PART-I

1.2 GENERAL INTRODUCTION:

Health care is an integral part of social welfare. Economic progress is not meaningful without social well being. This can be achieved by proper nutrition, potable water, adequate shelter, clothing, clean air and sanitary facilities. Pharmaceutical industry produces almost all medicines for preventing and curing ill health.

Medicines means, according to UNITED STATE PHARMACOPOEIA (USP).

- 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 man or other animals.

Most of the drugs fall into the following categories:

1. Which boost supplies of chemicals in the body that are too low.
2. Which suppress levels of chemicals or block their effects when they are out of control or have risen too high.
3. Which are designed to make the body to produce chemicals it would not normally make except under attack (vaccines).

Ever since the beginning of human life on the earth there have been various kinds of ailments which have bothered the human beings. There has been a continuous on going battle between the nature and human being in this regard with the mankind trying to develop drugs, which can cure ailments.

Over the years, the pharmaceutical industry has played a vital role in human battle against diseases, disability and sufferings at global level. Some of epidemic diseases which claimed millions of lives are malaria, bubonic plague, jaundice, tuberculosis, cancer and the recent among them being the AIDS. To counter...
these diseases mankind has tried methods of ayurveda, herbal therapy, homeopathy and different methods of allopathic such as chemotherapy, radiation and surgery as also psychotherapy and physiotherapy. Of all the therapies, chemotherapy has been used most widely nowadays due to the rate at which it provides the relief from the ailments. It also decides the success of treatment obtained through surgery and psychotherapy. Chemotherapy has also helped in improving the life expectancy of and over all health in newborn, infants and older children. This has highlighted the importance of the pharmaceutical industry.

Medicines constitute the most cost-effective segment of healthcare. Various classes of drugs such as antibiotics, antiinfective, antineoplastic, steroids, immunizing agents, biotechnological products and genetically engineered products where the focus has been more selective mode of drug action are available in the market today. They protected patients from prolonged sickness and premature death, reduce the need for hospitalization and improve productivity and quality of life. Prescription drug therapy often eliminates the need for other costly interventions in health care like surgery, hospitalization, physician visits, and nursing care, increased drug spending keeps asthma patients out of the hospital. A recent study sponsored by the National Institute of Health (NIH), USA found that treating stroke patients promptly with a clot busting drug not only reduces disability, but also saves health care costs.

The pharmaceutical Industry today faces some of its toughest challenges. It must produce “block–buster” drugs that are innovative, free of adverse effects and cost-effective. They must be the first to the market in a particular class of drugs that can improve the quality of life. Pharmaceutical research has assumed a great dimension not only in terms of finding a new molecule, which can be effective in countering the diseases but also should be safe and without any side effects. “in
short, the industry must get it right the first time, with zero defect. Scientific and socio-economic pressures demand predictability and progress”. More and more companies have realized the importance of pharmaceutical research and are spending about 3–10% of their turnover on research activities. Efforts are on in the pharmaceutical research to develop drugs with more and more focused mode of drug action in order to have the effect of the drug with minimum dosage or at the site of diseases. Some of the examples of these kinds are transdermal patches of nitroglycerin for the treatment of heart ailment and development of rotahaler devices for the drug delivery of various bronchodilators such as salbutamol sulphate. These kinds of drug delivery systems have helped in getting faster response time by making the drug available at the site where it is required as also helped in reducing the required dose. In the case of the latter, companies will increasingly need to demonstrate that making a new drug available at a given price will actually result in cost savings—be this in terms of comparisons with other treatments, reduced costs of hospital treatment or fewer working days lost through sickness. In fact, by the year 2000, cost–effectiveness studies could come to be regarded as an integral part of the drug development process.

1.2 **IMPORTANCE OF QUALITY CONTROL IN THE 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 the quality of a product to a defined set of standards. Quality control is a concept, which strives to produce perfect product by series of measures designed to prevent and eliminate errors at different stages of production. Quality control personnel’s are responsible for acceptance or rejection of incoming raw materials and
packaging components for a wide range of in-process tests and inspections/audit to ensure that systems are being controlled and monitored and finally for the approval or rejection of completed dosage forms.

The quality control function in an organization normally consists of at least two primary units:

I) Analytical control and II) Inspection control

The analytical control laboratory is responsible for testing and approving raw materials, work in process and finished products. Inspection control refers to the responsibilities assumed by quality control that is 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.

Quality assurance definition as per Mr. Sayle is “Integrated management systems that provide an assurance that the contractural and legal obligations of the company, to its customers and the community are being efficaciously fulfilled”. Quality assurance requires that there has to be documentary evidence with respect to all those systematic actions necessary to provide adequate confident that product, process or service will satisfy a given quality requirement. It is responsible for designing, implementing equipment and process validation protocols. One of the most important functions for quality assurance is Quality Audit. The responsibility of this function is normally separate from both production and control operations. It is the duty of the audit team through review and inspection to assure that written procedures and policies are available for each significant production and control operation.
Quality control and Quality assurance functions today have greater significance than ever and are no more considered being a liability on to a company’s expenditure plan. More and more companies are recognizing the importance of these two functions and are trying to distinguish them from each other. Over the past two decades various leading drug regulatory bodies such as US FDA, WHO, British Pharmacopoeial Commission etc. have taken very serious view about the quality of bulk drugs and pharmaceuticals which are prepared. These agencies in consultation and collaboration with various research organizations and industries have been monitoring the efficacy of various drugs and their generic equivalents. Center for drug evaluation and research in USA is one of the leading agency, which is issuing timely guidance as regard to various GMP norms, formulation design and bio study. US FDA regulations\(^{(2)}\) so far are supposed to be the most stringent in this regard and they focus not only on the physical and chemical attribute of the product but also on the bioequivalence of new generic products to the innovator product. In today’s context of a current Good Manufacturing Practice (cGMP) demanding a zero defective approach and stringent regulatory as well as ethical requirements it becomes equally important to monitor quality of these active pharmaceutical ingredients with respect to their identity, potency and purity (including chiral purity) at the time of release and at various stages of it’s shelf life. This can be possible only by using various analytical techniques.

1.3 **ANALYTICAL CHEMISTRY IN PHARMACEUTICAL INDUSTRY:**

1.3.1 **Analytical Chemistry and Various Analytical Techniques**

Analytical chemistry is a systematic branch of chemistry which provides various techniques for evaluating the quality of any product. Developments in Analytical chemistry is and will be a major contributing factor in deciding the
quality of pharmaceutical products\textsuperscript{(3,4)}. These developments have helped in setting more stringent specifications for pharmaceutical products which are easy to monitor qualitatively and quantitatively. Various examples which can be cited in this regard are development of various chromatographic methods to determine the chiral purity of any drug. Development of differential scanning calorimetry and X–ray diffraction spectroscopy has made it possible to differentiate the different polymorphic forms of the same compound. Application of the laser diffraction spectroscopy has helped in determining the particle size distribution of powder form. Introduction of dissolution test in the Pharmacoepias has made it possible to have a in vitro test which can be related to the in vivo bio availability of many drugs. Drug products which are chemically equivalent but bio non equivalent can be discrimanted using this technique along with various quantitation techniques such as UV–visible spectrophotometry, Spectroflorimetry and HPLC\textsuperscript{(5)}. An overview of different analytical techniques used in pharmaceutical analysis is given in \textit{table 1.1}. With the availability of equipment’s with superior design and high level of automation the productivity, precision and accuracy level are going up. Analysis which required couple of days can now be completed with in few minutes or hour.

Among various analytical techniques the instrumental techniques are widely used in Pharmaceutical Analysis due to high precision, selectivity, sensitivity and degree of automation available.
### TABLE 1.1
VARIous TECHNIQUES Used IN PHARMACEUTICAL ANALYSIS

<table>
<thead>
<tr>
<th>Technique</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CHEMICAL</strong></td>
<td></td>
</tr>
<tr>
<td>TITRIMETRY</td>
<td>ASSAY OF BULK DRUGS</td>
</tr>
<tr>
<td><strong>PHYSICO CHEMICAL</strong></td>
<td></td>
</tr>
<tr>
<td><strong>SPECTROPHOTOMETRIC</strong></td>
<td></td>
</tr>
<tr>
<td>X-ray</td>
<td>IDENTIFICATION, STRUCTURE ELUCIDATION</td>
</tr>
<tr>
<td>ULTRA–VISIBLE SPECTROPHOTOMETRY</td>
<td>ASSAY</td>
</tr>
<tr>
<td>INFRARED SPECTROPHOTOMETRY</td>
<td>IDENTIFICATION</td>
</tr>
<tr>
<td>NUCLEAR MAGNETIC RESONANCE</td>
<td>IDENTIFICATION</td>
</tr>
<tr>
<td>ATOMIC ABSORPTION SPECTROCOPY</td>
<td>ESTIMATION OF DIFFERENT ELEMENTS</td>
</tr>
<tr>
<td>POLARIMETER</td>
<td>STUDYING THE CHIRALITY OF THE MOLECULE</td>
</tr>
<tr>
<td><strong>THERMAL</strong></td>
<td></td>
</tr>
<tr>
<td>DIFFERENTIAL SCANING COLORIMETER</td>
<td>STUDYING POLYMORPHISM, DRUG EXCIPIENT INTERACTION</td>
</tr>
<tr>
<td><strong>ELECTRO ANALYTICAL</strong></td>
<td></td>
</tr>
<tr>
<td>COULOMETRY</td>
<td>MOISTURE DETERMINATION</td>
</tr>
<tr>
<td><strong>SEPARATION</strong></td>
<td></td>
</tr>
<tr>
<td>PAPER/THIN LAYER CHROMATOGRAPHY</td>
<td>IDENTIFICATION</td>
</tr>
<tr>
<td></td>
<td>IDENTIFICATION AND QUANTITATION</td>
</tr>
<tr>
<td>GAS CHROMATOGRAPHY</td>
<td>SEPARATION AND ESTIMATION OF COMPOUNDS</td>
</tr>
<tr>
<td>HIGH PERFORMANCE LIQUID</td>
<td>SEPARATION AND DETERMINATION OF DIFFERENT CLASS OF COMPOUNDS</td>
</tr>
<tr>
<td>CHROMATOGRAPHY</td>
<td></td>
</tr>
<tr>
<td>SUPER CRITICAL FLUID</td>
<td>SEPARATION AND DETERMINATION OF COMPOUNDS</td>
</tr>
<tr>
<td>CHROMATOGRAPHY</td>
<td></td>
</tr>
</tbody>
</table>
**1.3.2 Importance of Instrument Design.**

Instrument technology and design have undergone a sea change over the years and the trivial instruments of yester year have been replaced by most modern instruments of today. Instruments available today are much more compact and lighter in weight with many more features and flexibility packed into them. Mass spectrometers which used to occupy a big room are now available as bench top models. Filter Spectrometers have been replaced with grating Spectrophotometers providing higher wave length resolution, accuracy and higher spectral discrimination. Fourier transform spectrophotometers provide the luxury of recording a spectrum of the compound with much lesser quantity of sample than would be required by a conventional spectrophotometer. Recent developments in near infrared spectroscopy have opened a new avenue in qualitative and quantitative analysis without the need for any sample preparation. Improvement in the design of hardware for High performance liquid chromatography has made it possible to carry out very efficient separations of complex biological mixtures on very narrow bore column with lower particle size and detect them at very low level. Development of Super Critical Fluid Chromatography has resulted into combining the advantages of Gas Chromatography and Liquid Chromatography and is going to be an exciting field to work in the coming years. New developments in the field of instrumentation’s are regularly discussed at Pitcon and other conferences. Developments in Analytical instrumentation have provided a much needed boost for pharmaceutical research by helping to achieve a better selectivity and sensitivity.

**1.3.3 Automation in the Instrumentation.**

Dependence of pharmaceutical research and manufacturing activity on the feedback from analytical development and quality control is well established. The
pace at and the way in which the research and manufacturing activities go in today’s world it is not possible to delay the analytical input because of constrain of resources. The resources whichever are available have to be used to their fullest potential. Whereas the human resources can not be stretched beyond a certain level the instrumentation can be. Contemporary research in the field of instrumentation has been focused on automation which has been helping to simplify and speed up many activities and thus increasing the output per unit time. Auto sampler in spectroscopic and chromatographic techniques is becoming a very common feature. This makes it possible to carry out unattended instrument operation over night, tripling the output and enhancing the accuracy of the results. Diluters can be used instead of volumetric glassware’s to make many accurate dilutions in lesser time. Almost complete robotic analytical systems are available which are capable of sample preparation including extraction, dilution, filtration and forwarding the final processed sample onto the analytical system such as spectrophotometer or chromatograph\(^6\). In the countries where manpower is scarce and costly these robotic instruments are slowly replacing the analyst from the laboratory and a whole new concept of good laboratory practices is fast catching up. Availability of uninterrupted power supply systems has been a good boost in ensuring the continuous usage of this highly sophisticated equipment to obtain the results in lesser time.

1.4 **GLP IN ANALYTICAL LABORATORY**

Good Laboratory Practices (GLP), regulates operational management of laboratory facility with objectives to certify quality and reliability of data of the tests carried out in the laboratory. It helps to improve morale of the people by ensuring precise and accurate results. It helps to improve the over all productivity. Few important points which are discussed below:
1.4.1 Calibration of Analytical Instruments

To ensure precise and accurate results analytical instrument should be calibrated. Some of the common errors which can arise from the use of non calibrated instrument are shown in Table 1.2.

<table>
<thead>
<tr>
<th>No.</th>
<th>PARAMETER</th>
<th>EFFECT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Wavelength accuracy (In UV/HPLC)</td>
<td>Error in assay using E₁%</td>
</tr>
<tr>
<td>2</td>
<td>Pump flow (In HPLC)</td>
<td>Error in retention time</td>
</tr>
<tr>
<td>3</td>
<td>Temperature accuracy of oven</td>
<td>Error in LOD values</td>
</tr>
<tr>
<td>4</td>
<td>Temperature accuracy of Thermometer</td>
<td>Error in Melting point/Boiling point determination</td>
</tr>
<tr>
<td>5</td>
<td>Calibration of Burette</td>
<td>Error in titrimetric analysis</td>
</tr>
</tbody>
</table>

1.4.2 Validation of Analytical Methods.

The main objective of validation of an analytical procedure is to demonstrate that the procedure is suitable for its intended purpose. Due to their complex nature, analytical procedures for biological and biotechnological products in some cases may be approached differently than in this document. Various guidelines are available, among them one of the most widely followed is International Conference on Harmonization (ICH) guidelines. Some common parameters which need validation are listed in the Table 1.3 and discussed.
**TABLE 1.3**

**ANALYTICAL PARAMETERS**

<table>
<thead>
<tr>
<th>NO.</th>
<th>PARAMETERS</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>SPECIFICITY</td>
</tr>
<tr>
<td>II</td>
<td>LINEARITY AND RANGE</td>
</tr>
<tr>
<td>III</td>
<td>PRECISION</td>
</tr>
<tr>
<td>IV</td>
<td>ACCURACY</td>
</tr>
<tr>
<td>V</td>
<td>LIMIT OF DETECTION</td>
</tr>
<tr>
<td>VI</td>
<td>LIMIT OF QUANTIFICATION</td>
</tr>
<tr>
<td>VII</td>
<td>RUGGEDNESS</td>
</tr>
<tr>
<td>VIII</td>
<td>ROBUSTNESS</td>
</tr>
<tr>
<td>IX</td>
<td>SYSTEM SUITABILITY</td>
</tr>
</tbody>
</table>

**I) SPECIFICITY**

It is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these ensuring include non-interference of impurities, degradants, matrix etc. In case of lack of specificity of an individual analytical procedure such as spectrophotometry an interference of 0.5–1.0% may be permitted.

**II) LINEARITY AND RANGE**

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. Results of linearity test as analyzed by a method of linear regression and represented in terms of the coefficient of regression (\( \gamma \)) and the intercept (\( c \)) obtained from the graph of response versus concentration. Typical acceptance level are a value of more than 0.99 for \( \gamma \) and intercept value of not
more than 2.0% of the response obtained for the standard concentration (commonly referred as 100% standard theoretical concentration) which will be used for regular analysis.

The range of an analytical procedure is the interval between the upper and lower concentration (amounts) 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.

The following minimum specified ranges should be considered:

- For the assay of an active substance or a finished product: normally from 80 to 120 percent of the test concentration;
- For content uniformity, covering a minimum of 70 to 130 percent of the test concentration, unless a wider more appropriate range, based on the nature of the dosage form (e.g., metered dose inhaler), is justified;
- For dissolution testing: +/- 20% over the specified range; e.g., if the specifications for a controlled released product cover a region from 20%, after 1 hour, up to 90%, after 24 hours, the validated range would be 0–110% of the label claim.

III) PRECISION

The precision of an analytical procedure express the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility. The precision of a analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements.
IV) ACCURACY

Accuracy of the procedure relates to the closeness of the result obtained by the procedure to the true values. The accuracy of a test procedure can usually be determined by applying the procedure to the quantitatively prepared samples of material to be analysed.

V) LIMIT OF DETECTION

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. Usually this is achieved by measuring the signal to noise ratio for the analyte at different concentration. Concentration giving a signal to noise ratio of 3 is considered as limit of detection.

VI) LIMIT OF QUANTITATION

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices and is used particularly for the determination of impurities and/or degradation products. Usually this is achieved by measuring the signal to noise ratio for the analyte at different concentration. Concentration giving a signal to noise ratio of 10 is considered as limit of quantitation.

VII) RUGGEDNESS

Deliberately varying certain experimental condition such as analyst, pH of the solution, composition of mobile phase and temperature in chromatographic analysis, sample preparation using different volume of solvent and different time of sonication etc and determining it’s effect on the results is called as ruggedness of the method. Many such parameters can be varied judiciously depending upon
the complexity anticipated in carrying out that particular analysis and ensuring that a practical deviation from the originally specified condition does not significantly alter the results obtained.

**VIII) ROBUSTNESS**

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Examples of typical variations are:

- Stability of analytical solutions,
- Extraction time.

In case of liquid chromatography, examples of typical variations are

- Influence of variations of pH in a mobile phase,
- Influence of variations in mobile phase composition,
- Different columns (different lots and/or suppliers),
- Temperature,
- Flow rate.

In the case of gas–chromatography, examples of typical variations are

- Different columns (different lots and/or suppliers),
- Temperature,
- Flow rate.

**VIII) SYSTEM SUITABILITY**

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be
evaluated as such. System suitability test parameters to be established for a particular procedure depend on the type of procedure being validated.

1.4.3 **Validation of Analyst**

This ensures that analyst uses the method and machine judiciously in order to get the most accurate and reproducible results. This generally involves making an analyst familiar with the machine, methods and systems by providing him with a set of documented standard operating procedures and testing instructions.

1.4.4 **Importance of Documentation**

The documentation in the laboratory is a prime requirement in the present scenario as it gives track of product quality and help understand the trend. The documentation normally required in the laboratory may be divided into following broad categories.

1. General SOPs.
2. SOPs related to the handling of equipment’s.
3. Different analytical test procedures.
4. Installation Qualification of various equipment’s and their maintenance.
5. Test reports of samples

1.5 **CHROMATOGRAPHIC TECHNIQUES IN PHARMACEUTICAL ANALYSIS:**

The importance of QA / QC has been discussed in the preceding section. Therefore it is necessary, an analyst from QC requires competent, precise, sensitive, selective and reproducible methods of qualitative and quantitative analysis to measure the amounts of required active pharmaceutical ingredients and impurities, if any, in the raw materials, in-process products and finished products.

In the last four decades many instrumental techniques have been developed which have replaced laborious and lengthy classical analytical methods. These
instrumental methods of analysis are extremely sensitive and requirement of sample quantity is very less. It gives precise and detailed information about the samples.

Among the various instrumental techniques, the universally employed techniques for the pharmaceutical analysis nowadays are chromatographic techniques such as,

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

1.6 **SCOPE AND METHODOLOGY OF PRESENT WORK:**

To monitor various active pharmaceutical ingredients (APIS), there are lots of analytical techniques available but looking at various validation criteria on an analytical methods needs to meet in today’s stringent quality requirements. High Pressure Liquid Chromatography (HPLC), and High Pressure Thin Layer Chromatography (HPTLC) are considered as most versatile techniques \(^{(7-10)}\). HPLC has lots of applications because of availability of different types of stationary phases, unlimited choice of mobile phases, varieties of detectors to be chosen from a range. It is applicable for providing faster separations, good separation efficiency, reduced zone diffusion, higher sensitivity and accurate quantification for a wide variety of applications. Because of the optimized instrumentation with high levels of automation, HPTLC offers precise control over sample application, chromatographic development, derivatisation, detection and determination. The special feature of HPTLC is its ability to simultaneously \(^{(11-14)}\) spot, develop and automatically scan a number of samples on a single plate. The importance of both these techniques have been highlighted in many international symposia and conferences. It is therefore thought worth while to develop new methods of
analyzing active pharmaceutical ingredients using HPLC and HPTLC techniques.\textsuperscript{(15–20)} There are a number of books\textsuperscript{(21–30)} and reviews available on this subject\textsuperscript{(31–41)} discussing aspects of the technique in detail.

The work carried has been presented in the subsequent chapters:—

1. HPLC technique for the determination of some pharmaceutical preparations and bulk drugs.

2. HPTLC techniques used for the quantification of pharmaceutical preparations.
PART I

1.7 REFERENCES:


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