BIOAVAILABILITY AND BIOEQUIVALENT STUDIES

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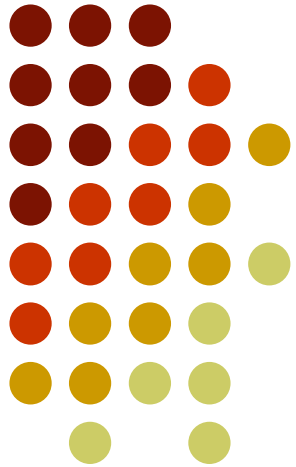
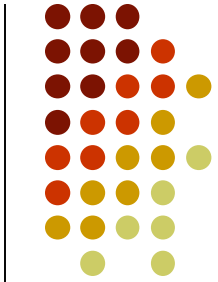


BIOAVAILABILITY :

Bioavailability is the rate and extent of drug that reaches systemic circulation in an unchanged form so as to provide desired therapeutic activity.

OBJECTIVES :



1. For development of preliminary formulation designing a new drug moiety (investigational pre formulative stage).

2. To check influence of various physicochemical factors, biological factors and excepients related factors on the drug in a particular dosage form.

3. It is mainly used to develop newer formulation for existing drug moieties.

Ex: Aspirin is available as ordinary compressed tablet , effervescent aspirin tablet and also as buffered aspirin tablet.

4.To improve quality control of dosage forms containing a drug moiety due to processing variables, storage problems and stability problems arise with dosage forms.

VARIOUS CONSIDERATIONS IN ASSESING BIOAVALABILITY

BIOAVAILABILITY –ABSOLUTE (VS) RELATIVE

Absolute bioavailability (F) :- it is the measure of systemic ability of drug moiety in comparison with the intravenously administered dosage form of the same drug.

$$F = \frac{\textbf{(AUC)}\text{oral}.\ \textbf{DOSE}\ \text{IV}}{\textbf{(AUC)}\text{IV}\ .\textbf{DOSE}\ \text{oral}}$$

Sigficance: to characterize a drugs inherent absorption property from the .E.v site.

VARIOUS CONSIDERATIONS IN ASSESING BIOAVALABILITY

Relative bioavailability (Fr) :- it is the measure of systemic availability of a particular drug from a test dosage form is in comparison with the standard dosage form of similar type.

$$F_r = \frac{\text{(AUC)test. DOSE std}}{\text{(AUC)std .DOSE test}}$$

Significance:

To characterize absorption of a drug from its formulation.

SINGLE DOSE (VS) MULTIPLE DOSE STUDIES

- **Single dose:-**

➢ These are simple.

Note:-

✓ difficult to predict-steady state concentration.

✓ lot of errors.

✓ inter subject variables.

✓ more samples are required.

MULTIPLE DOSE STUDIES

- More accurate.
- Steady state concentration is achieved.
- No. of subjects required is less.
- Inter subject variables are less.

DISADVANEAGES

- Need expertise.
- Costly.
- Greater exposure to test drug. (adverse reactions)

HUMANVOLUNTEERS- healthy subjects (vs.) patients

PATIENTS

ADVANTAGES

- The patient will be benefited.
- Reflects the therapeutic efficacy of the drug.
- Drug absorption pattern in disease state can be evaluated.
- Avoids the ethical quandary of administering of drugs to a healthy subjects.

DISADVANTAGES

- Disease, other drugs, physiological changes may modify the drug absorption pattern.

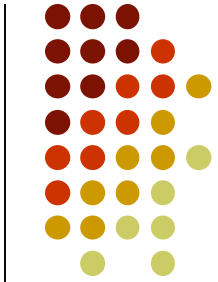
SELECTION OF HEALTHY HUMANS

- Adult healthy human of age 20-40,with uniform weight should be considered.
- For certain drugs adult human females of age 20-40 can be selected.
- The voluntaries selected should be clinically examined for any diseases.
- The human voluntaries selected should be kept under restricted dietary conditions prior to12hrs before drug administration.
- The voluntaries are advised not to undergo vigorous physical exercise during the testing.

SELECTION OF HEALTHY HUMANS

- voluntaries consent must be obtained before involvement in testing.
- The voluntaries should be insured.
- voluntaries must be advised to abstain from any meditation for at least one week and to fast over night prior to and for a minimum of 4hrs after dosing.
- A minimum of 10 half-lives should be allowed for any two studies on the same subject.

ESTIMATION / MEASUREMENT OF BIOAVAILABILITY



There are three methods to measure the bioavailability.

They are :

1) Pharmacokinetic methods
2) Pharmacodynamic methods
3) In vitro dissolution testing studies

- Pharmacokinetic methods and Pharmacodynamic methods are together called as in vivo methods.

- In vitro dissolution testing is the preliminary test carried on any dosage form for assessing rate and extent of drug release.

Pharmacokinetic Method

(Indirect method of estimation)

1.Plasma concentration Vs time profile studies which includes

a) single dose studies

b) multiple dose studies

2. Urinary Excretion studies which includes

a) single dose studies

b) multiple dose studies

Pharmacodynamic Method

(Direct method of estimation)

It includes two methods :

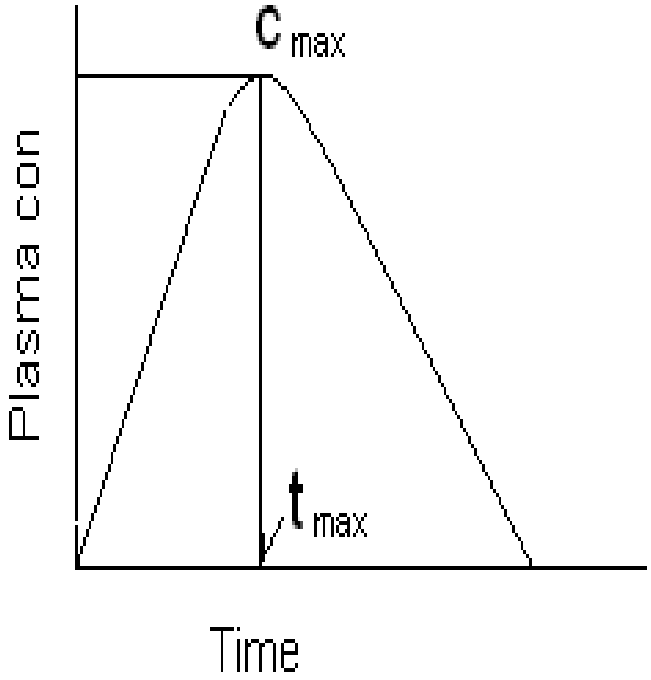
1. Acute pharmacological studies
2. Pharmacotherapeutic / Clinical response studies

PHARMACOKINETIC STUDIES

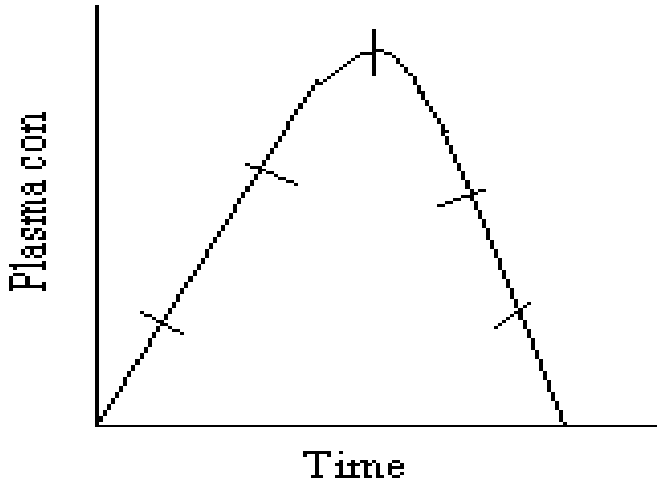
Plasma concentration Vs Time profile:

Single dose

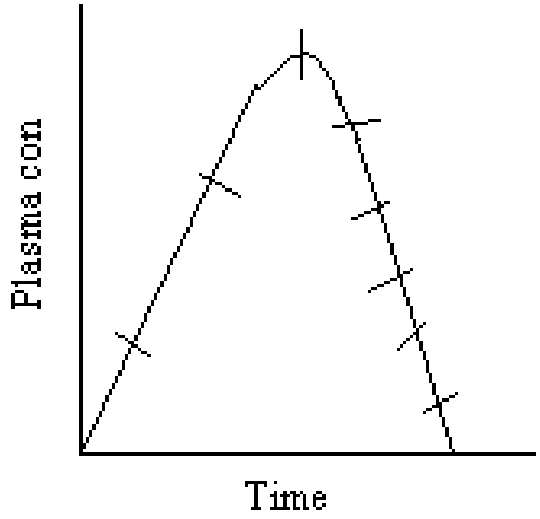
- In general single dose bioavailability studies are conducted.
- From the point administration of dosage form the samples are withdrawn for about 2-3 biological half lives.
- Number of samples to be withdrawn depends on the nature of the dosage form i.e., route of administration.
- From the graph AUC, Tmax, and Cmax were determined.



- In one compartment model 3 samples are taken on descending curve and 3 samples on ascending curve.
- In multi compartment model 3 samples are taken on ascending curve and 5-6 samples on descending curve.



One compartment



Multi compartment

- **Multiple dose is administered to attain steady state concentration.**

- **It is generally done to lessen the intersubject variability.**

- **Number of doses to be administered is to the extent of 5 to 6 biological half lives.**

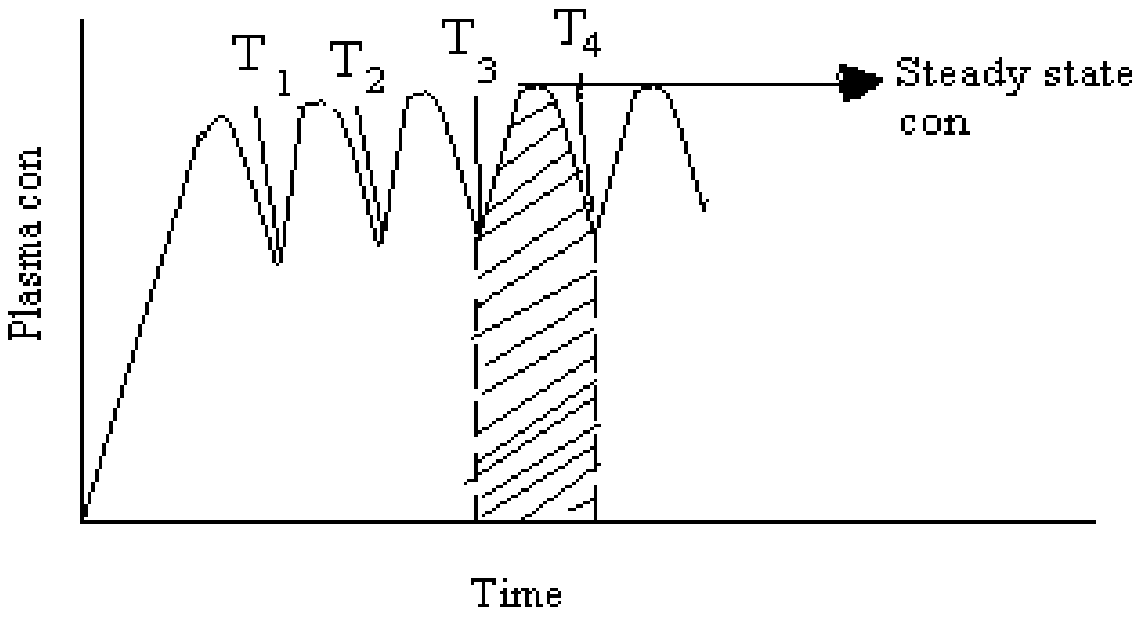
- **Sample is withdrawn prior to the administration of next dose.**
- **Cmax Tmax and Auc**

- **Samples are withdrawn at stipulated time intervals after attaining the steady state concentration.**

$$Fr = \frac{[AUC]\ test\ D_{std}\ \ Ttest}{[AUC]std\ \ Dtest\ Tstd}$$

- **Bioavailability can be assessed by Cmax at steady state concentration.**

$$Fr = \frac{(Cmax\ ss)test\ Dstd\ Ttest}{(Cmax\ ss)std\ Dtest\ \ Tstandard}$$



URINARY EXCRETION DATA

- Most of the drugs are excreted in unchanged form in urine.
- As a rule determination of bioavailability of urinary excretion data should be conducted only if at least 20% of administered dose is excreted unchanged in urine.

  Ex : Thiazide diuretics and sulphonamides

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 amount excreted.

CRITERIA TO OBTAIN VALID RESULTS:

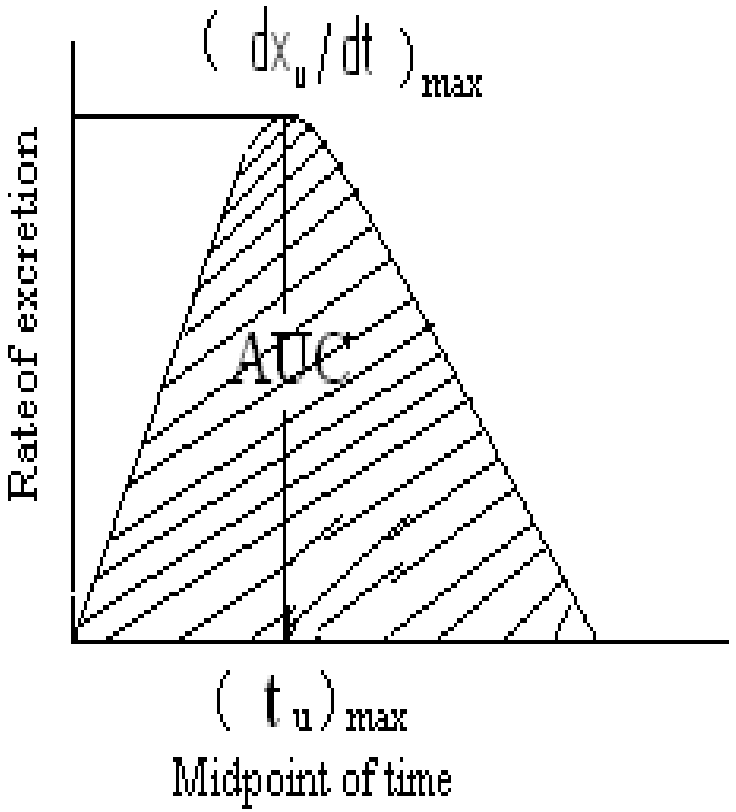
- At each sample collection total emptying of bladder is necessary to avoid errors resulting from addition of residual amount of next urine sample.

- Frequent sampling of urine is also essential in the beginning in order to compute correctly the rate of absorption.

- The fraction excreted unchanged in urine must remain constant.

Three Parameters Examined In Single Dose Study :

1. **The maximum urinary excretion rate (dXu/dt)max :** it is obtained from the peak of plot between rate of excretion Vs midpoint time of urine collection period. It is analogous to the Cmax .
2. **The time For maximum excretion rate (tu)max :** It is analogous to Tmax of plasma level data.
3. **The cumulative amount of drug excreted in the urine Xu~:** It is related to AUC of plasma level data.



- The extent of bioavailability can be calculated by using

$$F = \frac{(Xu)\text{oral Div}}{(Xu)\text{iv Doral}} \qquad Fr = \frac{(Xu)\text{test Dstd}}{(Xu)\text{std Dtest}}$$

- With multiple dose study to steady state the equation for computing bioavailability is :

$$Fr = \frac{(Xu, ss)\text{test Dstd Ttest}}{(Xu, ss)\text{std Dtest Tstd}}$$

PHARMACODYNAMIC STUDIES

- When bioavailability measurement by pharmacokinetic method is difficult , inaccurate or non reproducible, an acute pharmacological effect such as a change in ECG or EEG readings, pupil diameter etc is related to time course of related drug.
- Bioavailability can then be determined by construction of pharmacological effect-time curve as well as dose-response graphs.
- This method requires measurement of responses for atlease 3 biological half lives of the drug in order to obtain a good estimate of AUC.

THERAPEUTIC RESPONSE METHOD

- This method is based on observing the clinical response to a drug formulation given to patients suffering from disease for which it is intended to be used.

Drawbacks :

1. Quantitation of observed response is too improper to allow for reasonable assessment of bioavailability.
2. Bioequivalence studies are conducted using a crossover design and the physiological status of the subject should not change over duration of study.
3. Unless multiple dose protocols are employed a patient who required the drug for a disease would be able to receive only a single dose of drug.
4. Many patients receive more than one drug and the results obtained from a bioavailability study could be compromised because of drug-drug interaction.

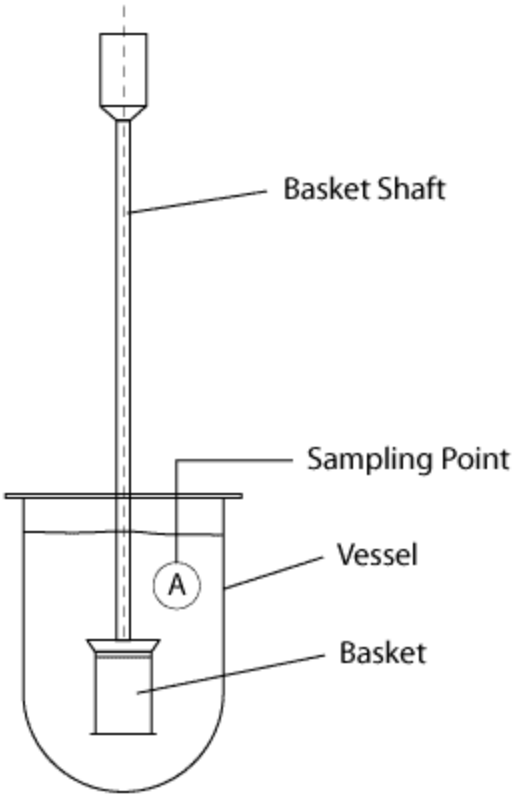
INVITRO DISSOLUTION TESTING

- The best available tool today which can at least quantitatively assure about the biological availability of a drug from its formulation is its in vitro dissolution test.

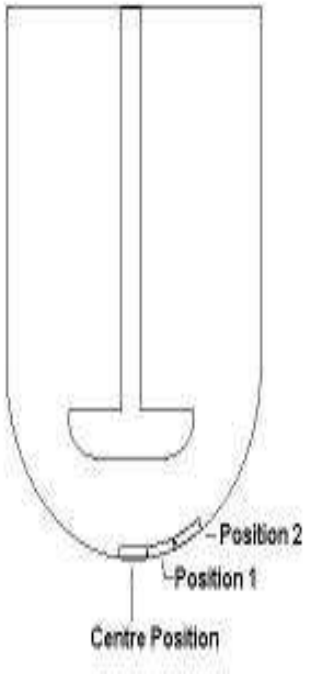
Factors Considered In Design Of Dissolution Test :

1. Factors relating to dissolution apparatus.
2. Factors relating to dissolution fluid.
3. Process parameters.

USP dissolution apparatus 1



USP dissolution apparatus 2



DISSOLUTION ACCEPTANCE CRITERIA

S1	6	No dosage unit is less than Q+5%
S2	6	Average of 12 dosage units (S1+S2) >Q% and no dosage unit is less than Q-15%
S3	12	Average of 24 dosage units (S1+S2+S3) > Q% and not more thatn 2 dosage units are less than Q-15% and no dosage unit is less than Q-25%

INVITRO - INVIVO CORRELATION

- It is defined as the predictive mathematical model that describes the relationship between an in-vitro property of a dosage form and an in-vivo response.

- The main objective of developing and evaluating an IVIVC is to enable the dissolution test to serve as a surrogate for in vivo bio availability studies in human beings.

Objectives:

1. To ensure batch to batch consistency in the physiological performance of a drug product by use of such in vitro values.

2. To serve as a tool in the development of a new dosage form with desired in vivo performance.

3. To assist in validating or setting dissolution specifications.

Approaches for invivo-invitro correlation:

1. By establishing a relationship usually linear between the in vitro dissolution and the in vivo bioavailability parameters.

- Correlations based on the plasma level data
- Correlations based on the urinary excreation data.
- Correlations based on the pharmacologic response.

2. By using data from previous bioavailability studies to modify the dissolution methodology in order to arrive at meaningful in vitro- in vivo correlation.

Correlations based on plasma level data :

Here linear relationships between dissolution parameters and plasma level data are established.

In vitro dissolution parameters

- Time for specific amount of drug to dissolve
- Amount dissolved at a specific time point
- Mean dissolution time
- Parameter estimated after modeling the dissolution process.

In vivo plasma data parameters

- AUC , Cmax
- Fraction absorbed , absorption rate constant Ka
- Mean residence time ,mean dissolution time, mean absorption time
- Concentration at time t, amount absorbed at time t.

Correlations based on urinary excretion data

- Here dissolution parameters are correlated to the amount of drug excreted unchanged in urine, cumulative amount of drug excreted as a function of time etc.,

Correlations based on pharmacological response :

- An acute pharmacological effect such as LD 50 in animals is related to any of the dissolution parameters.

- **Equivalence** is more a general,relative term that indicates a comparision of one drug product with another or with a set of established standards.Equivalence may be defined in several ways:

- **Chemical equivalence** indicates that two or more dosage forms contain the same labeled quantities (plus or minus specified range limits) of the drug.

- **Clinical equivalence** occurs when the same drug from two or more dosage forms gives identical *in vivo* effects as measured by a pharmacological response or by control of a symptom or disease.

- **Therapeutic equivalence** implies that two brands of a drug product are expected to yield the same clinical result. The FDA specifically uses the term therapeutic equivalence in the evaluation of multisource prescription drug products.

- **Bioequivalence** indicates that a drug in two or more similar dosage forms reaches the general circulation at the same relative rate and the same relativ extent(i.e, that the plasma level profiles of the drug obtained using the two dosage forms are the same).

- **Pharmaceutical equivalence** refers to two drug products with the same dosage form and same strength.

EXPERIMENTAL DESIGN:

Parallel Design:

- Two formulations are administered to two groups of volunteers.
- To avoid bias two formulations are administered randomly to the volunteers.

Disadvantages:

- inter-subject variation is not corrected.
- Inter-subject variation is greater than the variation between any formulations

EXPERIMENTAL DESIGN:

Cross- Over Design:

- Each subject receives the test drug product and the reference drug product
- Minimizes the effect of inter-subject variability in the study

3 Types:-

- Latin Square crossover Design
- Balanced Incomplete Block Design (BIBD)
- Replicated crossover design

Latin Square crossover Design:

- ✓ Each subject receives each formulation only once.
- ✓ Each formulation is administered only once in each study period.
- ✓ Each subject acts as his own control.

Table 11.2 Latin Square Designs

Two-Way Crossover		
Group No.	Subjects in Group	Treatment for Period No.
		I II
1.	1,2,3,4,5,6	A B
2.	7,8,9,10,11,12	B A
Three-Way Crossover		
Group No.	Subjects in Group	Treatment for Period No.
		I II III
1.	1,2,3,4,5,6	A C B
2.	7,8,9,10,11,12	B A C
3.	13,14,15,16,17,18	C B A
Four-Way Crossover		
Group No.	Subjects in Group	Treatment for Period No.
		I II III IV
1.	1,2,3,4,5,6	A B C D
2.	7,8,9,10,11,12	B D A C
3.	13,14,15,16,17,18	C A D B
4.	19,20,21,22,23,24	D C B A

Latin Square crossover Design:

Advantages

- ✓ Minimizes the carry-over effects.
- ✓ Minimizes the time effect on bioavailability.
- ✓ Requires less number of subjects to get good results.

Disadvantages

- ✓ Requires longer time to complete the study.
- ✓ Time to complete the study also depends on the number of formulations to be evaluated.
- ✓ Increased number of study periods leads to high subject drop outs.
- ✓ Medical ethics does not allow too many trials on a subject for a longer time.

Balanced incomplete Block Design (BIBD) :

- Each subject receives not more than 2 formulations
- Each formulation is administered same number of times
- Each pair of formulations occurs together in the same number of subjects

Table 11.3 Balanced incomplete block design (BIBD) for four formulations

Subject	Treatment for Period No. I	II
1	A	B
2	B	A
3	A	C
4	C	A
5	A	D
6	D	A
7	B	C
8	C	B
9	B	D
10	D	B
11	C	D
12	D	C

Replicated crossover design :

- To determine individual bio-equivalence.
- To estimate within-subject variance for both the Test and Reference drug products.
- To estimate subject-by-formulation interaction variance.
- Reference-Reference and Test-Test comparisons may be made.

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

Wash out period:

The time interval between two treatments

- 10 Half-lives
- Elimination of 99.9% of administered dose
- Elimination of metabolites

No: of washout periods:

- ✓ Study design used
- ✓ No: of formulations to be evaluated

Example:

- ✓ Digitoxin – Half-life = 6-9 days
- ✓ Most drugs – Half-life = 1-10 hrs

DRUG PRODUCTS:

Test Products

- Newdrug formulation
- New dosage form

Reference standard

- Any innovator's drug product
- RLD (reference listed drug)
- Approved drug products with therapeutic equivalence evaluations (Orange book)