

ANOVA

(Part 1/4)

Basic Concepts



Biostatistics & Research Methodology
B Pharm 8th Sem | M. Pharm. | PhD

ANOVA



- Most common and advance technique to analysis the parametric data.
- It is developed by **RA Fisher in 1920**
- It is used to measure the variance or difference between multiple groups
(Groups > 2)
- In ANOVA, level of significance is checked by **F-Ratio**

$$F \text{ Ratio} = \frac{\text{Variance between mean of groups}}{\text{Variance within the group}}$$

Types:

- One-Way ANOVA – 1 variable ✓
- Two-Way ANOVA – 2 Variable
- Three-Way ANOVA- 3 Variable

drug A vs drug B

	drug A	drug B
Time		
0h		
1h		
2h		

ANOVA



F Ratio = $\frac{\text{Variance between mean of groups}}{\text{Variance within the group}}$

$= \frac{V_b}{V_w}$

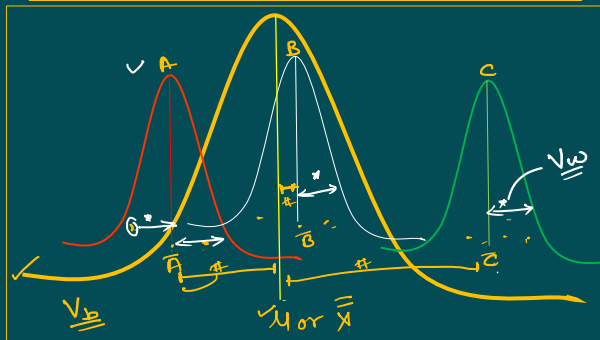
Variance b/w groups
* Variance within groups

group mean μ_j

A_i, V_{A_i}
 B_i, V_{B_i}

populatioⁿ df: N
Sample df: n-1

σ^2 , Variance = $\frac{\sum (X_i - \bar{X})^2}{df}$



$V_b = \frac{\sum (\bar{A} - \mu)^2 + \sum (\bar{B} - \mu)^2 + \sum (\bar{C} - \mu)^2}{df \text{ (no. of gp - 1)}}$

$V_w = \frac{\sum (A_i - \bar{A})^2 + \sum (B_i - \bar{B})^2 + \sum (C_i - \bar{C})^2}{df}$
df = $df_A + df_B + df_C$
 $(n-1)$

(F_{crit})

$F_{calculated} < F_{tabulated} < F_{calculated}$

H₀

$\bar{A} = \bar{B} = \bar{C}$

H_a

At least 2 groups should be differ

$\bar{A} \neq \bar{B} \neq \bar{C}, \bar{A} = \bar{B} \neq \bar{C}$
 $\bar{A} \neq \bar{B} = \bar{C}, \bar{A} = \bar{C} \neq \bar{B}$

ANOVA



ASSUMPTION

▣ Data (each population) are normally distributed. ✓

▣ Samples have equal variance or standard deviation

$\sigma_1^2 = \sigma_2^2 = \sigma_3^2 = \dots = \sigma_n^2$

▣ Samples are random and independent ✓

▣ Hypothesis

H₀ ($F_{cal} < F_{tab}$) $\bar{A} = \bar{B} = \bar{C}$ - similar

H₁ ($F_{cal} > F_{tab}$) $\bar{A} \neq \bar{B} \neq \bar{C}$ = significant difference

One-Way ANOVA

(Part 2/4)

- ✓ Manually calculation
- ✓ GraphPad Prism 5.0 Software
- ✓ MS Excel



GraphPad Prism 5
Project



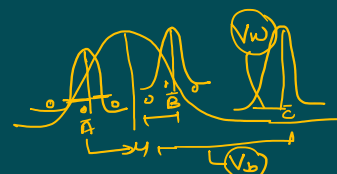
Microsoft Excel
Worksheet

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One-Way ANOVA

ASSUMPTION

- ▣ Data (each population) are normally distributed. *- parametric data*
- ▣ Samples have equal variance or standard deviation SD, σ^2
 $S_1^2 = S_2^2 = S_3^2 = S_n^2$
- ▣ Samples are random and independent
- ▣ Involve single variable
- ▣ No of Groups ≥ 2
- ▣ Compare the means or variance between multiple groups



$N < 30$ t test $N > 30$ Z test
group ≤ 2 group ≤ 2

Group ≥ 3 - ANOVA
(A) GB GC $n = 3$
AVB
AVC
AVC
3 times t test

$$F \text{ Ratio} = \frac{\text{Variance between mean of groups}}{\text{Variance within the group}} = \frac{V_b}{V_w}$$

$$\frac{F_{cal}}{F_{table}} = \frac{F_{cal}}{HT}$$

One-Way ANOVA



Question: Camper average marks of different batch, whether they are same or differ (5% level of significance, $F_{2,6} = 5.14$)

	A	B	C
1	2	3	4
2	4	5	6
3	6	7	8
Sum-	12	15	18
Mean	12/3 4	15/3 5	18/3 6

A. State the hypothesis

$H_0 = \bar{A} = \bar{B} = \bar{C} = \text{Similar}$
 $H_1 = \bar{A} \neq \bar{B} \neq \bar{C} = \text{difference}$
 $\bar{A} = \bar{B} \neq \bar{C}$
 $\bar{A} \neq \bar{B} = \bar{C}$
 $\bar{A} = \bar{C} \neq \bar{B}$

B. Calculation

1. Calculate the mean of each group

$\bar{A} = 4, \bar{B} = 5, \bar{C} = 6$

2. Calculate the Grand mean (\bar{X} or μ)

$\bar{X} \text{ or } \mu = \frac{\bar{A} + \bar{B} + \bar{C}}{3} = \frac{4 + 5 + 6}{3}$
 $\mu = 5$

3. Calculate the Variance

Variance = $\frac{\sum (X - \bar{X})^2}{df}$

$V_b = \frac{\sum (C\bar{A} - \mu)^2 + \sum (C\bar{B} - \mu)^2 + \sum (C\bar{C} - \mu)^2}{df}$, $df = \text{no. of gp} - 1$

$V_w = \frac{\sum (C\bar{A} - \bar{A})^2 + \sum (C\bar{B} - \bar{B})^2 + \sum (C\bar{C} - \bar{C})^2}{df}$, $df = df_A + df_B + df_C$
 $= (n-1) + (n-1) + (n-1)$
 $\text{no. of population} = N - 3$

One-Way ANOVA



3A. Variance between samples

a) Calculate the sum of square between sample (SSC)

	$\bar{A} - \mu$	$(\bar{A} - \mu)^2$	$\bar{B} - \mu$	$(\bar{B} - \mu)^2$	$\bar{C} - \mu$	$(\bar{C} - \mu)^2$
1	-1	1	0	0	1	1
2	-1	1	0	0	1	1
3	-1	1	0	0	1	1
		3		0		3

$\sum (C\bar{A} - \mu)^2 + \sum (C\bar{B} - \mu)^2 + \sum (C\bar{C} - \mu)^2$
 $= 3 + 0 + 3 = 6$ OR
 $SSC = n_A(\bar{A} - \mu)^2 + n_B(\bar{B} - \mu)^2 + n_C(\bar{C} - \mu)^2$
 $3(1) + 3(0) + 3(1)$
 $3 + 0 + 3 = 6$
 $SSC = 6$

b) Degree of Freedom (df) = (No. of Group - 1)

$df = 3 - 1$
 $= 2$

Variance b/w sample

Mean of sum of square (MSC) = SSC / df
 $= 6 / 2$
 $= 3$

One-Way ANOVA



3B. Variance within samples

a) Calculate the sum of square within sample (SSE)

	A-A	(A-A) ²	B-B	(B-B) ²	C-C	(C-C) ²
1	-2	4	-2	4	-2	4
2	0	0	0	0	0	0
3	2	4	2	4	2	4
		8		8		8

$$SSE = \sum(A-\bar{A})^2 + \sum(B-\bar{B})^2 + \sum(C-\bar{C})^2$$

$$= 8 + 8 + 8 = 24$$

$$SSE = 24$$

	A	B	C
1	2	3	4
2	4	5	6
3	6	7	8
M	4	5	6

b) Degree of Freedom (dfe) = df_a + df_B + df_c

$$df = (n_A - 1) + (n_B - 1) + (n_C - 1)$$

$$= (3 - 1) + (3 - 1) + (3 - 1)$$

$$= 2 + 2 + 2 = 6$$

or $N - 3$ $\therefore N = \text{not sample in population}$

$$9 - 3 = 6$$

Variance within sample

Mean of sum of square (MSE) = $\frac{SSE}{dfe}$

$$= \frac{24}{6}$$

$$= 4$$

One-Way ANOVA



F Ratio = $\frac{\text{Variance between mean of groups}}{\text{Variance within the group}} = \frac{V_b}{V_w} = \frac{3}{4} = 0.75$

Source of Variance	SS	df	Mean of SS	F value
Variance b/w sample	SSC = 6	dfc = 2	MSC = $\frac{SSC}{dfc} = \frac{6}{2} = 3$	F = MSC/MSE = $\frac{3}{4} = 0.75$
Variance within sample	SSE = 24	dfe = 6	MSE = $\frac{24}{6} = 4$	F _{cal}

4. Take a Design ($\alpha = 0.05, F_{2,6} = 5.14$)

$F_{cal} < F_{tab}$

- $\therefore F_{cal} < F_{tab}$
- $\therefore H_0$ is Accepted
- = There are no significant difference between batches

F(0.05)	df ₁ =1	2	3	4	5	6	7	8	9	10	12	15	20	24	30	40	60	120	∞
df ₂ =1	161.4476	199.5000	215.7073	224.5832	230.1619	233.9860	236.7684	238.8827	240.5433	241.8817	243.9060	245.9499	248.0131	249.0518	250.0951	251.1432	252.1957	253.2529	254.3144
2	18.5128	19.0000	19.1643	19.2468	19.2964	19.3295	19.3532	19.3710	19.3848	19.3959	19.4125	19.4291	19.4458	19.4541	19.4624	19.4707	19.4791	19.4874	19.4957
3	10.1280	9.5521	9.2766	9.1172	9.0135	8.9406	8.8867	8.8452	8.8123	8.7855	8.7446	8.7029	8.6602	8.6385	8.6166	8.5944	8.5720	8.5494	8.5264
4	7.7086	6.9443	6.5914	6.3882	6.2561	6.1631	6.0942	6.0410	5.9988	5.9644	5.9117	5.8578	5.8025	5.7744	5.7459	5.7170	5.6877	5.6581	5.6281
5	6.6079	5.7861	5.4095	5.1922	5.0503	4.9503	4.8759	4.8183	4.7725	4.7351	4.6777	4.6188	4.5581	4.5272	4.4957	4.4638	4.4314	4.3985	4.3650
6	5.9874	5.1433	4.7571	4.5337	4.3874	4.2839	4.2067	4.1468	4.0990	4.0600	3.9999	3.9381	3.8742	3.8415	3.8082	3.7743	3.7398	3.7047	3.6689
7	5.5914	4.7374	4.3468	4.1203	3.9715	3.8660	3.7870	3.7257	3.6767	3.6365	3.5747	3.5107	3.4445	3.4105	3.3758	3.3404	3.3043	3.2674	3.2298
8	5.3177	4.4590	4.0662	3.8379	3.6875	3.5806	3.5005	3.4381	3.3881	3.3472	3.2839	3.2184	3.1503	3.1152	3.0794	3.0428	3.0053	2.9669	2.9276
9	5.1174	4.2565	3.8625	3.6331	3.4817	3.3738	3.2927	3.2296	3.1789	3.1373	3.0729	3.0061	2.9365	2.9005	2.8637	2.8259	2.7872	2.7475	2.7067
10	4.9646	4.1028	3.7083	3.4780	3.3258	3.2172	3.1355	3.0717	3.0204	2.9782	2.9130	2.8450	2.7740	2.7372	2.6996	2.6609	2.6211	2.5801	2.5379
11	4.8443	3.9823	3.5874	3.3567	3.2039	3.0946	3.0123	2.9480	2.8962	2.8536	2.7876	2.7186	2.6464	2.6090	2.5705	2.5309	2.4901	2.4480	2.4045
12	4.7472	3.8853	3.4903	3.2592	3.1059	2.9961	2.9134	2.8486	2.7964	2.7534	2.6866	2.6169	2.5436	2.5055	2.4663	2.4259	2.3842	2.3410	2.2962
13	4.6672	3.8056	3.4105	3.1791	3.0254	2.9153	2.8321	2.7669	2.7144	2.6710	2.6037	2.5331	2.4589	2.4202	2.3803	2.3392	2.2966	2.2524	2.2064
14	4.6001	3.7389	3.3439	3.1122	2.9582	2.8477	2.7642	2.6987	2.6458	2.6022	2.5342	2.4630	2.3879	2.3487	2.3082	2.2664	2.2229	2.1778	2.1307
15	4.5431	3.6823	3.2874	3.0556	2.9013	2.7905	2.7066	2.6408	2.5876	2.5437	2.4753	2.4034	2.3275	2.2878	2.2468	2.2043	2.1601	2.1141	2.0658
16	4.4940	3.6337	3.2389	3.0069	2.8524	2.7413	2.6572	2.5911	2.5377	2.4935	2.4247	2.3522	2.2756	2.2354	2.1938	2.1507	2.1058	2.0589	2.0096
17	4.4513	3.5915	3.1968	2.9647	2.8100	2.6987	2.6143	2.5480	2.4943	2.4499	2.3807	2.3077	2.2304	2.1898	2.1477	2.1040	2.0584	2.0107	1.9604
18	4.4139	3.5546	3.1599	2.9277	2.7729	2.6613	2.5767	2.5102	2.4563	2.4117	2.3421	2.2686	2.1906	2.1497	2.1071	2.0629	2.0166	1.9681	1.9168
19	4.3807	3.5219	3.1274	2.8951	2.7401	2.6283	2.5435	2.4768	2.4227	2.3779	2.3080	2.2341	2.1555	2.1141	2.0712	2.0264	1.9795	1.9302	1.8780
20	4.3512	3.4928	3.0984	2.8661	2.7109	2.5990	2.5140	2.4471	2.3928	2.3479	2.2776	2.2033	2.1242	2.0825	2.0391	1.9938	1.9464	1.8963	1.8432
21	4.3248	3.4668	3.0725	2.8401	2.6848	2.5727	2.4876	2.4205	2.3660	2.3210	2.2504	2.1757	2.0960	2.0540	2.0102	1.9645	1.9165	1.8657	1.8117
22	4.3009	3.4434	3.0491	2.8167	2.6613	2.5491	2.4638	2.3965	2.3419	2.2967	2.2258	2.1508	2.0707	2.0283	1.9842	1.9380	1.8894	1.8380	1.7831
23	4.2793	3.4221	3.0278	2.7955	2.6400	2.5277	2.4422	2.3748	2.3201	2.2747	2.2036	2.1282	2.0476	2.0050	1.9605	1.9139	1.8648	1.8128	1.7570
24	4.2597	3.4028	3.0088	2.7763	2.6207	2.5082	2.4226	2.3551	2.3002	2.2547	2.1834	2.1077	2.0267	1.9838	1.9390	1.8920	1.8424	1.7896	1.7330
25	4.2417	3.3852	2.9912	2.7587	2.6030	2.4904	2.4047	2.3371	2.2821	2.2365	2.1649	2.0889	2.0075	1.9643	1.9192	1.8718	1.8217	1.7684	1.7110
26	4.2252	3.3690	2.9752	2.7426	2.5868	2.4741	2.3883	2.3205	2.2655	2.2197	2.1479	2.0716	1.9898	1.9464	1.9010	1.8533	1.8027	1.7488	1.6906
27	4.2100	3.3541	2.9604	2.7278	2.5719	2.4591	2.3732	2.3053	2.2501	2.2043	2.1323	2.0558	1.9736	1.9299	1.8842	1.8361	1.7851	1.7306	1.6717
28	4.1960	3.3404	2.9467	2.7141	2.5581	2.4453	2.3593	2.2913	2.2360	2.1900	2.1179	2.0411	1.9586	1.9147	1.8687	1.8203	1.7689	1.7138	1.6541
29	4.1830	3.3277	2.9340	2.7014	2.5454	2.4324	2.3463	2.2783	2.2229	2.1768	2.1045	2.0275	1.9446	1.9005	1.8543	1.8055	1.7537	1.6981	1.6376
30	4.1709	3.3158	2.9223	2.6896	2.5336	2.4205	2.3343	2.2662	2.2107	2.1646	2.0921	2.0148	1.9317	1.8874	1.8409	1.7918	1.7396	1.6835	1.6223
40	4.0847	3.2317	2.8387	2.6060	2.4495	2.3359	2.2490	2.1802	2.1240	2.0772	2.0035	1.9245	1.8389	1.7929	1.7444	1.6928	1.6373	1.5766	1.5089
60	4.0012	3.1504	2.7581	2.5252	2.3683	2.2541	2.1665	2.0970	2.0401	1.9926	1.9174	1.8364	1.7480	1.7001	1.6491	1.5943	1.5343	1.4673	1.3893
120	3.9201	3.0718	2.6802	2.4472	2.2899	2.1750	2.0868	2.0164	1.9588	1.9105	1.8337	1.7505	1.6587	1.6084	1.5543	1.4952	1.4290	1.3519	1.2539
∞	3.8415	2.9957	2.6049	2.3719	2.2141	2.0986	2.0096	1.9384	1.8799	1.8307	1.7522	1.6664	1.5705	1.5173	1.4591	1.3940	1.3180	1.2214	1.0000

Two-Way ANOVA

(Without Replication)

(Part 3/4)

- ✓ Manually calculation
- ✓ GraphPad Prism 5.0 Software
- ✓ MS Excel

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Two-Way ANOVA



ASSUMPTION

- ❑ Data (each population) are normally distributed.
- ❑ Samples have equal variance or standard deviation

$$S_1^2 = S_2^2 = S_3^2 = S_n^2$$
- ❑ Samples are random and independent
- ❑ Involve Two variable
- ❑ No of Groups > 2
- ❑ Compare the means or variance between multiple groups

Randomize Block Design $\checkmark V_1 - \text{Drug}$

	Drug Treatment			
	\checkmark a	\checkmark b	\checkmark c	\checkmark d
1H	2	3	4	5
2H	4	4	6	6
3H	6	5	8	8

 V_2
Time

$$\checkmark \text{ F Ratio} = \frac{\text{Variance between mean of groups}}{\text{Variance within the group}} \checkmark$$

Two-Way ANOVA



Question: analyse

1. Effects of different drugs are same in a time frame
 $[p < 0.05, \underline{F_{3,6}} = 4.76]$

2. Effect of each drug is same in different time frame
 $[p < 0.05, \underline{F_{2,6}} = 4.76]$

Randomize Block Design

	Drug Treatment			
	a	b	c	d
1H	2	3	4	5
2H	4	4	6	6
3H	6	5	8	8

Variance can be determine for-

- a) Between column (Column Factors) (a,b,c,d)-
- b) Between Row (Row factor)- (1H, 2H, 3H)
- c) Within the samples (Residual)

Two-Way ANOVA



1. Calculate Grand total and Correction factor

- a. Select a assume mid value- 5
- b) Calculate Grand total- by their difference

	Drug Treatment				Total
	a-5	b-5	c-5	d-5	
1H	-3	-2	-1	0	6
2H	-1	-1	1	1	0
3H	1	0	3	3	7
Total	-3	-3	3	4	1

Grand Total

$$\text{Grand Total (T)} = 1$$

c) Correction Factor = $\frac{(T)^2}{N}$

$$= \frac{1}{12}$$

$$= 0.08$$

	Drug Treatment			
	a	b	c	d
1H	2	3	4	5
2H	4	4	6	6
3H	6	5	8	8

2. Calculate Sum of square between Column (Column Factor, SSC)

$$\text{SSC} = \frac{(\sum A)^2}{n_a} + \frac{(\sum B)^2}{n_b} + \frac{(\sum C)^2}{n_c} + \frac{(\sum D)^2}{n_d} - c.f.$$

$$= \frac{(-3)^2}{3} + \frac{(-3)^2}{3} + \frac{3^2}{3} + \frac{4^2}{3} - 0.08$$

$$= \frac{9}{3} + \frac{9}{3} + \frac{9}{3} + \frac{16}{3} - 0.08$$

$$= 3 + 3 + 3 + 5.33 - 0.08$$

$$= 14.33 - 0.08 = 14.25$$

$V_b = \frac{\text{SSC}}{\text{df}_{\text{column}}}$ \Rightarrow $\text{df} = \text{no. of column} - 1$

$$= \frac{14.25}{3} = 4.75$$

	Drug Treatment				Total
	(a-5)	(b-5)	(c-5)	(d-5)	
	A	B	C	D	
1H	-3	-2	-1	0	-6
2H	-1	-1	1	1	0
3H	1	0	3	3	7
Total	-3	-3	3	4	1

$\sum A$ $\sum B$ $\sum C$ $\sum D$

Two-Way ANOVA



3. Calculate Sum of square between Row (Row Factor, SSR)

$$\begin{aligned}
 SSR &= \frac{(\sum H_1)^2}{n_{H_1}} + \frac{(\sum H_2)^2}{n_{H_2}} + \frac{(\sum H_3)^2}{n_{H_3}} - 0.08 \\
 &= \frac{(-6)^2}{4} + \frac{(0)^2}{4} + \frac{(7)^2}{4} - 0.08 \\
 &= \frac{36}{4} + 0 + \frac{49}{4} - 0.08 \\
 &= 9 + 0 + 12.25 - 0.08 \\
 &= 21.25 - 0.08 = 21.17
 \end{aligned}$$

$$\begin{aligned}
 \# V_{bRow} &= \frac{SSR}{df_R}, \quad df_R = n_0 \cdot q - 1 \\
 &= \frac{21.17}{2} = 10.59 \\
 V_{bRow} &= 10.59
 \end{aligned}$$

	Drug Treatment				Total
	(a-5)	(b-5)	(c-5)	(d-5)	
	A	B	C	D	
H1	-3	-2	-1	0	-6
H2	-1	-1	1	1	0
H3	1	0	3	3	7
Total	-3	-3	3	4	1

Two-Way ANOVA



4. Calculate Sum of square of Total (Total, SST) = $\sum (x_i)^2$

$$\begin{aligned}
 SST &= (-3)^2 + (-2)^2 + (-1)^2 + (0)^2 + (-1)^2 + (-1)^2 + (1)^2 + (1)^2 + (1)^2 + \\
 & (0)^2 + (3)^2 + (3)^2 - 0.08 \\
 &= 9 + 4 + 1 + 0 + 1 + 1 + 1 + 1 + 0 + 9 + 9 - 0.08 \\
 &= 37 - 0.08 = 36.92
 \end{aligned}$$

	Drug Treatment				Total
	(a-5)	(b-5)	(c-5)	(d-5)	
	A	B	C	D	
H1	-3	-2	-1	0	-6
H2	-1	-1	1	1	0
H3	1	0	3	3	7
Total	-3	-3	3	4	1

4. Calculate Sum of square of within sample (Residual, SSE)

$$\begin{aligned}
 SSE &= SST - (SSC + SSR) \\
 &= 36.92 - (14.25 + 21.17) \\
 &= 36.92 - 35.42 \\
 &= 1.5
 \end{aligned}$$

$$\begin{aligned}
 \# V_w &= SSE/df, \quad df = dfc \times dfr = 3 \times 2 = 6 \\
 &= 1.5/6 = 0.25
 \end{aligned}$$

Two-Way ANOVA



Source of Variance	SS	Df	MS	F	Fcrit
Column Factors ✓	SSC = <u>14.25</u>	Dfc = <u>3</u>	14.25/3 = <u>4.75</u>	F = MSC/MSE = 4.75/0.25 = <u>19</u>	F _{3,6} = <u>4.76</u>
Row Factor ✓	SSR = <u>21.17</u>	Dfr = <u>2</u>	21.17/2 = <u>10.59</u>	F = MSR/MSE = 10.59/0.25 = <u>42.33</u>	F _{2,6} = <u>5.14</u>
Residual ✓	SSE = <u>1.5</u>	Dfe = 3 x 2 = <u>6</u>	1.5/6 = <u>0.25</u>		
Total ✓	SST = 36.92	Dft = N-1 = 12-1 = <u>11</u>			

Ho reject
Ha Accept

Ha Accept
Ho reject
Significance difference

F(0.05)	df1=1	2	3	4	5	6	7	8	9	10	12	15	20	24	30	40	60	120	∞
2	161.4476	199.5000	215.7073	224.5832	230.1619	233.9860	236.7684	238.8827	240.5433	241.8817	243.9060	245.9499	248.0131	249.0518	250.0951	251.1432	252.1957	253.2529	254.3144
3	18.5128	19.0000	19.1643	19.2468	19.2964	19.3295	19.3532	19.3710	19.3848	19.3959	19.4125	19.4291	19.4458	19.4541	19.4624	19.4707	19.4791	19.4874	19.4957
4	10.1280	9.5521	9.2766	9.1172	9.0135	8.9406	8.8867	8.8452	8.8123	8.7855	8.7446	8.7029	8.6602	8.6385	8.6166	8.5944	8.5720	8.5494	8.5264
5	7.7086	6.9443	6.5914	6.3882	6.2561	6.1631	6.0942	6.0410	5.9988	5.9644	5.9117	5.8578	5.8025	5.7744	5.7459	5.7170	5.6877	5.6581	5.6281
6	6.6079	5.7861	5.4095	5.1922	5.0503	4.9503	4.8759	4.8183	4.7725	4.7351	4.6777	4.6188	4.5581	4.5272	4.4957	4.4638	4.4314	4.3985	4.3650
7	5.9874	5.1433	4.7571	4.5337	4.3874	4.2839	4.2067	4.1468	4.0990	4.0600	3.9999	3.9381	3.8742	3.8415	3.8082	3.7743	3.7398	3.7047	3.6689
8	5.5914	4.7374	4.3468	4.1203	3.9715	3.8660	3.7870	3.7257	3.6767	3.6365	3.5747	3.5107	3.4445	3.4105	3.3758	3.3404	3.3043	3.2674	3.2298
9	5.3177	4.4590	4.0662	3.8379	3.6875	3.5806	3.5005	3.4381	3.3881	3.3472	3.2839	3.2184	3.1503	3.1152	3.0794	3.0428	3.0053	2.9669	2.9276
10	5.1174	4.2565	3.8625	3.6331	3.4817	3.3738	3.2927	3.2296	3.1789	3.1373	3.0729	3.0061	2.9365	2.9005	2.8637	2.8259	2.7872	2.7475	2.7067
11	4.9646	4.1028	3.7083	3.4780	3.3258	3.2172	3.1355	3.0717	3.0204	2.9782	2.9130	2.8450	2.7740	2.7372	2.6996	2.6609	2.6211	2.5801	2.5379
12	4.8443	3.9823	3.5874	3.3567	3.2039	3.0946	3.0123	2.9480	2.8962	2.8536	2.7876	2.7186	2.6464	2.6090	2.5705	2.5309	2.4901	2.4480	2.4045
13	4.7472	3.8853	3.4903	3.2592	3.1059	2.9961	2.9134	2.8486	2.7964	2.7534	2.6866	2.6169	2.5436	2.5055	2.4663	2.4259	2.3842	2.3410	2.2962
14	4.6672	3.8056	3.4105	3.1791	3.0254	2.9153	2.8321	2.7669	2.7144	2.6710	2.6037	2.5331	2.4589	2.4202	2.3803	2.3392	2.2966	2.2524	2.2064
15	4.6001	3.7389	3.3439	3.1122	2.9582	2.8477	2.7642	2.6987	2.6458	2.6022	2.5342	2.4630	2.3879	2.3487	2.3082	2.2664	2.2229	2.1778	2.1307
16	4.5431	3.6823	3.2874	3.0556	2.9013	2.7905	2.7066	2.6408	2.5876	2.5437	2.4753	2.4034	2.3275	2.2878	2.2468	2.2043	2.1601	2.1141	2.0658
17	4.4940	3.6337	3.2389	3.0069	2.8524	2.7413	2.6572	2.5911	2.5377	2.4935	2.4247	2.3522	2.2756	2.2354	2.1938	2.1507	2.1058	2.0589	2.0096
18	4.4513	3.5915	3.1968	2.9647	2.8100	2.6987	2.6143	2.5480	2.4943	2.4499	2.3807	2.3077	2.2304	2.1898	2.1477	2.1040	2.0584	2.0107	1.9604
19	4.4139	3.5546	3.1599	2.9277	2.7729	2.6613	2.5767	2.5102	2.4563	2.4117	2.3421	2.2686	2.1906	2.1497	2.1071	2.0629	2.0166	1.9681	1.9168
20	4.3807	3.5219	3.1274	2.8951	2.7401	2.6283	2.5435	2.4768	2.4227	2.3779	2.3080	2.2341	2.1555	2.1141	2.0712	2.0264	1.9795	1.9302	1.8780
21	4.3512	3.4928	3.0984	2.8661	2.7109	2.5990	2.5140	2.4471	2.3928	2.3479	2.2776	2.2033	2.1242	2.0825	2.0391	1.9938	1.9464	1.8963	1.8432
22	4.3248	3.4668	3.0725	2.8401	2.6848	2.5727	2.4876	2.4205	2.3660	2.3210	2.2504	2.1757	2.0960	2.0540	2.0102	1.9645	1.9165	1.8657	1.8117
23	4.3009	3.4434	3.0491	2.8167	2.6613	2.5491	2.4638	2.3965	2.3419	2.2967	2.2258	2.1508	2.0707	2.0283	1.9842	1.9380	1.8894	1.8380	1.7831
24	4.2793	3.4221	3.0278	2.7955	2.6400	2.5277	2.4422	2.3748	2.3201	2.2747	2.2036	2.1282	2.0476	2.0050	1.9605	1.9139	1.8648	1.8128	1.7570
25	4.2597	3.4028	3.0088	2.7763	2.6207	2.5082	2.4226	2.3551	2.3002	2.2547	2.1834	2.1077	2.0267	1.9838	1.9390	1.8920	1.8424	1.7896	1.7330
26	4.2417	3.3852	2.9912	2.7587	2.6030	2.4904	2.4047	2.3371	2.2821	2.2365	2.1649	2.0889	2.0075	1.9643	1.9192	1.8718	1.8217	1.7684	1.7110
27	4.2252	3.3690	2.9752	2.7426	2.5868	2.4741	2.3883	2.3205	2.2655	2.2197	2.1479	2.0716	1.9898	1.9464	1.9010	1.8533	1.8027	1.7488	1.6906
28	4.2100	3.3541	2.9604	2.7278	2.5719	2.4591	2.3732	2.3053	2.2501	2.2043	2.1323	2.0558	1.9736	1.9299	1.8842	1.8361	1.7851	1.7300	1.6717
29	4.1960	3.3404	2.9467	2.7141	2.5581	2.4453	2.3593	2.2913	2.2360	2.1900	2.1179	2.0411	1.9586	1.9147	1.8687	1.8203	1.7689	1.7138	1.6541
30	4.1830	3.3277	2.9340	2.7014	2.5454	2.4324	2.3463	2.2783	2.2229	2.1768	2.1045	2.0275	1.9446	1.9005	1.8543	1.8055	1.7537	1.6981	1.6376
40	4.1709	3.3158	2.9223	2.6896	2.5336	2.4205	2.3343	2.2662	2.2107	2.1646	2.0921	2.0148	1.9317	1.8874	1.8409	1.7918	1.7396	1.6835	1.6223
60	4.0847	3.2317	2.8387	2.6060	2.4495	2.3359	2.2490	2.1802	2.1240	2.0772	2.0035	1.9245	1.8389	1.7929	1.7444	1.6928	1.6373	1.5766	1.5089
120	4.0012	3.1504	2.7581	2.5252	2.3683	2.2541	2.1665	2.0970	2.0401	1.9926	1.9174	1.8364	1.7480	1.7001	1.6491	1.5943	1.5343	1.4673	1.3893
∞	3.9201	3.0718	2.6802	2.4472	2.2899	2.1750	2.0868	2.0164	1.9588	1.9105	1.8337	1.7505	1.6587	1.6084	1.5543	1.4952	1.4290	1.3519	1.2539
	3.8415	2.9957	2.6049	2.3719	2.2141	2.0986	2.0096	1.9384	1.8799	1.8307	1.7522	1.6664	1.5705	1.5173	1.4591	1.3940	1.3180	1.2214	1.0000

Two-Way ANOVA

(With Replication)
(Part 4/4)



- ✓ Manually calculation
- ✓ GraphPad Prism 5.0 Software
- ✓ MS Excel

Biostatistics & Research Methodology
B Pharm 8th Sem | M. Pharm. | PhD

Two-Way ANOVA



ASSUMPTION

- ▣ Data (each population) are normally distributed.
- ▣ Samples have equal variance or standard deviation
$$S_1^2 = S_2^2 = S_3^2 = S_n^2$$

- ▣ Samples are random and independent
- ▣ Involve Two variable *s/factors*
 - ▣ 1. Different drug ✓
 - ▣ 2. Different Time frame ✓

Randomize Block Design

	Drug Treatment			
	DRUG A ✓		DRUG B ✓	
	<u>A1</u>	<u>A2</u>	<u>B1</u>	<u>B2</u>
✓ Day 1	2	3	4	5
✓ Day 7	4	4	6	6
✓ Day 14	6	5	8	8

$$\text{F Ratio} = \frac{\text{Variance between of groups}}{\text{Variance within the group}}$$

F-value

Two-Way ANOVA



Question: analyse

1. Do the drugs act differently or same ✓
2. Are time affects directly ✓
3. Is the interaction term significantly

Hypothesis:

1. H_0 - mean effect of two drugs is same or equal
2. H_a - mean effect of two drugs is different

Variance can be determine for-

- a) Between column (Column Factors) (Drug A & Drug B)
- b) Between Row (Row factor) - (1H, 2H, H) D_1, D_7, D_{14}
- c) Within the samples (Residual)

	Drug A	Drug B	
✓ D1 s_1	2 ✓	4 ✓	R1 = 14
	3 ✓	5 ✓	
D7	4	6	R2 = 20
	4	6	
D14	6	8	R3 = 27
	5	8	
	C1 = 24	C2 = 37	

Two-Way ANOVA



1. Calculate Correction factor

	Drug A	Drug B	
D1	2	4	R1 = 14
	3	5	
D7	4	6	R2 = 20
	4	6	
D14	6	8	R3 = 27
	5	8	
	C1 = 24	C2 = 37	T = 61

2. Sum of square of Total (SST)

$$\begin{aligned}
 SST &= \sum x_i^2 - CF \\
 &= (2^2 + 4^2 + 3^2 + 5^2 + 4^2 + 6^2 + 4^2 + 6^2 + 6^2 + 8^2 + 5^2 + 8^2) - 310 \\
 &= (4 + 16 + 9 + 25 + 16 + 36 + 16 + 36 + 36 + 64 + 25 + 64) - 310 \\
 &= 347 - 310 = 37 \text{ or } 347 - 310 \cdot 08 \\
 &= 36.92
 \end{aligned}$$

3. SS between column (SSC) = $\frac{\sum c_j^2}{n_c} - CF$

$$\begin{aligned}
 &= \frac{(24)^2}{6} + \frac{(37)^2}{6} - 310 \\
 &= \frac{576}{6} + \frac{1369}{6} - 310 \\
 &= 96 + 228.16 - 310 \\
 &= 324.16 - 310 = 14.16 \text{ or } 324.16 - 310 \cdot 08 = 14.08
 \end{aligned}$$

a) Grand Total (T) = $\sum x_i = 61$

c) Correction Factor = $(T)^2 / N = \text{No. of samples in population}$

$$\begin{aligned}
 &= (61)^2 / 12 \\
 &= 3721 / 12 \\
 &= 310.08 \text{ or } 310
 \end{aligned}$$

Two-Way ANOVA



	Drug A	Drug B	
D1	2	4	R1 = 14
	3	5	
D7	4	6	R2 = 20
	4	6	
D14	6	8	R3 = 27
	5	8	
	C1 = 24	C2 = 37	61

$$4. \text{ SS between Row (SSR)} = \frac{\sum R_i^2}{n_R} - CF$$

$$SSR = \frac{(14)^2}{4} + \frac{(20)^2}{4} + \frac{(27)^2}{4} - 310$$

$$= \frac{196}{4} + \frac{400}{4} + \frac{729}{4} - 310$$

$$= 49 + 100 + 182.25 - 310$$

$$= 331.25 - 310 = 21.25$$

$$331.25 - 310.00 = \underline{\underline{21.17}} \quad \text{SSR}$$

Two-Way ANOVA



	Drug A	Drug B		mean A	mean B
D1	2	4	2.5	2.5	4.5
	3	5			
D7	4	6	4	4	6
	4	6			
D14	6	8	5.5	5.5	8
	5	8			

	A-mean	B-mean	(A-mean) ²	(B-mean) ²
D1	-0.5	-0.5	0.25	0.25
	0.5	0.5	0.25	0.25
D7	0	0	0	0
	0	0	0	0
D14	0.5	0	0.25	0
	-0.5	0	0.25	0

5. SS within sample

$$SSE = 1.5$$

$$\sum (x_i - \bar{x})^2$$

$$0.25 + 0.25 + 0.25 + 0.25 + 0.25 + 0.25 = \underline{\underline{1.5}}$$

6. SS for interaction = $SST - (SSC + SSR + SSE)$

$$= 36.92 - (14.08 + 21.17 + 1.5)$$

$$= \underline{\underline{0.17}}$$



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