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A NEW RP HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF LUMEFANTRINE AND ARTEMETHER USING BULK AND PHARMACEUTICAL DOSAGE FOMS

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ABSTRACT

A simple and selective LC method is described for the determination of Lumefantrine and Artemether in tablet dosage forms. Chromatographic separation was achieved on a c_{18} column using mobile phase consisting of a mixture of Phosphate buffer (KH2PO4) Ph3.5: Methanol (30:70v/v),with detection of 238 nm. Linearity was observed in the range 16-24 µg /ml for Artemether (r^2 =0.995) and 72-168µg /ml for Lumefantrine (r^2 =0.997) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form.

Key Words: Lumefantrine, Artemether, RP HPLC method

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INTRODUCTION

A drug includes all medicines intended for internal or external use for or in the diagnosis, treatment, mitigation or prevention of disease or disorder in Human beings or animals, and manufactured exclusively in accordance with the formulae mentioned in authoritative books (1). Pharmaceutical analysis is a branch of chemistry involving a process of identification, determination, quantification, purification and separation of components in a mixture or determination of chemical structure of compounds. There are two main types of analysis – Qualitative and Quantitative analysis. Qualitative analysis is performed to establish composition of a substance. It is done to determine the presence of a compound or substance in a given sample or not. The various qualitative tests are detection of evolved gas, limit tests, color change reactions, determination of melting point and boiling point, mass spectroscopy, determination of nuclear half life etc. Quantitative

analysis techniques are mainly used to determine the amount or concentration of analyte in a sample and expressed as a numerical value in appropriate units. These techniques are based on suitable chemical reaction and either measuring the amount of reagent added to complete the reaction or measuring the amount of reaction product obtained the characteristic movement of a substance through a defined medium under controlled conditions, electrical measurement or measurement of spectroscopic properties of the compound (2).

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. Lumefantrine is a blood schizonticide active against erythrocytic stages of *Plasmodium falciparum*. It is thought that administration of lumefantrine with artemether results in cooperate antimalarial clearing effects. Artemether has a rapid onset of action and is rapidly cleared from the body. It is thus thought to provide rapid symptomatic relief by reducing the number of malarial parasites. Lumefantrine has a much longer half life and is believed to clear residual parasites. Lumefantrine is an antimalarial agent used to treat acute uncomplicated malaria. It is administered in combination with artemether for improved efficacy. This combination therapy exerts its effects against the erythrocytic stages of *Plasmodium spp*. and may be used to treat infections caused by *P. falciparum* and unidentified *Plasmodium* species, including infections acquired in chloroquine-resistant areas.

Artemether is an antimalarial agent used to treat acute uncomplicated malaria. It is administered in combination with lumefantrine for improved efficacy. This combination therapy exerts its effects against the erythrocytic stages of *Plasmodium spp*. and may be used to treat infections caused by *P. falciparum* and unidentified *Plasmodium* species, including infections acquired in chloroquine-resistant areas. In the body, artemether is metabolized into the active metabolite metabolitedihydroartemisinin. 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. Artemether and lumefantrine combination therapy is indicated for the treatment of acute uncomplicated malaria caused by *Plasmodium falciparum*, including malaria acquired in chloroquine-resistant areas. May also be used to treat uncomplicated malaria when the *Plasmodium* species has not been identified. Indicated for use in adults and children greater than 5 kg (1-5).

Aim is to develop new RP HPLC method for the simultaneous estimation of Lumefantrine and Artemether in pharmaceutical dosage form.

MATERIALS AND METHODS

Determination of Working Wavelength (λmax) (6-8)

In simultaneous estimation of two drugs isobestic wavelength is used. Isobestic point is the wavelength where the molar absorptivity is the same for two substances that are interconvertible. So this wavelength is used in simultaneous estimation to estimate both drugs accurately.

Preparation of standard stock solution of LUMEFANTRINE.

10 mg of lumefantrine.was weighed and transferred in to 100ml volumetric flask and dissolved in methanol and then make up to the mark with methanol and prepare 10 µg /ml of solution by diluting 1ml to 10ml with methanol.

Preparation of standard stock solution of ARTEMETHER

10mg of artemetherwas weighed in to 100ml volumetric flask and dissolved in Methanol and then dilute up to the mark with methanol and prepare 10 µg /ml of solution by diluting 1ml to 10ml with methanol.

Assay

Preparation of samples for Assay

Standard sample

Standard stock solutions of lumefantrine.and artemether (microgram/ml) were prepared by dissolving 120 mg of lumefantrine.and20 mg of artemether dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5min and dilute to 100 ml with

mobile phase. Further dilutions are prepared in 5 replicates of 120 µg/ml of lumefantrine.and20 µg/ml of artemether was made by adding 1 ml of stock solution to 10 ml of mobile phase.

Tablet sample

20 tablets (each tablet contains 120 mg of lumefantrine. and 20 mg of artemether) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of lumefantrine and artemether (µg/ml) were prepared by dissolving weight equivalent to120 mg of lumefantrine.and20 mg of artemether and dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 100ml with mobile phase. Further dilutions are prepared in 5 replicates of 120µg/ml of lumefantrine.and20 µg/ml of artemether was made by adding 1 ml of stock solution to 10 ml of mobile phase.

RESULTS AND DISCUSSION

The wavelength of maximum absorption (λ_{max}) of the drug, 10 µg/ml solution of the drugs in methanol were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against methanol as blank. The resulting spectra show characteristic absorption maxima at 238nm for the combination.

The amount of lumefantrine and artemether present in the taken dosage form was found to be 101.23 % and 101.73 % respectively (Table-1).

Table-1 Assay Results of lumefantrine and artemether

ARTEMETHER			**LUMEFANTRINE**	
	Standard Area	**Sample Area**	**Standard Area**	**Sample Area**
Injection-1	1333.404	1440.213	3698.718	3923.338
Injection-2	1342.346	1430.934	3648.405	3865.243
Injection-3	1440.28	1467.678	3929.997	3966.918
Injection-4	1422.858	1354.578	3843.489	3733.491
Injection-5	1357.828	1363.983	3691.504	3714.100
Average Area	1372.110	1411.477	3762.423	3840.618
Tablet average weight	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 %	

The % RSD for the retention times and peak area of artemether and lumefantrine were found to be less than 2%. The plate count and tailing factor results were found to be satisfactory and are found to be within the limit.

The correlation coefficient for linear curve obtained between concentration vs. Area for standard preparations of artemether and lumefantrine is 0.995 and 0.997. The relationship between the concentration of artemether and lumefantrine and area of artemether and lumefantrine is linear in the range examined since all points lie in a straight line and the correlation coefficient is well within limits (Fig-1, 2).

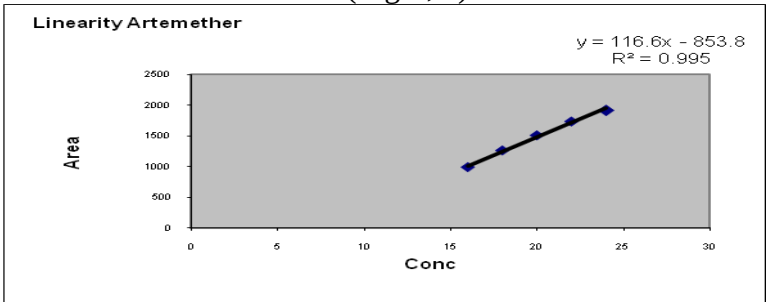

Fig-1 Linearity graph of artemether

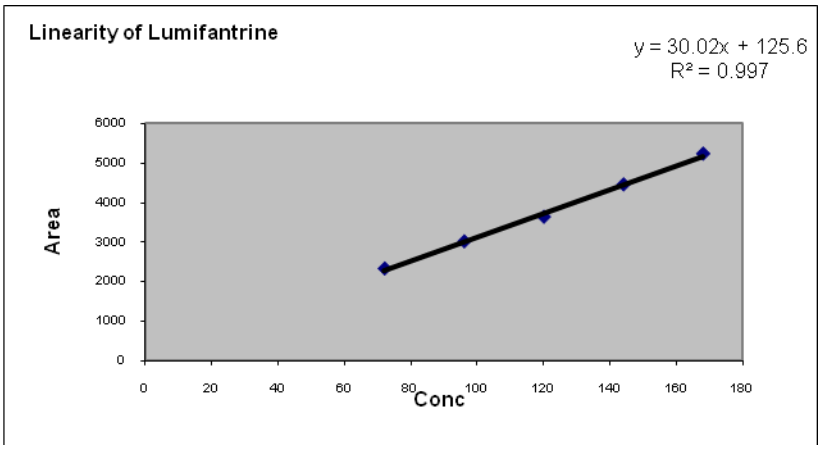

Fig-2 Linearity graph of lumefantrine

The percentage mean recovery of artemether and lumefantrine is 99.96% and 100.85% respectively (Table-2, 3).

Table-2 Recovery results for artemether

Recovery level	Accuracy Artemether					Average % Recovery
	Amount taken(mcg/ml)	Area	Average area	Amount recovered(mcg/ml)	%Recovery	
80%	20	1410.587	1388.557	19.86	99.32	99.96%
	20	1355.229				
	20	1399.855				
100%	22	1513.556	1518.778	21.64	98.37	
	22	1522.183				
	22	1520.594				
120%	24	1917.606	1918.536	24.82	101.76	
	24	1916.681				
	24	1921.321				

Table-3 Recovery results for lumefantrine

Recovery level	Accuracy lumefantrine					Average % Recovery
	Amount taken(mcg/ml)	Area	Average area	Amount recovered(mcg/ml)	%Recovery	
80%	120	3835.779	3790.400	120.60	100.50	100.85%
	120	3705.436				
	120	3829.984				
100%	144	4163.556	4160.090	141.32	98.14	
	144	4163.954				
	144	4152.761				
120%	168	1730.097	5217.299	168.99	100.59	
	168	1730.097				
	168	1736.576				

From the observation the % RSD between two analysts Assay values not greater than 2.0%, hence the method was rugged. It was found that the system suitability parameters were within limit at all variable conditions.

CONCLUSION

From the above experimental results and parameters it was concluded that, this newly developed method for the simultaneous estimation of Lumefantrine and Artemether was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in meant in industries, approved testing laboratories, bio-pharmaceutical and bio-equivalence studies and in clinical pharmacokinetic studies in near future.

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