The Design and Data Analysis of Two Stage Cross-Over Test for Medicament Bioavailability

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Abstract. In order to research the logistics bioavailability and sample difference, the two stage cross-over test design is introduced. The two stage cross-over design is explained. The two stage cross-over design of a medicament experiment was realized using SAS and the test data were gained. The test data was analyzed using variance analysis. The bioavailability and sample difference are gained. The study in this paper can be adopted in other fields of test design and data analysis.

1. Introduction

The medicament bioavailability presents the level and velocity of medicament active ingredient in metaboly, and is a key evaluation for quality, security and efficiency [1, 2].

In the test of medicament bioavailability, the two stage cross-over test is often be used. The two stage cross-over test has only 2 levels in every factors, and these 2 levels act on one same object in turn accord to speciality knowledge. The influence upon the measured response of one test facor with 2 levels and two block factors, sample difference and mesure process can be checked using two stage cross-over test. Two stage cross-over test often be used in medician: Qin Xiuping researches the two stage cross trial on woman chiliasm treatment with traditional Chinese medicine and the difference between therapeutic group and controlling group is examined [3]. Qu Yao researches the data analysis of cross trial using variance analysis and SPSS [4]. The cross test design and data analysis are realized in SAS, and the analysis process is present. The content in this paper has referring value and can be used in other fields for test design and analysis.

2. Two stage cross-over design

The two factors in two stage cross-over test have not cross interaction or the cross interaction can be cancelled. The influence of every level in factor is short and not consistent. It has a time interval betwen the tow stage and the accessary effect disappears. The measured index of sample has regressed to original level before next operation acted [5].

2.1. Foundation principle

In a data set of two stage cross test, the measured index is \( y_{ijk} \), the factor level is \( i \), the row block factor level is \( j \), and the column block factor level is \( k \). The value of \( i \) and \( k \) is 1 or 2, and the value of \( j \)
can be ,...2,1 . The standard data is for table 1. The factor C and its subscript are only hints and do not represent really process. Because the amount of factor level and measured process is same, so the index can be found only using $j$ and $k$.

| Table 1. The standard data table |
|----------------------------------|
| test sample | test factor C and measure index (measure unit) |
| measure phase | 1 | 2 |
| 1 | $C_2()$ | $C_1()$ |
| 2 | $C_1()$ | $C_2()$ |
| ... | ... | ... |
| $n$ | $C_1()$ | $C_2()$ |

2.2. Calculating process
The variance analysis of two stage cross test is for table 2.

| Table 2. Variance analysis |
|-----------------------------|
| Source | SS | DF | MS | $F$ |
| processing | $SS_{process}$ | 1 | $SS_{process}$ | $MS_{process} / MS_{error}$ |
| phase | $SS_{procedure}$ | 1 | $SS_{procedure}$ | $MS_{process} / MS_{error}$ |
| sample | $SS_{individual}$ | $n-1$ | $SS_{individual} / (n-1)$ | $MS_{individual} / MS_{error}$ |
| error | $SS_{error}$ | $n-2$ | $SS_{error} / (n-2)$ |  |
| total | $SS_{total}$ | $2n-1$ |  |  |

The calculating formula of every statistic value is for bellow [6] [7].

$$SS_{total} = \sum_{i=1}^{2} \sum_{j=1}^{n} \sum_{k=1}^{2} y_{ijk}^2 - \frac{y_{.}^2}{2n}$$

$$SS_{process} = \frac{1}{n} \sum_{j=1}^{n} y_{.j}^2 - \frac{y_{.}^2}{2n}$$

$$SS_{individual} = \frac{1}{n} \sum_{j=1}^{n} y_{j}^2 - \frac{y_{.}^2}{2n}$$

$$SS_{procedure} = \frac{1}{n} \sum_{k=1}^{2} y_{..k}^2 - \frac{y_{.}^2}{2n}$$

$$SS_{error} = SS_{total} - SS_{process} - SS_{individual} - SS_{procedure}$$

$$y_{..} = \sum_{i=1}^{2} \sum_{j=1}^{n} \sum_{k=1}^{2} y_{ijk}$$  

$$y_{.j} = \sum_{i=1}^{n} y_{ij}$$  

$$y_{j} = \sum_{k=1}^{2} y_{jk}$$  

$$y_{..k} = \sum_{j=1}^{n} y_{jk}$$  

It have repertory medicine B and its corrected medicine A. In order to check the bioavailability of A and B, the AUC is used for evaluation index. 24 samples are random divided into two group A and B are used for the first group in process 1 and 2, and B and An are used for the second group in process 1 and 2. The washing interval is set between two test processes. The SAS 9.2 and Windows XP
Professional are used for calculating. The main hardware consists of double Intel T7100 1.8GHz and RAM 2.47GB. The SAS program consists of data content and process content. The data content is for bellow:

```sas
Data data171031;
Do subject=1 to 24;
Do order=1 to 2;
Input drug $ y@@;
Output;
End; end;
Cards;
An 80.1800 B 67.3550
A 95.9500 B 88.0650
......
```

The process content is for bellow:

```sas
Proc anova;
Class order subject drug;
Model y=order subject drug;
......
The result is as in table 3-5.
```

**Table 3. Test result**

| order | test sample | AUC phase 1 | AUC phase 2 | order | test sample | AUC phase 1 | AUC phase 2 |
|-------|-------------|-------------|-------------|-------|-------------|-------------|-------------|
| 1     | 80.1800     | 67.3550     |             | 3     | 63.2700     | 60.4200     |
| 2     | 95.9500     | 88.0650     |             | 5     | 111.1500    | 101.9350    |
| 4     | 69.0650     | 94.0500     |             | 7     | 45.2200     | 51.8700     |
| 6     | 82.3650     | 75.9050     |             | 9     | 70.2050     | 61.9400     |
| 8     | 82.2700     | 67.0700     |             | 10    | 106.3050    | 108.6800    |
| 12    | 62.2250     | 44.3650     |             | 11    | 56.7150     | 54.7200     |
| 15    | 64.0300     | 51.8700     |             | 13    | 76.7600     | 71.1550     |
| 16    | 55.2900     | 84.6450     |             | 14    | 81.5100     | 92.5300     |
| 18    | 42.9400     | 50.2550     |             | 17    | 58.3300     | 43.7950     |
| 19    | 68.9700     | 63.1750     |             | 20    | 74.0050     | 59.1850     |
| 21    | 62.1300     | 59.8500     |             | 22    | 53.9600     | 58.4250     |
| 23    | 58.8050     | 49.3050     |             | 24    | 70.9650     | 72.2000     |

**Table 4. The output of ANOVA Procedure about model**

| Source        | DF | Sum of Squares | Mean Square | F Value | Pr>F   |
|---------------|----|----------------|-------------|---------|--------|
| Model         | 25 | 12882.14812    | 515.28592   | 6.86    | <.0001 |
| Error         | 22 | 1652.74975     | 75.12499    |         |        |
| Corrected     | 47 | 14534.89788    |             |         |        |
| Total         | 47 |                |             |         |        |
| Source | DF | Anova SS     | Mean Square | F Value | Pr>F |
|--------|----|--------------|-------------|---------|------|
| order  | 1  | 74.62547     | 74.62547    | 0.99    | 0.3298|
| subject| 23 | 12807.30530  | 556.83936   | 7.41    | <.0001|
| drug   | 1  | 0.21735      | 0.21735     | 0.00    | 0.9576|

According to table 4 and table 5, the AUC difference has not static sense in different test process and medicine. It has static sense in different test sample \((F=7.41, P<0.001)\). The bioavailability of A and B is same.

3. Conclusion
When the factor has only 2 levels and these 2 levels are used to same object one by one, this test design is two stage cross test design. Many test design can be transformed to cross design. Cross design is simple, has few process, can check two factors with two levels and block factors such as sample difference and measure phase. Cross design is practicable and can be widely used in logistics and test.

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