CHAPTER 1

INTRODUCTION
Introduction

Quality assurance and control of pharmaceutical and chemical formulations is essential to ensure the availability of safe and effective drugs to consumers. Hence, Pharmaceutical analysis is important to secure statutory certificates for drugs either to the industry, or for perusal by the regulatory authorities. The complex problems encountered in pharmaceutical analysis are in achieving the selection, speed, cost-effectiveness, simplification, precision and for ensuring accuracy of results. New methods of analysis are being quickly adopted by pharmaceutical industries and chemical laboratories depending upon the facilities available. Formulations containing various combinations of drugs for potentiating and complementing one another are key in therapy. Quite often the reported procedures need improvements or changes in view of the latest advances.

Among the several instrumental techniques eg., HPLC, GC, Fluorimetry, NMR, IR, UV and Visible spectroscopy available for the assay of drugs, and among all spectrophotometric technique is simple and cost-effective. UV and Visible spectrophotometric methods are highly versatile, sensitive and reproducible. An attempt is made here to develop new spectrophotometric methods for estimating the selected drugs from pharmaceutical preparations.
Section (i)

(a) Brief account of drugs

Drugs play a vital role in the process of curing diseases of humans and animals. The word “drug” is derived from the French word ‘drogue’, meaning ‘dry herb’. A drug may be defined as ‘a substance used in the prevention, diagnosis, treatment or cure of diseases in men or other animals’. According to World Health Organization (WHO), a drug has been defined as ‘any substance or product which is used or intended to be used for modifying or exploring physiological systems or pathological states for the benefit (physical, mental as well as economical) of the recipient’.

An ideal drug when administered to the ailing individual should be such that:

i) Its action should be localised at the site where it is desired to act
ii) It should act on a system with efficiency and safety
iii) It should not have any toxicity
iv) It should have minimum side effects
v) It should not injure host tissues or physiological processes
vi) It should not develop tolerance by the tissues even when administered for a long duration.

Very few drugs satisfy all the above conditions. However, the search for ideal drug continues.
(b) Dosage forms

In order to administer to the patient, drugs are prepared and supplied in a variety of pharmaceutical forms known as dosage forms.

1. Syrup

A 66% solution of sugar in water is called syrup. Flavoured syrup is used as a vehicle for more palatability and in some cases, masks the bitter taste of a few active ingredients (syrup chloroquine). Widely used ‘cough mixtures’ containing antihistamines, expectorants, decongestants, mucolytics or anti tussives are mostly in syrup base.

2. Tablets

These are disc shaped solid preparations for oral use and generally do not weigh more than 0.5g. In addition to the active ingredient, a tablet contains inert substances like disintegrator (calcium lactate, starch and binder gum). Some tablets are sugar-coated or with shellac or cellulose (enteric coated) to mask the bitter taste. Enteric coated tablets do not dissolve in the acidic juices of the intestine. Enteric coating prevents gastric irritation and protects some drugs from the hydrolytic action of gastric acid. It also helps to get the desired concentration of the drug in the small intestine.

3. Capsule

A capsule is a cylindrical envelope of gelatin in which a drug can be enclosed for oral administration. Drugs in powder form are filled in hard gelatin capsules where as spansules are time-release capsules where the granules of the drug have different
coatings, which dissolve, at different time intervals. Such differential release of the drug provides for uniform medication over a prolonged period.

4. Ointments

Ointments are semisolid grease-like preparations for local application. Ointments are used as astringents, antiseptics and protectives. If the medium or base is absorbable like animal fat, systemic effects may follow, suppositories, pessaries and bougies are solid preparations intended for insertion into cavities, viz., rectum, vagina and nasal cavities respectively. The base is either the oil of theobroma or glycerinated gelatin.

5. Injections

Liquid preparations meant for parenteral administration are called injections. They are to be sterile, and depending upon the volume, are supplied in sealed glass ampules, rubber capped multidose vials or large infusion bottles or in polypropylene pouches. Examples are adrenaline ampules, lignocaine vials and dextrose saline infusion bottles. Sometimes, vials contain powder, which has to be dissolved or suspended in colloidal form before injection by adding an adequate quantity of a suitable solvent.

6. Enema

Enema is a liquid preparation meant for administration for rectum. They are of two types: retention enemas and evacuant enemas.

Retention enemas may be used either for a local action in the large bowels as in the case of prednisolone in ulcerative colitis, or for a systemic action after
absorption of the active ingredient into the general circulation as in the case of paraldehyde enema. Usually 100-200 ml fluid is administered. The evacuant enemas are used principally to wash out the large bowels prior to surgery and to relieve constipation (soap-water enema). For diagnostic purposes, a suspension of barium sulphate is used as an enema in order to delineate pathological changes in the colon and rectum on an x-ray screen or film.

7. Tinctures

Tinctures are alcoholic or hydro-alcoholic extractive preparations of vegetable drugs; usually these represent 10 percent of the drug from which they are prepared.

8. Inhalations: Inhalations are medicaments meant to be inhaled or nebulised or aerosolized for their local action on the respiratory tract or for systemic effect after absorption.

(c) Drug quality

Safety and efficacy of pharmaceuticals are two fundamental issues of importance in drug therapy. The safety of a drug is determined by its pharmacological-toxicological profile as well as the adverse effects caused by the impurities in bulk and dosage forms. The impurities in drugs often possess unwanted pharmacological or toxicological effects by which any benefit from their administration may be outweighed. Therefore, it is quite obvious that the products intended for human consumption must be characterized as completely as possible. The quality and safety of a drug is generally assured by monitoring and controlling the impurities effectively. Thus, the analytical activities concerning impurities in drugs are among the most important issues in modern pharmaceutical analysis. This has become quite clear by the recent research articles on this topic.
Control is more important today than ever. Until the beginning of the 20th century, drug products were produced and sold, having no imposed control. Quality was generally poor. Many products were patent medicines of dubious value. Some were harmful and addictive. The impurities to be considered for new drugs are listed in regulatory documents of the Food and Drug Administration (FDA), International Conference on the Harmonization (ICH) of the Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and United States Pharmacopoeia (USP). Nevertheless, there are many drugs in existence, which have not been studied in such detail. The USP and National Formulary (NF) are the recognized standards for potency and purity of new drugs. These compendia have become official upon adoption of the first Food and Drug Act. They formulate legal standards of quality, purity and potency of new drugs. Good manufacturing practices provide minimum quality standards for production of pharmaceuticals as well as their ingredients.

The International Conference (ICH) which was held at Yokohama, Japan in 1995 has released new guidelines to control impurities in new drug products. These guidelines have a number of advantages, both for the industry and the regulatory authorities. The most critical aspect of elaboration of the guidelines includes the definition of the levels of impurities for identification and qualification. Qualification is the process of acquiring and evaluating data for establishing the biological safety of an individual impurity or a given impurity profile at the levels specified.

The level of any impurity present in a new drug substance that has been adequately tested in safety and clinical studies is considered qualified. A rationale for selecting impurity limits based on safety considerations has to be provided. Analytical procedures should be able to separate all the impurities from each other and the
method should be optimized to separate and quantify them in the dosage forms. Such methods are to be validated demonstrating the accuracy, precision, and specificity, limit of detection, quantification, linearity range and interferences.
Section (ii)

(a) The role of Analytical chemistry in Pharmacy

Analytical chemistry is a measurement science consisting of a set of powerful propositions and methods useful to all fields of science and medicine. An exciting illustration of the utility and significance of analytical chemistry is the pathfinder demonstration that took place on July 4th 1997. The pathfinder example demonstrated both the qualitative and the quantitative information required in an analysis. Qualitative analysis establishes the chemical identity of the species in the sample. Quantitative analysis determines the relative amounts of these species or analytes in numerical terms. Analytical chemistry is applied to the industry, medicine, and all the relevant sciences.

Quantitative analytical measurements also play a vital role in many research areas in chemistry, biochemistry, biology, geology, physics, and other sciences. For example, quantitative measurements of potassium, calcium and sodium ions in the body fluids of animals permit physiologists to study the role of these ions in nerve signal conduction as well as muscle-contraction and relaxation. Chemists unravel the mechanisms of chemical reactions through reaction-rate studies. The rate of consumption of reactants or formation of products in a chemical reaction can be calculated from quantitative measurements made at equal time intravels. Many medicinal chemists devote much time to the laboratory in gathering quantitative information about systems that are important and interesting to them.

Analytical chemistry has a similar function with respect to many other scientific fields. The interdisciplinary nature of chemical analysis has a pivotal role to
play in the fields of medicine, industrial, academic laboratories and in the
governments throughout the world.

A typical quantitative analysis involves a sequence of steps shown in the flow
diagram (figure-1.1). In some instances, one or more of these steps can be omitted.
There are a number of possible paths in quantitative analysis.

![Flow Diagram](image)

**Fig. 1.1. UV-Visible double beam spectrophotometer**

In the simplest example represented by the central vertical pathway, we select
a method, acquire and process the sample, dissolve the sample in a suitable solvent
measure property of the analyte, calculate the results, and estimate the reliability of
the results. Depending on the complexity of the sample and the chosen method,
various other pathways may be followed.

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(b) Drug validation

Analytical procedure, proof of suitability for the intended use, and validation are the important processes for drug registration of a new drug. Additional peak tailing, peak resolution and analyte recoveries are important in case of chromatographic methods. The ICH has harmonized the requirements in two guidelines. The first one summarizes and defines the validation characteristics needed for various types of test procedures; the second one extends the previous text to include the experimental data required and some statistical interpretations. These guidelines serve as a basis worldwide both for regulatory authorities and industry. Thus, the importance of a proper validation is brought to the attention of all those involved in the process of submission of drug master files. The Analytical Research and Development units in the pharmaceutical industry are responsible for preparation and validation of test methods.

Every country has a legislation on bulk drugs and their pharmaceutical formulations that sets standards and obligatory quality indices for them. These regulations are presented in separate articles, general and specific, relating to individual drugs and are published in the form of a book called Pharmacopoeia (e.g. Indian IP, United States USP, European EP, United Kingdom BP, Martindale Extra Pharmacopoeia, Merck Index, etc.).

In view of the foregoing discussion assaying and stability testing in pharmaceutical analysis plays a vital role to meet the requirement of statutory certification of drugs and their formulations by the industry. The complexity of problems encountered in pharmaceutical analysis coupled with the importance of achieving high selectivity, speed, cost, simplicity, sensitivity, precision and accuracy,
warrant the invention of new methods of analysis. The assaying and stability test results are being quickly absorbed by the pharmaceutical industry and chemical laboratories depending upon the facilities available at their end.

Among the several instrumental techniques (HPLC, GC, capillary electrophoresis, fluorimetry, NMR, mass spectroscopy, spectrophotometry covering IR, NIR, Raman, UV and visible regions) available for the assay of drugs, the visible Spectrophotometric technique is simple and less expensive.

The selectivity and sensitivity of the visible Spectrophotometric method depend only on the nature of chemical reactions involved in colour development and not on the sophistication of the equipment. In view of the above discussion, the author proposes to develop the visible Spectrophotometric methods for selected new drugs.

(c) Spectroscopy

Spectroscopy details with the transitions induced in a chemical species by its interaction with the photons of electromagnetic radiation or the measurement and interpretation of Electro Magnetic Radiation (EMR) absorbed or emitted when the molecule or atoms or ions of a sample move from one energy state to another. This change may be from ground state to excited state and vice versa. At ground state, the energy of a molecule is the sum of rotational, vibrational and electronic energy. In other words, spectroscopy measures the changes in rotational, vibrational and/or electronic energies. Spectroscopic techniques are generally employed to measure the energy difference between various molecular energy levels and to determine the atomic and molecular structures.
(i) Ultraviolet spectroscopy

Ultraviolet Spectroscopy is concerned with the study of absorption of UV radiation which ranges from 200 nm to 400 nm. Any molecule has either 'n', 'π' or 'σ' or a combination of these electrons. These bonding (σ and π) and non bonding (n) electrons absorb the characteristic radiation and undergo transition from ground state to excited state. By the characteristic absorption peaks, the nature of the electrons present and hence, the molecule structure can be elucidated.

(ii) Visible spectroscopy (colourimetry)

Colourimetry is concerned with the study of absorption of visible radiation whose wavelength ranges from 400 nm to 760 nm. Any coloured substance will absorb radiation in this wavelength region. Coloured substances absorb light of different wavelengths in differently and hence we get an absorption curve (absorbance Vs wavelength). In this absorption curve, the wavelength (λ) at which maximum absorption of radiation takes place is called as $\lambda_{\text{max}}$. This $\lambda_{\text{max}}$ is characteristic or unique for every coloured substance and this is a qualitative aspect, useful in identifying the substance.

(iii) Laws governing absorption of radiation

- **Beer's law (related to concentration of absorbing species)**
  
  'The intensity of a beam of monochromatic light decreases exponentially with increase in the concentration of absorbing species arithmetically'.

- **Lambert's law (related to thickness/path length of absorbing species)**
  
  'The rate of decrease of intensity of monochromatic light with the thickness of the medium is directly proportional to the intensity of incident light'.
(iv) Terms used in Absorption spectroscopy:

- **Transmittance (T)**: It is the ratio of intensity of transmitted light to that of incident light.

  \[ T = \frac{I_t}{I_0} \]

- **Absorbance (A)**: It is the negative logarithm of transmittance to the base 10.

  \[ A = -\log_{10} T = \log_{10} I_0 / I_t \]

  \[ A = abc \]

- **Molar absorptivity (\(\varepsilon\))**: When concentration ‘c’ in equation \(A = abc\) is expressed in mole/lit and cell length (b) in ‘cm’ then Absorptivity is called as molar absorptivity (\(\varepsilon\)).

  \[ \varepsilon = A / bc \]

- **Beer-Lambert's law**: It can be stated that as the intensity of beam of monochromatic light when passed through transparent medium decreases exponentially as the thickness and concentration of absorbing media increases arithmetically.

  \[ A = \log I_o / I_t = abc \]

Where,

- \(A\) = Absorbance of the solution at particular wavelength of the light beam
- \(I_o\) = Intensity of incident light beam
- \(I_t\) = Intensity of transmitted light beam
- \(a\) = Absorptivity of molecule at the wavelength of beam
- \(b\) = Path length of cell in cm
- \(c\) = Concentration of solution in moles/lit.
Beer's law is said to be obeyed over a concentration range, if a plot of concentration against absorbance passes through origin and is a straight line.

(v) Quantitative analysis by UV / Visible spectrophotometer

Single component analysis

When the absorption of each of a series of solutions of the same substance are measured at the same wavelength, temperature and solvent conditions, a graph of absorbance measured can be plotted against its concentration. If the graph is a straight line passing through the origin, then it is said to obey Beer's law over that concentration range. The slope of the line is equal to "ab", where "b" is the internal path length of the sample cell in cms and "a" is the absorptivity calculated which is constant. The concentration of a component in a sample which contains other absorbing substances may be determined by a simple Spectrophotometric measurement of absorbance as described above, provided that the other components have a sufficiently small or negligible absorbance at the wavelength of measurement. Once this is determined, the analysis of known samples of this substance can be easily done under the same experimental conditions. The absorbance is measured and from the Beer’s plot, the unknown concentration can be calculated.

(vi) Points to be considered before devising new analytical methods are

- Literature Survey

Existing analytical methods for the compound to be analyzed are scanned to avoid duplication of the method. Further, the information about the solubility, absorbance maxima and the molar absorptivities in various solvents of the individual component formulation are obtained.
• **Selecting a solvent**

Solvent mixtures, in which all the components in the formulation are soluble and stable, are chosen. Another point that needs consideration in selecting the solvent is the difference in absorbance maxima of the component in the particular solvent. Greater the difference in the absorbance, better the result.

• **Selecting the sampling wavelength**

Sample wavelengths are selected considering the peaks and valleys in the UV Spectra of the individual component and the other wavelengths in the various components show a difference in the absorbance.

• **Sample analysis and calculations**

The concentration of the sample solution is adjusted such that the absorbance at the wavelength in the scanning region is in the range of 0.5-1.5 Abs units. The analysis is repeated and the accuracy, and reproducibility, confirmed.

• **Type of instrument**

It is the crux of the analytical method because the more advanced the instrument, the greater the accuracy of results and hence, the confidence with which the results are reported.

• **Evaluation of reproducibility**

To ensure proper conditions prevail and no important variables are overlooked, a tentative method is to be critically evaluated with respect to Beer’s law.
(vii) Statistical Analysis

Statistical procedures and representative calculations

The consistency and suitability of the developed method is substantiated by means of statistical analysis utilizing statistical tools such as standard deviation, relative standard deviation and theoretical plates per meter.

- **For Accuracy**
- **Standard Deviation**

\[ \sigma = \sqrt{\frac{\sum (x - x_i)^2}{n-1}} \]

Where,

- \( x \) = Sample
- \( x_i \) = Mean value of samples,
- \( n \) = Number of samples

- **Relative Standard Deviation**

\[ \text{Relative Standard Deviation} = \frac{\sigma}{x_i} \times 100 \]

- **Molar extinction coefficient** (mol\(^{-1}\) cm\(^{-1}\))

\[ \text{Molar extinction coefficient} = \frac{A}{C \times L} \]

Where,

- \( A \) = Absorbance of drug
- \( C \) = Concentration of drug
- \( L \) = Path length

- **Sandell’s sensitivity** (\( \mu g / cm^2 / 0.001 \text{ absorbance units} \))

\[ \text{Sandell’s sensitivity} = \frac{C}{A \times 0.001} \]

Where,

- \( C \) = Concentration of drug
- \( A \) = Absorbance of drug
- **Coefficient of variance** ($\sigma$) = $\frac{\sum(x- \bar{x})^2}{n-1}$
- **Regression equation** $y = a + bx$
- **Slope** = $\frac{y}{x}$

Where,

$x$ = Concentration

$y$ = Absorbance

$a$ = Intercept

- **Limit of detection**: ($D_L$) = $3.3 \times \sigma / S$

Units- ($\mu g / ml$)

Where,

$\sigma$ = Standard deviation of the response.

$S$ = Slope of the calibration curve.

The slope ‘$S$’ may be estimated from the calibration curve of the analyte.

The estimate of $\sigma$ may be carried out in a variety of ways.

- **Limit of quantitation** ($Q_L$) = $10 \times \sigma / S$

Units- ($\mu g / ml$)

Where,

$\sigma$ = Standard deviation of the response

$S$ = Slope of the calibration curve

The slope ‘$S$’ may be estimated from the calibration curve of the analyte.

**(viii) Methodology in UV-Visible Spectrophotometry**

In developing a quantitative method for determining an unknown concentration of a given substance by absorption spectrophotometry, the first step is
the selection of analytical wavelength at which absorption measurements are made. The analytical wavelength can be chosen either from literature or experimentally by recording the absorption spectrum. In order to enhance the sensitivity of the method and signal to noise ratio, the wavelength of maximum absorbance is chosen as analytical wavelength. Absorption spectrum is a graphical representation of the amount of light absorbed by a substance at definite wavelengths. After selection of the analytical wavelength, the next important aspect is the chromogenic reagent and the absorbing product must be stable for a considerable period of time.

**Optimization of experimental conditions of analytical method**

In each reaction, the absorbance of coloured species formed or the final colour (absorbance) of the reaction mixture is measured. Thus, the sensitivity of the method, rate of colour formation and stability is affected by the concentration of the reagent in the solution, nature of solvent, temperature, pH of the medium, order of addition of reactants and intervals between additions. For simple systems having no interaction between variables, one variable at a time (OVAT) is simple, efficient and effective to establish optimum conditions. The OVAT approach requires all variables but one to be held constant, while a univariate study is carried out on the variable of interest.

**Selectivity of the method**

The determination of an analyte may be disturbed by matrix and interference effects. Some of the excipients, incipients and additives present in pharmaceutical formulations may sometimes interfere in the assay of drug and in such cases appropriate separation procedure is to be adopted initially. The selectivity of the method is determined by studying the effect, of a wide range of excipients and other additives usually present in the pharmaceutical formulations under optimal conditions.
Initially, interference studies are carried out by the determination of fixed concentration of the drug several times by the optimum procedure in the presence of a suitable (1 - 100 fold) molar excess of the foreign compound under investigation and its effects on the absorbance of the solution is investigated. The foreign compound is considered to be non-interfering at these concentrations, if it produces an error of less than ±3.0% in the absorbance produced in pure solution.

**Linearity and Sensitivity of the method**

Knowledge of the sensitivity of the colour is important and the following terms are commonly employed for expressing sensitivity.

According to Beer- Lambert's law, \( A = e \cdot b \cdot c \)

Where,

- 'e' is a constant called molar absorptivity and depends on the nature of the absorbing species and wavelength.
- 'b' is the thickness of the medium, usually kept constant at 1 cm.
- 'c' is the concentration of the analyte in moles/lit.

Thus, the absorbance (A) is proportional to the concentration (c) of the absorbing species. Beer's law limits and \( \varepsilon_{\text{max}} \) values are expressed as \( \mu g \text{ ml}^{-1} \) and 1 \( \text{mol}^{-1} \text{ cm}^{-1} \).

The sensitivity of the Spectrophotometric methods is often expressed in terms of Sandell's sensitivity, which represents the number of micrograms of the determine and per mole of a solution having an absorbance of 0.001 for a path length of 1 cm. Sandell's sensitivity is expressed as \( \mu g \text{ cm}^{-2} \).
(ix) Precision and Accuracy for Spectrophotometric methods

The purpose of carrying out a determination is to obtain a valid estimate of a 'true' value. Precision and accuracy together determine the error of an individual determination. They are the most important criterion for evaluating an analytical procedure.

**Precision**

Precision refers to the reproducibility of measurement within a set, that is, to the scatter of dispersion of a set about its mean value. The term 'set' is defined to be 'referring to a number (n) of independent replicate measurements of a property'. One of the most important statistical terms employed is the standard deviation.

Standard deviation is the square root of the sum of squares of deviations of individual results for the mean, divided by one less than the number of results in the set. The standard deviation $S$, is given by

$$S = \sqrt{\frac{1}{n-1} \sum_{i=1}^{n} (x_i - \bar{x})^2}$$

Standard deviation ‘$S$’ has the same units as that of the property being measured.

The square of standard deviation is called Variance ($S^2$). The relative standard deviation is the standard deviation expressed as fraction as a percent relative standard deviation. It is a more reliable expression of precision.

% Relative standard deviation  = $S \times 100/ \bar{x}$
**Accuracy**

Accuracy normally refers to the difference between the mean $\bar{x}$, of the set of results and the true value for the quantity measured. According to IUPAC accuracy relates to the difference between a measured value and the true value. For analytical methods, there are two ways of expressing the accuracy, absolute method and comparative method.

**Absolute method**

Taking different amounts of the analyte and proceeding according to specified instructions the test of accuracy of the method is carried out. "The difference between the mean of an adequate number of measurements and the amount of analyte actually present, usually expressed as parts per hundred (%) and is termed as % error. The constituents in question will be determined in the presence of other substances. This will be necessary to know the effect of the constituents upon the determination. This will require testing the influence of a large number of probable compounds in the chosen samples, each in varying amounts. In a few instances, the accuracy of the method is controlled by separations (usually solvent extraction or chromatography technique) involved.

**Comparative method**

In the analysis of pharmaceutical formulation samples of desired composition, the content of the analyte sought is determined by two or more (proposed and official or reference methods) accepted as “accurate” methods. These methods are usually accepted as indicative of the absence of an appreciable determinate error. The general procedure for the assay of commercial samples either in the proposed or reference
methods comprises various operations that include sampling preparation of solutions, separation of interfering ingredients, if any, and the method for quantitative assay.

**Evaluation of precision and accuracy by comparison of two procedures**

The accuracy of a method is often evaluated by comparing the results of the method under investigation with the results obtained from a known accurate method called reference method.

**Students ‘t’ test**

Student t-test involves comparison of the means of two related (paired) samples analysed by reference and test methods. It gives answer to the correctness of the null hypothesis with a certain confidence such as 95 % or 99 %. If the number of pairs \( n \) are smaller than 30, the condition of normality of \( x \) is required or at least the normality of the difference \( (d_i) \). If this is the case the quantity.

\[
t = \frac{d_i}{S_d / \sqrt{n}}
\]

has a student t - distribution with \( (n-1) \) degrees of freedom, where \( d_i = x_r \) (Reference method) – \( x_t \) (Test method) and \( S_d \) is the standard deviation.

**Applications of UV-Visible Spectroscopy**

- UV-Visible Spectroscopy has been mainly applied for the detection of functional groups (chromophore), the extent of conjugation, detection of polynuclear compounds by comparison, detection and study of chromophores, etc.
- Colourimetry method can be applied to highly coloured solutions which could not be determined by the usual visible indicators.
• Photometric method can also be used for precipitation titrations. The suspended particles diminish the radiant power by scattering. Hence titrations are carried to a condition of constant turbidity.

• The most significant application of UV spectroscopy is to study the extent of configuration, distinction between conjugated and non-conjugated compound, study of geometrical isomerism, study of tautomerism, study of structural feature in different solvents, distinction between equatorial and axial conformations.

• It is used for determination of molecular weight, detection of impurities and identification of unknown compounds etc.

• The UV/Visible spectrophotometer may be used as a detector for HPLC. The presence of an analyte gives a response which can be assumed to be proportional to the concentration.