UNIT I

1

Introduction to Pharmaceutical Analysis

DEFINITION

Pharmaceutical analysis is analytical chemistry that deals with process or series of processes to identify and quantify the drug or formulation and purification of substances, separation of the components from mixture or solutions, and determination of structure of chemical compounds.

SCOPE

Pharmaceutical analysis has wide scope in India because of having vast and growing pharmaceutical industries. Pharma industries require both qualitative and quantitative analysis to ensure the quality of raw materials and final product that meets certain specifications. Gardiner said "Analytical science is the broader umbrella, of which pharmaceutical analysis is a part.'' At every stage of the drug development process, the pharmaceutical analyst is called upon to use a range of techniques to characterize the constituents in a variety of samples collected during drug trials.

The job avenues for a pharmacist are in pharmaceutical industries, in the government departments as drug inspector or government analyst, in research and development sector, in academics, etc. The pharmaceutical field needs pharmaceutical analyst to check the quality of new drug entity and ensure the finished product in required specifications.

Further, geographical surveys require analysis to determine the composition of soil sample and numerous rock samples collected from the field. Most of the industrial processes give rise to pollutants which may cause health-related problems. So quantitative analysis of air, water and soil sample should be carried out to determine the level of pollution and to establish the safe limits for pollutants. To perform such analysis, the fundamental concept of analysis should be cleared and handling of analytical devices and skill is needed. And hence analytical chemistry helps to do all these experiments.

Duties and responsibilities of drug inspector or government analyst are to make sure that the manufactured and sold drugs are standard, safe and of good quality. Food and cosmetic industries are also open the doors of jobs for an analyst or one can employed within any other healthcare industry which requires the assurance of drug products for safety. Number of pharmacy graduates, postgraduates and doctorates are highly placed in the various departments of pharma industries, and or placed in reputed private and government institutes.

TECHNIQUES OF ANALYSIS

There are main two types of chemical analysis.

1. Qualitative (identification)
2. Quantitative (estimation)

Qualitative Analysis

Qualitative analysis gives an indication of the identity of the chemical species in the sample. This method is performed to establish composition of natural or synthetic substances. It gives the information whether the substance or compound is present in the sample or not. Qualitative analysis includes detection of evolved gas, formation of precipitates, limit tests; color change reactions, melting point and boiling point test, etc.

Melting point: The temperature at which a solid melts is known as the melting point. It is a physical property of solid substances. This method is used to identify substances.

Melting point determination is a simple, fast and economic method used to obtain a first impression of the purity of a substance. Pure crystalline substances give a clear, sharp melting point. This method can identify purity of compound as small amount of impurities present in sample can change the melting point, or enlarge its melting range. The capillary method is a standard method used for determination of melting point of a substance. In this method, thin glass capillary tubes filled with sample is tied to thermometer and placed in Thieles tube containing liquid paraffin and heat is provided until the substance is completely melted and melting point is noted.

Boiling point: Boiling point is the temperature at which the vapor pressure of a liquid equal to the atmospheric pressure or some other applied pressure. Boiling point is physical property of liquids for indicating the purity of solvents or liquids. The boiling point of a liquid varies depending upon the surrounding environmental pressure. A liquid in a partial vacuum has a lower boiling point than when that liquid is at atmospheric pressure.

Refractive index: Refractive index, also called index of refraction is a specific property of light. It gives information regarding behavior of light. The refractive index is measure of the bending of a ray of light when passing from one medium into another.

When light pass through any substance its velocity decreases and refractive index of increases due to the effect of interaction between molecules of substances on light. Refractometer is a device which is used to measure the refractive index of substances.

Mainly four types of refractometers are used to measure refractive index.

1. Traditional handheld refractometers
2. Digital handheld refractometers
3. Laboratory or Abbe refractometers
4. In-line process refractometers.

Rayleigh refractometer is also used (typically) for measuring the refractive indices of gases. A sodium lamp may be used to provide the light source at a known wavelength.

a. **Optical rotation:** Optical rotation or optical activity is the rotation of the plane of polarization of linearly polarized light as it travels through certain materials. Optical activity occurs only in chiral materials, those lacking microscopic mirror symmetry. Optical activity can be observed in fluids. This can include gases or solutions of chiral molecules, such as sugars, some proteins, and also chiral liquid crystals. The substance which rotates plane of polarization of light towards right or in clockwise direction are called **dextrorotatory** or right handed.

   The substance which rotates plane of polarization of light towards left or in anticlockwise directions are called **laevorotatory** or left handed. Polarimeter is used to measure the optical activity. To measure the sugar concentration in sugar industries, the polarimeter is used. In analytical chemistry, to measure the concentration or enantiomeric ratio of chiral molecules in solution polarimeter is used.

b. **Viscosity:** Viscosity is a measure of the resistance of a fluid which is being deformed by either shear stress or tensile stress. For example, honey, glycerin contains high viscosity while water is having low viscosity. Viscosity describes a fluid's internal resistance to flow and hence measure of fluid friction.

Quantitative Analysis

These techniques are mainly used to quantify any compound or substance in the sample or to determine the amount of each compound present in the sample.

Types of Quantitative analysis:

1. Chemical methods
   a. Volumetric or titrimetric methods
   b. Gravimetric methods
   c. Gasometric analysis
2. Electrical methods
3. Instrumental methods
4. Biological and microbiological methods.

1. Chemical Methods

a. Volumetric or titrimetric method: This method involves the measurement of volume of a solution of known concentration which is used to determine the concentration of the analyte. Volumetric method is a simple method and requires less apparatus and they are susceptible of high accuracy.

Basic terms involves in Titrimetric or volumetric method.

1. **Titrate:** A substance is to be titrated is called titrate.
2. **Titrant:** A solution of known concentration is called titrant. It is placed in burette.
3. **Indicator:** Indicator is a substance that show color change after completion of chemical reaction between titrate and titrant. It depends upon pH of solution.
4. **End point or equivalence point or stoichiometric point:** The equivalence point is the point in a titration at which the amount of titrant added is completely neutralizes analyte in solution.
5. **Standard solution:** A solution of known concentration is called standard solution.

Various types of **titrimetric methods** are:

i. Acid–base titrations (neutralization reactions)
ii. Complexometric titrations
iii. Precipitation titrations
iv. Oxidation reduction titrations
v. Non-aqueous titrations.

b. Gravimetric methods: It is quantitative method used to determine the mass of analyte. The analyte first is converted into an insoluble precipitate in the purest form, and then collected, dried and weighed. It is the time consuming process. In electrogravimetry, electrolysis of the sample is carried out on the electrodes is weighed after drying. In volatilization gravimetry, the analyte is separated from other constituents of a sample by conversion to a gas. Thermogravimetry (TG) records the change in weight, differential thermal analysis (DTA) records the difference in temperature between test

substance and an inert reference material, differential scanning calorimetry (DSC) records the energy needed to establish a zero temperature difference between a test substance and reference material.

c. Gasometric analysis: Gasometry involves measurement of the volume of gas evolved or absorbed in a chemical reaction. Examples of gases which are analyzed by gasometry are cyclopropane, carbon dioxide, ethylene, N_2, helium, etc.

2. Electrical Methods

Electrical methods of analysis involve the measurement of electric current, voltage or resistance in relation to the concentration of some species in the solution.

Electrical methods of analysis include:

a. Potentiometry

b. Conductometry

c. Polarography

d. Voltametry

e. Amperometry.

Potentiometry measures electrical potential of an electrode in equilibrium with an ion to be determined. Conductometry measures electrical conductivity of an electrode with a reference electrode while polarography, voltametry and amperometry measures electrical current at a microelectrode.

3. Instrumental Methods of Analysis

Instrumental methods are used to determine the concentration of component in sample. This method is employed to measure physical properties of substances like conductance, pH, fluorescence, absorption; transmittance, etc. Instrumental methods are preferred due to their selectivity, high speed, accuracy and simplicity of analysis.

Spectroscopic methods of analysis depend upon measurement of electromagnetic radiation of a particular wavelength emitted by the sample. Spectroscopic methods are

atomic absorption spectroscopy, atomic emission spectroscopy, ultraviolet-visible spectroscopy, X-ray fluorescence spectroscopy, infrared spectroscopy, Raman spectroscopy, nuclear magnetic resonance spectroscopy, photoemission spectroscopy.

Emission methods: Emission is the process by which a higher energy state of molecules converted to a lower state by the emission of a photon. Each element emits a characteristic set of discrete wavelengths according to its electronic structure, and by observing these wavelengths the elemental composition of the sample can be determined. Emission methods are based upon the measurement of these emitted radiations of molecules. Emission methods include emission spectroscopy, flame photometry, fluorimetry, etc.

Chromatographic techniques and electrophoretic methods These are methods of identification and separation of components from mixture. Examples of chromatographic techniques are gas chromatography, high performance liquid chromatography, thin layer chromatography, paper chromatography, column chromatography, etc.

Mass spectrometry involves vaporization of material using a high vacuum and the vapor is bombarded by a high energy electron beam. Vapor molecules undergo fragmentation to produce ions of varying size. These ions are differentiated by accelerating them in electrical field and then deflecting them in a magnetic field. Each kind of ion gives a peak in the mass spectrum.

4. Biological and Microbiological Methods

Biological methods are used when potency of a drug or its derivative cannot be properly determined by any physical or chemical methods. They are called bioassays. Microbiological methods are used to observe potency of antibiotic or antimicrobial agents. In antimicrobial assay, inhibition of growth of bacteria of the sample is compared with that of the standard antibiotic. These methods include cup plate method and turbidimetric analysis.

METHODS OF EXPRESSING CONCENTRATION

Percent Concentration

1. **Mass percentage:** The mass percentage of a component in a given solution is the mass of the component per 100 g of the solution. It is represent as %w/w

$$\text{Mass percentage of solute in solution} = \frac{\text{Mass of solute}}{\text{Mass of solution}} \times 100$$

2. **Volume percentage:** The volume percentage is defined as the volume of the solute per 100 parts by volume of solution. It is represent as %v/v

$$\text{Volume percentage of solute in a solution} = \frac{\text{Volume of solute}}{\text{Volume of solution}} \times 100$$

3. **Mass per Volume percentage:** Strength of a solution is defined as the amount of the solute in gms, present in 100 parts of the solution. It is expressed as % w/v

$$\text{Mass per volume percentage} = \frac{\text{Mass of solute in gram}}{\text{Volume of solution}} \times 100$$

4. **Normality:** The normality of a solution is the number of gram-equivalent weights of the solute contained in one liter of the solution.

$$\text{Normality of a solution} = \frac{\text{Number of gram-equivalent weights of solute}}{\text{Liter of solution}}$$

5. **Molarity:** The molarity of a solution is the number of moles of the solute contained in one liter of the solution.

$$\text{Molarity of a solution} = \frac{\text{Number of moles of solute}}{\text{Liter of solution}}$$

6. **Molality:** The molality of a solution is the number of moles of the solute per 1000 gm of solvent.

$$\text{Molality of a solution} = \frac{\text{Number of moles of solute}}{\text{Number of Kilograms of solvent}}$$

7. **Formality:** The formality of a solution is the number of gram-formula weights of the solute contained in one liter of the solution.

$$\text{Formality of a solution} = \frac{\text{Number of gram-formula of the solute}}{\text{Number of liter of solution}}$$

8. **Mole fraction:** The mole fraction of one component in a solution is defined as the number of moles of that component divided by the total number of moles of all components in the solution.

$$\text{Mole fraction of any component} = \frac{\text{Number of moles of that component}}{\text{Total number of moles of all components}}$$

9. **Parts per billion:** It is denotes as ppb. These are applicable when solute concentration is very small.

$$\text{ppb} = \frac{\text{Mass of solute}}{\text{Mass of solution}} \times 10^9$$

10. **Parts per million:** It is denoted as ppm. These are applicable when solute concentration is very small.

$$\text{ppm} = \frac{\text{Mass of solute}}{\text{Mass of solution}} \times 10^6$$

PRIMARY STANDARDS

A **primary standard** is a pure substance may be dissolved in a known volume of solvent to give a **primary standard solution**.

- **Properties of Primary Standards**
  a. High level of purity
  b. Low reactivity (high stability)
  c. High equivalent weight
  d. Nonhygroscopic
  e. Nontoxic.
  f. Inexpensive and readily available.

- **Examples of Primary Standards**
  - **a.** Sodium chloride (NaCl) is used as a primary standard for silver nitrate reactions.
  - **b.** Zinc powder may be used to standardize EDTA solutions after it has been dissolved in hydrochloric acid or sulfuric acid.
  - **c.** Potassium hydrogen phthalate or KHP may be used to standardize perchloric acid in an acetic acid solution.
  - **d.** Potassium bromate ($KBrO_3$) for standardization of sodium thiosulfate solutions.
  - **e.** Sodium carbonate for standardization of hydrochloric, sulfuric acid and nitric acid solutions.

SECONDARY STANDARD

A secondary standard is a standard that is prepared in the laboratory for calibration of control material which is required for analysis of unknown concentration of a substance. It is usually standardized against a primary standard. Generally volumetric solutions are known as secondary standards.

Examples: HCl, H_2SO_4, NaOH, KOH, $KMnO_4$, etc. are the secondary standard substances and solutions prepared from such substances are called secondary standard solutions.

Properties of Secondary Standards

a. It is less pure than primary standard

b. Less stable and more reactive than primary standard

c. Titrated against primary standard.

PREPARATION AND STANDARDIZATION OF SOLUTIONS

Oxalic Acid ($C_2H_2O_4.2H_2O$) (Mol. Wt: 126.065)

Preparation of 0.1 N Oxalic Acid

Procedure: Weigh accurately 6.3 g $(COOH).2H_2O$ and transfers it to a volumetric flask (1 liter), half-filled with distilled water. Shake well and make the volume up to the mark. Label it as 0.1 N oxalic acid solution.

Note: If anhydrous oxalic acid (COOH) is available then dissolve 4.5 g of the acid in one liter of distilled water to get 0.1 N oxalic acid solution.

Standardization of Oxalic Acid

Pipette 25 ml of 0.1 M NaOH and transfer it into clean conical flask. Add 2 drops of phenolphthalein indicator and titrate with 0.1 N oxalic acid solution until color changes from purple to colorless.

Factor: Each ml of 0.1 N *oxalic acid* is equivalent to 0.0040 g of NaOH.

Principle Reaction

$$H_2C_2O_4 + 2NaOH \rightarrow 2H_2O + Na_2C_2O_4$$

Sulfuric Acid (H_2SO_4) (Mol. Wt. 98.079)

Preparation of 0.5 M Sulfuric Acid

Add slowly, with stirring, 30 ml of sulfuric acid to about 1000 ml of *water*, allow to cool 25° and standardize against sodium carbonate.

Standardization of Sulfuric Acid

Weigh accurately about 1.5 g of anhydrous sodium carbonate, previously heated at about 270° for 1 hour. Dissolve it in 100 ml of *water* and add 0.1 ml of methyl red solution. Add the acid slowly from a burette, with constant stirring, until the solution becomes faintly pink. Heat the solution to boiling, cool and continue the titration. Heat again to boiling and titrate further as necessary until the faint pink color is no longer affected by continued boiling.

Factor: 1 ml of *0.5 M* Sulfuric acid is equivalent to 0.05299 g of Na_2CO_3.

Principle reaction:

$$Na_2CO_3 + H_2SO_4 \rightarrow Na_2SO_4 + H_2O + CO_2$$

Sodium Hydroxide (NaOH) (Mol. Wt. 40)

Preparation of 0.1 N Sodium Hydroxide

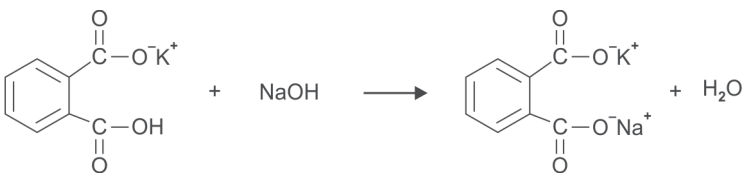
Procedure: Weigh accurately 4 g NaOH in a beaker (as it is hygroscopic) and dissolve it in distilled water (preferably CO_2 free). Transfer the contents and the washings to a volumetric flask (1 liter). Cool and then make volume up to the mark.

Standardization of Sodium Hydroxide

Weigh accurately about 5 g of potassium hydrogen phthalate, previously powdered and dried at 120° for 2 hours, and dissolve in 75 ml of carbon dioxide-free water. Add 0.1 ml of phenolphthalein solution and titrate with the sodium hydroxide solution until a permanent pink color is produced.

Factor: 1 ml of 0.*1 M sodium hydroxide* is equivalent to 0.02042 g of $C_8H_5KO_4$.

Principle reaction:



Hydrochloric Acid (HCl) (Mol.Wt. 36.5)

Preparation of 1M Hydrochloric Acid Solution

Procedure: Measure 85 ml of 37% hydrochloric acid and dilute to 1000 ml with water.

Standardization of Hydrochloric Acid

Weigh accurately about 1.5 g of anhydrous sodium carbonate, previously heated at about 270° for 1 hour. Dissolve it in 100 ml of water and add 0.1 ml of methyl red solution. Add the acid slowly from a burette, with constant stirring, until the solution becomes faintly pink. Heat the solution to boiling, cool and continue the titration. Heat again to boiling and titrate further as necessary until the faint pink color is no longer affected by continued boiling.

Factor: 1 ml of 1 M Hydrochloric acid is equivalent to 0.05299 g of Na_2CO_3.

Principle reaction:

$$Na_2CO_3 + 2HCl \rightarrow NaCl + H_2O + CO_2$$

Sodium Thiosulfate ($Na_2S_2O_3$) Mol.Wt. 158.11

Preparation of 0.1 M Sodium Thiosulfate Solution

Procedure: Dissolve 25 g of sodium thiosulfate and 0.2 g of sodium carbonate in carbon dioxide-free water and dilute to 1000 ml with the same solvent.

Standardization of Sodium Thiosulfate Solution

Dissolve 0.200 g of potassium bromate, weighed accurately, in sufficient water to produce 250.0 ml. To 50.0 ml of this solution add 2 g of potassium iodide and 3 ml of 2 M hydrochloric acid and titrate with the sodium thiosulfate solution using starch solution, added towards the end of the titration, as indicator until the blue color is discharged.

Factor: 1 ml of 0.1 M sodium thiosulfate is equivalent to 0.002784 g of KBrO3.

Principle reaction:

$$KBrO_3 + 5KI + 6\ HCl \rightarrow 3I_2 + 6\ KCl + 3\ H_2O$$

$$I_2 + 2Na_2S_2O_3 \rightarrow 2NaI + Na_2S_4O_6$$

Potassium Permanganate ($KMnO_4$) (Mol.Wt.158.034)

Preparation of 1 M Potassium Permanganate Solution

Procedure: Dissolve 158 g of potassium permanganate in 900 ml of water, heat on a water-bath for 1 hour, cool, filter through a sintered-glass filter and add sufficient water to produce 1000 ml.

Standardization of Potassium permanganate

To 25.0 ml of the solution in a glass-stoppered flask add 2 g of potassium iodide, followed by 10 ml of 1 M sulfuric acid.

Titrate the liberated iodine with 0.1 M sodium thiosulfate, using 3 ml of starch solution, added towards the end of the titration, as indicator. Perform a blank determination and make necessary correction.

Factor: 1 ml of 0.1 M sodium thiosulfate is equivalent to 0.003161 g of $KMnO_4$.

Principle reaction:

$$2KMnO_4 + 10KI + 16H^+ \rightarrow 2Mn^{2+} + 5I_2 + 8\ H_2O$$
$$I_2 + 2Na_2S_2O_3 \rightarrow 2NaI + Na_2S_4O_6$$

CERIC AMMONIUM SULFATE ($Ce\ (NH_4)_4(SO_4)_4.2H_2O$)

(Mol. Wt. 632.55)

Preparation of 0. 1M Ceric ammonium sulfate Solution

Procedure: Dissolve 65 gm of ceric ammonium sulfate with gentle heat in a mixture of 30 ml sulfuric acid and 500 ml of water. Cool, filter the solution if turbid and dilute to 1000 ml with water.

Standardization of Ceric Ammonium Sulfate

Weigh accurately about 0.2 gm of arsenic trioxide previously dried at 105° about an hour and transfer to a 500 ml conical flask. Wash down the inner walls of the flask with 25 ml of 8% w/v of sodium hydroxide solution, swirl to dissolve, add 100 ml of water and mix thoroughly. Then add 30 ml of dil. sulfuric acid, 0.15 ml of osmic acid solution, 0.1 ml of ferroin sulfate solution as indicator. Slowly titrate with ceric ammounium sulfate solution until pink color changes to very pale blue or yellowish green color, adding the titrant slowly towards the end point.

Factor: 1 ml of 0.1 M ceric ammonium sulfate is equivalent to 0.004946 g of As_2O_3.