Chapter 5

Analysis of a Three Treatments Crossover Design for the First Order Carryover Model

5.1 Introduction

Apart from design and model modifications, different procedures have been suggested to handle carryover effects at the analysis stage. Since, a crossover design is highly suitable for treatment comparison beyond elimination of carryover effects, the first step in analysis of crossover designs, has been the test of equality of carryover effects. In the context of $cod\{\text{AB, BA}\}$, Grizzle [16] developed F-test for testing equality of carryover effects, based on between sequence and within sequence sum of squares, then treatment comparison is made as per crossover design only if, carryover effects are not significantly different. Lehmacher [40] discussed an alternative approach consisting of testing multiple tests of hypothesis about equality of treatment effects, and /or equality of carryover effects using Hoteling T^2 test and t-test. Their test statistics are based on sequence total differences and within sequence treatment differences. Laird et al. [39] considered the data resulting from a crossover design as representing

longitudinal data from number of subjects, and developed GLS and REML estimators, considering two periods observations as bivariate normal observations. They advocated to employ *cod*{AB, BA, AA, BB} or *cod*{3AB, 3BA, AP, BP, PA, PB} instead of *cod*{AB, BA} to ensure the availability of adequate information on carryover effect.

From all the above discussion an idea emerges that, a crossover design suitable for analysis under carryover model should be the *cod*{AB, BA} expanded to accommodate placebo as an additional treatment. As per medical knowledge, when placebo is given to patient, in the patients' mind the psychology is that drug is given to them, but in reality it is placebo, so whatsoever psychological effects the patient' has, get measured as direct effects of the placebo, and in the next period, there are no carryover of the psychological effects. Therefore it is logical to assume no psychological carryover and obviously, there is no pharmacological carryover from a placebo treatment. Jones and Donev [28], Jones and Kenward [26], etc., discussed that when one of the treatments is a placebo, there is no reason to expect a pharmacological carryover effect into a following period as there should be no active drug ingredients in the placebo. Hence, a realistic model for pharmacological carryover effects in a trial that contains a placebo treatment, would not include a carryover parameter for placebo, but there may be carryover parameters for other active treatments.

In this chapter, we consider analyzing a three treatments, three periods, uniform, balanced crossover design in six sequences, $COD(3, 6, 3)$, as a crossover design in two treatments and a placebo, under carryover model assuming no carryover from placebo. There are two advantages of using one treatment as placebo. First, the efficiency of separability of treatment and carryover effects increases. Second, carryover effects are

estimated and tested independently prior to embarking upon the least square analysis. A test for testing the significance of carryover effect is developed. The estimation of model terms and ANOVAs are presented for three cases, (i) both active treatments have significant carryover, (ii) only one of the active treatments have carryover and (iii) none of the active treatments have significant carryover.

5.2 The model and characterization of $COD(2+1,6,3)$

The $COD(3,6,3)$ belongs to the series of uniform, minimum balanced $COD(t, t(t-1), t);\ t > 2$; prime power which has been considered by many authors (Martin and Eccleston[42], Hedayat and Yang[22], Yang and Stufken[55], Divecha and Gondaliya[9]). The said design is considered here as $COD(2+1,6,3)$, especially to represent a clinical trial design in two active treatments A and B and the third treatment P as a placebo, because in execution, this design is close to the design consisting of three replications of *cod*{AB, BA} and, in analysis it is possible to obtain a test for testing significance of carryover effects. The carryover effect tests can be generalized for the corresponding series. The design layout of $COD(2+1,6,3)$ is shown below.

	Subjects					
Periods	1	2	3	4	5	6
1	A	B	P	A	B	P
2	B	P	A	P	A	B
3	P	A	B	B	P	A

5.2.1 The model and notation

The model followed by data from $COD(2+1,6,3)$ is of 1.2.2 type, where each sequence is equally replicated and is given by

$$Y_{ijk} = \mu + \tau_{d(k,j)} + \gamma_{d(k-1,j)} + \pi_k + \xi_{ij} + \epsilon_{ijk}, \quad i = 1,\ldots,6; j = 1,\ldots,n; k = 1,2,3, \tag{5.2.1}$$

It is obvious that, there is no carryover effect in the first period, i.e. $\gamma_{d(0,j)} = 0$. We further assume that the placebo does not produce carryover effects, i.e. $\gamma_{d(k-1,j)} = 0$ if $d(k-1,j) = P$.

We use the notation G, T_A, T_B, T_P, R_A, R_B, P_k and U_{ij} to denote in order, the grand total, treatment total for A, B, P, residual total for A, B, period total for k^{th} period, $k = 1,2,3$ and total for j^{th} subject receiving i^{th} sequence, $j = 1,\ldots,n,\ i = 1,\ldots,6$.

5.2.2 Characterization of $COD(3,6,3)$ as $COD(2+1,6,3)$

$COD(2+1,6,3)$ retains all the characteristics of $COD(3,6,3)$ such as it is uniform, minimal balanced design and so on. An additional characteristic is that, there are 10 observations free from carryover effects in the former design instead of only 6 in the latter. This facilitates to divide the total 18 observations into three equal size sets of 6 observations, each complete in occurrence of treatment, period and subject factor levels. Exploiting this feature, tests of carryover effects are developed based on means of sets. Also, the additional 4, free from carryover effects observations results increase in ES. The ES of $COD(2+1,6,3)$ is 50% greater than that of $COD(3,6,3)$, the efficiency of separability of former is 63.5% and that of latter is 42.3%. Its analysis is discussed in the following Section.

5.3 Analysis

In practice, presence or suspect of carryover effects leads to lengthy clinical trials. It has a large impact on the estimation and testing of treatment effects. Undoubtedly, a crossover design that allows the test of significance of carryover effects, prior to its analysis for treatment effects should be useful in practice. For $COD(3, 6n, 3)$, either form of model followed by the experimental data can be determined or conformed, on the basis of conclusion from test of carryover effects.

5.3.1 Tests of carryover effect

A test for testing significance of carryover effect of a active treatment is developed in case of $COD(2+1, 6n, 3)$. Divide the $6n$ subjects from the above crossover design in three sets say, f_1, f_2 and f_3, consisting of specific observations respectively as $\{U_{11}, \ldots, U_{1n}, U_{51}, \ldots, U_{5n}\}$, $\{U_{21}, \ldots, U_{2n}, U_{61}, \ldots, U_{6n}\}$ and $\{U_{31}, \ldots, U_{3n}, U_{41}, \ldots, U_{4n}\}$. Define,

$$\begin{aligned} \overline{U}_m &= \frac{1}{2n} \sum_{l \in f_m} U_l;\ m = 1, 2, 3 \\ s_m^2 &= \frac{1}{2n-1} \sum_{l \in f_m} (U_l - \overline{U}_m)^2;\ m = 1, 2, 3 \end{aligned}$$

For the model, under the assumption that subject effects are independently normally distributed with mean 0 and variance σ_ξ^2, the sampling distributions of group means

are:

$$\begin{aligned}
\overline{U}_m &\sim N(\mu_m, \sigma_1^2/2n);\ m = 1,2,3;\ \text{Where } \sigma_1^2 = 3\sigma^2 + 3\sigma_\xi^2 \\
\overline{U}_1 - \overline{U}_2 &\sim N(\gamma_A, \sigma_1^2/n) \\
\overline{U}_1 - \overline{U}_3 &\sim N(\gamma_B, \sigma_1^2/n) \\
\frac{(2n-1)s_m^2}{\sigma_1^2} &\sim \chi^2_{(2n-1)};\ m = 1,2,3
\end{aligned}$$

Therefore, independent two sample t-tests with null hypothesis $H_0 : \gamma_A = 0$ against alternative $H_1 : \gamma_A \neq 0$ and with hypothesis $H_0 : \gamma_B = 0$ against $H_1 : \gamma_B \neq 0$ provide tests for testing significance of carryover effects of A, and carryover effects of B, defined respectively by 5.3.1 and 5.3.2.

$$\frac{(\overline{U}_1 - \overline{U}_2) - \gamma_A}{\sqrt{(s_1^2 + s_2^2)/2n}} \sim t_{(4n-2)} \tag{5.3.1}$$

$$\frac{(\overline{U}_1 - \overline{U}_3) - \gamma_B}{\sqrt{(s_1^2 + s_3^2)/2n}} \sim t_{(4n-2)} \tag{5.3.2}$$

In practice, clinical trial data from a crossover design may result in, both carryover effects of A as well as B are significant, or carryover from either A or B is significant, or carryover from neither A nor B is significant. Accordingly three cases of analysis of above crossover design arise. The analysis have been carried out under fixed effect model with varying assumptions of presence or absence of carryover effects.

5.3.2 Estimation in fixed effects carryover models

This section presents estimation of model parameters given by 5.2.1 in three cases about the two active treatments that, both treatments, only one treatment and none of the treatments possess significant carryover effects.

Case 1: Both active treatments have significant carryover effects.

The normal equations are:

$$
\begin{aligned}
18n\mu + 6n(\tau_A + \tau_B + \tau_P) + 4n(\gamma_A + \gamma_B) + 6n(\pi_1 + \pi_2 + \pi_3) + 3\sum_{i=1}^{6}\sum_{j=1}^{n}\xi_{ij} &= G \\
6n\mu + 6n\tau_A + 2n\gamma_B + 2n(\pi_1 + \pi_2 + \pi_3) + \sum_{i=1}^{6}\sum_{j=1}^{n}\xi_{ij} &= T_A \\
6n\mu + 6n\tau_B + 2n\gamma_A + 2n(\pi_1 + \pi_2 + \pi_3) + \sum_{i=1}^{6}\sum_{j=1}^{n}\xi_{ij} &= T_B \\
6n\mu + 6n\tau_P + 2n(\gamma_A + \gamma_B) + 2n(\pi_1 + \pi_2 + \pi_3) + \sum_{i=1}^{6}\sum_{j=1}^{n}\xi_{ij} &= T_P \\
4n\mu + 2n(\tau_B + \tau_P) + 4n\gamma_A + 2n(\pi_2 + \pi_3) + \sum_{j=1}^{n}\xi_{1j} + \sum_{j=1}^{n}\xi_{3j} + \sum_{j=1}^{n}\xi_{4j} + \sum_{j=1}^{n}\xi_{5j} &= R_A \\
4n\mu + 2n(\tau_A + \tau_P) + 4n\gamma_B + 2n(\pi_2 + \pi_3) + \sum_{j=1}^{n}\xi_{1j} + \sum_{j=1}^{n}\xi_{2j} + \sum_{j=1}^{n}\xi_{5j} + \sum_{j=1}^{n}\xi_{6j} &= R_B \\
6n\mu + 2n(\tau_A + \tau_B + \tau_P) + 6n\pi_1 + \sum_{i=1}^{6}\sum_{j=1}^{n}\xi_{ij} &= P_1 \\
6n\mu + 2n(\tau_A + \tau_B + \tau_P) + 2n(\gamma_A + \gamma_B) + 6n\pi_2 + \sum_{i=1}^{6}\sum_{j=1}^{n}\xi_{ij} &= P_2 \\
6n\mu + 2n(\tau_A + \tau_B + \tau_P) + 2n(\gamma_A + \gamma_B) + 6n\pi_3 + \sum_{i=1}^{6}\sum_{j=1}^{n}\xi_{ij} &= P_3 \\
3\mu + (\tau_A + \tau_B + \tau_P) + (\gamma_A + \gamma_B) + (\pi_1 + \pi_2 + \pi_3) + 3\xi_{1j} &= U_{1j} \\
3\mu + (\tau_A + \tau_B + \tau_P) + \gamma_B + (\pi_1 + \pi_2 + \pi_3) + 3\xi_{2j} &= U_{2j}
\end{aligned}
$$

$$
\begin{aligned}
3\mu + (\tau_A + \tau_B + \tau_P) + \gamma_A + (\pi_1 + \pi_2 + \pi_3) + 3\xi_{3j} &= U_{3j} \\
3\mu + (\tau_A + \tau_B + \tau_P) + \gamma_A + (\pi_1 + \pi_2 + \pi_3) + 3\xi_{4j} &= U_{4j} \\
3\mu + (\tau_A + \tau_B + \tau_P) + (\gamma_A + \gamma_B) + (\pi_1 + \pi_2 + \pi_3) + 3\xi_{5j} &= U_{5j} \\
3\mu + (\tau_A + \tau_B + \tau_P) + \gamma_B + (\pi_1 + \pi_2 + \pi_3) + 3\xi_{6j} &= U_{6j}
\end{aligned}
$$

The solutions of the normal equations with restrictions $\sum_{i=1}^{6}\sum_{j=1}^{n}\xi_{ij} = 0$, $\tau_A + \tau_B + \tau_P = 0$, $\gamma_A + \gamma_B = 0$ and $\pi_1 + \pi_2 + \pi_3 = 0$ are shown in Table 5.1.

Table 5.1: Estimates of model 5.2.1 parameters

Parameter	Estimates
μ	$G/18n$
γ_A	$(3(R_A - R_B) + (T_A - T_B) + \sum U_{2j} + \sum U_{6j} - \sum U_{3j} - \sum U_{4j})/16n$
γ_B	$(3(R_B - R_A) + (T_B - T_A) + \sum U_{3j} + \sum U_{4j} - \sum U_{2j} - \sum U_{6j})/16n$
τ_A	$T_A/6n - G/18n - \widehat{\gamma}_B/3$
τ_B	$T_B/6n - G/18n - \widehat{\gamma}_A/3$
τ_P	$T_P/6n - G/18n$
π_1	$P_1/6n - G/18n$
π_2	$P_2/6n - G/18n$
π_3	$P_3/6n - G/18n$
ξ_{1j}	$U_{1j}/3 - G/18n$
ξ_{2j}	$U_{2j}/3 - G/18n - \widehat{\gamma}_B/3$
ξ_{3j}	$U_{3j}/3 - G/18n - \widehat{\gamma}_A/3$
ξ_{4j}	$U_{4j}/3 - G/18n - \widehat{\gamma}_A/3$
ξ_{5j}	$U_{5j}/3 - G/18n$
ξ_{6j}	$U_{6j}/3 - G/18n - \widehat{\gamma}_B/3$

Case 2: Only one active treatment has significant carryover effect.

When only one active treatment has carryover effect, the normality restriction on carryover effects is unavailable, and hence, the normal equations containing the non-zero carryover effects parameter cannot be solved. The only way to analyze the model 5.2.1, is to substitute for the non-zero carryover effect terms by the empirical estimates. Define, four sets h_1, h_2, h_3 and h_4 consisting of model observations $\{Y_{1j2}, Y_{2j1}, Y_{3j3}, Y_{4j2}, Y_{5j3}, Y_{6j1}\}$, $\{Y_{1j3}, Y_{2j2}, Y_{3j1}, Y_{4j3}, Y_{5j1}, Y_{6j2}\}$, $\{Y_{1j3}, Y_{2j2}, Y_{3j1}, Y_{4j1}, Y_{5j2}, Y_{6j3}\}$ and $\{Y_{1j1}, Y_{2j3}, Y_{3j2}, Y_{4j2}, Y_{5j3}, Y_{6j1}\}$ and having means $\overline{Y}_1$, $\overline{Y}_2$, $\overline{Y}_3$ and $\overline{Y}_4$ respectively. When 5.3.1 results in γ_A to be non-zero, use sets h_1 and h_2 to estimate γ_A, $\widehat{\gamma}_A = 1.5(\overline{Y}_1 - \overline{Y}_2)$. Similarly, when 5.3.2 results in γ_B to be non-zero, use sets h_3 and h_4, and obtain $\widehat{\gamma}_B = 1.5(\overline{Y}_3 - \overline{Y}_4)$. Without loss of generality let us assume, γ_A is significant. Then model 5.2.1 can be transformed into a no carryover effects model given by,

$$Y'_{ijk} = \begin{cases} Y_{ijk} - \widehat{\gamma}_A, & \text{if } d(k-1, j) = A; \\ Y_{ijk}, & \text{otherwise.} \end{cases}$$

where,

$$Y'_{ijk} = \mu + \tau_{d(k,j)} + \pi_k + \xi_{ij} + \epsilon_{ijk}, \quad i = 1, \ldots, 6; j = 1, \ldots, n; k = 1, 2, 3, \qquad (5.3.3)$$

Then estimates of model 5.3.3 parameters are as shown in Table 5.2. Note that G, $\tau_{d(k,j)}$, p_k, and U_{ij} is calculated using observation Y'_{ijk} in place of Y_{ijk}.

Case 3: None of the active treatments have significant carryover effect.

When carryover effects are present, carryover effects are confounded with the treatment period interaction effects, therefore, the treatment period interaction term was

ignored in above two cases. Now, in the absence of carryover effects, there is an opportunity to analyze the following model,

$$Y_{ijk} = \mu + \tau_{d(k,j)} + \pi_k + \xi_{ij} + (\tau\pi)_{d(k,j)k} + \epsilon_{ijk}, \quad i = 1, \ldots, 6; j = 1, \ldots, n; k = 1, 2, 3, \tag{5.3.4}$$

where $(\tau\pi)_{d(k,j)k}$ is the treatment period interaction effects due to treatment $d(k, j)$ and period k and the remaining terms are as defined in 5.2.1. Under the side conditions, $\sum_{d(k,j)}(\tau\pi)_{d(k,j)k} = 0$ and $\sum_k(\tau\pi)_{d(k,j)k} = 0$, estimate of $(\tau\pi)_{d(k,j)k}$ is $[3(TP)_{d(k,j)k} - T_{d(k,j)} - P_k - V_{d(k,j)k} + 2G/3]/6n$, where $(TP)_{d(k,j)k}$ is sum of observations receiving the treatment $d(k, j)$ in period k and $V_{d(k,j)k}$ is the sum of subjects which receives treatment $d(k, j)$ in period k. Estimates of the remaining model terms are as shown in Table 5.2.

Table 5.2: Estimates of model 5.3.3 parameters

Parameter	Estimates
μ	$G/18n$
$\tau_{d(k,j)}$	$T_{d(k,j)}/6n - G/18n$
π_k	$P_k/6n - G/18n$
ξ_{ij}	$U_{ij}/3 - G/18n$

5.3.3 ANOVA for various effects of carryover models

Case 1: Both active treatments have significant carryover effect.

Analysis of *cod*{AB, BA} cannot test hypothesis about equality of treatment effects eliminating carryover. Grizzle [16] has suggested to use only first period data to test

treatment effects under a carryover model. Lehmacher [40] made a detailed study of all the possible hypothesis about treatment and carryover effects, and recommended that *cod*{AB, BA} can be useful for crossover trial, if no or only positive carryover is assumed. He suggested to test following set of hypothesis to conclude about treatment when carryover effects are present, $H_0 : (\tau_A - \tau_B) - (\lambda_A - \lambda_B) = 0$, $H_1 : \tau_A - \tau_B = 0$ (no treatment effects in first period), $H_2 : (\tau_A - \tau_B) - (\lambda_A - \lambda_B)/2 = 0$, $H_3 : \lambda_A - \lambda_B = 0$ and $H_4 : (\tau_A - \tau_B) - (\lambda_A - \lambda_B) = 0$ (no second period difference). Then, subject to rejection of H_0, if H_1 is rejected, treatment effects are concluded as significant, however, if fail to reject H_1 but H_3 and H_4 are rejected, imply either treatment effects are significantly positive or carryover effects are significantly negative. These set of hypothesis give proper conclusion when $\lambda_A = \lambda_B$ and erroneous otherwise.

Table 5.3: Analysis of variance of model 5.2.1 for treatment effects

Source of Variation	Sum of Squares Expression	Degrees of Freedom
Treatments	$\widehat{\tau}_A Q_A + \widehat{\tau}_B Q_B + \widehat{\tau}_P Q_P$	2
(eliminating carryover)		
Active Treatments	$(5(T_A - T_B) + 3(R_A - R_B) + \sum_j U_{2j} + \sum_j U_{6j}$	1
(eliminating carryover)	$- \sum_j U_{3j} - \sum_j U_{4j})^2/240n$	
Carryover of treatments	$3[(R_A + P_1/3 + \sum U_{2j}/3 + \sum U_{6j}/3 - 4G/9)^2$	1
(ignoring treatments)	$+(R_B + P_1/3 + \sum U_{3j}/3 + \sum U_{4j}/3 - 4G/9)^2]/10n$	
(eliminating subjects)		
Periods	$\sum_{i=1}^{3} P_i^2/6n - G^2/18n$	2
Subjects	$\sum_{i=1}^{6} \sum_{j=1}^{n} U_{ij}^2/3 - G^2/18n$	$6n - 1$
(ignoring carryover)		
Error	By Subtraction	$12n - 5$
Total	$\sum Y_{ijk}^2 - G^2/18n$	$18n - 1$
Where $Q_A = T_A - G/3 - 2n\widehat{\gamma}_B$; $Q_B = T_B - G/3 - 2n\widehat{\gamma}_A$; $Q_P = T_P - G/3$		

It is desirable to obtain test of treatment effects based on treatment effect estimates that have been adjusted for carryover effects. Such estimates of treatment effects for two treatment crossover designs have been discussed by Lucas [41] for *cod*{ABB, BAA}, Ebbutt [10] for *cod*{ABB, ABA}, Senn and Lambrou [51] for crossover design in four period, etc., but all have limited to estimation. Analysis of variance for COD(2+1,6n,3) provides tests of treatment effects, eliminating carryover effects as shown in Table 5.3. Here, the primary interest is to compare active treatments. Under $H_0 : \tau_A = \tau_B$, the sum of squares due to active treatments $(5(T_A - T_B) + 3(R_A - R_B) + \sum_j U_{2j} + \sum_j U_{6j} - \sum_j U_{3j} - \sum_j U_{4j})^2/240n$ normed by σ^2 follows chi-square distribution with one degree of freedom. As a result, active treatment effects eliminating carryover effects are also tested by analysis of variance shown in Table 5.3.

Table 5.4: Analysis of variance of model 5.2.1 for subject effects

Source of Variation	Sum of Squares Expression	Degrees of Freedom
Subjects		$6n-1$
(ignoring carryover)		
Groups	$(G_1^2+G_2^2)/9n - G^2/18n$	1
Subjects within group	$\sum_{i=1}^{3}\sum_{j=1}^{n} U_{ij}^2/3 - G_1^2/9n + \sum_{i=3}^{6}\sum_{j=1}^{n} U_{ij}^2/3 - G_2^2/9n$	$6n-2$
Periods	$\sum_{i=1}^{3} P_i^2/6n - G^2/18n$	2
Treatments	$(T_A^2+T_B^2+T_P^2)/6n - G^2/18n$	2
(ignoring carryover)		
Carryover of treatments	$\widehat{\gamma}_A R_A^{'} + \widehat{\gamma}_B R_B^{'}$	1
(eliminating treatments)		
(eliminating subjects)		
Error	By Subtraction	$12-5$
Total	$\sum Y_{ijk}^2 - G^2/18n$	$18n-1$

Where $R_A^{'} = 3(R_A - R_B) + (T_A - T_B) + \sum U_{2j} + \sum U_{6j} - \sum U_{3j} - \sum U_{4j}$;
$R_B^{'} = 3(R_B - R_A) + (T_B - T_A) + \sum U_{3j} + \sum U_{4j} - \sum U_{2j} - \sum U_{6j}$

Subject effects can be significantly different due to receiving of the different sequences of treatment or due to biological variation. Second analysis of variance shown in Table 5.4, is developed to test hypothesis about subject effects and get some idea about the cause of variation, when they are significant.

Case 2: Any one active treatment has significant carryover effect.

When any one active treatment has significant carryover effect, the tests procedures suggested in literature specifically by Grizzle [16], Lehmacher [40], etc. are not useful because of the assumption that, carryover of all treatments should be equal. Here, it is better to transform model 5.2.1 into the form 5.3.3 according to the procedure suggested in Case 2 of Section 5.3.2. Model 5.3.3 is free from carryover and hence, the hypothesis $H_0 : \tau_A = \tau_B = \tau_P$ can be tested using analysis of variance shown in Table 5.5.

Table 5.5: Analysis of variance of model 5.3.3

Source of Variation(SS)	Sum of Squares Expression	Degrees of Freedom
Treatments(SST)	$(T_A^2 + T_B^2 + T_P^2)/6n - G^2/18n$	2
Active Treatments (SSAT)	$(T_A - T_B)^2/8n$	1
Periods (SSP)	$\sum_{i=1}^{3} P_i^2/6n - G^2/18n$	2
Subjects (SSS)	$\sum_{i=1}^{6}\sum_{j=1}^{n} U_{ij}^2/3 - G^2/18n$	$6n-1$
Error	TSS-SST-SSP-SSS	$4(3n-1)$
Total (TSS)	$\sum Y_{ijk}^2 - G^2/18n$	$18n-1$

Note that, here primary objective is to compare active treatments. Under null hypothesis $H_0 : \tau_A = \tau_B$, the test statistics $(T_A - T_B)^2/8n$ normed by σ^2 follows chi-square distribution with one degree of freedom. Therefore, $H_0 : \tau_A = \tau_B$ can be tested by SSAT as shown in Table 5.5. Also one more advantage due to model transformation is that, period and subject effects are now orthogonal with the treatment effects and

hence, period and subject effects are also tested using analysis of variance shown in Table 5.5.

Case 3: None of the active treatments has significant carryover effect.

In absence of significant carryover of active treatments, sum of squares due to treatment period interaction can also be split from the total sum of squares. Total sum of squares for a model can be split into sum of squares due to treatments, periods, subjects and treatment period interaction. Also active treatments are tested by the test statistics $(T_A - T_B)^2/8n$. The analysis of variance of the model 5.3.4 for testing hypothesis about treatments, active treatments, periods, treatment period interaction and subjects is shown in Table 5.6.

Table 5.6: Analysis of variance of model 5.3.4

Source of Variation(SS)	Sum of Squares Expression	Degrees of Freedom
Treatments (SST)	$(T_A^2 + T_B^2 + T_P^2)/6n - G^2/18n$	2
Active Treatments (SSAT)	$(T_A - T_B)^2/8n$	1
Periods (SSP)	$\sum_{i=1}^{3} P_i^2/6n - G^2/18n$	2
Treatments × Periods (SSTP)	$\sum_{d(k,j)} \sum_k (TP)^2_{d(k,j)k}/2n - G^2/18n$−SST−SSP	4
Subjects (SSS)	$\sum_{i=1}^{6} \sum_{j=1}^{n} U_{ij}^2/3 - G^2/18n$	$6n-1$
Error	TSS-SST-SSP-SSTP-SSS	$3(4n-3)$
Total (TSS)	$\sum Y_{ijk}^2 - G^2/18n$	$18n-2$

5.4 Example

The data in Table 5.7 is used to illustrate computations involved in estimating effects, and performing analysis of variance for the cases, as both active treatments have significant carryover effects. Here $f_1 = \{54, 54, 54, 55\}$, $f_2 = \{52, 52, 52, 52\}$ and $f_3 = \{51, 51, 51, 51\}$. According to f_1, f_2 and f_3 the numerical values of the statistics

$\bar{U}_1$, $\bar{U}_2$, $\bar{U}_3$, s_1^2, s_2^2 and s_3^2 are respectively as 54.25, 52, 51, 0.25, 0 and 0. Calculated values of test statistics 5.3.1 and 5.3.2 are, respectively 9 and 13. Both test statistics 5.3.1 and 5.3.2 show that carryover effect of active treatments are significant at 5% as well as 1% level of significance. Estimates of the model parameters in this case, follows from Table 5.1, and, are shown in Table 5.8. It shows that active treatment A has 2.0625 unit more effects than B (i.e., $\widehat{\tau}_A - \widehat{\tau}_B = 2.0625$). Similarly analysis of variance in this case for treatments, active treatments and carryover effects follows from Table 5.3, is shown in Table 5.9. All three effects in this table are significant, specifically active treatment effects, as manipulated in the experiment.

Computations involved in estimating effects and performing analysis of variance, for the case, any one active treatment has significant carryover effect is illustrated using manipulated data and is shown in Table 5.10. Here, calculated values of test statistics 5.3.1 and 5.3.2 are 1 and 13 respectively. Test statistics 5.3.2 shows that carryover effect of active treatment B is significant whereas that of A is insignificant at 5% as well as 1% level of significance. The empirical estimate of $\hat{\gamma}_B$ from the sets $h_3 = \{18, 18, 15, 18, 21, 21, 18, 18, 15, 18, 21, 21\}$ and $h_4 = \{18, 18, 18, 15, 15, 15, 18, 18, 18, 15, 16, 15\}$ is $1.5 \times (18.5 - 16.583) = 2.875$. Now, transform the observations which are affected by carryover effects of treatment B. Transformed observations are shown in parenthesis in Table 5.10. Estimates of model parameters and analysis of variance for the model 5.3.3 are shown in Table 5.11 and Table 5.12 respectively. Active treatment A has 2.0417 unit more effects than B (i.e., $\widehat{\tau}_A - \widehat{\tau}_B = 2.0417$). Also, analysis of variance shows that treatment effects and active treatment effects are significant, whereas periods and subject effects are insignificant.

Table 5.7: Manipulated Data from COD(3,12,3)

Period	Treatment Sequence	Subject U_{11}	U_{12}	Treatment Sequence	Subject U_{21}	U_{22}	Treatment Sequence	Subject U_{31}	U_{32}
1	A	18	18	B	16	16	P	15	15
2	B	18	18	P	18	18	A	18	18
3	P	18	18	A	18	18	B	18	18
Period	Treatment Sequence	Subject U_{41}	U_{42}	Treatment Sequence	Subject U_{51}	U_{52}	Treatment Sequence	Subject U_{61}	U_{62}
1	A	18	18	B	16	16	P	15	15
2	P	17	17	A	21	21	B	16	16
3	B	16	16	P	17	18	A	21	21

Table 5.8: Estimates of model 5.2.1 for data in Table 5.7

Parameter	Estimates
μ	17.4722
γ_A	-0.4062
γ_B	0.4062
τ_A	1.3924
τ_B	-0.6701
τ_P	-0.7222
π_1	-1.1389
π_2	0.5278
π_3	0.6111

Table 5.9: Analysis of variance of model 5.2.1 for data in Table 5.7

Source of Variation	Sum of Squares	Degrees of Freedom	Means Squares	F_c
Treatments	34.9123	2	17.4562	22.9959
(eliminating carryover)				
Active Treatments	20.4185	1	20.4185	26.8987
(eliminating carryover)				
Carryover of treatments	16.6093	1	16.6093	21.8803
(ignoring treatments)				
(eliminating subjects)				
Periods	23.3889	2	11.6944	
Subjects	7.6389	11	0.6944	
(ignoring carryover)				
Error	14.4229	19	0.7591	
Total	96.9722	35		

Table 5.10: Manipulated Data from COD(3,12,3) when active treatment B has carryover effect

Period	Treatment Sequence	Subject U_{11}	U_{12}	Treatment Sequence	Subject U_{21}	U_{22}	Treatment Sequence	Subject U_{31}	U_{32}
1	A	18	18	B	16	16	P	15	15
2	B	16	16	P	18(15.125)	18(15.125)	A	18	18
3	P	18(15.125)	18(15.125)	A	18	18	B	16	16
Period	Treatment Sequence	Subject U_{41}	U_{42}	Treatment Sequence	Subject U_{51}	U_{52}	Treatment Sequence	Subject U_{61}	U_{62}
1	A	18	18	B	16	16	P	15	15
2	P	15	15	A	21(18.125)	21(18.125)	B	16	16
3	B	16	16	P	15	16	A	21(18.125)	21(18.125)

Table 5.11: Estimates of model 5.3.3 under $\gamma_B \neq 0$ for data in Table 5.10

Parameter	Estimates
μ	16.3889
τ_A	1.6528
τ_B	-0.3839
τ_P	-1.2639
π_1	-0.0556
π_2	-0.0139
π_3	0.0694

Table 5.12: Analysis of variance under $\gamma_B \neq 0$ for data in Table 5.10

Source of Variation	Sum of Squares	Degrees of Freedom	Means Squares	F_c
Treatments	53.7639	2	26.8819	1138.5294
Active Treatments	37.5156	1	37.5156	1588.8971
Periods	0.0972	2	0.04861	2.0589
Subjects	0.3472	11	0.3157	1.3369
Error	0.4722	20	0.0236	
Total	54.6806	35		