A REVIEW ON BIOAVAILABILITY AND BIOEQUIVALENCE TRIALS AND ITS NECESSITY

JIGAR PATEL*, KRITIKA ANEJA, RAVI TIWARI

School of Pharmacy and Technology Management Shirpur CampusMumbai Agra Road,Near Bank of Tapi River Babulde, Shirpur Dist. Dhule- 425405 (MS) India jigarpatel31187@gmail.com

ABSTRACT

Bioequivalence (BE) means the absence of a greater-than-allowable difference between the systemic bioavailability of a test product and that of a reference product. Studies to test the BE of drug products, and the statistical basis for their design, analysis and interpretation, have evolved over the last two decades. A crossover design is preferred over a parallel-group design as it segregates the inter-subject variation (which is not product-dependent) from the intra-subject variation (which is product-dependent). The value of testing two one-sided null hypotheses of non-equivalence at a significance level of 0.05, and the importance of estimating a 90% confidence interval of the ratio(test/reference) of mean AUC and Cmax values, and of the difference between mean T max values, are now recognized and form the current standards for BE. The number of subjects required for a BE study with the desired power (at least 0.80) and significance level (0.05), depends on the expected deviation of the test product from the reference product and the error variance associated with the bioavailability parameters (AUC, Cmax, Tmax etc.) of the drug substance. At present, according to the Indian regulatory authority, the number of subjects required to conduct a BE study is 12 which is inadequate for most drug substances by the current international standards and criteria. In this section we will also discuss some regulatory norms by ICH and their criteria's of selection.

Key words: Bioavailability & Bioequivalence Trials.

INTRODUCTION

Current international regulatory authorities[1-3] require that the final quality judgment of an oral dosage form be based on its in vitro dissolution profile and its in vivo bioavailability and/ or bioequivalence evaluation. The latter is based on the premise that the concentration of the drug in the systemic circulation is at equilibrium with the concentration of the drug at the site of action and that the therapeutic effect of the drug moiety is a function of its pha rmacodynamic -pharmacokinetic relationship.

Bioequivalence gained increasing attention during the last 40 years after it became evident that marketed products having the same amounts of the drug may exhibit marked differences in their therapeutic responses. Generally, these differences were well correlated to dissimilar drug plasma levels caused mainly by impaired absorption. Several examples illustrating.

This phenomenon are available in the literature, for example, researchers correlated the bioavailability and clinical effectiveness of USP thyroid products,[4-6] digoxin,[7-8] tolbutamide,[9,10] prednisone[11,12] and phenytoin.[13]Consequently, on the basis of simple pharmacokinetic concepts and parameters, bioavailability and bioequivalence studies have been established as acceptable surrogates for expensive, complicated and lengthy clinical trials, and are used extensively worldwide to establish and ensure consistent quality and a reliable, therapeutically effective performance of marketed dosage forms.

Bioavailability reflects the extent of the systemic availability of the active therapeutic moiety and is generally assessed by measuring the 'area under the concentration time curve' (AUC),the peak plasma concentration (Cmax) and the time to reach Cmax (Tmax). The extent of the systemic availability is determined by the extent of drug absorbed from the site of administration, and is influenced by the drug, the dosage form and the interaction of these with the complex environment of the absorption site. For a drug that obeys linear pharmacokinetics,the AUC and Cmax values increase proportionately with the dose.[14] Consequently, if two formulations / dosage forms of the same drug exhibit comparative AUC values, they are considered to have similar systemic availability. The bioavailability of an oral dosage form or a drug is generally compared with an intravenous solution (100% standard), to determine the absolute bioavailability. In case of drugs which obey non-linear kinetics, the changes in AUC and Cmax values are not proportional to the dose administered.[14] This is because either one or more of the processes which handle the drug i.e. absorption, distribution, metabolism and excretion are saturated i.e. their capacity has been exceeded within the therapeutic concentration range of the drug (substrate). In this situation, the plasma-concentration-time profile cannot be used as an indicator of absolute bioavailability. The latter has to be then assessed by measuring the extent of the drug and its metabolites excreted in the urine.

The comparative bioavailability assessment of two or more formulations of the same active ingredient to be administered by the same route is termed bioequivalence. Bioequivalence studies compare both the rate and extent of absorption of various multisource drug formulations with the innovator (reference)product, on the basis that if two formulations exhibit similar drug concentration-time profiles in the blood/plasma, they should exhibit similar therapeutic effects. For an unapproved generic dosage form to be marketed and accepted as therapeutically equieffective to the innovator product, it must establish bioequivalence with the innovator product, in vivo.Bioequivalence studies provide a quality control tool to monitor production and manufacturing changes.[15]

DEFINITIONS

Bioavailability

It refers to the relative amount of drug from an administered dosage form which enters into the systemic circulation and the rate at which the drug appears in systemic circulation.

Bioequivalence

Bioequivalence of a drug is achieved if its rate and extent of absorption is not statistically   significantly different from those of reference product when administered at same molar dose.

Clinical Trial

A clinical trial is a systematic study of pharmaceutical products in human subjects in order to discover and verify the clinical, pharmacological (including pharmacodynamic, pharmacokinetic), and/or adverse effects, with the object of determining their safety and/or efficacy.

Good Clinical Practices (GCP) Guidelines

Good clinical practices guidelines issued by Directorate General of Health Services, Ministry of Health and Family Welfare, Government of India.

Pharmaceutical Equivalents

Pharmaceutical equivalents are drug products that contain identical amount of identical active drug ingredient in identical dosage forms, but not necessarily containing the same inactive ingredients.

.Pharmaceutical Alternatives

Pharmaceutical alternatives are drug products that contain the identical therapeutic moiety but not necessarily in the same amount.

Pharmacodynamic Evaluation

Pharmaceutical evaluation is a measurement of the effect on patho-physiological process as a function of time, after administration of two different products to serve as a basis for bioequivalence assessment.

Pharmacokinetics

It refers to the absorption, distribution, metabolism and excretion the drug in the body.

Non-linear Pharmacokinetics

It refers to the change of one or more pharmacokinetics parameters during ADME due to increased dose size.

Reference Product

It refers to the pharmaceutical product which is licensed by the authority as a reference for comparing with the generic product in BE studies.

Supra-Bioavailability

It is the term used when the test product shows appreciable large bioavailability than the reference product.

.Steady State

Steady state is the state when the plasma concentration of drug at any time point during any dosing interval should be identical to the concentration at the same time during any other dosing interval.

Therapeutic Equivalent

Therapeutic equivalents are drug products that contain the same active substance or therapeutic moiety and, clinically show the same efficacy and safety.

PHARMACOKINETIC TERMS

Cmax

This is the maximum drug concentration achieved in systemic circulation following drug administration.

Cmin

This is the minimum drug concentration achieved in systemic circulation following multiple dosing at steady state.

Tmax

It is the time required to achieve maximum drug concentration in systemic circulation.

AUC 0-t

Areas under the plasma concentration - time curve from 0 h to the last quantifiable concentration to be calculated using the trapezoidal rule.

Kel

Apparent first-order terminal elimination rate constant calculated from a semi-log plot of the plasma concentration versus time curve.

T1/2

Elimination half life of a drug is the time necessary to reduce the drug concentration in the blood, plasma, or serum to one-half of its initial concentration.

GUIDELINES TO BE CONSIDERED FOR THE STUDIES

Bioavailability and Bioequivalence studies are required by regulations to ensure therapeutic equivalence between a pharmaceutically equivalent test product and a reference product.

Several in vivo and in vitro methods are used to measure product quality.

When bioequivalence studies are necessary and types of studies required

In- vivo studies

For certain drugs and dosage forms, in vivo documentation of equivalence, through either a bioequivalence study, a comparative clinical pharmacodynamics study, or a comparative clinical trial, is regarded as important. These include:

- Oral immediate release drug formulations with systemic action when one or more of the following criteria apply:

- indicated for serious conditions requiring assured therapeutic response;

- narrow therapeutic window/safety margin; steep dose-response curve;

- pharmacokinetics complicated by variable or incomplete absorption or absorption window, nonlinear pharmacokinetics, pre-systemic elimination/high first-pass metabolism >70%;

- unfavourable physicochemical properties, e.g., low solubility, instability meta-stable modifications, poor permeability, etc.;

- documented evidence for bioavailability problems related to the drug or drugs of similar chemical structure or formulations;

- where a high ratio of excipients to active ingredients exists.

- Non-oral and non-parenteral drug formulations designed to act by systemic absorption (such as transdermal patches, suppositories, etc.).

- Sustained or otherwise modified release drug formulations designed to act by systemic absorption.

- Fixed-dose combination products with systemic action.

- Non-solution pharmaceutical products which are for non-systemic use (oral, nasal, ocular, dermal, rectal, vaginal, etc. application) and are intended to act without systemic absorption. In these cases, the bioequivalence concept is not suitable and comparative clinical or pharmacodynamic studies are required to prove equivalence. There is a need for drug concentration measurements in order to assess unintended partial absorption.

Bioequivalence documentation is also needed to establish links between

- early and late clinical trial formulations,

- formulations used in clinical trials and stability studies, if different,

- clinical trial formulations and to be marketed drug products,

- other comparisons, as appropriate

- In each comparison, the new formulation or new method of manufacture shall be the test product and the prior formulation (or respective method of manufacture) shall be the reference product.

When bioequivalence studies are not necessary

In following formulations and circumstances, bioequivalence between a new drug and the reference product may be considered self-evident with no further requirement for documentation:

- When new drugs are to be administered parenterally (e.g., intravenous, intramuscular, subcutaneous, intrathecal administration etc.) as aqueous

- solutions and contain the same active substance(s) in the same concentration and the same excipients in comparable concentrations;

- When the new drug is a solution for oral use, and contains the active substance in the same concentration, and does not contain an excipient that is known or suspected to affect gastro-intestinal transit or absorption of the active substance;

- When the new drug is a gas;

- When the new drug is a powder for reconstitution as a solution and the solution meets either criterion (a) or criterion (b) above.

- When the new drug is an otic or ophthalmic or topical product prepared as aqueous solution and contains the same active substance(s) in the same concentration(s) and essentially the same excipients in comparable concentrations;

- When the new drug is an inhalation product or a nasal spray, tested to be administered with or without essentially the same device as the reference products, prepared as aqueous solutions, and contain the same active substance(s) in the same concentration and essentially the same excipients in comparable concentrations. Special in-vitro testing is required to document device performance comparison between reference inhalation product and the new drug product.

DESIGN AND CONDUCT OF STUDIES

Pharmacokinetic Studies

Study Design

The basic design of an in-vivo bioavailability study is determined by the following:

- What is the scientific question(s) to be answered.

- The nature of the reference material and the dosage form to be tested.

- The availability of analytical methods.

- Benefit-risk ratio considerations in regard to testing in humans.

The study should be designed in such a manner that the formulation effect can be distinguished from other effects. Typically, if two formulations are to be compared, a two-period, two-sequence crossover design is the design of choice with the two phases of treatment separated by an adequate washout period which should ideally be equal to or more than five half life's of the moieties to be measured.

Alternative study designs include the parallel design for very long half-life substances or the replicate design for substances with highly variable disposition.

Single-dose studies generally suffice. However situations as describe below may demand a steady-state study design:

- Dose or time-dependant pharmacokinetics.

- Some modified release products (in addition to single dos investigations)

- Where problems of sensitivity preclude sufficiently precise plasma concentration measurements after single dose administration.

- If intra individual variability in the plasma concentration or disposition precludes the possibility of demonstrating the bioequivalence in a reasonably sized single dose study and

- This variability is reduced at steady state 3.

Selection of the Number of Subjects

The number of subjects required for a study should be statistically significant and is determined by the following considerations:

- The error variance associated with the primary characteristic to be studied as estimated from a

- pilot experiment, from previous studies or from published data.

- The significance level desired: usually 0.05

- The expected deviation from the reference product compatible with bioequivalence.

- The required (discriminatory) power, normally =80% to detect the maximum allowable difference (usually± 20%) in primary characteristics to be studied.

The number of subjects recruited should be sufficient to allow for possible withdrawals from the study. However, the minimum number of subjects should not be less than 16 unless justified for ethical reasons. They should be tested under similar conditions and environments.

Sequential or add-on studies are acceptable in specific cases e.g. where a large number of subjects are required or where the results of the study do not convey adequate statistical significance.

SUBJECT SELECTION CRIETERIA

To minimize intra and inter individual variation subjects should be standardised as much as possible and acceptable. The studies should be normally performed on healthy adult volunteers with the aim to minimise variability and permit detection of differences between the study drugs. Subjects may be males or females; however the choice of gender should be consistent with usage and safety criteria.

Risks to women of childbearing potential should be considered on an individual basis. Women should be required to give assurance that they are neither pregnant, nor likely to become pregnant until after the study. This should be confirmed by a pregnancy test immediately prior to the first and last dose of the study. Women taking contraceptive drugs should normally not be included in the studies.

If the drug product is to be used predominantly in the elderly attempt should be made to include as many subjects of 60 years of age or older as possible. If the drug product is intended for use in both sexes attempt should be made to include similar proportions of males and females in the studies.

For a drug representing a potential hazard in one group of users, the choice of subjects may be narrowed, e.g., studies on teratogenic drugs should be conducted only on males.

For drugs primarily intended for use in only males or only females – volunteers of only respective gender should be included in the studies.

For drugs where the risk of toxicity or side effects is significant, studies may have to be carried out in patients with the concerned disease, but whose disease state is stable. They should be screened for suitability by means of a comprehensive medical examination including clinical laboratory tests, an extensive review of medical history including medication history, use of oral contraceptives, alcohol intake and smoking, use of drugs of abuse.

Depending on the study drugs therapeutic class and safety profile, special medical investigations may need to be carried out before, during and after the study.

STUDY CONDITIONS

Standardisation of the study environment, diet, fluid intake, post-dosing postures, exercise, sampling schedules etc. is important in all studies. Unless the study design requires, subjects should abstain from smoking, drinking alcohol, coffee, tea, xanthine containing foods and beverages and fruit juices during the study and at least 48 hours before its commencement.

Selection of Blood Sampling Points/Schedules

The blood-sampling period in single-dose trials of an immediate release product should extend to at least three-elimination half-lives. Sampling should be continued for a sufficient period to ensure that the area extrapolated from the time of the last measured concentration to infinite time is only a small percentage (normally less than 20%) of the total AUC. The use of a traced AUC is undesirable except in certain circumstances such as in the presence of entero- hepatic recycling where the terminal elimination rate constant cannot be calculated accurately.

There should be at least three sampling points during the absorption phase,three to four at the projected T_{max} and four points during the elimination phase. The number of points used to calculate the terminal elimination rate constant should be preferably determined by a semi-logarithmic plot.

Intervals between successive data/sampling points used to calculate the terminal elimination rate constant should, in general, not be longer than the half-life of the study drug.

Where urinary excretion is measured in a single-dose study it is necessary to collect urine for seven or more half-lives.

Fasting and Fed State Considerations

Generally, a single dose study should be conducted after an overnight fast (at least 10 hours), with subsequent fast of 4 hours following dosing. For multiple dose fasting state studies, when an evening dose must be given, two hours of fasting before and after the dose is considered acceptable.

However, when it is recommended that the study drug be given with food (as would be in routine clinical practice), or where the dosage form is a modified release product, fed state studies need to be carried out in addition to the fasting state studies.

Fed state studies are also required when fasting state studies make assessment of Cmax and Tmax difficult.

Studies in the fed state require the consumption of a high-fat breakfast before dosing. Such a breakfast must be designed to provide 950 to 1000 KCals. At least 50% of these calories must come from fat, 15 to 20% from proteins and the rest from carbohydrates. The vast ethnic and cultural variations of the Indian sub- continent preclude the recommendation of any single standard high fat breakfast. Protocol should specify the suitable and appropriate diet. The high fat breakfast must be consumed approximately 15 minutes before dosing.

Steady State Studies

In following cases – an additional "steady state study" is considered appropriate:

- Where the drug has a long terminal elimination half-life and blood concentrations after a single
- dose cannot be followed for a sufficient time.
- Where assay sensitivity is inadequate to follow the terminal elimination phase for an adequate
- period of time.
- For drugs, which are so toxic that ethically they should only be administered to patients for
- whom they are a necessary part of therapy, but where multiple dose therapy is required, e.g.
- many cytotoxics.
- For modified-release products where it is necessary to assess the fluctuation in plasma concentration over a dosage interval at steady state.
- For those drugs which induce their own metabolism or show large intra- individual variability.
- For enteric-coated preparations where the coating is innovative.
- For combination products where the ratio of plasma concentration of the individual drugs is important.
- For drugs that exhibit non-linear (i.e., dose- or time- dependent) pharmacokinetics.
- Where the drug is likely to accumulate in the body.

CHARACTERISTICS TO BE INVESTIGATED DURING BIOAVAILABILITY /BIOEQUIVALENCE STUDIES

In most cases evaluations of bioavailability and bioequivalence will be based upon the measured concentrations of the active drug substance(s) in the biological matrix. In some situations, however, the measurements of an active or inactive metabolite may be necessary. These situations include (a) where the concentrations of the drug(s) may be too low to accurately measure in the biological matrix, (b) limitations of the analytical method, (c) unstable drug(s), (d) drug(s) with a very short half-life or (e) in the case of prodrugs.

Racemates should be measured using an achiral assay method. Measurement of individual enantiomers in bioequivalence studies is recommended where all of the following criteria are met:

- the enantiomers exhibit different pharmacodynamic characteristics
- the enantiomers exhibit different pharmacokinetic characteristics
- primary efficacy/safety activity resides with the minor enantiomer
- non-linear absorption is present for at least one of the enantiomers

The plasma-time concentration curve is mostly used to assess the rate and extent of absorption of the study drug. These include pharmacokinetic parameters such as the Cmax ,Tmax, AUC 0-t,AUC0-8.

For studies in the steady state AUC0-1 Cmax, Cmin and degree of fluctuation should be calculated.[16]

BIOEQUIVALENCE TESTING

In determining bioequivalence, for example, between two products such as a commercially-available Brand product and a potential to-be-marketed Generic product, pharmacokinetic studies are conducted whereby each of the preparations are administered in a cross-over study to volunteer subjects, generally healthy individuals but occasionally in patients. Serum/ plasma samples are obtained at regular intervals and assayed for parent drug (or occasionally metabolite) concentration. Occasionally, blood concentration levels are neither feasible nor possible to compare the two products (e.g. inhaled corticosteroids), then pharmacodynamics endpoints rather than pharmacokinetic endpoints (see below) are used for comparison. For a pharmacokinetic comparison, the plasma concentration data are used to assess key pharmacokinetic parameters such as area under the curve (AUC), peak concentration (C_{max}), time to peak concentration (T_{max}), and absorption lag time (t_{lag}). Testing should be conducted at several different doses, especially when the drug displays non-linear pharmacokinetics.

In addition to data from bioequivalence studies, other data may need to be submitted to meet regulatory requirements for bioequivalence. Such evidence may include:

- analytical method validation

- in vitro-in vivo correlation studies[17]

Criteria for Bioequivalence

To establish Bioequivalence, the calculated 90% confidence interval for AUC and Cmax should fall within the bioequivalence range, usually 80-125%. This is equivalent to the rejection of two one sided-t tests with the null hypothesis of non- bioequivalence at 5% level of significance.

Tighter limits for permissible differences in bioavailability may be required for drugs that have:

- A narrow therapeutic index.

- A serious, dose-related toxicity

- A steep dose/effect curve, or

- A non-linear pharmacokinetics within the therapeutic dose range[15]

Three situations have thus been defined in which bioequivalence studies are required

- when the proposed marketed dosage form is different from that used in pivotal clinical trials,

- when significant changes are made in the manufacture of the marketed formulation and

- when a new generic formulation is tested against the innovator's marketed product.[16]

REGULATORY NORMS CONSIDERING IN STUDY

Australia

In Australia, the Therapeutics Goods Administration (TGA) considers preparations to be bioequivalent if the 90% confidence intervals (90% CI) of the transformed natural log ratios, between the two preparations, of C_{max} and AUC lie in the range 0.80-1.25. T_{max} should also be similar between the products. (Birkett2003)

There are tighter requirements for drugs with a narrow therapeutic index and/or saturable metabolism thus no generic products exist on the Australian market for digoxin or phenytoin for instance

Europe

According to European regulations EMEA-CPMP, Note for Guidance on the investigation of Bioavailability and Bioequivalence, London, July 2001 CPMP/EWP/QWP/1401/98 two medicinal products are bioequivalent if they are pharmaceutically equivalent or pharmaceutical alternatives and if their bioavailabilities after administration in the same molar dose are similar to such a degree that their effects, with respect to both efficacy and safety, will be essentially the same. This is considered demonstrated if the 90% confidence intervals (90% CI) of the transformed natural log ratios, between the two preparations, of Cmax and AUC lie in the range 0.80-1.25.

United States

The United States Food and Drug Administration (FDA) has defined bioequivalence as, "the absence of a significant difference in the rate and extent to which the active ingredient or active moiety in pharmaceutical equivalents or pharmaceutical alternatives becomes available at the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study."

The FDA considers two products bioequivalent if the 90% CI of the relative mean C_{max}, $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$ of the test (e.g. generic formulation) to reference (e.g. innovator brand formulation) should be within 80.00% to 125.00% in the fasting state. Although there are a few exceptions, generally a bioequivalent comparison of Test to Reference formulations also requires administration after an appropriate meal at a specified time before taking the drug, a so-called "food-effect" study. A food-effect study requires the same statistical evaluation as the fasting study, described above.[17]

CONCEPTS OF CROSS OVER DESIGN

As recommended by the US FDA (1992),[1] in most bioequivalence trials, a "test" formulation is compared with the standard/innovator "reference" formulation, in a group of normal, healthy subjects (18-55 yr), each of whom receive both the treatments alternately, in a crossover fashion (two-period, two-treatment crossover design), with the two phases of treatment separated by a "washout period" of generally a week's duration, but may be longer (a minimum time equivalent to 5half-lives) if the elimination half-life of the drug is very long.

The treatment is assigned to each subject, randomly, but an equal number of subjects receive each treatment in each phase, as depicted in Table 1. Thus, in case of two treatments A and B, one group gets the treatment in the order AB, and the second group in the reverse order. This is done to avoid the occurrence of possible sequence or period effects.15 A similar allocation is done in case of a three-treatment crossover design (three-period, three-treatment crossover design).

For several drugs a great inter-subject variability in clearance is observed. The intra-subject coefficient of variation (approximately 15%) is usually substantially smaller than that between subjects (approximately 30%), and therefore, crossover designs are generally recommended for bioequivalencestudies.[18,19]

The primary advantage of the crossover design is that since the treatments are compared on the same subject, the inter-subject variability does not contribute to the error variability. If the drug under investigation and/or its metabolites have an extremely long half life, a parallel group design may be indicated. In a parallel group design, subjects are divided randomly into groups, each group receiving one treatment only. Thus, each subject receives only one treatment In a parallel design, although one does not have to worry about sequence, period or carry over effects, or dropouts during the study, the inter-subject variability being very high, the sensitivity of the test is considerably reduced, thus requiring a larger number of subjects compared to a crossover design, to attain the same sensitivity.Inherent in both the crossover and parallel designs are the three fundamental statistical concepts of study design, namely randomization, replication and error control.[20,21] Randomization implies allocation of treatments to the subjects without selection bias. Consequently, randomization is essential to determine an unbiased estimate of the treatment effects. Replication implies that a treatment is applied to more than one experimental unit (subject) to obtain more reliable estimates than is possible from a single observation and hence provides a more precise measurement of treatment effects. The number of replicates (sample size) required will depend upon the degree of differences to be detected and inherent variability of the data. replication is used concomitantly 7variability.

The various pharmacokinetic parameters (AUC, Cmax) derived from the plasma concentration-time curve are subjected to ANOVA in which the variance is partitioned into components due to subjects, periods and treatments. The classical null hypothesis test is the hypothesis of equal means, H0: $\mu T = \mu R$ (i.e. products are bioequivalent), where μT and μR represent the expected mean bioavailabilities of the test and reference formulations, respectively. The alternate hypothesis therefore is H1: $\mu T \neq \mu R$ (i.e. products are bioinequivalent). There are two ways of designing the experiment: (i) Design 1 (parallel group design): divide the subjects into two groups and assign one treatment to each group and; (ii) Design 2 (crossover design): consider each subject as a block and then apply both the treatments to each block (subject) on two different occasions. In a parallel group design, only the variability due to the treatment is separated out, whereas in the crossover design,

variability due to treatment, block (subject) and period are separated out from error variability. Consequently, the error sum of squares (SSE) is greater in the parallel design for a specific sample size (Error sum of squares in Design 1 (SSE1) = Error sum of squares in Design 2 (SSE2) + (subjects sum of squares) SSS2 + (periods sum of squares) PSS2 . As degrees of freedom for SSE are the same in both the designs (for two-treatment case), the error mean sum of square for Design 1 (MSE1) will be greater than the error mean sum of square for Design 2 (MSE2),[21] i.e. error variability is greater in the parallel group design compared to the crossover design.

Table 1: Analysis of variance (ANOVA) table for t-period, t-treatment Cross over design

Sources of variance	Degree of freedom(DF)	Sum of squares(SS)	Mean sum of squares(MS)	F statistic
Treatment	T^a-1	SST	MST	MST/MSE
Subject	N^b-1	SSS	MSS	MSS/MSE
Period	t-1	SSP	MSP	MSP/MSE
Error	(t-1)(n-2)	SSE	MSE	
Total	tn-1			

T^a is number of treatments; N^b is number of subjects; SST-Sum of squares due to treatments; SSS-Sum of squares due to subjects; SSP-Sum of squares due to period; SSE-Sum of squares due to error; MST Mean sum of squares due to treatments; MSS-Mean sum of squares due to subjects; MSP-Mean sum of squares due to period; MSE-Mean sum of squares due to error.

Table 2: Design 1 A comparison of ANOVA for parallel group design and 2-treatment, 2-period crossover design with n subjects

Sources of variation	Sum of squares(SS)	Degree of freedom(DF)	Mean sum of squares(MSS)	F statistic
Between treatments	SST_1	1	MST_1	MST_1/MSE_1
Error	SSE_1	N^a-2	MSE_1	
Total		n-1		

N^a: Number of subjects

Design 2

In ANOVA, the mean sum of squares due to a factor is compared with the mean sum of squares due to error (e.g. F= MST / MSE), and if these are comparable, no difference between the levels of a factor is concluded, otherwise a difference is concluded. Suppose there is a difference in treatments i.e. the treatment mean sum of squares is larger than the error mean sum of squares. Then the chances of the treatment mean sum of squares being larger than the error mean sum of squares are more in Design 2 compared to Design 1, since $MSE_2 < MSE_1$. Therefore, chances of showing a statistically significant difference (when actually there is a difference) are higher in Design 2 compared to Design 1. This is equivalent to saying that Design 2 is more powerful than Design 1. This reveals how the power of a test is influenced by the design of the experiment. In ANOVA, the ratio of the formulations' mean sum of squares to the error mean sum of squares gives an F-statistic to test the null hypothesis H0⬛µT=µR . This provides a test of whether the mean amount of drug absorbed from the test formulation is identical to the mean amount of drug absorbed from the reference. The test of this simple null hypothesis of identity is of little interest in bioequivalence studies, since the answer is always negative. This is because we cannot expect the mean amounts of drug absorbed from two different formulations or two different batches of the same formulation to be identical. They may be very nearly equal, but not identical. Also, if the trial is run under tightly controlled conditions (resulting in a small error mean sum of squares in the analysis) and if the number of subjects is large enough, no matter how small the difference between the formulations, it will be detected as significant.Thus the detection of the difference (which as indicated above, will always exist) becomes simply a function of sample size, and since the probable magnitude of the difference is the critical factor, this gives rise to two anomalies:

- A large difference between two formulations which is nevertheless not statistically

- Significant if error variability is high and/or sample size not large enough.

- A small difference, probably of no therapeutic importance whatsoever, that is shown to be statistically significant if error variability is minimal and/or sample size adequately large.

The first case suggests a lack of sensitivity in the analysis, and the second an excess of it. Consequently, any practice that increases the variability of the study (sloppy designs, assay variability and within formulation variability) would reduce the chances of finding a significant difference and hence improve the chances of concluding bioequivalence.

The FDA[22] therefore, recognized that a finding of no statistical significance in the first case was not necessarily evidence of bioequivalence and consequently asked for a retrospective examination of the power of the test of null hypothesis. Specifically,

it was mandated that the test of equivalence have at least an 80% power of detecting a 20% difference between μT and μR (the 80/20 rule), where 20% was apparently arbitrarily chosen to represent the minimum difference that could be regarded as of therapeutic importance. However, there was no such criterion for the second case, and consequently if a very small difference was shown to be statistically significant, the conclusion that the difference was negligible and that the formulations could be considered bioequivalent was based solely on clinical judgment. It was therefore realized that the testing of the simple null hypothesis was inadequate and inappropriate and what was needed was not a test of whether the two formulations were identical but some degree of assurance that the mean amount of drug absorbed using the test formulation was close to the mean amount absorbed in case of the reference.The test hypothesis therefore, needed to be reformulated.

Table 3: Design 2 A comparison of ANOVA for parallel group design and 2-treatment, 2-period crossover design with n subjects

Sources of variation	Sum of squares(SS)	Degree of freedom(DF)	Mean sum of squares(MSS)	F statistic
Between treatments	SST_2	1	MST_2	MST_2/MSE_2
Between blocks(subjects)	SSS_2	N^a-1	MSS_2	
Between periods	SSP_2	1	MSP_2	
Errors	SSE_2	n-2	MSE_2	
Total		2n-1		

Another argument which favoured an alternate approach to ANOVA for bioequivalence determination was the magnitude of the manufacturer's risk versus the consumer's risk. Manufacturer's risk is defined as the probability of rejecting a formulation which is in fact bioequivalent. In other words, the manufacturer's risk is the probability α) of rejecting H0

When H0 is true (Type I error), and this risk was fixed at α =0.05 by the FDA (1977).[20] Similarly, the consumer's risk is defined as the probability (β) of accepting a formulation which is bioinequivalent, i.e. accepting H0 when H0 is false (Type II error).

By introducing the requirement that the power (1-β) of the test should be 80%, the FDA sought to restrict the consumer's risk "β" to 20%. This, however, was not a satisfactory solution for either the consumer or the regulatory agencies. In a regulatory environment, it makes sense that the regulatory authorities control the consumer's risk and let the pharmaceutical company decide how much manufacturer's risk they are willing to accept. As indicated, neither of these risks are formally identified or controlled when using the ANOVA F-test for treatments, even with the 80/20 rule. Also the FDA guidelines1 for bioavailability studies state that "Products whose rate and extent of absorption differ by 20% or less are generally bioequivalent". This implies that in the case of bioequivalence studies the interest is not in testing the null hypothesis of equality but in assessing the difference in two treatments. Bioequivalence is concluded if this difference is within 20% of the reference mean.[22]

APPLICATIONS OF BA/BE STUDIES

Comparative bioavailability: a universal approach

Most bioavailability studies, whether for a new or generic product, possess a common theme. A test is conducted to identify the quantitative nature of a specific product comparison. This comparison for a new drug may be, for example, to assess the performance of an oral formulation relative to that of an intravenous dose, or perhaps the performance of a modified-release formulation in comparison to a conventional capsule. For a generic product, it is typically a comparison of a competitive formulation with a reference product. Such commonality surrounding comparative bioavailability studies suggests a universal experimental approach.

Comparative bioavailability studies for new drugs (NDA)

The initial oral formulation for a new drug is frequently used to conduct early human studies of safety and efficacy. Often, early oral bioavailability information about the drug (and this initial formulation) is obtained by means of studies comparing it with an intravenous dose and/or a solution of the drug they employ the Universal Approach wherein the comparator is an intravenous dose or perhaps a solution of the drug.

Comparative bioavailability for generic drug products (ANDA)

When a manufacturer thereby wishes to gain therapeutic equivalence by introducing a competitive generic product into the marketplace, it is not necessary to conduct the full array of trials needed for the first(innovative) product. If equivalence has been demonstrated, according to prescribed study requirements appropriately determined metrics the generic product by inference is regarded as therapeutically equivalent to the innovative drug product.

Testing under fasting conditions

When the particular drug is not showing any expected results, then the drug is tested under fasting conditions using BE trials.

Testing under fed conditions

The drug can also be tested under fed conditions to meet all conditions as per regulatory norms.[23-30]

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