Chapter - 4

Chapter - 4. EXPERIMENTAL INVESTIGATIONS

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4. EXPERIMENTAL INVESTIGATIONS

4.1 ANALYTICAL METHODS

4.1.1 Estimation of Atorvastatin Calcium

A spectrophotometric method based on the measurement of absorbance at 246 nm in 6.8 pH phosphate buffer was used in the present study for estimation of Atorvastatin Calcium\textsuperscript{168-169}.

**Standard Solution:**

100 mg of Atorvastatin Calcium was weighed and dissolved in methanol in a 100 ml volumetric flask and the solution was made up to volume with methanol.

**Procedure:**

Standard solution of Atorvastatin Calcium was subsequently diluted with 6.8 pH phosphate buffer to obtain series of dilutions containing 2,4,6,8,10 µg of Atorvastatin Calcium per 1ml of solution. The absorbance of the above solutions were measured in Elico double beam UV spectrophotometer at 246 nm using 6.8 pH phosphate buffer as blank. Concentration of Atorvastatin Calcium and corresponding absorbance values were given in table 5.1 The absorbance values were plotted against concentration of Atorvastatin Calcium as shown in figure 5.1. The method obeys the Beer’s law in the concentration range of 0-20 µg/ml. Reproducibility of the method was tested by analyzing six separately weighed samples of Atorvastatin Calcium.
Thus the method was found to be suitable for the estimation of Atorvastatin Calcium in dissolution fluids. Calibration curve shown in figure 5.1 was used for the estimation of Atorvastatin Calcium.

### 4.1.2 Estimation of Rosuvastatin Calcium

A spectrophotometric method based on the measurement of absorbance at 248 nm in 6.8 pH phosphate buffer was used in the present study for the estimation of Rosuvastatin Calcium\textsuperscript{170-171}.

**Standard Solution:**

100 mg of Rosuvastatin Calcium was weighed and dissolved in methanol in a 100 ml volumetric flask and the solution was made up to volume with methanol.

**Procedure**

Standard solution of Rosuvastatin Calcium was subsequently diluted with 6.8 pH phosphate buffer to obtain series of dilutions containing 2,4,6,8,10 µg of Rosuvastatin Calcium per 1ml of solution. Absorbance of the above solutions were measured in Elicco double beam UV spectrophotometer at 248 nm using 6.8 pH phosphate buffer as blank. The concentration of Rosuvastatin Calcium and corresponding absorbance values are given in table 5.2. The absorbance values were plotted against concentration of Rosuvastatin Calcium as shown in figure 5.2. The method obeys the Beer’s law in the concentration range of 0-20 µg/ml. Reproducibility of the method
was tested by analyzing six separately weighed samples of Rosuvastatin Calcium. Thus the method was found to be suitable for the estimation of Rosuvastatin Calcium in dissolution fluids. Calibration curve shown in figure 5.2 was used for the estimation of Rosuvastatin Calcium.

4.1.3 Assay of Atorvastatin Calcium in Rabbit Plasma

The plasma concentration of Atorvastatin Calcium was estimated by HPLC method\textsuperscript{172-173}. Stock solutions of Atorvastatin Calcium and internal standard, Diclofenac sodium each 1mg/ml were prepared in methanol. Working standard solutions of Atorvastatin Calcium was prepared by serial dilutions of stock solutions by the mobile phase (methanol:water, 68:32 v/v) at a concentration range of 20-100 ng/ml keeping internal standard concentration 100 ng/ml. Blood samples were collected from rabbits and centrifuged at 3000 rpm, 4\textdegree C for 20 minutes. The plasma was collected and stored at -20\textdegree C until analysis. For sample preparation the plasma was thawed at room temperature and a volume of 200 µl was transferred to a glass tube and spiked with 50 µl of standard solutions containing concentrations ranging from 0.2-1µg/ml of Atorvastatin Calcium and a constant concentration of diclofenac sodium 1µg/ml as internal standard. Blank samples were spiked with 50µl of mobile phase. Atorvastatin Calcium was extracted from plasma samples using liquid liquid extraction technique. Ammonium acetate buffer was added to the samples for protein precipitation. The samples were then vortex-
mixed for 1 minute. Then methyl-tert-butyl ether 4 ml was added for extraction of Atorvastatin Calcium, then vortex-mixed for 2 minutes and centrifuged at 4100 rpm, 4°C for 10 minutes. The clear supernatant layer was separated in a glass test tube and evaporated to complete dryness under the gentle stream of nitrogen at 40°C. After drying the residue was reconstituted in 500 µl of mobile phase. Samples were vortex-mixed for 1 minute then placed in inser tubes inside the pre-labeled HPLC vials and 20 µl were injected into the HPLC column for analysis. The HPLC analysis was performed at room temperature using methanol:water (68:32 v/v) as a mobile phase. The mobile phase was always clarified by filtration through a nylon filter paper, with pore size equal to 0.45µm and degassed through a sonicator, the pumped at a flow rate of 1 ml/min, in an isocratic mode on hypersil BDS C18 column (250 mm x 4.6 mm, 5 µm). The peak response was monitored at a wavelength of 241 nm. The data was acquired using Chromquest workstation software. The plot of peak ratio of Atorvastatin Calcium to Diclofenac sodium against the concentration of Atorvastatin Calcium in plasma was linear in the concentration range of 20-200 ng/ml. The limit of detection and lower limit of quantitation of Atorvastatin Calcium with this method is 1.35 ng/ml and 10.3 ng/ml. The mean percent drug recovered for the above concentration range was 96.47% indicating the method is reproducible. It is given in table 5.3 and shown in figure 5.3.
4.1.4 Assay of Rosuvastatin Calcium in Rabbit Plasma

The plasma concentration of Rosuvastatin Calcium was estimated by HPLC method\textsuperscript{174-175}. Stock solutions of Atorvastatin Calcium and internal standard, Naproxen each 1mg/ml were prepared in methanol. Working standard solutions of Rosuvastatin Calcium was prepared by serial dilutions of stock solutions by the mobile phase (methanol:water, 68:32 v/v) at a concentration range of 20-200 ng/ml keeping internal standard concentration 400 ng/ml. Blood samples were collected from rabbits and centrifuged at 3000 rpm, 4$^\circ$C for 20 minutes. The plasma was collected and stored at -20$^\circ$C until analysis. For sample preparation the plasma was thawed at room temperature and a volume of 200 µl was transferred to a glass tube and spiked with 50 µl of standard solutions containing concentrations ranging from 0.2-1µg/ml of Rosuvastatin Calcium and a constant concentration of Naproxen 0.4µg/ml as internal standard. Blank samples were spiked with 50µl of mobile phase. Rosuvastatin Calcium was extracted from plasma samples using liquid liquid extraction technique. Ammonium acetate buffer was added to the samples for protein precipitation. The samples were then vortex-mixed for 1 minute. Then methyl-tert-butyl ether 4 ml was added for extraction of Atorvastatin Calcium, then vortex-mixed for 2 minutes and centrifuged at 4100 rpm, 4$^\circ$C for 10 minutes. The clear supernatant layer was separated in a glass test tube and evaporated to complete dryness under the gentle stream of nitrogen at 40$^\circ$C. After drying the
residue was reconstituted in 500 µl of mobile phase. Samples were vortex-mixed for 1 minute then placed in inser tubes inside the per labeled HPLC vials and 20 µl were injected into the HPLC column for analysis. The HPLC analysis was performed at room temperature using methanol:water (68:32 v/v) as a mobile phase. The mobile phase was always clarified by filtration through a nylon filter paper, with pore size equal to 0.45µm and degassed through a sonicator, the pumped at a flow rate of 1 ml/min, in an isocratic mode on hypersil BDS C18 column (250 mm x 4.6 mm, 5 µm). The peak response was monitored at a wave length of 241 nm. The data was acquired using Chromquest workstation software. The plot of peak ratio of Rosuvastatin Calcium to Naproxen against the concentration of Rosuvastatin Calcium in plasma was linear in the concentration range of 20-200 ng/ml. The limit of detection and lower limit of quantitation of Rosuvastatin Calcium with this method is 7.2 ng/ml and 8.5 ng/ml. The mean percent drug recovered for the above concentration range was 91.43% indicating the method is reproducible. It is given in table 5.4 and shown in figure 5.4.

4.2 SATURATED SOLUBILITY STUDIES

4.2.1 Saturated Solubility Studies of Atorvastatin Calcium

Saturated solubility studies of Atorvastatin Calcium was performed in different dissolution media. 500mg of Atorvastatin Calcium was weighed and transferred into different conical flasks. 50ml of different dissolution media were transferred into individual
conical flasks and were closed appropriately. All the conical flasks were placed in the REMI incubator shaker. The shaker was allowed to operate at 50 rpm at 37°C ± 1°C for 24 hrs. Then the conical flasks were removed from the incubator shaker and the samples were filtered by using whatman filter paper. The clear solution obtained by filtration was suitably diluted with appropriate dissolution media and the absorbance values were noted at 246 nm by using corresponding dissolution media as blank solutions. The solubility of Atorvastatin Calcium in different dissolution media were given in table 5.5.

4.2.2 Saturated Solubility Studies of Rosuvastatin Calcium

Saturated solubility studies of Rosuvastatin Calcium was performed in different dissolution media. 500mg of Rosuvastatin Calcium was weighed and transferred into different conical flasks. 50ml of different dissolution media were transferred into individual conical flasks and were closed appropriately. All the conical flasks were placed in the REMI incubator shaker. The shaker was allowed to operate at 50 rpm at 37°C ± 1°C for 24 hrs. Then the conical flasks were removed from the incubator shaker and the samples were filtered by using whatman filter paper. The clear solution obtained by filtration was suitably diluted with appropriate dissolution media and the absorbance values were noted at 248 nm by using corresponding dissolution media as blank solutions. The solubility of Rosuvastatin Calcium in different dissolution media were given in table 5.6.
4.3 PREPARATION OF SOLID DISPERSIONS

Solid dispersions of Atorvastatin Calcium and Rosuvastatin Calcium were prepared by using Polyethylene glycol-6000 as a carrier by employing different techniques. The carrier concentration was maintained constant in the investigation. The methods employed for the preparation of solid dispersions are:

4.3.1 Physical Mixing:

Specified quantity of drug and polyethylene glycol-6000 were weighed separately and passed separately through sieve no 80. The materials passed through sieve no. 80 were collected and transferred into a clean and dry glass mortar. Drug and PEG-6000 were triturated together and again screened through sieve no 100. The mixture passed through sieve no 100 was collected and packed in a wide mouthed amber coloured glass container and was hermetically sealed. Then the mixture was stored at an ambient conditions.

4.3.2 Fusion Method:

Specified quantity of PEG-6000 was taken in a china dish and it was heated at on a mantle until molten mass was formed. To the molten mass specified quantity of drug was added and triturated vigorously at room temperature. The mixture obtained was triturated thoroughly in a glass mortar and screened through sieve no. 100. Then the mixture was collected, packed in a wide mouthed amber coloured
glass container and was hermetically sealed. Then the mixture was stored at an ambient conditions\textsuperscript{178}.

**4.3.3 Solvent Evaporation:**

Specified quantity of drug was taken in a china dish and it was dissolved in few ml of methanol. To the methanolic solution, specified amount of PEG-6000 was added and the solvent was evaporated under vacuum using Rota flash evaporator. The mixture obtained was triturated thoroughly in a glass mortar and screened through sieve no.100. Then the mixture was collected, packed in a wide mouthed amber coloured glass container and was hermetically sealed. Then the mixture was stored at an ambient conditions\textsuperscript{179}.

**4.3.4 Lyophilisation:**

Specified quantity of drug and PEG-6000 were weighed added with minimum amount of water. This dispersion was rapidly solidified by freezing in the IlShin freeze drier (Shin Lab Co., Ltd). The solvent in the dispersion was sublimed under a pressure of 10M torr and condensed onto a -40°C condenser. After the solvent was completely removed, the powder residue appeared as a porous, light and puffy mass. The lyophilised preparations were stored in a dessicator at room temperature\textsuperscript{180}.

The composition of various solid dispersions were given in tables 5.7 & 5.8.

**4.4 EVALUATION OF SOLID DISPERSIONS**
Physical parameters such as Angle of Repose, Carr’s Index, Average particle size and Drug content were evaluated for prepared tablets as per the standards of official compendium.

4.4.1 Angle of Repose

The internal angle between the surface of the pile of blend and the horizontal surface is known as the angle of repose.

**Method:**

The Angle of repose was known by passing the blend through a funnel fixed to a burette stand at a particular height (4 cm). A graph paper was placed below the funnel on the table. The height and radius of the pile was measured. Angle of repose of the blend was calculated using the formula:

\[ \theta = \tan^{-1} \left( \frac{h}{r} \right) \]

Where, \( h = \) Height of the pile; \( r = \) Radius of the pile.

The flow properties of different batches of solid dispersions were given in tables 5.9 & 5.10

**Table 4.1 Specifications of Angle of Repose**

<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>

4.4.2 Carr’s Index:
It is the propensity of a powder to be compressed.

Method

It is measured by tapped density apparatus for 500, 750 and 1250 taps for which the difference should be not more than 2%\(^\text{182}\). Based on the apparent bulk density and tapped density the percentage compressibility of the blend was determined using the following formula.

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

Table: 4.2 Specifications of Carr’s Index

<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>

The flow properties of different formulations were shown in tables 5.9 & 5.10.

4.4.3 Particle Size Determination:

The average particle size of the prepared solid dispersions was analyzed by simple sieve analysis method\(^\text{183}\). The average particle size of different formulations were shown in tables 5.9 & 5.10.

4.4.4 Drug Content Uniformity:
**a) Atorvastatin Calcium:**

Solid dispersions of Atorvastatin Calcium from a batch were taken at random and was transferred into a 100ml volumetric flask and 70ml of methanol was added to it. It was shaken occasionally for about 30 minutes and the volume was made up to 100ml by adding methanol. 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 Whatmann filter. Then the filtrate was subsequently diluted with 6.8 pH phosphate buffer and the absorbance was measured at 246 nm. This test was repeated ten times (n=10) for each batch of solid dispersions. The amount of Atorvastatin Calcium estimated from different batches were depicted in table 5.9.

**b) Rosuvastatin Calcium**

Solid dispersions of Rosuvastatin Calcium from a batch were taken at random and was transferred into a 100ml volumetric flask and 70ml of methanol was added to it. It was shaken occasionally for about 30 minutes and the volume was made up to 100ml by adding methanol. 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 Whatmann filter. Then the filtrate was subsequently diluted with 6.8 pH phosphate buffer and the absorbance was measured at 248nm. This test was repeated
ten times (n=10) for each batch of solid dispersions. The amount of Rosuvastatin Calcium estimated from different batches were depicted in table 5.10.

4.5 DRUG RELEASE STUDIES FROM SOLID DISPERSIONS

4.5.1 Atorvastatin Calcium Solid Dispersions:

Dissolution studies on solid dispersions were performed in a calibrated eight stage dissolution rate test apparatus equipped with paddles employing 900 ml of 6.8 pH phosphate buffer as a medium. The paddles were operated at 50 rpm and the temperature was maintained at 37±0.5°C throughout the experiment. Samples were withdrawn at 5, 10, 15, 20, 30 minutes and replaced with equal volume to maintain the constant volume of dissolution medium throughout the experiment. Drug content of the samples was determined by Elico double beam UV spectrophotometer at 246 nm after suitable dilutions of the samples. The drug release from solid dispersions were conducted in triplicate. The dissolution profiles were depicted in tables 5.11 to 5.14 and shown in figures 5.5 to 5.8. The first order profiles were shown in figures 5.9 to 5.12. The release rate constants, T_{50}, T_{90}, DE_{20}% were given in table 5.19.

4.5.2 Rosuvastatin Calcium Solid Dispersions:

Dissolution studies on solid dispersions were performed in a calibrated eight stage dissolution rate test apparatus equipped with paddles employing 900 ml of 6.8 pH phosphate buffer as a medium.
The paddles were operated at 50 rpm and the temperature was maintained at 37±0.5°C throughout the experiment. Samples were withdrawn at 5, 10, 15, 20, 30 minutes and replaced with equal volume to maintain the constant volume of dissolution medium throughout the experiment. Drug content of the samples was determined by Elico double beam UV spectrophotometer at 248 nm after suitable dilutions of the samples. The drug release from solid dispersions were conducted in triplicate. The dissolution profiles were depicted in tables 5.15 to 5.18 and shown in figures 5.13 to 5.16. The first order profiles were shown in figures 5.17 to 5.20. The release rate constants, $T_{50}$, $T_{90}$, $DE_{20}$% were given in table 5.20.

### 4.6 Characterization of Solid Dispersions

Based on the dissolution studies performed on all the solid dispersions, some of the optimized solid dispersions of Atorvastatin Calcium and Rosuvastatin Calcium were selected and further characterized XRD, DSC IR and SEM studies.

#### 4.6.1 Differential Scanning Calorimetry (DSC)

A differential scanning calorimeter (DSC 60, Shimadzu) was used to obtain the DSC curves of solid dispersions prepared by fusion, solvent evaporation and lyophilisation methods representing the rate of heat uptake. About 10mg of sample was weighed in a standard open aluminium pans, and scanned from 20-300°C, at a heating rate of 10°C/minute while being purged with dry nitrogen. The DSC
thermograms of various solid dispersions were shown figures 5.21 to 5.24 & 5.33 to 5.36.

4.6.2 Powder X-Ray Diffractometry (PXRD)

Powder X-ray diffraction (PXRD) patterns were traced employing X-ray diffractometer Bruker AXS, DH Advance, Germany for all the samples using Ni filter, CuK (α) radiation, a voltage of kV, a current of 20mA and receiving slit of 0.2. The samples were analyzed over 2θ range of 5° to 60°, with scan step size of 0.020 °(2θ) and scan step time of 1 second. The DSC thermograms of various solid dispersions were shown figures 5.25 & 5.37.

4.6.3 Fourier Transform Infrared Spectroscopy (FTIR):

FTIR spectra of pure drug, carrier and optimized solid dispersions were obtained on a Bruker FTIR spectrometer (Bruker). Samples were prepared in KBr discs (2 mg sample in 200 mg KBr). The scanning range was 400 to 4000 cm\(^{-1}\) and the resolution 1cm\(^{-1}\). The FTIR spectras were shown in figures 5.26 to 5.29 & 5.38 to 5.41.

4.6.4 Scanning Electron Microscopy (SEM):

The samples were coated with a thin gold layer by sputter coater unit (SPI, Sputter, USA). Then, the SEM photographs were taken by a scanning electron microscope (Scanning electron microscope JSM-6390, Japan) operated at an accelerated voltage of 15kV. The SEM photographs were shown in figures 5.30 to 5.32 & 5.42 to 5.44.
4.7 PREPARATION OF TABLETS WITH SOLID DISPERSIONS

Among the solid dispersions prepared and based upon the dissolution studies performed, Four (AF 8, AL 16, RF 8, RL 16) optimized dispersions were selected for further preparation as tablets. The tablets of Atorvastatin Calcium and Rosuvastatin Calcium were prepared by direct compression process. The tablet formulations consisted of drug, carrier, super disintegrants and diluents. The ratio of drug and polymer were maintained constant while the superdisintegrant concentration was varied. The weights of all the tablet formulations were maintained uniformly by using directly compressible lactose as diluent. The compositions of various tablet formulations were given in tables 5.21 to 5.24.

The materials were individually weighed, passed through sieve no: 80 and blended for 15 minutes by using double cone blender. The powder mixture was then lubricated with 1% talc and magnesium stearate and directly compressed as tablets using Elite 10 station mini press. To minimize the processing variables all batches of tablets were compressed under identical conditions. The compressed tablets were further evaluated for their physical parameters such as weight uniformity, hardness, friability, wetting time, dispersion time and drug content.
4.8 EVALUATION OF PHYSICAL PARAMETERS OF TABLETS

Physical parameters such as Weight Uniformity, Hardness, Friability and Drug content were evaluated for prepared tablets as per the standards of official compendium.

4.8.1 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. The weight ranges of different batches of tablets were depicted in tables 5.25 to 5.28.

<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>
4.8.2 Hardness

Hardness of the tablets were determined by using Monsanto hardness tester (Tab-machines, Mumbai). The tablet to be tested is held between 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/cm$^2$ to break the tablet. Hardness of different batches of tablets was given in tables 5.25 to 5.28. 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.

4.8.3 Friability

Friability test was performed by using Roche friabilator (Remi Equipments, Mumbai). Ten tablets from 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 1.0%. 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 tables 5.25 to 5.28.
4.8.4 Wetting Time

A piece of tissue paper folded double was placed in clean and dry petri plates containing 10 ml of water. The tablet was placed on the paper and the time for complete wetting of the tablet was measured in seconds. The results obtained were given in tables 5.25 to 5.28.

4.8.5 Dispersion Time

Two tablets were placed in 100 ml of water and stirred till completely dispersed. The dispersion time was noted for different formulations and the results obtained were given in table 5.25 to 5.28.

4.8.6 Drug content uniformity

a) Atorvastatin Calcium Tablets:

One tablet of Atorvastatin Calcium from a batch was taken at random and was crushed to a fine powder. The powdered material was transferred into a 100 ml volumetric flask and 70 ml of methanol was added to it. It was shaken occasionally for about 30 minutes and the volume was made up to 100 ml by adding methanol. About 10 ml 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 Whatmann filter. Then the filtrate was subsequently diluted with 6.8 pH phosphate buffer and the absorbance was measured at 246 nm. This test was repeated ten times (n=10) for each batch of tablets. The amount of Atorvastatin Calcium estimated from different batches were depicted in tables 5.25 & 5.26.
b) Rosuvastatin Calcium Tablets:

One tablet of Rosuvastatin Calcium 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 methanol was added to it. It was shaken occasionally for about 30 minutes and the volume was made up to 100ml by adding methanol. 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 Whatmann filter. Then the filtrate was subsequently diluted with 6.8 pH phosphate buffer and the absorbance was measured at 248 nm. This test was repeated ten times (n=10) for each batch of tablets. The amount of Rosuvastatin Calcium estimated from different batches were depicted in tables 5.27 & 5.28.

4.9 DRUG RELEASE STUDIES FROM TABLETS

4.9.1 Atorvastatin Calcium Tablet Formulations

Dissolution studies on each tablet formulation were performed in a calibrated eight stage dissolution rate test apparatus equipped with paddles employing 900 ml of 6.8pH phosphate buffer as a medium. The paddles were operated at 50 rpm and the temperature was maintained at 37±0.5°C throughout the experiment. Samples were withdrawn at 5, 10, 15, 20, 30 minutes and replaced with equal volume to maintain the constant volume of dissolution medium throughout the experiment. Drug content of the samples was
determined by Elico double beam UV spectrophotometer at 246 nm after suitable dilutions of the samples. The drug release from solid dispersions were conducted in triplicate. The dissolution profiles were depicted in tables 5.29 to 5.36 and shown in figures 5.45 to 5.52. The first order release profiles were shown in figures 5.53-5.60. The release rate constants, $T_{50}$, $T_{90}$, $D_{E20}$% were given in tables 5.45 & 5.46.

**4.9.2 Rosuvastatin Calcium Tablet Formulations**

Dissolution studies on each tablet formulation were performed in a calibrated eight stage dissolution rate test apparatus equipped with paddles employing 900 ml of 6.8 pH phosphate buffer. The paddles were operated at 50 rpm and the temperature was maintained at 37±0.5°C throughout the experiment. Samples were withdrawn at 5, 10, 15, 20, 30 minutes and replaced with equal volume to maintain the constant volume of dissolution medium throughout the experiment. Drug content of the samples was determined by Elico double beam UV spectrophotometer at 248 nm after suitable dilutions of the samples. The drug release from solid dispersions were conducted in triplicate. The dissolution profiles were depicted in tables 5.37-5.44 and shown in figures 5.61-5.68. The first order release profiles were shown in figures 5.69 to 5.76. The release rate constants, $T_{50}$, $T_{90}$, $D_{E20}$% were given in tables 5.47 & 5.48.
4.10 PHARMACOKINETIC STUDIES OF ATORVASTATIN CALCIUM AND ROSUVASTATIN CALCIUM

4.10.1 Pharmacokinetic Studies

Male white rabbits weighing between 1.6 to 2.2 kg were used for in vivo studies of orally administered Atorvastatin Calcium and Rosuvastatin Calcium. Animals were housed at 25 ± 1°C in air conditioned room at a relative humidity of 60 ± 5% and were provided with water and standard rabbit feed. Animals were fasted for 24 hrs prior to the administration of the drug formulation, but had free access to water. The tablets containing solid dispersion mixture was administered in the suspension form with a soft plastic tube (Atorvastatin Calcium 10 mg/kg body weight) (Rosuvastatin Calcium 10 mg/kg body weight) to another group of animals. About 2.0 ml of blood was collected at 0, 0.25, 0.5, 1, 2, 4, 6, 8 and 12hrs, after oral solution administration from marginal ear vein into the tubes containing heparin and the plasma was separated immediately. The plasma samples were analyzed for plasma Atorvastatin Calcium and Rosuvastatin Calcium concentration by the HPLC method as described earlier. All the pharmacokinetic studies were conducted by taking permission from the CPCSEA.

4.10.2 Pharmacokinetic Parameters:

The pharmacokinetic parameters such as maximum plasma concentration \(C_{\text{max}}\), time to reach peak plasma concentration \(t_{\text{max}}\), \(\text{AUC}_{(0-t)}\), \(\text{AUMC}_{(0-t)}\), \(t\frac{1}{2}\) and MRT were calculated using the PK summit
solutions software. The data was given in tables 5.51 to 5.52 and shown in figures 5.79 to 5.80.

4.11 ACCELERATED STABILITY STUDIES

The tablet formulations which showed good \textit{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 fast dissolving tablets containing drugs.

The tablet formulations such as AT 13, AT 26, & RT 13, RT 26 were subjected to accelerated stability studies. The above said formulations were kept in petri dishes after preparation and stored in thermostated oven at a temperature and relative humidity of 25 ± 2\degree C, 60 ± 5\% RH for 6 months and 40 ± 2\degree C, 75 ± 5\% RH for 3 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 5.53 to 5.58 and shown in figures 5.81 to 5.84.