

LIMIT TESTS IN PHARMACEUTICALS

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INTRODUCTION

DEFINITIONS

Limit Test: Limit tests are quantitative or semi-quantitative tests designed to identify and control small quantities of impurities, which are likely to be present in the pharmaceutical substance.

Impurity: The impurities in pharmaceuticals are the unwanted chemicals that remain with the active pharmaceutical ingredients (APIs), or develop during formulation, or upon aging of both API and formulated APIs to medicines. The presence of these unwanted chemicals even in small amounts may influence the efficacy and safety of the pharmaceutical products.

TYPES OF INORGANIC IMPURITIES

1. **Toxic impurities:** This kind of impurities are very harmful and can even cause death when taken once or short period of time. **Example: Arsenic**
2. **Cumulative impurities:** This kind of impurities shows their toxicity when it taken over a period of time. **Example: Heavy Metals**
3. **Harmless impurities:** Some impurities may not cause harm to body but reduce the therapeutic activity of active ingredient when it is present in large quantities. **Example: Chlorides, Sulphates**

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

Impurity profiling (i.e., the identity as well as the quantity of impurity in the pharmaceuticals), is now getting receiving important critical attention from

regulatory authorities because practically it is impossible to remove all the impurities from any pharmaceuticals. Some remain in trace even after purification, so it is only desirable that the substance should be sufficiently pure and can be used safely. The different pharmacopoeias (IP, BP, USP etc.) specify the limits up to which various impurities can be tolerated in pharmaceuticals. The limit tests help to check and indicate the presence of various inorganic impurities in pharmaceuticals.

Obviously the quantity of any impurity present in official pharmaceuticals is often small, and therefore, the normal visible-reaction-response to any test for that impurity is also quite small. Hence, it is essential to design the individual test in such a manner so as to avoid possible errors in the hands of various analysts. This is accomplished by taking into consideration the following *three* cardinal factors.

1. **Specificity of the tests:** Any test used as a limit test must, of necessity, give some form of selective reaction with the trace impurity. Many tests used for the detection of inorganic impurities in official inorganic chemicals are based upon the separations involved in inorganic qualitative analysis. A test may be demanded which will exclude one specific impurity, but highly specific tests are not always the best; a less specific test, which limits several likely impurities, at once, is obviously advantageous, and in fact can often be accomplished.

An example of such a test is the heavy metals test applied to alum, which not only limits contamination by lead, but also other heavy metal contaminants precipitated by thioacetamide as sulphide at pH 3.5.

2. **Sensitivity:** The degree of sensitivity required in a limit test varies enormously according to the standard of purity demanded by the monograph. The sensitivity of most tests is dependent upon a number of variable factors all

capable of strict definition, and all favorable towards the production of reproducible results. Thus the precipitation of an insoluble substance from solution is governed by such factors as concentration of the solute and of the precipitating reagent, duration of the reaction, reaction temperature, and the nature and concentration of other substances unavoidably present in solution. As a general rule, cold dilute solutions give light precipitates, whereas more granular ones are obtained from hot concentrated solutions. Many of the limit tests, however, are concerned with very dilute solutions, which are often slow to react, and here sensitivity of the reaction can often be increased by extending the duration of the reaction or by raising the reaction temperature. Similar considerations apply in the design of colour and other tests employed as limit tests.

- 3. Control of personal errors:** It is essential to exclude all possible sources of ambiguity in the description of a test. Vague terms such as 'slight precipitate,' should be avoided as far as possible. The extent of the visible reaction to be expected under the specified test conditions should be clearly and precisely defined. This is usually accomplished in one of three ways.

(a) **Tests in which there is no visible reaction:** A definite statement is incorporated in the wording of the test, which states that there shall be no colour, opalescence or precipitate, whichever is appropriate to the particular test. One example of this type of requirement is the test for barium, and calcium in dilute hypo-phosphorus acid, where the additions of dilute sulfuric acid under precisely controlled condition shall produce 'no turbidity, or precipitate' within one hour. The time factor is used here as a means of increasing the

sensitivity of the test. Tests such as these which give negative results do not necessarily imply the complete absence of the impurity, the test as laid down merely indicating the absence of an undesirably large amount of the impurity

(b) Comparison methods: Tests of this type require a standard containing a definite amount of impurity, to be set up at the same time and under the same conditions as the test experiment. In this way the extent of the reaction is readily determined by direct comparison of the test solution with a standard of known concentration. The official limit tests for chlorides, sulfates, iron and heavy metals are based on this principle. The limit tests for lead and arsenic are, in practice, also comparison methods. They are, however, so designed that they can be readily applied as quantitative determinations.

(c) Quantitative determinations: Quantitative determination of impurities is only applied in special circumstances, usually in those cases where the limit is not readily susceptible to simple and more direct chemical determination. The method is used in the following different types of tests:

- i. Limits of insoluble matter
- ii. Limits of soluble matter
- iii. Limits of moisture, volatile matter, and residual solvents
- iv. Limits of non-volatile matter
- v. Limits of residue on ignition
- vi. Loss on ignition
- vii. Ash values
- viii. Precipitation methods.

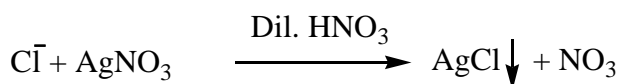
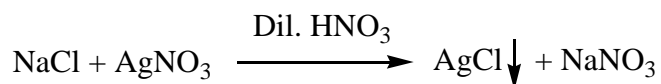
Differences between assay and limit test

1. The assay is quantitative whereas the limit test is semi-quantitative or qualitative test.
2. The assay result provides the exact amount of substances whereas the limit tests for range of impurities.
3. The assay will be done for substances as well as impurities whereas the limit tests particularly for impurities.

LIMIT TEST FOR CHLORIDES

Principle: It is based upon the chemical reaction between silver nitrate and soluble chlorides to obtain silver chloride in presence of dilute nitric acid. The silver chloride produced in the presence of dilute nitric acid makes the test solution turbid, the extent of turbidity/opalescence depending upon the amount of chloride present in the substance is compared with a standard turbidity/opalescence produced by 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. If the turbidity/opalescence from the sample has been less than the standard solution, the sample will pass the limit test and vice versa. Dilute nitric acid is used in the limit test of chloride to make solution acidic and which helps silver chloride precipitate to make solution turbid at the end of process.

Chemical Reaction:



Procedure:

Take two 50 mL Nessler Cylinders. Label one as “Test” and the other as ‘Standard’.

Test	Standard
Specific weight of compound is dissolved in distilled water or solution is prepared as directed in the pharmacopoeia and transfer to a Nessler cylinder	Take 10 mL of chloride standard solution (25 ppm Cl) and 5 mL of water
Add 1.0 mL of dilute nitric acid	Add 1.0 mL of dilute nitric acid
Dilute to 50 mL with water	Dilute to 50 mL with water

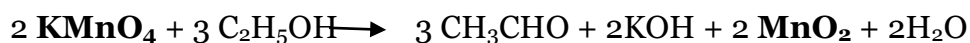
Add 1.0 mL of AgNO ₃ solution	Add 1.0 mL of AgNO ₃ solution
Stir immediately with glass rod and allow to stand for 5 minutes	

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 for chloride and vice-versa.

NOTE:

- 1. Chloride standard solution (25 ppm Cl):** Dilute 5 mL of 0.0824% w/v solution of sodium chloride to 100 mL with distilled water.
- 2. Dilute nitric acid:** Dilute 1.06 mL of conc. Nitric acid in sufficient distilled water to produce 100 mL.
- 3. 0.1 M Silver nitrate:** Dissolve 1.7 g of silver nitrate to 100 mL with distilled water.
- 4.** Insoluble substances like magnesium trisilicate or light kaolin are first boiled with a mixture of water and dilute nitric acid. The solution is filtered and the filtrate is subjected to the test.
- 5.** Coloured substances if present may be specially treated. For example, potassium permanganate is decolourised by boiling with ethanol, filtered to remove precipitated manganese dioxide and the filtrate is subjected to the test. The reducing substances which would otherwise react with silver nitrate in the chloride limit test are oxidized.

Reaction:

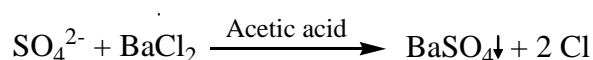
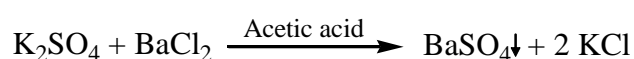


- 6.** The quantitative and semi-quantitative determination of chloride contamination is also possible for water-soluble organic compounds.
- 7.** This test is not used for water-immiscible liquids.
- 8.** The opalescence in the sample and standard solution is compared by keeping the Nessler cylinders against dark background and observing side by side.

LIMIT TEST FOR SULPHATES

Principle: The limit test for sulphate is carried out on the basis of the reaction between barium chloride and soluble sulphates in the presence of Acetic acid. Then, the comparison of the turbidity produced by a given amount of the substance is done with a standard turbidity obtained from a known amount of sulphates. The barium chloride 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. The ionic concentrations in the reagent has been so adjusted that the solubility product of barium sulphate gets exceeded, and the very small amount of barium sulphate present in the reagent acts as a seeding agent for precipitation of barium sulphate, if sulphate be present in the substance under test. Alcohol helps to prevent super-saturation and thus produces a more uniform opalescence/turbidity. Acetic acid helps to make solution acidic and barium sulphate precipitate formed is insoluble which gives turbidity/opalescence.

Reactions:



Procedure:

Take two 50 mL Nessler Cylinders. Label one as "Test" and the other as 'Standard'.

Test	Standard
Specific weight of compound is dissolved in water or solution is prepared as directed in the pharmacopoeia and transfer to a	Mix 15 mL of sulphate standard solution and 15 mL of distilled water in a Nessler cylinder

Nessler cylinder	
Add 0.15 mL of 5.0 M acetic acid	Add 0.15 mL of 5.0 M acetic acid
Add 2.5 mL of barium sulphate reagent	Add 2.5 mL of barium sulphate reagent
Add sufficient distilled water to produce 50 mL	Add sufficient distilled water to produce 50 mL
Stir immediately with glass rod and allow to stand for 5 minutes protected from light	

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 for sulphates and vice-versa.

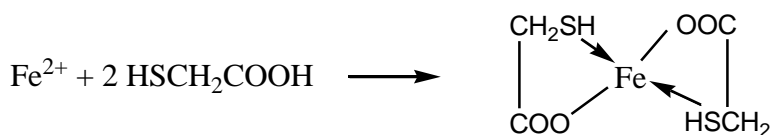
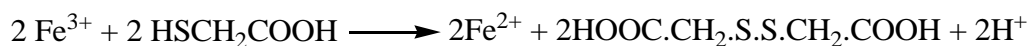
Note:

1. **Barium Sulphate Reagent:** Mix 10 mL of 25% w/v solution of barium chloride and 15 mL of ethanolic sulphate standard solution (10 ppm SO_4) and allow standing for 1 minute. It should always be prepared fresh.
2. **Sulphate standard solution (10 ppm SO_4):** Dilute 1.0 mL of a 0.181% w/v solution of potassium sulphate in distilled water to 100 mL with the same solvent.
3. **Ethanolic sulphate standard solution (10 ppm SO_4):** Dilute 1.0 mL of a 0.181% w/v solution of potassium sulphate in ethanol (30%) to 100 mL with the same solvent.
4. **5.0 M Acetic acid:** Dilute 28.5 mL of glacial acetic acid in sufficient distilled water to produce 100 mL.
5. This test is not used for water-immiscible liquids.
6. The opalescence in the sample and standard solution is compared by keeping the Nessler cylinders against dark background and observing side by side.

LIMIT TEST FOR IRON

Principle: It depends on the reaction of iron in an ammonical solution with thioglycollic acid in the presence of citric acid when a pale pink to deep reddish purple colour is produced. The colour is due to the formation co-ordination compound, ferrous thioglycollate which is stable in the absence of air but fades in air due to oxidation. Therefore, the colour should be compared immediately after the time allowed for full development of colour is over. Ferrous thioglycollate is colourless in neutral or acid solutions. The colour develops only in the presence of alkali. The original state of iron is immaterial, as thioglycollic acid reduces ferric (Fe³⁺) to ferrous (Fe²⁺) form. Citric acid forms a soluble complex with iron and prevents its precipitation by ammonia as ferrous hydroxide. Interference of other metal cations is eliminated by making use of citric acid, which forms complex with other metal cations.

Reactions:



Ferrous Thioglycollate
Complex

Procedure:

Take two 50 mL Nessler Cylinders. Label one as "Test" and the other as 'Standard'.

Test	Standard
Sample is dissolved in specific amount of distilled 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	Add 2 mL of 20 % w/v of citric acid

(iron free)	(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 with distilled water	Add ammonia to make the solution alkaline and adjust the volume to 50 mL with distilled water
Stir immediately with glass rod and allow standing for 5 minutes.	

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.

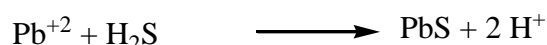
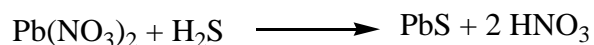
Note:

1. 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.
2. **Standard Iron Solution:** Weigh accurately 0.1726 g of ferric ammonium sulphate and dissolve in 10 mL of 0.1 N sulphuric acid and sufficient distilled water to produce 1 Litre. Each mL of this solution contains 0.02 mg of Fe.
3. The colour developed in the sample and standard solution is compared by keeping the Nessler's cylinder against dark background and observing side by side.

LIMIT TEST FOR HEAVY METALS

Principle: The limit test for heavy metals is based on the reaction of metallic impurities with hydrogen sulfide in acidic medium; the reaction product will be the sulphides of the respective metals. The sulphides so formed are distributed in colloidal state and produce brownish or black color 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.

Reactions:



Procedure:

The Indian Pharmacopoeia has adopted three methods for the limit test for heavy metals.

Method I: Use for the substance which gives clear colorless solution under the specific condition.

Test	Standard
Into a 50 mL Nessler cylinder place 25 mL of the solution prepared as per the test as directed in the individual monograph or dissolve the specified quantity of the substance under examination in sufficient distilled water to produce 25 mL	Take 1 mL of standard lead solution (20 ppm, Pb) and dilute to 25 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 dilute acetic acid or dilute ammonia solution
Dilute with water to about 35 mL and mix	Dilute with water to about 35 mL and mix
Add freshly prepared 10 mL of hydrogen sulphide solution and mix	Add freshly prepared 10 mL of hydrogen sulphide solution and mix
Dilute with water to 50 mL	Dilute with water to 50 mL
Allow to stand for 5 minutes	

Method II: Use for the substance which do not give clear colorless solution under the specific condition.

Test	Standard
<p>Weigh in a suitable crucible specific quantity of test substance, moisten with sulphuric acid and ignite carefully at a low temperature until thoroughly charred. Add 2 mL of nitric acid and 5 drops of sulphuric acid and heat cautiously until white fumes are no longer evolved.</p> <p>Ignite, preferably in a muffle furnace, at 500 °C to 600 °C, until the carbon is completely burnt off. Allow to Cool, add 4 mL of hydrochloric acid, digest on a water-bath for 15 minutes, uncover and slowly evaporate to dryness on water-bath.</p> <p>Moisten the residue with 1 drop of hydrochloric acid, add 10 mL of hot</p>	<p>Take 1 mL of standard lead solution (20 ppm, Pb) and dilute to 25 mL with water</p>

water and digest for 2 minutes. Add ammonia solution dropwise until the solution is just alkaline to litmus paper, dilute to 25 mL with water.	
Adjust the pH between 3 to 4 by adding dilute acetic acid or dilute ammonia solution and filter if necessary	Adjust the pH between 3 to 4 by adding dilute acetic acid or dilute ammonia solution
Dilute with water to about 35 mL and mix	Dilute with water to about 35 mL and mix
Add freshly prepared 10 mL of hydrogen sulphide solution and mix	Add freshly prepared 10 mL of hydrogen sulphide solution and mix
Dilute with water to 50 mL	Dilute with water to 50 mL
Allow to stand for 5 minutes	

Method III: Use for the substance which gives clear colorless solution in sodium hydroxide solution.

Test	Standard
Into a 50 mL Nessler cylinder place 25 mL of the solution prepared as per the test as directed in the individual monograph or dissolve the specified quantity of the substance under examination in sufficient in a mixture of 20 mL of distilled water and 5 mL of dilute sodium hydroxide solution	Take 1 mL of standard lead solution (20 ppm, Pb) and dilute to 25 mL with water
Dilute with water to 50 mL and mix	Add 5 mL dilute sodium hydroxide solution and dilute with water to 50 mL and mix
Add 5 drops of sodium sulphide solution and mix	Add 5 drops of sodium sulphide solution and mix
Allow to stand for 5 minutes	

Observation: 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 heavy metals and vice versa.

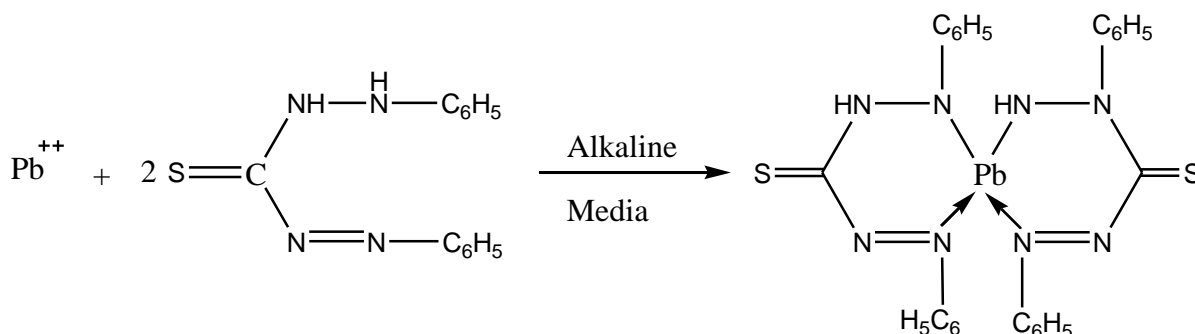
Note:

1. **Lead Standard Solution:** On the day of use, dilute 10 mL of lead nitrate stock solution with water to 100 mL. A control comparison solution prepared with 2.0 mL of lead standard solution contains, when compared to a solution representing 1.0 g of the substance under examination, the equivalent of 20 ppm lead.
2. **Lead Nitrate Stock Solution:** Dissolve 0.1598 g of lead nitrate in 100 mL of water to which has been added 1 mL of nitric acid, then dilute to 1000 mL with water.
3. **Dilute Acetic Acid:** Dilute 5.7 mL of glacial acetic acid to 100 mL with water.
4. **Dilute Ammonia Solution:** Dilute 42.5 mL of strong ammonia solution to 100 mL with water.
5. **Dilute Sodium Hydroxide:** A 5.0% w/v solution of sodium hydroxide.
6. The colour developed in the sample and standard solution is compared by keeping the Nessler cylinders against dark background and observing side by side.

LIMIT TEST FOR LEAD

Principle: Limit test of lead is based on the reaction between lead and diphenyl thiocabazone (dithizone) in alkaline solution to form lead-dithizone complex which is red in color. The original dithizone has a green colour in chloroform thus the lead-dithizone shows violet colour. 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 lead solution, treated in the same manner. In this method, the lead present as an impurity in the substances, is separated by extracting an alkaline solution with a dithizone extraction solution. The interference and influence of other metal ion etc., is eliminated by adjusting the optimum pH for the extraction, by using ammonium citrate, potassium cyanide, hydroxylamine hydrochloride reagents, etc.

Reactions:



Procedure:

Test	Standard
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 6 mL of ammonium citrate	Add 6 mL of ammonium citrate
Add 2 mL of potassium cyanide and 2	Add 2 mL of potassium cyanide and 2

mL of hydroxylamine hydrochloride	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 sec. with 30 mL of 1.0 % nitric acid and the chloroform layer is discarded	Combine dithizone extracts are shaken for 30 sec. with 30 mL of 1.0% 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 sec.	
Observe the colour developed	

Note:

1. All reagents and solutions used in the test must be free from lead.
2. **Lead Standard Solution:** On the day of use, dilute 10 mL of lead nitrate stock solution with water to 100 mL. A control comparison solution prepared with 2.0 mL of lead standard solution contains, when compared to a solution representing 1.0 g of the substance under examination, the equivalent of 20 ppm lead.
3. **Lead Nitrate Stock Solution:** Dissolve 0.1598 g of lead nitrate in 100 mL of water to which has been added 1 mL of nitric acid, then dilute to 1000 mL with water.
4. Lead has been one of the most undesirable impurities in medicinal substances. The chief sources of this impurity have been the sulfuric acid and

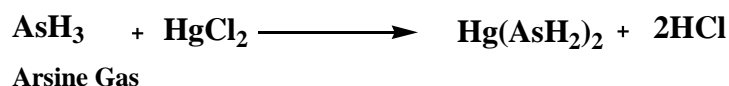
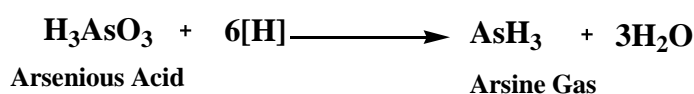
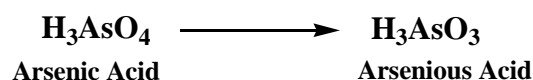
the lead-lined apparatus. Besides, glass bottles used for storage of chemicals have been dangerous sources of contamination with lead.

5. The colour in lead limit test has been due to colloidal particles of lead sulphide and has been governed by the degree of fineness and degree of dispersion of these particles. The dispersion of particles could be controlled by the magnitude of electric charges which are carried by them. Addition of other substances modified the charges on the particles and their dispersion, thereby altering intensity of the original colour. That is why it is usual to use two solutions in the Lead Limit Test, both having original substance in solution.
6. Phenol red is used as indicator to develop the color at the end of process
7. The colour developed in the sample and standard solution is compared by keeping the Nessler's cylinder against dark background and observing side by side.

LIMIT TEST FOR ARSENIC

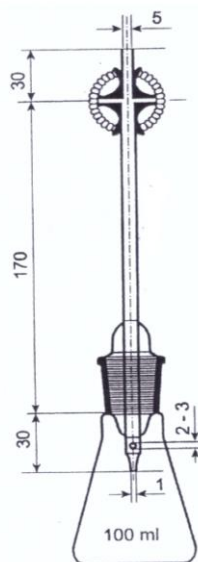
Principle: Arsenic is an undesirable and harmful type of impurity in pharmaceutical substances because it is toxic and cumulative in nature. The IP prescribes the limits for the presence of arsenic (**NMT 2 ppm**) as an impurity in various pharmaceutical substances [for example, NaCl should not contain more than 1 ppm]. When the sample is dissolved in acidic medium, the arsenic present in the sample is converted into arsenic acid. The arsenic acid is reduced by reducing agents (like Zinc and Hydrochloric acid, potassium iodide, stannous chloride) to arsenious acid. The nascent hydrogen (Zinc and Hydrochloric acid) produced in the reaction further reduces arsenious acid to arsine gas, which reacts with mercuric chloride paper producing yellow stain. The depth of yellow stain on mercuric chloride paper will depend upon the quantity of arsenic present in the sample. The intensity of stain produced varies according to the quantity of arsenic present in the sample. The stain is compared with that produced from a known amount of arsenic.

Reactions:



GUTZEIT'S ARSENIC APPARATUS

The apparatus consists of a 100 mL conical flask closed with a rubber or ground glass stopper through which passes a glass tube (about 20 cm × 5 mm). The lower part of the tube is drawn to an internal diameter of 1.0 mm, and 15 mm from its tip is a lateral orifice 2 to 3 mm in diameter. When the tube is in position in the stopper the lateral orifice should be at least 3 mm below the lower surface of the stopper. The upper end of the tube has a perfectly flat surface at right angles to axis of the tube. A second glass tube of the same internal diameter and 30 mm long, with a similar flat surface, is placed in contact with the first and is held in position by two spiral springs or clips. Into the lower tube insert 50 to 60 mg of lead acetate cotton, loosely packed, or a small plug of cotton and a rolled piece of lead acetate paper weighing 50 to 60 mg. between the flat surfaces of the tubes place a disc or a small square of mercuric chloride paper large enough to cover the orifice of the tube (15 mm × 15 mm).



(Dimensions in mm)

Apparatus for Limit Test For Arsenic

PROCEDURE

Test Solution: Dissolve 2.5 g of sample (ammonium chloride) in 50 mL of distilled water contain in the bottle of arsenic limit test apparatus labeled as test. To the above solution add 5 mL of 1.0 M Potassium Iodide AsT, 10 mL Stannated hydrochloric acid AsT and 10 g granulated zinc AsT. Immediately assemble apparatus and immerse the flask in a water-bath at a temperature such that a uniform evolution of gas is maintained for 40 minutes.

Standard Solution: Take 50 mL of distilled water in bottle of another arsenic limit test apparatus labeled as standard. Add 1.0 mL of standard arsenic solution and mix it. To the above solution, add 5 mL of 1.0 M Potassium Iodide AsT, 10 mL Stannated hydrochloric acid AsT, 10 g granulated zinc AsT. Immediately assemble apparatus and immerse the flask in a water-bath at a temperature such that a uniform evolution of gas is maintained for 40 minutes.

Observation: After 40 minutes any stain produced on the mercuric chloride paper is not more intense than that obtained by treating in the same manner 1.0 mL of arsenic standard solution.

Note:

1. All the reagents employed for the test should arsenic free and are designated as AsT in Pharmacopoeia.
2. **Arsenic Standard Solution (10 ppm):** Dissolve 0.33 g of arsenic trioxide, previously dried at 105°C for 1 hour and accurately weighed, in 5 mL of 2.0 M sodium hydroxide and dilute to 250 mL with water. Dilute 1.0 mL of this solution to 100 mL with water.

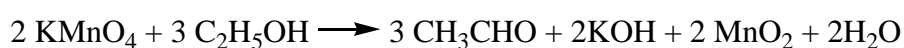
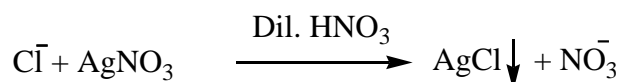
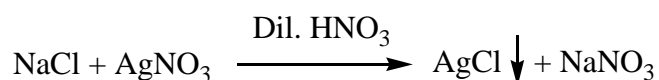
3. **2.0 M Sodium Hydroxide:** Dissolve 8.4 g of sodium hydroxide in sufficient carbon dioxide free water to produce 100 mL.
4. **1.0 M Potassium Iodide:** Dissolve 16.6 g of potassium iodide in sufficient water to produce 100 mL
5. **Stannated hydrochloric acid AsT (Stannous chloride AsT):** Dissolve 33 g of stannous chloride in 10 mL of hydrochloric acid and add sufficient water to produce 100 mL. (or) Dilute 60 mL of hydrochloric acid with 20 mL water, add 20 g of tin, heat gently until no more gas is evolved and add sufficient water to produce 100 mL. Store over a little of the undissolved tin remaining in the solution and protected from air.
6. The tube must be washed with arsenic free hydrochloric acid, rinsed with water and dried between successive tests.
7. Carry out the test and the standard simultaneously using approximately similar time period.
8. Mercuric chloride paper should be protected from sunlight during the test to avoid lighter or no stain. If the stain present in the filter paper becomes dark, the test should be repeated by using pure reagents.
9. The reaction may be expedited by the application of heat and 40°C is considered to be the most ideal temperature.
10. Cotton wool dipped in lead acetate solution is used to trap any hydrogen sulphide gas liberated with arsine gas.
11. Connect tightly the ground joints holding the mercuric chloride test paper so that the gas produced does not leak out.

MODIFIED LIMIT TEST FOR CHLORIDES

Principle: Modified limit test is performed; if the limit tests for a sample (Coloured compound) cannot be done by normal method. For example, potassium permanganate is de-colourised by boiling with ethanol, filtered to remove precipitated manganese dioxide and the filtrate is subjected to the test.

It is based upon the chemical reaction between silver nitrate and soluble chlorides to obtain silver chloride in presence of dilute nitric acid. The silver chloride produced in the presence of dilute nitric acid makes the test solution turbid, the extent of turbidity/opalescence depending upon the amount of chloride present in the substance is compared with a standard turbidity/opalescence produced by 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. If the turbidity/opalescence from the sample has been less than the standard solution, the sample will pass the limit test and vice versa. Dilute nitric acid is used in the limit test of chloride to make solution acidic and which helps silver chloride precipitate to make solution turbid at the end of process.

Reactions:



Procedure:

Preparation of Test Solution (KMnO₄): Dissolve 1.5 g in 50 mL of distilled water, heat on a water-bath and add gradually 6 mL of ethanol (95%), cool, dilute to 60 mL with distilled water and filter.

Take two 50 mL Nessler Cylinders. Label one as "Test" and the other as 'Standard'.

Test	Standard
Take 40 mL of the above test solution in Nessler cylinder.	Take 10 mL of chloride standard solution (250 ppm Cl) and 5 mL of water
Add 1.0 mL of dilute nitric acid	Add 1.0 mL of dilute nitric acid
Dilute to 50 mL with distilled water	Dilute to 50 mL with distilled water
Add 1.0 mL of 0.1 M AgNO ₃ solution	Add 1.0 mL of 0.1 M AgNO ₃ solution
Stir immediately with glass rod and allow to stand for 5 minutes protected from light	

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 for chloride and vice-versa.

NOTE:

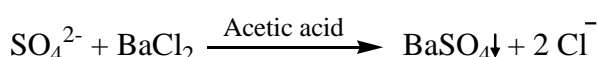
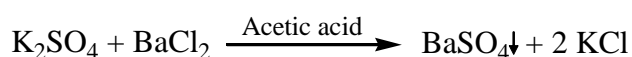
- 1. Chloride standard solution (250 ppm Cl):** Dilute 50 mL of 0.0824% w/v solution of sodium chloride to 100 mL with distilled water.
- 2. Dilute nitric acid:** Dilute 1.06 mL of conc. Nitric acid in sufficient distilled water to produce 100 mL.
- 3. 0.1 M Silver nitrate:** Dissolve 1.7 g of silver nitrate to 100 mL with distilled water.
- The quantitative and semi-quantitative determination of chloride contamination is also possible for water-soluble organic compounds.
- This test is not used for water-immiscible liquids.
- The opalescence in the sample and standard solution is compared by keeping the Nessler cylinders against dark background and observing side by side.

MODIFIED LIMIT TEST FOR SULPHATES

Principle: Modified limit test is performed; if the limit tests for a sample (Coloured compound) cannot be done by normal method. For example, potassium permanganate is de-colourised by boiling with ethanol, filtered to remove precipitated manganese dioxide and the filtrate is subjected to the test.

The limit test for sulphate is carried out on the basis of the reaction between barium chloride and soluble sulphates in the presence of acetic acid. Then, the comparison of the turbidity produced by a given amount of the substance is done with a standard turbidity obtained from a known amount of sulphate. The barium chloride 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. The ionic concentrations in the reagent has been so adjusted that the solubility product of barium sulphate gets exceeded, and the very small amount of barium sulphate present in the reagent acts as a seeding agent for precipitation of barium sulphate, if sulphate be present in the substance under test. Alcohol helps to prevent super-saturation and thus produces a more uniform opalescence/turbidity. Acetic acid helps to make solution acidic and barium sulphate precipitate formed is insoluble which gives turbidity/opalescence.

Reactions:



PROCEDURE

Preparation of Test Solution (KMnO₄): Dissolve 1.5 g in 50 mL of distilled water, heat on a water-bath and add gradually 6 mL of ethanol (95%), cool, dilute to 60 mL with distilled water and filter.

Take two 50 mL Nessler Cylinders. Label one as “Test” and the other as ‘Standard’.

Test	Standard
Take 10 mL of the above test solution in Nessler cylinder.	Mix 15 mL of sulphate standard solution and 15 mL of distilled water in a Nessler cylinder
Add 0.15 mL of 5.0 M acetic acid	Add 0.15 mL of 5.0 M acetic acid
Add 2.5 mL of barium sulphate reagent	Add 2.5 mL of barium sulphate reagent
Add sufficient distilled water to produce 50 mL	Add sufficient distilled water to produce 50 mL
Stir immediately with glass rod and allow to stand for 5 minutes protected from light	

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 for sulphates and vice-versa.

Note:

- Barium Sulphate Reagent:** Mix 10 mL of 25% w/v solution of barium chloride and 15 mL of ethanolic sulphate standard solution (10 ppm SO₄) and allow standing for 1 minute. It should always be prepared fresh.
- Sulphate standard solution (600 ppm SO₄):** Dilute 60 mL of a 0.181% w/v solution of potassium sulphate in distilled water to 100 mL with the same solvent.

3. **Ethanollic sulphate standard solution (10 ppm SO₄):** Dilute 1.0 mL of a 0.181% w/v solution of potassium sulphate in ethanol (30%) to 100 mL with the same solvent.
4. **5.0 M Acetic acid:** Dilute 28.5 mL of glacial acetic acid in sufficient distilled water to produce 100 mL.
5. This test is not used for water-immiscible liquids.
6. The opalescence in the sample and standard solution is compared by keeping the Nessler cylinders against dark background and observing side by side.