CHAPTER 1 : *Introduction*

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Analytical chemistry is a branch of chemistry dealing with separation and analysis of chemical substances. Analytical chemistry includes both qualitative and quantitative analysis. Qualitative analysis is concerned with "what is" present and quantitative analysis with "how much".

Analytical chemistry finds extensive applications in the analysis of organic compounds, pharmaceuticals, biochemicals, body fluids , soils and many other types of substances and in pollution control. Pharmaceutical analysis[1-8] is an important area of application of analytical chemistry; especially in industries.

1.1 IMPORTANCE OF PHARMACEUTICAL ANALYSIS

During the development of a new potential drug, detailed chemical studies must be made of raw materials, intermediates and final formulation. These studies must identify the types and levels of impurities, degradation products, degradation rate etc. The analytical methods should be capable of monitoring these factors. The information resulting from these studies, is used for establishing quality control procedures and specifications for the product.

In addition to the identification of the degradation products, it is necessary to measure the rates of degradation of the drug and its formulation under a variety of conditions. This information is needed to define conditions for storage and handling, that will assure potency throughout the expected shelf-life of the product. Stability studies are especially demanding of analytical precision and accuracy because changes of a few percent over a period of 5 years are considered significant and must be accurately quantitated. In order to detect such small

changes, analysis must be very precise and free from interference from the degradation products. Methods based on HPLC, spectrophotometry, fluorimetry and titrimetry are capable of adequate precision.

The set of analytical procedures developed to control the quality of formulation must include both qualitative and quantitative methods in order to assure the identity and purity of the product. All the procedures used during product development stage must be as simple and rapid as possible. However, control limits on both purity of the drug and the drug content of the final formulation are usually very tight. The preferred solution to the problem of quantitative assays is, therefore, to use HPLC or GC methods which afford simplicity, high speed, good specificity, precision and accuracy. An alternative that may be chosen when these instruments are not readily available, is to combine a precise but nonspecific quantitative assay with qualitative chromatographic test that shows the absence of interfering impurities. This approach was widely used in the compendia and in control procedures for older products. The quantitative analysis in these cases is often a titrimetric or spectrophotometric method and the qualitative test, thin layer chromotography.

1.2 AIM OF STUDY

The range of problems encountered in pharmaceutical analysis coupled with the importance of achieving the highest specificity, precision and accuracy, result in new techniques for organic analysis being adapted quickly in pharmaceutical industry. Various analytical methods are available for the drugs which are already existing in the market. For newer drugs, however, only a limited number of methods are available for analysis. The objective of the present work is to develop simple analytical techniques which are sensitive, selective, accurate, rapid and

relatively inexpensive and can serve as alternative to the existing pharmacopoeial methods, if any.

1.3 THE DRUGS SELECTED FOR THE STUDY

Four relatively new drugs, viz Diclofenac sodium (DFS) Diltiazem hydrochloride (DLZ), Famotidine (FMD) and Ketorolac tromethamine (KTR) were selected for the study. (Scheme 1)

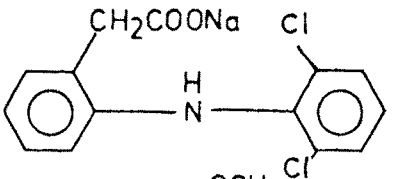
Diclofenac sodium is a synthetic, nonsteroidal anti-inflammatory and analgesic compound. Chemically the drug is monosodium 2-(2,6-dichloroanilino)phenylacetate. The drug has recently been incorporated in the Pharmacopoeia of Japan XII[9] which specifies a titrimetric method for the assay of raw material. Diclofenac sodium is a white to offwhite crystalline powder. It is freely soluble in methanol, ethanol, sparingly soluble in water and glacial acetic acid and practically insoluble in ether.

Diltiazem hydrochloride is an important coronary vasodilator drug of calcium channel blocker type, used in therapy of heart disease and hypertension. The compound is (+)-5[2-(Dimethyl amino)ethyl] -cis2,3-dihydro-3hydroxy-2-(p-methoxy phenyl)-1,5-benzothiazepin-4(5H)-one acetate monohydrochloride. The drug and its tablet formulations are official in The United States Pharmacopoeia[10], USP XXII wherein they are analysed by HPLC method. The drug is a white crystalline powder; freely soluble in methanol, water and chloroform but insoluble in ether.

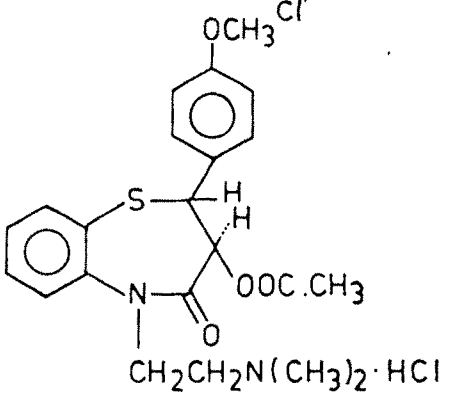
Famotidine is a new antagonist and chemically it is propanimidamide-N'-(aminosulfonyl)-3-[[[2-[diaminomethylene) amino]-4-thiazolyl]-methyl]thio]-. The drug is official in USP XXII which specifies nonaqueous titration for the assay

Scheme 1

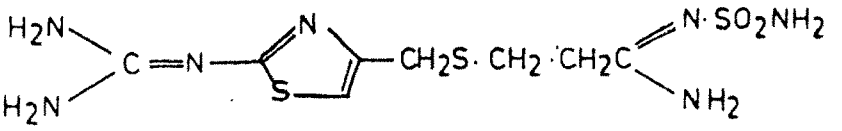
DICLOFENAC SODIUM



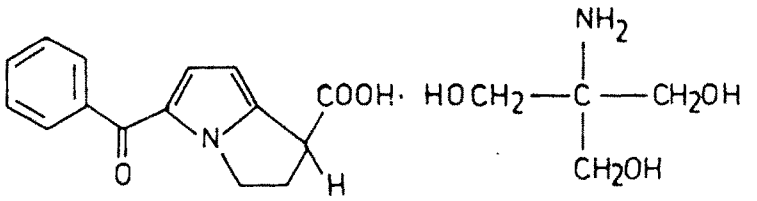
DILTIAZEM HCl



FAMOTIDINE



KETOROLAC TROMETHAMINE



of raw material and HPLC method for tablet analysis. The drug is a white crystalline powder, freely soluble in dimethyl formamide and in glacial acetic acid, slightly soluble in methanol, very slightly soluble in water and practically insoluble in acetone, alcohol and chloroform.

Ketorolac tromethamine is a new drug possessing analgesic and anti-inflammatory activities and chemically it is tromethamine salt of (±)-5-Benzoyl-2,3-dihydro-1H-pyrrolizine-1-carboxylic acid[11]. Presently KTR or its dosage forms are not found in any pharmacopoeia. KTR is a white crystalline powder, soluble in water, methanol and in glacial acetic acid.

1.4 LITERATURE SURVEY

Literature survey reveals various methods for the analysis of DFS[12]. DFS was analysed by spectrophotometric method using 3-methyl-2-benzothiazoline hydrochloride and ceric ammonium sulphate[13], potassium ferricyanide in presence of sodium hydroxide[14], sodium nitrite in presence of hydrochloric acid[15], N,N-dimethylphenylenediamine in presence of persulphate[16] and ion-pair complexation with methylene violet[17]. GC methods have been used for the analysis of DFS and its metabolites[18-23]. Another GC method describes the determination of DFS in tablets after converting the drug into its methyl ester[24]. DFS was analysed in biological fluids by HPLC[25-29]. Another HPLC method describes the determination of the drug in tablets using a cyanocolumn[30].

Diltiazem hydrochloride has been analysed by GC methods[31,32]. A TLC-spectrophotometric procedure[33] has also been reported. These methods either lack sensitivity or are time consuming. USP XXII specifies a HPLC method for the analysis of the raw material and tablets. A few other HPLC methods[34-40] have also

been reported for the determination of the drug in biological fluids which includes use of ion-pair techniques. The cost of ion-pair reagent is high, and more time is needed to stabilize the column in ion-pair HPLC. Also, the life of the column will shorten in ion-pair chromatography. An amperometric method has been utilized for the determination of DLZ[41].

Famotidine is official in USP XXII, which specifies nonaqueous titration using perchloric acid as titrant for the assay of raw material. For tablet analysis USP specifies a reverse phase HPLC with porous silica, mobile phase consisting of water, methanol and 0.01M monobasic potassium phosphate in the ratio of 31:6:3. The flow rate is one $ml.min^{-1}$ and detection wavelength is 254 nm. The other methods reported for the estimation of FMD include spectrophotometry[42-44], polarography[45] and HPLC[46-52].

HPLC methods have been reported for the analysis of ketorolac tromethamine in plasma[53-56] and also for the degradation studies[57].

1.5 TECHNIQUES INVOLVED IN THE PROPOSED METHODS

Analytical methods based on the most recent and rapid technique viz Flow Injection Analysis (FIA) have been developed for all the four drugs. Sensitive and selective High Performance Liquid Chromatographic (HPLC) methods have been optimised for the analysis of DFS, DLZ and FMD. The most widely used but relatively less selective spectrophotometric methods have been developed for all the four drugs. Also techniques like fluorimetry and titrimetry have been adapted for the determination of DLZ.

1.5.1 SPECTROPHOTOMETRY

Spectrophotometry is the most widely used technique in pharmaceutical industry. Among the various physicochemical methods, UV-Vis spectrophotometry is the most commonly used analytical tool[58-61]. As compared to other physical methods UV-Vis has several advantages: (a) High sensitivity in case of suitable chromophore (b) No special qualification is required for handling instrument (c) It can be combined with suitable separation techniques (HPLC, TLC, column) and (d) It is useful for quantitative examination of multicomponent mixture.

The main disadvantages are : (a) It cannot be generally applied for trace analysis and (b) The methods are often not selective.

The analysis of organic compound via functional groups represents a powerful tool for the analyst. All drug molecules possess one or more functional groups that can be analysed in some fashion. However, when a functional group method is employed it is specific only for that particular group. A drawback of this method is that degradation products of the drug may also possess the same functional group and hence will be analysed along with the parent molecule. In this case, the assay would not be stability indicating. If, on the other hand, the functional group being analysed is destroyed during the degradation of the drug, the assay may be stability indicating. For example, if diltiazem hydrochloride is analysed by a method utilizing the tertiary amino moiety, the hydrolysis product, viz desacetyl diltiazem hydrochloride, will react and interfere. If the method utilizes the ester linkage, only the intact drug will be analysed.

Spectrophotometric methods are based on the Lambert Beer's law and involve the derivatization of substance with a model compound exhibiting known

characteristic and stable absorption properties. The quantitative basis of spectrophotometry is that the amount of radiation absorbed at an appropriate wavelength is proportional to the concentration of the light absorbing substance in the sample. For samples which do not absorb significant amount of light, it can be treated with a suitable reagent to convert it into a new species that absorbs light intensely. Among the several techniques used to convert the sample into a absorbing species are : reactions such as oxidation, oxidative coupling, redox, charge transfer complexation, ion-pair complexation and complexation with metal ions.

1.5.2 FLOW INJECTION ANALYSIS

It is an interesting development that an old technique sometimes achieves new importance when it is modified with the incorporation of modern advances in instrumentation. A variety of approaches to the automation of wet chemical analysis has been introduced during recent years. However, because of the development of physical methods (e.g. spectroscopy and chromatography) over the same period, the application of wet chemical analysis has received little attention. In situations where physical methods are inapplicable, automated wet chemical methods have been actively pursued. This is particularly true of process control and clinical analysis where frequent tests are required on a large number of similar samples. The combination of specificity and the low cost of chemical methods with speed and reproducibility of instrumentation has led to extensive use of automated wet chemical analysis in these fields.

Flow injection analysis (FIA) is one such approach where sample is injected to a continuous flow of reagent solution. The sample and reagent are allowed to mix in a coil and passed through a detector. The detector response is recorded for quantification.

Conceptually, FIA can be viewed as HPLC without a column. In HPLC the column provides the specificity while in FIA, a combination of the chemistry and the detector may provide the required specificity. Much of the theory of chromatography, which is based on chemical engineering transport theory developed to describe mass transport and the dispersion in a narrow tube can be applied to FIA.

In recent years, FIA has proved to be relatively inexpensive and useful technique with practical applications in clinical chemistry[62-64] and other fields[65-66]. The features of FIA that make it attractive for pharmaceutical analysis[67,68] are the short start up time, simplicity of instrumentation and high sampling rate. The technique is suitable for batch type analysis where a few to hundreds of samples are to be analysed.

A sample injected into a carrier stream flowing through a narrow bore section of tube exists as a well defined plug and subsequently disperses and mixes with the carrier stream. Dispersion in a FIA system can be reduced by coiling the tube. The peak shape will be sharp for limited dispersion. The type of dispersion desired is dependent on the requirements of the analytical method and is obtained by varying the instrumental parameters such as reaction coil length, internal diameter of the coil, flow rate, reagent concentration etc. At high flow rates dispersion increases, residence time decreases and reagent consumption increases. Optimization of parameters is required to maximize the sensitivity and sample throughput which minimizes reagent and sample consumption.

A single FIA system has been designed that is suitable for the determination of three drugs viz DFS, FMD and KTR. The system was assembled by utilising the available components of HPLC viz, plunger pumps, six bore injector, spectrophotometric detector and computing integrator. Using a similar FIA manifold,

separate methods have been developed for FMD and DLZ. The benefits of the method have been demonstrated in content of uniformity tests and in monitoring dissolution in a representative case of DLZ.

1.5.3 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

High performance liquid chromatography (HPLC)[69-73] is increasingly becoming the premier choice for analysing pharmaceutical raw materials and their formulations, according to regulatory agencies and compendia. The advantages of HPLC include simplicity, accuracy, precision, versatility and most importantly the selectivity, especially to distinguish between constituents of a multicomponent system. The ability of HPLC to separate degradation products makes it very useful in the analysis of pharmaceutical formulations.

Liquid chromatography is basically a separation technique wherein various types of mixtures can be separated into their constituent components. The purpose of the separation may either be to quantitate each of the separated compounds in order to evaluate performance characteristics or to prepare large amounts of the samples for further use.

Over the past few years, HPLC has been one of the fastest growing analytical techniques. Unlike in GC analysis where sample must be volatile, the main criterion in HPLC is that the sample must be soluble. More than 80% of known organic compounds can be analysed by HPLC. A typical HPLC system consists of a solvent delivery system (pump) which is required to force the mobile phase through the column at a constant flow rate. Some kind of injection device is used to introduce a solution of the sample into the solvent stream. The sample and mobile phase travel through the heart of the system - the column. As the sample

components separate and elute from the column they will be passed through a selective detector. Choice of the detector depends on the particular compounds to be monitored and sensitivity levels required. A recording device can be used to record the detector signal as a function of time called the chromatogram.

There are two types of separation modes in HPLC. Normal phase, where a polar packing material such as silica and a relatively non-polar mobile phase such as hexane or chloroform are used. Reverse to this, where packing material is non-polar and mobile phase is polar, the system is called "reverse phase chromatography". It has been estimated that possibly 85% of the known separations, have been done by reverse phase chromatography.

Reverse phase HPLC methods have been developed for the determination of DFS, DLZ and FMD in their formulations using octadecyl silane column and different mobile phases. The preliminary kinetic investigation of degradation of FMD in acidic medium has been carried out to demonstrate the usefulness of this method in monitoring degradation.

1.5.4 FLUORIMETRIC ANALYSIS

Fluorescence analysis[74-78] is an analytical method closely related to spectrophotometry. A molecule can get excited from its ground electronic state to an excited electronic state by absorbing energy in the form of visible or ultraviolet light. Many molecules are capable of emitting this energy as radiation, thus returning to the ground state. The emitted radiation is called 'fluorescence' which is directly proportional to the concentration of the absorbing species. A necessary condition for fluorescence is a strong absorption by the molecule. Aromatic heterocyclic and highly conjugated structures, all of which cause intense absorption are therefore apt to impart fluorescent properties to a molecule.

Direct fluorimetric analysis is not possible as none of the drugs selected for the study possess natural fluorescence. An indirect fluorimetric method has been developed for DLZ by forming a derivative of the drug by attaching a fluorescent tag to the drug.

1.5.5 TITRIMETRIC METHODS

Titrimitry is a widely applicable approach for quantitative analysis[79-81]. One of the advantages of titrimetry as an analytical tool is that it is an absolute method of analysis. A titration is feasible when (a) the titration reaction is rapid compared to the speed of titration (b) it's equilibrium constant is large enough to give a sharp "break" at the end point and (c) a method of end point detection is available. The choice of the titration method depends upon many factors, including the sensitivity required, possible interfering substance that would also be titrated and alternative methods of analysis.

Quaternary ammonium compounds and certain tertiary amines react with alkyl sulphates such as sodium lauryl sulphate and dioctyl sodium sulphosuccinate to form stable water-soluble addition compounds. The titration is carried out biphasically between dilute sulphuric acid and chloroform using a basic dye indicator. Past the equivalence point, the excess titrant partitions into chloroform layer to react with the indicator. DLZ contains a tertiary amino moiety and therefore can be analyzed by the titrimetric method described above.