STUDY PROTOCOL

Protocol No: Pro/0408/006

Title: 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.

Test Product: Enalapril Maleate sustained release 20 mg
(Containing Enalapril Maleate 20 mg)
Cadila Pharmaceuticals Ltd., India

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

Version No: 01

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### LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AE</td>
<td>Adverse Events</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0-t&lt;/sub&gt;</td>
<td>Area under plasma concentration-time curve from time zero to last measurable concentration</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0-∞&lt;/sub&gt;</td>
<td>Area under plasma concentration-time curve from time zero to time infinity</td>
</tr>
<tr>
<td>BE</td>
<td>Bioequivalence</td>
</tr>
<tr>
<td>BP</td>
<td>Blood pressure</td>
</tr>
<tr>
<td>CC</td>
<td>Calibration Curve</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence Interval</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt;</td>
<td>Maximum plasma concentration</td>
</tr>
<tr>
<td>cm</td>
<td>Centimetre</td>
</tr>
<tr>
<td>CPL</td>
<td>Cadila Pharmaceuticals Limited</td>
</tr>
<tr>
<td>CPPU</td>
<td>Clinical Pharmacology and Pharmacokinetic Unit</td>
</tr>
<tr>
<td>CRF</td>
<td>Case Report Form</td>
</tr>
<tr>
<td>cu.mm</td>
<td>cubic millimetre</td>
</tr>
<tr>
<td>DBP</td>
<td>Diastolic Blood Pressure</td>
</tr>
<tr>
<td>ECG</td>
<td>Electrocardiogram</td>
</tr>
<tr>
<td>Ext</td>
<td>Extension</td>
</tr>
<tr>
<td>gm/dL</td>
<td>grams/ decilitre</td>
</tr>
<tr>
<td>GCP</td>
<td>Good Clinical Practice</td>
</tr>
<tr>
<td>gm</td>
<td>Gram</td>
</tr>
<tr>
<td>Hb</td>
<td>Haemoglobin</td>
</tr>
<tr>
<td>HBsAg</td>
<td>Hepatitis B surface Antigen</td>
</tr>
<tr>
<td>HCT</td>
<td>Hematocrit</td>
</tr>
<tr>
<td>HDL</td>
<td>High-density lipoprotein cholesterol</td>
</tr>
<tr>
<td>HIV</td>
<td>Human immunodeficiency virus</td>
</tr>
<tr>
<td>HPLC</td>
<td>High Performance Liquid Chromatography</td>
</tr>
<tr>
<td>HR</td>
<td>Heart Rate</td>
</tr>
<tr>
<td>hrs</td>
<td>Hours</td>
</tr>
<tr>
<td>ICF</td>
<td>Informed Consent Form</td>
</tr>
<tr>
<td>ICH</td>
<td>International Conference on Harmonization</td>
</tr>
<tr>
<td>ICU</td>
<td>Intensive Care Unit</td>
</tr>
<tr>
<td>IEC</td>
<td>Independent Ethics Committee</td>
</tr>
<tr>
<td>k&lt;sub&gt;el&lt;/sub&gt;</td>
<td>Apparent first order elimination</td>
</tr>
<tr>
<td>kg</td>
<td>Kilogram</td>
</tr>
<tr>
<td>LIC</td>
<td>Life Insurance Corporation of India</td>
</tr>
<tr>
<td>LCMS</td>
<td>Liquid Chromatography Mass Spectrometry</td>
</tr>
<tr>
<td>LOQ</td>
<td>Limit of Quantification</td>
</tr>
<tr>
<td>LSM</td>
<td>Least Square Means</td>
</tr>
<tr>
<td>Max</td>
<td>Maximum</td>
</tr>
<tr>
<td>MCV</td>
<td>Mean corpuscles volume</td>
</tr>
<tr>
<td>MCH</td>
<td>Mean corpuscles haemoglobin</td>
</tr>
</tbody>
</table>
**LIST OF ABBREVIATIONS (continued)**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCHC</td>
<td>Mean corpuscles haemoglobin concentration</td>
</tr>
<tr>
<td>mEq/L</td>
<td>Milli equivalents per litre</td>
</tr>
<tr>
<td>Min</td>
<td>Minimum</td>
</tr>
<tr>
<td>mm</td>
<td>Millimetre</td>
</tr>
<tr>
<td>MS/PC</td>
<td>Missing samples / Poor chromatography</td>
</tr>
<tr>
<td>PCV</td>
<td>Packed Cell Volume</td>
</tr>
<tr>
<td>PI</td>
<td>Principal Investigator</td>
</tr>
<tr>
<td>RBC</td>
<td>Red blood cells</td>
</tr>
<tr>
<td>RQA</td>
<td>Research Quality Assurance</td>
</tr>
<tr>
<td>rpm</td>
<td>Rotations per minute</td>
</tr>
<tr>
<td>SAE</td>
<td>Serious Adverse Event</td>
</tr>
<tr>
<td>SBP</td>
<td>Systolic Blood Pressure</td>
</tr>
<tr>
<td>SD</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>SGOT/AST</td>
<td>Serum glutamate oxaloacetate transaminase/ Aspartate Aminotransferase</td>
</tr>
<tr>
<td>SGPT/ALT</td>
<td>Serum glutamate pyruvate transaminase/ Alanine Aminotransferase</td>
</tr>
<tr>
<td>SOP</td>
<td>Standard Operating Procedure</td>
</tr>
<tr>
<td>t</td>
<td>Midpoint of each collection interval</td>
</tr>
<tr>
<td>t (_{1/2})</td>
<td>Apparent half-life</td>
</tr>
<tr>
<td>T(_{max})</td>
<td>Time to maximum plasma concentration/ Terminal order rate constant</td>
</tr>
<tr>
<td>VLDL</td>
<td>Very-low-density lipoprotein</td>
</tr>
<tr>
<td>WBC</td>
<td>White blood cells</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>WMA</td>
<td>World Medical Association</td>
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PROTOCOL SYNOPSIS

Title
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.

Study Objectives
1. To study comparative pharmacokinetic profile of immediate release Enalapril Maleate tablet 20mg with sustained release Enalapril Maleate Tablet 20mg
2. To estimate the duration of time of the plasma concentration of enalapril and enalaprilat above minimum effective concentration (for Enalaprilate: 10ng/ml to 50ng/ml) by single dose of Enalapril Maleate Sustained Release Tablet 20 mg
3. To study the food effect in Enalapril Maleate Sustained Release Tablet 20mg
4. To study the plasma levels of enalapril and enalaprilat in sustained release formulation and immediate release formulation during early morning hours (between 2:00 AM to 8:00AM)
5. To study the IVIVC of Enalapril Maleate Sustained Release Tablets 20mg

Secondary objective will be to monitor the safety of subjects.

Study Design
A randomized, open label, three treatment, parallel pharmacokinetics study of single dose of Envas 10 (2 tablets) (containing Enalapril Maleate 10 mg) or a single dose of Enalapril Maleate sustained release 20 mg tablet under fasting and fed condition allocated by randomization sheet.

The randomization scheme will not be made available to the subjects and analyst.

Screen Procedure
During screening procedure Demographic data, standard physical examination with Vital signs, Clinical laboratory tests on blood and urine samples, Electrocardiogram (ECG) and Chest X-ray will be done.

Sample Size
Eighteen (18) healthy, adult, male, human subjects.

Products

Test Product: Enalapril Maleate sustained release 20 mg
(Containing Enalapril Maleate 20 mg)
Cadila Pharmaceuticals Ltd. Ahmedabad

Reference Product: Envas 10 mg tablet
(Containing Enalapril Maleate 10 mg)
Cadila Pharmaceuticals Ltd. Ahmedabad
**Administration**

Single dose of 20 mg of test or reference product will be administered along with 240 ml of drinking water with allocated fasting and fed condition of participants in randomization sheet.

**Blood Sampling**

A total of 23 blood samples (5 mL each) will be collected, prior to drug administration (0.0) and at 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 3.0, 3.5, 4.0, 5.0, 6.0, 8.0, 12.0, 16.0, 18, 19, 20, 21, 22, 24.0, 36.0, and 48.0 hours thereafter.

The total volume collected per subject in this study will not exceed 140mL including 10 mL for screening, 5.0 mL for post study safety analysis and the 10 mL blood loss due to 0.5 mL discarded blood up to 24.0 hours.

**Admission and Stay**

Subjects will be housed in the Clinical Pharmacology and Pharmacokinetic Unit (CPPU) from at least 11 hrs before drug administration to 24 hrs after drug administration. Subjects will return to CPPU for ambulatory sampling at 36.0 and 48.0 hrs post-dose with a variation of ±60 minutes being accepted.

**Variables Assay**

Enalapril Maleate and its active metabolite Enalaprilat in plasma concentrations will be measured by a validated HPLC-UV / UPLC-PDA detection or LC-MS/MS method.

**Pharmacokinetic Parameters**

Pharmacokinetic analysis will be done on the plasma-concentration data of all the subjects who complete the study, obtained from bioanalytical laboratory using WinNonlin Pro-software Version 5.0.1, Pharsight Corporation, USA.

Following pharmacokinetic parameters will be calculated. Cmax, AUC0-t, AUC0-∞, Tmax, t1/2, and Kel

**Statistical Analysis**

Statistical analysis will be performed on the pharmacokinetic parameters using SAS, Statistical Software Version 9.1.3, SAS Institute. Inc., CARY, USA.

**Confidence Interval**

The confidence intervals are expressed as a percentage relative to the LSM of test to reference treatments. The confidence interval for ln-transformed pharmacokinetic parameters Cmax, AUC0-t and AUC0-∞ will be calculated.
1.0 BACKGROUNDS AND PHARMACOKINETICS

1.1 Drug description

Enalapril is the maleate salt of Enalapril, the ethyl ester of a long-acting angiotensin converting enzyme inhibitor, Enalaprilate. Enalapril is a pro-drug; following oral administration, it is bioactivated by hydrolysis of the ethyl ester to Enalaprilat, which is the active angiotensin converting enzyme inhibitor.

Enalapril maleate is chemically described as (S)-1-[N-[1-(ethoxycarbonyl) -3-phenylpropyl]-L-alanyl]-L-proline, (Z)-2-butenedioate salt (1:1). Its empirical formula is C_{20}H_{28}N_{2}O_{5}·C_{4}H_{8}O_{4}.

1.2 Mechanism of action

After oral administration of Enalapril, it hydrolysis to Enalaprilat, inhibits angiotensin-converting enzyme (ACE) in human subjects and animals. ACE is a peptidyl dipeptidase that catalyzes the conversion of angiotensin I to the vasoconstrictor substance, angiotensin II. Angiotensin II also stimulates aldosterone secretion by the adrenal cortex. The beneficial effects of Enalapril in hypertension and heart failure appear to result primarily from suppression of the renin-angiotensin-aldosterone system. Inhibition of ACE results in decreased plasma angiotensin II, which leads to decreased vasopressor activity and to decreased aldosterone secretion. Although the latter decrease is small, it results in small increases of serum potassium.

1.3 Contraindications

Enalapril is contraindicated in patients who are hypersensitive to this product and in patients with a history of angioedema related to previous treatment with an angiotensin converting enzyme inhibitor.

1.4 Indications and Usage

**Hypertension:** Tablets: Enalapril is indicated for the treatment of hypertension.

Enalapril is effective alone or in combination with other antihypertensive agents, especially thiazide-type diuretics. The blood pressure lowering effects of Enalapril and thiazides are approximately additive.

**Heart Failure:** Enalapril is indicated for the treatment of symptomatic congestive heart failure, usually in combination with diuretics and digitalis. In these patients Enalapril improves symptoms, increases survival, and decreases the frequency of hospitalization.

**Asymptomatic Left Ventricular Dysfunction:** In clinically stable asymptomatic patients with left ventricular dysfunction, Enalapril decreases the rate of development of overt heart failure and decreases the incidence of hospitalization for heart failure.

1.5 Dosages and Administration

**Hypertension:** In patients who are currently being treated with a diuretic, symptomatic hypotension occasionally may occur following the initial dose of Enalapril. The diuretic should, if possible, be discontinued for two to three days before beginning therapy with Enalapril to reduce the likelihood of hypotension.
The recommended initial dose in patients not on diuretics is 5 mg once a day. Dosage should be adjusted according to blood pressure response. The usual dosage range is 10 to 40 mg per day administered in a single dose or two divided doses.

Concomitant administration of Enalapril with potassium supplements, potassium salt substitutes, or potassium-sparing diuretics may lead to increases of serum potassium.

**Heart Failure:** Enalapril is indicated for the treatment of symptomatic heart failure, usually in combination with diuretics and digitalis. In the placebo-controlled studies that demonstrated improved survival, patients were titrated as tolerated up to 40 mg, administered in two divided doses.

The recommended starting dose is 2.5 mg. The recommended dosing range is 2.5 to 20 mg given twice a day. Doses should be titrated upward, as tolerated, over a period of a few days or weeks. The maximum daily dose administered in clinical trials was 40 mg in divided doses.

1.6 Absorption, Distribution, Metabolism and Excretion

Following oral administration of Enalapril, peak serum concentrations of Enalapril occur within about one hour. Based on urinary recovery, the extent of absorption of Enalapril is approximately 60 percent. Enalapril absorption is not influenced by the presence of food in the gastrointestinal tract. Following absorption, Enalapril is hydrolyzed to Enalaprilat, which is a more potent angiotensin converting enzyme inhibitor than Enalapril; Enalaprilat is poorly absorbed when administered orally. Peak serum concentrations of Enalaprilat occur three to four hours after an oral dose of Enalapril maleate. Excretion of Enalapril is primarily renal. Approximately 94 percent of the dose is recovered in the urine and feces as Enalaprilat or Enalapril. The principal components in urine are Enalaprilat, accounting for about 40 percent of the dose, and intact Enalapril. There is no evidence of metabolites of Enalapril, other than Enalaprilat.

The serum concentration profile of Enalaprilat exhibits a prolonged terminal phase, apparently representing a small fraction of the administered dose that has been bound to ACE. The amount bound does not increase with dose, indicating a saturable site of binding. The effective half-life for accumulation of Enalaprilat following multiple doses of Enalapril maleate is 11 hours.

1.7 Adverse Effect

**Body as a Whole:** Anaphylactoid reactions.

**Cardiovascular:** Cardiac arrest; myocardial infarction or cerebrovascular accident, possibly secondary to excessive hypotension in high risk patients; pulmonary embolism and infarction; pulmonary edema; rhythm disturbances including atrial tachycardia and bradycardia; atrial fibrillation; palpitation.

**Digestive:** Ileus, pancreatitis, hepatitis (hepatocellular (proven on rechallenge) or cholestatic jaundice), melena, anorexia, dyspepsia, constipation, glossitis, stomatitis, dry mouth.

**Musculoskeletal:** Muscle cramps.

**Nervous/Psychiatric:** Depression, confusion, ataxia, somnolence, insomnia, nervousness, peripheral neuropathy (e.g., paresthesia, dysesthesia).
**Respiratory:** Bronchospasm, rhinorrhea, sore throat and hoarseness, asthma, upper respiratory infection, pulmonary infiltrates.

**Skin:** Exfoliative dermatitis, toxic epidermal necrolysis, Stevens-Johnson syndrome, herpes zoster, erythema multiforme, urticaria, Pruritus, alopecia, flushing, diaphoresis, photosensitivity.

**Special Senses:** Blurred vision, taste alteration, anosmia, tinnitus, conjunctivitis, dry eyes, tearing.

**Urogenital:** Renal failure, oliguria, renal dysfunction flank pain, gynecomastia, impotence.

**Miscellaneous:**

A symptom complex has been reported which may include a positive ANA, an elevated erythrocyte sedimentation rate, arthralgia/arthritis, myalgia, fever, serositis, vasculitis, leukocytosis, eosinophilia, photosensitivity, rash and other dermatologic manifestations.

Angioedema: Angioedema has been reported in patients receiving Enalapril. Angioedema associated with laryngeal edema may be fatal. If angioedema of the face, extremities, lips, tongue, glottis and/or larynx occurs, treatment with Enalapril should be discontinued and appropriate therapy instituted immediately.

1.8 References

1.0 [www.Rxlist.com](http://www.Rxlist.com).
3.0 Therapeutic Drugs; second edition, edited by Dollery.

2.0 OBJECTIVES

1. To study comparative pharmacokinetic profile of immediate release Enalapril Maleate tablet 20mg with sustained release Enalapril Maleate Tablet 20mg
2. To estimate the duration of time of the plasma concentration of enalapril and enalaprilat above minimum effective concentration (for Enalaprilate: 10ng/ml to 50ng/ml) by single dose of Enalapril Maleate Sustained Release Tablet 20 mg
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3.0 STUDY DESIGN

3.1 Design

A randomized, open label, three treatment, parallel pharmacokinetics study of single dose of Envas 10 (2 tablets) (containing Enalapril Maleate 10 mg) or a single dose of Enalapril Maleate sustained release 20 mg tablet under fasting and fed condition allocated by randomization sheet

The randomization scheme will not be made available to the subjects and analyst.
3.1.1 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.

3.1.2 Number of Subjects

Eighteen (18) healthy, adult, male, Indian human subjects between the age group of 18-45 years will be enrolled in the study.

3.1.3 Blood samples

A total of 23 blood samples (5 mL each) will be collected, prior to drug administration (0.0) and at 0.25, 0.5, 0.75, 1, 1.5, 2.0, 3.0, 3.5, 4.0, 5.0, 6.0, 8.0, 12.0, 16.0, 18, 19, 20, 21, 22, 24.0, 36.0, and 48.0 hours thereafter.

The total volume collected per subject in this study will not exceed 140 mL including 10 mL for screening, 5.0 mL for post study safety analysis and the 10 mL blood loss due to 0.5 mL discarded blood up to 24.0.

3.1.4 Fasting/ Meals

Allocated fasting and fed condition of participants in randomization sheet subjects will be required to fast for at least 10 hrs (10 hrs fasting to be completed before test meal is served) and 30 minutes after start of high fat and high calorific test meal/ breakfast.

A standardized lunch, snack and dinner will be served to all the subjects at 4.0, 9.0, and 13.0 hrs, respectively, after dosing. A standardized high fat and high calorific test meal/ breakfast will be served 30 minutes before dosing. Non-compliance to 10 hrs pre-dose and 4 hrs post-dose fasting will be recorded as protocol deviation. Information on the amount of meals consumed will be recorded in the subjects “CRF”.

Drinking water will not be allowed from 1 hr before dosing until 2 hrs post-dose except while administration of the dose or as clinically indicated.

3.1.5 Housing

Subjects will be housed in the Clinical Pharmacology and Pharmacokinetic Unit (CPPU) from at least 11 hrs before drug administration to 24 hrs after drug administration. Subjects will return to CPPU for ambulatory sampling at 36.0 & 48.0 hrs post-dose with a variation of ±60 minutes being accepted.
3.2 Subject Selection and Restrictions

Healthy, adult, male, Indian human subjects will be selected on the following criteria

3.2.1 Screening Evaluation

Subjects will be screened within twenty one (21) days of the start of the study. The screening evaluation will consists of a complete medical history, clinical examination with vital signs, clinical laboratory evaluations 12-lead ECG and X-ray chest.

3.2.2 Inclusion Criteria

Subjects must fulfil all of the following criteria to be considered for inclusion into this study:

- Healthy, male, human subject aged from 18 to 45 years.
- Subject’s Body Mass Index (BMI) within normal limit of 18-25 kg/m^2.
- Willingness to sign statements of written informed consent form (for screening & study related procedures).
- No contraindications with the study medication with any previous medical or surgical history.
- Willingness to undergo pre- and post-study physical examinations and laboratory investigations.
- Normal general physical examination.
- Normal 12 lead ECG finding with PR intervals will be within normal range for heart rate and it will not exceed 190 msec during screening and study, or abnormalities which the Clinical Investigator/Physician does not consider a disqualification for participation in the study.
- Availability of subject for the entire study duration and willingness to adhere to protocol.
- Non-smokers or mild to moderate smokers (10 cigarettes/bidies/pipes, daily) and willing to discontinue smoking 48 hrs before initiation of study and during the study period.

3.2.3 Exclusion Criteria

The subjects will be excluded based on the following criteria:

- Subjects incapable of understanding the informed consent process/procedure.
- Evidence of psychiatric disorder, antagonistic personality, poor motivation, emotional or intellectual problems likely to limit the validity of consent to participate in the study, or limit the ability to comply with protocol requirements.
- History of hypotensive episodes, or systolic blood pressure reading of <100 mm Hg or a diastolic reading of <60 mm Hg at time of general Physical examination.
- Resting heart rate of >100 beats/min or <55 beats/min on the screening day.
- History of hypertension, or systolic blood pressure reading of >140 mm Hg or a diastolic reading >90 mm Hg at time of general Physical examination.
- The subject has any evidence of organ dysfunction or any clinically significant deviation from the normal, in physical or clinical determinations.
- Subject who have taken over the counter or prescribed medications, including any enzymes modifying (inducing or inhibiting) drugs or any systemic medication within the past four weeks prior to start of clinical period.
- The subject with known drug hypersensitivity or idiosyncratic reaction to Enalapril or any related drug.
- The subject with known history of clinically significant psychiatric or medical diseases or presence of cardiac, pulmonary, gastrointestinal, endocrine, neuromuscular, neurological, haematological, liver or kidney disease, or any condition know to interfere with the absorption, distribution, metabolism or excretion of drugs will be excluded from the study.
- History of or current alcohol abuse (>600 mL weekly) or history of exposure to other substance of abuse.
- Investigations with blood samples of the subject showing presence of disease marker of HIV 1 and 2, Hepatitis B & C viruses.
- Positive test for urinary screen testing of drugs of abuse (Amphetamines, Morphine, Benzodiazepines, Marijuana, Cocaine and Barbiturate).
- Investigations with blood sample of the subjects showing the presence of values which are clinically significantly different from normal reference range (defined as markedly abnormal values in Table-I) for haemoglobin, total white blood cells count, differential WBC count, platelet count and hematocrit values.
- Investigations with blood samples of the subjects showing the presence of values which are clinically significantly different from normal range (defined as markedly abnormal values in Table-I) for serum creatinine, blood urea, serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), serum alkaline phosphatase (ALP), serum bilirubin, plasma glucose (fasting), serum cholesterol.
- Investigations with urine sample of the subject showing clinically abnormal chemical and microscopic examination of urine defined as presence of RBC, WBC (>4/ HPF), epithelial cells (>4/ HPF), glucose (positive) and protein (positive) (unless Clinical Investigator/Physician considers the deviation to be irrelevant for the purpose of the study).
- Subject who participated in any other clinical investigation using experimental drug or had bleed more than 350 ml in the past 3 months.
- Xanthine-containing food or beverages and grape fruit or grape fruit juice consumption within 48 hrs prior to the study.
- Subject without adequate venous access in their left or right arm to allow collection of all samples via venous cannula during study period.
- X-ray chest finding suggesting of any abnormality/ies like cardiomegalia, pneumonia etc.

**Principal Investigator may withdraw a subject from the study for any of the following:**

- If subject suffers from significant inter-current illness or undergoes surgery during the course of study.
- If the subject is found to have entered the study in violation of this protocol.
- If subject requires any concomitant medication, which may interfere with the pharmacokinetic property of study medication.
- If it is felt in the investigator's opinion that it is not in the subject's best interest to continue.
- Subject on his own, wishes to withdraw consent.
- If the subject experiences adverse event when withdrawal would be in the best interest of the subject.
- Emesis occurs at or before 2 times median Tmax.

Any subject withdrawal during study along with reason thereof shall be documented. Subjects, who dropout/withdrawn from the study will not be replaced.

**3.2.4 Prohibitions**

**3.2.4.1 Smoking**

Subjects will be instructed not to smoke for at least 48 hrs before dosing and will be prohibited from smoking throughout their stay at CPPU.

**3.2.4.2 Medications**

Subjects will be asked about their medication history in past four weeks from screening date and will be instructed not to take any medication (either prescribed or OTC) from the date of screening till completion of the study.

If drug therapy other than that specified in the protocol is required prior to or during the study, decisions shall be taken by the Principal Investigators to continue or discontinue the subjects based on the following:

- Pharmacology and pharmacokinetics of the non-study medication.
- Likelihood of a drug-drug interaction, thereby affecting pharmacokinetics completion of the study medication.
- Time and duration of administration of the non-study medication.
3.2.4.3 Diet

All subjects will be asked to abstain from any xanthine-containing food or beverages (tea, coffee, chocolates, soft drinks like cola etc.) or alcoholic products for at least 48 hrs prior to dosing and will be prohibited from consuming above mentioned products, during their stay in the CPPU.

3.3 Clinical Procedures

3.3.1 Dosing

Prior to drug administration, the Research Quality Assurance in charge will verify the packing of the study medication and the randomization schedule.

After an overnight fast of at least 10 hours, subjects will receive single oral dose of test or references product with 240 mL of water at room temperature. Subjects would be instructed not to chew or crush the drug. Compliance will be assessed by conducting a thorough examination of the oral cavity using flashlight and spatula by trained study personnel after dosing and by measurement of drug concentration in plasma (during the analytical phase of the study). All subjects will be dosed at the fixed time and will be required to remain in sitting position for the first 2 hours following drug administration. Subjects report to facility for ambulatory sample and will be advised to avoid severe physical exertion during study period. First 2 hours post dose samples will be collected at bedside and remaining blood samples will be taken at sample collection area. The order of administration of the test and reference products to each subjects will be determined according to the randomization scheduled prepared at CPL, Dholka. The randomization will be balanced and the code will be kept under controlled access.

3.3.2 Blood Sampling

Blood samples will be withdrawn by an indwelling cannula placed in a forearm vein or fresh clean venipuncture using a disposable sterilized syringe and a needle in case of clotting of cannula. Blood samples will be collected in pre-labelled (label mentioning study number, subject number, period and sampling time point) test tubes containing K$_2$EDTA as an anticoagulant and at the times specified under Study Design Section.

The pre-dose blood sample will be collected within a period of 1 hr before dosing and post-dose samples will be within ±2 minutes of the scheduled time till 24.0 hour blood sample. Subjects will return to CPPU for ambulatory sampling at 48.0 hrs post-dose with a variation of ±60 minutes being accepted. The actual mid-point time of collection of each blood sample (to the nearest minute) will be recorded on the appropriate data sheet.

After collection, blood samples will be centrifuged with the help of cooling centrifuge at 4°C as soon as possible but not more than 30 minutes to separate plasma at 3000 rpm for 10 minutes. All plasma samples will be properly labelled and stored at –20°C or colder, till analysis.

3.3.3 Activity Restrictions

All subjects will be dosed at the fixed time and advised to remain sitting position for the first 2 hrs following drug administration.
3.3.4 Supplies

The drug products will be received by the Principal Investigator (PI) or a suitable designate from the concerned departments/ sponsors. Reference products will be supplied in the original manufacturer’s packing and the test products will be supplied with information like product name, strength, number of dosage units, manufacturer, lot number or batch number, expiry date in an appropriate package deemed to maintain the integrity of the products. The products may be briefly stored at the clinical facility under prescribed storage conditions. At the clinical facility, the drug products will be logged-in by the study monitor or a suitable designate and stored under prescribed storage conditions in a controlled access area. The Principal Investigator will be accountable for the study drug products. Study drugs will be dispensed according to the randomization schedule.

3.4 Safety Procedures

3.4.1 Medical Surveillance

A physician will be available on site through out the study period. A trained paramedic or physician will take the vitals prior to and after dosing as specified under section 3.4.4.

3.4.2 Clinical Laboratory Tests

**Haematology:** Haemoglobin, PCV, MCV, MCH, MCHC, RBC count, WBC count, Differential Leukocyte count and Platelet count.

**Blood Chemistry:** ALT, AST, Alkaline Phosphatase, Total Bilirubin, Total Protein, Blood Glucose, Urea, Creatinine, Serum albumin, Triglyceride, Cholesterol and Uric Acid.

**Urine analysis:** Colour, specific gravity, pH, Transparency, Protein, Ketoses, Glucose, Bilirubin, Blood, Urobilinogen and Microscopic examination.

**Serology:** For Hepatitis B & C, HIV 1 and 2.

**Urine Drugs Screen:** Urine Drug screen for Amphetamines, Morphine, Benzodiazepines, Marijuana, Cocaine and Barbiturates. Test for Drugs of abuse will be done before admission into the study.

The normal ranges of laboratory test-values utilized are in Table 1.

Hematology, blood chemistry, urine analysis and serology test will be carried out at pathology laboratory of Cadila Pharmaceuticals Ltd. Dholka. Or Ashish Pathology Laboratory, Ahmedabad.

In the event of unexplained or unexpected laboratory test investigation values abnormalities during or post study, the tests will be repeated and followed-up until the results returned to normal. The investigator should indicate on the laboratory test page of the CRF all laboratory test values, which are potentially clinically significant. These clinically significant abnormal laboratory results values should then be described and the relationship to treatment, if any, should be indicated.
3.4.3 Clinical Examination

Clinical examination will be done by the physician during screening, check in, check out and whenever required by physician during adverse event.

3.4.4 Vital Signs

Sitting blood pressure, radial pulse rate and oral temperature will be assessed at the pre-study screening. Further measurements of vitals including BP, pulse rate and oral temperature will be performed at the following time points: before check-in time, prior to administration of study drug and at 1.0, 2.0, 4.5, 6.0, 8.0, 12.0 and 24.0 hrs (check-out time).

3.4.5 ECG

A 12-lead ECG will be recorded at the pre-study screening after the subjects have been in the supine position for at least 5 min.

3.4.6 Eligibility Assessments

The following assessments will be conducted before entry of the subjects into the study:
- Demography- Gender, height, age, date of birth.
- Vital signs- Sitting Blood pressure, radial pulse and oral temperature.
- Alcohol and tobacco consumption patterns.
- Study participation in last 3 months.
- Allergies and Medication history.
- Current medication usage and concomitant therapy in the previous 30 days.
- Medical history and current status.
- The subject’s status as a healthy volunteer will be confirmed.
- A standard physical examination will be conducted. Clinically significant findings will be documented.
- Clinical laboratory tests on blood and urine samples.
- Electrocardiogram (ECG)
- Chest X-ray (PA view)

3.4.7 Trial Assessments

The following will be recorded during the conduct of the study:
- Times of dose administration.
- Target and actual blood sampling times
- Concomitant therapy changes
- Adverse events as described below
- Vital signs

3.4.8 Adverse Drug Reactions (ADR) Monitoring

Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse event (AE) can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding). Symptom or disease temporally associated with the
use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

The severity of the adverse events will be graded on a three-point scale as follows:

- **Mild:** Discomfort noticed but no disruption of normal daily activity
- **Moderate:** Discomfort sufficient to reduce or affect normal daily activity
- **Severe:** Inability to work or perform normal daily activity

The relationship of the adverse events encountered during the study will be reported in the CRF.

The study physician at Clinical Research Division would do the emergency management of all ADR’s. SAE requiring immediate transfer to a referral hospital would be done with the help of the on-call ambulance to the nearest specialty hospital (Sharda Hospital which is 8 km away from CPPU, Cadila Pharmaceuticals Ltd.)

### 3.4.8.1 Immediately Reportable Adverse Events

Any adverse event that is serious (including death or overdose) occurring during the course of the study, irrespective of the treatment received by the subject, must be reported to the sponsor and chairman, IEC immediately within 24 Hrs, by Phone/ Mail/ or Fax; followed by a detailed report within 07 working days by the Principle Investigator. If the sponsor is located outside India, the detailed report will be submitted within 14 working days, though summarized intimation will be done within 48 Hrs by Phone/ Mail/ or Fax. Any death or Serious Adverse Event will be reported to regulatory authorities.

A serious adverse event is any experience that suggests a significant hazard, contraindication, side effect or precaution. With respect to human clinical experience, this includes any experience which:

- Results in death
- Is life-threatening
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity (as per reporter’s opinion)
- Is a congenital anomaly/birth defect
- Other medically important condition

**Emergency Telephone Numbers:**

- **Dr. Bhaswat S. Chakraborty, B. Pharm., Ph.D.**
  Phone Nos. (O) 02714- 220312/221481/83/84 Ext No: 107

- **If you have any experience or study related enquiry, you may contact:**
  **Dr. Anil Patel, M.B.B.S., M.D.**
  Phone Nos. (O) 02714- 221481/83/84 Ext No: 180

- **Dr.R. M. Shah M.D.**
  Phone Nos. (O) 079-26576160
  (R) 079-26620290
3.4.9 Concomitant Medication and Treatment

During the course of the study, no other medications are allowed, if applicable unless, for the treatment of adverse events. If any treatment for adverse events becomes necessary, the medication(s) details will be reported noted on the adverse event section of the Case Report Forms including generic name, indication, total daily dose, route and time/duration of administration.

4.0 ANALYTICAL METHOD

Blood samples will be analyzed for the quantification of Enalapril and Enalaprilat concentrations in plasma using Liquid Chromatography with Mass Spectrometry (LC-MS/ MS) procedures developed at Cadila Pharmaceuticals Ltd., Ahmedabad. All subject samples will be analysed who have completed whole study and the subject who dropout or withdrawal (except by adverse events) from the study will not be analyzed.

The analysis of subject’s samples will be done using a calibration curve with quality control samples, distributed throughout each batch. The details for the preparation of the calibration curve and quality control samples and the analytical batch acceptance criteria will be discussed in the respective in-house procedure. Samples with drug concentrations greater than the upper limit of the validated range of the assay will be diluted with the appropriate drug-free biological fluid and re-assayed for those which are below the lower limit of this range will be reported as below LOQ. Repeat assays will be done, if required after proper justification and documentation. The analyst will not have access to the randomization schedule until analysis is completed.

Analytical results will be represented in tabular form in the final report and chromatographic and derived data will also be provided. Accuracy, precision and linearity data for each standard curve and all quality control samples will be presented. Representative chromatograms and standard curve graphs will be included in the final report.

All concentration values below the limit of quantification will be set to zero for all pharmacokinetic and statistical evaluation. Any missing samples will be reported as 'MS' and all unreportable concentration values due to poor chromatography will be reported as 'PC' and will not be included for pharmacokinetic and statistical analysis.
4.1 Pharmacokinetic Analysis

The following Pharmacokinetic parameters for Enalapril and Enalaprilat will be computed using non-compartmental model of WinNonlin Professional Software Version 5.0.1., Pharsight Corporation, USA:

- **Cmax**: Peak or maximum plasma concentration
- **AUC\(_{0-t}\)**: The area under the plasma concentration versus time curve from time zero to the last measurable concentration as calculated by linear trapezoidal method.
- **AUC\(_{0-\infty}\)**: The area under the plasma concentration versus time curve from time zero to infinity. Where \( AUC_{0-\infty} = AUC_{0-t} + C_t / Kel \), \( C_t \) is the last measurable concentration and \( Kel \) is the terminal elimination rate constant.
- **Tmax**: Time of the maximum measured plasma concentration. If the maximum value occurs at more than one time point, \( Tmax \) is defined as the first time point with the value.
- **Kel**: First order rate constant associated with the terminal (log-linear) portion of the curve. This is estimated via linear regression of time vs. log concentration.
- **t\(_{1/2}\)**: The elimination or terminal half-life will be calculated as \( 0.693 / Kel \).

For all the above computations, actual time points of the blood sample collection (samples collected before or after scheduled time) will be used for pharmacokinetic and statistical analysis.

5.0 STATISTICAL METHOD

Statistical analysis will be performed on the pharmacokinetic parameters using SAS, Statistical Software Version, 9.1.3, SAS Institute Inc., CARY, USA.

Summary Statistics

Mean, minimum, maximum, standard deviation and coefficient of variation will be calculated for plasma time concentration and pharmacokinetic parameters (Cmax, Tmax, AUC\(_{0-t}\), AUC\(_{0-\infty}\), Kel and t\(_{1/2}\)) of Enalapril and Enalaprilat. In addition, following statistical information will be provided for AUC\(_{0-t}\), AUC\(_{0-\infty}\) and Cmax for Enalapril and Enalaprilat:

(i) Geometric Mean
(ii) Ratios of Mean
(iii) 90% Confidence Intervals
5.1 Analysis of Variance

The ln-transformed pharmacokinetic parameters Cmax, AUC₀–t and AUC₀–∞ for Enalapril and Enalaprilat will be statistically analysed using SAS, Statistical Software Version, 9.1.3 SAS Institute Inc., CARY, USA. The factors included in this model will be the treatment received, and the subject effect nested within the treatment. Each analysis of variance will include calculation of least square mean (LSM). Two one-sided “t” test at 5% level of significance will be used to compare the average values of pharmacokinetic parameters determined after administration of test and reference products.

5.2 Confidence Intervals

Consistent with the two one-sided “t” tests for bioequivalence, 90% Confidence Interval for the difference between treatments least-square means will be calculated for ln-transformed pharmacokinetic parameters Cmax, AUC₀–t and AUC₀–∞. Confidence Intervals are expressed as a percentage relative to the LSM of the reference treatments.

5.3 Ratio Analysis

Ratio of Least Square Means of test and reference formulation will be computed for ln-transformed pharmacokinetic parameters Cmax, AUC₀–t and AUC₀–∞. Ratio analysis will be reported for ln-transformed pharmacokinetic parameters Cmax, AUC₀–t and AUC₀–∞ for Enalapril and Enalaprilat.

5.4 Power Test

Power of the study will be calculated and reported in the report.

5.5 Criteria for Exclusion of Subjects from Data Analysis

All subjects will be included for data analysis, who complete the study. However, in case of significant violation of inclusion or exclusion criteria affecting the results of the pharmacokinetics analysis, or if Cmax, AUC₀–t and AUC₀–∞ cannot be estimated, the subject might be excluded from the pertaining pharmacokinetics (Pk) analysis if the exclusion is justified.

Data from the subjects who vomit at or below 2 times median Tmax during the course of study will not be considered for statistical analysis. If a pharmacokinetic parameter cannot be determined, the corresponding subject will be excluded for that particular statistical comparison.

Statistical outliers are detected using Lund’s test. If the outlier is proved then data and results with and without outlier will be reported.
6.0 FACILITIES

6.1 Clinical, Pharmacokinetic and Statistical Services

Department of Clinical Pharmacology
Research and Development Centre
Cadila Pharmaceuticals Ltd.,
1389 Dholka – Trasad Road,
Dholka – 387 810
Ahmedabad, India

6.2 Bio analytical Service

Research and Development Centre
Cadila Pharmaceuticals Ltd.,
1389 Dholka – Trasad Road,
Dholka – 387 810
Ahmedabad, India

6.3 Pathology Laboratories

Pathology Laboratory
Research and Development Centre
Cadila Pharmaceuticals Ltd.,
1389 Dholka – Trasad Road,
Dholka – 387 810
Ahmedabad, India

6.4 Waste Management and Disposal

E-Coli Waste Management System
Ahmedabad, India

6.5 Quality Assurance

Research Quality Assurance
Cadila Pharmaceuticals Ltd.,
1389 Dholka – Trasad Road,
Dholka – 387 810
Ahmedabad, India

6.6 Radiology Services

Hi-Tech Imaging Centre,
Dholka, Ahmedabad
Phone: (02714220115)
7.0 REGULATORY AND ETHICAL CONSIDERATIONS

7.1 Basic Principles

The study will be carried out in accordance with the provisions of the current version of the ICH-Good Clinical Practice and the principle enunciated in the Declaration of Helsinki (WMA General Assembly, Tokyo, Japan 2004) and Directive 2001/20/EC.

7.2 Independent Ethics Committee

This protocol and Informed Consent Form (ICF) used to obtain informed consent of study subjects will be reviewed by an IEC. The study will commence only after obtaining the written approval of the study protocol by the IEC, with or without modifications.

7.3 Written Informed Consent Form

Medical person or with his/her presence, a designated clinical research personnel will be give information regarding study to the subjects in verbal and written form. Informed consent will be given in language, which is understandable to the subject (English/ Gujarati translation of ICF/ any other) before initiation of the study through an oral presentation regarding the purpose, procedures to be carried out, potential hazards and rights of the subjects during the course of the study (APPENDIX II). If subject is illiterate or is unable to read, medical person must explain the consent, point by point in the presence of an impartial witness.

Subject should be provided with enough time and opportunity to read consent form. The entire question should be answer prior to signing the ICF. When medical person obtaining ICF is satisfied that the subject is fully informed and understands what study participation entails, ask the subject for participation in the study. Subjects will be required to understand and sign the consent forms, after they have been asked to summarize the discussion held during administration of the ICF. One Copy of signed ICF of each subject will be retained at CPL-CRO and second copy will be given to subject.

7.4 Drug Accountability

Drug store in-charge of Clinical Pharmacology and Pharmacokinetic Unit, Cadila Pharmaceuticals Ltd. will maintain the record of the total medication received, storage conditions, and quantity of drug used and retained.

7.5 Confidentiality

The data identifying each study subject by name will be kept confidential and will be accessible only to the study personnel, Research Quality Assurance auditor during audits and if necessary, to Sponsor, IEC and various regulatory authorities.

7.6 Subjects Compensation

The subjects will be paid an adequate participation fees for the inconvenience caused due to their participation in the study. In case of drop-out/ withdrawal of a subject before completion of the study decision of IEC on participation fees of the withdrawn subjects will be final and will be binding on both, Cadila Pharmaceuticals Ltd. and the study subjects.
7.7 Study Documentation

All clinical data generated during the conduct of the study will be entered in the respective CRF’s. The computer-generated chromatograms will also be treated as raw data.

- Study protocol, with Amendments if any
- Written signed and dated ICF from each subject
- Completed CRF of each subject along with trial master file and with all source documents
- Drug Accountability Records
- Study correspondence and
- Chromatograms

7.8 Protocol Deviations and Amendments

Subjects must adhere to the protocol and instructions as stated in the subject ICF. Deviations from this protocol if any, during the study with reasons there of will be informed to the ethics committee and will be mentioned in the final report.

All amendments that affect subject safety or the study integrity must be approved by the IEC. Significant changes in any portion of the protocol made after approval will need to obtain IEC approval again prior to the start of the study and will be termed and documented as Protocol Amendments (Appendix I). A list of changes with reference to the previous version will be generated and included in the study report.

7.9 Source Data Accessibility

Sponsor, IEC and Regulatory Agencies will have the access to the CRF’s and other source documents during inspection and audits.

7.10 Quality Assurance Audits

The raw data generated during the course of the study, including the clinical operations and the final reports will be liable for inspection and quality audit for conformance to this protocol and all the governing SOPs by an auditor from the Research Quality Assurance Division of Cadila Pharmaceuticals Ltd.

During audit, Research Quality Assurance officers of Cadila Pharmaceuticals Ltd. will retrospectively check the data generated during the study.

7.11 Subject’s Insurance

The clinical trial liability Insurance is provided by The Oriental Insurance Co. Ltd., Divisional Office No. 5, II Floor, Amrut Jayanti, Ashram Road, Ahmedabad for the subject(s) for any study drug related injury or any health related problem/s caused by participation in the study.

Insurance Policy No.: 141500/48/2009/1613
Insurance Policy Period: 21-08-2008 to 20-08-2009
8.0 ARCHIVAL OF DATA

All the raw and final data generated in connection with this study and one copy of the final report will be retained until up to 15 years in the archives.

9.0 LIST OF TABLE AND APPENDICES

<table>
<thead>
<tr>
<th>TABLE</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>TABLE-IA</td>
<td>Clinical Safety Laboratory Evaluation Parameters</td>
</tr>
<tr>
<td>TABLE-IB</td>
<td>Clinical Safety Laboratory Parameter Abnormality Range</td>
</tr>
<tr>
<td>Appendix I</td>
<td>Study Event Flow Chart</td>
</tr>
</tbody>
</table>
### TABLE- 1A

**CLINICAL SAFETY LABORATORY EVALUATION PARAMETERS**

#### HAEMATOLOGICAL PARAMETERS

<table>
<thead>
<tr>
<th>SR. NO.</th>
<th>PARAMETER</th>
<th>UNITS</th>
<th>NORMAL VALUES</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Male</td>
</tr>
<tr>
<td>01</td>
<td>Total W.B.C.</td>
<td>103/mm³</td>
<td>3.54-9.06</td>
</tr>
<tr>
<td>02</td>
<td>Total R.B.C.</td>
<td>106/mm³</td>
<td>4.30-5.60</td>
</tr>
<tr>
<td>03</td>
<td>Haemoglobin</td>
<td>g/dl</td>
<td>13.3-16.2</td>
</tr>
<tr>
<td>04</td>
<td>HCT (PCV)</td>
<td>%</td>
<td>38.8-46.4</td>
</tr>
<tr>
<td>05</td>
<td>MCV</td>
<td>µ m³</td>
<td>79-93.3</td>
</tr>
<tr>
<td>06</td>
<td>MCH</td>
<td>pg/cell</td>
<td>26.7-31.9</td>
</tr>
<tr>
<td>07</td>
<td>MCHC</td>
<td>g/dl</td>
<td>32.3-35.9</td>
</tr>
<tr>
<td>08</td>
<td>Neutrophils</td>
<td>%</td>
<td>40-70</td>
</tr>
<tr>
<td>09</td>
<td>Lymphocytes</td>
<td>%</td>
<td>20-50</td>
</tr>
<tr>
<td>10</td>
<td>Eosinophils</td>
<td>%</td>
<td>0-6</td>
</tr>
<tr>
<td>11</td>
<td>Monocytes</td>
<td>%</td>
<td>4-8</td>
</tr>
<tr>
<td>12</td>
<td>Basophils</td>
<td>%</td>
<td>0-2</td>
</tr>
<tr>
<td>13</td>
<td>Platelets</td>
<td>103/mm³</td>
<td>165-415</td>
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#### BIOCHEMICAL PARAMETERS

<table>
<thead>
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<th>Sr. No.</th>
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<th>REFERENCE INTERVALS</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Male</td>
</tr>
<tr>
<td>01</td>
<td>Blood Glucose</td>
<td>mg/ dL</td>
<td>75-110</td>
</tr>
<tr>
<td>02</td>
<td>Triglyceride</td>
<td>mg/ dL</td>
<td>30-200</td>
</tr>
<tr>
<td>03</td>
<td>Cholesterol</td>
<td>mg/ dL</td>
<td>Upto 200</td>
</tr>
<tr>
<td>04</td>
<td>Urea</td>
<td>mg/ dL</td>
<td>7-20</td>
</tr>
<tr>
<td>05</td>
<td>Creatinine</td>
<td>ng/ dL</td>
<td>0.6-1.2</td>
</tr>
<tr>
<td>06</td>
<td>SGOT (ALT)</td>
<td>U/L</td>
<td>12-38</td>
</tr>
<tr>
<td>07</td>
<td>SGPT (AST)</td>
<td>IU/L</td>
<td>7-41</td>
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<tr>
<td>08</td>
<td>Alkaline Phosphatase (ALP)</td>
<td>µ/L</td>
<td>13-100</td>
</tr>
<tr>
<td>09</td>
<td>Total Bilirubin</td>
<td>mg/ dL</td>
<td>0.3-1.3</td>
</tr>
<tr>
<td>10</td>
<td>Total Protein</td>
<td>g/ dL</td>
<td>6.7-8.6</td>
</tr>
<tr>
<td>11</td>
<td>Uric acid</td>
<td>mg/ dL</td>
<td>3.1-7.0</td>
</tr>
<tr>
<td>12</td>
<td>S.Albimin</td>
<td>g/ dL</td>
<td>3.5-5.5</td>
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<tr>
<td>13</td>
<td>S.Sodium</td>
<td>meq/L</td>
<td>136-148</td>
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<tr>
<td>14</td>
<td>S.Chloride</td>
<td>meq/L</td>
<td>102-109</td>
</tr>
<tr>
<td>15</td>
<td>S.Calcium</td>
<td>mg/ dL</td>
<td>8.7-10.2</td>
</tr>
<tr>
<td>16</td>
<td>S.Potassium</td>
<td>meq/L</td>
<td>3.5-5.0</td>
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**SEROLOGY**

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<tr>
<th>SEROLOGY SCREENING</th>
<th>INDICATION</th>
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<tbody>
<tr>
<td>HIV-1 &amp; 2</td>
<td>HIV ANTIBODIES, AIDS</td>
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<tr>
<td>HBs Antigen &amp; HCV</td>
<td>HEPATITIS B &amp; HEPATITIS C VIRUS</td>
</tr>
</tbody>
</table>

**URINE ANALYSIS**

<table>
<thead>
<tr>
<th>PARAMETER</th>
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<tbody>
<tr>
<td><strong>Physical Examination</strong></td>
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<td>Colour</td>
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<tr>
<td>Odour</td>
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</tr>
<tr>
<td>Deposit</td>
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</tr>
<tr>
<td>Clarity</td>
<td></td>
</tr>
<tr>
<td>Reaction</td>
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<tr>
<td>Sp. Gravity</td>
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<td>Transparency</td>
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<tr>
<td><strong>Chemical Examination</strong></td>
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<tr>
<td>pH</td>
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<tr>
<td>Albumin</td>
<td></td>
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<tr>
<td>Bile Salts</td>
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<tr>
<td>Bile Pigments</td>
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<tr>
<td>Ketones</td>
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<tr>
<td>Protein</td>
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</tr>
<tr>
<td>Glucose</td>
<td></td>
</tr>
<tr>
<td>Bilirubin</td>
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<td>Occult Blood</td>
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<td>Urobilinogen</td>
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<td><strong>Microscopic Examination</strong></td>
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<tr>
<td>Pus Cells</td>
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<td>Red Cells</td>
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<td>Epithelial Cells</td>
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<tr>
<td>Casts</td>
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<tr>
<td>Crystals</td>
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<td>Amorphous</td>
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<td>Bacteria</td>
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## TABLE- 1B

### CLINICAL SAFETY LABORATORY PARAMETER ABNORMALITY RANGE

<table>
<thead>
<tr>
<th>Laboratory test</th>
<th>Clinically relevant direction*</th>
<th>Abnormality ≥%</th>
<th>Marked abnormality ≥ (%)</th>
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<tbody>
<tr>
<td><strong>HAEMATOLOGICAL PARAMETER</strong></td>
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<tr>
<td>HGB</td>
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<td>25</td>
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<tr>
<td>HCT</td>
<td>Decrease</td>
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<td>25</td>
</tr>
<tr>
<td>RBC</td>
<td>Either</td>
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<td>25</td>
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<tr>
<td>Platelets</td>
<td>Decrease</td>
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<td>25</td>
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<tr>
<td>WBC</td>
<td>Either</td>
<td>10</td>
<td>25</td>
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<tr>
<td>Neutrophil</td>
<td>Either</td>
<td>10</td>
<td>25</td>
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<tr>
<td>Eosinophil</td>
<td>Increase</td>
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<td>50</td>
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<tr>
<td>Basophil</td>
<td>Increase</td>
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<td>50</td>
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<tr>
<td>Lymphocyte</td>
<td>Either</td>
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<td>25</td>
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<tr>
<td>Monocyte</td>
<td>Increase</td>
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<td>50</td>
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<tr>
<td><strong>BIOCHEMICAL PARAMETER</strong></td>
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<td>Total Bilirubin</td>
<td>Increase</td>
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<td>50</td>
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<tr>
<td>Total Protein</td>
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<td>05</td>
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<tr>
<td>SGOT</td>
<td>Increase</td>
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<td>SGPT</td>
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<td>Blood Urea</td>
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<td>Creatinine</td>
<td>Increase</td>
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<td>50</td>
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<tr>
<td>Glucose (Fasting)</td>
<td>Either</td>
<td>10</td>
<td>50</td>
</tr>
</tbody>
</table>

* Only clinically relevant direction of abnormal value is considered and percent limits are applied to the limit on normal range closest to that abnormal value.

Source: Drug safety assessment in clinical trials. Edited by Gilbert GS.
Marcel Dekker, Inc., USA. Pages 222-223
APPENDIX-I

STUDY EVENT FLOW CHART

The following schedule will be observed for the study

<table>
<thead>
<tr>
<th>Study Events</th>
<th>Screening</th>
<th>Study Days in Period I</th>
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<tbody>
<tr>
<td>Activity</td>
<td>Within 21 days before dosing</td>
<td>Day-0 (Check-in)</td>
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<tr>
<td>Informed Consent for Screening</td>
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<td>Day-1 (Dosing)</td>
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<tr>
<td>Demographic Data</td>
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<td>Day 2 (Check-out)</td>
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<td>Drug Allergy/ Medication/</td>
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<td>Post Study Safety Analysis</td>
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<tr>
<td>Medical Histories</td>
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<tr>
<td>General Physical Examination</td>
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<tr>
<td>Vital Signs</td>
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<tr>
<td>ECG (12-lead)</td>
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<tr>
<td>X-ray chest</td>
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<td>Haematology</td>
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<tr>
<td>Serum biochemistry</td>
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<td>Serology</td>
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<td>Urine analysis</td>
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<tr>
<td>Informed Consent for Study</td>
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<td>Urine Drug Screen</td>
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<td>Record of Concomitant Medication</td>
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<tr>
<td>Check-in procedure</td>
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<tr>
<td>Housing in CPPU</td>
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<tr>
<td>PK blood sampling</td>
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<tr>
<td>Check-out procedures</td>
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</tr>
</tbody>
</table>

1. Subject will be housed in the CPPU from at least 11 hrs prior dosing to 24 hrs post dose.
2. Measurements of vitals including BP, pulse rate will be performed at the following time points: before check-in time, prior to administration of study drug and 1.0, 2.0, 4.5, 6.0, 8.0, 12.0 and at check-out time.
3. Urine drug screen test and alcohol breath test will be done during check-in time.
4. Blood samples will be taken prior to drug administration (0.0) followed by post-dose samples at different time points as per specified in protocol.