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Analytical Method Development and Validation of Artesunate and Amodiaquine Hydrochloride in Combined Tablet Dosage Form by High Performance Liquid Chromatography

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ABSTRACT

A simple, economic, specific and accurate reverse phase high performance liquid chromatographic method was developed for the simultaneous determination of Artesunate (ART) and Amodiaquine hydrochloride (AMO) in tablet dosage form. The column used was Agilent technologies 150 mm x 3.9 mm, 5µ particle size, C18 packing in isocratic mode, with mobile phase containing phosphate buffer and methanol (30: 70) (v/v), pH 3.0 and injection volume of 20µL, with a flow rate of 1.0ml/min. and the detection were carried out by Diode Array Detector with Variable Wavelength (DAD) at dual wavelengths, 225nm and 339 nm respectively for Artesunate and Amodiaquine HCl. The retention times of artesunate and amodiaquine hydrochloride were both 1.3 minutes but extracted at different wavelength. The linearity for Artesunate and Amodiaquine hydrochloride were in the range of range 10-60 µg/mL and 5-30 µg/ml respectively with correlation coefficient of r2 =0.996 and 0.995 for artesunate and amodiaquine respectively. The recoveries of artesunate and amodiaquine hydrochloride were found to be 102.9% and 102.9%, respectively. The % RSD from repeatability, intra and inter day precisions was found to be <2.0%. The proposed method was statistically evaluated and can be applied for routine quality control analysis of artesunate and amodiaquine hydrochloride in tablets. The method was validated as per ICH Q(2A) guideline.

KEY WORDS: Artesunate and Amodiaquine hydrochloride, HPLC, Method development, Analytical Method Validation, Forced degradation studies

INTRODUCTION

Artesunate belongs to artemesinin group effective in the treatment of malarial patients. Amodiaquine hydrochloride is an antimalarial agent like chloroquine in structure and activity which belongs to the class of 4-aminoquinoline widely used in both antimalarial and anti-inflammatory pharmaceutical formulations, alone or combination with other drugs. Artesunate is chemically (3R,5aS,6R,8aS,9R,10S,12R,12aR) - Decahydro - 3,6,9 - trimethyl-3, 12epoxy - 12H - pyrano [4,3- j] -1,2-benzodioxepin-10-ol,hydrogensuccinate, and amodiaquine hydrochloride is 4-[(7-chloro4-quinolyl)amino]-2-[(diethylamino)methyl] phenol dihydro chloride dihydrate was successfully used as one content in association with other drugs in the treatment of malaria (Rao et al, 2013). Literature survey revealed that various analytical methods have been reported for the determination of Artesunate and Amodiaquine hydrochloride in pure drug, pharmaceutical dosage forms and in biological samples using liquid chromatography either in single or in combined forms. Confirmation of the applicability of the developed method was validated according to the International Conference on Harmonization (ICH) for the simultaneous determination of Artesunate and Amodiaquine hydrochloride in bulk and in tablet dosage form (Odedara et al,2012).

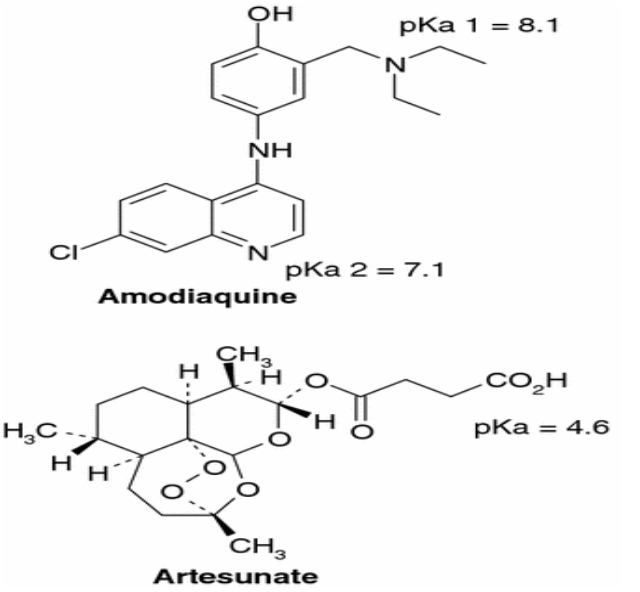

Figure 1 Structure of Amodiaquine and Artesunate (www.ijrpb.com)

MATERIALS AND METHODS

HPLC system, Agilent technologies, Model- AGILENT 1290 HPLC, and 150 x 3.9 mm, 5µ particle size, C18 packing column were used. The data acquisition was performed using Agilent Chemstation software. In addition, Mettler Toledo analytical balance (0.1mg sensitivity), Mettler Toledo digital pH meter, an ultrasonic bath (sonicator) and carbolite oven were used in this study.

Chemicals and reagents: Artesunate and Amodiaquine hydrochloride primary reference standards from United State Pharmacopeia Convention (USP) with lot: R07680 and R078LO respectively were used. Potassium dihydrogen phosphate salt and orthophosphoric acid were sourced from Merck. Other solvent like Methanol and Water (Milli-Q) were all HPLC grade.

Preparation of Buffer and mobile Phase

Phosphate Buffer: 2.72009g of potassium dihydrogen phosphate was weighed and dissolved in 1800mL of milli-Q water, it was then adjusted to a pH of 3.00 at 25.0 degree Celsius with orthophosphoric acid and then the volume was made up to 2 Litres and filtered through 0.45µm nylon filter membrane.

Mobile Phase: Isocratic mixture of 30 volume of phosphate buffer online A and 70 volumes of methanol online B.

Stock preparation of standards and Samples

Artesunate standard stock preparation: 10.0mg of USP Artesunate primary reference standard was accurately weighed into 100mL class A volumetric flask and dissolved in diluent (Methanol) and made-up to mark with diluent to obtain a concentration of100µg/mL.

Amodiaquine Hydrochloride standard stock preparation

10.00mg of USP Amodiaquine Hydrochloride primary standard was accurately weighed into 100ml class A volumetric flask and dissolved in diluent (Methanol) and was diluted to mark with diluent to obtain 100µg/mL

Calibration curve

Calibration curve was prepared by taking aliquots of 1.0, 2.0, 3.0, 4.0, 5.0, 6.0ml of stock solution of Artesunate standard and 0.5, 1.0, 1.5, 2.0, 2.5, 3.0ml of stock solution of Amodiaquine standard in 10ml class A volumetric flask and made-up to mark with diluent to obtain a concentration of 10.0, 20.0, 30.0, 40.0, 50.0, 60.0µg/ml of Artesunate and 5.0, 10.0, 15.0, 20.0, 25.0, 30.0µg/ml of Amodiaquine.

Sample Preparation (100mg/270mg Artesunate/ Amodiaquine HCl)

20 tablets were accurately weighed and triturated with glass mortar and pestle. The powder equivalent to 100mg was weighed and transferred into a 100mL class A volumetric flask, it was dissolved in the diluent and placed in the ultrasonic bath for 10 minutes, then made up to mark with diluent and the resultant solution was filtered through 0.45-micron filter. The solution was analysed under a well equilibrated and optimized chromatographic condition.

Procedure: The column was equilibrated for 30 minutes with the mobile phase at 1.0mL/minute, then 20µL of diluent (blank) was separately injected, five injections of standard solution and sample solution were introduced into the chromatographic system accordingly.

RESULT AND DISCUSSION

Method Validation

Accuracy:

Accuracy may often be expressed as percentage recovery. To a fixed amount of pre-analyzed sample (2.0 mL) mixture of Artesunate (100 µg/mL) and Amodiaquine (100 µg/ml) increasing amount of its working standard solution (1.0, 2.0, 3.0ml of 100 µg/ml of Artesunate and 100 µg/ml

Amodiaquine) was added in three different 10 ml volumetric flask and made up to mark with methanol. Samples were injected to the system and analyzed. The mean % recovery from peak areas of artesunate and amodiaquine hydrochloride were found to be 102.9% and 102.9%, respectively.

Specificity:

The Chromatograms of Standard and Sample are identical with nearly same retention time. There is no interference with blank to the drugs. Hence the proposed method was found to be specific.

Specificity is the ability of analytical method to measure accurately and specifically the analyte in the presence of components that may be expected to be present in the sample. The specificity of method was determined by injecting the diluent to observe any interference with the drug peak. However, the artesunate and amodiaquine peak eluted at the same retention time but extracted at different wavelength.

Linearity:

Linearity is the ability of the method to produce results that is directly proportional to the concentration of the analyte in samples with a specific range.

Table 1 Correlation Coefficient of Linearity

Injections	Artesunate		Amodiaquine HCl	
	Concentration	Peak Area	Concentration	Peak Area
1	10	431.577	5	86.938
2	20	893.995	10	177.713
3	30	1187.797	15	264.004
4	40	1537.702	20	347.195
5	50	1913.110	25	433.057
6	60	2177.308	30	484.964
r^2	0.996		0.995	

Acceptance criteria: Correlation Coefficient (r2): ≥ 0.99

An accurate amount of Artesunate and Amodiaquine were weighed (in duplicate) to prepare standard solution of Artesunate and Amodiaquine in range 10-60 µg/mL and 5-30 µg/ml respectively in a 10ml volumetric flask, 40mL of diluent was added and sonicated until totally dissolved. The sample was allowed to cool and diluted to volume with diluent.

20µL of each solution (2 injections per solution) was injected. A graph of average area versus concentration was plotted to determine the linearity co-efficient of correlation (r^2).

Precision: Precision is the degree of closeness of agreement among individual test results when the method is applied to multiple sampling of a homogeneous sample.

Precision was determined and expressed as Relative Standard deviation (% RSD) of the same homogeneous sample injected under the same prescribed conditions.

System precision: Six (6) replicates of Artesunate and Amodiaquine standard preparations were used to calculate the %RSD for the peak area and retention time.

Table 2 Percent Relative Standard Deviations of peak area and retention time

Inj.	Area		Retention Time	
	Artesunate	Amodiaquine	Artesunate	Amodiaquine
1	413.788	85.989	1.342	1.344
2	431.039	86.781	1.341	1.343
3	421.666	85.911	1.341	1.343
4	421.132	86.339	1.342	1.344
5	428.954	86.781	1.341	1.343
AVG	**423.3158**	**86.3602**	**1.3414**	**1.3434**
STDEV	**6.886707**	**0.416583**	**0.000548**	**0.000548**
%RSD	**1.63**	**0.48**	**0.04**	**0.04**
Acceptance Criteria (%RSD)	≤2.0%	NMT 2.0%	NMT 2.0%	NMT 2.0%

*NMT = Not More Than

Repeatability: Three different standard solutions of Artesunate (30, 40, and 50 µg/mL) and Amodiaquine (15, 20, and 25 µg/ml) was prepared from working standard solution and injected three times into the HPLC system with same chromatographic conditions and analyzed.

Table 3 Percent Relative Standard Deviations for Repeatability

Conc.	Peak Artesunate	Response%RSD ≤ 1.0%	Conc.	Peak Amodiaquine	Response%RSD ≤ 1.0%
30 µg/ml	1192.46	0.01	15 µg/ml	265.795	0.12
	1192.345			265.1656	
	1192.124			265.4695	
40 µg/ml	1544.736	0.003	20µg/ml	349.005	0.07
	1544.756			349.1674	
	1544.823			348.6848	
50 µg/ml	1915.226	0.06	25µg/ml	434.071	0.02
	1916.035			434.1219	
	1913.857			433.9863	

INTRADAY PRECISION: Standard solutions Artesunate (10-60 µg/ml) and Amodiaquine (5-30 µg/ml) was prepared from working standard solution and injected into the system with stated chromatographic conditions and analyzed, three times in a day.

Table 4 Percent Relative Standard Deviation for Intra-day precision

Injections	Artesunate			Amodiaquine HCl		
	Concentration	Mean Area	%RSD	Concentration	Mean Area	%RSD
1	10	418.8183	0.03392	5	85.56262	0.070444
2	20	885.6786	0.360247	10	176.703	0.169615
3	30	1193.389	0.30805	15	265.4951	0.238953
4	40	1544.905	0.02614	20	348.7892	0.029013
5	50	1916.603	0.106476	25	433.6548	0.113922
6	60	1.239014	0.0561	30	491.7065	0.021562

Acceptance Criteria: %RSD ≤ 2.0%

INTERDAY PRECISION: Standard solutions Artesunate (10-60 µg/ml) and Amodiaquine (5-30 µg/ml) was prepared from working standard solution and injected into the system with same chromatographic conditions and analyzed for five (5) consecutive days.

Table 5 Percent Relative Standard Deviation for Inter-day precision

Injections	Artesunate			Amodiaquine HCl		
	Concentration	Mean Area (Day 1-5)	%RSD	Concentration	Mean Area (Day 1-5)	%RSD
1	10	415.764	0.17905	5	85.66695	0.235416
2	20	886.3033	0.074985	10	177.4362	0.231357
3	30	1196.501	0.051691	15	267.5071	0.317172
4	40	1546.274	0.122449	20	350.1765	0.423075
5	50	1912.702	0.069556	25	433.799	0.078951
6	60	2209.597	0.031745	30	492.8544	0.109026

LOD and LOQ: LOD is the lowest concentration of analyte in a sample that can be detected but not quantified under experimental conditions. The LOD values were determined by the formulae LOD=3.3σ/s (where σ is the standard deviation of the responses and s is the mean of the slopes of the calibration curves).

LOQ is the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy under experimental conditions. It is a parameter of the quantitative determination of compounds in the mixtures. The LOQ values were determined by the formulae LOD=10σ/s. The results are tabulated below.

Table 6 LOD and LOQ for Artesunate and Amodiaquine

LOD and LOQ	ARTESUNATE (µg/ml)	AMODIAQUINE (µg/ml)
LOD (Limit of Detection)	0.9895	31.54
LOQ (Limit of Quantitation)	2.998	95.58

Method Ruggedity and Robustness: Forced degradation of Artesunate and Amodiaquine hydrochloride

Thermal degradation: Sample powder equivalent to 100mg of Artesunate and 12.5mg of Amodiaquine hydrochloride was taken and kept in a controlled temperature oven at 400 ºC for 12hrs. After 12hrs the powder was diluted with diluents to get a concentration of 10µg/ml solution and analysed to recorded chromatogram.

Base Degradation: Base degradation was determined by taking 5ml of stock solution in 10ml volumetric flask and to this 2ml of 0.1N NaoH was added and sonicate for 5min, kept aside for 12hrs at room temperature. After 12hrs the solution was neutralized with 0.1N HCl then diluted with diluents to get a concentration of 10µg/ml solution and analysed to recorded chromatogram.

Method Robustness: All the system suitability parameters are within limits when the drugs were subjected to stress conditions like thermal and base degradation. The results obtained were satisfactory and in good agreement as per the ICH (Q2A) guidelines.

Table 7 Optimized chromatogram conditions for Artesunate and Amodiaquine hydrochloride

Column	Agilent technologies 150 x 3.9 mm, 5µ particle size, C18 packing
Mobile phase	Phosphate Buffer Methanol (30:70)
Flow rate	1.0 ml/ min
Wavelength (DAD)	225nm(Artesunate) and 339nm(Amodiaquine)
Injection volume	20 Ml
Column temperature	Ambient
Run time	5 min

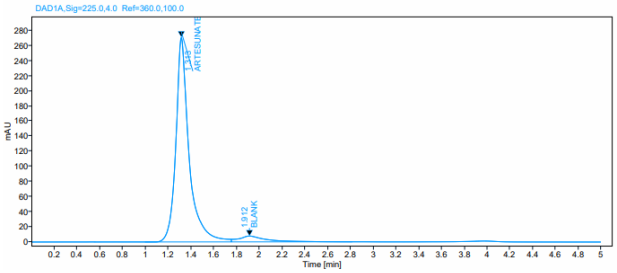

Figure 2 Chromatogram of Artesunate primary reference standard

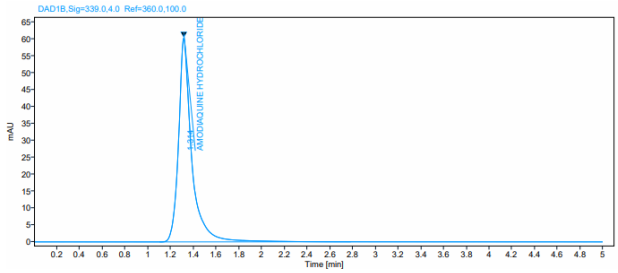

Figure 3 Chromatogram of Amodiaquine primary reference standard

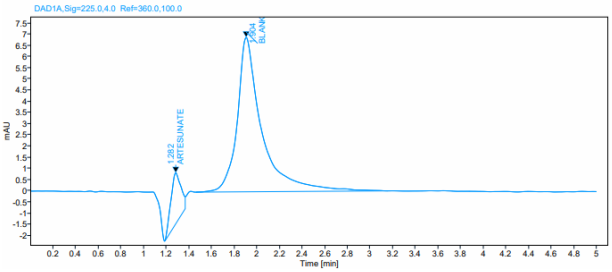

Figure 4 . Chromatogram of Blank

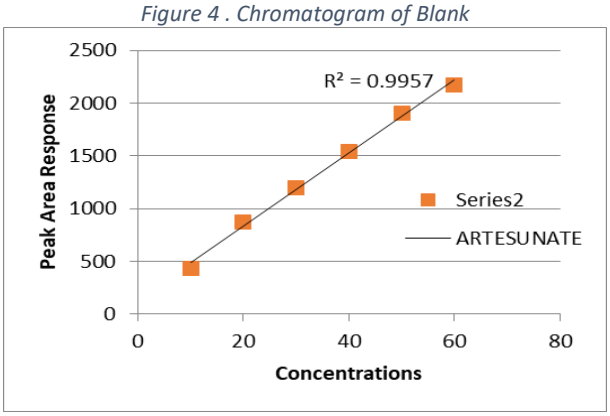

Figure 5 Linearity plot of Artesunate solution and the peak response

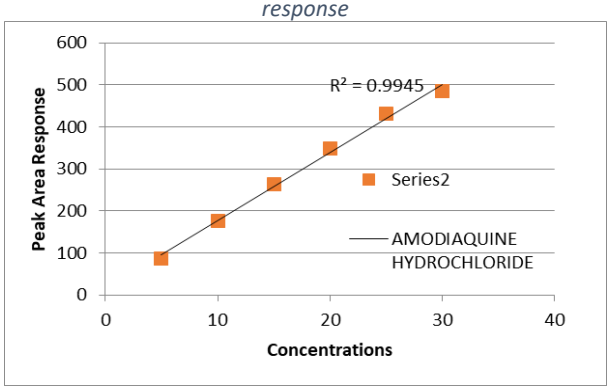

Figure 6 Linearity plot of Amodiaquine solution and the peak response

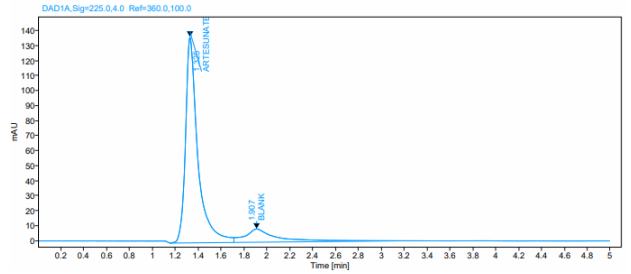

Figure 7 Chromatogram of Artesunate sample

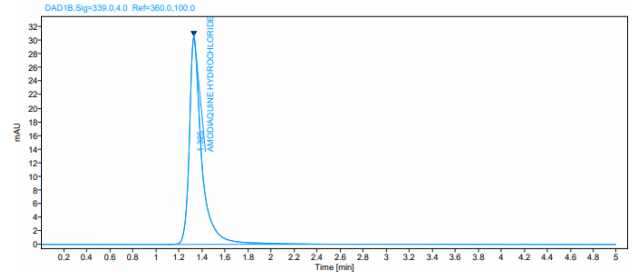

Figure 8 Chromatogram of Amodiaquine sample

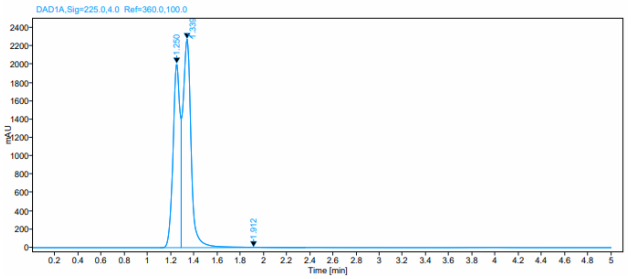

Figure 9 Chromatogram of forced degradation Artesunate sample

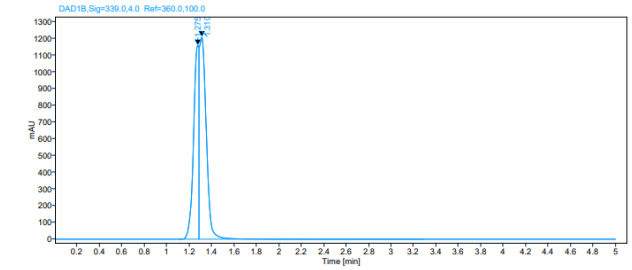

Figure 10 Chromatogram of forced degradation Amodiaquine sample

For the HPLC method different mobile phases were tried and the mobile phase containing Methanol and phosphate buffer (70:30, v/v) was found to be optimal for obtaining well defined and resolved peaks with mean retention times 1.34 minutes for both Artesunate and Amodiaquine HCl. The representative chromatogram of the standard solution of mixture is shown in Figure 1and 2. Results were found to be linear in the concentration range of 10–60 mg/mL for Artesunate and 5–30 mg/mL for Amodiaquine HCl. The correlation coefficients for the plots were 0.996 for Artesunate and 0.995 for Amodiaquine HCl. The method was found to be accurate and precise, as indicated by recovery studies with 102.9% recovery and % RSD not more than 2.0 for all precision performance characteristics. The summary of the method development and validation parameters of the HPLC methodology is given in tables above.

CONCLUSION

HPLC Isocratic elution using mobile phase of Methanol and phosphate buffer at pH 3.0 (70:30) v/v for a run time of 5 minutes was found to be ideal for the estimation of Artesunate and Amodiaquine HCl fixed-dose combination.

The proposed method is simple, sensitive and reproducible and hence can be used in routine determination of Artesunate and Amodiaquine HCl in pharmaceutical solid dosage preparations. Statistical analysis of the results has been carried out revealing high accuracy and good precision. The developed method can also be used for routine quantitative estimation of Artesunate and Amodiaquine HCl in pharmaceutical preparations.

All the parameters are within limits and meet the acceptance criteria of ICH (Q2A) guidelines for method validation. The proposed method was cheap, simple, accurate, specific, precise, robust, rugged and economical. Hence this method is validated and can be used for routine and stability sample analysis of artesunate and amodiaquine fixed-dose combination tablets.

REFERENCES

1. Odedara MH, Faldu SD, Dadhania KP, RPHPLC Method for Simultaneous Estimation of Artesunate and Amodiaquine HCL in their combined pharmaceutical dosage form, JPSBR, 2(3), 2012, 114-117.

2. P RajaRao, Nanda Kishore AgarwalIndian Journal of Research in Pharmacy and Biotechnology ISSN: 2321-5674(Print) ISSN: 2320 – 3471(Online) IJRPB 1(6) www.ijrpb.com November – December 2013

3. World Health Organisation (2006) Who Guidelines For The Treatment Of Malaria. Http://Www.Who.Int/Malaria/Docs/Treatmentguidelines2 006.Pdf. (Last Accessed February 2009).