Relative Potency Estimations in Multiple Bioassay Problems

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1. Introduction

2. Parallel–Line Assay

3. Simultaneous CI

4. Example

5. Remarks
Relative potency estimation is quite a standard problem in bioassays.

- Relative strength of a standard drug versus test drug(s)
- Parallel-line and slope-ratio assays
- Inferences for ratios of coefficients in the general LM
Aims:

- Ratio-based comparisons with a control in a normal one-way layout (Dilba et al., 2006)
- Mention applications in the general LM
- Relative Potency Estimations in Parallel-Line Assays
- Method Comparisons & Some Extensions
Model (2 preparations):

\[ Y_{ij} = \alpha_i + \beta D_{ij} + \epsilon_{ij}, \quad i = 0, 1; \quad j = 1, \ldots, n_i \]

\[ \theta = (\alpha_0 \quad \alpha_1 \quad \beta)' \]

Relative potency:

\[ \gamma = \frac{\alpha_1 - \alpha_0}{\beta} = \frac{(-1 \ 1 \ 0)\theta}{(0 \ 0 \ 1)\theta} \]

Fieller confidence interval
Multiple Assays

\[ Y_{ij} = \alpha_i + \beta D_{ij} + \epsilon_{ij}, \quad i = 0, \ldots, r; \quad j = 1, \ldots, n_i \]

- Relative potencies

\[ \gamma_i = \frac{\alpha_i - \alpha_0}{\beta}, \quad i = 1, \ldots, r \]

- Simultaneous CI for ratios of linear combinations of general LM coefficients

(Zerbe et al., 1982; Jensen, 1989; Dilba et al., 2006; Hare and Spurrier, 2007)
Simultaneous CI for Ratios in the GLM

- Model: \( Y = X\theta + \epsilon \)

- Parameters of interest

\[
\gamma_\ell = \frac{c_\ell^t \theta}{d_\ell^t \theta}, \quad \ell = 1, 2, \ldots, r
\]

- When \( r = 1 \), Fieller CI
SCI for Multiple Ratios

\[ Y = X\theta + \epsilon \]

- Parameters: \( \gamma_\ell = \frac{c'_\ell \theta}{d'_\ell \theta}, \quad \ell = 1, 2, \ldots, r \)

- Let \( L_\ell = (c_\ell - \gamma_\ell d_\ell)\hat{\theta} \), then
  \[
  T_\ell(\gamma_\ell) = \frac{L_\ell}{s_{L_\ell}} \sim t(\nu) \\
  (T_1, \ldots, T_r)' \sim Mt_r(\nu, R(\gamma))
  \]

- \( Mt_r(\nu, R(\gamma)) \) and hence \( C_{1-\alpha, R(\gamma)} \) depends on \( \gamma \)!
In a parallel-line assay with **one standard** and **two test** preparations

- $\theta = (\alpha_0, \alpha_1, \alpha_2, \beta)$

**Relative potencies:** $\gamma = \left(\frac{\alpha_1 - \alpha_0}{\beta}, \frac{\alpha_2 - \alpha_0}{\beta}\right)'$

**Test statistics:**

$$T_i(\gamma_i) = \frac{(\hat{\alpha}_i - \hat{\alpha}_0) - \gamma_i \hat{\beta}}{s \sqrt{a_i'(X'X)^{-1}a_i}} \sim t(\nu), \quad i = 1, 2$$

where $a_1 = (-1, 1, 0, -\gamma_1)$ and $a_2 = (-1, 0, 1, -\gamma_2)$
In a parallel-line assay with **one standard** and **two test** preparations

- \( \theta = (\alpha_0, \alpha_1, \alpha_2, \beta) \)

**Relative potencies:** \( \gamma = \left( \frac{\alpha_1-\alpha_0}{\beta}, \frac{\alpha_2-\alpha_0}{\beta} \right)' \)

**Test statistics:** \( T_i(\gamma_i) = \frac{(\hat{\alpha}_i-\hat{\alpha}_0) - \gamma_i \hat{\beta}}{s \sqrt{a'_i (X'X)^{-1} a_i}} \sim t(\nu), \quad i = 1, 2 \)

where \( a_1 = (-1, 1, 0, -\gamma_1) \) and \( a_2 = (-1, 0, 1, -\gamma_2) \)

**Jointly,** \( T_i(\gamma_i), \ i = 1, 2 \) has a **bivariate** \( t \)-distribution

**Correlation:** \( \rho = \frac{a'_1 (X'X)^{-1} a_2}{\left[ (a'_1 (X'X)^{-1} a_1) (a'_2 (X'X)^{-1} a_2) \right]^{1/2}} \)
Two-sided percentage point of a bivariate $t$-distribution with $\nu = 20$ and $\rho = 0.6$
Exact Simultaneous confidence sets for $\gamma$

- Using $R(\gamma)$ as it is

  $\iff$ Inverting tests point-wise over grids of $\gamma$

- Two-sided simultaneous $(1 - \alpha)100\%$ CS

  $\{ \gamma : -C_{1-\alpha,R(\gamma)} \leq T_\ell(\gamma_\ell) \leq C_{1-\alpha,R(\gamma)}, \ell = 1, \ldots, r \}$

- SCSs are not necessarily rectangular
1a. **Bounded** exact two-sided confidence set

\[ \hat{\theta} = (\hat{\alpha}_0, \hat{\alpha}_1, \hat{\alpha}_2, \hat{\beta})' = (10.7, 10.5, 11.7, 2.2)', \ s^2 = 18.4, \ \nu = 41 \ \text{and} \ \mathbf{X}'\mathbf{X} = \begin{bmatrix} 15 & 0 & 0 & 10.4 \\ 0 & 15 & 0 & 10.4 \\ 0 & 0 & 15 & 10.4 \\ 10.4 & 10.4 & 10.4 & 64.86 \end{bmatrix} \]
1b. **Bounded exact two-sided confidence set**

\[ \hat{\theta} = (\hat{\alpha}_0, \hat{\alpha}_1, \hat{\alpha}_2, \hat{\beta})' = (10.8, 11.5, 10.2, 2.5)' \]

\[ s^2 = 58.2, \quad \nu = 71 \quad \text{and} \quad X'X = \begin{bmatrix} 25 & 0 & 0 & 13.54 \\ 0 & 25 & 0 & 13.54 \\ 0 & 0 & 25 & 13.54 \\ 13.54 & 13.54 & 13.54 & 71.37 \end{bmatrix} \]
2a. **Unbounded** exact two-sided confidence set

\[ \hat{\theta} = (\hat{\alpha}_0, \hat{\alpha}_1, \hat{\alpha}_2, \hat{\beta})' = (9.2, 8.5, 9.7, 1.6)', \quad s^2 = 28.3, \quad \nu = 41 \text{ and } X'X = \begin{bmatrix} 15 & 0 & 0 & 10.4 \\ 0 & 15 & 0 & 10.4 \\ 0 & 0 & 15 & 10.4 \\ 10.4 & 10.4 & 10.4 & 64.86 \end{bmatrix} \]
2b. **Unbounded** exact two-sided confidence set

\[
\hat{\theta} = (\hat{\alpha}_0, \hat{\alpha}_1, \hat{\alpha}_2, \hat{\beta})' = (8.6, 10.6, 11.4, 0.9)', \quad s^2 = 9.6, \quad \nu = 41 \text{ and } X'X = \begin{bmatrix}
15 & 0 & 0 & 10.4 \\
0 & 15 & 0 & 10.4 \\
0 & 0 & 15 & 10.4 \\
10.4 & 10.4 & 10.4 & 64.86
\end{bmatrix}
\]
**Approximate** Simultaneous CI

- *Probability inequalities*
  - Boole’s inequality
  - Šidák (Jensen, 1989)
  - Scheffé (Scheffé, 1970; Zerbe *et al.*, 1982; Young *et al.*, 1997)

- *Estimating* $R(\gamma)$, MLE plug-in (Dilba *et al.*, 2006)

- *Min & Max* of exact SCS (Hare and Spurrier, 2007)
\[ A_\ell \gamma_\ell^2 + B_\ell \gamma_\ell + C_\ell \leq 0, \quad \ell = 1, 2, \ldots, r \]

where

\[
A_\ell = (d'_\ell \hat{\theta})^2 - q^2 S^2 d'_\ell M d_\ell
\]
\[
B_\ell = -2 \left[ (c'_\ell \hat{\theta})(d'_\ell \hat{\theta}) - q^2 S^2 c'_\ell M d_\ell \right]
\]
\[
C_\ell = (c'_\ell \hat{\theta})^2 - q^2 S^2 c'_\ell M c_\ell
\]
\[
M = (X'X)^{-1}
\]

\[
q = \begin{cases} 
  t_{1-\frac{\alpha}{2}} & \text{, Fieller} \\
  t_{1-\frac{\alpha}{2r}} & \text{, Boole's inequality} \\
  C_{1-\alpha, I_r} & \text{, Šidák inequality} \\
  C_{1-\alpha, R(\hat{\gamma})} & \text{, Plug-in}
\end{cases}
\]
Relative Potency Estimation
Software

**mratios:** R extension package, CRAN: [www.r-project.org](http://www.r-project.org)

- Two-sample (*t.test.ratio*)
  E.g., Fieller CI (homogeneous & hetero. variances)

- One-way layout (*simtest.ratio*)
  E.g., Ratio-based multiple tests for any contrast

- General linear model (*sci.ratio.gen*)
  E.g., Parallel-line & slope-ratio assays, Calibration
Example - Multiple PL Assay

Four animals were subjected to three concentrations of three tuberculin preparations (S, T1, T2). The response is diameter of irritated spots twenty-four hours after application (Finney, 1975; Zerbe et al., 1982).

<table>
<thead>
<tr>
<th>Animal</th>
<th>Stand 1/2500</th>
<th>Stand 1/500</th>
<th>Stand 1/100</th>
<th>Test 1 1/2500</th>
<th>Test 1 1/500</th>
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<th>Test 2 1/2500</th>
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<tbody>
<tr>
<td>1</td>
<td>36</td>
<td>52</td>
<td>64</td>
<td>45</td>
<td>40</td>
<td>65</td>
<td>33</td>
<td>44</td>
<td>70</td>
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<tr>
<td>2</td>
<td>41</td>
<td>48</td>
<td>62</td>
<td>38</td>
<td>42</td>
<td>65</td>
<td>36</td>
<td>57</td>
<td>63</td>
</tr>
<tr>
<td>3</td>
<td>44</td>
<td>48</td>
<td>100</td>
<td>45</td>
<td>62</td>
<td>57</td>
<td>33</td>
<td>54</td>
<td>78</td>
</tr>
<tr>
<td>4</td>
<td>48</td>
<td>52</td>
<td>59</td>
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Model:

\[ Y_{ijk} = \mu + \tau_i + \alpha_j + \beta D_k + \epsilon_{ijk}, \]

\[ i = 1, 2, 3, 4; \quad j = 0, 1, 2; \quad k = 1, 2, 3 \]
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\[ \gamma_j = (\alpha_j - \alpha_0)/\beta, \quad j = 1, 2 \]

Two-sided 95% simultaneous CI:

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<td>(-0.593, 0.218)</td>
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<td>(-0.593, 0.216)</td>
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<td>(-0.585, 0.211)</td>
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Random animal effects
Simultaneous CI for **ratios of fixed effect parameters** in LMM

\[ Y_i = X_i \theta + Z_i b_i + e_i, \]

- Asymptotically, \( \hat{\theta} \) is normal and LC of this estimator,

\[ U_j = (c_j - \gamma_j d)' \hat{\theta}, \quad j = 1, \ldots, r \]

are asymptotically \( r \)-**variate normal**, \( \mathcal{N}_r(0, \Sigma[\sigma_{ij}]) \), where

\[ \sigma_{ij} = \text{cov}(U_i, U_j) = (c_i - \gamma_i d)' \text{var}(\hat{\theta})(c_j - \gamma_j d)' \]

- Estimate of \( \text{var}(\hat{\theta}) \) can be extracted from the fitted LMM
• Plugging the MLEs of $\gamma_i$s in $\Sigma$, equi-coordinate percentage points can be computed for SCI estimations

• Simultaneous CI:

$$\frac{[c_i - \gamma_id]^2}{(c_i - \gamma_id)'\text{var}(\hat{\theta})(c_i - \gamma_id)'} = C_{1-\alpha, \hat{\Sigma}}^2$$
Remarks

- Software is available for *simultaneous inferences for ratios* of coefficients in the general LM

- The plug-in approach has **good coverage**

- **Extension to** linear mixed models with possible improvements over existing conservative approaches


