International Journal of Pharmacy and Analytical Research (IJPAR)

ISSN: 2320-2831

IJPAR |Vol.12 | Issue 1 | Jan - Mar -2023
www.ijpar.com

Research article ***Analytical research***

Analytical method development and validation for the simultaneous determination of artemether and lumefantrine in bulk and pharmaceutical dosage form by using RP-HPLC method

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Published on: 16.03.2023

ABSTRACT

A new simple, accurate, economic, rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Artemether and Lumefantrine, in its pure form as well as in pharmaceutical dosage form. Chromatography was carried out on X bridge C18 (4.6×150mm) 5 µ column using a mixture of Methanol: Phosphate Buffer pH-3.6 (30:70v/v) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 260nm. The retention time of the Artemether and Lumefantrine was 2.669, 3.855±0.02min respectively. The method produce linear responses in the concentration range of 10-50µg/ml of Artemether and 10-50µg/ml of Lumefantrine. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

Keywords: Artemether and Lumefantrine, RP-HPLC, Validation.

INTRODUCTION

Analysis may be defined as the science and art of determining the composition of materials in terms of the elements or compounds contained in them. In fact, analytical chemistry is the science of chemical identification and determination of the composition (atomic, molecular) of substances, materials and their chemical structure.

Chemical compounds and metallic ions are the basic building blocks of all biological structures and processes which are the basis of life. Some of these naturally occurring compounds and ions (endogenous species) are present only in very small amounts in specific regions of the body, while others such as peptides, proteins, carbohydrates, lipids and nucleic acids are found in all parts of the body. The main object of analytical chemistry is to develop scientifically substantiated methods that allow the qualitative and quantitative evaluation of materials with certain accuracy. Analytical chemistry derives its principles from various branches of science like chemistry, physics, microbiology, nuclear science and electronics. This method provides information about the relative amount of one or more of these components.[1]

Every country has legislation on bulk drugs and their pharmaceutical formulations that sets standards and obligatory quality indices for them. These regulations are presented in separate articles relating to individual drugs and are published in the form of book called "Pharmacopoeia" (e.g. IP, USP, and BP). Quantitative chemical analysis is an important tool to assure that the raw material used and the intermediate products meet the required specifications. Every year number of drugs is introduced into the market. Also quality is important in every product or service, but it is vital in medicines as it involves life.

There is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. This happens because of the possible uncertainties in the

continuous and wider usage of these drugs, report of new toxicities and development of patient resistance and introduction of better drugs by the competitors. Under these conditions standard and analytical procedures for these drugs may not be available in Pharmacopoeias. In instrumental analysis, a physical property of the substance is measured to determine its chemical composition. Pharmaceutical analysis comprises those procedures necessary to determine the identity, strength, quality and purity of substances of therapeutic importance. [2]

Pharmaceutical analysis deals not only with medicaments (drugs and their formulations) but also with their precursors i.e. with the raw material on which degree of purity and quality of medicament depends. The quality of the drug is determined after establishing its authenticity by testing its purity and the quality of pure substance in the drug and its formulations.

Quality control is a concept which strives to produce a perfect product by series of measures designed to prevent and eliminate errors at different stages of production. The decision to release or reject a product is based on one or more type of control action. With the growth of pharmaceutical industry during last several years, there has been rapid progress in the field of pharmaceutical analysis involving complex instrumentation. Providing simple analytical procedure for complex formulation is a matter of most importance. So, it becomes necessary to develop new analytical methods for such drugs. In brief the reasons for the development of newer methods of drugs analysis are:

1. The drug or drug combination may not be official in any pharmacopoeias.
2. A proper analytical procedure for the drug may not be available in the literature due to Patent regulations.
3. Analytical methods for a drug in combination with other drugs may not be available.
4. Analytical methods for the quantitation of the drug in biological fluids may not be available.
5. The existing analytical procedures may require expensive reagents and solvents. It may also involve cumbersome extraction and separation procedures and these may not be reliable. [1, 15]

MATERIALS AND METHODS

Artemether from Sura labs,Artemether from Sura labs, Water and Methanol for HPLC from LICHROSOLV (MERCK). Acetonitrile for HPLC from Merck, Phosphate buffer from Sura labs.

HPLC METHOD DEVELOPMENT

Mobile Phase Optimization

Initially the mobile phase tried was Water: Methanol and ACN: Methanol with varying proportions. Finally, the mobile phase was optimized to phosphate buffer (pH 3.6), Methanol in proportion 70:30 v/v respectively.

Optimization of Column

The method was performed with various columns like C18 column ODS column, Zodiac column, and Xterra C18 column. Xbridge C18 (4.6 x 150mm, 5µm) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

Optimized chromatographic conditions

Instrument used :Waters HPLC with auto sampler and PDA detector 996 model.
Column : X bridge C18 (4.6×150mm) 5 µ
Buffer : Phosphate buffer (pH-3.6)-Dissolve 1.1998g of anhydrous di hydrogen phosphate in sufficient water to produce 1000ml. Adjust the pH 3.6 by using ortho phosphoric acid.
pH : 3.6
Mobile phase:Methanol:Phosphate Buffer pH-3.6 (30:70v/v)
Flow rate: 1.0 ml per min
Wavelength:260 nm
Injection volume: 10 µl
Run time : 10 min.
Optimized chromatogram, blank, System suitability parameters are shown in the figure and the results are shown in Table.

Validation

Preparation of buffer and mobile phase

Preparation of Phosphate buffer (pH-3.6)

Dissolve 1.1998g of anhydrous di hydrogen phosphate dissolved in sufficient HPLC Grade water to produce 1000mL. Adjust the pH 3.6 by using ortho phosphoric acid.

Preparation of mobile phase

Accurately measured 300 ml (30%) of Methanol and 700 ml of Phosphate buffer (70%) were mixed and degassed in digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation

The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION

Mobile phase (30:70v/v)	:	Methanol: Phosphate Buffer pH3.6
Column	:	X bridge (4.6×150mm, 5 µ)
Flow rate	:	1.0 ml/min
Wavelength	:	260 nm
Column temp	:	Ambient
Injection Volume	:	10 µl
Run time	:	8 min

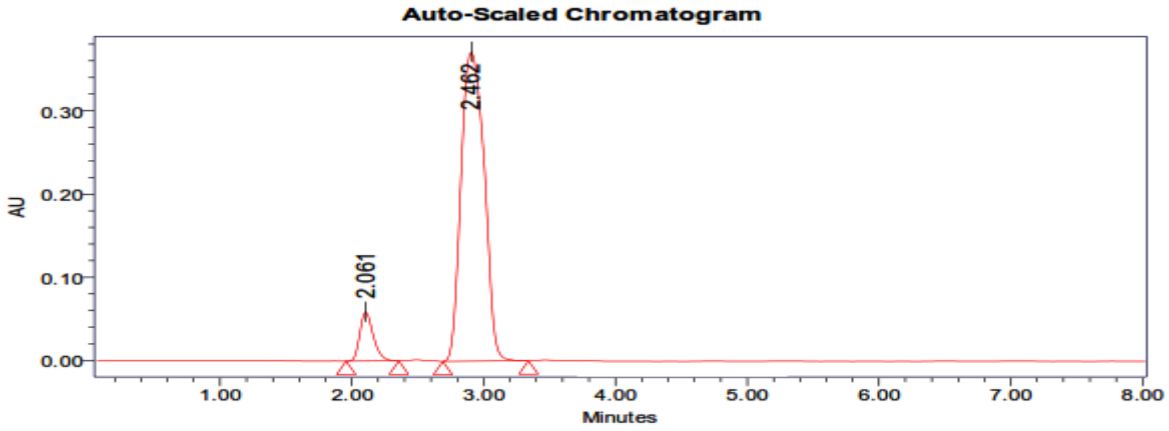

Fig 1: Optimized Chromatogram (Standard)

Table 1: Peak results optimized chromatogram

S. No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Artemether	2.669	986574	128672		1.5	3551.0
2	Lumefantrine	3.855	5365216	562209	1.7	1.4	4675.7

This trial shows improper separation sample peaks, baseline and show very less plate count in the chromatogram. So it's required more trials to obtain good peaks.

Optimized Chromatogram (Sample)

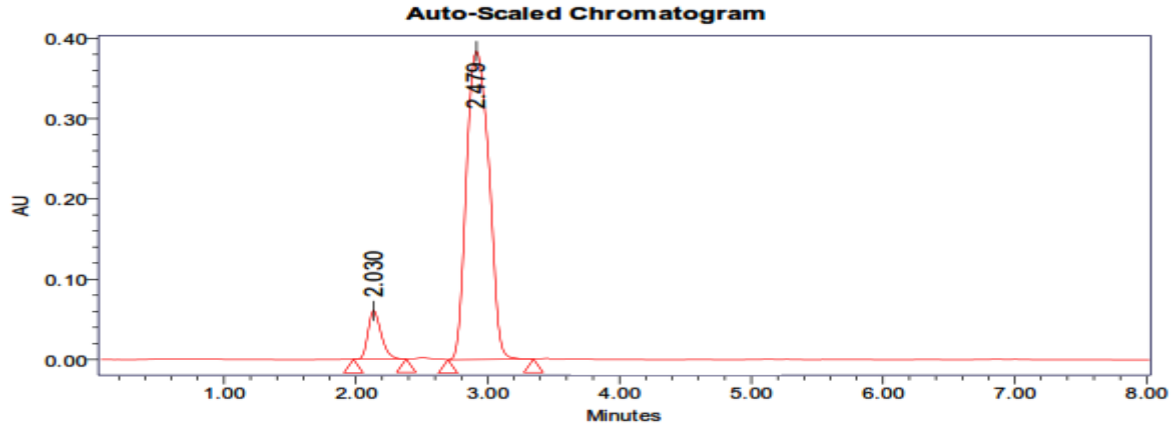

Fig 2: Optimized Chromatogram (Sample)

Table 2: Showing assay sample results

S.No.	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Artemether	2.669	988626	127854		1.6	3561	1
2	Lumefantrine	3.855	5387547	568541	1.7	1.4	4874	1
3	Artemether	2.651	989685	127841		1.5	3658	2
4	Lumefantrine	3.849	5392435	563524	1.7	1.4	4641	2
5	Artemether	2.621	989874	127856		1.5	3854	3
6	Lumefantrine	3.840	5389854	565412	1.7	1.4	4365	3

Assay (Standard)

Table 3: Results of system suitability parameters for Artemether and Lumefantrine

S.No	Name	Retention time(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	Artemether	2.669	979867	129658		1.6	3854
2	Lumefantrine	3.855	5356471	587452	1.8	1.9	4796

- Resolution between two drugs must be not less than 2.
- Theoretical plates must be not less than 2000.
- Tailing factor must be not less than 0.9 and not more than 2.
- It was found from above data that all the system suitability parameters for developed method were within the limit.

Assay (Sample)

Table 4: Showing assay results

S.No	Name of compound	%purity
1	Artemether	99 %
2	Lumefantrine	100%

The retention time of Artemether and Lumefantrine was found to be 2.669min and 3.855mins respectively. The % purity of Artemether and Lumefantrine in pharmaceutical dosage form was found to be 99% and 100% respectively.

Linearity

Linearity results: (for artemether)

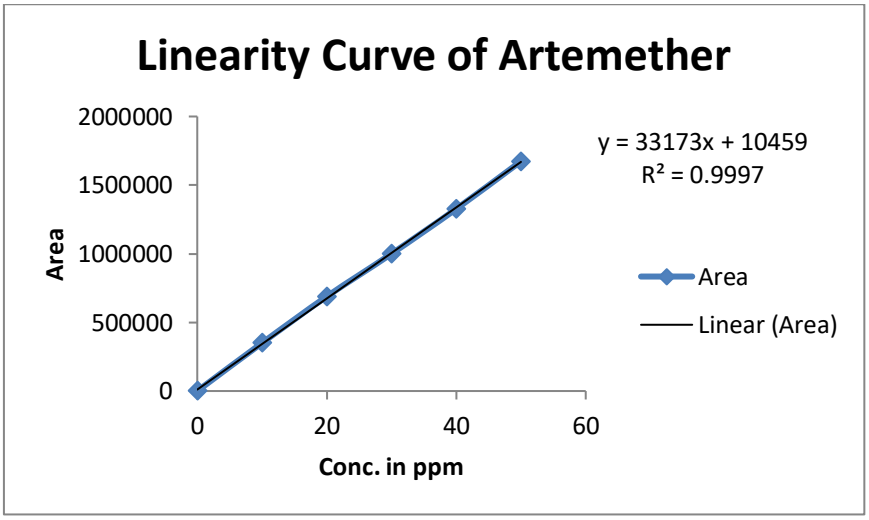

Fig 3: Calibration graph for Artemether

S.No	Linearity Level	Concentration(ppm)	Area
1	I	10	349877
2	II	20	688574
3	III	30	999895
4	IV	40	1326522
5	V	50	1673877
	Correlation Coefficient		0.999

Correlation coefficient should be not less than 0.999.

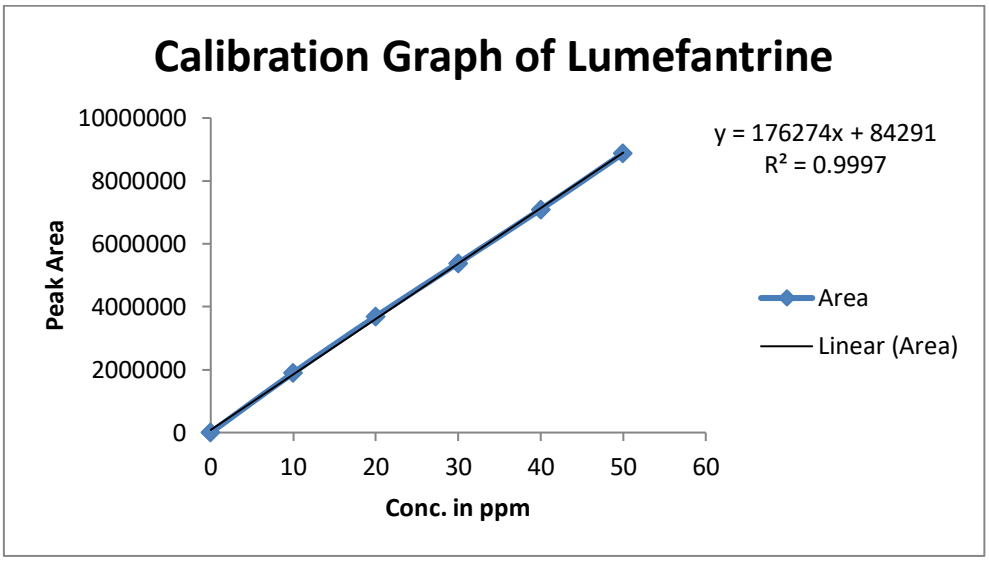

Fig 4: Calibration graph for Lumefantrine

Linearity Results: (forLumefantrine)

S.No.	Linearity Level	Concentration (ppm)	Area
1	I	10	1896545
2	II	20	3685798
3	III	30	5389557
4	IV	40	7096443
5	V	50	8878478
Correlation Coefficient			**0.999**

Correlation coefficient should be not less than 0.99.

Intermediate precision

Table 5: Results of intermediate precision Day 1 for Artemether

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Artemether	2.669	986857	128231	3653	1.5
2	Artemether	2.659	987854	129852	3541	1.5
3	Artemether	2.671	985474	128145	3635	1.5
4	Artemether	2.669	986589	129611	3595	1.5
5	Artemether	2.669	985213	128321	3698	1.5
Mean			986397.4			
Std. Dev			1075.302			
% RSD			0.109013			

Table 6: Results of intermediate precession for Day 1 Lumefantrine

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Lumefantrine	3.855	5378559	565621	4675	1.4	1.7
2	Lumefantrine	3.842	5386231	564587	4696	1.4	1.7
3	Lumefantrine	3.850	5385411	563651	4684	1.4	1.7
4	Lumefantrine	3.845	5369874	563544	4763	1.4	1.7
5	Lumefantrine	3.855	5389745	578547	4954	1.4	1.7
Mean			5381964				
Std. Dev			7880.279				
% RSD			0.14642				

- %RSD for sample should be NMT 2.

- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Table 7: Results of Intermediate precision day 2 for Artemether

S.no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Artemether	2.669	978985	128874	3686	1.5
2	Artemether	2.529	975686	128365	3654	1.5
3	Artemether	2.669	969876	128471	3536	1.5
4	Artemether	2.569	975487	128698	3682	1.5
5	Artemether	2.569	978546	128365	3598	1.5
6	Artemether	2.669	976898	128241	3536	1.5
Mean			975913			
Std. Dev			3286.897			
% RSD			0.336802			

Table 8: Results of Intermediate precision day 2 for Lumefantrine

S.No	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Lumefantrine	3.845	5352141	563658	4685	1.4	1.7
2	Lumefantrine	3.795	5365847	564587	4665	1.4	1.7
3	Lumefantrine	3.855	5378412	563652	4654	1.4	1.7
4	Lumefantrine	3.840	5378543	563547	4641	1.4	1.7
5	Lumefantrine	3.855	5363598	565811	4669	1.4	1.7
6	Lumefantrine	3.855	5386879	562541	4658	1.4	1.7
Mean			5370903				
Std. Dev			12656.43				
% RSD			0.235648				

- %RSD of five different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is rugged.

Accuracy

Table 9: accuracy (recovery) data for Artemether

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	509438	15	15.041	100.273%	
100%	1010974.3	30	30.160	100.533%	100.549%
150%	1515817	45	45.379	100.842%	

- The percentage recovery was found to be within the limit (98-102%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Table 10: Accuracy (recovery) data for Lumefantrine

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	347527	15	14.933	99.553%	
100%	609753	30	29.810	99.366%	99.935%
150%	884568	45	45.400	100.888%	

- The % Recovery for each level should be between 98.0 to 102.0%.

Robustness

Robustness results forArtemether

		System Suitability Results	
S.No	Flow Rate (ml/min)	USP Plate Count	USP Tailing
1	0.9	3569.2	1.5

2	1.0	3551.0	1.5
3	1.1	3584.4	1.5

***** Results for actual flow (1.0 ml/min) have been considered from Assay standard.

Robustness results for Lumefantrine

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.9	4864.2	1.4
2	1.0	4675.7	1.4
3	1.1	4524.9	1.4

***** Results for actual flow (1.0ml/min) have been considered from Assay standard

System suitability results for Artemether

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	4789.4	1.5
2	*Actual	3551.0	1.5
3	10% more	4635.6	1.5

System suitability results for Lumefantrine

S.No.	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	5865.8	1.4
2	*Actual	4675.7	1.4
3	10% more	5342.4	1.4

***** Results for actual mobile phase have been considered from Assay standard.

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Glipizide and Metformin in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Glipizide and Metformin was freely soluble in ethanol, methanol and sparingly soluble in water. Methanol: Phosphate Buffer pH 3.9 (55:45v/v) was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed inTablesfor RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Glipizide and Metformin in bulk drug and in Pharmaceutical dosage forms.

ACKNOWLEDGEMENT

The Authors are thankful to the Management and Principal, Department of Pharmacy, Surabhi Dayakar Rao College of Pharmacy, Rimmanaguda, for extending support to carry out the research work. Finally, the authors express their gratitude to the Sura Labs, Dilsukhnagar, Hyderabad, for providing research equipment and facilities.

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