LIMIT TEST

- ***Limit tests are quantitative or semi-quantitative tests designed to identify and control small amount of impurities, which are likely to be present in the substance.***
- ***They involve simple comparisons of opalescence, turbidity or colour produced in test with that of fixed standards.***
- ***Limit test is generally carried out to determine the inorganic impurities present in compound. In short, limit test is nothing but to identify the impurities present in the substance and compare it with standard.***
- ***Some of the limit tests are performed in a special apparatus known as Nessler cylinder. Nessler cylinders are matched tubes of clear, colourless glass with a uniform internal diameter and a flat, transparent base.***
- ***They have a nominal capacity of 50 ml. The overall height of Nessler cylinder is about 150 mm and the external height to the 50 ml mark is about 110 to 124 mm.***
- ***In general, limit tests are performed in an aqueous solution or sometimes the solution is prepared as specified in the monograph of the Pharmacopoeia.***
- ***An identical pair of Nessler cylinder should be used i.e. a pair made of the same glass, having same diameter and same height of the graduation mark from the base.***
- ***Comparison is made by placing the two Nessler cylinders side by side and viewing transversely against proper background in case of limit test chlorides, sulphates and iron. Whereas for heavy metals comparison is done by placing the two Nessler cylinders close together and viewing down through the solution against a light background.***

Importance of Limit tests:

- To find out the harmful amount of impurities.
- To find out the avoidable/unavoidable amount of impurities.
- To improve the quality of pharmacopoeial substance.

Limit Test of Chloride

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.

Procedure:

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 1ml of 0.05845 % W/V solution of sodium chloride in Nessler cylinder
Add 1ml of nitric acid	Add 1ml of nitric acid
Dilute to 50ml in Nessler cylinder	Dilute to 50ml in Nessler cylinder
Add 1ml of $AgNO_3$ solution	Add 1ml of $AgNO_3$ solution
Keep aside for 5 min	Keep aside for 5 min
Observe the Opalescence/Turbidity	Observe the Opalescence/Turbidity

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.

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.

Limit Test of Sulphate

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.

Procedure:

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 1ml of 0.1089 % W/V solution of potassium sulphate in Nessler cylinder
Add 2ml of dilute hydrochloric acid	Add 2ml of dilute hydrochloric acid
Dilute to 45 ml in Nessler cylinder	Dilute to 45 ml in Nessler cylinder
Add 5ml of barium sulphate reagent	Add 5ml of barium sulphate reagent
Keep aside for 5 min	Keep aside for 5 min
Observe the Turbidity	Observe the Turbidity

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

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.

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.

Modified limit test for Chloride and Sulphate

The limit test for Chloride and Sulphate cannot be performed for some substances, because of the interfering of opalescence by colour or precipitate produced by the particular substances. So before carrying out the limit test, we have to modify the procedures to remove those types of interferences.

For example, **potassium permanganate,**

Potassium permanganate in water produce dark violet colour, in presence of which the opalescence or turbidity cannot be seen. So before carrying out the limit test, it is necessary to destroy the colour by using some reducing agents like methanol, ethanol etc which will not interfere the limit test. The manganese dioxide colour is reduced by adding ethanol. The precipitation is removed by filtration and the clear colourless filtrate is used for performing limit test for chloride and sulphate.

$$2KMnO_4 + 3CH_3CH_2OH \qquad 2MnO_4 + 2CH_3CHO + 2H_2O$$

Procedure

Dissolve 0.5 ml of KMnO4 in 20 ml boiling water and add gradually 3 ml of 95% ethanol and dilute with 30 ml of distilled water and filter it. Then the 10 ml of colourless filtrate is used for the limit test chloride and sulphate by using the similar procedures.

Similarly **sodium benzoate** and **Sodium salicylate** are salts of organic acids, which are treated with nitric acid or HCl, the corresponding organic acids are precipitated which are not soluble in water. These precipitates are interfere the opalescence. In such cases, it is completely acidified using concentrated acids, filter the precipitate and the clear filtrate was used for limit tests by using similar procedures.

Limit Test of Iron

Principle:

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 which is pale pink to deep reddish purple in color.

Procedure:

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 40ml
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
Color developed is viewed vertically and compared with standard solution	Color developed is viewed vertically and compared with standard solution

Earlier ammonium thiocyanate reagent was used for the limit test of iron. Since thioglycolic acid is more sensitive reagent, it has replaced ammonium thiocyanate in the test.

Observation:

The purple color produce in sample solution should not be greater than standard solution. If purple color 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 helps precipitation of iron by ammonia by forming a complex with it.

Thioglycolic acid helps to oxidize iron (II) to iron (III).

Ammonia to make solution alkaline

Limit Test of Heavy Metals

Principle:

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

Procedure:

Test sample	Standard compound
Solution is prepared as per the monograph and 25 ml is transferred in Nessler's cylinder	Take 2 ml of standard lead solution and dilu ml with water
Adjust the pH between 3 to 4 by adding dilute acetic acid or dilute ammonia solution	Adjust the pH between 3 to 4 by adding dilut acid or dilute ammonia solution
Dilute with water to 35 ml	Dilute with water to 35 ml
Add freshly prepared 10 ml of hydrogen sulphide solution	Add freshly prepared 10 ml of hydrogen s solution
Dilute with water to 50 ml	Dilute with water to 50 ml
Allow to stand for five minutes	Allow to stand for five minutes
View downwards over a white surface	View downwards over a white surface

Observation:

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

Limit Test of 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 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.

Procedure:

Test sample	**Standard compound**
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 color	Observe the color

Observation:

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

Limit Test of Arsenic

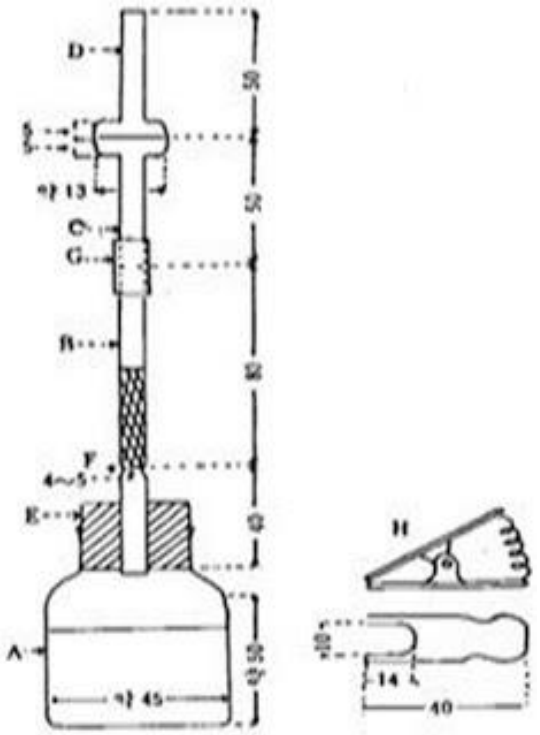
Principle:

The limit test for arsenic is based on the reduction of the arsenic in the arsenious state to the arsine gas (AsH_3) with zinc and hydrochloric acid. The arsine gas stains the mercuric chloride paper yellow. The sample is dissolved in acid whereby the arsenic present as impurity in the sample gets converted to arsenic acid. The arsenic acid is reduced to arsenious acid by reducing agents like stannous acid, potassium iodine etc. The nascent hydrogen formed during the reaction further reduced arsenious acid to the arsine gas. The arsine gas reacts with mercuric chloride paper to produce a yellow stain.

The depth of the yellow stain depending upon the amount of arsenic present in the sample, is compared with that of standard stain produced from a known amount of arsenic.

The Apparatus (I.P. 1996)

It is also called as Gutzeit test and requires special apparatus.



A : approximately 60 ml generator bottle with 40 ml indicating line.

B : glass tube with 6.5 mm inner diameter

C and D : a ground joint glass tube with 6.5 mm inner diameter and 18 mm outer diameter at the joint. Inner joint and the outer joint form a concentric circle.

E : rubber stopper

F : narrow part of the glass tube B. Glass wool is inserted up to this part.

G : rubber board (Lead acetate cotton plug)

H : clamp

Procedure:

Test solution:

The test solution is prepared by dissolving specific amount in water and stannated HCl (arsenic free) and kept in a wide mouthed bottle.

To this solution 1 gm of KI, 5 ml of stannous chloride acid solution and 10 gm of zinc is added (all this reagents must be arsenic free)

Keep the solution aside for 40 min and stain obtained on mercuric chloride paper is compared with standard solution.

Standard solution:

A known quantity of dilute arsenic solution is kept in wide mouthed bottle and rest procedure is followed as described in test solution.

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 pledger or papers are used to trap any hydrogen sulphide which may be evolved along with arsine.