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# Selective High Performance Liquid Chromatographic Determination of Amodiaquine and Artesunate in bulk and pharmaceutical formulation

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**ABSTRACT**

Aim of the study was to develop a new selective high-performance liquid chromatography (HPLC) method for the quantification of amodiaquine and artesunate in bulk and pharmaceutical dosage form. The HPLC analysis was performed on the LCGC Qualisil $C_8$ (5 µm, 250 mm X 4.6 mm i.d.) column in isocratic mode, at $30^0$C temperature using a mobile phase consisting of Acetonitrile: phosphate buffer (70:30, *v/v*) at a flow rate of 0.8 ml/min. The detection was carried out at 254nm for amodiaquine and 221nm for artesunate. The retention time for AMQ and ART were found to be 2.8 min. and 5.6 min. respectively. The method was validated for precision, recovery, robustness, specificity, and detection and quantification limits, in accordance with International Conference on Harmonization guidelines. Linearity was observed in the concentration range from 2-12 µg/ml ($r^2$=0.998) for AMQ and for ART 0.2-1.2 mg/ml ($r^2$=0.998). The limit of detection and quantification of AMQ were 0.07 µg/ml and 0.21 µg/ml respectively. While for ART it was 0.044 mg/ml and 0.133 mg/ml, respectively. The method has been successively applied for the determination of AMQ and ART in tablets. There was no interference from the excipients commonly present in the tablets. The drug content was found to be 100.83 % for AMQ and 98.63 for ART. Accuracy of the method was studied by the recovery studies at three different levels 80 %, 100 % and 120 %. The % recovery was found to be within the limits of the acceptance criteria with average recovery of 98.55–101.46% for AMQ and 99.48–101.60% for ART. The % RSD below 2.0 shows the high precision of proposed method. The above method was a rapid and cost-effective quality-control tool for routine analysis of amodiaquine and artesunate in bulk and in pharmaceutical dosage form.

## INTRODUCTION

Amodiaquine is chemically 4-(7-chloro-4-quinolyamino)-2-diethylaminoethyl) phenol Dihydrochloride dehydrate (Fig. 1), having molecular formula $C_{20}H_{22}ClN_3O$ with molecular weight 355. It is yellow crystalline powder with melting point 208-209$^0$C and soluble in water and alcohol **(**Martindale, 2007; Maryadele, 2006).

It is acts as an anti-malerial agent. Since AMQ still has a high degree of efficacy against all chloroquine-resistant strains, there has been a recent increase in its use. However, monitoring of effectiveness and surveillance for evidence of toxicity are still being maintained. Artesunate is a semi-synthetic derivative of artemisinin, a naturally occurring sesquiterpene endoperoxide. It is indicated chemically as 3R,5aS,6R,8aS,9R,- 10S,-12R,12aR)-decahydro-3,6,9-trimethyl-3,12-epoxy- 12H-pyrano(4,3-j)-1,2-benzodioxepin-10-ol hydrogen succinate (Fig. 1). The molecular formula is C19H28O8 with molecular weight of 384.42. It is a white crystalline powder with melting point range of 132–135$^0$C and slightly soluble in water**.**

Artesunate is an antimalarial agent and a hemi-succinate derivative of dihydroartemisinin. Artemisinin is a sesquiterpene lactone isolated from *Artemisia annua*, a herb that has traditionally been used in China for the treatment of malaria.

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Artesunate and its active metabolite dihydroartemisinin are potent blood schizonticides, active against the ring stage of the parasite. Various methods are reported for the analysis of individual drug and in combination but no method were reported for selective estimation of these two drugs in combined dosage form. Therefore, it was thought worthwhile to develop RP-HPLC methods for analysis of Amodiaquine and Artesunate in Pharmaceutical dosage form (Dua *et al*., 2004, Dongre *et al*., 2008, Minzi *et al*., 2003, Ntalea *et al*., 2007, Bell *et al*., 2007, Mount *et al*., 1986, Berguvist and Churchill, 1988, Xiaoyan *et al*., 2007, Taylor *et al*., 2000, Battya *et al*., 1986, Kotecha *et al*., 2003, Nabangchang *et al*., 2007, Gaudin *et al*., 2009, Lee *et al*., 2010, Lai *et al*., 2007).

Therefore, the objective of this study was, therefore, to develop a simple, economical, selective, precise, and reproducible high performance liquid chromatography (HPTC) method on both the drugs in bulk and tablet formulation.

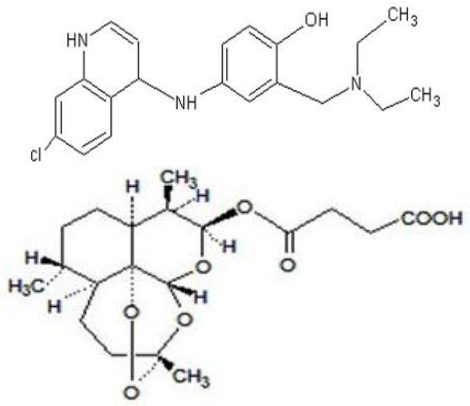


**Fig. 1:** Chemical structure of Amodiaquine (a) and Artesunate (b).

**Structured Abstract**

A new simple**,** precise, accurate and selective HPLC method has been developed and validated for estimation of Amodiaquine and Artisunate in pharmaceutical formulation. The detection was carried out at 254nm for amodiaquine and 221nm for artesunate.

The retention time for AMQ and ART were found to be 2.8 min. and 5.6 min. respectively. The method was validated for precision, recovery, robustness, specificity, and detection and quantification limits, in accordance with International Conference on Harmonization guidelines.

Linearity was observed in the concentration range from 2-12 µg/ml ($r^2$=0.998) for AMQ and for ART 0.2-1.2 mg/ml ($r^2$=0.998). The limit of detection and quantification of AMQ were 0.07 µg/ml and 0.21 µg/ml respectively. While for ART it was 0.044 mg/ml and 0.133 mg/ml, respectively.

The drug content was found to be 100.83 % for AMQ and 98.63 for ART. The % recovery was found to be within the limits of the acceptance criteria with average recovery of 98.55–101.46% for AMQ and 99.48–101.60% for ART. The % RSD below 2.0 shows the high precision of proposed method.

## SUBJECTS AND METHODS

### Reagent and chemicals

Amodiaquine (AMQ) and Artesunate (ART) were supplied as a gift sample by Mepro pharmaceutical Ltd. (Gujarat). These drugs were used as working standard. All the chemicals used of HPLC Grade (MERCK. Chem. Ltd., Mumbai) and double distilled water was used for mobile phase preparation.

### Selection of chromatographic parameters

#### *Selection of chromatographic mode*

The reverse phase HPLC was selected for separation because it was convenient and rugged than other forms of the liquid chromatography and was more likely to give good resolved peaks at a reasonable retention time at a specific pH.

#### *Selection of stationary phase*

On the basis of reversed phase HPLC mode and number of carbon present in molecule (analyte) stationary phase with $C_8$ bonded phase i.e. RP LCGC Qualisil $C_8$ (250 mm x 4.6 mm i.d.) with particle size 5 µm was selected.

#### *Preparation of standard stock solution*

Standard stock solutions were prepared by dissolving 10 mg of AMQ and 25 mg of ART in 10 ml mobile phase that gives concentration of 1000 µg/ml of AMQ and 2500 µg/ml of ART. Standard solutions were prepared by further dilution with the mobile phase to the required concentrations.

#### *Optimization of mobile phase strength*

The mobile phase was chosen after several trials with Acetonitrile and phosphate buffer in various proportions. A mobile phase consisted of Acetonitrile: phosphate buffer (70:30, v/v) was selected to achieve symmetrical peak and sensitivity. The effects of flow rates in the ranges of 0.5 to 1.5 ml/min were examined.

## METHOD VALIDATION

The proposed method was validated as per ICH guidelines. The solutions of the drugs were prepared as per the earlier adopted procedure given in the experiment.

### Linearity studies

From AMQ stock solution aliquots of 20, 40, 60, 80, 100 and 120 µl were taken in 10 ml volumetric flasks and diluted up to the mark with mobile phase such that the final concentration of AMQ in the range 2 – 12 µg/ml. From ART stock solution aliquots of 80, 160, 240, 320, 400 and 480 µl were taken in 10 ml volumetric flasks and diluted up to the mark with mobile phase such that the final concentration of ART in the range 0.2–1.2 mg/ml. Volume of 20 µL of each sample was injected with the help of Hamilton Syringe. All measurements were repeated five times for each concentration and calibration curve was constructed by plotting the peak area *vs* the drug concentration (Fig. 3)

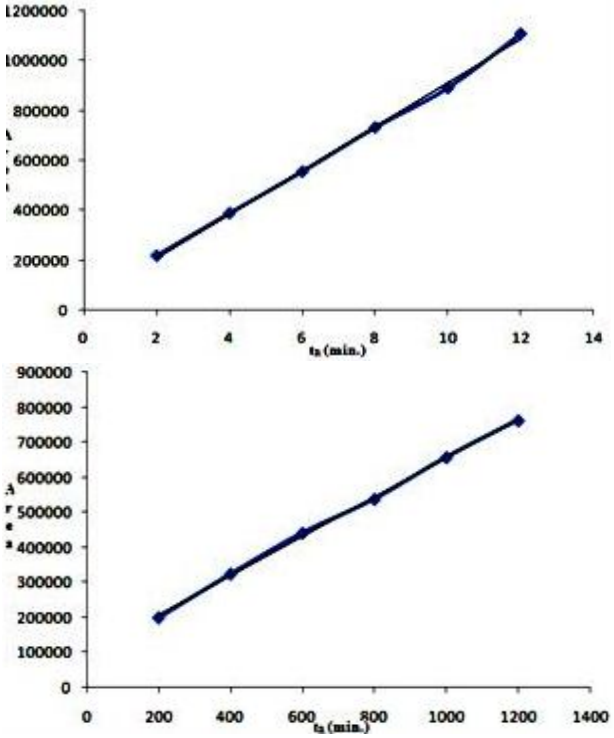


**Fig. 3:** Calibration curve of Amodiaquine (a) and Artesunate (b).

**Accuracy**

The accuracy was done by recovery study using standard addition method at 80%, 100% and 120 % level; known amount of standard AMQ and ART were added to preanalyzed sample (6 µg/ml of AMQ; 0.6 mg/ml of ART) and subjected them to the proposed HPLC method.

**Precision**

Precision was the measure of how close the data values are to each other for a number of measurements under the same analytical conditions.

**Intra – day and Inter – day Precision**

Intra – day precision were determined by analyzing, the three different concentrations 8 µg/ml, 12 µg/ml and 16 µg/ml of AMQ, 0.4 µg/ml, 0.6 µg/ml and 0.8 µg/ml of ART for three times in the same day. Day to day variability was assessed using above mentioned three concentrations analyzed on three different days, over a period of one week.

**Repeatability**

It was measured by multiple injections of a homogenous sample of 6 µg/ml of AMQ and 0.6 µg/ml of ART that indicates the performance of the HPLC instrument under chromatographic conditions.

**Robustness**

To evaluate robustness few parameters were deliberately varied. The parameters include variation of flow rate, percentage of methanol using 8 µg/ml solution of AMQ and 0.8 mg/ml of ART.

**Sensitivity**

Sensitivity of the proposed method was estimated in terms of Limit of Detection (LOD) and Limit of Quantitation (LOQ). LOD = 3.3 SD/S and LOQ = 10 S.D./S, where S.D. is the residual standard deviation and S is the slope of the line.

**Specificity and Selectivity**

The analytes should have no interference from other extraneous components and be well resolved from them. Specificity is a procedure to detect quantitatively the analyte in presence of component that may be expected to be present in the sample matrix, while selectivity is the procedure to detect qualitatively the analyte in presence of components that may be expected to be present in the sample matrix.

**Ruggedness**

From stock solutions, sample solutions of AMQ (6 µg/ml) and ART (0.6 mg/ml) were prepared and analyzed by two different analysts using similar operational and environmental conditions. Peak area was measured for same concentration solutions, six times.

**System suitability test**

System suitability testing is essential for the assurance of the quality performance of the chromatographic system. Earlier prepared solutions for chromatographic conditions were tested for system suitability testing.

## RESULTS AND DISCUSSION

**Optimization of mobile phase strength**

A mobile phase consisted of Acetonitrile: phosphate buffer (70:30, v/v) was selected to achieve symmetrical peak and sensitivity. A flow rate of 0.8 ml/min gave reasonable retention time; using reverse phase $C_8$ column, the retention times of AMQ and ART were observed as 2.87 and 5.6 min respectively. The total time of analysis was less than 7 min (Fig. 2).

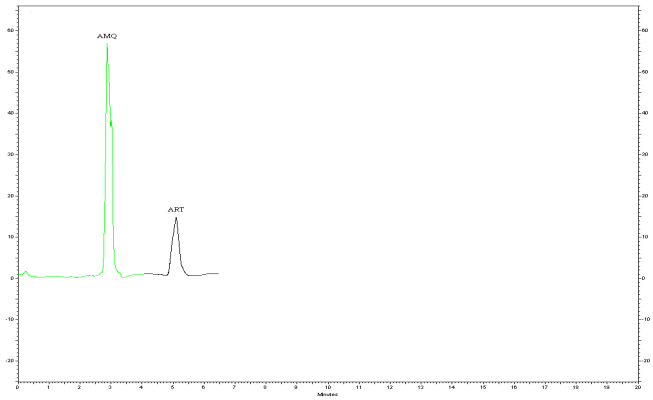


**Fig. 2:** HPLC Chromatogram of Amodiaquine and Artesunate.

**Method Validation**

The proposed method was validated as per ICH guidelines. The solutions of the drugs were prepared as per the earlier adopted procedure given in the experiment.

**Linearity studies**

The linear regression data for the calibration curves showed good linear relationship over the concentration range 2 – 12 µg/ml for AMQ and 0.2–1.2 mg/ml for ART. Linear regression equation was found to be Y = 87524x + 37305 ($r^2$ = 0.998) for AMQ and Y = 559.0x + 95402 ($r^2$ = 0.998) for ART respectively. The result is expressed in Table 1.

**Table. 1:** Linearity study of AMQ and ART .

| Parameters | AMQ | ART |
|---|---|---|
| Linearity range (µg/ml) | 2-12 | 0.2-1.2 |
| Calibration curve equation | Y = 87524x + 37305 | Y=559.0x+95402 |
| Correlation coefficient | 0.998 | 0.998 |

**Accuracy**

The proposed method when used for extraction and subsequent estimation of both the drug from pharmaceutical dosage forms after over spotting with 80, 100 and 120 % of additional drug; afforded recovery of 98.55 – 101.46% for AMQ and 99.48 –101.60 % for ART as listed in Table 2.

**Table. 2:** Results of Recovery Studies for AMQ and ART.

| Drug | Initial amount of drug [ µg/mL ] | Excess drug added to the analyte [%] | Amount added [ µg/mL ] | % Recovery [n = 3] | % R.S.D. |
|---|---|---|---|---|---|
| AMQ | 6 | 80 | 4.8 | 101.46 | 1.74 |
| | 6 | 100 | 6 | 98.55 | 1.04 |
| | 6 | 120 | 7.2 | 100.39 | 1.39 |
| ART | 0.6 | 80 | 0.48 | 99.48 | 1.91 |
| | 0.6 | 100 | 0.6 | 101.60 | 1.17 |
| | 0.6 | 120 | 0.72 | 99.71 | 1.22 |

*****average of six determinations

**Precision**

The precision of the developed HPTLC method was expressed in terms of % relative standard deviation (% RSD). The results depicted revealed high precision of the method is presented in Table 3.

**Table. 3:** Results of precision studies (Intra-day and Inter-day).

| Drug | Conc. (µg/mL) | Intraday Amount found (µg/mL) | | Interday Amount found (µg/mL) | |
|---|---|---|---|---|---|
| | | Mean ± S.D. | %R.S.D. n=3 | Mean ± S.D. | %R.S.D. n=3 |
| AMQ | 4 | 4.05 ± 2629 | 0.67 | 3.99 ± 8153 | 1.56 |
| | 6 | 5.97 ± 5561 | 1.11 | 5.89 ± 8012 | 0.97 |
| | 8 | 7.89 ± 5079 | 0.69 | 7.91 ± 4531 | 0.79 |
| ART | 0.4 | 0.403 ± 3818 | 0.18 | 0.41 ± 4269 | 1.31 |
| | 0.6 | 0.598 ± 5575 | 1.29 | 0.60 ± 3848 | 0.89 |
| | 0.8 | 0.793 ± 5534 | 1.03 | 0.796 ± 2957 | 0.54 |

**Intra – day and Inter – day Precision**

Day to day variability was assessed using above mentioned three concentrations analyzed on three different days, over a period of one week. These result shows reproducibility of the assay. The % R.S.D. values found to be less than 2, so that indicate this method precise for the determination of both the drugs in formulation (Table 3).

**Repeatability**

The % relative standard deviation (%RSD) was found to be less than 2% for the both the drugs AMQ and ART respectively. The results summarized in Table 4.

**Table . 4:** Results of Repeatability study.

| Drug | Concentration (µg/ml) | Amt found* Mean ± S.D. |
|---|---|---|
| AMQ | 6 | 98.85±9556.27 |
| | %R.S.D. | 1.71 |
| ART | 0.6 | 100.28±4527 |
| | %R.S.D. | 1.04 |

**Robustness**

The standard deviation of peak areas was calculated for each parameter and % R.S.D. was found to be less than 2 % (Table 5(A&B).

**Table. 5A:** Result robustness study for AMQ.

| S. no. | Parameter | S.D. of peak area | %R.S.D. |
|---|---|---|---|
| 1 | Mobile phase composition | | |
| | a) ACN: Buffer (65:35) | 8563.41 | 5880.84 |
| | b) CAN: Buffer(75:25) | 1.52 | 1.04 |
| 2 | Changing flow rate | | |
| | a) 0.7 ml/min | 2023.78 | 3821.54 |
| | b) 0.9 ml/min | 0.41 | 0.78 |
| 3 | Changing pH of mobile phase | | |
| | a) 3.1 | 7460.1 | 9656.86 |
| | b) 3.3 | 1.34 | 1.71 |
| 4 | Changing column temp. | | |
| | a) 25 | 7997.12 | 6804.61 |
| | b) 35 | 1.42 | 1.20 |

*****average of six determinations

**Table. 5B:** Result robustness study for ART.

| S. no. | Parameter | S.D. of peak area | %R.S.D. |
|---|---|---|---|
| 1 | Mobile phase composition | | |
| | c) ACN: Buffer (65:35) | 7669.59 | 6199.95 |
| | d) CAN: Buffer(75:25) | 1.86 | 1.51 |
| 2 | Changing flow rate | | |
| | c) 0.7 ml/min | 4124.97 | 4829.26 |
| | d) 0.9 ml/min | 0.95 | 1.12 |
| 3 | Changing pH of mobile phase | | |
| | c) 3.1 | 6467.74 | 4652.50 |
| | d) 3.3 | 1.53 | 1.08 |
| 4 | Changing column temp. | | |
| | c) 25 | 6490.24 | 4732.09 |
| | d) 35 | 1.51 | 1.09 |

*****average of six determinations

**Sensitivity**

The LOD and LOQ were found to be 0.069 and 0.21 µg/ml for AMQ; 0.044 and 0.133 µg/ml for ART, respectively.

**Specificity and Selectivity**

The method is quite selective. There were no other interfering peak around the retention time of AMQ and ART; also the base line did not show any significant noise.

**Ruggedness**

Peak area was measured for same concentration solutions, six times. The results are in the acceptable range for both the drugs. The results are given in Table 6(A&B).

**Table 6A: Results of Ruggedness study for AMQ**

| Analyst | %Amount found of AMQ (Mean ± S.D.) | %R.S.D. [n=3] |
|---|---|---|
| I | 99.78±1.73 | 1.74 |
| II | 99.09±1.23 | 1.24 |

**Table 6B: Results of Ruggedness study for ART**

| Analyst | %Amount found of ART (Mean ± S.D.) | %R.S.D. [n=3] |
|---|---|---|
| I | 99.47±0.95 | 0.95 |
| II | 100.35±1.30 | 1.30 |

**System suitability test**

The no. of high theoretical plate, peak symmetry ($N \geq 1$) and proper retention time indicates that proposed method was suitable for selective determination of AMQ and ART(Table 7(A&B).

**Table. 7A:** System suitability test for AMQ.

| System suitability parameters | Proposed method |
|---|---|
| Retention time ($t_R$) | 2.87 |
| Capacity factor (K') | 0.75 |
| Theoretical plate (N) | 5238 |
| Tailing factor (T) | 1.14 |

**Table. 7B:** System suitability test for ART .

| System suitability parameters | Proposed method |
|---|---|
| Retention time ($t_R$) | 6.12 |
| Capacity factor (K') | 0.45 |
| Theoretical plate (N) | 2324 |
| Tailing factor (T) | 1.63 |

## CONCLUSION

This densitometric HPTLC technique is quite simple, accurate, precise, reproducible, sensitive, and specific. HPTLC method has been developed for quantification of AMQ and ART in combined tablet formulation. The validation procedure confirms that this is an appropriate method for their quantification in the plant material and formulation. It is also used in routine quality control of the raw materials as well as formulations containing any or all of these compounds.

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