Detecting Outliers in Bioequivalence Studies for Crossover Design

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ABSTRACT

Outliers may affect the conclusion of the decision about the bioequivalence between test drug and reference drug. This paper aims to study the statistical methods to detect outliers in Bioequivalence study for a crossover design. Two methods of detecting outliers are considered, Principal Component Analysis (PCA) and cook's distance. The study is conducting using real data set to achieve the results.

Keywords: Bioequivalence Study, Principal Component Analysis, Cook's Distance.

INTRODUCTION

Bioequivalence studies are very important for any new treatment (Test drug, T) as alternative to the original drug (Reference drug, R). The T drug must be tested statistically to prove that it is no worse than (or equivalence) to the active control within defined than the original drug, R drug. Bioequivalence (BE) studies provide a statistical evidence of bioequivalence between two medicinal products (T drug and R drug) containing the same active substance. A common problem in BE studies is the occurrence of outliers, whether extremely large or small observations. The existing of such observation may affect the conclusion of the decision about the bioequivalence between T drug and R drug. The bioequivalence of two formulations of the same drug can be determined based on no significant differences in pharmacokinetic parameters.

The American Food and Drug Administration, FDA (1992), specified the rule that the BE studies must have at least 80% power of detecting a 10% difference between the parameters of interest. It was also suggested that 90% confidence intervals (CI) be used within the pre-specified range (80%, 125%).

The present study is an extension for the model which presented by Ibrahim (2017). They suggested a model to measure a BE between formulations using four pharmacokinetic parameter C_{max}, T_{max}, AUC_{0-t}, $AUC_{0-\infty}$, respectively where:

- C_{max}: Indicates to the maximum drug concentration achieved in systemic circulation following drug administration.
- T_{max}: the time which required to achieve maximum drug concentration in systemic circulation.
- AUC_{0-t}: Area under the plasma concentration-time curve to the last quantifiable concentration to be calculated using trapezoidal rule.
- $AUC_{0-\infty}$: Area under the plasma concentration-time curve from 0 to infinity to be calculated as the sum of AUC_{0-t} plus the ratio of the last measurable concentration to the elimination rate constant. [CDSCO, (2005)]

The real data which used in this study is obtained from Central Administration for Pharmaceutical Affairs (CAPA), in Egypt. The data are divided according to crossover design. A crossover design is a design used when collecting blood samples from study subjects such that pharmacokinetic parameters can be obtained. The present study is

concerned with detecting outliers in bioequivalent studies for 2x2 and 3x3 crossover design. This paper is organized as follows; section 2 is concerned with methods of detecting outliers in bioequivalence. In section 3, concluding remarks of the results from the data are obtained.

Methods of Detecting Outliers in Bioequivalence:

There are many methods used to detect outliers in bioequivalence studies. This study is concerned with some of outliers detection methods, These methods are Principal Component Analysis (PCA) and Cook's distance in some details for 2x2 Crossover Design and 3x3 Crossover Design, respectively:

Principal Component Analysis (PCA):

The objective of PCA is to discover or to reduce the dimensionality of the dataset and identify new meaningful underlying variables. In PCA number of (possibly), correlated variables are transformed into (smaller) number of variables which are uncorrelated called principal components. A large amount of variability is accounted by the first PC and each succeeding PC accounts for as much of the remaining variability as possible. With:

$$E\left(\sum_{i=1}^{p} \lambda_i X^2_{1,j}\right) = \sum_{i=1}^{p} \lambda_i = p \text{ And, } Var\left(\sum_{i=1}^{p} \lambda_i X^2_{1,j}\right) = 2 \sum_{i=1}^{p} \lambda^2_i$$

$\sum_{i=1}^{p} \lambda_i X^2_{1,j}$: The weighted sum of square distance to zero of the projected data into principal factorial plane.

λ_i : is called Eigen value denotes the variance of the i^{-th} principal component. For more details, see [Enachescu and Enachescu, (2009)].

Now the Observations with a square distance greater than Threshold (*m*) may be considered as outliers where

$$m = p + 2\sqrt{2 \sum_{i=1}^{3} \lambda^2_i}$$

For 2x2 Crossover Design:

In 2x2 crossover design, subjects' study are randomized into two groups then given a sequence of treatments at uniform intervals of time (periods). In the first sequence, each study subject receives, say, a test treatment in period 1, then a reference treatment in period 2 and this arrangement is reversed in the second sequence, reference treatment is given followed by the test treatment. [Lim *et al* (2017)]

In this article, the population study consisted of (*N* = 24) healthy, adult volunteers (subjects), according to real data obtained from Central Administration for Pharmaceutical Affairs (CAPA), in Egypt. The subjects were randomized to receive test and reference drugs in each period in order to eliminate any bias factors.

The study was crossover designed as two-treatment, two-sequence (groups, each group consists of 12 subjects) and two-period (PER I and PERII). Bioequivalence study is made with a washout period. Volunteers must receive the same amount of test and reference drugs. The first group received single oral doses of the test drug (90mg). The second group received three doses of the reference drug (30mg). Plasma levels of the chemical substance were measured by taking samples of blood from every volunteer during the following times per hour zero: 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 8, 10, 12, 24, 48 and 72. Then after wash out time, in period two, drugs were switched between volunteers as shown below:
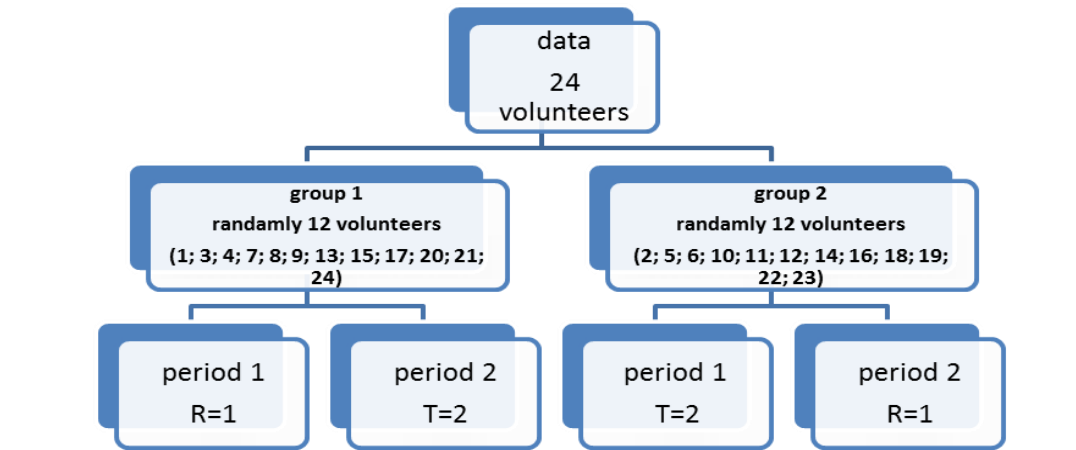
According to 2x2 Crossover Design, as two-treatment, two-sequence (groups, each group consists of 12 subjects) and two-period (PER I and PERII). The study employed the data described above for both Linear and Logarithmic Scales. SPSS Package is used to apply PCA method to detect outlier observations. The results are obtained from table (1-a) to table (4-b) for each parameter, C_{max}, T_{max}, $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$, respectively, reported in Appendix (A).

According to table (1-b), by comparing between Squared distance and Threshold (6.808) for linear scale and (6.8849) for Logarithmic Scale, it was found that, there are no outliers for the C_{max} parameter.
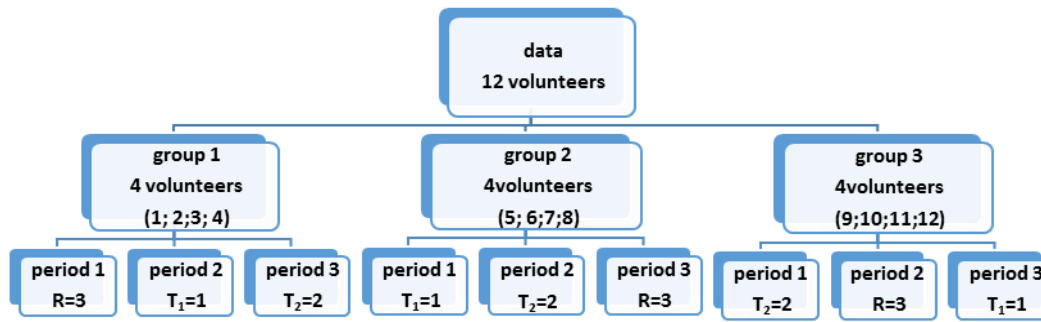
According to table (2-b), by comparing between Squared distance and Threshold (6.054) for linear scale and (6.08) for Logarithmic Scale, it was found that, there are two outlier observations for the T_{max} parameter, 1st and 7th subject respectively.

According to table (3-b), by comparing between Squared distance and Threshold (6.813) for linear scale and (6.75338) for Logarithmic Scale, it was found that, there are two outlier observations for the AUC_{0-t} parameter, 10th and 18th subject respectively. According to table (4-b), by comparing between Squared distance and Threshold (6.076) for linear scale and (6.43) for Logarithmic Scale, it was found that, there are two outlier observations for the $AUC_{(0-\infty)}$ parameter, 10th and 15th subject respectively.

For 3x3 Crossover Design:

In this section, the PCA techniques for detecting outliers for 3x3 Crossover Design is illustrated. For more details, see Kirtiraj (2014). The study used a new data contains of:

• Three drugs : two test (T1 and T2) and Reference (R) drugs.

• 12 healthy volunteers (subjects). Volunteers must receive the same amount of drugs.

• According to 3x3 crossover design, the data is divided into three groups (sequence) randomized. Each group consists of randomly 4 volunteers.

• Each group is divided into three periods and between the each periods a period of rest of the each treatment. Then after wash out time, drugs were switched between volunteers as shown below:

SPSS Package is used to detect outliers by PCA method for both Linear and Logarithmic scales. The results are obtained from table (5-a) to table (8-b) for each parameter, C_{max}, T_{max}, $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$, respectively, reported in Appendix (A).

According to table (5-b), by comparing between Squared distance and Threshold (8.44165) for Logarithmic Scale, there is no outlier was found. Table (5-b) shows that there is one outlier observation for the C_{max} parameter which is 7th subject.

According to table (6-b), by comparing between Squared distance and Threshold (8.32619) for linear scale, it was found that, there is no outlier observation for the T_{max} parameter. Also, for Threshold (8.37496) for Logarithmic Scale, there is no outlier was found.

According to table (7-b), by comparing between Squared distance and Threshold (10.0015) for linear scale, it was found that, there is no outlier observation for the AUC_{0-t} parameter. Also, for Threshold (10.37676) for Logarithmic Scale, there is no outlier was found.

According to table (8-b), by comparing between Squared distance and Threshold (10.244339) for linear scale, it was found that, there is no outlier observations for the $AUC_{(0-\infty)}$ parameter. Also, for Threshold (10.5203) for Logarithmic Scale, there is no outlier was found.

Cook's Distance:

Cook's distance technique (D_i) is applied to detect outlier observations. It is useful for identifying outliers. Observations are outliers if D_i is larger than three times the mean. D_i is a combination of each observation's leverage and residual values; the higher the leverage and residuals, the higher the Cook's distance The formula for Cook's distance is.

$$D_i = \frac{\sum_{j=1}^{n}(\widehat{Y}_j - \widehat{Y}_{j(i)})^2}{(p+1)\widehat{\sigma}^2}$$

Where:

- $\hat{y}_j$: The jth fitted response value.

- $\hat{y}_{j(i)}$: The jth fitted response value, where the fit does not include observation i.

- p : the number of coefficients in the regression model.

- A general rule of thumb is that observations with a Cook's D of more than 3 times the mean, μ, is a possible outlier.

- An alternative interpretation is to investigate any point over 4/n, where n is the number of observations. [Cook (1977)]

Cook's Distance for 2x2 Crossover Design:

The study employed the same data described in PCA method in subsection (2-1-2). In 2x2 crossover design, the number of observation were 48, where for each person he took both of two drugs, T and R drugs. SPSS Package is used to detect outliers by Cook's distance. The results are obtained in table 9, reported in Appendix (A).

Table 9 shows that there are outliers of pharmacological parameters but some of them affect and some are ineffective.

For parameter C_{max}, there are values greater than three times the mean, but the difference between them is not large. It is assumed that they do not affect the results.

For parameter T_{max}, there are values greater than three times the mean but there are values the difference between them is not large. It is assumed that they do not affect the results. There is one value difference large between them and three times the average can be said to be outliers, this is 8th subject.

For parameter AUC_{0-t}, there are some values greater than three times the mean but there are values the difference between them is not large it is assumed they do not affect the results. There is one value difference large between them and three times the average can be said to be outliers is 18th subject.

For parameter $AUC_{(0-\infty)}$, there are some values greater than three times the mean but there are values the difference between them is not large it is assumed they do not affect the results. There are two values difference large between them and three times the average can be said to be outliers are 10th and 15th subject.

Figures, from 1 to 4, reported in Appendix (B), show the results graphically, using SPSS Package, according to Cook's distance for 2x2 crossover design.

Cook's Distance for 3x3 Crossover Design:

The study employed the same data described in PCA method in subsection (2-1-2). In 3x3 crossover design, the number of observation were 36, where for each person he took three drugs, T1 and T2 and R drugs. SPSS Package is used to detect outliers for Cook's distance. The results are obtained in table 10, reported in Appendix (A).

Table 10 shows that there are outliers of pharmacological parameters but some of them affect and some are ineffective.

For parameter C_{max}, there are some values greater than three times the mean, but the difference between them is not large. It is assumed that they do not affect the results. For parameter T_{max}, there are some values greater than three times the mean but the difference between them is not large, it is assumed that they do not affect the results. There is one value difference large between them and three times the average can be said to be outliers is 12th subject.

For parameter AUC_{0-t}, there are values greater than three times the mean, but the difference between them is not large. It is assumed that they do not affect the results. There is one value difference large between them and three times the average can be said to be outliers is 10th subject.

For parameter $AUC_{(0-\infty)}$ there are values greater than three times the mean but there are values the difference between them is not large it is assumed they do not affect the results. There is one value difference large between them and three times the average can be said to be outliers is 5th subject.

Figures, from 5 to 8, reported in Appendix (B), show the results graphically, using SPSS package, according to cook's distance for 3x3 crossover design

CONCLUDING REMARKS

According to the present study, Test drugs should be as safe and effective as reference drugs to guarantee safety and effectiveness of test drugs. In practice, if test drugs never have the treatment formulations and exactly the same bioavailability of test drugs, an insignificant difference would be always exist between them. Based on the results obtained for bioequivalence in the presence of outliers in this study, it can be indicated that, although the presences of some outliers in the datasets, but the most of these outliers could not had too much effect on the results. But if these outliers have a clear presence in the datasets, in this case, these values should be removed. On the other hand, if these outliers cannot be removed, may be the investigator, for example, could replace these outlier values with the mean of the remaining data to avoid undesirable effect or bad effect of these values.

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APPENDIX (A)

PCA for 2x2 Crossover Design

For C_{max} parameter

Table (1-a): Eigen-Values for the Linear and Logarithmic Scales to C_{max}

	Linear Scale			*m*	Logarithmic Scale			*m*
	Eigen values	Proportion	Cumulative		Eigen values	Proportion	Cumulative	
1	1.6670	0.834	0.834	6.808	1.7010	0.850	0.850	6.8849
2	0.3330	0.166	1		0.2990	0.150	1	

Table (1-b): Threshold Value and the Squared Distance on Linear and Logarithmic Scales to C_{max}:

Subjects	Linear scale		Logarithmic Scale	
	Squared distance	*m*	Squared distance	*m*
1	0.292205	**6.808**	0.117841	**6.8849**
2	0.816273		0.536746	
3	0.659577		0.680656	
4	0.056346		0.006443	
5	1.320921		1.28478	
6	5.058621		3.279357	
7	2.642502		2.373858	
8	4.009222		2.922897	
9	0.442939		0.245346	
10	5.230638		3.756424	

11	0.400073		0.220627	
12	2.520026		3.275803	
13	3.653834		6.272092	
14	0.044758		0.137969	
15	1.853063		1.925527	
16	0.934129		1.195209	
17	1.760393		1.851076	
18	2.631723		3.411712	
19	0.231058		0.065552	
20	3.628451		3.051832	
21	1.206296		1.430743	
22	2.641721		4.291098	
23	1.341158		1.792048	
24	2.624074		1.874367	

For T_{max} parameter

Table (2-a): Eigen-Values for the Linear and Logarithmic Scales to T_{max}

	Linear Scale			M	Logarithmic Scale			m
	Eigen values	Proportion	Cumulative		Eigen values	Proportion	Cumulative	
1	1.1651	0.583	0.583	6.054	1.2013	0.601	0.601	6.0800
2	0.8349	0.417	1		0.7987	0.399	1	

Table (2-b): Threshold Value and the Squared Distance on Linear and Logarithmic Scales to T_{max}

	Linear scale		Logarithmic Scale	
Subjects	Squared distance	m	Squared distance	M
1	6.347855	**6.054**	4.388171	**6.0800**
2	2.297066		4.048853	

3	0.502297		0.568065	
4	0.565021		0.301535	
5	4.372961		2.527352	
6	1.764949		1.74148	
7	4.038462		6.820777	
8	2.45064		5.266462	
9	1.607051		1.54805	
10	1.367655		1.802514	
11	0.338866		0.247664	
12	0.026936		0.176717	
13	0.072167		0.046449	
14	1.913013		2.208554	
15	3.121881		2.724687	
16	0.93796		1.01109	
17	0.598727		0.558705	
18	0.415242		0.509174	
19	0.140014		0.337831	
20	0.406713		0.221234	
21	5.089844		3.297713	
22	5.669389		3.877557	
23	1.616426		1.521704	
24	0.338866		0.247664	

For AUC$_{(0-t)}$ parameter

Table (3-a): Eigen-Values for the Linear and Logarithmic Scales to AUC$_{(0-t)}$

Linear Scale			*m*	Logarithmic Scale			*m*
Eigen values	Proportion	Cumulative	6.6813	Eigen values	Proportion	Cumulative	6.75338

1	1.6079	0.804	0.804		1.6416	0.821	0.821
2	0.3921	0.196	1		0.3584	0.179	1

Table (3-b): Threshold Value and the Squared Distance on Linear and Logarithmic Scales to $AUC_{(0-t)}$

Subjects	Linear scale		Logarithmic Scale	
	Squared distance	m	Squared distance	M
1	1.130739	**6.6813**	1.166421	**6.75338**
2	0.130936		0.189553	
3	2.693126		1.787536	
4	0.828198		0.760176	
5	3.640664		3.004641	
6	1.315992		1.266076	
7	4.824435		3.587424	
8	1.672631		1.750794	
9	1.064299		0.92472	
10	9.755956		5.838646	
11	0.089366		0.288052	
12	0.211984		0.196616	
13	1.206321		0.967642	
14	0.048347		0.010055	
15	0.293516		0.448449	
16	0.033572		0.05923	
17	3.119882		4.216528	
18	4.871316		8.724557	
19	1.064342		0.937579	
20	1.634295		1.619637	
21	2.72691		3.514808	

22	2.437192		3.63084	
23	0.840124		0.820937	
24	0.365857		0.289085	

For AUC$_{(0-\infty)}$ parameter

Table (4-a): Eigen-Values for the Linear and Logarithmic Scales to AUC$_{(0-\infty)}$

	Linear Scale			*m*	Logarithmic Scale			*m*
	Eigen values	Proportion	Cumulative		Eigen values	Proportion	Cumulative	
1	1.1957	0.598	0.598	6.076	1.4762	0.738	0.738	6.43
2	0.8043	0.402	1		0.5238	0.262	1	

Table (4-b): Threshold Value and the Squared Distance on Linear and Logarithmic Scales to AUC$_{(0-\infty)}$

Subjects	Linear scale		Logarithmic Scale	
	Squared distance	*m*	Squared distance	*m*
1	3.925195	**6.076**	3.364347	**6.43**
2	0.186792		0.110908	
3	0.363777		0.611628	
4	0.229681		0.327462	
5	0.608863		1.302561	
6	0.499782		0.738279	
7	0.57144		1.399878	
8	0.304627		0.987484	
9	0.564519		1.061837	
10	12.32783		6.938618	
11	0.037797		0.100451	
12	0.196569		0.355952	

13	0.795239		1.169819	
14	0.148041		0.107392	
15	17.31843		9.200627	
16	0.295323		0.494621	
17	1.973091		5.71573	
18	1.959896		5.500872	
19	0.528605		0.577337	
20	0.397433		0.571075	
21	1.497086		3.369482	
22	0.236245		0.192456	
23	0.485104		1.212364	
24	0.548629		0.588821	

PCA for 3x3 Crossover Design

For C_{max} parameter

Table (5-a): Eigen-Values for the Linear and Logarithmic Scales for C_{max}

	Linear Scale			*m*	Logarithmic Scale			*m*
	Eigen values	Proportion	Cumulative		Eigen values	Proportion	Cumulative	
1	1.4902	0.497	0.497	8.22097	1.6489	0.550	0.550	8.44165
2	0.9080	0.303	0.799		0.8623	0.287	0.837	
3	0.6018	0.201	1.000		0.4889	0.163	1.000	

Table (5-b): Threshold Value and the Squared Distance on Linear and Logarithmic Scales

	Linear scale		Logarithmic Scale	
subjects	Squared distance	*m*	Squared distance	*M*
1	0.222558	**8.22097**	0.204182	**8.44165**
2	5.830684		6.384072	

3	1.47235		1.533581	
4	3.263167		2.713873	
5	1.351464		1.209467	
6	2.175122		1.797298	
7	7.082718		8.907234	
8	1.389955		1.250126	
9	1.352193		1.163977	
10	3.917405		3.274342	
11	3.031144		2.88769	
12	1.91124		1.674158	

For T_{max} parameter

Table (6-a): Eigen-Values for the Linear and Logarithmic Scales for T_{max}

	Linear Scale			m	Logarithmic Scale			m
	Eigen values	Proportion	Cumulative		Eigen values	Proportion	Cumulative	
1	1.5021	0.501	0.501	8.32619	1.5610	0.520	0.520	8.37496
2	1.0386	0.346	0.847		0.9834	0.328	0.848	
3	0.4594	0.153	1		0.4555	0.152	1	

Table (6-b): Threshold Value and the Squared Distance on Linear and Logarithmic Scales

Subjects	Linear scale		Logarithmic Scale	
	Squared distance	m	Squared distance	M
1	0.853127	**8.32619**	0.785971	**8.37496**
2	4.358666		4.766511	
3	3.932859		3.767538	
4	0.711192		0.633187	
5	2.173905		2.813442	

6	2.698289		2.757822	
7	3.735195		3.939105	
8	1.126286		1.28556	
9	7.412		5.977355	
10	1.126286		1.10388	
11	1.126286		1.10388	
12	3.745908		4.06575	

For AUC_{0-t} parameter

Table (7-a): Eigen-Values for the Linear and Logarithmic Scales for AUC_{0-t}

	Linear Scale			Threshold	Logarithmic Scale			Threshold
	Eigen values	Proportion	Cumulative		Eigen values	Proportion	Cumulative	
1	2.4308	0.810	0.810	10.0015	2.5881	0.863	0.863	10.37676
2	0.4532	0.151	0.961		0.3034	0.101	0.964	
3	0.1160	0.039	1		0.1085	0.036	1	

Table (7-b): Threshold Value and the Squared Distance on Linear and Logarithmic Scale

	Linear scale		Logarithmic Scale	
Subjects	Squared distance	m	Squared distance	m
1	0.30391	**10.0015**	0.256611	**10.376766**
2	4.858578		6.737077	
3	8.096736		6.476307	
4	1.124955		1.157936	
5	1.273431		1.094553	
6	0.1008		0.126238	
7	5.881876		8.683825	

8	0.398347		0.168179	
9	0.035312		0.024426	
10	7.350513		5.327044	
11	0.435668		0.417511	
12	3.139874		2.530292	

For AUC$_{(0-\infty)}$ parameter

Table (8-a): Eigen-Values for the Linear and Logarithmic Scales for $AUC_{(0-\infty)}$

	Linear Scale			m	Logarithmic Scale			m
	Eigen values	Proportion	Cumulative		Eigen values	Proportion	Cumulative	
1	2.5341	0.845	0.845	10.24434	2.6448	0.882	0.882	10.5203
2	0.3551	0.118	0.963		0.2531	0.084	0.966	
3	0.1109	0.037	1		0.1021	0.034	1	

Table (8-b): Threshold Value and the Squared Distance on Linear and Logarithmic Scales

subjects	Linear scale		Logarithmic Scale	
	Squared distance	m	Squared distance	m
1	0.330807	**10.244339**	0.243947	**10.5203**
2	4.507126		6.251101	
3	9.3847		7.246678	
4	1.047287		1.113829	
5	1.339328		1.098742	
6	0.13412		0.141676	
7	5.74696		8.605466	
8	0.468563		0.202925	
9	0.126915		0.033461	
10	6.504686		5.19097	

11	0.888945		0.77215	
12	2.520565		2.099056	

Cook's Distance for 2x2 Crossover Design

Table 9: Cook's distance Value for ln C_{max}, ln T_{max}, ln AUC_{0-t} and ln $AUC_{(0-\infty)}$

Drug	OBS	COOK'S C_{max}	*m*	COOK'S T_{max}	*m*	COOK'S AUC_{0-t}	*m*	COOK'S $AUC_{(0-\infty)}$	*m*
	1	0.0068	**0.0677**	0.1011	**0.0725**	0.0500	**0.0704**	0.0356	**0.0675**
	2	0.0206		0.0914		0.0368		0.0237	
	3	0.0273		0.0195		0.0100		0.0000	
	4	0.0018		0.0015		0.0004		0.0001	
	5	0.0219		0.0000		0.0205		0.0019	
	6	0.0569		0.0287		0.0082		0.0001	
	7	0.0051		0.0536		0.0251		0.0049	
	8	0.0026		0.0000		0.0063		0.0043	
	9	0.0016		0.0087		0.0088		0.0124	
T	10	0.0202		0.0033		0.0435		0.0111	
	11	0.0000		0.0036		0.0032		0.0000	
	12	0.0398		0.0004		0.0008		0.0023	
	13	0.0444		0.0010		0.0177		0.0160	
	14	0.0006		0.0297		0.0014		0.0006	
	15	0.0114		0.0221		0.0009		0.2023	
	16	0.0097		0.0021		0.0053		0.0034	
	17	0.0142		0.0038		0.0414		0.0648	
	18	0.0226		0.0010		0.1125		0.0377	
	19	0.0014		0.0000		0.0023		0.0006	

	20	0.0403		0.0055		0.0021		0.0056	
	21	0.0000		0.0199		0.0129		0.0154	
	22	0.0706		0.0512		0.0531		0.0142	
	23	0.0021		0.0017		0.0062		0.0518	
	24	0.0895		0.0099		0.0618		0.0000	
	25	0.0195		0.0876		0.0048		0.0879	
	26	0.0173		0.0053		0.0247		0.0202	
	27	0.0015		0.0016		0.0305		0.0217	
	28	0.0024		0.0001		0.0129		0.0114	
	29	0.0014		0.0816		0.0064		0.0066	
	30	0.0083		0.0072		0.0825		0.0541	
	31	0.0328		0.0620		0.0349		0.0169	
	32	0.0495		0.1159		0.0199		0.0105	
	33	0.0093		0.0120		0.0052		0.0007	
	34	0.0624		0.0479		0.0757		0.1190	
R	35	0.0047		0.0000		0.0001		0.0001	
	36	0.0129		0.0012		0.0004		0.0015	
	37	0.0913		0.0002		0.0051		0.0047	
	38	0.0048		0.0127		0.0000		0.0000	
	39	0.0299		0.0400		0.0351		0.0045	
	40	0.0281		0.0287		0.0028		0.0016	
	41	0.0244		0.0062		0.0288		0.0349	
	42	0.0288		0.0068		0.0799		0.0694	
	43	0.0000		0.0030		0.0103		0.0103	
	44	0.0664		0.0000		0.0013		0.0096	
	45	0.0358		0.1009		0.0135		0.0296	
	46	0.0000		0.0473		0.0102		0.0009	

	47	0.0372		0.0297		0.0826		0.0535	
	48	0.0035		0.0016		0.0276		0.0017	

Cook's Distance for 3x3 Crossover Design

Table 10: Cook's distance Values for C_{max}, T_{max}, AUC_{0-t} and $AUC_{(0-\infty)}$

Drug	OBS	COOK'S C_{max}	m	COOK'S T_{max}	m	COOK'S AUC_{0-t}	m	COOK'S AUC_{inf}	m
T1	1	0.030431	**0.0802**	0.000002	**0.0889**	0.031649	**0.0779**	0.000081	**0.0966**
	2	0.005612		0.021422		0.019380		0.020300	
	3	0.038988		0.020685		0.112348		0.044706	
	4	0.116609		0.028495		0.043810		0.024818	
	5	0.006914		0.012633		0.000812		0.000108	
	6	0.004686		0.060982		0.057662		0.016659	
	7	0.044037		0.018569		0.111962		0.095650	
	8	0.020028		0.002222		0.007089		0.021076	
	9	0.008404		0.052330		0.006920		0.000394	
	10	0.078773		0.007516		0.042691		0.019433	
	11	0.003062		0.007880		0.018826		0.019606	
	12	0.065642		0.055915		0.000194		0.003490	
T2	13	0.065695		0.006028		0.000331		0.002584	
	14	0.000050		0.116658		0.000016		0.000000	
	15	0.020431		0.001736		0.072012		0.026036	
	16	0.004410		0.004891		0.007800		0.000024	
	17	0.013448		0.037754		0.023566		0.020268	
	18	0.000226		0.002034		0.000312		0.000001	
	19	0.067868		0.015223		0.046745		0.021412	
	20	0.018985		0.039670		0.012072		0.003240	
	21	0.005514		0.086818		0.002038		0.007522	

	22	0.016779		0.006062		0.002485		0.001852
	23	0.000568		0.054741		0.029886		0.027730
	24	0.044377		0.003675		0.000333		0.002365
R	25	0.106304		0.036238		0.005994		0.027536
	26	0.002194		0.024310		0.031864		0.001406
	27	0.000254		0.024962		0.000052		0.000053
	28	0.001586		0.000005		0.024703		0.004705
	29	0.001733		0.065352		0.000035		0.644733
	30	0.000005		0.029744		0.002247		0.000828
	31	0.006277		0.029032		0.003701		0.011659
	32	0.019255		0.004571		0.004622		0.013353
	33	0.057899		0.000931		0.005528		0.002253
	34	0.081583		0.000529		0.171514		0.051552
	35	0.003709		0.000419		0.008198		0.006882
	36	0.000059		0.186388		0.025720		0.015436

APPENDIX (B)
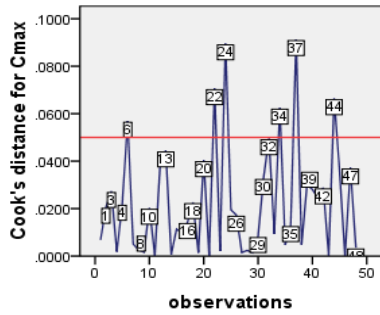
Figure1: Fit Outliers Plot for C_{max}
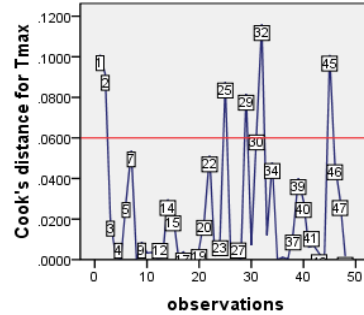
Figure2: Fit Outliers Plot for T_{max}
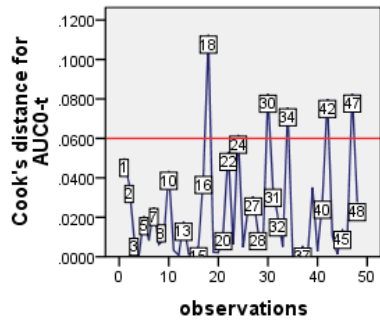
Figure 3: Fit Outliers Plot for $AUC_{(0-t)}$
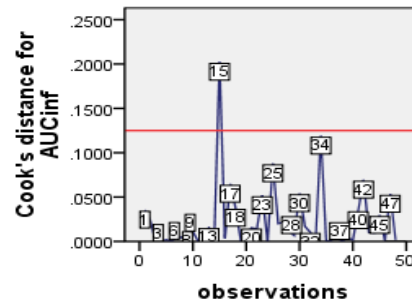

Figure 4: Fit Outliers Plot for $AUC_{(0-\infty)}$
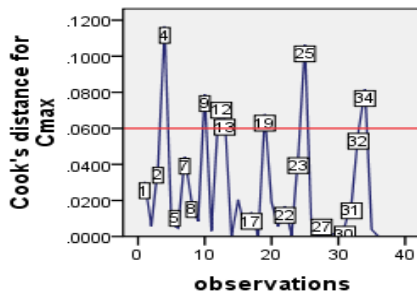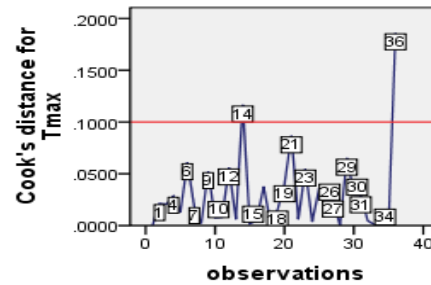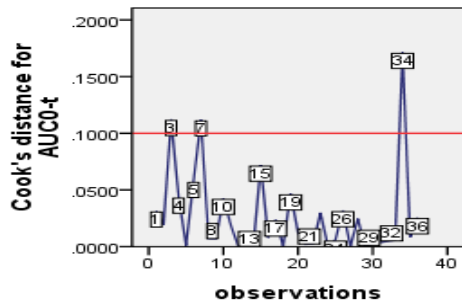

Figure 5: Fit Outliers Plot for C_{max}

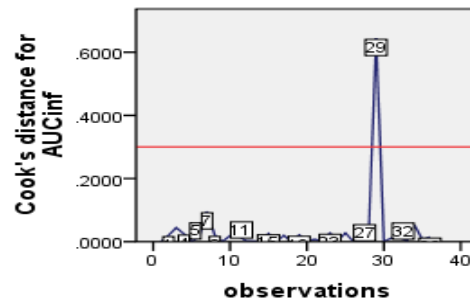**Figure 6:** Fit Outliers Plot for T_{max}

Figure 7: Fit Outliers Plot for $AUC(0-t)$

Figure 8: Fit Outliers Plot for $AUC_{(0-\infty)}$