**Pharmaceutical Impurities and Limit test**

In [chemistry](https://en.wikipedia.org/wiki/Chemistry) and [materials science](https://en.wikipedia.org/wiki/Materials_science), impurities are [chemical substances](https://en.wikipedia.org/wiki/Chemical_substance) inside a confined amount of [liquid](https://en.wikipedia.org/wiki/Liquid), [gas](https://en.wikipedia.org/wiki/Gas), or [solid](https://en.wikipedia.org/wiki/Solid), which differ from the [chemical composition](https://en.wikipedia.org/wiki/Chemical_composition) of the material or compound. Firstly, a pure chemical should appear thermodynamically in at least one [chemical phase](https://en.wikipedia.org/wiki/Phase_%28matter%29) and can also be characterized by its one-component-[phase diagram](https://en.wikipedia.org/wiki/Phase_diagram). Secondly, practically speaking, a pure chemical should prove to be [homogeneous](https://en.wikipedia.org/wiki/Homogeneous) (i.e., will show no change of properties after undergoing a wide variety of consecutive analytical chemical procedures). The perfect pure chemical will pass all attempts and tests of further separation and purification. Thirdly, and here we focus on the common chemical definition, it should not contain any trace of any other kind of chemical species. In reality, there are no absolutely 100% pure chemical compounds, as there is always some minute [contamination](https://en.wikipedia.org/wiki/Contamination). Indeed, as detection limits in analytical chemistry decrease, the number of impurities detected tends to increase.

Impurities are either naturally occurring or added during [synthesis](https://en.wikipedia.org/wiki/Chemical_synthesis) of a chemical or commercial product. During production, impurities may be purposely, accidentally, inevitably, or incidentally added into the substance.

The levels of impurities in a material are generally defined in relative terms. [Standards](https://en.wikipedia.org/wiki/Standardization) have been established by various organizations that attempt to define the permitted levels of various impurities in a manufactured product. Strictly speaking, then a material's level of purity can only be stated as being more or less pure than some other material.

A compound is said to be impure if it is having foreign matter i. e Impurities. A pure chemical compound refers to that compound which is having no foreign matter i.e. pure compounds. Chemical purity means freedom from foreign matter. Analytically 100 % pure substances are not available and traces of impurities must be present.

Normally undesirable foreign materials are present in the pharmaceutical substances.

• **Impurity is any material that affects the purity of the material of interest.**

• Presence of Impurities in the pharmaceutical substances may produce toxic effects on the body and may also lower down the active strength of the pharmaceutical substance.

• Impurities commonly in chemical substances include small quantities of lead, Arsenic, Iron, lead, heavy metals, Chloride and sulphate.

**The different sources of impurities in pharmaceuticals are listed below:**

**1) Raw material used in manufacture**

**2) Reagents used in manufacturing process**

**3) Method/ process used in manufacture or method of manufacturing**

**4) Chemical processes used in the manufacture**

**5) Atmospheric contamination during the manufacturing process**

 **6) Intermediate products in the manufacturing process**

**7) Defects in the manufacturing process**

 **8) Manufacturing hazards**

**9) Inadequate Storage conditions**

**10) Decomposition of the product during storage**

**11) Accidental substitution or deliberate adulteration with spurious or useless materials**

**Raw materials employed in manufacture:** Impurities known to be associated with these chemicals may be carried through the manufacturing process and contaminate the final product.

 **Example:** Rock salt---→ Calcium Sulphate (CaSO4) + Magnesium Chloride (MgCl2)= NaCl prepared Rock salt contains small amounts of Calcium sulphate and Magnesium chloride. Thus Sodium chloride prepared from this source will contain traces of Calcium and Magnesium compounds. Impurities such as Arsenic, Lead and Heavy metals are present in raw materials and hence are found in substances. So, it is necessary to use pure chemicals and substances as raw materials for the manufacturing process.

**Example:** Copper sulphate may be prepared by the action of sulphuric acid on copper turnings:

**Cu+ 2 H2SO4 ------------------------→ CuSO4 + 2 H2O + SO2**

Copper turnings are known to have Iron and Arsenic as impurities. If Large quantities of impurities are present in the raw material (e.g Copper turnings), they may enter the final product. (CuSO4 .5H2O) Due to this I.P. prescribes limit of tolerance for Arsenic as impurity to be not more than 8 parts per million in copper sulphates. Similarly it prescribes a limit of Iron as impurity.

**Reagents used in the manufacturing process:** If reagents used in the manufacturing process are not completely removed by washing, these may find entry into the final products.

**Example:** Ammoniated mercury may be prepared by adding a solution of Mercuric chloride to dilute ammonia solution.

**HgCl2+ 2NH4OH-------------→NH2HgCl + NH4Cl + 2 H2O**

Soluble Ammoniated mercury precipitate of Ammoniated mercury (Final Product) contains ammonium hydroxide. Thus, this precipitate is washed with cold water to remove ammonium hydroxide. If it is not removed completely by washing with water, the final product may contain in it Ammonium hydroxide as impurity.

**Method or the process used in the manufacture:**

* Many drugs and chemicals (usually organic) are manufactured from different raw materials, by using different methods or processes.
* Some impurities are incorporated into the materials during the manufacturing process.
* The type and amount of impurity present in the drug/ chemical varies.
* In certain drugs, a multiple-step-synthesis procedure is used, which produces intermediate compounds.
* The purification of intermediates is also important; otherwise the impurities present in the intermediate will get incorporated in the final product.
* Usually side reactions occur during the synthesis.
* Impurities of the product side reactions also occur in the substances. This may introduce new impurities due to contamination by reagents and solvents at various stages of the process as described below:

a) Reagents employed in the process

b) Reagents added to remove other impurities

c) Solvents

d) Action of solvents and reagents on reaction vessels.

**Reagents employed in the manufacturing process:** Soluble alkali in Calcium carbonate arises from sodium carbonate used in the process. Calcium carbonate is obtained by interaction of a soluble calcium salt and a soluble carbonate and therefore the product will contain traces of soluble alkali, which the washing process has failed to remove.

**Reagents added to remove other impurities:** Potassium bromide contains traces of Barium, which is added in the manufacturing process to remove excess of sulphate.

**Chemical process used in the manufacture:** For the synthesis of drugs, many chemical reactions such as Nitration, Halogenations, Oxidation, reduction, and hydrolysis are involved. In these chemical processes, different chemicals are used. Tap water is generally used in the various processes and it is often having Cl- , Mg+2, Ca+2 ions, which are generally found in the substance which is being manufactured.

**Atmospheric contamination during the manufacturing process**

* In the industrial areas, the atmosphere is contaminated with dust particles and some gases like Hydrogen sulphide, Sulphur dioxide, and black smoke.
* During the manufacture or purification of the pharmaceutical products, these impurities enter the final products.
* There are many pharmaceutical products which when manufactured are contaminated with atmospheric CO2 and water vapour. E. g NaOH absorbs atmospheric CO2.

 2NaOH + CO2 ----→ Na2CO3 + H2O

* Due to this reaction, NaOH should not be kept open for a longer time during its manufacture.
* Therefore, IP has prescribed that Sodium hydroxide should not contain more than 3% of sodium carbonate.

**Defects in the manufacturing process:** In many manufacturing processes, there are defects like imperfect mixing, incompleteness, non-adherence to proper temperature, pressure, pH or reaction conditions, which may give chemical compounds with impurities in them.

Example: Zinc oxide may be prepared by heating metallic zinc to bright redness in a current of air. The vapors of Zinc burn to form Zinc oxide which is collected as a fine white powder.

 • But if there is less heat or air or both, zinc metal is not completely converted to zinc oxide.

 • Thus the final product, Zinc oxide may still contain metallic zinc as impurity.

 • So, IP has prescribed a test for Zinc metal in zinc oxide.

**Intermediate products in the manufacturing process:**

* There are some intermediates which are produced during the manufacturing process. Sometimes these intermediates may be carried through to the final product as impurity.

Example: Potassium iodide is prepared by reacting Iodine with Potassium hydroxide.

**6KOH+ 3I2 -------------------- 5KI + KIO3 + 3H2O**

* The resulting solution is first evaporated and then heated with charcoal.

**KIO3 + 3C---------- KI + 3CO**

* In this process if the intermediate product (KIO3) is not completely converted into KI, then it may be carried through to the final product as an impurity.

**Manufacturing hazards:**

* Particulate contamination Process errors
* Cross contamination
* Microbial contamination
* Packing errors
* Particulate contamination
* The presence of unwanted particulate matter can arise due to dirt, dust, glass, porcelain or plastic fragments from sieves, granulating or tableting machines or from product containers.
* Ware and tare of equipment or improperly cleaned equipment may also cause particulate contamination.
* Clarity of solutions for injection is particularly important.

 E.g Metal particles which have been found in eye ointments packed in metal tubes.

**Process errors:**

* Gross errors arising from incomplete solution of a solute in a liquid preparation must be detected readily by the normal analytical control procedures.
* Minor errors arise if the manufacturing tolerance for the quantity of active ingredient in the product has been wide.

 **Cross contamination:**

* The handling of powders, granules, and tablets in large bulk creates air-borne dust, which leads to cross contamination of the product.
* So, face masks and special extraction equipment are used to protect operators from harmful effects of drugs.
* E.g penicillin preparation requires special handling during its manufacture.

**Microbial contamination:**

• Parenteral preparations and ophthalmic preparations require special care against microbial contamination.

 • Many liquid preparations and creams are liable to bacterial and fungal contamination. So care should be taken.

 Eg. Acacia, senna, tragacanth---→They should be controlled for Salmonellae.

**Packing errors:**

• Products of similar appearance such as tablets of same size, shape, colour packed in similar containers can constitute a potential source of danger.

• Improper labeling or destruction of stock of unused labels also constitutes a major packaging hazard.

**Storage Conditions:**

The chemical substances when prepared have to be stored in different types of containers depending upon:

✓ Nature of the material

✓ Batch size

✓ Quantity

Many types of materials are used for storage purpose like plastic, polythene, iron vessels, stainless steel and aluminium.

 **Leaching out effect:**  Alkalies stored in ordinary glass containers extract lead from it, which in found as impurity in the final product.

 Strong chemicals react with iron containers and extract Iron an impurity in final product.

**Inadequate storage and their effects are as follows:**

 a) **Filth**: Stored products may become contaminated with dust, bodies of insects, animal and insect excreta.

 b**) Chemical instability:** decomposition because of light, traces of acid or alkali, air oxidation, water vapour, CO2 and traces of metallic ions. e.g light sensitive materials should be stored in amber colored bottles.

c**) Reactions with container materials:** e.g salicylic acid ointment must not be stored in metal tubes.

d) **Physical changes:** The occurrence of changes in the physical form of drug like change in crystal size can lead to change in efficiency of product.

e) **Temperature effect**: Chemical and physical changes occur if materials are not stored at proper temperature.

**Decomposition of the product during storage**:

* Chemical decomposition, analysis or breakdown is the separation of a chemical compound into elements or simpler compounds. It is sometimes defined as the exact opposite of a chemical synthesis. Chemical decomposition is often an undesired chemical reaction.
* Some substances decompose on storing due to presence of air, light and oxygen. So, the final product is contaminated.
* Deliquescent substances absorb water from the atmosphere and get liquefied.
* Decomposition products appear as impurities in the substances.

 **Accidental substitution or deliberate adulteration with spurious or useless materials:**

* It is possible to avoid accidental substitution by storing the toxic substances together separately or in a locked cupboard.
* Many pharmaceutical chemicals are adulterated with cheaper substances.
* E.g The expensive potassium may be adulterated with sodium bromide.

**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 color, odour and taste.

**Test for purity:**

* Pharmacopoeia prescribes the “Test for purity” for pharmaceutical substances to check their freedom from undesirable impurities.
* Pharmacopoeia will decide and fix the limit of tolerance for these impurities.
* For certain common impurities for which pharmacopoeia prescribes the test of purity are:
* Colour, odour, taste
* Physicochemical constants (Iodine value, saponification value, melting point, refractive index etc.)
* Acidity, alkalinity, pH
* Humidity (Estimation of moisture)
* Cations and anions
* Ash
* Arsenic or lead
* Loss on drying
* Loss on ignition

**Limit Test**

Practically it is impossible to remove all the impurity from any substance. Some remain in trace even after purification. So it is only desirable that the substance should be sufficient pure and can be use safely.

The pharmacopoeia (IP, BP, USP) specify the limit up to which various impurity can be tolerated in pharmaceutical drugs and excipients. The limit tests help to check and indicate the presence of various impurities in substances.

The pharmacopoeia has fixed the limit of various impurities considering various factors.

**Limit tests are qualitative and semi quantitative test design to detect the limits of impurity commonly present in pharmaceutical substance.**

**Impurity:** Any foreign material present in drug or chemical is called as impurity.

**Pure compound:** A drug or chemical said to be pure if it is free from all impurity.

**Qualitative test:** This is the by which we can identify the compound, or it is identification test.

**Quantitative test:** The test by which the quantity of the substance is estimated.

In the limit test the impurity is identified and the presence of impurity is compared with the standard taking the specified amount of impurity.

The standard substances of limit test contain the maximum amount of impurity which can be allowed in pharmaceutical substance.

The comparison of sample with standard involves the physical changes like colour, Turbidity or opalescence etc.

**Types of Impurity**

**Toxic impurity:** The impurity which is very harmful and can cause death even when taken once or short period of time. Example Arsenic

**Cumulative impurity:** The impurity which may not cause immediate effect, but when taken for long period of time shows toxicity. Example Heavy metal

**Harmless impurity:** some impurity may not cause harm to body but if present in large quantity reduces the therapeutic activity of active ingredient. Example Chloride, Sulphate.

On considerations of above classification the pharmacopoeia has fixed the permissible limit for each impurity. For toxic impurity the permissible limit is as less as 5-10 PPM. Whereas cumulative impurity limit is the little high as 20 PPM. For harmless impurity the limits are still high.

**Difference between assay and limit test**

1. The assay is quantitative test where as limit test is semi quantitative or qualitative test.
2. The assay results provide the exact amount of substance where as limit test for range of impurity.
3. The assay will be done for substance as well as impurity where as limit test particularly for impurity.

**Limit Test of Chloride:**

**Principle**: The limit test for chloride is based upon the reaction between soluble chloride and silver nitrate reagent in presence of dilute nitric acid to produce insoluble silver chloride.

The opalescence produce by the sample is compared with that of standard opalescence produce by the specific amount of chloride ions.

The sample opalescence should not be greater than the standard opalescence for passing the limit test for chloride.

**Reaction**: Dil HNO3

 Sample Cl - + AgNO3 AgCl + NO3-

 Dil HNO3

 Standard NaCl + AgNO3 AgCl + NaNO3

**Procedure:**

|  |  |
| --- | --- |
| **Standard** | **Sample** |
| Pipette Out 1ml Of 0.05845% w/v of standard NaCl solution in a clean Nessler’s Nesselar cylinder. Add 10ml of dilute Nitric acid and make up the volume up to 50ml by adding distilled water. Add 1ml of 0.1M silver nitrate and allow to stand for 5min. | Dissolve the sample given in 25ml distilled water in a clean Nessler’s cylinder. Add 10ml of dilute Nitric acid and make up the volume up to 50ml by adding distilled water. Add 1ml of 0.1M silver nitrate and allow to stand for 5min. |

**Reason:** Dilute nitric acid is used to prevent the interference of other impurity like Carbonates, Bicarbonates, Phosphate, and Hydroxide etc which also react with silver nitrate to give their respective precipitates. And the precipitates other than the silver chloride are soluble in dilute nitric acid.

The dilute nitric acid also increases the sensitivity of the reaction by common ion effect. The nitrate ion of nitric acid and silver nitrate is common so the formation of precipitates of silver chloride from silver nitrate will produce fast.

**Question for viva and synopsis**

1. Explain principle involved in Chloride limit test.
2. Why HNO3 used in Chloride limit test?
3. What do you mean by pure compound?
4. What is Impurities?
5. What are Toxic impurities?
6. Define limit test?

**Limit Test of Sulphate:**

**Principle:** The limit test for Sulphate is based upon the reaction between soluble Sulphate and Barium chloride in the form of Barium sulphate reagent in presence of Dilute HCl; it produces the turbidity due to the formation of Barium sulphate. The turbidity is compared with that of standard using 0.1089%w/v potassium sulphate.

The sample Turbidity should not be greater than the standard turbidity for passing the limit test for sulphate.

**Reaction:**

 Sample SO4- + BaCl2 Dil. HClBaSO4 + 2Cl-

 Standard K2SO4+ BaCl2  Dil. HClBaSO4 + 2KCl

**Composition of Barium Sulphate Reagent:**

1. 15ml of 0.5M Barium Chloride used as precipitating agent.
2. 5ml of 0.1089%w/v potassium sulphate used as seeding agent and thereby increase sensitivity of the reaction.
3. 20 ml of sulphate free alcohol used to avoid super saturation of Barium sulphate.
4. Up to 100ml of distill water.

**Reason:** Dilute HCl is used to prevent the interference of other impurity like Carbonates, Chloride, Bicarbonates, Phosphate, and Hydroxide etc which also react with Barium chloride to give their respective precipitates. And also the other precipitates are soluble in dilute HCl.

The HCl acid also increases the sensitivity of the reaction by common ion effect. The Chloride ion of HCl and Barium chloride is common so the formation of precipitates of Barium sulphate from Barium Chloride will produce fast.

**Procedure:**

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| --- | --- |
| **Standard** | **Sample** |
| Pipette Out 1ml of 0.1089% w/v of standard potassium sulphate solution in a clean Nessler’s cylinder and dilute to 25ml with distilled water Add 2ml of dilute HCl and 5ml of Barium Sulphate reagent. Make up the volume up to 50ml by adding distilled water. Allow to stand for 5min. | Dissolve the specified amount of sample with distilled water in a clean Nessler’s cylinder. Add 2ml of dilute HCl and 5ml of Barium Sulphate reagent. Make up the volume up to 50ml by adding distilled water. Allow to stand for 5min. |

**Comparison:** Compare the sample Turbidity with standard Turbidity by viewing the Nesselar cylinder against white back ground.

**Question for viva and synopsis**

1. Explain principle involved in Sulphate limit test.
2. Why Dil. HCl used in Chloride limit test?
3. Write the composition of Barium sulphate reagent. Explain their use.
4. Why Alcohol used in Sulphate limit test.?

**Limit test of Iron**

**Principle:** The limit test for Iron is based upon the reaction between ferrous ion and Thioglycolic acid to produce pale pink color of reddish violet color due to the formation of ferrous thioglycolate in ammonia solution.

The oxidation state of Iron is not important because the Thioglycolic acid a strong reducing agent which reduce the Ferric ion to ferrous ion.

The color produce by the sample is compared with that of Standard color produced by specific amount of ferric ammonium sulphate solution under the same reaction condition.

For the sample to pass the limit test for Iron the sample color should not be more than that of standard.

**Reaction:**

 Fe +++ SH CH2 COOH Fe++

 Fe ++ + 2 SH CH2 COOH Fe (SHCH2COO) 2  + H2

 Thioglycolic acid ferrous thioglycolate

 **Reason:** Citric acid is added because it forms a soluble complex with iron and avoids precipitation as its hydroxide when ammonia is added.

Ammonia is added to make the solution alkaline because ferrous thioglycolate gives pink color only in basic media but remain color less in acidic or neutral media.

 **Procedure:**

|  |  |
| --- | --- |
| **Standard** | **Sample** |
| * Pipette out 2ml of standard ferric ammonium sulphate solution in to a clean Nessler’s cylinder and dilute to 25ml with distilled water.
* Add 2ml of 20%w/v citric acid and 0.1ml Thioglycolic acid.
* Make up the solution alkaline by adding ammonia and check with Litmus paper.
* Dilute to 50ml with distilled water, mixed and allowed to stand for 5 min.
 | * Dissolve the specified amount of sample with 25ml distilled water in a clean Nessler’s cylinder.
* Add 2ml of 20%w/v citric acid and 0.1ml Thioglycolic acid.
* Make up the solution alkaline by adding ammonia and check with Litmus paper.
* Dilute to 50ml with distilled water, mixed and allowed to stand for 5 min.
 |

**Preparation of Standard Ferric Ammonium Sulphate Solution:** Dissolve 0.173gm of Ferric ammonium sulphate [NH4Fe (SO4)2], 2H2O to 1.5ml HCl. Add sufficient water to make up the volume 1000ml.

**Comparison:** Compare the sample color with standard color by viewing the Nesselar cylinder against white back ground.

**Question for viva and synopsis**

1. Explain principle involved in Iron limit test
2. Write the use of ammonia and citric acid in iron limit test.
3. Write the use of Thioglycolic acid is in iron limit test.
4. Name the standard substance used in iron limit test.

**Limit test of Heavy Metals:**

**Principle**: The limit test for heavy metal is based upon the reaction between heavy metal ion and hydrogen sulphide (freshly prepared) or sodium sulphide to produce black or brown color precipitate of heavy metal sulphide at suitable pH around 3-4 which could be maintained by adding dilute acetic acid and ammonia.

The color produce by the sample is compared with that of Standard color produced by specific amount of lead nitrate solution under the same reaction condition.

For the sample to pass the limit test for heavy metal the sample color should not be more than that of standard.

**Reaction**:

HM + H2S HMS + H2

HM + Na2S HMS + H2

Pb(NO3)2 + H2S/ Na2S PbS + HNO3/ NaNO3

**Procedure:**

**Method A:** Thismethod is applied for those substances which gives clear colorless solution under the specified condition**.**

|  |  |
| --- | --- |
| **Standard** | **Sample** |
| Pipette out 2ml of lead nitrate solution in to a clean Nesselar cylinder and dilute to 25ml with distilled water.The ph of the solution is adjusted between 3-4 by adding dilute acetic acid and ammonia. Make up the volume to about 35 ml by adding water.Add 10 ml freshly prepared hydrogen sulphide solution and make up the volume to 50 ml with water. | Dissolve the specified amount of sample with 25ml distilled water in a clean Nesselar cylinder.The ph of the solution is adjusted between 3-4 by adding dilute acetic acid and ammonia. Make up the volume to about 35 ml by adding water.Add 10 ml freshly prepared hydrogen sulphide solution and make up the volume to 50 ml with water. |

**Reason:** The pH is maintained 3-4 because in this pH range the heavy metal sulphide precipitate stable.

The hydrogen sulphide solution is freshly prepared because on keeping the hydrogen sulphide gas escape.

**Method B:** This method is applied for those substances which do not give clear colorless solution under the specified condition as per method A.

 The sample solution has to be made in a different way but the standard solution is same as method A.

**Preparation of sample solution for method B**

1. Specified quantities of sample as per monograph are taken in a crucible.
2. Moistened with sulphuric acid and ignited until charred.
3. A few drops of nitric acid are added and the mixture is heated about 500oC.
4. It is then allowed to cool and the residue is digested with 10ml of HCl for 2-3 min.
5. The excess acid is neutralized by NH3 and dilute with water and filtered.
6. 35ml of the above solution is taken in a Nessler’s cylinders. Add 10ml of freshly prepared H2S solution and make up the volume with water up to 50ml.

**Reason:**

Heating the sample with acids is done to remove non metallic substances which will interfere with the limit test

**Method C:**It is for those substances which gives clean colorless solution in NaOH.

|  |  |
| --- | --- |
| **Standard** | **Sample** |
| Pipette out 2ml of lead nitrate solution in to a clean Nessler’s cylinder. The ph of the solution is adjusted between 3-4 by adding Add 5ml NaOH and 5 drops of Na2S solution and make up the volume to 50 ml with water. | Dissolve the specified amount of sample with 25ml distilled water in a clean Nesselar cylinder.Add 5ml NaOH and 5 drops of Na2S solution and make up the volume to 50 ml with water. |

**Comparison:** Compare the sample color with standard color by viewing the Nesselar cylinder against white back ground.

**Question for viva and synopsis**

1. Explain principle involved in Heavy metal limit test.
2. Why ammonia and citric acid are used in Heavy metal limit test?
3. Why Hydrogen sulphide solution has to prepare freshly in Heavy metal limit test?
4. When Na2S and H2S used as reagent in Heavy metal limit test?

**Limit Test of Lead:**

**Principle:** The limit test for lead is based upon the reaction between lead and diphenyl thiocarbazone or Dithiazone in chloroform is able to extract lead impurities from alkaline aqueous solution as a lead Dithiazone complex which is red in color. The original Dithiazone is having green color in chloroform while the lead Dithiazone having violet or the red color. The intensity of color complex is depending upon on the amount of lead in solution. The color of lead Dithiazone complex in chloroform is compared with the color produced by standard lead nitrate solution treated in a same manner.

**Reaction**:



**Procedure: For sample:**

* A specified amount of sample solution is prepared as directed in IP and is taken in a separating funnel.
* Add 6 ml of ammonium citrate,
* Add 2 ml hydroxyl amine hydrochloride,
* Add 2 drops of phenol red is added. The solution is made alkaline by adding ammonia
* Add 2 ml of potassium cyanide is added.
* The alkaline solution is extracted with 5ml portion of Dithiazone solution in chloroform.
* Extraction is continued until the color of Dithiazone layer remains green. The combined chloroform extract are shaken with 1% nitric acid, the Dithiazone layer is taken into a beaker.

**For Standard:**

Specified quantity of lead nitrate treated in the same manner as the sample solution.

**Reason:** Reagent like hydroxyl amine hydrochloride, KCN, is added to prevent the interference of other impurities.

Ammonia is added to make the solution alkaline which will indicate by phenol red indicator, at this pH the extraction is optimum.

**Comparison:** Compare the sample color with standard color by viewing the Nesselar cylinder against white back ground.

**Question for viva and synopsis**

1. Why the reagent like hydroxyl amine hydrochloride, KCN is added to lead limit test?
2. Explain the principle and reaction inlead limit test?
3. Write Dithiazone test.

**Gutzeit’s Apparatus:** It consists of awide mouthed glass bottle of 120 ml capacity fitted with rubber bung through which a delivery tube of 20 cm length is placed having external diameter 8mm and internal diameter of 6.5mm. The tube is constricted at lower end with a hole of 2mm diameter and also a side hole of 1mm diameter. The upper end of the tube is fixed with two rubber bung such a way that a mercuric chloride paper is sandwiched in between them. The two rubber bung are held together tightly with a clip or a screw clamp. Inside the tube, 2mm below the rubber bung lead acetate cotton is placed.

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**Principle:** The limit test for arsenic is based upon the conversion of arsenic impurity by a series of reaction to arsenic gas which react with mercuric chloride test paper to give yellow or brown color strain of mercuric arsenide.

 The strain produce by the sample is compared to the strain of standard produce from specific amount of arsenic trioxide under the same reaction condition.

For the sample to pass the limit test of arsenic the sample strain should not be more than that of standard strain.

**Reactions:**

1. Trivalent or pentavalent arsenic impurity is converting to arsenous acid and arsenic acid by dilute HCl.

As3+ Dil HCl H3AsO3 As5+ Dil HCl H3AsO4

1. the arsenic acid formed is reduce to Arsenous acid by reacting with stannous chloride , HCl and KI

 HCl / KI / SnCl2

 H3AsO4  H3AsO3

1. Arsenous acid is further reduce to arsines gas by reacting with nascent hydrogen obtain from zinc and HCl

Zn + 2 HCl ZnCl2 + 2[H]

 H3AsO3 6[H] AsH3 + 3H2O

1. Arsene gas react with mercuric chloride test paper to give yellow or brown stain of mercuric arsenide

 AsH3+ HgCl2 Hg (AsH2)2 + 2HCl

**Preparation of the Apparatus for the Arsenic Limit Test:**

1. The glass bottle and the tube are first washed with dilute HCl and rinsed with water.
2. The delivery tube is then tightly packed with lead acetate cotton.
3. The upper end of the tube is inserted into a rubber bung on which a square piece of mercuric chloride test paper is placed.
4. The second bung is placed over this and the two bungs are tightly attached with spring clip.
5. The lower end of the delivery tube is inserted into another rubber bung.

**Procedure for Standard:** Place 50ml of water in wide mouth bottle; add 10ml of standard HCl, 1g of KI. Pipette out 10ml of standard Arsenic trioxide and transfer to the bottle, add 10g of granulated Zinc and quickly place the prepare glass tube in its position. Allow to stand for 40min at 40oC.

**Procedure for Sample:** Place 50ml of water in wide mouth bottle, add 10ml of standard HCl, 1g of KI and specified amount of sample in to the bottle. Add 10g of granulated Zinc and quickly place the prepare glass tube in its position. Allow to stand for 40min at 40oC.

**Reagent And Chemicals Required:** All the reagents and chemicals used arsenic limit test must be completely free from arsenic and labeled as **AsT** except the sample and standard solution.

**Mercuric Chloride Test Paper:** It is smooth white filter paper, not less than 25mm in width, which is first shocked in saturated solution of hgcl2 and finally dried at 60oc in dark. The grade of filter paper must be such that the g/m2 should be between 65-120g. The thickness of 400 papers must be approximately equal.

 **Precaution:**

1. The apparatus must be properly washed with dilute HCl and the rinsed with water every time before and after the Arsenic limit test.
2. Mercuric chloride test paper must is freshly prepared by wetting the test paper in Mercuric chloride solution and drying under shade.
3. The delivery tube must not dip in to the mixture in the bottle, so that the Arsene gas allowed passing through it.
4. The reaction must be allowed to precede at 400Cfor 40min.
5. The standard strain and sample strain should be prepare simultaneously and compare immediately after the reaction.
6. Lead acetate cotton plug should be keep freshly prepared.

**Reasons:**

1. Stannous chloride is added because it acts as a reducing agent in presence of HCl. It converts arsenic acid to Arsenous acid.
2. Granulated zinc is added to produce the nascent hydrogen gas by reacting with HCl. The nascent hydrogen gas is more reactive which convert Arsenous acid to arsene gas.
3. Granulated zinc contains an impurity of sulphur which reacts with HCl and produce H2S gas. So lead acetate cotton is placed in the delivery tube to trap the H2S gas by reacting with them, which otherwise react with HgCl2 paper and produce black color and the stain produce by arsene gas will not be visible.

H2S + HgCl2 HgS + 2HCl

 H2S + Pb (CH3COO) 2 PbS + CH3COOH

1. A side whole is provide in the delivery tube to allow continues passage for arsene gas eve the lower whole blocked due to condensation of water vapor.
2. The strains are immediately compared because they fade on exposure to light. They can be preserved by dipping the test paper in molten paraffin wax and dry it.

**Question for viva and synopsis**

1. Explain principle involved in Arsenic limit test.
2. Draw a neat labeled diagram of Gutzeit’s apparatus.
3. Why KI used in arsenic limit test?
4. Why Zn/HCl used in arsenic limit test?
5. Why side has made in the delivery tube in Gutzeit’s apparatus?
6. Explain why lead acetate cotton use in arsenic limit test.
7. How to preserve the stain on test paper?
8. Why Mercuric chloride test paper has to prepare freshly?

**Special Procedure for Limit Test of Chloride and Sulphate for Potassium permanganate**

**Principle:** The potassium permanganate is highly coloured substances. If this limit test done in the usual way it will be difficult to make observation. So potassium permanganate is eliminating by reduction with alcohol. This can be called pretreatment. The sample is dissolve in water and heated on water bath; alcohol is added it is filtered to remove the precipitate manganese dioxide. The filtrate is colourless and can be used for performing limit test for chloride and sulphate in usual way.

**2KMnO4 + 3CH3CH2OH 2KOH + 2MnO2 + 3CH3CHO + 2H2O**

**Procedure:**

* Dissolve 1.5 gm of sample in a 50 ml of distilled water.
* Heat on water bath and add gradually 6 ml of ethanol 95%.
* Cool, then dilute to 60 ml with distilled water and filter. The filtrate (solution A) is colourless.

**For limit test for chloride:** Take 40 ml of solution A and do the limit tests for chloride.

**For limit test for Sulphate:** Take 10 ml of solution A and do the limit tests for sulphate.

**OBSERVATION:**

 The opalescence of the test solution is ------than the standard solution.

**REPORT:**  The limit test for chloride and sulphate is -------.

**Special Procedure for Limit Test of Chloride and Sulphate for Sodium Bicarbonate**

**Principle: For Sodium bicarbonate**

An alkaline substance when reacted with mineral acid produces carbon dioxide; if the produced gas is not removed then it may interference in the normal procedure. Hence alkaline substance are dissolved in sufficient acid stirred well until carbon dioxide effervesce ceases and then the prescribed limit test for chloride and sulphate is performed.

**NaHCo3 + HNO3 / HCl NaNo2 / NaCl + CO2 + H2O**

**Preparation of Sample: Sodium Bicarbonate**

**Chloride:** 1.25gm dissolved in 15ml of distilled water and 2 ml of nitric acid complies with the limit test for chloride **(200ppm).**

**Sulphate:** 1gm in 10ml of distilled water, neutralize with HCl and dilute to 15ml distilled water. The resulting solution complies with the limit test for sulphate **(150ppm).**

In this special procedure the acid is added to neutralize the alkaline carbonate and then the sulphate ion reacts with barium chloride as barium sulphate reagent.

**Procedure: For Sulphate:** Suspend 1gm of sample in 10ml of distilled water, neutralize with HCl and dilute to 15ml with distilled water

|  |  |
| --- | --- |
| **Standard** | **Sample** |
| Pipette Out 1ml Of 0.1089% w/v of standard potassium sulphate solution in a clean Nessler’s cylinder and dilute to 25ml with distilled water Add 2ml of dilute HCl and 5ml of Barium Sulphate reagent. Make up the volume up to 50ml by adding distilled water. Allow to stand for 5min. | Take above solution in a clean Nessler’s cylinder. Add 2ml of dilute HCl and 5ml of Barium Sulphate reagent. Make up the volume up to 50ml by adding distilled water. Allow to stand for 5min. |

**Comparison:** Compare the sample Turbidity with standard Turbidity by viewing the Nessler’s cylinder against white back ground.

**For Chloride:** 1.25gm in dissolve in 15ml of water adds 2ml of nitric acid.

|  |  |
| --- | --- |
| **Standard** | **Sample** |
| Pipette Out 1ml Of 0.05845% w/v of standard NaCl solution in a clean Nessler’s cylinder. Add 10ml of dilute Nitric acid and make up the volume up to 50ml by adding distilled water. Add 1ml of 0.1M silver nitrate and allow to stand for 5min | Above solution is taken in a clean Nessler’s cylinder. Add 10ml of dilute Nitric acid. Add 1ml of 0.1M silver nitrate and allow to stand for 5min |

**Comparison:** Compare the sample opalescence with standard opalescence by viewing the Nesselar cylinder against white back ground.