CHAPTER VI

STUDIES ON THE APPLICATION OF THE HPLC METHOD DEVELOPED FOR THE SIMULTANEOUS ESTIMATION OF LAMIVUDINE AND ZIDOVUDINE IN PHARMACOKINETIC STUDIES

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. In the present study a new HPLC method was developed and validated for the simultaneous estimation of lamivudine and zidovudine combination. The HPLC method developed for the simultaneous estimation of lamivudine and zidovudine is evaluated for its application in pharmacokinetic studies. 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. The results are presented in this Chapter.

EXPERIMENTAL

Materials

Lamivudine and zidovudine were gift samples from M/s Hetero Pharmaceuticals, Hyderabad.
Acetonitrile (HPLC Grade) procured commercially.

All other materials used were of Pharmacopoeial grade.

**Pharmacokinetic Studies:**

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. *In vivo* study protocols were approved by the Institutional Animal Ethics Committee (No. 516/01/a/CPCSEA).

**Pharmacokinetic Study Protocol:**

After collecting the 0 h blood sample (blank), the drugs in the study were administered orally in a capsule shell with the 10 ml of water. No food or liquid other than water was permitted until 4 h following the administration of the drugs. Blood samples (3 ml) were collected from marginal ear vein at 0.5,1,2,3,4,6,8,10 and 12 h after administration. The blood samples were collected into heparinized tubes and were centrifuged at 10000 rpm for 10 min and the plasma separated was collected into dry tubes. All the samples were stored under refrigerated conditions prior to assay on the same day.

Plasma concentrations of the lamivudine and zidovudine were determined by the HPLC method developed as follows.

Plasma (0.5 ml) collected at each sampling time was taken into a dry test tube. To each tube 1 ml of acetonitrile was added, mixed thoroughly and centrifuged
at 5000 rpm for 20 min. The organic layer (0.5 ml) was taken into a dry tube and the acetonitrile was evaporated. To the dried residue 0.5 ml of mobile phase (a mixture of phosphate buffer of pH 3.6: methanol [60:40 v/v]) was added and mixed for reconstitution. Subsequently 20 µl were injected into the column for HPLC analysis.

From the time _versus_ plasma concentration data, various pharmacokinetic parameters such as peak concentration (_C_{max}_), time at which peak occurred (_T_{max}_), area under the curve (_AUC_), elimination rate constant (_K_{el}_), biological half-life (_t_{1/2}_), percent absorbed to various times and absorption rate constant (_K_{a}_), were calculated in each case as per known standard methods.1,2.

**Determination of Pharmacokinetic Parameters:**

**Determination of _C_{max}_ and _T_{max}_:**

From the time versus plasma concentration curves, peak plasma concentration (_C_{max}_) and time at which peak occurred (_T_{max}_) were recorded.

**Determination of Elimination Rate Constant (_K_{el}_) and Biological half-life (_t_{1/2}_):**

Time versus plasma concentration data was plotted on a semi logarithmic graph paper. The elimination rate constant (_K_{el}_) was calculated from the slope of the linear line in the elimination phase (the ‘best fit’ linear regression line for the
points in the elimination phase was drawn by the method of least squares). The corresponding biological half-life was calculated using the equation,

\[ t_{1/2} = \frac{0.693}{K_{el}} \]

**Determination of Percentages Absorbed to Various Times and Absorption Rate Constant (K_a):**

Percentages absorbed to various times and absorption rate constant (K_a) were calculated from plasma concentration data by the method described by Wagner and Nelson.\(^1^2\). The equation developed for the determination of absorption rate from blood data is

\[
\frac{dA}{dt} = V_d \cdot \frac{dC_b}{dt} + K_{el} \cdot C_b
\]

Where, \( \frac{dA}{dt} \) = absorption rate, \( V_d \) = apparent volume of distribution \( \frac{dC_b}{dt} \) = rate of change of blood concentration (C_b) with respect to time t and \( K_{el} \) = elimination rate constant.

The equation may be integrated between the limits of \( t = 0 \) and \( t = T \) and divided by \( V_d \) to give,

\[
\frac{A_T}{V_d} = C_T + K_{el} \cdot \int_{t=0}^{t=T} C_b \, dt
\]

\[
\frac{A_T}{V_d} = C_T + K_{el} \cdot [AUC]_{t=0}^{t=T}
\]
where $A_T =$ amount of drug absorbed to time $T$, $C_T =$ blood concentration at time $T$ and the quantity under the integral sign is the area under the blood concentration versus time curve between the indicated limits. When the successive values of $A_T / V_d$ are calculated, a maximum or asymptotic value $[A_T/V_d]_\infty$ is obtained. The maximum asymptotic value is divided into successive values of $A_T / V_d$ to yield percentage absorbed data i.e.,

$$\frac{A_T/V_d}{[A_T/V_d]_\infty} \times 100 \quad \text{as a function of time}$$

A graph of log percent unabsorbed Vs time is a linear plot, the slope of which is equal to $-K_a / 2.303$ from which $K_a$ was calculated.

**Estimation of Area under the Curve [AUC]:**

The area under the time versus plasma concentration curve (AUC) for 12 hour period was estimated, from an arithmetic plot of time versus plasma concentration by applying trapezoidal rule. The remaining area from 12 hours to $\infty$ time was calculated using the following equation,

$$[\text{AUC}]_t^{\infty} = \frac{\text{concentration at 12}^{th} \text{ hour}}{K_{el}}$$

Then, $[\text{AUC}]_0^{\infty} = [\text{AUC}]_0^{12} + [\text{AUC}]_{12}^{\infty}$
Determination of Mean Residence Time:

The tendency of drugs and metabolites to remain in the body can be assessed by measuring the mean residence time (MRT). Assuming that the drug in the organs and elimination is always in equilibrium with drug in plasma, the mean residence time can be defined as the average amount of time spent by drug molecules in the body before being eliminated (under constant clearance conditions). If one considers time course of drug concentration in plasma as statistical distribution curve, it can be shown that

\[
\text{MRT} = \frac{\text{AUMC}}{\text{AUC}}
\]

Where the AUMC is the area under the ‘first movement curve’ and is obtained from a plot of the product of drug concentration in plasma and time versus time from zero to infinity.

\[
\text{AUMC} = \int_{0}^{\infty} tC(t)dt
\]

AUC is the area under the ‘zero’ moment curve and is obtained by plotting the drug concentration in plasma versus time (C vs. t) from zero to infinity.

\[
\text{AUC} = \int_{0}^{\infty} C(t)dt
\]

The MRT is considered as the statistical movement analogy to the half-life (\(t_{1/2}\)). Plots of time versus serum concentration (t vs. C) and time versus the
product of concentration and time (t vs. Ct) were plotted and the area under the corresponding curves i.e. AUC and AUMC respectively were computed. The mean residence time (MRT) in each case calculated as follows:

\[ \text{MRT} = \frac{\text{AUMC}}{\text{AUC}} \]

**EXPERIMENTAL RESULTS**

The results of the pharmacokinetic study on lamivudine and zidovudine combination are given in Tables 6.1-6.2 and shown in Fig. 6.1

**Table 6.1: Plasma Concentrations of Lamivudine and Zidovudine Following their Oral Administration to Healthy Rabbits**

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Plasma Concentration (µg/ml)</th>
<th>Lamivudine</th>
<th>Zidovudine</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.5</td>
<td>3.60 ± 0.9</td>
<td>1.68 ± 0.15</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>5.71 ± 1.3</td>
<td>2.52 ± 0.16</td>
<td></td>
</tr>
<tr>
<td>2.0</td>
<td>7.18 ± 1.5</td>
<td>4.28 ± 0.22</td>
<td></td>
</tr>
<tr>
<td>3.0</td>
<td>8.2 ± 0.8</td>
<td>3.26 ± 0.25</td>
<td></td>
</tr>
<tr>
<td>4.0</td>
<td>7.6 ± 0.7</td>
<td>2.82 ± 0.26</td>
<td></td>
</tr>
<tr>
<td>6.0</td>
<td>6.8 ± 1.2</td>
<td>1.56 ± 0.45</td>
<td></td>
</tr>
<tr>
<td>8.0</td>
<td>6.05 ± 1.3</td>
<td>0.85 ± 0.26</td>
<td></td>
</tr>
<tr>
<td>10.0</td>
<td>4.82 ± 1.2</td>
<td>0.48 ± 0.18</td>
<td></td>
</tr>
<tr>
<td><strong>12.0</strong></td>
<td><strong>3.52 ± 1.7</strong></td>
<td><strong>0.24 ± 0.11</strong></td>
<td></td>
</tr>
</tbody>
</table>
Fig. 6.1: Plasma Concentrations of Lamivudine and Zidovudine Following their Oral Administration to Healthy Rabbits

Table 6.2: Summary of Pharmacokinetic Parameters Estimated Following the Oral Administration of Lamivudine and Zidovudine in Rabbits (n=6)

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameter</th>
<th>Lamivudine</th>
<th>Zidovudine</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$ (µg/ml)</td>
<td>8.2</td>
<td>4.28</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (h)</td>
<td>3.0</td>
<td>2.0</td>
</tr>
<tr>
<td>$K_{\text{el}}$ (h$^{-1}$)</td>
<td>0.1466</td>
<td>0.3119</td>
</tr>
<tr>
<td>$t_{1/2}$ (h)</td>
<td>4.72</td>
<td>2.22</td>
</tr>
<tr>
<td>$(AUC)_0^\infty$ (µg.h/ml)</td>
<td>106.88</td>
<td>27.63</td>
</tr>
<tr>
<td>$K_{\text{a}}$ (h$^{-1}$)</td>
<td>0.469</td>
<td>1.1020</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>6.95</td>
<td>3.97</td>
</tr>
</tbody>
</table>
RESULTS AND DISCUSSION

Lamivudine and Zidovudine drug combination was administered orally to healthy rabbits (n = 6). Plasma samples were collected at different times after drug administration and were subjected to analysis. Plasma concentrations of lamivudine and zidovudine were determined simultaneously by the HPLC method developed. The results are given in Tables 6.1 -6.2 and shown in Fig. 6.1. Table 6.2 contains the summary of pharmacokinetic parameters estimated for lamivudine and zidovudine.

Elimination Characteristics of Lamivudine and Zidovudine Drug Combination

The elimination rate constant ($K_{el}$) for Lamivudine was found to be 0.1466 h$^{-1}$ 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 ($K_{el}$) 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 biological half life ($t_{1/2}$) is a characteristic pharmacokinetic parameter of a drug. The half life ($t_{1/2}$) 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$^3$ 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\textsuperscript{4} 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.

**Absorption Characteristics:**

The absorption rate constant (K_a) of lamivudine was found to be 0.469 h\textsuperscript{-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\textsuperscript{-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), which is a measure of drugs residence in the body 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.

REFERENCES