JOURNAL OF PHARMACEUTICAL SCIENCE AND BIOSCIENTIFIC RESEARCH (JPSBR)

(An International Peer Reviewed Pharmaceutical Journal that Encourages Innovation and Creativities)

# Analytical Method Development and Validation of Artesunate in Bulk and Pharmaceutical Dosage Form by using RP-UPLC with Evaporative Light Scattering Detector

**Patidar Khushwant*, Sarangdevot Y.S, Saraswat Nitin**

**Department of Quality Assurance, Bhupal Nobels' College of Pharmacy, Udaipur, Rajasthan, India.**

**ABSTRACT:**

A rapid sensitive, accurate, precise and reproducible validated alternative RP-UPLC method was developed for determination of less UV-active drug Artesunate in bulk and pharmaceutical dosage form using evaporative light scattering detection technique. The chromatographic estimation was carried out on Agilent 1290 series UPLC system with XBridge BEH C18 Column (50 x 2.1 mm, 2.5µm particle size) by using mobile phase Water (5mM Ammonium Acetate) and Acetonitrile with a gradient flow method of runtime as short as 3 min. The flow rate was 0.6 ml/min, temperature of the column compartment was maintained at ambient and detection was made by using Evaporative Light Scattering detector. The developed method was validated according to ICH guidelines with respect to linearity, accuracy, precision, specificity and robustness. The developed method was linear in concentration range of 100-300 ppm and the linear regression obtained was 0.9993. The proposed method was statistically evaluated and can be applied for routine quality control analysis of Artesunate in bulk and pharmaceutical dosage form.

**KEYWORDS:** Artesunate, RP-UPLC, ELSD, Analytical method development, artemisinin, Antimalarial.

**Article history:**

Received 20 Feb 2015
Revised 19 Oct 2015
Accepted 25 Oct 2015
Available online 01 Jan 2016

**Citation:**

Patidar K, Sarangdevot Y. S., Saraswat N. Analytical Method Development and Validation of Artesunate in Bulk and Pharmaceutical Dosage Form by using RP-UPLC with Evaporative Light Scattering Detector. **J Pharm Sci Bioscientific Res**. 2016 6(1):111-119

***For Correspondence:***

**Khushwant Patidar**

Department of Quality Assurance

Phone- 8003032456.

(www.jpsbr.org)

## 1. INTRODUCTION

Artesunate is a derivative of artemisinin and belongs to a class with antimicrobial properties. The compound is an active ingredient in the Chinese herb Artemisia annua and has been used in Malaria studies [1,2,3]. It acts by increasing the oxidant stress on the intra-erythrocytic plasmodia. Although the thresholds for in vitro sensitivity and resistance of Plasmodium falciparum have not been determined, artesunate is active against chloroquine- and mefloquine-resistant strains of P. falciparum [5]. Artesunate has now been analyzed for its anti-cancer activity against 55 cell lines of the Developmental Therapeutics Program of the National Cancer Institute, USA. ART was most active against leukemia and colon cancer cell lines (mean GI50 values: 1.11±0.56 µM and 2.13±0.74 µM, respectively) [5]. Chemically Artesunate is (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 (WHO, Ph. Int.)[6]. Refer fig. 1.1 for structure of Artesunate.

Literature survey revealed that very few methods have been reported for the estimation of Artesunate in bulk and pharmaceutical dosage form as it is very less detectable for PDA detector because artesunate lacks an intensive chromophore for UV absorption[7z]. The aim and objective of the present work is to develop a new simple, sensitive, accurate, precise RP-

UPLC method for the simultaneous estimation of Artesunate API in bulk and marketed formulation.

The method would help in estimation of drug in single run with shorter runtime which reduces the time of analysis. Very less conc. of sample required as compared to previous methods as ELSD is used as a detector which is more sensitive for Artesunate as compared to PDA detector. Mobile phase used is the blend of Water (5mM Ammonium Acetate buffer) and Acetonitrile in a ratio of 70:30 which is cost effective and suitable for about all types of column. Thus, the paper reports an economical, simple and accurate RP-UPLC method for the estimation of Artesunate in routine quality control analysis.

## 2. MATERIALS AND METHODS

Artesunate working standard was procured from Jubilant life Sciences, Noida, India. Commercially available Larinate®-50 Kit purchased from local pharmacy. Ammonium acetate and Acetonitrile HPLC grade were obtained from Sigma Aldrich, India. Water was prepared by using Millipore Milli-Q water purification system.

### 2.1 Instrumentation

This study was performed on Agilent 1290 series UPLC system with Binary pump besides ELSD detector. Instrument online software (chemstation) was used.

### 2.2 Selection of the column

XBridge BEH C18 (50 x 2.1mm, 2.5 µm particle size) column is selected for further study on Artesunate.

### 2.3 Selection of the Detector

Drug Artesunate was found less UV active. Evaporative Light Scattering Detector (ELSD) has chosen for better detection.

### 2.4 Mode of elution

The study was performed on the gradient elution method of mobile phase i.e. Aqueous (5mM Ammonium acetate) and Organic (Acetonitrile).

### 2.5 Flow rate programming

Flow rate was adjusted to 0.6 ml/min from column. All peaks were detected by the detector if flow rate is increased then the compounds are eluted earlier from the column.

### 2.6 Mobile phase selection

In mobile phase preparation following solvent has been taken:

a. Aqueous (5mM Ammonium Acetate)

b. Organic (Acetonitrile)

### 2.7 Preparation of 5mM Ammonium Acetate

Weighed approx. 385 mg of Ammonium Acetate, transferred to a 1000 ml of bottle, dissolved and volume made up to 1000 ml by using Milli-Q water and sonicated the solution for 3 min.

## 3. RESULT AND DISCUSSION

To optimize the RP-UPLC method, several mobile phase compositions and different chromatographic conditions were tried. A satisfactory peak symmetry and baseline was found in XBridge BEH C18 Column (50 x 2.1 mm, 2.5µm particle size) by using mobile phase Water (5mM Ammonium Acetate) and Acetonitrile with a gradient flow method of runtime as short as 3 min. The flow rate was 0.6 ml/min, temperature of the column compartment was maintained at ambient and detection was made by using Evaporative Light Scattering detector. Refer table 3.1 for description of optimized chromatographic conditions.

### 3.1 Specificity

The ICH documents define specificity as the ability to assess unequivocally the analyte in presence of components that may be expected to be present, such as impurities, the degradation products and matrix components [8]. The excipient compounds must not interfere with the analysis of the targeted analyte [9]. The % RSD for six replicate measurements of peak area response of standard preparation was found to be 0.49 % and % RSD for retention time of six replicate injections of standard preparation was found to be 0.12 %. Refer table 3.2 for observation table and figure 3.1 for chromatograms.

### 3.2 Linearity

The linearity of an analytical method is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range [10]. The correlation coefficient for six concentration

levels will be ≥ 0.999 for the range of 80 to 120% of the target concentration [9] and it was found to be 0.9993 for developed method. Refer table 3.3, fig 7.2 and 7.3 for data.

**3.3 Accuracy**

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness [11]. Acceptance criterion for accuracy is all the individual recoveries should be within 93.0% to 107.0% [12] and it found to be between 97.03 to 102.17 % for Artesunate. Refer table 3.4-3.5 and fig 3.4 to 3.7 for data and chromatograms.

**3.4. Precision**

ICH defines the precision of an analytical procedure as the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions [13]. Acceptance criteria for precision are the % RSD for six replicate preparations of standard should not more than 2.0% and the % RSD for two replicate measurements of peak area response of sample preparation should be not more than 2.0%.

**3.4.1 System Precision-** The % RSD for six replicate measurements of peak area response of standard preparation was found to be 0.79% for Artesunate, % RSD for retention time of six replicate injections of standard preparation was found to be 0.12 and % RSD for two replicate measurements of peak area response of sample preparation was found to be 0.28 for Artesunate. Refer table 3.6 and fig. 3.8 & 3.9 for data and chromatogram.

**3.4.2 Method Precision-** The relative standard deviation for six replicate measurements of peak area response of standard preparation was found to be 1.34 for Artesunate and the relative standard deviation for six replicate measurements of peak area response of sample preparation was found to be 1.11 for Artesunate. Refer table 3.7 & 3.8 and fig. 3.10 & 3.11 for data and chromatogram.

**3.5 Intermediate Precision**

Intermediate precision expresses within-laboratories variations: different days, different analysts, different equipment, etc [14].

**3.5.1 Intraday Precision-** The % RSD for six replicate measurements of peak area response of standard preparation was found to be 0.39% and % RSD for six replicate measurements of peak area response of sample preparation was found to be 0.95% Artesunate. Refer table 3.9 & 3.10 and fig. 3.12 & 3.13 for data and chromatogram.

**3.5.2 Interday Precision-** The % RSD for six replicate measurements of peak area response of standard preparation was found to be 1.45% and % RSD for six replicate measurements of peak area response of sample preparation was found to be 0.27%. Refer table 3.11 & 3.12 and fig. 3.14 & 3.15 for data and chromatogram.

**3.6 Solution Stability**

Chemical compounds can decompose prior to chromatographic investigations, for example, during the preparation of the sample solutions, extraction, cleanup, phase transfer or storage of prepared vials (in refrigerators or in an automatic sampler). Under these circumstances, method development should investigate the stability of the analytes and standards [13]. The stability of the stock solutions of drug and internal standards should be evaluated at room temperature for at least 6 hours [15] Acceptance criteria for solution stability are the %RSD for six replicate preparation of standard and sample should not more than 2.0% and the pattern of chromatography should remain same throughout solution stability study. The %RSD for six replicate measurements of peak area response of standard and sample preparation were found to be 1.97% and 1.84% respectively. The pattern of chromatography remained same throughout solution stability study. Refer table 3.13 & 3.14 and fig. 3.16 to 3.22 for data and chromatogram.

**3.7 Robustness**

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage [11]. Acceptance criteria for robustness are the %RSD for six replicate measurements of peak area response of standard should not more than 2.0% and % RSD for two replicate measurements of peak area response of sample preparation should not more than 2.0%.

**3.7.1 Change in column oven temperature from 40 ± 5˚C-** The % RSD for six replicate measurements of peak area response of standard preparation was found to be 1.57% for 45˚C and 1.03% for 35˚C and the relative standard deviation for two replicate measurements of

peak area response of sample preparation was found to be 0.02% for 45˚C and 0.65% for 35˚C for Artesunate. Refer table 3.15 & 3.16 for observation tables.

**3.7.2 Change in Buffer conc. from 5mM to 5±2 mM Ammonium Acetate-** The %RSD for six replicate measurements of peak area response of standard preparation was found to be 1.03% for 7mM AA and 1.79% for 3mM AA for Artesunate and %RSD for two replicate measurements of peak area response of sample preparation was found to be 0.65% for 7mM AA and 0.02% for 3mM AA for Artesunate. Refer table 3.17 & 3.18 for observation tables.

## 4. OBSERVATION TABLES

**Table: 3.1 Optimized chromatographic conditions**

| | |
|---|---|
| **Column** | **XBridge BEH-C18(50 x 2.1mm), 2.5 µm** |
| **Flow rate** | 0.6 ml/min. |
| **Injection volume** | 2 µm |
| **Sample Temperature** | 30° C |
| **Column Temperature** | 40° C |
| **Detector** | ELSD |
| **Diluent** | ACN : Water (80:20) |
| **Mobile phase** | Water (5mM AA) : ACN |
| **Run time** | 3 min. |
| **Gradient** | |

| TIME (Min) | AA % | ACN% |
|---|---|---|
| 0 | 70 | 30 |
| 0.1 | 70 | 30 |
| 2 | 2 | 98 |
| 2.5 | 2 | 98 |
| 2.7 | 50 | 50 |
| 3 | 70 | 30 |

**Table 3.2 Observation Table for Specificity**

| S.No. | ART-STD Area | ART-STD Retntion Time (min) |
|---|---|---|
| **INJ-01** | 1004.7 | 0.831 |
| **INJ-02** | 993.2 | 0.831 |
| **INJ-03** | 997.9 | 0.831 |
| **INJ-04** | 1001 | 0.833 |
| **INJ-05** | 992.9 | 0.832 |
| **INJ-06** | 979 | 0.831 |
| **MEAN** | **995** | **0.8** |
| **SD** | **4.87** | **0.00** |
| **%RSD** | **0.49** | **0.12** |

**Table 3.3: Observation Table for Linearity**

| Level | Concentration (ppm) | Area (mVxSec) | RT (Min) |
|---|---|---|---|
| **50%** | 100 | 326.8 | 0.857 |
| **75%** | 150 | 631.8 | 0.848 |
| **100%** | 200 | 977.0 | 0.846 |
| **125%** | 250 | 1336.3 | 0.843 |
| **150%** | 300 | 1666.6 | 0.842 |
| | MEAN | | 0.8 |
| | SD | | 0.01 |
| | %RSD | | 0.71 |

**Table 3.4: Observation Table for Accuracy of Standard**

| Replicate | RT | Standard Area |
|---|---|---|
| Replicate-1 | 0.83 | 950.7 |
| Replicate-2 | 0.84 | 954.5 |
| Replicate-3 | 0.83 | 973.4 |
| Replicate-4 | 0.83 | 968.4 |
| Replicate-5 | 0.83 | 976.4 |
| Replicate-6 | 0.83 | 974.9 |
| **Average** | 0.83 | 966.38 |
| **SD** | 0.00 | 11.08 |
| **%RSD** | **0.49** | **1.15** |

**Table 3.5: Observation Table for Recovery of Artesunate in tablet dosage form**

| Level | Sample area | Average area | Sample Wt. (mg) | Amount added (µg) | Amount recovered (µg) | % Recovery | Average % recovery | SD | %RSD |
|---|---|---|---|---|---|---|---|---|---|
| 50% | 468.103 | 469 | 10.12 | **101.20** | 98.04 | 96.88 | **97.03** | 0.223 | **0.24** |
| | 468.315 | | | | 98.08 | 96.92 | | | |
| | 470.12 | | | | 98.46 | 97.29 | | | |
| 100% | 976 | 969 | 20.1 | **201.00** | 203.00 | 101.00 | **100.31** | 0.60 | **0.60** |
| | 966 | | | | 200.92 | 99.96 | | | |
| | 966 | | | | 200.92 | 99.96 | | | |
| 150% | 1485 | 1481 | 29.95 | **299.50** | 306.82 | 102.44 | **102.17** | 0.25 | **0.24** |
| | 1480 | | | | 305.79 | 102.10 | | | |
| | 1478 | | | | 305.37 | 101.96 | | | |

**Table 3.6: Observation Table for System Precision**

| S.No | Replicate | RT | Standard Area | Sample | Sample Area |
|---|---|---|---|---|---|
| 1 | Replicate-1 | 0.831 | 942.8 | Sample 1 | 838.6 |
| 2 | Replicate-2 | 0.831 | 934.9 | | |
| 3 | Replicate-3 | 0.831 | 953.1 | | |
| 4 | Replicate-4 | 0.833 | 941.8 | Sample 2 | 835.3 |
| 5 | Replicate-5 | 0.831 | 969.5 | | |
| 6 | Replicate-6 | 0.831 | 955.8 | | |
| | **Average** | **0.831** | **949.7** | **836.95** | |
| | **SD** | **0.00** | **7.51** | | **2.33** |
| | **%RSD** | **0.12** | **0.79** | | **0.28** |

**Table 3.7: Observation Table for Method Precision for Standard**

| S.No. | RT | STD Area |
|---|---|---|
| 1 | 0.831 | 937.6 |
| 2 | 0.834 | 966.4 |
| 3 | 0.832 | 949.8 |
| 4 | 0.833 | 960.9 |
| 5 | 0.831 | 949.7 |
| 6 | 0.831 | 940 |
| **Mean** | **0.832** | **950.7** |
| **SD** | **0.00** | **12.75** |
| %RSD | 0.16 | 1.34 |

**Table 3.8: Observation Tables for Method Precision for Sample**

| Sample | Sample area | Average area | Sample wt. (mg) | % Assay |
|---|---|---|---|---|
| **1** | 937.3<br>955.8 | 947 | 108.1 | 98.2 |
| **2** | 893.8<br>965.2 | 930 | 108 | 96.5 |
| **3** | 953.9<br>906.2 | 930.05 | 107.9 | 96.5 |
| **4** | 912.5<br>932.4 | 922.45 | 108.1 | 95.7 |
| **5** | 932.6<br>919.4 | 926 | 107.93 | 96.1 |
| **6** | 938<br>953.1 | 945.55 | 108.13 | 98.1 |
| **Average** | | 934 | | 96.9 |
| **SD** | | 10.38 | | 1.05 |
| **%RSD** | | 1.11 | | 1.09 |

**Table 3.9: Observation Table of Standard for Intraday Precision**

| S.No. | RT | STD Area |
|---|---|---|
| **1** | 0.832 | 940 |
| **2** | 0.833 | 948.5 |
| **3** | 0.832 | 946.4 |
| **4** | 0.834 | 944.1 |
| **5** | 0.834 | 936.8 |
| **6** | 0.833 | 933.6 |
| **Mean** | 0.8 | 941.6 |
| **SD** | 0 | 3.64 |
| **%RSD** | 0.11 | 0.39 |

**Table 3.10: Observation Table of Sample for Intraday Precision**

| Sample | Sample | Average | Sample | % Assay |
|---|---|---|---|---|
| **1** | 943.3<br>930.8 | 937 | 108.1 | 98.5 |
| **2** | 921.2<br>913.1 | 917 | 108.2 | 96.4 |
| **3** | 905.2<br>938.4 | 921.8 | 107.92 | 96.9 |
| **4** | 920.9<br>938.7 | 929.8 | 107.98 | 97.8 |
| **5** | 948.8<br>934.9 | 941.8 | 108.12 | 99 |
| **6** | 937<br>937 | 931.5 | 108.14 | 97.9 |
| **Average** | | 930 | | 97.8 |
| **SD** | | 8.82 | | 0.93 |
| **%RSD** | | 0.95 | | 0.96 |

**Table 3.11: Observation Table of Standards for Interday Precision**

| S.No. | RT | STD Area |
|---|---|---|
| **1** | 0.832 | 995.5 |
| **2** | 0.83 | 998.2 |
| **3** | 0.83 | 1020.2 |
| **4** | 0.834 | 1023.7 |
| **5** | 0.833 | 991.3 |
| **6** | 0.832 | 1007.6 |
| Mean | **0.8** | **1006.1** |
| SD | **0.00** | **14.60** |
| %RSD | **0.23** | **1.45** |

**Table 3.12: Observation Table of Sample for Interday Precision**

| Sample | Sample area | Average area | Sample wt. (mg) | % Assay |
|---|---|---|---|---|
| **1** | 1006.8<br>986.6 | 997 | 108.1 | 98.7 |
| **2** | 942.2<br>962.3 | 992 | 107.85 | 95.3 |
| **3** | 989.4<br>992.6 | 991 | 108.13 | 98.1 |
| **4** | 987.9<br>995.7 | 991.8 | 107.95 | 98.2 |
| **5** | 1012.7<br>985.1 | 998.9 | 108.14 | 98.9 |
| **6** | 1006.7<br>984.9 | 995.8 | 107.97 | 98.6 |
| **Average** | | 994 | | 98 |
| **SD** | | 2.73 | | 1.54 |
| **%RSD** | | 0.27 | | 1.57 |

**Table 3.13: Observation Table of Standard for Solution Stability**

| Replicate | RT | Standard area |
|---|---|---|
| **Replicate-1** | 0.816 | 811.4 |

| | | |
|---|---|---|
| **Replicate-2** | 0.818 | 773.5 |
| **Replicate-3** | 0.824 | 808.1 |
| **Replicate-4** | 0.828 | 803.6 |
| **Replicate-5** | 0.827 | 789.8 |
| **Replicate-6** | 0.827 | 793.1 |
| **Average** | 0.8 | 794.9 |
| **SD** | 0.01 | 15.64 |
| **%RSD** | 0.67 | 1.97 |

**Table 3.14: Observation Table of Sample for Solution Stability**

| Hours | Sample | Area | Average Area | % Assay |
|---|---|---|---|---|
| **0 Hr** | 1 | 740.2 | 742 | 95.2 |
| | 2 | 743.2 | | |
| **1 Hr** | 1 | 756.2 | 755 | 96.9 |
| | 2 | 754.2 | | |
| **3 Hr** | 1 | 795.5 | 773.65 | 99.3 |
| | 2 | 751.8 | | |
| **6 Hr** | 1 | 752.1 | 747 | 95.8 |
| | 2 | 741.9 | | |
| **12 Hr** | 1 | 756.9 | 758.5 | 97.3 |
| | 2 | 760.1 | | |
| **24 Hr** | 1 | 742.5 | 745.05 | 95.6 |
| | 2 | 747.6 | | |
| **Average** | | | 754 | 96.7 |
| **SD** | | | 13.9 | 1.81 |
| **%RSD** | | | 1.84 | 1.87 |

**Table 3.15: Observation Table for Increased Temperature (45 0C)**

| Replicate | RT | Standard | Sample | % Assay |
|---|---|---|---|---|
| **Replicate-1** | 0.891 | 1356 | 1321.2 | 95.2 |
| **Replicate-2** | 0.888 | 1388.5 | | |
| **Replicate-3** | 0.889 | 1395.1 | | |
| **Replicate-4** | 0.892 | 1353 | 1320.8 | |
| **Replicate-5** | 0.888 | 1389.4 | | |
| **Replicate-6** | 0.886 | 1399.4 | | |
| **Average** | 0.9 | 1380.2 | 1321 | |
| **SD** | 0 | 21.74 | 0.28 | |
| **%RSD** | 0.21 | 1.57 | 0.02 | |

**Table 3.16: Observation Table for Decreased Temperature (35 0C)**

| Replicate | RT | Standard area | Sample area | % Assay |
|---|---|---|---|---|
| **Replicate-1** | 1.135 | 1760.8 | 1686.6 | 94.6 |
| **Replicate-2** | 1.136 | 1778.6 | | |
| **Replicate-3** | 1.136 | 1733.7 | | |
| **Replicate-4** | 1.133 | 1757.6 | 1671.2 | |
| **Replicate-5** | 1.132 | 1979.2 | | |
| **Replicate-6** | 1.134 | 1787.4 | | |
| **Average** | 1.1 | 1799.6 | 1678.9 | |
| **SD** | 0 | 18.46 | 10.89 | |
| **%RSD** | 0.12 | 1.03 | 0.65 | |

**Table 3.17: Observation Table for Increased Buffer Strength**

| Replicate | RT | Standard area | Sample | % |
|---|---|---|---|---|
| **Replicate-1** | 0.885 | 1760.8 | 1686.6 | 96.5 |
| **Replicate-2** | 0.883 | 1778.6 | | |
| **Replicate-3** | 0.882 | 1733.7 | | |
| **Replicate-4** | 0.885 | 1757.6 | 1671.2 | |
| **Replicate-5** | 0.883 | 1979.2 | | |
| **Replicate-6** | 0.881 | 1787.4 | | |
| **Average** | 0.9 | 1799.6 | 1678.9 | |
| **SD** | 0 | 18.46 | 10.89 | |
| **%RSD** | 0.17 | 1.03 | 0.65 | |

**Table 3.18: Observation Table for Decreased Buffer Strength**

| Replicate | RT | Standard area | Sample area | % Assay |
|---|---|---|---|---|
| **Replicate-1** | 0.884 | 1346.4 | 1283.1 | **96.3** |
| **Replicate-2** | 0.885 | 1301.1 | | |
| **Replicate-3** | 0.884 | 1305.9 | | |
| **Replicate-4** | 0.88 | 1342.4 | 1282.7 | |
| **Replicate-5** | 0.876 | 1341.2 | | |
| **Replicate-6** | 0.882 | 1341.1 | | |
| **Average** | **0.9** | **1329.7** | **1282.9** | |
| **SD** | **0** | **23.75** | **0.28** | |
| **%RSD** | **0.25** | **1.79** | **0.02** | |

## 5. FIGURES

**Fig.1.1- Structure of Artesunate**

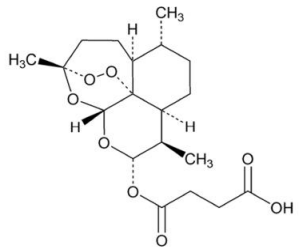



**Fig.3.1- Chromatograms of standard for specificity**

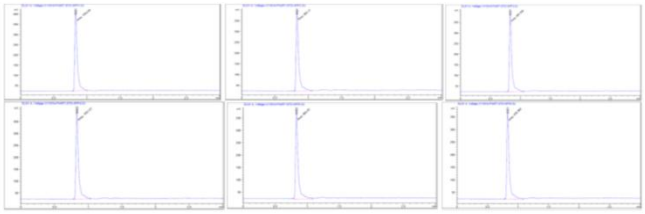


**Fig.3.2- Linearity Plot of Artesunate-**

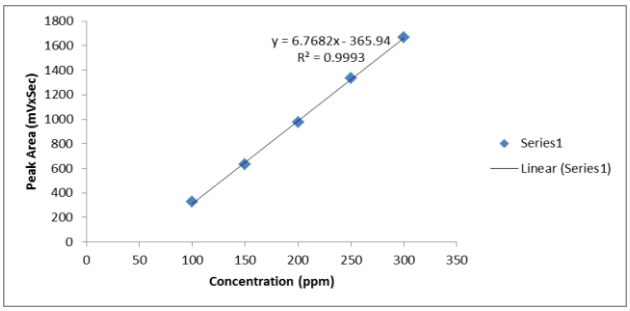


**Fig.3.3- chromatograms of standard for linearity**

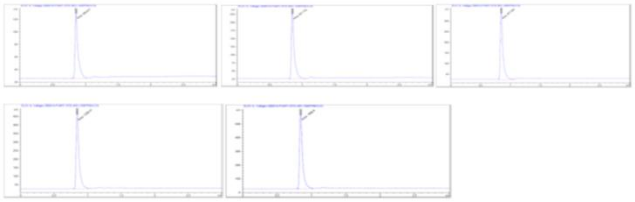


**Fig.3.4- chromatograms of standard for accuracy**

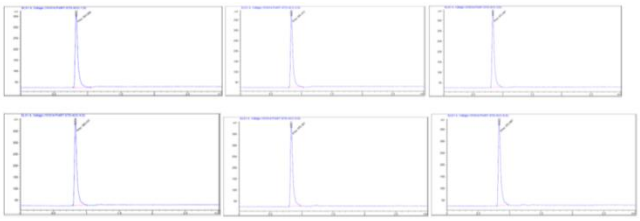


**Fig.3.5- chromatograms of sample for accuracy of 50% (100ppm)-**

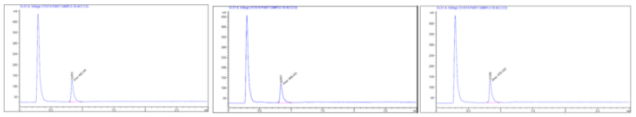


**Fig.3.6- chromatograms of sample for accuracy of 100% (200ppm)**

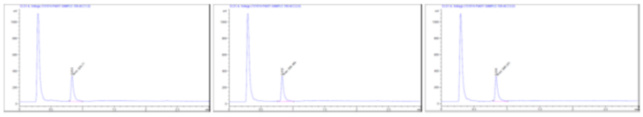


**Fig.3.7- chromatograms of sample for accuracy of 100% (200ppm)**

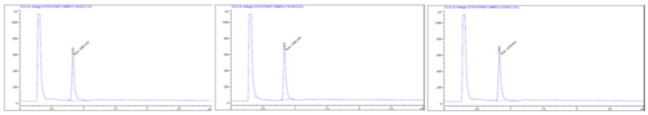


**Fig.3.8- Chromatograms of standard for system precision**

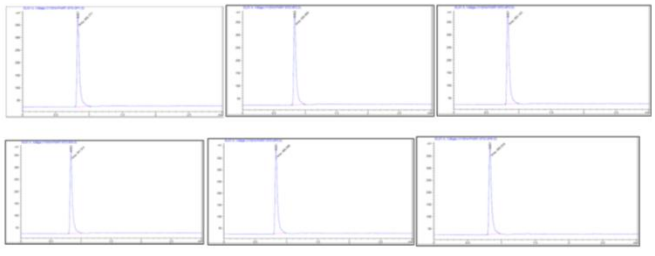


**Fig. 3.9- Chromatograms of sample for system precision**

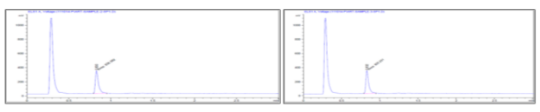


**Fig.3.10- Chromatograms of standards for method precision-**

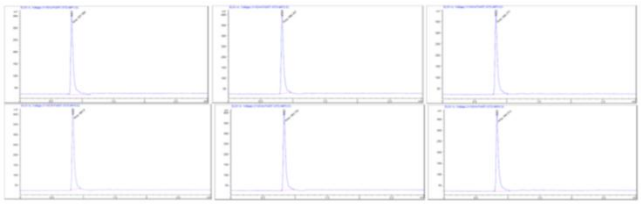


**Fig. 3.11- Chromatograms of samples for method precision**

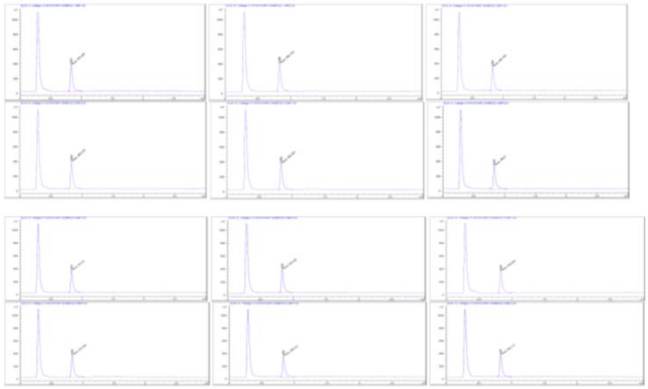


**Fig.3.12- Chromatograms of standard for intraday precision**

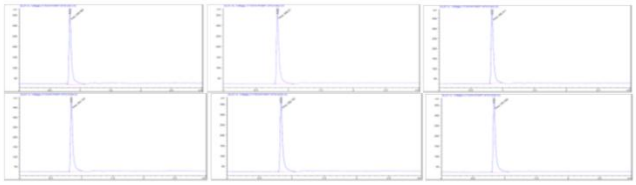


**Fig.3.13- Chromatograms of samples for intraday precision**

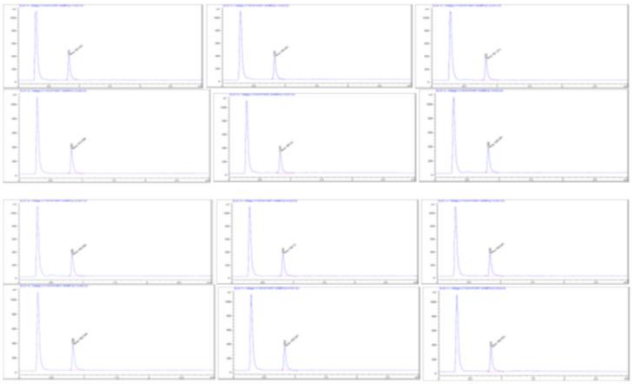


**Fig.3.14- Chromatograms of standard for Interday precision**

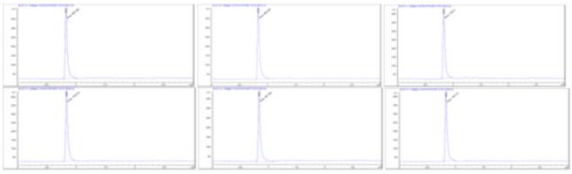


**Fig.3.15- Chromatograms of samples for Interday precision**

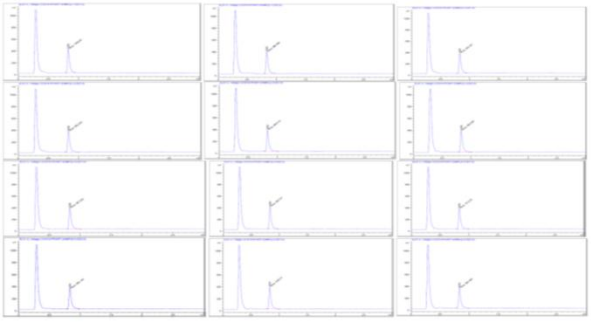


**Fig.3.16- Chromatograms of standard for solution stability**

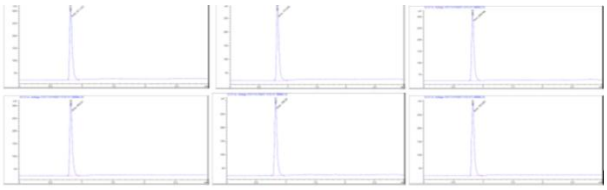


**Fig.3.17- Chromatograms of sample for solution stability at 0 hour**

**Fig.3.18- Chromatograms of sample for solution stability at 1 hour**

**Fig.3.19- Chromatograms of sample for solution stability at 3 hours**

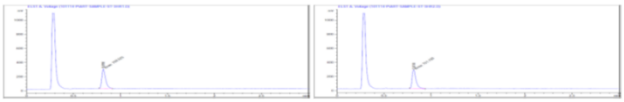


Fig.3.20- Chromatograms of sample for solution stability at 6 hour

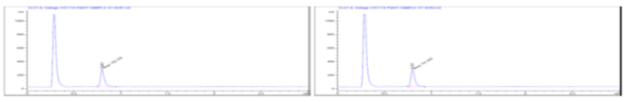
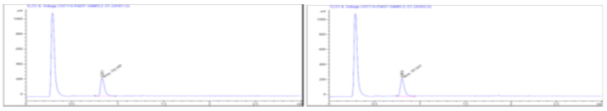


Fig.3.21- Chromatograms of sample for solution stability at 12 hours

**Fig.3.22- Chromatograms of sample for solution stability at 24 hours**



## 6. CONCLUSION

The proposed RP-UPLC method was found to be specific, precise, accurate, rapid and economical for estimation of Artesunate in bulk and Tablet dosage form. The developed method was validated in terms of accuracy, precision, linearity, robustness and ruggedness and results will be validated statistically according to ICH guidelines. The developed method can be used for routine quality control analysis of Artesunate.

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