Chapter 4

Material and Methods

4. Material Used

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Ingredients</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Enalapril Maleate</td>
<td>Active</td>
</tr>
<tr>
<td>2</td>
<td>Hypromellose (HPMC E-5)</td>
<td>Polymer</td>
</tr>
<tr>
<td>3</td>
<td>Isopropyl Alcohol</td>
<td>Granulating solvent</td>
</tr>
<tr>
<td>4</td>
<td>Dichloromethane</td>
<td>Granulating solvent</td>
</tr>
<tr>
<td>5</td>
<td>Corn starch</td>
<td>Binding agent</td>
</tr>
<tr>
<td>6</td>
<td>Lactose</td>
<td>Diluent</td>
</tr>
<tr>
<td>7</td>
<td>Maleic Acid</td>
<td>Diluent</td>
</tr>
<tr>
<td>8</td>
<td>Corn Starch (for paste)</td>
<td>Binder</td>
</tr>
<tr>
<td>9</td>
<td>Purified water</td>
<td>Solvent for starch paste</td>
</tr>
<tr>
<td>10</td>
<td>Hypromellose (HPMC K4M)</td>
<td>Release Retardant</td>
</tr>
<tr>
<td>11</td>
<td>Hypromellose (HPMC K15M)</td>
<td>Release Retardant</td>
</tr>
<tr>
<td>12</td>
<td>Zinc stearate</td>
<td>Lubricant</td>
</tr>
</tbody>
</table>

- *Enalapril maleate* was a gift sample from Cadila Pharmaceuticals, Ahmedabad. All other chemicals used were of high analytical grades procured from Sigma.
4.1 Pre-formulation study of Enalapril Maleate

4.1.1 Angle of Repose (θ)
Angle of repose has been used to characterize the flow properties of solids. It is a characteristic related to inter-particulate friction or resistance to movement between particles. This is the maximum angle possible between surface of pile of powder or granules and the horizontal plane. The angle of repose of granules was determined by the funnel method. The accurately weighed granules were taken in funnel. The height of the funnel was adjusted in such a way that the tip of the funnel just touched the apex of the heap of the granules. The granules were allowed to flow from the funnel on the surface. The diameter and height of the heap formed from the granules were measured. The angle of repose was calculated using following formula

\[ \tan \theta = \frac{h}{r} \]  
Equation 31

where,
\( \theta \) = angle of repose,
\( h \) = height of heap,
\( r \) = radius of base of heap circle.

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

4.1.2 Compressibility Index
The Carr’s Compressibility Index was calculated from Bulk density and tapped density of the granules.
A quantity of 2 g of granules from each formulation, filled into a 10 ml of measuring cylinder. Initial bulk volume was measured, and cylinder was allowed to tap from the height of 2.5 cm. The tapped frequency was 25±2 per min to measure the Tapped volume of the granules. The bulk density and tapped density were calculated by using the bulk volume and tapped volume. And the Carr’s Compressibility index was calculated by using following formula.\textsuperscript{130}

\[
\text{Carr’s Index (\%): } \frac{(\text{Tapped Density} - \text{Bulk Density}) \times 100}{\text{Tapped Density}}
\]

Compressibility and Flow properties: Compressibility is indirectly related to the flow rate, cohesiveness and particles size distribution of powder. Powders with compressibility values less than about 20 \% has been found to exhibit good flow properties.

In the present study the compressibility index of the granules were in the range of 16-23 and thus displayed a good flow property.

4.2 Preparation of tablets
The polymer used in this formulation was HPMC contains hydropropyl (hydrophilic) and methoxy groups and hence retard drug release, main aspects of HPMC govern its performance in Sustain Released matrix system is rapid formation viscous gel layer upon hydration. And once gel layer is formed, the viscosity of the gel layer regulates the overall rate of drug release. HPMC is semi-synthetic, non-ionic cellulose ether which is widely used in sustained release dosage forms because of its non toxic nature, its capacity to accommodate high levels of drug loading and its non pH –dependence. In the present study, the release profile of all formulation is dependent on the polymer concentration. Different tablets formulations were prepared by wet granulation technique.

3.3.1 Procedure for Preparation of Tablets:

i. Sifting of materials:
Enalapril Maleate and excipients were sifted through following sieves, manually
### ii. Granulation

**A) Drug part:**

A.1) **Binder solution preparation:**

Hypromellose was dissolved in IPA: DCM (1: 2 ratio)

A.2) **Granulation:**

Enalapril Maleate was granulated with Hypromellose binder solution

**B) Dummy part:**

B.1). **Dry mixing:**

Corn starch and Lactose were mixed in rapid mixer granulator (RMG) for 10 minutes at slow speed (about 75 rpm) of Impeller and chopper at off position.

**B.2) Starch paste preparation:**

B.2.1) **Starch slurry preparation:**

Corn Starch was dissolved in purified water

B.2.2) **Paste preparation:**

Purified water was boiled and starch slurry was added and paste was cooled to room temperature

**B 3) Granulation:**

Starch and Lactose was granulated with starch paste. The granulated mass was dried and milled.
C 1) Lubrication/Blending
The drug part and Dummy part were mixed geometrically for 35 minutes
Methocel K4M and Methocel K15M were mixed for 10 minutes
Zinc stearate was added and lubricated for 2 minutes

D 1). Compression
The lubricated granules were compressed into tablets.
Prior to compression, the granules were evaluated for several tests. In all formulations, the amount of the active ingredient is equivalent to 20 mg of Enalapril (Table 4.2 & Table 4.3)

**Table 4.2: Quantitative formula of Enalapril Maleate Sustained release tablets**

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Ingredients</th>
<th>Quantity (mg/tab)</th>
<th>Quantity (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Enalapril Maleate</td>
<td>20.00</td>
<td>8.50</td>
</tr>
<tr>
<td>2</td>
<td>Hypromellose (HPMC E-5)</td>
<td>0.21</td>
<td>0.10</td>
</tr>
<tr>
<td>3</td>
<td>Isopropyl Alcohol + Dichloromethane (1: 2 ratio)</td>
<td>q.s</td>
<td>q.s</td>
</tr>
<tr>
<td>4</td>
<td>Corn starch</td>
<td>50.00</td>
<td>21.20</td>
</tr>
<tr>
<td>5</td>
<td>Lactose</td>
<td>120.00</td>
<td>51.00</td>
</tr>
<tr>
<td>6</td>
<td>Maleic Acid</td>
<td>2.00</td>
<td>0.85</td>
</tr>
<tr>
<td>7</td>
<td>Corn Starch (for paste)</td>
<td>5.00</td>
<td>2.10</td>
</tr>
<tr>
<td>8</td>
<td>Purified water</td>
<td>q.s</td>
<td>q.s</td>
</tr>
<tr>
<td>9</td>
<td>Hypromellose (HPMC K4M)</td>
<td>Variable</td>
<td>Variable</td>
</tr>
<tr>
<td>10</td>
<td>Hypromellose (HPMC K15M)</td>
<td>Variable</td>
<td>Variable</td>
</tr>
<tr>
<td>11</td>
<td>Zinc stearate</td>
<td>8.00</td>
<td>3.40</td>
</tr>
</tbody>
</table>
Table 4.3: Quantitative formula of nalapril maleate sustained release tablets using various combinations of Hypromellose (HPMC K4M) and Hypromellose (HPMC K15M)

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Enalapril (mg)</th>
<th>HPMC K4M (mg)</th>
<th>HPMC K15M (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>20 mg</td>
<td>--</td>
<td>15</td>
</tr>
<tr>
<td>F2</td>
<td>20 mg</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>F3</td>
<td>20 mg</td>
<td>20</td>
<td>15</td>
</tr>
<tr>
<td>F4</td>
<td>20 mg</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>F5</td>
<td>20 mg</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>F6</td>
<td>20 mg</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>F7</td>
<td>20 mg</td>
<td>12.5</td>
<td>12.5</td>
</tr>
<tr>
<td>F8</td>
<td>20 mg</td>
<td>15</td>
<td>17.5</td>
</tr>
<tr>
<td>F9</td>
<td>20 mg</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>F10</td>
<td>20 mg</td>
<td>10</td>
<td>12.5</td>
</tr>
<tr>
<td>F11</td>
<td>20 mg</td>
<td>15</td>
<td>20</td>
</tr>
</tbody>
</table>

4.3 Evaluation of tablets

4.3.1 Hardness

Hardness indicates the ability of a tablet to withstand mechanical shocks while handling. The hardness of the tablets was determined using Digital hardness tester (Erweka GmbH, Germany). Ten tablets were randomly selected from each formulation and hardness of the same were determined. The average value was also calculated.

4.3.2 Friability

The friability of tablets was determined using Roche Friabilator. It is expressed in percentage (%). About 6.5 g tablets (Winitial) were transferred into Friabilator. The Friabilator was operated
at 25 rpm for 4 minutes or run up to 100 revolutions. The tablets were dedusted and weighed again (W\textsubscript{final}). The percentage friability was calculated by

\[ F = \frac{W\textsubscript{initial} - W\textsubscript{final}}{W\textsubscript{initial}} \times 100 \]  

\[ \text{Equation 33} \]

% Friability of tablets less than 1 % are considered acceptable.

#### 4.3.3 In vitro dissolution studies

In vitro drug release studies from the prepared matrix tablets were conducted using USP type II apparatus at 37\textdegree C at 50 rpm. Dissolution Medias were used as 900 ml of 1.0 N HCl and Phosphate buffer of PH 6.8. The release rates from matrix tablets were conducted in HCl solution (pH-1.2) for 2 hours and changed to phosphate buffer (pH-6.8) for further time periods. The samples were withdrawn at desired time periods from dissolution media and the same were replaced with fresh dissolution media of respective pH. The samples were analyzed by HPLC. The amounts of drug present in the samples were calculated with the help of appropriate calibration curves constructed from reference standards. Drug dissolved at specified time periods was plotted as percent release versus time curve.

#### 4.3.4 Independent-model method (data analysis)

**Data Analysis-Release Kinetics**

There are several Model dependent and Model independent methods for data analysis. Literature survey shows the different kinetics models as Zero order Kinetics, First order kinetics, Hixon-Crowell model, Korsmeyer-Peppas models applied to interpret release order and mechanism of drug release from matrix systems. When these models are used and analyzed in the preparation, the rate constant obtained from these models is an apparent rate constant. The release of drugs from the matrix tablets can be analysed by release kinetic theories.

To study the Kinetics of drug release from matrix system, the release data were fitted into Zero order as cumulative amount of drug release vs. time (Equation 34), first order as log cumulative percentage of drug remaining vs. time (Equation 35), Higuchi model as cumulative percent drug release vs. square root of time (Equation 36), Hixon-Crowell cube root law as cube root of percent drug remaining vs time (Equation 37). To describe the release behavior from the
polymeric systems, data were fitted according to well known exponential Korsmeyer –Peppas equation as log cumulative percent drug release vs log of time equation (Korsmeyer equation 38).

**Zero order kinetics,**

\[ Q_t = K_0 t \]  \hspace{1cm} \text{Equation 34}

Where,

- \( Q_t \): Amount of drug release in time \( t \)
- \( K_0 \): Zero order rate constant expressed in unit of concentration /time
- \( t \): Release time

**First order kinetics,**

\[ \log Q = \log Q_0 - k t / 2.303 \]  \hspace{1cm} \text{Equation 35}

Where,

- \( Q_0 \): is the initial concentration of drug
- \( k \): is the first order rate constant
- \( t \): release time

**Higuchi Kinetics,**

\[ Q = k t^{1/2} \]  \hspace{1cm} \text{Equation 36}

Where,

- \( k \): Release rate constant
- \( t \): release time, Hence the release rate is proportional to the reciprocal of the square root of time.

**Hixon Crowell cube root law,**

\[ Q_0^{1/3} - Q_t^{1/3} = K_{HC} t \]  \hspace{1cm} \text{Equation 37}

Where,

- \( Q_t \): the amount of drug release at time \( t \)
- \( Q_0 \): initial amount of drug in tablets
- \( K_{HC} \): rate constant for Hixon Crowell

**Korsmeyer peppas Model,**

First 60% in vitro release data was fitted in equation of Korsmeyer et al. to determine the release behavior from controlled release polymer matrix system. The equation is also called as power law,

\[ M_t / M_\infty = K t^n \]  \hspace{1cm} \text{Equation 38}

Where,
$M_t = \text{amount of drug released at time } t$

$M_\infty = \text{amount of drug released after infinite time}$

$M_t / M_\infty = \text{fraction solute release}$

$t = \text{release time}$

$K = \text{kinetic constant incorporating structural and geometric characteristics of the polymer system}$

$n = \text{diffusional exponent that characterizes the mechanism of the release of traces.}$

The magnitude of the release exponent “$n$” indicates the release mechanism (i.e. Fickian diffusion, Non-fickian, supercase II release). For matrix tablets, values of $n$ of near 0.5 indicates fickian diffusion controlled drug release, and an $n$ value of near 1.0 indicates erosion or relaxational control (case II relaxational release transport, non-fickian, zero order release).

Values of $n$ between 0.5 and 1 regarded as an indicator of both diffusion and erosion as overall release mechanism commonly called as anomalous release mechanism.

The values of $n$ and $k$ are inversely related. A very high $k$ values may suggest a burst drug release from the matrix.

**Similarity factor analysis (f2 factor):**

The dissolution profile was statistically analyzed by using dissolution similarity factor (f2). F2 was calculated by following formula:

$$f_2 = 50 \cdot \log \left( \frac{1 + \left( \frac{1}{n} \sum_{i=1}^{n} (R_i - T_i)^2 \right)^{0.5}}{} \right) \cdot 100$$

Where,

$n = \text{Number of dissolution time points, so that}$

$R_i = \text{Reference dissolution value at time } t$

$T_i = \text{Test dissolution value at time } t$

In vitro release profile of test formulation was compared with the desired theoretical dissolution profile. The $f2$ value between 50-100 ensures sameness and equivalence between two dissolution profiles.
4.4 Bioanalytical method development and validation

4.4.1 Bioanalytical Technique

Enalapril and Enalaprilat were extracted from human plasma by SPE Extraction Procedure. After elution the analytes were analysed by Reverse Phase UPLC coupled with mass detector.

4.4.2 Detection

This method is accurate and precise at 1.012 ng/mL and 1.012 ng/mL for Enalapril and Enalaprilat respectively.

4.4.3 Working standard

<table>
<thead>
<tr>
<th>Description</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enalapril</td>
<td>Analyte</td>
</tr>
<tr>
<td>Enalaprilat</td>
<td>Metabolite</td>
</tr>
<tr>
<td>Ramipril</td>
<td>Internal Standard</td>
</tr>
</tbody>
</table>

4.4.4 Biological matrix

Appropriate amount of human plasma specimen collected from all subjects.

4.4.5 Anticoagulant

K$_2$EDTA

4.4.6 Type of extraction

Enalapril and Enalaprilat are extracted from human plasma by SPE Extraction Procedure.

4.4.7 Linearity range

This method is linear in the range of 1.012 ng/mL to 502.10 ng/mL for Enalapril and 1.012 ng/mL to 502.000 ng/mL for Enalaprilat.

4.4.8 Method validation

The validation parameters were specifically, linearity, sensitivity, accuracy, precision, and matrix effects of the assay and the recovery and stability in human plasma, according to the US Food and Drug Administration (FDA) guidance for the validation of bioanalytical methods.

Selectivity was studied by comparing the chromatograms of six different lots of plasma obtained from six subjects, with the plasma samples having been spiked with ENP, ENPT, and IS. Calibration curves were prepared by assaying standard plasma samples at ENP and ENPT.
concentrations, ranging from 0.064 to 431.806 ng/mL for ENP and 0.064 to 431.720 ng/mL for ENPT.

The linearity of each method matched calibration curve was determined by plotting the peak area ratio (y) of ENP or ENPT to IS versus the nominal concentration (x) of ENP or ENPT, respectively.

The calibration curves were constructed by weighing (1/x^2) least squares linear regression. LLOQ for ENP or ENPT in human plasma was defined as the lowest concentration giving at least 10-fold, acceptable accuracy (80–120%), and sufficient precision (within 20%). Intra- and inter-day accuracy and precision for this method were determined at three different concentration levels on at least two different days, six replicates were analyzed with independently prepared calibration curves. The percentage accuracy was expressed as (mean observed concentration)/(nominal concentration) ×100, and the precision was the relative standard deviation (RSD, %).

The stability of ENP or ENPT in human plasma was assessed by analyzing six replicate samples spiked with LQC and HQC (high quality control) samples, respectively, under five different conditions: after short-term storage for 8.30 h at room temperature; after long-term storage of 6 months at −30 °C; after four freeze-thaw cycles; after 7 h on bench top, and after 48.30 h within the auto sampler. The concentrations obtained were compared with the nominal values of the QC samples.

**Method validation**

**Linearity**

Linearity of calibration standards was assessed by subjecting the spiked concentrations and the respective peak areas using 1/X^2 linear least-squares regression analysis. Linearity ranged from 0.064 to 431.806 ng/mL for ENP (r>0.990), 0.064 to 431.72 ng/mL for ENPT (r>0.990). In aqueous solution, accuracy of all calibration standards was within 85–115%, except LLOQ where it was 80–120%.
Specificity and selectivity
Six different lots of blank plasma, one each of haemolyzed and lipemic effects were analyzed to ensure that no endogenous interference took place with the mass transitions chosen for ENP, ENPT, and IS. Six LLOQ level samples along with plasma blank from the respective plasma lot were prepared from six different lots of plasma and analyzed. In all six plasma blanks, the response at the retention time of ENP and ENPT was < 20% of LLOQ response and at the retention time of IS, the response was < 5% of mean IS response in LLOQ.

Limit of quantitation
To check the reproducibility of the method at the highest and lowest concentration level, six injections at each level was injected. For ENP the % accuracy at LLOQ and ULOQ (upper limit of quantification) level was 99.90 and 106.19, and the % CV (% coefficients of variation) was 4.15 and 0.37. Whereas for ENPT the % accuracy at LLOQ and ULOQ level was 108.79 and 102.54, and the % CV was 11.26 and 0.46.

Accuracy and precision
For the validation of the assay, QC samples were prepared at three concentration levels of low, medium, and high. Six replicates of each QC samples were analyzed together with a set of calibration standard. The accuracy of each sample preparation was determined by injection of calibration samples and three QC samples in six replicate for at least two days. Obtained accuracy and precision (inter- and intra-day) are presented in Table 1A for ENP and Table 1B for ENPT. The results show that the analytical method is accurate, as the accuracy is within the acceptance limits of 100 ± 15% at all concentration levels. The precision around the mean value was never greater than 15% at any of the concentration studied.

Recovery study
A recovery study was performed by comparing processed QC samples of three different levels in six replicate with aqueous samples of same level. The recovery of ENP at LQC level was 87.87%, medium quality control (MQC) level was 83.80% and for high quality control (HQC) level was 84.54%. The mean recovery of ENP was 85.40%; % CV of mean recovery of all three QCs were 2.54. The recovery of ENPT at LQC level was 90.94%, medium quality control (MQC) level was 89.94%, and for high quality control (HQC) level was 92.17%. The mean
recovery of ENPT was 91.02%; % CV of mean recovery of all three QCs were 1.23. Recovery of IS was 91.73%.

4.5 In vivo study of optimized sustained release formulation

4.5.1 Study Design
A randomized, three-treatment, single dose, parallel pharmacokinetics study on Enalapril Maleate sustained release 20 mg tablet (containing Enalapril Maleate 20 mg) of Cadila Pharmaceuticals Ltd., India compared with Envas 10 mg tablet (2 tablets) (containing Enalapril Maleate 10 mg) of Cadila Pharmaceuticals Ltd., India in 18 healthy, adult, male, human subjects under fasting and fed condition allocated by randomization sheet.

4.5.2 Treatments
Test product: Enalapril Maleate 20 mg tablet
(Containing Enalapril Maleate 20 mg)
Cadila Pharmaceuticals Ltd., India.

References product: Envas 10 mg tablet
(Containing Enalapril Maleate 10 mg)
Cadila Pharmaceuticals Ltd., India.

4.5.3 Number of Subjects
Eighteen (18) healthy, adult, male, Indian human subjects between the age group of 18-45 years were enrolled in the study. (Annexure I – Study Protocol)

4.6 IVIVC:
The relationship between % drug released in vitro and the percent absorbed sustained release Enalapril Maleate Tablets 20 mg formulations was assessed using different modelling approaches:

1) Compartmental approach:
Compartmental model was tried develop using first order input and zero order input on In vivo release data of immediate release Enalapril Maleate Tablets.
By using in vivo profile of Enalapril Maleate immediate release tablets, the data was tried to fit in one compartment model and two compartment model by using first order input and zero order input.

2) **Nelson-Wagner approach:**
The fraction absorbed concentration-time data by deconvolution using the Nelson-Wagner method as described in

\[
F_{\text{abs}}(t) = \frac{[C(t) + k_e \times \text{AUC}_{0-\infty}]}{[k_e \times \text{AUC}_{0-\infty}]}.
\]

With the Nelson-Wagner equation, the pharmacokinetic profile is deconvoluted to obtain the *in vivo* absorption as a function of time and is plotted alongside the *in vitro* release data to assess the superimposability of the two profiles. If the two curves are superimposable and a linear relationship is obtained, it suggests a strong correlation between *in vivo* and *in vitro* drug release.

3) **Fractional AUC approach:**
The area under the curve (AUC) was calculated using the trapezoidal rule. The fractional AUC was determined by dividing cumulative AUC at time “t” with cumulative AUC(0–last), as described in previous publications \(^3, 29\) and plotted along with the % drug released *in vitro*. In a manner similar to the Nelson-Wagner approach, the superimposability of the *in vivo* and *in vitro* drug release was compared.

4) **Classical (Numerical Deconvolution) approach:**
A conventional method was based on a numeric deconvolution, where whole *in vitro* profiles of cumulative fraction dissolved were taken into account.
In order to calculate fraction of drug absorbed in time, simulated *in vivo* profile was used as an equivalent to the unit impulse response (UIR).
5) **Weibull Model:**

In Vitro release profiles of slow, fast and medium sustained released Enalapril maleate Tablets were analyzed by Weibull model. Predicted Enalapril Maleate concentrations were obtained based on Weibull Model.

6) **IVIVC Model development:**

A proper IVIVC model should be established using formulations with different release rates. At minimum three (slow, medium, and fast dissolving) dosage forms should be designed, where the extreme ones are used to build a model able to predict *in vivo* bioperformance of the “middle” formulation. Such an approach is common when sustained-release dosage forms are tested.

For Enalapril Maleate Sustained release Tablets, level A model , level B and Multiple linear Level C model of IVIVC were tried to attempt.

i) Basic Level A model was tried to develop through correlation of percent *In vivo* input and Percent *In vitro* dissolution data obtained. Level A IVIVC was developed by drawing a plot between the percentage drug absorbed (along x axis) of a formulation and its percentage drug dissolved (along y-axis) followed by the regression analysis of each curve to evaluate the strength of correlation determining whether the curve is linear or non-linear.

ii) Level B IVIVC is developed by plotting the values of MDT (along x-axis) against MRT (along y-axis) of a formulation followed by the regression analysis of the curve.

i) Multiple Linear level C IVIVC models were developed using dissolution data at 0.25, 0.50, 1.0, 1.5, 2.0 and 4.0 h and in vitro mean dissolution time (MDT).

7) **IVIVC Model Predictability evaluation:**

a) **External Predictability:** External predictability can be evaluated when one formulation is used as testing formulation, and other two are used for model building. Here the external predictability was evaluated by comparing the linearity of IVIVC model curves of testing formulation i.e. Moderate sustained released Enalapril maleate Tablets 20 mg and other two used for model building viz. Slow and fast sustained released Enalapril Maleate Tablets.
b) **Internal Predictability**: In case of internal validation, testing formulation was included in model building phase. Whole or partial area under the curve (pAUC) prediction error (PE) (1) and $C_{\text{max}}$ prediction error (2) was calculated using following formulae’s:

$$pAUC_{\text{PE}}[\%] = \left[ \left| \frac{pAUC_{\text{observed}} - pAUC_{\text{predicted}}}{pAUC_{\text{observed}}} \right| \right] \times 100$$

$$C_{\text{max}}_{\text{PE}}[\%] = \left[ \left| \frac{C_{\text{max,observed}} - C_{\text{max,predicted}}}{C_{\text{max,observed}}} \right| \right] \times 100$$