# Simplified Computation of Confidence Intervals for Relative Potencies Using Fieller's Theorem

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**Confidence intervals for relative potencies in bioassays are usually calculated by using Fieller's theorem, but the procedures presented in standard texts are computationally cumbersome. It is shown that Fieller's formula can be expressed in an alternative form which takes advantage of calculated quantities from the analysis of variance (ANOV) and thus simplifies computations. Slope ratio assays and parallel line assays are discussed, and 2 examples illustrate the use of the proposed alternatives.**

Confidence intervals for relative potencies in bioassays based on normally distributed responses are calculated from the formula derived by Fieller (1) which, for present purposes, is restated as follows. Suppose that the ratio estimate of $\rho = \mu/\gamma$ is

$$R = u/v \qquad \text{(Eq. 1)}$$

where $u$, the unbiased estimate of $\mu$, and $v$, the unbiased estimate of $\gamma$, are linear combinations of variates which are normally distributed with variance $\sigma^2$. Suppose also that the variance and covariance estimates are

$$V(u) = a_u s^2, \quad V(v) = a_v s^2, \quad CV(u, v) = a_{uv} s^2 \qquad \text{(Eq. 2)}$$

where $s^2$, with $f$ degrees of freedom, is the unbiased estimate of $\sigma^2$, and $a_u$, $a_v$, and $a_{uv}$ are known constants depending on the construction of $u$ and $v$. The usual derivation of Fieller's theorem leads to $R_L$ and $R_U$, the lower and upper 100 $(1 - \alpha)\%$ confidence limits on $\rho$, as

$$R_L, R_U = \frac{Rv^2 - s^2F_c a_{uv}}{v^2 - s^2F_c a_v} \mp \frac{\sqrt{s^2F_c\{a_u v^2 - 2a_{uv}uv + a_v u^2 - s^2F_c(a_u a_v - a_{uv}^2)\}}}{v^2 - s^2F_c a_v} \qquad \text{(Eq. 3)}$$

where $F_c$, the $100(1 - \alpha)\%$ tabulated critical value from the $F$-distribution, with 1 and $f$ degrees of freedom, has replaced $t^2$ in the usual formulation.

Users (2) know that, as usually presented, the formula is computationally cumbersome, and it will be shown that the formula can be thrown into a simpler alternative form. The beneficial results for slope ratio and, particularly, parallel line assays are exemplified.

## SLOPE RATIO ASSAYS WITH ONE TEST PREPARATION

For slope ratio assays with responses at the zero-dose level and at doses $x_{Si}$ and $x_{Tj}$, $i = 1, 2, \ldots, k_1$ and $j = 1, 2, \ldots, k_2$, for the standard and test preparations, respectively, the relative potency estimate, $R$, is obtained as

$$R = b_T/b_S \qquad \text{(Eq. 4)}$$

where $b_S$ and $b_T$ are the estimated slopes of the $(x, y)$ dose-response lines for the standard and test preparations, respectively. The 2 slopes are given by

$$b_S = \frac{1}{\Delta}\{(\Sigma' x_T^2)(\Sigma' x_S y) - (\Sigma' x_S x_T)(\Sigma' x_T y)\}$$

$$b_T = \frac{1}{\Delta}\{-(\Sigma' x_S x_T)(\Sigma' x_S y) + (\Sigma' x_S^2)(\Sigma' x_T y)\} \qquad \text{(Eq. 5)}$$

where, if $\Sigma$ denotes summation over all the observations for each preparation, $\Sigma'$ denotes "corrected" summation so that the quantities in Eq. 5 are obtained as follows:

$$\Sigma' x_S^2 = \Sigma x_{Si}^2 - \frac{(\Sigma x_{Si})^2}{N}, \quad \Sigma' x_T^2 = \Sigma x_{Tj}^2 - \frac{(\Sigma x_{Tj})^2}{N}, \quad \Sigma' x_S x_T = \frac{-(\Sigma x_{Si})(\Sigma x_{Tj})}{N} \qquad \text{(Eq. 6)}$$

$$\Sigma' x_S y = \Sigma x_{Si} y_{Si} - \frac{G(\Sigma x_{Si})}{N}, \quad \Sigma' x_T y = \Sigma x_{Tj} y_{Tj} - \frac{G}{N}(\Sigma x_{Tj}) \qquad \text{(Eq. 7)}$$

in which $N$ is the total number of observations, $G$ is the grand total of all the responses, and

$$\Delta = (\Sigma' x_S^2)(\Sigma' x_T^2) - (\Sigma' x_S x_T)^2 \qquad \text{(Eq. 8)}$$

Hence, by comparison with Eqs. 1 and 2

$$u = b_T, \quad v = b_S \qquad \text{(Eq. 9)}$$

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and Finney (3) shows that

$$a_u = \Sigma' x_S^2/\Delta, \quad a_v = \Sigma' x_T^2/\Delta, \quad a_{uv} = -\Sigma' x_S x_T/\Delta \quad \text{(Eq. 10)}$$

If now quantities $F_r$ and $F_S$ are defined as

$$2F_r = (\text{regressions sum of squares})/s^2 \quad \text{(Eq. 11)}$$

$$F_S = b_S^2/a_v s^2 \quad \text{(Eq. 12)}$$

then it is shown in the *Appendix* that the confidence interval on the ratio, $\rho$, can be expressed as

$$R_L, R_U = \frac{RF_S - F_c(a_{uv}/a_v) \mp \sqrt{F_c(2F_r - F_c)/\Delta a_v^2}}{(F_S - F_c)} \quad \text{(Eq. 13)}$$

Using Eq. 10, Eq. 13 can be put into the alternative form

$$R_L, R_U = \frac{R(\Delta b_S^2/s^2) + F_c(\Sigma' x_S x_T) \mp \sqrt{F_c(2F_r - F_c)\Delta}}{(\Delta b_S^2/s^2) - F_c(\Sigma' x_T^2)} \quad \text{(Eq. 14)}$$

With $\Delta$ from Eq. 8, the alternatives given in Eqs. 13 and 14 are already more convenient than the usual form in the general case described. Further advantages occur in applications to the common symmetrical assays for which explicit values of $a_u$, $a_v$, and $a_{uv}$ are available.

Thus, for the symmetrical 5-point assay with a zero dose and coded doses of 0.5 and 1 for each of the standard and test preparations,

$$\Sigma' x_S^2 = \Sigma' x_T^2 = \frac{16N}{100} \text{ and } \Sigma' x_S x_T = \frac{-9N}{100} \quad \text{(Eq. 15)}$$

so that, from Eqs. 8 and 10,

$$\Delta = 175N^2/10^4, \; a_u = a_v = 64/7N, \; a_{uv} = 36/7N \quad \text{(Eq. 16)}$$

Substitution in Eq. 13 leads to the very simple expression of the confidence limits as

$$R_L, R_U = \frac{16F_S R - 9F_c \mp \sqrt{175F_c(2F_r - F_c)}}{16(F_S - F_c)} \quad \text{(Eq. 17)}$$

where, from Eq. 12,

$$F_S = 7Nb_S^2/64s^2 \quad \text{(Eq. 18)}$$

Taking $175/256 \doteq 0.6836$, Eq. 17 becomes

$$R_L, R_U = \frac{F_S R - (0.5625)F_c \mp \sqrt{(0.6836)F_c(2F_r - F_c)}}{(F_S - F_c)} \quad \text{(Eq. 19)}$$

In microbiological assays the quantity $F_c/F_S$, which is $g$ as used by Finney (3), is often small enough to be neglected. In this case Eqs. 17 and 19 reduce to the very convenient approximate form

$$R_L, R_U = R \mp \frac{20}{Nb_S^2} \sqrt{F_c(\text{regressions sum of squares})s^2/7} \quad \text{(Eq. 20)}$$

**Numerical Example.**—Values from the assay by Wood (4), analyzed in Finney (3), will be used to illustrate the computational procedures. The numerical quantities required are

$$N = 20, \quad b_S = 118.629, \quad R = 0.6847, \quad s^2 = 14.43 \text{ (15 d.f.)}$$

From the ANOV, the regressions sum of squares is 31456.9, while, for 95% confidence limits, $F_c = F_{1,15}(0.05) = 4.54$ from standard tables. Since $F_S$ from Eq. 18 will plainly be large relative to $F_c$, the approximate formula can safely be used in this case. Hence, from Eq. 20

$$R_L, R_U = 0.6847 \mp \frac{1}{(118.629)^2} \sqrt{(4.54)(31456.9)(2.06)} = 0.6462, 0.7232$$

which compare well with the accurate results that are obtained below and given in Finney (3). To obtain the accurate values, $2F_r$ and $F_S$ are first computed as

$$2F_r = 31456.9/14.43 = 2179.9653$$

and, from Eq. 18,

$$F_S = (7)(20)(118.629)^2/(64)(14.43) = 2133.3566$$

so that, from Eq. 19,

$$R_L, R_U = \frac{1}{2128.8166}[(2133.3566)(0.6847) - (0.5625)(4.54) \mp \sqrt{(0.6836)(4.54)(2175.4253)}] = 0.6464, 0.7236$$

## PARALLEL LINE ASSAYS

The logarithm of the relative potency estimate in parallel line assays is obtained by Finney (3) as $M$ where

$$M = \bar{x}_S - \bar{x}_T - \frac{(\bar{y}_S - \bar{y}_T)}{b} \quad \text{(Eq. 21)}$$

in which $\bar{x}_S$ and $\bar{x}_T$ are the mean log-doses, $\bar{y}_S$ and $\bar{y}_T$ are the mean responses for the standard and test preparations, respectively, and $b$ is the estimated common slope of the log-dose, response lines. Since $\bar{x}_S$ and $\bar{x}_T$ are taken as fixed, the confidence interval is therefore obtained for the ratio quantity

$$M - \bar{x}_S + \bar{x}_T = -(\bar{y}_S - \bar{y}_T)/b \quad \text{(Eq. 22)}$$

Here the numerator and denominator on the right hand side are statistically independent so that, from Eq. 2, $a_{uv} = 0$. It is shown in the *Appendix* that the confidence limits can be represented as

$$R_L, R_U = \frac{1}{(F_r - F_c)} \left\{ RF_r \mp \sqrt{\frac{a_p}{a_r} F_c(F_p + F_r - F_c)} \right\} \quad \text{(Eq. 23)}$$

where

$$a_p = \left(\frac{1}{n_S} + \frac{1}{n_T}\right), \; a_r = \left\{ \sum_S (x - \bar{x})^2 + \sum_T (x - \bar{x})^2 \right\}^{-1} \quad \text{(Eq. 24)}$$

and

$F_p$ = the F-ratio for preparations in the ANOV
$F_r$ = the F-ratio for regression in the ANOV
(Eq. 25)

and $n_S$ and $n_T$ are the total number of responses to the standard and test preparations, respectively.

Some simplification occurs if $F_c/F_r$ is small enough to be neglected but, in practice, the gain is so slight that the use of Eq. 23 may be generally recommended. Simplified forms can, however, be presented for the common, balanced 4- and 6-point parallel assays using explicit values for $a_p$ and $a_r$ as follows.

**Four-Point Parallel Line Assay.**—If $S_1$, $S_2$, are the total responses to the lower and upper doses of the standard preparation, respectively, and $T_1$ and $T_2$ are the corresponding response totals for the test preparation, and linear contrasts $L_p$ and $L_r$ are defined as

$$L_p = -(S_1 + S_2) + (T_1 + T_2)$$
$$L_r = -(S_1 + T_1) + (S_2 + T_2) \quad \text{(Eq. 26)}$$

the logarithm of the relative potency is calculated from the ratio of $R = dL_p/L_r$, where $d$, as used by Finney (3), is the logarithm of the ratio between successive doses, this ratio being the same for both preparations. Since $V(L_p) = V(L_r)$ from Eq. 26, the confidence limits for $dL_p/L_r$ are found by using Eq. 23 with $a_p = a_r$.

$$\left(\frac{dL_p}{L_r}\right)_L, \left(\frac{dL_p}{L_r}\right)_U = \frac{d}{(F_r - F_c)}\left\{\frac{L_p}{L_r} F_r \mp \sqrt{F_c(F_p + F_r - F_c)}\right\} \quad \text{(Eq. 27)}$$

where, as before, $F_c$ is the $100(1 - \alpha)\%$ tabulated $F$-value with 1 and $f$ degrees of freedom, $f$ being the number of degrees of freedom for $s^2$ in the ANOV, $F_p$ and $F_r$ are as defined in Eq. 25.

If $g$ is computed as

$$g = F_c s^2/(\text{regression mean square}) \quad \text{(Eq. 28)}$$

an alternative computational form of Eq. 27 is then

$$\left(\frac{dL_p}{L_r}\right)_L, \left(\frac{dL_p}{L_r}\right)_U = \frac{d}{(1 - g)} \times \left\{\frac{L_p}{L_r} \mp \sqrt{g\left(1 - g + \frac{\text{preparations mean square}}{\text{regression mean square}}\right)}\right\} \quad \text{(Eq. 29)}$$

which is perhaps the most expedient formulation.

**Six-Point Parallel Line Assay.**—If $L_p$ and $L_r$ are the usual contrasts for the 6-point assay, corresponding to those in Eq. 26, the confidence limits for $\bar{y}_T - \bar{y}_S/b = 4dL_p/3L_r$ are obtained from Eq. 23 as

$$\left(\frac{4dL_p}{3L_r}\right)_L, \left(\frac{4dL_p}{3L_r}\right)_U = \frac{4d}{3(F_r - F_c)} \times \left\{\frac{L_p}{L_r} F_r \mp \sqrt{\frac{3}{2} F_c(F_p + F_r - F_c)}\right\} \quad \text{(Eq. 30)}$$

$$= \frac{4d}{3(1 - g)} \times \left\{\frac{L_p}{L_r} \mp \sqrt{\frac{3g}{2}\left(1 - g + \frac{\text{preparations mean square}}{\text{regression mean square}}\right)}\right\} \quad \text{(Eq. 31)}$$

with all quantities as previously defined.

**Numerical Example.**—To illustrate the identification of the required quantities the 4-point assay of oestrin by Bülbring and Burn (5) as used by Finney (3) is taken. In that example $L_p = -42$, $L_r = 448$ and the ANOV was essentially

| | d.f. | m.s. |
|---|---|---|
| Preparations | 1 | 63 |
| Regression | 1 | 7168 |
| Divergence | 1 | 240 |
| Residual error | 13 | 551.15 |

where the term "divergence" is introduced in preference to "parallelism" or, uglier, "antiparallelism." Hence, for 95% limits,

$$F_c = F_{1,\,13}(0.05) = 4.67$$

and

$$g = (4.67)(551.15)/7168 = 0.3591$$

The ratio of upper to lower doses was 2 so that, with $d = \log 2$, from Eq. 29,

$$\left(\frac{dL_p}{L_r}\right)_L, \left(\frac{dL_p}{L_r}\right)_U = \frac{0.30103}{0.6409} \times \left\{-\frac{42}{448} \mp \sqrt{\left(0.3591(0.6409 + \frac{63}{7168}\right)}\right\}$$
$$= -0.2709,\ 0.1828$$

The basic doses were 0.2 mcg. and 0.0075 ml., for the standard and test preparations, respectively, so that the limits for the actual relative potency estimate $\hat{\rho}_p$ are,

$$(\hat{\rho}_p)_L, (\hat{\rho}_p)_U = \frac{0.2}{0.0075} \text{ antilog } (\bar{1}.7291,\ 0.1828)$$
$$= 14.29,\ 40.60 \text{ mcg./ml.}$$

## PRECISION

The formulas derived above are useful in discussions of the precision of bioassays. For example, from Eqs. 29 and 30 the squared length of the confidence interval for 4- and 6-point parallel line assays is seen to depend on the quantity,

$$\frac{1}{(1 - g)^2}\left\{g\left(1 - g + \frac{\text{preparations mean square}}{\text{regression mean square}}\right)\right\}$$
$$= \frac{g}{1 - g} + \frac{g}{(1 - g)^2}\left\{\frac{\text{preparations mean square}}{\text{regression mean square}}\right\} \quad \text{(Eq. 32)}$$

From the definition of $g$ in Eq. 28, it therefore simply follows that the basic requirements are small values for $F_c$, $s^2$, and the mean square for preparations, and a large value for the regression mean square.

## CONCLUSION

Detailed presentations of the above formulas have been made because of the assurance [Schultz (6)] that they give considerable computational advantage. In particular, the gain arises because values in the necessarily computed ANOV do double duty. The exact rather than the approximate formulas may be recommended, for parallel line assays at least, because once $F_c/F_r$ has been calculated for arbitration, its retention involves little extra labor.

## APPENDIX

**Slope Ratio Assay.**—Using the values for $a_u$, $a_v$, and $a_{uv}$ for a slope ratio assay as given in Eq. 10, the expression $a_u v^2 - 2a_{uv}uv + a_v u^2$ under the radical of Eq. 3 can be written as

$$a_u v^2 - 2a_{uv}uv + a_v u^2 = v(a_u v - a_{uv}u) + u(a_v u - a_{uv}v)$$
$$= \frac{1}{\Delta}\{b_S(b_S\Sigma' x_S^2 + b_T\Sigma' x_S x_T) + b_T(b_T\Sigma' x_T^2 + b_S\Sigma' x_S x_T)\}$$
$$= \frac{1}{\Delta}\{b_S\Sigma' x_S y + b_T\Sigma' x_T y\} \quad \text{(Eq. 33)}$$

This follows from the normal equations of which Eq. 5 is the solution. Further, the numerator on the right of Eq. 33 is the sum of squares for regression in the ANOV. Also,

$$a_u a_v - a_{uv}^2 = \frac{1}{\Delta^2}\{(\Sigma' x_S^2)(\Sigma' x_T^2) - (\Sigma' x_S x_T)^2\} = \frac{1}{\Delta} \quad \text{(Eq. 34)}$$

Substituting from Eqs. 33 and 34 into Eq. 3, the confidence limits can therefore be written as

$$R_L, R_U = \frac{1}{b_S^2 - s^2F_c a_v}[Rb_S^2 - s^2F_c a_{uv} \mp \sqrt{s^2F_c(\text{regressions sum of squares} - s^2F_c)/\Delta}] \quad \text{(Eq. 35)}$$

Hence, with $F_r$ and $F_S$ defined as in Eqs. 11 and 12, the confidence limits can be expressed as

$$R_L, R_U = \frac{RF_S - F_c(a_{uv}/a_v) \mp \sqrt{F_c(2F_r - F_c)/\Delta a_v^2}}{F_S - F_c} \quad \text{(Eq. 36)}$$

as given in Eq. 13.

**Parallel Line Assay.**—For a parallel line assay, Eq. 3 with $a_{uv} = 0$ reduces to

$$R_L, R_U = \frac{Rv^2 \mp \sqrt{s^2F_c(a_u v^2 + a_v u^2 - s^2F_c a_u a_v)}}{v^2 - s^2F_c a_v}$$
$$= \frac{1}{\left(\frac{v^2}{a_v s^2} - F_c\right)} \times \left\{\frac{Rv^2}{a_v s^2} \mp \sqrt{\frac{a_u}{a_v} F_c\left(\frac{u^2}{a_u s^2} + \frac{v^2}{a_v s^2} - F_c\right)}\right\}$$
$$= \frac{1}{(F_v - F_c)} \times \left\{RF_v \mp \sqrt{\frac{a_u}{a_v} F_c(F_u + F_v - F_c)}\right\} \quad \text{(Eq. 37)}$$

where $F_u$ and $F_v$ are defined as

$$F_u = u^2/a_u s^2 \text{ and } F_v = v^2/a_v s^2 \quad \text{(Eq. 38)}$$

For the general parallel line assay, comparison of Eqs. 1 and 22 shows that the above result can be applied to obtain the confidence interval for $M - \bar{x}_S + \bar{x}_T$ if

$$u = (\bar{y}_S - \bar{y}_T) \text{ and } v = b \quad \text{(Eq. 39)}$$

If $n_S$ and $n_T$ are the total number of responses to the standard and test preparations, respectively, the variance of $(\bar{y}_S - \bar{y}_T)$ is estimated as

$$V(u) = V(\bar{y}_S - \bar{y}_T) = \left(\frac{1}{n_S} + \frac{1}{n_T}\right)s^2 = a_u s^2 \quad \text{(Eq. 40)}$$

so that, from Eq. 38,

$$F_u = \frac{(\bar{y}_S - \bar{y}_T)^2}{\left(\frac{1}{n_S} + \frac{1}{n_T}\right)s^2} \quad \text{(Eq. 41)}$$

which itself is the F-ratio for preparations in the ANOV. Similarly

$$F_v = \frac{b^2}{s^2}\left\{\sum_S (x - \bar{x})^2 + \sum_T (x - \bar{x})^2\right\} \quad \text{(Eq. 42)}$$

which is the F-ratio for regression in the ANOV. If then $F_p$ and $F_r$ are defined as in Eq. 25, the confidence interval from Eq. 37 is

$$R_L, R_U = \frac{1}{(F_r - F_c)}\left\{RF_r \mp \sqrt{\frac{a_p}{a_r} F_c(F_p + F_r - F_c)}\right\} \quad \text{(Eq. 43)}$$

which establishes the result given in Eq. 23.

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