Statistics for "Pharmacokinetic" Studies

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PhUSE SDE, Trivandrum (30-July-2016)

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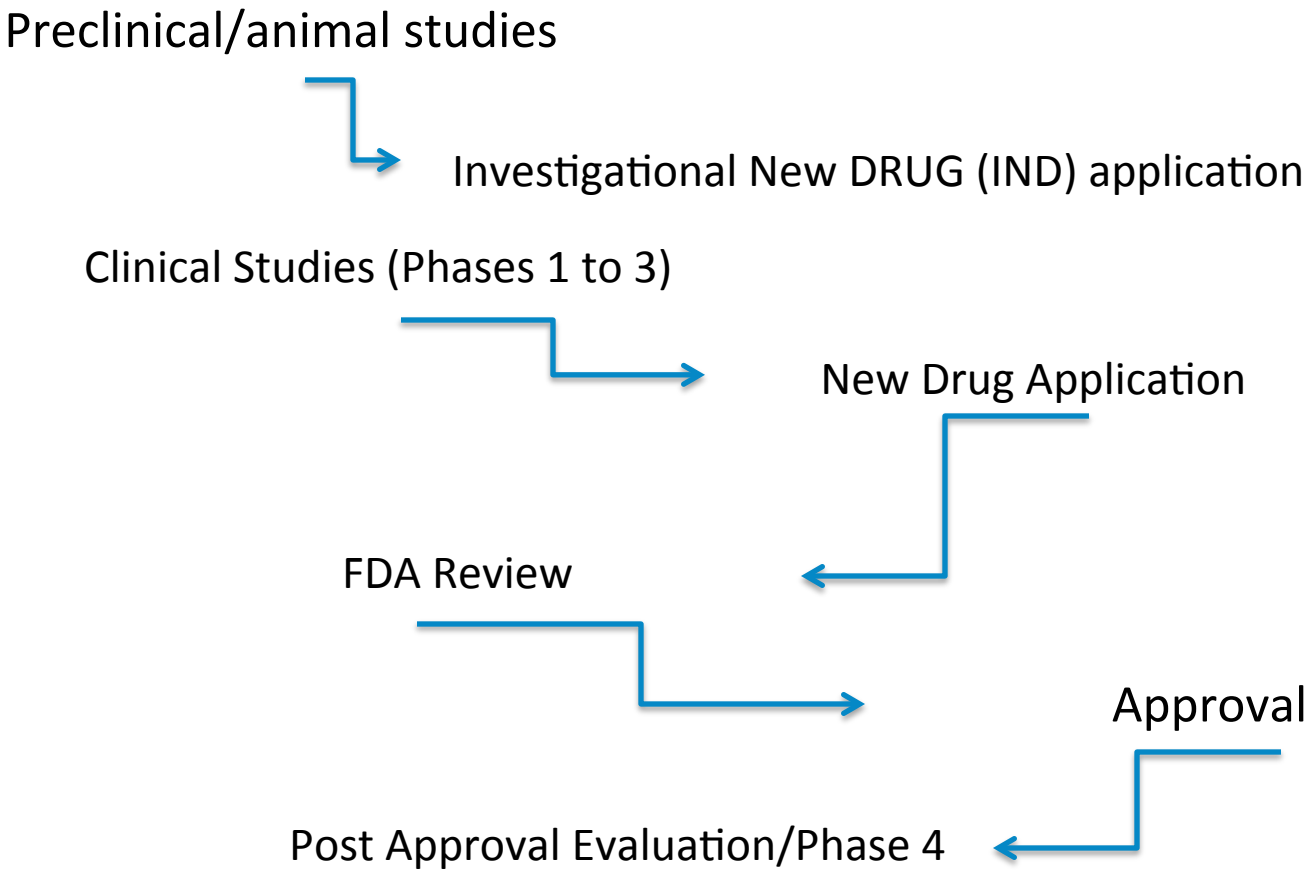
DRUG DEVELOPMENT

Flow Diagram



INTRODUCTION TO PHARMACOKINETIC

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What is Pharmacokinetic?

Pharmacokinetics, also abbreviated as **PK**, (from Ancient Greek pharmakon "drug" and kinetikos "to do with motion"; see chemical kinetics) is a branch of pharmacology dedicated to the determination of the fate of substances administered externally to a living organism. In practice this discipline is applied mainly to drug substances, though in principle it concerns itself with all manner of compounds ingested or otherwise delivered externally to an organism, such as nutrients, metabolites, hormones, toxins, etc.

How dose the drug concentration change as it moves through the different compartments of the body

We are interested in ADME:

- ✓ **A**bsorption
- ✓ **D**istribution
- ✓ **M**etabolism
- ✓ **E**xcretion

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Components of PK

Absorption

- Before a drug can exert a pharmacological effect in tissues, it has to be taken into the blood stream. Absorption is the process that involves drug movement from the site of entry into the bloodstream.

Distribution

- Distribution is the process by which a drug is transported in body fluids from the bloodstream to the tissues of the body.

Metabolism

- Metabolism is the process by which a drug is chemically inactivated (broken down by enzymes) so that it can be excreted from the body.

Excretion

- Excretion is the process by which a drug is removed from the site of action and eliminated from the body. Immediately after a dose of drug is administered, the body begins to eliminate by hepatic metabolism, renal excretion, or both.

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PK Outcome

- Select the compounds that have maximum potential of reaching the target (PK)
- Select the appropriate route of administration to deliver the drug
- Understand how the blood (or plasma) levels relate to efficacy (PK-PD) or toxicity in order to select safe dose
- Decide on the frequency and duration of dosing in order to sustain drug at target for disease modification

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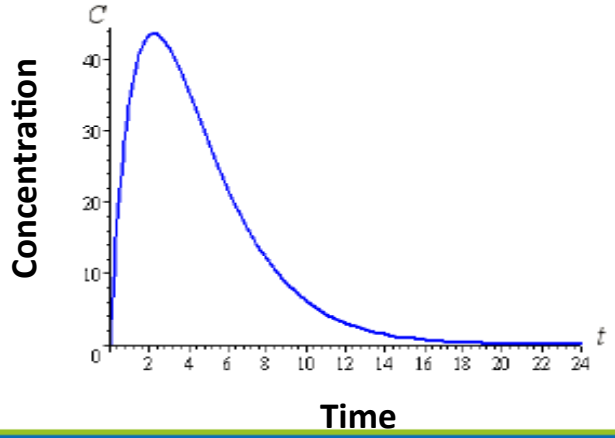
Right Drug

Right Patients

Safe and
Effective
Personalized
Medicine

Right Dose



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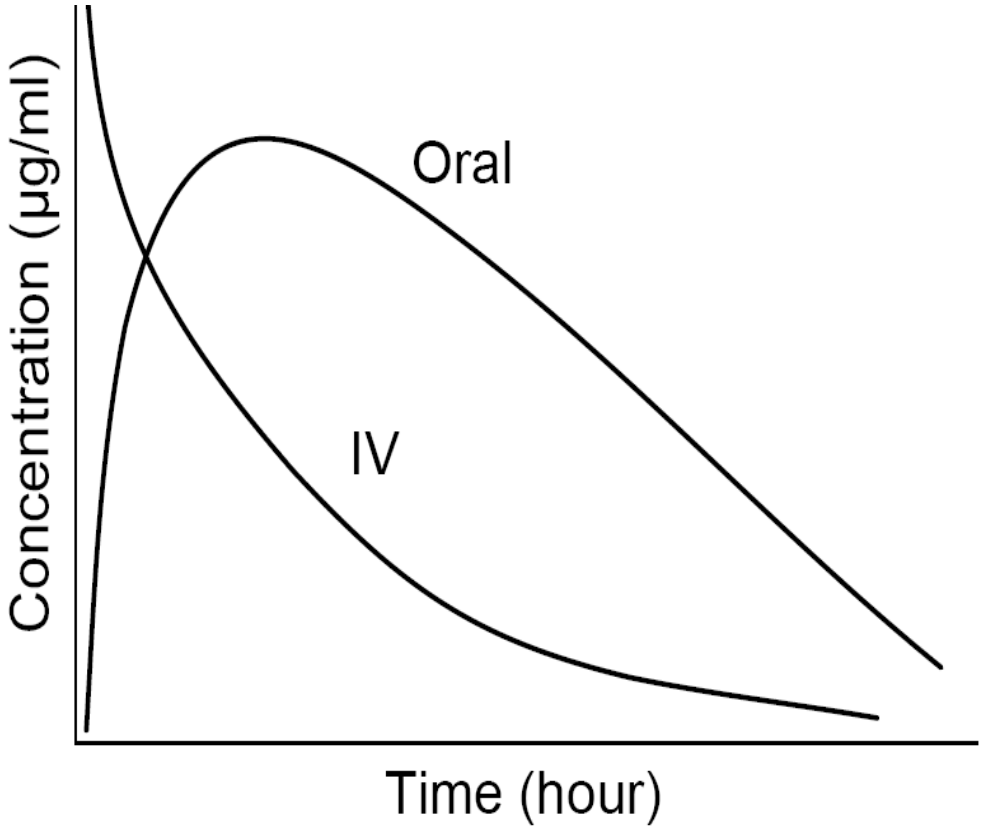
Rate of absorption can be affected by the route of drug administration. Drug given orally need to be absorbed into the blood via GUT whereas those given intravenously do not.

Schematic representation of pharmacokinetic profiles for intravenous and oral doses.



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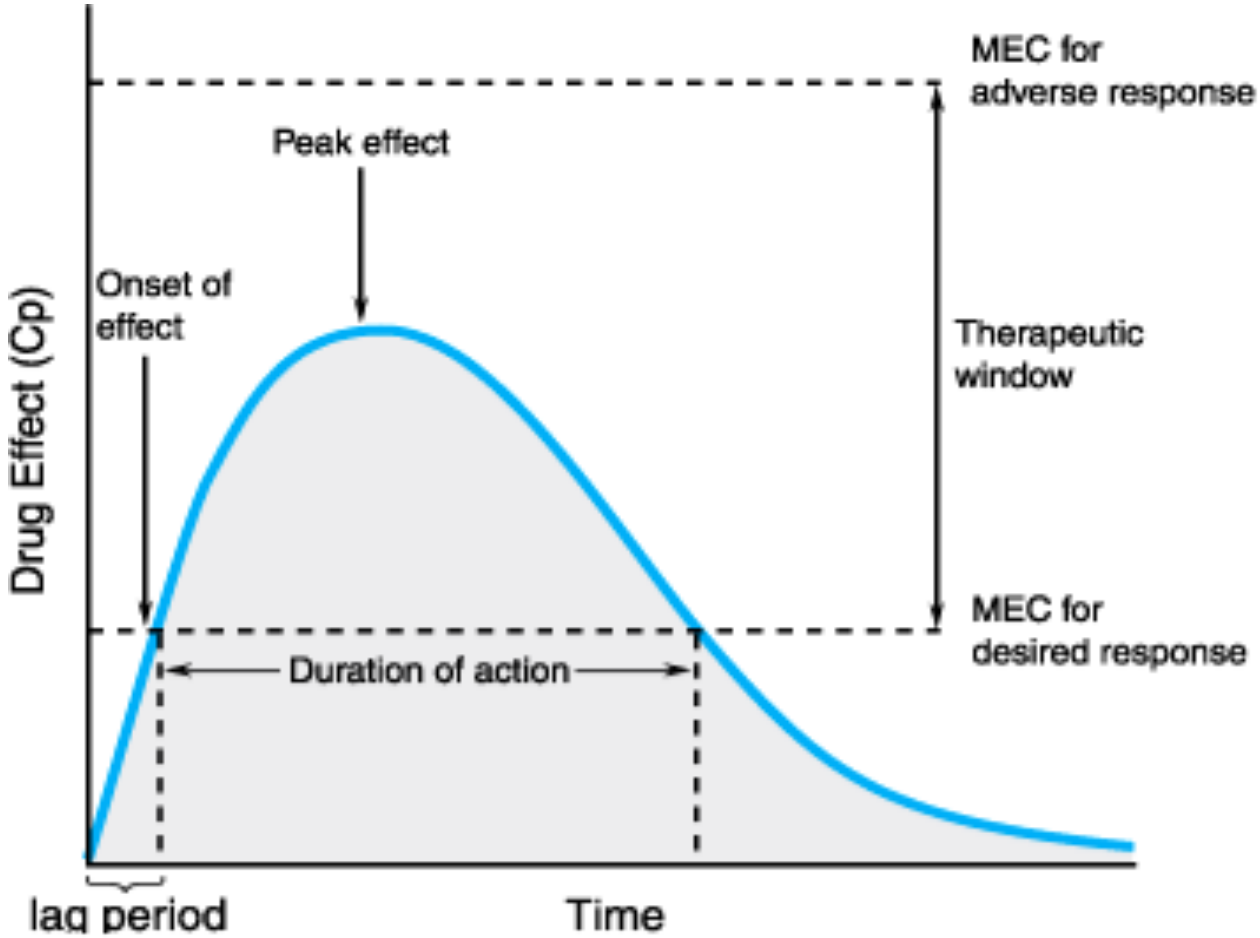
Representative PK Profile



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Type of PK studies

- Single dose/multiple dose PK
- Mass balance (ADME)
- Bioavailability/Bioequivalence
- Food effect (Oral formulation)
- Specific populations
- Drug-drug interactions
- Thorough QTc (cardiac depolarization)

INTRODUCTION TO PHARMACOKINETIC

Single dose/Multiple dose Escalation Studies

- Typically the first-in-human study (or studies)
- Randomized, placebo-controlled, healthy volunteers (or patients, in certain cases)
- Starting dose determined by preclinical toxicology studies
- Information gained:
  - Safety/tolerability, identify maximum tolerated dose (MTD)
  - General PK characteristics, variability, linearity, dose proportionality
  - Steady-state parameters (accumulation, time-dependency)
  - Preliminary exploration of drug elimination (urine PK, metabolite identification)

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ADME (Mass Balance) Study

- Objective: To understand the routes of elimination of a drug and to identify its circulatory and excretory metabolites
- Typically single dose, healthy males (n=4-6), at intended route of administration
- Radio-labeled (^{14}C) drug molecule
- Measure concentrations of parent and metabolite(s) and determine amt of radioactivity in plasma, urine, feces
- Information gained:
  - Primary mechanism(s) of elimination and excretion from the body
  - Proportion of parent drug converted to metabolite(s)

INTRODUCTION TO PHARMACOKINETIC

Bioavailability (BA) and Bioequivalence (BE)

- Objective: To evaluate the rate (Cmax, Tmax) and extent (AUC) of absorption of drug from a test formulation (vs. reference formulation)
- BA: Typically, crossover, single dose (if linear PK) study in healthy subjects; measure blood/plasma conc. of parent drug
- BE: crossover study in fasted healthy subjects given single doses of test & reference products administered at same molar doses; measure blood/plasma conc. of parent drug only
- BE acceptance criteria: 90% CI of the geometric mean ratios of Cmax & AUC between test and reference fall within 80-125%
  - Information gained: Relative BA, Absolute BA of drug from a formulation
  - BE (no significant difference in BA) of test vs. reference

Food Effect Study

- Administration of food with oral drug products may affect the BA and/or BE
- Food can have influences on the release of the drug substance from the drug product as well as the absorption of the drug substance
- Usually a single-dose, two-period, two-treatment, two-sequence crossover study comparing a high-fat high- calorie meal to the fasted state
- High-fat high-calorie meal = 150, 250, and 500-600 calories from protein, carbohydrate, and fat, respectively

Specific Populations

- Evaluates the impact of an intrinsic factor on the PK of the drug
  - Age (elderly and pediatrics)
  - Gender (male and female subjects)
  - Renal or hepatic impairment
  - Obesity
  - Pregnancy
- Usually a single-dose study
- Recommended to perform study prior to Phase 3
- Supports dose adjustments of the drug in the specific population

INTRODUCTION TO PHARMACOKINETIC

Drug Interaction Studies

- Evaluates the potential of the drug acting as either a pharmacokinetic perpetrator or a victim when two drugs are co-administered
- Supports dose adjustments when drug interactions observed (either perpetrator or victim drug)
- Usually performed in healthy male and female subjects
- Study design depends on the mechanisms of drug interactions, half life, clinical dosing regimen, etc

INTRODUCTION TO PHARMACOKINETIC

Thorough QT Study (TQT)

- In vivo safety study required for all systemically available NMEs (regardless of *in vitro or non-clinical findings)*
- Objective: To identify drugs that prolong QT (95% CI upper bound ≥ 10 ms) that need a more thorough ECG monitoring in pivotal trials; TQT study conducted prior to Phase 3 trials
- Usually, single dose study in healthy subjects; evaluate therapeutic and "supratherapeutic" doses of drug versus positive control (e.g., moxifloxacin)
- ICH Guidelines, E14: The Clinical Evaluation of QT/QTc Interval Prolongation and Proarrhythmic Potential for Non-Antiarrhythmic Drugs
  - Recommendations for design, conduct, analysis, and interpretation of clinical studies

INTRODUCTION TO PHARMACOKINETIC

PK Data Analysis

Non-Compartmental Analysis:

- Derived from plasma PK concentration data without using any modeling techniques.
- Subject wise estimates C_{max}, T_{max}, AUCs, $t_{1/2}$ can be obtained.

Compartmental Analysis:

- A model is fit (for e.g., one-compartmental, two-compartmental,…) and PK parameters (Ka, CL, V, …) estimated
- Population analysis and influence of subject covariates
  - Two-stage approach
  - Non-linear mixed-effects modeling approach

INTRODUCTION TO PHARMACOKINETIC

PK parameters derived from plasma and/or blood concentration data to access bioavailability and bioequivalence are:

- **$AUC_{(0-t)}$:** This is the Area Under the Curve from 0 to t, where t is the time corresponding to last observed blood or plasma concentration of the chemical or metabolite.
- **$AUC_{(0-\tau)}$:** This is the area under the plasma concentration curve C(t) in the interval $[0,\tau]$ where $0<\tau<t$. Typically, τ corresponds to the dosing interval.
- **$AUC_{(0-\infty)}$ or AUC_{Inf}:** This the area under the plasma concentration curve C(t) in the interval $[0,\infty)$.
- **C_{max}:** This is the maximum plasma concentration. It is used assess the rate of absorption.
- **T_{max}:** This is the time at which Cmax is attained. It is also used assess the rate of absorption.
- **K_{el}:** This is the apparent first order elimination or terminal rate.
- **$t_{1/2}$:** This is the time required for the reduction to one half of the initial drug dose via excretion or metabolism or a combination of both. It is also known as the half-life of the drug.

BIOAVAILABILITY STUDIES

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Definition

Bioavailability (F): rate and extent to which the active ingredients or active moiety is absorbed from a drug product and becomes available at site for auction.

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

It is expressed as a portion of the dose and so it can take values from 0 to 1 (or from 0% to 100%, if it is measured in percents).

F = 100% for intravenously administered drugs

F ≤ 100% for orally administered drugs

BIOAVAILABILITY STUDIES

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Need of Bioavailability Studies:

- To evaluate the absolute systemic availability of an oral, topic, Intramuscular, or any other dosage form.
- To determine if bioavailability parameters are linear over the proposed clinical dose range .
- To estimate the inter and intra subject variability.
- To study food effects .
- To define the effect of changes in the physicochemical properties of the drug substance and the effect of the drug product (dosage form) on the pharmacokinetics of the drug.

BIOAVAILABILITY STUDIES

Factors that affect F

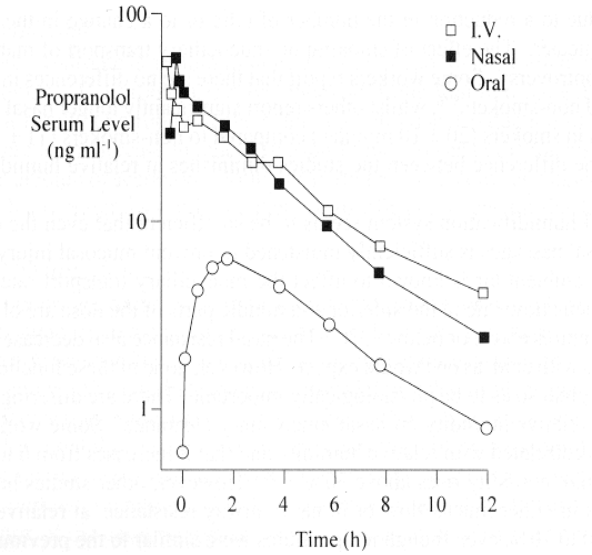
- Physical or formulation properties (molecular weight, polarity, dissolution rate, stability in gastric acid)
- Gastric emptying rate
- Metabolism in intestinal wall (CYP3A4)
- Drug efflux from intestinal wall (Pgp)
- Hepatic first pass metabolism

BIOAVAILABILITY STUDIES

Type of Bioavailability

Absolute Bioavailability: It is the absolute part from the submitted dose, which gets to the systemic blood circulation, from the particular drug form (after another than intravenous submission).

It is obtained via comparison of AUC for drug submission in it's usual form to AUC for intravenous drug submission.



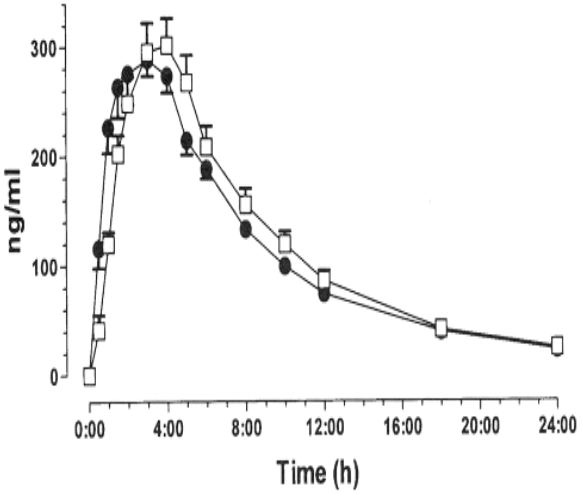
$$F = AUC_{po} / AUC_{iv}$$

$$F = \frac{(AUC/dose)_{po}}{(AUC/dose)_{iv}}$$

BIOAVAILABILITY STUDIES

Type of Bioavailability

Relative Bioavailability: It is relative part of drug dose, which gets into systematic blood Circulation, when comparing two drugs form (the tested one with another), both non-intravenous.



For evaluation of relative bioavailability, also the speed of getting the active form of the drug into systemic blood circulation is important (it influences maximal concentration).

$$F = \frac{AUC_{\text{form A}}}{AUC_{\text{formB}}}$$

Bioavailability comprises the quantitative aspect (volume of bioavailability) and speed (speed of bioavailability). Hence, the volume and speed of bioavailability of the drug in the tested form is compared to another, reference form.

BIOEQUIVALENCE STUDIES

Definition

- The absence of a significant difference in the rate and extent to which the active ingredient or active moiety becomes available at the site of action when administered at the same molar dose under similar conditions
- Studies are conducted to assess the sameness of two drug products, different dosing regimens, etc (eg., formulation change, food effects, crushed tablets)

BIOEQUIVALENCE STUDIES

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Why BE studies are pivotal?

- For New Drug Applications (NDA):
  - When the to-be-marketed formulation is different than the clinical trial formulation
  - When changes are made to the marketed formulation (eg., switch to capsule, suspension or ER formulation)
- For Abbreviated New Drug Applications (ANDA):
  - To compare the generic versus the innovator formulation

BIOEQUIVALENCE STUDIES

Study Design

Standard Design:

- Randomized, 2 sequence, two period crossover
  - Treatment sequence is randomized
    - Every subjects gets both treatments
    - Half get generic drug first, half get brand drug first (minimizes the sequence effect)
  - Crossover design
    - Each subject serves as his/her own control (minimizes inter-subject variability)
    - Fewer subjects required to achieve adequate statistical power
  - Treatment periods separated by a washout
    - At least 5 drug half-lives
    - Minimizes the carryover (residual) effect

BIOEQUIVALENCE STUDIES

Statistical Analysis

- Statistical comparison of generic to brand C_{max} and AUC using ANOVA
  - Evaluates potential confounding effect of 4 factors:
    - Sequence (treatment order)
    - Period
    - Subject
    - Treatment (formulation)
  - C_{max} and AUC are log-transformed prior to analysis
    - ANOVA is based on the assumption of normal distribution of data
    - Most pharmacokinetic parameters have a skewed distribution (log transformation normalizes the distribution)

Fundamental Assumption

Independence:

- The random variables implied in the analysis are independent.
- In a parallel group : the (observations obtained on) subjects are independent.
- In a cross-over :
  - the subjects are independent.
  - the difference of observations obtained in each subject with the different formulations are independent.

Homoscedasticity:

- Maybe the most important assumption
- Analysis of variance is not robust to heteroscedasticity
- More or less easy to check in practice :
  - graphical inspection of data (residuals)
  - multiple comparisons of variance (Cochran, Bartlett, Hartley…).

Fundamental Assumption

Homoscedasticity:

- Crucial for the bioequivalence problem : the width of the confidence interval mainly depends on the quality of estimation of the variance.

Normality:

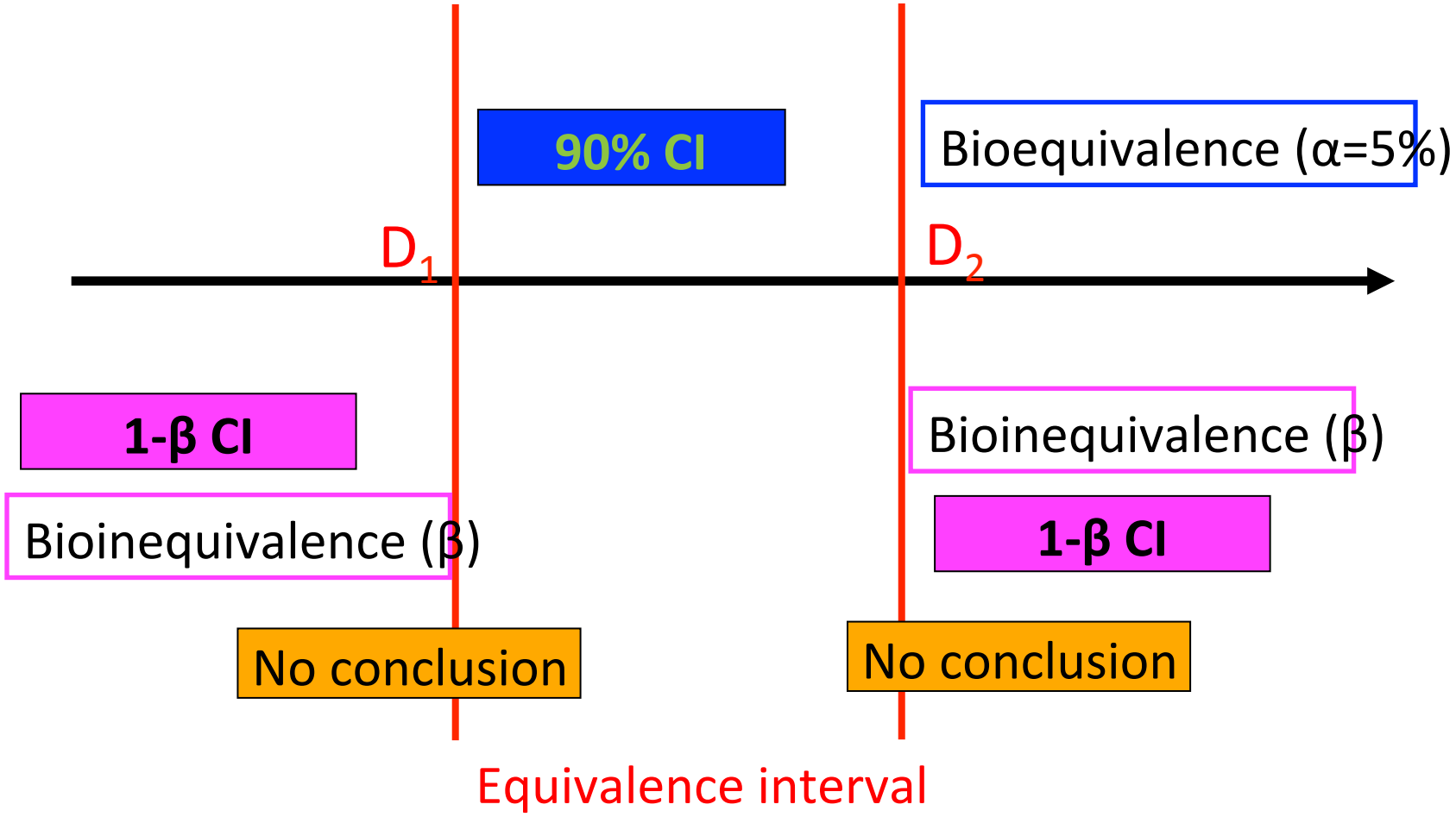
- The random variables implied in the analysis are normally distributed.
- In parallel group: the observations of each formulation come from a Gaussian Distribution
- In crossover: the observations obtained in each subject for each formulation are assumed to be normally distributed.
- The analysis of variance is robust to non normality.
- Checked using:
  - Graphical inspections of the residuals (PP plot)
  - Kolmogorov-Smirnov, Chi-Square test…

BIOEQUIVALENCE STUDIES

Statistical Analysis

- Log Transformation of AUC and C_{max}. T_{max} not required.
- Enough subjects must be tested to permit detection of a 20% difference in log-transformed parameters with 80% power
  - Report the following for each parameter (C_{max}, AUC_{0-t} , AUC_{∞}, or $AUC_{0-\tau}$): arithmetic mean, geometric mean, geometric mean ratio (generic/brand) and 90% confidence interval (CI)
  - With log transformation the ± 20% rule translates to a mean ratio and 90% CI for each parameter of 80-125%

Confidence Interval - Summary



SAS code for the Crossover Design

```
proc mixed data=xxx method=reml;
class sequence subject period trt;
model log_AUC = sequence period trt / ddfm=kenwardroger;
random subject(sequence);
estimate 'T-R' trt -1 1 /cl alpha=0.1;
ods output estimates=test;
run;

data test;
set test;
low_limit=exp(lower);
upper_limit=exp(upper);
run;
```

EXAMPLE

Study Title: A Phase I, Open-Label, Randomized, Single Dose, 2-way crossover bioequivalence (BE) study of investigation product (IP) in healthy subjects under fasted condition.

Indication: Hypertension

Objectives:

- Primary: To establish bioequivalence between IP manufactured at Site A (Test) and Site B (Reference).
- Secondary: To describe safety and tolerability of IP

Study Design:

Sequence	Period 1	Washout 7 days	Period 2
1 (n = 36)	Trt A		Trt B
2 (n = 36)	Trt B		Trt A

PK Parameter: AUC_{inf}, AUC_{last}, C_{max}, T_{max}, and $t_{1/2}$

EXAMPLE

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Statistical analysis:

Natural log transformed PK parameters (AUC_{inf}, AUC_{last}, and C_{max}) were analyzed using a mixed effect model with sequence, period and treatment as fixed effects and subject within sequence as a random effect. Estimates of the adjusted mean differences (Test-Reference) and the corresponding 90% CIs were obtained from the model. The adjusted mean differences and the 90% CIs for the differences were exponentiated to provide estimates of the ratio of adjusted geometric means (Test/Reference) and the 90% CIs for the ratios.

Bioequivalence of the Test treatment (Treatment B) relative to Reference treatment (Treatment A) was concluded if the 90% CIs for the ratio of adjusted geometric means of Treatment B relative to Treatment A for both AUC_{last}, and C_{max} fell wholly within (80%, 125%).

EXAMPLE

Descriptive Statistics of PK Parameter Values:

Parameters	Treatment A	Treatment B
AUC_{inf}	3364 (41)	3501 (43)
AUC_{last}	3241 (40)	3361 (41)
C_{max}	189.4 (36)	197.5 (30)
T_{max}	5.00 (3.98 – 9.98)	5 (3.98 – 8.00)
$t_{1/2}$	7.989 ± 2.923	8.181 ± 2.984

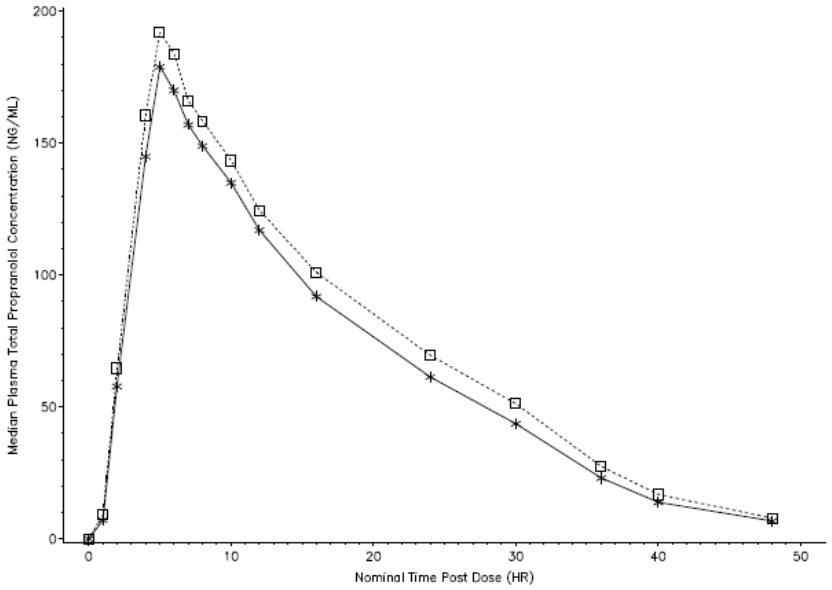
Statistical Summary of Treatment Comparison for PK Parameters:

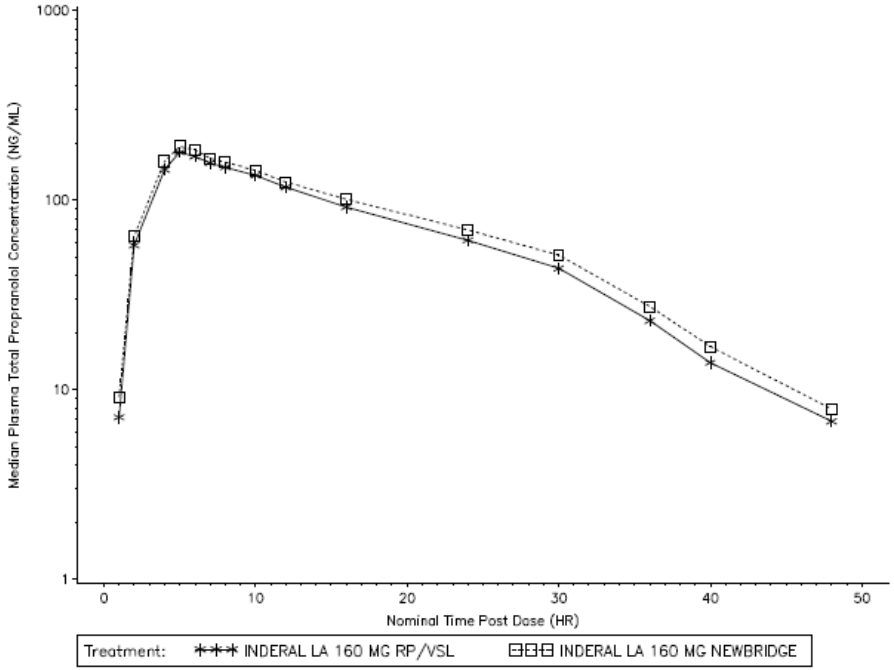
Parameters	Treatment A	Treatment B	Ratio (Test/ Reference)[a]	90% CI[a]
AUC_{inf}	3501	3415	102.54	(98.64, 106.58)
AUC_{last}	3361	3285	102.32	(98.38, 106.40)
C_{max}	197.5	190.7	103.58	(99.34, 108.01)

a: The ratios and 90% CIs are expressed as percentages

EXAMPLE

Median Plasma Total IP Concentration –Time Profile – Linear Scale





Median Plasma Total IP Concentration –Time Profile – Semi-Logarithmic Scale

EXAMPLE

Conclusion:

C_{max} were reached with a median time for C_{max} (T_{max}) of 5 hours for both treatments.

The median plasma total IP concentration-time profiles were nearly super imposable and the overall exposures (as assessed by geometric mean C_{max} and AUC values) were similar.

Mean terminal $t_{½}$ values were 8.0 hours and 8.2 hours respectively.

The 90% CIs fell wholly within the acceptance range of (80%, 125%) for bioequivalence. Thus, the 2 treatments were concluded to be bioequivalent.

Questions?

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THANK YOU!