Original Article

Mathematical Analysis of a Five Periods Crossover Design for Two Treatments

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Introduction: A cross-over design is a repeated measurements design such that each experimental unit receives different treatments during different time periods. Lower order cross-over designs such as the two treatments, two periods and two sequences C (2, 2) design have been discovered to be inefficient and erroneous in their analysis of treatments efficacy. In this regard, higher order cross-over designs have been recommended and developed like: the two treatments, three periods and four sequence C (2, 3, 4) design; and the two treatments, four periods and four sequence C (2, 4, 4) designs. However, there still exists more efficient higher order cross-over designs for two treatments which can be used in bioequivalence experiments. This study gives a new design and analysis for two treatments, five periods and four sequence C (2, 5, 4) cross-over design that gives more precise estimates and provides estimates for intra subject variability.

Method: A hypothetical case study was considered on 160 experimental units which are assumed to be randomly selected from a given population. A cross over design of two treatments (A, B) in five periods whose sequences are given by BABAA, ABABB, BAABA and ABBAB were used. Each of the experimental units was used as its own control. The estimates for both direct treatments and treatments carry-over effects were obtained using best linear unbiased estimation method (BLUE). We simulated data for two treatments in five periods and four sequences and used it to test the null hypotheses of no significant differences in both the direct treatments and treatments carry-over effects using the t− test. The subject profiles plots were used to determine the general trend so as to enable an experimenter make a decision on which of the two treatments under consideration was more efficacious.

Results: In testing the null hypothesis of no significant difference in carry-over effects for the two treatments (A&B), the calculated value was found to be 0.55 which was less than the tabular value at 156 degrees of freedom at 95% confidence level, hence the null hypothesis was not rejected. Similarly, in testing the null hypothesis of no significant difference in treatment effects for A&B, the calculated value was found to be 11.73 which was higher than the tabular value at 156 degrees of freedom at 95% confidence level hence the null hypothesis was rejected, and it was concluded that there was indeed a significant difference in the treatment effects. The mean subject profiles plots for a majority of periods and their respective sequences indicated that the general trend implied that treatment B was more effective as compared to treatment A.

Conclusion: In cross-over designs, the presence carry-over effects affect the precision of treatments effects estimates in an experiment. Apart from increasing the washout periods, increasing the number of periods in cross-over designs can help in eliminating the carry-over effects. The C (2, 5, 4) design in this study gives more precise estimates and can provide estimates for intra subject variability. The simulated data indicated that there was significant difference in the treatment effects, and in comparison of the two treatments, treatment B was more effective as compared to treatment A.

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Introduction

A cross-over design is a repeated measurements design such that each experimental unit receives different treatments during the different time periods. In cross-over designs, a direct treatment effect is the effect of treatment at the time of its application, while a carry-over treatment effect is the effect of a treatment that persist after the end of a treatment period. Carry-over effects appear when the response to a current treatment is affected by the treatment that was applied in the previous period.

Cross-over designs are popular for comparing several non-curative treatments for their efficacy. The use of cross-over designs to compare the efficacy of two or more treatments has the advantage that each individual is used as its own control [1]. Additionally, a cross-over trial has the advantage that fewer participants are needed than the equivalent parallel group trial, and, from a clinical point of view, the experimental treatments are tested within each subject which eliminates many of the confounding factors that might occur in studies with a different design[2].

For convenience, a cross-over design with \( t \) treatments, \( p \) periods and \( s \) sequences is denoted as \( C(t, p, s) \) [3].

The most common cross-over design that has been widely studied is the \( C(2, 2, 2) \) [4]. Designs that have two treatments and two periods were frequently utilized by researchers, but it has been shown that these designs lack the structure to test for carry-over and also produce biased direct treatment effects under the presence of carry-over effects [5].

Critiques of the \( C(2, 2, 2) \) with sequences \( AB \) and \( BA \) allude that the carry-over effects is confounded with sequence by period effects leading to erroneous analyses[6,7]. The carry-over effects may arise for a variety of reasons such as; an inadequate washout period, a change in physiological or psychological state of the patients caused by the treatment in the first period, or if the treatment effect depends on the mean levels [5]. Potential solutions to these problems have been considered, but these designs are not normally recommended in practice [8]. Two strategies can be used to obtain higher order cross-over designs which are used to overcome the problems inherent in the \( C(2, 2, 2) \) design. The first one is to extend the number of sequences such as Baalam’s \( C(2, 2, 4) \) design [4]. Secondly, the design can be extended by adding a third period or more and repeating one of the two treatments [9]. In this regard, higher order designs that involve more than two periods are preferable and are becoming more widely used in practice [10].

In higher order five period cross-over designs with two treatments, thirty two possible treatment sequences can result; AAAAA, BAAAA, ABAAA, AABAA, AAABA, AAAAB, BBAAA, BABAA,BAABA, BAAAB, ABAAA, AABAA, ABBAB, AABAB, AABBA, AABAB, AABBB and their duals. This paper considered the most optimum and robust for missing data design \((D_1)\) with sequences BABAA, ABABB, BAABA, and ABBAB.

It outlines the BLUE method of estimating direct treatments and first order carry-over effects in the set of five period designs, assuming a traditional model that specifies first order carry-over effect. The unbiased estimates of treatment and carry-over effects were formulated using a strategy outlined by [6, 9, and 11]. The null hypotheses of no significant differences in both the direct treatments and treatments carry-over effects were analyzed using the \( t \) – test, from the simulated data. In order to indicate the general trend so as to enable an experimenter make a decision on which of the two treatments under consideration was to be favored, the subject profiles plots were used.

Methods

Assume that the primary goal is to compare two treatments A and B used in a study. By estimating the treatment contrasts \( \tau_A - \tau_B \) and period effects \( \pi_1 \) and \( \pi_2 \); first order carry-over effects \( \lambda_A \), \( \lambda_B \) and \( \mu \) are regarded as nuisance parameters. Also assume that the response variable is continuous and that there is one response from each subject in each period. Finally, it is assumed that each treatment has simple first order carry-over effect that does not interact with direct effect of the treatment in the subsequent
period. This model then assumes the following for the response of individual \( y_{ij} \).

If \( y_{ijk} \) denotes the observed response of subject \( j(j = 1,2,\ldots,n) \) in period \( i(i = 1,\ldots,p) \), Then,

\[
y_{ijk} = \mu + \pi_i + \tau_{d(ij)} + \lambda_{d(i-1,j)} + \beta_j + e_{ij}, (1)
\]

Where \( \pi_i \) the effect of the first period is, \( \tau_{d(ij)} \) is the effect of treatment A and B, and \( \lambda_{d(i-1,j)} \) is the simple first order carry-over effect of treatment A. It is assumed that all effects are fixed effects. \( \beta_j \) is the effect of patient j and \( e_{ij} \) is the error term. The random subject effect \( \beta_j \), and the experimental error, \( e_{ij} \) are assumed to be mutually independently distributed as \( N(0,\sigma^2) \).

Consider the estimation of contrasts among direct and residual treatment effects under (1) let \( \tau_A - \tau_B \), and \( \lambda_A - \lambda_B \) be the direct treatment effects and carry-over effects to be estimated respectively, their best linear unbiased estimators can be written as linear combinations of cell means; for example,

\[
\tau_A - \tau_B = \sum \sum a_{ij} \bar{y}_{ij}
\]

(2)

and

\[
\lambda_A - \lambda_B = \sum \sum b_{ij} \bar{y}_{ij}
\]

(3)

The estimability of \( \tau_A - \tau_B \) and \( \lambda_A - \lambda_B \) ensures that

\[
\sum_{i=1}^{p} a_{ij} = 0
\]

(4)

And

\[
\sum_{i=1}^{p} b_{ij} = 0,
\]

(5)

For \( j = 1,\ldots,s \).

2.1: Student’s t-Test

2.1.1: Determination of Variance for the C (2, 5, 2) Cross-Over Design

Let the \( k \)th subject in group 1 have, \( k = 1,2,\ldots,n_1 \), the \( k \)th subject in group 2, \( k = 1,2,\ldots,n_2 \), the \( k \)th subject in group 3, \( k = 1,2,\ldots,n_3 \), and the \( k \)th subject in group 4, \( k = 1,2,\ldots,n_4 \).

Assuming that \( s_{11}^2 \) is the variance of the first group and \( s_{21}^2 \) is the variance of the second group, the pooled variance for the first two groups is given by,

\[
s_1^2 = \frac{(n_1 - 1)s_{11}^2 + (n_2 - 1)s_{21}^2}{(n_1 + n_2 - 2)}
\]

(6)

Similarly, assuming that \( s_{31}^2 \) is the variance of the third group and \( s_{41}^2 \) is the variance of the fourth group, the pooled variance for the two groups is given by,

\[
s_2^2 = \frac{(n_3 - 1)s_{31}^2 + (n_4 - 1)s_{41}^2}{(n_3 + n_4 - 2)}
\]

(7)

2.1.2 Determination of Variance for the C (2, 5, 4) Cross-Over Design

Let \( (\tau_A - \tau_B)_1 = \frac{1}{k_1} (f_1 - f_2) \), and \( (\tau_A - \tau_B)_2 = \frac{1}{m_1} (f_3 - f_4) \)

(8)

Similarly,

Let \( (\lambda_A - \lambda_B)_1 = \frac{1}{p_1} (f_5 - f_6) \), and \( (\lambda_A - \lambda_B)_2 = \frac{1}{q_1} (f_7 - f_8) \)

(9)

Where: \( f_1, f_2, f_3, f_4, f_5, f_6, f_7 \& f_8 \) are treatment

Contrasts for groups 1, 2, 3 and 4 respectively,

\[
V(\tau_A - \tau_B)_1 = \frac{s_{21}^2}{k_1^2} \left[ \frac{1}{n_1} + \frac{1}{n_2} \right]
\]

(10)

\[
V(\tau_A - \tau_B)_2 = \frac{s_{21}^2}{m_1^2} \left[ \frac{1}{n_3} + \frac{1}{n_4} \right]
\]

(11)
Note that \( n_1, n_2, n_3, \& n_4 \) are the sample sizes for groups 1, 2, 3 & 4 respectively.

A combined estimator of \((\tau_A - \tau_B)_W\) can be obtained by taking a weighted average of the two estimators where the weights are taken to be inversely proportional to the variances of the estimators. That is,

\[
W_1 = \frac{1}{\text{Var}(\tau_A - \tau_B)_1} \quad (12)
\]

\[
W_2 = \frac{1}{\text{Var}(\tau_A - \tau_B)_2}
\]

Using (8), (9), (12) and (13), the combined estimator for treatment effects is given by,

\[
(\tau_A - \tau_B)_W = \frac{W_1(\tau_A - \tau_B)_1 + W_2(\tau_A - \tau_B)_2}{W_1 + W_2} \quad (14)
\]

Thus the variance of (14) which forms the combined variance estimator is given by [1] as:

\[
V(\tau_A - \tau_B)_W = \left(\frac{W_1}{W_1 + W_2}\right)^2 \text{Var}(\tau_A - \tau_B)_1 + \left(\frac{W_2}{W_1 + W_2}\right)^2 \text{Var}(\tau_A - \tau_B)_2 \quad (15)
\]

The same procedure can be used to obtain \((\lambda_A - \lambda_B)_W\) and \(V(\lambda_A - \lambda_B)_W\).

From (14) and (15), the calculated \( t \) values for treatment effects and carry-over effects are given by,

\[
t_\tau = \frac{(\tau_A - \tau_B)_W}{\sqrt{V(\tau_A - \tau_B)_W}} \quad , \quad (16)
\]

\[
t_c = \frac{(\lambda_A - \lambda_B)_W}{\sqrt{V(\lambda_A - \lambda_B)_W}} \quad (17)
\]

A simple approximation to the degrees of freedom of the estimated variance of the combined estimator is obtained using the result given by [12].

\[
f_w = \frac{(a_1 V_1 + a_2 V_2)^2}{\frac{1}{f_1} + \frac{1}{f_2}} \quad (18)
\]

Where, \( a_1 = \frac{W_1}{W_1 + W_2} \), \( a_2 = \frac{W_2}{W_1 + W_2} \), \( V_1 = \text{Var}(\tau_A - \tau_B)_1, V_2 = \text{Var}(\tau_A - \tau_B)_2 \), and

\[
V_W = \text{Var}(\tau_A - \tau_B)_W \quad (19)
\]

In this case, \( f_1, f_2 \) and \( f_w \) are assumed to be the degrees of freedom respectively for the estimates of \( V_1, V_2 \& V_W \). By comparing the tabulated value at \( f_w \) degrees of freedom in (18) with the calculated value from (16) and (17), the null hypothesis is rejected if the
calculated values are greater than the tabulated values at 95% confidence level.

2.2: Plotting the Data

2.2.1: Subject Profiles Plot
The objective of cross-over trial is to focus attention on within-individual treatment differences. A good plot for displaying these differences is the subject profiles plot. In this case, subject profiles graphs are plotted for each group to represent the change in each individual’s response over two treatments periods.

For each value of \( k \), the pairs of points \((y_{11k}, y_{12k}, y_{13k}, y_{14k}, y_{15k}), (y_{21k}, y_{22k}, y_{23k}, y_{24k}, y_{25k})\), and \((y_{31k}, y_{32k}, y_{33k}, y_{34k}, y_{35k}), (y_{41k}, y_{42k}, y_{43k}, y_{44k}, y_{45k})\), are plotted.

This plot helps to identify the general trend and ascertain the effectiveness of the new treatment (B) with regard to the standard treatment (A).

3.1 Construction of Design 1: BABAA, BAABA and their duals

| SEQ     | \( p_1 \)                  | \( p_2 \)                  | \( p_3 \)                  | \( p_4 \)                  | \( p_5 \)                  |
|---------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| BABAA   | \( \mu + \pi_1 + \tau_B \)  | \( \mu + \pi_2 + \tau_A \)  | \( \mu + \pi_3 + \tau_B \)  | \( \mu + \pi_4 + \tau_A \)  | \( \mu + \pi_5 + \tau_A \)  |
|         | + \lambda_B                 | + \lambda_A                 | + \lambda_B                 | + \lambda_A                 | + \lambda_A                 |
| ABABB   | \( \mu + \pi_1 + \tau_A \)  | \( \mu + \pi_2 + \tau_B \)  | \( \mu + \pi_3 + \tau_A \)  | \( \mu + \pi_4 + \tau_B \)  | \( \mu + \pi_5 + \tau_B \)  |
|         | + \lambda_B                 | + \lambda_A                 | + \lambda_B                 | + \lambda_A                 | + \lambda_B                 |
| BA ABA  | \( \mu + \pi_1 + \tau_B \)  | \( \mu + \pi_2 + \tau_A \)  | \( \mu + \pi_3 + \tau_A \)  | \( \mu + \pi_4 + \tau_B \)  | \( \mu + \pi_5 + \tau_B \)  |
|         | + \lambda_B                 | + \lambda_A                 | + \lambda_A                 | + \lambda_B                 | + \lambda_B                 |
| ABBAB   | \( \mu + \pi_1 + \tau_A \)  | \( \mu + \pi_2 + \tau_B \)  | \( \mu + \pi_2 + \tau_A \)  | \( \mu + \pi_4 + \tau_B \)  | \( \mu + \pi_5 + \tau_B \)  |
|         | + \lambda_B                 | + \lambda_A                 | + \lambda_B                 | + \lambda_A                 | + \lambda_A                 |

The Contrasts \( f_1, f_2, f_3 \) and \( f_4 \) are chosen in such a way that (2) and (4) are satisfied to give;

\[
f_1 = \frac{1}{4} (Y_{11} - Y_{12} - Y_{13} + Y_{14} + Y_{15}),
\]
\[
f_2 = \frac{1}{4} (Y_{21} - Y_{22} - Y_{23} + Y_{24} + Y_{25}),
\]

\[
f_3 = \frac{1}{12} (Y_{31} - Y_{32} + Y_{33} - Y_{34} + Y_{35})
\]

and

\[
f_4 = \frac{1}{12} (Y_{41} - Y_{42} + Y_{43} - Y_{44} + Y_{45})
\]

respectively, whose expected values are given by;
\[ E(f_1) = \frac{1}{4} [\mu + (\pi_1 - \pi_2 - \pi_3 + \pi_4 + \pi_5) + \tau_A], \quad (24) \]
\[ E(f_2) = \frac{1}{4} [\mu + (\pi_1 - \pi_2 - \pi_3 + \pi_4 + \pi_5) + \tau_B], \quad (25) \]
\[ E(f_3) = \frac{1}{12} [\mu + (\pi_1 - \pi_2 - \pi_3 - \pi_4 + \pi_5) + 3\tau_A], \quad (26) \]
\[ E(f_4) = \frac{1}{12} [\mu + (\pi_1 - \pi_2 + \pi_3 - \pi_4 + \pi_5) + 3\tau_B] \quad (27) \]

The linear combination of \((f_1 - f_2) + (f_3 - f_4)\) from (24), (25), (26), and (27) forms an unbiased estimate of the treatment effect \(\tau_A - \tau_B\).

Thus,
\[ \tau_A - \tau_B = 2[(f_1 - f_2) + (f_3 - f_4)] \quad (28) \]

Similarly, the Contrasts \(f_5, f_6, f_7\) and \(f_8\) are chosen in such a way that (3) and (5) are satisfied to give;
\[ f_5 = \frac{1}{2} (Y_{11} - 2Y_{12} - Y_{13} + Y_{14} + Y_{15}) \]
\[ f_6 = \frac{1}{2} (Y_{21} - 2Y_{22} - Y_{23} + Y_{24} + Y_{25}) = f_7 = \frac{1}{2} (Y_{31} + Y_{32} + Y_{33} - Y_{34} - Y_{35}) \quad \text{and} \quad f_8 = \frac{1}{2} (Y_{41} + Y_{42} + Y_{43} - Y_{44} - Y_{45}) \]
respectively, whose expected values are given by;

\[ E(f_5) = \frac{1}{2} [(\pi_1 - 2\pi_2 - \pi_3 + \pi_4 + \pi_5) - \lambda_B] \quad (29) \]
\[ E(f_6) = \frac{1}{2} [(\pi_1 - 2\pi_2 - \pi_3 + \pi_4 + \pi_5) - \lambda_A] \quad (30) \]
\[ E(f_7) = \frac{1}{2} [\mu + (\pi_1 + \pi_2 - \pi_3 - \pi_4 + 2\pi_5) - \lambda_B] \quad (31) \]
\[ E(f_8) = \frac{1}{2} [\mu + (\pi_1 + \pi_2 + \pi_3 - \pi_4 - 2\pi_5) - \lambda_A] \quad (32) \]

The linear combination of \((f_5 - f_6) + (f_7 - f_8)\) thus,
from (29), (30), (31), and (32) forms an unbiased estimate of the treatment effect \(\lambda_A - \lambda_B\).

\[ \lambda_A - \lambda_B = (f_5 - f_6) + (f_7 - f_8) \quad (33) \]

3.2: Data analysis

Table 2: Hypothetical experimental data.

| Sequences | Periods | Treatments | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | Mean(\(\mu_i\)) | \(\sigma^2\) |
|-----------|---------|------------|-----|-----|-----|-----|-----|-----|-----|-----|-----------------|-------------|
| 1         | 1       | B          | 2.4 | 7.1 | 8.0 | 2.3 | 2.9 | 6.4 | 7.0 | 2.9 | 4.8750          |             |
| 1         | 2       | A          | 4.1 | 7.6 | 9.7 | 1.8 | 2.7 | 5.6 | 5.5 | 2.4 | 4.9250          |             |
| 1         | 3       | B          | 1.9 | 0.5 | 0.6 | 8.7 | 15.7| 5.3 | 3.7 | 9.8 | 5.7750          |             |
| 1         | 4       | A          | 6.4 | 0.5 | 2.8 | 3.8 | 9.5 | 5.4 | 4.6 | 5.8 | 4.8500          |             |
| 1         | 5       | A          | 0.1 | 5.2 | 6.2 | 4.4 | 2.4 | 7.5 | 2.1 | 4.2 | 4.0125          | 10.1057     |
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| Sequence | period₁ | period₂ | period₃ | period₄ | period₅ |
|----------|---------|---------|---------|---------|---------|
| BABAA    | E(Y₁₁) = 4.875 | E(Y₁₂) = 4.925 | E(Y₁₃) = 5.775 | E(Y₁₄) = 4.850 | E(Y₁₅) = 4.0125 |
| ABABB    | E(Y₂₁) = 4.525 | E(Y₂₂) = 4.750 | E(Y₂₃) = 4.763 | E(Y₂₄) = 5.113 | E(Y₂₅) = 1.700 |
| BAABA    | E(Y₃₁) = 1.475 | E(Y₃₂) = 3.613 | E(Y₃₃) = 6.488 | E(Y₃₄) = 4.263 | E(Y₃₅) = 6.250 |
| ABBAB    | E(Y₄₁) = 6.163 | E(Y₄₂) = 5.913 | E(Y₄₃) = 2.500 | E(Y₄₄) = 3.275 | E(Y₄₅) = 2.688 |

The variances of the four groups from table (2) are given by; Substituting the variances $s^2_{11} = 10.1057$ in table (2) to (6) and (7) gives,

\[ s^2_1 = 10.8752 \]  
\[ s^2_2 = 8.24235 \]  \hspace{2cm} \text{And} \hspace{2cm} \text{(34)}  
\[ s^2_{31} = 7.6876 \]  \hspace{2cm} \text{and} \hspace{2cm} \text{(35)}

Substituting (34) and (35) on (10) and (11) respectively using the contrasts given in 24, 25, 26 & 27 gives,

\[ V(\tau_A - \tau_B)_1 = 0.033985 \]  \hspace{2cm} \text{(36)}
\[ V(\tau_A - \tau_B)_2 = 0.00286 \]  \hspace{2cm} \text{(37)}

Substituting the values of table 3 on the contrasts given in 24, 25, 26 & 27 gives,

\[ f_1 = 8.8125, \; f_2 = 1.55, \; f_3 = 6.337, \; f_4 = 2.163 \]  \hspace{2cm} \text{(38)}
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Substituting (38) on (8) gives,

\[(\tau_A - \tau_B)_1 = 3.63125 \quad (39)\]
\[(\tau_A - \tau_B)_2 = 0.34783 \quad (40)\]

Substituting (36) and (37) to (12) and (13) gives,

\[W_1 = 29.42474621 \quad \text{and} \quad W_2 = 349.6503497 \quad (41)\]

Substituting (39), (40), & (41) to (14) and (15) respectively gives,

\[(\tau_A - \tau_B)_w = 0.602697235 \quad (42)\]
\[V(\tau_A - \tau_B)_w = 0.002637999729 \quad (43)\]

Similarly, substituting (34) and (35) on (10) and (11) respectively using the contrasts given in 29, 30, 31 & 32 gives,

\[V(\lambda_A - \lambda_B)_1 = 0.13594 \quad (44)\]
\[V(\lambda_A - \lambda_B)_2 = 0.10303 \quad (45)\]

Substituting the values of table 3 on the contrasts given in 29, 30, 31 & 32 gives,

\[f_5 = -0.94375, \ f_6 = -1.4625, \ f_7 = 0.5281, \ f_8 = 0.18025 \quad (46)\]

Substituting (46) on (9) gives,

\[(\lambda_A - \lambda_B)_1 = 0.259375 \quad (47)\]
\[(\lambda_A - \lambda_B)_2 = 0.17392 \quad (48)\]

Substituting (44) and (45) to (12) and (13) gives,

\[W_3 = 7.3562 \quad \text{and} \quad W_4 = 9.706 \quad (49)\]

Substituting (47), (48), & (49) to (14) and (15) respectively gives,

\[(\lambda_A - \lambda_B)_w = 0.098936 \quad (50)\]
\[V(\lambda_A - \lambda_B)_w = 0.0333404 \quad (51)\]

3.1.1 $t$-Test For Treatment Effects

The hypothesis to be tested was,

\[H_0: [\tau_A - \tau_B]_W = 0\]
\[H_1: [\tau_A - \tau_B]_W \neq 0\]

Substituting (34) and (35) on (16) gives

\[t_\tau = 11.73442533 \quad (52)\]

3.1.2 Degrees of Freedom for Treatment Effects

Let,

\[a_1 = \frac{W_1}{W_1 + W_2} \quad (53)\]
\[ a_2 = \frac{W_2}{W_1 + W_2} \]  
(54)

Substituting (41) to (37) and (38) respectively gives
\[ a_1 = 0.077622472, \]  
(55)
and
\[ a_2 = 0.922377527. \]  
(56)

Substituting (36), (37), (55), and (56) to (18) with \( f_1 = f_2 = 78 \) degrees of freedom gives;
\[ f_w = 156 \]  
(57)

By comparing the tabulated value at 156 degrees of freedom given in (57) with the calculated value from (52), the calculated value is greater than the tabulated value at 95\% level of significance hence the null hypothesis is rejected

3.1.3 \( t \)-Test For Carry-Over Effects

The hypothesis to be tested was,
\[ H_0: (\lambda_A - \lambda_B)_W = 0 \]
\[ H_1: (\lambda_A - \lambda_B)_W \neq 0 \]

Substituting (50) and (51) to (17) gives
\[ t_C = 0.541837436 \]  
(58)

3.1.4 Degrees Of Freedom for Carry-Over Effects

Let,
\[ a_3 = \frac{W_3}{W_3 + W_4} \]  
(59)
\[ a_4 = \frac{W_4}{W_3 + W_4} \]  
(60)

Substituting (49) to (43) and (44) respectively gives
\[ a_1 = 0.431140496, \]  
(61)
and
\[ a_2 = 0.568859503 \]  
(62)

Substituting (45), (46), (61), and (62) to (18) with \( f_1 = f_2 = 78 \) degrees of freedom gives
Then \( f_w = 155.4 \)  
(63)

By comparing the tabulated value at 155.4 degrees of freedom given in (63) with the calculated value from (58), the calculated value is less than the tabulated value at 95\% confidence level hence the null hypothesis is not rejected.

3.2: Subject Profiles Plots
There were high between individuals variability since there are the low mean values of some individuals in group 3. However, from figures 1, 2, 3, 4, 5, and 6, the general trend implies a direct treatment effect in favor of treatment B, this implies that treatment B is more efficacious as compared to treatment A.

**Discussion**

In testing the null hypothesis of no significant difference in carry-over effects for the two treatments (A&B), the calculated value was found to be 0.55 which was less than the tabular value at 156 degrees of freedom at 95% confidence level, hence the null hypothesis was not rejected. Similarly, In
testing the null hypothesis of no significant difference in treatment effects for A&B, the calculated value was found to be 11.73 which was higher than the tabular value at 95% confidence level hence the null hypothesis was rejected, and it was concluded that there was indeed a significant difference in the treatment effects. The mean subject profiles plots for a majority of periods and their respective sequences indicated that the general trend implied that treatment B was more effective as compared to treatment A. This analysis confirms the assertion by Hills and Armitage in the year 1979, that higher order cross-over designs possess the structure to test for carry-over effects and also produce biased direct treatment effects under the presence of carry-over effects [2,10].

The main problem with clinical trials practitioners who apply cross-over designs is the presence of carry-over effects is that, in any given period, an observation from an experimental unit can be affected not only by the treatment effect in which it is applied, but also by the effect of a treatment applied in the preceding period.

One way to avoid the impact of carry-over is to insert a washout period between two successive periods with the aim of eliminating the carry-over effect. The washout periods effectively increases the interval between the observed periods and can help in overcoming the carry-over effect if the carry-over effect is not expected to persist. Alternatively, the design can be designed in such a way that the difference in treatment effects may be estimated after adjusting for the presence of possible carry-over effects. More precise estimates can be achieved if the two approaches can be applied in cross-over designs concurrently, like in this study.

Like the C(2,4,4) cross-over designs, the C (2, 5, 4) in this study gives; more precise estimates, allows treatment effects to be estimated even in the presence of carry-over effects, and can provide estimates for intra-subject variability [13]. The C (2, 5, 4) designs have an advantage of using the subjects as their own control. Additionally, the designs require fewer subjects for the same number of observations than the non-cross-over designs [7]. In this regard, the designs are efficient in situations where the experimental subjects are scarce and are expensive to recruit and maintain. Moreover, it is possible to estimate important treatment contrasts in such designs even when the carry-over effects are assumed in the overall model.

Conclusion
This article considered C (2, 5, 4) design for a simple one period carry-over effect model. The design presented is ideal because the design efficiency is optimal.

In cross-over designs, the presence carry-over effects affect the precision of treatments effects estimates in an experiment. Apart from increasing the washout periods, increasing the number of periods in cross-over designs can help in eliminating the carry-over effects. Just like the other higher order cross-over designs, the C (2, 5, 4) design in this study gives; more precise estimates, allows treatment effects to be estimated even in the presence of carry-over effects, provides estimates for intra-subject variability and can draw inference on the carry-over effect.

References
1. Jones B, Kenward MG. Design and analysis of cross-over trials. CRC Press; 2014 Oct 8.
2. Godolphin PJ, Godolphin EJ. Robustness of cross-over trials against subject dropout-Examples of perpetually connected designs. Statistical methods in medical research. 2019 Mar; 28(3):788-800.
3. Reed III JF. Higher order C (t, p, s) cross-over designs. Journal of modern applied statistical methods. 2011; 10(2):2017.
4. Balaam LN. A two-period design with \( t^2 \) experimental units. Biometrics. 1968 Mar 1:61-73.
5. Hills M, Armitage P. the two period cross-over clinical trial. British journal of clinical pharmacology. 1979 July; 8(1):7-20.
6. Reed III JF. Extension of grizzle’s classic cross-over design. Journal of modern applied statistical methods. 2011; 10(1):25.
7. Reed III JF. Four period cross-over designs. Journal of modern applied statistical methods 2012; 11(1):25.
8. Fleiss JL. A critique of recent research on the two treatment crossover design. Controlled clinical trials. 1989 Sep 1; 10(3):237-243.
9. Matthews JN. Multi-period cross-over trials. Statistical methods in medical research. 1994 Dec; 3(4):383-405.
10. Godolphin PJ, Godolphin EJ. Robust assessment of two treatment higher order cross-over designs against missing values. Computational statistics and data analysis. 2019 April 1; 132:31-45.
11. Laska E, Meissner M, Kushner HB. Optimal cross-over designs in the presence of carry-over effects. Biometrics. 1983 Dec 1:1087-1091.
12. Satterthwaite FE. An approximate distribution of estimates of variance components. Biometrics bulletin. 1946 Dec 1; 2(6): 110-114.
13. Chow SC, Liu JP. Design and analysis of bioavailability and bioequivalence studies. CRC Press; 2008 Oct 15.