UDC 615.513

# **Bun-ichi Tamaoki**: On the Analysis of $m \times n$ Point Assay.

(*Pharmaceutical Institute, Medical Faculty, University of Tokyo**)

In the field of biological assay, the $2 \times n$ point assay design has been widely used, where 2 and $n$ respectively denotes two preparations (say, standard preparation and unknown to be examined), and the number of dosage level of each preparation. When more than two preparations are simultaneously assayed and the interest of experimenter is in the relative potency ratios between any pair of preparations, such as in the case of screening test, it would be tiresome and time-consuming to apply the above-mentioned $2 \times n$ point assay to all the possible combinations of pairs among the preparations, as the number of combinations $n(n-1)/2$ increases very rapidly with the increase of the number of preparation $n$.

For the explanation, three preparations (A, B, and C) and two dosage levels (high and low) of each preparation, or $3 \times 2$ point assay design could be described as shown in Table I, using a one-way lay-out.

TABLE I. Design of $3 \times 2$ Point Assay

| | Preparation | | | | | |
|---|---|---|---|---|---|---|
| | A | | B | | C | |
| | H | L | H | L | H | L |
| 1 | $x_{11}$ | $x_{21}$ | $x_{31}$ | $x_{41}$ | $x_{51}$ | $x_{61}$ |
| 2 | $x_{12}$ | $x_{22}$ | $x_{32}$ | $x_{42}$ | $x_{52}$ | $x_{62}$ |
| 3 | $x_{13}$ | $x_{23}$ | $x_{33}$ | $x_{43}$ | $x_{53}$ | $x_{63}$ |
| ⋮ | ⋮ | ⋮ | ⋮ | ⋮ | ⋮ | ⋮ |
| ⋮ | ⋮ | ⋮ | ⋮ | ⋮ | ⋮ | ⋮ |
| n | $x_{1n}$ | $x_{2n}$ | $x_{3n}$ | $x_{4n}$ | $x_{5n}$ | $x_{6n}$ |
| Total | $T_1$ | $T_2$ | $T_3$ | $T_4$ | $T_5$ | $T_6$ |

Here, each ratio of high dose to low of the three preparations should be equal, and the equal number of animals (n) should be allocated to each treatment of the six. For the test of schedasticity, the method of Bartlett[1] or the simplified ones[2~3] could be used. When the heteroschedasticity is found by any of those methods, the calculation of the potency ratios could not be carried out any further. When the homoschedasticity is proved, the analysis of variance and test of validity of the assay could be done as follows:

$$S_{\text{Total}} = x_{11}^2 + x_{12}^2 + \cdots + x_{6n}^2 - T^2/6n$$

where, $T = T_1 + T_2 + \cdots + T_6$

$$S_{\text{Treatment}} = \frac{1}{n}(T_1^2 + T_2^2 + \cdots + T_6^2) - T^2/6n$$

$$S_{\text{Error}} = S_{\text{Total}} - S_{\text{Treatment}}$$

For further analysis of the treatment into the so-called "preparation," "regression," and "parallelism," the coefficients indicated in Table II are used.

For the calculation of "preparation," etc., the subtotals of the response, $T_i$ are respectively multiplied by the corresponding coefficient, then squared, and divided by the divisor, similar to the usage of polynomial orthogonal coefficients for $2 \times n$ point assay.

* Hongo, Tokyo (玉置文一).

1) M. S. Bartlett: Suppl. J. Roy. Stat. Soc., **4**, 137(1937).
2) The United States Pharmacopoeia XV, Mark Publ. Co., U. S. A. (1955).
3) B. Tamaoki: This Bulletin, **2**, 161(1954).

Table II. Coefficients for 3×2 Point Assay

| | A | | B | | C | | Divisor |
|---|---|---|---|---|---|---|---|
| | H | L | H | L | H | L | |
| Preparation | 1 | 1 | −1 | −1 | 0 | 0 | 12n |
| | 0 | 0 | 1 | 1 | −1 | −1 | |
| | −1 | −1 | 0 | 0 | 1 | 1 | |
| Regression | 1 | −1 | 1 | −1 | 1 | −1 | 6n |
| Parallelism | 1 | −1 | −1 | 1 | 0 | 0 | 12n |
| | 0 | 0 | 1 | −1 | −1 | 1 | |
| | −1 | 1 | 0 | 0 | 1 | −1 | |
| Total | $T_1$ | $T_2$ | $T_3$ | $T_3$ | $T_4$ | $T_5$ | |

$$V_{\text{Prep.}}=\frac{1}{12n}[\{(T_1+T_2)-(T_3+T_4)\}^2+\{(T_3+T_4)-(T_5+T_6)\}^2+\{(T_5+T_6)-(T_1+T_2)\}^2]$$

$$V_{\text{Reg.}}=\frac{1}{6n}\{(T_1-T_2)+(T_3-T_4)+(T_5-T_6)\}^2$$

$$V_{\text{Para.}}=\frac{1}{12n}\{(T_1-T_2-T_3+T_4)^2+(T_3-T_4-T_5+T_6)^2+(-T_1+T_2+T_5-T_6)^2\}$$

Without using these coefficients, the sum of squares of "preparation" and "parallelism" could be calculated by the ordinary method as follows:

$$S_{\text{Prep.}}=\frac{1}{2n}\{(T_1+T_2)^2+(T_3+T_4)^2+(T_5+T_6)^2\}-T^2/6n \qquad (f=2)$$

$$S_{\text{Para.}}=S_{\text{Treat.}}-(S_{\text{Prep.}}+S_{\text{Reg.}}) \qquad (f=2)$$

Both results of computation are quite identical with each other. Then, the analysis of variance of Table I could be summarized as in Table III.

Table III. Analysis of Variance for 3×2 Point Assay

| Factor | $SS$ | $f$ | $V$ |
|---|---|---|---|
| Preparation | $S_{\text{Prep.}}$ | 2 | $V_{\text{Prep.}}$ |
| Regression | $S_{\text{Reg.}}$ | 1 | $V_{\text{Reg.}}$ |
| Parallelism | $S_{\text{Para.}}$ | 2 | $V_{\text{Para.}}$ |
| Treatment | $S_{\text{Treat.}}$ | 5 | $V_{\text{Treat.}}$ |
| Error | $S_{\text{Error}}$ | 6(n−1) | $V_{\text{Error}}$ |
| Total | $S_{\text{Total}}$ | 6n−1 | |

The test of validity could be carried out, calculating the ratio, $F_0=V_{\text{Para.}}/V_{\text{Error.}}$ When the deviation from parallelism could not be found significant, the condition of similarity is satisfied, and the potency ratios of any pair of three preparations and their fiducial limits could be computed by the formulae,[4] using the $V_{\text{Error}}$ as common error term. When the deviation from parallelism is significant, the three contributors such as $(T_1-T_2-T_3+T_4)$, $(T_3-T_4-T_5+T_6)$, and $(-T_1+T_2+T_5-T_6)$ should be examined in comparison with the error terms, of which these three are not independent.

This principle of the extension of 2×2 point assay to 3×2 could be applied, in general, to $m\times n$, such as 3×3, 3×4, 4×2, 4×3, 4×4, etc. point assay.

For example, this 4×2 point assay analysis is applied to the experimental result of synthetic estrogen assay.[5]

As previously reported, the homoschedasticity was confirmed by Bartlett's method. The analysis of the result is shown in Table IV, using the 4×2 point assay coefficients as shown in Table V.

4) D. J. Finney: "Statistical Method in Biological Assay," C. Griffin Co., London, (1952).
5) Y. Ito, B. Tamaoki, M. Egusa, H. Sakamoto: J. Pharm. Soc. Japan, **72**, 1292(1952).

Table IV. Analysis of Variance for 4×2 Point Assay Result

| Factor | *SS* | *f* | *V* |
|---|---|---|---|
| Preparation | 129.1 | 3 | 43.1 |
| Regression | 228.8 | 1 | 228.8 |
| Parallelism | 22.1 | 3 | 7.9 |
| Treatment | 380.0 | 7 | 54.3 |
| Error | 332.9 | 56 | 5.9 |
| Total | 712.9 | 63 | |

Table V. Coefficients for 4×2 Point Assay

| | A | | B | | C | | D | | Divisor |
|---|---|---|---|---|---|---|---|---|---|
| | H | L | H | L | H | L | H | L | |
| Preparation | 1 | 1 | −1 | −1 | 0 | 0 | 0 | 0 | 24n |
| | 1 | 1 | 0 | 0 | −1 | −1 | 0 | 0 | |
| | 1 | 1 | 0 | 0 | 0 | 0 | −1 | −1 | |
| | 0 | 0 | 1 | 1 | −1 | −1 | 0 | 0 | |
| | 0 | 0 | 1 | 1 | 0 | 0 | −1 | −1 | |
| | 0 | 0 | 0 | 0 | 1 | 1 | −1 | −1 | |
| Regression | 1 | −1 | 1 | −1 | 1 | −1 | 1 | −1 | 8n |
| Parallelism | 1 | −1 | −1 | 1 | 0 | 0 | 0 | 0 | 24n |
| | 1 | −1 | 0 | 0 | −1 | 1 | 0 | 0 | |
| | 1 | −1 | 0 | 0 | 0 | 0 | −1 | 1 | |
| | 0 | 0 | 1 | −1 | −1 | 1 | 0 | 0 | |
| | 0 | 0 | 1 | −1 | 0 | 0 | −1 | 1 | |
| | 0 | 0 | 0 | 0 | 1 | −1 | −1 | 1 | |

In addition, the coefficients for 3×3 point assay are described in Table VI.

Table VI. Coefficients for 3×3 Point Assay

| | A | | | B | | | C | | | Divisor |
|---|---|---|---|---|---|---|---|---|---|---|
| | H | M | L | H | M | L | H | M | L | |
| Preparation | −1 | −1 | −1 | 1 | 1 | 1 | 0 | 0 | 0 | 18n |
| | 0 | 0 | 0 | −1 | −1 | −1 | 1 | 1 | 1 | |
| | 1 | 1 | 1 | 0 | 0 | 0 | −1 | −1 | −1 | |
| Regression | 1 | 0 | −1 | 1 | 0 | −1 | 1 | 0 | −1 | 6n |
| Parallelism | 1 | 0 | −1 | −1 | 0 | 1 | 0 | 0 | 0 | 12n |
| | 0 | 0 | 0 | 1 | 0 | −1 | −1 | 0 | 1 | |
| | −1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | −1 | |
| Curvature | 1 | −2 | 1 | 1 | −2 | 1 | 1 | −2 | 1 | 18n |
| Difference of Curvature | 1 | −2 | 1 | −1 | 2 | −1 | 0 | 0 | 0 | 36n |
| | 0 | 0 | 0 | 1 | −2 | 1 | −1 | 2 | −1 | |
| | −1 | 2 | −1 | 0 | 0 | 0 | 1 | −2 | 1 | |

Therefore, this method of analysis for $m \times n$ point assay could be effectively used to calculate the potency ratios among more than two preparations which are assayed simultaneously under a balanced design.

The author is greteful to Dr. P. Armitage, Department of Biostatistics, London School of Hygiene and Tropical Medicine, for his suggestions on this work.

(Received April 13, 1957)