CHAPTER

1

Common Impurities in Official Compounds

An understanding of the nature and sources of impurities in pharmaceuticals is necessary to apply various tests for their detection and control. Impurities in chemical/pharmaceutical compounds are introduced from starting materials, intermediates, reagents, solvents, catalysts, etc., used in their manufacture and through the use of metallic plants (equipment, apparatus, reaction vessels, etc.). In these the solvents act on the metals like silver, copper, lead, cast iron, steel, iron, aluminium and other alloys of which the apparatus, vessels, pans, etc., are made. In order to find the nature and magnitude of impurities in a chemical/pharmaceutical compound, one has to have the knowledge of raw materials, intermediates, reagents, etc. and the process of manufacture involved in obtaining the compound. However, it is out of scope of this book to go into details about the occurrence, causes and sources of impurities in pharmaceutical compounds. In short it can be stated that the methods of manufacture of a pharmaceutical compound can always afford to point out the source and types of expected impurities.*

The most common qualitative official limit tests for impurities such as chloride, sulphate and iron and the quantitative tests for arsenic and lead are given below. There are general qualitative and quantitative limit tests which are applicable to almost all the pharmaceutical compounds, sometimes in special cases with little modification. Special data concerning description, solubility, identification, test for purity, and also other standards, such as melting point, distillation range, specific gravity and methods of assay of each pharmaceutical compound will be given under each compound, along with the method of preparation, storage, uses and doses.

Limit Tests for Chlorides, Sulphates and Iron

These tests are based on the production of an opalescence, turbidity or colour and comparing any of these with that obtained in standard solutions.

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* Impurities in commercial products are common and vary much in their proportion. Sometimes being so small that for a layman they may not be of any significance. They are likely to be higher in commercial products compared with those which are produced on smaller scales in laboratories. The commonest source of impurities in pharmaceutical products is the material used for plant construction and decomposition of substances on storage. Sometimes imurities can creep in due to the nature and properties of substances, e.g. impurity of sodium carbonate in sodium hydroxide due to highly deliquescent nature of sodium hydroxide. Impurities may also occur due to the process of manufacture followed for obtaining a particular substance and due to adulteration with cheaper materials.

Limit Test for Chlorides

The test is performed by dissolving a specified quantity of the substance (to be tested for Cl ion) in water and transferring the solution to a Nessler glass.* To the solution in Nessler glass is added 1 ml of nitric acid (HNO_3) and sufficient water to make the volume up to 50 ml. 1 ml of a solution of silver nitrate is then added to the Nessler glass and the solution after stirring with a glass rod is set aside for five minutes. An opalescence, due to the formation of silver chloride, is produced which is compared with that produced by adding silver nitrate to a standard solution containing a specified quantity of hydrochloric acid.

Standard opalescence: This is produced by measuring 1 ml of 0.01 N (N/100) hydrochloric acid (HCl) and 1 ml of nitric acid (HNO_3) in a Nessler glass, adding sufficient water to make 50 ml. 1 ml of a solution of silver nitrate is then added and the solution is stirred with a glass rod and set aside for five minutes. The reaction involved is as follows:

$$NaCl + AgNO_3 \xrightarrow{HNO_3} \underset{\text{(white curdy ppt. or white turbidity)}}{AgCl\downarrow} + NaNO_3$$

Test solution

Specified substance (1 g) +
10 ml of water
+
1 ml of HNO_3 ⟶
diluted to 50 ml in Nessler glass
+ 1 ml $AgNO_3$ sol.
↓
Opalescence / turbidity

Standard solution

1 ml of 0.01 N HCl
+
1 ml of HNO_3 ⟶
diluted to 50 ml in Nessler glass
+ 1 ml of $AgNO_3$ sol.
↓
Opalescence / turbidity

If the turbidity appears to be greater in test solution than that in standard solution, the Cl impurity is greater and hence the substance does not pass the limit test for chloride.

Note: Sometimes the solution, to be tested, is to be prepared by special method and instruction to this effect, if given, should be followed for preparing the test solution.

Limit Test for Sulphates

In this case also the turbidity produced in the test solution is compared with that produced in standard solution.

Test solution: A specified quantity of the substance (to be tested for SO_4 ions) is dissolved in water and transferred to Nessler glass. To the Nessler glass is added 1 ml of hydrochloric acid (if HCl is not already used for preparing the test solution) and the volume is made up with water upto 50 ml. 1 ml of

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* **Nessler glass/cylinder:** They are tubes of clear, colourless glass with uniform internal diameter and a flat, transparent base. They are made of transparent glass with a capacity of 50 ml. They measure 150 × 124 × 3.0 mm. They are used for comparative tests.

a solution of barium chloride ($BaCl_2$) is added to the Nessler glass and the solution stirred immediately with glass rod and set aside for five minutes. The turbidity produced is compared with that produced by the standard solution simultaneously.

Standard solution: This solution for standard turbidity is prepared by taking 2.5 ml of N/100 H_2SO_4 and 1 ml of HCl into a Nessler glass and diluting the solution to 50 ml by adding sufficient water. To this standard solution is then added 1 ml of $BaCl_2$. The solution is immediately stirred with a glass rod and set aside for five minutes.

The reaction involved is as follows :

$$H_2SO_4 + BaCl_2 \xrightarrow{HCl} \downarrow BaSO_4 + 2HCl$$

Test solution
Specified substance (1 g) + 10 ml
water + 1 ml HCl diluted to 50 ml
+ 1 ml sol. of $BaCO_2$
↓
Turbidity

Standard solution
2.5 ml 0.01 $(NH_4)_2SO_4$ + 1 ml
HCl + H_2O diluted to 50 ml
+ 1 ml sol. of $BaCl_2$
↓
Turbidity

The turbidity produced in the test solution should not be greater than that produced in the standard solution.

Limit Test for Iron

In this test, colour produced in the test solution is compared with that produced in the standard solution.

Test solution: A specified quantity of substance (to be tested for iron) is dissolved in 40 ml of water to which is added 2 ml of a 20% w/v solution of iron free citric acid in water and two drops of thioglycollic acid. The solution is mixed, made alkaline with (iron-free) solution of ammonia and diluted with water to make the volume up to 50 ml in Nessler glass. The solution is allowed to stand for 5 minutes and the colour developed in Nessler glass is compared by viewing vertically with that developed in the standard solution.

Standard solution: Two ml of standard solution of iron (prepared as follows) is diluted with 40 ml of water and to this 2 ml of a 20% w/v solution of iron-free citric acid in water and 2 drops of thioglycollic acid are added. The solution is mixed, made alkaline with (iron-free) solution of ammonia and diluted with sufficient amount of water to make the volume up to 50 ml in Nessler glass. The solution is allowed to stand for 5 minutes and the colour developed is compared with that developed in the test solution by viewing vertically.

The colour developed in test solution should not be deeper than that developed in the standard solution.

Preparation of standard solution of iron: It is prepared by adding 0.173 g of ferric ammonium sulphate ($NH_4Fe(SO_4)_2 \cdot 12H_2O$) to 1.5 ml of HCl and adding sufficient water to produce 1000 ml. Each ml of solution contains 0.02 mg of iron.

Test solution	**Standard solution**
Sample + 40 ml of water + 2 ml of 20% w/v (iron-free) citric acid + 2 drops of thioglycollic acid; solution mixed, made alkaline with ammonia and volume adjusted to 50 ml; allowed to stand. Colour developed is viewed vertically and compared with standard solution.	2 ml of standard sol. of iron + 40 ml of water + 2 ml of 20% w/v citric acid + 2 drops of thioglycollic acid; solution mixed, made alkaline with ammonia and volume adjusted to 50 ml; allowed to stand and develop colour.

In ammonical solution thioglycollic acid ($HS.CH_2CO_2H$) gives with iron salts a pale pink to deep reddish colour which is due to the formation of ferrous compound:

$$(HS.CH_2.COO)_2Fe$$

The citric acid is added to prevent the precipitation of iron by ammonia.

Note: All the reagents used in the limit test for iron should themselves be iron-free. Hence they themselves should conform to the limit tests for iron.

Quantitative test for arsenic

Arsenic impurity in a substance is expressed as parts per million and the principle involved in its detection and determination is based on the reduction of arsenic compound to gaseous arsenious hydride or arsine (AsH_3) by nascent hydrogen. The reduction is achieved by using zinc and dilute hydrochloric acid.

The substance to be tested is dissolved in acid (in case the substance is in the form of an extract or if it is to be dissolved in water, it is acidified) whereby the arsenic present in the sample is converted to arsenic acid. The solution, when treated with a reducing agent, converts arsenic acid to arsenious acid.

$$\underset{\text{Arsenic acid}}{H_3AsO_4} \longrightarrow \underset{\text{Arsenious acid}}{H_3AsO_3}$$

The nascent hydrogen reduces arsenious acid to arsine.

$$\underset{\text{Arsenious acid}}{H_3AsO_3} + 6[H] = \underset{\text{Arsine}}{AsH_3\uparrow} + 3H_2O$$

Arsine gas then comes into contact with mercuric chloride paper and stains it yellow. The depth of yellow stain on mercuric chloride paper will depend upon the quantity of arsenic present in the sample.

Apparatus: It consists of a wide mouth glass bottle of 120 ml capacity, fitted with a rubber bung through which passes a glass tube, 20 cm long, having an external diameter of about 0.8 cm and internal diameter of 0.65 cm. The upper end of the tube is rounded off, while the lower end of the tube, towards the bottom of the glass bottle, is drawn out to a diameter of about 1 mm and a hole, not less than 2 mm in diameter, is blown in the side near the lower end where the constriction begins.

The upper end of the glass tube is to be fitted with two rubber bungs, each with a hole bored centrally and exactly 6.5 mm in diameter. One of the bungs is fitted to the upper end of the tube, while the second bung is required to be fitted upon the first bung in such a way that the mercuric chloride paper is exactly sandwiched between the central perforation of the two. The bungs are then kept in close contact with the help of rubber band or spring clip in such a way that gas evolved from bottle must pass through the 0.65 mm internal circle of mercuric chloride paper.

The glass tube with the lower rubber bung should be so arranged that it, on being fitted to the glass bottle containing 70 ml of liquid, should in no case touch the liquid and its side hole should be below the bung (Fig. 1.1).

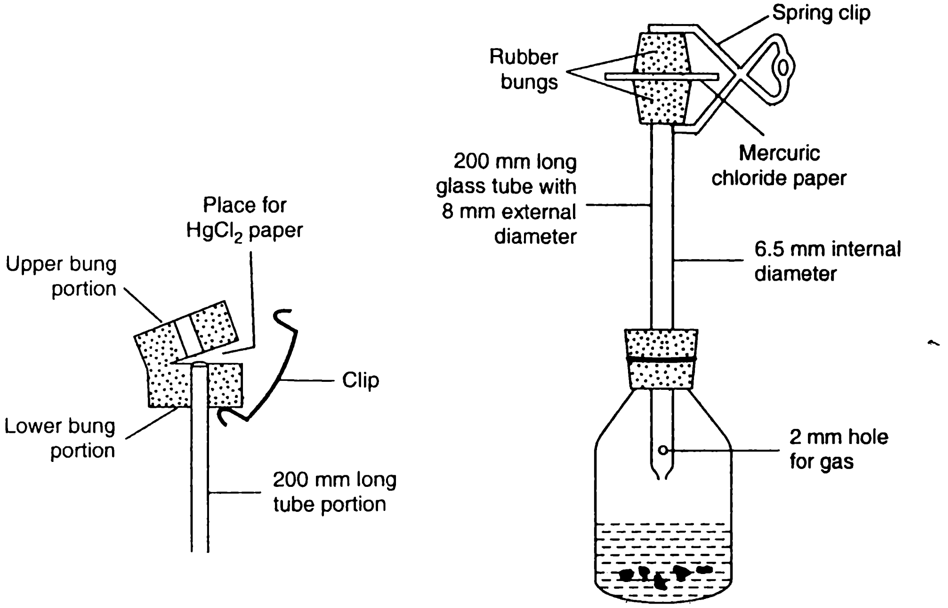

Fig. 1.1. Apparatus used for arsenic limit test; on the left is an alternative device for placing mercuric chloride paper.

Reagents: All the special reagents that are used in the limit test for arsenic are marked and distinguished by letter 'As T', meaning that they all should be arsenic free and should themselves conform to limit test for arsenic. Of course, dilute and strong arsenic solutions which are used for standard stains are exceptions.

Hydrochloric Acid 'As T'

HCl should comply with the following additional tests:

(i) Dilute 10 ml with sufficient water to produce 50 ml, add 5 ml of solution of ammonium thiocyanate and stir immediately. No colour is produced.

(ii) To 50 ml add 0.2 ml of bromine solution 'As T', evaporate on a water bath until reduced to 16 ml, adding more bromine solution 'As T', if necessary, in order that an excess, as indicated by the colour, may be present throughout the evaporation, add 50 ml of water and 5 drops of stannous chloride solution 'As T' and apply the general test. The stain produced is not deeper than 0.05 part per million.

Mercuric chloride paper: Smooth white filter paper, not less than 25 mm in width, soaked in a saturated solution of mercuric chloride, pressed to remove superfluous solution and dried at about 60° in the dark. The grade of the filter paper shall be such that the weight in g per sq. m. shall be between 65 and 120 g, the thickness in mm of 400 papers shall be approximately equal, numerically, to the weight in g per sq. m.

Note: Mercuric chloride paper should be stored in a stoppered bottle in the dark. Paper which has been exposed to sunlight or to the vapour of ammonia, affords a lighter coloured stain or no stain at all when employed in the quantitative test for arsenic.

Potassium Iodide 'As T'

Potassium iodide which complies with the following additional test:

Dissolve 10 g in 25 ml of hydrochloric acid 'As T' and 25 ml of water, add drops of stannous chloride solution 'As T' and apply the general test; no visible stain is produced.

Zinc 'As T'

Granulated zinc complies with the following additional test:

Add 10 ml of stannated hydrochloric acid 'As T' to 50 ml of water, and apply the general test using 10 g of the zinc, but allowing the action to continue for one hour; no visible stain is produced (limit of arsenic). Repeat the test with the addition of 0.1 ml of dilute arsenic solution As T; a faint but distinct yellow stain is produced (test for sensitivity).

Nitric Acid 'As T'

Nitric acid which complies with the following additional test:

Heat 20 ml in a procelain dish with 2 ml of sulphuric acid 'As T' until white fumes are given off. Cool, add 2 ml of water and again heat until white fumes are given off. Cool, add 50 ml of water and 10 ml of stannated hydrochloric acid 'As T' and apply the general test; no visible stain is produced.

Potassium Chlorate 'As T'

Potassium chlorate which complies with the following additional test:

Mix 5 g in the cold with 20 ml of water and 22 ml of hydrochloric acid 'As T'; when the first reaction has subsided, heat gently to expel chlorine, remove the last traces with a few drops of stannous chloride solution 'As T', add 20 ml of water, and apply the general test; no visible stain is produced.

Sodium Carbonate Anhydrous 'As T'

Anhydrous sodium carbonate which complies with the following additional test:

Dissolve 5 g in 50 ml of water, add 20 ml of brominated hydrochloric acid 'As T', remove the excess of bromine with a few drops of stannous chloride 'As T' and apply the general test; no visible stain is produced.

Stannated Hydrochloric Acid 'As T'

Prepared from solution of stannous chloride by adding an equal volume of hydrochloric acid, boiling down to the original volume and filtering through a fine-grained filter paper.

It complies with the following test:

To 10 ml add 6 ml of water and 10 ml of hydrochloric acid 'As T', distil, and collect 16 ml. To the distillate add 50 ml of water and a few drops of stannous chloride solution 'As T' and apply the general test; the stain produced is not deeper than a 1 ml standard stain showing that the proportion of arsenic present does not exceed 1 part per million.

Stannated Hydrochloric Acid 'As T'

Stannous chloride solution 'As T'	1 ml
Hydrochloric Acid 'As T'	100 ml

Sulphuric Acid 'As T'

Sulphuric acid which complies with following additional test:

Dilute 10 g with 50 ml of water, add 0.2 ml of stannous chloride solution 'As T' and apply the general test; no visible stain is produced.

Arsenic Solution Dilute 'As T'

Strong arsenic solution As T	1 ml
Water, sufficient to produce	100 ml

Dilute arsenic solution As T must be freshly prepared.

1 ml contains 0.01 mg of arsenic As T.

Arsenic Solution Strong 'As T'

Arsenic trioxide	0.132 g
Hydrochloric acid	50 ml
Water, sufficient to produce	100 ml

Brominated Hydrochloric Acid 'As T'

Bromine solution 'As T'	1 ml
Hydrochloric acid 'As T'	100 ml

Bromine Solution 'As T'

Bromine	30 g
Potassium bromide	30 g
Water, sufficient to produce	100 ml

METHODS FOR TESTING PURITY FOR ARSENIC

There are three methods which can be used for testing purity for arsenic:

1. Gutzeit Test; 2. Marsch Test; 3. Fileitmann Test.*

Note: For those substances which are soluble (simple organic compounds and large number of inorganic acids and salts) the solution under examination is prepared with water and stannated HCl

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* In Gutzeit test arsine stains mercuric chloride paper yellow (as explained above). In Marsch test arsenic is decomposed to As mirror, while in Fileitmann test arsenic stains $AgNO_3$ paper greyish-black.

'As T'. But if the substances to be examined are insoluble, like $BaSO_4$, bentonite or kaolins, they are diffused in water.

The solution of substances, like metallic carbonates, etc., which effervesce with acids is obtained with brominated HCl As T. I.P. gives the methods of preparation of solutions of most of the substances which are required to be tested for arsenic impurity. Special reagents required for making solutions are given in I.P.

Procedure for Test for Arsenic

The glass tube (Fig. 1.1) is first lightly packed with cotton wool, which has been previously moistened with a solution of lead acetate and dried (because if impurity of H_2S is present, it will be trapped by lead acetate present in cotton, which otherwise itself would stain the mercuric chloride paper). The cotton is so arranged in the tube that the upper surface of the cotton is not less than 2.5 cm below the top of the tube and is lightly packed to allow the gas to quit efficiently.

The upper end of the tube is then inserted into the narrow end of one of the bungs and the two bungs secured by means of the rubber band after placing the mercuric chloride paper in between them as described above (Fig. 1.1).

The solution to be examined and prepared as specified is placed in the wide-mouthed bottle and to this is added 1 g of KI 'As T' and 10 g of Zinc 'As T'. The glass tube is placed in position quickly. The action is allowed to go on for 40 minutes. A yellow stain which is produced on the $HgCl_2$ paper, if arsenic is present, is compared in daylight with the standard stains which are produced by operating in a similar manner with known quantities of dil. arsenic sol. 'As T' (the action may be hastened by placing the glass bottle on warm surface).

The comparison of the stains is made immediately at the completion of the test and the standard stains used for comparison are prepared freshly as they fade on keeping. As a matter of fact both these tests should be performed simultaneously.

The proportion of arsenic in a substance can be determined by matching the intensity of colour with that of the standard stain.

A stain equivalent to 7 ml standard stain produced by operating on 10 g of a substance indicates that the proportion of arsenic is 1 part per million.

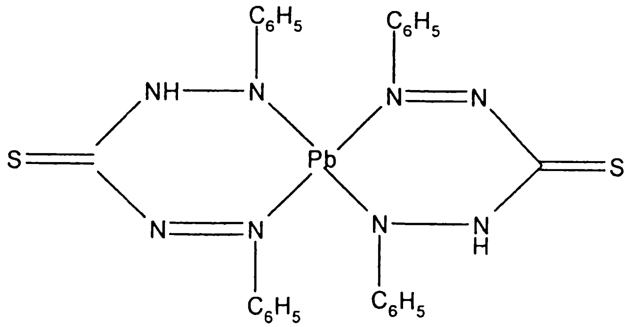
Standard Stains

Solutions are prepared by adding to 50 ml of water, 10 ml of stannated hydrochloric acid As T and quantities of dilute arsenic solution As T varying from 0.2 ml to 1 ml. The resulting solutions, when treated as described in the general test, yield stains on the mercuric chloride paper which are referred to as the standard stains.

Quantitative Test for Lead

The test is based on a reaction between lead and dithizone (diphenylthiocarbazone).

Dithizone in chloroform has the property of extracting lead (Pb) from alkaline aqueous solution as lead dithizonate, which is coloured red in chloroform solution. Since dithizone itself gives a green colour in chloroform, the resulting colour is a violet shade, which is compared with the standard colour.



Diphenylthiocarbazone lead complex
(Lead dithizonate)

The test known as Diphenylthiocarbazone test for lead in I.P. involves the use of large number of reagents, which should be as lead-free as obtainable and are designated as Sp. The I.P. requires that all glasswares should be well rinsed with warm dilute nitric acid (1 in 2), followed by water before use.

Following are the reagents, which are prepared as follows:

Ammonia Solution Sp.

The heavy metals limit of solution of ammonia used in this test shall not exceed 1 part per million when determined as directed in the monograph for dilute ammonia solution.

Ammonium Citrate Solution Sp.

Dissolve 40 g of citric acid in 90 ml of water. Add 2 or 3 drops of a solution of phenol red, then cautiously add ammonia solution until the solution acquires a reddish colour. Remove any lead that may be present by extracting the solution with 20 ml portions of diphenylthiocarbazone extraction solution, until the diphenylthiocarbazone solution retains its orange-green colour.

Potassium Cyanide Solution Sp.

Dissolve 50 g of potassium cyanide (deadly poison, handle with care) in sufficient water to make 100 ml. Remove the lead from this solution by extraction with successive portions of Diphenylthiocarbazone Extraction Solution as described under ammonium citrate solution above. Then extract any diphenylthiocarbazone remaining in the cyanide solution by shaking with chloroform. Finally dilute the cyanide solution with sufficient water so that each 100 ml contains 10 g of potassium cyanide.

Ammonium Cyanide Solution Sp.

Dissolve 2 g of potassium cyanide in 15 ml of ammonia solution and dilute with water to 100 ml.

Hydroxylamine Hydrochloride Solution Sp.

Dissolve 20 g of hydroxylamine hydrochloride in sufficient water to make approximately 65 ml. Transfer to a separator, add a few drops of a solution of thymol blue; then add ammonia solution Sp., until the solution assumes a yellow colour. Add 2 ml of a 4 per cent solution of sodium diethyldithiocarbamate, mix well and allow to stand for 5 minutes. Extract this solution with two successive 10 to 15 ml portions of chloroform until a 5 ml portion of the chloroform extract does not assume a yellow colour when shaken with a dilute copper sulphate solution. Add dilute hydrochloric acid until the solution is pink, and then dilute with sufficient water to make 100 ml.

Nitric Acid 1 Per Cent

Dilute 10 ml of nitric acid with sufficient water to make 1000 ml.

Diphenylthiocarbazone Extraction Solution

Dissolve 30 mg of diphenylthiocarbazone in 1000 ml of chloroform and add 5 ml of alcohol. Store the solution in a refrigerator.

Before use shake a suitable volume of the diphenylthiocarbazone extraction solution with about half its volume of 1 per cent nitric acid.

Standard Diphenylthiocarbazone Solution

Dissolve 10 mg diphenylthiocarbazone in 1000 ml of chloroform. Keep the solution in a glass-stoppered, lead-free bottle, suitably wrapped to protect it from light and store in a refrigerator.

Lead Solution

Dilute 10 ml of standard lead solution (containing 10 micrograms of lead per ml) with sufficient 1 per cent nitric acid, make to 100 ml. This solution contains 1 microgram of lead per ml (1 part per million).

Sodium Diethyldithiocarbamate $(C_2H_5)_2N.CS.SNa.3H_2O$

Description: White or colourless crystals.

Sensitivity: Add 10 ml of 0.1 per cent w/v solution to 50 ml of water containing 0.002 g of copper, previously made alkaline with dilute ammonia solution. A yellow-brown colour should be apparent in the solution when compared with a blank containing no copper.

Procedure for Test for Lead

Transfer the volume of the prepared sample directed in the monograph to a separator and unless otherwise directed in monograph, add 6 ml of ammonium citrate solution Sp., 2 ml of potassium cyanide solution Sp. and 2 ml of hydroxylamine hydrochloride solution Sp. (for the determination of lead in iron salts use 10 ml of the ammonium citrate solution Sp.). Add 2 drops of a solution of phenol red and make the solution just alkaline (red colour) by the addition of ammonia solution Sp. Immediately extract the solution with 5 ml portions of diphenylthiocarbazone extraction solution, draining off each extract into another separating funnel, until the diphenylthiocarbazone extraction solution retains its green colour. Shake the combined diphenylthiocarbazone solution for 30 seconds with 30 ml of a 1 per cent nitric acid solution and discard the chloroform layer. Add to the acid solution exactly 5 ml of standard diphenylthiocarbazone solution and 4 ml of ammonium cyanide solution Sp., and shake for 30 seconds, the colour of the chloroform layer is of no deeper violet shade than that of a control made with a volume of lead solution equivalent to the amount of lead permitted in the sample under examination.

Test for Heavy Metals

This test is performed to detect the amount of all those metallic impurities which get coloured by hydrogen sulphide under the condition of the test. The Indian Pharmacopoeia includes a limit test for many official substances. The Heavy Metals' limit is also expressed as parts of lead per million parts of the substance by weight. The proportion of such impurities is expressed as the quantity required to produce a colour of equal depth as in a standard comparison solution.

The test is based on the reaction between hydrogen sulphide and certain heavy metals, like lead, iron, copper, nickel, cobalt and bismuth, the reaction products being the sulphides of the respective metals. The sulphides so formed are distributed in colloidal state and produce brownish solutions with soluble sulphides. The usual limit for heavy metals prescribed in I.P. is 20 parts per million. It is also indicated in the individual monographs in terms of ppm i.e. the parts of lead, Pb, per million parts (by weight) of the substance under examination.

The test requires the use of a large number of reagents which are designated as Sp. The reagents and their methods of preparation are given below:

Dilute Acetic Acid Sp.

Dilute acetic acid which complies with the following additional test:

Evaporate 20 ml in a porcelain dish nearly to dryness on a water bath. Add to the residue 2 ml of the acid and dilute with water to make 25 ml, then add 10 ml of a solution of hydrogen sulphide. Any dark colour produced is not darker than a control made with 0.04 mg of Pb and 5 ml of the dilute acetic acid.

Hydrochloric Acid Sp.

Hydrochloric acid which complies with the following additional test:

Evaporate 17 ml of the acid in a beaker to dryness on a water bath. Dissolve the residue in 2 ml of dilute acetic acid Sp., dilute to make 40 ml with water and add 10 ml of a solution of hydrogen sulphide till any darkening produced is not darker than that in a blank to which 0.02 mg of Pb has been added (1 part per million).

Acetic Acid Sp.

Acetic acid which complies with the following additional test:

Make 25 ml alkaline with dilute ammonia solution Sp., add 1 ml of solution of potassium cyanide Sp., dilute to make 60 ml with water and add two drops of solution of sodium sulphide; no darkening is produced.

Dilute Ammonia Solution Sp.

Dilute ammonia solution which complies with the following additional test: To 20 ml add 1 ml of a solution of potassium cyanide Sp. dilute to make 50 ml with water and add two drops of solution of sodium sulphide, no darkening is produced.

Stock Solution of Lead Nitrate

Dissolve 159.8 mg of lead nitrate in 100 ml of water, to which has been added 1 ml of nitric acid, then dilute to make 1000 ml with water. This solution must be prepared and stored in glass containers free from soluble lead salts.

Standard Lead Solution

Dilute 10 ml of the stock solution of lead nitrate, accurately measured, to 100 ml with water.

This solution must be freshly prepared. Each ml of this standard lead solution contains the equivalent of 0.01 mg of lead. When 0.1 ml of standard lead solution is employed to prepare the solution to be compared with a solution of 1 g of the substance being tested, the comparison solution thus prepared contains the equivalent of 1 part of lead per million parts of the substance being tested:

Bromine Solution Sp.

Bromine	30 g
Potassium bromide	30 g
Water, sufficient to produce	100 ml

Dissolve and mix. Evaporate 10 g in a porcelain dish to dryness on a water bath. Add 10 ml of water and again evaporate to dryness.

Repeat the process till all the bromine is driven off. Add 10 ml of water and 2 ml of dilute acetic acid Sp. and make up to 25 ml with water. Add 10 ml of a solution of hydrogen sulphide, the resulting solution is not darker than a blank to which 0.01 mg of lead has been added.

PROCEDURE FOR TESTING CHEMICALS

Solution A: Introduce into a 50 ml Nessler tube, 2 ml of dilute acetic acid Sp. and exactly the same quantity of standard lead solution containing the lead equivalent to heavy metals limit specified for the substance to be tested and make up to 25 ml with water.

Solution B: This consists of 25 ml of solution prepared for this test according to specific directions given in each monograph.

Transfer solutions A and B to matching 50 ml Nessler tubes and add 10 ml of solution of hydrogen sulphide to each tube; allow to stand for ten minutes; then view downwards over a white surface, the column of Solution B will be darker than that of Solution A.

The latest I.P. 2007 has described the limit test for heavy metals by use of four methods: methods (i) A, (ii) B, (iii) C and (iv) D. Different methods are used for different kinds of substances, e.g.

(i) this method for one which affords clear and colourless solutions during a particular test condition;

(ii) this method is utilized for such substances which fail to give clear and colourless solutions as per the conditions similar to the first one;

(iii) this method is used for substances which due to their complex nature interfere with the precipitation of substance's metals by sulphide ion;

(iv) this method is used for those substances which impart clear colourless solutions with sodium hydroxide solution.