CHAPTER 4: PHARMACOKINETICS

4.1 INTRODUCTION

The duration of drug therapy ranges from a single dose of a drug taken for relieving an acute condition such as headache to drugs taken lifelong for chronic condition such as hypertension, diabetes, asthma or epilepsy. The frequency of administration of a drug in a particular dose is called as dosage regimen. Depending upon the therapeutic objective to be attained, the duration of drug therapy and the dosage regimen are decided.

Rational and optimal therapy with a drug depends upon:

1. Choice of a suitable drug, and
2. A balance between the therapeutic and the toxic effects.

Both, the therapeutic and the toxic effects, depend upon the concentration of drug at the site of action which is difficult to measure. However, it corresponds to a specific concentration of drug in plasma which can be measured with accuracy. The drug fails to elicit a therapeutic response when the concentration is below the effective level and precipitates adverse reactions when above the toxic level. The plasma drug concentration between these two limits is called as the therapeutic concentration range or therapeutic window (the ratio of maximum safe concentration to minimum effective concentration of the drug is called as the therapeutic index). Thus, in order to achieve therapeutic success, plasma concentration of the drug should be maintained within the therapeutic window. For this, knowledge is needed not only of the mechanisms of drug absorption, distribution, metabolism and excretion, but also of the kinetics of these processes i.e. pharmacokinetics. Pharmacokinetics is defined as the kinetics of drug absorption, distribution, metabolism and excretion (KADME) and their relationship with the pharmacologic, therapeutic or toxicologic response in man and animals. The applications of pharmacokinetic principles in the safe and effective management of individual patient is called as clinical pharmacokinetics.
Plasma Drug Concentration – Time profile

A direct relationship exists between the concentration of drug at the biophase (site of action) and the concentration of drug in plasma. A typical plasma drug concentration-time curve obtained after a single oral dose of a drug and showing various pharmacokinetic and pharmacodynamic parameters is depicted in Fig. 4.1. Such a profile can be obtained by measuring the concentration of drug in plasma samples taken at various intervals of time after administration of a dosage form and plotting the concentration of drug in plasma (y-axis) versus the corresponding time at which the plasma sample was collected (X-axis).

Fig 4.1 Atypical plasma concentration-time profile showing pharmacokinetics and pharmacodynamics parameters. Obtained after oral administration of single dose of a drug.

The three important **pharmacokinetic parameters** that describe the plasma level-time curve and useful in assessing the bioavailability of a drug from its formulation are:
Peal Plasma Concentration ($C_{\text{max}}$)

The point of maximum concentration of drug in plasma is called as the peak and the concentration of drug at peak is known as peak plasma concentration. It is also called as peak height concentration and maximum drug concentration. $C_{\text{max}}$ is expressed in mcg/mL. The peak level depends upon the administered dose and rate of adsorption and elimination. The peak represents the point of time when absorption rate equals elimination rate of drug. The portion of curve to the left of peak represents absorption phase, i.e. when the rate of absorption is greater than the rate of elimination. The section of curve to the right of peak generally represents elimination phase, i.e. when the rate of elimination exceeds rate of absorption. Peak concentration is often related to the intensity of pharmacologic response and should ideally be above minimum effective concentration (MEC) but less than the maximum safe concentration (MSC).

Time of peak Concentration ($t_{\text{max}}$)

The time for drug to reach peak concentration in plasma (after extravascular administration) is called as the time of peak concentration. It is expressed in hours and is useful in estimating the rate of absorption. Onset time and onset of action are dependent upon $t_{\text{max}}$. The parameter is of particular importance in assessing the efficacy of drugs used to treat acute conditions like pain and insomnia which can be treated by a single dose.

Area Under the Curve (AUC)

It presents the total integrated area under the plasma level-time profile and expresses the total amount of drug that comes into the systemic circulation after its administration. AUC is expressed in mcg/mL X hours. It is the most important parameter in evaluating the bioavailability of a drug from its dosage form as it represents the extent of absorption. AUC is also important for drugs that are administered repetitively for the treatment of chronic conditions like asthma or epilepsy.

The various Pharmacodynamic parameters are:

1. Minimum Effective Concentration (MEC)
It is defined as the minimum concentration of drug in plasma required to produce the therapeutic effect. It reflects the minimum concentration of drug at receptor site to elicit the desired pharmacologic response. The concentration of drug below MEC is said to be in the subtherapeutic level.

In case of antibiotics, the term minimum inhibitory concentration (MIC) is used. It describes the minimum concentration of antibiotic in plasma required to kill or inhibit the growth of microorganisms.

2. Maximum Safe Concentration (MSC)

Also called as minimum concentration (MTC), it is the concentration of drug in plasma above which adverse or unwanted effects are precipitated. Concentration of drug above MSC is said to be in the toxic level.

3. Onset of Action

The beginning of pharmacologic response is called as onset of action. It occurs when the plasma drug concentration just exceeds the required MEC.

4. Onset Time

It is the time required for the drug to start producing pharmacologic response. It corresponds to the time for the plasma concentration to reach MEC after administration of drug.

5. Duration of Action

The time period for which the plasma concentration of drug remains above the MEC level is called as duration of drug action.

6. Intensity of Action

It is the maximum pharmacologic response produced by the peak plasma concentration of drug. It is also called as peak response.

7. Therapeutic Range

The drug concentration between MEC and MSC represents the therapeutic range.
Rate, Rate Constants and Orders of Reactions

**Pharmacokinetics** is the mathematical analysis of processes of ADME. The movement of drug molecules from the site of application to the systemic circulation, through various barriers, their conversion into another chemical form and finally their exit out of the body can be expressed mathematically by the rate at which they proceed, the order of such processes and the rate constants.

The velocity with which a reaction or a process occurs is called as its rate. Consider the following chemical reaction:

\[
\text{drug A} \rightarrow \text{drug B} \tag{4.1}
\]

The rate of forward reaction is expressed as:

\[
\frac{-dA}{dt} \tag{4.2}
\]

Negative sign indicates that the concentration of drug A decreases with time \( t \). As the reaction proceeds, the concentration of drug B increases and the rate of reaction can also be expressed as:

\[
\frac{dB}{dt} \tag{4.3}
\]

Experimentally, the rate of reaction is determined by measuring the decrease in concentration of drug A with time \( t \).

The manner in which the concentration of drug (or reactants) influence the rate of reaction or process is called as the order of reaction or order of process. If \( C \) is the concentration of drug A, the rate of decrease in \( C \) of drug A as it is changed to B can be described by a general expression as function of time \( t \).

\[
\frac{dc}{dt} = -K C^n \tag{4.4}
\]

Where \( K = \text{rate constant} \)

\( n = \text{order of reaction} \)
if $n=0$, it’s a zero-order process. If $n=1$, it is a first-order process and so on. The three commonly encountered rate processes in a physiologic system are – zero process, first-order process and mixed-order process. The pharmacokinetics of most drugs can be adequately described by zero-and first-order processes of which the later are more important.

**Zero-order Kinetics (Constant Rate Processes)**

If $n=0$, equation 9.4 becomes:

$$\frac{dc}{dt} = -K_0 C^0 = -K_0$$

(4.5)

Where $K_0 = $ zero order rate constant (in mg/min)

From equation 4.5 the zero-order process can be defined as the one whose rate is independent of the concentration of drug undergoing reaction. I.e the rate of reaction cannot be increased further by increasing the concentration of reactants.

Rearrangement of equation 4.5 yields:

$$dC = -K_0 \, dt$$

(4.6)

Integration of equation 4.6 gives:

$$C - C_0 = -K_0 \, t$$

(4.7)

Or simply

$$C = C_0 - K_0 \, t$$

Where $C_0 =$ concentration of drug at $t =0$, and

$$C = \text{Concentration of drug yet to undergo reaction at time } t.$$

Equation 4.7 is that of a straight line and states that the concentration of reactant decreases linearly with time. A plot of $C$ versus $t$ yields such a straight line having slope $-K_0$ and y-intercept $C_0$ (Fig. 4.2).
Zero-Order Half-Life

Half-Life \((t_{1/2})\) or half time is defined as the time period required for the concentration of drug to decrease by one-half. When \(t = t_{1/2}\), \(C = C_0/2\) and the equation 4.7 becomes:

\[
\frac{c_0}{2} = C_0 - K_0 \cdot t_{1/2}
\]

Solving 4.8, We get:

\[
t_{1/2} = \frac{c_0}{2K_0} = \frac{0.5c_0}{K_0}
\]

Equation 4.9 shows that the \(t_{1/2}\) of a zero-order is not constant but proportional to the initial concentration of drug \(C_0\) and inversely proportional to the zero-order rate constant \(K_0\). Since the zero-order \(t_{1/2}\) changes with the decline in drug concentration. It is of little practical importance. Zero-order equations do not require logarithmic transformations.

Examples of zero-order processes are:

1. Metabolism/protein-drug binding/enzyme or carrier-mediated transport under saturated conditions. The rate of metabolism, binding or transport of drug remains constant as long as its concentration is in excess of saturating concentration.
2. Administration of a drug as a constant rate i.v. infusion
3. Controlled drug delivery such as that from i.m. implants or osmotic pumps.

4.2 First-order kinetics (Linear Kinetics)

If \( n = 1 \), equation 4.4 becomes:

\[
\frac{dc}{dt} = -KC \tag{4.10}
\]

Where \( K \) = first-order rate constant (in time\(^{-1}\) or per hour)

From equation 4.10, it is clear that a first-order process is the one whose rate is directly proportional to the concentration of drug undergoing reaction i.e. greater the concentration, faster the reaction. It is because of such a proportionality between rate of follow linear kinetics (Fig. 4.3).

\[
\frac{dc}{c} = -Kdt \tag{4.11}
\]

Integration of equation 4.11 gives:

\[
\ln C = \ln C_0 - Kt \tag{4.12}
\]

Equation 4.12 can also be written in exponential form as:
\[ C = C_0 e^{kt} \quad (4.13) \]

Where \( e \) = natural (Naperian) log base.

Since equation 4.13 has only one exponent, the first-order process is also called as **monoexponential rate process**. Thus, a first-order process is characterized by **logarithmic** or **exponential kinetics** i.e. constant fraction of drug undergoes reaction per unit time.

Sincs \( \ln = 2.303 \log \), equation 4.12 can be written as:

\[ \log C = \log C_0 - \frac{Kt}{2.303} \quad (4.14) \]

or

\[ \log C = \log C_0 - 0.434 Kt \quad (4.15) \]

A semilogarithmic plot of equation 4.14 yields a straight line with slope = -\( K/2.303 \) and y-intercept = \( \log C_0 \) (Fig. 4.4.).

![Graph showing regular and semilog graphs of first-order kinetics](image)

**4.3 First – Order Half – Life**

Substituting the value of \( C = C_0/2 \) at \( t_{1/2} \) in equation 4.14 and solving it yields:

\[ t_{1/2} = \frac{0.693}{K} \quad (4.16) \]

Equation 4.16 shows that, in contrast to zero-order process, the halflife of a first-order process is a constant and independent of initial drug concentration i.e. irrespective of what the initial drug
concentration is, the time required for the concentration to decrease by one – half remains the same ( see Fig. 4.4 ). The \( t_{1/2} \) of a first – order process is an important pharmacokinetic parameter.

Most pharmacokinetic processes viz. absorption, distribution and elimination follow first – order kinetics.

**4.4 Mixed – Order Kinetics ( Nonlinear Kinetics )**

In some instances, the kinetics of a pharmacokinetic process change from predominantly first – order to predominantly zero – order with increasing dose or chronic medication. A mixture of both first – order and zero – order kinetics is observed in such cases and therefore the process is said to follow **mixed – order kinetics**. Since deviations from an originally linear pharmacokinetic profile is observed, the rate process of such a drug is called as **nonlinear kinetics**. Mixed order kinetics is also termed as **dose dependent kinetics** as it is observed at increased or multiple doses of some drugs. Nonlinearities have been observed in absorption (e.g. vitamin C ), distribution ( e.g. naproxen ) and elimination ( e.g. riboflavin ) characteristics of few drugs. The phenomena is seen when a particular pharmacokinetic process involves presence of carriers of enzymes which are substrate specific and have definite capacities and can get saturated at high drug concentration ( i.e. capacity – limited ). The kinetics of such capacity – limited processes can be described by the **Michaelis – Menten Kinetics**.

**4.5 CASE STUDY FOR BA/BE**

**STUDY TITLE:** An Open-Label, Balanced, Randomized, Two-Treatment, Two-Sequence, Two-Period, Crossover, Single-Dose Comparative Oral Bioavailability Study Of Felodipine Extended Release Tablets 10 mg And ‘PLENDIL’ Extended Release Tablets 10 mg (Felodipine Extended Release Tablets 10 mg) In Healthy Adult Human Subjects Under Fed Conditions.

**DRUG:** Felodipine

**INDICATION:** It is indicated for the treatment of hypertension.
DESIGN: An Open-label, randomized, two-period, two-treatment, two-sequence, crossover, balanced, single dose comparative oral bioavailability study in healthy, adult, human subjects under fed conditions.

STUDY DURATION: The time from first subject dosed to when the last subject completed was 18 days.

STUDY TYPE: An Open-label, randomized, two-period, two-treatment, two-sequence, crossover, balanced, single dose comparative oral bioavailability study in healthy, adult, human subjects under fed conditions.

OBJECTIVE: The objective of this study was to compare the oral bioavailability of Felodipine extended release tablets 10 mg with that of ‘PLENDIL’ extended release 10 mg tablets (Felodipine extended release 10 mg tablets) in healthy adult human subjects under fed conditions and to monitor safety of subjects.

METHODOLOGY: This open-label, balanced, randomized, two-treatment, two-sequence, two-period, crossover, single-dose comparative oral bioavailability study in healthy, adult, human subjects under fed conditions was conducted to compare the oral bioavailability of two formulations of Felodipine extended release 10 mg tablets. The study was conducted with 60 (57 completed) healthy, adult, human subjects in accordance with Project No. FELO/10 (Version#00). In each study period, investigational product of 10 mg was administered at about 30 minutes after serving of high fat and high calorie breakfast. The test formulation was Cadila Healthcare Limited, INDIA’s Felodipine extended release 10 mg tablets and the reference formulation was Merck & co., Inc., Whitehouse station, NJ, 08889, USA’s ‘PLENDIL’ extended release 10 mg tablets (Felodipine extended release 10 mg tablets). The subjects received the test product in one study period and the reference product in the other period; the order of administration was according to the randomization schedule. There was a 14-day interval between treatments.

Blood samples were collected pre-dose and at intervals over 96.0 hours after administration of each dose. The plasma samples for all subjects of the study were delivered to the analytical laboratory at BA Research India Ltd., BA Research House, Opp. Pushparaj Towers, Nr. Judges Bungalows, Bodakdev, Ahmedabad-380 054, Gujarat, India for the determination of Felodipine.

During the course of study safety parameters assessed were vital signs, physical examination, medical history, clinical laboratory safety tests (haematology, biochemistry, immunological tests, urine analysis, X ray and ECG) at baseline. Laboratory parameters (excluding some) were reassessed at 96.0 hours post dose of the last study period.
Statistical analysis was performed at BA Research India Ltd., BA Research House, Opp. Pushparaj Towers, Nr. Judges Bungalows, Bodakdev, Ahmedabad-380 054, Gujarat, India to evaluate the relative bioavailability of the test formulation to that of the reference product.

**NUMBER OF SUBJECTS:** A total of 64 healthy, adult, human subjects consenting to participate and meeting the Inclusion & Exclusion criteria as mentioned in the protocol were enrolled into the study to ensure the dosing of 60 subjects. Remaining four extra subjects who were not dosed were checked out from the facility after the completion of dosing in Period I. 57 subjects completed the study.

**MAIN DIAGNOSIS FOR ENTRY:** Diagnosis was not required for this study. All subjects were asymptomatic, healthy, adult, human subjects who met the inclusion/exclusion criteria for this study.

**TEST PRODUCT:** Felodipine Extended Release tablets USP 10 mg, Cadila Healthcare Ltd., India.

**REFERENCE PRODUCT:** ‘PLENDIL®’ extended release tablets 10 mg (Felodipine extended release tablets 10 mg), Manufactured by: Merck & co., Inc., Whitehouse station, NJ, 08889, USA. Manufactured For: Astra Zeneca LP Wilmington DE 19850.

**ROUTE OF ADMINISTRATION:** Oral

**DURATION OF TREATMENT:** In each period, single oral dose of Felodipine extended release tablet 10 mg was administered to fed subjects. Each dose was separated by a 14-day interval. Total duration of the treatment was 18 days.

**PRIMARY EFFICACY VARIABLE:** Not applicable.

**SECONDARY EFFICACY VARIABLE:** Not applicable.

**SAFETY ANALYSIS:** Adverse events were collected and tabulated.

Clinical Lab tests: The safety related laboratory tests were carried out on the study subjects during screening and reassessed (excluding some) at 96 hours post dose of the last study period. The laboratory tests for values found significantly elevated were repeated until they were reported normal/clinically non-significant in the follow ups.
STATISTICAL METHODS: The analytical data was used to calculate the pharmacokinetic parameters: Cmax, AUCt, AUCi, Tmax, Kel and tHalf using a non-compartmental analysis of WinNonlin® professional software.

The pharmacokinetic parameters were evaluated statistically using the PROC MIXED procedure from SAS® statistical software.

SUMMARY OF RESULTS: For the log transformed Felodipine data, the 90% confidence intervals about the ratio of the Test geometric mean to Reference geometric mean are within the 80% to 125% limits for AUCt, AUCi and Cmax. (set by FDA, Guidance for Industry, Bioavailability and Bioequivalence Studies for Orally Administered Drug Products—General Considerations, Center for Drug Evaluation and Research [CDER], March, 2003).

CONCLUSION: Based on these results, the Felodipine Extended Release tablets USP 10 mg, Cadila Healthcare Limited, India and ‘PLENDIL®’ extended release tablets 10 mg (Felodipine extended release tablets 10 mg), Manufactured by: Merck & co., Inc., Whitehouse station, NJ, 08889, USA., Manufactured For: Astra Zeneca LP Wilmington DE 19850 are bioequivalent under Fed conditions.

4.5 STUDY DESIGN AND PLAN DESCRIPTION

This was an open label, balanced, randomized, two-treatment, two-sequence, two-period, crossover, single-dose comparative oral bioavailability study in healthy adult human subjects under fed conditions comparing equal doses of the test and reference products. Blood samples were collected at pre-dose and at intervals over 96 hours after dosing in each period. Subjects were confined at the clinical facility from at least 11 hours prior to dosing until after the 24 hours post dose. The interval between doses was 14 days.

4.6 SELECTION OF STUDY DESIGN

Cadila Healthcare Limited, India is seeking approval of a generic version of Felodipine Extended Release tablets USP 10 mg, for which demonstration of bioequivalence to the reference listed product ‘PLENDIL®’ extended release tablets 10 mg (Felodipine extended release tablets 10 mg), Manufactured by: Merck & co., Inc., Whitehouse station, NJ, 08889, USA., Manufactured For: Astra Zeneca LP Wilmington DE 19850 is required.

After considering bioequivalence standards, intrasubject variability and possibility of drop outs, the sponsor requested that the protocol should be written with sufficient number of subjects to be enrolled to ensure dosing of 60 subjects in this study.
Data from subjects who complete both the periods in the study would be used in the statistical determination of bioequivalence according to the protocol. In order to minimize any possibility of a carry-over effect, at least 14 days and not more than 21 days washout period was selected for this study. Bioequivalence was determined by statistical comparison of AUCt, AUCi and Cmax for the test and reference products for Felodipine. The protocol was reviewed and approved by the sponsor prior to commencement of the study.

The study was designed based on the known pharmacokinetics of Felodipine and general accepted standards for the conduct of bioequivalence studies.

4.7 SELECTION OF STUDY POPULATION

A total of 64 healthy, adult, human subjects who met study inclusion and exclusion criteria were enrolled in the study to ensure the dosing of 60 subjects. Remaining four extra subjects who were not dosed were checked out from the facility after the completion of dosing in Period I. 57 subjects completed the study.

**Inclusion Criteria**

1. The subjects should be healthy human between 18 and 45 years.
2. The subjects should be screened within 21 days prior to the administration of first dose of the study drug.
3. The subjects should have a BMI between 18.5 and 24.9 weight in kg/ height² in meter.
4. The subjects should be able to communicate effectively with study personnel.
5. The subjects should be able to give written informed consent to participate in the study.

If subject is a female volunteer and

6. Is of child bearing potential practicing an acceptable method of birth control for the duration of the study as judged by the investigator(s), such as condoms, foams, jellies, diaphragm, intrauterine device (IUD), or abstinence or
7. Is postmenopausal for at least 1 year or
8. Is surgically sterile (bilateral tubal ligation, bilateral oophorectomy, or hysterectomy has been performed on the subject).

**Exclusion Criteria**
1. The subjects who have a history of allergic responses to Felodipine or other related drugs.
2. The subjects who have significant diseases or clinically significant abnormal findings during screening, medical history, physical examination, laboratory evaluations, ECG and X-ray recordings.
3. The subjects who have any disease or condition which might compromise the haemopoietic, gastrointestinal, renal, hepatic, cardiovascular, respiratory, central nervous system, diabetes, psychosis or any other body system.
4. The subjects who have a history or presence of bronchial asthma.
5. The subject who have used enzyme-modifying drugs within 30 days prior to receiving the first dose of study medication.
6. The subjects who have history of drug dependence, recent history of alcoholism or of moderate alcohol uses.
7. The subjects who are smokers who smoke more than or equal to 10 cigarettes per day or more than or equal to 20 biddies per day or those who cannot refrain from smoking during study period.
8. The subjects with a history of difficulty with donating blood or difficulty in accessibility of veins.
9. The subjects who have donated 1 unit (350 ml / 450 ml) blood within 90 days prior to receiving the first dose of study medication.
10. The subjects who have a positive hepatitis screen (includes subtypes A, B, C & E).
11. The subjects who have a positive test result for HIV antibody and / or syphilis (RPR/VDRL).
12. The subject who receives an investigational product, or has participated in a drug research study within a period of 90 days prior to the first dose of the study medication administration.
13. Female volunteers demonstrating a positive pregnancy screen.
14. Female volunteers who are currently breast-feeding.
15. Female volunteers not willing to use contraception during the study.

**Removal of Subjects from the Study**

Subjects were advised that they were free to withdraw from the study at any time for any reason or, if necessary, the Investigator could withdraw a subject from the study for any of the following reasons:

- The subjects suffer from significant intercurrent illness or underwent surgery during the course of the study, or have any significant symptoms or signs during the course of the study.
- Any subject found to have entered the study in violation of the protocol. This would include pre-study directions regarding alcohol and drug use, fasting or if the subject is uncooperative during the study.
- Any subject who required the use of an unacceptable concomitant medication.
- If it was felt in investigator’s opinion that it is not in the subject’s best interest to continue.
Any other justifiable reason, which should be adequately documented. The subject withdrawal during the study was handled as per in house procedure with adequate documentation.

4.8 TREATMENTS ADMINISTERED

In each study period, after an overnight fasting of at least 10 hours subjects were served high fat and high calorie breakfast 30 minutes prior to administration of investigational product. The test formulation was of Cadila Healthcare Ltd., India’s Felodipine extended release tablets 10 mg and the reference formulation was of Merck & co., Inc., Whitehouse station, NJ, 08889, USA’s ‘PLENDIL®’ extended release 10 mg tablets (Felodipine extended release 10 mg tablets). The subjects received the test product in one study period and the reference product in the other period. The order of administration was according to the randomization schedule. There was a 14-day interval between treatments.

4.9 STATISTICAL PLANS

Concentration data of subjects, which are received after analysis of samples, are to be included in the final data analysis. Data from subjects with missing concentration values (missed blood samples, lost samples, samples unable to be quantitated) may be used if pharmacokinetic parameters can be estimated using remaining data points, otherwise data from these subjects are to be excluded from the final analysis.

Pharmacokinetic parameters: The following pharmacokinetic parameters are to be determined from the time and concentration data using a non-compartmental analysis by WinNonlin® professional software (Version: 4.1 or higher; Pharsight Corporation, USA).

AUCt: The area under the plasma concentration versus time curve is to be calculated using the linear trapezoidal rule from the zero time point to the last quantifiable concentration.

AUCi: The area under the plasma concentration versus time curve from zero to infinity is to be calculated by adding Ct/Kel to AUCt, where Ct is the last quantifiable concentration and Kel is the elimination rate constant.

Cmax: The maximum observed plasma concentration is to be obtained by inspection.

Tmax: The time to maximum plasma concentration is to be obtained by inspection. If the maximum plasma concentration occurs at more than one time point, the first is chosen as Tmax.

Kel: The terminal elimination rate constant is to be obtained from the slope of the line, fitted by linear least squares regression, through the terminal points of the log (base e) of the concentration versus time plot for these points.

tHalf: The half-life is to be calculated by the equation tHalf = 0.693/ Kel.
No values of \( K_e \), AUC\(_i\) or t\(_{\text{Half}}\) are to be reported for cases that do not exhibit a terminal log-linear phase in the concentration versus time profile.

Statistical analysis: Statistical analysis is to be performed on pharmacokinetic data of the subjects by using SAS\textsuperscript{®} statistical software (Version 9.1 or higher; SAS Institute Inc, USA).

Descriptive statistics: Mean, Standard deviation, coefficient of variation, Median, Maximum and Minimum for all pharmacokinetic parameters is to be calculated.

Analysis of Variance: Ln-transformed data of C\(_{\text{max}}\), AUC\(_t\) & AUC\(_i\) and Untransformed data of T\(_{\text{max}}\), \( K_e \) and t\(_{\text{Half}}\) are to be evaluated statistically by PROC MIXED from SAS\textsuperscript{®} for difference due to treatments, period and sequence as a fixed effects and subject within sequence as a random effect.

All main effects are to be tested at 5% level of significance using the Mean Square Error as the error term.

Two One-Sided test for bioequivalence: Two one-sided 90% confidence interval for the ratio of means between drug formulations is to be calculated for untransformed data of T\(_{\text{max}}\), \( K_e \) and t\(_{\text{Half}}\) and Ln-transformed data of C\(_{\text{max}}\), AUC\(_t\) and AUC\(_i\).

Power: The power of ANOVA test to detect a 20% mean difference between test and reference formulations are to be reported.

The 90 % confidence interval of the relative mean AUC\(_t\), AUC\(_i\) and C\(_{\text{max}}\) of the test to reference formulation for Ln- transformed data should be within 80 % to 125 % for Felodipine to establish bioequivalence.

Outliers in a data set are defined as observations that appear to be inconsistent with the rest of the data. They can be identified as the values, which completely distort descriptive statistics. Subjects who exhibit extremely high or low bioavailability relative to the reference formulation are to be detected using statistical method namely Lund’s method (using statistical package SAS\textsuperscript{®} 9.1 or higher version). A valid clinical or physiological reason is to be explored for such an outlier, if found, and is to be reported if identified by the Principal Investigator of the study. However, to avoid the biasedness in the results, the statistical analysis are to be performed on both the data sets i.e. including as well as excluding the outliers if the outlier is justified clinically as well.

Plasma samples are to be assayed by validated method developed at BA Research India Ltd., Ahmedabad which is specific for the determination of Felodipine.

Samples of all subjects who complete both the periods of study are to be analyzed. Incase of dropouts, samples of such subjects are not to be taken for analysis. The criteria for repeat analysis, as defined in the respective in-house procedure are to be followed.

Study conducted to establish validity including accuracy, precision, reproducibility, specificity, recovery and frozen stability of the analytical method is to be reported in the final report.

4.10 DATA SETS ANALYZED

Sixty four (64) subjects were enrolled for this study to ensure the dosing of 60 subjects. Fifty seven (57) subjects completed the clinical portion of the study in it’s entirely.
The plasma samples from 57 subjects were assayed for Felodipine. There are 57 sets of Felodipine data for this study.
### Summary of Statistical Analysis of Felodipine Data

**FELODIPINE EXTENDED RELEASE TABLETS 10 mg FED STUDY**  
**CADILA HEALTHCARE LIMITED FELO/10**  
**SUMMARY OF STATISTICAL ANALYSIS OF UN-TRANSFORMED DATA**

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>REFERENCE LEAST SQUARE MEANS</th>
<th>TEST LEAST SQUARE MEANS</th>
<th>RATIO OF LEAST SQUARE MEANS</th>
<th>90% CI OF UN TRANSFORMED DATA</th>
<th>POWER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kel</td>
<td>0.034</td>
<td>0.037</td>
<td>106.56%</td>
<td>(97.12%; 116.00%)</td>
<td>0.9667</td>
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<tr>
<td>Tmax</td>
<td>5.436</td>
<td>6.366</td>
<td>117.10%</td>
<td>(102.38%; 131.82%)</td>
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<tr>
<td>tHalf</td>
<td>25.925</td>
<td>26.887</td>
<td>103.71%</td>
<td>( 97.77%; 109.65%)</td>
<td>0.9999</td>
</tr>
</tbody>
</table>

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**SUMMARY OF STATISTICAL ANALYSIS OF LOG-TRANSFORMED DATA**

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>REFERENCE LEAST SQUARE MEANS</th>
<th>TEST LEAST SQUARE MEANS</th>
<th>REFERENCE GEOMETRIC MEAN</th>
<th>TEST GEOMETRIC MEAN</th>
<th>CV(%)</th>
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</thead>
<tbody>
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<td>AUCi</td>
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<td>76.644</td>
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<tr>
<td>Cmax</td>
<td>2.275</td>
<td>2.304</td>
<td>9.731</td>
<td>10.012</td>
<td>31.481</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>RATIO OF GEOMETRIC MEANS</th>
<th>90% CI OF LOG TRANSFORMED DATA</th>
<th>POWER</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUCi</td>
<td>102.23%</td>
<td>(97.87%; 106.78%)</td>
<td>1.0000</td>
</tr>
<tr>
<td>AUCt</td>
<td>102.23%</td>
<td>(97.82%; 106.85%)</td>
<td>1.0000</td>
</tr>
<tr>
<td>Cmax</td>
<td>102.89%</td>
<td>(93.42%; 113.31%)</td>
<td>0.9839</td>
</tr>
</tbody>
</table>
Linear Mean Plot of Felodipine

R: Reference; T: Test

Semi Logarithmic Mean Plot of Felodipine

R: Reference; T: Test
4.11 EFFICACY CONCLUSIONS

The analysis of the plasma Felodipine data resulted in statistically significant, \( \alpha = 0.05 \), differences between dosing period and dosing sequence for log-transformed Cmax & AUCt.

The analysis of the plasma Felodipine data resulted in no statistically significant, \( \alpha = 0.05 \), differences between product for log-transformed Cmax & AUCt.

The analysis of the plasma Felodipine data resulted in statistically significant, \( \alpha = 0.05 \), differences between dosing period for log-transformed AUCi.

The analysis of the plasma Felodipine data resulted in no statistically significant, \( \alpha = 0.05 \), differences between product and dosing sequence for log-transformed AUCi.

The differences between the LSMEAN Test values and the corresponding LSMEAN Reference values of plasma Felodipine relative to reference LSMEAN are 17.1% for Tmax, 8.8% for Kel and 3.7% for tHalf.

The differences between the GEOMEAN Test values and the corresponding GEOMEAN Reference values of plasma Felodipine relative to reference GEOMEAN are 2.9% for Cmax, 2.2% for AUCt and 2.2% for AUCi.

For the log transformed Felodipine data, the 90% confidence intervals about the ratio of the Test geometric mean to Reference geometric mean are within the 80% to 125% limits for AUCt, AUCi and Cmax. (Set by FDA, Guidance for Industry, Bioavailability and Bioequivalence Studies for Orally Administered Drug Products– General Considerations, Center for Drug Evaluation and Research [CDER], March, 2003).

Based on these results, Felodipine Extended Release tablets USP 10 mg, Cadila Healthcare Limited, India and ‘PLENDIL® extended release tablets 10 mg (Felodipine extended release tablets 10 mg), Manufactured by: Merck & co., Inc., Whitehouse station, NJ, 08889, USA. Manufactured For: Astra Zeneca LP Wilmington DE 19850 are bioequivalent under fed conditions.