PHARMACEUTICAL INORGANIC CHEMISTRY

UNIT I

- **Impurities in pharmaceutical substances:** History of Pharmacopoeia, Sources and types of impurities, principle involved in the limit test for Chloride, Sulphate, Iron, Arsenic, Lead and Heavy metals, modified limit test for Chloride and Sulphate

WHAT IS PHARMACEUTICAL CHEMISTRY?

Pharmaceutical chemistry is a science that makes use of the general laws of chemistry to study drugs, *i.e.*, their preparation, chemical nature, composition, structure, influence on an organism and studies, the physical and chemical properties of drugs, the methods of quality control and the conditions of their usage. In other words, it is the chemistry of drugs. Drugs mainly exert action depending upon the biochemical pathways.

Pharmaceutical chemistry is a specialized science which depends on other chemical disciplines such as inorganic, organic, analytical; physical and colloid chemistry and also on medico-biological disciplines such as pharmacology, physiology, biological chemistry, etc.
Pharmaceutical chemistry occupies the most important place among the related sciences such as drug technology, toxicological chemistry, pharmacognosy, the economy and organization of pharmacy.

INORGANIC PHARMACEUTICAL CHEMISTRY

Inorganic pharmaceutical chemistry is a science that makes use of the laws of chemistry to study inorganic substances as drugs, *i.e.*, their preparation, chemical nature, composition, structure, influence on an organism, etc.

The main source of inorganic drugs happens to be the natural source such as minerals. There are many reports on the use of minerals in medicine. Some interesting examples of these include mineral waters, salts, partially purified inorganic chemicals, calcined inorganics and chemically transformed inorganics. At a later stage, many of these were found to be poisonous substances but are being still used cautiously by some doctors in sufficiently small quantities. Thus, the ancient physicians were quite familiar with the use of antimony, arsenic and mercury.

In ancient Indian medicine system mercury was supposed to occupy a dominant position for sometime and whole treatises were written giving its therapeutic and other properties. Many noble metals like gold and copper were also used in medicine. The use of minerals as medicinal agents was not restricted to any single country but all ancient cultures were quite familiar with their medicinal uses.

Although many inorganic chemicals are rarely employed today, yet few inorganic chemicals are being still used in modern medicine. Synthetic organic chemicals arc able to replace the more toxic inorganic pharmaceuticals.

Most of these belong to one or other of the following categories:

(1) Those that find use for replacing or *replenishing* the normal content of the body. These are required for normal physiological processes and are required in diet. But disease conditions are able to deplete their amounts in the body. Hence they have to be replenished or replaced. Calcium, sodium, potassium, magnesium, iron, chloride, phosphate, bicarbonate, oxygen, etc.

(2) Those that are used for changing *reactions* of body fluids, *i.e.,* acidify or alkalise. Mineral acids, antacids, alkalis etc.

(3) Those that find use as *medicinal and therapeutic* agents in disease conditions. These may be able to change physical *(e.g.,* topical agents, protectants), physiological and/or biochemical processes *(e.g.,* astringents, respiratory stimulants, hypnotics, expectorants). Some may be used as correctives or in infections *(e.g.,* dental products, antidotes, antimicrobials etc.).

(4) Those that find use as *pharmaceutical aids.* Bentonite, talc, antioxidants, pigments etc. are examples of this category.

(5) Those that find use in *analytical and quality control* processes. Most of inorganic chemicals included in the Pharmacopoeia belong to this category. Titrants such as permanganate, dichromate, iodine, bromate etc,, buffers like phosphoric acid salts, boric acid salts, ammonium salts etc. are useful examples of this group.

(6) Those that are used as *reagents* for carrying out other reactions. Some inorganic substances find use as catalysts (platinum, nickel, charcoal etc.), some as oxidizing or reducing agents (chromic the acid, lithium aluminium hydride etc.), some as adsorbents in chromatography (alumina, silica gel etc.) and in other several ways.

The term *'pharmaceuticals',* is used for any chemical that finds use in the preparation of medicament for internal administration of the human body. Some find use only in the laboratory during the preparation but may not be present in the final product. These are also incorporated under pharmaceuticals. All pharmaceuticals must be carefully controlled for their quality. For this purpose, specifications of quality have been listed for each pharmaceutical. A collection of such descriptions has been reported in a *Pharmacopoeia.*

PHARMACOPOEIA

Pharmacopoeia:
(Gr. Pharmakon — a drug, and poiein — to make) is a book of directions and requirement for the preparation of medicine; it is generally published by an authority. Thus pharmacopoeia is a legislation of a country which sets standards and obligatory quality indices for drugs, raw materials used to prepare them and various pharmaceutical preparations. These regulations are presented separately in general and specific articles.

Monograph:
is a complete description of a specific pharmaceutical, which includes nomenclature, classification, physical characteristics, dosage, purity, limits for impurities, identification, assay and conditions for storage. The appendices may also include standards for apparatus, other chemicals, techniques, processes etc. related to the said pharmaceutical.

HISTORY OF PHARMACOPOEIA

Every country has legislation on pharmaceutical preparations that sets standards and obligatory quality indices for medicaments, raw materials, and preparations employed in the manufacture of drugs. These regulations are presented in separate articles — general and specific — relating to individual drugs, and are published in the form of a book called a Pharmacopoeia.

The first British Pharmacopoeia (B.P.) was published in 1864. It was including monographs on benzoic acid, gallic acid, tartaric acid, tannic acid, camphor, lactose, sucrose and seven alkaloids along with their salts. The first United State Pharmacopoeia (U.S.P.) was released on 15th December, 1820.

INDIAN PHARMACOPOEIA

In India, in the pre-independence days, B.P. was employed as the official book of standards. The first edition of the Indian Pharmacopoeia (I.P.) was published in 1955. It was having a large number of crude drugs and their preparations. The third edition of the I.P. got published in 1985, Addendum I to third edition has been published in 1989.

The Addendum I to the Pharmacopoeia of India 1985 amends the Indian Pharmacopoeia 1985 and constitutes a part of Indian Pharmacopoeia, third edition.

Indian Pharmacopoeia Commission (IPC) is an autonomous institution of the Ministry of Health and Family Welfare which sets standards for all drugs that are manufactured, sold and consumed in India. The set of standards are published under the title **Indian Pharmacopoeia (IP)** which has been modelled over and historically follows from the British Pharmacopoeia.

I.P., the abbreviation of 'Indian Pharmacopoeia' is familiar to the consumers in the Indian sub-continent as a mandatory drug name suffix. Drugs manufactured in India have to be labelled with the mandatory non-proprietary drug name with the suffix I.P. This is similar to the B.P. suffix for British Pharmacopoeia and the U.S.P. suffix for the United States Pharmacopeia.

The actual process of publishing the first Pharmacopoeia started in the year 1944 under the chairmanship of Col. R. N. Chopra. The I. P. list was first published in the year 1946 and was put forth for approval and first Edition of Indian Pharmacopoeia is in the year 1955.

Edition	Year	Addendum/Supplement
1st Edition	**1955**	Supplement 1960
2nd Edition	**1966**	Supplement 1975
3rd Edition	**1985**	Addendum 1989
		Addendum 1991

4th Edition	**1996**	Addendum 2000
		Vet Supplement 2000
		Addendum 2002
		Addendum 2005
5th Edition	**2007**	Addendum 2008
6th Edition	**2010**	Addendum 2012
7th Edition	**2014**	Addendum 2015
		Addendum 2016
8th Edition	**2018**	Addendum 2019

The General Notices and Appendices included in the Indian Pharmacopoeia 1985 and as amended in this Addendum apply both to the matter contained in the Indian Pharmacopoeia 1985 and to the matter contained in this Addendum.

Indian Pharmacopoeia, 1996, 2 volumes.

The edition of the Indian Pharmacopoeia was published in 1996. Under the Drugs and Cosmetics Act, 1940, the Indian Pharmacopoeia is the legally recognized book of standards for the quality of drug substances and their preparations included therein.
Contents: Volume I: Notices, Preface, Acknowledgements, Introduction, General notices, Monographs (A-O).
Volume II: Monographs (P-Z), Infra-red reference spectra, Appendices, Index.
The Indian Pharmacopoeia is the legally recognized book of standards for the quality of drug substances and preparations included therein. This new edition, which supersedes the 1985 edition and its addenda, includes many new drugs and their dosage forms and omits others as also amends the specifications and updates standards of others keeping in view the advances in pharmaceutical sciences.

Pharmacopoeial monographs have been organized as described below:

(1) Title: The title is stated in English and refers to the official name of the compound. Sometimes sub-titles are given. These are synonyms and could be used in place of the main title *e.g.,* calcium carbonate can also be called *precipitated chalk,* iron and ammonium citrate can also be called *ferric ammonium citrate;* milk of magnesia can also be called *magnesium hydroxide mixture.*

(2) Formula weight and molecular weight: Following the title has been the chemical formula of the pure compound, with its molecular weight, e.g., $MgCl_2.6H_2O$ Mol. wt. 202.30. These two items are not given, provided the correct chemistry is not known or the compound is of indefinite composition. For example, for iron ammonium citrate, formula and mol.wt. are not given.

(3) Category: This describes the therapeutic or pharmacologic or pharmaceutical application of the compound. Usually this is the main application, although the compound may be having other applications. Some main categories for inorganic pharmaceuticals mentioned in the Pharmacopoeia include haematinic; antacid; laxative; pharmaceutical aid; astringent, etc.

(4) Dose: are the quantities for the guidance of the prescriber or the physician to achieve the desired therapeutic effects in adults. It can be altered as and when required, *e.g.,* $CaCO_3$ dose 1 to 5 gm.
This is omitted for substances not used for internal administration. *Usual strength* may be given for pharmaceutical dosage forms, like injection etc., which is the most commonly marketed dosage strength.

(5) Description: This gives a physical description of the substance like crystalline or amorphous nature, colour, odour, taste, etc. These properties help in the preliminary evaluation of the integrity of an article and not themselves the standards or tests of purity, *e.g.,* $CaCO_3$, fine, white macrocrystalline powder, odourless, tasteless.

(6) Solubility: Solubility is described in popular terms, which are defined in the Pharmacopoeia under general notices. This is usually given in water, sometimes in hot or boiling water, in alcohol, in glycerol, in solvent ether and sometimes in other organic solvents, acids or alkalis.
Solubility for an article given in the monograph may be considered primarily as information, but if it is given under the quantitative solubility test, then it is a standard. If the exact solubility of the article is not known, a descriptive term is used to indicate its solubility. The following table reveals the meaning of such term.

(7) Standard: It is an important part of monograph, which specifies the quantitative purity of the title compound, where the compound is of definite composition, *e.g.,* (1) Potassium bromide is having not less than 98.0 per cent of KBr, calculated with reference to the dried substance, *e.g.,* (2) Hydrogen peroxide solution is an aqueous solution of hydrogen peroxide. It contains not less than 5.0 per cent *w/v* and not more than 7.0 per cent *w/v* of H2O2 corresponding to about 20 times its volume of available oxygen. It is having not more than 0.025 per cent *w/v* of a suitable stabilizing agent.

(8) Identification: This usually involves specific chemical test or tests for identifying the substance. Colour reactions, precipitating tests, and gas evolving reactions are commonly used for inorganic pharmaceuticals.
Identification tests are not absolute proof of identity. It provides a means of verification only, *e.g.,* phenol.

Phenol + FeCl3 solution gives violet colour.

(9) pH: The pH values given in the monograph are for the guidance of manufacturing pharmacist to develop various dosage forms and to avoid physiological complications, *e.g.,* calcium amino salicylate. 2 percent w/v solution gives pH 6.0-8.0.

(10) Limits for impurities: For different chemicals different limit tests have been included, as also different amounts of such impurities permissible for that chemical. The various tests that are included could be any of these: *acidity or alkalinity, pH, specific impurities* (like phosphates, sulphides, magnesium, barium etc.), arsenic, heavy metals, chloride, sulphate etc. These will be dealt with in another chapter on, Quality control and Tests of purity.

Limit tests for impurities are generally represented in parts per million by weight (ppm) or as a percentage. These are approximate values.

(11) Assay: It is a step-by-step description of a chemical analytical method for the active substance. If the assay method described in the Pharmacopoeia is applied to the chemical, the standards prescribed in the monograph earlier should be realised. For most inorganic pharmaceuticals, *titrimetric or gravimetric* methods are used.

(12) Storage: This is the last item under the monograph. These directions are helpful in preserving the activity of the chemical. These are usually brief and general, intended more as guidance then as a condition.

The container is the device that holds the article. The immediate container is that which is in direct contact with the article at all times. The closure is a part of the container.

(a) well-dosed containers; this implies the substance is stable and gets protected from dust, dirt, insects etc. getting into the container,

(b) tightly-closed containers; the substance in such cases gets affected by atmospheric oxygen or moisture or carbon dioxide *(e.g.,* reducing agents, hygroscopic substances, strong bases etc.); it may also include such compounds as are volatile or contain dissolved gases etc. it may also include such compounds as are volatile or contain dissolved gases etc.

(c) light-resistant containers; substances which are affected by light are stored in amber or dark-coloured containers

(d) *cool place;* this is applicable to substances which are affected by warm climate *(e.g.,* thermolabilc substances); for some solutions, freezing is to be avoided

(e) single-dose containers; this is generally prescribed for some injectables, which once opened, should not be used again.

Precautions to be taken where there is a possibility of effect of atmosphere, moisture, heat and light are indicated where appropriate in the monograph. In certain specified instances when additional protection against light is necessary, the bottle must further be covered with a black paper.

Quality Control of Drugs and Pharmaceuticals

Importance of Quality Control

Quality control is vital in the case of drugs and pharmaceuticals. There cannot be any compromise in this regard and one cannot even think of any "Seconds Quality" in drugs and pharmaceuticals. If a person puts on clothing of any "Seconds quality" or of any inferior quality, no harm can come to him as far as his life is concerned. Similarly using of any furniture of seconds quality/inferior quality cannot be harmful. But presence of very small quantities (even few parts per million) of toxic impurities such as arsenic or lead in drugs/ pharmaceuticals can be very harmful to the patients because these are cumulative and toxic. Medicines of substandard quality which do not contain the required quantity of the active ingredients are not fully effective in the cure of the diseases. Therefore, the pharmaceuticals must conform to the prescribed standards not only at the time of their manufacture but must also retain their strength in terms of potent ingredients without acquiring undue toxicity when stored over a long period (shelf life). To give some idea about the desirability of quality control one can cite an example of sodium chloride. This is sold as common salt as an article of food at the rate of about Rs. 5.00 per Kg. But sodium chloride which is used as a drug in the preparation of intravenous glucose saline injections cost about Rs. 75 per Kg. This great difference in its cost is due to the fact that sodium chloride which is used as a drug has to be of much greater purity as compared to the common salt. Similarly, sodium bicarbonate which is sold as 'Baking Soda' or as 'Mitha Soda' is much cheaper as compared to sodium bicarbonate which is used in the preparation of pharmaceuticals. As drugs and pharmaceuticals are used in the treatment of diseases, therefore, it is very important to maintain their proper quality. Hence, 'quality control' becomes vital for drugs and pharmaceuticals. Standards for drugs and methods of quality control are monographed in Pharmacopœias which are official publications made in various countries under the authorities of their respective Governments. For example in our country 'Indian Pharmacopoeia' is official and substances which are prepared and purified keeping in view the requirements

of Indian Pharmacopœia are labelled as I.P. and these when tested or analysed, must conform to the standards of quality prescribed for them.

The Pharmaceutical manufacturer is responsible for the quality of his products during its total life (shelf-life). A sub-standard product apart from being less efficacious, could be harmful directly or can cause undesirable effects or incompatibilities during dispensing with other drugs. The regulatory measures (*i.e.* enforcement of Drugs and Cosmetic Acts and Rules and other relevant Acts) which are often claimed as 'key to health care', alone are not sufficient to ensure quality control because regulatory authority can only monitor the quality by random sampling and testing. Quality of drug has to be built in the product during plant construction, product development, procurement of raw-materials, manufacture, in-process controls, testing, labelling, storage and distribution. The manufacturer should aim at drug quality with zero defect (no defect). In matter of drugs, the consumers (patients), treating physicians and regulatory authorities cannot accept any compromise on the quality. Today, the consumer is well aware of his rights and he expects safe, effective and quality medicines at a reasonable cost. The responsibility of supplying quality drugs to the public is solely that of industry. It is primarily in the interest of manufacturer to adhere quality assurance because rejection of any batch or lot due to lack of quality will hit the manufacturer economically apart from loss of goodwill or reputation of the organisation.

Although, the terms 'Quality Control' and 'Quality Assurance' are generally used interchangeably, yet a clear difference can be made out. *Quality Control* refers to day-to-day control of quality within an organisation, by technically competent and qualified staff, responsible for the acceptance or rejection of incoming raw materials, packaging components, in-process tests and inspections (to ensure that systems are being controlled and monitored) and finally, for the approval or rejection of ultimate dosage forms. It includes not only the analytical testings but also the monitoring of all the operations from receipt of raw materials to processing, production and packaging operations, finished product testing, documentation, surveillance and distribution. *Quality Assurance* refers to the responsibility of an organisation to ensure that systems, facilities and written procedures are adequate and are followed in order to ensure that the final products will meet all the applicable specifications in the final dosage forms.

To be effective, quality assurance must be supported by a team effort. Quality must be built into drug product, during product and process design, space, ventilation, cleanliness with environmental and microbiologic control and sanitation; during routine production. The product and process design includes preformulation, physical, chemical, therapeutic and toxicologic considerations. It considers raw-materials, in-process and

product control, including specifications and tests for active ingredients, the excipients (inactive materials), and the final product itself, specific stability procedures for the products, protection from microbial contamination, proper storage of the product and containers, packaging and labelling to ensure that container closure system provide protection against moisture, air (oxygen), light, volatility and drug package interaction. There should be a provision for cross reference system to trace any batch of a product from its raw materials to its final destination in event of unexpected difficulties. The aim of the Drugs and Cosmetic Act and Rules (DCAR) is to provide safe and effective quality drugs. Recent requirements specified under the Schedule M of the DCAR is to enforced Good Manufacturing Practices (GMP). These rules are being further tightened to ensure only quality products. In current era of globalization quality assurance has become imperative for the pharmaceutical industry in order to survive and grow in the globally competitive environment.

IMPURITIES IN PHARMACOPŒIAL SUBSTANCES

Chemical purity means freedom from foreign matters. However, it is very very costly to manufacture chemical substances of absolute purity. For example, the best level of purity reached out during mass-production of sugar and/or rock-salt (even as vacuum salt) is around 99.9% of sucrose/sodium chloride. Therefore, it is necessary to strike a balance (compromise) for obtaining a Pharmacopœial substance whose cost is reasonable and which is tolerably pure for medicinal purposes. In other words, the product may not be very costly and yet it must be safe for medicinal use. The Pharmacopœias prescribe various tests for purity for each substance and also fix limits of tolerance for certain impurities such as for arsenic, lead, heavy metals, iron, chlorides and sulphates, etc. These impurities are commonly present in Pharmacopœial substances, though, in traces *i.e.* in very very small amounts. Arsenic and lead are cumulative *i.e.* these are neither excreted not catabolised in the body and, therefore, these keep on accumulating in the body. If these are present in more amounts than their prescribed limits of tolerance, then these may prove harmful as these are toxic substances. Therefore, Pharmacopœias fix limits of tolerance for these impurities in many compounds depending upon their dose and frequency of doses *i.e.* depending upon the quantity of the drug which is to be administered daily for its therapeutic action.

Sources of Impurities in Pharmaceutical Chemicals

If we know the process which is employed in the manufacture of a substance, the composition of raw materials used, the properties of the substances itself and its behaviour in normal and abnormal conditions of storage, we can think of impurities which may possibly be present in it.

Sources of impurities in pharmaceutical chemicals are as follows:

1. Raw materials : If impurities are present in the raw materials (ores, metals, etc.) which are used in the preparation of pharmaceutical chemicals then these may be carried through during the manufacturing process to the final products. Thus, the final compound may be contaminated with these impurities.

Example .1 : Copper sulphate is prepared by the action of sulphuric acid on copper turnings.

$$Cu + 2H_2SO_4 \longrightarrow CuSO_4 + 2H_2O + SO_2$$

Copper turnings do contain iron and arsenic as impurities. These may be present in negligible amounts or in more quantities. If these impurities are present in appreciable amounts in the raw material (copper turnings in this case) then these are likely to be carried through to the final products ($CuSO_4 \cdot 5H_2O$) in more amounts. The I.P., therefore, prescribes limit of tolerance for arsenic as impurity to be not more than 8 parts per million in copper sulphate. Similarly, it prescribes a limit for iron as impurity.

Example 2 : Zinc sulphate is prepared from either zinc metal or from zinc oxide (raw materials):

$$Zn + H_2SO_4 \longrightarrow ZnSO_4 + H_2$$

$$ZnO + H_2SO_4 \longrightarrow ZnSO_4 + H_2O$$

Zinc metal is known to contain aluminium, copper, magnesium, manganese, nickel, arsenic and iron as impurities. Similarly, commercial zinc oxide may contain these impurities. If these are present in appreciable amounts in the raw materials (zinc metal or zinc oxide, as the case may be) then these impurities are likely to be carried through in the manufacturing process in appreciable amounts to the final product ($ZnSO_4 \cdot 7H_2O$).

2. Reagents used in the manufacturing process : If reagents which are used in the manufacturing process are not completely removed by washing, these may still be present in the final products.

Example 1 : Ammoniated mercury is prepared by adding a solution of mercuric chloride to dilute ammonia solution.

$$\underset{\text{(soluble)}}{HgCl_2} + \underset{\text{(soluble)}}{2NH_4OH} \longrightarrow \underset{\substack{\text{ammoniated}\\\text{mercury}\\\text{(precipitate)}}}{NH_2HgCl} + \underset{\text{(soluble)}}{NH_4Cl} + 2H_2O$$

The precipitate of ammoniated mercury (final product) has to be washed with cold water to completely remove ammonium hydroxide. If, therefore, the precipitate of ammoniated mercury is not completely washed, then the final product may contain in it ammonium hydroxide as impurity.

Example 2 : Precipitated calcium carbonate is prepared by the interaction of solutions of calcium chloride and sodium carbonate :

$$\underset{\text{(soluble)}}{CaCl_2} + \underset{\text{(soluble)}}{Na_2CO_3} \longrightarrow \underset{\text{(ppt.)}}{CaCO_3} + \underset{\text{(soluble)}}{2NaCl}$$

The precipitate of calcium carbonate (final product) has to be washed thoroughly to completely remove excess of sodium carbonate and the soluble chlorides. If the precipitate is not properly washed, these may be present as impurities. The Pharmacopœias, therefore, prescribe limits of tolerance for the soluble alkali (Na_2CO_3) and for the chlorides as impurities in this case.

3. Intermediate products in the manufacturing process : Sometimes an intermediate substance produced during the manufacturing process may be carried through, to the final product. For example, potassium iodide is prepared by the interaction of potassium hydroxide and iodine.

$$6KOH + 3I_2 \longrightarrow 5KI + KIO_3 + 3H_2O$$

The resulting solution is evaporated to dryness and the residue is heated with charcoal:

$$KIO_3 + 3C \longrightarrow KI + 3CO$$

In this preparation, potassium iodate is an intermediate product and if it is not completely converted into KI, then it may be present as an impurity in the final product which is not desirable and hence Pharmacopœias prescribe a test for iodate in potassium iodate.

4. Defects in the manufacturing process : Defects such as imperfect mixing, incompleteness of reaction, non adherence to proper temperature, pressure pH or reaction conditions etc. may result in the production of chemical compounds with impurities in them.

Example 1 : Calcium chloride I.P. ($CaCl_2 \cdot 6H_2O$) is manufactured by adding pure calcium carbonate in slight excess to hot dilute hydrochloric acid followed by stirring and filtration. The filtrate is then concentrated to obtain crystals of $CaCl_2 \cdot 6H_2O$.

$$\underset{\text{(insoluble)}}{CaCO_3} + \underset{\text{(soluble)}}{2HCl} \longrightarrow \underset{\text{(soluble)}}{CaCl_2} + H_2O + \underset{\text{(gas)}}{CO_2}$$

The purpose of adding slight excess of calcium carbonate is to completely consume hydrochloric acid so that it does not go to the filtrate. But if these ingredients ($CaCO_3$ and HCl) are not perfectly mixed, then some hydrochloric acid may still remain unreacted which shall in that case pass to the filtrate and then to the crystals of calcium chloride. Therefore, the Indian Pharmacopœia prescribes a test for acidity in this case.

Example 2 : One of the methods to manufacture zinc oxide involves in heating metallic zinc to bright redness in a current of air. The vapours of the metal burn to form zinc oxide which is collected as a fine white powder.

$$2Zn + O_2 \longrightarrow 2ZnO$$

If, however, zinc metal is not completely converted into zinc oxide, (due to lesser heat or air) a small amount of zinc metal may still remain as such (*i.e.* as impurity) in the final product. Therefore, Indian Pharmacopœia prescribes a test for zinc metal in zinc oxide.

5. Solvents : Water is the cheapest solvent and is commonly used especially in the manufacture of inorganic chemicals.

(*a*) Tap water contains calcium, magnesium, sodium, chlorides, sulphates and carbonates as impurities though in very small amounts. Hence traces of such impurities may still remain in the final products even after good deal of washing.

(*b*) Softened water is prepared by passing tap water through the sodium form of zeolite which removes the divalent cations (cal-

cium, magnesium) from tap water in exchange for sodium. In other words, softened water is free from calcium and magnesium salts but contains more quantity of sodium salts than in the tap water. Therefore, with softened water, calcium and magnesium shall not be present as impurities but the final products may still contain sodium salts as impurities.

(*c*) De-mineralised water is prepared by passing tap water through columns containing ion-exchange resins and is free from calcium, magnesium, sodium, chlorides, sulphates and carbonates, etc. It is a better solvent than tap water or softened water but may contain organic impurities.

(*d*) Distilled water is free from all the inorganic and organic impurities including albuminoid ammonia and is, therefore, the best as a solvent. But it is quite costly. As it is itself free from impurities, therefore, it does not pass on any impurities to the final products.

6. Action of solvents and reagents on reaction vessels : Some solvents and some reagents may react with the metals of the reaction vessels during the manufacturing processes and may dissolve these metals which appear as impurities in the final products. Metallic reaction vessels such as a copper, iron, galvanized iron, tinned iron, aluminium and now-a-days more usually of stainless steel are generally used in the manufacture of inorganic chemicals. Iron contains arsenic and, therefore, the inorganic compounds which are manufactured in the iron vessels are likely to contain in them arsenic and iron as impurities. Therefore, Pharmacopœias prescribe limit tests for arsenic and iron for most of the inorganic chemicals. Substances prepared in galvanised iron vessels may contain in them zinc as impurity because galvanized iron itself contains zinc which is likely to get dissolved by the action of solvents and reagents. Reaction vessels made of soda glass may release some alkali as impurity during the manufacturing process. However, reaction vessels made of hard glass do not release any impurities but these are very expensive.

7. Atmospheric contamination during the manufacturing process : Atmosphere at many places especially in the industrial areas may contain dust particles (aluminium oxide, silica glass particles, porcelain particles, plastic fragments etc.) and some gases such as sulphur dioxide, hydrogen sulphide and black smoke (soot). These may enter the product during the process of its manufacture or during purification including crystallisation, with the result that the final product may be contaminated with these impurities. Some substances get contaminated with atmospheric air or carbon dioxide and water vapour during their preparation. For example, sodium hydroxide readily absorbs carbon dioxide:

$$2NaOH + CO_2 \longrightarrow Na_2CO_3 + H_2O$$

Therefore, sodium hydroxide should not be exposed to atmosphere for a long time during its manufacture. The Indian Pharmacopœia prescribes that sodium hydroxide should not contain more than 3% of sodium carbonate.

8. Defective storage of final products : Some pharmaceutical chemicals undergo chemical decomposition if these are not properly stored.

Example 1 : Ferrous sulphate I.P. ($FeSO_4 \cdot 7H_2O$) when exposed to moist air, readily undergoes slow oxidation and gets coated with brown basic ferric sulphate which is an impurity. Therefore, it should be stored in well-closed containers.

Example 2 : Iodine reacts with cork, rubber and some metals. Therefore, it has to be stored in glass bottles fitted with glass stoppers. Iodine may contain impurities if it is stored in containers made of metals with which it reacts or if stoppers of the containers are of rubber or cork.

Example 3 : Potassium iodide is a deliquescent substance and it gets liquefied *i.e.* it shall become a liquid if it is exposed to moist air for a long time. Hence Indian Pharmacopœia mentions that it should be stored in well closed containers.

9. Adulteration : Some pharmaceutical chemicals may be adulterated with cheaper substances. For example, potassium bromide may be adulterated with sodium bromide. Potassium bromide is more expensive than sodium bromide. Some people may mix sodium bromide in potassium bromide. Potassium salts are more expensive than their corresponding sodium salts. Hence, to avoid the chances of adulteration of potassium salts, Pharmacopœias prescribe a test for sodium in them. Likewise, crude drugs, essential oils, fats and other substances in which analytical control is difficult, may be deliberately adulterated.

From the foregoing sources of impurities, it is clear that in order to appreciate the type of impurities likely to be found in a medicinal substance, it is important to have the knowledge of composition of raw materials, process employed in its manufacture, the chemical properties of the substance (especially its behaviour under normal and abnormal conditions of storage) and possibility of adulteration with cheaper materials. It would be a wasteful exercise to list every possible impurity in each medicinal substance.

Effect of impurities The impurities when present in a medicinal substance may

(*i*) have a toxic effect and be dangerous when present beyond certain limits;

(*ii*) cause a change in the physical and chemical properties of the substance (making it unsuitable for medicinal/pharmaceutical use);

(*iii*) cause an incompatibility with other substances;

(*iv*) lead to technical difficulties in the formulation; and

(*v*) decrease the shelf-life of the substances.

In addition, the impurities present *even in traces* (*e.g.* Pb, As, etc.) may exert a cumulative toxic effect when they accumulate to a certain level. The otherwise *harmless impurities* may cause changes in physical properties (*e.g.* odour, colour, taste, etc.), thus, making the use of the substance dangerous and less effective.

Fixing the limits for impurities Having realised the difficulties in achieving a 100% pure product by manufacture and the consequences of the presence of impurities in the pharmaceutical substances, it is necessary to strike a balance between the two situations. In fixing such limits for the tolerance of impurities, the over-riding principles are:

(*i*) how much of the impurity is likely to be harmful, or to cause undesirable results in dispensing; and

(*ii*) the practicability of obtaining a reasonable standard of purity in a commercial substance. It would not be practicable to fix unattainable limits or which can only be attained at a very high cost.

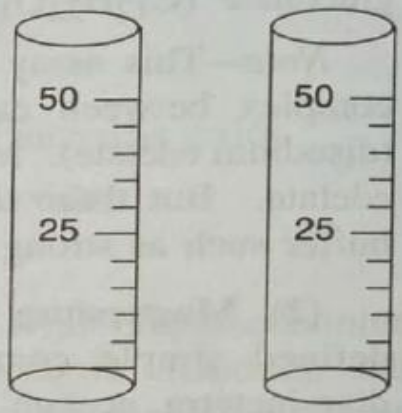
In general, the pharmaceutical substance should not only be free from toxic or objectionable impurities, but also be of a reasonable good commercial quality. The Pharmacopœia fixes limits of tolerance and accordingly prescribes tests for impurities as the case may be. For example, it is not necessary that solvent ether should be of such a high standard of purity as that of an anaesthetic ether. Similarly, presence of a trace of iron in copper sulphate is not as objectionable as the same amount would in sodium salicylate. Thus, for the impurities like lead and arsenic which are so common and dangerous (because of their toxicity and cumulative nature), very small limits have to be fixed for their presence in all substances intended for internal use. While considering the harmless impurities, the concept of practicability upholds. The substance is required not to contain impurities in exceeding limits than those which can be easily and reasonably controlled up to certain limits during appropriate manufacturing process to obtain a product of good commercial quality. The substance susceptible for adulterations are required to be free from adulterants.

LIMIT TESTS FOR IMPURITIES

Limit tests involve simple comparisons of opalescence, turbidity or colour with fixed standards.

Limit tests for chlorides, sulphates, iron, lead and heavy metals are carried out in Nessler cylinders which are made of colourless glass and have uniform diameter and height as per specifications given in the Pharmacopœias. Two similar kinds of cylinders are required each time *i.e.* one for the 'Test' (Sample) and other for the 'Standard' to make comparision in identical manner. The Standards are kept constant but the quantities of the samples are varied according to the limits of impurities prescribed in the Pharmacopœias.

The Pharmacopœia does not give any numerical value to the limits in these test, as it is not practicable. The amount of impurity necessary to cause a certain opalescence, turbidity or colour is affected to a great extent by the relatively large quantities of other substances present. In general, limit tests are performed in an aqueous solution/ extract or in media as specified in the monograph of the Pharmacopœia.



Note—In performing limit tests only *distilled water* or *purified water* is used because ordinary tap water contains number of ions to vitiate the test.

Limit Test for Chlorides

Limit test for chlorides depends upon the interaction of chlorides with silver nitrate in the presence of nitric acid. This results in the precipitation of chlorides as silver chloride. When only very small quantity of chloride ions are present, silver chloride appears as opalescence (semi-translucent white) and not as precipitate. Chlorides as impurity are present in pharmaceutical substances in very small quantities and, therefore, silver chloride appears as opalescence which is compared under uniform conditions of illumination with standard opalescence in Nessler cylinders.

$$\underset{\text{(soluble)}}{\text{Chlorides}} + \underset{\text{(soluble)}}{AgNO_3} \longrightarrow \underset{\text{(precipitate)}}{AgCl} + \underset{\text{(soluble)}}{\text{Nitrates}}$$

$$NaCl + AgNO_3 \longrightarrow AgCl + NaNO_3$$

As per IP 2010, the following method is prescribed for the limit test of chlorides.

Dissolves the specified quantity of the substance under examination in *water*, or prepare a solution as directed in the individual monograph and transfer to a Nesser cylinder. Add 10 ml of *dilute nitric acid*, except when nitric acid is used in the preparation of the solution, dilute to 50 ml with water and add 1 ml of 0.1 M *silver nitrate solution*. Stir immdiately with a glass rod and allow to stand for 5 minutes protected from light. When viewed transversely against a black background any opalescence produced is not more intense than that obtained by treating a mixture of 10.0 ml of *Chloride Standard Solution* (*25 ppm Cl*) and 5ml of *water* in the same manner.

The standard opalescence is produced by the action of silver nitrate solution with 10 ml of *Chloride Standard Solution* (*25 ppm Cl*). This is prepared by diluting 5 volumes of 0.0824 percent w/v solution of sodium chloride to 100 volumes with water. The quantities of samples are varied according to whether the prescribed limit of the impurity (chlorides) is less or more in them but the standard is kept constant. If the opalescence produced in the 'Test' (sample) is more as compared to in the 'Standard' then it means that sample contains more quantity of chloride (impurities) than the prescribed limit.

Note—Nitric acid is added to prevent precipitation of other acid radicals such as phosphate, sulphate etc. with silver nitrate solution, because, in presence of nitric acid other precipitates are not produced and only chlorides get precipitated. It produces common ion effect, so that the dissociation of silver ions is suppressed.

Limit Test for Chlorides in Magnesium Sulphate I.P.

S.No.	*Test*	*Standard*
1.	Dissolve 1 gm of magnesium sulphate (sample) in about 10 ml of distilled water and transfer it to a Nessler cylinder labelled as 'Test'.	Place 1 ml of a 0.0585% w/v solution of sodium chloride in Nessler cylinder labelled as 'Standard'. Add about 9 ml of distilled water and mix.
2.	Add 10 ml of dilute nitric acid.	Add 10 ml of dilute nitric acid.
3.	Dilute to 50 ml mark with distilled water.	Dilute to 50 ml mark with distilled water.
4.	Add 1 ml of 5% solution of silver nitrate.	Add 1ml of 5% solution of silver nitrate.

Stir immediately each solution with a glass rod and allow to stand for 5 minutes protected from light. Compare the opalescence transversely against a black background to have a better contrast for comparison.

Limit Test for Sulphates

Limit test for sulphates depends upon the interaction of sulphates with barium chloride in the presence of hydrochloric acid. This results in the precipitation of sulphates as barium sulphate.

$$\underset{\text{(Soluble)}}{BaCl_2} + \underset{\text{(Soluble)}}{\text{Sulphates}} \longrightarrow \underset{\text{(Precipitate)}}{BaSO_4} + \underset{\text{(Soluble)}}{\text{Chlorides}}$$

$$BaCl_2 + Na_2SO_4 \longrightarrow BaSO_4 + 2NaCl$$

Hydrochloric acid is added to prevent precipitation of other acid radicals by common ion effect with barium chloride solution so that less barium ions are formed and precipitation of other acid radicals such as phosphate, oxalate, etc. is prevented.. However, in the presence of hydrochloric acid, only sulphates are precipitated.

When only very small quantity of sulphate ions are present, barium sulphate appears as turbidity which is compared under uniform conditions of illumination with a standard turbidity in Nessler cylinders. If the turbidity produced by the 'Test' (sample) is less than the turbidity produced by the 'Standard' then it means that sample contains less quantity of sulphates (as impurity) than permissible limit. The standard turbidity is produced by 1 ml of 0.1089% w/v solution of potassium sulphate.

Quantities of the samples are varied according to whether the prescribed limit of the impurity (sulphates) is less or more but the 'Standard' is kept constant.

Note—According to I.P. 1985 '*Barium sulphate reagent*' was used instead of solution of barium chloride alone in this test. The so-called 'Barium sulphate reagent' is actually solution of barium chloride containing in it alcohol and a very small quantity of potassium sulphate (0.905 mg in 100 ml). The "Barium sulphate reagent" is used for producing the turbidity in the limit test for sulphates instead of solution of barium chloride alone due to following reasons:—

(*a*) Presence of very small proportion of potassium sulphate increases sensitivity of this test by giving ionic concentrations in the reagent which just exceeds the solubility of $BaSO_4$.

(*b*) The presence of alcohol helps to prevent supersaturation and keeps precipitated barium sulphate in the form of turbidity.

However as pr IP 2010, the method is revised as under:

Note—The solutions used for this test should be prepared with distilled water.

To 1.0 ml of a 25.0 per cent w/v solution of barium chloride in a Nessler cylinder add 1.5 ml of ethanolic sulphate standard solution (10

ppm SO_4), mix and allow to stand for 1 minute. Add 15 ml of the solution prepared as directed in the monograph or a solution of the specified quantity of the substance under examination in 15 ml of water and 0.15 ml of 5M acetic acid. Add sufficient water to produce 50 ml, stir immediately with a glass rod and allow to stand for 5 minutes. When viewed transversely against a black background any opalescence produced is not more intense than that obtained by treating in the same manner 15 ml of *sulphate standard solution* (*10 ppm SO_4*) in place of the solution under examination.

Note: The solutions used in the test should be prepared with the distilled water. *Ethanolic Sulphate Standard Solution* (10 ppm SO_4) is also refered as Sulphate Standard Solution (10 ppm SO_4). It is prepared by diluting 1 volume of 0.181 per cent w/v solution of potassium sulphate in ethanol (30 per cent) to 100 volumes with ethanol (30 per cent). This solution is not referred as Barium Sulphate Reagent as was used under IP 1985, although purpose of addition of ethanol and potassium sulphate are essentially same as stated under the "*Barium Sulphate Reagent*".

Limit Test for Sulphate in Sodium Chloride I.P.

S.No.	*Test*	*Standard*
1.	Dissolve 2 gm of sodium chloride (sample) in about 10 ml distilled water and transfer it to a Nessler cylinder labelled as 'Test'.	Place 1 ml of 0.1089 w/v solution of potassium sulphate in Nessler cylinder labelled as 'Standard'. Add about 9 ml of distilled water.
2.	Add 2 ml of dilute hydrochloric acid.	Add 2 ml of dilute hydrochloric acid.
3.	Dilute to 45 ml with distilled water.	Dilute to 45 ml with distilled water.
4.	Add 5 ml of 'Barium sulphate reagent'.	Add 5 ml of 'Barium sulphate 'reagent'.

Stir immediately each solution with a glass rod and allow to stand for 5 minutes. Compare the turbidity transversely against a black background.

From IP 1996 onwards, limit test for sulphate has been modified to a great extent. It has done away the requirement of Barium Sulphate Reagent. However, it still uses alcohol along with barium chloride to produce comparable turbidity.

Limit Test for Iron

Limit test for iron depends upon the interaction of thioglycollic acid with iron in the presence of citric acid and in the ammoniacal alkaline medium. This results in the formation of purple coloured ferrous salt of thioglycollic acid.

$$\underset{\text{Thioglycollic acid}}{2HSCH_2COOH} + Fe^{++} \longrightarrow \underset{\text{Ferrous thioglycollate}}{Fe(HSCH_2COO)_2} + 2H^+$$

Thioglycollic acid performs the following two functions:

(*i*) Iron impurity may be present in the trivalent ferric form or in the divalent ferrous form. If it is present in the ferric form then thioglycollic acid reduces it to the ferrous form.

$$Fe^{3+} + 2HSCH_2COOH \longrightarrow Fe^{2+} + 2HSCH_2COO^- + 2H^+$$

(*ii*) Thioglycollic acid produces purple colour with the ferrous iron in the ammoniacal alkaline medium.

Citric acid prevents precipitation of iron with ammonia as iron hydroxides. It keeps iron in the solution form even in the presence of ammonia by forming a complex.

$$2Fe + 10NH_3 \longrightarrow 2Fe(NH_2)_5 + 5H_2$$

$$2C_6H_8O_4 + Fe \longrightarrow Fe(C_6H_6O_4)_2 + 2H_2$$

All the reagents which are used for this test must be completely free from iron. These are labelled as 'FeT'.

Dissolve the specified quantity of the substance under examination in *water*, or prepare a solution as directed in the monograph, and transfer to a Nessler cylinder. Add 2 ml of a 20 per cent w/v solution of *iron-free citric acid* and 0.1 ml of *thioglycollic acid*, mix, make alkaline with *iron-free ammonia solution*, dilute to 50 ml with *water* and allow to stand for 5 minutes. Any colour produced is not more intense than that obtained by treating in the same manner 2.0 ml of *iron standard solution* (*20 ppm Fe*) in place of the solution under examination.

The limit test for iron is carried out in two Nessler cylinders, one for the 'Test' and other for 'Standard'. The intensity of purple colour produced in the two is compared by viewing vertically downwards. If the intensity of colour is more in the 'Test' (sample) than in the 'Standard' it means that the sample contains more quantity of iron impurity than the permissible limit and hence the sample is declared as not of standard quality.

Limit Test for Iron in Sodium Chloride I.P.

S.No.	Test	Standard
1.	Place 1 gm of sodium chloride in a Nessler cylinder labelled as 'Test' and dissolve it in 40 ml of distilled water.	Place 2 ml of the standard iron solution (equal to 0.04 mg Fe) in a Nessler cylinder labelled as 'Standard' and add 40 ml of distilled water.
2.	Add 2 ml of 20% solution of iron-free citric acid.	Add 2 ml of 20% solution of iron-free citric acid.
3.	Add 0.1 ml of thioglycollic acid.	Add 0.1 ml of thioglycollic acid.
4.	Make the solution alkaline with iron-free ammonia solution.	Make the solution alkaline with iron-free ammonia solution.
5.	Dillute to 50 ml mark with distilled water.	Dilute to 50 ml mark with distilled water.

Stir each solution with a glass rod and allow to stand for 5 minutes. Compare the colour in the two Nessler cylinders by viewing vertically downwards.

Limit Test for Lead

As impurity, lead is most undesirable in medicinal substances. The most common source of lead (impurity) in the medicinal substances is due to its presence in sulphuric acid and in the apparatus which are used for their manufacture. Defective storage can also increase the lead impurities in medicinal substances. If the substances are stored in ordinary lead glass bottles, these are likely to contain more quantity of lead as impurity specially when these are opened repeatedly in moist conditions or are kept without stoppers.

There are two methods for the quantitative test for lead (limit test for lead) as follows:

(1) B.P. method
(2) I.P./U.S.P. method.

B.P. Method

Concentrated solutions of lead salts when treated with sodium sulphide give a black precipitate due to the formation of lead sulphide. However, very dilute solution (for example solutions containing 10 parts per million of lead) produce with sodium sulphide brown coloration and not black precipitate.

$$\text{Lead salt} + Na_2S \longrightarrow \text{Sodium salt} + PbS \text{ (brown coloration)}$$

$$Pb(NO_3)_2 + Na_2S \longrightarrow 2NaNO_3 + PbS \text{ (brown coloration)}$$

Other metals such as copper and iron also produce black precipitate or brown coloration with sodium sulphide, which would, therefore, interfere with this test. This difficulty is overcome by doing the test in the presence of ammonia and potassium cyanide which form complex cyanide and thus prevent the precipitation of copper and iron as sulphides.

The intensity of the brown colour produced in the 'Test' (sample) is compared with the 'Standard'. If it is more in the 'Test', it means that the sample contains more quantity of lead (as impurity) than the permissible limit and hence it is non-standard.

The test is carried out in Nessler cylinders which must be made of lead-free clear glass. All the reagents to be used in this test must be completely free from lead and are labelled as 'PbT'.

For this test the following two solutions are prepared:

(*i*) *Primary solution* (*Test*) : This contains a definite quantity of the test sample.

(*ii*) *Auxiliary solution* (*Standard*) : This contains a very small quantity of the test sample and specified volume of the standard lead solution *i.e.* solution of lead nitrate.

The primary solution is taken in one Nessler cylinder and the auxiliary solution is taken in another Nessler cylinder. Excess of ammonia and a small quantity of potassium cyanide are then added to each cylinder. At this stage solution in the two Nessler Cylinders are examined for any difference in colour. If there is any difference in the two, this is removed by adding solution of burnt sugar to the solution which has less intensity of colour. Volume in the two Nessler cylinders is then made upto 50 ml mark with distilled water. Two drops of sodium sulphide solution are then added to each and mixed. After 5 minutes the colour in the two is compared by looking vertically from top into the two cylinders held side by side over a white tile.

For calculation, quantity of the sample in the auxiliary solution (Standard) is subtracted from that in the primary solution (Test). For example, if 12 gms of the substance is taken in the primary solution and 2 gms of the substance is taken in the auxiliary solution, then the test is regarded to be made on 10 gms of the substance.

Note—The reason for having small quantity of the substance (Test sample) in the auxiliary solution (Standard) is to enable the comparison to be made under as comparable conditions as possible.

Example : Limit test for lead in ammonium chloride by B.P. Method. As prescribed in B.P. ammonium chloride should not contain lead more than 2.5 parts per million in it.

S.No.	*Primary (Test)*	*Auxiliary (Standard)*
1.	Place 12 gm of ammonium chloride (sample) in Nessler cylinder labelled as 'Primary'.	Place 2 gm of ammonium chloride (sample) in Nessler cylinder labelled as 'Auxiliary'.
2.	Dissolve it in hot distilled water containing 5 ml of acetic acid.	Dissolve it in hot distilled water containing 5 ml of acetic acid.

3.	Dissolve it in hot water containing 5 ml of acetic acid.	Add 5 ml of 'dilute solution of lead'.
4.	Make alkaline with solution of ammonia.	Make alkaline with solution of ammonia.
5.	Add 1 ml of potassium cyanide solution (from burette).	Add 1 ml of potassium cyanide solution (from burette).

Compare intensity of colour in the two Nessler cylinders. In case there is any noticeable difference in colour it should be removed by adding a very dilute solution of burnt-sugar.

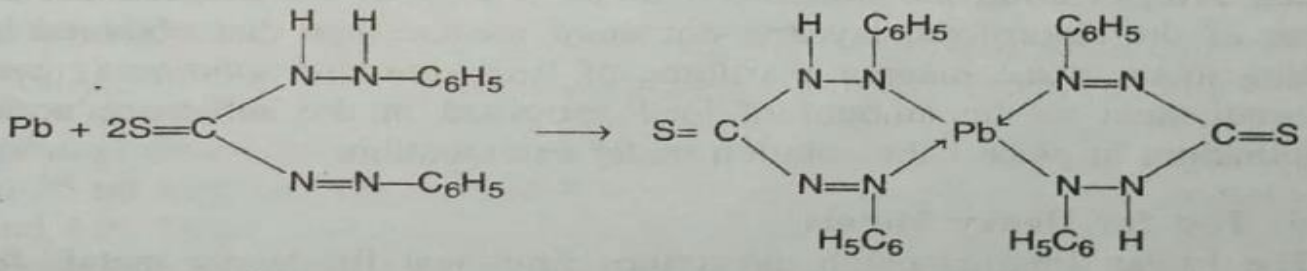
6.	Dilute the solution with distilled water to make 50 ml.	Dilute the solution with distilled water to make 50 ml.
7.	Add two drops of sodium sulphide solution.	Add 2 drops of sodium sulphide solution.

Stir each solution with a glass rod and allow to stand for 5 minutes. Compare intensity of colour in the two Nessler cylinders by viewing vertically downwards.

In this example the test is considered to be made on 10 gm of ammonium chloride.

I.P./U.S.P. Method

The limit test for lead as described in the latest I.P. is based on the reaction between lead (impurity) and dithizone (diphenyl thiocarbazone) which results in the formation of lead-dithizonate. This test is not carried out in Nessler cylinders but involves double extraction method with the help of separating funnels.



Dithizone Lead dithizonate

$$\text{Lead} + \text{dithizone} \xrightarrow[\text{medium}]{\text{alkaline}} \text{Lead dithizonate}$$

Dithizone is soluble in chloroform and forms a green coloured solution. This is called the dithizone-chloroform extraction solution and this is used for the quantitative extraction of lead (impurity) from the alkaline aqueous solution of the substance (Test sample).

The final product (lead-dithizonate) in chloroform is red in colour. However, mixture of these two (green coloured solution and red coloured solution) appears as violet in colour. Intensity of the final violet colour

produced in the chloroform medium in the case of the test sample should not be more than that in the Standard.

The limit for lead under IP 2010 is indicated in the individual monograph in terms of ppm, i.e., the parts of lead, Pb, per million parts (by weight) of the substance under examination.

The following method is based on the extraction of lead by solutions of dithizone.

All reagents used for the test should have as low a content of lead as practicable. All reagent solutions should be stored in containers of borosilicate glass. Glassware should be rinsed thoroughly with warm dilute nitric acid followed by water.

Limit Test for Heavy Metals

The Indian Pharmacopœia prescribes limit test for heavy metals for some drugs. These heavy metals (impurities) may be lead, antimony, bismuth, tin, cobalt, manganese, etc., but these are grouped together for the purposes of testing their presence in Pharmacopœial substances. Their quantity is expressed in terms of lead (for heavy metals) that is parts by weight of lead in one million parts by weight of the substance.

The latest Indian Pharmacopœia (2010) prescribes four methods (A, B, C and D) for the limit test of heavy metals.

Methods A and B

Method A is for the colourless substances whereas method B is for the coloured substances. Both the methods are based on the reaction between H_2S (freshly prepared saturated solution of hydrogen sulphide in water) and heavy metals in an acidic medium to produce the metal sulphides. These remain distributed in a colloidal state and produce brownish coloration. The 'Test' is compared against the 'Standard' which is prepared by using solution of lead nitrate (as heavy metal).

$$\text{Heavy metals} + H_2S \xrightarrow[\text{medium}]{\text{acidic}} \underset{\text{(brown coloration)}}{\text{Sulphides of heavy metals}}$$

Method A

Standard solution : Into a 50 ml Nessler cylinder pipette 1.0 ml of *lead standard solution (20 ppm Pb)* and dilute with water to 25 ml.

Adjust with *dilute acetic acid* or dilute ammonia solution to a pH between 3.0 and 4.0, dilute with *water* to about 35 ml and mix.

Test solution : Into a 50-ml Nessler cylinder place 25 ml of the solution prepared for the test as directed in the individual monograph or dissolve the specified quantity of the substance under examination in sufficient water to produce 25 ml. Adjust with dilute acetic acid or dilute ammonia solution to a pH between 3.0 and 4.0 dilute with water to about 35 ml and mix.

Procedure : To each of the cylinders containing the standard solution and test solution respectively add 10 ml of freshly prepared *hydrogen sulphide solution*, mix, dilute to 50 ml with *water*, allow to stand for 5 minutes and view downwards over a white surface; the colour produced with the test solution is not more intense than that produced with the standard solution.

METHOD A

S.No.	*Test*	*Standard*
1.	Place 25 ml of the solution of the test sample prepared according to directions of I.P. in a 50 ml. Nessler cylinder labelled as 'Test'.	Place 2 ml of standard lead solution (equal to 0.02 mg lead) in a 50 ml Nessler cylinder labelled as 'Standard' and dilute with distilled water to 25 ml.
2.	Adjust with dilute acetic acid or ammonia to a pH between 3.0 to 4.0. Dilute with distilled water to about 35 ml and mix.	Adjust with dilute acetic acid or ammonia to a pH between 3.0 and 4.0. Dilute with distilled water to about 35 ml and mix.
3.	Add 10 ml of freshly prepared saturated solution of hydrogen sulphide. Make up the volume to 50 ml mark with distilled water and mix. Allow to stand for 5 minutes.	Add 10 ml of freshly prepared saturated solution of hydrogen sulphide. Make up the volume to 50 ml mark with distilled water. Allow to stand for 5 minutes.

METHOD B

It is similar to method A except that in this case the coloured substance (Test sample) is given special treatment (sulphuric acid, ignition, nitric acid, ignition, hydrochloric acid and finally digestion with water etc.) to make it colourless before preparing its solution,

Procedure : Proceed as directed under Method A.

METHOD C

Method C is used for those substances which form clear and colourless solutions with sodium hydroxide. The test by this method is based upon reaction between sodium sulphide and heavy metals in an alkaline medium to produce heavy metals sulphides. This results in the production of colour which is compared in Nessler cylinders.

$$\text{Heavy metals} + Na_2S \xrightarrow[\text{medium}]{\text{alkaline}} \underset{\text{(dark-brown coloration)}}{\text{Sulphides of heavy metals}}$$

Standard solution : Into a 50 ml Nessler cylinder pipette 1.0 ml of *lead standard solution* (*20 ppm Pb*), add 5 ml of *dilute sodium hydroxide solution*, dilute with *water* to 50 ml and mix.

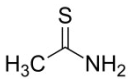
Test solution : Into a 50 ml Nessler cylinder place 25 ml of the solution prepared for the test as directed in the individual monograph, or dissolve the specified quantity of the substance under examination in a mixture of 20 ml of *water* and 5 ml of *dilute sodium hydroxide solution*. Dilute with *water* to 50ml and mix.

Adjust with *dilute acetic acid* or dilute ammonia solution to a pH between 3.0 and 4.0, dilute with *water* to about 35 ml and mix.

Test solution : Into a 50-ml Nessler cylinder place 25 ml of the solution prepared for the test as directed in the individual monograph or dissolve the specified quantity of the substance under examination in sufficient water to produce 25 ml. Adjust with dilute acetic acid or dilute ammonia solution to a pH between 3.0 and 4.0 dilute with water to about 35 ml and mix.

Procedure : To each of the cylinders containing the standard solution and test solution respectively add 10 ml of freshly prepared *hydrogen sulphide solution*, mix, dilute to 50 ml with *water*, allow to stand for 5 minutes and view downwards over a white surface; the colour produced with the test solution is not more intense than that produced with the standard solution.

S.No.	*Test*	*Standard*
1.	Place 25 ml of the solution of the sample prepared according to I.P. in a Nessler cylinder labelled as 'Test'.	Place 2 ml of standard lead solution (equal to 0.02 mg of lead) in a Nessler cylinder labelled as 'Standard' and dilute to 25 ml with distilled water.
2.	Add 5 ml of dilute sodium hydroxide solution.	Add 5 ml of dilute sodium hydrooxide solution.
3.	Add distilled water to make 50 ml and mix.	Add distilled water to make 50 ml and mix.
4.	Add 5 drops of sodium sulphide solution and mix.	Add 5 drops of sodium sulphide solution and mix.

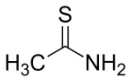


Method D

Standard solution : Into a smaller Nessler cylinder pipette 10.0 ml of either *lead standard solution* (*1 ppm Pb*) or *lead standard solution* (*2 ppm Pb*).

Test solution : Prepare as directed in the individual monograph and pipette 12 ml into a small Nessler cylinder.

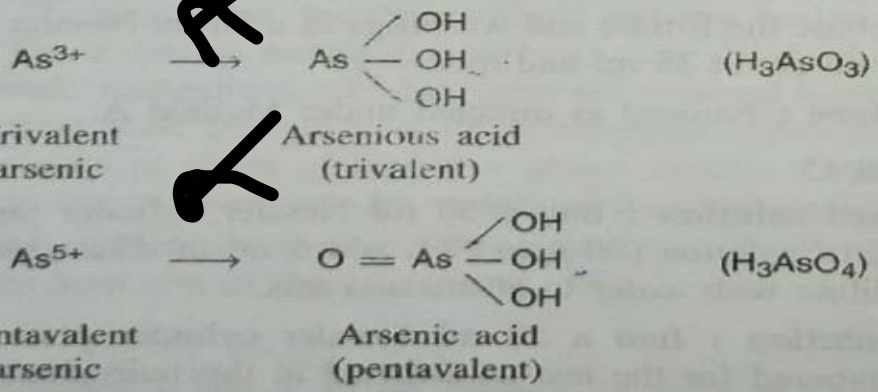
Procedure : To the cylinder containing the standard solution add 2.0 ml of the test solution and mix. To each of the cylinders add 2 ml of *acetate buffer pH 3.5*, mix, add 1.2 ml of *thioacetamide reagent*, allow to stand for 2 minutes and view downwards over a white surface; the colour produced with the test solution is not more intense than that produced with the standard solution.

Heavy Metals +  **Sulphides of Heavy Metals**

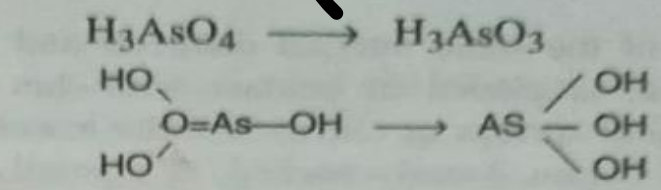
Limit Test for Arsenic

The presence of arsenic in drugs even in the traces is not desirable because it is toxic and cumulative nature. The Indian Pharmacopœia prescribes the limits for the presence of arsenic as an impurity in various drugs. For example, sodium citrate should not contain arsenic more than 2 parts per million. Similarly, for sodium chloride the limit is 1 part per million.

Principle The sample is dissolved in acid (or its aqueous extract is acidified) which convert the arsenic impurity into arsenious acid or arsenic acid depending upon the valency state of arsenic present in the drug sample:



This solution is treated with a reducing agent to convert the pentavalent arsenic acid into the trivalent arsenious acid.



Arsenic acid Arsenious acid

The arsenious acid is then converted into gaseous arsenious hydride (arsine gas) with the help of nascent hydrogen (which is produced by Zn + HCl).

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

Arsine gas is carried through the tube by the stream of hydrogen and out through the mercuric chloride paper. A reaction occurs between arsine and mercuric chloride which may be represented as follows:

$$2AsH_3 + HgCl_2 \longrightarrow Hg(AsH_2)_2 + 2HCl$$

(Yellow in colour)

This results in the formation of a yellow or brown stain on the mercuric chloride paper. The intensity of the colour is proportional to the quantity of arsenic.

In the same manner a standard stain is separately produced for the corresponding quantity of arsenic *i.e.* for the permissible limit.

The intensity of the two stains is compared. If the intensity of stain in the case of 'Test' (sample) is more than that of the 'Standard' then the sample contains more arsenic than the limit and hence the sample is non-standard.

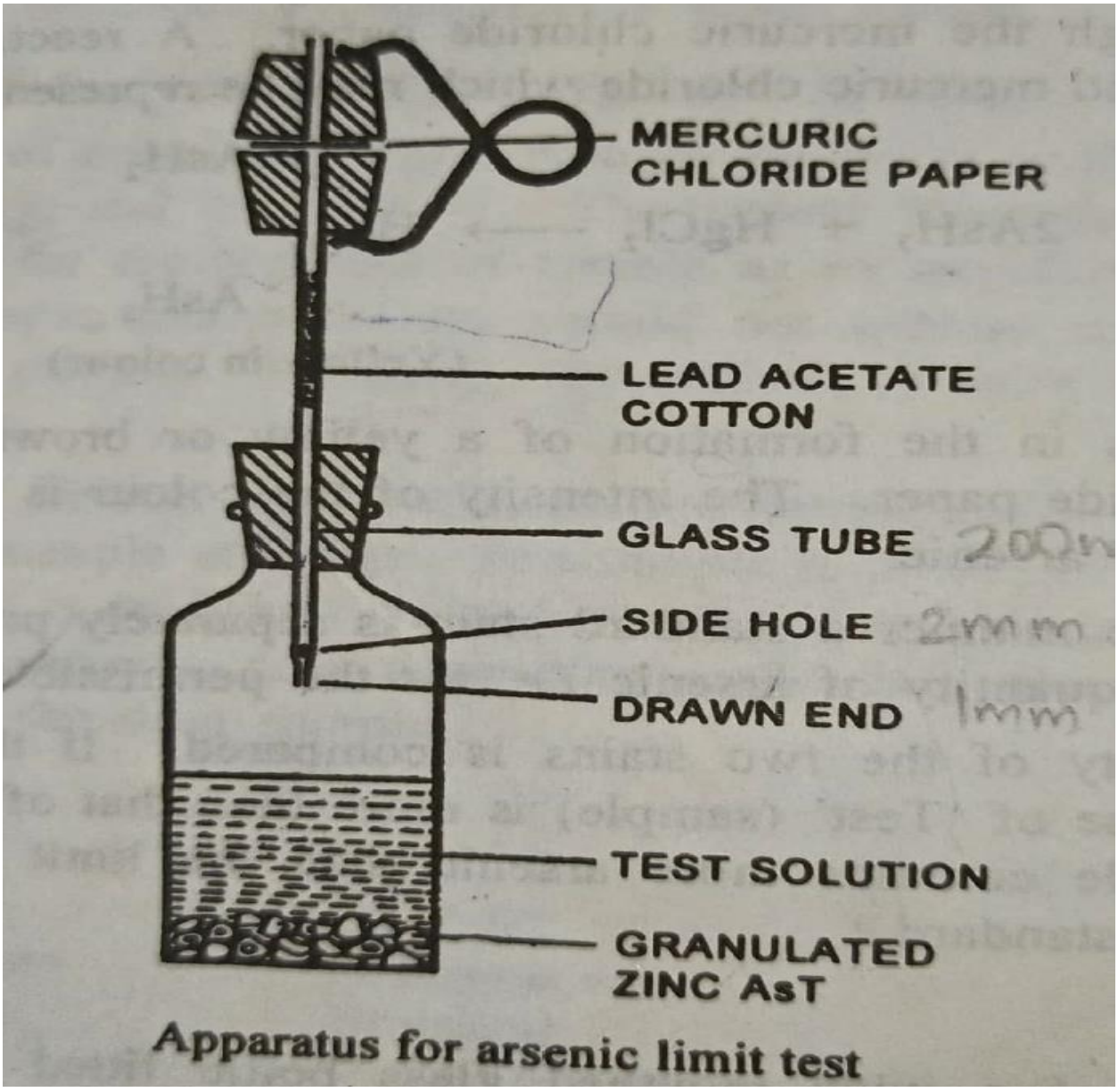
The limit test for arsenic is indicated in the individual monographs in terms of ppm i.e. the parts of arsenic as per million parts (by weight) of the substance under examination.

All reagents used for the test should have as low content of arsenic as possible.

Apparatus

The apparatus consists of a 100 ml bottle or conical flask closed with a rubber or ground glass stopper through which passes a glass tube (about 20 cm × 5mm). 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 the 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 surface 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).



Apparatus for arsenic limit test

The purpose of lead acetate cotton wool is to trap any hydrogen sulphide (H_2S) gas which would otherwise interfere with this test as it also gives some stain with mercuric chloride paper. The tube is fitted at its upper end with two rubber bungs as shown in figure. A piece of dry mercuric chloride paper is placed flat on the top of the bung and the other bung is placed over it and secured by means of clips in such a manner that the borings of the two bungs meet to form a true tube of the same diameter (6.5 mm) interrupted by a diaphragm of mercuric chloride paper.

Separate apparatus is used for the 'Test' and for the 'Standard'.

Notes

1. This test is a modification of the Gutzeit test and is, therefore, called Modified Gutzeit test.
2. All the reagents which are used for this test should have as low content of arsenic as possible free from arsenic impurity.
3. Potassium iodide is used because it helps in the reduction of pentavalent arsenic acid into trivalent arsenic acid. Potassium iodide is first converted into hydroiodic acid (HI) which helps in this reduction process.
4. Granulated zinc is used instead of ordinary zinc because evolution of nascent hydrogen is steady and prolonged with granulated zinc.
5. The arsenic impurity is expressed in terms of p.p.m. (parts per million). One p.p.m. is 1 mg in 1 kg *Or* 1 mcg in 1 g.

Limit test for Arsenic in Ammonium Chloride I.P.

According to I.P. 2010 ammonium chloride should not contain more than 4 parts per million (4 p.p.m.) of arsenic in it.

S.No.	*Test*	*Standard*
1.	Dissolve 2.5 g in 50 ml water and place in the bottle of an arsenic test apparatus labelled as 'Test'	Dilute 1.0 ml of arsenic standard solution (10ppm As) with 50 ml water and place ion the bottle of an arsenic test apparatus labelled as 'standard'.
2.	Add 5 ml of 1 M potassium iodide solution and 10 g of Zinc AsT.	Add 5 ml of 1 M potassium iodide solution and 10 g of Zinc AsT.
3.	Immediately assemble the apparatus and immerse the flask in a water bath at a temperature such that a uniform evolution of gas is maintained.	Immediately assemble the apparatus and immerse the flask in a water bath at a temperature such that a uniform evolution of gas is maintained.

Place the prepaed glass tube quickly in position in both the cases and allow the action to proceed for 40 minutes in the dark. Compare the yellow stain in the two, in day-light without delay.

After 40 minutes any stain produced on the mercuric chloride paper in Test is not more intense than that obtained by "Standard"

MODIFIED PROCEDURES FOR CERTAIN LIMIT TESTS

1. CHLORIDE AND SULPHATE IN POTASSIUM PERMANGANATE

Principle : If these limit tests are done in the usual way, it will be difficult to make any observation, since the sample itself (potassium permanganate) is highly coloured. So potassium permanganate is eliminated by reduction with alcohol. This can be called as *pretreatment.*

The sample is dissolved in water and heated on a water bath. Alcohol is added. It is filtered to remove the precipitated manganese dioxide. The filtrate is colourless and can be used for performing the limit tests for chloride and sulphate in the usual way.

$$2KMnO_4 + 3CH_3CH_2OH = 2KOH + 2MnO_2 + 3CH_3CHO + 2H_2O$$

Procedure

a. *Dissolve 1.5 g of the sample, accurately weighed, in 50 ml of distilled water.*

b. *Heat on a water bath and add gradually 6 ml of ethanol 95%.*

c. *Cool, dilute to 60 ml with distilled water and filter. The filtrate (solution A) is colourless.*

For Limit Test for Chlorides : Take 40 ml of solution A and do the limit test for chlorides.

For Limit Test for Sulphates : Take 10 ml of solution A and do the limit test for sulphates.

2. CHLORIDE AND SULPHATE IN SODIUM BICARBONATE

solution for the particular limit test. In the case of limit test for chlorides, the sample is dissolved in distilled water and neutralised with nitric acid.

$$NaHCO_3 + HNO_3 = NaNO_3 + CO_2\uparrow + H_2O$$

In the case of limit test for sulphates, the sample is suspended in distilled water and neutralised with hydrochloric acid.

$$NaHCO_3 + HCl = NaCl + CO_2\uparrow + H_2O$$

In both cases, the solutions should be stirred well and the effervescence should be allowed to subside.

Procedure

For Limit Test for Chlorides

a. *Weigh accurately 1.25 g of the sample and dissolve it in 15 ml of distilled water.*
b. *Add 2 ml of concentrated nitric acid.*
c. *Apply the limit test for chlorides to this solution.*

For Limit Test for Sulphates

a. *Weigh accurately 1g of the sample and suspend it in 10 ml of distilled water.*
b. *Neutralise with concentrated hydrochloric acid adding it gradually till the effervescence ceases.*
c. *Dilute to 15 ml with distilled water.*
d. *Do the limit test for sulphates with this solution.*

3 . LIMIT TEST FOR CHLORINATED COMPOUNDS IN SODIUM BENZOATE

Principle : Chlorinated compounds as impurity may be due to benzoic acid being manufactured from chlorinated derivatives of toluene. It is done by dissolving the sample in sodium carbonate solution, evaporating to dryness and heating the residue at a temperature below 400°C till it is charred. The organic matter has been destroyed and the chlorine present is converted to sodium chloride. It is extracted with

water and dilute nitric acid and filtered. The filtrate must comply with the limit test for chlorides.

Procedure

a. Dissolve 0.33 g of the sample, accurately weighed, in 5 ml of 0.5M sodium carbonate.
b. Evaporate to dryness and heat the residue till it is completelv charred, keeping the temperature below 400°C.
c. Extract the residue with a mixture of 10 ml of distilled water and 12 ml of dilute nitric acid. Filter.
d. The filtrate complies with the limit test for chlorides (do the limit test).

4. CHLORIDE AND SULPHATE IN SODIUM SALICYLATE

Principle : For doing these two limit tests, a solution of sodium salicylate in carbon dioxide-free water (solution A) should be prepared. For chloride limit test, nitric acid is added which decomposes the sodium salicylate and releases salicylic acid which is filtered. The limit test for chlorides is then applied to the filtrate.

Procedure

Pretreatment : Weigh 10 g of the sample accurately and dissolve it in enough quantity of carbon dioxide-free water and making up to 100 ml with more carbon dioxide-free water (carbon dioxide-free water may be prepared by boiling distilled water and cooling it, keeping the container closed.)

For Limit test for Chloride :

a. *Take 25 ml of solution A and add 15 ml of distilled water.*
b. *Add 10 ml of 2M nitric acid and filter.*
c. *Do the limit test for chlorides on the filtrate.*

For Limit test for Sulphates :

a. *Take 2.5 ml of solution A and add 125 ml of distilled water.*
b. *Do the limit test for sulphates.*