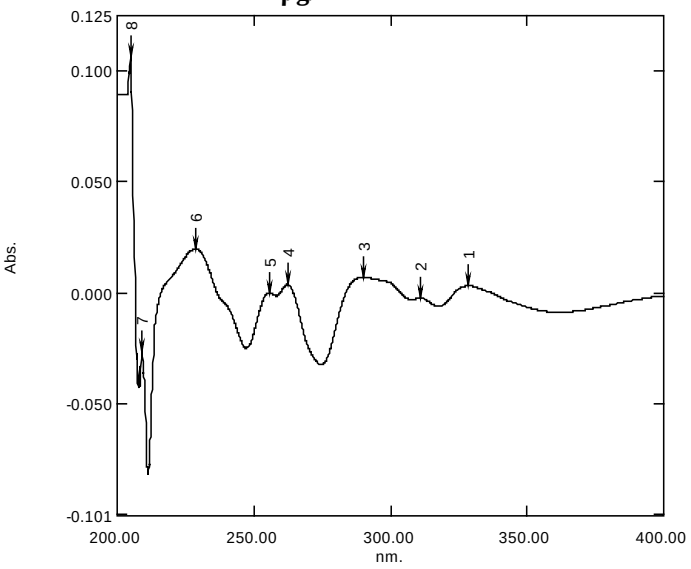


Figure 12: Area Under curve of Lumefantrine 36 µg/ml

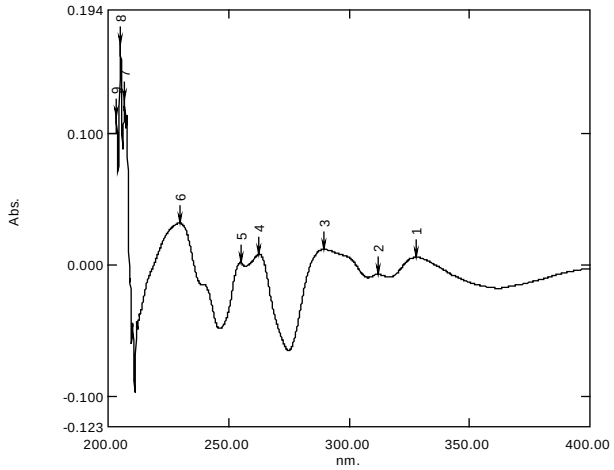


Figure 13: Area Under curve of Lumefantrine 40 µg/ml

The concentration range over which the drugs followed linearity was chosen as an analytical concentration rang i.e. 10 to 30µg/ml for Artemether and 24 to 44µg/ml for Lumefantrine.

Estimation of drug from dosage form:

Twenty tablets of a commercial brand were weighed, and finely powdered. A quantity of powder sample equivalent to 100mg of Artemether and 100mg of Lumefantrine was taken in volumetric flask and dissolved in ethanol. Further dilution was made to get concentration of 25µg/ml of Artemether and Lumefantrine. These concentrations were scanned at different wavelength between 210 to 214nm for Artemether and 260 to 264nm for Lumefantrine in First derivative mode with N=5. (Table 2)

Accuracy (Recovery Test):

Accuracy of the method was studied by recovery experiments. The recovery experiments were performed by adding known amounts to tablet. The recovery was performed at three levels, 80, 100 and 120% of Artemether and Lumefantrine standard concentration. The recovery samples were prepared. Three samples were prepared for each recovery level. The solutions were then analyzed, and the percentage recoveries were calculated using formula:

$$\%\,\textbf{recovery} = \frac{\textbf{Observed amount of compound in sample}}{\textbf{Amount of all compound present in sample}} \times 100$$

The details are given in Table 3 and Table 4

Precision Study:

The dilution was made to get concentration of 25µg/ml of Artemether and Lumefantrine these concentrations were scanned at different wavelength i.e.212nm for Artemether and 262nm for Lumefantrine in Area under curve mode by four different analysts. The precision values are summarized in the Table 5 and Table 6.

Table 2: Assay of Artemether and Lumefantrine in Tablet formulation

Drug	Label Claim (mg/tab)	Amount Found (mg/tab)	% of Label Claim	Mean %	SD	CV
Artemether	80	81.41	101.77	100.29	0.8760	0.7719
	80	80.68	100.85			
	80	79.96	99.95			
	80	80.16	100.20			
	80	79.60	99.50			
	80	79.60	99.51			
Lumefantrine	480	479.52	99.90	100.10	0.4049	0.1639
	480	479.90	99.98			
	480	479.76	99.95			
	480	480.62	100.13			
	480	484.32	100.90			
	480	478.94	99.78			

Table 3: Results of accuracy parameter of Artemether

Level of % Recovery	Amount present (µg/ml)	Amount of standard added (µg/ml)	Total amount recovered (µg/ml)	% Recovery	% mean Recovery	SD	CV
80	80	64	145.29	100.90	100.24	0.5729	0.3283
80	80	64	143.81	99.87			
80	80	64	143.92	99.95			
100	80	80	161.31	100.82	100.26	0.4808	0.2312
100	80	80	159.92	99.95			
100	80	80	160.04	100.03			
120	80	96	176.63	100.36	100.43	0.3404	0.1159
120	80	96	176.22	100.13			
120	80	96	177.40	100.80			

Table 4: Results of accuracy parameter of Lumefantrine

Level of % Recovery	Amount present (µg/ml)	Amount of standard added (µg/ml)	Total amount recovered (µg/ml)	% Recovery	% mean Recovery	SD	CV
80	480	384	857.60	99.26	99.67	0.4801	0.2305
80	480	384	865.72	100.20			
80	480	384	860.19	99.56			
100	480	480	950.20	98.98	99.70	0.6335	0.4014
100	480	480	959.71	99.97			
100	480	480	961.53	100.16			
120	480	576	1,054.73	99.88	99.98	0.9493	0.9010
120	480	576	1,066.34	100.98			
120	480	576	1,046.39	99.09			

Table 5: Data for precision of Artemether

Sample Number	Assay of Artemether as % of Labeled amount			
	Analyst-I	Analyst-II	Analyst-III	Analyst-IV
1	99.62	100.12	99.97	100.10
2	101.08	101.21	100.98	101.03
3	100.16	100.04	99.95	99.98
4	99.56	99.85	100.02	99.92
5	99.89	100.02	99.96	100.20
6	99.88	99.93	99.86	100.23
Mean%	**100.03**	**100.19**	**100.12**	**100.24**
S.D	**0.5568**	**0.05059**	**0.4228**	**0.4038**
CV	**0.3100**	**0.2559**	**0.1788**	**0.1630**

Table 6: Data for precision of Lumefantrine

Sample Number	Assay of Artemether as % of Labeled amount			
	Analyst-I	Analyst-II	Analyst-III	Analyst-IV
1	99.45	99.72	99.68	99.61
2	100.65	99.97	100.52	99.98
3	99.78	99.63	99.82	99.56
4	99.92	100.05	99.96	99.92
5	99.20	99.48	99.63	99.68
6	99.16	99.36	99.40	99.48
Mean%	**99.69**	**99.70**	**99.83**	**99.70**
S.D	**0.5587**	**0.2708**	**0.3847**	**0.2015**
CV	**0.3122**	**0.0729**	**0.1480**	**0.0406**

RESULTS:

The standard solutions of Artemether and Lumefantrine in Ethanol (10µg/ml each) subjected to a scan individually at the series of wave-lengths of 200nm to 400nm at AUC mode and the AUC spectra were taken at N=5 using Shimadzu 1800 spectronic UV Visible spectrophotometer. λ max of Artemether and Lumefantrine was found to be at 212nm and 262nm respectively. Therefore, 212nm was selected as λmax of Artemether and 262nm was selected as λmax of Lumefantrine for the present study. The calibration curve of Artemether was found to be linear in the concentration range of 10 to 30µg/ml in an area between 210nm to 214nm and the calibration curve of Lumefantrine was found to be linear in the range of 24 to 44µg/ml in an area between 260nm to 264nm. Therefore, it was clear that Artemether and Lumefantrine can be determined in presence of each other with no intervention of any irrelevant substance in multi component combination pharmaceutical products.

With the intention of determining the practicability of the developed technique for the assessment of commercially available brands of medicinal formulations, the technique was initially attempted on bulk drugs in their synthetic mixture sample and concentrations were estimated. Then the technique was subjected to the assay of tablets in two marketed dosage brand that is Brand-A and Brand-B and adequate results were attained within the acceptable limits as per the content of the label claim for Artemether and Lumefantrine. The recovery experiments were conducted by adding known amounts to tablet. The recovery was performed at three levels, 80, 100 and 120% of Artemether and Lumefantrine standard concentration. Three samples were framed for each recovery level. The

solutions were then analyzed, and the percentage recoveries were found to be satisfactory within the acceptable limits as per the content of the label claim for tablet formulations.

The newly developed method was validated as per the ICH guidelines and parameters. The novel method for the quantitative investigation of Artemether and Lumefantrine was subjected to different validation parameters like specificity and selectivity in presence of formulation additives and excipients, studied for Linearity and range at different levels of concentrations and calibration standards where the determination range was optimized, accuracy was proved by recovery studies at different concentration levels, precision for Artemether and Lumefantrine was established through the analysis of sample by four different analyst using same instrument and same laboratory

DISCUSSION

The method was developed successfully for Artemether and Lumefantrine in their multi component dosage forms by Area under curve method. The newly developed method is an able method for routine analysis of Artemether and Lumefantrine in presence of each other and has clear advantage over economical factors compared to the application of reported HPLC methods for routine determinations. The method is better than absorption factor correction method and simultaneous equation methods which rely upon mathematical corrections.

CONCLUSION

From the experimental studies it can be concluded that Area Under Curve method is developed for Artemether and Lumefantrine in bulk and combined dosage form and first order derivative is developed for Artemether and Etodolac in bulk and single dosage form. The proposed method for the selected drugs was found to be accurate and precise. However, this method is more reproducible. The most striking features of spectrophotometric method are their simplicity and rapidity. Results of validation parameters demonstrate that these analytical procedures are suitable for its intended purpose and meet the criteria defined in ICH Q2A/B.

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