Limit Test

Introduction

- Limit tests are quantitative or semiquantitative tests designed to identify & control small quantities of impurities likely to present in the substance.
- It involved comparison of opalescence, turbidity or color produced in a test with that of standard.
- To design the limit test following three factors are consider.

1. Specificity of the test
2. Sensitivity
3. Selectivity

Specificity of the test: A test employed as a limit test should imply some salt of selective reaction with the traced impurity.

e.g. contamination of lead and other heavy metals in alum is precipitated by thioacetamide at PH 2.5 thus less specific test which limits several impurities in single test.

Sensitivity: It depends on reproducibility of result.

e.g. in precipitation of insoluble sub. The factors such as concentration of solute, precipitating agent, duration of reactions, temp. are likely to be consider.

- Usually cold dilute solutions give light precipitate where as hot conc. Solution gives granular precipitate.

Selectivity: it depends on personal error and chemical nature.

- **Importance:**
- To find out harmful amount of impurity.
- To find out permissible or impermissible amount of impurities.

Nessler's cylinder

- Special apparatus design to carryout limit test.
- These are match tubes of clear colorless glass with uniform in diameter & flat transparent base.
- They have normal capacity of 50ml, height is 150mm & external height to the 50ml mark is about 110mm
- The comparison is made by placing the tubes Nessler's cylinder side by side & view transparently against proper background in case of limit test of chloride, sulphate & iron.

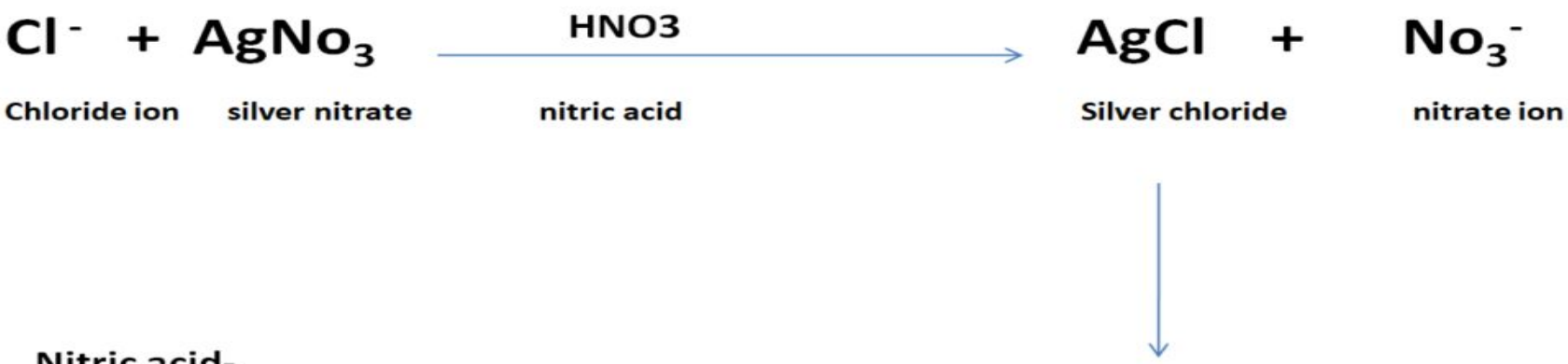

100
ml
100
ml
50
50

Limit test for chlorides

Principle:

1. It is used to control chloride impurity in the inorganic sub.
2. It involves the reaction between silver nitrate & soluble chloride to give insoluble silver chloride in presence of dil. Nitric acid.
3. Insoluble chloride makes the solution opalescence & the extent of opalescence depends upon the amount of chloride present in sub. i.e. compared with standard opalescence produced in standard solution.
4. If the turbidity from the sample has been less than the standard turbidity, the sample will pass the limit test and vice versa.

Reaction



Nitric acid-

Provide acidic condition for reaction.
Prevents ppt. of other radicals.

Opalescence produces

Procedure:

- **For standard solution:**

- 1. Take 50 ml of Nessler's cylinder, label as standard.
- 2. Placed 10ml of sodium chloride solution in N. cylinder. And 5ml water.
- 3. Add 10ml of water.
- 4. Add 1 ml of HNO_3 (Nitric acid).
- 5. Dilute to 50ml in Nessler cylinder.
- 6. Add 1 ml of 0.1 M $AgNO_3$(Silver Nitrate)
- 7. Stir and allow it to stand for 5 min.

For Test solution:

- 1. Placed specified quantity of substance being examined.
- 2. Dissolve in 10ml water.
- 3. Add 1ml of HNO_3(Nitric acid)
- 4. Dilute to 50 ml in Nessler cylinder.
- 5. Add 1 ml of $AgNO_3$ (silver nitrate) solution
- 6. Stir and allow it to stand for 5 min.

- Compare the opalescence of test solution with standard opalescence transversely by keeping Nessler cylinder against black background.
- Opalescence produced in test solution should not be more intense than standard opalescence.

Method for limiting test for chloride

Compliance: The opalescence of the test solution with standard opalescence transparency by keeping Nessler's cylinder against black background it shows the test solution not be more intense than standard opalescence.

- **Use:** This limit test is utilized to find out chloride impurities in water, food particles, pesticides and pharmaceuticals.

Modified limit test for chloride:

- **Principle:** limit test of chloride is based on the precipitation reaction, in case of general chloride limit test there is occurrence of reaction between chloride impurity or sodium chloride and silver nitrate in presence of dilute nitric acid with the reference to the international pharmacopoeia 2016.
- The limit test of chloride has been modified in contest of standard solution preparation. Early year the standard solution of chloride was prepared by dissolving sodium chloride but now it has been modified by using hydrochloric acid instead of sodium chloride.

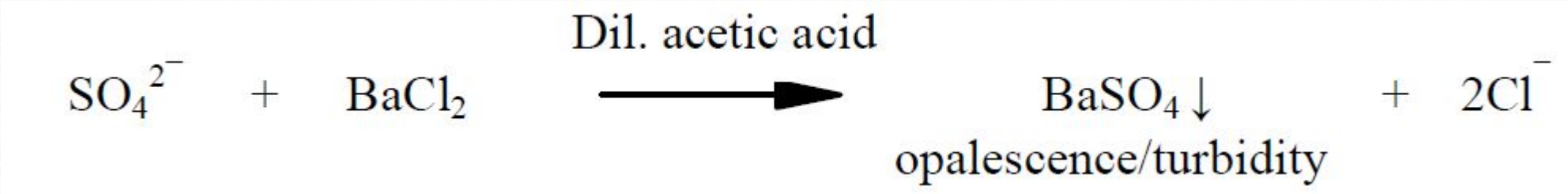
- In the past, the chlorine solution was prepared through the dissolution of sodium chloride (NaCl, known as Cl- impurity), but now it has been modified by substituting sodium chloride (NaCl) for hydrochloric acid (HCl).
- $HCl + AgNO_3 \rightarrow AgCl + HNO_3$
- **Conclusion:** When the opalescence produced in the sample solution is lower than that produced in the standard solution, the sample will pass the limitation test for chloride and vice versa.

Limit test for sulphate

Principle:

- This test is designed to control sulphate impurity in inorganic substance.
- This test depends on simple reaction between barium chloride and soluble sulphate in the presence of acetic acid to give insoluble barium sulphate.
- The opalescence produced in the test is compared with that of standard opalescence obtained from standard sulphate solution containing known amount of sulphate produced in the same manner. If the opalescence is less intense than that of standard, the sample passes the limit test for sulphate and vice versa.
- The solubility of barium sulphate precipitate is affected by the concentration of the acid. acidity of the solution is controlled by using acetic acid.

- **Reaction:**



- **Method (IP2007, 2014)**
- **Preparation of 25% w/v barium chloride solution-** dissolve 25 gm of barium chloride in distilled water to produced 100ml.
- **Preparation of ethanolic sulphate solution- (10ppm SO4)**
- Dilute 1 volume of 0.181 %w/v solution of potassium sulphate in ethanol (30%) to the 100 vol. with ethanol (30%)
- **Preparation of sulphate standard solution-** dilute 1 vol. of a 0.181%w/v solution of potassium sulphate in distilled water to 100 volume with distilled water.

Preparation Of test solution	Preparation of standard solution
1. Take 1ml of 25% w/v solution of barium chloride in Nessler's cylinder.	1. Take 1ml of 25% w/v solution of barium chloride in Nessler's cylinder.
2. Add 1.5ml of ethanolic sulphate standard solution(10ppm SO4)	2. Add 1.5ml of ethanolic sulphate standard solution(10ppm SO4)
3. Mix and allow to stand for 1 minute.	3. Mix and allow to stand for 1 minute.
4. Add 15ml solution of specified quantity of substance in water or 15ml solution prepared as per individual monograph.	4. Add 15ml of sulphate standard solution (10 ppm SO4)
5. Add 0.15 ml of 5M acetic acid	5. Add 0.15 ml of 5M acetic acid
6. Dilute to 50ml with distilled water.	6. Dilute to 50ml with distilled water.
7. Stir immediately with glass rod & allow it stand for 5min.	7. Stir immediately with glass rod & allow it stand for 5min.

- **Compliance:** The opalescence of the test solution with standard opalescence transparency by keeping Nessler's cylinder against black background it shows the test solution not be more intense than standard opalescence.

Modified limit test for sulphate

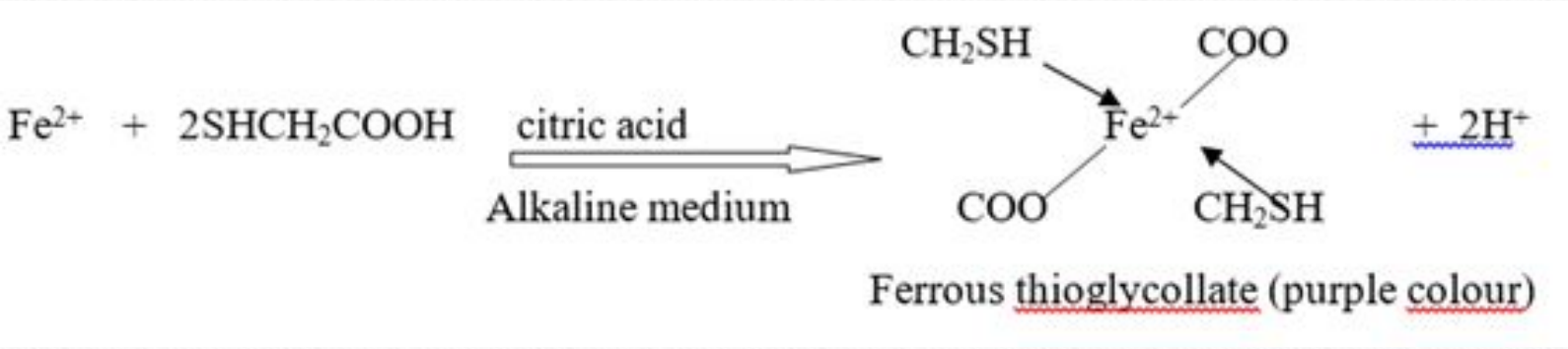
- **Principle:** The limit test for sulfates uses the precipitation method as its basic principle. As a result of reacting with barium chloride in the presence of hydrochloric acid, the sulfates precipitate as barium sulfate. In the presence of hydrochloric acid, only sulfate precipitates as other acid radicals do not react with barium chloride as hydrochloric acid prevents the reaction of different acid radicals with barium chloride.
- **$SO_4^{2-} + BaCl_2 (HCl) \rightarrow BaSO_4 + 2Cl^-$**
- Precipitates form in the solution, leaving it turbid, with the degree of turbidity being dependent on the amount of sulfate present. In order for the test results to indicate that the sample contains sulfates within prescribed limits, the sample's turbidity must be less than that of the standard.

- **Procedure:** Modified sulfate limit test - From I.P.1996 on, limit tests for sulfate have undergone an extensive modification. By doing so, it eliminated the need for barium sulfate reagents. While turbidity is comparable through the use of alcohol and barium chloride, the method still uses alcohol.

- **Conclusion:** According to the I.P.1996 standard, the sample has passed the limit test if the opalescence produced by the standard exceeds the opalescence of the test.

Limit test for iron

- **Principle-** the limit test of iron is based on formation of pale pink to deep reddish-purple color by reaction of iron with thioglycolic acid in presence of citric acid in a solution made alkaline with ammonia solution.
- The color is due to formation of coordination compound, ferrous thioglycolate Fe(HSCH2COO)2.



Preparation of iron standard solution(20 ppm Fe)

- Dilute 1 volume of a 0.1726 % w/v solution of ferric ammonium sulphate in 0.05M sulphuric acid to 10 vol. with distilled water.

Procedure:

Test solution	Standard solution
Dissolve the given sample in 20 ml water and transfer into Nessler's cylinder.	Transfer 2.0 ml of iron standard solution (20 ppm Fe) to a Nessler's cylinder.
Add 2 ml of 20 % w/v of iron free citric acid.	Add 2 ml of 20 % w/v of iron free citric acid.
Add 0.1 ml of thioglycollic acid.	Add 0.1 ml of thioglycollic acid.
Then make the solution to alkaline with iron free ammonia solution.	Then make the solution to alkaline with iron free ammonia solution.
Dilute to 50 ml with water and allowed to stand for 5 min and observe the color transversely.	Dilute to 50 ml with water and allowed to stand for 5 min and observe the color transversely.

Compare any color produced (pale pink to deep reddish purple) in test solution with standard by viewing transversely through the Nessler's cylinder.
The color produced in test solution not be more intense than standard opalescence.

Citric acid is added in the official process to prevent the precipitation of iron by ammonia. Iron gets precipitated in the form of Fe(OH)2 & Fe(OH)3. Citric acid forms a soluble complex with iron, hence ammonia is not able to precipitate iron. It only provide alkaline medium.

- Samples to be tested for the iron limit test-
- Calcium carbonate
- Sodium bicarbonate
- Zinc oxide

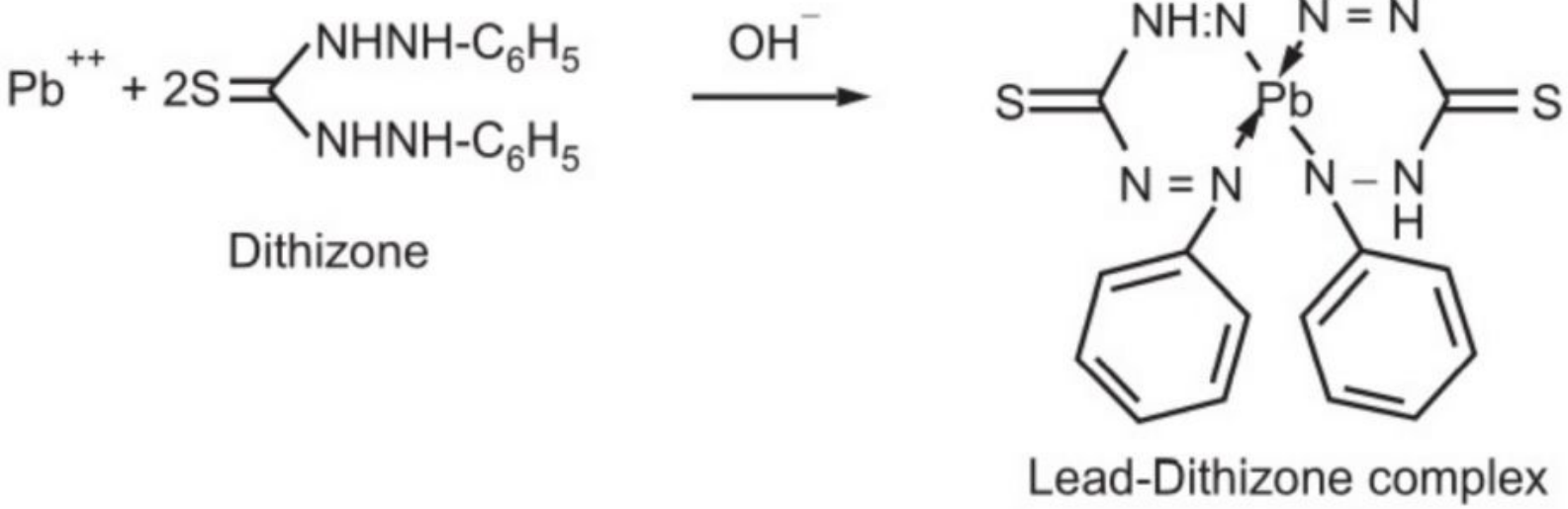
Limit test for lead

- **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 read in color.

  Dithizone is green in color in chloroform and lead-dithizone complex is violet in color, so the resulting color at the end of process is red.

- Reaction:



Dithizone

Lead-Dithizone complex

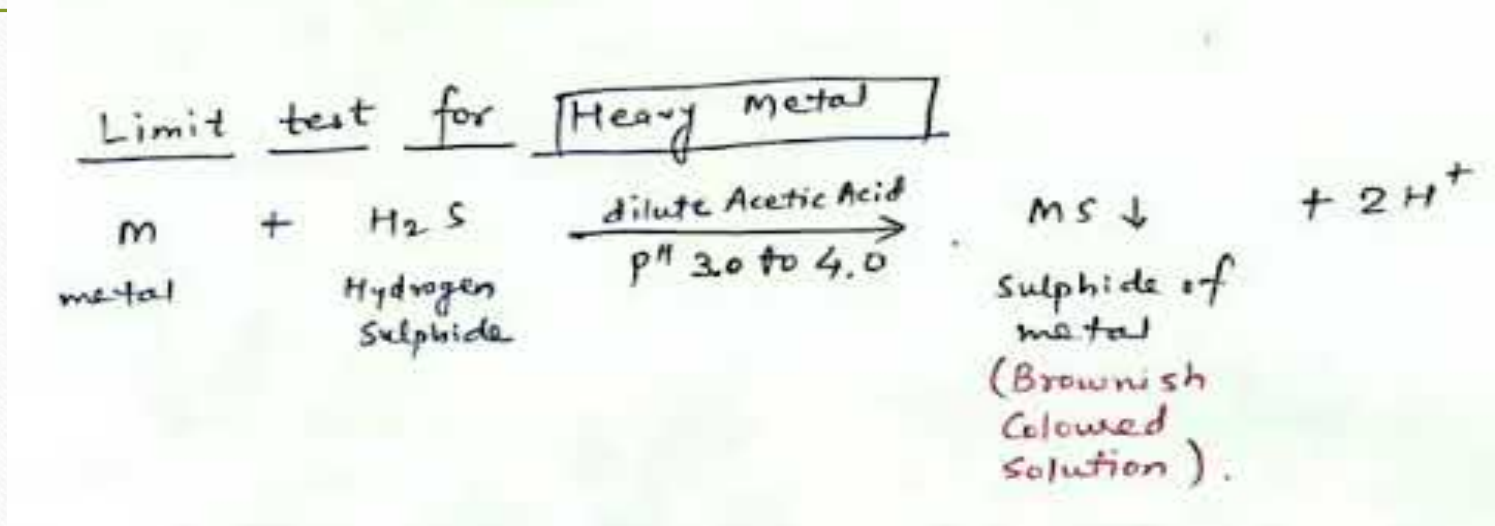
Test sample	Standard compound
1. A known quantity of sample solution is transferred in a separating funnel	1. A standard lead solution is prepared equivalent to the amount of lead permitted in the sample under examination
2. Add 6ml of ammonium citrate	2. Add 6ml of ammonium citrate
3. Add 2 ml of potassium cyanide and 2 ml of hydroxylamine hydrochloride Add 2 drops of phenol red	3. Add 2 ml of potassium cyanide and 2 ml of hydroxylamine Hydrochloride Add 2 drops of phenol red
4. Make solution alkaline by adding ammonia solution. Extract with 5 ml of dithizone until it becomes green	4.Make solution alkaline by adding ammonia solution. Extract with 5 ml of dithizone until it becomes green
5. Combine dithizone extracts are shaken for 30 mins with 30 ml of nitric acid and the chloroform layer is discarded	5.Combine dithizone extracts are shaken for 30 mins with 30 ml of nitric acid and the chloroform layer is discarded
6. To the acid solution add 5 ml of standard dithizone solution Add 4 ml of ammonium cyanide	6.To the acid solution add 5 ml of standard dithizone solution Add 4 ml of ammonium cyanide
7. Shake for 30 mins	7.Shake for 30 mins

Violet color of the chloroform layer in test solution should not be more intense than that of standard solution.

Limit test for heavy metals

- Limit test for heavy metals is designed to determine the content of metallic impurities that are colored by hydrogen sulphide or sodium sulphide under the condition of the test. The heavy metals may be iron, copper, lead, nickel, cobalt, bismuth, antimony etc. the limit for heavy metals is indicated in the individual monograph in the term of ppm of lead i.e., the parts of lead per million part of the substance being examined. The Indian pharmacopoeia adopted four methods (A, B, C & D) for the limit test for heavy metals.

Reactions



$$Pb + H_2S \longrightarrow \underset{\text{colour (brown)}}{PbS\downarrow} + 2H^+$$

Method C: (for the substances that gives clear colorless solution in sodium hydroxide)

Test solution	Standard solution
The sample solution is prepared as per the monograph and 25 ml of solution is transferred into a Nessler's cylinder.	Transfer 1.0 ml of standard lead solution and dilute to 25 ml with water.
Add dilute ammonia or acetic acid solution to adjust the pH between 3 to 4	Add dilute ammonia or acetic acid solution to adjust the pH between 3 to 4
Dilute to 35 ml with distilled water and mix well.	Dilute to 35 ml with distilled water and mix well.
Add 10 ml of freshly prepared hydrogen sulphide solution, mix, dilute to 50 ml with water.	Add 10 ml of freshly prepared hydrogen sulphide solution, mix, dilute to 50 ml with water.
Allow to stand for 5 min and view downwards over a white background.	Allow to stand for 5 min and view downwards over a white background.

Limit test for Arsenic

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

$$\underset{\text{Arsenic acid}}{H_3AsO_4} + H_2SnO_2 \rightarrow \underset{\text{Arsenious acid}}{H_3AsO_3} + H_2SnO_3$$

$$\underset{\text{Arsenious acid}}{H_3AsO_3} + 3H_2 \rightarrow \underset{\text{Arsine}}{AsH_3} + 3H_2O$$

Gutzuit's Apparatus

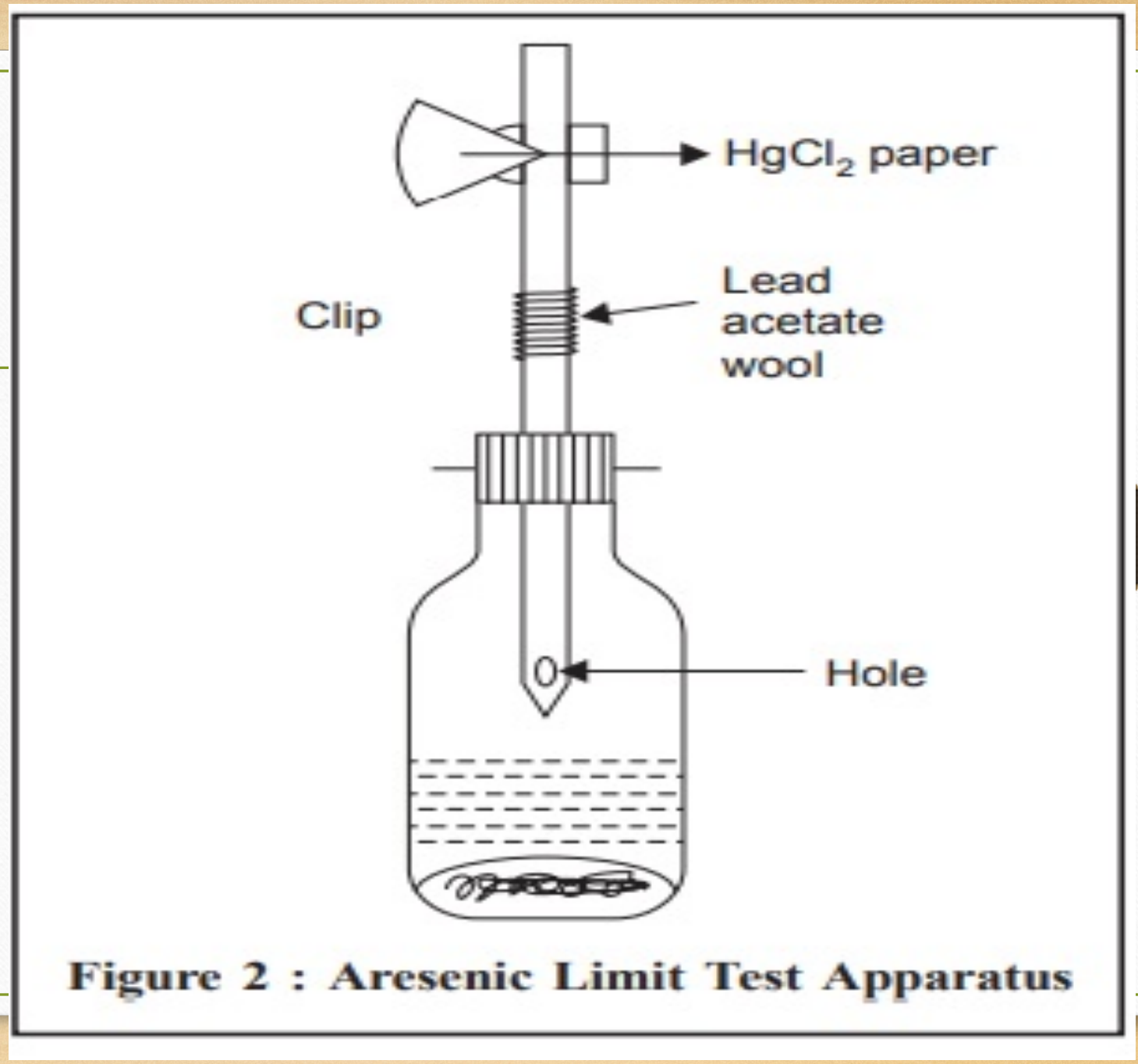


Figure 2 : Aresenic Limit Test Apparatus

Procedure

Standard Preparation	Test Preparation
1. Take a specified volume of standard arsenic solution in a wide mouthed bottle as prescribed in the pharmacopoeia.	1. Dissolve specific amount of sample in specified volume of water as prescribed in the pharmacopoeia.
2. Add 10 ml of stannated HCl (arsenic free).	2. Add 10 ml of stannated HCl (arsenic free).
3. Add 1 gm of KI and 10 gm of zinc dust.	3. Add 1 gm of KI and 10 gm of zinc dust.
4. Keep the solution aside for 40mins and compare the stain obtained on mercuric chloride paper.	4. Keep the solution aside for 40mins and compare the stain obtained on mercuric chloride paper.