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# Concurrent process validation: A case study for Artesunate and Amodiaquine tablets

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**Abstract**
Process validation is a requirement of Current Good Manufacturing Practices (cGMP). The essence of process validation is to ascertain the quality of a product throughout its production life cycle. The various steps in manufacturing processes must be validated as it is essential for quality to be built into the manufacturing processes of drugs. In this study, we discuss the Process validation of Artesunate (Art) tablets and Amodiaquine (Amod) tablets. The following critical tests were performed on the blend; Assay, blend uniformity studies, blend characteristics and flowability properties (Bulk Density, Tap Density, Compressibility Index, Hausner Ratio), resistance to Segregation studies, Loss on Drying and Blend hold-time studies. The following analytical tests were performed on the compressed tablets; Identification, weight variation, Disintegration, Hardness, Average weight, Loss on Drying, Friability, Assay, Dissolution, Thickness and Uniformity of Dosage Unit. The following were performed on the packaged finished products; leak test, Print quality, carton seal integrity. Percentage yield was also determined. The following statistical tools were used in the data evaluation; F-test, Shapiro-Wilk test, Kurtosis, Skewness, T-test emanating from regression analysis, Tests for Normality and Tests for Significant differences. Results indicated an acceptable level of homogeneity within a batch and a high level of consistencies between batches. The manufacturing process was concluded to be capable and stable to assure quality and safe products.

**Keywords:** Artesunate, Amodiaquine, Statistics, Process Validation

## 1. Introduction
The US FDA in its January 2011 Guidance for Industry (Process Validation: General Principles and Practices) defines Process Validation as the collection and evaluation of data, from the process design stage through commercial production, which establishes scientific evidence that a process is capable of consistently delivering quality product.
Effective process validation contributes significantly to assuring drug quality. The basic principle of quality assurance is that a drug should be produced that is fit for its intended use. This principle incorporates the understanding that quality, safety, and efficacy are designed or built into the product.
The guidance describes process validation activities in three stages.

- **Stage 1:** Process Design: The commercial manufacturing process is defined during this stage based on knowledge gained through development and scale-up activities.
- **Stage 2:** Process Qualification: During this stage, the process design is evaluated to determine if the process is capable of reproducible commercial manufacturing.
- **Stage 3:** Continued Process Verification: Ongoing assurance is gained during routine production that the process remains in a state of control [1].

Process validation is a requirement of current cGMP. The essence of process validation is to establish through scientific/designed data collection and analysis that the defined manufacturing process is capable of reliably and repeatedly rendering a product of the required quality that consistently meets all quality and design specifications. cGMP has identified three different ways of validating a process; Prospective, Concurrent and Retrospective validations. Revalidation is also performed after significant changes in a manufacturing process.
According to cGMP, Qualification and validation should establish and provide documentary evidence that a specific process will consistently produce a product meeting its predetermined specifications and quality attributes (process validation or PV, also called performance qualification or PQ) [2].

### 1.1 Types of process validation

1.1.1 Prospective Validation

This form of validation which should be performed for new or substantially modified manufacturing processes, is essential for restricting the risk of errors occurring in advance; e.g. the preparation of injectable products requires this form of validation.

### 1.1.2 Concurrent Validation

This is the form of validation carried out during the normal production of a product to ensure that a process produces products with desired characteristics as the manufacturing process is implemented.

### 1.1.3 Retrospective Validation

It is the form of validation which demonstrates process consistency and involves looking back into past experiences obtained during production; i.e. establishment of documented evidence that a process will continuously produce a product of desired characteristics from a review of historical data, based on the precondition that composition, procedures and equipment remain unchanged, and that facility, experience and the results from in-process and final control tests are evaluated.

### 1.1.4 Revalidation

This is needed to ensure that changes in the process and/or in the process environment, whether introduced intentionally or unintentionally, do not adversely affect process characteristics and product quality [3].

## 2. Materials and Methods

The following production and analytical equipment were utilized during production and quality control processes; Moisture Analyzer, Analytical Balance, Dissolution Tester, Disintegration Tester, Weighing Balance, Vernier Caliper, Friability Tester, Tablet Compression Machine, Mechanical Mixer, Dispensing Booth, Stop Watch.

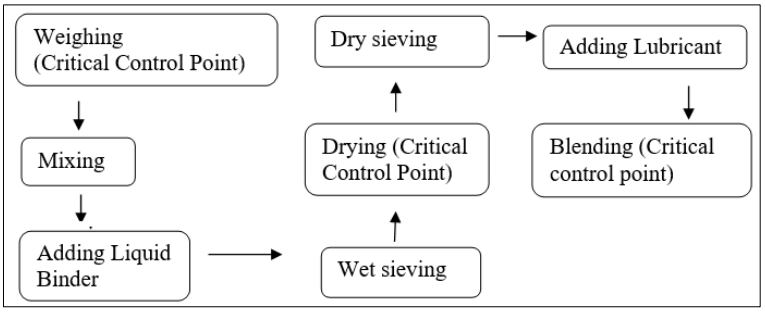


**Fig 1:** Schematic Diagram of Manufacturing Process (Wet granulation)

**Table 1:** Product Compositions

| Raw Material/Ingredient | |
|---|---|
| Artesunate Tablet | Amodiaquine Tablet |
| Artesunate BP | Amodiaquine hydrochloride BP |
| Lactose granules Super Tab 24 AN BP | Lactose BP |
| Sodium lauryl sulphate BP | Starch BP |
| Magnesium stearate BP | P.V.P. K30 BP |
| Starch BP | Nipasept sodium BP |
| Sodium starch glycolate BP | Sodium starcy glycolate BP |
| | Magnesium stearate BP |
| | Cab-O-sil BP |
| | Stearicacid BP |
| | Talc BP |

### 2.1 Study Design

The validation design was based on the study of the effect of critical operation variables on the quality of intermediate and finished products at identified critical control points. The design considered sampling from ten points.

The blender employed was U-shaped. Cluster sampling was adopted for the blend. Top samples from the middle region were identified as $T_5$ and those from the bottom were represented by $B_5$. Samples represented by $T_1$, $T_2$, $T_3$ and $T_4$ were sampled about 6 cm away from the walls of the blender and equidistance to each other and the middle sampling points. Samples from the bottom region were represented as follows; $B_5$, $B_1$, $B_2$, $B_3$ and $B_4$.

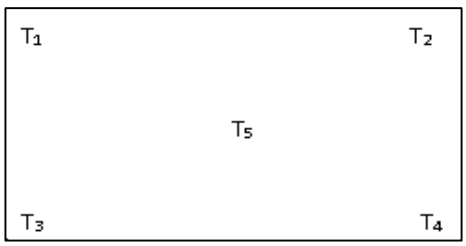


**Fig 2:** Sampling plan for Top region

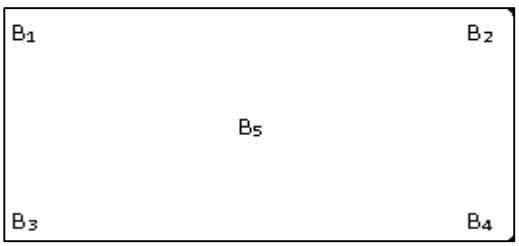


**Fig 3:** Sampling Plan for Bottom region

NB: The following figures; 1,2,3,4,5,6,7,8,9 and 10 were used to represent the following cluster sampling points respectively; $T_1$,$T_2$,$T_3$,$T_4$,$T_5$,$B_1$,$B_2$,$B_3$,$B_4$ and $B_5$, for the purposes of statistical analysis. For compression and packaging, the expected operations and performance of the compression machine was verified. 100 tablets for each batch were sampled during compression at the beginning, middle and end of each process. The blister packaging machine was qualified and all packing materials were pretested. 100 tablets were taken at initial, midway and closing stages of blister packaging operations. Complete quality control tests were performed on the blistered and packed samples. Results obtained were evaluated and suitable conclusions were drawn.

**Table 2:** Critical Control Variables

| No. | Identified Critical Control Point | Control Variables | | Critical Test |
|---|---|---|---|---|
| | | Variable | Defined Value | |
| 1. | After Mixing and lubrication; Artesunate | Mixing times after Lubrication | 3 minutes | Blend Uniformity and Characteristic Study (Assay, Appearance, Bulk density, tap density, hausner ratio, compressibility, index, LOD) |
| | | Mixer capacity | 5 L – 1200 L | |
| | | Mixing speed | Low speed | |
| | Amodiaquine | Mixing times after Lubrication | 3 minutes | |
| | | Mixer capacity | 5 L – 1200 L | |
| | | Mixing speed | Low speed | |
| 2. | Transfer of blend into holding drums | - | - | Blend segregation study (Assay) |
| 3. | After Holding blend for one month | Storage Temp | NMT 30 ºC | Blend Hold-Time Study (Assay, Appearance, Bulk density, Tap density, hausner ratio, compressibility, index, LOD) |
| | | Storage Hum. | NMT 75 % RH | |
| 4. | Tablets Compression; Artesunate | Compression speed | 36 rpm | Compression Process Quality and Consistency (Assay, content uniformity, dissolution, Weight variation, LOD, DT, friability, hardness, diameter, thickness) |
| | | Punch size | 12 mm | |
| | | Embossment | Lower punch = 'Break line' Upper punch = 'LS 100' | |
| | Amodiaquine | Compression speed | 36 rpm | |
| | | Punch size | 12 mm | |
| | | Embossment | Lower punch = 'Break line' Upper punch = LQS 300 | |
| 5. | Blister | Output | 240 blisters/minute | Sealing and Blistered product Quality (Leak test, Print/embossment quality, Assay, dissolution, LOD, DT, friability) |
| | | Forming/Film temperature | 172 ºC | |
| | | Sealing Temperature | 225 ºC | |
| 6. | Packaging | - | - | Carton seal integrity (Print/embossment quality, Assay, dissolution, LOD, DT, friability, Average weight, Hardness, Weight variation, Identification) |

## 2.2 Statistical Tools

Statistical tools used are shown in table using SPSS 13 [4].

**Table 3:** Formulas for Statistical Analysis

| No. | Tool | Formula |
|---|---|---|
| 1. | Mean | Sum of sample/number of sample |
| 2. | Standard deviation | $s_N = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(x_i - \overline{x})^2},$ |
| 3. | T-test | $t = \frac{\overline{x_1} - \overline{x_2}}{\sqrt{\frac{s_1^2}{N_1} + \frac{s_2^2}{N_2}}}$ |
| 4. | F-test | $F$ = explained variance / unexplained variance |
| 5. | Cpk | USL – LSL/6*SD, USL: upper specification limit, LSL: lower specification limit, SD: standard deviation |
| 6. | CpU | USL – μ/3*SD, USL: upper specification limit, LSL: lower specification limit, SD: standard deviation; μ: Mean |
| 7. | CpL | μ – LSL/3*SD, USL: upper specification limit, LSL: lower specification limit, SD: standard deviation; μ: Mean |
| 8. | Skewness | $\gamma_1 = \mathrm{E}\left[\left(\frac{X-\mu}{\sigma}\right)^3\right] = \frac{\mu_3}{\sigma^3} = \frac{\mathrm{E}\left[(X-\mu)^3\right]}{(\mathrm{E}\left[(X-\mu)^2\right])^{3/2}} = \frac{\kappa_3}{\kappa_2^{3/2}},$ |
| 9. | ketosis | $\operatorname{Kurt}\left(\sum_{i=1}^{n} X_i\right) = \frac{1}{n^2}\sum_{i=1}^{n}\operatorname{Kurt}(X_i),$ |
| 10. | Confidence Interval | Mean ± t * s / √N; s = standard deviation; N = sample size; t = Constant from t-distribution table |
| 11. | Total Yield (%) | Actual Yield/Expected Yield * 100 |

## 3. Results and Discussions
### 3.1 Blend Analysis
#### 3.1.1 Blend Uniformity Studies

**Table 4:** Blend uniformity studies

| Artesunate: 90.0 – 110.0 %; Amodiaquine: 90.0 – 110.0 % | | | | | | | |
|---|---|---|---|---|---|---|---|
| No. | Sampling Point | Mixing Time = 3 minutes | | | | | |
| | | Artesunate | | | Amodiaquine | | |
| | | Batch 1 | Batch 2 | Batch 3 | Batch 1 | Batch 2 | Batch 3 |
| 1 | $T_1$ | 97.91 | 103.92 | 98.49 | 98.47 | 100.65 | 99.73 |
| 2 | $T_2$ | 99.78 | 101.01 | 101.18 | 99.23 | 101.87 | 99.73 |
| 3 | $T_3$ | 102.35 | 101.38 | 96.98 | 98.47 | 97.72 | 99.58 |
| 4 | $T_4$ | 103.2 | 99.68 | 98.4 | 100.18 | 101.67 | 99.44 |
| 5 | $T_5$ | 102.02 | 106.23 | 97.85 | 98.93 | 100.23 | 99.33 |
| 6 | $B_1$ | 102.94 | 106.99 | 106.31 | 99.29 | 98.57 | 99.47 |
| 7 | $B_2$ | 100.6 | 97.97 | 97.95 | 98.42 | 98.52 | 99.87 |
| 8 | $B_3$ | 102.24 | 98.54 | 104.97 | 98.77 | 98.73 | 100.16 |
| 9 | $B_4$ | 103.99 | 104.5 | 97.35 | 98.48 | 98.74 | 99.82 |
| 10 | $B_5$ | 98.92 | 104.99 | 102.88 | 98.60 | 101.12 | 99.36 |
| CI at 99 % | | 101.40 | 102.52 ± | 100.24 | 98.88 | 99.78 | 99.65 |
| Probability level | | ± 2.05 | 3.32 | ± 3.48 | ± 0.57 | ± 1.54 | ± 0.27 |

#### 3.1.1.1 Graphical Representation of Regression Analysis for Blend Uniformity Studies

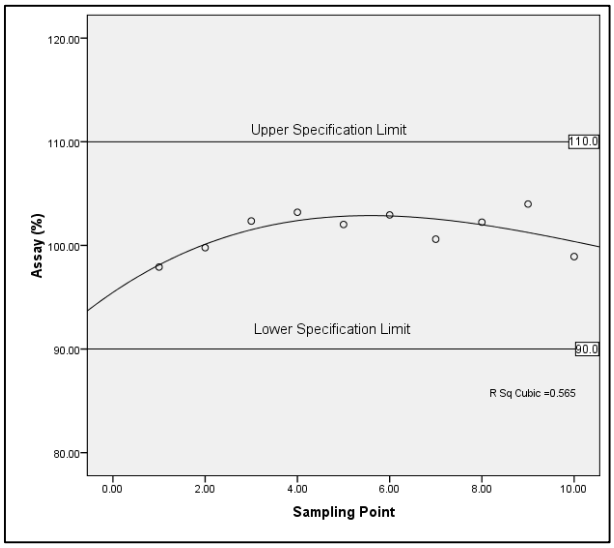


**Fig 4:** Regression analysis for Batch 1(Art)

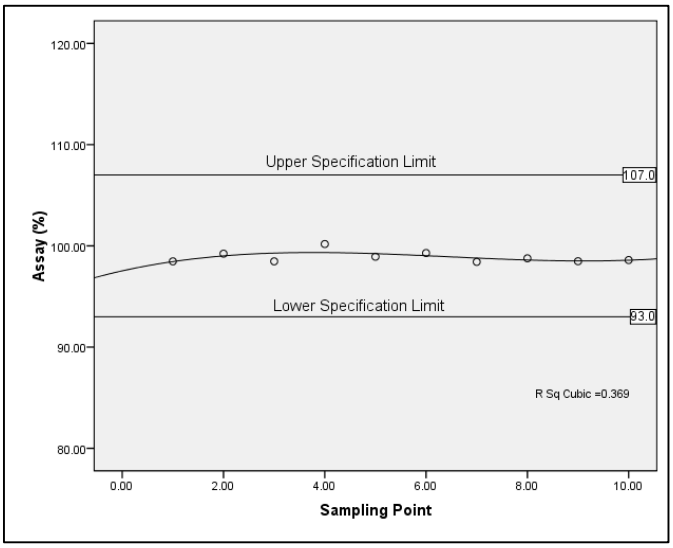


**Fig 5:** Regression analysis for Batch 1(Amod)

Regression Analysis (Cubic) and Test for Normality on each batch was performed. Null Hypothesis: The data are normally distributed. The null hypothesis is rejected if the p-value is below 0.05 [5]. A histogram for each Batch was plotted. Also included was Boxplot for each batch showing the Content Distribution of Artesunate and Amodiaquine. Skewness and Kurtosis were determined. For normal distribution, Kurtosis = 3.0. If Kurtosis is greater than 3.0, it is heavily tailed. If Kurtosis is less than 3.0, data set is slightly tailed.

Skewness: If the skewness is between -0.5 and 0.5, the data are fairly symmetrical. If the skewness is between -1 and –0.5 or between 0.5 and 1, the data are moderately skewed. If the skewness is less than -1 or greater than 1, the data are highly skewed [6].

#### 3.1.1.2 Statistical Data for Blend Uniformity

**Table 5:** Statistical Descriptives of Test for Normality (Blend Uniformity of Artesunate)

| | | Batch 1 | | Batch 2 | | Batch 3 | |
|---|---|---|---|---|---|---|---|
| | | Statistic | Std. Error | Statistic | Std. Error | Statistic | Std. Error |
| Mean | | 1.0140E2 | 0.63164 | 1.0252E2 | 1.02152 | 1.0024E2 | 1.07215 |
| 95% Confidence Interval for Mean | Lower Bound | 99.9661 | | 1.0021E2 | | 97.8106 | |
| | Upper Bound | 1.0282E2 | | 1.0483E2 | | 1.0266E2 | |
| 5% Trimmed Mean | | 1.0144E2 | | 1.0253E2 | | 1.0008E2 | |
| Median | | 1.0213E2 | | 1.0265E2 | | 98.4450 | |
| Variance | | 3.990 | | 10.435 | | 11.495 | |
| Std. Deviation | | 1.99741 | | 3.23032 | | 3.39043 | |
| Minimum | | 97.91 | | 97.97 | | 96.98 | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Maximum | 103.99 | | 106.99 | | 106.31 | |
| Range | 6.08 | | 9.02 | | 9.33 | |
| Interquartile Range | 3.44 | | 5.90 | | 5.68 | |
| Skewness | -0.583 | 0.687 | -0.080 | 0.687 | 0.905 | 0.687 |
| Kurtosis | -0.837 | 1.334 | -1.540 | 1.334 | -0.746 | 1.334 |

**Table 6:** Tests of Normality

| **Specification: Null hypothesis is rejected if p-value is less than 0.05 for Shapiro-Wilk test. p-value is labeled as "sig" in SPSS.** | | | | | | |
|---|---|---|---|---|---|---|
| | **Shapiro-Wilk** | | | | | |
| | Artesunate | | | Amodiaquine | | |
| | Statistic | df | Sig. | Statistic | df | Sig. |
| Batch 1 | 0.936 | 10 | 0.509 | 0.814 | 10 | 0.021 |
| Batch 2 | 0.936 | 10 | 0.504 | 0.899 | 10 | 0.215 |
| Batch 3 | 0.843 | 10 | 0.048 | 0.943 | 10 | 0.584 |

**3.1.1.2.1** Histogram and Normal Distributive Curve showing the Content Distribution

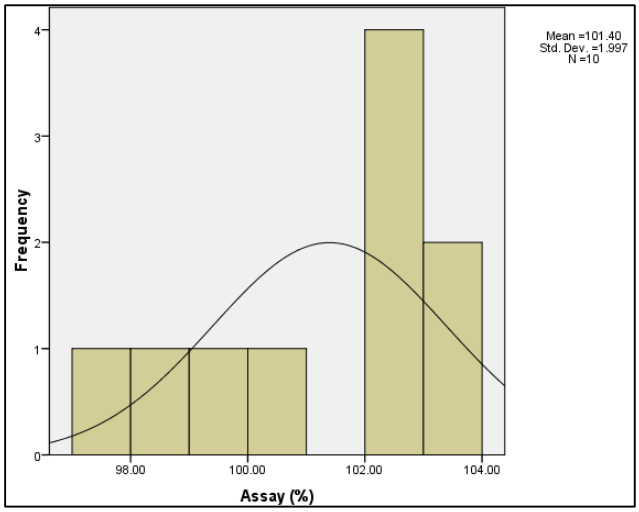


**Fig 6:** Normal distributive curve for Batch 1 of (Art)

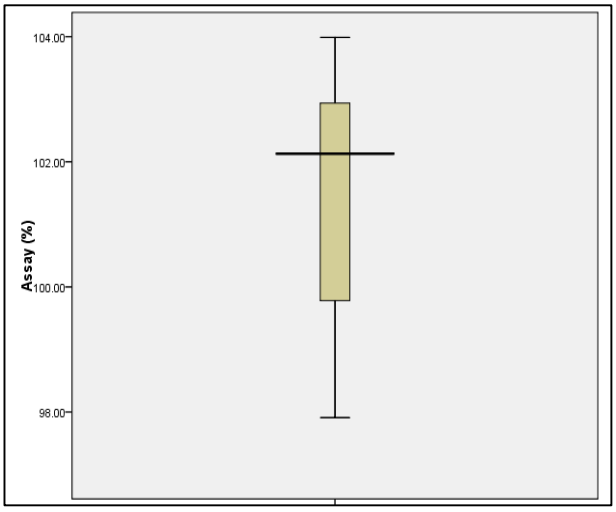


**Fig 7:** Boxplot for Batch 1 of (Art)

**Artesunate**

The p-values for Batches 1, 2 and 3 were 0.509, 0.504 and 0.048 respectively. The null hypothesis is accepted for Batches 1 and 2 as their p-values are greater than 0.05 for the Shapiro-Wilk test. For Batch 3, the p-value of 0.048 implies the batch fell slightly short of significance (p>0.0167).

In terms of skewness, data for batches 1, 2 and 3 were -0.583, -0.080 and 0.905 respectively. Batch 3 is between 0.5 and 1 implying that, the data is moderately skewed. Batch 1 is between-1 and – 0.5 implying that the data is moderately skewed. Batch 2 is between -0.5 and 0.5 implying that, the data is fairly symmetrical. For kurtosis, batches 1, 2 and 3 were -0.837, -1.540 and -0746 respectively which are all less than 3 implying that, all the sets of data had lighter tails.

**Amodiaquine**

The p-values for Batches 1, 2 and 3 were 0.457, 0.057 and 0.690 respectively. The null hypothesis is accepted as all batches are greater than 0.05 for the Shapiro-Wilk test. This implies the data for all batches are not numerically significant. In terms of skewness, data for Batches 1, 2 and 3 were 1.579, 0.170 and 0.606 respectively. Batch 1 is greater than 1 that, the data is highly skewed. Batch 2 is between -0.5 and 0.5 implying that the data is moderately skewed. Batch 3 is between 0.5 and 1 implying that, the data is moderately symmetrical.

For kurtosis, batches 1, 2 and 3 were 2.519, -1.703 and -0.091 respectively which are all less than 3 implying that, all the sets of data had lighter tails.

**3.1.1.3 Inter-batch Analysis**

Null Hypothesis: There are no significant differences between batches 1, 2 and 3 at 0.1 % significance level for Artesunate and Amodiaquine.

**Table 7:** Results of inter-batch analysis of Artesunate

| **Specification: F– calculated for inter batch samples should be NMT 3.18 at 95 % confidence level; RSD %: NMT 5.00 %** | | | | | | | |
|---|---|---|---|---|---|---|---|
| No. | **Sampling Point** | **Artesunate** | | | | | |
| | | **Batch 1** | **Batch 2** | **Batch 1** | **Batch 3** | **Batch 2** | **Batch 3** |
| 1 | $T_1$ | 97.91 | 103.92 | 97.91 | 98.49 | 103.92 | 98.49 |
| 2 | $T_2$ | 99.78 | 101.01 | 99.78 | 101.18 | 101.01 | 101.18 |
| 3 | $T_3$ | 102.35 | 101.38 | 102.35 | 96.98 | 101.38 | 96.98 |
| 4 | $T_4$ | 103.2 | 99.68 | 103.2 | 98.4 | 99.68 | 98.4 |
| 5 | $T_5$ | 102.02 | 106.23 | 102.02 | 97.85 | 106.23 | 97.85 |
| 6 | $B_1$ | 102.94 | 106.99 | 102.94 | 106.31 | 106.99 | 106.31 |
| 7 | $B_2$ | 100.6 | 97.97 | 100.6 | 97.95 | 97.97 | 97.95 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 8 | $B_3$ | 102.24 | 98.54 | 102.24 | 104.97 | 98.54 | 104.97 |
| 9 | $B_4$ | 103.99 | 104.5 | 103.99 | 97.35 | 104.5 | 97.35 |
| 10 | $B_5$ | 98.92 | 104.99 | 98.92 | 102.88 | 104.99 | 102.88 |
| SD | | 2.00 | 3.23 | 2.00 | 3.39 | 3.23 | 3.39 |
| Variance | | 3.99 | 10.43 | 3.99 | 11.49 | 10.43 | 11.49 |
| RSD % | | 1.97 | 3.15 | 1.97 | 3.38 | 3.15 | 3.38 |
| F-test at 0.1 % significance level | | 2.62 | | 2.88 | | 1.10 | |

For Artesunate, the F-calculated values of 2.62, 2.88 and 1.10 were lower than the F-tabulated value of 10.11 at 0.1 % significance level indicating insignificant differences between the batches.
For Amodiaquine, the F-calculated values of 7.25, 4.50 and 8.32 were lower than the F-tabulated value of 10.11 at 0.1 % significance level indicating insignificant differences between the batches.

### 3.1.1.4 Blend Characterization and Flowability Properties

A Bulk and tap density apparatus was used to determine the following parameters, bulk density, tap density, compressibility index and hausner ratio [7].

**Table 8:** Scale of Flowability

| Compressibility index (%) | Flow Character | Hausner Ratio |
|---|---|---|
| ≤ 10 | Excellent | 1.00 – 1.11 |
| 11 – 15 | Good | 1.12 – 1.18 |
| 16 – 20 | Fair | 1.19 – 1.25 |
| 21 – 25 | Passable | 1.26 – 1.34 |
| 26 – 31 | Poor | 1.35 – 1.45 |
| 32 – 37 | Very poor | 1.46 – 1.59 |
| > 38 | Very, very poor | > 1.60 |

**Artesunate**

The compressibility indices of Batches 1, 2 and 3 were 14.943 ± 1.275, 15.454 ± 0.161 and 14.506 ± 0.122 respectively. This implies that the compressibility characteristics of all the batches were good (11-15). Hausner ratio values for Batches 1, 2 and 3 were 1.176 ± 0.018, 1.182 ± 0.002 and 1.171 ± 0.002 respectively. This implies that the flow character for all the batches were good (1.12 – 1.18). Bulk and tap densities were used to predict the flow and the compressibility character of the powders.

**Amodiaquine**

The compressibility indices of Batches 1, 2 and 3 were 11.969±0.078, 13.651±0.063 and 13.860±0.040 respectively. This implies that the compressibility characteristics of all the batches were good (11-15). Hausner ratio values for Batches 1, 2 and 3 were 1.133±0.001, 1.161±0.008 and 1.140±0.011 respectively. This implies that the flow character for all the batches were good (1.12 – 1.18). Bulk and tap densities were used to predict the flow and the compressibility character of the powders.

### 3.1.1.5 Loss on Drying (LOD) (%)

LOD (%) for the granules was determined after 3 minutes of mixing with sampling from all 10 sampling points. Data was recorded and regression analysis was performed on the data obtained for each batch.
For Artesunate, the Loss on drying gave the following values for Batches 1, 2 and 3 respectively; 1.41 ± 0.44 %, 1.30 ± 0.17 % and 0.98 ± 0.07 %. These values are less than the specified upper limit of 5.00 %. For Amodiaquine, the Loss on drying gave the following values for Batches 1, 2 and 3 respectively; 1.51 ± 0.10 %, 1.53 ± 0.11 % and 1.86 ± 0.07 %. These values are less than the specified upper limit of 2.00 %.

#### 3.1.1.5.1 Graphical Representation for LOD (%)

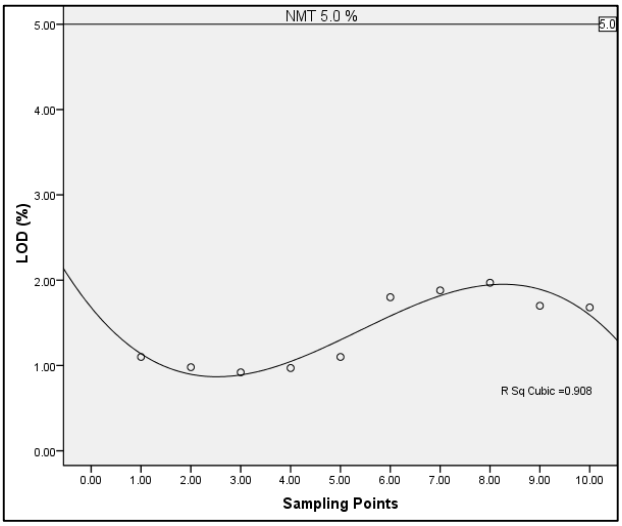


**Fig 8:** Regression analysis of LOD (Art, Batch 1)

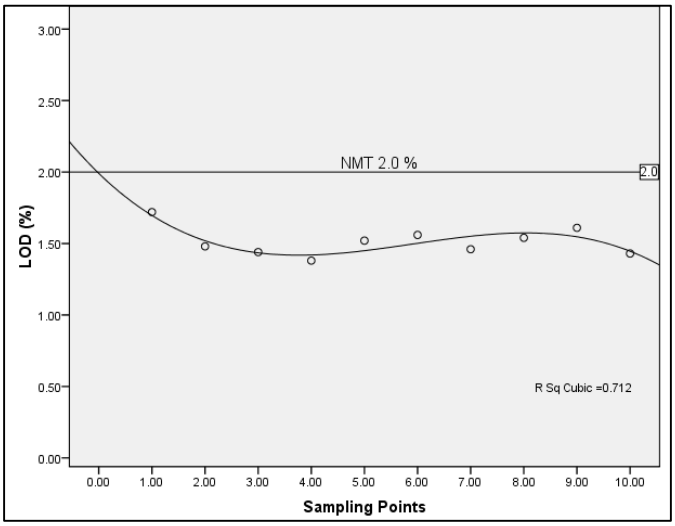


**Fig 9:** Regression analysis of LOD (Amod, Batch 1)

### 3.1.1.6 Resistance to Segregation Studies

Statistical evaluation of Data obtained from holding drums as against data from Blend Uniformity studies was performed for statistical differences. T-test (Two tailed test); Critical value at 99 % probability level = 9.925.
**Null Hypothesis:** There is no significant difference between the assay results obtained from the blend uniformity studies and those obtained from the holding drums.

**Table 9:** Assay results of holding drums for Artesunate

| Holding Drum | Batch No. 1 | Batch Bo. 2 | Batch No. 3 |
|---|---|---|---|
| Drum 1 | 99.51 | 101.55 | 104.52 |

**Table 10:** Mean values of sampling positions1 for Artesunate

| Sampling Positions | Batch No. 1 | Batch No. 2 | Batch No. 3 |
|---|---|---|---|
| $T_1$ | 97.91 | 103.92 | 98.49 |
| $B_1$ | 102.94 | 106.99 | 106.31 |
| Mean ± SEM | 100.43±2.52 | 105.46±1.53 | 102.40±3.91 |
| $T_2$ | 99.78 | 101.01 | 101.18 |
| $B_2$ | 100.6 | 97.97 | 97.95 |
| Mean ± SEM | 100.19±0.41 | 99.49±1.52 | 99.57±1.62 |
| $T_3$ | 102.35 | 101.38 | 96.98 |
| $B_3$ | 102.24 | 98.54 | 104.97 |
| Mean ± SEM | 102.30±0.05 | 99.96±1.42 | 100.98±4.00 |
| $T_4$ | 103.20 | 99.68 | 98.4 |
| $B_4$ | 103.99 | 104.5 | 97.35 |
| Mean ± SEM | 103.60±0.40 | 102.09±2.41 | 97.88±0.53 |
| $T_5$ | 102.02 | 106.23 | 97.85 |
| $B_5$ | 98.92 | 104.99 | 102.88 |
| Mean ± SEM | 100.47±1.55 | 105.61±0.62 | 100.37±2.51 |

**Table 11:** Statistical evaluation of Data obtained from holding drums as against data from Blend Uniformity (Artesunate)

| Specification: T-critical value = 9.925 at 99 % probability level. | | | | | | |
|---|---|---|---|---|---|---|
| | Artesunate Batch 1 | | | | | |
| | Drum 1 | $T_1$ and $B_1$ | $T_2$ and $B_2$ | $T_3$ and $B_3$ | $T_4$ and $B_4$ | $T_5$ and $B_5$ |
| Mean Assay (%) | 99.51 | 100.43 | 100.19 | 102.30 | 103.60 | 100.47 |
| T-Test | | 0.414 | 0.884 | 4.886 | 2.208 | 0.540 |
| Artesunate Batch 2 | | | | | | |
| Mean Assay (%) | 101.55 | 105.46 | 99.49 | 99.96 | 102.09 | 105.61 |
| T-Test | | 1.095 | 0.799 | 0.727 | 0.325 | 1.757 |
| Artesunate Batch 3 | | | | | | |
| Mean Assay (%) | 104.52 | 102.40 | 99.57 | 100.98 | 97.88 | 100.37 |
| T-Test | | 0.506 | 1.203 | 0.647 | 2.443 | 0.882 |

T-test was carried out to prove that, there is no significant difference between the assay results obtained from the analysis of the blend transferred into the holding drums and the assay results obtained at the various sampling positions during the uniformity of blend studies. The t-values were less than the t-critical (tabulated) value of 9.925 at 99 % probability level. Hence, there is no significant difference between the mean assay results. The null hypothesis is therefore accepted implying that, no segregation takes place when blends are transferred into holding drums.

For Amodiaquine, T-test was carried out to prove that, there is no significant difference between the assay results obtained from the analysis of the blend transferred into the holding drums and the assay results obtained at the various sampling positions during the uniformity of blend studies. The t-values were less than the t-critical (tabulated) value of 9.925 at 99 % probability level. Hence, there is no significant difference between the mean assay results. The null hypothesis is therefore accepted implying that, no segregation takes place when blends are transferred into holding drums.

### 3.1.1.7 Blend Hold-Time studies

**Table 12:** Results from Blend hold-time studies for Artesunate

| Specification: Must be within specifications for Appearance, Assay, LOD, Bulk density, tap density, Compressibility index and Hausner ratio. | | | | | | |
|---|---|---|---|---|---|---|
| Test Parameter | Study time: Initial | | | Study time: 30th Day | | |
| | Batch No. 1 | Batch No. 2 | Batch No. 3 | Batch No. 1 | Batch No. 2 | Batch No. 3 |
| Appearance | Granular | Granular | Granular | Granular | Granular | Granular |
| Assay (%) | 102.35 | 104.68 | 97.85 | 101.29 | 102.84 | 98.08 |
| LOD (%) | 1.20 | 1.18 | 0.99 | 1.17 | 1.21 | 1.08 |
| Bulk Density | 0.551 | 0.546 | 0.554 | 0.561 | 0.558 | 0.564 |
| Tap Density | 0.647 | 0.645 | 0.646 | 0.645 | 0.646 | 0.648 |
| Comp. Index | 14.835 | 15.217 | 14.285 | 14.845 | 15.381 | 14.981 |
| Hausner Ratio | 1.174 | 1.179 | 1.166 | 1.149 | 1.158 | 1.149 |

A simulated container was adopted for the blend holding time studies. The blends were held for one month in a polyethylene rubber inserted in High Density Poly Ethylene (HDPE) bottles. Assay, LOD, Bulk density, Compressibility index, Hausner ratio and Appearance were the parameters tested at initial and at the 30th day. Results show insignificant differences between results obtained at initial and those obtained on the 30th day. Thus, holding the blend for a period

of one month in HDPE drums and also at the required storage condition (below 30 ºC and NMT 60 % RH) will insignificantly affect the blend with respect to the tested parameters

### 3.1.1.8 Tablet Compression Process Performance and Product Quality Studies

Tests for Hardness, Thickness and Diameter were performed on the compressed tablets at beginning, middle and end of the compression process for all three batches of Artesunate and Amodiaquine. With Artesunate have a specification of Not less than 2.5 for hardness, all tested tablets were within specification and that of Amodiaquine were also within specification of Not less than 1.5. For Thickness test, the specification for Artesunate was 4.30 – 4.50 mm and all tested tablets were within specification. For diameter, results were within specification of 9.96 – 10.02 for Artesunate and 12.76 – 12.82 for Amodiaquine.

### 3.1.1.9 Uniformity of Dosage Unit (Weight Variation (WV))

**Artesunate**

Dose and ratio of Drug substance implies ≥25 mg and ≥25% [8]

**Table 13:** Assay Results (Artesunate)

| Batch | Average weight (mg) of 20 Tablets | Assay (%) |
|---|---|---|
| Batch No. 1 | 355.0 | 103.65 |
| Batch No. 2 | 354.0 | 106.98 |
| Batch No. 3 | 352.0 | 103.72 |

**Table 14:** Sample Uniformity of Dosage Unit (Batch No. 1; 10 Determinations)

| Specification: AV of first 10 dosage units is ≤ L1 %; L1 = 15 | | | | | | |
|---|---|---|---|---|---|---|
| Batch No. 1 (Assay =103.65) | | | | | | |
| Beginning | | | Middle | | End | |
| | Individual Weights (mg) | Individual Estimated Content (%) | Individual Weights (mg) | Individual Estimated Content (%) | Individual Weights (mg) | Individual Estimated Content (%) |
| Mean ± SEM | 350.4 ± 1.25 | 105.55 ± 0.36 | 348.2 ± 1.36 | 101.66 ± 0.4 | 350.3 ± 0.63 | 102.28 ± 0.18 |
| SD | 3.95 | 1.15 | 4.29 | 1.25 | 2 | 0.58 |
| RSD % | - | 1.13% | - | 1.23% | - | 0.57% |
| AV = IM-XI + ks; k = 2.4; SD = 1.19 ; X = 105.55; T = 100 %; M(Case 1); X > 101.5 %, AV = X – 101.5 + ks | | | AV = IM-XI + ks; k = 2.4; SD = 0.41 ; X = 104.89; T = 100 %; M(Case 1); X > 101.5 %, AV = X – 101.5 + ks | | AV = IM-XI + ks; k = 2.4; SD = 0.60; X = 105.52; T = 100 %; M(Case 1); X > 101.5 %, AV = X – 101.5 + ks | |
| AV = 3.57 | | | AV = 3.17 | | AV = 2.18 | |

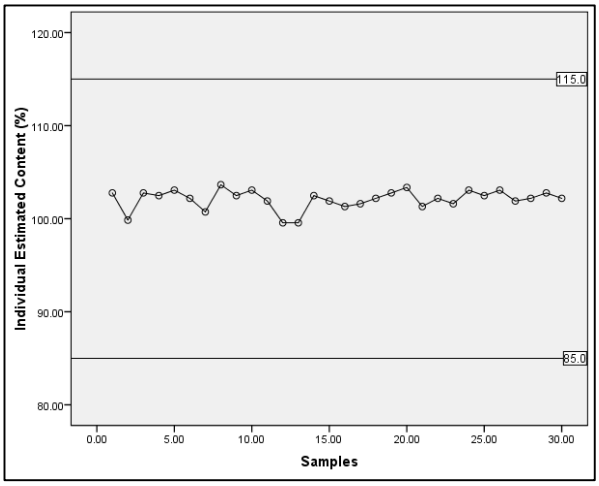


**Fig 10:** Regression analysis for Art Batch 1 (WV)

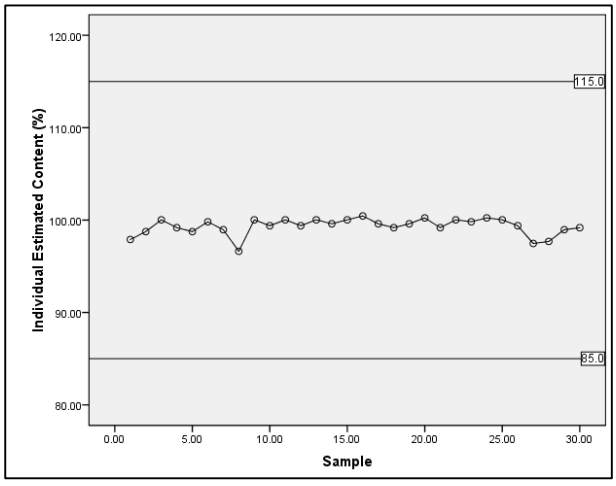


**Fig 11:** Regression analysis for Amod Batch 1 (WV)

**Artesunate**

Acceptance values for Batch 1 at the beginning, middle and end of compression were 3.57, 3.17 and 2.18 respectively. Those of Batch 2 at the beginning, middle and end of compression were 7.11, 6.96 and 5.70 respectively whereas Batch 3 at the beginning, middle and end of compression were 3.28, 4.01 and 3.10 respectively. The acceptance values are all less than L1 = 15.0 [8] which indicates consistent uniformity in the dosage units of all the batches.

**Amodiaquine**

Acceptance values for Batch 1 at the beginning, middle and end of compression were 2.52, 0.96 and 2.29 respectively. Those of Batch 2 at the beginning, middle and end of compression were 2.17, 1.15 and 1.31 respectively whereas Batch 3 at the beginning, middle and end of compression were 2.00, 3.01 and 1.83 respectively. The acceptance values are all less than L1 = 15.0 which indicates consistent uniformity in the dosage units of all the batches.

## 3.2 Finished Products Analysis

### 3.2.1 Quality Control Tests

**Table 15:** Complete analysis of Compressed Tablets (Artesunate)

| No. | Test | Specification | Reference | Result | | |
|---|---|---|---|---|---|---|
| | | | | Batch 1 | Batch 2 | Batch 3 |
| 1 | Identification By HPLC | The retention time of Artesunate in Chromatograms obtained from both standard and test solutions are comparable. | United States Pharmacopeia (USP) | Complies | Complies | Complies |
| 2 | Weight variation | Not more than 2 of the individual masses deviates from average mass by more than the percentage deviation of 7.5 % and none deviates by more than 15 %. | British Pharmacopeia (BP) | Complies | Complies | Complies |
| 3 | Disintegration | Not more than 15 minutes | In-House (IH) | 5 | 4 | 4 |
| 4 | Hardness (Kp) | Not Less than 2.5 | IH | 5.46 | 4.86 | 4.47 |
| 5 | Average weight (mg) | 332.50 – 367.50 (mg) | IH | 349 | 351 | 351 |
| 6 | Loss on Drying (by moisture analyzer at 105 $^{o}$C) | Not more than 5.00 % | IH | 1.68 | 1.61 | 1.20 |
| 7 | Friability | Not more than 1.00 % | BP | 0.56 | 0.59 | 0.68 |
| 8 | Assay | 90.0 % - 110.0 % | Ph. Int | 106.59 | 106.76 | 103.11 |
| 9 | Dissolution | Not less than 75 % (Q) | Ph. Int | 86.49 | 90.96 | 95.28 |

**Table 16:** Complete analysis of Compressed Tablets (Amodiaquine)

| No. | Test | Specification | Reference | Result | | |
|---|---|---|---|---|---|---|
| | | | | Batch 1 | Batch 2 | Batch 3 |
| 1 | Identification<br>a) By UV<br>Or<br>b) By HPLC | The spectra of Amodiaquine obtained from both test and standard solutions are comparable<br>The retention times of Amodiaquine in both chromatograms obtained from both standard and test solutions are comparable | IH<br>USP | Complies | Complies | Complies |
| 2 | Weight variation | Not more than 2 of the individual masses deviates from average mass by more than the percentage deviation of 7.5 % and none deviates by more than 15 %. | BP | Complies | Complies | Complies |
| 3 | Disintegration | Not more than 15 minutes | USP | 4 | 4 | 6 |
| 4 | Hardness (Kp) | Not Less than 1.5 | IH | 6.80 | 5.35 | 4.91 |
| 5 | Average weight (mg) | 455.70 – 474.30 (mg) | IH | 464 | 468 | 461 |
| 6 | Loss on Drying (by moisture analyzer at 105 $^{o}$C) | Not more than 2.00 % | IH | 1.63 | 1.56 | 1.75 |
| 7 | Friability | Not more than 1.00 % | BP | 0.52 | 0.53 | 0.42 |
| 8 | Assay | 93.0 % - 107.0 % | USP | 98.79 | 98.56 | 99.48 |
| 9 | Dissolution | Not less than 75 % (Q) | USP | 96.94 | 97.41 | 94.47 |

### 3.2.2 Process Capability

**Table 17:** Process Capability Index of Batches 1, 2 and 3 of Artesunate and Amodiaquine with sampling at beginning middle and End of process

| Specifications: Cpk< 1 -not capable; Cpk= 1 -marginally capable; Cpk> 1 -capable [9] | | | | | | |
|---|---|---|---|---|---|---|
| | Artesunate | | | Amodiaquine | | |
| | Hardness Test | Thickness | Diameter | Hardness Test | Thickness | Diameter |
| μ ± SEM | 5.57 ± 0.09 | 4.41 ± 0.01 | 10.00 ± 0.01 | 6.68 ± 0.12 | 4.60 ± 0.01 | 12.01 ± 0.01 |
| SD | 0.89 | 0.10 | 0.10 | 1.14 | 0.11 | 0.08 |
| LSL | 2.5 | 3.00 | 8.5 | 2 | 3.50 | 11.00 |
| 3*sd | 2.67 | 0.29 | 0.29 | 3.41 | 0.32 | 0.23 |
| CpL | 1.15 | 4.85 | 5.25 | 1.37 | 3.39 | 4.49 |

**Table 18:** Process capability index for Uniformity of Dosage Units (Art); 10 Determinations

| | Art | Amod |
|---|---|---|
| SD | 1.40 | 0.94 |
| LSL | 85 | 85 |
| USL | 115 | 115 |
| 6SD | 8.40 | 5.64 |
| Cp | 3.57 | 5.32 |

**Artesunate**

Process capability indices for Hardness, Thickness, Diameter and Uniformity of Dosage units tests were 1.15, 4.85, 5.25 and 3.57 respectively, which are greater than 1. This implies that, the manufacturing process is reproducible as well as capable of consistently delivering quality products.

**Amodiaquine**

Process capability indices for Hardness, Thickness, Diameter and Uniformity of Dosage units tests were 1.37, 3.39, 4.49 and 5.32 respectively, which are greater than 1. This implies that, the manufacturing process is reproducible as well as capable of consistently delivering quality products.

### 3.2.3 Yield Analysis

**Table 19:** Yield Analysis of Artesunate

| Parameter | Granulation (95 % - 105 %) (IH) | | | Compression (95 - 105 %) (IH) | | |
|---|---|---|---|---|---|---|
| | Batch 1 | Batch 2 | Batch 3 | Batch 1 | Batch 2 | Batch 3 |
| Expected Yield | **Artesunate** | | | | | |
| | 35.00 | 35.00 | 35.00 | 100000 | 100000 | 100000 |
| | **Amodiaquine** | | | | | |
| | 46.41 | 46.50 | 46.50 | 100000 | 100000 | 100000 |
| Actual yield | **Artesunate** | | | | | |
| | 34.84 | 35.06 | 35.08 | 96800 | 95485 | 97314 |
| | **Amodiaquine** | | | | | |
| | 45.41 | 46.02 | 45.64 | 95763 | 96817 | 96623 |
| % Yield / Reconciliation | **Artesunate** | | | | | |
| | 99.54 | 100.17 | 100.23 | 96.80 | 95.49 | 97.31 |
| | **Amodiaquine** | | | | | |
| | 97.85 | 98.97 | 98.15 | 95.73 | 96.82 | 96.62 |

The percentage yields obtained from the compression and granulation stages for all three batches were within specification for both Artesunate and Amodiaquine.

## 4. Conclusion

Results obtained indicated that, there was an acceptable level of homogeneity within a batch and consistency between batches. All critical variables are therefore valid indicating that, the manufacturing processes for Artesunate and Amodiaquine tablets had been robustly designed enough to meet predetermined standards and quality attributes. The manufacturing process as a result is capable and stable to assure quality and safe products. Quality control tests carried out on the compressed tablets of Artesunate and Amodiaquine were within the acceptance criteria for Identification, Weight variation, Disintegration, Friability, Assay, Hardness, Dissolution, Average weight and Loss on Drying.

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