PREFACE

To day, modern pharmaceutical analysis entails much more than the analysis of active pharmaceutical ingredients or the formulated products. There are many reasons for this change, not the least of which is our ability to better understand physiochemical properties of pharmaceutical compounds through the use of advanced instrumental methods. Furthermore, there is a need for quality assurance of pharmaceutical products throughout their shelf life. This requires study of interactions of the drug substance with the excipients in the presence of residual solvents, as well as other potential degradation reactions that may occur in the formulated product over a period of time under various stress conditions (these include conditions they may be subjected to during storage or shipment in the final package configuration).

Pharmaceutical analysis provides assurance with respect to identity, safety, efficacy, purity and quality of a drug product. The need for pharmaceutical analysis is driven largely by regulatory requirements. This stems from the fact that regulatory considerations loom large when a commercial product does not meet its purported quality. Moreover the pharmaceutical industry is under increased scrutiny from the government and public interest groups to contain costs and yet consistently deliver to market safe, efficacious products that fulfill unmet medical needs. As part of the crusade to hold the
market on prescription drugs, the pharmaceutical industry focusing seriously on quality of the drug substances and drug products.

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

Development of analytical methods which can separate and quantitate the related components (i.e. impurities) in pharmaceutical compounds is a key challenge. The developed methods should be stability indicating. The legal requirements of stability are aimed at ensuring that the drug product remains within specifications established to ensure its identity, strength, quality, and purity. It is necessary to conduct stability studies to predict, evaluate, and ensure drug product safety. Stability indicating analytical methods are important to monitor the stability of Active pharmaceutical ingredients and pharmaceutical dosage forms during the investigational phase of drug development, and, once the drug is marketed, for the ongoing stability studies which must be conducted. The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of atmospheric factors such as temperature, humidity and light, enables recommended
storage conditions, re-test periods and shelf lives to be established. “Stress testing of the drug substance can help identify the likely degradation products, which can turn help, establish the degradation pathways and the intrinsic stability of the molecules and validate the stability indicating power of the analytical procedures used”.

For the drug to be manufactured in pure form, it is important to keep a strict vigilance on quality at all stages of its manufacture and market distribution. The origin of impurities in drugs is from various sources and phases of the synthetic process and preparation of pharmaceutical dosages forms. As per ICH (International conference on harmonization of technical requirements for registration of pharmaceuticals for human use), impurities are classified as organic impurities, inorganic impurities and residual solvents. Of the above three types, the number of possible inorganic impurities and residual solvents is limited. These are easily identified and their physiological effects and toxicity are well known. For this reason the limits set by the pharmacopoeias and the ICH guidelines can guarantee that the harmful effects of these impurities do not contribute to the toxicity or the side effects of the drug substances. The situation is different with the organic impurities. Drugs prepared by multi-step synthesis results in various impurities, their number and the variety of their structures are almost unlimited and highly dependent on the route and reaction conditions of the synthesis and several other factors such as the purity of the starting material, method of isolation, purification, conditions of storage etc. In addition, toxicity is unknown or not easily predictable.
For this reason the ICH guideline set threshold limit above which the identification of the impurity is obligatory. Beyond the stated three types of impurities, enantiomeric impurities are frequently found in chiral new drug substances. Moreover the growing number of chiral new drug substances requires increasing efforts in developing enantioselective methods. According to International conference on Harmonization guidelines, one should quantify the enantiomeric impurity of 0.15% relative to the major constituent.

By keeping in view the importance of the development of analytical methods for the determination of related components in pharmaceutical compounds, research work was proposed and developed high performance liquid chromatographic (HPLC) methods. The HPLC methods were developed using UV detection. LC methods were developed to determine the related components in different classes of pharmaceutical compounds which includes Nateglinide (anti-diabetic drug), Ranolazine (anti anginal), Brimonidine Tartrate (for the treatment of open-angle glaucoma), Tolterodine tartrate (for the treatment of urinary urge incontinence and other symptoms related to unstable bladder), Zafirlukast (for the treatment of asthma) and Tadalafil (for the treatment of erectile dysfunction). The literature survey reveals that there is no stability indicating liquid chromatographic methods for estimation of related components in Nateglinide, Ranolazine, Brimonidine tartrate, Tolterodine tartrate. Moreover literature survey reveals the absence of methods for
enantiomeric purity determination of Nateglinide, separation of meta and para isomers of Zafirlukast from Zafirlukast, a method for separation of (R, R)- Tadalafil from its enantiomer. The developed methods were validated in accordance to regulatory requirements. Long term and accelerated stability studies were also carried out on by using the developed HPLC methods.

In the literature survey, no attempt has been made for determination of related components through development and validation of stability indicative methods by Liquid Chromatography for the above cited Active Pharmaceutical ingredients (API’s / drug substance) and pharmaceutical dosage forms. Hence the present investigation has been attempted for determination of related impurities in pharmaceutical compounds through stability indicative methods by high performance liquid chromatography (HPLC).

This thesis is divided into seven chapters:

**Chapter 1** deals about the “Importance of Development and validation of analytical methods for the determination of related components in pharmaceutical compounds using chromatography technique”. This chapter highlights the need for development of analytical methods for determination of related components in pharmaceuticals, the source of impurities, different kind of impurities, adverse effects by the presence of impurities in final dosage form, control of impurities, stability testing of drug substance and drug product, stress testing route to the development of SIAMs (stability indicating analytical methods), steps
involved during the development of SIAMs, techniques employed in literature for development of SIAMs, role of mass balance during SIAM development and application of SIAM’s with LC contents of method validation parameters and discussion on the general methodology for HPLC was discussed. Significant discussion has also been provided regarding the ICH that has attempted to harmonize the requirements by regulatory authorities in United State, Europe, and Japan.

Chapter 2 was subdivided into two parts as A and B. Part-A deals with development and validation of a novel LC method for determination of related components in Nateglinide where as Part-B delts with a validated reverse phase chiral liquid chromatographic method for the enantiomeric purity determination of Nateglinide in bulk drug samples and pharmaceutical dosage forms.

Chapter 3 deals with “Development and Validation of a New Analytical Method for the Determination of Related Components and Assay of Ranolazine in Bulk Drug and Pharmaceutical Dosage Forms by LC”

Chapter 4 explains “Development and Validation of a New LC Method for Analysis of Brimonidine Tartrate and Related Compounds”

Chapter 5 focuses on “Development and Validation of a New Analytical Method for the Determination of Related Components in Tolterodine Tartarate Using LC”. In this chapter a new, fast and effective microwave
assisted degradation technique was designed and employed for hydrolytic forced degradation study along with conventional reflux method for stress studies. The mass balances of both the studies were compared.

**Chapter 6** deals with “LC Separation of para and meta Isomers of Zafirlukast in Bulk Drug Samples and Pharmaceutical Dosage Forms Using a Chiral Stationary Phase”.

**Chapter 7** deals with “Chiral Separation of (R,R)-Tadalafil and Its Enantiomer in Bulk Drug Samples and Pharmaceutical Dosage Forms by Chiral RP-LC”

At the end of the thesis summary of the study and list of publications were captured.

In each chapter (other than Chapter-1) brief discussion about the molecule, its pharmacology, survey of literature, development of novel HPLC method for determination of related components, method validation, stability studies (chapter-2B, Chapter-6 & chapter-7) stress testing according to ICH, Mass balance (chapter-2A, chapter-3, chapter-4 and chapter-5) were produced. Along with this, at the end of each chapter references are also provided. The developed methods are successfully implemented during the quality monitoring and also well employed for the assessment of quality during its storage and stability.