Chapter

1

Pharmaceutical Analysis: Definition, Different Techniques of Analysis and Scope

Chemicals as nutrition or medicines are routinely used by humans for survival. How much and what is consumed is determined by the subject, analytical chemistry. The concerned subject, pharmaceutical analysis, is a version of analytical chemistry which evaluates the drugs both as bulk drug substances and as dosage formulations.

Various analytical chemistry methods are broadly categorised into two types:

1. **Qualitative analysis:** This type of method determines, what is present; means what constituent or substance is present in the sample. This can be determined either by physical methods or chemical methods at preliminary level. Physical methods include determination of boiling/melting point, refractive index, specific rotation, solubilities. Chemical method generally includes the chemical reactions. The analyte, which is to be qualitatively determined, is reacted with reagent to obtain the product that is either known or could be easily distinguished based on colors, boiling/melting point, refractive index, smell, etc.
2. **Quantitative analysis:** This determines the quantity. It means, how much amount of individual substance or constituent is present in the sample. Various techniques are used for the quantitative analysis, which are illustrated in the coming text.

DIFFERENT TECHNIQUES OF ANALYSIS

Various techniques used for qualitative as well as for quantitative analysis are introduced as follows:

A. Classical Methods

Methods under this category determine the change in weight or volume. Some examples of classical methods are given here.

1. Volumetric Methods

These methods are simple and precise in comparison to other methods. These involve the determination of volume of titrant required to react with analyte (which is to be analysed or determined). Based on the type of reaction, these are further classified into four basic methods as follows:

a. ***Neutralization titrations or acid–base titrations:*** In this type of titration, either acidic substance (analyte) is analysed by titration with base (titrant) or basic substance (analyte) is analysed by titration with acid (titrant). In both types, resulting product is salt and water.

Measurement of strength of acidic substances (by titration with base) is called **acidimetry.** In contrast, **alkalimetry** is measurement of strength of base (analyte) by titrating against a standard solution of acid.

Neutralization titrations are of two types:

i. **Non-aqueous titrations:** These are performed in non-aqueous solvent, means other than water. Glacial acetic acid, alcohol, dimethylformamide, etc are some examples of solvents, used in non-aqueous titration. These are used when:
   a. Analyte is insoluble in water or
   b. Either weak acid or weak base in comparison to water (amphoteric), making difficult to observe the endpoint with the use of indicator.

ii. **Aqueous titrations:** These types of titrations are performed in water (aqueous solvent). These are preferred for ionic analyte which easily dissociated completely. The endpoint is determined with the help of indicator by observing change in color.

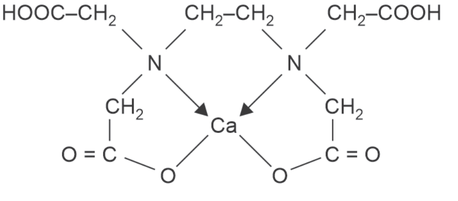
b. ***Precipitation or argentometric titrations:*** In these types of titrations, there is formation of precipitate by the reaction of analyte with precipitate forming reagent. The most common example of determination is analysis of sodium chloride. The estimation of sodium chloride is based on presence of halide ion (Cl^-). This reacts with standard silver nitrate solution (precipitate forming reagent) to form insoluble silver chloride (white colored precipitate). The endpoint is determined with the use of indicator. Here, potassium chromate is used which yields brownish-red colored precipitate at the endpoint.

$$\underset{\substack{\text{Sodium chloride}\\ \text{(Analyte)}}}{NaCl} + \underset{\substack{\text{Silver nitrate}\\ \text{(Reagent)}}}{AgNO_3} \longrightarrow \underset{\substack{\text{Silver chloride}\\ \text{(White precipitate)}}}{AgCl\downarrow} + NaNO_3$$

These types of titrations are used for analysis of halide ions (chloride, bromide, iodide), divalent anions (S^{2-}) **and** mercaptans (e.g. CH_3SH). Endpoint is easily determined by either observing the appearance/disappearance of turbidity or with use of indicator which yields different colored precipitate.

c. ***Complexometric titrations:*** These involve the formation of complex or chelate (complexation) on reaction with complex forming reagent. The sodium salt of ethylene-diaminetetraacetic acid (EDTA) is most common complex forming reagent. This contains several electron donating groups to form covalent bonds with metallic ions (e. g. magnesium, zinc, calcium, etc). Thus, it has a ability to form complex with several metallic ion like calcium. The structure of calcium–EDTA complex is given below. The endpoint is determined with the use of metallic indicator, like eriochrome black T.

Complexometric titrations are widely used to measure the various metallic ions, like calcium, aluminium, magnesium, zinc, etc.



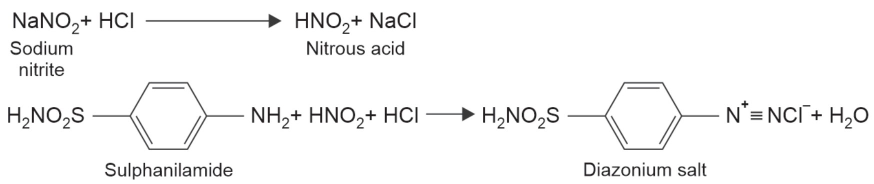
Calcium–EDTA complex

d. ***Oxidation–reduction titrations:*** These are also referred to as redox titrations due to involvement of oxidation–reduction reaction. It is important to remind that oxidation is loss of electron while reduction is gain of electron. By this type of titrations, reducing or oxidising substances can be determined. For example, ferrous sulphate (reducing substance) can be determined by titration with potassium permanganate (oxidising substance.)

$$\underset{\substack{\text{Pot. Permanganate}\\\text{(Oxidising agent)}}}{2KMnO_4} + \underset{\substack{\text{Ferrous sulphate}\\\text{(Reducing agent)}}}{10FeSO_4} + 8H_2SO_4 \longrightarrow 2MnSO_4 + K_2SO_4 + 8H_2O + \underset{\text{Ferric sulphate}}{5Fe_2(SO_4)_3}$$

In this case ferrous sulphate is oxidized into ferric sulphate in acidic media and endpoint is determined by appearance of pink color due to slight excess of potassium permanganate (self-indicator).

e. ***Diazotisation titration:*** These types of titrations are specific to drug/analyte having primary amino group. The primary amino group in drug like sulphanilamide form the diazo compound on titration with nitrous acid (generated from sodium nitrite on acidification). The endpoint is determined by using starch paper. The overall reaction is given here:



2. Gravimetric Technique

It involves the determination of weight as a parameter for analysis. In one of the techniques, analyte is determined by extraction with solvent which is then evaporated to dryness to constant weight. This weight is then determined for quantitative analysis.

In another technique, analyte is converted into the precipitate (insoluble form) by adding precipitate forming reagent. The weight of precipitate is co-related with the actual analyte for quantitative analysis. For example, sodium sulphate (soluble analyte) is analysed by adding barium chloride solution (precipitate forming reagent) to convert it into barium sulphate (precipitate). The weight of barium sulphate is co-related with quantity of sodium sulphate for quantitative analysis.

$$\underset{\substack{\text{Sodium}\\\text{sulphate}}}{Na_2SO_4} + BaCl_2 \longrightarrow 2NaCl + \underset{\substack{\text{Barium}\\\text{sulphate}}}{BaSO_4}\downarrow$$

3. Gasometric Techniques

These are mainly meant for analysis of medicinal gases like carbon dioxide, oxygen, nitrous, etc., in which techniques, volume of gas either absorbed or evolved are measured.

B. Instrumental Methods

These methods are preferred method due to their accuracy, sensitivity, selectivity and high speed. These involve the measurement of changes in physical property. This change is analysed by observing the changes in absorbance, potential, current strength, charge to mass ratio, etc. These are classified as:

1. Electroanalytical Method

These methods involve the measurement of changes in the potential, strength of electric current, etc. The changes in the electrical parameters are co-related with the concentration of analyte.

a. ***Potentiometry:*** It involves the determination of potential difference between two electrodes—indicator electrode and reference electrode. The potential of indicator electrode depends on concentration of analyte while potential of reference electrode is fixed. Hence, change in potential difference is co-related to concentration of analyte in the sample.

b. ***Conductometry:*** It is based on the measurement of conductivity difference. Two ions differ in the conductivity, hence when one type of ions is replaced by another ions, there is a change in conductivity. This change is measured graphically by having the changes in conductance on one axis versus volume of titrant on the other axis.
For example, in the estimation of HCl by conductometric titration with NaOH solution, decrease in the conductance is plotted against the volume of NaOH. At the endpoint, there is a steep rise (Fig. 1.1) in the conductance due to replacement of hydrogen ion by sodium ion.

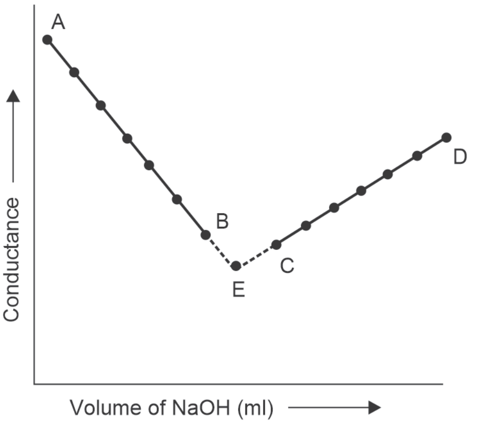


Fig.1.1. Conductometric titration of HCl with NaOH

c. ***Polarography:*** It involves the analysis of reducible or oxidizable substances. In this, analyte is subjected to diffusion-controlled electrolysis using indicator and reference electrode and range of voltage is applied. The diffusion current is measured and co-related to analyte concentration. It is also useful for qualitative analysis with the determination of half wave potential.

d. ***Amperometry:*** It is similar to polarographic technique with only difference is maintenance of constant voltage. The change in diffusion current is recorded on adding the titrant. This change is co-related to concentration of analyte.

2. Spectroscopic Methods

In these methods, intensity of radiant energy of specific wavelength either absorbed or emitted out by the analyte is measured. These are of two types:

a. ***Absorption spectroscopic methods:*** Ultraviolet (UV), infrared (IR) and nuclear magnetic (NMR), atomic absorption (AA), spectroscopic method, etc., are examples of absorption spectroscopy. All of these methods involve the measurement of absorption of energy.

b. ***Emission spectroscopic methods:*** Flame photometry, fluorometry, atomic emission spectroscopy are the examples of emission spectroscopic methods, which analyse the emitted light.

3. Chromatographic Methods

These are basically separation techniques, often utilized for qualitative and quantitative analysis. There are two types of chromatographic techniques: (a) Absorption chromatography: In this, different constituents of a mixture are separated, based on their differential adsorption ability between stationary (absorbent) and mobile (solvent) phase. Column chromatography, thin layer chromatography, gas–solid chromatography, etc., are examples of absorption chromatography, (b) Partition chromatography: Different constituents of a mixture are separated, based on their partitioning abilities (partition coefficient) between two liquid phases. The mobile phases may be either liquid or gas. The paper chromatography, gas–liquid chromatography, etc., are examples of partition chromatography.

4. Electrophoretic Methods

These methods are mostly utilized for analysis of proteins. Different types of proteins/amino acids in a mixture are separated on the basis of their size and charge when allowed to move under the influence of electrical field. Positively and negatively charged molecules migrate toward cathode and anode respectively, which result in separation of individual amino acid mixture.

5. Mass Spectrometric Methods

Mass spectrometry is a sensitive analytical technique, used to identify as well as to quantify unknown compound. In this, sample molecule is fragmented by providing energy (e.g. electron beam); each fragment after separation gives response in the form of peak (mass spectrum). The position and intensity of peak are helpful in the identification and quantification of the compound respectively. Each fragment is characterized by its mass to charge ratios (m/e) and relative abundances.

6. Polarimetric Methods

These techniques are used for optically active compounds, for qualitative and quantitative analysis. The extent of rotation of polarized light is measured, when passed through optically active substance in a polarimeter. The term, saccharimeter is used when sugar solution is analysed. In both saccharimeter and polarimeter, extent of rotation of polarized light is measured.

7. Kinetic Methods of Analysis

These methods are sensitive and simple; use the rate of a chemical or physical process to evaluate an analyte's concentration. In a simple case, where analyte 'X' decomposes to form the product 'Y'.

$$X \longrightarrow Y$$

The progress or rate of reaction can be determined by either measuring the disappearance of reactant or formation of product. It is proportional to either product or reactant, at time 't'. The concentration of analyte or product may be determined by physical (e.g. conductometry) or chemical method

8. Thermal Methods

These methods measure the specific parameter as a function of temperature. Thermogravimetric analysis (TGA), differential thermal analysis (DTA), differential scanning calorimetry (DSC), etc., are some common examples of thermal method of analysis.

9. Hyphenated Techniques

In this, two or more techniques are clubbed together with the aid of interphase to increase the sensitivity and utility. For example, GC-MS, gas chromatography is clubbed with mass spectrometry in which gas chromatography separates out the individual component from a mixture while mass spectrometer (MS) identifies and quantifies the component in a mixture. LC-MS, LC-NMR, LC-MS-MS, GC-NMR, etc., are other examples of hyphenated techniques.

C. Biological and Microbiological Methods

These are only used, when other physical or chemical methods are not applicable, especially when present in a complex mixture. In biological methods, concentration of analyte is determined by its effect on living cells or tissues. For example, vitamins are assayed by measuring the growth of some specific microorganism. In microbiological methods, inhibition of growth of some microorganism is compared with that of standard/reference antibiotic.

SCOPE OF ANALYTICAL CHEMISTRY

Scopes of the analytical chemistry are as follows:

Agricultural Applications

Analytical chemistry is used in testing soil, water and harvested crops. Various nutritional components like carbohydrate, proteins, etc., in the harvested crops can be qualitatively and quantitatively determined. Even caloric value of harvested crop can be determined.

Clinical Applications

Analytical chemistry form the basis of clinical diagnosis to detect various abnormalities in human body. For example, kidney profile is taken by analysing the creatinine, urea, etc. level in the blood to diagnose abnormalities in renal function. Similarly, liver profile is analysed by analysing the level ALT, AST, ALP, etc., in the blood to evaluate the liver functioning. Analytical chemistry is also helpful in monitoring the progress during drug therapy.

Pharmaceutical Applications

Analytical chemistry also check the presence of impurities in the pharmaceutical and indicate the safety of these pharmaceuticals. This increases the safety and efficacy of pharmaceuticals. With the use of classical or non-classical methods, raw materials and formulations can be analysed qualitatively and quantitatively.

Applications in Forensic Science

Analytical chemistry is also helpful in evaluation of biological samples, taken during criminal investigation.

Quality Issues or Product Failures

Sometimes due to presence of toxic substances like lead, off-color or discoloration or bad smell, customer file a complaint which results in shut down of production. For example, Maggi brand noodles was banned in many Indian states due to presence of lead concentration beyond toxic level. This even leads to legal disputes. In these conditions, manufacturer may approach analytical lab for further confirmation.

Applications in Public Health

Public health is a major problem related to water and air pollution. Hence, air also require analysis to check the permitted limit of various noxious substances.

EXERCISE

MCQs

Tick Mark the Appropriate Choice

1. Which one of these is not an electroanalytical method?

a. Potentiometric b. Amperometric
c. Polarographic d. Polarimetric

2. Non-aqueous titrations are performed when drug is ---

a. Weak acid or weak basic in comparison to water
b. Insoluble in water
c. Not reactive
d. Options ′a′ and ′b′

3. In which analytical method, rate of reaction is determined?

a. Kinetic method
b. Thermal method
c. Mass spectrometry
d. Conductometry

4. In which growth of microorganism is observed?

a. Biological method
b. Chromatographic methods
c. Microbiological methods
d. Hyphenated methods

5. Which method can determine the analyte in presence of impurities by observing the effect on living tissues?

a. Biological method
b. Chromatographic methods
c. Microbiological methods
d. Kinetic methods

SHORT AND LONG QUESTIONS

1. What is analytical chemistry? Why is it important in pharmacy?
2. What do you mean by qualitative and quantitative analysis? Explain in brief.
3. What is the concept of complexometric titration? Illustrate it.
4. Discuss the classical method of analysis with appropriate examples.
5. Illustrate the scope of analytical chemistry.
6. What are hyphenated techniques? Give some examples.
7. What are electroanalytical methods? Enlist them.
8. What is measured in thermal methods? Enlist a few thermal method, used in pharmacy.
9. Describe the various methods of volumetric analysis in detail.

ANSWER TO MCQs

1. (d) 2. (d) 3. (a) 4. (c) 5. (a)