Journal of Scientific Research & Reports

27(9): 123-130, 2021; Article no.JSRR.74000
ISSN: 2320-0227

Bayesian Analysis of an Optimal Five Period Cross-over Design

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JSRR/2021/v27i930440
Editor(s):
(1) Shunpu Zhang, University of Nebraska, USA.
Reviewers:
(1) Constance Izuchukwu Amannah, Ignatius Ajuru University of Education, Nigeria.
(2) Deebom, Zorle Dum, Nigeria.
Complete Peer review History: https://www.sdiarticle4.com/review-history/74000

Original Research Article

Received 10 July 2021
Accepted 20 September 2021
Published 12 October 2021

ABSTRACT

A crossover design is a repeated measurements design such that each experimental unit receives different treatments during the different time periods. In a majority of bioequivalence studies, design and analysis of cross-over using classical methods such as analysis of variance (ANOVA) and t –test are normally associated with erroneous results. The Bayesian method is desirable in the analysis of crossover designs to eliminate errors associated with carryover effects. The objective of this study was to compare the Bayesian and the t- test analysis methods on treatments and carryover effects for an optimal two treatments, five periods and four sequence C (2, 5, 4) design. The treatments and residual estimates were obtained using Best Linear Unbiased Estimation (BLUE) method. In the Bayesian method of analysis, the posterior quantities were obtained for the mean intervals of treatments and carry-over effects and the highest posterior density (HPD) graphs were plotted and interpreted using conditional probability statements. For validation purposes, the Bayesian method results were compared with the existing t-tests results. From the Bayesian analysis, the probability of significant treatment difference in the presence of carryover effects was 1, while from the t-test, the calculated t –value of 11.73 was greater than the two sided tabulated value at 95% level of significance. The two analysis methods implied significant differences in the treatment effects. In conclusion, it was established that Bayesian method of analysis can be used for bioequivalence analysis even when the carry-over effects are present and hence it is highly recommended for bioequivalence studies.

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Keywords: Bio-equivalence; carryover effects; Bayesian methods.

1. INTRODUCTION

A cross-over trial is a longitudinal study in which subjects receive a sequence of different treatments. Crossover designs are popular for comparing several non-curative treatments for their efficacy. It is regularly implemented in medical scientific experiments and has a rich history stretching back to 19th century [1].

The most common design is that with two periods and two treatments with these frequently used in trials of neurology, psychiatry and pain treatments [2]. However, the design is associated with confounded carryover effect with sequence by period effects leading to erroneous analyses [3].

Grizzle [4] proposed a preliminary test for the residual or carryover effects from treatment administration in the first period and gave the analysis of variance for the C (2, 2, 2) crossover design. He observed that the test should strictly test for equality of carryover effects , and if the preliminary test statistics is not significant at the 0.1 or 0.15 level of significance, then the carryover effects are ignored and the hypothesis of no treatment difference is tested by ANOVA or the t-test [5].

Several authors [6-7] [1] have suggested that the classical hypothesis testing techniques of t-test and ANOVA are inappropriate in crossover designs. In this regard, they have identified an alternative method which makes use of the confidence interval approach [8]. In his research on design and analysis of comparative blood levels in the year 1973, Westlake states that, instead of testing for just the presence of the difference, the difference should be large enough in order to matter [9]. A biologically meaningful bio-equivalence measure should be the posterior probability difference that the mean difference is less than a specified fraction, such as $\frac{1}{5}$ of the standard. This probability can only be approximated by the confidence interval approach because the mean of the standard is unknown and hence is a meaningless parameter.

In this paper, the Bayesian method is illustrated by a two treatments, five periods, and four periods C (2, 5, 4) design. We calculate the approximate posterior probabilities, first under the assumption that there are no carry-over effects and then incorporating the carryover effects in the model. The highest posterior density (HPD) graphs are used to represent the posterior direct treatment and treatment carryover effect distributions under the two assumptions.

2. METHODS

2.1 Estimation of Direct Treatment Effects

In sequence BABAA, the expected value of its contrast $c_1 = \frac{1}{4}(Y_{11} - Y_{12} - Y_{13} + Y_{14} + Y_{15})$ is given by $\frac{1}{4}[\mu + (\pi_1 - \pi_2 - \pi_3 + \pi_4 + \pi_5) + \tau_A]$ while it's dual of sequence ABABB of contrast $c_2 = \frac{1}{4}E(Y_{21} - Y_{22} - Y_{23} + Y_{24} + Y_{25})$ has an expected value of $\frac{1}{4}[\mu + (\pi_1 - \pi_2 - \pi_3 + \pi_4 + \pi_5) + \tau_B]$. Similarly, sequence BAABA with contrast $c_3 = \frac{1}{12}(Y_{31} - Y_{32} + Y_{33} - Y_{34} + Y_{35})$ has an expected value of $\frac{1}{12}[\mu + (\pi_1 - \pi_2 + \pi_3 - \pi_4 + \pi_5) + 3\tau_A]$ whereas it's dual of sequence ABBAB with contrast $c_4 = \frac{1}{12}(Y_{41} - Y_{42} + Y_{43} - Y_{44} + Y_{45})$ has an expected value of $\frac{1}{12}[\mu + (\pi_1 - \pi_2 + \pi_3 - \pi_4 + \pi_5) + 3\tau_B]$. The linear combination of $(c_1 - c_2) + (c_3 - c_4)$ forms an unbiased estimate of the treatment effect denoted by $\tau_A - \tau_B$. Thus,

$$\tau_A - \tau_B = (c_1 - c_2) + (c_3 - c_4) \quad (1)$$

2.2 Estimation of Treatment Carryover Effects

Sequence BABAA has expected values of contrast $d_1 = \frac{1}{2}[(Y_{11} - 2Y_{12} - Y_{13} + Y_{14} + Y_{15})]$ given by $\frac{1}{2}[(\pi_1 - 2\pi_2 - \pi_3 + \pi_4 + \pi_5) - \lambda_B]$ while it's dual of sequence ABABB of contrast $d_2 = \frac{1}{2}[(Y_{21} - 2Y_{22} - Y_{23} + Y_{24} + Y_{25})]$ has an expected value of $\frac{1}{2}[(\pi_1 - 2\pi_2 - \pi_3 + \pi_4 + \pi_5) - \lambda_A]$. Similarly, sequence BAABA with contrast $d_3 = \frac{1}{2}[(Y_{31} + Y_{32} + Y_{33} - Y_{34} - Y_{35})]$ has an expected value of $\frac{1}{2}[\mu + (\pi_1 + \pi_2 + \pi_3 - \pi_4 - 2\pi_5) - \lambda_B]$ whereas it's dual of sequence ABBAB with contrast $d_4 = \frac{1}{2}E[(Y_{41} + Y_{42} + Y_{43} - Y_{44} - Y_{45})]$ has an expected value of $\frac{1}{2}[\mu + (\pi_1 + \pi_2 + \pi_3 - \pi_4 - 2\pi_5) - \lambda_A]$. The linear combination of $(d_1 - d_2) + (d_3 - d_4)$ forms an unbiased

estimate of the treatment carry-over effect denoted by $\lambda_A - \lambda_B$. Thus,

$$\lambda_A - \lambda_B = (d_1 - d_2) + (d_3 - d_4) \quad (2)$$

2.3 Bayesian Analysis of C (2, 5, 4) Design 1

Consider the following data simulated to demonstrate the analysis of efficacy for two treatments in five periods and four sequences.

Let the k^{th} subject in sequence 1 have , $k = 1,2,\dots,n_1$,the k^{th} subject in sequence 2 , have $k = 1,2,\dots,n_2$, the k^{th} subject in sequence 3 have $k = 1,2,\dots,n_3$, and the k^{th} subject in sequence 4 have $k = 1,2,\dots,n_4$ respectively.

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

$$s_1^2 = \frac{(n_1-1)s_{11}^2+(n_2-1)s_{21}^2}{(n_1+n_2-2)} \quad (3)$$

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

$$s_2^2 = \frac{(n_3-1)s_{31}^2+(n_4-1)s_{41}^2}{(n_3+n_4-2)} \quad (4)$$

From table 2, s_{11}^2, s_{21}^2, s_{31}^2 and s_{41}^2 are given by; 10.1057, 11.6447, 7.6876 and 8.7971 respectively,
Substituting these values to equations (3) and (4) gives

$$s_1^2 = 10.8752 \quad (5)$$

And

$$s_2^2 = 8.24235 \quad (6)$$

The direct treatments effects difference for the two pairs of sequences are given by

$$(\tau_A - \tau_B)_1 = \frac{1}{k}(d_{11} - d_{21}) \quad (7)$$

And

$$(\tau_A - \tau_B)_2 = \frac{1}{m}(d_{31} - d_{41}) \quad (8)$$

Where $d_{11}, d_{21}, d_{31} \& d_{41}$, are the treatment contrasts for sequences 1, 2, 3 and 4 respectively?

Substituting the expected values of the treatment contrasts given in Table 3 to equations (7) and (8)

gives;

$$(\tau_A - \tau_B)_1 = 3.63725 \quad (9)$$

And

$$(\tau_A - \tau_B)_2 = 0.34783 \quad (10)$$

The variances of (7) and (8) are given by;

$$V(\tau_A - \tau_B)_1 = \frac{s_1^2}{k^2}\left[\frac{1}{n_1} + \frac{1}{n_2}\right] \quad (11)$$

And

$$V(\tau_A - \tau_B)_2 = \frac{s_2^2}{m^2}\left[\frac{1}{n_3} + \frac{1}{n_4}\right] \quad (12)$$

Note that $n_1, n_2, n_3, \& n_4$ are the sample sizes for sequences 1, 2, 3&4 respectively, and m and k are constants.

Substituting (5) and (6) to (11) and (12) for $n_1 = n_2 = n_3 = n_4 = 40$ gives,

$$V(\tau_A - \tau_B)_1 = 0.033985 \quad (13)$$

And

$$V(\tau_A - \tau_B)_2 = 0.00286 \quad (14)$$

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

$$W_1 = \frac{1}{V(\tau_A-\tau_B)_1} \quad (15)$$

And

$$W_2 = \frac{1}{V(\tau_A-\tau_B)_2} \quad (16)$$

Using (9), (10), (15) and (16), the combined estimator for treatment effects is given by,

$$(\tau_A - \tau_B)_W = \frac{W_1(\tau_A-\tau_B)_1+W_2(\tau_A-\tau_B)_2}{W_1+W_2} \quad (17)$$

Substituting the calculated values of (7), (8), (15), and (16) to (17) gives,

$$(\tau_A - \tau_B)_W = 0.602697235 \quad (18)$$

Thus the variance of (17) which forms the combined variance estimator is given by,

$$V(\tau_A - \tau_B)_W = \left(\frac{W_1}{W_1+W_2}\right)^2 V(\tau_A - \tau_B)_1 + \left(\frac{W_2}{W_1+W_2}\right)^2 V(\tau_A - \tau_B)_2 = 0.00264 \quad (19)$$

We employed an approximation proposed by [10], who fits a scaled t distribution to the t distribution.

It is shown by Patil that t is approximately distributed as $t\ [\delta, a^2\left(\frac{s_1^2}{n_1}+\frac{s_2^2}{n_2}\right), b]$

Where,

$$\delta = [\tau_A - \tau_B]_W , \quad (20)$$

$$a = \sqrt{\left(\frac{b-2}{b}\right) f_1} , \quad (21)$$

$$b = 4 + \frac{f_1^2}{f_2} , \quad (22)$$

$$f_1 = \left(\frac{v_2}{v_2-2}\right)\cos^2\theta + \left(\frac{v_1}{v_1-2}\right)\sin^2\theta \quad (23)$$

$$f_2 = \frac{v_1^2}{(v_2-2)^2(v_2-4)}\cos^4\emptyset + \frac{v_2^2}{(v_2-2)^2(v_2-4)}\sin^4\emptyset, \quad (24)$$

Where

$$\cos^2\emptyset = \frac{\frac{s_2^2}{n_2}}{\left(\frac{s_1^2}{n_1}+\frac{s_2^2}{n_2}\right)} \quad (25)$$

v_1 & v_2 , Are the degrees of freedom given by $n_1 - 2$ and $n_2 - 2$ respectively, where, $n_1 = n_{11} + n_{21}$ and $n_2 = n_{31} + n_{41}$.

From (25), $\sin^2\emptyset = 1 - \cos^2\emptyset$ (26)

To this degree of approximation, the difference of the mean values $[\tau_A - \tau_B]_W$ and $[\lambda_A - \lambda_B\ ]_W$ are distributed a posterior as

$$t\left[(\tau_A - \tau_B)_W , a^2\left(\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}\right), b\right] \quad (28)$$

The (1- ∝) H.P.D intervals for treatments effects are given by;

$$(\tau_A - \tau_B)_W \pm (a)\left(\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}\right)^{\frac{1}{2}} t_{\frac{\alpha}{2}(b,95\%)} , \quad (29)$$

The same procedure can be followed for the carry-over effects and treatment effects given the carry-over effects to give the intervals;

$(\tau_A - \tau_B)_W$ = (0.5607-0.6453), $(\lambda_A - \lambda_B)_W$ = (-0.008-0.21), and

$$(\tau_A - \tau_B)_W/(\lambda_A - \lambda_B)_W = (11.86\text{-}11.92), \quad (30)$$

For treatments, carryover and treatments given carry-over effects respectively.

2.4 Hypothesis Testing

The following null hypotheses were tested,

i. H_0: prob $(\lambda_A - \lambda_B) > 0 = 0$,
ii. H_0: Prob $(\tau_A - \tau_B) > 0 = 0$,
and
iii. H_0: prob$((\tau_A - \tau_B)/(\lambda_A - \lambda_B)) > 0 = 0$ (31)

The strategy was to use a non-informative prior to produce the posterior distribution which was used to obtain the highest posterior density (H.P.D) interval and to test the null hypotheses as given in [10].

Different values of $(\tau_A - \tau_B)_W$, $(\lambda_A - \lambda_B)_W$ and $(\tau_A - \tau_B)_W/\ (\lambda_A - \lambda_B)_W$ were tested and a directional hypothesis tests and a probabilistic statements regarding the parameter estimates were given and the whole posterior distribution was used. The null hypothesis of H_0: $(\lambda_A - \lambda_B)_W$ = 0, H_0: $(\tau_A - \tau_B)_W$ = 0, and H_0: $(\tau_A - \tau_B)_W/(\lambda_A - \lambda_B)_W$ = 0, were tested at **α**=5%.

Table 1. Expected values for C (2× 5 × 4) Design 1

SEQ	P1	P2	P3	P4	P5
BABAA	$\mu + \pi_1 + \tau_B$	$\mu + \pi_2 + \tau_A + \lambda_B$	$\mu + \pi_3 + \tau_B + \lambda_A$	$\mu + \pi_4 + \tau_A + \lambda_B$	$\mu + \pi_5 + \tau_A + \lambda_A$
ABABB	$\mu + \pi_1 + \tau_A$	$\mu + \pi_2 + \tau_B + \lambda_A$	$\mu + \pi_3 + \tau_A + \lambda_B$	$\mu + \pi_4 + \tau_B + \lambda_A$	$\mu + \pi_5 + \tau_B + \lambda_B$
BA ABA	$\mu + \pi_1 + \tau_B$	$\mu + \pi_2 + \tau_A + \lambda_B$	$\mu + \pi_3 + \tau_A + \lambda_A$	$\mu + \pi_4 + \tau_B + \lambda_A$	$\mu + \pi_5 + \tau_A + \lambda_B$
ABBAB	$\mu + \pi_1 + \tau_A$	$\mu + \pi_2 + \tau_B + \lambda_A$	$\mu + \pi_2 + \tau_B + \lambda_B$	$\mu + \pi_4 + \tau_A + \lambda_B$	$\mu + \pi_5 + \tau_B + \lambda_A$

Table 2. Experimental data for two treatments (A and B)

Sequence	Period	Treatment	1	2	3	4	5	6	7	8	Mean(μ_i)	σ^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
2	1	A	1.0	3.0	6.9	7.0	5.9	5.1	4.9	2.4	4.5250	
2	2	B	1.6	0.8	1.5	7.8	13.1	2.4	2.2	8.6	4.7500	
2	3	A	1.5	0.7	1.5	7.8	13.2	2.5	2.2	8.7	4.7625	
2	4	B	2.9	3.3	2.0	7.5	8.2	2.5	5.1	9.4	5.1125	
2	5	B	1.4	3.4	0.6	0.7	0.2	3.4	3.0	0.9	1.7000	11.6447
3	1	B	0.5	2.1	1.1	0.5	0.6	1.9	4.2	0.9	1.4750	
3	2	A	3.7	1.2	2.1	4.1	3.6	3.9	2.8	7.5	3.6125	
3	3	A	7.2	3.7	4.8	6.8	6.3	5.8	3.9	13.4	6.4875	
3	4	B	2.3	5.1	7.2	2.7	5.3	6.7	3.6	1.2	4.2625	
3	5	A	5.7	6.6	8.1	5.2	6.7	8.4	7.4	1.9	6.2500	7.6876
4	1	A	3.6	4.3	6.0	12.3	10.7	2.7	5.9	3.8	6.1625	
4	2	B	13.3	3.6	2.64	8.6	9.2	1.5	4.7	3.8	5.9125	
4	3	B	2.0	4.5	3.8	1.8	1.3	1.5	3.6	1.5	2.5000	
4	4	A	2.0	5.3	5.4	1.3	2.2	2.5	5.3	2.2	3.2750	
4	5	B	4.7	1.4	2.9	2.0	3.2	2.4	1.5	3.4	2.6875	8.7971

If in the 95 % HPD interval, the probability of the effects of interest greater than zero higher than 0.2, the null hypothesis was rejected.

3. RESULTS

The treatments, carryover and treatments given carry-over effects intervals in (30) above are used to plot their respective HPD graphs as shown in Figs 1,2 and 3 below.

4. DISCUSSION

The null hypothesis of no significant difference in the carry-over effects was tested. From Fig. 1, the range of the carry-over effects comprises of a zero value. This implies that there was a likelihood for absence of carry-over effects. However, $\mathrm{p}\,(\lambda_{\mathrm{A}}-\lambda_{\mathrm{B}})>0=0.9633$ implied that the carry-over effects difference was significant, since it is greater than 0.2. Thus, the null hypothesis was rejected. Consequently, the null hypothesis of no significant difference in the treatment effects was tested. From Fig. 2, the $rob(\tau_{\mathrm{A}}-\tau_{\mathrm{B}})>0=1$. This implies that the treatment effects were significant, thus, the null hypothesis was rejected. Finally, the null hypothesis of no significant difference in the treatment effects given carry-over effects was tested. From Fig. 3 $\mathrm{prob}((\tau_{\mathrm{A}}-\tau_{\mathrm{B}})/(\lambda_A-\lambda_B))>0=1$. This implies that the treatment effects were significant, thus, the null hypothesis was rejected. The HPD graphs indicate that it is possible to test for treatment effects even when the carry-over effects are present using the Bayesian method as shown in Fig. 3. In comparison to the t-test done for the same data by [11]. The null hypothesis of no significant difference in treatment effects at 156 degrees gave the same results at 95% level of significance leading to rejection of the null hypothesis. However, the test for carry-over effects was different since at 156 degrees of freedom, the calculated value was less than the tabulated value leading to acceptance of the null hypothesis. This difference can be attributed to the fact that sometimes the classical analysis methods like the $t-$ test give erroneous results [6].

Table 3. Expected values for design D_1

Sequence	p_1	p_2	p_3	p_4	p_5
BABAA	$E(Y_{11})$ =4.875	$E(Y_{12})$ =4.925	$E(Y_{13})$ =5.775	$E(Y_{14})$ =4.850	$E(Y_{15})$ =4.0125
ABABB	$E(Y_{21})$ =4.525	$E(Y_{22})$ =4.750	$E(Y_{23})$ =4.763	$E(Y_{24})$ =5.113	$E(Y_{25})$ =1.700
BAABA	$E(Y_{31})$ =1.475	$E(Y_{32})$ =3.613	$E(Y_{33})$ =6.488	$E(Y_{34})$ =4.263	$E(Y_{35})$ =6.250
ABBAB	$E(Y_{41})$ =6.163	$E(Y_{42})$ =5.913	$E(Y_{43})$ =2.500	$E(Y_{44})$ =3.275	$E(Y_{45})$ =2.688

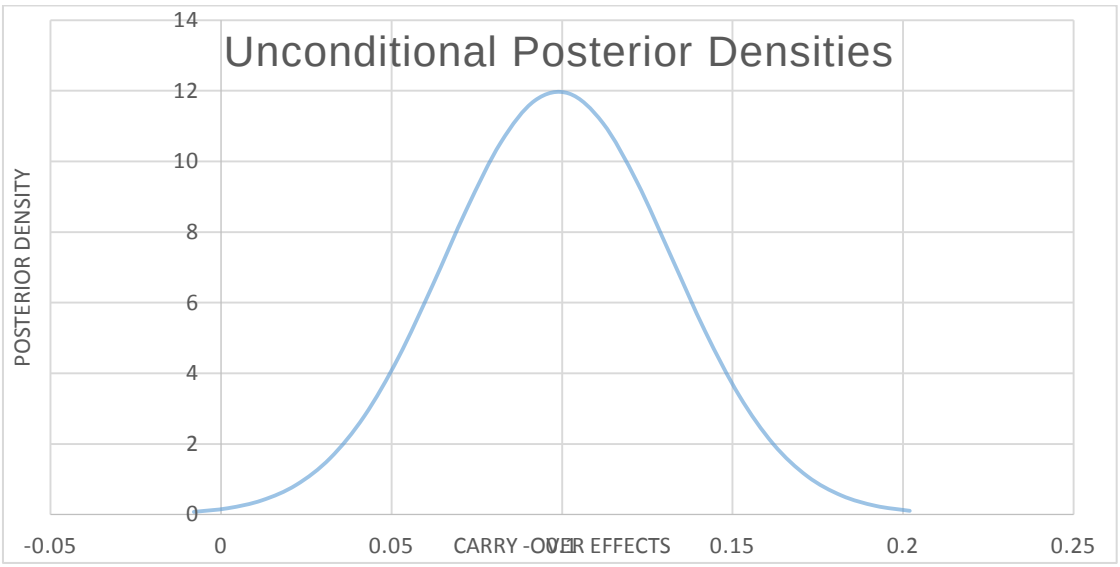

Fig. 1. HPD for carry-over effects

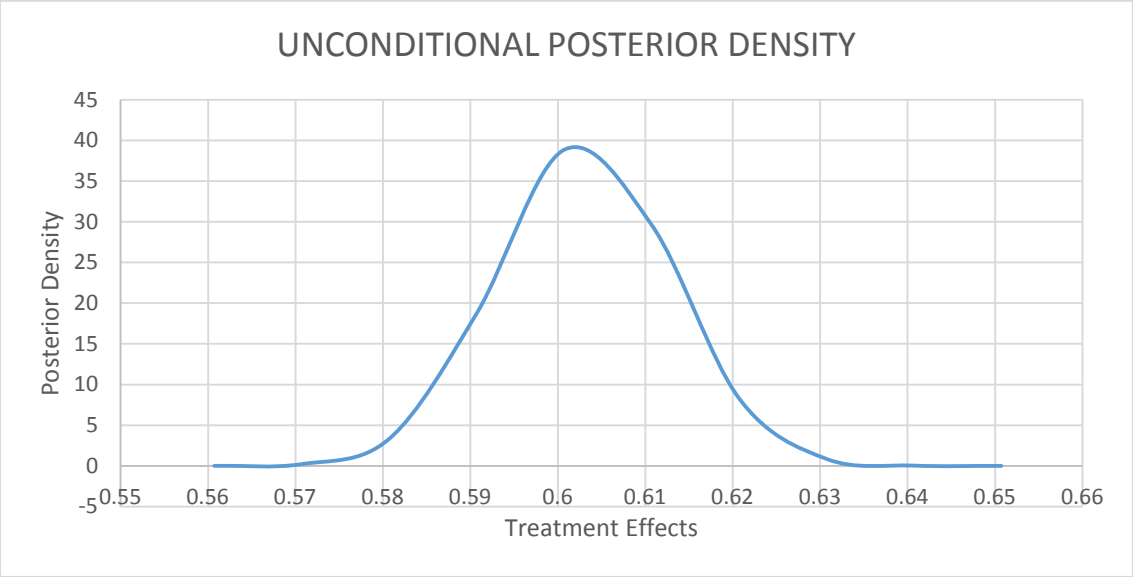

Fig. 2. HPD for treatment effects

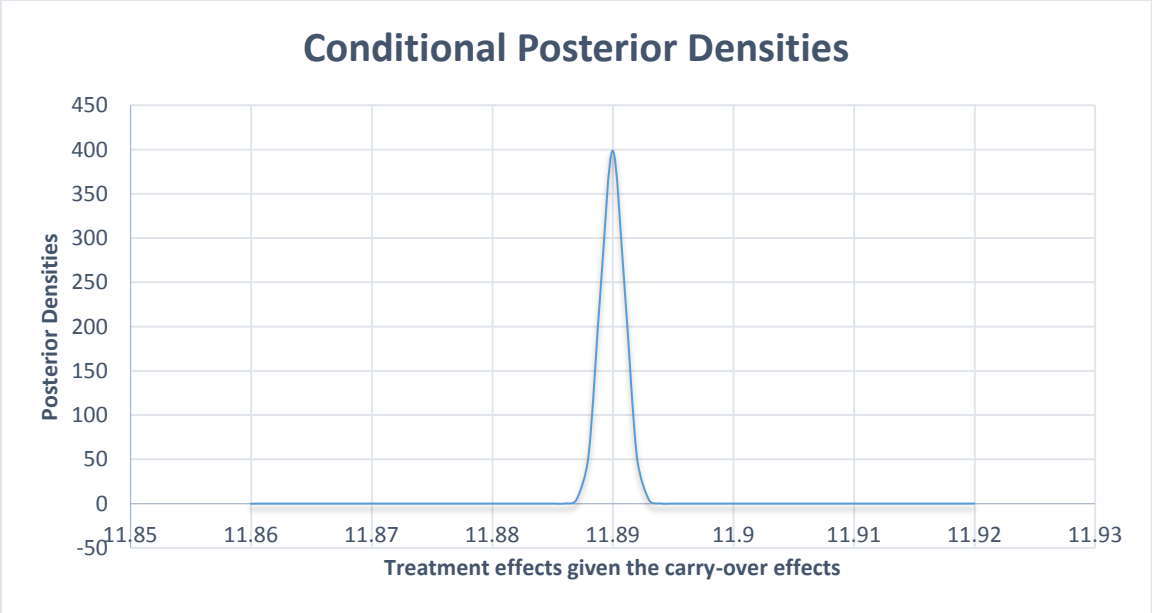

Fig. 3. HPD for Treatment effects in the presence of Carry-over effects

5. CONCLUSION

In this study, an optimal five period cross-over design for two treatments constructed by [11] was considered. The treatment effects difference and the carry-over effects difference for the design is obtained by the best linear unbiased estimation (BLUE) method. The design was analyzed hypothetically by the Bayesian method. The results from the Bayesian method are compared with those obtained from the classical $t-$test. The study gave the same results as those obtained by [11] except for the carry-over effects. The difference in the result is attributed to the fact that the classical methods of analysis are sometimes erroneous. The Bayesian method is thus highly recommended in bioequivalence studies since it gives more accurate results as compared to the classical methods of analysis such as, the $t-$test and ANOVA. The Bayesian method should also be preferred in bioequivalence studies due to the fact that it can test for treatment effects difference even in the presence of carry-over effects.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Jones B, Kenward GM.Design and analysis of cross-over trials, 3rd edn. 2015;138.CRC Press.
2. Reed III J. Extension of grizzle's classic cross-over design. Journal of Modern Applied Statistical Methods. 2011;10(1): 29.
3. Reed III J. Four period cross-over designs. Journal of Modern Applied Statistical Methods. 2012;11(1):25.
4. Grizzle J. The two period change-over design and it's use in clinical trials, Biometrics. 1965;1(1):467-480.
5. Novick M, Grizzle J. A Bayesian approach to the analysis of data from clinical trials. Journal of the American Statistical Association. 1965;60(309);81-96.
6. Westlake W. The design and analysis of comparative blood level trials. Swarbick J.Ed. current concepts in the pharmaceutical sciences, dosage form design and bioavailability. Philadelphia: Lea and Febier,149-179. 1973;1(1):149-179.
7. Westlake W. Symmetrical confidence interval for bioequivalence trials. Biometrics. 1976;1(1):741-744.
8. Patil V. Approximation to the Behren's fisher distributions. Biometrika. 165;52(1/2):267-271.
9. Metzler C. Bioavailability -A problem in Equivalence. Biometrics. 1965;1(1):309-3171.
10. Patil V. The Beherens Fisher Problem andits Bayesian Solution. Journal of the Indian Statistical Association. 1964;2(1):21-31.
11. Nyakundi CN, Koske JK, Mutiso JM, Tum IK. Mathematical Analysis of a Five periods Cross-over design for two treatments. Journal of Biostatistics and Epidemiology. 2021;7(1):48-59.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle4.com/review-history/74000