**Research Article**

# Development and Validation of a Dissolution Test Method for Artemether and Lumefantrine in Tablets

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## Abstract

**Purpose:** To develop and validate a dissolution test method for dissolution release of artemether and lumefantrine from tablets.

**Methods:** A single dissolution method for evaluating the in vitro release of artemether and lumefantrine from tablets was developed and validated. The method comprised of a dissolution medium of 1000 ml of 2 %w/v of Myrj 52 in 0.005M HCl per vessel with the paddle rotating at 100 rpm for 120 min. The dissolution samples were analysed using a Waters HPLC system with Waters symmetry column (C-18 column of 250mm x 4.6mm i.d., 5 μ particle size). The mobile phase was a mixture of 20 volumes of 0.5 %v/v of triethylamine in water (adjusted to a pH of 3.0 with orthophosphoric acid) and 80 volumes of acetonitrile. The detection wavelength was set at 216 nm and 100 μl of each sample was injected. The HPLC method used for the determination of drug release was validated for the parameters of accuracy, precision, linearity, specificity, filter validation, solution stability and robustness.

**Results:** The dissolution test provided sink conditions for artemether and lumefantrine and was able to discriminate between tablet formulations of different hardness and different composition. Application of Mann-Whitney U test for significant difference between samples at various time points during the dissolution test yielded z values > 1.96 (1.96 = critical z value at p = 0.05) for the various formulations tested, indicating the discriminatory power of the dissolution test.

**Conclusion:** This validated dissolution test may be used as a single dissolution test for artemether and luminfantrine in tablet formulations.

**Keywords:** Artemether, Lumefantrine, Tablet formulations, Single dissolution test, HPLC

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# INTRODUCTION

Artemether is a drug used for the treatment of malaria [1,2]. Artemether and lumefantrine combination is a fixed dose artemisinin-based combination therapy (ACT) combining artemether, an artemisinin derivative, and lumefantrine, a synthetic antimalarial drug for the treatment of malaria [3,4]. Artemether is a fast acting drug with a short half-life. Lumefantrine acts slowly and has a longer half-life. On oral administration, the plasma concentration of artemether reaches peak level after 2 h while the peak plasma concentration of lumefantrine is attained after 6 - 8 h [ 5 ].

The International Pharmacopoeia monograph of artemether and lumefantrine tablet does not include a dissolution test [6]. No common single dissolution test method for simultaneous dissolution testing of artemether and lumefantrine tablets has been reported in the literature although separate dissolution methods for artemether and lumefantrine have been developed [7]. Artemether is poorly soluble in water while lumefantrine is practically insoluble in water. In general, developing an appropriate single dissolution method of discriminatory nature to evaluate the dissolution release of poorly soluble drugs from dosage forms has been a lingering challenge. [8-11]

In the present study, a single dissolution test method for the dissolution release of artemether and lumefantrine which will be very useful for quality control of combination tablets has been designed and validated in terms of the discriminatory power of the dissolution test. The HPLC method used for analysis has been validated at our laboratory.

# EXPERIMENTAL

## Reagents and materials

The working standards of artemether (99.4 % purity) and lumefantrine (98.3 % purity) were obtained from Micro Labs Ltd, India. Three reference products (A, B and C), each containing 20 mg of artemether and 120 mg of lumefantrine were obtained from Indian market. The test tablet product used was manufactured in-house. Water purified with Millipore Water Purification System (Elix 10 C model) was used for the preparation of buffers. Sodium acetate trihydrate, glacial acetic acid AR, potassium dihydrogen phosphate and sodium hydroxide pellets were sourced from Merck, India. PTFE membrane filters (0.45 μ, syringe filter – 25mm GD/X- Whatman) were used for filtration of standard and sample solutions. Cellulose acetate filter (0.45 μ, Sartorium stedim) was used for the filtration of the mobile phase.

## Instrumentation

The dissolution test was carried out using an Electrolab Dissolution Test System (Model TDT-08L). A Waters HPLC system equipped with a 2695 solvent delivery system, Waters auto injector, thermostatted column compartment and Waters 2998 photo diode array detector was used. Waters Symmetry column (C-18 column of 250mm x 4.6mm i.d., 5 μ particle size) was used for the analysis.

## *Determination of solubility of artemether and lumefantrine*

The solubility of artemether and lumefantrine was determined in the various dissolution media as follows. Artemether (500 mg) and lumefantrine (500 mg) were transferred into separate 100 ml conical flasks. Dissolution medium (50 ml) was added to each flask and the flasks were closed with Teflon-lined screw caps. These flasks were sonicated for 2 h at 37 °C with intermittent shaking. The solutions were each filtered, through a 0.45 μ Whatman membrane filter. Each filtrate was analysed separately by HPLC to determine the solubility of the drug in the particular dissolution medium.

## Evaluation of stability of the drug solutions

The stability of artemether and lumefantrine in different dissolution media was evaluated at room temperature by calculating the change in peak area of samples at different time points which was then compared with that of the same sample immediately after injection.

## Stirring rate and dissolution medium

Dissolution experiments were performed using standard USP dissolution apparatus 1 (basket) and apparatus 2 (paddle). Stirring rates of 100 rpm for basket, and 50, 75 and 100 rpm for paddle were evaluated. All the dissolution media used were de-aerated by sonication followed by filtration using membrane filter under vacuum.

## Sample preparation and determination

The dissolution test was carried out using 1, 1.5 and 2 %w/v of Myrj 52 (polyoxyl 40 stearate) in 0.005M hydrochloric acid. Sample aliquots of 5.0 ml were withdrawn at different time points up to 120 min. After each withdrawal, a volume of dissolution medium equal to the volume of sample withdrawn was added to each vessel to maintain the volume in each vessel. The samples were filtered and analysed by HPLC and the percent drug release at each dissolution time point calculated.

## Selection of sample filtration procedure

The filtration procedure for standard and sample solutions was evaluated using 2.5 µ pore filter paper (Whatman), 0.45 µ PTFE syringe filter (Whatman) and 0.45 µ nylon syringe filter (Advanced Microdevices, India)

## Discriminatory power

The discriminatory power of the dissolution test was evaluated by (a) comparison of the dissolution results of reference products A, B, C and in-house test product (b) comparison of the dissolution results of in-house test

product of various hardness (c) comparison of test results of the test products stored at 40 ºC and 75% RH with those of the test products stored at room temperature and humidity (23 °C / 60% RH)

## Chromatographic conditions

The dissolution samples were analysed using Waters HPLC system, Symmetry column (C-18 column of 250mm x 4.6mm i.d., 5 µ particle size) with a mobile phase of a mixture of buffer and acetonitrile (20:80, v/v) at a flow rate of 1.0 ml/min. The buffer for mobile phase was prepared by diluting 5 ml of triethylamine to 1000 ml with water and adjusting the pH to 3.0 ± 0.05 using orthophosphoric acid. The detection wavelength was set at 216 nm and 100 µl of each sample was injected.

## Preparation of lumefantrine standard stock solution

About 240 mg of lumefantrine working standard was accurately weighed and transferred into a 100 ml volumetric flask. About 25 ml of mobile phase and one drop of orthophosphoric acid were added, the solution sonicated to dissolve the drug and the volume made up to 100 ml with mobile phase**.**

## Artemether standard stock solution

About 20 mg of artemether working standard was accurately weighed and transferred into a 100 ml volumetric flask. About 5 ml of acetonitrile was added and the solution was sonicated to dissolve the substance; the volume was then made up to 100 ml with dissolution medium.

## Standard solution

Lumefantrine standard stock solution (5 ml) and 10 ml of artemether standard stock solution were transferred into a 100 ml volumetric flask and made up to volume with dissolution medium to obtain a concentration of 120 µg of lumefantrine and 20 µg of artemether per ml. This solution was filtered through 0.45 µ PTFE membrane filter

(Whatman) and the filtrate used as standard solution.

## Sample solution

During the dissolution test, 5 ml aliquots were withdrawn from the dissolution medium at various time points up to 120 min and replaced with an equal volume of fresh dissolution medium after each withdrawal  to maintain a constant volume of the dissolution medium in the vessel. The samples were filtered through 0.45 µ PTFE membrane filter (Whatman) and the filtrate used as sample solution.

## System suitability parameters

System suitability parameters of not less than 5.0 resolution between artemether and lumefantrine peaks, not more than 2.0 tailing factor for each peak, not less than 2000 theoretical plates for each peak and a %RSD of not more than 2.0 for five replicates of standard injection were specified as the system suitability requirements for the analytical method.

## Method validation

The HPLC method was validated for specificity, linearity, precision, accuracy, filter validation, solution stability and robustness according to USP and ICH guidelines [12,13].

## Statistical analysis

The dissolution values of the various formulations at different time points were compared using similarity factor (f2) and difference factor (f1) which were calculated using Eqs 1 and 2..

$$f_1 = \{[\sum_{t=1}^{n} (R_t - T_t)] / [\sum_{t=1}^{n} R_t]\} \times 100 \quad \text{............ (1)}$$

$$f_2 = 50 \times \log \{[1 + (1/n)\sum_{t=1}^{n} (R_t - T_t)^2]^{-0.5} \times 100\} \quad \text{..... (2)}$$

where $R_t$ and $T_t$ are the cumulative percentage dissolved at each of the selected 'n' time points of the reference and test product respectively

The calculation was performed with Microsoft Office Excel 2003. The difference between the dissolution values of various formulations was evaluated using Mann-Whitney U test and the calculated z values were compared with the critical z value of 1.96 ($p$ = 0.05). This calculation was carried out using a web calculator [14]. *The %RSD value and the linear regression analysis by the method of least squares were calculated using Excel program.*

# RESULTS

The solubility and stability data for artemether and lumefantrine in various commonly used dissolution media are presented in Tables 1 and 2 on the basis of which the most suitable dissolution medium was selected.

The dissolution values of reference product A in 0.005M HCl containing 1.0, 1.5  and 2.0% w/v of Myrj 52 are presented in Table 3 while those of the reference products A, B, C and the test product in the selected dissolution medium of 2% w/v Myrj 52  in 0.005N hydrochloric acid at various time points are presented in Table 4. The dissolution results of a formulation at a particular time point were different from those of other formulations at the same time point as per the Mann-Whitney U test.   The difference factor (f1) values for the various formulations indicate significant difference in the release pattern of the formulations when compared with reference product A.

## Validation of the HPLC method

The validation results for the HPLC method used for the determination of drug release are as follows.

### *Accuracy*

The accuracy of the method in the range of 25 to 150 % of test concentration of artemether (20 µg/ml of artemether) and lumefantrine (120 µg/ml of lumefantrine) was satisfactory.   Recovery (% was between 98.52 to 99.49% for artemether and 98.48 to 99.73% for lumefantrine.

**Table 1:** Solubility of artemether and lumefantrine in various dissolution media (n = 3)

| Medium | Concentration (µg/ml, mean ± SD) | |
|---|---|---|
| | Artemether | Lumefantrine |
| Water | 133 ± 4 | Insoluble |
| 0.1N Hydrochloric acid | 127 ± 5 | Insoluble |
| pH 4.5 Acetate buffer | 130 ± 4 | Insoluble |
| pH 6.8 Phosphate buffer | 119 ± 6 | Insoluble |
| 1% w/v SLS in Water | 4107 ± 12 | 16 ± 2 |
| 1% w/v SLS in 0.1N Hydrochloric acid | 2633 ± 21 | 216 ± 3 |
| 1% w/v SLS in pH 4.5 Acetate buffer | 3896 ± 11 | 238 ± 4 |
| 1% w/v SLS in pH 6.8 Phosphate buffer | 3511 ± 9 | 79 ± 10 |
| 1% w/v BKC in Water | 1230 ± 8 | 11 ± 2 |
| 1% w/v BKC in 0.1N Hydrochloric acid | 1055 ± 17 | 119 ± 3 |
| 1% w/v BKC in pH 4.5 Acetate buffer | 1246 ± 12 | 6 ± 1 |
| 1% w/v BKC in pH 6.8 Phosphate buffer | 1275 ± 11 | 3 ± 1 |
| 1% w/v CTAB in Water | 2544 ± 6 | 11 ± 2 |
| 1% w/v CTAB in 0.1N Hydrochloric acid | 2469 ± 16 | 175 ± 3 |
| 1% w/v CTAB in pH 4.5 Acetate buffer | 2555 ± 9 | 23 ± 2 |
| 1% w/v CTAB in pH 6.8 Phosphate buffer | 2589 ± 5 | 3 ± 1 |
| 1% w/v Tween 80 in Water | 643 ± 6 | 11 ± 1 |
| 1% w/v Tween 80 in 0.1N Hydrochloric acid | 639 ± 23 | 272 ± 5 |
| 1% w/v Tween 80 in pH 4.5 Acetate buffer | 641 ± 8 | 25 ± 2 |
| 1% w/v Tween 80 in pH 6.8 Phosphate buffer | 661 ± 6 | 7 ± 1 |
| 1% w/v Myrj 52 in Water | 536 ± 7 | 34 ± 2 |
| 1% w/v Myrj 52 in 0.1N Hydrochloric acid | 503 ± 19 | 813 ± 4 |
| 1% w/v Myrj 52 in pH 4.5 Acetate buffer | 524 ± 8 | 19 ± 2 |
| 1% w/v Myrj 52 in pH 6.8 Phosphate buffer | 528 ± 7 | 7 ± 1 |
| 1% w/v Myrj 52 in 0.005N Hydrochloric acid | 603 ± 4 | 463 ± 5 |
| 1.5% w/v Myrj 52 in 0.005N Hydrochloric acid | 897 ± 4 | 616 ± 4 |
| 2 % w/v Myrj 52 in 0.005N Hydrochloric acid | 1077 ± 5 | 732 ± 6 |

### Precision

The intra-day and inter-day precision, compared in terms of %RSD, were close to each other indicating satisfactory level of precision of the method. %RSD for intra-day precision was 1.33 and 1.51 for artemether and 1.23 and 1.33 for lumefantrine on first and second day of analysis, respectively while %RSD for inter-day precision was 1.37 for artemether and 1.27 for lumefantrine

### Linearity

The analytical method was linear for artemether and lumefantrine in the concentration range of 5 – 150 % of test concentration of the drugs. The test concentration of artemether was 20 µg/ml

and that of lumefantrine 120 µg/ml . The regression analysis of the linearity data yielded correlation coefficient values above 0.9990 for each drug (see Table 5).

### Specificity

The specificity of the method was ascertained from the absence of peaks in the chromatogram of the dissolution medium at the retention times of artemether (Rt = 8.03 min) and lumefantrine (Rt = 5.34 min) as well as from the purity angle values. For artemether, the purity angle was 0.849 and 0.824 while the purity threshold was 1.269 and 1.312 in standard and sample solutions, respectively. Similarly, for lumefantrine the purity angle was 1.656 and 1.632 while the purity threshold was 9.897 and 9.832 in

**Table 2:** Stability of artemether and lumefantrine  in various dissolution media (n = 3)

| Medium | Percent peak area reduction* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Artemether | | | | Lumefantrine | | | |
| | At 0 hour | After 2 hours | After 6 hours | After 10 hours | At 0 hour | After 2 hours | After 6 hours | After 10 hours |
| Water | 0 | 0.61 | 1.12 | 1.96 | | Insoluble | | |
| 0.1N HCl | 0 | 26.19 | 57.41 | 74.33 | | Insoluble | | |
| pH 4.5 Acetate buffer | 0 | -0.26 | 2.03 | 0.79 | | Insoluble | | |
| pH 6.8 Phosphate buffer | 0 | -0.36 | 1.15 | 1.31 | | Insoluble | | |
| 1% w/v SLS in Water | 0 | -2.33 | 1.38 | -0.86 | 0 | 0 | 0.00 | 0.04 |
| 1% w/v SLS in 0.1N Hydrochloric acid | 0 | 54.27 | 88.39 | 100 | 0 | 0.01 | 0.01 | -0.04 |
| 1% w/v SLS in pH 4.5 Acetate buffer | 0 | -0.14 | 0.98 | 0.35 | 0 | 0.18 | 0.04 | 0.80 |
| 1% w/v SLS in pH 6.8 Phosphate buffer | 0 | 0.43 | -0.29 | 0.57 | 0 | 3.30 | 7.02 | 9.10 |
| 1% w/v BKC in Water | 0 | 0.69 | 0.37 | 0.39 | 0 | 0.13 | 0.48 | 0.96 |
| 1% w/v BKC in 0.1N Hydrochloric acid | 0 | 1.73 | 12.16 | 17.74 | 0 | 0.06 | 0.05 | 0.05 |
| 1% w/v BKC in pH 4.5 Acetate buffer | 0 | 1.27 | 0.85 | 0.28 | 0 | -0.05 | -0.01 | 0.01 |
| 1% w/v BKC in pH 6.8 Phosphate buffer | 0 | 0.00 | -0.14 | 0.28 | 0 | 4.67 | 27.42 | 51.53 |
| 1% w/v CTAB in Water | 0 | -0.86 | 0.13 | -0.57 | 0 | -0.08 | 0.11 | 0.26 |
| 1% w/v CTAB in 0.1N HCl | 0 | 1.49 | 7.08 | 9.86 | 0 | 0.03 | 0.34 | 0.45 |
| 1% w/v CTAB in pH 4.5 Acetate buffer | 0 | 0.14 | -0.14 | 0.95 | 0 | 0.03 | -0.14 | 0.30 |
| 1% w/v CTAB in pH 6.8 Phosphate buffer | 0 | 0.14 | 0.42 | 0.42 | 0 | 0.16 | 1.70 | 8.72 |
| 1% w/v Tween 80 in Water | 0 | -0.15 | 0.45 | 0.75 | 0 | -0.07 | 0.41 | 0.50 |
| 1% w/v Tween 80 in 0.1N HCl | 0 | 9.61 | 19.47 | 29.94 | 0 | 1.50 | 2.76 | 6.24 |
| 1% w/v Tween 80 in pH 4.5 Acetate buffer | 0 | 0.42 | -0.28 | 0.85 | 0 | 0.01 | 0.02 | 0.55 |
| 1% w/v Tween 80 in pH 6.8 Phosphate buffer | 0 | 0.28 | 0.42 | 0.14 | 0 | 0.02 | 0.19 | 0.61 |
| 1% w/v Myrj 52 in Water | 0 | -0.56 | 0.56 | 0.14 | 0 | 0.02 | 0.02 | 0.32 |
| 1% w/v Myrj 52 in 0.1N Hydrochloric acid | 0 | 9.60 | 20.47 | 31.81 | 0 | 0.23 | 0.57 | 0.68 |
| 1% w/v Myrj 52 in pH 4.5 Acetate buffer | 0 | 0.14 | 0.70 | 0.14 | 0 | 0.28 | 0.31 | 0.58 |
| 1% w/v Myrj 52 in pH 6.8 Phosphate buffer | 0 | 0.14 | -0.28 | 0.28 | 0 | 0.16 | 2.60 | 9.16 |
| 1% w/v Myrj 52 in 0.005N HCl | 0 | 0.10 | 0.21 | 0.43 | 0 | 0.02 | 0.01 | 0.02 |
| 1.5% w/v Myrj 52 in 0.005N HCl | 0 | 0.10 | 0.20 | 0.41 | 0 | 0.01 | 0.02 | 0.03 |
| 2 % w/v Myrj 52 in 0.005N HCl | 0 | 0.12 | 0.22 | 0.44 | 0 | 0.02 | 0.03 | 0.05 |

*SLS= Sodium lauryl sulphate    BKC= Benzalkonium chloride   CTAB= Cetyl trimethylammonium bromide*

**Table 3:** Dissolution profile of reference product A in 0.005M HCl containing 1,1.5 and 2.%w/v of Myrj 52 (n = 24)

| Time (min) | Basket 100 RPM | Paddle 50 RPM | Paddle 75 RPM | Paddle 100 RPM |
|---|---|---|---|---|
| | *1% w/v Myrj 52 in 0.005M hydrochloric acid* | | | |
| | *%Artemether release (mean ± SD)* | | | |
| 30 | 35.2 ± 2.6 | 39.6 ± 2.2 | 44.2 ± 3.2 | 53.4 ± 2.8 |
| 60 | 47.5 ± 2.1 | 55.3 ± 2.9 | 64.6 ± 2.6 | 78.4 ± 2.1 |
| 90 | 62.5 ± 1.8 | 73.2 ± 2.7 | 82.3 ± 1.9 | 95.4 ± 1.4 |
| 120 | 74.7 ± 1.3 | 80.1 ± 2.3 | 89.4 ± 1.4 | 98.5 ± 1.1 |
| | *% Lumefantrine release (mean ± SD)* | | | |
| 30 | 19.5 ± 1.3 | 21.4 ± 1.7 | 26.3 ± 2.1 | 35.4 ± 1.5 |
| 60 | 35.3 ± 1.7 | 37.5 ± 2.6 | 44.4 ± 2.1 | 53.4 ± 2.2 |
| 90 | 52.4 ± 2.5 | 56.6 ± 2.9 | 60.6 ± 2.2 | 65.4 ± 3.1 |
| 120 | 62.9 ± 2.4 | 64.5 ± 2.3 | 68.3 ± 3.1 | 73.7 ± 2.9 |
| | *1.5% w/v Myrj 52 in 0.005M hydrochloric acid* | | | |
| | *% Artemether release (mean ± SD)* | | | |
| 30 | 37.2 ± 2.8 | 45.4 ± 3.1 | 54.4 ± 2.4 | 58.4 ± 2.9 |
| 60 | 57.3 ± 3.1 | 65.1 ± 2.8 | 77.4 ± 2.5 | 84.6 ± 2.1 |
| 90 | 74.6 ± 2.6 | 83.7 ± 1.9 | 94.4 ± 1.9 | 98.6 ± 1.7 |
| 120 | 79.6 ± 2.1 | 88.6 ± 1.4 | 97.8 ± 1.4 | 100.8 ± 1.1 |
| | *% Lumefantrine release (mean ± SD)* | | | |
| 30 | 22.3 ± 1.3 | 23.4 ± 1.1 | 36.8 ± 1.2 | 41.4 ± 1.8 |
| 60 | 38.7 ± 3.3 | 44.4 ± 2.4 | 55.3 ± 2.8 | 60.4 ± 2.9 |
| 90 | 55.4 ± 2.7 | 61.1 ± 3.1 | 66.4 ± 3.1 | 76.3 ± 2.7 |
| 120 | 64.1 ± 3.3 | 67.2 ± 2.9 | 71.7 ± 2.8 | 84.3 ± 2.5 |
| | *2% w/v Myrj 52 in 0.005M hydrochloric acid* | | | |
| | *% Artemether release (mean ± SD)* | | | |
| 30 | 46.7 ± 2.7 | 55.8 ± 2.7 | 58.5 ± 3.2 | 69.4 ± 1.9 |
| 60 | 65.2 ± 2.4 | 79.7 ± 2.2 | 84.9 ± 2.6 | 94.5 ± 0.9 |
| 90 | 82.6 ± 1.7 | 92.7 ± 1.8 | 99.2 ± 1.5 | 100.1 ± 0.9 |
| 120 | 86.5 ± 1.8 | 98.9 ± 1.3 | 100.2 ± 1.2 | 100.8 ± 0.7 |
| | *% Lumefantrine release (mean ± SD)* | | | |
| 30 | 34.4 ± 2.2 | 36.4 ± 1.5 | 40.3 ± 2.2 | 47.8 ± 2.2 |
| 60 | 53.4 ± 2.7 | 54.3 ± 2.2 | 59.8 ± 2.6 | 68.5 ± 2.0 |
| 90 | 62.7 ± 2.7 | 64.4 ± 2.6 | 75.7 ± 2.6 | 93.4 ± 1.2 |
| 120 | 67.6 ± 2.5 | 69.3 ± 2.3 | 83.4 ± 1.9 | 100.7 ± 1.1 |

standard and sample solution, respectively. The purity angle value, being less than the purity threshold value, indicates that the peaks are spectrally pure in standard and sample solutions.

### Filter validation

The filtration procedure for artemether and lumefantrine standard and sample solutions was evaluated using three different types of filters, namely, 0.45µ mdi nylon filter (A), 0.45µ Whatman PTFE (B) filter and No. 42 Whatman filter paper (C).

The centrifuged solutions of the standard and sample were compared with the filtered solutions in terms of % recovery. The percentage peak area difference between the centrifuged solutions and solutions filtered through filters A, B and C was less than 1 % indicating that loss during filtration is within the acceptable limits.

### Stability of solution

The stability of standard and sample solutions was studied for 24 h at room temperature. The standard and sample solutions were stable in 2 %w/v Myrj 52 in

**Table 4:** Dissolution profile of reference products A, B, C and test product in 2% w/v Myrj 52 in 0.005M hydrochloric acid using paddle at 100 rpm (n = 24)

| Time (min) | Reference Product A | Reference Product B | Reference Product C | Test Product T-1 (Hardness : About 80 N) | Test Product T-2 (Hardness: About 40 N) | Test Product T-1 40℃/75% RH 15 days | Test Product T-2 40℃/75% RH 15 days |
|---|---|---|---|---|---|---|---|
| *% Artemether release (mean ± SD)* | | | | | | | |
| 15 | 58.3 ± 2.2 | 66.2 ± 2.3 | 72.3 ± 3.6 | 46.2 ± 2.4 | 67.6 ± 2.9 | 33.4 ± 3.1 | 54.7 ± 3.4 |
| 30 | 69.4 ± 1.9 | 76.4 ± 2.1 | 85.4 ± 2.9 | 58.9 ± 2.8 | 76.4 ± 2.4 | 49.5 ± 3.4 | 65.9 ± 3.1 |
| 60 | 94.5 ± 0.9 | 95.1 ± 1.9 | 98.4 ± 1.4 | 71.4 ± 1.9 | 90.3 ± 1.7 | 59.6 ± 2.7 | 81.7 ± 2.8 |
| 90 | 100.1 ± 0.9 | 99.8 ± 1.1 | 100.1 ± 1.1 | 79.5 ± 1.5 | 100.5 ± 1.2 | 64.8 ± 2.9 | 89.8 ± 2.7 |
| 120 | 100.8 ± 0.7 | 100.5 ± 0.9 | 101.3 ± 0.8 | 88.3 ± 1.7 | 100.5 ± 0.9 | 74.7 ± 2.4 | 98.5 ± 1.5 |
| f1 (Difference factor) | | 6.98 | 15.26 | 18.62 | 9.23 | 33.35 | 9.37 |
| f2 (Similarity factor) | | 60.43 | 45.12 | 39.01 | 57.09 | 26.98 | 53.15 |
| *% Lumefantrine release (mean ± SD)* | | | | | | | |
| 15 | 38.7 ± 1.8 | 29.2 ± 1.2 | 37.4 ± 1.6 | 23.4 ± 1.3 | 30.1 ± 3.1 | 17.8 ± 2.1 | 26.3 ± 2.9 |
| 30 | 47.8 ± 2.2 | 46.3 ± 1.8 | 59.3 ± 2.4 | 35.7 ± 1.8 | 44.9 ± 2.8 | 26.2 ± 2.6 | 39.4 ± 3.1 |
| 60 | 68.5 ± 2.0 | 72.1 ± 2.1 | 82.1 ± 2.8 | 51.9 ± 2.9 | 74.3 ± 2.4 | 39.7 ± 2.8 | 67.6 ± 2.9 |
| 90 | 93.4 ± 1.2 | 87.4 ± 2.8 | 96.4 ± 1.1 | 69.4 ± 2.5 | 92.7 ± 2.0 | 55.7 ± 2.9 | 83.7 ± 2.5 |
| 120 | 100.7 ± 1.1 | 96.3 ± 2.6 | 100.2 ± 0.9 | 78.7 ± 2.7 | 99.8 ± 1.2 | 65.9 ± 3.0 | 92.4 ± 1.9 |
| f1 (Difference factor) | | 8.29 | 11.84 | 25.78 | 7.25 | 41.19 | 11.37 |
| f2 (Similarity factor) | | 60.98 | 52.03 | 36.58 | 63.03 | 26.46 | 52.61 |

0.005M hydrochloric acid for a minimum period of 24 h.

**Robustness**

The robustness of the method was evaluated by the varying chromatographic conditions of wavelength, flow rate, pH and composition of mobile phase of the analytical method. As per the standard test method, the wavelength was 216 nm, flow rate 1.0ml/min, pH of mobile phase 3.0 and composition of mobile phase 20:80 buffer:acetonitrile. To evaluate the robustness of the method, the chromatograms were recorded with detection wavelengths of 213 nm and 219nm, flow rate of 0.8 ml/minute and 1.2 ml/minute, mobile phase pH of 2.8 and 3.2, and mobile phase

composition of 22 : 78 and 18 : 82 v/v of buffer : acetonitrile. The results of the chromatographic test under varied conditions indicated that the accuracy and precision are within the specified limits of 98 - 102 % for accuracy and %RSD of ≤ 2.0 for precision. No significant change was observed in system suitability parameters such as resolution, tailing factor, number of theoretical plates and %RSD for replicate injections of the standard.

## DISCUSSION

The sink condition for artemether (a minimum solubility of 60 µg/ml) may be achieved in all the dissolution media listed in Table 1 where-

**Table 5:** Linearity of response of artemether and lumefantrine

| Concentration (%) | Artemether | | Lumefantrine | |
|---|---|---|---|---|
| | *Concentration (µg/ml)* | *Area Response* | *Concentration (µg/ml)* | *Area Response* |
| 5 | 1.02 | 3078 | 6.03 | 1691399 |
| 10 | 2.05 | 6187 | 12.05 | 3349978 |
| 25 | 5.03 | 15781 | 30.07 | 8464426 |
| 50 | 10.07 | 30993 | 60.11 | 16760389 |
| 75 | 15.06 | 45454 | 90.06 | 25010957 |
| 100 | 20.11 | 60697 | 120.04 | 33369990 |
| 125 | 25.08 | 75497 | 150.12 | 42557238 |
| 150 | 30.12 | 90809 | 180.04 | 50499341 |
| Slope | 3004.7 | | 281157 | |
| Intercept | 295.32 | | 79052 | |
| Linear equation | y = 3004.7x + 295.32 | | y = 281157x - 79052 | |
| Correlation coefficient | 0.9999 | | 0.9999 | |

as the sink condition for lumefantrine (a minimum solubility of 360 µg/ml) may be achieved only in HCl containing more than 1 %w/v of Myrj 52. Lumefantrine is practically insoluble in 0.1M HCl, pH 4.5 acetate buffer, pH 6.8 phosphate buffer and in water. Addition of a solubilising agent to these media resulted in various levels of solubility of lumefantrine in these media. Although the solubility of lumefantrine in HCl containing Myrj 52 was satisfactory, it was observed that the stability of lumefantrine in HCl in the presence of Myrj 52 increased as the molarity of HCl decreased.   On this basis, the dissolution medium of 1 %w/v of Myrj 52 in 0.005M HCl was stable and provided sink conditions for artemether and lumefantrine.

To verify the suitability of 1 %w/v of Myrj 52 in 0.005M HCl as dissolution medium, dissolution release from reference product A was checked in three different dissolution media of 1, 1.5 and 2 %w/v Myrj 52 in 0.005M HCl. The test was performed with a volume of 1000ml of each dissolution medium using USP dissolution apparatus type 1 (basket) and type 2 (paddle) with different rates of stirring. At a stirring rate of 100 rpm of paddle, the release of artemether was above 90 % in all the three media but the release of lumefantrine was above 90 % only in 2 %w/v of Myrj 52 in 0.005M HCl. Lumefantrine release was about 73 % in 1

%w/v of Myrj 52 in 0.005M HCl and 84 % in 1.5 %w/v of Myrj 52 in 0.005N HCl.

The results obtained show that the medium of 2 %w/v Myrj 52 in 0.005M HCl with a stirring rate of 100 rpm for paddle was able to release 100.8 and 100.7 % of artemether and lumefantrine, respectively, from reference product A in 120 min.

Therefore, this dissolution medium using paddle at 100 rpm for 120 minutes was selected for the dissolution test. The discriminating power of the selected method was verified by analyzing the three reference products (A, B and C), two in-house prepared test products with different hardness and in-house test products exposed to 40 °C/75 % RH conditions for 15 days in an open dish. The dissolution results presented show that the proposed dissolution test has satisfactory discriminating power to distinguish the release profiles of the different reference products, the in-house products of different hardness and the in-house products which were exposed to heat and humidity. Mann-Whitney U test was applied to the data to find out whether each group of dissolution release values at a particular time point is different for different formulations. For example, the dissolution release values at 15 min of reference product A were  compared with the dissolution release values of test product 1

(T1)  using Mann-Whitney U test. Each of the z values obtained was > 1.96. The z value of 1.96 is the critical value for a two-tailed test at the level of significance of 5 % ($p$ = 0.05). Since the z values were > 1.96, it was inferred that the groups of values being compared are different from each other at $p$ = 0.05. This showed that the dissolution method is able to produce results which are different for different formulations at the various time points.

Typical acceptance criteria for dissolution release of drugs from immediate release tablets is about 80 % of labeled amount in 45 minutes [15]. Using 2 %w/v Myrj 52 in 0.005M HCl with paddle at 100rpm,  95 % of artemether was released in 60 min from each of the reference products (A, B and C) whereas above 95 % of lumefantrine was released only around 120 min. Hence, with 2 %w/v Myrj 52 in 0.005M HCl  as dissolution medium and with a stirring rate of 100 rpm with paddle, the suggested dissolution release acceptance limit would not be less than 85 % of artemether in 60 min and not less than 85 % of lumefantrine in 120 min. A single time point of not less than 85 % release of each drug in 120 min may be considered for quality control test.  However, the in-house limit for a particular tablet formulation of artemether and lumefantrine ought to be fixed on the basis of its dissolution performance using the developed method.

The HPLC method used for the determination of dissolution release of artemether and lumefantrine from tablets were validated in terms of accuracy, precision, linearity, specificity, filter validation, solution stability and robustness. The results of validation showed that all the validation parameters are satisfactory. The peak areas of artemether and lumifantrine peaks were linear with respect to concentration. The correlation coefficient values of 0.9999 indicate excellent correlation between response (peak area) and concentration.

## CONCLUSION

The proposed dissolution test method for artemether and lumefantrine in tablets provides sink condition and is able to satisfactorily discriminate drug release between formulations differing in terms of hardness, exposure to heat and moisture and composition of excipients. The analysis was carried out by a HPLC method which was validated. Hence the proposed dissolution method may be used as a single test method for the routine dissolution testing of tablets containing artemether and lumefantrine.

## ACKNOWLEDGEMENT

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