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Row-column designs for investigational products vs. control comparisons in veterinary trials

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ABSTRACT

Veterinary trials are generally conducted for drug testing which uses experimental designs like parallel group designs, incomplete block designs, row-column designs, crossover designs, etc. In trials that are specially planned for making comparisons between investigational products with control(s), row-column designs eliminating variation due to periods and animals are most suitable. The present study demonstrated 2 methods of constructing small and efficient row-column designs for investigational products vs. control comparisons. The first method provides designs (Series-I) for number of investigational products ≤ 20 along with variance of estimated elementary contrasts pertaining to various comparisons of interest. The limitation of these designs is that these designs cannot be obtained for comparing odd number of investigational products with one (two) control(s). The second method (Series-II) yields designs for comparing all odd prime number of investigational products with one (two) control(s) and fills almost all the gaps left by Series-I. Thus, both series together provide a wide range of small and efficient row-column designs suitable for comparing a group of investigational products with one (two) control(s). The canonical efficiency factor of both the series of designs in comparison to an orthogonal design having the same number of investigational products has also been calculated considering a nested model with observational units nested within experimental units. These designs are found to be highly efficient.

Key words: Active control, Canonical efficiency, Double diagonal Latin square, Nested model, Row column designs, Veterinary trials

The process of drug development includes testing them on animals in controlled situations. Commonly used experimental units are fish, cats, dogs, rabbits, guinea pigs, cattle, etc. For ethical and economic reasons, it is important to use an efficient design, to analyze the data correctly and to use the minimum number of animals to achieve the objectives. Due to inherent physiological or genetic variations, it is difficult to group or form blocks in animals. Hence, drugs are given to individual animals over varying periods of time leaving sufficient rest periods in between. Thus, row-column designs (RCDs) eliminating 2 cross-classified sources of variation, one source due to animals and the other due to periods, are most suitable for such situations. Many a times, experimenters are interested in making different types of comparisons among the formualtions (EMEA 2001) in different types of trials like superiority trials, equivalence trials and non-inferiority trials. Many active control trials are designed to show that the efficacy of an investigational product is no worse than that of the active comparator and hence fall into the category of non-inferiority studies. Using a RCD for investigational product vs. control comparisons will prove worthy for any of these situations. Freeman (1975) gave methods for constructing such designs. Ture (1994a, 1994b) studied the problem of identification, construction and cataloguing of optimal RCDs for test vs. control comparisons. Majumdar and Tamhane (1996) proposed a class of balanced treatment vs. control RCDs. Majumdar (1996) reviewed the work done on RCDs for test vs. control comparisons. Parsad and Gupta (2001) also gave some methods of construction of such designs.

In some experimental situations where each treatment group is kept together in one location, the location rather than the animal becomes the experimental unit; but observations are taken from individual animals. Considering a nested set up in the model with observational units nested under experimental units, is advisable for such situations. Other situations for considering a nested model are described in EMEA (2001) and Bailey (2009) like animals kept in a cage, udder of a cow, microscopic fields within histological sections from an animal, different bones of same animal, a

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fish tank, chickens housed in groups of hundreds (layers) or many thousands (broilers), litter of pigs (sow plus 10-12 piglets) or weaner pool (25-50) or fattening group (pens of 10-40).

In this study, 2 methods of constructing small and efficient RCDs for showing the superiority or equivalence or non-inferiority of investigational products with one (two) active control(s) are obtained. The first method (Series-I) yields designs for comparing all even numbers of investigational products with one (two) control(s) whereas the second method (Series-II) provides designs for comparing all odd prime numbers of investigational products with one (two) control(s). The two active controls situation can be considered as a trial with one active control and placebo, as active control equivalence or non-inferiority trials may also incorporate a placebo, thus pursuing multiple goals in one trial like establishing superiority to placebo and at the same time allowing comparisons of the investigational products with an active control. Considering a nested model, the variance of estimated elementary contrasts pertaining to various comparisons of interest has been computed and the canonical efficiency of these designs as compared to an orthogonal design with the same number of investigational products has also been studied.

MATERIALS AND METHODS

Seires-I designs: Let v (an even number) represents the number of investigational products. Consider a Latin square with (v+1) symbols (denoted by 1, 2, 3, …, v+1) arranged in (v+1) × (v+1) array in standard order. Symbols in the main diagonal of such an array are always distinct as (v+1) is an odd number. Deleting the first column and last row of the array, results in a v×v array. In the backward diagonal, symbol (v+1) is occurring v times. All other symbols appear in the array (v-1) times. This is a RCD for comparing v investigational products with an active control. Now in the forward diagonal, each of the v symbols, except the symbol (v+1), is appearing exactly once. Replacing these forward diagonal elements by a new symbol, say (v+2), it will also be occurring v times. The final array results in a RCD in v rows and v columns for comparing v investigational products with two active controls.

Let v = 6. Consider the following 7×7 Latin square in standard form:

1	2	3	4	5	6	7
2	3	4	5	6	7	1
3	4	5	6	7	1	2
4	5	6	7	1	2	3
5	6	7	1	2	3	4
6	7	1	2	3	4	5
7	1	2	3	4	5	6

Deleting first column and last row would result in a RCD for comparing 6 investigational products with an active control (denoted by 7) in 6 rows and 6 columns as shown below:

2	3	4	5	6	7
3	4	5	6	7	1
4	5	6	7	1	2
5	6	7	1	2	3
6	7	1	2	3	4
7	1	2	3	4	5

Further, replacing the diagonal elements by another control (denoted by 8), the following arrangement can be obtained:

8	3	4	5	6	7
3	8	5	6	7	1
4	5	8	7	1	2
5	6	7	8	2	3
6	7	1	2	8	4
7	1	2	3	4	8

The above array is a RCD for comparing 6 investigational products (represented by 1, 2, 3, 4, 5, 6) with 2 active controls (denoted by 7, 8) in 6 rows and 6 columns having $r_1 = 4$ and $r_2 = 6$. The properties of the designs obtained have been studied and it was seen that the investigational products in these designs follow a two-associate class partially balanced association scheme (Das and Giri 1986).

Series-II designs: When v is an odd integer, one cannot easily obtain a Latin square for (v+1) symbols with distinct main diagonal elements and may not be able to obtain a RCD for comparing v investigational products with one (two) active control(s) using the method discussed in Series I. In such situations, one may consider a double diagonal Latin square for the purpose. A double diagonal Latin square is one in which each symbol i ($1 \leq i \leq v+1$) occurs exactly once in each row, each column, on the main diagonal and also on the back diagonal.

Let v be an odd prime number ≥ 5. A double diagonal Latin square of order v can be constructed by developing an initial column obtained using the following steps:

Step 1: Fill the entries in the alternative rows of the first column in decreasing order starting from v.

Step 2: When all the odd number of rows of the first column are filled, go back and fill the remaining symbols in all even number of rows, starting from the second row.

Develop the initial column in a decreasing order cyclically mod v to the right hand side to obtain the other (v-1) columns. The final array is a v×v double diagonal Latin square i.e., in the main as well as back diagonal all the v elements appear exactly once.

Now, replacing all the main diagonal elements by a

control, say (v+1), results in a RCD for comparing v investigational products with a control. Here, the investigational products are replicated (v - 1) times and the control is replicated v times.

Further, one can obtain a RCD for comparing v investigational products with two controls by replacing the back diagonal entries by another control, say (v + 2). Since v is an odd prime number, the intersection element at [(v+1)/2, (v+1)/2]th position, of main and back diagonal is common. So [(v-1)/2, (v+3)/2] and [(v+1)/2, (v+1)/2]th positions are left undisturbed and instead the positions [(v-1)/2, (v+1)/2] and [(v+1)/2, (v+3)/2] are replaced by the control (v + 2), to let the main diagonal pass unbroken. The elements removed from positions [(v-1)/2, (v+1)/2] and [(v+1)/2, (v+3)/2] have to be placed in positions [(v-3)/2, (v+3)/2] and [(v+3)/2, (v+1)/2], so that in the resultant array all the investigational products are equally replicated. This yields a RCD for comparing v investigational products with two controls replicated (v-2) and v respectively.

Let v =7. A double diagonal Latin square of order 7×7 can be obtained as follows:

7	6	5	4	3	2	1
3	2	1	7	6	5	4
6	5	4	3	2	1	7
2	1	7	6	5	4	3
5	4	3	2	1	7	6
1	7	6	5	4	3	2
4	3	2	1	7	6	5

Now, replacing all the main diagonal elements by a control, say 8, results in a RCD for comparing 7 investigational products with a control as given below:

8	6	5	4	3	2	1
3	8	1	7	6	5	4
6	5	8	3	2	1	7
2	1	7	8	5	4	3
5	4	3	2	8	7	6
1	7	6	5	4	8	2
4	3	2	1	7	6	8

In the above array, replace the back diagonal elements by another control, say 9. However, the [3, 5] and [4, 4]th positions, i.e., 2 and 8 are left undisturbed and instead the positions [3, 4] and [4, 5] positions, i.e., 3 and 5 are replaced by the second control 9, to let the main diagonal pass unbroken.

8	6	5	4	3	2	9
3	8	1	7	6	9	4
6	5	8	9	2	1	7
2	1	7	8	9	4	3
5	4	9	2	8	7	6
1	9	6	5	4	8	2
9	3	2	1	7	6	8

Now, 3 and 5 have to be placed in place of 2 and 6 in positions [5, 4] and [2, 5] respectively. This results in a RCD for comparing 7 investigational products with 2 controls (8 and 9) replicated 5 and 7 times respectively.

8	6	5	4	3	2	8
3	8	1	7	5	9	3
6	5	8	9	2	1	6
2	1	7	8	9	4	2
5	4	9	3	8	7	5
1	9	6	5	4	8	1
9	3	2	1	7	6	9

Model

Many times, there are many observational units within each experimental unit. For example, few animals from different farms are considered for the experiment and same investigational product/placebo/active control is given to all the selected animals in a given farm over various periods of time. Here farms are experimental units while selected animals are the observational units. Number of animals in each farm may or may not be a constant. Appropriate nested model with observational units nested within experimental units (given below) is considered for such situations.

$$y_{ijlm} = \mu + \Pi_i + \phi_j + \alpha_{l(j)} + \tau_m + \varepsilon_{ijlm} \quad (1)$$
$$i = 1,2,\ldots,p;\ j = 1,2,\ldots,f;\ l = 1,2,\ldots,k;\ m = 1,2,\ldots,v$$

where y_{ijlm} is the observation taken from the l^{th} observational unit (animal) nested within the j^{th} experimental unit (farm) in i^{th} row (period) receiving m^{th} investigational product/placebo/active control. The μ, Π_i, ϕ_j, $\alpha_{l(j)}$ and τ_m represent the general mean, effect of period i, effect of j^{th} farm, effect of the l^{th} animal within the j^{th} farm, effect of treatment m; respectively. ε_{ijlm} are random errors assumed to be identically and independently distributed as $N(0,\sigma^2)$.

Considering the nested model (1), the canonical efficiency of the designs obtained under Series-I as compared to an orthogonal design with same number of investigational products has been studied by developing a SAS code in PROC IML and listed along with other parameters (for designs having investigational products ≤ 20) in Table 1. In a similar manner, the canonical efficiency of the designs obtained under Series-II (for designs having investigational products < 20) has been studied under a nested set up using the same program and these efficiencies are presented along with other parameters in Table 2 (Supplementary files available online).

In these tables, v = number of investigational products, c = number of active control(s) / placebo, p = number of periods under investigation, f = number of farms, k = number of observational units considered under each experimental unit, $\bar{V}_{(i,i)}$ = average variance of the estimated elementary contrasts pertaining to all possible comparisons among

investigational products, $\bar{V}_{(i,i)}$ = average variance of the estimated elementary contrasts pertaining to all possible comparisons between investigational products and active control(s) / placebo and Ec = Canonical efficiency factor of the row-column designs for making comparisons of investigational products with active control(s)/ placebo under a nested model in comparison to an orthogonal design with same number of treatments.

RESULTS AND DISCUSSION

Designs of Series-I are obtained when the number of investigational products is even when there is (are) one control (two controls / one control and placebo) in the study. Designs in Series-II are constructed when the number of investigational products is odd prime and there is (are) one control (two controls / one control and placebo). Thus, designs in Series-II are complementary to those of Series-I. Both the series together yields a wide range of small and efficient RCDs, suitable for comparing v investigational products with one (two) control(s).

It is observed from the tables that as the number of investigational products increases, there is a reduction in the average variance of the estimated elementary contrasts pertaining to comparisons among investigational products as well as comparisons between investigational products and control(s). Further, the average variance of the estimated elementary contrasts pertaining to comparisons between investigational products and active control(s) / placebo is less than the average variance of the estimated elementary contrasts pertaining to comparisons among investigational products. Hence, these designs can be advantageously used for comparing a set of investigational products with one (two) control(s).

Except for the case of 4 investigational products and 2 controls, the efficiency factor is more than 0.90 indicating the appropriateness of using a nested model. This efficiency factor increases and attains the value close to unity as the number of investigational products exceeds 10.

The number of observational units within each experimental unit should be at least 2 to use a nested model. However, the number of observational units in an experimental unit does not affect the canonical efficiency of the designs as shown in Tables 1 and 2 (As Annexure II and III: Available online).

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