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A new RP-HPLC method development and validation for Simultaneous estimation of lumefantrine & artemether using bulk and Pharmaceutical Dosage Forms

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A B S T R A C T

A simple, rapid, accurate and precise isocratic reversed phase high performance liquid chromatographic method has been developed and validated for Simultaneous Estimation of Lumefantrine and Artemether in Combined Dosage Form. The chromatographic separation was carried out on Kromosil column (250×4.6mm× 5µ) with a mixture of Phosphate buffer (KH_2PO_4): Methanol (30:70, v/v) as a mobile phase at a flow rate of 1.0mL/min. PDA detection was performed at 238 nm. The retention times were 3.800 minutes and 2.343 minutes for Lumefantrine and Artemether respectively. Calibration plots were linear (r^2=0.999 for both Lumefantrine and Artemether respectively) over the concentration range of 72-168µg/mL for Lumefantrine and 16-24 µg/mL for Artemether. The method was validated for linearity, precision, accuracy, ruggedness and robustness. The proposed method was successfully used for simultaneous estimation of Lumefantrine and Artemether in combined dosage form. Validation studies revealed that the proposed method is specific, rapid, reliable and reproducible. The high % recovery and low % RSD confirms the suitability of the proposed method for routine quality control analysis of Lumefantrine and Artemether in bulk and tablet dosage forms.

Keywords: Lumefantrine, Artemether, Estimation, HPLC

A R T I C L E I N F O

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C O N T E N T S

1. Introduction

Lumefantrine chemically 2-(dibutylamino)-1-[(9Z)-2,7-dichloro-9-[(4- chlorophenyl) methylidene]-9H- fluoren-4yl]ethanl-ol has the molecular formula $C_{30}H_{32}Cl_3NO$. The Antimalarial Agent, Lumefantrine exerts its antimalarial effect is unknown. However, available data suggest that lumefantrine inhibits the formation of β-hematin by forming a complex with hemin and inhibits nucleic acid and protein synthesis. (Fig.1).[1] Artemether chemically known as (1R,4S, 5R,8S, 9R,10S,12R,13R)-10-methoxy-1,5,9-trimethyl-11, 14, 15, 16tetraoxatetracyclo [10.3.1.0^{4,13}. 0^{8,13}] hexadecane has the molecular formula $C_{16}H_{26}O_5$. It is antimalarial agent used to treat acute uncomplicated malaria. It is metabolized into the active metabolite metabolite dihydroartemisinin. The drug works against the erythrocytic stages of *P. falciparum* by inhibiting nucleic acid and protein synthesis. Artemether is

administered in combination with lumefantrine for improved efficacy. Artemether has a rapid onset of action and is rapidly cleared from the body. It is thought that artemether provides rapid symptomatic relief by reducing the number of malarial parasites. Lumefantrine has a much longer half life and is believed to clear residual parasites. (Fig.2).[2]

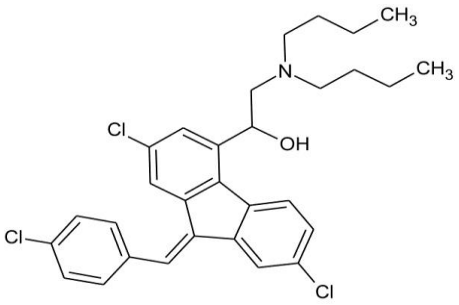

Fig. 1: Molecular structure of Lumefantrine

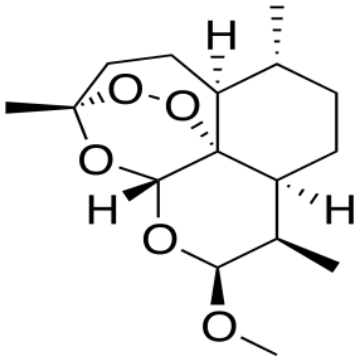

Fig. 2: Molecular structure of Artemether

Review of literature reveals that a lot of work has been carried out for the routine analysis of drugs and pharmaceuticals of marketed as well as existing formulations & bulk drugs.[3-7] A number of references are available for the present study to develop analytical methods. A preliminary survey of literature for suitable method development for newer drugs has been made. Further survey of literature will be done by referring chemical abstracts, Publish Sed papers, and international research journals and on internet. It is attempt to develop a novel, rapid, accurate and precise RP-HPLC method for simultaneous estimation of Lumefantrine and Artemether in tablet dosage form and validated in accordance with ICH guidelines.

2. Materials and Methods

Instrumentation:

To develop a high performance liquid chromatographic method for simultaneous estimation of Lumefantrine and Artemether using Shimadzu (LC 20 AT VP) on Kromosil ODS column,C18 (250 mm x 4.6 mm I.D., 5 µm particle size) column was used. The instrument is equipped with an auto sampler and PDA detector. A 20µL rheodyne injector port was used for injecting the samples. Data was analyzed by using Empower 2 software. A AD102U pH meter was used for pH measurements.

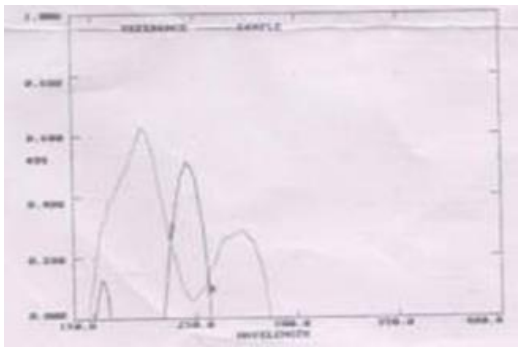

Fig. 3: Overlain UV Spectra for Lumefantrine and Artemether

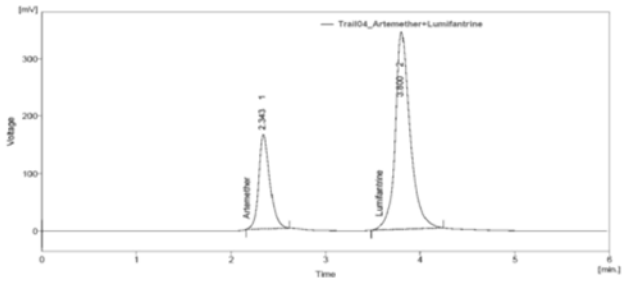

Fig. 4: Typical chromatogram of standard for Lumefantrine and Artemether

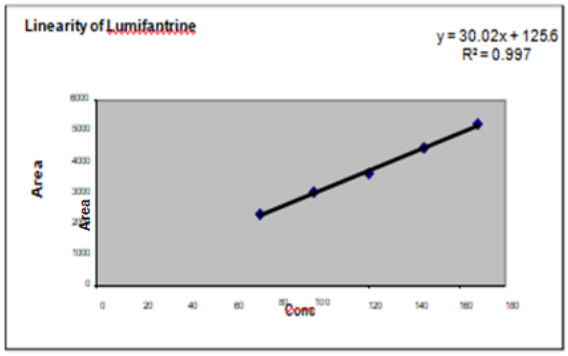

Fig. 5: Linearity graph of Lumefantrine

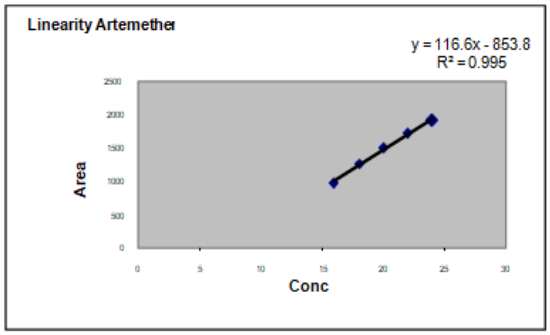

Fig. 6: Linearity graph of Artemether

Chemicals and solvents:

The working standards of Lumefantrine and Artemether were provided as gift samples from Chandra labs, Hyderabad, India. The marketed formulation of Lumefantrine and Artemether tablets (Lumefantrine 120 mg and Artemether 20 mg) were procured from local market. HPLC grade water, methanol and acetonitrile were

purchased from E.Merck (India) Ltd., Mumbai, India. Ortho phosphoric acid of LR grade was obtained from Standard reagents, Hyderabad, India.

Detection Wavelength by UV Spectroscopy: Accurately weighed and transferred about 10 mg each of Lumefantrine and Artemether working standard into a 10 mL volumetric flask separately, then added to it about 6 mL of methanol and sonicated for 10 minutes to dissolve and diluted up to mark with methanol. This produced standard stock solution (1mg/mL). Further 1mL of above solution was transferred into 10mL volumetric flask and volume was made up with methanol. This produced solutions of concentration 100µg/mL. Finally diluted 1mL of above solution to 10mL using methanol and mixed well. The concentration of the working solution thus produced was 10µg/mL. The working standard solutions of Lumefantrine and Artemether (10µg/mL) were scanned over the range of 190-400nm. By observing the overlain spectra of standard solutions λ 238.0 was taken for trials to develop HPLC method.

Chromatographic conditions: Phosphate buffer (KH_2PO_4): Methanol (30:70) was found to be the most suitable mobile phase for ideal chromatographic separation for simultaneous estimation of Lumefantrine and Artemether. The solvent mixture was filtered through 0.45µm membrane filter and sonicated before use. It was pumped through the column at a flow rate of 1.0 mL/min. Injection volume was 10 µL and the column was maintained at a temperature of 25^0C. The column was equilibrated by pumping the mobile phase through the column for at least 30 minutes prior to the injection of the drug solution. The detection of the drug was monitored at 238 nm. The run time was set as 6 minutes.

Preparation of Phosphate buffer (20mM): 2.72gm of potassium di hydrogen phosphate (KH_2PO_4) was weighed and dissolved in 100mL of water and volume was made up to 1000mL with water. Adjust the pH to 3.5 using ortho phosphoric acid. The buffer was filtered through 0.45µ filters to remove all fine particles and gases.

Preparation of mobile phase and diluent:

A mixture of 30 volumes of Phosphate buffer (KH_2PO_4) pH 3.5 and 70 volumes of Methanol. The mobile phase was sonicated for 10min to remove gases.

Preparation of mixed standard stock solution:

Weigh accurately 120 mg of Lumefantrine and 20 mg of Artemether in 100 mL of volumetric flask and dissolve in 100mL of mobile phase and make up the volume with mobile phase. From above stock solution 120 µg/mL of Lumefantrine.and20 µg/mL of Artemether is prepared by diluting 1mL to 10 mL with mobile phase. This solution is used for recording chromatogram.

Preparation of sample preparation: Twenty commercial tablets were weighed, powdered and weighed accurately the tablet powder equivalent to 120 mg of Lumefantrine and 20 mg of Artemether, transferred in to 100 mL volumetric flask and was dissolved in 70 mL of the diluent. Sonicated the solution for few minutes and dissolved the drugs completely and make up to the final volume with diluent. Then it was filtered through 0.45µm filter. From this stock solution 1 mL was transferred into 10 mL volumetric flask and diluted up to the mark with diluent.

Procedure: The column was maintained at a temperature of 25^0C. The run time was set at 6 minutes. The column was equilibrated by pumping the mobile phase through the column for at least 30 minutes prior to the injection of the drug solutions. Inject 10 µL of the standard and sample solutions six times into the chromatographic system at a flow rate of 1.0 mL/min and the corresponding chromatograms were obtained. From these chromatograms, the average area under the peak of each dilution was computed.

Method Validation

Linearity: Several aliquots of standard solutions of Lumefantrine and Artemether were taken in six different 10 mL volumetric flasks and diluted up to the mark with diluent such that the final concentrations were in the range of 72-168 µg/mL for Lumefantrine and 16-24 µg/mL for Artemether. The above solutions were injected into the HPLC system keeping the injection volume constant. The drugs were eluted with PDA detector at 238 nm, peak areas was recorded for all the peaks. The linearity curves were constructed by plotting concentration of the drugs against peak areas. The regression equation of this curve was computed. This regression equation was later used to estimate the amount of drugs in tablet dosage forms.

Precision:

Precision for Lumefantrine and Artemether were. Every sample was injected six times. The measurements for peak areas were expressed in terms of % RSD.

Accuracy:

The accuracy of the method was assessed by recovery studies of Lumefantrine and Artemether at three concentration levels 50%, 100% and 150%. Fixed amount of pre-analyzed sample was spiked with known amount of Lumefantrine and Artemether. Each level was repeated three times. The % recovery of Lumefantrine and Artemether were calculated.

System suitability: The system suitability parameters like retention time, theoretical plates and tailing factor were evaluated by six replicate analysis of Lumefantrine and Artemether and compared with standard values. The acceptance criteria are % RSD of peak areas not more than 2%, theoretical plates numbers (N) at least 3000 per each peak and tailing factors not more than 2.0 for Lumefantrine and Artemether.

Limit of detection and limit of quantification:

The limit of detection (LOD) and limit of quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solutions of Lumefantrine and Artemether using the developed HPLC method. LOD and LOQ were estimated from signal-to-noise ratio. LOD and LOQ were calculated using 3.3 σ/s and 10 σ/s formulae, respectively. Where, σ is the standard deviation of the peak areas and S is the slope of the corresponding calibration curve.

Robustness: The robustness of the method was determined by making small deliberate changes in method like variation of flow rate, mobile phase ratio and temperature.

Assay: Standard preparations are made from the bulk drug and sample preparations are made from formulation. Both standard and sample solutions were injected in five homogeneous samples. 10 µL of sample solution was injected and from the peak areas of Lumefantrine and Artemether, amount of each drug in the sample were computed. The results were compared with the label claim of Lumefantrine and Artemether in tablet dosage forms. From the results the average % Assay was calculated.

Table 1: Optimized chromatographic conditions

S. No.	Parameter	Condition
1	Mobile phase	Phosphate buffer(KH_2PO_4):Methanol (30:70, v/v)
2	Diluent	Phosphate buffer(KH_2PO_4):Methanol (50:50, v/v)
3	Column	Kromosil ODS column,C18 (250 mm x 4.6 mm, 5 µm)
4	Column temperature	25^0C
5	Wave length	238 nm
6	Injection volume	10 µL
7	Flow rate	1.0 mL/min.
8	Run time	6 min.

Table 2: Linearity of Lumefantrine

S. No.	Conc.(µg/ml)	Area
1	72	2331.916
2	96	3014.296
3	120	3632.436
4	144	4445.894
5	168	5219.285

Table 3: linearity of Artemether

S.No.	Conc.(µg/ml)	Area
1	16	987.272
2	18	1258.337
3	20	1503.539
4	22	1728.237
5	24	1918.878

Table 4: Precision data of Lumefantrine

S. No.	Rt	Area
1	4.093	3866.918
2	4.047	3896.611
3	4.007	3865.243
4	3.933	3786.432
5	3.910	3806.319
6	3.993	3796.59
Avg	3.997	3836.352
SD	0.069	45.552
%RSD	1.72	1.19

Table 5: Precision data of Artemether

S. No.	Rt	Area
1	2.507	1467.687
2	2.483	1429.284
3	2.46	1430.934
4	2.4	1391.463
5	2.403	1401.169
6	2.397	1415.644
Avg	2.4417	1422.697
SD	0.0480	26.922

%RSD	1.97	1.89

Table 6: Accuracy studies of Lumefantrine

% Concentration level	Conc. added (µg/mL)	Conc. found (µg/mL)	% Recovery
50%	120	120.60	100.50%
100%	144	141.32	98.14%
150%	168	168.99	100.59%

Table 7: Accuracy studies of Artemether

% Concentration level	Conc. added (µg/mL)	Conc. found (µg/mL)	% Recovery
50 %	20	19.86	99.32%
100%	22	21.64	98.37%
150%	24	24.82	101.76%

Table 8: System suitability parameters of proposed method

S. No.	Parameters	Lumefantrine	Artemether
1	Linearity (µg/mL)	120-168	16-24
2	Correlation coefficient	0.997	0.995
3	Retention time (min.)	3.800	2.343
4	Resolution	---	6.122
5	Tailing factor	1.465	1.484
6	Theoretical plates (N)	3259	2000
7	LOD (µg/mL)	4.17	0.090
8	LOQ (µg/mL)	12.64	0.27

Table 9: Assay results of marketed formulations

S. No.	Lumefantrine		Artemether	
	Standard Area	Sample Area	Standard Area	Sample Area
1.	3698.718	3923.338	1333.404	1440.213
2.	3648.405	3865.243	1342.346	1430.934
3.	3929.997	3966.918	1440.28	1467.678
4.	3843.489	3733.491	1422.858	1354.578
5.	3691.504	3714.100	1357.828	1363.983
Average Area	1372.110	1411.477	3762.423	3840.618
Tablet averageweight	230.1 mg		230.1 mg	
Standard weight	20.1 mg		120.02 mg	
Sample weight	230.2 mg		230.2 mg	
Label amount	20 mg		120 mg	
std. purity	98.5		99.2	
Amount found in mg	20.27 mg		122.13 mg	
Assay(%purity)	101.73 %		101.23 %	

3. Results and Discussion

The HPLC procedure was optimized with a view to develop an accurate, precise and reproducible method for simultaneous estimation of Lumefantrine and Artemether in tablet dosage form using Kromosil ODS column,C18 (250 mm x 4.6 mm, 5 µm) in isocratic mode with mobile phase composition of Phosphate buffer(KH_2PO_4):Methanol (30:70, v/v). The use of Phosphate buffer (KH_2PO_4): Methanol (30:70, v/v) resulted in peak with maximum separation, good shape and resolution. Flow rates between 0.8 to 1.2 mL/min were studied. A flow rate of 1.0 mL/min gave an optimum signal-to-noise ratio with reasonable separation time, the retention times for Lumefantrine and Artemether were found to be 3.800 minutes and 2.343 minutes respectively. Total run time was 6 minutes. The drug components were measured with PDA detector at 238 nm. The results of optimized chromatographic conditions were shown in Table 1. Linearity was obtained in the range of 72-168 µg/mL for Lumefantrine and 16-24 µg/mL for Artemether. The correlation coefficient (r^2) was found to be 0.999 for both Lumefantrine and Artemether respectively. The regression equation of the linearity plot of concentration of Lumefantrine over its peak area was found to be y = 30.02x + 125.6, where x is the concentration of Lumefantrine (µg/mL) and y is the corresponding peak area. The regression equation of the linearity plot of concentration of Artemether over its peak area was found to

be y = 116.6x - 853.8, where x is the concentration of Artemether (µg/mL) and y is the corresponding peak area. The results show that an excellent correlation exists between peak area and concentration of drugs within the concentration range indicated. The linearity data of Lumefantrine and Artemether were furnished in Table 2 and Table 3.

The % RSD for method precision for Lumefantrine were found to be 1.97% and 1.72% respectively (limit % RSD<2.0%) and hence the method is precise. The precision data of Lumefantrine and Artemether were furnished in Table 4 and Table 5. The % recovery of the drugs Lumefantrine and Artemether were found to be 98.14 to 100.59% and 98.37 to 101.76% respectively and the high percentage of recovery of Lumefantrine and Artemether indicates that the proposed method is highly accurate.

The results of accuracy studies of Lumefantrine and Artemether were shown in Table 6 and Table 7. The retention times for the drugs Lumefantrine and Artemether was 3.800 minutes and 2.343 minutes respectively. The number of theoretical plates calculated for Lumefantrine and Artemether was 3259 and 2000 respectively. The tailing factor for Lumefantrine and Artemether was 1.484 and 1.465 respectively, which indicates efficient performance of the column. The limit of detection (LOD) and limit of quantification (LOQ) for Lumefantrine were found to be 4.17 µg/mL and 12.64 µg/mL; 0.090 µg/mL and 0.27 µg/mL for Artemether respectively, which indicate the sensitivity of the method. The summary of system suitability parameters and validation parameters were shown in Table 8.

The robustness studies indicated that no considerable effect on the determination of the drugs. Therefore the test method is robust for the quantification of the drugs. In all deliberately varied conditions, the % RSD for replicate injections of Lumefantrine and Artemether were found to be within the acceptable limits. Validated method was applied for the simultaneous estimation of Lumefantrine and Artemether in commercial tablet dosage forms. The % Assay of Lumefantrine and Artemether were found to be 101.23 % and 101.73 % respectively.

The results for the drugs assay showed good agreement with label claims. No interfering peaks were found in the chromatogram of the tablet formulation within the run time indicating that excipients used in tablet formulation did not interfere with the simultaneous estimation of the drugs Lumefantrine and Artemether by the proposed HPLC method. The assay results are shown in Table 9. The chromatograms were checked for appearance of any extra peaks under optimized conditions, showing no interference from common tablet excipients and impurities. Also the peak areas were compared with standard and were found to be within limits. As shown in chromatogram, two analytes are eluted by forming symmetrical peaks. The typical chromatogram of Lumefantrine and Artemether standard were shown in Fig. 4.

4. Conclusion

The proposed HPLC method is rapid, sensitive, precise and accurate for the simultaneous estimation of Lumefantrine and Artemether and can be reliably adopted for routine quality control analysis of Lumefantrine and Artemether in its tablet dosage forms.

Source of Support: None
Conflict of Interest: Nil

5. References

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