

PHARMACEUTICAL ANALYSIS

Theoretical Basis of Analysis:

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Keywords

Method of analysis, Error, Precision, Calibration

Scope and Significance of Pharmaceutical Analysis

Pharmaceutical companies rely upon both qualitative and quantitative chemical analysis to ensure that the raw material used meet all the desired specifications, and also to check the quality of the final product. The examination of raw material is carried out to ensure that there is no unusual substance present which might deteriorate the manufacturing process or appear as a harmful impurity in the final product. The quantity of required ingredient in raw material is determined by a procedure known as *Assay*.

The final manufactured product is subjected to quality control to ensure that desired components are present within a range and impurities do not exceed certain specified limits.

Some specific use of analysis is under mentioned:

- (i) Quantitative analysis of air, water and soil samples is carried out to determine the level of pollution.
- (ii) Chemical analysis is widely used to assist in the diagnosis of illness and in monitoring the condition of patients.
- (iii) In farming, nature of soil and level of fertilizer application is analyzed
- (iv) In geology, composition of the rock and soil is carried out.

In general analysis is divided into two major part:

- (a) Qualitative analysis (what substances are present in the given sample)
- (b) Quantitative analysis (to determine the quantity of each component in the given sample)

Types of Analysis

The factors which must be taken into account when selecting an appropriate method of analysis are:

- (a) The nature of the information sought
- (b) The size of sample available and the proportion of the constituent to be determined
- (c) The purpose for which the analytical data is required.

Different types of chemical analysis may be classified as:

- (i) **Proximate Analysis:** the amount of each element in a sample is determined with no concern as to the actual components present.
- (ii) **Partial analysis:** deals with the determination of selected constituents in the sample,
- (iii) **Trace constituent analysis:** a specialized form of partial analysis in which determination of specified components present in very minute quantity,
- (iv) **Complete Analysis:** when the proportion of each component of the sample is determined.

Different Techniques of Analysis

The main techniques are based upon:

- 1 The quantitative performance of suitable chemical reactions
- 2 Appropriate electrical measurements
- 3 Measurement of certain optical properties and

- 4 Combination of optical and electrical measurement followed by quantitative chemical reaction eg. Amperometry

1. Methods Based on Chemical Analysis: These are based on traditional method of analysis and may be divided as:

- (a) Titrimetry
- (b) Gravimetry
- (c) Volumetry

(a) Titrimetric Analysis (also termed as volumetric analysis) in this technique the substance to be determined is allowed to react with an appropriate reagent added as a standard solution, and the volume of solution needed for completion on reaction is determined. Following are the types of titrimetric analysis:

- (i)** Neutralization (acid-base) reactions
- (ii)** Complexometric titrations
- (iii)** Precipitation titrations
- (iv)** Oxidation-reduction titrations

(b) Gravimetric Analysis in this technique substance under determination is converted into an insoluble precipitate which is collected and weighed.

In a special case of gravimetric analysis, electrolysis of the substance is carried out and the material deposited on one of the electrodes is weighed, this technique is called as **electrogravimetry**.

(c) Volumetry Analysis is concerned with measuring the volume of gas evolved or absorbed in a chemical reaction.

2. Electrical Methods of Analysis: These involve the measurement of current voltage or resistance in relation to the concentration of a certain species in a solution. These methods are of following types:

- i. **Voltametry:** It is the measurement of current at a microelectrode at a specified voltage.
- ii. **Coulometry:** It is the measurement of current and time needed to complete an electrochemical reaction or to generate sufficient material to react completely with a specified reagent.
- iii. **Conductometry:** It is the measurement of electrical conductivity of a solution. The ionic reactions in which there is a sudden change in conductance after completion of reaction, can act as a basis of conductometric titration method.
- iv **Potentiometry:** It is the measurement of the potential of an electrode in equilibrium with an ion to be determined..

3. Optical Methods of Analysis: The optical methods of analysis depend upon:

- i. Measurement of the amount of radiant energy of a particular wavelength absorbed by the sample.
- ii. The emission of radiant energy and measurement of the amount of energy of a particular wavelength emitted.

Several analytical techniques have been developed which involve the measurement of radiant energy. These are:

- i. Emission spectrography
- ii. Colorimetry
- iii. Fluorimetry
- iv. Turbidimetry & Nephelometry
- v. Spectrophotometry
- vi. Flame photometry
- vii. Atomic absorption spectroscopy
- viii. Polarimetry

The optical methods are basically of two types:

- i. Absorption methods**
- ii. Emission methods.**

Absorption methods are usually classified according to wavelength involved:

- i. Visible spectrophotometry
- ii. Ultraviolet spectrophotometry
- iii. Infrared spectrophotometry
- iv. Atomic absorption spectroscopy

In **emission** method sample is subjected to heat or electrical treatment so that the atoms are raised to excited states causing them to emit-energy; and the intensity of this emitted energy is measured. The emission spectroscopy includes flame photometry and fluorimetry as common excitation techniques.

In emission spectroscopy the sample is subjected to an electric arc or spark plasma and the light emitted is examined. The flame photometry involves the solution of the sample, injected into a flame while in fluorimetry a suitable substance in solution is excited by irradiation with visible or ultraviolet radiation.

4. Other Methods: Beside chromatography, X-ray, radioactivity and kinetic methods, other methods also includes Mass spectrometry, Thermal method of analysis, NMR spectroscopy and X-ray diffraction analysis etc.

- i. **Chromatography:** It is separative process, which is very useful for separating molecular mixtures. In this technique differential absorption of different components of a mixture by an adsorbent is observed. For example, suppose a petroleum ether extract of green leaves is allowed to flow down through a glass tube filled with CaCO_3 powder. The eluted ether extract will be a

mixture of different components derived from green leaves such as green pigments (chlorophylls), yellow pigments (xanthophylls) and another yellow substance (carotenes). The green pigments are more strongly absorbed and so are more firmly held by the powder consequently they are not able to move much down the column and remain near the top of the column. Yellow pigments are less strongly adsorbed so they move further down the column. The adsorption material has the least adsorption tendency for carotenes; hence they pass through the column without being adsorbed. The various molecular components present in green leaves can thus be separated by means of a very simple method. The adsorption column along with its coloured material is called a chromatogram and this technique of separation is known as chromatography.

ii. **X-Ray Methods:** Primary X-rays are produced by the collision of high-speed electrons with a solid target. By obtaining emission peaks resulting from the displacement of an electron from inner electron shell of an atom in the target, it is possible to identify the elements present in the target sample by relating the wavelengths of peaks with the atomic numbers of the elements producing them.

Similarly, secondary X-rays may be produced by the attack of primary X-rays on a solid target, which help in deciding the sample composition.

iii **Radioactivity:** Radioactivity methods involve the measurement of intensity of radiation from a naturally radioactive material and are used:

- i. In the determination of trace elements.
- ii. In examining geological specimens
- iii. In quality control in semiconductor manufacturing.

iv. **Kinetic Methods:** Kinetic methods involve the study of increase in speed of a reaction by adding a small amount of catalyst to the reaction mixed.

These methods are found to be useful:

- i. In determining sub microgram amount of appropriate organic substances.
- ii. In determining the amount of a substance in solution.
- iii. In clinical chemistry.

Significant Figures

A figure of digit denotes any one of the ten numerals (0,1,2,3,4,5,6,7,8,9). A digit alone or in combination serves to express a number.

A significant figure is a digit having some practical meaning, *i.e.* it is a digit, which denotes the amount of the quantity in the place in which it stands.

For example in 0.456, 4.56 and 546 there are three significant figures in each number.

Zero may or may not be a significant figure. A zero is a significant figure except when it serves to locate the decimal point, while it is a significant figure when it indicates that the quantity in

place in which *i.e.* in 1.3680 and 1.0082, zero is significant but in 0.0035, zeros are not the significant figures as they serve only to locate the decimal point. Thus, first two numbers contain five but the third one contains two significant figures.

Computation Rules

Rule 1 → In expressing an experimental measurement, never retain more than one doubtful digit. Eliminate all the digits that are not significant.

Rule 2 → Retain as many significant figures in a result or in any data as will give only one uncertain figure. *e.g.* a volume between 30.5 ml and 30.7 ml should be written as 30.6 ml. and not as 30.60 as it would be between 30.59 and 30.61.

Rule 3 → Two rules are given for rejecting superfluous digits.

1. When the last digit dropped is greater than 5, the last digit retained is increased by one. *e.g.* in rejecting the last digit in 8.942, the new value will be 8.94 as 2 is smaller than 5. But when 4.863 is rounded up to two digits, it gives 4.9 as the first digit discarded is 6 which is greater than 5. This is known as rounding up.

2. If the first digit discarded is less than 5, leave the last digit unchanged. It is known as rounding down. *e.g.* when the number 5.64987 is rounded to two digits, we get 5.6 as the first digit, discarded is 4, which is less than 5. Rounding never changes the power of 10. Thus, it is better to express numbers in exponential notation before rounding. *e.g.* in rounding 57832 to four figures, result 5.783×10^4

Rule 4. In addition or subtraction, there should be in each number only as many significant figures as there are in the least accurately known number. *e.g.* sum of three values 35.6, 0.162 and 71.41 should be reported only to the first decimal place as the value 35.6 is known only to the first decimal place. Thus, the answer 107.172 is rounded to 107.2

Rule 5. In multiplication or division, retain in each factor one more significant figure than is contained in the factor having the largest uncertainty. The percentage precision of a product or quotient cannot be greater than the percentage precision of the least precise factor entering into the calculation. *e.g.* the product of the three figures 0.0121, 25.64 and 1.05782 is $0.0121 \times 25.6 \times 1.06 = 0.328$

In a product or quotient of experimental numbers, the final result will have only as many significant figures as the factor with smallest number of significant figures.

e.g. in the calculation,
$$\frac{(0.0181057)(197.15)(0.218)}{0.4970}, \text{least number of significant figures}$$

(3) is in 0.218. Thus, the answer should also be expressed in three significant figures.

When a calculation involves both addition or subtraction and multiplication or division, addition is done first so as to determine the number of significant figures in the answer.

Rule 6 Computation involving a precision not greater than one fourth of 1 % should be made with a 10-inch slide rule. For greater precision, logarithm tables should be used.

Slide rule is a good method for checking the calculations made by logarithms. Use of logarithms has been recommended where a large number of multiplications and divisions are to be made.

Errors, Statics and Sampling

In quantitative analysis, when numerical data and numerical results are measured with the greatest exactness. It has been observed that the results of successive determination differ among themselves to a greater or lesser extent. Evidently not all and perhaps none, of the values obtained are correct within the possible limits of measurements. The average value of a series of measurements is accepted as the most probable value. It should, however, be noted that the average value may not always be the true value. In some cases difference may be small and in others, it may be so large that the result is unacceptable. Thus, the reliability of the result depends upon the magnitude of the difference between the average value and the true value.

Classification of Errors: Errors in any set of measurements can be divided into the following categories:

1. Systematic, determinate or constant errors.
2. Random or indeterminate errors.
3. Errors in measurements.
4. Gross errors.
5. Other errors.

1. Systematic, determinate or constant errors: These errors can be avoided and their magnitude can be determined, thereby correcting the measurements.

Determinate errors are characterized by the fact that it affects the results of a series of determination to the same degree. These errors occur with definite regularity owing to the faulty methods of technique or measuring instruments.

Types of determinate errors: Determinate errors may be of different types :

- I. **Personal errors:** These errors are not connected with the method or procedure but the individual analyst is responsible for them. This type of errors may arise due to the inability of the individual making observations. Some important personal errors are:
 - A. Inability in judging colour change sharply in visual titrations.
 - B. Error in reading a burette.
 - C. Mechanical loss of material in various steps of an analysis.
 - D. Failure to wash and ignite a precipitate properly.
 - E. Insufficient cooling of crucible before weighing.
 - F. Using impure reagents.
 - G. Ignition of precipitate at incorrect temperatures.
 - H. Errors in calculations.

II. **Operational errors:** These errors are mostly physical in nature and occur when sound analytical technique is not followed.

III. **Instruments and reagent errors:** following factors are responsible for such errors:

- A. Balance arms of unequal lengths.
- B. Uncalibrated or improperly calibrated weights.
- C. Incorrectly graduated burettes.
- D. Attack of foreign materials upon glasswares.
- E. Loss in weight of platinum crucibles on strong heating.
- F. Impure reagents.

These errors can be avoided by using calibrated weights, glasswares and pure reagents.

IV. **Methodic errors:** These are the most serious types of errors encountered in chemical analysis. Some examples involving methodic errors are:

- A. Solubility of precipitate in medium and in wash liquid.
- B. Decomposition or volatilization of weighing forms of precipitates on ignition or on heating.
- C. Hygroscopicity of the weighing forms of the precipitates.
- D. Co-precipitation.
- E. Post-precipitation
- F. Failure of a reaction to achieve completion.
- G. Occurrence of side reactions.

These errors can be eliminated or reduced to a small magnitude by employing the proper technique.

V **Additive and proportional errors:** Absolute error is independent of the amount of the constituent present in the determination e.g., loss in weight of a crucible.

On the other hand, the magnitude of proportional error depends upon the quantity of the constituent. e.g., impurity present in a standard substance.

2. Random or Indeterminate Errors: These errors are accidental and analyst has no control over them. They may be of two types.

(i) **Variation within determinate errors:** These cannot be prevented from variation e.g., in igniting a precipitate of Al(OH)_3 to constant weight, an analyst may obtain successive values, varying without a definite trend.

(ii) **Erratic errors:** Analyst has no control over such errors. Important examples of erratic errors are:

- A. Vibration in balance while handling it.
- B. Accidental loss of material during analysis.

The mathematic model that satisfies the magnitude of random error and the frequency of its occurrence is called normal distribution.

Following information can be obtained from this curve:

- A. Small errors occur more frequently than large ones.
- B. Positive and negative errors of the same numerical magnitude are equally likely to occur.
- C. Narrow peaked curve with slopes indicates a relatively high degree of precision.
- D. A broad curve indicates a relatively low degree of precision.

2. Errors in Measurements:

- (i) Errors in weighing may be due to the insensitivity of balance.
- (ii) Wrong suspension of ring-riders
- (iii) Placing the weights at edge of the pan.
- (iii) Using non-calibrated weights.
- (iv) Difference in temperature between the object weighed and the balance.
- (v) Errors in measuring solutions may be due to the incorrect use of glass wares.

3. Gross errors: Common gross errors are:

- (i) Use of numerically incorrect conversion factors.
- (ii) Wrong selection of method.
- (iii) Unsuitable storage of samples.

4. Other errors: Besides above, there may be present some other types of errors, as

- (i) Errors in radiometric analysis.
- (ii) Errors in chromatography.
- (iii) Photometric errors.

Minimization of Errors: The determinate error may be minimized by using following methods:

1. **Running a blank determination:** Errors arising from the introduction of impurities through the reagents and vessels are accomplished by running a blank. Such a procedure involves going through all the analysis, using the same solvent and reagent in the same quantities, but omitting the unknown component. Thus, in making a blank, sample is omitted; otherwise the details of the procedure are followed exactly as far as possible.
2. **Calibration of apparatus and application of corrections:** All instruments, such as burettes, pipettes, weights, measuring flasks, etc. must be properly calibrated and the appropriate corrections must be applied to the original measurements.
3. **Running a controlled determination:** It consists in carrying out a determination under identical experimental conditions as far as possible upon a quantity of a standard substance, which contains the same weight of the constituent as it contained in the unknown sample.

The weight of the constituent x in the unknown can then be calculated.

$$\frac{\text{Result found for standard}}{\text{Result found for unknown}} = \frac{\text{Wt. of constituent in standard}}{x}$$

4. **Running of parallel determination:** Parallel determinations serve as a check in the result of a single determination and indicate only the precision of the analysis. The values obtained in

parallel determination should agree well among themselves. These values should not vary by more than three parts per thousand. If larger variations are shown the determination must be repeated until satisfactory concordance is obtained. A good agreement between, duplicate and triplicate determinations does not justify the conclusion that the result is correct, but it merely shows that the accidental errors or variations of the determinate errors are same in parallel determinations.

5. **Standard addition:** A known amount of the constituent being determined is added to the sample, which is then analysed for the total amount of constituent present. The difference between the analytical results for samples with and without the added constituent gives the recovery of the amount of added constituent. If the recovery is satisfactory, accuracy of the procedure is enhanced. This procedure is especially applied to physico-chemical processes, as polarography and spectrophotometry.
6. **Isotopic dilution:** It consists in adding a known amount of pure component containing a radioactive isotope to the unknown, now the element so isolated is obtained in pure form usually as a compound. Its activity is determined with the help of an electroscope. The activity is compared with the added element. The weight of element in the unknown sample can then be calculated.
7. **Use of independent method of analysis:** Sometimes the complete analysis has to be carried out in an entirely different manner to get accuracy of results. e.g. strength of HCl may be determined by two methods :
 - i. Titrating it with a standard solution of a strong base.
 - ii. Precipitation with AgNO_3 and weighing as AgCl .

If the results obtained by the two methods are in good agreement, it may be said that the values are correct within small limits of errors.

8. **Internal Standards:** It involves the addition of a fixed amount of a reference material, *i.e.* the internal standard to a series of known concentrations of the sample to be determined. The ratios of the physical value of the internal standard and the series of known concentrations are plotted against the concentration values. It should give a straight line. Any unknown concentration can then be determined by adding the same quantity of internal standard and finding where the ratio obtained falls on the concentration scale. The method is of particular interest in spectroscopic and chromatographic determinations.

Rejection of Doubtful Values, Mean Deviation, Standard Deviation and Statistical Treatment of Small Data Set

When a series of similar measurements are made, it has been observed that one or more of the numerical values stand out as being considerably different from the others and to get the mean value, this value is rejected.

It can be assumed that higher the number of samples, the greater is the possibility of obtaining the true answer by taking average of these numbers. Thus, it is desirable to obtain as many samples or measurements as possible. But there is a practical limit of taking a certain number of measurements into consideration. Now, the question arises which measurement should be accepted and which should be rejected. To solve this problem, following tests may be considered:

1. **Average deviation:** After the average deviation for a series of measurements have been obtained, omit the doubtful value and determine the mean and average deviation of the retained values as usual. If the deviation of the suspected value from the mean is at least four times the average deviation, *i.e.* if $x \geq 4d$, then the rejection is justified.

2. **Standard deviation:** A normal distribution curve is plotted for 100 measurements of a sample.

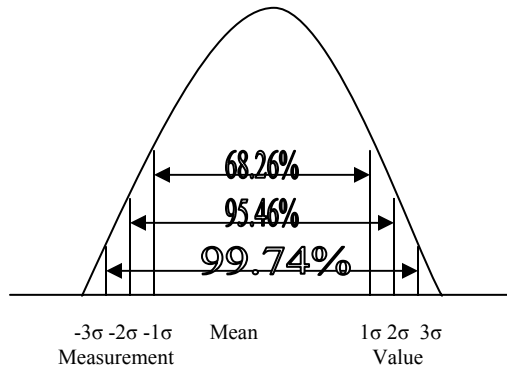


Fig.1: Normal distribution curve

3. **The Q test (a test for rejection of discordant data):** In some groups of five replicates, one value can be rejected. There is a test for this called the Q test, which is valid on samples of 3 to 10 replicates. To perform the Q test, calculate the quantity Q, which is the ratio of [the difference between the value under suspicion and the next closest value] to [the difference between the highest and lowest value in the series]. Compare Q with the critical value for Q (below), which for 5 observations is 0.64. If Q is greater than 0.64, the suspect measurement may be rejected. Otherwise, it must be retained.

For example, for values 1,2,3,4,9, if we wanted to test "9", we would take $(9-4)/(9-1)=5/8=0.625$, which is less than 0.64, so we'd have to keep the 9.

On the other hand, for the values, 3,3,4,4,9, if we wanted to test "9", we would take $(9-4)/(9-3)=5/6=0.833$, which is greater than 0.64, so we can throw the 9 out.

Only one value in a small (defined as 3-10 values) group can be removed by this test. If you have more than one "wild" value, you just have a group of data with a lot of scatter, and you need to keep them all.

Confidence Interval Tests of Significance

These tests may be used to define confidence intervals for the true value of a given phenomenon. Important of these tests are:

I.The T-Test : The t-test assesses whether the means of two groups are *statistically* different from each other. This analysis is appropriate whenever you want to compare the means of two groups, and especially appropriate as the analysis for the post test-only two-group randomized experimental design.

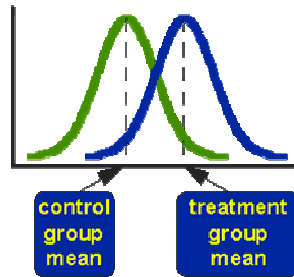


Fig. 2: Idealized distributions for treated and comparison group posttest values

Figure 2 shows the distributions for the treated (blue) and control (green) groups in a study. Actually, the figure shows the idealized distribution -- the actual distribution would usually be depicted with a histogram or bar graph. The figure indicates where the control and treatment group means are located. The question the t-test addresses is whether the means are statistically different.

What does it mean to say that the averages for two groups are statistically different? Consider the three situations shown in Figure 3. The first thing to notice about the three situations is that ***the difference between the means is the same in all three***. But, you should also notice that the three situations don't look the same -- they tell very different stories. The top example shows a case with moderate variability of scores within each group. The second situation shows the high variability case, the third shows the case with low variability. Clearly, we would conclude that the two groups appear most different or distinct in the bottom or low-variability case. Why? It is because there is relatively little overlap between the two bell-shaped curves. In the high variability case, the group difference appears least striking because the two bell-shaped distributions overlap so much.

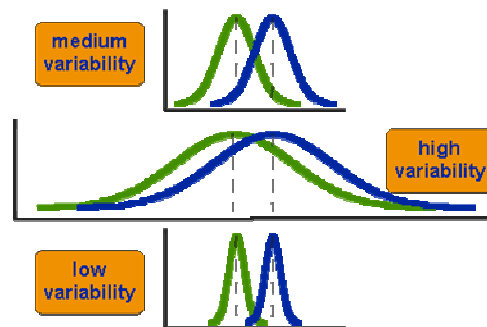


Fig. 3: Three scenarios for differences between means

This leads us to a very important conclusion: when we are looking at the differences between scores for two groups, we have to judge the difference between their means relative to the spread or variability of their scores. The t-test does just this.

Statistical Analysis of the t-test: The formula for the t-test is a ratio. The top part of the ratio is just the difference between the two means or averages. The bottom part is a measure of the variability or dispersion of the scores. This formula is essentially another example of the signal-to-noise metaphor in research: the difference between the means is the signal that, in this case, we think our program or treatment introduced into the data; the bottom part of the formula is a measure of variability that is essentially noise that may make it harder to see the group difference. Figure 4 shows the formula for the t-test and how the numerator and denominator are related to the distributions.

$$\begin{aligned}
 \frac{\text{signal}}{\text{noise}} &= \frac{\text{difference between group means}}{\text{variability of groups}} \\
 &= \frac{\bar{X}_T - \bar{X}_C}{SE(\bar{X}_T - \bar{X}_C)} \\
 &= \text{t-value}
 \end{aligned}$$

Fig. 4: Formula for the t-test

The top part of the formula is easy to compute -- just find the difference between the means. The bottom part is called the **standard error of the difference**. To compute it, take the variance for each group and divide it by the number of people in that group. Add these two values and then take their square root. The specific formula is given in Figure 5:

$$SE(\bar{X}_T - \bar{X}_C) = \sqrt{\frac{\text{var}_T}{n_T} + \frac{\text{var}_C}{n_C}}$$

Fig. 5: Formula for the Standard error of the difference between the means

Remember, that the variance is simply the square of the standard deviation. The final formula for the t-test is shown in Figure 6:

$$t = \frac{\bar{X}_T - \bar{X}_C}{\sqrt{\frac{\text{var}_T}{n_T} + \frac{\text{var}_C}{n_C}}}$$

Fig. 6: Formula for the t-test

The t-value will be positive if the first mean is larger than the second and negative if it is smaller. Once you compute the t-value you have to look it up in a table of significance to test whether the ratio is large enough to say that the difference between the groups is not likely to have been a chance finding. To test the significance, you need to set a risk level (called the alpha level). In most social research, the "rule of thumb" is to set the alpha level at .05. This means that five times out of a hundred you would find a statistically significant difference between the means even if there was none (i.e., by "chance"). You also need to determine the degrees of freedom (df) for the test. In the t-test, the degree of freedom is the sum of the persons in both groups minus 2. Given the alpha level, the df, and the t-value, you can look the t-value up in a standard table of significance (available as an appendix in the back of most statistics texts) to determine whether the t-value is large enough to be significant. If it is, you can conclude that the difference between the means for the two groups is different (even given the variability). Fortunately, statistical computer programs routinely print the significance test results and save you the trouble of looking them up in a table.

II. Chi-Square test (χ^2 –test) : Generally speaking, the chi-square test is a statistical test used to examine differences with categorical variables. The chi-square test is used in two similar but distinct circumstances:

- a. for estimating how closely an observed distribution matches an expected distribution - we'll refer to this as the **goodness-of-fit test**
- b. For estimating whether two random variables are independent.

The Goodness-of-Fit Test: The steps used in calculating a goodness-of-fit test with chi-square are:

1. Establish hypotheses.
2. Calculate chi-square statistic. Doing so requires knowing:
 - o The number of observations
 - o Expected values
 - o Observed values
3. Assess significance level. Doing so requires knowing the number of degrees of freedom.
4. Finally, decide whether to accept or reject the null hypothesis.

The key idea of the chi-square test is a comparison of observed and expected values. How many of something was expected and how many were observed in some process? In this case, we would expect 10 of each number to have appeared and we observed those values in the left column.

With these sets of figures, we calculate the chi-square statistic as follows:

$$\chi^2 = \sum \frac{(\text{observed} \times \text{frequency} - \text{expected} \times \text{frequency})}{(\text{expected} \times \text{frequency})}$$

Lastly, to determine the significance level we need to know the "degrees of freedom." In the case of the chi-square goodness-of-fit test, the number of degrees of freedom is equal to the number of terms used in calculating chi-square minus one; finally compare the value calculated in the formula above to a standard set of tables.

Testing Independence: The other primary use of the chi-square test is to examine whether two variables are independent or not.

More generally, we say that variable Y is "not correlated with" or "independent of" the variable X if more of one is not associated with more of another. If two categorical variables are correlated their values tend to move together, either in the same direction or in the opposite.

The steps used in calculating a goodness-of-fit test with chi-square:

1. Establish hypotheses
2. Calculate expected values for each cell of the table.
3. Calculate chi-square statistic. Doing so requires knowing:
 - a. The number of observations
 - b. Observed values
4. Assess significance level. Doing so requires knowing the number of degrees of freedom
5. Finally, decide whether to accept or reject the null hypothesis.

1. Establish Hypotheses: As in the goodness-of-fit chi-square test, the first step of the chi-square test for independence is to establish hypotheses. The null hypothesis is that the two variables are independent.

Cautionary Note: It is important to keep in mind that the chi-square test only tests whether two variables are independent. It cannot address questions of which is greater or less.

2. Calculate the expected value for each cell of the table: As with the goodness-of-fit example described earlier, the key idea of the chi-square test for independence is a comparison of observed and expected values. How many of something was expected and how many were observed in some process? In the case of any tabular data, expected values are calculated based on the row and column totals from the table.

The expected value for each cell of the table can be calculated using the following formula:

$$\frac{\text{Row total} \times \text{Column total}}{\text{Total } n \text{ for table}}$$

The first step, then, in calculating the chi-square statistic in a test for independence is generating the expected value for each cell of the table.

3. Calculate Chi-square statistic: With these sets of figures, we calculate the chi-square statistic as follows:

$$\text{Chi - square} = \text{Sum of } \frac{(\text{observed} \times \text{frequency} - \text{expected} \times \text{frequency})^2}{(\text{expected} \times \text{frequency})}$$

4. Assess significance level: Lastly, to determine the significance level we need to know the "degrees of freedom." In the case of the chi-square test of independence, the number of degrees

of freedom is equal to the number of columns in the table minus one multiplied by the number of rows in the table minus one.

Then compare the value calculated in the formula above to a standard set of tables. If the value returned from the table is $p < 20\%$ we cannot reject the null hypothesis.

III. F – Test: The F -test is used to test for differences among sample variance. Like the Student's t , one calculates an F and compares this to a table value. The formula for F is simply

$$F = \frac{s_1^2}{s_2^2}$$

The variance are arranged so that $F > 1$. That is; $s_1^2 > s_2^2$.

We use the F -test as the Student's t test; only we are testing for significant differences in the variances.

- 1) Invoke the null hypothesis that states that the two variances we are comparing are from the same population. (*i.e.*, they are not statistically different)
- 2) Calculate the F value (the ratio of the two variances)
- 3) Look up the table value of F for the degrees of freedom used to calculate both variances and for a given confidence level.
- 4) If the calculated F is greater than the table value, then the null hypothesis is not correct. Else, the two could have come from the same population of measurements.

Precision and Accuracy

Accuracy: Absolute error, which is defined as the difference between the observed or measured value and the true or most probable value, is a measure of accuracy of the quantity measured.

Accuracy is defined as the degree of agreement between a measured value and the most probable or true value.

Practically since no measurement is completely accurate, true value is never known except within certain limits. Therefore, accuracy of result is never known except within those limits. Thus, accurate results are assumed to be those measured values which have been obtained by using instruments of best quality.

Precision: Precision may be defined as the concordance of a series of measurement of the same quality.

Accuracy expresses the correctness of a measurement, while precision the reproducibility of a measurement. Accuracy without precision is impossible, but precision does not imply accuracy.

When a student is able to reproduce two or more measurements with slight differences in the results, his work may be regarded as to be precise. Let, a substance was known to contain 49.10 ± 0.02 % of a constituent x. Two analysts obtained results using same substance and the same analytical method. Their results were as:

Analyst 1: % X $\longrightarrow$ 49.01, 49.08, 49.14

The results range from 49.01% to 49.25 %. Their arithmetic mean is 49.12%, which is very close to the amount of X present in the substance.

Analyst 2: % X $\longrightarrow$ 49.40, 49.44, 49.42, 49.42.

The results range from 49.40 % to 49.44 % and the arithmetic mean is 49.42 %, which is very much different from the actual amount of X present in the substances.

Thus, it is clear from the above example that although the results of analyst 2 were précised, yet was not accurate, while results obtained by analyst-1 were not précised to such a great degree, but was accurate.

Precision Measures

1. Mean or average or relative mean deviation is a measure of precision. Mean or average is obtained by dividing the sum of a set of measurements by the number of individual results in the set

$$\text{Mean, } m = \frac{\sum Mn}{n}$$

Where, M is the individual measurement and n is the total number of measurements.

2. Median is a value about which all the other values are equally distributed. Half of the values are smaller and other half is larger than the median value. Mean and median may or may not be the same.
3. Absolute error is the difference between the observed or measured and the true or the most probable value.

Absolute error = Observed value – true or most probable value.

4. Relative error is the absolute error divided by the true or most probable value. It is expressed in parts per thousand.

$$\text{Relative error} = \frac{\text{Absolute error}}{\text{Most probable value}} \times 1000 \text{ parts per thousand.}$$

5. Mean deviation of a single measurement is the mean of the deviations of all the individual measurements. It can be calculated in three steps:

- i. Determining the arithmetic mean of results.
- ii. Calculating the deviation of each individual measurement from the mean.
- iii. Dividing the sum of deviations (regardless of sign), by the number of measurements.

$$\text{Mean deviation, } d^- = \frac{\sum [M_n - m]}{N}$$

Where, $d^- \rightarrow$ mean deviation

$[M_n - m] \rightarrow$ absolute value of the deviation of the M_n th number from the mean.

6. Relative mean deviation is the mean deviation divided by the mean. It is expressed in percentage or parts per thousand.

$$\text{Relative mean deviation} = \frac{\text{Mean deviation}}{\text{Mean}} \times 100 \%$$

$$= \frac{\text{Mean deviation}}{\text{mean}} \times 1000 \text{ parts per thousand.}$$

As in the above example, relative mean deviation

$$= \frac{0.070}{46.627} \times 100 = 0.15\%$$

7. Average deviation of the mean is equal to the average deviation (d^-) of the single measurement divided by the square root of the number of measurement made.

$$\text{Average deviation of the mean } D = \frac{d^-}{\sqrt{n}}$$

Where, $d^- \rightarrow$ average deviation

$n \rightarrow$ total number of measurements

$$\begin{aligned} \text{As in the above example, } D &= \frac{0.070}{\sqrt{9}} \\ &= 0.023 \end{aligned}$$

8. Standard deviation is obtained by extracting the square root of the quotient obtained by dividing the sum of the square of individual deviations of the number of measurements made.

$$\text{Standard deviation } \sigma = \sqrt{\frac{\sum (M_n - m)^2}{N}}$$

When number of determinations is small, the above equation is modified as,

$$S = \sqrt{\frac{\sum (M_n - m)^2}{(n-1)}}$$

As, in the example discussed above,

$$S = \frac{\sqrt{(0.007)^2 + (0.157)^2 + \dots}}{9-1} = 0.091$$

9. Variance is the square of standard deviation variance $V=S^2$

$$10. \text{ Coefficient of variance } C.V. = \frac{S \times 100}{X}$$

Where, $S \rightarrow$ Standard deviation
 $X \rightarrow$ mean

Calibration of Analytical Equipments

There are three general approaches to the calibration of volumetric glassware.

- 1. Direct, absolute calibration:** In this, volume or water delivered by the burette or pipette or contained in volumetric flask is obtained directly from the weight of the water and its density.
- 2. Calibration by Comparison (Indirect):** In this approach, the volumetric glassware is calibrated by using previously calibrated vessel. It is especially convenient if many pieces of glassware's are to be calibrated.
- 3. Relative calibration:** Sometimes it is necessary to know only the relationship between two items of glassware's without knowing the absolute volume of either one.

Calibration of Burettes: A burette is a long calibrated glass tube with a fine end tip and a glass stopcock to allow controlled flow of volume. It affords greater precision, typically 0.1 to 0.2 %. Burette is principally used in titrations for the accurate delivery of a standard solution to the sample solution until the end point is reached. The conventional burettes for macro titrations are marked in 0.1 ml increments and are available in capacities of 10, 25, 50 and 100 ml. Micro-burettes are available in capacities of down to 2 ml where the volume is marked in 0.01 ml increments.

Burettes can be calibrated by the following method

- Clean the burette thoroughly and lubricate the stopcock properly.
- Fill with water and test for leakage and wait for at least 5 minutes.
- During waiting period weigh a suitable receiver (generally an Erlenmeyer flask) to the nearest milligram. Record this weight.
- Fill the burette with distilled water. Measure and record the temperature. Sweep any air bubble from the tip of burette. Now, withdraw water more slowly until meniscus is at or slightly below the zero mark on burette. After drainage is complete read the burette to the nearest 0.01 ml. Record the initial reading. Remove the hanging drop.
- Now run about 10 ml of water from burette into a previously weighed flask. Quickly stopper the flask and weigh it to the nearest milligram. Record this weight.
- Read the burette reading after allowing the time for drainage and record the final reading. Calculate the correction as –

Initial B.R.	Final B.R.	App. Vol ^m	Initial wt.	Final wt.	Act. Vol.	Temp.	Correction

Now, refill the burette and obtain another initial reading. Run about 20 ml of water into the flask. Obtain the final burette reading and reweigh the flask. Note that we are calibrating the burette in 10 ml intervals but starting each time from the initial reading as titration starts generally from zero.

This process is repeated for 30-40-50 ml volumes.

The difference between actual volume and apparent volume is used as correction.

Calibration of burette is repeated, as a check on work and the duplicate results should agree within 0.04 ml.

For intermediate value, calibration is better by graphical methods. Plot correction against interval of 10,20 ml ...Connect the points to get a straight line so as to obtain linear interpolations by simple inspection of graph.

The tolerances on capacity for burettes as per Indian pharmacopoeia are given below:

BURETTES			
Normal capacity ml.	10	25	50
Subdivision ml.	0.05	0.05	0.1
Tolerance ± ml.	0.01	0.03	0.05

Calibration of Pipette: The pipette is used to transfer quantitatively a known volume of solution from one container to another. There are two common types of pipettes, the volumetric (transfer) pipette and measuring pipette.

Volumetric pipette are used for high accuracy analytical work. They are calibrated to deliver a specified volume at a given temperature and are available in sizes from 0.5 to 200 ml.

Measuring pipette are straight bore pipette that are marked at 6 different volume intervals. They are convenient for delivering various volumes with reasonable 0.5 to 0.01 ml accuracy. They are not as accurate because of non-uniformity of the internal diameter. Pipette must be calibrated if higher accuracy is required.

Pipette can be calibrated as follows:

- Clean and rinse the pipette thoroughly and try it.
- Weigh to nearest milligram a receiver generally Erlenmeyer flask.
- Fill the pipette to a level above the itched line using distilled water at the laboratory temperature. Remove any liquid on outside and release the pressure to allow the liquid to fall to itched line.
- Discharge the contents of pipette to a previously weighed receiver. Allow the pipette to drain completely for 20 to 30 seconds.
- Stopper the container and reweigh it. Calculate the volume of water delivered by the pipette from the weight and see the apparent volume from table.
- Calibration of pipette is repeated, as a check on work and duplicate results should not differ by more than 1 mg.

The tolerances on capacity to pipettes as per Indian pharmacopoeia are given below:

One mark Pipette			
Normal capacity ml.	10	25	50
Tolerance $\pm$ ml.	0.02	0.03	0.05
Graduated Pipette			
Normal capacity ml.	1	5	10
Subdivision ml.	0.01	0.05	0.10
Tolerance $\pm$ ml.	0.006	0.03	0.05

Calibration of volumetric flasks: Volumetric flasks are used in the preparation of standard solution and are available with capacities from 5 to 1000 ml. They are normally calibrated to contain a specified volume at 27°C when filled to the line etched on the neck. Volumetric flask must be thoroughly cleaned and rinsed with the pure solvent before calibration.

Calibration of a volumetric flask is necessary only for work of the highest accuracy and following ways can do it:

- Volumetric flask is cleaned, rinsed and then clamped in an inverted position to dry it.
- Stopper the flask and weigh to nearest milligram and record this weight.

- Fill the flask with distilled water at room temperature. Adjust the lower meniscus of water to the etched level mark by means of pipette or dropper.
- Stopper the flask and reweigh to the nearest milligram. Difference in weight gives the apparent volume of water contained and from the weight of water. Calculate the actual volume.
- Calibration should, be checked by repeating the procedure. Duplicate results should agree within 0.3 ml for the flask.

The tolerances on capacity for volumetric flask as per Indian Pharmacopoeia are given below:

One Mark Pipette				
Normal capacity ml.	10	25	50	100
Tolerance $\pm$ ml.	0.02	0.03	0.04	0.06

Suggested Readings:

- i. K A Connors, A Text Book of Pharmaceutical Analysis, Wiley-Intersciences, New York.
- ii. A H Backett and J B Stenlake, Practical Pharmaceutical Chemistry, Vol.I and II, The Athlone Press of The University of London.
- iii. Pharmacopoeia of India, Govt.of India, Ministry of Health, Delhi.
- iv. J Bassett, R C Denney, G H Jeffery, J Mendham, Vogel's Textbook of Quantitative Inorganic Analysis, The ELBS and Longman, London.