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Research Article

Stability indicating Ultra Performance Liquid Chromatography method development and validation for simultaneous estimation of artemether and lumefantrine in bulk and pharmaceutical dosage form

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ABSTRACT

Ultra performance liquid chromatography method was developed for the simultaneous estimation of the Artemether (AMT) and Lumefantrine (LFT) in Tablet dosage form. Chromatogram was run through X-bridge C18 100 x 2.1 mm, 3.5µ. Mobile phase containing Buffer 0.01N KH_2PO_4 (3.5pH): Acetonitrile taken in the ratio 55:45 was pumped through column at a flow rate of 0.3ml/min. Buffer used in this method was 0.01N KH_2PO_4. Temperature was maintained at 30°C. Optimized wavelength selected was 215nm. Retention time of AMT and LFT were found to be 0.787 min and 1.572min. %RSD of the AMT and LFT were and found to be 0.7 and 0.6 respectively. %Recovery was obtained as 99.49% and 100.22% for AMT and LFT respectively. LOD, LOQ values obtained from regression equations of AMT and LFT were 0.03, 0.08 and 0.095, 0.288 respectively. Regression equation of AMT is y = 19308x + 1509 and y = 36919x + 11566 of LFT. The developed method was simple and economical that can be adopted in regular Quality control test in Industries.

Keywords: Artemether (AMT), Lumefantrine (LFT), Acetonitrile, UPLC.

INTRODUCTION

AMT [1,2] is an antimalarial agent used to treat acute uncomplicated malaria. Chemical name of AMT is 3,12-Epoxy-12H-pyrano[4,3-j]-1,2-benzodioxepin, decahydro-10-methoxy-3,6,9-trimethyl-, (3R,5aS,6R,8aS,9R,10S,12R,12aR). It is administered in combination with LFT 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 [3].

LFT is an antimalarial agent used to treat acute uncomplicated malaria. Chemical name of LFT 2-(dibutylamino)-1-[(9Z)-2,7-dichloro-9-[(4-chlorophenyl) methylidene] -9H-fluoren-4-yl] ethan-1-ol. It is administered in combination with AMT 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 [4].
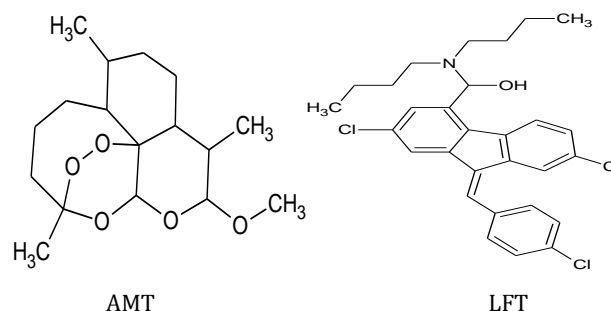
Structure:

Figure.1: Structures for AMT and LFT

Literature review [7-19] reveals that different methods RP-HPLC, UV, LCMS for its analysis in formulations. Hence our present plan is to develop a new, sensitive, robust& accurate method for its analysis in formulation, after a detailed study, a new UPLC method was decided to be developed and validated as per ICH norms[5,6].

MATERIALS AND METHODS

Instruments Used:

Electronics Balance-Denver, p^H meter -BVK enterprises, India, Ultra sonicator-BVK enterprises, WATERS UPLC 2695 SYSTEM equipped with quaternary pumps, Photo Diode Array detector and Auto sampler integrated with Empower 2 Software. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2 mm and 10mm and matched quartz cells integrated with UV win 6 Software was used for measuring absorbances of AMT and LFT solutions.

Drug samples:

AMT 20mg and LFT 120mg.

Reagents and Solutions:

Distilled water, Acetonitrile, Phosphate buffer, Methanol, Ortho-phosphoric acid [All are HPLC grade], Potassium dehydrogenate ortho phosphate buffer [AR].

Analytical methodology:

Diluent: Based up on the solubility of the drugs, diluent was selected, Acetonitrile and Water taken in the ratio of 50:50.

Preparation of Standard stock solutions: Accurately weighed 5 mg of AMT, 30 mg of LFT and transferred to individual 25 ml volumetric flasks separately. 3/4th of diluents was added to both of these flasks and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution 1and 2. (200µg/ml of AMT and 1200µg/ml of LFT).

Preparation of Standard working solutions (100% solution): 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (20µg/ml AMT of and 120µg/ml of LFT).

Preparation of Sample stock solutions: 5 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 50 ml volumetric flask, 25ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (400µg/ml of AMT and 2400µg/ml of LFT).

Preparation of Sample working solutions (100% solution): 0.5ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (20µg/ml of AMT and 120µg/ml of LFT).

Preparation of buffer:

0.1% OPA Buffer: 1ml of Conc. Ortho Phosphoric acid was diluted to 1000ml with water.

Mobile phase: Mobile phases used for HPLC are typically mixtures of organic solvents and water or aqueous buffers.

RESULTS AND DISCUSSION

Method Development

Optimized method: Trials were performed for the method development and the best peak with least fronting factor was found to be with RT=0.783 min for AMT and 1.573min for LFT.

Table 1: Chromatographic conditions

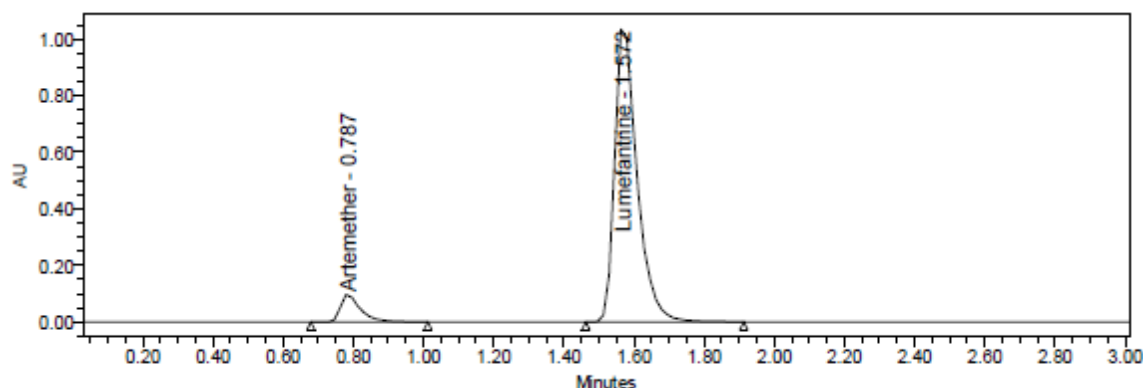| Mobile phase	55% 0.01N KH$_2$PO$_4$ buffer: 45% Acetonitrile
Flow rate	1ml/min
Column	X-bridge C18 (4.6 x 100mm, 5µm)
Detector wavelength	215nm
Column temperature	30°C
Injection volume	0.5µL
Run time	3.0 min
Diluent	Water and Acetonitrile in the ratio 50:50
Results	Both peaks have good resolution, tailing factor, theoretical plate count and resolution.

Figure 2: System suitability chromatogram

System Suitability: According to ICH guidelines plate count should be more than 2000, tailing factor should be less than 2 and resolution must be more than 2. All the system suitable parameters were passed and were within the limits.

Table 2: System suitability parameters for AMT and LFT

S. No Injection	AMT			LFT			
	RT (min)	USP Plate Count	Tailing	RT (min)	USP Plate Count	Tailing	Resolution
1	0.786	2017	1.45	1.568	3312	1.33	7.4
2	0.786	2013	1.47	1.568	3520	1.39	7.3
3	0.786	2962	1.47	1.570	3479	1.37	7.4
4	0.787	2041	1.47	1.570	3349	1.41	7.0
5	0.787	2934	1.59	1.570	2798	1.42	6.8
6	0.788	2837	1.62	1.572	2834	1.42	6.8

Methods for Validation

Linearity: Six linear concentrations of AMT (5-30µg/ml) and LFT (30-180µg/ml) were injected in a duplicate manner. Average areas were mentioned above and linearity equations obtained for AMT was y = 19308x + 1509.4 and of LFT was y = 36919x + 115664 Correlation coefficient obtained was 0.999 for the two drugs.

Table 3: Linearity table for AMT and LFT

AMT		LFT	
Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area
0	0	0	0
5	102290	30	1269437
10	200320	60	2442112
15	279892	90	3439710
20	385202	120	4553166
25	487724	150	5653184
30	582440	180	6711237

y = 19308x + 1509.4
R² = 0.9992
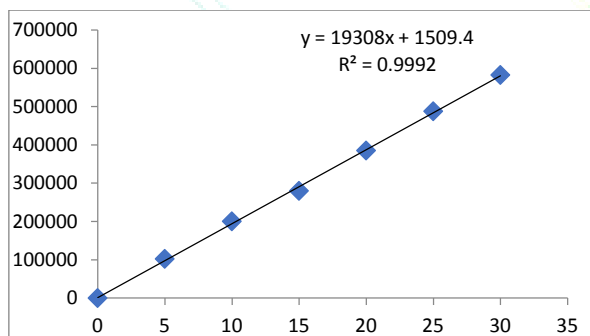
Figure 3: Calibration curve of AMT
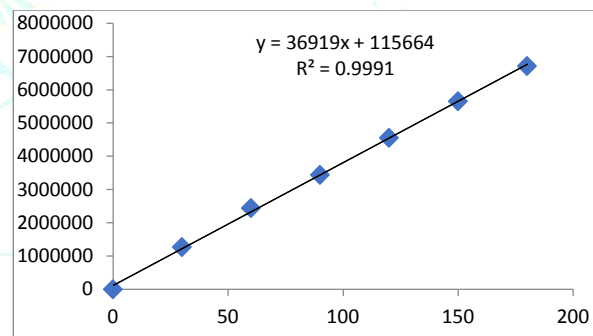
y = 36919x + 115664
R² = 0.9991

Figure 4: Calibration curve of LFT

Precision: From a single volumetric flask of working standard solution six injections were given and the obtained areas were mentioned above. Average area, standard deviation and % RSD were calculated for two drugs. % RSD obtained as 0.3% and 0.3% respectively for AMT and LFT. As the limit of Precision was less than "2" the system precision was passed in this method.

Table 4: System precision table of AMT and LFT

S. No	Area of AMT	Day-Day precision Peak area	Area of LFT	Day-Day precision Peak area
1.	380088	374570	4595070	4590553
2.	380169	371816	4585652	4515867
3.	378427	374545	4591200	4506563
4.	380085	377682	4586047	4596058
5.	380944	376553	4611663	4538194
6.	381169	370952	4610550	4545747
Mean	380147	374353	4596697	4548830
S.D	964.0	2608.0	11697.6	37328.2
%RSD	0.3	0.7	0.3	0.8

Accuracy: Three levels of Accuracy samples were prepared by standard addition method. Triplicate injections were given for each level of accuracy and mean %Recovery was

obtained as 99.49% and 98.71% for AMT and LFT respectively.

Table 5: Recovery studies for AMT and LFT

%Concentration	AMT			LFT		
	50%	100%	150%	50%	100%	150%
Trail-I	96.88	96.88	99.66	100.53	100.10	99.90
Trail-II	98.71	98.71	99.80	99.26	98.97	98.71
Trail-III	99.20	99.20	100.54	101.35	101.56	101.64
AVG (%Recovery)	98.3	100.2	100.00	100.38	100.21	100.08
SD	1.22	1.13	0.47	1.051	1.2990	1.4752
%RSD	1.24	1.13	0.47	1.05	1.30	1.47

Robustness: Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus (55B:45A), mobile phase plus (45B:55A), temperature minus (25°C) and temperature plus (35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

Table 6: Robustness data for AMT and LFT

S. No.	Condition	%RSD of AMT	%RSD of LFT
1	Flow rate (-) 0.9ml/min	0.6	0.6
2	Flow rate (+) 1.1ml/min	1.7	1.6
3	Mobile phase (-) 55B:45A	0.5	0.1
4	Mobile phase (+) 45B:55A	1.1	0.2
5	Temperature (-) 25°C	0.1	0.3
6	Temperature (+) 35°C	0.9	0.3

LOD and LOQ: LOD and LOQ were estimated from the signal-to-noise ratio. The LOD for AMT and LFT were found to be 0.03 and 0.08μg/ml and the LOQ were 0.095 and 0.0288μg/ml respectively.

Table 7: LOD AND LOQ of AMT and LFT

Molecule	LOD	LOQ
AMT	0.03	0.08
Lumefantrine	0.095	0.288

Degradation data: Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation.

Table 8: Degradation data of AMT and LFT

Type of degradation	AMT			LFT		
	AREA	%RECOVERED	% DEGRADED	AREA	%RECOVERED	% DEGRADED
Acids	356108	93.30	6.70	4377916	95.05	4.95
Base	365252	95.70	4.30	4338107	94.19	5.81
Peroxide	370534	97.08	2.92	4339047	94.21	5.79
Thermal	371099	97.23	2.77	4469922	97.05	2.95
Uv	375429	98.36	1.64	4479189	97.25	2.75
Water	379345	98.36	1.64	4550018	98.79	1.21

CONCLUSION

The UPLC method was developed and validated. The developed method was system suitability, precision, accuracy, limit of detection and limit of quantization and degradation studies of the simultaneous estimation of AMT and LFT in bulk and pharmaceutical dosage form. The combination of these drugs is easy to administer and may improve adherence in the treatment of uncomplicated malaria caused by plasmodium falciparum. UPLC gives increased resolution, speed and sensitivity for liquid chromatography therefore due to UPLC new chemistry and instrumentation technology can provide more information per unit of work. UPLC has main advantage over others is reduction of analysis time which also helps to reduce solvent consumption. A negative aspect of UPLC could be the higher back pressure than in conventional HPLC. This back pressure can be reduced by increasing the column temperature. But it seems that UPLC can offer significant improvements in speed, sensitivity and resolution compared with conventional HPLC.

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