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## RP-HPLC Method for Simultaneous Estimation of Artesunate and Amodiaquine HCL in their Combined Pharmaceutical Dosage Form

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### ABSTRACT:

A Simple, precise, accurate and rapid RP-HPLC method developed and validated for the simultaneous estimation of Artesunate and Amodiaquine HCl in pure and pharmaceutical dosage form. The quantification was carried out using symmetry C18 column, 250 x 4.6 mm, i.d, 5µm particle size in isocratic mode, with mobile phase compressing of buffer and methanol in the ratio of 30:70 (v/v), pH 3 ± 0.5. The flow rate was 1 ml/min and the detection was carried out by UV detector dual i.e, 225 and 339 nm. The retention times were 2.39 and 3.99 mins for Amodiaquine HCl and Artesunate, respectively. The percentage recovery was found to be 99.40 and 99.83 % for Amodiaquine HCl and Artesunate, respectively. The method was validated as per ICH guideline.

**Key words:** HPLC system, Amodiaquine HCl and Artesunate , UV detector.

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### INTRODUCTION:

Artesunate, chemically, (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, is an antimalarial drug. Amodiaquine HCl, chemically, 4-[(7-Chloro-4-quinolyl) amino]-α-(diethylamino)-o-cresol dihydrochloride dihydrate; 4-[(7-chloro-4-quinolyl) amino]-2-[(diethylamino)-methyl] phenol dihydrochloride dehydrate, is an anti malarial drug(8, 9). The literature survey indicates that Artesunate and Amodiaquine HCl were estimation by UV, TLC, HPTLC and HPLC in different pharmaceutical dosage forms (10, 11). This combination of Artesunate and Amodiaquine HCl is used in the treatment of malaria which is due to Plasmodium falciparum parasite (1-7). There is no method has been reported for estimation of Artesunate and Amodiaquine HCl, thus an attempt was made to simultaneous estimate Artesunate and Amodiaquine HCl by using RP- HPLC.

Branded Drugs: Larimal tablet, Larither injection - 3 x 1ml, Larither capsules -Strip of 6 capsules. Lumerax tablets, Riamet, Coartem.

### MATERIALS AND METHOD:

#### Chemicals and Reagents

ARTE and AMQ bulk powder was kindly gifted by JAY RADHE SALES, Ahmedabad, India. The commercial fixed dose combination product ( LARIMAL) was procured from the local market. Methanol AR Grade was procured from S. D. Finar Chemicals Ltd., Ahmedabad, India.

#### Instrumentation

HPLC system, Company-Shimadzu, Model-Pump-LC 20AD, Columns available: Symmetry C18, 250 x 4.6 mm, 5µ particle size.

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### Chromatographic conditions

Stationary phase: Symmetry C18, 250 x 4.6 mm, 5 $\mu$  particle size.

Mobile phase: Phosphate Buffer : methanol 30:70 (v/v) (pH of Phosphate Buffer was adjusted to 3.0 using 0.5% O-phosphoric acid)

Temperature: ambient

Flow rate: 1.0 ml/min

Wave length: For Artesunate- 225 nm and Amodiaquine- 339 nm.

Run time: 10 min.

### EXPERIMENTAL

All chemicals and reagents used were of AR/HPLC grade.

#### Preparation of buffer

A 1.36 gm of potassium dihydrogen orthophosphate was dissolved in 900 ml of Milli-Q water. Then the pH was adjusted to 3.0 with ortho phosphoric acid. Then the volume was made up to 1000 ml and was filtered through 0.45 $\mu$ m nylon membrane filter and degassed.

#### Preparation of mobile phase

A degassed mixture of Buffer and Acetonitrile in the ratio of 30:70 (v/v) was prepared and the mixture was filtered through 0.45  $\mu$  membrane filters and it was degassed.

#### Preparation of Artesunate stock solution

Accurately weighed artesunate 10 mg was transferred into 100 mL volumetric flask and dissolved in methanol and diluted up to the mark with methanol to give a stock solution having strength 100  $\mu$ g/ml.

#### Preparation of Amodiaquine HCl stock solution

Accurately weighed Amodiaquine HCl 10 mg was transferred into 100 mL volumetric flask and dissolved in methanol and diluted up to the mark with methanol to give a stock solution having strength 100  $\mu$ g/ml.

#### Calibration curve

Calibration curves were prepared by taking appropriate aliquots 1, 2, 3, 4, 5, 6 ml of stock solution of Artesunate and 0.5, 1, 1.5, 2, 2.5, 3 ml stock solution of Amodiaquine HCl in 10 ml vol. flask and dilute up to the mark by Methanol to give 10-60  $\mu$ g/ ml of ARTE and 5-30  $\mu$ g/ ml of AMQ. Representative Chromatogram of calibration curve for Artesunate (10-60  $\mu$ g/ml) and Amodiaquine (5-30  $\mu$ g/ml) is shown in Figure 2.

#### Procedure for analysis of tablet formulation

Total 20 tablets were accurately weighted and triturated with

glass mortar and pestle. The powder equivalent to 50 mg of Artesunate and 150 mg of Amodiaquine HCl was taken in 100 ml volumetric flask; methanol was added and the flask was kept in an ultrasonic bath for 10 min. The volume was made up to mark and the solution was filtered through 0.2 micron nylon membrane filter. The diluted solution was analyzed under optimized chromatographic conditions.

### VALIDATION OF RP-HPLC METHOD

#### Linearity

Aliquots of standard solutions of Artesunate and Amodiaquine in range 10-60  $\mu$ g/mL and 5-30  $\mu$ g/ml respectively, was prepared from working standard solution and injected to system with stated chromatographic conditions and analyzed. The graph of peak area obtained versus respective concentration was plotted. The mean area with its standard deviation and % relative standard deviation of peak were calculated.

#### PRECISION

##### 1. Repeatability

Three different standard solutions of Artesunate (30, 40, and 50  $\mu$ g/ml) were prepared from working standard solution and injected three times to system with stated chromatographic conditions and analyzed.

Three different standard solutions of Amodiaquine (15, 20, and 25  $\mu$ g/ml) were prepared from working standard solution and injected three times to system with stated chromatographic conditions and analyzed.

##### 2. Intraday precision

Standard solutions Artesunate (10-60  $\mu$ g/ml) and Amodiaquine (5-30  $\mu$ g/ml) were prepared from working standard solution and injected in to system with stated chromatographic conditions and analyzed, three times in a day.

##### 3. Interday precision

Standard solutions Artesunate (10-60  $\mu$ g/ml) and Amodiaquine (5-30  $\mu$ g/ml) were prepared from working standard solution and injected in to system with stated chromatographic conditions and analyzed, five days.

#### ACCURACY

Accuracy may often be expressed as percentage recovery. The accuracy was determined by standard addition method.

To a fixed amount of pre-analyzed sample (2.0 ml) mixture of Artesunate (100 $\mu$ g/ml) and Amodiaquine (75  $\mu$ g/ml) increasing amount of its working standard solution (1.0, 2.0, 3.0ml of 100 $\mu$ g/ml of Artesunate and 75  $\mu$ g/ml Amodiaquine)

**Table 1** Accuracy for Artesunate and Amodiaquine HCl

Conc. Sample (µg)		Conc. Added (µg/mL)		Total conc (µg/mL)		Conc Recovered (µg/mL)		% Recovery	
ARTE	AMQ	ARTE	AMQ	ARTE	AMQ	ARTE	AMQ	ARTE	AMQ
20	15	0	0	20	15	19.81	14.99	99.05	99.94
20	15	10	7.5	30	22.5	29.76	22.41	99.22	99.62
20	15	20	15	40	30	39.60	29.87	99.02	99.58
20	15	30	22.5	50	37.5	50.17	37.57	100.34	100.21

were added in in three different 10 ml volumetric flask and made up to mark with methanol. Samples were injected to system and analyzed. The mean % recovery from of peak areas calculated (Table 1).

**LIMIT OF DETECTION (L.O.D.)**

The L.O.D. was estimated from the set of 5 calibration curves is shown in eq. 1.

$$LOD = 3.3 \times (S.D./Slope) \dots\dots\dots 1$$

Where,

S.D. = Standard deviation of the Y- intercepts of the 5 calibration curves.

Slope = Mean slope of the 5 calibration curves.

**LIMIT OF QUANTIFICATION (L.O.Q.)**

The L.O.Q. was estimated from the set of 5 calibration curves is shown in eq. 2.

$$LOQ = 10 \times (S.D./Slope) \dots\dots\dots 2$$

Where,

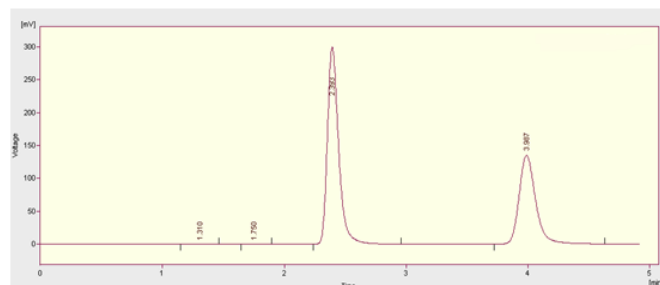
S.D.= Standard deviation of the Y- intercepts of the 5 calibration curves.

Slope = Mean slope of the 5 calibration curves.

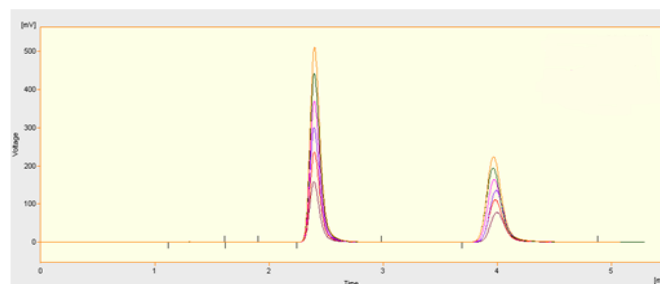
**RESULT AND DISCUSSION**

For RP-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 2.39 min and 3.99 min for AMQ and ARTE respectively. The representative chromatogram of the standard solution of mixture is shown in Figure 1.

Results were found to be linear in the concentration range of 10–60 mg/mL for ATN and 5–30 mg/mL for AMQ. The correlation coefficients for the plots were 0.999 for ATN and 0.999 for AMQ. The proposed method was also evaluated by the assay of commercially available tablets containing ATN and AMQ. The % assay was found to be 99.40 ± 0.542 for ATN and 99.83 ± 0.709 for AMQ (mean ± S.D., n = 6). The method



**Figure 1** Representative chromatogram obtained for standard mixture containing AMQ (15 mg/ml, 2.38 min) and ATN (50 mg/ml, 3.99 min)



**Figure 2** Representative Chromatogram of calibration curve for Artesunate (10-60 µg/ml) and Amodiaquine (5-30 µg/ml)

**Table 2** Summary of Validation parameters for Artesunate and Amodiaquine

Sr. no.	Parameters	Results	
		ARTE	AMO
1	Linearity Range (µg/ml)	10-60	5-30
2	Correlation coefficient	0.999	0.999
3	Precision (%C.V.)		
	1. Repeatability	0.164-0.273	0.120-
	2. Intraday precision	0.354-0.987	0.271
	3. Interday precision	0.325-0.988	0.190-0.732
4	Accuracy (%recovery)	99.40%	99.83%
5	Limit of Detection (L.O.D.) (µg/ml)	3.24	9.84
6	Limit of Quantitation (L.O.Q.) (µg/ml)	15.71	46.61

was found to be accurate and precise, as indicated by recovery studies and % RSD not more than 2. The summary of validation parameters of proposed HPLC method is given in Table 2.

## CONCLUSION

The proposed method is simple, sensitive and reproducible and hence can be used in routine for determination of Artesunate and Amodiaquine HCl in pharmaceutical preparations. Statistical analysis of the results has been carried out revealing high accuracy and good precision. The developed method can be used for routine quantitative estimation of Artesunate and Amodiaquine HCl in pharmaceutical preparation. The mobile phase Methanol and phosphate buffer Ph 3.0 (70:30) was found to be ideal for estimation of Artesunate and Amodiaquine HCl. The elution was as followed ( For Arte RT-3.99 and Amo RT-2.39).The mean recovery was (For Arte 99.40% and Amo 9.83%).

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