CHAPTER-1
Introduction: Drugs-Importance and Assay

The word drug is derived from the French word drogue, which means a dry herb. In a general way, a drug may be defined as a substance used in the prevention, diagnosis, treatment or cure of disease in man or other animals. According to WHO, a drug may be defined as any substance or product which is used or intended to be used for modifying or exploring physiological systems or pathological states for the benefit of the recipient and it is presumed that this refers to total benefit-physical, mental as well as economical. An ideal drug should satisfy the following requirements:

i) When administrated to the ailing individual or host, its action should be localised at the site where it is desired to act. In actual practice, there is no drug, which behaves in this way. It generally tends to distribute itself anywhere in the tissues of the host.

ii) It should act on a system with efficiency and safety.

iii) It should not have any toxicity.

iv) It should have minimum side effects.

v) It should not injure host tissues or physiological processes.

vi) The cells should not acquire tolerance or resistance to the drug after some time. In actual practice, the cells which were originally
susceptible to the action of a particular drug may after some time acquire tolerance or resistance to that drug.

Very few drugs satisfy all the above conditions. However, the search for ideal drug continues.

(a) Historical evolution of drugs:

To understand science, one has to know its history and development. Since the dawn of civilization, mankind has been concerned about their protection from the evils of diseases and sufferings. Conquering these afflictions often determined their survival but the current state of knowledge did not permit rational use of drugs, additional help was sought from supernatural powers. This was especially true of ancient Greeks and Indians who believed that the Gods dispensed prosperity or pestilence. There was a recognition that a regularity prevailed in the natural world that was independent of supernatural whim or will. This was a giant step in the making and formation of scientific medicine.

Human beings began to believe that nature alone could provide the means to remove pain and disease and thus they sought remedies in nature, i.e., in plants, minerals and animals. A variety of medicinal agents were collected on the basis of their symbolic qualities as well as their astrological signs and portents, e.g. Since the sword symbolized strength and power, the
early Greek Physicians attempted to use iron therapy against weakness and anemia. The observation that the horn of rhinoceros is powerful, led Chinese physicians to prescribe it as a potent aphrodisiac.

One should keep in mind that these practitioners brought forth their explanations in good faith. Many of their drugs were added to the therapeutic armamentarium only after considerable trial and error and application of clinical judgement. We should not automatically brand their explanations as silly and as having no basis for to-day’s rational standards. These early drug users were just as intelligent as we are. In the light of the knowledge then available they had good reasons for what they said and did. The answer to the question, what did they consider good reasons, is not a simple one. It must take into consideration their entire intellectual, ethical and cultural background.

The earliest references about medicine preparations in writing come from India or Rigveda and from China in their Materia Medica 2500-3000 B.C. In India, later on, a large number of medicine preparations including Ayurveda were described by physicians such as Chark, Sushruta, Vagbhata and others. All the drugs used were of vegetable origin. As a large number of drugs have been found to be listed on papyrus, and many clay tablets are discovered in Egypt, it is evident that the Western
medicinal system comes from Egypt and from the kingdoms of Assyria and Babylonia.

It was the Greek physician, Hippocrates (450 B.C.) who laid the foundation of modern medicine. According to him, a disease is a pathological process and its treatment with drugs is not a magic. The medicine system introduced by Hippocrates had a scientific basis, i.e.; it was based on observation, analysis and deductions. However, on the whole until the nineteenth century it was believed that the treatment of diseases was mainly based on the combination of guess work and experience. This resulted quack doctors in many countries. These doctors used therapeutic preparations comprised of a limited number of substances extracted from herbs and animals or from the earth in the form of minerals. Some 500 years later the Roman, Galen of pergamon, besides conducting impressively detailed investigations of animal anatomy, was a convinced herbalist who also used certain metallic salts, copper and zinc ores, iron sulphate and cadmium oxide, and introduced the assaying of preparations in efforts to control the quantity and quality of his dosages. The next major advances were introduced by two Persians named Rhazes and Avrienna, who, in the tenth and eleventh century respectively, introduced opium pills for coughs and extracts of wild autumn crocus (colchicum) seeds to treat gout. Both of
these remedies are still used in modern medicine. In Europe in the early
sixteenth century theophrastus paracelsus extrolled the virtues of antimony
salts as cure-alls, and, for a period, metal therapy dominated herbal recipes.
Theophrastus paracelsus, originally named Bombastus von Hohenheim, was
ironically frustrated by unsuccessful attempts during his life time to
introduce the useful laudanum (morphine tincture) for relief of pain and
tartar emetic, still a useful antimonial, for the dread schistosomiasis.

One of the greatest herbal remedies of all was introduced into Europe
in the seventeenth century by Jesuit missionaries who had accompanied the
Spanish consquistadors on their exploration of central and South America.
This was an extract of the cinchona bark obtained from South America.
 Indians who had long used it as an against chills and malarias in Europe.
In eighteenth century in England, Withering introduced the use of an extract
of the fox-glove plant for the treatment of dropsy, a heart condition
characterised by excessive accumulation of liquid in the lower limbs of the
afflicted. He used this extract on the personal recommendation of country
folk who had been using the elixir for untold years, a fine example of an
enquiring medical practitioner following up and developing a lead from folk
culture. The active material digitals, is still used today for threatened heart
failure and is even now obtained by extraction from the foxglove.
By the middle of the 19th century, modern medicine had brought to the fight against diseases only one effective weapon, i.e.; immunisation against smallpox. In quick succession came surgical anaesthesia and antisepsis. The last quarter of the 19th century marked the identification of the causative organisms of few diseases like malaria, plague, cholera, typhoid and dysentery so that areas of the globe till then mastered by them changed hands to man. Insect vectors were identified. Vigilant students of preventive medicine contributed more than their mite. Organic and biochemistry gave a tremendous fillip to the study of hormones and vitamins. During the last decade intensive work in chemotherapy has given us such valuable aids as the sulpha drugs and antibiotics.

In the subsequent years, the knowledge of the chemistry of natural substances particularly of enzymes increased. This was made possible with the help of new physical, chemical and biological techniques. It is widely accepted that the enzymes play some role in drug action.

In the last fifty years, there had occurred more spectacular advances in medicinal chemistry, particularly with the discovery of sulpha drugs and antibiotics. Recent years have seen the creation of new therapeutic agents by medicinal chemists working usually as part of interdisciplinary terms. All their names and contributions are too numerous to mention here.
The systematic research in pharmaceutical laboratories has led to the introduction of more and more synthetic drugs in the modern times. The synthetic work is carried out more or less along the following lines.

i) Compounds are synthesised whose structures are more or less similar to naturally occurring substances. This sometimes produces drug whose price is much less than the naturally occurring one.

ii) Attempts are made to prepare the compounds with simplified structure based on the structures of natural drugs. For example, the structure of a compound having specific physiological activity is varied systematically. This work sometimes leads to the discovery of new drug with simplified structure.

iii) Attempts are made to synthesize new drugs, which have the properties of certain natural products but have no relation to them in structure.

iv) Attempts are made to synthesize new drugs, which are unrelated in structure and properties to natural products.

Sometimes it is found that a drug discovered poses certain serious problems. For example, phthalidomide was at one time considered to be an ideal hypnotic due to its low toxicity. Later on, this was found to be
responsible for the birth of thousands of deformed children. Thus, this is not
used at all. Certain drugs are known which are abused on the whole it is
found that the beneficial effects of drugs are much more than the problems
created. However, these problems could be overcome.

(b) Sources of drugs:

1. Plants: Medicinal plants have been used to treat various diseases from
time immemorial. Crude medicinal preparations made from plants are called
‘galenicals’ because they were extensively used and popularized by Galen,
the famous Greek physician. They owe their actions to biologically active
ingredients contained in them. The important classes of active ingredients in
plants and their characteristics are as follows.

(a) Alkaloids: These are plant bases containing nitrogen which form salts
with acids. They are insoluble in water. Acid salts of alkaloids are freely
soluble in water. To day hundreds of synthetic alkaloids are being produced.
Examples are as follows:

Morphine (narcotic analgesic) from unripe capsule of papaver
somniferum. Ephedrine (bronchodilator) from plant Ephedra vulgaris.
Atropine (anticholinergic) from leaves of Atropa belladonna. Quinine
(antimalarial) from bark of cinchona. Reserpine (antihypertensive) from root
of Rauwolfia serpentina.
(b) **Glycosides**: They are ether-like combination of sugars and non-sugar moiety (cyclopentane-phenanthrene steroid nucleus). If a glycoside is boiled with mineral acid, it is hydrolysed and it splits off the sugar. The non-sugar residual part of the glycoside is called aglycone. Examples of glycoside are digoxin, a cardiac stimulant obtained from the leaves of Digitalis Lanata.

(c). **Oils**: They are immiscible with water but dissolve readily in solvents like ether chloroform and alcohol. Oils of medicinal value can be divided into three classes.

i) **Fixed oils**: Chemically these are esters of fatty acids and glycerol. Most of them are edible oils e.g., ground nut oil, coconut oil, mustard oil, olive oil. Some have pharmacological actions e.g., castor oil (purgative) and cod liver oil (rich source of vitamin A and D).

ii) **Volatile oils**: These are terpenes or their polymers. They are also called essential or flavouring oils. Most of them are liquids. For example, clove oil (anodyne-relieves pain when applied locally specially in toothache), eucalyptus oil, coriander oil, dill oil, ginger oil (carminative, for expulsion of gas from the stomach), methyl salicylate (oil of wintergreen) and terpentine oil (counter irritant, applied locally to relieve pain in arthralgia). Few volatile oils exist
in a solid form and they are known as stearoptenes e.g. camphor and menthol.

iii) Mineral oils: They are hydrocarbon by chemical nature and are obtained from petroleum e.g. liquid paraffin (lubricant and laxative).

(d) Resins: They are formed by oxidation or polymerization of volatile oils, e.g. podophyllum, colocynth, jalap. They are more of toxicological importance than pharmacological.

(e) Gums: They are secretory products of plants chemically they are related to polysaccharides. They form thick mucilage when mixed with water. Some gums are pharmacologically inert and are mainly used as emulsifying agents e.g. gum acacia, gum tragacanth; while other gums are active e.g. agar (bulk purgative), gum guggul (hypolipidemic).

(f) Tannins: They are non-nitrogenous compounds characterized by their astringent action on the mucous membrane, i.e. they precipitate proteins from the cells of the mucous membrane and have a protective action.
2. **Animals:** Some drugs are obtained from animals. Examples are as follows: Insulin (hypoglycemic) from pancreas of sheep, oxen and pigs. Thyroid extract (for hypothyroidism) from thyroid gland of oxen. Gonadotropins (sex hormone) from serum of pregnant mares. Pepsin (enzyme) from stomach of oxen and pigs.

3. **Human:** Some drugs are available from human source. Examples are as follows. Immunoglobulins from blood. Growth hormone from anterior pituitary. Chorionic gonadotropin from urine of pregnant women.

4. **Microbes:** They are mainly the source of antibiotics, i.e. chemical substances produced by one type of microorganism and lethal to others. Apart from antibiotics, certain other drugs have also been derived from microorganisms e.g. the enzyme streptokinase (fibrinolytic) as obtained from streptococcus. Examples of antibiotics are as follows. Penicillin from the fungus penicillium chrysogenum. Streptomycin, neomycin and actinomycin from Actinomycetaceae. Griseofulvin from penicillium griseofulvum. Nystatin from streptomyces nouresi.

5. **Minerals:** Some elementary substances like iron, iodine and sulphur are used in the treatment of diseases. Some metallic compounds like antimony salts for kalaazar and bismuth salts for peptic ulcer are valuable drugs.
Similarly magnesium and aluminium salts are widely used in antacid preparations. Some important examples are as follows:


6. Synthetic: Many new drugs today are synthetic. Even some of the drugs that were originally obtained from natural sources can be synthesized now. e.g. chloramphenicol. A study of the structure-activity relationship of known drugs often helps to make purposeful modification of the molecules so as to develop new drugs with desirable action. New semisynthetic penicillins, corticosteroids, cardiovascular drugs and diuretics etc., are few of the important examples.

(c) Some important terms used in chemistry of drugs:

1. Medicinal chemistry: Medicinal chemistry has been aptly defined by Dr. Glenn ullyuot as a field, which applies the principles of chemistry and biology to the creation of knowledge leading to the introduction of new therapeutic agents. Hence, the medicinal chemist must not only be a
competent organic chemist but also that he must have a basic background in the biological sciences, especially biochemistry and pharmacology.

2. **Pharmacy:** The clinician does not administer a pure compound, but a complex formulation of which the active constituent agent forms only a small part. Pharmacy is the study of the formulation of an active chemical entity, which is also known as the active principle. The drug so formed is the vehicle which is then considered most appropriate for the administration of a particular therapy; such vehicles are in the forms of tablets, capsules, powders, suppositories and aerosols.

   In general the tablet form is preferred because this package is usually the simplest to manufacture, transport, handle and imbibe, and is frequently the most suitable form for long-term storage. The active principle is only a small proportion of the whole tablet, whose bulk is composed of fillers and binders designed to hold the tablet together, and agents which are added to break up the tablet efficiently in the patient’s gastro-intestinal tract.

3. **Pharmacology:** This science is the study of the effects of pharmaca or biologically active substances on the animal system. It is restricted to therapeutic agents or drugs because it is also applicable to all active agents; that is, fungicides, insecticides, toxins, etc., which affect the living body. The word pharmacology is derived from the Greek words pharmakon (drug)
and logos (a discourse or a treatise) and hence includes such allied fields, as pharmacy, pharmacognosy, toxicology, posology, chemotherapy, therapeutics and materia medica.

4. Bacteria: These are a group of micro-organisms, which are unicellular and surrounded by rigid, complex, protein cell wall. These may be free living, saprophytic or parasitic; some are pathogenic to man, animals and plants. Bacteria are classified into two types, i.e.; grampositive and gramnegative according to a method developed by Christian Gram, which is as follows:

In this method, the fixed bacterial, smear is first treated with a solution of crystal violet and then with iodine solution, which reacts with the dye and the cell constituents. The smear is then washed with alcohol (decolourising agents) and safranin or some other counter stain is added.

The bacteria, which retain the colour of crystal violet and appear deep violet (in colour) are called Gram-positive bacteria, whereas those, which lose the violet colour and get counterstained by safranin and appear red in colour, are called gram-negative bacteria. The following are some of the disease causing bacteria classified in this manner.
<table>
<thead>
<tr>
<th>Gram +ve bacteria</th>
<th>Gram –ve bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diphtheria bacillus</td>
<td>Coli and typhoid bacillus</td>
</tr>
<tr>
<td>Leprocy bacillus</td>
<td>Gonococcus</td>
</tr>
<tr>
<td>Pneumococcus</td>
<td>Meningococcus</td>
</tr>
<tr>
<td>Staphylococcus</td>
<td>Plague bacillus</td>
</tr>
<tr>
<td>Streptococcus</td>
<td>Spirochaetes</td>
</tr>
<tr>
<td>Tubercle bacillus</td>
<td>Vibrios (v.cholerae)</td>
</tr>
</tbody>
</table>

5. Virus: These are very small micro-organisms, which are parasitic within living cells. These differ from bacteria in having only one kind of nucleic acid, either DNA or RNA, in lacking the apparatus necessary for energy production and protein synthesis and by not reproducing by binary fission but by independent synthesis of their component parts, which are then assembled. These can multiply in a living tissue or tissue culture but not in artificial culture medium. Virus may cause many kinds of acute and chronic diseases in man and can also cause tumours in animals.

6. Fungi (Singular-fungus): It is a low form of vegetable life including many microscopic organism. It does not contain chlorophyll and generally grows on organic matter like leather, stale food, sugar, fruit etc. It causes many superficial and systemic diseases in living beings.
7. Chemotherapy: The treatment of infectious disease by using a chemical agent is called chemotherapy. The substance so employed is referred to as chemotherapeutic agent. These agents are designed in such a way that they kill or destroy the diseases producing organisms without any harmful effect on the cells in which organisms are present.

(d) Biological and medical terms used in the study of drugs:

1. Antibiotics: Antibiotics are specific chemical substances derived from or produced by living organisms, which in small concentrations are capable of inhibiting the life processes of microorganisms.

2. Antibacterial: Antibacterial agents are the drugs used in the treatment of infections caused by bacteria. According to the effect produced, antibacterial agents can be bacteriostatic (inhibit growth of bacteria) or bactericidal (kill the bacteria).

3. Antifungal: Antifungal agents are the drugs used against the infection caused by fungi. They can be either fungistatics or fungicides. Fungi are parasites.

4. Anti-inflammatory: Anti-inflammatory drugs modify the inflammatory response to diseases but are not curative and do not remove the underlying cause of the disease. Any ideal anti-inflammatory drug should affect only aberrant, uncontrolled inflammation and not interfere with the normal
inflammatory response, which is a part of the body's vital defence mechanisms to invading micro-organisms.

5. CNS drugs: Central nervous system (CNS) composed of complex network of sub-units, which act as conducting pathways between peripheral nervous system, receptors and effectors. These drugs produce depressing effect on the central nervous system as their principal pharmacological action. These include general anaesthetics, hypnotics, sedatives and tranquilizers. Anaesthetics, hypnotics and sedatives produce depressing effect on central nervous system in the decreasing order. Sedatives exert milder depression on central nervous system. Hypnotics induce sleep while anaesthetics induce different degrees of depression finally leading to unconsciousness. Tranquilizers are the central nervous system selective depressants having skeletal muscle relaxant properties. Central nervous system is subjected to depression by these drugs in the following order depending upon dosage.

\[\text{Sedation} \leftrightarrow \text{Hypnosis} \leftrightarrow \text{Anaesthesia} \leftrightarrow \text{Coma} \rightarrow \text{Death}\]

6. Cardio-vascular drugs: These are the drugs, which influence hearts mechanism (either stimulate or depress the heart by different mechanism). They produce direct action on the heart or on the other parts of the vascular (blood vessels) system. These drugs affect heart muscles.
7. **Anti-viral drugs:** They are selective inhibitors of one or more unique steps of the replicate cycle of viruses. They improve antibody formation and activity. They are selectively active against either RNA containing or DNA containing viruses.

8. **Anti cancer drugs:** Cancer is a form of abnormal development, transforming normal cells into cancerous cells. It is a tumor, which means an unusual amount of growth or enlargement of a tissue due to unlimited and uncontrolled repeated divisions of cells. Anticancer drugs are used for the treatment of cancer in combinations, they interfere with cell division. Various alkylating agents react with DNA leading to cross linking, dipurination and scission, these agents interfere with enzymes required for biosynthesis of nicotinamide adenine dinucleotide (NAD), since the NAD content of tumour cell gets diminished after the treatment with alkylating agents.

9. **Vitamins:** They are comparatively simple organic compounds, which are required in small quantities by animals for their maintenance and normal growth of life. Except vitamin D, animal body cannot synthesise any other vitamin. They are mainly supplied by the food we take. If the diet lacks any one or more vitamins, a deficiency disease results. There are about 25
vitamins known. Of these, vitamins B and C are water soluble while vitamin A, D, E and K are fat soluble.

10. **Hormones:** They are chemical substances produced in certain specific parts of the body called ductless glands also known as endocrine glands. These glands deliver the hormones in small amounts directly into the bloodstream. These substances then exert physiological effect at a site of action, which is remote from its origin. They are required in small amount and are specific in their action. A deficiency of a particular hormone leads to a specific disease, which can be cured by the administration of that hormone.

(e) **Dosage forms**

For administration to patients, drugs are prepared and supplied in a variety of pharmaceutical forms known as dosage forms. Different dosage forms are:

1. **Syrup:** A 66% solution of sugar in water is called syrup. Flavoured syrup is used as a vehicle for active ingredients. It is more palatable and in some cases masks the bitter taste of a few active ingredients (syrup chloroquine). Widely used 'cough mixtures' containing antihistaminics, expectorants, decongestants, mucolytics or antitussives are mostly in syrup base.

2. **Tablets:** These are disc shaped solid preparations for oral use and generally do not weigh more than 0.5g. In addition to the active ingredient, a
tablet contains inert substances like disintegrator (calcium lactate, starch and binder gum). Some of these tablets are coated with sugar (sugar-coated) or with shellac or cellulose (enteric coated) to mask the bitter taste. Enteric coated tablets do not dissolve in the acidic juices of the intestine. Enteric coating prevents gastric irritation and protects some drugs from the hydrolytic action of gastric acid. It also helps to get the desired concentration of the drug in the small intestine.

3. Capsule: A capsule is a cylindrical envelope of gelatin in which a drug can be enclosed for oral administration. Drugs in powder form are filled in hard gelatin capsules where as spansules are time-release capsules where the granules of the drug have different coatings, which dissolve, at different time intervals. Such differential release of the drug provides uniform medication over a prolonged period, i.e. sustained release.

4. Ointments: Ointments are semisolid grease-like preparations for local application. Ointments are used as astringents, antiseptics and protective. If the medium or base is absorbable like animal fat, systemic effects may follow, suppositories, pessaries and bougies are solid preparations intended for insertion into cavities, viz; rectum, vagina and nasal cavities respectively. The base is either the oil of theobroma or glycerinated gelatin.
5. **Injections:** Liquid preparations meant for parenteral administration are called injections. They have to be sterile and depending upon the volume, are supplied in sealed glass ampules, rubber capped multidose vials or large infusion bottles or in polypropylene pouches. Examples are adrenaline ampules, lignocaine vials and dextrose saline infusion bottles. Sometimes vials contain powder, which has to be dissolved or suspended in colloidal form before injection by adding an adequate quantity of a suitable solvent.

6. **Enema:** Enema are liquid preparations meant for administration per rectum. They are of two types: retention enemas and evacuant enemas.

Retention enemas may be used either for a local action in the large bowel as in the case of prednisolone in ulcerative colitis, or for a systemic action after absorption of the active ingredient into the general circulation as in the case of paraldehyde enema. Usually 100-200 ml fluid is administered.

Evacuant enemas are used principally to washout the large bowel prior to surgery and to relieve constipation (soap-water enema). For diagnostic purposes, a suspension of barium sulphate is used as an enema in order to delineate pathological changes in the colon and rectum on an x-ray screen or film.
7. **Tinctures**: Tinctures are alcoholic or hydro-alcoholic extractive preparations of vegetable drugs; usually these represent 10 percent of the drug from which they are prepared.

8. **Inhalations**: Inhalations are medicaments meant to be inhaled or nebulised or aerosolized for their local action on the respiratory tract or for systemic effect after absorption.

(f) **The role of Analytical chemistry in Pharmacy**:

Analytical chemistry is a measurement science consisting of a set of powerful ideas and methods that are useful in all fields of science and medicine. An exciting illustration of the power and significance of analytical chemistry occurred on July 4th 1997.

The pathfinder example demonstrates that both qualitative information and quantitative information are required in an analysis. Qualitative analysis establishes the chemical identity of the species in the sample. Quantitative analysis determines the relative amounts of these species or analytes in numerical terms.

Analytical chemistry is applied throughout industry, medicine, and all the sciences. For example, analysis of steel during its production permits adjustment in the concentration of such elements as carbon, nickel, and
chromium to achieve a desired strength, hardness, corrosion, resistance and ductility.

Quantitative analytical measurements also play a vital role in many research areas in chemistry, biochemistry, biology, geology, physics, and other sciences. For example, quantitative measurements of potassium, calcium and sodium ions in the body fluids of animals permit physiologists to study the role these ions in nerve signal conduction as well as muscle contraction and relaxation. Chemists unravel the mechanisms of chemical reactions through reaction rate studies. The rate of consumption of reactants or formation of products in a chemical reaction can be calculated form quantitative measurements made at equal time in travels. Many chemistry and medicinal chemists devote much time in the laboratory gathering quantitative information about systems that are important and interesting to them.

Analytical chemistry is a similar function with respect to the many other scientific fields. The interdisciplinary nature of chemical analysis makes it a vital role in medicine, industrial, government and academic laboratories throughout the world.

A typical quantitative analysis involves the sequence of steps shown in the flow diagram of figure-1. In some instances, one or more of these
steps can be omitted. A flow diagram is showing the steps in a quantitative analysis. There are a number of possible paths through the steps in a quantitative analysis. In the simplest example represented by the central vertical pathway, we select a method, acquire and process the sample, dissolve the sample in suitable solvent measure a property of the analyte, calculate the results, and estimate the reliability of the results. Depending on the complexity of the sample and the chosen method, various other pathways may be necessary.

(g) Assay of drugs:

The measure of the biological activity of a drug is called its potency. Assay is the estimation of the potency of the active principle in the unit quantity of medicinal preparation.

1. **Chemical assay:** In this case, the potency of the active principle in the drug preparation is determined by chemical methods. The choice of chemical method depends upon the nature of the functional group present in the drug and its chemical properties. For instance the drug having hydroxyl group can be estimated by acetylation, the drug having ester or amide group can be estimated by hydrolysis, the drug having carboxylic group can be estimated by direct titration with an alkali, the drug having amino group can be estimated by acetylation or if the drug has aromatic amino group, it is
estimated by diazotisation, and soon. Certain special methods have been
developed for the estimation of individual drugs, e.g., penicillin is estimated by iodometry.

2. Instrumental assay: More recent instrumental analysis such as various spectroscopic, chromatographic and photometric techniques have been advantageously employed for drug assay. These methods are rapid, require smaller quantities of substance, can be readily compared with standards can be obtained from various pharmacopoeias such as I.P, U.S.P or B.P and the assay reports can be preserved.