World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org

CARRYOVER EFFECT ANALYSIS IN BIOEQUIVALENCE STUDIES FOR CROSSOVER DESIGN

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Article Received on 27/08/2015 **Article Revised on 16/09/2015** **Article Accepted on 06/10/2015**

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ABSTRACT

The crossover design to study the differences in treatments yields a more efficient comparison of treatments than a parallel design. Subjects are on their own controls. The within-patient variation is less than between-patient variation. Crossover design requires a smaller sample size than a parallel design, but achieves same level of statistical power and precision. However, there might be a potential carryover effect in crossover design. Generally more bias is occurred in cross over design compare to parallel design. Thus the effective analysis is done using SAS procedures.

KEYWORDS: Cmax, Subject, Sequence, Period, Treatment, P-value, carry over effect or sequence effect, SAS 9.1.3, Grizzle's model.

INTRODUCTION

A crossover design is one in which individual subjects are given a sequence of treatments rather than one treatment at any time. The subjects cross over from one treatment to another treatment during the course of the trial. This is in contrast to a parallel design where subjects are randomized to a treatment and remain on that treatment throughout the whole trial. Crossover design is commonly used in the early phase trials such as – Bioequivalence studies

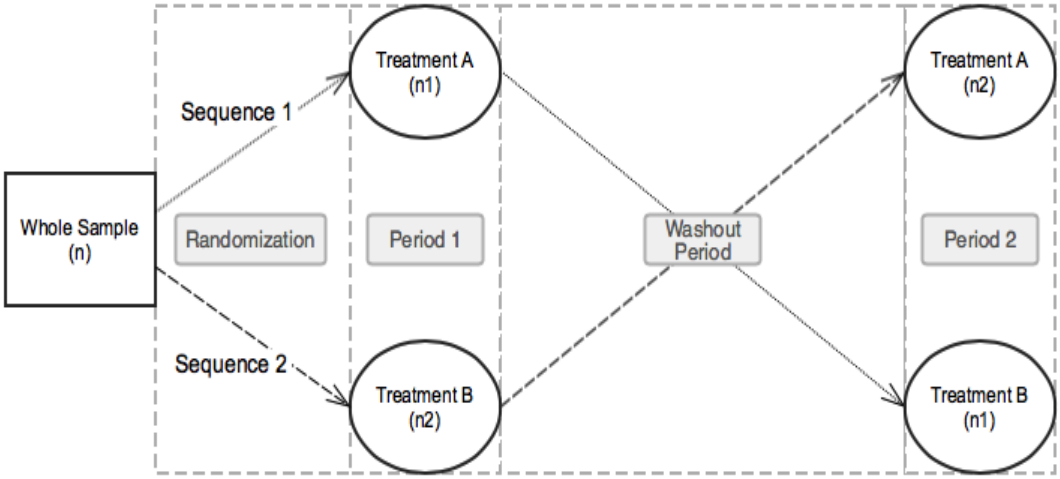
Let the particular disease is devolved among the patients and to apply the different types of treatment (drug) for healing disease. If one treatment cures the patient disease in the first period, then the other treatment will have no chance to demonstrate its effectiveness in the

second period. It's not recommended for crossover design when either treatment is expected to be a cure or the condition disappears in a short period. The crossover design would increase the precision. Each patient serves his or her own control, so the comparisons are usually based on within-patient variability (which is usually less than the between-subject variability). The increased power with greater precision to estimate the treatment differences reduces the sample size.

Model and Design

The order of drug treatment in a crossover study is called a sequence and the time of a treatment is called a period. The treatment is represented with capital letters, such as A, B, etc. The most common crossover design is AB/BA.

Flow chart of cross over design



Subjects (Patients) should be randomized to the sequence. If all subjects receive the two treatments in the same order, the difference between treatments would be confounded with any other changes, which may occur overtime.

For example, in a study of treatment effect of blood sugar level, subjects may change their diet and exercise behavior for as a result of awareness of health issues. This would likely manifest itself as a decrease in blood sugar levels over the later portion of the study and might end up being attributed to the second treatment. In randomization, half of subjects are assigned to receive A/B while other half receive B/A. Any change that favors B over A in one sequence will favor A over B in the other sequence and cancel out of the treatment comparison.

CARRYOVER EFFECTS or SEQUENCE EFFECTS

The potential problem in crossover design is that carryover effects may bias the direct treatment effects. Carryover (or sequence) effect is defined as the effect of the treatment from the previous time period on the response at the current time period. It occurs when the effect of a treatment given in the first time period persists into the second period and distorts the effect of the second treatment. The incorporation of washout period in the design can diminish the impact of carryover effects. The washout period is defined as the time between treatment periods. If the carryover effects for A and B are equivalent in the AB|BA crossover design, then this carryover effect is not aliased with the treatment difference. While differential carryover effects may occur is in clinical trials where an active drug or new drug (A) is compared to placebo or standard drug (B). The subjects in the AB sequence might experience a strong A carryover during the second period, whereas the subjects in BA sequence might experience a weak B carryover during the second period. A washout period should be long enough to minimize the carryover effects. Separate baseline is also helpful to eliminate the carryover effects.

Crossover designs are planned so that each treatment is given an equal number of times in each period. This is most efficient and yields unbiased estimates of treatment differences if a period effect is present. The blood is analyzed for each subject with both first and second periods analyzed concurrently (the same day)

After the blood assays are completed, the blood level Vs time curves analyzed for the derived parameters, AUC_t, $AUC_{0-\infty}$, t_{max}, Kele, $t_{1/2}$.

STATISTICAL DETAILS

Statistical analysis of cross over design is more complex than parallel design; possibility of carryover effects poses added concern in the analysis which is not present in the randomized parallel group design. To apply Dr. James Grizzle's (1965) model to calculate carry over effect. The model is given below:

$$Y_{ijk} = \mu + \alpha_k + \delta_m + \lambda_m + \beta_{ij} + e_{ijk} \quad \text{----------} \quad (1)$$

Where i = sequence (i = 1, 2), j = patient (j=1, 2, 3 , …. ,n), k = period (k=1, 2) and m = treatment (m=1, 2).

μ: overall mean

α_k: effect of k^{th} period

δ_m: direct effect of m^{th} drug treatment

λ_m: residual effect (carryover effect) of m^{th} drug treatment

β_{ij}: effect of j^{th} patient with i^{th} sequence and is ~$N(0, \sigma^2_\beta)$

e_{ijk}: random error and is ~$N(0, \sigma^2_e)$

Effect of Sequence AB/BA.

	Period 1	**Period 2**	**Sum**	**Difference**
Sequence AB	$\mu + \alpha_1 + \delta_{1\,(Y\,1.1)}$	$\mu + \alpha_2 + \delta_2 + \lambda_{1\,(Y\,2.1)}$	Y1.1 +Y2.1	Y1.1 - Y2.1
Sequence BA	$\mu + \alpha_1 + \delta_{2\,(Y\,1.2)}$	$\mu + \alpha_2 + \delta_1 + \lambda_{2\,(Y\,2.2)}$	Y1.2 +Y2.2	Y1.2 - Y2.2

Estimate - Steps:

Step-1: Estimate the Carry over effect.

H_0: $\lambda_1 = \lambda_2$

The effect sum for each sequence can be used for the hypothesis test.

H_0: Y1.1+ Y2.1 = Y1.2+ Y2.2

i.e., $\mu + \alpha_1 + \delta_1 + \mu + \alpha_2 + \delta_2 + \lambda_1 = \mu + \alpha_1 + \delta_2 + \mu + \alpha_2 + \delta_1 + \lambda_2$

Finally $\lambda_1 = \lambda_2$;

If the null hypothesis cannot be rejected, then go to step - 2, otherwise go to step - 3.

Step- 2. Estimate the treatment effect of 2 periods.

H_0: $\delta_1 = \delta_2$

The effect crossover difference for each sequence can be used for the hypothesis test.

H0: (Y1.1- Y2.1)/2 = (Y1.2 - Y2.2)/2

i.e., $(\mu + \alpha_1 + \delta_1 - \mu - \alpha_2 - \delta_2 - \lambda_1)/2 = (\mu + \alpha_1 + \delta_2 - \mu - \alpha_2 - \delta_1 - \lambda_2)/2$

$(\mu + c_1 + d_1 - \mu - c_2 - d_2 - \lambda_1)/2 = (\mu + c_1 + d_2 - \mu - c_2 - d_1 - \lambda_2)/2$

⇨ $\delta_1 - \delta_2 - \lambda_1 = \delta_2 - \delta_1 - \lambda_2$

⇨ $2\,\delta_1 - \lambda_1 = 2\delta_2 - \lambda_2$

assume $\lambda 1 = \lambda 2$

then $\delta_1 = \delta_2$

Step 3. Estimate the treatment effect of period 1.

H0: $\delta_1 = \delta_2$

If carryover effect is significant, then data from period 1 only is used.

H0: Y1.1 = Y1.2

$\mu + \alpha_1 + \delta_1 = \mu + \alpha_1 + \delta 2$

that is $\delta_1 = \delta_2$

DATA ANALYSIS

The analysis of crossover studies is more complex than parallel group designs. The following is an example of AB/BA crossover study. The output using SAS 9.1.3 statistical software.

Randomization Schedule for Cross over design of 12 Subjects.

PATIENT ID	SEQUENCE	PERIOD 1	PERIOD 2
001	2	B	A
002	1	A	B
003	2	B	A
004	1	A	B
005	2	B	A
006	2	B	A
007	1	A	B
008	1	A	B
009	2	B	A
010	2	B	A
011	1	A	B
012	1	A	B

Thus the following data is given below for statistical analysis:

SUBJECT WITHOUT SEQUENCE			
Subject	Period	Treatment	Cmax
1	1	2	470.00
2	1	1	456.00
3	1	2	397.00
4	1	1	440.00
5	1	2	533.20
6	1	2	575.70
7	1	1	304.80
8	1	1	335.00
9	1	2	465.00
10	1	2	467.80
11	1	1	569.70
12	1	1	423.80
1	2	1	454.00

SUBJECT WITH SEQUENCE				
Subject	Sequence	Period	Treatment	Cmax
1	2	1	2	470.00
2	1	1	1	456.00
3	2	1	2	397.00
4	1	1	1	440.00
5	2	1	2	533.20
6	2	1	2	575.70
7	1	1	1	304.80
8	1	1	1	335.00
9	2	1	2	465.00
10	2	1	2	467.80
11	1	1	1	569.70
12	1	1	1	423.80
1	2	2	1	454.00

2	2	2	468.00
3	2	1	292.00
4	2	2	297.00
5	2	1	458.60
6	2	1	558.60
7	2	2	389.60
8	2	2	404.90
9	2	1	491.00
10	2	1	590.08
11	2	2	482.60
12	2	2	404.00

2	1	2	2	468.00
3	2	2	1	292.00
4	1	2	2	297.00
5	2	2	1	458.60
6	2	2	1	558.60
7	1	2	2	389.60
8	1	2	2	404.90
9	2	2	1	491.00
10	2	2	1	590.08
11	1	2	2	482.60
12	1	2	2	404.00

The above table col.-1 = Subject (Patient's Id) which are randomly selected, total 12 subjects, Sequence (AB/BA = 1/2), Period (1 and 2), treatment (Code; Standard drug = A = 1, New drug = B = 2), Cmax.

For using software, used code 1 and 2 respectively A and B, 1 for AB and 2 for BA (sequence) Analysis Cmax value of GLM **without sequence** of 12 subjects using SAS software;

GLM without Sequence effect of Cmax data

```
/* Use the following statements to produce Outputs 11.30 and 11.31. */;
options ls = 80;
title "SAS 9.1.3 Version - Grizzles Model by PROC GLM";
data msubject;
input subject treatment  period $ Cmax;
datalines;
1 1 1 470.00
2 1 1 456.00
3 1 2 397.00
4 1 2 440.00
5 1 1 533.20
6 1 1 575.70
7 1 1 304.80
8 1 1 335.00
9 1 2 465.00
10 1 2 467.80
11 1 2 569.70
```

12	1	1	423.80
1	2	2	454.00
2	2	2	468.00
3	2	1	292.00
4	2	1	297.00
5	2	2	458.60
6	2	2	558.60
7	2	2	389.60
8	2	2	404.90
9	2	1	491.00
10	2	1	590.08
11	2	1	482.60
12	2	2	404.00

```
ods rtf;
title "SAS 9.1.3 Version -Grizzles Model by PROC GLM";
proc glm data = msubject;
class treatment subject period;
model Cmax = period  subject treatment;
means subject;
lsmeans Period Treatment /pdiff stderr;
estimate 'Treatment' Treatment 1-1;
run;
title "SAS 9.1.3 Version -Grizzles Model by PROC GLM";
proc glm data = mtreatment;
class treatment subject period;
model Cmax = period subject treatment;
means treatment;
lsmeans Period  Treatment /pdiff stderr;
estimate 'Treatment' Treatment 1-1;
run;
/*Use these statements to produce Outputs 11.34 through 11.36*/;
title "SAS 9.1.3 Version -Grizzles Model by PROC GLM";
proc glm method = msubject;
```

```
class treatment  period;
model Cmax = period;
random subject period treatment;
lsmeans treatment = treatment ;
estimate 't1 vs t2 at  period 1-1;
estimate 't1 vs t2 at period 1-1;
estimate 't1 vs t2 at treatment 1'period 1-1;
estimate 't1 vs t2 at treatment 2' period 1-1;
estimate 't1 vs t2 at subject 1' period 1-1;
estimate 't1 vs t2 at subject 2' period 1-1;
estimate 't1 vs t2 at subject 3' period 1-1;
estimate 't1 vs t2 at subject 4' period 1 -1;
estimate 't1 vs t2 at subject 5' period 1-1;
estimate 't1 vs t2 at subject 6' period 1-1;
estimate 't1 vs t2 at subject 7' period 1-1;
estimate 't1 vs t2 at subject 8' period 1-1;
estimate 't1 vs t2 at subject 9' period 1-1;
estimate 't1 vs t2 at subject 10' period 1-1;
estimate 't1 vs t2 at subject 11' period 1-1;
estimate 't1 vs t2 at subject 12' period 1-1;

title "SAS 9.1.3 Version -Grizzles Model by PROC GLM";
proc glm method=msubject;
class treatment subject period;
model Cmax =msubject;
random subject period treatment;
lsmeans treatment = treatment;
estimate 'subject 1 vs treatment 1 at period 1-1;
estimate 'subject 1 vs treatment 2 at period 1-1;
estimate 'subject 2 vs treatment 1 at period 1-1;
estimate 'subject 2 vs treatment 2 at period 1-1;
estimate 'subject 3 vs treatment 1 at period 1-1;
estimate 'subject 3 vs treatment 2 at period 1-1;
estimate 'subject 4 vs treatment 1 at period 1-1;
```

estimate 'subject 4 vs treatment 2 at period 1-1;

estimate 'subject 5 vs treatment 1 at period 1-1;

estimate 'subject 5vs treatment 2 at period 1-1;

estimate 'subject 6 vs treatment 1 at period 1-1;

estimate 'subject 6 vs treatment 2 at period 1-1;

estimate 'subject 7 vs treatment 1 at period 1-1;

estimate 'subject 7 vs treatment 2 at period 1-1;

estimate 'subject 8 vs treatment 1 at period 1-1;

estimate 'subject 8 vs treatment 2 at period 1-1;

estimate 'subject 9 vs treatment 1 at period 1-1;

estimate 'subject 9 vs treatment 2 at period 1-1;

estimate 'subject 10 vs treatment 1 at period 1-1;

estimate 'subject 10 vs treatment 2 at period 1-1;

estimate 'subject 11 vs treatment 1 at period 1-1;

estimate 'subject 11 vs treatment 2 at period 1-1;

estimate 'subject 12 vs treatment 1 at period 1-1;

estimate 'subject 12 vs treatment 2 at period 1-1;

Run;

Quit;

ods rtf close;

GLM Procedure without Sequence effect output of Cmax data.

Class Level Information		
Class	**Levels**	**Values**
treatment	2	1 2
subject	12	1 2 3 4 5 6 7 8 9 10 11 12
period	2	1 2

Number of Observations Read	24
Number of Observations Used	24

Dependent Variable: C_{max}.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	13	130678.6176	10052.2014	2.81	0.0543
Error	10	35785.0528	3578.5053		
Corrected Total	23	166463.6704			

R-Square	Coeff Var	Root MSE	Cmax Mean
0.785028	13.38221	59.82061	447.0158

Source	DF	Type I SS	Mean Square	F Value	Pr > F
period	1	907.9860	907.9860	0.25	0.6254
subject	11	129755.9362	11795.9942	3.30	0.0352
treatment	1	14.6954	14.6954	0.00	0.9502

Source	DF	Type III SS	Mean Square	F Value	Pr > F
period	1	907.9860	907.9860	0.25	0.6254
subject	11	129755.9362	11795.9942	3.30	0.0352
treatment	1	14.6953	14.6953	0.00	0.9502

Level of subject	N	Cmax	
		Mean	Std Dev
1	2	462.000000	11.313708
2	2	462.000000	8.485281
3	2	344.500000	74.246212
4	2	368.500000	101.116270
5	2	495.900000	52.750166
6	2	567.150000	12.091526
7	2	347.200000	59.962655
8	2	369.950000	49.426764
9	2	478.000000	18.384776
10	2	528.940000	86.465017
11	2	526.150000	61.589001
12	2	413.900000	14.000714

period	Cmax LSMEAN	Standard Error	H0:LSMEAN=0 Pr > |t|	H0:LSMean1=LSMean2 Pr > |t|
1	453.166667	17.268722	<.0001	0.6254
2	440.865000	17.268722	<.0001	

treatment	Cmax LSMEAN	Standard Error	H0:LSMEAN=0	H0:LSMean1=LSMean2
			Pr > |t|	Pr > |t|
1	447.798333	17.268722	<.0001	0.9502
2	446.233333	17.268722	<.0001	

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Treatment	1.56500000	24.4216614	0.06	0.9502

Similarly, we can use GLM Procedure with Sequence effect of Cmax data

```
/* Use the following statements to produce Outputs 11.30 and 11.31. */;
options ls=80;
title "SAS 9.1.3 Version -Grizzles Model by PROC GLM";
data msequence;
input subject sequence period treatment $ Cmax;
```

datalines;

1	2	1	2	470.00
2	1	1	1	456.00
3	2	1	2	397.00
4	1	1	1	440.00
5	2	1	2	533.20
6	2	1	2	575.70
7	1	1	1	304.80
8	1	1	1	335.00
9	2	1	2	465.00
10	2	1	2	467.80
11	1	1	1	569.70
12	1	1	1	423.80
1	2	2	1	454.00
2	1	2	2	468.00
3	2	2	1	292.00
4	1	2	2	297.00
5	2	2	1	458.60
6	2	2	1	558.60
7	1	2	2	389.60
8	1	2	2	404.90
9	2	2	1	491.00
10	2	2	1	590.08
11	1	2	2	482.60
12	1	2	2	404.00

ods rtf;

proc print data = msequence;

run;

proc sort data = msequence;

by sequence period;

proc means noprint data = msequence;

by sequence period treatment; var Cmax;

output out = averages mean median stadev cv min max DATA = Cmax _mean;

```
run;
proc print data = averages;
run;
/*Use these statements to produce Output 11.32*/;
title "SAS 9.1.3 Version -Grizzles Model by PROC GLM";
proc glm data = msequence;
class sequence treatment subject period;
model Cmax = period sequence subject(sequence) treatment;
means period;
lsmeans Period Sequence Treatment /pdiff stderr;
estimate 'Treatment' Treatment 1 -1;
test h=Sequence e = Subject(Sequence);
run;
title "SAS 9.1.3 Version -Grizzles Model by PROC GLM";
proc glm data=msubject;
class sequence treatment subject period;
model Cmax =period sequence   subject(sequence) treatment;
means subject;
lsmeans Period Sequence Treatment /pdiff stderr;
estimate 'Treatment' Treatment 1 -1;
test h = Sequence e = Subject(Sequence);
run;
title "SAS 9.1.3 Version -Grizzles Model by PROC GLM";
proc glm data = mtreatment;
class sequence treatment subject period;
model Cmax = period sequence subject(sequence) treatment;
means treatment;
lsmeans Period Sequence Treatment /pdiff stderr;
estimate 'Treatment' Treatment 1 -1;
test h=Sequence e=Subject(Sequence);
run;
/*Use these statements to produce Output 11.33*/;
title "SAS 9.1.3 Version -Grizzles Model by PROC GLM";
proc glm data = msequnce;
```

```
class sequence treatment period;
model Cmax = sequence subject (sequnce) period treatment;
means period;
lsmeans Period Sequence Treatment /pdiff stderr;
estimate 'Treatment' Treatment 1 -1;
test h=Sequence e=Subject (Sequence);
run;
/*Use these statements to produce Outputs 11.34 through 11.36*/;
title "SAS 9.1.3 Version -Grizzles Model by PROC GLM";
proc glm method = msequnce;
class sequnce treatment  period;
model Cmax =period;
random sequence subject(sequence) period treatment;
lsmeans treatment = treatment;
estimate 't1 vs t2 at sequence 1' period 1-1;
estimate 't1 vs t2 at sequence 2' period 1-1;
estimate 't1 vs t2 at treatment 1'period 1-1;
estimate 't1 vs t2 at treatment 2' period 1-1;
estimate 't1 vs t2 at subject 1' period 1-1;
estimate 't1 vs t2 at subject 2' period 1-1;
estimate 't1 vs t2 at subject 3' period 1-1;
estimate 't1 vs t2 at subject 4' period 1-1;
estimate 't1 vs t2 at subject 5' period 1-1;
estimate 't1 vs t2 at subject 6' period 1-1;
estimate 't1 vs t2 at subject 7' period 1-1;
estimate 't1 vs t2 at subject 8' period 1-1;
estimate 't1 vs t2 at subject 9' period 1-1;
estimate 't1 vs t2 at subject 10' period 1-1;
estimate 't1 vs t2 at subject 11' period 1-1;
estimate 't1 vs t2 at subject 12' period 1-1;
estimate 't1 vs t2 at subject(sequence)  1' period 1-1;
estimate 't1 vs t2 at subject(sequence)  2' period 1-1;
estimate 't1 vs t2 at subject(sequence)  3' period 1-1;
estimate 't1 vs t2 at subject(sequence)  4' period 1-1;
```

```
estimate 't1 vs t2 at subject(sequence) 5' period 1-1;
estimate 't1 vs t2 at subject(sequence)  6' period 1-1;
estimate 't1 vs t2 at subject(sequence)  7' period 1-1;
estimate 't1 vs t2 at subject(sequence)  8' period 1-1;
estimate 't1 vs t2 at subject(sequence)  9' period 1-1;
estimate 't1 vs t2 at subject(sequence)  10' period 1-1
estimate 't1 vs t2 at subject(sequence)  11' period 1-1;
estimate 't1 vs t2 at subject(sequence)  12' period 1-1;

title "SAS 9.1.3 Version -Grizzles Model by PROC GLM";
proc glm method=msequnce;
class sequnce treatment subject period;
model Cmax =subject;
random sequence subject (sequence) period treatment;
lsmeans treatment = treatment;
estimate 'subject 1 vs treatment 1 at sequence 1' period 1-1;
estimate 'subject 1 vs treatment 2 at sequence 2' period 1-1;
estimate 'subject 2 vs treatment 1 at sequence 1' period 1-1;
estimate 'subject 2 vs treatment 2 at sequence 2' period 1-1;
estimate 'subject 3 vs treatment 1 at sequence 1' period 1-1;
estimate 'subject 3 vs treatment 2 at sequence 2' period 1-1;
estimate 'subject 4 vs treatment 1 at sequence 1' period 1-1;
estimate 'subject 4 vs treatment 2 at sequence 2' period 1-1;
estimate 'subject 5 vs treatment 1 at sequence 1' period 1-1;
estimate 'subject 5vs treatment 2 at sequence 2' period 1-1;
estimate 'subject 6 vs treatment 1 at sequence 1' period 1-1;
estimate 'subject 6 vs treatment 2 at sequence 2' period 1-1;
estimate 'subject 7 vs treatment 1 at sequence 1' period 1-1;
estimate 'subject 7 vs treatment 2 at sequence 2' period 1-1;
estimate 'subject 8 vs treatment 1 at sequence 1' period 1-1;
estimate 'subject 8 vs treatment 2 at sequence 2' period 1-1;
estimate 'subject 9 vs treatment 1 at sequence 1' period 1-1;
estimate 'subject 9 vs treatment 2 at sequence 2' period 1-1;
estimate 'subject 10 vs treatment 1 at sequence 1' period 1-1;
```

estimate 'subject 10 vs treatment 2 at sequence 2' period 1-1;

estimate 'subject 11 vs treatment 1 at sequence 1' period 1-1;

estimate 'subject 11 vs treatment 2 at sequence 2' period 1-1;

estimate 'subject 12 vs treatment 1 at sequence 1' period 1-1;

estimate 'subject 12 vs treatment 2 at sequence 2' period 1-1;

Run;

Quit;

ods rtf close;

GLM Procedure with Sequence effect output of Cmax data.

Class Level Information		
Class	**Levels**	**Values**
sequence	2	1 2
treatment	2	1 2
subject	12	1 2 3 4 5 6 7 8 9 10 11 12
period	2	1 2

Number of Observations Read	24
Number of Observations Used	24

Dependent Variable: C_{max}.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	13	130678.6176	10052.2014	2.81	0.0543
Error	10	35785.0528	3578.5053		
Corrected Total	23	166463.6704			

R-Square	Coeff Var	Root MSE	Cmax Mean
0.785028	13.38221	59.82061	447.0158

Source	DF	Type I SS	Mean Square	F Value	Pr > F
period	1	907.9860	907.9860	0.25	0.6254
sequence	1	25192.9440	25192.9440	7.04	0.0242
Subject (sequence)	10	104562.9922	10456.2992	2.92	0.0529
treatment	1	14.6954	14.6954	0.00	0.9502

Source	DF	Type III SS	Mean Square	F Value	Pr > F
period	1	907.9860	907.9860	0.25	0.6254
sequence	1	25192.9440	25192.9440	7.04	0.0242
Subject (sequence)	10	104562.9922	10456.2992	2.92	0.0529
treatment	1	14.6954	14.6954	0.00	0.9502

Level of period	N	Cmax	
		Mean	Std Dev
1	12	453.166667	83.0179645

Level of period	N	Cmax	
		Mean	Std Dev
2	12	440.865000	90.3246054

period	Cmax LSMEAN	Standard Error	H0:LSMEAN=0 Pr > |t|	H0:LSMean1=LSMean2 Pr > |t|
1	453.166667	17.268722	<.0001	0.6254
2	440.865000	17.268722	<.0001	

sequence	Cmax LSMEAN	Standard Error	H0:LSMEAN=0	H0:LSMean1=LSMean2
			Pr > |t|	Pr > |t|
1	414.616667	17.268722	<.0001	0.0242
2	479.415000	17.268722	<.0001	

treatment	Cmax LSMEAN	Standard Error	H0:LSMEAN=0 Pr > |t|	H0:LSMean1=LSMean2 Pr > |t|
1	447.798333	17.268722	<.0001	0.9502
2	446.233333	17.268722	<.0001	

Tests of Hypotheses Using the Type III MS for subject(sequence) as an Error Term					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
sequence	1	25192.94402	25192.94402	2.41	0.1517

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Treatment	1.56500000	24.4216614	0.06	0.9502

RESULT AND DISCUSSION

Statistical Analysis Report

The above analysis of without sequence effect and with sequence effect in both cases the total sum of squares = 166463.6704 and model is not statistically significant at 5% level i.e., P-value = 0.0543 (P.<0.05) and total degrees of freedom 23 i.e., n-1 = 24-1 = 23 (where n = subject)

All the above analysis without sequence effect and with sequence effect are same except subject effect. This subject effect is most important for analysis in sequence effect and without sequence effect.

In the analysis, total subjects 12 in both standard drug (A=1) and new drug (B=2), For GLM model the subject degrees of freedom should be 11 i.e., n-1 = 12-1 =11 (i.e., occurred in without sequence effect) which (between the subjects) is statistically significant at 5% level, P-value = 0.0352 (P<0.05).

But in cross over design important point, all patients are taken both the drugs in period 1 and period 2, the drug effect is important among the patients, so we can arrange with pooled group effect only due to carry over effect or sequence effect. In pooled group for 12 subjects (or patients), each group 6 subjects and taken 5 degrees of period i.e., n-1 formula = 6-1=5, both sides it will be 5+5=10 degrees of freedom, But without sequence effect 11 subjects are there, 1 will be deducted from 11 due to sequence effect.

Now, we can compare with sequence effect the subjects (10 degrees of freedom) is not statistically significant at 5% level (F –value = 2.92, P-value = 0.0529 >0.05). So it is fully specified that both the drugs are equally effectible among the patients. Here sequence is statistically significant at 5% level i.e P – value = 0.0242. That change only the cause of effect among the subjects due to sequence.

CONCLUSION

The feature of crossover seems to make the design preferable to parallel group study. It is widely used in pharmacokinetics and bioequivalence studies. However, there are some potential problems in using crossover design, especially the carryover effect. The ideal crossover design should be uniform and strongly balanced. The test for carryover effect should be performed. If the carryover effect is not significantly between two sequences, then the data from two periods can be combined and analyzed. Otherwise, the data only from period 1 can be used to estimate the treatment effect. Carry over effect or sequence effect will more preferable than without sequence effect for statistical analysis report in Bioequivalence study in cross over design.

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