

DEPTH OF BIOLOGY

LIMIT TEST



- ❖ Test being used to identify the impurity.
- ❖ It is quantitative or semi-quantitative test design to identify and control small quantity of impurities (which is likely to be present in the substance).
- ❖ Limit test → Generally carried out to determine the inorganic impurities present in compound.

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LIMIT TEST

- Lead and Heavy Metal
- Sulphate
- Chloride
- Arsenic
- Iron
- Lead
- Modified Limit test for Sulphate
- And modified Limit test for chloride and sulphate

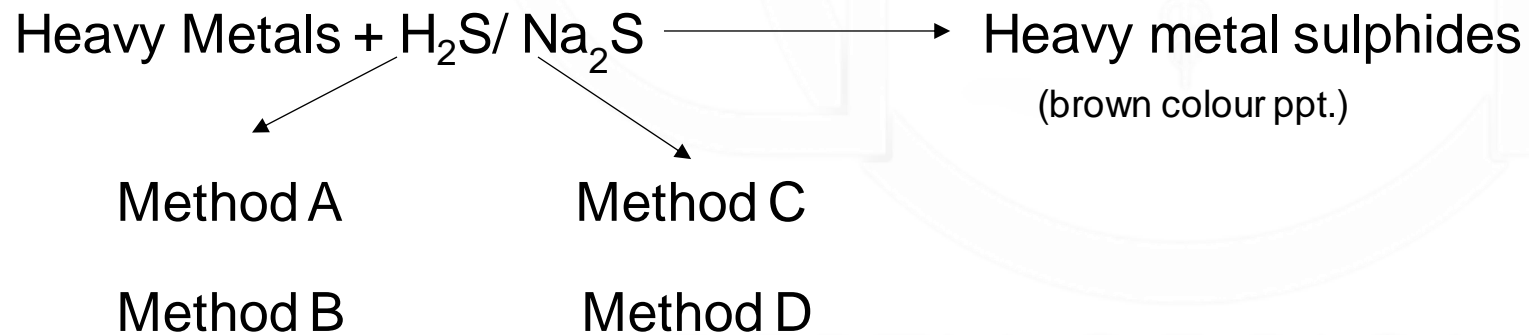


LIMIT TEST FOR HEAVY METALS

Heavy Metal like \longrightarrow Co, Sn, Mn, Pb, Ag, Sb

PRINCIPLE:-

Limit test for heavy metal is based on the reaction of metallic impurities with Hydrogen, Sulphide or Sodium sulphide in acidic medium to produce metal sulphides which gives Brown colour. [LEAD IS USED AS STAND]



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Method A = For the substance give clear colourless solution under specific condition give in monograph (pharmacopeia).

Method B = For the substance not give clear colourless solution.

Method C = For the substance who give clear colourless solution in NaOH solution.

Method D = For the substance not give clear colourless solution.

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LIMIT TEST FOR SULPHATE

Means, here we check impurities of sulphate in sample with the help of Limit Test.

Here we use 2 Nessler cylinder

- Test solution
- Standard solution

Chemical required :-

Potassium Sulphate , Distilled water, Barium sulphate reagent, Dilute HCl



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Test solution

1. Take 1 ml of a 25 % w/v solution of barium chloride in a Nessler's cylinder
2. Add 1.5 ml of ethanolic sulphate standard solution(10 ppm SO₄) Mix and allow to stand for 1min.
3. Take 15ml of specific amount of test substance dissolve in distilled water or 25ml of test solution prepared as directed in monograph in IP.
4. Add 0.15ml of 5M acetic acid
5. Make up to 50ml with distilled water.
6. Stir with glass rod and allow to stand for 5min
7. Observe the turbidity and compare with standard solution.

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STANDARD SOLUTION (HCl)

1. Take 1.0ml of a 25% w/v solution of barium chloride in a Nessler's cylinder
2. Add 1.5ml of ethanolic sulphate standard solution (10ppm SO₄) Mix and allow to stand for 1min.
3. Take 15ml of sulphate standard solution (10 ppm SO₄)
4. Add 0.15ml of 5M acetic acid
5. Make up to 50ml with distilled water
6. Stir with glass rod and allow to stand for 5min.
7. Compare with sample solution

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❖ PRINCIPLE

- . BaSO_4 reagent react with sulphate
- . In the presence of dilute HCl
- . And form BaSO_4
- . Lead to formation of white ppt.
- . Lead to turbidity in solution



LIMIT TEST FOR CHLORIDE

Means, here we check impurities of chloride in sample with the help of Limit test.

For Limit Test, we use Nessler cylinder [2]

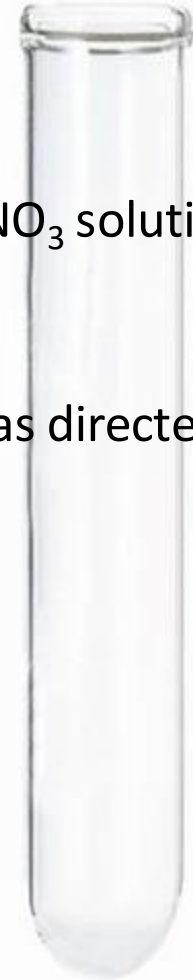
Test solution

Standard solution

Chemical required :- Standard NaCl solution – 0.05845%, distilled H₂O, Dilute HNO₃, AgNO₃ solution- 5%

TEST OR SAMPLE SOLUTION

1. Specific amount of test substance dissolve in distilled water or solution is prepared as directed in monograph and take it into Nessler's cylinder.
2. Add 10ml dilute Nitric acid
3. Dilute upto 50ml with distilled water
4. Add 1ml of AgNO₃ solution
5. Keep aside for 5mins
6. Observe the opalescence and compare with standard solution



STANDARD SOLUTION(25 PPM)

1. Take 10ml standard chloride solution + 5ml distilled water in Nessler's cylinder
2. Add 10ml dilute Nitric acid
3. Dilute upto 50ml with distilled water
4. Add 1ml AgNO₃ solution
5. Keep aside for 5mins
6. Compare with sample solution
7. Now, compare both test and standard solution
8. If, test solution less turbidity as compared to standard then test pass
9. [test solution > standard solution (turbidity) test fail

PRINCIPLE

– Limit Test of chloride

Here we add AgNO₃

→ this react with chloride in the presence of dilute HNO₃ form AgCl

This leads to turbidity



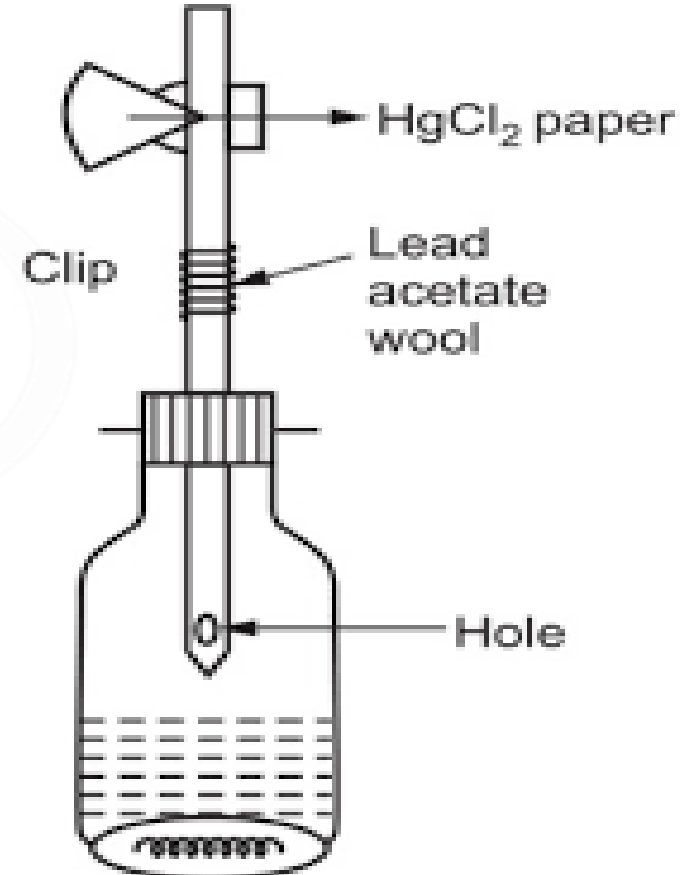
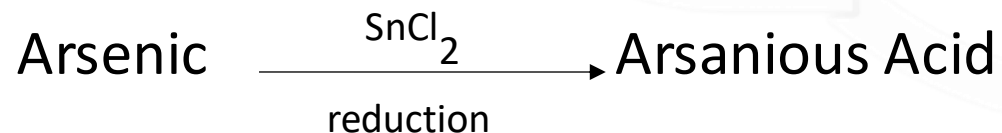
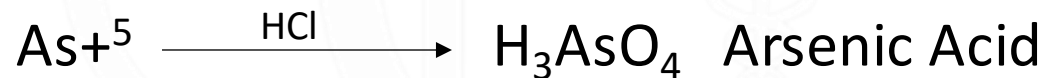
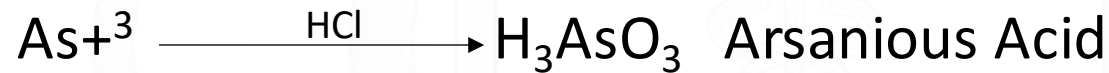
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LIMIT TEST FOR ARSENIC

Arsenic easily reduce into Arsine gas If we use Nessler cylinder then gas release out , so we use Gutzeit apparatus.

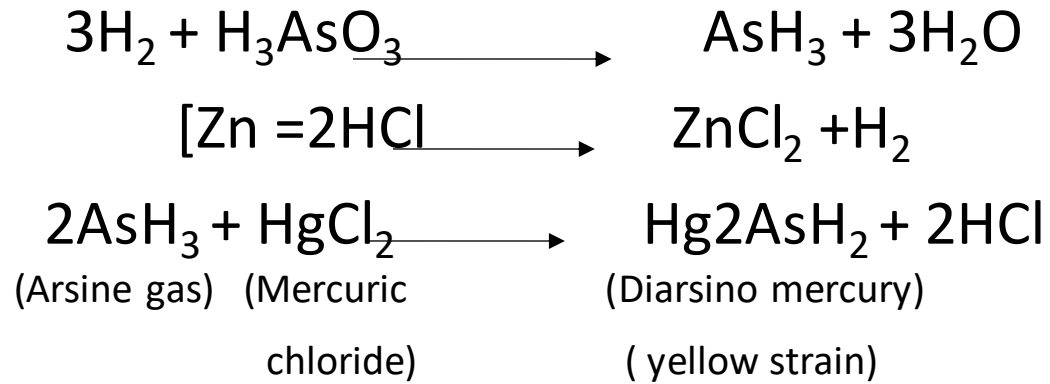
As may be present as As^{+3} (Trivalent)

As^{+5} (Pentavalent)



Standard Solution	Test Solution
Standard solution of Arsenic.	Test solution of Arsenic.
Add 10gm KI	Add 10gm KI
10gm granulated Zn	10gm granulated Zn
5ml of SnCl ₂	5ml of SnCl ₂
Kept solution for 40 min.	Kept solution for 40 min.
Arsenic gas release	Arsenic gas release

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If,

[test solution < standard solution (yellow strain)] → Test Pass

But,

[standard solution < test solution (yellow strain)] → Test Fail

Principle

Arsine Gas react with HgCl_2 paper

Form Diarsino Mercury which leads to yellow strain.



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LIMIT TEST FOR IRON

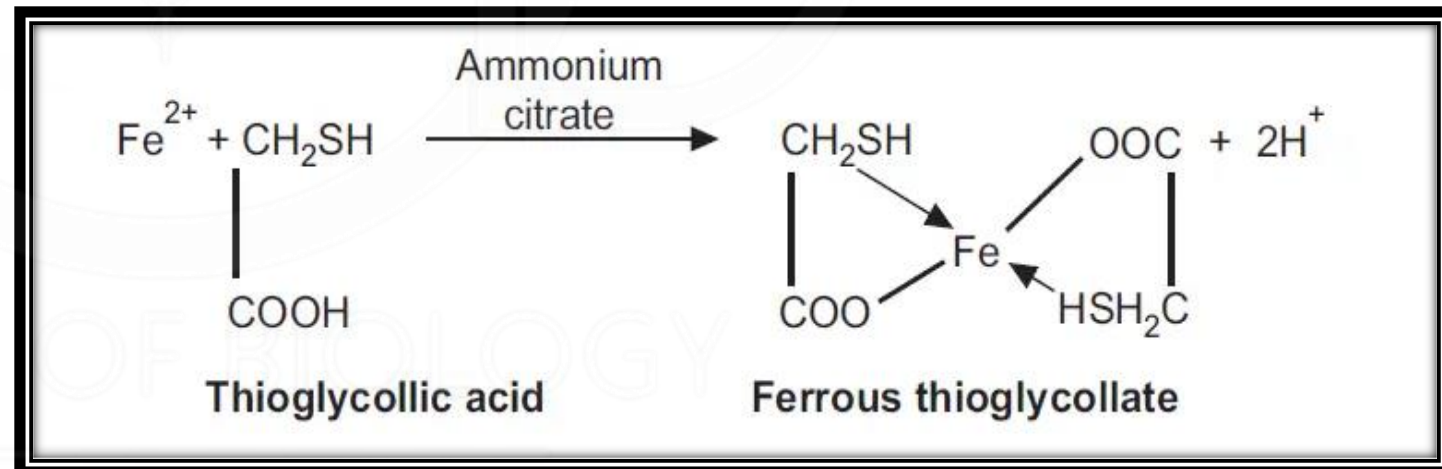
Small amount of Inorganic impurity are detect.

Here, we check impurity of Fe so we perform limit test and called limit test for Iron.

Use 2 Nessler cylinder

Chemical required:-

- Standard Ferric ammonium sulphate solution =20ml
- Citric acid solution = 20% w/v
- Ammonia solution
- Thioglycolic acid



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

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Now, compare the color of Test solution with Standard solution.

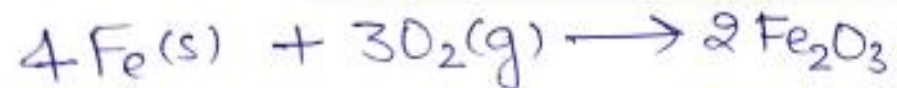
If , Test solution have less color as compared to standard then test pass

[standard < test solution (color) Test Fail]

Principle

- 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 to reddish purple color is visible only in the alkaline media. The color is not visible in acidic media as ferrous thioglycolate complex decomposes in high acidic media.

The balanced chemical equation for
Synthesis of Iron(III) oxide from its elements



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LIMIT TEST FOR LEAD

Most undesirable impurity in medical compound and come through use of H_2SO_4

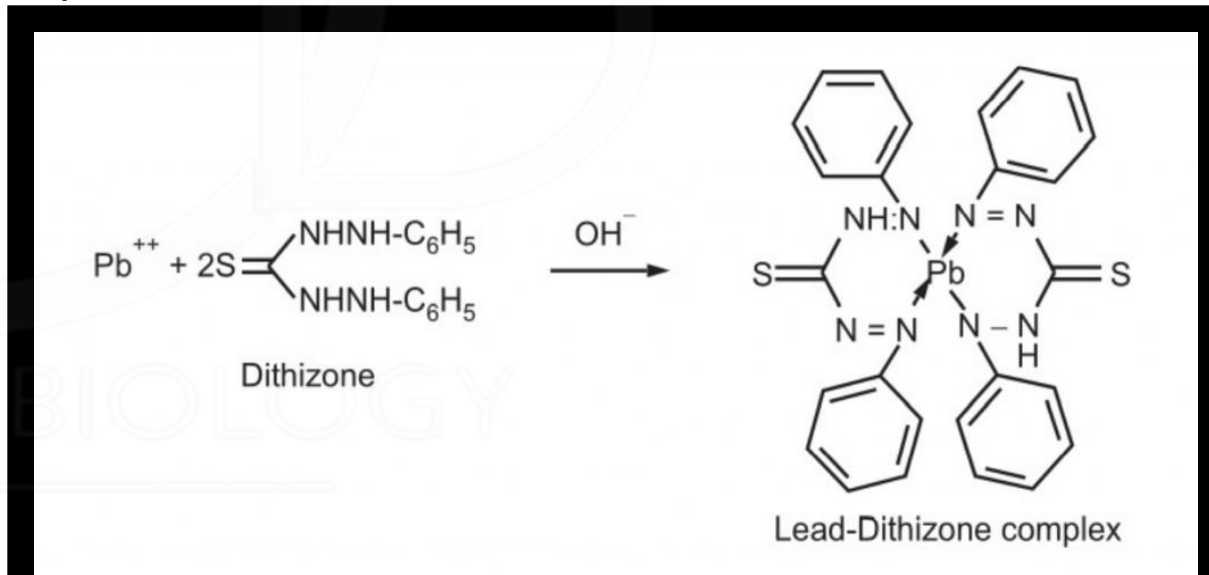
- Lead line apparatus
- Glass bottles used for storage of chemicals

Principle

Based on the reaction of lead and diphenyl thiocarbazon (dithizone) in alkaline solution to form lead dithizone complex (Red color).

Dithizone → is green color in chloroform and lead dithizone complex is violet in color.

So, the resulting color at the end of the process is red.



Test solution :- A known quantity of sample solution is transferred in a separating funnel	Standard solution :- 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 2ml of potassium cyanide and 2ml of hydroxylamine hydrochloride	Add 2ml of potassium cyanide and 2ml of hydroxylamine hydrochloride
Add 2 drops of phenol red and make solution alkaline by adding ammonia solution	Add 2 drops of phenol red and make solution alkaline by adding ammonia solution
Extract with 5ml of dithizone until it becomes green	Extract with 5ml of dithizone until it becomes green
Combine dithizone extracts are shaken for 30mins with 30ml of nitric acid and the chloroform layer is discarded	Combine dithizone extracts are shaken for 30mins with 30ml of nitric acid and the chloroform layer is discarded
To the acid solution add 5ml of standard dithizone solution	To the acid solution add 5ml of standard dithizone solution
Add 4ml of ammonium cyanide	Add 4ml of ammonium cyanide
Shake for 30 mins and observe the color	Shake for 30 mins and observe the color

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OBSERVATION

The intensity of the color of complex, is depends on the amount of lead in the solution.

[Standard > Test solution (Red color) Test Pass]

[Test > Standard solution (Red color) Test Fail]

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 color 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.



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MODIFIED LIMIT TEST FOR SULPHATE

Principle :- Used for those color compound who does not pass normal limit test.

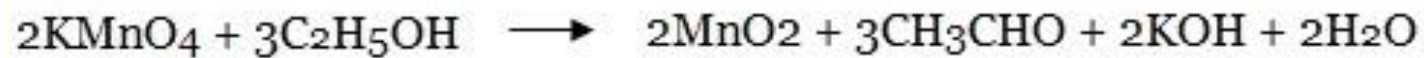
It carried out on the basis of reaction between BaCl_2 and soluble in the presence of Acetic acid .

Leads to formation of BaSO_4

Here reagent used \rightarrow BaCl_2 , sulphate free alcohol and a solution of Potassium sulphate (is added to increase sensitivity of test) and here alcohol is helps in prevention from supersaturation and produce uniform turbidity.

Here Acetic acid makes acidic solution \rightarrow BaSO_4 ppt. form (insoluble) which give turbidity.

Chemicals: Potassium sulphate (K_2SO_4), potassium permanganate (KMnO_4), barium chloride (BaCl_2), ethanol, hydrochloric acid (HCl), glacial acetic acid (CH_3COOH).



(Sample) (ethanol)

Test solution

Take 1 ml 25% w/v barium chloride in Nessler's cylinder and add 1.5 ml of ethanolic sulphate standard solution (10 ppm SO_4^{-2}). Mix and allow to stand for 1 minutes

Transfer prepared test solution and add 0.15 ml of 5 M acetic acid.

Add sufficient distilled water to produced 50 ml. Stirred it immediately and allow standing for 5 minutes.

Standard solution

Take 1 ml 25% w/v barium chloride in Nessler's cylinder and add 1.5 ml of ethanolic sulphate standard solution (10 ppm SO_4^{-2}). Mix and allow to stand for 1 minutes

Add 15 ml of standard sulphate solution (10 ppm SO_4^{-2}) and 0.15 ml of 5M acetic acid.

Add sufficient distilled water to produced 50ml. Stirred it immediately and allow standing for 5 minutes.



[Standard solution > Test solution (Turbidity) Test Pass]
[Standard solution < Test solution (Turbidity) Test Fail]

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MODIFIED LIMIT TEST FOR CHLORIDE

Used for color compound which cannot be tested with normal test.

Principle → based upon the chemical reaction between silver nitrate and soluble chloride to obtain AgCl (makes the test solution turbid) in the presence dilute HNO₃



Limit Test for Chlorides



Procedure	Test solution	Standard solution
1.5gm of KMnO_4	Take 40ml of the test solution in Nessler cylinder	10ml standard chloride solution
50ml distilled H_2O and heat on a water bath	Add 0.1ml dilute HNO_3	Add 5ml H_2O and add 1ml dilute HNO_3
Now gradually add 6ml of Ethanol (95%)	50ml distilled H_2O	50ml distilled H_2O
Now, cool the solution and dilute to 60ml with distilled water and then filter	Now add 1ml 0.1M AgNO_3 solution	0.1M AgNO_3 solution
Take two 50ml Nessler cylinder	Stir immediately with glass rod	Stir with glass rod
Labelled one test	And placed it for 5min (protected from light)	And placed it for 5min
And other on standard		

Reagent used:- Dilute $\text{HNO}_3 \rightarrow$ makes solution Acidic which helps in formation of AgCl ppt. (Leads to turbidity)
 This test is not used for H_2O immiscible liquid.