Chapter 1

Introduction

1. INTRODUCTION

Combination antiretroviral therapy prolongs life and prevents progression of disease caused by human immunodeficiency virus (HIV). The pharmacotherapy of HIV infection is a rapidly moving field. In 2004, there were 20 antiretroviral drugs available in the United States. Since three-drug combinations are the minimum standard of care for this infection, current agents constitute at least 1140 possible regimens. The long-term management of a patient on antiretroviral therapy can be daunting, even for an experienced healthcare provider. Knowing the essential features of the pathophysiology of this disease and how chemotherapeutic agents affect the virus and the host is critical in developing a rational approach to therapy.

The Joint United National Program on HIV/AIDS estimated that by the end of 2005, a total of 40.3 million people worldwide were living with HIV/AIDS, the majority having been infected through heterosexual contact. It is estimated that in 2005, more than 3.1 million people died of AIDS, and 4.9 million new cases of HIV were diagnosed, including more than 700,000 children (WHO 2005). Increasingly, the public health impact of this epidemic has shifted to those regions least able to afford treatment. Fewer than 5% of those who would benefit from combination antiretroviral therapy were receiving it, even though such treatment is known to reduce the complications of infection and prolong life (Lee et al., 2001). The

causes of maldistribution of effective antiretroviral therapy are complex, including social and economic inequities, patient and provider choice, and shortcomings in the convenience and tolerability of available regimens. Regardless, these drugs have the capacity to improve the quality of human health substantially and to produce near-normal life expectancies for some patients with a previously lethal disease (Lee et al., 2001).

As quest for new antiretroviral drugs continue, the need for a new analytical technique exists for rapid qualitative and quantitative analysis of drug substances, drug products, raw materials and biological samples. Today, pharmaceutical analysis is employed throughout all phases of drug development, from research to quality control and therapeutic drug monitoring. Pharmaceutical formulations are complex mixtures, in addition to one or more active medicinal agents contains with various other excipients such as diluents, binders, disintegrants, glidants, lubricants, colours, flavours and sweeteners. In order to assure quality and stability of the final product, the pharmaceutical analyst must be able to separate these mixtures into individual components prior to quantitative analysis. Chromatography is one of the sensitive and efficient techniques available to the analyst for the resolution of compound mixtures.

Once a chromatography method is developed, it has to be validated. Method validation is carried out to ensure that an analytical method is

accurate, precise, specific, sensitive, reproducible and robust over the specified range that an analyte will be analyzed. As defined by the USP, method validation provides an assurance of reliability during normal use, and is sometime referred to as "the process of providing documented evidence that the method does what it is intended to do". Regulated laboratories must perform method validation in order to be in compliance with government or other regulators and the data generated becomes part of the method validation package submitted to regulatory agencies such as the US Food and Drug Administration.

The development and validation of an analytical test method are in and of themselves unrelated to specifications. It is only when one evaluates the interrelationship between the process that is used to make the product and the biological relevance of the impurities detected by the specific method that can begin to appreciate the impact that assay validation has on the design of specification. For example, one may develop and validate an exquisite method for the detection and quantitation of a specific product impurity. However, if the process that is used to make the product is validated to consistently and reproducibly remove the impurity, then the utility of the specific method as part of the specification and control; system needs to be challenged. In addition, the information gained in clinical trials can impact the types of methods and ultimately the specifications that are necessary. For example, the presence of a product-related impurity may be allowable at relatively high levels

even if this causes reduced specific activity as long as an appropriate dose response has been demonstrated in clinical trials and the product is shown to be efficacious. Therefore, focuses less on how to validate specific methods and more on how to validate specific methods and more on how that validation influences the design of specifications. Analytical method validation criteria are the elements that, to some extent, dictate what type of specification may be considered for a product. They include the following: specificity, detection and quantitation of limits, precision, accuracy, range, linearity, robustness, and stability indicating properties, as described in the ICH documents on assay validation (ICH 1994, 1996).

Accuracy and Precision are two criteria that are often confused. Accuracy expresses the closeness of agreement between the value that is accepted (either as conventional true value or as an accepted reference value) and the value found. Precision expresses the closeness of agreement among a series of measurement obtained from multiple sampling of the same homogeneous sample under prescribed condition. Therefore one can describe four type of assay that may be developed. The most desirable is the accurate and precise method, which may be described as a cluster of analytical results very close to intended target value. These types of assays lend themselves to establishment of quantitative specifications. Examples of these are chromatographic or spectrophotometric based assays. Another categ of assays is the accurate but impressive assay, in which the analytical esult are very close to the intended target but the variation

around the target is large. These types of assays lend themselves to the establishment of limit based specifications. Examples of these are animal bioassays and immunoassays. Another category of assays is one that is inaccurate but very precise. An assay of this type may make it difficult to establish specification unless the inaccuracy is extremely reproducible and therefore correctable by some predetermined factor or the magnitude of the inaccurate is relatively small. The least desirable assays are ones that are inaccurate and imprecise. In fact, there may be no value in this type of assay because it would be difficult, if not possible, to validate it.

Specificity is a critical attribute of an assay. Specificity addresses the ability of an assay to assess the analyte in the presence of components. These components that may be impurities, degradation compounds or excipients that are likely to be found in the product. The ability of methods to resolve product related impurities adequately is crucial for the evaluation of the overall purity of the product. One may have to rely on these methods to resolve product impurities that are only slightly different from the intended product. These methods may be relied upon to purify adequate quantities of these product related impurities to assess biological activity as related to the intended product.

Subjecting the API (Active Pharmaceutical Ingredients) or drug product to common stress conditions provides insight into the stability of the analyte under different conditions. The common stress conditions include acidic, basic, different temperature, oxidation and photo

degradation. These studies help to determine the significant related substances to be used in method development and to determine the sample solvent that gives the best sample solution stability. Physical (e.g., solubility) and chemical (e.g., UV activity, stability, pH effect) properties of the sample matrix will help to design sample preparation scheme.

Highly active antiretroviral therapy has been the standard for the treatment of patients infected with the human immunodeficiency virus (HIV). Three classes of drugs, the nucleoside reverse transcriptase inhibitors (NRTIs), the non-nucleoside reverse transcriptase inhibitors (NNRTIs), and protease inhibitors (PIs) are primarily used for the treatment of patients with HIV infection. During the few years, increasing interest has been shown in the possible role of monitoring antiretroviral drugs pharmacokinetics in the field of HIV infection. Therapeutic drug monitoring (TDM) has been suggested if clinically significant drug-drug interactions exist, no virological response is observed or concentration dependent toxicities occur. TDM of NNRTs and PIs is straightforward and reproducible. Assay validations and their impact on the rationale for specification design, specifically some methods used to monitor the quality of Lamivudine, Zidovudine and Nevirapine. NRTI Zidovudine, the first antiviral drug approved for the treatment of HIV infection in 1987 and Lamivudine is a synthetic dideoxynucleoside derivative with potent activity against HIV. Nevirapine a highly potent inhibitor was the first NNRT approved by the FDA.

The present study intends to concentrate developing simple, efficient and cost effective analytical procedure for Lamivudine, Zidovudine and Nevirapine by using HPLC. There have been few studies which have indicated the validation of number of antiretroviral drugs. However, the present study aims at the fulfilling the lacunas.

The lacunas are;

1. Individual or Simultaneous methods available for Lamivudine, Zidovudine, Neviriapine whereas, absence of single method available for these drugs.

2. There is no short run (Retention time) time for these drugs and most of these based on buffer solution. It may chance to growth of microorganism and reduce the lifetime of the columns.

3. Preparations of buffer solutions and ion pair agent in the mobile phase which its addition reduces the column life, increase the time for preparations and cost of the assay, which may not be affordable in developing countries.

4. Absence of Pharmacokinetic studies (*invitro)* with a different pH levels (i.e.) gastric, Small intestine, and mouth.

Therefore, monitoring of the plasma levels of HIV drugs (therapeutic drug monitoring (TDM) has been considered necessary and a useful tool towards improving therapy. On the other hand, the importance of the adequate reference pharmacokinetic profiles for the NRTs Zidovudine, Lamivudine and NNRTs Nevirapine the usefulness of a concentration-controlled therapy has been demonstrated. According to request through local HIV treatment centres, analytical procedures have been established

recently. In this study, single drug levels from routine clinical plasma samples were determined to evaluate the significance of a single measurement. Hence the proposed work is a new RP-HPLC method development and validation with UV detection for assaying antiretroviral drugs Lamivudine, Zidovudine and Nevirapine in bulk and tablet dosage forms. The major advantage of this method is it can be carried out with shorter chromatographic run time, faster preparation steps with a lower requirement of toxic organic solvents and reduced time consuming in contrast to other methods. The performance of proposed method has complies in spirit of ICH guidelines for the validation of a analytical method in terms of linearity, precision, accuracy and recovery, robustness, LOD and LOQ. After method development and validation were determined stability of the method.

The present study was carried out to set up a simple, efficient, and cost-effective analytical procedure for monitoring plasma concentrations under steady state conditions of a number of antiretroviral drugs with high-performance liquid chromatography (HPLC). A total of three drugs, the NRTIs Lamivudine and Zidovudine, the NNRTIs Nevirapine, were analyzed.

It is hoped that it might be helpful in obtaining a cheaper, more acceptable method of validation of antiretroviral compounds thereby helping to improve their therapeutic success in the management of HIV infections.