International Journal of Innovative Research and Advanced Studies (IJIRAS)
Volume 5 Issue 7, July 2018

ISSN: 2394-4404

Development And Validation Of Antimalarial Drugs In Combined Dosage Form Of Artesunate And Lumefantrine By Derivative Spectroscopic Method

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Abstract: ***UV Spectroscopic method it is a most powerful tool available for the study of atomic and molecular structure and is used in the analysis of wide range of samples. Objective of new method to develop simple, rapid and sensitive UV Spectrophotometry and First order derivative methods have been developed and validation of that method on Artesunate and Lumefantrine in combined dosage form. To knowledge, no analytical methods have been reported for analysis and estimation of artesunate and lumefantrine in combined dosage form. Therefore, it has created a need for new analytical methods for their simultaneous determination. Methanol and chloroform was used as a common solvent. In first order derivative method 263 nm wavelength used for artesunate and 368 nm wavelength used for Lumefantrine. The first order derivative method gives excellent recoveries 99.84 % for artesunate and 100.38 % for lumefantrine. LOD and LOQ are 0.21µg/ml and 0.65µg/ml for artesunate and 0.15µg/ml and 0.47µg/ml for lumefantrine respectively. Assay results were in good agreement with label claim. These method was developed and validated statistically. The method is simple and suitable for the artesunate and lumefantrine in combined dosage form.***

Keywords: Artesunate, Lumefantrine, First order derivative.

I. INTRODUCTION

Derivative spectrophotometry involves the conversion of a normal spectrum to its first, second or higher derivative spectrum. The transformations that occur in derivative spectra are understood by reference to a Gaussian band which represents an ideal absorption band. In this context of derivative spectrophotometry, the normal absorption spectrum is referred to as fundamental, zero order orD^0 spectrum. Conventional absorption spectrum is a plot of A vs. λ. In this technique plot of A vs. λ is transformed into plot of dA/dλ vs. λ (first derivative of the absorption spectrum) $d^2A/d^2\lambda$ vs. λ (second derivative) or higher derivative.

Derivative spectra often yield a characteristic profile where changes of gradient and curve nature in the normal (zero order) spectrum are observed as distinctive bipolar features. The derivative of an absorption spectrum represent the gradient at all points of the spectrum and can be used to locate hidden peak, since dA/dλ=0 at peak maxima. However, second and even order derivative are potentially more useful in analysis.

Here absorbance (A) of a sample is differentiated with respect to wavelength λ to generate first, second or higher order derivatives.

[A] = f (λ): Zero order,
[dA/dλ] = f (λ): First order,
$[d^2A/d^2\lambda]$ = f(λ): Second order, etc.

Zero order derivative yields smoothing of spectra, first order derivative spectra represents the gradient at all points of the spectrum and can be used to locate 'hidden' peaks while second and even higher order derivatives are potentially more use full in analysis. The method to generate derivative spectra are optical method and wavelength modulation method, first or second derivative spectra of individual component is generated, provided that peaks and valleys of X and Y are dissimilar. At wavelength of zero order crossing of derivative

spectra of X, the component Y should show some [dA/dλ] or [d^2A/d^2 λ] and vice-versa. Since the values [dA/dλ] or [d^2A/d^2 λ] also obey Beers –Lamberts law. First or second derivative spectra of various known concentration of mixture X and Y are analysed, taking the zero crossing wavelength of X to measure Y and vice- versa. Then calibration curve of [dA/dλ] or [d^2A/d^2 λ] vs concentration is prepared for each compound.

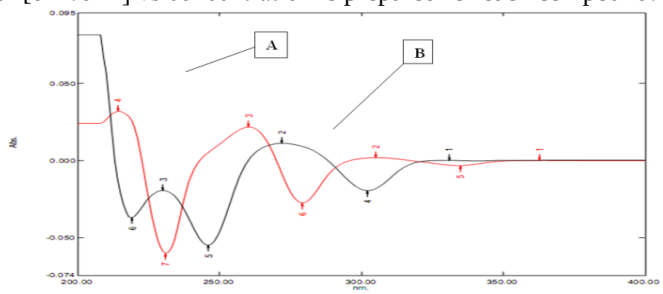

Figure 1: Derivative Spectra of Two Substances A and B

II. MATERIAL AND METHODS

- ✓ *Method of analysis used for the experimental work:* First order derivative UV spectrophotometric method
- ✓ *Materials:* The materials required for the present work were procured from diverse sources. Artesunate and Lumefantrine drugs and Methanol, chloroform, and other chemicals were used for the development validation of analytical method. All the other ingredients used were of analytical grade, Artesunate was procured from vital laboratory, Gujrat and Lumefantrine was procured from IPCA laboratory, Mumbai. Analytical grade chemicals were obtained from research laboratory Mumbai.

III. EXPERIMENTAL WORK

Instrument: UV Visible double beam spectrophotometer, JASCO UV-V-550 with matched pair quartz cells corresponding to 1 cm path length, with spectral bandwidth of 2nm.

Experimental work was carried by following ways-

A. SELECTION OF COMMON SOLVENT

Chloroform is selected as a common solvent for developing spectral characteristics of drugs. The selection was made after assessing the solubility of both the drugs in different solvents.

B. PREPARATION OF STANDARD DRUG SOLUTION

Standard stock solution containing Artesunate and Lumefantrine was prepared by dissolving 24 mg of Artesunate in 10 ml of chloroform and 4 mg lumefantrine separately in 10 ml of chloroform to get stock solution containing 2400 µg/ml of Artesunate and 400µg/ml of Lumefantrine in different 10ml volumetric flasks.

C. PROCEDURE FOR DETERMINING THE SAMPLING WAVELENGTH FOR SIMULTANEOUS ANALYSIS

By appropriate dilution of both standard drug solutions with chloroform, solutions containing 10µg/ml of Artesunate and 10µg/ml of Lumefantrine were scanned separately in a wavelength range 200-400 nm against chloroform as blank to determine the wavelength of maximum absorption for the drugs. The first derivative spectra were obtained by instrumental electronic differentiation in the range of 200-400 nm. A signal at 263 nm first derivative spectrum was selected for quantification of Artesunate, while a signal at 368 nm was selected for quantification of Lumefantrine, where both the drugs does not interfere each other.

D. PROCEDURE FOR PLOTTING CALIBRATION CURVE

From standard stock solutions, serially diluted standard solutions containing Artesunate and Lumefantrine at a concentration of 10, 20, 30, 40 and 50 µg/ml and 5, 10, 15, 20 and 25µg/ml respectively were prepared. The absorbances of above solutions were measured at the selected wavelength and the calibration curves were constructed by plotting the absorbance against the concentration for both drugs. Calibration curve for Artesunate was plotted by recording absorbance at the selected wavelength i.e. 263 nm and calibration curve for Lumefantrine was plotted by recording absorbance at 368 nm. Both the drugs obeyed Beer's law in the concentration range of 10-50µg/ml for Artesunate and 5-25µg/ml for Lumefantrine. By using quantitative modes of instrument, slope, intercept and correlation coefficient values for calibration curve was obtained for all the drugs. For Artesunate, the concentration in sample solution was calculated by using formula Abs=A+B*C, where A=intercept and B=slope and C=concentration of Artesunate. For Lumefantrine the concentration in sample solution was calculated by using formula Abs=A+B*C, where A=intercept and B=slope and C=concentration of Lumefantrine.

E. ANALYSIS OF MARKETED FORMULATION

A marketed tablet formulation containing Artesunate 240 mg and Lumefantrine 40 mg was used for sample preparation. Twenty tablet were weighed accurately, finely powdered and powder equivalent to 240 mg of Artesunate and 40 mg of Lumefantrine was weighed accurately and dissolved up to 100 ml of chloroform, solution was sonicated for 20 min, allowed to cool and then filter through filter paper. Final volume was made up to the mark with chloroform to get stock solution containing 2400µg/ml of Artesunate and 400µg/ml of Lumefantrine. The absorbances of standard and sample solutions were measured at 263 and 368 nm using solvent as a blank. The results were calculated by the formula generated. The statistical data obtained after replicate determination (n=3).

F. RECOVERY STUDY

Recovery study was determined by performing spiking addition of stock solution and make different concentrations of pure drug in the pre-analyzed sample. Results of recovery studies are shown in Table No. 4.

G. METHOD VALIDATION

The method was validated in terms of linearity, accuracy, precision and LOD and LOQ of the sample applications. The linearity of the method was investigated by serially diluting the stock solutions and measured the absorbance at 263 and 368 nm. Calibration curves were constructed by plotting the absorbance against the concentration. All the drugs show linearity in the concentration range. Method was validated as per ICH Q2B guidelines. Results of validation were shown in Table No. 7.

IV. RESULT AND DISCUSSION

CALIBRATION CURVE FOR ARTESUNATE

Sr.No	Concentration (µg/ml)	Mean absorbance	± SD	% RSD
1.	10	0.002097	0.00003112	1.4
2.	20	0.003418	0.00002930	0.85
3.	30	0.005052	0.00002219	0.43
4.	40	0.006704	0.00002022	0.30
5.	50	0.008436	0.00002863	0.33

Table 1: Concentrations for Calibration curve of Artesunate

CALIBRATION CURVE FOR ARTESUNATE

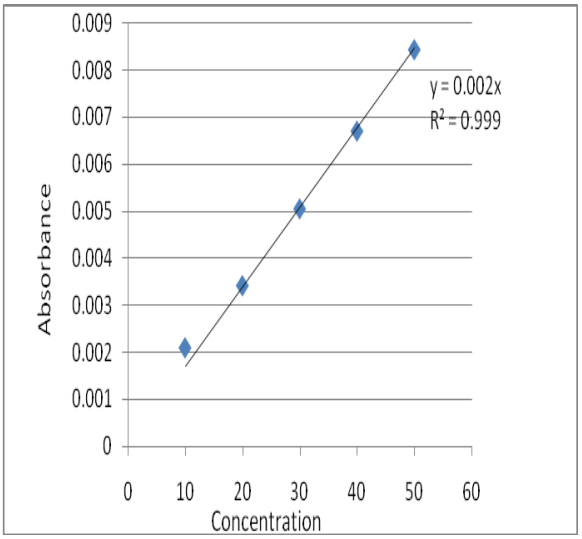

Figure 2: Calibration curve of Artesunate

RESULT OF CALIBRATION CURVE FOR LUMEFANTRINE

- ✓ Calibration curve : linear
- ✓ Expression : Abs=A+B*Conc.
- ✓ Factor : A=0.000
  B=0.002
- ✓ Coefficient : 0.999

Sr.No	Concentration (µg/ml)	Mean absorbance	± SD	% RSD
1.	5	0.001914	0.00002722	1.42
2.	10	0.003272	0.0000366	1.11
3.	15	0.004995	0.00001652	0.33
4.	20	0.006679	0.00002015	0.30
5.	25	0.008386	0.00001901	0.22

Table 2: Concentrations for Calibration curve of Lumefantrine

CALIBRATION CURVE OF LUMEFANTRINE

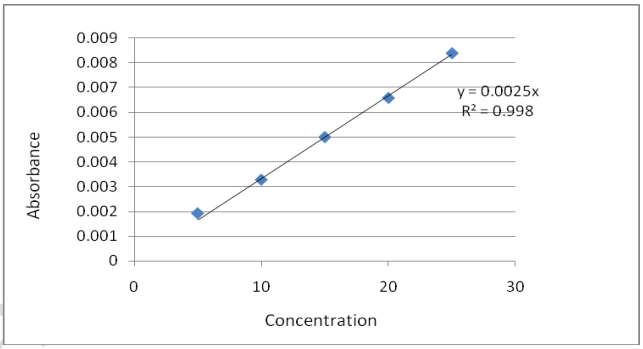

Figure 3: Calibration curve of Lumefantrine

RESULT OF CALIBRATION CURVE FOR LUMEFANTRINE

- ✓ Calibration curve : linear
- ✓ Expression : Abs= A+B* Conc.
- ✓ Factor : A =0.000
  B =0.0025
- ✓ Coefficient : 0.998

Proposed First Order Derivative UV Spectrophotometric method was validated as per ICH guidelines. Linearity of artesunate and lumefantrine was determined by using different concentrations. Artesunate was showed linearity in range of 10-50 µg/ml and Lumefantrine was showed linearity in the range of 5-30 µg/ml with correlation coefficient 0.999 for artesunate and 0.998 for lumefantrine.

ANALYSIS OF MARKETED FORMULATION

A marketed formulation containing artesunate 240 mg and lumefantrine 40 mg was used for analysis. The obtained statistical data after replicate determinations (n=3) was shown in Table No.3.

Drug	Amt. Present(mg/Tab)	Amt found (mg/Tab)	Mean	± S.D	% Label Claim estimated	% RSD
Artesunate	240	239.87 240.04 239.96	239.95	0.0051	99.75	0.021

Lumefantrine	40	39.94 41.07 39.93	39.90	0.0022	99.66	0.055

Table 3: Result of Analysis of Marketed Formulation

Marketed formulation was analysed and amount of drug determined by proposed method was in good agreement with the labelled claim. The results for the formulations were found to be 99.75 and 99.66 for artesunate and lumefantrine respectively and Result for analysis of formulation showed % relative standard deviation values in the range which indicates good repeatability of the method.

RECOVERY STUDIES

Accuracy was determined by performing recovery studies by spiking addition of pure drug in different concentrations in the pre-analyzed sample. Results of recovery studies are shown in Table No.4.

Drug	Conc.	Mean absorbance	±S.D.	%RSD	% Drug Recovery
Artesunate	50%	0.003499	0.00002	0.57	99.37%
	100%	0.004339	0.000033	0.76	98.61%
	150%	0.005603	0.000024	0.42	98.90%
Lumefantrine	50%	0.001124	0.000037	0.33	98.21%
	100%	0.001503	0.000030	0.20	101.59%
	150%	0.001836	0.000076	0.96	99.76%

Table 4: Recovery study of Artesunate and Lumefantrine

The recovery of sample artesunate and lumefantrine was expressed in terms of ±SD and %RSD. Percentage recovery values were found in the range of 98.21 to 101.59% with very small S.D.

PRECISION

PRECISION FOR ARTESUNATE AT 263NM WAVELENGTH

Concentration	Absorbance
10 µg/ml	0.0022
	0.002281
	0.00181
Mean	0.002097
±SD	0.000029
%RSD	1.45

Table 5: Precision study of Artesunate

PRECISION FOR LUMEFANTRINE AT 368 NM WAVELENGTH

Concentration	Absorbance
5 µg/ml	0.001953
	0.00181
	0.00198
Mean	0.001914
±SD	0.000012
%RSD	0.62

Table 6: Precision study of Lumefantrine

System reproducibility was determined by three replicate applications and three times measurement of a formulation at the analytical concentration. The reproducibility of sample was expressed in terms of ±SD and % RSD. There was no interference from the common excipients present in tablets.

Precision study of Artesunate and Lumefantrine showed that the method which developed is precise and the % RSD was found to be within the limit i.e.<2%. The result showed that the %RSD value was found to be less than 2.0%. The result signifies the proposed method is precise.

METHOD VALIDATION

All parameters are validated as per ICH guidelines. Accuracy, precision and linearity were checked. Result of validation was shown in table **7**.

Parameter	Artesunate	Lumefantrine
λ_{max}	263 nm	368 nm
Beer's law limit	10-60 µg/ml	5-30 µg/ml
Accuracy	99.84	100.38
Limit of detection	0.21	0.15
Limit of Quantification	0.65	0.47

Table 7: LOD and LOQ of Artesunate and Lumefantrine

Limit of detection and Limit of quantification were determined by standard deviation of response and slope of calibration curve. LOD and LOQ were found to be 0.21, 0.65 for Artesunate and 0.15, 0.47 for Lumefantrine respectively.

V. CONCLUSION

The proposed first order derivatives spectroscopic method is simple, reliable and selective provide satisfactory results. Moreover the shorter duration of analysis for Artesunate and Lumefantrine will make this method useful for routine quantitative analysis in pharmaceutical dosage forms. The analysis of tablet formulation containing both the drugs gives satisfactory results. The result indicated excellent recoveries 99.84 % for artesunate and 100.38 % for lumefantrine. LOD and LOQ are 0.21µg/ml and 0.65µg/ml for artesunate and 0.15µg/ml and 0.47µg/ml for lumefantrine respectively. Excellent statistical parameters and recovery data indicated that the method can be employed for efficient, rapid analysis of both drugs from tablet formulation. The results of analysis clearly indicated absence of interference from excipients in the formulation.

The proposed method for simultaneous estimation of artesunate and lumefantrine in combined dosage form was found to be simple, accurate and rapid. It can be employed for estimation of pharmaceutical formulations in quality control departments.

Method Parameter	**HPTLC methods for Arte and Lume**	
Analyte	**Artesunate**	**Lumefantrine**
Scanning Wavelength	263 nm	368 nm
Correlation Coefficient	0.999	0.998

LOD(µg/ml)	0.21	0.15
LOQ(µg/ml)	0.65	0.47
Mean recovery	99.84 %	100.38 %

Table 8: Summary

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