



Tutorials and worked examples for simulation,  
 curve fitting, statistical analysis, and plotting.  
<http://www.simfit.org.uk>

Experimental design seeks to optimize ANOVA in order to eliminate systematic effects, and one such design is the Latin square. This requires a  $m$  by  $m$  matrix of observations, but also a corresponding  $m$  by  $m$  Latin square matrix of treatments, so that

- each row of the Latin square contains all of the  $m$  treatments;
- the order of treatments is different within every row; and
- the observations are arranged according to the pattern dictated by the Latin square.

Note that SIMFIT provides the option to generate random Latin squares to avoid systematic effects with repeated experiments.

From the SIMFIT main menu choose [Statistics], [ANOVA], then [Latin squares] and open the test file `anova3.tf1` which contains a 10 by 5 data matrix as follows.

5	4	1	3	2
2	5	4	1	3
3	2	5	4	1
1	3	2	5	4
4	1	3	2	5
6.67	7.15	8.29	8.95	9.62
5.40	4.77	5.40	7.54	6.93
7.32	8.53	8.50	9.99	9.68
4.92	5.00	7.29	7.85	7.08
4.88	6.16	7.83	5.38	8.51

Here the upper 5 by 5 matrix colored red is the Latin square, which corresponds to the lower 5 by 5 matrix of observations. In other words, observation( $5 + i, j$ ) corresponds to treatment( $i, j$ ) for  $i = 1, 2, \dots, 5$  and  $j = 1, 2, \dots, 5$ . For example, observation(1, 1) which is 6.67 resulted from treatment 5, observation(1, 2) which is 7.15 resulted from treatment 4, observation(5, 4) which is 5.38 resulted from treatment 2, etc.

Analysis leads to the following table.

Three Way Analysis of Variance: Grand mean 7.186  
 Data file: `anova3.tf1`  
 Data title: Latin square ANOVA Data ... see NAG routine G04ADF

Source	NDOF	SSQ	MSQ	F	p
Rows	4	29.42	7.356	9.027	0.0013
Columns	4	22.99	5.749	7.055	0.0037
Treatments	4	0.5423	0.1356	0.1664	0.9514
Error	12	9.779	0.8149		
Total	24	62.74	2.614		

  

Mean Values					
Row means	8.136	6.008	8.804	6.428	6.552
Column means	5.838	6.322	7.462	7.942	8.364
Treatment means	7.318	7.244	7.206	6.900	7.260

## Theory for Latin square ANOVA

The linear model for a  $m$  by  $m$  Latin square ANOVA is

$$y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k + e_{ijk}$$
$$\sum_{i=1}^m \alpha_i = 0$$
$$\sum_{j=1}^m \beta_j = 0$$
$$\sum_{k=1}^m \gamma_k = 0$$

where  $\alpha_i$ ,  $\beta_j$  and  $\gamma_k$  represent the row, column and treatment effect, and  $e_{ijk}$  is assumed to be normally distributed with zero mean and variance  $\sigma^2$ . The sum of squares partition is now

$$\text{Total } SSQ = \text{Row } SSQ + \text{Column } SSQ + \text{Treatment } SSQ + \text{Residual } SSQ$$

where the  $m^2$  observations are arranged in the form of a  $m$  by  $m$  matrix so that every treatment occurs once in each row and column. This design, which is used for economical reasons to account for row, column, and treatment effects, leads to the three variance ratios

$$F_R = \frac{\text{Row } MS}{\text{Residual } MS}$$
$$F_C = \frac{\text{Column } MS}{\text{Residual } MS}$$
$$F_T = \frac{\text{Treatment } MS}{\text{Residual } MS}$$

to use in  $F$  tests with  $m - 1$ , and  $(m - 1)(m - 2)$  degrees of freedom. Note that SIMFIT data files for Latin square designs with  $m$  treatment levels have  $2m$  rows and  $m$  columns, where the first  $m$  by  $m$  block identifies the treatments, and the next  $m$  by  $m$  block of data are the observations. When designing such experiments, the particular Latin square used should be chosen randomly if possible as described later. For instance, study the test file `anova3.tff1`, which should be consulted for details, noting that integers (1, 2, 3, 4, 5) are used instead of the usual letters (A, B, C, D, E) in the data file header to indicate the position of the treatments.

Note that in the Latin square results table there are now three  $p$  values for significance testing between rows, columns, and treatments.

### Generating random Latin squares

This section describes how to generate a data file so that SIMFIT can perform Latin square analysis. This involves three procedures.

1. Generate a random Latin square
2. Organize the observations according to the design
3. Fuse the Latin square and Observations to make a data matrix

From the SIMFIT main menu choose [A/Z], open **rannum**, choose random permutations, then generate a Latin square like this next one.

Random  $k$  by  $k$  Latin square:  $k = 5$

```
5 2 4 1 3
3 5 2 4 1
4 1 3 5 2
2 4 1 3 5
1 3 5 2 4
```

Equivalent alphabetical representation

```
E B D A C
C E B D A
D A C E B
B D A C E
A C E B D
```

Now proceed as follows.

- Save the  $k$  by  $k$  data from **rannum** to a file in integer format, not alphabetical format.
- Create a  $k$  by  $k$  data-only file with the observations arranged according to the saved Latin square
- Fuse the two files to make a  $2k$  by  $k$  data file where the upper matrix contains the Latin square integers.

In order to fuse the two files you can use **editmt** to combine the two files but it is very easy to proceed as follows.

- Open the Latin square matrix in a text editor such as Notepad
- Select the  $k$  by  $k$  Latin square integer matrix and copy to the clipboard
- Open the  $k$  by  $k$  data matrix in a text editor
- Paste the  $k$  by  $k$  Latin square integer matrix before the data section
- Edit the edited data matrix at line 1 to indicate the changes
- Change the matrix dimension on line 2 from  $k \quad k$  into  $2k \quad k$
- Save the edited data file.

Alternatively, as data files copied from the clipboard into **SIMFIT** do not require titles and array dimensions, you can easily paste the following data matrix directly into **SIMFIT** from the clipboard.

```
5      4      1      3      2
2      5      4      1      3
3      2      5      4      1
1      3      2      5      4
4      1      3      2      5
6.67  7.15  8.29  8.95  9.62
5.40  4.77  5.40  7.54  6.93
7.32  8.53  8.50  9.99  9.68
4.92  5.00  7.29  7.85  7.08
4.88  6.16  7.83  5.38  8.51
```