

BioEQuivalance study Designs and Protocol

Crossover Design, Case Report, Case Series, Cohort Study



**BIOEQUIVALANCE STUDY DESIGNS AND PROTOCOL**

**Bioequivalence** is defined as the absence of a significant difference in the rate and extent to which the active ingredient or active moiety becomes available at the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study.

**Bioequivalence studies** are performed to compare the bioavailability of the test or generic drug product to the reference or brand-name product

**The basic design for a bioequivalence study is determined by**

1. The scientific questions and objectives to be answered
2. The nature of the reference material and the dosage form to be tested
3. The availability of analytical methods
4. The pharmacokinetics and pharmacodynamics of the drug substance
5. The route of drug administration
6. Benefit-risk and ethical considerations with regards to testing in humans

For bioequivalence studies, the test and reference drug formulations must contain the same dose strength and in similar dosage form (eg, immediate release or controlled release), and must be given by the same route of administration.

Once bioequivalence is established, it is likely that both the generic and brand- name dosage forms will produce the same therapeutic effect.

The basic guiding principle in performing studies is ***do not do unnecessary human research.***

Generally, the study is performed in normal, healthy male and female volunteers who have given informed consent to be in the study.

Bioequivalence studies involve both a **clinical component** and an **analytical component**.

The objective of a typical bioequivalence study is to demonstrate that the test and reference products achieve a similar pharmacokinetic profile in plasma, serum and/or urine.

Formulations to be tested are administered either as a single dose or as multiple doses. Sometimes formulations can be labelled with a radioactive component to facilitate subsequent analysis.

In a bioequivalence study, serial samples of biologic fluid (plasma, serum, or urine) are collected just before and at various times after dose administration. These samples are later analyzed for drug and/or metabolite concentrations. The study data are used in subsequent pharmacokinetic analyses to establish bioequivalence.

Nearly all crossover designs have "balance", which means that all subjects should receive the same number of treatments and that all subjects participate for the same number of periods. In most crossover trials, in fact, each subject receives all treatments.

**CROSSOVER STUDY DESIGNS:**

In crossover study design, each subject receives the test drug product and the reference product.

**Latin Square Design**

A Latin square is a square array of objects/treatments (letters A, B, C, …) such that each object appears once and only once in each row and each column with adequate time between medications for the elimination of the drug from the body.

In this design, each subject is his own control, and subject tom subject variation is reduced.

Example - 4 x 4 Latin Square.

|  |
| --- |
| ABCD |
| BCDA |
| CDAB |
| DABC |

The treatments are assigned to row-column combinations using a Latin-square arrangement.

Period refers to the time period in which a study is performed.

A two-period study is a study that is performed on two different days (time-period) separated by a washout period during which most of drug is eliminated from the body; generally, about 10 elimination half-lives.

A sequence refers to the number of different orders in the treatment groups in a study.

For Example,

A two-sequence, two-period study would be designed as follows:

|  |  |  |
| --- | --- | --- |
|  | **Period 1** | **Period 2** |
| **Sequence 1** | T | R |
| **Sequence 2** | R | T |

Where R = reference and T = treatment

***Latin-Square Crossover Design for a Bioequivalency Study of 4 Drug Products in 16 Human Volunteers***

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **DRUG PRODUCT** | | | |
| **Subject** | **Period 1** | **Period 2** | **Period 3** | **Period 4** |
| 1 | A | B | C | D |
| 2 | B | C | D | A |
| 3 | C | D | A | B |
| 4 | D | A | B | C |
| 5 | A | B | D | C |
| 6 | B | D | C | A |
| 7 | D | C | A | B |
| 8 | C | A | B | D |
| 9 | A | C | B | D |
| 10 | C | B | D | A |
| 11 | B | D | A | C |
| 12 | D | A | C | B |
| 13 | A | C | D | B |
| 14 | C | D | B | A |
| 15 | D | B | A | C |
| 16 | B | A | C | D |

Randomized, balanced, crossover Latin square designs are commonly used for bioequivalence studies.

**Latin Square Design Pros:**

•Minimizes the inter subject variability in plasma drug levels

•Minimizes variations due to time effect

•Minimizes the carry over effects intra subject

•Treatments can be studied from a small-scale experiment

**Latin Square Design Cons:**

•Does not give an estimate of intrasubject variability

•Study takes a long time as appropriate washout period is required which will be long if drug has long half-life

•When the number of formulations to be tested is more; the study becomes difficult and also the subject dropouts are high.

**Replicated Crossover Study Design**

By giving the same drug twice to the same subject, the replicate design provides a measure for with-in subject variability. Replicate design studies may be used for highly variable drugs and for narrow therapeutic index drugs.

Replicated crossover designs are used for the determination of individual bioequivalence, to estimate within-subject variance for both the test and reference drug products, and to provide an estimate of the subject-by-formulation interaction variance. A four-period, two-sequence, two-formulation design is shown below:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Period 1** | **Period 2** | **Period 3** | **Period 4** |
| **Sequence 1** | T | R | T | R |
| **Sequence 2** | R | T | R | T |

where R = reference and T = treatment.

In this design, the same reference and the same test are each given twice to the same subject. Other sequences are possible.

Narrow therapeutic index (NTI) drugs, also referred to as critical dose drugs, are drugs in which small changes in dose or concentration may lead to serious therapeutic failures or serious adverse drug reactions in patients. The FDA currently recommends that bioequivalence studies of narrow therapeutic index drugs should employ *a four-way, fully replicated, crossover study design.*

In this design, reference-to- reference and test-to-test comparisons may also be made.

Recently *a three-sequence, three-period, two-treatment partially replicated crossover design* for bioequivalence studies of highly variable drugs has been recommended by the FDA (This design is usually used for highly variable drugs with within-subject variability ≥30%).

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Period 1** | **Period 2** | **Period 3** |
| **Sequence 1** | T | R | R |
| **Sequence 2** | R | T | R |
| **Sequence 3** | R | R | T |

where R = reference and T = treatment.

**Multiple-dose Crossover Study Design**

A bioequivalence study may be performed using a multiple-dose study design. Multiple doses of the same drug are given consecutively to reach steady-state plasma drug levels. *The multiple-dose study is designed as a steady-state, randomized, two-treatment, two-way, crossover study* comparing equal doses of the test and reference products in healthy adult subjects. Each subject receives either the test or the reference product separated by a “washout” period, which is the time needed for the drug to be completely eliminated from the body. To ascertain that the subjects are at steady state, three consecutive trough concentrations (Cmin) are determined. Blood sampling is then performed over one dosing interval. The area under the curve during a dosing interval at steady state should be the same as the area under the curve extrapolated to infinite time after a single dose.

There are several **disadvantages** of using the multiple-dose crossover method for the determination of bioequivalence.

1. The study takes more time to perform, because steady-state conditions must be reached.
2. A longer time for completion of a study leads to greater clinical costs and the possibility of a subject dropping out and not completing the study.
3. More plasma samples must be obtained from the subject to ascertain that steady state has been reached and to describe the plasma level–time curve accurately.
4. Small differences in the rate of drug absorption may not be observed with steady-state study comparisons

**CASE REPORT STUDY DESIGN**

The following clinically very relevant question raised: does equivalence of average bio-availability, which they termed average bioequivalence, ensure that the bioavailability of two drug products is equivalent in individual patients? In other words, does average bioequivalence imply switchability of drug products in individual patients?

The case report is a specific type of research design that reports on an aspect of the management of one or two patients. It is the first piece of research writing in the health field and represents the most basic type of study design.

• Generally, report a new or unique finding e.g.:

– unexpected new therapeutic effect

– adverse events

– An unexpected event in the course of observing or treating a patient.

• The case may be an individual, an event, a policy, etc.

The general format/template:

* Background
* Case presentation
* Discussion
* Consent

**Case report:** inability to achieve a therapeutic dose of tacrolimus in a pediatric allogeneic stem cell transplant patient after generic substitution

**CASE SERIES:**

Observations are made on a series of individuals, usually all receiving the same intervention, before and after an intervention but with no control group. Consequently, a case series cannot be comparative.

Case series exist in 2 types:

1. Sampling is based on a specific outcome and presence of a specific exposure.

2. Selection is based only on a specific outcome, and data are collected on previous exposures. Cases are reported regardless of whether they have specific exposures. This type of case series can be seen as the case group from a case–control study.

**For Example:**

* Clinical Experience of the Use of CT-P13, a Biosimilar to Infliximab in Patients with Inflammatory Bowel Disease: A Case Series
* Symptom relapse following switch from Celexa to generic citalopram: an anxiety disorders case series

**COHORT STUDY**

A study in which a defined group of people (the cohort) is followed over time, to examine associations between different interventions received and subsequent outcomes.

There are two fundamental types of cohort studies based on when and how the subjects are enrolled into the study:

1. Prospective Cohort Studies:

In prospective cohort studies the investigators conceive and design the study, recruit subjects, and collect baseline exposure data on all subjects, before any of the subjects have developed any of the outcomes of interest. The subjects are then followed into the future in order to record the development of any of the outcomes of interest. The follow up can be conducted by mail questionnaires, by phone interviews, via the Internet, or in person with interviews, physical examinations, and laboratory or imaging tests. Combinations of these methods can also be used.

1. Retrospective Cohort Studies

Retrospective studies also group subjects based on their exposure status and compare their incidence of outcomes. However, in this case both exposure status and outcome are ascertained retrospectively.

**For Example:**

Clinical Outcomes After Conversion from Brand-Name Tacrolimus (Prograf) to a Generic Formulation in Renal Transplant Recipients (A Retrospective Cohort Study)

There are three general types of comparison groups for cohort studies.

1. An internal comparison groups
2. A comparison cohort
3. The general population

**For Example:**

Generic switching of warfarin and risk of excessive anticoagulation: a Danish nationwide cohort study

**STUDY PROTOCOL**

A bioequivalence study should be carried out in accordance with a protocol agreed upon and signed by the investigator and the sponsor. The protocol and its attachments and/or appendices should state the aim of the study and the procedures to be used, the reasons for proposing the study to be undertaken in humans, the nature and degree of any known risks, assessment methodology, criteria for acceptance of bioequivalence, the groups from which it is proposed that trial subjects be selected and the means for ensuring that they are adequately informed before they give their consent. The investigator is responsible for ensuring that the protocol is strictly followed. Any change(s) required must be agreed on and signed by the investigator and sponsor and appended as amendments, except when necessary to eliminate an apparent immediate hazard or danger to a trial subject. The protocol, attachments and appendices should be scientifically and ethically appraised by one or, if required by local laws and regulations, more review bodies (e.g. institutional review board, peer review committee, ethics committee or NRA) constituted appropriately for these purposes and independent of the investigator(s) and sponsor.

**ELEMENTS OF BIOEQUIVALENCE STUDY PROTOCOL**

**I. Title**

1. Principal investigator (study director)
2. Project/protocol number and date

**II. Study objective**

**III. Study design**

1. Design
2. Drug products
3. Test product(s)
4. Reference product
5. Dosage regimen
6. Sample collection schedule
7. Housing/confinement
8. Fasting/meals schedule
9. Analytical methods

**IV. Study population**

1. Subjects
2. Subject selection
3. Medical history
4. Physical examination
5. Laboratory tests
6. Inclusion/exclusion criteria
   1. Inclusion criteria
   2. Exclusion criteria
7. Restrictions/prohibitions

**V. Clinical procedures**

1. Dosage and drug administration
2. Biological sampling schedule and handling procedures
3. Activity of subjects

**VI. Ethical considerations**

1. Basic principles
2. Institutional review board
3. Informed consent
4. Indications for subject withdrawal
5. Adverse reactions and emergency procedures

**VII. Facilities**

**VIII. Data analysis**

1. Analytical validation procedure
2. Statistical treatment of data

**IX. Drug accountability**

**X. Appendix**

**Analytical Methods:**

The results of the method validation should be available before the initiation of study sample analysis, with the possible exception of the evaluation of the long-term stability of the analyte in matrix. However, these results should be available before the study report is issued and should be submitted with the validation report in the application.

Analytical methods used in an in-vivo bio availability, bio equivalence, or pharmacodynamic studies must be validated for accuracy, precision, sensitivity, specificity, and robustness. The use of more than one analytical method during a bio equivalence study may not be valid because different methods may yield different values.

**Subject Selection:**

* Healthy adult volunteers
* age 18-45years
* age/sex representation corresponding to therapeutic and safety profile
* weight with in normal limits
* women-pregnancy test period to first and last dose of study

**Selection of Number of Subjects:**

* Sample size estimated by Pilot Experiments Previous Studies Published Data
* Significance level desired usually 0.05
* Power of study normally 80% or more
* Minimum 16 subjects unless ethical justification
* Allow for drop outs

**Exclusion Criteria:**

* H/o allergy to test drug
* H/o liver or kidney disfunction
* H/o jaundice in past 6 months
* Chronic diseases E.g.: asthma, arthritis
* Psychiatric illness

**Ethics:**

**Independent ethics committee**

Trials must be approved by an independent ethics committee (IEC) (or equivalent) before any study is conducted, according to WHO operational guidelines for ethics committees that review biomedical research (6), and to the legislation in force. This Committee must be independent from the sponsor, the investigator and the CRO. Detailed minutes should be kept of the discussions, recommendations and decisions of the IEC meetings.

**Informed consent**

The following points should be borne in mind in relation to informed consent.

■■ Information for study participants should be given to them in a language and at a level of complexity appropriate to their understanding, both orally and in writing.

■■ Informed consent must always be given by the subject and documented in writing before the start of any trial-related activities, in accordance with GCP. If informed consent is also recorded by video, this recording should be retained in accordance with local legal requirements.

■■ The information must make clear that participation is voluntary and that the subject has the right to withdraw from the study on his or her own initiative at any time, without having to give a reason. If subjects who withdraw from the study offer their reasons for doing so, those reasons should be included in the study records.

■■ The subject must have access to information about insurance and other procedures for compensation or treatment should he or she be injured or disabled by participating in the trial or during screening.

■■ The volunteers or subjects should be given the opportunity to discuss with a physician their concerns regarding potential side effects or reactions from the use of the investigational products before participating in the trial. They should also be given the opportunity and sufficient time to discuss their concerns about participating in the trial with individuals outside the CRO, such as friends and family members, if they wish.

■■ If the ICF is available in several languages (e.g. in English and in the local language, or in several vernacular languages) care should be taken to ensure that all versions of the form contain the same information.

**Administration of drug products:**

Administration of drug products to the should be based on randomization. After the administration of drug products, blood samples are withdrawn from the subjects at fixed time points. It takes some to take a sample from each subject, and the total time difference between first and last subject by range from 10 to 20 minutes depending upon the number of subjects and technicians in the study. This 10 to 20-minute difference would represent a substantial change in the drug concentrations observed in the blood.

If under these conditions treatments are administered to the subjects in a sequential manner( such as treatment A to the first 6 volunteers, treatment B to volunteers 7 to 12, and treatment C to Volunteers 13 to18),the error between the time of administration and sampling will gradually increase from treatment group to treatment group. This is because of sequential administration of drug products to different treatments.

**Sampling:**

The biological sample to be used in the study as to be decided before the commencement of a bioavailability study. If the bioavailability of a given dosage form is to be evaluated by a blood level study, some estimate of the area under the serum concentration v/s time curve, peak serum concentration, time of peak concentration must be obtained from the study. These factors can markedly influence the ‘apparent’ results obtained in a given study.

The sampling scheme should frequent enough to define the absorption phase, the peak, and the elimination phase during a drugs time course in the body.

The absorption rate, volume of distribution, elimination rate, all influence the apparent drug concentration one obtains in a given sample. It is necessary to see that all these factors influence each dosage form equally.

To estimate the AUC from the data, sampling as to be carried out till the concentration of the drug reaches the linear elimination phase. In the case of urinary excretion studies, the same principles apply.

**The advantages of urinary excretion studies are**

1. It involves non-invasive method of sampling.
2. The drug concentration in the urine is greater than blood/serum allowing easy estimation of the drug.
3. The amount of drug excreted in urine is obtained directly. In the case of a blood level study, the amount of drug in the body is estimated using pharmacokinetic parameters.

**The urinary excretion method has several disadvantages**

urinary excretion studies are not useful in estimating the drug absorption rate.

In some cases, the metabolites of the drug are also concentrated in the sample that interferes with the estimation of unchanged drug in the urine sample.

**Evaluation of data:**

Pharmacokinetic evaluation of the data for single dose studies, including a fasting study or a food intervention study, the pharmacokinetic analyses include calculation for each subject of the area under the curve to the last quantifiable concentration (AUC0) and to infinity (AUC0), tmax and Cmax .Additionally ,the elimination rate constant-k, the elimination half-life (t1/2)

**Statistical evaluation of the data:**

Bioequivalence is generally determined using a comparison of population averages of a bioequivalence metric, such as AUC and Cmax. This approach, termed average bioequivalence, involves for the ratio of averages of the test and reference drug products.

Statistical Analysis for Average Bio equivalence:

* Based on log transformed data
* Point estimates of the mean ratios
* Test / reference for AUC and Cmax are between 80% -125% 
* AUC and Cmax -90% confident intervals must fit between 80%-125%

Statistical model typically includes factors accounting for following sources of variations: Sequence, subjects, nested in sequences, period in treatment