# UNIT 1

**Pharmaceutical Analysis – Definition and Scope**

**Definition:**
Pharmaceutical analysis is the branch of practical chemistry that deals with the qualitative and quantitative determination of chemical compounds and pharmaceutical substances. It plays a vital role in the identification, purification, determination of structure, and estimation of the constituents in both raw materials and formulated products. According to *Beckett & Stenlake* and *Vogel's Textbook of Quantitative Chemical Analysis*, it involves the application of various analytical procedures to ensure the safety, efficacy, purity, and quality of pharmaceutical products.

It includes both **qualitative analysis** (which identifies the chemical components or functional groups in a sample) and **quantitative analysis** (which determines the exact amount of a substance present). Analytical techniques may be classical (like titrimetry and gravimetry) or instrumental (like spectrophotometry, chromatography, and electrochemical methods).

**Scope of Pharmaceutical Analysis:**

1. **Quality Control and Quality Assurance:**
   Pharmaceutical analysis ensures that drugs meet the standards laid down by pharmacopeias (like IP, BP, USP).

2. **Raw Material Analysis:**
   Before production, pharmaceutical raw materials such as active pharmaceutical ingredients (APIs) and excipients must be analyzed for identity, purity, and strength to prevent formulation errors and ensure safety.

3. **In-process and Finished Product Testing:**
   Throughout the manufacturing process, analytical methods monitor product integrity.

4. **Detection of Impurities:**
   Pharmaceutical analysis is used to detect and quantify impurities or degradation products which may affect safety and efficacy. Techniques like HPLC and LC-MS are used for impurity profiling.

5. **Stability Studies:**
   To determine the shelf-life and proper storage conditions of pharmaceuticals, analysis is done over time under different environmental conditions.

6. **Research and Development:**
   Analytical methods support the development of new drug substances and formulations by characterizing their chemical and physical properties

7. **Regulatory Compliance:**
   Pharmaceutical analysis is essential for compliance with national and international regulatory agencies like CDSCO, USFDA, EMA, and ICH.

8. **Pharmacokinetics and Bioavailability Studies:**
   Quantification of drugs in biological fluids (plasma, urine, etc.) during pharmacokinetic studies requires highly sensitive analytical methods like LC-MS/MS.

**Different Techniques of Analysis**

Pharmaceutical analysis employs a wide range of techniques to ensure the identity, purity, potency, and quality of raw materials and finished pharmaceutical products. These techniques are broadly classified into classical (chemical) and instrumental methods based on the nature of the procedure and tools used.

**1. Classical (Chemical) Methods of Analysis**

a. **Gravimetric Analysis**
This is a quantitative method where the analyte is converted into a stable, insoluble compound which is then isolated and weighed. It includes:

- Precipitation gravimetry
- Volatilization gravimetry
  Gravimetric methods are highly accurate but time-consuming.

b. **Titrimetric (Volumetric) Analysis**
These methods involve the measurement of the volume of a standard solution required to react completely with the analyte. It includes:

- **Acid-base titrations** (e.g., HCl vs. NaOH)
- **Redox titrations** (e.g., $KMnO_4$ vs. $Fe^{2+}$)
- **Complexometric titrations** (e.g., EDTA with $Ca^{2+}$ or $Mg^{2+}$)
- **Precipitation titrations** (e.g., Mohr's method for $Cl^-$)
  Titrimetry is widely used for routine QC due to its simplicity and precision.

c. **Qualitative Chemical Tests**
These are used for the identification of ions, functional groups, and certain compounds using color changes, precipitation reactions, and flame tests.

**2. Instrumental Methods of Analysis**

These methods offer higher sensitivity, specificity, and speed and are essential for modern pharmaceutical analysis.

a. **Spectroscopic Techniques**
These are based on the interaction of electromagnetic radiation with matter.

- **UV-Visible Spectrophotometry:** Measures absorbance of UV or visible light; useful for analyzing chromophoric drugs and dissolution studies.
- **Infrared (IR) Spectroscopy:** Identifies functional groups by their characteristic vibrations.
- **Nuclear Magnetic Resonance (NMR) Spectroscopy:** Provides structural information based on magnetic properties of nuclei.
- **Mass Spectrometry (MS):** Identifies molecular weights and structures by ionizing molecules and measuring their mass-to-charge ratio.
- **Atomic Absorption Spectroscopy (AAS):** Determines metal ions in samples by measuring the absorption of light by free metallic ions.

- **Fluorimetry and Flame Photometry:** Used for trace analysis of elements and some fluorescent drugs.

b. **Chromatographic Techniques**
These techniques separate mixtures into individual components.

- **Thin Layer Chromatography (TLC):** A qualitative technique used for identification and purity checking.
- **High Performance Liquid Chromatography (HPLC):** A powerful method for quantification and separation of components in mixtures.
- **Gas Chromatography (GC):** Used for volatile compounds; coupled with MS for advanced applications.
- **Paper Chromatography:** Used for separating small amounts of organic compounds in educational settings.
- **Column Chromatography and Ion Exchange Chromatography:** Used in separation and purification processes.

c. **Electrochemical Techniques**
These are based on the measurement of electrical properties.

- **Potentiometry:** Measures the potential of electrochemical cells; used in pH determination.
- **Conductometry:** Measures the electrical conductivity of solutions; applied in titrations.
- **Polarography and Voltammetry:** Techniques involving current-voltage measurements to determine trace elements.

d. **Thermal Analysis Techniques**
Used to study changes in physical and chemical properties as a function of temperature.

- **Differential Scanning Calorimetry (DSC):** Measures heat flow associated with phase transitions.
- **Thermogravimetric Analysis (TGA):** Measures changes in weight as temperature changes.

e. **X-ray Techniques**

- **X-ray Diffraction (XRD):** Determines crystal structure and polymorphism of drug substances.
- **X-ray Fluorescence (XRF):** Determines elemental composition in solid samples.

**3. Bioanalytical Techniques**
Used for the analysis of drugs and metabolites in biological samples like blood and plasma.

- **LC-MS/MS:** Combines high-performance liquid chromatography with mass spectrometry for highly sensitive and specific detection.
- **ELISA (Enzyme-Linked Immunosorbent Assay):** A biochemical technique to detect antigens or antibodies.

**Methods of Expressing Concentration**

Concentration refers to the amount of solute present in a given quantity of solvent or solution. In pharmaceutical analysis, accurate expression of concentration is essential for preparing standard solutions, performing titrations, calculating dosages, and maintaining consistency in formulations. The concentration of a solution can be expressed in several ways, depending on the requirement of the analytical method or formulation.

**1. Percentage Concentration**
This method expresses the amount of solute in 100 parts of solution or mixture and is commonly used in pharmaceutical formulations.

a. **% w/w (weight/weight):**
Grams of solute in 100 grams of solution.
Used for ointments, creams, and solids.
Example: 2% w/w salicylic acid means 2 g of salicylic acid in 100 g of product.

b. **% w/v (weight/volume):**
Grams of solute in 100 mL of solution.
Commonly used for oral and injectable solutions.
Example: 5% w/v glucose means 5 g of glucose in 100 mL solution.

c. **% v/v (volume/volume):**
Milliliters of solute in 100 mL of solution.
Used when both solute and solvent are liquids.
Example: 70% v/v ethanol means 70 mL of ethanol in 100 mL of solution.

d. **% v/w (volume/weight):**
Milliliters of solute in 100 g of solution.
Less commonly used but applicable in certain liquid-solid systems.

**2. Parts Per Notation**
Used for expressing very dilute solutions, especially in toxicology and environmental analysis.

a. **Parts per million (ppm):**
1 part of solute in 1 million parts of solution.
1 ppm = 1 mg/L (for aqueous solutions).

b. **Parts per billion (ppb):**
1 part of solute in 1 billion parts of solution.
1 ppb = 1 µg/L (for aqueous solutions).

**3. Molarity (M)**
Molarity is the number of moles of solute per liter of solution.
M = moles of solute / volume of solution in liters.
It is temperature-dependent as volume varies with temperature.
Example: A 1 M NaCl solution contains 58.44 g of NaCl in 1 L of solution.

**4. Molality (m)**
Molality is the number of moles of solute per kilogram of solvent.
m = moles of solute / mass of solvent in kg.
It is temperature-independent and used in colligative property studies.

**5. Normality (N)**
Normality is the number of gram equivalents of solute per liter of solution.
N = equivalents of solute / volume of solution in liters.
Used mainly in titration calculations.
Example: 1 N $H_2SO_4$ has 49.04 g (1 equivalent) of $H_2SO_4$ per liter.

**6. Mole Fraction (χ)**
The ratio of the number of moles of a component to the total number of moles in the solution.
χA = moles of component A / total moles of all components.
Used in thermodynamic calculations.

**7. Milliequivalents (mEq) and Millimoles (mmol)**
Used in clinical and pharmacological calculations, especially for electrolytes and ions.

- **mEq = (mg × valency) / molecular weight**
- **mmol = mg / molecular weight**

These units provide a more practical approach in prescribing and preparing injections and IV fluids.

**8. Volume Strength**
Used especially for expressing concentration of hydrogen peroxide and similar oxidizing agents.
It represents the volume of oxygen (at NTP) liberated from 1 volume of solution.
Example: 10-volume hydrogen peroxide liberates 10 volumes of $O_2$ per volume of solution.

**Primary and Secondary Standards**

In pharmaceutical analysis, the accurate preparation of standard solutions is crucial for titrations and quantitative determinations. A **standard substance** is one of known high purity used to prepare a solution of known concentration. Standards are categorized into **primary** and **secondary** based on their purity, stability, and role in analytical procedures.

---

**Primary Standard**

A **primary standard** is a highly pure chemical substance that can be directly used to prepare a solution of accurately known concentration without the need for standardization against another substance.

**Characteristics of a Good Primary Standard:**

1. High purity (typically ≥ 99.9%)
2. Stability (non-hygroscopic, does not decompose or oxidize)
3. Soluble in the chosen solvent, usually water
4. High molecular weight (to reduce weighing error)
5. Easily and accurately weighed
6. Readily and completely reacts in a known and stoichiometric manner

**Examples of Primary Standards:**

- Sodium carbonate ($Na_2CO_3$) – used to standardize HCl

- Potassium hydrogen phthalate (KHP) – used to standardize NaOH
- Oxalic acid dihydrate ($H_2C_2O_4 \cdot 2H_2O$) – used to standardize $KMnO_4$
- Silver nitrate ($AgNO_3$) – used in chloride estimations
- Arsenic trioxide ($As_2O_3$) – used in iodometry

**Uses:**

- Direct preparation of standard solutions
- Calibration of secondary standards and analytical instruments
- Establishment of reference concentrations in pharmaceutical analysis

---

**Secondary Standard**

A **secondary standard** is a chemical substance whose exact concentration is determined by standardizing it against a primary standard solution.

**Characteristics of Secondary Standards:**

1. May not be highly pure
2. May be hygroscopic or unstable
3. Cannot be weighed accurately due to instability or reactivity
4. Concentration must be determined by titration with a primary standard

**Examples of Secondary Standards:**

- Hydrochloric acid (HCl) – standardized using sodium carbonate
- Sodium hydroxide (NaOH) – standardized using KHP or oxalic acid
- Potassium permanganate ($KMnO_4$) – standardized using oxalic acid or sodium oxalate
- Iodine solution – standardized using arsenic trioxide
- Sulfuric acid ($H_2SO_4$) – standardized using sodium carbonate

**Uses:**

- Frequently used in volumetric titrations due to ease of preparation
- Required in cases where the reagent degrades or absorbs moisture/$CO_2$ from the air
- Suitable for routine laboratory titrations after proper standardization

### Sodium Thiosulphate Solution Preparation

- Take about 100 ml of water in a cleaned and dried 1000 ml volumetric flask.
- Add about 25 gm of Sodium Thiosulphate with continues stirring.
- Add about 0.2 gm of Sodium Carbonate with continues stirring.
- Add more about 700 ml of water, mix.
- Make up the volume 1000 ml with water. Mix solution thoroughly.
- Keep the solution for at least one hour and then carry out the standardization.

### Sodium Thiosulphate Solution Standardization

- Accurately weigh about 210 mg of primary standard Potassium Dichromate, previously pulverized and dried at 120°C for 4 hours.
- Dissolve in 100 ml of distilled water in a glass-stoppered, 500 ml conical flask.
- Swirl to dissolve the solid, remove the stopper, and quickly add 3 g of Potassium Iodide, 2 g of Sodium Bicarbonate, and 5 ml of hydrochloric acid.
- Insert the stopper gently in the flask, swirl to mix, and allow to stand in the dark for exactly 10 minutes.
- Rinse the stopper and the inner walls of the flask with distilled water, and titrate the liberated iodine with the Sodium Thiosulfate solution until the solution is yellowish green in color.
- Add 3 ml of starch indicator solution, and continue the titration until the blue color is discharged.
- Perform a blank determination.
- Calculate the molarity by the following formula:

$$M = \frac{K_2Cr_2O_7 \text{ in mg}}{49.04 \times Na_2S_2O_3 \text{ in ml}}$$

### Hydrochloric acid Solution Preparation

- Take about 100 ml of water in a cleaned and dried 1000 ml volumetric flask.
- Add about 8.5 ml of Conc. Hydrochloric acid with continuous stirring.
- Add more about 700 ml of water, mix and allow to cool to room temperature.
- Make up the volume 1000 ml with water. Mix solution thoroughly.
- Keep the solution for at least one hour and then carry out the standardization.

### Hydrochloric acid Solution Standardization

- Accurately weigh about 0.5 g of THAM (Tris (hydroxymethyl)-amino methane (tromethamine), previously dried at 105° for 3 hours and cooled in a desiccator, transfer to a conical flask.
- Dissolve in 50 ml of distilled water.
- Add 2 drops of Bromocresol Green indicator.
- Titrate with 0.1M Hydrochloric acid to a pale yellow endpoint.
- Each 121.14 mg of tromethamine is equivalent to 1 ml of 0.1M hydrochloric acid.
- Calculate the molarity by the following formula:

$$M = \frac{THAM \text{ in mg}}{121.14 \times HCl \text{ in ml}}$$

### Sulphuric Acid Solution Preparation

- Add slowly, with stirring, 6 ml of Sulphuric acid to about 800 ml of purified water.
- Makeup to 1000 ml with purified water.
- Allow cooling at 25°C.

### Sulphuric Acid Solution Standardization

- Weigh accurately about 0.2 g of anhydrous Sodium Carbonate, previously heated at about 270°C 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.
- 1 ml of 0.1 M sulphuric acid is equivalent to 0.0098 g of $Na_2CO_3$.
- Calculate the molarity of solution by the following formula:

$$M = \frac{Na_2CO_3 \text{ in mg}}{H_2SO_4 \text{ in ml} \times 9.8}$$

### Sodium Hydroxide Solution Preparation

- Take about 100ml of distilled water in a cleaned and dried 1000 ml volumetric flask.
- Add about 4.2 gm of Sodium hydroxide with continues stirring.
- Add more about 700ml of distilled water, mix and allow to cool to room temperature.
- Make up the volume 1000 ml with distilled water. Mix solution thoroughly.
- Keep the solution for at least an hour and then carry out the standardization.

### Sodium Hydroxide Solution Standardization

- Accurately weigh about 0.5 g of potassium biphthalate, previously crushed lightly and dried at 120° for 2 hours.
- Dissolve in 75 ml of carbon dioxide free water.
- Add 2 drops of phenolphthalein, and titrate with the sodium hydroxide solution to the production of a permanent pink color.
- Each 20.42 mg of potassium biphthalate is equivalent to 1 ml of 0.1N sodium hydroxide.

**Calculation**

$$M = \frac{\text{Wt. in gm of potassium biphthalate}}{0.20423 \times \text{ml NaOH solution}}$$

### Potassium Permanganate Solution Preparation

- Dissolve 3.2 g of potassium permanganate in 1000 ml of water.
- Heat on a water-bath for 1 hour.
- Allow to stand for 2 days and filter through glass wool.
- Standardize the solution in the following manner.

### Potassium Permanganate Solution Standardization

- 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 sulphuric acid.
- Titrate the liberated iodine with 0.1 M sodium thiosulphate, using 3 ml of starch solution, added towards the end of the titration, as an indicator.
- Perform a blank determination and make the necessary correction.
- Store protected from light.
- 1 ml of 0.1 M sodium thiosulphate is equivalent to 0.003161 g of $KMnO_4$.

### Ceric Ammonium Sulphate Solution Preparation

- Dissolve 65 g of ceric ammonium sulfate with the aid of gentle heat, in a mixture of 30 ml of sulphuric acid and 500 ml of water.
- Cool, filter the solution, if turbid, and dilute to 1000 ml with water.
- Standardize the solution in the following manner.

### Ceric Ammonium Sulphate Solution Standardization

- Weigh accurately about 0.2 g of arsenic trioxide, previously dried at 105° for 1 hour, and transferred to a 500 ml conical flask.
- Wash down the inner walls of the flask with 25 ml of a 8.0 % w/v solution of sodium hydroxide, swirl to dissolve, add 100 ml of water and mix.
- Add 30 ml of dilute sulphuric acid, 0.15 ml of osmic acid solution, 0.1 ml of ferroin sulfate solution.
- Titrate with the ceric ammonium sulfate solution until the pink color is changed to a very pale blue, adding the titrant slowly towards the end-point.
- 1 ml of 0.1 M ceric ammonium sulfate is equivalent to 0.004946 g of $As_2O_3$.
- Calculate the molarity of the solution by the following formula:

$$M = \frac{As_2O_3 \text{ in mg}}{\text{Ceric Ammonium Sulphate in ml} \times 4.946}$$

**Preparation and Standardization of Oxalic Acid Solution (Simple Format)**

**Molecular weight of oxalic acid dihydrate ($H_2C_2O_4 \cdot 2H_2O$):** 126.07 g/mol
**Equivalent weight (for redox reactions):** 63.035 g/eq (2 electrons involved)

---

**Preparation of 0.1 M Oxalic Acid Solution:**

- Weigh **12.607 g** of oxalic acid
- Dissolve in some distilled water
- Transfer to 1 L volumetric flask
- Make up to 1000 mL with distilled water
- Mix well

---

**Preparation of 0.1 N Oxalic Acid Solution:**

- Since 1 mole = 2 equivalents → 0.1 N = 0.05 M
- Weigh **6.3035 g** of oxalic acid
- Dissolve and make up to 1 L with water

---

**Standardization of $KMnO_4$ using Oxalic Acid:**
**Reaction:**
$2\ KMnO_4 + 5\ H_2C_2O_4 + 3\ H_2SO_4 \rightarrow 2\ MnSO_4 + 10\ CO_2 + K_2SO_4 + 8\ H_2O$

**Steps:**

1. Take 25.00 mL of 0.1 N oxalic acid
2. Add 10 mL dilute $H_2SO_4$
3. Heat to 60–70°C
4. Titrate with $KMnO_4$ till a light pink color persists
5. Use formula:
   $N_1V_1 = N_2V_2$ to calculate exact normality of $KMnO_4$

**Errors in Pharmaceutical Analysis**

In pharmaceutical analysis, **errors** refer to the difference between the measured (observed) value and the true (actual) value. Errors can affect the accuracy, precision, and reliability of analytical results, making it essential to understand their **types, sources, and minimization strategies**.

---

**1. Sources of Errors**

**a) Instrumental Errors:**
Due to imperfections or malfunction in analytical instruments.
Example: improper calibration of balances, drift in spectrophotometer readings.

**b) Personal Errors:**
Arise from the analyst's carelessness or incorrect technique.
Example: parallax error in reading burette levels, incorrect pipetting.

**c) Method Errors:**
Due to improper or non-ideal analytical procedures or assumptions.
Example: using a method not specific to the analyte, side reactions.

**d) Reagent Errors:**
Caused by impure or unstable reagents.
Example: impure sodium hydroxide, degraded $KMnO_4$ solution.

**e) Environmental Errors:**
Due to external factors such as temperature, humidity, and dust.
Example: hygroscopic substances absorbing moisture from air during weighing.

---

**2. Types of Errors**

**a) Systematic Errors (Determinate Errors):**

These are reproducible errors that occur in the same direction (either high or low).
They can be identified and corrected.
**Causes:**

- Faulty instruments
- Improper standardization
- Consistent technique flaws

**Types of Systematic Errors:**

- **Instrumental error** – miscalibrated balance
- **Operational error** – consistent over-titration
- **Method error** – interfering substances in method

**Effect:** Affects **accuracy**.
**Correction:** Identify and eliminate the cause.

---

**b) Random Errors (Indeterminate Errors):**

Unpredictable and unavoidable variations during measurement.
Caused by uncontrollable factors like fluctuations in temperature or observer's judgment.

**Effect:** Affects **precision**.
**Correction:** Cannot be completely eliminated but can be minimized by repeated measurements and statistical treatment.

---

**c) Gross Errors:**

Large, often obvious errors usually due to human mistakes.
**Examples:**

- Using the wrong reagent
- Misreading instruments by large amounts
- Recording incorrect values

**Effect:** Makes data unusable.
**Correction:** Avoid by careful procedure and cross-checking.

---

**3. Minimization of Errors**

- **Calibrate instruments** regularly
- **Use primary standards** wherever possible
- **Train personnel** on proper techniques
- **Conduct blank determinations**
- **Use proper storage** of reagents and samples
- **Repeat experiments** to identify anomalies
- **Perform statistical analysis** to detect random errors

**1. Accuracy**

Accuracy refers to **how close** a measured or experimental value is to the **true or accepted value**. It represents the **correctness** of a result.

**Formula (if true value is known):**
Accuracy (%)=(True Value/Measured Value)×100

**Example:**
If the actual concentration of a solution is 100 mg/mL and your analysis shows 98 mg/mL, then the result is close, hence **accurate**.

**Factors affecting accuracy:**

- Systematic errors
- Impure reagents
- Faulty calibration
- Methodological flaws

**Improvement:**

- Use of primary standards
- Calibration of instruments
- Performing recovery studies

---

**2. Precision**

Precision refers to the **closeness among repeated measurements** under the same conditions. It is the **reproducibility** or **repeatability** of a method.

**It does not guarantee accuracy.** A result can be precise (consistent) but still inaccurate if it consistently deviates from the true value.

**Types of precision:**

- **Repeatability (Intra-assay precision):** Same analyst, same equipment, short time
- **Intermediate precision:** Different analysts, different instruments, same lab
- **Reproducibility:** Different labs (inter-laboratory precision)

**Measured using:**

- Standard deviation (SD)
- Relative standard deviation (RSD) or coefficient of variation (CV)
  RSD (%)=(Mean/Standard Deviation)×100

  **Improvement:**
- Strict procedure adherence
- Use of automated instruments
- Environmental control

---

**3. Significant Figures**

Significant figures are the digits in a number that **convey meaningful information** about the precision of a measurement.

**Rules for identifying significant figures:**

1. All **non-zero digits** are significant.
   Example: 456 has 3 significant figures.
2. **Zeros between non-zero digits** are significant.
   Example: 405.2 has 4 significant figures.
3. Leading zeros (before the first non-zero digit) are **not** significant.
   Example: 0.0034 has 2 significant figures.

4. Trailing zeros:
    - In a number **with a decimal**, trailing zeros **are** significant.
      Example: 45.00 has 4 significant figures.
    - In a number **without a decimal**, trailing zeros are **not necessarily significant**.
      Example: 1500 has 2 or 3 or 4 depending on context. Use scientific notation to clarify:
        - $1.50 \times 10^3$ → 3 significant figures
        - $1.500 \times 10^3$ → 4 significant figures

**Use in analysis:**

- Reflects the precision of instruments used
- Avoids overstating the accuracy of results
- Essential in reporting analytical data and rounding

Limit tests are **qualitative or semi-quantitative** procedures designed to **identify and control small quantities of impurities** that are likely to be present in pharmaceutical substances. These impurities, even in trace amounts, can be **toxic** or affect the **quality** of the product.

---

**1. Limit Test for Lead (Pb)**

**Principle:**
Lead is detected by forming a **brown complex** with **hydrogen sulfide** or by comparison with a standard using **thioacetamide** or **dithizone** in modern methods. In Indian Pharmacopoeia, the **color produced is compared with a standard lead solution**.

**Reagents Used:**

- Lead nitrate (standard solution)
- Acetic acid
- Potassium cyanide
- Hydrogen sulfide or dithizone solution
- Buffer solution (to maintain pH)

**Procedure (IP method):**

- Dissolve the sample in water and acidify with acetic acid.
- Add potassium cyanide to avoid interference.
- Pass hydrogen sulfide gas or use dithizone reagent.
- Observe and compare color with standard lead solution.

**Result Interpretation:**
If the color produced in the test solution is **not more intense** than that in the standard, the sample passes the test.

---

**2. Limit Test for Arsenic (As)**

**Principle:**
Arsenic present in the sample is converted into **arsine gas ($AsH_3$)** by reaction with **zinc and acid**, and the gas reacts with **mercuric chloride paper**, producing a **yellow to brown stain**.

**Reagents Used:**

- Arsenic-free zinc
- Stannous chloride
- Mercuric chloride paper
- Hydrochloric acid
- Potassium iodide
- Arsenic standard solution

**Apparatus:**
A special glass apparatus with a reaction flask and a fitted glass tube holding **mercuric chloride paper**.

**Procedure (IP method):**

- Mix the sample with hydrochloric acid and potassium iodide.
- Add arsenic-free zinc to generate arsine gas.
- Gas passes through the apparatus and reacts with mercuric chloride paper.
- After 40 minutes, the paper is compared with that of standard.

**Result Interpretation:**
If the yellow stain on the mercuric chloride paper in the test is **not more intense** than the standard, the sample passes the test.

---

**3. Limit Test for Iron (Fe)**

**Principle:**
Iron reacts with **ammonium thiocyanate** in an acidic medium to form a **blood-red ferric thiocyanate complex**. The color intensity is compared with that of a standard solution.

**Reagents Used:**

- Ferric ammonium sulfate (standard iron solution)
- Ammonium thiocyanate

- Nitric acid
- Distilled water

**Procedure (IP method):**

- Dissolve the sample in water and acidify with nitric acid.
- Add ammonium thiocyanate solution.
- Make up to volume and allow the color to develop.
- Compare with standard iron solution prepared similarly.

**Result Interpretation:**
If the red color in the test solution is **not more intense** than that of the standard, the sample complies with the IP specification.

---

**4. Limit Test for Chloride ($Cl^-$)**

**Principle:**
Chloride reacts with **silver nitrate** in the presence of nitric acid to form **white turbidity** of **silver chloride**, which is compared with a standard.

**Reagents Used:**

- Silver nitrate
- Nitric acid
- Standard sodium chloride solution
- Distilled water

**Procedure (IP method):**

- Dissolve the sample in water and acidify with nitric acid.
- Add silver nitrate solution.
- Mix well and allow turbidity to develop.
- Compare the turbidity with that produced by a standard chloride solution.

**Result Interpretation:**
If the turbidity in the test solution is **not greater** than in the standard, the sample passes the test.