SLOPE RATIO BIOASSAY USING SAS

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ABSTRACT

Slope ratio bioassay is considered, leading to the development of a SAS macro which produces an appropriate statistical analysis. In contrast to less flexible approaches, the routine presented here handles a rather general case which allows for the use of either applied (i.e., pre-specified) dosages or measured dosage intake as the dose metamer and, in addition, accepts any number of test preparation groups. Although the analysis can be obtained through multiple use of GLM augmented by further hand computations, a more convenient approach is via the MATRIX procedure. The analysis technique is discussed and illustrated with results that include estimates of relative potency, appropriate variance estimates and fiducial limits, and a detailed analysis of variance with the associated tests of hypotheses.

1. INTRODUCTION

The subject of biological dilution assays has a long history and the associated statistical analyses are rather well-defined. Finney (1978), for example, in his comprehensive work has treated many methods in detail and has set out the computational algorithms required. Of concern here is that area dealing with slope ratio bioassay and the description of a SAS macro to handle the statistical analysis of a somewhat general case.

Generally, the purposes of a slope ratio assay include estimation of relative potency of a test preparation with respect to a standard preparation, assessment of precision in the form of fiducial limits, and testing for fundamental validity. In a multiple assay, several test preparations are incorporated simultaneously with a single standard preparation.

In contrast to the standard procedure of using applied (pre-specified) dosages to form the dose metamer, Hegsted and Worcester (1966) utilized the actual measured values of uptake in a study to assess relative nutritive value of proteins, thus generalizing to some degree the assay procedure. Regest, et al., (1968) reported the development of a computer program which would provide the statistical analysis of such an assay.

The SAS MATRIX procedure provides a convenient means for developing a macro in which each portion of the analysis may be easily obtained by utilizing simple linear models. The routine presented here will provide the statistical analysis of a slope ratio assay with any number of test preparations and for either the applied dosage (symmetric or asymmetric design) or measured uptake situation, with or without zero-dose data.

2. SLOPE RATIO ASSAY - GENERAL

Finney (1978) discussed the concept of potency of a test preparation relative to a standard preparation as the ratio of those doses of the two which have the same expected response. In his notation, an arbitrary dose-response relation is represented by \( U = E(u|z) = F(z) \) where \( z \) is the dose variable and \( u \) is the observed response variable. The potency ratio is required to be independent of \( U \) so that \( F_T(z) = F_S(pz) \) for all \( z \), where \( F_T \) and \( F_S \) are the dose-response relations for the test and standard preparations, respectively (called the "condition of similarity").

With the intent of achieving a linear dose-response relation, the response metameter is defined as \( Y = f^{-1}(U) \), where \( f \) represents a specified function (usually known from prior knowledge) possessing a single-valued inverse over the dosage range. In addition, the family of transformations due to Box and Cox (1964) is utilized to provide the dose metameter

\[
X = \begin{cases} z^\lambda, & \lambda \neq 0 \\ \log z, & \lambda = 0 \end{cases}
\]

where \( \lambda \) is a predetermined quantity. (The case of \( \lambda = 0 \) produces parallel line assay whereas \( \lambda \neq 0 \) yields slope ratio assay.) In the simplest application, no transformation of either variable is required to establish linearity.

For the case \( \lambda \neq 0 \), the relations between the response metameter and the dose metameter for the standard preparation and the test preparation are, respectively,

\[
Y_S = \alpha + \beta_S x = \alpha + \beta_S z^\lambda \\
Y_T = \alpha + \beta_T x = \alpha + \beta_T z^\lambda
\]

Thus, the similarity condition implies that

\[
p = \left( \frac{\beta_T}{\beta_S} \right)^{1/\lambda}
\]

We consider only the case where \( \lambda = 1 \). The estimate \( R \) of relative potency is obtained by fitting a multiple regression model of the form

\[
E(y) = \alpha + \beta_S X_S + \beta_T X_T
\]

and then computing \( R = \beta_T / \beta_S \). Fiducial limits on \( \alpha \) are then obtained by utilizing Fieller's
theorem. Generalization to the case of more than one test preparation is straightforward.

Assessment of the validity of an assay is performed by partitioning the sum of squares among dose classes and testing relevant hypotheses. We may subdivide SS(Doses) into parts - that due to the linear regression, SS(Regr), and that due to deviations from the linear regression, SS(Dev from regr). The latter provides a composite test of linearity and common intersection for the regression lines. SS(Dev from regr) is further subdivided into components due to "Blanks," "Intersection," and "Curvature." "Blanks" provides a test of whether the common intersection point differs from the value obtained using zero-dose data. "Intersection" is used to test for a common intersection point among all preparation lines. "Curvature" yields a test for departure from linearity. A significant result in any of these cases is evidence of invalidity.

Such an orthogonal decomposition is achievable only when the observations are classified into dose classes and the dose meteraters are constant within each such group. If some form of measured uptake is used to form the doses (e.g., amount of dietary substance consumed), a similar analysis may be computed by utilizing quadratic terms (see references).

Details of the computational steps are given in the following section.

3. COMPUTATIONAL METHODS

The estimation of relative potency follows immediately from the fitting of the multiple regression model

$$E(y) = \alpha + \beta_1 x_1 + \beta_2 x_2 + \ldots + \beta_k x_k$$

where the subscript s refers to the standard preparation and 1, 2, ..., k refer to k test preparations. There is, of course, a relative potency associated with each of the test preparations, so that the i-th estimate $R_i = \beta_i/\beta_s$. (In the case where "Blanks" is significant, Finney suggests computing the potency estimates on the basis of data with blanks (i.e., zero-dose data) excluded; both types of estimates are included in the output.)

Fieller's theorem (c.f., Finney, 1978), is used to provide fiducial limits:

$$R_L, R_U (i) = \frac{\hat{b}_s}{\hat{b}_s} \left( V_{ii} - 2R_i V_{ii} + \frac{1}{\text{MSE}} \right)$$

$$R_L^2 V_{ss} - g(V_{ii} - V_{ii}^2)(1/2) (1-g)^{-1},$$

where $g = t^2(\text{MSE}) V_{ss}/\hat{b}_s^2, t = t_{c, 1-1/2, \text{MSE}}$.

The calculations for the regression model above, as well as for the various other models used to obtain the analysis of variance sums of squares, are carried out directly with the MATRIX procedure as in the general linear model of full rank. In particular, given $E(y) = X\beta$ where $X$ is a design matrix and $\beta$ a parameter vector, the least squares solution is simply $\hat{\beta} = (X'X)^{-1}X'y$. The reduction in sum of squares due to fitting the model is $SSR = y'X\hat{\beta} - y'\bar{y}$ and the residual sum of squares is $SSE = y'y - y'X\hat{\beta}$.

When using applied dosages, the analysis of variance is constructed as in Table 1. The "Among doses" sum of squares is formed by using as grounds each of the preparation-dosage (preparation-dose) levels, there being one dose for blanks, $n_s$ doses for the standard preparation, and $n_i$ doses for the i-th test group. By fitting the regression model (1), SS(Doses) is decomposed into that due to "Regression" and that due to "Deviations from regression." SS(Blanks) may be obtained as the additional reduction over SS(Regr) obtained from fitting the model

$$E(y) = \alpha + \beta_1 x_1 + \beta_2 x_2 + \ldots + \beta_k x_k$$

where $x_0$ takes the value unity if the observation is a blank, and zero otherwise. SS(Regression) is the sum over all preparations of the sum of squares of deviations from the simple linear model $E(y) = \alpha + \beta x$. For any particular preparation $j$, its term may be computed as $SSR_j(y = \text{DOSE}) - SSR_j(y = \alpha + \beta x)$. SS(Intersection) is then the remaining portion of the "Deviations from regression" sum of squares.

Table 1. APPLIED DOSAGES ANALYSIS

<table>
<thead>
<tr>
<th>Source</th>
<th>D.F.</th>
<th>Sum of Squares</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among Doses</td>
<td>m-1</td>
<td>SSR(y = DOSE)</td>
</tr>
<tr>
<td>Regression</td>
<td>k+1</td>
<td>SSR1</td>
</tr>
<tr>
<td>Day from regression</td>
<td>m-k-2</td>
<td>SSR(Doses) - SSR1</td>
</tr>
<tr>
<td>Blanks</td>
<td>1</td>
<td>SSR2 - SSR1</td>
</tr>
<tr>
<td>Intersection</td>
<td>k</td>
<td>SSR(Dev from regr) - SSR(Blanks) - SSR(Curvature)</td>
</tr>
<tr>
<td>Curvature</td>
<td>m-2k-3</td>
<td>$\sum SSR_j(y = \text{DOSE}) - SSR_j(y = \alpha + \beta x)$</td>
</tr>
<tr>
<td>Residual</td>
<td>N-m</td>
<td>SS(Total) - SS(Doses)</td>
</tr>
<tr>
<td>Total</td>
<td>N-1</td>
<td>$\sum (y - \bar{y})^2$</td>
</tr>
</tbody>
</table>

$k$ = number of test preparations

$m = 1 + n_1 + n_2 + \ldots + n_k = \text{total number of doses}$

$SSR_1 = SSR(y = \alpha + \beta_1 x_1 + \beta_2 x_2 + \ldots)$

$SSR_2 = SSR(y = \alpha + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \ldots)$
If dosage represents a measured, variable quantity, such as intake, and there is no dosage classification criterion, the sum of squares for doses is not computed. SS(Regr) is computed as above; so is SS(Blanks). Following Hegsted and Worcester, SS(Curvature) is restricted to the additional reduction obtained by fitting a quadratic polynomial rather than a linear one for each preparation, see Table 2. Although higher degree models could be fitted, the information regarding invalidity gained through the use of a quadratic term should be adequate. The residual sum of squares is computed as the sum over all preparations of the residual sum of squares associated with fitting quadratic polynomials to each preparation individually, plus the sum of squares of deviations of the blank responses from the blank mean.

Table 2. MEASURED INTAKE ANALYSIS

<table>
<thead>
<tr>
<th>Source</th>
<th>D.F.</th>
<th>Sum of Squares</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
<td>k+1</td>
<td>SSR1</td>
</tr>
<tr>
<td>Dev from regression</td>
<td>2k+2</td>
<td>SS(Total) - SS(Residual) - SSR1</td>
</tr>
<tr>
<td>Blanks</td>
<td>1</td>
<td>SSR2 - SSR1</td>
</tr>
<tr>
<td>Interaction</td>
<td>k</td>
<td>SS(Dev from reg) - SS(Blanks) - SS(Curvature)</td>
</tr>
<tr>
<td>Curvature</td>
<td>k+1</td>
<td>(\sum (\text{SSR}(y_1 + \beta_1 + \beta_2 x_1 + \beta_3 x_2)))</td>
</tr>
<tr>
<td>Residual</td>
<td>N-3k-4</td>
<td>(\sum (y_i - \hat{y})^2 + \sum \text{SSR}(y_1 + \beta_1 + \beta_2 x_1 + \beta_3 x_2))</td>
</tr>
<tr>
<td>Total</td>
<td>N-1</td>
<td>(\sum (y_i - \hat{y})^2)</td>
</tr>
</tbody>
</table>

4. EXAMPLE AND PROGRAM USAGE

The input data upon which the slope ratio assay analysis is to be performed is assumed to be the most recently created data set. Each observation must consist of four variables in order as follows.

The first character variable specifies the preparation group. For blank observations, its value must be either 'B' or 'BLANK'; for standard preparation observations, its value must be either 'S', 'STD', or 'STANDARD'. For test preparations, any identifying values, exclusive of the above, are permitted. Observations for the standard preparation and at least one test preparation are required.

The first numeric variable specifies the dose class. If there are no such classes, any values (e.g., missing) may be used as long as they are constant within each preparation group.

The second numeric variable specifies the dose meter value. For the applied dosage situation, these values must be constant within prep-dose classes. The case of measured intake is recognized by inequality of these values within prep-dose classes.

The third numeric variable specifies the response metameter value.

The observations are sorted within the macro program, eliminating the need for sorting prior to entry. On the output, 'TEST 1' refers to the first occurring test preparation of the input data set, 'TEST 2' the next, and so on.

As an illustration of the SAS macro as applied to a typical slope ratio assay, consider the data in Figure 1. The input data are measures of hemoglobin gain (Y) by anemic rats in response to iron intake (X) for a standard and two test preparation diets. Each diet consisted of three dosage levels with six animals being randomly assigned to each of the nine dietary groups. In addition, a group of six animals received a diet containing no iron. The animals were housed individually with food and deionized water being provided ad libitum. Since the amount of iron intake per animal was computed as a function of the amount of food each animal consumed, iron intake varied between animals within each dietary group. Therefore, this represents a case of measured intake with dose classes specified.

The macro program, SRASSAY, produces the output given in Figures 2, 3, and 4. Figure 2 has two sets of values showing, for each test preparation relative potency estimates \(R\), 95% fiducial limits on \(R\), and standard errors of the estimates. The potency estimates and fiducial limits in the first set are obtained by fitting model (1) to the non-blank data only. The second set of values is derived from fitting (1) to the complete data set.

Fitting model (2) to all data produces the same coefficient estimates as does fitting model (1) to the data with blanks excluded. Figure 3 displays the multiple regression coefficient estimates and their standard errors which result from fitting each of the linear models (1) and (2) to the complete data set. For the first set of estimates, it should be noted that the standard errors of the regression coefficients differ from those which would be obtained using model (1) on the non-blank data only. The potency estimates are ratios of the corresponding regression coefficients.

The analysis of variance presented in Figure 4 permits assessment of fundamental validity of the assay. Interpretation of these tests is in accord with those described by Finney (1978). The "Among doses" sum of squares is calculated using the dose classes given in Figure 1.

Since in the case of measured intake this sum of squares is not partitioned, the associated residual is calculated separately. The single and double asterisks denote significance at the .05 and .01 levels, respectively; these are added only for the "deviations" tests. When there are no blanks or no dose classes, the table is modified accordingly.
Figure 1.

<table>
<thead>
<tr>
<th>PREP</th>
<th>DOSE</th>
<th>X</th>
<th>Y</th>
<th>PREP</th>
<th>DOSE</th>
<th>X</th>
<th>Y</th>
</tr>
</thead>
<tbody>
<tr>
<td>BLANK</td>
<td>0.000</td>
<td>0.445</td>
<td>1.172</td>
<td>0.830</td>
<td>BLANK</td>
<td>0.000</td>
<td>-0.084</td>
</tr>
<tr>
<td>BLANK</td>
<td>0.000</td>
<td>0.270</td>
<td>1.116</td>
<td>0.649</td>
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<td>0.000</td>
<td>0.263</td>
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<tr>
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<td>0.000</td>
<td>0.533</td>
<td>1.836</td>
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<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>STD 1</td>
<td>0.822</td>
<td>0.680</td>
<td>1.183</td>
<td>0.604</td>
<td>STD 1</td>
<td>0.828</td>
<td>0.281</td>
</tr>
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<td>0.822</td>
<td>0.680</td>
<td>1.183</td>
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<td>0.604</td>
<td>STD 1</td>
<td>0.828</td>
<td>0.281</td>
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<tr>
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<td>0.822</td>
<td>0.680</td>
<td>1.183</td>
<td>0.604</td>
<td>STD 1</td>
<td>0.828</td>
<td>0.281</td>
</tr>
</tbody>
</table>

Figure 2.

**STATISTICAL ANALYSIS SYSTEM**

**SLOPE RATIO ANALYSIS**

**POWER VS. SLOPE INTERVAL**

<table>
<thead>
<tr>
<th>TEST</th>
<th>PREPARATION</th>
<th>R</th>
<th>LOWER LIMIT</th>
<th>UPPER LIMIT</th>
<th>STANDARD ERROR OF R</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.8071450</td>
<td>0.7800907</td>
<td>0.8080890</td>
<td>0.0009090</td>
<td>0.0000909</td>
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<tr>
<td>2</td>
<td>0.9071450</td>
<td>0.8800907</td>
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<td>0.0000909</td>
</tr>
</tbody>
</table>

**NOTE:** ESTIMATES ABOVE ARE COMPUTED ON THE BASIS OF DATA EXCLUDING BLANKS

<table>
<thead>
<tr>
<th>TEST</th>
<th>PREPARATION</th>
<th>R</th>
<th>LOWER LIMIT</th>
<th>UPPER LIMIT</th>
<th>STANDARD ERROR OF R</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.7971450</td>
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<tr>
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<td>0.8600907</td>
<td>0.9080890</td>
<td>0.0009090</td>
<td>0.0000909</td>
</tr>
</tbody>
</table>

**NOTE:** ESTIMATES ABOVE ARE COMPUTED ON THE BASIS OF DATA EXCLUDING BLANKS

Figure 3.

**STATISTICAL ANALYSIS SYSTEM**

**SLOPE RATIO ANALYSIS**

**MULTIPLE REGRESSION COEFFICIENT ESTIMATES**

<table>
<thead>
<tr>
<th>COEFFICIENT</th>
<th>ESTIMATE</th>
<th>STANDARD ERROR</th>
</tr>
</thead>
<tbody>
<tr>
<td>INTERCEPT</td>
<td>-0.0474208</td>
<td>0.0000644</td>
</tr>
<tr>
<td>BLANK</td>
<td>0.0020260</td>
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</tr>
<tr>
<td>STANDARD</td>
<td>0.0020260</td>
<td>0.0000644</td>
</tr>
<tr>
<td>TEST 1</td>
<td>0.0476287</td>
<td>0.0000644</td>
</tr>
<tr>
<td>TEST 2</td>
<td>0.0476287</td>
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**NOTE:** ESTIMATES ABOVE ARE COMPUTED ON THE BASIS OF DATA EXCLUDING BLANKS

<table>
<thead>
<tr>
<th>COEFFICIENT</th>
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<tr>
<td>BLANK</td>
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<tr>
<td>TEST 1</td>
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<td>0.0000644</td>
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<tr>
<td>TEST 2</td>
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<td>0.0000644</td>
</tr>
</tbody>
</table>

**NOTE:** ESTIMATES ABOVE ARE COMPUTED ON THE BASIS OF DATA EXCLUDING BLANKS

Figure 4.

**STATISTICAL ANALYSIS SYSTEM**

**SLOPE RATIO ANALYSIS**

**ANALYSIS OF VARIANCE - SLOPE RATIO TEST**

<table>
<thead>
<tr>
<th>SOURCE OF VARIATION</th>
<th>DF</th>
<th>SUM OF SQUARES</th>
<th>MEAN SQUARE</th>
<th>F</th>
<th>P VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERROR</td>
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<td>1.07472</td>
<td>0.13793</td>
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<td>0.0000</td>
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<td>STANDARDIZE</td>
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<td>1.0000</td>
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<td>0.00000</td>
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<td>1.0000</td>
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</tr>
</tbody>
</table>

**NOTE:** SLOPE RATIO IS BASED ON SLOPE ESTIMATES AND SLOPE INTERVALS

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**REFERENCES**


