6. MATERIALS AND METHODS

6.1 MATERIALS

Table 12 List of materials used in the experiments throughout the work

<table>
<thead>
<tr>
<th>Drug/Excipient</th>
<th>Gifted by / Procured from</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valsartan &amp; Telmisartan</td>
<td>Aurobindo Pharmaceuticals, Hyderabad, India</td>
</tr>
<tr>
<td>Avicel PH102</td>
<td>Signet Chemicals Corporation, Mumbai, India</td>
</tr>
<tr>
<td>Pluronic F68</td>
<td>Sigma-Aldrich Co., USA</td>
</tr>
<tr>
<td>Eudragit E100</td>
<td>Evonik Industries AG, Germany</td>
</tr>
<tr>
<td>Gelucire 50/13 &amp; Transcutol HP</td>
<td>Gattefosse India Pvt. Ltd., Mumbai, India</td>
</tr>
<tr>
<td>Tween 20, Tween 80, Propylene glycol (PG) Polyethylene glycol (PEG 200, 400, 600, 4000 and 8000) Sodium hydroxide, Magnesium stearate Potassium dihydrogen orthophosphate</td>
<td>Sd Fine-Chem Ltd., Mumbai, India</td>
</tr>
<tr>
<td>Aerosil 200, Lactose mono hydrate Dicalcium phosphate (DCP) di hydrate Croscarmellose sodium</td>
<td>Nehal traders, Hyderabad, India</td>
</tr>
<tr>
<td>Acetonitrile, Methanol (HPLC grade)</td>
<td>Merck India ltd., India</td>
</tr>
<tr>
<td>Valzaar 40 mg (Torrent Pharmaceutical Ltd., Ahmadabad) Telma 20 mg (Glenmark Pharmaceutical Ltd., Himachal pradesh)</td>
<td>Local Pharmacy</td>
</tr>
</tbody>
</table>
6.2 METHODS

The study was divided into four sections

**Experiment I:** Liquisolid compacts of valsartan and telmisartan

**Experiment II:** Melt dispersion granules of valsartan and telmisartan

**Experiment III:** Polymeric solid dispersions of valsartan and telmisartan

**Experiment IV:** In vivo studies on selected formulations

**Experiment I: Liquisolid Compacts**

Liquisolid compacts of valsartan and telmisartan were prepared by direct compression. The drug excipient interactions and solid state characterization was performed by the using FT-IR, DSC and XRD analysis. The prepared tablets were evaluated for hardness, friability, content uniformity, disintegration and in vitro dissolution. The dissolution profile of the optimized formulation was compared with plain drug and marketed formulation. The optimized liquisolid formulations were subjected to accelerated stability studies for 3 months.

**Experiment II: Melt dispersion granules**

Melt dispersion granules of valsartan and telmisartan were prepared using carriers, PEG 8000, Pluronic F68 and Gelucire 50/13 and lactose as surface adsorbent. The prepared granules were characterized by FT-IR, DSC, XRD and in vitro dissolution studies. The dissolution profiles of the optimized formulations were compared with that corresponding physical mixture, plain drug and marketed formulations. The optimized formulations were subjected to accelerated stability studies for 3 months.

**Experiment III: Polymeric solid dispersions**

Polymeric solid dispersions of valsartan and telmisartan were prepared using Eudragit E 100. The prepared dispersions were characterized by FT-IR, DSC, XRD and in vitro dissolution studies. The dissolution profiles of optimized formulations were compared with physical mixture, plain drug and marketed formulation. The optimized polymeric dispersions were subjected to accelerated stability studies for 3 months.
Experiment IV: In vivo studies on selected formulations

The optimized formulations of valsartan were subjected to in vivo pharmacokinetic studies. The formulations and plain drug was administered to male Wistar rats. The plasma drug concentration versus time profiles were obtained and bioavailability of the formulations was calculated and compared relative to plain drug. All animal experimentation were carried out with prior approval from institutional animal ethical committee.
6.3. ANALYTICAL METHOD DEVELOPMENT

Analytical method to quantify the samples were developed for valsartan and telmisartan by UV spectroscopy. HPLC method was also developed to quantify the valsartan in plasma samples.

6.3.1 UV SPECTROSCOPIC METHOD FOR VALSARTAN

Calibration curves of valsartan was prepared in different media i.e., 0.1 N hydrochloric acid (HCl), 0.001 N HCl, acetate buffer pH 4.5 and phosphate buffer pH 6.8. All solutions were freshly prepared before use.

6.3.1.1 Preparation of media/buffer solutions

Preparation of 0.1 N HCl

Aliquots of 8.5 ml of concentrated HCl was transferred into a 1000 ml of volumetric flask and diluted to 1000 ml with distilled water\textsuperscript{173}.

Preparation of 0.001 N HCl

Aliquots of 10 ml of 0.1 N HCl was pipetted into a 1000 ml volumetric flask and diluted to 1000 ml with distilled water.

Preparation of acetate buffer pH 4.5

Accurately weighed 2.99 g of sodium acetate trihydrate was placed in a 1000 ml volumetric flask. Aliquot of 14 ml of 2 N acetic acid was added and volume was made up to 1000 ml with distilled water.

Preparation of 0.2 M potassium dihydrogen phosphate solution

Accurately weighed 27.218 g of potassium dihydrogen phosphate was dissolved in some amount of water and diluted with distilled water to 1000 ml.

Preparation of 0.2 M sodium hydroxide solution

About 8 g of sodium hydroxide (NaOH) was dissolved in some amount of water and diluted with distilled water to 1000 ml.
Preparation of phosphate buffer pH 6.8

Aliquots of 250 ml of 0.2 M Potassium dihydrogen orthophosphate was transferred to a 1000 ml of volumetric flask and 112 ml of 0.2 M NaOH was added to it. The volume was made up to 1000 ml with distilled water. The pH was adjusted to 6.8 using 0.2 M Potassium dihydrogen orthophosphate or sodium hydroxide.

6.3.1.2 Preparation of standard solutions of valsartan

Stock solution – I: Accurately weighed amount (100 mg) of valsartan was placed in a 100 ml volumetric flask and small amount of methanol was added to dissolve the drug. The volume was made up to 100 ml using methanol to give 1000 μg/ml solution.

Stock solution – II: One ml aliquot from stock solution -I was taken and diluted to 10 ml with 0.1N HCl in a volumetric flask to get 100 μg/ml.

Stock solution – III: One ml aliquot from stock solution -II was taken and diluted to 10 ml with 0.1N HCl in a volumetric flask to get 10 μg/ml.

The same procedure was repeated using 0.001 N HCl, acetate buffer pH 4.5 and phosphate buffer pH 6.8.

6.3.2 UV SPECTROSCOPIC METHOD FOR TELMISARTAN

Calibration curves of telmisartan was prepared in different media i.e., 0.1 N HCl, acetate buffer pH 4.5 with 0.5 % sodium lauryl sulphate (SLS) and phosphate buffer saline pH 7.4 with 0.5 % SLS. All the solutions were freshly prepared before use.

6.3.2.1 Preparation of media/buffer solutions

Preparation of acetate buffer with 0.5 % SLS

Accurately weighed 5 g of sodium lauryl sulphate was dissolved in some amount of acetate buffer and the solution was made up to 1000 ml with buffer.
**Preparation of phosphate buffer saline (PBS) pH 7.4**

Dissolve 2.38 g of disodium hydrogen phosphate, 0.19 g of potassium dihydrogen phosphate and 8.0 g of sodium chloride in sufficient water to produce 1000 ml. The pH was adjusted if necessary.

**Preparation of PBS with 0.5 % SLS**

About 5 g of sodium lauryl sulphate was dissolved in some amount of PBS and the solution was made up to 1000 ml with PBS.

**6.3.2.2 Preparation of standard solution of telmisartan**

Stock solution – I: Accurately weighed amount (100 mg) of telmisartan was placed in a 100 ml volumetric flask and small amount of methanol was added to dissolve the drug. The volume was made up to 100 ml using methanol to give 1000 μg/ml solution.

Stock solution – II: One ml aliquot from stock solution -I was taken and diluted to 10 ml with 0.1 N HCl in a volumetric flask to get 100 μg/ml.

Stock solution – III: One ml aliquot from stock solution -II was taken and diluted to 10 ml with 0.1 N HCl in a volumetric flask to get 10 μg/ml.

The same procedure was repeated using acetate buffer pH 4.5 and PBS pH 7.4 containing 0.5 % SLS.

**6.3.2.3 Determination of absorption maxima (λ_{max})**

A 10 μg/ml standard solutions (stock solution III) of valsartan and telmisartan was scanned on a double beam spectrophotometer against their respective media blanks in the range of 200-400 nm. The wavelength where it shows maximum absorption was determined.
6.3.2.4 Preparation of calibration curve

Valsartan

From the valsartan stock solution-II containing (100 μg/ml), aliquots of 0.5, 1, 1.5, 2, 2.5 and 3 ml were diluted to 10 ml with 0.1 N HCl to obtain concentrations 5, 10, 15, 20, 25 and 30 μg/ml. The absorbance of solutions was determined at respective $\lambda_{\text{max}}$ against corresponding media blank. The experiment was repeated 6 times and mean with standard deviation was reported. Similar procedure was repeated using 0.001 N HCl, acetate buffer pH 4.5 and phosphate buffer pH 6.8.

Telmisartan

From the telmisartan stock solution-II (100 μg/ml), aliquots of 0.4, 0.6, 0.8, 1, and 1.2 ml were diluted to 10 ml with 0.1N HCl to obtain concentrations 4, 6, 8, 10 and 12 μg/ml. The absorbance of solutions was determined at respective $\lambda_{\text{max}}$ against corresponding media blank. The experiment was repeated 6 times and mean with standard deviation was reported. The same procedure was repeated using acetate buffer pH 4.5 and PBS pH 7.4 containing 0.5 % SLS.

6.3.3 HPLC METHOD FOR VALSARTAN

HPLC analysis was developed for plasma samples to determine the concentration of valsartan from the plasma samples.

6.3.3.1 Apparatus and chromatographic conditions

HPLC analysis was performed on Schimadzu system equipped with isocratic pump, UV detector and a rheodyne injector holding 20 μL loop. The separation of the compounds was carried out using Grace C18 column (250×4.6mm; 5μm) with isocratic elution. The mobile phase consisted of a mixture of acetonitrile and water in ratio of (60:40 % v/v) and pH was adjusted to 3.2 with dilute orthophosphoric acid or triethanolamine. The eluents were monitored at a wavelength of 225 nm and 250 nm at a flow rate of 0.7 ml/min. Data acquisition was performed using Shimadzu LC solution software.
6.3.3.2 Preparation of mobile phase

The mobile phase was freshly prepared, filtered through 0.45 µ membrane filter and degassed prior to use. Acetonitrile and water of HPLC grade were mixed in 60:40 v/v ratios and pH was adjusted to 3.2 using orthophosphoric acid or triethanolamine.

6.3.3.3 Preparation of plasma samples

Prior to HPLC analysis, plasma samples were removed from frozen storage, allowed to equilibrate to room temperature and processed using acetonitrile protein precipitation method\textsuperscript{174,175}. A measured volume of plasma 40 µl was transferred to a micro centrifuge tube and spiked with 10 µl of internal standard (10 µg/ml diclofenac sodium solution) was mixed and vortexed for 2 min. A volume of 450 µl of acetonitrile was added to precipitate the proteins and vortexed for 5 min. The mixture was centrifuged at 5000 rpm for 5 min. The supernatant was separated and filtered through 0.45 µ filter and 20 µl of the this solution was injected into the system \textsuperscript{176}.

6.3.3.4 Preparation of calibration curve of valsartan by HPLC method

About 10 mg of valsartan was dissolved in 100 ml of mobile phase prepared as discussed in section 6.3.3.2 to obtain a concentration of 100 µg/ml. From this stock solution, concentrations of 10, 20, 30, 40, 50 and 60 µg/ml were prepared by appropriate dilution with mobile phase. These solutions were spiked with 40 µl of plasma containing 10 µl internal standard (10 µg/ml diclofenac sodium solution) and processed as described in section 6.3.3.3 to yield concentrations of 200,400,600,800, 1000 and 1200 ng/ml. Plot of ratio of areas of valsartan and internal standard versus valsartan concentration was obtained wherein each value as a mean of 3 experiments.
6.4 EXPERIMENT I: LIQUISOLID COMPACTS

Liquisolid compacts of valsartan and telmisartan were prepared according to the procedure given by Spireas and Bolton\textsuperscript{40}.

6.4.1 SOLUBILITY STUDIES

To select the suitable non-volatile solvent for dissolving or suspending the drug, solubility studies of valsartan and telmisartan were conducted in different non-volatile solvents like propylene Glycol (PG), Polyethylene Glycol (PEG) 200, 600, Transcutol HP, Tween 20 and Tween 80. The solubility of valsartan and telmisartan in the given non-volatile solvents was carried out by equilibrium solubility method\textsuperscript{177}. An excess amount of either valsartan or telmisartan was added to vials containing selected vehicles. After sealing, the mixture was vortexed on a vertex mixer for 10 min and subjected to shaking on the incubator shaker (JEIOTECH, Korea) for 48 h at 25 ± 1°C. After this period, the solutions were centrifuged and supernatant was separated. The supernatant was filtered through a 0.45 µm Millipore filter, diluted with phosphate buffer and analysed by UV-spectrophotometer (JASCO V-650, Japan) at respective wavelength against blank. The blank samples were prepared with same concentration of solvent without drug. The determinations were carried out in triplicate and mean values were reported along with standard deviation for each drug in each solvent.

6.4.2 SELECTION OF CARRIER MATERIAL

Preliminary experiments were carried to determine the binding capacity of the carrier materials. Binding capacity is defined as the capacity of powder excipients to hold liquid with no change in their flow properties. This was determined by following technique. About 1 g of the different powder excipients (Avicel PH102, lactose and DCP) were taken in a mortar and non-volatile liquid was added in increments of 0.01 ml. The mixture was triturated after each addition to help distribution of the liquid throughout the powder particles. The amount of liquid required to wet the complete powder was determined.
6.4.3 CALCULATION OF LOAD FACTOR

Load factor was calculated from flowable liquid-retention potential value using equation (6). Elkordy et al. has given a procedure to calculate the flowable liquid retention potential based on angle of slide measurements. Accurately weighed quantity of Avicel (5 g) was mixed with increasing amounts of liquid vehicle. The resulting liquid/powder admixture was placed on one end of a polished metal plate. The metal plate was tilted gradually until the powder mixture with liquid starts to slide. The angle formed by the plate during the slide (angle of slide) was noted. The load factor was calculated based on the following formula at 33° was considered as optimal flowable liquid-retention potential according to the literature.

\[
\text{Load factor} = \frac{\text{Weight of non-volatile liquid vehicle}}{\text{weight of solid}} \quad (6)
\]

6.4.4 PREPARATION OF LIQUISOLID COMPACTS

The drug was dissolved or dispersed in different non-volatile solvents depending on the solubility in respective vehicle. Then calculated amount of carrier and coating materials were added to the liquid medication under constant mixing in a mortar and pestle to produce a dry free-flowing powder mixture. About 5% w/w of super disintegrant was added to the above powder and mixed thoroughly. Finally, 1% w/w lubricant was added to the liquisolid systems in the mortar and mixed until a homogeneous powder mixture was obtained. The resulting powder mixture was compressed into tablets using multi-station rotary punching machine. The compression force was adjusted depending on the weight of tablet and ingredients in the formulation as all the formulations are different in weight and composition.

6.4.5 EVALUATION OF LSC

6.4.5.1 Flow properties of liquisolid powders

The prepared liquisolid systems were evaluated for flow properties by angle of repose measurements using fixed funnel and free standing cone method. A funnel was fixed at a given height ‘H’, above a graph paper placed on a flat horizontal surface. The powders were carefully poured through the funnel until the apex of the conical
pile just touches the tip of the funnel. The mean radius ‘R’, of the base of the conical pile was determined and the tangent of the angle of repose was given by

\[ Tan \alpha = \frac{H}{R} \quad \text{Where } \alpha \text{ is angle of repose} \]

The flow properties of powders were concluded based on Table 13.

**Table 13** Relationship between angle of repose and flow property

<table>
<thead>
<tr>
<th>Angle of repose</th>
<th>Type of Flow</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 – 30</td>
<td>Excellent</td>
</tr>
<tr>
<td>31 – 35</td>
<td>Good</td>
</tr>
<tr>
<td>36 – 40</td>
<td>Fair – aid not needed</td>
</tr>
<tr>
<td>41 – 45</td>
<td>Passable — may hang up</td>
</tr>
<tr>
<td>46 – 55</td>
<td>Poor — must agitate, vibrate</td>
</tr>
<tr>
<td>56 – 65</td>
<td>Very poor</td>
</tr>
<tr>
<td>&gt; 66</td>
<td>Very very poor</td>
</tr>
</tbody>
</table>

**6.4.5.2 FTIR spectroscopy studies**

The spectra of valsartan, telmisartan, Avicel PH102, Aerosil 200, placebo (formulation without drug) and optimized formulation were separately recorded on a Perkin Elmer spectrophotometer using KBr pellet from 4000 cm\(^{-1}\) to 400 cm\(^{-1}\) range. The pellets were prepared by mixing 5 mg of sample with 100 mg potassium bromide and compacted at a pressure of about 12,000 psi for 3 minutes under vacuum.

**6.4.5.3 X-ray powder diffraction (XRD) studies**

The XRD patterns of valsartan, telmisartan, placebo and optimized formulation were obtained using Philips PW 3710 X-ray diffractometer. Samples were exposed to Cu radiation of wavelength 1.540 Å and analysed over the 2θ range of 2\(^{0}\) to 50\(^{0}\).
6.4.5.4 Differential scanning calorimetry (DSC) studies

DSC studies were performed using a Mettler DSC 1 (Mettler Toledo, Germany). The instrument was calibrated with an indium standard. Accurately weighed Samples (5-10 mg) were placed in a closed, pierced, flat bottom aluminium pans. DSC scans were recorded at a constant heating rate of 10 °C/min. DSC scan of valsartan was recorded from 30 to 200 °C and 30 to 350 °C for telmisartan. Nitrogen gas was pumped at a flow rate of 80 ml/min\textsuperscript{181}. The melting point, peak maxima, appearance of any new peak and change in peak shape was noted.

6.4.5.5 Hardness, friability, disintegration time and drug content

The prepared liquisolid tablets of valsartan and telmisartan were evaluated for hardness, friability, disintegration time and drug content by methods reported in the official compendium\textsuperscript{182}. Hardness was determined by Pfizer hardness tester that measures the pressure required to break diametrically placed tablets by applying pressure with coiled spring. The friability values of tablets were determined by Roche friabilator. The disintegration time was measured using USP disintegration tester (Electro lab, India). All the studies were done in triplicate and mean values with standard deviation are reported.

Ten tablets were selected randomly and weighed. Then drug equivalent to 10 mg was weighed and mixed with 100 ml of methanol. The mixture was vortexed for 10 min and sonicated. Insoluble residue was separated by filtration and absorbance was recorded using UV spectrophotometer after appropriate dilutions with phosphate buffer pH 6.8.

6.4.5.6 In vitro dissolution studies

The USP paddle method was used for all in vitro dissolution studies. Dissolution studies were performed in different media as shown in Table 14. The dissolution was maintained at 37.5 ± 0.5 °C. At appropriate intervals (5, 10, 15, 30, 45, 60, 90 and 120 min), 5 ml of sample was taken and filtered through a 0.45 micron filter. The samples were analyzed at respective wavelength by UV-Visible spectrophotometer. The mean of three determinations was used to calculate the drug release from each of the formulations.
Table 14 Summary of dissolution parameters used in the experiment

<table>
<thead>
<tr>
<th>Dissolution parameter</th>
<th>For valsartan</th>
<th>For telmisartan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dissolution media</td>
<td>0.1 N HCl (pH 1.2), 0.001 N HCl (pH 3.2), acetate buffer (pH 4.5) and phosphate buffer (pH 6.8)</td>
<td>0.1 N HCl (pH 1.2), acetate buffer with 0.5% SLS (pH 4.5) and PBS with 0.5% SLS (pH 7.4)</td>
</tr>
<tr>
<td>Volume</td>
<td>1000 ml</td>
<td>900 ml</td>
</tr>
<tr>
<td>rpm</td>
<td>50(^{183})</td>
<td>75(^{183})</td>
</tr>
</tbody>
</table>

6.4.5.7 Dissolution data treatment

For comparison of dissolution profiles, percentage of drug dissolved in 15 min \((Q_{15\text{min}})\), 30 min \((Q_{30\text{min}})\), Mean Dissolution Time (MDT) and dissolution efficiency (DE) at 30 min were calculated.

Mean Dissolution Time\(^{184}\) was calculated as follows:

\[
MDT = \frac{\sum_{i=0}^{n} t \Delta M_i}{\sum_{i=0}^{n} \Delta M_i} \quad (7)
\]

Where, ‘i’ is the sample number, ‘n’ is the number of dissolution sample times, ‘t’ is the time at the midpoint between \(t\) and \(t-1\) and \(\Delta M_i\) is the additional amount of drug dissolved between \(t\) and \(t-1\).

Dissolution efficiency DE is given by formula,

\[
DE = \frac{\int_{0}^{t} Y \, dt}{Y_{100} \, t} \times 100 \% \quad (8)
\]

Where ‘Y’ is the percent of drug released as a function of time, \(t\) is the total time of drug release and \(Y_{100}\) is 100 % drug release\(^{185}\).
The in vitro release profiles were further compared using similarity factors, \( f_2 \), as defined by the following equation.\(^{186}\)

\[
f_2 = 50 \log \left\{ 1 + \frac{1}{n} \sum_{t=1}^{n} (R_t - T_t) \right\}^{-0.5}
\] (9)

Where, ‘n’ is number of time points at which % dissolved was determined, ‘R_t’ is the % dissolved of one formulation and ‘T_t’ is the % dissolved from second formulation at a given time point. The similarity factor has the value between 0 and 100. The value will be 100 when the test and reference profiles are identical and approaches 0 as the dissimilarity increases. An \( f_2 \) above 50 points out that the two profiles are similar.

6.4.5.8 Stability studies

To obtain information on the stability of liquisolid systems, the effects of storage on the release profile and the crushing strength of LSC were investigated. The tablets were stored at 40\(^\circ\)C/75\% RH (relative humidity) for a period of 3 months and evaluated for hardness and % drug release and compared with those of freshly prepared tablets.

6.4.5.9 Statistical analysis

The difference in the dissolution rate of drugs from different formulations, physical mixture and plain drug in vitro were evaluated by one way ANOVA or paired t-test at a level of \( p = 0.05 \).
6.5 EXPERIMENT II: MELT DISPERSION GRANULES

6.5.1 PREPARATION OF MELT DISPERSION GRANULES

Melt dispersion granules of valsartan and telmisartan were prepared by combining fusion and adsorption process\textsuperscript{187-190}. The carriers selected for the study were Gelucire 50/13, PEG 8000 and Pluronic F-68 in four ratios (1:1, 1:2, 1:3 and 1:4). Total 12 formulations were prepared as shown in Table 15. The dispersions obtained with valsartan were represented as VGSD1 – VGSD4 (Gelucire), VP8SD1 – VP8SD4 (PEG) and VPOSD1 – VPOSD4 (Pluronic). Similarly, dispersion obtained with telmisartan were represented as TGSD1 – TGSD4 (Gelucire), TP8SD1 – TP8SD4 (PEG) and TPOSD1 – TPOSD4 (Pluronic). The carrier was melted in a petri dish, on water bath, at a temperature 5°C higher than its melting point. The drug was then dispersed in the molten carrier with continuous stirring and allowed to cool to room temperature. During the cooling process, the adsorbent was added to the molten mass and mixed properly to get a homogenous product. The blend was passed through a sieve (22 mesh) and stored in desiccators until subsequent analysis\textsuperscript{95}.

6.5.2 PREPARATION OF PHYSICAL MIXTURE

All the carriers were pulverised to fine particles before preparing the physical mixtures. Physical mixtures (PMs) of were prepared by slightly triturating valsartan in a mortar for 10 minutes with different carriers at same ratios as that of solid dispersions. To study the effect of each excipient used in the formulation on dissolution three types of physical mixtures were prepared at four ratios as follows. Total physical mixture (TPM1 – TPM4) consists of valsartan, carrier and adsorbent. Physical mixture containing valsartan and different carriers GPM1 – GPM4 with Gelucire, P8PM1 – P8PM4 with PEG and POPM1 – POPM4 with Pluronic. Physical mixture containing valsartan and lactose (LPM1- LPM4). Similar procedure was repeated with telmisartan.

6.5.3 CHARACTERIZATION STUDIES

The following studies were conducted to investigate the mechanism of improved dissolution properties and interactions if any between drug and excipients.
6.5.3.1 Flow properties of melt dispersion granules

The prepared valsartan and telmisartan granules were evaluated for flow properties by angle of repose measurements using fixed funnel and free standing cone method as per procedure described in section 6.4.5.1 under section flow properties of liquisolid compacts.

Table 15 Composition of different melt dispersion granule formulations

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Drug (mg)</th>
<th>Polymer (mg)</th>
<th>Lactose (mg)</th>
<th>Drug: carrier ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Valsartan</td>
<td>Telmisartan</td>
<td>Drug: carrier</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gelucire 50/13</td>
<td>PEG 8000</td>
<td>Pluronic F 68</td>
<td></td>
</tr>
<tr>
<td>VGSD1</td>
<td>100</td>
<td>100</td>
<td>-</td>
<td>-</td>
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<tr>
<td>VGSD2</td>
<td>100</td>
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<td>-</td>
<td>-</td>
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<tr>
<td>VGSD4</td>
<td>100</td>
<td>400</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>VP8SD1</td>
<td>100</td>
<td>-</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>VP8SD2</td>
<td>100</td>
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<td>200</td>
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<td>300</td>
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<tr>
<td>VPOS4</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>400</td>
</tr>
</tbody>
</table>
6.5.3.2 Determination of drug content

Accurately weighed quantities of ternary systems of drug, carrier and adsorbent were dissolved in methanol\(^1\). The solutions were vortexed and sonicated for 5 min using bath sonicator. The resulting solutions were filtered through 0.45 micron filter. The filtrate was diluted appropriately with suitable media and analyzed by UV spectroscopy (JASCO V-650, Japan). The drug content was determined from the respective calibration curves.

6.5.3.3 FTIR spectroscopy

The FTIR spectra of valsartan, telmisartan, Gelucire 50/13, PEG 8000, Pluronic F 68, lactose, physical mixture and optimised formulation were recorded on Perkin Elmer spectrophotometer as per procedure described in section 6.4.5.2 under FTIR spectroscopy studies.

6.5.3.4 X-ray powder diffraction (XRD) studies

The XRD patterns of valsartan, telmisartan, Gelucire 50/13, PEG 8000, Pluronic F 68, lactose, physical mixture and optimised formulation were obtained using Philips PW 3710 X-ray diffractometer as described in section 6.4.5.3 under X-ray powder diffraction studies.

6.5.3.5 Differential scanning calorimetry (DSC)

DSC studies were performed on valsartan, telmisartan, Gelucire 50/13, PEG 8000, Pluronic F 68, lactose, physical mixture and optimised formulation as per procedure described in section 6.4.5.4 using Mettler DSC 1 (Mettler Toledo, Germany).

6.5.3.6 In vitro drug release

In vitro dissolution studies of valsartan, telmisartan and their physical mixtures/formulations in different carriers was determined using USP dissolution apparatus II as described in section 6.4.5.6. The dissolution studies were performed in 0.1 N HCl for valsartan and in 0.1 N HCl and PBS with 0.5 % SLS for telmisartan formulations.
6.5.3.7 Dissolution data treatment

The dissolution data obtained from in vitro dissolution studies was analyzed by using different parameters like, percentage of drug dissolved in 15 min (Q_{15\text{ min}}), 30 min (Q_{30\text{ min}}), Mean Dissolution Time (MDT) and dissolution efficiency (DE) at 30 min as described in section 6.4.5.7.

6.5.3.8 Stability studies

The effects of storage on the release profile of melt dispersion granules were investigated. The optimized formulation was stored at 40°C / 75% RH for a period of 3 months and evaluated for % drug release and compared with those of freshly prepared formulations.

6.5.3.9 Statistical analysis

The difference in the dissolution rate of drugs from different formulations, physical mixture and plain drug were evaluated by one way ANOVA or paired t-test at a level of p = 0.05.
6.6 EXPERIMENT III: POLYMERIC SOLID DISPERSIONS

Polymeric solid dispersions of valsartan and telmisartan were prepared using Eudragit E 100 as a carrier. Various proportions of Eudragit based dispersions were prepared and evaluated for the role of polymer on the dissolution rate of both the drugs. The optimized formulations were characterized by FTIR and DSC analysis.

6.6.1 PREPARATION OF POLYMERIC DISPERSIONS

Polymeric solid dispersions were prepared by solvent evaporation method at various weight ratios of drug to the carrier (Table 16). Eudragit and drug was dissolved in a mixture of dichloromethane (DCM) and ethanol and stirred for 10 min to obtain clear solution. The solvent was evaporated under reduced pressure at 40 °C using rotary evaporator and the resulting residue was dried under vacuum for 3 h. The mixture was stored overnight in a desiccator. The hardened mixture was powdered in a mortar, sieved through a 100 mesh screen, and stored in a screw-cap vial at room temperature until further use.

6.6.2 PREPARATION OF PHYSICAL MIXTURE

The granules of Eudragit E 100 was crushed to fine particles before preparing the physical mixture. Physical mixtures (PMs) of valsartan and telmisartan in Eudragit were prepared at same ratios as that of solid dispersions by grinding them in a mortar for 10 min followed by sieving through 100 mesh. The mixtures were stored in a screw-cap vial at room temperature until use.

6.6.3 CHARACTERIZATION STUDIES

The following studies were conducted to investigate the mechanism of improved dissolution properties and interactions if any between drug and excipients.

6.6.3.1 Determination of drug content

Accurately weighed quantities of SD was dissolved in methanol and solutions were vortexed and sonicated for 5 min using bath sonicator. The resulting solutions were filtered through 0.45 micron filter. The filtrate was diluted appropriately with suitable
media and analysed by UV spectroscopy. The drug content was determined from the respective calibration curves.

**Table 16** Composition of Eudragit E100 solid dispersions

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Drug (mg)</th>
<th>Eudragit E100 (mg)</th>
<th>Drug to Eudragit ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valsartan</td>
<td>Telmisartan</td>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td>VF1</td>
<td>TF1</td>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td>VF2</td>
<td>TF2</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>VF3</td>
<td>TF3</td>
<td>100</td>
<td>200</td>
</tr>
<tr>
<td>VF4</td>
<td>TF4</td>
<td>100</td>
<td>300</td>
</tr>
</tbody>
</table>

**6.6.3.2 FTIR spectroscopy**

The FTIR spectra of valsartan, telmisartan, Eudragit E 100, physical mixture and optimised formulation were recorded on Perkin Elmer spectrophotometer as per procedure described in *section 6.4.5.2* under FTIR spectroscopy studies.

**6.6.3.3 Differential scanning calorimetry (DSC)**

DSC studies were performed on valsartan, telmisartan, Eudragit E 100, physical mixture and optimised formulation as per procedure described in *section 6.4.5.4* using Mettler DSC 1 (Mettler Toledo, Germany).

**6.6.3.4 *In vitro* dissolution studies**

In vitro dissolution studies of valsartan, telmisartan, physical mixtures and their formulations was determined using USP dissolution apparatus II as described in *section 6.4.5.6*. 
6.6.3.5 Dissolution data treatment

The dissolution data obtained from in vitro dissolution studies was analyzed by using different parameters like, percentage of drug dissolved in 15 min (Q_{15 min}), 30 min (Q_{30 min}), Mean Dissolution Time (MDT) and dissolution efficiency (DE) at 30 min as described in section 6.4.5.7.

6.6.3.6 Kinetic analysis of dissolution data

*In vitro* drug release data were fitted to various release kinetic models viz. zero-order, first-order, Higuchi and Korsemeyer–Peppas model using the following set of equations\(^\text{195}\).

Zero order equation

\[
M_0 - M_t = K_0 t \quad (10)
\]

First order equation

\[
\ln \left( \frac{M_0}{M_t} \right) = K_1 t \quad (11)
\]

Higuchi equation

\[
M_t = K_h \sqrt{t} \quad (12)
\]

Korsemeyer–Peppas model

\[
\frac{M_t}{M_\infty} = t^n \quad (13)
\]

Where, \(M_0\), \(M_t\) and \(M_\infty\) correspond to the drug amount taken at time equal to zero, dissolved at a particular time, \(t\), and at infinite time, respectively. \(K_0\), \(K_1\), and \(K_h\) refer to the release kinetic constants obtained from the linear curves of zero-order, first-order and constant incorporating the surface–volume relation, respectively.

For Korsemeyer–Peppas models, the data taken was within 10 - 60\% drug release\(^\text{196}\). The ‘\(n\)’ value is used to characterize different release patterns from Peppas model. In this model, \(0.45 \leq n\) corresponds to a Fickian diffusion mechanism, \(0.45 < n < 0.89\) to non-Fickian transport, \(n = 0.89\) to Case II (relaxational) transport, and \(n > 0.89\) to super case II transport\(^\text{197}\).
6.6.3.7 Stability studies

The effects of storage on the release profile of solid dispersions were investigated. The optimized formulation was stored at 40°C / 75% RH for a period of 3 months and evaluated for % drug release and compared with those of freshly prepared formulations.

6.6.3.8 Statistical analysis

The difference in the dissolution rate of drugs from different formulations, physical mixture and plain drug were evaluated by one way ANOVA and paired t-test at p = 0.05.
6.7 EXPERIMENT IV: IN VIVO STUDIES OF SELECTED VALSARTAN FORMULATIONS

6.7.1 BIOAVAILABILITY STUDIES

All animal experiments were carried out according to the protocol (IAEC No: NIP/04/2012/PE/11) approved by Institutional Animal Ethics Committee of National Institute of Pharmaceutical Educational and Research (NIPER), Hyderabad, India. The experimental procedures were in accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines for the safe use and care of experimental animals.

Bioavailability of valsartan from solid dispersion in Gelucire 50/13 and Eudragit E100 was compared with the valsartan plain drug dispersed in by triturating drug with sodium carboxy methyl cellulose. Male Wistar rats weighing 200 - 220 g were used in the study. The animals were kept in the animal house at a temperature of 25 ± 2°C with 12 h of dark and light cycles. Animals were fasted overnight but allowed free access to water. Eighteen rats were divided into three groups. The first group received valsartan plain drug, second group received valsartan melt dispersion granules in Gelucire 50/13 and third group of animals were administered with valsartan Eudragit E 100 solid dispersion formulation equivalent to a dose of 10 mg/kg body weight orally. Blood samples (0.5 ml) were collected by retro-orbital venous plexus puncture with aid of glass capillary at 0.25, 0.5, 1, 2, 4, 6 and 8 h post oral dose. All samples were collected in EDTA coated Eppendorf tubes. Plasma was separated by centrifuging at 5000 rpm for 5 min. Plasma samples were stored at -20°C until further analysis. Valsartan concentration in plasma was determined using calibration curve of valsartan prepared by HPLC method as per procedure described in section 6.3.2.4.

6.7.2 PHARMACOKINETIC ANALYSIS

Pharmacokinetic parameters were calculated using Kinetica software (version 5 demo, Thermo Fisher Scientific) by standard non compartmental model. Area under the concentration-time curve (AUC$_{0-6}$) was calculated by the trapezoidal method for 0 to 6 hrs. The peak plasma concentration (C$_{max}$) and time to attain maximum concentration (t$_{max}$) were directly obtained from the plasma concentration time plots.
The relative bioavailability of valsartan formulation was calculated against valsartan suspension using the formula mentioned below.

\[
\text{% Relative bioavailability} = \frac{\text{AUC (test)} / \text{AUC (reference)}}{\text{Dose (reference)} / \text{Dose (test)}} \times 100
\]

6.7.3 STATISTICAL ANALYSIS

The data obtained from plasma concentration time profile of formulations and plain drug were compared by paired t-test at a level of significance of P = 0.05 for calculated parameters.