Bioavailability and Bioequivalence Studies

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Objectives

- Discuss the pharmacokinetic processes that determine absolute bioavailability
- Discuss the basic FDA requirements for approval of ANDAs submitted by generic drug manufacturers
- Discuss the basic study methods for bioequivalence
  - Study design
  - Statistical analysis
Bioavailability (F)

- (21 CFR 320.1): “rate and extent to which the active ingredient or active moiety is absorbed from a drug product and becomes available at the site of action”
- AUC (total amount of unchanged drug that reaches the systemic circulation) is used to calculate $F$

\[
\text{absolute } F = \frac{(\text{Dose}_{\text{IV}} \cdot \text{AUC}_{\text{oral,}\infty})}{(\text{Dose}_{\text{oral}} \cdot \text{AUC}_{\text{IV,}\infty})}
\]
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
Bioequivalence

(21 CFR 320.1): “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”
Definitions

- **Pharmaceutical alternatives**
  - Same active ingredient
  - May differ in salt, ester, dosage form or strength
  - *Example: different dosage forms or strengths within a single manufacturers' product line*

- **Pharmaceutical equivalents**
  - Same active ingredient, dosage form and strength
  - Same USP standards (strength, quality, purity, identity)
  - May differ in shape, color, excipients, release mechanism, packaging, labeling and expiration date
  - *Example: brand name and generic version of a drug*
Therapeutic equivalents

  - Permits pharmaceutical equivalents to be considered “therapeutically equivalent” (will have the same clinical effect and safety profile)
  - Allows generic manufacturers to gain FDA approval via ANDA (abbreviated NDA) submission
  - ANDAs do not include pre-clinical data or proof of safety/efficacy; *only have to prove bioequivalence*
# Normal drug approval process

<table>
<thead>
<tr>
<th>Pre-clinical testing</th>
<th>FDA review</th>
<th>Phase I</th>
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<th>Phase III</th>
<th>FDA review</th>
<th>Sponsor addresses reviewer comments</th>
<th>Final approval</th>
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<tr>
<td>Synthesis &amp; purification</td>
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<td>Animal testing</td>
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<tr>
<td>Short-term toxicity</td>
<td>3-4 years</td>
<td>1 year</td>
<td>1-2 years</td>
<td>3 years</td>
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<td>Long-term toxicity</td>
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- **IND**
- **NDA**
Investigational new drug (IND)

- Under the Federal Food, Drug & Cosmetic Act no drug can be distributed across state lines prior to final FDA approval
  - IND grants an exemption to the sponsor in order to conduct clinical trials
  - Three types: Investigator IND, Emergency Use IND, Treatment IND
  - Must include: (1) animal pharmacology/toxicology; (2) manufacturing information; (3) detailed clinical testing plan (protocols/investigator qualifications)
NDA

- Submitted after clinical testing; must include:
  - “Substantial evidence” of safety and efficacy (results of both animal and human testing)
  - Proposed labeling, package insert
  - Proof of adequate “Good Manufacturing Practices” to maintain USP standards for drug identity, strength, quality, and purity
  - Duration of FDA review
    - Regular review must be completed within 12 months
    - Fast-track review is completed within 6 months
ANDA (generic drugs)

- Active ingredient was already approved under the original sponsor NDA
  - Generic manufacturer only required to demonstrate bioequivalence (Phase I)
  - Typically 24-36 healthy volunteers in a double-blind, randomized, crossover trial (or trials)
    - Single-dose fasting
    - Steady-state fasting
    - Single-dose fed/fasting
Study design

- Randomized, two-sequence, two-period crossover
  - Treatment sequence is randomized
    - Every subject 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 intersubject 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
Sampling of biologic specimens

- Blood (plasma) samples are recommended
  - 12-18 samples (including predose) in each subject, spanning at least 3 drug elimination half-lives
  - Sampling should be spaced to allow accurate estimation of $C_{\text{max}}$ and $\lambda_z$
  - Minimum of 3-4 samples during terminal log-linear phase to accurately estimate $\lambda_z$
Required studies

- Oral solutions, elixirs, syrups, tinctures
  - Bioequivalence studies are generally waived
- Oral suspensions and immediate-release solid oral dosage forms
  - Single-dose fasting (highest strength)
- Extended-release solid oral dosage forms
  - Single-dose fasting (all strengths)
  - Multiple-dose steady-state (highest strength)
  - Single-dose fed/fasting (highest strength)
Single-dose fasting

- PK parameters to be submitted
  - Subject, period, sequence, treatment
  - Plasma concentrations and time points
  - Measures of systemic exposure (reflect rate and extent of absorption):
    - Early exposure – partial AUC (truncated at population median $T_{\text{max}}$ value)
    - Peak exposure – $C_{\text{max}}$
    - Total exposure – $\text{AUC}_{0-t}$, $\text{AUC}_{\infty}$
  - Also report $T_{\text{max}}$, $\lambda_z$, $t_{1/2}$
- Statistical analysis of $\text{AUC}_{0-t}$, $\text{AUC}_{\infty}$, and $C_{\text{max}}$
Multiple-dose (steady-state)

- PK parameters to be submitted:
  - Subject, period, sequence, treatment
  - Plasma concentrations and time points
  - Total systemic exposure – \( \text{AUC}_{0-\tau} \) (\( \tau = \text{dosing interval} \))
  - Also report
    - \( C_{\text{max}} \)
    - \( T_{\text{max}} \)
    - \( C_{\text{min}} \)
    - \( C_{\text{avg},ss} \) (\( \text{AUC}_{0-\tau}/\tau \))
    - \( D_{\text{F,ss}} \) (degree of fluctuation, or \( [C_{\text{max}} - C_{\text{min}}]/C_{\text{avg,ss}} \))
    - Swing (\( [C_{\text{max}} - C_{\text{min}}]/C_{\text{min}} \))

- Statistical analysis of \( \text{AUC}_{0-\tau} \) and \( C_{\text{max}} \)
Single-dose fed/fasting

- PK parameters to be submitted
  - Subject, period, sequence, treatment
  - Plasma concentrations and time points
  - Measures of systemic exposure (reflect rate and extent of absorption):
    - Early exposure – partial AUC (truncated at population median $T_{\text{max}}$ value)
    - Peak exposure – $C_{\text{max}}$
    - Total exposure – $\text{AUC}_{0-t}$, $\text{AUC}_{\infty}$
  - Also report $T_{\text{max}}$, $\lambda_z$, $t_{1/2}$
- Statistical analysis of $\text{AUC}_{0-t}$, $\text{AUC}_{\infty}$, and $C_{\text{max}}$
Demonstrating bioequivalence

- Statistical comparison of generic to brand $C_{\text{max}}$ and AUC using ANOVA
  - Evaluates potential confounding effect of 4 factors:
    - Sequence (treatment order)
    - Period
    - Subject
    - Treatment (formulation)
  - $C_{\text{max}}$ 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)
80/20 rule

- 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 ($C_{\text{max}}$, $AUC_{0-t}$, $AUC_{\infty}$, or $AUC_{0-\tau}$): arithmetic mean, geometric mean, geometric mean ratio (generic/brand) and 90% confidence interval (CI).
  - With log transformation the $\pm$ 20% rule translates to a mean ratio and 90% CI for each parameter of 80-125%.
FDA Orange Book

- [http://www.fda.gov/cder/ob](http://www.fda.gov/cder/ob)
- "A" – therapeutically equivalent with no known or anticipated bioequivalence problems: AA (oral), AN (soln/powder for aerosolization), AO (injectable oil), AP (injectable aqueous soln), AT (topical)
- "AB" – recognized as therapeutically equivalent based on ANDA approval
- "B" – not yet proven therapeutically equivalent: B* (require further FDA review), BX (therapeutically inequivalent), BC (extended-release caps.tabs/injectables), BD (known bioequivalence problems), BE (oral delayed-release), BN (soln/powder for nebulization), BP (potential bioequivalence problems), BR (suppositories/enemas), BS (drug standard deficiencies), BT (topical)
Example

- In a single-dose randomized, crossover study 6 healthy volunteers are each given a 50 mg oral dose of a generic drug (Treatment A) and its brand name reference drug (Treatment B). $C_{max}$ and $AUC_{0-t}$ values are given below. For each parameter, calculate the mean ratio and 90% confidence interval. Based on these criteria, does the generic drug meet the requirements for bioequivalence?
## Evaluation of $C_{\text{max}}$

<table>
<thead>
<tr>
<th>Subject</th>
<th>Treatment A</th>
<th>Treatment B</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$C_{\text{max}}$ (ng/mL)</td>
<td>ln $C_{\text{max}}$</td>
<td>$C_{\text{max}}$ (ng/mL)</td>
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<tr>
<td>1</td>
<td>11.4</td>
<td>2.4336</td>
<td>12.9</td>
</tr>
<tr>
<td>2</td>
<td>10.6</td>
<td>2.3609</td>
<td>10.1</td>
</tr>
<tr>
<td>3</td>
<td>12.1</td>
<td>2.4932</td>
<td>9.4</td>
</tr>
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<td>4</td>
<td>11.7</td>
<td>2.4596</td>
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<td>5</td>
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<td>2.4159</td>
<td>10.8</td>
</tr>
<tr>
<td>6</td>
<td>12.1</td>
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<td>12.3</td>
</tr>
<tr>
<td>Mean</td>
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<tr>
<td>SEM</td>
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</tbody>
</table>

**mean $C_{\text{max}}$ ratio** = $100 \cdot e^{(\text{mean } \ln C_{\text{max}} \text{ difference})} = 100 \cdot e^{(0.0402)} = 104.09$

**90% CI**

$100 \cdot e^{(\text{mean } \ln C_{\text{max}} \text{ difference } \pm t_{df,0.05} \cdot \text{SEM}_{\text{difference}})} = 93.76 \text{ to } 115.56$

1. degrees of freedom (df) = # of groups $\cdot$ # of treatments $\cdot$ $(n - 1) = 2 \cdot 2 \cdot (6 - 1) = 20$
2. at alpha = 0.05 and 20 df, $t = 2.086$
3. lower limit = $100 \cdot e^{(0.0402 - (2.086 \cdot 0.0501))} = 93.76$
4. upper limit = $100 \cdot e^{(0.0402 + (2.086 \cdot 0.0501))} = 115.56$
## Evaluation of AUC$_{0-t}$

<table>
<thead>
<tr>
<th>Subject</th>
<th>Treatment A</th>
<th>Treatment B</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AUC$_{0-t}$ (ng·hr/mL)</td>
<td>ln AUC$_{0-t}$</td>
<td>AUC$_{0-t}$ (ng·hr/mL)</td>
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<tr>
<td>1</td>
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<td>5.0389</td>
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<td>5</td>
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<td>5.0857</td>
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<tr>
<td>6</td>
<td>144.4</td>
<td>4.9726</td>
<td>154.7</td>
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</tbody>
</table>

- **Mean**: 0.0425
- **SD**: 0.0824
- **SEM**: 0.0336

**mean AUC$_{0-t}$ ratio** = 100 · e^{(mean ln AUC$_{0-t}$ difference)} = 104.34

**90% CI** = 100 · e^{(mean ln AUC$_{0-t}$ difference ± t$_{df,0.05}$ · SEM$_{difference}$)} = 97.28 to 111.92

(1) df = 2 · 2 · (6-1) = 20
(2) at alpha = 0.05 with 20 df, t = 2.086
(3) lower limit = 100 · e^{[0.0425 - (2.086 · 0.0336)]} = 97.2788
(4) upper limit = 100 · e^{[0.0425 + (2.086 · 0.0336)]} = 111.9173
References


- From idea to market: the drug approval process. *J Am Board Family Med* 2001;14(5)