Name of Unit	Elimination & Bioavailability and Bioequivalence.
Course/Subject Name	Biopharmaceutics and Pharmacokinetics
Course/Subject Code	BP604T
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Learning Outcome of Unit-2

LO	Learning Outcome(LO)	Course Outcome Code
LO1	Students will learn about the various aspects of metabolism and elimination.	BP604.2
LO2	Students will learn about the different routes of excretion other than renal excretion.	BP604.2
LO3	Students will learn about the objectives and study designs of bioavailability and bioequivalence	BP604.3

CONTENT OF MODULE

Topics
Elimination • Drug metabolism and basic understanding metabolic pathways. • Renal excretion of drugs. • Factors affecting renal excretion of drugs. • Renal clearance. • Non-renal routes of drug excretion of drugs. **Bioavailability and Bioequivalence** • Definition and Objectives of bioavailability. • Absolute and Relative bioavailability, • Measurement of bioavailability, • In-vitro drug dissolution models, • In-vitro-In-vivo correlations, • Bioequivalence studies, • Methods to enhance the dissolution rates and bioavailability of poorly soluble drugs.

ELIMINATION

The decline from peak plasma concentrations after drug administration results from drug elimination or removal by the body. The elimination of most drugs from the body involves the processes of both metabolism (biotransformation) and renal excretion. For many drugs, the principal site of metabolism is the liver. However, other tissues or organs, especially those tissues associated with portals of drug entry into the body, may also be involved in drug metabolism. These sites include the lung, skin, gastrointestinal mucosal cells, microbiological flora in the distal portion of the ileum, and large intestine. The kidney may also be involved in certain drug metabolism reactions.

Whether a change in drug elimination is more likely to be affected by renal disease, hepatic disease, or a drug–drug interaction may be predicted by measuring the fraction of the drug that is eliminated by either metabolism or excretion. Drugs that are highly metabolized (such as phenytoin, theophylline, and lidocaine) often demonstrate large intersubject variability in elimination half-lives and are dependent on the intrinsic activity of the biotransformation enzymes, which may vary by genetic and environmental factors. Intersubject variability in elimination half-lives is less for drugs that are eliminated primarily by renal drug excretion. Renal drug excretion is highly dependent on the *glomerular filtration rate* (GFR) and blood flow to the kidney. Since GFR is relatively constant among individuals with normal renal function, the elimination of drugs that are primarily excreted unchanged in the urine is also less variable.

Biotransformation of drugs is defined as the chemical conversion of one form to another. The term is used synonymously with **metabolism**. The chemical changes are usually affected enzymatically in the body and thus, the definition excludes chemical instability of a drug within the body; for e.g. conversion of penicillin to penicilloic acid by the bacterial penicillinase and mammalian enzymes is metabolism but its degradation by the stomach acid to penicillenic acid is chemical instability.

Biotransformation –

Normally results in pharmacological inactivation of drugs, i.e. it results in formation of metabolites with little or no pharmacological activity; e.g. conversion of phenytoin to p-hydroxy phenytoin.

Occasionally yields metabolites with equal activity; e.g. conversion of phenylbutazone to oxyphenbutazone.

Rarely leads to toxicological activation of drugs, i.e. it results in formation of metabolites with high tissue reactivity; e.g. conversion of paracetamol to reactive metabolites that cause hepatic necrosis.

Inactive drugs (prodrugs) also depend upon biotransformation for activation, the process being called as pharmacological activation; e.g. conversion of enalapril to enalaprilat. A change in pharmacological activity of the drug on metabolism has also been observed.

Drug Metabolizing Organs

Liver is the primary site for metabolism of almost all drugs (and other xenobiotics) because of its relative richness in possessing a large variety of enzymes in large amounts. Metabolism by organs other than liver (called as **extrahepatic metabolism**) is of minor importance since lower level of drug metabolising enzymes are present in such tissues. The decreasing order of drug metabolising ability of various organs is:

Liver > Lungs > Kidneys > Intestine > Placenta > Adrenals > Skin

Brain, testes, muscles, spleen, etc. also metabolise drugs but to a small extent.

CHEMICAL PATHWAYS OF DRUG BIOTRANSFORMATION

R.T.Williams, the leading pioneer in drug biotransformation research, divided the pathways of drug metabolism reactions into two general categories—

a) Phase reactions, and
b) Phase II reactions.

Phase I Reactions

These reactions generally precede phase II reactions and include oxidative, reductive and hydrolytic reactions. By way of these reactions, a polar functional group is either *introduced* or *unmasked* if already present on the otherwise lipid soluble substrate, e.g. -OH, -COOH, -NH2 and -SH. Thus, *phase I reactions are also called as* **functionalisation reactions**. These transformations are also called as **asynthetic reactions**, opposite to the synthetic phase II reactions. The resulting product of phase I reaction is susceptible to phase II reactions.

Phase II Reactions

These reactions generally involve covalent attachment of small polar endogenous molecules such as glucuronic acid, sulphate, glycine, etc. to either unchanged drugs or phase I products having suitable functional groups viz. -OH, -COOH, -NH2 and -SH and form highly water-soluble conjugates which are readily excretable by the kidneys (or bile). Thus, these reactions are called as **conjugation reactions**. Since the outcome of such processes are generally products with increased molecular size (and altered physicochemical properties), they are also called as **synthetic reactions**. Quite often, a phase I reaction may not yield a metabolite that is sufficiently hydrophilic or pharmacologically inert but conjugation reactions generally result in products with total loss of pharmacological activity and high polarity. Hence, phase II reactions are better known as **true detoxification reactions**. Since these reactions generally involve transfer of moieties to the substrate to be conjugated, the enzymes responsible are called as transferases.

PHASE I REACTIONS		
A. Oxidative Reactions		
1.	Oxidation of aromatic carbon atoms	(M)
2.	Oxidation of olefins (C=C bonds)	(M)
3.	Oxidation of benzylic. allylic carbon atoms and carbon atoms alpha to carbonyl and imines	(M)
4.	Oxidation of aliphatic carbon atoms	(M)
5.	Oxidation of alicyclic carbon atoms	(M)
6.	Oxidation of carbon-heteroatom systems:	
a.	Carbon-Nitrogen systems (aliphatic and aromatic amines):	
i.	N-Dealkylation	(M)
ii.	Oxidative deamination	(M), (N)
iii.	N-Oxide formation	(M)
iv.	N-Hydroxylation	(M)
b.	Carbon-Sulphur systems:	
i.	S-Dealkylation	(M)
ii.	Desulphuration	(M)
iii.	S-oxidation	(M)
c.	Carbon-Oxygen systems (O-dealkylation)	(M)
7.	Oxidation of alcohol, carbonyl and acid functions	(M)
8.	Miscellaneous oxidative reactions	(M), (N)

The biotransformation of drug metabolites, particularly the glutathione conjugates which are excreted *via* bile in the gut, by the intestinal microflora, is considered by few researchers as Phase III Reactions.

TABLE - Chemical Pathways of Drug Biotransformation — (M) and (N) Indicate Reactions Catalysed by Microsomal and Non-microsomal Enzymes

B. Reductive Reactions		
1.	Reduction of carbonyl functions (aldehydes/ketones)	(M), (N)
2.	Reduction of alcohols and C=C bonds	(M)
3.	Reduction of N-compounds (nitro, azo and N-oxide)	(M), (N)
4.	Miscellaneous reductive reactions	
C. Hydrolytic Reactions		
1.	Hydrolysis of esters and ethers	(M),(N)
2.	Hydrolysis of amides	(M),(N)
3.	Hydrolytic cleavage of non-aromatic heterocycles	(M),(N)
4.	Hydrolytic dehalogenation	
5.	Miscellaneous hydrolytic reactions	

	PHASE II REACTIONS	
1.	Conjugation with glucuronic acid	(M)
2.	Conjugation with sulphate moieties	(N)
3.	Conjugation with alpha amino acids	(N)
4.	Conjugation with glutathione and mercapturic acid formation	(N)
5.	Acetylation reactions	(N)
6.	Methylation reactions	(N)
7.	Miscellaneous conjugation reactions	(N)

RENAL EXCRETION OF DRUGS:

Excretion is defined as the process whereby drugs and/or their metabolites are irreversibly transferred from internal to external environment. Excretion of unchanged or intact drug is important in the termination of its pharmacological action. The principal organs of excretion are kidneys. Excretion of drug by kidneys is called as **renal excretion.**

Almost all drugs and their metabolites are excreted by the kidneys to some extent or the other. Some drugs such as gentamicin are exclusively eliminated by renal route only.

Agents that are excreted in urine are –

1. Water-soluble.
2. Non-volatile.
3. Small in molecular size (less than 500 Daltons).
4. The ones that are metabolised slowly.

The basic functional unit of kidney involved in excretion is the **nephron**. Each kidney comprises of one million nephrons. Each nephron is made up of the glomerulus, the proximal tubule, the loop of Henle, the distal tubule and the collecting tubule.

The **principal processes** that determine the urinary excretion of a drug are –

1. Glomerular filtration.
2. Active tubular secretion.
3. Active or passive tubular reabsorption.

Factors affecting renal excretion of drugs:

Glomerular Filtration

Glomerular filtration is a non-selective, unidirectional process whereby most compounds, ionised or unionised, are filtered except those that are bound to plasma proteins or blood cells and thus behave as macromolecules. The glomerulus also acts as a negatively charged selective barrier promoting retention of anionic compounds. The driving force for filtration through the glomerulus is the hydrostatic pressure of the blood flowing in the capillaries. Out of the 25% of cardiac output or 1.2 litres of blood/min that goes to the kidneys via renal artery, only 10% or 120 to 130 ml/min is filtered through the glomeruli, the rate being called as the **glomerular filtration rate** (GFR). Though some 180 litres of protein and cell free ultrafiltrate pass through the glomeruli each day, only about 1.5 litres is excreted as urine, the remainder being reabsorbed from the tubules.

The GFR can be determined by an agent that is excreted exclusively by filtration and is neither secreted nor reabsorbed in the tubules. The excretion rate value of such an agent is 120 to 130 ml/min. Creatinine, inulin, mannitol and sodium thiosulphate are used to estimate GFR of which the former two are widely used to estimate renal function.

Active Tubular Secretion

It is a carrier-mediated process which requires energy for transportation of compounds against the concentration gradient. The system is capacity-limited and saturable. Two active tubular secretion mechanisms have been identified:

System for secretion of organic acids/anions like penicillins, salicylates, glucuronides, sulphates, etc. It is the same system by which endogenous acids such as uric acid are secreted.

System for secretion of organic bases/cations like morphine, mecamylamine, hexamethonium and endogenous amines such as catecholamines, choline, histamine, etc.

Both the systems are relatively non-selective and independent of each other but both can be bidirectional i.e. agents may both be secreted as well as reabsorbed actively, for example, uric acid.

Tubular Reabsorption

Tubular reabsorption occurs after the glomerular filtration of drugs. It takes place all along the renal tubule. Reabsorption of a drug is indicated when the excretion rate values are less than the GFR of 130 ml/min. An agent such as glucose that is completely reabsorbed after filtration has a clearance value of zero. *Contrary to tubular secretion, reabsorption results in an increase in the half-life of a drug*.

Tubular reabsorption can either be an:

1. Active process, or
2. Passive process.

Active tubular reabsorption is commonly seen with high threshold endogenous substances or nutrients that the body needs to conserve such as electrolytes, glucose, vitamins, amino acids, etc. Uric acid is also actively reabsorbed (inhibited by the uricosuric agents). Very few drugs are known to undergo reabsorption actively e.g. oxopurinol.

Passive tubular reabsorption is common for a large number of exogenous substances including drugs. The driving force for such a process i.e. the concentration gradient is established by the back diffusion or reabsorption of water along with sodium and other inorganic ions.

Understandably, if a drug is neither secreted nor reabsorbed, its concentration in the urine will be 100 times that of free drug in plasma due to water reabsorption since less than 1% of glomerular filtrate is excreted as urine.

The primary determinant in the passive reabsorption of drugs is their lipophilicity. Lipophilic substances are extensively reabsorbed while polar molecules are not. Since a majority of drugs are weak electrolytes (weak acids or weak bases), diffusion of such agents through the lipoidal tubular membrane depend upon the degree of ionisation which in turn depends on three factors:

1. pH of the urine.
2. pKa of the drug.
3. Urine flow rate.

Urine pH: It is an important factor in the sense that it is not constant like the plasma pH but varies between 4.5 to 7.5, the two extremes. Thus, a large pH gradient may exist between urine and plasma.

The pH of the urine is dependent upon diet, drug intake and pathophysiology of the patient. Food rich in carbohydrates result in higher urinary pH whereas proteins lower it. Drugs such as acetazolamide and antacids such as sodium bicarbonate produce alkaline urine while ascorbic acid makes it acidic. More significant alteration in urine pH is brought about by i.v. infusion of solutions of sodium bicarbonate and ammonium chloride which are used in the treatment of acid- base imbalance. Respiratory and metabolic acidosis and alkalosis result in acidification and alkalinisation of the urine respectively.

Renal clearance:

It can be defined as the volume of blood or plasma which is completely cleared of the unchanged drug by the kidney per unit time. It is expressed mathematically as:

$$Cl_R = \frac{\text{Rate of urinary excretion}}{\text{Plasma drug concentration}}$$

Renal clearance is the ratio of “sum of rate of glomerular filtration and active secretion minus rate of reabsorption” to “plasma drug concentration C”.

$$Cl_R = \frac{\text{Rate of filtration} + \text{Rate of secretion} - \text{Rate of reabsorption}}{C}$$

NON-RENAL ROUTES OF DRUG EXCRETION

Drugs and their metabolites may also be excreted by routes other than the renal route, called as the **extrarenal** or **nonrenal routes of drug excretion**. The various such excretion processes are:

1. Biliary excretion
2. Pulmonary excretion
3. Salivary excretion
4. Mammary excretion
5. Skin/dermal excretion
6. Gastrointestinal excretion
7. Genital excretion

Biliary Excretion of Drugs—Enterohepatic Cycling

The hepatic cells lining the bile canaliculi produce bile. The production and secretion of bile are active processes. The bile secreted from liver, after storage in the gall bladder, is secreted in the duodenum. In humans, the bile flow rate is a steady 0.5 to 1 ml/min. Bile is important in the digestion and absorption of fats. Almost 90% of the secreted bile acids are reabsorbed from the intestine and transported back to the liver for re-secretion. The rest is excreted in faeces.

Being an active process, bile secretion is capacity-limited and subject to saturation. The process is exactly analogous to active renal secretion. Different transport mechanisms exist for the secretion of organic anions, cations and neutral polar compounds. A drug, whose biliary concentration is less than that in plasma, has a small biliary clearance and vice versa. In some instances, the bile to plasma concentration ratio of drug can approach 1000 in which cases, the biliary clearance can be as high as 500 ml/min or more.

Compounds that are excreted in bile have been classified into 3 categories on the basis of their bile/plasma concentration ratios:

Group A compounds whose ratio is approximately 1, e.g. sodium, potassium and chloride ions and glucose.

Group B compounds whose ratio is >1, usually from 10 to 1000, e.g. bile salts, bilirubin glucuronide, creatinine, sulphobromophthalein conjugates, etc.

Group C compounds with ratio < 1, e.g. sucrose, inulin, phosphates, phospholipids and mucoproteins.

Drugs can fall in any of the above three categories. Several factors influence secretion of drugs in bile –

Physicochemical Properties of the Drug

The most important factor governing the excretion of drugs in bile is their **molecular weight**. Its influence on biliary excretion is summarized in the Table 6.3.

Polarity is the other physicochemical property of drug influencing biliary excretion. Greater the polarity, better the excretion. Thus, metabolites are more excreted in bile than the parent drugs because of their increased polarity. The molecular weight threshold for biliary excretion of drugs is also dependent upon its polarity. A threshold of 300 Daltons and greater than 300 Daltons is necessary for organic cations (e.g. quaternaries) and organic anions respectively. Nonionic compounds should also be highly polar for biliary excretion, e.g. cardiac glycosides.

The ability of liver to excrete the drug in the bile is expressed by biliary clearance.

$$\text{Biliary clearance} = \frac{\text{Biliary clearance rate}}{\text{Plasma drug concentration}} = \frac{\text{Bile flow x Biliary drug clearance}}{\text{Plasma drug concentration}}$$

Pulmonary Excretion

Gaseous and volatile substances such as the general anaesthetics (e.g. halothane) are absorbed through the lungs by simple diffusion. Similarly, their excretion by diffusion into the expired air is possible. Factors influencing pulmonary excretion of a drug include pulmonary blood flow, rate of respiration, solubility of the volatile substance, etc. Gaseous anaesthetics such as nitrous oxide which are not very soluble in blood are excreted rapidly. Generally intact gaseous drugs are excreted but metabolites are not. Compounds like alcohol which have high solubility in blood and tissues are excreted slowly by the lungs. The principle involved in the pulmonary excretion of benzene and halobenzenes is analogous to that of steam distillation.

Salivary Excretion

Excretion of drugs in saliva is also a passive diffusion process and therefore predictable on the basis of pH-partition hypothesis. The pH of saliva varies from 5.8 to 8.4. The mean salivary pH in man is 6.4. Unionised, lipid soluble drugs at this pH are excreted passively in the saliva.

Mammary Excretion

Excretion of a drug in milk is important since it can gain entry into the breast-feeding infant. Milk consists of lactic secretions originating from the extracellular fluid and is rich in fats and proteins. About 0.5 to 1 litre/day of milk is secreted in lactating mothers

Excretion of drugs in milk is a passive process and is dependent upon pH-partition behaviour, molecular weight, lipid solubility and degree of ionisation. The pH of milk varies from 6.4 to 7.6 with a mean pH of 7.0. Free, unionised, lipid soluble drugs diffuse into the mammary alveolar cells passively. The extent of drug excretion in milk can be determined from milk/plasma drug concentration ratio (M/P). Since milk is acidic in comparison to plasma, as in the case of saliva, weakly basic drugs concentrate more in milk and have M/P ratio greater than 1. The opposite is true for weakly acidic drugs. It has been shown that for acidic drugs, excretion in milk is inversely related to the molecular weight and partition coefficient and that for basic drugs, is inversely related to the degree of ionisation and partition coefficient.

Table-Excretion Pathways, Transport Mechanisms and Drugs Excreted

Excretory Route	*Mechanism*	*Drugs Excreted*
Urine	Glomerular filtration, active secretion, active/passive reabsorption	Free, hydrophilic, unchanged drugs/metabolites/conjugates of MW < 500
Bile	Active secretion	Hydrophilic, unchanged drugs/metabolites/conjugates of MW ≥ 500
Lung	Passive diffusion	Gaseous and volatile, blood and tissue insoluble drugs
Saliva	Passive diffusion, active transport	Free, unionised, lipophilic drugs, some polar drugs
Milk	Passive diffusion	Free, unionised, lipophilic drugs (generally basic)
Sweat	Passive diffusion	Free, unionised, lipophilic drugs
Intestine	Passive diffusion	Water-soluble, ionised drugs

BIOAVAILABILITY AND BIOEQUIVALENCE

Bioavailability is defined 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. Bioavailability data provide an estimate of the fraction of drug absorbed from the formulation, and provide information about the pharmacokinetics of the drug.

Bioavailability studies are drug product performance studies used to define the effect of changes in the physicochemical properties of the drug substance, the formulation of the drug, and manufacture process of the drug product (dosage form). Bioavailability is a key indicator of drug absorption. It represents the administered dose fraction which achieves success in reaching the systemic circulation when administered orally or through any other extravascular dosing route. Intravenous dosing is considered 100% bioavailable since the drug is administered directly to the bloodstream, also termed the central compartment or systemic circulation. However, if a drug has some different route of administration, oral being most commonly employed, its bioavailability may be limited. For oral doses, bioavailability limitations are typically due to the first-pass metabolism produced by the liver as well as incomplete absorption in the gut. Thus, it holds its importance as an essential pharmacokinetic tool and contributes widely towards the calculation of dosage for the different routes of administrations.

Bioequivalence studies are used to compare the bioavailability of the same drug (same salt or ester) from various drug products. Bioavailability and bioequivalence can be considered as performance measures of the drug product *in vivo*. If the drug products are pharmaceutically equivalent, bioequivalent, and therapeutically equivalent (as defined by the regulatory agency such as the FDA), then the clinical efficacy and the safety profile of these drug products are assumed to be similar and may be substituted for each other.

Bioavailability—Absolute versus Relative

Absolute bioavailability: It compares the bioavailability of the active drug in the systemic circulation following extravascular administration with the bioavailability of the same drug following intravenous administration . Intravenous drug administration is considered 100% absorbed. The route of extravascular administration can be inhaled, intramuscular, oral, rectal,

subcutaneous, sublingual, topical, transdermal, etc. The absolute bioavailability is the dose-corrected AUC of the extravascularly administered drug product divided by the AUC of the drug product given intravenously. Thus, for an oral formulation, the absolute bioavailability is calculated as follows:

Fabs = [AUC]oralDiv / [AUC]ivDoral

*F*abs is the fraction of the dose absorbed, expressed as a percentage; AUCoral is the AUC following oral administration;

*D*iv is the dose administered intravenously;

AUCiv is the AUC following intravenous administration; and

*D*oral is the dose administered orally.

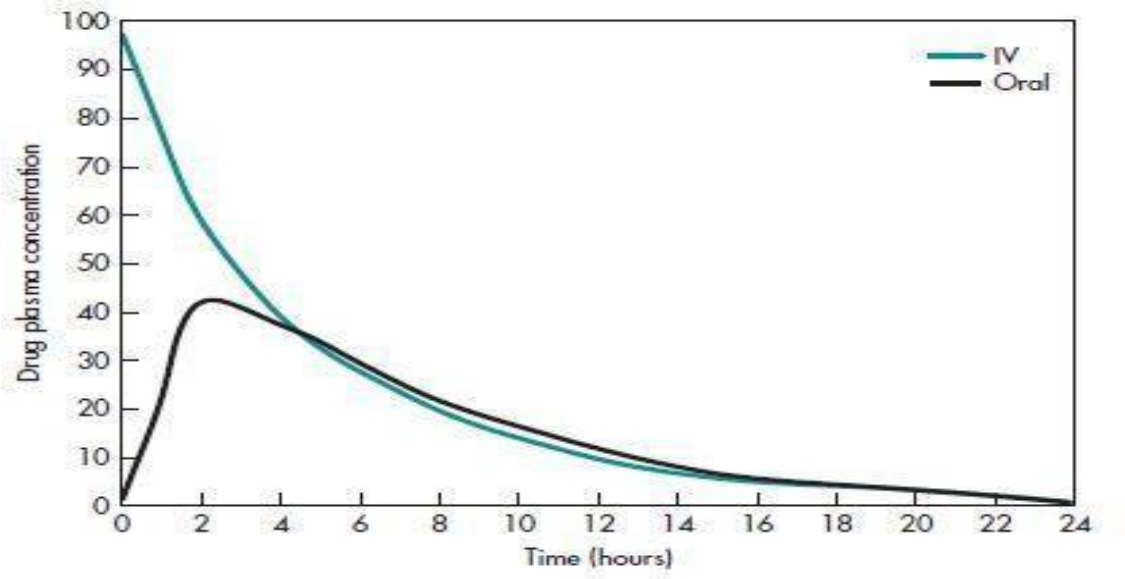

Fig. *Relationship between plasma drug concentration-versus-time profiles for anintravenously administered formulation versus an orally administered formulation. In an absolute bioavailability study, the systemic exposure profile of a drug administered by the oral route (black curve) is compared with that of the drug administered by the intravenous route (green curve).*

Relative Bioavailability: When the systemic availability of a drug after oral administration is compared with that of an oral standard of the same drug (such as an aqueous or non-aqueous

solution or a suspension), it is referred to as relative or comparative bioavailability. In contrast to absolute bioavailabiliy it is used to characterize absorption of a drug from its formulation.

Methods for assessing bioavailability:

Direct and indirect methods may be used to assess drug bioavailability. 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. The design of the bioavailability study depends on the objectives of the study, the ability to analyze the drug (and metabolites) in biological fluids, the pharmacodynamics of the drug substance, the route of drug administration, and the nature of the drug product.

TABLE: Methods for Assessing Bioavailability and Bioequivalence

***In vivo* measurement of active moiety or moieties in biological fluids**

Plasma drug concentration

Time for peak plasma (blood) concentration (*t*max) Peak plasma drug concentration (*C*max)

Area under the plasma drug concentration–time curve (AUC)

Urinary drug excretion

Cumulative amount of drug excreted in the urine (*D*u) Rate of drug excretion in the urine (*dD*u/*dt*)

Time for maximum urinary excretion (*t*)

***In vivo* pharmacodynamic (PD) comparison**

Maximum pharmacodynamic effect (*E*max) Time for maximum pharmacodynamic effect

Area under the pharmacodynamic effect–time curve Onset time for pharmacodynamic effect

Clinical endpoint study

Limited, comparative, parallel clinical study using predetermined clinical endpoint(s) and performed in patients

***In vitro* studies**

Comparative drug dissolution, *f*2 similarity factor

In vitro binding studies

Examples: Cholestyramine resin—*In vitro* equilibrium and kinetic binding studies

Any other approach deemed acceptable (by the FDA)

a. Plasma Drug Concentration:

Measurement of drug concentrations in blood, plasma, or serum after drug administration is the most direct and objective way to determine systemic drug bioavailability. ***C*max:** The *peak plasma drug concentration*, *C*max, represents the maximum plasma drug concentration obtained after oral administration of drug. For many drugs, a relationship is found between the pharmacodynamic drug effect and the plasma drug concentration. *C*max provides indications that the drug is sufficiently systemically absorbed to provide a therapeutic response. In addition, *C*max provides warning of possibly toxic levels of drug. **AUC:** The area under the plasma level-time curve that gives a measure of the extent of absorption or the amount of drug that reaches the systemic circulation. The AUC reflects the total amount of active drug that reaches the systemic circulation.

***t*max:** The *time of peak plasma concentration*, *t*max, corresponds to the time required to reach maximum drug concentration after drug administration. At *t*max, peak drug absorption occurs and the rate of drug absorption exactly equals the rate of drug elimi- nation.

b. Urinary Excretion Studies

This method of assessing bioavailability is *based on the principle that the urinary excretion of unchanged drug is directly proportional to the plasma concentration of drug*. As a rule of thumb, determination of bioavailability using urinary excretion data should be conducted only if at least 20% of administered dose is excreted unchanged in the urine. The study is particularly useful for

- Drugs extensively excreted unchanged in the urine – for example, certain thiazide diuretics and sulphonamides.
- Drugs that have urine as the site of action - for example, urinary antiseptics such as nitrofurantoin and hexamine.

Concentration of metabolites excreted in urine is never taken into account in calculations since a drug may undergo presystemic metabolism at different stages before being absorbed. The method involves –

- Collection of urine at regular intervals 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 and cumulative amount excreted.

For obtaining valid results, following criteria must be met further –

- ✓ At each sample collection, total emptying of the bladder is necessary to avoid errors resulting from addition of residual amount to the next urine sample.
- ✓ Frequent sampling of urine is also essential in the beginning in order to compute correctly the rate of absorption

The three major parameters examined in urinary excretion data obtained with a single dose study are:

(dXu/dt)max: The ***maximum urinary excretion rate,*** it is obtained from the peak of plot between rate of excretion versus midpoint time of urine collection period. It is analogous to the *Cmax* derived from plasma level studies since the rate of appearance of drug in the urine is proportional to its concentration in systemic circulation. Its value increases as the rate of and/or extent of absorption increases (*see* Fig. 11.2).

$(t_u)_{max}$: The ***time for maximum excretion rate***, it is analogous to the t_{max} of plasma level data. Its value decreases as the absorption rate increases.

$X^{\infty}u$: The ***cumulative amount of drug excreted in the urine,*** it is related to the *AUC* of plasma level data and increases as the extent of absorption increases.

IN VIVO PHARMACODYNAMIC (PD) COMPARISON:

In some cases, the quantitative measurement of a drug in plasma is not available or *in vitro* approaches are not applicable. The following criteria for a PD endpoint study are important:

i. A dose–response relationship is demonstrated.

ii. The PD effect of the selected dose should be at the rising phase of the dose–response curve.

iii. Sufficient measurements should be taken to assure an appropriate PD response profile. (iv)All PD measurement assays should be validated for specificity, accuracy, sensitivity, and precision.

For locally acting, nonsystemically absorbed drug products, such as topical corticosteroids, plasma drug concentrations may not reflect the bioavailability of the drug at the site of action. An acute pharmacodynamic effect,4 such as an effect on forced expiratory volume, FEV1 (inhaled bronchodilators), or skin blanching (topical corticosteroids) can be used as an index of drug bioavailability. In this case, the acute pharmacodynamic effect is measured over a period of time after administration of the drug product. Measurements of the pharmacodynamic effect should be made with sufficient frequency to permit a reasonable estimate for a time period at least three times the half-life of the drug. This approach may be particularly applicable to dosage forms that are not intended to deliver the active moiety to the bloodstream for systemic distribution.

Bioequivalence studies, design, evaluation of bioequivalence studies: Bioequivalence is established if the *in vivo* bioavailability of a test drug product (usually the generic product) does not differ significantly (ie, statistically not significant) from that of the reference listed drug (usually the brand-name product approved through the NDA route) in the product's rate and extent of drug absorption. Bioequivalence is determined by comparison of measured parameters (eg, concentration of the active drug ingredient in the blood, urinary excretion rates, or pharmacodynamic effects), when administered at the same molar dose of the active moiety under similar experimental conditions, either single dose or multiple dose.

DESIGN AND EVALUATION OF BIOEQUIVALENCE STUDIES:

Objective: The main objective for a bioequivalence study is that the drug bioavailability from test and reference products is not statistically different when administered to patients or subjects at the same molar dose from pharmaceutically equivalent drug products through the same route of administration under similar experimental conditions.

Study Considerations

The basic design for a bioequivalence study is determined by

(1) The scientific questions and objectives to be answered,

(2) The nature of the reference material and the dosage form to be tested,

(3) The availability of analytical methods,

(4) The pharmacokinetics and pharmacodynamics of the drug substance,

(5) The route of drug administration, and

(6) benefit– risk and ethical considerations with regard to testing in humans. Once bioequivalence is established, it is likely that both the generic and brand-name dosage forms will produce the same therapeutic effect.

TABLE -Elements of a Bioavailability Study Protocol

I. Title
   A. Principal investigator (study director)
   B. Project/protocol number and date
II. Study objective
III. Study design
   A. Design
   B. Drug products
      1. Test product(s)
      2. Reference product
   C. Dosage regimen
   D. Sample collection schedule
   E. Housing/confinement
   F. Fasting/meals schedule
   G. Analytical methods
IV. Study population
   A. Subjects
   B. Subject selection
      1. Medical history
      2. Physical examination
      3. Laboratory tests
   C. Inclusion/exclusion criteria
      1. Inclusion criteria
      2. Exclusion criteria
   D. Restrictions/prohibitions
V. Clinical procedures
   A. Dosage and drug administration
   B. Biological sampling schedule and handling procedures
   C. Activity of subjects
VI. Ethical considerations
   A. Basic principles
   B. Institutional review board
   C. Informed consent
   D. Indications for subject withdrawal
   E. Adverse reactions and emergency procedures
VII. Facilities
VIII. Data analysis
   A. Analytical validation procedure
   B. Statistical treatment of data
IX. Drug accountability
X. Appendix

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

the study, the *Institutional Review Board* (IRB) of the clinical facility in which the study is to be performed must approve the study. The IRB is composed of both professional and lay persons with diverse backgrounds who have clinical experience and expertise as well as sensitivity to ethical issues and community attitudes. The IRB is responsible for all ethical issues including safeguarding the rights and welfare of human subjects.

The basic guiding principle in performing studies is *do not do unnecessary human research*. Generally, the study is performed in normal, healthy male and female volunteers who have given informed consent to be in the study. Critically ill patients are not included in an *in vivo* bioavailability study unless the attending physician determines that there is a potential benefit to the patient. The number of subjects in the study will depend on the expected intersubject and and. intrasubject variability. Patient selection is made according to certain established criteria for inclusion in, or exclusion from, the study.

Regulatory Recommendations for Optimizing Bioavailability Study Design:

1. The FDA lists a number of recommendations to consider in designing clinical relative bioavailability studies in drug development. These recommendations include the following: 1)Use of a randomized crossover design whenever possible
2. Enrolling both male and female subjects whenever possible
3. Administering single doses rather than multiple doses, as single-dose studies are more sensitive, although multiple-dose studies may be more suitable in some cases 4)Conducting the studies under fasting and fed conditions6
4. Measuring the parent drug rather than metabolites, unless the parent cannot be reliably measured. Presystemically formed metabolites that contribute meaningfully to safety and efficacy should also be measured.

Factors Influencing Bioavailability and Impact on Drug Development:

Physicochemical properties of the drug and formulation: Formulations can be designed to improve the bioavailability of poorly soluble drugs, extend the absorption phase by slowing the rate of release of drugs (controlled-release formulations), or prevent dissolution in the gastric lumen for drugs that are destroyed by gastric acidity.

Drug stability and pH effects: Acid-labile drugs potentially have low bioavailability, as they are subject to acid-induced degradation in the low pH conditions of the stomach. For such drugs to achieve therapeutic plasma concentrations, it is necessary to deliver them by formulations that protect against acid-induced degradation, such as buffered products or enteric-coated products. Enteric-coated formulations are used to deliver acid-labile drugs such as didanosine, a purine nucleoside analog indicted to treat HIV disease, and omeprazole and lansoprazole, which are proton pump inhibitors indicated to treat acid reflux.

Presystemic and first-pass metabolism: The effects of presystemic metabolism on oral bioavailability is illustrated by propranolol, a nonselective beta adrenergic receptor blocking agent used as an antihypertensive, antianginal, and antiarrhythmic, presystemic metabolism. Propranolol is almost completely absorbed after oral administration, but due to extensive first-pass metabolism in the liver, only about 25% of the parent drug reaches the systemic circulation.

Prodrugs: that undergo rapid presystemic metabolism can be used to improve bioavailability, as illustrated by valacyclovir, a prodrug of the nucleoside analog antiviral compound acyclovir.

Valacyclovir undergoes rapid presystemic conversion to acyclovir. Both valacyclovir and acyclovir are effective in treating herpes infections. However, because acyclovir bioavailability is greatly enhanced when delivered by its prodrug valacyclovir, for treating herpes zoster, it is only necessary to administer Valtrex® (valacyclovir) tablets administered once daily, compared to 5 times daily for Zovirax® (acyclovir) capsules.

Food effects: Food can either decrease drug bioavailability or increase bioavailability, or have no effect on bioavailability. Food can influence bioavailability in a number of ways, such as affect- ing gastrointestinal pH, gastric emptying, intestinal transit, splanchnic blood flow, and first-pass metabolism. Food can also affect bioavailability by physical or chemical interactions. Most food effects on drug bioavailability are not considered clinically significant, and, consequentially, most drug products are labeled to be administered without regard to meals. If the food effects on drug bioavailability are clinically significant, then the drug product labeling will provide instructions about how to achieve the optimal dosing regimen—

either to take the drug only on an empty stomach, or only with food, depending on the nature of the bioavailability effect and clinical consequences.

Effects of drug–drug interactions:

Changes in drug bioavailability due to drug–drug interactions can occur via a variety of mechanisms, such as inhibition of metabolizing enzymes, induction of metabolizing enzymes, inhibitor of transporters, and induction of transporters.

Disease state:

The bioavailability of drugs eliminated primarily through renal excretory mechanisms is likely to increase in patients with impaired renal function . The FDA recommends that, where appropriate, drug pharmacokinetics be characterized in patients with varying degrees of renal impairment. The results of such studies are used to determine how doses can be adjusted in patients with renal impairment in order to achieve the same systemic drug bioavailability as in patients with normal renal function . Similarly, it may be advisable to conduct pharmacokinetic studies of drugs that are primarily cleared by the liver in patients with varying degrees of hepatic impairment. The results of pharmacokinetic studies in hepatic-impaired patients can be useful in determining whether dose adjustments are required in such patients to achieve the same systemic drug bioavailability as in patients with normal liver function.

STUDY DESIGNS:

For many drug products, the FDA, Division of Bioequivalence, Office of Generic Drugs, provides guidance for the performance of *in vitro* dissolution and *in vivo* bioequivalence studies.

Generally, two bioequivalence studies are required for solid oral dosage forms, including

1. a fasting study and
2. a food intervention study.

Other study designs such as parallel design, replicate design, and multiple-dose (steady-state) bioequivalence studies have been proposed by the FDA. Proper study design and statistical evaluation are important considerations for the determination of bioequivalence. Some of the designs listed above are summarized here:

Fasting Study:

Bioequivalence studies are usually evaluated by a single-dose, two-period, two-treatment, two-sequence, open-label, randomized crossover design comparing equal doses of the test and reference products in fasted, adult, healthy subjects. This study is requested for all immediate-release and modified-release oral dosage forms. Both male and female subjects may be used in the study. Blood sampling is performed just before (zero time) the dose and at appropriate intervals after the dose to obtain an adequate description of the plasma drug concentration–time profile. The subjects should be in the fasting state (overnight fast of at least 10 hours) before drug administration and should continue to fast for up to 4 hours after dosing.

No other medication is normally given to the subject for at least 1 week prior to the study.

Food Intervention Study:

Coadministration of food with an oral drug product may affect the bioavailability of the drug. Food intervention or food effect studies are generally conducted using meal conditions that are expected to provide the greatest effects on GI physiology so that systemic drug availability is maximally affected. Food effects on bioavailability are generally greatest when the drug product is administered shortly after a meal is ingested. The nutrient and caloric contents of the meal, the meal volume, and the meal temperature can cause physiological changes in the GI tract in a way that affects drug product transit time, luminal dissolution, drug permeability, and systemic availability.

Meals that are high in total calories and fat content are more likely to affect the GI physiology and thereby result in a larger effect on the bioavailability of a drug substance or drug product. For bioequivalence studies for generic drugs, drug bioavailability from both the test and reference products should be affected similarly by food. The usual study design uses a single-dose, randomized, two-treatment, two-period, crossover study comparing equal doses of the test and reference products. Following an overnight fast of at least 10 hours, subjects are given the recommended meal 30 minutes before dosing. The meal is consumed over 30 minutes, with administration of the drug product immediately after the meal. The drug product is given with 240 mL (8 fluid oz) of water. No food is allowed for at least 4 hours post dose. This study is requested for all modified-release dosage forms and may be requested for immediate-release dosage forms if the bioavailability of the active drug ingredient is known to be affected by food (eg, ibuprofen, naproxen).

3. Cross Over Study Design:

a. **Latin-square design:** Subjects who meet the inclusion and exclusion study criteria and have given informed consent are selected at random. A complete crossover design is usually employed, in which each subject receives the test drug product and the reference product. Examples of ***Latin-square crossover designs*** for a bioequivalence study in human volunteers, comparing three different drug formulations (A, B, C) or four different drug formulations (A, B, C, D), are described in Tables given below. The Latin-square design plans the clinical trial so that each subject receives each drug product only once, with adequate time between medications for the elimination of the drug from the body. In this design, each subject is his own control, and subject-to-subject variation is reduced. Moreover, variations due to sequence, period, and treatment (formulation) are reduced, so that all patients do not receive the same drug product on the same day and in the same order. The order in which the drug treatments are given should not stay the same in order to prevent any bias in the data due to a residual effect from the previous treatment. Possible carryover effects from any particular drug product are minimized by changing the sequence or order in which the drug products are given to the subject. Thus, drug product B may be followed by drug product A, D, or C. After each subject receives a drug product, blood samples are collected at appropriate time intervals so that a valid blood drug level–time curve is obtained. The time intervals should be spaced so that the peak blood concentration, the total area under the curve, and the absorption and elimination phases of the curve may be well described.

TABLE - Latin-Square Crossover Design for a Bioequivalence Study of Three Drug Products in Six Human Volunteers

	Drug Product		
Subject	Study Period 1	Study Period 2	Study Period 3
1	A	B	C
2	B	C	A
3	C	A	B
4	A	C	B
5	C	B	A
6	B	A	C

Period refers to the time period in which a study is performed. A two-period study is a study that is performed on two different days (time periods) separated by a *washout period* during which most of the drug is eliminated from the body—generally about 10 elimination half-lives.

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

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

	Drug Product			
Subject	Study Period 1	Study Period 2	Study Period 3	Study Period 4
1	A	B	C	D
2	B	C	D	A
3	C	D	A	B
4	D	A	B	C
5	A	B	D	C
6	B	D	C	A
7	D	C	A	B
8	C	A	B	D
9	A	C	B	D
10	C	B	D	A
11	B	D	A	C
12	D	A	C	B
13	A	C	D	B
14	C	D	B	A
15	D	B	A	C
16	B	A	C	D

Replicated Crossover Study Designs

The standard bioequivalence criterion using the two-way crossover design does not give an estimate of within subject (intrasubject) variability. By giving the same drug product twice to the same subject, the replicate design provides a measure for within-subject variability. Replicate design studies may be used for highly variable drugs and for narrow therapeutic index drugs. In the case of highly variable drugs (%CV greater than 30), a large number of subjects (>80) would be needed to demonstrate bioequivalence using the standard two-way crossover design. Drugs with high within-subject variability generally have a wide therapeutic window and despite high variability, these products have been demonstrated to be both safe and effective. Replicate designs for highly variable drugs/products require a smaller number of subjects and, therefore, do not unnecessarily expose a large number of healthy subjects to a drug when this large number of subjects is not needed for assurance of bioequivalence.

In this design, the same reference and the same test are each given twice to the same subject. Other sequences are possible. In this design, reference-to-reference and test-to-test comparisons may also be made.

	Period 1	Period 2	Period 3	Period 4
Sequence 1	T	R	T	R
Sequence 2	R	T	R	T

Where R = reference and T = treatment

(c) Parallel Study Designs:

A nonreplicate, parallel design is used for drug products that contain drugs that have a long elimination half-life or drug products such as depot injections in which the drug is slowly released over weeks or months. In this design, two separate groups of volunteers are used. One group will be given the test product and the other group will be given the reference product. It is important to balance the demographics of both groups of volunteers. Blood sample collection time should be adequate to ensure completion of gastrointestinal transit (approximately 2–3 days) of the drug product and absorption of the drug substance. *C*max and a suitably truncated AUC, generally to 72 hours after dose administration, can be used to characterize peak and total drug exposure, respectively. For drugs that demonstrate low intrasubject variability in distribution and clearance, an AUC truncated at 72 hours (AUC^{72} hours) can be used in place of $AUC0^{t}$ or $AUC0^{\infty}$. This design is not recommended for drugs that have high intrasubject vari- ability in distribution and clearance.

PHARMACOKINETIC EVALUATION OF THE DATA:

For single-dose studies, including a fasting study or a food intervention study, the pharmacokinetic analyses include calculation for each subject of the area under the curve to the last quantifiable concentration (AUC $)0^{t}$ and to infinity (AUC $)0^{\infty}$, *t*max, and *C*max. Additionally, the elimination rate constant, *k*, the elimination half-life, *t*1/2, and other parameters may be estimated. For multiple-dose studies, pharmacokinetic analysis includes

calculation for each subject of the steady-state area under the curve,$(AUC)_{\infty}^{t}$, *t*max, *C*min, *C*max, and the percent fluctuation [100 × (*C*max − *C*min)/*C*min]. Proper statistical evaluation should be performed on the estimated pharmacokinetic parameters.

Statistical Evaluation of the Data

Bioequivalence is generally determined using a comparison of population averages of a bioequivalence metric, such as AUC and Cmax. This approach, termed average bioequivalence, metrics for the test and reference drug products involves the calculation of a 90% confidence interval for the ratio of averages (population geometric means) of the bioequivalence.

After the data has been collected, statistical methods must be applied to determine the level of significance of any observed difference in the rate and/or extent of absorption in order to establish bioequivalence between two or more drug products. The commonly adopted approaches to determine statistical differences are –

Analysis of variance (ANOVA) is a statistical procedure used to test the data for differences within and between treatment and control groups. A statistical difference between the pharmacokinetic parameters obtained from two or more drug products is considered statistically significant if there is a probability of less than 1 in 20 or 0.05 (p 0.05). The probability p is used to indicate the level of statistical significance. If p 0.05, the differences between the two drug products are not considered statistically significant. A bioequivalent product should produce no significant difference in all pharmacokinetic parameters tested. The parameters tested statistically usually include $AUC0^{t}$, $AUC0^{\infty}$, and *C*max obtained for each treatment or dosage form.

Confidence interval approach – Also called as **two one-sided test procedure**, it is used to demonstrate if the bioavailability from the test product is too low or high in comparison to the reference product. The 90% confidence limits are estimated for the sample means based on Student's t distribution of data. A 90% confidence interval about the ratio of means of the two drug products must be within 20% for bioavailability parameters such AUC or Cmax i.e. the difference between the bioavailabilities of the test product should not be greater than ± 20% of the average of reference product (between 80 and 120%). When log transformed data are used, the 90% confidence interval is set at 80-125%. These confidence limits are also termed as

bioequivalence interval. For most drugs, up to a 20% difference in AUC or *C*max between two formulations would have no clinical significance.

STUDY SUBMISSION AND DRUG REVIEW PROCESS:

The contents of New Drug Applications (NDAs) and Abbreviated New Drug Applications (ANDAs) are similar in terms of the quality of manufacture. The submission for an NDA must contain safety and efficacy studies as provided by animal toxicology studies, clinical efficacy studies, and pharmacokinetic/bioavailability studies. For the generic drug manufacturer, the bioequivalence study is the pivotal study in the ANDA that replaces the animal, clinical, and pharmacokinetic studies.

An outline for the submission of a completed bioavailability to the FDA is shown in table. The investigator should be sure that the study has been properly designed, the objectives are clearly defined, and the method of analysis has been validated (ie, shown to measure precisely and accurately the plasma drug concentration). The results are analyzed both statistically and pharmaco kinetically. These results, along with case reports and various data supporting the validity of the analytical method, are included in the submission. The FDA reviews the study in detail. If necessary, an FDA investigator may inspect both the clinical and analytical facilities used in the study and audit the raw data used in support of the bioavailability study. For ANDA applications, the FDA Office of Generic Drugs reviews the entire ANDA. If the application is incomplete, the FDA will not review the submission and the sponsor will receive a Refusal to File letter.

IMPORTANT QUESTIONS

VERY SHORT ANSWER TYPE QUESTIONS (2Marks)

1. Explain renal clearance of drugs.
2. How do you determine renal clearance of drugs ?
3. Explain hepatic extraction ratio and its importance.
4. Explain various non-renal routes of excretion.
5. Explain hepatic clearance.
6. Explain glucuronic acid conjugation.
7. Explain phase I reactions.
8. What is biotransformation and explain its importance.
9. Explain the hepatic metabolism of drugs.
10. Explain the pre systemic metabolism of drugs.
11. List out the various factors affecting biotransformation and discuss any two.
12. List out the various factors affecting excretion and discuss any two.

SHORT ANSWER TYPE QUESTIONS (5 Marks)

1. Define bioavailability. Mention the objectives of bioavailability studies.
2. Define bioequivalence. Explain various types of equivalence.
3. Explain about the subject selection criterion in bioavailability studies.
4. Discuss the various study designs in for performing bioavailability.
5. Explain two ways cross over design.
6. Discuss the various considerations for bioequivalence studies.
7. Explain any two methods to calculate AUC.
8. Explain how bioavailability is measured using plasma data.
9. Explain how bioavailability is measured using urinary data.
10. List out the various methods of assessment of bioavailability and explain any two.
11. What are the various methods of enhancement of bioavailability?

LONG ANSWER TYPE QUESTIONS (10 Marks)

1. Give in detail various levels of IVIVC.
2. Explain in detail methods of enhancing bioavailability of poorly soluble drugs.