USING THE NLIN PROCEDURE TO COMBINE ESTIMATES OF RELATIVE POTENCY

S. Altan, The R.W.Johnson Pharmaceutical Research Institute
J. Natarajan, The R.W.Johnson Pharmaceutical Research Institute

Abstract

The estimation of relative potency from indirect biological assays, assuming parallel line model has been thoroughly discussed by Finney (1978). Finney has also given a method for combining estimates of relative potency when the same test preparation is evaluated over s (s>1) assays. Finney’s formula is not applicable when pooling estimates from k (k>1) replicates of the same test preparation from a single assay. In this case, the replicates are correlated due to a common standard. The current paper discusses two methods for finding the appropriate weights for the pooled estimate of relative potency in the case of correlated estimates. The first method uses the formulae for approximate standard errors of functions of random variables (Stuart & Ord, 1987) to obtain the covariance matrix, while the second method looks at a non-linear reparameterization of the model. A numerical example is also given using the NLIN procedure to produce all necessary parameter estimates.

1. Introduction

The estimation of relative potency from indirect biological assays, assuming a parallel line model has been thoroughly discussed by Finney (1978). The experimental design involves c (c>1) unknown test preparations, whose potency is to be compared against a known standard preparation. Each preparation is tested at d (d>2) specified doses, applied to n (n>1) subjects. The choice of doses depends on knowledge of the dose-response relation, ideally limited to the linear region. The estimation procedure given by Finney permits tests on the modelling assumptions, as well as biological considerations implicit in the conditions of similarity.

Finney has also given a method for combining estimates of relative potency when the same test preparation is evaluated over k (k>1) assays. The estimates of the relative potency from each of the k assays are pooled together to give a weighted estimate, m, with the weights equal to the inverse of the variance. The expression is defined by

\[ m = \sum_{i=1}^{k} \left( \frac{m_i}{\text{Var}(m_i)} \right) / \sum_{i=1}^{k} \frac{1}{\text{Var}(m_i)} \]  

(1.1)

where \( m_i \) = log relative potency estimate from the i-th assay, i=1,2,...,k,

\( \text{Var}(m_i) \) = variance of \( m_i \).

Tests for evaluating homogeneity of the relative potency estimates and variances are also given.

Formula 1.1 is not applicable when pooling estimates from k (k>1) replicates of the same test preparation from a single assay. In this case, the replicates are correlated due to a common standard. In the following sections, two methods are discussed for finding the appropriate weights for calculating the pooled estimate of relative potency in the case of correlated estimates. A straight-forward generalization of formula 1.1 is given for calculating the pooled estimate of relative potency. A numerical example is also given using the NLIN procedure to produce all necessary parameter estimates.

2. Estimation of relative potency from k replicates of a test preparation

Suppose k replicates \( T_1, T_2, \ldots, T_k \) of the same test preparation are assayed against a standard preparation, S. The relative potency of the test preparation can be estimated from the model given below, in which k+2 parameters are estimated from a single parallel line model.

Model:

\[ y_{ij} = \alpha_i + \beta x_j + \epsilon_{ij} \]  

for the standard, and

\[ y_{ij} = \alpha_i + \beta x_j + \epsilon_{ij} \]  

for replicate \( T_i, i=1,2, \ldots, k \), 

(2.1)
where

\[ y_{ij} \] is the response of the \( i \)-th subject with the \( j \)-th dose, using the standard preparation,

\[ y_{ij} \] is the response of the \( i \)-th subject with the \( j \)-th dose, from the \( i \)-th replicate of the test preparation,

\[ \alpha_i \] is the intercept for the \( i \)-th replicate, \( i = 1, 2, \ldots, k \),

\[ \beta \] is the common slope,

\[ \varepsilon_{ij} \] and \( \varepsilon_y \) are random error terms with the usual assumptions of independence, homogeneity and normality.

This model gives \( k \) estimates of log relative potency of the test preparation, which can be pooled to obtain a single point value. If \( \alpha_i, \alpha_r, \) and \( \beta \) are the estimates of \( \alpha_i, \alpha_r, \) and \( \beta \) respectively, then the estimated log relative potency of the \( i \)-th replicate is given by

\[ m_i = \frac{(\alpha_i - \alpha_r)}{\beta} \quad i = 1, 2, \ldots, k. \quad (2.2) \]

Due to the correlation between the estimates \( \alpha_i \) (\( i = 1, 2, \ldots, k \)), \( \alpha_r \), and \( \beta \), the estimates \( m_1, m_2, \ldots, m_k \) are also correlated. Suppose that the estimated covariance matrix of \( m_1, m_2, \ldots, m_k \) is given by \( \mathbf{W} \). Then the estimates \( m_1, m_2, \ldots, m_k \) can be pooled to give a weighted estimate of the log relative potency, namely,

\[ \bar{m} = (\mathbf{J}' \mathbf{W}^{-1} \mathbf{J})^{-1} \mathbf{J}' \mathbf{W}^{-1} (m_1, m_2, \ldots, m_k)' \quad (2.3) \]

with

\[ \text{Var}(\bar{m}) = (\mathbf{J}' \mathbf{W}^{-1} \mathbf{J})^{-1} \quad (2.4) \]

where \( \mathbf{J} \) is a \( k \times 1 \) vector of unities, the superscript \( t \) denotes the transpose of a matrix, and \( (m_1, m_2, \ldots, m_k) \) is a \( 1 \times k \) vector of the estimates of log relative potency of the \( k \) replicates. When the estimates are uncorrelated, as in the case of estimates obtained from several independent assays, expression (2.3) reduces to (1.1).

3. Using the NLIN procedure to obtain the estimated covariance matrix of the correlated estimates of log relative potency:

Assuming model (2.1), the estimated covariance matrix of the estimates of log relative potency can be obtained by either one of the following two methods.

i. Method 1 - Estimating the covariance matrix of non-linear functions of random variables

Following Stuart and Ord (1987), we approximate the estimate of the standard errors using a Taylor Series expansion of a function of random variables. Let \( \theta = (\alpha_1, \alpha_2, \ldots, \alpha_r, \beta)' \) be the \( (k+2) \times 1 \) vector of regression coefficients in model (2.1). Then

\[ \mu = H(\theta) = ((\alpha_1 - \alpha_r)/\beta, \ldots, (\alpha_k - \alpha_r)/\beta)' = (h_1(\theta), \ldots, h_k(\theta))' \]

is the \( k \times 1 \) vector of log relative potencies of replicates \( T_1, \ldots, T_k \). Let \( H'(\theta) \) be the \( k \times (k+1) \) matrix of partial first derivatives of \( H(\theta) \),

\[ H'(\theta) = (\delta h_1(\theta)/\delta \theta_1, \ldots, \delta h_k(\theta)/\delta \theta_1)' \]

If \( \hat{\theta} \) and \( \hat{\mu} = H(\hat{\theta}) \) are estimates of \( \theta \) and \( \mu \) respectively, then the covariance matrix of \( \hat{\mu} \) is approximately equal to

\[ \text{Var}(\hat{\mu}) = \sigma^2 H'(\hat{\theta}) \mathbf{S} (H'(\hat{\theta}))' \quad (3.1) \]

where \( \sigma^2 \mathbf{S} = \text{covariance matrix of } \hat{\theta} \). The proof can be found in Stuart & Ord (1987).

If \( \alpha_1, \alpha_2, \ldots, \alpha_r, \) and \( \beta \) are the least squares estimates of \( \alpha_1, \alpha_2, \ldots, \alpha_r, \) and \( \beta \) respectively, then \( \hat{\theta} = (\alpha_1, \alpha_2, \ldots, \alpha_r, \beta)' \) and \( \hat{\mu} = (m_1, m_2, \ldots, m_k)' \). Carrying out the matrix multiplication given in (3.1), we get the approximate formula for the variance of \( m_i \) to be

\[ w_i = \text{Var}(m_i) = (v_{\alpha} - 2m_i v_{\alpha^2} + m_i^2 v_{\alpha^4})/b^2 \quad (3.2) \]

where \( v_{\alpha} = \text{variance of } (\alpha_1 - \alpha_r), v_{\alpha^2} = \text{covariance } ((\alpha_1 - \alpha_r), \beta), \) and \( v_{\alpha^2} = \text{variance of } \beta \). The approximate formula for the covariance of \( m_i \) and \( m_j, i \neq j \) is

\[ w_{ij} = \text{Cov}(m_i, m_j) = (v_{\alpha} - m_i m_j v_{\alpha^2} + m_i m_j v_{\alpha^3})/b^2 \quad (3.3) \]

where \( v_i = \text{covariance } ((\alpha_1 - \alpha_r), (a_i - a_r)). \]

1392
The covariance matrix of $b$, $a_i$, and $a_i$, $i=1,2,...,k$ can be obtained using the GLM procedure, and simple manipulation of the matrix using the IML procedure yields the covariance matrix of the estimates of log relative potency. Further calculations to produce the pooled estimate as given in formula 2.2 can easily be carried out using the IML procedure code.

ii. Method 2 - Non-linear reparameterization of model 2.1

Since the true log relative potency of replicate $T_i$ is given by $M_i = (a_i - a_o)/b$, we can rewrite the model (2.1) as

$$y_{ij} = c_i + b \chi_j + \epsilon_{ij}$$ for the standard,

and

$$y_{ij} = c_i + b \chi_j + b M_i + \epsilon_{ij}$$ for replicate $T_i$, $i=1,2,...,k$, with parameters $c_i$, $b$, $M_i$, $i=1,2,...,k$. The normal equations for the above non-linear model are equivalent to the normal equations from the linear model (2.2). Hence, the solution to the normal equations (or the estimates of $c_i$, $b$, $M_i$, ..., $M_k$) are identical to those obtained from the linear model (2.2). The NLIN procedure can be used to obtain the estimates as well as the covariance matrix of the estimates $m_1$, ..., $m_k$ directly without further manipulations of the matrices as required in method 1. Computationally, this is an easier approach than Method 1 since all the necessary estimates are obtained in a single step.

4. Numerical Example

Three replicates $T_1$, $T_2$, and $T_3$ of a test preparation $T$ were assayed against a known standard preparation $S$. All preparations were assayed at doses 0.025u/ml, 0.50u/ml, and 0.10u/ml corresponding to previously determined linear region of the dose-response relationship. The sample size used was 10 animals per each dose-replicate (or standard) combination. In addition 10 animals were observed as the vehicle control group, yielding a total of 130 animals in the study.

Prior to performing regression analysis, descriptive measures for the data were obtained and seven animals were excluded from further analysis due to the following reasons: i. Six animals were excluded owing to biological considerations; ii. One animal was found to be a statistical outlier based on Dixon's test.

Method 1: Linear Modelling

As a first step in the regression analysis, the GLM procedure was used to test for the parallel line model (common slope). The interaction term of the model

$$\text{RESPONSE = TEST LDOSE LDOSE*TEST}$$

where LDOSE = Log (Base 2) of Dose and TEST is a CLASS variable with levels $S$, $T_1$, $T_2$, and $T_3$, was found to be not significant at a 10% level, indicating the appropriateness of the parallel line model. Next, model (2.1) was fit to the data using the REG procedure and the parameters and the estimated covariance matrix of $a_i$, $a_o$, $a_i$, $a_o$, and $b$ were obtained using the commands OUTEST and COVOUT. The IML procedure code was utilized to calculate the variance-covariance terms as per formulae (3.3) and (3.2). The estimated slope of the parallel line model was found to be 0.842 and the estimates of log (to the base 2) of the relative potencies of $T_1$, $T_2$, and $T_3$ were -0.0627, -0.1516, and 0.197 respectively. The covariance matrix was estimated to be

$$
\begin{bmatrix}
T_1 & T_2 & T_3 \\
T_1 & 0.0193 & 0.0093 & 0.0093 \\
T_2 & 0.0093 & 0.0194 & 0.0092 \\
T_3 & 0.0093 & 0.0092 & 0.0185
\end{bmatrix}
$$

The results from the linear models are given in Appendix A. The three estimates of log relative potency were combined using formula (2.3) and the IML procedure. The pooled estimate of log relative potency was found to be .00050 with an estimated variance of 0.0125.

Method 2: Non-linear reparameterization of the model

Using the NLIN procedure, model (3.4) was fit to the data as follows: initial values were selected as 5 for the intercept, 1 for the slope, and 1 for log relative potencies. Derivative statements were included and Gauss-Newton method (default when derivatives are included) was utilized to fit the model. The estimates of log (base 2) of the relative potencies of $T_1$, $T_2$, and $T_3$ and the estimated covariance matrix were found to be identical to those obtained by method 1. The results from the NLIN procedure are given in Appendix B.
5. Discussion

A non-linear reparameterization of the usual parallel line model is a convenient way to approach the problem of combining estimates of relative potency. The NLIN procedure can be used to produce the necessary parameter estimates, and the covariance matrix. An extension of Finney’s (1978) method for combining estimates is then easily carried out using the IML procedure code.

6. References


APPENDIX A

SAS
PARALLEL LINE METHOD SECTION
TABLE III.0.1 - GLM TEST OF PARALLELISM
GENERAL LINEAR MODELS PROCEDURE
CLASS LEVEL INFORMATION
CLASS LEVELS VALUES
TEST 4 STND T1 T2 T3
NUMBER OF OBSERVATIONS IN DATA SET = 123
NOTE: ALL DEPENDENT VARIABLES ARE CONSISTENT WITH RESPECT TO THE PRESENCE OR ABSENCE OF MISSING VALUES. HOWEVER, ONLY 113 OBSERVATIONS CAN BE USED IN THIS ANALYSIS.
DEPENDENT VARIABLE: LPeT

<table>
<thead>
<tr>
<th>SOURCE</th>
<th>DF</th>
<th>SUM OF SQUARES</th>
<th>MEAN SQUARE</th>
<th>F VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>MODEL</td>
<td>8</td>
<td>1788.25441179</td>
<td>223.53180147</td>
<td>1182.83</td>
</tr>
<tr>
<td>ERROR</td>
<td>105</td>
<td>19.84294922</td>
<td>0.18898047</td>
<td>PR &gt; F</td>
</tr>
<tr>
<td>UNCORRECTED TOTAL</td>
<td>113</td>
<td>1808.09736102</td>
<td></td>
<td>0.0</td>
</tr>
<tr>
<td>R-SQUARE C.V.</td>
<td>0.989026</td>
<td>11.1058</td>
<td>0.43471884</td>
<td>3.91432402</td>
</tr>
<tr>
<td>ROOT MSE</td>
<td>4.34183242</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LPET MEAN</td>
<td>3.91432402</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SOURCE</th>
<th>DF</th>
<th>TYPE III SS</th>
<th>F VALUE</th>
<th>PR &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEST</td>
<td>4</td>
<td>224.56079042</td>
<td>299.71</td>
<td>0.0001</td>
</tr>
<tr>
<td>LOOSE</td>
<td>1</td>
<td>54.62453262</td>
<td>289.05</td>
<td>0.0001</td>
</tr>
<tr>
<td>LOOSE*TEST</td>
<td>3</td>
<td>0.81473107</td>
<td>1.44</td>
<td>0.2362</td>
</tr>
</tbody>
</table>


Authors:

Stan Altan
Preclinical Biostatistics
RWJ-PRI
Raritan, NJ 08869
908-704-4083 (Tel)

Jaya Natarajan
Preclinical Biostatistics
RWJ-PRI
Raritan, NJ, 08869
908-704-4652 (Tel)
PARAMETER ESTIMATES

| VARIABLE | DF | PARAMETER ESTIMATE | STANDARD ERROR | T FOR HO: PARAMETER=0 | PROB > |T| |
|-----------|----|--------------------|----------------|-----------------------|--------|---|
| T1        | 1  | 7.56034200         | 0.23196133     | 32.593                | 0.0001 |
| T2        | 1  | 7.50755685         | 0.22968943     | 32.686                | 0.0001 |
| T3        | 1  | 7.43271164         | 0.23313087     | 31.882                | 0.0001 |
| T4        | 1  | 7.72601975         | 0.22987340     | 35.610                | 0.0001 |
| LOOSE     | 1  | 0.84209782         | 0.04987580     | 16.884                | 0.0001 |

ESTIMATES OF LOG RELATIVE POTENCY FROM LINEAR REGRESSION MODEL

<table>
<thead>
<tr>
<th>TEST</th>
<th>ESTIMATED LOG RELATIVE POTENCY</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>-0.06268</td>
</tr>
<tr>
<td>T2</td>
<td>-0.15156</td>
</tr>
<tr>
<td>T3</td>
<td>0.19675</td>
</tr>
</tbody>
</table>

ESTIMATED COVARIANCE MATRIX OF LOG RELATIVE POTENCY FROM LINEAR REGRESSION

<table>
<thead>
<tr>
<th>TEST</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>0.0193</td>
<td>0.0092966</td>
<td>0.0093085</td>
</tr>
<tr>
<td>T2</td>
<td>0.0092966</td>
<td>0.0194</td>
<td>0.0091763</td>
</tr>
<tr>
<td>T3</td>
<td>0.0093085</td>
<td>0.0091763</td>
<td>0.0185</td>
</tr>
</tbody>
</table>

APPENDIX B

NON-LINEAR LEAST SQUARES ITERATIVE PHASE

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>ESTIMATE</th>
<th>ASYMPTOTIC STD. ERROR</th>
<th>ASYMPTOTIC 95% CONFIDENCE INTERVAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>7.560342E+00</td>
<td>2.319613E-01</td>
<td>[7.100552E+00, 7.100552E+00]</td>
</tr>
<tr>
<td>B1</td>
<td>8.42088E+00</td>
<td>4.987580E-02</td>
<td>[7.432247E+00, 7.432247E+00]</td>
</tr>
<tr>
<td>R2</td>
<td>-6.26837E-02</td>
<td>1.38895E-03</td>
<td>[-3.380002E-01, -3.380002E-01]</td>
</tr>
<tr>
<td>R3</td>
<td>-1.51664E+00</td>
<td>1.39190E-03</td>
<td>[-4.27427E+00, -4.27427E+00]</td>
</tr>
<tr>
<td>R4</td>
<td>1.96748E-01</td>
<td>1.35554E-03</td>
<td>[-7.87157E-02, -7.87157E-02]</td>
</tr>
</tbody>
</table>
## ESTIMATES OF LOG RELATIVE POTENCY FROM NON-LINEAR REGRESSION MODELLING

<table>
<thead>
<tr>
<th>Intercept for std slope</th>
<th>Common Slope</th>
<th>M1</th>
<th>M2</th>
<th>M3</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.56034</td>
<td>0.842088</td>
<td>-0.062684</td>
<td>-0.15156</td>
<td>0.196746</td>
</tr>
</tbody>
</table>

## ESTIMATED COVARIANCE MATRIX OF LOG RELATIVE POTENCIES FROM NON-LINEAR REGRESSION MODELLING

<table>
<thead>
<tr>
<th></th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>0.0192919</td>
<td>0.0092966</td>
<td>0.0093085</td>
</tr>
<tr>
<td>T2</td>
<td>0.0092966</td>
<td>0.0193750</td>
<td>0.0091763</td>
</tr>
<tr>
<td>T3</td>
<td>0.0093085</td>
<td>0.0091763</td>
<td>0.0184802</td>
</tr>
</tbody>
</table>