MATERIALS
AND
METHODS
3.1 MATERIALS AND METHODS

The Drugs selected for studies were Verapamil Hydrochloride and Losartan Potassium procured from M/S PELLET PHARMA Ltd, Hyderabad and M/S AUROBINDO Pharma Ltd, Hyderabad respectively as gift samples. The polymers such as (Polyethylene Oxide (Polyox WSR n-301) and Polyethylene Oxide (Polyox WSR n-303) were used as controlled release polymers obtained from M/S Colorcon Asia Pvt. Ltd, Mumbai, as gift samples. The Microcrystalline Cellulose (Tabulose), Lactose and other inorganic pharmaceutically acceptable electrolytes like Dried Aluminium Hydroxide, Aluminium Sulphate, Calcium Carbonate, Light Magnesium Carbonate, Sodium Bicarbonate and Sodium Carbonate were procured commercially from S.D.Fine Chemicals Ltd., Mumbai of Analytical grade for this investigation.

3.2 ANALYTICAL METHODS

3.2.1 Estimation of Verapamil Hydrochloride:

Several Spectrophotometric and Chromatographic methods have been reported for the estimation of Verapamil Hydrochloride in its pure form and in dosage forms (Walash M, 2006).

In the present investigation a simple, sensitive spectrophotometric method (Tan L, 1995) was used based on the measurement of absorbance at a wavelength (\(\lambda_{max}\)) of 278 in 0.1N HCl and 6.8 pH phosphate buffer media.

Preparation of standard solution:

100 mg of Verapamil Hydrochloride was accurately weighed and dissolved in 0.1N HCl and 6.8 pH phosphate buffer separately in 100ml volumetric flasks and the solutions were made up to volume with the same media to get the 1000 \(\mu\)g/ml stock solution as standard.
Analytical Procedure:

The standard solutions of Verapamil Hydrochloride were subsequently diluted with 0.1N HCl and 6.8 pH phosphate buffer separately to obtain a series of dilutions having 2, 4, 6, 8 and 10 µg of drug per ml of solution. The absorbance of the above standard dilutions were measured in ELICO double beam UV spectrophotometer (SL-159 model) at 278 nm using 0.1N HCl and 6.8 pH phosphate buffer solutions as blank solution. The concentrations of Verapamil Hydrochloride and the corresponding absorbance values are given in table 4.1. The absorbance values were plotted against concentrations of Verapamil Hydrochloride as shown in graph 4.1.

3.2.2 Estimation of Losartan Potassium:

Several Spectrophotometric and Chromatographic methods have been reported for the estimation of Losartan Potassium in its pure form and in dosage forms (Londhe S, 2010). A spectrophotometric method based on the measurement of absorbance at wavelength of 205 nm in 0.1N HCl and 6.8 pH phosphate buffer media was used (Bienert A, 2006).

Preparation of standard solution:

100 mg of Losartan Potassium was accurately weighed and dissolved in 0.1N HCl and 6.8 pH phosphate buffer separately in 100 mL volumetric flasks and the solutions were made up to volume with the same media to get the 1000 µg/ml stock solution as standard.

Analytical Procedure:

The standard solutions of Losartan Potassium were subsequently diluted with 0.1N HCl and 6.8 pH phosphate buffer separately to obtain a series of dilutions having 2, 4, 6, 8 and 10 µg of drug per ml of solution. The absorbance of the above standard dilutions were measured in ELICO double beam UV spectrophotometer (SL-159 model) at 205 nm using 0.1N HCl and 6.8 pH phosphate buffer solutions as blank solution. The concentrations of Losartan Potassium and the corresponding absorbance values are given in table 4.2. The absorbance values were plotted against concentrations of Losartan Potassium as shown in graph 4.2.
3.2.3 Assay of Verapamil Hydrochloride in Plasma:

Verapamil Hydrochloride in rabbit plasma was estimated by HPLC method (Dobovisek J, 2005) was used in the present investigation. A working standard Verapamil Hydrochloride solution (20 μg/ml) and internal standard solution diclofenac sodium (20 μg/ml) in methanol were prepared. To 0.5 ml pooled plasma, 0.1 ml of internal standard solution was added and vortexed for 30 seconds in graduate centrifuge tubes. The standard drug solutions containing 10, 25, 50, 75, 100, 200, 300, 400 and 500 ng in 0.1 ml of methanol were added to the tubes and vortexed for 60 seconds. 0.2 ml aliquots of 1M diclofenac sodium solution were added to plasma containing Verapamil Hydrochloride and again vortex-mixed for another 60 sec. 5 ml of chloroform was added and the solution was again vortex-mixed for about 5 minutes and then centrifuged at 3000 rpm for 10 minutes. 4 ml of the Organic layer was separated and evaporated to dryness under a stream of nitrogen. The residue was reconstituted with 0.5 ml of methanol. A volume of 50 μl of reconstituted solution was injected into the HPLC system (Agilant technologies 1120 LC 10AS pump, 10A UV-Vis detector, SCL-10A system controller and class CR 10 software as data processor on-line with reverse phase C18 column, Inert-sil ODS-2, 4.6x250 nm, 5 μm particle size and pre column). Mobile phase consisted of methanol, water and Triethylamine (67:33:0.4, Ph 6.7) was used. The flow rate was 1 ml/min. Analysis was monitored at 278 nm with a sensitivity of 0.1000 aufs. The plot of peak area ratio of Verapamil Hydrochloride to diclofenac sodium against the concentration of Verapamil Hydrochloride in plasma was linear upto 500 ng/ml.

3.2.4 Assay of Losartan Potassium in Plasma:

The plasma concentration of Losartan Potassium was estimated by HPLC method (Deanne L, 2002). A working standard of Losartan Potassium (20 μg/ml) and internal standard solution diclofenac sodium (20 μg/ml) in distilled water were prepared. To 0.5 ml pooled plasma, 0.1 ml of internal solution was added and vortexed for 30 sec in graduated centrifuge tubes. The standard drug solution containing 10, 25, 50, 75, 100, 200, 300, 400 and 500 ng in 0.1 ml of distilled water were added to the tubes and vortexed for 60 sec. A 0.2 ml aliquots of 1 M sodium hydroxide solution was added to plasma containing Losartan Potassium and again vortex mixed for
another 60 sec. 5 ml of chloroform was added and the solution was again vortex-mixed for about 5 minutes and then centrifuged at 3000 rpm for 10 minutes. 4 ml of the organic layer was separated and evaporated to dryness under a stream of Nitrogen. The residue was reconstituted with 0.5 ml of distilled water. A volume of 50 µl of reconstituted solution was injected into the HPLC system (Agilent technologies, 1120 LC 10AS pump, 10A UV-Vis detector, SCL-10A system controller and class CR 10 software as data processor on-line with reverse phase C18 column, Inert-sil ODS-2, 4.6x250 nm, 5 µm particle size and pre column). Mobile phase consisted of phosphate buffer (pH 4.3), acetonitrile (75:25) was used. The flow rate was 0.9 ml/min. Analysis was monitored at 205 nm with a sensitivity of 0.1000 aufs. The plot of peak area ratio of Losartan Potassium against the concentration of Losartan Potassium in plasma was linear upto 500 ng/ml.

3.3 PREPARATION OF MATRIX TABLETS

The controlled release of matrix tablets of Verapamil Hydrochloride and Losartan Potassium were prepared by direct compression process.

The controlled release matrix tablet formulations were mainly composed of polymer, drug and electrolytes. The ratio of drug and polymer were maintained constant while the electrolyte concentration was varied. The composition of various tablet formulation were given in tablets 4.3 to 4.14.

The materials were individually passed through sieve no. 60 and blended for 15 minutes in a double cone blender. The powder mixture was then lubricated with 1% talc and Magnesium.stearate and directly compressed as matrix tablets using Clit 10 station mini press. To minimize processing variables all batches of tablets were compressed under identical conditions. The powder mixtures were tested for flow properties such as Angle of Repose and compressibility index before they were subjected to Direct Compression as Matrix tablets. The compressed Matrix tablets were further evaluated for their physical parameters such as weight uniformity, friability, Hardness, Drug content and Swelling index.
3.4 EVALUATION OF FLOW PROPERTIES

3.4.1 Carr's Index:

A simple test was used to evaluate the flowability of a powder by comparing the poured density and the tapped density of a powder and the rate at which it is packed down. A useful empirical guide is given as carr's compressibility index (Leon LAchman, 1990).

\[
\text{Carr's index} = \frac{\text{Tapped density} - \text{poured density}}{\text{Tapped density}} \times 100
\]

This is a simple index that can be determined on small quantities of powders and may be interpreted as in table-No: 4.15 to 4.18.

<table>
<thead>
<tr>
<th>CARR'S INDEX</th>
<th>TYPE OF FLOW</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-15</td>
<td>Excellent</td>
</tr>
<tr>
<td>12-16</td>
<td>good</td>
</tr>
<tr>
<td>18-21</td>
<td>Fair to passable</td>
</tr>
<tr>
<td>23-35</td>
<td>poor</td>
</tr>
<tr>
<td>33-38</td>
<td>Very poor</td>
</tr>
<tr>
<td>&gt;40</td>
<td>Extremely poor</td>
</tr>
</tbody>
</table>

3.4.2 Angle of Repose:

The powder flow properties were determined to know the good or bad material flow by conducting this process in which powder is taken into a funnel through which the powder to be calculated to its angle of repose is poured through funnel below this graph sheet is placed and allowed it to flow during this process material will form a heap like structure for which we can measure its radius and its height of the heap by using the formula (Leon LAchman, 1990).

\[
\theta = \tan^{-1}(h/r)
\]

The angle of repose is given in the table may be used as a guide to know the flow and the results were shown in the table-No: 4.15 to 4.18

<table>
<thead>
<tr>
<th>Angle of repose(degrees)</th>
<th>Type of flow</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;20</td>
<td>Excellent</td>
</tr>
<tr>
<td>20-30</td>
<td>good</td>
</tr>
<tr>
<td>30-34</td>
<td>Passable</td>
</tr>
<tr>
<td>&gt;40</td>
<td>Very poor</td>
</tr>
</tbody>
</table>
3.5 EVALUATION OF PHYSICAL PARAMETERS OF MATRIX TABLETS
(Herbert A. Liebermann et al, 2005)

Physical parameters such as weight Uniformity, Hardness, Friability and Drug content were evaluated for prepared tablets as per the standards of official compendium. The data obtained was given in Table No. 4.19 to 4.22.

3.5.1 Weight Uniformity:

Twenty tablets from each batch at random were taken and weighted. The average weight was calculated, then each tablet was weighed individually and weights of each tablet were noted. The weights of individual tablets were then compared with the average weight that was already calculated. The deviation if any in the weight of individual tablets from the average weight was checked. This test highly describes that all tablets of a particular batch should be uniform in weight. If any weight variation is there, that should be within the I.P limits. The test was considered correct if not more that two tablets fall outside the I.P limits out of twenty tablets taken for the test.

<table>
<thead>
<tr>
<th>Average Weight</th>
<th>Percentage Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>80 mg or less</td>
<td>10</td>
</tr>
<tr>
<td>More than 80mg but less than 250mg</td>
<td>7.5</td>
</tr>
<tr>
<td>250 mg or more</td>
<td>5</td>
</tr>
</tbody>
</table>

3.5.2 Hardness:

Hardness of the tablets were determined by using Monsanto hardness tester (Tab-machines, Mumbai). The tablet to be tested is held in fixed and moving jaw and reading of the indicator adjusted to zero. Then force to the edge of the tablet was gradually increased by moving the screw knob forward until the tablet breaks. The reading was noted from the scale which indicates the pressure required in kg/cm² to break the tablet. Hardness of
different batches of tablets were given in Table No: 4.19 to 4.22. The hardness of tablet depends on the weight of the material used, space between the upper and lower punches at the time of compression and pressure applied during compression.

3.5.3 Friability:

Friability test was performed by using Roche friabilator (Remi Equipments, Mumbai). Twenty tablets of a batch were weighted and placed in a friabilator chamber and it was allowed to rotate for 100 revolutions. During each revolution these tablets fall from a distance of six inches to undergo shock. After completion of 100 revolutions, tablets were again weighed and the loss in weight indicated friability. The acceptance limits of weight loss should not be more than 0.8%. This test was performed to evaluate the ability of the tablets to withstand abrasion in packing, handling and transporting. These friability values were given in Table No: 4.19 to 4.22.

3.5.4 Drug Content Uniformity:

Matrix tablet of Verapamil Hydrochloride from a batch was taken at random and was crushed to a fine powder. The powdered material was transferred into a 100ml volumetric flask and 70ml of distilled water was added to it. It was shaken occasionally for about 30 minutes and the volume was made up to 100ml by adding distilled water. About 10ml of the solution from the volumetric flask was taken and centrifuged. The supernatant solution from the centrifuge tube was collected and again filtered by using Millipore filter. Then the filtrate was subsequently diluted and the absorbance was measured at 278nm. This test was repeated six times (N=6) for each batch of tablets. The amounts of Verapamil Hydrochloride estimated from different batches were depicted in Table No: 4.19 to 4.22.
3.6 SWELLING INDEX

The swelling behavior of a dosage unit was measured by studying its weight gain. The swelling index of tablets was determined by placing the tablets in the basket of dissolution apparatus using dissolution medium 0.1 N HCl at 37±0.5°C. After 0.5, 1, 2, 3, 4, 5, 6, 7 and 8hr’s, each dissolution basket containing tablet was withdrawn and blotted with tissue paper to remove the excess water and weighed on the analytical balance (Shimadzu, Ax 120). The experiment was performed in triplicate for each time point. Swelling index was calculated by using the following formula [Parade B, 1996]. The swelling index for various selected formulations of matrix tablets were shown in Table No: 4.23.

\[
\text{Swelling index} = \frac{\text{Wet weight of tablet} - \text{Dry weight of tablet}}{\text{Dry weight of tablet}}
\]

3.7 DRUG RELEASE STUDIES

3.7.1 Verapamil Hydrochloride Matrix Tablets:

Dissolution studies on each matrix tablet formulations were performed on a calibrated 8 station LABINDIA dissolution apparatus equipped with paddles employing 900ml of 0.1N Hydrochloric acid for first 2 hours and then replaced with 6.8 pH phosphate buffer for the rest of the studies as media. The paddles were operated to rotate at 100 rpm and the temperature of the medium was maintained at 37±1°C throughout the studies. Dissolution samples were withdrawn at regular intervals upto 12 hrs and replaced with equal volume to maintain the constant volume of the dissolution medium throughout the studies. The drug content in the samples was determined by measuring the absorbance at 278nm by ELICO double beam uv spectrophotometer after suitable dilution of the samples. Necessary corrections were made for the loss of drug due to each sampling and plotted the cumulative % amount of drug released Vs time. The drug release experiments were performed 6 times for each batch of formulation as per I.P Dissolution acceptance criteria. And the average of 6 values were taken for studies (n=6).
The dissolution profiles were depicted in tables 4.24 to 4.30 & 4.38 to 4.44 and shown in graphs 4.3 to 4.9 & 4.17 to 4.23. The square root plots and their corresponding release rate constants were given in respective tabular columns from next pages.

3.7.2 Losartan Potassium Matrix Tablets:

Dissolution studies on each matrix tablet formulations were performed on a calibrated 8 station LABINDIA dissolution apparatus equipped with paddles employing 900ml of 0.1N Hydrochloride acid for first 2 hours and then replaced with 6.8 pH phosphate buffers for the rest of the studies as media. The paddles were operated to rotate at 100 rpm and the temperature of the medium was maintained at 37±1°C throughout the studies. Dissolution samples were withdrawn at regular intervals upto 12 hrs and replaced with equal volume to maintain the constant volume of the dissolution medium throughout the studies. The drug content in the samples was determined by measuring the absorbance at 205nm by ELICO double beam UV spectrophotometer after suitable dilution of the samples. Necessary corrections were made for the loss of drug due to each sampling and plotted the cumulative % amount of drug released Vs time. The drug release experiments were performed 6 times for each batch of formulation as per I.P Dissolution acceptance criteria and the average of 6 values was taken for studies. (n=6).

The dissolution profiles were depicted in tables 4.31 to 4.37 & 4.45- 4.51 and shown in graphs 4.10 – 4.16 & 4.24 to 4.30. The square root plots and their corresponding release rate constants were given in respective tabular columns from next pages.

3.8 ESTIMATION OF INVITRO DRUG RELEASE KINETICS

From the dissolution studies of various matrix tablets containing Verapamil Hydrochloride and Losartan Potassium the following invitro kinetics were evaluated.

3.8.1 First Order-Release Rate Constant:

The graphs were plotted with log% undissolved on Y-axis and time in hours on X-axis. The plots were shown as graphs 4.31 to 4.58. From the slope values obtained with the graphs the ‘K’ value, (first order release rate constants) for all the formulations were calculated by multiplying the slope value with 2.303. The correlation coefficient (R²) values were calculated for each and every plot to check the linearity of the drug release.
The first order release rate constant and correlation coefficient values were given in Table: 4.52 to 4.55.

3.8.2 Higuchi’s Diffusion Constant:

The graphs were plotted with cumulative amount of drug release on Y-axis and square root of time on X-axis. The graphs were plotted based on the Higuchi’s diffusion rate equation. The plots were shown as graphs 4.59 to 4.86.

The dissolution rate constants (Higuchi’s constants) were calculated as slope values obtained from the plots. The correlation coefficient values (R²) were calculated for each and every plot to check the linearity of the drug release by diffusion process. The Higuchi’s constant and respective values were given in Table 4.52 to 4.55.

3.8.3 Korsermeyer-Peppas Constant:

The graphs were plotted with Mt/Ma on Y-axis and time ‘t’ on X-axis. The plots were shown as graphs 4.87 to 4.114.

The peppas constant was obtained by calculating the slope from graphs. The n values were obtained from Y-intercept. The correlation coefficient (R²) values were calculated for each and every plot to check the linearity of the drug release. The n values, peppas constant values and correlation coefficient values were given in Tables: 4.52 to 4.55.

3.9 I.R. SPECTRAL STUDIES

I.R Spectral studies were carried out on some selected matrix tablets by using BRUKER FOURIER TRANSFER INFRARED SPECTROSCOPY.

These studies on matrix tablets were performed before they are subjected to dissolution studies to check the structural variation if any arised between the drug and electrolytes used in the matrix tablets. The I.R. spectra of various tablet formulations were shown as figure 2 – 3.
3.10 IN VIVO PHARMACOKINETIC STUDIES OF VERAPAMIL HYDROCHLORIDE

Animals: Male Newzealand white rabbits weighing between 1.5 to 2.0 Kg were used for in vivo studies of orally administered Verapamil Hydrochloride. Animals were housed at 25±1°C in air conditioned room at a relative humidity of 60±2% and were provided with water adlibitum and standard rabbit feed obtained from M/S RAYANS Bio Tech. Pvt. Ltd., Hyderabad.

Animals were fasted for 24hrs prior to the administration of the drug formulation, but had free access to water. The matrix tablet was administered orally with a soft plastic tube. Drug solution was also administered orally with a soft plastic tube (Verapamil Hydrochloride 10mg/rabbit) to another group of animals. About 2.0 ml of blood was collected at 0.5, 1.0, 2.0, 3.0, 4.0, 6.0, 8.0 and 12.0 hr’s after oral solution administration and 0, 1, 2, 4, 6, 8, 12, 16, 20 and 24 hr’s after matrix tablets administration from marginal ear vein into the heparinized tubes and the plasma was separated immediately and frozen at -20°C.

In case of Verapamil Hydrochloride, the blood was collected into clean test tubes and allowed to clot. The serum was separated by centrifugation and frozen at -20°C. The concentration of the Verapamil Hydrochloride in plasma was analyzed by HPLC method as described earlier.

Pharmacokinetic Parameters: The pharmacokinetic parameters such as maximum plasma/serum concentration (Cmax), time to reach peak plasma/serum concentration (tmax) were taken directly from the individual plasma/serum concentration – time profiles. The other pharmacokinetic parameters like biological half-life (t1/2), mean residence time (MRT) and area under the curve (AUC0-a) were calculated by model independent computer programme (“PK SOLUTIONS” 2.0, provided by summit research services, USA) Which measures the t1/2 from the regression of the terminal phase of the concentration – time plot. MRT was calculated by dividing the AUMC0-a by AUC0-a whereas, AUC0-a was calculated by linear trapezoidal rule. The data was given in table 4.56 and depicted in graph 4.115.
3.11. *INVIVO* PHARMACOKINETIC STUDIES OF Losartan Potassium

**Animals:** Male Newzealand white rabbits weighing between 1.5 to 2.0 Kg were used for in vivo studies of orally administered Losartan Potassium. Animals were housed at 25±1°C in air conditioned room at a relative humidity of 60±2% and were provided with water adlibitum and standard rabbit feed obtained from M/S RAYANS Bio Tech. Pvt. Ltd., Hyderabad.

Animals were fasted for 24hr’s prior to the administration of the drug formulation, but had free access to water. The matrix tablet was administered orally with a soft plastic tube. Drug solution was also administered orally with a soft plastic tube (Losartan Potassium potassium 10mg/rabbit) to another group of animals. About 2.0 ml of blood was collected at 0.5, 1.0, 2.0, 3.0, 4.0, 6.0, 8.0 and 12.0 hr’s after oral solution administration and 1, 2, 4, 6, 8, 12, 16, 20 and 24 hr’s after matrix tablets administration from marginal ear vein into the heparinized tubes and the plasma was separated immediately and frozen at -20°C.

In case of Losartan Potassium, the blood was collected into clean test tubes and allowed to clot. The plasma was separated by centrifugation and frozen at -20°C. The concentration of the Losartan Potassium in plasma was analysed by HPLC method as described earlier.

**Pharmacokinetic Parameters:** The pharmacokinetic parameters such as maximum plasma/serum concentration (C_{max}), time to reach peak plasma/serum concentration (t_{max}) were taken directly from the individual plasma/serum concentration – time profiles. The other pharmacokinetic parameters like biological half-life (t_{1/2}), mean residence time (MRT) and area under the curve (AUC_{0-\alpha}) were calculated by model independent computer programme (“PK SOLUTIONS” 2.0, provided by summit research services, USA) which measures the t_{1/2} from the regression of the terminal phase of the concentration – time plot. MRT was calculated by dividing the AUMC_{0-\alpha} by AUC_{0-\alpha} whereas; AUC_{0-\alpha} was calculated by linear trapezoidal rule. The data was given in table 4.57 and depicted in graph 4.116.
3.12 ACCELERATED STABILITY STUDIES

The formulation, which showed good in vivo performance were subjected to accelerated stability studies. These studies were carried out by investigating the effect of temperature on the physical properties of tablets and chemical stability of matrix tablets containing drugs. The matrix tablet formulations such as VP3, VPA6 and VPC44 containing Verapamil Hydrochloride and LP3, LPA6 and LPS55 containing Losartan Potassium were subjected to accelerated stability studies.

The above said formulations were kept in screw mouthed bottles after preparation and stored in thermostated ovens at a temperature and relative humidity of 25\(^{0}\pm 2^{0}\)C, 60\(^{0}\pm 5\%\) RH for 12 months and 40\(^{0}\pm 2^{0}\)C, 75\(^{0}\pm 5\%\) RH for 6 months. Then the samples of each type of formulations were evaluated for the earlier mentioned physical parameters. The tablets were evaluated for physical parameters and drugs were analyzed for drug content uniformity by a known Spectrophotometric method as described earlier. Further these were subjected to drug release studies as stated earlier. The data was given in tables 4.58 to 4.59.