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A STABILITY INDICATING ANALYTICAL METHOD FOR SIMULTANEOUS QUANTIFIATION OF ARTEMETHER AND LUMEFANTRINE IN COMBINED DOSAGE FORMS BY RP-HPLC

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ABSTRACT

A new high performance liquid chromatographic method was developed for the simultaneous determination of Artemether and Lumefantrine in pharmaceutical dosage form. Stability indicating studies have been performed under various stress conditions. The reported method adopts Symmetry C18 (4.6 x 150mm, 5µm, Make XTerra) column as stationary phase and a mobile phase consisting of Acetonitrile: Phosphate buffer in the ratio of 80:20 (v/v) pH adjusted to 2.5 with ortho-phosphoric acid, employing UV detection at 274 nm.Peaks eluted at a retention time of 2.003 min and 5.067 min was found to be Artemether and Lumefantrine respectively, where flow was monitored at a rate of 0.8mL/min. Linear calibration curves for proposed method are arrived in the concentration range of 25-125 µg/ml for both the drugs(r^2>0.999).The method is validated in terms of precision, ruggedness, robustness and accuracy. The limit of quantification [s/n 10.05(ART) &10.14(LUM)]shows the method meets the regulatory criteria. The proposed method successfully separated the drug from its degradation products when they were exposed to various stress conditions like photolytic, aqueous acid, base, thermal and peroxide conditions.High percentage of recovery shows that the method is free from the interference of excipients used in the formulation. Hence the method can be used in the routine quality control of these drugs.

INTRODUCTION

Artemether[(3R, 5as, 6R, 8as, 9R, 10S, 12R, 12ar)-decahydro-10-Methoxy-3, 6, 9-trimethyl-3, 12-epoxy-12H-pyrano [4, 3-j] -1, 2-benzodioxepin] is a medication used for the treatment of malaria.Its mechanism of action involves interaction of the peroxide-containing drug with heme, a hemoglobin degradation byproduct, derived from proteolysis of hemoglobin. This interaction is believed to result in the formation of a range of potentially toxic oxygen. The injectable form is specifically used for severe

malaria rather than quinine. It is also available by mouth in combination with lumefantrine, known as artemetherlumefantrine. Animal studies[1-5] on acute toxicity shows the LD50 of Artemether in mice is a single i.g. administration of 895mg/kg & a single i.m injection of 296mg/kg dose; in rats, the LD50 is a single i.m. injection of 597mg/kg dose.Lumefantrine, chemically it is 2-(dibutylamino)-1-[(9Z)-2, 7-dichloro-9-[(4-chlorophenyl) methylidene] fluoren-4-yl] ethanolalso an antimalarial drug,used only in combination with artemether("co-artemether"). 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 has a much longer half-life compared to artemether, and is therefore thought to clear any residual parasites that remain after combination treatment.Literature reveals few spectrometric[6-7], HPLC[8-10] and HPTLC[11-12]methods reported for estimation of ART and LUM either in single or combined dosage form.In the present study the authors report aneconomic, rapid, sensitive, accurate and precise stability indicating RP-HPLC method for the estimation of ART and LUM in pure and combined dosage form.

Materials and methods

Instrumentation: ARTand LUM were separated on Symmetry C18 (4.6 x 150mm, 5µm, Make XTerra) fixed to HPLC (make & model: waters 2695) installed with Empower version 2.0 employing PDA detector.

Chemicals used

Artemether and Lumefantrine were obtained as gift samples from KP labs, Hyderabad.Aarnet and Lumerax (marketed formulations) were purchased locally. HPLC grade water, methanol [make: lichrosolv (Merck)] and Acetonitrile (make: molychem) were used all along the experimental work.KH_2PO_4 waspurchased from FINER chemical LTD.

Standard Solution Preparation: Accurately weighed amount of 50mg Artemether and 50 mg Lumefantrine were taken to a 100 ml clean and dry volumetric flask. This was then diluted with 70 ml of diluent and was sonicated. The volume was made to100 ml with the same solvent. This was taken as standard stock solution. Further, 1.5 ml of above stock solution was diluted to 10ml with the diluent to get final concentration of 75µg/ml.

Sample Solution Preparation

Weight equivalent to 50 mg of Artemether and Lumefantrine sample were weighed this was taken into a 100 ml clean dry volumetric flask and about 70ml of diluent was added and sonicated to dissolve it completely and volume made up to the mark with the same solvent. This was taken as sample stock solution. Further, 1.5 ml of above stock solution was diluted to 10ml with diluent to get final concentration of 75µg/ml.

Results and discussion

Optimized chromatographic conditions

Method was developed by conditioning the system with freshlyprepared buffer and acetonitrile 80:20 (v/v) which were filtered through 0.45µ membrane filter and sonicated to degas before use.Flow rate of mobile phase was maintained at 0.8 ml per min. ambient column oven temperature was maintained throughout the analysis. Detection was carried outat 274nm.Injection volume was 20µl and retention time of ARTand LUM was found to be 2.003 minand5.067 min respectively(Fig.1).

Method validation[13]

Standard solution in single injection was analysed to evaluate system suitability parameters like USP plate count, separation factor and USP tailing for ART and LUM and the results are given in Table 1.

Method Precision: Five replicate injections of standard solution were analyzed to measure the %relative standard deviation and the values are depicted in Table 2 and 3 for ART and LUM respectively.

Intermediate Precision/Ruggedness: ART and LUM present in the standard solution were evaluated for ruggedness of the method

by considering %relative standard deviation. Values obtained are presented in Table 4 and 5 for ART and LUM respectively.

Accuracy: Labeled amounts of formulation werespiked with ART and LUM API at a level of 50%, 100%&150%.Triplicate injections of each spike level were analyzed to obtain the %recovery and tabulated in Table 6.

Linearity: Linearity of the method was performed by pippeting 0.5,1.0,1.5,2.0,2.5 ml of standard solution to obtain a final concentration ranging from 25-125 µg/ml. Peak areas obtained were tabulated (Table 7) , a straight line obtained in the calibration curve (Fig 2&3) shows the method is linear. Regression analysis (r^2=0.999) by the least square method(Table 8) meets acceptance criteria. This regression equation was later used to estimate the amount of ART and LUM in combined dosage forms.

LOD and LOQ: The Minimum concentration level at which the analyte can be reliably detected (LOD) and quantified (LOQ) were generated by the instrument method using empower 3.0.obtained results are furnished in Table 9 for ART and LUM respectively.

ROBUSTNESS: Deliberate changes were made to the method parameters flow rate (±0.1ml) and mobile phase composition (±10%) and %RSD for ART and LUM were calculated (Table 10 & 11) for the same.

Stress studies

Acid degradation: A precisely measured 10 mg of unadulterated API was weighed and transferred to a clean and dry round bottomed flask. 30 ml of 0.1 N HCl was added to it and it was refluxed in a water at 60 $^0C$for 4 hours. Permitted to cool to room temperature.The sample was then neutralized using dilute NaOH solution & final volume of the sample was made up to 100ml with water to prepare 100 µg/ml solution. It was injected into the HPLC system against a blank (after optimizing the mobile phase compositions). This experiment was repeated several times using same concentration of HCl (0.1N) and observed its degradation profile.

Base degradation: A precisely measured 10 mg of unadulterated medication was exchanged to a clean and dry round bottomed flask. 30 ml of 0.1N NaOH was added and refluxed in a water bath at 60^0C for 4 hours. Allowed to cool to room temperature. The sample was than neutralized using 2N HCl solution & final volume of the sample was made up to 100ml to prepare 100 µg/ml solution. It was injected into the HPLC system against a blank after optimizing the mobile phase compositions. This experiment was repeated several times using same concentration of NaOH such as 0.1N to observe its degradation profile.

Thermal degradation: Accurately weighed 10 mg of pure drug was transferred to a clean & dry round bottom flask. 30 ml of HPLC water was added to it. Then, it was refluxed in a water bath at 60^0C for 6 hours uninterruptedly. After the reflux was over, the drug became soluble and the mixture of drug & water was allowed to cool to room temperature. Final volume was made up to 100 ml with HPLC water to prepare 100 µg/ml solution. It was injected into the HPLC system against blank.

Photolytic degradation: Approximately 10 mg of pure drug was taken in a clean & dry Petri dish. It was kept in a UV cabinet at 254 nm wavelength for 24 hours without interruption. Accurately weighed 1 mg of the UV exposed drug was transferred to a clean & dry 10 ml volumetric flask. First the UV exposed drug was dissolved in methanol & made up to the mark with mobile phaseto get 100 µg/ml solution.Finally this solution was injected into the HPLC system against blank.

Oxidation with (3%) H_2O_2

Accurately weighed 10 mg of pure drug was taken in a clean & dry 100 ml volumetric flask. 30 ml of 3% H_2O_2 and a little methanol was added to it to make it soluble & then kept as such in dark for 24 hours. Final volume was made up to 100 ml using water to give 100 µg/ml solution. The above sample was injected into the HPLC system. Results are depicted in Table 12

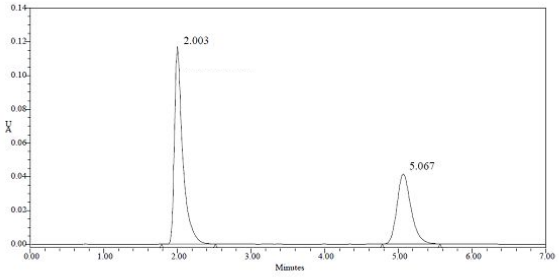

Fig.1: Chromatogram for Artemether and Lumefantrine

Table 1: RESULTS OF SYSTEM SUITABILITY PARAMETERS FOR ARTEMETHER ANDLUMEFANTRINE

S. No	Name	Retention time(min)	Area(µV sec)	USP resolution	USP tailing	USP plate count
1	Artemether	2.003	920101	1.5	1.6	2711.8
2	Lumefantrine	5.067	552058	11.0	1.3	3428.2

Table 2: RESULTS OF METHOD PRECISION FOR ARTEMETHER

S. No	Sample area	Standard area	% purity
1	983375	971536	101.04
2	985049	973007	101.03
3	982956	975717	100.54
4	985219	978909	100.44
5	994145	981422	101.09
Average	986149	9763118	100.84
%RSD	0.5	0.4	0.3

Table 3: RESULTS OF METHOD PRECISION FOR LUMEFANTRINE

S.No	Sample area	Standard area	% purity
1	592403	577531	101.36
2	592352	580381	101.85
3	592357	577723	102.32
4	592323	582190	101.44
5	596525	583378	101.09
Average	593192	580240	101.61
%RSD	0.3	0.4	0.5

Table 4: RESULTS OF INTERMEDIATE PRECISION FOR ARTEMETHER

S. No	Sample area	Standard area	Percentage purity
1	979556	984395	99.30
2	982467	984039	99.64
3	979717	983976	99.36
4	978909	984278	99.28
5	981432	973915	100.57
Average	980416	982121	99.63
%RSD	0.2	0.5	0.5

Table5: RESULTS OF INTERMEDIATE PRECISION FOR LUMEFANTRINE

S. No	Sample area	Standard area	Percentage purity
1	583416	593403	99.12
2	583657	594352	99.01
3	584731	593357	99.52
4	583594	592673	99.61
5	597649	593671	99.12
Average	586609	593491	99.28
%RSD	1.1	0.1	0.3

Table 6: ACCURACY RESULTS

Sample concentration	Sample set no	Sample area		Assay		% Recovery	
		ART	LUM	ART	LUM	ART	LUM
50%	1	460064	276931	24.9	25.0	99.8	100
	2	460124	276694	24.6	24.9	99.6	99.6
	3	460216	276891	24.8	24.9	99.8	99.6
	Average Recovery					99.7%	99.7%
100%	1	923429	554156	49.9	50.0	99.8	100
	2	923654	554897	49.8	49.9	99.6	99.8
	3	923742	556371	49.8	49.9	99.6	99.8
	Average recovery					99.6%	99.8%
150%	1	1387901	828113	74.8	75.0	99.8	100
	2	1385360	828794	74.9	74.9	99.8	99.8
	3	1386984	828349	74.6	74.8	99.6	99.8
	Average recovery					99.7%	99.8%

Table 7: Linearity Data

Concentration (µg/ml)	Peak area of ART	Peak area of LUM
25	296800	179891
50	653819	387781
75	983775	599708
100	1342535	799619
125	1694286	1019614

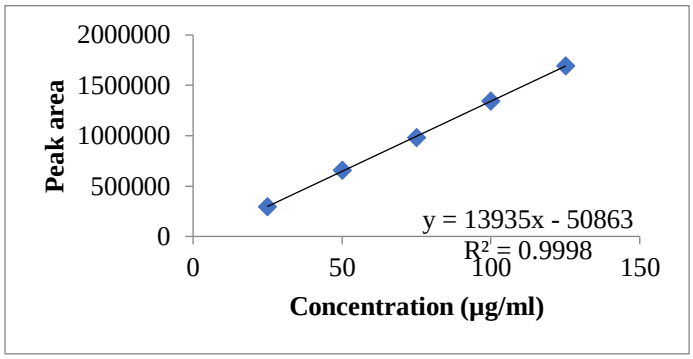

Fig.2: Linearity plot of Artemether API

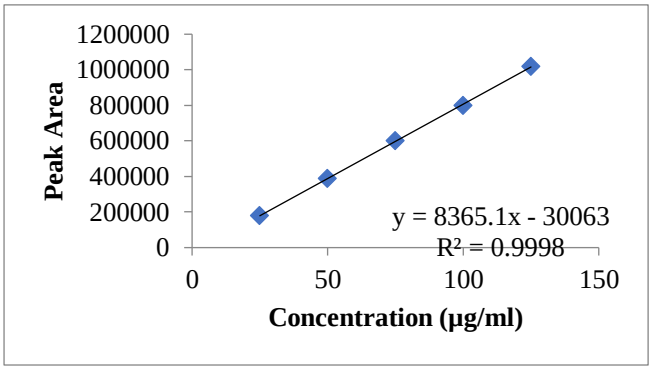

Fig.3: Linearity plot of Lumefantrine API

Table 8:LINEARITY DATA

Parameters	Artemether	Lumefantrine
Slope (m)	13935	8365
Intercept (c)	-50863	-30063
Correlation coefficient (R^2)	0.999	0.999

Table 9: RESULTS OF LOQ & LOD

	LOQ		LOD	
	ART	LUM	ART	LUM
Signal (µV)	563	558	176	154
Baseline noise(µV)	56	56	56	56
S/N ratio	10.05	10.14	3.14	2.75

Table 10:RESULTS FOR EFFECT OF VARIATION IN FLOW

S. No	peak area for Less flow (0.7 ml/min)		peak area for More flow (0.9 ml/min)	
	Artemether	**Lumefantrine**	**Artemether**	**Lumefantrine**
1	983465	575351	971563	592641
2	985134	580381	973021	592352
3	983467	587724	975674	595471
4	985217	583190	978974	594416
5	994245	584468	984542	583453
Mean	986306	582223	976755	591667
%RSD	0.45	0.80	0.53	0.80

Table 11:RESULTS FOR EFFECT OF VARIATION IN MOBILE PHASE COMPOSITION

S. No	Peak area for Less organic(70%)		Peak area for More organic (90%)	
	Artemether	**Lumefantrine**	**Artemether**	**Lumefantrine**
1	984565	574371	981565	593761
2	986134	585481	983527	592462
3	984268	587627	985489	594491
4	986216	585362	987954	596316
5	995247	585448	994672	587353
Mean	987286	583658	986641	592877
%RSD	0.45	0.90	0.51	0.57

Table 12:RESULTS OF STRESS STUDIES

Stress Condition	ART			LUM		
	Area	%Assay	%Degradation	Area	%Assay	%Degradation
Acidic	120473	91.1	8.7	395751	92.4	8.3
Alkaline	124364	92.0	12.8	348779	81.7	12.8
Photolytic	113269	87.2	13.7	352292	87.4	12.4
Thermal	104474	96.3	14.5	352323	85.4	11.5
Oxidative	106734	94.3	11.2	392423	95.1	11.3

SUMMARY AND CONCLUSION

The proposed stability indicating RP-HPLC method is rapid, specific, accurate and precise for the quantification of Artemether and Lumefantrine in pharmaceutical dosage form. The method provides great sensitivity, adequate linearity and repeatability. High percentage of recovery shows that the method is free from the interference of excipients used in the formulation. So the method can be useful in the routine quality control of these drugs.

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