# Analytical method development and validation of Artesunate and Amodiaquine hydrochloride in tablet dosage form by RP-HPLC

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## ABSTRACT

A simple, specific and accurate reverse phase high performance liquid chromatographic method was developed for the simultaneous determination of Artesunate (ART) and Amodiaquine hydrochloride (AMO) in pharmaceutical dosage form. The column used was inertsil ODS C18 (250*4.6mm,5µ) in isocratic mode, with mobile phase containing phosphate buffer- acetonitrile-methanol (50:30:20) adjusted to pH 5.8 using ortho phosphoric acid was used and injection volume of 20µL, with a flow rate of 1.0ml/min. and effluents were monitored at 208 nm. The retention times of artesunate and amodiaquine hydrochloride were 5.03 min and 2.77 min, respectively. The linearity for Artesunate and Amodiaquine hydrochloride were in the range of 15-35 mcg/ml and 45.9-107.1 mcg/ml respectively with correlation coefficient of $r^2$=0.999 for both.The assay of the proposed method was found to be 98.56% and 99.08%. The recoveries of artesunate and amodiaquine hydrochloride were found to be 99.81% and 99.3%, respectively. The % RSD from reproducibility was found to be <2%. The proposed method was statistically evaluated and can be applied for routine quality control analysis of artesunate and amodiaquine hydrochloride in bulk and in Pharmaceutical dosage form.

**Key Words:**, Artesunate & Amodiaquinehydrochloride RP-HPLC, Inertsil ODS, Validation, Forced degradation studies.

## INTRODUCTION

Artesunate belongs to artemesinin group effective in the treatment of malarial patients. Amodiaquinehydrochloride is an antimalarial agent similar to 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-chloro-4-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. Literature survey revealed that a 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.

## MATERIALS AND METHODS

UV-3000 LABINDIA double beam with UV win 5software UV-VISIBLE spectrophotometer with 1cm matched quartz cells. Schimadzu HPLC equipped with SPD 20A UV-VIS detector and the column used was INERTSIL ODS C18 (250*4.6mm, 5µ). The data acquisition was performed by using LC solutions software. In addition an analytical balance (DENVER 0.1mg sensitivity), digital pH meter (Eutech pH 510), a sonicator (Unichrome associates UCA 701) were used in this study.

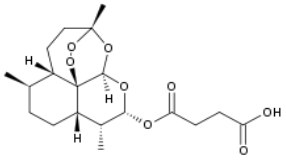

**Figure.1.Structure of Artesunate**

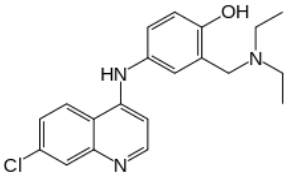

**Figure.2. Structure of Amodiaquine hydrochloride**

**Chemicals and reagents:** Artesunate and Amodiaquinehydrochloride pure sample was taken as a gift sample from local labs and dosage form "falcinil" manufactured by zuventus was purchased from local pharmacy. Other chemicals all are of HPLC grade.

**Preparation of mobile phase:** Potassium dihydrogen phosphate was weighed (2g) and dissolved in 1000 ml of water. Finally the pH was adjusted to 5.8 with ortho phosphoric acid (0.1 M). The solution was sonicated for 10 minutes and filtered using Whatman filter paper (No.1) and used. Then mix the buffer, acetonitrile and methanol in 50:30:20 compositions respectively.

**Preparation of stock solutions:**

**Artesunate standard stock solution:** An accurately weighed quantity of artesunate 25 mg was transferred to the 100ml volumetric flask add 30ml of diluents (buffer:ACN:methanol 50:30:20), sonicate to dissolve, dilute upto the mark with diluent and mix well.(Concentration of artesunate is about 250 µg/ml).

**Amodiaquine hydrochloride standard stock solution:** An accurately weighed quantity of Amodiaquine hydrochloride 76.5 mg was transfer to the 100ml volumetric flask add 30ml of diluent, sonicate to dissolve, dilute up to the mark with diluent and mix well. (Concentration of Amodiaquinehydrochloride is about 765 µg/ml).

**Preparation of standard:** Take 10 ml solution from standard stock solution of Artesunate and 10ml solution from standard stock solution of Amodiaquine hydrochloride in 100 ml volumetric flask and make up the volume upto the mark with diluents.

(Concentration of Artesunate is about 250 µg/ml).
(Concentration of Amodiaquine hydrochloride is about 765µg/ml).

**Preparation of the sample solution:**

The powder equivalent to 25 mg of Artesunate and 76.5mg of Amodiaquine hydrochloride were weighed and taken into a 100mL volumetric flask. To this 25mL of diluents was added and sonicated for 15min to dissolve the drugs then made up the volume to required volume with the diluents. From this solution 10ml was taken into a 100mL flask and made up to final volume with diluents to get concentration of Artesunate is about 250µg/ml, concentration of Amodiaquine hydrochloride is about 765µg/ml and filtered through 0.45µ filter under vacuum filtration. From this stock solution further dilutions were made for the validation of the method developed.

## RESULTS AND DISCUSSION

**Method Validation:**

**Specificity:** 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 spiking possible impurities at specific level to standard drug solution (100ppm). The diluent and placebo solutions were also injected to observe any interference with the drug peak. The results are tabulated in the table no-2 and the chromatogram was shown in the figure no- 2, 3.

**Linearity:** Linearity is the ability of the method to produce results that is directly proportional to the concentration of the analyte in samples with given range. The linearity of ARTESUNATE was in the concentration range of 15- 35 %, for AMODIAQUINEHYDROCHLORIDE 45.9-107.1%. From the linearity studies calibration curve was plotted and concentrations were subjected to least square regression analysis to calculate regression equation. The regression coefficient was found to be 0.999 and shows good linearity for both the drugs. The results are tabulated in the table no-3 and the chromatogram was shown in the figure no-.4, 5.

**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. Study was carried out by injecting six replicates of the same sample preparations at a concentration of Artesunate 25 ppm/ml & Amodiaquinehydrochloride 76.5 ppm/ml. The results are tabulated in the table no-5.

**Accuracy:** Accuracy is the closeness of results obtained by a method to the true value. It is the measure of exactness of the method. Accuracy of the method was evaluated by standard addition method. Recovery of the method was determined by spiking an amount of the pure drug (80%,100% ,120%) at three different concentration levels in its solution has been added to the pre analyzed working standard solution of the drug. The results are tabulated in the table n-5.

**LOD & 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 in the table no-5

**Forced degradation of Artesunate and Amodiaquine hydrochloride**

**Acid degradation:** Acid degradation was determined by taking 5ml of stock solution in 10ml volumetric flask and to this 2ml of 0.1N HCl was added and sonicate for 5min, kept aside for 12hrs at room temperature. After 12hrs the solution was neutralized with 2ml of 0.1N NaoH then 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.

**Oxidative degradation:** Oxidative degradation was determined by taking 5ml of stock solution in 10ml volumetric flask and diluted up to the mark with 5% H2O2 and kept aside for 12hrs. After 12hrs the solution was diluted with diluents to get a concentration of 10µg/ml solution and analysed to recorded chromatogram.

**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 $80^0_c$ for 12hrs. After 12hrs the powder was diluted with diluents to get a concentration of 10µg/ml solution and analysed to recorded chromatogram.

**Photolytic degradation:** The Artesunate and Amodiaquine hydrochloride powder and solutions of both were prepared and exposed to light to determine the irradiation of light on the stability of solution and powder form of drugs. Approximately 100mg of drug powder and 1mg/ml solution were spread on a glass dish in a layer that was less than 2mm thickness and were placed in a light cabinet and exposed to UV light at 300-400nm for 12hrs. After 12hrs the samples are removed and diluted with diluents to get a concentration of 10µg/ml solution and analysed to recorded chromatogram.

Several trials has made until getting good peak resolution, acceptable plate count and tailing factor. Method was optimized and the retention times of Artesunate and Amodiaquine hydrochloride was reported as 5.03 &2.77

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

**Linearity:** From the Linearity data it was observed that the method was showing linearity in the concentration range of 15-35µg/ml for Artesunate and 45.9-107.1 µg/ml for Amodiaquinehydrochloride Correlation coefficient was found to be 0.999 for both the compounds.

**Accuracy:** The recoveries of pure drug from the analyzed solution of formulation were 99.81% for Artesunate and 99.30% for Amodiaquinehydrochloride, which shows that the method was accurate.

**Precision:** The %RSD for the sample chromatograms of method precision were found to be 0.21 &1.48 (Rt & Area) for Artesunate and 0.24 &0.73 (Rt & Area) for Amodiaquinehydrochloride. Hence it passes method precision.

**Robustness:** All the system suitability parameters are within limits for variation in flow rate (±0.2 ml). Hence the allowable flow rate should be within 0.8 ml to 1.2 ml. All the system suitability parameters are within limits for variation (±2nm) in wavelength. Hence the allowable variation in wavelength is ± 2nm

**LOD & LOQ:** LOD and LOQ of Artesunate was found to be 2.07, 6.27 and for Amodiaquine hydrochloride was found to be 1.58, 4.78 respectively. All the system suitability parameters are within in the limits when the drugs are subjected to stress conditions like acid, base peroxide, thermal and photolysis. The results obtained were satisfactory and good agreement as per the ICH guidelines.

**Table.1.Details of marketed Formulation**

| Brand name | Content | Mfg.Company |
|---|---|---|
| FALCINIL AQ | ART & AMO (100mg & 306mg Respectively) | Zuventus |

**Table.2.Optimized chromatogram conditions for Artesunate and Amodiaquine hydrochloride**

| **Column** | **Inertsil ODS C18 (250*4.6mm,5µ)** |
|---|---|
| Mobile phase | Phosphate Buffer pH 5.8:ACN:Methanol(50:30:20) |
| Flow rate | 1.0 ml/ min |
| Wavelength | 208 nm |
| Injection volume | 20 µl |
| Column temperature | Ambient |
| Run time | 8 min |

**Table.3.Specificity Data for Artesunate and Amodiaquine hydrochloride**

| | **Artesunate** | | | **Amodiaquine hydrochloride** | | |
|---|---|---|---|---|---|---|
| **Standard Injection** | **Retention time** | **Area** | **Theoretical Plates** | **Retention time** | **Area** | **Theoretical Plates** |
| | 5.03 | 315.58 | 4028 | 2.777 | 4586.64 | 2808 |
| | 5.04 | 324.64 | 4039 | 2.780 | 4549.66 | 2973 |
| | 5.05 | 298.92 | 3919 | 2.783 | 4538.27 | 2821 |
| **Sample Injection** | 5.05 | 319.49 | 4060 | 2.783 | 4587.39 | 2980 |
| | 5.03 | 304.96 | 4338 | 2.770 | 4512.74 | 2952 |
| | 5.06 | 309.21 | 4220 | 2.790 | 4564.21 | 2995 |
| **Blank injection** | - | - | - | - | - | - |

**Table.4.Linearity data for Artesunate and Amodiaquine hydrochloride**

| **For Artesunate** | | | **For Amodiaquine hydrochloride** | | |
|---|---|---|---|---|---|
| **Mcg/ml** | **Area** | **$R_t$** | **Mcg/ml** | **Area** | **$R_t$** |
| 15 | 214.518 | 5.060 | 45.9 | 2981.917 | 2.787 |
| 20 | 277.385 | 5.023 | 61.2 | 3680.566 | 2.740 |
| 25 | 337.549 | 5.053 | 76.5 | 4650.723 | 2.777 |
| 30 | 409.413 | 5.063 | 91.8 | 5266.344 | 2.790 |
| 35 | 463.805 | 5.060 | 107.1 | 6014.413 | 2.787 |
| Slope | 12.61 | | Slope | 50.66 | |
| Correlation coefficient | 0.9987 | | Correlation Coefficient | 0.9954 | |
| Intercept | 25.233 | | Intercept | 663.41 | |

**Table.5.Summary of validation parameters**

| **Parameter** | **Artesunate** | | **Amodiaquine hydrochloride** | |
|---|---|---|---|---|
| Linearity | 15-35µg/ml | | 45.9-107.1µg/ml | |
| Precision(% RSD) | 0.21 (Rt) | 1.48(Area) | 0.24 (Rt) | 0.73(Area) |
| Accuracy | 99.81% | | 99.3% | |
| LOD & LOQ | 2.07,6.27 | | 1.58, 4.78 | |
| Assay | 98.56% | | 99.08% | |

**Table.6.Summary of Forced degradation data for Artesunate and Amodiaquinehydrochloride**

| **Stress Condition** | **Time(hrs)** | **Retention Time** | **Time(hrs)** | **Retention Time** |
|---|---|---|---|---|
| As such | 12hrs | 5.033 | 12hrs | 2.777 |
| Acid Hydrolysis (0.1 N, at RT) | 12hrs | 5.033 | 12hrs | 2.777 |
| Base Hydrolysis (0.1N at RT) | 12hrs | 5.033 | 12hrs | 2.777 |
| Oxidation (5% $H_2O_2$ at RT) | 12hrs | 5.053 | 12hrs | 2.783 |
| Photolysis(UV Light and sunlight) | 12hrs | 5.053 | 12hrs | 2.783 |
| Thermal (at $80^0$c) | 12hrs | 5.040 | 12hrs | 2.780 |

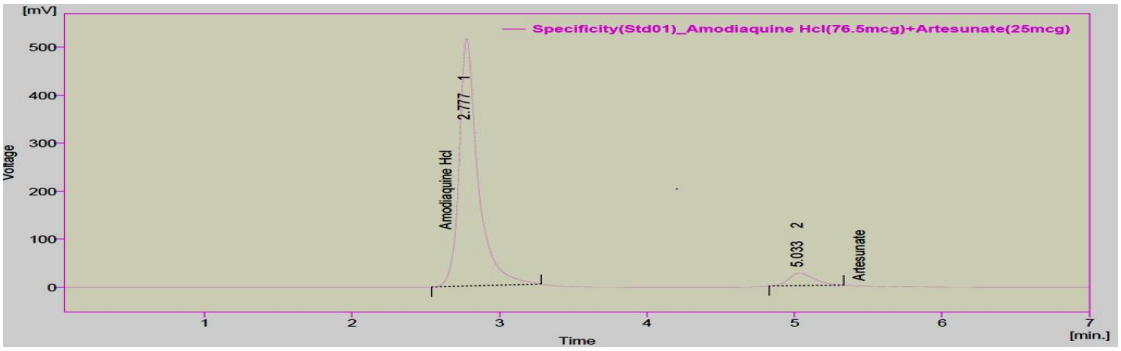


**Figure.1.Chromatogram of standard drug**

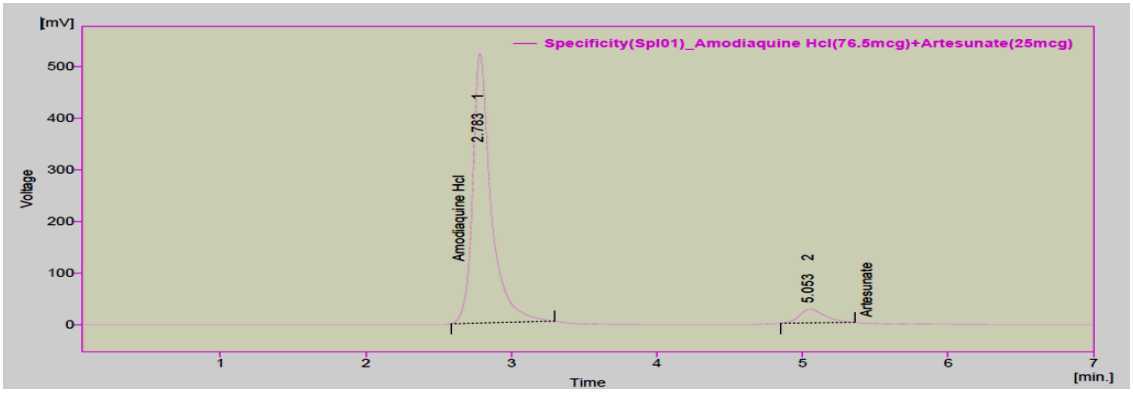


**Figure.2.Chromatogram for specificity sample**

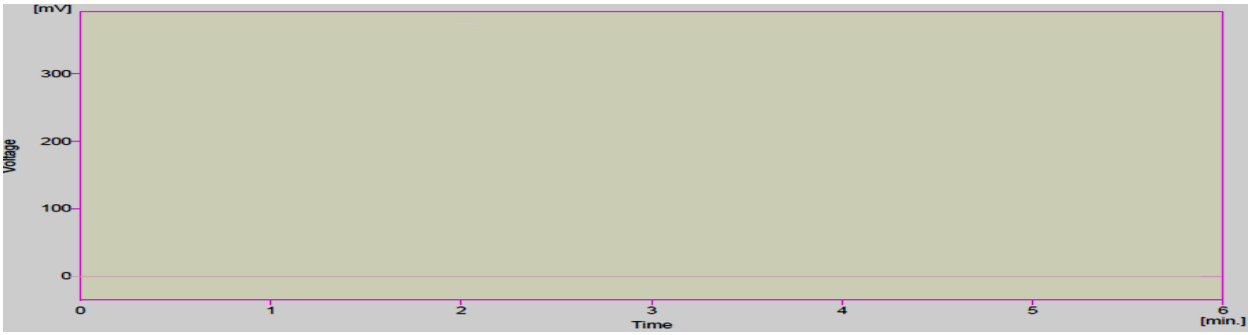


**Figure.3.Chromatogrphy for blank**

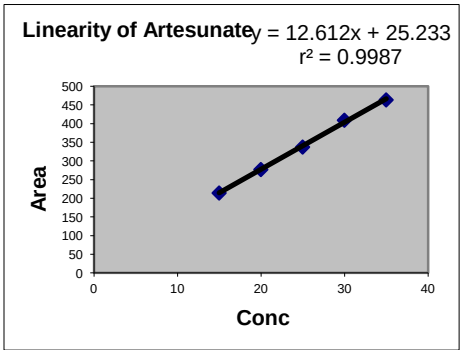


**Figure.4. Linearity plot for Artesunate**

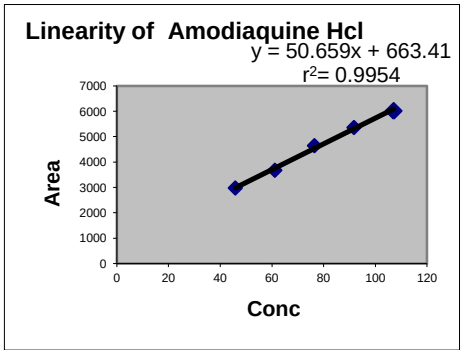


**Figure.5.Linearity plot for Amodiaquine hydrochloride**

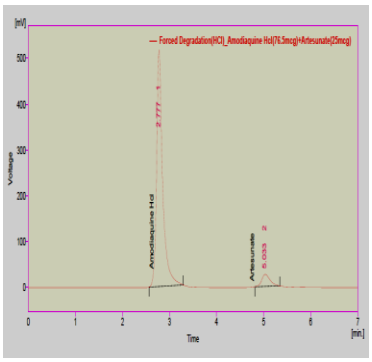


**Figure.6. Acid degradation**

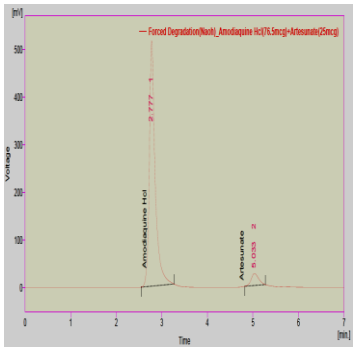


**Figure.7. Base degradation**

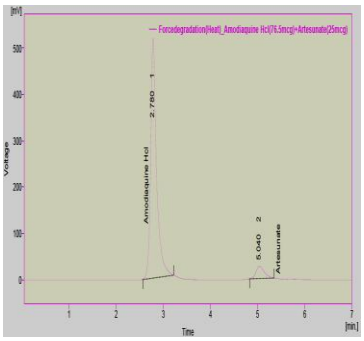


**Figure.8.Thermal Degradation**

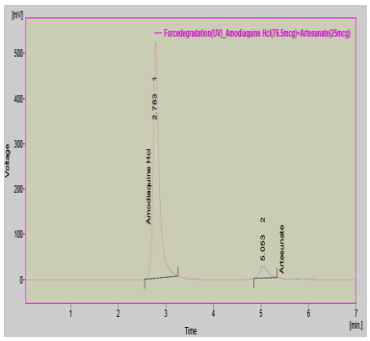


**Figure.9.Photolytic Degradation**

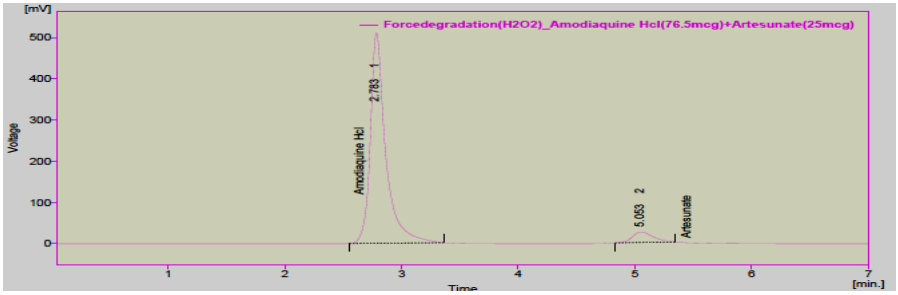


**Figure.10.Peroxide degradation**

## CONCLUSION

Finally it concludes that all the parameters are within the limits and meet the acceptance criteria of ICH guidelines for method validation. The proposed method was simple, accurate, specific, precise, robust, rugged and economical. Hence this method is validated and can be used for routine and stability sample analysis

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