UNIT 1

Impurities in Pharmaceutical Substances

INTRODUCTION

Pharmaceutical chemistry is a branch of chemistry that deals with the chemical, biochemical and pharmacological aspects of drugs. It includes synthesis/isolation, identification, structural elucidation, structural modification, Structural Activity Relationship (SAR) studies, study of the chemical characteristics, biochemical changes after drug administration and their pharmacological effects. It is specialized science which depends on other chemical disciplines, such as inorganic, organic, analytical, physical chemistry and also on medicobiological disciplines, such as pharmacology, physiology and biological chemistry, etc.

Inorganic chemistry is the study of all the elements and their compounds except carbon and its compounds (which is studied under organic chemistry). Inorganic chemistry describes the characteristics of substances such as nonliving matter and minerals which are found in the earth except the class of organic compounds. The distinction between the organic and inorganic are not absolute, and there is much overlap, especially in the organometallic chemistry, which has applications in every aspect of the pharmacy, chemical industry including catalysis in drug synthesis, pigments, surfactants and agriculture.

Inorganic pharmaceutical chemistry is a science which makes use of the laws of chemistry to study inorganic substances as drugs. It deals with the study of both non-essential and essential elements about their preparation, chemical nature, structure, standards of purity, test for identification, limit tests, storage, assay methods and uses of inorganic agents used as pharmaceutical aids and as therapeutic and diagnostic agents.

Compounds being synthesized by the geological systems and lack of hydrocarbon (carbon-hydrogen) are known as inorganic compounds while organic compounds are

those found in biological systems. In 19th century, inorganic compounds are inanimate described by Berzelius chemist. The first important synthetic inorganic compound was ammonium nitrate for soil fertilization. Medicinally useful substances are derived from either organic or inorganic sources in which inorganic chemicals contributing significantly in some of the ailments, even after the development of many drugs from synthetic and plant sources. Many of the inorganic salts (antimony, arsenic and mercury) are known to be poison but still they are used in medicine cautiously.

The word 'Pharmaceutical' is used for any chemical substance which is useful in preventive or therapeutic or finds used in the preparation of medicament. Quality of all these pharmaceuticals must be carefully controlled. Hence, specifications of quality are mentioned for each pharmaceutical and their descriptions are reported in the pharmacopeia.

PHARMACOPEIA

The word pharmacopeia is derived from Greek words '*pharmakon*' means a drug (both remedy and poison) and '*poiein*' means to make or create. Pharmacopeia is a book containing directions for the identification of samples and the preparation of compound medicines and published by the authority of a government or a medical or pharmaceutical society. Pharmacopeia is a legislation of a nation which sets standards and mandatory quality indices for drugs, raw materials used to prepare them and various pharmaceutical preparations. Knowledge of the history of pharmacy would help us better to understand the development of pharmacopeias.

MONOGRAPH

Monographs are complete descriptions of pharmaceutical preparations which include chemical formulae, atomic and molecular weight, definition, statement of content, category, dose, description, solubility, identification tests, assay, other test, limits for impurities, quantities and conditions for storage. The appendices also include standards for apparatus, reagents and solutions, indicators, reference substances, test animals, calculation of results, other chemicals techniques, processes, etc. of the concerned pharmaceuticals. In another way it is a reference work for pharmaceutical drug specifications. By the direction of the council of the pharmaceutical society of the certain nations, the world's most comprehensive source of drug information in a single volume is published periodically in the society's department of pharmaceutical sciences. It is the traditional activity, to help the practicing pharmacists and physicians aiming to provide unbiased concise reports on the actions and uses of most of the world's drugs and medicines.

By reflecting clinical practice, every publication of pharmacopeia monographs is accurately organized based on the updated needs of today's pharmacist. Details are provided about new compounds in the form of new monographs accompanied by some of the previous monographs are deleted which are not in continued use. The overall effect is to provide an increase in the average of drugs with typographical improvements to assist the reader in locating sections of a monograph.

With the search for an effective treatment of diseases a few of the developing therapeutics are revised continuously in pharmacopeia such as anti-HIV agents. In

pharmacopeia, the drug's distinguished features are updated, renewed and discussed for the treatment of infections and development of antiviral, antiprotozoal and antibacterial therapy. Along with novel approaches in the treatment advances in the cardiovascular group of drugs are included. The other areas like antimalarial drugs, anti-neoplastic agents, anti-parkinsonism drugs, etc. are also included in pharmacopeia.

Pharmacopeia is divided into three different major parts based on the published information. Each part is comprised of several chapters.

Part I: Generally, the drugs that have similar use or actions are bringing together by part I of pharmacopeia. The cross references is used to guide reader to find out the drug that may be of interest. Many of the chapters are providing background information with an introduction on that group of drugs having many common actions.

Part II: It includes monographs of new drugs, drugs under investigation, drugs which are not easily classified and obsolescent drugs still of interest are presented in this part. It also provides details regarding effects of required drug therapy.

Part III: Composition of the proprietary medicines that are advertised to the public in different countries and herbal medicines which have been omitted are included in part III of pharmacopeia.

Only the pharmaceuticals which are commonly and currently in use are included in the pharmacopeia; whereas the substances which are found to be undesirable and are not currently in use are excluded. Moreover part of pharmacopeia may also comprise the pharmaceuticals which are used for application or internal consumption by human beings.

In the pharmacopeia only minimum standards are prescribed for pharmaceuticals, but with more stringent standards the manufacturer may supply these substances. Hence a drug has to obey strictly the standards prescribed by anyone of the pharmacopeias. The medication may be considered as substandard if it does not obey these standards and usually it is not prescribed by medical practitioners.

HISTORY OF PHARMACOPEIA

Every country has legislation on pharmaceutical preparations which sets a standards and required quality indices for medicament, raw materials and preparations employed in the manufacture of drugs. These regulations are presented in separate articles. General and specific matters relating to individual drugs are published in the form of a book called a Pharmacopeia. On 15th December 1820, the first United State Pharmacopeia (USP) was released. The first British Pharmacopoeia (BP) was published in 1864 with inclusion of monographs on camphor, lactose, sucrose, benzoic acid, gallic acid, tartaric acid, tannic acid and seven alkaloids along with their salts.

Indian Pharmacopoeia

British Pharmacopoeia was utilised as the official book of standards in India before independence. The government passed Drugs and Cosmetics Act in 1940. The Drugs and Cosmetics Act 1940 stated that the Indian Pharmacopoeia is the book of standards for drugs included therein would be official. If considered necessary, these standards can be amended and the secretary of the Indian Pharmacopoeia Committee is authorized to issue such amendments. The general notices and appendices included

in the Indian Pharmacopoeia and as amended in addendum apply both to the matter contained in the Indian Pharmacopoeia and to the matter contained in this Addendum.

- The actual process of publishing the first Indian Pharmacopoeia started in the year **1944** under the chairmanship of Col. R. N. Chopra.
- The list of drugs was published in the year **1946** and was put forth for approval.
- The government of India constituted a permanent Indian Pharmacopoeia Committee in **1948** for the preparation of the Indian Pharmacopoeia and established a central Indian Pharmacopoeia Laboratory at Ghaziabad, Uttar Pradesh to keep it up to date.
- The first edition of the Indian Pharmacopoeia (IP) was published in the year **1955** under the chairmanship of Dr BN Ghosh. Ministry of Health and Family Welfare, Government of India publishes Indian Pharmacopoeia based on the recommendation of Indian Pharmacopoeia Committee (in accordance with Drugs and Cosmetics Act, 1940, Dangerous Drugs Act, 1930, and Poisons Act, 1919 and the rules framed thereunder).
- Supplement for first edition of Indian Pharmacopoeia was published in the year **1960**. It contained both western and traditional system drugs commonly used in India.
- After eleven years, under the chairmanship of Dr. B. Mukherji the second edition of Indian Pharmacopoeia was released in **1966** with some modification.
- The supplement to the second edition of Indian Pharmacopoeia was published in **1975**.
- There had been a phenomenal growth and development of Indian pharma industry especially from early 1970 both in the range of active pharmaceutical ingredients (APIs) and the dosage forms produced. In view of these rapid advances, it was decided to publish a new edition of the Pharmacopoeia and its addenda at regular and shorter intervals for which the Indian Pharmacopoeia Committee was reconstituted in **1978**.
- Third edition of Indian Pharmacopoeia were in two volumes published in **1985** under the chairmanship of Dr. Nityanand. In this Pharmacopoeia inclusion of traditional system of drugs was limited. However, most of the new drugs manufactured and/or marketed were included while only those herbal drugs which had definite quality control standards had got place in it.
- Addendum/supplement I and II to third edition has been published in **1989** and **1991** respectively.
- Fourth edition of the Indian Pharmacopoeia was published in two volumes under the chairmanship Dr. Nityanand in **1996** which omitted many lesser used and obsolete product monographs and added monographs based on the therapeutic merit, medicinal need and extent of use of such articles in the country.
- Addendum to fourth edition has been published initially in **2000** followed by in **2002**. In addition, supplement for veterinary products are also released.
- Third addendum was published in **2005** which included a large number of antiretroviral drugs, and raw plants commonly used in making medicinal products not covered by any other.
- The Indian Pharmacopoeia Commission (IPC) has been established in the year 2005.

- It is the 5th edition of IP in **2007**. The IPC provided systematic approach and practices for publication of Indian Pharmacopoeia 2007 with focus on those drugs and formulations that cover the National Health Care Programmes and the national essential medicines. It contained monographs on antiretroviral, anticancer, antitubercular and herbal drugs. It also emphasized on biological monographs, such as vaccines, immunosera for human use, blood products, biotechnological and veterinary (biological and non-biological) preparations.
- Addendum 2008 to the IP 2007 was published which had taken care of the amendments to Indian Pharmacopoeia 2007 and also incorporated 72 new monographs.
- Government of India declared Indian Commission, an autonomous institution under the Ministry of Health and Family Welfare by its resolution of 6th May 2008 and declared Central Indian Pharmacopoeia Laboratory, Ghaziabad as subordinate office since 1st Jan 2009.
- The **6th edition** of IP published in **2010.** The sixth edition of IP published in accordance with the principles and designed plan decided by the scientific body of the IPC. To establish transparency in setting standards for this edition, the contents of new monographs, revised appendices and other information have been published on the website of IPC.
- The IPC secretariat and Indian Pharmacopoeia laboratory staff, with the support of different advisory expert committee, and expert members of the scientific body have examined the suitability of the standards. In order to make Indian Pharmacopoeia 2010 user friendly, the existing formatting pattern has been suitably revised.
- The Indian Pharmacopoeia 2010 has been considerably revised and improved in respect of the requirements of monographs, appendices and testing protocols by introducing advanced technology. The contents of appendices are by and large revised in consonance with those adopted internationally. The monographs of special relevance disease of this region have been given special attention.
- National Formulary of India 4th edition was published in the same year and it meant for the guidance of the members of the medical profession such as medical students, nurses and pharmacists working in hospitals and other areas.
- The **seventh edition** of the IP 2014 has been published in **Nov 2013** by the Indian Pharmacopoeia Commission (IPC). It is presented in four volumes. The scope of the Pharmacopoeia has been extended to include products of biotechnology, indigenous herbs and herbal products, veterinary vaccines and additional antiretroviral drugs and formulations, inclusive of commonly used fixed-dose combinations. Standards for new drugs and drugs used under National Health Programmes are added and the drugs as well as their formulations not in use nowadays are omitted from this edition.
- The IP 2014 incorporates 2548 monographs of drugs out of which 577 are new monographs consisting of APIs, excipients, dosage forms, antibiotic monographs, insulin products and herbal products, etc. 19 New radiopharmaceutical monographs and 1 general chapter is first time being included in this edition.
- First Addendum 2015 of IP 2014 has been released on **2014**. It incorporates 82 new monographs consisting of 57 chemical monographs, 13 herbal monographs, 02 human vaccines monographs and 10 radiopharmaceutical monographs, etc.

- Second Addendum 2016 of IP 2014 has been released on **2015**. It incorporates 89 new monographs consisting of 64 chemical monographs, 14 herbal monographs, 3 vaccines and immunosera for human use, 3 radiopharmaceuticals monographs, 1 blood related products, 4 biotechnology products monographs and 2 general chapters being included in this addendum.
- The **latest edition** of the IP 2018 has been published in **29th Sep 2017.** IP 2018 has been brought out in 4 volumes incorporating 220 new monographs (chemical monographs (170), herbal monographs (15), blood and blood related products (10), vaccines and immunosera for human use monographs (2), radio-pharmaceutical monographs (3), biotechnology derived therapeutic products (6), veterinary monographs (14)), 366 revised monographs and 7 omissions.

Salient Features of Indian Pharmacopoeia 2018

Keeping in view the essential requirement for harmonization of analytical methods with those accepted internationally, steps have been taken for monitoring drug standards.

- It is effective from 1st January, 2018
- Presented in 4 hard bound volumes with DVD
- Total monographs 2761, 220 new monographs included.
- Veterinary product monographs are the integral part of this edition
- Use of chromatographic methods has been greatly extended
- Obsolete monographs have been omitted
- Herbal drug monographs have been added
- General chemical tests and thin layer chromatography (TLC) for identification of an article have been almost eliminated and more specific infrared, ultraviolet spectrophotometer and HPLC tests have been given emphasis. The concept of relying on published infrared spectra as a basis for identification has been continued.
- Most of the existing assays and related substances test methods are upgraded by liquid chromatographic in view to harmonize with other International Pharmacopoeia.
- Pyrogen test has been replaced by bacterial endotoxin test (BET) in parenteral preparations and other monographs.
- For ease of access to make pharmacopoeia more user-friendly, index has been incorporated in volume I along with that already existing in volume IV of IP.
- 53 new fixed dose combination (FDC) monographs have been included, out of which 25 FDC monographs are not available in any pharmacopoeia.
- 10 new general chapters on pharmaceutical, microbiological and biological have been incorporated.
- General chapters on volumetric glassware, conductivity, dissolution test, disintegration test, dimensions of hard gelatin capsule shells, etc. have been revised.
- For controlling the microbial quality of the entire medicinal product, general chapter on maintenance, identification, preservation and disposal of microorganism have been revised.
- IP 2018 has been incorporated with a security feature to avoid counterfeiting.

Extra Pharmacopoeia (Martindale)

Pharmacopoeia possesses wealth of information with no explanation. The person must familiarize himself with the general notices and the various appendices of pharmacopoeia to consult the pharmacopoeia. One can obtain most complete information from Extra Pharmacopoeia (Martindale) on every type of pharmaceutical or drug. A practicing pharmacist William Martindale in the year 1883 published the Extra Pharmacopoeia. This book was rich especially with therapeutic and clinical information of the drugs.

For inorganic pharmaceuticals there are several other useful literature references are included. With an aim to provide practical and up-to-date information concerning drugs and gelenicals included in the British Pharmacopoeia. In the span of three years four editions of Martindale were published. Due to the accumulation of information up to the year 1910 the subject matter to be divided into two volumes in the initial editions of Martindale. The first double volume edition was published in 1912.

In December 1933, the Pharmaceutical Society of Great Britain acquired the copy right of the Extra Pharmacopoeia upon the death of Dr W.H Martindale son of William Martindale. Thereafter the society is continuing to issue it under the editorship of the Director of its Department Pharmaceutical Sciences. 23rd edition of Volume II was published in 1955 and the 24th edition of Volume I was published in 1958. Supplement for 24th edition was published in 1961. In February 1967 the 25th edition was published by the Pharmaceutical Society of Great Britain while 26th edition was released in July 1972. In 30th edition of Martindale contains up-to-date authoritative information on drugs and medicine which are used throughout the world was published in 1993. It is written for all those involve in use of drugs and medicines including practicing pharmacists and physicians.

In order to meet the requirements of today's reader the latest edition of Martindale has been markedly changed. It includes a significant shift to a more clinical emphasis an increase in the number of referenced reviews and massive increase information on proprietary medicines. In addition usual period between editions was shortening to meet the need for up-to-date information.

British Pharmacopoeia

Medical Act, 1858 under Section 54 was stressed the need of publication of a book having a list of medicines and compounds about their manner of preparing them together with true weights and measures by which they are to be prepared and mixed. Hence, the British Pharmacopoeia was decided to publish.

- In the year 1864 the first British Pharmacopoeia was published by combining the three old and reputed Pharmacopoeias, namely Pharmacopoeia Londinensis (1618), Edinburgh Pharmacopoeia (1699) and Dublin Pharmacopoeia (1807). New editions and addendum were released quickly.
- The 2nd edition was released in 1867.
- The 3rd and 4th editions were published in the year 1885 and 1898 respectively.
- Addendum to 2nd and 3rd editions was released in the year 1874 and 1890 respectively.
- Separate parts such as preparation of compounds are included in the year 1864 British Pharmacopoeia. In this edition contents had been arranged alphabetically. A gap in revision belated the next edition of British Pharmacopoeia until 1914.

- Further edition was published in 1928 and 1932.
- A range of diagnostic materials was included in 1932 revision. An important addition was inclusion of standards and tests for antitoxins and insulin.
- Thereafter the commission was recommended to revise the BP every 10 years once.
- Seven addenda covered the interim between 1932 and next edition of 1948.
- In this 1948 edition (7th), for substances newly introduced into medicine, generic names were provided. Methods of analysis such as disintegration tests for tablets and sterilization methods were expanded. Many new monographs related to sex hormones and penicillin's were included.
- Due to the rapid development of pharmaceutical and pharmacological progress at this time it was decided that the normal interval between new editions should be 5 instead of 10 years.
- The next edition was released in the year 1953. It incorporates the titles of drugs and preparations were given in English instead of Latin.
- The 9th edition (1958) contains 160 new monographs. Spectrophotometric analysis and inclusion of tranquillizing drugs are the other features of this edition.
- The next, i.e. tenth edition was published in 1963. The duties of the British Pharmacopoeia Commission were defined clearly in medicines order 1970.

The first edition of British Pharmacopoeia that was prepared strictly under the provisions of Medicines Act was the thirteenth edition which was published in the year 1980. Due to an expansion of drug information latter the British Pharmacopoeia was decided to publish in two volumes.

Authoritative standard for the quality of many substances preparation and articles used in medicine and pharmacy for some 130 years was provided in 1993 edition of British Pharmacopoeia. For the convenience of user this edition consolidates and extends the 1988 edition with its 1989, 1991, and 1992 addenda. Moreover monographs of the European Pharmacopoeia were also included in this particular edition.

The last year edition of the British Pharmacopoeia (BP), i.e. British Pharmacopoeia 2013 comprises six volumes which contain nearly 3,000 monographs for drug substances, excipients and formulated preparation, together with supporting general notices, appendices (test methods, reagents, etc.) and reference spectra used in the practice of medicine. All are comprehensively indexed and cross-referenced for easy reference. Items used exclusively in veterinary medicine in the UK are included in the BP (veterinary).

The volume I and II deals with medicinal substances, whereas volume III describes about formulated preparations, blood related preparations, immunological products, radiopharmaceutical preparations, surgical materials and homeopathic preparations. The volume IV contains appendices, infrared reference spectra and index. The volume V is for veterinary purpose, i.e. British Pharmacopoeia (veterinary). The volume VI is the CD-ROM version of British Pharmacopoeia, British Pharmacopoeia (veterinary) and British approved names. The 2013 edition of British Pharmacopoeia is available as a printed volume and electronically in both on line and CD-ROM versions, the electronic products use sophisticated search techniques to locate information quickly. For example, pharmacists referring to a monograph can immediately link to other related substances and appendices referenced in the content by using 1,30,000 plus hypertext links within the text.

The British Pharmacopoeia 2013 package comprises five volumes and a single volume of the British Pharmacopoeia (veterinary) 2013, along with a fully searchable CD-ROM and online access which provided flexible resources. The British Pharmacopoeia 2013 was legally effective from 1 January 2013 and contains 41 new British Pharmacopoeia monographs, 40 new European Pharmacopoeia monographs, 619 amended monographs, 6 new and 1 amended infrared reference spectra and European Pharmacopoeia 7th edition material up to and including Supplement 7.5. In addition updates in January, April and July to harmonize with the European Pharmacopoeia was also provided. The current edition of the British Pharmacopoeia, i.e. British Pharmacopoeia 2014 comprises five volumes and a single volume of the British Pharmacopoeia (veterinary) 2014, along with a fully searchable CD-ROM and online access to provide with flexible resources. The latest edition was published in 2018 which includes around 4000 monographs spread out in six printed volumes.

European Pharmacopoeia

An official book of standards adopted by Germany, France, Italy, Netherlands, Switzerland and Belgium is European Pharmacopoeia. The council of Europe issued an order to frame out European Pharmacopoeia in July 1964. In 1969 onwards in the respective member countries it was appeared as official standard book for medicinal substances and other drugs. Later on it was revised continuously to keep the information up-to-date.

PHARMACOPEIA INTERNATIONALS

In various countries there are no uniformity in terminology and strengths of pharmaceutical preparations used. In the year 1874, a view had been expressed that uniformity in the standards for potent drugs must necessary to overcome various problems. In 1936, the Health Organization of the League of Nations established a Technical Commission of Pharmacopeial Experts. The work was undertaken by the WHO after the World War II which was ended in 1946. Finally volume I of the long awaited International Pharmacopeia was published in 1951 by Latin with translation into English and French. This International Pharmacopeia contains monographs for over 200 drugs and chemicals, with appendices on reagents tests and biological assays. Second volume was published in 1955. In 1967 the second edition followed by a supplement in 1971. Third edition was published in 1979 spread out in several volumes. The pharmacopeial authorities of all countries are expected to give due considerations to its standards so as for achieving uniformity of standards as far as practicable even though the International Pharmacopeia cannot be imposed legally on any country.

United States Pharmacopeia (USP)

United States Pharmacopeia and the National Formulary (USP-NF) are recognized as official compendia for determining standard of pharmaceutical products. The first USP was published in 1820 with 217 drugs. National formulary was published in 1888 under the authority of American Pharmacists Association. After 1975, USP and NF are published in combined volume as USP-NF by United State Pharmaceutical Convention. It was published at an interval of five years. After 2000, USP-NF has been published annually. The current version of USP-NF standards deemed official by USP

are enforceable by the US Food and Drug Administration for medicines manufactured and marketed in the United States. The USP 42-NF 37 becomes official on 1st May 2019.

PHARMACOPEIAL DESCRIPTION/PRESENTATION

Most of the pharmacopeias including Indian Pharmacopoeia consist of the three major sections, namely (a) introduction including general notices, (b) monographs of the official drugs, (c) appendices.

(a) Introduction

It is useful information to pharmaceutical progress since last edition. It summarizes the different changes including additions/deletions in the current edition compared to last edition. To avoid misinterpretation, misunderstanding of later parts of the text, attention should be paid to general notices at the outset.

(b) Monographs

The general monographs for dosage forms of active pharmaceutical ingredients (APIs) are grouped together at the beginning of volume II of IP 2010. The written study of a subject was implied by the word 'monograph' (mono—single, graph—to write). These are considered as very important because medicinal substances are used for the cure and/or prevention of diseases. Therefore their written studies appear as monographs. Monographs are arranged in the alphabetical order of their names and are somewhat stereotyped in style.

Contents of Individual Monographs

An official monograph for a drug and pharmaceutical substance generally includes the following.

1. **Title:** The official name of the compound in English is stated in the title. Sometimes common names or synonyms are also mentioned, e.g. calcium carbonate can also be called precipitated chalk, milk of magnesia can also be called magnesium hydroxide mixture.
2. **Chemical formulae:** When the chemical structure of an official compound is known, the graphic, molecular formulae and molecular weight are given at the beginning of the monograph.
3. **Chemical names:** Sometimes the International Union of Pure and Applied Chemists (IUPAC). IUPAC name of the substance is also given in the monograph.
4. **Atomic and molecular weight:** The atomic and molecular weight is shown, as and when appropriate at the top right hand corner of the monograph.
5. **Category:** This part of monograph expresses the pharmacological or therapeutic or pharmaceutical application of the compound. Although the compound may have other applications usually this part describes the main application. Analgesics, antibiotic, antacid, laxative, etc. are some of the main categories for inorganic pharmaceuticals in the pharmacopeia.
6. **Dose:** Dose mentioned in the IP is intended merely for general guidance and represent the average range of quantities regarded as suitable for adults when administered by mouth.

7. **Description:** It illustrates physical and organoleptic properties of the substance such as amorphous nature or crystalline, odor, color and taste, etc. It helps in the preliminary evaluation of the integrity of an article and should not be considered as analytical requirements.
8. **Solubility:** The solubility of the substance given in the monograph is primarily for the information and should not be regarded as standards or test for purity. The term "partly soluble" is used to describe a mixture where only some of the components dissolve. If the exact solubility of the substance is not known, the approximate solubility of the substance is indicated by the descriptive terms. The solubility mentioned in Indian Pharmacopoeia is the approximate solubility at a temperature between 15°C and 30°C.

Descriptive term	*Part of solvent required for part of solute to dissolve*
Very soluble	Less than 1 part
Freely soluble	From 1 to 10 parts
Soluble	From 10 to 30 parts
Sparingly soluble	From 30 to 40 parts
Slightly soluble	From 100 to 1000 parts
Very slightly soluble	From 1000 to 10000 parts
Insoluble or practically insoluble	More than 10000 parts

9. **Standards:** IP prescribes the standard of purity and strength of all official substances. A substance is not deemed to be of standard quality unless it complies with the requirements stated under this of its monograph.
10. **Identification test:** It is to ensure the correct labelling of substances. Identification tests are specific, but they are not necessarily sufficient in establishing the absolute proof of identity of the substance. This usually involves specific chemical test or tests for identifying the substance. It provides a means of verifying that the identity of the material under examination is in accordance with the label on the container. There is substantial overlap between identification and 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. Physical constants, such as boiling point, melting point, solubility, refractive index, viscosity, optical rotation, etc. have characteristic values for a given substance. It can be used in identification, checking quality and maintaining standard of purity.
11. **Tests for purity:** Tests for purity are tests for the presence of impurities in the substance and fix the limits of tolerance for undesirable impurities.
12. **Assay:** Assay is used for the quantitative determination of active ingredients of the official substance and their preparations.
13. **Storage:** It contains information regarding the storage conditions of official substance so they can be protected against possible contamination and deterioration. The precautions need to be taken with the effect of atmosphere, moisture, heat and light are also indicated in the monograph. In case of some drugs or pharmaceutical substances, lower or higher temperatures produce undesirable effects.

The storage conditions are defined by the following terms:

a. Store in a dry, well-ventilated place at a temperature not exceeding 30°C
b. Store in a refrigerator (2°C to 8°C) and do not freeze
c. Store in a freezer (–2°C to –18°C)
d. Store in a deep freezer (below –18°C).

The storage conditions not related to temperature are indicated in the following terms:

a. Store protected from light
b. Store protected from light and moisture

where no specific storage directions or limitations mentioned, it is to be understood that the storage conditions include protection from moisture, freezing and excessive heat (any temperature above 40°C).

14. **Storage containers:** The storage containers in the pharmacopeia are indicated in the following terms:
    a. Well-closed containers: This implies the substance is stable and gets protected from dust, dirt, insects, etc.
    b. Tightly closed container: The substances in such cases get affected by atmospheric oxygen or moisture or carbon dioxide. For example, reducing agents, hygroscopic substances, strong bases, etc. must be stored in tightly closed containers. It may also include such compounds are volatile or contain dissolved gases, etc.
    c. Light resistant container: Substances which are affected by light are stored in amber or dark colored containers.
    d. Single dose containers: This is generally prescribed for some injectables which once opened should not be used again.
15. **Labeling:** The labeling statements may appear on the container, the package, a leaflet accompanying the package or certificate of analysis associated with the article, as decided by the competent authority.

(c) Appendices

A comprehensive section of appendices are presented followed by the general notices and monographs.

Appendix 1: It describes the apparatus that are needed for various pharmacopeial tests and assays

Appendix 2: It describes biological tests and assays

Appendix 3: It describes the details of various chemical tests and assays

Appendix 4: It describes the details of microbiological tests and assays

Appendix 5: It describes some physical tests and determinations like loss on drying, determination of pH, melting range, etc.

Appendix 6: It describes includes the useful directions on cleaning glassware.

Appendix 7: It describes the reagents and solutions needed for the various tests and assays, their method of preparation, standards, etc.

Appendix 8: Describes reference substances

Appendix 9: It describes the names, symbols used in the pharmacopeia and their atomic weights.

IMPURITY

In pharmaceuticals, impurity is defined as any other material besides the drug substance such as intermediates or starting material or by products, interaction products or degradation products due to any side reactions.

Substances which are used in pharmaceutical field must be pure so that they can be used safely. But it is very difficult to obtain an almost pure substance. Impurities defined as a foreign particle that affects the purity of a substance.

An impurity in a drug substance as defined by the International Conference on Harmonisation (ICH) Guidelines is any component of the drug substance that is not the chemical entity defined as the drug substance and affects the purity of active ingredient or drug substances.

Effect of Impurities

As we know that almost pure substances are difficult to get and some amount of impurity is always present in the material. So, the impurities which are present in the substances may have the following effects:

- Impurities may bring about incompatibility with other substances
- Impurities may lower the shelf life of the substances
- Impurities may cause difficulties during formulations and use of the substances
- Sometimes impurities changes the physical and chemical properties of the substances
- Therapeutic effect can be decreased
- Shows toxic effect after a certain period
- Injurious when present above certain limits
- It may change odor, color, taste of the substance.

Sources of Impurities

To prevent these impurities many test such as limit test are carried out to lower the impurities to make the pharmaceuticals safer.

A substance having foreign materials is termed to be impure. The cause of impurities in drugs is from various sources and phases of the synthetic process. Many of the impurities may arise from starting materials, by products, synthetic intermediates, synthetic route of manufacturing process and degradation products. The pharmaceutical preparation should be free from toxic and other impurities.

The impurities commonly found in pharmaceutical substances are:

- Raw materials employed in manufacture
- Method or the process used in manufacture
- Chemical processes and the plant materials employed in the processes
- Due to color and flavoring substances
- Incompatibility of active ingredient with other substance
- Storage conditions
- Decomposition
- Impurities due to humidity and temperature.

The various sources of impurities in pharmaceutical substances are as follows:

1. **Raw materials employed in the pharmaceutical process:** Pharmaceutical substances are either isolated from natural sources or synthesized from chemical starting materials which have impurities. Impurities associated with the raw materials may be carried through the manufacturing process to contaminate the final product, e.g. rock salt used for the preparation of sodium chloride is contaminated with small amounts of calcium and magnesium chlorides, many sulfide ores containing lead and heavy metals as impurities.
2. **Method or manufacture process:** The process of manufacture may introduce new impurities. Due to impure reagents, catalysts and solvents, reaction vessels and reaction intermediates employed at various stages.
   a. *Reagents employed in the manufacturing process*: If reagents are employed in the process are not completely removed and these reagents may be present in the final products, e.g. calcium carbonate contains 'soluble alkali' as impurity. Anions like Cl^- and SO_4^{2-} are common impurities in many substances because of the use of hydrochloric acid and sulfuric acid respectively. Barium ion may be an impurity in hydrogen peroxide.
   b. *Reagents used to eliminate other impurities*: Barium is used to remove sulfate from potassium bromide, which can be found, itself (barium) as impurity at the end of process.
3. **Solvents:** In pharmaceutical substances, solvents employed in preparation and purification of the product and it may also contaminate the product. Water is the most commonly used solvent in the pharmaceuticals which can be the major source of impurities. Different types of water are as follows.
   a. *Distilled water*: It is free from all inorganic and organic impurities and best solvent for pharmaceutical preparation.
   b. *Demineralized water*: It is free from magnesium, calcium, sodium, sulfates, chlorides, carbonates impurities and prepared by ion exchange method. It contains bacteria, pyrogens and organic impurities.
   c. *Tap water*: It contains magnesium, calcium, sodium, sulfates, chlorides, carbonates as impurities.
   d. *Softened water*: It is prepared from tap water and it contains sodium and chloride ions as impurities.
4. **Intermediates:** An intermediate substance produced during the manufacturing process may contaminate the final product, e.g. sodium bromide is prepared by reaction of sodium hydroxide and bromine in slight excess.

$$6NaOH + 3Br_2 \rightarrow NaBrO_3 + 5NaBr + 3H_2O$$

The sodium bromate an intermediate product is reduced to sodium bromide by heating the residue with charcoal.

$$NaBrO_3 + 3C \rightarrow NaBr + 3CO$$

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

5. **Atmospheric contamination during the manufacturing process:** Atmosphere may contain dust (aluminium oxide, sulfur, silica, soot, etc.) and some gases like carbon dioxide, sulfur dioxide, arsine and hydrogen sulfide. These may contaminate the final product during the manufacturing process, e.g. sodium hydroxide readily absorbs atmospheric carbon dioxide when exposed to atmosphere.

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

6. **Chemical process:** Various chemical reactions such as oxidation, reduction, halogenation and hydrolysis are involved in the synthesis of pharmaceuticals. In these reactions chemical and solvents are used which may found in the product as a impurities.
7. **Manufacturing hazards:** Sometimes certain manufacturing hazards which can lead to product contamination.
   a. *Contamination from the particulate matter*: The unwanted particulate matter can arise by accidental introduction of dirt or glass, porcelain, plastic or metallic fragments from sieves, granulating, filling machines and the product container.
   b. *Cross-contamination of the product*: It can occur by airborne dust arising out of handling of powders, granules and tablets in bulk. If two or more products are manufactured in same time this type of contamination is possible.
   c. *Contamination by microbes*: Microbes like bacteria, fungi, algae, etc. can contaminate the final product. Many liquid preparations and creams intended for topical applications are liable to contamination by microbes from the atmosphere during manufacturing.
   d. *Errors in the packaging*: Similar looking products such as tablets of the same size, shape and color are packed in similar containers can result in mislabeling of either or both of the products.

8. Instability of the product:
   a. *Chemical instability*:
      - Many pharmaceutically important substances undergo chemical decomposition when storage conditions are inadequate.
      - Chemical decomposition is often catalyzed by light, traces of acid or alkali, traces of metallic impurities, air oxidation, carbon dioxide and water vapors.
      - Impurities can also arise during storage because of chemical instability.
   b. *Reaction with container material*: The reaction between the container material and the contents can affect the stability. Preparations susceptible to reaction with metal surfaces, e.g. salicylic acid ointment must not be packed in metal tubes.
   c. *Temperature*: The rate of chemical decomposition and physical changes of stored products depends upon the temperature.

To minimize and prevent impurities many test such as limit test carried out to diminish the impurities and make the pharmaceuticals safer.

LIMIT TEST

Limit test is defined as **quantitative** or **semiquantitative** test designed to identify and control small quantities of impurity which is likely to be present in the substance.

Limit test for chlorides, sulfates, iron, lead and heavy metal are carried out in Nessler cylinders (Fig. 1.1). It is made up of borosilicate glass having fixed diameter and length as per IP. Two similar kinds of cylinders are required for test and standard to make the comparison in identical manner. No numerical values for the limits in these tests are prescribed in pharmacopeia as it is not practicable. The sample quantity may vary according to the limits while standard remains constant.

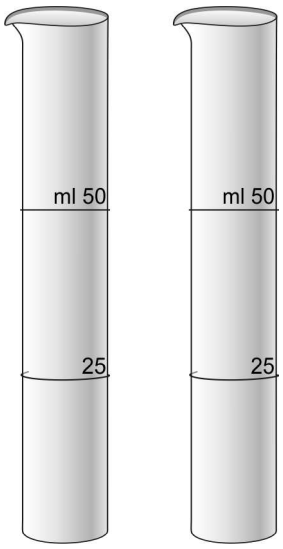


Fig. 1.1: Nessler cylinders

In these tests, the **test opalescence/turbidity/color/stain** produced by the reaction of specified amount of impurity in the test sample with the reagent is compared with the **standard opalescence/turbidity/color/stain** produced by the reaction of known amount of impurity [standard] with the reagent. It is generally carried out to determine the inorganic impurities present in compound.

Importance of limit tests:

- To find out the harmful amount of impurities
- To find out the avoidable/unavoidable amount of impurities.

Limit Test for Chloride

Principle

Limit test of chloride is based on the simple reaction between silver nitrate and soluble chlorides in presence of dilute nitric acid to give opalescence of silver chloride.

$$Cl^- + AgNO_3 \rightarrow AgCl\downarrow + NO_3^-$$

A comparison **Limit Test** is made of the opalescent solution so obtained with the standard opalescence containing a known amount of chloride ions.

Preparation of Solutions

Chloride standard solution (25 ppm Cl): Dilute 5 ml of 0.0824% w/v solution of sodium chloride in 100 ml of water.

Silver nitrate solution (0.1 M): 0.1 M silver nitrate was prepared by dissolving 17 g of silver nitrate in sufficient water to produce 1000 ml.

Nitric acid, dilute: Contains approximately 10% w/w of HNO_3. Dilute 106 ml of nitric acid to 1000 ml with water.

Procedure

Take two (50 ml) Nessler cylinders and label it one as 'Standard' and other as 'Test'.

Test solution	*Standard solution*
Dissolve the specified quantity of the substance under examination in water and transfer to a Nessler cylinder	Take 1 ml of chloride standard solution (25 ppm Cl^-) in a Nessler cylinder
Add 10 ml of dilute nitric acid	Add 10 ml of dilute nitric acid
Dilute to 50 ml with distilled water	Dilute to 50 ml with distilled water
Add 1 ml of 0.1 M silver nitrate	Add 1 ml of 0.1 M silver nitrate
Stir immediately with a glass rod and keep aside for 5 minutes protected from light	Stir immediately with a glass rod and keep aside for 5 minutes protected from light
Observe the opalescence/turbidity	Observe the opalescence/turbidity

Comparison of Opalescence

Both the Nessler cylinder viewed transversely against a black background for comparison of opalescence (Fig. 1.2).

Observation: The opalescence produced in test solution should not be greater than standard solution. If opalescence produces in test solution is less than the standard solution, the sample will pass the limit test of chloride and *vice versa*.

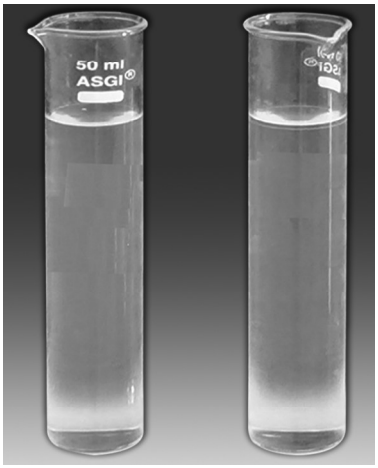


Fig. 1.2: Comparison of limit test for chloride

Reason for adding nitric acid:

- It extracts a common ion effect by furnishing nitrate ions and thereby suppression of dissociation of silver chloride.
- Dilute nitric acid is used to dissolve other impurities if present and helps silver chloride precipitate to make solution turbid at the end of process.

Precautions:

- Distilled water must be used because chloride present in the tap water will interfere the result
- Same glass rod should not be used because it will affect your observation.
- Silver nitrate is photosensitive store it in amber color bottle.

Limit Test for Sulfate

Principle

Limit test of sulfate is based on the reaction of soluble sulfate with barium chloride in presence of dilute hydrochloric acid to form barium sulfate which appears as solid particles (turbidity) in the solution.

$$SO_4^- + BaCl_2 \rightarrow BaSO_4\downarrow + 2Cl^-$$

A comparison is made of the turbid solution so obtained with the standard turbidity containing a known amount of sulfate ions.

Preparation of Solutions

Barium chloride solution (25% w/v): It is prepared by dissolving 25 gm of barium chloride in sufficient quantity of water and volume was adjusted to 100 ml.

Acetic acid solution (5M): 5M acetic acid solution prepared by diluting 185 ml of glacial acetic acid with sufficient water to produce 1000 ml.

Ethanolic sulfate standard solution (10 ppm): Dilute 1 volume of 0.181% w/v solution of potassium sulfate in ethanol (30%) to 100 volume with ethanol (30%).

Sulfate standard solution (10 ppm SO_4): Dilute 1 volume of a 0.181% w/v solution of potassium sulfate in distilled water to 100 volumes with the same solvent.

Procedure

Test solution	*Standard solution*
To 1 ml of a 25% w/v solution of barium chloride chloride in a Nessler cylinder add 1.5 ml of ethanolic sulfate standard solution (10 ppm SO_4), mix and allow to stand for 1 minute	To 1 ml of a 25% w/v solution of barium chloride in a Nessler cylinder, add 1.5 ml of ethanolic sulfate standard solution (10 ppm SO_4), mix and allow to stand for 1 minute
Dissolve the given sample in 15 ml of water and 0.15 ml of 5M acetic acid	Add 15 ml of sulfate standard solution (10 ppm SO_4) and 0.15 ml of 5M acetic acid
Add sufficient water to produce 50 ml	Add sufficient water to produce 50 ml
Stir immediately with a glass rod and keep aside for 5 minutes	Stir immediately with a glass rod and keep aside for 5 minutes.
Observe the opalescence/turbidity	Observe the opalescence/turbidity

Observation: The opalescence/turbidity produced in test solution should not be greater than standard solution. If opalescence/turbidity produces in test solution is less than the standard solution, the sample will pass the limit test of chloride and *vice versa*.

Reason for adding:

- Hydrochloric acid helps to make solution acidic
- Potassium sulfate is used to increase the sensitivity of the test by giving ionic concentration in the reagent.

Modified Limit Test for Chloride

In modified limit test for chloride, as $KMnO_4$ gives purple color in aqueous solution that interferes in the comparison of opalescence and turbidity, so the aqueous solution first be decolorized. $KMnO_4$ is an oxidizing agent and ethanol is reducing agent. When $KMnO_4$ is treated with ethanol in presence of heat, redox reaction will takes place which reduces $KMnO_4$ to manganese dioxide (precipitate) and the filtrate is colorless to proceed the limit chloride.

Method: Weighed amount of test substance after treated with suitable reducing agent dissolve it in water. Transfer the solution to Nessler cylinder and add 10 ml dilute of nitric acid, except when nitric acid is used in the preparation of solution and make up the volume to 50 ml with water. Then add 0.1 ml of silver nitrate, mix well and allow it stand for 5 minutes protected from light. On viewing transversely against a black background, any opalescence produced in the test solution should not be greater than that formed by treating a mixture of 10 ml of standard chloride solution (25 ppm Cl) and 5 ml of water in the similar manner.

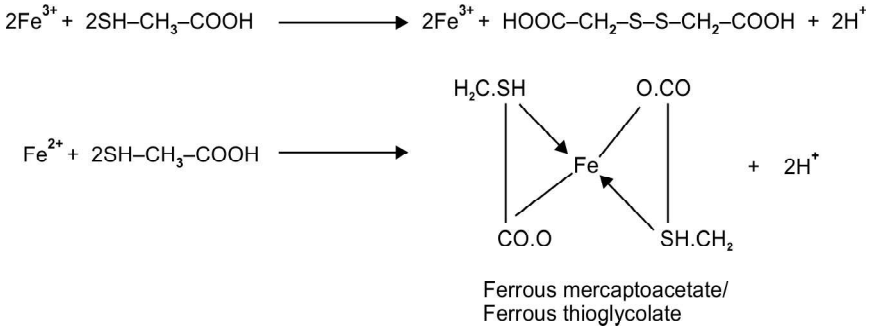
Modified Limit Test for Sulfate

Method: Take 1 ml of 25% w/v barium chloride solution in a Nessler cylinder; add 1.5 ml of standard ethanolic sulfate solution (10 ppm), mix well and allowed to stand for 1 minute. Then add 15 ml of the test solution prepared as specified in monograph and 0.15 ml of 5 M acetic acid after treatment with suitable reducing agent. Dilute the solution up to the mark (50 ml) with water, stir well immediately with a glass rod and allowed to stand for 5 minutes. On viewing transversely against a black background, any opalescence produced in the test solution should not be greater than that formed by treating a mixture of 15 ml of standard sulfate solution (10 ppm SO_4) in the similar manner.

Limit Test for Iron

Principle

Limit test of iron is based on the reaction of iron impurities with thioglycolic acid to form ferrous thioglycolate which produce purple color in the solution.



A comparison is made of the color solution so obtained with the standard color containing a known amount of iron.

Preparation of Solutions

Iron-free citric acid solution (20% w/v): It is prepared by dissolving 20 gm of iron free citric acid in sufficient quantity of water and volume was adjusted to 100 ml.

Iron-free ammonia solution: Contains approximately 10% w/w of NH_3. Dilute 425 ml of strong ammonia solution to 1000 ml.

0.05 M sulfuric acid: It is prepared by careful adding 2.7 ml sulfuric acid to equal volume of water and further diluting 1000 ml with water.

Iron standard solution (20 ppm Fe): Dilute 1 volume of a 0.1726% w/v solution of ferric ammonium sulfate in 0.05 M sulfuric acid to 10 volumes with water. Contains iron in ferric state.

Procedure

Test solution	*Standard solution*
Dissolve the specified quantity of the substance in water and then volume is made up to 40 ml	Take 2 ml of iron standard solution (20 ppm Fe) diluted with water up to 40 ml
Add 2 ml of a 20% w/v solution of citric acid (iron-free)	Add 2 ml of a 20 % w/v solution of citric acid (iron-free)

(Contd.)

Test solution	*Standard solution*
Add 0.1 ml of thioglycolic acid	Add 0.1 ml of thioglycolic acid
Add ammonia to make the solution alkaline	Add ammonia to make the solution alkaline
Adjust the volume to 50 ml	Adjust the volume to 50 ml
Keep aside for 5 minutes	Keep aside for 5 minutes
Color developed is viewed vertically and compared with standard solution	Color developed is viewed vertically and compared with standard solution

Observation: The purple color produce in sample solution should not be greater than standard solution. If purple color produces in sample solution is less than the standard solution, the sample will pass the limit test of iron and *vice versa*.

Reason for adding:

- Citric acid (iron free) is used to complex metal cations other than iron if present
- Thioglycolic acid helps to oxidize iron (II) to iron (III)
- Ammonia to make solution alkaline.

Precautions

- Distilled water must be used during preparation of all the solution if required.
- Same glass rod should not be used because it will affect your observation.
- Iron free ammonia and citric acid are used during preparation of reagents.

Limit Test for Heavy Metals

Principle

Limit test of heavy metals is based on the reaction of heavy metals impurities with saturated solution of hydrogen sulfide to forms sulfides, which produce color (brownish) in the solution. A comparison is made of the color solution so obtained with the standard color (reaction of known amount of lead with saturated solution of hydrogen sulfide).

$$\text{Heavy metal} + H_2S/Na_2S \rightarrow \underset{\text{(Brownish color)}}{\text{Heavy metals sulfides}} + 2H^+$$

Metals that response to this test are lead, mercury, bismuth, arsenic, antimony, tin, cadmium, silver, copper, and molybdenum. The metallic impurities in substances are expressed as parts of lead per million parts of the substance. The usual limit as per Indian Pharmacopoeia is 20 ppm.

Preparation of Solutions

Dilute acetic acid solution (approx. 6% w/w): It is prepared by diluting 57 ml of glacial acetic acid to 1000 ml with water.

Dilute ammonia solution (approx. 10% w/w): It is prepared by diluting 425 ml of strong ammonia solution to 1000 ml with water.

Lead standard solution (0.1% Pb): Dissolve 0.400 gm of lead nitrate in water containing 2 ml nitric acid and add sufficient quantity of water to produce 250 ml.

Lead standard solution (100 ppm): Dilute 1 volume lead standard solution (0.1 % Pb) to 10 volumes with water.

Lead standard solution (20 ppm): Dilute 1 volume lead standard solution (100 ppm Pb) to 5 volumes with water.

Procedure

Indian Pharmacopoeia provided four methods depending on resulting solution substance (i.e. based on solubility, color, etc.)

Method A: It is used for the substance which gives clear colorless solution under the specific condition.

Test solution	*Standard solution*
Solution is prepared as per the monograph and 25 ml is transferred in Nessler's cylinder	Take 2 ml of standard lead solution (20 ppm Pb) and dilute to 25 ml with water
Adjust the pH between 3 to 4 by adding dilute acetic acid or dilute ammonia solution	Adjust the pH between 3 to 4 by adding dilute acetic acid or dilute ammonia solution
Dilute with water to 35 ml	Dilute with water to 35 ml
Add freshly prepared 10 ml of hydrogen sulfide solution	Add freshly prepared 10 ml of hydrogen sulfide solution
Dilute with water to 50 ml	Dilute with water to 50 ml
Allow to stand for 5 minutes	Allow to stand for 5 minutes
View downwards over a white surface	View downwards over a white surface

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

Method B: It is used for the substance which does not give clear colorless solution under the specific condition. In this method hydrogen sulfide is used after igniting the substance.

Test solution	*Standard solution*
Weigh in a suitable crucible the quantity of the substance specified in the individual monograph, add sufficient sulfuric acid to wet the sample, ignite carefully at a low temperature until thoroughly charred. Add to the charred mass 2 ml of nitric acid and 5 drops of sulfuric acid and heat cautiously until white fumes are no longer evolved. Ignite, preferably in a muffle furnace at 500 to 600°C, until the carbon is completely burnt off. Cool and add 4 ml of hydrochloric acid cover, digest on a water bath for 15 minutes, uncover and slowly 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	Take 2 ml of standard lead solution (20 ppm Pb) and dilute to 25 ml with water
Add ammonia solution dropwise until the solution is just alkaline to litmus paper, dilute to 25 ml with water and adjust with dilute acetic acid to a pH between 3.0 and 4.0. Filter, if necessary dilute with water to 35 ml	Adjust the pH between 3 to 4 by adding dilute acetic acid or dilute ammonia solution Dilute with water to 35 ml
Add freshly prepared 10 ml of hydrogen sulfide solution	Add freshly prepared 10 ml of hydrogen sulfide solution
Dilute with water to 50 ml	Dilute with water to 50 ml
Allow to stand for 5 minutes	Allow to stand for 5 minutes
View downwards over a white surface	View downwards over a white surface

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

Method C: Use for the substance which gives clear colorless solution and used sodium sulfide solution after treating the substance with sodium hydroxide solution.

Test solution	*Standard solution*
Dissolve the specified quantity of the substance under examination in a mixture of 20 ml of water and add 5 ml of dilute sodium hydroxide solution	Take 2 ml of standard lead solution (20 ppm Pb)
Make up the volume to 50 ml with water	Add 5 ml of dilute sodium hydroxide solution and make up the volume to 50 ml and mix
Add 5 drops of sodium sulfide solution	Add 5 drops of sodium sulfide solution
Mix and allow to stand for 5 minutes	Mix and allow to stand for 5 minutes
View downwards over a white surface	View downwards over a white surface

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

Method D:

Test solution	*Standard solution*
Prepare as directed in the individual monograph and pipette 12 ml into a small Nessler cylinder	Pipette 10.0 ml of either lead standard solution (1 ppm Pb) or lead standard solution (2 ppm Pb) and add 2.0 ml of the test solution
Add 2 ml of acetate buffer pH 3.5	Add 2 ml of acetate buffer pH 3.5
Add 1.2 ml of thioacetamide reagent	Add 1.2 ml of thioacetamide reagent
Allow to stand for 2 minutes	Allow to stand for 2 minutes
View downwards over a white surface	View downwards over a white surface

Observation: The color produce in test solution is not more intense than standard solution. If color produces in test solution is less than the standard solution, the sample will pass the limit test of heavy metals and *vice versa*.

Reasons for adding: Dilute acetic acid and ammonia solution is added to maintain the pH between 3.0 to 4.0 so that precipitate formed is colloidal and uniform.

Precautions

- Distilled water must be used during preparation of all the solution if required.
- Same glass rod should not be used because it will affect your observation.

Limit Test for Arsenic

Principle

The principle is based on converting any arsenic impurity present in the sample to arsine gas by a series of reaction. The arsine gas is made to come in contact with mercuric chloride test paper when by it produces a yellow or brown stain due to the formation of mercuric arsenide. It is also called Gutzeit test and requires special apparatus.

The arsenic impurity is converted in acidic medium into arsenious acid or arsenic acid depending upon the valency state of arsenic.

$$\underset{\text{Pentavalent}}{As^{5+}} \longrightarrow \underset{\text{Arsenic acid}}{O{=}As(OH)_3 \text{ or } H_3AsO_4}$$

$$\underset{\text{Trivalent}}{As^{3+}} \longrightarrow \underset{\text{Arsenious acid}}{As(OH)_3 \text{ or } H_3AsO_3}$$

Any arsenic acid formed is converted into arsenious acid by reduction with stannous chloride and hydrochloric acid

$$\underset{\text{Arsenic acid}}{H_3AsO_4} \xrightarrow{SnCl_2/HCl} \underset{\text{Arsenious acid}}{As(OH)_3 \text{ or } H_3AsO_3}$$

The arsenic acid is further reduced to arsine gas with the help of nacent hydrogen obtained in the reaction between zinc and hydrochloride acid

$$\underset{\text{Arsenious acid}}{H_3AsO_3} \xrightarrow{Zn/HCl} \underset{\text{Arsine}}{AsH_3} + 3H_2O$$

Arsenic gas reacts with mercuric choride test paper to produce yellow to brown stain due to formation of mercuric arsenide

$$\underset{\text{Arsine}}{AsH_3} + HgCl_2 \longrightarrow \underset{\text{Mercuric arsenide}}{Hg(AsH_2)_2} + 2HCl$$

The stain (yellow or brown) produce by the sample is compare to a standard stain produced by standard.

Preparation of Solutions

Potassium iodide, 1 M: Dissolve 166 g of potassium iodide in sufficient water to produce 1000 ml.

Sodium hydroxide, 2 M: Dissolve 80 g of sodium hydroxide in sufficient water to produce 1000 ml.

Arsenic standard 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.

Lead acetate solution: A 10% w/v solution of lead acetate in carbon dioxide-free water.

Stannous chloride solution: May 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 produce 1000 ml.
2. Dilute 60 ml of hydrochloric acid with 20 ml of water, add 20 g of tin, heat gently until no more gas is evolved and add sufficient water to produce 100 ml. Store over a little of the undissolved tin remaining in the solution and protected from air.

Procedure

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

To this solution 1 gm of KI, 5 ml of stannous chloride acid solution and 10 gm of zinc is added (all this reagents must be arsenic-free). Keep the solution aside for 40 min and stain obtained on mercuric chloride paper is compared with standard solution.

Standard solution: Transfer 1 ml of arsenic standard solution (10 ppm As) diluted to 50 ml with water. Add 10 ml of stanneted hydrochloric acid, 5 ml of 1 M potassium iodide and 10 g of zinc AsT. Immediately assemble the apparatus and immerse the

flask in a water bath at a temperature such that a uniform evolution of gas is maintained. Keep aside for 40 minutes observe the stain produced on the mercuric chloride paper.

Observation: Stain produced by test sample is not more intense than that obtained by standard sample or equals to the standard one, passes the limit test. If stain produced by test sample is more intense than that obtained by standard sample which fails the limit test for arsenic as per IP.

Arsenic apparatus: This apparatus details are as follows:

- It 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 × 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.
- A second glass tube of the same internal diameter and 30 mm long is placed in contact with the first and held in position by two spiral springs or clips.
- Into the lower tube insert 50 to 60 mg of *lead acetate cotton, loosely packed*.
- 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.

The arsenic apparatus is shown in Fig. 1.3.

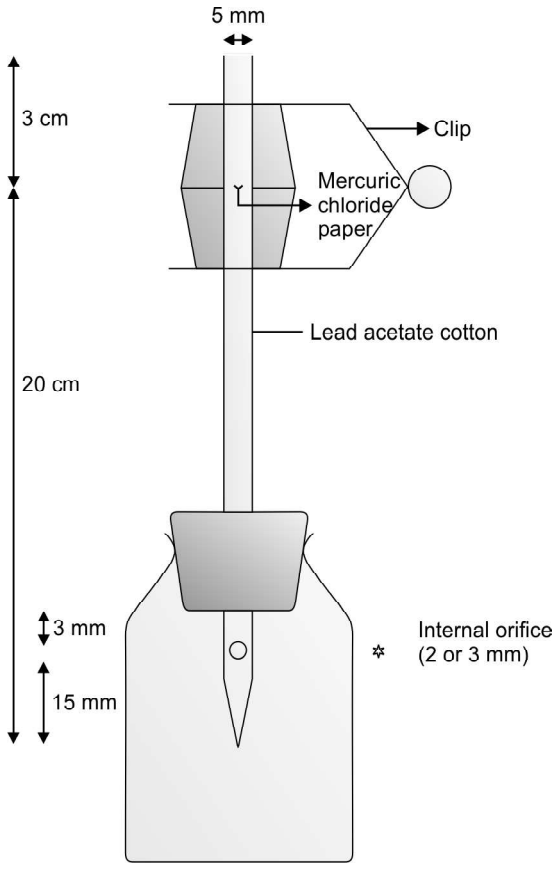


Fig. 1.3: Arsenic apparatus

Reason for adding:

- Lead acetate papers are used to trap any hydrogen sulphide which may be evolved together with arsine
- Stannous chloride is used for complete evolution of arsine
- HCl is used to make the solution acidic
- Zinc, potassium iodide and stannous chloride is used as a reducing agent.

Precautions

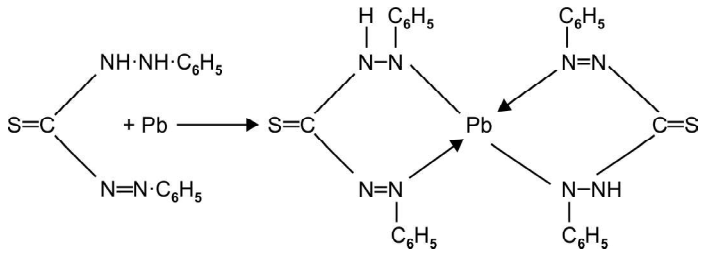
- The most suitable temperature for carry out this test is 40°C.
- Care must be taken that the filter paper remains quite dry during the reaction.

- During the succeeding tests the tube must be washed with HCl AsT rinsed with water and dried.
- All the reagents used for this test should be free from arsenic and mentioned as AsT.

Limit Test for Lead

Principle

It is based on the violet color produced in chloroform due to the reaction between lead impurity and dithizone (diphenyl thiocarbazone) which results in the formation of lead dithizonate. The intensity of final violet color produced in the chloroform medium is compared with standard.



Lead dithizonate complex

Preparation of Solutions

Preparation of standard lead solution (1 ppm Pb): Dissolve 0.4 g of lead nitrate in water containing 2 ml of dilute nitric acid and add sufficient water to produce 250 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.

Procedure

Test solution	*Standard solution*
A known quantity of sample solution is transferred in a separating funnel	A standard lead solution is prepared equivalent to the amount of lead permitted in the sample under examination
Add 6 ml of ammonium citrate	Add 6 ml of ammonium citrate
Add 2 ml of potassium cyanide and 2 ml of hydroxylamine hydrochloride	Add 2 ml of potassium cyanide and 2 ml of hydroxylamine hydrochloride
Add 2 drops of phenol red	Add 2 drops of phenol red
Make solution alkaline by adding ammonia solution	Make solution alkaline by adding ammonia solution
Extract with 5 ml of dithizone until it becomes green	Extract with 5 ml of dithizone until it becomes green

(Contd.)

Test solution	*Standard solution*
Combine dithizone extracts are shaken for 30 mins with 30 ml of nitric acid and the chloroform layer is discarded	Combine dithizone extracts are shaken for 30 mins with 30 ml of nitric acid and the chloroform layer is discarded
To the acid solution add 5 ml of standard dithizone solution	To the acid solution add 5 ml of standard dithizone solution
Add 4 ml of ammonium cyanide	Add 4 ml of ammonium cyanide
Shake for 30 mins	Shake for 30 mins
Observe the color	Observe the color

Comparison of stain: Compare the voilet color of the chloroform layer.

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

Reason for adding:

- 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.
- Lead present as an impurities in the substance, gets separated by extracting an alkaline solution with a dithizone extraction solution.
- Phenol red is used as indicator to develop the color at the end of process.

Precautions

- 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.

IMPORTANT QUESTIONS/ANSWERS

I. Multiple Choice Questions

1. Indian Pharmacopoeia is published by:
   a. Indian Pharma Commission
   b. Indian Pharmacopoeia Commission
   c. Indian Patent Commission
   d. None of the above
2. Impurities may be present in pharmaceutical substances because of:
   a. Raw material
   b. Chemical instability
   c. Manufacturing process
   d. All of the above
3. Why HCl is used in the limit test of sulfate?
   a. Forms precipitate
   b. Clear the solution
   c. Remove the impurities of sulfate
   d. All of the above
4. Limit test is performed in:
   a. Round bottom flask
   b. Nessler cylinder
   c. Volumetric flask
   d. Conical flask
5. ..... is also called Gutzeit test.
   a. Limit test of sulfate
   b. Limit test of chloride
   c. Limit test of lead
   d. Limit test of arsenic

6. In pharmacopeia, directions and specifications of drugs mentioned which are intended for medicinal use is known as:
   a. Test for impurity
   b. Monograph
   c. Limit test
   d. Quality parameters
7. Indian Pharmacopoeia update frequency of addendum is once in every ..... years.
   a. Two
   b. Four
   c. Three
   d. Five
8. Which reagent is added to prevent supersaturation of barium sulfate in the limit test of sulfate?
   a. Ethanol
   b. HCl
   c. Methanol
   d. Butanol
9. In limit test of heavy metals, the metal sulfides formed are in ..... color.
   a. Orange
   b. Yellow
   c. Brownish
   d. Green
10. Limit test for iron, formation of ..... produce purple color.
    a. Citric acid
    b. Thioglycolic acid
    c. Arsine
    d. Nitric acid
11. Limit test for lead is based on the reaction between lead and ..... in an alkaline medium.
    a. Diphenylamine
    b. Aluminum chloride
    c. Methanol
    d. Diphenylthiocarbazone
12. The limit test for lead is based upon reaction of lead with ..... in an alkaline media.
    a. Ethanol
    b. HCl
    c. Methanol
    d. Butanol

Answers

1. b 2. d 3. c 4. b 5. d 6. b 7. b 8. a 9. c 10. b 11. d 12. a

II. Fill in the Blanks

1. The standard and test solution used for limit test are prepared in .....
2. The limit test for arsenic is based upon ..... test.
3. In limit test for arsenic ..... is converted into arsenous acid/arsine gas.
4. Arsine gas is carried and comes into contact with ..... in produces a yellow or brown stain.
5. The function of granulated Zn in limit test for arsenic is .....
6. Limit test for sulfate has been based upon the precipitate of sulfate with ..... in the presence of .....
7. In limit test for sulfate to prevent the supersaturation of $BaSO_4$ a small amount of ..... has been added in the reagent.
8. Limit test for iron is based upon reaction of Fe with ..... in presence of a ..... solution buffered with ammonium citrate.
9. Limit test for iron purple color is due to formation of .....
10. In limit test for iron ..... prevent the precipitate of iron as Fe(OH) solution.
11. In limit test for iron ferrous thioglycolate has stable pink to reddish purple colour in ..... medium.

12. Limit test is ..... test designed to identify and control small quantities of impurities.
13. Limit test for chloride has been based open reaction between ..... and ..... to obtain silver chloride.
14. Limit test for Pb has been based upon reaction between ..... and ..... to form complex.
15. Ppm is ..... and one ppm is .....

Answers

1. Distilled water
2. Gutzeit test
3. Arsenious acid
4. Mercuric chloride
5. Slow and prolonged evolution of nascent H_2 gas
6. Barium chloride, dilute hydrochloric acid
7. Alcohol
8. Thioglycolic acid, citric acid
9. Iron thioglycolate
10. Citric acid
11. Alkaline
12. Quantitative
13. Silver nitrate and soluble chloride
14. Lead and diphenyl thiocarbazone
15. Parts per million, 1 mg in 1 kg

III. Short Answer Questions

1. Define pharmaceutical inorganic chemistry.
2. Write a note on sources of impurities in pharmaceutical preparation.
3. Explain the importance of inorganic chemistry in pharmacy.
4. Write the salient features of recent edition of Indian Pharmacopoeia.
5. What is the role of citric acid, thioglycolic acid and ammonia in the limit test of iron?
6. What is the use of stannous chloride in limit test of arsenic?

IV. Long Answer Questions

1. Briefly explain the history of Indian Pharmacopoeia.
2. Discuss about various pharmacopeias.
3. Define the term monograph and explain with any one official drug.
4. Explain the development of pharmacopeias.
5. Discuss limit test of iron.
6. Explain the principle and procedure for the limit test of sulfate.
7. Discuss in detail about the apparatus used in limit test of arsenic.
8. Discuss the principle of limit test of sulfate, arsenic and chloride.
9. Give complete principle and method as per IP of limit test of lead.
10. Explain the limit test of heavy metals.