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SIMULTANEOUS ESTIMATION OF ARTEMETHER AND LUMEFANTRINE IN FORMULATION BY DERIVATIZED SPECTROPHOTOMETRIC METHOD

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ABSTRACT

Determination of Artemether and Lumefantrine (ATM-LUM) in combined pharmaceutical dosage form is difficult because of instability and absence of chromophore in Artemether molecule. For quality control and quality assurance checks Choice of an appropriate solvent to extract artemether and lumefantrine in final dosage form was a difficult challenge. There is a need to develop a reliable fast derivatized UV Spectroscopic method for estimation of combined ATM-LUM commercial Formulations. In the present analytical development Artemether and Lumefantrine were derivatized using methanolic hydrochloric acid. Both Artemether and Lumefantrine have absorbance maxima of 252 nm and 235 nm, respectively, and both satisfy Beer's law in concentration ranges of 4-20 µg/ml for Artemether and 2-10 µg/ml for Lumefantrine. The developed method was validated as per ICH guidelines. Without the interference of common excipients, the method was effectively used to estimate Artemether and Lumefantrine in suspension dosage form.

Keywords: - Artemether, Lumefantrine, Derivatization, Simultaneous, UV spectroscopy.

INTRODUCTION

An artemisinin-based combination therapy (ACT) combining artemether (ATM) and lumefantrine (LUM) is used to treat acute uncomplicated malaria caused by Plasmodium falciparum. Many Fixed dose combinations of ATM and LUM are commercially For Quality control of such formulations many HPLC (Reference) HPTLC methods are available. To avoid complexities tedious and requirement of costly solvents, fast and reliable Derivative UV method is developed to estimate ATM and LUM in commercial dosage forms as both the drug were having maximum wavelength near UV spectrum which is 210 and 218 which make estimation of both the drug difficult in the near UV region.

ATM is a chemical compound (3R, 5aS, 6R, 8aS, 9R, 10S, 12R, 12aR) Decahydro-10methoxy 1, 2 benzodioxepin, 3, 6, 9 trimethyl 3, 12 epoxy 12H pyrano [4, 3 j] Artemether is an antimalarial medication that is used to treat multidrug-resistant falciparum malaria and Plasmodium vivax. The antimicrobial therapy (AMT) is effective against blood flukes. Artemether has been found to have anticancer and antitumor properties.

2, 7-Dichloro-9-[(Z)-p-chlorobenzylidine] is the chemical formula for LUM. [(dibutylamino) methyl] - alpha - fluorene-4-methanol (fluorene-4-methanol). The parasites are killed by both ATM and LUM act. Figures 1 and 2 show the structures of ATM and LUM, respectively.

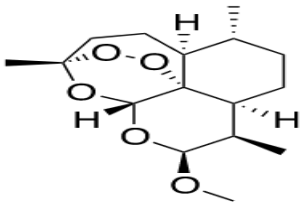

Figure no 1: Structure of Artemether

Over the range of 200-400 nm, a UV-spectrophotometer UV-1800 (Shimadzu, Japan) with a spectral bandwidth of 2 nm and 10 mm matched quartz cells was utilised to create an analytical procedure.

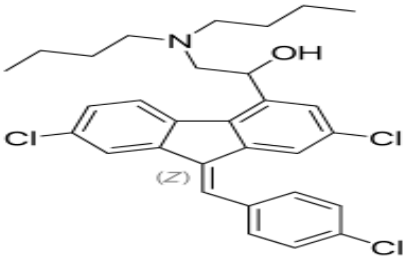

Figure no 2 : Structure of lumefantrine

UV spectroscopy is a versatile and well-established method for analysing pharmaceuticals, plant ingredients, food, biomolecules, environmental, and it has been one of the most extensively used procedures for determining the quality, authenticity, and purity of raw materials, crude pharmaceuticals, and market formulations for over 35 years. Other techniques could be used in some cases, but none compare to UV-Visible spectrometry in terms of simplicity, adaptability, speed, accuracy, and cost effectiveness.

Therefore, we developed and Validated a simultaneous method for estimation of ATM & LUM in the solid and liquid dosage forms.

MATERIALS AND METHODS

Materials:-

Artemether and lumefantrine were obtained as a gift sample from Medley Pharmaceuticals Ltd. All solvents used for analysis were of analytical grade and were procured from Vishal Chem. India.

Method:-

Derivatization of Artemether and Lumefantrine: -

Selection of solvent system for Derivatization

The solubility of artemether and lumefantrine was checked in different solvents viz., methanol, Acetonitrile (ACN), ethanol, DMSO, DMF, Chloroform, dilute HCl and Concentrated methanolic HCL at different temperatures (50, 60, 70). The drugs were found to be soluble in both dilute HCl and

prepared concentrated Methanolic HCL at 60°C. Therefore, prepared concentrated methanolic HCl and a temperature of 60°C was selected for derivatization.

Solvent	40°C	60°C	70°C
Methanolic HCl (0.01N)	Dissolve more than 4 hrs	3 hrs	Not stable
methanolic HCl (0.5N)	Dissolve upto 2 hrs	1 hour	Not stable

Preparation of methanolic HCL (0.5 M) system :-

About 21.5 ml of conc. HCl was pipetted in a 500 ml volumetric flask and mixed with 20 ml of distill water. The solution was then made upto 500 ml with methanol.

Standardization of solvent system :-

A Weighted quantity of dry anhydrous sodium carbonate was dissolved in conc. methanolic Hcl and heated in constant temperature bath for 270°C for 1 hour. while preparing 500 mL of Concentrated Methanolic HCL in a burette and a 10 mL solution of anhydrous sodium carbonate in a calibrated iodometric flask. As an indicator, methylene red reagent was utilised.

In an iodimetric flask, 3 drops of methylene red indicator were added, and titration began. A pink colour was created, but the titration continued until the pink colour disappeared. When the pink colour has faded, take notes on the readings. This technique was repeated three times, with average readings recorded. Calibration preparation

Procedure:-reference

Preparation of standard derivatized stock solution :-

The standard derivatized stock solution was made by transferring approximately but accurately weighed 5 mg of artemether and lumefantrine into a 25ml calibrated volumetric flask, adding 10 ml of prepared 1N concentrated methanolic HCL, and shaking the flask at 60 C for 1 hour. The solutions were allowed to cool at room temperature, and the volume was made up to mark with methanol. Different dilutions were made from this stock solution, ranging from 4 µg/ml to 20 µg/ml for artemether and from 2 µg/ml to 10 µg/ml.

Blank was made by heating 5 mL of methanolic HCL to the same temperature as the blank and diluting with methanol up to 10 ml.

Dilution absorbances were scanned against a blank at two wavelengths (252 and 235). To obtain absorbance at both wavelengths, these dilutions were also scanned in the UV range 200-400nm.

Selection of analytical wavelengths :-

From the standard stock solution, appropriate dilutions were made for each drug and scanned in the spectrum mode from 400 nm to 200 nm. Absorbance maxima were observed at 252 nm (Figure no 3) and 235 nm (Figure no 4) for ATM and LUM, respectively.

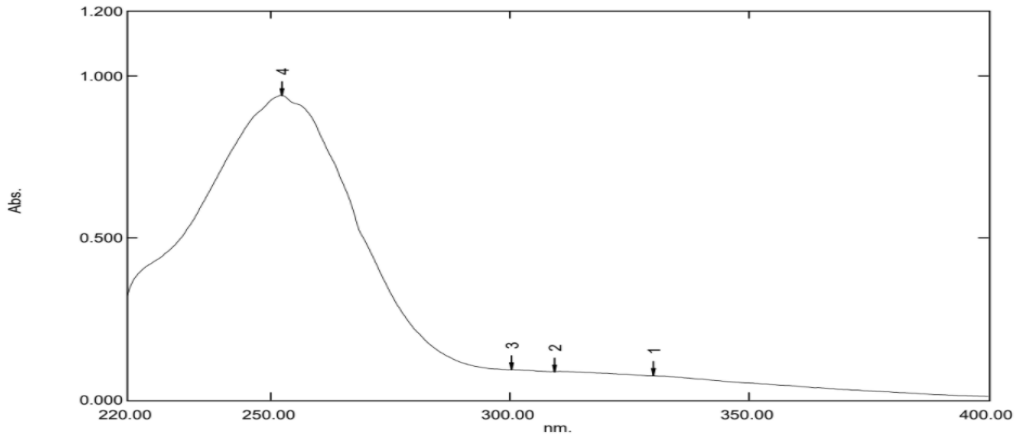

Figure no 3: UV spectrum of ATM

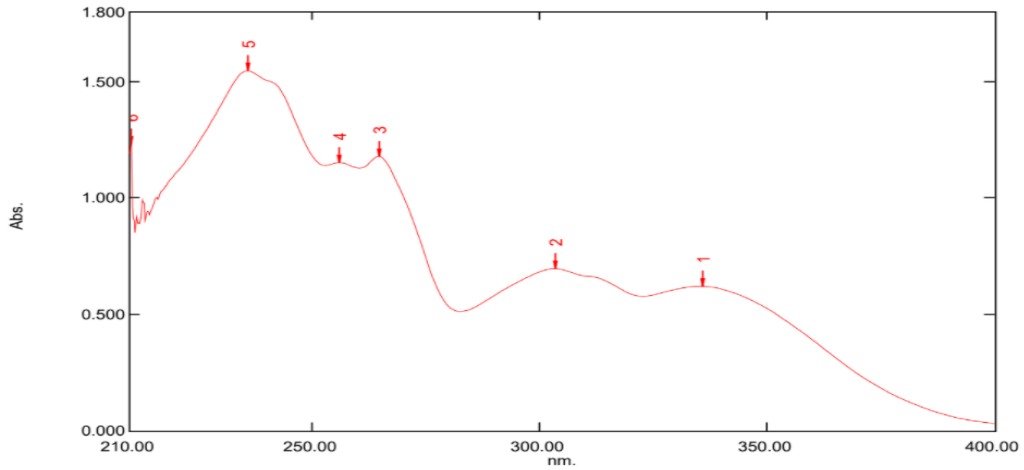

Figure no 4: UV spectrum of LUM

Preparation of calibration curve of artemether :-

The calibration curve was created by comparing the absorbance of different concentrations of artemether to a blank of 1N methanolic Conc HCL (methanol). The graph was made in order to investigate the linear relationship between absorbance and concentration. Different dilutions were made from this stock solution, ranging from 4 µg/ml to 20 µg/ml.

Sr no	Concentration(mcg/ml)	Absorbance
1	BLANK	0.00
2	4	0.170
3	8	0.359
4	12	0.558
5	16	0.701
6	20	0.853

Table no 1: Standard calibration curve table for ART at 252 nm

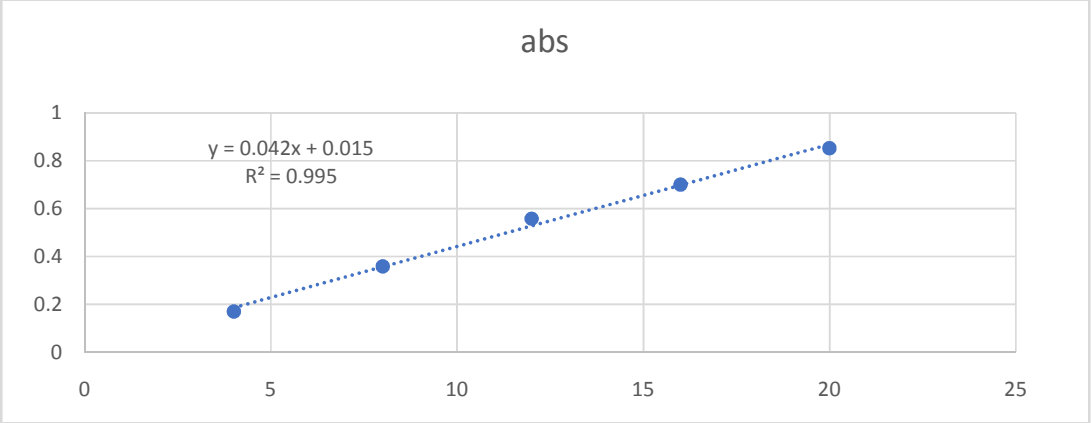

Figure no 5 : Calibration curve of ART

Figure no 5: Artemether shows the linearity range of 4 – 20 µg/ml having the line equation of y=0.0427x and regression value of 0.995.The regression value indicates that the point lies near the line approaches 1.

Sr no	Concentration(mcg/ml)	Absorbance
1	BLANK	0.00
2	4	0.174
3	8	0.374
4	12	0.590
5	16	0.721
6	20	0.887

Table no 2: Linearity range for ART at 252 nm

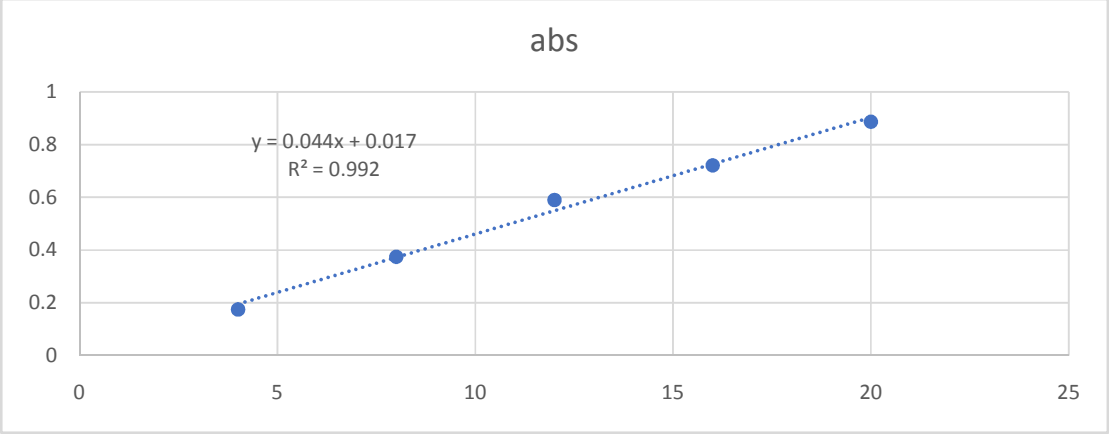

Figure no 6: Linearity of ART

Figure no 6: Artemether shows the linearity range of 4 – 20 µg/ml having the line equation of y=0.0443 and regression value of 0.9924.The regression value indicates that the point lies near the line approaches 1

Preparation of calibration curve of lumefantrine

The calibration curve was created by comparing the absorbance of v

arious lumefantrine concentrations against a blank of 1N methanolic conc HCL (methanol). The graph was made in order to investigate the linear relationship between absorbance and concentration**.** Different dilutions were made from this stock solution, ranging from 2 µg/ml to 10 µg/ml

Sr no	Concentration(mcg/ml)	Absorbance
1	BLANK	0.00
2	2	0.184
3	4	0.287
4	6	0.457
5	8	0.596
6	10	0.744

Table no 3: Standard calibration curve table for LUM at 235 nm

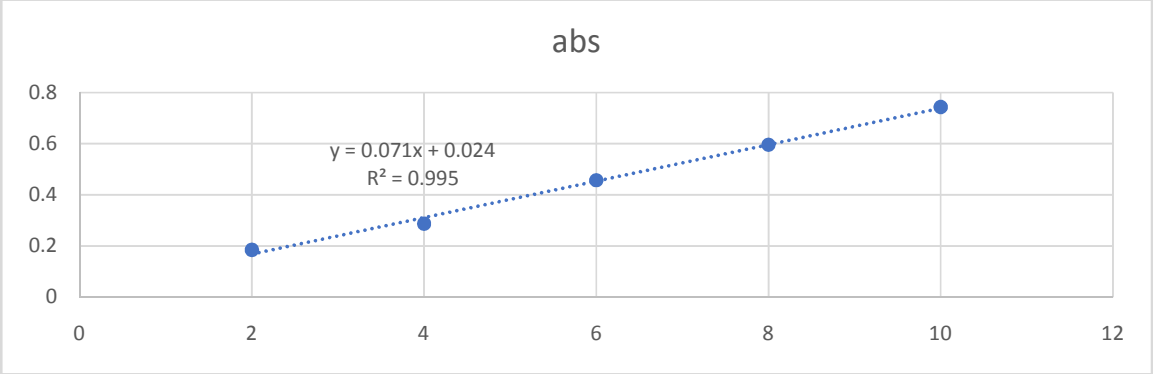

Figure no 7: Calibration curve of LUM

Figure no 7: Lumefantrine shows the linearity range of 2 – 10 µg/ml having the line equation of y=0.0715x and regression value of 0.9958.The regression value indicates that the point lies near the line approaches 1

Sr no	Concentration(mcg/ml)	Absorbance
1	BLANK	0.00
2	2	0.138
3	4	0.256
4	6	0.445
5	8	0.577
6	10	0.753

Table no 4: Linearity table for ART at 235 nm

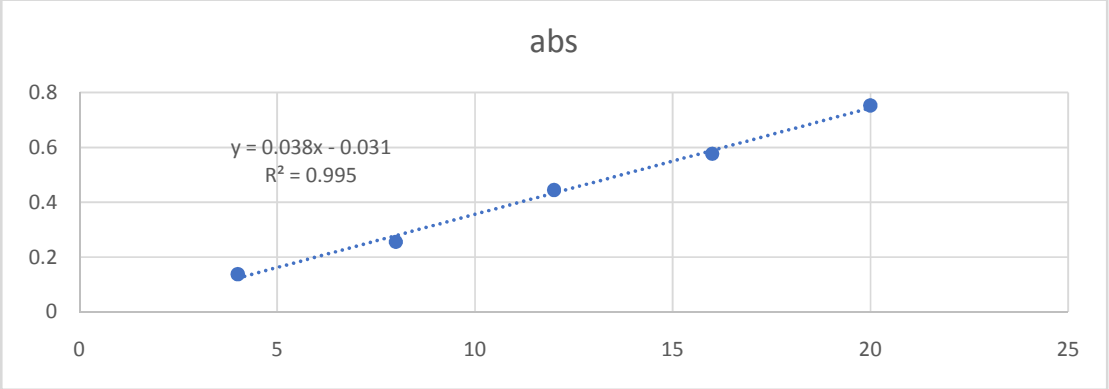

Figure no 8: Linearity of LUM

Figure no 8: Lumefantrine shows the linearity range of 2 – 10 µg/ml having the line equation of y=0.0388x and regression value of 0.9956.The regression value indicates that the point lies near the line approaches 1

Molar absorptivity and percent absorptivity :-

Molar absorptivity (ε) it is a measurement of how strongly a chemical species absorb light at a given wavelength. It is an intrinsic property of the species7.The actual absorbance (A) of a sample is dependent on the path length (l) and the concentration (c) and is given as-

[A = ε cl]

The unit used to describe the molar absorptivity is L/mol/cm8.

Calculation of molar absorptivity:-

Molar absorptivity (ε) = Absorbance/molar concentration

Calculation of percent absorptivity:-

% Absorptivity = Absorbance/concentration in mg/ml

Wavelength (nm)	Molar absorptivity of (ε) artemether	Molar absorptivity (ε)of lumefantrine
252	0.047	0.0571
235	0.294	0.0771

Table no 5 : Molar absorptivity of artemether and lumefantrine.

RESULTS AND DISCUSSION :-

Validation of developed method

The developed methods for simultaneous estimation of artemether and lumefantrine were validated as per ICH guidelines (ICH 1996)

S. NO	PARAMETERS	OFFICIAL IN

1	Specificity	USP,ICH
2	Selectivity	ISO 17025
3	Precision	USP,ICH
4	Repeatability	ICH,ISO 17025
5	Intermediate Precision	ICH
6	Reproducibility	ICH
7	Accuracy	USP,ICH,ISO 17025
8	Linearity	USP,ICH,ISO 17025
9	Limit Of Detection	USP,ICH,ISO 17025
10	Limit Of Quantitation	USP,ICH,ISO 17025
11	Robustness	USP included in ICH,ISO
12	Range	USP,ICH

Table no 6: Parameters for method validation with references to ICH, USP and ISO guidelines.

Linearity

The linearity of proposed method was determined from the calibration curve data of both the drugs that is artemether and lumefantrine. Artemether shows linear response between 4 - 20 µg/ml and lumefantrine shows linear response between 2 – 10 µg/ml. Acceptance criteria usually involve a Goodness of Fit test. A high correlation coefficient (r2) of 0.99 is often used as criterion of linearity. However this is not sufficient to prove that a linear relationship exists, and a method with a coefficient of determination of less than 0.99 may still fit for process**.**

Slope = 0.0456x for artemether

Slope = 0.0737x for lumefantrine

(r2) for artemether = 0.998

(r2) for lumefantrine = 0.9984

Precision:

Intraday Precision

Solutions containing 4-20 µg/ml of ART and 2-10 µg/mL of LUM were analyzed 3 times on the same day and % RSD was calculated.

Interday Precision

Solutions containing 4-20 µg/ml of ART and 2-10 µg/mL of LUM were analyzed 3 times on three consecutive days and % RSD was calculated.

Range :-

The range of an analytical technique is defined by the ICH as the interval between the higher and lower concentrations of analyte in a sample for which the analytical procedure has been proved to have a satisfactory level of precision, accuracy, and linearity. The range was calculated using data from a study of mixed standard medicines in various ratios. The method's range is between 1-6 g/ml.

Limit of detection :-

The lowest concentration of analyte in a sample that can be detected but not necessarily quantified using a certain method under the proper experimental conditions is known as the limit of detection. This limit is expressed in terms of analyte concentration in the sample.

% RSD = σ ×mean/100, CV= σ 2

Limit of detection of artemether

[LOD = 3.3 × σ/s]

Where, Standard error = 0.01438, Slope (s) = 0.0445 , LOD = 3.3×0.01438/0.0445 = 1.066766 µg/ml.

LOD of lumefantrine

Where, Standard error =0.019808, Slope (s) = 0.0718, LOD = 3.3 × 0.019808/0.0718 = 0.910407 µg/ml.

Limit of quantitation The Limit of quantitation is the lowest concentration of analyte in a sample which can quantitatively determine with suitable accuracy and precision under the stated operational condition of the method. Limit of quantitation can vary with the type of method employed and the nature of the sample. It is based on the standard deviation of the response and the slope.

Limit of quantitation of artemether

[LOQ = 10 × σ/ s]

Where, Standard Error =0.01438, Slope (s) = 0.0445, LOQ = 10 ×0.01438 /0.0445 = 3.232624.

Limit of quantitation of lumefantrine

Where, Standard Error = 0.019808, Slope (s) = 0.0718, LOQ = 10 × 0.019808/0.0718 =2.758809.

S.NO	PARAMETER	FOR ARTEMETHER	FOR LUMEFANTRINE
1	Beers Law	4 – 20	2 – 10
2	Λmax	252 nm	235 nm
3	Molar Absorptivity	AT 252 nm AT 235 nm	AT 252 nm AT 235 nm
4	%Absorptivity	AT 252 nm AT 235 nm	AT 252 nm AT 235 nm

Table no 7 : Optical characteristics of the developed method

Accuracy (Recovery Test):-

Accuracy of the method was studied by recovery experiments. The recovery experiments were performed by adding known amounts to suspension. 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:

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

Level of % Recovery	Formulation (ppm)	Amount of standard added (ppm)	Total amount recovered	%Recovery	% mean Recovery
80	8	6.4	14.25	100.39	101.26%
80	8	6.4	14.3	102.30	
80	8	6.4	14.38	101.9	
100	8	8.0	15.2	100	100.27%
100	8	8.0	15.3	100.40	
100	8	8.0	15.34	100.41	
120	8	9.6	17.65	98	99.5%
120	8	9.6	17.61	101	
120	8	9.6	17.74	100	

Table no 8: Results of accuracy parameter of Artemether

Level of % Recovery	Formulation (ppm)	Amount of standard added (ppm)	Total amount recovered	% Recovery	% mean Recovery
80	4	3.2	6.8	90	90.51%
80	4	3.2	6.89	100.25	
80	4	3.2	6.90	80.26	
100	4	4	7.91	98.98	99.63%
100	4	4	7.99	99.97	
100	4	4	8.01	99.16	
120	4	4.8	8.78	99	98.23%
120	4	4.8	8.88	102	
120	4	4.8	8.71	95	

Table no: 9: Results of accuracy parameter of Lumefantrine

S.NO	PARAMETERS	FOR ARTEMETHER	FOR LUMEFANTRINE
1	Linearity	4 – 20 µg/ml.	2 – 10 µg/ml.
2	Range	1 – 6 µg/ml.	1 – 6 µg/ml.
3	Precision (%RSD)		
	Intraday	0.02-0.6	0.03-0.5
	Interday	0.06-0.5	0.05-0.3
4	Correlation Coefficient (r2)	0.9933	0.9958
5	Intercept (b)	0.0098	0.0249
6	Slope (m)	0.04255	0.035725
7	RSD	0.2291653333	0.21184767
9	LOD	1.066766	0.910407
10	LOQ	3.232624	2.758809

Table no 10 : Summary of validated method

The current paper discusses the use of UV spectrophotometry to estimate and validate artemether and lumefantrine in suspension dose form. Following a review of the literature, it was discovered that artemether and lumefantrine can be estimated separately using several HPLC, other analytical methods, and that only a few individual methods for estimation of artemether and lumefantrine by UV spectrophotometry are available. Since there are relatively few methods for estimation of artemether and lumefantrine in combined dosage form, it was felt that developing a specific method for their combination was necessary. Simultaneous spectroscopic estimation of artemether and lumefantrine was performed using the simultaneous equation approach, in which separate forms of artemether and lumefantrine were estimated using 0.5 N concentrated methanolic HCL as a solvent and a calibration curve. The absorption maxima for artemether are at 252 and for lumefantrine are at 235. Beer's rule is followed across a concentration range of 4–20 µg/ml. , and 2–10 µg/ml. for lumefantrine. The devised approach has been validated in accordance with ICH criteria from 1996

CONCLUSION :-

The UV spectroscopic approach devised for the quantitative determination of artemether and lumefantrine is quick, cheap, linear, repeatable, specific, and economical. The method was validated, and all of the method validation parameters were found to be satisfactory. The suggested method can be used to analyse in-process quality control and marketed artemether and lumefantrine samples on a regular basis.

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