EXPERIMENTAL SECTION

ANALYTICAL METHODS

Estimation of diltiazem hydrochloride

A simple sensitive and accurate spectrophotometric method was used for the measurement of Diltiazem Hydrochloride at $\lambda_{\text{max}}$ 238 nm. The absorbances of standard dilutions were measured at 238 nm. (Lakshmana et al, 2009))

Preparation of Standard Solution

100mg of Diltiazem HCl was dissolved in distilled water in 100 ml volumetric flask and the solution was made up with distilled water.

Procedure

The standard solutions of Diltiazem HCl were subsequently diluted with distilled water to obtain series of dilutions containing 2,4,6,8 and 10 μg of Diltiazem HCl/ml of solution. The absorbances of above dilutions were measured in ELICO double beam UV spectrophotometer (SL-218) at 238 nm using distilled water as a blank. The concentration and the corresponding absorbance values were given in table 5. The absorbance values were plotted against concentration of diltiazem HCl as shown in graph 1. Reproducibility of method was tested by analyzing six separately weighed samples of diltiazem hydrochloride. The method obeys beers law in the concentration range 0 – 10 μg/ml. The method was found to be suitable for the estimation of diltiazem HCl in distilled water.
Estimation of verapamil hydrochloride

A spectrophotometric method based on the measurement of absorbance at 277 nm in buffer solutions, was used in the present study for the estimation of verapamil HCl (Bistra et. al, 2007).

Preparation of Standard Solution

100 mg of verapamil HCl was dissolved in 0.1 N HCl and 7.5 pH phosphate buffer in 100 ml volumetric flasks separately and the solutions were made up to volume with the same buffer solutions.

Procedure

The standard solutions of verapamil HCl were subsequently diluted with 0.1N HCl to obtain series of dilutions of containing 10,20,30,40 and 50 µg of verapamil HCl per ml of solution. The absorbances of the above solutions were measured by ELICO UV spectrophotometer (SL 218) at 278 nm using 0.1N HCl and pH 7.5 phosphate buffer as blank. The concentrations of verapamil HCl and the corresponding absorbance values are given in table 6. The absorbance values were plotted against concentrations of verapamil hydrochloride in graph 2 and 3. The method obeys Beers law in the concentration range of 0-50µg /ml. Reproducibility of method was tested by analyzing six separately weighed samples of verapamil hydrochloride. Thus the method was found to be suitable for the estimation of verapamil HCl in dissolution fluids. Verapamil HCl was also estimated in pH 7.5 phosphate buffer in the similar manner.
Analytical Method for the Estimation of Diltiazem Hydrochloride in the Rabbit Serum

The serum concentration of diltiazem hydrochloride was estimated by HPLC method (Choudhary et al, 1993). A working standard diltiazem solution (5μg/ml) and internal standard solution (diazepam, 5pg/ml) in methanol were prepared. To 0.5 ml pooled serum 0.1 ml of internal solution was added and vortexed for 30 seconds in a graduated 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. A 0.2 ml aliquots of 1 M sodium hydroxide solution was added to serum containing diltiazem and again vortex-mixed for another 60 seconds. Five ml of chloroform was added and the solution was again vortex-mixed for about five minutes and then centrifuged at 3000 rpm for 10 minutes. Four 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 Agilent made HPLC instrument with C18 Column. Mobile phase consisted of methanol: 0.1% acetic acid (70:30). The flow rate was 1.5 ml/min. Analysis was monitored at 270 nm with a sensitivity of 0.1000 aufs. The plot of peak area ratio of diltiazem against the concentration of diltiazem in serum was linear up to 500 ng/ml. It was given in table 7 and shown in graph 4.
Analytical Method for the Estimation of Verapamil Hydrochloride in the Rabbit Plasma

The plasma concentration of verapamil hydrochloride was estimated by HPLC method (Yalcin et.al, 2000). A working standard verapamil HCl solution (5μg/ml) in ethanol were prepared. To 0.5ml pooled plasma the standard drug solutions containing 10, 25, 50, 75, 100, 200, 300, 400 and 500 ng in 0.1ml of ethanol were added to the tubes and vortexed for 60 seconds. A 0.2 ml aliquots of 1 M sodium hydroxide solution was added to serum containing verapamil and again vortex-mixed for another 60 seconds. Five ml of chloroform was added and the solution was again vortex-mixed for about five minutes and then centrifuged at 3000 rpm for 10 minutes. Four 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 Agilent made HPLC instrument with C 18 Column was used for the analysis. The mobile phase composition used for the estimation of verapamil hydrochloride in the rabbit plasma is a mixture of 0.05 M potassium dihydrogen phosphate: acetonitrile: orthophosphoric acid (69.5:30:0.5) (pH adjusted to 3.6). The flow rate was 1.5 ml / min. Analysis was monitored at 278 nm with a sensitivity of 0.1000 aufs. The plot of peak area for verapamil HCl with the concentration in serum was linear upto 1000 ng/ml. It was given in table 8 and shown in graph 5.
Preparation of Diltiazem HCl and Verapamil HCl Controlled Release Pellets by Pan Coating Technique

Equal quantities of diltiazem HCl / verapamil HCl and crosscarmellose sodium were taken in to bowl and mixed with gloved hand. To the mixer another equivalent quantity of diltiazem HCl / verapamil HCl was added and mixed with the help of gloved hand and remaining quantity of drug was loaded in to the blender and mixed with the powder for 10 mins.

- **Preparation of Povidone Solution**

  Isopropyl alcohol, PVP K-30 and Tween 80 were taken into stainless steel the tank and switched on the propeller type stirrer and mixed for 10mins. The solution was filtered through nylon cloth in to stainless steel tank.

**Drug loading**

Sugar pellets were loaded into the pan. On to the sugar pellets diltiazem HCl / verapamil HCl and crosscarmellose blends prepared, earlier were loaded while spraying the povidone solution by using 1.2 mm spray gun nozzle. Pan was allowed to rotate for about 10 mins until uniform drug loading occurs. The coating pan was operated at an rpm of 15-20. The pellets bed was agitated to avoid sticking to coating pan. The pellets were kept under rotation for 30 minutes to avoid sticking.

**Drying**

The drug loaded pellets from the pan were spread on to the trays uniformly and dried at 60°C temperature for about 3hrs. After drying the pellets
were sifted by using vibro sifter to remove fines and collected the uniform sized pellets.

- **Preparation of HPMC E5 Solution**

  HPMC E5 and water were taken into the stainless steel tank and mixed for 10mins with propeller type stirrer. The solution was filtered through nylon cloth into SS tank.

**Sub Coating**

The drug loaded pellets were loaded in to the pan and coated with HPMC E5 polymer solution by using 1.2 mm spray gun nozzle. Coating of the pellets was done under specified conditions like inlet temperature of 40°C and outlet temperature of 35°C. Flow rate rpm was adjusted to 15-20 rpm. Coating pan was allowed to rotate for 10 mins until uniform coating was applied.

**Drying**

The drug loaded pellets from the pan were spread on to the trays uniformly and dried at 60°C temperature for about 3hrs. After drying the pellets were sifted by using vibro sifter to remove fines and collected the uniform sized pellets.

- **Preparation of HPMCP Solution**

  HPMCP, cetyl alcohol, acetone and isopropyl alcohol were taken in to the tank and mixed for for 10 mins at 1300 rpm by using propeller type stirrer and filtered through nylon cloth in to SS tank.
**Polymer Loading**

HPMC coated pellets were loaded in to the pan and coated with HPMCP by using 1.2 mm spray gun nozzle. Coating of the pellets was done under specified conditions like inlet temperature of 40°C and outlet temperature of 35°C. Coating pan was allowed to rotate for 10 mins until uniform coating was applied.

**Drying**

The drug loaded pellets from the pan were spread on to the trays uniformly and dried at 60°C temperature for about 3hrs. After drying the pellets were sifted by using vibro sifter to remove fines and collected the uniform sized pellets.

- **Preparation of EC Solution**

Ethyl cellulose, Diethyl phthalate, talc, IPA and acetone were taken in to the SS tank. They were mixed in homogenizer for 15 mins and filtered through nylon cloth into SS tank.

**Polymer loading**

HPMCP coated pellets were loaded in to the pan and coated with ethyl cellulose solution by using 1.2 mm spray gun nozzle. After coating with EC solution coating pan was allowed to rotate for 10 mins until uniform coating was applied. The finally coated pellets were dried at ambient conditions for 2hrs and sifted through vibro sifter to collect uniform sized pellets. The composition of various diltiazem hydrochloride and verapamil hydrochloride controlled release pellets were given in the tables 9-16.
Table 3: Coating Pan Process Parameters

<table>
<thead>
<tr>
<th>S.No</th>
<th>Process Controls</th>
<th>Specifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Batch Size</td>
<td>100 G</td>
</tr>
<tr>
<td>2</td>
<td>Inlet Air temperature</td>
<td>40°C</td>
</tr>
<tr>
<td>3</td>
<td>Product Temperature</td>
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<tr>
<td>4</td>
<td>Air Flow</td>
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</tr>
<tr>
<td>5</td>
<td>No. of Spray Guns</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>Nozzle Aperture</td>
<td>1.2 mm</td>
</tr>
<tr>
<td>7</td>
<td>Atomization Air Pressure</td>
<td>20 psi</td>
</tr>
<tr>
<td>8</td>
<td>Spray time</td>
<td>10 min</td>
</tr>
<tr>
<td>9</td>
<td>Secondary drying</td>
<td>60°C</td>
</tr>
</tbody>
</table>

Preparation of Diltiazem HCl and Verapamil HCl Controlled Release Pellets by Fluid Bed Coating

General Procedure

Equal quantities of diltiazem HCl / verapamil HCl and crosscarmellose sodium were taken in to bowl and mixed with gloved hand. To the mixer another equivalent quantity of diltiazem HCl / verapamil HCl was added and mixed with help of gloved hand and remaining quantity of drug was loaded in to the blender and mix with the powder for 10 mins.
• **Preparation of Povidone Solution**

Isopropyl alcohol, PVP K-30 and Tween 80 were taken into stainless steel the tank and switched on the propeller type stirrer and mixed for 10mins. The solution was filtered through nylon cloth in to SS tank.

**Drug Loading**

Sugar pellets were charged in to fluidization basket. The drug and crosscarmellose powder blends were also charged in to the fluidized basket and povidone solution was atomized on to the materials while the air is allowed to circulate in to the basket at an air flow rate of 2000 - 4500 cfm to keep the materials under fluidized state. The process of fluidization was continued for 10 mins.

**Drying**

The drug loaded pellets from the FBC were spread in to the trays uniformly and dried at 60°C temperature for about 3hrs. After drying the pellets were sifted by using vibro sifter to remove fines and collected the uniform sized pellets.

• **Preparation of HPMC E5 Solution:**

HPMC E5 and water were taken into the stainless steel tank and mixed for 10mins with propeller type stirrer. The solution was filtered through nylon cloth into stainless steel tank.
Sub Coating

The drug loaded pellets were charged into a fluidization basket. HPMCE₅ polymer solution was atomized onto the materials while the air is allowed to circulate in to the basket at an air flow rate of 2000 - 4500 cfm to keep the materials under fluidized state. The process of fluidization was continued for 10 mins. Coating of the pellets was done under specified conditions like inlet temperature of 40°C and outlet temperature of 35°C, with an air pressure 2.5 Kg/cm². Damper was adjusted such that pellets should not hit the upper screen. Flow rate rpm was adjusted to 18-22 rpm.

Drying

The drug loaded pellets from the FBC were spread onto the trays uniformly and dried at 60°C temperature for about 3hrs. After drying the pellets were sifted by using vibro sifter to remove fines and collected the uniform sized pellets.

- Preparation of HPMC Solution

HPMCP, cetyl alcohol, acetone and isopropyl alcohol were taken into the tank and mixed for 10 mins at 1300 rpm by using propeller type stirrer and filtered through nylon cloth in to SS tank.

Polymer Loading

The HPMC coated pellets were charged into the fluidization basket. Polymer solution was atomized onto the materials while the air is allowed to circulate in to the basket at an air flow rate of 2000 - 4500 cfm to keep the materials under
fluidized state. The process of fluidization was continued for 10 mins. Pellets were coated under specified conditions like inlet temperature of 40 °C and outlet temperature of 35 °C, with an air pressure of 2.5 Kg/cm². Damper was adjusted such that pellets should not hit the upper screen. Flow rate rpm was adjusted to 24-28 rpm.

**Drying**

The drug loaded pellets from the FBC were spread on to the trays uniformly and dried at 60°C temperature for about 3hrs. After drying the pellets were sifted by using vibro sifter to remove fines and collected the uniform sized pellets.

- **Preparation of EC Solution**

  Ethyl cellulose, Diethyl phthalate, talc, IPA and acetone were taken in to the SS tank. They were mixed in homogenizer for 15 mins and filtered through nylon cloth into SS tank.

- **Polymer loading**

  The HPMCP coated pellets were charged in to fluidization basket. EC Polymer solution was atomized on to the materials while the air is allowed to circulate in to the basket to keep the materials under fluidized state. The process of fluidization was continued for 10 mins. The finally coated pellets were dried at ambient conditions for 2hrs and sifted through vibro sifter to collect uniform sized pellets. The composition of various diltiazem hydrochloride and verapamil hydrochloride controlled release pellets were given in tables 9-16.
Table 4: Fluid Bed Coating Process Parameters

<table>
<thead>
<tr>
<th>S.No</th>
<th>Process Controls</th>
<th>Specifications</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Batch Size</td>
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</tr>
<tr>
<td>2</td>
<td>Inlet Air temperature</td>
<td>40(^\circ)C</td>
</tr>
<tr>
<td>3</td>
<td>Outlet Air temperature</td>
<td>35(^\circ)C</td>
</tr>
<tr>
<td>4</td>
<td>Product Temperature</td>
<td>36(^\circ)C</td>
</tr>
<tr>
<td>5</td>
<td>Chamber Humidity</td>
<td>60 % RH</td>
</tr>
<tr>
<td>6</td>
<td>Air Flow</td>
<td>2000 - 4500 cfm</td>
</tr>
<tr>
<td>7</td>
<td>Nozzle Aperture</td>
<td>1.2 mm</td>
</tr>
<tr>
<td>8</td>
<td>No. of Spray Guns</td>
<td>1</td>
</tr>
<tr>
<td>9</td>
<td>Spray Direction</td>
<td>Bottom Spray</td>
</tr>
<tr>
<td>10</td>
<td>Spray Pressure</td>
<td>2.5 Kg/cm(^2)</td>
</tr>
<tr>
<td>11</td>
<td>Spray time</td>
<td>10 min</td>
</tr>
<tr>
<td>12</td>
<td>Secondary drying</td>
<td>60 (^\circ)C</td>
</tr>
</tbody>
</table>

Evaluation of Physical Parameters of Controlled Release Pellets Prepared By Pan Coating and Fluid Bed Coating Techniques

Percentage yield

All the batches of controlled release diltiazem hydrochloride and verapamil hydrochloride pellets prepared by both pan coating and fluid bed
coating were evaluated for percentage yield of the pellets. The actual percentage yields of pellets were calculated by using the following formula. The % yields of various batches of pellets were given in table 17-18.

\[
\text{Percentage yield of pellets} = \frac{\text{Practical yield of pellets}}{\text{Theoretical yield of pellets}} \times 100
\]

**Particle Size Determination**

The average particle size of the pellet formulations of diltiazem hydrochloride and verapamil hydrochloride pellets were analyzed by simple sieve analysis method. The particle size of various batches of pellets was given in tables 17-18.

**Friability**

The friability of the core pellets of diltiazem hydrochloride and verapamil hydrochloride (Santos *et al.*, 2002) was determined as % weight loss after 100 revolutions of 10 g of pellets in a friabilator. The friability values of various pellets formulations were given in tables 17-18.

**Drug Content**

One gram of diltiazem hydrochloride and verapamil hydrochloride pellets from each batch were taken at random and were 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 supernatent 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 238 nm and 278 nm for diltiazem hydrochloride and verapamil hydrochloride respectively. This test was repeated six times (N=6) for each batch of pellets. The drug content of various batches of pellets were given in tables 17- 18.

**In Vitro Dissolution Studies of Controlled Release Diltiazem HCl Pellets**

120 mg equivalent weight of diltiazem hydrochloride containing pellets were collected and weighed at random from each batch of pellet formulation and dissolution studies were performed in a calibrated 8 station dissolution test apparatus (Disso 2000), equipped with paddles (USP apparatus II method) employing 900ml of distilled water as a medium. The paddles were operated at 100 rpm and the temperature was maintained at 37± 0.5°C throughout the experiment. 5ml of samples were withdrawn at regular intervals up to 16 hours and replaced with equal volume of fresh dissolution medium to maintain a constant volume of dissolution medium throughout the experiment. Samples withdrawn at various time intervals were suitably diluted with same dissolution medium and the amount of drug released was estimated by ELICO double beam spectrophotometer at 238nm. The dissolution studies on each formulation were conducted in three times. Necessary corrections were made for the loss of drug
due to each sampling. The dissolution profiles were depicted in tables 19 to 22 and shown in graphs 6 to 9.

**In Vitro Dissolution Studies of Controlled Release Verapamil HCl Pellets**

120 mg equivalent weight of verapamil hydrochloride pellets were collected and weighed at random from each batch of pellet formulation and dissolution studies were performed in a calibrated 8 station dissolution test apparatus (Disso 2000) equipped with paddles employing 900 ml of 0.1 N HCl for first 2 hrs and pH 7.5 phosphate buffer for remaining period of time as dissolution medium. The paddles were operated at 50 rpm and the temperature was maintained at 37±0.5°C through out the experiment. 5ml of samples were withdrawn at regular intervals up to 16 hours and replaced with equal volume of fresh dissolution medium to maintain a constant volume of dissolution medium through out the experiment. Samples were suitably diluted and drug content was determined by measuring the absorbance at 278 nm using double beam UV spectrophotometer (Elico Model SL-218). Necessary corrections were made for the loss of drug due to each sampling. The dissolution profiles were depicted in tables 23 to 26 and shown in graphs 10 to 13 (USP., 2004<711>).

**In Vitro Drug Release Parameters**

The *in vitro* drug release parameters such as first order rate constant, Higuchi constant and Peppas constants were calculated from the dissolution data obtained from various formulations. A plot of cumulative percent drug released
Vs time (hrs) was plotted and the zero order release rate constant ($K_O$) was calculated from the slope. A plot of log% undissolved Vs time (hrs) was plotted for all the formulations and the first order release rate constant ($K_1$) were obtained by multiplying slope with 2.303. A plot of cumulative amount of drug released Vs square root of time was plotted for all the formulations and the higuchi constant was calculated from the slope. A plot of log $M^t/M^α$ Vs log time was plotted for all the formulations and the peppas constant was obtained by the slope and the ‘n’ values were noted from y-intercept of the straight line. The *in vitro* dissolution parameters were given in the tables 27-30. The following mathematical expressions were used to calculate pharmacokinetic parameters.

**First order equation,**

\[
\text{Log % drug unreleased} = K_1 t
\]

Where, $K_1$ = first order rate constant.

**Higuchi equation,**

\[
\text{Cumulative amount of drug released} = K_H t^{1/2}
\]

Where, $K_H$ = higuchi constant

**Korser Mayer Peppas Constant,**

\[
\log \frac{M^t}{M^α} = \log K_P + n \log t
\]

Where, $n$ = release exponent.
Comparison of Dissolution Profiles by Model-Independent Method

The dissolution profiles comparison was done using dissimilarity factor $f_1$ and similarity factor $f_2$ to compare the dissolution profile of diltiazem HCl and verapamil HCl pellet formulations with marketed pellet formulations. The dissolution profiles of all the pellet formulations for diltiazem hydrochloride and verapamil hydrochloride were compared with the marketed pellet formulation of diltiazem hydrochloride SR Pellets (Meenaxi Pharma Ltd) and verapamil hydrochloride sustained-release pellets (Verelan capsules) by using a model independent approach of difference factor, $f_1$ and similarity factor, $f_2$, with all time points included in the in vitro dissolution studies (Marcel Dekker: 2004, Moore et al, 1996) . The equation for calculating difference factor is

\[
f_1 = \frac{\sum_{t=1}^{n} |R_t - T_t|}{\sum_{t=1}^{n} R_t} \times 100
\]

The percent error is zero when the test formulation and reference formulation drug release profiles are identical and increase proportionally with the dissimilarity between the two dissolution profiles. Similarity factor, $f_2$ is a logarithmic reciprocal square root transformation of the sum of squared error and is a measurement of the similarity in the percent (%) dissolution between the two curves of marketed and test formulations. The equation for calculating similarity factor is
\[
N \quad f_2 = 50 \times \log \left\{ \frac{1}{n} \sum_{j=1}^{n} \left| R_t - T_t \right|^2 \right\}^{0.5} \times 100
\]

Where 'n' is the number of dissolution time and \( R_t \) and \( T_t \) are the reference (theoretical) and test dissolution values at time 't'. Dissolution profile was considered satisfactory if \( f_1 \) values lies below 15 (nearing zero) and \( f_2 \) value lies more than 50. Two dissolution profiles are considered similar when the \( f_2 \) value is 50 to 100. The similarity factor values were given in the tables 31 and 32.

**Characterization**

Based on the dissolution studies performed on all the pellet formulations, some of the optimized pellet formulations of diltiazem hydrochloride and verapamil hydrochloride were further investigated by DSC and SEM studies.

**Differential Scanning Calorimetry (DSC)**

A differential scanning calorimeter (DSC 60, Shimadzu) was used to obtain the DSC curves of selected diltiazem hydrochloride and verapamil hydrochloride pellet formulations prepared by pan coating technique and fluid bed coating representing the rate of heat uptake. About 10mg of sample was weighed in a standard open aluminium pans, were scanned from 20-300°C, at a heating rate of 10°C/minute while being purged with dry nitrogen. The DSC thermograms were shown in the figures 14-16 and 23-25.
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 of pan coating and fluid bed coating pellets were shown in figures 17-19 and 29-31.

FTIR Spectral Studies

I.R Spectral studies were carried out on some selected pellet formulations of diltiazem HCL and verapamil HCl by using Bruker Fourier Transfer Infrared Spectrometer. These studies on pellet formulations were performed before they are subjected to dissolution studies to check the drug excipients interactions arised in the formulation of pellets. The I.R. spectra of various pellet formulations were shown in figures 20-22 and 26-28.

Pharmacokinetic Studies of Diltiazem Hydrochloride and Verapamil Hydrochloride Controlled Release Pellet Formulations.

Animals: Male New Zealand whit rabbits weighing between 1.5 to 2.0 kg were used for in vivo studies of orally administered diltiazem and verapamil. 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 obtained from M/s Hindustan lever Ltd. Mumbai. Animals were fasted for 24 hrs prior to the administration of the pellet formulation, but had free access to water. The pellet
formulations FDL6, FDL12, FVL6 and FVL12 were administered orally with a soft plastic tube to different groups of rabbits separately. Drug solution was also administered orally with a soft plastic tube (diltiazem 10 mg/rabbit) (verapamil 10 mg / rabbit) to another group of animals. About 2 ml of blood was collected at 0, 0.5, 1.0, 2.0, 3.0, 4.0 and 6.0 hrs after oral solution administration and 0, 1, 2, 4, 6, 8, 12, 16, 20 and 24 hrs after pellet formulation administration from marginal ear vein in heparinized tubes and plasma was separated immediately by centrifugation and frozen at –20 °C. The plasma samples were analyzed for diltiazem and verapamil hydrochloride by HPLC methods as described earlier.

**Pharmacokinetic parameters**

The pharmacokinetic parameters such as maximum plasma concentration ($C_{max}$), time to reach peak plasma concentration ($t_{max}$), $AUC_{(0 to t)}$, $AUMC_{(0-t)}$, $K_e$, and MRT were calculated using software by PK summit solutions software and results are given in the tables 34 and 36.

**Accelerated Stability Studies**

The formulations which showed good *in vitro* performance were subjected to accelerated stability studies. These studies were carried out by investigating the effect of temperature on the physical properties of pellets and chemical stability of diltiazem HCl and verapamil HCl Pellets. Diltiazem HCl pellets formulations such as FDL 6 and FDL12 verapamil hydrochloride pellet formulations such as FVL 6 and FVL12, 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°C, 60 ± 5% RH for 12 months and 40 ± 2°C, 75 ± 5% RH for 6 months. Then the samples of each type of formulations were evaluated for the earlier mentioned physical parameters. The pellets 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 37-42 and in graphs 42-45.