Program	:	B. Pharmacy
Semester	:	1st (First)
Subject /Course	:	Pharmaceutical Inorganic Chemistry (Theory) BP104T
Subject/Course ID	:	BP104 T
Module No.	:	1
Module Title	:	Impurities in Pharmaceutical Substances:
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Learning Outcome of Module-1

LO	Learning Outcome (LO)	Course Outcome Code
LO1	To know history regarding pharmacopoeia and its editions.	
LO2	To learn about the sources of impurities and methods/ procedures of limit test of impurities in inorganic pharmaceuticals.	

Module Content Table

No.	Topic
1	History of Pharmacopoeia
2	Sources and types of impurities
3	Principle involved in the limit test for Chloride
4	Principle involved in the limit test for Sulphate
5	Principle involved in the limit test for Iron
6	Principle involved in the limit test for Arsenic
7	Principle involved in the limit test for Lead and Heavy metals
8	Principle involved in the limit test for modified limit test for Chloride and Sulphate.

Contents: Impurities in pharmaceutical substances:

Impurity is any unwanted substance present in desired substance in less than or near to 5% of desired substance, e.g. a pinch of sodium chloride present in 100mg of sugar is known as impurity. Pharmaceutical substance is any required material including API (active pharmaceutical ingredient) for manufacturing of pharmaceutical dosage form. The limit of impurities present in pharmaceutical substance is listed in Pharmacopoeia and also method or procedure of assay related this impurity detection / identification is already written under each pharmaceutical substance monograph.

1. HISTORY OF PHARMACOPOEIA: PHARMACOPOEIA

The word Pharmacopoeia is derived from Greek words 'pharmakon' means a drug (both remedy and poison) and 'poiein' means to make or create. Pharmacopoeia 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. For this reason Pharmacopoeia 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.

Monograph

In simple way monographs are descriptions of pharmaceutical preparations. In broader way it is a reference work for pharmaceutical drug specifications. It is a complete description of a specific pharmaceutical, which includes chemical formulae, atomic and molecular weight, definition, statement of content, category, dose, usual strength, description, solubility, identification tests, assay, other test, limits of impurities, quantities, and conditions for storage. The appendices include standards for apparatus, reagents and solutions, indicators, reference substances, test animals, calculation of results, other chemicals techniques, processes etc. of the concerned pharmaceuticals.

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 Pharmacopoeia monographs are accurately organized based on the updated needs of today's pharmacist. In the form of new monographs the details are provided for new compounds and some of the previous monographs which are not in continued use are deleted. The overall effect is to provide an increase in the average of drugs with typographically 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 Pharmacopoeia. Example: Anti HIV agents. In Pharmacopoeia 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 anti-malarial drugs, anti-neoplastic agents, anti-parkinsonism drugs etc. are also included in Pharmacopoeia.

Based on the published information, Pharmacopoeia is divided in to three different major parts. 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 Pharmacopoeia. In related chapters to guide reader the cross references is used to find out the drug that may be of interest. The common actions of the groups of drugs are provided as background information in many of the chapters.

Part II: Monographs of new drugs, drugs under investigation, drugs which are not easily classified and obsolescent drugs still of interest are presented in part II of Pharmacopoeia. 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 are documented with omission of herbal medicine in part III of Pharmacopoeia.

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

In the Pharmacopoeia 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 any one of the Pharmacopoeias. 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 Pharmacopoeia

Each 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 Pharmacopoeia.

On 15th December 1820, the first United State Pharmacopoeia (U.S.P) was released. In 1864, the first British Pharmacopoeia (B.P) was published with inclusion of monographs on benzoic acid,

gallic acid, tartaric acid, tannic acid, camphor, lactose, sucrose and seven alkaloids along with their salts.

INDIAN PHARMACOPOEIA

British Pharmacopoeia was utilized as the official book of standards in India before independence. The actual process of publishing the first Indian Pharmacopoeia started in the year 1944 under the chairmanship of Col. R. N. Chopra. The Indian Pharmacopoeia list was first 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 Indian Pharmacopoeia is published in fulfillment of the requirements of the Drugs and Cosmetics act, 1940 and rules there under.

The drugs and cosmetics act 1940 stated that the Indian Pharmacopoeia is the book of standards for drugs included therein and the standards as included in the Indian Pharmacopoeia 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.

Government of India, Ministry of Health and Family welfare publishes Indian

Pharmacopoeia based on the recommendation of Indian Pharmacopoeia committee (in accordance with Drugs and Cosmetics Acts 1940, Dangerous Drugs Act 1930, and Poisons

Act 1919 and the rules framed there under). In general, 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.

After independence, the first edition of the Indian Pharmacopoeia (I.P) was published in the year 1955 under the chairmanship of Dr. B. N. Ghosh. Supplement for first edition of

Indian Pharmacopoeia was published in the year 1960. This Pharmacopoeia contained both western and traditional system drugs commonly used in India. The same policy was continued while preparing the Indian Pharmacopoeia 1966. 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. The third edition of the Indian

Pharmacopoeia got published in 1985 under the chairmanship of Dr. Nityanand.

Addendum/supplement I and II to third edition has been published in 1989 and 1991 respectively. 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.

In view of the continuing rapid increase in the range of drugs produced in India eleven year later the fourth edition of the Indian Pharmacopoeia was published under the chairmanship Dr. Nityanand in 1996. Addendum to fourth edition has been published initially in 2000 followed by in 2002 and 2005. In addition, supplement 2000 for veterinary products are also released. The addendum 2005 was published by the Indian Pharmacopoeia

Committee which included a large number of antiretroviral drugs, and raw plants commonly used in making medicinal products not covered by any other Pharmacopoeias and attracted much global attention. The Indian Pharmacopoeia Committee decide to delete the obsolete or less used product monographs and added monographs based on the therapeutic merit, medicinal need and extent of use of such articles in the country.

The Indian Pharmacopoeia Commission (IPC) has been established in the year 2005. 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 programs and the national essential medicines. It contained monographs on antiretroviral, anticancer, anti-tubercular and herbal drugs. It further 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 Indian

Pharmacopoeia 2007 was published which had taken care of the amendments to Indian Pharmacopoeia 2007 and also incorporated 72 new monographs.

The sixth edition of Indian Pharmacopoeia 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, besides following conventional approach of obtaining comments. The feedback and inputs were reviewed by the relevant expert committee to ensure the feasibility and practicability of the standards and methods revised. The principle of openness, justice and fairness is kept in mind during compiling and editing the contents of this edition.

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 standards prescribed in this edition are encouraged to adhere with the concept of harmonization, keeping in view the technological status for manufacture and analysis of drugs and pharmaceuticals in the country without compromising with the quality of the products. It strives to update the existing monographs as well as incorporating the new monographs of drug substances based on clinical use of medicines in India and improving their test protocols.

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.

In addition emphasis has been put to bring out harmonization in appendices to establish a sound connection between individual monographs and the relevant appendices, so as to make this edition precise and well structured. Number of monographs and appendices are expanded further to incorporate the latest technological advancement and regulatory compliance. Constant efforts have been made to unify the national drug standards and to bring them in line with the international standards progressively, by addition of monographs of new drugs and adopting current methodology.

Indian Pharmacopoeia (I.P.):

The actual process of publishing the first Pharmacopoeia started in the year 1944 under the chairmanship of Col. R. N. Chopra. The I. P. list was first published in the year 1946 and was put forth for approval. The titles are suffixed with the respective years of publication, e.g. IP 1996.

The following table describes the publication history of the Indian Pharmacopoeia

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

4th Edition	**1996**	Addendum 2000
		Vet Supplement 2000
		Addendum 2002
		Addendum 2005
5th Edition	**2007**	Addendum 2008
6th Edition	**2010**	Addendum 2012
7th Edition	**2014**	Addendum 2015
		Addendum 2016
8th Edition	**2018**	Addendum 2019
9th Edition	**2022**	

PHARMACEUTICAL CHEMISTRY:

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.

Inorganic Chemistry

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. Branches of inorganic chemistry include coordination chemistry, bioinorganic chemistry, organometallic compounds and synthetic inorganic chemistry. 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. In short, Inorganic chemistry is the branch of chemistry that deals with inorganic compounds. In other words, it is the chemistry of compounds that do not contain hydrocarbon radicals.

Inorganic Compounds

These are traditionally viewed as compounds being synthesized by the geological systems and lack hydrocarbon (carbon-hydrogen). In contrast, organic compounds are those found in biological systems. In general organic chemists say any molecule containing carbon as an organic compound and hence this means that inorganic chemistry deals with the compounds or molecules which lack carbon atom. Berzelius, the 19th century chemist, described inorganic compounds as inanimate. The first important synthetic inorganic compound was ammonium nitrate for soil fertilization. Inorganic compounds are found in nature as minerals. Soil contain iron sulfide as pyrite or calcium sulfate as gypsum. They are also found multitasking as biomolecules: As electrolytes (sodium chloride), in energy storage (ATP) or in construction (the polyphosphate backbone in DNA).

Inorganic compounds are synthesized for use as drugs such as cisplatin, magnesium hydroxide, catalysts such as vanadium (V) oxide and titanium (III) chloride, or as reagents in organic chemistry such as lithium aluminium hydride.

Medicinally useful substances are derived from either organic or inorganic sources.

Naturally obtained compounds attracted the attention of humans always, 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, still they are used in medicine cautiously. Some of them are replaced by the organic medicines.

Study of pharmaceutical applications of the inorganic compounds led to the establishment of a new avenue called Pharmaceutical inorganic chemistry, which deals with the study of both non-essential and essential elements about their preparation, standards of purity, test for identification, limit tests to be performed for determining the quality and extent of purity, storage, different formulations and their storage conditions and therapeutic uses.

The term ‘Pharmaceutical’ is used for any chemical substance useful in preventive or therapeutic or which finds use in the preparation of medicament. Some find use only in the laboratory during the preparation but may not be present in the final product, these are also incorporated under pharmaceuticals. Quality of all these pharmaceuticals must be carefully controlled. For this reason specifications of quality are mentioned for each pharmaceutical.

These descriptions are reported in the pharmacopoeia.

Importance of Inorganic Pharmaceuticals

Inorganic pharmaceuticals are useful in any of the following ways.

1. Useful medicinally for their therapeutic purpose. Example: Astringents and antimicrobials etc.

2. Useful as pharmaceutical aids. Example: Bentonite, talc etc.

3. To change the reaction of body fluid. To acidify or alkalise. Example: Antacids, alkalis, mineral acids.

4. Replacing or replenishing the normal content of body fluids. Example: Sodium, potassium, calcium, chloride, phosphate etc.

5. Useful as reagents to carry out the reactions. Example: Catalysts (platinum, nickel) oxidizing and reducing agents (lithium aluminium hydride).

6. Useful in Pharmaceutical analysis. Example: Titrants such as potassium permanganate etc.

2. SOURCES OF IMPURITIES:

The various sources of impurity in pharmaceutical products are — reagents, heavy metals, ligands, catalysts, other materials like filter aids, charcoal, and the like, degraded end products obtained during \ after manufacturing of bulk drugs from hydrolysis, photolytic cleavage, oxidative degradation, decarboxylation, enantiomeric impurity, and so on.

1. Impurities originating from drug substance synthetic processes
2. Starting materials and intermediates
3. Impurities in the starting materials
4. Reagents, ligands and catalysts
5. By-products of the synthesis
6. Products of over-reaction
7. Products of side reactions
8. Impurities originating from degradation of the drug substance.
9. Enantiomeric impurities: Most therapeutic chiral drugs used as pure enantiomers is natural products. The high level of enantio selectivity of their biosynthesis excludes the possibility of the presence of enantiomeric impurities.

In the case of synthetic chiral drugs, the racemates which are usually marketed, if the pure enantiomer is administered, the antipode is an impurity. The reason for its presence can be either the incomplete enantio selectivity of the syntheses or incomplete resolution of the enantiomers of the racemate.

Methods to identify impurity in pharmaceutical substance:

Various methods are used to isolate and characterize impurities in pharmaceuticals, such as,

1. Capillary Electrophoresis,
2. Electron Paramagnetic Resonance,
3. Gas–Liquid Chromatography,
4. Gravimetric Analysis,
5. High Performance Liquid Chromatography,
6. Solid-Phase Extraction Methods,
7. Liquid–Liquid Extraction Method,
8. Ultraviolet Spectrometry,
9. Infrared Spectroscopy,
10. Supercritical Fluid Extraction
11. Column Chromatography,
12. Mass Spectrometry,
13. Nuclear Magnetic Resonance (Nmr) Spectroscopy,
14. Raman Spectroscopy.

Among all hyphenated techniques, the most exploited techniques for impurity profiling of drugs are Liquid Chromatography (LC)-Mass Spectroscopy (MS), LC-NMR, LC-NMR-MS, GC-MS, and LC-MS. This reveals the need and scope of impurity profiling of drugs in pharmaceutical research.

Methods to control impurity in pharmaceutical substance:

1. Sublimation
2. Drying

3. Crystallization
4. Evaporation

Effects of impurity in pharmaceutical substance:

1. Impurities may bring about incompatibility with other substances.
2. Impurities may lower the shelf life of the substances.
3. Impurities may cause difficulties during formulations and use of the substances.
4. Sometimes Impurities changes the physical and chemical properties of the substances.
5. Therapeutic effect can be decreased.
6. Shows toxic effect after a certain period.
7. Injurious when present above certain limits.
8. It may change odour, colour, taste of the substance

Limit test:

Limit test is defined as quantitative or semi quantitative **test** designed to identify and control small quantities of impurity which is likely to be present in the substance. **Limit test** is generally carried out to determine the inorganic impurities present in compound.

In Chemistry, Limit means a value or amount that is likely to be present in a substance and test means to examine or to investigate. Thus, limit test is nothing but to identify the impurities in the substance and compare it with standard. In general, limit test is defined as quantitative or semi quantitative test designed to identify and control small quantities of impurity which is likely to be present in the substance.

Limit test is generally carried out to determine the inorganic impurities present in compound. Limit test of chloride is based on the reaction of soluble chloride with silver nitrate in presence of dilute nitric acid to form silver chloride, which appears as solid particles (Opalescence) in the solution.

Limit test of sulphate is based on the reaction of soluble sulphate with barium chloride in presence of alcohol and potassium sulphate to form barium sulphate, which appears as solid particles (turbidity) in the solution. Here alcohol is added to prevent super saturation.

Limit test of heavy metals is based on the reaction of metallic impurities with hydrogen sulfide in acidic medium to form colored solution. Metals that response to this test are lead, mercury, bismuth, arsenic, antimony, tin, cadmium, silver, copper, and molybdenum.

Limit test of lead is based on the reaction of lead and diphenyl thiocabazone (dithizone) in alkaline solution to form lead dithizone complex which is read in color.

Limit test of Iron is based on the reaction of iron in ammonical solution with thioglycollic acid to form iron thioglycolate which is pink-reddish purple in color.

Limit test of Arsenic is based on the reaction of arsenic gas with hydrogen ion to form yellow stain on mercuric chloride paper in presence of reducing agents like potassium iodide. It is also called as Gutzeit test and requires special apparatus.

VARIOUS LIMIT TEST

3. Limit test for Chlorides

Principle:

Limit test of chloride is based on the reaction of soluble chloride with silver nitrate in presence of dilute nitric acid to form silver chloride, which appears as solid particles (Opalescence) in the solution.

Procedure:

TABLE :1

Test sample	**Standard compound**
Specific weight of compound is dissolved in water or solution is prepared as directed in the pharmacopoeia and transferred in Nessler's cylinder	Take 1ml of 0.05845 % W/V solution of sodium chloride in Nessler's cylinder
Add 1ml of nitric acid	Add 1ml of nitric acid
Dilute to 50ml in Nessler's cylinder	Dilute to 50ml in Nessler's cylinder
Add 1ml of $AgNO_3$ solution	Add 1ml of $AgNO_3$ solution
Keep aside for 5 min	Keep aside for 5 min
Observe the Opalescence/Turbidity	Observe the Opalescence/Turbidity

Observation:

The opalescence produce in sample solution should not be greater than standard solution. If opalescence produces in sample solution is less than the standard solution, the sample will pass the limit test of chloride and visa versa.

Reasons:

Nitric acid is added in the limit test of chloride to make solution acidic and helps silver chloride precipitate to make solution turbid at the end of process.[3-5]

Limit test for Sulphates

Principle:

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

Procedure:

TABLE :2

Test sample	Standard compound
Specific weight of compound is dissolved in water or solution is prepared as directed in the pharmacopoeia and transferred in Nessler cylinder	Take 1ml of 0.1089 % W/V solution of potassium sulphate in Nessler cylinder
Add 2ml of dilute hydrochloric acid	Add 2ml of dilute hydrochloric acid
Dilute to 45 ml in Nessler cylinder	Dilute to 45 ml in Nessler cylinder
Add 5ml of barium sulphate reagent	Add 5ml of barium sulphate reagent
Keep aside for 5 min	Keep aside for 5 min
Observe the Turbidity	Observe the Turbidity

Barium sulphate reagent contains barium chloride, sulphate free alcohol and small amount of potassium sulphate.

Observation:

The turbidity produce in sample solution should not be greater than standard solution. If turbidity produces in sample solution is less than the standard solution, the sample will pass the limit test of sulphate and vice versa.

Reasons:

Hydrochloric acid helps to make solution acidic. Potassium sulphate is used to increase the sensitivity of the test by giving ionic concentration in the reagent Alcohol helps to prevent super saturation.

Limit test for Iron

Principle:

Limit test of Iron is based on the reaction of iron in ammonical solution with thioglycollic acid in presence of citric acid to form iron thioglycolate which is pale pink to deep reddish purple in color.

Procedure:

TABLE :3

Test sample	Standard compound
Sample is dissolved in specific amount of water and then volume is made up to 40 ml	2 ml of standard solution of iron diluted with water upto 40ml
Add 2 ml of 20 % w/v of citric acid (iron free)	Add 2 ml of 20 % w/v of citric acid (iron free)
Add 2 drops of thioglycollic acid	Add 2 drops of thioglycollic acid
Add ammonia to make the solution alkaline and adjust the volume to 50 ml	Add ammonia to make the solution alkaline and adjust the volume to 50 ml
Keep aside for 5 min	Keep aside for 5 min
Color developed is viewed vertically and compared with standard solution	Color developed is viewed vertically and compared with standard solution

Earlier ammonium thiocyanate reagent was used for the limit test of iron. Since thioglycolic acid is more sensitive reagent, it has replaced ammonium thiocyanate in the test.

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.

Reasons:

Citric acid helps precipitation of iron by ammonia by forming a complex with it.

Thioglycolic acid helps to oxidize iron (II) to iron (III).

Ammonia is to make solution alkaline.

Limit test for Heavy Metals

Principle:

Limit test of heavy metals is based on the reaction of metallic impurities with hydrogen sulfide in acidic medium to form brownish colour solution. Metals that response to this test are lead, mercury, bismuth, arsenic, antimony, tin, cadmium, silver, copper, and molybdenum. The metallic impurities in substances are expressed as parts of lead per million parts of the substance. The usual limit as per Indian Pharmacopoeia is 20 ppm

Procedure:

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

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

TABLE :4

Observation:

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

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

TABLE :5

Test sample	**Standard compound**
Weigh specific quantity of test substance, moisten with sulphuric acid and ignite on a low flame till completely charred Add few drops of nitric acid and heat to 500 °C Allow to cool and add 4 ml of hydrochloric acid and evaporate to dryness Moisten the residue with 10 ml of hydrochloric acid and digest for two minutes Neutralize with ammonia solution and make just acid with acetic acid	Take 2 ml of standard lead solution and dilute to 25 ml with water
Adjust the pH between 3 to 4 and filter if necessary	Adjust the pH between 3 to 4 by adding dilute acetic acid ‘Sp’ or dilute ammonia solution ‘Sp’
Dilute with water to 35 ml	Dilute with water to 35 ml
Add freshly prepared 10 ml of hydrogen sulphide solution	Add freshly prepared 10 ml of hydrogen sulphide solution
Dilute with water to 50 ml	Dilute with water to 50 ml
Allow to stand for five minutes	Allow to stand for five 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 III: Use for the substance which gives clear colorless solution in sodium hydroxide solution.

TABLE :6

Test sample	**Standard compound**
Solution is prepared as per the monograph and 25 ml is transferred in Nessler's cylinder or weigh specific amount of substance and dissolve in 20 ml of water and add 5 ml of dilute sodium hydroxide solution	Take 2 ml of standard lead solution
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 with water
Add 5 drops of sodium sulphide solution	Add 5 drops of sodium sulphide solution
Mix and set aside for 5 min	Mix and set aside for 5 min
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.

Limit test for Lead

Lead is a most undesirable impurity in medical compounds and comes through use of sulphuric acid, lead lined apparatus and glass bottles use for storage of chemicals.

Principle:

Limit test of lead is based on the reaction of lead and diphenylthiocabazone (dithizone) in alkaline solution to form lead dithizone complex which is read in color.

Dithizone is green in color in chloroform and lead-dithizone complex is violet in color, so the resulting color at the end of process is red.

Procedure:

TABLE :6

Test sample	Standard compound
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 6ml of ammonium citrate	Add 6ml 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
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

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.

Reasons:

Ammonium citrate, potassium cyanide, hydroxylamine hydrochloride is used to make pH optimum so interference and influence of other impurities have been eliminated.

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

Limit test for Arsenic

Principle:

Limit test of Arsenic is based on the reaction of arsenic gas with hydrogen ion to form yellow stain on mercuric chloride paper in presence of reducing agents like potassium iodide. It is also called as Gutzeit test and requires special apparatus. Arsenic, present as arsenic acid in the sample is reduced to arsenious acid by reducing agents like potassium iodide, stannous acid, zinc, hydrochloric acid, etc. Arsenious acid is further reduced to arsine (gas) by hydrogen and reacts with mercuric chloride paper to give a yellow stain.

$$H_3AsO_4 + H_2SnO_2 \rightarrow H_3AsO_3 + H_2SnO_3$$

Arsenic acid Arsenious acid

$$H_3AsO_3 + 3H_2 \rightarrow AsH_3 + 3H_2O$$

Arsenious acid Arsine

The depth of yellow stain on mercuric chloride paper will depend upon the quality of arsenic present in the sample.

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:

A known quantity of dilute arsenic solution is kept in wide mouthed bottle and rest procedure is followed as described in test solution (Gutzeit apparatus shown in Fig.1).

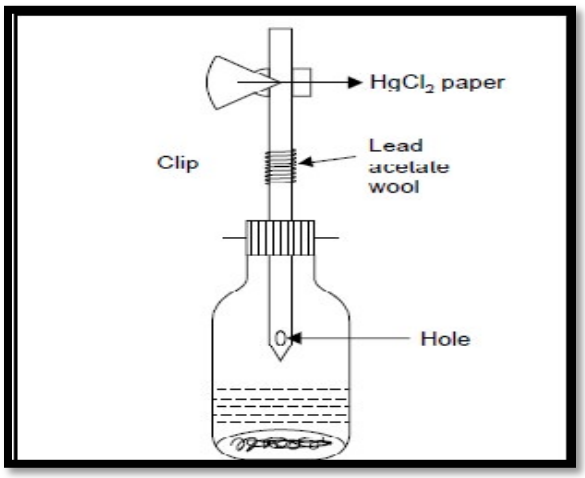

Figure: Gutzeit apparatus.

A : Approximately 60 ml generator bottle with 40 ml indicating line.

B : Glass tube with 6.5 mm inner diameter

C and D : a ground joint glass tube with 6.5 mm inner diameter and 18 mm outer diameter at the joint. Inner joint and the outer joint form a concentric circle.

E : rubber stopper

F : narrow part of the glass tube B. Glass wool is inserted up to this part.

G : rubber board (Lead acetate cotton plug)

H : clamp

Reasons:

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

Important points to remember

1. **Pharmacopoeia**; History of Pharmacopoeia. Monograph
2. **Inorganic Chemistry**
   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.
3. Impurity is any unwanted substance present in desired substance in less than or near to 5% of desired substance, e.g. a pinch of sodium chloride present in 100mg of sugar is known as impurity.

The various sources of impurity in pharmaceutical products are — reagents, heavy metals, ligands, catalysts, other materials like filter aids, charcoal, and the like, degraded end products obtained during \ after manufacturing of bulk drugs from hydrolysis, photolytic cleavage, oxidative degradation, decarboxylation, enantiomeric impurity, and so on.

4. Impurities originating from drug substance synthetic processes
5. Starting materials and intermediates
6. Impurities in the starting materials
7. Reagents, ligands and catalysts
8. By-products of the synthesis
9. Products of over-reaction
10. Products of side reactions
11. Impurities originating from degradation of the drug substance.
12. Enantiomeric impurities: Most therapeutic chiral drugs used as pure enantiomers is natural products. The high level of enantio selectivity of their biosynthesis excludes the possibility of the presence of enantiomeric impurities.

Methods to control impurity in pharmaceutical substance:

1. Sublimation
2. Drying
3. Crystallization
4. Evaporation

Effects of impurity in pharmaceutical substance:

1. Impurities may bring about incompatibility with other substances.
2. Impurities may lower the shelf life of the substances.
3. Impurities may cause difficulties during formulations and use of the substances.
4. Sometimes Impurities changes the physical and chemical properties of the substances.
5. Therapeutic effect can be decreased.
6. Shows toxic effect after a certain period.
7. Injurious when present above certain limits.
8. It may change odour, colour, taste of the substance.

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

Limit test is generally carried out to determine the inorganic impurities present in compound.

VARIOUS LIMIT TEST

Principle involved in the limit test for

1. Chloride,
2. Sulphate,
3. Iron,
4. Arsenic,
5. Lead and Heavy metals,
6. Modified limit test for Chloride and Sulphate

References for more learning

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You may use website links or you tube links for self-learning

IMPORTANT QUESTION

MCQ

I. Multiple Choice Questions **(10)**

Each of the following questions have four alternatives. Only one of them is correct. Choose the correct answer.

1. Impurities in pharmaceutical preparation may be due to following sources:
   (a) Raw material (b) Manufacturing process
   (c) Chemical instability (d) All of the above
   Ans. (d)
2. Pharmaceutical buffer system could be categorizes into
   (a) 1 (b) 2 (c) 3 (d) none of these
   Ans. (b)
3. Fluoride inhibits caries formation via
   (a) Increase acid solubility of enamel (b) Bacterial inhibition
   (c) Both the above (d) Decrease acid solubility of enamel
   Ans. (d)
4. In Bronsted-Lowry concept acid is
   (a) Proton donor (b) electron donor (c) proton accepter (d) electron accepter
   Ans. (a)
5. Hypochloremia can be caused by
   (a) salt losing nephritis (b) metabolic acidosis
   (c) both (a) and (b) (d) metabolic alkalosis
   Ans. (c)
6. In physiological acid-base imbalance K excretion will be decreased
   (a) the amount of Na reaching distal tubule is low
   (b) the proton secretion by kidney tubule is increased
   (c) both (a) and (b)
   (d) none of the above
   Ans. (c)
7. Calcium gluconate is prepared by
   (a) lactic acid and $CaCO_3$ (b) oxalic acid and $CaCO_3$
   (c) gluconic acid and $CaCO_3$ (d) gluconic acid and $Ca(OH)_2$
   Ans. (c)

8. Which one of the followings is used as systemic alkalizer?
   (a) Sodium chloride (b) Sodium bicarbonate
   (c) Sodium sulphate (d) Sodium acetate
   Ans. (b)
9. The principle function of chloride is
   (a) maintenance of proper hydration (b) maintenance of osmotic pressure
   (c) normal electrolytic balance (d) all of the above
   Ans. (d)
10. The advantage of sodium lactate over sodium bicarbonate
   (a) rapidly metabolized (b) it may be sterilized by boiling
   (c) both of the above (d) none of the above
   Ans. (c)

2marks

1. Why distilled water is used in limit tests instead of tap water.
2. Why lead acetate cotton plug is used in arsenic limit test.
3. What are the methods used to control impurities in pharmaceutical substance?
4. Why dilute HNO_3 is used in limit test of chloride.
5. Why dilute HCl is used in limit test of sulphate.
6. Define physiological ion and their role in body.
7. Define the term acidosis and alkalosis.
8. Classify the acidifiers.
9. Define antimicrobial agents with its examples.
10. Define protective and adsorbent with examples.
11. Define expectorant and write its examples.
12. Define emetics and write its examples.
13. Define haeminitics and write its examples.
14. Define astringents and write its examples.
15. What are the units used to measure the radioactive substance.

5 marks

1. Write the functions of thioglycollic acid, citric acid and NH_3 in iron limit test. 5
2. Write procedure of lead limit test and give solution to overcome the problem related to dense precipitation in lead limit test. 5
3. Write procedure of sulphate limit test. Write procedure to prepare BaSO4 reagent. Why alcohol is added in BaSO4 reagent in sulphate limit test.
4. Explain in brief about the mechanism of action of expectorants.
5. Explain in brief about the mechanism of action of emetics.
6. What are storage and handling conditions of radiopharmaceuticals

7. Define dental products and write the mechanism of acid base balance.
8. What are the regulatory mechanisms of acid base balance?
9. Define gastrointestinal agents. Write their classification with examples.

10 Marks

1. Define the term impurity and write its sources. Describe procedure and apparatus used in arsenic limit test. 10
2. Define the term limit test, its types and methods used to control of limit tests. Describe procedure used in chloride limit test. 10
3. Define replacement therapy. Explain in detail about types of fluid systems in body. Write about importance of acid base balance and its related problems. 10