CHAPTER 1

INTRODUCTION TO PHARMACEUTICAL CHEMISTRY, SOURCES & TYPES OF ERRORS AND IMPURITIES IN PHARMACEUTICALS

1.1 INTRODUCTION TO PHARMACEUTICAL CHEMISTRY

❖ Introduction

- Pharmaceutical Chemistry is the branch of chemical science that deals with the study of drugs, and it involves drug development.
- This includes drug discovery, delivery, absorption, metabolism, and more.
- Pharmaceutical chemistry involves cures and remedies for disease, analytical techniques, pharmacology, metabolism, quality assurance, and drug chemistry.
- Pharmaceutical chemistry leads to careers in drug development, biotechnology, pharmaceutical companies, research facilities, and more.

❖ The subject is further subdivided into various branches.

Pharmaceutical inorganic chemistry- Pharmaceutical inorganic chemistry is the branch of pharmaceutical chemistry that deals with the study of preparation, standards of purity, limit test for determining quality, purity and storage conditions of all inorganic compounds.

Pharmaceutical Organic chemistry- Pharmaceutical organic chemistry is the main branch of organic chemistry deals with the study of preparation, structure and reactions of organic (Hydrocarbon) compounds.

Pharmaceutical Analytical chemistry- Pharmaceutical Analytical chemistry is the branch of pharmaceutical chemistry that deals science of obtaining, processing, and communicating information about the composition and structure of matter.

Pharmaceutical Physical chemistry- Pharmaceutical Physical pharmacy is the branch of pharmaceutical chemistry that concentrates on the applications of physics and chemistry to the study of pharmacy.

Pharmaceutical Phytochemistry- Deals with study of phytochemicals, which are chemicals derived from plants.

Pharmaceutical Medicinal Chemistry- deals with the discovery, design, development and both pharmacological and analytical characterization of drug substances.

Pharmaceutical Biochemistry- Pharmaceutical biochemistry is the branch of biochemistry that studies how molecules in the body interact with drugs.

1.1.1 Scope of Pharmaceutical Chemistry

- Pharmaceutical Chemistry allows for collaboration with researchers from other disciplines like microbiologist, theoretical chemists, pharmacologist, toxicologist, biologists, and bio-pharmacists in developing new drugs as it is a stimulating discipline which links many scientific disciplines.
- By having a strong background in organic chemistry and medicinal chemistry, interested candidates can place themselves in a competitive position in this career field.

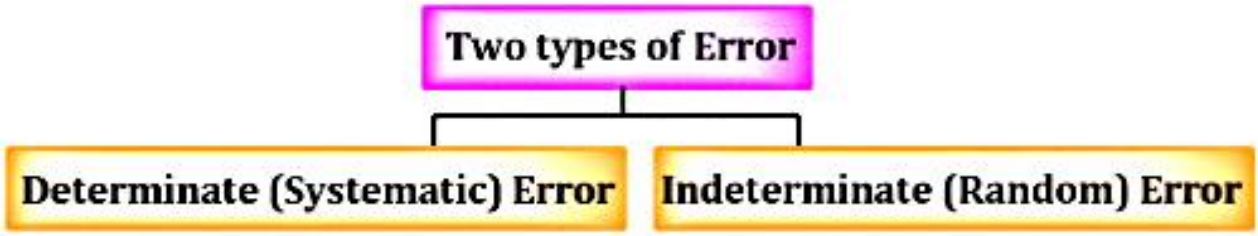
➢ Scopes in Industries

- Drug Discovery and Development.
- Computer Aided Drug Design.
- Organic and Medicinal Chemistry Division.
- Pharmaceutical Manufacturing Division.
- Pharmaceutical Bulk Drug Division.
- Pharmaceutical Analytical R and D.

1.1.2 Objectives of Pharmaceutical Chemistry

- The Scope and Objective of study of Pharmaceutical Chemistry is very vast and upgrading day by day to deal with modern medical requirement to meet current Health challenges.
- To improve the knowledge base required for **synthesis, isolation, purification and characterization of various pharmaceuticals**.
- To improve skills for effective **handling of chemicals, Glassware and analytical instruments.**
- To provide enduring atmosphere and encourage students & faculty for research activity.
- To **train faculty** & students in safe **handling of chemicals** & creating awareness about hazardous effects of chemicals.
- To provide students with the appropriate qualities & skills required to fulfill job responsibilities as chemists in pharmaceutical, chemical and biomedical industries.

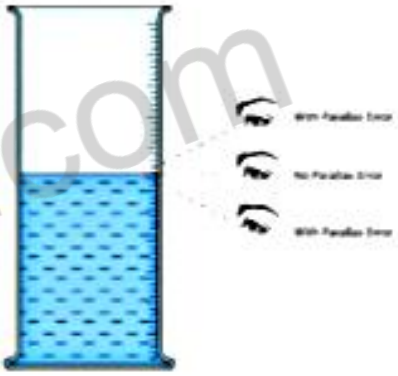
1.2.1 Classification of Errors



➢ **Determinant or Systemic or Constant Error-**

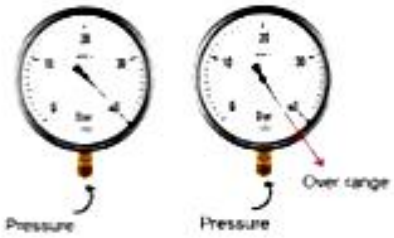
These are ascertainable errors that can be either avoided or corrected. The error may be constant as in the case of weighing with uncalibrated weights or in measuring a volume using burette or pipette. Such measurable determinate errors are categorized as systematic errors.

- **The most vital errors having a place with this specific class are:**



Personal error- These errors occur by persons who are handling the method of analysis. The error may be resulted due to carelessness or ignorance and even by unskilled persons. This error is also called operative error

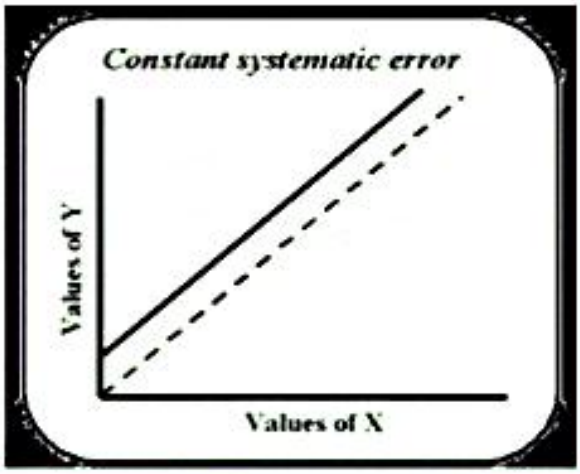
Instrumental errors- These errors are caused by faulty equipments or low quality equipments which do not perform well.



Chemical errors- These errors are resulted by using chemicals and reagents with impurities or contaminants which may interfere with the reactions, thus affects the result.

Constant Errors- Multiple measurements show the same constant error.

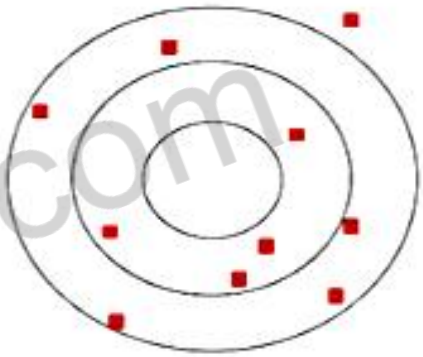
Example- if a scale of 15 cm actually measures 14.8 cm. Then it is measuring 0.2 cm more in every observation. This type of error will be same in all measurements done by the scale.



Errors due to Methodology- This is a most serious error in analysis as the error arises due to faulty method, e.g. co-precipitation of impurities, slight solubility of precipitate, incomplete reactions etc. Errors of this category are usually detectable and can be eliminated to a large extent.

➢ **Indeterminate or accidental or random Error**

- These are often **called accidental or random errors**, which represent experimental uncertainty that occurs in any measurements.
- These errors are shown by small differences in successive measurements made by the same analyst under almost similar conditions.
- These errors **cannot be predicted or determined**. However, they can be considerably reduced by careful work and by the increase in the number of repeated determinations.
- This kind of errors mainly affect the precision of measurement.



Random Error

✓ **Examples:**

- During the course of series of replicate measurements, the temperature might fluctuate slightly. This would cause slight variations in the volumes associated with the use of pipette or burette.
- Instrumental uncertainty is the major source of random error.

- Experienced chemist touches the pipette against the side of the conical flask and permit it to drain for a set time. However, the way in which they do this and the time which is allotted for drainage may vary, even if only slightly. This variation will cause a variation in the volume delivered.

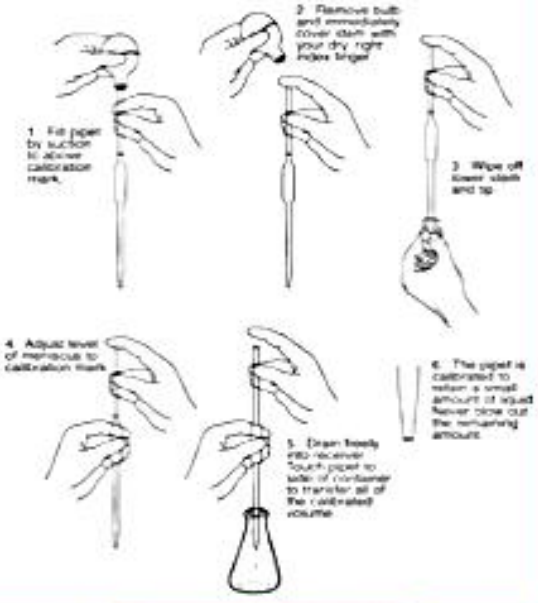


Table 1.2 - Difference between Determinant and In-determinant Error

S.NO.	DETERMINANT ERRORS	INDETERMINANT ERRORS
1.	These are also known as Systemic or non-random or Constant errors.	These are also known as Non-systematic/ Random/accidental errors.
2.	These errors are recognized by the lack of agreement between the mean of a series of replicate determinations and the correct value.	These errors are recognized by variability in the replicate determinations, i.e., by the scatter of result about their mean.
3.	These errors are determinable and can be eliminated.	These errors can never be determined and eliminated but can be minimized by careful work.
4.	These errors are quantified by a measure of accuracy such as absolute error or the relative error of the mean.	These errors are quantified by a measure of precision such as standard deviation or the relative standard deviation.
5.	The sources of these errors may be personal, instrumental and methodological bias.	The sources of these errors may be personal, instrumental uncertainties.

1.2.2 Methods to Minimize Errors

- Errors can be minimized by understanding the source and type of errors thoroughly.
- The predictable errors can be minimized by correcting the factor directly whereas the unpredictable errors can be minimized by following the standard protocols and good laboratory practices (GLP) strictly.

✓ **Some of the methods to minimize the errors are discussed as follows:**

1. Instrumental Errors: These errors can be minimized by checking thoroughly the equipments used for the analysis before starting of any analysis. Proper calibration should be performed to ensure the performance of equipments. Faulty equipments should be corrected by experts and rechecked for accuracy of results. If the performance is not satisfied then replacement should be done.

2. Personal Errors: Skilled persons should be employed or the knowledge of the operator to perform analysis is to be ensured prior to analysis. Regular reporting and monitoring of analysis can be done.

3. Chemical Errors: Standard chemicals from authentic source without impurities must be used for the analysis. The quality of chemicals and reagents can be checked periodically as per the standard guidelines.

4. Errors in Methodology: These errors can be avoided by following the standard methods with proper references. Continuous monitoring of reactions by skilled persons can be employed to minimize these errors.

5. Indeterminate Errors: Since indeterminate errors are not predictable, the entire procedure of analysis should be carried out in a well-planned manner considering all factors which affect the accuracy and precision of the results.

1.2.3 Accuracy and Precision

Accuracy- The term '**accuracy**' refers to the agreement of experimental value with the true value and it is usually expressed in terms of error.

The absolute error is a measure of accuracy. Absolute Error (E_{abs}) the absolute error of a determination is the ***difference between the observed or measured value*** and the true or most probable value of the quantity measured. The absolute error is a measure of the accuracy of the measurement.

The absolute error may be positive or negative (if sign is positive than measurement result is high and if the sign is negative measurement result is low).

$$\textit{Absolute Error } (E_{abs}) = (X - T)$$

Where, X is the observed or measured value and T is accepted the true value.

Precision- "The degree of agreement between various results of the same quantity". In other words, it is the reproducibility of result. It refers to the closeness of the set of values obtained from identical measurements of a quantity. Or it is the degree of agreement between replicate measurements of the same quantity, ie, repeatability of results. It represents the reproducibility of a measurement. The ***standard deviation*** is a measure of precision.

Higher the degree of precision, the greater the chance of obtaining true value. It is useless to hope that a value is accurate if the precision is poor and the analytical chemist must try repeatable results to assure the highest possible accuracy.

Standard deviation/root mean square deviation (S/S×D) - It is one of the most common statistical term employed in analytical chemistry. The Standard Deviation is a measure of how spread-out numbers is, in other words, it is a measure of precision. It is abbreviated as S or SD.

$$SD = \sqrt{\frac{\Sigma(x - \bar{X})^2}{n - 1}}$$

Where, n is the number of measurements.

1.2.4 SIGNIFICANT FIGURES

- The number of significant figures is the number of digits which are necessary to express results which are consistent with the precision of the measurement. A significant figure denotes which figures are really giving information about how precise our measurements are. The significant digits are indicated by underlining only those digits which are significant.

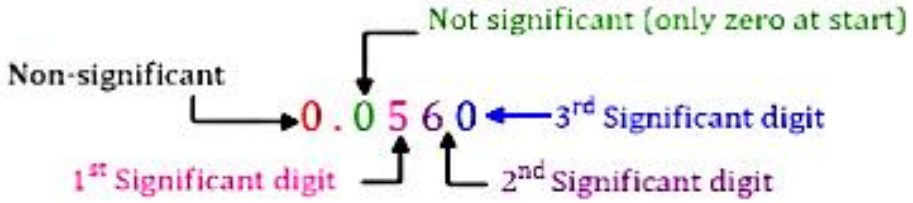
- **SIGNIFICANT FIGURES RULES**

SIGNIFICANT

- ✓ All non-zeros are significant E.g., 1, 2, 3, 4, 5, 6, 7, 8, 9.
- ✓ Trapped zeros/zeros appearing in between non- zero digits are significant E.g., 101, 2001, 0.01002, 90.001.
- ✓ Ending zeros with a decimal point are significant. E.g., 6.300, 50.000, 96.60.

NON-SIGNIFICANT

- ✓ All starting zeros are non-significant E.g.,0.0123, 0.00001
- ✓ All ending zeros without decimal point are non-significant E.g., 100, 55600, 50100.



- Exact numbers have an infinite amount of sig digs.
- Trailing zeros in a number containing a decimal point are significant. For example, 12.2300 has six significant figures: 1, 2, 2, 3, 0 and 0.
- The number 0.000122300 still has only six significant figures (the zeros before the 1 are not significant).
- **For example,** in **6575 cm** there are four significant figures and in **0.543** there are three significant figures. If any zero precedes the non-zero digit then it is not significant.

Table 1.3 - Example of Significant Figures

Number	Significant Digits Identified
15.20	4
01	1
0.004	1
0.02	1
35.5	3
1.200	4
1.35×10^5	3
501.562	6
26.025	5
64000	2

1.3 IMPURITIES IN PHARMACEUTICALS

❖ Impurities

- It is defined as foreign particle that affect the purity of the substance.

❖ Pharmaceutical Impurities

- Impurities in pharmaceuticals are the unwanted chemicals that remain with the active pharmaceutical ingredients (APL), or develop during formulation.
- The presence of these unwanted chemicals even in small amounts may influence the efficacy and safety of the pharmaceutical products.

1.3.1 Sources of Impurities in Pharmaceuticals

- Chemical compounds manufactured on commercial state contain different types of impurities, although the amount of purity may be very small.
- These impurities commonly present in pharmaceuticals include raw material, dust particle and moisture.

✓ From Raw materials

- The raw material required for manufacturing of pharmaceuticals are either synthesized or obtained from natural sources like; plants, animals, etc.
- During this process, along with desired substance traces of some other substances (impurities) are also get mixed with the desired substances.
- Hence, it is always necessary to use the raw materials available in the pure form.

Pharmaceutical	Raw material	Impurities Present in Raw Material
Copper compounds	Copper turnings	Arsenic and iron
Sodium compounds	Sodium chloride/rock salt	Chloride, calcium and magnesium

✓ Method or the Process used in Manufacture

- There are a number of drugs and chemicals, which are manufactured from different raw materials by adopting different methods or processes.
- Some impurities get incorporated into the materials during the manufacturing process.

- The type and amount of impurity present in the drugs or chemicals varies. Furthermore, for certain drugs a multiple-step-synthesis procedure is used, which produces intermediate compounds.

Chemical Processes and Plant Materials employed the Process

- In the synthesis of drugs, many chemical reactions like nitration, halogenations, oxidation, reduction, hydrolysis etc. are involved.
- In these chemical processes, different solvents, chemicals are used. When chemical reactions are carried out in iron, copper, tin, aluminium vessels, the solvents and chemicals in the vessels react with the metals, thereby forming reaction products.
- These reaction products derived from the plant material occur as impurities in the final product. Thus, impurities of iron, lead, heavy metals, copper etc., in substances are due to the above-mentioned reasons.

✓ Storage Condition

- The chemicals, substances when prepared are stored in different types of containers, depending upon the nature of the material, batch size and the quantity. Various types of materials are used for storage purpose.
- These could be plastic, polythene, iron vessels, stainless steel, aluminium, copper etc.
- Reaction of these substances with the material of the storage vessel takes place and the products formed, occur as impurities in the stored material.
- The reaction may take place directly or by the leaching out effect on the storage vessel. Alkalis stored in ordinary glass containers, extract lead from it, which occurs in the final product. Similarly, strong chemicals react with iron containers, and extract iron.

✓ Decomposition

- Some substances **decompose and the decomposition is greater in the presence of light, air or oxygen.**
- The result of decomposition causes contamination of the final product. Many substances lose water of crystallization when kept open, while deliquescent substances absorb water from the atmosphere, and get liquefied.
- Crude vegetable drugs are especially susceptible to decomposition.

- A number of organic substances get spoiled, because of decomposition on exposure to the atmosphere, **e.g. amines, phenols, potent drugs etc.**
- The decomposition products thus appear as impurities in the substances.

1.3.2 Effect of Impurities

- Almost pure substances are difficult to get and some amount impurity is always present in the material. So, the impurities which are present in the substances may have the following effects.

✓ Impurities may bring about incompatibility with other substances.
✓ Impurities may lower the shelf life of the substances.
✓ Impurities may cause difficulties during formulation and use of the substances.
✓ Sometimes Impurities changes the physical and chemical properties of the substances.
✓ Therapeutic effects can be decreased.
✓ Shows toxic effect after a certain period.
✓ Injurious when present above certain limits.
✓ It may change odour, colour, taste of the substance.

- To prevent these impurities many test such as limit test are carried out to lower the impurities to make the pharmaceuticals safer.

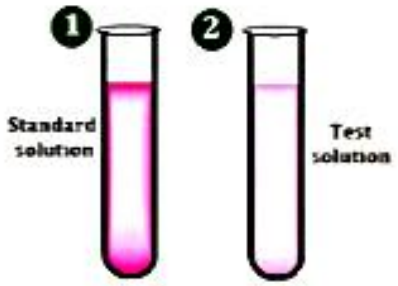
1.3.3 How We Can Minimize Pharmaceutical Impurities

- Evaluating starting material purity.
- Minimizing impurity levels in synthesis and manufacturing processes.
- Identifying impurity structures.
- Isolating and synthesizing impurities for qualification in toxicology studies.
- Monitoring the stability of APIs and DPs to detect degradation products.
- Storage and Stability Analysis.

1.4 LIMIT TESTS

- Limit tests are defined as, quantitative or semi-quantitative tests which are performed to identify and control small quantity of impurities which are likely to be present with the substances to be analysed. Generally, limit tests are carried out to identify the inorganic impurities present in a pharmaceutical substance.

- In these tests, the turbidity/opalescence/colour intensity produced by the test sample is compared with turbidity/opalescence/colour intensity produced by the standard.



❖ Importance of Limit Test

- To find out the harmful amount of impurities.
- To find out the avoidable/unavoidable amount of impurities.
- To determine the inorganic impurities, present in compound.
- To determine the total quantity of impurity in the substance.

1.4.1 LIMIT TESTS OF CHLORIDE

Principle - This test is based on the reaction in between soluble chloride as an impurity and silver nitrate which result in the formation of a precipitate of insoluble silver chloride in the presence of dilute HNO_3 giving an opalescence to the final solution.

Chemical reaction

$$Cl^- + AgNO_3 \xrightarrow{\text{dil. } HNO_3} AgCl + NO_3^-$$

Chloride ions + Silver nitrate → Silver chloride + Nitrate ions

- **Role of HNO_3 -** It is used to dissolve other impurities like; carbonate and phosphate, which otherwise will precipitate along with silver chloride and unnecessarily intensify the opalescence of the test solution.

$$2HNO_3 + CHO_3^{--} \longrightarrow H_2CO_3 + 2NO_3^-$$

Dil. Nitric acid + Carbonic acid → Carbonate ions + Nitrate ions

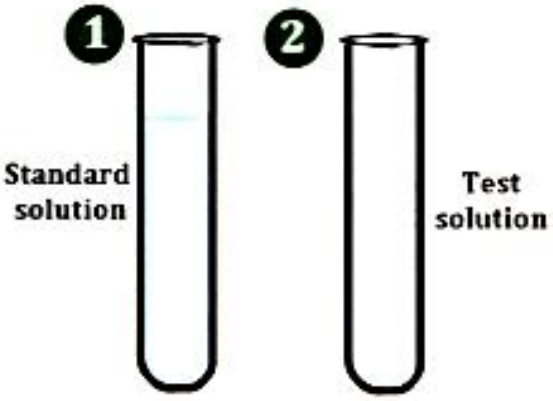
$$3HNO_3 + PO_3^{--} \longrightarrow H_3PO_4 + 3HNO_3^-$$

Dil. Nitric acid + Phosphate ions → Phosphoric acid + Nitrate ions

Procedure-

Standard Solution	Test Solution
• Place 1 ml of 10 ppm standard chloride solution and add 10 ml of dilute HNO_3 in Nessler's cylinder, mark 'S' diluting up to 50 ml with water and add 1 ml of 0.1 N $AgNO_3$ solution. • Stir immediately with a glass rod and allow it to stand for 5 minutes.	• Dissolve the specified quantity of test substance being examined in water or prepare a solution as directed in the monograph and transfer it to the Nessler's cylinder, mark 'T'. • Add 10 ml of dilute HNO_3 except when HNO_3 is used in the preparation of solution in Nessler's cylinder, diluting up to 50 ml with water and add 1 ml of 0.1 N $AgNO_3$ solution stir immediately with a glass rod and allow it to stand for 5 minutes.

- Protect the opalescence from the light and view it transversely against a black background and compare the opalescence produced by a test with that of standard opalescence.
- If test opalescence is less than or equal to Standard opalescence, then Limit test for chloride for given sample complies/passes with the official standards of I.P.
- If test opalescence is more than the Standard opalescence, then Limit test for chloride for a given sample doesn't comply/fails with the official standards of I.P.



1.4.2 LIMIT TEST OF SULPHATE

Principle-

This test is based on the reaction between soluble sulphate as an impurity and barium chloride in the presence of dilute HCl. Barium chloride reacts with sulphate impurity and produces turbidity due to precipitation of sulphate as barium sulphate. Alcohol prevent supersaturation and more uniform turbidity develops.

Chemical reaction-

$$\underset{\text{Sulphate ions}}{SO_4^{2-}} + \underset{\text{Barium chloride}}{BaCl_2} \xrightarrow{\text{Dil. HCl}} \underset{\text{Barium sulphate}}{BaSO_4\downarrow} + \underset{\text{Chloride ions}}{2Cl^-}$$

- **Role of dilute HCl** It is used to dissolve the carbonate impurities which otherwise will precipitate along with sulphate impurities and unnecessarily intensify the turbidity of the test solution.

$$\underset{\text{Dil. Hydrochloric acid}}{2HCl} + \underset{\text{Carbonate ions}}{CO_3^{--}} \longrightarrow \underset{\text{Carbonic acid}}{H_2CO_3} + \underset{\text{Chloride ions}}{2Cl^-}$$

Procedure

• Place 1 ml of alcoholic K_2SO_4 solution, add 2 ml of dilute HCl in Nessler's cylinder, mark 'S' and dilute it with 45 ml of water then add 5 ml of $BaSO_4$ reagent. • Stir immediately with a glass rod and allow it to stand for 5 minutes	• Dissolve the specified quantity of water being examined in water or prepare a solution as directed in the monograph and transfer it to the Nessler's cylinder, mark 'T'. • Add 2 ml of dilute HCl except when HCl is used in the preparation of the solution. Dilute with 45 ml of water add 5 ml of $BaSO_4$ reagent. • Stir immediately with a glass rod and allow it to stand for 5 minutes.

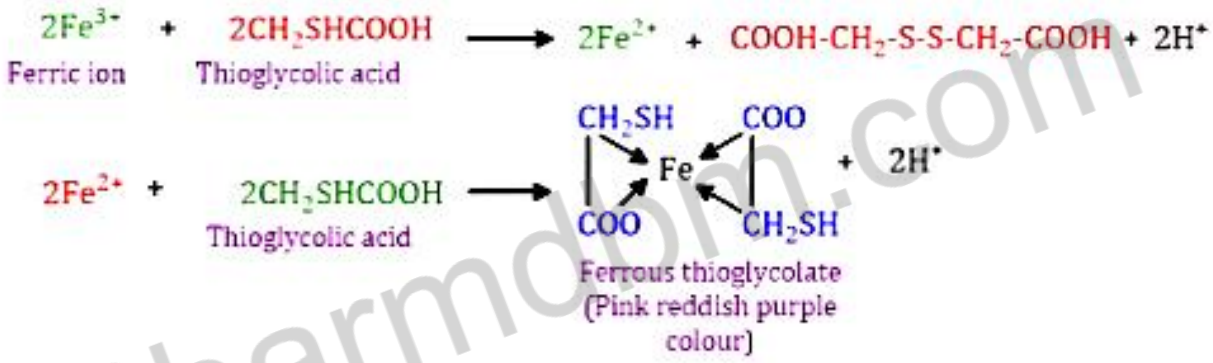
- Protect the turbidity from the light and view it transversely against a black background and compare the turbidity produced by a test with that of standard turbidity.
- If test turbidity is less than or equal to Standard turbidity, then Limit test for sulphate for given sample complies/passes with the official standards of I.P.

1.4.3 LIMIT TEST OF IRON

Principle-

This test is based on the reaction between the iron and thioglycollic acid in the presence of strong ammonia solution and citric acid. The reaction produces a pale pink to deep reddish purple colour, which is due to the formation of ferrous thioglycolate.

Chemical reaction

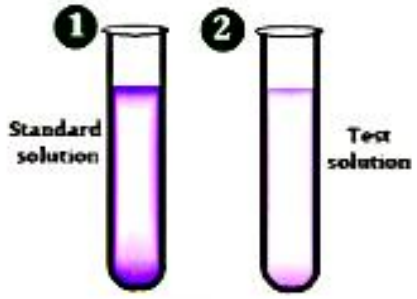


- **Role of strong ammonia solution (iron free)-** Formation of ferrous thioglycolate takes place only in the presence of strong alkali or certain oxidizing agents; therefore, strong ammonia solution is used to provide an alkaline medium which forms ammonium citrate for the pH maintenance.
- The state of oxidation of iron (ferrous or ferric) is immaterial because ferric ions are reduced to ferrous ions by thioglycollic acid.
- **Role of citric acid (iron free)-** Ammonia solution if used alone and has a tendency to precipitate iron which will not be able to react with thioglycolic acid therefore citric acid is used that prevents the precipitation of iron with ammonia by forming its own complex with ammonia.
- **Role of thioglycolic acid-** Iron impurities may be present in trivalent ferric form or in the divalent ferrous form. If it is in ferric form thioglycolic acid converts ferric form of impurity into ferrous form.
- It also forms the coloured complex with iron (ferrous) and produces pink reddish purple colour.

Procedure-

Standard solution	Test solution
• Dilute 2 ml of standard iron solution (20 ppm) with 40 ml of water in Nessler's cylinder, mark 'S'. • Then add 2 ml of 20% w/v solution of iron-free citric acid and 0.1 ml of thioglycolic acid mix and make it alkaline with iron-free ammonia solution. • Dilute up to 50 ml with water and allow it to stand for 5 minutes after stirring.	• Dissolve the specified quantity of test substance being examined in 40 ml water or use 10 ml solution prescribed by monograph and transfer it to the Nessler's cylinder, mark 'T'. • Add 2 ml of 20% w/v solution of iron-free citric acid and 0.1 ml of thioglycolic acid, mix and make it alkaline with iron-free ammonia solution. • Dilute up to 50 ml with water and allow it to stand for 5 minutes after stirring.

- Protect the colour from the light and view it transversely against a black background and compare the colour produced by a test with that of standard opalescence.
- If the test colour is less than or equal to Standard colour, then Limit test for iron for given sample complies/passes with the official standards of I.P.
- If the test colour is more than the Standard colour, then Limit test for iron for a given sample doesn't comply/fails with the official standards of I.P.

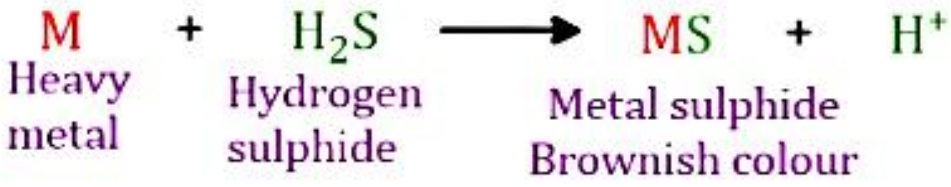


1.4.4 LIMIT TEST OF HEAVY METALS

Principle –

Limit test of heavy metals is based on the reaction of metallic impurities with hydrogen sulfide in acidic medium to form brownish colour solution. Metals that response to this test are lead, mercury, bismuth, arsenic, antimony, tin, cadmium, silver, copper, and molybdenum. The metallic impurities in substances are expressed as parts of lead per million parts of the substance. The usual limit as per Indian Pharmacopoeia is 20 ppm.

Chemical reaction-



Procedure -

The Indian Pharmacopoeia has adopted three methods for the limit test of heavy metals

Method I: Use for the substance which gives clear colorless solution under the specific condition.

Standard solution	Test solution
Take 2 ml of standard lead solution and dilute to 25 ml with water.	Solution is prepared as per the monograph and 25 ml is transferred in Nessler's cylinder.
Adjust the pH between 3 to 4 by adding dilute acetic acid 'Sp' or dilute ammonia solution 'Sp'.	Adjust the pH between 3 to 4 by adding dilute acetic acid 'Sp' or dilute ammonia solution 'Sp'.
Dilute with water to 35 ml.	Dilute with water to 35 ml.
Add freshly prepared 10 ml of hydrogen. Sulphide solution.	Add freshly prepared 10 ml of hydrogen sulphide solution.
Dilute with water to 50 ml.	Dilute with water to 50 ml.
Allow to stand for five minutes.	Allow to stand for five minutes.
Observe the Opalescence/Turbidity	Observe the Opalescence/Turbidity

Observation:

The colour produce in sample solution should not be greater than standard solution. If colour produces in sample solution is less than the standard solution, the sample will pass the limit test of heavy metals and vice versa.

Method II: Use for the substance which do not give clear colorless solution under the specific condition.

Standard solution	Test solution
Take 2 ml of standard lead solution and dilute to 25 ml with water.	Weigh specific quantity of test substance, moisten with Sulphuric acid and ignite on a low flame till completely charred. Add few drops of nitric acid and heat to 500 °C. Allow to cool and add 4 ml of hydrochloric acid and evaporate to dryness. Moisten the residue with 10 ml of hydrochloric acid and digest for two minutes. Neutralize with ammonia solution and make just acid with acetic acid
Adjust the pH between 3 to 4 by adding dilute acetic acid 'Sp' or dilute ammonia solution 'Sp'.	Adjust the pH between 3 to 4 and filter if necessary
Dilute with water to 35 ml.	Dilute with water to 35 ml.
Add freshly prepared 10 ml of hydrogen sulphide solution.	Add freshly prepared 10 ml of hydrogen sulphide solution.
Dilute with water to 50 ml.	Dilute with water to 50 ml.
Allow to stand for five minutes.	Allow to stand for five minutes.
Observe the Opalescence/Turbidity	Observe the Opalescence/Turbidity

Observation:

- The color produce in sample solution should not be greater than standard solution. If color produces in sample solution is less than the standard solution, the sample will pass the limit test of heavy metals and vice versa.

Method III: Use for the substance which gives clear colorless solution in sodium hydroxide solution.

Standard solution	Test solution
Take 2 ml of standard lead solution.	Solution is prepared as per the monograph and 25 ml is transferred in Nessler's cylinder or weigh specific amount of substance and dissolve in 20 ml of water and add 5 ml of dilute sodium hydroxide solution.
Add 5 ml of dilute sodium hydroxide solution and make up the volume to 50 ml with water.	Make up the volume to 50 ml with water
Add 5 drops of sodium sulphide solution.	Add 5 drops of sodium sulphide solution.
Mix and set aside for 5 min.	Mix and set aside for 5 min.
Observe the Opalescence/Turbidity	Observe the Opalescence/Turbidity

Observation

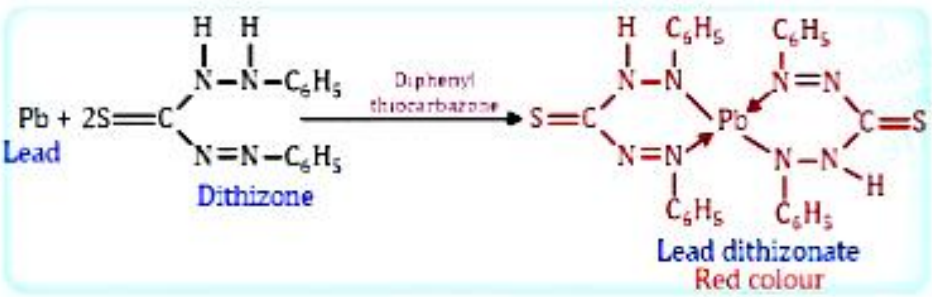
The colour produce in sample solution should not be greater than standard solution. If colour produces in sample solution is less than the standard solution, the sample will pass the limit test of heavy metals and vice versa.

1.4.5 Limit Test of Lead

Principle-

Limit Test for Lead is based upon the chemical reaction between lead and diphenyl thiocarbazone (dithizone) in an alkaline solution to form lead dithizone, which is red. Dithizone is green in colour in chloroform and lead-dithizone complex is violet in colour, so the resulting colour at the end of process is red. To avoid interference by other metals and make the pH optimum, reagents like ammonium citrate, KCN, and $NH_2OH.HCl$ is employed.

Chemical reaction

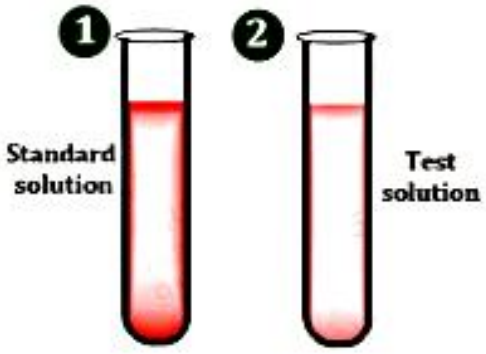


Procedure

Standard solution	Test solution
• A standard lead solution is prepared equivalent to the amount of lead permitted in the sample under examination. • Add 6 ml of ammonium citrate. • Add 2 ml of potassium cyanide and 2 ml of hydroxylamine hydrochloride. • Add 2 drops of phenol red. • Make solution alkaline by adding ammonia solution. • Extract with 5 ml of dithizone until it becomes green. • Combine dithizone extracts are shaken for 30 mins with 30 ml of nitric acid and the chloroform layer is discarded. • To the acid solution add 5 ml of standard dithizone solution Add 4 ml of ammonium cyanide. • Shake for 30 mins. • Observe the colour.	• A known quantity of sample solution is transferred in a separating funnel. • Add 6 ml of ammonium citrate. • Add 2 ml of potassium cyanide and 2 ml of hydroxylamine hydrochloride. • Add 2 drops of phenol red. • Make solution alkaline by adding ammonia solution. • Extract with 5 ml of dithizone until it becomes green. • Combine dithizone extracts are shaken for 30 mins with 30 ml of nitric acid and the chloroform layer is discarded. • To the acid solution add 5 ml of standard Dithizone solution. • Add 4 ml of ammonium cyanide. • Shake for 30 mins. • Observe the color.

Observation-

The intensity of the colour of complex, is depends on the amount of lead in the solution. The colour produce in sample solution should not be greater than standard solution. If colour produces in sample solution is less than the standard solution, the sample will pass the limit test of lead and vice versa.



Reasons-

Ammonium citrate, potassium cyanide, hydroxylamine hydrochloride is used to make pH optimum so interference and influence of other impurities have been eliminated. Phenol red is used as indicator to develop the colour at the end of process. Lead present as an impurity in the substance, gets separated by extracting an alkaline

1.4.6 Limit Test of Arsenic

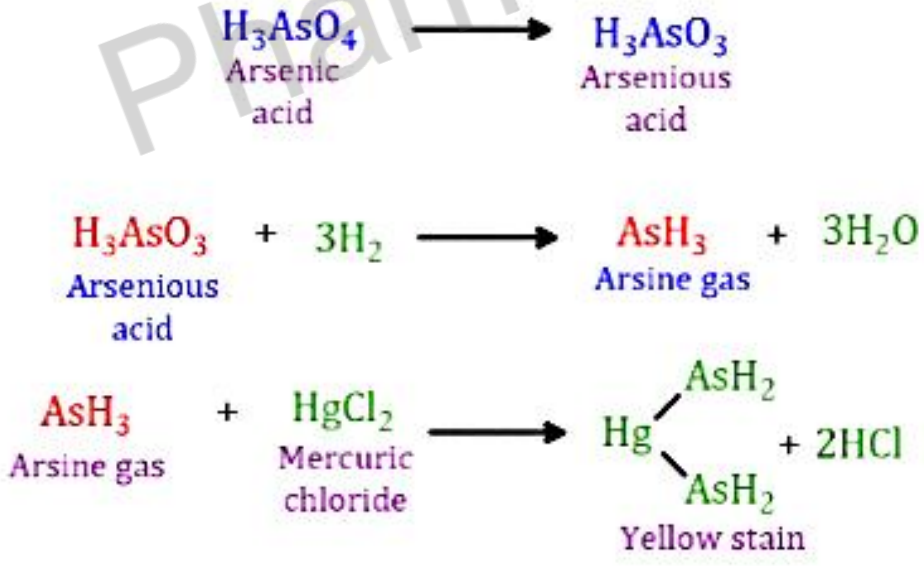
Principle-

This test has based the modification of Gutzeit test in which all the arsenic impurities are converted into arsine gas by reduction with zinc and stannous hydrochloric acid.

The arsine gas thus produced is allowed to react with mercuric chloride paper and produces yellow to brown coloured stain.

The depth and intensity of the stain are depended upon the concentration of arsenic impurities in the test sample. The stain produced by the test sample is compared with the stain produced by using standard, dilute arsenic solution.

Chemical reaction



Role of potassium iodide (KI)-

It is used along with HCl to reduce pentavalent arsenic to trivalent arsenic.

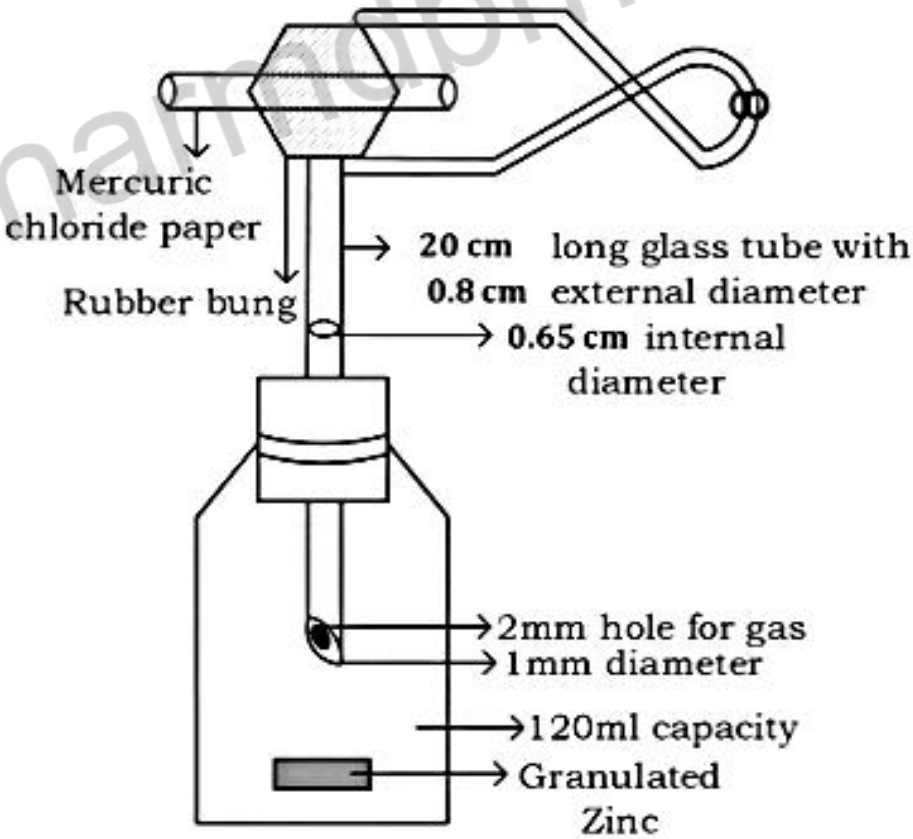
Role of Stannous chloride- It is used for the complete evolution of arsine gas.

Role of lead acetate cotton plug-

- Metallic zinc contains traces of sulphide, sulphite and thiosulphate which can be reduced in the presence of HCl into hydrogen sulphide which travels along with arsine gas and may react with mercuric chloride paper producing dark stain and thus interfere in the intensity of the actual stain of mercuric arsenide.

Gutzeit Apparatus

- Wide mouthed **120ml** glass bottle with **2.5cm** diameter of mouth.
- Bottle fitted with **20cm** long Glass tube with **0.65cm** inner diameter & **0.8cm** external diameter.
- The tube is constricted at its lower end extremity to about **1mm** diameter and there is blown a hole, not less than **2mm** in diameter, in the side of the tube near the constricted part.
- The upper end of the glass tube has been fitted with two rubber bungs **(about 25 mm×25 mm)**, each having a hole bored centrally and exactly **6.5 mm in diameter**. One of the bungs has been fitted to the upper end of the tube, while the second bung has to be fitted upon the first bung in such a way that the **mercuric chloride paper** gets exactly sandwiched between the central perforations of the two.



Procedure-

Take two 50 ml of Arsenic LT apparatus bottles. Label one as "**Test**" and the other as "**Standard**".

Standard solution	Test solution
A known amount of dilute arsenic solution is kept in the wide mouthed bottle of the apparatus.	**Test solution:** Dissolving specific amount of sample in water and stannate HCl (as free) and kept in the wide mouthed bottle of the apparatus.
To this solution, 1 gm of KI, 5 ml of stannous chloride and 10 gm of zinc is added (all these reagents should be arsenic free).	To this solution, 1 gm of KI, 5 ml of stannous chloride and 10 gm of zinc is added (all these reagents should be arsenic free).
Keep the solution aside for 40 minutes.	Keep the solution aside for 40 minutes.
Compare the stain obtained on the mercuric chloride paper with that in the apparatus containing test solution.	Compare the stain obtained on the mercuric chloride paper with that in the apparatus containing standard solution.

Observation–

If the sample show stain lesser intensity than that of the standard stain the sample passed the limit test for arsenic as per IP.

Reasons-

Zinc, potassium iodide and stannous chloride is used as a reducing agent. Hydrochloric acid is used to make the solution acidic. Lead acetate pledger or papers are used to trap any hydrogen sulphide which may be evolved along with arsine.