Relative Potency Estimations in Multiple Bioassay Problems

Gemechis Dilba

Institute of Biostatistics, Leibniz University of Hannover, Germany

5^{th} International Conference on Multiple Comparison Procedures

July 2007, Vienna

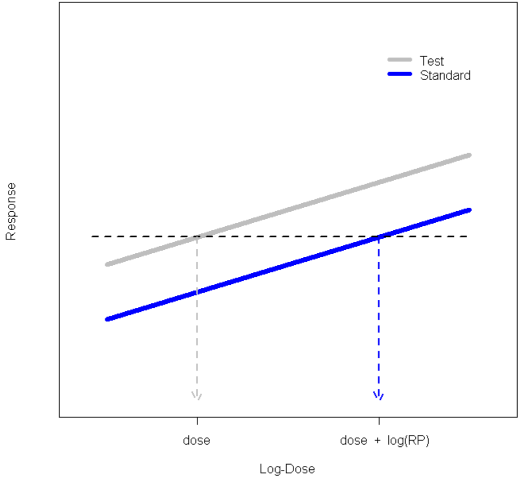
Introduction

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**


Test
Standard
Response
dose
dose + log(RP)
Log-Dose

- Model (**2 preparations**):

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

$$\boldsymbol{\theta} = (\alpha_0 \ \ \alpha_1 \ \ \beta)'$$

- Relative potency: $\gamma = \frac{\alpha_1 - \alpha_0}{\beta} = \frac{(-1 \ \ 1 \ \ 0)\boldsymbol{\theta}}{(\ \ 0 \ \ 0 \ \ 1)\boldsymbol{\theta}}$

- **Fieller** confidence interval

Multiple Assays

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

- 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: $\mathbf{Y} = \mathbf{X}\boldsymbol{\theta} + \boldsymbol{\epsilon}$

- Parameters of interest

$$\gamma_\ell = \frac{\mathbf{c}_\ell'\boldsymbol{\theta}}{\mathbf{d}_\ell'\boldsymbol{\theta}}, \quad \ell = 1, 2, \ldots, r$$

- When $r = 1$, Fieller CI

SCI for Multiple Ratios

$$\mathbf{Y} = \mathbf{X}\boldsymbol{\theta} + \boldsymbol{\epsilon}$$

- Parameters: $\gamma_\ell = \frac{\mathbf{c}_\ell'\boldsymbol{\theta}}{\mathbf{d}_\ell'\boldsymbol{\theta}}, \quad \ell = 1, 2, \ldots, r$
- Let $L_\ell = (\mathbf{c}_\ell - \gamma_\ell \mathbf{d}_\ell)'\widehat{\boldsymbol{\theta}}$, then

$$T_\ell(\gamma_\ell) = \frac{L_\ell}{S_{L_\ell}} \sim t(\nu)$$

$$(T_1, \ldots, T_r)' \sim Mt_r(\nu, \mathbf{R}(\gamma))$$

- $Mt_r(\nu, \mathbf{R}(\gamma))$ and hence $C_{1-\alpha,\mathbf{R}(\gamma)}$ depends on γ**!**

In a parallel-line assay with **one standard** and **two test** preparations

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

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

- Test statistics: $T_i(\gamma_i) = \frac{(\widehat{\alpha}_i - \widehat{\alpha}_0) - \gamma_i \widehat{\beta}}{S\sqrt{\mathbf{a}_i'(\mathbf{X}'\mathbf{X})^{-1}\mathbf{a}_i}} \sim t(\nu), \quad i = 1, 2$

  where $\mathbf{a}_1 = (-1, 1, 0, -\gamma_1)$ and $\mathbf{a}_2 = (-1, 0, 1, -\gamma_2)$

In a parallel-line assay with **one standard** and **two test** preparations

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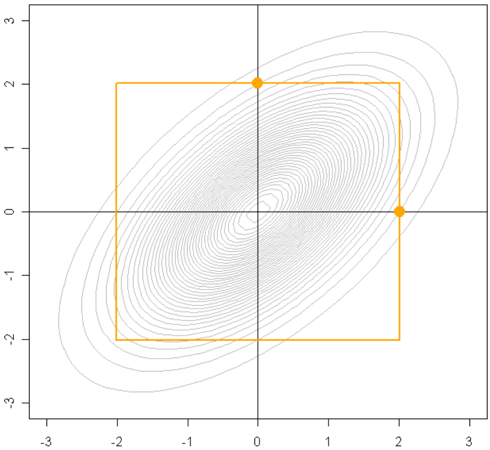
where $\mathbf{a}_1 = (-1, 1, 0, -\gamma_1)$ and $\mathbf{a}_2 = (-1, 0, 1, -\gamma_2)$

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

- Correlation: $\rho = \frac{\mathbf{a}_1'(\mathbf{X}'\mathbf{X})^{-1}\mathbf{a}_2}{\left[(\mathbf{a}_1'(\mathbf{X}'\mathbf{X})^{-1}\mathbf{a}_1)(\mathbf{a}_2'(\mathbf{X}'\mathbf{X})^{-1}\mathbf{a}_2)\right]^{\frac{1}{2}}}$



Two-sided percentage point of a bivariate t-distribution with $\nu = 20$ and $\rho = 0.6$

Exact Simultaneous confidence sets for γ

- Using $\mathbf{R}(\gamma)$ as it is

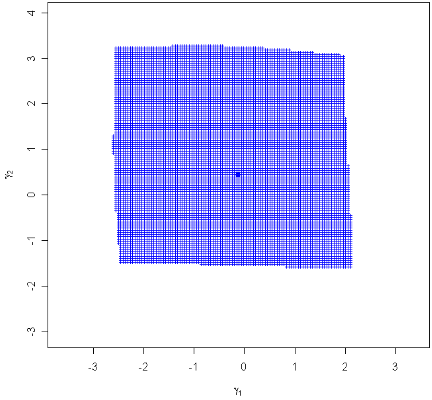
  $\Leftrightarrow$ Inverting tests point-wise over grids of γ

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

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

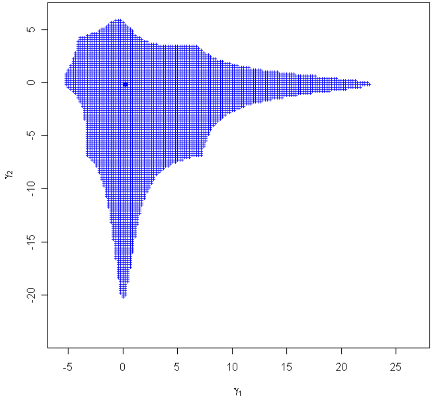
- SCSs are **not necessarily rectangular**

1a. **Bounded** exact two-sided confidence set



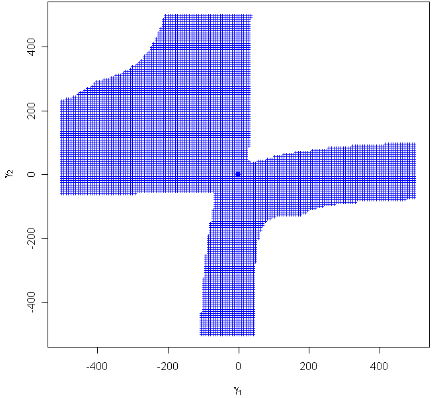
$$\widehat{\boldsymbol{\theta}} = (\widehat{\alpha}_0, \widehat{\alpha}_1, \widehat{\alpha}_2, \widehat{\beta})' = (10.7, 10.5, 11.7, 2.2)',\ s^2 = 18.4,\ \nu = 41 \text{ and } X'X = \begin{bmatrix} 15 & 0 & 0 & 10.4 \\ 0 & 15 & 0 & 10.4 \\ 0 & 0 & 15 & 10.4 \\ 10.4 & 10.4 & 10.4 & 64.86 \end{bmatrix}$$

1b. **Bounded** exact two-sided confidence set



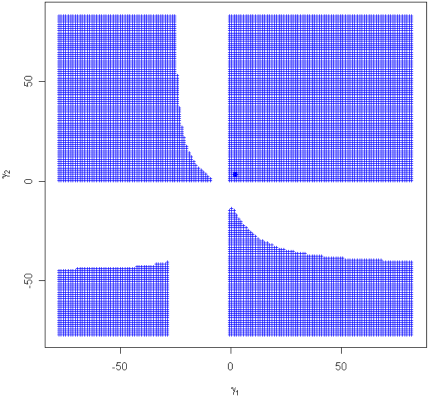
$$\widehat{\boldsymbol{\theta}} = (\widehat{\alpha}_0, \widehat{\alpha}_1, \widehat{\alpha}_2, \widehat{\beta})' = (10.8, 11.5, 10.2, 2.5)',\ s^2 = 58.2,\ \nu = 71 \text{ and } X'X = \begin{bmatrix} 25 & 0 & 0 & 13.54 \\ 0 & 25 & 0 & 13.54 \\ 0 & 0 & 25 & 13.54 \\ 13.54 & 13.54 & 13.54 & 71.37 \end{bmatrix}$$

2a. **Unbounded** exact two-sided confidence set



$$\widehat{\boldsymbol{\theta}} = (\widehat{\alpha}_0, \widehat{\alpha}_1, \widehat{\alpha}_2, \widehat{\beta})' = (9.2, 8.5, 9.7, 1.6)',\ s^2 = 28.3,\ \nu = 41 \text{ and } X'X = \begin{bmatrix} 15 & 0 & 0 & 10.4 \\ 0 & 15 & 0 & 10.4 \\ 0 & 0 & 15 & 10.4 \\ 10.4 & 10.4 & 10.4 & 64.86 \end{bmatrix}$$

2b. **Unbounded** exact two-sided confidence set



$$\widehat{\boldsymbol{\theta}} = (\widehat{\alpha}_0, \widehat{\alpha}_1, \widehat{\alpha}_2, \widehat{\beta})' = (8.6, 10.6, 11.4, 0.9)',\ s^2 = 9.6,\ \nu = 41 \text{ and } X'X = \begin{bmatrix} 15 & 0 & 0 & 10.4 \\ 0 & 15 & 0 & 10.4 \\ 0 & 0 & 15 & 10.4 \\ 10.4 & 10.4 & 10.4 & 64.86 \end{bmatrix}$$

Approximate Simultaneous CI

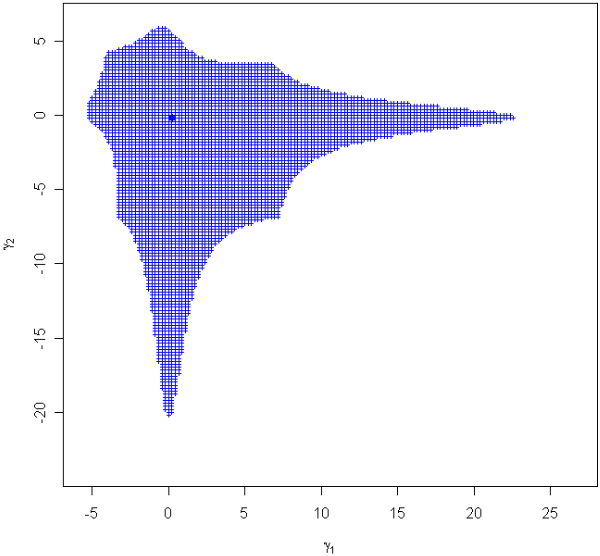
- *Probability inequalities*
  - Boole's inequality
  - Šidák (Jensen, 1989)
  - Scheffé (Scheffé, 1970; Zerbe *et al.*, 1982; Young *et al.*, 1997)
- *Estimating* $\mathbf{R}(\gamma)$, MLE plug-in (Dilba *et al.*, 2006)
- *Min & Max* of exact SCS (Hare and Spurrier, 2007)

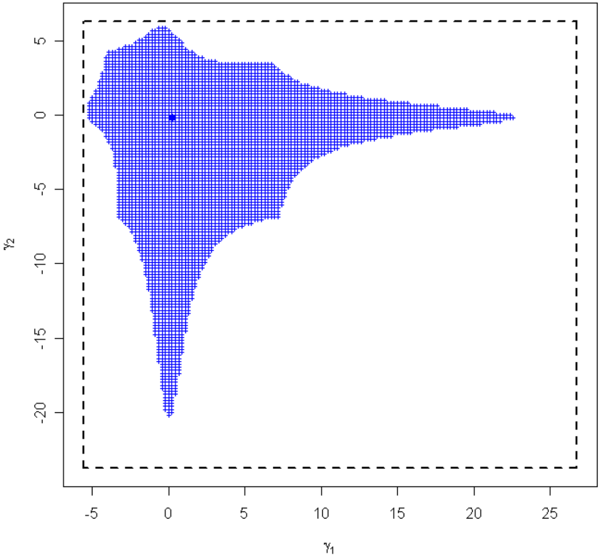
$$A_\ell\gamma_\ell^2 + B_\ell\gamma_\ell + C_\ell \leq 0, \quad \ell = 1, 2, \ldots, r$$

where

$$\begin{aligned}
A_\ell &= (\mathbf{d}_\ell'\widehat{\theta})^2 - q^2 S^2 \mathbf{d}_\ell' \mathbf{M} \mathbf{d}_\ell \\
B_\ell &= -2\left[(\mathbf{c}_\ell'\widehat{\theta})(\mathbf{d}_\ell'\widehat{\theta}) - q^2 S^2 \mathbf{c}_\ell' \mathbf{M} \mathbf{d}_\ell)\right] \\
C_\ell &= (\mathbf{c}_\ell'\widehat{\theta})^2 - q^2 S^2 \mathbf{c}_\ell' \mathbf{M} \mathbf{c}_\ell \\
\mathbf{M} &= (\mathbf{X}'\mathbf{X})^{-1}
\end{aligned}$$

$$q = \begin{cases} t_{1-\frac{\alpha}{2}} & \text{, Fieller} \\ t_{1-\frac{\alpha}{2r}} & \text{, Boole's inequality} \\ C_{1-\alpha,\mathbf{I}_r} & \text{, Šidák inequality} \\ C_{1-\alpha,\mathbf{R}(\widehat{\gamma})} & \text{, Plug-in} \end{cases}$$


5
0
-5
-10
-15
-20
γ_2
-5
0
5
10
15
20
25
γ_1



5
0
-5
-10
-15
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γ_2
-5
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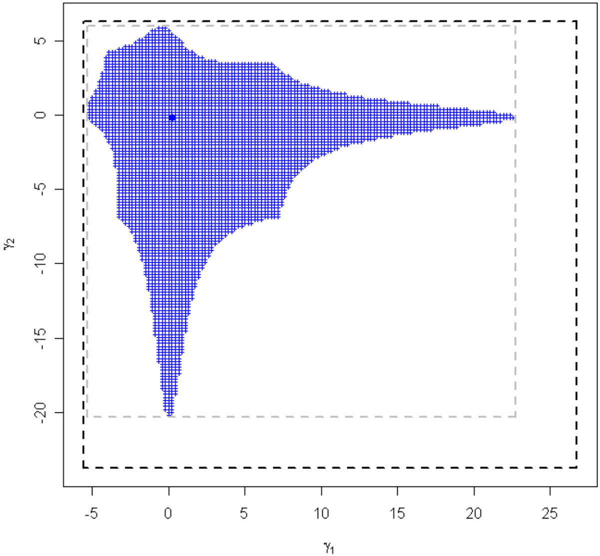
5
10
15
20
25
γ_1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20
25
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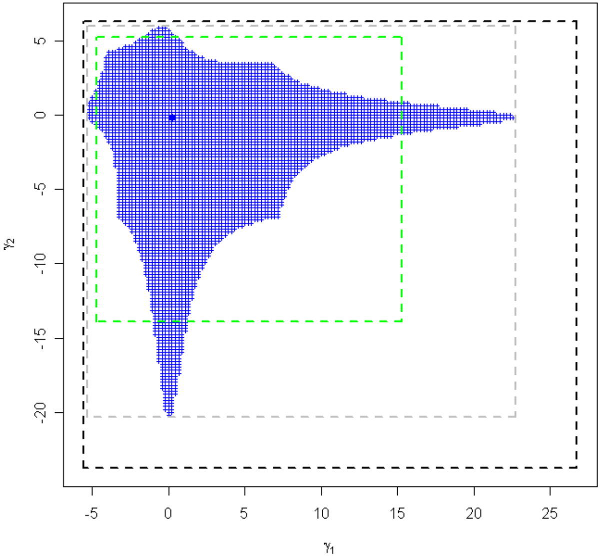
5
0
-5
-10
-15
-20
γ_2
-5
0
5
10
15
20
25
γ_1

Software

mratios: R extension package, CRAN: **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).

	Stand			**Test 1**			**Test 2**		
Animal	1/2500	1/500	1/100	1/2500	1/500	1/100	1/2500	1/500	1/100
1	36	52	64	45	40	65	33	44	70
2	41	48	62	38	42	65	36	57	63
3	44	48	100	45	62	57	33	54	78
4	48	52	59	40	42	70	37	61	70

- Model:

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

$i = 1, 2, 3, 4;\ j = 0, 1, 2; \quad k = 1, 2, 3$

- Model:

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

$i = 1, 2, 3, 4;\ j = 0, 1, 2; \quad k = 1, 2, 3$

- $\gamma_j = (\alpha_j - \alpha_0)/\beta, \quad j = 1, 2$

Two-sided 95% simultaneous CI:

Method	γ_1	γ_2
Bonferroni	(-0.593, 0.218)	(-0.481, 0.324)
Šidák	(-0.593, 0.216)	(-0.479, 0.322)
Plug-in	(-0.585, 0.211)	(-0.474, 0.317)

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- **Random** animal effects

Linear Mixed Model

Simultaneous CI for **ratios of fixed effect parameters** in LMM

$$\mathbf{Y}_i = \mathbf{X}_i\theta + \mathbf{Z}_i\mathbf{b}_i + \mathbf{e}_i,$$

- Asymptotically, $\widehat{\boldsymbol{\theta}}$ is normal and LC of this estimator,

$$U_j = (\mathbf{c}_j - \gamma_j\mathbf{d})'\widehat{\boldsymbol{\theta}}, \quad j = 1, \ldots, r$$

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

$$\sigma_{ij} = \mathrm{cov}(U_i, U_j) = (\mathbf{c}_i - \gamma_i\mathbf{d})'\mathrm{var}(\widehat{\boldsymbol{\theta}})(\mathbf{c}_j - \gamma_j\mathbf{d})'$$

- Estimate of $\mathrm{var}(\widehat{\boldsymbol{\theta}})$ can be extracted from the fitted LMM

- Plugging the MLEs of γ_is in $\boldsymbol{\Sigma}$, equi-coordinate percentage points can be computed for SCI estimations

- Simultaneous CI:

$$\frac{[\mathbf{c}_i - \gamma_i \mathbf{d}]^2}{(\mathbf{c}_i - \gamma_i \mathbf{d})' \widehat{\mathrm{var}(\widehat{\theta})} (\mathbf{c}_i - \gamma_i \mathbf{d})'} = C^2_{1-\alpha, \widehat{\boldsymbol{\Sigma}}}$$

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

Dilba, G., Bretz, F. and Guiard, V. (2006). Simultaneous confidence sets and confidence intervals for multiple ratios. *Journal of Statistical Planning and Inference* 136, 2640-2658.

Dilba, G. and Schaarschmidt, F. (2007). mratios: Inferences for ratios of coefficients in the general linear model. R package version 1.2.3.

Finney, D.J. (1978). *Statistical methods in biological assay*. 3rd ed. Macmillan, New York.

Hare, D.R. and Spurrier, J.D. (2007). Simultaneous inference for ratios of linear combinations of general linear model parameters. *Biometrical Journal* 49(4), in press.

Jensen, G.R. (1989). Joint confidence sets in multiple dilution assays. *Biometrical Journal* 31, 841-853.

Scheffé, H. (1970). Multiple testing versus multiple estimation, improper confidence sets, estimation of directions and ratios. *The Annals of Mathematical Statistics* 41, 1-29.

Young, D.A., Zerbe, G.O. and Hay, W.Jr. (1997). Fieller's Theorem, Scheffé's simultaneous confidence intervals, and ratios of parameters of linear and nonlinear mixed-effect models. *Biometrics* 53, 835-847.

Zerbe, G.O., Laska E., Meisner, M. and Kushner, H.B. (1982). On multivariate confidence regions and simultaneous confidence limits for ratios. *Communications in Statistics-Theory and Methods* 11, 2401-2425.