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Trend resistant designs for bioequivalence assessment of veterinary medicinal products

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ABSTRACT

In veterinary medicinal trials, formulations are to be applied to the animals sequentially over time due to scarcity of homogeneous and healthy animals for experimentation, leading to carryover effects. Further, in such trials, many a times it may be required to compare some new (test) formulations to a previously well established (reference) formulation. Bioequivalence trials, using designs balanced for carryover effects, are advantageous for such situations. As experimental units are used sequentially over periods, there is a possibility that a systematic effect, or trend, influences the observations in addition to the experimental unit effect, formulation effect and carryover effect. Condition have been derived for designs for bioequivalence trials balanced for carryover effects to be trend-free and a method of constructing a class of such designs had also been developed. When trend effects are suspected, trend free designs are to be selected for experimentation and data need to be analyzed accordingly.

Keywords: Bioequivalence trials, Carry over effects, Control formulation, Repeated measurements designs, Test formulation, Trend-free designs

Bioequivalence is defined as the degree to which clinically important outcomes after receiving a new preparation known as test formulation resemble those of a previously well established preparation called reference formulation (Liu and Chow 2000, Oh *et al*. 2003). Evaluation of veterinary medicinal products is one of the important areas where bioequivalence trials are conducted. A veterinary medicinal product is a finished dosage form that contains the active ingredient with or without inactive ingredients. An important aspect in planning a bioequivalence trial is the choice of a good design. Designs that are often considered for bioequivalence studies include some families of parallel designs, crossover designs (CODs) and row-column designs with incomplete columns. However, all these designs give equal importance to all pair-wise comparisons of formulation effects. But, in many bioequivalence studies, the experimenters are more interested in the comparison of several test formulations to an established standard or reference formulation rather than in all pair-wise comparisons. The main interest here lies in making test formulation vs. reference comparisons with as much precision as possible and comparisons within test formulations with less precision. The statistical problem is to obtain suitable arrangements such that the test formulation vs. reference comparisons are estimated with maximum precision.

In bioequivalence trials, as experimental units receive formulations over one after another, it is natural for these units to exhibit time trend over periods. In many animal experiments where observations are recorded over periods, experimental units may exhibit time trend. For example, in dairy cattle, where experimenter wants to study the effect of calcium supplement (Ostocalcium) on the milk yield of dairy cows, the milk yield within lactation exhibits time trend. Therefore, it is necessary to account for these possible trends while carrying out analysis of data and/or designing experiments for such situations. However, appropriate designs for bioequivalence trials seem to be not available which allow estimation of contrasts among formulation effects orthogonal to trend effects. So there is need to obtain robust designs for bioequivalence trials in the presence of systematic trend.

Afsarinejad (2001) investigated the existence and non-existence of trend-free repeated measurement designs. Bhowmik *et al.* (2014) studied block model with neighbour effects from adjacent experimental units incorporating trend component and derived the necessary and sufficient condition for a block design with neighbour effects to be trend free. Subsequently, Bhowmik *et al*. (2015) studied block model with second order neighbour effects in the presence of systematic trend. Trend resistance designs have also been obtained for this situation. Sarkar *et al.* (2017) obtained trend resistant neighbour balanced block designs for test vs. control comparisons.

Bhowmik *et al*. (2018) derived conditions to nullify the effects of trend component when they are present in the

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experimental material considering the model under two-way blocking structure incorporating systematic trend component. These trend effects are generally neglected but they may have significant impact on the precision of the experiments.

MATERIALS AND METHODS

A design for bioequivalence trial is said to be trend-free, if the sum of squares due to treatments under the model considering trend effects besides direct, residual and subject effects, is same as that obtained under the model considering direct, residual and subject effects ignoring trend effects. In these designs direct as well as residual effects contrasts are estimated orthogonal to trend effects. It is assumed that the experimental units exhibit time trend over the periods and the trend effects are represented by orthogonal polynomials of various degrees.

Conditions for a design to be trend-free: Consider a COD for v treatments in p (≤) periods and n experimental units. We assume that the experimental units exhibit the same trend over the periods which can be adequately represented by q (p-1) orthonormal polynomials and the period effects are non-existent. Besides the direct effects and first-order residuals effects of treatments, the observations contain the experimental unit effects. Thus, we have the following additive fixed effects model for the observations:

$$Y = \mu 1 + D_1 \tau + D_2 \rho + S \psi + Z\alpha + \varepsilon \qquad ...(1)$$

where Y, a (np × 1) vector of observations; 1, column vector of unities and D_1, D_2, S and Z are respectively, the design matrices for direct effects, first-order residual effects, experimental unit effects and trend effects; μ is the general mean, τ, ρ, Ψ and α are the column vectors of v direct effects, v first-order residual effects, n unit effects and q (≤ p-1) trend effects, respectively. And ε is the column vector of independently, identically distributed normal with mean zero and variance σ^2.

In order to derive the conditions for the design to be trend free, we rewrite the Model (1) as:

$$Y = X_1 \theta_1 + X_2 \theta_2 + \varepsilon \qquad ...(2)$$

with $X_1 = [D_1 D_2]$, and the corresponding coefficient vector of parameters of interest $\theta_1 = [\tau\ \rho]'$, $X_2 = [S\ Z\ 1]$, and the coefficient vector of other factors $\theta_2 = [\Psi\ \alpha\ \mu]'$. The matrix Z can be written as

$$Z = 1_n \otimes \xi_{p\times q}$$

where $\xi_{p\times q}$ is the p×q matrix of orthonormal polynomials so that $\xi'_{p\times q}\, \xi_{p\times q} = I_q$, an identity matrix of order q.

In absence of time trend, the additive fixed effects model is

$$Y = X_1 \theta_1 + X_3 \theta_3 + \varepsilon \qquad ...(3)$$

where $X_3 = [S\ 1]$ and $\theta_3 = [\Psi\ \mu]'$.

The design is said to be trend-free, if the sum of squares due to fitting of θ_1 after eliminating the effect of θ_2 under Model (2), R (θ_1 | θ_2) is the same as the sum of squares due to fitting of θ_1 after eliminating the effect of θ_3 under Model (3), R (θ_1 | θ_3), i.e.

$$R(\theta_1 \mid \theta_2) = R(\theta_1 \mid \theta_3) \qquad ... (4)$$

Evidently,

$$R(\theta_1 \mid \theta_2) = Y' H_1 X_1 C_1^- X'_1 H_1 Y \text{ and}$$
$$R(\theta_1 \mid \theta_3) = Y' H_2 X_1 C_2^- X'_1 H_2 Y \qquad ... (5)$$

Here,

$$H_i = I - X_{i+1} (X'_{i+1} X_{i+1})^- X'_{i+1},\ C_i = X'_i H_i X_1$$

with C_i^- and $(X'_{i+1} X_{i+1})^-$ being the generalized inverses of C_i and $(X'_{i+1} X_{i+1})$, respectively (i = 1, 2).

Thus, in view of (5), the condition (4) becomes

$$Y' H_1 X_1 C_1^- X'_1 H_1 Y = Y' H_2 X_1 C_2^- X'_1 H_2 Y$$

for all values of Y, implying

$$H_1 X_1 C_1^- X'_1 H_1 = H_2 X_1 C_2^- X'_1 H_2 \qquad ... (6)$$

Pre- and post-multiplication of both sides of equation (6) by X'_1 and X_1 respectively, gives

$$X'_1 (H_1 - H_2) X_1 = 0,$$

since $C_1 = X'_1 H_1 X_1$ and $C_2 = X'_1 H_2 X_1$ and using $AA^-A = A$; A^- being a g-inverse of A.

That is,

$$X'_1 [X_2 (X'_2 X_2)^- X'_2 - X_3 (X'_3 X_3)^- X'_3] X_1 = 0 \qquad ... (7)$$

Now, because

$$X'_2 X_2 = \begin{bmatrix} S'S & S'Z & S'1 \\ Z'S & Z'Z & Z'1 \\ 1'S & 1'Z & 1'1 \end{bmatrix} = \begin{bmatrix} pI_n & 0_{n\times q} & p1_n \\ 0'_{q\times n} & nI_q & 0_{q\times 1} \\ p1'_n & 0_{1\times q} & np \end{bmatrix}$$

$$(X'_2 X_2)^- = \begin{bmatrix} \frac{I_n}{n} & 0 & 0 \\ 0 & \frac{I_q}{n} & 0 \\ 0 & 0 & 0 \end{bmatrix} \text{ and}$$

$$X_2 (X'_2 X_2)^- X'_2 = \frac{SS'}{p} + \frac{ZZ'}{n} \qquad ... (8)$$

Similarly,

$$X_3 (X'_3 X_3)^- X'_3 = \frac{SS'}{p} \qquad ... (9)$$

In view of (8) and (9), the condition (7) becomes

$$X'_1 ZZ' X_1 = 0$$

giving

$$\begin{bmatrix} D'_1 ZZ' D_1 & D'_1 ZZ' D_2 \\ D'_1 ZZ' D_1 & D'_2 ZZ' D_2 \end{bmatrix} = 0$$

That is,

$$\left.\begin{matrix} D'_1 Z = 0 \\ D'_2 Z = 0 \end{matrix}\right\} \qquad ... (10)$$

A design for bioequivalence trial is said to be robust against trend or trend free design, if the sum of squares due to formulations under the model considering trend effects besides direct, residual and subject effects, is same as that obtained under the model considering direct, residual and subject effects ignoring trend effects. In these designs direct as well as residual effects contrasts are estimated orthogonal to trend effects. Hence, the conditions given in (10) ensure that design for bioequivalence trial is trend free.

RESULTS AND DISCUSSION

Method of construction of trend-free designs for bioequivalence trials: Let the number of formulations (test + control) be v–1, where v be an odd prime number. Denote the test formulations by the symbols 1, 2 and so on; v–2 and the control by 0. Juxtapose (v–1) initial sequences with contents {0, 1, 2, and so on; (v–1)}; one after another. Develop the first initial sequence, up to r rows ($2 \le r \le v$), by adding one to each preceding row; second initial sequence by adding 2; third by adding 3; …(v–1)th by adding (v–1); Take mod (v–1) whenever it exceeds (v–1) and then replace each (v–1) by the control 0. The final arrangement has r rows and v (v–1) columns. Now, treat rows as periods (number of periods $p \le v$, as per the resources available with the experimenter) and columns as experimental units. Considering the first period as pre-period (observations are not to be recorded from this period; however it brings smoothness in deriving the conditions for the design to be trend free), this array will constitute a design for bioequivalence trials balanced for carry over effects.

Example 1: Let v = 5. The two-period trend free design for four test treatments and one control treatment having 20 experimental units (Table 1) can be obtained as follows:

$$S=\begin{bmatrix} 1 & 0 & 0 & . & . & 0 & 0 \\ 1 & 0 & 0 & . & . & 0 & 0 \\ 0 & 1 & 0 & & & . & . \\ 0 & 1 & 0 & . & & . & . \\ . & . & 1 & & . & . & . \\ . & . & 1 & & & 1 & 0 \\ . & . & . & & & 1 & 0 \\ 0 & 0 & . & . & . & 0 & 1 \\ 0 & 0 & . & . & . & 0 & 1 \end{bmatrix}_{40\times 20} \qquad Z=\begin{bmatrix} 1 \\ 1 \\ 1 \\ . \\ . \\ . \\ 1 \\ 1 \\ 1 \end{bmatrix}_{20\times 1} \otimes \begin{bmatrix} \frac{1}{\sqrt{2}} \\ -\frac{1}{\sqrt{2}} \end{bmatrix} = \frac{1}{\sqrt{2}}\begin{bmatrix} 1 \\ -1 \\ 1 \\ -1 \\ . \\ . \\ . \\ 1 \\ -1 \end{bmatrix}_{40\times 1}$$

$S'S = 2I_{20}$, $S'Z = 0_{20\times 1}$, $S'1 = 21_{20\times 1}$, $Z'Z = 20I_{1\times 1}$, $Z'1 = 0_{1\times 1}$ and $1'1 = 40$

$$X_2'X_2=\begin{bmatrix} 2I_{20} & 0_{20\times 1} & 21_{20} \\ 0'_{1\times 20} & 20I_{1\times 1} & 0_{1\times 1} \\ 21'_{1\times 20} & 0_{1\times 1} & 40 \end{bmatrix} \text{ with } (X_2'X_2)^- = \begin{bmatrix} \frac{I_{20}}{2} & 0 & 0 \\ 0' & \frac{1}{20} & 0 \\ 0' & 0' & 0 \end{bmatrix}$$

Therefore

$$X_2(X_2'X_2)^- X_2' = \frac{SS'}{p} + \frac{ZZ'}{n}$$

Again,

$$X_3'X_3 = \begin{bmatrix} 2I_{20} & 21_{20} \\ 21'_{20} & 40 \end{bmatrix}$$

and

$$(X_3'X_3)^- = \begin{bmatrix} \frac{I_{20}}{2} & 0 \\ 0' & 0 \end{bmatrix}$$

similarly,

$$X_3(X_3'X_3)^- X_3' = \frac{SS'}{p}$$

$$X_1'[X_2(X_2'X_2)^- X_2' - X_3(X_3'X_3)^- X_3']X_1 = 0$$

$$X_1' Z Z' X_1 = 0$$

giving

$$\begin{bmatrix} D_1'ZZ'D_1 & D_1'ZZ'D_2 \\ D_1'ZZ'D_1 & D_2'ZZ'D_2 \end{bmatrix} = 0$$

That is,

$$D_1' Z = 0$$
$$D_2' Z = 0$$

Example 2: Let v = 5. The three-period trend free design for four test treatments and one control treatment having 20 experimental units (Table 2) can be obtained as follows:

Giving rise to

$$\begin{bmatrix} D_1'ZZ'D_1 & D_1'ZZ'D_2 \\ D_1'ZZ'D_1 & D_2'ZZ'D_2 \end{bmatrix} = 0$$

Hence, condition for design to be trend free is satisfied. That is,

$$D_1' Z = 0$$
$$D_2' Z = 0$$

Hence, the considered design is trend resistant indicating that even in the presence of trend between observations in successive experimental periods within a unit, the design remains equally efficient as it performs in the absence of any systematic trend.

The proposed designs are useful for situations where experimental units exhibit a systematic time trend over

Table 1. Two period trend free design for 4 test treatments and 1 control treatment.

Periods	Experimental units																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
0	0	1	2	3	0	0	1	2	3	0	0	1	2	3	0	0	1	2	3	0
I	1	2	3	0	0	2	3	0	0	1	3	0	0	1	2	0	0	1	2	3
II	2	3	0	0	1	0	0	1	2	3	1	2	3	0	0	3	0	0	1	2

Table 2. Three period trend free design for 4 test treatments and 1 control treatment

Periods	Experimental units																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
0	1	2	3	0	0	0	1	2	3	0	0	1	2	3	0	0	1	2	3	0
I	1	2	3	0	0	2	3	0	0	1	3	0	0	1	2	0	0	1	2	3
II	2	3	0	0	1	0	0	1	2	3	1	2	3	0	0	3	0	0	1	2
III	3	0	0	1	2	1	2	3	0	0	0	0	1	2	3	2	3	0	0	1

periods. It allows the estimation of formulation effects and carryover effects orthogonal to trend effects. This type of effects should be taken into account both when the experiment is planned and when the results are analyzed. By adopting a trend resistant design in the planning stage will ensure the accuracy of results obtained even if a systematic trend exists within the observations taken from an experimental unit over various periods.

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