CHAPTER 1

INTRODUCTION
1.1 INTRODUCTION

It was truly said by prehistoric philosophers that well people make the nation strong and prosperous. People in developed countries are very health conscious compared to developing countries. Lack of health awareness, malnutrition, mental stress sanitary facilities, basic hygiene, pollutes air and water invites many diseases. To overcome such diseases mankind has developed different systems for treatment of illness. These are mainly Ayurveda, Homeopathy, Unani and Allopathy. Amongst this Allopathy is the most popular science due to its reliable, rapid and safe cure of diseases. Allopathy makes use of drugs and drug products, which are proved to be of good quality, safe and effective as compared to any other traditional systems of medicines. Pharmaceutical industry has developed dramatically to make allopathic medicines palatable and very easy to administer giving a fairly good compliance rate.

The 21st century offers a bright vision of good health for all. It holds the prospect not merely of longer life, but superior quality of life, with less disability and diseases. Quality is important in every product or service but it is vital in medicines, as it involves life. Unlike in consumer’s goods there is no “second quality” in medicines. Hence it is a paramount responsibility of drug manufacturers to produce drugs, which are effective, safe and economically affordable to the common public.

1.2 Health care in India

Access to health care is a fundamental human right. Health care encompasses everything that prevents ill health and promotes well-being. Therefore, total living environment such as proper nutrition, potable water, adequate shelter, clothing, clean air, hygiene, sanitary facilities, medical care and MEDICINES (Drugs) are all needed to promote good health.

1.2.1 Different Medicinal systems

Ever since the dawn of turbulent history man has evolved several ways of coping with illness. Different societies have looked for different substances and ways that may ease pain and elevate spirits. All ancient civilizations have thus developed their own medical systems which reflect not only specific philosophies but also appear to be influenced by the then existing social beliefs and practices.
In India Ayurvedic systems appeared to be the most formal and organized amongst the traditional systems.

Global estimate indicates that 75% of the five billion world population cannot afford modern drugs. As a part of the strategy to reduce financial burden of developing countries which spend over 40-50% of their total health budget on drugs. World Health Organization (WHO) currently encourages, recommends and promotes inclusion of traditional remedies in National Health Care Programs because such drugs are easily available at low cost, are safe and as such are time tested. Traditional systems of medicines continue to be widely practiced in India and the annual production of traditional drugs is about 350 million. The biggest stumbling block that the traditional drug industry faces today is the standardization and purity control of such drugs. It is the need of the hour that like modern drugs traditional drugs should also be analyzed and proper quality control techniques should be developed to verify the quality and quantity of these drugs. Allopathic medicines often give rapid cure medications which are essentially symptomatic, for example, antipyretic and analgesic agents. The pharmaceutical industry has also developed dramatically to make the allopathic medicines palatable and very easy to administer giving a fairly good compliance rate.

1.2.2 Medicines and their classification

According to United state Pharmacopeias (USP), Medicines means

- “Articles intended for use in the diagnosis, cure, mitigation, treatment or prevention of disease in man or other animals”.
- “Articles (other than food) intended to affect the structure or any function of the body of the man or other animals”.

Most drugs fall into the following categories.

- Which boost supplies of chemicals in the body that are too low?
- Which suppress levels of chemicals or block their effects when they are out of control or have risen too high?
- Which are designed to make the body to produce chemicals it would not normally make except under attack?
Medicines (Vaccines) are classified in various ways. However, on the pharmacological basis of therapeutics they are classified as follows.

- Medicines acting at synaptic and neuroeffector junctional site. (Psychotropics)
- Medicines acting on the Central nervous system. (Antiparkinson, Antimuscarinic, Anticonvulsant)
- Autacoids, Drug therapy of inflammation (Anti inflammatory).
- Medicines affecting renal and cardiovascular system. (Antihypertensive and cardiovascular)
- Medicines affecting gastrointestinal function. (Antacids, Anti cholinergic, Antispasmodics)
- Chemotherapy of microbial diseases (Antibiotic and Antibacterials)
- Chemotherapy of neoplastic diseases (Anticancer)
- Medicines used for immunomodulation (Immunomodulator)
- Medicines used for blood and blood forming organs.
- Hormones and hormones antagonists.
- The vitamins.
- Dermatologic.
- Ophthalmic.
- Toxicologic

1.3 **Role of pharmaceutical Industry:**

Medicines are needed to promote good health. The role of pharmaceutical industry is limited to providing Medicines as an important component of health care package. Over the years, the pharmaceutical industry has played a vital role in human battle against diseases, disability and sufferings at global level. Besides servings at global level, besides serving the nation, the Indian pharmaceutical industry has also made significant contributions in elevating the economy of the country. For most of the century, doctors and drug industry have tried to make medicines in the laboratory out of synthetic chemicals and to maintain more control over Quality and Quantities of their product.
Safe and effective drug therapy however is possible only with strict observation of Current Good Manufacturing Practice (CGMP) of drugs and careful quantity monitoring of their active ingredients in pharmaceutical preparations and in bulk drugs.

"Quality" and "Cost effectiveness" are the buzzwords in today’s changed environment of globalized and liberalized economy and the only way to survive in this competitive world is by maintaining a constant upgradation of quality. Quality is important in every product or service but it is vital in medicine as it involves life. Unlike ordinary consumer’s goods there is no "Second" qualities in drugs. Quality is the sum of all the factors which contribute directly or indirectly to the safety, efficiency or acceptability of the product.

With signing of GATT (General Agreement of Trade and Treaty) and agreeing to TRIPS (Trade Related Intellectual Property Rights) the concept of global economy has finally taken off and the pharmaceutical industries can no longer rely on their traditional market. The key word to stay ahead of the competition is quality. It has been proved beyond doubt that the quality cannot be achieved by end product testing alone but quality has to be built into the product as well as in the system.

This involves,

- Defined project execution i.e. design and construction of production and laboratory area.
- Development of a stable, safe and reliable product.
- Development of document and validation of processes.
- Control of raw materials, packaging components, intermediate product, finished products and trained motivated workforce.

The purpose of any manufacturer is to sell a targeted customer, a right product at the right price and right quality aspects. In the case of pharmaceutical industry business, the right product is the one (various dosage forms) which conforms to all its requirements in term of physical, chemical and microbiological parameters throughout its shelf life. The functions, responsible to achieve these objectives are called as Quality Control.
1.4 Quality Control in pharmaceutical industry:

Quality as per the dictionary definition is the degree of excellence which thing possesses. Quality control is defined as the management function to control quality of a product to a defined set of standards.

The quality control (QC)\(^{[5,6]}\) function is an organization which normally consists of at least two primary units. Analytical control and Inspection control

The analytical control laboratory is responsible for testing and approving of raw materials, work in process and finished products.

Inspection control refers to the responsibilities assumed by QC that are ancillary to the analytical testing. These include sampling and inspection of incoming raw materials, packaging and labeling components, the physical inspection of product at various intermediate stages- packaging line inspection and batch production document review.

Only after the production document review has been completed satisfactorily, the batch may be released for distribution. Government legislation in America and in Britain in 70’s brought about what is called as “Good Manufacturing Practice” (GMP).

GMP can be defined as a set of detailed procedures during product manufacturing documentation of all relevant aspects encountered during manufacturing including quality control. In our country recently in 1988, GMP has been made statutory and a set of guidelines to be followed during manufacturing has been included, also a new schedule called “Schedule M” in “Drug and Cosmetic act and rules”. Thus mere testing of raw materials and finished products gave way to detailed in process checks during manufacturing and compliance to GMP’s\(^{[7]}\). There was more assurance of product quality in last two decades than mere control of the 60’s and the department was renamed as “Quality Assurance department”, in place of “Quality control” department.

1.5 Quality Assurance in Pharmaceutical Industry:

Drugs being a very important component of health care system need special attention with regards to their quality, efficacy and safety. This is achieved in pharmaceutical industry by
maintaining high standards of good manufacturing practices and good laboratory practices. The overall assurance of quality is governed and maintained by Quality Assurance Department.

Quality Assurance (Q.A.) \[^{8,9,10}\] may be defined as the responsibility of an organization to determine the systems, facilities and written procedure. Quality assurance is responsible for designing, implementing equipment and process validation protocols. Normally, Standard operating procedures (S.O.P’s) are developed, which when followed by properly trained operators will help to assure the quality and integrity of the product. In recent years ISO 9000 certification is very important for EXPORT. ISO 9000 is a Quality Audit for system. It follows very simple principle “Write what you do and do what you write”. Thus quality management can be simply defined as a function which is concerned with preventing problems from occurring by creating the right attitudes and controls.

In today’s context the emphasis of a “Current Good Manufacturing Practice” (C.G.M.P.) demands a zero defective approach to pharmaceutical preparations and in bulk productions, as quoted by John Ruskin “Quality is never an accident. It is always the result of intelligent effort. There must be the will to produce a superior thing”. To maintain the quality of drugs and pharmaceutical products, it is essential that Quality Assurance department must adopt “Current Good Laboratory Practice” (C.G.L.P) to ensure reliability and accuracy of the results given out by them.

1.6 Good Laboratory Practices in Pharmaceutical Industry:

In practice the quality of medicines or pharmaceutical products is assured through quality control. It is therefore essential that quality assurance department must adopt “Good Laboratory Practice” \[^{11}\] to ensure reliability and accuracy of the results given out by them. The assurance of the quality and reliability of pharmaceuticals, together with their careful control are our moral obligations.

The attainment of this “Quality” objective requires involvement and commitment of all concerned at all stages.

All these efforts taken by an organization with respect to Quality Assurance, Good Manufacturing Practice and Quality Assurance, Good Manufacturing Practice and Quality Control lead us to a better quality and acceptability of the products.
In India, code of Good Laboratory Practice (GLP) is yet to be standardized. In America, GLP is mandatory and hence, regulatory compliance process presumes that testing procedures are in accordance with GLP. The final test reports of the drugs have the integrity to allow accountable decision on end product use.

Obviously, the organizational process and the conditions under which laboratory studies are planned, performed, monitored, recorded, and reported makes compliance procedures imperative. These include onsite audits of a test facility and its procedures, in order to determine whether these are in conformance with GLP.

Broadly, GLP strives to promote the quality and validity of test data and provides a solid working basis for authorities and industry studying the properties and risks of the drugs. The results obtained by any analytical method may not yield useful information unless enough amount of validation is carried out before employing the method. GLP contributes to minimize technical trade barriers. Uniform quality of test results formalized under GLP constitutes a prerequisite for the acceptance of data by authorities of other countries. Such reciprocal acceptance avoids duplication of tests and thus saves materials and labor.

As per GLP, it is mandatory to maintain a record of the qualification, training, experience and job description for each professional and technical individual, and also, by records, prove that person clearly understands the functions they are to perform and wherever necessary, have been given training for these functions. It also helps to show that health and safety precautions are applied according to current regulations.

But, above all that, regulatory authorities want appropriate Standard Operating Procedures (SOPs) established and followed; and a historical file of all SOPs maintained. Each laboratory unit should have readily available SOPs relevant to the activities being performed. Published text books and manuals may be used as supplements to these SOPs. SOPs must be available at the very least for the following categories of the laboratory activities.

- Tests for Reference Substances

Receipt, identification, labeling, handling, sampling, storage of reference standards, Validation of analytical method and instruments.
1.7 Importance of analytical methodology in the drug development process

During the drug development process, key decisions are based upon data obtained from analytical test methods. Results generated from these test methods are expected to be both accurate and reliable. In later stages of development, the methodology is expected to be robust because the methods will be ultimately transferred to a control laboratory. Throughout the development process, individual test methods are used in a variety of laboratory settings in the evaluation of:

- Product safety.
- The dosage form's bioavailability.
- The setting of specifications for the drug substance, intermediates and drug product.
- The shelf life of the product (Stability).
- Determining optimum formulation.
- Identification, quantitation and "qualification" of impurities and degradants.
- Determining optimum crystalline and salt form of the drug substance, testing support to process
- Development and validation.
- Support to preclinical and clinical studies (Product safety and efficacy)

In addition, data from analytical test methods are required to support the regulatory filings. In brief, the chemistry, manufacturing and control section of a U.S. regulatory filing is required to contain the following information directly related to analytical testing.

- Method validation.
- Physical description and characterization of the drug substance and drug product — Proof of the chemical structure.
• In-process controls.
• Characterization of reference standards.
• Specifications and description of analytical methods.
• Evaluation of container/ closure system for storage.
• Drug development and drug substance stability.
• Justification of drug product development
• References to compendial test methods for inactive ingredients.
• Bioequivalence.
• Pharmaceutical development report.

1.8 Validation of analytical methods:

Modern medicines for human use are required to meet exacting standards which relate to their quality, safety and efficacy. Therefore, the overall purity of a medicine must be accessed throughout its storage, distribution and use. This objective can be achieved if the specifications applied are based on a validated procedure. It is therefore imperative that, analytical procedure proposed for analysis of a particular active ingredient or its dosage form, be systematically evaluated so as to demonstrate that the method is the process by which it is established, by laboratory studies, that the performance characteristics of the method meet the requirements for the intended analytical applications. Internationally various guidelines are available but amongst guideline laid by International Conference on Harmonization (ICH), Q2A and Q2B [12,13] is widely used.

The typical validation performance characteristics that should be considered for validation are specificity, precision, accuracy, linearity, range, limit of detection, limit of quantification and robustness.

1.8.1 Specificity / Selectivity

The analyte should have no interference from other extraneous components and be well resolved from them. A representative high performance liquid chromatography (HPLC) chromatogram or profile should show that the extraneous peaks either by addition of known compounds or samples from stress testing are baseline resolved from the parent analyte.
1.8.2 Accuracy

The accuracy of the analytical method is a measure of the systematic error or bias and is defined as the agreement between the measured value and the true value. Accuracy is the best reported as percentage bias, which is calculated from the expression.

\[
\%\text{Bias} = \left(\frac{\text{Measured value} - \text{true value}}{\text{true value}}\right) \times 100
\]

Since the true value is not known for real samples, an approximation is obtained, based on spiking drug-free matrix to a nominal concentration. The accuracy of the analytical method is then determined at each concentration by assessing the agreement between the measured and nominal concentration of the analytes in the spiked drug-free matrix samples.

Accuracy studies for drug substance and drug product are recommended to be performed at the 80, 100 and 120% levels of label claim as stated in the Guideline for Submitting Samples and Analytical Data for Methods Validation.

For the drug product, this is performed frequently by the addition of known amounts of drug by weight or volume (dissolved in diluent) to the placebo formulation working in the linear range of detection of the analyte. This would be a true recovery for liquid formulations. For formulations such as tablet, suppository, transdermal patch, this could mean evaluating potential interaction of the active drug with the excipients in the diluent. From a practical standpoint, it is difficult to manufacture a single unit with known amount of active drug to evaluate recovery. This test evaluates the specificity of the method in the presence of the excipients under the chromatographic conditions used for the analysis of the drug product. It will pick up recovery problems that could be encountered during the sample preparation and the chromatographic procedures. However, it does not count the effect of the manufacturing process.

At each recommended level studied, replicate samples are evaluated. The relative standard deviation (RSD) of the replicates will provide the analysis variation or how precise the test method is. The mean of the replicates, expressed as % label claim, indicates how accurate the test method is.
1.8.3 Detection Limit and Quantitation Limit

These limits are normally applied to related substances in the drug substance or drug product. Specifications on these limits are submitted with the regulatory impurities method relating to release and stability of both drug substance and drug product.

Detection limit is the lowest concentration of analyte in a sample that can be detected, but not necessarily quantitated, under the stated experimental conditions.

Quantitation limit is the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions.

With ultra violet (UV) detectors, it is difficult to assure the detection precision of low level compounds due to potential gradual loss of sensitivity of detector lamps with age, or noise level variation by detector manufacturer. At low levels, assurance is needed that the detection and quantitation limits are achievable with the test method each time. With no reference standard for a given impurity or means to assure detectability, extraneous peak(s) could "disappear/appear." A crude method to evaluate the feasibility of the extraneous peak detection is to use the percentage claimed for detection limit from the area counts of the analyte. For example, detection limit claim of 0.01% for the analyte integrated area count of 50,000 will give an area count of 5 that is not detectable. Though USP expresses detection limit and quantitation limit in terms of 2 or 3, and 10 times noise level respectively, this concept is not very practical. Noise level on a detector during the method development phase may be different when samples are assayed on different detectors, etc. The use of standard(s) in the test method at the quantitation limit level (proposed by the applicant) is assurance that the impurity can be observed and quantitated.

Detector sensitivity can vary with the model number and/or manufacturer for the analysis of a compound by two commercial detectors. The data should not be taken as the expected ratio of sensitivity of the two detectors. It is not known if other parameters which can also play a part, e.g., age of lamp, column, were considered when setting these limits.

1.8.4 Linearity

In chromatographic methods of analysis peak area or peak height may be used as the response function to define the linear relationship with concentration known as the calibration model. It is
essential to verify the calibration model, selected to ensure that it adequately describes the relationship between response function (Y) and concentration (X). The difference between the observed Y-values and the fitted Y-value or residual should be examined for a minimum six unique concentrations. A plot of studentised residual (raw residual / standard error) V/S log concentration will then show how well the model describes the data.

1.8.5 Precision

Precision is the measure of how close the data values are to each other for a number of measurements under the same analytical conditions. International Conference on Harmonization (ICH) has defined precision to contain three components: repeatability, intermediate precision and reproducibility. Ruggedness as defined in USP 27, incorporates the concepts described under the terms "intermediate precision", "reproducibility" and robustness" of this guide.

1.8.5.1 Repeatability

1.8.5.1a Injection Repeatability

Sensitivity is the ability to detect small changes in the concentration of the analyte in the sample. Sensitivity can be partially controlled by monitoring the specification for injection reproducibility (system suitability testing).

The sensitivity or precision as measured by multiple injections of a homogeneous sample (prepared solution) indicates the performance of the HPLC instrument under the chromatographic conditions and day tested. The information is provided as part of the validation data and as a system suitability test. The specification, as the coefficient of variation in % or relative standard deviation (RSD), set here will determine the variation limit of the analysis. The tighter the value, the more precise or sensitive to variation one can expect the results. This assumes that the chromatograph does not malfunction after the system suitability testing has been performed. Keeping in mind, however, that it does not consider variations due to the drug product manufacturing and laboratory sample preparation procedures. The set of four duplicate samples were injected sequentially. Variations in peak area and drift of retention times are noted.
1.8.5.1b Analysis Repeatability

Determination, expressed as the RSD, consists of multiple measurements of a sample by the same analyst under the same analytical conditions. For practical purpose, it is often combined with accuracy and carried out as a single study.

1.8.5.2 Intermediate Precision

Intermediate precision was previously known as part of ruggedness. The attribute evaluates the reliability of the method in a different environment other than that used during development of the method. The objective is to ensure that the method will provide the same results when similar samples are analyzed once the method development phase is over. Depending on time and resources, the method can be tested on multiple days, analysts, instruments, etc. Intermediate precision in the test method can be partly assured by good system suitability specifications. Thus, it is important to set tight, but realistic, system suitability specifications.

1.8.5.3 Reproducibility

Reproducibility expresses the precision between laboratories.

(Collaborative studies usually applied to standardization of Methodology)

1.9 Range

The range of an analytical procedure is the interval between the upper and lower concentration of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

1.10 Robustness

ICH defines robustness as a measure of the method's capability to remain unaffected by small, but deliberate variations in method parameters. Robustness can be partly assured by good system suitability specifications. Thus, it is important to set tight, but realistic, system suitability specifications.
1.11 System Suitability Specifications and System Suitability Testing

System suitability test parameters and acceptance criteria are based on the concept that the equipment, electronics, analytical operations, and samples to be analyzed constitute an integrated system. System suitability testing ensures that the system is working properly at the time of analysis. Appropriate system suitability criteria should be defined and included in the analytical procedure. All chromatographic analytical procedures should include system suitability testing and criteria. Parameters typically used in system suitability evaluations are defined and discussed in the Center for Drug Evaluation & Research (CDER) reviewer guidance on Validation of Chromatographic Methods (November 1994).

System suitability testing is recommended as a component of any analytical procedure, not just those that involve chromatographic techniques. Regardless of the type of analytical procedure, testing should be used to confirm that the system will function correctly independent of the environmental conditions.

1.12 Sample Solution Stability

Solution stability of the drug substance or drug product after preparation according to the test method should be evaluated according to the test method. Most laboratories utilize auto samplers with overnight runs and the sample will be in solution for hours in the laboratory environment before the test procedure is completed. This is of concern especially for drugs that can undergo degradation by hydrolysis, photolysis or adhesion to glassware.

1.13 Stressed conditions / Forced degradation studies

Degradation information obtained from stress studies (e.g., products of acid and base hydrolysis, thermal degradation, photolysis, and oxidation) for the drug substance and for the active ingredient in the drug product should be provided to demonstrate the specificity of the assay and analytical procedures for impurities. The stress studies should demonstrate that impurities and degradants from the active ingredient and drug product exipients do not interfere with the quantitation of the active ingredient. Stress studies are described in various Food and Drugs Administration (FDA) guidance’s relating to the stability of drug products. The design of the stress studies and the results should be submitted to the stability section of the application. Representative
instrument output (e.g., chromatograms) and/or other appropriate data (e.g., degradation information obtained from stress studies) should be submitted in the sections on analytical procedures and controls

1.14 SCOPE AND METHODOLOGY OF PRESENT WORK

The need for expeditious and reliable testing has been increasing in the field of medicinal formulations. Today in pharmaceutical industry, drug analysis plays a vital role in deciding the quality of the product. The selection of analytical method used to determine the active ingredients of the drugs and impurities in bulk drugs and in formulations is a challenging problem. The method should be sensitive, accurate, rapid, precise, and reproducible, stable (stability indicating) and free from the interferences and for the excipients used in the formulations.

Analytical chemistry in its broadest sense encompasses the theory and practice of acquiring the information about the composition of matter, qualitatively and quantitatively. An analyst from laboratory therefore requires precise, selective, sensitive and rapid method of qualitative and quantitative analysis of the drugs for their purities and impurities if any.

In the last four decades many instrumental techniques have been developed which have been replaced the laborious and lengthy classical analytical methods. The instrumental methods of analysis are extremely sensitive and they provide precise and detailed information for a very small amount of samples. Amongst the various instrumental techniques, the widely employed techniques for the pharmaceutical analysis now days are chromatographic technique such as,

High Performance Liquid Chromatography (HPLC)
High Performance Thin Layer Chromatography (HPTLC)
Gas Chromatography (GC)

In the present study, an attempt has been made to explore most widely acceptable High Performance Liquid Chromatography (HPLC) technique for the analysis of various medicinal products. The drugs chosen for the present study are recently introduced new drugs Tegaserod Maleate, Eprosartan Mesylate, Ranolazine, Acipimox, Rufinamide Rifaximin and Levobetaxolol for which no analytical method have been reported in pharmacopeias.
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