SECTION 1.0

INTRODUCTION

The desire for protection against the evils of pain, disease and suffering was the concern of the man since the dawn of civilization. The early Greeks believed that Gods dispensed prosperity and pestilence. This naturally created a bond between supernatural powers, religion and the use of drugs. The ancient Indian and Chinese literature describe many vegetable and metallic preparations as remedies. The Vedic (Rigveda) writings describe about medicinal uses. It was however, Charaka, a renowned ancient Indian physician and later Sushruta and Vagbhata who described various medicinal preparations included in Ayurveda, the science of life. As the process of cultural evolution took place, man began to search in nature for remedies for pain and suffering. Thus plants, minerals and animals became the source of remedies for human suffering. For example, since sword symbolized strength and power, the early Greek physicians attempted to use iron therapy against weakness and anaemia. Earliest prescriptions are recorded in a Sumerian table of 2100 B. C. describing ointments and medicines containing asafoetida, thyme (source of Thymol), sodium chloride and potassium nitrate. The Ebers Papyrus written in 1550 B. C. contains prescriptions of castor oil, opium, colchicum and other drugs, which are still in use. This historic document was found in a tomb of a mummy in Thebes in Egypt, and is now preserved at the University of Leipzig. Perhaps, Chinese Medicine was about the earliest, dated at about 2500 B. C. Ephedra or Ma Huang was used even in those early days. Ayurveda or Indian Medicine is about equally ancient, and it has given some useful remedies to modern medicine[1-10].

Some giants in the history of medicine were Hippocrates, Aristotle, Theophrastus, Pliny, Dioscordies and Galen. Hippocrates (460-370 B. C.) is referred to as the “father of medicine’, and many of his writings dealt with anatomy and physiology. The “Hippocratic Oath” of modern day medicine reflects the high esteem with which this Greek physician is regarded. Hippocrates was the first to recognize disease as an abnormal reaction of the body rather than a visitation from the Gods. Aristotle (384 – 322 B.C.), a student of Plato, is considered to be the most influential Greek Philosopher. He attempted to separate superstition from fact. Theophrastus (370 – 287 B.C.), a student of Aristotle, utilized his teacher’s scientific reasoning, and
applied it to the plant kingdom. Dioscorides, a Greek physician of the first century B. C. described several plants of medicinal value. Later, Pliny (23 - 70 A.D.) compiled 37 volumes of natural history, which served as reference data for many years. The Hippocratic concept of disease was then lost and did not reappear until the Renaissance (15th Century A.D. marking the transition from the Middle Ages to the modern world). During this dark period, the concept of Galen (131 – 201 A.D.) and his dogmatic system of polypharmacy prevailed. Galen, a Greek pharmacist physician, who lived in Rome, described many formulae containing plant and animal drugs. He compiled this knowledge in 20 books. Galen’s name is retained in the term “gallenical” pharmacy, which includes preparation of crude vegetable drugs [2-10].

Paracelsus (1493-1541 A.D.) an outstanding leader of the Renaissance reintroduced free thought and critical enquiry. He attacked the Galenic system of polypharmacy, and introduced the use of simple chemicals for treating disease. He introduced mercurials in the treatment of syphills. During his time, the first official pharmacopoeia was printed [1].

Experimental medicine and therapeutics had to wait long for the advances in physiology, which during the decade (1850 –1860) became an experimental science. Virchow, the German physiologist in 1855 propounded the concept of “all cells from cells”. Later, with advances in the knowledge of the physiology of circulation, central nervous system, the hormones and enzymes in the body, the study of pharmacology and therapeutics was stabilized. In fact, pharmacology originated as a branch of physiology. The application of scientific method to studies on drugs was generated in France by Francois Megendie (1783 – 1855), and was expanded by Claude Bernard (1813 – 1921) and is associated with the development of experimental pharmacology in Germany, and John Jacob Abel (1857 – 1938) played a similar role in the United States of America. In India Sir Ram Nath Chopra (1882-1973) was responsible for the development of pharmacology as a well-defined discipline. He was the founder director of the Drug Research Laboratory of the Council of Scientific and Industrial Research (CSIR), located at Jammu-Tawi. Sir Chopra has been widely acclaimed as the Father of Indian Pharmacology, because of his investigations on Indian indigenous drugs. The growth of pharmacology was further stimulated by the development of synthetic organic chemistry, which has provided many new therapeutic agents. Today we live in an era of a “drug explosion” due to the introduction of a large number of
drugs, and the knowledge of clinical pharmacology aims at the rational and safe use of drugs in man.

The word “drug” is derived from the French word “drogue” (herb) [1]. It is defined as any substance used for the purpose of diagnosis, prevention, relief or cure of a disease in man or animals. A drug may have useful action as in the treatment of disease (therapeutic effect) or it may show unwanted reactions (side effects or toxicity).

Sources of Drugs

The main sources [1] of drugs are:

Natural sources: Plants and animals are the main sources of drugs that are used even today. The plant products which are still in use are atropine (belladonna), cocaine (coca), digitalis (digitalis leaf), morphine (opium), quinine (cinchona bark), reserpine (rauwolfia), curare (strychnos species), ephedrine (ephedra), nicotine (tobacco) and pencillins (mold cultures). These pharmacologically active constituents in medicinal plants are different chemical entities like alkaloids, glycosides, neutral and bitter principles, cellulose, oils and tannins. The examples of animal products are hormones (insulin), seras and vaccines. Vitamins and enzymes may also be obtained from naturals sources.

Semi-synthetic substances: These are obtained by chemical modification of the naturally occurring compounds. For example, morphine derivatives (heroine, diacetylmorphine), semi-synthetic penicillin’s or fluorinated corticosteroids. These compounds show superiority over the parent compounds with respect to potency, specificity and duration of action.

Synthetic compounds: This is the most recent class. The examples are aspirin, tolbutamide, barbiturates, indomethacin and several other drugs. The potency of synthetic compounds is generally greater than crude plant drugs. Their toxicity is also more as compared to the ancient drugs. The development of semi-synthetic and synthetic compounds is based on structure-activity relationship studies.

Biotechnology products: The term biotechnology encompasses any technique that uses living organisms (microorganisms) in the production or modification of products. Recombinant deoxyribonucleic acid (DNA) and monoclonal antibody technologies
have provided new and exciting approaches for the production of drugs, and diagnostics. The production of therapeutic agents through recombinant DNA has had significant impact on the pharmaceutical industry. Although production of human insulin was the first biotechnology approach, today more than 10 biotechnology products are either in the market or under developmental process in the United States of America. These include human growth factor, beta-interferon, interleukin-1, and hepatitis-B vaccine recombinant. The future will see the development of more protein-based pharmaceuticals from biotechnology strategies and ‘gene-therapy’ will be becoming popular for the treatment of generic disorders.

The continued widespread use of synthetic organic therapeutic drugs in therapy has given rise to a new area in chemical analysis—pharmaceutical analysis.

Methods of measuring drugs in biological media are increasingly important to many societal groups. Problems related to bioavailability and bioequivalents, new drug development, drug abuse, chemical pharmacokinetics, and drug research are highly dependent on biopharmaceutical analytical methodology.

Until recently, bioavailability of drug was not emphasised. It was more or less assumed that if the physical and chemical integrities of a drug product were assured, satisfactory pharmacological performance would be observed. It is now recognized that formulation factors can also influence the biological availability of medicament from a dosage unit in mammalian systems. Consequently, it has become common practice to establish bioavailability by measurement of blood levels of drugs following administration of dosage forms. These analyses permit graphic representation of bioavailability. Comparative bioavailability studies permit judgments as to the bioequivalence of drugs. These determinations may, in turn, lead to important decisions related to drug product selection by pharmacists.

Pharmacological testing would indicate whether a given drug possesses potent effects on an organ system. If the drug is poorly absorbed after oral administration or has a short half-life in the body, it may not be of practical benefit. Methods of analyzing the drug in biological fluid are indispensable when determining the kinds of information required to making these judgments.

A number of therapeutically essential drugs possess narrow therapeutic indices. That is, the ratios of toxic dose to therapeutic dose are less than ten. In recent years, dosing regimens have been individually and rationally established by carefully
adjusting patient’s dosages through the use of blood level monitoring. This activity, commonly known as clinical pharmacokinetics, cannot be accomplished without biophamaceutical analysis [11-15].

Pharmaceutical analysis is currently performed by an array of sophisticated and expensive instrumental techniques such as HPLC, GC, GC-MS, LC-MS, LC-MS-MS, spectrofluorimetry and capillary electrophoresis. These instruments are not within the reach of most industrial quality control, clinical and research laboratories located in developing and underdeveloped countries. It would be interesting and an advantage if such determinations can be accomplished through the use of simple and inexpensive techniques such as titrimetry, UV and visible spectrophotometry. It is proposed to extend these approaches to new drugs in pharmaceuticals in addition to the most widely used technique, HPLC.

Commercially available immunoassays such as fluorescence polarization immunoassay (FPIA), enzyme multiplied immunoassay technique (EMIT), radio immunoassay (RIA) and enzyme linked immunoabsorbent assay (ELISA), give quick qualitative results and, in some cases, a semi-quantitative result in plasma for a variety of substances or groups of compounds. Their limitations in terms of specificity and sensitivity must always be considered when interpreting results.

Quantitative assays in serum or plasma are rarely needed urgently and are usually reserved for cases with medico legal implications. Routine analysis of drugs of abuse in urine also forms part of drug-dependence treatment programmer in which laboratory tests are used to assess the drug-taking pattern of new patients and subsequently to monitor their compliance with treatment. For routine drug-dependence screening programmer, in which large batches of urine samples are analysed daily, the analytical protocol usually comprises rapid automated immunoassay screening using a clinical chemistry analyses followed by re-examination of positive samples using a more selective chromatographic technique such as TLC, HPLC and HPLC-MS [14]. Since LC-MS requires shortest analysis time, most of the earlier methods have been replaced by LC-MS-MS technique.

**SCOPE OF THE PRESENT WORK**

Pharmaceutical analysis, in general terms, comprises those procedures necessary to determine the “identity, strength, quality and purity” of drugs. However, for practical reasons, it is proper to broaden the scope of this definition to include the
analysis of raw materials and intermediates in the manufacture of drugs. Analytical chemists in the pharmaceutical industry as well as in those chemical industries that produce pharmaceutical raw materials must perform such analyses.

Pharmaceutical analysis plays a key role not only for the quality assurance of drugs and their formulations, but also to guarantee to the consumer a safe and reliable product. Devising accurate procedures for each ingredient of complex dosage formulations containing several therapeutically and chemically compatible drugs with very similar chemical nature is a tough task. The presence of additives, excipients and decomposition products further complicates the development of analytical procedures. The increasing complexities of pharmaceutical preparations and the marked emphasis on quality control by the ethical manufacturers have placed a greater load on the ingenuity of the quality control chemist.

An analytical chemist who is in need of an analytical method may be obliged to survey tremendous amount of literature in order to select one procedure which may appear to suit his need and facilities available, but when he subjects the selected procedure to actual test, he may find that he cannot reproduce the method. It is, therefore essential that that practicing analytical chemist should have access to analytical procedures, which are simple, reliable and responsible under routine laboratory conditions.

Analytical techniques called instrumental approaches currently used in pharmaceutical analysis rely increasingly on complex techniques of analysis such as LC-MS, GC-MS, FIA-spectrofluorimetry, FIA-spectrophotometry, stripping voltammetry and capillary electrophoresis that require expensive equipments and highly specialized personnel. Such techniques are not of much help in countries lacking these resources. For most part, modern analytical techniques merely permit analysis to be carried out more rapidly than the conventional method of analysis. Even if a manufacturer is ready to procure and install modern instruments/equipments, their maintenance in the fittest condition is an Herculean task. Non-availability of trained analytical personnel and simple, accurate precise and cost-effective methods of analysis is a major handicap for pharmaceutical manufacturers in developing countries.
It is true that these instrumental approaches make possible a great many things that could not be accomplished previously using conventional techniques. They also make possible, in some cases, faster analysis than was possible with the chemical methods. However, even with these, powerful instrumental approaches, the chemical methods not only still persist but have continued to flourish, if the number of publications dealing with pharmaceutical/analytical/chemical methods, is any indication. The new analytical instruments serve to enlarge the analytical tool kit but do not displace the older analytical approaches.

The reason for the persistence of the chemical types of analysis can be summarized as follows:

There are many chemical situations which are better handled by chemical, rather than instrumental methods. The broad spectrum of reactions available gives the analyst quite versatility. For e.g., we find that the analysis of complex systems relies heavily on conventional chemical analysis, since specific reactions are generally available for classes of organic compounds of pharmaceutical importance. In addition, the area of trace analysis relies heavily on chemical methods to develop specific colours for the materials in question.

Another advantage of the conventional chemical analysis, particularly titrimetry, can be stated as follows: Most instrumental analyses are dependent on calibration curves which require pure samples of the compounds in question. Titrimetry does not require such calibrations. Hence, this approach is the most practical when the analytical laboratory is faced with problem of pure samples.

The cost of equipment of conventional analysis is generally quite low [16].

Taking into consideration the technical and economical constraints, it is necessary that the recommended methods should permit their use by pharmaceutical industries located in the developing countries. It is significant to stress that even simple techniques like titrimetry and spectrophotometry result in sensitive and accurate measurements with clear advantages of speed, simplicity, cost-effectiveness and zero/or easy maintenance. The large volume of literature devoted to their application in almost every field of scientific research constitutes irrefutable evidence of their utility. It is, therefore, reasonable to assume that the analytical procedures
involving the use of such simple techniques will find wider applications in the field of pharmaceutical analysis as well.

Further more, majority of the methods currently in use in pharmaceutical analysis, like HPLC, LC-MS, TLC, HPTLC, GC, GC-MS, Capillary-electrophoresis, etc., are purely physical methods. Hence, there is a widespread sense that “the chemistry was going out of analytical chemistry”. More recently, it has been recognized that a combination of physical and chemical approaches to the analysis can often accomplish more than either of them alone.

Keeping the above trends in view, the author has carried out detailed investigation and developed new analytical methods based on chemical reactions for the assay of the following five organic oxidisable therapeutic drugs:

1) Zidovudine – antiviral drug.
2) Raloxifene hydrochloride – anti osteoporotic drug.
3) Gatifloxacin sesquihydrate – anti bacterial drug
4) Pantoprazole sodium sesquihydrate – anti ulcerative drug
5) Olanzapine – antipsychotic drug

All the methods are based on a variety of chemical reactions, the finish to the determination being made with techniques such as titrimetry, and spectrophotometry. To add variety to the techniques employed, a purely physical method, HPLC for the determination of drug in dosage forms and LC-MS-MS for the determination of the drug in human urine sample, has also been used in the study.

All the developed methods were validated as per the ICH guidelines [17]. The parameters such as specificity, precision, accuracy, LOD, LOQ, solution stability, robustness, ruggedness and recovery were studied and the results were compared statistically with those obtained by the reference/literature method by applying Student’s t- test for accuracy and variance ratio F- test for precision.
REFERENCES


