Detecting Outliers in Replicated Crossover Bioequivalence Studies

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Abstract

Outlier detection is an important part in a bioequivalence study. The existence of outliers could have severe influence on the result of bioequivalence. The existing outlier detection methods mainly focus on the standard 2×2 crossover design and limited work has been done about the fully replicated 2x4 (ABAB, BABA) and the partially replicated 3x3 (BAA, ABA, AAB) designs. Additionally a large proportion of bioequivalence studies received by FDA are replicated crossover studies.

Goal: Develop an outlier detection method for the replicated crossover studies.

Bioequivalence and Outliers

Bioequivalence (BE): Two pharmaceutical products are bioequivalent if there is an absence of a significant difference in the rate and extent of absorption of the active ingredient after administration of the same molar dose under the similar conditions. (21 CFR 314.3)

Pharmacokinetic (PK) parameters:

- Maximum blood or plasma concentration (C_{max})
- Area under the blood concentration-time curve (AUC)

Formulations: Test (T), Reference (R)

Types of outliers:

- Single-data-point outlier: due to product or process failure
- Subject-by-formulation outlier: because of the unusual reaction of a single subject to one of the formulations
- Subject outlier: as a result of the unusual reaction of a single subject to both formulations

Potential influence of outliers:

- Bias the point estimate of the relative bioavailability
- Inflate the standard error of the point estimate of relative bioavailability

Deletion of outliers:

- Subject-by-formulation outliers: discouraged
- Single-data-point outliers: acceptable

Goals: Develop methods that

1) detect the outliers
2) find the types of outliers

Fully Replicated Crossover design

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

Statistical Model

$$y_{hijk} = \mu + \xi_h + \pi_m + \tau_j + s_{hij} + e_{ijk}$$

y_{hijk}: the observation for the kth replicate ($k = 1,2$) of the jth formulation ($j = R, T$) on the ith subject ($i = 1, \dots, n_h$) in sequence $h = 1,2$

μ: the overall mean

ξ_h: the fixed effect of the hth sequence with $\sum_h \xi_h = 0$

π_m: the fixed effect of the mth period ($m = 1, 2, 3, 4$) with $\sum_m \pi_m = 0$

τ_j: the fixed effect for the jth formulation with $\sum_j \tau_j = 0$

s_{hij}: the (i, j)th random subject-by-formulation effect in sequence h

e_{hijk}: the random error with variance σ^2_{WT} for T and σ^2_{WR} for R

$\boldsymbol{s}_{hi} = (s_{hiR}, s_{hiT})^T$ and e_{hijk} are assumed mutually independent

Distributional Derivations

Schall et at. (2010) proposed four contrasts of the four observations for each subject

- $c_{hi1} = y_{hi\cdot\cdot} = \frac{y_{hiT1}+y_{hiT2}+y_{hiR1}+y_{hiR2}}{4}$ ⟵ Subject outlier
- $c_{hi2} = y_{hiT\cdot} - y_{hiR\cdot} = \frac{y_{hiT1}+y_{hiT2}}{2} - \frac{y_{hiR1}+y_{hiR2}}{2}$ ⟵ Subject-by-formulation outlier
- $c_{hi3} = \frac{y_{hiT1}-y_{hiT2}}{\sqrt{2}}$ ⟵ Single-data-point outlier (T)
- $c_{hi4} = \frac{y_{hiR1}-y_{hiR2}}{\sqrt{2}}$ ⟵ Single-data-point outlier (R)

Un-studentized residuals: Subtract from the previous contrasts the corresponding sequence averages

- $r_{S_{hi}} = c_{hi1} - c_{h\cdot1} = y_{hi\cdot\cdot} - y_{h\cdot\cdot\cdot}$
- $r_{SF_{hi}} = c_{hi2} - c_{h\cdot2} = (y_{hiT\cdot} - y_{hiR\cdot}) - (y_{h\cdot T\cdot} - y_{h\cdot R\cdot})$
- $r_{DT_{hi}} = c_{hi3} - c_{h\cdot3} = \frac{[(y_{hiT1}-y_{hiT2})-(y_{h\cdot T1}-y_{h\cdot T2})]}{\sqrt{2}}$
- $r_{DR_{hi}} = c_{hi4} - c_{h\cdot4} = \frac{[(y_{hiR1}-y_{hiR2})-(y_{h\cdot R1}-y_{h\cdot R2})]}{\sqrt{2}}$

Our method is based on the un-studentized residuals. Use r_{DT} as an example to show the derivation of the test statistic.

$r_{DT_{hi}} = c_{hi3} - c_{h\cdot3}$, $\text{var}(c_{hi3}) = \sigma^2_{WT}$, $\text{var}(c_{h\cdot3}) = \frac{\sigma^2_{WT}}{n_h}$.

Let $x_1, \dots, x_{n_h} \sim$ i.i.d. $N(a, \sigma^2_{WT})$, where a is an unknown parameter. Under the normality assumption:

- $\left(r_{DT_{h1}}, \dots, r_{DT_{hn_h}}\right)$ has the same distribution as $\left(x_1 - \bar{x}_{n_h}, \dots, x_{n_h} - \bar{x}_{n_h}\right)$, where $\bar{x}_{n_h} = \frac{1}{n_h}\sum_{i=1}^{n_h} x_i$
- $T_{DT_{ht}} = \frac{r^2_{DT_{ht}}}{\sum_{i=1}^{n_h} r^2_{DT_{hi}}} = \frac{\left(x_{n_h} - \bar{x}_{n_h}\right)^2}{\sum_{i=1}^{n_h}\left(x_i - \bar{x}_{n_h}\right)^2}$
$= \frac{\left(\frac{n_h-1}{n_h}\right)^2\left(x_{n_h} - \bar{x}_{n_h-1}\right)^2}{\sum_{i=1}^{n_h-1}\left(x_i - \bar{x}_{n_h-1}\right)^2 + \frac{n_h-1}{n_h}\left(x_{n_h} - \bar{x}_{n_h-1}\right)^2}$
$\sim \frac{n_h - 1}{n_h} beta\left(\frac{1}{2}, \frac{n_h - 2}{2}\right)$

Hypothesis and Testing Procedure

Hypothesis:

H_0: $\frac{(y_{hiT1} - y_{hiT2})}{\sqrt{2}} \sim N(a, \sigma^2_{WT})$, for all $i = 1, \dots, n_h$

H_a: $\frac{(y_{hiT1} - y_{hiT2})}{\sqrt{2}} \sim N(+\delta_i, \sigma^2_{WT})$, for at least one $i, \delta_i \neq 0$.

Generate the sampling distribution of the test statistic and obtain the the αth upper quantile, denoted by $b_{n_h,1-\alpha}$

Examine the first p extreme T_{DT} statistics and do a sequential step-up test to ensure the outliers would not contaminate the normal observations. The testing procedure is:

1) Calculate $\{T_{DT_{ht}}\}$ from the data. Let $T_{DT_{(1)}}, \dots, T_{DT_{(n_h)}}$ be the order statistics of $\{T_{DT_{ht}}\}$, and $H_{0_{(t)}}$ the corresponding order null sub-hypotheses. Let $p = l$.
2) Remove l residuals that have the l largest T_{DT} statistics.
3) Add back the residual that has the smallest T_{DT} statistics from the removed residual pool.
4) Starting from $T_{DT_{(n_h-l+1)}}$, get the $\frac{\alpha}{p}$ quantile of the sampling distribution of the maximum of the correlated beta variables, denoted by $b_{n_h,1-\frac{\alpha}{p}}$, compare it with $T_{DT_{(n_h-l+1)}}$. Reject $H_{0_{(n_h-l+1)}}$ if $T_{DT_{(n_h-l+1)}} > b_{n_h,1-\frac{\alpha}{p}}$.
5) If $H_{0_{(n_h-l+1)}}$ is rejected, remove the residual.
6) Subtract 1 from l. Repeat procedure 2 to 5 until $l = 0$.

Data Simulation and Replicated Crossover Studies

Generate random sample $\{x_{ik}\}$ as $x_{ik} = \sqrt{0.5}(z_{i0} + z_{ik})$, where z_{i0} and $z_{ik} \sim N(0,1)$ i.i.d. $(i = 1, \dots, n; k = 1, 2, 3, 4)$

- z_{i0} and z_{ik} are for between-subject and within-subject variabilities, respectively

Generate random samples: $y_{hijk} = cx_{ik} + \mu_j + \pi_m + \xi_h$.

- c: the degree of variability. $c = 2\ln(0.2^2 + 1)$
- $\mu_R = \ln(100)$, $\mu_T = \ln(105)$
- $\pi_m = \xi_h = 0$

Generate outliers: adding qs_f to the response of the T formulation

- q: outlying degree, $q = 3, 4, \dots, 10$
- s_f: the standard deviation of the test formulation

Method Performance

Figure 1: Type 1 error rate assessment in replicated 2×4 and 3×3 design

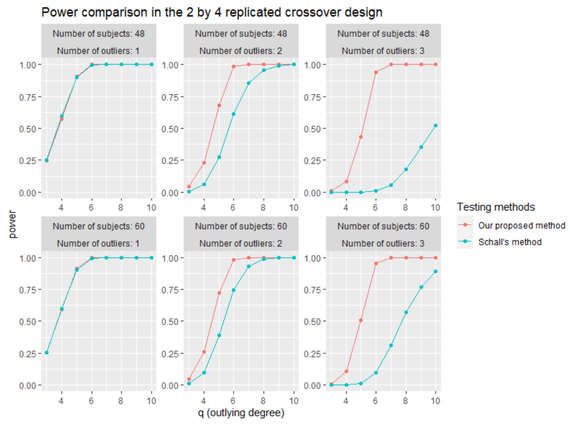

Figure 2: Power comparison in the 2×4 replicated crossover design for our method and an existing outlier detection method

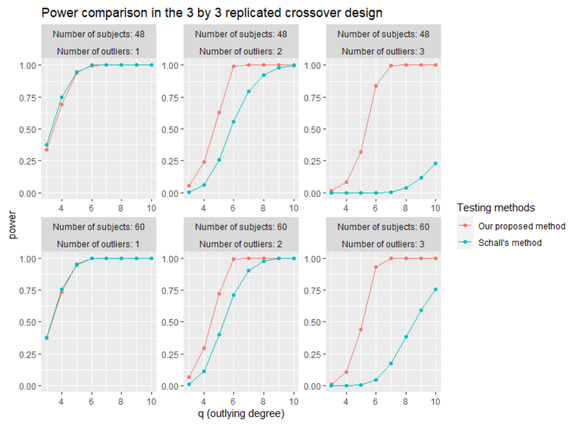

Figure 3: Power comparison in the 3×3 replicated crossover design for our method and an existing outlier detection method

Reference

Food and Drug Administration. (2001). *Statistical Approaches to Establishing Bioequivalence*. Guidance for Industry. Rockville, MD: Center for Drug Evaluation and Research.

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