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UNIT-1

Pharmaceutical analysis

Pharmaceutical analysis is a branch of practical chemistry that involves a series of process for identification, determination, quantification and purification of a substance, separation of the components of a solution or mixture, or determination of structure of chemical compounds.

The substance may be a single compound or a mixture of compounds and it may be in any of the dosage form. The substance used as pharmaceuticals are animals, plants, microorganisms, minerals and various synthetic products.

The different pharmaceutical agents are as follows:

1. Plants
2. Microorganisms
3. Minerals
4. Synthetic compounds

Pharmaceutical analysis is traditionally defined as analytical chemistry dealing with drugs both as bulk drug substances and as pharmaceutical products (formulations). However, in academia, as well as in the pharmaceutical industry, other branches of analytical chemistry are also involved, viz. bioanalytical chemistry, drug metabolism studies and analytical biotechnology. The development of drugs in the pharmaceutical industry is a long-term process, often taking more than a decade from the start of a research project to the appearance of a drug on the market. That process involves several decision points, such as the choice of the candidate drug after the preclinical screening phase, the investigational new drug (IND) application before testing the compound for the first time in man, and finally the new drug application (NDA) which summarizes the data obtained from all the studies needed for marketing approval of the drug as a medicine. In all these steps, especially the IND and NDA, the amount of data generated is enormous. Analytical chemists take part in many of the

studies that constitute this documentation. Substance quality and its specifications are based on substance analysis, and that knowledge is later used for quality control during full-scale production. Product analysis involves dealing with the various formulations and starts after the IND has been approved. The results from such work lead to specifications that form the basis for the quality control of the product. For both substances and formulations there is an increasing interest in the introduction of process analytical chemistry.

The sample to be analysed is called as analyse.

- Quality control and quality assurance
- Chromatographic techniques
- Quantitative and qualitative analysis
- Validation methods
- Stoichiometry between reactants & products

Scope of Pharmaceutical Analysis

- Pharmaceutical Analysis is one of the most sort after specializations in masters of pharmacy. People specialised in pharmaceutical analysis are indispensable to the manufacturing, quality control and analytical manifestations of the industry.
- They can work in quality control department which oversees the purity, qualitative aspects and the matching of the stringent regulatory limits required by a finished product.
- Research and development has huge implications on the results of the analysis and detection of new compounds. More and more companies are stressing on a separate analytical R&D department.
- Pharmaceutical analysis students also find takers in the medical devices companies, equipment companies, regulatory agencies etc.
- Always remember, no matter what compounds you discover or formulation you make nothing is valid until it is evaluated, analysed and validated.
- Based upon the determination type, there are mainly two types of analytical methods. They are as follows:

Qualitative analysis:
Quantitative analysis:

1. Qualitative analysis

This method is used for the identification of the chemical compounds. Qualitative analysis is performed to establish composition of natural/synthetic substances. These tests are performed to indicate whether the substance or compound is present in the sample or not.

2. Quantitative analysis

This method is used for the determination of the amount of the sample. Quantitative analytical techniques are mainly used to quantify any compound or substance in the sample. There are various methods to find out the quantity of a substance in a product.

Various types of Qualitative analysis:

1.Chemical methods

a) volumetric or titrimetric methods

b) gravimetric methods

c) gasometric analysis

2.Electrical methods

3.Instrumental methods

4.Biological and microbiological

Methods of Expressing Concentration of Solution

Concentration of solution is the amount of solute dissolved in a known amount of the solvent or solution. The concentration of solution can be expressed in various ways as discussed below,

(1) **Percentage:** It refers to the amount of the solute per 100 parts of the solution. It can also be called as parts per hundred (pph). It can be expressed by any of following four methods,

(i)**Weight to weight percent**

$$\% \text{ w/w} = \frac{\text{Wt of solute}}{\text{Wt of solution}} \times 100$$

(ii) **Weight to volume percent**

$$\% \text{ w/v} = \frac{\text{Wt of solute}}{\text{Volume of solution}} \times 100$$

(iii) **Volume to volume percent**

$$\% \text{ v/v} = \frac{\text{Volume of solute}}{} \times 100$$

Volume of solution

(iv)**Volume to weight percent**

$$\textbf{\%}\ v/w = \frac{\text{Volume of solute}}{\text{Wt of solution}} \times 100$$

(2) Parts per million (ppm) and parts per billion (ppb):

When a solute is present in trace quantities, it is convenient to express the concentration in parts per million and parts per billion. It is the number of parts of solute per million (10^1) or per billion (10^9) parts of the solution. It is independent of the temperature.

$$Ppm = \frac{\text{mass of solute component}}{\text{Total mass of solution}} \times 10^1$$

$$Ppb = \frac{\text{mass of solute component}}{\text{Total mass of solution}} \times 10^9$$

(3) Normality (N)

It is defined as the number of gram equivalents (equivalent weight in grams) of a solute present per litre of the solution. Unit of normality is gram equivalents litre^{-1}. Normality changes with temperature since it involves volume. When a solution is diluted x times, its normality also decreases by x times. Solutions in term of normality generally expressed as,

N= Normal solution; 5N= Penta normal,

10N= Deca normal;N/2= semi normal

N/10= Deci normal; N/5= Penti normal

N/100 or 0.01N= centinormal,

N/1000 or 0.001= millinormal

Mathematically normality can be calculated by following formula

$$\text{Normality (N)} = \frac{\text{Number of gm eq. of solute}}{\text{Volume of solution (l)}}$$

(* 1 equivalent = 1000 mill equivalent or meq.)

(4) Molarity

The number of moles of solute per litter of solution OR the molar concentration of a solution usually expressed as the number ofmoles of solute per liter of solution.

It is also known as molar concentration, is the number of moles of a substance per litter of solution. Solutions libelled with the molar concentration are denoted with a capital M. A 1.0 M solution contains 1 mole of solute per litter of solution.

$$\text{Molarity (M)} = \frac{\text{Mole of solute}}{\text{Litres of solution}}$$

Molarity - M → moles per litter solution

(5) Molality

The number of moles of solute per kilogram of solvent. It is important the mass of solvent is used and not the mass of the solution. Solutions labelled with molal concentration are denoted with a lower case m. A 1.0 m solution contains 1 mole of solute per kilogram of solvent.

$$\text{Molality (m)} = \frac{\text{Mole of solute}}{\text{Kg of solvent}}$$

Molality - m → moles per kilogram solvent

Preparation and standardization of solutions of various molar and normal solutions

Oxalic acid $(COOH)_2$

Oxalic acid is available in pure state and its standard solutions can, therefore, be prepared by the direct method. Eq. wt. of hydrated oxalic acid ($C_2H_2O_4.2H_2O$), being 63 its N/10 solution would contain 6.3 gm/litre, and N/20 solution would contain 3.15 gm/litre. These standard solutions are employed to find the strength of solutions of alkalies (NaOH and KOH) whose standard solutions cannot be prepared by the direct method.

Preparation of oxalic acid solution

Standard solutions are prepared by using standard substances. Here a known quantity of standard substances depending upon the requirement is dissolved in a known amount of water and desired volume is made. Since, these substances have a constant weight, high purity, non-

hygroscopic property, so the solution obtained is of known and definite concentration. The examples of such solutions are as follows.

Standardization N/10 oxalic acid solution (Primary standard)

To prepare N/10 solution of oxalic acid, weigh 6.3 gm of oxalic acid & dissolve in distilled water & finally make up the volume to one liter in a volumetric flask. The standard solution of oxalic acid (Primary standard) is used to find the strength of solutions of alkalies like NaOH, KOH (Secondary standards) whose standard solutions cannot be made by direct weighing. Secondary standard substances. Those substances or reagents which cannot be obtained in a sufficient pure state, e.g. NaOH, KOH, HCl, H_2SO_4 are called secondary standard substances.

Preparation of Sodium Hydroxide (NaOH) solutions
N/10 NaOH

Prepare concentrated stock solution (say 50%) of NaOH by dissolving equal parts of NaOH pellets (50 gm) & water (50 gm) in a flask. Keep it tightly stoppered for 3-4 days. Use the clean, supernatant liquid for preparing N/10 solution. Approximately 8 ml of this stock solution (50%) is required per litre of distilled water. This will give approximate solution. Now take 10 ml of standard N/10 oxalic acid (primary standard) solution in conical flask and add 2- 3 drops of phenolphthalein indicator. Take unknown solution i.e. approximate N/10 NaOH solution in burette and add to the conical flask containing standard oxalic acid solution by continuous mixing by swirling the flask till the appearance of pink color. NaOH is taken in burette and standard oxalic acid in conical flask. Note down the volume of approximate N/10 NaOH solution used in the titration of 10 ml of standard oxalic acid. Calculate the normality of the unknown sodium hydroxide solution by using following equation.

$$N_1V_1 = N_2V_2$$
$$(\text{Base}) = (\text{Acid})$$

N1 = Normality of NaOH solution. (ml)
V1 = Volume of NaOH solution used (ml)
N2 = Normality of standard oxalic acid solution (0.1 N)

V2 = Volume of standard oxalic acid solution (10 ml)

If the volume of approximate NaOH used in the titration is less than 10 ml, means the solution is strong and its normality is not N/10, so dilute the basic solution and again standardize with standard oxalic acid solution till normality of approximate solution is same as that of standard solution.

Preparation of Sodium Hydroxide Concentrated acids (HCl)

Preparation of Concentrated acids

Prepare approximately 0.1 N solutions on the basis of strength diluting it 120 times with distilled water. Then standardize it against standard N/10 Na_2CO_3 using methyl orange as an indicator.

Standardization of Concentrated acids (HCl)

Prepare approximetly 0.1N solution on the basis of the strength given on the label by diluting it 120 times with distilled water. Then standardize it against standard N/10 NaOH which is already

Standardized against N/10 oxalic acid using phenolphthalein indicators.

Preparation of sulphuric acid (H_2SO_4)

Concentrated H2So4 is very corrosive in nature; therefore, it should be handled carefully. And always remember add acid to water under cold condition this is done to avoid pumping due to the heat generated.

Standardization of Sulphuric Acid H_2SO_4

For the preparation of N/10 H_2SO_4, take 10 ml of concentrated H_2SO_4 (usually about 36 N), dilute 36 times by adding acid in small quantity to distilled water in a cold water bath, to make it 1N and then dilute this 1N solution further 10 times to make it N/10. Then standardize against standard N/10 NaOH or N/10 KOH using phenolphthalein indicator.

Preparation of nitric acid (HNO3)

Take 10 ml of concentrated nitric acid HNO3 (about 16N), and dilute 16 times by adding acid to distilled water to make it 1N and then dilute this 1N solution futher 10 tiimes to make it N/10 then standerize agaianst standard N/10 KOH using phenolphthalein indicator.

Preparation of 0.1N sodium thiosulphate solution ($Na_2SO_3.5H_2O$)

Dissolve approximately 24.8 gm of sodium thiosulphate crystals in previously boiled and cooled distilled water and make the volume to 1000 ml. Store the solution in a cool place in a dark colored bottle. After storing the solution for about two weeks, filter if necessary and standardize as follows.Weigh accurately about 5.0 gm of finely ground potassium dichromate which has been previously dried to a constant weight at 105 ± 2° in to a clean 1.0 litre volumetric flask. Dissolve in water make up to the mark; shake thoroughly and keep the solution in dark place. Pipette 25.0 ml of this solution into a clean glass stoppered 250 ml conical flask. Add 5.0 ml of concentrated hydrochloric acid and 15.0 ml of 10% potassium iodide solution. Allow to stand in dark for 5 minutes and titrate the mixture with the solution of sodium thiosulphate using starch solution as an indicator towards the end. The end point is taken when blue color changes to green. Calculate the normality (N) of the sodium thiosulphate as follows:

$$N = \frac{25W}{49.03\ V}$$

W= Weight in g of the potassium dichromate

V= Volume in ml of solution thiosulphate solution required for the titration

Preparation of 0.1N ceric ammonium sulphate $(NH_4)_4Ce(SO_4)_4 2\ H_2O$

66gm of ceric ammonium sulphate was dissolved with gentle heat in a mixture of 30 ml of sulphuric acid and 500 ml of water. The mixture was cooled and filtered. The resulting solution was diluted to 1000ml with water

Standardisation of 0.1 N Ceric Ammonium Sulphate

1. About 0.2 gm of Arsenic trioxide which was previously dried for about an hour was accurately weighed and transferred into a 500 ml conical flask.
2. The inner walls of the flask were washed with 100 ml of water and mixed thoroughly
3. Then 300 ml of dil. sulphuric acid , 0.15 ml of osmic acid, 0.1 ml of ferroinsulphate indicator were added

4. Titration was carried out until pink colour of solution changed to pale blue or yellowish green colour
5. Each ml of 0.1 N ceric ammonium sulphate ~ 0.6326 gm of ceric ammonium sulphate ~ 4.946 grams of arsenic trioxide

Preparation and standardization of EDTA solutions

1. Preparation of 0.01 M EDTA solution: Dissolve 3.8 g of disodium ethylene diamine dihydrogen tetraacetate (EDTA, M.Wt. 372.25) in distilled water and volume is made to 1 litre. Mix it well, store in polyethylene reagent bottle. It is standardized against 0.01 M $CaCO_3$ or $CaCl_2$.
2. Preparation of 0.01 M $CaCl_2$ solution: Prepare standard Ca solution (1 ml = 1 mg $CaCO_3$, M.wt. 100) by weighing 1 g $CaCO_3$ into 500 ml conical flask or beaker and adding dilute HCl through funnel until $CaCO_3$ is dissolved. Add 20 ml water, boil to expel CO_2 and cool. Add few drops of methyl red indicator and adjust colour intermediate orange (brownish red) with dilute NH_4OH or HCl as required. Transfer quantitatively to 1 L volumetric flask and make up volume to the mark. Shake it well and store it well and store in air-tight reagent bottle.
3. Erichrome Black T indicator: Dissolve 0.5 g of Erichrome black T in 100 ml of triethanolamine. Or 0.4 g in 100 ml methanol.
4. Buffer solution: Dissolve 16.9 g NH_4Cl in 143 ml NH_4OH, and dilute to 250 ml with water. Store in tightly stoppered Pyrex of plastic bottle. Dispense from bulb-operated pipette. Discard after 1 month or when 1-2 ml added to sample fails to produce pH 10.0±0.1 at end point titration.

Standardization of EDTA solution

Rinse and then fill burette with prepared EDTA solution. Pipette 25 ml of standard $CaCO_3$ solution into 250 ml Erlenmeyer flask, add 1 ml ammonia buffer (to raise the pH as reaction takes place at high pH) and 3-4 drops of Erichrome black T indicator. Titrate the EDTA solution until colour changes from wine red to dark blue with no reddish tinge remaining. Calculate the molarity of EDTA ($M_1V_1 = M_2V_2$), if excess follows the procedure for the standardization, recheck the molarity and it should be 0.01 M.

Preparation of Potassium Permanganate

Potassium Permanganate 0.1 N: Dissolve 3.3 g of reagent grade potassium permanganate (KmnO4) in 1 L of purified water and heat on a steam bath for two hrs. Cover and allow to

stand for 24 hrs. Filter through a fine porosity sintered glass crucible, discarding the first 25 mL. Store in a glass-stoppered, amber-colored bottle. Avoid exposure to direct sunlight; cover the neck of the bottle with a small beaker as a protection against dust. If manganese dioxide precipitates on standing, refilter and restandardize before use.

Standardization of Potassium Permanganate

Potassium Permanganate 0.1 N: Weigh accurately 0.2-0.3 g sodium oxalate (Na2 C2 O4) (dried 2 hrs., 105-110 °C) National Institutes of Science and Technology, U. S. Department of Commerce. Cool in a desiccator and transfer quantitatively to a 600 mL beaker. Add 250 mL of purified water (freshly boiled and cooled) and 10 mL sulfuric acid (96% H2 SO4 , sp g 1.84). Add rapidly from a buret about 95% of the theoretical quantity of potassium permanganate solution needed; stir until the solution is clear. Heat the solution to 55-60 °C (Maintain temperature range during titration.) and complete the titration by slow dropwise addition until the appearance of a pink color which persists for 30 secs. Determine and subtract a blank titration run at 55-60 °C on a mixture of 250 mL of purified water (freshly boiled and cooled) and 10 mL of concentrated sulfuric acid.

Pharmacopoeia

Pharmacopoeia the word derives from the ancient Greek pharmakopoiia from (pharmako-) ″drug″, followed by the verb-stem (poi-) ″make″ and finally the abstract noun ending -ια (-ia). These three elements together can be rendered as ″drug-mak-ing″ or ″to make a drug″. A pharmacopoeia, pharmacopeia, or pharmacopoeia, in its modern sense, is a legally binding collection, prepared by a national or regional authority, of standards and quality specifications for medicines used in that country or region. A quality specification is composed of a set of appropriate tests that will confirm the identity and purity of the product, ascertain the strength (or amount) of the active substance and, when needed, its performance characteristics. Reference substances, i.e. highly-characterized, physical specimens, are used in testing to help ensure the quality, such as identity, strength and purity, of medicines. The texts cover pharmaceutical starting materials, excipients, intermediates and finished pharmaceutical products (FPPs). General requirements may also be given in the pharmacopoeia on important subjects related to medicines quality, such as analytical methods, microbiological purity, dissolution testing, stability, etc. (1). The role of a modern pharmacopoeia is to furnish quality specifications for active pharmaceutical ingredients (APIs), FPPs and general requirements, e.g. for dosage forms. The existence of such specifications and requirements is necessary for the proper functioning or regulatory control

of medicines. Pharmacopoeial requirements form a base for establishing quality requirements for individual pharmaceutical preparations in their final form. According to the information available to the World Health Organization (WHO), 140 independent countries are at present employing some 30 national as well as the African, European and International Pharmacopoeias (2). Compared to national and regional pharmacopoeias, The International Pharmacopoeia (Ph. Int.) is issued by WHO as a recommendation with the aim to provide international standards – including less technically demanding alternatives where needed - for adoption by Member States and to help achieve a potentially global uniformity of quality specifications for selected pharmaceutical products, excipients and dosage forms. After discussion with many representatives of world pharmacopoeias and in response to feedback, WHO has initiated steps based on WHO's first attempts during various meetings of the International Conference of Drug Regulatory Authorities (ICDRA), especially the 10th ICDRA meeting held in Hong Kong in 2002 and a further discussion among regulators during the 11th ICDRA meeting held in Madrid in 2004, to organize an official meeting entitled International meeting of world pharmacopoeias for participation of all WHO Member States' pharmacopoeias worldwide, be they national, regional or international. The aim was to discuss topics of common interest and challenges. The meeting dates were 29 February–2 March 2012. In order to prepare for the meeting in a timely manner, WHO sent a preliminary agenda and Questions to pharmacopoeias in advance to receive feedback and enable comprehensive input to the agenda. The questions, participant's presentations and the meeting report are shown on the meeting web site (3). This document presents a summary of the answers to the Questions to pharmacopoeias provided by representatives of world pharmacopoeias participating in the international meeting, and of other related information received from those that were unable to actively participate in this meeting. History and background Overwhelming empirical knowledge of mankind gained during cent

Indian Pharmacopoeia Commission (**IPC**) is an autonomous institution of the Ministry of Health and Family Welfare which sets standards for all drugs that are manufactured, sold and consumed in India. The set of standards are published under the title **Indian Pharmacopoeia** (**IP**) which has been modelled over and historically follows from the British Pharmacopoeia. The standards that are in effect since 1 December 2010 is the *Indian Pharmacopoeia 2010 (IP 2010).* The Pharmacopoeia 2014 was released by Health Minister Ghulam Nabi Azad on 4 November 2013.

I.P., the abbreviation of 'Indian Pharmacopoeia' is familiar to the consumers in the Indian sub-continent as a mandatory drug name suffix. Drugs manufactured in India have to be labelled with the mandatory non-proprietary drug name with the suffix *I.P*. This is similar to

the *B.P.* suffix for British Pharmacopoeia and the *U.S.P.* suffix for the United States Pharmacopeia.

The IPC was formed according to the Indian *Drugs and Cosmetics Act* of 1940 and established by executive orders of the Government of India in 1945.

History of Pharmacopoeia

The actual process of publishing the first Pharmacopoeia started in the year 1944 under the chairmanship of Col. 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.

History and background Overwhelming empirical knowledge of mankind gained during centuries and constant effort to establish better health care possibilities have led to the creation of a list of origin, preparation and healing properties of medicines. The term Pharmacopoeia first appears as a distinct title in a work published in Basel, Switzerland in 1561 by Dr A. Foes, but does not appear to have come into general use until the beginning of the 17th century. Today's pharmacopoeias focus mainly on assurance of quality of products by various tools of analytical sciences. The aim to achieve a wide global harmonization of quality specifications for selected pharmaceutical products, excipients and dosage forms came with increased globalization and reciprocal collaboration. History of these approaches goes back to 1902–1925 when agreements established a ″Unified″ Pharmacopoeia. In 1929 the "Brussels Agreement" stipulated the League of Nations to carry out related administrative functions. Eight years later, in 1937, the first meeting of the ″Technical Commission of Pharmaceutical Experts″ was held. An important date in the history of quality assurance of medicines is 1948, when the First World Health Assembly (WHA) approved the Expert Committee on Unification of Pharmacopoeias to continue this work. One year later, the WHA renamed it the Expert Committee on International Pharmacopoeia.

Current Issues

- Amendments to IP 2007 have been published in IP Addendum 2008. Further amendments are to be taken care of in IP 2009 edition.
- Publication of IP 2009 by Dec. 2009 is the immediate priority. Work is in full swing.
- 159 Drug molecules have been short listed for the IP 2009. The APIs and their formulations monographs are being prepared at the IPC.

- Monograph inclusion/deletion criteria and the Monograph Inclusion Form have been uploaded on the website of the Commission

Indian National Formulary

1st Edition 1960

2nd Edition 1966

3rd Edition 1979

It is a reliable reference book on drugs formulations for the practicing physicians/ clinicians, pharmacists, clinical pharmacists,nurses and others engaged in healthcare profession

- PHAMACOPOEIA ➔ OFFICIAL DRUGS
- PHARM.CODEX ➔ OFFICIAL+UNOFFICIAL DRUGS
- EXTRAPHARACOPOEIA➔DETAILED INFORMATION OF OFFICIAL+UNOFFICIAL DRUGS
- FORMULARY➔REGISTERED DRUGS FOR MARKETING

Table: 2 TYPES OF PHARMACOPOEIAS

Edition	Year	Addendum/Supplement
1st Edition	**1955**	Supplement 1960
2nd Edition	**1966**	Supplement 1975
3rd Edition	**1985**	Addendum 1989
		Addendum 1991
4th	**1996**	Addendum 2000

Edition		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

Sources of Impurities in Medicinal agent

The origin of impurities in drugs is from various sources and phases of the synthetic process and preparation of pharmaceutical dosage forms. Majority of the impurities are characteristics of the synthetic route of the manufacturing process. There are several possibilities of synthesizing a drug; it is possible that the same product of different sources may give rise to different impurities. According to the ICH impurities are classified as organic impurities, inorganic impurities and residual solvents**.** Organic impurities may arise from starting

materials, by products, synthetic intermediates and degradation products. Inorganic impurities may be derived from the manufacturing process and are normally known and identified as reagents, ligands, inorganic salts, heavy metals, catalysts, filter aids and charcoal etc. The number of inorganic impurities and residual solvents are limited. These are easily identified and their physiological effects and toxicity are well known. For this reason the limits set by the pharmacopoeias and the ICH guidelines can guarantee that the harmful effects of these impurities do not contribute to the toxicity or the side effects of the drug substances. The situation is different with the organic impurities. Drugs prepared by multi-step synthesis results in various impurities, their number and the variety of their structures are almost unlimited and highly dependent on the route and reaction conditions of the synthesis and several other factors such as the purity of the starting material, method of isolation, purification, conditions of storage etc. In addition, toxicity is unknown or not easily predictable. For this reason the ICH guidelines set threshold limit above which the identification of the impurity is obligatory.

Sources of organic impurities:

- Impurities originating from drug substance synthetic processes
- Starting materials and intermediates
- Impurities in the starting materials
- Reagents, ligands and catalysts
- By-products of the synthesis
- Products of over-reaction
- Products of side reactions
- Impurities originating from degradation of the drug substance.

Limit Test

Limit = a value or amount that is likely to be present in a substance
Test = to examine or to investigate
Impurities = a foreign matter present in a compound

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 short,

limit test is nothing but to identify the impurities present in the substance and compare it with standard.

Importance of Limit tests

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

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:

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 0.05845 % W/V solution of sodium chloride in Nessler cylinder
Add 1ml of nitric acid	Add 1ml of nitric acid
Dilute to 50ml in Nessler cylinder	Dilute to 50ml in Nessler 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.

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:

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 colour.

Procedure:

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 aamoniumthiocyanate 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.

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.

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

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. [7-9]

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:

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