Chapter 4

Experimental Designs and Their Analysis

Design of experiment means how to design an experiment in the sense that how the observations or measurements should be obtained to answer a query in a valid, efficient and economical way. The designing of the experiment and the analysis of obtained data are inseparable. If the experiment is designed properly keeping in mind the question, then the data generated is valid and proper analysis of data provides the valid statistical inferences. If the experiment is not well designed, the validity of the statistical inferences is questionable and may be invalid.

It is important to understand first the basic terminologies used in the experimental design.

Experimental unit:

For conducting an experiment, the experimental material is divided into smaller parts and each part is referred to as an experimental unit. The experimental unit is randomly assigned to treatment is the experimental unit. The phrase "randomly assigned" is very important in this definition.

Experiment:

A way of getting an answer to a question which the experimenter wants to know.

Treatment

Different objects or procedures which are to be compared in an experiment are called treatments.

Sampling unit:

The object that is measured in an experiment is called the sampling unit. This may be different from the experimental unit.

Factor:

A factor is a variable defining a categorization. A factor can be fixed or random in nature. A factor is termed as a fixed factor if all the levels of interest are included in the experiment.

A factor is termed as a random factor if all the levels of interest are not included in the experiment and those that are can be considered to be randomly chosen from all the levels of interest.

Replication:

It is the repetition of the experimental situation by replicating the experimental unit.

Experimental error:

The unexplained random part of the variation in any experiment is termed as experimental error. An estimate of experimental error can be obtained by replication.

Treatment design:

A treatment design is the manner in which the levels of treatments are arranged in an experiment.

Example: (Ref.: Statistical Design, G. Casella, Chapman and Hall, 2008)

Suppose some varieties of fish food is to be investigated on some species of fishes. The food is placed in the water tanks containing the fishes. The response is the increase in the weight of fish. The experimental unit is the tank, as the treatment is applied to the tank, not to the fish. Note that if the experimenter had taken the fish in hand and placed the food in the mouth of fish, then the fish would have been the experimental unit as long as each of the fish got an independent scoop of food.

Design of experiment:

One of the main objectives of designing an experiment is how to verify the hypothesis in an efficient and economical way. In the contest of the null hypothesis of equality of several means of normal populations having the same variances, the analysis of variance technique can be used. Note that such techniques are based on certain statistical assumptions. If these assumptions are violated, the outcome of the test of a hypothesis then may also be faulty and the analysis of data may be meaningless. So the main question is how to obtain the data such that the assumptions are met and the data is readily available for the application of tools like analysis of variance. The designing of such a mechanism to obtain such data is achieved by the design of the experiment. After obtaining the sufficient experimental unit, the treatments are allocated to the experimental units in a random fashion. Design of experiment provides a method by which the treatments are placed at random on the experimental units in such a way that the responses are estimated with the utmost precision possible.

Principles of experimental design:

There are three basic principles of design which were developed by Sir Ronald A. Fisher.

(i) Randomization

(ii) Replication

(iii) Local control

(i) Randomization

The principle of randomization involves the allocation of treatment to experimental units at random to avoid any bias in the experiment resulting from the influence of some extraneous unknown factor that may affect the experiment. In the development of analysis of variance, we assume that the errors are random and independent. In turn, the observations also become random. The principle of randomization ensures this.

The random assignment of experimental units to treatments results in the following outcomes.

a) It eliminates systematic bias.
b) It is needed to obtain a representative sample from the population.
c) It helps in distributing the unknown variation due to confounded variables throughout the experiment and breaks the confounding influence.

Randomization forms a basis of a valid experiment but replication is also needed for the validity of the experiment.

If the randomization process is such that every experimental unit has an equal chance of receiving each treatment, it is called **complete randomization**.

(ii) Replication:

In the replication principle, any treatment is repeated a number of times to obtain a valid and more reliable estimate than which is possible with one observation only. Replication provides an efficient way of increasing the precision of an experiment. The precision increases with the increase in the number of observations. Replication provides more observations when the same treatment is used, so it increases precision. For example, if the variance of x is σ^2 than variance of the sample mean $\overline{x}$ based on n observation is $\frac{\sigma^2}{n}$. So as n increases, $Var(\overline{x})$ decreases.

(ii) Local control (error control)

The replication is used with local control to reduce the experimental error. For example, if the experimental units are divided into different groups such that they are homogeneous within the blocks, then the variation among the blocks is eliminated and ideally, the error component will contain the variation due to the treatments only. This will, in turn, increase the efficiency.

Complete and incomplete block designs:

In most of the experiments, the available experimental units are grouped into blocks having more or less identical characteristics to remove the blocking effect from the experimental error. Such design is termed as **block designs**.

The number of experimental units in a block is called the **block size**.

If

size of block = number of treatments

and

each treatment in each block is randomly allocated,

then it is a **full replication** and the design is called a **complete block design**.

In case, the number of treatments is so large that a full replication in each block makes it too heterogeneous with respect to the characteristic under study, then smaller but homogeneous blocks can be used. In such a case, the blocks do not contain a full replicate of the treatments. Experimental designs with blocks containing an incomplete replication of the treatments are called **incomplete block designs**.

Completely randomized design (CRD)

The CRD is the simplest design. Suppose there are v treatments to be compared.

- All experimental units are considered the same and no division or grouping among them exist.
- In CRD, the v treatments are allocated randomly to the whole set of experimental units, without making any effort to group the experimental units in any way for more homogeneity.
- Design is entirely flexible in the sense that any number of treatments or replications may be used.
- The number of replications for different treatments need not be equal and may vary from treatment to treatment depending on the knowledge (if any) on the variability of the observations on individual treatments as well as on the accuracy required for the estimate of individual treatment effect.

Example: Suppose there are 4 treatments and 20 experimental units, then

- the treatment 1 is replicated, say 3 times and is given to 3 experimental units,
- the treatment 2 is replicated, say 5 times and is given to 5 experimental units,
- the treatment 3 is replicated, say 6 times and is given to 6 experimental units

and

- finally, the treatment 4 is replicated [20-(6+5+3)=]6 times and is given to the remaining 6 experimental units.

- All the variability among the experimental units goes into experimented error.
- CRD is used when the experimental material is homogeneous.
- CRD is often inefficient.
- CRD is more useful when the experiments are conducted inside the lab.
- CRD is well suited for the small number of treatments and for the homogeneous experimental material.

Layout of CRD

Following steps are needed to design a CRD:

- Divide the entire experimental material or area into a number of experimental units, say n.
- Fix the number of replications for different treatments in advance (for given total number of available experimental units).
- No local control measure is provided as such except that the error variance can be reduced by choosing a homogeneous set of experimental units.

Procedure

Let the v treatments are numbered from 1,2,...,v and n_i be the number of replications required for i^{th} treatment such that $\sum_{i=1}^{v} n_i = n.$

- Select n_1 units out of n units randomly and apply treatment 1 to these n_1 units.

  (**Note**: This is how the randomization principle is utilized is CRD.)
- Select n_2 units out of $(n-n_1)$ units randomly and apply treatment 2 to these n_2 units.
- Continue with this procedure until all the treatments have been utilized.
- Generally, the equal number of treatments are allocated to all the experimental units unless no practical limitation dictates or some treatments are more variable or/and of more interest.

Analysis

There is only one factor which is affecting the outcome – treatment effect. So the set-up of one-way analysis of variance is to be used.

y_{ij} : Individual measurement of j^{th} experimental units for i^{th} treatment $i = 1,2,...,v$, $j = 1,2,...,n_i$.

y_{ij} : Independently distributed following $N(\mu+\alpha_i,\sigma^2)$ with $\sum_{i=1}^{v} n_i\alpha_i = 0$.

μ : overall mean

α_i : i^{th} treatment effect

$H_0 : \alpha_1 = \alpha_2 = ... = \alpha_v = 0$

$H_1 :$ All $\alpha_i's$ are not equal.

The data set is arranged as follows:

Treatments			
1	2	...	v
y_{11}	y_{21}	...	y_{v1}
y_{12}	y_{22}	...	y_{v2}
$\vdots$	$\vdots$	$\ddots$	$\vdots$
y_{1n_1}	y_{2n_2}	...	y_{vn_v}
T_1	T_2	...	T_v

where $T_i = \sum_{j=1}^{n_i} y_{ij}$ is the treatment total due to i^{th} effect,

$G = \sum_{i=1}^{v} T_i = \sum_{i=1}^{v} \sum_{j=1}^{n_i} y_{ij}$ is the grand total of all the observations.

In order to derive the test for H_0, we can use either the likelihood ratio test or the principle of least squares. Since the likelihood ratio test has already been derived earlier, so we choose to demonstrate the use of the least-squares principle.

The linear model under consideration is

$y_{ij} = \mu + \alpha_i + \varepsilon_{ij}, \quad i = 1,2,...,v, j = 1,2,...,n_i$

where $\varepsilon_{ij}'s$ are identically and independently distributed random errors with mean 0 and variance σ^2. The normality assumption of $\varepsilon's$ is not needed for the estimation of parameters but will be needed for deriving the distribution of various involved statistics and in deriving the test statistics.

Let $S = \sum_{i=1}^{v} \sum_{j=1}^{n_i} \varepsilon_{ij}^2 = \sum_{i=1}^{v} \sum_{j=1}^{n_i} (y_{ij} - \mu - \alpha_i)^2.$

Minimizing S with respect to μ and α_i, the normal equations are obtained as

$$\frac{\partial S}{\partial \mu} = 0 \Rightarrow n\mu + \sum_{i=1}^{v} n_i \alpha_i = 0$$

$$\frac{\partial S}{\partial \alpha_i} = 0 \Rightarrow n_i \mu + n_i \alpha_i = \sum_{j=1}^{n_i} y_{ij} \quad i = 1,2,..,v.$$

Solving them using $\sum_{i=1}^{v} n_i \alpha_i = 0$, we get

$\hat{\mu} = \bar{y}_{oo}$

$\hat{\alpha}_i = \bar{y}_{io} - \bar{y}_{oo}$

where $\bar{y}_{io} = \frac{1}{n_i}\sum_{j=1}^{n_i} \bar{y}_{ij}$ is the mean of observation receiving the i^{th} treatment and $\bar{y}_{oo} = \frac{1}{n}\sum_{i=1}^{v}\sum_{j=1}^{n_i} \bar{y}_{ij}$ is the mean of all the observations.

The fitted model is obtained after substituting the estimate $\hat{\mu}$ and $\hat{\alpha}_i$ in the linear model, we get

$$y_{ij} = \hat{\mu} + \hat{\alpha}_i + \hat{\varepsilon}_{ij}$$

or $y_{ij} = \bar{y}_{oo} + (\bar{y}_{io} - \bar{y}_{oo}) + (y_{ij} - \bar{y}_{io})$

or $(y_{ij} - \bar{y}_{oo}) = (\bar{y}_{io} - \bar{y}_{oo}) + (y_{ij} - \bar{y})$.

Squaring both sides and summing over all the observation, we have

$$\sum_{i=1}^{v}\sum_{j=1}^{n_i}(y_{ij} - \bar{y}_{oo})^2 = \sum_{i=1}^{v} n_i(\bar{y}_{io} - \bar{y}_{oo})^2 + \sum_{i=1}^{v}\sum_{j=1}^{n_i}(y_{ij} - \bar{y}_{oo})^2$$

$$\downarrow \qquad\qquad \downarrow \qquad\qquad \downarrow$$

or $$\begin{pmatrix}\text{Total sum}\\ \text{of squares}\end{pmatrix} = \begin{pmatrix}\text{Sum of squares}\\ \text{due to treatment}\\ \text{effects}\end{pmatrix} + \begin{pmatrix}\text{Sum of squares}\\ \text{due to error}\end{pmatrix}$$

or $$TSS = SSTr + SSE$$

- Since $\sum_{i=1}^{v}\sum_{j=1}^{n_i}(y_{ij} - \bar{y}_{oo}) = 0,$ so TSS is based on the sum of $(n-1)$ squared quantities. The TSS carries only $(n-1)$ degrees of freedom.

- Since $\sum_{i=1}^{v} n_i(\bar{y}_{io} - \bar{y}_{oo}) = 0,$ so $SSTr$ is based only on the sum of $(v\text{ -}1)$ squared quantities. The $SSTr$ carries only (v -1) degrees of freedom.

- Since $\sum_{i=1}^{n_i} n_i(\bar{y}_{ij} - \bar{y}_{io}) = 0$ for all i = 1,2,...,v, so SSE is based on the sum of squaring n quantities like $(y_{ij} - \bar{y}_{io})$ with v constraints $\sum_{j=1}^{n_i}(y_{ij} - \bar{y}_{io}) = 0,$ So SSE carries $(n - v)$ degrees of freedom.

- Using the Fisher-Cochran theorem,

  $TSS = SSTr + SSE$

  with degrees of freedom partitioned as

  $(n - 1) = (v - 1) + (n - v)$.

Moreover, equality in $TSS = SSTr + SSE$ has to hold exactly. To ensure that the equality holds exactly, we find one of the sums of squares through subtraction. Generally, it is recommended to find SSE by subtraction as

$$SSE = TSS - SSTr$$

$$TSS = \sum_{i=1}^{v}\sum_{j=1}^{n_i}(y_{ij} - \bar{y}_{io})^2$$

$$= \sum_{i=1}^{v}\sum_{j=1}^{n_i} y_{ij}^2 - \frac{G^2}{n}$$

where

$$G = \sum_{i=1}^{v}\sum_{j=1}^{n_i} y_{ij}.$$

$$SSTr = \sum_{j=1}^{n_i} n_i(\bar{y}_{io} - \bar{y}_{oo})^2$$

$$= \sum_{i=1}^{v}\left(\frac{T_i^2}{n_i}\right) - \frac{G^2}{n}$$

where $T_i = \sum_{j=1}^{n_i} y_{ij}$

$\frac{G^2}{n}$: correction factor.

Now under $H_0 : \alpha_1 = \alpha_2 = ... = \alpha_v = 0$, the model become

$$Y_{ij} = \mu + \varepsilon_{ij},$$

and minimizing $S = \sum_{i=1}^{v}\sum_{j=1}^{n_i}\varepsilon_{ij}^2$

with respect to μ gives

$$\frac{\partial S}{\partial \mu} = 0 \Rightarrow \hat{\mu} = \frac{G}{n} = \bar{y}_{oo}.$$

The SSE under H_0 becomes

$$SSE = \sum_{i=1}^{v}\sum_{j=1}^{n_i}(y_{ij} - \bar{y}_{oo})^2$$

and thus $TSS = SSE$. This TSS under H_0 contains the variation only due to the random error whereas the earlier $TSS = SSTr + SSE$ contains the variation due to treatments and errors both. The difference between the two will provides the effect of treatments in terms of the sum of squares as

$$SSTr = \sum_{i=1}^{v} n_i(\bar{y}_i - \bar{y}_{oo})^2 .$$

- **Expectations**

$$
\begin{aligned}
E(SSE) &= \sum_{i=1}^{v}\sum_{j=1}^{n_i} E(y_{ij} - y_{io})^2 \\
&= \sum_{i=1}^{v}\sum_{j=1}^{n_i} (\varepsilon_{ij} - \bar{\varepsilon}_{io})^2 \\
&= \sum_{i=1}^{v}\sum_{j=1}^{n_i} E(\varepsilon_{ij}^2) - \sum_{i=1}^{v} n_i E(\bar{\varepsilon}_{io.}^2) \\
&= n\sigma^2 - \sum_{i=1}^{v} n_i \frac{\sigma^2}{n_i} \\
&= (n-v)\sigma^2
\end{aligned}
$$

$$
E(MSE) = E\left(\frac{SSE}{n-v}\right) = \sigma^2
$$

$$
\begin{aligned}
E(SSTr) &= \sum_{i=1}^{v} n_i E(\bar{y}_{io} - \bar{y}_{oo})^2 \\
&= \sum_{i=1}^{v} n_i E(\alpha_i + \bar{\varepsilon}_{io} - \bar{\varepsilon}_{oo})^2 \\
&= \sum_{i=1}^{v} n_i \alpha_i^2 + \left[\sum_{i=1}^{v} n_i \bar{\varepsilon}_{io}^2 - n\bar{\varepsilon}_{oo}^2\right] \\
&= \sum_{i=1}^{v} n_i \alpha_i^2 + \left[\sum_{i=1}^{v} n_i \frac{\sigma^2}{n_i} - n\frac{\sigma^2}{n}\right] \\
&= \sum_{i=1}^{v} n_i \alpha_i^2 + (v-1)\sigma^2
\end{aligned}
$$

$$
E(MSTr) = E\left(\frac{SStr}{v-1}\right) = \frac{1}{v-1}\sum_{i=1}^{v} n_i \alpha_i^2 + \sigma^2.
$$

In general $E\left(MSTr\right) \neq \sigma^2$ but under H_0, all $\alpha_i = 0$ and so $E(MSTr) = \sigma^2$.

Distributions and decision rules:

Using the normal distribution property of $\varepsilon_{ij}'s$, we find that $y_{ij}'s$ are also normal as they are the linear combination of $\varepsilon_{ij}'s$.

- $-\frac{SSTr}{\sigma^2} \sim \chi^2(v-1)$ under H_0
- $-\frac{SSE}{\sigma^2} \sim \chi^2(n-v)$ under H_0
- $-SSTr$ and SSE are independently distributed
- $-\frac{MStr}{MSE} \sim F(v-1, n-v)$ under H_0.
- – Reject H_0 at α^* level of significance if $F > F_{\alpha^*;v-1,n-v.}$

[Note: We denote the level of significance here by α^* because α has been used for denoting the factor]

The analysis of variance table is as follows

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F
Between treatments	v - 1	$SSTr$	$MSTr$	$\frac{MSTr}{MSE}$
Errors	n - v	SSE	MSE	
Total	n - 1	TSS		

Randomized Block Design

If a large number of treatments are to be compared, then a large number of experimental units are required. This will increase the variation among the responses and CRD may not be appropriate to use. In such a case when the experimental material is not homogeneous and there are v treatments to be compared, then it may be possible to

- group the experimental material into blocks of sizes v units.
- Blocks are constructed such that the experimental units within a block are relatively homogeneous and resemble to each other more closely than the units in the different blocks.
- If there are b such blocks, we say that the blocks are at b levels. Similarly, if there are v treatments, we say that the treatments are at v levels. The responses from the b levels of blocks and v levels of treatments can be arranged in a two-way layout. The observed data set is arranged as follows:

		Blocks						Block Totals
		1	2		i		b	
Treatments	1	y_{11}	y_{21}	…	y_{i1}	…	y_{b1}	$B_1 = y_{o1}$
	2	y_{12}	y_{22}	…	y_{i2}	…	y_{b2}	$B_2 = y_{o2}$
	. . .	. . .	. . .		. . .		. . .	. . .
	j	y_{1j}	y_{2j}	…	y_{ij}	…	y_{bj}	$B_j = y_{oj}$
	. . .		. . .		. . .		. . .	. . .
	v	y_{1v}	y_{2v}	…	y_{iv}	…	y_{bv}	$B_b = y_{ob}$
Treatment Totals		$T_1 = y_{1o}$	$T_2 = y_{2o}$	…	$T_i = y_{io}$	…	y_{vo}	**Grand Total** G= y_{oo}

Layout:

A two-way layout is called a randomized block design (RBD) or a randomized complete block design (RCB) if, within each block, the v treatments are randomly assigned to v experimental units such that each of the $v!$ ways of assigning the treatments to the units has the same probability of being adopted in the experiment and the assignment in different blocks are statistically independent.

The RBD utilizes the principles of design - randomization, replication and local control - in the following way:

1. Randomization:

- Number the v treatments 1,2,…,v.
- Number the units in each block as 1, 2,...,v.
- Randomly allocate the v treatments to v experimental units in each block.

2. Replication

Since each treatment is appearing in each block, so every treatment will appear in all the blocks. So each treatment can be considered as if replicated the number of times as the number of blocks. Thus in RBD, the number of blocks and the number of replications are same.

3. Local control

Local control is adopted in RBD in the following way:

- First form the homogeneous blocks of the experimental units.
- Then allocate each treatment randomly in each block.

The error variance now will be smaller because of homogeneous blocks and some variance will be parted away from the error variance due to the difference among the blocks.

Example:

Suppose there are 7 treatments denoted as $T_1, T_2, .., T_7$ corresponding to 7 levels of a factor to be included in 4 blocks. So one possible layout of the assignment of 7 treatments to 4 different blocks in an RBD is as follows

Block 1	T_2	T_7	T_3	T_5	T_1	T_4	T_6
Block 2	T_1	T_6	T_7	T_4	T_5	T_3	T_2
Block 3	T_7	T_5	T_1	T_6	T_4	T_2	T_3
Block 4	T_4	T_1	T_5	T_6	T_2	T_7	T_3

Analysis

Let

y_{ij} : Individual measurements of j^{th} treatment in i^{th} block, $i = 1,2,...,b,\ j = 1,2,...,v$.

y_{ij}'s are independently distributed following $N(\mu + \beta_i + \tau_j, \sigma^2)$

where μ : overall mean effect

β_i : i^{th} block effect

τ_j : j^{th} treatment effect

such that $\sum_{i=1}^{b} \beta_i = 0,\ \sum_{j=1}^{v} \tau_j = 0$.

There are two null hypotheses to be tested.

- related to the block effects

$$H_{0B}: \beta_1 = \beta_2 = ... = \beta_b = 0.$$

- related to the treatment effects

$$H_{0T}: \tau_1 = \tau_2 = ... = \tau_v = 0.$$

The linear model, in this case, is a two-way model as

$$y_{ij} = \mu + \beta_i + \tau_j + \varepsilon_{ij},\ i = 1,2,..,b;\ j = 1,2,..,v$$

where ε_{ij} are identically and independently distributed random errors following a normal distribution with mean 0 and variance σ^2.

The tests of hypothesis can be derived using the likelihood ratio test or the principle of least squares. The use of likelihood ratio test has already been demonstrated earlier, so we now use the principle of least squares.

$$\text{Minimizing } S = \sum_{i=1}^{b}\sum_{j=1}^{v} \varepsilon_{ij}^2 = \sum_{i=1}^{b}\sum_{j=1}^{v} (y_{ij} - \mu - \beta_i - \tau_j)^2$$

and solving the normal equation

$$\frac{\partial S}{\partial \mu} = 0,\ \frac{\partial S}{\partial \beta_i} = 0,\ \frac{\partial S}{\partial \tau_j} = 0 \text{ for all } i = 1,2,..,b,\ j = 1,2,..,v.$$

the least squares estimators are obtained as

$$\hat{\mu} = \bar{y}_{oo},$$
$$\hat{\beta}_i = \bar{y}_{io} - \bar{y}_{oo},$$
$$\hat{\tau}_j = \bar{y}_{oj} - \bar{y}_{oo}.$$

The fitted model is

$$\begin{aligned} y_{ij} &= \hat{\mu} + \hat{\beta}_i + \hat{\tau}_j + \hat{\varepsilon}_{ij} \\ &= \bar{y}_{oo} + (\bar{y}_{io} - \bar{y}_{oo}) + (\bar{y}_{oj} - \bar{y}_{oo}) + (y_{ij} - \bar{y}_{io} - \bar{y}_{oj} + \bar{y}_{oo}). \end{aligned}$$

Squaring both sides and summing over i and j gives

$$\sum_{i=1}^{b}\sum_{j=1}^{v} (\bar{y}_{ij} - \bar{y}_{oo})^2 = v\sum_{i=1}^{b} (\bar{y}_{io} - \bar{y}_{oo})^2 + b\sum_{j=1}^{v} (\bar{y}_{oj} - \bar{y}_{oo})^2 + \sum_{i=1}^{b}\sum_{j=1}^{v} (y_{ij} - \bar{y}_{io} - \bar{y}_{oj} + \bar{y}_{oo})^2$$

or $TSS = SSBl + SSTr + SSE$

with degrees of freedom partitioned as

$$bv - 1 = (b-1) + (v-1) + (b-1)(v-1).$$

The reason for the number of degrees of freedom for different sums of squares is the same as in the case of CRD.

Here $TSS = \sum_{i=1}^{b}\sum_{j=1}^{v}(y_{ij} - \bar{y}_{oo})^2$

$$= \sum_{i=1}^{b}\sum_{j=1}^{v} y_{ij}^2 - \frac{G^2}{bv}$$

$\frac{G^2}{bv}$: correction factor.

$G = \sum_{i=1}^{b}\sum_{j=1}^{v} y_{ij}$: Grand total of all the observation.

$$SSBl = v\sum_{i=1}^{b}(\bar{y}_{io} - \bar{y}_{oo})^2$$

$$= \sum_{i=1}^{b}\frac{B_i^2}{v} - \frac{G^2}{bv}$$

$B_i = \sum_{j=1}^{v} y_{ij}$: i^{th} block total

$$SSTr = b\sum_{j=1}^{v}(\bar{y}_{oj} - \bar{y}_{oo})^2$$

$$= \sum_{j=1}^{v}\frac{T_j^2}{b} - \frac{G^2}{bv}$$

$T_j = \sum_{i=1}^{b} y_{ij}$: j^{th} treatment total

$$SSE = \sum_{i=1}^{b}\sum_{j=1}^{v}(y_{ij} - \bar{y}_{io} - \bar{y}_{oj} + \bar{y}_{oo})^2.$$

The expectations of mean squares are

$$E(MSBl) = E\left(\frac{SSBl}{b-1}\right) = \sigma^2 + \frac{v}{b-1}\sum_{i=1}^{b}\beta_i^2$$

$$E(MSTr) = E\left(\frac{SSTr}{v-1}\right) = \sigma^2 + \frac{b}{v-1}\sum_{j=1}^{v}\tau_j^2$$

$$E(MSE) = E\left(\frac{SSE}{(b-1)(v-1)}\right) = \sigma^2.$$

Moreover,

$$(b-1)\frac{SSBl}{\sigma^2} \sim \chi^2(b-1)$$

$$(v-1)\frac{SSTr}{\sigma^2} \sim \chi^2(v-1)$$

$$(b-1)(v-1)\frac{SSE}{\sigma^2} \sim \chi^2(b-1)(v-1).$$

Under $H_{0B}: \beta_1 = \beta_2 = ... = \beta_b = 0,$

$$E(MSBl) = E(MSE)$$

and $SSBl$ and SSE are independent , so

$$F_{bl} = \frac{MSBl}{MSE} \sim F((b-1,(b-1)(v-1)).$$

Similarly, under $H_{0T}: \tau_1 = \tau_2 = ... = \tau_v = 0,$ so

$$E(MSTr) = E(MSE)$$

and $SSTr$ and SSE are independent, so

$$F_{Tr} = \frac{MSTr}{MSE} \sim F(v-1),(b-1)(v-1)).$$

Reject H_{0B} *if* $F_{be} > F_{\alpha}((b-1),(b-1)(v-1)$

Reject H_{0T} *if* $F_{Tr} > F_{\alpha}((v-1),(b-1)(v-1))$

If H_{0B} is accepted, then it indicates that the blocking is not necessary for future experimentation.

If H_{0T} is rejected then it indicates that the treatments are different. Then the multiple comparison tests are used to divide the entire set of treatments into different subgroup such that the treatments in the same subgroup have the same treatment effect and those in the different subgroups have different treatment effects.

The analysis of variance table is as follows

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F
Blocks	b - 1	$SSBl$	$MSBl$	F_{Bl}
Treatments	v - 1	$SSTr$	$MSTr$	F_{Tr}
Errors	(b - 1)(v - 1)	SSE	MSE	
Total	bv - 1	TSS		

Latin Square Design

The treatments in the RBD are randomly assigned to b blocks such that each treatment must occur in each block rather than assigning them at random over the entire set of experimental units as in the CRD. There are only two factors – block and treatment effects – which are taken into account and the total number of experimental units needed for complete replication are bv where b and v are the numbers of blocks and treatments respectively.

If there are three factors and suppose there are b, v and k levels of each factor, then the total number of experimental units needed for a complete replication are bvk. This increases the cost of experimentation and the required number of experimental units over RBD.

In Latin square design (LSD), the experimental material is divided into rows and columns, each having the same number of experimental units which is equal to the number of treatments. The treatments are allocated to the rows and the columns such that each treatment occurs once and only once in each row and in each column.

In order to allocate the treatment to the experimental units in rows and columns, we take help from Latin squares.

Latin Square:

A Latin square of order p is an arrangement of p symbols in p^2 cells arranged in p rows and p columns such that each symbol occurs once and only once in each row and in each column. For example, to write a Latin square of order 4, choose four symbols – A, B, C and D. These letters are Latin letters which are used as symbols. Write them in a way such that each of the letters out of A, B, C and D occurs once and only once in each row and each column. For example, as

A	B	C	D
B	C	D	A
C	D	A	B
D	A	B	C

This is a Latin square.

We consider first the following example to illustrate how a Latin square is used to allocate the treatments and in getting the response.

Example:

Suppose different brands of petrol are to be compared with respect to the mileage per litre achieved in motor cars.

Important factors responsible for the variation in mileage are

- the difference between individual cars.
- the difference in the driving habits of drivers.

We have three factors – cars, drivers and petrol brands. Suppose we have

- 4 types of cars denoted as 1, 2, 3, 4.
- 4 drivers that are represented by a, b, c, d.
- 4 brands of petrol are indicated as A, B, C, D.

Now the complete replication will require $4 \times 4 \times 4 = 64$ the number of experiments. We choose only 16 experiments. To choose such 16 experiments, we take the help of the Latin square. Suppose we choose the following Latin square:

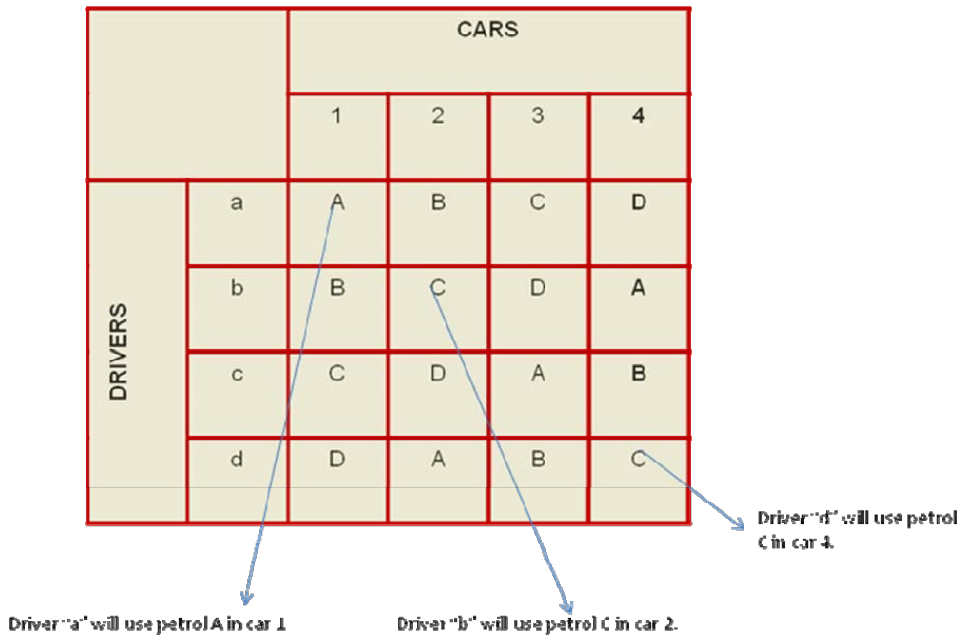
A B C D

B C D A

C D A B

D A B C

Write them in rows and columns and choose rows for drivers, columns for cars and letter for petrol brands. Thus 16 observations are recorded as per this plan of treatment combination (as shown in the next figure) and further analysis is carried out. Since such design is based on Latin square, so it is called as a Latin square design.



Another choice of a Latin square of order 4 is

C B A D

B C D A

A D C B

D A B C

This will again give a design different from the previous one. The 16 observations will be recorded again but based on different treatment combinations.

Since we use only 16 out of 64 possible observations, so it is an incomplete 3-way layout in which each of the 3 factors – cars, drivers and petrol brands are at 4 levels and the observations are recorded only on 16 of the 64 possible treatment combinations.

Thus in an LSD,

- the treatments are grouped into replication in two-ways
  - once in rows and
  - and in columns,
- rows and columns variations are eliminated from the within treatment variation.
  - In RBD, the experimental units are divided into homogeneous blocks according to the blocking factor. Hence it eliminates the difference among blocks from the experimental error.
  - In LSD, the experimental units are grouped according to two factors. Hence two effects (like as two block effects) are removed from the experimental error.
  - So the error variance can be considerably reduced in LSD.

The LSD is an incomplete three-way layout in which each of the three factors, viz, rows, columns and treatments, is at v levels each and observations only on v^2 of the v^3 possible treatment combinations are taken. Each treatment combination contains one level of each factor.

The analysis of data in an LSD is conditional in the sense it depends on which Latin square is used for allocating the treatments. If the Latin square changes, the conclusions may also change.

We note that Latin squares play an important role is an LSD, so first we study more about these Latin squares before describing the analysis of variance.

Standard form of Latin square

A Latin square is in the standard form if the symbols in the first row and first columns are in the **natural order** (Natural order means the order of alphabets like A, B, C, D,…).

Given a Latin square, it is possible to rearrange the columns so that the first row and first column remain in a natural order.

Example: Four standard forms of 4×4 Latin square are as follows.

A B C D	A B C D	A B C D	A B C D
B A D C	B C D A	B D A C	B A D C
C D B A	C D A B	C A D B	C D A B
D C A B	D A B C	D C B A	D C B A

For each standard Latin square of order p, the p rows can be permuted in $p!$ ways. Keeping a row fixed, vary and permute $(p - 1)$ columns in $(p - 1)!$ ways. So there are $p!(p - 1)!$ different Latin squares.

For illustration

Size of square	Number of Standard squares	Value of $p!(1 - p)!$	Total number of different squares
3 × 3	1	12	12
4 × 4	4	144	576
5 × 5	56	2880	161280
6 × 6	9408	86400	812851250

Conjugate:

Two standard Latin squares are called conjugate if the rows of one are the columns of other.

For example

A B C D		A B C D
B C D A	and	B C D A
C D A B		C D A B
D A B C		D A B C

are conjugate. In fact, they are **self conjugate**.

A Latin square is called **self conjugate** if its arrangement in rows and columns are the same.

Transformation set:

A set of all Latin squares obtained from a single Latin square by permuting its rows, columns and symbols is called a transformation set.

From a Latin square of order p, $p!(p-1)!$ different Latin squares can be obtained by making $p!$ permutations of columns and $(p-1)!$ permutations of rows which leaves the first row in place. Thus

Number of different Latin squares of order p in a transformation set = $p!(p-1)!$ X number of standard Latin squares in the set

Orthogonal Latin squares

If two Latin squares of the same order but with different symbols are such that when they are superimposed on each other, every ordered pair of symbols (different) occurs exactly once in the Latin square, then they are called orthogonal.

Graeco-Latin square:

A pair of orthogonal Latin squares, one with Latin symbols and the other with Greek symbols form a Graeco-Latin square.

For example

A B C D	$\alpha\ \beta\ \gamma\ \delta$
B A D C	$\delta\ \gamma\ \beta\ \alpha$
C D A B	$\beta\ \alpha\ \delta\ \gamma$
D C B A	$\gamma\ \delta\ \alpha\ \beta$

is a Graeco-Latin square of order 4.

Graeco Latin squares design enables to consider one more factor than the factors in Latin square design. For example, in the earlier example, if there are four drivers, four cars, four petrol and each petrol has four varieties, as α, β, γ and δ, then Graeco-Latin square helps in deciding the treatment combination as follows:

		Cars			
		1	2	3	4
Drivers	a	$A\alpha$	$B\beta$	$C\gamma$	$D\delta$
	b	$B\delta$	$A\gamma$	$D\beta$	$C\alpha$
	c	$C\beta$	$D\alpha$	$A\delta$	$B\gamma$
	d	$D\gamma$	$C\delta$	$B\alpha$	$A\beta$

Now

$A\alpha$ means: Driver 'a' will use the α variant of petrol A in Car 1.

$B\gamma$ means: Driver 'c' will use the γ variant of petrol B in Car 4

and so on.

Mutually orthogonal Latin square

A set of Latin squares of the same order is called a set of mutually orthogonal Latin square (or a hyper Graeco-Latin square) if every pair in the set is orthogonal. The total number of mutually orthogonal Latin squares of order p is at most (p - 1).

Analysis of LSD (one observation per cell)

In designing an LSD of order p,

- choose one Latin square at random from the set of all possible Latin squares of order p.
- Select a standard Latin square from the set of all standard Latin squares with equal probability.
- Randomize all the rows and columns as follows:
  - Choose a random number, less than p, say n_1 and then 2nd row is the n_1^{th} row.
  - Choose another random number less than p, say n_2 and then 3rd row is the n_2^{th} row and so on.
  - Then do the same for the column.
- For Latin squares of the order less than 5, fix the first row and then randomize rows and then randomize columns. In Latin squares of order 5 or more, need not to fix even the first row. Just randomize all rows and columns.

Example:

Suppose following Latin square is chosen

A B C D E

B C D E A

D E A B C

E A B C D

C D E A B

Now randomize rows, e.g., 3rd row becomes 5th row and 5th row becomes 3rd row . The Latin square becomes

A B C D E

B C D E A

C D E A B

E A B C D

D E A B C.

Now randomize columns, say 5th column becomes 1st column, 1st column becomes 4th column and 4th column becomes 5th column

E B C A D

A C D B E

D A B E C

C E A D B

B D E C A

Now use this Latin square for the assignment of treatments.

y_{ijk} : Observation on k^{th} treatment in i^{th} row and j^{th} block, $i = 1,2,...,v,\ j = 1,2,...,v,\ k = 1,2,...,v$.

Triplets (I ,j, k) take on only the v^2 values indicated by the chosen particular Latin square selected for the experiment.

y_{ijk} 's are independently distributed as $N(\mu + \alpha_i + \beta_j + \tau_k, \sigma^2)$.

Linear model is

$$y_{ijk} = \mu + \alpha_i + \beta_j + \tau_k + \varepsilon_{ijk}, i = 1,2,...,v; j = 1,2,...,v; k = 1,2,...,v$$

where ε_{ijk} are random errors which are identically and independently distributed following $N(0,\sigma^2)$.

with $\sum_{i=1}^{v} \alpha_i = 0,\ \sum_{j=1}^{v} \beta_i = 0,\ \sum_{k=1}^{v} \tau_k = 0,$

α_i : main effect of rows

β_j : main effect of columns

γ_k : main effect of treatments.

The null hypothesis under consideration are

$H_{0R} : \alpha_1 = \alpha_2 = ... = \alpha_v = 0$

$H_{0C} : \beta_1 = \beta_2 = ... = \beta_v = 0$

$H_{0T} : \tau_1 = \tau_2 = ... = \tau_v = 0$

The analysis of variance can be developed on the same lines as earlier.

Minimizing $S = \sum_{i=1}^{v}\sum_{j=1}^{v}\sum_{k=}^{v} \varepsilon_{ijk}^2$ with respect to μ, α_i, β_j and τ_k given the least-squares estimate as

$\hat{\mu} = \bar{y}_{ooo}$

$\hat{\alpha}_i = \bar{y}_{ioo} - \bar{y}_{ooo} \qquad i = 1,2,...,v$

$\hat{\beta}_j = \bar{y}_{ojo} - \bar{y}_{ooo} \qquad j = 1,2,...,v$

$\hat{\tau}_k = \bar{y}_{ook} - \bar{y}_{ooo} \qquad k = 1,2,...,v.$

Using the fitted model based on these estimators, the total sum of squares can be partitioned into the mutually orthogonal sum of squares *SSR, SSC, SSTr* and *SSE* as

$TSS = SSR + SSC + SSTr + SSE$

where

TSS: Total sum of squares = $\sum_{i=1}^{v}\sum_{j=1}^{v}\sum_{k=1}^{v}(y_{ijk} - \bar{y}_{ooo})^2 = \sum_{i=1}^{v}\sum_{j=1}^{v}\sum_{k=1}^{v} y_{ijk}^2 - \frac{G^2}{v^2}$

SSR: Sum of squares due to rows = $v\sum_{i=1}^{v}(\bar{y}_{ioo} - \bar{y}_{ooo})^2 = \frac{\sum_{i=1}^{v} R_i^2}{v} - \frac{G^2}{v^2}$; where $R_i = \sum_{j=1}^{v}\sum_{k=1}^{v} y_{ijk}$

SSC: Sum of squares due to column = $v\sum_{j=1}^{v}(\bar{y}_{ojo} - \bar{y}_{ooo})^2 = \frac{\sum_{i=1}^{v} C_j^2}{v} - \frac{G^2}{v^2}$; where $C_j = \sum_{i=1}^{v}\sum_{k=1}^{v} y_{ijk}$

SSTr : Sum of squares due to treatment = $v\sum_{k=1}^{v}(\bar{y}_{ook} - \bar{y}_{ooo})^2 = \frac{\sum_{i=1}^{v} T_k^2}{v} - \frac{G^2}{v^2}$; where $T_k = \sum_{i=1}^{v}\sum_{j=1}^{v} y_{ijk}$

Degrees of freedom carried by *SSR, SSC* and *SSTr* are $(v - 1)$ each.

Degrees of freedom carried by *TSS* is $v^2 - 1$.

Degree of freedom carried by *SSE* is $(v - 1)(v - 2)$.

The expectations of mean squares are obtained as

$$E(MSR) = E\left(\frac{SSR}{v-1}\right) = \sigma + \frac{v}{v-1}\sum_{i=1}^{v}\alpha_i^2$$

$$E(MSC) = E\left(\frac{SSC}{v-1}\right) = \sigma^2 + \frac{v}{v-1}\sum_{j=1}^{v}\beta_j^2$$

$$E(MSTr) = E\left(\frac{SSTr}{v-1}\right) = \sigma^2 + \frac{v}{v-1}\sum_{k=1}^{v}\tau_k^2$$

$$E(MSE) = E\left(\frac{SSE}{(v-1)(v-2)}\right) = \sigma^2.$$

Thus

- under H_{0R}, $F_R = \dfrac{MSR}{MSE} \sim F((v-1),(v-1)(v-2))$

- under H_{0C}, $F_C = \dfrac{MSC}{MSE} \sim F((v-1),(v-1)(v-2))$

- under H_{0T}, $F_T = \dfrac{MSTr}{MSE} \sim F((v-1),(v-1)(v-2))$.

Decision rules:

Reject H_{0R} at level α if $F_R > F_{1-\alpha;v(-1),(v-1)(v-2)}$

Reject H_{0C} at level α if $F_C > F_{1-\alpha;(v-1),(v-1)(v-2)}$

Reject H_{0T} at level α if $F_T > F_{1-\alpha;(v-1),(v-1)(v-2)}$.

If any null hypothesis is rejected, then use multiple comparison test.

The analysis of variance table is as follows

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F
Rows	v - 1	*SSR*	*MSR*	F_R
Columns	v - 1	*SSC*	*MSC*	F_C
Treatments	v - 1	*SSTr*	*MSTr*	F_T
Error	$(v - 1)(v - 2)$	*SSE*	*MSE*	
Total	$v^2 - 1$	*TSS*		