# CHAPTER – 1 (C) Impurities in Pharmaceuticals

## 1C.1 INTRODUCTION

Impurity Means presence of unwanted foreign particle other than active drugs which may be or may not be toxic and is found in pharmaceutical substances.

## 1C.2 SOURCES OF IMPURITIES IN PHARMACEUTICALS

Sources of Impurities in Pharmaceuticals:

1. Raw Material Employed in Manufacture.
2. Reagents used in the Manufacturing Process.
3. Methods/Process used in Manufacture.
4. Intermediate products in manufacturing process.
5. Atmospheric contamination during the Manufacturing Process.
6. Decomposition of the Product during Storage.
7. Accidental substitution & Deliberate Adulteration Type and Amount of impurity present in the chemicals or pharmaceutical substances, depends on factors.

**Note:-** Substances used in pharmaceutical field should be Almost pure. Official pharmacopoeias prescribe limits for particular impurities like sulphate, chloride, iron, heavy metals and arsenic.

## 1C.3 TYPES OF IMPURITIES

**Four types of Impurities**

1. Impurity which produce toxic effect on the body is present beyond the limit **e.g.:** Impurities of lead and arsenic
2. Impurities which are harmless but, if present beyond the prescribed limit in Pharmaceutical substance will change the effect of active ingredient. **e.g.:** Impurities of Na and K salts
3. Impurities which present beyond the limit will affect the storage property of pharmaceuticals. **e.g.:** presence of moisture leads to decompose the product (LOD)
4. Impurities will alter the colour, odour and taste **e.g.:** Phenolic impurities present in sodium salicylate will alters the colour of the solution.

## 1C.4 FACTORS RESPONSIBLE FOR PHARMACEUTICAL IMPURITIES

**1. Raw Material Employed in Manufacture:**

• The raw materials, from which these are prepared, often contain impurities.

• It is therefore necessary to employ pure chemicals and substances as raw materials.

E.g: Presence of tin, lead, silver, copper, cobalt and gold in bismuth salts.

**2. Method used in Manufacture:**

Some impurities get incorporated into the materials during the manufacturing process.

**A) Intermediates** For certain drugs a multiple-step-synthesis procedure is involved, which produces intermediate compounds. The purification of the intermediates is essential, otherwise impurities present in the intermediates will get into the final product. (e.g.) Potassium iodide is prepared by treating potassium hydroxide with iodine. The intermediate potassium iodate (KIO3) formed is reduced to iodide. If the iodide is not reduced completely, the final product potassium iodide, will contain traces of potassium iodate as impurity.

**B) Reagents used in the Process**

The final product may contain unreacted reagents as impurities, if it is not washed properly.

E.g., 1. Lead as an impurity may result from the sulphuric acid used as reagent.

2. Soluble alkali may be an impurity in calcium carbonate if the calcium carbonate is made by reacting calcium chloride and sodium carbonate and not properly washed.

**C) Solvents**

• Water is a common solvent in large scale manufacturing of pharmaceuticals.

• This can give rise to trace impurities such as sodium, calcium, magnesium, carbonate, chloride and sulphate ions.

• These impurities can be avoided by using purified water.

**D) Catalyst**

• Generally, catalysts are used to induce the reaction.

• There may be possibility of incorporation of traces of catalyst in the final products.

e.g., 1. Presence of palladium catalyst in phenanthrene.

2. Presence of copper chloride in the synthesis of phenol.

**E) The Reaction Vessels**

• The vessels used in manufacturing process are made of metals like copper, iron, aluminium, zinc, tin though these days many of these metals are replaced by stainless steel. Traces of these metal ions may contaminate the final products.

• Metal particles of aluminium containers may contaminate the products like ointments and pastes stored in it.

**F) Atmospheric contaminants**

• Dust, sulphur dioxide, hydrogen sulphide, arsenic and water vapour from atmosphere may affect a drug.

• Presence of carbon dioxide, carbon monoxide and hydrogen cyanide from environment also affect the drug products if it is not manufactured under controlled conditions.

**G) Decomposition of the Product during Storage**

• Many drugs undergo changes due to improper storage conditions.

• These decompositions may be due to light, water vapour, air, carbon dioxide and metallic ions.

E.g, 1. Ferrous sulphate slowly changed into insoluble ferric oxide by air and moisture.

2. Bismuth carbonate turns black on exposure to sunlight for a long period.

## 1C.5 TESTING FOR IMPURITIES

LIMIT TESTS

• Limit tests are quantitative tests or semi-quantitative tests which are designed to detect and limit / control small quantities of impurities present in the substance.

• All the limit tests that are prescribed in the pharmacopoeias are based on the comparison of standard turbidity or colour with that of the sample under test.

• Usually the limits are prescribed in parts per million (PPM).

• The amount of test samples to be taken is mentioned in the individual monograph of the pharmacopoeias.

• Amount of impurity present in actually determined and compared with the numerical limit given in Pharmacopoeia.

**Quantitative Determination**

1. Limits of soluble matter
2. Limits of Insoluble matter
3. Limits of non-volatile matter
4. Limits of moisture and volatile matter
5. Limits of residue on ignition
6. Loss on ignition
7. Ash values.

| 1C.6 | LIMIT TEST |
|---|---|

Limit tests are quantitative or semi quantitative test designed to identify and control small quantities of impurities which are likely to be present in the substances.

- Tests being used to identify the impurity.
- Tests being used to control the impurity.

1. Limit Test for Chlorides
2. Limit Test for Sulphate
3. Limit Test for Iron
4. Limit Test for Heavy Metals
5. Limit Test for Arsenic

**1. Limit test for IRON:**

- Limit test of Iron is based on the reaction of iron in ammonical solution with thioglycollic acid in presence of citric acid to form iron thioglycolate (Ferrous thioglycolate complex) which produces pale pink to deep reddish-purple colour in alkaline media.
- Thioglycolic acid is used as reducing agent.
- The colour of the Ferrous thioglycolate complex fades in the presence of air due to oxidation.
- Also, the colour is destroyed in presence of oxidizing agents and strong alkalis.
- The purple colour is developed only in alkaline media. So ammonia solution is used.
- But ammonia reacts with iron, forms precipitate of ferrous hydroxide.
- Thus citric acid is used which prevents the precipitate of iron with Ammonia by forming a complex with iron as iron citrate.

Test sample Standard compound Sample is dissolved in specific amount of water and then volume is made up to 40 ml 2 ml of standard solution of iron diluted with water upto 40 ml Add 2 ml of 20 % w/v of citric acid (iron free) Add 2 ml of 20 % w/v of citric acid (iron free) Add 2 drops of thioglycollic acid Add 2 drops of thioglycollic acid Add ammonia to make the solution alkaline and adjust the volume to 50 ml Add ammonia to make the solution alkaline and adjust the volume to 50 ml Keep aside for 5 min Keep aside for 5 min Colour developed is viewed vertically and compared with standard solution Colour developed is viewed vertically and compared with standard solution Procedure.

Note: All the reagents used in the limit test for Iron should themselves be iron free.

**Observation:** The purple colour produce in sample solution should not be greater than standard solution. If purple colour produces in sample solution is less than the standard solution, the sample will pass the limit test of iron and vice versa.

**Reasons:**

- Citric acid forms complex with metal cation and helps precipitation of iron by ammonia by forming a complex with it.
- Thioglycolic acid helps to oxidize iron (II) to iron (III).
- Ammonia is added to make solution alkaline. The pale pink colour is visible only in the alkaline media. The colour is not visible in acidic media as ferrous thioglycolate complex decomposes in high acidic media.

**2. Limit test for Chloride:** The test is used to limit the amount of Chloride as an impurity in inorganic substances.

**Principle:** Limit test of chloride is based on the reaction of soluble chloride with silver nitrate in presence of dilute nitric acid to form silver chloride, which appears as solid particles (Opalescence) in the solution.

$$Cl^- + AgNO_3 \text{ ----------◊ } AgCl + NO_3^-$$

Soluble chloride present as impurity

The silver chloride produced in the presence of dilute Nitric acid makes the test solution turbid, the extent of turbidity depending upon the amount of Chloride present in the substance is compared with the standard opalescence produced by the addition of Silver nitrate to a standard solution having a known amount of chloride and the same volume of dilute nitric acid as used in the test solution.

Test sample Standard compound Specific weight of compound is dissolved in water or solution is prepared as directed in the pharmacopoeia and transferred in Nessler cylinder Take 1 ml of 0.05845 % W/V solution of sodium chloride in Nessler cylinder Add 1 ml of nitric acid Add 1 ml of nitric acid Dilute to 50 ml in Nessler cylinder Dilute to 50 ml in Nessler cylinder Add 1 ml of AgNO3 solution Add 1 ml of AgNO3 solution Keep aside for 5 min Keep aside for 5 min Observe the Opalescence/Turbidity Observe the Opalescence/Turbidity

**Observation:** The opalescence produce in sample solution should not be greater than standard solution. If opalescence produces in sample solution is less than the standard solution, the sample will pass the limit test of chloride and vice- versa.

**Reasons:** Nitric acid is added in the limit test of chloride to make solution acidic and helps silver chloride precipitate to make solution turbid at the end of process as Dilute HNO3 is insoluble in AgCl.

Pharmacopoeia does not prescribe any numerical value of limit test for chlorides, sulphate and iron because limit test is based on the simple comparison of opalescence or colour between the test and standard solution prescribed according to pharmacopoeia.

- In this type of limit test, the extent of turbidity or opalescence or colour produced in influenced by the presence of other impurities present in the substance and also by variation in time and method of performance of test.
- Thus the pharmacopoeia does not prescribe any numerical value of the limit test. Pharmacopoeia not prescribe any numerical value for limit test for chlorides, sulphate and iron because limit test is based on simple comparison of opalescence or colour between test and standard solution prescribed according to pharmacopoeia.
- The variation in the permissible limits for various substances is obtained by taking varying quantities of the substances under test. In this type of limit test, the extent of opalescence or turbidity or colour produced is influenced by the presence of other impurities present in the substance and also by variation in time and method of performance of tests and hence the pharmacopoeia do not prescribe any numerical values for the limit test in these test.

**3. Limit test for sulphate:** The Sulphate Limit Test is designed to determine the allowable limit of sulphate contained in a sample.

**Principle:** Limit test of sulphate is based on the reaction of soluble sulphate with barium chloride in presence of dilute hydrochloric acid to form barium sulphate which appears as solid particles (turbidity) in the solution. Then comparison of turbidity is done with a standard turbidity obtained from a known amount of Sulphate and same volume of dilute Hydrochloric acid have been added to both solutions. The barium chloride test solution in the IP has been replaced by Barium sulphate reagent which is having barium chloride, sulphate free alcohol and a solution of potassium sulphate. Potassium sulphate has been added to increase the sensitivity of the test.

$$SO4^{-2} + BaCl_2 \text{ ----------◊ } BaSO4 + 2Cl^-$$

Test sample Standard compound Specific weight of compound is dissolved in water or solution is prepared as directed in the pharmacopoeia and transferred in Nessler cylinder Take 1 ml of 0.1089 % W/V solution of potassium sulphate in Nessler cylinder Add 2 ml of dilute hydrochloric acid Add 2 ml of dilute hydrochloric acid Dilute to 45 ml in Nessler cylinder Dilute to 45 ml in Nessler cylinder Add 5 ml of barium sulphate reagent Add 5 ml of barium sulphate reagent Keep aside for 5 min Keep aside for 5 min Observe the Turbidity Observe the Turbidity Procedure.

Barium sulphate reagent contains barium chloride, sulphate free alcohol and small amount of potassium sulphate.

**Observation:** The turbidity produce in sample solution should not be greater than standard solution. If turbidity produces in sample solution is less than the standard solution, the sample will pass the limit test of sulphate and vice versa.

**Reasons:** Hydrochloric acid helps to make solution acidic. Potassium sulphate is used to increase the sensitivity of the test by giving ionic concentration in the reagent. Alcohol helps to prevent super saturation and so produces a more uniform opalescence.

**4. Limit test for Arsenic:**

- Arsenic is a well-known undesirable and harmful impurity which is present in medicinal substances.
- All pharmacopoeias prescribe a limit test for it.
- Pharmacopoeial method is based on the Gutzeit test.
- All the special reagents used in the limit test for Arsenic are marked and distinguished by letter 'As T', which means that they all should be Arsenic free and should themselves conform to the test for Arsenic.

**Principle:** Limit test of Arsenic is based on the reaction of arsenic gas with hydrogen ion to form yellow stain on mercuric chloride paper in presence of reducing agents like potassium iodide. It is also called as Gutzeit test and requires special apparatus.

- Arsenic, present as arsenic acid ($H_3AsO_4$) in the sample is reduced to arsenious acid ($H_3AsO_3$) by reducing agents like potassium iodide, stannous acid, zinc, hydrochloric acid, etc.
- Arsenious acid is further reduced to arsine (gas) ($AsH_3$) by hydrogen and reacts with mercuric chloride paper to give a yellow stain.

Substance + dil HCl ----------◊ $H_3AsO_4$ (contains Arsenic impurity) Arsenic acid

$H_3AsO_4 + H_2SnO_2$ ----------→ $H_3AsO_3 + H_2SnO_3$ Arsenic acid Arsenious acid

$H_3AsO_3 + 6[H]$ ----------→ $AsH_3 + 3H_2O$ Arsenious acid nascent hydrogen Arsine gas The depth of yellow stain on mercuric chloride paper will depend upon the quantity of arsenic present in the sample.

- When the sample is dissolved in acid, the Arsenic present in the sample gets converted to Arsenic acid.
- By action of reducing agents like Potassium iodide, stannous acid etc., Arsenic acid gets reduced to arsenious acid.
- The nascent hydrogen formed during the reaction, further reduces Arsenious acid to Arsine gas, which reacts with mercuric chloride paper, giving a yellow stain.

**Apparatus:** It is having a wide mouthed glass bottle of 120 mL capacity having mouth of about 2.5 cm in diameter. This bottle is fitted with a rubber bung through which passes a glass tube, 20 cm long.

External diameter=0.8 cm

Internal diameter=0.65 cm

The tube is constricted at its lower end extremity to about 1 mm diameter and there is blown a hole, not less than 2 mm in diameter, in the side of the tube near the constricted part. The upper end of the glass tube is fitted with two rubber bungs (25 mm x 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 perforation of the two. The bungs are kept in close contact by using rubber band or spring clip in such a manner that the gas evolved from the bottle must have to pass through the 0.65 mm internal circle of mercuric chloride paper. During the test, the evolved gases have been passing through the side hole, the lower hole serving as an exit for water which condenses in the constricted part of the tube. An important feature has been the standardization of the area of Mercuric chloride paper which is exposed to the reaction of arsine gas.

The test solution is prepared by dissolving specific amount in water and stannated HCl (arsenic free) and kept in a wide mouthed bottle. A known quantity of dilute arsenic solution in water and stannated HCl (arsenic free) is kept in wide mouthed bottle. 1 g of KI 1 g of KI 5 ml of stannous chloride acid solution 5 ml of stannous chloride acid solution 10 g of granulated zinc is added (all these reagents must be arsenic free). Keep the solution aside for 40 min 10 g of zinc is added (all these reagents must be arsenic free). Keep the solution aside for 40 min Stain obtained on mercuric chloride paper is compared with standard solution. Standard stain must be freshly prepared as it fades on keeping.

**Inference:** If the stain produced by the test is not deeper than the standard stain, then sample complies with the limit test for Arsenic.

**Reasons:** Stannous chloride is used for complete evolution of arsine. Zinc, potassium iodide and stannous chloride is used as a reducing agent. Hydrochloride acid is used to make the solution acidic Lead acetate paper are used to trap any hydrogen sulphide which may be evolved along with arsine.

**Limit test for heavy metals:**

- The limit test for heavy metals is designed to determine the content of metallic impurities that are coloured by hydrogen sulphide or sodium sulphide under the condition of the test should not exceed the heavy metal limits given under the individual monograph.
- The heavy metals (metallic impurities) may be iron, copper, lead, nickel, cobalt, bismuth, antimony etc.. The limit for heavy metals is indicated in the individual monograph in term of ppm of lead i.e. the parts of lead per million parts of the substance being examined
- In substances the proportion of any such impurity (Heavy metals) has been expressed as the quantity of lead required to produce a colour of equal depth as in a standard comparison solution having a definite quantity of lead nitrate.
- The quantity is stated as the heavy metal limit and is expressed as parts of lead (by weight) per million parts of the test substance.
- The limit test for heavy metals has been based upon the reaction of the metal ion with hydrogen sulphide, under the prescribed conditions of the test causing the formation of metal sulphides.
- These remain distributed in colloidal state, and give rise to a brownish coloration.
- I.P limit for heavy metals in 20 ppm.
- The test solution is compared with a standard prepared using a lead solution (as the heavy metal). The metallic impurities in substance are expressed as parts of lead per million parts of substance.

**IP has adopted 3 methods for this:**

**Method I:** The method is applicable for the samples which give clear colourless solutions under specified conditions of test.

**Method II:** The method is applicable for the samples which DO NOT give clear colourless solutions under specified conditions of test.

**Method III:** Used for substances which give clear colourless solutions in sodium hydroxide medium.

Limit test for lead: Lead is a most undesirable impurity in medical compounds and comes through use of sulphuric acid, lead lined apparatus and glass bottles use for storage of chemicals.

**Principle:** Limit test of lead is based on the reaction of lead and diphenyl thiocabazone (dithizone) in alkaline solution to form lead dithizone complex which is red in colour.

Dithizone in chloroform, is able to extract lead from alkaline aqueous solutions as a lead dithizone complex (Red in colour)

The original dithizone is having a green colour in chloroform while the lead- dithizone is having a violet colour. So, resulting colour at the end of the process is read. The intensity of the colour of complex is dependent upon the amount of lead in the solution. The colour of the lead-dithizone complex in chloroform has been compared with a standard volume of lead solution, treated in the same manner. In this method, the lead present as an impurity in the substances, gets separated by extracting an alkaline solution with a dithizone extraction solution.

The interference and influence of the other metal ions has been eliminated by adjusting the optimum pH for the extraction by employing Ammonium citrate/ potassium cyanide.

**Method:**

• Sample solution is transferred to a separating funnel.

• To it 6 ml of ammonium citrate, 2 ml potassium cyanide and 2 ml of hydroxalamine HCl are added.

• 2 drops of phenol red

• Solution is made alkaline by adding ammonia solution.

• This is then extracted with 5 ml portions of dithizone solution until it becomes green.

• The combined dithizone extracts are shaken for 30 seconds with 30 ml of nitric acid and chloroform layer is discarded.

• To the acid solution 5 ml of standard dithizone solution is added and 4 ml ammonium cyanide and solution is shaken for 30 sec.

• Similarly prepare standard.

**Observation:** The intensity of the colour of complex, is depends on the amount of lead in the solution. The colour produced 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 impurities in the substance, gets separated by extracting an alkaline solution with a dithizone extraction solution.

A known quantity of sample solution is transferred in a separating funnel A standard lead solution is prepared equivalent to the amount of lead permitted in the sample under examination Add 6ml of ammonium citrate Add 6ml of ammonium citrate Add 2 ml of potassium cyanide and 2 ml of hydroxylamine hydrochloride Add 2 ml of potassium cyanide and 2 ml of

hydroxylamine hydrochloride Add 2 drops of phenol red Add 2 drops of phenol red Make solution alkaline by adding ammonia solution. Make solution alkaline by adding ammonia solution. Extract with 5 ml of dithizone until it becomes green 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 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 To the acid solution add 5 ml of standard dithizone solution Add 4 ml of ammonium cyanide Add 4 ml of ammonium cyanide Shake for 30 mins Shake for 30 mins Observe the colour Observe the colour

Aq. Ammonia is added in limit test of lead:

Pb+ S----------◊PbS

- In limit test of lead, PbS is produced by addition of standard H2S , to the solution containing lead.
- pH 3-4 is necessary for the precipitation of PbS. So aq. Ammonia / acetic acid is added to maintain that pH.

## 1C.7 EFFECT OF IMPURITIES

The impurities present in the substances may give following effects:

- Impurities having toxic effects may be injurious to health, if present above certain limits.
- Traces of impurities, may exert a cumulative toxic effect after a certain time.
- Impurities may lower the active strength of the substance.
- Impurity may decrease shelf life of substance.
- Impurity may cause incompatibility with other substances.
- Impurities may cause a physical or chemical change in the properties of the substance, so making the substance medicinally useless.
- May cause change in colour, odour and taste.