

Chemometrics

MA4605

Week 10. Lecture 18. Two-way ANOVA with interactions

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Two-way ANOVA

- In the previous approach, two factors possibly affecting the results of observed variable have been assumed to enter the model in a linear fashion.
- In particular, an increase in the observed values due to a change of levels of one of the factors, has been assumed independent of the levels set for the other factor.
- In reality, there maybe more complicated relations between factors and their levels - there can be interaction between factors.

Interactions

We can extend two-way ANOVA to include an interaction term.

- Two factors.
- One factor **A** with k levels (called treatments), another factor **B** with b levels (called blocks).
- One factor **AxB** called the interaction term.
- Four sources of variation: treatments, blocks, interaction and experimental variation.

Two-way ANOVA with interactions sum of squares

The total variability is partitioned into four components:

- the variability due to the different treatments (k)
- the variability due to the different blocks (b)
- the variability due to the interaction between treatments and blocks
- the error variability (residuals)

Adding the interacting terms requires replicating the measurements in each cell. Instead of one single measurement in a cell we will need $n=2,3,\dots$.

Two-way ANOVA is most powerful when the experiment has the same number of replicates in each group defined by the pair of factors. This is called a **balanced design**.

$$SS_{Total} = SS_A + SS_B + SS_{A \times B} + SS_{Residuals}$$

$$SS_{Total} = \sum_{i=1}^b \sum_{j=1}^k \sum_{l=1}^n (y_{ijl} - \bar{\bar{y}})^2$$

$$SS_A = nb \sum_{j=1}^k (\bar{y}_{.j} - \bar{\bar{y}})^2$$

$$SS_B = nk \sum_{i=1}^b (\bar{y}_{i.} - \bar{\bar{y}})^2$$

$$SS_{A \times B} = n \sum_{i=1}^b \sum_{j=1}^k (y_{ij} - \bar{y}_{i.} - \bar{y}_{.j} + \bar{\bar{y}})^2$$

$$SS_{Residual} = SS_{Total} - SS_A - SS_B - SS_{A \times B}$$

Degrees of freedom

Source of variation	Degrees of freedom
Total	$n \cdot k \cdot b - 1$
Between treatments	$b - 1$
Between blocks	$k - 1$
Interactions	$(k - 1) \cdot (b - 1)$
Residuals	$(n - 1) \cdot k \cdot b$

Example

In an inter-laboratory collaborative experiment on the determination of arsenic in coal, samples of coal from three different regions were sent to each of three laboratories. Each lab performed a duplicate analysis on each sample.

Sample	1	2	3
A	5.1,5.1	5.3,5.4	5.3,5.1
B	5.8,5.4	5.4,5.9	5.2,5.5
C	6.5,6.1	6.6,6.7	6.5,6.4

Two-way ANOVA with interactions in R

To perform a two-way analysis of variance in *R* we need to code the new table structure.

```
y <- c(5.1,5.1, 5.3,5.4, 5.3,5.1,  
5.8,5.4, 5.4,5.9, 5.2,5.5,  
6.5,6.1, 6.6,6.7, 6.5,6.4)
```

```
A <- factor(rep(1:3,3,each=2))
```

A

```
1 1 2 2 3 3 1 1 2 2 3 3 1 1 2 2 3 3
```

```
B <- factor(rep(1:3,each=6))
```

B

```
1 1 1 1 1 1 2 2 2 2 2 2 3 3 3 3 3 3
```

```
model <- lm(y~A+B+A:B)
```

```
anova(model)
```


Analysis of Variance Table

Response: y

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
A	2	0.1878	0.09389	2.3151	0.1545
B	2	5.0678	2.53389	62.4795	5.281e-06
A:B	4	0.1022	0.02556	0.6301	0.6533
Residuals	9	0.3650	0.04056		

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Three separate statistical tests are performed (based on the F statistic), comparing

- the variability due to first factor,
- the variability due to second factor
- the variability due to the interaction between factors

to the error variability.

The error variability is estimated from the within-cell variation and it equals 0.365 with $(n - 1)bk = 1 * 3 * 3 = 9$ degrees of freedom. The residual mean square is obtained as the ratio between the residual sum of square and its corresponding degrees of freedom: $MS_{Residuals} = \frac{SS_{Residuals}}{df} = \frac{0.3650}{9} = 0.04056$

Each source of variation is compared with the residual mean square to test whether it is significant.

Interactions effect. The interaction effect is not significant as the p -value 0.6533. Since the interaction term is not significant, we can interpret the separate effects of factor A and B.

Between-column effect The effect of factor A is not significant since the p -value $0.1545 > 0.05$.

Between-row effect The effect of factor B is significant since the p -value $5.281\text{e-}06$ is less than 0.05 making this source of variation very significant.

Interaction plot

We can visually inspect the sample-laboratory interactions from the following plot in which the results are grouped by the two factors: laboratory and sample.

