# REVERSE PHASE-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD FOR THE SIMULTANEOUS ESTIMATION OF ARTEMETHER AND LUMEFANTRINE IN PURE FORM AND TABLET DOSAGE FORM

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## ABSTRACT

A simple, accurate, robust, specific and precise Reverse phase HPLC method was developed for the simultaneous estimation of the Artemether and Lumefantrine in pure and pharmaceutical dosage form as per ICH Guidelines. Chromatogram was run through Phenomenex Luna C18 (4.6mm×150mm, 5µm) Particle size column and Mobile phase containing Methanol: Tri Ethyl Amine Buffer (35:65% v/v)was pumped through column at a flow rate of 1.0ml/min. Temperature was maintained at 38°C. Optimized wavelength selected was 261nm. Retention time of Artemether and Lumefantrinewere found to be 2.256min and 5.427min. The developed method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantification. The proposed method optimized and validated as per ICH guidelines. The

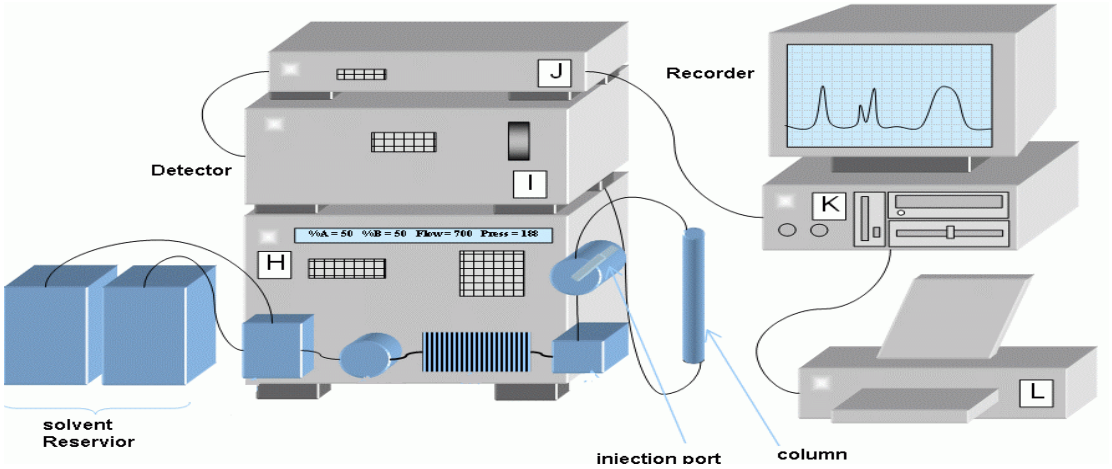
Precision of the %RSD of the Artemether and Lumefantrine were and found to be 0.212 and 0.064 respectively. %Recovery was obtained as 100.36% and 100.15% for Artemether and Lumefantrine respectively. LOD, LOQ values obtained from regression equations of Artemether and Lumefantrine were found to be 2.63, 3.84 and 7.92, 11.54 respectively. Regression equation of Artemether is y = 10511x + 9597 and y = 6120x + 29119 of Lumefantrine. The proposed method can be used for the estimation of these drugs in pure and its pharmaceutical combined dosage forms.

**Keywords:**Artemether, Lumefantrine, RP-HPLC, Validation, ICH Guidelines.

## INTRODUCTION

### Introduction to HPLC [1-2]

High Performance Liquid Chromatography (HPLC) is a technique that has arisen from the application to liquid chromatography the use of an instrumentation that was originally developed for gas chromatography. High Pressure Liquid Chromatography was developed in the mid-1970 and was improved with the development of column packing material and the additional convenience of on-line detectors. The various components of HPLC are pumps (solvent delivery system), mixing unit, gradient controller and solvent degasser, injector (manual or automatic), guard column, analytical columns, detectors, recorders and/or integrators. Recent models are equipped with computers and software for data acquisition and processing. The mobile phase in HPLC refers to the solvent being continuously applied to the column or stationary phase at a flow rate of 1-5 cm3/min. The mobile phase acts as a carrier for the sample solution. The chemical interactions of the mobile phase and sample with the column determine the degree of migration and separation of components contained in the sample. The mobile phase can be altered in order to manipulate the interactions of the sample and the stationary phase.




**Advantages of HPLC** [2]

1) It provides specific, sensitive and precise method for analysis of the different complicated sample.

2) There is ease of sample preparation and sample introduction.

3) There is speed of analysis.

4) The analysis by HPLC is specific, accurate and precise.

5) It offers advantage over gas chromatography in analysis of many polar, ionic substances, high molecular weight substances, metabolic products and thermo labile as well as nonvolatile substances.

**Applications of HPLC** [2]

a) Natural Products: HPLC is an ideal method for the estimation of various components in plant extracts which resemble in structure and thus demand a specific and very sensitive method e.g., analysis of digitalis, cinchona, liquorice, and ergot extracts.

b) Stability studies: HPLC is now used for ascertaining the stability of various pharmaceuticals. With HPLC the analysis of the various degradation products can be done and thus stability indicating HPLC systems have been developed.

c) Bioassays and its complementation: Complex molecules as antibiotics and peptide hormones are mainly analyzed by bioassay which suffers from high cost, necessity replicates, poor

precision and length of time required. Also bioassay gives an overall estimate of potency and gives no guidance about the composition. Thus HPLC can be used to complement bioassays and give an activity profile. It has been used for analysis of chloramphenicol, penicillins and clotrimoxazole, sulfas and peptides hormones.

d) HPLC has also been used in the cosmetic industry for quality control of various cosmetics.

## MATERIALS

**Instruments used:** HPLC-WATERS Alliance 2695 separation module. 996 PDA detector, software: Empower 2, pH meter-LabIndia, Weighing machine- Sartorius,Digital ultra sonicator-Labman.

**Chemicals used:** Artemether, Lumefantrine- Sura labs, Water and Methanol for HPLC-LICHROSOLV (MERCK),Acetonitrile for HPLC – Merck, Provided by Sura Labs, Dilsukhnagar, India

## METHODOLOGY

### HPLC METHOD DEVELOPMENT

**Preparation of standard solution:**

Accurately weigh and transfer 10 mg of Artemether and Lumefantrine working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette required amount of the above Artemether and Lumefantrine stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

**Preparation of Sample Solution:**

Take average weight of the Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight ofArtemether and Lumefantrine sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette required amount of Artemether and Lumefantrine above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

**Preparation of mobile phase:**

Accurately measured 350ml (35%) of Methanol, 650ml of Tri Ethyl Amine Buffer (65%) were mixed and degassed in digital ultra sonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration.

**Diluent Preparation:**

The Mobile phase was used as the diluent.

**Optimized Chromatographic Conditions**

Mobile phase : Methanol: Tri Ethyl Amine Buffer (35:65% v/v)

Column : Phenomenex Luna C18 (4.6mm×150mm, 5µm) Particle size

Flow rate : 1 ml/min

Wavelength : 261 nm

Column temp : 38ºC

Injection Volume : 10 µl

Run time : 10 minutes

**METHOD VALIDATION PARAMETERS**

**System Suitability**

**Procedure:**

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

**Specificity**

**Procedure:**

Inject the three replicate injections of standard and sample solutions and calculate the assay by using formula:

%ASSAY =

$$\frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

**Linearity :** prepare various levels of concentrated solutions of drugs Artemether in the range of 60-140ppm and Lumefantrine in the range of 100-500ppm

**Procedure:**

Inject each level into the chromatographic system and measure the peak area.

Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

**Precision**

***Repeatability:*** The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

***Intermediate precision:*** To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.

**Procedure:**

***DAY 1:***

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

***DAY 2:***

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

**Accuracy:**

***For preparation of 50% Standard stock solution:***pipette out 0.5ml of Artemether and 1.5ml of Lumefantrine from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents

***For preparation of 100% Standard stock solution:***pipette out 1ml of Artemether and 3ml of Lumefantrine from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

***For preparation of 150% Standard stock solution:***pipette 1.5ml of Artemether and 4.5ml of Lumefantrine from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

**Procedure:**

Inject the Three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Artemetherand Lumefantrine and calculate the individual recovery and mean recovery values.

**Robustness:**

The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results. .

***Effect of Variation of flow conditions:***

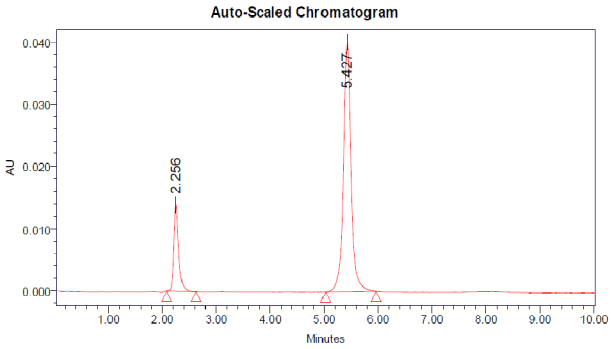
The sample was analyzed at 0.9 ml/min and 1.1 ml/min instead of 1ml/min, remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded

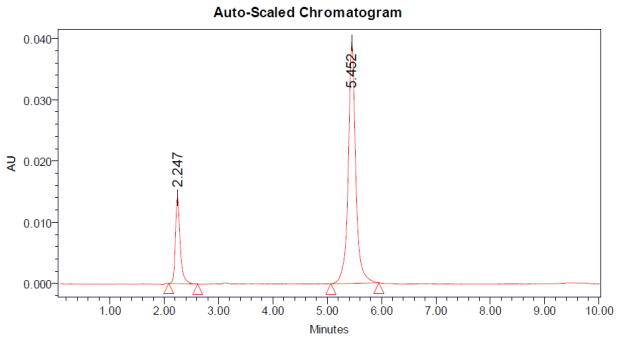
***Effect of Variation of mobile phase organic composition:***

The sample was analyzed by variation of mobile phase i.e. Methanol: Tri Ethyl Amine (35:65% v/v)was taken in the ratio and 40:60, 30:70 instead (35:65% v/v) remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

## RESULTS AND DISCUSSION

### Standard Optimized ChromatogramOptimized Chromatogram (Sample)




## METHOD VALIDATION

### System Suitability:

**Table-: Results of System Suitability for Artemether**

| S.No. | Name | Rt | Peak Area | Height | USP plate Count | USP Tailing |
|---|---|---|---|---|---|---|
| 1 | Artemether | 2.247 | 105698 | 18652 | 7592 | 1.08 |
| 2 | Artemether | 2.246 | 105874 | 18754 | 7584 | 1.09 |
| 3 | Artemether | 2.248 | 105698 | 18698 | 7562 | 1.08 |
| 4 | Artemether | 2.252 | 105465 | 18689 | 7549 | 1.08 |
| 5 | Artemether | 2.248 | 105236 | 18695 | 7591 | 1.09 |
| Mean | | | 105594.2 | | | |
| Std. Dev | | | 247.4049 | | | |
| % RSD | | | 0.234298 | | | |

**Table-: Results of System Suitability for Lumefantrine**

| S.No. | Name | Rt | Area | Height | USP Plate Count | USP Tailing | USP Resolution |
|---|---|---|---|---|---|---|---|
| 1 | Lumefantrine | 5.452 | 1856985 | 63659 | 6359 | 1.05 | 5.86 |
| 2 | Lumefantrine | 5.484 | 1856754 | 63598 | 6384 | 1.04 | 5.85 |
| 3 | Lumefantrine | 5.491 | 1856985 | 63845 | 6395 | 1.05 | 5.86 |
| 4 | Lumefantrine | 5.482 | 1856574 | 63989 | 6345 | 1.04 | 5.86 |
| 5 | Lumefantrine | 5.491 | 1854735 | 63895 | 6395 | 1.05 | 5.85 |
| Mean | | | 1856407 | | | | |
| Std. Dev | | | 950.2696 | | | | |
| % RSD | | | 0.051189 | | | | |

**SPECIFICITY**

%ASSAY =

$$\frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

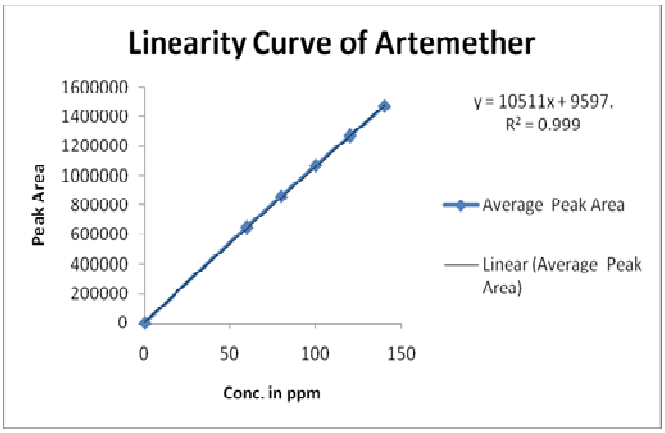
The % purity of Artemether and Lumefantrine in pharmaceutical dosage form was found to be 99.72%.

**LINEARITY**

**CHROMATOGRAPHIC DATA FOR LINEARITY STUDY:**

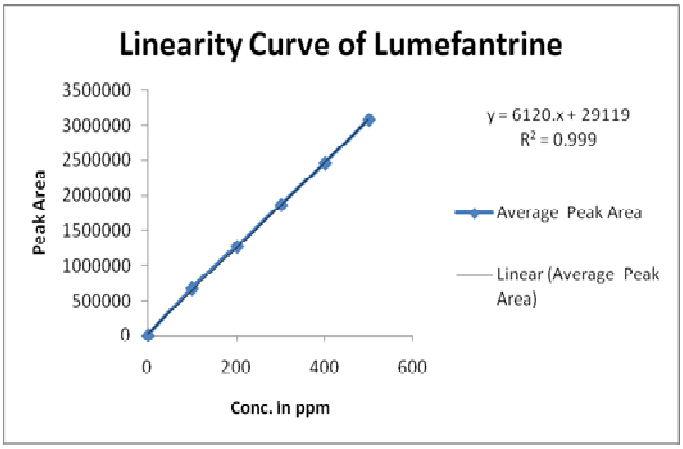
**Artemether:**

| Concentration µg/ml | Average Peak Area |
|---|---|
| 60 | 648743 |
| 80 | 856982 |
| 100 | 1068542 |
| 120 | 1268984 |
| 140 | 1469853 |




**Lumefantrine**

| Concentration µg/ml | Average Peak Area |
|---|---|
| 100 | 667564 |
| 200 | 1268547 |
| 300 | 1868598 |
| 400 | 2465487 |
| 500 | 3085864 |




**PRECISION:**

***Repeatability***

**Table-: Results of Repeatability for Artemether:**

| S.No. | Name | Rt | Area | Height | USP plate count | USP Tailing |
|---|---|---|---|---|---|---|
| 1 | Artemether | 2.269 | 105698 | 18569 | 7598 | 1.08 |
| 2 | Artemether | 2.255 | 105684 | 18547 | 7546 | 1.09 |
| 3 | Artemether | 2.252 | 105421 | 18594 | 7549 | 1.09 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 4 | Artemether | 2.267 | 105879 | 18574 | 7538 | 1.08 |
| 5 | Artemether | 2.260 | 105326 | 18563 | 7582 | 1.08 |
| **Mean** | | | 105601.6 | | | |
| **Std. Dev** | | | 224.5023 | | | |
| **% RSD** | | | 0.212594 | | | |

**Table-: Results of Method Precision for Lumefantrine**

| S. No. | Name | Rt | Area | Height | USP Plate Count | USP Tailing | USP Resolution |
|---|---|---|---|---|---|---|---|
| 1 | Lumefantrine | 5.274 | 1856985 | 63598 | 6359 | 1.05 | 5.86 |
| 2 | Lumefantrine | 5.266 | 1857458 | 63579 | 6357 | 1.04 | 5.85 |
| 3 | Lumefantrine | 5.265 | 1854795 | 63547 | 6358 | 1.04 | 5.86 |
| 4 | Lumefantrine | 5.278 | 1857469 | 63592 | 6357 | 1.05 | 5.86 |
| 5 | Lumefantrine | 5.305 | 1857685 | 63569 | 6345 | 1.04 | 5.85 |
| **Avg** | | | 1856878 | | | | |
| **Std. Dev** | | | 1192.4 | | | | |
| **% RSD** | | | 0.064215 | | | | |

***Intermediate precision:***

**Table-: Results of Intermediate Precision for Artemether**

| S. No. | Name | Rt | Area | Height | USP Plate Count | USP Tailing |
|---|---|---|---|---|---|---|
| 1 | Artemether | 2.248 | 115246 | 19685 | 7698 | 1.09 |
| 2 | Artemether | 2.245 | 116985 | 19654 | 7685 | 1.09 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 3 | Artemether | 2.242 | 115847 | 19675 | 7645 | 1.09 |
| 4 | Artemether | 2.239 | 116985 | 19682 | 7682 | 1.09 |
| 5 | Artemether | 2.243 | 115848 | 19654 | 7691 | 1.09 |
| 6 | Artemether | 2.246 | 116582 | 19647 | 7642 | 1.10 |
| **Mean** | | | **116248.8** | | | |
| **Std. Dev** | | | **710.3091** | | | |
| **% RSD** | | | **0.611025** | | | |

**Table-: Results of Intermediate Precision for Lumefantrine**

| S.No. | Name | Rt | Area | Height | USP Plate Count | USP Tailing | USP Resolution |
|---|---|---|---|---|---|---|---|
| 1 | Lumefantrine | 5.284 | 1948592 | 64582 | 6459 | 1.05 | 5.96 |
| 2 | Lumefantrine | 5.293 | 1958245 | 64256 | 6475 | 1.06 | 5.95 |
| 3 | Lumefantrine | 5.306 | 1947584 | 64598 | 6498 | 1.05 | 5.96 |
| 4 | Lumefantrine | 5.319 | 1948675 | 64785 | 6472 | 1.06 | 5.95 |
| 5 | Lumefantrine | 5.346 | 1959854 | 64585 | 6493 | 1.05 | 5.96 |
| 6 | Lumefantrine | 5.352 | 1958246 | 64924 | 6438 | 1.06 | 5.96 |
| **Mean** | | | **1953533** | | | | |
| **Std. Dev** | | | **5792.661** | | | | |
| **% RSD** | | | **0.296522** | | | | |

**Table-: Results of Intermediate precision Day 2 for Artemether**

| S.No. | Name | Rt | Area | Height | USP Plate Count | USP Tailing |
|---|---|---|---|---|---|---|
| 1 | Artemether | 2.255 | 102658 | 62584 | 6259 | 1.03 |
| 2 | Artemether | 2.260 | 102856 | 62359 | 6276 | 1.02 |
| 3 | Artemether | 2.242 | 102658 | 62451 | 6215 | 1.03 |
| 4 | Artemether | 2.245 | 102698 | 62584 | 6285 | 1.02 |
| 5 | Artemether | 2.260 | 102451 | 62758 | 6235 | 1.03 |
| 6 | Artemether | 2.255 | 102368 | 62154 | 6298 | 1.02 |
| **Mean** | | | **102614.8** | | | |
| **Std. Dev** | | | **176.9592** | | | |
| **% RSD** | | | **0.17245** | | | |

**Table-: Results of Intermediate precision for Lumefantrine**

| S.No. | Name | Rt | Area | Height | USP plate count | USP Tailing | USP Resolution |
|---|---|---|---|---|---|---|---|
| 1 | Lumefantrine | 5.266 | 1798952 | 62859 | 6265 | 1.03 | 5.42 |
| 2 | Lumefantrine | 5.265 | 1789854 | 62985 | 6289 | 1.02 | 5.43 |
| 3 | Lumefantrine | 5.306 | 1798659 | 62895 | 6279 | 1.03 | 5.42 |
| 4 | Lumefantrine | 5.293 | 1789898 | 62785 | 6285 | 1.02 | 5.43 |
| 5 | Lumefantrine | 5.265 | 1796856 | 62354 | 6249 | 1.03 | 5.42 |
| 6 | Lumefantrine | 5.266 | 1798568 | 62589 | 6245 | 1.02 | 5.43 |
| Mean | | | **1795465** | | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Std. Dev | | | **4390.879** | | | | |
| % RSD | | | **0.244554** | | | | |

**ACCURACY:**

**Table-: The Accuracy Results for Artemether**

| **%Concentration (at specification Level)** | **Area** | **Amount Added (ppm)** | **Amount Found (ppm)** | **% Recovery** | **Mean Recovery** |
|---|---|---|---|---|---|
| 50% | 539070 | 50 | 50.373 | 100.746% | 100.36% |
| 100% | 1063578 | 100 | 100.274 | 100.274% | |
| 150% | 1587149 | 150 | 150.085 | 100.056% | |

**Table-: The accuracy results for Lumefantrine**

| **%Concentration (at specification Level)** | **Area** | **Amount Added (ppm)** | **Amount Found (ppm)** | **% Recovery** | **Mean Recovery** |
|---|---|---|---|---|---|
| 50% | 949127 | 150 | 150.328 | 100.218% | 100.15% |
| 100% | 1867824 | 300 | 300.441 | 100.147% | |
| 150% | 2785321 | 450 | 450.359 | 100.079% | |

**LIMIT OF DETECTION**

**LOD= 3.3 × σ / s**

**Result:**

**Artemether** =2.63µg/ml ; **Lumefantrine** =3.84µg/ml.

**LIMIT OF QUANTITATION**

**LOQ=10×σ/S**

**Result:**

**Artemether** = 7.92µg/ml ; **Lumefantrine** = 11.54µg/ml.

## ROBUSTNESS

**Table-: Results for Robustness**

**Artemether:**

| Parameter used for sample analysis | Peak Area | Retention Time | Theoretical plates | Tailing factor |
|---|---|---|---|---|
| Actual Flow rate of 1.0 mL/min | 105265 | 2.256 | 7589 | 1.08 |
| Less Flow rate of 0.9 mL/min | 109898 | 2.505 | 7256 | 1.05 |
| More Flow rate of 1.1 mL/min | 102365 | 2.046 | 7469 | 1.07 |
| Less organic phase | 101548 | 2.505 | 7358 | 1.06 |
| More organic phase | 104645 | 2.046 | 7659 | 1.02 |

**Lumefantrine:**

| Parameter used for sample analysis | Peak Area | Retention Time | Theoretical plates | Tailing factor |
|---|---|---|---|---|
| Actual Flow rate of 1.0 mL/min | 1858475 | 5.427 | 6354 | 1.04 |
| Less Flow rate of 0.9 mL/min | 1925684 | 5.599 | 6253 | 1.05 |
| More Flow rate of 1.1 mL/min | 1863525 | 4.576 | 6248 | 1.03 |

| | | | | |
|---|---|---|---|---|
| Less organic phase | 1825471 | 5.599 | 6415 | 1.02 |
| More organic phase | 1836594 | 4.576 | 6529 | 1.06 |

## CONCLUSION

The analytical method was developed by studying different parameters.First of all, maximum absorbance was found to be at 261nm and the peak purity was excellent. Injection volume was selected to be 10µl which gave a good peak area.

The column used for study was Phenomenex Luna C18 (4.6mm×150mm, 5µm) Particle sizebecause it was giving good peak.38˚C temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0ml/min because of good peak area and satisfactory retention time. Mobile phase is Methanol: Tri Ethyl Amine Buffer (35:65% v/v)was fixed due to good symmetrical peak. So this mobile phase was used for the proposed study.

Run time was selected to be 10 min because analyze gave peak around 2.256, 5.427 ±0.02min respectively and also to reduce the total run time.The percent recovery was found to be 98.0-102 was linear and precise over the same range. Both system and method precision was found to be accurate and well within range.

The analytical method was found linearity over the range 60-140mg/ml of Artemetherand 100-500mg/ml of Lumefantrine of the target concentration. The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory.

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