आईएफटीएम विश्वविद्यालय, मुरादाबाद, उत्तर प्रदेश

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NAAC ACCREDITED

E-Content

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INTRODUCTION

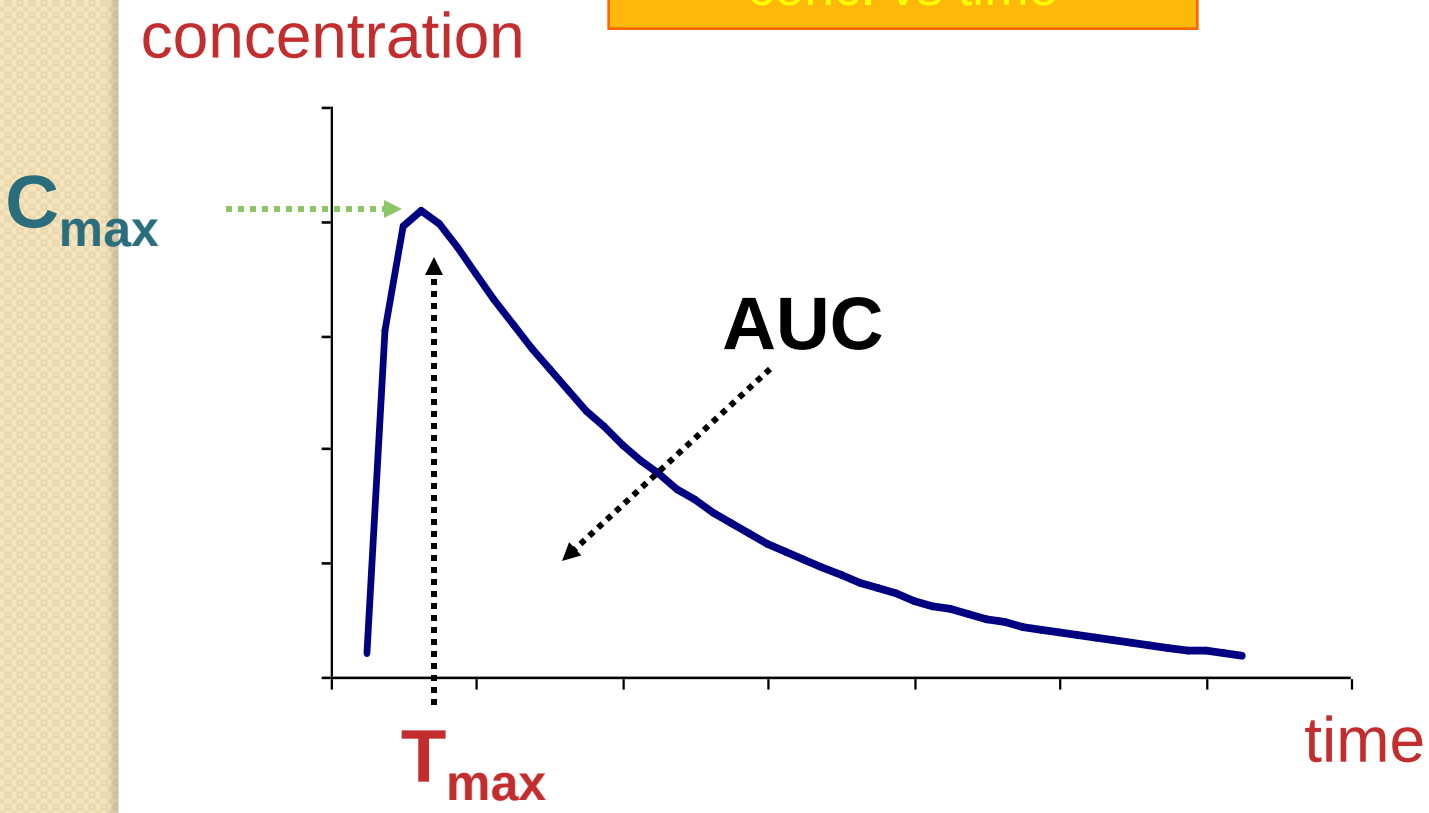
- Ensuring uniformity in standards of quality, efficacy of pharmaceutical products.
- BA/BE focus on the release of the drug from the dosage form and absorption in to the systemic circulation.
- BE for the comparison of the two drug, several test method are given to determine the equivalence.

Important Pharmacokinetic Parameters

- AUC: area under the concentration-time curve $\Rightarrow$ measure of the extent of bioavailability
- C_{max}: the observed maximum concentration of drug $\Rightarrow$ measure of both the rate of absorption and the extent of bioavailability
- t_{max}: the time after administration of drug at which C_{max} is observed $\Rightarrow$ measure of the rate of absorption

Plasma concentration time profile

Pharmacokinetics
conc. vs time



DEFINITION

- BIOAVAILABILITY: It is relative amount of drug from an administered dosage form which enters the systemic circulation and rate at which the drug appears in the systemic circulation.
- The extent and rate at which its active moiety is delivered from pharmaceutical form and becomes available in the systemic circulation

Scheme of Oral Dosage Form

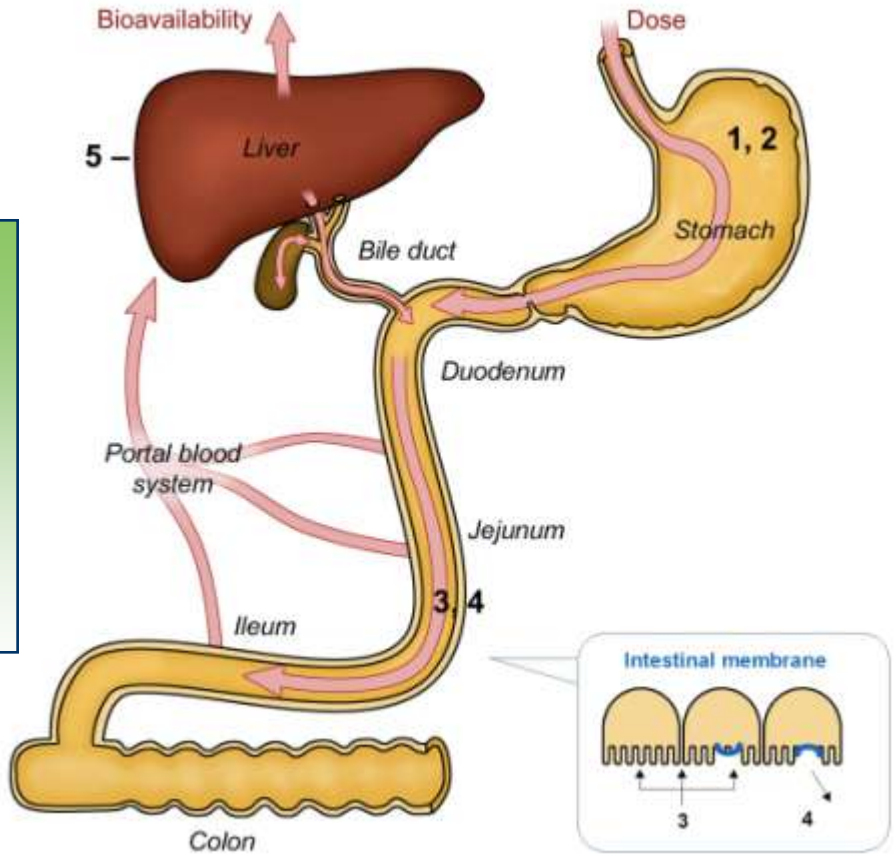
Human Intestinal Absorption (*HIA*)

1,2 – Stability + Solubility

3 – Passive + Active Tr.

4 – Pgp efflux + CYP 3A4

Oral Bioavailability (%*F*)



Why do we care about BIOAVAILABILITY?

- The “true dose” is not the drug swallowed;

  BUT is the drug available to exert its effect.

- Dissolution
- Absorption
- Survive metabolism

Why do we care about BIOAVAILABILITY?

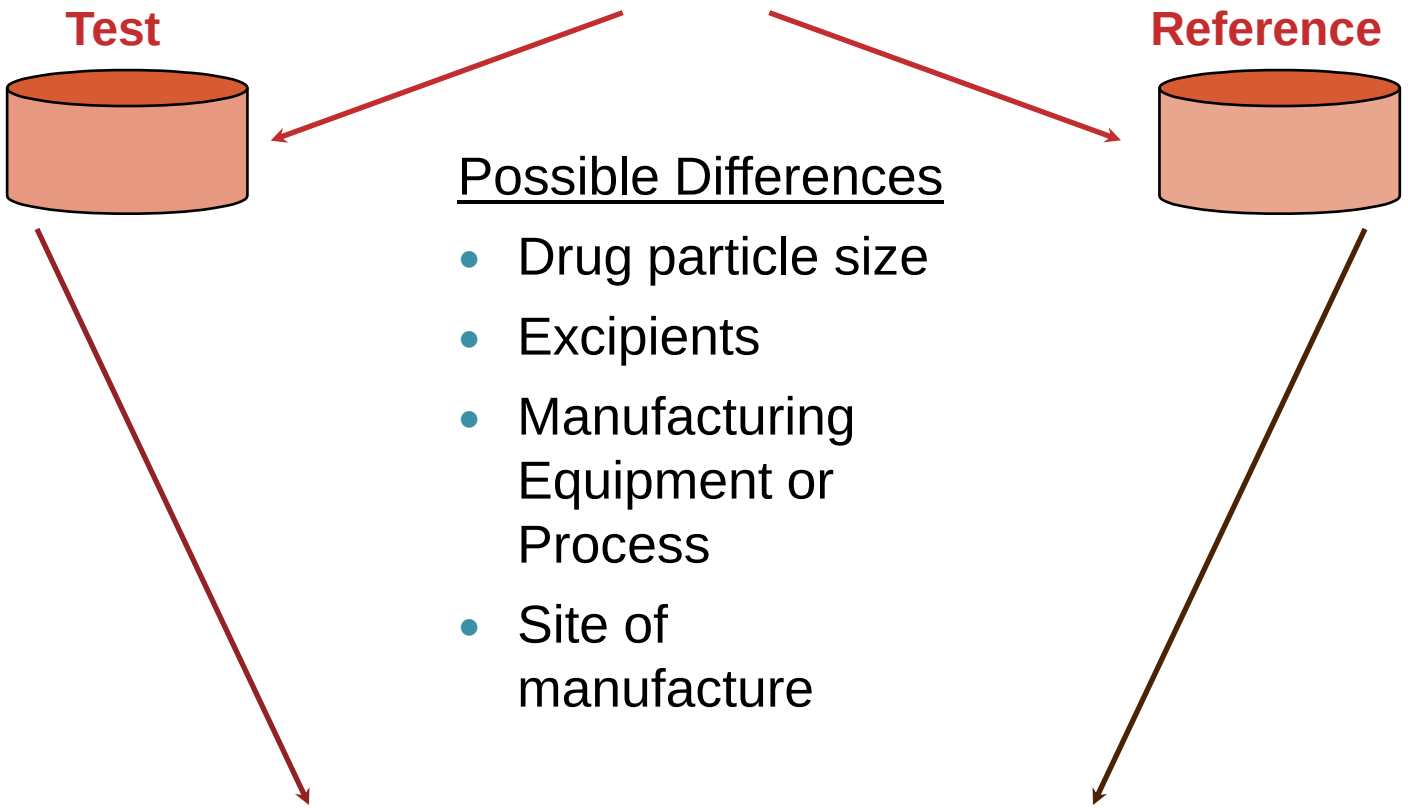
- May have a drug with very low bioavailability.
- Dosage form or drug may not dissolve readily.
- Drug may not be readily pass across biological membranes (i.e. be absorbed).
- Drug may be extensively metabolized during absorption process (first-pass, gut wall, liver).
- Important component of overall variability i.e. Variable bioavailability may produce variable exposure.

Pharmaceutical Equivalents

- contain the same amount of the same active substance in the same dosage form
- meet the same or comparable standards
- intended to be administered by the same route

Pharmaceutical equivalence by itself does not
necessarily imply therapeutic

Pharmaceutical Equivalents



Could lead to differences in product performance *in vivo*

⇒ Possible Bioinequivalence

Bioequivalence

Two products are bioequivalent if

- they are pharmaceutically equivalent
- bioavailabilities (both rate and extent) after administration in the same molar dose are similar to such a degree that their effects can be expected to be essentially the same

Therapeutic equivalence

Two products are therapeutically equivalent if

- pharmaceutically equivalent
- their effects, with respect to both efficacy and safety, will be essentially the same as derived from appropriate studies
  - bioequivalence studies
  - pharmacodynamic studies
  - clinical studies
  - *in vitro* studies

Significance of Bioavailability

- Drugs having **low therapeutic index,** e.g. cardiac glycosides, quinidine, phenytoin etc. and **narrow margin of safety** e.g. antiarrythmics, antidiabetics, adrenal steroids, theophylline .
- Drugs whose **peak levels are required** for the effect of drugs, e.g. phenytoin, phenobarbitone, primidone, sodium valporate, anti-hypertensives, antidiabetics and antibiotics.
- Drugs that are absorbed **by an active transport,** e.g. amino acid analogues, purine analogues etc.
- In addition, any **new formulation** has to be tested for its bioavailability profile.

- Drugs which are **disintegrated in the alimentary canal and liver,** e.g.chlorpromazine etc. or those which under go **first pass metabolism.**
- Formulations that give **sustained release of drug,** formulations with smaller disintegration time than dissolution rate and drugs used as **replacement therapy** also warrant bioavailability testing.
- Drugs with **steep dose response relationship** i.e. drugs **obeying zero order kinetics / mixed order elimination kinetics** (e.g. warfarin , phenytoin, digoxin, aspirin at high doses, phenylbutazone)

Absolute Bioavailability (F)

- **Definition**

"When the systemic availability of a drug administered orally is determined in comparison to its intravenous administration ,is called as absolute bioavailability".

$$\% \text{ Absorption} = \frac{\text{Dose}_{(iv)} \times \text{AUC}_{(oral)}}{\text{Dose}_{(oral)} \times \text{AUC}_{(iv)}} \times 100$$

- It is denoted by symbol **F.**
- Its determination is used to characterize a **inherent absorption properties from the e.v. Site.**

Relative Bioavailability (Fr)

- **Definition**

"When the systemic availability of the drug after **oral** administration is compared with that of **oral standard of same drug** (such as aqueous or non aqueous solution or a suspension) is referred as Relative Bioavailability or comparative ".

e.g. comparison between **cap**. Amox and **susp.** Amox

- It is used to characterize absorption of a drug from its formulation.
- It is denoted by symbol **Fr.**

$$Fr = \frac{AUC\ A}{AUC\ B}$$

Single Dose vs Multiple Dose Studies

- **Single dose study**

Advantages

- More common
- Easy
- less tedious
- Less exposure to drug.

Disadvantages

- Difficult to predict steady state characteristics.

Multiple dose study

Advantages

- ✓ Accurate.
- ✓ Easy to predict the peak & valley characteristics of drug.
- ✓ Few blood samples required.
- ✓ Ethical.
- ✓ Small inter subject variability .
- ✓ Better evaluation of controlled release formulations.
- ✓ Can detect non linearity in pharmacokinetics.
- ✓ Higher blood levels (d/t cumulative effect).
- ✓ Eliminates the need for long wash out period between doses.

Disadvantages

- ✓ Poor subject compliance .
- ✓ Tedious , time consuming.
- ✓ More drug exposure.
- ✓ More difficult and costly.

Human Volunteers

Healthy Subjects vs. Patients

- ✓ **Patients :** used in multiple dose studies.
- ✓ **Advantages**

1. Patient gets benefited from the study.
2. Reflects better therapeutic efficacy.
3. Drug absorption pattern in disease states evaluated.
4. Avoids ethical quandary.

- ✓ **Disadvantages**

1. Disease states , other drugs affects study
2. Difficult to follow stringent study conditions.

❑ Healthy human volunteers

i. Young

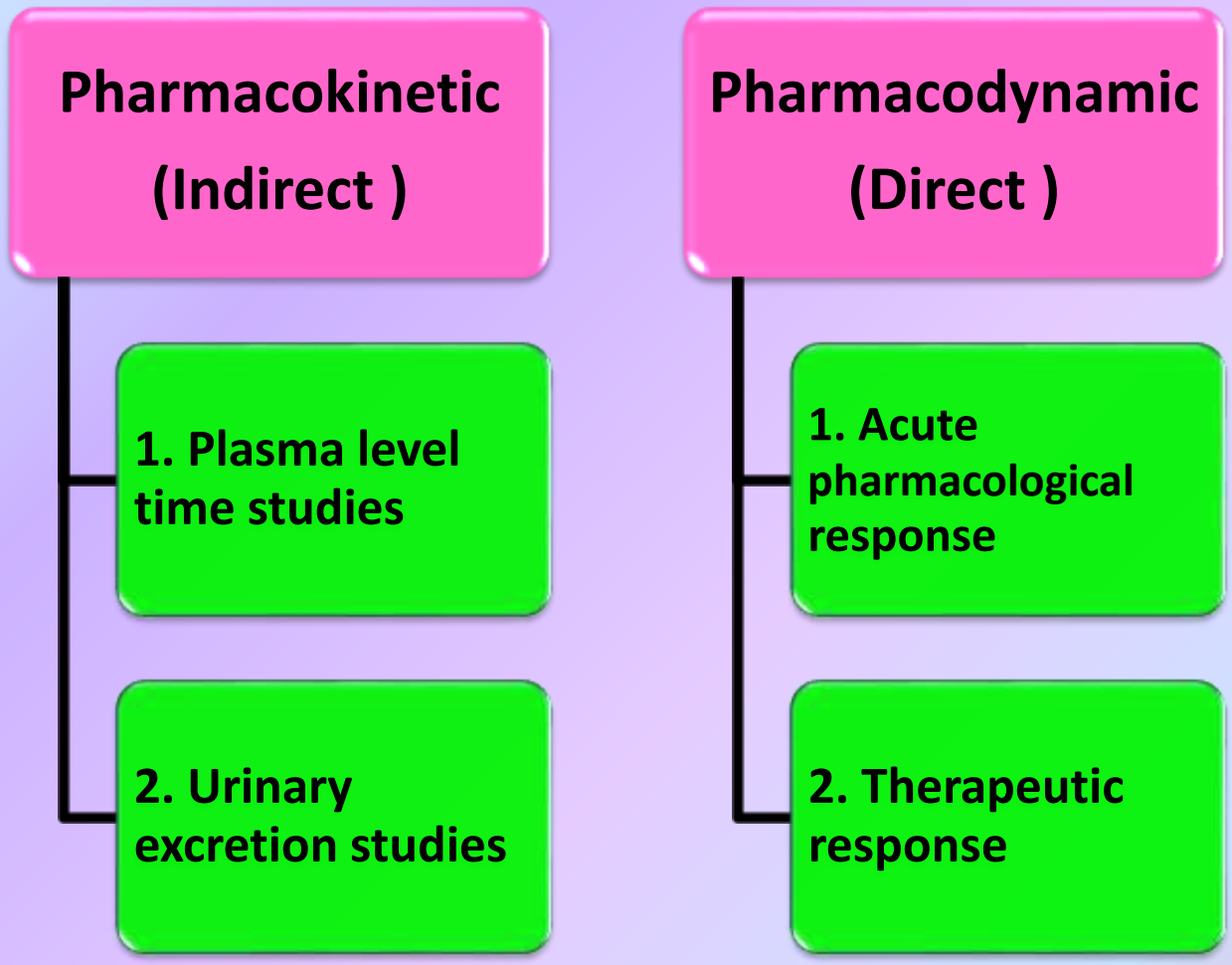
ii. Healthy

iii. Male (females : e.g. OC pills study)

iv. Body wt. within narrow range.

v. Restricted dietary & fixed activity conditions.

Measurement of Bioavailability



- **Pharmacokinetic method**

1. Based on assumption that the pharmacokinetic profile **reflects** the therapeutic effectiveness of drug.
2. These are **indirect** method.

- **Pharmacodynamic method**

1. Involves **direct measurement** of drug effect on a (patho) physiological process as a function of time.
2. It is direct measurement.

1. Plasma level-time studies

✓With single dose study

- The method is based on the **assumption of 2 dosage forms that exhibit superimposable plasma level time profiles** in a group of subject should result in identical therapeutic activity.
- With single does study, the method **requires collection of serial blood samples for a period of 2 to 3 biological half lives** after drug administration, their analysis for drug concentration and making a plot of **concentration versus corresponding time** of sample collection to obtain the plasma level – time profile.
- **With i.v.** Does, **sampling should start within 5 minutes of drug administration** and subsequent samples taken at **15 minute intervals.**

- The three parameters of plasma level time studies which are considered important for determining bioavailability are:

1. **AUC:** The **AUC** is proportional to the **total amount of drug reaching the systemic circulation,** and thus characterizes the extent of absorption.
2. **C_{max}:** Gives indication whether drug is **sufficiently absorbed systemically to provide a therapeutic response.** It is a function of both the rate and extent of absorption**. C_{max} will increase with an increase in the dose, as well as with an increase in the absorption rate.**
3. **T_{max}:** The **T_{max}** reflects the **rate of drug absorption, and decreases as the absorption rate increases**.

- The extent of bioavailability can be determined by following equation:

$$F = \frac{[AUC]_{oral}\, D_{iv}}{[AUC]_{iv}\, D_{oral}}$$

$$Fr = \frac{[AUC]_{test}\, D_{std}}{[AUC]_{test}\, D_{std}}$$

Where, D = dose administered and subscript iv and oral indicates the route of administration.

Subscript **test and std** indicates the test and standard doses of same drug .

✓With multiple dose administration

- The method involves drug administration for atleast 5 biological half lives a dosing interval equal to or greater than the biological half life (i.e. Adminstration of at least 5 doses) to reach the steady state.
- The extent of bioavailability is given as:

$$Fr = \frac{[AUC]_{test}\, D_{std}\, \tau_{test}}{[AUC]_{test}\, D_{std}\, \tau_{std}}$$

where, τ = dosing interval

- Bioavailability can also be determined from peak plasma concentration at steady state Css,max according to following equation:

$$Fr = \frac{[Css,max]_{test}\ D_{std}\ \tau_{test}}{[Css,max]_{test}\ D_{std}\ \tau_{std}}$$

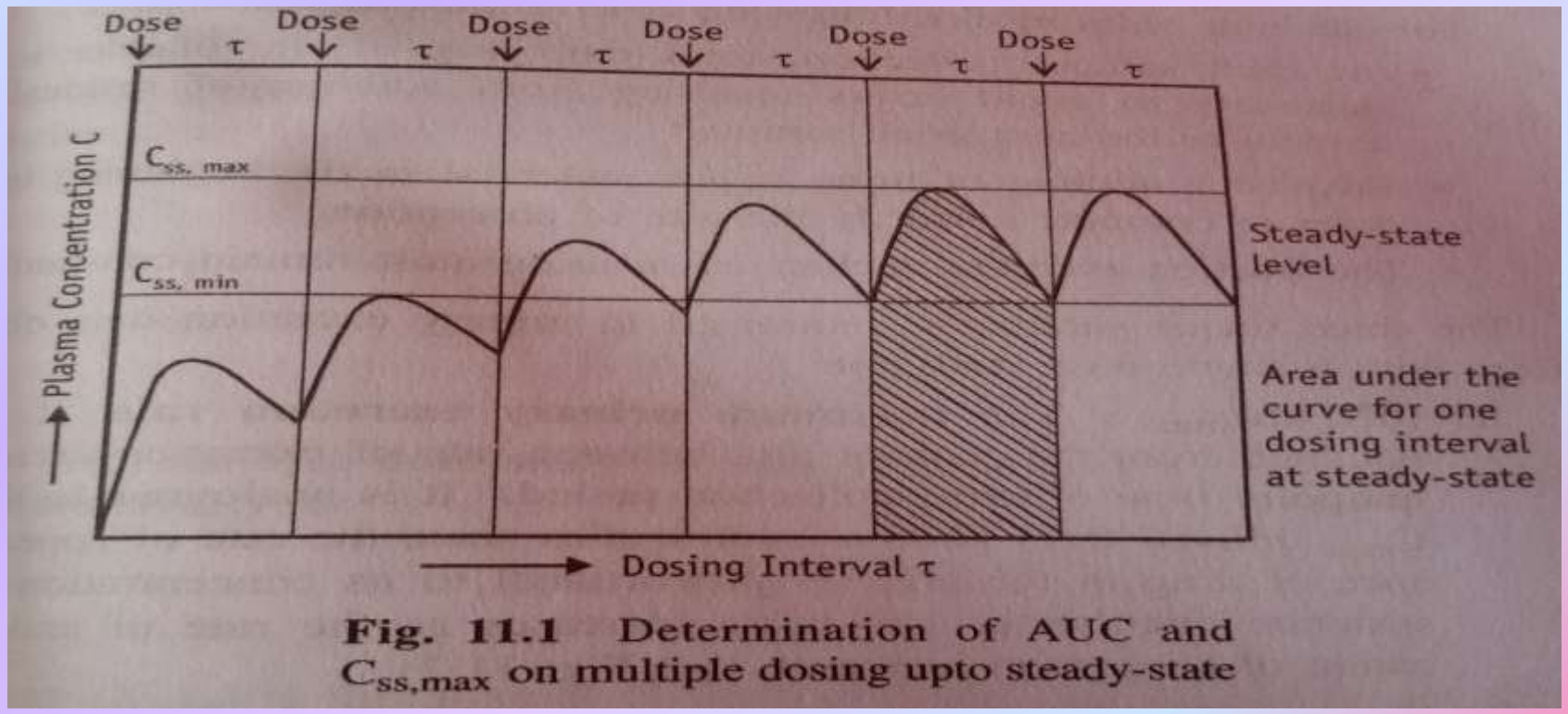


Fig. 11.1 Determination of AUC and $C_{ss,max}$ on multiple dosing upto steady-state

2. Urinary Excretion studies

- These studies are based on the premise that urinary excretion of the **unchanged drug is directly proportional to the plasma concentration of total 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

1. Drugs that extensively excreted **unchanged** in the urine.

   For example: thiazide diuretics, sulfonamides.

2. Drug that have **urine as the site of action.**

   For example: Urinary antiseptics : nitrofurantoin, Hexamine.

✓ **The method involves**

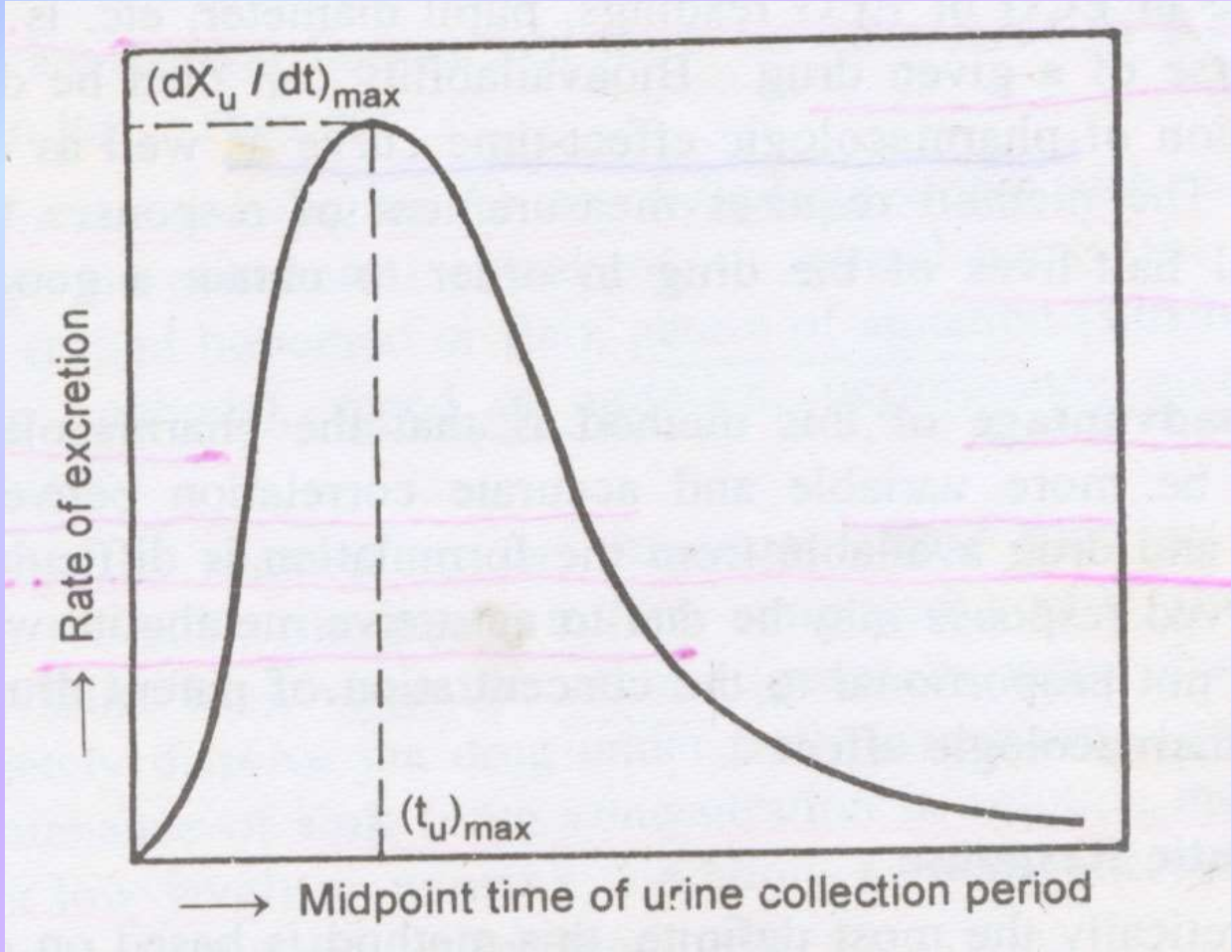
1. Collection of urine at regular intervals for a time **span equal to 7 biological half lives.**
2. Analysis of **unchanged drug** in the collected sample.
3. Determination of the **amount of drug excreted** in each interval and cumulative excreted.

✓ **For obtaining valid result following criteria must be followed**

1. At each sample collection, **total emptying of the bladder** is necessary to avoid errors.
2. **Frequent sampling** of urine is also essential in the beginning.
3. The fraction excreted unchanged in urine must remain **constant**.

- ✓ **The three major parameters examined in urinary excretion data are as follow:**

1. **$(dXu/dt)_{max}$:** It gives 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
2. **$(tu)_{max}$:** It is analogous to the plasma level data, its value **decreases as the absorption rate increases.**
3. **Xu :** It is related to the **AUC of plasma level data** and increases as the extent of absorption increases.



Plot of urinary excretion rate versus time.

✓ The extent of bioavailability is calculated from equation

$$F = \frac{[Xu\infty]oral \times D\ iv}{[Xu\infty]iv \times D\ oral}$$

$$Fr = \frac{[Xu\infty]test \times D\ std}{[Xu\infty]std \times D\ test}$$

✓ With multiple dose study

$$Fr = \frac{[Xu,ss]test \times D\ std \times \tau_{test}}{[Xu,ss]std \times D\ test \times \tau_{std}}$$

✓ Where (Xu,ss) is the **amount of drug excreted unchanged** during a single interval at steady state.

B. Pharmacodynamic methods

1) Acute Pharmacological Response :

✓ Used when pharmacokinetic methods are difficult, inaccurate & non reproducible an acute pharmacological effect such as

- E.g. 1. Change in ECG/EEG readings.

2. Pupil diameter.

<u>Disadvantages</u> :

1. **More variable** and accurate correlation between measured response and available from the formulation is **difficult**.
2. **Active metabolite interferes** with the result.

2) Therapeutic Response :

- ✓ Measurement of clinical response to a drug formulation given to **patients suffering from disease** for which it is intended to be used.

- ✓ **Disadvantages :**

1. Improper quantification of observed response.
2. Bioequivalence studies are usually conducted using a **crossover design.**

LIMITATIONS OF BA/BE STUDIES

- Difficult for drugs with a **long elimination half life.**
- **Highly variable drugs** may require a far greater number of subjects
- Drugs that are administered by **routes other than the oral route** drugs/dosage forms that are intended **for local effects** have minimal systemic bioavailability.

  E.g. ophthalmic, dermal, intranasal and inhalation drug products.
- **Biotransformation** of drugs make it difficult to evaluate the bioequivalence of such drugs: e.g. stereoisomerism

Bioequivalence (BE):

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

Need of bioequivalence studies

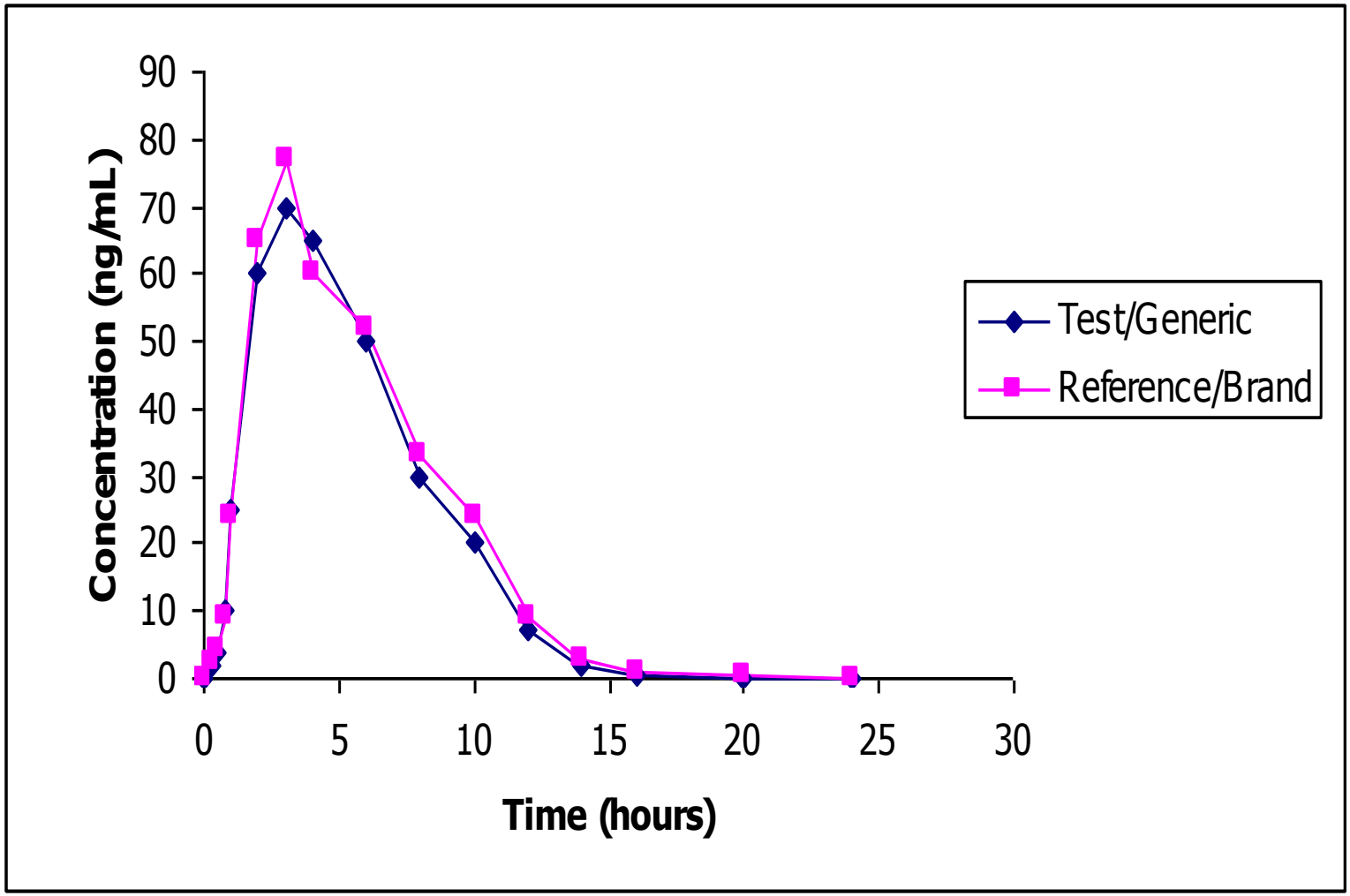
- No clinical studies have been performed in patients with the Generic Product to support its Efficacy and Safety.
- With data to support similar in vivo performance (= Bioequivalence) Efficacy and Safety
  data can be extrapolated from the Innovator Product to the Generic Product.

Bioequivalence (BE):

Ultimate: Bioequivalence studies 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

- Establish that a new formulation has therapeutic equivalence in the rate and extent of absorption to the reference drug product.
- Important for linking the commercial drug product to clinical trial material at time of NDA.
- Important for post-approval changes in the marketed drug formulation.

Bioequivalence



Goals of BE

Ultimate: Bioequivalence studies 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

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NDA vs. ANDA Review Process

Innovator and generic drug

Original Drug

NDA Requirements

1. Chemistry
2. Manufacturing
3. Controls
4. Labeling
5. Testing
6. Animal Studies
7. Clinical Studies

(Bioavailability/Bioequivalence)

Generic Drug

ANDA Requirements

1. Chemistry
2. Manufacturing
3. Controls
4. Labeling
5. Testing
6. Bioequivalence Study (In Vivo, In vitro)

NDA vs. ANDA Review Process

- Note: Generic drug applications are termed **"abbreviated"** because they are generally

not required to include preclinical (animal) and **clinical (human) data to establish safety and effectiveness**.

- Instead, generic applicants must scientifically demonstrate that their product is bioequivalent

(i.e., performs in the same manner as the original; drug).

Current BE* Requirements

Major Regulatory Agencies

- U.S. Food and Drug Administration (FDA)
- Health Canada
- Committee for Proprietary Medicinal Products (CPMP), Europe
- National Institute of Health Sciences (NIHS), Japan

*BE = Bioequivalence

Current BE Requirements
FDA*

- AUC: 90% Confidence Interval Limits 80-125%
- C_{max}: 90% Confidence Interval Limits 80-125%
- Criteria applied to drugs of low and high variability

Current BE Requirements
Health Canada

- AUC: 90% Confidence Interval Limits 80-125%
- C_{max}: Mean T/R ratio (point estimate) between 80-125% (T=test, R=reference)
- Criteria judged flexible enough to deal with highly variable drugs*

Current BE Requirements
CPMP*

- AUC: 90% Confidence Interval Limits 80-125%
- C_{max}: 90% Confidence Interval Limits 80-125%
- C_{max}: “In certain cases a wider interval is acceptable (*e.g.*, 75-133%)

Current BE Requirements
NIHS (Japan)*

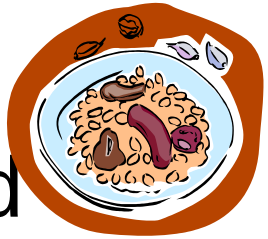
- AUC: 90% Confidence Interval Limits 80-125%
- C_{max}: 90% Confidence Interval Limits 80-125%
- In cases of failure, add-on studies are acceptable (provided other criteria are met)

Study Design: Basic design consideration

- Minimize variability to formulations
- Minimize bias

To compare performance of two products!!!

Study Designs



- Single-dose, two-way crossover, fasted
- Single-dose, two-way crossover, fed

<u>Alternative</u>

- Single-dose, parallel, fasted (Long half-life)
- Single-dose, replicate design (Highly Variable Drugs)
- Multiple-dose, two-way crossover, fasted (Less Sensitive, non-linear kinetic)

Study Designs

- Duration of washout period for cross-over design
- should be approximately > 5 times the plasma apparent terminal half-life
- However, should be adjusted accordingly for drugs with complex kinetic model

Study Designs

- Sample size determination(dose)
  - significant level ($\alpha = 0.05$)
  - 20% deviation from the reference product
  - power > 80%
- Sample time determination
  - adequate data points around t_{max}
  - 3 or more time of $t_{1/2}$ to around $AUC_{0\text{-}t}$ = at least 80% $AUC_{0\text{-}inf}$

Study Designs

- Subjects? (Inclusion/exclusion criteria)

LABEL

- Healthy subjects (male and female)
- 18-55 years old,
- Non-smokers/without a history of alcohol or drug abuse Medical history/Clinical Lab test values must be within normal ranges
- Contraindication
- Refrain from the concomitants use of any medications or food interact with GI, renal, liver function from 28 days prior study Day1 through the safety.

Study Design:

- Crossover Design
- x Crossover design
- A single-dose bioequivalence study is performed in normal, healthy, adult volunteers.
- 18 subjects are hired (Male or Female?)
- The subjects are randomly selected for each group and the sequence of drug administration is randomly assigned.
- One-week washout periods
- Fasted or Fed?

Statistical Analysis (Two one-sided Tests Procedure)

- AUC (Extent) and C_{max} (Rate) – Log transformation

i.e. 90% Confidence Intervals (CI) of the difference in Log (AUC_t) –Log (AUC_R) must fit between 80%-125%

Bioanalytical Method Validation

Method Validation should include

Accuracy

Precision

Sensitivity

Specificity

Recovery

Stability

Bioanalytical Method Validation

- Accuracy

Closeness of determined value to the true

Value

The acceptance criteria is mean value < 15% deviation from the true value.

At LOQ(limit of quantification), 20% deviation is acceptable

Bioanalytical Method Validation

- Precision

The closeness of replicate determinations of

a sample by an assay

The acceptance criteria is < 15% CV,(closeness value) at

20% LOQ(limit of quantification)

Bioanalytical Method Validation

- Sensitivity

The limit of quantitation is the lowest concentration which can be measured with acceptable accuracy and precision

- Selectivity

Ability of the method to measure only what it

is intended to measure in the presence of other components in the sample. Blank samples of the biological matrix should be tested for the interfering peak.

Bioanalytical Method Validation

- Recovery

The extraction efficiency of an analytical process, reported as an percentage of the known amount of an analyte. Recovery does not have to be 100% but the extent of recovery of internal standard and analyte should be consistent.

Bioanalytical Method Validation

- Stability

During, sample collection , sample storage

and sample analysis process, the stability of

drug in matrix should be conducted

Conclusion

- Establish that a new formulation has therapeutic equivalence in the rate and extent of absorption to the reference drug product.
- Important for linking the commercial drug product to clinical trial material at time of NDA.
- Important for post-approval changes in the marketed drug formulation.

References

- D.M.Brahmankar, S.B.Jaiswal Biopharmaceutics and Pharmacokinetics A Treatise Vallabh Prakashan pg.no.315-363.