Experimental design and analysis for bioequivalence trials

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A reference book for experts

- Provides a complete presentation of the latest progress of activities and results in bioavailability and bioequivalence on regulatory requirements, scientific and practical issues, and statistical methodology.
Change in phenytoin excipients results in epidemic toxicity

The EMEA guideline on bioequivalence 01/11/2011

11 April 2011
EMA/CVMP/016/00-Rev.2
Committee for Medicinal Products for Veterinary Use (CVMP)

Guideline on the conduct of bioequivalence studies for veterinary medicinal products
Bioequivalence: FDA

Guidance for Industry
Statistical Approaches to Establishing Bioequivalence

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
January 2001
Bioequivalence

• Definition and Assumptions
• Types of Bioequivalence
• The Bioequivalence trial
• Analytical techniques
• Statistical analysis
Bioequivalence: Definition and assumptions
Bioequivalence: Definition 2011

- For two products, pharmacokinetic equivalence (i.e. bioequivalence) is established if the rate and extent of absorption of the active substance investigated under identical and appropriate experimental conditions only differ within acceptable predefined limits.
- Rate and extent of absorption are typically estimated by $C_{\text{max}}$ (peak concentration) and $AUC$ (total exposure over time), respectively, in plasma.

Bioequivalence studies are often part of applications for generic veterinary medicinal products to allow bridging of safety and efficacy data associated with a reference veterinary medicinal product.
Bioequivalence: The basic assumption
Bioequivalence: The basic assumption

The basic assumption underlying the bioequivalence concept is that similar plasma time courses lead to essentially the same effects (pharmacological, toxic, therapeutic).

Classical objections

• Plasma concentration is not biophase concentration
• there is no (univocal) relationships between exposure and effect
Why to use bioavailability outcomes to demonstrate bioequivalence
Basic assumption to bioequivalence

Similar plasma concentration profile $\Rightarrow$ same effect? Why?

Substance property (efficacy)

$$\text{Effect} = \frac{E_{max} \times Dose}{ED_{50} + Dose}$$

Hybrid substance and formulation properties (Potency)
Basic assumption to bioequivalence

\[ ED_{50} = \frac{\text{Clearance} \times EC_{50}}{\text{Bioavailability}} \]

Substance property

Formulation property
Does similar plasma time curve leads to essentially the same effect whether toxic or therapeutic?
PK/PD relationship to discuss bioequivalence acceptance criteria

Exposure

\[ \Delta = 20\% \]

Effect

Drug with a large margin of safety
Dose may be selected in the asymptotic part of the dose-effect relationship curve and a \( \Delta \) of 20% for exposure is generally irrelevant in terms of effect
PK/PD relationship to discuss bioequivalence acceptance criteria

Drug with a narrow margin of safety
Dose cannot be selected in the asymptotic part of the dose-effect relationship curve and a Δ of 20% for exposure may be very relevant in term of effect depending of the slope of the curve
Consequence of a ± 20 % of the exposure on the therapeutic (blue curve) and of the toxic effect (red curve)
3-The three possible definitions of Bioequivalence
Different types of bioequivalence

• Average (ABE) : mean
• Population (PBE) : prescriptability
• Individual (IBE) : switchability
Average bioequivalence

• Test and reference are bioequivalent if the means are “sufficiently similar” with regard to AUC and Cmax

• Sufficiently similar
  – $0.80 \leq \text{CI of } (\mu_T / \mu_R) \leq 1.25$
  – log scale $\log (0.8) \leq \mu_T - \mu_R \leq \log (1.25)$
Average bioequivalence

Same mean

Average BE do not account for difference in inter-or intra subject variability between formulations
Average bioequivalence

Average B.E. refers to the location parameters

Average B.E. may not be sufficient to guarantee that an individual patient could be switched from a reference to a generic formulation

(e.g., more than 50 % of subjects may be outside the B.E. range when the average B.E. is actually demonstrated)
Average BE and therapeutic window (TW)

YES
All subjects in the TW

NO
Some subject outside the TW

Exposure (AUC)

Figure 2. Équivalence en moyenne et en variabilité (variances).
Prescribability

• Refer to the clinical setting in which a practitioner prescribes a drug product to a patient for the first time
• he has no information on his patient
• the prescriber needs to know the comparability of the 2 or n formulations in the population

Assessed by population bioequivalence
“Test” and “reference” are bioequivalent if the entire population distribution (mean and variability) are sufficiently similar with regard to AUC and Cmax.

The case of collective treatment
Population bioequivalence
Population dosage regimen

Yes

Pigs that eat less:
Possible underexposure

No

Pigs that eat more
Possible overexposure
The types of bioequivalence: summary

**Average**
- Only guarantees on the mean

**Population**
- Guarantees an overall distribution (mean and variance)

**Individual**
- Test of no interaction between patient and formulation guarantees an individual BE
Switchability between generics
The Bioequivalence trial
Types of Bioequivalence trials
Types of bioequivalence trials

• Order of preference
  1 - Pharmacokinetic
  2 - Pharmacodynamic
  3 - Clinical
  4 - In vitro studies
Types of bioequivalence trials

- Metabolite
- Drug C (t)
- Drug in urine
- PD₁
- PD₂
- PD
- Clinical efficacy
- Dissolution
- Dose
- abs
- PK
- PD
- Clinical
- in vitro testing
- in vivo testing
Types of Bioequivalence trial
Pharmacodynamic endpoints

**T and R are bioequivalent**

**Response A**
(e.g.; of clinical interest)

**Response B**
(e.g.; a surrogate)

**Systemic exposure**

**Test**

**Reference**

**T and R are not bioequivalent**

**AUC**
In vitro approach (biowaiver)
In vitro equivalence

• The disintegration vs. the absorption phase

• The logic to support an *in vitro* testing
  
  – to waive in vivo study rather than to demonstrate a bioequivalence

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**Figure 1 -** Dissolution profile of chloramphenicol palmitate obtained from products A, B, C, D, E, F, G and H, for each time interval, in HCl 0.01 N environment. *Each data point represents the mean of 12 units.*
Computation of a similarity factor
The similarity factor

- If there is no difference in mean cumulative percentage at all time points, the value of $f_2$ is 100 (log is $\log_{10}$) and the range is between 0 and 100

\[ f_2 = 50 \cdot \log \left( \frac{100}{\sqrt{1 + \frac{n}{\sum_{t=1}^{n} [R(t) - \bar{T}(t)]^2}}} \right) \]

$f_2$ should be $>50$ to claim that the two profiles are similar; this is the value that is obtained if all differences are of 10% at all sampling times.
Bioequivalence trial:
test subjects
Selection of animals

- EU: Animals used in bioequivalence studies should be **clinically healthy** representatives of the target population.

- US: Ordinarily, studies should be conducted with **healthy animals** representative of the species, class, gender, and physiological maturity for which the drug is approved.
  - The bioequivalence study may be conducted **with a single gender** for which the pioneer product is approved, unless there is a known interaction of formulation with gender.
Bioequivalence : test subject

• Remind : B.E. trial is not to document bioavailability variability

• The selected subjects must be as homogeneous as possible (age, sex, weight)
Sex, bioavailability and bioequivalence

A sex effect

Sex effect
Frequent in human medicine because Body Weight is not considered for dosage regimen!
A sex effect (or any other possible effect as healthy status, age, body weight, breed etc) is not an issue for a BE trial.

Sex effect

Frequent in human medicine because Body Weight is not considered!
The two formulations are BE in male but not in female; thus it exist an interaction sex by formulation.
Sex, bioavailability and bioequivalence

• Question: do we need to test both sexes?
  – Bioavailability
    yes: possible sex effect frequent in human medicine because BW is not taken into account for dosage regimen
  – Bioequivalence
    no: interaction formulation*sex unlikely

Possible Interaction

• For oral route: ruminant vs pre-ruminant
Dose to be tested
Dose to be tested

- The approved dose must be tested
- For drugs with multiple claims involving different doses, different trials should be performed
Single dose *or* multiple doses

steady state studies?
Single dose vs. multiple dose steady state studies

• Two bio-inequivalent formulations (single dose) may become bioequivalent in steady-state condition
Single dose vs. multiple dose steady state studies

2 products that are not bioequivalent after a single dose may appear to be bioequivalent in a multiple dose administration.
Bioequivalence: Experimental design
Bioequivalence: experimental design

- Parallel design
- Cross-over design
Parallel design

Groups and formulations are confounded

Example:
- growing animals
- small animals (fish, chicken,…) (blood sampling)
- long half-life (washout)
Bioequivalence : Parallel design

- Advantage
  • no washout period (appropriate for long-acting drug)
  • possible unequal numbers of subjects per treatment group
  • statistical analysis is still possible when subjects (animals) are lost during the experiment

- Limits
  • more subjects are required because comparison is made based on the inter- and the intra-subject variabilities
Bioequivalence: parallel design

- Drug with very long terminal $t_{1/2}$
  - With a crossover, risk of dropouts due to the long washout period
Bioequivalence: experimental design

- 2x2 crossover

- other crossover
  e.g.: AB, BA, AA, BB (BALAAM design)
The washout period

• The washout period is defined as the rest period between the two administrations (periods)
  – Should be long enough to guarantee that the first treatment period does not carry over the second
  – Related to any changes
    • **PK**: residual concentration of the drugs
    • **PD**: effect as induction/inhibition (or any pharmacological effect) triggered at the first period and able to influence the second period
Bioequivalence:
2x2 crossover design (I)

• **Advantage**
  - decrease in the residual error, therefore reduction in the number of subjects

• **Limits**
  - washout period required
  - risk of an unequal carryover effect
  - difficulties in analyzing the design if subjects are lost during the experiment
Bioequivalence:
The *a priori* Bioequivalence range
**A priori** Bioequivalence range

- These are the two limits (θ₁ and θ₂) between which the **90 % CI interval of the ratio** of the two product should be located in order to accept average B.E.
Acceptance limits (EMEA)

- In studies to determine bioequivalence after a single dose, the parameters to be analysed are AUCt and Cmax.

- For these parameters the 90% confidence interval for the ratio of the test and reference products should be contained within the acceptance interval of 80-125%.
The 70-143% rule for Cmax

- Only EMA (but challenged)
- Should be prospectively specified
- Justification should be given in terms of efficacy and safety
Decision procedures in bioequivalence trials

80%  

BE accepted

BE not accepted

$\mu_T / \mu_R$  

Ratio of test and reference formulation

The 90% CI of the ratio
A priori Bioequivalence range

- For drug with a narrow therapeutic index
  
  0.90 - 1.11 (multiplicative model)
Bioequivalence sample size
Bioequivalence: sample size (I)

• The number of subjects has not to be justified if the appropriate risk is controlled (consumer risk, 5 %)

• For economical and ethical reasons, the appropriate number of subjects must be calculated to avoid an excessively high producer risk
Bioequivalence : sample size (II)

Information required to calculate the sample size

\( \Delta \) : The bioequivalence range (80-125 %)

\( \alpha \) : The consumer risk (5 %)

\( \beta \) : The producer risk (e.g., 20 %)
  (the probability of rejecting bioequivalence when products are actually bioequivalent. Power is used only in planning the experiment, not as part of the statistical test)

\( \sigma^2 \) : The error / (residual) variance
Bioequivalence: sample size: multiplicative model

\( \alpha = 5\% - \text{Power 80\%} \)

\( \Theta_1 = 0.80 \) \( \Theta_2 = 1.25 \)

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<th>( \mu_T / \mu_R )</th>
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\( CV_{intra} = \sqrt{\exp(\sigma_w^2) - 1} \)
Bioequivalence:

Characteristics to be investigated
BE Characteristics to be investigated

- AUC & $C_{\text{max}}$, (no longer $T_{\text{max}}$)

- How to calculate or obtain these relevant parameters
  - Curve: trapezoidal rule (no fitting)
  - $C_{\text{max}}$: observed
Bioequivalence:

Analytical techniques
Bioequivalence: analytical technique

- Must be validated (GLP)
- Case of a chiral drug
  - An enantioselective assay may have to be used
- Pooled approach as a preliminary analysis
Statistical analysis

• The test problem

• Data analysis
  - Distribution
  - Outliers
  - Logarithmic transformation
  - $2 \times 2$ crossover / the carryover effect
The test problem
Bioequivalence : the test problem

From a regulatory point of view the producer risk of erroneously rejecting bioequivalence is of no importance

The primary concern is the protection of the patient (consumer risk) against the acceptance of BE if it does not hold true
Bioequivalence: the test problem

Classical test of null hypothesis (I)

\[ H_0 : \mu_T - \mu_R = 0 \quad \text{or} \quad \mu_T = \mu_R \]

\[ H_1 : \mu_T - \mu_R \neq 0 \quad \text{or} \quad \mu_T \neq \mu_R \]

\( \mu_T \) and \( \mu_R \): population mean for test and reference formulation respectively

Decision on the BE cannot be based on the classical null hypothesis
Classical statistical hypothesis: drawback

Statistically different for $p \leq 0.000$ but actually therapeutically equivalent
Classical statistical problem: the drawback

Not statistically different with \( p \geq 0.05 \) but actually not therapeutically equivalent
Bioequivalence : the test problem

Classical test of null hypothesis

• Can be totally misleading

• Acceptance of B.E. despite clinically relevant difference between R and T formulation

• Rejection of B.E. despite clinically irrelevant difference between R and T
Bioequivalence : the test problem
Classical test of null hypothesis

Use of the classical null hypothesis would encourage poor trials, with few subjects, under uncontrolled conditions to answer an irrelevant question

Usual hypothesis testing for equality are not appropriate for BE assessment
Bioequivalence: the test problem

• The appropriate hypothesis

\[ H_{01} \text{(Ref/test)} \]
\[ H_{02} \text{(Ref/test)} \]
\[ \theta_1 \quad \theta_2 \]
\[ \text{inequivalent} \]

\[ H_1 \text{(Ref -test)} \]
\[ \theta_1 \quad \theta_2 \]
\[ \text{equivalent} \]
The decomposed two-one sided hypothesis

- $H_01: \theta \leq \theta_1$ vs. $H_{a1}: \theta_1 < \theta$
- $H_02: \theta \geq \theta_2$ vs. $H_{a2}: \theta < \theta_2$
Bioequivalence: the test problem

• The appropriate hypothesis

Can we reject $H_01$?

Can we also reject $H_02$?

YES

Bioequivalent

YES

two unilateral "t" tests

$\theta_1$ (Ref/test) $\theta_2$

$H_01$ $H_02$

$\leq 5\%$ $\leq 5\%$

$\leq 5\%$
Bioequivalence : The test problem

The interval hypothesis (multiplicative scale)

\[ H_0 : \frac{\mu_t}{\mu_r} \leq \delta_l \text{ or } \frac{\mu_t}{\mu_r} \geq \delta_u \]

\[ H_a : \delta_l < \frac{\mu_t}{\mu_r} < \delta_u \]

Where \( \delta_l = EXP(\theta_1) \) and \( \delta_u = EXP(\theta_2) \)

\( \mu_T \) and \( \mu_R \) : the expected medians for test and reference respectively

\( \theta_1 \) and \( \theta_2 \) : lower and upper limits of the bioequivalence range

i.e. domain -0.2231 and +0.2231 in the Ln domain)
The Schuirmann’s Two one-sided test procedure

\[ T_L = \frac{(\bar{Y}_T - \bar{Y}_R) - \theta_L}{\hat{\sigma}_d \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}} > t(\alpha, n_1 + n_2 - 2) \]

\[ T_U = \frac{(\bar{Y}_T - \bar{Y}_R) - \theta_U}{\hat{\sigma}_d \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}} < -t(\alpha, n_1 + n_2 - 2) \]

The two one-sided \( t \) test procedure is operationally equivalent to the classic (shortest) confidence interval approach.
The shortest 90% confidence interval

• If a \((1-2\alpha)\times 100\%\) confidence interval for the ratio \((\mu_t/\mu_r)\) is within the acceptance limits as recommended by the regulatory agency, then it is conclude that the test and reference formulation are BE
Decision procedures in bioequivalence trials

Regulatory point of view

only the 90 % CI

A priori B.E. Range

BE accepted

Conclusion : BE rejected

(administrative bioinequivalence)

Industrial point of view

the 90 and 95% CI

BE accepted

No conclusion
(Lack of power for any decision)

Biological Bioinequivalence

Biological Bioinequivalence

A priori B.E. Range

only the 90 % CI