UNIT 7 SLOPE RATIO BIOASSAY

Structure

7.1 INTRODUCTION

You have studied in Units 5 and 6 that assays in fact are the experiments where we intentionally apply a stimulus at different potencies or strengths or intensities to see the effect and compare it with the effect of a standard intervention. The effect of an induced stimulus is technically called the response. In case of assays, we apply same intervention at different potencies assuming that as the intensity increases, the response will be higher. This particularly applies to the poisons such as insecticides and pesticides. Larger the dose, more insects will die. In this case, response is the death which is called a quantal response as it occurs or does not occur. There is nothing in between. We will discuss this in the next unit. For a quantitative assay, we may consider the time elapsed before death in place of death.

For example, if you give 2 mg of poison to rats, how much time does it take for a rat to die, and if you give 4 mg, then how much time is taken. For the same dose, say 4 mg, some rats will take 4 hours and some will take 5 hours to die. Different rats will take different time to die but here the response is not either dead or alive, but it is measured in quantitative terms, i.e., hours taken to die a rat. When the comparison is with a standard poison, this is called quantitative assay. The primary objective of an assay is to estimate the relative potency of the test intervention compared to the standard intervention for achieving a particular outcome.

You have studied in Unit 6 that an assay is called parallel-line when plot of the response verses dose falls in a straight line for both test preparation as well as standard preparation but these lines are parallel to each other. This means that difference in the responses remains constant as the dose escalates. In the example of rat, if the difference in time to death is 4 hours between test preparation and standard preparation at a dose of 2 mg, this difference, i.e., 4 hours also remains same at other doses, say, 3 mg, 4 mg, etc. In place of actual dose (d), you can also use transformation such as $x = \phi(d)$ (where ϕ is a real valued function) which is called the dose-metameter. Sometimes this kind of transformation is also used to achieve the response look like in parallel lines.

Quite often, the difference in responses at different doses of the standard and test preparation will not be constant. The difference in time to death could be 4 hours at dose of 2 mg but 6 hrs at dose of 3 mg and 8 hours at dose of 4 mg. As the dose is increased by 1 mg, the difference increases by 2 hours each time in this example. It does not remain constant. This kind of response gives rise to, what are called, **slope-ratio assays**. This unit introduces you to slope-ratio

assays and provides details of how to analyse data obtained from such assays. In this unit, we apprise you about the slope-ratio assays, under what conditions you would apply and analyse these assays.

The essentials of slope-ratio assays which make it clear to you what a slope-ratio assay is and why this is called a slope-ratio assay are explained in Sec.7.2. The general layout of a slope-ratio assay is presented in Sec. 7.3. We discuss the regression approach in case of slope ratio assay in Sec. 7.4. The primary objective of the bioassay is to estimate the relative potency of the test preparation compared with the standard preparation. However, this estimate of the relative potency is valid under certain conditions. How to check these conditions is mentioned in Sec. 7.5. The methods for obtaining the relative potency and fiducial limits of the relative potency are described in Sec. 7.6.

Objectives

After studying this unit, you should be able to:

- identify situations where slope-ratio assays are applicable;
- analyse data from simple slope-ratio assays, particularly to check the validity conditions;
- estimate the relative potency in slope-ratio assays; and
- compute the fiducial limits of the relative potency.

7.2 SETUP OF A SLOPE RATIO ASSAY

The basic setup in slope-ratio assays is that the response for test and standard preparations is same at some baseline but increases (or decreases) at a faster rate for one preparation than the other as the dose increases. However, the trend in both preparations should be linear. Let us illustrate this with the help of an example. The data considered here are an artificial and not a real data.

Example 1. Egg is a standard source of dietary protein. However, its digestibility is supposed to be low. Rice is consumed by a large part of population and this also has a good source of quality protein. An experiment was conducted to determine the digestibility score of rice protein compared with the digestibility score of egg protein. The digestibility score measures how much protein is really absorbed by the body proteins. Other diets are also standardised for all. For this study, a group of 30 male adults of similar weight were randomly divided to receive as much egg or rice to provide the desired doses of protein. Both were given in doses of 0 gram, 5 grams, 10 grams, 15 grams and 20 grams to three persons each. Note that these are protein doses, not egg or rice doses. For example, one cup of cooked rice may have to be eaten to get 5 grams of protein). The data obtained are shown in Table 1.

Table 1: Digestibility score for different doses of egg and rice protein

Person	Egg Protein (Standard)					Rice Protein (Test)				
	Blank 0 gm	Dose 1 5 gm	Dose 2 10 gm	Dose 3 15 gm	Dose 4 20 gm	Blank 0 gm	Dose 1 5 gm	Dose 2 10 gm	Dose 3 15 gm	Dose 4 20 gm
1	5	8	10	13	14	7	11	16	23	25
2	6	8	12	15	17	3	9	15	20	24
3	4	7	11	13	15	5	10	14	21	27
Mean score	5.0	7.7	11.0	13.7	15.3	5.0	10.0	15.0	21.3	25.3

Note that Person-1 receiving egg is not the same as person-1 receiving rice.

They are different persons so that the total persons are 30 in this experiment. A positive digestibility score for 0 gm of dose is because of other diet constituents. Let us denote the i^{th} dose by d_{si} and d_{ti} for the standard and test preparations, respectively. As discussed in Unit 6, we consider the following transformation in the slope-ratio assays:

$$x_{si} = (d_{si})^{\lambda} \text{ and } x_{ti} = (d_{ti})^{\lambda};\ \lambda > 0 \qquad \ldots (1)$$

For the sake of simplicity, let us consider $\lambda = 1$. If we consider dose-metameters $(x_{si} \text{ and } x_{ti})$ as dose-itself in this example, i.e., $x_{si} = d_{si} \text{ and } x_{ti} = d_{ti}$, the approximate relationship between response and dose for rice and egg preparations can be shown in Fig. 7.1.

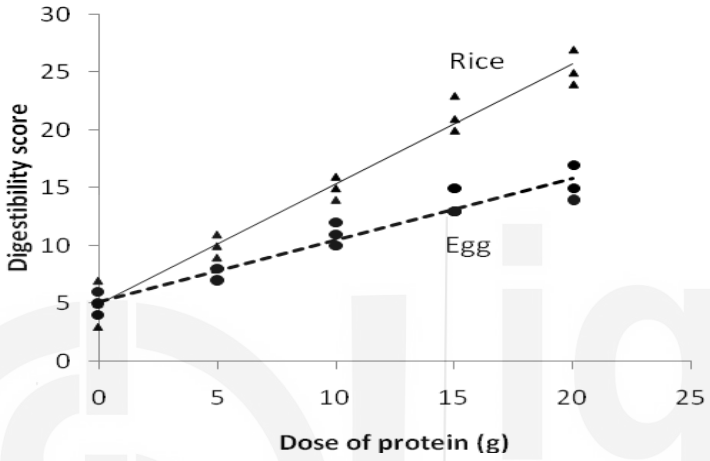

Fig. 7.1: Dose-response plot for the data given in Table 1

Remember that the regression lines shown in Fig. 7.1 are for the purpose of rough idea but the actual regression may or may not be linear. We will check this subsequently. Beside linearity, an important feature of the regression lines shown in Fig. 7.1 is that both lines have same intercept but slope for the rice is steeper than the egg. This condition will also be checked later in Sec. 7.5.

In this example, egg is considered as a standard preparation and rice, a test preparation.

You may observe the following features of Example 1:

1. In Example 1, dose itself is being used in regression and not its log or any other transformation. Thus, dose-metameter is the dose itself in this case. Similarly same is true for the response (digestibility score). In general, you may have to use a dose-metameter and in some cases response-metameter, to get a linear regression. You will study in Block 2 of MSTE-004 how to fit a linear regression.
2. The doses considered in this example are equi-spaced, i.e., 0, 5, 10, 15, and 20 grams. In actual experiments, this may not be so. Somebody may receive, 12 grams, some 14 grams, some 17.5 grams, etc. This does not change the setup.
3. The dose of 0 gm is called **blank dose**. In Table 1, this dose is given to both groups. Actually, this dose is the same for both groups although the digestibility scores differ. Thus, factually we can say that the blank dose is given to 6 persons in this example.
4. In this example, doses of the test and standard preparations are same. In general, they may also have different doses.

5. The number of doses under trial are same for both preparations in this example. Such an assay is called **symmetric assay** as discussed in Sec. 6.3 of Unit 6. There are 4 doses for each preparation plus one blank dose (although blank dose appears in both groups). Thus, this is a 9-point assay. In general, they can differ. For example, standard preparation can be tried for 3 dose levels and test for 5 dose levels. At least 3 doses of the standard preparation and 3 doses of the test preparation are needed to validate the linearity condition (see point 8) and a blank dose is needed to check the equality of intercepts (see point 7).
6. In this example, each dose has been given to 3 persons. In practice, one dose may be given to 4 persons, another dose to 7 persons, third dose to only 2 persons, etc. These numbers can vary. That also does not change the basic structure of the experiment and the regression lines can be obtained as usual. However, equal number of subjects for each dose level relatively simplifies the computations. As you have already studied, when the number of subjects is same for each dose, this is called the **balanced assay**.
7. Fig.7.1 shows that the intercepts of two regression lines are same for the given data. In practice, you will be required to conduct a statistical test to check that the intercepts are significantly different or not. We will discuss this later in this unit. Once they are found not to be different, then only the ratio of slope will provide correct estimate of the relative potency.
8. In Fig. 7.1, both regression lines are clearly linear. In practice, you will have to check that they are really linear or not. For this, the deviation from linearity must be statistically non-significant. You will learn how to test this in Sec. 7.5.
9. The relative potency in case of slope-ratio assays is the ratio of the slopes of the regression lines provided the conditions such as equality of intercepts and linearity of regression models are not violated. Once you have determined such regression lines, you can easily obtain the ratio of slopes to compute the relative potency.

Now, you can try the following exercises.

E1) Which of the followings are basic requirements for a slope-ratio assay:

i) Equality of slopes
ii) Equality of intercepts
iii) Linearity in dose-response relationship
iv) Equal number of subjects for the test and standard preparations
v) Dose levels must be equi-spaced
vi) A blank dose is necessarily needed

E2) When a slope-ratio is called balanced and symmetric? How does this help?

7.3 GENERAL LAYOUT OF A SLOPE RATIO ASSAY

Suppose blank dose is given to total M_b subjects. If we have K doses of a standard preparation and the i^{th} dose is given to M_{si} (for i=1, 2, …, K) subjects.

In the same way, there are L doses in a test preparation and total M_{ti} (for i=1, 2, …, L) subjects receive the i^{th} dose. Let $y_{bij}, y_{sij}, y_{tij}$ denote the responses of the j^{th} subject who receive the i^{th} dose of the blank, standard and test groups, respectively. For example, in Table 1, the response of the 3rd subject (i.e., j = 3) who receive the 2nd dose (i.e., i = 2) of the standard preparation is $y_{s23} = 11$. The general layout of a (K+L+1)-point slope-ratio assay is shown in Table 2.

Table 2: Layout of a (K + L+1)-point assay

Dose	**Blank**	**Standard preparation**				**Test preparation**			
	($d_b = 0$)	d_{s1}	d_{s2}	…	d_{sK}	d_{t1}	d_{t2}	…	d_{tL}
Dose-metameter	x_b	x_{s_1}	x_{s_2}	…	x_{s_K}	x_{t_1}	x_{t_2}	…	x_{t_L}
Response	y_{b1}	$y_{s_1 1}$	$y_{s_2 1}$	…	$y_{s_K 1}$	$y_{t_1 1}$	$y_{t_2 1}$	…	$y_{t_L 1}$
	y_{b2}	$y_{s_1 2}$	$y_{s_2 2}$	…	$y_{s_K 2}$	$y_{t_1 2}$	$y_{t_2 2}$	…	$y_{t_L 2}$
	…	…	…	…	…	…	…	…	…
	…	…	…	…	…	…	…	…	…
	y_{bM_b}	$y_{s_1 M_{s1}}$	$y_{s_2 M_{s2}}$	…	$y_{s_K M_{sK}}$	$y_{t_1 M_{t1}}$	$y_{t_2 M_{t2}}$	…	$y_{t_L M_{tL}}$
Total	y_b	y_{s1}	y_{s2}	…	y_{sK}	y_{t1}	y_{t2}	…	y_{tL}

You can see that the notations are already becoming complex. To keep notations simple and easy to understand, we may restrict ourselves to same number of subjects for each dose. This makes it a balanced assay. Let us denote the number of subjects for each dose by M. For example, in Table 1, we have M = 3. Also, for convenience, let the number of doses of the standard preparation be same as for the test preparation. This makes it a symmetric assay. Let us denote the number of doses of each preparation by K. In Table 1, K = 4, since there are 4 doses of the standard preparation and 4 doses of the test preparation.

In the simplified case of equal doses (i.e., K doses of standard and test preparations) and equal number of subjects (say, M) receiving blank dose, each dose of the standard and test preparations. In this way, the total number of subjects will be (M +2KM) and it is known as (K + K + 1) = **(2K + 1)-point assay** which is the total number of doses in this experiment.

Note that those who receive standard preparation do not receive test preparation and vice-versa. For example, when standard dose of 7 grams of egg protein is given to a subject, the dose for rice protein is 0 gram for that subject. So, we can say that for blank or zero dose, both $x_s = 0$ and $x_t = 0$. For all subjects in the standard preparation group, dose of the test preparation $x_t = 0$ and for all subjects in the test preparation group, dose of the standard preparation $x_s = 0$.

Thus, we can represent the layout of a slope-ratio assay given in Table 2 to simplify the notations for hand calculations as shown in Table 3.

Remember to keep track of the notations as they become quite messy for the data obtained from this kind of assay. To help you understand these notations, examples are given and small exercises are assigned to evaluate your understanding. You are advised to do these exercises and check with the given answers.

Table 3: General layout of a (K+L+1)-point assay

Subject No.	Group	Blank dose (x_b)	Standard dose (x_{si})	Test dose (x_{ti})	Response (y_i)
1	Blank	1	0	0	y_{b1}
. . .		. . .	. . .	. . .	. . .
M_b		1	0	0	y_{bM_b}
1	Standard	0	x_{s_1}	0	$y_{s_1 1}$
. . .		. . .	. . .	. . .	. . .
M_{s1}		0	x_{s_1}	0	$y_{s_1 M_{s1}}$
… … …		… … …	… … …	… … …	… … …
1		0	x_{s_K}	0	$y_{s_K 1}$
. . .		. . .	. . .	. . .	. . .
M_{sK}		0	x_{s_K}	0	$y_{s_K M_{sK}}$
1	Test	0	0	x_{t_1}	$y_{t_1 1}$
. . .		. . .	. . .	. . .	. . .
M_{t1}		0	0	x_{t_1}	$y_{t_1 M_{t1}}$
… … …		… … …	… … …	… … …	… … …
1		0	0	x_{t_L}	$y_{t_L 1}$
. . .		. . .	. . .	. . .	. . .
M_{tL}		0	0	x_{t_L}	$y_{t_L M_{tL}}$

The data given in Table 1 and corresponding Fig. 7.1 may have made the setup of slope-ratio assays clear to you. Before learning how to estimate the relative potency, let us extend this to a general situation so that most of the restrictive features mentioned in Points 1 to 9 in Sec. 7.2 are removed. Table 4 given in the following example is a modification of Table 1.

Example 2: In a slope-ratio assay, blank dose is given to 5 subjects. A standard preparation is considered in 4 doses (5 gm, 7 gm, 11 gm, and 20 gm) and test preparation in 3 doses (3 gm, 10 gm, and 18 gm). The number of subjects receiving different doses are also varied in both preparations and shown in Table 4.

Table 4: An assay with unequal number of subjects and doses

Subject	Blank Dose (0 gm)	Receiving Egg Protein (Standard)				Receiving Rice Protein (Test)		
		Dose 1 (5 gm)	Dose 2 (7 gm)	Dose 3 (11 gm)	Dose 4 (20 gm)	Dose 1 (3 gm)	Dose 2 (10 gm)	Dose 3 (18 gm)
1	5	8	10	10	14	8	16	25
2	6	8	8	9	17	9	15	24
3	3	7	12	12		7	14	21
4	7		11			10		23
5	4		9			6		
Total	25	23	50	31	31	40	45	93
Mean	$\bar{y}_{01}$ =5	$\bar{y}_{s1}$ =7.6667	$\bar{y}_{s2}$ = 10	$\bar{y}_{s3}$ =10.3333	$\bar{y}_{s4}$ =15.5	$\bar{y}_{t1}$ = 8	$\bar{y}_{t2}$ =15	$\bar{y}_{t3}$ =23.25

Represent this data in the same way as given in Table 3.

Solution: In the data given in Table 4, we observe that 3 subjects receive dose 1 of the standard preparation and 4 subjects receive dose 3 of the test preparation. If you carefully see Table 4, you can confirm, for example, response of the 2nd person receiving the 3rd dose of standard preparation is 9. This is shown in a box in Table 4. Note that when dose of the test preparation is given, dose of the standard preparation is considered as zero dose. Similarly, when dose of the standard preparation is given, dose of the test preparation is considered as zero dose. In this way, you can say that conceptually each person has received both preparations. Thus, the data given in Table 4 can also be represented as shown in first seven columns of Table 5. The last eight columns will be discussed in Sec. 7.4.

Table 5: General representation of data given in Table 4

Subject No.	Group	Dose	Blank dose (x_b)	Standard dose (x_s)	Test dose (x_t)	Response (y)	x_b^*	$x_s \times x_t$	$y \times x_s$	$y \times x_t$	$x_s \times x_s$	$x_t \times x_t$	$y \times y$	$y \times x_b^*$	$x_b^* \times x_b^*$
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)
1	Blank	$x_b = 0$	0	0	0	5	1	0	0	0	0	0	25	5	1
2			0	0	0	6	1	0	0	0	0	0	36	6	1
3			0	0	0	3	1	0	0	0	0	0	9	3	1
4			0	0	0	7	1	0	0	0	0	0	49	7	1
5			0	0	0	4	1	0	0	0	0	0	16	4	1
Total	**$M_b = 5$**		0	0	0	25	5	0	0	0	0	0	135	25	5
6	Standard	$x_{s1} = 5$	0	5	0	8	0	0	40	0	25	0	64	0	0
7			0	5	0	8	0	0	40	0	25	0	64	0	0
8			0	5	0	7	0	0	35	0	25	0	49	0	0
9		$x_{s2} = 7$	0	7	0	10	0	0	70	0	49	0	100	0	0
10			0	7	0	8	0	0	56	0	49	0	64	0	0
11			0	7	0	12	0	0	84	0	49	0	144	0	0
12			0	7	0	11	0	0	77	0	49	0	121	0	0
13			0	7	0	9	0	0	63	0	49	0	81	0	0
14		$x_{s3} = 11$	0	11	0	10	0	0	110	0	121	0	100	0	0
15			0	11	0	9	0	0	99	0	121	0	81	0	0
16			0	11	0	12	0	0	132	0	121	0	144	0	0
17		$x_{s4} = 20$	0	20	0	14	0	0	280	0	400	0	196	0	0
18			0	20	0	17	0	0	340	0	400	0	289	0	0
Total	**$M_s = 13$**		0	123	0	135	0	0	1426	0	1483	0	1497	0	0

19	Test	$x_{t1} = 3$	0	0	3	8	0	0	0	24	0	9	64	0	0
20			0	0	3	9	0	0	0	27	0	9	81	0	0
21			0	0	3	7	0	0	0	21	0	9	49	0	0
22			0	0	3	10	0	0	0	30	0	9	100	0	0
23			0	0	3	6	0	0	0	18	0	9	36	0	0
24		$x_{t2} = 10$	0	0	10	16	0	0	0	160	0	100	256	0	0
25			0	0	10	15	0	0	0	150	0	100	225	0	0
26			0	0	10	14	0	0	0	140	0	100	196	0	0
27		$x_{t3} = 18$	0	0	18	25	0	0	0	450	0	324	625	0	0
28			0	0	18	24	0	0	0	432	0	324	576	0	0
29			0	0	18	21	0	0	0	378	0	324	441	0	0
30			0	0	18	23	0	0	0	414	0	324	529	0	0
Total	**$M_t = 12$**		0	0	117	178	0	0	0	2244	0	1641	3178	0	0
Grand Total	N=30		0	123	117	338	0	0	1426	2244	1483	1641	4810	25	5
Mean			0	4.1	3.9	11.2667	0	0	47.5333	74.80	49.4333	54.70	160.3333	0.8333	0.1667

Now test if you have understood the notations clearly. Check your answers with those given at the end of this unit.

E3) In Table 4, what is the value of (i) M_b for blank dose, (ii) M_{s1}, M_{s2}, etc. for the standard preparation (egg), and (iii) M_{t1}, M_{t2}, etc. for the test preparation (rice), where M with subscripts are the numbers of subjects in that group. Also write the values of y_{b2}, y_{s24} and y_{t13}.

7.4 REGRESSION APPROACH IN SLOPE RATIO ASSAY

As discussed in Sec. 7.3, we have only one dose in blank preparation, K doses in standard and L doses in test preparations. If we have total M_b, $M_s = \sum_{i=1}^{K} M_{si}$ and $M_t = \sum_{i=1}^{L} M_{ti}$ subjects (responses) for the blank, standard and test preparations, respectively, the total number of responses in all preparations are $N = M_b + M_s + M_t$.

Let x_{sij} is the dose-metameter for the j^{th} subject receiving the i^{th} dose of the standard preparation and x_{tij} is the dose-metameter for the j^{th} subject who receives the i^{th} dose of the test preparation. For example, in Table 4, if we consider dose-metameter as the dose itself, the dose-metameter for the 2^{nd} subject receiving the 3^{rd} dose of the test (rice) preparation is $x_{t32} = 18$ grams. For blank preparation, $x_{bj} = 0$ for all M_b subjects in this group. It means that the amount of dose is zero for each subject who receive the blank dose. This would be like this in almost all slope-ratio situations but the blank dose can be different from 0 gram in some experiments. We will not discuss it here as it is out of the scope of this course.

Let y_s is the response of standard preparation and y_t of the test preparation. If doses of the standard and test preparations under trial are denoted by x_s and x_t, respectively, the dose-response regression models, assuming that they are linear, can be stated as:

For the standard preparation

$$\hat{y}_s = \hat{\alpha} + \hat{\beta}_s x_s \quad \ldots (2)$$

For the test preparation

$$\hat{y}_t = \hat{\alpha} + \hat{\beta}_t x_t \quad \ldots (3)$$

where, α is the intercept; β_s and β_t are the slopes of the regression lines. $\hat{\alpha}$, $\hat{\beta}_s$ and $\hat{\beta}_t$ are the estimates of intercept and slopes, respectively, as you have already studied in Unit 6.

Note that both regression lines have the same intercepts, i.e., $\alpha_s = \alpha_t = \alpha$ as given in equations (2) and (3). However, the slopes $(\beta_s \text{ and } \beta_t)$ differ in case of slope-ratio assay. You may have noted in Unit 6 that in case of parallel line assays, intercepts were different but slopes were the same.

In bioassay, we fit regression models to assess the dose-response relationship and to estimate the relative potency. For this purpose, we need to compute the sum of squares and cross-products (i) groupwise and (ii) combined. The sum of squares and cross-products required in fitting of the regression models as well as in computing the relative potency are as follows:

(i) Groupwise computation

Let us first define the various terms which are required for groupwise computation of each preparation separately. The means, sum of squares and cross-products of the response and doses calculated separately for the blank, standard and test preparations are defined as:

For Blank dose:

$$\bar{y}_b = \frac{1}{M_b}\sum_{j=1}^{M_b} y_{bj}\,,\ \bar{x}_b = 0\,, \text{ and } \ S_{y_b y_b} = \sum_{j=1}^{M_b}\left(y_{bj} - \bar{y}_b\right)^2 = \sum_{j=1}^{M_b} y_{bj}^2 - M_b \bar{y}_b^2$$

For standard preparation:

$$\bar{y}_s = \frac{1}{M_s}\sum_{i=1}^{K}\sum_{j=1}^{M_{si}} y_{sij}\,,\ \bar{x}_s = \frac{1}{M_s}\sum_{i=1}^{K}\sum_{j=1}^{M_{si}} x_{si} = \frac{1}{M_s}\sum_{i=1}^{M_s} M_{si} x_{si}\,,$$

$$S_{y_s y_s} = \sum_{i=1}^{K}\sum_{j=1}^{M_{si}}\left(y_{sij} - \bar{y}_s\right)^2 = \sum_{i=1}^{K}\sum_{j=1}^{M_{si}} y_{sij}^2 - M_s \bar{y}_s^2\,,$$

$$S_{x_s x_s} = \sum_{i=1}^{K}\sum_{j=1}^{M_{si}}\left(x_{si} - \bar{x}_s\right)^2 = \sum_{i=1}^{K}\sum_{j=1}^{M_{si}} x_{si}^2 - M_s \bar{x}_s^2\,, \text{ and}$$

$$S_{y_s x_s} = \sum_{i=1}^{K}\sum_{j=1}^{M_{si}}\left(y_{sij} - \bar{y}_s\right)\left(x_{si} - \bar{x}_s\right) = \sum_{i=1}^{K}\sum_{j=1}^{M_{si}} y_{sij} x_{si} - M_s \bar{y}_s \bar{x}_s$$

For test preparation:

$$\bar{y}_t = \frac{1}{M_t}\sum_{i=1}^{L}\sum_{j=1}^{M_{ti}} y_{tij}\,,\ \bar{x}_t = \frac{1}{M_t}\sum_{i=1}^{M_t} M_{ti} x_{ti}\,,$$

$$S_{y_t y_t} = \sum_{i=1}^{L}\sum_{j=1}^{M_{ti}}\left(y_{tij} - \bar{y}_t\right)^2 = \sum_{i=1}^{L}\sum_{j=1}^{M_{ti}} y_{tij}^2 - M_t \bar{y}_t^2$$

$$S_{x_t x_t} = \sum_{i=1}^{L}\sum_{j=1}^{M_{ti}} (x_{ti} - \bar{x}_t)^2 = \sum_{i=1}^{L}\sum_{j=1}^{M_{ti}} x_{ti}^2 - M_t \bar{x}_t^2$$

$$S_{y_t x_t} = \sum_{i=1}^{L}\sum_{j=1}^{M_{ti}} (y_{tij} - \bar{y}_t)(x_{ti} - \bar{x}_t) = \sum_{i=1}^{L}\sum_{j=1}^{M_{ti}} y_{tij} x_{ti} - M_t \bar{y}_t \bar{x}_t$$

(ii) Combined computation

We now define various required formulae based on all N observations of all preparations shown in Table 4 as:

$$\bar{y}' = \frac{1}{N}\sum_{h=1}^{N} y_h \,,\ \bar{x}_s' = \frac{1}{N}\sum_{h=1}^{N} x_{sh} \,,\ \bar{x}_t' = \frac{1}{N}\sum_{h=1}^{N} x_{th}$$

$$S'_{yy} = \sum_{h=1}^{N} (y_h - \bar{y}')^2 = \sum_{h=1}^{N} y_h^2 - N\bar{y}'^2$$

Note that we are denoting the sum of squares and cross-products with S′ if we use all N observations in the computation and with S if we consider groupwise observations separately for each preparation.

$$S'_{x_s x_s} = \sum_{h=1}^{N} (x_{sh} - \bar{x}_s')^2 = \sum_{h=1}^{N} x_{sh}^2 - N\bar{x}_s'^2$$

$$S'_{x_t x_t} = \sum_{h=1}^{N} (x_{th} - \bar{x}_t')^2 = \sum_{h=1}^{N} x_{th}^2 - N\bar{x}_t'^2$$

$$S'_{x_s x_t} = \sum_{h=1}^{N} (x_{sh} - \bar{x}_s')(x_{th} - \bar{x}_t') = \sum_{h=1}^{N} x_{sh} x_{th} - N\bar{x}_s'\bar{x}_t' = -N\bar{x}_s'\bar{x}_t'$$

$$S'_{yx_s} = \sum_{h=1}^{N} (y_h - \bar{y}')(x_{sh} - \bar{x}_s') = \sum_{h=1}^{N} y_h x_{sh} - N\bar{y}'\bar{x}_s'$$

$$S'_{yx_t} = \sum_{h=1}^{N} (y_h - \bar{y}')(x_{th} - \bar{x}_t') = \sum_{h=1}^{N} y_h x_{th} - N\bar{y}'\bar{x}_t'$$

We need the estimates of β_s and β_t which are denoted by $\hat{\beta}_s$ and $\hat{\beta}_t$, respectively, to determine the relative potency. β_s and β_t are the slopes of regression lines for the standard and test preparation, respectively.

Remember that the subjects who receive the test dose will not receive the standard dose, and vice-versa. Also, who receive the blank dose will not receive both standard and test doses. So one or both (standard and test) doses will be zero in slope-ratio for all subjects. For estimating the common intercept (α) and slopes (β_s and β_t), we redefine the dose-response relationship in the form of a multiple linear regression model as:

$$y = \hat{\alpha} + \hat{\beta}_s x_s + \hat{\beta}_t x_t \qquad \ldots (4)$$

You will study how to estimate the values of regression coefficients in case of multiple linear regression in Unit 7 of MSTE-004. So, without going into the mathematics here, the estimates $\hat{\beta}_s$ and $\hat{\beta}_t$ are obtained by solving the following two linear equations which are known as normal equations:

$$S'_{x_s x_s}\beta_s + S'_{x_s x_t}\beta_t = S'_{yx_s} \qquad \ldots (5)$$

$$S'_{x_s x_t}\beta_s + S'_{x_t x_t}\beta_t = S'_{yx_t} \qquad \ldots (6)$$

or, you can directly obtain the values of $\hat{\beta}_s$ and $\hat{\beta}_t$ as:

$$\hat{\beta}_s = \frac{S'_{yx_s} S'_{x_t x_t} - S'_{yx_t} S'_{x_s x_t}}{S'_{x_s x_s} S'_{x_t x_t} - S'^2_{x_s x_t}} \quad \ldots (7)$$

$$\hat{\beta}_t = \frac{S'_{yx_t} S'_{x_s x_s} - S'_{yx_s} S'_{x_s x_t}}{S'_{x_s x_s} S'_{x_t x_t} - S'^2_{x_s x_t}} \quad \ldots (8)$$

The value of $\hat{\alpha}$ is obtained as:

$$\hat{\alpha} = \bar{y}' - \hat{\beta}_s \bar{x}'_s - \hat{\beta}_t \bar{x}'_t \quad \ldots (9)$$

In slope-ratio assay, we also fit the regression model including the blank dose. We consider a variable say x_b^* corresponding to blank dose which contains value 1 corresponding to subjects who receive blank dose and 0 for the subjects receiving standard and test doses. We then fit a multiple linear regression model including blank dose as:

$$y = \alpha' + \beta'_b x_b^* + \beta'_s x_s + \beta'_t x_t \quad \ldots (10)$$

We can obtain the values of these regression coefficients (β'_s, β'_t and β'_b) by solving the following three normal equations:

$$S'_{x_s x_s}\beta'_s + S'_{x_s x_t}\beta'_t + S'_{x_s x_b}\beta'_b = S'_{yx_s} \quad \ldots (11)$$

$$S'_{x_t x_s}\beta'_s + S'_{x_t x_t}\beta'_t + S'_{x_t x_b}\beta'_b = S'_{yx_t} \quad \ldots (12)$$

$$S'_{x_b x_s}\beta'_s + S'_{x_b x_t}\beta'_t + S'_{x_b x_b}\beta'_b = S'_{yx_b} \quad \ldots (13)$$

The value of $\hat{\alpha}$ is obtained as:

$$\hat{\alpha}' = \bar{y} - \hat{\beta}'_b \bar{x}_b^{*'} - \hat{\beta}'_s \bar{x}'_s - \hat{\beta}'_t \bar{x}'_t \quad \ldots (14)$$

where

$$\bar{x}_b^{*'} = \frac{1}{N}\sum_{h=1}^{N} x_{bh}^*,\ S'_{x_s x_b} = -N\bar{x}'_s\bar{x}_b^{*'},\ S'_{x_t x_b} = -N\bar{x}'_t\bar{x}_b^{*'},\ S'_{yx_b} = \sum_{h=1}^{N} y_h x_{bh}^* - N\bar{y}'\bar{x}_b^{*'}$$

and $S'_{x_b x_b} = \sum_{h=1}^{N} x_{bh}^{*2} - N\bar{x}_b^{*'2}$

Hopefully, you know how to solve these linear equations. Following illustration will help you to recollect the procedure used to solve two and three simultaneous equations and fit the regression models for slope-ratio assay.

Example 3: For the data given in Table 5, fit regression models for the standard and test preparation with or without involving the blank dose in the model.

Solution: We consider a variable x_b^* in Column (8) of Table 5 having value 1 for the blank dose and 0 for the standard as well as test doses. We compute the $x_s x_t, yx_s, yx_t, x_s x_s, x_t x_t$, yy, yx_b^* and x_b^* and arrange in Columns 9 to 16 of Table 5. From Table 5, we obtain the required summations for blank, standard and test preparations given as follows:

For blank dose

$$M_b = 5,\ \sum_{j=1}^{M_b} y_{bj} = 25, \sum_{j=1}^{M_b} y_{bj}^2 = 135, \sum_{j=1}^{M_b} x_{bj} = 0$$

We determine the sum of squares $S_{y_b y_b}$ as:

$$S_{y_b y_b} = 135 - 5\left(\frac{25}{5}\right)^2 = 135 - 125 = 10$$

For Standard preparation

$$M_s = 13,\ \sum_{j=1}^{M_s} y_{sj} = 135,\ \sum_{j=1}^{M_s} x_{sj} = 123,\ \sum_{i=1}^{K}\sum_{j=1}^{M_s} y_{sij}^2 = 1497,\ \sum_{j=1}^{M_s} y_{sj}x_{sj} = 1426,$$

$$\sum_{i=1}^{K} x_{si}^2 = 1483$$

We obtain the sum of squares and cross-products for standard preparation as:

$$S_{x_s x_s} = 1483 - 13\left(\frac{123}{13}\right)^2 = 1483 - 1163.7692 = 319.2308$$

$$S_{y_s y_s} = 1497 - 13\left(\frac{135}{13}\right)^2 = 1479 - 1401.9231 = 95.0769$$

$$S_{y_s x_s} = 1426 - 13\left(\frac{135}{13}\right)\left(\frac{123}{13}\right) = 1426 - 1277.3077 = 148.6923$$

For test preparation

$$M_t = 12,\ \sum_{j=1}^{M_t} y_{tj} = 178,\ \sum_{j=1}^{M_t} x_{tj} = 117,\ \sum_{i=1}^{L}\sum_{i=1}^{M_t} y_{tij}^2 = 3178,\ \sum_{j=1}^{M_t} y_{tj}x_{tj} = 2244,$$

$$\sum_{i=1}^{L} x_{ti}^2 = 1641$$

The sum of squares and cross-products for test preparation are computed as:

$$S_{y_t y_t} = 3178 - 12\left(\frac{178}{12}\right)^2 = 3178 - 2640.3333 = 537.6667$$

$$S_{x_t x_t} = 1641 - 12\left(\frac{117}{12}\right)^2 = 1641 - 1140.75 = 500.25$$

$$S_{y_t x_t} = 2244 - 12\left(\frac{178}{12}\right)\left(\frac{117}{12}\right) = 2244 - 1735.5 = 508.5$$

For combined data

$$N = M_b + M_s + M_t = 5 + 13 + 12 = 30$$

$$\sum_{h=1}^{N} y_h = 338,\ \sum_{h=1}^{N} x_{sh} = 123,\ \sum_{h=1}^{N} x_{th} = 117,\ \sum_{h=1}^{N} y_h^2 = 4810,\ \sum_{h=1}^{N} y_h x_{sh} = 1426,$$

$$\sum_{h=1}^{N} y_h x_{th} = 2244,\ \sum_{h=1}^{N} x_{sh}^2 = 1483,\ \sum_{h=1}^{N} x_{th}^2 = 1641,\ \sum_{h=1}^{N} x_{sh}x_{th} = 0,$$

$$\sum_{h=1}^{N} y_h x_{bh}^* = 25,\ \sum_{h=1}^{N} x_{bh}^{*2} = 5,\ \bar{y}' = 11.2667,\ \bar{x}_s' = 4.1,\ \bar{x}_t' = 3.9 \text{ and } \bar{x}_b^{*'} = 0.1667$$

We now compute the sum of squares and cross-products for combined data as:

$$S'_{yy} = 4810 - 30\left(\frac{338}{30}\right)^2 = 4810 - 3808.1333 = 1001.8667$$

$$S'_{yx_s} = 1426 - 30\left(\frac{338}{30}\right)\left(\frac{123}{30}\right) = 1426 - 1385.38 = 40.2$$

$$S'_{yx_t} = 2244 - 30\left(\frac{338}{30}\right)\left(\frac{117}{30}\right) = 2244 - 1318.2 = 925.8$$

$$S'_{x_sx_s} = 1483 - 30\left(\frac{123}{30}\right)^2 = 1483 - 504.3 = 978.7$$

$$S'_{x_tx_t} = 1641 - 30\left(\frac{117}{30}\right)^2 = 1641 - 456.3 = 1184.7$$

$$S'_{x_sx_t} = 0 - 30\left(\frac{123}{30}\right)\left(\frac{117}{30}\right) = -479.7$$

$S'_{x_sx_b} = -30 \times 4.1 \times 0.1667 = -20.5$, $S'_{x_tx_b} = -30 \times 3.9 \times 0.1667 = -19.5$,

$S'_{yx_b} = 25 - 30 \times 338 \times 0.1667 = -31.3333$ and

$S'_{x_bx_b} = 5 - 30 \times (0.1667)^2 = 4.1667$

These calculations have been done to several decimal places and you may find slight difference because of approximation when you do calculations by hand.

(i) We define the normal equations for multiple regression model given in equation (4) as:

$$978.7\beta_s - 479.7\beta_t = 40.2 \quad \ldots (15)$$

$$-479.7\beta_s + 1184.7\beta_t = 925.8 \quad \ldots (16)$$

Let rewrite equation (15) as:

$$\beta_s = (40.2 + 479.7\beta_t)/978.7 \quad \ldots (17)$$

After Substituting the value of β_s from equation (17) to equation (16), we obtain

$$-479.7 \times (40.2 + 479.7\beta_t)/978.7 + 1184.7\beta_t = 925.8$$

or, $-19.7036 + 949.5799\beta_t = 925.8$

or, $\hat{\beta}_t = (925.8 + 19.7036)/(949.5799) = 0.9957$

The value of $\hat{\beta}_s$ is obtained after substituting the value of $\hat{\beta}_t$ in equation (15) as:

$$\hat{\beta}_s = (40.2 + 479.7 \times 0.9957)/978.7 = 0.5291$$

We can also obtain the values of $\hat{\beta}_s$ and $\hat{\beta}_t$ directly using equations (7) and (8) as:

$$\hat{\beta}_s = \frac{40.2 \times 1184.7 - 925.8 \times (-479.7)}{978.7 \times 1184.7 - (-479.7)^2} = \frac{491731.2}{929353.8} = 0.5291$$

$$\hat{\beta}_t = \frac{925.8 \times 978.7 - 40.2 \times (-479.7)}{978.7 \times 1184.7 - (-479.7)^2} = \frac{925364.4}{929353.8} = 0.9957$$

The value of $\hat{\alpha}$ is obtained from equation (9) as:

$$\hat{\alpha} = 11.2667 - 0.5291 \times 4.1 - 0.9957 \times 3.9 = 5.2141$$

A multiple linear regression model without blank dose can be defined as:

$$y = 5.2141 + 0.5291 x_s + 0.9957 x_t$$

For the standard preparation, the regression line is obtained by substituting $x_t = 0$ as:

$$y_s = 5.2141 + 0.5291 x_s$$

For the test preparation (i.e., $x_s = 0$), the regression line is given as:

$$y_t = 5.2141 + 0.9957 x_t$$

(ii) From equations (11) to (13), the normal equations for multiple regression model with blank given equation (10) are defined as:

$$978.7\beta'_s + -479.7\beta'_t + -20.5\beta'_b = 40.2 \quad \ldots (18)$$

$$-479.7\beta'_s + 1184.7\beta'_t + -19.5\beta'_b = 925.8 \quad \ldots (19)$$

$$-20.5\beta'_s + -19.5\beta'_t + 4.1667\beta'_b = -31.3333 \quad \ldots (20)$$

The value of $\hat{\beta}'_b$, $\hat{\beta}'_s$ and $\hat{\beta}'_t$ are obtained after solving these normal equations as:

$$\hat{\beta}'_b = -0.3798,\ \hat{\beta}'_s = 0.5154 \text{ and } \hat{\beta}'_t = 0.9839$$

The value of $\hat{\alpha}'$ is obtained from equation (14) as:

$$\hat{\alpha}' = 11.2667 + 0.3798 \times 0.1667 - 0.5154 \times 4.1 - 0.9839 \times 3.9 = 5.3798$$

A multiple linear regression model including blank dose given is given as:

$$y = 5.3798 - 0.3798 x_b + 0.5154 x_s + 0.9839 x_t$$

The estimation of relative potency is valid only when the data obtained from the experiment follow certain validity conditions which will be discussed in the next section.

If you have understood these computations, you should be able to do the following exercise.

E4) In a 7-point slope-ratio assay, one blank dose, three doses of standard and test drugs were applied on 3 guinea pigs each. The experimenter computed the following values for recorded responses and doses:

For blank dose

$$\sum_{j=1}^{M_b} y_{bj} = 20.5,\ \sum_{j=1}^{M_b} y_{bj}^2 = 141.25,\ \sum_{j=1}^{M_b} x_{bj}^* = 3,\ \sum_{j=1}^{M_b} y_{bj} x_{bj}^* = 20.5,\ \sum_{j=1}^{M_b} x_{bj}^{*2} = 3$$

For Standard preparation

$$\sum_{j=1}^{M_s} y_{sj} = 216.5, \sum_{j=1}^{M_s} x_{sj} = 90, \sum_{i=1}^{K}\sum_{j=1}^{M_s} y_{sij}^2 = 5487.75, \sum_{j=1}^{M_s} y_{sj}x_{sj} = 2365,$$

$$\sum_{i=1}^{K} x_{si}^2 = 1050$$

For test preparation

$$\sum_{j=1}^{M_t} y_{tj} = 317, \sum_{j=1}^{M_t} x_{tj} = 72, \sum_{i=1}^{L}\sum_{i=1}^{M} y_{tij}^2 = 12307.5, \sum_{j=1}^{M_t} y_{tj}x_{tj} = 2866,$$

$$\sum_{i=1}^{L} x_{ti}^2 = 672$$

(i) Determine the sum of squares and cross-products for individual as well as combined data.

(ii) Also fit regression models with or without considering the blank dose in the model.

7.5 VALIDITATION OF CONDITIONS FOR A SLOPE RATIO ASSAY

As already mentioned, the relative potency computed for a slope-ratio assay is valid only after certain statistical tests are done. We check the significance of (i) regression (equal slopes), (ii) intercept and (iii) linearity of dose-response relationship. For carrying out these tests, we need to set up an analysis of variance (ANOVA) table similar to one we have prepared for parallel line assay. We are interested to test the following null hypotheses to check the validity assumptions of a slope-ratio assay:

Note the following points:

- The sum of squares for deviation from linearity (SSL) is based only on the standard and test preparations which is excluding the blank dose.
- We cannot determine the sum of squares for non-linearity in case of two-dose assay.

(i) Regression or Equal slope:

H_0: There is no significant difference between slopes of the standard and test preparations.

(ii) Equal intercepts or Interaction:

H_0: There is no significance difference between intercepts of the standard and test preparations, i.e., there exists a common intercept.

(iii) Deviation from Linearity:

H_0: There is no significant deviation from linearity between both regression models, i.e., both models are linear.

(iv) Difference between Doses:

H_0: There is no significant difference among the doses.

(v) Blank Dose:

H_0: There is no significance invalidity of the blank dose.

The computation and formulae of total sum of squares (TSS), sum of squares between doses (SSD) and sum of squares due to error (SSE) are same as explained in Unit 6. Let us briefly define these sums of squares and their respective degrees of freedom as:

- **Total Sum of Squares (TSS)**

  Degrees of freedom = (N−1)

$$TSS = S'_{yy} = \sum_{h=1}^{N} y_h^2 - N\bar{y}'^2 \quad \ldots (21)$$

- **Sum of Squares between Doses (SSD)**

  Degrees of freedom = (K + L)

$$SSD = \left(M_b \bar{y}_b^2 + \sum_{i=1}^{K} M_{si} \bar{y}_{si}^2 + \sum_{i=1}^{L} M_{ti} \bar{y}_{ti}^2 \right) - N\bar{y}'^2 \quad \ldots (22)$$

- **Sum of Squares due to error or within doses (SSE)**

  Degrees of freedom= [N – (K + L) – 1]

  SSE = TSS – SSD … (23)

- **Sum of Squares due to Regression or equal slopes**

  Degree of freedom = 1

$$SSR = S'_{yx_s} \hat{\beta}_s + S'_{yx_t} \hat{\beta}_t \quad \ldots (24)$$

- **Sum of squares for Blanks (SSB)**

  If we are considering any blank dose in the assay, we also use an additional sum of squares which is for this blank dose. We consider a multiple linear regression model with blank dose defined in equation (10) to obtain the sum of squares for blanks. The sum of squares for blank with 1 degree of freedom is computed as:

$$SSB = \left(S'_{yx_b} \hat{\beta}'_b + S'_{yx_s} \hat{\beta}'_s + S'_{yx_t} \hat{\beta}'_t \right) - SSR \quad \ldots (25)$$

- **Sum of Squares due to equal intercepts or Interaction**

  Degrees of freedom = 1

  SSI = SSD – SSR – SSL – SSB … (26)

- **Sum of Squares due to Deviation from Linearity**

  Degrees of freedom = (K + L – 4)

$$SSL = \left(\sum_{i=1}^{K} M_{si} \left(\bar{y}_{si} - \bar{y}_s \right)^2 + \sum_{i=1}^{L} M_{ti} \left(\bar{y}_{ti} - \bar{y}_t \right)^2 \right) - \left[\frac{\left(S_{y_s x_s} \right)^2}{S_{x_s x_s}} + \frac{\left(S_{y_t x_t} \right)^2}{S_{x_t x_t}} \right]$$

$$= \left(\sum_{i=1}^{K} M_{si} \bar{y}_{si}^2 - M_s \bar{y}_s^2 \right) + \left(\sum_{i=1}^{L} M_{ti} \bar{y}_{ti}^2 - M_t \bar{y}_t^2 \right) - \left[\frac{\left(S_{y_s x_s} \right)^2}{S_{x_s x_s}} + \frac{\left(S_{y_t x_t} \right)^2}{S_{x_t x_t}} \right]$$

… (27)

The mean sum of squares, F values and interpretation will be same as explained in Unit 6. All calculations and formulae explained in this section can be summarise in the form of ANOVA table shown in Table 6.

Table 6: ANOVA for (K + L+1)-point slope-ratio assay

Source of Variation	d.f.	Sum of squares (SS)	Mean Sum of Squares $\mathbf{MS} = \left(\frac{\mathbf{SS}}{\mathbf{df}}\right)$	F_{cal}
Regression (Equal Slopes)	2	SSR	MSR	$F_P = \frac{MSR}{MSE}$
Blanks	1	SSB	MSB	$F_P = \frac{MSB}{MSE}$
Equal intercepts (Interaction)	1	SSI	MSI	$F_R = \frac{MSI}{MSE}$
Deviation from Linearity	(K + L) – 4	SSL	MSL	$F_L = \frac{MSL}{MSE}$
Between Doses	(K + L)	SSD	MSD	$F_D = \frac{MSD}{MSE}$
Error (within doses)	N – (K + L) – 1	SSE	MSE	
Total	N – 1	TSS		

Remember to check the validity assumptions before estimating the relative potency.

Decision about null hypotheses

If $F_{cal} \geq F_{tab}$, we may reject H_0 at α % level of significance, otherwise we do not reject H_0. It implies that we do not have enough evidence against H_0.

Let us solve an example so that you may comprehend how to apply ANOVA method to test the validity conditions in a (4+3+1)-point slope-ratio assay.

Example 4: Consider the data given in Example 3 and test the validity conditions necessary to estimate the relative potency of rice preparation for a slope-ratio assay.

Solution: From the solution of Example 3, we have

$$S'_{yy} = 1001.8667$$

The total sum of squares is obtained as:

$$TSS = S'_{yy} = 1001.8667$$

We obtain $M_b \bar{y}_b^2 = 5 \times 5^2 = 125$,

$$\sum_{i=1}^{L} M_{ti} \bar{y}_{ti}^2 = 5 \times 8^2 + 3 \times 15^2 + 4 \times 23.25^2 = 3157.25$$

$$\sum_{i=1}^{K} M_{si} \bar{y}_{si}^2 = 3 \times 7.6667^2 + 5 \times 10^2 + 3 \times 10.3333^2 + 2 \times 15.5^2 = 1477.1667$$

We obtain the sum of squares between doses as:

$$SSD = \left(M_b \bar{y}_b^2 + \sum_{i=1}^{K} M_{si} \bar{y}_{si}^2 + \sum_{i=1}^{L} M_{ti} \bar{y}_{ti}^2 \right) - N \bar{y}'^2$$

$$= (125+1477.1667+3157.25) - (30 \times 11.2667^2)$$

$$= 4759.4167 - 3808.1333 = 951.2833$$

We compute the sum of squares due to deviation from linearity as:

Note the following points:

- The sum of squares for deviation from linearity (SSL) is based only on the standard and test preparations which is excluding the blank dose.
- We cannot determine the sum of squares for non-linearity in case of two-dose assay.

$$SSL = \left(\sum_{i=1}^{K} M_{si}\bar{y}_{si}^2 - M_s\bar{y}_s^2\right) + \left(\sum_{i=1}^{L} M_{ti}\bar{y}_{ti}^2 - M_t\bar{y}_t^2\right) - \left[\frac{\left(S_{y_s x_s}\right)^2}{S_{x_s x_s}} + \frac{\left(S_{y_t x_t}\right)^2}{S_{x_t x_t}}\right]$$

$$= (1477.1667 - 13(10.3846)^2) + (3157.25 - 12(14.8333)^2)$$

$$- \left[\frac{(148.6923)^2}{319.2308} + \frac{(508)^2}{500.25}\right]$$

$$= (75.2436+516.9167) - (69.2584 +516.8861) = 592.1603 - 586.1444$$

$$= 6.0158$$

We obtain the sum of squares due to regression or equal slopes as:

$$SSR = S'_{yx_s}\hat{\beta}_s + S'_{yx_t}\hat{\beta}_t = 40.2\times0.5291 + 925.8\times0.9957 = 943.0961$$

We determine the sum of squares for blank with 1 degree of freedom as:

$$SSB = \left(S'_{yx_b}\hat{\beta}'_b + S'_{yx_s}\hat{\beta}'_s + S'_{yx_t}\hat{\beta}'_t\right) - SSR$$

$$= \left[(-31.3333)\times(-0.3798) + 40.2\times0.5154 + 925.8\times0.9839\right] - 943.0961$$

$$= 0.4065$$

The sum of squares for equal intercepts is obtained as:

SSI = SSD – SSR – SSL – SSB

= 951.2833–943.0961 – 6.0158 – 0.4065 = 1.7649

We compute the sum of squares due to error as:

SSE = TSS – SSD =1001.8667 – 951.2833 = 50.5833

We are now ready to build an ANOVA table for the data given as follows:

Table 7: ANOVA for slope-ratio assay

Source of Variation	df	SS	MSS	F_{cal}	F_{tab}	Decision
Regression or Slopes	2	943.0961	471.5481	205.0884	3.4434	Reject
Blank	1	0.4065	0.4065	0.1768	4.3009	Do not reject
Intercepts	1	1.7649	1.7649	0.7676	4.3009	Do not reject
Linearity	(4+3) – 4 = 3	6.0158	2.0053	0.8721	3.0491	Do not reject
Between Doses	4+3 = 7	951.2833	135.8976	59.1054	2.4638	Reject
Error	29 – 7 = 22	50.5833	2.2992			
Total	30 – 1 = 29	1001.8667				

Interpretation:

1. **Slopes**: Since F_{cal}= 205.0884 >3.4434, which is too high and statistically significant. This indicates that slope of regression for the test preparation is different from slope of the standard preparation.

2. **Intercepts**: The F_{cal} is 0.7676, which is less than the tabulated value 4.3009. This hypothesis may not be rejected. Thus, the regression lines for standard and test preparations can be considered to have same intercept.
3. **Linearity**: In the same way, $F_{cal} = 0.8721 < 3.0491$. Thus, the null hypothesis for linearity cannot be rejected and it can be safely concluded that fitted regression models of the test and standard preparations are linear.
4. In the same way, we can infer that the blank and difference among the doses are also significant.

Since all these conditions of validity of the slope-ratio assay are fulfilled, we can safely say that the estimate of relative potency computed in Sec. 7.6 is a valid estimate.

Note that if slopes are equal and intercepts are different, this becomes a parallel line assay. The different slopes make it a valid slope-ratio assay.

The solution of this example of the slope-ratio assay given in Table 7 is for unequal subjects and unequal doses. The calculations for equal subjects, i.e., $M_{si} = M_{ti}$ and equal doses, i.e., K = L are relatively simple. Now, let us try the following exercise.

To ensure that you have understood this method clearly, solve the following exercise.

E5) For the results of a slope-ratio assay given in **E4,** the following values are also given:

$$M_b\bar{y}_b^2 = 140.0833,\ \sum_{i=1}^{K} M_{si}\bar{y}_{si}^2 = 5475.5833 \text{ and } \sum_{i=1}^{L} M_{ti}\bar{y}_{ti}^2 = 12303.8333$$

Check validity conditions of the relative potency at 5% level of significance.

7.6 RELATIVE POTENCY AND ITS FIDUCIAL LIMITS

Towards the end of this unit, let us discuss how to calculate the relative potency and its fiducial limits for a slope-ratio assay. If we consider regression model with blank, standard and test preparations, the values of slopes and sum of squares considered in the formulae defined for relative potency and fiducial limits will be corresponding to the regression model with blank dose. For the sake of simplicity, let us assume the regression model given in equation (4) without including the blank dose to compute the relative potency.

If we consider the dose-metameter $x = d^{\lambda};\ \lambda > 0$ in a slope-ratio assay, the estimated relative potency is defined as ratio of slopes of regression models of the test and standard preparations as:

$$R = \left(\frac{\hat{\beta}_t}{\hat{\beta}_s}\right)^{\frac{1}{\lambda}} \qquad \ldots (28)$$

In the same way, we can also determine the relative potency including the

blank dose in the model which is given in equation (10) as:

$$R = \left(\frac{\hat{\beta}'_t}{\hat{\beta}'_s}\right)^{\frac{1}{\lambda}} \quad \ldots (29)$$

As discussed in Units 5 and 6, the relative potency is a ratio and the fiducial limits of the relative potency can be calculated based on the Fieller's theorem. The (1–α)100% fiducial limits for the relative potency in case of slope-ratio assay can be determined as:

$$(R_L, R_U) = \frac{R - g\frac{S'_{x_s x_s}}{S'_{x_s x_t}} \mp \frac{ts}{\hat{\beta}_s}\sqrt{\frac{(1-g)}{S'_{x_t x_t}} - \frac{2R}{S'_{x_s x_t}} + \frac{R^2}{S'_{x_s x_s}} + g\frac{S'_{x_s x_s}}{S'^2_{x_s x_t}}}}{(1-g)} \quad \ldots (30)$$

For the sake of simplicity, we are assuming that the standard and test doses are independent i.e., $S_{x_s x_t} = 0$. In this case, we obtain the (1–α)100% fiducial limits for the relative potency as:

$$(R_L, R_U) = \frac{R \mp \frac{ts}{\hat{\beta}_s}\sqrt{\frac{(1-g)}{S'_{x_t x_t}} + \frac{R^2}{S'_{x_s x_s}}}}{1-g} \quad \ldots (31)$$

where

- $g = \frac{t^2 s^2}{\hat{\beta}_s^2 S'_{x_s x_s}}$ (In most of the cases, g is a small number and can be neglected)
- $\hat{\beta}_s$ is the estimated regression coefficient for the standard preparation,
- s^2 is the mean sum of squares for error which can be obtained from ANOVA Table 6, i.e., $s^2 = MSE$, and
- $t = t_{\upsilon,\alpha/2}$ is a two-tailed tabulated value of Student's t distribution at (1 – α)100% confidence with ν =(N – K – L – 1) which is the degrees of freedom of the mean sum of squares due to error (MSE).

The variance and covariance of the regression coefficients are defined as:

$$Var(\hat{\beta}_s) = \frac{s^2}{S'_{x_s x_s}},$$

$$Var(\hat{\beta}_t) = \frac{s^2}{S'_{x_t x_t}}, \text{ and}$$

$$Covar(\hat{\beta}_s, \hat{\beta}_t) = \frac{s^2}{S'_{x_s x_t}}$$

Let us solve an example to get a better idea about the computation of relative potency and its fiducial limits.

Example 5: For the data of a slope-ratio assay given in Example 2, obtain the relative potency and its 95% fiducial limits.

Solution: From the solution of Example 3, we have

$\hat{\beta}_s = 0.5291,\ \hat{\beta}_t = 0.9957,\ \hat{\beta}'_s = 0.5154,\ \hat{\beta}'_t = 0.9839,\ S'_{x_s x_s} = 978.7$, and $S'_{x_t x_t} = 1184.7$

Since we considered dose-metameter as dose itself, i.e., $x = d$ for $\lambda = 1$, we estimate the relative potency using the formulae given in equations (28) and (29) as:

Without blank dose in regression model:

$$R = \frac{\hat{\beta}_t}{\hat{\beta}_s} = \frac{0.9957}{0.5291} = 1.8819$$

With blank dose in regression model:

$$R = \frac{\hat{\beta}'_t}{\hat{\beta}'_s} = \frac{0.9839}{0.5154} = 1.9091$$

It is clear that the relative potency with or without blank dose are only slightly varying. We can say that the test preparation is nearly twice ($\cong 1.9$ times) as potent as the standard preparation in this experiment. In other words, the rice protein is 1.9 times as effective in raising digestibility score as egg protein for the given data.

Note that if x is a dose-metameter such as $x = (\text{dose})^{\lambda}$, the relative potency will have to be converted back to the original units by taking $R = \left(\frac{\hat{\beta}_t}{\hat{\beta}_s}\right)^{1/\lambda}$

You can find the values of degrees of freedom and MSE from ANOVA table. From Table 7, we have

MSE = 2.2992 and (N – K – L – 1) = 22

Since $\alpha = 0.05$, we obtain $t = t_{22,0.025} = 2.074$ from Table 1 of Appendix given at the end of this block. Let us compute the fiducial limits considering the regression model without blank dose.

The value of g is computed as:

$$g = \frac{t^2 s^2}{\hat{\beta}_s^2 S'_{x_s x_s}} = \frac{(2.074)^2 \times 2.2992}{(0.5291)^2 \times 978.7} = 0.0361$$

We determine 95% fiducial limits using equation (31) as:

$$(R_L, R_U) = \frac{R \mp \frac{ts}{\hat{\beta}_s}\sqrt{\frac{(1-g)}{S'_{x_t x_t}} + \frac{R^2}{S'_{x_s x_s}}}}{1-g}$$

$$= \frac{1.8819 \pm \frac{2.074 \times \sqrt{2.2992}}{0.5291} \times \sqrt{\frac{(1-0.0361)}{1184.7} + \frac{(1.8819)^2}{978.7}}}{1-0.0361}$$

$$= \frac{1.8819 \mp 5.9433 \times \sqrt{0.0044}}{0.9639}$$

$$= \frac{1.8819 \mp 0.3957}{0.9639}$$

$$(R_L, R_U) = (1.5418,\ 2.3628)$$

The chances are less than 5% that the relative potency is less than approximately 1.54 or more than 2.36.

Now, you must try the following exercise for practice.

E8) Calculate 95% fiducial limits of the relative potency in a slope-ratio assay for the data given in **E4** assuming that the validity conditions are fulfilled.

Let us now summarise what you have learnt in this unit.

7.7 SUMMARY

1. The slope-ratio assay is applicable when (i) dose-response relationship is linear for both test and standard preparations, and (ii) response increases faster with one preparation compared with the other preparation.

2. In slope-ratio assay, (i) both regression lines have different slopes, and (ii) regression line of the standard preparation has same intercept as the test preparation.

3. The validity of these conditions can be checked by setting up an appropriate ANOVA table. When all these conditions are validated, the relative potency is obtained as ratio of the slope of test preparation to the slope of standard preparation as:

$$R = \left(\frac{\hat{\beta}_t}{\hat{\beta}_s}\right)^{\frac{1}{\lambda}} \quad ; \quad \lambda > 0$$

4. Under the validity conditions, (1–α)100% fiducial limits for the relative potency can be obtained by using Fieller's theorem as:

$$(R_L, R_U) = \frac{R \mp \frac{ts}{\hat{\beta}_s}\sqrt{\frac{(1-g)}{S'_{x_t x_t}} + \frac{R^2}{S'_{x_s x_s}}}}{1-g}$$

where $g = \frac{t^2 s^2}{\hat{\beta}_s^2 S'_{x_s x_s}}$

7.8 SOULTIONS/ANSWERS

E1) Basic requirements for a slope-ratio assay:

i) Equality of slopes – No, this is required for parallel line assay

ii) Equality of intercepts – Yes

iii) Linearity in dose-response relationship – Yes

iv) Equal number of subjects for test and standard preparations – This is not required but helps in calculations

v) Dose levels must be equi-spaced – No

vi) A blank dose is necessarily needed – Yes

E2) A slope-ratio assay is balanced when the number of subjects receiving different doses of the test as well as standard preparations are the same. It is symmetric when number of doses of the test preparation is the same as of the standard preparation. This makes calculations relatively simple and additionally helps in achieving best efficiency in results in terms of reliability of results.

E3) In Table 4, we observe that

- $M_b = 5$ since 5 subjects have received the blank dose.

- For standard preparation: $M_{s1} = 3$, $M_{s2} = 5$, $M_{s3} = 3$ and $M_{s4} = 2$.
- For test preparation: $M_{t1} = 5$, $M_{t2} = 3$ and $M_{t3} = 4$. These are the number of subjects who received various doses.
- $y_{b2} = 6$ which is the response in second (j = 2) subject receiving blank dose.
- $y_{s24} = 11$ which is the response of forth subject (j = 4) receiving second dose (i = 2) of the standard preparation.
- $y_{t13} = 7$ which is the response of third subject (j = 3) receiving first dose (i = 1) of the test preparation.

E4) We determine the sum of squares $S_{y_b y_b}$ as:

$$S_{y_b y_b} = 141.25 - 3\left(\frac{20.5}{3}\right)^2 = 1.1667$$

We obtain the sum of squares and cross-products for standard preparation as:

$$S_{x_s x_s} = 1050 - 9\left(\frac{90}{9}\right)^2 = 150\,,\ S_{y_s y_s} = 5487.75 - 9\left(\frac{216.5}{9}\right)^2 = 279.7222$$

$$S_{y_s x_s} = 2365 - 9\left(\frac{216.5}{9}\right)\left(\frac{90}{9}\right) = 200$$

The sum of squares and cross-products for test preparation are computed as:

$$S_{y_t y_t} = 12307.5 - 9\left(\frac{317}{9}\right)^2 = 1142.0556\,,\ S_{x_t x_t} = 672 - 9\left(\frac{72}{9}\right)^2 = 96$$

$$S_{y_t x_t} = 2866 - 9\left(\frac{317}{9}\right)\left(\frac{72}{9}\right) = 330$$

From the given data, we can determine the following values for combined data for blank, standard and test preparations as:

$$\sum_{h=1}^{N} y_h = \sum_{j=1}^{M_b} y_{bj} + \sum_{j=1}^{M_s} y_{sj} + \sum_{j=1}^{M_t} y_{tj} = 20.5 + 216.5 + 317 = 554$$

$$\sum_{h=1}^{N} x_{sh} = \sum_{j=1}^{M_s} x_{sj} = 90\,,\ \sum_{h=1}^{N} x_{th} = \sum_{j=1}^{M_t} x_{tj} = 72\,,$$

$$\sum_{h=1}^{N} y_h^2 = \sum_{j=1}^{M_b} y_{bj}^2 + \sum_{i=1}^{K}\sum_{j=1}^{M_s} y_{sij}^2 + \sum_{i=1}^{L}\sum_{i=1}^{M_t} y_{tij}^2 = 141.25 + 5487.75 + 12307.5 = 17936.5,$$

$$\sum_{h=1}^{N} y_h x_{sh} = \sum_{j=1}^{M_s} y_{sj} x_{sj} = 2365\,,\ \sum_{h=1}^{N} y_h x_{th} = \sum_{j=1}^{M_t} y_{tj} x_{tj} = 2866\,,$$

$$\sum_{h=1}^{N} x_{sh}^2 = \sum_{i=1}^{K} x_{si}^2 = 1050\ ,\ \sum_{h=1}^{N} x_{th}^2 = \sum_{i=1}^{L} x_{ti}^2 = 672\,,\ \sum_{h=1}^{N} x_{sh} x_{th} = 0\,,$$

$$\sum_{h=1}^{N} y_h x_{bh}^{*} = \sum_{j=1}^{M_b} y_{bj} x_{bj}^{*} = 20.5\,,\ \sum_{h=1}^{N} x_{bh}^{*2} = \sum_{j=1}^{M_b} x_{bj}^{*2} = 3\,,$$

$$\bar{y}' = 26.3810, \bar{x}_s' = 4.2857, \bar{x}_t' = 3.4286 \text{ and } \bar{x}_b^{*'} = 0.1429$$

We now compute the sum of squares and cross-products for combined data as:

$$S'_{yy} = 17936.5 - 21\left(\frac{554}{21}\right)^2 = 3321.4524,$$

$$S'_{x_s x_s} = 1050 - 21\left(\frac{90}{21}\right)^2 = 664.2857$$

$$S'_{yx_s} = 2365 - 21\left(\frac{554}{21}\right)\left(\frac{90}{21}\right) = -9.2857,$$

$$S'_{x_s x_t} = 0 - 21\left(\frac{90}{21}\right)\left(\frac{72}{21}\right) = -308.5714$$

$$S'_{yx_t} = 2866 - 21\left(\frac{554}{21}\right)\left(\frac{72}{21}\right) = 966.5714,$$

$$S'_{x_t x_t} = 672 - 21\left(\frac{72}{21}\right)^2 = 425.1429,$$

$$S'_{x_s x_b} = -21 \times 4.2857 \times 0.1429 = -12.8571,$$

$$S'_{x_b x_b} = 3 - 21 \times (0.1429)^2 = 2.5714$$

$$S'_{x_t x_b} = -21 \times 4.2857 \times 3.4286 = -10.2857 \text{ and}$$

$$S'_{yx_b} = 20.5 - 21 \times 26.3810 \times 0.1429 = -58.6429$$

(i) The values of $\hat{\beta}_s$ and $\hat{\beta}_t$ using equations (7) and (8) are calculated as:

$\hat{\beta}_s = 1.5722$ and $\hat{\beta}_t = 3.4146$

The value of $\hat{\alpha}$ obtained from equation (9) is:

$\hat{\alpha} = 7.9359$

A multiple linear regression model without blank dose is given as:

$y = 7.9359 + 1.5722\,x_s + 3.4146\,x_t$

For the standard preparation, the regression line is:

$y_s = 7.9359 + 1.5722\,x_s$

For the test preparation, the regression line is:

$y_t = 7.9359 + 3.4146\,x_t$

(ii) We obtain the values of $\hat{\beta}'_b$, $\hat{\beta}'_s$ and $\hat{\beta}'_t$ by solving the normal equations defined in equations (11) to (13) as well as the value of $\hat{\alpha}'$ using equation (14) as:

$\hat{\alpha}' = 9.2222$, $\hat{\beta}'_b = -2.3889$, $\hat{\beta}'_s = 1.4619$ and $\hat{\beta}'_t = 3.2768$

A multiple linear regression model including blank dose given is:

$y = 9.2222 - 2.3889\,x_b + 1.4619\,x_s + 3.2768\,x_t$

E5) From the solution of E4, the total sum of squares is calculated as:

$$TSS = S'_{yy} = 3321.4524$$

We have $M_b\bar{y}_b^2 = 140.0833$, $\sum_{i=1}^{K} M_{si}\bar{y}_{si}^2 = 3304.4524$ and

$$\sum_{i=1}^{L} M_{ti}\bar{y}_{ti}^2 = 12303.8333$$

We obtain the sum of squares between doses as:

$$SSD = (140.0833 + 5475.5833 + 12303.8333) - 21\times(26.3810)^2$$

$$= 3304.4524$$

We compute the sum of squares due to deviation from linearity as:

$$SSL = \left(3304.4524 - \frac{(216.5)^2}{9}\right) + \left(12303.8333 - \frac{(317)^2}{9}\right) - \left[\frac{(200)^2}{150} + \frac{(330)^2}{96}\right]$$

$$= 4.9028$$

We obtain the sum of squares due to regression or equal slopes as:

$$SSR = -9.2857\times1.5722 + 966.5714\times3.4146 = 3285.8622$$

We determine the sum of squares for blank with 1 degree of freedom as:

$$SSB = [(-58.6429)\times(-2.3889) + (-9.2857)\times1.4619 + 966.5714\times3.2768]$$

$$-3285.8622 = 7.9017$$

The sum of squares for equal intercepts is obtained as:

$$SSI = SSD - SSR - SSL - SSB$$

$$= 3304.4524 - 3285.8622 - 3285.8622 - 7.9017 = 5.7857$$

We compute the sum of squares due to error as:

$$SSE = TSS - SSD = 3321.4524 - 3304.4524 = 17$$

We can now construct the ANOVA table by computing the other values as:

Table 8: ANOVA for slope-ratio assay

Source of Variation	df	SS	MSS	F_{cal}	F_{tab}	Decision
Regression or Slopes	2	3285.8622	1642.9311	1353.0021	3.7389	Reject
Blank	1	7.9017	7.9017	6.5073	4.6001	Reject
Linearity	2	4.9028	2.4514	2.0188	3.7389	Do not reject
Intercepts	1	5.7857	5.7857	4.7647	4.6001	Reject
Between Doses	6	3304.4524	550.7421	453.5523	2.8477	Reject
Error	14	17	1.2143			
Total	20	3321.4524				

Interpretation:

1. The slopes of regression models for the standard and test preparation are not same.
2. The regression lines for standard and test preparations do not have same intercept.
3. The null hypothesis for linearity cannot be rejected and it can be concluded that fitted regression models of the test and standard preparations are linear.
4. In the same way, we can infer that the blank dose is not significant while the difference among the doses is significant.

Note that all validity condition of the relative potency are not fulfilled for the given slope-ratio assay as intercept for the standard and test preparations are not same.

E6) From the solution of **E4**, we have

$\hat{\beta}_s = 1.5722$, $\hat{\beta}_t = 3.4146$, $\hat{\beta}'_s = 1.4619$, $\hat{\beta}'_t = 3.2768$, $S'_{x_s x_s} = 664.2857$ and $S'_{x_t x_t} = 425.1429$

If we assume that the validity conditions are fulfilled, the relative potency can be computed using the formulae given in equations (28) and (29) as:

Without blank dose in the regression model:

$$R = \frac{\hat{\beta}_t}{\hat{\beta}_s} = \frac{3.4146}{1.5722} = 2.1719$$

With blank dose in the regression model:

$$R = \frac{\hat{\beta}'_t}{\hat{\beta}'_s} = \frac{3.2768}{1.4619} = 2.2414$$

The test preparation is more than twice (2.2 times) as potent as the standard preparation for this slope-ratio assay.

From Table 8, we have

s^2 = MSE = 1.2143 and (N – K – L – 1) = 17

Since $\alpha = 0.05$, we obtain $t = t_{17,0.025} = 2.145$ from Table 1 of the Appendix.

The value of g is computed as:

$$g = \frac{t^2 s^2}{\hat{\beta}_s^2 S'_{x_s x_s}} = \frac{(2.145)^2 \times 1.2143}{(1.5722)^2 \times 664.2857} = 0.0034$$

We determine 95% fiducial limits using equation (31) without using blank dose as:

$$(R_L, R_U) = \frac{2.1719 \mp \frac{2.145 \times \sqrt{1.2143}}{1.5722} \sqrt{\frac{(1-0.0034)}{425.1429} + \frac{2.1719^2}{664.2857}}}{1-0.0034}$$

$$(R_L, R_U) = (2.0327,\ 2.3259)$$