



Quantitative Methods in Chemistry

Week 4, Lecture 1&2

NPTEL



Last week we learnt about:

- Z-test
- T-test
- Hypothesis testing
- Errors in Hypothesis testing
- Finding out outliers in data using Q-test

This week we will learn about:

- Need for conducting Analysis of Variance (ANOVA)
- Introduction to ANOVA
- Protocol for doing “single factor ANOVA”
- Obtaining “Least Significant Difference” (LSD) of means for multiple samples
- Examples of applying ANOVA and LSD

NPTTEL



The need for conducting ANOVA

T-tests compare differences between two samples or populations.

Question: Can we extend this to comparing n different samples?

Answer: No, because of the Error in Hypothesis testing. Each pair of data set compared brings about Type I or Type II error. We learnt that at 95% Confidence Level, there is 5% chance of committing Type I error i.e. rejecting H_0 when it is true. As the number of data sets increase, the chance of committing this error increases.

If we are performing t-test on 10 samples at 95% CL, the overall probability of committing Type I error will be given by:

$$1-(0.95)^{10} = 1 - 0.599 \sim 0.4.$$

So, there's 40% chance of committing Type I error when applying t-test on 10 samples at 95% CL.

Applying ANOVA reduces this error by condensing the comparison to two numbers.



Introduction to ANOVA

Presumptions when applying ANOVA:

- Normality of data sets – All the populations being compared are presumed to follow Gaussian Distribution
- Independence of Errors – The samples are randomly selected and are independent of one another
- Equivariance – The variances (SD^2) of the populations being compared are similar. In other words, the largest SD of a sample should not be greater than 2 x smallest SD.

ANOVA compared the fluctuations in variance between the samples with those within the samples.

NPTTEL



Comparison of Variances using F-test

F-statistics is named after Ronald A. Fisher who made seminal contributions to the Statistics.

Protocol:

1. Establish a Null Hypothesis – The population variances being compared are equal. In other words, $s_1^2 = s_2^2$.
2. Apply test statistics : $F_{\text{test}} = s_1^2 / s_2^2$ (Note the square of std. dev.!). The **larger SD** is kept on **numerator**.
3. Compare the F_{test} with F_{critical} at a particular Confidence Level. There will be two degrees of freedom involved, for sample 1 and sample 2, respectively.

NPTEL

F Distribution: Critical Values of F (5% significance level)

v_1	1	2	3	4	5	6	7	8	9	10	12	14	16	18	20
1	161.45	199.50	215.71	224.58	230.16	233.99	236.77	238.88	240.54	241.88	243.91	245.36	246.46	247.32	248.01
2	18.51	19.00	19.16	19.25	19.30	19.33	19.35	19.37	19.38	19.40	19.41	19.42	19.43	19.44	19.45
3	10.13	9.55	9.28	9.12	9.01	8.94	8.89	8.85	8.81	8.79	8.74	8.71	8.69	8.67	8.66
4	7.71	6.94	6.59	6.39	6.26	6.16	6.09	6.04	6.00	5.96	5.91	5.87	5.84	5.82	5.80
5	6.61	5.79	5.41	5.19	5.05	4.95	4.88	4.82	4.77	4.74	4.68	4.64	4.60	4.58	4.56
6	5.99	5.14	4.76	4.53	4.39	4.28	4.21	4.15	4.10	4.06	4.00	3.96	3.92	3.90	3.87
7	5.59	4.74	4.35	4.12	3.97	3.87	3.79	3.73	3.68	3.64	3.57	3.53	3.49	3.47	3.44
8	5.32	4.46	4.07	3.84	3.69	3.58	3.50	3.44	3.39	3.35	3.28	3.24	3.20	3.17	3.15
9	5.12	4.26	3.86	3.63	3.48	3.37	3.29	3.23	3.18	3.14	3.07	3.03	2.99	2.96	2.94
10	4.96	4.10	3.71	3.48	3.33	3.22	3.14	3.07	3.02	2.98	2.91	2.86	2.83	2.80	2.77
11	4.84	3.98	3.59	3.36	3.20	3.09	3.01	2.95	2.90	2.85	2.79	2.74	2.70	2.67	2.65
12	4.75	3.89	3.49	3.26	3.11	3.00	2.91	2.85	2.80	2.75	2.69	2.64	2.60	2.57	2.54
13	4.67	3.81	3.41	3.18	3.03	2.92	2.83	2.77	2.71	2.67	2.60	2.55	2.51	2.48	2.46
14	4.60	3.74	3.34	3.11	2.96	2.85	2.76	2.70	2.65	2.60	2.53	2.48	2.44	2.41	2.39
15	4.54	3.68	3.29	3.06	2.90	2.79	2.71	2.64	2.59	2.54	2.48	2.42	2.38	2.35	2.33
16	4.49	3.63	3.24	3.01	2.85	2.74	2.66	2.59	2.54	2.49	2.42	2.37	2.33	2.30	2.28
17	4.45	3.59	3.20	2.96	2.81	2.70	2.61	2.55	2.49	2.45	2.38	2.33	2.29	2.26	2.23
18	4.41	3.55	3.16	2.93	2.77	2.66	2.58	2.51	2.46	2.41	2.34	2.29	2.25	2.22	2.19
19	4.38	3.52	3.13	2.90	2.74	2.63	2.54	2.48	2.42	2.38	2.31	2.26	2.21	2.18	2.16
20	4.35	3.49	3.10	2.87	2.71	2.60	2.51	2.45	2.39	2.35	2.28	2.22	2.18	2.15	2.12
21	4.32	3.47	3.07	2.84	2.68	2.57	2.49	2.42	2.37	2.32	2.25	2.20	2.16	2.12	2.10
22	4.30	3.44	3.05	2.82	2.66	2.55	2.46	2.40	2.34	2.30	2.23	2.17	2.13	2.10	2.07
23	4.28	3.42	3.03	2.80	2.64	2.53	2.44	2.37	2.32	2.27	2.20	2.15	2.11	2.08	2.05
24	4.26	3.40	3.01	2.78	2.62	2.51	2.42	2.36	2.30	2.25	2.18	2.13	2.09	2.05	2.03
25	4.24	3.39	2.99	2.76	2.60	2.49	2.40	2.34	2.28	2.24	2.16	2.11	2.07	2.04	2.01
26	4.22	3.37	2.98	2.74	2.59	2.47	2.39	2.32	2.27	2.22	2.15	2.09	2.05	2.02	1.99
27	4.21	3.35	2.96	2.73	2.57	2.46	2.37	2.31	2.25	2.20	2.13	2.08	2.04	2.00	1.97
28	4.20	3.34	2.95	2.71	2.56	2.45	2.36	2.29	2.24	2.19	2.12	2.06	2.02	1.99	1.96
29	4.18	3.33	2.93	2.70	2.55	2.43	2.35	2.28	2.22	2.18	2.10	2.05	2.01	1.97	1.94
30	4.17	3.32	2.92	2.69	2.53	2.42	2.33	2.27	2.21	2.16	2.09	2.04	1.99	1.96	1.93
35	4.12	3.27	2.87	2.64	2.49	2.37	2.29	2.22	2.16	2.11	2.04	1.99	1.94	1.91	1.88
40	4.08	3.23	2.84	2.61	2.45	2.34	2.25	2.18	2.12	2.08	2.00	1.95	1.90	1.87	1.84
50	4.03	3.18	2.79	2.56	2.40	2.29	2.20	2.13	2.07	2.03	1.95	1.89	1.85	1.81	1.78
60	4.00	3.15	2.76	2.53	2.37	2.25	2.17	2.10	2.04	1.99	1.92	1.86	1.82	1.78	1.75
70	3.98	3.13	2.74	2.50	2.35	2.23	2.14	2.07	2.02	1.97	1.89	1.84	1.79	1.75	1.72
80	3.96	3.11	2.72	2.49	2.33	2.21	2.13	2.06	2.00	1.95	1.88	1.82	1.77	1.73	1.70
90	3.95	3.10	2.71	2.47	2.32	2.20	2.11	2.04	1.99	1.94	1.86	1.80	1.76	1.72	1.69
100	3.94	3.09	2.70	2.46	2.31	2.19	2.10	2.03	1.97	1.93	1.85	1.79	1.75	1.71	1.68
120	3.92	3.07	2.68	2.45	2.29	2.18	2.09	2.02	1.96	1.91	1.83	1.78	1.73	1.69	1.66
150	3.90	3.06	2.66	2.43	2.27	2.16	2.07	2.00	1.94	1.89	1.82	1.76	1.71	1.67	1.64
200	3.89	3.04	2.65	2.42	2.26	2.14	2.06	1.98	1.93	1.88	1.80	1.74	1.69	1.66	1.62
250	3.88	3.03	2.64	2.41	2.25	2.13	2.05	1.98	1.92	1.87	1.79	1.73	1.68	1.65	1.61
300	3.87	3.03	2.63	2.40	2.24	2.13	2.04	1.97	1.91	1.86	1.78	1.72	1.68	1.64	1.61
400	3.86	3.02	2.63	2.39	2.24	2.12	2.03	1.96	1.90	1.85	1.78	1.72	1.67	1.63	1.60
500	3.86	3.01	2.62	2.39	2.23	2.12	2.03	1.96	1.90	1.85	1.77	1.71	1.66	1.62	1.59
600	3.86	3.01	2.62	2.39	2.23	2.11	2.02	1.95	1.90	1.85	1.77	1.71	1.66	1.62	1.59
750	3.85	3.01	2.62	2.38	2.23	2.11	2.02	1.95	1.89	1.84	1.77	1.70	1.66	1.62	1.58
1000	3.85	3.00	2.61	2.38	2.22	2.11	2.02	1.95	1.89	1.84	1.76	1.70	1.65	1.61	1.58



F Distribution: Critical Values of F (1% significance level)

v_1	1	2	3	4	5	6	7	8	9	10	12	14	16	18	20
1	4052.18	4999.50	5403.35	5624.58	5763.65	5858.99	5928.36	5981.07	6022.47	6055.85	6106.32	6142.67	6170.10	6191.53	6208.73
2	98.50	99.00	99.17	99.25	99.30	99.33	99.36	99.37	99.39	99.40	99.42	99.43	99.44	99.44	99.45
3	34.12	30.82	29.46	28.71	28.24	27.91	27.67	27.49	27.35	27.23	27.05	26.92	26.83	26.75	26.69
4	21.20	18.00	16.69	15.98	15.52	15.21	14.98	14.80	14.66	14.55	14.37	14.25	14.15	14.08	14.02
5	16.26	13.27	12.06	11.39	10.97	10.67	10.46	10.29	10.16	10.05	9.89	9.77	9.68	9.61	9.55
6	13.75	10.92	9.78	9.15	8.75	8.47	8.26	8.10	7.98	7.87	7.72	7.60	7.52	7.45	7.40
7	12.25	9.55	8.45	7.85	7.46	7.19	6.99	6.84	6.72	6.62	6.47	6.36	6.28	6.21	6.16
8	11.26	8.65	7.59	7.01	6.63	6.37	6.18	6.03	5.91	5.81	5.67	5.56	5.48	5.41	5.36
9	10.56	8.02	6.99	6.42	6.06	5.80	5.61	5.47	5.35	5.26	5.11	5.01	4.92	4.86	4.81
10	10.04	7.56	6.55	5.99	5.64	5.39	5.20	5.06	4.94	4.85	4.71	4.60	4.52	4.46	4.41
11	9.65	7.21	6.22	5.67	5.32	5.07	4.89	4.74	4.63	4.54	4.40	4.29	4.21	4.15	4.10
12	9.33	6.93	5.95	5.41	5.06	4.82	4.64	4.50	4.39	4.30	4.16	4.05	3.97	3.91	3.86
13	9.07	6.70	5.74	5.21	4.86	4.62	4.44	4.30	4.19	4.10	3.96	3.86	3.78	3.72	3.66
14	8.86	6.51	5.56	5.04	4.69	4.46	4.28	4.14	4.03	3.94	3.80	3.70	3.62	3.56	3.51
15	8.68	6.36	5.42	4.89	4.56	4.32	4.14	4.00	3.89	3.80	3.67	3.56	3.49	3.42	3.37
16	8.53	6.23	5.29	4.77	4.44	4.20	4.03	3.89	3.78	3.69	3.55	3.45	3.37	3.31	3.26
17	8.40	6.11	5.18	4.67	4.34	4.10	3.93	3.79	3.68	3.59	3.46	3.35	3.27	3.21	3.16
18	8.29	6.01	5.09	4.58	4.25	4.01	3.84	3.71	3.60	3.51	3.37	3.27	3.19	3.13	3.08
19	8.18	5.93	5.01	4.50	4.17	3.94	3.77	3.63	3.52	3.43	3.30	3.19	3.12	3.05	3.00
20	8.10	5.85	4.94	4.43	4.10	3.87	3.70	3.56	3.46	3.37	3.23	3.13	3.05	2.99	2.94
21	8.02	5.78	4.87	4.37	4.04	3.81	3.64	3.51	3.40	3.31	3.17	3.07	2.99	2.93	2.88
22	7.95	5.72	4.82	4.31	3.99	3.76	3.59	3.45	3.35	3.26	3.12	3.02	2.94	2.88	2.83
23	7.88	5.66	4.76	4.26	3.94	3.71	3.54	3.41	3.30	3.21	3.07	2.97	2.89	2.83	2.78
24	7.82	5.61	4.72	4.22	3.90	3.67	3.50	3.36	3.26	3.17	3.03	2.93	2.85	2.79	2.74
25	7.77	5.57	4.68	4.18	3.85	3.63	3.46	3.32	3.22	3.13	2.99	2.89	2.81	2.75	2.70
26	7.72	5.53	4.64	4.14	3.82	3.59	3.42	3.29	3.18	3.09	2.96	2.86	2.78	2.72	2.66
27	7.68	5.49	4.60	4.11	3.78	3.56	3.39	3.26	3.15	3.06	2.93	2.82	2.75	2.68	2.63
28	7.64	5.45	4.57	4.07	3.75	3.53	3.36	3.23	3.12	3.03	2.90	2.79	2.72	2.65	2.60
29	7.60	5.42	4.54	4.04	3.73	3.50	3.33	3.20	3.09	3.00	2.87	2.77	2.69	2.63	2.57
30	7.56	5.39	4.51	4.02	3.70	3.47	3.30	3.17	3.07	2.98	2.84	2.74	2.66	2.60	2.55
35	7.42	5.27	4.40	3.91	3.59	3.37	3.20	3.07	2.96	2.88	2.74	2.64	2.56	2.50	2.44
40	7.31	5.18	4.31	3.83	3.51	3.29	3.12	2.99	2.89	2.80	2.66	2.56	2.48	2.42	2.37
50	7.17	5.06	4.20	3.72	3.41	3.19	3.02	2.89	2.78	2.70	2.56	2.46	2.38	2.32	2.27
60	7.08	4.98	4.13	3.65	3.34	3.12	2.95	2.82	2.72	2.63	2.50	2.39	2.31	2.25	2.20
70	7.01	4.92	4.07	3.60	3.29	3.07	2.91	2.78	2.67	2.59	2.45	2.35	2.27	2.20	2.15
80	6.96	4.88	4.04	3.56	3.26	3.04	2.87	2.74	2.64	2.55	2.42	2.31	2.23	2.17	2.12
90	6.93	4.85	4.01	3.53	3.23	3.01	2.84	2.72	2.61	2.52	2.39	2.29	2.21	2.14	2.09
100	6.90	4.82	3.98	3.51	3.21	2.99	2.82	2.69	2.59	2.50	2.37	2.27	2.19	2.12	2.07
120	6.85	4.79	3.95	3.48	3.17	2.96	2.79	2.66	2.56	2.47	2.34	2.23	2.15	2.09	2.03
150	6.81	4.75	3.91	3.45	3.14	2.92	2.76	2.63	2.53	2.44	2.31	2.20	2.12	2.06	2.00
200	6.76	4.71	3.88	3.41	3.11	2.89	2.73	2.60	2.50	2.41	2.27	2.17	2.09	2.03	1.97
250	6.74	4.69	3.86	3.40	3.09	2.87	2.71	2.58	2.48	2.39	2.26	2.15	2.07	2.01	1.95
300	6.72	4.68	3.85	3.38	3.08	2.86	2.70	2.57	2.47	2.38	2.24	2.14	2.06	1.99	1.94
400	6.70	4.66	3.83	3.37	3.06	2.85	2.68	2.56	2.45	2.37	2.23	2.13	2.05	1.98	1.92
500	6.69	4.65	3.82	3.36	3.05	2.84	2.68	2.55	2.44	2.36	2.22	2.12	2.04	1.97	1.92
600	6.68	4.64	3.81	3.35	3.05	2.83	2.67	2.54	2.44	2.35	2.21	2.11	2.03	1.96	1.91
750	6.67	4.63	3.81	3.34	3.04	2.83	2.66	2.53	2.43	2.34	2.21	2.11	2.02	1.96	1.90
1000	6.66	4.63	3.80	3.34	3.04	2.82	2.66	2.53	2.43	2.34	2.20	2.10	2.02	1.95	1.90





Example of applying F-test to compare the precision of two samples

Question: A standard method based on 1001 data points for estimation of Na ions has a SD value of 0.18 ppm. A new method based on 21 data points had SD value of 0.21 ppm. Can we say at 95% confidence level that the new method is more imprecise than the standard method?

Answer:

1. Apply null hypothesis : $H_0 = SD1 \sim SD2$.
2. Calculate $F_{\text{test}} = (0.21)^2 / (0.18)^2 = 0.0441/0.0324 = 1.36$.
3. Compare with $F_{\text{critical}} (95\%, 20, 1000) = 1.58$.
4. Since $F_{\text{test}} < F_{\text{critical}}$, null hypothesis cannot be rejected. That means, at 95% CL, the two methods being compared have similar precision.

NPTEL



Single Factor ANOVA

Questions that we want to address:

1. Does the **yield** of a reaction change with **temperature**?
2. Does the **spectral profile** of the molecule change with **pH**?

Factor – the variable that is being changed.

Level – Individual measurements within each factor.

Response – The change in the value of measurement being performed.

Single factor ANOVA implies that there is **only one factor** that is **being varied**.

NPTTEL



Protocol for undertaking ANOVA

Presumptions: Normality, Independence of Errors and Equivariance

If we are analysing I different samples:

Samples	1	2	3	...	I
Data points	N_1	N_2	N_3	...	N_I
Means	x_1	x_2	x_3	...	x_I
Std. Dev.	s_1	s_2	s_3	...	s_I
Variance	$(s_1)^2$	$(s_2)^2$	$(s_3)^2$...	$(s_I)^2$

INPTEL



1. Calculate the **Grand Average** of the data set. It is a weighted average of the individual sample means.
2. Obtain the square of **deviations between the group means and the grand average**. This is called Sum of Squares due to Factor (**SSF**). Degrees of Freedom = $I - 1$
3. Obtain the square of **deviations within the groups**. This is called the Sum of Squares due to errors (**SSE**). Degrees of Freedom = $N - I$
4. Calculate **Sum of Squares Total (SST)** = $SSF + SSE$. Degrees of Freedom = $N - 1$
5. Calculate the **Mean Square Factor (MSF)** = $SSF / (I - 1)$. MSF is an estimate of variance due to error plus variance between the groups i.e. $\sigma_E^2 + \sigma_F^2$.
6. Calculate the **Mean Square Error (MSE)** = $SSE / (N - I)$. MSE estimates only the variance due to error, i.e. σ_E^2 .
7. Calculate $F_{\text{test}} = \text{MSF} / \text{MSE}$ and compare with F_{crit} .

NPTEL



ANOVA Table

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squared Values	F
Between the groups (Factor Effect)	SSF	$I - 1$	$MSF = SSF / I - 1$	$F_{\text{test}} = MSF / MSE$
Within the group (Error Effect)	SSE	$N - I$	$MSE = SSE / N - I$...
Total	SST	$N - 1$

NPTEL



Q. The mean potassium levels of three groups of volunteers considered either Healthy, Pre-clinical and Clinical were compared. The following data was obtained:

Healthy	Pre-clinical	Clinical
120	100	89
100	110	65
98	70	110
90	80	90
75	50	40
80	70	35

NPTTEL

Is there a statistically significant difference in the mean potassium levels of the three groups at 95% Confidence Level?