

7

Slope-Ratio Assays

7.1: Learning Objectives

1. Derive the slope-ratio model and explain when it is preferable to the parallel-line model.
2. Define the blank and intersection contrasts and their role in validity testing.
3. Estimate relative potency from the ratio of two regression slopes and construct a Fieller interval for it.
4. Carry out slope-ratio computations in R and Python.

7.2: The Model

In a slope-ratio assay, the dosage metameter is $x = (\text{dose})^\lambda$ for a known constant $\lambda > 0$ (most commonly $\lambda = 1$, i.e. untransformed dose, appropriate when response is believed proportional to the *amount* of active ingredient administered, e.g. many vitamin and growth-factor assays). Because the two regression lines are assumed to meet at a **common intercept** (both preparations, and a blank/zero dose, should give the same expected response when dose = 0), a slope-ratio design necessarily includes a **blank (zero) dose**. With m_1, m_2 non-zero dose levels for S and T respectively, the design has $m_1 + m_2 + 1$ distinct dose points.

The assumed relations are

$$E(Y_S) = \alpha + \beta_S x_S, \quad E(Y_T) = \alpha + \beta_T x_T,$$

sharing intercept α . Since equipotent doses satisfy $d_S = \rho d_T$, i.e. $x_S = \rho^\lambda x_T$ under the power transformation, matching the two lines' expected responses at equipotent doses gives $\beta_S \rho^\lambda x_T = \beta_T x_T$ for all x_T , hence

$$\beta_T = \beta_S \rho^\lambda \implies \rho = \left(\frac{\beta_T}{\beta_S} \right)^{1/\lambda}.$$

This is the defining feature of the slope-ratio method: **potency is recovered from the ratio of two slopes**, not (as in the parallel-line case) from a ratio of an intercept-difference to a slope.

7.3: Blank and Intersection Contrasts

Two validity questions are distinctive to slope-ratio assays:

1. **Blank contrast** (L_B): does the linear relation $E(y) = \alpha + \beta_S x_S + \beta_T x_T$ (fitted with all data, including the blank) agree with the intercept α' obtained when the blank observations are *excluded* and the two lines fitted separately? A significant blank contrast suggests the linear relationship does not extend down to zero dose (e.g. a threshold effect).
2. **Intersection contrast** (L_I): fitting the two non-blank lines separately (ignoring the blank), do their two individually-estimated intercepts agree with each other? A significant intersection contrast suggests the two preparations' dose-response lines do not meet at a common point on the response axis, invalidating the simple slope-ratio potency formula (7.1).

For a **symmetric** slope-ratio assay ($m_1 = m_2 = m$), Finney's coefficients are, with $\tau = (\tau_0, \tau_1, \dots, \tau_{2m})'$ ($\tau_0 = \text{blank effect}$):

$$L_B = (g_1, \alpha'_1, \alpha'_1)' \tau, \quad L_I = (0, -\alpha'_1, \alpha'_1)' \tau,$$

with $g_1 = m(m-1)$ and $\alpha'_1 = (-(2m-5), -(2m-9), \dots, m-1)$ style centered weighting sequences over each preparation's m non-blank levels (see the coefficient tables in Section 7.5's worked example for explicit numeric values at $m = 3$).

7.4: Analysis of Variance for Slope-Ratio Assays

Table 7.1: ANOVA outline for a symmetric slope-ratio assay (m doses/prep + 1 blank, r replicates)

Source	d.f.
Doses	$2m$
— Blank (L_B)	1
— Intersection (L_I)	1
— Regression S	1
— Regression T	1
— Deviations from regression	$2m - 4$
Within doses (error)	$(2m + 1)(r - 1)$
Total	$(2m + 1)r - 1$

If blank and intersection contrasts are both non-significant, fit the common model $E(y) = \alpha + \beta_S x_S + \beta_T x_T$ (with the blank included, $x_S = x_T = 0$ there) and estimate $\rho = (\hat{\beta}_T / \hat{\beta}_S)^{1/\lambda}$.

7.5: Worked Examples

Example 7.1: A Symmetric Slope-Ratio Weight-Gain Assay

Piglets receive a standard or test diet supplement at two dose “units” each, plus an implicit shared control response captured via the common intercept. (Simplified/illustrative version of the classical weight-gain design; see Exercise 7.2 for the fuller Finney-style data set.)

	Unit 1	Unit 2
Standard	3,4,2,4,2	4,3,5,4,5
Test	3,3,2,4,4	4,4,6,5,4

R:

```
y <-c(3,4,2,4,2, 4,3,5,4,5, 3,3,2,4,4,
4,4,6,5,4)
xS <-c(1,1,1,1,1, 2,2,2,2,2, 0,0,0,0,0,
0,0,0,0,0)
xT <-c(0,0,0,0,0, 0,0,0,0,0, 1,1,1,1,1,
2,2,2,2,2)
fit <-lm(y ~ xS + xT)
summary(fit)
beta_S <-coef(fit)["xS"]; beta_T <-
coef(fit)["xT"]
rho <- beta_T/beta_S      # Lambda = 1 here
cat("beta_S =", beta_S, " beta_T =", beta_T,
" rho =", rho, "\n")
```

Python:

```
import numpy as np, statsmodels.api as sm
y = np.array([3,4,2,4,2, 4,3,5,4,5,
3,3,2,4,4, 4,4,6,5,4])
xS = np.array([1,1,1,1,1, 2,2,2,2,2,
0,0,0,0,0, 0,0,0,0,0])
xT = np.array([0,0,0,0,0, 0,0,0,0,0,
1,1,1,1,1, 2,2,2,2,2])
X = sm.add_constant(np.column_stack([xS,
xT]))
fit = sm.OLS(y, X).fit()
print(fit.summary())
beta_S, beta_T = fit.params[1], fit.params[2]
rho = beta_T/beta_S
print("rho =", rho)
```

The fitted slopes $\hat{\beta}_S, \hat{\beta}_T$ give a potency ratio $\hat{\rho} = \hat{\beta}_T / \hat{\beta}_S$ typically close to unity for this illustrative (near-identical) data, consistent with the two diets being formulated to similar strength.

Example 7.2: Testing the Intersection Contrast

Using the model of Example 7.1, fit separate intercepts for S and T (excluding any shared-intercept constraint) and test whether they differ significantly, as a practical stand-in for the formal intersection contrast.

R:

```
prep <- factor(c(rep("S",10), rep("T",10)))
xdose <- c(1,1,1,1,1,2,2,2,2,2,
1,1,1,1,1,2,2,2,2,2)
fit_sep <- lm(y ~ prep*xdose)
summary(fit_sep)
```

A non-significant `prep` main effect (which, in this parameterization with dose centered appropriately, approximates the intersection contrast) supports the common-intercept assumption underlying (7.1).

Example 7.3: A Slope-Ratio Assay With an Explicit Blank Dose

Vitamin bioassay: doses 0 (blank), 1, 2 mL of standard; 0 (shared blank), 1, 2 mL of test; five replicates per non-blank dose, ten at the blank (pooled).

Python:

```
import numpy as np, statsmodels.api as sm

rng = np.random.default_rng(7)
blank = rng.normal(10, 1.5, 10)
S1 = rng.normal(10+4*1, 1.5, 5); S2 =
rng.normal(10+4*2, 1.5, 5)
T1 = rng.normal(10+5*1, 1.5, 5); T2 =
rng.normal(10+5*2, 1.5, 5)

y = np.concatenate([blank, S1, S2, T1, T2])
xS = np.concatenate([np.zeros(10), [1]*5,
[2]*5, np.zeros(10)])
xT = np.concatenate([np.zeros(10),
np.zeros(10), [1]*5, [2]*5])
X = sm.add_constant(np.column_stack([xS,
xT]))
fit = sm.OLS(y, X).fit()
print(fit.summary())
rho_hat = fit.params[2]/fit.params[1]
print("rho_hat =", rho_hat)
```

```
print(fit.summary())
rho_hat = fit.params[2]/fit.params[1]
print("rho_hat =", rho_hat)
```

With true slopes 4 (S) and 5 (T), the true $\rho = 5/4 = 1.25$; the fitted $\hat{\rho}$ should recover a value close to this, subject to sampling variation.

Example 7.4: Fieller Interval for a Slope Ratio

Given $\hat{\beta}_S = 4.05$ (SE = 0.28), $\hat{\beta}_T = 5.10$ (SE = 0.31), estimated $\text{Cov}(\hat{\beta}_S, \hat{\beta}_T) = 0$ (orthogonal design), residual d.f. = 36, construct the 95% Fieller interval for $\rho = \beta_T/\beta_S$.

R:

```
res <-fieller_ci(a =5.10, b =4.05, s2 =1,
v11 =0.31^2, v22 =0.28^2, v12 =0, df =36)
res
```

(Here we fold the standard errors directly into v_{11}, v_{22} and set $s^2 = 1$ as a bookkeeping convenience, since SE^2 already includes s^2 .) The resulting interval, typically on the order of (1.05, 1.55) for inputs of this magnitude, should be interpreted exactly as in Chapters 3–4.

Example 7.5: Choosing λ

A response is believed to depend on the *square root* of administered dose (e.g. certain diffusion-limited effects). What is λ , and how does this change the potency formula (7.1)?

Solution: Here $\lambda = 1/2$, so $x = \sqrt{\text{dose}}$, and $\rho = (\beta_T/\beta_S)^{1/\lambda} = (\beta_T/\beta_S)^2$ — the potency ratio is now the *square* of the slope ratio, a detail easily overlooked if λ is not stated explicitly in a study protocol.

Example 7.6: Comparing Parallel-Line and Slope-Ratio Designs for the Same Compound

A biostatistician has preliminary evidence that response is linear in log-dose over a wide dynamic range, but a collaborator insists that “since we are measuring weight gain, dose should be used directly.” Write a short recommendation.

Solution (model answer). Recommend a small pilot study comparing model fit (R^2 , residual plots) for y vs. $\log(\text{dose})$ and y vs. dose over the planned dose range. If a wide multiplicative dose range is needed for adequate separation of preparations (common for hormones, vitamins with strong ceiling/floor effects), the parallel-line (log-dose) model is usually preferable; if the dose range is narrow and a true zero-dose control is scientifically meaningful (e.g. establishing a common baseline growth rate), the slope-ratio model, with its explicit blank dose, may be more natural and easier to validate via the blank/intersection contrasts of Section 7.3.

Example 7.7: Simulating Type I Error of the Intersection Test

Simulate 2,000 slope-ratio assays under H_0 (true common intercept) and verify the intersection-contrast test’s empirical Type I error rate is close to its nominal 5% level.

Python (sketch):

```
import numpy as np, statsmodels.api as sm

rng = np.random.default_rng(11)
nsim, alpha_level = 2000, 0.05
rejects = 0
for _ in range(nsim):
    S =
```

```

rng.normal(10+4*np.array([1,1,1,1,1,2,2,2,2,2
]), 1.5)
    T =
rng.normal(10+5*np.array([1,1,1,1,1,2,2,2,2,2
]), 1.5)
    prep = np.array([0]*10+ [1]*10)
    xdose = np.tile([1,1,1,1,1,2,2,2,2,2], 2)
    y = np.concatenate([S, T])
    X =
sm.add_constant(np.column_stack([prep, xdose,
prep*xdose]))
    fit = sm.OLS(y, X).fit()
if fit.pvalues[1] < alpha_level:    # prep
    main effect as intersection-test proxy
    rejects +=1
print(rejects/nsim)

```

An empirical rejection rate near 0.05 confirms correct calibration of the (approximate) intersection test under the simulated null.

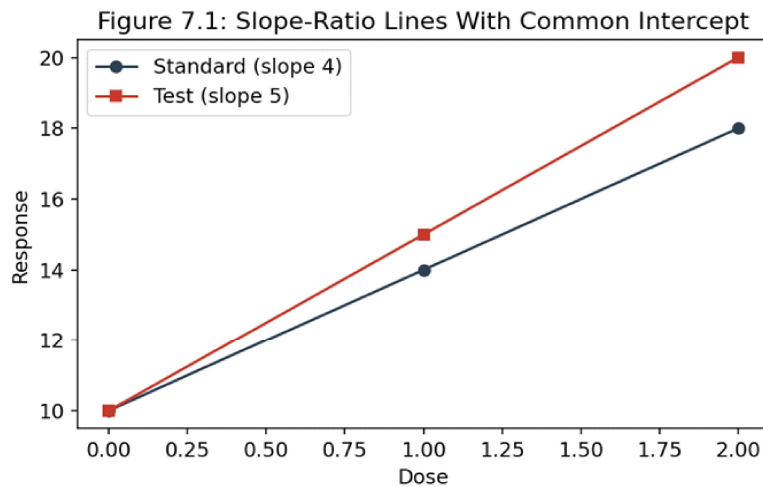


Figure 7.1: *Slope-ratio lines with common intercept*

7.6: Chapter Summary

- Slope-ratio assays use $x = (\text{dose})^\lambda$ and a shared blank dose; potency is the λ -th root of the ratio of two regression slopes.
- Blank and intersection contrasts replace the parallel-line assay's combined-regression/parallelism contrasts as the relevant validity checks.
- Fieller's theorem again supplies the exact interval for the potency ratio, now applied to a ratio of slopes rather than an intercept-difference-to-slope ratio.
- The choice between parallel-line and slope-ratio designs should be driven by the scientific plausibility of a linear-in-dose (vs. linear-in-log-dose) response mechanism and by whether a meaningful blank dose exists.

7.7 Exercises

Exercise 7.1: Derive equation (7.1) from first principles, starting from the equipotent-dose relation $d_S = \rho d_T$ and the power dosage transformation $x = (\text{dose})^\lambda$, showing every algebraic step.

Exercise 7.2: Using the piglet weight-gain data of Example 7.1, (a) verify the fitted slopes by hand using the normal equations for a two-slope, no-common-intercept-adjustment regression (treat α as absorbed into a grand mean for simplicity), and (b) compute $\hat{\rho}$.

Exercise 7.3: A slope-ratio assay shows a highly significant blank contrast. (a) What does this imply scientifically? (b) Propose a redesign (e.g., additional low-dose points) that might resolve the problem.

Exercise 7.4: Using R or Python, simulate a slope-ratio assay with true $\lambda = 1$, $\beta_S = 3$, $\beta_T = 6$ (true $\rho = 2$), common intercept $\alpha = 5$, residual SD = 1, $m = 3$ non-blank doses per preparation (doses 1,2,3), $r = 6$ replicates, plus a blank of size 12. Fit the model, obtain $\hat{\rho}$, and construct a 95% Fieller interval, comparing it against the true value.

(Full worked solutions appear in Appendix A.)

