Chapter

1

Introduction to Pharmaceutical Analysis

The goal of a pharmaceutical analysis is to either identify or measure one or more compounds in a sample of pharmaceutical relevance. The material or materials of interest in pharmaceutical analysis are often active pharmaceutical compounds, pharmaceutical excipients, pollutants, or drug metabolites. The term 'analyte' refers to a material that has to be recognized or measured. Pharmaceutical raw materials, completed pharmaceutical products, or biological samples such as human blood or urine containing one or more drug compounds are the usual samples used in pharmaceutical analysis. The samples are made up of one or more analytes and the remainder of the sample, known as the sample matrix. Identification is meant to verify the analytes' identities. Qualitative analysis is another name for identification. The goal of a quantitative analysis is to determine the precise amount or concentration of the analyte in a particular sample. Another name for a quantitative analysis is a determination. For instance, before being released from production, 500 mg of paracetamol each tablet must be monitored. Pharmaceutical analysis is used to achieve this. The remainder of the tablet, which is made up of various pharmaceutical excipients, is the sample matrix, while paracetamol is the analyte. While a quantitative analysis is done to quantify the amount of paracetamol and ensure that the result is precisely or nearly 500 mg per tablet, identification of the paracetamol in the tablets is done to ensure that the tablets contain the correct active pharmaceutical ingredient. In the latter scenario, the amount of paracetamol is determined. The figure depicts the different steps in a typical procedure in pharmaceutical analysis (Fig. 1.1).

Pharmaceutical analysis is a domain of practical chemistry that includes a number of procedures for identifying, determining, quantifying, and purifying a drug, separating the constituents of a

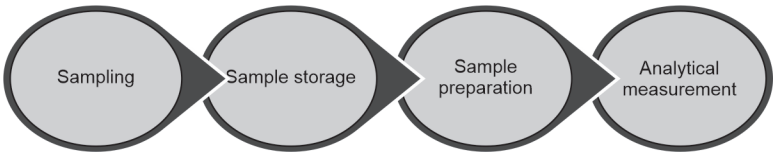


Fig. 1.1: Steps involved in process of pharmaceutical analysis

solution or mixture, or determining out the structure of chemical compounds. The substance could be a single component or a combination of compounds, and it could be in any dose form. Animals, plants, microbes, minerals, and a variety of synthetic materials are all employed as medications. The sample to be analyzed is referred to as an analyze, and it can be categorized as macro (0.1 g or more), semi-micro (0.01 g to 0.1 g), micro (0.001 g to 0.01 g), sub-micro (0.0001 g to 0.001 g), ultramicro (below 10^{-4} g), or trace analysis (100 to 10000 ppm) based on its size. The semi-microanalysis is the most popular of all.

TYPES OF PHARMACEUTICAL ANALYSIS

Two main types of pharmaceutical analysis are there:

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

1. Qualitative

It is carried out to determine the composition of synthetic and natural materials. These tests are carried out to determine whether or not the compound or substance is present in the sample. Detection of evolved gas, precipitate formation, limit tests, color change reactions, melting and boiling point tests, and more are examples of qualitative testing.

2. Quantitative

The primary purpose of these approaches is to quantify any component or compound present in the sample. These methods are based on the following:

a. The quantitative performance of a suitable chemical reaction and either measuring the amount of reagent added to complete the reaction or measuring the amount of reaction product obtained.
b. The characteristic movement of a substance through a defined medium under controlled conditions.

c. Electrical measurement
d. Measurement of some spectroscopic properties of the compound.

Different types of qualitative 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

1. **Chemical methods**:
   a. **Titrimetric (volumetric method):** It involves determining the volume of solution required to complete the reaction by reacting the substance to be determined with a suitable reagent as a reference solution. Volumetric procedures are capable of high precision and need minimal equipment. Titrimetric approaches come in a variety of forms.
      i. Titrations of acid and base (neutralization reactions)
      ii. Titrations using complexometry
      iii. Titrations for precipitation
      iv. Titrations for oxidation reduction
      v. Non-aqueous titrations
   b. **Gravimetric techniques**: In gravimetric analysis, a material to be determined is transformed into the purest form of an insoluble precipitate, which is afterwards obtained and weighed. The process takes a lot of time. In electrogravimetry, the material is electrolyzed on the electrodes and then weighed after drying. Differential thermal analysis (DTA) measures the temperature difference between a test substance and an inert reference material; thermogravimetry (TG) measures the change in weight; and differential scanning calorimetry (DSC) measures the energy required to achieve a zero-temperature difference between a test substance and reference material.
   c. **Gasometric analysis**: Measuring the amount of gas evolved or absorbed during a chemical reaction is known as gasometry. Gasometry is used to analyze a variety of

gases, including CO_2, N_2O, cyclopropane, amyl nitrate, ethylene, N_2, helium, and more.

2. **Electrical methods**: Electric current, voltage, or resistance are measured in relation to the concentration of certain species in the solution using electrical methods of analysis:
   a. Potentiometry
   b. Conductometry
   c. Polarography are examples of electrical methods of analysis
   d. Amperometry
   e. Voltametry.

   Potentiometry determines an electrode's electrical potential when it is in equilibrium with an ion. While polarography, voltametry, and amperometry measure electrical current at a microelectrode, conductometry measures the electrical conductivity of an electrode with a reference electrode.
3. **Instrumental method of analysis:** Measuring a compound's or substance's physical characteristics is part of the instrumental technique. These techniques are used to determine the element's trace or minor concentration in the sample. Because of their selectivity, speed, precision, and ease of analysis, instrumental approaches are favored. Measurements of absorbance, specific rotation, refractive index, migration difference, charge to mass ratio, etc. are used to identify any changes in the system's characteristics. Spectroscopic analysis depends on measuring the amount of radiant energy at a specific wavelength that the sample emits. UV, visible, infrared, atomic absorption, nuclear magnetic resonance spectroscopy, and other techniques all include radiation absorption. In emission procedures, the sample is heated or electrically treated to cause the atoms to become excited and release energy. The intensity of this energy is then measured. Flame photometry, flourimetry, and emission spectroscopy are a few examples of emission techniques. In addition to being separation techniques for chemical mixtures, chromatographic and electrophoretic technologies are also used to identify compounds in mixtures. GC, HPLC, TLC, HPTLC, PC, and other chromatographic techniques are among them. Material is vaporized using a high vacuum in mass spectrometry, and the vapor is then subjected to a high-energy electron beam. Ions of different sizes are created by

the fragmentation of vapor molecules. These ions are separated by first accelerating them in an electrical field and then deflecting them in a magnetic field. Every type of ion contributes a peak to the mass spectrum.

4. **Biological and microbiological methods:** When no physical or chemical method can accurately determine the potency of a medication or its derivative, biological methods are employed. We refer to them as bio-assays. Antimicrobial or antibiotic agents' potency is measured using microbiological techniques. The antimicrobial assay compares the sample's bacterial growth suppression to that of a reference antibiotic. Turbidimetric analysis and the cup plate method are two of these techniques.

Analytical techniques may also be classified as:

Analytical techniques

Volumetric analysis:
1. Acid–base titration (aqueous and non-aqueous titration)
2. Redox titration
3. Complexometric titration
4. Precipitation titration

Electrochemical analysis:
1. Potentiometric titration
2. Conductometric titration
3. Amperometric titration
4. Polarography

Spectroscopic analysis:
1. Ultraviolet-visible (UV) spectroscopy
2. Infra-red (IR) spectroscopy
3. Nuclear magnetic resonance (NMR) spectroscopy
4. Mass spectrometry

Chromatographic analysis

Fig. 1.2: Classification of analytical techniques

APPLICATIONS

Both qualitative and quantitative analysis are necessary for manufacturing sectors to verify that their raw materials fulfill specified requirements and to assess the quality of the finished output. Raw materials must be examined to make sure that all necessary components are present within the specified range of composition and that no unexpected chemicals are present that could disrupt the manufacturing process or show up as a hazardous contaminant

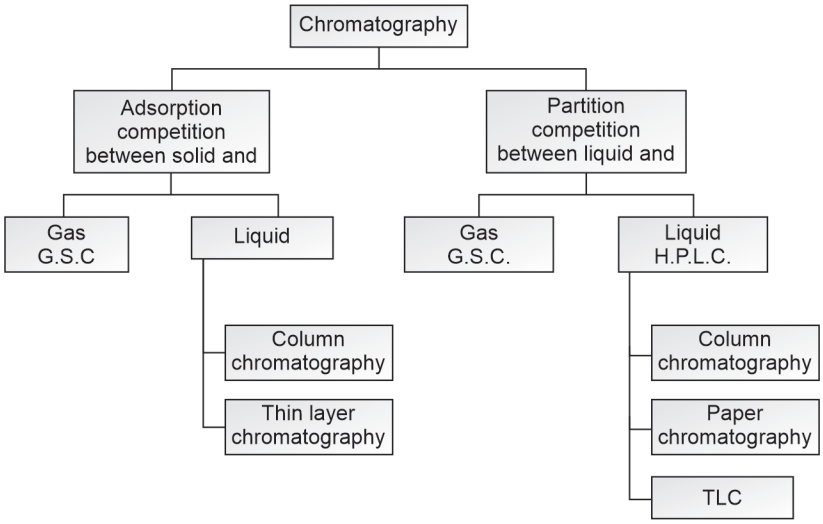


Fig. 1.3: Flowchart of chromatography

in the finished product. Determining the composition of a combination that exhibits the ideal properties for which the material has been designed is essential when creating new products that incorporate mixtures other than pure materials.

Analysis is necessary for geographical surveys in order to ascertain the composition of soil samples and multiple rock samples that are taken from the field.

The majority of industrial operations produce contaminants that can lead to health issues. Therefore, quantitative examination of soil, water, and air samples should be done in order to define safe limits for pollutants and to determine the level of contamination.

Methods of Expressing Strength of Solutions

Different ways of expressing concentration are: 1. Normality, 2. Molarity, 3. Molality, 4. Percentage solution, 5. Parts per million and parts per billion, 6. Formal concentration.

1. **Normality (N):** It is number of gram equivalents of solute in one liter of solution. It is Eq/L. It is commonly used in calculations of titrations.

$$\text{Equivalent weight} = \frac{\text{gram equivalent weight of solute}}{\text{number of replaceable } H^+ \text{ or } OH^-}$$

2. Molarity (M): Solute moles per liter of solution (mol/L). Because volume varies with temperature, it is temperature-dependent.

$$M = \frac{\text{gram molecular weight of solute}}{\text{1000 ml of solution}}$$

3. Molality (m): Solute moles per kilogram of solvent (mol/kg). It is helpful in stability investigations and is not affected by temperature.

$$m = \frac{\text{number of molecular weight of substance}}{\text{1000 gm of solution}}$$

4. Percentage of solution (% w/w, % w/v, % v/v): The amount of solute in 100 parts of a solution or mixture is represented as a percentage.

$$\%\frac{w}{w} = \frac{\text{weight of solute}}{\text{weight of solution}} \times 100$$

$$\%\frac{w}{v} = \frac{\text{weight of solute}}{\text{volume of solution}} \times 100$$

$$\%\frac{v}{v} = \frac{\text{volume of solute}}{\text{volume of solution}} \times 100$$

5. Parts per concentration (ppm, ppb): For expressing the concentration of impurities in pharmaceuticals, ppm and ppb are used. ppm: mg of solute per kg (L) of solution. Ppb is µg if solute per kg (L) of solution.
6. Formal concentration (F): Certain compounds remain in their ionic form both in the solid state and in solution; they do not exist in molecule form. In these situations, a solution is prepared using grams of molecular weight, formula weight, and its concentration is indicated in terms of formality.

$$F = \frac{\text{weight of solute in gram}}{\text{weight of solution in litre}} \times \text{formula weight}$$

PRIMARY STANDARD

The term 'standard' in pharmaceutical analysis refers to a chemical of interest having a known concentration. This can be expressed using specific numbers in the appropriate units. A primary standard has some characteristics:

1. **Extremely pure**: A primary standard material should have a high degree of purity, ideally 99.98%, meaning that it should be exceptionally pure. Chemicals of various purity levels are encountered in a pharmaceutical analysis laboratory. A number with a percentage that is referred to as purity will be seen if we look at the label. Therefore, a chemical is appropriate for use as a primary standard when its purity is 99.98% or above.
2. **Highly stable**: It should either have very low reactivity or be extremely stable, which indicates that it typically does not react readily when retained in its pure state. This is crucial because unstable and unreliable compounds can never be used as standards if a reagent reacts readily with water or ambient oxygen or alters its properties over time.
3. **Anhydrous in nature**: It should be anhydrous, meaning that its molecular structure should not include any water molecules. For instance, magnesium sulphate ($MgSO_4$), often known as Epsom salt, is a compound that we encounter in a pharmaceutical analysis lab with varying numbers of water molecules connected to it. The chemical Epsom salt, which is available in pharmacies, has the formula $MgSO_4 \cdot 7H_2O$. Therefore, we should buy anhydrous magnesium sulfate ($MgSO_4$) that is at least 99.98% pure and of analytical reagent grade if we wish to make a primary standard.
4. **Less hygroscopic**: The chemical should ideally be less hygroscopic, meaning that it should not collect water molecules from the atmosphere when the container is opened.
5. **Very high molecular weight**: When compared to other related forms, its molecular weight is extremely high. Epsom salt, for instance. To create a main standard, use one gram of $MgSO_4$ and designate it as salt A. For everyday use, take one gram of $MgSO_4 \cdot 7H_2O$ and label it salts B. Now, if we create a standard solution for both compounds using the real weight (molecular weight) of magnesium sulphate, we found that $MgSO_4$ (108) $MgSO_4 \cdot 7H_2O$ (234). First, a salt molecule 108 atomic mass units will be the exact weight of $MgSO_4$. However, in the second scenario, the weight of the actual $MgSO_4$ molecule B will make up 108 of its total weight, which is 234 atomic mass units. 108 grams of $MgSO_4$ will be produced from 108 grams of salt A. Therefore, 1 gram of $MgSO_4$ salt will yield 108/108 = 1 gram of $MgSO_4$; while, 234 grams of

salt B ($MgSO_4 \cdot 7H_2O$) will yield 108 gram of $MgSO_4$. Thus, 1 gram of $MgSO_4 \cdot 7H_2O$ salt will yield 108/234 = 0.461 gram of $MgSO_4$. As a result, if we accidentally create a standard using salt B, we are actually computing 0.461 grams of $MgSO_4$ as one gram. Therefore, the result will be lower than the real concentration due to this flawed standard calculation of $MgSO_4$ in other unknown solutions. Therefore, it is crucial that main standards have a large molecular weight and are anhydrous.

6. **Easily weighted**: Because it is so pure, its weight accurately reflects the number of moles that make up its actual weight, making it easy to weigh.
7. It should be ready to available and use.
8. Should be preferable non-toxic.
9. Should not be expensive.

Uses

In order to standardize a volumetric solution, or to standardize solution titration, primary standards are utilized. It can be used to titrate both bases and acids. Trisaminomethane [$(CH_2OH)_3CNH_2$], sodium carbonate (Na_2CO_3), and other basic chemical standards are frequently used in pharmaceutical analysis labs for acid titration. Potassium hydrogen phthalate [(KHP): $KHC_8H_4O_4$] etc., for base titration, etc. The primary standards for redox titration are frequently sodium oxalate ($Na_2C_2O_4$) and potassium dichromate ($K_2Cr_2O_7$). The primary standard is used to validate a particular method or to calibrate a secondary standard.

Secondary Standard

A secondary standard is used in the manufacture of kits and reagents or in labs that create quality control materials for other labs. They make use of the major standard as the main reference or calibrator. In a laboratory setting, secondary standards are used to calibrate control materials for the purpose of analyzing unknown drug concentrations. Secondary standards are essentially used for external quality control in laboratories. Therefore, standardizing the secondary standard versus the primary standard is necessary. The aqueous solution used to prepare the secondary standard solution needs to be extremely pure. When using water as an aqueous solvent, it needs to be deionized. The properties of secondary standard are as follows:

a. The secondary standard is less pure than the primary standard.
b. It is more reactive and less stable than the primary standard
c. The secondary standard solution is stable for an extended period of time.
d. The secondary standard is titrated against the primary standard.

For example:

1. **Sodium hydroxide anhydrous (NaOH):** It is a very hygroscopic substance. NaOH becomes wet as soon as the container is opened because it takes in moisture from the surrounding air. Let's put it practically: Take an analytical balance, put a Petri dish inside, and set its weight to zero (Tare). Now open the NaOH container, put a small NaOH crystal on the Petri dish, and record the weight. Keep the analytical balance's glass windows open for a few minutes, and then see how its weight gradually increases. This is due to the water molecules being absorbed from the air by the NaOH crystals.
2. **$KMnO_4$, or potassium permanganate, is frequently employed as a secondary standard**: It is reactive and therefore less stable since it is an effective oxidizing agent. The content is contaminated by manganese oxide (MnO_2), its own oxidized product. As a result, it cannot serve as a primary standard.

Smaller labs that actually analyze unknown samples employ secondary standards as calibrators.

Calibration

Calibration is the process of determining if an instrument is operating correctly, that is, whether it is providing accurate measurements. Although the terms calibration and standardization are synonymous, we use calibration when referring to instruments and standardization when referring to solutions.

This method involves comparing the measurements made by a primary standard or instrument with those made by a secondary standard or instrument. By doing this, we attempt to eliminate any fluctuation or discrepancy in measurement made using an instrument or secondary standard.

KEY POINTS

- Pharmaceutical analysis involves identification, determination, quantification, and purification of drugs.
- **Main goal:** To identify or measure compounds in pharmaceutical samples.
- **Analyte:** Substance to be identified or measured.
- **Sample matrix:** Remaining components of the sample except analyte.
- Examples of samples:
  - Raw materials
  - Finished products (tablets, capsules)
  - Biological samples (blood, urine)
- **Types of analysis:**
  Qualitative analysis (identification): Confirms presence/identity of drug.
  Examples:
  - Color change
  - Precipitate formation
  - Melting/boiling point
  - Gas evolution

  Quantitative analysis (estimation): Determines amount/concentration
  Example: Ensuring 500 mg paracetamol per tablet
- **Classification based on sample size:**
  - Macro: ≥ 0.1 g
  - Semi-micro: 0.01–0.1 g (most common)
  - Micro: 0.001–0.01 g
  - Sub-micro and ultra-micro: Very small amounts
  - Trace analysis: ppm/ppb level
- **Methods of pharmaceutical analysis:**
  A. **Chemical methods:**
  Titrimetric (volumetric):
  - Acid–base, redox, precipitation, complexometric
  - High accuracy, simple equipment

  Gravimetric:
  - Convert analyte $\rightarrow$ insoluble precipitate $\rightarrow$ weigh
  - Time-consuming but accurate

  Gasometric: Measure gas evolved/absorbed (e.g. CO_2, N_2)

B. **Electrical methods:** Based on electrical properties:
   – Potentiometry (voltage)
   – Conductometry (conductivity)
   – Polarography, voltammetry, amperometry (current)

C. **Instrumental methods:**
   – Measure physical properties
   – Advantages: Fast, accurate, sensitive

   Examples:
   – Spectroscopy (UV, IR, NMR)
   – Chromatography (HPLC, GC, TLC)
   – Mass spectrometry

D. **Biological and microbiological methods:**
   – Used when chemical methods are not suitable
   – Measure drug potency

   Example: Antibiotic assay (cup plate method)

- **Applications:**
  – Quality control of drugs
  – Raw material testing
  – Detection of impurities
  – Environmental analysis (air, water, soil)
  – Drug development
- **Expression of concentration:**
  – Normality (N): Eq/L
  – Molarity (M): mol/L (temperature dependent)
  – Molality (m): mol/kg (temperature independent)
  – Percentage: % w/w, w/v, v/v
  – ppm/ppb: Trace impurities
  – Formality (F): for ionic compounds
- **Primary standard:** Characteristics:
  – Highly pure (≈ 99.98%)
  – Stable and non-reactive
  – Anhydrous
  – Non-hygroscopic
  – High molecular weight
  – Easily weighable

  Examples: Sodium carbonate (Na_2CO_3), potassium dichromate ($K_2Cr_2O_7$), potassium hydrogen phthalate (KHP)

- **Secondary standard:**
  - Less pure, less stable
  - Hygroscopic/reactive
  - Standardized using primary standard

  Examples: Sodium hydroxide (NaOH), potassium permanganate ($KMnO_4$)
- Calibration
  - Process of checking accuracy of instruments
  - Comparison with standard reference
  - Ensures reliable results

PRACTICE QUESTIONS

Long Answer Type Questions

1. Define pharmaceutical analysis. Discuss its aims, scope, and importance in ensuring drug quality and safety.
2. Explain the concept of analyte and sample matrix. Describe different types of samples used in pharmaceutical analysis with examples.
3. Describe in detail the qualitative and quantitative analysis. Add principles, methods, and applications.
4. Classify pharmaceutical analysis based on their sample size.
5. Describe biological and microbiological methods. Explain their importance in determining drug potency with examples.

Short Answer Type Questions

1. Define pharmaceutical analysis.
2. What is an analyte?
3. Define sample matrix.
4. What is qualitative analysis?
5. What is quantitative analysis?
6. Define calibration.
7. What is ppm?
8. Define primary standard.
9. Define secondary standard.
10. Write differences between qualitative and quantitative analysis.

Multiple Choice Questions

1. Pharmaceutical analysis mainly deals with:
   a. Manufacturing drugs
   b. Identification and quantification of drugs
   c. Marketing of drugs
   d. Packaging of drugs
2. The substance to be analyzed is called:
   a. Matrix b. Solvent
   c. Analyte d. Excipient
3. The remaining part of a sample excluding analyte is called:
   a. Solute b. Matrix
   c. Reagent d. Indicator
4. Identification of a compound is also known as:
   a. Quantitative analysis b. Qualitative analysis
   c. Volumetric analysis d. Gravimetric analysis
5. Determination of amount of substance is called:
   A. Qualitative analysis b. Physical analysis
   c. Quantitative analysis d. Biological analysis
6. Which of the following is an example of qualitative test?
   a. Titration b. Weighing
   c. Color change d. Calculation
7. Which method involves measurement of volume of solution?
   a. Gravimetric b. Titrimetric
   c. Gasometric D. Spectroscopic
8. Which of the following is an electrical method?
   a. Chromatography b. Spectroscopy
   c. Potentiometry d. Titration

Answers

1. b
2. c
3. b
4. b
5. c
6. c
7. b
8. c