Journal of Pharma Research
Available online through
www.jprinfo.com

Research Article
ISSN: 2319-5622

Development and Validation of UV Spectrophotometric Methods for Simultaneous Estimation of Artemether and Lumefantrine in Synthetic Mixture and Tablet Dosage form

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Received on: 24-04-2014; Revised and Accepted on: 03-05-2014

ABSTRACT

The novel UV spectrophotometric methods were developed and validated for simultaneous estimation of Artemether and Lumefantrine in synthetic mixture and tablet dosage form. Simultaneous Equation method, Absorption correction method and first order derivative spectrophotometric methods were developed and validated. For all three methods Artemether showed good linearity over the range of 3-5 µg/ml and Lumefantrine showed linearity over the range of 18–30 µg/ml with r^2 greater than 0.9985.The wavelengths selected for Artemether were 254 nm, 254 nm and 236 nm and for Lumefantrine were 236 nm, 338 nm and 220.55 nm forSimultaneous Equation Method, Absorption Correction Method and First order derivative Method respectivelyThe percentage recoveries of Artemether and Lumefantrine for all three methods were found to be in the range of 98.28-101.71%and 98.08-102.00% respectively. Validation of the proposed methods was carried out for its accuracy and precision according to ICH guidelines.

Keywords: *Artemether, Lumefantrine, UV Spectrophotometric methods, Validation.*

INTRODUCTION

Artemether 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 [1] It is from sesquiterpene lactone endoperoxide class. The Endoperoxide Bridge in its molecule appears to interact with haeme in the parasite. Iron-mediated cleavage of the bridge releases highly reactive free radical species that binds to membrane proteins causes lipid peroxidation, damages endoplasmic reticulum, inhibits protein synthesis and ultimately results in lysis of the parasite [2, 3].

Lumefantrine is 2-Dibutylamino-1-[2,7-dichloro-9-(4-chlorobenzylidene)-9H-fluoren-4-yl]-ethanol.[4] It is from amino alcohol class. Lumefantrine is an orally active, high efficacy, long-acting erythrocytic schizontocide, related chemically and in mechanism of action tohalofantrine and mefloquine. It acts in the food vacuole of plasmodia to inhibit haeme polymerization. Additionally nucleic acid and protein synthesis of the parasite is affected [2, 3].

Literature review reveals that UV method was developed for Artemether [7, 8] and Lumefantrine [9] seperately and HPLC [10-13] method and RP-HPLC [14, 15] was developed for both drugs in combination. The present paper describes a simple, accurate and precise method for simultaneous estimation of Artemether and Lumefantrine in synthetic mixture and tablet dosage form. The proposed method is optimized and validated as per the International Conference on Harmonization (ICH) guidelines [15].

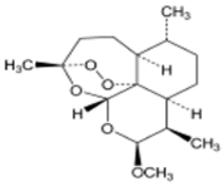

Fig. 1: Structure of Artemether (ART)

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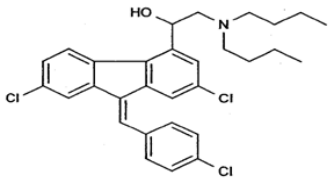

Fig. 2: Structure of Lumefantrine (LUM)

MATERIALS AND METHODS

Instrumentation: UV -Visible spectrophotometer Shimadzu-1800

Chemicals and reagents: Artemether and Lumefantrine working standards were procured from Mangalam Drugs and Organics Ltd., and the tested pharmaceutical formulations (Lumerax-80 Artemether 80mg and Lumefantrine 480mg) were procured from commercial pharmacy. Methanol and Conc. HCl were of suitable analytical grade.

Preparation of standard solution:

Standard stock solutions of pure drug containing 1000µg/ml of Artemether and Lumefantrine were prepared separately in 0.1M methanolic HCl. Standard stock solutions were further diluted to get working standard solutions of analytes in the concentration range of 3-5 µg/ml and 18-30 µg/ml of Artemether and Lumefantrine, respectively. In all flasks of working standard solutions add 2ml of 5N HCl and make to 10 ml with 0.1M methanolic HCl and scanned in the range of 400-200nm.

Preparation of synthetic mixture:

Pure API of Artemether and Lumefantrine were taken in a ratio of 7+42 mg, 8+48 mg, 9+54 mg Artemether and Lumefantrine respectively.

Preparation of sample solution:

Twently tablets were weighed and powdered. The average weight of powderwas calculated. The tablet powder equivalent to 8 mg of Artemether, 48 mg Lumefantrine was transferred to a 100 ml volumetric flask, dissolved in 0.1M methanolic HCl diluted up to the mark. The solution was filtered through Whatman filter paper no.42 and first few ml of filtrate were

discarded. 1 ml of this filtered solution was diluted to 10 ml with 0.1M methanolic HCl.5 ml of this solution was pipetted out and 2ml 5N HCl was added. Volume was made upto to 10 ml with 0.1M methanolic HCl.

Justification for use of 5N HCl:

Artemether has no chromophore group so it does not give in UV absorption spectra. Thus for that quantification of Artemether, its acid decompotition product α β unsaturated decalone is used which absorb UV light at 254 nm. The incubation period for acid decomposition product formation was assessed by adding 2 ml of 5N HCl. After 4 hr. the absorbance was measured at 254 nm.

Lumefantrine UV spectra were not affected by using 5N HCl. Because Lumefantrine acidic degradation product Desbenzyl keto dvt form at stressed condition and it wavelength maxima is 266.6 nm. A Lumefantrine wavelength maximum is 236 nm. Thus in 5N HCl Lumefantrine absorbance maxima is not changed.

RESULT AND DISCUSSION

- **Selection of wavelengths for Simultaneous eq. method:**

For Simultaneous equation method wavelength maxima of both the drugs were selected.

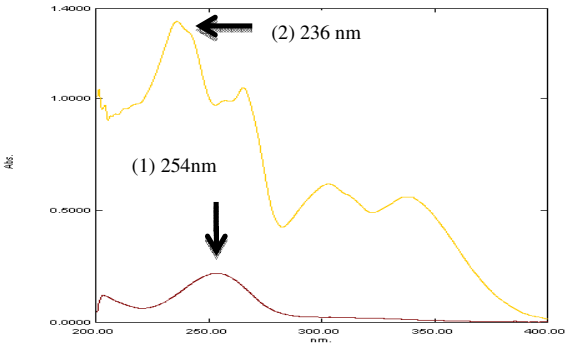

Fig. 3: Overlain spectra of (1) Artemether (5µg/ml) and (2) Lumefantrine (30µg/ml)

- **Selection of wavelengths for Absorption Correction method:**

In absorption correction method overlain spectra showed that Artemether has zero absorbances at 338 nm whereas Lumefantrine has subtantial absorbance. Thus Lumefantrine was estimated directly at 338 nm without interference of Artemether.

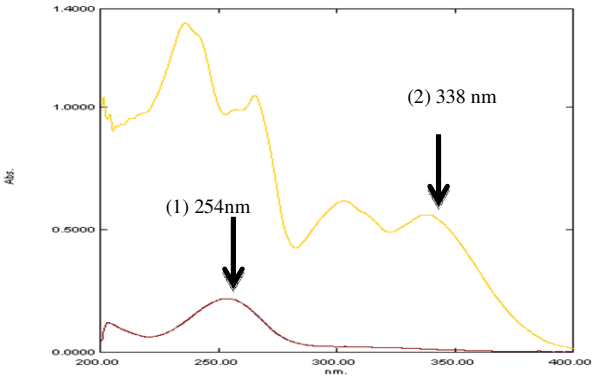

Fig. 4: Overlain spectra of (1) Artemether (5µg/ml) and (2) Lumefantrine (30µg/ml)

- **Selection of wavelength for First order derivative method:**

In First order derivative method overlain spectra showed thatZero crossing point for Lumefantrine was found at 236nm. So, Artemether was measured at 236nm.Zero crossing point (ZCP) for Artemether was found at 220.55 nm. So, Lumefantrine was measured at 220.55 nm.

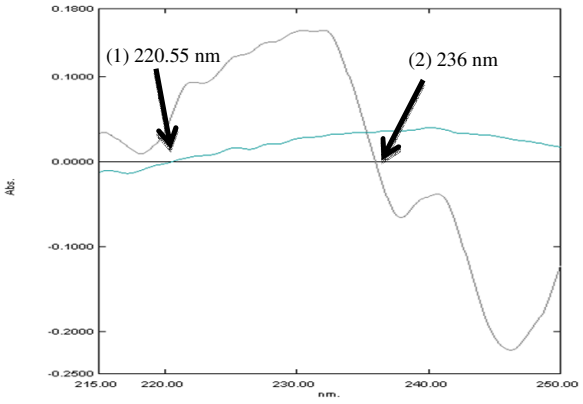

Fig. 5: Overlain spectra of (1) Artemether (5µg/ml) and (2) Lumefantrine (30µg/ml)

- **Calibration curve of Artemether:**

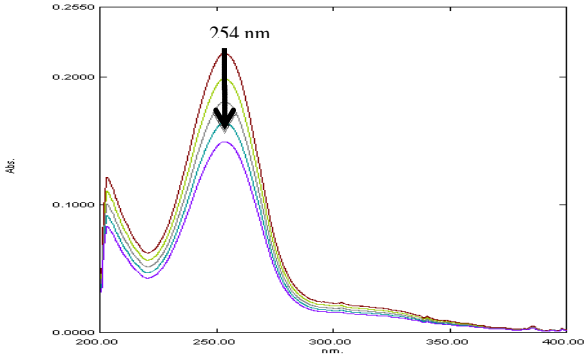

Fig. 6: Overlain spectra of Artemether (3-5µg/ml)

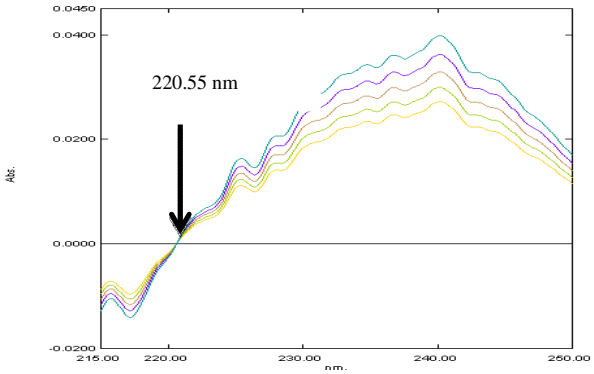

Fig. 7: Overlain first order derivative spectra of Artemether (3-5µg/ml) ZCP at 220.55nm

- **Calibration curve of Lumefantrine:**

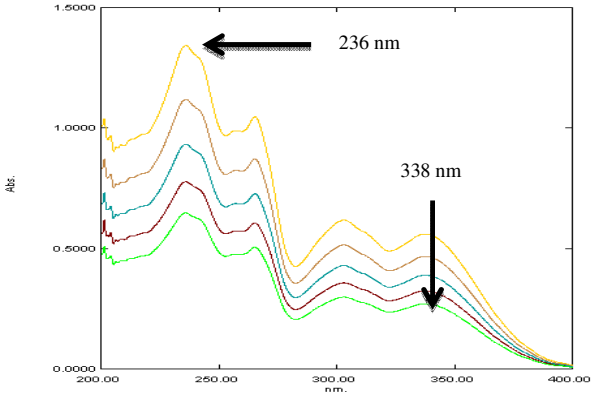

Fig. 8: Overlain spectra of Lumefantrine (18-30µg/ml)

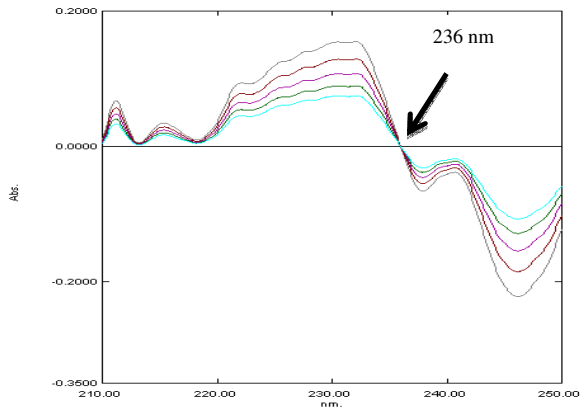

Fig. 9: Overlain first order derivative spectra of Lumefantrine (18-30µg/ml) ZCP at 236nm

Validation Parameters:

1. Linearity & Range:

Linearity was foud in the range of 3-5µg/ml for Artemether and 18-30µg/ml for Lumefantrine for all three methods. Co-relation Co-efficient was found to be greater than 0.9986 for all three methods.

2. Precision:

The precision expressed as standard deviation or relative standard deviation.

Intraday precision:

Pure API of Artemether and Lumefantrine were taken in a ratio was analyzed at three levels of concentration for three times in a day. Absorbances of the solutions were measured. The % RSD for Artemether and Lumefantrine were found to be less than 2% for all three methods.

Interday precision:

Pure API of Artemether and Lumefantrine were taken in a ratio was analyzed at three levels of concentration for three consecutive days. Absorbances of the solutions were measured. The % RSD for Artemether and Lumefantrine were found to be less than 2% for all three methods.

3. Accuracy:

The accuracy of the method was established using recovery technique i.e external standard addition method. The known amount of standard was added at three different levels to preanalyzed sample. Each determination was performed in triplicate. The result of recovery study is presented in **Table 1, 2,3.**

Table No. 1: % Recovery of Artemether and Lumefantrine using Simultaneous Equation Method

Assay Level	Tablet content taken eq. to (mg)		Standard added (mg)		Total drugs recoverd (mg)		%Recovery of standard added (n=3)	
	Art	Lum	Art	Lum	Art	Lum	Art	Lum
Blank	8	48	0	0	7.88	48.29	0	0
80%	8	48	6.4	38.4	14.20	86.12	98.75	98.51
100%	8	48	8	48	15.76	96.80	98.50	101.06
120%	8	48	9.6	57.6	17.55	105.20	100.72	98.80

Table No. 2: % Recovery of Artemether and Lumefantrine using Absorption Correction Method

Assay Level	Tablet content taken eq. to (mg)		Standard added (mg)		Total drugs recoverd (mg)		%Recovery of standard added (n=3)	
	Art	Lum	Art	Lum	Art	Lum	Arte	Lum
Blank	8	48	0	0	7.89	47.95	0	0
80%	8	48	6.4	38.4	14.35	86.64	100.93	101.01
100%	8	48	8	48	15.82	97.01	99.12	102.00
120%	8	48	9.6	57.6	17.65	104.75	101.66	98.61

Table No. 3: % Recovery of Artemether and Lumefantrine using First order Derivative Method

Assay Level	Tablet content taken eq. to (mg)		Standard added (mg)		Total drugs recoverd (mg)		%Recovery of standard added (n=3)	
	Art	Lum	Art	Lum	Art	Lum	Art	Lum
Blank	8	48	0	0	7.90	48.77	0	0
80%	8	48	6.4	38.4	14.35	86.55	100.80	98.40
100%	8	48	8	48	15.85	96.92	99.46	100.32
120%	8	48	9.6	57.6	17.34	106.83	98.40	100.80

4. LOD and LOQ:

Table No. 4: LOD & LOQ for all three methods

	Simultaneous Eq. Method		Absorption Correction Method		First order derivative	
	Arte	Lume	Arte	Lume	Arte	Lume
LOD (µg/ml)	0.29	2.04	0.29	1.64	0.38	1.75
LOQ(µg/ml)	0.88	6.19	0.88	4.97	1.17	5.32

5. Assay:

Table No. 5: Assay result for all three methods

Tablet content taken eq. to mg)		Amount found (mg)		Assay (% Estimated) (n=3)	
Art	Lum	Art	Lum	Art	Lum
Simultaneous Eq. Method:					
8	48	7.88	48.29	98.37%	100.61%
Absorption Correction Method:					
8	48	7.89	47.95	98.64%	99.90%
First Order Derivative Mehtod:					
8	48	7.90	48.77	98.77%	101.61%

CONCLUSION

The validated UV methods proved to be simple, less expensive, fast, accurate, and precise and thus can be used for routine analysis of Artemether and Lumefantrine in synthetic mixture and tablet dosage forms.

ACKNOWLEDGEMENTS

The authors are grateful to Mangalam Drugs and Organics Ltd., Vapi for providing gift samples of Artemether and Lumefantrine.

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Conflict of interest: The authors have declared that no conflict of interest exists.

Source of support: Nil