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COMPARATIVE STUDY OF THE POTENCY OF ANTIMALARIAL DRUGS USED ON MALE AND FEMALE PATIENTS USING BIOASSAY ANALYSIS METHOD

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ABSTRACT

Background: Bioassay remains essential for evaluating antimalarial drug potency in resource-limited settings, particularly for Plasmodium malariae infections which contribute significantly to malaria morbidity in sub-Saharan Africa. Establishing parallelism between dose-response curves is a critical prerequisite for valid relative potency estimation.

Objective: This study assessed the potency of four generic antimalarial brands (Drugs A, B, C, D) relative to a standard formulation using clinical recovery time data with gender-stratified analysis.

Methods: An indirect assay design measured time to recovery (hours) post-treatment. Parallelism was tested using F-test, χ^2-test, and equivalence testing via JMP software version 17. A 4-parameter logistic (4PL) model characterized dose-response relationships. One-way ANOVA assessed formulation variation, with bioequivalence declared when 90% confidence intervals for growth rate, inflection point, and asymptotes fell within decision limits.

Results: All formulations demonstrated significant variation (ANOVA $p < 0.0001$). However, parallelism testing failed comprehensively: overall F-test ($p = 0.0094$), χ^2-test ($p = 0.0001$); male patients F-test ($p < 0.0001$), χ^2-test ($p = 0.0001$); female patients F-test ($p < 0.0001$), χ^2-test ($p = 0.0001$). Equivalence testing confirmed non-equivalence across all drugs versus standard for all 4PL parameters in both genders. Graphical analysis revealed inconsistent logarithmic dose spacing and variable horizontal shifts (Δ), violating parallelism assumptions.

Conclusion: The consistent failure of parallelism testing across all analyses prevented relative potency estimation, indicating potential degradation of the reference standard, formulation inconsistencies, or assay design flaws. This underscores the critical importance of parallelism validation in bioassays and highlights methodological challenges in antimalarial potency assessment.

KEYWORDS: Bioassay, Parallelism testing, Relative potency, Antimalarial drugs, 4-parameter logistic model, Dose-response analysis.

INTRODUCTION

Worldwide malaria cases are estimated at 228 million with 93% cases occurring in sub-Saharan Africa. Malaria accounted for approximately 405,000 deaths globally in 2018.[1] Global efforts toward malaria elimination would be futile if treatments of the neglected non-*falciparum Plasmodium* species, such as *Plasmodium malariae*, are ineffective. *P. malariae* is widespread in sub-Saharan Africa and the southeast area of the Pacific region where its prevalence has surpassed 30%.[2-5] Like *Plasmodium falciparum*, *P. malariae* infection is also reported to be associated with a high burden of anaemia and can result in chronic infection if not well treated. *P. malariae* infections can also cause a high burden of morbidity associated with severe illness and possibly death.[6-7] Therefore, the clinical impacts of *P.*

malariae malaria require that it be given a priority in the context of disease-elimination strategies, e.g. as reported for *Plasmodium vivax.*[8]

A recent report indicated a decline of *P. falciparum* infection in 2016 yet a 2- to 6-fold increase of *P. malariae* and *Plasmodium ovale spp.* infections, respectively, when compared with 2010.[2] Thus, it is important to tackle *P. malariae* in order to reach the elimination milestone. Bioassay is defined as the quantitative estimation of biologically active substances by the amount of their actions in standardized conditions on living organisms or part of organisms. As for the purpose of this study, 'Bio assays' are the methods for the estimation of the nature, constitution, or potency or strength of an 'agent' or 'stimulus' by utilizing the response or effect or reaction caused by its application to biological material or experimental living 'subjects'.[9] A bioassay is an analytical method to determine concentration or potency of a substance by its effect on living cells or tissues. Bioassays are used to estimate the potency of agents by observing their effects on living animals (*in-vivo*) or tissues (*in-vitro*).[10] The best experimental design and the statistical analysis will be impacted by the difference in purpose, even though the experimental technique may be the same as that used in a strictly comparison experiment.[9]

However, the significance of this study will provide valuable information concerning the potency of the drug products. It will also provide techniques for researchers to follow in order to determine the potency or efficacy of malarial products.

METHODOLOGY

Data Source

Secondary data were obtained from an Aligned national MDA programme datasets. Structurally harmonized datasets were used.

Methods of Data Analysis

The ANOVA test was used to check for variation in the different Anti-malarial drugs used for the study with the aid of the JMP software Version 17. In order to check for potency, the dataset was tested for parallelism using the F-test, χ^2- test and equivalence with the aid of the JMP software Version 17.

Parallelism

The parallelism test is found in the Fit Curve platform for Sigmoid Curves using the JMP software. In this study, a dataset from Federal Medical Centre Abakiliki, Ebonyi State was used. A logistic 4 Parameters model was fitted to the different formulations. The curves were analyzed and tested for parallelism.

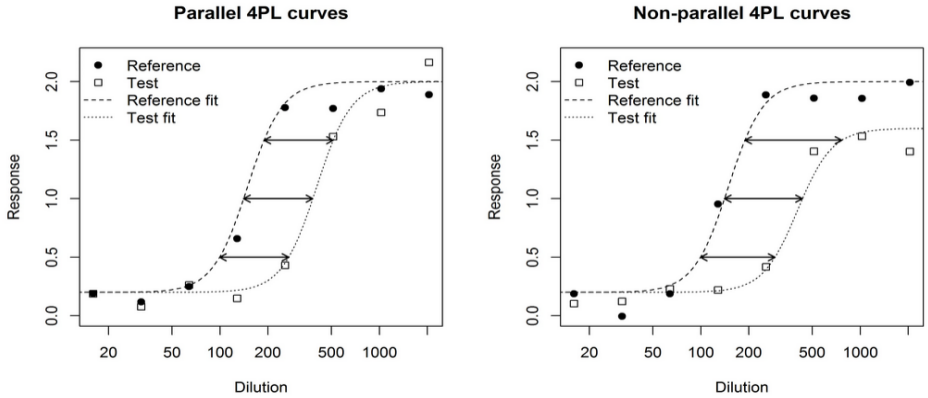

Figure 1: An illustration of parallel (a) and non-parallel (b) 4PL dose-response curves.

Decision Rule for Parallelism

For the concentration response curves to be parallel, if p-value obtained is greater than the critical value of α = 0.05 we conclude that parallelism exists between the pair of concentration response curves of the standard and test samples. Otherwise, parallelism do not exist, hence relative potency cannot be calculated.

χ^2- test

The RSSE (Chi-Square) method in parallelism is a direct measure of the similarity between the weighted residuals of the individual concentration of the two curves. This method measures the difference in the residual sum of squares error (RSSE) of non-parallel curves and parallel curves to the same shape to determine parallelism. This is given as

$$RSSE = RSS_p - RSS_q \quad equ\ 3$$

Equ. 3 follows χ^2_{q-p} distribution only if the model has been correctly weighted such that the variance of the weighted response is 1 across the range of the assay, for both test and standard, and if the response is normally distributed. A set of historical assays must be used to estimate the relationship between the response and its variance; this provides the weighting. The set significance level (usually 5%). Again, the probability of correctly concluding that truly non-parallel curves are non-parallel depends on the true difference to be detected, and on the underlying variance of the data.[11]

Equivalence Test

In the Fit Curve platform, the Equivalence Test option gives an analysis for testing the equivalence of models

across levels of the grouping variable. In the Bioassay data used for this study, five different drugs are compared and tested whether they are equivalent. A Logistic 4P showed a fit for four Parameters (Growth Rate, Inflection Point, Lower and Upper Asymptote).[11] If all the confidence intervals are inside the decision lines, then the two groups are practically equal. If a single interval falls outside the lines, then we cannot conclude that the groups are equal in terms of potency.

Relative Potency for Indirect Assay

In Indirect Assays, the dose is fixed and the response is random; and that response could be a measurement or the occurrence of an event. The Relative Potency in JMP is found below the parallelism test. Five different formulations are compared: Drug A, Drug B, Drug C, Drug D and Standard.

Decision Rule for Relative potency

If the relative potency of Drug A is greater than Drug B been compared. It is concluded that the Drug A is more potent while the Drug B is less potent.

Ethical Consideration

The study used secondary, aggregated programme data with no personal identifiers. Ethical approval was not required as the analysis posed no risk to individuals and complied with accepted standards for public health programme evaluation. And for the purpose of confidentiality, the drug products brand names used for the study were represented with codes such as (Drug A, Drug B, Drug C, Drug D and Standard).

Statistical Software

All analyses were conducted using SPSS and MATHLAB statistical software. Data management and visualization were performed within the same environment to ensure reproducibility.

RESULTS AND DISCUSSION

Relative Potency of Antimalarial Drugs

Table 1: Parallelism F-Test.

Parallel Fit SSE	Full SSE	NDF	DDF	F Ratio	Prob > F
1149.5738	764.47358	12	60	2.519	0.0094*

Source: *JMP software output*

Table 1 revealed that the p-value obtained (0.0094) is less than the critical value of α = 0.05. Thus, we recall from the decision rule of parallelism that if the p-value obtained is less than the critical value of α = 0.05, we conclude that the assay failed the F-test parallelism test which is the case in this study.

Decision Rule

H_0 = No significance different amongst different drug samples if P< a = 0.05, otherwise H_1

H_1 = There is significance amongst different drug samples, atleast one is different.

Conclusion; P <α = 0.05, We reject the H_0.

Table 2: Parallelism Chi-Square Test.

ChiSquare	DF	Prob>ChiSq
385.100	12	<.0001*

Source: JMP software output

Table 2 also revealed that the p-value obtained (0.0001) is less than the critical value of α = 0.05. Thus, we recall from the decision rule of parallelism that if the p-value obtained is less than the critical value of α = 0.05, we conclude that the assay failed the Chi-Square parallelism test which is the case in this study.

Decision Rule

H_0 = No significance different amongst different drug samples if P< a = 0.05, otherwise H_1

H_1 = There is significance amongst different drug samples, atleast one is different.

Conclusion; P <α = 0.05, We reject the H_0.

Parallelism by Gender

Parallelism Test for Male Patients

Table 3: Parallelism F Test for Male Patients.

Parallel Fit SSE	Full SSE	NDF	DDF	F Ratio	Prob > F
362.01849	4.5603962	12	20	130.639	<.0001*

Source: *JMP software output*

Table 3 revealed that the p-value obtained (<.0001*) is less than the critical value of α = 0.05. Thus, we recall from the decision rule of parallelism that if the p-value obtained is less than the critical value of α = 0.05, we conclude that the assay failed the F-test parallelism test which is the case in this study.

Table 4: Parallelism Chi-Square Test.

Chi Square	DF	Prob>ChiSq
357.458	12	<.0001*

Source: *JMP software output*

Table 4 also revealed that the p-value obtained (0.0001) is less than the critical value of α = 0.05. Thus, we recall from the decision rule of parallelism that if the p-value obtained is less than the critical value of α = 0.05, we conclude that the assay failed the Chi-Square parallelism test.

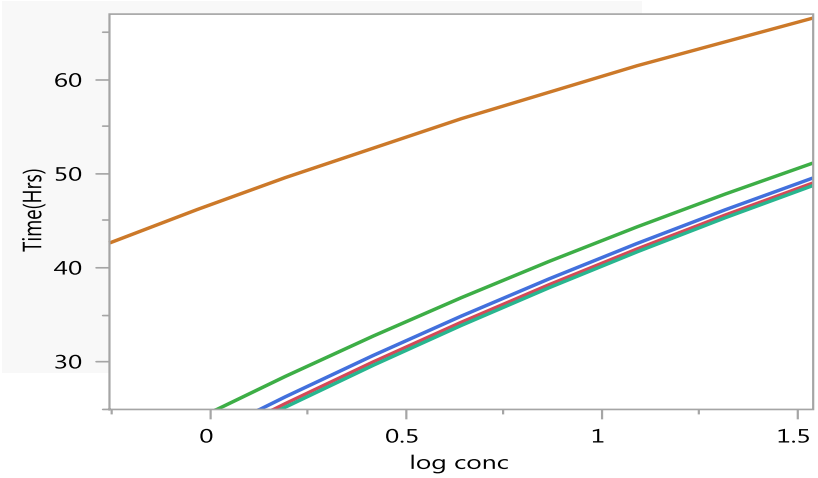

Figure 2: Parallelism Test for Male patients.

(Notice that log conc. horizontal shift Δ is not the same at all doses)

Figure 2 showed the graphical representation of each drug when compared to the standard on male patients. It can be seen clearly that it failed the parallelism test as the number of dose levels of the test and standard preparations are not the same.

Equivalence Test Results for Male Patients

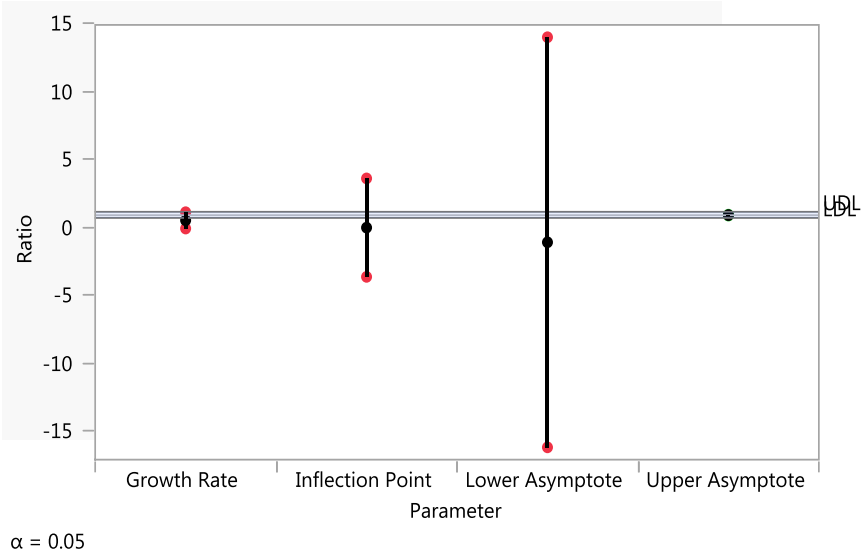

Figure 3: Equivalence between Drug A and standard for Male Patients Figure 3: Equivalence between Drug A and standard for Male Patients.

Figure 3 showed that when drug A was compared to the standard on male patients, it has been demonstrated that Drug A is not equivalent to the standard as the growth rate, inflection points and lower asymptote were not within the acceptable limit.

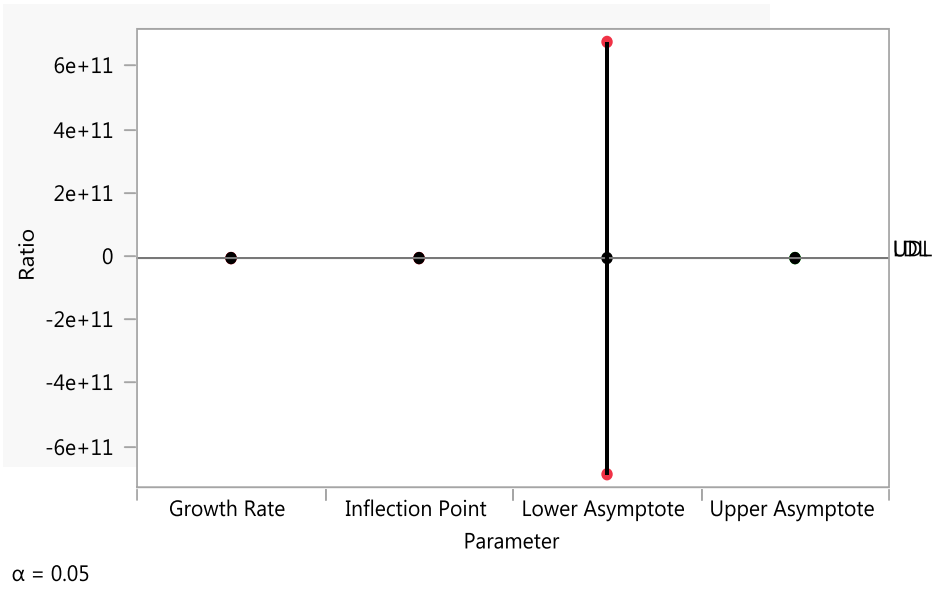

Figure 4: Equivalence between Drug B and standard for Male Patients.

Figure 4 showed that when drug B was compared to the standard on male patients, it revealed that Drug B is not equivalent to the standard as the growth rate, inflection points, Upper and Lower asymptote were not within the acceptable limit.

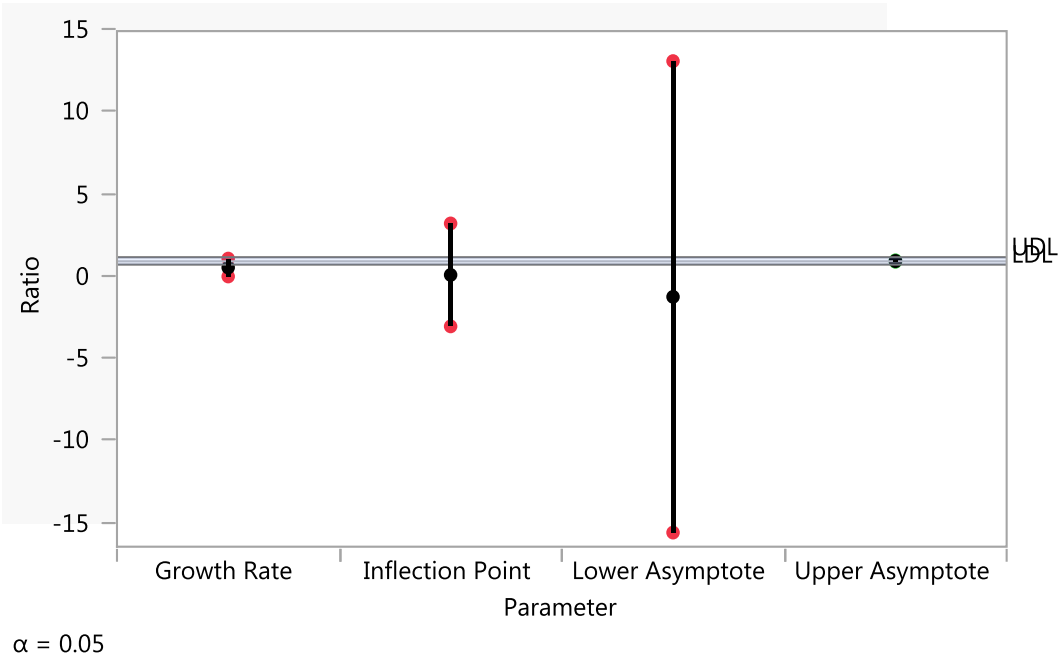

Figure 5: Equivalence between Drug C and standard for Male Patients.

Figure 5 showed that when drug C was compared to the standard on male patients, this test revealed that Drug C is not equivalent to the standard as the growth rate, inflection points, Upper and Lower asymptote were not within the acceptable limit.

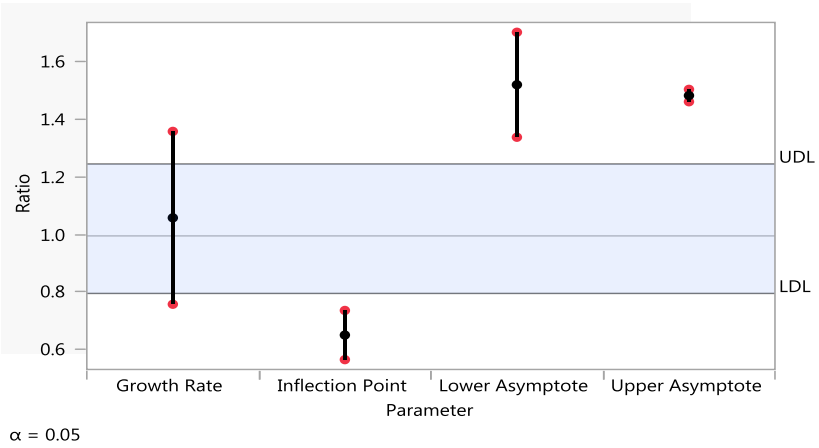

Figure 6: Equivalence between Drug D and standard for Male Patients.

Figure 6 showed that when drug D was compared to the standard on male patients, it revealed that Drug D is not equivalent to the standard as the growth rate, inflection points, Upper and Lower asymptote were not within the acceptable limit.

Parallelism Test for Female Patients

Table 5: Parallelism F Test for Female Patients.

Parallel Fit SSE	Full SSE	NDF	DDF	F Ratio	Prob > F
159.46703	2.8840484	12	20	90.488	<.0001*

Source: *JMP software output*

Table 5 revealed that the p-value obtained (<.0001*) is less than the critical value of α = 0.05. Thus, we recall from the decision rule of parallelism that if the p-value obtained is less than the critical value of α = 0.05, we conclude that the assay failed the F-test parallelism test.

Table 6: Parallelism Chi-Square Test for Female patients.

ChiSquare	DF	Prob>ChiSq
156.583	12	<.0001*

Source: *JMP software output*

Table 6 also showed that the p-value obtained (0.0001) is less than the critical value of α = 0.05. Thus, we recall from the decision rule of parallelism that if the p-value obtained is less than the critical value of α = 0.05, we conclude that the assay failed the Chi-Square parallelism test.

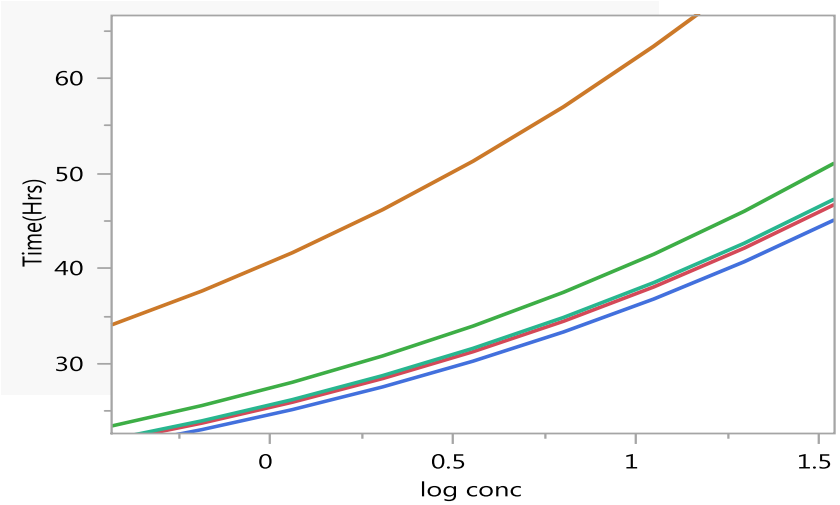

Figure 7: Parallelism Test for Female patients.

(Notice that log conc. horizontal shift Δ is not the same at all doses)

Figure 7 showed the graphical representation of each drug when compared to the standard on female patients. It can be seen clearly that it failed the parallelism test as the number of dose levels of the test and standard preparations are not the same.

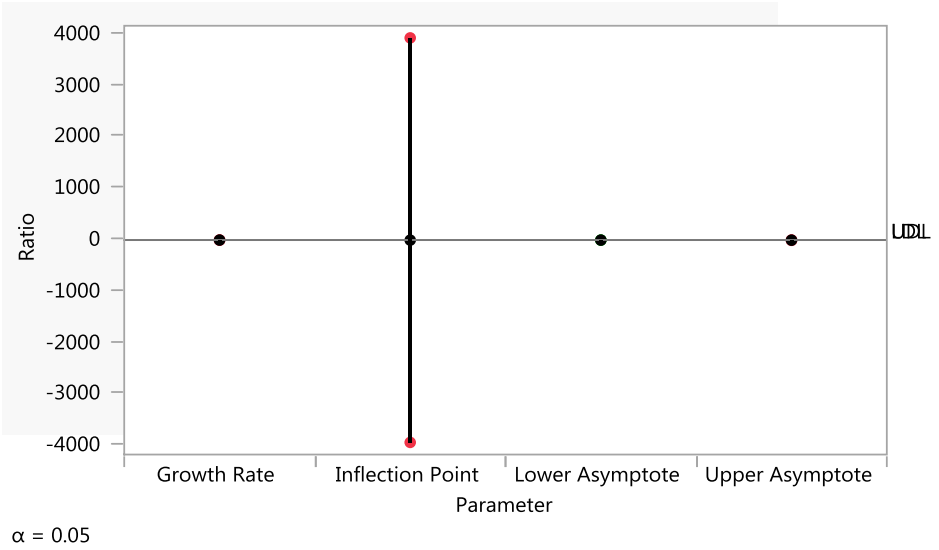

Figure 8: Equivalence between Drug A and standard for Female Patients.

Figure 8 showed that when drug A was compared to the standard on female patients, it revealed that Drug A is not equivalent to the standard as the growth rate, inflection points, Upper and Lower asymptote were not within the acceptable limit.

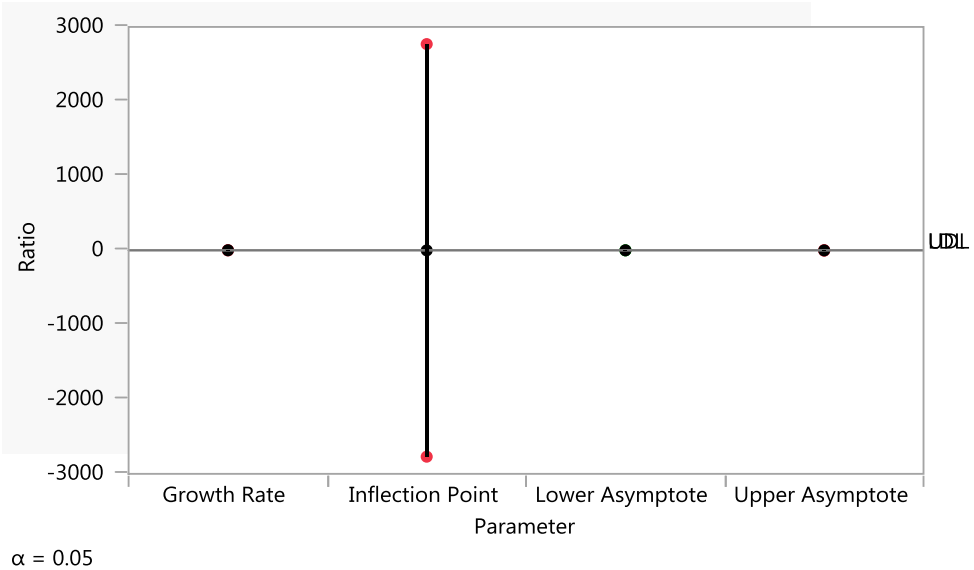

Figure 9: Equivalence between Drug B and standard for Female Patients.

Figure 9 showed that when drug B was compared to the standard on female patients, it revealed that Drug B is not equivalent to the standard as the growth rate, inflection points, Upper and Lower asymptote were not within the acceptable limit.

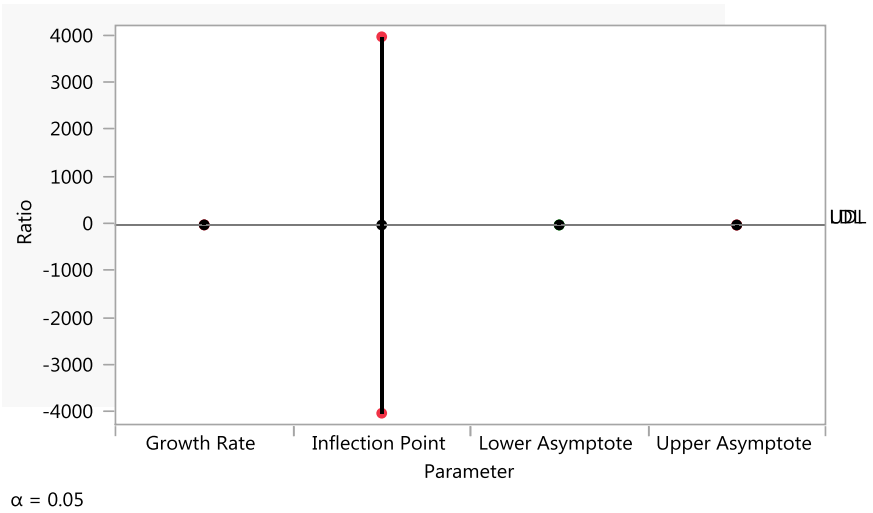

Figure 10: Equivalence between Drug C and standard for Female Patients.

Figure 10 showed that when drug C was compared to the standard on female patients, it revealed that Drug C is not equivalent to the standard as the growth rate, inflection points, Upper and Lower asymptote were not within the acceptable limit.

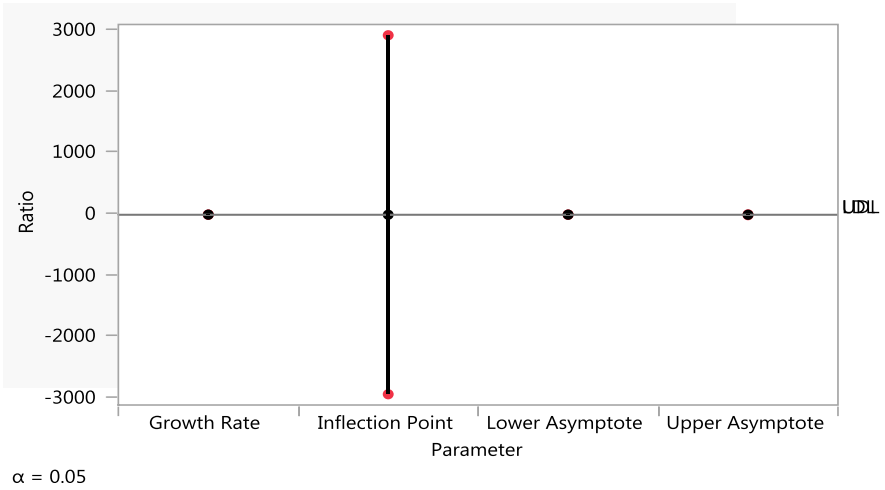

Figure 11: Equivalence between Drug D and standard for Female Patients.

Figure 11 showed that when drug D was compared to the standard on female patients, it revealed that Drug D is not equivalent to the standard as the growth rate, inflection points, Upper and Lower asymptote were not within the acceptable limit.

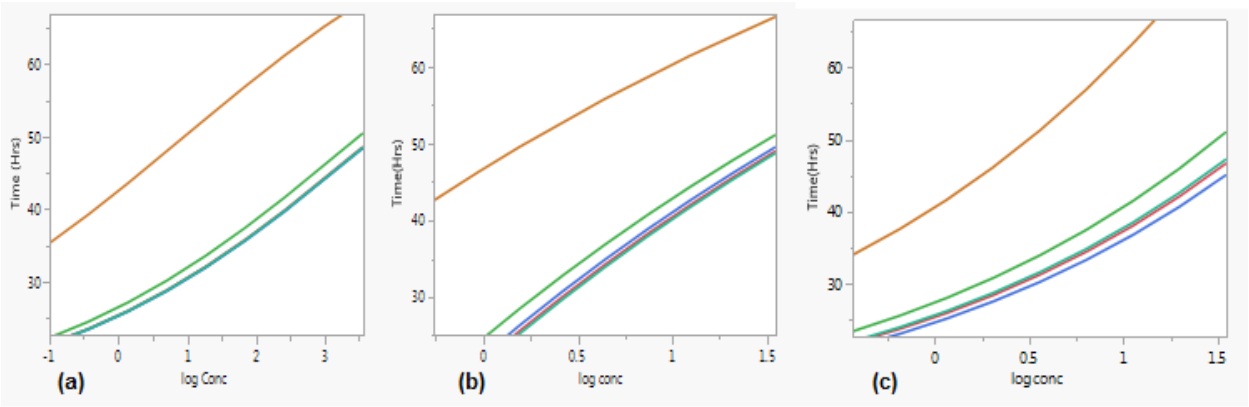

Figure 12: Parallelism comparison between all patients; male and female.

Figure 11 showed that log concentration horizontal shift Δ is not the same at all doses for All patients (a), Male patients (b) and Female patients (c). Thus, in all cases failed parallelism.

DISCUSSION

Parallelism testing failure

The comprehensive failure of parallelism testing represents the study's primary finding. Both F-test ($p = 0.0094$) and χ^2-test ($p = 0.0001$) rejected the null hypothesis of parallel dose-response curves at $\alpha = 0.05$. The F-test assesses variance homogeneity across parallel vs. non-parallel models, while the χ^2-test evaluates residual sum of squares differences. These statistically significant results ($p < 0.01$) indicate fundamental differences in dose-response profiles among test drugs and standard.

Figure 12 visually confirms non-parallelism through inconsistent horizontal shifts (Δ) across dose levels. Parallel curves maintain constant Δ (log potency difference), but observed variable shifts suggest differing pharmacological behaviours potentially due to.

a) Reference standard degradation
b) Formulation inconsistencies (excipient differences)
c) Dose preparation errors (non-logarithmic spacing)

Gender-Stratified Analysis

Male Patients (Tables 3-4; Figures 2-6)

a) F-test: $p < 0.0001$
b) χ^2-test: $p = 0.0001$

Female Patients (Tables 5-6; Figures 7-11)

a) F-test: $p < 0.0001$
b) χ^2-test: $p = 0.0001$

Equivalence Test: Identical failure pattern across all parameters.

ANOVA Results Interpretation

The One-way ANOVA ($p < 0.0001$) confirms significant variation among formulations, supporting the parallelism test findings.

This indicates.

a. Real pharmacological differences exist
b. Multiple sources of variation: formulation, patient factors, assay conditions
c. No single formulation dominates (all differ from each other and standard)

4PL Model Fit Quality

Despite parallelism failure, the 4PL model demonstrated excellent fit:

a. $R^2 = 93.3\%$: Explains 93.3% of response variance
b. AIC = 465.53: Good model selection criterion

CONCLUSION

This comprehensive bioassay evaluation of four generic antimalarial formulations against a standard reference demonstrated consistent failure of parallelism testing across all statistical methods and patient subgroups. The F-test ($p = 0.0094$ overall; $p < 0.0001$ by gender), χ^2-test ($p = 0.0001$), and equivalence testing uniformly rejected dose-response curve parallelism, preventing valid relative potency estimation. The assay failure most likely reflects reference standard degradation or critical assay design flaws. No test drugs demonstrated bioequivalence to the standard, indicating none are reliably interchangeable for uncomplicated malaria treatment. This study underscores the fundamental importance of parallelism validation in bioassays and highlights quality assurance challenges in antimalarial drug evaluation within resource-limited settings.

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