CHAPTER 1

Bioavailability

Introduction

Biopharmaceutics is the study of the interrelationship of the physicochemical properties of the active pharmaceutical ingredient (API), and its pharmacokinetic and pharmacodynamic behavior. Pharmacokinetics is "What the body does with the drug", while the pharmacodynamics is "what the drug does to the body". Biopharmaceutics also considers the formulation of the drug product including excipients, the method of manufacturing, and the route of drug administration. In terms of regulatory product quality attributes, results from bioequivalence (BE) studies and certain bioavailability (BA) studies may be viewed as "product quality performance specifications". A specific regulatory challenge is to validate the methods used to assess the results from these studies to assure that the BA and BE data generated relate in a well-defined and meaningful way to safety and efficacy of the drug product.

Bioavailability is defined in various ways. For drugs and other substances that act within the body (as contrasted to within the gut), it is

generally considered to be the quantity or fraction of an administered dose of a substance that gets into the circulation and then is not metabolized, complexed or excreted before it can exert its intended biological effect.

Bioavailability is defined in § 320.1 of US FDA guidelines for industry as: *the rate and extent to which the active ingredient or active moiety is absorbed from a drug product and becomes available at the site of action. For drug products that are not intended to be absorbed into the bloodstream, bioavailability may be assessed by measurements intended to reflect the rate and extent to which the active ingredient or active moiety becomes available at the site of action.*

Usually, the "bioavailability" is the fraction of an extravascular dose that goes to the central blood compartment. But the exceptions exist e.g. Topical dosing (bioavailability is then drug delivered to site of action). Intravenous (IV) doses are 100% bioavailable and are the basis for absolute bioavailability calculations.

If the BA of two or more products are found almost similar (on comparison), they are called as Bioequivalent (BE). More precisely, 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.

Importance of Studying Bioavailability

Except the parenterals, the "true dose" is not the drug administered, but is the drug available to exert its effect. The drug becomes available to the systemic circulation for exerting the effect after dissolution (dissolving into the gastrointestinal fluid), absorption (permeation across the biological membranes) and surviving metabolism. The drug may show very low bioavailability due to one (or more) of the following reasons.

- Dosage form or drug may not dissolve readily
- Drug may not be readily pass across biological membranes (or be absorbed)
- Drug may be extensively metabolized during absorption process (first-pass, gut wall, liver)

So it can be understood that a variable bioavailability may produce variable exposures and thus variable effects.

Concept of Bioavailability

A schematic illustration of the steps involved in the release and absorption of a drug taken as an oral solid dosage form is presented in Fig. 1.1.

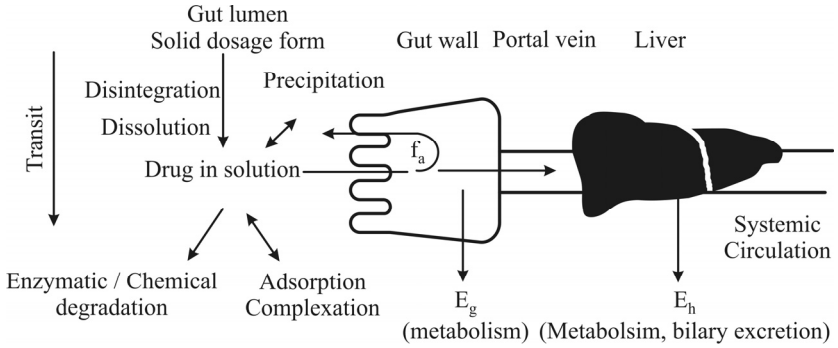

Fig. 1.1 Processes influencing the bioavailability of orally administered drugs.

The oral bioavailability can be divided into three major determinants, according to the following equation:

$$F = f_a . (1 - E_g) . (1 - E_h)$$

where f_a is the fraction of the dose that is absorbed across the apical cell membrane of the enterocyte and E_g and E_h are the extraction of the drug over the gut and liver, respectively.

The f_a may be limited by all the reactions that may happen in the lumen and at the apical membrane. This includes the dissolution of the drug in the gastrointestinal (GI) tract, since in order to be absorbed in the GI, a drug has to be dissolved. This can be a problem with poorly water-soluble substances, for which the dissolution often limits the absorption after oral administration. Most of the new substances in drug development today are highly lipophilic, and the solubility and dissolution rates in gastric and intestinal fluids (IF) are therefore often critical for the oral bioavailability.

Biopharmaceutical Classification System (BCS)

The Biopharmaceutical Classification System (BCS), which was proposed by Amidon et al. in 1995, classifies drugs into four different groups (Table 1.1), depending on their solubility and permeability. BCS

is a drug development tool that allows estimation of the contribution of three fundamental factors including dissolution, solubility and intestinal permeability, which govern the rate and extent of drug absorption from solid oral dosage forms. Drug dissolution is the process by which the drug is released, dissolved and becomes ready for absorption. Permeability is referred to the ability of the drug molecule to permeate through a membrane into the systemic circulation. The intention of the system (BCS) was to set up a theoretical basis for correlating the *in vitro* dissolution profiles with *in vivo* bioavailability of drugs. BCS is also a fundamental guideline for determining the conditions under which *in vitro in vivo* correlations (IVIVCs) are expected. It is also used as a tool for developing the *in vitro* dissolution specification. The BCS can be employed as a tool to develop a strategy for improving the bioavailability of new chemical entities. Additionally, the system provides information about whether a compound's BA is solubility or permeability limited.

Table 1.1 The biopharmaceutical classification system

Class	Solubility	Permeability	General properties of drugs of the class	Examples of the Class
I	High	High	Water Soluble, (high C_S value resulting in a high C_{Aq} value); well absorbed from GIT (larger P values); lipophilic with a MW ≤ 500Da and aqueous solubility ≥ 1 mg/mL; D : S ≤ 250mL	Paracetamol, piroxicam*, propranolol, theophylline, rofecoxib
II	Low	High	relatively lipophilic and water insoluble drugs (C_S ≤ 0.1 mg/mL); well absorbed from GIT (large P values); D : S ≥ 250 mL	Carbamazepine, digoxin, cinnarizine, glibenclamide, miconazole, nimesulide, nifedipine, phenytoin, spironolactone, tolbutamide, Itraconazole
III	High	Low	Water Soluble (high C_S and high C_{Aq}); do not readily permeate biomembranes (low P); D : S ≤ 250mL	Acyclovir, atenolol, ranitidine, diphenhydramine

Table* 1.1 *contd…

Class	Solubility	Permeability	General properties of drugs of the class	Examples of the Class
IV	Low	Low	water-insoluble (Low C_S and low C_{Aq}); do not readily permeate biomembranes (low P); **D : S** ≥ 250mL	Furosemide, cyclosporine A

***** *piroxicam is practically insoluble in water but is a potent drug with low enough* ***D : S*** *ratio to be classified as a class I drug.*

C_S is saturation solubility of the drug in the aqueous fluid; C_{Aq} is the drug concentration in the aqueous exterior immediately adjacent to the mucosal surface; P is permeability coefficient of the drug through the lipophilic mucosa; D:S is dose to solubility ratio.

When a drug shows a dose to solubility ratio (D:S) of 250 ml or lower at 37 °C over a **pH range of 1.2–6.8**, it can be classified as "**highly soluble**". The pH was decreased from 7.5 in the FDA guidance to 6.8 in WHO Expert Committee on Specifications for Pharmaceutical Preparations, 40th Report, 2006, this reflects the need to dissolve the drug before it reaches the mid-jejunum to ensure absorption from the gastrointestinal tract. A drug is classified as "**highly permeable"** if the fraction absorbed is **> 85 %** (from solution). In WHO revisions to the criteria for BCS classification, the permeability criterion was relaxed from 90% in the FDA guidance (40th Report, 2006) to 85%, which shifted some BCS class III drugs to class I drugs e.g. paracetamol, acetylsalicylic acid, allopurinol, lamivudine and promethazine.

Modeling of BCS and Key Parameters

The BCS is based on a simple absorption model, in which the intestine is a cylindrical tube where absorption occurs; particles are spheres of the same size; there are no reactions (i.e., there is no metabolism) in the intestine; solubility is independent of the particle size and the intestinal pH gradient; and no aggregation occurs. Amidon et al. have demonstrated that the key parameters controlling drug absorption are three dimensionless numbers: an Absorption Number, A_n; a Dissolution Number, D_n; and a Dose Number, D_o; representing the fundamental processes of membrane permeation, drug dissolution and dose, respectively:

Absorption Number (A_n)

The Absorption Number (A_n) is the ratio of the Mean Residence Time (T_{res}) to the Mean Absorption Time (T_{abs}) and is calculated by equation 1.

$$A_n = T_{res} / T_{abs} \quad \text{.....(1)}$$

or $$A_n = (P_{eff} / R)\, T_{res}$$

where T_{res} is the mean residence time (~180 min), P_{eff} is the effective permeability, and R is the radius of the intestinal segment.

Dissolution Number (D_n)

The Dissolution Number (D_n) is the ratio of T_{res} to Mean Dissolution Time (T_{diss}) and could be estimated using equation 2.

$$D_n = T_{res} / T_{diss} \quad \text{.....(2)}$$

T_{diss} is the time required for a drug particle to dissolve

Dose Number (D_o)

The Dose Number (D_o) is calculated using equation 3.

$$D_o = (M_o/V_o)/ C_s \quad \text{.....(3)}$$

where M_0 is the dose of drug administered, V_0 is the initial gastric volume (~250 ml), C_s is the saturation solubility,

Class I compounds such as metoprolol exhibit a high absorption (A_n) and a high Dissolution (D_n) number. The rate-limiting step to drug absorption is drug dissolution or gastric emptying rate if dissolution is very rapid.

Class II drugs such as phenytoin has a high absorption number, A_n, but a low dissolution number, D_n. *In vivo* drug dissolution for Class II drugs is, therefore, a rate limiting factor in drug absorption (except at very high dose number, D_o) and consequently absorption is usually slower than Class I and takes place over a longer period of time.

Class III drugs, such as cimetidine, are rapidly dissolving and permeability is the rate controlling step in drug absorption.

Class IV drugs are low solubility and low permeability drugs. This class of drugs exhibit significant problems for effective oral delivery. It is anticipated that inappropriate formulation of drugs fall in class IV, as in the case of class II drugs, could have an additional negative influence on both the rate and extent of drug absorption.

Types of Bioavailability

Bioavailability Dose: Dose available to the patient to give therapeutic effect is called as bioavailable dose, which is always less than the administered dose.

Systemic bioavailability: The amount of drug that reaches the systemic circulation is known as 'Systemic bioavailability'.

Bioavailable fraction: It refers to the fraction of administered dose.

F = Bioavailable dose / Administered dose

Absolute bioavailability: Comparison between systemic availability of orally administered drug with IV administered one. (Denoted by F).

or "*Absolute bioavailability*" *compares an extravascular formulation to an IV formulation*

Relative bioavailability: Comparison between systematic availability of orally administered drug with an oral standard of same drug. Denoted by F_r.

Or "Relative bioavailability" compares 2 extravascular formulations

Bioavailability Calculations	
Systemic clearance	CL(iv) = Dose(iv) / AUC(iv)
Apparent (oral) clearance	CL(oral) = CL(iv) / F = Dose(oral) / AUC(oral)
Set CL(iv) equivalent	CL(iv) = Dose(iv) / AUC(iv) = (Dose(oral) • F)/ AUC(oral)
Absolute bioavailability	F = [Dose(iv) •AUC(oral)] / [Dose(oral) •AUC(iv)]

Objectives of Bioavailability Studies

Bioavailability studies are done in clinical, academic, and regulatory interest. The latter includes agencies that approve the sale of products in their nation(s), as well as regulatory agencies. Applications from manufacturers seeking regulatory approval for a new drug (New Drug Application (NDA) must furnish exhaustive information about a drug's pharmacokinetics. Typically, such evidence involves studies wherein the drug has been orally administered. While such trials may broadly be viewed as bioavailability studies, many are apparently designed to assess the drug's safety and efficacy via strategies of dose escalation and chronic administration. The more pertinent interest in bioavailability relates to questions about absolute extent of absorption (absolute bioavailability), the importance of product formulation changes that are made during a new drug's development process, the comparability of different oral

dosage forms (e.g. modified-release versus conventional products), and whether the products can be administered with meals. Therefore, objective of BA studies can be summarized as followed.

1. Development of new drug entity.
2. Determination of influence of
    - Excipients.
    - Patient related factors.
    - Possible interaction with other drugs.
3. Development of new formulations.
4. To control the quality of drug.
5. To determine the
    - Processing factors.
    - Storage
    - Stability on drug absorption.

Factors Affecting Bioavailability

Bioavailability following oral doses may vary because of either patient-related or dosage-form-related factors. Patient factors can include the nature and timing of meals, age, disease, genetic traits and gastrointestinal physiology. The dosage form factors include:

1. the chemical form of the drug (salt vs. acid),
2. its physical properties (crystal structure, particle size), and
3. an array of formulation (non-active ingredients) and manufacturing (tablet hardness) variables. Some important factors affecting BA are described as followed.

- **Food effects**

  Co-administration of food with oral drug products may influence drug BA and/or BE. Food effect BA studies focus on the effects of food on the release of the drug substance from the drug product as well as the absorption of the drug substance. BE studies with food focus on demonstrating comparable BA between test and reference products when co-administered with meals. Usually, a single-dose, two-period, two-treatment, two-sequence crossover study is recommended for both food-effect BA and BE studies. ***-FDA***

  (i) Food may increase, decrease, or have no effect on the rate and/or the extent of absorption.
    - May affect rate and extent independently

- Food affects GI motility and also can increase solubilization of drugs
- Change may depend on content of meal

(ii) Food may mitigate nausea. Vomiting tends to decrease bioavailability

(iii) Dose time and food: Timing of dose with respect to food also affects the bioavailability of administered drugs.

- **Physiology related factors**

Bilayer structure of cell membranes

A drug when administered to the body, first dissolves into the gastric fluid (hydrophilic environment) and then it permeates across the biological membranes (lipophilic environment), finally reaching into the blood. For good bioavailability, a drug must have an adequate hydrophilicity (for dissolution into gastrointestinal fluid) as well as an adequate lipophilicity (to permeate across the lipidic biomembrane). So, the drugs too lipophilic won't dissolve while the drugs too hydrophilic won't transverse lipid outer layer of cell. Thickness and blood supply of membranes also play the role in BA.

GI transit time

How much time a drug spends in transit through GIT is responsible factor in BA. Acetaminophen is a useful probe drug to assess GI transit

pH environment

GIT shows a variety of pH (pH 6.6 (buccal), pH 1.2 (stomach), pH 6.8 duodenum, pH 7-8 (small intestine)) throughout its length from oral cavity to colon. Depending on pKa, drug may be charged or uncharged in different regions and its absorption and hence the BA may vary (pH partition hypothesis: Un-ionized drug is absorbed through membranes; Charges species don't get through easily; Ionization of drug molecule depends on pH of the site e.g. weakly acidic drugs are unionized at acidic pH of stomach and hence absorbed from the gastric region). For acids, a pH below the pKa enhances absorption, while for bases; a pH above the pKa enhances absorption.

Metabolic activity (induction, inhibition or first pass metabolism)

Several drugs selectively increase or decrease the activity of cytochrome P450, these are called enzyme inducer and enzyme inhibitors, respectively (Table 1.2). Enzyme induction usually occurs within several days and increases liver weight, microsomal protein content and biliary secretion. Enzyme induction usually increases the activity of glucuronyl transferase, and thus enhances drug conjugation. In some instances, drugs may induce their own metabolism (auto-induction). On the other hand enzyme inhibition may increase plasma concentrations of other concurrently used drugs, resulting in drug interactions.

Table 1.2 Drugs inducing or inhibiting Cytochrome P450

Inducers	Inhibitors
Barbiturates	Imidazoles (cimetidine, etomidate,
Phenytoin	ketoconazole, omeprazole)
Carbamazepine	Macrolide antibiotics (erythromycin,
Rifampicin	clarithromycin)
Griseofulvin	Antidepressants
Alcohol (chronic consumption)	HIV protease inhibitors
Polycyclic hydrocarbons	Cyclosporin
(tobacco Amiodarone smoke,	Gestodene
grilled meat)	

Another most common metabolic factor governing the bioavailability of a large number of drugs is first pass or presystemic metabolism. After oral administration, some drugs are extensively metabolized by the gut wall (e.g. chlorpromazine, dopamine) or by the liver (e.g. lidocaine, pethidine) before they enter the systemic circulation. In these conditions, oral administration may not produce adequate plasma concentrations in the systemic circulation and may result in an impaired response to drugs. As hepatic, renal and cardiac diseases are important factors affecting the variable response to drugs, the pathological changes may also affect the metabolism and clearance of drugs in an unpredictable manner. In severe

hepatic disease (e.g. cirrhosis or hepatitis), the elimination of drugs that are primarily metabolized may be impaired.

Surface area for absorption

Mucosal surface area is more extensive in the upper small intestine than the stomach, and hence most drugs, whether acids or bases, are predominantly absorbed from the duodenum.

- **Drug related factors**

Particle size of drug

In general reduction of particle size of a drug increases the effective surface area (surface area of drug available for dissolution) and hence the bioavailability. But particle size reduction in hydrophobic drugs (like aspirin) results in decrease in effective surface area and hence the (lowered) absorption.

Crystal structure

In general the amorphous drugs are more soluble than the crystalline one in aqueous (gastric) fluids. So the amorphous drugs are more absorbed.

Polymorphic forms

If a drug shows polymorphism (existence of more than one crystalline forms of a drug), some of its ploymorphs show more bioavailability than the others. Chloramphenicol palmitate is found in 3 polymorphic forms (A, B, C). Out of the three forms form B shows best bioavailability while the form A is virtually inactive biologically.

Drug-Drug interactions

Drug interactions which occur mainly due to enzyme induction or inhibition, change in gastric motility and gastric pH affect the bioavailability of concomitant drugs. Enzyme induction (in the gut or liver) lowers the bioavailability, while enzyme inhibition increases the bioavailability of a drug. On the other hand, drugs affecting gastric motility can modify drug dissolution, and influence the rate, but not the extent, of drug absorption. In particular, drugs that slow gastric emptying (e.g. atropine, morphine) decrease the rate of drug absorption. Other drug interactions, as between tetracyclines and iron, or colestyramine and digoxin, may affect the extent of drug absorption and thus modify systemic bioavailability. Drug interaction may also

occur due to change in gastric or urinary pH. For example, the bioavailability of a drug which is predominantly absorbed in gastric pH may be reduced due to concomitant administration of antacids.

Instability of the drug

The drug itself may have instability in the GI tract either due to chemical instability in acidic environment or due to extent of metabolism via enzymes in gut. This instability can have impact on bioavailable fraction of the drug.

- **Pharmaceutical factors**

Pharmaceutical factors like particle size, chemical formulation, the inclusion of inert fillers and the outer coating of the tablet influence the dissolution of tablets and capsules. In these circumstances, proprietary or generic preparations of the same drug may have different dissolution characteristics and thus produce a range of plasma concentrations after oral administration. At one time, differences in the potency of digoxin tablets suspected from clinical observations were eventually traced to variations in the dissolution of different preparations of the drug. Similarly, toxic effects were produced by diphenylhydantoin (phenytoin) tablets when an excipient (calcium sulphate) was replaced by lactose. In these conditions, dissolution was more rapid, resulting in faster and more extensive absorption, and higher blood levels of the drugs. Manufacturing process may also affect the bioavailability, for example tablets with more hardness may show less bioavailability.

Methods of Improving Bioavailability

There are three major approaches in overcoming the bioavailability problems.

1. ***The pharmaceutical approach:*** It involve modification of formulation manufacturing process or the physicochemical properties of drugs without changing the chemical structure (Table 1.1).
2. ***The pharmacokinetic approach:*** In which the pharmacokinetics of the drug is altered by modifying its chemical structure.
3. ***The biological approach:*** In this approach the route of drug administration may be changed such as changing from oral to parenteral.

Various methods have been investigated for improvement in bioavailability. Table 1.3 gives a summary of all these methods.

Table 1.3 Methods of improving bioavailability

Methods	Mechanism involved	Methodology	Examples
Use of co-solvent	Increase in solubility of drug	Addition of co-solvent ethanol, propylene, glycol, glycerin	Analgesic syrups of paracetamol
Hydrotrophy method	Addition of large amounts of a second solute results in an increase in the aqueous solubility of another solute	Adding hydrotropic agents like urea, nicotinamide etc.	Paracetamol
By addition of polar group	Increasing water solubility by increasing hydrogen bonding and interaction with water	Addition of polar group in the structure of drug (carboxylic acid and amine)	--
Use of solid solution	Improving solubility by preparing sol-gel form of the drug	Fusion, melting	Succinic acid
Solid dispersion	Decreasing the drug's particle size changes the microenvironment of the drug particle which increases the dissolution rate and absorption	Prepared by fusion, solvent evaporation,	Paracetamol-urea
Eutectic mixture	When exposed to water the soluble carrier dissolves leaving the drug in micro crystalline state which solubilize rapidly	Fusion	Paracetamol-camphor
Micronization	Increasing the effective surface area of drug by decreasing particle size	By spray drying and by use of fluid energy mill	Griesiofulvin and several steroidal and sulpha drugs

Table 1.3 *Contd...*

Methods	Mechanism involved	Methodology	Examples
Use of surfactants	By promoting wetting and penetration of dissolution fluid into the drug	Addition of suitable surfactants (polysorbate)	Spironolactone is a drug whose bioavailability is increased by this method
Alternation of pH of solvent	Changing the pH of drug in solution	Salt formation, addition of buffer	Buffered tablets of aspirin
Use of metastable polymorphs	Metastable forms show better solubility than stable form	Converting the stable form to metastable form	Using B form of chlorom-phenicol than A and C forms
Solvates formation	Powder of submicron size having increased surface area show the improved solubility	Freeze drying of solute with organic solvent	Benzene solvate
Selective absorption on insoluble carrier	Weak physical interaction between adsorbate and adsorbant through hydration and swelling of clay in aqueous media improves solubility	Use of highly active adsorbant, clay like bentonite	Indomethacin, Prednisone
Cyclodextrin complexation	Inclusion of hydrophobic groups of drug in the core of cyclodextrin cavity and thereby improving solubility	Formation of drug Complex with cyclodextrin (α, β, γ) or its derivatives	Meloxicam, phenytoin,
Phospholipid complexation	Drug-Lipid complexes improve amorphous nature of drug in the complexes and being amphiphilic in nature the complex show improved solubility and dissolution	The phospholipid complexes of drug without the presence of covalent bond are formed.	aceclofenac, aspirin, curcumin, silybin etc.

Bioavailability Study Characteristics

With recently introduced products properly conducted bioavailability studies should have been performed before the product is allowed to be

marketed. However, products which were approved sometime ago may not have been tested as thoroughly. It is therefore helpful to be able to evaluate the testing which may have been undertaken.

The evaluation of a drug product bioavailability study involves the consideration of various factors. Some are:

1. **Drug**
   (a) The drug substance in each product must be the same
   (b) Bioavailability studies are conducted to compare two or more products
   (c) Different chemical substances cannot be compared
   (d) Compare the drug products with the same drug in each dosage form
2. **Drug product**
   (a) Comparison is made between two or more similar products containing exactly the same chemical substance.
   (b) Different dosage form can be compared when they contain same drug.
3. **Subjects**
   (a) Health
      (i) Subjects of similar kinetic characteristics have taken, so major variations are not introduced.
      (ii) Medical examination will be used to confirm their medical state.
      (iii) For some drugs there may be special disease state which causes exclusion of volunteers.
   (b) Age
      Age can have a significant effect on drug pharmacokinetic.
      Subject between the ages of 18-35 year are preferred.
   (c) Weight
      To better match the subjects with normal weights are preferred.
   (d) Enzyme status
      Smokers or subjects taking certain drug having altered enzyme activity or having drug-drug interaction may be excluded from the study. If these subjects are included, their effect adds complications to study. Therefore, an attempt is usually made to minimize these factors.

(e) Number

Usually 20-20 subjects are used.

(f) Assay

Same assay method should be used far all phases. Assay method should be sensitive and specific.

(g) Design

Usually Cross over design is used.

Bioavailability Studies

Bioavailability studies are designed to determine either an absolute BA (relative to an IV formulation) or relative BA (with an alternate reference dosage form with good absorption characteristics). They can be used to compare different route of administration.

Ex: oral versus iv, ip versus im.

The bioavailability study should be carried out in patient for whom the drug is intended to be used. Because of the following advantages-

- The patient will be benefitted from the study
- Reflects better the therapeutic efficacy of the drug
- Drug absorption in disease states can be evaluated
- Avoids the side effects of the drug to healthy one

There are some drawbacks of using patient volunteer for study like:

- Disease state, other drugs etc. modify the drug absorption,
- Establishing a standard set of conditions necessary for bioavailability study is difficult with patients as volunteer

So healthy patients are taken for bioavailability study to avoid inter subject variability.

Study are performed in young healthy male adult, age 20-40 and body weight within narrow range of ±10%, under restricted dietary and fixed activity condition.

***Note*:** Drug wash out period for a minimum of ten biological half lives must be allowed for between any two studies in the same subject

Measurement of Bioavailability

It is divided into two categories:

1. Pharmacokinetic method

(a) Plasma level time studies
(b) Urinary excretion studies

2. Pharmacodynamic method
   (a) Acute pharmacologic response
   (b) Therapeutic response

Plasma Level Time Studies

Principle: The method is based on the assumption that two dosage forms that exhibit super imposable plasma level time profiles in a group of subjects should result in identical therapeutic activity (and they would be termed as bioequivalent). These studies cab be single dose or multiple dose studies (Table 1.4).

Single Dose Study

Following steps are involved in a single dose study.

- Collection of serial blood samples for period of 2-3 biological half lives after drug administration.
- Analysis for drug concentration.
- Making a plot of plasma concentration versus time of sample collection.
- Obtain plasma level time profile by this plot.

***Note*:**

- *For IV dose sampling should start within 5 minutes of drug taken. At least 3 sample point should be taken*
- *For oral at least 3 sample point*

FDA Guidelines on Collection of Blood Samples

1. When comparison of the test product and the reference material is to be based on blood concentration time curves, unless some other approach is more appropriate for valid scientific reasons, blood samples should be taken with sufficient frequency to permit an estimate of both:
   (i) The peak concentration in the blood of the active drug ingredient or therapeutic moiety, or its metabolite(s), measured; and
   (ii) The total area under the curve for a time period at least three times the half-life of the active drug ingredient or therapeutic moiety, or its metabolite(s), measure

2. In a study comparing oral dosage forms, the sampling times should be identical.
3. In a study comparing an intravenous dosage form and an oral dosage form, the sampling times should be those needed to describe both:
   (i) The distribution and elimination phase of the intravenous dosage form; and
   (ii) The absorption and elimination phase of the oral dosage form.
4. In a study comparing drug delivery systems other than oral or intravenous dosage forms with an appropriate reference standard, the sampling times should be based on valid scientific reasons.

Table 1.4 Single dose verses multiple dose study

Type of Study	Advantages	Disadvantages
Single Dose Study	• Easy to conduct. • Offer less exposure to drug. • Less tedious.	• Dose does not give any idea of drug/metabolites. • Difficult to predict the steady state characteristics of the drugs.
Multiple Dose Study*:	• Easy to predict the peak and valley characteristics of drug • Fewer blood samples requirements • Less sensitive analytical method • Performed in patients because of the therapeutic benefits to the patients • Small inter subject variability • Better evaluation of the performance • Nonlinearity in pharmaco-kinetics, easily detected	• Difficult to control • Highly tedious • Time consuming • Drugs having long half lives require longer period to achieve steady state, • Exposes the subject to more drug

* For this study the drug should be administered for 5-6 elimination half lives before collecting the blood.

Basic Pharmacokinetic Parameters of Plasma level time Studies

Pharmacokinetics provides a mathematical basis to assess the time course of drugs and their effects in the body. It enables the following processes to be quantified:

Absorption, Distribution, Metabolism, and Excretion.

These pharmacokinetic processes often referred to as ADME; determine the drug concentration in the body when medicines are prescribed. A fundamental understanding of these parameters is required to design an appropriate drug regimen for a patient.

The effectiveness of a dosage regimen is determined by the concentration of the drug in the body. Ideally, the concentration of drug should be measured at the site of action of the drug; that is, at the receptor. However, owing to inaccessibility, drug concentrations are normally measured in whole blood from which serum or plasma is generated. Other body fluids such as saliva, urine and cerebrospinal fluid (CSF) are sometimes used. It is assumed that drug concentrations in these fluids are in equilibrium with the drug concentration at the receptor.

Upon oral (or IV) administration, when plasma concentration of drug is plotted against the time, a plasma level time profile curve (Fig. 1.2) can be plotted. A plasma level time profile curve shows the following pharmacokinetic parameters, which should be studied to assess the bioavailability.

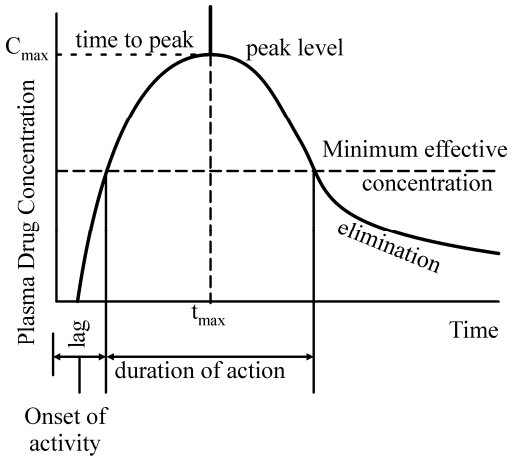

Fig. 1.2 A plasma level time profile curve of a orally administered drug.

Various parameters which should be reported as per FDA guidelines in a BA study are as followed:

$AUC_{0\text{-}t}$, $AUC_{0\text{-}\infty}$, Cmax, Tmax, λz, and $t_{1/2.}$

Area under the Concentration vs Time Curve (AUC)

Area under the curve of plasma concentration of a drug versus the time after single-dose administration. This denotes the bioavailability.

$$\mathbf{BA = AUC_{oral}/AUC_{iv}}$$

$AUC_{0\text{-}t}$

It is area under the plasma concentration-time curve from 0 hr to the last quantifiable concentration to be calculated using the trapezoidal rule (Fig. 1.3).

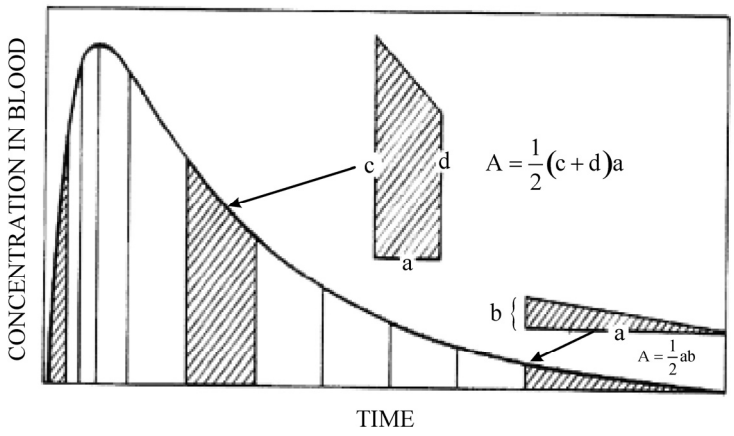

Fig. 1.3 AUC estimated by numerical integration techniques; for example, trapezoidal rule (smaller rectangular areas are integrated) as shown.

$AUC_{0\text{-}\infty}$

Area under the plasma concentration-time curve from zero to infinity is calculated as the sum of $AUC_{0\text{-}t}$ plus the ratio of the last measurable concentration to the elimination rate constant.

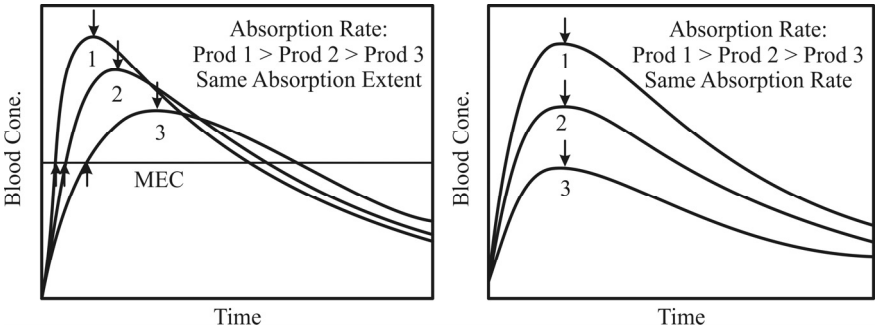

Fig. 1.4 AUC in understanding bioavailability.

- Rate of drug absorption mainly affects the time to onset of action (↑), as well as timing and magnitude of maximal effect (↓).
- Extent of absorption affects maximal effect and overall exposure as measured by area under the blood concentration-time curve (AUC).

Maximum Concentration (Cmax)

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

Time of Cmax (Tmax)

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

Terminal or elimination rate constant (λ_z)

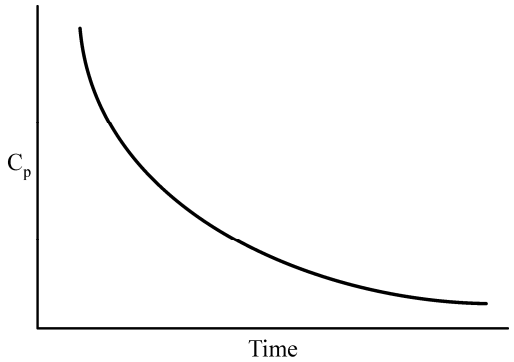

Fig. 1.5 Plasma concentration (Cp) versus time profile of a drug (one-compartment model).

Consider a single IV bolus injection of drug X (see Fig. 1.5). As time proceeds, the amount of drug in the body is eliminated. Thus the rate of elimination can be described (assuming first-order elimination) as:

$$dX/dt = -k X$$

Hence

$$X = X_0 \exp(-kt)$$

where X = amount of drug X, X_0 = dose and *k* =first-order elimination rate constant.

Half-life ($t_{1/2}$)

The time required to reduce the plasma concentration to one half its initial value is defined as the *half-life* ($t_{1/2}$)

$$t_{1/2} = 0.693/ k \qquad \text{(for first order reaction)}$$

where, k = first-order elimination rate constant.

Multiple Dose Study

Some drugs may be used clinically on a single-dose basis, although most drugs are administered continually over a period of time. When a drug is administered at a regular dosing interval (orally or IV), the drug accumulates in the body and the serum concentration will rise until steady-state conditions have been reached, assuming the drug is administered again before all of the previous dose has been eliminated (see Fig. 1.6).

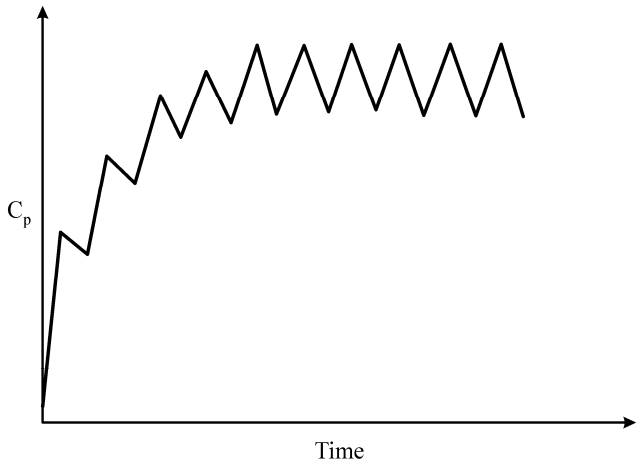

Fig. 1.6 Time profile of multiple IV doses.

At steady state the rate of drug administration is equal to the rate of drug elimination. At steady state the plasma concentrations of the drug (C^{ss}_{p}) at any time during any dosing interval, as well as the peak and trough, are similar. The time to reach steady-state concentrations is dependent on the half-life of the drug under consideration.

Following steps are involved in a multiple dose study

- Administration of drug for at least 5 biological half lives (administration of at least 5 doses) to reach steady state.
- Collection of blood sample at the end of previous dose interval.
- 8-10 sampling after administration of next dose.

Steady state plasma concentrations $C_P^{SS} = \dfrac{D}{\tau \times CL}$

where **D** is dose, τ is dosing interval and CL is Clearance

$$\text{Bioavailability} = \frac{[AUC]_{test}\ D_{std}\ T_{test}}{[AUC]_{std}\ D_{test}\ T_{std}}$$

§ 320.27 FDA Guidelines on the design of a multiple-dose *in vivo* bioavailability study

(a) Basic Principles

1. In selected circumstances it may be necessary for the test product and the reference material to be compared after repeated administration to determine steady-state levels of the active drug ingredient or therapeutic moiety in the body.
2. The test product and the reference material should be administered to subjects in the fasting or nonfasting state, depending upon the conditions reflected in the proposed labeling of the test product.
3. A multiple-dose study may be required to determine the bioavailability of a drug product in the following circumstances:
   (i) There is a difference in the rate of absorption but not in the extent of absorption.
   (ii) There is excessive variability in bioavailability from subject to subject.
   (iii) The concentration of the active drug ingredient or therapeutic moiety, or its metabolite(s), in the blood

resulting from a single dose is too low for accurate determination by the analytical method.

(iv) The drug product is an extended release dosage form.

(b) Study Design

1. A multiple-dose study should be crossover in design, unless a parallel design or other design is more appropriate for valid scientific reasons, and should provide for a drug elimination period if steady-state conditions are not achieved.
2. A multiple-dose study is not required to be of crossover design if the study is to establish dose proportionality under a multiple-dose regimen or file of a new drug product, a new drug delivery system, or a extended release dosage form.
3. If a drug elimination period is required, unless some other approach is more appropriate for valid scientific reasons, the drug elimination period should be either:
   (i) At least five times the half-life of the active drug ingredient or therapeutic moiety, or its active metabolite(s), measured in the blood or urine; or
   (ii) At least five times the half-life of decay of the acute pharmacological effect.

(c) Achievement of steady-state conditions

Whenever a multiple-dose study is conducted, unless some other approach is more appropriate for valid scientific reasons, sufficient doses of the test product and reference material should be administered in accordance with the labeling to achieve steady state conditions.

(d) Collection of blood or urine samples

1. Whenever comparison of the test product and the reference material is to be based on blood concentration time curves at steady state, appropriate dosage administration and sampling should be carried out to document attainment of steady state.
2. Whenever comparison of the test product and the reference material is to be based on cumulative urinary excretion-time curves at steady state, appropriate dosage administration and sampling should be carried out to document attainment of steady state.
3. A more complete characterization of the blood concentration or urinary excretion rate during the absorption and

elimination phases of a single dose administered at steady-state is encouraged to permit estimation of the total area under concentration-time curves or cumulative urinary excretion-time curves and to obtain pharmacokinetic information, e.g., half-life or blood clearance, that is essential in preparing adequate labeling for the drug product.

(e) Steady-state parameters

1. In certain instances, e.g., in a study involving a new drug entity, blood clearances at steady-state obtained in a multiple dose study should be compared to blood clearances obtained in a single-dose study to support adequate dosage recommendations.
2. In a linear system, the area under the blood concentration-time curve during a dosing interval in a multiple dose steady-state study is directly proportional to the fraction of the dose absorbed and is equal to the corresponding ''zero to infinity'' area under the curve for a single-dose study. Therefore, when steady-state conditions are achieved, a comparison of blood concentrations during a dosing interval may be used to define the fraction of the active drug ingredient or therapeutic moiety absorbed.
3. Other methods based on valid scientific reasons should be used to determine the bioavailability of a drug product having dose-dependent kinetics (non-linear system).

Various parameters which should be reported as per FDA guidelines in a BA (multiple dose) study are as followed: $AUC_{0\text{-}tau}$, Cmin, Tmax, C_{min} and C_{pd}

$AUC_{0\text{-}\tau}$

Area under the plasma concentration-time curve from time zero to time tau over a dosing interval at steady state ($AUC_{0\text{-}\tau)}$, where tau is the length of the dosing interval.

$AUC_{0\text{-}\tau\,(ss)}$

Area under the plasma concentration-time curve over one dosing interval in multiple dose study at steady state.

Time of Cmax (Tmax)

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

Cmin

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

Cpd

This is the pre dose concentrations determined immediately before a dose is given at steady state.

Urinary Excretion Studies

Principle: The urinary excretion of unchanged drug is directly proportional to the plasma concentration of drug.

The study is particularly useful for drugs extensively excreted unchanged in the urine.

Example: thiazides, sulphonamides, urinary antiseptics, hexamine etc.

Method

- Collection of urine at regular interval for a time span equal to 7 biological half lives.
- Analysis of unchanged drug in the collected sample.
- Determination of the amount of drug excreted in each interval.

***Note*:** At each sample collection total emptying of bladder is necessary to avoid errors resulting from addition of residual amount to the next urine.

A curve of drug excretion rate against the time is plotted and following parameters are studied in urinary excretion studies:

1. $(dx\ u/dt)max$ = maximum urinary excretion rate
2. $(tu)max$ = time for maximum excretion rate
3. Xu = cumulative amount of drug excreted in urine.

Bioavailability

$$F = \frac{Xu_{oral}\ D_{iv}}{Xu_{iv}\ D_{oral}}$$

$$Fr = \frac{Xu_{test}\ D_{std}}{Xu_{std}\ D_{test}}$$

With multiple dos study:

$$Fr = \frac{Xu,\ SS_{test}\ D_{std}\ T_{test}}{Xu,\ SS_{std}\ D_{test}\ T_{std}}$$

where,

Xu, ss = amount of drug excreted unchanged during single dosing interval at steady state

Acute Pharmacologic Response

It may include measurement of one or more of the following pharmacological reponses:

- Change in ECG or EEG reading.
- Pupil diameter etc are related to time course of a given drug.
- Bioavailability can be then determined by plotting the pharmacologic effect- time curve as well as dose response graph.
- Require measurement of response at least 3 biological half lives of drug.

Disadvantages

- Variable pharmacologic response.
- Accurate correlation between measured response and drug available is difficult.

Measurement of an acute pharmacological effect: When comparison of the test product and the reference material is to be based on acute pharmacological effect-time curves, measurements of this effect should be made with sufficient frequency to demonstrate a maximum effect and a lack of significant difference between the test product and the reference material. **(21CFR (320.27 f) FDA)**

Therapeutic Response

Principle: Observing the clinical response to a drug formulation given to patient suffering from disease for which it is intended to be used. But the method is associated with the fact that the response observed is often too improper.

Pharmacodynamic studies are not recommended for orally administered drug products when the drug is absorbed into the systemic circulation and a pharmacokinetic approach can be used to assess systemic exposure and establish BA/BE. However, in those instances where a pharmacokinetic approach is not possible, suitably validated pharmacodynamic methods can be used to demonstrate BE. ***FDA***

Bioequivalence Studies (Comparative Bioavailability Studies)

According to the FDA Orange Book (Approved Drug Products with Therapeutic Equivalence Evaluations), test and reference products are said to be bioequivalent, if the rate and extent of absorption of the test drug do not show a significant difference from the rate and extent of absorption of the reference drug, when administered at the same molar dose, of the therapeutic ingredient under similar experimental conditions in either single dose or multiple doses.

Bioequivalence studies are designed to compare dug products. The objective is to determine if these products are bioequivalent. The dosage forms should be similar, especially the route of administration. For example, tablet versus tablet, or may be tablet versus capsule, given orally. These studies may be necessary before a generic period may be marketed. In general a relative bioavailability is determined which may be close to 100%.

Types of Equivalence

Equivalence is a relative term that compares drug products with respect to a specific characteristic or function or to a defined set of standards. There are several types of equivalences.

Chemical Equivalence

It indicates that two or more drug products contain the same labeled drug substance as an active ingredient in the same amount.

Pharmaceutical Equivalence

Pharmaceutical equivalent means drug products that contain identical amounts of the identical active drug ingredient, i.e., the salt or ester of the same therapeutic moiety, in identical dosage forms, but not necessarily containing the same inactive ingredients.

- Central Drug Standard Control Organization (*CDSCO), India.*

"Drug products are considered pharmaceutical equivalents if they contain the same active ingredient(s), are of the same dosage form, route

of administration and are identical in strength or concentration (*e.g., chlordiazepoxide hydrochloride, 5 mg capsules*). Pharmaceutically equivalent drug products are formulated to contain the same amount of active ingredient in the same dosage form and to meet the same or compendial or other applicable standards (i.e., strength, quality, purity, and identity), but they may differ in characteristics such as shape, scoring configuration, release mechanisms, packaging, excipients (including colors, flavors, preservatives), expiration time, and, within certain limits, labeling." *-FDA CDER 2004*

Pharmaceutical equivalents are the same drug entity, the same type of dosage form, the same dose and meet the same compendial requirements. For example Aspirin Tablets, U.S.P. of a particular strength. Although the U.S.P. monograph includes dissolution and chemical assay requirements there are no bioavailability requirements (at least not in U.S.P. XX). Thus all Aspirin U.S.P. tablets of a particular dose would be pharmaceutical equivalents. Capsules of aspirin would not and neither would tablets of a different dose. Dosage forms containing different salt forms, esters or other chemical form are not pharmaceutical equivalents.

Pharmaceutical Alternatives

Pharmaceutical alternatives are drug products that contain identical therapeutic moiety or its precursor but not necessarily, in the same amount or dosage form or as the same salt or ester.

- CDSCO, India.

"Drug products are considered pharmaceutical alternatives if they contain the same therapeutic moiety, but are different salts, esters, or complexes of that moiety, or are different dosage forms or strengths (*e.g., tetracycline hydrochloride, 250 mg capsules vs. tetracycline phosphate complex, 250 mg capsules; quinidine sulfate, 200 mg tablets vs. quinidine sulfate, 200 mg capsules*). Data are generally not available for FDA to make the determination of tablet to capsule bioequivalence. Different dosage forms and strengths within a product line by a single manufacturer are thus pharmaceutical alternatives, as are extended release products when compared with immediate- or standard-release formulations of the same active ingredient."

- FDA CDER 2004

Pharmaceutical alternatives are drug products that can provide the same therapeutic moiety. Different dosage forms, doses and even salts can be pharmaceutical alternatives.

Therapeutic Equivalence

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

- CDSCO, India

Drug products are considered to be therapeutic equivalents only if they are pharmaceutical equivalents and if they can be expected to have the same clinical effect and safety profile when administered to patients under the conditions specified in the labeling.

- FDA CDER 2004

Thus, pharmaceutical equivalents that have been shown to be bioequivalent (and the same by other determinations of clinical effect and safety profile) are therapeutic equivalents. Therapeutic equivalents would be expected to produce identical drug concentration time profiles and therapeutic response when administered under the same conditions. This is not the same as two pharmacologically similar (equivalent) compounds that may produce the same therapeutic response in some individuals ("e.g., propoxyphene hydrochloride vs. pentazocine hydrochloride for the treatment of pain").

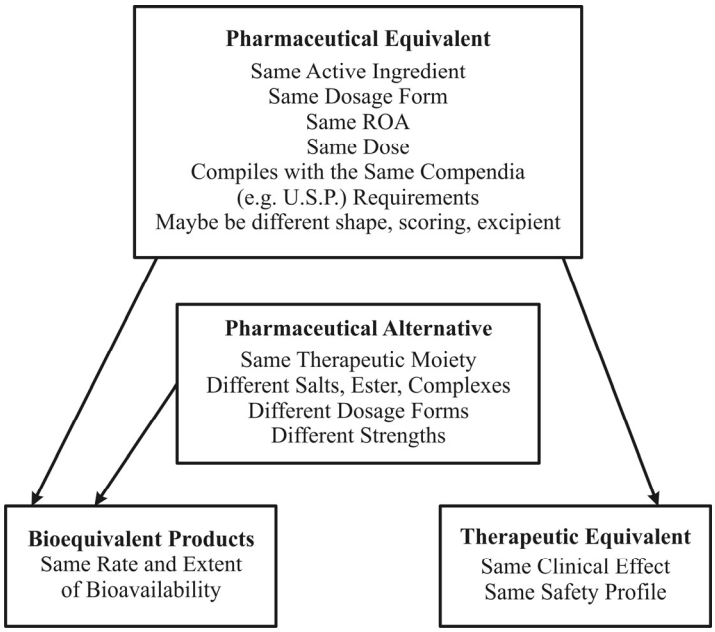

Fig. 1.7 Summary of bioavailability definitions.

Bioequivalence

It is a relative term which denotes that the drug substance in two or more identical dosage forms, reaches the systemic circulation at the same relative rate and to the same relative extent i.e. their plasma concentration-time profiles will be identical without significant statistical differences.

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

- CDSCO, India.

Bioequivalent Drug Products means pharmaceutical equivalents or pharmaceutical alternatives whose rate and extent of absorption do not show a significant difference when administered at the same molar dose of the therapeutic moiety under similar experimental conditions, either single dose or multiple dose.

Some pharmaceutical equivalents or pharmaceutical alternatives may be equivalent in the extent of their absorption but not in their rate of absorption and yet may be considered bioequivalent because such differences in the rate of absorption are intentional and are reflected in the labeling, are not essential to the attainment of effective body drug concentrations on chronic use, or are considered medically insignificant for the particular drug product studied as Therapeutic Equivalents.

Bioequivalence is defined in 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."

-21CFR 320.1 (FDA)

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.

Reasons for Bioequivalence Requirements

The FDA may decide to require bioavailability studies for a variety of reasons including:

- Results from clinical studies indicate that different drug products produce different therapeutic results.

- Results from bioavailability studies indicate that different products are not bioequivalent.
- Drug has a narrow therapeutic range.
- Low solubility and/ or large dose.
- Absorption is considerably less than 100%.
- Bioequivalence studies assess *in vivo* impact of changes to the dosage form/process after pivotal studies commence to ensure product on the market is comparable to that upon which the efficacy is based

Biowaivers

The term biowaiver is applied to a regulatory drug approval process when the dossier (application) is approved based on evidence of equivalence other than through *in vivo* equivalence testing.

WHO Expert Committee on Specifications for Pharmaceutical Preparations (40th Report 2006)

Under certain circumstances, product quality BA and BE can be documented using *in vitro* approaches (21 CFR 320.24(b) and 21 CFR 320.22(d)). For highly soluble, highly permeable, rapidly dissolving, and orally administered drug products, documentation of BE using an *in vitro* approach (dissolution studies) is appropriate based on the biopharmaceutics classification system. This approach may also be suitable under some circumstances in assessing BE during the IND period, for NDA and ANDA submissions, and in the presence of certain post approval changes to approved NDAs and ANDAs.

Diagrams depicting the products eligible (revised criteria) for the biowaiver procedure under the HHS-FDA guidance and those eligible according to the WHO "Multisource document" are presented in Fig. 8 (WHO Expert Committee on Specifications for Pharmaceutical Preparations, 40th Report, 2006).

Thus, the eligibility criteria (Fig. 1.8) according to WHO (40th Report, 2006) are:

1. **The BCS classification** (according to the revised criteria) of the API.
2. **Risk assessment:** only if the risk of an incorrect biowaiver decision and an evaluation of the consequences (of an incorrect, biowaiver-based equivalence decision) in terms of public health and risks to individual patients is outweighed by the potential

benefits accrued from the biowaiver approach, the biowaiver procedure may be applied.

(a) according to FDA

(b) according to WHO

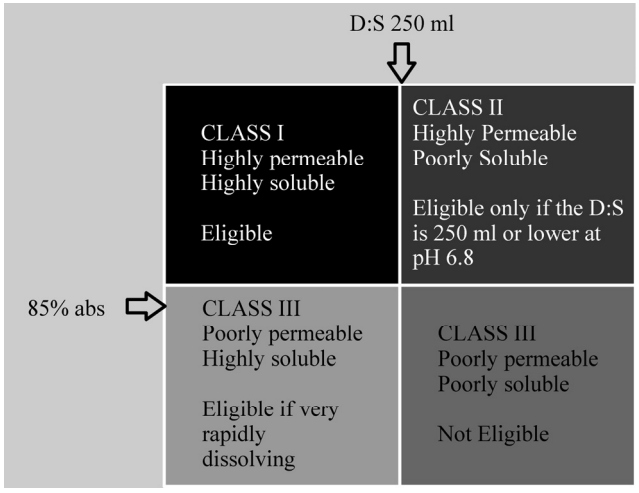

Fig. 1.8 Eligibility for the biowaiver procedure based on solubility and permeability characteristics of the active pharmaceutical ingredient.

3. **Dissolution requirements** for the pharmaceutical product:
   - very rapidly dissolving (release of > 85% of the labelled amount of drug in 15 minutes) in standard media at pH 1.2, 4.5 and 6.8, at a rotational speed of 75 rpm in the paddle apparatus or 100 rpm in the basket apparatus (applies to pharmaceutical

products containing Class III Active Pharmaceutical Ingredients or APIs);

- rapidly dissolving (release of > 85% of the labelled amount of drug in 30 minutes) in standard media at pH 1.2, 4.5 and 6.8, at a rotational speed of 75 rpm in the paddle apparatus or 100 rpm in the basket apparatus (applies to pharmaceutical products containing Class I APIs and/or Class II APIs which are weak acids and meet the 250 ml dose: solubility requirement at pH 6.8).

Dissolution testing is also used to assess batch-to-batch quality, where the dissolution tests, with defined procedures and acceptance criteria, are used to allow batch release. USFDA recommends that dissolution testing is also used to provide process control and quality assurance, and assess whether further BE studies relative to minor post-approval changes be conducted, where dissolution can function as a signal of bioinequivalence. *In vitro* dissolution characterization is encouraged for all product formulations investigated (including prototype formulations), particularly if *in vivo* absorption characteristics are being defined for the different product formulations. Such efforts may enable the establishment of an *in vitro-in vivo* correlation.

If an appropriate dissolution method has been established, and the dissolution results indicate that the dissolution characteristics of the product are not dependent on the product strength, then dissolution profiles in one medium are usually sufficient to support waivers of *in vivo* testing.

A simple model independent approach for dissolution profile comparison uses a difference factor (f_1) and a similarity factor 1 (f_2) to compare dissolution profiles. The difference factor (f_1) calculates the percent (%) difference between the two curves at each time point and is a measurement of the relative error between the two curves:

$$\mathbf{f_1 = [\{\sum_{t=1}^{n} | R_t - T_t | \}/\{\sum_{t=1}^{n} R_t \}] \times 100}$$

where *n* is the number of time points, R_t is the dissolution value of the reference t (pre-change) batch at time t, and T_t is the dissolution value of the test (post-change) batch t at time t.

US FDA recommends that the f_2 test be used to compare profiles from the different strengths of the product. The similarity factor (f_2) is a logarithmic reciprocal square root transformation of the sum of squared error and is a measurement of the similarity in the percent (%) dissolution

between the two curves. An f_2 value (similarity factor) > 50 indicates a sufficiently similar dissolution profile such that further *in vivo* studies are not needed. The similarity factor f_2 is to be computed using the equation:

$$f_2 = 50 \times \log [\{1 + (1/n) \sum_{t=1}^{n} (R_t - T_t)^2\}^{-0.5} \times 100]$$

where R_t and T_t are the cumulative percentage of the drug dissolved at each of the selected n time-points of the comparator (reference) and multisource (test) product respectively.

(If the comparator and multisource products are very rapidly dissolving, i.e. at least 85% dissolution in 15 minutes or less, in all media (pH 1.2, 4.5 and 6.8 buffer), using the recommended test method, a profile comparison is not necessary.)

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