SHREE H. N. SHUKLA INSTITUTE OF PHARMACEUTICAL EDUCATION AND RESEARCH

B.PHARM

(SEMESTER –I)

SUBJECT NAME: PHARMACEUTICAL ANALYSIS -I

UNIT 1: PHARMACEUTICAL ANALYSIS

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Content

Pharmaceutical analysis- Definition and scope

i. Different techniques of analysis
ii. Methods of expressing concentration
iii. Primary and secondary standards
iv. Preparations and standardization of various molar and normal solutions-

Oxalic acid, sodium hydroxide, hydrochloric acid, sodium thiosulphate, sulphuric acid, potassium permanganate and ceric ammonium sulphate

(b) Errors: Source of errors, types of errors, methods of minimizing errors. Accuracy, precision and significant figures.

(c) Pharmacopoeia, Sources of impurities in medical agents, limit tests

Introduction

It is defined as the application of process or series of processes for identification and quantification of a substance or components in the drugs and to determine its purity, safety and quality of drugs.

When a completely unknown sample is presented to an analyst, the first requirement is usually to check what substances are present in it. Some impurities are also present in the sample. Identification of substances and impurities are present in the sample is known as **Qualitative analysis**.

When analyst determine how much of each component is present, such determination is known as **Quantitative analysis.**

Scope of Pharmaceutical Analysis

Pharmaceutical industries rely upon the quantitative and qualitative analysis to ensure their raw materials meet certain specification and to check the quality of final product.

- **Determination of active ingredient**

  Quantitative analysis is performed to establish the proportion of the active ingredient. This procedure is called assay.

  The final manufactured product is to be checked to ensure its active ingredient are present with in a predetermined range of composition.

- **Determination of impurities**

  Raw material may contain some impurities. Presence of these impurities are determined and ensure that they present in their specified limit.

- **To study the stability of Product**

  Analysis is to be done to study the stability of the any pharmaceutical product.

- **Development of new products**

  The development of the new product is a very long process. This whole process involves many analytical studies. It will be necessary to check the composition of the mixture which shows the optimum characteristics for the purpose of development of the product.

- **Biopharmaceutical analysis**

  Rate of drug from its formulation is determined. Concentration of drug in plasma or biological fluids is calculated. Determine pKa values, partition coefficients, solubility and stability of drugs under development.

Methods of expressing concentration

These methods are used for the quantitative analysis.

The solution as diluted or concentrated can be express by Concentration.

$$Concentration = \frac{Amount\ of\ solute}{Amount\ of\ solvent}$$

Concentration can be measured by various ways.

- Molarity (M)
- Molality (m)
- Normality (N)
- Parts per million (ppm)
- Percentage composition
- Mole fraction

Molarity (M)

It is defined as the number of moles of solute dissolved in one liter of a solution.

$$Molarity\ (M) = \frac{Number\ of\ Moles\ of\ Solute}{Volume\ of\ Solution\ (L)}$$

Molarity of a solution will change as volume changes and volume is temperature dependent. Therefore Molarity can change with temperature.

Example:

- 1 mole (40 gm) of NaOH is dissolved in 1 liter of solution is said to be 1 molar (M) Solution. Similarly if only half mole (20 gm) of NaOH is dissolved in 500 mL of solution then the solution is said to be 1 molar.

- Similarly only half mole (20 gm) of NaOH is dissolved in 1 liter of solution then the solution is said to be half molar (0.5 M).

Molality (m)

It is defined as the number of moles of solute dissolved per 1000 g (1 kg) of solvent.

$$Molality\ (M) = \frac{No.of\ Moles\ of\ Solute}{Kilogram\ of\ the\ solvent}$$

Molality of solution is independent of temperature.

Example:

- 1 mole of Na_2CO_3 i.e. its gram of molecular mass (106 g) dissolved in 1000gm of solvent is said to be 1 molal (1M) solution.
- Only half of the mole i.e. 53 gm of Na_2CO_3 is dissolved in 1000 gm of solvent, the solution is said to be one half molal (i.e. 0.5 m).

Normality (N)

The normality of solution is a given by the number of equivalents of solute per litre of solution.

$$N = \frac{Equivalent\ weight\ of\ Solute}{Volume\ of\ the\ solvent}$$

Equivalent weight is the weight of the substance that contains or reacts with 1 mole of hydrogen (1.0078 g), 1 mole of oxygen (8 g) or 1 mole of chlorine (35.45 g). Equivalent weight is simplified below:

$$\text{Equivalent weight} = \frac{\text{Molecular weight}}{\text{Basicity or Acidity}}$$

Formality

Some substances do not exist in molecular form, whether in solid or in solution form, they remain in ionic form in solid state as well as in solution. In such cases instead of molecular weight, formula weight is used in preparation of solution and its concentration is expressed in term of formality.

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

Formal solutions generally show changes in formality where volume changes associated with temperature.

Parts per million (ppm)

It is used to express the concentration of very dilute solutions. It can also be expressed as mg/L. this term is also employed to express the concentration of impurities in pharmaceuticals.

1 ppm = 1µg/mL

Percentage composition or gram/liter

Concentration is many time expressed in terms of percent. Percent composition of a solution can be expressed as:

1. Percentage w/w = $\frac{mass\ of\ solute}{mass\ of\ solution} \times 100$
2. Percentage v/v = $\frac{volume\ of\ solute}{volume\ of\ solution} \times 100$
3. Percentage w/v = $\frac{mass\ of\ solute}{volume\ of\ solution} \times 100$

Mole Fraction (X)

Mole fraction of any component in a solution is the number of moles of the component divided by total number of moles making up a solution.

$$Mole\ fraction = \frac{Moles\ of\ component}{Total\ number\ of\ moles\ making\ up\ the\ solution}$$

Example:

A solution is prepared by dissolving 1 mole of ethyl alcohol (C_2H_5OH) in 3 moles of water. Where nA and nB represent the number of moles of ethyl alcohol and water respectively.

Mole fraction of ethyl alcohol = $XA = \frac{nA}{nA+nB} = \frac{1}{1+3} = \frac{1}{4} = 0.25$

Mole fraction of water = $X_B = \frac{nA}{nA+nB} = \frac{3}{1+3} = \frac{3}{4} = 0.75$

Sum of mole fractions is always equal to 1.

Different techniques of analysis

There are various methods of Quantitative analysis. These techniques are used for the estimation of quantity.

1. Chemical Methods
   a) Volumetric
   b) Gravimetric

c) Gasometric

2. Instrumental Methods
3. Microbiological Methods
4. Biological Methods

1. Chemical Methods

a) Volumetric methods: Volumetric or titrimetric methods are better than the gravimetric methods. As name suggest, in these

The solution of the accurately known strength is called the standard solution. The weight of the substance to be determined is calculated from the volume of the standard solution used and the chemical equation and relative molecular masses of the reacting compound.

In titrimetric analysis the reagent of known concentration is called the **titrant** and the substance being titrated is called as the **titrand**.

The standard solution is usually added from a long graduated tube called a burette. The process of adding the standard solution until the reaction is just complete is called a titration. The point at which this occurs is called the **equivalent point** or the **theoretical or stoichiometric end point**.

The completion of the titration is detected methods assay is based on the measurement of volume of solution of known strength that is required to react completely with the substance to be analysed.by some physical change, produced by the standard solution itself or by the addition of a reagent, known as indicator.

After the reaction between the substance and the standard solution is practically complete, the indicator should give a clear visual change that is either a colour change or the formation of turbidity in the liquid being titrated. The point at which this occur is called the **visual end point** of the titration.

The reagent used in this method fulfil the following requirements.

i. The reagent should react rapidly
ii. The reaction should be complete, and
iii. The completion of the reaction should be detected by a suitable method.

On the bases of the type of reactions involved, volumetric method is divided into the following.

a. Neutralization titrations: It involves neutralization reaction in presence of water as solvent.

b. Non-aqueous titrations: It involves the reaction between acid and base in presence of non-aqueous i.e. organic solvents.

c. Precipitation titrations: It involves the reaction leading to precipitate formation. It includes the methods where the reacting substance and standard solution react to give a precipitate or a slightly soluble salt as the primary reaction product.

d. Oxidation-reduction (Redox) titrations: These titrations involves simultaneous oxidation-reduction reaction. It include all the methods where reacting substance is oxidized or reduced by the standard solution.

e. Complexometric titrations: It includes all the methods where the reacting substance and the standard solution react to form a soluble but very slightly dissociated complex substance. For e.g. EDTA titrations.

b) Gravimetric methods: These methods are based on weight analysis. In these methods sample is isolated in its pure form and then weighing of that isolated compound is done.

Compound isolation is based on precipitation and volatilization methods or electro-analytical methods.

Although gravimetric analysis is time consuming, these methods are helpful for examination of impurities and correction can be applied if necessary.

c) Gasometric methods: These methods involve the measurement of the volume of gases. They measure the:

1. Volume of gas liberated in the given chemical reaction under the conditions that are described in the process
2. The reduction in volume of gas in the presence of agents absorbing one of the gases, thus, lowering the standard conditions of temperature and pressure.

Following gases are determined by gasometric analysis in pharmacopoeia:
Cyclopropane, carbon dioxide, nitrous oxide, oxygen, octyl nitrate, nitrogen, amyl nitrate, ethylene and helium etc.

The measurement of volume of gas is generally made in gas burettes or by nitro meters.

b) Instrumental Methods

In instrumental methods the difference between the physicochemical properties of the standard compound and the sample compound under test is utilized for analysis.

Any changes in properties of the system are detected by measurement of current, potential, electrical conductivity, optical density, refractive index, etc., through sensitive instruments.

Physical Properties	Instrumental methods
Electrical Potential	Potentiometry
Electrical conductance	Conductometry

Electrical current	Polarography, Voltaetry
Absorption of radiation	Spectrophotometry Colorimetry Atomic absorption spectroscopy
Emission of radiation	Emmision spectroscopy Flame photometry Fluorimetry
Scattering of radiation	Turbidimetry, nephelometry
Refraction of radiation	Refractometry
Rotation of plane Polarized light	Polarimetry Optical rotatory dispersion
Thermal properties	Thermal method
Mass to charge ratio	Mass spectroscopy

In addition to above methods, **chromatographic methods** are also available. Chromatography is the procedure by which active principles, excipients and impurities are separated by passage of a mixture through a fixed porous bed possessing varying but reversible affinity for individual components.

Thus chromatography is a separation technique or a device by which a mixture of substances is separated into its various components.

Chromatographic methods include following techniques:

1. Column chromatography
2. Paper chromatography
3. Thin Layer chromatography
4. Gas chromatography
5. Ion exchange chromatography
6. HPLC chromatography

c) Microbiological methods

Chemical and Physical methods are not as much as useful for the detection of change in the stability of antibiotics. In such cases, microbiological methods are used for the analysis of these type of drugs. In these methods microbial growth is inhibited under standardized conditions by such antibiotic drugs to be analyzed and potency of the drug is to be calculated.

Generally antibiotics give antimicrobial activity which is detected by the microbiological assays. The microbiological assay is based upon a comparison of the inhibition of the growth of bacteria by both the sample and analyte.

Here comparison is done between the activity of concentration of antibiotics to be examined (analyte) and known concentration of standard preparation of the antibiotic having a known activity.

d) Biological methods

Biological assays (bioassays) are prescribed where the potency of drug or its preparation cannot be adequately determined by chemical or physical means, but it is possible to observe the biological effect of the drug on some type of living matter.

The principle of such assay is to compare how much of the sample being tested produces the same biological effect as a given quantity of standard preparation.

It is also important that conditions under which the sample and the standard preparation are tested, are identical in all respects of time, environmental factors and biological media used.

ACCURACY OF VOLUMETRIC ANALYSIS

Volumetric methods of analysis are mainly affected by following three factors.

1. Substance of known purity for preparation of standard solution (Standardization)
2. End point detection
3. Calibrated volumetric glassware

1. Standardization Process:

When the reagent is not available in pure form, as in cases of most alkali hydroxides, some inorganic acids and various deliquescent substances, solutions of highly pure substance is to be prepared for finding out the actual molar concentration of that reagent.

In this process, solutions corresponding approximately to the molar strength required are to be prepared. These are then standardize by titration against a solution of a pure substance of a known concentration. This procedure is known as standardization of that reagent.

Primary and Secondary standards: In titrimetry certain chemicals are used frequently in defined concentrations as reference solutions. They are known as primary standards or secondary standards.

Primary Standard: The substance of high purity used in preparation of standard solution are known as primary standard substances. Primary standard solution can be prepared by direct weighing of a quantity of that substance and dilute it to give defined volume of solution.

Primary standard substances should satisfy the following requirements:

1. It must be easy to obtain, to purify, to dry and to preserve in pure state.

2. It should be 100 % pure although 0.01 % to 0.02 % impurity is tolerable if accurately known.
3. It should be stable to atmospheric conditions. It should not be decompose or be hygroscopic, or deliquescent.
4. It should show a high equivalent weight in order to reduce the effect of weighing errors. In weighing a greater amount of substance, the relative error will be smaller than that for a smaller amount.
5. The reaction with standard solution should be stoichiometric and practically instantaneous.
6. The reaction is to be capable to use simple indicator to determine the end point of the titration.
7. There should not be any difference between end point and theoretical equivalence point i.e. titration error should not be there.
8. It should be readily soluble under the conditions in which it is to be employed.
9. It should not have water of hydration, so that the composition of the solid does not change with variation in relative humidity.

It is not always possible to satisfy all the requirements of a primary standard but the requirements mentioned above should be met at all times as closely as possible. In practice ideal primary standard is difficult to obtain and hence, a compromise between above requirements is usually necessary.

These substances of known purity whose known accurately weighed quantity is used in the standardization of solution of unknown strength.

- The commonly used primary standards are:

  Acid-base Titrations: Sodium carbonate, potassium hydrogen phthalate, succinic acid, benzoic acid, oxalic acid and adipic acid.

  Redox titrations: Potassium dichromate, potassium bromate, potassium iodate, sodium oxalate, arsenious oxide.

  Precipitation titration: Sodium chloride, potassium chloride, potassium bromide, silver nitrate.

  Complexometric titration: Various pure metals such as zinc, magnesium, manganese and salt such as lead nitrate, calcium carbonate etc.

Secondary Standard:

As the number of the primary standard substances is limited, a substance with less but known purity is used in standardization process.

A compound whose concentration is estimated by its titration against primary standard compound called secondary standard compound.These substances are known as secondary standards.

2. End point Detection: The main object of the end point detection is to find out the volume of standard solution which reacts with the analyte completely. If the end point is not detected accurately, the quantitative estimation will not be accurate.

In volumetric methods, the end point is usually detected by use of some external substance which shows sharp change in its colour at the end point of the reactions. This external substance which detects end point is called as visual indicator. This detection of end point is affected by various factors such as:

1. **Indicator selection:** Indicator should be selected depending upon the properties of the substance to be analysed. For example, in neutralization titrations transition interval of the indicator should be close to and around the equivalence point of titration.

2. **Colour change detection:** Colour change in the titration by using visual indicator is nothing but the first deviation from the original colour which can be clearly detected. For visual detection of the colour the chemist must relay on its own ability to detect the colour and it is quite possible that differences from one individual to another are possible.

3. **Quantity of indicators:** Generally, indicators are added in very small quantity approximately 0.0001 – 0.0004 % of the solution being titrated. If large quantity of indicator is added then large quantity of titrant will be needed to show the colour change of indicator.

4. **Presence of colloidal substances:** Colloidal particles are having property to absorb ions on their surface and this property leads to the problems in detection of end point.

5. **Effect of temperature:** Change in temperature has many effects on the end point detection in the titration. Change in temperature leads to dissociation of substances to be anlysed and in addition it will also affect the indicator leading to change in colour of indicator.

3. Calibration of volumetric Glass wares:

Volumetric glasswares generally used are burettes, pipettes, volumetric flasks for measurement of accurate volume. Reliability of these volumetric glasswares depends upon the volume actually contained/delivered by that glassware.

The calibration is the measurement of volumes actually glasswares contained/ delivered and if it is lacking, it provides correction in the volume.

The temperature for the glassware calibration is the 27 °C as per the Indian Standards Institutions (ISI). Glasswares should meet the requirements as per ISI and such glasswares should be purchased in anlytical laboratory.

Preparation and standardization of various molar and normal solutions

1. Sodium Hydroxide (NaOH):

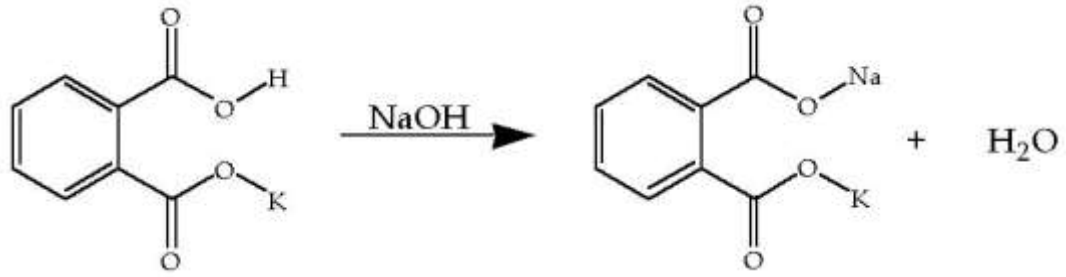
Principle:

The principle involved in the standardization of sodium hydroxide is **acid base neutralization titration**. NaOH is very deliquescent substance. Therefore, when we prepare solution of NaOH, it is not actual molar concentration as we prepared.

It absorbs moisture and CO_2 when exposed to air so, approximate concentration of NaOH is prepared by dissolving specified amount of it in carbon dioxide free water and this solution is standardized against acidic primary standard potassium hydrogen phthalate ($C_8H_5KO_4$).

When we titrate NaOH which is base with the acid, neutralization occurs when mole of the acid and mole of base are equal, pH of the solution become neutral and it is end point for the reaction.

End point is detected by the phenolphthalein added as an indicator in the solution which shows by the colour change from colourless to permanent light pink.



Preparation of 1 M NaOH:

For the preparation of 1 M NaOH, first dissolve 40 gm of NaOH in 1000 mL of CO_2 free water. Store this solution with stoppers to prevent from atmospheric CO_2.
Here CO_2 free water is used because in the presence of CO_2, following reaction take place.

$$2\ NaOH\ (s) + CO_2\ (g) \longrightarrow Na_2CO_3 + H_2O$$

Standardization of 1 M NaOH:

Potassium hydrogen phthalate is used as a primary standard for the standardization of Sodium Hydroxide.

Weigh 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.

1 ml of 1 M sodium hydroxide is equivalent to 0.2042 g of $C_8H_5KO_4$ (KHP).

Volumetric solutions of sodium hydroxide must be restandardize frequently.

Factor: Each ml of 0.1 M NaOH equivalent to 0.020422 g of $C_8H_5KO_4$.

Derivation of Factor:

1 mole of NaOH $\cong$ 1 mole of potassium hydrogen phthalate (KHP)

1000 ml of 1 M NaOH $\cong$ 204.22 g of $C_8H_5KO_4$

Each ml of 1 M NaOH $\cong$ 0.20422 g of $C_8H_5KO_4$

2. Sulphuric acid (H_2SO_4):

Principle: This is direct **acid base neutralization titration** in which sulphuric acid reacts with sodium carbonate to form carbon dioxide and water. As sodium carbonate which (base) is reacted with the sulphuric acid (acid), carbon dioxide formed in reaction can change colour of indicator before equivalence point.

It is advisable to boil solution to perform the errorless titration.

$$\mathbf{Na_2CO_3 + H_2SO_4 \longrightarrow Na_2SO_4 + H_2CO_3 \longrightarrow Na_2SO_4 + CO_2 + H_2O}$$

Preparation of 0.5 M Sulphuric acid:

For the preparation of 0.5 M H_2SO_4, add slowly with stirring, 27 ml of H_2SO_4 in 1000 mL of CO_2 free water. Allow to cool at 25 °C. Store this solution with stoppers to prevent from atmospheric CO_2.

Standardization of 0.5 M H_2SO_4:

Anhydrous Sodium carbonate is used as a primary standard for the standardization of Sulphuric 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. Add 0.1 ml of methyl red solution and titrate with the sulphuric acid solution.

Add the acid slowly from a burette, with continuous 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 colour is no longer affected by continued boiling.

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

Derivation of factor:

1 mole of H_2SO_4 $\cong$ 1 mole of Na_2CO_3

1000 ml of 1 M H_2SO_4 $\cong$ 105.9888 g of Na_2CO_3

1000 ml of 0.5 M H_2SO_4 $\cong$ 52.9944 g of Na_2CO_3

Each ml of 0.5 M H_2SO_4 $\cong$ 0.05299 g of Na_2CO_3

3. Hydrochloric acid (HCl):

Principle: Hydrochloric acid is prepared by the reaction between sodium chloride and sulphuric acid. Commercially available Hcl is 35 – 38 % pure. The principle involved in the standardization of Hcl is based on **acid base neutralization titration**.

Two primary standard compound sodium carbonate and borax can be used for the standardization of Hcl.

Sodium carbonate containing varying amount of carbon dioxide and sodium bicarbonate and hence it is heated to 270 for 1 hour to remove moisture and to convert sodium bicarbonate to sodium carbonate.

The standardization of Hcl should be carried out in boiling condition.

- During standardization, carbon dioxide is liberated as a result of neutralization reaction it makes solution acidic and
- Presence of carbon dioxide affect the colour change of methyl red which is due to the formation of weak carbonic acid which introduces error in the titration.

Preparation of 0.1 M HCl:

Pipette out 8.5 ml of concentrated hydrochloric acid in 1000 ml volumetric flask containing some amount of distilled water and make up the volume up to the mark with distilled water.

Standardization of 0.1 M HCl:

Anhydrous Sodium carbonate is used as a primary standard for the standardization of Hydrocloric 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. Add 0.1 ml of methyl red solution and titrate with the hydrochloric acid solution.

Add the acid slowly from a burette, with continuous 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 colour is no longer affected by continued boiling.

Factor:

1 ml of 0.1 M hydrochloric acid is equivalent to 0.005299 g of Na_2CO_3.

Derivation of factor:

2 mole of Hcl $\cong$ 1 mole of Na_2CO_3

2(1000 ml of 1 M Hcl) $\cong$ 105.9888 g of Na_2CO_3

1000 ml of 1 M Hcl $\cong$ 52.9944 g of Na_2CO_3

Each ml of 1 M Hcl $\cong$ 0.05299 g of Na_2CO_3

Each ml of 0.1 M Hcl $\cong$ 0.005299 g of Na_2CO_3

4. Sodium Thiosulphate ($Na_2S_2O_3$):

Principle: Sodium thiosulphate requires standardization due to several reasons:

1. There is always some doubt as to the exact water content of sodium thiosulphate crystals. It is generally available as pentahydrate (i.e. $Na_2S_2O_3.5H_2O$).

2. Solution of sodium thiosulphate when prepared in distill water, it absorbs carbon dioxide forming carbonic acid which causes slow decomposition of sodium thiosulphate and form thiosulphuric acid ($H_2S_2O_3$) which is further readily decompose into sulphurous acid (H_2SO_3) with the formation of sulphur.

$$\mathbf{H_2CO_3 + Na_2S_2O_3 \longrightarrow H_2S_2O_3 + Na_2CO_3}$$

$$\mathbf{H_2S_2O_3 \longrightarrow H_2SO_3 + S}$$

3. The solution of sodium thiosulphate is also decomposed by bacterial growth. Bacterial activity is least when pH of the solution is in the range of 9-10, therefore a small amount of sodium carbonate or borax is added to sodium thiosulphate solution to maintain these pH range.

4. The solution is decomposed by light.

Thus, it is usual to standardize thiosulphate by such substances as potassium iodate, potassium bromate or potassium dichromate, all of which can be obtained in a high state of purity.

The principle of standardization of sodium thiosulphate is based on redox iodometric titration with potassium bromate as primary standard. Iodometric titrations are those titrations in which iodine liberated during the reaction.

Potassium bromate, a strong oxidizing agent, is treated with excess potassium iodide (reducing agent) in acidic media (here Hcl), and the liberated iodine is titrated with sodium thiosulphate solution.

The standardization with potassium bromate depends upon the reactions expressed by the following equations:

$$\mathbf{KI + HCl \longrightarrow HI + KCl}$$

$$KBrO_3 + H1 \longrightarrow HIO_3 + KBr$$

$$1O_3^- + 51^- + 6H^+ \longrightarrow 31_2 + 3H_2O$$

$$1_2 + 2\ Na_2S_2O_3 \longrightarrow Na_2S_4O_6 + 2NaI$$

Reaction between potassium iodate and excess of KI in acid solution results in liberation of iodine which can be titrated with sodium thiosulphate.

Preparation of 0.1 M Sodium Thiosulphate:

Dissolve 25 g of sodium thiosulphate and 0.2 g of sodium carbonate in carbon dioxide free water and dilute to 1000 ml with the carbon dioxide free water. Standardize the solution in following manner.

Standardization of 0.1 M Sodium Thiosulphate:

Weigh accurately and dissolve 0.2 g of potassium bromate, in sufficient water to produce 250 ml.

To 50 ml of this solution add 2 g of potassium iodide and 3 ml of 2 M hydrochloric acid and titrate with the sodium thiosulphate 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 thiosulphate is equivalent to 0.002784 g of $KBrO_3$.

Derivation of Factor:

1 mole of $KBrO_3$ $\cong$ 3 mole of I_2 $\cong$ 6 mole of $Na_2S_2O_3$

6 (1000 ml 1 M $Na_2S_2O_3$) $\cong$ 167.02 g of $KBrO_3$

1000 ml 1 M $Na_2S_2O_3$ $\cong$ 27.84 g of $KBrO_3$

Each ml of 1 M $Na_2S_2O_3$ $\cong$ 0.02784 g of $KBrO_3$

Each ml of 0.1 M $Na_2S_2O_3$ $\cong$ 0.002784 g of $KBrO_3$

5. Potassium Permanganate ($KMnO_4$):

Principle: The principle of standardization of $KMnO_4$ based on **redox iodometric titration** using standardized sodium thiosulphate. Potassium permanganate is reacted with potassium iodide (reducing agent) in acidic media which liberates iodine.

Liberated iodine is then titrated with sodium thiosulphate using starch indicator which is added near the end point.

Permanganate ion in acidic media acts as a strong oxidizing agent which is reduced to Mn^{+2} ion (reduction product).

Sulphuric acid is the most suitable acid for providing acidic media, as it has no action upon permanganate in dilute solution. Hydrochloric acid is not used, as there are chances of the reaction and some permanganate may be consumed in the formation of chorine resulting in error.

Starch solution is added as an indicator at the end of titration because in high iodine concentration starch forms starch iodide water insoluble complex which causes introduction of error in titration.

The starch solution should be freshly prepared because upon standing for a considerable period of a time it undergoes decomposition and become insensitive for detection of iodine. Compound like mercuric iodide can be added to preserve the stability of starch solution.

The standardization depends upon the reaction represented by following.

$$2\,[MnO_4 + 8H^+ + 5e^- \longrightarrow Mn^{+2} + 4\,H_2O]$$

$$5\,[2I^- \longrightarrow I_2 + 2e^-]$$

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

Preparation and Standardization of sodium thiosulphate solution:

Prepare and standardize the sodium thiosulphate solution as per previous method given.

Preparation of 0.02 M Potassium permanganate solution:

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.

Standardization of 0.02 M Potassium permanganate solution:

To 25 ml of potassium permanganate solution taken in iodine flask, 2 g of potassium iodide, dissolve and add 10 ml of 1 M sulphuric acid.

Titrate the liberated iodine with 0.1 M sodium thiosulphate solution, using 3 ml of starch solution as indicator which is added towards the end of the titration and blue colour is discharged. Perform a blank titration and make any necessary correction.

Factor:

1 ml of 0.1 M sodium thiosulphate is equivalent to 0.003161 g of $KMnO_4$.

Derivation of Factor:

2 mole of $KMnO_4 \cong 5$ mole of $I_2 \cong 10$ mole of $Na_2S_2O_3$

10 (1000 ml of 1 M $Na_2S_2O_3$) $\cong$ 2 mole of $KMnO_4$

10 (1000 ml of 1 M $Na_2S_2O_3$) $\cong$ 2 × 158 (M.W. of $KMnO_4$) g of $KMnO_4$

1000 ml of 1 M $Na_2S_2O_3$ $\cong$ 31.61 g of $KMnO_4$

Each ml of 1 M $Na_2S_2O_3$ $\cong$ 0.03161 g of $KMnO_4$

Each ml of 0.1 M $Na_2S_2O_3$ $\cong$ 0.003161 g of $KMnO_4$

Here we get amount of potassium permanganate in 25 ml of solution. Depending on that we can calculate amount of potassium permanganate present in1000 ml sample. Calculate molarity of potassium permanganate solution from following manner.

$$\text{Molarity} = \frac{mole\ (gm\ of\ Molecular\ weight)}{1000\ ml}$$

1000 ml of 1 M $KMnO_4$ = 158 gm of $KMnO_4$
1000 ml of X M $KMnO_4$ = A gm of $KMnO_4$

$$\frac{1000 \times M \times 158}{1000 \times 1 \times A}$$

$$M = \frac{A}{158}$$

Ceric ammonium sulphate ($Ce(NH_4)_4(SO_4)_4$):

Principle: It is standardized using redox titration where arsenic trioxide is used as a primary standard.

Here ceric ammonium sulphate is strong oxidizing agent and Arsenic trioxide is a reducing agent.

In that reaction, Arsenic trioxide is converted into arsenious acid (H_3AsO_3). Cerric ion (Ce^{+4}) oxidizes arsenious acid to arsenic acid and ceric ion is reduced to cerrous ion (Ce^{+3}) in the reaction.

As_2O_3 (Arsenic trioxide) + $NaOH$ (Sodium hydroxide) $\longrightarrow$ $NaAsO_2$ (Sodium arsenite)

$NaAsO_2$ (Sodium arsenite) + $2H_2O$ (Water) $\longrightarrow$ $NaH_2AsO_4 + 2H^+ + 4e^-$ (Sodium arsenate)

$4 \times [Ce^{4+} + e^- \longrightarrow Ce^{3+}]$

Preparation of 0.1 M Ceric Ammonium Sulphate:

Dissolve 65 g of ceric ammonium sulphate, 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.

Standardizaion of 0.1 M Ceric Ammonium Sulphate:

Weigh accurately about 0.2 g of arsenic trioxide, previously dried at 105° for 1 hour, and transfered to a 500 ml conical flask. Wash down the inner walls of the flask with 25 m1 of 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 sulphate solution and slowly titrate with the ceric ammonium sulphate solution until the pink colour is changed to a very pale blue, adding the titrant slowly towards the end-point.

Factor:

1 ml of 0.1 M ceric ammonium sulphate is equivalent to 0.004946 g of As_2O_3.

Factor Derivation:

4 mole of ceric ammonium sulphate $\cong$ 1 mole of As_2O_3

4 (1000 ml of 1 M ceric ammonium sulphate $\cong$ 197.841 gm of As_2O_3

1000 ml of 1 M ceric ammonium sulphate $\cong$ 49.4602 g of As_2O_3

Each ml of 1 M ceric ammonium sulphate $\cong$ 0.004946 g of As_2O_3

(b) ERRORS

Error can be defined as the difference between the measured and the true value.

It is impossible to completely eliminate an error while making measurements, even when specialized hand and apparatus are employed.

Source of errors:

1. Weighing an object displaces some amount of air from the area occupied by the substance on the balance.
2. Sometimes even standard weights have error.
3. Fluctuation of scale reading (up or down) due to air.
4. Friction in the moving particle distributing the scale equilibrium.
5. Dust particles in air may adhere to the balance causing error in reading.
6. Delay in calibration or wrong calibration due to the effect of circuit's accuracy or temperature.
7. Atmospheric moisture affect the weighing of hygroscopic substance.

Classification of errors:

a) Systematic (determinate) errors

b) Random (indeterminate) errors

(a) Systematic (determinate) errors: These are errors which can be avoided, or whose magnitude can be determined. The most important of them are operational and personal errors, instrumental and reagent errors, and errors of method.

1. Operational and personal errors
   These are due to factors for which the individual analyst is responsible and are not connected with the method or procedure. The errors are mostly physical in nature and sound analytical technique is not followed.
   Examples-
   - incomplete drying of analytical samples before weighing
   - Mechanical loss of materials during sample dissolution from effervescence or from bumping
   - Incorrect technique involving the transfer of solutions
   - Lack of reproducibility in solvent extraction methods
   - Sample treatment before measurement

   Personal errors may arise from the constitutional inability of an individual to make certain observations accurately. Thus some persons are unable to judge colour changes sharply in visual titrations, which may result in slight overstepping of the end point.

2. Instrumental and reagent errors
   These arise from the –
   - Faulty construction of the balances
   - The use of uncalibrated or impropery calibrated weights, graduated glassware and other instruments
   - The attack of reagent upon glassware, porcelain etc.

   These are resulting in the introduction of foreign materials, and the use of the reagents containing impurities.

3. Errors of method
   These are the most serious errors because often they can be difficult to detect.
   Examples-
   - pH meter that has be wrongly standardized
   - Background absorption in atomic absorption spectroscopy
   - Faulty detector response in chromatographic and spectroscopic method
   - In gravimetry, solubility of precipitates and the decomposition or volatilization on ignition of weighing forms
   - Errors may arise in titrimetry, if there are differences between the observed end point and the stoichiometric equivalence point of a reaction.

- Errors due to "Matrix effects" when there is a difference between the bulk composition and the analyte sample solution and the composition of the standard solutions used to establish the calibration graph.

(b) Random (indeterminate) errors:

These errors manifest themselves by the slight variation that occur in successive measurements made by the same observer with the greatest care under as nearly identical conditions are possible.

The random errors is also known as accidental errors. They are due to causes over which the analyst has no control, and which are so intangible that they are incapable of analysis.

Examples-

- Unpredictable fluctuation in the readings of a measurement apparatus or in the experimenter's interpretation of the instrumental reading

Methods for Errors Minimization

1. Calibration of instruments, apparatus and application of necessary corrections

Analytical instruments like UV spectrophotometer, IR spectrophotometer, single pan electric balance, pH meter, turbidimeter, polarimeter, refractometer, etc. should be calibrated before use to avoid errors.

Similarly, all the apparatus like pipettes, burettes, volumetric flask, thermometers, weights etc. should also be calibrated and appropriate corrections applied to the original measurements.

2. Running a blank determination:

It is a separate analysis without the sample. Blank determination is performed in which sample is not added and same experiment is performed under same conditions with other reagents added in same manner in the solution.

The object for performing blank determination is to find out the effect of impurities in a regent or vessels and exact amount of standard solution is needed to obtain the specific end point for unknown sample.

3. Performing a control determination

In this method, estimation is done with standard having similar quantity of component as present in the unknown sample. Determination is done separately under same experimental conditions. Comparison is done between the standard solutions of known concentration with the analyte sample of unknown concentration. Determination of the unknown sample is known as the normal determination and determination of standard is known as the control determination.

Thus, the weight of the component present in the unknown sample may be calculated with the help of the following expression:

$$\frac{Wt.of\ Component\ in\ Stndard\ Substance}{X} = \frac{Result\ obtained\ for\ standard\ substance}{Result\ obtained\ for\ unknown\ sample}$$

Where, X = Weight of the component present in the unknown sample.

4. Use of independent methods of analysis

In some instances the accuracy of result may be established by carrying out the analysis in an entirely different manner.

For example, in water hardness, calcium and magnesium concentrations determined by atomic absorption may be compared with the results obtained by complexometric titration.

If the results obtained by the two radically different methods are compatible with each other, it is highly probable that the values are correct with in small limits of error.

5. Running parallel determinations

These serve as a check on the result of a single determination and indicate only the precision of the analysis.

If large variations are shown, the determinations must be repeated until satisfactory concordance is obtained. The procedure is repeated for 2 to 3 times. Duplicate or triplicate determinates are sufficient. Good agreement between duplicate and triplicate determinations does not justify the conclusion that the result is correct, a constant error may be present.

The agreement shows that the accidental errors, or variations of the determinate errors, are the same or nearly the same in the parallel determinations.

6. Method of Standard Addition

In this method, quantitative determination of the component present in a sample under examination is done by adding a small amount of standard sample.

The difference between the analytical results obtained for samples with and without addition of standard sample gives the recovery of the amount of added compound.

If the recovery is satisfactory our confidence in the accuracy of the procedure is enhanced. The method is usually applied to physicochemical procedures such as polarography and spectrophotometry.

7. Methods of Internal Standards:

This method is valuable in chromatography and spectroscopy also.

In this method, a known amount of reference material or the internal standard is incorporated into a series of measured quantity of the substance to be analysed.

A graphical represent about ratio of peak size of the internal standard and the series of known concentration are plotted against the concentrations values. This should give a straight line.

Any unknown concentration can then be determined by adding the same quantity of internal standard and finding where the ratio obtained falls on the concentration scale.

8. Amplification method

In determinations in which a very small amount of material is to be measured. In these cases if the small amount of material can be reacted in such a way that every molecule produces two or more molecules of some other measurable material, the resultant amplification may then bring the quantity to be determined within the scope of the instruments.

ACCURACY AND PRECISION

Accuracy

Accuracy may be defined as the concordance between the data and the true value or most probable value.

It relates to the closeness of test results to true or actual value i.e. measure of exactness of analytical method.

The true value is usually not known, the mean calculated from results obtained from several different analytical methods which are very precise and in close agreement with one another may be considered the true value in practical sense.

It is expressed as % recovery by the assay of known amount of analyte in the linearity range.

For analytical methods, there are two possible ways of determining the accuracy:

1. Absolute Method
2. Comparative Method

1. Absolute Method:

Systemic samples containing known amounts of the constituents is used.

Known amount of constituents can be obtained by weighing out pure elements or compounds of known stoichiometric composition.

These substances, primary standards, may be available commercially or they may be prepared by the analyst and subjected to rigorous purification by recrystallization, etc. the substances must be of known purity.

The test of the accuracy of the method under consideration is taken out by taking different amount of the constituent and proceeding according to specified instructions. The amount of the constituent must be differ because the determinate errors in the procedure may be a function of the amount used.

The difference between the mean of an adequate number of results and the amount of the constituent actually present, usually expressed as part per thousand, is a measure of accuracy of the method in the absence of foreign substance.

The constituent in question will usually have to be determined in the presence of other substances.

Comparative method

Sometimes, as in the analysis of mineral it may be impossible to prepare solid synthetic samples of the desired composition.

It is then necessary to resort to standard samples of the mineral in question (mineral, ore, alloy, etc) in which the content of the constituent sought has been determined by one or more supposedly 'accurate' methods of analysis.

This comparative method involving secondary standards, is obviously not altogether satisfactory from the theoretical standpoint, but is very useful in applied analysis. Standard samples can be obtained from various sources.

If several fundamentally different methods of analysis for a given constituent are available, e.g. gravimetric, titrimetric, spectrophotometric, the agreement between at least two methods of essentially different character can usually be accepted as indicating the absence of an appreciable systemic error in either of each method.

Precision

Precision may be defined as the degree of agreement between the replicate measurements of the same quantity.

The method is allow to repeat for several times and depending on results from these repeated methods, the precision of an analytical method is obtained.

The precision is usually expressed as:

- Standard deviation (SD)
- Relative Standard deviation (RSD)

It is a measure of degree of repeatability or reproducibility under normal conditions.

If analyst perform the determination on the same day in repeating manner, results of every performance are known as the repeatability.

While, analyst perform this same determination on another day where, laboratory conditions may vary, this set of results would be defined as 'reproducibility'.

Significant figures:

It is important to perform accurate measurement, but it is also significant how accurately numbers are displayed or used during calculations.

This can be achieved by noting the results to the significant numbers. Significant figures reflect the uncertainty of measurement.

Normally significant numbers are the digits which are known exactly plus one digit whose value is uncertain. A significant factor shows the quantity in place in which it stands.

Rules for using Zero as significant figure:

i. All nonzero digits are called significant figures.

For e.g. 2.13, there are three significant figures

ii. Zero to the left of nonzero digits (Leading zeros) are nonsignificant

For e.g. 1.8 gms, there are two sifnificant figures

0.0018 kgs (two significant figures)

iii. Zero between the digits are significant

For e.g. three significant figures are present in 1.08

iv. Zeros right of the decimal point (trailing zeros) is considered significant.
For e.g. 12.2300 contain six significance digits, i.e.1, 2, 2, 3, 0 and 0 are present. In 0.000122300 only six significant digits i.e. 1, 2, 2, 3, 0 and 0 are present.

Rules for using significant figures

i. Retain all the digit which give only one uncertain figure
e.g. During titration, burette reading between 10.4 ml and 10.6 ml is noted as 10.5 ml, but note as 10.50 ml which is indicate that the reading lies between 10.49 ml to 10.51 ml.

ii. During rounding off,

Add one digit to the last figure retained if the first number of rejected figure is 5 or above it.

For e.g. 3.456 if it is to be displayed with 3 significant figure than it is written as 3.46.

When the first number of rejected figure is less than 5, the figure is rounded off unchanged.

For e.g. 3.454 is to be displayed with 3 significant figure than it is written as 3.45.

When first number of rejected figure is 5 and there are no figure or only zero beyond it, the figure in the last place to be retained in increased by 1 if it is odd and left as it is if it is even.

For e.g. 1.325 is written as 1.32, while 1.335 is written as 1.34.

When first number of the rejected figure is 5 and there are other numbers beyond it, the figure in the last place to be retained is increased by 1 whether it is odd or even.

For e.g. 1.3254 is written as 1.33 (three significant figure)

iii. During addition and subtraction, round of the figures to the last decimal which is significant for each measurement or round of the figures to the same decimal places as the number with least decimal places.
e.g. 25.6788 + 0.345, which should be written as 25.679 + 0.345

iv. During multiplication also, significant figures of answer should be same as the significant factors present in one of the number used in calculation which have least significant numbers.

$1.69 \times 2.09 = 3.5321$.

The final answer is written by rounding off as 3.53.

$4.522 \times 15.4 = 69.6388$ written as 69.6

$4.522 \times 15 = 67.83$ written as 68

(c) PHARMACOPOEIAS

The word pharmacopoeia comes from two Greek words, Pharmakon = drugs/medicine and Poiein = to make.

For preparation of medicines thee is requirements of specific direction, which is written in the book called **Pharmacopoeia.** Pharmacopoeia is a legalized book of standard issued under the authority of government and it contains list of drugs/medicines, their descriptions/identification test, physical and chemical tests, formula, assays, tests for purity, dose, uses and official formulations (Tablet, Capsule, Injection, Syrup, Elixir, Powder)

This book is published by Government Authority of the country's council nominated by the government e.g.

Indian Pharmacopoeia (I.P): Published by Government of India and Ministry of Health & Family welfare. It was first published in 1955, then 1966, 1985, 1996, 2007, 2010, 2014 and 2018.

British Pharmacopoeia (B.P): It is published by General Medicinal Council of Great Britain.

United States Pharmacopoeia (U.S.P): It is published by private organization in U.S.A.

International pharmacopoeia: It is published by World Health Organization (WHO) in 1955.

British Pharmaceutical Codex (B.P.C): It is published by Concil of the Pharmaceutical Society of Great Britain.

National Formulary (N.F): It is published by private organization in U.S.A.

Indian Pharmacopoeia (I.P): Indian Pharmacopoeia (LP) is an official text responsible for the quality control and assurance of pharmaceuticals in India. This book does so by contributing in their safety, efficacy, and affordability. A plethora of official analytical procedures and drug specifications are present in IP. This book and its subsequent parts have got approval under the Second Schedule of the Drugs & Cosmetics Act, 1940 and Rules 1945. The standards for identity, purity, and strength of drugs with respect to the health of humans and animals are mentioned in IP

After independence, the Indian Pharmacopoeia Committee was constituted in 1948 for the publication of I.P. as its main function. The Indian Pharmacopoeia editions are:

1) Indian Pharmacopoeia, 1955: First edition, followed by its supplement in 1960.

2) Indian Pharmacopoeia, 1966: Second edition, followed by its supplement in 1975.

3) Indian Pharmacopoeia, 1985: Third edition, followed by its addendum in 1989 and 1991.

4) Indian Pharmacopoeia, 1996: Fourth edition, followed by its addendum in 2000 and 2002.

5) Indian Pharmacopoeia, 2007: Fifth edition, followed by its addendum in 2008. It is presented in the following three volumes:

i. **Volume 1** contains the general notices, preface, and structure of the I.P.C., acknowledgement, introduction, and the general chapters.
ii. **Volume 2** deals with the general monographs on drug substances dosage forms, and pharmaceutical aids (from A-M).
iii. **Volume 3** contains monographs on drug substances, dosage forms, and pharmaceutical aids (from N-Z) followed by monographs on vaccines and immunisers for human use, herbs and herbal products, blood and blood related products, biotechnology products, and veterinary products.

6) Indian Pharmacopoeia, 2010: Sixth edition, followed by its addendum in 2012. It is presented in the following three volumes:

i. **Volume I** contains the general notices, preface, and constituents of the P.C., acknowledgement, introduction, and the general chapters.
ii. **Volume II** consist of general notices and monographs related to dosage forms, drug substances and pharmaceutical aids (from A-M).
iii. **Volume III** contains monographs related to drug substances, dosage forms, and pharmaceutical aids (from N-Z). This volume also contains monographs of vaccines and immunosera for human use, herbs and herbal products, blood and blood related products, biotechnology products, and veterinary products.

7) Indian Pharmacopoeia, 2014 with DVD: Seventh edition, followed by its addendum in 2015.

The Indian Pharmacopoeia, 2014 was introduced according to the principal and plans designed and decided as per the Scientific Body of the I.P.C. The monographs, appendices, as well as other revised information are made public on the website of I.P.C.

It was done in order to gain transparency in setting standards as well as for getting feedback from the public. These feedbacks are reviewed by the expert members of the committee for testing viability and practicality of methods and standards.

The I.P. 2014 was scheduled to be effective from **1st January, 2014**, but came into effect from **1st April, 2014**. The reason behind the delay was to give the much needed time to industries so as to adopt the changes and policies under the I.P. 2014.

The 7th edition I.P. 2014 includes:

1) A total of 2548 monographs, out of which 577 new monographs were added.

2) 19 monographs and a general chapter related to radiopharmaceuticals.

3) 4 volumes which are in hard bound, along with a DVD.

4) Monographs related to veterinary.

5) A uniform pattern was followed for presenting a subject matter.

6) Use of chromatographic methods.

7) Many monographs related to herbs.

8) Certain monographs not present in other major pharmacopoeias globally.

9) A certificate to prove its authenticity.

10) The format is quite easy and simple to understand.

11) Cross referencing was eliminated.

12) Traditional tests were replaced with more specific tests, such as IR and UV spectrophotometry.

13) Pyrogen testing was eliminated virtually

14) Irrelevant monographs were eliminated.

7) Indian Pharmacopoeia, 2018: The Indian Pharmacopoeia Commission (IPC) has released the Eighth edition of Indian Pharmacopoeia (IP-2018). It was released by the Secretary, Ministry of Health & Family Welfare, and Government of India.

IP-2018 has been brought out in **4 Volumes** incorporating 220 new monographs *(Chemical Monographs (170), Herbal Monographs (15), Blood and Blood related products (10), Vaccines and Immunosera for Human use monographs (02), Radiopharmaceutical monographs (03), Biotechnology Derived Therapeutic Products (06), Veterinary monographs (14))*, 366 revised monographs and 7 omissions.

- Salient Features of IP-2018 are:
  - General Chemical tests & Thin Layer Chromatography (TLC) for identification of an article have been almost eliminated; and more specific infrared, ultraviolet spectrophotometer and HPLC tests have been emphasized (given special importance).
  - Most of the existing Assays and Related Substances Test methods have been upgraded by **liquid chromatography** to harmonize with other International Pharmacopoeias.
  - Pyrogen test has been replaced by Bacterial Endotoxin test (BET) in parenteral preparations and other monographs.
  - For ease of access to Pharmacopoeia, an Index has been incorporated in Volume-I along with the already existing index in Volume-IV of IP.
  - 53 New Fixed Dose Combination (FDCs) monographs have been included, out of which 25 FDC monographs are not available in any Pharmacopoeia.
  - General Chapters on Volumetric Glassware, Conductivity, Dissolution test, Disintegration test, Dimensions
  - of Hard Gelatin Capsule Shells etc. have been revised.

British Pharmacopoeia (B.P.)

The British Pharmacopoeia (B.P.) is published annually and comprises of the standards required for maintaining quality of medical substances of United Kingdom (U.K.). B.P. is normally employed by professionals as well as organizations related to pharmaceutical research, development, manufacturing, and testing.

In **1864**, the **1st edition** of British Pharmacopoeia was published. This edition was compiled with the help of three old and reputed pharmacopoeias which were **Pharmacopoeia Londinensis 1618**. **Edinburgh Pharmacopoeia 1699**, and **Dublin Pharmacopoeia 1807**.

The further editions and their addendums were followed in quick progression, in the year **1867**. The **2nd edition** of B.P. was published in **1874**. The **3rd edition** of B.P. was published in the year **1885** with its addendum in **1890**. The year **1898** marked the coming of **4th edition**.

The **5th edition** was published in **1914**, which was little delayed. After the publication of this edition there arose a need for different setup for the preparation of pharmacopoeias because of the increased technical complexicity in drug specifications. As a result, commission was made in 1928 which published the **6th edition** in **1932.**

In the **6th edition** 1932, variety of diagnostic materials was integrated; apart from that, standard tests for antioxidants, and insulin were also included.

In the year **1948**, **7th edition** containing the generic names for the newly introduced substances in medicines was published. Various analytical methods, such as disintegration tests in case of tablets and methods of sterilization were also added. Monographs related to sex hormones and penicillin was also added.

In **1953** the **8th edition** was published, in which all the titles of drugs, as well as preparations were given in English in place of Latin, but still Latin was used in abbreviated form for the synonyms. Capsules were also added for the first time and the implant methods along with standard tests for sex hormones were also included.

160 new monographs were added in the **9th edition** which was published in **1958**. Tranquilizers and spectrophotometry were also included.

In the year **1963**, **10th edition** of B.P. was published

In **1970**, Medicines Order was passed which defined the duties of the British Pharmacopoeia Commission. The **13th edition** of British Pharmacopoeia which was published in **1980** was the first pharmacopoeia which was prepared as per the provisions mentioned under Medicines Act.

From this edition the two volumes of B.P. were published due to inclusion of more number of drugs and their information.

Editions and volumes of B.P.

1. **Volume I and II:** it consists of medicinal substances.

2. **Volume III:** It consists of:
   i. Formulated preparations
   ii. Blood related preparations

iii. Immunological products
iv. Radiopharmaceutical products
v. Surgical materials
vi. Homeopathic preparations

3. **Volume IV:** It consist of
   i. Appendices
   ii. Infrared reference spectra
   iii. Index
4. **Volume V:** It consists of British Pharmacopoeia (veterinary).
5. Volume VI: It consists of
   i. CD-ROM version
   ii. British Pharmacopoeia
   iii. British Pharmacopoeia (veterinary), and
   iv. British Approved Names

- **Latest edition- British Pharmacopoeia, 2016**

Last edition became effective from **1st January, 2016**. This Pharmacopoeia includes:

1. 37 new monographs
2. 142 amended monographs
3. One new B.P. appendix for DNA identification of herbs.
4. All European Pharmacopoeia monographs integrated

United States Pharmacopoeia (U.S.P.)

The U.S.P is published in a combined volume with the National Formulary as the U.S.P.-NF. If a drug ingredient or drug product has an applicable U.S.P quality standard (in the form of a USP-NF monograph), it must conform in order to use the designation "U.S.P." or "NF".

Drugs subject to U.S.P. standards include both human drugs (prescription over-the-counter, or otherwise), as well as animal drugs. The U.S.P. also sets standards for dietary supplements, and food ingredients.

In all of the United States, the U.S.P. acts as an official public standard for setting an authority over all the prescriptions. OTC drugs, healthcare products which are either manufactured or sold. The standards for food ingredients and dietary supplements are also regulated by U.S.P. The regulation of these standards ensures quality, purity, strength, and consistency of the products which are being manufactured for the consumption by the general public.

In the year 1820, the very **1st edition of U.S.P.** was compiled, edited, and published. It contains 217 drugs and covers around 272 pages. The consequent editions were published after a decade. The 9th edition was published in 1905, but was titled U.S.P. VIII as it was the 8 revision.

The U.S.P. and NF (National Formulary) got unified on **5th July, 1974** and thereafter a single volume is published for both. The U.S.P. covers all the pharmaceutical substances and their products, while the NF exclusively covers the pharmaceutical ingredients. **In 1990, the 22nd edition** of U.S.P. (U.S.P. 22) was combined with the 17th edition of NF (NF 17).

The U.S.P. 37 and NF 32 edition was published on November 2013 and was officially adopted in May 2014. It included more than 4700 monographs having specifications related to identity, strength, quality, purity, packaging, and labelling of substances and dosage forms incorporated in it. There are above 270 general chapters which provide clear, simple guidance for assays, tests, and other procedures included.

- **Latest Edition - U.S.P. 39-NF 34, 2016**

The United States Pharmacopeia and the National Formulary (USP-NF) is a book of public pharmacopoeial standards for chemical and biological drug substances, dosage forms, compounded preparations, excipients, medical devices, and dietary supplements.

The latest edition of U.S.P.-NF includes:

1) More than 4,900 monographs with specifications for identity, strength, quality, purity, packaging, and labelling for substances, and dosage forms

2) More than 300 general chapters providing clear, step-by-step guidance for assays, tests, and procedures

3) Focus-specific charts and a combined index to find the information needed

4) Helpful sections on reagents, indicators, and solutions, along with reference tables.

Impurities in Pharmaceuticals

Various types of impurities are generally found in Pharmaceuticals.

- Some of the impurities produces **toxic effects** on body, if present beyond prescribed limit, e.g. Lead and Arsenic; those which are harmless but if present beyond the limit will lower down active strength of the medicinal compound, e.g., sodium and potassium salt.
- The impurities if present beyond the limit, which affect the storage property of pharmaceuticals, e.g. presence of moisture may affect the free flowing property and may decompose the substance.
- Some impurities such as those who alter taste, odour, colour or appearance and are easily detectable, e.g., phenolic impurities present sodium salicylate alter its odour.

Sources for Impurities in Medicinal Agents

The impurities in drug products may be obtained from various sources. Starting materials, byproducts, residual solvents, degradants formed and long-term storage are the main sources of impurities.

Heat, light changes in formulation pH, interaction with packaging and other components also results in impurities.

Sources of impurities in pharmaceutical preparations are:

i. **Raw Materials used in the Manufacturing Process:**
   Impurities of raw materials may affect the process of manufacturing and contaminate the product.

a) Majority of sodium containing compounds are prepared from sodium chloride and therefore, traces of chloride ions may be present in final production.

b) Metallic Zinc may be present as an impurity in zinc oxide sample, as it is prepared by metallic zinc.

c) For example, Sodium chloride is prepared from rock salts, which also contains traces of calcium sulphate and magnesium chloride so that a small quantity of calcium and magnesium will be present in the sodium chloride.

ii. Reagents used in the Manufacturing process:

The impurities from the reagents may contaminate the final product if they are not washed away properly.

For e.g. water is a cheapest solvent that contains chloride, magnesium, calcium etc., ions and these may be present as impurities.

In acids, chloride and sulphate ions may be present as impurities.

iii. Processes used in the manufacturing process:

Different manufacturing procedure and raw materials are used for producing many drugs and chemicals. During these processes of manufacturing some impurities get access into the materials.

For some drugs, multiple-step synthesis procedure is used for the production of intermediate compound. This intermediate compound need to be purified or else the final product will be contaminated by the impurities present.

- Impurities may access the final product at various stages of reaction:

➢ During the formulation process, the drug goes through various reactions, resulting in its degradation and detrimental reactions. For e.g. Solutions or suspensions are more likely to degrade due to hydrolysis or solvolysis.

➢ During formulation, due to exposure to heat, light, pH change, solvents etc., impurities are produced. A product may degrade if it is dried by heating.

➢ Impurities may present in the solvents employed during synthesis. These impurities can further undergo reaction with the chemicals that produce impurities in the synthesis reaction.

➢ Some chemical processes like nitration, halogenation, oxidation, reduction, hydrolysis etc., are the various reaction involved in the synthesis of drugs. During theses chemical reactions some impurities are formed. For e.g. when nitrogenous organic matter and alkalies are burned together, sea weed cyanides are formed.

iv. Material of Plant/Equipments:

The equipment used in the manufacturing process is made up of metals like copper, aluminum, iron, nickel and stainless steel. Due to solvent action on such equipments, traces of metals are introduced as impurities. For e.g. water and steam pipes may contain lead and other heavy metals which may be present as an impurity.

v. Inadequate storage condition:

Storage conditions adversely affect the stability of every drug. If drug is not stored properly, impurities get added to the drug and hence its activity deteriorates or toxicity increases.

a) Stored product may contaminated with dust, bodies of insects, etc.

b) Upon storage, the most common decomposition reactions in medical products are oxidation and phytochemical reactions followed by absorption of light by the molecules. Atmospheric oxygen can initiate oxidation of a medicament even present in a low concentartions.

c) Such reactions are catalyzed by traces of metals and ultraviolate radiation from light sources. E.g., chloroform, ether, violate oils, fat vitamin A, vitamin C, vitamin D and many other organic drugs are degraded by action of oxygen.

d) The photo-oxidation of tablet coatings is particularly occurs in the pharmaceutical industries. E.g. ferrous sulphate is converted into insoluble ferric oxide by air and moisture, surgical solution of chlorinated soda rapidly degradate when exposed to heat and ight. Therefore, it should be prevented in lead-free-air-tight bottles of green glass.

e) The compound which when exposed to atmosphere, loses its water or crystallization and is converted to anhydrous powder form, is known as an efflorescent compound. For e.g. ferrous sulphate.

f) The compound which absorb water from the atmosphere to such an extent that a concentrated solution of the solid eventually forms, is known as deliquescent compound. These types of compounds upon storage may contaminate with some impurities present in atmospheric water or moisture.

vi. Environmental related impurities:

Environmental conditions such as temperature, atmospheric gases, moisture content, light and microorganisms are the worst enemies of drugs.

Certain chemical reaction always take place inside the drug molecules whether it is in tablet, powder, syrup or liquid system than in dry state.

Therefore solutions are less stable than suspensions which in turn are less stable than solid dosage forms. Most of drugs are tendency to degrade.

LIMIT TESTS

Limit test is defined as a quantitative or semi-quantitative test designed to identify and control small quantity of impurity that is likely to be present in any pharmaceutical.

These identification is done by simple comparison of opalescence, turbidity, colour or precipitates with the standard as prescribed by the respective pharmacopoeias. The extent of opalescence, turbidity, precipitates or colour produced is influenced by presence of other impurities, variation in time or that of method of performance, due to which, **pharmacopoeias do not prescribe numerical values for the limit test.**

The limit tests are those, which are performed to know whether the impurities in the substance are below or beyond the limits prescribed.

Limit Test for Chloride

Principle

It is based on well-known reaction between soluble chloride and silver nitrate reagent resulting in the formation silver chloride which make the solution opalescence.

$$NaCl + AgNO_3 \longrightarrow AgCl + NaNO_3$$

Sample may contain impurities like carbonates, sulphides, phosphates, chromates and dichromates which gives precipitates with silver nitrate forming salts like Ag_2CO_3, AgS, Ag_3PO_4, Ag_2CrO_4, $Ag_2Cr_2O_7$. Dilute nitric acid dissolves all the above precipitates whereas silver chloride is insoluble. This insoluble silver chloride is responsible of opalescence solution.

Amount of chloride present in the test sample influences the degree of turbidity. With a known amount of chloride ions, the opalescent solution obtained and is compared with a standard opalescent solution.

By viewing transversely through both the solutions against a dark background in Nessler's cylinder, opalescence is compared. The sample passes the limit test if the test solution is less turbid than the standard solution and fails in vice versa condition.

Procedure

In this limit test, a standard solution and test solution is prepared and then the appearance of these two solutions is compared:

1) Test Solution: 1.0 gm of sample is accurately weighed and transferred to Nessler cylinder A after dissolving in 10 ml of distilled water. 10 ml of dilute nitric acid is added to this solution and volume is made up to 50 ml with distilled water. 1 ml of 0.1 M silver nitrate is then added to the solution with stirring and the resultant solution is set aside for 5 minutes protected from light, after which turbidity develops.

2) Standard Solution: Take 1ml of 0.05845 % w/v solution of sodium chloride in Nessler cylinder. Add 10.0 ml dilute nitric acid and dilute to 50 ml with water. 1 ml of 0.1 M silver nitrate solution is then added stir immediately with glass rod and allow to stand for 5 minutes which produces turbidity after 5 minutes.

Compare the opalescence produced in the test solution with that of standard. The opalescence produced in test should not be greater than that of standard. The sample passes the limit test if it is less opalescent or turbid than the standard opalescence.

Test Solution	**Standard Solution**
Specified substance +10 ml of water + 10 ml of dilute HNO_3	1 ml of 0.05845 % w/v solution of sodium chloride + 10 ml of dilute M HNO_3
↓	↓
Diluted to 50 ml in Nessler cylinder A + 1 ml of 0.1 M $AgNO_3$ solution	Diluted to 50 ml in Nessler cylinder B + 1 ml of 0.1 M $AgNO_3$ solution
↓	↓
Opalescence/turbidity	Opalescence/turbidity

Limit Test for Sulphate

This test is carried out for controlling sulphate impurities in inorganic substances.

Principle

Limit test of sulphate is based on the reaction between soluble sulphate (present as an impurity) and barium chloride in presence of dilute hydrochloric acid resulting in formation of barium sulphate precipitate which cause the turbidity.

$$\mathbf{SO_4^{-2} + BaCl_2 \longrightarrow BaSO_4 + 2Cl^-}$$

Here, the samples may contain impurities of Cr_3^{-2}, S^{-2}, PO_4^{-3}, CrO_4^{-2} and $Cr_2O_7^{-2}$ which give precipitates with barium sulphate forming respective salts like $BaCO_3$, BaS, $Ba_3(PO_4)_2$, $BaCr_2O_7$. Dilute hydrochloric acid dissolves all the above precipitated, whereas barium sulphate is insoluble.

Thus, the turbidity produced in the solution because of insoluble barium sulphate in dilute hydrochloric acid.

This turbidity produced in test solution is compared with that of the standard.

Procedure:

Preparation of Barium Sulphate solution:

(a) Test Solution: 1 ml of sample is weighed and 2 ml of dilute hydrochloric acid is added to dissolve in a nessler cylinder. The resultant solution is diluted with 45 ml of water. Then 5 ml of $BaSO_4$ reagent is added to the solution. Stir immediately with glass rod and allow to stand for 5 minutes.

(b) Standard Solution: Dilute 1 ml of a 0.1089 % w/v solution of potassium sulphate into a Nessler cylinder. Add 2 ml of dilute hydrochloric acid. Then 45 ml of water is added to dilute the solution.

Then 5 ml of Barium sulphate reagent is added to the solution. Stir immediately with glass rod and allow to stand for 5 minutes.

Compare the turbidity produced in test solution with that of the turbidity produced in the standard solution. Turbidity should not be greater than that of the standard.

Barium Sulphate reagent: Barium Sulphate Reagent: 15ml of 0.5M barium chloride (122.1gm/1000ml) is mixed with 55 ml of water, 20 ml of sulphate free alcohol and 5ml of 0.0181% w/v solution of potassium sulphate. The resultant solution is diluted up to 100ml with water.

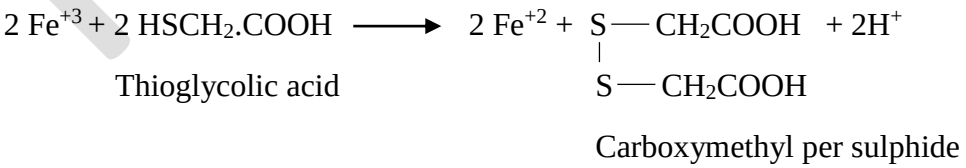
Test Solution	**Standard Solution**
Specified substance (1 gm) + 2 ml of diluted HCl + Diluted to 45 ml of water + 5 ml of $BaSO_4$ reagent ↓ Opalescence/turbidity	1 ml of a 0.1089 % w/v solution of potassium sulphate + 2 ml of diluted HCl + Diluted to 45 ml of water + 5 ml of $BaSO_4$ reagent ↓ Opalescence/turbidity

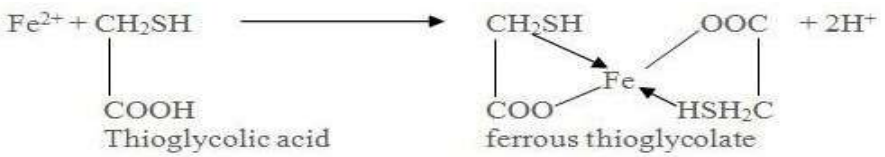
Limit Test of Iron

This test is carried out for controlling iron impurity in inorganic substances.

Principle:

Limit test for iron is based on the reaction between iron and thioglycolic acid in presence of citric acid, resulting the formation for ferrous-thioglycolate complex, which gives pale pink to deep reddish purple colour in basic media provided by ammonia.





Thioglycolic acid act as a reducing agent and complexing agent. Thioglycolic acid reduces ferric ion into the ferrous ion.

The colour of ferrous thioglycolate complex is stable in absence of air but fades after sometimes due to oxidation. The coulour is destroyed in presence of oxidizing agents (acids) and strong alkalis.

As purple colour is developed only in basic media, ammonia solution is used. But ammonia reacts with iron forming precipitate of ferrous hydroxide, therefore citric acid is used which prevents the precipitation of iron with ammonia by forming a complex with iron as iron citrate.

Procedure

Sr No	**Test Solution**	**Standard Solution**
1	Dissolve the specified quantity of substance in 40 ml water or use 10.0 ml of solution as prescribed in the monograph and transfer to Nessler cylinder	Dissolve 2.0 ml of standard iron solution with 40 ml of water into Nessler Cylinder.
2	Add 2.0 ml of 20 % w/v solution of iron free citric acid.	Add 2.0 ml of 20 % w/v solution of iron free citric acid.
3	Add 0.1 ml of thioglycolic acid and mix well.	Add 0.1 ml of thioglycolic acid and mix well.
4	Make alkaline with iron-free ammonia solution	Make alkaline with iron-free ammonia solution
5	Dilute to 50 ml with water	Dilute to 50 ml with water
6	Mix well and allow to stand for 5 minutes	Mix well and allow to stand for 5 minutes

Compare the colour produced in test solution with that of standard. The colour produced in test solution should be more intense than that of the standard.

Some essential points that should be kept in mind while performing the limit test for iron are as follows:

1. Colour is developed and not turbidity
2. The solution should be compared immediately after 5 minutes, else the colour fades away due to oxidation, making the test unreliable.
3. This test is very sensitive.

Limit Test for Arsenic

Arsenic is an undesirable and harmful impurity in medicinal substances, and all pharmacopoeias prescribe a limit test for it. There are many qualitative and quantitative tests for arsenic. The pharmacopoeial method is based on the **Gutzeit test**.

Principle:

In this test, arsenic is converted in to gaseous arsenious hydride or arsine (AsH_3), which when passed over a mercuric chloride test paper, produces a yellow stain. The intensity of the stain is proportional to the amount of arsenic present. This is to be compared with standard stain produced by the definite amount of arsenic.

The chemical reaction involved in the method are given below:

The substance to be tested is dissolved in acid, the arsenic present in the sample is converted to arsenic acid. The arsenic acid is reduced to arsenious acid, by reducing agents (like potassium iodide, stannous chloride etc.).

$$\underset{\text{Arsenic Acid}}{H_3AsO_4} + 6H^+ \longrightarrow \underset{\text{Arsenious acid}}{H_3AsO_3}$$

The nascent hydrogen produced during the reaction, further reduces arseniuos acid to arsine (gas), which reacts with mercuric chloride paper, producing a yellow stain.

$$\underset{\text{Arsenious acid}}{H_3AsO_3} + 3H_2 \longrightarrow \underset{\text{Arsine (gas)}}{AsH_3} + 2H_2O$$

To carry out the test, a specified apparatus is used, In order to convert arsenic into arsine gas, various reducing agents like zinc and hydrochloric acid, stannous chloride, and potassium iodide are employed.

The rate of evolution of gas is maintained by using a particular size of zinc, and controlling the concentration of acids and other salts of the reaction medium, besides temperature.

Any impurity coming along with the gas is trapped by placing a lead acetate soaked cotton plug in the apparatus. All the reagents employed for the test should be arsenic-free.

Apparatus:

The apparatus used for limit test for arsenic is known as **"Gutzeit Apparatus".**

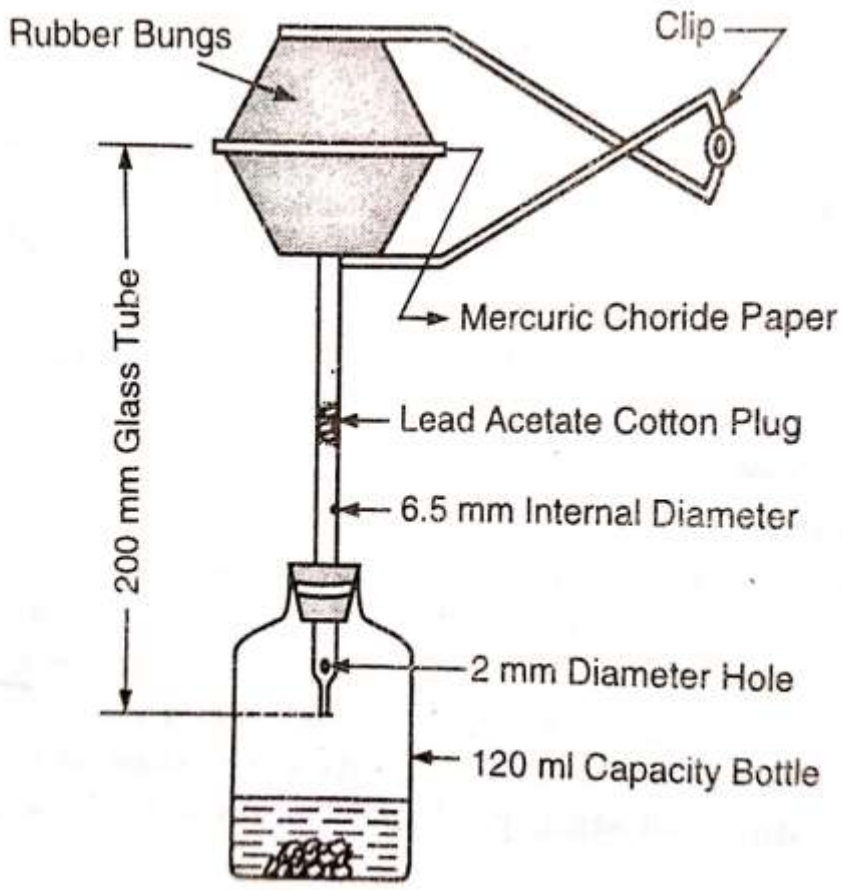
A wide mouth bottle of 100 ml capacity fitted with rubber bung carrying a glass tube 200 mm long and 5 mm internal diameter.

The lower part of the glass tube have a hole of 1 mm internal diameter and 15 mm from its tip is a lateral orifice 2 to 3 mm in diameter, which removes water vapour. During the test, the hole of the glass tube should be at least 3 mm below the lower surface of the stopper.

Another glass tube of the same internal diameter but 30 mm in length is placed in contact with the first and is held by two spiral springs or clips.

Into the lower tube insert 50 to 60 mg of cotton wool soaked with lead acetate solution. Insert a disc or small square of mercuric chloride paper large enough to cover the orifice of the tube, between the two tubes.

Two rubber bungs, each with a hole of 6.5 mm in diameter at the centre and are fitted with tube. These two rubber bungs are held tightly together by clip. Mercuric chloride paper is sandwiched between the rubber bungs. During the test, the gas released passes through mercuric chloride paper, produces stain on paper.



Gutzeit Apparatus –Limit test for Arsenic

Procedure:

Test solution: Into the bottle or conical flask introduce the test solution prepared as directed in the individual monograph, add 5 ml of 1 M potassium iodide, 5 ml of stannous chloride acid solution and 10 g of zinc AsT.
Immediately assemble the apparatus and immerse the flask in a water-bath at a temperature such that a uniform evolution of gas is maintained.

The reaction is allowed to continue for 40 minutes at about 40 °C. After 40 minutes yellow stain produced on the mercuric chloride paper.

Standard Solution: Prepare arsenic standard solution by Dissolve 0.33 g of arsenic trioxide in 5 ml of 2 M sodium hydroxide and dilute to 250.0 ml with water. Dilute 1 volume of this solution to 100 volumes with water.

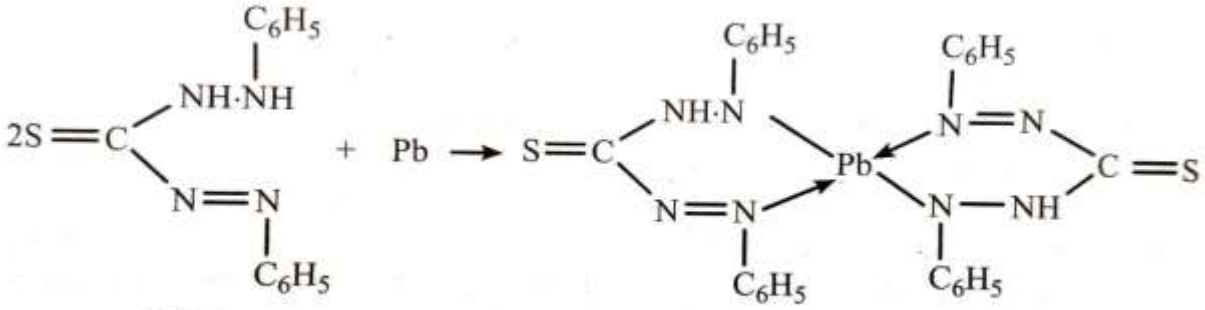
Add 5 ml of 1 M potassium iodide, 5 ml of stannous chloride acid solution and 10 g of zinc AsT. The reaction is allowed to continue for 40 minutes at about 40 °C. After 40 minutes yellow stain produced on the mercuric chloride paper.

Stain produced by test solution is not more intense than that obtained by 1.0 ml of arsenic standard solution when treating in the same manner.

Limit test for Lead

Principle

The limit test for lead relies on the formation of a complex by the reaction between lead and diphenylthiocarbazone (dithizone). A chloroform solution of dithizone is prepared, which extracts lead from alkaline aqueous solutions, forming a red-coloured lead dithizone complex.



Dithizone

Dithizone-lead complex (violet colour)

Amount of lead present in the solution influences the intensity of the complex. The colour of lead-thizone complex in chloroform and standard lead solution is compared.

Procedure

Test Solution: The sample solution of known quantity is prepared as prescribed in the monograph and transferred in a separating funnel. Then 6 ml of ammonium citrate, 2 ml of potassium cyanide, and 2 ml of hydroxylamine hydrochloride are added, followed by 2 drops of phenol red. Ammonia solution is added to make the resultant solution alkaline.

5 ml of dithizone solution is used to extract the above solution until it becomes green. With 20 ml of 1 % nitric acid, the combined dithiazone extracts are shaken for 30 seconds, converting all the lead dithiazone into nitrate. The remaining chloroform layer is discarded and again extracted with 5 ml of dithiazone extraction solution. Therefore, 4 ml of ammonium cyanide is added, the solution is shaken for 30 seconds, and the colour is observed.

Standard Solution: A standard solution is prepared similarly with volume of dilute standard lead solution equivalent to the amount of lead permitted in the sample being tested and containing all other reagents in the same quantity as in the test solution.

- The intensity of the colour of complex depends on the amount of lead in the solution. The colour produced in sample solution should not be greater than standard solution. If colour produced in sample solution is less than the standard solution, the sample passes the limit test of lead and vice versa.
- Ammonium citrate, potassium cyanide, hydroxylamine hydrochloride solutions are used to make the pH optimum, so that other impurities that influence and interfere with the test are removed, Phenol red is used as indicator to develop the colour at the end of process.
- Some essential points that should be kept in mind while performing the limit test of lead are as follows:
    1. All the reagents and solutions used should be free from lead.
    2. Glassware should be washed properly with warm dilute HNO_3 and then with water.

Limit test for Heavy Metals

The limit for heavy metals is indicated in the individual monographs in terms of ppm, i.e., the parts of lead, Pb, per million parts (by weight) of the substance under examination.

1. Method A

Standard solution: Into a 50-ml Nessler cylinder pipette 1.0 ml of lead standard solution (20 ppm Pb) and dilute with water to 25 ml. Adjust with dilute acetic acid or dilute ammonia solution to a pH between 3.0 and 4.0, dilute with water to about 35 ml and mix.

Test Solution: Into a 50-ml Nessler cylinder place 25 ml of the solution prepared for the test as directed in the individual monograph or dissolve the specified quantity of the substance under examination in sufficient water to produce 25 ml. Adjust with dilute acetic acid or dilute ammonia solution to a pH between 3.0 and 4.0, dilute with water to about 35 ml and mix.

Procedure: To each of the cylinders containing the standard solution and test solution respectively add 10 ml of freshly prepared hydrogen sulphide solution, mix, dilute to 50 ml with water, allow to stand for 5 minutes and view downwards over a white surface; the colour produced with the test solution is not more intense than that produced with the standard solution.

2. Method B

This method is suitable for substances not producing clear and colorless solutions under the test conditions prescribed for method A or for substances which hinder the precipitation of metals by sulphide ion due to their complex nature.

Standard solution: Into a 50-ml Nessler cylinder pipette 1.0 ml of lead standard solution (20 ppm Pb) and dilute with water to 25 ml. Adjust with dilute acetic acid or dilute ammonia solution to a pH between 3.0 and 4.0, dilute with water to about 35 ml and mix.

Test solution: Weigh in a suitable crucible the quantity of the substance specified in the individual monograph, add sufficient *sulphuric acid* to wet the sample, ignite carefully at a low temperature until thoroughly charred. Add to the charred mass 2 ml of *nitric acid* and 5 drops of *sulphuric acid* and heat cautiously until white fumes are no longer evolved. Ignite, preferably in a muffle furnace, at 5000 to 6000, until the carbon is completely burnt off. Cool, add 4 ml of *hydrochloric acid,* cover" digest on a water-bath for 15 minutes, uncover and slowly evaporate to dryness on a water-bath. Moisten the residue with 1 drop of *hydrochloric acid,* add 10 ml of hot *water* and digest for 2 minutes. Add *ammonia solution* dropwise until the solution is just alkaline to *litmus paper,* dilute to 25 ml with *water* and adjust with *dilute acetic acid* to a pH between 3.0 and 4.0. Filter, if necessary, rinse the crucible and the filter with 10 ml of *water,* combine the filtrate and washings in a 50-ml Nessler cylinder, dilute with *water* to about 35 ml and mix.

Procedure: Proceed as directed under Method A.

3. Method C

Standard solution: Into a 50-ml Nessler cylinder pipette 1.0 ml of *lead standard solution (20 ppm Pb),* add 5 ml of *dilute sodium hydroxide solution,* dilute with *water* to 50 ml and mix.

Test solution: Into a 50-ml Nessler cylinder place 25 ml of the solution prepared for the test as directed in the individual monograph, or dissolve the specified quantity ofthe substance under examination in a mixture of 20 ml of *water* and 5 ml of *dilute sodium hydroxide solution.* Dilute with *water* to 50 ml and mix.

Procedure: To each of the cylinders containing the standard solution and the test solution respectively add 5 drops of *sodium sulphide solution*, mix, allow to stand for 5 minutes and view downwards over a white surface; the colour produced with the test solution is not more intense than that produced with the standard solution.

4. Method D

Standard solution: Into a small Nessler cylinder pipette 10.0 ml of either *lead standard solution* (1 *ppm Pb)* or *lead standard solution* (2 *ppm Pb).*

Test solution: Prepare as directed in the individual monograph and pipette 12 ml into a small Nessler cylinder.

Procedure: To the cylinder containing the standard solution add 2.0 ml of the test solution and mix. To each of the cylinders add 2 ml of *acetate buffer pH* 3.5, mix, add 1.2 ml of *thioacetamide reagent*, allow to stand for 2 minutes and view downwards over a white surface; the colour produced with the test solution is not more intense than that produced with the standard solution.