CHEM 463
Current Microanalytical Methods

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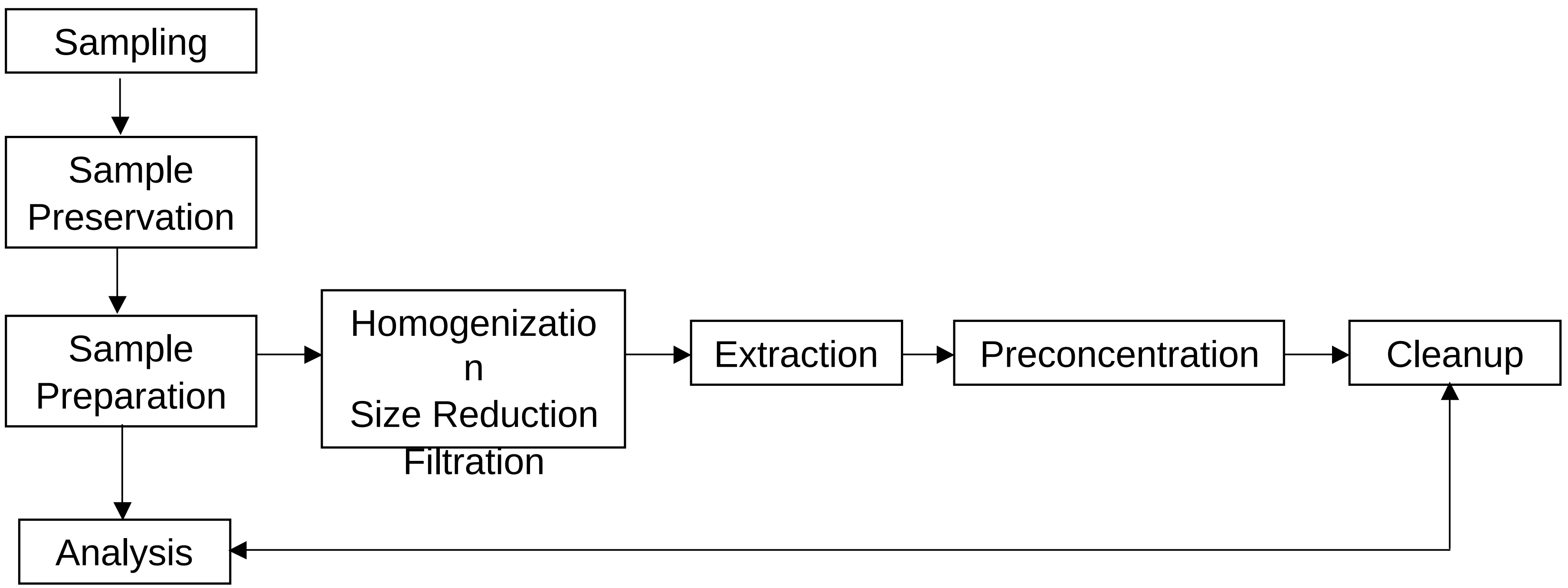
Objectives:

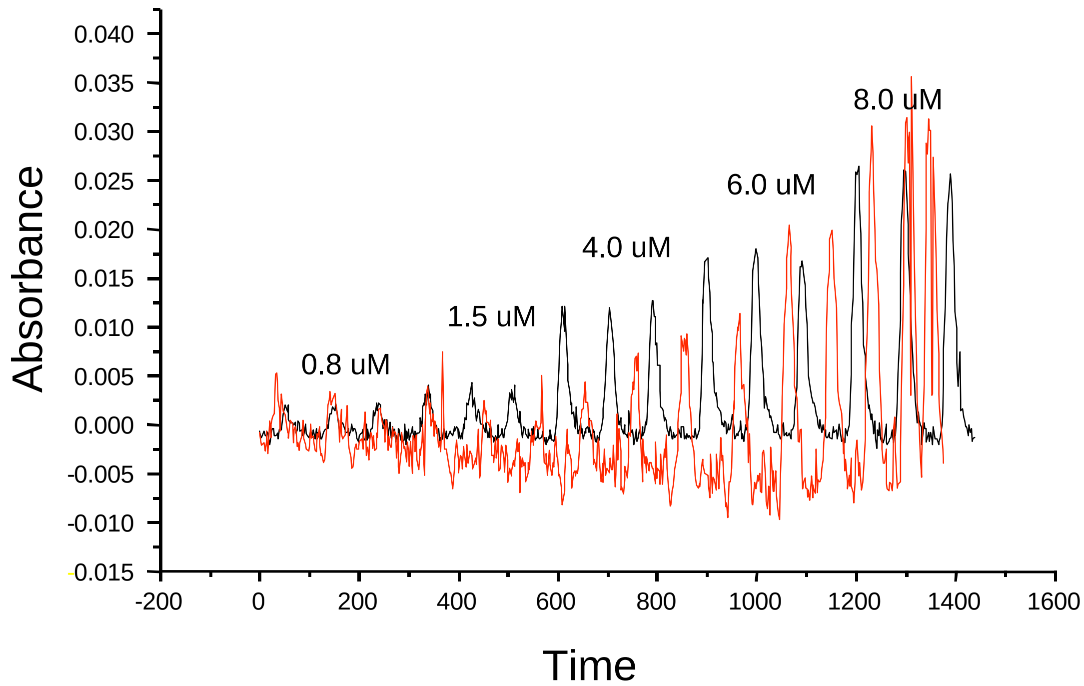
1. To learn both classical and modern instrumental aspects of microanalysis;
2. To learn the analytical processes as applied to basic laboratory and field research;
3. To become familiar and later master the elements of good laboratory practice;
4. To ultimately apply his/her knowledge of microanalysis in an independent manner.

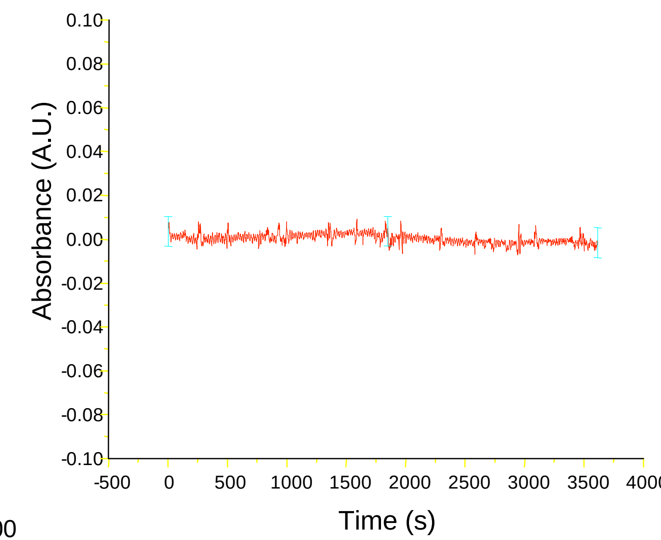
The Measurement Process



The Measurement Process

1. Methods of Quantitation
   a. Calibration Curves
      → relationship between a detector signal and an analyte.





The Measurement Process

Determining Concentration from Calibration Curve

Basic steps:
(1) Make a series of dilutions of known concentration for the analyte.
(2) Analyze the known samples and record the results.
(3) Determine if the data is linear.
(4) Draw a line through the data and determine the line's slope and intercept.
(5) Test the unknown sample in duplicate or triplicate. Use the line equation to determine the concentration of the analyte: y = mx + b

$$\text{Conc}_{\text{analyte}} = \frac{\text{reading} - \text{intercept}}{\text{slope}}$$

Statistical Analysis of Data

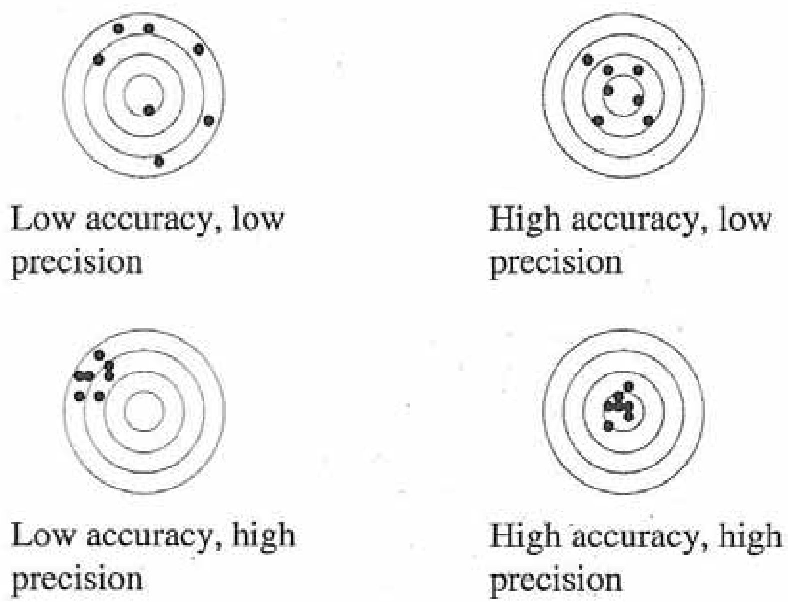
- No analysis is free of error!
- Types of errors
  1. Gross – readily described, errors that are obvious.
     Instrument breakdown, dropping a sample, contamination (gross)
  2. Random
  3. Systematic

Student	Results (mL)					Comments
A	10.08	10.11	10.09	10.10	10.12	Precise, biased
B	9.88	10.14	10.02	9.80	10.21	Imprecise, unbiased
C	10.19	9.79	9.69	10.05	9.78	Imprecise, biased
D	10.04	9.18	10.02	9.97	10.04	Precise, unbiased

Titration – each student performs an analysis in which exactly 10.00 mL of exactly 0.1 M NaOH is titrated with exactly 0.1M HCl.

Statistical Analysis of Data

- Accuracy – How far a result is from the true value.
- Precision – How close multiple determinations are to each other.



Statistical Analysis of Data

1. Basic Statistics → Descriptive

Spectrophotometric measurement (Abs) of a sample solution from 15 replicate measurements.

Measurement	Value	Measurement	Value
1	0.3410	9	0.3430
2	0.3350	10	0.3420
3	0.3470	11	0.3560
4	0.3590	12	0.3500
5	0.3530	13	0.3630
6	0.3460	14	0.3530
7	0.3470	15	0.3480
8	0.3460		

Statistical Analysis of Data

Descriptive statistics for the spectrophotometric measurements.

Parameter	Value
Sample #, n	15
Mean	0.3486
Median	0.347
Std Dev	0.00731
RSD %	2.096
Std error	0.00189
Max value	0.363
Min value	0.335

Statistical Tests – Student's *t*-test, *F*-test, tests for outliers

Distributions – Gaussian, Poisson, binominal

Statistical Analysis of Data

1. Statistics of Repeated Measurements

$$Mean\ \bar{x} = \frac{\sum x_i}{n}$$

$$Standard\ deviation\ \ s = \sqrt{\sum (x - \bar{x})^2 / (n-1)}$$

Student A

	x_i	$(x_i - \bar{x})$	$(x_i - \bar{x})^2$
	10.08	-0.02	0.0004
	10.11	0.01	0.0001
	10.09	-0.01	0.0001
	10.10	0.00	0.0000
	10.12	0.02	0.0010
Total	50.50	0	0.0010

Statistical Analysis of Data

$$\overline{x} = \frac{\sum x_i}{n} = \frac{50.50}{5} = 10.1 mL$$

$$s = \sqrt{\sum (x - \overline{x})^2 / (n-1)} = \sqrt{0.001/4} = 0.0158 mL$$

Statistical Analysis of Data

2. Distribution of Repeated measurements
    1. Gaussian distribution
        - Bell-shaped curve for the frequency of the measurements

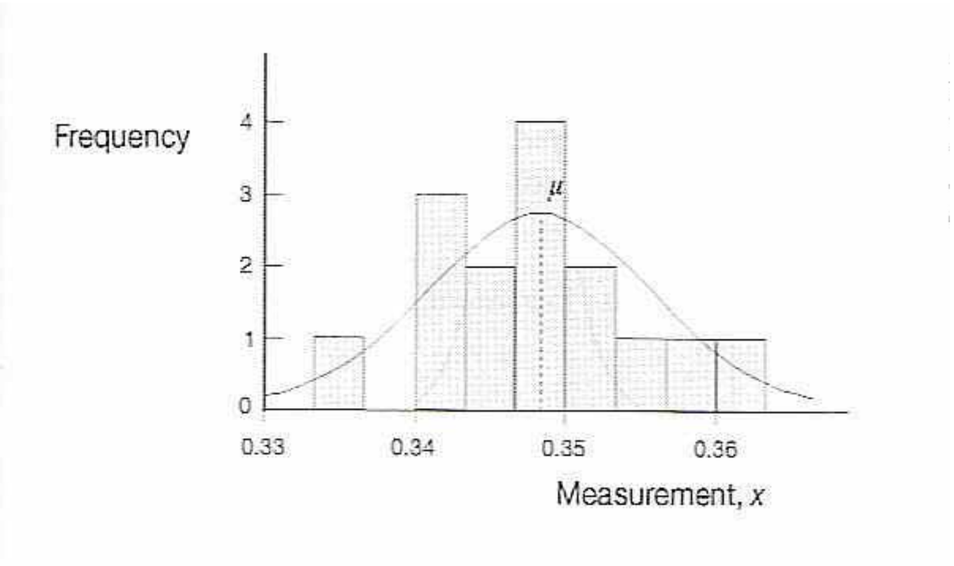


Fig. 1 – Histogram for the measurements of spectrophotometric data. Theoretical distribution with the Gaussian curve in the solid line.

Statistical Analysis of Data

A. Gaussian Distribution

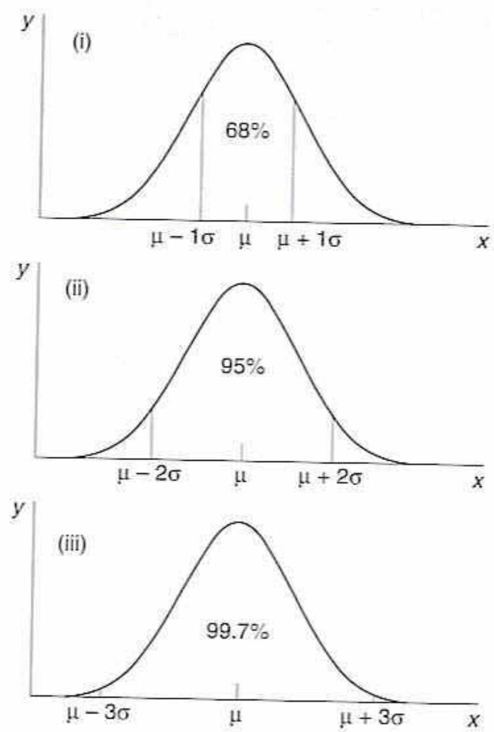


Figure 2.4 Properties of the normal distribution: (i) approximately 68% of values lie within $\pm 1\sigma$ of the mean; (ii) approximately 95% of values lie within $\pm 2\sigma$ of the mean; (iii) approximately 99.7% of values lie within $\pm 3\sigma$ of the mean.

Statistical Analysis of Data

3. Significance Tests in Analytical Measurements

- Testing the truth of the hypothesis (null hypothesis, H_o)
- Null = implies that no difference exists between the observed and known values $\bar{x}$

Assuming H_o is true, stats can be used to calculate the probabilty that the difference between and true value, μ, arises solely as a result of random errors.

Is the difference significant?

$$t = \frac{|\bar{x} - \mu|}{s}\sqrt{n} \qquad \text{one variable } t-\text{test (student's } t\text{)}$$

where s = estimate of the standard deviation

n = number of parallel measurements

Statistical Analysis of Data

3. Statistical tests
   - A. Student's *t*
     - ➢ If |*t*| exceeds a certain critical value, then the H_o is rejected,

Table A.2 The *t*-distribution

Value of t for a confidence interval of *Critical value of* |t| *for P values of number of degrees of freedom*	*90%* *0.10*	*95%* *0.05*	*98%* *0.02*	*99%* *0.01*
1	6.31	12.71	31.82	63.66
2	2.92	4.30	6.96	9.92
3	2.35	3.18	4.54	5.84
4	2.13	2.78	3.75	4.60
5	2.02	2.57	3.36	4.03
6	1.94	2.45	3.14	3.71
7	1.89	2.36	3.00	3.50
8	1.86	2.31	2.90	3.36
9	1.83	2.26	2.82	3.25
10	1.81	2.23	2.76	3.17
12	1.78	2.18	2.68	3.05
14	1.76	2.14	2.62	2.98
16	1.75	2.12	2.58	2.92
18	1.73	2.10	2.55	2.88
20	1.72	2.09	2.53	2.85
30	1.70	2.04	2.46	2.75
50	1.68	2.01	2.40	2.68
∞	1.64	1.96	2.33	2.58

The critical values of |*t*| are appropriate for a *two*-tailed test. For a *one*-tailed test the value is taken from the column for *twice* the desired *P*-value, e.g. for a one-tailed test, $P = 0.05$, 5 degrees of freedom, the critical value is read from the $P = 0.10$ column and is equal to 2.02.

Statistical Analysis of Data

3. Statistical tests

   A. Student's *t*

      Ex: The following results were obtained in the determination of Fe^{3+} in water samples.

      504 ppm 50.7 ppm 49.1 ppm 49.0 ppm 51.1 ppm

      $\bar{x} = 50.06$ $s = 0.956$

      Is there any evidence of systematic error?

      H_o → no systematic error , i.e., $\mu = 50$ and using equations

      Table: critical value is $t_4 = 2.78$ (p=0.05).

      NO significant difference since the observed $|t|$ is less than the critical value (H_o retained)

Statistical Analysis of Data

3. Statistical tests

B. Two-sided *t*-test

- A comparison of two sample means: $\bar{x}_1 - \bar{x}_2$

$$t = \frac{|\bar{x}_1 - \bar{x}_2|}{s_d} \sqrt{\frac{n_1 n_2}{n_1 + n_2}}$$

where : n_1, n_2 = # of parallel determinations for $\bar{x}_1$ *and* $\bar{x}_2$

s_d = weighted averaged standard deviation:

$$s_d = \sqrt{\frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{n_1 + n_2 - 2}}$$

Note: The null hypothesis is accepted if $\bar{x}_1$ *and* $\bar{x}_2$ are different only randomly at risk level *P*, i.e. if the calculated *t*-value is lower than the tabulated value for *t*.

Statistical Analysis of Data

3. Statistical test

   C. *F*- test

   - Used to compare the standard deviations of two random samples

$$F = \frac{s_1^2}{s_2^2} \qquad (where\; s_1^2 > s_2^2)$$

Note : The null hypothesis is accepted if s_1^2 *and* s_2^2 differ randomly, i.e., if the calculated *F*-value is lower than *F*-distribution from the Table with $v_1 + v_2$ degrees of freedom.

Statistical Analysis of Data

3. Statistical test

   F- test

Table A.3 Critical values of F for a one-tailed test ($P = 0.05$)

ν_2	ν_1												
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*12*	*15*	*20*
1	161.4	199.5	215.7	224.6	230.2	234.0	236.8	238.9	240.5	241.9	243.9	245.9	248.0
2	18.51	19.00	19.16	19.25	19.30	19.33	19.35	19.37	19.38	19.40	19.41	19.43	19.45
3	10.13	9.552	9.277	9.117	9.013	8.941	8.887	8.845	8.812	8.786	8.745	8.703	8.660
4	7.709	6.944	6.591	6.388	6.256	6.163	6.094	6.041	5.999	5.964	5.912	5.858	5.803
5	6.608	5.786	5.409	5.192	5.050	4.950	4.876	4.818	4.772	4.735	4.678	4.619	4.558
6	5.987	5.143	4.757	4.534	4.387	4.284	4.207	4.147	4.099	4.060	4.000	3.938	3.874
7	5 591	4.737	4.347	4.120	3.972	3.866	3.787	3.726	3.677	3.637	3.575	3.511	3.445
8	5.318	4.459	4.066	3.838	3.687	3.581	3.500	3.438	3.388	3.347	3.284	3.218	3.150
9	5.117	4.256	3.863	3.633	3.482	3.374	3.293	3.230	3.179	3.137	3.073	3.006	2.936
10	4.965	4.103	3.708	3.478	3.326	3.217	3.135	3.072	3.020	2.978	2.913	2.845	2.774
11	4.844	3.982	3.587	3.357	3.204	3.095	3.012	2.948	2.896	2.854	2.788	2.719	2.646
12	4.747	3.885	3.490	3.259	3.106	2.996	2.913	2.849	2.796	2.753	2.687	2.617	2.544
13	4.667	3.806	3.411	3.179	3.025	2.915	2.832	2.767	2.714	2.671	2.604	2.533	2.459
14	4.600	3.739	3.344	3.112	2.958	2.848	2.764	2.699	2.646	2.602	2.534	2.463	2.388
15	4.543	3.682	3.287	3.056	2.901	2.790	2.707	2.641	2.588	2.544	2.475	2.403	2.328
16	4.494	3.634	3.239	3.007	2.852	2.741	2.657	2.591	2.538	2.494	2.425	2.352	2.276
17	4.451	3.592	3.197	2.965	2.810	2.699	2.614	2.548	2.494	2.450	2.381	2.308	2.230
18	4.414	3.555	3.160	2.928	2.773	2.661	2.577	2.510	2.456	2.412	2.342	2.269	2.191
19	4.381	3.522	3.127	2.895	2.740	2.628	2.544	2.477	2.423	2.378	2.308	2.234	2.155
20	4.351	3.493	3.098	2.866	2.711	2.599	2.514	2.447	2.393	2.348	2.278	2.203	2.124

ν_1 = number of degrees of freedom of the numerator and ν_2 = number of degrees of freedom of the denominator.

Statistical Analysis of Data

3. Statistical test
   - Ex. Determination of titanium content (absolute %) by two laboratories.

Lab 1	Lab2
0.470	0.529
0.448	0.490
0.463	0.489
0.449	0.521
0.482	0.486
0.454	0.502
0.477	
0.409	

Statistical Analysis of Data

Compare the standard deviations between the two laboratories:

$$F = \frac{s_1^2}{s_2^2} = \frac{0.0229^2}{0.0182^2} = 1.58 \quad \text{Critical Value from the table} = 6.85$$

Calculated value is lower than the tabulated, hence the test result is not significant (only random differences).

Statistical Analysis of Data

3. Significance Tests

   D. Dixon's *Q*-test

   → Test for the determination of outliers in data sets

$$Q = \frac{|x_2 - x_1|}{|x_n - x_1|} \quad and \quad Q_n = \frac{|x_n - x_{n-1}|}{|x_n - x_1|}$$

   → The H_o , i.e., that no outlier exists, is accepted if the quantity ***Q*<*Q*(1-*P*;*n*)**

Statistical Analysis of Data

Table Critical values for the *Q*-test at H_o at the 1% risk level:

n	*Q(0.99; n)*
3	0.99
4	0.89
5	0.76
6	0.70
7	0.64
8	0.59
9	0.56
10	0.53
11	0.50
12	0.48
13	0.47
14	0.45
15	0.44
20	0.39

Statistical Analysis of Data

3. Significance Tests

Ex: Trace analysis of PAH's in soil reveals benzo [a] pyrene in the following amounts ($mg\text{-}kg^{-1}$)*

5.30 5.00 5.10 5.20 5.10 6.20 5.15

→ Use the Q test to determine if the largest and smallest are outliers

$$Q = \frac{|5.10 - 5.00|}{|6.20 - 5.00|} = 0.083 \qquad Q_n = \frac{|6.20 - 5.30|}{|6.20 - 5.00|}\ 0.75$$

Critical values from the Table, $Q(1\text{-P} = 0.99;\ n = 7) = 0.64$

For the smallest, $Q_1 < Q_{calc}$, thus the value cannot be an outlier

For the largest, $Q_1 > Q_{calc}$, then the outlier exists

Why is this determination important?

Statistical Analysis of Data

3. Significance Tests

   E. Analysis of variance (ANOVA)

→ Separate and estimate the causes of variation, more than one source of random error. Example?

Note: There are numerous chemometric tools which can be used with ANOVA.

→ We will cover them in this course!

Statistical Analysis of Data

E. ANOVA

→ Within sample variation

Conditions	Replicate Measurements	Mean
A. Freshly prepared	102, 100, 101	101
B. Stored for 1 hr in the dark	101, 101, 104	102
C. Stored for 1 hr in the subdued light	97, 95, 99	97
D. Stored for 1 hr in bright light	90, 92, 94	97

For each sample, the variance can be calculated: $\sum \frac{(x_i - \overline{x})^2}{(n-1)}$

Statistical Analysis of Data

E. ANOVA

Sample A = $$\frac{(102-101)^2+(100-101)^2+(101-101)^2}{3-1}=1$$

Sample B = $$\frac{(101-102)^2+(101-102)^2+(104-102)^2}{3-1}=3$$

C + D = 4

Sample Mean variance

$$\frac{(101-98)^2+(102-98)^2+(97-98)^2+(92-98)^2}{3-1}=62/3$$

Note: This estimate has 3 degrees of freedom since it is calculated from 4 samples

Statistical Analysis of Data

E. ANOVA

Summarizing our calculations:

within – sample mean square = 3 with 8 d.f.

between – sample mean sample = 62 with 3 d.f.

$F = 62/3 = 20.7$

From Table – the critical value of F= 4.066 (P=0.05)

- since the calculated value of F is greater than this, the null hypothesis is rejected: the sample means <u>DO</u> differ significantly.