Statistical Considerations of Bioequivalence Studies Using *In-Vitro* Permeation Test (IVPT) Data

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Abstract

For topical dermatological drug products, establishing bioequivalence (BE) between a generic (Test) and a reference listed drug (RLD) product based on the in vitro permeation test (IVPT) consists of comparing the rate and extent of drug permeation through excised human skin. The IVPT data analysis uses a reference-scaled approach and two statistical endpoints, those of cumulative amount penetrated (AMT) and maximum flux (Jmax). Commonly arising issues in this analysis can be the presence of aberrant data values, or outliers that such values pose to the IVPT review.

Goal: Study the impact of different sources of outliers on BE outcomes

Bioequivalence

Bioavailability (BA): Measurement of the extent of a therapeutically active medicine available at the site of action

- ❑Oral drug products: pharmacokinetic (PK) sampling of blood at multiple time points
- ❑Topical dermatological drug products: Locally acting, need to evaluate the concentration near the site of action

Bioequivalence (BE): If two drugs are bioequivalent, there is no clinically significant difference in their bioavailability

In Vitro Permeation Test (IVPT):

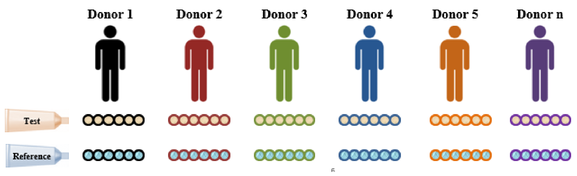
- ❑ Uses excised human skin in conjunction with diffused cells to measure drug concentration
- ❑ The rate of drug delivery (flux) is measured by sampling at specific, pre-selected time-points in a way analogous to that used in blood (or plasma) concentration sampling in PK studies

In vitro cutaneous PK endpoints:

- ❑ Maximum flux (Jmax) at the peak of the acyclovir flux profile
- ❑ Cumulative total permeation (AMT) of acyclovir across the study duration

IVPT Study design

- parallel, single dose, multiple replicate per treatment group,
- n donors and two treatment groups, each for the test product and reference product



Balanced data: same number of replicates
Unbalanced data: different number of replicates

IVPT Statistical Analysis of BE

Statistical approaches were introduced with Acyclovir guidance (2016). The mixed criterion uses the estimated within-reference variability (Swr) as a cut-off point for BE analysis

- ❑ When Swr ≤ 0.294, Average Bio-Equivalence (ABE) used
- ❑ When Swr ≥0.294, Scaled ABE (SABE) used

SABE approach for BE:

- 95% upper confidence bound must be less than or equal to zero
- point estimate of the T/R geometric mean ratio must fall within [0.8, 1.25]

ABE approach for BE: 90% CI for T/R geometric mean ratio must fall within [0.8, 1.25]

Outliers

Outliers: extreme replicate values within donors per treatment

- Standard practices used in PK-studies (standardized residuals) do not apply here
- Small sample tests such as Dean-Dixon test and Grubbs test considered because of the small sample size of replicate values within one donor
- Three possible outlier sources considered:
  - ❑ R outliers only
  - ❑ T outliers only
  - ❑ Both R and T outliers

Simulation design

Generate balanced IVPT data:

$$T_{ij} \sim N(\mu_T, \sigma^2_{WT}) \text{ and } R_{ij} \sim N(\mu_R, \sigma^2_{WR})$$

T_{ij} and R_{ij} : log-transformed Jmax/AMT for test (T) and reference (R) product for the ith replicate ($i = 1, \dots, r$) and jth donor ($i = 1, \dots, n$)
μ_R and μ_T: population means of the T and R products
σ^2_{WR} and σ^2_{WT}: within-subject variance of R and T products
Assume, $\mu_T = \mu_R + \ln(GMR); \mu_R = 5$
$\sigma^2_{WR} = \sigma^2_{WT}$
GMR: true geometric mean ratio of T/R

Generate outliers: Suppose we want to generate $m(< n)$ outliers in reference group:

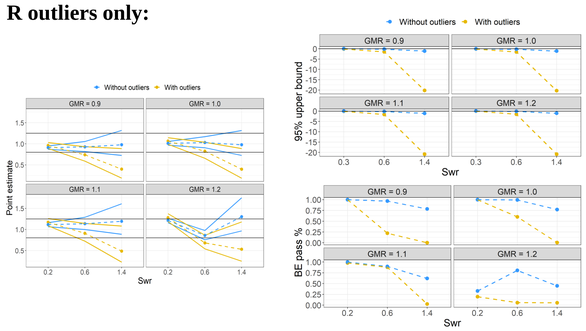
- ❑ Select m donors out of n donors in reference group
- ❑ For each of the m donors, take the largest replicate
- ❑ Multiply qs_f where q: outlying degree = 3.5 &
  s_f: standard deviation for that donor in reference group

- Perform BE test on the balanced data with the outliers
- Use Dixon test or Grubbs test to detect outliers (α=0.05)
- Perform BE test on the unbalanced data without the outliers
- Exclusion: any BE studies if the outlier test resulted in detecting different number of outliers than the pre-specified

Impact on BE outcome

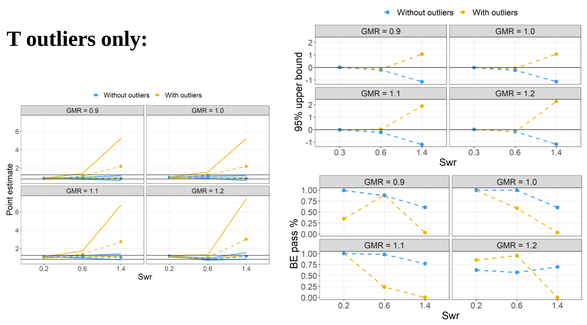
- n = 20, r = 6
- We report mean point estimate with mean 95% confidence intervals, mean 95% upper bound and proportion of cases where BE is true

R outliers only:



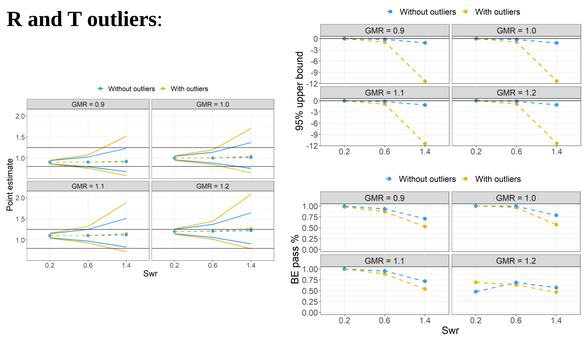
Number of reference outliers is 4

T outliers only:



Number of test outliers is 4

R and T outliers:



Number of R outliers is 2
Number of T outliers is 2

Performance

Hypothesis to be tested:

$$H_0 = \text{BE} \text{ vs. } H_1 = \text{not BE}$$

- Power is computed at **GMR = 0.95 &** type-I error rate is computed at **GMR = 1.251 and 1.29**
- Swr is fixed at 0.6 and r = 6
- Number of R outliers is 2, number of test outliers is 2

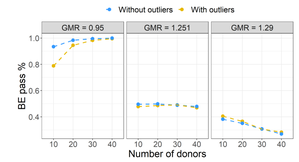
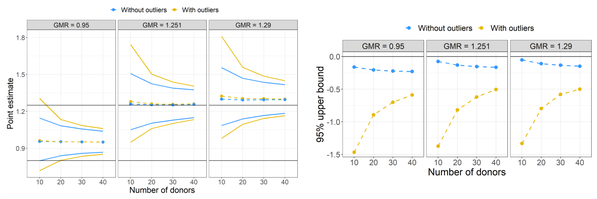


Figure: Power of the BE test at GMR = 0.95 (left) and type-I error rate at GMR = 1.251 and GMR = 1.29 (right)



Conclusion

- For R outliers only, the BE outcome seem to be affected markedly when Swr is high.
- For T outliers only, BE result is affected for all choices of Swr and GMR.
- When both R and T outliers are present simultaneously, BE outcome is affected less than when only R outliers or only T outliers are present.

Reference

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