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Bioequivalence trial with two generic drugs in 2×3 crossover design with missing data

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Abstract

The 2×3 crossover design, a modified version of the 3×3 crossover design, is considered to compare the bioavailability of two generic candidates with a reference drug. The 2×3 crossover design is more economically favorable due to decrease in the number of sequences, rather than conducting a 3×3 crossover trial or two separate 2×2 crossover trials. However, when using a higher-order crossover trial, the risk of drop-outs and withdrawals of subjects increases, so the suitable statistical inferences for missing data is needed. The bioequivalence model of a of 2×3 crossover trial with missing data is defined and the statistical procedures of assessing bioequivalence is proposed. An illustrated example of the 2×3 trial with missing data is also presented with discussion.

Keywords: average bioequivalence, 3×3 crossover design, 3×2 crossover design, missing at random, missing data

1. Introduction

A statistical procedure based on the 3×3 crossover design for assessing average bioequivalence of an original drug and two generic ones at once is proposed. This 3×3 bioequivalence trial has an advantage over conducting two separate 2×2 trials in terms of time and cost. The Korea Food and Drug Administration (KFDA, currently MFDS; Ministry of Food and Drug Safety) officially announced the partial acceptance for the regulation of the bioequivalence results from the 3×3 crossover design under the conditions that generic drugs contain the same active ingredients and are produced by the same company. Lee *et al.* (1998) proposed a model for assessing bioequivalence using a 3×3 crossover design which is a simple extension of the standard 2×2 design and Oh *et al.* (1999) discussed Lee's work from a statistical point of view.

We believe that the 3×3 crossover design can be further modified to a 2×3 crossover design. The 2×3 crossover comprises two sequences with three periods, rather than three sequences in the case of the 3×3 crossover design. Such a deletion of a sequence would allow for a precise estimate of treatment effect while utilizing the same or less number of subjects; therefore, more ethically and economically favorable. Recently, Lim *et al.* (2005) proposed a statistical method of assessing average bioequivalence in 3×3 crossover design when some data are missing. Lim's method is a modified version of Chow and Shao (1997) in 3×3 crossover design. Inferred from these statistical methods,

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Table 1: 3×3 crossover design

Sequence	Period		
	1	2	3
1	R	T2	T1
2	T2	T1	R
3	T1	R	T2

Table 2: 2×3 crossover design

Sequence	Period		
	1	2	3
1	R	T2	T1
2	T1	R	T2

the 2×3 crossover design could prove to be more efficient and is worthy to explore as a statistical method for assessing average bioequivalence.

In Section 2, we illustrate the statistical model for the 2×3 crossover design and develop simultaneous confidence intervals for drug effects. Next, we discuss the statistical method of constructing simultaneous confidence intervals for drug effects in Section 3 when dropouts occur in the later periods. In Section 4, we provide an example of this method and its results. Lastly in Section 5, concluding remarks are reported.

2. 2×3 crossover design

The standard form of 3×3 crossover trial is given in Table 1, where R, T1, and T2 stand for the reference drug and two generic drugs.

One weakness of the 3×3 crossover design are its difficulty to manage in trial due to the increased number of sequences and periods compared to the 2×2 crossover trial. One may want to reduce some sequence or period to conduct a trial more efficiently like Table 2. It may be more advantageous to reduce the sequence that can adjust the sample size. Let us say the second row is deleted.

The statistical model for the 2×3 crossover design can be written as

$$y_{ijk} = \mu + G_k + P_j + F_{(j,k)} + S_{ik} + \epsilon_{ijk}, \quad (k = 1, 2;\ j = 1, 2, 3;\ i = 1, 2, \ldots, n_k), \tag{2.1}$$

where μ is the overall mean, G_k is the fixed effect of the k^{th} sequence with $\Sigma G_k = 0$, P_j is the fixed effect of the j^{th} period with $\Sigma P_j = 0$, $F_{(j,k)}$ is the fixed effect of the formulation in the k^{th} sequence administered in the j^{th} period such that

$$F_{(j,k)} = \begin{cases} F_R, & (j,k) = (1,1), (2,2), \\ F_{T1}, & (j,k) = (3,1), (1,2), \\ F_{T2}, & (j,k) = (2,1), (3,2), \end{cases}$$

with $\Sigma F_{(j,k)} = 0$, and the subject variability $S_{ik} \overset{\text{iid}}{\sim} N(0, {\sigma_s}^2)$, and the drug variability $\epsilon_{ijk} \overset{\text{iid}}{\sim} N(0, {\sigma_e}^2)$ and they are also assumed to be independent each other.

From the usual ANOVA construction given by Chow and Liu (2008) and Park (2014) assuming the equal sample sizes, we can obtain Table 3.

Table 4 provides the coefficients for estimates of pairwise formulation effects in order to draw a statistical inference on the drug effects.

Table 3: ANOVA table for 2×3 crossover design

Sources	Degrees of freedom	Expected mean squares
Between SS	$2n-1$	
Sequence SS	1	${\sigma_e}^2 + {\sigma_s}^2 + 3n\left({G_1}^2 + {G_2}^2\right)$
Residual SS (Inter)	$2n-2$	${\sigma_e}^2 + 3{\sigma_s}^2$
Within SS		
Period SS	2	${\sigma_e}^2 + \frac{n}{2}\left\{(P_1-P_2)^2 + (P_1-P_3)^2 + (P_2-P_3)^2\right\}$ $+\frac{n}{12}\left\{(F_R-F_{T1})^2 + (F_R-F_{T2})^2 + (F_{T2}-F_{T1})^2\right\}$ $-n\{P_1F_{T2} + P_2F_{T1} + P_3F_R\}$
Drug SS	2	${\sigma_e}^2 + \frac{n}{4}\left\{(F_R-F_{T1})^2 + (F_{T1}-F_{T2})^2 + (F_R-F_{T2})^2\right\}$
Residual SS (Intra)	$4n-4$	${\sigma_e}^2$
Total SS	$6n-1$	

Table 4: Coefficients for estimates of pairwise formulation effects in 2×3 crossover design

Sequence	$\theta_{1R} = F_{T_1} - F_R$				$\theta_{2R} = F_{T_2} - F_R$				$\theta_{21} = F_{T_2} - F_{T_1}$			
	Period			$\Sigma {c_{jk}}^2$	Period			$\Sigma {c_{jk}}^2$	Period			$\Sigma {c_{jk}}^2$
	1	2	3		1	2	3		1	2	3	
1	-1	2	-1	6/9	-2	1	1	6/9	-1	-1	2	6/9
2	1	-2	1	6/9	2	-1	-1	6/9	1	1	-2	6/9
Variance				$\frac{4}{3n}{\sigma_e}^2$				$\frac{4}{3n}{\sigma_e}^2$				$\frac{4}{3n}{\sigma_e}^2$

Coefficients are multiplied by 3; Variance when $n = n_k$ for $k = 1, 2$.

Denoting $\theta_{1R} = F_{T_1} - F_R$, $\theta_{2R} = F_{T_2} - F_R$, and $\theta_{21} = F_{T_2} - F_{T_1}$, the unbiased estimators of these parameters can be obtained through:

$$\hat{\theta}_{1R} = -\frac{1}{6}\bar{Y}_{\cdot 11} + \frac{2}{6}\bar{Y}_{\cdot 21} - \frac{1}{6}\bar{Y}_{\cdot 31} + \frac{1}{6}\bar{Y}_{\cdot 12} - \frac{2}{6}\bar{Y}_{\cdot 22} + \frac{1}{6}\bar{Y}_{\cdot 32},$$
$$\hat{\theta}_{2R} = -\frac{2}{6}\bar{Y}_{\cdot 11} + \frac{1}{6}\bar{Y}_{\cdot 21} + \frac{1}{6}\bar{Y}_{\cdot 31} + \frac{2}{6}\bar{Y}_{\cdot 12} - \frac{1}{6}\bar{Y}_{\cdot 22} - \frac{1}{6}\bar{Y}_{\cdot 32},$$
$$\hat{\theta}_{21} = -\frac{1}{6}\bar{Y}_{\cdot 11} - \frac{1}{6}\bar{Y}_{\cdot 21} + \frac{2}{6}\bar{Y}_{\cdot 31} + \frac{1}{6}\bar{Y}_{\cdot 12} + \frac{1}{6}\bar{Y}_{\cdot 22} - \frac{2}{6}\bar{Y}_{\cdot 32}.$$

Now we can assess average bioequivalence by Dunnett's $(1-2\alpha)\times 100\%$ simultaneous confidence intervals of $\theta_{1R} = F_{T_1} - F_R$ and $\theta_{2R} = F_{T_2} - F_R$

$$\hat{\theta}_{1R} \pm d(\alpha, 2, 4n-4)\sqrt{\frac{4\mathrm{MS}_{\text{intra}}}{3n}},$$
$$\hat{\theta}_{2R} \pm d(\alpha, 2, 4n-4)\sqrt{\frac{4\mathrm{MS}_{\text{intra}}}{3n}},$$

where $d(\alpha, a, b)$ is the critical values for Dunnett's test (a is number of treatment means and b is the degrees of freedom) and $\mathrm{MS}_{\text{Intra}}$ is obtained from Table 3.

One can claim the bioequivalence among drugs if the calculated $(1-\alpha)\times 100\%$ simultaneous confidence intervals based on log transformed scale belong to the pre-assigned limit $(\log(0.8), \log(1.25))$.

3. 2×3 crossover design with missing data

The 2×3 crossover design consists of three periods of testing periods with washouts between the periods. Responses are often not obtained properly for various reasons, such as protocol violations,

Table 5: 2×3 Crossover design with dropouts

Sequence	Period		
	1	2	3
1	R $Y_{111}, Y_{211}, \ldots Y_{n_1 11}$	T1 $Y_{121}, Y_{221}, \ldots, Y_{m_{11}21}$ $(n_1 - m_{11})$ missing	T2 $Y_{131}, Y_{231}, \ldots, Y_{m_{12}31}$ $(n_1 - m_{12})$ missing
2	T2 $Y_{112}, Y_{212}, \ldots Y_{n_2 12}$	R $Y_{122}, Y_{222}, \ldots, Y_{m_{21}32}$ $(n_2 - m_{21})$ missing	T1 $Y_{132}, Y_{232}, \ldots, Y_{m_{22}32}$ $(n_2 - m_{22})$ missing

failure of assay methods, or missed visits. The unobserved responses are considered as dropouts. In this design, the subjects are likely to drop out in the second or the third period. Dropouts in the second period would result in missing data in both the second and third period; whereas dropouts in the third period would result in missing data only in the third period.

When we have some dropouts, we might try Grizzle (1965)'s idea after deleting subjects with dropouts. But it may cause some significant loss of information in statistical inference, if subjects with missing data are deleted and statistical analysis is performed. Chow and Shao (1997) proposed a general statistical method to compare a generic drug to the reference one in a two sequence, three period crossover design with unbalanced or incomplete data. Lim *et al.* (2005) extended their method in a 3×3 crossover design and this paper also modifies Lim *et al.*'s way in a 2×3 crossover design.

Without loss of generality, we assume that in the k^{th} sequence, the first m_{k2} subjects have the data for all three periods; the next $m_{k1} - m_{k2}$ subjects have data for period 1 and period 2 like Table 5. The sample sizes $m_{kl}(l = 1, 2)$ may be random and assume whether or not Y_{ijk} is independent of the measurement error ϵ_{ijk} (Chow and Shao, 1997).

One can express the model (2.1) as

$$\mathbf{Y} = \mathbf{X}\beta + \mathbf{ZS} + \epsilon, \tag{3.1}$$

here X is a design matrix, $\beta' = (\mu, G_1, P_1, P_2, F_R, F_{T_1})$ is a fixed parameter vector, $\mathbf{S}' = (S_{11}, \ldots, S_{n_1 1}; S_{12}, \ldots, S_{n_2 2})$ is a vector of random subject effects, $\mathbf{Z}$ is the corresponding subject effect design matrix, and ϵ is a error vector. $\mathbf{S}$ and ϵ are assumed to be mutually independent with $N(\mathbf{0}, {\sigma^2}_s\mathbf{I})$ and $N(\mathbf{0}, {\sigma^2}_e\mathbf{I})$, respectively.

Consider the linear transformation $\mathbf{HY}$ with $\mathbf{HZ}$, where $\mathbf{H}$ is some suitably defined matrix. Since

$$\mathbf{HY} = \mathbf{HX}\beta + \mathbf{H}\epsilon \tag{3.2}$$

the conditional distribution of $\mathbf{HY}$, given $m_{kl}(l = 1, 2)$, is still normal if ϵ is normal and independent of m'_{kl}s. This kind of transformation was used by Fuller and Batesse (1973).

Under model (3.2), we obtain the unbiased estimator P_1, P_2, F_R, F_T by considering a special transformation $\mathbf{H}$ which is equivalent to taking within-subject differences (between two periods). The within-subject differences $d_{i1k}(1 \le i \le m_{k1})$ and $d_{i2k}(1 \le i \le m_{k2})$ are defined as follows

$$\begin{aligned}
d_{i11} &= Y_{i11} - Y_{i21} = P_1 - P_2 + F_R - F_{T_1} + \epsilon_{i11} - \epsilon_{i21'}, \quad 1 \le i \le m_{11},\\
d_{i21} &= Y_{i21} - Y_{i31} = P_2 - P_3 + F_{T_1} - F_{T_2} + \epsilon_{i21} - \epsilon_{i31'}, \quad 1 \le i \le m_{12},\\
d_{i12} &= Y_{i12} - Y_{i22} = P_1 - P_2 + F_{T_2} - F_R + \epsilon_{i12} - \epsilon_{i22'}, \quad 1 \le i \le m_{21},\\
d_{i22} &= Y_{i22} - Y_{i32} = P_2 - P_3 + F_R - F_{T_1} + \epsilon_{i22} - \epsilon_{i32'}, \quad 1 \le i \le m_{22}.
\end{aligned}$$

Let $\mathbf{d}$ be the vector of these differences and then $\mathbf{d}$ is independent of $\mathbf{S}$ and $\mathbf{d} = \mathbf{HY}$ for some $\mathbf{H}$ satisfying $\mathbf{HZ} = \mathbf{0}$. Assuming that m_{kl}'s are independent of ϵ, we obtain

$$\mathbf{d} \sim N\left(\mathbf{W}\theta, \sigma^2{}_e\mathbf{G}\right), \tag{3.3}$$

where

$$\theta' = (P_1 - P_2, P_2 - P_3, F_R - F_{T_1}, F_R - F_{T_2}),$$

$$\mathbf{W} = \begin{bmatrix} \mathbf{1}_{m_{12}} \otimes \begin{pmatrix} 1 & 0 & 1 & 0 \\ 0 & 1 & -1 & 1 \end{pmatrix} \\ \mathbf{1}_{m_{11}-m_{12}} \otimes \begin{pmatrix} 1 & 0 & 1 & 0 \end{pmatrix} \\ \mathbf{1}_{m_{22}} \otimes \begin{pmatrix} 1 & 0 & 0 & -1 \\ 0 & 1 & 1 & 0 \end{pmatrix} \\ \mathbf{1}_{m_{21}-m_{22}} \otimes \begin{pmatrix} 1 & 0 & 0 & -1 \end{pmatrix} \end{bmatrix},$$

$$\mathbf{G} = \mathbf{diag}\{\mathbf{I}_{m_{12}} \otimes \mathbf{B}, 2\mathbf{I}_{m_{11}-m_{12}}, \mathbf{I}_{m_{22}} \otimes \mathbf{B}, 2\mathbf{I}_{m_{11}-m_{22}}\},$$

and

$$\mathbf{B} = \begin{pmatrix} 2 & -1 \\ -1 & 2 \end{pmatrix}.$$

Where $\otimes$ is the Kronecker product, $\mathbf{1}_v$ is the v-vector of ones, $\mathbf{I}_v$ is the identity matrix of order v, and $\mathbf{0}$ is the matrix of 0s of an appropriate order.

Under model (3.3), the maximum likelihood estimator of $\hat{\theta}$,

$$\hat{\theta} = \left(\mathbf{W}'\mathbf{G}^{-1}\mathbf{W}\right)^{-1}\mathbf{W}'\mathbf{G}^{-1}\mathbf{d}. \tag{3.4}$$

The estimator of the covariance matrix of $\hat{\theta}$ can be obtained by the least square methods:

$$\hat{\sigma}_e^2\left(\mathbf{W}'\mathbf{G}^{-1}\mathbf{W}\right)^{-1}, \tag{3.5}$$

where

$$\hat{\sigma}_e^2 = \frac{\mathbf{d}'\left[\mathbf{G}^{-1} - \mathbf{G}^{-1}\mathbf{W}\left(\mathbf{W}'\mathbf{G}^{-1}\mathbf{W}\right)^{-1}\mathbf{W}'\mathbf{G}^{-1}\right]\mathbf{d}}{m_{11} + m_{21} + m_{12} + m_{22} - 4}. \tag{3.6}$$

By using equations (3.4), (3.5), and (3.6) we can then construct an exact confidence interval for $l'\theta$ with a fixed vector l because

$$\frac{l'\hat{\theta} - l'\theta}{\hat{\sigma}_e^2\sqrt{l'\left(\mathbf{W}'\mathbf{G}^{-1}\mathbf{W}\right)^{-1}l}}$$

has a t-distribution with $m_{11} + m_{21} + m_{12} + m_{22} - 4$ degrees of freedom.

From this we can calculate Dunnett's test $(1-2\alpha)\times 100\%$ confidence intervals for $F_R - F_{T_1}$, $F_R - F_{T_2}$ for two drug formulations with the reference one. The bioequivalence among drugs if the calculated 90% simultaneous confidence intervals based on log transformed scale belong to pre-assigned limit $(\log 0.8, \log 1.25)$.

Table 6: AUC value for Ondansetron example adopted from Lim *et al.* (2005)

Sequence Period	Sequence	Period 1	Period 2	Period 3
1		12176	11424	14319
2		10913	12114	11640
3		11004	11802	11234
4	1	14377	15322	14700
5		15110	18308	18598
6		21644	23917	(24176)
7		11367	(10524)	(13224)
8		12153	9771	12794
9		14121	12292	18396
10		6339	7860	7907
11	2	20062	17667	23253
12		12306	17170	(15114)
13		19123	(15472)	(17058)
14		20043	(15816)	(19540)

( ): missing data

4. An illustrative example

Table 6 shows Ondansetron example data given by Lim *et al.* (2005), but with one sequence deleted. Ondansetron is used to prevent nausea and vomiting caused by cancer chemotherapy, radiation therapy, anesthesia, and surgery. Ondansetron comes as the brand name drug Zofran and a company developed generic drugs Vominon 4 mg and Vomonon 8 mg for Zofran. The 21 healthy Korean male subjects selected from the well defined protocol were randomized and received each formulation. The plasma concentrations of Ondansetron were monitored by the high performance liquid chromatography for over a 12 hour period after administration. In this experiment, all planed-for data are actually observed and both test drugs were found to be bioequivalent to Zofran. We will use first and second sequences data. However, pretend that some of the subjects of period 3 in each sequence are dropped out for the purpose of demonstrating the results.

According to MFDS's standard on pharmaceutical equivalence test (2018), bioequivalence analysis should be based on the log-transformed data rather than original one. When the dropouts occur, the common way is to exclude the corresponding subjects' data and analyze the bioequivalence study with the rest of the data. In this case the deletion of subjects with dropouts leads $m_{11} = 6$, $m_{12} = 5$, $m_{21} = 5$, and $m_{22} = 4$. The study loses significant amount of information due to the dropouts and it may cause to declare bioinequivalence.

The 90% confidence intervals $F_R - F_{T_1}$, $F_R - F_{T_2}$ based on the proposed method are

$$F_R - F_{T_1} : (-0.026973, 0.1985201),$$
$$F_R - F_{T_2} : (-0.091839, 0.1336536).$$

The confidence intervals are compared to preassigned limit ($\log 0.8 = -0.22314, \log 1.25 = 0.22314$) and the tested two drugs are claimed to be bioequivalent with the reference drug since both intervals given by the proposed method are within this limit. It is noted that the lengths of intervals based on the proposed method are shorter than ones based on the complete case. Consequently, there is a minor loss of information due to missing data when the proposed method is applied.

5. Conclusion

When determining the bioequivalence of multiple test drugs and a reference drug, performing several

separate 2×2 crossover trials proves less efficient than performing one higher-order crossover trial (Lim *et al.*, 2005). We proposed the statistical inference of 2×3 crossover design with missing data to assess the bioequivalence between two generic drugs with the reference drug. The 2×3 crossover design may grant pharmaceutical companies some economical advantages such as benefits from a decreased trial duration and a financial expenditure from possible decreased number of subjects required to demonstrate bioequivalence. This study may be more meaningful and practical as it includes the statistical procedure and an illustrated example to assess the bioequivalence, especially when missing data occur during the trial. However, it may be needed to show the efficiencies of the 2×3 design and the method of handling missing data under more general circumstances like the well-designed simulation studies in further studies.

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