DOKUZ EYLÜL UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

SAMPLE SIZE DETERMINATION IN BIOEQUIVALENCE STUDIES UNDER 2x2 CROSSOVER DESIGN

by

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June, 2012
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SAMPLE SIZE DETERMINATION IN BIOEQUIVALENCE STUDIES UNDER 2x2 CROSSOVER DESIGN

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M.Sc THESIS EXAMINATION RESULT FORM

We have read the thesis entitled **"SAMPLE SIZE DETERMINATION IN BIOEQUIVALENCE STUDIES UNDER 2X2 CROSSOVER DESING"** completed by **HAILE MEKONNEN FENTA** under supervision of **PROF. DR. MEHMET N. ORMAN** and we certify that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science.

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SAMPLE SIZE DETERMINATION IN BIOEQUIVALENCE STUDIES UNDER 2x2 CROSSOVER DESIGN

ABSTRACT

In bioequivalence studies, approximate formulas for sample size determination are derived based on Schuirmann's (1987) two one-sided tests (TOST) in bioequivalence studies. In clinical trials, crossover trials are experiments in which patients/volunteer are allocated a series of treatments with the objective of comparing the different treatments or different doses of the same treatment. This design attracts clinicians because it eliminates **between subjects** variability**.**

Sample size calculation plays an important role in bioequivalence trials. In practice, a bioequivalence study is usually conducted under a crossover design or a parallel design with raw data or log-transformed data. The purpose of this work is to determine the **number of subjects/sample size** required to conduct a clinical trial in order to compare the efficacy or futility of a new produced drug/treatment with that of the reference drug in case of heterogeneous variability. A simulation study was carried out to construct two-one sided (1-2alpha)x100 percent confidence intervals for ratios of the test and reference formulations of a drug product to assess whether the test and the reference drug products are bioequivalence or not. Finally, the simulation is performed through R 2.14.0 statistical software.

Keywords: Crossover design, sequential design, bioequivalence studies, power and sample size.

2X2 ÇAPRAZ TASARIMI ALTINDAKİ BİYOEŞDEĞERLİK ÇALIŞMALARINDA ÖRNEK BOYUTUNUN TANIMLANMASI

ÖZ

Biyoeşdeğerlik çalışmalarında örneklem büyüklüğü Schuirmann (1987)'ın iki tek yönlü testine (TOST) dayanılarak elde edilir. Biyoeşdeğerlik çalışmaları için çarpımsal ve toplamsal modeller kullanılır. En yaygın tasarım 2 dizi, 2 dönem ve 2 tedavi içeren 2x2 çarpımsal tasarım modelidir. Çapraz tasarımda gönüllülere/hastalara farklı tedaviler ya da aynı tedavide farklı dozlar uygulanır ve sonuçlar karşılaştırılır. Bu tasarım bireyler arası değişkenliği yok ettiği için klinisyenler tarafından tercih edilmektedir.

Örneklem büyüklüğü klinik çalışmalarda önemli bir rol oynar. Gerçek veriler (dönüşüm uygulanmamış) ya da Logaritmik dönüşüm uygulanmış veriler, biyoeşdeğerlik çalışmalarında, paralel ya da çapraz tasarımlar altında kullanılır. Bu çalışmanın amacı heterojen varyanslılık durumunda test ve referans ilacının etkinliğini karşılaştırmak için gerekli örneklem büyüklüğünü belirlemektir. Test ve referans ilaçlarının biyoeşdeğer olup olmadığını belirlemek için iki tek yönlü test yapısı kullanılarak %(1-2α)x100 güven aralığında simulasyon çalışması yapılmıştır. Son olarak simulasyon çalışması R 2.14.0 paket programı kullanılarak yapılmıştır.

Anahtar kelimeler: Çapraz tasarım, ardışık tasarım, biyoeşdeğerlik çalışması, güç ve örneklem büyüklüğü.

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CHAPTER ONE
INTRODUCTION

The sequential approach has been a natural way to proceed throughout the history of experimentation. Perhaps the earliest proponent was Noah, who on successive days released a dove from the Ark in order to test for the presence of dry land during the subsidence of the flood (Turnbull, B.C., & Jennison, C., 2000). Sequential design is an adaptive design this allows for pre mature termination of a trial due to **efficacy** or **futility**, based on the interim analyses. According to Gould, A.L. (1995), the concept of sequential statistical methods was originally motivated by the need to obtain clinical benefits under certain economic constraints, that is, for a trial for a positive result, early stopping ensures that a new drug product can exploited sooner. While negative results indicated, early stopping avoids wastage of resources, referred to as "abandoning a lost cause". That is the right drug at the right time for the right patient. In general; Sequential methods typically lead to savings in sample-size, time, and cost when compared with the classic design with a fixed sample-size.

Bioavailability (BA) of a drug is defined as the rate and extent to which the active drug ingredient is absorbed and becomes available at the site of the drug action. Bioavailability (BA) and Bioequivalence (BE) studies are performed based on the requirements set forth in part 320 of section 21 of the Code of Federal Regulation (CFR) and guidance given by US Food and Drug Administration's (FDA) Center for Drug Evaluation and Research (CDER)

Bioequivalence; "The absence of significant difference in the rate(C_{max}) and extent (AUC) to which the active ingredient or active moiety in pharmaceutical equivalents or pharmaceutical alternatives become available at the site of drug action when administered the same molar dose under the similar conditions in an appropriately designed study. Or in a broad definition: Two different drugs or formulations of the same drug are called bioequivalent if they are absorbed into the blood and become available at the drug action site at about the same rate and concentration. Bioequivalence trials (FDA, guidance 1992, 2000*b* and 2001) play a

crucial role in the drug development processes. Under this approach, to minimize both inter and intra individual variation eligible subjects (typically, normal healthy volunteers, preferably nonsmokers and without a history of alcohol and drug abuse) are randomized to one of two treatment sequences, either Test followed by reference (TR) or reference followed by test (RT). Subjects may be males or females. However, risk to women of childbearing potential should be considered an individual basis. Women should be neither pregnant, nor likely to become pregnant until after the study. Additionally, women taking contraceptive drugs should not include in the studies.

Exception: If the investigated active substance is known to have adverse (negative or harmful) effects, it may be necessary to use patients instead under suitable precautions and supervision.And the two drugs are said to be average bioequivalence (ABE) if and only if the $(1-2\alpha)\times 100\%$ confidence interval for the ratio of test to reference formulation is contained within the regulatory limits of θ_1, θ_2 , specifically according to some regulatory agencies, like FDA, $0.8-1.25$ or $\text{-}0.2231436-0.2231436$ for both AUC and $C_{\max}$ (Anonymous, 2001a).

There are two commonly used experimental study designs in clinical research: parallel and crossover.

In parallel study design, each subject is randomized to one and only one treatment. Most large clinical studies adopt this approach. While a crossover design is a repeated measurements design such that each experimental unit (patient) receives different treatments during the different periods of time, i.e., the patient's crossover from one treatment to another during the course of the trial. In a crossover trial subjects are randomly allocated to study groups where each group consists of a sequence of two or more treatments given consecutively. Subjects allocated to the RT study group receive the reference treatment R first, followed by the test treatment T, and vice versa in the TR group. Crossover trials allow the response of a subject to treatment R to be contrasted with the same subject's response to treatment T.

Removing patient variation in this way makes crossover trials potentially more efficient than similar sized, parallel group trials in which each subject is exposed to only one treatment. In theory, treatment effects can be estimated with greater precision given the same number of subjects.

Even if there are so many types of crossover designs, the most popular crossover design is the 2-sequence, 2-period, 2-treatment crossover design, sometimes called the 2x2 crossover design. Crossover designs have been the most popular designs of choice in many clinical and pharmaceutical trials. Many diseases and conditions are studied using a crossover design in a clinical trial, Chow, S.C., & Liu, J.P. (2009). A crossover design is a study that compares two or more treatments or interventions in which subjects, on completion of a course of one treatment, are switched to another. This implies that each subject acts as his/her own control. The fundamental assumption of a crossover design is that patients usually have a chronically stable condition that will not vary between when they are taking the first and the second treatments. Therefore, crossover trials are, by necessary, short term trials. Typically, each treatment is administered for a selected period of time and, often, there is a "washout" or "re-stabilization" period between the last administration of one treatment and the first administration of the next treatment, allowing the effect of the preceding treatment to wear off. Where possible, allocation of the treatment sequences in crossover trial is randomized, blinded process.

It is widely recognized among statisticians that the evaluation of sample size and power is a crucial element in the planning of any research venture (Chow, S.C., Shao, J., & Wang, H., 2003). Consider a clinical trial to study the efficacy and safety of new drug where patients are randomized to receive either a treatment with the new drug or a control with a reference or existing treatment. A key design element is to determine the required sample size (Julious, S. A., 2010).

Power and sample size estimations are measures of how many patients are needed in a study (Schuirmann, D.J.A., 1987). Nearly all clinical studies entail studying a sample of patients with a particular characteristic rather than the whole population.

We then use this sample to draw inferences about the whole population. Power and sample size estimations are used by researchers to determine how many subjects are needed to answer the research (Anonymous, 2001).

Sample size determination is important for the following main reasons:

Economic reasons:

An undersized study may result in a waste of resources due to their incapability to yield useful results. Recall that without a large enough a sample, an important relationship or effect/difference may exist, but the collected data not be sufficient to detect it. An oversized study can result in unnecessary waste of resources, while at the same time yielding significant results that may not have much practical importance. Note that if a study is based on a very large sample, it will almost always lead to statistically significant results (Altman, D. G., 1982).

Ethical reasons:

An undersized study can expose subjects to unnecessary (sometimes potentially harmful or futile) treatments without the capability to advance knowledge. An oversized study has the potential to expose an unnecessarily large number of subjects to potentially harmful or futile treatments. Generally, overall sample size calculation is an important part of the study design to ensure validity, accuracy, reliability and, scientific and ethical integrity of the study (Altman, D.G., 1980).

This thesis consists of six chapters and the first chapter includes the general information about the study. The aim of the study, its content and the steps, which will be followed, are explained and also sequential designs, parallel and crossover designs, bioequivalence studies, features of the crossover designs, power and sample size determinations are shortly touched in this chapter.

The second chapter explains about the general concept of sequential design and the theoretical aspects of this design will be also stated in detail. In addition, adaptive design and sample size re-estimation will be seen.

The third chapter is about designs, the most important types of designs, i.e. crossover and parallel, types of effects in BE study, washout periods and the role of statisticians in clinical study. The advantageous and disadvantageous of crossover designs over parallel design will be touched.

The forth chapter is about bioequivalence (BE) and bioavailability (BA), pharmacokinetics and pharmacodynamics parameters are discussed in detail and some decision rules and regulatory aspects used to determine BE studies. Additionally, applications of group sequential design in BE studies will be touched.

The principal topic of the fifth chapter is, the statistical considerations for the assessment of average bioequivalence studies (BE) and methods used to evaluate BE will be considered. Some of the methods are Two One-Sided Test (TOST), confidence interval method and hypothesis testing methods will be considered in detail. Power and sample size determination for clinical study is also the main concern for this chapter.

Finally, in chapter six, simulation methodologies, formulas, conclusions of this work will be touched, which is the important of this paper work.

CHAPTER TWO
SEQUENTIAL DESIGN

A principal reasoning for conducting a group sequential test is discussed in detail in Pocock (1977) and O'Brien and Fleming's(1979), and its aim is simply to decrease the sample size of the study units. Interim analyses also enable management to make appropriate decisions regarding the allocation of limited resources for continued development of a promising treatment. In clinical trials, it is desirable to have a sufficient number of subjects in order to achieve a desired power for correctly detecting a clinically meaningful difference if such a difference truly exists (Chow, S.C., 2007).

2.1 Two Stage Design

According to Potvin, D., et al. (2008), first initial group of subjects are treated and data are analyzed, if bioequivalence are not demonstrated an additional subject can be employed and the results from both groups combine for final statistical analyses. In general, two stage group sequential design with interim look after n_1 subject's complete and final look after $N(=n_1+n_2)$ subjects complete. Here we have the following potential decisions.

1. In stage one (for n_1 subjects)
   a. Stop and claim bioequivalence
   b. Continue the trial in second stage
2. In stage two (for $n=n_1+n_2$)
   a. Stop and claim bioequivalence
   b. Stop and don't claim bioequivalence.

2.1.1 Sample size re-estimation methods

A sample size re-estimation (SSR) refers to an adaptive design that allows for sample size adjustment or re-sampling based on the review of interim analyses results. The sample size requirements for the trial are sensitive to the effect size and its variability (Schuirmann, D.J.A., 1987). That is inaccurate estimation of the effect size and its variability leads overpowered or underpowered results, neither of which is desirable. If a trial is underpowered, if the variance used in the power calculation is too low or the chosen effect size overly optimistic, it will not be able to detect a clinically meaningful difference, and consciously prevent a potentially effective drug from being delivered to patients. On the other hand, if the trial is overpowered, it could lead to unnecessary exposure of many patients to a potentially harmful compound when the drug, in fact, is not effective (Lenth, R.V., 2001).

The required sample size to compare two populations means μ_1 and μ_2 against a 2-sided alternative with common variance σ^2 can be derived as

$$n \geq \frac{2\left(z_{1-\alpha/2}+z_{1-\beta}\right)^2}{\left(\frac{\mu_1-\mu_2}{\sigma}\right)^2} = \frac{2\left(z_{1-\alpha/2}+z_{1-\beta}\right)^2}{\left(\frac{\delta}{\sigma}\right)^2} \qquad 2.1$$

Where $\delta = \mu_1 - \mu_2$

n : The number of subjects (patients) to be sampled.

$Z_{\frac{\alpha}{2}}$: The critical value

σ^2 and δ are the variance and the effect size respectively.

α and β are type one and type two errors respectively (Chow, S.C., 2007).

Our aim here is to **increase the power** by minimizing both type one and type two errors, but from (eq.2.1) and Figure 2.1, it is impossible to minimize these two errors simultaneously, for a constant sample size n, as a result the only way to increase the power, is increasing the sample size.

In short the effect size and its variability should be estimated correctly in order to get the appropriate results. And the sample size re-estimation depends on the effect size or the variance or both.

Table 2.1 The relationship between sample size, power and Type one error.

Tail(S)=one,Effect size=0.2

		α err prob = 0.05	α err prob = 0.1
#	Power (1−β err prob)	Total sample size	Total sample size
1	0.600000	87.8581	57.3932
2	0.627000	94.4058	62.7096
3	0.654000	101.355	68.3984
4	0.681000	108.770	74.5169
5	0.708000	116.735	81.1377
6	0.735000	125.356	88.3551
7	0.762000	134.772	96.2935
8	0.789000	145.174	105.122
9	0.816000	156.829	115.081
10	0.843000	170.128	126.520
11	0.870000	185.683	139.991
12	0.897000	204.531	156.427
13	0.924000	228.649	177.618

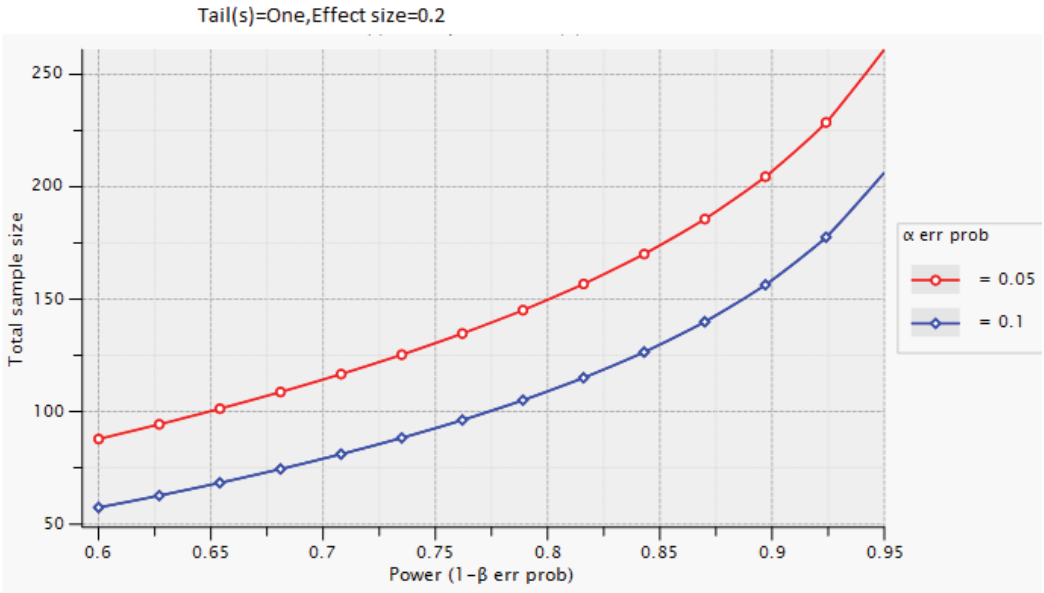

Figure 2.1 The relationship between type one error, power and sample size.

2.1.2 Adaptive sample size sequential methods

In a group sequential trial, interim analysis are conducted on the data available at one or more intermediate stages, when the sample size n_i and allowed type I error rate (α_i), at each stage are pre-established according to some rules. The utilization of adaptive trial designs can increase the probability of success, reduce the cost, reduce the time to market and deliver the right drug to the right patient at the right time. Commonly used adaptive trials include standard group sequential design, sample size re-estimation, drop-loser design (Jones, B. & Kenward, M.G., 2003 and Chow, S.C., 2007).

The benefits of monitoring clinical data are:

Economical: Savings in time and money can result if the answers to the research questions become evident early before the planned conclusion of the trial. By permitting early stopping, group sequential approaches provide some protection against unnecessary use of resources if the planned total sample size was based on an overestimated variance.

Ethical: In a trial comparing a new treatment with a control, it may be unethical to continue subjects on the control (or reference) arm once it is clear that the new treatment is effective. Likewise if it becomes apparent that the treatment is ineffective, inferior, or unsafe, and then the trial should not continue. Interim analysis in sequential trials allows making conclusions on efficacy and safety before the planned end of the trial is reached.

In the basic two treatment comparison, a maximum number of groups" k ", and a group size" m ", are chosen, subjects are allocated to treatments according to a constrained randomization scheme which ensures m subjects receive each treatment in every group and the accumulating data are analyzed after each group of $2\times m$ responses. For each $K=1...k,$ a standardized statistic Z_k is computed from the first k groups of observations, and the test terminates with rejection of

H_0 if $|Z_k|$ is greater than critical value C_k

Here H_0, Z_k and C_k are respectively the null hypothesis, test statistic and the critical values. If the test continues to the K^{th} analysis and the $Z_k < C_k$ then it stops at that point and H_0 is accepted.

Here the sequence C_k are $C_1, C_2, C_3, ...C_k$, chosen to achieve a specified type 1 error and different types of group sequential test give rise to different sequence (O'Brien, P.C., & Fleming, T.R., 1979). Shortly, the following can be achieved.

After group $k = 1...k-1$

if $|Zk| \geq Ck$ stop, reject H_0 and otherwise

Continue to group $k+1$

After group k

If $|Zk| \geq Ck$. Stop and reject H_0 , otherwise,

Stop and report fail to reject H_0 ,"accept" H_0 .

Or simply let T_K be the test statistic and a_k and b_k be the lower and upper limits then the stopping rule can be rewritten as:

$$\begin{cases} \textit{Stop} \text{ for efficacy if } T_k \leq b_k \\ \textit{Stop} \text{ for futility if } T_k \geq a_k \\ \text{Continue to second stage if } a_k < T_k < b_k \end{cases}$$

The major imputes to group sequential testing came with papers of Pocock's (1977), O'Brien and Fleming's (1979) and Turnbull and Jonnison (2000).

The minimum sample size for stage two is 2 (if the decision rule determined that the study should continue to stage 2) and there is no upper limit to the size of stage 2. This can be expressed as: Sample size for stage 2 is [2 , ∞) and here equal sample size assumption is also under consideration.

CHAPTER THREE
TYPES OF DESIGNS

We can split research studies in to two broad classes. That is experimental/ interventional and observational studies. There are two commonly used experimental study designs in clinical research: parallel and crossover (Hinkelmann, K. & Kempthorne, O., 1994).

3.1 Parallel Design

Parallel study design, each subject is randomized to one and only one treatment (Jones, B., Kenward, M.G., 2003).

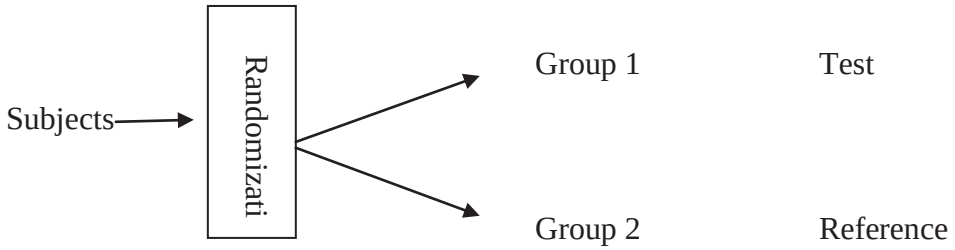

Figure 3.1 Two group parallel design

Parallel design may not be an appropriate for bioavailability and bioequivalence studies. This is because the variability in observations (e.g., AUC) consists of the inter-subject and intra-subject variabilities and the assessment of bioequivalence between formulations is usually made based on the intra-subject variability. Even if the bioequivalence in average bioavailability between formulations can still be established through this design, the comparison is made based on the inter-subject and intra-subject variabilities. In crossover design an adequate length of washout period is important in order to eliminate the possible carry over effects and as a result, the study may take considerable time. This, in turn, may increase the number of drop outs and make the completion of a study difficult. In addition, if the study is conducted with very ill patients, a parallel design is recommended over that of a

crossover design so that the study can be completed quickly. Generally a parallel design is recommended over a crossover design for the following conditions:

1. The drug is potentially toxic or has a very long elimination half-life.
2. The population of interest consists of very ill patients.
3. The cost increasing the number of subjects is much less than that of adding an additional treatment period.

3.2 Crossover Designs and Statistical Inferences for a Standard 2x2 Crossover Design

A crossover design is a repeated measurements design such that each experimental unit (patient) receives different treatments during the different periods of time, i.e., the patient's crossover from one treatment to another during the course of the trial (Brown, B., 1980). Generally, a crossover design is a modified randomized block design in which each block receives more than one formulation of a drug at different time periods and a block may be subjects or a group of subjects. Jones, B. & Kenward, M.G. (2003). A crossover trial is a study that compares two or more treatments or interventions in which subjects, on completion of a course of one treatment, are switched to another. This effectively means that each subject acts as his/her own control. Senn, S. (2002) the fundamental assumption of a crossover trial is that, patients usually have a chronically stable condition that will not vary between when they are taking the first and second treatments. Therefore, crossover trails are, by necessity, short-term trials.

3.2.1 Classification of crossover trials

Crossover trials are classified according to the number of treatments given to a subject and according to whether a given subject receives all (complete crossover) or just some (incomplete crossover) of the study treatments. For simplification, as usual, let's represent T for the test drug and R for the reference drug.

The simplest crossover design is two sequence, two period, two treatment crossover design, in which subjects receives either test (T) and reference(R) treatment in the first study period and the alternative treatment in the succeeding period, commonly called the 2×2 crossover design (Jones, B. & Kenward, M.G., 2003).

Table 3.1 Crossover design (2x2)

Design 1	period 1	period 2
Sequence TR	T	R
Sequence RT	R	T

Table 3.2 Higher-order crossover design

Design type	Order	Treatment sequence
Two-sequence dual design	2x3	TRR,RTT
Double design	2x4	RRTT,TTRR
Balaam's design	4x2	TT,RR,TR,RT
Four-sequence design	4x4	TTRR,RRTT,TRRT,RTTR
Williams' design with three treatments	6x3	TRT,TAR,RTA,RAT,ATR,ART
3x3 Latin square design	3x3	TRA,RAT,ATR
4x4 Latin square design	4x4	TRBA,RATB,ABRT,BTAR

Where: TR means for the assumption of equal number of subjects for the two groups, the first group receives treatment T in period 1 and after a certain period of time (sufficient washout period), this group receives treatment R in period 2 and the result is recorded. While RT stands for the reverse, first treatment R and after a certain period of time this group receives treatment T and the results are recorded. T=for test, R= reference and other two test drugs A and B for two other drugs.

3.2.2 Washout period

According to Carriere, K.C. & Huang, R., (2000), the washout period is defined as the rest period between two treatment periods for which the effect of one formulation (the first treatment) administered at one treatment period does not carry over to the next in other words, to eliminate the effect of the first treatment to the second time. In a crossover design, the washout period should be long enough for the formulation effects to diminish so that there is no carryover effect from one treatment period to the next.

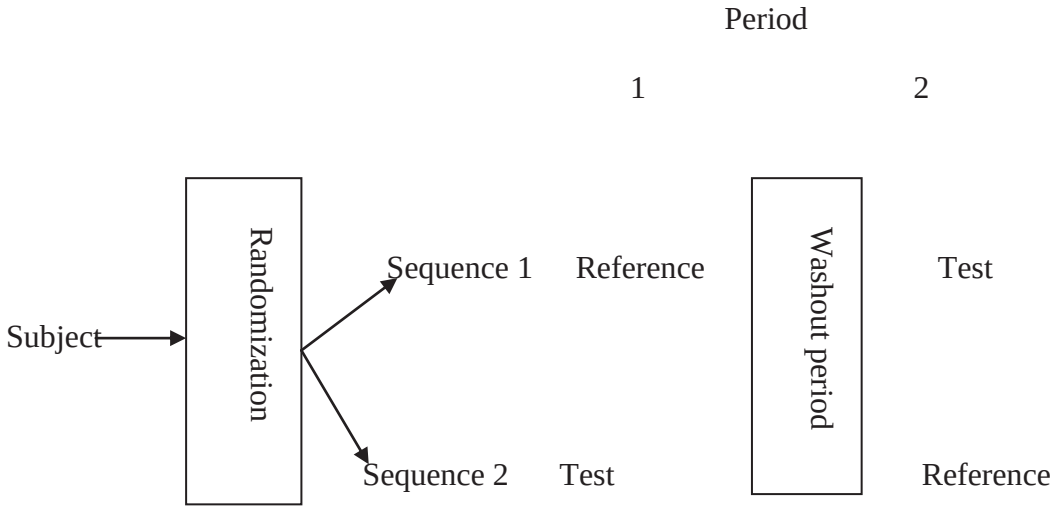

Figure 3.2 A standard 2x2 crossover designs.

3.2.3 Two-treatment crossover study

The typical study design employed in bioequivalence studies is the two-treatment, two-period, and two sequence crossover design given in (table 3.1). In this study design, subjects are randomly separated into two groups of equal number. The test formulation is administered to group '1' in the first study period, and the reference formulation is administered to group '2' in the first period. During the second study period, group '1' receives the reference formulation and group '2' receives the test formulation. The first and second study periods are separated by a washout period, which is designed to be of sufficient duration to allow elimination of the drug

administered in the first period (Jones, B. & Kenward, M.G., 2003). An example of a crossover experiment is one in which laboratory animals are treated sequentially with more than one drug and blood levels of certain metabolites are measured for each drug.

A two-period crossover design is commonly used in blood-level studies. The use of crossover design eliminates a major source of study variability: between-subject differences in the rates of drug absorption, drug clearance, and the volume of drug distribution. In a typical two-period crossover design, subjects are randomly assigned to either sequence T or sequence R with the restriction that equal numbers of subjects are initially assigned to each sequence. A crucial assumption in the two-period crossover design is that of equal residual effects. Unequal residual effects may result, for example, from an inadequate washout period. Another assumption of the crossover design is that there is no subject by formulation interaction. In other words, the assumption is that all subjects are from a relatively homogeneous population and will exhibit similar relative bioavailability of the test and reference products (Brown, B., 1980.

3.2.4 The role of statisticians in clinical trials

Statistics has been called the technology of the scientific method yet medical research is often criticized for ignorance and misuse of statistics. Examples include incorrect use of statistical methods, inadequate sample sizes and poor reporting of study design and analysis (Jones, B., 2006). In epidemiological research and clinical research based on populations there is a particularly strong need for good statistical input. For these reasons it is unwise for epidemiologists and clinical researchers to get on alone upon such research or to seek insufficient statistical advice. Additionally, statistician in clinical study is to use randomization (to eliminate the systematic error), replication, blocking, and blinding in study design and proper application of models to ensure that the statistics for the parameters we are interested in are accurate and precise. In short, statisticians are the **backbone** of any field of study. For example, suppose a standard 2x2 crossover design is to be conducted with 24 healthy volunteers to access bioequivalence between a test and reference

formulations of a drug product (Chow, S.C., & Liu, J.P., 2009). Here we have two sequence of formulations (RT and TR), implies 12 subjects are assigned for each sequence for equal number of subjects for each group assumptions. And finally one group will receive the first sequence of formulations (TR) and the second group receives formulations in reverse order (RT). And the main thing here is we have to assign 12 subjects for each sequence randomly, means that we first generate a set of random numbers from 1 to 24 using appropriate statistical software like, R, Minitab, SPSS, SAS else (Jones, B. & Kenward, M.G., 2003).

Table 3.3 Randomization of numbers

Sequence1	20	4	18	21	9	5	2	22	14	11	19	12
sequence2	10	24	15	1	13	7	23	8	16	3	6	17

Then, the subjects are sequentially assigned a number from 1 to 24. Subjects with numbers in the first half of the above random order are assigned to the first sequence RT and the rest are assigned to the second sequence TR.

Table 3.4 Randomization codes for the standard crossover design

Sequence 1		Sequence 2	
Subject	Formulation	Subject	Formulation
2	TR	1	RT
4	TR	3	RT
5	TR	6	RT
9	TR	7	RT
11	TR	10	RT
12	TR	11	RT
14	TR	13	RT
18	TR	15	RT
19	TR	16	RT
20	TR	17	RT
21	TR	23	RT
22	TR	24	RT

3.2.5 Linear model for 2x2 cross-over data

In order to define the linear model, let Y_{ijk} denote the response (e.g. AUC, **log AUC** or $\log C_{\max}$) in period j, in subject i on sequence k,

where;

i=1,2,..,n, j=1,2 and k=1,2 and n_i is the number of subjects in group k. The total number of subjects in the trial is $n = n_1 + n_2$. The systematic effects we anticipate are due to the periods and formulations (Chow, S.C., 200). As the subjects are allocated randomly to the two groups, there should be no sequence effect. However, it is traditional to include such an effect and we will do so here.

After each subject is assigned to either treatment sequences TR or RT in each period, we can construct a general linear model as follows:

$$Y_{ijk} = \mu + S_{ik} + \pi_j + F_{j,k} + \lambda_{j-1,k} + e_{ijk} \text{ (Additive model)} \qquad 3.2.1$$

$$X_{ijk} = \mu . S_{ik} . \pi_j . F_{j,k} . \lambda_{j-1,k} . e_{ijk} \text{ (Multiplicative model)}$$

$Y_{ijk} = \log(X_{ijk})$ (and the multiplicative model can be changed in to additive model.),

where

μ =the overall mean;

S_{ik} =the random effect of the i^{th} subject in the k^{th} sequence, i=1, 2,…, n_k and k=1,2

π_j =the fixed effect of the j^{th} period, where, j=1, 2.

$F_{i,k}$ =the direct fixed effect of the formulation or drug product administered at period j in sequence k .In the standard 2x2 crossover design there are only two formulations (Jones, B. & Kenward, M.G., 2003). This is because the formulation administered at the first period in the first sequence, as shown in table 3.5 below, is the test formulation, then

$$F_{(j,k)} = \begin{cases} F_T & \text{if k=j ,j,k=1,2} \\ F_R & \text{if k} \neq \text{j} \end{cases}, \qquad 3.2.2$$

$\lambda_{i-1,k}$ = the residual effect carried over from the $(j-1)^{th}$ period to the j^{th} period in sequence k.

e_{ijk} =the (within subject) random error in observing y_{ijk} .

For the standard 2x2 crossover design, the carry over effects can be occurring at the second period. Let us represent the carry over effect of the test formulation from period 1which exists in period 2 at sequence 1 by λ_T . Thus

$$\lambda_{(j-1,k)} = \begin{cases} \lambda_T & \text{if j=2, k=1} \\ \lambda_R & \text{if k,j=2} \end{cases} \qquad 3.2.3$$

Table 3.5 The fixed effects in the full model.

Group	Sequence	Period 1	Data for period 1	Period 2	Data for period 2
1	TR	$\mu_{11} = \mu + \pi_1 + F_T$	For T drug(Y_{i11})	$\mu_{12} = \mu + \pi_2 + F_R + \lambda_T$	For R drug(Y_{i21})
2	RT	$\mu_{21} = \mu + \pi_1 + F_R$	For R drug(Y_{i12})	$\mu_{22} = \mu + \pi_2 + F_T + \lambda_R$	For T drug(Y_{i22})

$$\mu_{jk} = E(Yijk)$$

Where; $\pi_1 + \pi_2 = 0$ $\qquad (3.2.4)$

$$F_T + F_R = 0$$

$$\lambda_1 + \lambda_2 = 0$$

Based table 3.5, for comparison of the bioavailability of these two formulation effects we have to separate and estimate each effect from drug (treatment effect). In general for bioavailability and bioequivalence studies in crossover design, it is commonly assumed that:

1. There are no period effects

2. No carry over effects

This is due to

a. A well conducted study can eliminate the possible period effect.
b. The residual effects from the previous dosing period, carry- over effect, can be eiminated by giving sufficient length of washout (drug free) period.

But these two effects may be still present and especially the present of the carry over effect strongly increases the complexity of statistical analysis for the **assessment of average bioequivalence**. In conclusion, before the comparison of average bioavailability between two formulations, we have to test the presence/absence of both the period and the carry over effect (Carriere, K.C. & Huang, R., 2000). It was common practice to follow the advice of Grizzle (1965) when testing for the carryover difference. Grizzle suggests two important things about the carry over effect: If the test for a carry-over effect is not significant, then the t-test based on the **within-subject** difference is used. While if the carry-over effect is significant, then the treatments are compared using only the period 1 data, as in case of a parallel group design. In short if there is carryover effect, period 2 is discarded.

3.2.6 Types of Effects and assumptions

In any design the following effects are common to appear.

1. Carryover effect
2. Treatment effect
3. Period effect
4. The period by treatment effects/interaction effect

Statistical inferences for these effects can be done from the model given in equation (3.2.1) and we have to consider the following assumptions additionally (Chow, S.C., 2007). But from our study we assume that there is no carry-over, period and interaction effect in addition to the following assumptions.

i. $\{S_{ik}\}$ i.i.d with normal with mean 0 and variance σ_s^2.

ii. $\{e_{ijk}\}$ i.i.d with normal with mean 0 and variance σ_e^2

iii. $\{S_{ik}\}$ and $\{e_{ijk}\}$ are mutually independent. And

σ_s^2 and σ_e^2 are the inter and intra subject variabilities respectively.

3.2.6.1 Carry over effects

Carriere, K.C. & Huang, R.(2000), the effect of the treatment from the previous time on the response at the current period is called carryover effect. In other words, if a patient receives treatment T during the first period and treatment R during the second period, then measurements taken during the second period could be a result of the direct effect of treatment R administered during the second period, and/or the carryover or residual effect of treatment T administered during the first period. There are a few types of carryover effects for example **first-order carryover** effects which stay one period beyond application. Second-order carryover effects stay two periods beyond application, and generally kth-order carryover effects stay for k periods beyond application. These carryover effects yield statistical bias. In short, the possibility is that the effect of a treatment given in one period might still be present at the start of the next period.

Let $\lambda = \lambda_T - \lambda_R$. Then λ can be used to assess the carry over effect. Under the constraint of $\lambda_T + \lambda_R = 0$, carry over effects are equal for the two formulations, that is $\lambda = 0$ if and only if $\lambda_T = \lambda_R$. Therefore a test for carry over effect means a test for equal carry over effects. When there are no carry over effects, the direct treatment effect $\left(F = F_T - F_R\right)$ can be estimated the data from both periods.

Let's see the test for the present of the carry over effect.

$$H_0 : \lambda = 0 \ \left(or\ \lambda_T = \lambda_R\right) \quad \text{Versus} \quad H_a : \lambda \neq 0 \ \left(or\ \lambda_T \neq \lambda_R\right) \qquad 3.2.5$$

As usual the rejection of the null hypotheses leads to the presence of the carry over effect.

From statistical point of view, if the confidence interval contains **zero**, then there is no enough information to reject the null hypothesis and we can conclude that no carry over effect. Generally, there is a reasonable assumption that the washout period can be chosen to be long to eliminate the possible carry-over effect.

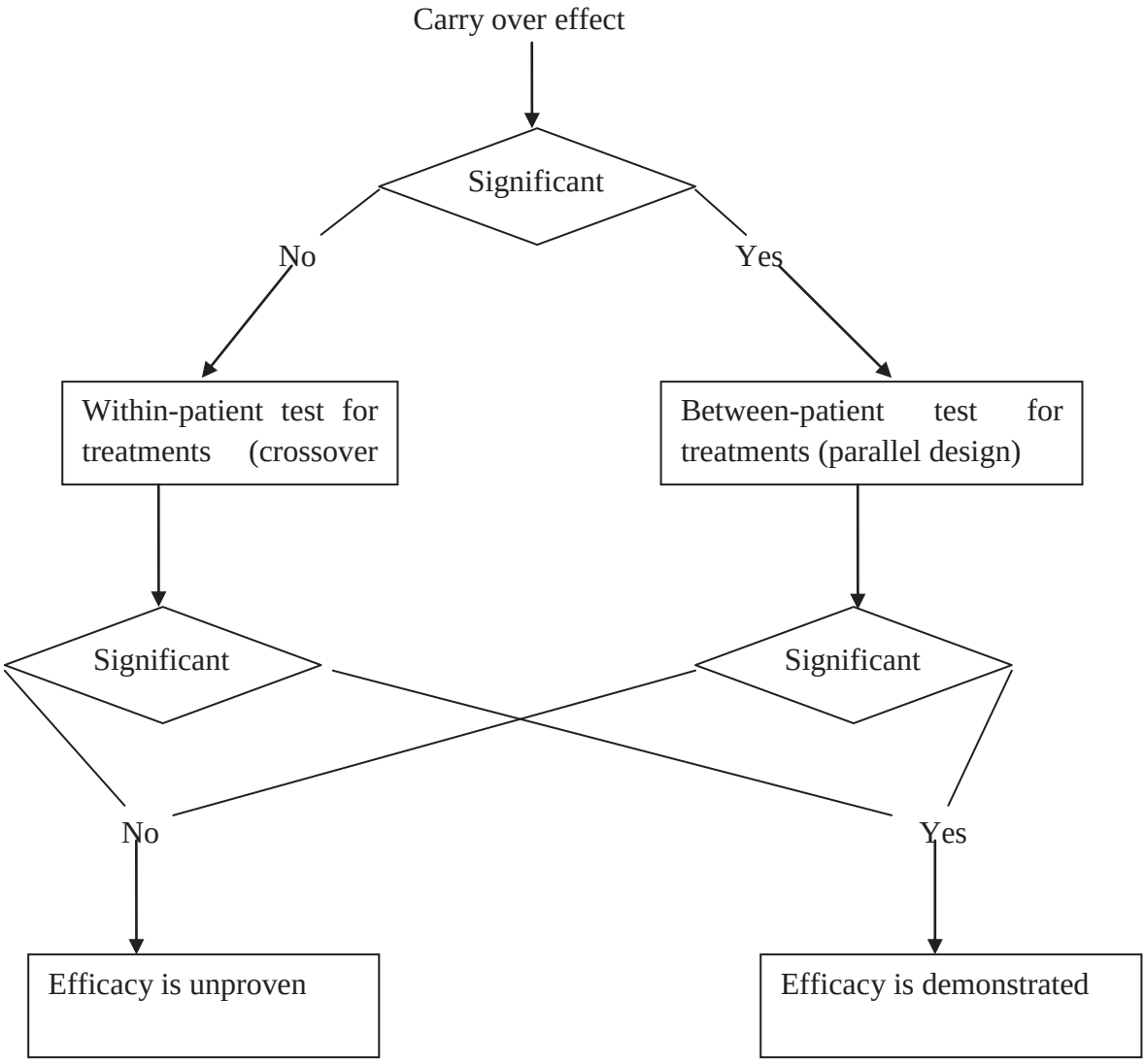

Figure 3.3 The impact of the carry-over effects

3.2.6.2 Direct Treatment effects

Unlike carry over effect, here in case of treatment effect, it is helpful to start with the period difference for each subject with in each sequence which is defined as follows:

$$d_{ik} = \tfrac{1}{2}\left(y_{i2k} - y_{i1k}\right),\ i = 1,2,...,n_k;\ k = 1,2 \qquad 3.2.6$$

And the expected value and the variances of the period differences are given by:

$$E\left(d_{ik}\right) = \begin{cases} \tfrac{1}{2}\left[\left(\pi_2 - \pi_1\right) + \left(F_T - F_R\right) + \lambda_R\right] \\ \tfrac{1}{2}\left[\left(\pi_2 - \pi_1\right) + \left(F_R - F_T\right) + \lambda_T\right] \end{cases} \qquad 3.2.7$$

$$and\ \operatorname{var}\left(d_{ik}\right) = \sigma_d^2 = \tfrac{\sigma_e^2}{2}$$

From this we can see that the variance of the period difference only involves the **intra-subject** variability which reflects the merits of the crossover design in comparing the direct drug effects. However, the expected value of d_{ik} consists of both the period and the carryover effects.

In short,

$$H_o : F_T = F_R$$
$$H_1 : F_T \neq F_R \qquad 3.2.8$$

Denote the period effect and the direct drug effect by $\pi = \pi_2 - \pi_1$ and $F = F_R - F_T$, respectively. To draw statistical inference on F, consider the sample means of the period differences for each sequence (Chow, S.C., & Liu, J.P., 2009). That is

$$\bar{d}_{.k} = \tfrac{1}{n_k}\sum_{i=1}^{n_k} d_{ik},\ \text{k=1,2.} \qquad 3.2.9$$

The difference between sequences $\left(i.e., \bar{d}_{.1} - \bar{d}_{.2}\right)$ is clearly not an unbiased estimator of F unless there are no unequal carry-over effects $\left(i.e., \lambda_R = \lambda_R\right)$ since

$$E\left(\bar{d}_{.1}-\bar{d}_{.2}\right)=\left(F_T-F_R\right)+\left(\lambda_R-\lambda_T\right)/2 \qquad 3.2.10$$
$$=\text{F-}\lambda/2$$

,

where $\lambda=\lambda_T-\lambda_R$.

As a result, if $\lambda_T \neq \lambda_R$, there exists no unbiased estimator for *F* based on the data from both periods. On the other hand, if $\lambda_T=\lambda_R$, then

$$\begin{aligned} F &= \bar{d}_{.1}-\bar{d}_{.2} \\ &= \tfrac{1}{2}\left[\left(\bar{Y}_{.21}-\bar{Y}_{.11}\right)-\left(\bar{Y}_{.22}-\bar{Y}_{.12}\right)\right] \\ &= \bar{Y}_T-\bar{Y}_R \end{aligned} \qquad 3.2.11$$

is MVUE of F, in where

$$\bar{Y}_R=\tfrac{1}{2}\left(\bar{Y}_{.11}+\bar{Y}_{.22}\right) \text{ and } \bar{Y}_T=\left(\bar{Y}_{.21}+\bar{Y}_{.12}\right). \qquad 3.2.12$$

$\bar{Y}_T$ and $\bar{Y}_R$ are the least squares (LS) means for the tests and the reference formulations, respectively.

A test for a direct treatment effect can be obtained easily as follows:

$$T_d=\frac{\hat{F}}{\hat{\sigma}_d\sqrt{\frac{1}{n_1}+\frac{1}{n_2}}} \qquad 3.2.13$$

Where $\hat{\sigma}_d^2$ is the pooled sample variance of period difference from both sequences and unbiased estimator of σ_d^2, which is given by;

$$\hat{\sigma}_d^2=\frac{1}{n_1+n_2-2}\sum_{k=1}^{2}\sum_{i=1}^{n_k}\left(d_{ik}-d_{.k}\right)^2 \qquad 3.2.14$$

And reject the null hypothesis that no direct drug /treatment effect of if and only if $\left|T_d\right|>t\left(\alpha/2,n_1+n_2-2\right)$.

And a $\left(1-\alpha\right)\times 100\%$ confidence interval for $F=F_T-F_R$ is given by

$$\hat{F}_u \pm\left(t_{\alpha/2},n_1+n_2-2\right)\hat{\sigma}_d\sqrt{\tfrac{1}{n_1}+\tfrac{1}{n_2}} \qquad 3.2.15$$

3.2.6.3 Period effects

According to ,Carriere, K.C. & Huang, R. (2000), the presence of a period effect can be studied by testing the following hypothesis.

$H_o : \pi_1 = \pi_2$
$\mathrm{H}_a : \pi_1 \neq \pi_2$ Using a t-test.

The null hypothesis of no period effect is rejected at the alpha significant level if,

If $\left|T_p\right| > t\left(\alpha / 2, n_1 + n_2 - 2\right).$

A $100\left(1-\alpha\right)\times 100\%$ confidence interval for $\pi = \pi_1 - \pi_2$ is given by

$$\hat{\pi} \pm \left(t_{\alpha/2}, n_1 + n_2 - 2\right)\hat{\sigma}_d \sqrt{\tfrac{1}{n_1} + \tfrac{1}{n_2}} \qquad 3.2.16$$

.

3.2.6.4 Period by treatment effects

This is also known as Direct-treatment by period interaction. As the name suggests, different conditions may be present in different periods and this might have an effect on patients. For example, certain diseases and conditions depend on the weather. Let say a trial is conducted from December to February for period 1 and March to May for period two. If the trial is applied to patients with an asthmatic problem, it is possible that the patients under treatment are being affected by the weather conditions (Chow, S.C., & Liu, J.P., 2009).

3.3 Analysis of variance for 2x2-crossover design

Although we can test all the hypothesis of interest by using two-sample t-tests, it is important to note that we can also test these hypotheses using F-tests obtained from ANOVA table. Here the main thing is the variability in the observed data by partitioning the total sum of squares (TSS) of the observations into components of the fixed effects and random errors (Senn, S., 2002)**.**

For 2x2 crossover design, we would partition the total sum of squares of the $2\ n_1+n_2$ observations into components for the carry-over effects, the period effects, the direct treatment effects, and the error. Let $\overline{Y}_{...}$ be the grand mean of all observations. Then the total corrected sum of squares is given by;

$$\begin{aligned} SS_{Total} &= \sum_{k=1}^{2}\sum_{j=1}^{2}\sum_{i=1}^{n_k} Y_{ijk} - \overline{Y}_{...}{}^2 \\ &= \sum_{k=1}^{2}\sum_{j=1}^{2}\sum_{i=1}^{n_k} Y_{ijk} - \overline{Y}_{i.k} + \overline{Y}_{i.k} + \overline{Y}_{...}{}^2 \\ &= \sum_{k=1}^{2}\sum_{j=1}^{2}\sum_{i=1}^{n_k} Y_{ijk} - \overline{Y}_{i.k}{}^2 + 2\sum_{k=1}^{2}\sum_{i=1}^{n_k} Y_{ijk} - \overline{Y}_{...}{}^2 \\ &= SS_{Within} + SS_{Between} \end{aligned} \qquad 3.2.17$$

Where $\overline{Y}_{i.k=\frac{1}{2}\sum_{J=1}^{2} Y_{ijk}}$ and SS_{Within} is the sum of squares for the within subject and $SS_{Between}$ is the sum of squares due to subjects (between subjects). Since there are $2\ n_1+n_2$ observations, SS_{Total} has $2\ n_1+n_2\ -1$ degrees of freedom. And there are n_1+n_2 subjects in both sequences. Thus, $SS_{Between}$ and SS_{Within} have n_1+n_2-1 and n_1+n_2 degrees of freedom, respectively (Jones, B., 2006).

3.4 Crossover design is appropriate over parallel design.

A crossover design is preferred over a parallel-group design as it segregates the inter-subject variation from the intra-subject variation (Jones, B. & Kenward, M.G., 2003). The main advantageous that the treatments are compared “with-in subjects”. That is every subject provides a direct comparison of a treatments she/he has received. For example, in case of 2x2 crossover design, each subject provides two measurements: one on T and the other on R. The difference between these measurements removes any ‘subject-effect’ from the comparison. The main advantageous and disadvantages will be highlighted below.

3.4.1 Advantages and disadvantages of crossover design

i. **Advantages**

Each subject serves as his/her own control. It allows a within-subject comparison between formulations, there is an assessment of both (all) treatments in each subject. It removes the inter-subject variability from the comparison between formulations. As there is usually less variability within than between different subjects, there is an increase in the precision of observations. Therefore, fewer numbers of subjects are required to detect a treatment difference (Chow, S.C., & Liu, J.P., 2009).

In short, since within-subject variation is almost certainly less than between – subject variation, a crossover should produce more precise result than a parallel group study of the same size.

ii. Some drawbacks of a crossover design:

There may be a carryover effect of the first treatment continuing into the next treatment period;

The experimental unit may change over time (for example, extreme weather changes may make the second part of the crossover design different from the first.)

In animal or human experiments, the treatment introduces permanent physiological changes; the experiment may take longer

In medical clinical trials, the disease should be chronic and stable, and the treatments should not be total cures but only alleviate the disease condition. If treatment A cures the patient during the first period, then treatment B will not have the opportunity to demonstrate its effectiveness when the patient crosses over to treatment B in the second period. Therefore this type of design works only for those conditions that are chronic, (such as asthma, diabetes, hypertension, migraine,

arthritis) where there is no cure and the treatments attempt to improve quality of life simply (Jones, B. & Kenward, M.G., 2003).

CHAPTER FOUR
BIOEQUIVALENCE AND BIOAVAILABILITY

4.1 Introduction

The term bioavailability (BA) is a contraction for "biological availability" (Chow, S.C., 2007). Both bioequivalence (BE) and BA are discussed in literature review in detail and here precisely. A comparative bioavailability study refers to the comparisons of bioavailability of different formulations of the same drug or different drug products (Anonymous, 2001a and Anonymous, 1994).

Bioequivalence is usually studied by administering dosages to subjects and measuring concentration of the drug in the blood just before and at set times after the administration. On the other hand, in precise the concentration of drug that is in the blood is referred to us bioavailability and two drugs, which have the same bioavailability is called bioequivalence. There are a number of reasons why trials are under taken to show two drugs are bioequivalent (Jones, B., 2006). Among them are:

1. When different formulations of the same drug are to be marketed, for instance in solid tablet or liquid capsule forms.
2. When a generic version of an innovator drug is to be marketed.
3. When production of drug is scaled up and the new production processes needs to be shown to produce drugs of at least equivalent strength and effectiveness to the original process.

For a text on bioequivalence studies in pharmaceutical trials, we refer the reader to (O'Brien, P.C., & Fleming, T.R, 1979).

4.2 Pharmacokinetic and pharmacodynamics parameters

Pharmacokinetic and pharmacodynamics parameters are explained in detail in 320 of section 24 of the Code of Federal regulation (CFR) and guidance given by US Food and Drug Administration's (FDA) Center for Drug Evaluation and Research

(CDER). Some of the pharmacokinetic parameters are plasma or blood concentration time curve (AUC), maximum concentration C_{max} , time to achieve maximum concentration T_{max} (Jones, B., 2006).

Pharmacokinetics; What the Body Does to the Drug (Absorption, Distribution, Metabolism and Elimination)

Pharmacodynamics; What the Drug Does to the Body (Wanted Effects: Efficacy or Unwanted Effects: Toxicity) (Anonymous, 2001).

Among the pharmacokinetic parameters, AUC is the primary measure of the extent of absorption or the amount of drug in the body which is often used to access bioequivalence between drug products. AUC is often used to measure the extent of absorption or total amount of drug absorbed in the body. This measure is most frequently estimated using the linear trapezoidal rule. Other Several methods exist for estimating the AUC from zero time until time t (trapezoidal rules, See for example, Chow, S.C. (2007), Patterson, S., & Jones, B. (2006), at which the last blood sample is taken. Let $C_0, C_1, C_2 ... C_k$ be the plasma or blood concentrations obtained at a time $0, t_1, ..., t_k$ respectively. The AUC from 0 to t_k, is obtained by AUC_{0-t_k} .

The area of a trapezoid is the sum of the area of a triangle and the rectangle. That is from each part of an AUC is we can extract a triangle and a rectangle at same time.

The area of a trapezoid is obtained by adding the area of a rectangle and a triangle.

$$A = \Delta x \; y_0 + \tfrac{1}{2} \; y_1 - y_0 \qquad 4.2.1$$

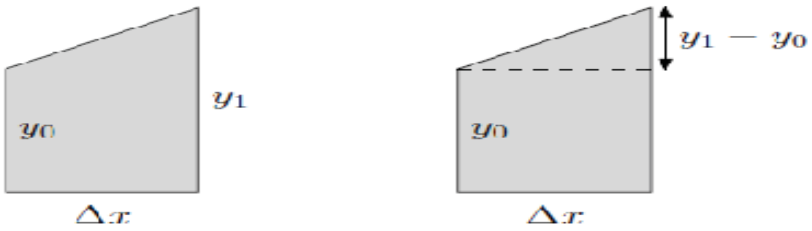

Figure 4.1 Computations of pharmacokinetics parameters like (AUC)

$$AUC_{0-t_k} = \sum_{t=2}^{k} \frac{C_{i\ 1}+C_i}{2} \quad t_i - t_{ti-1} - 1 \qquad 4.2.2$$

The AUC should be calculated from 0 to ∞, not just to the time of the blood sample, as is so often done. The remaining area from t_k to ∞ could be large if the blood level at t_k is substantial. The AUC from t_k to ∞, denoted by $AUC_{0-\infty}$, can be estimated as follows, Bonate, P.L. & Howard, D, R. (2011) and Chow, S.C. (2007).

$$AUC_{0-\infty} = AUC_{0-t_k} + AUC_{t_k-\infty} \Rightarrow AUC_{0-t_k} + \frac{C_k}{\lambda} \qquad 4.2.3$$

where; C_k is the concentration at the last measured sample after drug administration λ is the terminal or elimination rate constant, which can be estimated as the slope of the terminal portion of the log concentration-time curve multiplied by -2.303.

In addition the AUC, the absorption rate constant is usually studied during the absorption phase. Under the single-compartment model, the absorption rate constant can be estimated based on the following equation using the method of residuals (Chow, S.C., Shao, J., & Wang, H., 2003).

$$C_t = \frac{K_a F D_0}{V\ K_a - K_e}\ e^{-K_e t} - e^{-K_a t} \qquad 4.2.4$$

where;

K_a and K_e are the absorption and elimination rate constants, respectively.

D_0 is the dose administered.

V is the volume of distribution.

F is the fraction of the dose that reaches the systemic circulation.

Given equation 4.2.4 , C_{max} and T_{max} can similarly be obtained as follows:

$$T_{max} = \frac{2.303}{K_a - K_e} \log \frac{Ka}{Ke} \qquad 4.2.5$$

$$C_{max} = \frac{K_a F D_0}{V\ K_a - K_e}\ e^{K_e t_{max}} - e^{Kat_{max}} \qquad 4.2.6$$

Thus, C_{max} is estimated directly from the observed concentrations. That is, C_{max} =max $C_0, C_1, ..., C_k$. Similarly, t_{max} is estimated as the corresponding time point at which the C_{max} occurs. During the elimination phase, the pharmacokinetic parameters that are often studied are the elimination half-life $t_{\frac{1}{2}}$ and rate constant k_e . The plasma elimination half-life is the time taken for the plasma concentration to fail by half (Chow, S.C., 2007). Assume that the decline in plasma concentration is of first order, the $t_{\frac{1}{2}}$ can be obtained by considering

$$Log_D = \log D_0 - \frac{k_e t}{2.303} \qquad 4.2.7$$

D is the amount of drug in the body. Thus, at D= $\frac{D_0}{2}$, i.e. $t = t_{\frac{1}{2}}$, we have

$$\log \frac{1}{2} - \frac{k_e t_{\frac{1}{2}}}{2.303} \Rightarrow t_{\frac{1}{2}} = \frac{0.693}{k_e}$$

Where $k_e = -2,303 \frac{d \log D}{dt}$

4.3 Assessment of Bioequivalent and bioavailability

4.3.1 Decision rules and regulatory aspects

The association between bioequivalence limits and clinical difference is difficult to assess in practice. Suppose AUC and C_{max} are the primary systematic exposure measures of the extent and rate of absorption. For each parameter, the following decision rules for assessment of average bioequivalence are applied (Anonymous, 2001).

75/75 Rule

Bioequivalence is claimed if at least 75% individual subject ratios (relative individual bioavailability of the test formulation to the reference formulation) are within $(75\%,125\%)$ limits. Even if this rule has some advantageous like; it is easy to apply, it compares the relative variability within each subjects and removes the effect of heterogeneity of inter-subject variability from the comparison between the formulations, and it is not viewed favorably by FDA owing to some undesirable statistical properties.

In a simulation study, Chow, S.C. (2007) showed that the 75/757 rule is very sensitive for drugs that have large inter- or intra-subject variabilities; even in the situation where the mean AUC's for the test and reference formulations are exactly the same. Provided an analytic evaluation of the 75/75 rule relative to the ±20 rule. The results suggest that the 75/75 rule will never be met when the intra-subject variability is large (say 20%) for any given true ratio of means.

80/20 Rule

If the average of the test product is not statistically significantly different from that of the reference product, and if there is at least 80% of power for detection of a 20% difference of reference average bioequivalence is concluded. 80/20 Rule is considered only as a pre-study power calculation for sample size determination in the

planning stage of study protocol. In other words the idea proposed for testing bioequivalence was to simply test to see whether the formulations were different, and if the test did not demonstrate a significant difference of 20%, then one would accept bioequivalence.

$\pm 20\%$ **Rule**

Bioequivalence is concluded if the average bioavailability of the test formulation is within $\pm 20\%$ of that of the reference formulation with a certain assurance (Chow, S.C., & Liu, J.P., 2009).

$80/125$ **Rule (Current Regulation Criteria of Bioequivalence)**

At present, the regulatory authorities, recommended analysis of the data after logarithmic transformation for $C_{\max}$ and AUC and bioequivalence is concluded if the average bioavailability of the test formulation is within $(80,125\%)$ of that of the reference formulation with a certain assurance. To achieve this equivalence, geometric mean ratios (like AUC test/AUC reference), as well as their projected (1-α2)x100% confidence intervals for the population mean ratio, must be located within in 80% to 125%. From a multiplicative model for pharmacokinetic responses postulated by Potvin, D.et al. (2008), the logarithmic transformation is suggested for $\text{AUC}(0-\infty)$ or $\text{AUC}(0-t_{last})$ and $C_{\max}$ in the guidance of (Anonymous, 2001). As a result, the Division of Bioequivalence, the FDA suggested use of an equivalence criterion of $80\%-125\%$ for assessment of bioequivalence based on the ratio of average bioavailability. This criterion is not symmetric about 1 on the original scale where the maximum probability of concluding average bioequivalence occurs. However, on the logarithmic scale, the criterion has a range of -0.2231 to 0.2231, which the symmetric about 0 where the probability of concluding average bioequivalence is at maximum.

4.4 Application of group sequential design in the assessment of bioequivalence

Application of group sequential approaches to the BE studies differs from their application to most other types of clinical studies because the former generally involves crossover designs, testing of equivalence hypotheses, and testing based on t-distributions, whereas the later generally involves parallel designs with testing of difference hypotheses (Gould, A.L., 1995). At the i^{th} stage of a group sequential BE trial, data are analyzed from the first n_i of planned maximum number of subjects n, and the trial is stopped and BE is concluded if and only if the $1-2\alpha \times 100\%$ CI for the test to reference ratios are entirely contained within the interval [80, 125%]for both C_{max} (maximum drug concentration) and (the area under the drug concentration verses curve (Hauck, W.W.,et al., 1997). AUC is often used to measure the extent of absorption or the total amount of drug absorbed in the body). Otherwise the trial continues to the second stage (Potvin, D. et al., 2008).

CHAPTER FIVE
STATISTICAL METHODS FOR AVERAGE BIOEQUIVALENCE

To claim average bioequivalence (ABE), for untransformed/raw data should be established if the 90% confidence interval for $\mu_T - \mu_R$ is entirely within the interval of $-0.2\mu_R, 0.2\mu_R$ (Chow,S.C., & Shao, J., 1990). The sponsor and FDA determine the acceptable bounds for confidence limits for the particular drug and formulation during protocol development (Anonymous, 2001b). Generally, if we keep the risk of a particular patients at (5%), the risk of the entire population of patients (<80% and >125% is $2\times\alpha(10\%)$.That is 90% confidence interval comes from (CI=1-2α).Generally, the statistical methods of choice at present are the two one-sided test procedure, Schuirmann, D.J.A. (1987), or to derive a parametric or nonparametric $(1-2\alpha)\times 100\%$ confidence interval for the ratio (or difference) between the test and reference product pharmacokinetic variable averages (Liu, J. P. & Weng, C.S., 1993). Alpha is set at 5% leading, in the parametric case, to the shortest (conventional) 90% confidence interval based on an analysis of variance or, in the nonparametric case, to the 90% confidence intervals (Lindley, D.V., 1998).

Consider a 2x2 crossover trial where we wish to compare R and T using two sequences of treatment (RT and TR) given in two periods. Let n_1 and n_2 subjects be allocated to the two sequences, respectively (assume $n_1 = n_2$). Also assume that $\bar{Y}_T$ and $\bar{Y}_R$ are the Test and Reference means, respectively, estimated from these $n_1 + n_2$ subjects.

Two statistical approaches are suggested in literature for testing bioequivalence between T and R. These are:

• Two One Sided Hypothesis Tests (TOST) procedure at α significance level (Westlake, W.J., 1972 and Schuirmann, D.J.A. 1987)

• $1-2\alpha \times 100\%$ Confidence Interval procedure.

5.1 TOST procedure

Let θ_L and θ_U are two known clinically meaningful bioequivalence limits and θ be the parameter of interest (Schuirmann, D.J.A., 1987). In TOST procedure two sided bioequivalence test divided in to two one-sided tests in the following manner:

$$\begin{aligned} &\text{Test1}, H_0^+ : \theta \le \theta_L \text{ versus } H_1^+ : \theta > \theta_L \\ &\text{Test2}, H_0^- : \theta_L \ge \theta \text{ versus } H_1^- : \theta < \theta_U \end{aligned} \qquad 5.1.1$$

Under the normality assumptions, the two sets of one-sided hypothesis can be tested with ordinary one-sided t test. We conclude that μ_T and μ_R are bioequivalent if;

$$\begin{aligned} T^+ &= \frac{\bar{Y}_T - \bar{Y}_R - \theta_L}{\sqrt{\hat{V}(\bar{Y}_T - \bar{Y}_R)}} > t(\alpha, n_1 + n_2 - 2) \text{and} \\ T^- &= \frac{\bar{Y}_T - \bar{Y}_R - \theta_U}{\sqrt{\hat{V}(\bar{Y}_T - \bar{Y}_R)}} < -t(\alpha, n_1 + n_2 - 2) \end{aligned} \qquad 5.1.2$$

Where; $\widehat{V}(\bar{Y}_T - \bar{Y}_R) = \frac{\alpha^2 e}{2}(\frac{1}{n1} + \frac{1}{n2})$ 5.1.3

Equation (5.1.3) is the estimate of variance of mean treatment difference. $\alpha^2 e$ MSE (Mean square error) from ANOVA of population measures (or its logarithmic transformation in ratio hypotheses) considering sequence, period and treatment as fixed factors and subject as random factor.

5.2 Confidence interval approach

For a two-period crossover study, the ANOVA model used to calculate estimates of the error variance and the least square means are identical for both transformed and untransformed data. The procedural difference comes after the lower and upper $(1-2\alpha)\times 100\%$ confidence intervals are found by formulas based on Student's t-distribution. A test for the null hypothesis of the equality of the two formulations of a drug product was derived under a standardized 2x2 crossover design indicate that the method of confidence interval is an appropriate method of assessing bioequivalence. Based on the confidence interval approach, Westlake, W.J. (1972), suggested the following action for decision-making:

If a $(1-2\alpha)\times 100\%$ confidence interval for the difference $(\mu_T - \mu_R)$ or the ratio $\left(\frac{\mu_T}{\mu_R}\right)$ is within the acceptance limits as recommended by the regulatory agency like (Anonymous, 2001a), then accept the test formulation (that is the test formulation is equivalent to the reference formulation), and otherwise reject it. In the Confidence Interval Approach, there are several methods for constructing a $(1-2\alpha)\times 100\%$ confidence interval for $\left(\frac{\mu_T}{\mu_R}\right)$ has been proposed under a raw data (untransformed) model. Among others the following have been included:

- ✓ The classical confidence interval which is also known as the shortest confidence interval.
- ✓ Westlake's symmetric confidence interval.
- ✓ Confidence interval for $(\mu_T - \mu_R)$ based on Filler's theorem (Vuorinen, J. & Tuominen, J., 1994).
- ✓ Chow and Shoa's joint confidence region for (μ_T, μ_R)

5.2.1 The classical (shortest) interval method

The FDA advocates the use of $(1-2\alpha)\times 100\%$ confidence intervals, as the best available method for evaluating BE study data. The confidence interval approach should be applied to the individual parameters of interest (e.g., AUC and $C_{\max}$). The sponsor may use untransformed or log-transformed data.

5.2.1.1 Untransformed data

If we let $\overline{X}_{T1}$ the mean of the test drug in period 1, $\overline{X}_{T2}$ be the mean of the test drug in period 2, and $\overline{X}_{R1}$ the mean of the reference drug in period 1, $\overline{X}_{R2}$ be the mean of the reference drug in period 2, then the estimates for drug averaged over both periods are

$$\overline{X}_T = \frac{1}{2}\left(\overline{X}_{T1} + \overline{X}_{T2}\right)$$

$$\overline{X}_R = \frac{1}{2}\left(\overline{X}_{R1} + \overline{X}_{R2}\right) \qquad 5.2.1$$

Or $\overline{X}_T$ and $\overline{X}_R$ be the respective least square means for the test and formulations respectively, which can be obtained from the sequence by period means stated above. That is the halves of subjects are considered in RT and the other are in TR sequence for first and second period respectively. The classical $(1-2\alpha)\times 100\%$ confidence interval can then be obtained based on the following t-statistic.

$$T = \frac{\left(\overline{X}_T - \overline{X}_R\right) - \left(\mu_T - \mu_R\right)}{\hat{\sigma}_d \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}} \qquad 5.2.2$$

Where n_1 and n_2 are the number of subjects in sequence 1 and sequence 2 and $\hat{\sigma}_d$ given in chapter three from direct treatment effect which is $\hat{\sigma}_d{}^2 = \frac{\sigma_e^2}{2}$, where σ_e^2 is the **intra-subject** variance. Under normality assumptions, T follows a central Student's t- distribution with $n_1 + n_2 - 2$ degrees of freedom (Locke, C. S.,

1984). Thus, the classical $(1-2\alpha)\times 100\%$ confidence interval for $\mu_T - \mu_R$ can be obtained as follows.

$$L_1 = \left(\overline{X}_T - \overline{X}_R\right) - t\left(\alpha, n_1 + n_2 - 2\right)\hat{\sigma}_d \sqrt{\tfrac{1}{n_1} + \tfrac{1}{n_2}} \qquad 5.2.3$$

$$U_1 = \left(\overline{X}_T - \overline{X}_R\right) + t\left(\alpha, n_1 + n_2 - 2\right)\hat{\sigma}_d \sqrt{\tfrac{1}{n_1} + \tfrac{1}{n_2}} \qquad 5.2.4$$

5.2.1.2 Logarithmically transformed data

In the previous discussion we have seen so many statistical methods for the assessment of bioequivalence. Most of the methods are derived under a row data. But one of the difficulties commonly encountered in bioavailability studies, however is, whether the assumption of normality is valid. In many cases distribution of the response are positively skewed and exhibit the luck of homogeneity of variances (Liu, J. P. & Weng, C.S., 1993). In this situation a log-transformation on the response is often considered in order to reduce the skewness and to achieve an additive model with relatively homogeneous variances. This leads to a multiplicative (log-transformed) model. Based on the transformed data, the methods introduced above can be applied directly (Hauschke, D., Steinijans, V.W., Diletti, E. & Burke, M., 1992).

Shortly, FDA guidance's (1) recommended log transformation before BE study. Then for log-transformed data, the BE can be established if the 90% CI for $\mu_T - \mu_R$ is entirely located in the interval (80%, 125%). It should be noted that (log 1.25 $=-\log\ 0.8$, which is $0.231 = -(-0.231)$). In other words, the BE limit for the log-transformed data is symmetric about 0.

This section discusses how the $(1-2\alpha)\times 100\%$ confidence interval approach should be applied to log-transformed data. In this situation, the individual animal AUC and $C_{\max}$ values are log-transformed and the analysis is done on the transformed data. For a two-period crossover study, the ANOVA model used to calculate

estimates of the error variance and the least square means are identical for both transformed and untransformed data. The procedural difference comes after the lower and upper $(1-2\alpha)\times 100\%$ confidence intervals are found by formulas based on Student's t-distribution. The lower and upper confidence bounds of the log-transformed data will then need to be back-transformed in order to be expressed on the original scale of the measurement. One thing to keep in mind when moving between the logarithm scale and the original scale is that the back-transformed mean of a set of data that has been transformed to the logarithm scale is not strictly equivalent to the mean that would be calculated from the data on the original scale of measurement. This back-transformed mean is known instead as the geometric mean.

Bioequivalence studies measure and compare statistically AUC, C_{max} and T_{max} of the formulations. In case of AUC and C_{max}, the regulatory authorities recommend that they should be logarithmically transformed before further statistical analysis. The use of log transformed values for AUC and C_{max} is recommended for several reasons (Anonymous, 2001).

Clinical rationale: In a meeting in September 1991, the Generic Drugs Advisory Committee (GDAC) concluded that the primary comparison of interest in a bioequivalence study was the ratio rather than the difference between average parameter data from the test and reference formulations. This is achieved statistically by using log transformation.

Pharmacokinetic rationale: In the crossover design, the usual assumption is that the observation is a function of additive effects due to subject, period and treatment. But pharmacokinetic equations are of multiplicative character.

Statistical rationale: Many biological data correspond more closely to a log normal distribution. AUC and C_{max} tend to be skewed and their variances increase with the means. Log transformation makes the variances independent of the mean and the frequency distribution is made more symmetrical.

5.3 Methods of interval hypothesis

As we have seen before the assessment of bioequivalence is based on the comparison of bioavailability profiles between treatment formulations. Schuirmann, D.J.A. (1987), first introduced the concept of interval hypothesis for the assessment of average bioequivalence based on the two-one sided tests (TOST).

Westlake, W.J. (1972), pointed out that a statistically significant difference in the comparison of bioavailability between drug products does not necessarily imply that there is a clinically significant difference between drug products. For example, the AUC for the test product may exhibit 80% bioavailability compared to the reference product. The 20% difference in AUC, which may be statistically significant, however, may not be of clinically significance in terms of therapeutic effect.

The statistical confidence interval hypotheses given below, is to show average bioequivalence by rejecting the null hypothesis of average bioinequivalence. The interval hypothesis for untransformed data/additive hypothesis of average bioequivalence can be formulated as

$$H_0 : \mu_T - \mu_R \leq \theta_L \text{ or } \mu_T - \mu_R \geq \theta_U$$
$$H_1 : \theta_l < \mu_T - \mu_R < \theta_U \text{ BE} \qquad 5.3.1$$

Or interms of Union and intersection,

$$H_{01} : \mu_T - \mu_R \leq \theta_L$$
$$H_{a1} : \mu_T - \mu_R > \theta_L \qquad 5.3.2$$

And

$$H_{02} : \mu_T - \mu_R \geq \theta_U$$
$$H_{a2} : \mu_T - \mu_R < \theta_U \qquad 5.3.3$$

The first set of hypothesis is to verify that the bioavailability of the tests formulation is not too low, while the second set of the hypothesis is to verify that the bioavailability of the test formulation is not too high (Berger, R. L. & Hsu, J. C., 1996).

Multiplicative bioequivalence tests of hypothesis/ after logarithmic transformation of the given data, the hypothesis of the above can be written as follows:

$$H_0' : \frac{\mu_T}{\mu_R} \leq \delta_L \text{ or } \frac{\mu_T}{\mu_R} \geq \delta_U \qquad 5.3.4$$

vs.

$$H_1' : \delta_L < \frac{\mu_T}{\mu_R} < \delta_U \qquad 5.3.5$$

And this becomes an additive model after ln transformation:

$$H_o : \ln \mu_T - \ln \mu_R \leq \ln \delta_L \text{ or } \ln \mu_T - \ln \mu_R \geq \ln \delta_L$$
$$H_1 : \ln \delta_L < \ln \mu_T - \ln \mu_R < \delta_U \quad , \qquad 5.3.6$$

where

μ_T and μ_R are respectively the mean of the test and reference treatments. When population measures distributed log normally, θ is considered as ratio and data is analyzed after logarithmic transformation. Logarithmic transformation ratio hypothesis is converted to difference hypothesis in the following way.

$$\text{Log}\theta = \frac{\text{Log}\theta_T}{\text{Log}\theta_R} = \text{Log}\theta_T - \text{Log}\theta_R \qquad 5.3.7$$

And here to keep integrity, BE limits also converted to their logarithmic values when θ is ratio. For example, when the actual BE limits are 0.8 and 1.25, one should use $\text{Log } 0.8 = -0.223$ and $\text{Log } 1.25 = 0.223$ (this is very imperative concept to change the given data to symmetry) as lower and upper BE limits, respectively for testing purpose. It is clear from the above discussion that ratio

hypotheses can be well converted into the hypotheses of difference. For simplicity, we will refer the corresponding bioequivalence hypotheses as ratio bioequivalence hypotheses and difference bioequivalence hypotheses when θ is ratio and difference, respectively (Chow, S.C., 2007). And finally we conclude that μ_T and μ_R are equivalent if

$$T^{+} = \frac{\overline{Y}_T - \overline{Y}_R - \theta_L}{\sqrt{\text{var}\ \overline{Y}_T - \overline{Y}_R}} > t\ \alpha, n_1 + n_2 - 2$$
$$T^{-} = \frac{\overline{Y}_T - \overline{Y}_R - \theta_U}{\sqrt{\text{var}\ \overline{Y}_T - \overline{Y}_R}} < -t\ \alpha, n_1 + n_2 - 2 \qquad 5.3.8$$

Where, $V\ \overline{Y}_T - \overline{Y}_R = \frac{\alpha^2 e}{2}\ \frac{1}{n1} + \frac{1}{n2}$, so from $\hat{\sigma}_d^2 = \frac{\alpha^2 e}{2}$ therefore the test statistics can be simplified as follows.

$$T^{+} = \frac{T\overline{Y} - R\overline{Y} - \theta_L}{\hat{\sigma}_d \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}} > t\ \alpha, n_1 + n_2 - 2\ \text{and}$$
$$T^{-} = \frac{T\overline{Y} - R\overline{Y} - \theta_U}{\hat{\sigma}_d \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}} < -t\ \alpha, n_1 + n_2 - 2 \qquad 5.3.9$$

5.4 Power and sample size Determination in clinical design

During the planning stage of BA/BE study, the clinicians and the statisticians are able to answer the following questions. How many subjects are needed in order to achieve a desired power (commonly 80%) to established BE between two formulations within clinically/may not be statistically important limits (±20% of the reference mean)? If only small number of subjects is available in hand due to limited resources/budget or some medical considerations, what we have to do? In order to answer the above critical questions, a statistical approach for sample size determination is employed. And the most commonly used approach is to perform a pre-study power calculation based on an estimate of the intra-subject variability from previous study (Chow, S.C., 2007) and (Phillips, K.F., 1990).

5.4.1 Type I and type II errors

In fact, two types of errors occur when testing hypotheses. As usual when the null hypothesis is rejected when it is true, then type I error has occurred. While, when the null hypothesis is not rejected when it is false, then a type II error has been made (Schuirmann, D.J.A., 1987) and (Phillips, K.F., 1990).

And the probability of making the above two types of errors is summarized below.

$$\begin{aligned}\alpha &= P(type\ \text{I error}) \\ &= \text{p}(reject\ H_o\ when\ H_o\ is\ true) \\ \beta &= p(type\ \text{II error}) \\ &= \text{p}(\ fail\ \text{to}\ reject\ H_o\ when\ H_o\ is\ \text{false})\end{aligned} \qquad 5.4.1$$

The probability of making a type I error, α, is called the level of significance, commonly called the patient risk. And the probability of making a type II error, β, is called commonly called the producers risk (Chow, S.C., 2007).

Table 5.1 Type one and two errors for traditional case /General case

H_o (no difference)		
	True/ No difference	False /Difference
Fail to reject	No error	Type II error
Reject	Type I error	Power

Table 5.2 Type one and two errors for Bioequivalence trials

H_o (bioinequiavalnce)		
	True/Bioinequivalent	False/Bioequivalent
Fail to reject	Right decision	Type II error/ producers risk
Reject	Type I error/patients risk	Right decision/power

5.4.2 Hypothesis setting

In practice, the null hypothesis H_o and the alternative hypothesis H_a are sometimes reversed and evaluated for different interests. Generally, the choice of the null and the alternative hypotheses may have some impact on the parameter to be tested (Chow, S.C., 2007).

Choose H_o based on the importance of a type I error. Under this rule, it is believed that a type I error is more important and serious than that of a type II error. We would like to control the chance of making a type I error at a tolerable limit.

For example in case of **bioequivalence-bioinequivalence,** the following two errors occur in assessment of bioequivalence when comparing two formulations in average bioavailabilities:

i. We conclude bioequivalence when in fact the test formulation is not bioequivalent to that of the reference formulations
ii. We conclude bioinequivalence when in fact the test formulation is bioequivalent to the reference formulation.

In the interest of controlling the chance of making type I error, the FDA may consider (i) is more important than (ii) and consequently prefer the following hypotheses:

H_o : Bioinequivalence

H_1 : Bioequivalence

CHAPTER SIX
APPLICATIONS AND CONCLUSIONS

6.1 Statement of the problem

Every clinical trial should be planned. This plan should include the objectives of the given trial, primary and secondary end-points, and method of collecting data, sample to be included, sample size with scientific justification, method of handling data, statistical methods and assumptions. This plan is termed as clinical trial protocol. One of the key aspects of this protocol is sample size estimation. The aim of our work is **to determine the minimum sample size to detect a clinically important difference in bioequivalence studies under 2x2 crossover design**. The number of patients in a clinical trial should always be large enough to provide a reliable answer to the questions addressed, but should also be the minimum necessary to achieve this aim. This number is usually determined by the primary efficacy objective of the trial. In any experimental study, neither under estimation nor over estimation of sample size is risky. It is explained in detail from literature review.

6.2 Simulation methodologies and formulas

The goal of simulation is to learn important statistical information about the processes .and it is performed based on random numbers. This random numbers form a basic tool for simulation studies. The following are important points for a simulation study.

- ✓ In our simulation work, the missing value is substituted by the mean of the other observations, if existed. And it is expected that there is no neither outliers nor the influential observations exist.
- ✓ In R, the ‘seed. Set’ declares the seed for random number generator. And if we use this command before random number generating statement, we are able to retain same number each time we provide same seed.
- ✓ The for-loop (see introduction to R):

For (var in vector)

{Statements we want to simulate}

In our simulation study, we want to determine the required sample size to conduct a clinical study in crossover design. The minimum sample size in BE study is 12.

- First let us see the traditional approach

Sample size determinations based on the rules and regulations for the assessment of bioequivalence and bioavailability of two drug treatments.

- The confidence interval approach
- Interval hypothesis testing approach
- Power approach

First the values of AUC for Test and References drugs were generated from the statistical model for a standard crossover design under normality assumption distributed with the given mean and standard deviation as follows. For simplicity, it is assumed that there were no carryover and period effects. For the standard 2 sequence, 2 period, 2 treatment crossover designs, Schuirmann's **TOST** procedure is still valid when the intra-subject varaibilities differ from formulation to formulations of a drug product. The confidence interval can be computed as follows:

$$L_1 = \left(\overline{X}_T - \overline{X}_R\right) - t\left(\alpha, n_1 + n_2 - 2\right)\hat{\sigma}_d\sqrt{\tfrac{1}{n_1} + \tfrac{1}{n_2}}$$
$$U_1 = \left(\overline{X}_T - \overline{X}_R\right) + t\left(\alpha, n_1 + n_2 - 2\right)\hat{\sigma}_d\sqrt{\tfrac{1}{n_1} + \tfrac{1}{n_2}} \quad \text{For difference} \qquad 6.2.1$$

$$L_1 = \frac{\overline{X}_T}{\overline{X}_R} - t\left(\alpha, n_1 + n_2 - 2\right)\hat{\sigma}_d\sqrt{\tfrac{1}{n_1} + \tfrac{1}{n_2}}$$
$$U_1 = \frac{\overline{X}_T}{\overline{X}_R} + t\left(\alpha, n_1 + n_2 - 2\right)\hat{\sigma}_d\sqrt{\tfrac{1}{n_1} + \tfrac{1}{n_2}} \quad \text{For ratio} \qquad 6.2.2$$

In this study design, ANOVA is to be used to identify the source contributions by factors including subjects, period, formulation and potential interactions. The geometric mean ratio together with the ANOVA residual mean error term are used to identify the statistical basis for the 90% confidence interval for the ratio of the population means (Test/Reference) of the identified metrics (e.g. AUC, Cmax).

For traditional approach

As we may know, from statistical point of view, test for equality of two treatments the hypothesis is stated as:

$$\begin{aligned} H_o &: \mu_T - \mu_R = 0 \\ H_1 &: \mu_T - \mu_R \neq 0 \end{aligned} \quad \text{For difference} \qquad 6.2.3$$

$$\begin{aligned} H_o &: \frac{\mu_T}{\mu_R} = 1 \\ H_1 &: \frac{\mu_T}{\mu_R} \neq 1 \end{aligned} \quad \text{For ratio} \qquad 6.2.4$$

And if the confidence in case of the difference contains zero and in case of the ratio contains one the equality of the two means can be conclude otherwise the null hypothesis is rejected for the given level of significant. The simulation for the traditional approach /method is performed based on this theoretical concept. For each random sample, the lower and upper (L_i, U_i) values are computed and finally the proportion of (L_i, U_i) which contains 0 and 1 for difference and ratio are calculated, if the proportion is $\geq (1-2\alpha)\times 100\%$, then the two treatments are said to be equivalent.

Some rules and regulations for bioequivalent studies

The confidence interval approach

As we have stated in the literature part of this paper in detail, if the computed confidence interval is contained in (0.8, 1.25) and (-0.223, 0.2231) for the difference and ratio logarithmic respectively, then bioequivalence is concluded. For each simulation step, the confidence interval is calculated for the difference and ratio and finally we compute the **proportion** of the confidence intervals contained in the two

values stated by FDA and some other regulatory agencies. For this approach BE was evaluated using two one-sided-t-test (Lindley, D.V., 1998).

$$L_1 = \left(\overline{X}_T - \overline{X}_R\right) - t\left(\alpha, n_1 + n_2 - 2\right)\hat{\sigma}_d\sqrt{\tfrac{1}{n_1} + \tfrac{1}{n_2}}$$
$$U_1 = \left(\overline{X}_T - \overline{X}_R\right) + t\left(\alpha, n_1 + n_2 - 2\right)\hat{\sigma}_d\sqrt{\tfrac{1}{n_1} + \tfrac{1}{n_2}} \quad \text{For difference case} \qquad 6.2.5$$

Interval hypothesis testing approach

Here in each simulation step, we perform a statistical test (t-test) on the 5% significant level for **TOST**. And both the simulated p-values of TOST are compared with the significant level of $\alpha = 5\%$, finally the proportion of rejecting the null hypothesis is evaluated. Actually, this approach is similar to the shortest confidence level.

Power approach for assessment of BE

An appropriate sample is chosen to meet the desired power for the assessment of bioequivalence within clinically important limits.

$$H_o : BIE$$
$$H_1 : BE \qquad 6.2.6$$

Here we have to see two important steps

1. If H_o is not rejected at α the level of significant, then we cannot conclude that the two formulations are bioequivalent.
2. But if the null hypothesis is rejected, we proceed to whether the power for detection of a difference of $\Delta = 0.2\mu_R$ is greater than 80%.

And the power was calculated based on the modification of (Hauschke et al., 1992).

$$1-\beta = F_t\left(\frac{\ln(1.25/\theta)}{s\sqrt{2/n}} - t_{1-\alpha}, DF, DF\right) - F_t\left(\frac{-\ln(1.25*\theta)}{s\sqrt{2/n}} + t_{1-\alpha}, DF, DF\right) \qquad 6.2.7$$

Where; $1-\beta$ is the power, DF is the degrees of freedom associated with the error, the $F_t(x, DF)$ is the cumulative distribution functions of student's t-distribution with DF degrees of freedom, and lastly, $t_{1-\alpha}, DF$ is the $(1-\alpha)th$ percentile of a student's t-density function. *S* is the sample standard deviation (estimate of σ) which is calculated from ANOVA on the ln(Test/Reference)=ln(Test)-ln(Reference) differences (from all the given data) using stage/sequence, and stage*sequence effects in the model (since only one stage is conducted this model reduces to just a sequence effect) (Potvin, D.,2008).

Generally; the simulation were performed using statistical software R, version 2.14.0 a different randomly selected seed was used for each scenario as shown in table 6.1 below. A scenario was defined as a specified combination **of ratio of geometric means (GMR), intra-subject coefficient of variation (CV), and sample size**.

Table 6.1 The given values parameters for this simulation study.

μ_T	μ_R	$\theta = \frac{\mu_T}{\mu_R}$	Sample size (n)	σ_e^2	CV%= $\frac{\sigma_R}{\mu_R}\times 100\%$
85	100	0.85	**12**	100	10
90		0.9	16	200	15
95		0.95	20	400	20
100		1.0	.	580	25
105		1.05	.	850	30
110		1.10	.	1200	35
115		1.15	100	1600	40
120		1.20	110	2000	45
			120	2500	50
			130	3000	55
			140		
			150		

μ_T = The true test mean

μ_R = The true references mean which constant.

θ = Geometric mean ratio, thus, the equivalence limits for the difference are -20 to $+20$ and for the ratio are 0.8 to 1.25.

σ_e^2 =Intra-subject variability and for specified values of sample size;

Test~N(μ_T, σ_T) and

Reference~N(μ_R, σ_R),

where, n, μ, σ are respectively the sample size, the true mean and the true standard deviation for the test and the reference drug products.

One million simulation studies were performed at $\alpha = 0.05$ significant level, and all approaches are evaluated. Note that, in our simulation results, the missing value is likely to be produced sometimes, if it is the case it is replaced by the **arithmetic mean** of the other simulated data produced in each step. For all stages we Evaluate BE based on the power approach for the given CV and GMR. Let us summarize this in the following table.

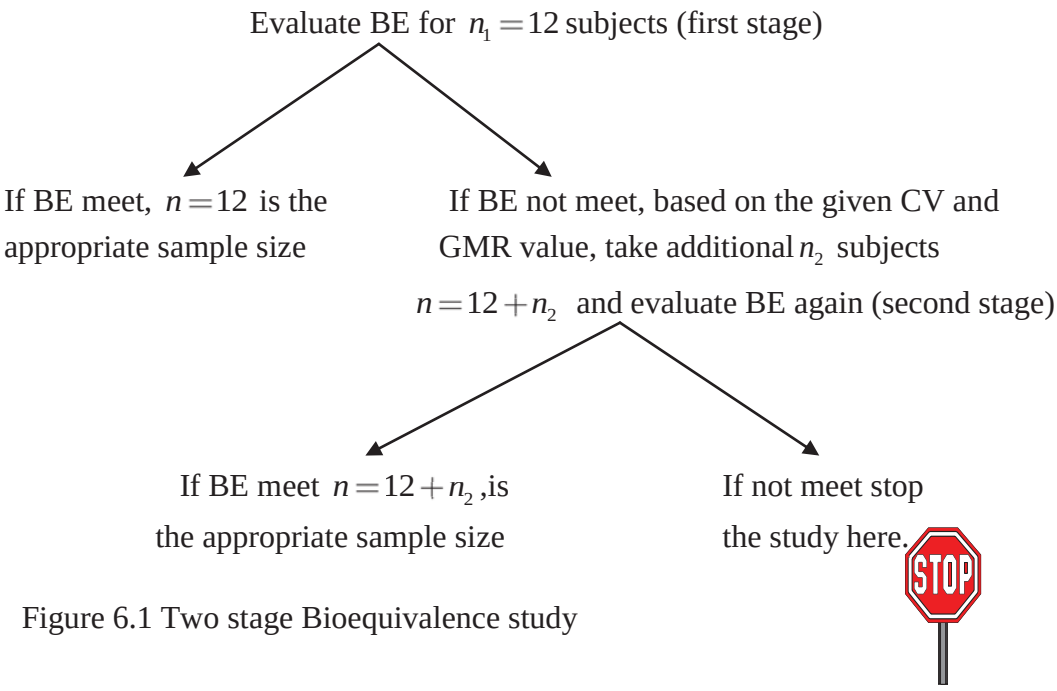

Figure 6.1 Two stage Bioequivalence study

6.3 Results and conclusions

The analysis conducted at the adjusted significance levels (with the confidence interval accordingly using an adjusted coverage probability which will be greater than 90% for significant level 0.05) the proportion of the simulated value for all

approaches should at least 90% to conclude BE and the corresponding sample size to be sufficient, this is our aim for this study. Since the minimum sample size required to conduct a clinical study is, 12, which is also the initial sample size in our simulation study, 6 for each group.

The simulation results of the sample size for **GMR=0.85** and the corresponding coefficient of variation, is given in table 6.2 below. For CV=10, we need additional 52subjects, in addition to the initial sample size n=12. While for second stage, CV=15, additional 60 subjects are required to demonstrate BE (n=12+60).

But generally, we can observe that when CV increased from 10 to15, we need additional 8 subjects. While when the coefficient of variation increased from 10 to 20 the sample size is extremely increased to120, further more we need additional 56 subjects.

Table 6.2 Simulation results for GMR=0.85

GMR=0.85										
CV%	10	15	20	25	30	35	40	45	50	55
Sample size=n	64	72	120	-	-	-	-	-	-	-

Table 6.2 shows the simulation results when the ***GMR=0.90***. And CV=10, additional 8 subjects in which 4 subjects for each group, are important to achieve BE between the test and the reference drugs. For second stage when CV= 20, only 24 in addition to the initial12 subjects, which is almost one **third of the sample** size required in case of GMR= 0.85 for same CV value to demonstrate BE. This implies that the sample size is highly affected by GMR, in addition to CV value. In short, for small values of CV < 30 , a maximum of 48 subjects/ sample size is needed. And for high values of CV ≥ 30 , a minimum of 72 subjects are needed to conduct a BE study.

Table 6.3 Simulation results for GMR=0.90

GMR=0.90										
CV%	10	15	20	25	30	35	40	45	50	55
Sample size=n	20	24	36	48	72	110		-	-	-

Here in table 6.3 above, we can observe that for GMR= 0.90 , when CV values larger than 35, proceeding to the next step is not important. It is harmful both ethically and economically.

If the ***GMR=0.95***, the minimum number of sample size is achieved compared to the above two GMR values for a constant CV values. Even, for CV=10,15 , BE is achieved in the first stage, which is not for the above two GMR values. For example, for CV = 20, only 4 subjects are needed, 2 for each group. And here we go for other CV values.

As shows below in table 6.4, to summarize, for small values of CV a maximum of 24 subjects are required, and for high values of CV, at least 36 subjects are needed.

Table 6.4 Simulation results for GMR=0.95

GMR=0.95										
CV%	10	15	20	25	30	35	40	45	50	55
Sample size=n	12	12	16	24	36	52	88	-	-	-

When the ***GMR=1.0,*** and CV value is small, the simulation result shows similar sample size value with that of ***GMR=0.95***. Furthermore, we stop the study when CV > 40 , which is the critical value of CV with the corresponding sample size = 88 .

But for small values of CV, this GMR value is more appropriate in terms of sample size.

Table 6.5 Simulation results for GMR=1.0

GMR=1.0										
CV%	10	15	20	25	30	35	40	45	50	55
Sample size=n	12	12	16	20	40	72	88	-	-	-

For ***GMR=1.05***, for small values of CV, almost similar results of sample size is required with that of ***GMR=0.95, 1.0***. And for large values of CV, adding additional sample size is unimportant, in other words we have to stop the study here and must use other alternatives.

Table 6.6 Simulation results for GMR=1.05

GMR=1.05										
CV%	10	15	20	25	30	35	40	45	50	55
Sample size=n	12	12	20	40						

And when the ***GMR=1.10***, for small values of CV given in table 6.7, the simulation result shows that, approximately two times the number of subjects needed for GMR $= 0.95$, 1.0, and 1.05. Nevertheless, for large CV values no need of conducting any trial for the given GMR value, it is also wastage of time, resource and may be risky ethically.

Example here for CV$= 25$, we need 60 additional number of samples to conduct BE study for the second stage.

Table 6.7 Simulation results for GMR=1.10

GMR=1.10										
CV%	10	15	20	25	30	35	40	45	50	55
Sample size=n	12	20	44	72	-	-	-	-	-	-

For ***GMR=1.15,*** the simulation result shows that, for the second stage, additional $12, 32, 98$ subjects are required in addition to the 12 initial subjects.

Table 6.8 Simulation results for GMR=1.15

GMR=1.15										
CV%	10	15	20	25	30	35	40	45	50	55
Sample size=n	24	44	110	-	-	-	-	-	-	-

For simplicity, when CV is 20, we have to take additional 98 subjects / sample size in addition to the first 12.

Finally for ***GMR=1.20***, for $CV = 10$, the sample size is $n = 100$, means that additional 88 sample are important. But for $CV > 10$, no need of taking additional sample size.

Table 6.9 Simulation results for GMR=1.20

GMR=1.20										
CV%	10	15	20	25	30	35	40	45	50	55
Sample size=n	100	-	-	-	-	-	-	-	-	-

Table 6.10 Summary of sample size from simulation results

GMR= μ_T / μ_R

CV%	0.85	0.90	0.95	1.0	1.05	1.10	1.15	1.20
10	64	20	12	12	12	12	24	100
15	72	24	12	12	12	20	44	
20	120	36	16	16	20	44	110	
25		48	24	20	40	72		
30		72	36	40	72			
35		110	52	72				
40			88	88				
45			130					
50								
55								

From our simulation study shown in the above table, we can understand that, for highly variable drugs (CV>=30), the appropriate GMR value is (0.95, 1.05), which is also very important for low variable drugs to achieve the minimum sample size required to conduct a clinical trials. For GMR values less than 0.95 and more than 1.05, we need maximum number of subjects even for low variable drugs. Finally from our simulation result given in the appendix, we observe that the when the sample size increases, the proportion of $(1-2\alpha)x100\%$ Confidence interval contained in $0.8,1.25$ is highly increased, even for large values of CV and any values of GMR, but the value of the power, which is very important to detect meaningful clinical difference is decreased. As a result, based on the power approach, demonstrating BE and determining the corresponding sample size for highly variable drugs and for GMR values out of the range $0.95,1.05$, is very difficult. As the intrasubject coefficient of variation CV increases, the power decreases and larger sample sizes are needed to achieve a given power. Table 6.10 demonstrates the influence of the intra-subject/within-subject coefficient of variation CV, where the sample sizes necessary to attain a power of at least 80% are given. As conclusion, the appropriate GMR to conduct BE study is $0.95,1.05$

REFERENCES

Altman, D. G. (1982). Statistics in Medical Journals. *Statistics in Medicine*, 1, 59-71.

Anderson, S. Hauck, W.W. (1983). A New Procedure for Testing Equivalence in Comparative Bioavailability and Other Clinical Trials. *Communications in Statistics*, 12, 2663–2692.

Anonymous,(1994).*Sağlık Bakanlığından Farmasötik Müstehzarların Biyoyararlanım ve Biyoeşdeğerliliğinin Değerlendirilmesi Hakkında Yönetmelik*, Sağlık Bakanlığı, Türkiye.

Anonymous, (2001a). *Food and Drug Administration (FDA), Guidance for Industry: Statistical Approaches to Establishing Bioequivalence*, USA.

Anonymous, (2001b). *The European Agency for the Evaluation of Medicinal Products* (EAEM), Europe Union.

Berger, R. L. & Hsu, J. C. (1996). Bioequivalence Trials, Intersection-Union Tests and Equivalence Confidence Tests. *Statistical Science* 11(4): 283-319.

Bonate, P.L. & Howard, D, R. (2011).*Pharmacokinetics in Drug Development* (3rd ed.). Springer, Heidelberg Dordrecht London.

Brown, B. (1980). The Crossover Experiment for Clinical Trials: *Biometrics*, 36, 69-79

Carriere, K.C. & Huang, R., (2000). Crossover Designs for Two-Treatment Clinical Trials. *Journal of Statistical Planning and Inference* 87, 125-134.

Chow, S.C. (2007). *Adaptive Design Methods in Clinical Trials*. Chapman & Hall/CRC: Boca Raton.

Chow, S.C. & Liu, J.P. (2009). *Design and Analysis of Bioavailability and Bioequivalence Studies* (3rd ed.). Chapman & Hall/CRC: Boca Raton.

Chow, S.C., Shao, J., & Wang, H. (2003). *Sample Size Calculation in Clinical Research* (2nd Ed.). Marcel Dekker; New York.

Deletti, E., Hauschke, D., & Steinjans, V.W. (1991). Sample Size Determination for Bioequivalence Assessment by Means of Confidence Intervals. *Int. J.Clin. Pharm. Ther. Toxicol, 29(1),* 1-8.

Gould, A.L. (1995). Group Sequential Extensions of a Standard Bioequivalence Testing Procedure. *J. of Pharmacokinetics and Biopharmaceutics*, 23, 57-86.

Grizzle, J.E. (1965). The Two-period Cross-over Design and Its Use in Clinical Trials: *Biometrics*, 21, 467-480.

Hauck, W.W., Preston, P.E., & Bois, F.Y. (1997). A Group Sequential Approach to Crossover Trials for Average Bioequivalence. *Journal of Biopharmaceutical Statistics*, 7, 87-96.

Hauschke, D., Steinijans, V.W., Diletti, E. & Burke, M. (1992). Sample Size Determination for Bioequivalence Assessment Using a Multiplicative Model. *Journal of Pharmacokinetics and Biopharmaceutics*, 20, 557–561.

Hinkelmann, K. & Kempthorne, O. (1994). *Design and Analysis of Experiments: Introduction to Experimental Design* (1st Ed.). Canada:

Hsu, J. C. Hwang, J. T. G., Liu, H.K. & Ruberg, S. J.(1994). Confidence Intervals Associated With Tests for Bioequivalence, *Biometrika*, 81, 103-114.

Jones, B. (2006). *Bioequivalence and Statistics in Clinical Pharmacology*. Chapman & Hall/CRC: Boca Raton.

Jones, B., Kenward, M.G. (2003). *Design and Analysis of Cross-over Trials*, (2nd Ed.). London: Chapman and Hall/CRC.

Julious, S. A. (2010). *Sample Sizes for Clinical Trials*. Chapman & Hall/CRC, Boca Raton.

Karian, Z. A. & Dudewicz, E.J. (2010). *Hand Book of Fitting Statistical Distributions with R* (3rd ed.). Chapman & Hall/CRC: Boca Raton.

Lenth, R.V. (2001). Some Practical Guidelines for Effective Sample Size Determination. *The American Statistician*, 55,187–193.

Lindley, D.V. (1998). Decision Analysis and Bioequivalence Trials. *Statistical Science*, 13,136 –141.

Liu, H.K. (1990).Confidence intervals in bioequivalence assessment, *American Statistical Association, Proceedings of the Biopharmaceutical* Section, 51-54.

Liu, J. P. & Weng, C.S. (1993). Evaluation of parametric and nonparametric two one-sided tests procedures for assessing bioequivalence of average bioavailability. *Journal of Biopharmaceutical Statistics*, 3, 85-102.

Locke, C. S. (1984). An Exact Confidence Interval from Untransformed Data for the Ratio of Two Formulations Means. *Journal of Pharmacokinetics and Biopharmaceutics*, 12, 649-655.

O'Brien, P.C., & Fleming, T.R (1979). A Multiple Testing Procedure for Clinical Trials. *Biometrika*, 35, 549-556

Patterson, S., & Jones, B. (2006). *Bioequivalence and Statistics in Clinical Pharmacology*. Chapman & Hall/CRC: Boca Raton.

Phillips, K.F. (1990). Power of the Two-One Sided Tests Procedure in Bioequivalence study. *J.Pharmacokin.Biopharm.* 18, 137-144.

Pocock SJ.(1977). Group sequential methods in the design and analysis of clinical trials. *Biometrika*, 64,191–199. Kkkd

Potvin, D., Diliberti, C.E., Hauck, W.W., Parr, A.F., Schuirmann, D.J. & Smith, R.A. (2008). Sequential Design Approach for Bioequivalence Studies with Crossover Designs. *Pharmaceutical Statistics*, 7, 245-262.

Schuirmann, D.J.A. (1987).Comparison of the Two One-Sided Tests Procedure and the Power Approach for Assessing the Equivalence of Average Bioavailability. Journal of *Pharmacokinetics and Biopharmaceutics*, 15,657–680.

Senn, S. (2002). *Crossover Trials İn Clinical Research (2nd Ed.).* Charater: John Wiley & Sons.

Turnbull, B.C., & Jennison, C. (2000). *Group Sequential Methods with Applications to Clinical Trials*. Chapman & Hall/CRC: Boca Raton.

Vuorinen, J. & Tuominen, J. (1994). Fiellers Confidence Intervals for the Ratio of Two Means in the Assessment of Average Bioequivalence from Crossover Data, *Statistics in Medicine*, 13, 2531-2545.

APPENDIX

The whole values of the simualtion results.

Proportions of Confidence interval values for all mean ratios

Sample Size	CV%	85	90	95	100	105	110	115	120
12	10	0.337132	0.79513	**0.980211**	**0.999023**	**0.989675**	**0.907737**	0.63172	0.25143
	15	0.288505	0.6942	**0.939016**	**0.979359**	**0.917381**	0.733534	0.44006	0.17596
	20	0.237764	0.54664	0.791602	0.828852	0.697624	0.484785	0.27145	0.11821
	25	0.199152	0.42707	0.61646	0.648073	0.534291	0.360519	0.2043	0.09399
	30	0.137941	0.27285	0.390426	0.422639	0.360349	0.251608	0.14867	0.07541
	35	0.078729	0.14673	0.212633	0.241876	0.222583	0.169149	0.10955	0.06157
	40	0.042952	0.07755	0.113579	0.13575	0.135424	0.114181	0.08233	0.05159
	45	0.021422	0.04464	0.066492	0.082956	0.088025	0.080853	0.06376	0.04425
	50	0.01082	0.02594	0.038625	0.041	0.05641	0.056074	0.04961	0.03728
	55	0.00600	0.02592	0.02505	0.033337	0.039236	0.041613	0.0393	0.03191
16	10	0.41769	0.8981	**0.99694**	**0.99998**	**0.99856**	**0.98548**	0.75036	0.31395
	15	0.36152	0.81775	**0.98402**	**0.99695**	**0.97047**	0.78491	0.53519	0.20858
	20	0.30562	0.68579	**0.91906**	**0.92776**	0.80637	0.76024	0.31864	0.12871
	25	0.2742	0.58973	0.80135	0.79429	0.64332	0.42879	0.22734	0.09728
	30	0.21809	0.4383	0.59088	0.58388	0.45522	0.29414	0.15718	0.07283
	35	0.14612	0.2694	0.36656	0.37499	0.30136	0.20103	0.11394	0.05702
	40	0.0854	0.15439	0.21419	0.23131	0.20025	0.14671	0.08906	0.04755
	45	0.05216	0.09094	0.13048	0.14976	0.13933	0.11049	0.07379	0.04326
	50	0.86303	0.005101	0.07708	0.09324	0.09584	0.0839	0.06104	0.03988
	55	0.8619	0.031182	0.04841	0.06345	0.06822	0.06504	0.05162	0.03794
20	10	0.48932	**0.94869**	0**.99941**	**1**	**0.99989**	**0.98936**	0.83487	0.36118
	15	0.4264	0.89318	**0.99589**	**0.99954**	**0.99058**	**0.90878**	0.61905	0.23328
	20	0. 36462	0.78657	**0.96881**	**0.96905**	0.87563	0.66227	0.36592	0.13448
	25	0.33611	0.70863	0**.90035**	0.89976	0.72089	0.49117	0.25469	0.09662
	30	0.29348	0.57603	0.72917	0.68611	0.52256	0.32973	0.16597	0.06785
	35	0.22	0.40157	0.50774	0.47456	0.35296	0.217	0.11454	0.05047
	40	0.14312	0.25137	0.32398	0.3138	0.2433	0.15578	0.0879	0.04143
	45	0.09126	0.15967	0.21163	0.22037	0.17851	0.12344	0.07364	0.03781
	50	0.03494	0.09522	0.1323	0.14676	0.13243	0.09899	0.06445	0.03754
	55	0.07877	0.06123	0.08918	0.10361	0.10069	0.08297	0.06008	0.03763

Corresponding power values for all values of mean ratio.

Sample Size	CV%	85	90	95	100	105	110	115	120
12	10	0.337132	0.56249	***0.980211***	***0.999023***	***0.989675***	***0.907737***	0.63172	0.25143
	15	0.009471	0.26495	***0.980722***	***0.998079***	***0.992461***	***0.844657***	0.22483	0.00154
	20	0.002156	0.06563	***0.841966***	***0.950614***	***0.888476***	0.492769	0.05747	0.00015
	25	0.000307	0.002389	0.436652	0.633913	0.488147	0.140131	0.00774	1e-05
	30	9e-05	0.00703	0.22278	0.375481	0.25279	0.052589	0.00208	1e-05
	35	8e-05	0.00221	0.089261	0.171257	0.102133	0.015858	0.00073	0
	40	5e-06	0.00227	0.033896	0.071018	0.038863	0.004897	0.00015	0
	45	1e-06	0.00042	0.014677	0.032707	0.016765	0.001948	4e-05	0
	50	0	0.00037	0.004971	0.012	0.005353	6e-04	0	0
	55	0	0.00032	0.003308	0.007886	0.003838	0.000428	0	0
16	10	0.01318	0.85141	***0.99694***	***1***	***0.99998***	***0.98546***	0.44059	0.00136
	15	0.0022	0.50486	***0.98402***	***0.99794***	***0.9912***	0.78368	0.11077	1e-05
	20	0.00014	0.12521	***0.91906***	***0.8791***	0.76352	0.27329	0.01046	0
	25	2e-05	0.03821	0.40993	0.62199	0.64567	0.09368	0.00196	0
	30	0	0.00875	0.16369	0.30804	0.18558	0.02369	0.00026	0
	35	0	0.00187	0.05552	0.12366	0.06451	0.00536	7e-05	0
	40	0	0.00061	0.02019	0.0513	0.02428	0.00165	2e-05	0
	45	0	0.00026	0.0097	0.02574	0.01151	0.00073	1e-05	0
	50	0	0.00012	0.00489	0.01346	0.00611	0.00028	1e-05	0
	55	0	6e-05	0.00291	0.00897	0.00393	0.00016	0	0
20	10	0.02289	***0.97568***	***1***	***1***	***1***	***0.99971***	0.70234	0.000134
	15	0.00289	0.75710	***0.99931***	***0.99999***	***0.99972***	***0.95005***	0.21705	3e-05
	20	0.00013	0.23392	***0.90237***	***0.97442***	***0.92576***	0.46772	0.01658	0
	25	1e-05	0.07019	0.63045	***0.82361***	0.67898	0.1737	0.00024	0
	30	0	0.01374	0.28196	0.48554	0.31975	0.03858	0	0
	35	0	0.00267	0.09389	0.20612	0.10731	0.0078	0	0
	40	0	0.00063	0.03211	0.08147	0.03708	0.00191	0	0
	45	0	0.00023	0.01402	0.03909	0.01611	0.00065	0	0
	50	0	0.00013	0.00657	0.01901	0.00029	0	0	0
	55	0	8e-05	0.00387	0.01143	0.00396	0.00021	0	0

24	10	0.55189	**0.97516**	0.**999936**	**10.999991**	**0.996757**	0.89393	0.41241	0.04385	***0.99852***	***1***	***1***	***1***	***0.999999***	***0.89465***	0.00242	
	15	0.48627	**0.93734**	**0.999045**	**0.999953**	**0.997017**	**0.948593**	0.69014	0.25995	0.0451	***0.92375***	***0.999979***	***1***	***0.999995***	***0.993572***	0.37952	4e-05
	20	0.4216	0.83383	**0.988332**	**0.986356**	**0.922052**	0.726902	0.40889	0.14127	8e-05	0.39902	***0.975339***	***0.995362***	***0.982824***	0.678704	0.03043	0
	25	0.3927	0.79003	**0.951962**	**0.9362**	0.781209	0.540547	0.27745	0.09836	0	0.12569	***0.808073***	***0.995363***	***0.840534***	0.295894	0.00387	0
	30	0.35945	0.6838	0.826074	0.754612	0.57049	0.35641	0.17514	0.06565	0	0.02316	0.432194	0.653015	0.473073	0.06907	0.00028	0
	35	0.29602	0.51933	0.616631	0.544549	0.387763	0.231894	0.11528	0.04655	0	0.00367	0.154681	0.313356	0.176845	0.01234	3e-05	0
	40	0.21059	0.35519	0.424296	0.380494	0.272415	0.163781	0.08439	0.03684	0	0.00073	0.051792	0.130473	0.060602	0.00762	0	0
	45	0.14332	0.24128	0.297172	0.278279	0.207903	0.130415	0.07056	0.03262	0	0.00019	0.020824	0.060623	0.024726	0.002512	0	0
	50	0.08877	0.15249	0.20125	0.199643	0.160872	0.108572	0.06314	0.03212	0	7e-05	0.028617	0.010787	0.000825	0.000293	0	0
	55	0.05838	0.10283	0.18561	0.14968	0.130716	0.096126	0.05981	0.03375	0	3e-05	0.016423	0.005839	0.000293	0.000146	0	0
28	10	0.60985	0.98842	**0.99999**	**1**	**1**	**0.999**	**0.93151**	0.45924	0.08418	***0.99996***	***1***	***1***	***1***	***1***	***0.97824***	0.00408
	15	0.54074	**0.96535**	**0.99977**	**1**	**0.99919**	**0.97061**	0.74738	0.14885	0.00784	***0.98559***	***1***	***1***	***1***	***0.99967***	0.58388	3e-05
	20	0.47253	**0.90387**	**0.9957**	**0.99417**	**0.95226**	0.77964	0.44886	0.10047	0.00018	0.59061	***0.99456***	***0.99913***	***0.99656***	***0.84323***	0.05511	0
	25	0.44435	0.85175	**0.97732**	**0.95192**	0.8276	0.59037	0.30202	0.06395	1e-05	0.21803	***0.91387***	***0.97445***	***0.92995***	0.45261	0.00625	0
	30	0.41656	0.76628	0.88644	0.80508	0.61639	0.38623	0.18315	0.04269	0	0.04015	0.58908	0.78471	0.62521	0.11671	0.00035	0
	35	0.36386	0.61905	0.70046	0.59714	0.41522	0.23998	0.11503	0.03298	0	0.00567	0.23487	0.4354	0.26392	0.01964	0	0
	40	0.27993	0.45374	0.50988	0.42847	0.28965	0.16526	0.0811	0.0288	0	0.00074	0.08049	0.19403	0.09431	0.00381	0	0
	45	0.20419	0.32657	0.37342	0.3202	0.22341	0.13136	0.06615	0.02765	0	0.00021	0.03176	0.09033	0.03782	0.00099	0	0
	50	0.13734	0.22176	0.26704	0.24289	0.17769	0.11019	0.05874	0.026	0	9e-05	0.01346	0.0427	0.015	0.00023	0	0
	55	0.09361	0.15735	0.19685	0.19134	0.15197	0.09917	0.05744	0	0	3e-05	0.00698	0.02349	0.00745	0.00015	0	0
32	10	0.66483	**0.99079**	1	1	1	**0.99958**	**0.95797**	0.50271	0.15689	***1***	***1***	***1***	***1***	***1***	***0.99724***	0.00742
	15	0.59433	**0.98111**	**0.99992**	**1**	**0.99976**	**0.98455**	0.79775	0.30845	0.01451	***0.99817***	***1***	***1***	***1***	***1***	0.77113	8e-05
	20	0.52356	**0.93668**	**0.99839**	**0.99742**	**0.97011**	0.82531	0.48643	0.15712	0.00018	0.76157	***0.99878***	***0.9997***	***0.99913***	***0.93714***	0.10002	0
	25	0.49457	**0.89626**	**0.98887**	**0.96863**	0.868	0.63343	0.32637	0.10214	1e-05	0.33786	***0.963***	***0.98898***	***0.97078***	0.61272	0.01109	0
	30	0.47068	0.82858	**0.92288**	0.84339	0.65626	0.41319	0.19394	0.06267	0	0.06656	0.71805	***0.87095***	0.74882	0.18586	0.00062	0
	35	0.42738	0.70286	0.75771	0.64193	0.44069	0.25428	0.11622	0.04007	0	0.00852	0.33091	0.55203	0.36734	0.03269	2e-05	0
	40	0.35056	0.54126	0.57389	0.63822	0.30619	0.16928	0.0797	0.02903	0	0.00122	0.12074	0.55089	0.13811	0.00552	1e-05	0
	45	0.26944	0.40796	0.43804	0.63406	0.23381	0.13121	0.06293	0.02539	0	0.00033	0.04781	0.55076	0.05629	0.00139	1e-05	0
	50	0.19319	0.29246	0.32334	0.62015	0.18839	0.10847	0.05541	0.02419	0	6e-05	0.01907	0.53210	0.02242	3e-04	0	0
	55	0.13765	0.21473	0.24869	0.55401	0.16482	0.1019	0.05291	0.02561	0	4e-05	0.00905	0.25664	0.01174	0.00017	0	0
36	10	0.707958	**0.99739**	**1**	**1**	**1**	**0.999903**	**0.97347**	0.54625	0.263753	***1***	***1***	***1***	***1***	***1***	***0.99977***	0.01315
	15	0.637613	0.9892	**0.999989**	**1**	**0.99987**	**0.991753**	0.83675	0.33564	0.027538	***0.99983***	***1***	***1***	***1***	***0.999997***	***0.90215***	7e-05
	20	0.567312	**0.95776**	**0.999401**	**0.998902**	**0.98212**	0.862019	0.52543	0.16528	0.000346	***0.88541***	***0.999578***	***0.999873***	***0.99974***	***0.978222***	0.17142	0
	25	0.538578	**0.92546**	**0.994441**	**0.98028**	0.89835	0.672854	0.34554	0.10392	5e-06	0.48738	***0.984359***	***0.995091***	***0.98727***	0.7531	0.02005	0
	30	0.47068	0.87163	**0.92288**	0.84339	0.65626	0.41319	0.19394	0.06267	0	0.11288	0.71805	***0.87095***	0.74882	0.18586	0.00062	0
	35	0.484338	0.76703	0.806469	0.672334	0.47011	0.264885	0.11567	0.03816	0	0.01438	0.437992	0.656194	0.47434	0.052066	6e-05	0
	40	0.415692	0.61744	0.627598	0.493342	0.32254	0.17305	0.07555	0.02667	0	0.00181	0.173676	0.352129	0.19769	0.008747	3e-05	0
	45	0.335686	0.48727	0.491997	0.381844	0.24339	0.132191	0.05904	0.02242	0	0.00031	0.071183	0.182467	0.08216	0.002095	1e-05	0
	50	0.251044	0.36304	0.37816	0.300935	0.19714	0.109281	0.05231	0.02096	0	0.00011	0.027972	0.087753	0.03328	0.000542	0	0
	55	0.18772	0.27846	0.303106	0.253997	0.1746	0.101106	0.05085	0.02254	0	4e-05	0.013599	0.048259	0.01604	0.000185	0	0
40	10	0.7502	**0.99889**	**1**	**1**	**1**	**0.99999**	**0.9831**	0.5853	0.40993	**1**	***1***	***1***	***1***	***1***	***1***	0.02568
	15	0.68001	**0.99374**	**1**	**1**	**0.99999**	**0.99559**	0.871	0.35941	0.05043	**0.99997**	***1***	***1***	***1***	***1***	0.96782	0.00019
	20	0.60885	**0.97223**	**0.99981**	**0.999963**	**0.98979**	0.89247	0.56021	0.17381	6e-04	**0.95277**	***0.99975***	***0.99996***	***0.9999***	***0.99314***	0.27256	0

	25	0.58218	0.94783	**0.99708**	**0.98753**	**0.92236**	0.70952	0.36749	0.10601	3e-05	0.63027	***0.99242***	***0.99709***	***0.99415***	0.85496	0.0338	0
	30	0.56319	**0.90592**	**0.96373**	**0.89864**	0.73085	0.46213	0.20664	0.06013	0	0.17234	***0.88261***	***0.95293***	***0.90023***	0.3813	0.00138	0
	35	0.53679	0.81713	0.84177	0.70606	0.49548	0.27474	0.11504	0.03581	0	0.023	0.54087	0.74184	0.57542	0.07948	5e-05	0
	40	0.47443	0.68169	0.67062	0.52179	0.33523	0.17592	0.07237	0.02454	0	0.0027	0.23651	0.43972	0.26134	0.0135	0	0
	45	0.39885	0.55559	0.53298	0.40471	0.25282	0.13347	0.05634	0.02047	0	0.00063	0.10237	0.24016	0.11581	0.00315	0	0
	50	0.31195	0.43335	0.42505	0.32098	0.2029	0.10909	0.0485	0.01971	0	0.00013	0.03973	0.11961	0.04846	0.00071	0	0
	55	0.24151	0.34126	0.34935	0.27708	0.18024s	0.0995	0.04728	0.02083	0	4e-05	0.013599	0.048259	0.01604	0.000185	0	0
44	10	0.78418	**0.99963**	**1**	**1**	**1**	**1**	**0.98993**	0.61873	0.57511	**1**	***1***	***1***	***1***	***1***	***1***	0.04627
	15	0.71757	**0.99695**	**1**	**1**	**0.99999**	**0.99765**	**0.89553**	0.38101	0.08885	**1**	***1***	***1***	***1***	***1***	***1***	0.00041
	20	0.64745	**0.98285**	**0.99985**	**0.99972**	**0.99337**	**0.9154**	0.59192	0.17898	0.00094	**0.98214**	***0.99985***	***0.99995***	***0.99986***	***0.9977***	***0.99177***	0
	25	0.61992	**0.96472**	**0.99839**	**0.99145**	**0.93983**	0.7428	0.38382	0.10704	6e-05	0.7543	***0.99573***	***0.99851***	***0.99666***	***0.92011***	0.39584	0
	30	0.60197	**0.93289**	**0.97436**	**0.91658**	0.75802	0.48691	0.21141	0.05844	0	0.25204	***0.88261***	***0.95293***	***0.90023***	0.3813	0.00138	0
	35	0.58346	0.8625	0.86951	0.73192	0.51611	0.28338	0.11458	0.03269	0	0.03654	***0.92691***	***0.96919***	***0.93441***	0.49202	0.05804	0
	40	0.53248	0.73953	0.70781	0.54378	0.34601	0.17708	0.0708	0.02161	0	0.00427	0.63335	***0.80514***	0.66569	0.11644	0.00234	0
	45	0.46099	0.61458	0.57453	0.42133	0.25808	0.12946	0.05407	0.01763	0	0.00017	0.30523	0.52191	0.33689	0.02102	7e-05	0
	50	0.37049	0.49228	0.46133	0.33899	0.20482	0.10661	0.04548	0.01623	0	7e-059e-05	0.13856	0.30373	0.1565	0.00466	0	0
	55	0.29824	0.4007	0.3887	0.29401	0.18203	0.09847	0.04465	0.01733	0	5e-05	0.05724	0.15821	0.06626	0.00108	0	0
48	10	0.81394	0.99974	**1**	**1**	**1**	**1**	**0.99393**	0.65231	0.72788	**1**	***1***	***1***	***1***	***1***	***1***	0.08375
	15	0.75086	0.99839	**1**	**0.999909**	**0.999995**	**0.998821**	**0.92035**	0.40229	0.15107	**1**	***1***	***0.999976***	***0.999955***	***1***	***0.99836***	0.00054
	20	0.68181	0.98885	**1**	**0.994769**	**0.996215**	**0.933325**	0.62252	0.18774	0.00147	**0.99378**	***0.933396***	***0.99995***	***0.998855***	***0.997774***	0.53565	0
	25	0.65521	**0.97558**	**0.99922**	**0.994745**	**0.954724**	0.771552	0.40629	0.10901	3e-05	**0.85112**	***0.99737***	***0.998867***	***0.957303***	***0.957423***	0.09375	0
	30	0.64015	**0.94994**	**0.98189**	0.756989	0.78452	0.509412	0.22126	0.05731	0	0.34197	***0.95042***	***0.855929***	0.737758	0.600509	0.00437	0
	35	0.62628	0.89139	0.89213	0.713154	0.537016	0.295954	0.1179	0.03197	0	0.05562	0.71323	0.59957	0.410842	0.16571	7e-05	0
	40	0.58247	0.78258	0.74022	0.44018	0.35812	0.181556	0.072	0.02073	0	0.00719	0.38023	0.371708	0.204785	0.030633	0	0
	45	0.51594	0.66646	0.6077	0.352957	0.265712	0.131034	0.05353	0.01708	0	0.00133	0.18207	0.203751	0.09018	0.006763	0	0
	50	0.4295	0.54475	0.49535	0.308769	0.210612	0.104963	0.04439	0.01569	0	0.00023	0.07794	0.119491	0.045163	0.001442	0	0
	55	0.35178	0.45299	0.42219	0.308078	0.186781	0.096056	0.04329	0.01611	0	7e-05	0.03986	0.119175	0.000459	0	0	0
52	10	0.84245	**0.99992**	**1**	**1**	**1**	**1**	**0.9962**	0.68124	0.85501	***1***	***1***	***1***	***1***	***1***	***1***	0.14013
	15	0.78072	**0.99908**	**1**	**1**	**1**	**0.99935**	**0.93586**	0.42376	0.35904	***1***	***1***	***1***	***1***	***1***	***0.99995***	0.00196
	20	0.75201	**0.99243**	**1**	**1**	**1**	**0.9552**	0.59991	0.48576	0.35904	***0.99772***	***1***	***1***	***1***	***1***	***0.99995***	0.00196
	25	0.68838	**0.98196**	0**.99949**	**0.9965**	**0.96564**	0.79826	0.42313	0.11075	7e-05	***0.91286***	***0.99885***	***0.99954***	***0.99894***	***0.98694***	0.21732	0
	30	0.674	**0.96308**	**0.98713**	**0.94683**	0.81007	0.53209	0.22813	0.05566	0	0.44107	***0.96674***	***0.98544***	***0.97101***	0.69552	0.00717	0
	35	0.66327	**0.91713**	**0.91051**	0.78204	0.56045	0.30355	0.11748	0.02951	0	0.08335	***0.82485***	***0.91971***	***0.84216***	0.28917	0.00038	0
	40	0.62756	0.82138	0.76642	0.59107	0.37127	0.18379	0.06944	0.01861	0	0.01051	0.52509	0.72923	0.54908	0.06416	1e-05	0
	45	0.56649	0.71163	0.63783	0.46166	0.27198	0.13151	0.04951	0.01483	0	0.00177	0.28511	0.50868	0.31312	0.01466	0	0
	50	0.4295	0.59208	0.49535	0.308769	0.210612	0.104963	0.04439	0.01569	0	0.00034	0.07794	0.119491	0.045163	0.001442	0	0
	55	0.40632	0.50197	0.45163	0.32348	0.19123	0.09508	0.04025	0.01389	0	0.00011	0.06782	0.19304	0.07981	0.00114	0	0
56	10	0.86664	0.99997	**1**	**1**	**1**	**1**	**0.99764**	0.71291	0.93311	***1***	1	1	1	1	1	0.22305
	15	0.80901	**0.99952**	**1**	**1**	**1**	**0.99981**	**0.94826**	0.44852	0.24004	***1***	***1***	***1***	***1***	***1***	***0.9997***	0.00105
	20	0.74565	**0.9953**	0**.99999**	**0.99999**	**0.99869**	**0.96083**	0.67904	0.20105	0.00283	***0.99919***	***0.99997***	***1***	***0.99998***	***0.99955***	0.66895	0
	25	0.72062	0.98847	**0.99966**	**0.9978**	**0.97339**	0.8243	0.44498	0.11319	6e-05	***0.95028***	***0.99842***	***0.99914***	***0.99856***	***0.97705***	0.14688	0
	30	0.70593	0.97385	**0.99091**	**0.95667**	0.82704	0.55571	0.23602	0.05658	0	0.54069	***0.96674***	***0.98544***	***0.97101***	0.69552	0.00717	0
	35	0.69778	**0.93702**	**0.92444**	0.8012	0.57638	0.31772	0.11839	0.02919	0	0.11708	0.77683	***0.89212***	0.79793	0.22134	0.00024	0

	40	0.67193	0.85202	**0.89091**	**0.89567**	0.82001	0.45317	0.15002	0.02018	0	0.01578	***0.595874***	***0.78444***	***0.75230***	0.24952	0	0
	45	0.66761	0.74875	0.78851	0.6076	0.37917	0.19159	0.0685	0.01746	0	0.00271	0.455	0.67028	0.48639	0.0446	0	0
	50	0.53177	0.63415	0.54967	0.8117	0.21712	0.10449	0.04035	0.01156	0	0.00042	0.10244	0.25431	0.11927	0.0022	0	0
	55	0.45748	0.54447	0.47911	0.33372	0.19049	0.09413	0.03828	0.01257	0	7e-05	0.05134	0.15514	0.06118.45748		0	0
60	10	0.88535	***0.99998***	**1**	**1**	**1**	**1**	**0.9989**	0.73834	***0.97437***	***1***	***1***	***1***	***1***	***1***	***1***	33486
	15	0.83143	**0.99982**	**1**	**1**	**1**	**0.99984**	**0.96207**	0.46747	0.48896	***1***	***1***	***1***	***1***	***1***	***1***	0.00372
	20	0.77228	**0.99724**	**1**	**1**	**0.99913**	**0.96868**	0.70541	0.20761	0.01003	***0.99961***	***0.99999***	***0.99999***	***0.99999***	***0.99986***	***0.87129***	0
	25	0.74671	**0.99208**	**0.99976**	**0.99858**	**0.98033**	0.84363	0.4656	0.11302	0.00013	***0.97246***	***0.99911***	***0.99971***	***0.99936***	***0.99227***	0.30297	0
	30	0.73592	**0.97862**	**0.99368**	**0.96475**	0.84919	0.57564	0.24502	0.05468	0	0.68574	***0.98371***	***0.99248***	***0.98535***	***0.83533***	0.01881	0
	35	0.7294	**0.95256**	**0.93463**	0.81918	0.59883	0.32549	0.1193	0.0264	0	0.15964	***0.86387***	***0.93951***	***0.88***	0.35806	0.00039	0
	40	0.70664	0.87888	0.80726	0.62482	0.39523	0.19042	0.06668	0.01533	0	0.02279	0.58799	0.77618	0.62238	0.08585	1e-05	0
	45	0.65823	0.78282	0.68261	0.48544	0.28658	0.13228	0.01731	0.01237	0	0.0037	0.3427	0.57143	0.37423	0.02028	0	0
	50	0.57793	0.67256	0.57083	0.39128	0.18957	0.10165	0.03803	0.01091	0	0.00012	0.16871	0.36389	0.18957	0.00442	0	0
	55	0.50386	0.58661	0.50235	0.34023	0.1952	0.09141	0.03692	0.01174	0	6e-05	0.09037	0.2349	0.10309	0.00144	0	0
64	10	***0.90375***	**0.99999**	**1**	**1**	**1**	**1**	**0.99927**	0.76108	***0.99228***	***1***	***1***	***1***	***1***	***1***	***1***	0.46317
	15	0.85843	**0.99991**	**1**	**1**	**1**	**0.999919**	**0.96896**	0.48696	0.62426	***1***	***1***	***1***	***1***	***1***	***1***	0.00707
	20	0.79849	**0.99825**	**1**	**0.999989**	**0.999531**	**0.976095**	0.72614	0.2124	0.01668	***0.99972***	***0.99999***	***0.999994***	***0.99998***	***0.999903***	***0.928***	0
	25	0.77526	0**.9944**	**0.99981**	**0.998927**	**0.98504**	0.861692	0.48141	0.11353	0.00028	***0.98421***	***0.99937***	***0.99967***	***0.999479***	***0.995055***	0.39943	0
	30	0.76504	0.98621	**0.9951**	**0.971512**	0.865827	0.595137	0.25215	0.05251	0	0.71755	***0.98744***	***0.994379***	***0.988988***	***0.87909***	0.02943	0
	35	0.7598	**0.96317**	**0.94516**	0.836332	0.617003	0.334812	0.11834	0.02439	0	0.20931	***0.89344***	***0.953177***	***0.906331***	0.430839	0.00055	0
	40	0.7409	**0.96317**	0.82713	0.642115	0.405925	0.193223	0.06675	0.01441	0	0.0331	0.65086	***0.819331***	0.678637	0.114226	2e-05	0
	45	0.69559	**0.89974**	0.70354	0.501982	0.292823	0.132766	0.0453	0.01065	0	0.00596	0.40162	0.630569	0.433816	028432	0	0
	50	0.62274	0.81017	0.58984	0.422918	0.224565	0.101636	0.0453	0.00919	0	0.00097	0.20628	0.422918	0.232744	0.006234	0	0
	55	0.55057	0.62613	0.52096	0.197278	0.091386	0.03557	0.01061	00.11276	0	0.00024	0.283201	0.131007	0.18012	0.001834	0	0
68	10	***0.91713***	***1***	**1**	**1**	**1**	**1**	**0.99968**	0.78462	***0.998***	***1***	***1***	***1***	***1***	***1***	***1***	***0.59635***
	15	0.87052	**0.99986**	**1**	**1**	**1**	**0.99997**	**0.97615**	0.5062	0.74827	***1***	***1***	***1***	***1***	***1***	***1***	0.01174
	20	0.81514	**0.99886**	**0.99999**	**0.99998**	**0.99974**	**0.9812**	0.74741	0.21959	0.02887	***0.99988***	***0.99997***	***0.99999***	***1***	***0.99992***	***0.96207***	1e-05
	25	0.79244	0.99713	**0.99987**	**0.99921**	**0.98894**	0.878	0.4964	0.11605	0.00057	***0.99909***	***0.99948***	***0.99971***	***0.99963***	***0.99664***	0.50136	0
	30	0.78173	0.99023	**0.99631**	**0.97715**	0.88155	0.61497	0.25638	0.05181	0	0.78399	***0.99098***	***0.99577***	***0.99189***	***0.91104***	0.04435	0
	35	0.77959	**0.9712**	**0.95425**	0.85127	0.63548	0.34576	0.11937	0.02385	0	0.26321	***0.91853***	***0.96505***	***0.92831***	0.50397	0.00094	0
	40	0.76374	**0.91556**	0.84108	0.65923	0.41853	0.19723	0.06449	0.01365	0	0.04636	0.70185	***0.85628***	0.72946	0.1491	2e-05	0
	45	0.72383	0.83435	0.72085	0.51562	0.2998	0.13291	0.04367	0.0103	0	0.0082	0.46186	0.68449	0.49371	0.03715	0	0
	50	0.65532	0.73614	0.61044	0.41031	0.22839	0.09892	0.03404	0.0088	0	0.00124	0.25092	0.48184	0.28081	0.00821	0	0
	55	0.58642	0.65803	0.54025	0.33884	0.20001	0.09004	0.03202	0.00988	0	0.00043	0.14268	0.33368	0.16291	0.0022	0	0
72	10	0**.93084**	**1**	**1**	**1**	**1**	**1**	**0.99976**	**0.80158**	***0.99956***	***1***	***1***	***1***	***1***	***1***	***1***	0.72502
	15	**0.88971**	**0.99999**	**1**	**1**	**1**	**0.99997**	**0.98089**	**0.5236**	***0.84485***	***1***	***1***	***1***	***1***	***1***	***1***	0.02008
	20	0.83845	**0.99939**	**1**	**0.99999**	**0.99986**	**0.98584**	0.76696	0.22508	0.04714	***0.99992***	***1***	***1***	***0.99999***	***0.99995***	***0.98183***	0
	25	0.81704	**0.99764**	**0.99995**	**0.99942**	**0.99144**	**0.89339**	0.5176	0.1176	0.011	***0.99366***	***0.99962***	***0.9998***	***0.99966***	***0.99753***	0.60135	0
	30	0.80765	**0.99316**	**0.99724**	**0.98107**	**0.89472**	0.63218	0.26519	0.05211	0	***0.83848***	***0.99962***	***0.99662***	***0.99393***	***0.93448***	0.06283	0
	35	0.80717	**0.97878**	**0.96273**	**0.90053**	0.65111	0.35534	0.12121	0.02293	0	0.32899	***0.93691***	***0.97307***	***0.94341***	0.56968	0.00169	0
	40	0.79397	**0.93082**	0.85804	0.86744	0.42579	0.19988	0.0635	0.01324	0	0.06319	0.75266	***0.88454***	0.77456	0.18625	5e-05	0
	45	0.75869	0.85452	0.74043	0.5297	0.30368	0.13306	0.04235	0.00969	0	0.01209	0.51886	0.73453	0.55078	0.05015	0	0

	50	0.69427	0.76183	0.62757	0.4205	0.22985	0.10138	0.03316	0.00829	0	0.00204	0.29943	0.53781	0.32779	0.01221	0	0
	55	0.62772	0.68752	0.55998	0.37068	0.19937	0.08999	0.03164	0.00878	0	0.00057	0.17478	0.38975	0.1961	0.0034	0	0
76	10	**0.941389**	**1**	**1**	**1**	**1**	**1**	**0.99986**	**0.82315**	***0.999929***	***1***	***1***	***1***	***1***	***1***	***1***	***0.82744***
	15	**0.903109**	**0.99999**	**1**	**1**	**1**	**0.999992**	**0.98551**	0.54568	***0.916921***	***1***	***1***	***1***	***1***	***1***	***1***	0.3598
	20	0.854882	**0.99963**	**1**	**0.999997**	**0.99992**	**0.989405**	0.78574	0.23338	0.075659	***0.99992***	***1***	***0.999997***	***0.99999***	***0.999964***	***99109***	1e-05
	25	0.835149	**0.99828**	**0.99997**	**0.999673**	**0.99316**	**0.905642**	0.53175	0.11977	0.001655	***0.99571***	***0.99978***	***0.99988***	***0.99973***	***0.99809***	0.69436	0
	30	0.82639	**0.99502**	**0.99771**	**0.984813**	0.51765	0.649329	0.27067	0.05114	4e-06	***0.87737***	***0.99416***	***0.997578***	***0.951197***	0.09218	0	0
	35	0.826015	**0.98461**	**0.96851**	**0.90809**	0.3558	0.363995	0.1207	0.02226	0	0.39164	***0.9502***	***0.97943***	0.632465	0.00247	0	0
	40	0.816043	**0.94239**	0.87328	0.66699	0.24639	0.202272	0.06178	0.01204	0	0.085	0.79589	***0.907369***	0.228611	5e-05	0	0
	45	0.784115	0.87488	0.75846	0.54475	0.1822	0.133663	0.0412	0.00834	0	0.01721	0.57749	0.777873	0.067375	0	0	0
	50	0.724977	0.78619	0.64498	0.433911	0.13146	0.09986	0.03181	0.00727	0	0.0027	0.34817	0.39358	0.016032	0	0	0
	55	0.660672	0.71415	0.57587	0.377881	0.10176	0.088159	0.03054	0.0781	0	7e-04	0.20985	0.440828	0.00488	0	0	0
80	10	***0.95039***	***1***	**1**	**1**	**1**	**1**	**0.999**	**0.83967**	***0.99998***	***1***	***1***	***1***	***1***	***1***	***0.90387***	***0.88421***
	15	**0.91744**	**0.99999**	**1**	**1**	**1**	**1**	**0.98922**	**0.56073**	***0.95841***	***1***	***1***	***1***	***1***	***1***	0.05866	0.58411
	20	0.8726	**0.99969**	**1**	**0.99999**	**0.99998**	**0.99994**	0.80543	0.24064	0.11608	***0.9999***	***0.99998***	***0.99999***	***0.99993***	***0.99597***	0	0.0541
	25	0.8531	**0.99881**	**0.99998**	**0.99967**	**0.99225**	**0.99507**	0.55231	0.1205	0.00277	***0.99658***	***0.99982***	***0.99989***	***0.99863***	0.99756	0	0.00021
	30	0.84322	**0.99655**	**0.99841**	**0.98812**	**0.91784**	**0.918**	0.27974	0.05045	0	***0.90611***	***0.99569***	***0.99817***	***0.96318***	0.1292	0	0
	35	0.84477	**0.98822**	**0.9724**	**0.89027**	0.66662	0.68535	0.12378	0.02124	0	0.45751	***0.95941***	***0.98371***	0.68661	0.00388	0	0
	40	0.83742	**0.95328**	0.88225	0.71084	0.37353	0.45414	0.06162	0.01082	0	0.10651	***0.82844***	***0.9271***	0.27626	2e-05	0	0
	45	0.80922	0.89117	0.77147	0.55685	0.20602	0.31824	0.04035	0.00815	0	0.02247	0.62836	***0.81577***	0.0863	0	0	0
	50	0.75731	0.80768	0.66102	0.44383	0.13345	0.23947	0.0306	0.0654	0	0.0039	0.3998	0.64436	0.02143	0	0	0
	55	0.69512	0.73874	0.59119	0.38581	0.08735	0.20847	0.02779	0.0727	0	0.00104	0.25085	0.4934	0.00679	0	0	0
84	10	***0.95934***	**1**	**1**	**1**	**1**	**1**	**0.99995**	**0.85311**	***1***	***1***	***1***	***1***	***1***	***1***	***1***	***0.95258***
	15	**0.92984**	**1**	**1**	**1**	**1**	**0.99999**	**0.9921**	**0.58099**	***0.98314***	***1***	***1***	***1***	***1***	***1***	***1***	0.09131
	20	0.88763	**0.9998**	**1**	**1**	**0.99996**	**0.99345**	0.82143	0.24799	0.16812	***0.99995***	***1***	***1***	***1***	***0.99998***	***0.99787***	0
	25	0.86993	**0.99918**	**1**	**0.99985**	**0.99594**	**0.92749**	0.56419	0.12236	0.00428	***0.99776***	***0.99988***	***0.99996***	***0.99991***	***0.99885***	***0.84015***	0
	30	0.861	**0.99741**	**0.99898**	**0.99002**	**0.92735**	0.67984	0.28289	0.05022	0	***0.93***	**0.99676**	**0.99842**	**0.9969**	**0.97165**	0.17356	0
	35	0.86292	**0.99048**	**0.9766**	**0.90203**	0.69925	0.38069	0.12301	0.02052	0	0.52222	***0.96941***	***0.98753***	***0.97227***	0.7365	0.00627	0
	40	0.86292	**0.96099**	**0.9766**	**0.90203**	0.69925	0.38069	0.12301	0.02052	0	0.13999	***0.85627***	***0.94149***	***0.87351***	0.32377	9e-05	0
	45	0.83207	**0.90359**	0.78596	0.56942	0.32469	0.13369	0.03927	0.00705	0	0.03019	0.67755	***0.84606***	0.70457	0.10876	1e-05	0
	50	0.7859	0.82388	0.67461	0.45441	0.24293	0.09852	0.02818	0.00624	0	0.00505	0.45135	0.69164	0.48467	0.02886	0	0
	55	0.72551	0.75979	0.607	0.3962	0.20901	0.08439	0.0268	0.00656	0	0.00134	0.29441	0.54787	0.32382	0.00919	0	0
88	10	**0.96535**	**1**	**1**	**1**	**1**	**1**	**0.99998**	0.86857	***1***	***1***	***1***	***1***	***1***	***1***	***1***	***0.9789***
	15	**0.93664**	**1**	**1**	**1**	**1**	**1**	**0.99338**	0.59315	***0.99378***	***1***	***1***	***1***	***1***	***1***	***1***	***0.13935***
	20	**0.89633**	**0.99989**	**1**	**1**	**0.99998**	**0.99549**	0.83674	0.25154	0.2362	***0.99996***	***1***	***1***	***1***	***0.99999***	***0.99883***	0
	25	0.87979	**0.99958**	**0.99999**	**0.99984**	**0.99703**	**0.93623**	0.58158	0.12329	0.00799	***0.99832***	***0.99995***	***0.99992***	***0.99985***	***0.99925***	***0.88684***	0
	30	0.87505	**0.99828**	**0.99897**	**0.99202**	**0.93471**	0.69424	0.29067	0.04985	2e-05	***0.94631***	***0.99898***	***0.99729***	***0.99773***	***0.97673***	0.22133	0
	35	0.87681	**0.99323**	**0.9811**	**0.91056**	0.7106	0.38709	0.12289	0.01874	0	0.58012	***0.99007***	***0.97634***	***0.97842***	0.77736	0.00774	0
	40	0.87275	**0.9674**	**0.905**	**0.90738**	0.4693	0.20974	0.05848	0.00913	0	0.1715	***0.95365***	***0.88406***	***0.89716***	0.37515	0.00017	0
	45	0.84779	**0.91698**	0.80056	0.58285	0.32891	0.13213	0.03614	0.00687	0	0.03928	***0.87244***	0.41904	0.74216	0.13287	1e-05	0
	50	0.80168	0.84113	0.6923	0.46545	0.24458	0.0977	0.02752	0.00564	0	0.00733	0.73741	0.50391	0.53161	0.03716	0	
	55	0.74655	0.77951	0.62094	0.40392	0.21091	0.0852	0.02625	0.00589	0	0.00169	0.59984	0.33797	0.37064	0.01157	0	0

92	10	**0.97135**	**1**	**1**	**1**	**1**	**1**	**0.99998**	**0.88101**	***1***	***1***	***1***	***1***	***1***	***1***	***1***	***0.99223***
	15	**0.9465**	**1**	**1**	**1**	**1**	**1**	**0.99511**	**0.61018**	***0.99765***	***1***	***1***	***1***	***1***	***1***	***1***	***0.20145***
	20	**0.91146**	**0.99996**	**1**	**1**	**1**	**0.99658**	0.84986	0.25766	0.31766	***0.99999***	***1***	***1***	***1***	***1***	***0.99939***	1e-05
	25	0.89622	**0.99973**	**0.99997**	**0.99992**	**0.99753**	**0.94524**	0.59385	0.12539	0.01259	***0.99855***	***0.99988***	***0.99996***	***0.99996***	***0.99936***	***0.92271***	0
	30	0.89017	**0.99899**	**0.99931**	**0.99351**	**0.94283**	0.71072	0.29442	0.04839	3e-05	***0.9579***	***0.99767***	***0.99915***	***0.9982***	***0.98171***	0.2815	0
	35	0.8914	**0.9949**	**0.98354**	**0.92048**	0.72386	0.40032	0.12294	0.01831	0	0.6351	***0.98115***	***0.99274***	***0.983***	***0.81475***	0.01244	0
	40	0.88784	**0.97279**	**0.91242**	0.74972	0.48245	0.21447	0.05818	0.00879	0	0.20736	***0.90405***	***0.96433***	***0.91626***	0.42389	0.00034	0
	45	0.89852	**0.92719**	0.81128	0.59661	0.33934	0.13646	0.03528	0.00598	0	0.05035	0.75943	***0.89782***	0.78284	0.16559	1e-05	0
	50	0.82618	**0.91718**	0.70362	0.47591	0.24966	0.098	02597	0.00523	0	0.01008	0.5507	0.77715	0.58156	0.04749	0	0
	55	0.77484	0.85674	0.63462	0.4128	0.21216	0.0858	0.02474	0.00555	0	0.00356	0.3855	0.64634	0.41545	0.01583	0	0
96	10	**0.977227**	**1**	**1**	**1**	**1**	**1**	**0.99999**	**0.89323**	***1***	***1***	***1***	***1***	***1***	***1***	***1***	***0.9975***
	15	**0.95461**	**1**	**1**	**1**	**1**	**1**	**0.99662**	**0.6254**	***0.99937***	***1***	***1***	***1***	***1***	***1***	***1***	***0.27908***
	20	**0.92163**	**0.99999**	**1**	**1**	**1**	**0.9972**7	0.86498	0.26373	0.4064	***0.99999***	***1***	***1***	***1***	***0.99996***	***0.99959***	5e-05
	25	**0.90698**	**0.99981**	**0.99997**	**0.99991**	**0.99823**	**0.95191**	0.61328	0.12672	0.02032	***0.99895***	***0.9999***	***0.99999***	***0.99996***	***0.99949***	***0.94786***	0
	30	**0.90108**	**0.99915**	**0.99949**	**0.99466**	**0.9505**	0.72231	0.30379	0.048	4e-05	***0.9677***	***0.9986***	***0.99928***	***0.99852***	***0.9858***	0.34796	0
	35	**0.90313**	**0.99582**	**0.98728**	**0.92733**	0.73953	0.40579	0.1259	0.01796	0	0.68845	***0.98529***	***0.99422***	***0.98648***	***0.84436***	0.01803	0
	40	**0.90104**	**0.97719**	**0.9214**	0.76203	0.48964	0.21416	0.05733	0.0087	0	0.24679	***0.92376***	***0.97182***	***0.93036***	0.47215	0.00041	0
	45	0.88428	**0.93548**	0.8257	0.60993	0.34055	0.13477	0.03522	0.00588	0	0.06491	0.79503	**0.9192**	**0.81637**	0.19296	0	0
	50	0.84667	0.86874	0.7168	0.48398	0.25385	0.0972	0.02761	0.00458	0	0.01249	0.59905	***0.81268***	0.6299	0.05915	0	0
	55	0.79591	0.81283	0.64706	0.42203	0.21833	0.0848	0.02413	0.0051	0	0.00337	0.43341	0.6948	0.4669	0.02002	0	0
100	10	**0.98**	**1**	**1**	**1**	**1**	**1**	**0.99999**	**0.90456**	***1***	***1***	***1***	***1***	***1***	***1***	***1***	***0.99923***
	15	**0.96023**	**1**	**1**	**1**	**1**	**1**	**0.99705**	0.642	***0.99979***	***1***	***1***	***1***	***1***	***1***	***1***	0.37285
	20	**0.92987**	**0.99999**	**1**	**0.999999**	**1**	**0.99785**	0.87481	0.2717	0.50172	***0.99999***	***1***	***0.999999***	***1***	***1***	***0.99969***	1e-04
	25	**0.91686**	**0.99987**	**0.9997**	**0.99994**	**0.99864**	**0.95775**	0.62337	0.1289	0.02948	***0.9991***	***0.99988***	***0.99998***	***0.99994***	***0.99956***	***0.96314***	0
	30	**0.91177**	**0.99939**	**0.9996**	**0.99574**	**0.95601**	0.73492	0.31109	0.04782	9e-05	***0.97473***	***0.99882***	***0.99954***	***0.99886***	***0.9887***	0.41238	0
	35	**0.91082**	**0.9972**	**0.98842**	**0.93533**	0.75365	0.41574	0.12666	0.01766	0	0.73332	***0.98811***	***0.995252***	***0.98957***	***0.87165***	0.02497	0
	40	**0.90861**	**0.98185**	**0.92843**	0.77518	0.50268	0.21702	0.05614	0.00789	0	0.28758	***0.93765***	***0.9784***	***0.9452***	0.52331	0.00054	0
	45	0.89422	**0.94335**	0.83421	0.61771	0.34982	0.13658	0.03315	0.005	0	0.08113	***0.82752***	***0.93408***	***0.84592***	0.22855	1e-05	0
	50	0.85799	0.88014	0.72667	0.494	0.25702	0.09708	0.02563	0.00449	0	0.01668	0.64425	***0.84536***	0.67139	0.07363	0	0
	55	0.81385	0.82457	0.6584	0.42795	0.21918	0.02543	0.02383	0.00459	0	0.00476	0.47879	0.7352	0.51502	0.002543	0	0
110	10	**0.98692**	**1**	**1**	**1**	**1**	**1**	**1**	**0.92623**	***1***	***1***	***1***	***1***	***1***	***1***	***1***	***0.99999***
	15	**0.9718**	**1**	**1**	**1**	**1**	**1**	**0.99841**	0.67541	***0.99999***	***1***	***1***	***1***	***1***	***1***	***1***	***0.62349***
	20	**0.94776**	**1**	**1**	**1**	**0.99997**	**0.99889**	**0.89908**	0.28757	0.73312	***0.99999***	***1***	***1***	***1***	***0.99999***	***0.99985***	0.0.00033
	15	**0.93658**	**0.99996**	**0.99999**	**1**	**0.9995**	**0.9704**	0.65587	0.134	0.08006	***0.99951***	***0.99996***	***1***	***0.99999***	***0.99976***	***0.98524***	0
	30	**0.93261**	**0.99967**	**0.99982**	**0.99721**	**0.96686**	0.7681	0.3255	0.04816	4e-04	***0.98503***	***0.99941***	***0.99979***	***0.99934***	***0.99299***	0.57451	0
	35	**0.93421**	**0.99827**	**0.9924**	**0.95035**	0.77931	0.43842	0.12775	0.01578	0	***0.82061***	***0.99355***	***0.99781***	***0.99444***	***0.91769***	0.05332	0
	40	**0.93328**	**0.98795**	**0.94524**	**0.80213**	0.52384	0.226	0.05422	0.00682	0	0.40369	***0.96404***	***0.98909***	***0.96826***	0.63356	0.00168	0
	45	0.92404	**0.95821**	0.85835	0.64668	0.36473	0.13903	0.03182	0.00434	0	0.13549	***0.8884***	***0.96336***	***0.9009***	0.32607	1e-05	0
	50	0.89519	**0.90554**	0.75631	0.51887	0.26597	0.09729	0.02256	0.00335	0	0.032231	0.74584	0.90402	0.77095	0.11967	0	0
	55	0.85763	0.85656	0.68821	0.45017	0.22364	0.08427	0.0204	0.00399	0	0.00824	0.59661	0.82452	0.6284	0.04657	0	0

120	10	**0.98692**	**1**	**1**	**1**	**1**	**1**	**1**	**0.92623**	***1***	***1***	***1***	***1***	***1***	***1***	***1***	***1***
	15	**0.9718**	**1**	**1**	**1**	**1**	**1**	**0.99841**	0.67541	***1***	***1***	***1***	***1***	***1***	***1***	***1***	***0.8349***
	20	**0.94776**	**1**	**1**	**1**	**0.99997**	**0.99889**	**0.89908**	0.28757	***0.88763***	***1***	***1***	***1***	***1***	***1***	***0.99992***	0.00133
	25	**0.93658**	**0.99996**	**0.99999**	**1**	**0.9995**	**0.9704**	0.65587	0.134	0.17437	***0.99969***	***0.99997***	***0.99999***	***1***	***0.99991***	***0.99313***	0
	30	**0.93261**	**0.99967**	**0.99982**	**0.99721**	**0.96686**	0.7681	0.3255	0.04816	0.00126	***0.9905***	***0.99957***	***0.99989***	***0.99956***	***0.99555***	0.71564	0
	35	**0.93421**	**0.99827**	**0.9924**	**0.95035**	0.77931	0.43842	0.12775	0.01578	0	***0.87882***	***0.99655***	***0.99873***	***0.99659***	***0.94746***	0.09856	0
	40	**0.93328**	**0.98795**	**0.94524**	**0.80213**	0.52384	0.226	0.05422	0.00682	0	0.51437	***0.97904***	***0.99415***	***0.98274***	0.73053	0.00316	0
	45	**0.92404**	**0.95821**	0.85835	0.64668	0.36473	0.13903	0.03182	0.00434	0	0.20538	***0.93169***	***0.97986***	***0.9399***	0.42967	0.00011	0
	50	0.**89519**	**0.90554**	0.75631	0.51887	0.26597	0.09729	0.02256	0.00335	0	0.05494	**0.82774**	**0.94182**	**0.84636**	0.18307	1e-05	0
	55	0.85763	0.85656	0.68821	0.45017	0.22364	0.08427	0.0204	0.00399	0	0.01648	0.70057	***0.89119***	0.73147	0.07527	0	0
130	10	**0.99494**	**1**	**1**	**1**	**1**	**1**	**1**	**0.95695**	***1***	***1***	***1***	***1***	***1***	***1***	***1***	***1***
	15	**0.98747**	**1**	**1**	**1**	**1**	**1**	**0.99952**	**0.73984**	**1**	***1***	***1***	***1***	***1***	***1***	**1**	**0.8349**
	20	**0.97262**	**1**	**1**	**1**	**1**	**0.99986**	**0.93675**	0.31671	***0.88763***	***1***	***1***	***1***	***1***	***1***	***0.99992***	0.00133
	25	**0.96559**	**0.99997**	**1**	**0.99999**	**0.99971**	**0.98557**	0.7168	0.14	0.17437	***0.99975***	***0.99997***	***0.99999***	***1***	***0.99991***	***0.99313***	0
	30	**0.96332**	**0.99993**	**0.99997**	**0.99896**	**0.9826**	0.82105	0.35746	0.04431	0.00126	***0.99428***	***0.99957***	***0.99989***	***0.99956***	***0.99555***	0.71564	0
	35	**0.96629**	**0.99958**	**0.9972**	**0.97109**	0.83092	0.47895	0.13041	0.01369	0	***0.92166***	***0.99655***	***0.99873***	***0.99659***	***0.94746***	0.09856	0
	40	**0.96686**	**0.99539**	**0.96759**	0.85031	0.57178	0.24134	0.0525	0.00534	0	0.61774	***0.97904***	***0.99415***	***0.98274***	0.73053	0.00316	0
	45	**0.9611**	**0.9777**	**0.90244**	0.70066	0.39479	0.14285	0.02866	0.00323	0	0.2914	***0.93169***	***0.97986***	***0.9399***	0.42967	0.00011	0
	50	**0.9445**	**0.94265**	0.8108	0.56222	0.28336	0.09527	0.01997	0.0025	0	0.09072	***0.82774***	***0.94182***	***0.84636***	0.18307	1e-05	0
	55	**0.91491**	**0.90415**	0.74187	0.48855	0.23649	0.08041	0.01805	0.00229	0	0.03002	0.78881	***0.93485***	***0.81055***	0.11801	0	0
140	10	**0.99665**	**1**	**1**	**1**	**1**	**1**	**1**	**0.96772**	***1***	***1***	***1***	***1***	***1***	***1***	***1***	***1***
	15	**0.99088**	**1**	**1**	**1**	**1**	**1**	**0.99982**	0.76793	***1***	***1***	***1***	***1***	***1***	***1***	***1***	***0.94943***
	20	**0.97992**	**1**	**1**	**1**	**1**	**0.9999**	**0.95025**	0.33228	***0.96354***	***1***	***1***	***1***	***1***	***0.99999***	***0.99996***	0.00045
	25	**0.97412**	**1**	**1**	**1**	**0.99988**	**0.98952**	0.74384	0.14268	0.31707	***0.99983***	***1***	***1***	***1***	***0.99994***	***0.99633***	0
	30	**0.97206**	**0.99999**	**0.99995**	**0.99946**	**0.98709**	0.8445	0.37126	0.04376	0.00352	***0.99653***	***0.99978***	***0.99994***	***0.99998***	***0.99737***	***0.81752***	0
	35	**0.97336**	**0.99982**	**0.99814**	**0.97801**	0.84959	0.49584	0.13138	0.01197	1e-05	***0.94908***	***0.99798***	***0.99944***	***0.99974***	***0.96708***	0.16339	0
	40	**0.97393**	**0.99721**	**0.97476**	0.86973	0.59197	0.24786	0.05029	0.00429	0	0.71167	***0.98869***	***0.99684***	***0.99009***	***0.80704***	0.00739	0
	45	**0.96968**	**0.98483**	**0.91747**	0.72283	0.40788	0.14339	0.02691	0.00267	0	0.38156	***0.95874***	***0.99***	***0.96393***	0.53232	0.00031	0
	50	**0.95667**	**0.95352**	0.83028	0.58215	0.29128	0.09414	0.01824	0.00223	0	0.13776	***0.88588***	***0.96851***	***0.89943***	0.25537	1e-05	0
	55	**0.92612**	**0.91946**	0.76382	0.50546	0.24373	0.07969	0.01604	0.00218	0	0.04811	0.70057	***0.89119***	0.73147	0.07527	0	0
150	10	**0.99811**	**1**	**1**	**1**	**1**	**1**	**1**	**0.97634**	***1***	***1***	***1***	***1***	***1***	***1***	***1***	***1***
	15	**0.99403**	**1**	**1**	**1**	**1**	**1**	**0.99992**	0.79296	***1***	***1***	***1***	***1***	***1***	***1***	***1***	***0.99864***
	20	**0.9858**	**1**	**1**	**1**	**1**	**0.99993**	**0.99091**	0.34727	***0.99759***	***1***	***1***	***1***	***1***	***1***	***0.99999***	0.03543
	25	**0.98159**	**1**	**1**	**1**	**0.99994**	**0.99316**	0.76564	0.14857	0.66565	***0.99987***	***0.99999***	***1***	***1***	***0.99997***	***0.9983***	1e-05
	30	**0.98009**	**0.99999**	**0.99999**	**0.99962**	**0.99094**	0.86268	0.38576	0.04288	0.02197	***0.99657***	***0.99995***	***0.99998***		***0.9989***	***0.92682***	0
	35	**0.98089**	**0.9994**	**0.99891**	**0.98368**	0.86805	0.51673	0.13577	0.01157	2e-05	***0.94788***	***0.99944***	***0.9999***	***0.99995***	***0.98662***	0.34984	0
	40	**0.98076**	**0.99723**	**0.98179**	0.88632	0.61578	0.25603	0.05015	0.00407	0	0.71414	***0.99663***	***0.99916***	***0.99958***	***0.90667***	0.02793	0
	45	**0.97835**	**0.984**	**0.93013**	0.74467	0.42661	0.14512	0.02632	0.00218	0	0.38166	***0.9861***	***0.99721***	***0.99689***	0.71191	0.0014	0
	50	**0.96691**	**0.9558**	0.84709	0.60225	0.30261	0.09521	0.01753	0.00164	0	0.13744	***0.9566***	***0.97882***	***0.98886***	0.43301	6e-05	0
	55	**0.95059**	**0.91645**	0.78544	0.52405	0.25088	0.07877	0.01559	0.00181	0	0.04854	***0.90774***	***0.95321***	***0.96278***	0.24025	0	0

R CODES

```
a <- sample (1:24, 24) # simply for randomization purpose.
sequence1 <- a [1:12]#  group1 taking the treatment in RT order.
sequence2 <- a[13:24]# group 2 taking the treatments in TR order.

# This the R codes for the simulation

sr=c(100,200,400,580,850,1200,1600,2000,2500,3000)#sr=variances for reference
st=100#variances for test
cv=ceiling(sqrt(sr)/mur*100)#ceiling()function rounds the vector entries up to the nearest integer
#cv
 data.frame(sr,cv)
#muT=seq(85,120,5)# is the assumed mean of the test drug
muT=85# can be changed here easly
muR=100# which assumed to be constant
sigmaT=sqrt(100)# is the variance of the test drug which is assumed to be constant.
#sigmaR=c(100,200,400,580,850,1200,1600,2000,2500,3000)
sigmaR=sqrt(100)# can be changed here easly
nsim=1000000# which is the number of simulation to be done
#n=c(seq(12,100,4),seq(110,150,10)) is the sample size
n=12  #n is the initial sample size and can be changed here
alpha=0.05

#1. Coverage of the Confidence interval

l90=rep(NA,nsim)# list of 90% lower Confidence interval values.
r90=rep(NA,nsim)# list of 90% upper Confidence interval values.
sequence=c(rep(1,1,n/2),rep(2,1,n/2))# to assign the sequence
stage1=rep(1,1,n)# here to assign the stages
for (i in 1:nsim)
{
```

```
xT=rnorm(n,muT,sigmaT)
xR=rnorm(n,muR,sigmaR)
#Ratio=log(xT)-log(xR)
ratio=(xT/xR)
T=log(xT)
R=log(xR)
Ratio=log(ratio)# equal to the log(xT)-log(xR)
Ratio[which(is.na(Ratio)==TRUE)] = mean(Ratio,na.rm = TRUE)# in case, if missing value is exist,
#it should be replaced by the mean of the remaining values.
mu=mean(Ratio)
lmfit=lm(Ratio~stage1+sequence+stage1*sequence)#$resid for s2^2=sserror+ss.. # sserrors=sresiduals
anova(lmfit)

resissq=anova(lmfit)["Residuals", "Sum Sq"] # here how to extract sum of squares of a residual
#from ANOVA table
 df=anova(lmfit)[,"Df"]
  names(df)=c("seq","Res")#only one stage here no stage effect
  df=df["Res"]
 var= (resissq)/(2*df)

data.frame(Ratio,sequence,stage1)# how to write the treatments order

l90[i]=mu-qt(1-alpha,df)*sqrt(var*2/n)# 1,000,000  lower values of confidence interval.
r90[i]=mu+qt(1-alpha,df)*sqrt(var*2/n)#1,000,000  upper values of confidence interval.
}
# here we are intersted to calculate the coverage of the confidence interval
CI=mean((l90>=-0.2231436)&(r90<=0.2231436)) #Rule of FDA that is log (0.8,1.25)

#2. tTesting statistical hypothesis (p-values are simulated)
```

```
pv1=rep(NA,nsim)# list of 90% lower p-values values.
pv2=rep(NA,nsim)# list of 90% lower p-valuesl values.
sequence=c(rep(1,1,n/2),rep(2,1,n/2))# to assign the sequence
stage1=rep(1,1,n)# here to assign the stages
for (i in 1:nsim)
{
xT=rnorm(n,muT,sigmaT)
xR=rnorm(n,muR,sigmaR)
#Ratio=log(xT)-log(xR)
ratio=(xT/xR)
T=log(xT)
R=log(xR)

pv1[i]=t.test(T,R,alternative="greater",mu=-0.2231436,paired=TRUE)$p.value
            #1,000,000 lower p-values (first one-sided).
pv2[i]=t.test(T,R,alternative="less",mu=0.2231436,paired=TRUE)$p.value
            #1,000,000 upper p-values (for the second one-sided test)
}

mean((pv1<alpha)&(pv2<alpha))# to compute the proportion of rejecting the null H. for TOST.



#3. The values of  power computaions

power=rep(NA,nsim)
sequence=c(rep(1,1,n/2),rep(2,1,n/2))# to assign the sequence
stage1=rep(1,1,n)# here to assign the stages
for (i in 1:nsim)
{
xT=rnorm(n,muT,sigmaT)
xR=rnorm(n,muR,sigmaR)
#Ratio=log(xT)-log(xR)
```

```
ratio=(xT/xR)
T=log(xT)
R=log(xR)
Ratio=log(ratio)# equal to the log(xT)-log(xR)
Ratio[which(is.na(Ratio)==TRUE)] = mean(Ratio,na.rm = TRUE)# in case if missing value is exist,
#it should be replaced by the mean of the remaining values.
mu=mean(Ratio)
lmfit=lm(Ratio~stage1+sequence+stage1*sequence)#$resid for s2^2=sserror+ss.. # sserrors=sresiduals
anova(lmfit)

resissq=anova(lmfit)["Residuals", "Sum Sq"] # here how to extract sum of squares of a residual
#from ANOVA table
 df=anova(lmfit)[,"Df"]
  names(df)=c("seq","Res")#only one stage here no stage effect
  df=df["Res"]
 var= (resissq)/(2*df)
s1=sqrt(var)# how to compute the standard deviation
theta=muT/muR # the ratio of the means

power[i]=pt((log(1.25/theta)/(s1*sqrt(2/n)))-qt(1-alpha,(n-2)),(n-2))-pt(-
(log(1.25*theta)/(s1*sqrt(2/n)))
+qt(1-alpha,(n-2)),(n-2))
}

mean(power>=0.8)# the values of power which is greater the the minimum requeirment.
```