An open label balance randomized two treatment two secevence two period, singledose crose over oral biogelivalence study of trimebutine 200mg and digedrat in 48 healthy adult human subject under fasting conditions

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INTRODUCTION

In pharmacology Bioavailability (BA) is a subcategory of absorption and is the fraction of an administered dose of unchanged drug that reaches the systemic circulation which is one of the principal pharmacokinetic properties of drugs. By definition when a medication is administered intravenously its bioavailability is 100% [1] however when a medication is administered via other routes (such as oral) its bioavailability generally decreases (due to incomplete absorption and first-pass metabolism) or may vary from patient to patient. Bioavailability is one of the essential tools in pharmacokinetics as bioavailability must be considered when calculating dosages for non-intravenous routes of administration.

Bioequivalence study (BE) is a comparative study of bioavailability among drug products that contain the same active agents. Bioavailability and bioequivalence of drug products and drug product selection have emerged as critical issues in pharmacy and medicine during the last three decades. Concern about lowering health costs is resulted in tremendous written increase in the use of generic drug products currently about one half of all prescriptions written are for drugs that can be substituted with a generic drug.

This circumstantial growth of generic pharmaceutical industry [1] and the abundance of multisource products have prompted some questions among healthy professionals and scientists regarding the therapeutic equivalency of these products. Inherent in the currently accepted guidelines for product substitution is the assumption that a generic drug considered to be equivalent to a brand name drug would elicit the same clinical effect.

Numerous papers in the literature indicate that there is concern that the current standards for approval of generic drugs may not always ensure therapeutic equivalence. The availability of different formulations of the same drug substance given at the same strength and in the same dosage form poses a special challenge to health care professionals.

This study is comparative bioequivalence study of *Trimebutine* 200 mg capsules and *Digedrat*® 200 mg capsules of Reference brand in 48 healthy, adult, human, male and non-pregnant female subjects under fasting conditions.

Trimebutine maleate is used to regulating effects of on lower gastrointestinal tract and to treat the irritable bowel syndrome.

BIOAVAILABILITY (BA):

Bioavailability is defined as: "The rate and extent to which the active ingredient or active moiety is absorbed from a drug product and becomes available at the site of action."[2]

Bioavailability[3]:

- It is the fraction of unchanged drug reaching the systemic circulation following administration by any route.
- To exert an optimal therapeutic action, an active moiety should be delivered to its site of action in an effective concentration for the desired period
- This defines how much drug needs to be administered to achieve therapeutic effect.
- The influence of route of administration on drug's bioavailability is:

  Parenteral > oral > rectal > topical

- Intravenous injection of a drug results in 100% bioavailability as the absorption process is bypassed. In such cases the dose available to the patient called as the bioavailable dose is often less than the administered dose.
- Estimation of bioavailability is a means of predicting the clinical efficacy of a drug.
- Bioavailability testing measuring the rate and extent of drug absorption is a way to obtain evidence of the therapeutic utility of a drug product.

Fraction of administered dose that enters the systemic circulation

$$F = \frac{\textbf{Bioavailable dose}}{\textbf{Administered dose}}$$

It ranges from 0 to 1 Bioavailability normally expressed as %.

TYPES OF BIOAVAILABILITY [4][5]:

Absolute bioavailability (F):

- When systemic availability of drug administered orally is determined in comparison to its intravenous administration is called absolute bioavailability.
- Its determination is used to characterize a drug's inherent absorption properties from the extra vascular site.
- Absolute bioavailability = $\frac{[AUC]ev\ /(Dose)ev}{[AUC]iv\ /(Dose)iv}$

(ev-extravascular & iv- intravenous)

Relative Bioavailability (Fr)

- When systemic availability of drug after oral administration is compared with that of an oral standard of same drug (such as an aqueous or non aqueous solution or suspension) it is referred as relative bioavailability.
- It is used to characterize absorption of drug from its formulation

$$\text{Relative Bioavailability} = \frac{[AUC]test\ /(Dose)test}{[AUC]std/(Dose)std}$$

- Before the therapeutic effect of an orally administered drug can be realized the drug must be absorbed

Supra bioavailability [6]:

Supra bioavailabilty is a term used when a test product displays larger bioavailability than the reference product. Such formulations are usually not to be accepted as therapeutically equivalent to the existing reference product.

General Objectives Of Bioavailability Studies

Bioavailability studies are important in the determination of influence of excipients patient related factors and possible interaction with other drugs on the efficiency of absorption.

i. Development of new formulations of the existing drugs e.g. innovator vs generic. Bioequivalence study looking for similarity of F and ka values between products.

ii. one type of dosage form with another e.g. tablet versus intravenous dosage form or regular tablet with sustained release tablet. Bioavailability study

where ka and F are to be determined. Changes in ka may be intentional (slow release) whereas F values should be similar.[7]

Factors Influencing Bioavailability

The absolute bioavailability of a drug when administered by an extra vascular route is usually less than one (i.e. F<1). Various physiological factors reduce the availability of drugs prior to their entry into the systemic circulation. [8]

Such factors may include but are not limited to:

- Physical properties of the drug (hydrophobicity, pKa, solubility)
- The drug formulation (immediate release, excipients used, manufacturing methods, modified release - delayed release, extended release, sustained release, etc.)
- If the drug is administered in a fed or fasted state
- Gastric emptying rate
- Circadian differences
- Enzyme induction/inhibition by other drugs/foods.
- Disease state eg: Hepatic insufficiency, poor renal function

Each of these factors may vary from patient to patient (inter-individual variation) and indeed in the same patient over time (intra-individual variation). Whether a drug is taken with or without food will affect absorption other drugs taken concurrently may alter absorption and first-pass metabolism, intestinal motility alters the dissolution of the drug and may affect the degree of chemical degradation of the

drug by intestinal micro flora. Disease states affecting liver metabolism or gastrointestinal function will also have an effect.

Absolute bioavailability compares the bioavailability (estimated as area under the curve, or AUC) of the active drug in systemic circulation following non-intravenous administration (i.e., after oral, rectal, transdermal, subcutaneous administration) with the bioavailability of the same drug following intravenous administration. [9]

Relative bioavailability is extremely sensitive to drug formulation. Relative bioavailability is one of the measures used to assess bioequivalence between two drug products as it is the Test/Reference ratio of AUC. The maximum concentration of drug in plasma or serum (C_{max}) is also usually used to assess bioequivalence.

If the size of the dose to be administered is same then bioavailability of a drug from its dosage form depends upon three major factors:[10]

- Pharmaceutical factors related to physiochemical properties of the drug and characteristics of dosage form.
- Patient related factors.
- Route of administration.

METHODS FOR ASSESSING BIOAVAILABILITY [10]:

The methods useful in quantitative evaluation can be divided into 2 categories

I.Pharmacokinetic methods (indirect method)

These are very widely used and based on the assumption that the pharmacokinetic profile reflects the therapeutic effectiveness of a drug. Thus these are indirect methods**.**

There are 2 methods in this type

A) Plasma level-time studies

B) Urinary excretion studies.

II. Pharmacodynamic methods (direct method)

These methods are complementary to pharmacokinetic approaches and involve direct measurement of drug effect on patho physiological process as a function of time.

The two pharmacodynamic methods are:

A) Acute pharmacological response

B) Therapeutic response.

PHARMACOKINETIC PARAMETERS[10]

C_{max} – Maximum measured plasma concentration after the administration of single dose of the drug expressed in terms of μg/ml or ng/ml.

AUC_{0-t} – The area under the plasma concentration versus time curve from time zero to the last time point with measurable concentration calculated by the linear trapezoidal method.

$AUC_{0-\infty}$ – The area under the plasma concentration versus time curve from time zero to time infinity. $AUC_{0-\infty}$ is calculated as the sum of the AUC_{0-t} plus the ratio of the last measurable concentration to the elimination rate constant.

T_{max}: Time of maximum measured plasma concentration. If the maximum value occurs at more than one point T_{max} is defined as the first point with this value in each period. Gives indication of the rate of absorption expressed in terms of hours or minutes.

K_{el}: Apparent first order elimination or terminal rate constant calculated from a semi-log plot of the plasma concentration versus time curve.

$T_{1/2}$: Apparent first-order terminal elimination half life will be calculated as $0.0693/K_{el.}$

The various pharmacodynamic parameters which influence the above mentioned pharmacokinetic parameters are:

Minimum Effective Concentration (MEC):

It is defined as the minimum concentration of drug in plasma required to produce the therapeutic effect. It reflects the minimum concentration of the drug at the receptor site to elicit the desired pharmacologic response. The concentration of drug below MEC is said to be in the sub therapeutic level.

Maximum Safe Concentration (MSC)

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

Onset of Action

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

Onset Time

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

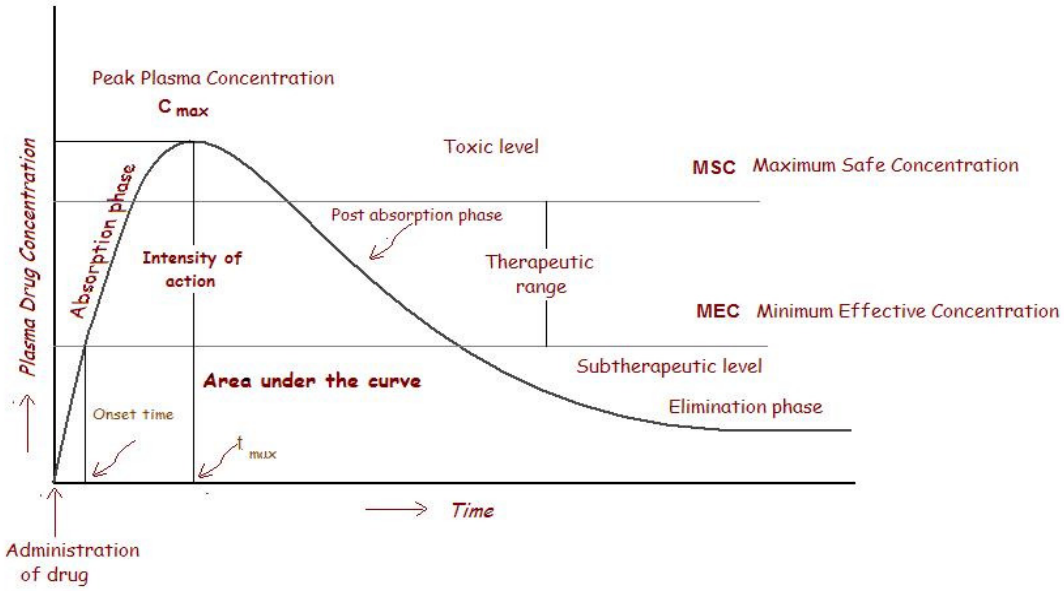
Duration of Action

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

Therapeutic Range

The drug concentration between MEC and MSC represents the Therapeutic range

FIGURE 1: Pharmacokinetic parameters.



Bioavailability is needed for

- Drugs having low therapeutic index e.g. cardiac glycosides, quinidine, phenytoin etc.

- Drugs whose peak levels are required for the effect of drugs e.g. phenytoin, phenobarbitone, primidone, sodium valporate, anti-hypertensives, antidiabetics and antibiotics.

- Drugs that are absorbed by an active transport e.g. amino acid analogues, purine analogues etc.

- Drugs which are disintegrated in the alimentary canal and liver e.g.chlorpromazine etc., or those which under go first pass metabolism.

- Formulations that give sustained release of drug formulations with smaller disintegration time than dissolution rate and drugs used as replacement therapy also warrant bioavailability testing. In addition any new formulation has to be tested for its bioavailability profile.

BIOEQUIVALENCE (BE) [8] :

Chemical equivalents which when administered to the same individuals in the same dosage regimen will result in comparable bioavailability. Bioequivalence gained increasing attention during the last 40 years after it became evident that marketed products having the same amounts of the drug may exhibit marked differences in their therapeutic responses. When drug products are administered to individuals the investigator inevitably finds differences in one or more of the variables measured. These differences are due partly to factors related to dosage form and partly to biological factors unique to each individual since each person has his own characteristics for absorption, metabolism and excretion of each drug. Through appropriate use of statistical procedures it is possible to identify the variations that result from differences among individuals and thus to isolate those that result from

differences in the bioavailability of the drug products. Generally these differences were well correlated to dissimilar drug plasma levels caused mainly by impaired absorption. Now a considerable body of evidence has accumulated indicating that drug response is better correlated with the plasma concentration or with the amount of drug in the body than with the dose administered. Consequently on the basis of simple pharmacokinetic concepts and parameters bioavailability and bioequivalence studies have been established as acceptable surrogates for expensive complicated and lengthy clinical trials and are used extensively worldwide to establish and ensure consistent quality and a reliable therapeutically effective performance of marketed dosage forms

Bioequivalence studies compare both the rate and extent of absorption of various multisource drug formulations with the innovator (reference) product on the basis that if two formulations exhibit similar drug concentration-time profiles in the blood/plasma they should exhibit similar therapeutic effects.

Three situations have thus been defined in which bioequivalence studies are required

- When the proposed marketed dosage form is different from that used in pivotal clinical trials. (Edwards., 1990) When significant changes are made in the manufacture of the marketed formulation.
- When a new generic formulation is tested against the innovator's marketed product. Comparative evidence may require not only studies in a fasting condition but following a specified meal. The latter permit drug formulations to be evaluated under "stressed conditions". If it is shown that competitive products are bioequivalent under both fasting and fed conditions there is greater confidence that they are therapeutically equivalent when used in patients. Bio-equivalent simply means that one brand or dosage

form of a drug or supplement is equivalent to a reference brand or dosage form of the same drug or supplement in terms of various bioavailability parameters measured via in-vivo testing in human subject.

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FACTORS INFLUENCING BIOEQUIVALENCE [12]:

- Delayed gastric emptying,
- Stimulation of bile flow,
- Change in gastrointestinal (GI) pH,
- Increase splanchnic blood flow,
- Change in luminal metabolism of a drug substance,
- Physical or chemical interaction with a dosage form or a drug substance.
- Food can change the BA of a drug and hence can influence the BE between test and reference products.

TYPES OF BIOEQUIVALENCE [4],[13]:

- **Chemical equivalence:**

It indicates that two or more drug products that contain the same labeled chemical substance as an active ingredient in the same amount.

- **Pharmaceutical equivalence:**

It is a relative term which denotes that the drug substance in two or more forms are identical in strength, quality, purity, content uniformity, disintegration and

dissolution characteristics. They may however differ in containing different excipients.

- **Therapeutic equivalence:**

It indicates that two or more drug products that contain the same therapeutically active ingredient elicit identical pharmacological effects and can control the disease to the same extent.

TYPES OF BIOEQUIVALENCE STUDIES:

- In vivo studies
- In vitro studies

In Vivo Studies:

The following points are used in assessing the need for in vivo studies:

- Oral immediate release products with systemic action.
  - Indicated for serious conditions requiring assured response.
  - Narrow therapeutic margin.
  - Pharmacokinetics complicated by absorption lesser than 70% or non linear kinetics, presystemic elimination greater than 70%.
  - Unfavourable physiochemical properties like low solubility, metastable conditions, instability,etc.
- Non- oral immediate release products.
- Modified release products with systemic action.

In Vitro Studies [14]:

If none of the above criteria is applicable comparative in vitro dissolution studies will suffice. In vitro studies are conducted in cases where

- The product is intended for topical administration (Cream, ointment gel) for local effect.
- The product is for oral administration but not intended to be absorbed (antacid or opaque medium).
- The product is administered by inhalation as a gas or vapor.

GENERAL CONCEPTS OF DESIGN AND CONDUCT OF STUDIES

The design and conduct of the study should follow EC-rules for Good Clinical Practice including reference to an Ethics Committee [15]

As recommended by the US FDA (1992) in most bioequivalence trials a test formulation is compared with the standard / innovator reference formulation in a group of normal healthy subjects (18-45 yr) each of whom receive both the treatments alternately in a crossover fashion (two-period, two-treatment crossover design) with the two phases of treatment separated by a washout period of generally a week duration but may be longer (a minimum time equivalent to 5 half-lives) if the elimination half-life of the drug is very long. The treatment is assigned to each subject randomly but an equal number of subjects receive each treatment in each phase. Thus in case of two treatments A and B one group gets the treatment in the order AB and the second group in the reverse order BA. This is done to avoid the occurrence of possible sequence or period effects. A similar allocation is done in case of a three-treatment crossover design (three-period, three-treatment crossover design).

For several drugs a great inter-subject variability in clearance is observed. The intra-subject coefficient of variation (approximately 15%) is usually substantially smaller than that between subjects (approximately 30%) and therefore crossover designs are generally recommended for bioequivalence studies [16]

The primary advantage of the crossover design is that since the treatments are compared on the same subject the intersubject variability does not contribute to the error variability. If the drug under investigation and/or its metabolites has an extremely long half-life a parallel group design may be indicated.

In a parallel group design subjects are divided randomly into groups each group receiving one treatment only. Thus each subject receives only one treatment. In a parallel design although one does not have to worry about sequence period or carry over effects or dropouts during the study the inter-subject variability being very high the sensitivity of the test is considerably reduced thus requiring a larger number of subjects compared to a crossover design to attain the same sensitivity.

Inherent in both the crossover and parallel designs are the three fundamental statistical concepts of study design namely

- Randomization
- Replication and Error control.

- **Randomization**

It implies allocation of treatments to the subjects without selection bias. Consequently randomization is essential to determine an unbiased estimate of the treatment effects.

- **Replication**

It implies that a treatment is applied to more than one experimental unit (subject) to obtain more reliable estimates than is possible from a single observation and hence provides a more precise measurement of treatment effects. The number of replicates (sample size) required will depend upon the degree of differences to be detected and inherent variability of the data. Replication is used concomitantly with Error control to reduce the experimental error or error variability.

More commonly used replicated crossover designs to compare two formulations are:

- **Four-sequence and two-period design (Balaam's design)**
- **Two-sequence and four-period design**
- **Four-sequence and four-period design**
- **Two-sequence and three-period design**
- **Crossover design for three medications (Williams' design)**
- **Crossover design for four medications (Williams' design)**

Crossover design for two medications (t – test; r -- reference)

2x2 crossover design:

This is a conventional not-replicated design with two formulations, two periods, two sequences that may be represented as follows:

	Period	
Sequence	1	2
1	R	T
2	T	R

Each individual is randomly assigned to RT or TR sequence in two dosage periods. That is, individuals assigned to RT (TR) sequence receive formulation R (T) in the first dosage period and formulation T (R) in the second dosage period.

Randomization for a 2x2 crossover study may be carried out through tables of random numbers or randomization procedures implemented by statistical software.

Replicated crossover design

This design is recommended for bioequivalence studies of formulations with modified-release dosage or highly variable products (intra-individual variation

coefficient ≥30%), including the quick-release, and modified-release ones and other oral administration products.

The same test and reference formulation batches shall be used for this design for replicated administration. The periods shall be sufficiently spaced (washout) to assure non-existence of carryover effects.

More commonly used replicated crossover designs to compare two formulations are

Four-sequence and two-period design (Balaam's design)

In this design test (T) and reference (R) will be taken in two period and four sequence pattern in order to compare two formulations of a drug.

	Period	
Sequence	1	2
1	T	T
2	R	R
3	R	T
4	T	R

Two-sequence and four-period design

In this design there will be four periods and two sequences. To know the typical drug concentration variations in human subjects regarding test and references, number of periods can be increased.

	Period			
Sequence	1	2	3	4
1	T	R	R	T
2	R	T	T	R

Four-sequence and four-period design

Here the design consists of four periods and four sequences. It was a most typical replicated crossover design.

	Period			
Sequence	1	2	3	4
1	T	T	R	R
2	R	R	T	T
3	T	R	R	T
4	R	T	T	R

Two-sequence and three-period design

In this design subjects will undergo three period and two sequence design.

	Period		
Sequence	1	2	3
1	T	R	T
2	R	T	R

Crossover design for three medications (Williams' design)

(Williams' design with T1 = test 1, T2 = test 2, R = reference)

In order to compare three formulations of a drug, there are a total of three possible comparison pairs among formulations: formulation 1 versus formulation 2, formulation versus formulation 3, and formulation 2 versus formulation 3.

	Period		
Sequence	1	2	3
1	R	T2	T1
2	T1	R	T2
3	T2	T1	R
4	T1	T2	R
5	T2	R	T1
6	R	T1	T2

TYPES OF BA/BE STUDIES :

FASTING STUDY

After a overnight fast of at least 10 hrs subjects are made to continue t**o** fast for upto 4 hrs after dosing.

FED STUDY

After a overnight fast of at least 10 hrs subjects are given a high calorie-high fat breakfast 60 min prior to administration of the drug product.

QUALITY CONTROL AND QUALITY ASSURANCE

QUALITY CONTROL:

The principal investigator by careful planning, assigning responsibilities to well qualified study personnel, through continuous review, verifies and maintains desired level of quality in the study.

QUALITY ASSURANCE

Review will be carried out by the QA department to confirm that deviations if any from approved protocol or sop are adequately documented.

EHICAL CONSIDERATIONS

BASIC PRINCIPLES

The study will be carried out in accordance with the provisions of current versions of ICH guidance for Good clinical practices ICMR guidance for biomedical research on human subjects.

INSTITUTIONAL REVIEW BOARD

The protocol and informed consent will be submitted to the IRB/IEC for review. Upon approval.

The study will be conducted as per approved protocol.

INFORMED CONSENT FORM

A process by which a subject voluntarily confirms his or her willingness to participate in a particular trial, after having been informed of all aspects of the trial that are relevant to the subject's decision to participate. Informed consent is documented by means of a written, signed and dated informed consent form. A reference copy of the form shall be given to the respective subjects.

TERMINATION OF STUDY

The sponsor reserves the right to discontinue the study at any time, upon IRB approval. Reasons for the termination will be provided to the subjects and IRB. The investigator reserves the right to discontinue the study at anytime for the reasons of subject safety and welfare. The institutional review board (IRB) may terminate the study, if there are major violations of ethics or due to any serious adverse effects.

SUBJECT COMPENSATION

The subjects will be paid an adequate compensation by the IRB on account of their time participation in the trail and for any inconvenience caused. In case of drop outs of subject before completion of study the compensation will be paid according to the compensation policy.

INSURANCE POLICY

The study will be covered by an insurance contract where in all subjects participation in any study is covered for indemnity and medical expenses.

DRAWING AND DISPOSAL OF PLASMA SAMPLES

The plasma samples should be drawn only when a proper validated bio analytical method is available and disposed after submission of study report.

STUDY TERMS & DEFINITIONS

ADVERSE DRUG REACTION (ADR)

In the pre-approval clinical experience with a new medicinal product or its new usages, particularly as the therapeutic dose (s) may not be established: all noxious and unintended responses to a medicinal product related to any dose should be considered adverse drug reactions. The phrase responses to a medicinal product means that a causal relationship between a medicinal product and an adverse event is at least a reasonable possibility, i.e. the relationship cannot be ruled out.

Regarding marketed medicinal products: a response to a drug which is noxious and unintended and which occurs at doses normally used in man for prophylaxis, diagnosis, or therapy of diseases or for modification of physiological function (see the

ICH Guideline for Clinical Safety Data Management: Definitions and Standards for Expedited Reporting).

ANOVA (ANALYSIS OF VARIANCE)

It is a statistical technique to identify sources of variance and estimate the degree of variability. In most bioavailability studies, there are three readily identified sources of variance namely formulation, subject and period. Hence it is a 3-way cross over.

ADVERSE EVENT (AE)

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 unfavorable 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 (see the ICH Guideline for Clinical Safety Data Management: Definitions and Standards for Expedited Reporting).

APPROVAL (IN RELATION TO INSTITUTIONAL REVIEW BOARDS)

The affirmative decision of the IRB that the clinical trial has been reviewed and may be conducted at the institution site within the constraints set forth by the IRB, the institution, Good Clinical Practice (GCP), and the applicable regulatory requirements.

AUDIT

A systematic and independent examination of trial related activities and documents to determine whether the evaluated trial related activities were conducted, and the data were recorded, analyzed and accurately reported according to the protocol, sponsor's

standard operating procedures (SOPs), Good Clinical Practice (GCP), and the applicable regulatory requirement(s).

AUDIT CERTIFICATE

A declaration of confirmation by the auditor that an audit has taken place.

AUDIT REPORT

A written evaluation by the sponsor's auditor of the results of the audit.

AUDIT TRAIL

Documentation that allows reconstruction of the course of events.

BLINDING/MASKING

A procedure in which one or more parties to the trial are kept unaware of the treatment assignment(s). Single-blinding usually refers to the subject(s) being unaware and double-blinding usually refers to the subject(s), investigator(s), monitor, and in some cases data analyst(s) being unaware of the treatment assignment(s).

CASE REPORT FORM (CRF)

A printed optical or electronic document designed to record all of the protocol required information to be reported to the sponsor on each trial subject

COMPARATOR (PRODUCT)

An investigational or marketed product (i.e., active control), or placebo, used as a reference in a clinical trial.

CONFIDENTIALITY

Prevention of disclosure to other than authorized individuals of a sponsor's proprietary information or of a subject's identity.

CONTRACT

A written, dated, and signed agreement between two or more involved parties that sets out any arrangements on delegation and distribution of tasks and obligations and if appropriate on financial matters. The protocol may serve as the basis of a contract.

COORDINATING INVESTIGATOR

An investigator assigned the responsibility for the coordination of investigators at different centre's participating in a multicentre trial.

CONTRACT RESEARCH ORGANIZATION (CRO)

A person or an organization (commercial, academic, or other) contracted by the sponsor to perform one or more of a sponsor's trial-related duties and functions

DOCUMENTATION

All records in any form (including, but not limited to, written, electronic, magnetic, and optical records, and scans, x-rays, and electrocardiograms) that describe or record the methods, conduct, and/or results of a trial the factors affecting a trial and the actions taken.

GOOD CLINICAL PRACTICE (GCP)

A standard for the design, conduct, performance, monitoring, auditing, recording, analyses, and reporting of clinical trials that provides assurance that the data and reported results are credible and accurate and that the rights, integrity and confidentiality of trial subjects are protected.

INSPECTION

The act by a regulatory authority of conducting an official review of documents, facilities, records, and any other resources that are deemed by the authority to be

related to the clinical trial and that may be located at the site of the trial at the sponsor's and/or contract research organizations (CRO's) facilities or at other establishments deemed appropriate by the regulatory authority.

INSTITUTIONAL REVIEW BOARD (IRB)

An independent body constituted of medical, scientific, and non-scientific members, whose responsibility is to ensure the protection of the rights, safety and well-being of human subjects involved in a trial by among other things reviewing, approving, and providing continuing review of trial protocol and amendments and of the methods and material to be used in obtaining and documenting informed consent of the trial subjects.

INVESTIGATOR

A person responsible for the conduct of the clinical trial at a trial site. If a trial is conducted by a team of individuals at a trial site, the investigator is the responsible leader of the team and may be called the principal investigator. See also Sub investigator.

INVESTIGATOR'S BROCHURE

A compilation of the clinical and nonclinical data on the investigational product(s) which is relevant to the study of the investigational product(s) in human subjects

PROTOCOL

A document that describes the objective(s), design, methodology, statistical considerations, and organization of a trial. The protocol usually also gives the background and rationale for the trial, but these could be provided in other protocol

referenced documents. Throughout the ICH GCP Guideline the term protocol refers to protocol and protocol amendments.

PROTOCOL AMENDMENT

A written description of a change(s) to or formal clarification of a protocol

PHARMACEUTICAL ALTERNATIVES

Medicinal products are pharmaceutical alternatives if they contain the same active moiety but differ in chemical form (salt, ester, etc)

PHARMACEUTICAL EQUIVALENTS

Products that contain the same amount of the same active substance(s) in the same dosage form, meet the same or comparable standards

REGULATORY AUTHORITIES

Bodies having the power to regulate. In the ICH GCP guideline the expression Regulatory Authorities includes the authorities that review submitted clinical data and those that conduct inspections. These bodies are sometimes referred to as competent authorities

SPONSOR

An individual, company, institution, or organization which takes responsibility for the initiation, management, and/or financing of a clinical trial

STANDARD OPERATING PROCEDURES (SOPS)

Detailed, written instructions to achieve uniformity of the performance of a specific function.

SUBINVESTIGATOR

Any individual member of the clinical trial team designated and supervised by the investigator at a trial site to perform critical trial-related procedures and/or to make important trial-related decisions (e.g., associates, residents, research fellows). See also Investigator.

SUBJECT/TRIAL SUBJECT

An individual who participates in a clinical trial, either as a recipient of the Investigational product(s) or as a control.

THERAPEUTIC EQUIVALENTS

Pharmaceutically equivalent products whose effects with respect to both safety and efficacy are essentially the same when administered.

LITERATURE REVIEW

Review of literature is an essential component for a worthwhile study in any field of knowledge. It helps the investigator to gain information on what has been done previously and to gain deeper insight into previous research problem. It also helps to plan and conduct the study in a systemic way.

Study has been done under various divisions like gastric emptying in patients with non-ulcer dyspepsia, smooth muscle relaxants, QT interval prolongation of pro arrhythmia, gastro intestinal modulator.

Delvaux M *et.al.,*(1997) has reported "*Trimebutine*: mechanism of action, effects on gastrointestinal function and clinical results".[26] The actions of *trimebutine* [3,4,5-trimethoxybenzoic acid 2-(dimethylamino)-2-phenylbutylester] on the gastrointestinal tract are mediated via (i) an agonist effect on peripheral mu, kappa and delta opiate receptors and (ii) release of gastrointestinal peptides such as motilin and modulation of the release of other peptides, including vasoactive intestinal peptide, gastrin and glucagon. *Trimebutine* accelerates gastric emptying, induces premature phase III of the migrating motor complex in the intestine and modulates the contractile activity of the colon. Recently, *trimebutine* has also been shown to decrease reflexes induced by distension of the gut lumen in animals and it may therefore modulate visceral sensitivity. Clinically, t*rimebutine* has proved to be effective in the treatment of both acute and chronic abdominal pain in patients with functional bowel disorders, especially irritable bowel syndrome, at doses ranging

from 300 to 600 mg/day. It is also effective in children presenting with abdominal pain.

Ayse aktas M.D *et.al.,*(1999) has reported "The effect of *trimebutine* maleate on gastric emptying in patients with non-ulcer dyspepsia", To investigate the effect of *trimebutine,*a drug used in both hyperkinetic and hypo kinetic motility disorders, on gastric emptying in patients with non ulcer dyspepsia having prolonged gastric emptying rates and to compare the parameters used for the determinations of the lag period observed during the emptying of solid foods from the stomach. Gastric emptying was measured by the radionuclide technique. 20 normal volunteers and 43 patients with non-ulcer dyspepsia participated in the study. Radio nuclide imaging was performed by using a solid meal labeled with Tc- tin colloid of the patients with non –ulcer dyspepsia,20 had prolonged gastric emptying .they were given 3 weeks of oral treatment with *trimebutine* maleate and had their radio nuclide gastric emptying study repeated .treatment with *trimebutine* maleate resulted in reduction in duration of the lag period and less retention of food and 100 minutes($P<0.0005$).after treatment with *trimebutine* maleate ,no significant difference has been observed in the mean symptom score of patients with prolonged gastric emptying. Among the parameters used for the determination, of the lag period, lag period determined by a mathematical equation (TLAG) has been found to be longer than the lag period determined by visual inspection of the images (VLAG) and there was correlation between the two parameters when the lag time was short.

Poynard T *et.al.,* (2001) has reported "Meta analysis of smooth muscle relaxants in the treatment of irritable bowel syndrome" To up date previous

overview of placebo controlled double blind trails assessing the efficacy and tolerance of smooth muscle relaxants in irritable bowel syndrome method: a total of 23 randomized clinical trails were selected for meta analysis of their efficacy and tolerance. Six drugs were analysed : climetropium bromide (five trails), hyosine butyle bromide (three trails),Mebeverine (five trails) otilium bromide (four trails), pinaverium bromide(two trails), *Trimebutine* (four trails). The total number of patients included was 1888, of which 945 received an active drug and 943 a placebo. As resulted the mean percentage of patients with global improvements was 38% in the placebo group (n=925) and 56% in the myorelaxnt group (n=927) in favour of myorelaxants with a mean odds ratio of 2.13 P<0.001 (95% CI : 1.77 -2.58) and a mean risk difference of 22% P<0.001(95%CI: 13-32%) the percentage of patients with pain improvement was 41% in the placebo group (n=568) and 53% in myorelaxant group (n=567). Odds ratio 1.65, P<0.001 (95% CI : 1.30-2.10) and risk difference 18%, P<0.001 (95% CI: 7-28%). There was no significant difference for adverse events .concludely myorelaxants are superior to placebo in the management of the irritable bowel syndrome.

Miuria Y *et.al*.,(2005) has reported as "studies of metabolic pathways of *trimebutine* by simultaneous administration of *trimebutine* and its deuterium-labeled metabolite." *Trimebutine* maleate (I), (+-)-2-dimethylamino-2-phenylbutyl 3,4,5-trimethoxybenzoate hydrogen maleate, and a deuterium-labeled sample of its hydrolyzed metabolite, 2-dimethylamino-2-phenylbutanol-d3 (II-d3), were simultaneously administered to experimental animals at an oral dose of 10 or 50 mumol/kg, and distribution ratios of the two alternative initial metabolic steps, i.e., ester hydrolysis and N-demethylation, were estimated by determining the composition

of the urinary alcohol-moiety metabolites, II, and its mono- and di-demethylated metabolites, III and IV, by GC/MS. In dogs, the order of quantities of the metabolites from II-d3 was II much greater than III much greater than IV, showing predominance of conjugation over N-demethylation. However, this order was reversed when the amounts of the metabolites from I were compared, indicating that I was preferentially metabolized by N-demethylation followed by ester hydrolysis and conjugation in this order. In rats, a considerable proportion of I was presumed to be metabolized by ester hydrolysis before N-demethylation. In *in-vitro* experiments employing the liver microsomes and homogenates of liver and small intestine from rats and dogs, it was found that both ester-hydrolizing and N-demethylating activities were higher in rats than in dogs, and the conjugating activity was higher in dogs than in rats. It was also found that I, having a high lipophilicity, was more susceptible to N-demethylation than less lipophilic II. These results from the *in-vitro* experiments could account for the species differences in the distribution ratio of the metabolic pathways of I in vivo.

Patel S.M.*et.al*.,(2005) has reported on "The placebo effect in irritable bowel syndrome trials: a meta-analysis"[24], [25] .Despite the apparent high placebo response rate in Randomized placebo-controlled trials (RCT) of patients with irritable bowel syndrome(IBS), little is known about the variability and predictors of this response. To describe the magnitude of response in placebo arms of IBS clinical trials and to identify which factors predict the variability of the placebo response. performed a meta-analysis of published English language, RCT with 20 or more IBS patients who were treated for at least 2 weeks. This analysis is limited to studies that assessed global response (improvement in overall symptoms).The variables considered as potential placebo modifiers were study

design, study duration, use of a run-in phase, Jadad score, entry criteria, number of office visits, number of office visits/study duration, use of diagnostic testing, gender, age and type of medication studied Forty-five placebo-controlled RCTs met the inclusion criteria. The placebo response ranged from16.0 to 71.4% with a population-weighted average of40.2%, 95% CI (35.9–44.4). Significant associations with lower placebo response rates were fulfilment of the Rome criteria for study entry (P = 0.049) and an increased number of office visits (P =0.026). Placebo effects in IBS clinical trials measuring a global outcome are highly variable .Entry criteria and numbers of office visits are significant predictors of the placebo response. More stringent entry criteria and an increased number of office visits appear to in dependently decrease the placebo response.

Schiariti. M *et.al.,* (2009) has reported on "QT Interval Prolongation and Atypical Proarrhythmia:Monomorphic Ventricular Tachycardia with *Trimebutine*"[17],[18],[19]A 59-year old woman was admitted at emergency for palpitation and dizziness. Medication history showed *Trimebutine* 450 mg daily, because of meteorism, increased to 450 mg TID a week earlier. At admittance, sustained monomorphic ventricular tachycardia was interrupted by 100 mg intravenous lidocaine and a largely prolonged QTc (523 ± 12 ms) was seen. Discontinuation of *Trimebutine* achieved normalisation of QTc (420 ± 10 ms, $p<0.001$). This is the first report in man to illustrate a probable proarrhythmic action of *trimebutine.* A weak inhibitory effect on both rapid and slow components of the delayed rectifier in guinea-pig ventricular myocytes calls for further investigations in human myocardial tissues. *Trimebutine* inhibition of Na+ and Ca++ channels in cardiac tissues of rabbits and guinea-pigs also call for further studies in human myocardial tissues.

Hyun-thai lee *et.al.,* (2011) has reported as "*Trimebutine* as a modulator of gastrointestinal motility" *Trimebutine* has been used for treatment of both hypermotility and hypomotility disorders of the gastrointestinal (GI) tract, such as irritable bowel syndrome. In this issue, Tan et al. (2011) examined the concentration-dependent dual effects of *trimebutine* on colonic motility in guinea pig. The authors suggested that *trimebutine* attenuated colonic motility mainly through the inhibition of L-type Ca^{2+} channels at higher concentrations, whereas, at lower concentrations, it depolarized membrane potentials by reducing BK_{ca} currents, resulting in the enhancement of the muscle contractions. *Trimebutine* might be a plausible modulator of GI motility, which gives an insight in developing new prokinetic agents. Further studies to elucidate the effects of *trimebutine* on the interstitial cells of Cajal, the pacemaker in GI muscles would promote the therapeutic benefits as a GI modulator.

Kountouras J, *et.al*.,(2012)has reported on "*Trimebutine* as a potential antimicrobial agent: a preliminary *in-vitro* approach".[20],[21],[22] .The aim of this preliminary study was to investigate the *in-vitro* effect of "non-antibiotic" *trimebutine* against reference strains Staphylococcus aureus ATCC 29213, ATCC 25923, Escherichia coli ATCC 25922, ATCC 35218, Pseudomonas aeruginosa ATCC 27853 and Enterococcus faecalis ATCC 29212; microbiota that are potentially involved in the patho physiology of post-infectious functional gastrointestinal disorders. *Trimebutine* activity was assessed by the broth micro dilution method according to Clinical and Laboratory Standards Institute recommendations against reference strains S. aureus ATCC 29213 and ATCC 25923, E. coli ATCC 25922 and ATCC 35218, P. aeruginosa ATCC 27853 and E. faecalis ATCC 29212. Bactericidal

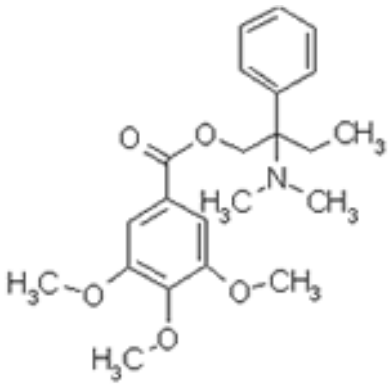
activity of the compound was determined by spreading a 10 µL aliquot on Mueller-Hinton agar from each dilution showing non-visible growth. All tests were carried out in triplicate. *Trimebutine* was active against all strains tested presenting with MIC ranging from 1024 to 4000 mg/L. MIC and MBC were similar for E. coli ATCC 25922 and P. aeruginosa ATCC 27853 whereas for Gram-positive isolates and E. coli ATCC 35218 the MBC was higher. Concludely they demonstrated the *in-vitro* bacteriostatic/bactericidal activity of *trimebutine* against bacteria frequently colonizing the gastrointestinal tract and potentially involved in human gastrointestinal infections that might trigger post-infectious functional gastrointestinal disorders.

DRUG PROFILE

NAME : *Trimebutine*

CHEMICAL NAME : 2-dimethyl amino-2-phenyl-butyl 3,4,5-trimethoxybenzoate

STRUCTURAL FORMULA:



EMPIRICAL FORMULA : $C_{22}H_{29}NO_5$

MOLECULAR WEIGHT : 387.5

CATEGORY : lower gastro intestinal tract motility regulator

SUB CLASS : non-competitive spasmolytic agent

PHYSICAL PROPERTIES : Appearance - white to off-white powder

Solubility - sparingly soluble in water

Melting point - 128°- 134°C

USE : To Treat spastic colon, Irritable Bowel Syndrome.

CLINICAL PHARMACOLOGY:

MECHANISM OF ACTION

The mode of actions of *trimebutine* [3,4,5-trimethoxybenzoic acid 2-(dimethyl amino)-2-phenylbutylester] on the gastrointestinal tract are mediated via (i) an agonist effect on peripheral mu (μ), kappa and delta (ɣ) opiate receptors and (ii) release of gastrointestinal peptides such as motilin and modulation of the release of other peptides, including vasoactive intestinal peptide, gastrin and glucagon. *Trimebutine* accelerates gastric emptying, induces premature phase III of the migrating motor complex in the intestine and modulates the contractile activity of the colon.[27],[28]

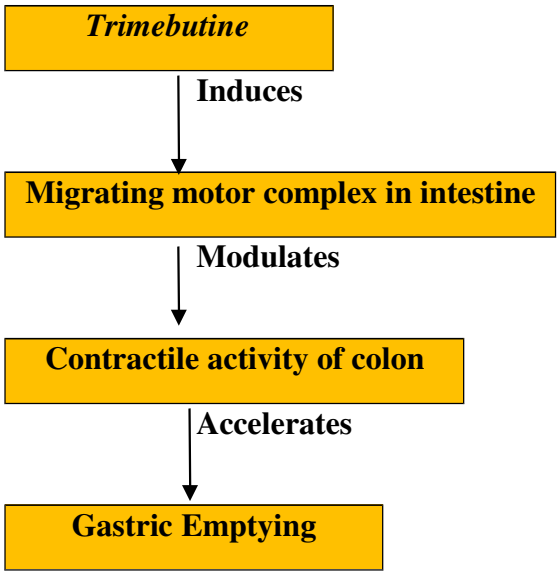
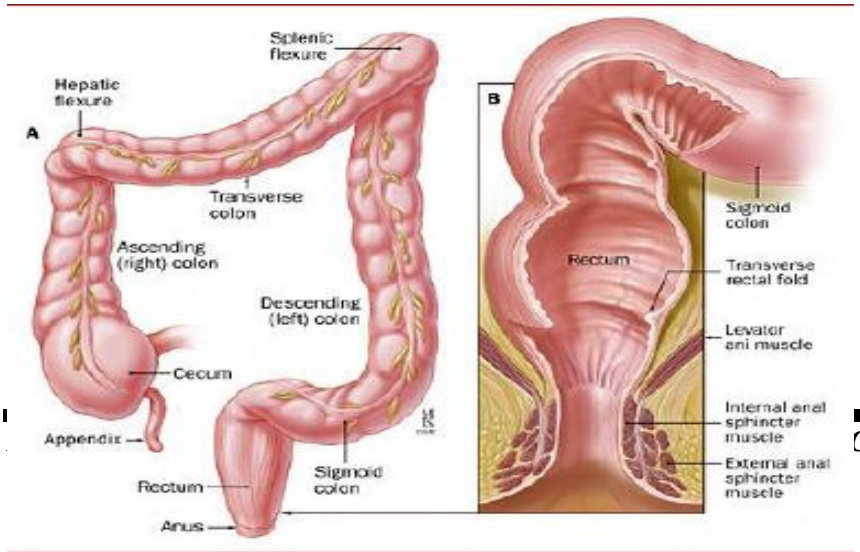


FIGURE 2: The above figure shows the mechanism of action of *Trimebutine* .



Mediation of gastro intestinal tract:

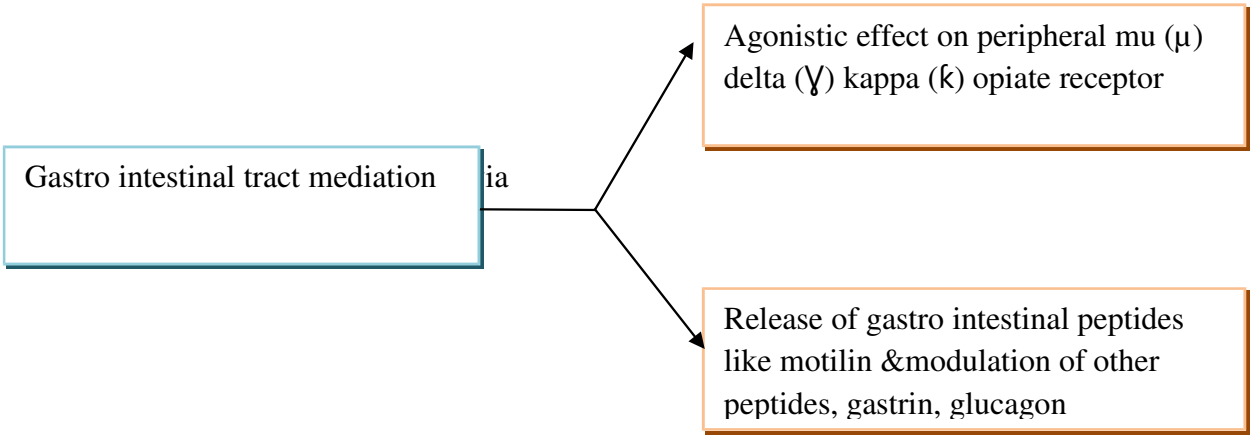


FIGURE 3: The above figures shows colon and the mediation of GIT

PHARMACOKINETICS

Absorption

Trimebutine maleate or its free base is rapidly absorbed after oral administration. Peak plasma concentrations of radioactivity were observed within one hour in man and rat and within 2-4 hours in the dog. Plasma radioactivity in man indicated a kinetic model with central and peripheral compartments and a mean distribution half-life of 0.66 hour.

Distribution

Tissue distribution studies showed high concentration of the radio labeled drug in the stomach and the intestinal walls of rat and in the major organs of metabolism and

excretion in mice. Placental transfer without teratogenic effect was observed in the rat. Protein-binding was less than 5% *in vivo* (rat plasma) and *in vitro* (bovine serum albumin).

Metabolism

Trimebutine undergoes N-demethylation process which is an oxidative reaction of phase I (N-dealkylation), here methyl groups are directly attached to the nitrogen atom which yields amines and amides after metabolized. However, two alternative initial metabolic steps, i.e., ester hydrolysis and N-demethylation which gives mono- and di-demethylated metabolites,

Trimebutine is extensively metabolised, although the metabolites appear to be pharmacologically inactive in man.

Elimination

Urine was the main route of elimination in all species while a small percentage (5-12%) of radioactivity was detected in the faeces. The plasma half-life of *Trimebutine* was short, but the elimination half-life of radioactivity was approximately 10-12 hours in man and rat. In the rat, an entero-hepatic circulation was also demonstrated. Extensive metabolism of the parent compound was indicated since less than 2.4% of the urinary radioactivity was found as unchanged drug in all species

Drug Interactions

Animal studies have shown that *trimebutine* maleate increases the duration of d-tubocurarine-induced curarization. No other drug interactions have been observed during clinical trials or otherwise reported**.**

Adverse drug reactions

In clinical studies, adverse effects of mild to moderate nature occurred in 7% of the patients treated with Modulon (*trimebutine* maleate). No single side effect occurred in more than 1.8% of the patients and some of these might have been related to the patient's condition rather than the medication. The commonly reported adverse effects are as follows:

a) Gastrointestinal: Dry mouth, foul taste, diarrhea, dyspepsia, epigastric pain, nausea and constipation were reported in total of 3.1% of the patient population;

b) CNS: Drowsiness, fatigue, dizziness, hot/cold sensations and headaches were reported in 3.3%;

c) Allergic reactions: Rash in 0.4% of the patients; and

d) Miscellaneous effects: Menstrual problems, painful enlargement of breast, anxiety, urine retention and slight deafness were also infrequently reported.

Dosage and administration

Trimebutine is available in the brand name of Modulon, Debridat, digedrat :

Tablets: Digedrate® 200 mg: each white, round, biconvex tablet, bisected on one side contains 200 mg of *trimebutine* maleate per tablet; bottles of 100.

The adult recommended dose is up to 600 mg daily in divided doses. It is administered as one 200 mg tablet three times daily before meals.

Contraindication

Trimebutine maleate is contraindicated in patients with known hypersensitivity to trimebutine maleate or any of the excipients. Although teratological studies have not shown any drug related adverse effects on the course and outcome of pregnancy in laboratory animals by both oral and parenteral routes, the use of Modulon *(trimebutine* maleate) in pregnant women is not recommended.

Children: Not recommended for use in children under 12 years of age.

DISEASE SPECIFIC LITERATURE REVIEW

IRRITABLE BOWEL SYNDROME

INTRODUCTION

Irritable Bowel Syndrome (IBS) is one of the most common ailments of the bowel (intestines) and affects an estimated 15% of people in the US. The term, irritable bowel, is not a particularly accurate one since it implies that the bowel is responding irritably to normal stimuli, and this may or may not be the case. The several terms used for IBS, including spastic colon, spastic colitis, and mucous colitis, attest to the difficulty of getting a descriptive handle on the ailment. Moreover, each of the other names is itself as problematic as the term IBS.

Irritable Bowel Syndrome (**IBS** or **spastic colon**) is a symptom-based diagnosis characterized by chronic abdominal pain discomfort, bloating, and alteration of bowel habits. As functional gastro intestinal disorder (FGID), IBS has no known organic cause.[1]

CLASSIFICATION

IBS can be classified as 3 sub classes

a) Diarrhea-predominant (IBS-D),
b) Constipation-predominant (IBS-C), or with
c) Alternating stool pattern (IBS-A) or pain-predominant.[51]In some individuals,

IBS may have an acute onset and develop after an infectious illness characterized by two or more of the following: fever, vomiting, diarrhea, or positive stool culture. This post-infective syndrome has consequently been termed "post-infectious IBS" (IBS-PI).

ETIOLOGY

The cause of IBS is unknown; several hypotheses have been proposed. The risk of developing IBS increases sixfold after acute gastrointestinal infection. Post infection, further risk factors are young age, prolonged fever, anxiety, and depression. Theories of the cause of IBS include abnormal input from intestinal sensory nerves, abnormal processing of input from the sensory nerves, and abnormal stimulation of the intestines by the motor nerves. Causes of IBS usually stress. Genetic tendency, drug addiction, other infections and some food sensitivity food allergic reactions all these may leads to abnormal stimulation of the intestinal motility. The causes of IBS are summarized below:

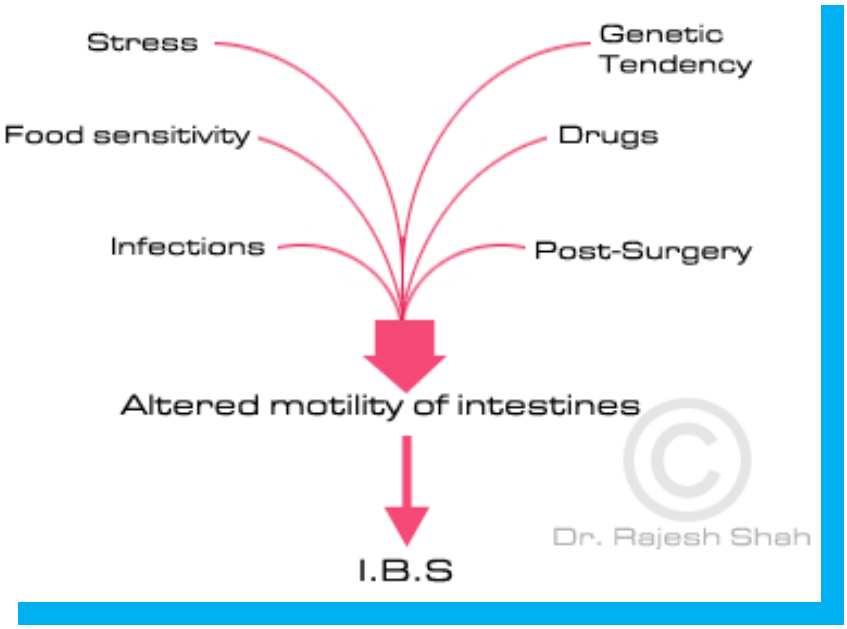


Figure 4 : shows causes of Inflammatory Bowel Syndrome

PATHO PHYSIOLOGY

A system of nerves runs the entire length of the gastrointestinal tract from the esophagus to the anus in the muscular walls of the organs. These nerves communicate

with other nerves that travel to and from the spinal cord. Nerves within the spinal cord, in turn, travel to and from the brain. (As an organ system, the gastrointestinal tract is exceeded only by the spinal cord and brain in the numbers of nerves it contains.) Thus, the abnormal function of the nervous system in IBS may occur in a gastrointestinal muscular organ, the spinal cord, or the brain. "Brain-gut response to stress and cholinergic stimulation in IBS" The nervous system that controls the gastrointestinal organs, as with most other organs, contains both sensory and motor nerves. The sensory nerves continuously sense what is happening within the organ and relay this information to nerves in the organ's wall. From there, information is relayed to the spinal cord and brain. The information is received and processed in the organ's wall, the spinal cord, or the brain. Then, based on this sensory input and the way the input is processed, commands (responses) are sent to the organ through the motor nerves. Two of the most common motor responses in the intestine are contraction or relaxation of the muscle of the organ and secretion of fluid and/or mucus into the organ. the role of brain-gut "axis" appeared in the 1990s, such as the study "Brain-gut response to stress and cholinergic stimulation in IBS"

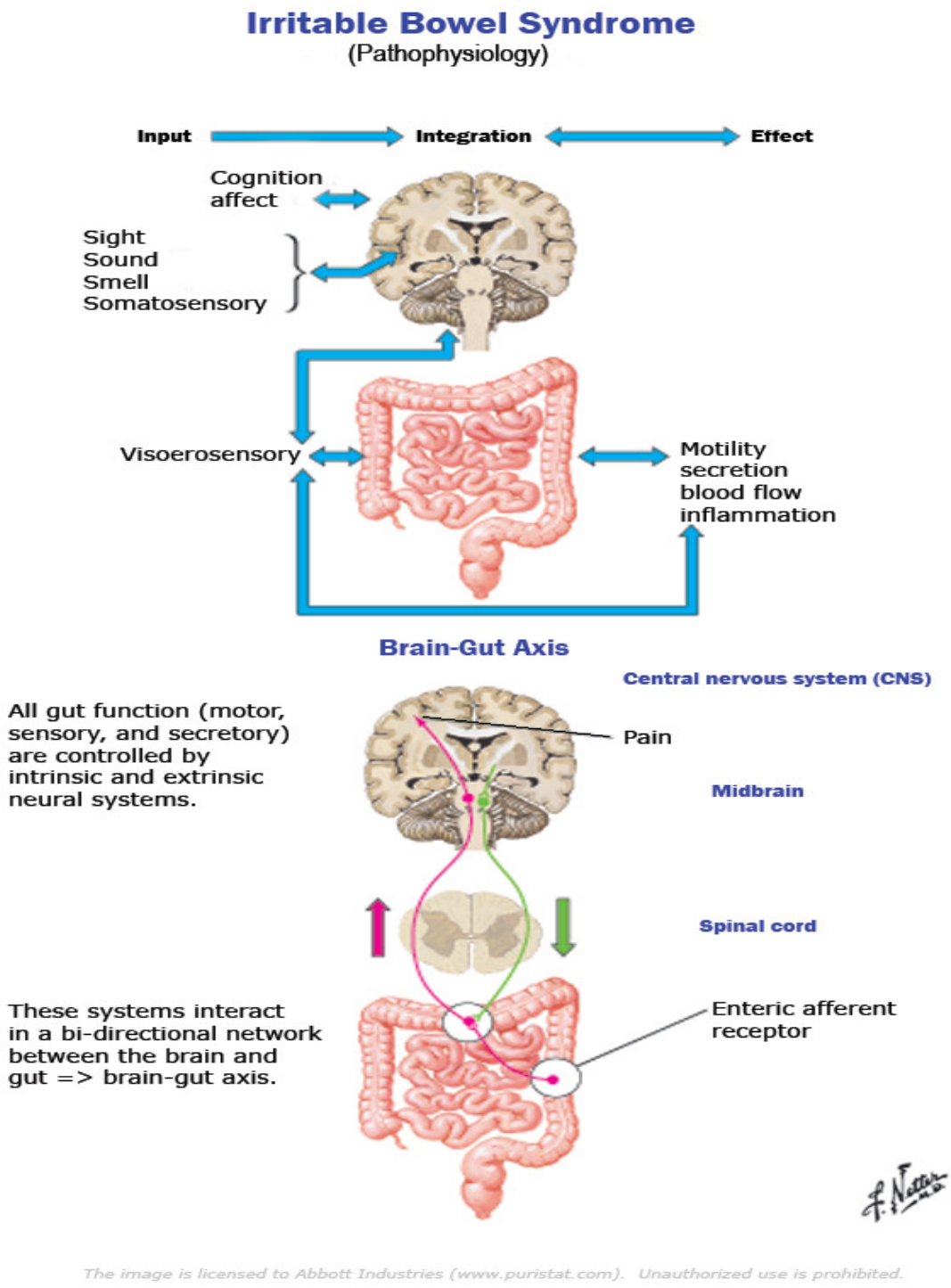


Figure 5: shows the pathophysiology of Inflammatory Bowel Syndrome

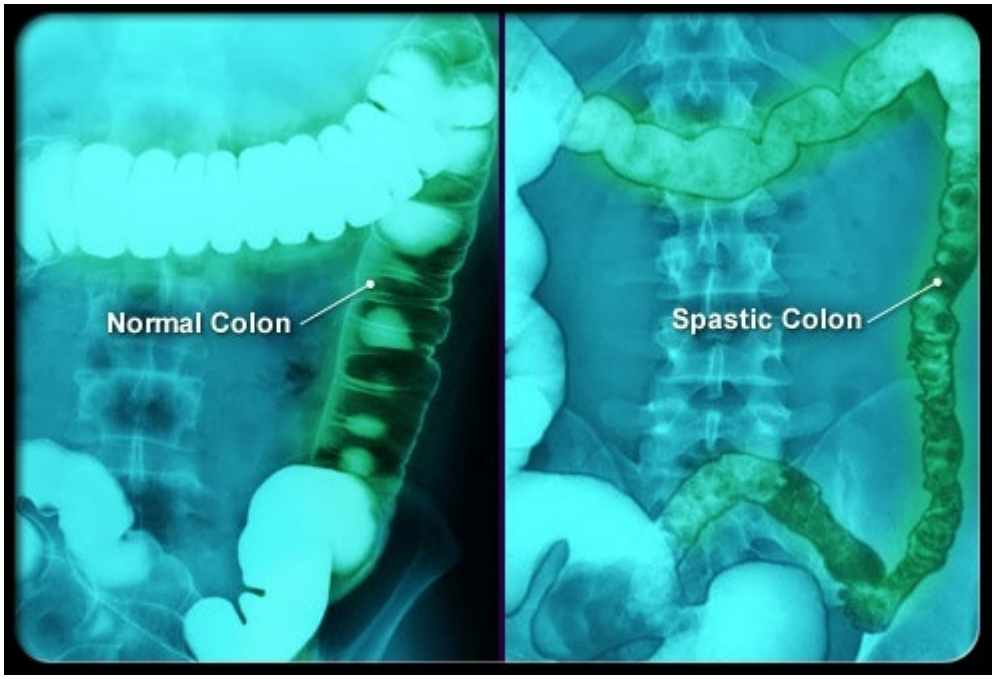


Figure 6: shows the differences of normal colon and spastic colon

SIGNS AND SYMPTOMS

The primary symptoms of IBS are abdominal pain or discomfort in association with frequent diarrhea or constipation and a change in bowel habits.[47]There may also be urgency for bowel movements, a feeling of incomplete evacuation (tenesmus), bloating, or abdominal distension[24]In some cases, the symptoms are relieved by bowel movements.[7] People with IBS, more commonly than others, have gastro esophageal reflux, symptoms relating to the genitourinary system, chronic fatigue syndrome, fibromyalgia, headache, backache and psychiatric symptoms such as depression and anxiety.[48][49] Some studies indicate that up to 60% of persons with IBS also have a psychological disorder, typically anxiety or depression.[50]

DIAGNOSIS

There is no specific laboratory or imaging test that can be performed to diagnose irritable bowel syndrome**.** Investigations are performed to exclude other conditions:

Stool microscopy and culture (to exclude infectious conditions)

- Blood tests: Full blood examination, Liver function tests, Erythrocyte sedimentation rate, serological testing for coeliac disease
- Abdominal ultrasound (to exclude gallstones and other biliary tract diseases)
- Endoscopy and biopsies (to exclude peptic ulcer disease, coeliac disease, inflammatory bowel disease, malignancies)
- Hydrogen breath testing (to exclude fructose and lactose malabsorption)

TREATMENT

A number of treatments have been found to be better than placebo, including fiber, antispasmodics, and peppermint oil

Proper diet plan can reduce symptoms in functional GI disorders such as IBS by 60-80%

Drugs classifications:

- Absorbants - Ispaghula, Psyllium
  Methyl cellulose.
- Anti secretory - Sulfasalazine mesalazine, Olsalazine
  Balsalazine, Bismuth sub salicylate, Racecodrotil
- Corticosteroids – Prednisolone.
- Immunosuppressants – Azathioprine , Methotrexate, Cyclosporine, Infliximab
- Anti motility – Codeine, diphenoxylate , loperamide .
- Spasmolytic agents - met chlorpramide.
- GIT regulators - Trimebutine,
- Seratonergic drugs – prucalopride

- Anti inflammatory agents and mast cell stabilizers –ketotifin
- Antibiotics - rifaximin
- Probiotics
- Other new drugs - Furiox,

OBJECTIVES OF STUDY

PRIMARY:

To investigate the bioequivalence of *Trimebutine* 200 mg capsules (Product Test).and Digedrat® (Product Reference) 200 mg capsules in 48 healthy, adult, human, male and non-pregnant female subjects under fasting conditions.

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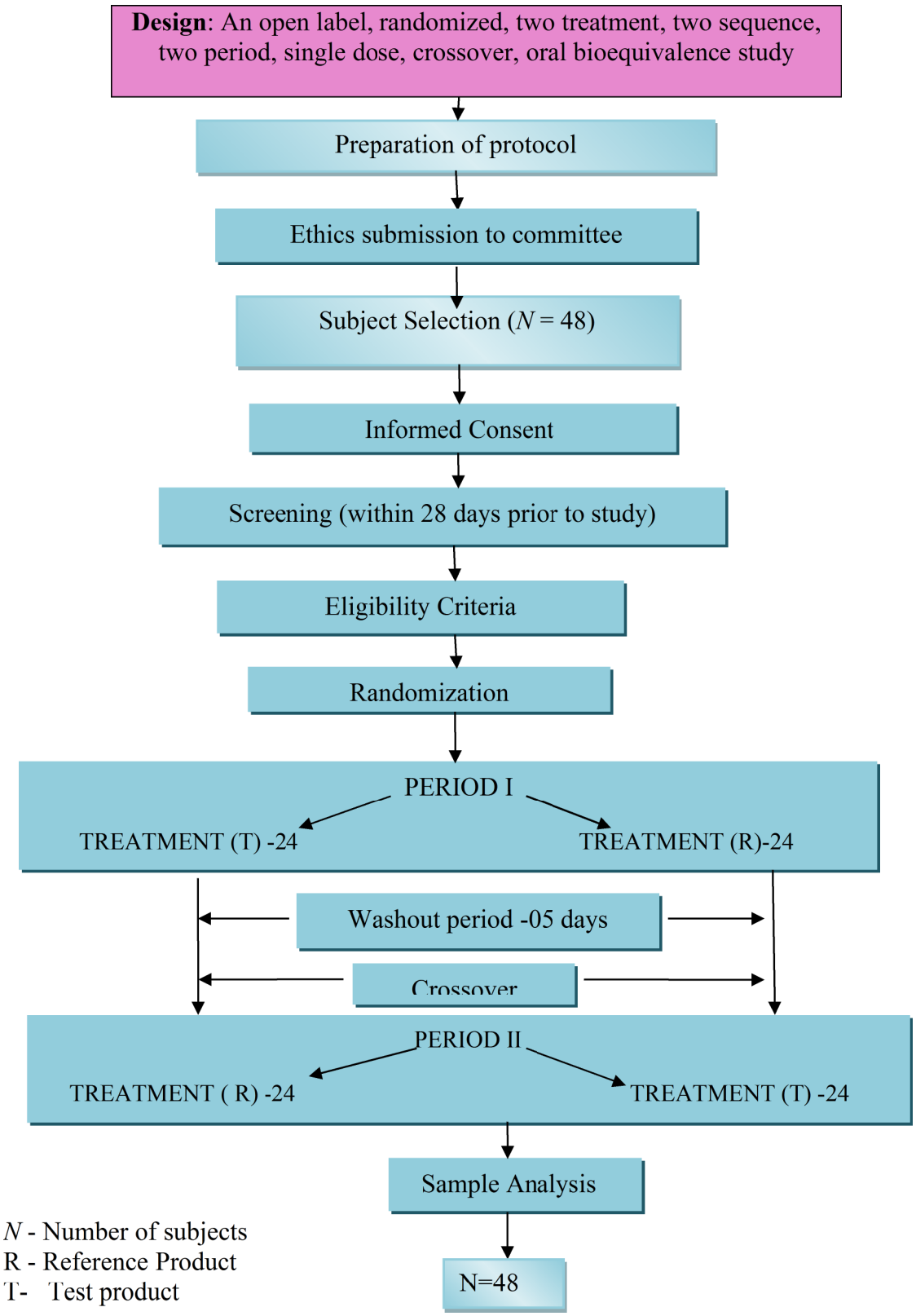
SECONDARY:

To monitor adverse events and ensure safety of the subjects.

Plan of work:



N - Number of subjects
R - Reference Product
T- Test product

MATERIALS AND METHODS

STUDY DESIGN:

An open label, randomized, two treatment, two sequences, two period, single dose, crossover, oral bioequivalence study under fasting conditions.

STUDY CENTRE:

Azidus laboratories Ltd., Rathnamangalam, Vandalur, Chennai-48.

STUDY POPULATION:

Healthy, adult, human, male and non-pregnant female volunteers

SAMPLE SIZE:

48 subjects (24 male and 24 non-pregnant female)

STUDY TYPE:

Fasting study.

NUMBER OF PERIODS:

02

STUDY DURATION:

09days

CLINICAL PROCEDURE:

STUDY DESIGN

An open label, balanced, randomized, two treatment, two sequence, two period, single dose, cross over, oral bioequivalence study of *Trimebutine* 200 mg capsules (Test)and Digedrat® (Product Reference) 200 mg capsules in 48 healthy, adult, human, male and non-pregnant female subjects under fasting conditions.

Open label: It is a condition where both the physician and the participating subjects know about the treatment administered.

Balanced: In the randomization schedule the blocks will be balanced.

Two treatments: One test product and one reference product.

Two sequence: One sequence is TR and the other RT.

R=Reference; **T**=Test

Single dose: *Trimebutine* 200 mg capsules.

Number of subjects: 48 Healthy adult human volunteers will be included in the study.

Washout period: At least 05 days will be given as the wash out period for each dosing.

Blinding: This study is an open labeled study the subjects and the investigator will not be blinded towards the identity of the study treatments.

Test and Reference products

Test(T): *Trimebutine* 200 mg capsules

Reference(R): Digedrat® 200 mg capsules

Receipt and storage of Investigational products

The sponsor supplied sufficient quantity of the investigational products to the testing facility along with their certificate of analysis (COA) for conduct of the study. The test and reference products supplied in sealed packages or stripes labeled with product name, strength, Number of dosage units, Manufacturer name, Lot number or Batch number, Expiration date and storage conditions.

After receipt of the investigational products, test and reference products has kept in separate storage box (labeled with project NO., Name of IP, Type of IP, Batch NO, and Expiry date and storage conditions) and stored in Refrigerator located in pharmacy.

Upon completion or termination of the study, the unused investigational products will be returned to sponsor.

Accountability of the Investigational products

The record of the total medications received and the quantity of drug used and retained with or returning to the sponsor will be maintained in the pharmacy.

Selection and with drawl of subjects

Subject screening

Each subject had undergone screening procedure for health assessment which consists of a complete medical history, physical examination with vital signs, clinical laboratory evaluations,12-lead ECG and chest x-ray PA view (if not done in the past 6 months or if clinically indicated).The physical examination findings, ECG and the laboratory tests can be considered valid maximum upto 28 days prior to the dosing in first period of the study.

Clinical laboratory tests

Screening Laboratory tests				
Biochemistry	**Hematology**	**Urine (routine analysis)**	**Serology**	**Others**
• Blood Sugar-Random • Serum Urea • Creatinine • Total Cholesterol • Triglycerides • Serum Bilirubin (Total) • Serum Bilirubin (Direct) • Serum Bilirubin (Indirect) • Uric acid • ALT • AST • Alkaline Phosphatase • Total Protein	• Total W.B.C. count • Differential leukocyte count • Total R.B.C. count • Hemoglobin • Haematocrit (PCV) • Platelets count • ESR (1st Hour) • Blood Grouping &	• Turbidity • Colour • RBCs • Bilirubin • Urobilinogen • Ketone • Protein • Nitrite • Glucose • pH • Specific Gravity • Microscopic Examination	• HIV 1 & 2 • Hepatitis B surface antigen Hepatitis C antibody • Syphilis	• Chest X ray PA view • ECG in 12 leads • Urine drugs of abuse* • Alcohol breath analysis* • Urine pregnancy test(in case of females)*

• Albumin • Globulin • A/G Ratio • LDH • Gamma Glutamyl Transferase • Sodium • Potassium • Chloride • Calcium • Serum pregnancy test(in case of females)	Rh Typing	(Leucocytes & Epithelial cells)		

INCLUSION CRITERIA

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

- **Gender**: Healthy male and/or non-pregnant female volunteers.
- **Age**: 20 - 45 yrs (both years inclusive)
- Willing to give informed written consent and comply with the study requirements.
- Female volunteers practicing an acceptable method of birth control as judged by the investigator(s), such as condom with spermicide, diaphragm with spermicide, intrauterine device (IUD), or abstinence throughout the duration

of the study; or of postmenopausal (no menses) status of at least 1 year; or surgically sterile (bilateral tubal ligation, bilateral oophorectomy, or hysterectomy).

- Subject should be able to communicate effectively.
- Non-smokers.
- Body Mass Index **(BMI**) 18.50-24.90 Kg/m^2 for males, 18.50-30.00 Kg/m^2 for females, and body weight not less than 50 kg.
- Healthy individuals as evaluated by personal history, medical history and general clinical examination.
- **Vital parameters** –

  BP: 100 – 139 mmHg systolic and 60 – 89 mmHg diastolic.

  Pulse rate: 60 – 100 / min.

  Oral temperature: between 97.8˚ F and 99.0 ˚ F.

  Respiratory rate: 14-18/min.

- Normal biochemical, hematological and urinary parameters.
- Normal Chest X - ray PA view & ECG in 12 leads.
- Negative for HIV 1 & 2, Hepatitis B, Hepatitis C, and Syphilis tests.
- Negative urine test for drugs of abuse for morphine, barbiturates, benzodiazepines, amphetamine, THC & cocaine (to be performed on the day of check in during each period).

- Negative serum test for pregnancy during screening and Negative urine pregnancy test on the day of check in during each period (for female volunteers).
- Negative alcohol breath analysis (to be performed on the day of check in during each period)

EXCLUSION CRITERIA

. The subjects will be excluded based on the following criteria:

- Subject incapable of understanding the informed consent.
- History of any major surgical procedure in the past 3 months.
- History of diabetes mellitus, tuberculosis and systemic hypertension.
- History suggestive of cardiac, gastrointestinal, respiratory, hepatic, renal, endocrine, neurological, metabolic, psychiatric or hematological systems, judged to be clinically significant.
- History of dysphasia.
- History of any medical disorder that is of significance in the investigator's opinion.
- Present or past history of smoking, alcohol intake or drug abuse.
- History of consumption of tobacco containing products.
- History of hypersensitivity to *Trimebutine* and related drugs or excipients in the formulation (if the excipients are known).

- History of allergy to vegetables and / or food substances and / or any other manifestations suggestive of hypersensitivity reactions.
- Present or past history of intake of drugs* which potentially modify kinetics / dynamics of *Trimebutine* or any other medication judged to be clinically significant by the investigator.
- Consumption of grapefruit / its products within 48 hours prior to the start of study.
- Intake of any prescription drug within 14 days or over-the counter (OTC) drugs within 7 days prior to study and / or intake of any drug* in the past that could affect the kinetics or dynamics of *Trimebutine* in view of investigator.
- Subject with clinically significant abnormal values of laboratory parameters.
- Pregnant, Lactating Females.
- Subject who had participated in any other clinical study during the last 6 months.
- Subject who had bled in the past 6 months from the date of start of study either for blood donation or for any other reason.
- History of habituation to coffee, tea or other xanthine containing products and inability to

  Withhold the intake during the - in house - stay.
- Drugs that can potentially affect the hepatic metabolism* of other drugs are as listed below (not limited to the list, though)

*Hepatic microsomal enzyme inducers (which can reduce the systemic bioavailability):- Barbiturates, Carbamazepine, Ethanol (chronic), Inhalational anaesthetics, Griseofulvin, Phenytoin, Primidone, Rifampicin.

CRITERIA FOR DISCONTINUATION OR WITHDRAWAL FROM THE STUDY.

Subjects will be withdrawn or may discontinue from the study for any of the following reasons. In case of withdrawal, the details must be documented in the appropriate Case Report Form (CRF).

- The subject withdraws consent.
- Development of intolerable adverse event.
- Emesis occurred at or before two times reported median Tmax and/or significant diarrhea, in any period.
- Development of an inter current illness or condition for which the subject requires concomitant medications which may interfere with the kinetics of the study medication.
- Discovery that the subject entered the study in violation of the protocol or occurrence of a significant protocol violation during the study.
- The investigator feels that in the best interest of the subject's health, the subject is to be withdrawn from the trial.
- Data not known before starting the trial become available and raise concern about the safety of the study drug so that continuation would pose potential risk to any particular subject.

STUDY PROCEDURE:

The study was conducted after obtaining approval from the Independent Ethics Committee (IEC). Prospective volunteers were explained about the study procedure and purpose. After obtaining written informed consent from willing participants, they were subjected to screening by History, Clinical examination and laboratory investigations.

HOUSING:

In all periods, the subjects were housed in the clinical facility, minimum 12 hours prior to dosing until 48 hours post dose. All subjects were maintained in a fasting state for a minimum 8 hours prior to dosing.

- ✓ Subjects were admitted at least 12 hours before the proposed time of drug administration.
- ✓ After drug administration, subjects had to stay for at least 48 hrs. The duration of stay in each period (from subject check-in to check-out) was 60 hrs, spreading over a period of 4 days.

DIET& WATER:

- ✓ Being a fasting study, subjects were not served any breakfast on the day of dosing.
- ✓ Subjects received standard food, approximately at 4, 8 and 12,24,28,32 hours post-dose for lunch, snacks, dinner, Breakfast, lunch and snacks respectively.
- ✓ During the study, meal plans were identical for all the subjects.

RANDOMIZATION:

Study subjects received any of the two investigational (test/reference) products in each of the study periods. The order of receiving the test and reference product for each subject was according to the randomization schedule. The randomization schedule was generated by using SAS®.

Each study subject were randomly assigned to one of the following dosing sequences.

	Period I	**Period II**
Sequence I	T	R
Sequence II	R	T

DISPENSING

The designated study personnel dispensed a quantity of the test and reference product a day before dosing in each period of the study. The doses transferred to the drug dispensing sachets as randomization schedule and properly labeled with the study number, randomization code and period.

PERIOD I:

Pre- dosing day:

- ✓ The subjects enrolled were instructed to come one day prior to the study day for admission.
- ✓ On admission, the vital signs were recorded.

- ✓ Dinner was provided and the subjects fasted overnight for at least 10 hours prior to dosing.

Study day:

- ✓ **7** AM: An intravenous cannula was inserted in forearm vein for blood collection and pre-dose blood sample was collected for estimating the baseline biochemical parameters .vital signs were recorded.
- ✓ 8 AM: Dosing was done (with 2 mts interval for each subject) as per randomization schedule. One tablet of *Trimebutine* 200 mg capsule was administered with 200 ml of water under direct observation .Dosing were done 4 stations (from S1 to S12 in Ist station , S13 to S24 in II nd station ,S25 to S36 in IIIrd station, S37 to S48 IV th station)
- ✓ The subjects were dosed in sitting posture and they remained in erect posture for four hours.
- ✓ They were not permitted to drink water for one hour prior to dosing and one hour post dosing .At all other times, drinking water was made available.
- ✓ Subjects were instructed to report if they had any side effects.
- ✓ A total of 22 blood samples (each 5ml) were collected from each subject at specific time points.
- ✓ Vital signs were recorded at .00, 04.00, 08.00, 12.00 and 24.00hours post-dose.
- ✓ The subjects were closely monitored for the adverse events of *Trimebutine.*
- ✓ At the end of 48 hours, the volunteers were discharged.

WASH OUT PERIOD

- ✓ The subjects were asked to come again for admission after a 5-days wash out period

PERIOD II:

Pre dosing day:

- ✓ The subjects enrolled were instructed to come one day prior to the study day for admission.
- ✓ On admission, the vital signs were recorded.
- ✓ Dinner was provided and the subjects fasted overnight for at least 10 hours prior to dosing.

Study day:

- ✓ **7** AM: An intravenous cannula was inserted in forearm vein for blood collection and pre-dose blood sample was collected for estimating the baseline biochemical parameters .vital signs were recorded.
- ✓ 8 AM: Dosing was done (with 2 mts interval for each subject) as per randomization schedule. One tablet of *Trimebutine* 200 mg capsule was administered with 200 ml of water under direct observation .Dosing were done 4 stations (from S1 to S12 in Ist station , S13 to S24 in II nd station ,S25 to S36 in IIIrd station,S37 to S48 in IV th station).
- ✓ The remaining procedures were followed as same in period I.
- ✓ At the end of 48, hour's volunteers were discharged.
- ✓ After completion of the study, blood samples were collected for post drug analysis to confirm that the drug has been eliminated completely from the physiological system.
- ✓ The laboratory post study values were compared with pre-study laboratory test values .if any clinically significant deviations were seen, the subjects were followed up and appropriate medical care provided.

DRUG ADMINISTRATION:

A single oral dose of test or reference product was administered to study subjects with 2 minutes gap between each subject in all the two periods in sitting posture at fixed time points with 200 ml of water at ambient temperature in each period. The order of receiving test and reference products will be as per

randomization schedule. This activity will be followed by a mouth check to assess compliance to dosing.

POSTURAL RESTRICTIONS:

Subjects will be administered with study medications in sitting posture. Subjects will remain upright (sitting) for the first 04 hrs post dose except for any procedural reason or when the subject experienced the adverse event or in case of any natural exigency.

No exercise or strenuous physical activities are permitted during the in-house stay.

METHODS OF BLOOD SAMPLE COLLECTION:

Before dosing:

Blood samples were collected through an indwelling cannula placed in forearm vein. If there was difficulty in obtaining through intravenous cannula, blood samples were obtained by direct venous puncture.

While collecting blood sample, after insertion of the cannula and after withdrawal of every sample of blood, 0.5 ml of heparinised saline (10 IU/ml) was injected to maintain the potency of cannula. After discarding the initial 0.5 ml of blood, the required volume of blood was collected. Blood samples were collected using pre-labeled vials containing K_3 EDTA.

In each period 22 samples were collected from each subject as per the following schedule. The pre-dose samples will be collected within 01 hour prior to drug dosing.

After dosing:

The post-dose samples were collected at 00.17, 00.33, 00.50, 00.67, 00.83, 01.00, 01.25, 01.50, 01.75, 02.00, 02.33, 02.67, 03.00, 04.00, 06.00, 08.00, 12.00, 18.00, 24.00, 36.00 and 48.00 hours post dose (Total of 22 samples - 5 mL each). All 22 samples will be collected as in-house samples.

The total volume of blood draw from each subject during the study will not exceed 258.0 mL.

[22x5mLx2 = 220 mL] + [0.5 mL discarded for first 22 in-house blood draws 22x0.5mLx2 = 22 mL] + [screening =10 mL] + [post study safety evaluation = 6 mL] = 258.0 mL.

SAMPLE STORAGE

After collection, blood samples were centrifuged at 4000 rpm for 10 minutes at 4°C ± 2°C to separate plasma. Following centrifugation, plasma will be separated into two aliquots the first aliquot will have 1.5 ml of plasma and the second aliquot, the remaining plasma stored at -20°C. After completion of the study, the plasma samples of two periods were transferred to the Bioanalytical department for analysis. While transferring the samples, adequate measures were taken for maintaining the temperature and integrity of samples.

SAFETY ASSESSMENT

In each period, vital signs measurement and subject well being assessment will be done during subject check-in and at 00.00 hrs (pre dose) and at 02.00, 04.00, 08.00, 12.00, 24.00 hours post-dose and during check-out. If 00.00 hours vitals found to be abnormal, decision will be taken by the Principal Investigator regarding exclusion of the subject from the study.

Post study procedure:

Following tests were performed at the end of period II

1. Hematology— Hemoglobin in %, Platelet Count,PCV.

2. Liver functions— Serum Glutamate Oxaloacetate Transaminase, Serum Glutamate Pyruvate Transaminase

3. Serum Chemistry--Random Blood Sugar, Urea, Creatinine. Serum Pregnancy test.

4.others --ECG

Physical examination, vital parameter assessment and wellbeing assessment were performed to all study subjects during each period & during check-out, to ensure safety of the study.

The laboratory post study values were compared with pre-study laboratory test values. If any clinically significant deviations were seen, the subjects were followed up and appropriate medical care was provided.

REPORTING OF AE AND SAE

In case of Serious Adverse Events (SAE), the event should be reported to the sponsor immediately by telephone and then the copy of SAE form to be faxed or scanned & sent by email in 24 hrs & the same shall be sent through post. In turn the sponsor should report to the regulatory authority with in 10 calendar days. The

investigator should inform the ethics committee about SAE in within 24hours in writing.

In case of AEs, the investigator needs to complete the AE form and may send the copies to the sponsor and IEC in his/her own discretion.

FOLLOW UP OF AE AND SAE

After the initial AE / SAE report, the investigator is required to proactively follow each subject and provide further information to the sponsor on the subject's condition.

All AEs and SAEs will be followed until resolution, until the condition stabilizes, until the event is otherwise explained or until the subject is lost from follow up. The investigator will ensure that follow up includes any supplemental investigations as may be indicated to elucidate the nature and / or causality of the AE or SAE. This may include additional laboratory tests or investigations, histo pathological examinations or consultation with other health care professionals.

DOCUMENTATION OF DATA:

PROTOCOL DEVIATIONS AND AMENDMENTS

All important deviations related to study inclusion or exclusion criteria, conduct of the study, subject management were informed to the sponsor and were mentioned in the final report.

BIOANALYTICAL METHODOLOGY

A validated Liquid Chromatography and Mass Spectroscopy (LC-MS/MS) method was employed for the estimation of *Trimebutine* in plasma. Samples with drug concentration greater than upper limit of the validated range of the analysis were diluted using the appropriate drug free biological fluid and reanalyzed by dilution integrity testing.

Incurred sample analysis was carried out for a minimum 10% of total study samples. Samples, which were below the lower limit of quantification (LLOQ) was reported as below limit of quantification (BLQ).

INSTRUMENT

Mass Spectrometric Conditions

Agilent Infinity 1290-Binary Pump, Auto Sampler, Degasser, Agilent 6460 Triple Quad LC/MS.

S.No	Parameter	
1	Ion Source	ESI+Agilent Jet Stream
2	Polarity	Positive
3	Gas Temp (°C)	300
4	Gas Flow (l/min)	8
5	Nebulizer (psi)	30
6	Sheath Gas Temp (°C)	350
7	Sheath Gas Flow (1/min)	7
8	Capillary (V)	4000
9	Nozzle Voltage (V)	500

Multiple Reacting Mode Conditions

S.No.	Parameter	Trimebutine	Verapamil (IS)
1	Precursor Ion	388.2	455.3
2	Product Ion	343.2	165.1
3	Fragmentor (V)	98	157
4	Collision Energy (V)	5	26

Chromatographic Conditions:

S.No.	Parameters	
1	Column	GeminiC18,50x2.00mm,3µ
2	Mobile Phase	Acetonitrile:2mM Ammonium acetate (90:10)
3	Flow Rate	0.2 mL/minute
4	Auto sampler Temperature	6°C
5	Column Oven Temperature	30°C
6	Injection Volume	5 µL
7	Run Time	2.5minutes
8	Retention time	1.4 ±0.5minutes for Analyte and 1.2 ±0.5minutes for IS

VALIDATION METHOD OF *TRIMEBUTINE*:

Bioanalytical procedure:

Materials Required:

- *Trimebutine* (Working Standard)
- verapamil (Internal Standard)
- Methanol (HPLC Grade)
- Di Sodium Hydrogen Ortho Phosphate (General Reagent)
- . Tert Butyl Methyl Ether
- . Acetonitrile (HPLC Grade)
- . Milli Q Water
- Formic acid (General Reagent)
- Ammonium Acetate (HPLC Grad

Preparation of Reagents and Solutions

Preparation of Diluent : (Methanol: Water(50:50)v/v)

Transfer 250mL of Methanol in 500 mL reagent bottle containing 250mL of MilliQ Water and mix well. Fill the respective 'SOP Forms' and label the solution as per relevant SOP.

Preparation of Buffer I :(2mM Ammonium acetate)

Weigh accurately about 0.1542 gm of Ammonium acetate and transfer into a 1000mL Reagent bottle. Dissolve it in Water and make up the volume with the same to produce a 1000mL solution. Mix well and sonicate it for 5 minutes and filter through 0.22µ membrane filter. Fill the respective 'SOP Forms' and label the solution as per relevant SOP.

Preparation of Mobile Phase:

Preparation of Acetonitrile: Buffer I(90:10) v/v)

Transfer 900mL of Acetonitrile into a clean 1000mL reagent bottle containing 100 mL of 2 mM Ammonium acetate. Mix well and sonicate for 5 minutes. Fill the respective 'SOP Forms' and label the solution as per relevant SOP.

Preparation of 0.1M Di Sodium Hydrogen Ortho Phosphate :

Weigh accurately about 1.4196gm of Di Sodium Hydrogen Ortho Phosphate and Transfer to 100 mL Standard flask. Make up the volume to 100 mL with Milli Q water, mix well and sonicate it for 5 minutes and filter through 0.22µ membrane filter. Fill the respective 'SOP Forms' and label the solution as per relevant SOP.

Preparation of Rinsing solution: (Methanol: Water (90:10) v/v)

Transfer 900mL of Methanol in 1000mL reagent bottle containing 200mL of Milli Q-Water. Mix well the reagent bottle. Fill the respective 'SOP Forms' and label the solution as per relevant SOP.

Preparation of *Trimebutine* Stock Solution:

Weigh accurately about 2mg of *Trimebutine* maleate and transfer into a 2mL volumetric flask. Dissolve it in Methanol and make up the volume with the same to produce a solution of 1mg/mL. Fill the respective 'SOP Forms' and label the solution as per relevant SOP.

Preparation of CC Samples Stock Dilutions:

From *Trimebutine* stock solutions ,Stock Dilutions ranging from 262.4549ng/mL to 30031.3643ng/mL with (Methanol: Water / 50:50), as provided in the Table No.1

Table No. 1 - Stock Dilution for Calibration Standards

S.NO	Initial Stock Conc. (ng/mL)	Volume of Stock taken (mL)	Volume of Diluent added (mL)	Total Volume of Final Solution (mL)	Final Stock Conc. (ng/mL)
SS1	79029.9060	3.800	6.200	10	30031.3643
SS2	30031.3643	8.000	2.000	10	24025.0914
SS3	24025.0914	6.300	3.700	10	15135.8076
SS4	15135.8076	6.000	4.000	10	9081.4846
SS5	9081.4846	3.400	6.600	10	3087.7048
SS6	3087.7048	5.000	5.000	10	1543.8524
SS7	1543.8524	3.400	6.600	10	524.9098
SS8	524.9098	5.000	5.000	10	262.4549

Preparation of Calibration Standards and Quality Control Samples

***Trimebutine* Calibration Standards in Human Plasma:**

From *Trimebutine* CC Stock Dilutions, Calibration Standards in Human K_3ETDA plasma ranging from 5.2491ng/mL – 600.6273ng/mL for *Trimebutine* as provided in the Table No.2

Table No. 2 – *Trimebutine* Calibration Standards in Human plasma

S.NO	**Stock Concns of *Trimebutine* (ng/mL)**	**Volume of Stock taken (mL)**	**Volume of Plasma added (mL)**	**Total Volume of Final Solution(mL)**	**Final *Trime-butine* Conc. In human plasma (ng/mL)**
SS1	30031.3643	0.200	9.800	10.000	600.6273
SS2	24025.0914	0.200	9.800	10.000	480.5018
SS3	15135.8076	0.200	9.800	10.000	302.7162
SS4	9081.4846	0.200	9.800	10.000	181.6297
SS5	3087.7048	0.200	9.800	10.000	61.7541
SS6	1543.8524	0.200	9.800	10.000	30.8770
SS7	524.9098	0.200	9.800	10.000	10.4982
SS8	262.4549	0.200	9.800	10.000	5.2491

Stock concentrations may vary depending upon the amount of compound weighed and the purity of the compound.

Preparation of Verapamil (IS) Stock Solution:

Weigh accurately about 2mg of Verapamil hcl and transfer into a 2mL volumetric flask. Dissolve it in Methanol and make up the volume with the same to produce a solution of 1mg /mL strength of Verapamil.

Preparation of Verapamil (IS) Standard Stock Solution Dilutions:

From Verapamil hcl Stock Solution, working Concentration of the Internal Standard Solution (about 100**n**g/mL) were prepared as described in the Table No.3 (below)

TABLE 3 - Preparation of Internal Standard Stock Solution

Initial Stock Conc. (µg/mL)	Volume taken (µl)	Volume of Diluent (mL)	Final Volume(mL)	Final Stock Conc. (µg/mL)
1000000.0000	0.020	1.980	2.000	10000.000
10000.000	1.000	99.000	100.000	100.000

QUALITY CONTROL (QC) SAMPLES:

Preparation of *Trimebutine* QC Samples Dilutions:

From *Trimebutine* Stock Solution, Stock Dilutions ranging 266.4016ng/mL to 23708.9718ng/mL with (Methanol: Water / 50:50), as provided in the Table No.4

Table No. 4 - Stock Dilution *Trimebutine* Quality Control Samples.

Initial Stock Conc. (ng/mL)	Volume of Stock taken (mL)	Volume of Diluent added (mL)	Total Volume of Final Solution (mL)	Final Stock Conc. (ng/mL)
79029.9060	3.000	7.000	10	23708.9718
23708.9718	5.100	4.900	10	12091.5756
12091.5756	2.400	7.600	10	2901.9781
2901.9781	2.700	7.300	10	783.5341
783.5341	3.400	6.600	10	266.4016

Preparation of *Trimebutine* Quality Control Samples in Human Plasma:

From *Trimebutine* QC Stock Dilutions , Quality Control samples in Human K_3ETDA plasma ranging 5.3280ng/mL -474.1794ng/mL for *Trimebutine* as provided in the Table No.5

Table 5- ***Trimebutine*** Quality Control Samples In Human Plasma

Initial Stock Conc. (ng/mL)	**Volume of Stock taken (mL)**	**Volume of Plasma added(mL)**	**Total Volume of Final Solution(mL)**	**Final Stock Conc. (ng/mL)**
23708.9718	0.200	9.800	10.000	474.1794
12091.5756	0.200	9.800	10.000	241.8315
2901.9781	0.200	9.800	10.000	58.0396
783.5341	0.200	9.800	10.000	15.6707
266.4016	0.200	9.800	10.000	5.3280

Sample Preparation:

Extraction Type – Solid Phase Extraction

- Withdraw the CC/QC samples from intended storage area and keep for thawing at room temperature.
- Aliquot 100µL of sample to labeled RIA vials.
- Add 50µL of internal standard (Verapamil, 100ng/mL) into all the samples except blank and vortex.
- Add 100 µL of 0.1M Disodium hydrogen ortho phosphate to all the samples and vortex.
- Add 3 mL of Tert Butyl Methyl Ether into all the samples and cap them.
- The samples were kept in vibramax for 10 minutes at 2000 rpm.
- Then The samples were Centrifuged at 4000 rpm at 4 ºC for 10 min.
- 250 µL of organic layer was transfered into respective labeled RIA vials.
- Kept the samples in Nitrogen evaporator at 40º C and 15 psi until dryness.
- The sample were Reconstitute with 0. 1mL of Mobile phase and vortex.
- The sample was Transfer into inserts in appropriately labeled auto sampler vials and load the samples into LC-MS/MS

***Trimebutine* – Analytical Data Processing**

The unknown concentration of blood sample is calculated from the following equation by utilizing linear regression with $1/x^2$ as weighting factor.

$$\mathbf{Y = mX + b}$$

Where, Y = Peak area ratio of *Trimebutine*

X = Concentration of *Trimebutine*

m = Slope of the Calibration Curve *Trimebutine*

b = Intercept of the Calibration Curve *Trimebutine.*

PHARMACOKINETIC ANALYSIS

Pharmacokinetic analysis was done by Non- compartmental method of analysis using the WinNonlin® Version 5.3.

The following pharmacokinetic parameters were calculated–

Primary pharmacokinetic parameters:

C_{max} :

Maximum measured plasma concentration over the time span specified.

AUC_{0-t} :

The area under the plasma concentration versus time curve, from time zero to the last time point with measurable concentration, calculated by the linear trapezoidal method.

$AUC_{0-\infty}$:

The area under the plasma concentration versus time curve, from time zero to time infinity. $AUC_{0-\infty}$ is calculated as the sum of the AUC_{0-t} plus the ratio of the last measurable concentration to the elimination rate constant.

Secondary Pharmacokinetic parameters:

T_{max}:

Time of maximum measured plasma concentration. If the maximum value occurs at more than one point, T_{max} is defined as the first point with this value in each period.

K_{el}:

Apparent first order elimination or terminal rate constant calculated from a semi-log plot of the plasma concentration versus time curve.

$T_{1/2}$:

Time required for the plasma drug concentration to decrease by one half of the drug.

If pre-dose concentration was found to be less than or equal to 5% of mean $C_{max,}$ the value was considered as such for calculation. If the pre-dose concentration of a subject is more than 5% of C_{max}, the respective subject will be dropped from bioequivalence analysis.

While Pharmacokinetic data is reported, the number of points of the terminal log-linear phase used to estimate the terminal rate constant (K_{el}) will be reported.

AUC_{0-t} shall cover at least 80% of $AUC_{0-\infty}$. Subjects will not be excluded from the statistical analysis if AUC_{0-t} covers less than 80% of $AUC_{0-\infty}$.

STATISTICAL ANALYSIS

The descriptive statistics such as mean, standard deviation, geometric mean and coefficient of variation were reported for the relevant pharmacokinetic parameters, C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ and secondary parameters, T_{max}, $t_{1/2}$ nad K_{el} were estimated for both Test and Reference products.

Analysis of variance (ANOVA):

ANOVA was performed using the SAS® statistical software (version: 9.2) General linear model (GLM) procedure. The Ln-transformed pharmacokinetic parameters (C_{max}, AUC_{0-t} and $AUC_{0-\infty}$) were analyzed using an ANOVA model with the main effects of treatment, period and sequence as fixed effects and subjects nested within sequence as random

A separate ANOVA model was used to analyze each of the parameters. The sequence effect was tested at the 0.10 level of significance using the subjects nested

within the sequence mean square from the ANOVA as the error term. As other main effects were tested at the 0.05 level of significance against the residual error (mean square error) from the ANOVA as the residual error.

Sum of squares (Type III) was reported and probability values (P) were derived from it. For all analyses, effects were considered statistically significant, if the probability associated with "F" was less than 0.05.

90 % Confidence Intervals (CI):

Consistent with the two one-sided tests for bioequivalence, 90% confidence intervals for the difference between the test and reference means was calculated for the untransformed data and log transformed data.

The confidence limits were expressed as percentages of the least square mean (LSM) of the reference product. Using the confidence limits of the above CI and the LSM of the reference product, an approximate 90% CI for the ratio of the test and reference product means was calculated.

Intra-subject variability:

The intra-subject variability for each of the pharmacokinetic parameters reflect the residual variability after accounting for the difference between the subjects, periods, and treatments and was reported in terms of the overall co-efficient of variation (CV%), and separately, from the ANOVA results using both untransformed and log-transformed data using the formula,

Untransformed data = $100^{*} (EMSE-1)^{1/2}$

Where, MSE is mean square error.

Bioequivalence criteria:

Based on the statistical results of 90% confidence intervals of the ratios of the means (Test/Reference) for Ln-transformed pharmacokinetic parameters C_{max}, AUC_{0-t} and $AUC_{0-\infty}$, conclusion was drawn to find whether the test product is bioequivalent to the reference product or not.

Bioequivalence was concluded, if the Test to Reference (T/R) ratios and the 90% confidence interval for the ratios for the means fall within the acceptance range of 80% -125% for the pharmacokinetic parameters, C_{max}, AUC_{0-t} and $AUC_{0-\infty}$.

RESULTS

This single oral dose comparative BA/BE study was undertaken to evaluate the bioequivalence and also, to monitor the adverse events of test product *Trimebutine* 200 mg capsules in healthy subjects.

The study was designed to evaluate and compare the relative bioavailability of test and reference products of *Trimebutine*

In this two period two way cross over study, 48 subjects who met the study inclusion and exclusion criteria were enrolled.

There was a washout period of 5 days between the two periods. The overall duration of the study was 09 days including the wash out period. Blood samples were collected at the predetermined time points to elicit the pharmacokinetic profiles of *Trimebutine.*

In this study, Test and Reference product containing *Trimebutine* were evaluated for the safety upon single dose administration to normal healthy adult male and non- pregnant female subjects under fasting conditions.

Vital parameters measured at the scheduled time intervals were normal and within the acceptable range for all study subjects.

There was no death or serious adverse event reported in this study.

The Plasma concentration level of *Trimebutine* was determined by a validated LC-MS/MS method.

Pharmacokinetic analysis of test and reference products were evaluated based on measured plasma concentration of the drugs using Non compartmental Model of

WinNonlin® v 5.3. Primary pharmacokinetic parameters like C_{max}, AUC_{0-t}, and secondary pharmacokinetic parameters like $AUC_{0-\infty}$, $T_{max,}V_{D,}CL$, K_{el}, and $T_{1/2}$ were calculated.

The statistical analysis was performed using the SAS® statistical software (version:9.2)

Pharmacokinetic Parameters

The pharmacokinetic parameters were estimated by using WinNonlin Software version 5

TABLE-6: Mean values of various pharmacokinetic parameters for Trimebutine

Parameters (Units)	***Trimebutine* (Mean ± SD)**	
	Test	**Reference**
C_{max} (ng/ml)	107.674± 121.571	107.162± 61.015
AUC_{0-t} (ng.h/ml)	139.194± 149.903	135.307± 79.670
$AUC_{0-\infty}$ (ng.h/ml) 155.939± 164.702	.150.772± 83.149	
t_{max} (hr)	0.998± 0.423	0.991 ± 0.497
VD (ml)	3506236.721± 2887193.223	3584623.509±2647885.163
CL (ml/hr)	2196302.925±1805242.163	1824528.769±1196714.197
K_{el} (hr^{-1})	0.711±0.512	0.564 ± 0.232
$T_{1/2}$ (hr)	1.635 ±1.625	1.625±1.396
AUC0-t /AUC0-∞ X 100	12.319±9.400	12.717± 9.609

Trimebutine:

- ✓ **C_{max}-- Peak or maximal Plasma concentration:**

Mean values (±SD) of C_{max} for *Trimebutine* , treatment Test was 107.674± 121.571 ng/ml & for Reference was 107.162± 61.015 ng/ml.

The Test / Reference (T/R) ratio of least square mean of log transformed C_{max} was 104.96 %

with 90% confidence interval LCL=100.79 % and UCL= 109.29 %.

- ✓ **AUC_{0-t} --Area under the concentration-time curve**

Mean values (±SD) of C_{max} for *Trimebutine* , treatment Test was 139.194± 149.903 ng/ml & for Reference was 135.307± 79.670 ng/ml

The Test / Reference (T/R) ratio of least square mean of log transformed AUC_{0-t} was 108.77 % with 90% confidence interval LCL= 106.25 % and UCL= 111.34 %.

- ✓ **$AUC_{0-\infty}$ -- Area under the Concentration-Time curve**

Mean values (±SD) of $AUC_{0-\infty}$ for *Trimebutine,* treatment T was 2077.516± 707.812 ng.h/ml & for Reference was 1907.487± 597.092 ng.h/ml.

The Test / Reference (T/R) ratio of least square mean of log transformed AUC_{0-t} was 108.18 % with 90% confidence interval LCL= 105.63 % and UCL= 110.80 %.

- ✓ **T_{max}:**

Mean values (±SD) of T_{max} for *Trimebutine,* treatment T was 0.998± 0.423 hr & for Reference was 0.991 ± 0.497 hr.

- ✓ **VD(ml):**

Mean values (±SD) of VD for *Trimebutine,* treatment T were 3506236.721±2887193.223 & for treatment R were 3584623.509±2647885.163 ng.h/ml .

- ✓ **CL(ml/hr):**

Mean values (±SD) of CL for *Trimebutine,* treatment T were 2196302.925±1805242.163& for treatment R were 1824528.769±1196714.197 ng.h/ml.

- ✓ **Kel(hr-1):**

Mean values (±SD) of Kel for *Trimebutine,* treatment T were 0.711±0.512 & for treatment R were 0.564 ± 0.232.

- ✓ **T1/2(hr):**

Mean values (±SD) of T1/2 for *Trimebutine,* treatment T were 1.635 ±1.625 & for treatment R were 1.625±1.396.

- ✓ **AUC0-t /AUC0-∞ X 100 % :**

Mean values (±SD) of AUC0-t /AUC0-∞ X 100 for *Trimebutine,*

Treatment T were 12.319±9.400 & for treatment R were 12.717± 9.609 .

TABLE 7- LATIN SQUARE DESIGN: ANOVA TABLE

Maximum Plasma Concentration (C_{max}):

Source	DF	Type III SS	Mean Square	F Value	Pr > F
FORMULATION	1	0.14659822	0.14659822	0.90	0.3479
SEQUENCE	1	0.02781272	0.02781272	0.04	0.8455
PERIOD	1	0.14013784	0.14013784	0.86	0.3587
SUBJECT(SEQUENCE)	45	32.59297900	0.72428842	4.45	<.0001

Table-7 shows the Latin square design using ANOVA for $C_{max.}$ The Subject (Sequence) effect show statistically significant difference between the test and reference products. The sequence effect, period effect and formulation effect does not show any statistically significant difference between the test and reference products.

Formulation effects was non-significant (at 5 % level of significance), when ANOVA was applied on Ln-transformed data for C_{max} of *Trimebutine.*

Sequence effects was non-significant (at 10 % level of significance), when ANOVA was applied on Ln-transformed data for C_{max} of *Trimebutine.*

Period effects was non-significant (at 5 % level of significance), when ANOVA was applied on Ln-transformed data for C_{max} of *Trimebutine.*

Subject (Sequence) effects was significant (at 5 % level of significance), when ANOVA was applied on Ln-transformed data for C_{max} of *Trimebutine*. Such significant effects do not have impact on the study results.

TABLE 8

C_{max}

	Mean	**SD**	Min	Max
Test				
Reference				

Table 8 shows maximum, minimum, mean and SD values of C_{max} for both Test and Reference products.

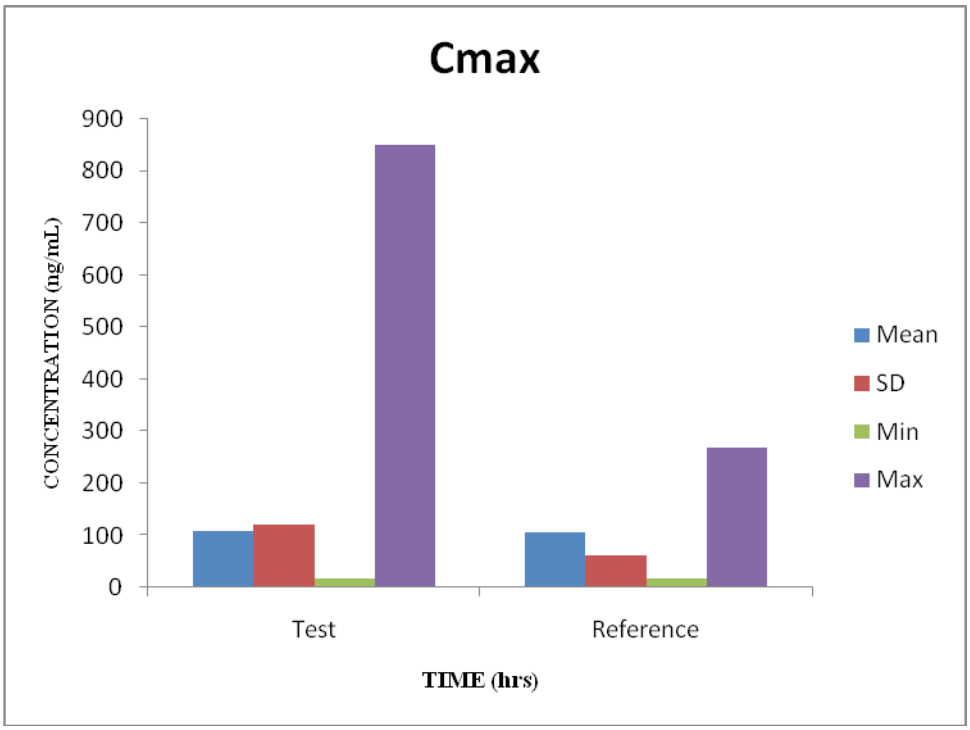


Figure 7 shows the graphical representation of table 8

TABLE 9- LATIN SQUARE DESIGN: ANOVA TABLE

Area under the curve (AUC_{0-t}):

Source	DF	Type III SS	Mean Square	F Value	Pr > F
FORMULATION	1	0.18986900	0.18986900	1.58	0.2158
SEQUENCE	1	0.03913736	0.03913736	0.04	0.8361
PERIOD	1	0.96165336	0.96165336	7.98	0.0070
SUBJECT(SEQUENCE)	45	40.66622420	0.90369387	7.50	<.0001

Table-9 shows the Latin square design using ANOVA for $AUC_{0-t.}$ The period effect and Subject (Sequence) effect show statistically significant difference between the test and reference products. Formulation effect and Sequence effect does not show any statistically significant difference between the test and reference products.

Formulation effects was non-significant (at 5 % level of significance), when ANOVA was applied on Ln-transformed data for AUC_{0-t} of *Trimebutine.*

Sequence effects was non-significant (at 10 % level of significance), when ANOVA was applied on Ln-transformed data for AUC_{0-t} of *Trimebutine.*

Period effects was significant (at 5 % level of significance), when ANOVA was applied on Ln-transformed data for AUC_{0-t} of *Trimebutine*. Such significant effects do not have impact on the study results.

Subject (Sequence) effects was significant (at 5 % level of significance), when ANOVA was applied on Ln-transformed data for AUC_{0-t} of *Trimebutine*. Such significant effects do not have impact on the study results.

TABLE 10

AUC_{0-t}

	Mean	**SD**	Min	Max
Test				
Reference				

Table 10 shows maximum, minimum, mean and SD values of AUC_{0-t} for both Test and Reference products.

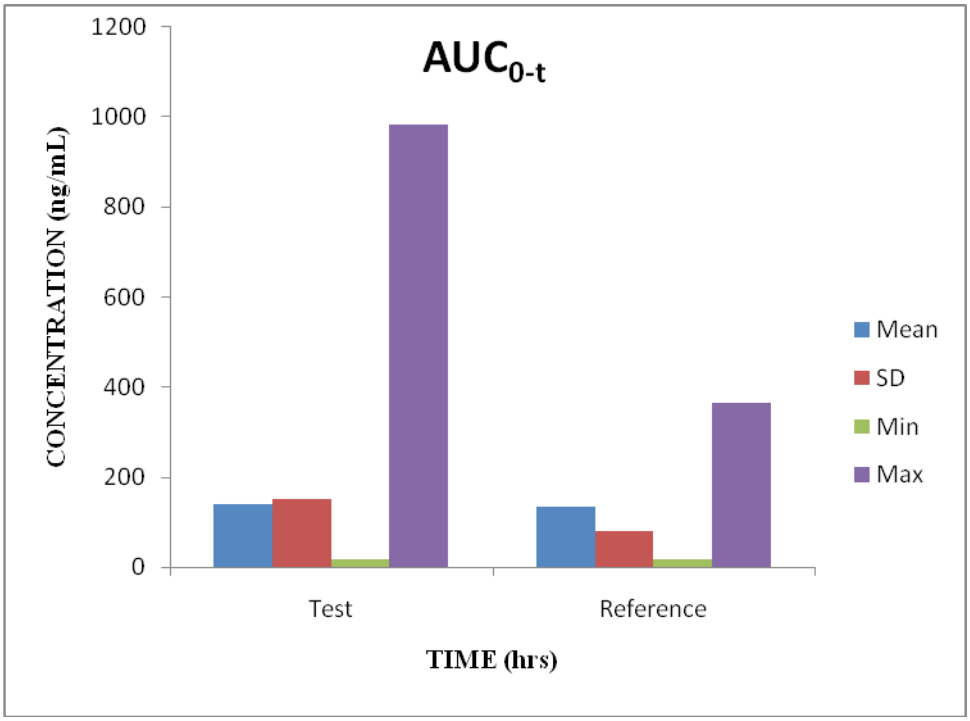


Figure 8 shows the graphical representation of table 10

TABLE 11

$AUC_{0-\infty}$

	Mean	**SD**	Min	Max
Test				
Reference				

Table 11 shows maximum, minimum, mean and SD values of $AUC_{0-\infty}$ for both Test and Reference products.

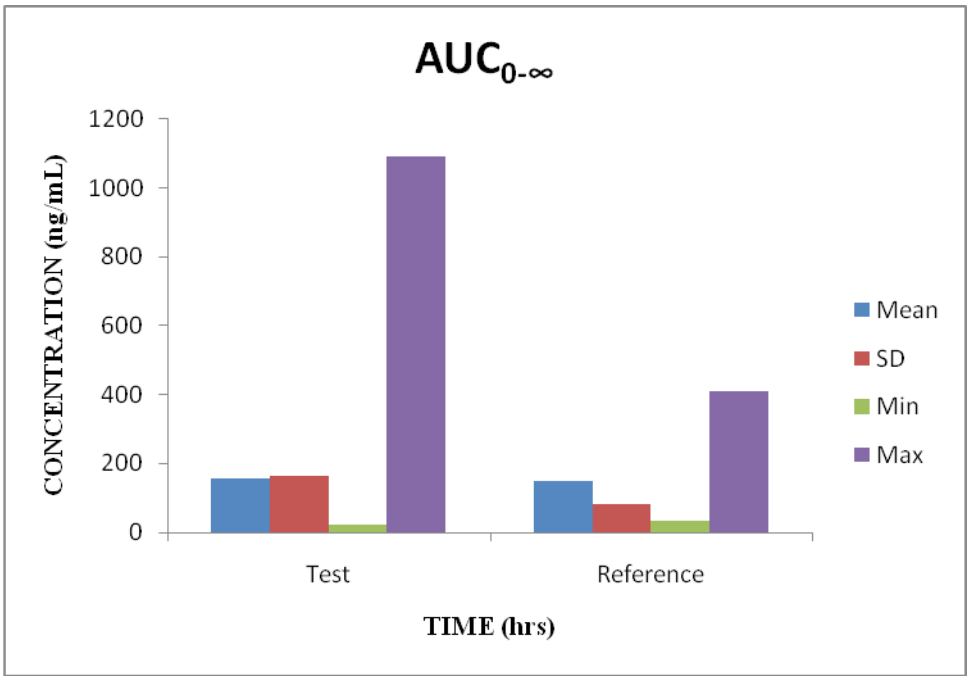


Figure 9 shows the graphical representation of table 11

TABLE 12

TIME OF MAXIMUM MEASURED PLASMA CONCENTRATION

Tmax

	Mean	**SD**	Min	Max
Test				
Reference				

Table 12 shows maximum, minimum, mean and SD values of T_{max} for both Test and Reference products.

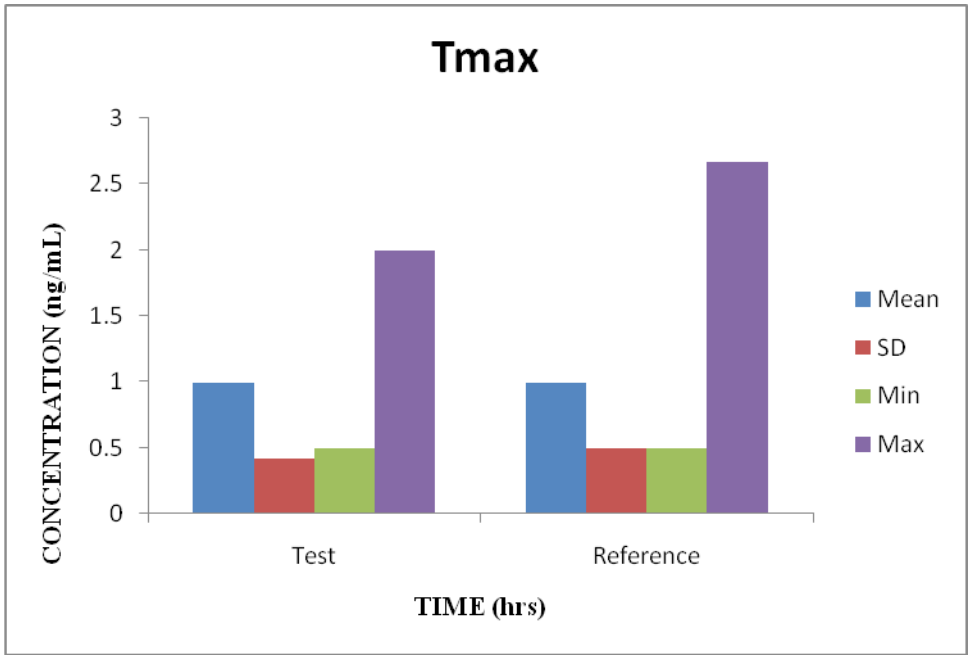


Figure 10 shows the graphical representation of table 12

TABLE 13

Volume of Distribution (VD)

	Mean	**SD**	Min	Max
Test				
Reference				

Table 13 shows maximum, minimum, mean and SD values of VD for both Test and Reference products.

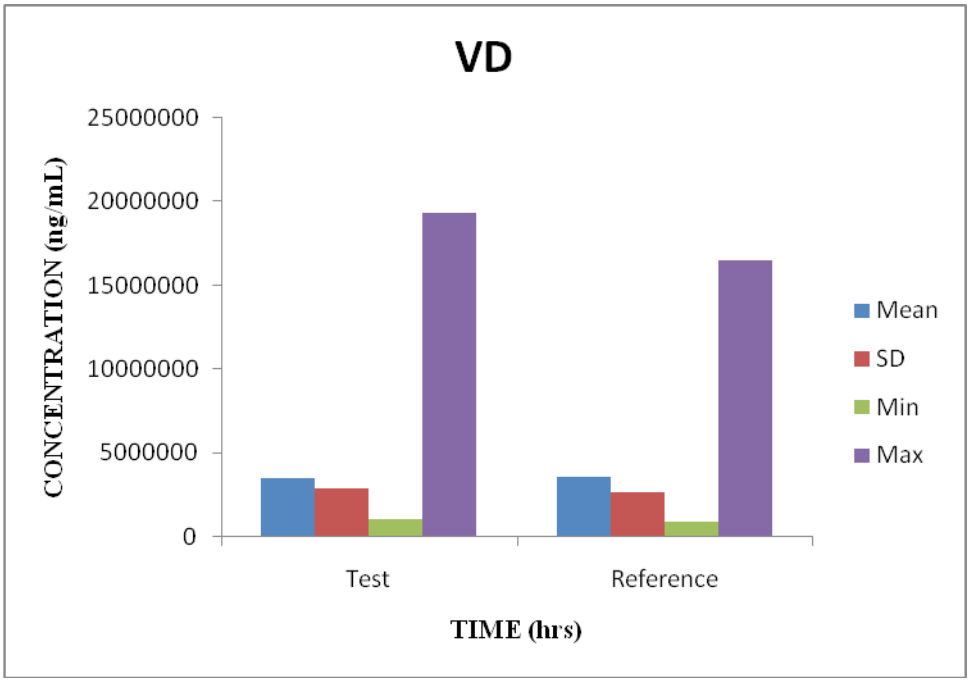


Figure 11 shows the graphical representation of table 13

TABLE 14

Clearance (CL)

	Mean	**SD**	Min	Max
Test	2196302.925	1805242.163	183390.68	8623189.61
Reference	1824528.769	1196714.197	489692.34	6169700.36

Table 14 shows maximum, minimum, mean and SD values of CL for both Test and Reference products.

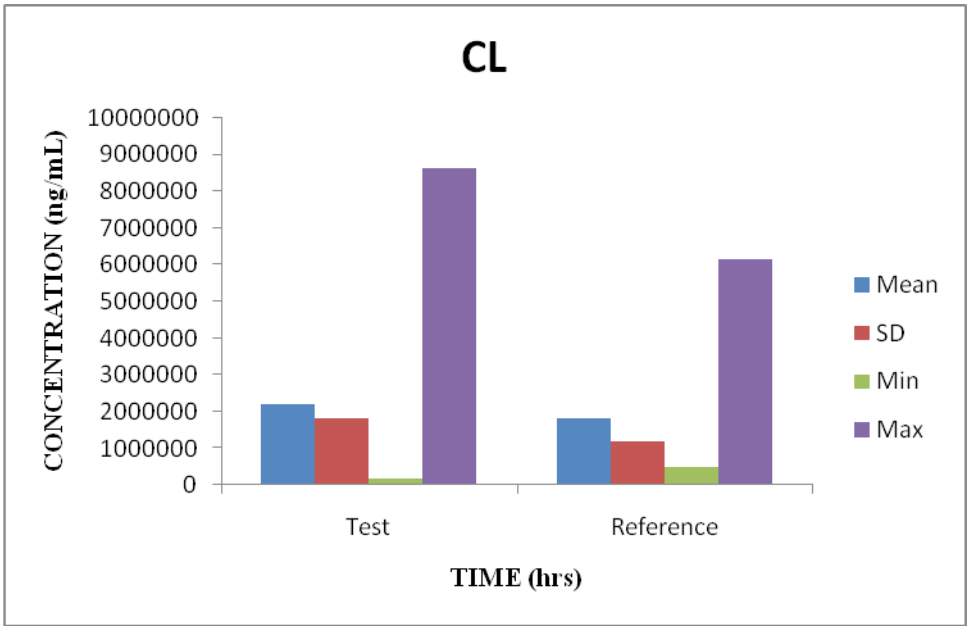


Figure 12 is the graphical representation of table 14

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TABLE 15

Kel

	Mean	**SD**	Min	Max
Test				
Reference				

Table 15 shows maximum, minimum, mean and SD values of Kel for both Test and Reference products.

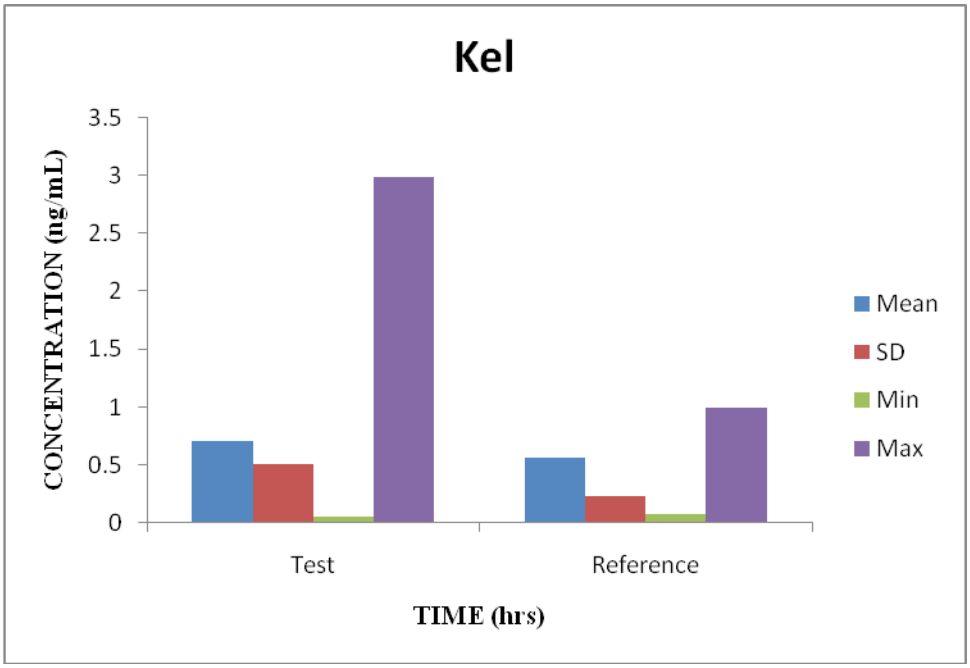


Figure 13 shows the graphical representation of table 15

TABLE 16

Thalf

	Mean	**SD**	Min	Max
Test				
Reference				

Table 16 shows maximum, minimum, mean and SD values of T_{half} for both Test and Reference products.

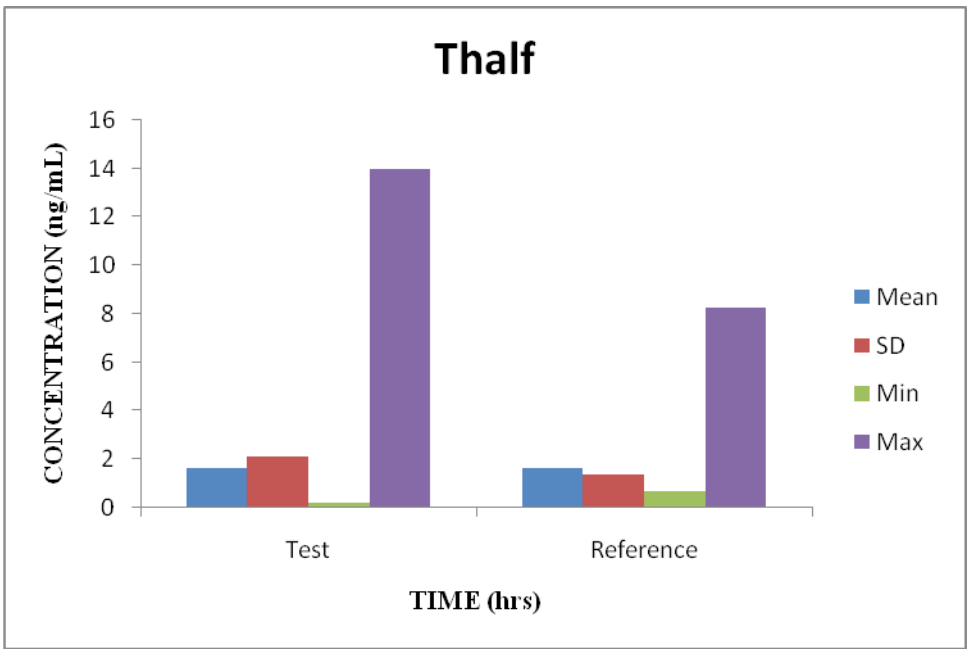
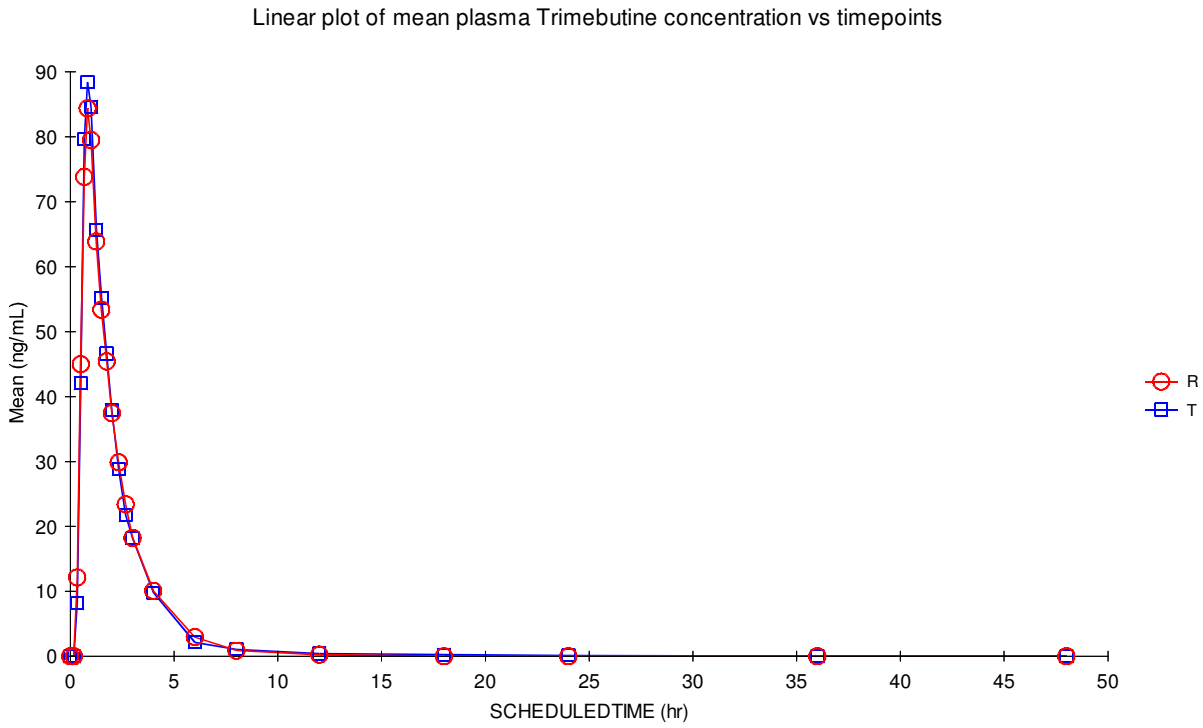


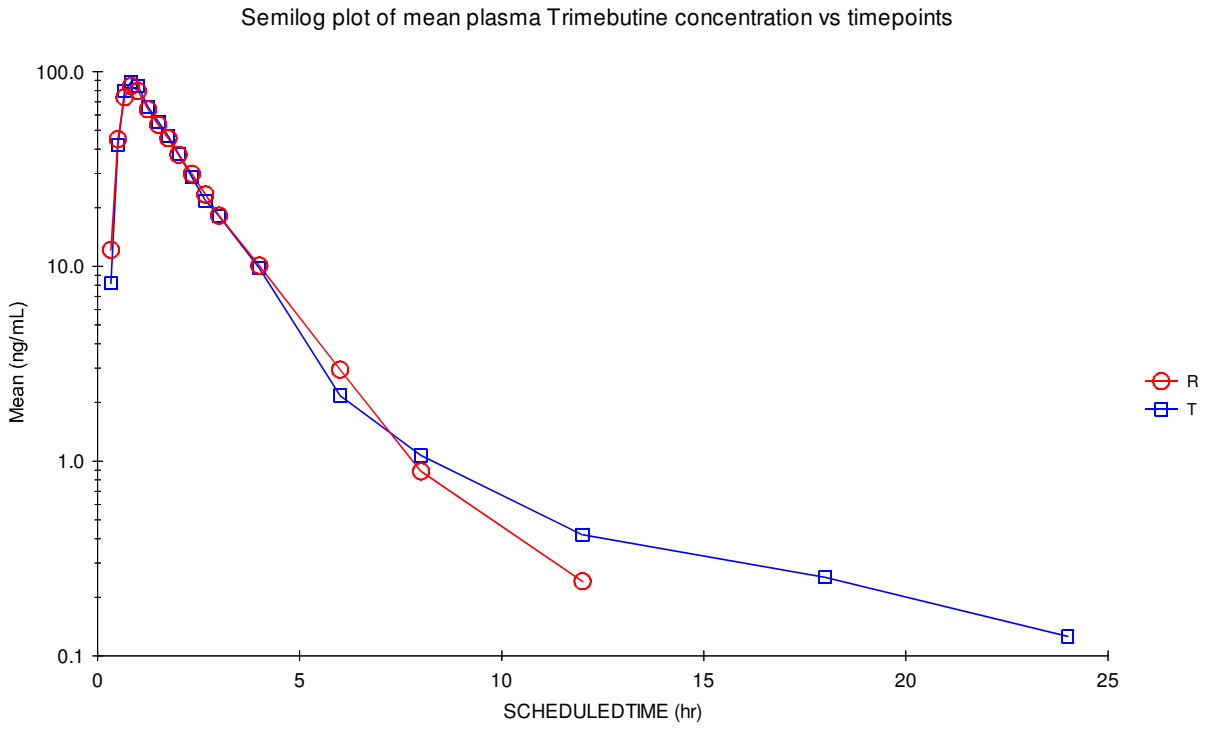
Figure 14 shows the graphical representation of table 16

Figure 15: LINEAR PLOT OF MEAN PLASMA *TRIMEBUTINE* CONCENTRATION VS TIMEPOINTS



The above Figure-15 shows the Means Plasma Concentration Vs various time points of both the Test and Reference products (*Trimebutine*).

Figure 16: SEMILOG PLOT OF MEAN PLASMA *TRIMEBUTINE* CONCENTRATION VS TIME POINTS



The above Figure-16 shows the Semilog plot of Mean Plasma Concentration Vs various time points of both the Test and Reference products (*Trimebutine*).

MEAN PLASMA CONCENTRATION:

Time (hours)	**TEST**	**REFERENCE**
0.00	0.0000	0.0000
0.17	0.0000	0.0000
0.33	7.3773	10.9368
0.50	37.8521	40.4967
0.67	71.7015	66.4532
0.83	79.6067	75.9546
1.00	76.1783	71.5594
1.25	59.1450	57.4993
1.50	49.7126	48.0618
1.75	41.9555	40.8818
2.00	34.1117	33.7060
2.33	25.9538	26.8831
2.67	19.5778	21.0533
3.00	16.3626	16.3978
4.00	8.8304	9.0843
6.00	1.9514	2.6540
8.00	0.9628	0.7977
12.00	0.3765	0.2172
18.00	0.2279	0.0000
24.00	0.1132	0.0000
36.00	0.0000	0.0000
48.00	0.0000	0.0000

DISCUSSION

Bioequivalance studies are fundamentally satisfied through single dose administration. The focus is on the rate and extent of absorption of the active ingredient. They are conducted in healthy normal subjects under fasting or fed conditions. Most comparative bioavailability studies are conducted to identify the quantitative with intravenous dose, modified release with conventional preparation and for a generic product, test and reference.

Nowadays, bioequivalence studies are a pivotal part of registration dossiers. These studies measure the bioavailability of two more formulations of the same active ingredient. The purpose of the study is to show the bioavailability of the formulations under investigation is equal. Based on the conclusion that the therapeutic qualities of these formulations are identical, these formulations can be interchangeable.

In this study, Test and Reference product containing *Trimebutine* 200 mg capsules were evaluated for the safety upon single dose administration to normal healthy adult male and non pregnant females' subjects under fasting conditions.

There was a washout period of 5 days between the two periods. The overall duration of the study was 09 days including the wash out period. Blood samples were collected at the predetermined time points to elicit the pharmacokinetic profiles of *Trimebutine*

Vital parameters measured at the scheduled time intervals were normal and within the acceptable range of all study subjects.

This study was conducted with *Trimebutine* 200 mg (test) and Digedrat 200 mg (reference) to establish the drugs concentration versus time profile and the results of the pharmacokinetic analysis of *Trimebutine* of the test (T) product were compared with the reference(R) product.

Analysis of variance for Ln-transformed pharmacokinetic parameters revealed that there was no significant variation between test and reference formulation for these three primary pharmacokinetic parameters C_{max} and $AUC_{0-t,}$ & $AUC_{0-\infty.}$

C_{max}:

The statistical analysis did not show any significant difference between the groups. The geometric mean ratio was within the limits of reference confidence interval which was found to be 100.79 % to109.29 %. (80 to 125%). This confirms the bioequivalence of the products.

AUC $_{0-t}$:

The statistical analysis did not show any significant difference between the groups. The geometric mean ratio was within the limits of reference confidence interval that was found to be 106.25 % to 111.34 % (80 to 125%). Therefore, bioequivalence can be concluded.

AUC $_{0-\infty}$:

The statistical analysis did not show any significant difference between the groups. The geometric mean ratio was within the limits of reference confidence

interval which was found to be 105.63 % to110.80 % (80 to 125%), which confirms bioequivalence.

90% Confidence Interval:

The 90% confidence intervals of the T/R ratio of Ln- transformed C_{max} and $AUC_{0-t,}$ $AUC_{0-\infty}$ were within the bioequivalence range of 80%-125%.

Post study assessment of the hematological and biochemical parameters showed no significant changes on comparing with the respective baseline parameters.

The above parameters are similar which suggested that *Trimebutine* (Test) and Digedrat (Reference) were bioequivalent.

SUMMARY AND CONCLUSION

The study, *Trimebutine* 200 mg (test) and Digedrat 200 mg (reference) capsules were evaluated for the safety upon single dose administration to normal healthy adult male and non pregnant females subjects under fasting conditions.

There was a washout period of 5 days between the two periods. The overall duration of the study was 09 days including the wash out period.

This study was conducted to establish the drugs concentration versus time profile and the results of the pharmacokinetic analysis of *Trimebutine* of the test(T) product were compared with the reference(R) product.

The Means Plasma Concentration Vs various time points of both the Test and Reference products (*Trimebutine*), were similar,at all the scheduled time points.

Analysis of variance for Ln-transformed pharmacokinetic parameters revealed that there was no significant variation between test and reference formulation for these three primary pharmacokinetic parameters C_{max} and $AUC_{0-t,}$ & $AUC_{0-\infty.}$

The 90% confidence intervals of the T/R ratio of Ln- transformed $C_{max,}$ and AUC_{0-t}, $AUC_{0-\infty}$ were within the bioequivalence range of 80%-125%.

In conclusion, in the present study, *Trimebutine* 200 mg capsule is bioequivalent to Digedrat (*Trimebutine)* 200 mg tablet in 48 healthy subjects under fasting conditions. *Trimebutine* 200 mg capsule was well tolerated.

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