CHAPTER-I

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Pharmaceutical analysis is vital for successful drug development. It is used to determine a drug structure, level of active ingredient and to identify contaminants. Pharmaceutical analysts use a range of techniques to examine the constituents of various samples throughout the drug development process. These samples include raw materials used in manufacturing and body fluids collected during drug trials.

The most remarkable change in the pharmacopoeias in the past 25 years has been the increasing importance of purity tests. At the beginning of this period very limited number of monographs contained tests related to impurities. The developments of TLC and HPLC, at present, the overwhelming majority of the monographs on bulk drugs and in fairly high proportion of those on formulations contain these tests. The impurity profile has become the most informative indicator of the quality of bulk-drug materials. Pharmaceutical analysis plays an important role in statutory certification of drugs and their formulations either by the industry or by the regulatory authorities. The use, abuse and misuse of drugs for various purposes have been on the increase and this requires a constant updating in the methods for their analysis. The pharmaceutical methods have come to be supplemented by other alternative analytical methods due to the complexity of problems encountered. There is a constant need for the development of methods, which are simple, sensitive, inexpensive, rapid and specific.

As a consequence of the tendencies towards globalization and harmonization, the necessity of increasing the safety of drug therapy, validation of the analytical methods has come to the fore-front: moreover, it has become one of the most important issues in contemporary drug analysis.
In order to bring a drug product from the discovery stage to the commercial market, many analytical methods must be employed. The analytical chemist develops methodology for quality control, stability testing, pharmacokinetics, identification and clinical studies. Managing the implementation of new technology in pharmaceutical development environment has provided challenges and opportunities to obtain benefits from technologies, e.g. laboratory automation. Successful application of new techniques requires a dedicated resource. Within pharmaceutical technologies, this was initially a single person, who has since evolved into a team dedicated to the investigation and development of robotics and non-invasive analytical techniques. Pharmaceutical development is an important interface between research and commercial manufacturing. In research, the success of genomics and combinatorial chemistry will result in a significant increase in the number of development of compounds, and this, combined with the desire of commercial manufacturing to move towards parametric release, puts an emphasis on the need for rapid analytical methods.

Technological developments in the latter part of this century have placed an increasing demand on chemist to develop analytical methods, which are inexpensive (in terms of outlay and running costs), reliable, rapid, simple to operate, accurate, sensitive, amenable to automation, and use of portable equipment. Few techniques possess sufficient generic applicability to enable their detection amongst the huge number of potential interferences that can be encountered within environmental, food, industrial and biological samples. As such, a large number of protocols, encompassing almost all major analytical methodologies have been developed to overcome the peculiarities of the various sample matrices.
Technological and scientific revolution is associated with either a new material (bio-active compounds) or a new investigating analytical tool (methods). Even amongst these two, comparatively the instrumentation plays a dominant role since ultimately analytical tool (methods) is the key to characterize the material.

The analytical instrumentation has evolved from physical, inorganic and organic chemistry, biochemistry, physics, surface science, neurobiology, kinetics, optimal spectroscopy, superlative sciences and many other active areas of scientific research. The three major areas of analytical instrumentation are: 1. Chromatographic techniques. 2. Spectroscopic techniques and 3. Conductometric techniques. Some of the important spectroscopic techniques are; UV-VIS, IR/FTIR/NIR and atomic absorption spectrophotometers etc. All these instruments have acquired important position as analytical tools in the fields of organic, inorganic and biochemistry.

Sophisticated analytical instrumentation is now ubiquitous in our every day life. Techniques such as GC, HPLC, GC-MS, LC-MS, UV-VIS-IR and AAS permit chemists to identify minute quantities of substances at ppm or ppb levels. The detection of ‘stimulating’ or ‘performance enhancing drugs’ in the biological samples of professional athletics is perhaps the most high profile example of the application of analytical instrumentation in modern life.

Thus analytical techniques provide invaluable data on soils, plant nutrients, quality of agro inputs and pesticide residues, interpretation of these data effectively and evolving appropriate management strategies are the ultimate aim towards giving enough food, and providing water and environment that are safe to living beings.

The efficacy, safety and economy of drug therapy are extremely important issues not only from the point of view of public health, but their financial, more over
political, aspects are also immense. As a consequence of this, pharmaceutical and biomedical analysis is among the most important branches of applied analytical chemistry. To fulfill the rapidity increasing demands as regards the number and the quality of analytical measurements, great efforts have been made and are being made to apply, moreover further development in this field, the latest achievements of analytical chemistry.

The drugs and their formulations are determined by means of physical, chemical, physico-chemical and biological methods. Physico-chemical methods [1-3] depend on the physical phenomenon that occurs as a result of chemical reactions. An important feature of modern pharmaceutical chemistry is the introduction of more refined and reliable analytical techniques for the analysis of therapeutic agents. The new methods of analysis include those based on emission and fluorescence spectroscopic techniques, photometry including photocolorimetry and spectrophotometry covering UV-visible and IR-regions., potentiometry, amperometry, coulometry and polarography. Most valuable chromatographic techniques [4-9] such as column, thin-layer, gas-liquid, HPLC and HPTLC are noteworthy for their scope, speed of analysis and reliability in quality control laboratories. Methods based on nuclear magnetic resonance (NMR), and proton magnetic resonance (PMR) spectroscopic techniques are used specially for the analysis of individual components of mixture. The combination of LC-MS is one of the most powerful tools available for the analysis of bio-active compounds. The chemical methods of analysis include the gravimetry and titrimetry.

With the increase in the manufacture of newer chemotherapeutics and the modification of existing ones followed by a corresponding increase in their abuse, increasing need is felt for newer methods for their analysis. Spot tests, colour tests,
titrimetry, UV-VIS spectrophotometry and thin layer chromatography (TLC) continue to be the most valuable and effective tools for rapid analysis in law enforcement laboratories. Occasionally, sophisticated and expensive techniques like gas chromatography (GC), high-pressure liquid chromatography (HPLC) and mass spectrometry (MS) may also be necessary for specialized analysis.

Drug analysis needs improvements in the analytical procedures from time to time due to increasing number of bio-active compounds being marketed every year. Of the various criteria used in selection of an appropriate analytical method, sensitivity, accuracy, precision and selectivity are of prime importance. Other important considerations are scope, sampling, standard requirements, cost of equipment and time of analysis. Due to multiplicity of problems, many of the analytical methods described in the pharmacopoeias, which have long been regarded as specific, suffer from other limitations. In view of this, there is a need to develop an entirely new range of analytical procedures, which would over come the existing inadequacies in the determination of some drugs of pharmaceutical interest. This thesis mainly focuses on the analytical methods development, optimization of HPLC, UPLC, spectrophotometric and titrimetric conditions and other important perception during method development and validation.

Method validation is a procedure obtaining experimentally justified evidence of the technique to give the results characterized by the required accuracy and precision. In view of this, development and validation of new analytical methods for the pharmaceutical has been proposed. The development and validation of a new analytical method for the pharmaceutical industry is carried out by using HPLC, UPLC, spectrophotometric and titrimetric methods.
The thesis entitled “To develop new methods involving chemical and chromatographic techniques for the determination of pharmaceutical drugs containing heterocyclic moieties” consists of seven chapters.

Heterocyclic compounds are one which have cyclic ring(s) in which carbon atom is attached to nitrogen, oxygen or sulphur etc. Or this can also defined as “A heterocyclic compound or ring structure is a cyclic compound that has atoms of at least two different elements as members of its ring(s)” The below compounds are selected to develop the methods.

**Pinaverium Bromide** :- Pinaverium bromide is chemically known as 4-(2-Bromo-4,5-dimethoxy-benzyl)-4-[2-[2-(6,6-dimethyl-bicyclo[3.1.1]hept-2-yl)-ethoxy]-ethyl]-morpholin-4-ium. It acts as a spasmolytic agent of the digestive tract. It acts upon inhibition of calcium ion entrance into smooth muscle cells. In humans, pinaverium bromide facilitates gastric emptying and decreases intestinal transit time in patients with constipation.

**Valacyclovir** :- It is chemically known as L-valine 2-[(2-amino-1,6-dihydro-6-oxo-9H-purin-9-yl)methoxy] ethyl ester, monohydrochloride. Valacyclovir is an antiviral drug used for the treatment of the herpes simplex viruses and the varicella zoster virus.

**Losartan Potassium**:-It has chemically known as mono potassium salt of 2-butyl-4-chloro-1-[p-(o-1H-tetrazol-5-ylphenyl) benzyl] imidazole-5-methanol. It is non-peptide AT1 receptor antagonist utilized for the treatment of hypertension.

**Midazolam**:-It is chemically known as 8-chloro-6-(2-fluorophenyl)-1-methyl-4Himidazo[1,5-a]. It is a potent, short acting benzodiazepine, which is commonly used clinically as a probe substrate for drug–drug interaction studies.
In the second chapter of the thesis, determination of pinaverium bromide (PNB), a spasmolytic agent was developed and validated. In the first section (section 2.1), two titrimetric methods were developed. Method A is based on the diphasic ion association complex formation with sodium lauryl sulphate (SLS) using dimethyl yellow as indicator. Method B is based on the stoichiometric reaction between \textit{in situ} generated bromine and PNB, wherein unreacted bromine corresponds to the concentration of the PNB reacted. The methods have been validated in-accordance with the ICH guidelines. However, both the methods are sensitive at microgram determination of pinaverium bromide. Therefore, another advanced method using ultra high performance liquid chromatography (UPLC) was developed and validated for the determination of pinaverium bromide. The advantage over previous method is rapidity. Since UPLC is a physical technique, the method selectiveness needs to be confirmed by considering various possible impurities such as process related impurities, forced degradation impurities and other extraneous peaks that may interfere. So, the selectiveness of the method was confirmed by performing stressed degradation study and standard addition technique. Further, this method has been validated in-accordance with the ICH guidelines.

In chapter 3.1, for the first time, a titrimetry method for the determination of valacyclovir (VLC) using N-bromosuccinimide (NBS) as oxidizing agent. The unreacted NBS was quantitatively determined, which turn quantifies VLC, iodometrically. Using the same chemistry, two spectrophotometric methods were also developed by quantifying the unreacted NBS using either erglaucin (EG) and measuring the absorbance at 630nm (Method B) or metacresol purple (MCP) and measuring the absorbance at 540nm (Method C). In another section 3.2, two more spectrophotometric methods were developed for the determination of VLC in bulk
drug and in dosage forms using NBS. The methods are based on the bromination reaction of VLC with a known excess of NBS in acid medium followed by the determination of residual NBS by iodometry. In the first the liberated iodine (I$^3$-) is either measured at 360nm (method A) or reacted with starch and the starch-iodide complex measured at 570nm (method B). UV spectrophotometric is known for its simplicity and sensitivity. In section 4.1, development and validation of two simple, inexpensive, accurate, reproducible, and stability-indicating UV-spectrophotometric methods for VLC are described. The methods are based on the measurement of absorbance of VLC solution either in 0.1M NaOH at 262nm (method A) or in methanol at 254nm (method B). The methods were also used to study the degradation of the drug under stress conditions as per the ICH guidelines. In comparison with the existing visible spectrophotometric methods for the quantification of VLC, the proposed methods using NBS are sensitive, require no heating or no pH control, no extraction step, and use an inexpensive instrumental setup, and eco-friendly chemicals and aqueous system.

A stability indicating HPLC method for the determination of VLC is presented in section 4.2. A validated method capable of selectively resolving all degradation impurities of VLC with wide linear dynamic range was achieved. The stability indicating power of the method was established by comparing the chromatograms obtained under optimized conditions before forced degradation with those after degradation via acidic, basic, oxidative, thermal and photolytic stress conditions. The optimization parameters and the validation results in detail are presented in section 4.2.

The emergence of fast separation technique such as ultra-high performance liquid chromatography (UPLC) in the year 2004 by Waters™ has revolutionized
separation science. They exploited the advantages of small column particle size resulting in higher separation factor, theorized by Van Deemter. The only modification required is system capable of withstanding high back pressure from the column. Currently, the lowest particle size column for UPLC system is 1.7 micron.

In section 5.1, a UPLC method for the determination of losartan potassium, mono potassium salt of 2-butyl-4-chloro-1-[p-(o-1H-tetrazol-5-ylphenyl) benzyl] imidazole-5-methanol, a non-peptide AT1 receptor antagonist utilized for the treatment of hypertension, has been developed and validated.

Although different analytical techniques have been reported for the determination of losartan potassium (LOS) in pharmaceutical and biological fluids, the speed of analysis in all the reported methods are often long and tedious. In this regard, authors have made an attempt to develop a faster chromatographic technique UPLC to reduce the analysis time but without compromising on accuracy, precision and sensitivity. The developed method was validated as per the regulations of current ICH guidelines. The method was successfully applied for the determination of LOS in its tablets form without getting any additional peaks from the inactive ingredients in the chromatogram and almost zero interference was observed. The proposed method is simple (isocratic), precise, accurate, linear, robust and specific. Additionally, the retention time of LOS at 2.40min enabled rapid determination of the drug which is important in routine analysis.

In section 5.2, a UV-spectrophotometric method for the determination of LOS was developed and validated as per the ICH guidelines. Once again in order to confirm the method specificity, a forced degradation of LOS was performed and tested. Based on the stress study result presented therein, the method is found to be
selective and free from degradation interferences. The precision was found to be 0.95 to 1.85% RSD. From the report on studies above, it can be concluded that the developed method is very simple, precise, accurate and more economical and this method can be implemented successfully for the estimation of LOS in bulk and formulation. Due to the low cost technique couple with high selectivity and rapidity, the proposed method can be employed in small scale industries for routine analysis in quality control laboratory.

Finally, in section 6.1, UPLC method for the determination of midazolam (MDZ), 8-chloro-6-(2-fluorophenyl)-1-methyl-4imidazo[1,5-a], a potent short acting benzodiazepine, was developed and validated. In this chapter, a simple, rapid and stability indicating UPLC method for the determination of MDZ in bulk and dosage form. The method has been validated using ICH guidelines. The forced degradation study conducted using the developed method results showed that the method is free from interferences such as degradation products of MDZ. Besides, the method is rapid with minimal sample preparation.

Section 7.1 gives the conclusion of all the methods developed and their advantages over existing methods in the pharmacopeia.
REFERENCES


