Model Answer
B.Pharm. VII sem, Examination 2013
Bioavailability and Therapeutic Drug Monitoring (BTDM)
Paper code: AS-2536

Section A: Short Answer

Ans. 1.

1.1. The point of maximum concentration of drug in plasma is called as the peak and the concentration of drug at peak is known as peak plasma concentration (Cmax).

1.2. The concentration of drug which is required to produce therapeutic action without any adverse effect is called as therapeutic dose.

1.3. The time period for which the plasma concentration of drug remains above the minimum effective concentration level is called as duration of drug action.

1.4. Maximum safe concentration (MSC) is the concentration of the drug in plasma above which adverse effects are precipitated. Concentration of drug above MSC is said to be in the toxic level.

1.5. Bioavailability is defined as the rate and extent of absorption of unchanged drug from its dosage form.

1.6. Noyes–Whitney equation relates the rate of dissolution of solids to the properties of the solid and the dissolution medium. It is an important equation in pharmaceutical science. The relation is given by:

$$\frac{dW}{dt} = \frac{DA(C_s - C)}{L}$$

Where:

dW/dt is the rate of dissolution.

A is the surface area of the solid.

C is the concentration of the solid in the bulk dissolution medium.

C_s is the concentration of the solid in the diffusion layer surrounding the solid.

D is the diffusion coefficient.

L is the diffusion layer thickness.

1.7. Therapeutic equivalence indicates that two or more drug products that contain the same therapeutically active ingredient produce identical pharmacologic effects and can control the disease to the same extent.

1.8. Most pharmacokinetic processes like absorption, distribution, metabolism and elimination follow first order kinetics.

1.9. The plasma drug concentration between the minimum effective concentration (MEC) and maximum safe concentration (MSC) is called as therapeutic window.

1.10. Branded drug: A drug that has a trade name and is protected by a patent (can be produced and sold only by the company holding the patent).

Generic drug: A drug product that is comparable to brand/reference listed drug product in dosage form, strength, route of administration, quality and performance characteristics and intended use. When the patent protection for a brand-name drug expires generic versions of the drug can be offered for sale if the FDA agrees; "generic drugs are usually cheaper than brand-name drugs"

1.11. Half-life ($t_{1/2}$) is defined as the time period required for the concentration of drug to decrease by one-half.

For zero order kinetics,

$$t_{1/2} = 0.5\ C_0/K_0$$

Where,

C_0 is the initial drug concentration.

K_0 is the zero order rate constant.

1.12. Clearance is defined as the theoretical volume of body fluid containing drug from which the drug is completely removed in a given period of time.

Ans. 2. The two major pharmacokinetic methods for the determination of bioavailability are

- Plasma level-time studies.
- Urinary excretion studies.

Discuss in detail procedure, principle involved, parameters derived, equations involved for determination of bioavailability, advantages and disadvantages and schematics available for both methods

i) Plasma level-time studies

a) C_{max} b) t_{max} c) AUC

ii) Urinary excretion studies

a) Maximum urinary excretion rate $(dXu/dt)_{max}$

b) Time for maximum excretion rate $(tu)_{max}$

c) Cumulative amount of drug excreted in the urine (X_u)

Ans.3. Bioequivalence is a term in pharmacokinetics used to assess the expected in vivo biological equivalence of two proprietary preparations of a drug. This term describes pharmaceutical equivalent or pharmaceutical alternative products that display comparable bioavailability when studied under similar experimental conditions. For systemically absorbed drugs, the test (generic) and reference listed drug (brand-name) shall be considered bioequivalent if: (1) 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 a single dose or multiple doses; or (2) the extent of absorption of the test drug does not show a significant difference from the extent of absorption of the reference drug when administered at the same molar dose of the therapeutic ingredient under similar experimental conditions in either a single dose or multiple doses and the

difference from the reference drug in the rate of absorption of the drug is intentional, is reflected in its proposed labeling, is not essential to the attainment of effective body drug concentrations on chronic use, and is considered medically insignificant for the drug.

Study design of Bioequivalence Studies

Bioequivalence studies are performed to compare the bioavailability of the generic drug product to the brand-name product. Statistical techniques should be of sufficient sensitivity to detect differences in rate and extent of absorption that are not attributable to subject variability.

The basic design for a bioequivalence study is determined by:

- The scientific questions to be answered,
- The nature of the reference material and the dosage form to be tested,
- The availability of analytical methods, and
- Benefit–risk and ethical considerations with regard to testing in humans.

Elements of a Bioequivalence 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

Discuss in detail above protocol for bioequivalence.

Study Designs

Currently, three different studies may be required for solid oral dosage forms, including (1) a fasting study, (2) a food intervention study, and/or (3) a multiple-dose (steady-state) study. Proper study design and statistical evolution 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 required 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. In some cases, a parallel design may be more appropriate for certain drug products, containing a drug with a very long elimination half-life. A replicate design may be used for a drug product containing a drug that has high intrasubject variability.

Food Intervention Study

Co-administration 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. The test meal is a high-fat (approximately 50% of total caloric content of the meal) and high-calorie (approximately 800–1000 calories) meal. A typical test meal is two eggs fried in butter, two strips of bacon, two slices of toast with butter, 4 ounces of brown potatoes, and 8 ounces of milk. This test meal derives approximately 150, 250, and 500–600 calories from protein, carbohydrate, and fat, respectively.

For bioequivalence studies, drug bioavailability from both the test and reference products should be affected similarly by food. The 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 ounces) of water. No food is allowed for at least 4 hours post dose. This study is required for all modified-release dosage forms and may be required for immediate-release dosage forms if the bioavailability of the active drug ingredient is known to be affected by food (eg, ibuprofen, naproxen). For certain extended-release capsules that contain coated beads, the capsule contents are sprinkled over soft foods such as apple sauce, which is taken by the fasted subject and the bioavailability of the drug is then measured. Bioavailability studies might also examine the affects of other foods and special vehicles such as apple juice.

Multiple-Dose (Steady-State) Study

In a few cases, a multiple-dose, steady-state, randomized, two-treatment, two-way crossover study comparing equal doses of the test and reference products may be performed in adult, healthy subjects. For these studies, three consecutive trough concentrations (C min) on three consecutive days should be determined to ascertain that the subjects are at steady state. The last morning dose is given to the subject after an overnight fast, with continual fasting for at least 2 hours following dose administration. Blood sampling is performed similarly to the single-dose study.

Crossover Designs

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 . 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, variation 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. 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.

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

Ans. 4

4.a. According to Dilling formula:

$$Dose\ for\ the\ child = \frac{Age\ in\ years}{20} \times Adult\ dose$$

$$Dose\ for\ the\ child = \frac{16}{20} \times 500\ mg$$

$$Dose\ for\ the\ child = \frac{16}{20} \times 500\ mg$$

$$Dose\ for\ the\ child = 400\ mg$$

According to Cowling formula:

$$Dose\ for\ the\ child = \frac{Age\ in\ years + 1}{24} \times Adult\ dose$$

$$Dose\ for\ the\ child = \frac{16 + 1}{24} \times 500\ mg$$

$$Dose\ for\ the\ child = \frac{17}{24} \times 500\ mg$$

$$Dose\ for\ the\ child = 354.17\ mg$$

According Bastedo's formula:

$$Dose\ for\ the\ child = \frac{Age\ in\ years + 3}{30} \times Adult\ dose$$

$$Dose\ for\ the\ child = \frac{16 + 3}{30} \times 500\ mg$$

$$Dose\ for\ the\ child = \frac{19}{30} \times 500\ mg$$
$$Dose\ for\ the\ child = 316.67\ \text{mg}$$

4.b.Individualization of Drug Dosage Regimens

Because of reasonable homogeneity in humans, the dosage regimens are calculated on population basis. However, same dose of a drug may produce large differences in pharmacologic response in different individuals. This is called as intersubject variability. In other words, it means that the dose required produce a certain response varies from individual to individual. Rational drug therapy requires individualization of dosage regimen to fit a particular patient's needs. The two sources of variability in drug responses are:

Pharmacokinetic variability which is due to differences in drug concentration at the site of action because of intersubject variability in drug absorption, distribution, metabolism and excretion.

Pharmacodyanamic variability which is attributed to differences in effect produced by a given drug concentration.

The main objective of individualization is aimed at optimizing the dosage regimen. An inadequate therapeutic response calls for a higher dosage whereas drug related toxicity calls for a reduction in dosage. Thus, in order to aid individualization, a drug must be made available in dosage forms of different dose strengths. The number of dose strengths in which a drug should be made available depends upon two major factors:

- The therapeutic index of the drug, and
- The degree of intersubject variability.

Smaller the therapeutic index and greater the variability, more the number of dose strengths required. Based on the assumption that all patients require same plasma drug concentration range for therapeutic effectiveness, the steps involved in the individualization are:

- Estimation of pharmacokinetic parameters in individual patient and determining their deviation from the population values to evaluate the variability.
- Attributing the variability to some measurable characteristics such as hepatic or renal disease, age, weight etc.
- Designing the new dosage regimen from collected data.

The design of new dosage regimen involves:

- Adjustment of dosage, or
- Adjustment of dosage interval, or
- Adjustment of both dosage and dosing interval.

Individualized Dose calculations are required and following point need to discuss with suitable examples and equations involved

1. **Dosing of drugs in obese patients.**

2. **Dosing of drugs in neonates, infants and children.**

$$Child's\ dose = \frac{Surface\ area\ of\ child\ in\ m^2}{1.73} \times Adult\ dose$$

$$Child's\ dose = \frac{Surface\ area\ of\ child\ in\ m^2}{1.73} \times Adult\ dose$$

3. **Dosing of drugs in elderly.**

$$Patient's\ dose = [\frac{Weight\ of\ child\ in\ Kg}{70}]^{0.7} \times Adult\ dose$$

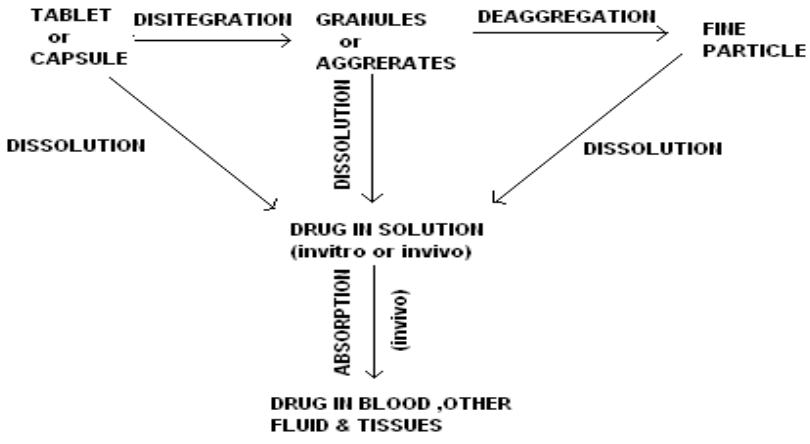
4. **Dosing of drugs in hepatic disease.**

5. **Dosing of drugs in renal disease.**

Ans. 5.

Dissolution process: - It is the transfer of solute molecules from the surface of solid into the bulk of solution. It is the process of dissolving solid part (solute) in the solvent (liquid).

Dissolution rate may be defined as amount of drug substance that goes in the solution per unit time under standard conditions of liquid/solid interface, temperature and solvent composition. It can be considered as a specific type of certain heterogeneous reaction in which a mass transfer results as a net effect between escape and deposition of solute molecules at a solid surface.



(Schematic illustration of dissolution process of solid dosage forms)

Factor affecting dissolution rate

1. Physicochemical Properties of Drug
    - Solubility
    - Particle size and effective surface area of drug
    - Polymorphism and amorphism
    - Salt form of drug
    - Hydrate and solvates
2. Factors relating to the dosage forms
    - Pharmaceutical excipients i.e. vehicles, diluents, lubricants, binders, surfactants, colorants, polymers etc.
    - Manufacturing process i.e. method of granulation, intensity of packing of capsule contents, compression force, drug excipient interactions etc.
3. Processing Factors
    - Method of introduction of dosage form
    - Sampling Technique
    - Changing the dissolution fluid
4. Factors Relating Dissolution Apparatus
    - Design of container
    - Size of container
    - Shape of container
    - Nature of agitation
    - Speed of agitation
    - Performance precision of apparatus
    - Temperature control
5. Factors related to dissolution fluid
    - Composition, viscosity, volume, Temperature, sink condition

Discuss in detail each factor with suitable examples

Ans. 6.

THERAPEUTIC DRUG MONITORING

In administering potent drugs to patients the physician must maintain the plasma drug level within a narrow range of therapeutic concentrations. Usually the initial dosage regimen is calculated empirically or estimated after a careful consideration of the known pharmacokinetics for the drug the pathological condition of the patient and patient's drug history.

Due to inter patient variability in drug absorption, distribution and elimination as well as changing pathophysiological conditions in the patient, therapeutic drug monitoring (TDM) services have been established in many hospitals to evaluate the response of patient to the recommended dosage regimen. The functions of a TDM service are listed below.

- Selection of drug

- Designing of dosage regimen
- Evaluation of patient's response
- Determining the need for measuring serum drug concentration
- Assay of drug

  The assay of drugs in serum should be validated with respect to the following

  Specificity, sensitivity, linearity, precision, accuracy and stability.
- Performing pharmacokinetic evaluation of drug levels

Serum concentration lower than anticipated

- Patient compliance
- Error in dosage regimen
- Wrong drug product
- Poor bioavailability
- Rapid elimination
- Enlarged apparent volume of distribution
- Steady state not reached
- Timing of blood sample

Serum concentration higher than anticipated

- Patient compliance
- Error in dosage regimen
- Wrong drug product
- Rapid bioavailability
- Slow elimination
- Smaller than anticipated apparent volume of distribution

Serum concentration higher than anticipated

- Altered receptor sensitivity
- Drug interaction at receptor site

- Readjustment of dosage regimen
- Monitoring serum drug concentration
- Recommending special requirements

Discuss above functions of TDM process in detail with suitable examples.

Ans. 7

a. Apparent volume of distribution:

$$X \propto C$$
$$X = Vd\ C$$

Where, C concentration of drug in plasma, X amount of drug in the body and Vd= proportionality constant having the unit of volume and popularly called as **Apparent volume of distribution.** It is **defined** as the hypothetical volume of the body fluid into which a drug is dissolved or distributed.

Apparent volume of distribution = amount of drug in the body/plasma drug concentration

b. ***In vitro-In vivo* Correlation**

IVIVC simply means a mathematical model that can describe the relationship between in vitro and in vivo properties of a drug product, so that in vivo properties can be predicted from its in vitro behavior.

According to FDA, "IVIVC is a predictive mathematical model describing relationship between in vitro properties of dosage form and relevant in vivo response. Generally the in vitro property is rate or extent of drug dissolution or release while in vivo response is the plasma drug concentration or amount absorbed.

Levels of Correlation : discuss with suitable graphical presentation

Five levels of correlation can be found in FDA guidelines. Each level denotes its ability to predict in vivo response of dosage form from its in vitro property. Higher the level better is the correlation.

1. Level A correlation.
2. Level B correlation.
3. Level C correlation.
4. Multiple level C correlation.
5. Level D correlation.

1. Level A correlation

It represents the relationship between in vitro dissolution and in vivo in put rate (in vivo absorption of drug from dosage form). A hypothetical level model showing relationship between fraction absorbed and fraction dissolved,

The *in vitro* properties like percent drug dissolved or fraction of drug dissolved can be used while *in vivo* properties like percent drug absorbed or fraction of drug absorbed can be used respectively.

Level A IVIVC is considered as predictive model for relationship between the entire *in vitro* release time course and entire *in vivo* response time course.

Most commonly there should exist a linear correlation but sometimes non-linear correlation may be appropriate. However no formal guidance on non-linear IVIVC has been established. When *in vitro* curve and *in vivo* curve are superimposable the relationship is called as 1:1 relationship. But if scaling factor is required to make curve superimposable the relationship is called as point-to-point relationship. It is the highest level of correlation and most preferred to achieve, since this allows biowaiver for changes in manufacturing site, raw material suppliers, and minor changes in formulation.

2. Level B correlation

In this level of correlation (Figure No. 2), the mean *in vitro* dissolution time (MDT *in vitro*) is compared with either the mean *in vivo* residence time (MRT *in vivo*) or mean *in vivo* dissolution time (MDT *in vivo*) derived by using principle of statistical moment analysis. Even though it utilizes all *in vitro* and *in vivo* data, it is not considered as point-to-point correlation, since number of *in vivo* curves can produce similar residence time value. Hence this correlation becomes least useful for regulatory purposes.

3. Level C correlation

It is a single point correlation that is established in between one dissolution parameter like t50% and one of the pharmacokinetic parameters like tmax, Cmax or AUC. It does not reflect the complete shape of plasma drug concentration time curve, which is the critical factor that defines the performance of drug product.

However it can be helpful in early stages of formulation development when pilot formulations are being selected.

4. Multiple level C correlation

Multiple level C correlation reflects the relationship between one or several pharmacokinetic parameters of interest and amount of drug dissolved at several time point of dissolution profile. It should be based on at least three dissolution time points that includes early, middle and late stage of dissolution profile. When multiple level C correlation is developed there are more chances of development of level A correlation, which should be preferred.

5. Level D correlation

It is a rank order and semi quantitative correlation and is not considered useful for regulatory purpose.

Development of level a correlation

At least three formulations should be developed with different release rates that is slow, medium and fast release rate (at least differ by ±10%) so that comparable difference between *in vivo* property (tmax, Cmax or AUC) of these formulation is possible. *In vitro* data should be obtained from optimized *in vitro* dissolution study, generally by using official dissolution apparatus.

Optimization of dissolution testing should be done to get best *in vivo* simulations and higher possible correlation. Once dissolution testing method is developed, same method should be used for all the formulations. The dissolution testing should be performed on 12 individual dosage forms from each lot and mean values should be considered.

To obtain *in vivo* data, bioavailability (BA) study in sufficient number of healthy volunteers (6-12) should be performed. The crossover study design is generally preferred; if not possible parallel study design is also acceptable. The drug product should be administered in fasting state, however if intolerable it can be administered in fed state and the effect of food should be considered.

Generally two methods are used for development of correlations (as shown in Figure No.5)-

1) Two stage deconvolution approach: It involve estimation of *in vivo* absorption profile from plasma drug concentration time profile using Wagner Nelson or Loo-Riegelman method, subsequently the relationship with *in vitro* data is evaluated.

2) One stage convolution approach: It computes the *in vivo* absorption and simultaneously models the *in vitro – in vivo* data.

FACTORS TO BE CONSIDERED WHILE DEVELOPING IVIVC

1. Stereochemistry
2. First Pass Effect
3. Food Effect

APPLICATIONS OF IVIVC

1. Early Development of Drug Product and Optimization
2. Biowaiver for Minor Formulation and Process Changes
3. Setting Dissolution Specifications