Chapter 1

INTRODUCTION TO PHARMACEUTICAL CHEMISTRY

CONTENTS

- Introduction to Pharmaceutical chemistry: Scope and objectives
- Sources and types of errors: Accuracy, precision, significant figures
- Impurities in Pharmaceuticals: Sources and effect of impurities in Pharmacopoeial substances, the importance of the limit test, Principle and procedures of Limit tests for chlorides, sulfates, iron, heavy metals and arsenic.

♦ LEARNING OBJECTIVES ♦

After completing this chapter, the student should be able to understand:

1. Scope and objectives of Pharmaceutical Chemistry.
2. Sources and types of errors.
3. Accuracy, precision, significant figures.
4. Impurities in Pharmaceuticals
5. Different sources of impurities
6. Principles and procedures of various Limit tests.

1.1 INTRODUCTION TO PHARMACEUTICAL CHEMISTRY

Pharmaceutical chemistry involves drug chemistry, quality assurance and analysis of drugs and study of various analytical techniques used, drug metabolism, pharmacology, as well as cures and remedies for diseases. **[Fig.1.1].**

Under the broad scope of Pharmaceutical Chemistry, various branches studied are – Pharmaceutical Inorganic chemistry, Pharmaceutical Organic Chemistry, Pharmaceutical Biochemistry, Pharmaceutical Analysis, Pharmaceutical Medicinal Chemistry, etc. Even new drug discovery, pharmacokinetics, and quality assurance as part of pharmaceutical chemistry. Thus, the scope of Pharmaceutical Chemistry is very broad, and these components are taught to more or less extent in various courses of Pharmacy.

To Summarize- "Pharmaceutical Chemistry deals with the Drugs' Structure, Synthesis, Analysis, Medicinal Effect, Mechanism of Action, Metabolism and Quality Assurance".

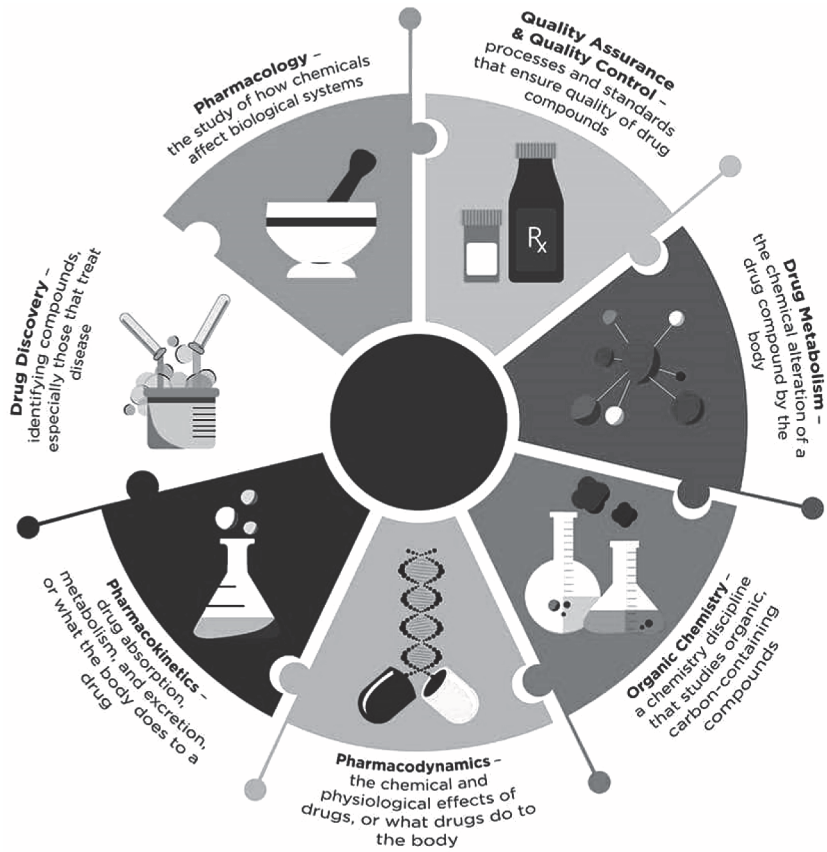


Fig. 1.1 Scope of Pharmaceutical Chemistry

1.2 SOURCES AND TYPES OF ERRORS, ACCURACY, PRECISION, SIGNIFICANT FIGURES

1.2.1 Common sources of errors

These include instrumental, environmental, procedural, and human errors. An error is the major aspect of laboratory science in physical and chemical testing, and its test findings are the primary scientific basis for assessing product quality. Physical and chemical laboratory experiments include three primary sources of errors: **systematic *error, random error*** and ***human error***. These sources of errors in the laboratory should be studied well before any further action.

1.2.1.1 System Errors in laboratory experiments- It applies to the repeated measuring of the same object under repeated conditions of measurement. The amount of the error

value is either positive or negative, which is called the **fixed system error** in laboratory experiments and laboratory tests.

The error is caused primarily by:

- The incorrect method of measurement in laboratory experiments
- The incorrect method of using the instrument in laboratory experiments
- The failure of the measuring instrument in laboratory experiments
- The performance of the testing tool itself in laboratory experiments
- The inappropriate use of the standard material and the changing environmental conditions in laboratory experiments

With certain steps and proper laboratory equipment**,** these sources of errors can be minimised and corrected.

Different types of system errors are:

1.2.1.1.1 Method error in laboratory experiments

The method error in laboratory experiments refers to the error created by the **very process** or **procedure** of physical and chemical examination. This error is inevitable, so the test result is often low or high. This error can be minimised through method validation.

1.2.1.1.2 Instrument error in laboratory experiments

The instrument error in test labs is caused primarily by **laboratory instrument inaccuracy**. For instance, if the meter dial or the zero point is inaccurate, the measurement result would be too small or too big. This error can be minimised through instrument calibration at regular intervals.

1.2.1.1.3 Reagent error in laboratory experiments

The reagent error in lab tests is caused primarily by the **impure reagent** or the **inability to meet the experimental provisions**; such as the existence of impurities in the reagent used in the physical and chemical testing phase; or the existence of contaminated water or reagent contamination that may influence the results of the examination; or the storage or operating climate.

1.2.1.2 Random error in laboratory experiments

Error caused by various **unknown factors** is known as random error. This error poses erratic changes at random, primarily due to a variety of small, independent, and accidental factors. The random error is atypical from the surface. Since it is accidental, the random error is often called an **unmeasurable** or **accidental error**.

1.2.1.3 Human Error in laboratory experiments

Human error in laboratory experiments and laboratory tests primarily refers to the mistake in the **physical and chemical inspection phase** caused by the factors of the **inspector**; particularly in the following three aspects:

a. **Operational error in laboratory experiments:** This is due to a lack of operation precautions. e.g., weighing a hygroscopic substance without effective moisture or humidity control can cause an error in weight.

b. **Subjective error in laboratory experiments:** It is caused by differences in subjective observations and perceptions from person to person regarding physical and chemical tests.

   e.g., different perceptions of sharpness in colour change at the endpoint

c. **Negligence error in laboratory experiments:** refers to the mistake caused during the physical and chemical examination by the analyst, e.g., reading mistake, operation error, measurement error etc.

1.2.2 Accuracy

Accuracy is the closeness of the measurement to its true or accepted value; any deviation from this value is expressed by the **error**. It measures agreement between a result and its actual true value. We can never determine accuracy precisely because the true value of a measured quantity can never be known exactly. We need to use an accepted value. Accuracy is expressed in terms of either *absolute* or *relative* error.

Precision, on the other hand, describes the agreement (or closeness or less deviation in values) among several results that have been obtained similarly. **(Fig. 1.2)**

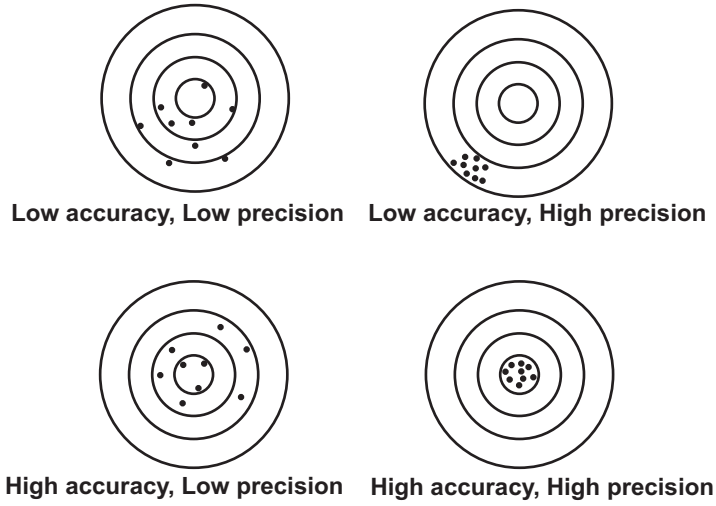


Fig. 1.2 Illustration of Accuracy and Precision showing their distribution in and around the Bull's Eye of a Dart Board

To determine accuracy, we have to know the true value, and this value is exactly what we are trying to obtain through the analysis. Do we know the answer precisely (precision), also, do we know it accurately (accuracy)?

1.2.3 Precision

Precision describes the reproducibility of measurements, i.e., the closeness of results to each other. Precision is determined by repeating the measurement on replicate samples.

Precision is a function of the deviation from the mean d_i, or just the deviation, which is defined as:

$$d_i = \left| X_i - \bar{X} \right|$$

The lesser the deviation, the higher the precision. In other words, precision is the closeness of results to others that have been obtained in precisely the same way.

Three terms to describe the precision of a set of replicate data are standard deviation, variance and coefficient of variation.

1.2.4 Significant Figures

One of the best ways of indicating reliability is to give a confidence interval at the 90% or 95% confidence level. Another method is to report the absolute standard deviation or the coefficient of variation of the data.

A less satisfactory but more common indicator of the quality of data is the **significant figure** convention:

- A simple way of indicating the probable uncertainty associated with an experimental measurement is to round the result so that it contains only significant figures.
- The significant figures in a number are all the certain digits plus the first uncertain digit.
- A zero may or may not be significant depending on its location in a number.
- A zero surrounded by other digits is always significant (such as 30.24 ml).
- Zeros that only locate the decimal point for us are not significant.

1.3 IMPURITIES IN PHARMACEUTICALS

Pharmaceutical chemicals and formulations must maintain a very high degree of purity. A compound is considered impure if it contains foreign matter/impurities. These impurities affect its potency. Although it is almost impossible to attain 100% purity, technically as well as cost-wise, it is still possible to achieve a high degree of purity.

Although, Purity can be achieved through the process of purification, it is often economically less viable. Alternatively, a reasonably acceptable purity can be achieved by controlling various sources or reasons that add to the impure nature of an active pharmaceutical ingredient or drug and excipients used in pharmaceutical formulations. Pharmacopoeias have fixed the limit for these impurities.

1.3.1 Sources and Effects of Impurities in Pharmacopoeial Substances

Impurities may enter or are formed in a drug substance during any of the following three stages during;

1. Manufacturing.
2. Purification and processing.
3. Storage and packaging.

1. During Manufacturing:

(a) Raw materials employed: Impurities present in raw materials may be carried through the manufacturing process to contaminate the final product. Impurities such as; As, Pb, heavy metals, chlorides associated with sodium compounds, sulfuric acid with copper sulfate and hydrochloric acid with iron chloride, are some common examples. Likewise, many elements accompany others in traces.

(b) Reagents used in the manufacturing process: The quality and purity of reagents used for manufacturing the drug substances are very important. If reagents used in the manufacturing process contain some impurities, these may find entry into the final product. For example, Sulphuric acid is used in many chemical processes. This acid often has lead present in it. Anions like Cl^- and SO_4^{-2} are common impurities in many substances because hydrochloric acid and sulphuric acid are used in processing.

(c) Solvents used in the manufacturing process: The manufacturing processes may involve single or multiple steps (unit operations). Naturally, solvents play an important role next to the main reagents as most of the chemical reactions involved in these processes are solvent based. If proper quality/purity of solvents is not assured, they may add to the impurities. Solvents like toluene and *n*-butanol contain water as an azeotrope. Alcoholic solvents also may be contaminated with water, and ethyl acetate can contain acetic acid in small amounts. Thus, the quality of solvents needs to be assured and controlled.

(d) Reaction vessels: The reaction vessels employed in the manufacturing process may be metallic (cast iron, mild steel, stainless steel) or mild steel with a glass lining. Nowadays, wooden or other metallic vessels are not used in the pharma industry. Some solvents and reagents employed in the process may react with the metals of the reaction vessels, leading to their corrosion and passing traces of metal impurities into the solution, contaminating the final product. Similarly, glass vessels may leach traces of alkali into the solvent. Even if acids like HCl, if by

chance, contain a small amount of fluoride, it can itch the glass lining and begin the metallic contamination. Lead antimony, bismuth etc., can crop up as impurities from the vessels.

(e) Intermediate products in the manufacturing process: Some intermediates which are produced during the manufacture may be carried out through the final product as impurities. Intermediates are products of (i) incomplete conversion of reactants to final products or (ii) side or competing reactions, or (iii) decomposition of products formed due to poor process control. In the manufacturing process of KI, the intermediate iodate is the main impurity. Similarly, sodium bromate is the impurity in NaBr.

(f) Defects in manufacturing process: Poor mixing and non-adherence to optimum reaction conditions (proper temperature, pressure and pH) may lead to impurities. E.g., Improper heating (failing to achieve bright red temperatures) in the process of manufacturing Zinc Oxide can lead to unoxidised metallic Zn as an impurity.

$$\mathbf{2\ Zn + O_2 \rightarrow 2\ ZnO + Zn\ (impurity)}$$

(g) Manufacturing hazards: In industrial areas, the atmosphere is contaminated with dust particles (Al_2O_3, silica glass, carbon, gases like; H_2S, SO_2, CO_2, CO, etc.). While manufacturing pharmaceutical products, these impurities may enter the final product. Accidental inclusion of dirt or glass or porcelain or silica or carbon or fibre particles due to poor manufacturing practices and facilities unable to check atmospheric and cross contaminations can lead to unwanted particulate matters in the product in many ways. These need to be checked and controlled. Wear and tear of machinery may shed metallic particles.

2. During Purification and Processing:

If not properly controlled, impurities are often added during the purification processes, mainly through the purifying reagents, solvents or vessels used.

(a) Reagents used to remove other impurities: Some chemicals are sometimes added to remove or precipitate another substance. This may also give rise to a source of impurity. For example, $BaCl_3$ is added to remove excess sulphate in $AlCl_3$. Hence, $AlCl_3$ is likely to contain Ba as an impurity.

(b) Solvents used in the process of purifications: Often, the solvents used for purification can be sources of impurities. These solvents range from organic solvents to acids (organic as well as mineral) and, of course, water. Water is the cheapest solvent and most widely used. Therefore, it is known as the universal solvent.

Types of water used are:

(i) **Tap water:** It contains impurities of Na^+, Ca^{2+}, Mg^{2+}, CO_2^{-3}, and $SO_4^{-2,}$ which, when used, appear as impurities in the final product.

(ii) **Softened water:** It contains Na^+ and Cl^- ions as impurities that may appear as impurities in the final product when used.

(iii) **Demineralised water:** Though it is free from all the above inorganic ion impurities, it still contains organic impurities like; salts of carboxylic acids, N and S, etc.

(iv) **Distilled water:** Considered to be the best. It is pure water and free from all inorganic and organic impurities, but its production cost is very high.

(c) **Contamination due to vessels and equipment used for purification:** During the purification processes, if the vessels are defective or not perfectly cleaned and dried, they may add impurities like; metallic ions, rust, glass particles, moisture, etc. The other equipment, mainly the filters, centrifuges, dryers, etc., also need to be cleaned and dry.

3. During Storage and Packaging:

(a) **Errors in the packaging or use of substandard packaging material:** During the process of packaging or filling and sealing, whether applicable for solid dosage forms or liquid dosage forms or API, proper material which can ensure complete foolproof packaging without access to the atmosphere and light will ensure the stability of the product. Thus, the quality and strength of packaging material is very important. For example, if the aluminium foil for tablet strip or cap for a liquid formulation bottle is of substandard quality, it can add to impurities. This may lead to recalls of entire batches from the market. This is very critical for parenteral formulations.

(b) **Faulty packaging processes:** Most pharmaceutical packaging processes are assembly lined automated, generally involving pressing and sealing with heat. If the process parameters are not optimised or are tampered with, then it may lead to contaminations. For example: Nowadays, most parenteral products are in polymer containers using FFS (Form-Fill-Seal) processes which involve proper heating, filling, sealing and congealing cycles. Any changes in process parameters can be hazardous.

(c) **Microbial contamination:** Microbial contaminations, mainly in the form of fungal and bacterial growth, may be due to the result of improper storage conditions as well as faulty packaging. The products for parenteral administration and ophthalmic preparations have to undergo sterility tests.

1.3.2 Effects of Impurities on Pharmaceuticals

1. Some impurities, if present beyond certain tolerance limits, can cause untoward side effects that can lead to unpleasant reactions—for example, Heavy metals like; Pb, Fe and As salts.
2. Some impurities which are otherwise harmless in nature and without any therapeutic effect, if present in considerable proportions, dilute the active strength or potency of the drug substance—for example, Na, K, Cl, SO_4, CO_3 salts.
3. Some impurities may be able to catalyse the degradation, thereby shortening the shelf life of the drug substance.
4. Some impurities, by their chemical nature, can interact with the drug substance to affect its purity and potency. Such impurities are said to be incompatible with the drug substance/s.
5. Some impurities, by virtue of their unstable nature like; hygroscopic nature, oxidisable nature, etc., can bring about change in the physical properties like; change in appearance, taste, odour, stability, etc., of the drug substance causing technical difficulties in its use as well as formulation.

1.4 LIMIT TESTS

Limit Tests are quantitative or semi-quantitative tests designed to identify or control small quantities of impurities. These tests should be specific and sensitive.

Limit = A value or amount that is likely to be present in a substance.

Test = To examine or to investigate.

Impurity =A foreign matter present in a compound.

Definition:

A limit test is defined as "a quantitative or semi-quantitative test designed to identify and control small quantities of impurities which are likely to be present in the substance".

1.4.1 Importance of Limit Tests

1. To find out the harmful amount of impurities.
2. To find out avoidable/unavoidable amounts of impurities.

Types of Limit Tests:

1. Comparison method.
2. Quantitative determination.
3. Test in which there is no visible reaction.

General Principles:

1. If the sample is lighter (in colour/turbidity/opalescence) than the standard solution, then it is within the pharmacopoeial limit (accepted).

2. If the sample is darker/heavier than the standard solution, it is above the pharmacopoeial limit (rejected).
3. **Specificity of a Limit Test:** A given limit test for a trace impurity should involve some selective reaction of the reagent with the trace impurity under consideration/detection specifically characteristic only to it.
4. **Sensitivity of a Limit Test:** As most limit tests involve dilute solutions and results are based on the concentration of the trace impurity, the results may take longer to become observable or appreciable. Thus, consideration of the duration of the test needs to be of prime consideration in designing the limit test.

Nesslers' Cylinder (IP appendix VII A127):

It is a clear glass cylinder with a normal capacity of 50 ml. However, some of Nessler's cylinders are of 100 ml capacity. The overall height is about 15 cm, the external height to the 50 ml mark is 11.0 to 12.4 cm, and the thickness of the wall is around 1.0 to 1.5 mm, while the thickness of the base is about 1.0 to 3.0 mm. The external height to the 50 mark of cylinders used for the test must not differ by more than 1 mm in the given pair.

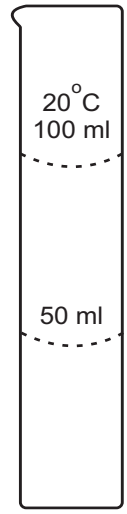


Fig. 1.3 Nessler's Cylinder

General Precautions:

1. The liquid used must be clean and filtered, if necessary.
2. The Nessler's cylinder must be made of colourless glass and have the same inner diameter in the given pair.
3. Detecting opalescence or colour development must be performed in daylight.
4. Comparison of turbidity should be done against a black background.
5. Comparison of colour should be done against a white background.

1.4.2 Principle and Procedure of Limit Test for Chloride

Aim: To perform the limit test for Chloride.

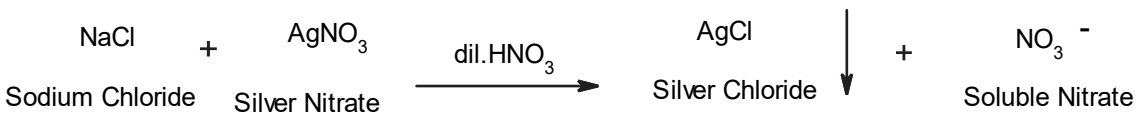
Apparatus: Pair of Nessler's cylinders, Glass rods, Stand, Measuring cylinder, Spatula, Pipette.

Chemicals: Dilute Nitric acid (10% v/v), 0.1 M Silver nitrate, Test sample, Distilled water.

Preparations:

1. **0.1 M Silver Nitrate:** Dissolve 1.7 gm of silver nitrate in 10 ml and make up the volume of 100 ml with water.
2. **Chloride Standard Solution (25 ppm Cl^-):** Dilute 5 volumes of a 0.0824% w/v sodium chloride solution to 100 volumes with water.

Principle: The limit test for chloride involves the chemical reaction between Silver nitrate and soluble chlorides in the presence of dilute nitric acid to form precipitation of Silver chloride, which is insoluble in dilute nitric acid. The turbidity (opalescence) formed in the test solution is compared with the standard solution in Nessler's glass cylinder by viewing them transversely against a dark background. If the opalescence in the sample is less than the standard, it passes the test. If it is more than the standard, it fails the test.



Procedure: Take two 50 ml Nessler's cylinders. Label one as "Test" and the other as 'Standard'.

(A) Test Solution:

1. Dissolve the specified quantity of substance under examination in distilled water or prepare a solution as directed in an individual monograph and transfer it to a Nessler's cylinder.
2. Add 10 ml of dil. HNO_3 (However, when nitric acid is used in the preparation of the solution, then dil. HNO_3 should not be added).
3. Dilute to 50 ml with distilled water and add 1 ml of 0.1 M Silver nitrate solution. Stir the mixture immediately with a glass rod and allow it to stand for 5 minutes.

(B) Standard Solution:

1. Take the mixture of 10 ml of Chloride Standard solution (25 ppm Cl^-) and 5 ml of distilled water and transfer to a Nessler's cylinder.
2. Add 10 ml of dil. HNO_3.
3. Dilute to 50 ml with water and add 1 ml of 0.1 M Silver nitrate solution. Stir the mixture immediately with a glass rod and allow it to stand for 5 minutes.

Table 1.1 Procedure for Preparation of Standard and Test Solution

Test Solution	Standard Solution
• Dissolve the specified quantity of substance in distilled water or prepare a solution as directed in individual monograph, in a Nessler's cylinder.	• Take mixture of 10 ml of Chloride Standard solution (25 ppm Cl^-) and 5 ml of distilled water and transfer to a Nessler's cylinder.
• Add 10 ml of dil. HNO_3.	• Add 10 ml of dil. HNO_3.
• Dilute to 50 ml with distilled water.	• Dilute to 50 ml with distilled water.
• Add 1 ml of 0.1 M Silver nitrate solution.	• Add 1 ml of 0.1 M Silver nitrate solution.
• Stir immediately with a glass rod.	• Stir immediately with a glass rod.
• Allow to stand for 5 minutes.	• Allow to stand for 5 minutes.

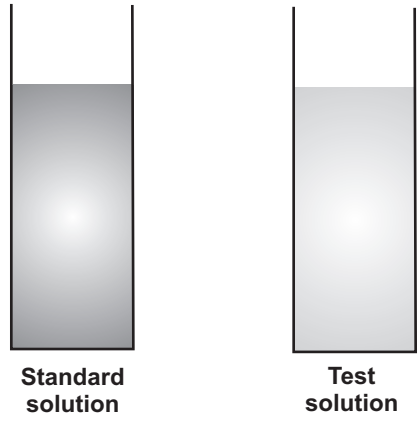


Fig. 1.4 Comparison of Standard and Test Solutions

Observation and Conclusion: The opalescence in STD is more than that of the test. Thus, the sample passes the limit test for Chloride.

Note: *Sometimes, the solution to be tested has to be prepared by a special method and instruction to this effect, if given, must be followed for preparing the test solution. The opalescence in the sample and the standard solution is compared by keeping the Nessler's cylinder against the proper background and observing side by side.*

1.4.3 Principle and Procedure of Limit Test for Sulphate

Aim: To perform the limit test for Sulphate.

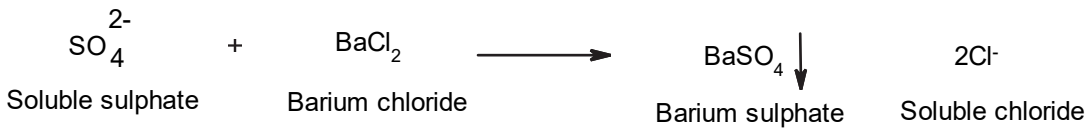
Apparatus: Pair of Nessler's cylinder, Glass rod, Stand, Measuring cylinder, Spatula, and Pipette.

Chemicals: Dilute hydrochloric acid, Barium chloride, Ethanolic sulphate standard solution (10 ppm), 5 M Acetic acid, Sulphate Standard solution (10 ppm), Distilled water.

Preparation:

1. **Ethanolic Sulphate Standard solution (10 ppm):** Dilute 1 volume of a 0.181% w/v solution of potassium sulphate in ethanol (30%) to 100 volumes with ethanol (30%).
2. **5M Acetic acid:** Dilute 285 ml of glacial acetic acid to 1000 ml with distilled water.
3. **Sulphate Standard Solution (10 ppm):** Dilute 1 volume of a 0.181% w/v solution of Potassium sulphate in distilled water to 100 volumes with the same solvent.
4. **Barium chloride (25%w/v):** Dissolve 25 gm of Barium chloride in 100 ml of distilled water.

Principle: It is based upon the chemical reaction between Barium chloride and soluble Sulphate. The turbidity produced is compared with the standard solution. The small quantity of Potassium sulphate increases the sensitivity of the test. Alcohol prevents supersaturation, and more uniform turbidity develops. If the turbidity produced in the test is more intense than the standard turbidity, it fails the test. Acetic acid is added to prevent the precipitation of other anions like; phosphate, oxalate, borate, etc., with a solution of Barium chloride. Therefore, Acetic acid precipitates only sulphate ions.



Procedure: Take two 50 ml Nessler's cylinders. Label one as "Test" and the other as 'Standard'.

(A) Test Solution:

1. Take 10 ml of 25% w/v solution of Barium chloride in a Nessler's cylinder.
2. Add 1.5 ml of Ethanolic sulphate standard solution (10 ppm SO_4^{-2}), mix and allow to stand for 1 minute.
3. Add 15 ml of the solution prepared as directed in the monograph or solution of the specified quantity of the substance under examination in 15 ml of water.
4. Add 0.15 ml of 5M Acetic acid.
5. Add a sufficient quantity of distilled water to produce 50 ml. Stir immediately with a glass rod and allow it to stand for 5 minutes.

(B) Standard Solution:

1. Take 10 ml of 25% w/v solution of Barium chloride in a Nessler's cylinder.
2. Add 1.5 ml of Ethanolic sulphate standard solution (10 ppm SO_4^{-2}), mix and allow to stand for 1 minute.
3. Add 15 ml of Sulphate standard solution (10 ppm SO_4^{-2}).
4. Add 0.15 ml of 5M Acetic acid.

5. Add a sufficient quantity of distilled water to produce 50 ml. Stir immediately with a glass rod and allow it to stand for 5 minutes.

Table 1.2 Procedures for Preparation of Test and Standard Solutions

Test Solution	Standard Solution
• Transfer 1 ml of 25% w/v solution of Barium chloride in a Nessler's cylinder.	• Transfer 1 ml of 25% w/v solution of Barium chloride in a Nessler's cylinder.
• Add 1.5 ml of Ethanolic sulphate standard solution (10 ppm SO_4^{-2}).	• Add 1.5 ml of Ethanolic sulphate Standard solution (10 ppm SO_4^{-2}).
• Add 15 ml of Test solution.	• Add 15 ml of Sulphate Standard solution (10 ppm SO_4^{-2}).
• Add 0.15 ml of 5M Acetic acid.	• Add 0.15 ml of 5M Acetic acid.
• Dilute to 50 ml with distilled water and allow to stand for 5 minutes.	• Dilute to 50 ml with distilled water and allow to stand for 5 minutes.
• Observe the turbidity developed and compare with that of the standard.	• Observe the turbidity developed and compare with that of the sample.

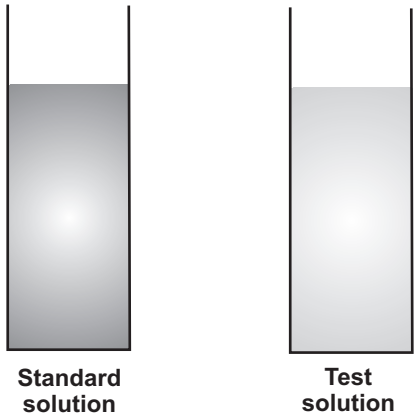


Fig. 1.5 Comparison of Standard and Test Solution

Observation and Conclusion: The turbidity in STD is seen more than that of the Test. Thus, the sample

1.4.4 Principle and Procedure of Limit Test for Iron

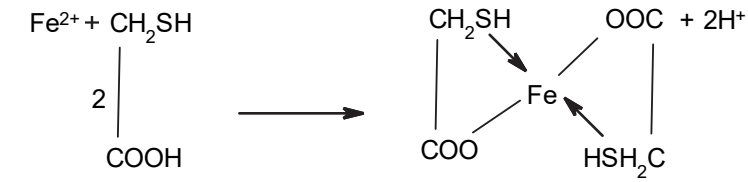
Aim: To perform the limit test for Iron.

Apparatus: Pair of Nessler's cylinders, Glass rods, Stand, Measuring cylinder, Spatula, Pipette.

Chemicals: 20% Iron free Citric acid, Thioglycollic acid, Iron Standard solution (20 ppm), Iron-free ammonia solution.

Preparation: Iron Standard Solution (20 ppm): Dilute 1 volume of 0.172% w/v solution of Ferric ammonium sulphate in 0.05 M Sulphuric acid to 10 volumes with water. Contains iron in the ferric state.

Principle: The test involves the chemical reaction between ferrous iron and thioglycollic acid in the presence of citric acid and ammoniacal alkaline medium. Wherein a pale pink to deep reddish purple colour is obtained. Ferric iron is reduced to ferrous iron by the thioglycollic acid, and ferrous thioglycollate is produced. Citric acid forms a soluble complex with iron and prevents its precipitation by ammonia as ferrous hydroxide. Ferrous thioglycollate is colourless in neutral or acid solutions. The colour due to the ferrous compound gets destroyed by oxidising agents. To avoid the interference of other ions, 20% iron-free citric acid is used. Citric acid forms a complex with other metal cations. The colour produced from the test substance is compared by viewing vertically with a standard solution (Ferric ammonium sulphate). If the colour of the test solution is less dark than the standard solution, then the test sample passes the test.



Thioglycolic acid — Ferrous thioglycolate

Procedure: Take two 50 ml Nessler's cylinders. Label one as 'Test' and the other's as 'Standard'.

(A) Test Solution:

1. Dissolve the specified quantity of substance under examination in distilled water or prepare a solution as directed in an individual monograph and transfer it to a Nessler's cylinder.
2. Add 2 ml of 20% w/v solution of iron-free citric acid and 0.1 ml of Thioglycollic acid. Mix, and make alkaline with iron-free ammonia solution.
3. Dilute to 50 ml with distilled water and allow it to stand for 5 minutes.

(B) Standard Solution:

1. Take 2 ml of Iron Standard solution (20 ppm) in 20 ml distilled water and transfer to a Nessler's cylinder.
2. Add 2 ml of 20% w/v solution of iron-free Citric acid and 0.1 ml of Thioglycollic acid. Mix, and make alkaline with iron-free ammonia solution.
3. Dilute to 50 ml with distilled water and allow it to stand for 5 minutes.

Table 1.3 Preparation of Test and Standard Solutions

Test Solution	Standard Solution
• Dissolve the specified quantity of substance under examination in distilled water and transfer in a Nessler's cylinder.	• Take 2 ml of Iron Standard Solution (20 ppm) in 20 ml water and transfer to a Nessler's cylinder.
• Add 2 ml of 20% w/v solution of iron free Citric acid and 0.1 ml of Thioglycollic acid.	• Add 2 ml of 20% w/v solution of iron free Citric acid and 0.1 ml of Thioglycollic acid.
• Make alkaline with iron free ammonia solution.	• Make alkaline with iron free ammonia solution.
• Dilute to 50 ml with distilled water and allow to stand for 5 minutes.	• Dilute to 50 ml with distilled water and allow to stand for 5 minutes.
• Observe the intensity of the purple, colour developed by viewing vertically and compare with that of the standard.	• Observe the intensity of the purple, colour developed by viewing vertically and compare with that of the sample

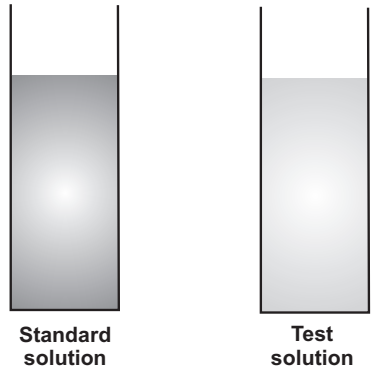


Fig. 1.6 Comparison of Standard and Test Solution

Observation and Conclusion: The intensity of the colour in STD is seen more than that of the Test. Thus, the sample passes the limit test for iron.

Note: *All the reagents used in the limit test for iron should themselves be iron-free. Hence, they themselves should conform to the limit.*

Table 1.4 Examples of Test Samples

Sample	Preparation
Sodium chloride	Dissolve 1 gm of Sodium chloride in distilled water. Use resulting solution for the limit test.
Sodium acetate	10 gm of Sodium acetate + 100 ml of distilled water. Use 20 ml of resulting solution for the limit test.
Magnesium sulphate	5.0 gm of Magnesium sulphate + 50 ml of distilled water. Use the 2 ml of resulting solution for the limit test.

Observation: The purple colour produced in the sample solution should not be greater than the standard solution. If the purple colour produced in the sample solution is less than the standard solution, then the sample will pass the limit test for iron and *vice versa*.

Reasons: Citric acid helps in the precipitation of iron by ammonia by forming a complex with it. Iron-free citric acid is used for complex metal cations other than iron, if any. Thioglycolic acid helps to oxidise Iron (II) to Iron (III). Ammonia is used to make the solution alkaline.

Result: Given sample passes/fails the limit test for iron.

1.4.5 Principle and Procedure of Limit Test for Arsenic

Aim: To perform the limit test for Arsenic.

Apparatus: Lead acetate, Cotton, Glass rod, Stand, Spatula, Measuring cylinder, Pipette (1 ml and 5 ml).

Apparatus for Limit Test for Arsenic:

1. A 120 ml capacity, wide-mouthed bottle fitted with a rubber bung through which passes a glass tube of height approximately 20 cm and diameter 6-8 mm is used. One end of this tube is constricted like that of a pipette with a 1 mm diameter having a hole of 2 mm diameter.
2. When the rubber bung is inserted into the bottle containing 70 ml of liquid, the constricted end of the tube should be above the surface of the liquid, and the hole inside should be below the bottom of the bung.
3. The upper end of the tube is cut off square and is either slightly rounded or ground smooth.
4. The rubber bungs (about 25 mm × 25 mm), each with a hole bored centrally and through, exactly 6.5 mm in diameter, are fitted with a rubber band or spring clip for holding them tightly in place.
5. The glass tube is lightly packed with cotton wool, previously moistened with Lead acetate solution and dried so that the upper surface of the cotton wool is not less than 25 mm below the top of the tube.
6. The upper end of the tube is then inserted into the narrow end of one of the pair of rubber bungs; to a depth of 10 mm (the tube must have a rounded-off end).
7. A piece of Mercuric chloride paper is placed flat on the top of the bung, and the other bung placed over it and secured by means of the spring clip in such a manner that the holes of the two bungs meet to form a true tube of 6.5 mm diameter interrupted by a diaphragm of Mercuric chloride paper.

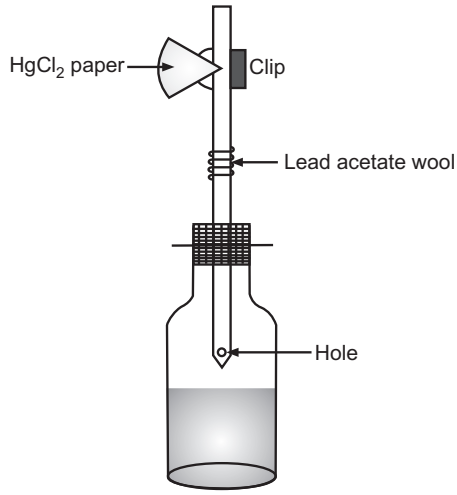


Fig. 1.7 Apparatus for Limit Test for Arsenic

Chemicals: Arsenic Standard Solution (10 ppm As), 1M Potassium Iodide, 10 g of Zinc AsT, Mercuric chloride test paper, Distilled water.

Preparation:

1. **Arsenic Standard Solution (10 ppm As):** Dissolve 0.33 gm of Arsenic Trioxide in 5 ml of 2M Sodium hydroxide and dilute to 250 ml with distilled water. Dilute 1 volume of this solution to 100 volumes with distilled water.
2. **1 M Potassium Iodide (AsT):** Dissolve 166 gm of Potassium Iodide in sufficient distilled water to produce 1000 ml.
3. **2 M Sodium Hydroxide Solution (AsT):** Dissolve 40 gm of Sodium hydroxide in distilled water to make the volume to 1000 ml with the same solvent.
4. **Lead Acetate Cotton Wool (AsT):** Immerse absorbent cotton in a mixture of 10 volumes of Lead Acetate solution and 1 volume of 2M Acetic acid. Drain off the liquids by absorbing them with filter paper and dry them at the R.T. store in a tightly-closed container.
5. **Mercuric Chloride Paper:** Prepared by impregnating filter paper in Saturated Mercury Chloride solution and drying at 60°C, avoiding contact with metal and in the dark. The paper used should be smooth, white and not less than 25 mm.
6. **Standard Hydrochloride Acid (AsT):** Add 1 ml of Stannous Chloride (AsT) solution to 100 ml of Hydrochloride Acid (AsT).

Principle: This test is based on the **Gutzeit test**. The limit test for Arsenic involves the reduction of all Arsenic into Arsine gas due to the combined action of Zinc and Potassium iodide. When Arsine gas comes in contact with dry Mercuric chloride paper, it produces a yellow to brown stain. The intensity and length of the stain is directly proportional to the amount of Arsenic present in the test substance.

$$Zn + 2HCl \rightarrow ZnCl_2 + 2(H)$$

$$2As + 6(H) \rightarrow 2AsH_3^{-}\uparrow$$

$$HgCl_2 + AsH_3 \rightarrow HgCl_2{\cdot}AsH_3$$

Yellow complex

Procedure: Take two sets of Arsenic Test Apparatus. Label one as 'Test' and the other as 'Standard'.

Table 1.5 Preparation of Standard and Test Solution

Test Solution	Standard Solution
• Dissolve given sample in 50 ml distilled water and add 10 ml of Stannated Hydrochloric Acid and transfer into the Arsenic Test apparatus bottle.	• Transfer 10 ml Arsenic Standard solution into the Arsenic Test apparatus bottle. Add 10 ml of Stannated Hydrochloric Acid.
• Add 5 ml of 1 M Potassium Iodide (AsT) and 10 gm of Zinc (AsT).	• Add 5 ml of 1 M Potassium Iodide (AsT) and 10 gm of Zinc (AsT).
• Immediately assemble the apparatus and keep the solution aside for 40 minutes.	• Immediately assemble the apparatus and keep the solution aside for 40 minutes.
• Compare the stain obtained on the Mercuric Chloride paper with that in the apparatus containing Standard Solution.	• Compare the stain obtained on the Mercuric Chloride paper with that in the apparatus containing Test Solution.

Observation and Conclusion: If the intensity of colour of the stain in the STD is seen more than that of the test, then the sample passes the limit test for arsenic.

Notes:

1. Lead acetate pledgers or papers are used to trap any Hydrogen sulphide which may be evolved along with Arsine gas.
2. Stannous chloride is essential for the complete evolution of Arsine. In the Arsenic Limit test, preference is given to Stannous salts because they reduce Arsenic to an Arsenious state and sometimes to the metallic state, whereas, Cadmium salts in themselves are not reducing agents.
3. Care must be taken that the Mercuric chloride paper remains quite dry during the test.
4. The most suitable temperature for running the test is generally about 40°C.
5. The tube must be washed with Hydrochloric acid (AST), rinsed with distilled water and dried between succeeding tests.

1.4.6 Principle and Procedure of Limit Test for Heavy Metals

Aim: To perform the limit test for Heavy Metals.

Apparatus: Nessler's cylinders, Glass rods, Stand, Measuring cylinder, Spatula, Pipette.

Principle: It is based on the reaction between the solution of heavy metals (i.e., Lead, Mercury, Bismuth, Tin, Cobalt, Manganese, etc.) and a saturated solution of Hydrogen sulphide. In acidic media, it produces metal sulphides, which are distributed in a colloidal state and produce reddish/black colour. The Colour intensity of the test solution is compared with the Standard Lead nitrate solution.

$$\text{Heavy Metals} + H_2S \longrightarrow \text{Heavy Metal Sulphide}$$

$$Pb(NO_3)_2 \xrightarrow{H_2S\ (excess)} (Pb^{+}) + H_2S{\cdot}PbS + 2HNO_3$$

$$PbCl_2 + Na_2S \longrightarrow PbS^{-} + 2NaCl$$

Chemicals: Lead Standard Solution (20 ppm Pb), Dilute Acetic Acid, Dilute Ammonia Solution, Hydrogen Sulphide solution, Distilled water.

Preparation:

1. **Lead Standard Solution (20 ppm Pb):** Dilute 1 volume of lead standard solution to 5 volumes with distilled water.
2. **Hydrogen Sulphide Solution:** Freshly prepared saturated solution of hydrogen sulphide in distilled water.
3. **Dilute Acetic Acid:** Dilute 57 ml of Glacial Acetic acid to 1000 ml with distilled water.
4. **Dilute Ammonia Solution:** Dilute 425 ml of Strong Ammonia solution to 1000 ml with distilled water.

Procedure:

Table 1.6 Preparation of Standard and Test Solutions

Standard Solution	Test Solution
• Pipette out 1 ml of Lead Standard Solution (20 ppm Pb) and transfer to 50 ml Nessler's cylinder. Dilute with distilled water to 25 ml.	• Place 25 ml of Test solution which is prepared as directed in individual monograph or dissolves the specified quantity of the substance in distilled water to produce 25 ml, in a Nessler's cylinder.
• Adjust a pH between 3 to 4 by using dilute acetic acid or dilute ammonia solution.	• Adjust a pH between 3 to 4 by using dilute acetic acid or dilute ammonia solution.
• Dilute with distilled water to about 35 ml and mix.	• Dilute with distilled water to about 35 ml and mix.
• Add 10 ml of freshly prepared hydrogen sulphide solution and mix.	• Add 10 ml of freshly prepared hydrogen sulphide solution and mix.
• Dilute to 50 ml with distilled water, allow standing for 5 minutes and viewing downwards over a white surface.	• Dilute to 50 ml with distilled water, allow standing for 5 minutes and viewing downwards over a white surface.

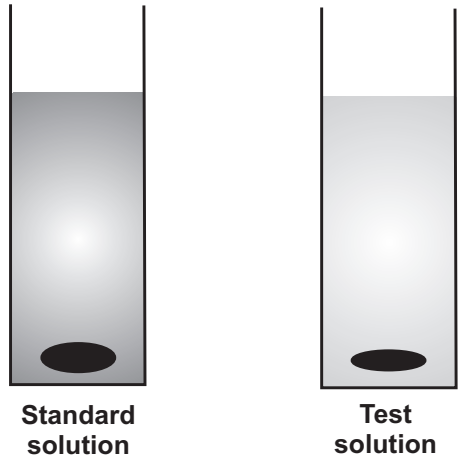


Fig. 1.8 Comparison of Standard and Test Solutions

Observation and Conclusion: The quantity of the black ppt of Lead Sulphide in STD solution is seen more than that of the Test solution. Thus, the sample passes the limit test for heavy metals.

QUESTION BANK

A. MULTIPLE CHOICE QUESTIONS

1. Which of the following error is caused by poor calibration of the instrument?
   A. Random error
   B. Gross error
   C. System error
   D. Precision error

Answer: C

2. Random errors in a measurement system are due to …….
   A. Environmental changes
   B. Use of un calibrated instrument
   C. Poor cabling practices
   D. Unpredictable effects

Answer: D

3. If the quantity to be measured remains constant while taking the repeated measurements, then the random errors can be eliminated by …….
   A. Calculating the mean of the number of repeated measurements
   B. Calculating the median of the number of repeated measurements

C. Calculating the sum of the numbers of repeated measurements
D. Either (a) or (b)

Answer: A

4. The error between the mean of finite data set and the mean of infinite data set is known as …….
   A. True error of the mean
   B. Standard error of the mean
   C. Finite error
   D. Infinite error

Answer: B

5. Which of the following statements is NOT true?
   A. Accuracy expresses the correctness of measurement
   B. Precision represents reproducibility of measurement
   C. High degree of precision implies a high degree of accuracy also
   D. High degree of accuracy implies a high degree of precision also

Answer: C

6. Which error affects the precision of measurement?
   A. Human error
   B. Systematic (or determinate) errors
   C. Random error
   E. Gross error

Answer: C

7. The closeness of the measurement to its true or accepted value is expressed by the error is known as …….
   A. Accuracy
   B. Precision
   C. Mean
   D. Median

Answer: A

8. The middle result when replicate data are arranged according to increasing or decreasing value is called as …….
   A. Median
   B. Mean
   C. Precision
   D. Accuracy

Answer: A

9. PPM means .....
   A. percent per million,
   B. percent parts per million,
   C. percent purity in millions,
   D. parts per million

Answer: D

10. Impurities in pharmaceutical preparations may be due to the following sources .....
    A. Raw materials,
    B. Manufacturing process,
    C. Chemical instability,
    D. All of the above

Answer: D

11. Limit test for Chloride has been based upon the reaction between ..... and ..... to obtain turbidity.
    A. Chloride and silver nitrate
    B. Iron and silver nitrate,
    C. Chloride and sulphuric acid
    E. None of these

Answer: A

12. The principle behind the limit test of Sulphate is .....
    A. To compare Opalescence of test solution with standard,
    B. To compare the Purple colour of the test solution with the standard,
    C. To compare the Pink colour of the test solution with the standard,
    D. None of the above

Answer: A

13. If the sample is lighter (in colour/ turbidity/ opalescence) than the standard solution, then it is .....
    A. Accepted
    B. Rejected,
    C. Rechecked
    D. None of above

Answer: A

14. What is the role of alcohol in the limit test of Sulphate?
    A. Prevents supersaturation and develops uniform turbidity
    B. Increases the sensitivity of the test

C. Prevents precipitation
D. All of these

Answer: A

15. In the limit test of Arsenic, Mercuric Chloride used produces a ..... stain.
    A. Pink
    B. Black
    C. Red
    D. Yellow

Answer: D

16. Limit test of Iron, ferric iron is reduced to ferrous iron by .....
    A. Ammonia
    B. Thioglycollic acid
    C. Chloroform
    D. Acetic acid

Answer: B

B. SHORT ANSWER QUESTIONS

1. Define the following terms:
    (a) Error
    (b) Accuracy
    (c) Precision
    (d) Significant figures
    (e) Mean
    (f) Median
    (g) Random error
    (h) Systematic error
2. Enlist the different types of errors.
3. What do you mean by Impurity?
4. Enlist various sources of impurities.
5. What are limit tests, and what is their significance?
6. Write specifications of Nessler's cylinder as per Pharmacopoeia.
7. Why Nessler's cylinder is required for the limit test?
8. Why only distilled water is used in the limit test?
9. Write the principle behind the limit test for Chlorides.
10. What is the modified limit test for Chlorides?
11. Write the reaction involved in the limit test for Chlorides.
12. What is the difference between a normal Chloride limit test and a modified limit test for Chloride?

13. How 0.1 M Silver Nitrate solution is prepared?
14. Discuss the principle of the limit test for Chloride.
15. Discuss limit test of Chloride for Potassium permanganate.
16. How standard Chloride solution is prepared?
17. What is the concentration of Chloride standard solution?
18. Why is nitric acid added to the Chloride limit test?
19. Why is silver nitrate used in limit tests for Chlorides?
20. What is the importance of the Chloride limit test?
21. Write the principle behind the limit test for Sulphate.
22. Write the reaction involved in the limit test for Sulphate.
23. Discuss the limit test for Sulphate in brief.
24. Why Potassium Sulphate in a small concentration is required in the limit test of Sulphate?
25. What is the role of Ethanolic Potassium sulphate in the limit test for Sulphate?
26. How standard Ethanolic Potassium sulphate solution is prepared?
27. What is the concentration of Sulphate in standard solution?
28. How Barium chloride solution is prepared?

C. LONG ANSWER QUESTIONS

1. Classify and discuss various types of errors with pertinent examples.
2. What are errors? Enlist sources of errors and explain types of errors with suitable examples.
3. What steps should be taken to minimise the following errors?
   (a) Instrumental errors (b) Method errors (c) Personal errors
4. Give the principle involved in the limit test for Iron as per I.P and describe it.
5. Write in detail raw materials as a source of impurity.
6. Enlist various sources of impurities. Discuss manufacturing hazards in detail.
7. Write in detail the Limit Test of Arsenic with its modifications.
8. Enlist sources of impurities in pharmaceutical substances and explain their effects on pharmaceutical substances.
9. Describe the limit test for Iron.
10. Discuss various sources of impurities in pharmaceutical substances.

11. Discuss the limit test for sulphate.
12. Explain the principle involved in the limit test for Lead I.P.
13. Illustrate the sources of impurities in pharmaceutical substances.
14. What is the effect of impurities on the quality of pharmaceuticals? Explain.

■■■