METHODS
Manufacturing Methods

Osmotic Pump

Osmotic pump is nothing but a single layered or bilayered-coated tablet with a orifice drilled in drug layer. Various steps of its fabrication are as follows.

**Milling** - Sodium chloride was milled in comminuting mill (Cadmill, Cadmach) using 0.25 mm screen keeping machine rpm at 4000 (fast) and impact forward.

**Blending** - All the other excipients including drug were sifted through 0.425 mm mesh and blended in machines depending on the batch sizes as given below in table 5.1. In case where red oxide of iron was used it was passed through 0.25 mm and geometricaly diluted with sodium CMC.

<table>
<thead>
<tr>
<th><strong>Batch Size</strong> (Tablets)</th>
<th><strong>Machine Type</strong></th>
<th><strong>Capacity</strong> (liter)</th>
<th><strong>Make</strong></th>
<th><strong>Mixing time (min.)</strong></th>
<th><strong>Rpm</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 500</td>
<td>Planetary Mixer</td>
<td>1.0</td>
<td>Gansons Ltd</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>500 - 2000</td>
<td>Planetary Mixer</td>
<td>4.0</td>
<td>Gansons Ltd</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>2000 - 5000</td>
<td>Rapid Mixing Granulator</td>
<td>10.0</td>
<td>Saral Engineering</td>
<td>10</td>
<td>100 (Blender)</td>
</tr>
<tr>
<td>5000 - 10000</td>
<td>Rapid Mixing Granulator</td>
<td>30.0</td>
<td>Saral Engineering</td>
<td>10</td>
<td>100 (Blender)</td>
</tr>
</tbody>
</table>

**Binder Solution Preparation** - Povidone (PVP K-30) was used as a binding polymer for granules of both the layers. However, mixture of isopropyl alcohol and water (75:25 v/v) were used as solvent for granules of drug compartment and for granules of push compartment methylene chloride was used as solvent. Povidone was slowly added
in solvent mixture/solvent while stirring (IKA stirrer, Germany) till a clear solution was obtained.

**Wet Granulation** - Binder solution was added to blended powder to get a wet mass of desired consistency. Depending on the batch sizes different machines were used for this purpose details of which are given in table 5.2. Extra solvent/solvent mixture was added as per the requirement and volume of extra solvent/solvent mixture added was recorded in the process sheet.

<table>
<thead>
<tr>
<th>Batch Size (Tablets)</th>
<th>Machine Type</th>
<th>Capacity (Litre)</th>
<th>Make</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 500</td>
<td>Planetary mixer</td>
<td>1.0</td>
<td>Gansons Ltd.</td>
</tr>
<tr>
<td>500 - 2000</td>
<td>Planetary mixer</td>
<td>4.0</td>
<td>Gansons Ltd.</td>
</tr>
<tr>
<td>2000 - 5000</td>
<td>Rapid Mixing Granulator</td>
<td>10.0</td>
<td>Saral Engineering</td>
</tr>
<tr>
<td>5000 - 10000</td>
<td>Rapid Mixing Granulator</td>
<td>30.0</td>
<td>Saral Engineering</td>
</tr>
</tbody>
</table>

**Wet Milling** - After granulation, wet mass was passed manually through 2.0 mm mesh for the batch size up to 2000 tablets. In case of batch size bigger than 2000 tablets, the wet mass was passed through comminuting mill (Cadmill, Cadmach) using 12.7 mm screen at machine rpm 2000 (slow) and keeping knives forward.

**Drying of Granules** - Wet granules were dried in Tray dryer (V.M. Industries) (for batch size up 2000 tablets) or fluid bed dryer (Bombay Engineering) (for batch size more than 2000 tablets). Drying duration in tray dryer was 90-120 minutes and that in fluid bed dryer was 30-60 minutes. Temperature of the inlet air was kept between 55-65°C. Granules were periodically checked for loss on drying using halogen moisture...
balance (Mettler Toledo, HG53, Switzerland) at 105°C in auto mode. Drying was stopped when loss on drying was between 1.0-2.0 %.

**Sizing of Granules** - Dried granules were sifted through 0.85 mm mesh manually or by sifter (Cip Machinery, India) (Depending on batch size). Oversize granules were passed through oscillating granulator (Cadmach) having 0.85 mm sieve.

**Lubrication** - Lubricants were passed through sieve 0.25 mm and lubrication was either done manually by mixing in polyethylene bag or in octagonal blender (Gansons, India).

**Storage of Lubricated Granules and Un-coated Tablets** - These granules have tendency to pick-up moisture so they were packed in double polyethylene bags and stored in controlled humidity and temperature environment till further processed.

**Analysis of Lubricated Granules**

**Bulk Density** - Tapped density and apparent bulk density of granules were determined by bulk density apparatus (Electrolab, ETD 2, India) as per USP23, Supp. 8. Method I(Graduated cylinder) and Method II(3 mm dropping distance, 250 droppings/min) were used to determine apparent density and tapped density respectively

**Angle of Repose** - Angle of repose of granules was determined by funnel method. A SS funnel was fixed vertically so that lower end of its neck was 2 cm above the surface. Granules were slowly poured from the top of the funnel by a spatula till the top of the heap of granules touched the lower tip of the funnel. Circumstance of the heap was
drawn carefully on a paper and its diameter was measured. Angle of repose was calculated from the formula

\[ \theta = \tan^{-1}\left(\frac{2h}{a}\right) \]

Where \( h \) = height of the heap (i.e. 2 cm)  
\( a \) = diameter of the heap.

**Sieve Analysis** - Sieve analysis of the granules was done using laboratory vibratory sieve shaker (Fritsch, analysette 3", Germany). Weighed quantity of granules were kept on the top sieve of set of the sieves arranged in decreasing order of mesh sizes. Then the vibratory sieve shaker was run at 2 mm amplitude and for a duration of 2 min. and the quantity of granules retained on each sieve was weighed and percentage was calculated. Different sieves (Fritsch, Germany) used for this purpose were 1.18 mm (16#), 0.85 mm (20#), 0.60 mm (30#), 0.425 mm (40#), and 0.25 mm (60#).

**Compression of Osmotic Tablets**

Both the single layer and double layered osmotic tablets were compressed on 10.32 mm diameter round standard concave punches (D type of tooling).

**Single Layer Osmotic Tablets** - Single layered osmotic tablets were compressed on 16 station rotary compression machine (Cadmach, CMD3, India). In case of smaller batch size only two sets of punches were used and rest of the die cavities were filled with dummy (plain) dies.

**Bilayered Osmotic Tablets - (a)** For batch size up to 2000 tablets:- In this case osmotic tablets were compressed on 16 station rotary compression machine (Cadmach, CMD3,
India) but modified dies were used. These dies were slightly tapered at the upper end, which prevented breaking the edges during bilayer tablet compression. Bilayered tablets were made in two stages. In first stage granules of drug layer were compressed into soft tablets (hardness -10 to 15 N). In next stage only two sets of punches placed at position 8 and 16 (in order to keep maximum distance between them) were used and dummy dies were fixed at the remaining positions. Granules of push compartment were fed into feed frame, which were then filled into die cavities by the movement of the turret. A soft tablet (drug layer) was then kept manually with the help of a forceps on the die cavity already filled with the granules of the push compartment and then machine was operated to complete the compression cycle. Due to the tapered die the soft tablet (drug layer) was pushed inside, without breaking the edges, by the upper punch and formed a bilayered tablet. During this compression the machine was run into inching mode to facilitate proper placement (which was done manually) of the soft tablet (drug layer) over the die cavity filled with the granules of push compartment. The whole operation is shown through flow diagram in fig 5.1 (page 84).

**Bilayered Osmotic Tablets - (b)** For batch size more than 2000 tablets - Bilayered osmotic tablets for the scale-up batches were compressed on double layer rotary compression machine (Presscota, Cadmach, India). Presscota machine is built of two sixteen station rotary compression machine equipped with a vacuum-cup arrangement which transfers one layer of the bilayered tablet (partially compressed) from one machine to other machine and places it just overtop the die filled with granules of other layer. Then the partially compressed layer is pushed gently inside the die cavity and
then compressed into a double layer tablets. Once the weights of both the layers are adjusted, the bilayered tablets are compressed automatically.

**Coating of Osmotic Tablets**

Osmotic tablets were coated in automatic perforated coating pan machine (Neocota, 15A India) fitted with spray gun (Binks 460M, England) and spray solution/suspension was pumped through peristaltic pump (Watson-Marlow, 505S, England). Coating suspension/solution tank was kept on a balance to monitor spray rate throughout the coating operation. Whenever the pan load was less than 1.5 kg dummy tablets were mixed to make the total pan load to 1.5 kg.

**Preparation of Cellulose Acetate Coating Solution** - Cellulose acetate was dissolved in mixture of acetone and methylene chloride (80:20 %v/v). This was done by adding slowly the polymer into solvent mixture while stirring vigorously. Plasticiser was added (where applicable) and solution was stirred for 15 min and filtered through 0.25 mm mesh.

**Preparation of Drug Coating Suspension** - Weighed quantity of drug, HPMC and Aerosil were mixed in polythene bag and then slowly added into water while stirring vigorously. The solution was then stirred at slow speed for 15 min and filtered through 0.425 mm mesh.

**Preparation Coloured Film-coating Suspension** - At first red oxide of iron was milled with water into colloid mill (Cip Machinery, India) for 5 min. Then in this, slurry of pigment, a ready mix coating powder containing HPMC (TRC Coat A, Torrent) was
added while stirring vigorously (Care was taken to avoid air entrapping) this was stirred for 30 minutes at slow speed and filtered through 0.425 mm mesh.

*Coating Parameters -*

**I - For cellulose Acetate Coating**

<table>
<thead>
<tr>
<th>Particulars</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preheating</td>
<td>15 min at 60° C inlet air temperature</td>
</tr>
<tr>
<td>Pan rpm</td>
<td></td>
</tr>
<tr>
<td>for first 20% coating</td>
<td>8 - 10</td>
</tr>
<tr>
<td>for rest of the coating</td>
<td>10 - 12</td>
</tr>
<tr>
<td>Inlet air temperature (°C)</td>
<td>60 ± 10</td>
</tr>
<tr>
<td>Outlet air temperature (°C)</td>
<td>40 ± 5 °C</td>
</tr>
<tr>
<td>Spray rate (g/min/kg)</td>
<td></td>
</tr>
<tr>
<td>for first 20% coating</td>
<td>8 ± 2</td>
</tr>
<tr>
<td>for rest of the coating</td>
<td>15 ± 2</td>
</tr>
<tr>
<td>Post heating</td>
<td>30 min at 50° C inlet air temperature</td>
</tr>
<tr>
<td>Atomization Pressure</td>
<td>2.0</td>
</tr>
</tbody>
</table>

**II - For Diltiazem HCl Coating**

<table>
<thead>
<tr>
<th>Particulars</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preheating</td>
<td>15 min at 60° C inlet air temperature</td>
</tr>
<tr>
<td>Pan rpm</td>
<td></td>
</tr>
<tr>
<td>for first 20% coating</td>
<td>6 - 8</td>
</tr>
<tr>
<td>for rest of the coating</td>
<td>8 - 10</td>
</tr>
<tr>
<td>Inlet air temperature (°C)</td>
<td>60 ± 10</td>
</tr>
<tr>
<td>Outlet air temperature (°C)</td>
<td>45 ± 5 °C</td>
</tr>
<tr>
<td>Spray rate (g/min/kg)</td>
<td></td>
</tr>
<tr>
<td>for first 20% coating</td>
<td>4 ± 2</td>
</tr>
<tr>
<td>for rest of the coating</td>
<td>8 ± 2</td>
</tr>
<tr>
<td>Post heating</td>
<td>30 min at 50° C inlet air temperature</td>
</tr>
<tr>
<td>Atomization Pressure</td>
<td>2.0</td>
</tr>
</tbody>
</table>
### III - For Coloured Film Coating

<table>
<thead>
<tr>
<th>Particulars</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preheating</td>
<td>15 min at 60°C inlet air temperature</td>
</tr>
<tr>
<td>Pan rpm</td>
<td></td>
</tr>
<tr>
<td>for first 20% coating</td>
<td>8-10</td>
</tr>
<tr>
<td>for rest of the coating</td>
<td>10-12</td>
</tr>
<tr>
<td>Inlet air temperature (°C)</td>
<td>60 ± 10</td>
</tr>
<tr>
<td>Outlet air temperature (°C)</td>
<td>45 ± 5°C</td>
</tr>
<tr>
<td>Spray rate (g/min/kg)</td>
<td></td>
</tr>
<tr>
<td>for first 20% coating</td>
<td>4 ± 2</td>
</tr>
<tr>
<td>for rest of the coating</td>
<td>6 ± 2</td>
</tr>
<tr>
<td>Post heating</td>
<td>30 min at 50°C inlet air temperature</td>
</tr>
<tr>
<td>Atomization Pressure</td>
<td>2.0</td>
</tr>
</tbody>
</table>

Tablet weight, hardness, thickness and diameter were measured on tablet multi-check machine (Erweka, TBH 30 MD, Germany) in which all four parameters of the same tablet are measured automatically.

**Drilling Orifice into Coated Tablet**

*Mechanical Drilling* - Electrically operated, mechanical drilling machine (hanging machine, Unident, India) was used to drill orifice into coated tablet. Carbide drilling burs of various sizes were fixed into hand set (W&H 434, Austria) attached to drilling machine. The hand set was fixed vertically on a stand with the help of elastic so as to facilitate its up & down movement. Drilling machine was operated at 1500-2000 rpm and coated tablet (drug layer facing drilling motor) was hold between fingers and a orifice was drilled into it by lowering the hand set. Care was taken to drill orifice in the centre of the tablet and depth was kept just sufficient to drill a orifice in coating and not in core of the tablet.
**Laser Drilling** - High power CO\(_2\) laser drilling machine was used to drill orifice in coated tablet. Tablets were placed under the laser source and the machine was operated using following parameters. No charring was observed.

<table>
<thead>
<tr>
<th>Particulars</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Power</td>
<td>100 W</td>
</tr>
<tr>
<td>Exposure duration</td>
<td>200 m sec.</td>
</tr>
<tr>
<td>Diameter of laser spot</td>
<td>0.5 mm and 0.8 mm</td>
</tr>
<tr>
<td>Laser wave length</td>
<td>10.064 (\mu m)</td>
</tr>
</tbody>
</table>

Orifice drilled in cellulose acetate film using mechanical and laser drilling device is shown in photograph number 5.1

**Tablet Friability (un-coated tablet)**

Friability test was performed on uncoated tablets as per method given in USP-23 NF-18 using USP friability test apparatus (Electrolab, EF 1W, India), whole tablets, carefully dedusted, (corresponding to 6.5 g) were accurately weighed and placed into the drum. Drum was rotated for 100 times, tablets were removed, dedusted and accurately weighed. Loss in the weight of the tablet was calculated.

Above test was repeated twice to check the adhesive strength of the bilayered tablet.

**Uniformity of Dosage Form**

To check uniformity of the dosage form weight variation test was performed as per method given in USP-23, NF-18. Ten tablets were weighed individually and range
of tablet weight (min. and max) was determined and relative standard deviation was also calculated.

**Measurement of Coating Thickness and Coating Uniformity**

Coated film was carefully peeled off from the coated tablets and washed with water to remove and adherent material and dried with the help of tissue paper. Coating thickness at edges and surface was measured using a digital thickness meter (Mitutoyo) have accuracy up to 0.001 mm.

To check uniformity of the coating thickness, coated film from 10 tablets were peeled off and their thickness was measured and standard deviation was calculated.

**Measurement of Orifice Diameter**

Orifice diameter was measured with the help of micrometer fitted in a stereozoom microscope (Carl Zeiss, Singapore). Tablets were placed under stereo microscope and was illuminated by an external light source, then the orifice was observed through it by adjusting focal length. Orifice diameter was calculated using stage micrometer.

To check roundness of the orifice, diameters at perpendicular positions were measured and the values were compared. To check the uniformity of the orifice diameter, orifice diameter of 20 tablets were checked and standard deviation was calculated.
Measurement of Tablet Surface Area and Volume

Surface area of the tablet was measured by using the following formulas in which tablet is considered as cylinder:

\[ S = \pi * [d * h + \{d^2/2\}] \]
\[ V = (\pi * h * d^2)/2 \]

Where \( S \) = surface area of the tablet, \( V \) = volume of the tablet, \( d \) = diameter of the tablet, \( h \) = thickness of the tablet (above formulas are taken from Eudragit Polymers information Booklet(1997), Rohm GmbH, Pharma Polymers, Germany)

Procedure for Assay and Related Impurities (RI)

Assay of Diltiazem HCL in osmotic pump was determined according to method of assay for Diltiazem HCl extended release capsules given in USP (USP 23-NF 18 through supp. 10). Some minor modification were made in that procedure. Details of instruments and parameters is given in table 5.3

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Isocratic HPLC (LC 10, Shimadzu)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>Hypersil, C8, 25 cm X 4.6 mm, 5p</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>Acetonitrile : Phosphate buffer pH 3 (50 : 50)</td>
</tr>
<tr>
<td>Flow rate (ml/min.)</td>
<td>1.0</td>
</tr>
<tr>
<td>Sensitivity (AUFS)</td>
<td>0.005</td>
</tr>
<tr>
<td>Injection volume (μL)</td>
<td>20.0</td>
</tr>
<tr>
<td>Wavelength (nm)</td>
<td>240</td>
</tr>
<tr>
<td>Diluent</td>
<td>Methanol</td>
</tr>
<tr>
<td>Retention time (min.)</td>
<td>3.5 to 4.5</td>
</tr>
</tbody>
</table>

Preparation of Standard Solution – Accurately weighed 60 mg Diltiazem HCl working standard was taken 50 ml volumetric flask. Thirty ml of diluent was added in it and it was sonicated for 10 min. to dissolve it. Volume was made with diluent. Two ml of this solution was transferred in a 100 ml volumetric flask. Two ml of Deacetyl Diltiazem
HC1 (0.02 mg/ml concentration) was added in it and volume was made with mobile phase. Deacetyl Diltiazem HCl (DAD) is considered as RI.

**Preparation of Sample Solution** – Ten osmotic pumps were weighed accurately and crushed to fine powder in mixer (Sumeet). This powder was passed through sieve 0.25 mm. This powder equivalent to 300 mg Diltiazem HCl was weighed accurately and transferred in to a 250 ml volumetric flask, 150 ml of diluent was added in and this was sonicated for 30 minutes. This was cooled to room temperature and volume was made with the diluent. Resultant solution was filtered through Whatman no. 3 filter paper. Two ml of filtered sample solution was transferred to 100 ml volumetric flask and volume was made with mobile phase.

**Procedure** – Mobile phase was injected as blank and the chromatogram was recorded. Five replicate injections of standard were performed and chromatograms were recorded. Duplicate injections of sample were given and chromatograms were recorded. Responses were measured for the analyte peak.

**Calculation** – Percentage assay and related impurity were calculated using the following formula-

\[
\frac{\text{Av. Sample area}}{\text{Av. Std area}} \times \frac{\text{Std. wt. taken}}{\text{Sample wt. taken}} \times \% \text{ purity of std}.
\]

\[
\% \text{ Assay (w/w)} = \frac{\text{Av. Sample area}}{\text{Av. Std area}} \times \frac{\text{Std. wt. taken}}{\text{Sample wt. taken}} \times \% \text{ purity of std}.
\]

\[
\frac{\text{Av. Sample area}}{\text{Av. Std area}} \times \frac{\text{DAD conc.}}{\text{Sample conc.}} \times 100
\]

\[
\% \text{ RI (DAD content in w/w)} = \frac{\text{Av. Sample area}}{\text{Av. Std area}} \times \frac{\text{DAD conc.}}{\text{Sample conc.}} \times 100.
\]
Water Content

Karl Fischer Auto Titrater (Metroohm, 701 KF Titrino) was used to determine water content of the osmotic pump. Approximately 100 mg of accurately weighed powdered sample was added to auto titrater after its conditioning which automatically gave the end point of the potentiometric titration and the % w/w water content.

Dissolution Study

Dissolution studies of the Osmotic Pumps were performed in USP-23 tablet dissolution apparatus II (paddle) (Electrolab, TDT 06P, India) and conditions are given in table 5.4.

TABLE 5.4 : Parameters for dissolution study of osmotic pumps.

<table>
<thead>
<tr>
<th>Particular</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dissolution media</td>
<td>Distilled water</td>
</tr>
<tr>
<td>Paddle rpm</td>
<td>100</td>
</tr>
<tr>
<td>Temperature</td>
<td>37 ± 0.5°C</td>
</tr>
<tr>
<td>Volume of media</td>
<td>900 ml.</td>
</tr>
<tr>
<td>Sampling volume</td>
<td>10 ml</td>
</tr>
<tr>
<td>Sampling duration</td>
<td>2 hr.</td>
</tr>
</tbody>
</table>

Dissolution was also performed in 0.1 N HCl, 6.8 pH phosphate buffer, pH 7.4 phosphate buffer solutions. Equal amount of dissolution media was added into each bowl after each sample withdrawal.

The entire buffer solutions used in dissolution study were prepared according to method given in USP 23.

Samples were filtered through No.1 whatman filter paper and sufficiently diluted before taking absorbance in UV spectrophotometer (Jasco, 550, Japan) % dissolution was calculated using formula -
Packing of Osmotic Pumps

Osmotic pumps (for stability study) were packed in PVC blister using blister packing machine (Precision Gear, TR 100). Thickness of Aluminum foil was 0.02 mm and that of PVC foil was 0.25 mm. Osmotic pump (CA film Coated, With orifice, colour film coated and packed in PVC blister) is shown in photograph no. 5.2.

Blister Pack Weight Checks

At the initiation of the test, 10 identified blister packs for each designated storage condition were weighed and mean was calculated. These packs were again weighed at subsequent examination intervals and any change in mean weight as a % of initial weight was recorded.

Leak Test of PVC Blister

Blister pack seal integrity was tested by means of a dye bath vacuum test. A glass dessicator filled with dye solution (0.1% brilliant blue aqueous solution) was connected to a vacuum pump (Millipore). Blisters to be tested for seal integrity were dipped into dye solution with the help of a perforated plastic sheet, lid of the dessicator was placed in position to make it air tight and vacuum was applied. Vacuum of 15 psig for 30 seconds was maintained and then it was released slowly. Blisters were removed, dried with the help of a tissue paper and observed for any cavity filled with dye solution.
Accelerated Stability Study

To determine stability and shelf life of the formulation, osmotic pumps packed in PVC blister were kept in humidity ovens (Newtronics, India) set at different conditions of temperature and humidity for different duration. At regular intervals samples were withdrawn and analysed for assay, dissolution and water content, stability protocol is given in table 5.5.

TABLE 5.5: Stability protocol (✓ to be tested, X→ not to be tested)

<table>
<thead>
<tr>
<th>Condition</th>
<th>3 Month</th>
<th>6 month</th>
<th>9, 12, 18, 24 month</th>
</tr>
</thead>
<tbody>
<tr>
<td>60°C</td>
<td>✓</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>25°C, 60% RH</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>40°C, 75% RH</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Fridge (2-8°C)</td>
<td></td>
<td></td>
<td>X</td>
</tr>
</tbody>
</table>

Estimation of Diltiazem HCl and DAD in Plasma

Preparation of Stock Solutions:

Diltiazem 0.1 mg/ml
Ten mg diltiazem of working standard was taken in 100 ml volumetric flask. Approx. 30 ml of methanol was added in it and sonicated to dissolve. Made up the volume with methanol.

DAD 0.1 mg/ml
Ten mg DAD of working standard was taken in 100 ml volumetric flask. Approx. 30 ml of methanol was added in it and sonicated to dissolve. Made up the volume with methanol.
Verapamil 0.1 mg/ml (internal standard)  
Ten mg Verapamil of working standard was taken in 100 ml volumetric flask. Aapprox.
30 ml of methanol was added in it and sonicated to dissolve. Made up the volume with Methanol.

Diltiazem & DAD 0.1 µg/ml  
Five ml each of solution (a) & (b) in was taken in 50 ml volumetric flask and diluted upto mark with water.

Verapamil 15000 ng/ml  
7.5 ml of solution (c) was taken in 50ml volumetric flask and diluted upto mark with 0.05M H₂SO₄

Verapamil 7500 ng/ml  
One ml of solution (e) was taken in clean test tube and 1ml of 0.05M H₂SO₄ was added in it.. This solution was used for spiking in plasma samples.

Preparation of Spiked Plasma Samples

4.5 ml of Heparinised Human Plasma was taken in a clean 15 ml capacity stoppered test tube and spiked with 0.5 ml of stock solution (d) to get concentration of 1000 ng/ml. It was further diluted to get the concentration of 200, 100, 50, 25 and 12.5 ng/ml.

Preparation of Quality Control Samples

QC high: (150 ng/ml)
4.5 ml of Heparinised Human Plasma was taken in a clean 15 ml capacity stoppered test tube and spiked with 0.5 ml of stock solution (d) to get concentration of 1000ng/ml, and further diluted to get the concentration of 150 ng/ml.
QC medium: (75 ng/ml)
Two ml of QC high solution was diluted with 2 ml of blank plasma to get concentration of 75 ng/ml.

QC low: (37.5 ng/ml)
Two ml of QC medium solution was taken and 2 ml of blank plasma was added in it to get concentration of 37.5 ng/ml.

Extraction of Drug from Plasma Samples
A 0.5 ml aliquot of plasma was pipetted into a 15 ml centrifuged tube, and 50 µl (equivalent to 375 ng) of internal standard was added, followed by the addition of 5 ml TBME. After the tubes were capped with glass stopper, the mixture was vortexed for 2 min. The tubes were centrifuged for 5 min and the organic layer was transferred into a 10 ml conical clean centrifuge tube. The organic extract was evaporated to dryness at savant vacuum drier at high drying rate for 35 minutes, the residue was reconstituted in 200 µl of 0.05M H₂SO₄, and 20 µl was injected on LC/MS.

Instrument Used

HPLC : Shimadzu LC10 AD VP
MS detector : Finnigan Mat LCQ

Instrument Parameters

HPLC parameters

Chromatographic conditions:

Column : C 18, Hypersil; ODS,BDS 150mm x 4.6mm, 5µ
Mobile phase : Buffer : Acetonitril : Methanol
                     50  33  20   v/v
Buffer : 0.04M ammonium acetate
pH of buffer-5.2 adjusted with acetic acid

Injection Volume : 20 μl
Flow rate : 0.8 ml/min

MS parameters

Ionisation mode : Electrospray ionisation (ESI)

MS scan parameter

Run time : 8.5 min
Scan segment : 1
Scan event : 1
Scan event settings :
  Scan Power : MS
  Polarity : Positive
  Scan Mode : Full
  Mass range : 300-500 m/z

ESI probe parameter

  Capillary temp : 200°C
  Capillary voltage : 20
  Tube lens voltage : 26
  Sheath gas flow rate : 0.8 L/min
  Auxiliary gas flow rate: 0.2 L/min

Divert valve : In use during run
Flow divered up to 2.75 min. to waste and afterwards to the MS detector.

Flow Splitter: In use during run.
Mobile flow from the column outlet was split with help of flow splitter in the ratio of 80 : 20 (80% going to waste and 20% going to the MS detector).

**LC-MS Procedure for Injection and Quantification**

20μl of reconstituted solution was injected on Column, C18, Hypersil; 150mm x 4.6mm, 5 μm. Drug diltiazem, DAD and internal standard were eluted using mobile phase.
MS was scanned from 300 to 500 Dalton. Mass chromatograms for molecular ions of diltiazem (415), DAD (373) and verapamil (455) were constructed and area under the peak is calculated for quantification.

The ratio of area of diltiazem & DAD to that of verapamil was calculated and plotted against the concentration to construct the calibration curve. Calibration curve was constructed every day before analysis of volunteer samples and used for calculation of concentration of diltiazem & DAD in volunteer samples. Quality control samples were also prepared and analyzed along with volunteer samples to ensure smooth functioning of extraction procedure as well as system.

Following equation for straight line was used for calculation

\[ X = \frac{(y - a)}{b} \]

Where,

\[ Y = \text{Ratio of area of diltiazem & DAD to Area of verapamil} \]

\[ A = \text{Intercept} \]

\[ B = \text{Slope} \]

\[ X = \text{Concentration of diltiazem & DAD in ng/ml} \]

All the calculations were performed on windows-95 based excel of office-97

Method was found to be linear from 6.25 ng/ml to 250 ng/ml (LOQ = 6.25 ng/ml, LOD + 3.125 ng/ml).

**Obstacles Faced During Formulation and Development of Osmotic Pump Of DL and Their Resolution**

**Compression of bilayered tablets**

To make bilayer tablet of small trial batches the process was divided into two parts. In the first part a soft tablet of first laye was compressed and then in
second part this tablet was to be placed on the die filled with the granules of second layer and then the compression of both the layers. But during this stage the first layer tablet was not completely pushed into die by the upper punch due to slightly high diameter of the tablets due to its expansion after ejection. To solve this problem, modified dies were used in which the upper part of the dies were of slightly high diameter of the tablet (tapered) to facilitate smooth insertion of tablet into the die during second compression stage.

The other problem associated with the compression of bilayered tablet was separation or lamination of both the layers. This problem was solved by optimization of quantity of binder in both the layers, keeping optimum hardness of the soft tablet (first layer) and finally the proper adjustment of the compression pressure at the time of making bilayered tablet.

Problem of lamination was more pronounced at the time of compression of bilayered tablet on Presscota / Drycota compression machine. The hardness of the soft tablet of first layer was optimized in such a way that it does not break during transfer from one compression stage to another and it does not laminate during second compression stage.

Sticking was observed during the compression of drug and push compartment. Increasing the lubrication concentration did not help much but compression in controlled humidity and temperature (RH < 40%, temp. < 25°C) solved the problem.

The other problem was mixing of some granules (fragments) of first soft layer into the granules of second layer. This happened due to the generation of small
fragments during bilayered tablet compression and their subsequent transfer to the feed frame filled with granules of second layer along with the movement of turret. This mixing was prevented by brushing off the small fragments during small trials and by applying suction units in scale up trials.

Coating of semipermeable membrane

Problems were - poor adhesion of film on tablet surface, cracking of the film on edges of tablet, breaking edges of tablets, formation of opaque film, non-uniform distribution of film on edges of the tablet.

All the problems referred above were solved by optimization of coating parameters like atomization pressure, spray rate, distance between gun and tablet bed, coating pan rpm, inlet and outlet air temperature, CFM of inlet and exhaust blower, selection the proper size of dummy tablets, etc.

Coating of drug layer

Due to very smooth surface of the semipermeable membrane, poor adhesion of drug coating was observed and at high build up that led to cracking of the film. Binder concentration was increased and coating was done at low coating pan rpm to solve these problems.

Orifice drilling

There were two choices for mechanical drilling - air operated drilling machine and electrically operated drilling machine. The later machine gave better results with respect to variation in diameter due to less ply then the former so it was selected.
Horizontal hand set was uncomfortable for large scale orifice drilling operation so the choice was vertical hand set.

Drilling bars with less than 0.5 mm tip diameter, which can be fitted in the drilling machine selected were not available, however, drilling bars with required tip diameter for the other types of machines were available. To make it possible to fit them to the selected machine, a special clutch was fabricated to grip these small diameter drilling burrs and then the clutch was fitted in the handsets.

Formation of orifice at the time of compression was also attempted by making an indent (orifice) in the uncoated tablet during the compression according to the method of Luschen and Gatos (1981). This avoided an extra step of orifice formation after the coating.

To do this, a tapered metal piece (length 1.5 mm, diameter 0.75 mm) was coupled to the center of the tip of the upper punch so that at the time of the tablet compression it will make an orifice on the upper surface of the un-coated tablet. Further during coating it was observed that orifice of the 5-10% of the tablets was covered partially or fully during the coating of the semipermeable membrane. Later on when the dissolution study was done, cracks were developed in such tablets due to generation of excess hydrostatic pressure inside the semipermeable membrane. So further trials were not taken to make such tablets.

**Large number of coating trials**

To reduce large number of coating trials of the same coating composition to be given to different batches, a mark of different colour of permanent marker was made to each batch and they were coated together. This not only saved the time and
chemicals but also maintained identical coating conditions for different batches. This avoided the one major variable while comparing different batches with same coating and different coating composition.

**Film formation**

Initial trials of CA film casting produced opaque to while films with wavy surface. To eliminate this, the entrapped air was removed by sonication, suspended particles were removed by filtration, vibration in oven was minimised and a inverted tunnel was placed on glass moulds for uniform drying.

**Film permeability study**

During the measurement of rate of water permeability from the CA membranes, inspite of grounded glass joints of diffusion cells and tight clamp, leakage from joints was observed which may be due to osmotic pressure generated or the plastic nature of the film. To stop this leakage neoprene rubber gaskets with a thin layer of silicon grease were used. They were placed on both the surfaces of the film and then clamped.

**Assay**

The CA film did not crush and uniformly mixed with the other tablet excipients when the tablets were crushed for assay. To solve this problems films were separated and their weights were reduced from the total weight of the tablets.
FIGURE 5.1 - Flow diagram depicting compression procedure of bilayered osmotic tablet.
Photograph 5.1: Photograph showing orifice drilled in cellulose acetate film using laser (A) and mechanical (B) drilling machines. The diameter of the orifice is 0.5 mm (left) and 0.8 mm (right).

Photograph 5.2: Photograph showing different stages of osmotic pumps. Cellulose acetate film coated (left), cellulose acetate film coated with orifice (lower middle), coloured film coated (upper middle) and packed in PVC blister (right).