CHAPTER VII

SUMMARY AND CONCLUSIONS

The thesis describes studies carried out on development and validation of a new RP-HPLC method for the simultaneous estimation of Lamivudine and zidovudine combination and its application in formulation development and pharmacokinetic studies.

The thesis consists of seven chapters.

Introduction and objectives of the investigation are described in Chapter I. The human immunodeficiency virus (HIV) infects cells of the immune system, destroying these cells as well as the immune system’s ability to fight off the invaders. The aim of antiretroviral therapy (ART) is to keep the amount of HIV in the body at a low level. This stops any weakening of the immune system and allows it to recover from any damage that HIV might have caused already. Several antiretroviral drugs (ARVs), which can significantly delay the progression from HIV to AIDS are available since 1996.

Lamivudine and zidovudine are two widely prescribed anti retroviral drugs. Lamivudine is an analogue of cytosine. It is given orally, is well absorbed and excreted unchanged in the urine. Zidovudine is an analogue of thymidine. It can prolong life in HIV-infected individuals and diminish HIV-associated dementia. Lamivudine and zidovudine are official in IP and USP. Lamivudine and
zidovudine combination has significant therapeutic importance. The combination treatment is known as highly active antiretroviral therapy (HAART). Using a HAART protocol, HIV replication is inhibited, the presence of HIV-RNA in the plasma is reduced to undetectable levels and patient survival is greatly prolonged. Zidolam tablets (a commercial brand) contain lamivudine (150 mg) and zidovudine (300 mg). Zidolam tablets are used in antiretroviral combination therapy for the treatment of HIV infection. Zidolam tablet reduces the amount of HIV in the body and keeps it at a low level. It also increases CD4 cell counts. CD4 cells are a type of white blood cells that plays an important role in maintaining a healthy immune system to fight against infection.

In the present investigation studies were carried out on lamivudine and zidovudine drug combination with the following objectives:

1. To develop a simple, sensitive, precise and accurate RP-HPLC method for the simultaneous estimation of lamivudine and zidovudine combination in bulk and in dosage forms.
2. To validate the developed RP-HPLC method as per ICH guidelines.
3. Formulation development of combined drug tablets containing lamivudine and zidovudine and evaluation of the tablets for various physical characteristics and dissolution rate.
4. To evaluate the application of the developed HPLC method for the simultaneous estimation of lamivudine and zidovudine in the dissolution study.
5. To evaluate the application of the developed HPLC method for the simultaneous estimation of lamivudine and zidovudine in plasma samples in the pharmacokinetic studies.

Chapter II contains an introduction to HPLC method development and validation. Principles, instrumentation of HPLC, HPLC method development and validation are discussed in this chapter. Literature on drugs investigated along with past research work on estimation of lamivudine and zidovudine combination are given in Chapter III.

Studies carried out on method development and validation of a new RP-HPLC method for the simultaneous estimation of lamivudine and zidovudine combination is described in Chapter IV.

A new RP-HPLC method was developed for the simultaneous estimation of lamivudine and zidovudine combination in Zidolam tablets and it was validated as per ICH guidelines. The chromatograms for lamivudine and zidovudine were found to be satisfactory on symmetry C-18 (4.6×150mm, 5µ Thermosil column) using mobile phase composed of 60:40%v/v phosphate buffer of pH 3.6 and methanol at a flow rate of 0.8ml/min. The HPLC system which was used consisted of a water alliance equipped with an Auto sampler and UV detector. The content of lamivudine and zidovudine in Zidolam tablets was found to be 151.57 mg and 304.06 mg per tablet respectively. The retention time of lamivudine and zidovudine was found to be 2.338 and 3.415 min respectively.
The system suitability parameters proved that the proposed method is suitable for estimation of lamivudine and zidovudine in Zidolam tablets. Tailing factor for the peaks due to lamivudine was 1.7 and for zidovudine also the tailing factor was 1.7. Theoretical plates for lamivudine and zidovudine was found to be 2252.5 and 2830.7 respectively. The %RSD for lamivudine and zidovudine was found to be 0.10 and 0.14 respectively.

The method was found to be linear in the range of 10-50µg/ml for lamivudine and for zidovudine it is 20-100 µg/ml. The precision of the method was good and the recovery of drugs is well within the acceptance limits of 80-120%. The LOD for lamivudine and zidovudine was found to be 0.024 µg/ml and 0.049 µg/ml respectively. The LOQ for lamivudine and zidovudine was found to be 0.08 µg/ml and 0.16µg/ml respectively.

The proposed RP HPLC method was found suitable for the simultaneous determination of lamivudine and zidovudine combination.

The developed method for the simultaneous estimation of lamivudine and zidovudine combination in formulations is simple, selective, reproducible and accurate with good precision and can be successfully applied to routine analytical purpose.

Studies carried out on formulation development of tablets of lamivudine and zidovudine combination are described in Chapter V.
Combined drug therapy with anti-retroviral drugs is more efficient for treating HIV related diseases. Combination of lamivudine and zidovudine is a preferred option for treating HIV and AIDS. Though this combination is used widely, there are only a few combined drug formulations available in the market. One of the objectives of the present study is to develop tablet formulations containing lamivudine and zidovudine combination and to evaluate the prepared tablets for various physical characteristics including dissolution rate. The HPLC method developed for the simultaneous estimation of lamivudine and zidovudine is used in the dissolution rate study to estimate the two drugs simultaneously.

Tablets each containing lamivudine (150 mg) and zidovudine (300 mg) were formulated employing commonly used tablet excipients. A total of six tablet formulations were prepared by wet granulation method. All the prepared tablets were evaluated for drug content, hardness, friability, disintegration time and dissolution rate. Dissolution rate of (i) lamivudine and (ii) zidovudine from the tablets prepared was studied in water (900 ml) employing USP 8 station Dissolution Rate Test Apparatus (M/s Labindia Disso 8000) with a paddle stirrer at 50 rpm as prescribed in IP 2010. A temperature of 37±1°C was maintained throughout the study. One tablet containing 150 mg of lamivudine and 300 mg of zidovudine was used in each run. Samples of dissolution fluid (5 ml) were withdrawn through a filter (0.45 µm) at different intervals of time, suitably diluted with the mobile phase and assayed for lamivudine and zidovudine by the HPLC
method developed. Dissolution samples (2.0 ml) were suitably diluted with the mobile phase and injected into the HPLC column for the simultaneous determination of lamivudine and zidovudine. Dissolution data were subjected to analysis as per zero order and first order kinetics. Three dissolution parameters namely PD_{10} (Percent dissolved in 10 min), T_{50} (Time for 50 % dissolution) and K_1 (First order dissolution rate constant) were calculated from the dissolution data. From the results obtained the following conclusions are drawn.

1. Hardness of the tablets was in the range 4.5 – 6.5 Kg/sq.cm. Friability of the tablets was less than 1.05% in all the cases. Tablet weight variation was within ±2.5%. The tablets contained both the drugs, lamivudine and zidovudine within 100±3 % of the labeled claim.

2. Tablets formulated with superdisintegrants, crospovidone and crosscarmellose sodium disintegrated rapidly within 1 min 30 seconds. Whereas the tablets formulated employing potato starch as disintegrant disintegrated relatively slowly and the disintegration time of these tablets was in the range 2 min 40 seconds - 4 min 50 seconds.

3. All the tablets prepared as well as commercial tablets fulfilled the official (IP 2010) specifications of uncoated tablets with regard to weight variation, hardness, friability, drug content and disintegration time.

4. The dissolution of both the drugs from the tablets formulated depended on the binder and disintegrant used.
5. Lamivudine and zidovudine dissolution from the tablets followed first order kinetics with correlation coefficient (r) values in the range 0.9284 – 0.9804.

6. Among the four binders PVP K30 gave relatively rapid and higher dissolution and starch paste (gelatinized starch) gave low dissolution. The order of increasing dissolution rate ($K_1$) observed with various binders is as follows:

   PVP K30 > Sucrose > Acacia > Starch paste

   The same order was observed with both the drugs.

7. Tablets formulated employing superdisintegrants gave faster dissolution of the two drugs than those formulated with potato starch as disintegrant.

8. IP 2010 described a dissolution of NLT 70% in 30 min for lamivudine and NLT 80% in 30 min for zidovudine. All formulated tablets except formulation F4 and commercial formulation tested fulfilled the official (IP 2010) dissolution rate test specification.

9. Overall, formulations F3, F5 and F6 gave very rapid dissolution of both the drugs and as such they are considered as best combined drug formulations developed. Formulation F3 contains PVP K30 as binder and potato starch as disintegrant. Formulation F5 contains acacia as binder and crospovidone as disintegrant. Formulation F6 contains acacia as binder and crosscarmellose sodium as disintegrant. The dissolution characteristics of these formulations are better than those of commercial formulations tested.
10. Based on the results PVP K30, sucrose and acacia are recommended as binders and crospovidone and crosscarmellose sodium as disintegrants for the formulation development of combined drug tablets containing lamivudine and zidovudine.

Studies carried out on the application of the new RP-HPLC method developed for the simultaneous estimation of lamivudine and zidovudine in pharmacokinetic studies are described in Chapter VI. The method developed was used for the estimation of lamivudine and zidovudine in plasma samples following their oral administration to healthy rabbits. Based on the plasma concentrations of lamivudine and zidovudine estimated, the pharmacokinetic parameters were calculated and compared with the literature values. Pharmacokinetic studies were done in healthy rabbits weighing 1.5-2.5 kg of either sex (n=6). The drugs were administered orally at a dose of 15 mg of Lamivudine and 30 mg of Zidovudine. From the time versus plasma concentration data, various pharmacokinetic parameters such as peak concentration (C<sub>max</sub>), time at which peak occurred (T<sub>max</sub>), area under the curve (AUC), elimination rate constant (K<sub>el</sub>), biological half-life (t<sub>1/2</sub>), percent absorbed to various times and absorption rate constant (K<sub>a</sub>), were calculated in each case as per known standard methods.

The elimination rate constant (K<sub>el</sub>) for Lamivudine was found to be 0.1466 h<sup>-1</sup> and the corresponding half-life was found to be 4.72 h. The mean residence time (MRT) was found to be 6.95 h. The elimination rate constant (K<sub>el</sub>) for
Zidovudine was found to be \(0.3119\ h^{-1}\) and the corresponding half-life was found to be 2.22 h. The mean residence time (MRT) was found to be 3.97 h.

The half lives (\(t_{1/2}\)) of lamivudine and zidovudine estimated in the present study are in good agreement with the literature values. The literature (\(t_{1/2}\)) value of lamivudine was 4-6 h and in the present study the estimated half life was 4.72 h. Similarly, the literature (\(t_{1/2}\)) value of zidovudine was 0.5 – 3.0 h and in the present study the estimated half life was 2.22 h. The close agreement of the estimated \(t_{1/2}\) values with the literature values with both the drugs indicated that the HPLC method developed was suitable for the simultaneous estimation of lamivudine and zidovudine.

The absorption rate constant (\(K_a\)) of lamivudine was found to be 0.469 h\(^{-1}\). A \(C_{\text{max}}\) of 8.2 ± 0.8 µg/ml was observed at 3.0 h after oral administration of lamivudine and zidovudine drug combination. Later the plasma concentrations were decreased rapidly. The absorption rate constant (\(K_a\)) of zidovudine was found to be 1.1020 h\(^{-1}\). A \(C_{\text{max}}\) of 4.28 ± 0.22 µg/ml was observed at 2.0 h after oral administration of lamivudine and zidovudine drug combination. Later the plasma concentrations were decreased rapidly.

These results indicated that zidovudine was rapidly adsorbed and also rapidly eliminated when compared to lamivudine. The mean residence time (MRT) is 6.95 h and 3.97 h respectively for lamivudine and zidovudine indicating longer stay of lamivudine in the body.
The results of the pharmacokinetic study, thus, indicated that the HPLC method developed for the simultaneous estimation of lamivudine and zidovudine was suitable for pharmacokinetic studies.

**Significant Contribution**

The investigation resulted in the development of a new RP – HPLC method for the simultaneous estimation of the lamivudine and zidovudine combination in bulk and in formulations. The method is simple, selective, reproducible and accurate with good precision and can be used for routine pharmaceutical analysis. The method was also found suitable for estimation of lamivudine and zidovudine combination in dissolution rate studies in formulation development of combined drug formulations. Though combination of lamivudine and zidovudine is a preferred option for treating HIV and AIDS, there are only a few combined drug formulations available in the market and the literature on formulation of combined drug tablets is rather scanty. In the present investigation tablets of lamivudine and zidovudine combination fulfilling the official requirements were developed.

The new HPLC method developed for the simultaneous estimation of lamivudine and zidovudine was also found suitable for pharmacokinetic studies, for the simultaneous estimation of these drugs in plasma samples.