RESEARCH METHODOLOGY

Data Analyses

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Outline

- Selection of problem/topic for research.
- Formulation of hypothesis.
- Formulation of objectives of the study.
- Preparation for collection of data.
- Collection of data.
- Presentation and analysis of data.
- Evaluation of data and testing of hypothesis.
- Interpretation of result.
- Writing the report.

Introduction

What is research?

- Research is a quest for knowledge through diligent search or investigation or experimentation aimed at the discovery and interpretation of new knowledge.
- A systematized effort to gain new knowledge
  A careful investigation or inquiry specially through search for new facts in any branch of knowledge.
- Research is an art of scientific investigation.

> "It is the theory that decides what can be observed."
> **Albert Einstein**

Why Research?

- India's future is in evolving into a knowledge society
- If we do not find solutions, no body would do it for us
- Foundation of great civilization is innovation.

Introduction

What is research methodology

- Is an integral part of learning, development and innovations in any subject.
- Deals specifically with the manner in which data is collected, analyzed and interpreted.

Method

- A research method is a technique for (or way of proceeding in) gathering evidence.

Types of Research

- Basic, fundamental or pure – May not have immediate application but is necessary for future development.
- Applied Research- Immediate application , can be problem oriented, goal oriented, developmental or operational.
- Empirical Research- based on observation & experience more than upon theory and abstraction.

Stages of Study: Typical case

Stage 1: Design of study

- Setting up the protocol
- Study population, inclusion and exclusion criteria
- Sample size and statistical plan

Stage 2: Conduct of the study

- Monitoring of the study
- Data capture, database design and data entry

Stage 3: Statistical analysis and reporting

Stages of Chemical Analyses

Formulating the question or defining the problem
Faultless Plan/Choice of methods

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Sampling
Sampling and storage, representative sample

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Preparation of Analyte
Convert representative sample into suitable form of analysis

↓

Intermediate process
Separation, masking, pre-concentration

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Measurements
Calibration of glasswares, instruments

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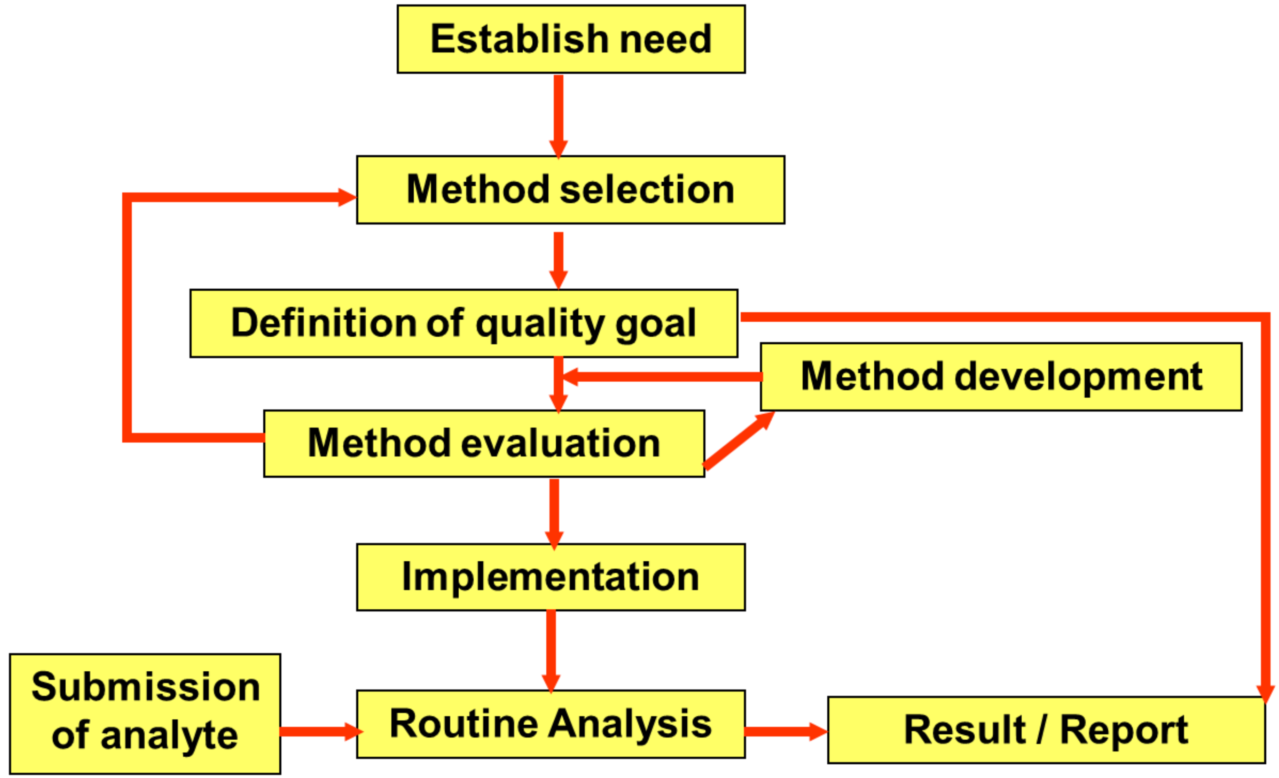
Results

↓

Preparation of data
Assessing data (statistical), method validation, Documentation

Inter-relationship of Methodology & Results



Experimental Research

Aspects of Experimental Research

- Tests the validity of Hypothesis
- Identifies a cause-and-effect relationship
- Seen as more objective, less subjective
- Can be predictive

What makes experimental Research Good?

- Validity
- Reliability
- Replicability
- Trustworthiness
- Rigor

Method Validation Criteria

Where do Robustness and Ruggedness fit in?

Validation Criteria

- Specificity
- Linearity
- Range
- Accuracy
- Precision
- Detection Limit
- Quantitation Limit
- Robustness

– Precision

– Repeatability

  - Single Lab: one day, analyst, instrument

– Intermediate Precision

  - Single Lab: multiple days, analysts, instruments

– Reproducibility "Ruggedness"

  - Multiple labs, days, analysts , instruments, etc.

- Validity and Reliability: The relationship between reliability and validity is a fairly simple one to understand:
- A measurement can be reliable, but not valid. However, a measurement must first be reliable before it can be validated. Thus reliability is a necessary, but not sufficient, condition of validity.
- In other words, a measurement may consistently assess a phenomena (or outcome), but unless that measurement tests what you want it to, it is not valid.

Measurement system components

- Equipment/ Instrument
  - Unit of measurement
  - Analyst and operating instructions

Measurement error

- Measurement error is considered to be the difference between a value measured and the true value.

Terminology in Data Analysis

- **Measured Value:** The observed value of weight, volume, meter-reading or other quantity, found in the analysis of a substance/ materials.
- **True value:** The correct value for a measurement. Remains to be unknown except standard samples
- **Result:** The final value reported for a measured or computed quantity, after performing a measuring procedure including all sub procedures and evaluations.
- **Variable:** The quantity or characteristic measured or computed. Two types - Dependent (y, always with some error) and independent (x, without any error) variables**.**
- **Errors:** The difference between true or accepted value and the measured value

Every measurement that is made is subject to a number of errors.

'If you cannot measure it, you cannot know it'

A. Einstein

Measured Value

Measured value = f (TV + Ac + Rep + Rpr)

TV	=	True value
Ac	=	Method accuracy
Rep	=	Repeatability
Rpr	=	Reproducibility

Accuracy and Precision: There is a difference

Accuracy

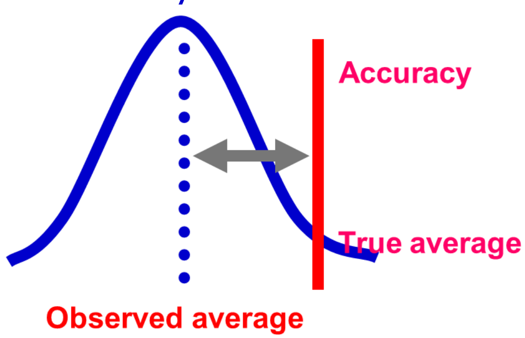
- Degree of agreement/closeness of a measured value (X_i) to the true or accepted value (X_t)
- Depends on the person measuring
- Often expressed as either absolute error or relative error (percent, parts per thousand, or parts per million)

Precision

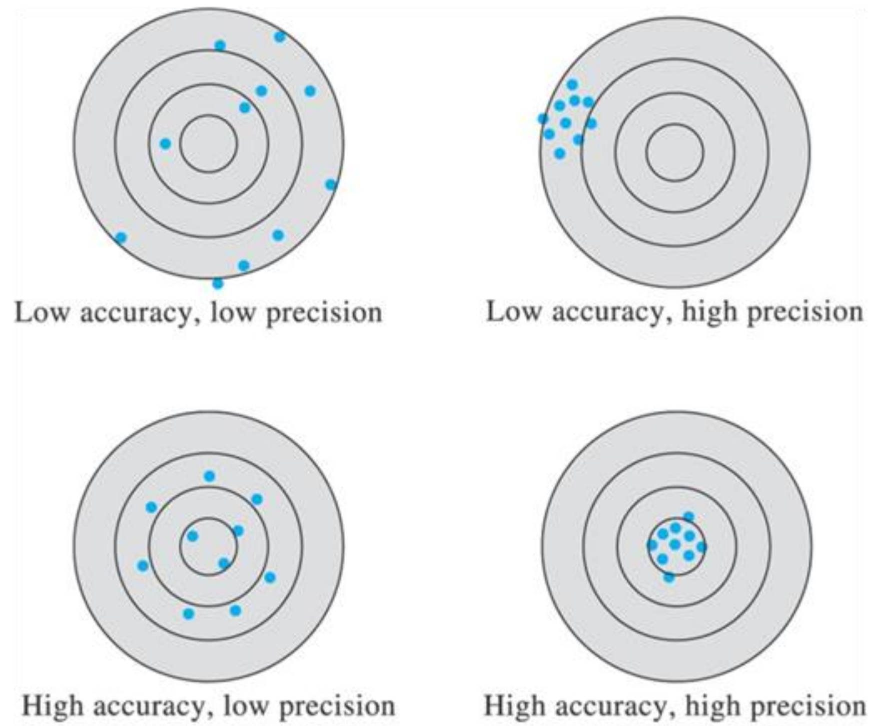
- Describes the reproducibility of measurements (closeness of results to others) obtained in exactly the same way.
- Depends on the measuring tools
- Determined by the number of significant digits
- Three terms widely used to describe precision include standard deviation, variance, and coefficient of variation. All are functions of deviation from the mean.
- Deviation from Mean (δi) = $|X_i$ – Mean $|$
- Three terms to describe the precision of a set of replicate data: standard deviation, variance, and coefficient of variation.

Accuracy and Precision: We need both

- Accuracy: Measurements are said to be accurate if their tendency is to center around the actual value of the entity being measured.
- Precision: Measurements are precise if they differ from one another by a small amount.



- Difference between the true average and the observed average.
- (True average may be obtained by using a more precise measuring tool)

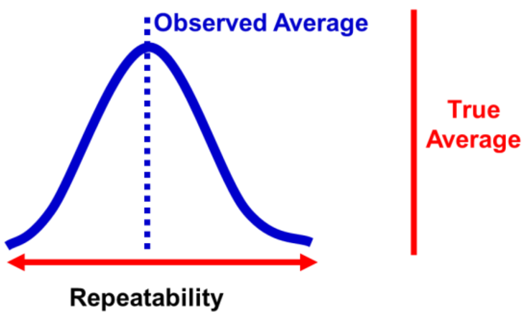


- Accuracy is how close you get to the bullseye. Precision is how close the repetitive shorts are to one another.
- Good precision does not guarantee good accuracy

Repeatability and Reproducibility:
Often confused?

Repeatability

➢ Closeness of agreement between independent results obtained with the same method on identical test material, under the same conditions (same operator, same apparatus, same laboratory and after short intervals of time).

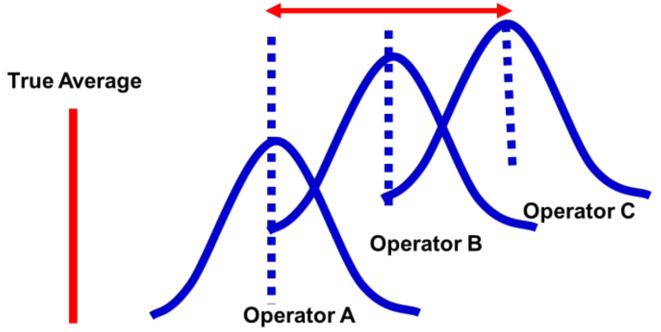


➢ Determinations in quick succession

➢ Data within run precision

Reproducibility

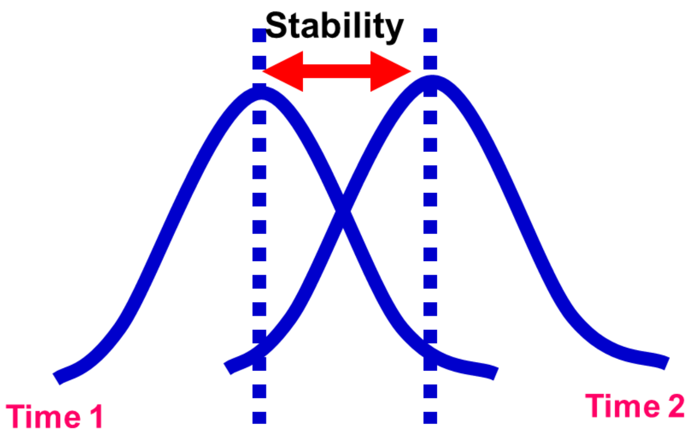
➢ Closeness of agreement between independent results obtained with the same method on identical test material but under different conditions (different operators, different apparatus, different laboratories and/or after different intervals of time).



➢ Data in between run precision

Stability

The difference in the average of at least 2 sets of measurements obtained with an instrument over time.



Errors in Measured Values

- All scientific measures are subject to error.
- These errors are reflected in the number of figures reported for the measurement.
- These errors are also reflected in the observation that two successive measures of the same quantity are different.
- The word error in science has a different significance than in general human experience.

Errors may be of

- **Static Error:** An error that does not vary with time and is inherent in most instruments.
  - A typical static error is the zero setting of the needle in an analog display.
- **Dynamic Error:** A dynamic error can occur when the quantity being measured fluctuates as a function of time.
  - An instrument may not give the correct reading if it is used to measure a voltage that varies slowly with time.

Errors in Analyses

- **Errors**
  - **Expression of errors**
    - **Absolute**
    - **Relative**
  - **Types of errors**
    - **Systematic or Determinate**
    - **Random or Indeterminate**
    - **Gross error**
    - **Static and Dynamic**

❖ Absolute Error (E): Difference between experimental/measured value and the true value (expected value) in the same unit (= X_i - X_t)

The sign of the error tells whether the measured value is lower (E is –ve) or higher (E is +ve) than the true value

❖ Relative Error (E_r): Expressed as percentage = $((X_i - X_t) / X_t) \times 100\ \%$

Relative error is also expressed in parts per thousand (ppt)

Ex: Er = {(19.8 – 20.0)/20} x 100% = - 0.1% or – 10 ppt

To obtain reliable results from analytical method, sources of error must identified and minimised/eliminated.

Errors in Analyses

Systematic or Determinate – Affect the Accuracy of result

Systematic or Determinate errors

a. Causes the mean of data set to differ the accepted value
b. Have a definite value with an assignable cause and are same magnitude for replicate measurement made in the same way.
c. Leads to bias in measurement technique. Bias leads to data in a set approximately in the same way and bears a sign
d. Are called as procedural errors, magnitude can be identified/determined and can be minimized or avoided.
e. Cannot be reduced by statistical method

Sources of systematic error: Three types

- Instrumental errors: Caused by imperfections in measuring devices and instabilities in their components.
- Methods errors: Most serious, often difficult to detect and remove which are inherent with the method
- Personal errors: Arises from the carelessness, inattention, or personal limitations of the analyst. Often systematic & unidirectional.

Systematic Errors

Determinate errors can be more serious than indeterminate errors for three reasons

- ❖ There is no sure method for discovering and identifying them just by looking at the experimental data.
- ❖ Their effects can *not* be reduced by averaging repeated measurements.
- ❖ A determinate error has the same size and sign for each measurement in a set of repeated measurements, so there is no opportunity for positive and negative errors to offset each other

Effects of Systematic Errors

- Constant Errors: does not depend on the size of the quantity measured
- Proportional Errors: decrease or increase in proportion to the size of the sample taken for analysis. A common cause of proportional errors is the presence of interfering contaminants in the sample

Errors in Analyses

Random or Indeterminate – Affect the Precision of result

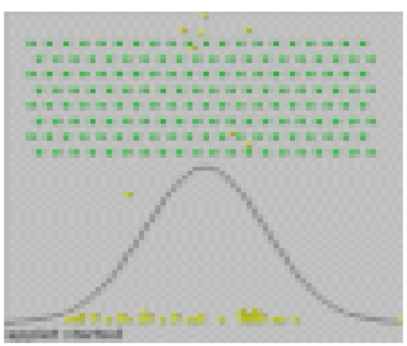
- All measurements contain random errors.
- Arises due to several experimental uncertainty (many uncontrollable variable like fluctuating experimental and environmental conditions) which can not be predicated or estimated.
- The errors are accumulative.
- The analyst has hardly any control to avoid the error but can be reduced by taking repeated measurements rather calculating their average.

Sources of random errors in Chemical analysis

- visual judgments, such as the level of the water with respect to the marking on the pipet and the mercury level in the thermometer
- variations in the drainage time and in the angle of the pipet as it drains
- temperature fluctuations, which affect the volume of the pipet, the viscosity of the liquid, and the performance of the balance
- vibrations and drafts that cause small variations in the balance readings.

Random Errors in Analyses

- Suppose there are four small random errors combine to give an overall error.
- Let us assume that each error has an equal probability of occurring and that each can cause the final result to be high or low by a fixed amount $\pm U$.
- The possible ways the four errors can combine to give the indicated deviations from the mean value.

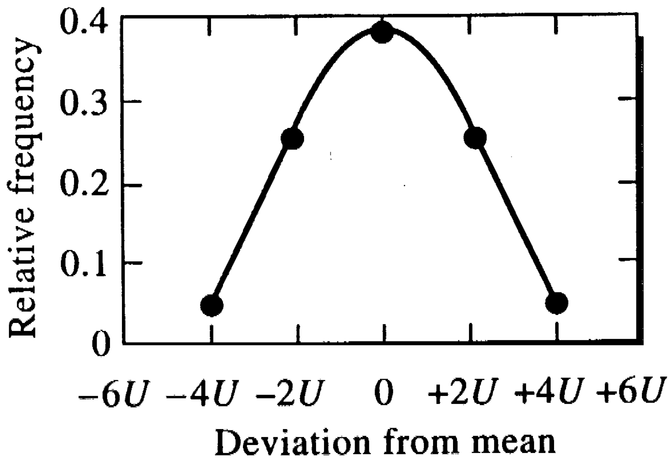


Dropping balls through series of pins

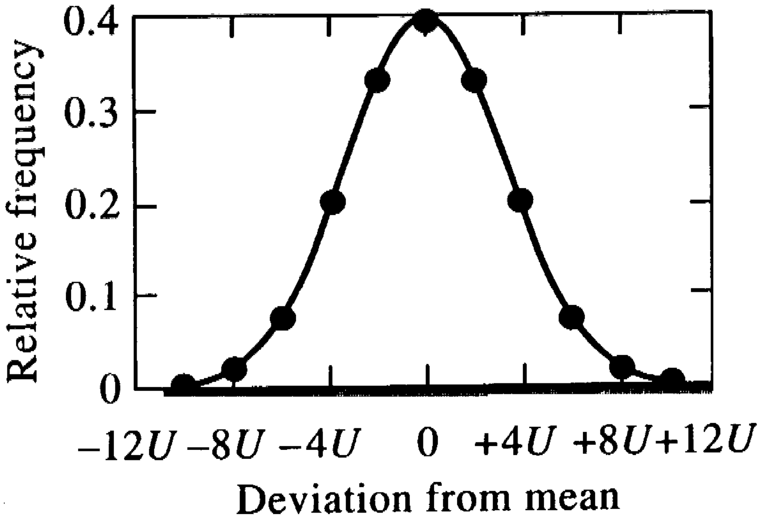
Combination of Random Errors

	Total Error	No.	Relative Frequency
+U+U+U+U	+4U	1	1/16 = 0.0625
-U+U+U+U	+2U	4	4/16 = 0.250
+U-U+U+U			
+U+U-U+U			
+U+U+U-U			
-U-U+U+U	0	6	6/16 = 0.375
-U+U-U+U			
-U+U+U-U			
+U-U-U+U			
+U-U+U-U			
+U+U-U-U			
+U-U-U-U	-2U	4	4/16 = 0.250
-U+U-U-U			
-U-U+U-U			
-U-U-U+U			
-U-U-U-U	-4U	1	1/16 = 0.01625

Frequency Distribution for Measurements Containing Random Errors

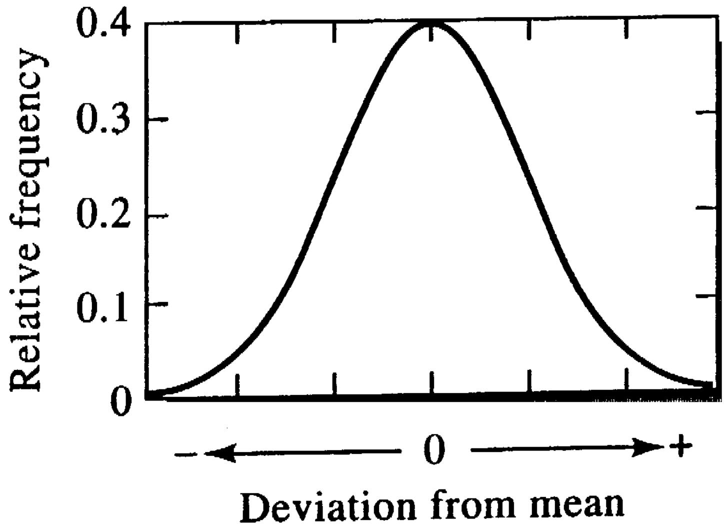


4 random uncertainties



10 random uncertainties

A very large number of random uncertainties



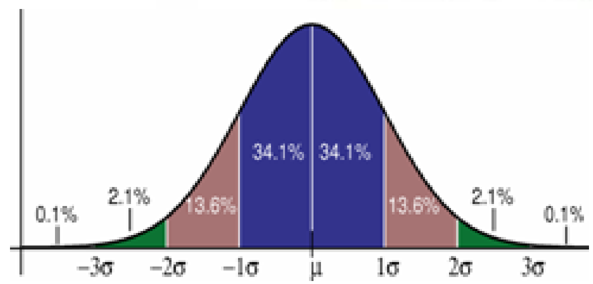
- Results from a number of analysis from a single sample follows the bell-shaped curve.
- This is a **Gaussian** or **normal error** curve.
- Symmetrical about the mean.

Treating Random Errors with Statistics

Gaussian Distribution

- Statistics only reveal information that is already present in a data set.
- The random, or indeterminate, errors in the results of an analysis can be evaluated by the methods of statistics.
- These errors ordinarily follow a normal distribution or Gaussian, hence, mathematical laws of probability can be applied to arrive at some conclusion regarding the most probable result of a series of measurements.

The Gaussian (normal) distribution was historically called the *law of errors*. It was used by Gauss to model errors in astronomical observations, which is why it is usually referred to as the Gaussian distribution.



The Gaussian Distribution

Main properties of Gaussian curve

- Symmetric bell-shaped curve representing the distribution of experimental data
- Characterized by mean (μ) and standard deviation (σ). The equation for a Gaussian curve expressed in terms of μ and σ as:

$$y = \frac{e^{-(x-\mu)^2/2\sigma^2}}{\sigma\sqrt{2\pi}}$$

Sample and Population mean in statistics

- Sample = finite number of observations (different from sample used in chemical analysis).
- Population = total (infinite no. of data) number of observations.
- Properties of Gaussian curve defined in terms of population. Then see where modifications needed for small samples of data
- Remember, sample mean (x) defined for small values of N. The difference between x and μ decreases rapidly as N reaches over 20 to 30 (i.e. sample mean ≈ population mean when N ≥ 20) .

The Gaussian Distribution

Main properties of Gaussian curve

The Population Mean μ and the Sample Mean $\overline{X}$

Sample mean $\bar{x} = \dfrac{\sum_{i=1}^{N} x_i}{N}$

Population mean
When N $\rightarrow \infty$ $\mu = \dfrac{\sum_{i=1}^{N} x_i}{N}$

Sample Standard Deviation (s): For a finite number of observations (N)
A measure of the width of the distribution.

$$s = \sqrt{\frac{\sum_{i=1}^{N} d_i^2}{N-1}} = \sqrt{\frac{\sum_{i=1}^{N} \left(x_i - \bar{x}\right)^2}{N-1}}$$

The Population Standard Deviation (σ) measure of precision of a population of data

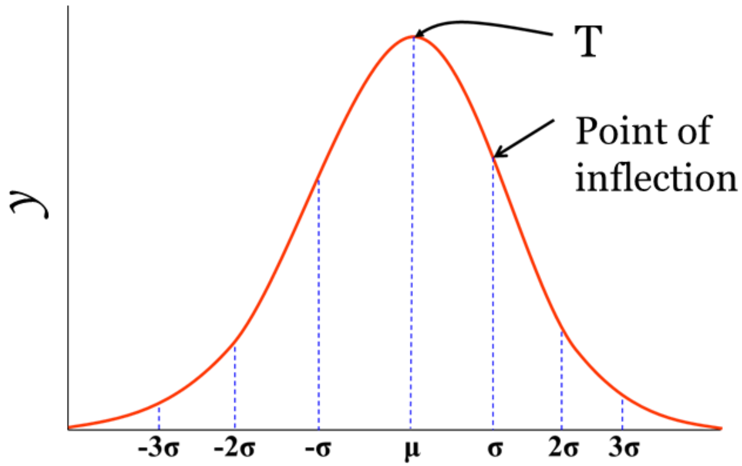
$$\sigma = \sqrt{\frac{\sum_{i=1}^{N} (x_i - \mu)^2}{N}}$$

The Gaussian Distribution

Main properties of Gaussian curve

Quantify random errors

The Gaussian distribution and statistics are used to determine how close the average value of measurements is to the true value



- The mean (μ) occurs at the position of the center of the peak
- σ is a measure of the width of the curve (standard deviation). Symmetrical distribution of +ve & -ve deviations about the maximum & exponential decrease in frequency as magnitude of the deviations increases.
- T (or x_t) is the accepted value, the larger the random error the broader the distribution.
- μ = T in the absence of systematic error
- There is a difference between the values obtained from a finite number of measurements (N) & those obtained from infinite number of measurements

The Gaussian Distribution

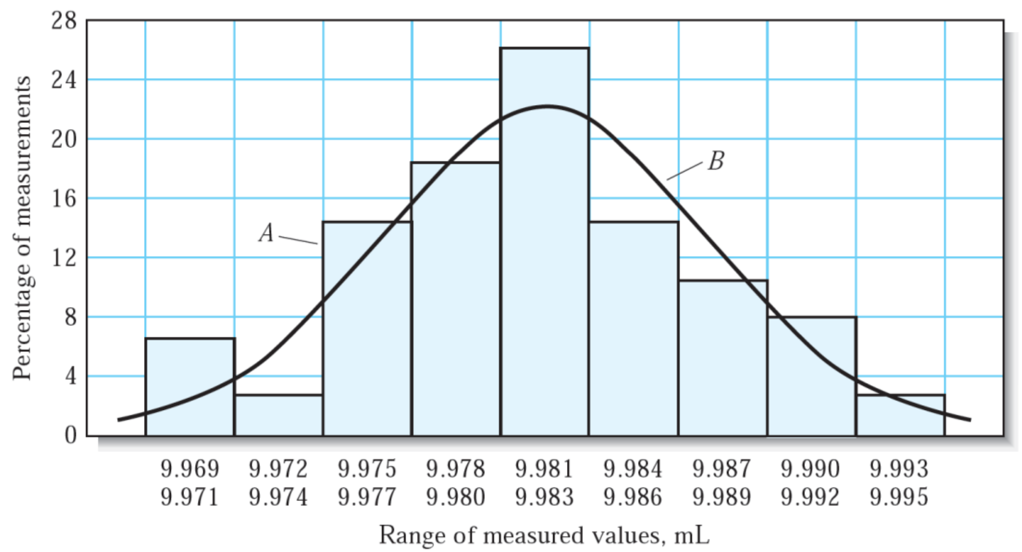
Quantifying random errors

Probability

- The percentage of measurements lying within the given range
- (one, two, or three standard deviation on either side of the mean) The average measurement is reported as: mean ± standard deviation.
- For example from the distribution curve we say: 68.3% of measurements of xi will fall within $x = \mu \pm \sigma$ (i.e. 68.3% of the area under the curve lies in the range of x)
- Mean and standard deviation should have the same number of decimal places

Range	Gaussian Distribution (%)
$\mu \pm 1\sigma$	68.3
$\mu \pm 2\sigma$	95.5
$\mu \pm 3\sigma$	99.7

Gaussian distribution and Calibration data in graphical form



A histogram (A) showing distribution of the 50 calibration results of 10 ml pipette and a Gaussian curve (B) for data having the same mean (9.982 ml) and same standard deviation (0.0056 ml) as the data in the histogram.

Gross Errors in Analyses

- They usually occur only occasionally, are often large, and may cause a result to be either high or low.
- Gross error leads to outliers. This error causes the result differs significantly from the rest of the results.
- Detectable by carrying out sufficient replicate measurements.

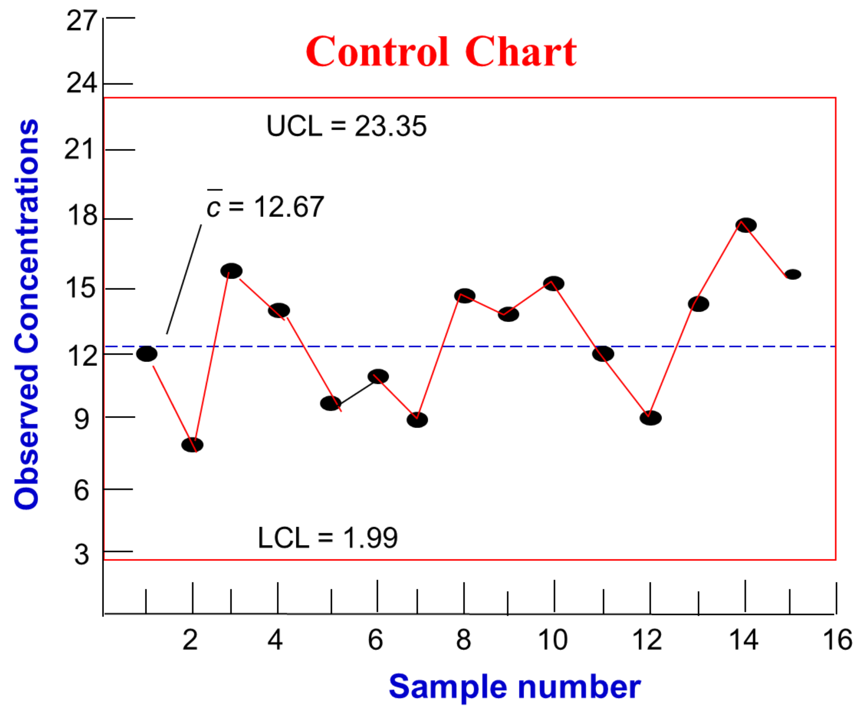
Minimisation of Errors

Control chart functions

- Control charts are decision-making tools - they provide an economic basis for deciding whether to alter a method
- Control charts are problem-solving tools - they provide a basis on which to formulate improvement actions.

Control chart components

- Centreline: shows where the method average is centered or the central tendency of the data
- Upper control limit (UCL) and Lower control limit (LCL): describes the method spread



Significant Figures

- Defined as the number of digits necessary to express the results of a measurement consistent with measured precision.
- The number of digits reported in a measurement reflect the accuracy of the measurement and the precision of the measuring device.
- Final report of result should only contain figures that are certain, plus the first uncertain number
    - Ex: The value of a result obtained is 45.2%.
    - If the error is less than 1%, we would only write 45%
    - If the error larger than 0.05% or would write 45.23%

Significant Figures

The number zero may or may not be a significant figure

- Final zeros after the decimal point are always significant figures. Ex: 9.8g to the nearest mg is reported as 9.800g (43.00, 4 sig. fig; 1.010, 4 sig. fig)
- Zeros before a decimal point with other preceding digits are significant. With no other preceding digit, a zero before the decimal point is not significant (Ex - 0.453 has 3 significant figures)
- If there are no digits preceding a decimal point, the zero after the decimal point but preceding other digits are not significant. These zeros only indicate the position of the decimal point (Ex 0.00024, 2 significant figures)
- Final zeros in a whole number may or may not be significant. For ex. A value of 100 ms/m for conductivity does not necessarily imply 100±1 ms/m (300, 1 significant figure)
- Every non-zero digit and zeros in between non-zeros are always significant

Values	Sig. Fig.
1 g	1
2.54 m	3
15,000 kg	2
1.500×10^3	4
0.0002 kg	1
221.001 V	6

Significant Figures

Addition and subtraction

- In addition or subtraction of several numbers, the number of significant figures should be equal to the number containing least accurately known
  - Ex - 168.11 + 7.045 + 0.6832.
  - Should be written as 168.11 + 7.06 + 0.68 (Fewest decimal places)
- When errors are known say

  $R \pm r = (A \pm a) + (B \pm b) + (C \pm c)$ then $r^2 = a^2 + b^2 + c^2$
  - Ex: Calculate the error in the MW of FeS from the following atomic weights:
  - Fe:55.847 $\pm$0.004 and S:32.064 $\pm$0.003
  - $r = (0.0042 + 0.0032)^{1/2}$ hence MW = 87.911 $\pm$0.00

Significant Figures

Multiplication and division

- In multiplication or division retain one more digit in each value than that in the value with fewest significant figures.
  - 1.26 x 1.236 x 0.683 x 24.8652. Should be written as 1.26 x 1.236 x 0.683 x 24.865.
- With known errors - add squares of **relative** uncertainties
  - $r/R = [(a/A)^2 + (b/B)^2 + (c/C)^2]^{1/2}$

Logarithms and Antilogarithms

- Only figures in the mantissa (after the decimal point) are significant figures
- In a logarithm of a number, keep as many digits to the right of the decimal point (mantissa) as there are significant figures in the original number. Ex: Say pH = 2.45   has 2 significant figures.
- In an antilogarithm of a number, keep as many digits as there are digits to the right of the decimal point in the original number.

Confidence Limit – How sure are you ?

- Unless there is large number of data, the std. deviation does not provide information about how close the experimentally determined mean ($\bar{x}$) might be to the true mean value (μ)
- However statistically, the range within which the true value might fall can be estimated within a given probability using experimental mean and std. deviation.
- The range is called confidence interval and the limit of this range is called confidence limit.
- The likelihood that true value fall within the range is called the probability (0-1) or confidence level (0-100%). CL is usually expressed in percentage. (5% CL implies a probability equal to 0.95.

- Definition: A numerical interval around the mean of a set of replicate analytical results within which the population mean can be expected to lie with a certain probability. This interval is called the *confidence interval*.
- Determining the number of replicate measurements required to ensure at a given probability that an experimental mean falls within a certain confidence interval.

Confidence Limit – How sure are you ?

- Confidence limits define a numerical interval around that contains μ with a certain probability.
- A confidence interval is the numerical magnitude of the confidence limit.

For a single measurement: CL for m = x ± zs

For the sample mean (x) of N measurements the equivalent expression is:

$$\text{CL for } \mu = \bar{x} \pm {}^{z\sigma}\!/\!_{\sqrt{N}}$$

CL,%	z
50	0.67
68	1.0
90	1.64
95	1.96
99.9	3.29

Confidence Interval When σ Is Unknown

$$t = \frac{x - \mu}{s}$$

Use of statistics

Statistics type: Used to quantify random errors	
Deductive statistics describe a complete data set (ex-Population)	*Inductive* statistics deal with a limited amount of data (ex-Sample)
Parameters: μ, σ, σ^2	Statistics: x, s, s^2

Descriptive statistics	
Measures of Central Tendency Describes the centre position of the data Mean Median Mode	Measures of Dispersion Describes the spread of the data Range Variance Standard deviation
Parameters: μ, σ, σ^2	Statistics: x, s, s^2

Measures of central tendency: Mean

Data Set: $x_1, x_2, x_3, \ldots, x_N$ Sample = finite number of observations

Population = total (infinite) number of observations

Mean: Arithmetic average of repeated set of results Where *xi* = individual values of *x* and *N* = number of replicate measurements	$\bar{x} = \frac{\sum_{i=1}^{N} x_i}{N}$

Weighted mean: If in a series of observations a statistical weight (w_i) is assigned to each value, then weighted mean is given by

$$\bar{x}_w = \frac{\Sigma w_i x_i}{\Sigma w_i}$$

Geometric (Logarithmic) mean: The n-th root of the product of the absolute values of the observations, taken with the proper sign

Harmonic mean: The number **of** observations, divided by the sum of reciprocals of the observation

Quadratic mean: The square root of the expression, in which the sum of squared observations is divided by the number ***n***

Mean deviation: Mean or average deviation is the mean of the deviations of all the individual measurements

Avg. deviation = $\Sigma|M_n - x| / N$ ($|M_n - x|$ is the abs. value of deviation of M_n^{th} number from the mean (x)

Measures of central tendency:
Median and Mode

Median: The middle value a replicate set of results. Sometime used instead of mean in statistical analysis

- For a set *n* values arranged in ascending order,
- the median is ½(n + 1) if *n* is odd or
- the avg. of value ½n and ½(n+1)

Characteristics of median

- Median can be calculated graphically while mean cannot be
- Median is not affected absolute value

Mode: The value of the variable occurring with the greatest frequency in the series of observations. The use of mode when reporting results of chemical analysis is generally not recommended.

100 91 85 84 75 72 72 69 65 : Mode is 72

Measures of dispersion: range

Spread or Range: The numerical difference between highest and lowest results in a set of result

2 4 6 8 10 12 14

Range = 14 - 2 = 12

Deviation: Numerical difference, w.r.t. sign, between an individual results and the mean or median of the set. Expressed as absolute or relative value

$$d_i = \left| x_i - \bar{x} \right|$$

Measures of dispersion: standard deviation

The most common statistical term

Standard Deviation (SD): For an infinite set of data (N) the SD (σ) is given by This Eq. holds strictly only as N $\rightarrow \infty$ **μ** is the mean of infinite number of measurements	$\sigma = \sqrt{\frac{\sum_{i=1}^{n}(x_i - \mu)^2}{n}}$
In practice we have limited/finite set of measurements, for which it is assumed that x $\rightarrow$ μ. In such case, the μ is replaced by mean of finite set of data (x) and one degree of freedom is lost. Hence SD (*s*) is given by As N increases s becomes a better estimator of σ. Typically when N>20, s is considered to be a good estimator of σ	$s = \sqrt{\frac{\sum_{i=1}^{N}(x_i - \bar{x})^2}{N-1}}$
The standard deviation of the mean = standard error of the mean	$s_m = \frac{s}{\sqrt{N}}$
Variance: The square of the standard deviation	$s^2 = \frac{\sum_{i=1}^{N}(\bar{x} - x_i)^2}{N-1}$
Coefficient of variance (CV) or Relative Standard Deviation (RSD): The standard deviation divided by the mean of the series and express as percentage	$CV = (\frac{s}{\bar{x}}) \times 100\%$

Commonly used statistical tests

Student *t* – Test

- Frequently used to decide whether the replicate set of data from two different methods are statistically different or not?
- One of them will be the *test method* and the other will be an *accepted method.*

Different ways in which *t*-test can be used

- *t-test when an accepted value is known (for this* student's *t is given by (test for systematic error)*

$$\pm t = (\bar{x} - \mu)\frac{\sqrt{N}}{s}$$

Values of *t* for ν degrees of freedom for various confidence level				
ν	CL=90	95	99	99.5%
4	2.132	2.776	4.604	5.598
5	2.015	2.571	4.032	4.773
9	1.833	2.262	3.250	3.690
∞	1.645	1.960	2.576	2.807
ν = N - 1 = Degrees of freedom				

Let the avg. of set of 5 values in a new method is found 10.8 ppm with a std. deviation of ± 0.7 ppm. The listed (true/accepted) value is 11.7. Does the method give a statistically correct value at the 95% Conf. level.

$$\pm t = (10.8 - 11.7)\sqrt{5} / 0.7 = 2.9$$

For 4 degree of freedom, the tabulated value at 95% CL is 2.776 and less than the calculated t value (i.e. $t_{cal} > t_{tab}$), hence, there is a systematic error in the new method or there is a 95% probability that the difference between the reference value and the measured value is not due to chance.

Student t – Test

- To test comparison of the means of two samples

The second use for Student's t is to test for significance. In this case we have two or more data sets that we wish to compare. We want to know whether or not they came from the same parent population (same object, etc.). To do this

1) Let assume the two results come from sampling the same parent population.

2) If this is true, then the two results have the same μ and σ.

3) Using two Student's t formulas, calculate t_{cal}

$$t_{calc} = \frac{|\bar{x}_1 - \bar{x}_2|}{s} \sqrt{\frac{N_1 N_2}{N_1 + N_2}} \quad \text{Where,} \quad s^2 = \frac{s_1^2(N_1 - 1) + s_2^2(N_2 - 1)}{N_1 + N_2 - 2}$$

4) we then compare t_{calc} to that of the table value for $(N_1 + N_2 - 2)$ degrees of freedom.

5) If $t_{calc} < t_{table}$, then the assumption is not wrong, else if $t_{calc} > t_{table}$, then the null hypothesis is wrong, and the two results are different.

Test of Significance: *F* - Test
Is there any difference ?

- Used to compare two methods (method 1 and method 2).
- Determines if the two methods are statistically different in terms of precision. The two variances (σ_1^2 and σ_2^2) are compared.
- *F* - function is defined in terms of variances of two methods as:

$$F = \frac{\sigma_1^2}{\sigma_2^2}$$

- The variance are arranged so that $F > 1$ i.e. $\sigma_1^2 > \sigma_2^2$
- F values are found in tables (make use of two degrees of freedom)
- $F_{cal} > F_{tab}$ implies there is a significant difference between the two methods
- F_{cal} = calculated F value and F_{tab} = tabulated F value

F – Test : An Example

Estimation of Glucose in blood serum by **method-1** and comparison with standard **method-2**. Two sets of replicate results for same sample are

Glucose in blood serum

	New method	Std. method
	127	130
	125	128
	123	131
	130	129
	131	127
	126	125
	129	
Mean	127	128

$\sigma_1^2 =$ 8.3 $\sigma_2^2 =$ 4.8

F = 8.3/4.8 = 1.7

Table for *F*-Test: Values of *F* at the 95% Confidence level

ν_2	$\nu_1 = 2$	3	4	5	6	7	8 …
2	19.0						
3							
4							
5	5.79	5.41	5.19	5.05	**4.95**	4.88	4.82
6	5.14	4.76	4.53	4.39	4.28	4.21	4.15
7							
8 ….							

- The variances are arranged so that the F values is >1.
- On comparison with tabulated value for $\nu_1 = 6$ and $\nu_2 = 5$ (i.e. 4.95), the calc. value is less indicating there is significant difference in the precision of two methods i.e. std. deviation are from random error alone and do not depend on the sample .

Rejection of a Result

Outlier

- A replicate result that is out of the line
- A result that is far from other results
- Is either the highest value or the lowest value in a set of data
- There should be a justification for discarding the outlier
- The outlier is rejected if it is $> \pm 4\sigma$ from the mean
- The outlier is not included in calculating the mean and
- standard deviation
- A new σ should be calculated that includes outlier if it is $< \pm 4\sigma$

Rejection of a Result : *Q* – Test

- Among wide variety of statistical tests to reject the a data in a set of data, the Q-test is most statistically correct for fairly small no. of observations.
- 90% CL is typically used
- Arrange data in increasing order
- Calculate range = highest value – lowest value
- Calculate gap = |suspected value – nearest value|
- Calculate Q ratio = gap/range
- The ratio is computed and compared with tabulated values. If $Q_{cal} > Q_{tab}$ then the suspected value can be rejected

Rejection quotient (Q) at different CL

Obs	Q_{90}	Q_{95}	Q_{99}
3	0.941	0.970	0.994
4	0.765	0.829	0.926
6	**0.560**	**0.625**	**0.740**
7	0.507	0.568	0.680
9	0.437	0.493	0.598

An Example

Data - 43.3, 44.7, 45.1, 46.2, 46.5, 69.2

GAP= 69.2 – 46.5 = 22.7

RANGE = 69.2 – 43.3 = 25.9

Q_{Calc} = 0.876,

$Q_{Calc}(0.876) > Q_{Table}(0.56)$ at 90% Conf. Level

Hence the data can be rejected

Rejection of a Result : Grubbs Test

- Used to determine whether an outlier should be rejected or retained
- Calculate mean, standard deviation, and then G

$$\boxed{G = \frac{|\text{outlier} - \bar{x}|}{s}}$$

- Reject outlier if $G_{cal} > G_{tab}$
- G tables are available

Linear Regression

When a set of standards are used to obtain a working curve, the data can be used to predict a straight line that will aid us in determining the analyte value in the unknown (sample) measurement.

The equation for the line is $y = mx + b$

Linear Regression Formulas

$$\sigma_x^2 = \frac{1}{N}\sum_{i=1}^{N}(x_i - \bar{x})^2 = \sum_{i=1}^{N} x_i^2 - N\bar{x}^2,$$

$$\sigma_{xy}^2 = \frac{1}{N}\sum_{i=1}^{N}(x_i - \bar{x})(y_i - \bar{y}) = \bar{y}\sum_{i=1}^{N} x_i^2 - \bar{x}\sum_{i=1}^{N} x_i y_i$$

The regression results for the slope and intercept A are

$$m = \frac{\sigma_{xy}^2}{\sigma_x^2} \quad b = \bar{y} - m\bar{x}$$

C C $$\sigma^2 = \frac{1}{N}\sum_{i=1}^{N}[y_i - (mx_i + b)]^2$$

Variance $$s_m^2 = N\frac{\sigma^2}{\sigma_x^2} \quad s_b^2 = \frac{\sigma^2}{N^2\sigma_x^2}\sum_{i=1}^{N} x_i^2$$

“Knowing is not enough,
we must apply;
Willing is not enough,
we must do.”

Goethe..

Thank you