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Review Article

Review On Comparative Oral Bioavailability Studies

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ABSTRACT

The registered chemist prepared research dosages according to the randomization schedule in the presence of quality assurance staff, under the guidance of the trained study staff. Investigational products were dispensed and then put into containers with the proper labels. To ensure proper administration of investigational products, an additional label was made available for affixing to each administration form for investigational products. As retention samples, the remaining investigational goods were kept in their original packaging. The doses of the supplied experimental products were transferred to the containers used for dispensing investigational products.

Key Words: *Bioavailability Studies, Bioequivalence, Biomedical Research.*

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INTRODUCTION

Bioavailability [1]

Rate and extent to which the active pharmaceutical ingredient is absorbed from the pharmaceutical dosage form and becomes available at the site of Action in the general circulation.

Bioequivalence [1]

Two pharmaceutical products are bio-equivalent if they are pharmaceutical Equivalent, and their biovailability in terms of peak (C_{max}, T_{max}) and total Exposure (AUC) after administration of same molar dose under same Conditions are similar

Criteria for Bioequivalence [2]

The 90 % confidence interval for C_{max}, AUC(0-t) and AUC(0-∞) of telmisartan will form the basis for concluding the bioequivalence of telmisartan in product R and T. If the confidence intervals are entirely included in the range of 80.00 – 125.00 % for C_{max}, AUC0-t and AUC0-∞ log-transformed then the products will be claimed to be bioequivalent.

Reasons for conducting BE studies [2]

- New formulation of a medicine is chosen to replace an old formulation (e.g. new excipients or new salt of the medicine are used).
- New dosage form of a medicine replaces an older one (e.g. tablets replace capsules) or extends the product line.
- New physical characteristics of a medicine product exists (e.g. increased density of a tablet due to higher compression used during manufacturing).
- An older supply of the medicine is to be compared with newer one in order to determine the effect of age and natural changes in the overall composition.
- Two lots of an active medicine are compared in which differences are shown to exist.
- A new manufacturing method is used to prepare the medicine or a new manufacturing site is preparing the medicine and question of bioequivalence is raised.
- A new manufacturer (may be a generic manufacturer or a new supplier of a brand name product) of a medicine desires to compare the new product with a standard.

General principles: [3]

These 12 principles laid down under General Principles are common to all areas of biomedical research. Any research using the human beings as participants shall follow the principles given below-

I. Principles of essentiality whereby the research entailing the use of human participants is necessary for the advancement of knowledge and for the benefit of all members of the human species and for the ecological and environmental well being of the planet.

II. Principles of voluntariness, informed consent and community agreement whereby research participants are fully apprised of the research and the impact and risk of such research on the research participant and others.

III. Principles of non-exploitation whereby as a general rule, research participants are remunerated for their involvement in the research or experiment. Each research shall include an in-built mechanism for compensation for the human participants.

IV. Principles of privacy and confidentiality whereby the identity and records of the human participants of the research or experiment are as far as possible kept confidential.

V. Principles of precaution and risk minimization whereby due care and caution is taken at all stages of the research and experiment to ensure that the research participants are put to the minimum risk and generally, benefit from and by the research or experiment.

VI. Principles of professional competence whereby the research is conducted at all times by competent and qualified persons who act with total integrity and impartiality.

VII. Principles of accountability and transparency whereby the research or experiment will be conducted in a fair, honest, impartial and transparent manner after full disclosure is made by those associated with the research or experiment of each aspect of their interest in the research and any conflict of interest that may exist.

VIII. Principles of the maximization of the public interest and of distributive justice whereby the research or experiment and its subsequent applicative use are conducted and used to benefit all human kind and not just those who are socially better off but also the least advantaged.

IX. Principles of institutional arrangements whereby there shall be a duty on all persons connected with the research to ensure that all the procedures required to be complied with and all institutional arrangements required to be made in respect of the research are duly made in a bonafide and transparent manner.

X. Principles of public domain whereby the research results are generally made known through scientific and other publications subject to such rights as are available to the researcher and those associated with the research under the law in force at that time.

XI. Principles of totality of responsibility whereby the professional and moral responsibility, devolves on all those directly or indirectly connected with the research or experiment including the researchers, those responsible for funding or contributing to the funding of the research, the institution or institutions where the research is conducted and the various persons, groups or undertakings who sponsor, use or derive benefit from the research, market the product (if any) or prescribe its use so that.

XII. Principles of compliance whereby, there is a general and positive duty on all persons, conducting, associated or connected with any research entailing the use of a human participant to ensure that both the letter and the spirit of these guidelines, as well as any other norms, directions and guidelines which have been specifically laid down or prescribed and which are applicable for that area of research or experimentation, are scrupulously observed and duly complied.

GENERIC DRUG [4, 5]

A generic must contain the same active ingredients as the original formulation. According to the U.S. Food and Drug Administration (FDA), generic drugs are identical or within an acceptable bioequivalent range to the brand name counterpart with respect to pharmacokinetic and pharmacodynamic properties.

By extension, therefore, generics are considered (by the FDA) identical in dose, strength, route of administration, safety, efficacy, and intended use. The FDA's use of the word identical is very much a legal interpretation, and is not literal. In most cases, generic products are available once the patent protections afforded to the original developer have expired. When generic products become available, the market competition often leads to substantially lower prices for both the original brand name product and the generic forms. In the US, drug patents give twenty years of protection, but they are applied before clinical trials are began, so that the effective life of a drug patent tends to be between seven and twelve years.

Properties of generic drug:

Generic drugs must contain the same active ingredients that their brand name counterparts do and are tested to assure that they are therapeutically equivalent, but they may contain different inactive ingredients from those found in the brand name medications[6].

Characteristics of generic drug:

Non proprietary name of its active ingredients or under a generally descriptive name rather than under a brand or trade name.

Abbreviated New Drug Application (ANDA)

An **Abbreviated New Drug Application** (ANDA) is an application for a U.S. generic drug approval for an existing licensed medication or approved drug.

The ANDA contains data which when submitted to FDA's Center for Drug Evaluation and Research, Office of Generic Drugs, provides for the review and ultimate approval of a generic drug product. Once approved, an applicant may manufacture and market the generic drug.

Product to provide a safe, effective, low cost alternative to the American public. ANDAs have grown by 70% since November 2008.

A generic drug product is one that is comparable to an innovator drug product in dosage form, strength, and route of administration, quality, performance characteristics and intended use. All approved products, both innovator and generic, are listed in FDA's Approved Drug Products with Therapeutic Equivalence Evaluations (Orange Book).

Generic drug applications are termed "abbreviated" because they are generally not required to include preclinical (animal) and clinical (human) data to establish safety and effectiveness. Instead, generic applicants must scientifically demonstrate that their product is bioequivalent (i.e., performs in the same manner as the innovator drug). One way scientists demonstrate bioequivalence is to measure the time it takes the generic drug to reach the bloodstream in 24 to 26 healthy volunteers. This gives them the rate of absorption, or bioavailability, of the generic drug, which they can then compare to that of the innovator drug. The generic version must deliver the same amount of active ingredients into a patient's bloodstream in the same amount of time as the innovator drug.

Using bioequivalence as the basis for approving generic copies of drug products was established by the Drug Price Competition and Patent Term Restoration Act of 1984, also known as the Hatch-Waxman Act. This Act expedites the availability of less costly generic drugs by permitting FDA to approve applications to market generic versions of brand-name drugs without conducting costly and duplicative clinical trials. At the same time, the brand-name companies can apply up to five additional years longer patent protection for the new medicines that they develop to make up for time lost while their products were going through FDA's approval process. Brand-name drugs are subject to the same bioequivalence tests as generics upon reformulation[7].

Table.1; Generics approval process

Type	Patent Certification	ANDA Filing
Paragraph I	The drug has not been patented.	If a generic drug manufacturer certifies I & II, then the FDA starts processing the generic ANDA right away
Paragraph II	The patent has already expired.	
Paragraph III	The generic drug will not go on the market until the day of expiry of the patent	If a generic drug manufacturer certifies 2, then the FDA starts processing the ANDA, and gives approval when the patent expires
Paragraph IV	The patent is not infringed or is invalid	ANDA filer notifies patent holder within 20 days Patent holder must sue for infringement within 45 days – If the patent holder sues, FDA must withhold approval for 20 months (one time only) – If the patent holder does not sue, FDA may approve ANDA at any time – If a court rules that the patent is not infringed or invalid, FDA may Proceed after decision. – If first generic ANDA files will gets 180 days exclusivity (per product)

General study procedure [8, 9, 10]

Adult volunteers who are medically fit and had passed certain inclusion and exclusion criteria are recruited by the principal investigator for a BE study. A minimum 12 volunteers must be recruited for a BE study. The most common study design is 2-way cross over, double blind and randomised study- 2 products, 2 sequences, 2 periods. (So that the treatment effect can be distinguished and other variability/bias can be minimized).

At Visit 1:

All participating volunteers must undergo a full medical check-up by a certified medical doctor before the study. Healthy volunteers who have passed the inclusion and exclusion criteria will be selected at this screening process. Then

the principal investigator will explain to the volunteers the experimental aspects of the research, the purpose, the procedures involved, the foreseeable risks and other important elements of informed consent. After that, an informed consent agreement will be obtained from each willing participating volunteer.

At Study Period 1:

All volunteers will stay for one or more days in the study wards. The volunteers will take one dose of the medicinal product (either Brand A or Brand B) and allow the clinical research team to collect their blood samples at specific time intervals. Doses are administered under close supervision and in a randomised method. After this is completed, the subjects will return home.

At Study Period 2:

After a period of wash-out determined by the principal investigator, the volunteers will check into the study ward to repeat the same procedures as in Study Period 1 but with a different Brand product (which they did not take during the first study period). The blood samples collection will be repeated at the same specific time intervals.

At Visit 4:

All the volunteers are required to come back to the study ward for a brief follow-up session one week after Study Period 2. This is to ensure the volunteers are healthy and are not adversely affected by the medicinal products. After the completion of the clinical phase, all the collected blood samples from Study period 1 and 2 will be send to a pharmacokinetics laboratory to conduct the concentration analysis phase. This analytical phase will determine whether the two medicinal products are Bioequivalent or not.

Study Design of BA/BE study

An open label, balanced, analyst blind, randomized, two-treatment, two-period, two-sequence, single dose, crossover bioequivalence study on healthy, adult, human subjects under fasting/Fed condition.

Explanation of study design

Open-label: The subjects and the investigators were not blinded towards the identity of the study medication.
Two-treatment: There were two treatments one test and one reference.
Two-period: There were two periods, period I and period II.
Two-sequence: Two sequences as AB and BA were used for assigning the Treatment to each subject
Single dose: Subject were dosed according to randomization schedule only at once in each period.
Crossover Design: Every subject receives the each of one treatment as per Randomization schedule. This design is used to minimize the inter-subject variability.

Table.2 ; Crossover Design

	Sequence A(TR)	**Sequence B(RT)**
Period I	Test	Reference
Period II	Reference	Test

Pre-study screening

Volunteer enrollment in database:

Enrollment of volunteers in database is carried out during first visit for the screening.

Following documents required for the enrollment in the data base.

- Volunteer's Identity Card (Election card, PAN card Deriving license etc)
- Documents in support of date of birth (birth date certificate, school leaving certificate, high school mark sheet etc.)

Enrolled volunteers are eligible for the screening.

Volunteers screening:

The screening was carried out after taking an initial informed consent from volunteers for study screening procedures and included the following:

- Demographic data, including sex, completed age, height and weight, BMI, diet, history of tobacco use, intake of abusive/recreational drugs, alcohol intake, history of blood donation and history of participation in a drug research study.
- Medical history including relevant past medical/ surgical history, family history, history of allergies (food/ drug/ any other), past medication history in the last 90 days.
- Present complaints (if any), medical examination including recording of vital signs (B.P, Pulse, Temperature and respiration), general examination, physical and systemic examination.
- 12-lead ECG for heart rate, rhythm and specific finding (if any).
- Blood and Urine analysis.
- Chest X-ray (PA view).

Medical examination:[8]

The Medical examination of volunteers was conducted to know the current and previous health status. If the volunteer was found normal then only he/she was allowed to participate in the study.

The following criteria were used.

The volunteer must be free from:

- Ear, Nose and Throat diseases
- Gastro- Intestinal diseases
- Respiratory diseases
- Cardiovascular diseases
- Central Nervous diseases
- Dermatology diseases
- Prolong Hospitalization
- Major surgery
- Family history of diseases
- Allergic to any drug or food

Table. 3 ; Normal ranges for hematology parameters

Specified tests/Examination performed	Normal Range	Unit
Leucocyte Count (WBC)	4.00 – 10.00	x1000 / ul
Erythrocyte count (RBC)	Male 4.50 – 5.50 million Female 3.80 – 4.80 million	x million / ul
Haemoglobin (Hb)	Male 13.0 – 17.0 Female 12.0 – 15.0	g/dL
Hematocrit (HCT)	Male 40.0 – 50.0 Female 36.0 – 46.0	%
Platelet Count (PLT)	150 – 400	x1000 / ul
Neutrophils	40 – 80	%
Eosinophils	1 – 6	%
Lymphocytes	20 – 40	%
Monocytes	2 – 10	%
Basophils	0 – 2	%
ESR	Male < 15, Female < 20	Mm/hr

During post study safety assessment the acceptable limit of the Hemoglobin was NLT 11.5 g/dl and for Haematocrit (PCV) was NLT 34% due approximately loss of 250-350 ml of blood during pharmacokinetics sampling.

Table. 4 ; Normal ranges for biochemical parameters

Biochemical Parameters	Normal Range	Unit
Fasting Blood Sugar	Fasting 74.0 – 106.0 Post Prandial Less than 140.0 Random 70.0 – 150.0	mg/Dl
Total Cholesterol	Less than 200.0	mg/dL
Triglyceride	Less than 150.0	mg/dL
Blood Urea Nitrogen	6.00 – 20.00	mg/dL
Serum Creatinine	0.60 – 1.20	mg/dL
Alkaline Phosphatase	20.0 – 130.0	U/L
SGPT	4.0 – 36.0	U/L
SGOT	8.0 – 33.0	U/L
Calcium	8.60 – 10.00	mg/dL
GGT	5.0 – 40.0	U/L
Bilirubin Total	0.10 – 1.2	mg/dL
Bilirubin Direct	< 0.30	mg/dL
Bilirubin Indirect	0.1 – 1.0	mg/dL
Sodium	136.0 – 145.0	mmol/L
Potassium	3.50 – 5.10	mmol/L

All biochemical parameters given in normal ranges with units at the screening time the volunteers should pass in these ranges.

Table. 5; Drug of abuse

Drugs	Ranges ng/mL
Cocaine (COC)	300
Amphetamine (AMP)	1000
Marijuana (THC)	50
Morphine (MOP)	300
Barbiturates (BAR)	300
Benzodiazepines (BZO)	300

Table. 6; Routine examination of urine

Laboratory parameters	Normal Range	Unit
Physical Examination		
Colour	Colourless to Yellow	--
Appearance	Clear	--
Deposits	Absent	--
pH	5.0 – 9.0	--
Specific Gravity	1.001-1.035	--
Chemical Examination		
Protine	Negative	--
Glucose	Negative	--
Ketones	Negative	--
Occult Blood	Negative	--
Bile pigments (Bilirubin)	Negative	--
Bile Salts	Absent	--
Urobilinogen	Normal	--
Microscopic Examination		
Red Blood cell/hpf	0-2	--
Leucocytes or Pus cells/hpf	2-3	--
Epithelial cells/hpf	2-5	--
Casts	Absent	--
Crystals	Absent	--
Amorphous material	Absent	--
Trichomonas Vaginalis	Absent	--
Bacteria	Absent	--

At the screening time in urine examination (physical, chemical ,and microscopical) the volunteers should pass above these ranges.

Table. 7; Urine pregnancy test

Specified tests/Examination performed	Normal Range
Urine Pregnancy Test	Negative

For female volunteers urine pregnancy test will be carried out and they should pass this test.

Table. 8; Normal range for vital parameter

Vital Signs	Normal Range
Respiratory rate	16-20 breaths per minute
Blood pressure	Systolic pressure (100-140mmHg)
	Diastolic pressure (60-90mm Hg)
Pluse rate	60-100 pulse per minutes
Body temperature	96.0-99.0 ˚F

Inclusion criteria[15]

The subjects were included based on the following criteria:

- Healthy volunteers within the age range of 18 to 45 years.
- Non-tobacco users.
- Willingly provided the written informed consent to participate in the study.
- Body mass index of ≥ 18.50 kg/m2 and ≤ 29.99 kg/m2, with body weight not less than 50 kg for males and 45 kg for females.
- Absence of significant disease or clinically significant abnormal laboratory values or laboratory evaluation, medical history or physical examination during the screening.
- Had a normal 12-lead ECG or one with abnormality considered as clinically insignificant.
- Had a normal chest X-ray PA view.
- Comprehension of the nature and purpose of the study and compliance with the requirement of the distributed ICF.
- Volunteer is regularly menstruating / Volunteer is in postmenopausal phase for at least 1 year / is surgically sterile (for females).
- Volunteer 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, and intrauterine device (IUD) or abstinence etc. except hormonal contraceptives (for females).

Exclusion Criteria:

The subjects were excluded based on the following criteria:

- Personal history of allergy or hypersensitivity to or allied drugs.
- Any major illness in the past 90 days or any clinically significant ongoing chronic medical illness e.g. Congestive Cardiac Failure (Heart failure), Hepatitis, Hypotensive episodes, Hyperglycemia etc.
- Presence of any clinically significant abnormal values during screening e.g. significant abnormality of liver function test, renal (kidney) function test etc.
- Severe cardiac, renal or liver impairment, gastro-intestinal disease or other conditions, any other organ or system impairment.
- History of seizures, epilepsy or any kind of Neurological disorders.
- Past history of Anaphylaxis or angioedema.
- Presence of disease markers of HIV and hepatitis B, C virus.
- History of chronic consumption of any kind of alcoholic beverages for more than 2 years or having consumed alcohol within 48 hours prior to dosing.
- Consumption of Xanthine containing derivatives (coffee, tea, cola – drinks, chocolate) or grape fruit juice or tobacco products within 48 hours prior to dosing.
- Used any recreational drug or a history of drug addiction.
- Participation in any clinical trial within the past 90 days.
- History of difficulty with donating blood or difficulty in accessibility of veins in left or right arm.
- Donation of blood (one unit or 350 mL) within 90 days prior to receiving the first dose of study medication.
- Consumption of any other prescription drug or over the counter (OTC) drugs (including vitamins and medicinal products from natural origin) within two weeks prior to receiving the first dose of study medication or repeated use of drugs within the last four weeks.
- An unusual diet for whatever reason e.g. low sodium diet, for two weeks prior to receiving any medication and throughout subject's participation in the study.
- Recent history of dehydration from diarrhoea, vomiting or any other reason within a period of 24 hours prior to the study.
- Known hypersensitivity to heparin.
- Use of oral contraceptive in last 90 days (for females)
- Pregnant / lactating volunteers (for females)

Subject's withdrawal /termination: [6,11]

Subjects were free to withdraw their participation from the study of their own at any time without giving any reason thereof. The Principal Investigator could withdraw a subject if he considered that there could be a risk to the health of the subject, or for any of the following reasons:

- Any adverse event because of taking the Investigational Product.
- Any abnormal laboratory test considered to be of clinical significance.
- Any serious protocol violation.
- Lack of co-operation from the subject.
- Inter-current illness requiring treatment.

The decision was dependant on the nature of the illness, the Investigational Product used, and the treatment required.

Ethics[11]

Ethics Committee:

The protocol was submitted to the Independent Ethics Committee (IEC) for approval. The subsequent amendments (if any) to the protocol prior to commencement of the study subject to the written approval of the IEC and Sponsor. During the course of the study, the Principal Investigator reserves the right to make minor administrative amendments to protocol, without changing the meaning of the content of protocol. The same was conveyed to the IEC and the Sponsor.

Ethical conduct of the study:

This study was conducted in accordance with the principles of the Declaration of Helsinki, 'ICH GCP', National Regulations (ICMR Guidelines), 'Indian GCP', USFDA guidelines and Schedule Y of Indian Drugs and Cosmetics Act.

Compensation to subjects:

In case of dropout/ withdrawal of a subject before completion of the study, the amount of proportionate compensation to the dropout / withdrawal subject will be as follows:

Table.9; Compensation criteria

Sr. No.	Reasons of Withdrawal from the Study	Compensation
1.	Principal Investigator / Medical Officer withdraw the subjects from the study based on medical decision.	Full payment
2.	After the initiation of the study, subject drop out on his/her own free.	Proportionate participation dues
3.	The subject was withdrawn from the study on humanitarian grounds, with the permission of the Principal Investigator / Medical Officer.	Proportionate participation dues
4.	The subject was withdrawn from the study due to violation of requirements of the study by the Principal Investigator / Medical Officer after signing the Informed Consent Form but before receiving any medications.	No payment
5.	The subject was withdrawn from the study by the Principal Investigator / Medical Officer because of willful misinformation on present and /or past medical illness/history.	No payment
6.	The subject was withdrawn from the study by Principal Investigator / Medical Officer for misconduct during the study.	No payment

Insurance and financing

Subject was covered by an appropriate insurance policy.

Information of Investigational Products

Table.10; Investigational products Details

Criteria	Innovators Details	Test Product
Dosage form type	Oral (Tablet)	Oral (Tablet)
Brand name	ABC	XYZ
Manufacturer	ABC Pharmaceuticals	XYZ Pharmaceuticals
Exp. Date	DD/MM/YYYY	DD/MM/YYYY
Mfg. Date.	DD/MM/YYYY	DD/MM/YYYY
No. of Units	xx	yy
Storage Condition	As per protocol	As per protocol

Selection of doses:[10]

Dose should be selected on the basis of codes of ethics. It should be safe and adequate to measure the concentration in plasma.

Justification of choice of reference product:

Choice of reference drug should be existed drug in market and approved by FDA.

Supply of investigational products:

An adequate number of investigational products (properly labeled according to the requirements of cGMP for administration along with certificate of analysis (COA) was provided by the sponsor and was received at Bioequivalence department of contract research organization (CRO) by the registered pharmacist.

Dispensing and labeling of investigational product:

Under the supervision of the trained study personnel, the registered pharmacist prepared doses for the study as per the randomization schedule in presence of Quality Assurance personnel. After dispensing investigational products was

place into appropriately labeled containers. An additional label was made available for attachment to each investigational product administration form at the time of dosing to confirm correct administration of investigational products. Remaining investigational products were store in their original container as retention samples. The issued investigational products were transfer to the investigational product dispensing containers as doses.

Investigational product accountability (receipt, inventory and return):

An adequate number of Investigational Products along with certificate of analysis (COA) received at Bioequivalence department of respective pharmaceuticals by the registered pharmacist.

Sequence and duration of study periods:

Overall Study Plan:

- Subjects from the pool of healthy volunteers who were screened within 21 days prior to the first dosing day will be considered as potential participants in the study.
- The inclusion and exclusion criteria were apply to all subjects as a condition of admission into the study.
- Before admission of volunteers into the Clinical Pharmacology Unit (CPU), on the pre-study day, the volunteers were given full details of the study.
- Volunteers required to give their written consent for participating in the study by signing with date the informed consent form.
- Tests for drugs of abuse, urine pregnancy test (for female volunteer only) and alcohol consumption by breath alcohol analyzer was carry out followed by pre – entry vital signs, medical examination, inclusion and exclusion criteria check.
- There was e a supervised overnight fasting period of at least 10 hours before dosing.
- A pre-dose sample was taken before dosing thereafter a series of blood samples will be taken, till 24 hour (or specified in protocol) post-dose with the subject remaining in the CPU.
- After the 24-hour post-dose blood sample collection, the subjects were allow to leave CPU after checking their vital signs and medical examination.
- Subjects were visit the centre for ambulatory blood sample collections, schedule specified in the study protocol.
- After a washout period specified in protocol, the same procedure was repeat in subsequent period except for the Inform Consent Form (ICF) presentation and obtaining of written informed consent.
- Post-study safety assessment was done after last ambulatory blood collection of study period II.

Study restrictions:

- No medication whether prescribed or over-the-counter products (including vitamins and medicinal products from natural origin) was allow within 14 days prior to dosing.
- Subjects were instructed to abstain from alcohol and products containing xanthine derivative (chocolates, tea, coffee or cola drinks), grape fruit juice, cigarettes and tobacco products, for at least 48 hours, prior to dosing, during their participation in the study including the ambulatory visit.
- Subjects were instructed to abstain from an unusual diet for whatever reason e.g. low sodium diet, for two weeks prior to receiving the first investigational product and throughout their participation in the study including the ambulatory visit.
- Procedures on pre-study and study day:
- Eligible volunteers were attended the ICF presentation on pre-study day.
- Volunteers were given full details about the study on this occasion, written informed consent was obtain from all willing volunteers.
- Urine samples of all the volunteers who have given written informed consent will be tested for drugs of abuse which include; Cocaine (COC),
- Amphetamines (AMP), Marijuana (THC), Morphine (MOP), Barbiturates (BAR), Benzodiazepine (BZO), followed by alcohol consumption by breath alcohol analyser, vital signs and subject questionnaire, medical examination inclusion and exclusion criteria check.
- For female volunteers urine pregnancy test carried out. The group of eligible and willing volunteers who comply with all the inclusion and exclusion criteria was recruited into the study.
- A standardised meal served at scheduled time to have at least 10 hours fasting prior to dosing.
- On the first study day, vital signs and subject questionnaire was done; pre dose sample will be taken within one hour prior to administration of the drug product.
- Water was not allowed 1.00 hour prior to dosing and until 1.00 hour post-dose except at time of dosing or as specified in protocol. Also no food permitted except meals specified in protocol..
- Subjects were dose while in sitting posture and instructed to remain seated or be ambulatory (avoiding any strenuous activity) for first two hours following the drug administration (except during recording of vitals).
- During this interval, under supervision, subjects were permit to use the washroom facilities.
- Thereafter the subjects were allowed to engage only in normal activities while avoiding severe physical exertion. However should any adverse event occur at any time during housing the subjects were place in an appropriate posture.

Dose and Route of Administration:

An oral dose of Reference product (R) or Test product (T) administered at 0.00 hour during each period with 240 mL (about 8 oz) of water at room temperature as per the randomization schedule under the supervision of the medical officer where end time of the dosing recorded in investigational product administration forms. The dosing intervals between two consecutive subjects were 2 minutes.

Blood sampling schedule:[12]

Blood samples (1 × x ml) were collected in x ml blood collection tube containing EDTA solution as anticoagulant. The venous blood samples were withdrawn pre-dose and at specified time points post-dose. (Time points being relative to the investigational product dosing).

Samples were collected through an indwelling cannula placed in a forearm vein. The pre-dose samples were collect within one hour prior to drug dosing.

Intravenous indwelling cannula would be kept in place as long as required by injecting not more than 0.5 mL of 5 IU/mL of heparin in normal saline solution during the collection of multiple samples.

Total blood loss:

For each subject, the total number of blood draws should not be more than 350 ml.

Handling of blood samples:

Each blood sample (1 × x mL) was collect into x mL blood collection tube containing K_2EDTA solution as anticoagulant. The blood samples collected at each time point was centrifuge between 4 to 8° °C and 4000 rpm for 10 minutes to separate plasma, after receiving the blood samples from all the subjects (For ambulatory samples, the samples collected till the scheduled time of last subject was centrifuge together and the samples collected later was centrifuge separately according to their collection time). Blood samples were centrifuge within 30 minutes after collection of last blood sample; if there is any delay in centrifugation then sample kept in cold condition. The separated plasma transferred in prelabelled polypropylene tubes during each period. These tubes were labelled with study number, period number, subject number, sample no, time point (hrs), and aliquot number.

These tubes then be transfer to a deep freezer for storage until analysis.

Termination of the study:

The principal investigator reserves the right to terminate the study at any time for safety reasons or in the best interest of the subject's welfare. The ethics committee can also cancel the study for major ethical violations. The subjects would be briefed on the reasons for the termination and compensated adequately.

Randomisation procedures:

This was a two-treatment, two-period, two-sequence, crossover bioequivalence study. In each period, subjects administered either test or reference product, according to the randomization schedule.

CONCLUSION

All recordings relating to each subject in the study made directly in the relevant forms or on the designated documentation related to the study. All reports from external sources will be countersigned by the principal investigator or designated physician responsible for screening the volunteers, to confirm they have seen and approved the reports. These reports were including those from, ECG tracings and the external clinical laboratory results. Any other information relevant to the study and considered to be source data filed with the study documentation.

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