Paper SD04

Crossover Designs and Proc Mixed In SAS

Abstract

Crossover designs are commonly used in pharmaceutical and human/animal nutrition studies. This design is used to reduce error variance on the one hand and to meet different experimental situations like limitation on experimental resources on the other. Error variation mainly arises due to variation in experimental units under identical treatments. As a result, a type of design known as crossover designs (or switchover designs) could be evolved to use the same experimental unit for different treatments in an experiment but in different periods. In a crossover design each treatments is applied to each of a number of experimental units in different time periods. The sequences in which the treatments are applied to an experimental unit (or a subject) may have some influence on the effects of the different treatments. Accordingly, different treatment sequences are taken to eliminate sequence effects. In this study, a crossover design example will be analyzed using Procures Mixed in SAS. In addition the Proc Mixed output will be compared with the GLM output in analyzing the crossover design example.

Introduction

There are quite numbers of types of designs. Such variation in types of designs is mainly due to efforts to reduce error variance on the one hand and to meet different experimental situations like limitation on experimental resources on the other. Error variation mainly arise due to variation in experimental units under identical treatments. It was, therefore, proposed to use the same experimental unit for different treatments in an experiment but in different periods. As a result a type of design known as crossover designs (or switchover designs) could be evolved (Cox and Reed, 2000; Friedman et al., 1998; Littell et al., 2002).

In a crossover design each subject receiving a sequence of experimental treatments. The aim is to compare the effects of individual treatments, not the sequences themselves. There are many possible sets of sequences that might be used in a design, depending on the number of treatments, the length of the sequences and the aims of the trial. The simplest design is the two-period two-treatment or 2 x 2 design. The feature that distinguishes the crossover design from other experimental designs, which compare treatments, is that measurements on different treatments are obtained from each subject. This feature brings with it advantages and disadvantages.

In crossover design, there are as many treatment periods as there are treatments to be compared, and each subject receives every treatment. If there are no missing data, then a conventional least squares analysis fitting treatment, period and subject effects is fully efficient. Whenever there are missing data, some of the within-subject treatment comparisons are unavailable for every subject. Therefore, additional between-subject information can be utilized.

The main advantage is that the treatments are compared within-subjects. The aim of the crossover design is to remove from the treatment comparisons any component that is related to the differences between the subjects. That is, the subject effect is removed from the comparison. In clinical trials it is usually the case that the variability of measurements taken on different subjects is far greater than the variability of repeated measurements taken on the same subject. The crossover design aims to exploit this feature by making sure that whenever possible, important comparisons of interest are estimated using differences obtained from the within-subject measurements.

The possible disadvantage of a crossover design is that the effect of a treatment given in one period might still be present at the start of the following period. That is, previous treatment allocation is a confounding factor for later periods and means that we cannot justify our conclusions about the comparative effects of individual treatments (rather than sequences of treatments) from the randomization alone. The phenomenon known as carry-over or residual effect may depend on the design, the setting, the treatment, and response. The results of carry-over effect in second or subsequent treatment periods may be influenced by treatment administered in earlier periods. In the simple two-period, crossover design, there is no possibility of estimating carry-over. In all of the remaining designs, carry-over effects can be estimated. Through appropriate choice of design and analysis, the impact of this disadvantage can be reduced, especially in trials with more than two periods (Jones and Donev, 1996; Jones and Kenward, 1989).

Let there be p experimental treatments. In a crossover design each of these treatments is applied to each of a number of experimental units in p different time periods. The sequences in which the treatments are applied to a experimental unit (or a subject) may have some influence on the effects of the different treatments. Accordingly, different treatment sequences are taken to eliminate sequence effects. These sequences are so chosen that each treatment appears in them in each period equally often. One experimental unit is required for each sequence.

The experimental units suitable for such experiments are usually animals or humans as different treatments can be applied to the same experimental unit in different periods without much difficulty. The lengths of the periods depends on the objectives of the experiment and

other experimental situations. The treatment sequences are usually formed out of the rows or columns of one or more latin squares with as many treatments. That is, in a cross over design the number of time periods and the number of sequences are the same.

Example:

Twelve males volunteered to participate in a study to compare the durations effects of three different formulations of a drug product. Formulation 1 was a 50–mg tablet (Treatment 1 or T_1), formulation 2 was a 100–mg tablet (Treatment 2 or T_2), and formulation 3 was a sustained–release formulation capsule (Treatment 3 or T_3). A three–period crossover design was used, with four volunteers assigned to each of the three treatment sequences (Sequence 1: T_1, T_2, T_3; Sequence 2: T_2, T_3, T_1; and Sequence 3: T_3, T_1, T_2). On each treatment day, volunteers were given their assigned formulation and were observed to determine the duration effect (blood pressure lowering). There was a 1–week washout between each treatment period of the study. The sample data are shown here (Ott & Longnecker, 2001).

		Period		
Sequence	subject	1	2	3
1	n = 4	T_1	T_2	T_3
2	n = 4	T_2	T_3	T_1
3	n = 4	T_3	T_1	T_2

		Period		
Sequence	subject	1	2	3
1	1	1.5	2.2	3.4
	2	2.0	2.6	3.1
	3	1.6	2.7	3.2
	4	1.1	2.3	2.9
2	1	2.5	3.5	1.9
	2	2.8	3.1	1.5
	3	2.7	2.9	2.4
	4	2.4	2.6	2.3
3	1	3.3	1.9	2.7
	2	3.1	1.6	2.5
	3	3.6	2.3	2.2
	4	3.0	2.5	2.0

With this design, 12 subjects are randomly assigned to the sequences (rows) of the design, 4 to each sequence. The periods correspond to the order in which the compounds are taken. In this example, the compounds (or treatments) is factor A, subject is factor B, period is factor C, and sequence is factor D. Given that factor B is random and factors A, C, and D are fixed, the appropriate statistical model is:

$$Y_{ijkl} = \mu + \delta_l + \beta_{i(l)} + \alpha_j + \gamma_k + \alpha\gamma_{jk} + \epsilon_{ijkl}$$

we assume the following:

1. Y_{ijkl} is the response due to subject i, compound j, and period k, and sequence l.
   (In this example: i = 1, 2, 3, 4, j = 1, 2, 3; k = 1, 2, 3; and l = 1, 2, 3.)
2. μ is an overall mean.
3. δ_l is a fixed effect due to sequence l; $\Sigma\delta_l = 0$.
4. $\beta_{i(l)}$ is a random effect due to subject i nested within sequence l; $\beta_{i(l)} \sim$ N(0, σ^2_β).
5. $\beta_{i(l)}$s are independent.
6. α_j is a fixed effect due to compound (or treatment) j; $\Sigma\alpha_j = 0$.
7. γ_k is a fixed effect due to period k; $\Sigma\gamma_k = 0$.
8. $\alpha\gamma_{jk}$ is a fixed interaction effect due to compound j and period k; $\Sigma\alpha\gamma_{jk} = 0$.
9. ϵ_{ijkl} is the random error; $\epsilon_{ijkl} \sim$ N(0, σ^2_ϵ).
10. The ϵ_{ijkl}s are independent.
11. The random components $\beta_{i(l)}$ and ϵ_{ijkl} are independent.

An ANOVA Table for a Three–Period Crossover Design. Factor A (compound), factor C (period), and factor D (sequence) are fixed and factor B (subject) is random.

				EMS
Source	SS	df	MS	Mixed Effects
A	SSA	a-1	MSA	$\sigma^2_\epsilon + \mathrm{bd}\theta_\mathrm{A}$
B(D)	SSB(D)	d(b-1)	MSB(D)	$\sigma^2_\epsilon + \mathrm{d}\sigma^2_\beta$
C	SSC	c-1	MSC	$\sigma^2_\epsilon + \mathrm{bd}\theta_\mathrm{C}$
AC	SSAC	(a-1)(c-1)-(d-1)	MSAC	$\sigma^2_\epsilon + \mathrm{b}\theta_\mathrm{AC}$
D	SSD	d-1	MSD	$\sigma^2_\epsilon + \mathrm{d}\sigma^2_\beta + \mathrm{bd}\theta_\mathrm{D}$
Error	SSE	d(a-1)(b-1)	MSE	σ^2_ϵ
Total	TSS	a^2b-1		

In this example: a = 3, b = 4, c = 3, d = 3, and n = abc = 50.

An important distinction needs to be made between those models in which the subject effects (β_i) are assumed to be unknown fixed parameters and those in which they are assumed to be realizations of random variables, usually with zero mean and variance σ^2_β . The random effects models can have advantages over fixed effects models in the context of crossover designs. In balanced situations, with normally distributed data, the results of both analyses will generally be similar. In unbalanced situations, however, the random effects models will lead to smaller standard errors of the estimates of treatment differences. If the degree of imbalance is slight (e.g. few missing observations in a balanced design) and if the subject variance component

is large compared with the residual variance component, this reduction in the size of the standard error will be modest. In addition, the benefits of using a random effects model are much more pronounced in models where carry-over is being estimated. On the other hand, there are situations where the random effects methods may not be sufficiently robust. This is of particular concern when we are dealing with non-normal data or fairly small samples (Brown and Prescott, 1999; DeMets, 2002).

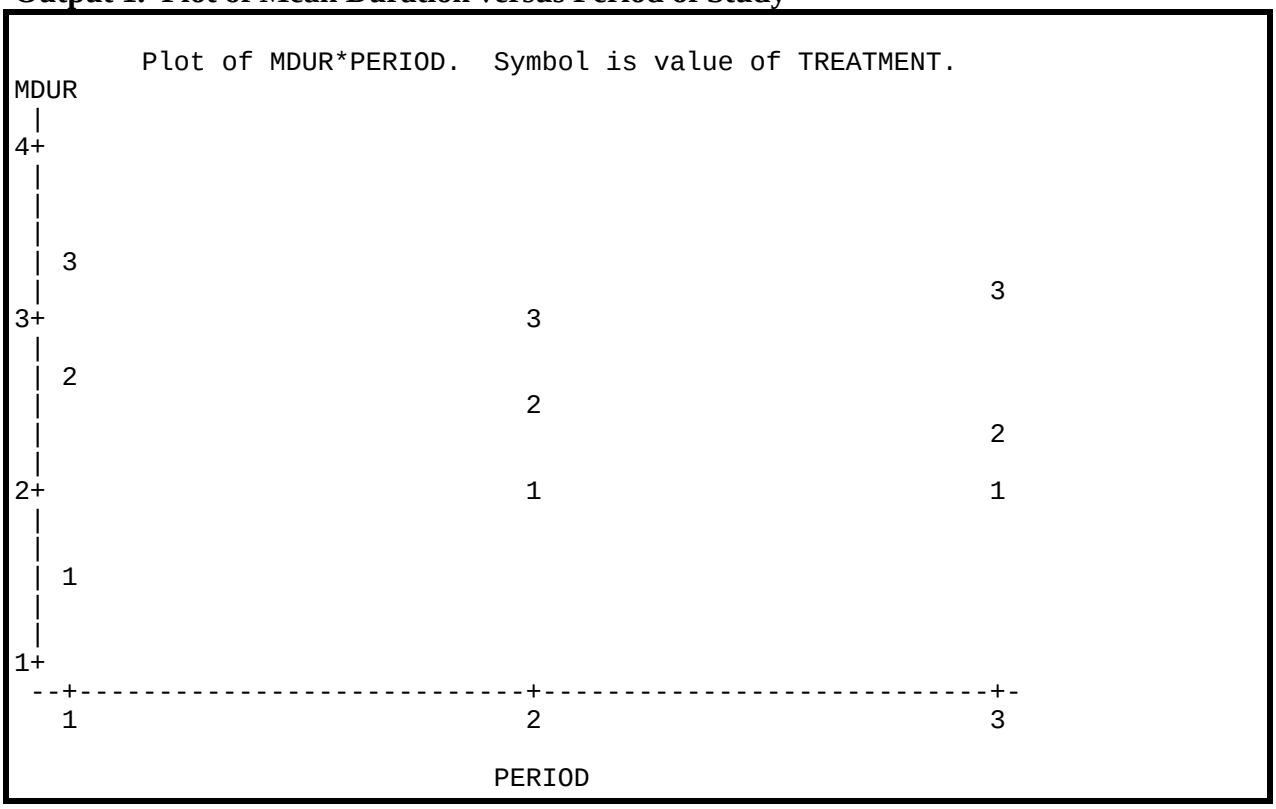
I. Using PROC GLM

The SAS code for analyzing this data set and resulting output are shown below. All four sources of variation (sequence, treatment, period, and subject) must appear in the CLASS statement in PROC GLM. In the TEST statement, the options HTYPE = 1 and ETYPE = 1 are specified. HTYPE = 1 and ETYPE = 1 are sum of squares to use for the hypothesis and the error term, respectively

SAS STATEMENTS:

```
DATA COD;
   DO SEQUENCE = 1 TO 3;
      DO SUBJECT= 1 TO 4;
         DO PERIOD = 1 TO 3;
            INPUT TREATMENT DURATION @@;
            OUTPUT;
         END;
      END;
   END;
DATALINES;
1 1.5 2 2.2 3 3.4 1 2.0 2 2.6 3 3.1
1 1.6 2 2.7 3 3.2 1 1.1 2 2.3 3 2.9
2 2.5 3 3.5 1 1.9 2 2.8 3 3.1 1 1.5
2 2.7 3 2.9 1 2.4 2 2.4 3 2.6 1 2.3
3 3.3 1 1.9 2 2.7 3 3.1 1 1.6 2 2.5
3 3.6 1 2.3 2 2.2 3 3.0 1 2.5 2 2.0
;
OPTIONS LS=78 PS=60;
PROC SORT; BY TREATMENT PERIOD;
PROC MEANS MEAN NOPRINT;
   VAR DURATION;
   BY TREATMENT PERIOD;
   OUTPUT OUT=A MEAN=MDUR;   /* MDUR = Mean Duration */
PROC PLOT DATA=A;
   PLOT MDUR*PERIOD=TREATMENT / HPOS=60 VPOS=20;
PROC GLM;
   CLASS SEQUENCE SUBJECT PERIOD TREATMENT;
   MODEL DURATION = SEQUENCE SUBJECT(SEQUENCE) TREATMENT PERIOD
                    TREATMENT*PERIOD;
   TEST  H = SEQUENCE  E = SUB(SEQUENCE) / HTYPE=1 ETYPE=1;
   LSMEANS TREAT / PDIFF CL E;
RUN;
```

Output 1. Plot of Mean Duration versus Period of Study



As the above plot (Output 1) indicates, the longest duration effects, on the average, were observed with formulation 3 followed by formulation 2 and then 1.

Because this data set is balanced, the Type I and III SS results are identical (Output 2). The ANOVA results in Output 2 shows that the treatment effect has a significant p-value of < 0.0001, which indicates a departure from the null hypothesis of equal treatment means. Neither the sequence effect nor the period effect are significant ($p = 0.2595$ and $p = 0.9279$, respectively).

As indicated in Output 3, there are significant difference in duration between treatment 1 & 2 ($p = 0.0005$), 1& 3 ($p = < 0.0001$) and 2 & 3 ($p = 0.0001$). In addition, all the confidence intervals in mean differences between treatments are conclusive.

Output 2. GLM for a Crossover Design

The GLM Procedure

Class Level Information

Class	Levels	Values
SEQUENCE	3	1 2 3
SUBJECT	4	1 2 3 4
PERIOD	3	1 2 3
TREATMENT	3	1 2 3

Number of observations 36

Dependent Variable: DURATION

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	17	11.08638889	0.65214052	5.69	0.0003
Error	18	2.06333333	0.11462963		
Corrected Total	35	13.14972222			

R-Square	Coeff Var	Root MSE	DURATION Mean
0.843089	13.55786	0.338570	2.497222

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SEQUENCE	2	0.23388889	0.11694444	1.02	0.3804
SUBJECT(SEQUENCE)	9	0.66916667	0.07435185	0.65	0.7425
TREATMENT	2	9.51722222	4.75861111	41.51	<.0001
PERIOD	2	0.01722222	0.00861111	0.08	0.9279
PERIOD*TREATMENT	2	0.64888889	0.32444444	2.83	0.0853

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SEQUENCE	0	0.00000000	.	.	.
SUBJECT(SEQUENCE)	9	0.66916667	0.07435185	0.65	0.7425
TREATMENT	2	9.51722222	4.75861111	41.51	**<.0001**
PERIOD	2	0.01722222	0.00861111	0.08	0.9279
PERIOD*TREATMENT	2	0.64888889	0.32444444	2.83	0.0853

Tests of Hypotheses Using the Type I MS for SUBJECT(SEQUENCE) as an Error Term

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SEQUENCE	2	0.23388889	0.11694444	1.57	0.2595

Output 3. GLM Least Square Means for a Crossover Design

TREATMENT	DURATION LSMEAN	LSMEAN Number
1	1.88333333	1
2	2.46666667	2
3	3.14166667	3

Least Squares Means for effect TREATMENT
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: DURATION

i/j	1	2	3
1		0.0005	<.0001
2	**0.0005**		0.0001
3	**<.0001**	**0.0001**	

TREATMENT	DURATION LSMEAN	95% Confidence Limits	
1	1.883333	1.677996	2.088671
2	2.466667	2.261329	2.672004
3	3.141667	2.936329	3.347004

Least Squares Means for Effect TREATMENT

i	j	Difference Between Means	95% Confidence Limits for LSMean(i)-LSMean(j)	
1	2	-0.583333	-0.873724	-0.292943
1	3	-1.258333	-1.548724	-0.967943
2	3	-0.675000	-0.965391	-0.384609

NOTE: To ensure overall protection level, only probabilities associated with pre-planned comparisons should be used.

II. Using PROC MIXED

Model fitting and inference for fixed subject-effect models will follow conventional ordinary least squares (OLS) procedures and for random subject-effect models the restricted maximum likelihood (REML) analyses for linear mixed models can be applied (Verbeke and Molenberghs, 2000, Brown and Prescott, 1999). The modeling component of SAS PROC

MIXED can be illustrated very simply for both fixed and random subject-effects models. The SAS codes using PROC MIXED for a random-effects model for the example in this paper is:

```
PROC MIXED;
   CLASS SEQUENCE SUBJECT PERIOD TREATMENT;
   MODEL DURATION = TREATMENT PERIOD;
   RANDOM SUBJECT(SEQUENCE);
   LSMEANS TREATMENT / PDIFF CL E;
```

Output 4: MIXED for a Crossover Design

The Mixed Procedure

Model Information

Data Set	WORK.COD
Dependent Variable	DURATION
Covariance Structure	Variance Components
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Containment

Class Level Information

Class	Levels	Values
SEQUENCE	3	1 2 3
SUBJECT	4	1 2 3 4
PERIOD	3	1 2 3
TREATMENT	3	1 2 3

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
TREATMENT	2	20	40.80	**<.0001**
PERIOD	2	20	0.07	0.9291

The test of significance for TREATMENT and PERIOD in "Type 3 Tests of Fixed Effects" in Output 4 is similar to the test from GLM in Output 3. As a result, the ordinary least-squares analyses, as performed by GLM, can be equivalent to generalized least-squares analyses, as performed by MIXED. The phenomenon occurs in this example because the within-subject effects are orthogonal to the between-subject effects. Likewise, the results of Least Square Means using PROC MIXED (Output 5) are similar to those obtained using PROC GLM (Output 3)

Output 5. MIXED Least Square Means for a Crossover Design

Coefficients for TREATMENT Least Squares Means

Effect	PERIOD	TREATMENT	Row1	Row2	Row3
Intercept			1	1	1
TREATMENT		1	1		
TREATMENT		2		1	
TREATMENT		3			1
PERIOD	1		0.3333	0.3333	0.3333
PERIOD	2		0.3333	0.3333	0.3333
PERIOD	3		0.3333	0.3333	0.3333

Least Squares Means

Effect	TREATMENT	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha
TREATMENT	1	1.8833	0.09858	20	19.10	<.0001	0.05
TREATMENT	2	2.4667	0.09858	20	25.02	<.0001	0.05
TREATMENT	3	3.1417	0.09858	20	31.87	<.0001	0.05

Least Squares Means

Effect	TREATMENT	Lower	Upper
TREATMENT	1	1.6777	2.0890
TREATMENT	2	2.2610	2.6723
TREATMENT	3	2.9360	3.3473

Differences of Least Squares Means

Effect	TREATMENT	_TREATMENT	Estimate	Standard Error	DF	t Value	Pr > |t|
TREATMENT	1	2	-0.5833	0.1394	20	-4.18	**0.0005**
TREATMENT	1	3	-1.2583	0.1394	20	-9.03	**<.0001**
TREATMENT	2	3	-0.6750	0.1394	20	-4.84	**<.0001**

Differences of Least Squares Means

Effect	TREATMENT	_TREATMENT	Alpha	Lower	Upper
TREATMENT	1	2	0.05	-0.8742	-0.2925
TREATMENT	1	3	0.05	-1.5492	-0.9675
TREATMENT	2	3	0.05	-0.9658	-0.3842

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