

PHARMACEUTICAL CHEMISTRY

Impurities in Pharmaceutical Substances

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Introduction

Chemically a compound is impure if it contains undesirable foreign matter i.e. impurities. Thus chemical purity is freedom from foreign matter. It is virtually impossible to have absolutely pure chemical compounds and even analytically pure chemical compounds contain minute trace of impurities. The chemical purity may be achieved as closely as desired provided sufficient care is observed at different levels in the manufacturing of a pharmaceutical. The level of purity of the pharmaceutical substance depends partly on the cost-effectiveness of the process employed, methods of purification, and stability of the final product. Setting higher standards of purity for pharmaceutical substances than that of desirable and pharmacologically safe level will unduly result in wastage of money, material, labour and time. Purification of chemical compounds is a very expensive process hence one has to strike a balance in order to obtain a pharmaceutical substance at reasonable cost yet sufficiently pure for all pharmaceutical purposes.

The word 'pure' is a relative term. Hence, different pharmacopoeias prescribe Test for purity for different substances. 'Purified Water' of the Indian Pharmacopoeia may be prepared by distillation or by means of ion-exchange and it may have pyrogens. 'Sterile Water for Injection' on the other hand must be free from pyrogens and must be sterile. It may have slightly acidic pH or may be very slightly alkaline. Hence, different limits are prescribed for impurities in waters prepared for different purposes.

Sources of Impurities

A list of impurities which are likely to be present in a given pharmaceutical substance can be easily compiled from the knowledge of the raw materials employed, the manufacturing process and stability of the final product. Impurities may also arise from physical contamination and improper storage conditions. The various sources of impurities in pharmaceutical substances are as follows:

1. Raw materials employed in the manufacturing of the pharmaceutical substance:

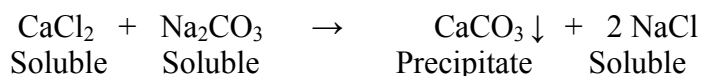
Pharmaceutical substances are either isolated from natural sources or synthesized from chemical starting materials. The natural sources include mineral sources, plants, animals and microbes. It is essential to verify the identity of the source material and to establish its quality otherwise impurities associated with the raw materials may be carried through the manufacturing process to contaminate the final product. In nature minerals rarely occurs in a reasonably pure form. Almost always mixtures of closely related substances occur together e.g., aluminum ores are usually accompanied by alkali and alkaline earth compounds, barium and magnesium impurities are found in calcium minerals, zinc accompanies magnesium or iron compounds, lead and heavy metals are found as impurities in many sulphide ores, among the acid radicals or anions, bromides and iodides are often found as impurities in chlorides, bismuth salts contains silver copper and lead as impurities.

Rock salt used for the preparation of sodium chloride is contaminated with small amounts of calcium and magnesium chlorides, so that sodium chloride prepared from rock salt will definitely contain traces of calcium and magnesium compounds impurities.

2. Method of Manufacture : The Process or method of manufacture may introduce new impurities into the final product arising due to contamination by reagents, catalysts and solvents

employed at various stages of the manufacturing process. The new impurities may also arise from the reaction vessels and reaction intermediates.

(A) Reagents employed in the manufacturing process: Calcium carbonate contains ‘soluble alkali’ as impurity which arises from the sodium carbonate (Na_2CO_3) employed in the process. Calcium carbonate is prepared by the interaction of a soluble calcium salt with a soluble carbonate. Therefore, the final product (CaCO_3) is liable to contain small amount of ‘soluble alkali’ as impurities which were not removed by the washing process.



Anions like Cl^- and SO_4^{2-} are common impurities in many substances because of the use of hydrochloric acid and sulphuric acid respectively in processing. Barium ion may be an impurity in hydrogen peroxide so, hydrogen peroxide employed as reagent in the manufacturing process can contaminate the final product.

(B) Regents used to eliminate other impurities: Barium is used in the preparation of potassium bromide to remove sulphate which in turn arise from the bromine used in the process. It is likely that potassium bromide will now be contaminated by traces of barium.

(C) Solvents: Most of the pharmaceutical substances are prepared in solvated crystalline form. Small amounts of solvents employed in preparation, and purification of reaction intermediates or the final product may also result in the contamination of the pharmaceutical substances. Water is the cheapest solvent available and is used quite frequently in the preparation of inorganic pharmaceuticals. Water can be the major source of impurities as different types of water containing different types and amount of impurities are available. Various types of water which are available are

(i) Tap water: Containing impurities of Ca^{2+} , Mg^{2+} , Na^+ , Cl^- , CO_3^{2-} and SO_4^{2-} in trace amounts. The use of tap water on large scale will lead to the contamination of the final product with these impurities because the impurities will remain in the product even after washings.

(ii) Softened water: It is almost free from divalent cations (Ca^{2+} , Mg^{2+}) but contains more of Na^+ and Cl^- ions as impurities because of the usual chemical water softening process. Therefore, the final products obtained using softened water as solvent will not have Ca^{2+} and Mg^{2+} impurities but still contain Na^+ and Cl^- impurities.

(iii) Demineralized water: It is prepared by means of ion-exchange and is free from Na^+ , Ca^{2+} , Mg^{2+} , Cl^- , SO_4^{2-} and CO_3^{2-} etc. It may have pyrogens, bacteria and organic impurities. So, it is a better solvent than tap water or softened water but the economic factors discourage its use on large scale.

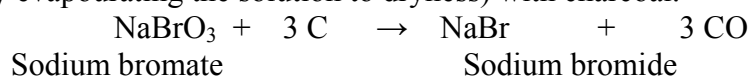
(iv) Distilled water: It is free from all organic and inorganic impurities and is therefore the best as a solvent but it is quite expensive. As it is free from all impurities, it does not pass on any impurities to the final products.

(D) Reaction vessels: The reaction vessels employed in the manufacturing process may be metallic such as copper, iron, cast iron, galvanized iron, silver, aluminium, nickel, zinc and lead. Glass and silica are also used in the construction of the chemical plants but these days many of these are replaced by stainless steel and variety of other alloys. Some solvents and reagents employed in the process may react with the metals of reaction vessels, leading to their corrosion and passing traces of metal impurities into the solution, contaminating the final product. Similarly, glass vessels may give traces of alkali to the solvent. Lead (Pb) may be found as impurity in commercial sulphuric acid which has been manufactured by lead chamber process. Also, substances prepared by same electrolytic process, may contain electrode material as an undesirable impurity e.g. antimony, bismuth etc.

(E) Intermediates: Sometimes, an intermediate substance produced during the manufacturing process may contaminate the final product e.g. Sodium bromate is prepared by reaction of sodium hydroxide and bromine in slight excess.

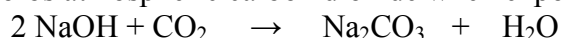


The sodium bromate an intermediate product is reduced to sodium bromide by heating the residue (obtained by evaporating the solution to dryness) with charcoal.

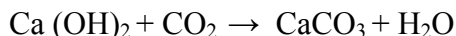


If sodium bromate is not completely converted to the sodium bromide then it is likely to be present as an impurity.

(F) Atmospheric contamination during the manufacturing process: Atmosphere may contain dust (aluminium oxide, sulphur, silica, soot etc.) and some gases like carbon dioxide, sulphur dioxide, arsine and hydrogen sulphide. These may contaminate the final product during the manufacturing process. Some substances which are susceptible to action by atmospheric carbon dioxide and water may get contaminated with them during their preparation e.g. sodium hydroxide readily absorbs atmospheric carbon dioxide when exposed to atmosphere.



Calcium hydroxide solutions can absorb carbon dioxide from the atmosphere to form calcium carbonate.



(G) Manufacturing hazards: If the manufacturer is able to control and check impurities from the all above mentioned sources there exists certain manufacturing hazards which can lead to product contamination. The various manufacturing hazards can lead to:

(i) Contamination from the particulate matter: The unwanted particulate matter can arise by a number of ways, such as accidental inclusion of dirt or glass, porcelain, plastic or metallic fragments from sieves, granulating, tableting and filling machines and the product container. The particulate contamination mainly arises from the wear and tear of the equipments. It may also arise from the bulk materials used in the formulation or from dirty or improperly maintained

equipments e.g. metal particles found in eye ointments packed in metal tubes made up of tin and aluminum.

(ii) Cross-contamination of the product: This manufacturing hazard has to be considered in the preparation of solid dosage forms. Cross-contamination of product can occur by air-borne dust arising out of handling of powders, granules and tablets in bulk. Cross-contamination is dangerous particularly in case of steroidal and other synthetic hormones and therefore, it should be carefully controlled. Precautions, such as use of face mask and special extraction equipment can minimize these undesirable contaminations.

(iii) Contamination by microbes: Many products, like liquid preparations and creams intended for topical applications are liable to contamination by microbes from the atmosphere during manufacturing. For all products intended for parenteral administration and ophthalmic preparations, sterility testing is done and it provides an adequate control for microbial contaminations in such preparations. Microbial contamination can be controlled by adding suitable antimicrobial and antifungal agents.

(iv) Errors in the manufacturing process: Sometimes in a liquid preparation, there is incomplete solution of the solute. This ought to be detected by the normal analytical methods as it can lead to major error. A proper check on the efficiency of mixing, filling, tableting, sterilization etc. should be exercised in order to obtain a product of maximum purity and desired quality. Special precautions are required to be observed to avoid mixing and filling errors in the preparation of low dosage forms ($\geq 5\text{mg}$) such as tablets and capsules containing highly potent medicaments.

(v) Errors in the packaging: Similar looking products, such as tablets of the same size, shape and colour, packed in similar containers can result in mislabeling of either or both of the products. Adequate care should be taken to avoid the handling of such products in the close proximity.

3. Instability of the product:

(A) Chemical instability: Impurities can also arise during storage because of chemical instability of the pharmaceutical substance. Many pharmaceutically important substances undergo chemical decomposition when storage conditions are inadequate. This chemical decomposition is often catalyzed by light, traces of acid or alkali, traces of metallic impurities, air oxidation, carbon dioxide and water vapours. The nature of the decomposition can easily be predicted from the knowledge of chemical properties of the substance. All such decompositions can be minimized or avoided by using proper storage procedures and conditions. The photosensitive substances should be protected from light by storing them in darkened glass or metal containers thereby inhibiting photochemical decomposition. Materials susceptible to oxidation by air or attack by moisture should be stored in sealed containers and if necessary the air from the containers can be displaced by an inert gas such as Nitrogen. Oxidation can also be prevented by adding suitable antioxidants which are capable of undergoing oxidation at the expense of the substances.

(B) Changes in physical properties: Pharmaceuticals may undergo changes in physical properties during storage. There can be changes in crystal size and shape, sedimentation, agglomeration and caking of the suspended particles. These physical changes are not always avoidable and may result in significant changes in the physical appearance, pharmaceutical and therapeutic effects of the product. Particle size and consequently surface area is a critical factor in determining the bioavailability of the low solubility drug such as griseofulvin. Physical changes such as sedimentation and claying in case of multidose suspension may constitute a safety hazard leading to the possibility of under dosage and later to overdosage of the drugs. Similarly increase in the globule size of the injectable emulsions on storage may lead to fat embolism.

(C) Reaction with container material: The possibility of reaction between the container material and the contents can not be ruled out as it constitutes a safety hazard. Preparations susceptible to reaction with metal surfaces e.g. salicylic acid ointment must not be packed in metal tubes. Solutions of substances which are alkali-sensitive e.g. atropine sulphate injection must be packed in glass ampoules which comply with the test of hydrolytic resistance therefore such preparations must not be packed in containers made from soda glass. Plastic containers and closures must be carefully evaluated because of their tendency to give undesirable additives, such as plasticizers, particularly in the presence of non-aqueous solvents. Plastic containers intended for injectables should be sufficiently translucent to allow visual inspection of the contents and if they are having higher than 500 ml capacity, they must also comply with the test limiting animal toxicity in the cat, ether-soluble extractive and metal additives with special reference to barium and heavy metals like lead, tin and cadmium. Rubber closures are more susceptible to absorb medicaments, antioxidants and bactericides from solution, unless they are appropriately pretreated by immersion in solutions of the concerned compounds.

(D) Temperature: The rate of chemical decomposition and physical changes of stored products depends upon the temperature. The susceptible substances may have temperature storage requirements assigned to them in order to protect them against undesirable decomposition.

Control of Impurities

Pharmacopoeial methods: Official monographs for pharmaceutical substances provide description and information in addition to prescribing standards for the product and its storage conditions. An official monograph for a pharmaceutical substance generally includes the following:

1. Title: It is the official name of the substance. Sometimes the common names or synonyms are also mentioned.

2. Chemical formulae: When the chemical structure of the compound is known, the graphic and molecular formulae and the molecular weight are given following the title. A chemical formula refers to the chemically pure substance and is not an indication of purity of the substance.

3. Chemical names: Sometimes the IUPAC name of the substance is also given.

4. Category: It is indicative of the medical or pharmaceutical application of the substance. It is generally the more common application, representing the main pharmacological action of the substance or its active ingredient and the substance may possess other uses or activities also.

5. Dose: The doses mentioned in the pharmacopoeia are for the general guidance and represents the average range of quantities regarded suitable for adults when administered orally.

6. Description: It gives information regarding the general physical and organoleptic properties of the substance. It helps in the preliminary evaluation of the integrity of the article and should not be considered as the analytical requirements.

7. Solubility: The solubility of the substance given in the monograph is primarily for information and should not be regarded as standards or Test for purity but if a quantitative solubility test is given under 'STANDARDS' then the substance should comply with the given requirement. If the exact solubility of the substance is not known, the approximate solubility of the substance is indicated by the descriptive terms. Following table gives the meaning of such descriptive terms for substances at 20 to 30 °C.

Descriptive term	Approximate volume of the solvent for 1 part of the solute
very soluble	less than 1 part
freely soluble	from 1 to 10 parts
soluble	from 10 to 30 parts
sparingly soluble	from 30 to 100 parts
slightly soluble	from 100 to 1000 parts
very slightly soluble	from 1000 to 10,000 parts
insoluble or practically insoluble	more than 10,000 parts

8. Storage: It contains information regarding the storage conditions of pharmaceutical substances so that they can be guarded against possible contamination and deterioration. The precautions need to be taken regarding the effect of atmosphere, moisture, heat and light are also indicated where appropriate in the individual monograph. The temperature conditions related to the storage of pharmaceutical substances are specified in some monographs. The following terms are used in the IP for defining the conditions of temperature.

(a) Cold: Any temperature not exceeding 8°C and usually between 2°C and 8°C. A refrigerator is a cold place in which the temperature is maintained thermostatically between 2°C and 8°C.

(b) Cool: Any temperature between 8°C and 25°C. An article directed to be stored in cool place, may, alternatively be stored in a refrigerator, unless otherwise specified in the monograph.

(c) Room temperature: The temperature prevailing in the working area.

(d) Warm: Any temperature between 30°C and 40°C.

(e) Excessive heat: Any temperature above 40°C.

(f) Protection from freezing: The label of container bears this instruction where, in addition to the risk of breaking of the container, freezing results in a loss of strength or potency or in destructive alteration of the characteristics of an article.

(g) Storage under non-specific conditions: When no specific storage conditions are indicated in the monograph, the storage conditions include protection from moisture, freezing and excessive heat.

9. Standards as determined by the assay: It specifies the quantitative purity of the official compound. If an article does not comply with all the stated requirements it is not of pharmacopoeial quality. These requirements are applicable only to those articles that are intended for medicinal use and not to articles that may be marketed under the same name for other purposes.

10. Identification test: It includes various chemical tests to verify the identity of the substance. They are not absolute proof of identity.

11. Test for purity including limits tests: Different limits for impurities are prescribed for different substances. Test for purity are tests for the presence of impurities in the substance and fix the limits of tolerance for undesirable impurities.

12. Assay: It describes the official method for the quantitative determination of the active ingredient of the pharmaceutical substance and its preparation.

Identification Test

The purpose of identification test is to ensure the correct labeling of the substances, Identification tests are specific but they are not necessary sufficient in establishing the absolute proof of identity of the substances. If an article taken from a labeled container do not meet to the requirements of a prescribed identification test indicates that the article is either mislabeled or substituted. In some monographs, more than one identification tests are given. In such cases, if the article complies with either one or the other identification test, is sufficient to verify the identity of the article.

Identification tests are generally based upon the combination of simple chemical test and measurement of the appropriate physical constants. There is considerable overlap between identification tests and the limit tests. Limit tests are designed to ensure that the undesirable impurities are within the prescribed limits. Identification tests whether physical or chemical, provided they are sufficiently specific, can be used as the basis of a quantitative estimation or in

the design of specific limit tests. Practically, a single identification test may contribute to identification as well as standardization of the substance.

Chemical tests, used for identification are basically qualitative confirming to the presence of the substance under investigation. They may be far too general or lack specificity but can be considered sufficiently specific when used in conjugation with the other requirement of the monograph.

Physical constants such as melting point, boiling point, solubility, weight per ml, refractive index, optical rotation, viscosity etc have characteristic values for a given substance. They can be used in identification, checking quality and maintaining standard of purity.

Test for purity

‘Test for purity’ for substances have been prescribed by the pharmacopoeias of the various countries in order to ensure reasonable freedom from the undesirable impurities. The so called ‘Test for purity’ are infact the tests for the presence of impurities in the substance and fix the limits of tolerance for these undesirable impurities. Test for purity are not framed to guard against all the possible impurity rather they provide appropriate limitation of the potential impurities only.

The guiding factor for fixing a limit of tolerance for the various impurities is the amount of impurity that is likely to be harm. Arsenic and lead are quite dangerous even in trace amounts therefore very small limits of tolerance have been fixed for their presence in all pharmaceutical substances. Another factor is the practicability of the commercial method of production of the substance meeting the requirements of a particular standard of purity. It would be useless to fix the limits of tolerance which can only be attained at a very high cost. There are cases in which the limits fixed in the pharmacopoeia were later relaxed because they were found to be too difficult to attain by the available methods of manufacturing.

The ultimate objective is that the pharmaceutical substances if not completely free from the undesirable or toxic impurities should be of reasonable good purity ensuring therapeutic safety. The presence of sodium bromide (NaBr) in the more expensive potassium bromide (KBr) is not likely to cause any harm to the patient but at the same time the KBr should be of sufficiently good pharmaceutical quality and purity not containing excess amount of sodium bromide. Some of the tests which may be undertaken to ascertain the purity of a substance are:

(a) Clarity of solution: The degree of clarity or opalescence of solution is measured by direct comparison with a reference solution having standard opalescence. The comparison is against a black background by viewing vertically downward under diffused light. A solution is considered clear if its clarity is the same as that of water or of the solvent employed in the preparation of the solution being examined.

(b) Colour of Solution: In Indian Pharmacopoeia, the colour standards are based on three primary colorimetric solutions: yellow, red and blue prepared from ferric chloride, cobaltous chloride and cupric sulphate respectively. These primary solutions are mixed in various proportions with or without 1% w/v hydrochloric acid to give five reference color solutions

which are yellow (YS), greenish yellow (GYS), brownish yellow (BYS), brown (BS), and red (RS). The colour of the solution is compared with reference colour solution by viewing vertically downwards through the columns of liquids in diffused light. A solution may be considered as colorless if it has the same appearance as water or as the solvent employed in the preparation of the solution being examined.

(c) Acidity or alkalinity: Pharmaceutical substances prepared using chemical reactions involving acids and alkalies may possess some degree of acidity or alkalinity resulting from improper purification by inadequate washings after their separation. The limits for acid or alkali impurities are fixed for various pharmaceutical substances and the test for acidity and alkalinity is of great help in determining the extent of such impurities.

(d) Loss on ignition: It is the loss of weight in % w/w resulting from a volatile part of any test material that is driven off under specified conditions. It is applied to thermostable substances which contain thermolabile impurities that decompose and lose a volatile product e.g., zinc carbonate decomposes losing carbon dioxide. The substance is heated, cooled and weighed repetitively until a constant weight is attained. The loss on ignition in this case should not be more than 2% w/w.

(e) Loss on drying: It is the loss of weight in % w/w resulting from water and volatile matter that is lost under specified conditions. The temperature to which the substance is subjected varies considerably according to the nature of the substance. The temperature applied should not be so high as to cause decomposition of the substance but at the same time it should be sufficiently high to produce the desired results within a reasonable time. It is usually applied by drying the substance to constant weight at 105°C.

(f) Moisture content: Sometimes the determination of the moisture content of the substance is a good measure of the purity of the substance especially in case of crude drugs.

(g) Ash values: The determination of ash values in crude drugs, organic compounds and certain inorganic compounds provides valuable information regarding the extent of heavy metals and minerals impurities.

Assay

An assay method should be specific for the substance or chemical species being examined. Nevertheless non-specific assay methods are quite commonly employed particularly in acid-base titrations. Many inorganic salts are assayed by simply determining the content of one of the ions present e.g., sodium sulphate is assayed by determining its sulphate content by precipitating the sulphate as barium sulphate. Although non-specific an assay method can be considered as sufficiently specific when used in conjunction with other requirements of the monograph.

Assay Tolerances

Assay tolerances play an important role in fixing standards for pharmaceutical substances. They include the limits of error of the actual assay process for the active pharmaceutical ingredients, the limits of tolerance of manufacturing process for the particular dosage form and the sampling errors. The limits of error of the assay process depend upon the method employed.

Volumetric and gravimetric methods are very accurate and have quite narrow limits of error whereas spectrophotometric assays are not that accurate and have much wider limits of error.

Limit Tests

Limit tests are quantitative or semi-quantitative tests designed to identify and control small amount of impurities, which are likely to be present in the substance. They involve simple comparisons of opalescence, turbidity or colour produced in test with that of fixed standards.

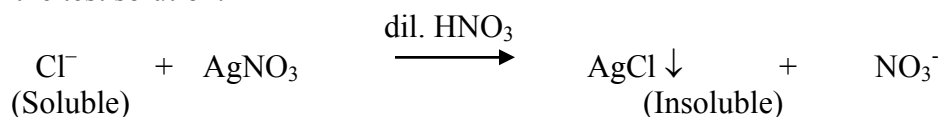


Nessler cylinder

Some of the limit tests are performed in a special apparatus known as Nessler cylinder. Nessler cylinders are matched tubes of clear, colourless glass with a uniform internal diameter and a flat, transparent base. They have a nominal capacity of 50 ml. The overall height of Nessler cylinder is about 150 mm and the external height to the 50 ml mark is about 110 to 124 mm. In general, limit tests are performed in an aqueous solution or sometimes the solution is prepared as specified in the monograph of the Pharmacopoeia. An identical pair of Nessler cylinder should be used i.e. a pair made of the same glass, having same diameter and same height of the graduation mark from the base. Comparison is made by placing the two Nessler cylinders side by side and viewing transversely against proper background in case of limit test chlorides, sulphates and iron. Whereas for heavy metals comparison is done by placing the two Nessler cylinders close together and viewing down through the solution against a light background.

1.Limit Test for Chloride

Principle: This test, which is mainly used to control chloride impurity in inorganic substance, depends upon the simple reaction between silver nitrate and soluble chlorides to give insoluble silver chloride in the presence of dilute nitric acid. The insoluble silver chloride makes the solution opalescent and the extent of opalescence depending upon the amount of chloride present in the substance, is compared with a standard opalescence produced in a standard solution having a known amount of chloride by adding silver nitrate and same volume of dilute nitric acid as used in the test solution.



If the opalescence produced in the test is less intense than that of standard opalescence, the sample passes the limit test for chloride and vice versa.

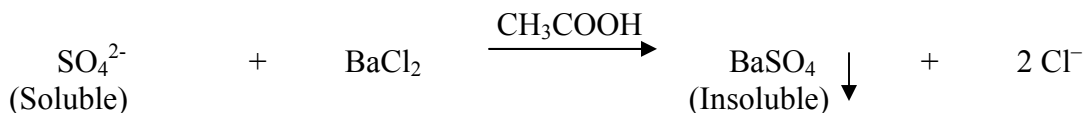
Dilute nitric acid is used to prevent the precipitation of other acid radicals with silver nitrate solution. It acts by providing common ion i.e. nitrate.

Method (I.P. 1996): Specified quantity of the substance to be examined is dissolved in water or a solution is prepared as directed in the individual monograph and transferred to a Nessler cylinder. To this solution 10 ml of dilute nitric acid is added, except when nitric acid is used in the preparation of the solution and the volume is made up to 50 ml with water. Then, 1 ml of 0.1 M silver nitrate is added and the solution is stirred immediately and allowed to stand for 5 minutes protected from light. Any opalescence produced when viewed transversely against a black background is not more intense than that obtained by treating a mixture of 10.0 ml of chloride standard solution (25 ppm Cl) and 5 ml of water in the same manner.

Preparation of standard chloride solution (25 ppm Cl): Dilute 5 volumes of a 0.0824% w/v solution of sodium chloride to 100 volumes with water.

2. Limit Test for Sulphate

Principle: This test is designed to control sulphate impurity primarily in inorganic substance and is based upon the simple reaction between barium chloride and soluble sulphate in the presence of acetic acid to give insoluble barium sulphate.



The opalescence produced in the test is compared with that of standard opalescence obtained from a standard sulphate solution containing known amount of sulphate produced in the same manner. If the test opalescence is less intense than that of standard opalescence, the sample passes the limit test for sulphate and vice versa.

Note: The solubilities of barium sulphate precipitates are very much affected by the concentration of the acid. The acidity of the solution is controlled by using acetic acid. Ethanolic sulphate standard solution (10 ppm SO_4^{2-}) is added to increase the sensitivity of the test. The ionic concentration is adjusted in such a manner that the solubility product of barium sulphate gets exceeded and traces of barium sulphate present assist rapid and complete precipitation by seeding. Ethanol prevents supersaturation and helps in producing a more uniform opalescence.

Method (I.P. 1996): Take 1.0 ml of a 25.0% w/v solution of barium chloride in a Nessler cylinder. To this add 1.5 ml of ethanolic sulphate standard solution (10 ppm SO_4^{2-}), mix and allow to stand for 1 minute. Add a solution of the specified quantity of the substance being examined in 15 ml of water or 15 ml of the solution prepared as directed in the individual monograph and 0.15 ml of 5M acetic acid. Make up the volume to 50 ml with water, stir immediately with a glass rod and allow to stand for 5 minutes. Any opalescence produced when viewed transversely against a black background is not more intense than that obtained by treating 15 ml of sulphate standard solution (10 ppm SO_4^{2-}) in the same manner.

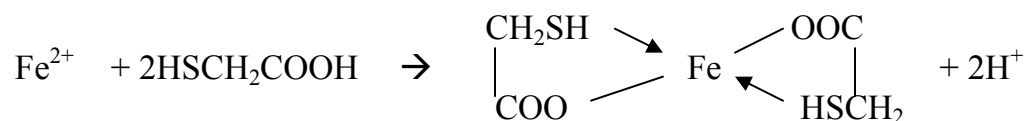
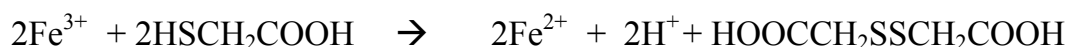
Preparation of 25% w/v barium chloride solution: Dissolve 25g of barium chloride in sufficient distilled water to produce 100 ml.

Preparation of ethanolic sulphate standard solution (10 ppm SO_4^{2-}): Dilute 1 volume of a 0.181% w/v solution of potassium sulphate in ethanol (30%) to 100 volumes with ethanol (30%).

Preparation of sulphate standard solution (10 ppm SO_4^{2-}): Dilute 1 volume of a 0.181% w/v solution of potassium sulphate in distilled water to 100 volumes with distilled water.

3. Limit Test for Iron

Principle: The limit test for iron is based on the formation of pale pink to deep reddish purple colour by reaction of iron with thioglycollic acid in the presence of citric acid in a solution made alkaline with ammonia solution. The colour is due to the formation of the co-ordination compound, ferrous thioglycolate $\text{Fe}(\text{HSCH}_2\text{COO})_2$. The original state of oxidation of the iron is immaterial since thioglycollic acid also reduces ferric iron to the ferrous state.



The colour produced is compared with the standard colour containing a definite quantity of iron by viewing transversely through the Nessler cylinders.

Citric acid is added in the official process to prevent the precipitation of iron by ammonia. Iron gets precipitated in the form of $\text{Fe}(\text{OH})_2$ and $\text{Fe}(\text{OH})_3$ depending upon the oxidation state of the iron in the presence of ammonia. Citric acid makes soluble complex with iron and ammonia will not be able precipitate iron and it will just provide alkaline medium.

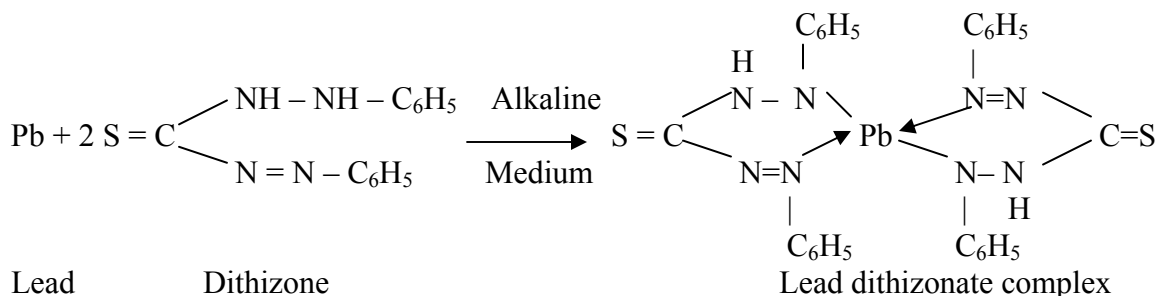
Method (I.P. 1996): The specified quantity of the substance being examined is dissolved in water or a solution is prepared as directed in the individual monograph and transferred to a Nessler cylinder. 2 ml of a 20% w/v solution of iron-free citric acid and 0.1 ml of thioglycollic acid are added and mixed. The solution is made alkaline with iron-free ammonia solution and diluted to 50 ml with water. The solution is stirred with a glass rod and allowed to stand for 5 minutes. Any colour produced is not more intense than that obtained by treating 2.0 ml of iron standard solution (20 ppm Fe) in the same manner in place of the solution being examined.

Preparation of iron standard solution (20 ppm Fe): Dilute 1 volume of a 0.1726% w/v solution of ferric ammonium sulphate in 0.05M sulphuric acid to 10 volumes with distilled water. It contains iron in the ferric state.

4. Limit Test for Lead

Principle: The limit test for lead is based on the reaction between lead and diphenylthiocarbazone (dithizone) in an alkaline medium to form lead–dithizonate complex. The lead present as an impurity in the substance is separated by extracting an alkaline solution with dithizone extraction solution. The interference by other metal ions is eliminated by adjusting the optimum pH for the extraction by using reagents like ammonium citrate, potassium cyanide and hydroxylamine hydrochloride.

The original colour of dithizone in chloroform is green while the lead-dithizonate complex is violet in colour. The intensity of the violet colour of the complex depending upon the quantity of lead present in the solution is compared with that of standard colour produced by treating standard solution containing definite amount of lead in the similar manner.



Method (I.P. 1996): Prepare the sample as directed in the monograph and transfer to a separator. Unless otherwise directed in the monograph, add 6 ml of ammonium nitrate solution and 2 ml hydroxylamine hydrochloride solution. To this, add two drops of phenol red solution and the make the solution alkaline (red in colour) by adding strong ammonia solution. If necessary, cool the solution and add 2 ml of potassium cyanide solution. Immediately extract the solution with 5 ml portions of dithizone extraction solution, until it retains green colour. Combine the dithizone extracts and transfer to a second separator. Shake the combined dithizone extracts for 30 seconds with 30 ml of 1% v/v nitric acid solution and discard the chloroform layer. To the nitric acid solution, add exactly 5 ml of dithizone standard solution and shake for 30 seconds. The violet colour of the chloroform layer should not be more intense than that obtained by treating a volume of standard lead solution (1 ppm Pb) equivalent to the amount of lead permitted in the substance being examined in the same manner as that of solution being examined.

Preparation of standard lead solution (1 ppm Pb): Dissolve 0.400 g of lead nitrate in water containing 2 ml of dilute nitric acid and add sufficient water to produce 250.0 ml. This gives **standard lead solution (1% Pb)**. Standard lead solution (1 ppm Pb) is prepared by diluting 1 volume of standard lead solution (1% Pb) to 1000 volumes with water.

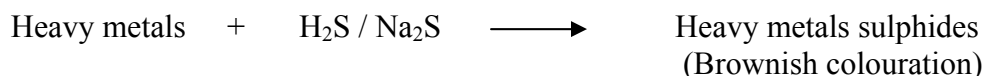
Preparation of dithizone extraction solution: Dissolve 30 mg of dithizone in 1000 ml of chloroform and add 5 ml of ethanol (95%). The solution is stored in refrigerator. Before use, the solution is shaken with about half of its volume of 1% v/v nitric acid solution and acid is discarded.

Preparation of Dithizone standard solution: Dissolve 10 mg of dithizone in 1000 ml of chloroform.

5. Limit Test for Heavy Metals

The limit test for heavy metals is designed to determine the content of metallic impurities that are coloured by hydrogen sulphide or sodium sulphide under the condition of the test. The heavy metals (metallic impurities) may be iron, copper, lead, nickel, cobalt, bismuth, antimony etc. The limit for heavy metals is indicated in the individual monograph in term of ppm of lead i.e. the parts of lead per million parts of the substance being examined. The Indian pharmacopoeia had adopted four methods (methods A, B, C, and D) for the limit test for heavy metals.

Principle: Method A, B and C are based upon the reaction of the heavy metal ion with hydrogen sulphide (in method A and B) or sodium sulphide (in method C) leading to the formation of heavy metal sulphides. The metal sulphides remain distributed in a colloidal state and give rise to a brownish colouration. The colour produced in the test solution is compared with that of standard solution containing a definite amount of the lead.



Method D is based upon the precipitation of relatively insoluble and characteristically coloured sulphides of heavy metals when aqueous solutions are treated with alkali metal sulphides (NaSH). NaSH is generated immediately before use by heating thioacetamide with sodium hydroxide solution. In this test there is a formation of brown colour because of the precipitation of metal sulphides at about pH 3.5 in colloidal form which is stabilized by the glycerin.

The colour is compared by keeping the two Nessler cylinders side by side and viewing vertically downwards against a white background. The usual limit for heavy metals as per I.P. is 20 ppm.

Method (I.P. 1996):

Method A: It is applicable for the substance, which give clear colourless solution under specified conditions of test.

Standard solution: Pipette 1.0 ml of standard lead solution (20 ppm Pb) into a Nessler cylinder labeled as “Standard” and dilute to 25 ml with water. Adjust the pH between 3.0 and 4.0 with dilute acetic acid or dilute ammonia solutions, dilute to 35 ml with water and mix.

Test solution: Take 25 ml of the solution prepared as directed in the individual monograph into a Nessler cylinder labeled as “Test” or dissolve the specified quantity of the substance in water to produce 25 ml. Adjust the pH between 3.0 and 4.0 with dilute acetic acid or dilute ammonia solution, dilute to 35 ml with water and mix.

Procedure: Add 10 ml of freshly prepared hydrogen sulphide to each of the Nessler cylinder containing test solution and standard solution respectively. Mix, dilute to 50 ml with water and allow to stand for 5 minutes. Compare the colour by viewing vertically downwards over a white surface. The colour produced with the test solution is not more intense than that produced with the standard solution.

Preparation of standard lead solution (20 ppm Pb): Dilute 1 volume of standard lead solution (0.1% Pb) to 50 volumes with water.

Method B: It is applicable for those substances, which do not give a clear colourless solution under the specified conditions.

Standard solution: Same as for Method A

Test solution: Weigh the quantity of the substance as specified in the individual monograph in a crucible, wet the sample by adding sufficient sulphuric acid, and ignite at low temperature to thorough charring. To the charred mass add 2 ml of nitric acid and 5 drops of sulphuric acid and heat until white fumes are no longer evolved. Ignite at 500°C to 600°C until the carbon is completely burnt off. Cool, add 4 ml of dilute hydrochloric acid, digest on a water-bath for 15 minutes and evaporate to dryness on a water bath. Moisten the residue with 1 drop of hydrochloric acid, add 10 ml of hot water and digest for 2 minutes. Make the solution just alkaline to the litmus paper by dropwise addition of ammonia solution. Dilute to 25 ml with water and adjust the pH between 3.0 and 4.0 with dilute acetic acid. Filter, rinse the crucible and filter with 10 ml of water. Combine the filtrate and washing to Nessler cylinder labeled as “Test”, dilute to 35 ml with water and mix.

Procedure: Same as for Method A.

Method C: It is applicable to those substances that give clear colourless solution in sodium hydroxide.

Standard solution: Pipette 1.0 ml of standard lead solution (20 ppm Pb) into a Nessler cylinder labeled as “Standard”, add 5 ml of dilute sodium hydroxide solution, dilute to 50 ml with water and mix.

Test solution: Place 25 ml of the solution prepared as directed in the individual monograph into a Nessler cylinder labeled as ‘Test’ or dissolve the specified quantity of the substance in 20 ml of water and 5 ml of dilute sodium hydroxide solution. Dilute to 50 ml with water and mix.

Procedure: Add 5 drops of sodium sulphide solution to each the Nessler cylinder containing the standard solution and the test solution respectively. Mix and allows to stands for 5 minutes and compare the colour by viewing vertically down words over a white surface. The colour produced with the test solution is not more intense than that produced with the standard solution.

Method D :

Standard Solution: Pipette 10.0 ml of either standard lead solution (1 ppm Pb) or standard lead solution (2 ppm Pb) into a small Nessler cylinder labeled as “Standard”. Add 2.0 ml of the test solution and mix.

Test Solution: Prepare as directed in the individual monograph and pipette 12 ml into a small Nessler cylinder labeled as “Test”.

Procedure: Add 2 ml of acetate buffer pH 3.5 to each of the above Nessler cylinders, mix, add 1.2 ml of thioacetamide reagent and allow to stand for 2 minutes. Compare the colour by viewing vertically downwards over a white surface. The colour produced with the test solution is not more intense that than produced with the standard solution.

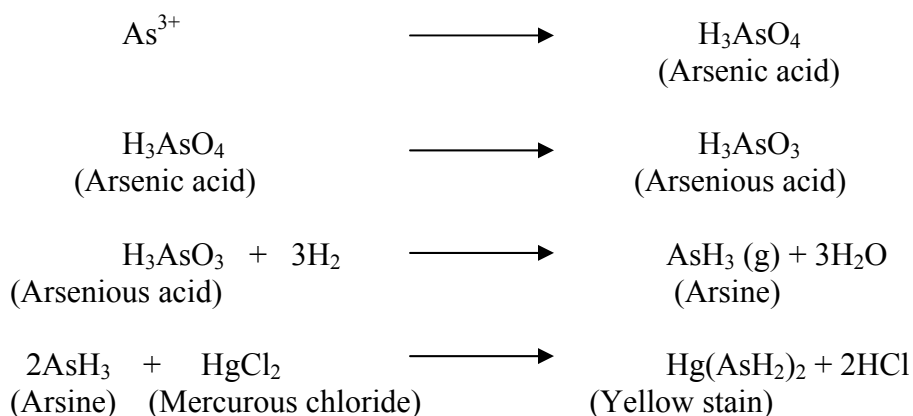
Preparation of standard lead solution (2 ppm Pb): Dilute 1 volume of standard lead solution (20 ppm Pb) to 10 volumes with water.

Preparation of thioacetamide reagent: To 0.2 ml of 4% w/v thioacetamide solution, add 1 ml of a mixture of 15 ml of 1M sodium hydroxide, 5 ml of water and 20 ml of glycerin (85%). Heat on a water bath for 20 seconds, cool and use immediately.

Preparation of acetate buffer pH 3.5: Dissolve 25 g of ammonium acetate in 25 ml of water and add 38 ml of 7M hydrochloric acid. Adjust the pH to 3.5 by using 3M hydrochloric acid or 6M ammonia and dilute to 100 ml with water.

Quantitative Test for Arsenic

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



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

The Apparatus (I.P. 1996)

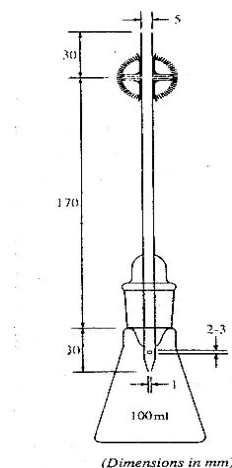


Fig. Apparatus for limit test for arsenic

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

Preparation: The solution of water soluble substances is prepared with water and stannated hydrochloric acid AsT. The solution of substances such as metallic carbonates, which effervesce with acids, is obtained with brominated hydrochloric acid AsT. The substances which are insoluble e.g., BaSO₄, bentonite or kaolins are diffused in water.

Method: The solution of the substances to be examined is prepared as specified in the individual monograph and transferred to the wide mouthed bottle. To this add 1 g of potassium iodide (5 ml of 1M KI), 5 ml of stannated hydrochloric acid solution and 10 g of zinc AsT. Immediately place the glass tube in position and immerse the bottle in a water-bath at a temperature such that a uniform evolution of gas is maintained. The most suitable temperature for the test is about 40°C. The reaction is allowed to continue for 40 minutes. After 40 minutes, the yellow stain produced on the HgCl₂ paper is compared with the standard stain produced by treating 1.0 ml of the **arsenic standard solution (10 ppm As)** diluted to 50 ml with water in the same manner. If the intensity of the yellow stain produced in the test solution is less than that of standard stain, the sample passes the limit test for arsenic and vice-versa. The stain produced on paper fades on keeping and therefore the stains should be compared immediately.

Hydrochloric acid AsT: Hydrochloric acid, low in arsenic, commercially available.

Bromine solution: Dissolve 9.6 ml of bromine and 30 g of potassium bromide in sufficient amount of water to make 100 ml.

Brominated hydrochloric acid AsT: It is prepared by adding 1 ml of bromine solution to 100 ml of hydrochloric acid.

Stannous chloride solution: It can be prepared by either of the following two methods.

1. Dissolve 330 g of stannous chloride in 100 ml of hydrochloric acid and add sufficient water to make 1000 ml.
2. Dissolve 60 ml of hydrochloric acid with 20 ml of water, add 20 g of tin and heat gently until evolution of gas ceases. Add sufficient water to make 100 ml. Store over a little of the undissolved tin remaining in the solution and protected from air.

Stannous chloride solution AsT: It is prepared by adding stannous chloride solution to an equal volume of hydrochloric acid AsT, reducing to the original volume by boiling and filtering through a fine-grain filter paper.

Stannated hydrochloric acid AsT: It is prepared by adding 1 ml of stannous chloride solution AsT to 100 ml of hydrochloric acid AsT.

Preparation of standard arsenic solution (10 ppm As): Dissolve 0.330 g of arsenic trioxide in 5 ml of 2M sodium hydroxide and dilute to 250.0 ml with water. Dilute 1 volume of this solution to 100 volumes with water.

Zinc AsT: It is the granulated zinc which complies with the following additional test:
To 10 g of the granulated zinc add 15 ml of the stannous chloride solution AsT and 5 ml of 0.1M potassium iodide. Apply the general test but allow the reaction to continue for one hour. No visible stain should be produced on the mercuric chloride paper. Repeat the test by adding 0.1 ml of standard arsenic solution (10 ppm As); a faint but distinct yellow stain is produced.

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