REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF ARTESUNATE IN TABLET DOSAGE FORM

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Abstract
Artesunate is a new potent antimalarial drug and is a synthetic analogue of artemisinin. A simple, economical, fast, and precise reverse phase high performance liquid chromatographic method has been developed for the determination of Artesunate in tablet dosage form. The present method was developed on C-18 Hypersil (5 micron 25 cm× 4.6 mm) column, in isocratic mode with mobile phase acetonitrile: methanol (70: 30 v/v) at a constant flow rate of 1.0 ml/min and the detection wavelength was 211 nm. Proposed method was validated for precision, accuracy, linearity range, recovery, robustness and ruggedness. The developed method can be used for assay of Artesunate in pharmaceutical preparations.

Keywords: Antimalarial, Artesunate, Reverse Phase high performance liquid chromatographic

Introduction
The World Health Organization (WHO) estimates that 300–500 million cases occur each year; this leads to more than one million deaths due to malaria. Artemisinin is a sesquiterpene isolated from Artemisia annua L., used in traditional medicine for the treatment of fever and chills,Artemisinin 1, dihydro-epideoxyarteannuin B 2 and deoxyartemisinin 3 were isolated from the sesquiterpene lactone-enriched fraction obtained from the crude ethanolic extract of Artemisia annua L.and shows antiulcerogenic activity. ¹. Artesunate is a new potent antimalarial drug and is a synthetic analogue of artemisinin. Artemisinin shows activity against Plasmodium falciparum and Plasmodium vivax ². Artesunate and artemether are semi synthetic derivatives of artemisinin. In addition to their antimalarial activity, artemisinin and its derivatives are also active against cancer cells ³⁻⁷. It is concentrated in parasitized erythrocytes where it is activated by parasite haem,
generating free radicals. It rapidly clears parasitaemia faster than any other antimalarial drug. Literature survey revealed estimation of Artesunate by HPLC, colorimetric, polarographic techniques.

Material and Methods

Chemicals and Reagents: Standard Artesunate drug was procured from Glenmark Pharma. Ltd. Tal. Sinnar Dist. Nasik, M. S. India as a gift sample. Acetonitrile (Ranbaxy Pvt. Ltd.), methanol and water (RFCL Ltd., New Delhi) were of HPLC grade. The pharmaceutical preparation of Artesunate tablet was FALCIGO 50 mg tablet (Zydus Cadila, Ahmedabad).

Instrumentation: High performance liquid chromatograph (Milton Roy) equipped with CM 4000 spectromonitor 3100 of variable wavelength detector, chromatograph I/F module from instrument, injector manual, 20 ml loop and Shimadzu UV-1201 spectrophotometer were used.

Chromatographic conditions: The mobile phase containing Acetonitrile: methanol (70: 30 v/v), was found to resolve Artesunate. The mobile phase was filtered on a 0.45 micron membrane filter and then ultrasonicated for 30 min. The flow rate was set to 1.0 ml/min. Artesunate drug showed good absorbance at 211 nm, which was selected as wavelength for further analysis. All determinations were performed at constant column temperature (28 ± 2°C).

Preparation of Stock Solutions: Standard stock solution containing Artesunate was prepared dissolving 10 mg in 50 ml of mobile phase. It was then sonicated for 10 minutes. The above stock solution was further diluted with mobile phase to get the concentration ranging from 10 µg/ ml to 50 µg/ ml.

Calibration curve: Calibration curves were prepared by taking appropriate aliquots of standard Artesunate stock solutions in different 10 ml volumetric flask and diluted up to the mark with mobile phase to obtain final concentrations of 10, 20, 30, 40, 50 µg/ml of Artesunate. Standard solutions (n=6) were injected through 20 µl loop system and chromatograms were obtained using 1.0 ml/min. flow rate. The effluent was monitored at 211 nm. Calibration curve was constructed by plotting average peak area against concentration and regression equation was computed.

Validation of the method: The developed method was validated in terms of linearity, accuracy, recovery and specificity. Linearity of a method is a measure of how
well a calibration plot of response vs. concentration approximates a straight line. Linearity can be assessed by performing single measurements at several analyte concentrations. The data are then processed using a linear least-squares regression. The resulting plot slope, intercept, and correlation coefficient provide the desired information on linearity. An example of this approach is shown in fig 6. The numerical value of the slope and intercept will depend on the response measured, but intercepts greater than 2% (relative to the target level response) are typically expected with well-designed HPLC methods for major component analysis. A linearity correlation coefficient above 0.999 is acceptable for most methods, especially for major components in assay methods.

Specificity can be defined as the ability to measure accurately the concentration of an analyte in the presence of all other sample materials. The determination of method specificity can be achieved in two ways, first and most desirable; all potential interfering compounds can be tested to demonstrate their separation from the peak(s) of interest with a specified resolution). A second method for achieving specificity is the use of selective detectors.

Precision can be defined as “the degree of agreement among individual test results when the procedure is applied repeatedly to multiple sampling of a homogenous sample”. A more comprehensive definition proposed by the ICH divides precision into three types: Repetability, Intermediate precision, Reproducibility.

Recovery at each level is determined is comparison to the known amount added. For a major component assay, spiked levels typically should be at 50, 75, 100, 125 and 150 % of the levels expected for the analyte in a normal assay. A minimum of three replicate measurements should be performed at each level. Other spiked concentration levels may also be appropriate (such as 75, 100 and 125 % or 80, 90, 100, 110 and 120 %) but the critical factor is to bracket the expected concentration range for the final product. An injection of the blank matrix should be made to determine matrix background effects.

**Sample Preparation:** Total 20 tablets were accurately weighted and triturated with glass mortar and pestle. The powder equivalent to 10 mg of Artesunate was taken in 100 ml volumetric flask; mobile phase 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 and chromatogram is shown in Figure 1.

**Results and Discussion**

To develop a precise, accurate and suitable RP- HPLC method for the Artesunate, different mobile phases were tried and the proposed chromatographic conditions were found to be appropriate for the quantitative determination. The result obtained by the assay of marketed formulation is summarized in Table 1. System suitability tests were carried out as per USP XXIV and parameters are summarized in Table 2.

**Method Validation:** The proposed HPLC method was validated as per ICH guidelines.

**Specificity:** The peak purity Artesunate was assessed by comparing the retention time (RT) of standard Artesunate. Good correlation was obtained between the retention time of standard and sample of Artesunate (Figure 1).

**Linearity:** Linearity was studied by preparing standard solutions at different concentration levels. The linearity range for Artesunate was found 10-50 µg/ml. The regression equation for Artesunate was found to be \( y = 14670x + 0.6823 \) with coefficient of correlation \( (r) \) 0.9993 (Table 2).

**Precision:** Precision was evaluated by carrying out six independent sample preparation of a single lot of formulation. The sample solution was prepared in the same manner as described in sample preparation. Percentage relative standard deviation (%RSD) was found to be less than 2% for within a day and day to day variations, which proves that method is precise.

**Accuracy (Recovery studies):** To check the degree of accuracy of the method, recovery studies were performed in triplicate by standard addition method at 50%, 100% and 150%. Known amounts of standard Artesunate was added to pre-analyzed samples and were subjected to the proposed RP- HPLC method. Results of recovery studies are shown in Table 1.

**Robustness of method:** To evaluate the robustness of the developed RP-HPLC method, small deliberate variations in the optimized method parameters were done. The effect of change in flow rate, pH and mobile phase ratio on the retention time and tailing factor were studied. The method was found to be unaffected by small changes like ± 0.1 change in pH, ± 0.1 change in flow rate and ± 1 change in mobile phase.

**Conclusion**

The proposed method is simple, sensitive and reproducible and hence can be used in routine for determination of Artesunate in bulk as well as 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 in multicomponent pharmaceutical preparations. During the present work, High performances liquid chromatographic method was studied in great detail with Artesunate drug in perform as well as in formulations. Various experiments were carried out to establish the method. The mobile phase Acetonitrile and Methanol(70:30) was found to be ideal for estimation of Artesunate. The elution was as followed (RT-3.63).The mean recovery was (100.05). The values of percent recovery and standard deviation show that the proposed method was reproducible, accurate and precise.

REFERENCES


Figure 1: Linearity of Detector Response for Artesunate.

Figure 2: A typical chromatogram of Artesunate

Figure 3: Structure of Artesunate drug.
### Table 1: Recovery studies

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### Table 2: System suitability parameter

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<td>Linearity range (µg/ml)</td>
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<td>Correlation coefficient</td>
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<td>Retention time (min.)</td>
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